Courtois et al., http://www.jcb.org/cgi/content/full/jcb.201202135/DC1

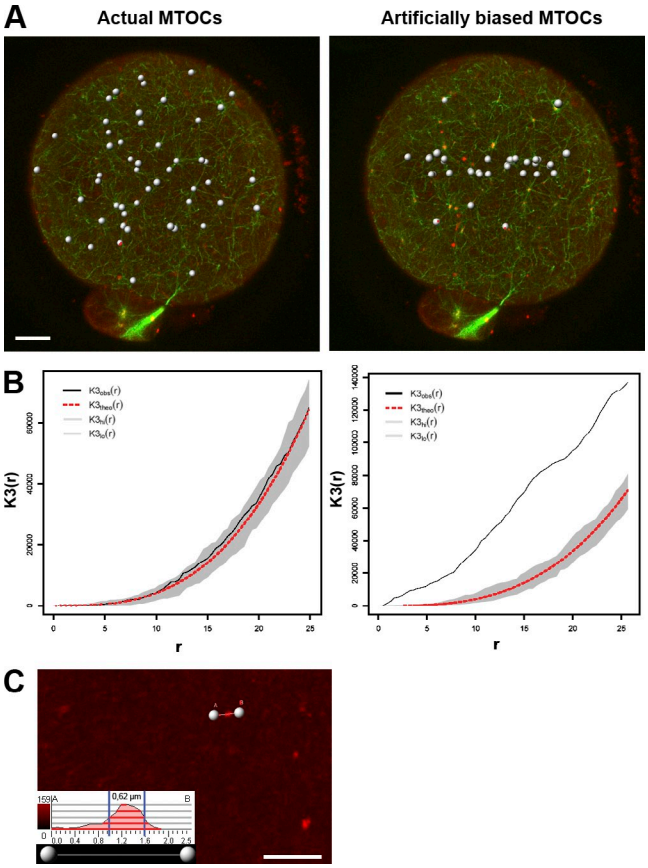


Figure S1. **Measurement of distribution and size of MTOCs in the mouse zygote.** Related to Fig. 1. (A) Immunostaining of a zygote fixed at 18 h after hCG and stained for microtubules (green) and pericentrin (red). Projection of the confocal sections in which positions of actual (left) and artificially biased (right) MTOCs are marked with gray balls. Bar, 10 µm. (B) Graphic representation of the K function (y) of a 3D point pattern against radius r (x). The observed curve (black), theoretical curve (broken red), and confidence interval (gray) are plotted for the actual (left) and artificially biased (right) MTOC positions. Similar results were obtained from three independent zygotes (see Materials and methods for detailed methods). (C) MTOC size was defined as the diameter of the circle at which the intensity of the immunostained pericentrin signal becomes half of the maximal intensity based on the fluorescence intensity profile (between two blue bars in the inset). Bar, 1 µm.

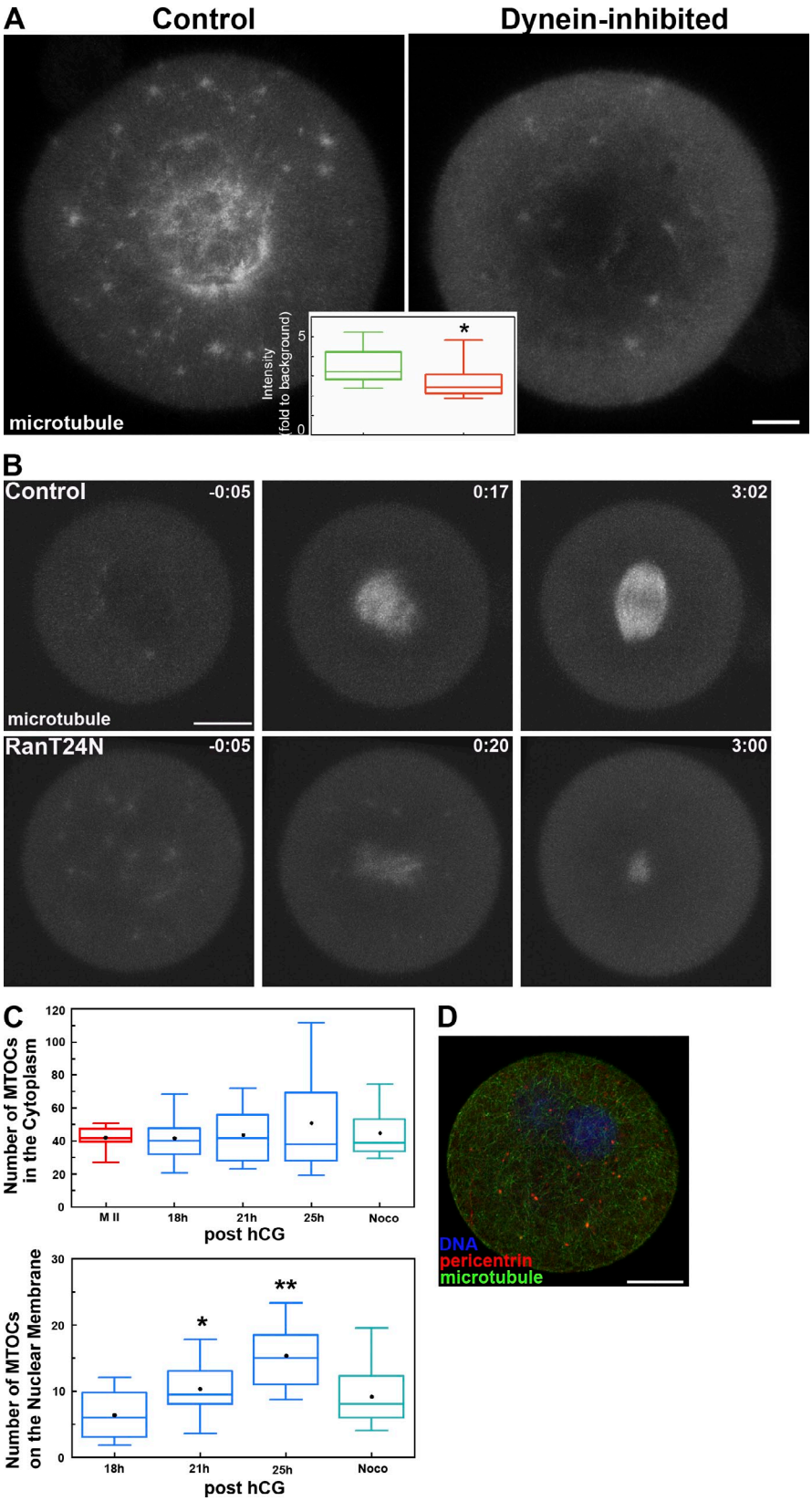


Figure S2. **Dynein is essential for MTOC maturation, whereas RanGTP facilitates spindle assembly.** Related to Fig. 2. (A) Live imaging of the mouse zygote at NEBD of the first division under inhibition of dynein by P150-CC1. Z-projected images of confocal sections (3 µm thick) showing microtubules (EGFP-MAP4; gray). Maturation of MTOCs is significantly reduced in the dynein-inhibited embryo (asterisk in inset; See Materials and methods). (B) RanGTP facilitates the enhanced microtubule polymerization upon NEBD. Live imaging of mouse zygotes injected with BSA (control) or RanT24N during the first division. Before the protein injection, zygotes were injected with mRNA encoding EGFP-MAP4 (gray) to visualize microtubules. Time is given in hours and minutes after NEBD. Projected image of 3-µm confocal sections. (C and D) Preferential MTOC accumulation on the pronuclear membrane depends on microtubules. (C) Whisker box plot of the number of MTOCs in the cytoplasm (top) and on the pronuclear membrane (bottom) in MII oocytes (n = 12; only for the cytoplasm) in zygotes at 18 h (n = 27), 21 h (n = 26), and 25 h (n = 37) after hCG injection and in zygotes treated with nocodazole (Noco) from 18 to 25 h after hCG (n = 17; black dots indicate mean value). The number of MTOCs on the pronuclear membrane differs significantly between the zygotes at 18 and 21 h (asterisk), 21 and 25 h (double asterisk), and 25 h after hCG and nocodazole-treated zygotes ($P < 0.05$, Welch two-sample t test). The lines near the middle of the boxes represent the median (50th percentile). The bottom and top of the boxes are the 25th and 75th percentile, respectively. The end of the bottom whisker is the 5th percentile, and the end of the top whisker is the 95 percentile. (D) Projected image of 180 0.45-µm stacks of a nocodazole-treated embryo immunostained for DNA, microtubules, and pericentrin. Bars: (A) 10 µm; (B and D) 20 µm.

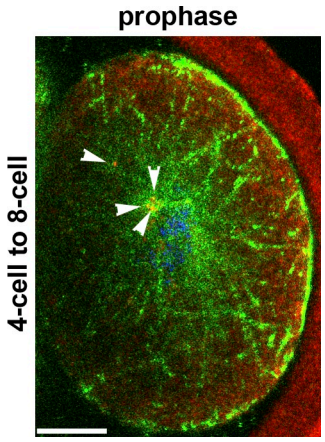


Figure S3. **Presence of multiple MTOCs at prophase during four- to eight-cell division.** Related to Fig. 3. Immunostaining of mouse embryos fixed at prophase of the third (four to eight cell) division. A single-section image of confocal scans showing microtubules (green), pericentrin (red), and DNA (blue). Note multiple MTOCs (arrowheads) around nucleus and in the cytoplasm. Bar, 10 µm.

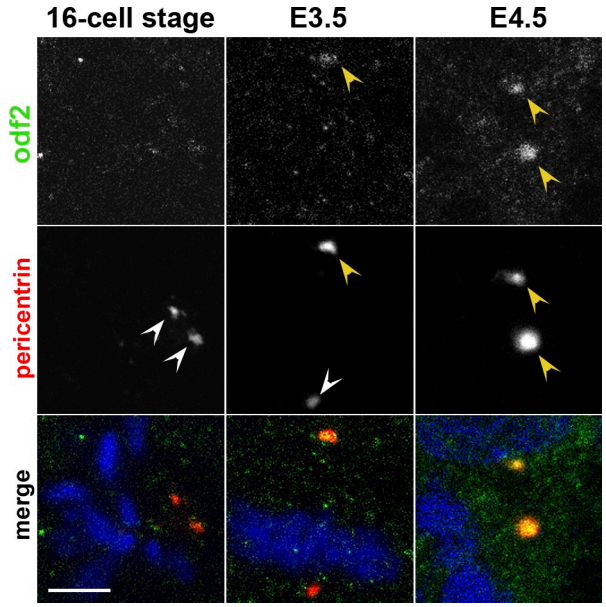


Figure S4. **Progressive emergence of odf2, a centrosome marker.** Related to Fig. 4. Immunostaining of mouse embryos fixed at the 16-cell stage, E3.5 (between 32- and 64-cell stages), and E4.5 (with >128 cells) and stained for DNA (blue), odf2 (single-channel image shown in grayscale), and pericentrin (single-channel image in grayscale). At the 16-cell stage, MTOCs are negative for odf2. At E3.5, only some MTOCs are positive for odf2 (yellow arrowheads; white arrowheads mark those negative for odf2), whereas at E4.5, all MTOCs are positive for odf2. Confocal single-section images. Bar, 5 µm.

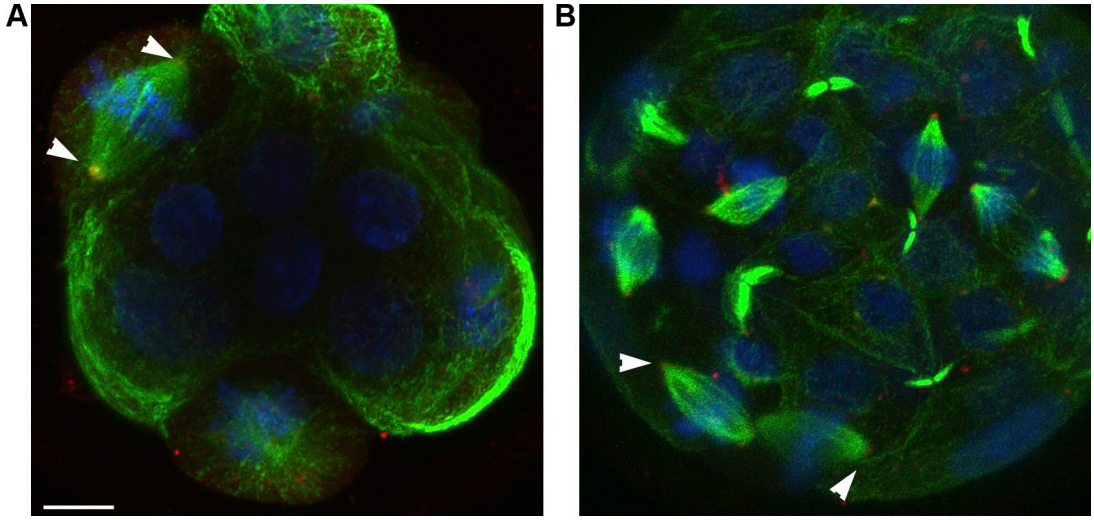
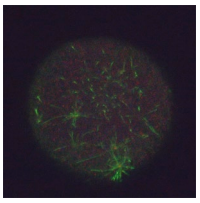


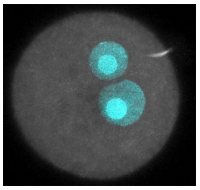
Figure S5. **Progressive focusing of spindle poles at 16- to 64-cell mouse embryos.** Related to Fig. 6. (A and B) Immunostaining of mouse embryos fixed at fifth (16–32 cell; A) and sixth (32–64 cell; B) divisions showing a metaphase spindle. Pictures are projected images of 0.38-µm confocal stacks. Embryos are stained for DNA (blue), microtubules (green), and pericentrin (red). Note the well-focused spindle poles at metaphase (arrowheads). Bar, 10 µm.

Table S1. **Comparison of the experimental conditions in this study to those used in Schuh and Ellenberg (2007)**

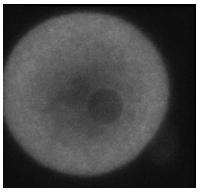
Experiments	Inhibitor	Concentrations	
		Schuh and Ellenberg, 2007	This study
Culture	Nocodazole	3 µg/ml	90 µg/ml (0.3 µM)
Culture	Monastrol	100 µM	100 µM
Culture	Cytochalasin	3 µg/ml (cytochalasin D)	5 µg/ml (cytochalasin B)
Microinjection	RanT24N	268 µM	270 µM
Microinjection	P150-CC1	Not applicable	24 mg/ml



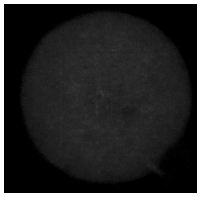
Video 1. **Multipolar spindle during the first division of the mouse zygote.** Related to Fig. 1 F. Immunofluorescence staining of the mouse zygote fixed at early prometaphase. 121 images of confocal sections (0.5 µm thick) were acquired using a laser-scanning confocal microscope (LSM 780), showing microtubules (green), pericentrin (red), and DNA (blue). Numbers refer to the z coordinate of the confocal section.



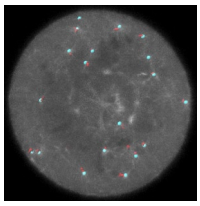
Video 2. **Live imaging of the mouse zygote during the first division.** Mouse zygote expressing EGFP-MAP4 (microtubule; gray) and H2B-mRFP1 (DNA; cyan) during the first division. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were taken every 2 min for 5 h and 40 min. See also Fig. 1 J. Time is given in hours and minutes relative to NEBD (at 00:00).



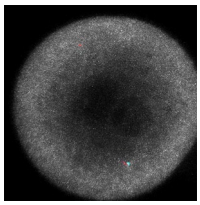
Video 3. **Live imaging of the dynein-inhibited mouse zygote during the first cleavage.** Mouse zygote expressing EGFP-MAP4 (microtubule; gray) during the first division under inhibition of dynein by P150-CC1. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were taken every 2.5 min for 5 h. See also Fig. 2 A. Time is given in hours and minutes relative to NEBD (at 00:00).



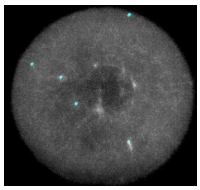
Video 4. **Live imaging of the kinesin-5–inhibited mouse zygote during the first cleavage.** Mouse zygote expressing EGFP-MAP4 (microtubule; gray) during the first division under inhibition of kinesin-5. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were taken every 2 min for 6 h. See also Fig. 2 B. Time is given in hours and minutes relative to NEBD (at 00:00).



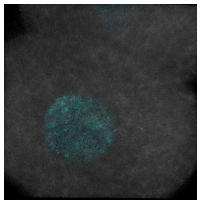
Video 5. **Tracking of MTOCs during the first division.** Mouse zygote expressing EGFP-MAP4 (microtubule; gray) during the first division. Main visible asters are tracked, represented as a blue sphere, using Imaris software. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were taken every minute for 1 h and 10 min. See also Fig. 2 (C–E). Time is given in hours and minutes relative to NEBD (at 00:00).



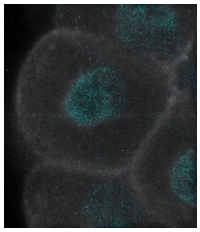
Video 6. **Tracking of MTOCs in the dynein-inhibited mouse zygote during the first cleavage.** Mouse zygote expressing EGFP-MAP4 (microtubule; gray) during the first division under inhibition of dynein by P150-CC1. Main visible asters are tracked, represented as a blue sphere, using Imaris software. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were taken every 2.5 min for 3 h and 15 min. See also Fig. 2 (C–E). Time is given in hours and minutes relative to NEBD (at 00:00).



Video 7. **Tracking of MTOCs in the kinesin-5–inhibited mouse zygote during the first cleavage.** Mouse zygote expressing EGFP-MAP4 (microtubule; gray) during the first division under inhibition of kinesin-5. Main visible asters are tracked, represented as a blue sphere, using Imaris software. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were taken every minute for 55 min. See also Fig. 2 (C–E). Time is given in hours and minutes relative to NEBD (at 00:00).



Video 8. **Live imaging of the mouse embryo during the second division.** Mouse embryo expressing EGFP-MAP4 (microtubule; gray) and H2B-mRFP1 (DNA; cyan) during the second division. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were taken every 5 min for 2.5 h. See also Fig. 3 A. Time is given in hours and minutes relative to NEBD (at 00:00).



Video 9. **Live imaging of the mouse embryo during the fourth division.** Mouse embryo expressing EGFP-MAP4 (microtubule; gray) and H2B-mRFP1 (DNA; cyan) during the fourth division. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were taken every 2 min for 1.5 h. See also Fig. 3 B. Time is given in hours and minutes relative to NEBD (at 00:00).