Supporting Information

Houghtaling et al. 10.1073/pnas.0902317106

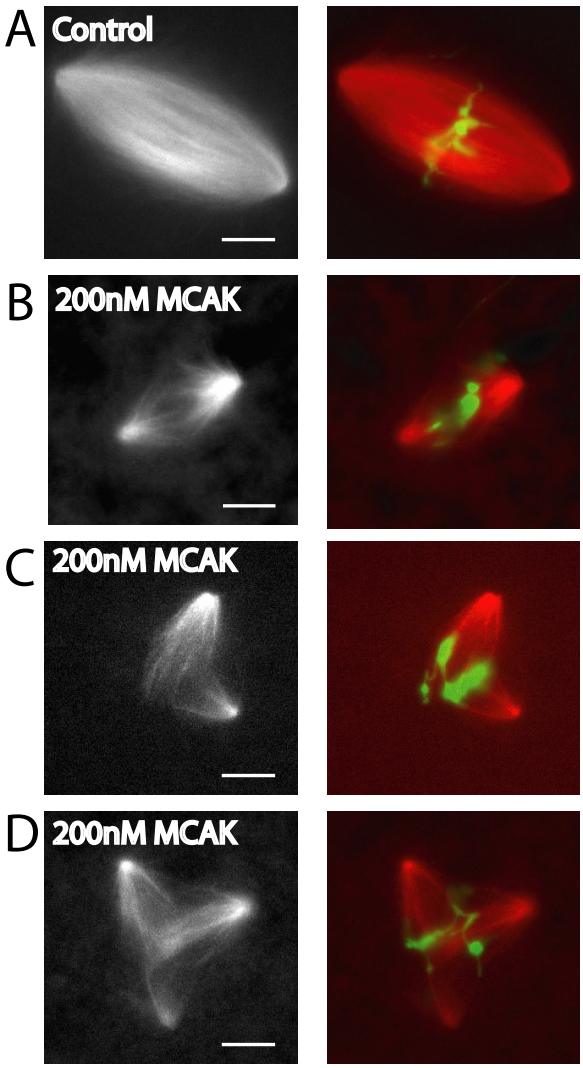


Fig. S1. Increasing MCAK concentration decreases spindle length. (*A–D*) 0.2 μM X-rhodamine tubulin was added to interphase *Xenopus laevis* egg extract along with either buffer alone, or 200 nM recombinant full-length MCAK, as indicated. Following spindle formation, spindles were fixed and imaged. (*Left*) Widefield images of labeled tubulin. (*Right*) Merged images of labeled tubulin (red) and DNA (green). (Scale bar: 10 μm.)

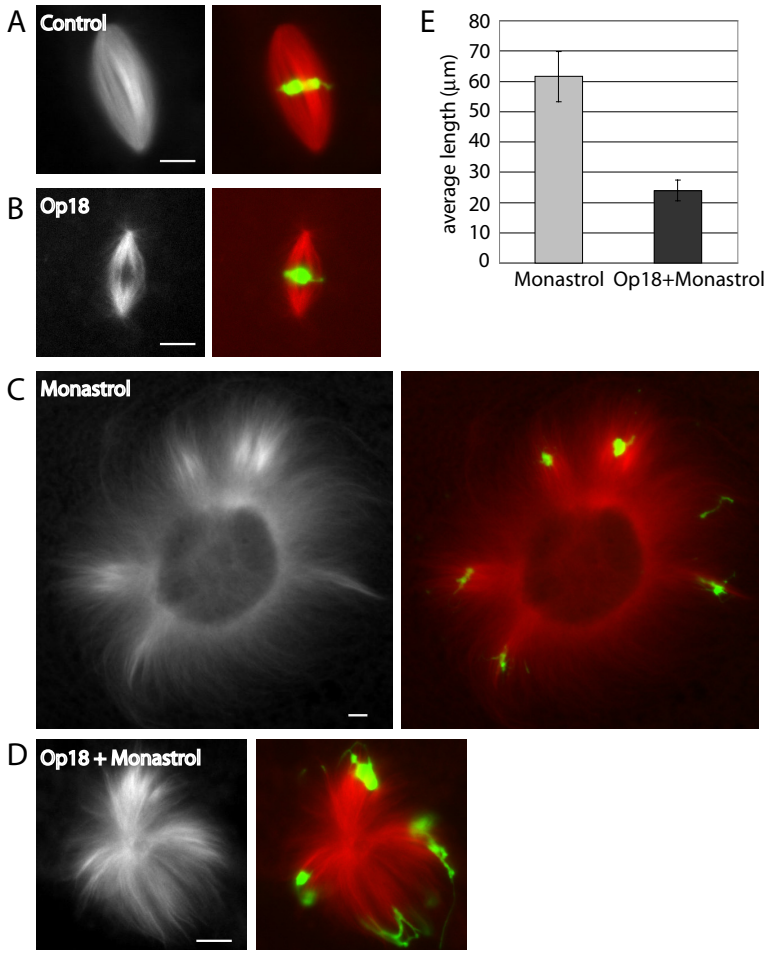


Fig. S2. Spindles assembly reactions performed in the presence of 100 μM monastrol and 15 μM Op18. (*A–D*) 0.2 μM X-rhodamine tubulin was added to interphase extract along with DMSO alone (*A*), 15 μM Op18 (*B*), 100 μM monastrol (*C*), or 15 μM Op18 and 100 μM monastrol (*D*), and extract was cycled into M phase. Following formation of control spindles, reactions were fixed and imaged. (*Left*) Widefield images of tubulin. (*Right*) Merged images of tubulin (red) and DNA (green). (*E*) Bar graph reporting the average length of microtubules in the monopolar structures formed with either 100 μM monastrol alone or 100 μM monastrol and 15 μM Op18. Length was measured in a straight line from the perimeter of the central ''hole'' (as in panel *C*) or the midpoint (as in panel *D*) to the microtubule end. $N = >15$ structures for each condition. Error bars represent one standard deviation. (Scale bar: 10 μm.)

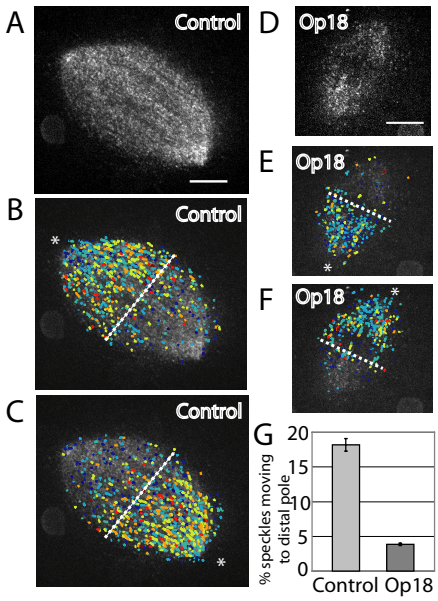


Fig. S3. Antiparallel microtubules are reduced in Op18-treated spindles. (*A*) Single-frame confocal image from a time-lapse series of a control spindle labeled with 9.6 nM X-rhodamine tubulin for qFSM. (*B* and *C*) Speckles from image series of spindle in Fig. 3*A* were detected and tracked using automated software. Tracks were separated based on direction of motion toward one pole (*B*) or the other (*C*). Asterisk represents the proximal pole (i.e., toward which tracks are moving). Dashed line represents spindle equator, determined by measuring the pole-to-pole length, and dividing by 2. For each image, the number of tracks in each spindle half were counted to quantify the number of speckles moving toward the distal or proximal pole. Track coloring reflects velocity, with blue colors being slower than red colors. (*D–F*) Same as panels *A–C*, except spindle was treated with 15 μM Op18. (*G*) Bar graph reflecting the percentage of total speckles in control spindles ($n = 5$) and Op18-treated spindles ($n = 12$) that move toward the distal pole. Error bars represent one standard deviation. (Scale bar: 10 μm.)

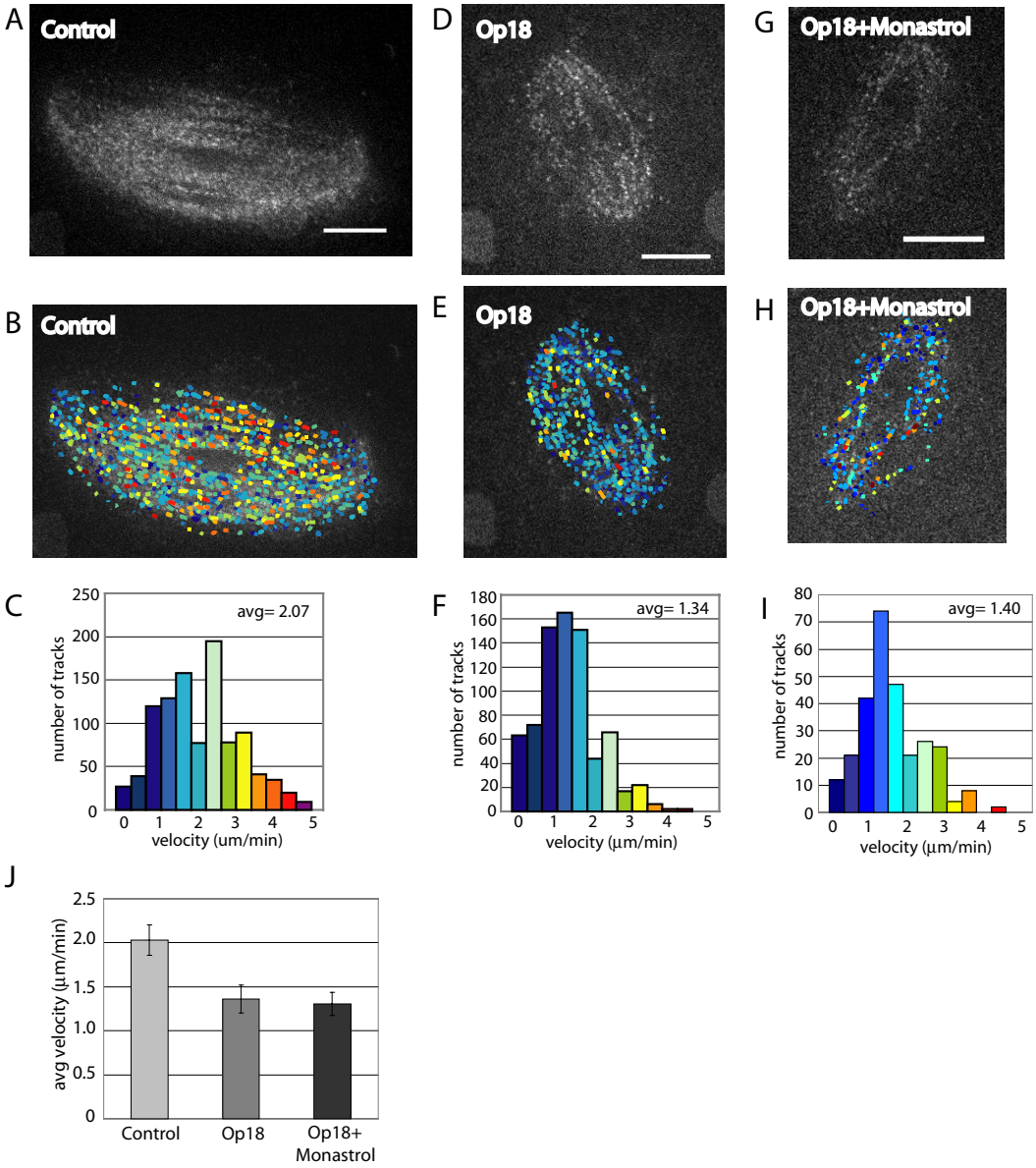


Fig. S4. Whole spindle flux rates in spindles treated with 15 μM Op18 with and without 100 μM monastrol using FSM. (*A*) Confocal single image of 9.6 nM X-rhodamine tubulin in control spindle. (*B*) Speckle tracks detected using automated software within the spindle in panel *A* were overlaid onto a single-frame image of X-rhodamine tubulin. Track colors reflect velocity, as shown in panel *C*. (*C*) Histogram of track velocities detected in control spindle shown in panel *A*. Average speckle velocity = 2.07 μm/min. (*D* and *E*) Same as panels *A* and *B*, conducted on a spindle treated with 15 μM Op18. (*F*) Histogram of track velocities detected in spindle shown in panel *D*. Average speckle velocity = 1.34 μm/min. (*G* and *H*) Same as panels *A* and *B*, conducted on a spindle treated with 15 μM Op18 for 15 min followed by addition of 100 μM monastrol. (*I*) Histogram of track velocities detected in spindle shown in panel *G*. Average speckle velocity = 1.40 μm/min. (*J*) Bar graph reporting the average velocity of speckles in control spindles (average speckle velocity = 2.03 μm/min, n = 5 spindles, 6,973 total tracks, SD = 0.17 μm/min), spindles treated with 15 μM Op18 (average speckle velocity = 1.36 μm/min, n = 12 spindles, 7,009 total tracks, SD = 0.16 μm/min), and spindles treated with 15 μM Op18 and 100 μM monastrol (average speckle velocity = 1.31 μm/min, n = 10 spindles, 4,734 total tracks, SD = 0.13 μm/min). Error bars represent one standard deviation between average velocities for all spindles in each condition. (Scale bar: 10 μm.)

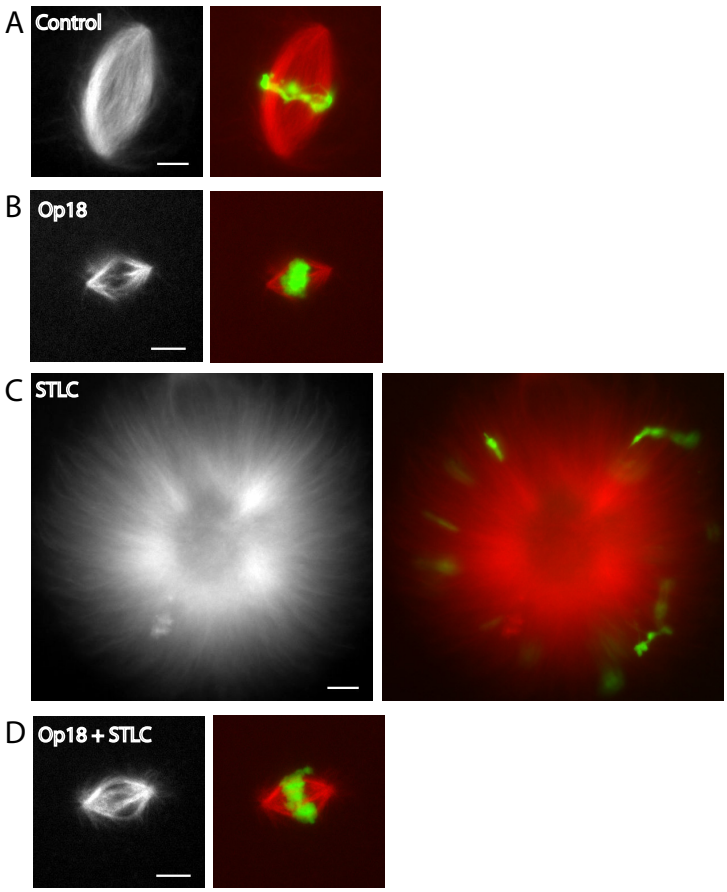
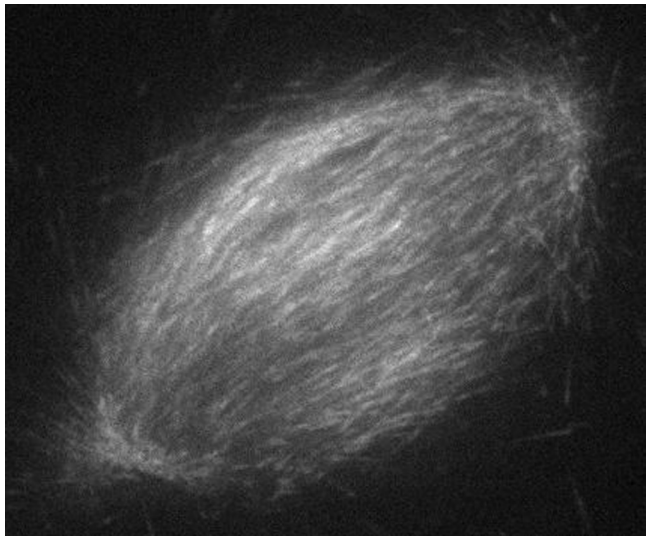
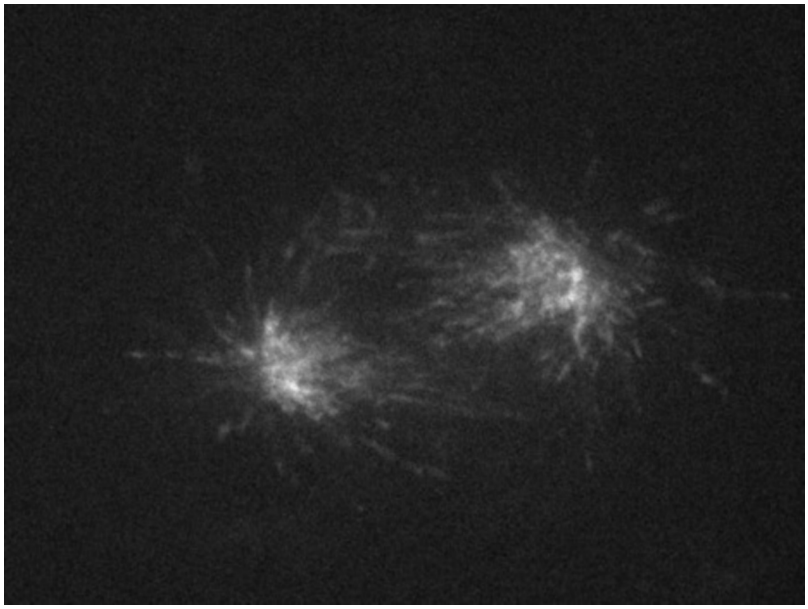


Fig. S5. Spindles treated with Op18 and S-trityl-L-cysteine retain bipolarity. (*A–D*) Spindles were formed in extract, labeled with 0.2 μM X-rhodamine tubulin, and treated with DMSO control (*A*), 15 μM Op18 for 45 min (*B*), 200 μM S-trityl-L-cysteine for 45 min (*C*), or 15 μM Op18 for 10 min followed by 200 μM S-trityl-L-cysteine for 45 min (*D*). (*Left*) Widefield images of labeled tubulin in black and white. (*Right*) Merged widefield images of tubulin (red) and DNA (green). (Scale bar: 10 μm.)



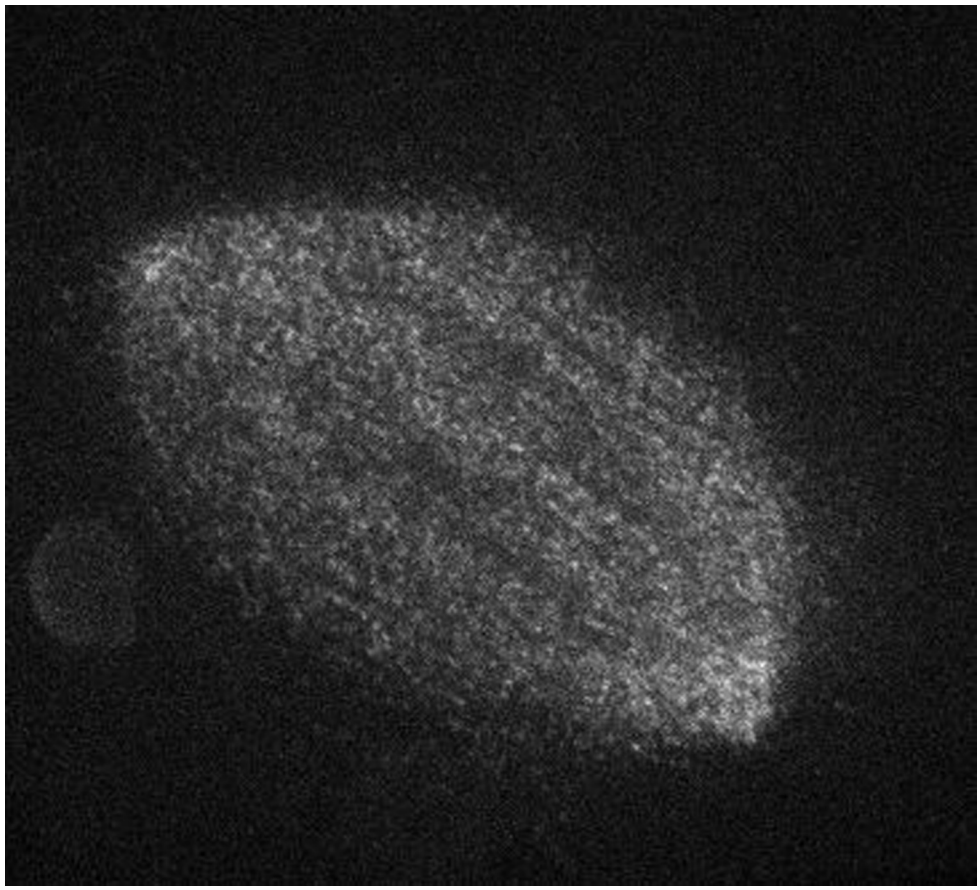
Movie S1. Control spindle labeled with 0.5 μM Alexa^{488} EB1, imaged over time with confocal microscopy. Time interval was 2 sec, for 84 sec. Scale bar located on the single frame shown in Fig. 2*A*.

Movie S1 (MOV)



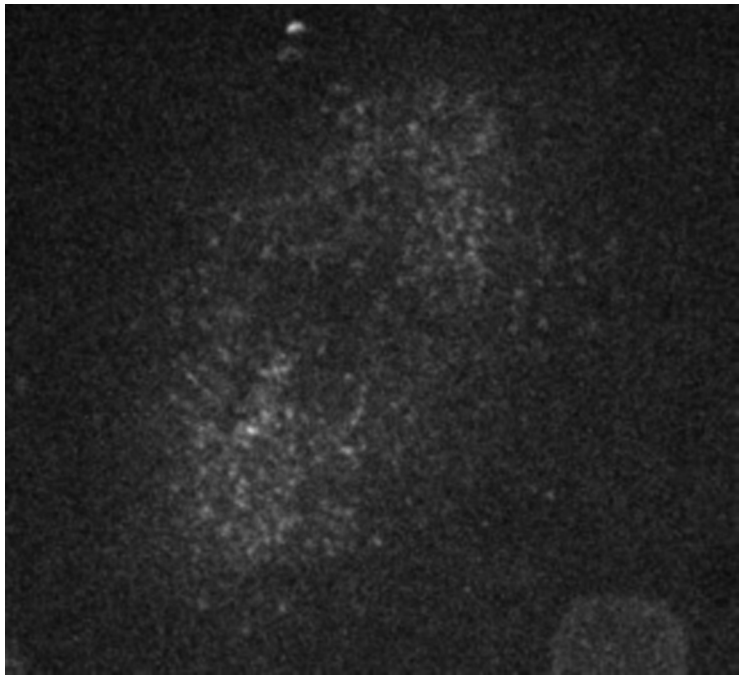
Movie S2. Preformed spindle was treated with 15 μM Op18 and, following a 15-min incubation, labeled with 0.5 μM Alexa^{488} EB1 and imaged with confocal microscopy. Time interval was 2 sec for 70 sec. Scale bar is located on the single frame shown in Fig. 2*B*.

Movie S2 (MOV)



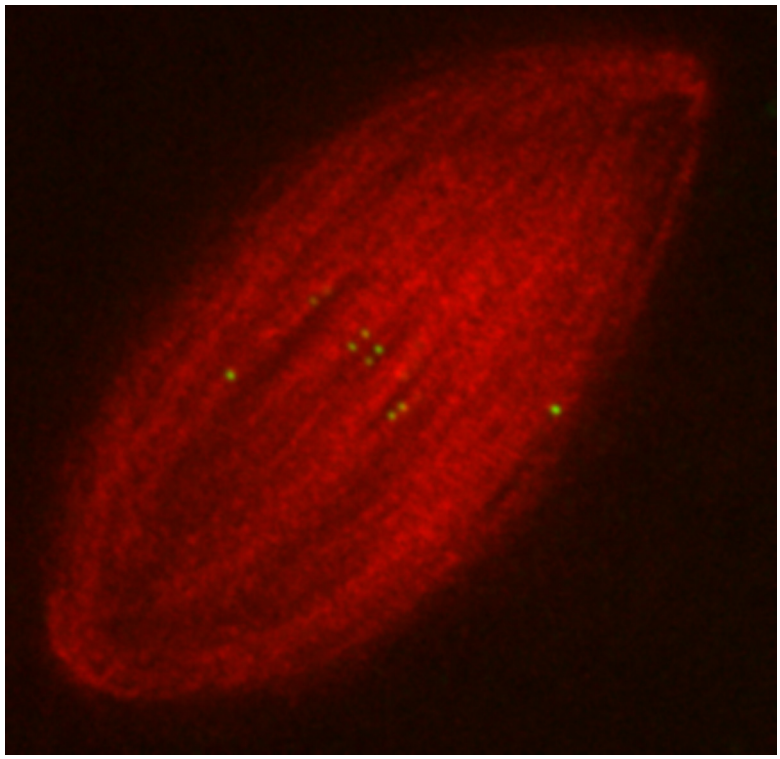
Movie S3. Preformed control spindle, labeled with 9.6 nM X-rhodamine tubulin, was imaged over time with confocal microscopy for automated qFSM analysis. Time interval was 4 sec for 100 sec. Scale bar is located on the single frame shown in Fig. S3*A*.

Movie S3 (MOV)



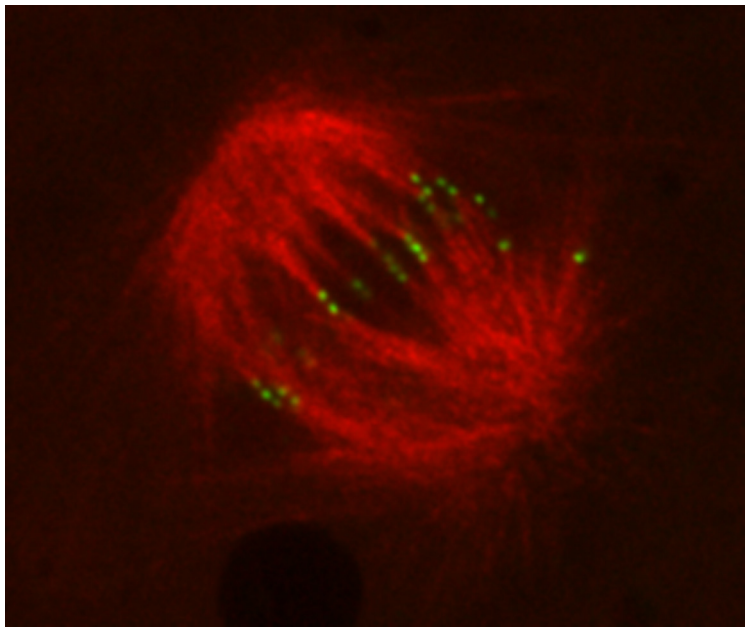
Movie S4. Preformed spindle, treated with 15 μM Op18 for 15 min, was labeled with 9.6 nM X-rhodamine tubulin, imaged over time with confocal microscopy, aligned, and used for automated qFSM analysis. Time interval was 4 sec for 100 sec. Scale bar is located on the single frame shown in Fig. S3*D*.

Movie S4 (MOV)



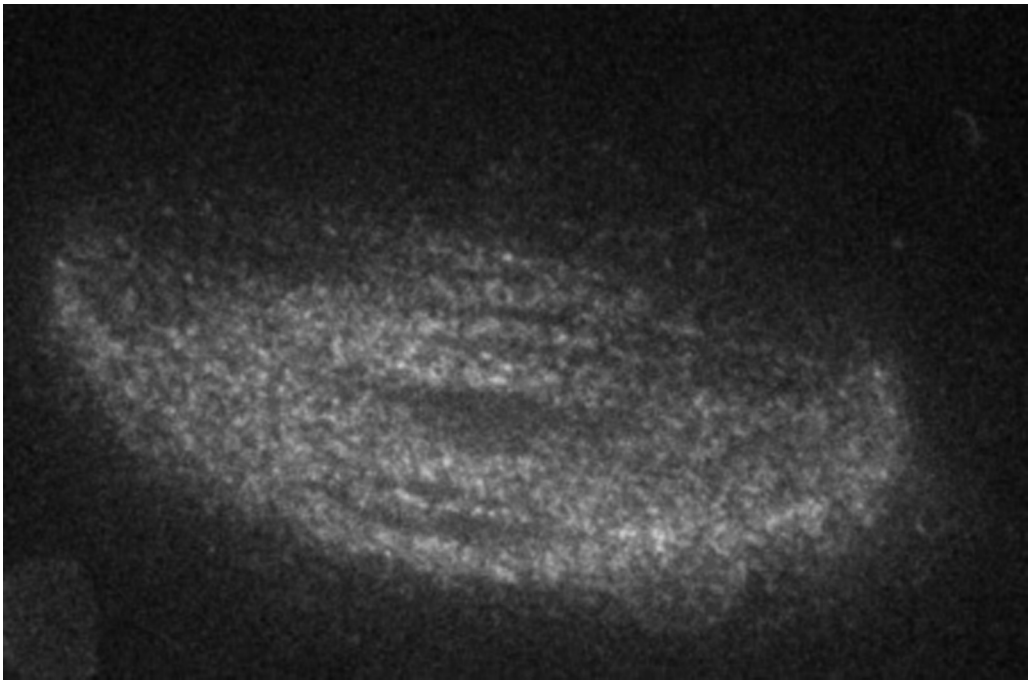
Movie S5. Many microtubules traverse the spindle equator without apparent association with kinetochores in a control spindle. Control spindle, labeled with 75 nM X-rhodamine tubulin (red) and CENP-A kinetochore marker (green) imaged over time using dual-mode confocal microscopy. Images from both channels were acquired every 6 sec, for 96 sec, and merged. Scale bar is located on the single frame shown in Fig. 3*A*.

Movie S5 (MOV)



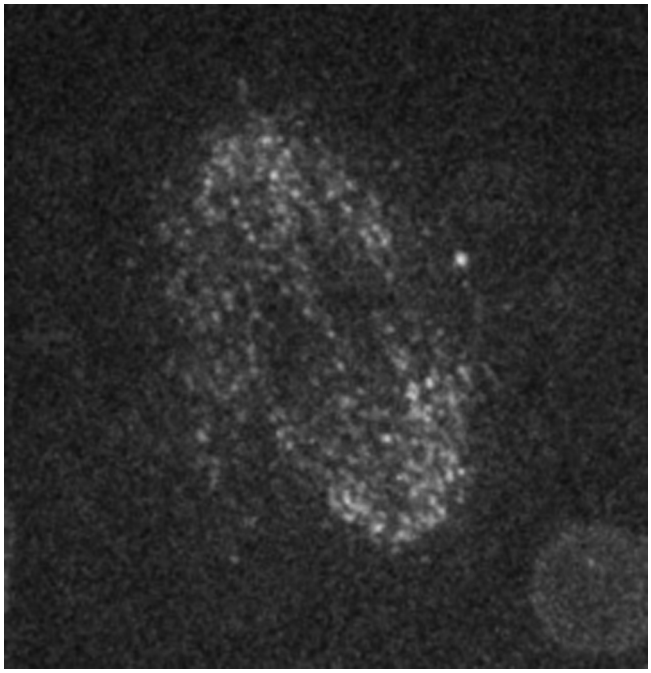
Movie S6. Kinetochore microtubules persist in spindle treated with 15 μM Op18. Preformed spindle treated with 15 μM Op18, labeled with 75 nM X-rhodamine tubulin (red), and CENP-A kinetochore marker (green) imaged over time using dual-mode confocal microscopy. Images from both channels were acquired every 6 sec, for 96 sec, and merged. Scale bar is located on the single frame shown in Fig. 3*B*.

Movie S6 (MOV)



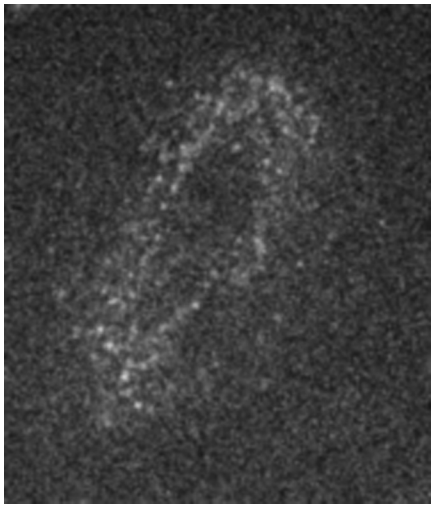
Movie S7. Control spindle, labeled with 9.6 nM X-rhodamine tubulin, imaged over time with confocal microscopy for automated qFSM analysis. Images were acquired every 4 sec for 68 sec. Scale bar is located on the single frame shown in Fig. 4*A*.

Movie S7 (MOV)



Movie S8. Preformed spindle was treated with 15 μM Op18 for 15 min, labeled with 9.6 nM X-rhodamine tubulin, and imaged over time with confocal microscopy for automated qFSM analysis. Images were acquired every 4 sec for 64 sec. Scale bar is located on the single frame shown in Fig. 4*E*.

Movie S8 (MOV)



Movie S9. Preformed spindle was treated with 15 μM Op18 for 15 min followed by 100 μM monastrol for 40 min, labeled with 9.6 nM X-rhodamine tubulin, and imaged over time with confocal microscopy for automated qFSM analysis. Images were acquired every 4 sec for 100 sec. Scale bar is located on the single frame shown in Fig. S4*G*.

Movie S9 (MOV)