Ellefson and McNally, http://www.jcb.org/cgi/content/full/jcb.201104008/DC1

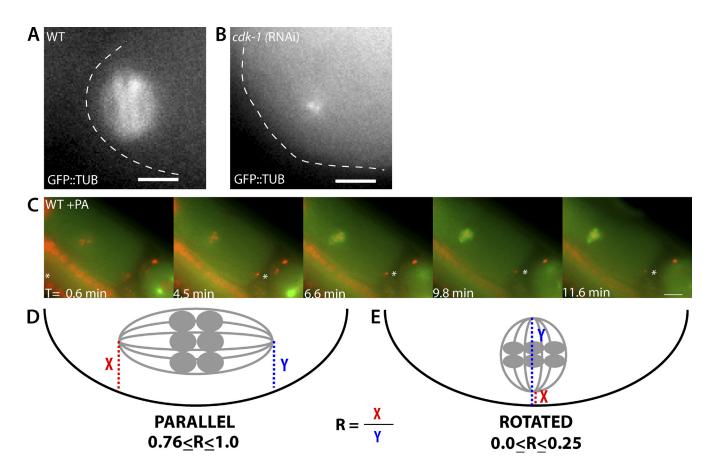


Figure S1. cdk-1(RNAi) prevents meiotic spindle assembly. (A and B) A single time point from a live time-lapse sequence of embryos expressing GFP::tubulin showing an example of a parallel metaphase-I meiotic spindle in a wild-type (WT) worm (A) and a cdk-1(RNAi) embryo in which the metaphase-I meiotic spindle does not form (B). Identical results were obtained for five out of five cdk-1(RNAi) worms. The embryo cortex is outlined for clarity. (C) A live time-lapse sequence of a wild-type meiotic embryo expressing GFP::tubulin and mCherry::histone treated with PA in which nuclear envelope breakdown already occurred but the embryo fails to ovulate. T = 0 is when the film started, and the asterisks mark the spermatheca. Bars, 5 µm. (D and E) Schematics depicting the calculation of the rotation index (R). Measurements are taken of the distance between each spindle pole and closest point on the cortex (X and Y). The rotatio of those distances (X/Y), in which X is the smaller distance measured, is the rotation index. (D) A parallel spindle's pole-to-cortex distances are such that X and Y are similar and will give a value for R closer to 1, with the cutoff for a parallel spindle being a spindle in which $0.76 \le R \le 1.0$. (E) A rotated spindle's pole-to-cortex distances are such that one spindle pole is much closer to the cortex than the other, giving a much smaller distance for X compared with Y, which results in a value for R closer to 0, with the cutoff for a rotated spindle being a spindle in which $0.00 \le R \le 0.00$.

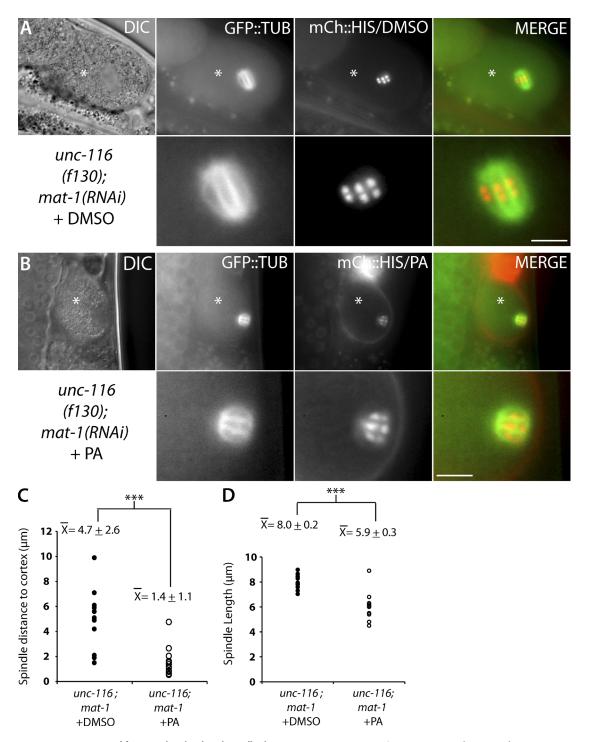


Figure S2. **UNC-116** is not required for purvalanol-induced spindle shortening or rotation. (A and B) Live images of unc-116(f130);mat-1(RNAi) embryos expressing mCherry::histone and GFP::tubulin in utero after treatment with DMSO (A) or PA (B). The bottom panels are magnified images of the top panels. Asterisks mark the +1 embryo. DIC, differential interference contrast. Bars, $5 \mu m$. (C) Quantification of distance between the meiotic spindle and embryo cortex in unc-116(f130);mat-1(RNAi) embryos after treatment with DMSO (closed circles; n=12) or PA (open circles; n=16). The y axis is the distance (micrometers) from the meiotic spindle to the closest point of the embryo cortex. Each data point represents a different embryo. (D) Quantification of meiotic spindle length in unc-116(f130);mat-1(RNAi) embryos after treatment with DMSO (closed circles; n=12) or PA (open circles; n=13). Each data point represents a different meiotic spindle. The \pm SEM is rounded to the nearest tenth. ***, P < 0.0005.

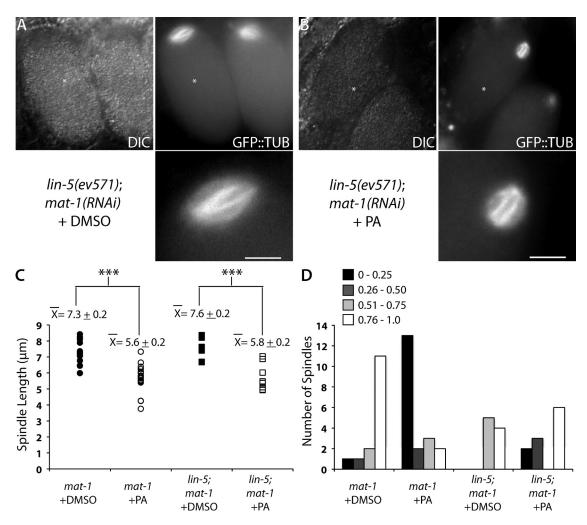


Figure S3. **LIN-5** is required for PA-induced meiotic spindle rotation. (A and B) Live images of lin-5(ev571);mat-1(RNAi) embryos expressing GFP::tubulin in utero after treatment with DMSO (A) or PA (B). The bottom panels are magnified images of the top panels. Asterisks mark the +1 embryo. DIC, differential interference contrast. Bars, $5 \, \mu m$. (C) Quantification of meiotic spindle length in lin-5(ev571);mat-1(RNAi) meiotic embryos after treatment with DMSO (closed squares; n=9) or PA (open squares; n=11) compared with mat-1(RNAi) embryos. Data for mat-1(RNAi) embryos are from Fig. 1. Each data point represents an individual meiotic spindle. The \pm SEM is rounded to the nearest tenth. ***, P < 0.0005. (D) Quantification of the rotation index in lin-5(ev571);mat-1(RNAi) meiotic embryos after treatment with DMSO (n=9) or PA (n=11) compared with mat-1(RNAi) embryos. The y axis is the number of meiotic embryos. The graph represents the rotation indices calculated for all of the spindles imaged in multiple experiments.

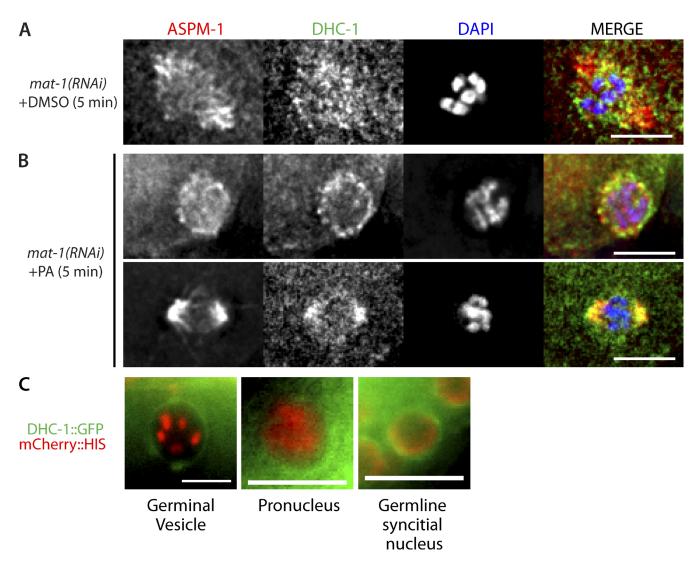


Figure S4. **DHC-1 relocalizes to meiotic spindle poles after 5 min of PA treatment.** (A and B) Immunostaining in fixed meiotic mat-1(RNAi) embryos of ASPM-1 and DHC-1::GFP costained with DAPI showing their localization to meiotic spindles after 5 min of incubation in DMSO (A) or PA (B). (A) Metaphase-I-arrested meiotic embryos with a 5-min incubation in DMSO showed no colocalization of ASPM-1 and DHC-1 (n = 5/5), and treatment with PA resulted in colocalization of ASPM-1 and DHC-1 (B; n = 7/7). However, the 5-min incubation in PA resulted in the colocalization of ASPM-1 and DHC-1 in a pattern resembling the circular nuclear envelope in six out of seven embryos, with only one embryo showing colocalization specifically at the spindle poles (B, bottom). (C) Examples of DHC-1::GFP localization to the nuclear periphery of different nuclei within a wild-type worm. Bars, 5 μ m.

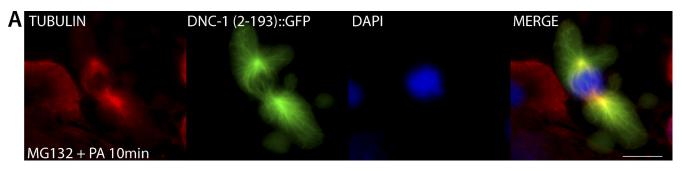


Figure S5. Inhibition of CDK-1 leads to mitotic exit in MG132-arrested cells. (A) Still images of an MG132 metaphase-arrested Xenopus A6 cell transfected with DNC-1(2–193)::GFP, treated with PA for 10 min, and also stained for tubulin and DAPI. n = 4/4. Bar, $5 \mu m$.