Supplemental Materials

Molecular Biology of the Cell

Connolly et al.

Supplemental Figure Legends:

Figure S1: TS gain-of-function mutant *bmk-1(or627*ts*)* has a defective meiotic spindle. (A) Partial sequence alignments of orthologs from *C. elegans Homo sapiens (Hs), Drosophila melanogaster (Dm),* and *Saccharomyces cerevisiae (Sc)* with the wild-type and mutant *Caenorhabditis elegans (Ce)* BMK-1. Arrowheads indicate altered residues, with wild-type amino acids in black and mutant amino acids in red. **(**B) Nomarski image of one-cell stage mutant embryos. Embryo is positioned with the anterior (maternal) and posterior (paternal) pronuclei to the left and right. Note the presence of extra maternal pronuclei in *bmk-1(or627)* (arrowheads). (C) One cell Time-lapse spinning disc confocal images from immobilized worms were recorded during Meiosis I in wild-type and mutant zygotes expressing mCherry::Histone2B and GFP::β-tubulin to mark chromosomes and microtubules, respectively, from ovulation to polar body extrusion. Anterior is to the left, times indicated are relative to ovulation, and a white dashed line marks the edge of the zygote plasma membrane. In this and subsequent figures, each image shown is a projection of 6 consecutive frames taken at 1.5 µm intervals in a z-stack for each time point. See the text for details.

Figure S2. Assembly of monopolar oocyte meiotic spindles in *klp-18* mutant requires both the microtubule severing activity of MEI-1 and ASPM-1. Spinning disc confocal images were recorded over time during Meiosis I in live mutant embryos expressing mCherry:Histone2B and GFP:β-tubulin translational fusions to mark chromosomes and microtubules, respectively. Indicated time points begin at ovulation.

A white dashed line marks the edge of the plasma membrane. The 5 μm bar is drawn to scale.

Figure S3. *mei-1* is required to recruit Dynein to the spindle poles. Spinning disc confocal images weres recorded over time during Meiosis I in live mutant embryos expressing GFP:DHC-1 translational fusion to mark dynein (O'Rourke et al., 2007). Indicated time points begin at ovulation. A white dashed line marks the edge of the plasma membrane. The 5 μm bar is drawn to scale.

Supplemental Movie 1: Wild type oocytes assemble small bipolar spindles that compact into a rosette structure before rotating toward the plasma membrane and segregating have the chromosomal content during anaphase. In this and all subsequent supplemental movies, unless otherwise noted, spinning disc confocal images were recorded over time during Meiosis I beginning at ovulation in live oocytes expressing mCherry:Histone2B and GFP:β-tubulin translational fusions to mark chromosomes and microtubules, respectively; images were collected every 10 seconds and are played back in 7x real time.

Supplemental Movie 2: *aspm-1(or645*ts*)* oocytes assemble long bipolar oocyte meiotic spindles with unfocused pole ends and aberrantly organized chromosomes.

Supplemental Movie 3: *aspm-1(RNAi)* oocytes assemble long bipolar oocyte meiotic spindles with unfocused pole ends and aberrantly organized chromosomes.

Supplemental Movie 4: Wild type embryos expressing GFP:MEI-1 and mCherry::Histone2B translational fusions to mark spindle poles and chromosomes, respectively.

Supplemental Movie 5: *aspm-1(RNAi)* mutant embryos express GFP:MEI-1 and mCherry:Histone2B translational fusions to mark spindle poles and chromosomes, respectively. *aspm-1(RNAi)* spindle poles initially appear fragmented but later coalesce into more focused poles resembling those observed in wild-type embryos.

Supplemental Movie 6: *mei-1(or1178)* oocytes assemble disorganized and apolar spindles, frequently extruding the all of the chromosomes into the Meiosis I polar body.

Supplemental Movie 7: *mei-1(RNAi)* oocytes assemble disorganized and apolar spindles, frequently extruding the all of the chromosomes into the Meiosis I polar body.

Supplemental Movie 8: *klp-18(or447*ts*)* oocytes assemble monopolar spindles, with chromosomes encompassing a large area upon ovulation before moving into a small area, frequently extruding all of the chromosomes into the Meiosis I polar body. This strain expresses GFP:H2B Histone only.

Supplemental Movie 9: *klp-18(RNAi)* oocytes assemble monopolar spindles, with chromosomes encompassing a large area upon ovulation before moving into a small area, frequently extruding all of the chromosomes into the Meiosis I polar body.

Supplemental Movie 10: Wild type embryos expressing GFP:ASPM-1 and mCherry::Histone2B translational fusions to mark spindle poles and chromosomes, respectively.

Supplemental Movie 11: In *mei-1(RNAi)* oocytes, GFP:ASPM-1 is largely absent with no spindle pole detected. Spinning disc confocal images were taken over time during Meiosis I in live oocytes expressing GFP:ASPM-1 and mCherry:Histone2B translational fusions to mark spindle poles and chromosomes, respectively

Supplemental Movie 12: In *klp-18(RNAi)* oocytes, GFP:ASPM-1 marks a single pole. Spinning disc confocal images taken over time during Meiosis I in live oocytes expressing GFP:ASPM-1 and mCherry:Histone2B translational fusions to mark spindle poles and chromosomes, respectively.

Supplemental Movie 13: *mei-1(or1178);klp-18(RNAi)* oocytes assemble disorganized and apolar spindles, frequently extruding the all of the chromosomes into the Meiosis I polar body. These double mutants resemble *mei-1(-)* single mutants in phenotype.

Supplemental Movie 14: *aspm-1(or645);klp-18* oocytes assemble monopolar spindles, with chromosomes encompassing a large area upon ovulation before moving into a small area, and all chromosomes are frequently extruded into the Meiosis I polar body. These double mutants resemble *klp-18(-)* single mutants in phenotype.

Supplemental Movie 15: *mei-1(ct46ct103);klp-18(RNAi)* mutant oocytes assemble defective spindles with a phenotype intermediate to those of *klp-18(-)* and *mei-1(-)* mutants.

Supplemental Movie 16: *tba-2(sb27);tbb-2(sb26); klp-18(RNAi)* mutant oocytes assemble defective spindles with a phenotype intermediate to those of *klp-18(-)* and *mei-1(-)* mutants.

Supplemental Movie 17: *mei-1(ct46ct103);aspm-1(RNAi);klp-18(RNAi)* mutant oocytes assemble defective spindles that closely resemble the apolar spindle phenotype of *mei-1(-)* mutants.

Supplemental Movie 18: *aspm-1(RNAi);tba-2(sb27);tbb-2(sb26);klp-18(RNAi)* mutant oocytes assemble defective spindles that closely resemble the apolar spindle phenotype of *mei-1(-)* mutants.

Supplemental Movie 19: Microtubule severing defective *mei-1(ct46ct103)* mutant oocytes assemble abnormal but bipolar oocyte meiotic spindles.

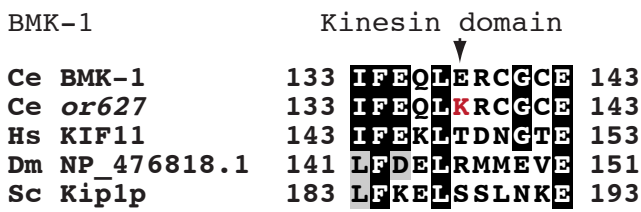
Supplemental Movie 20: Microtubule severing defective *tba-2(sb27);tbb-2(sb26)* mutant oocytes assemble abnormal but bipolar oocyte meiotic spindles.

Supplemental Movie 21: *mei-1(ct46ct103);aspm-1(RNAi)* mutants assemble defective spindles that closely resemble the apolar spindle phenotype of *mei-1(-)* mutants.

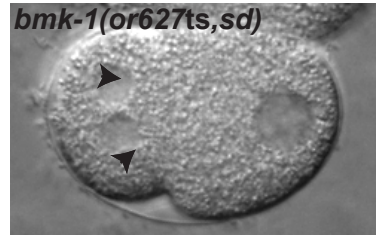
Supplemental Movie 22: *tba-2(sb27);tbb-2(sb26);aspm-1(RNAi)* mutants assemble defective spindles that closely resemble the apolar spindle phenotype of *mei-1(-)* mutants.

Figure S1

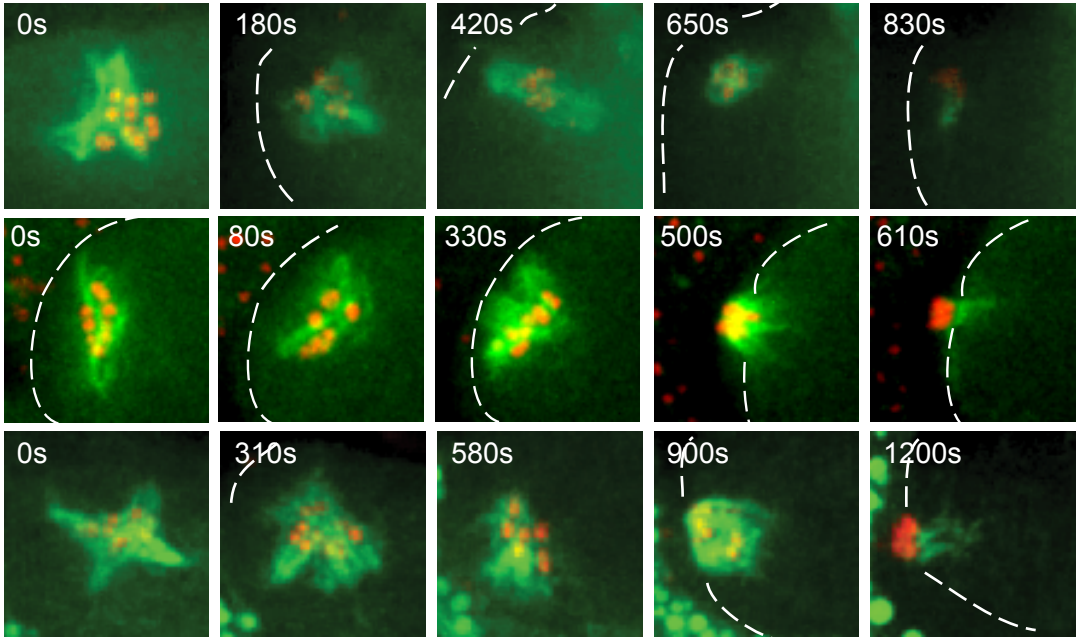
A



B



C



GFP:β-tubulin mCherry:H2B histone

Figure S2

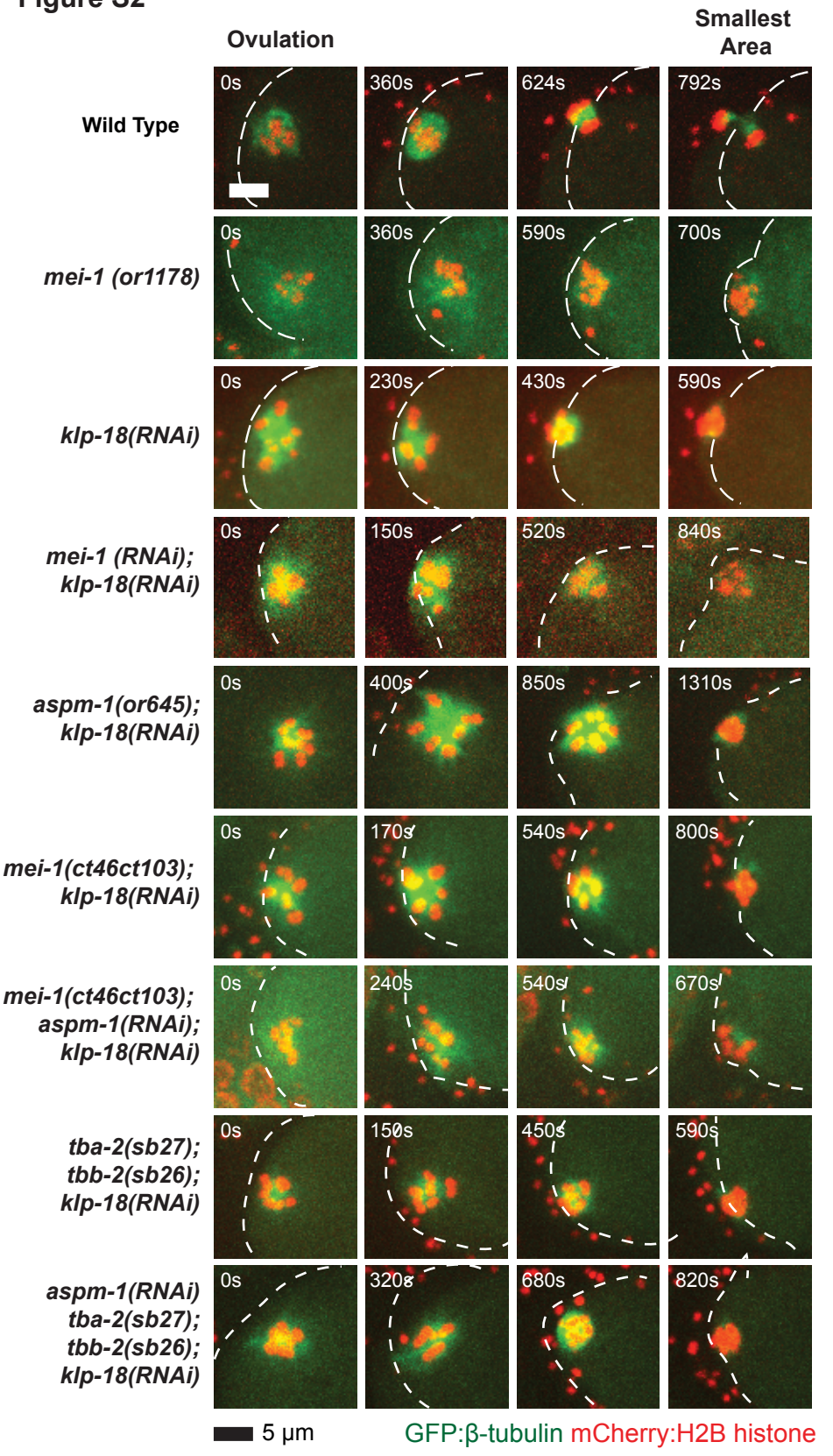
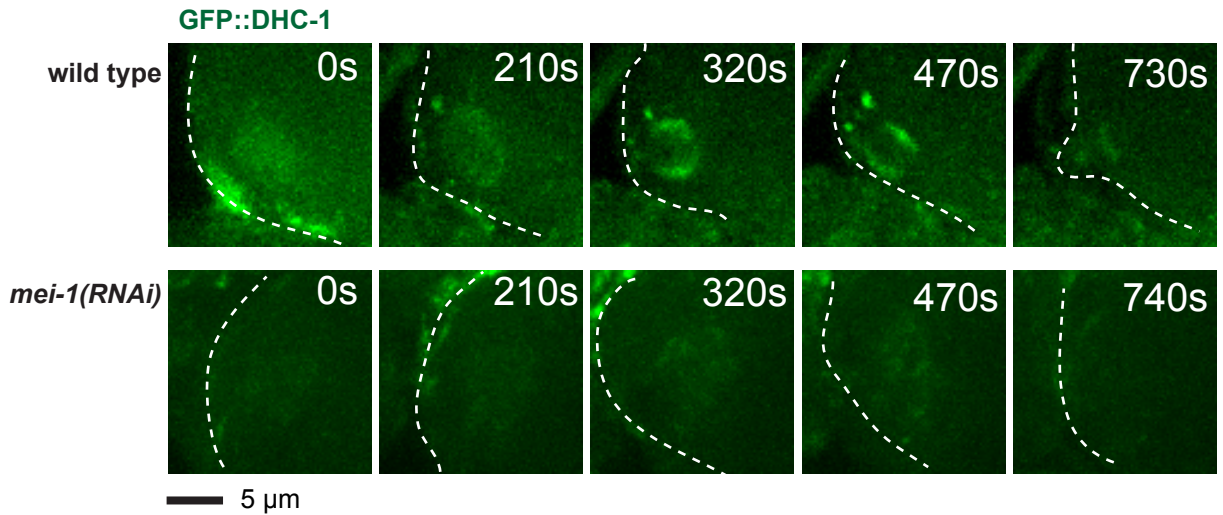

Ovulation
Smallest Area
Wild Type
0s
360s
624s
792s
mei-1 (or1178)
0s
360s
590s
700s
klp-18(RNAi)
0s
230s
430s
590s
mei-1 (RNAi); klp-18(RNAi)
0s
150s
520s
840s
aspm-1(or645); klp-18(RNAi)
0s
400s
850s
1310s
mei-1(ct46ct103); klp-18(RNAi)
0s
170s
540s
800s
mei-1(ct46ct103); aspm-1(RNAi); klp-18(RNAi)
0s
240s
540s
670s
tba-2(sb27); tbb-2(sb26); klp-18(RNAi)
0s
150s
450s
590s
aspm-1(RNAi) tba-2(sb27); tbb-2(sb26); klp-18(RNAi)
0s
320s
680s
820s
5 µm
GFP:β-tubulin mCherry:H2B histone

Figure S3


GFP::DHC-1
wild type
0s
210s
320s
470s
730s
mei-1(RNAi)
0s
210s
320s
470s
740s
5 µm