Supplemental Materials

Molecular Biology of the Cell

Heath and Wignall

SUPPLEMENTARY MATERIALS

6 Supplemental Figures

Supplemental Movie Legends

Figure S1

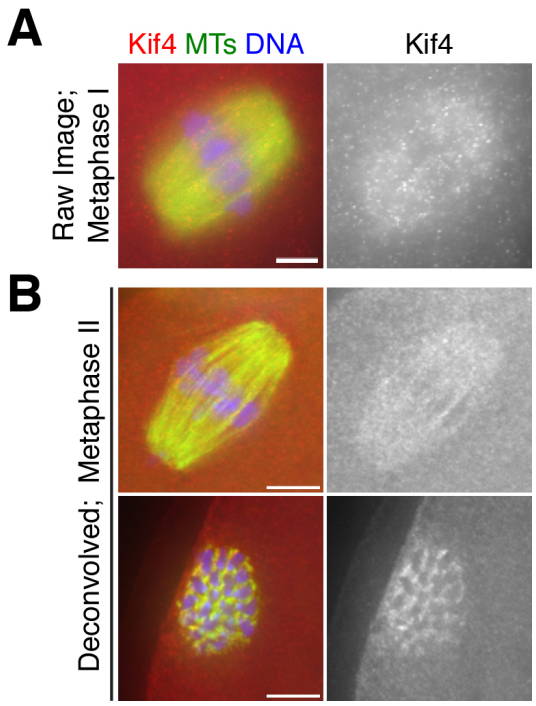

Figure S1. Fixation of oocytes with formaldehyde does not preserve Kif4's chromosomal localization.

A) Metaphase I spindle fixed with formaldehyde and stained for Kif4 (red), microtubules (green), and DNA (blue), showing diffuse Kif4 localization to the spindle. A single z-slice from a raw (not deconvolved) image is shown since the signal is weak and largely goes away with normal deconvolution steps. Chromosomal staining is not apparent in spindles fixed with this method.

B) Metaphase II spindles fixed with formaldehyde and stained for Kif4 (red), microtubules (green), and DNA (blue). Staining on Metaphase II spindles withstood the deconvolution algorithms and deconvolved images are shown. However, no chromosomal staining was apparent with this fixation, and despite Kif4's diffuse localization it is noticeably absent from the chromosomes. Two examples of multiple z-slices from the center of the stack are shown: one side view and one end-on view. Bars = 5 μm.

Figure S2

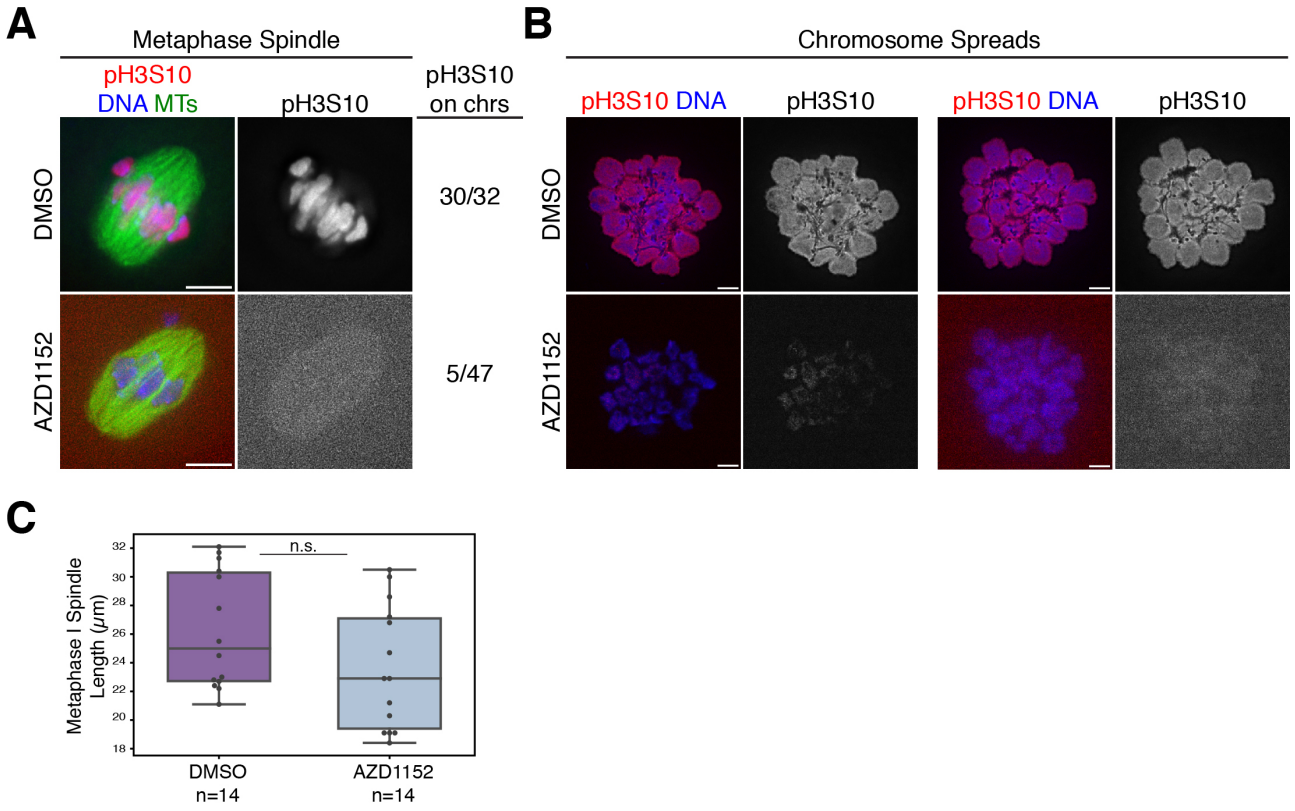

Figure S2. Validation of AZD1152 inhibitor in mouse oocytes.

A) Metaphase I spindles stained for phospho-Histone H3 Serine 10 (pH3S10; red), microtubules (green), and DNA (blue). pH3S10 chromosomal staining is markedly reduced following treatment with 100nM AZD1152. Numbers at right indicate the number of images with pH3S10 staining on chromosomes.

B) Prometaphase I chromosome spreads stained for pH3S10 (red) and DNA (blue), showing a reduction in chromosomal pH3S10 following AZD1152 treatment. Bars = 5 μm.

C) Quantification of Metaphase I spindle lengths treated with DMSO (vehicle control) or AZD1152. Spindles are not significantly shorter following AZD1152 treatment ($p > 0.05$, denoted by n.s.).

Figure S3

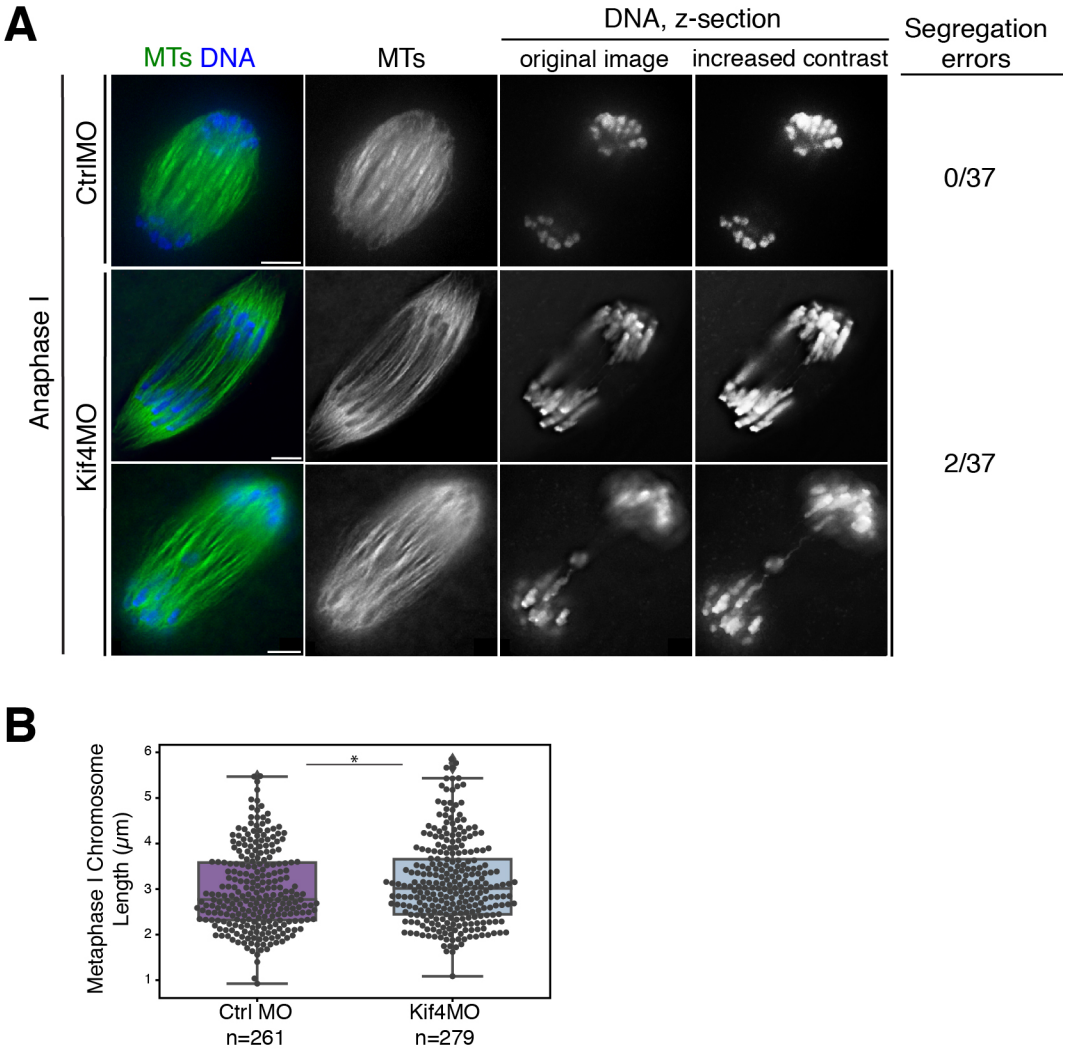

Figure S3. Chromosome segregation defects in a small number of Kif4-depleted oocytes.

A) Anaphase I spindles stained for microtubules (green) and DNA (blue). Kif4 knockdown can cause defects in chromosome segregation such as lagging chromosomes and anaphase bridges (two oocytes with these defects are shown). The first two columns represent full projections; for the DNA alone images, a partial projection is shown to highlight these defects. Quantification of 37 control and 37 Kif4 MO Anaphase/Telophase I spindles for errors in anaphase is shown to the right of the images. Bars = 5 μm.

B) Box plots with overlaid scatter plots for Metaphase I chromosome length measurements, demonstrating that Metaphase I chromosome lengths are not dramatically affected by Kif4 depletion. Average length of control is 2.9 μm +/- 0.1 (n= 261 chromosomes from 14 spindles), average length of Ki4MO is 3.1 μm +/- 0.1 (n=279 chromosomes from 15 spindles). p = 0.03 between conditions, denoted by asterisk.

Figure S4

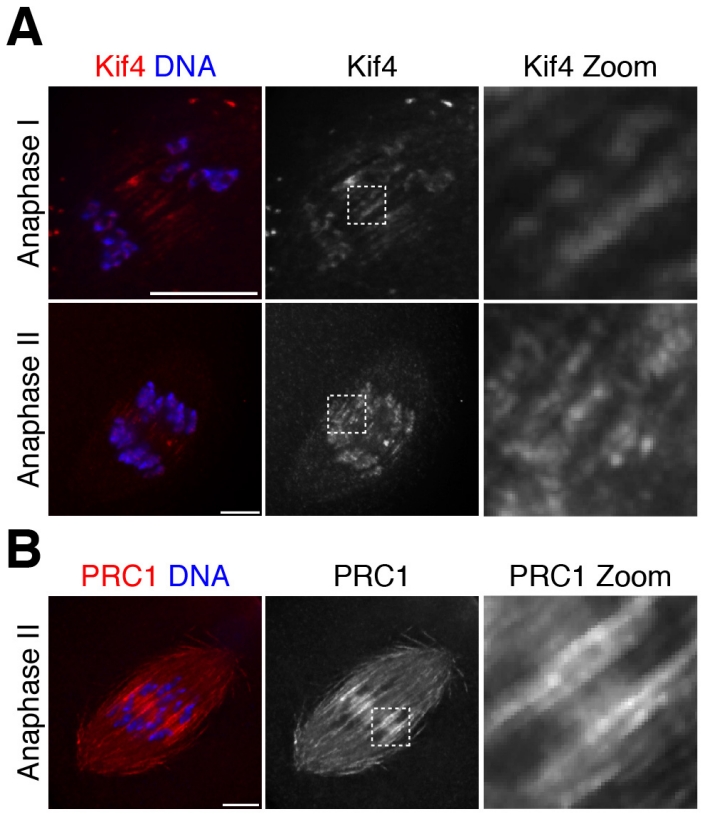

Figure S4. Kif4 and PRC1 accumulate on microtubules between segregating chromosomes in early anaphase.

A) Anaphase I & II spindles stained for Kif4 (red) and DNA (blue). Zooms highlight the localization of Kif4 between the segregating chromosomes. Anaphase II spindle (second row) is the same image shown for Early Anaphase II in Figure 1, but with a different zoom to highlight the localization of Kif4 to the spindle midzone between segregating chromosomes.

B) Anaphase II spindle stained for PRC1 (red) and DNA (blue). Zoom highlights the localization of PRC1 stretches between segregating chromosomes. Bars = 5 μm.

Figure S5

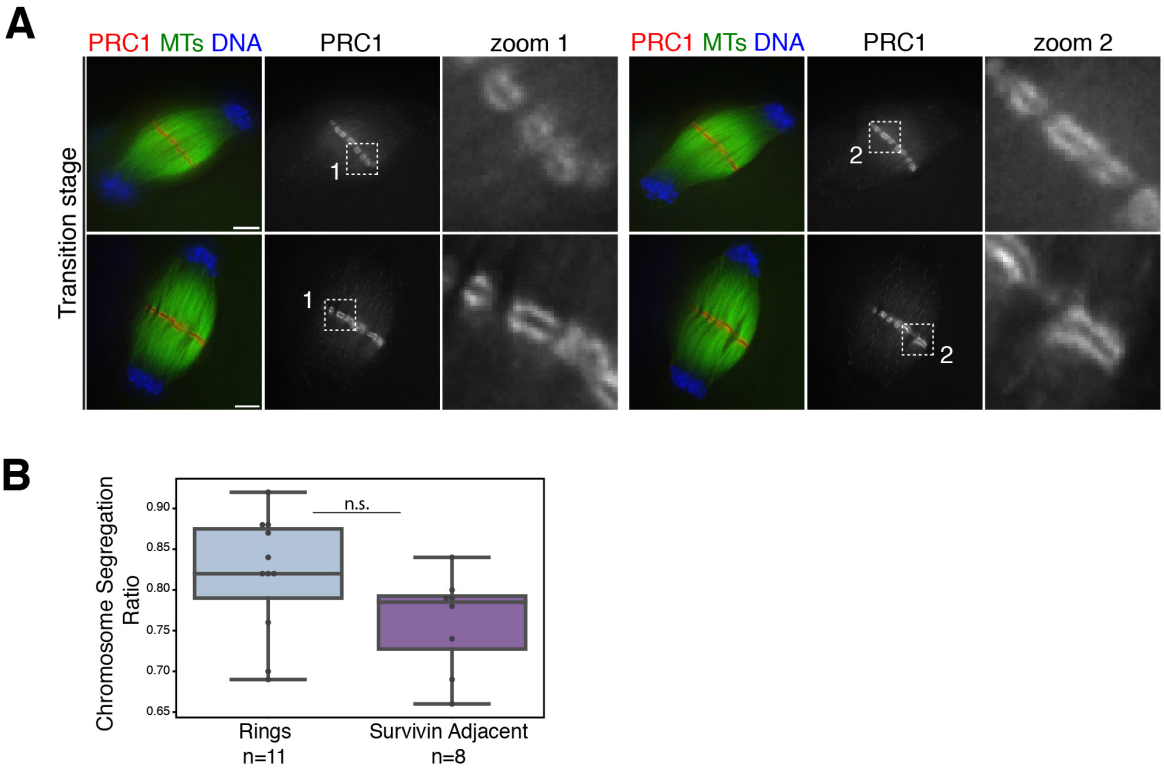

Figure S5. Additional characterization of midzone organization.

A) Anaphase II spindles stained for PRC1 (red), microtubules (green), and DNA (blue) showing that, similar to Kif4, we observed examples of intermediates between the ring stage and the plate stage, suggesting that the PRC1-containing rings fuse as anaphase progresses. Note how some of the microtubules seem to be cinched together where the plates appear to be forming. Bars = 5 μm.

B) Data supporting our classification of spindles as “ring stage” in our Survivin localization experiments (to support Figure 5C). The spindles that we called “ring stage” in the Survivin analysis (graphed on right) had a similar chromosome segregation ratio as those judged to be “ring stage” by Kif4 staining (graphed on left). Average for Survivin-stained spindles is 0.76 μm +/- 0.02 (n=8); these spindles are not significantly different from the ring stage.

Figure S6

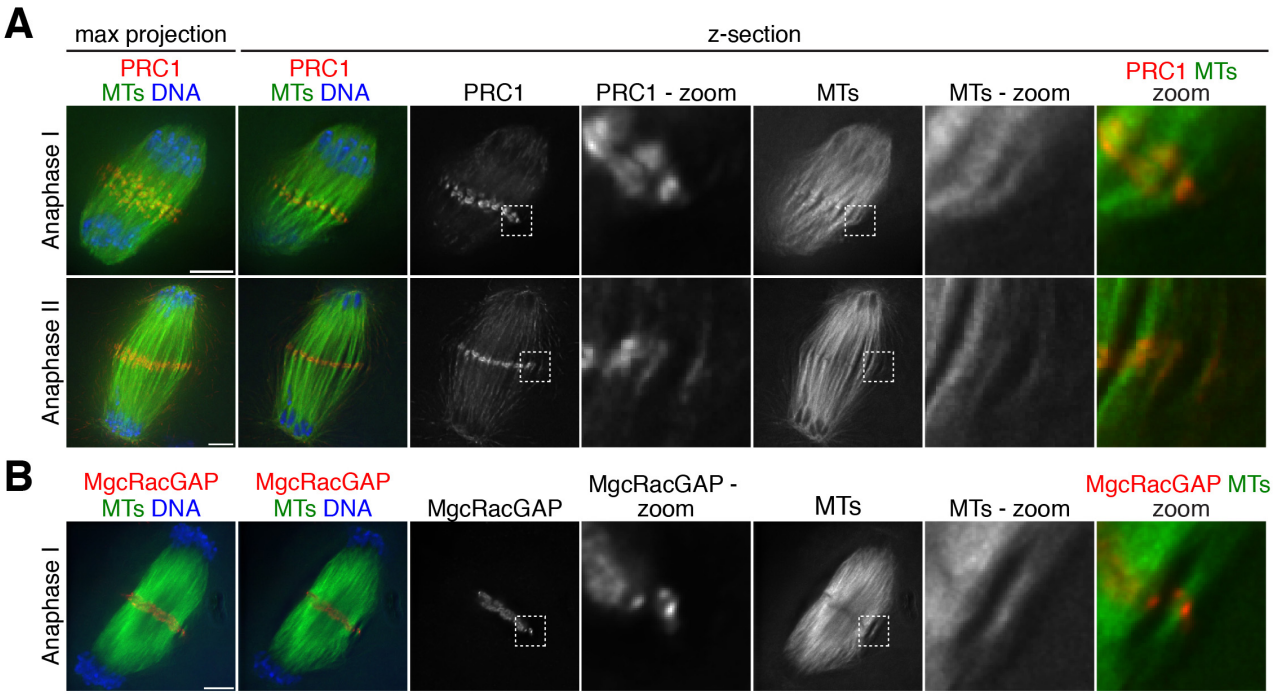

Figure S6. Midzone components localize along microtubule bundles that are outside the dense midzone region.

A) Anaphase I and II spindles stained for PRC1 (red), microtubules (green), and DNA (blue). Splayed microtubule bundles show PRC1 accumulation on continuous microtubule bundles, rather than bridging discontinuous bundles; this supports the idea that the “break” that is sometimes visible in the microtubule channel (examples in Figs. 4 and 5) is due to antibody exclusion in the dense midzone region.

B) Anaphase I spindle stained for MgcRacGAP (red), microtubules (green), and DNA (blue). Splayed microtubule bundle shows MgcRacGAP accumulation on a continuous microtubule bundle. For A and B, the first column is a z-stack comprising the majority of the spindle. Other columns are partial projections with different channels and zooms to demonstrate splayed microtubule bundle morphology. Bars = 5 μm.

SUPPLEMENTAL MOVIE LEGENDS

Movie S1
Movie S1 is a z-stack animation of an Anaphase I spindle in side view, with a midzone in the "ring stage" labeled with Kif4 (red), microtubules (green), DNA (blue) and corresponds to Figure 4A, row 1. Microtubule channel blinks in and out for clarity. Bar = 5 μm.

Movie S2
Movie S2 is a z-stack animation of an Anaphase II spindle in end-on view, with a midzone in the "ring stage" labeled with Kif4 (red), microtubules (green), and DNA (blue). Bar = 5 μm.

Movie S3
Movie S3 is a z-stack animation of an Anaphase I spindle in side view, with a midzone in the "plate stage" labeled with Kif4 (red), microtubules (green), and DNA (blue) and corresponds to Figure 4A, row 2. Bar = 5 μm.

Movie S4
Movie S4 is a z-stack animation of an Anaphase I spindle in an angled view, with a midzone in the "plate stage" labeled with Kif4 (red), microtubules (green), and DNA (blue). Bar = 5 μm.

Movie S5
Movie S5 is a z-stack animation of an Anaphase I spindle in side view, with a midzone in the "transition stage" labeled with Kif4 (red), microtubules (green), and DNA (blue) and corresponds to Figure 4B. Microtubule channel blinks in and out for clarity. Bar = 5 μm.

Movie S6
Movie S6 is a z-stack animation of an Anaphase I spindle in end-on view, with a midzone in the "ring stage" labeled with MgcRacGAP (red), microtubules (green), and DNA (blue) and corresponds to Figure 5B, row 1. Microtubule channel blinks in and out for clarity. Bar = 5 μm.

Movie S7
Movie S7 is a z-stack animation of an Anaphase II spindle in side view, with a midzone in the "transition stage" labeled with PRC1 (red), microtubules (green), DNA (blue) and corresponds to Supplemental Figure 5A, row 1. Microtubule channel blinks in and out for clarity. Bar = 5 μm.