

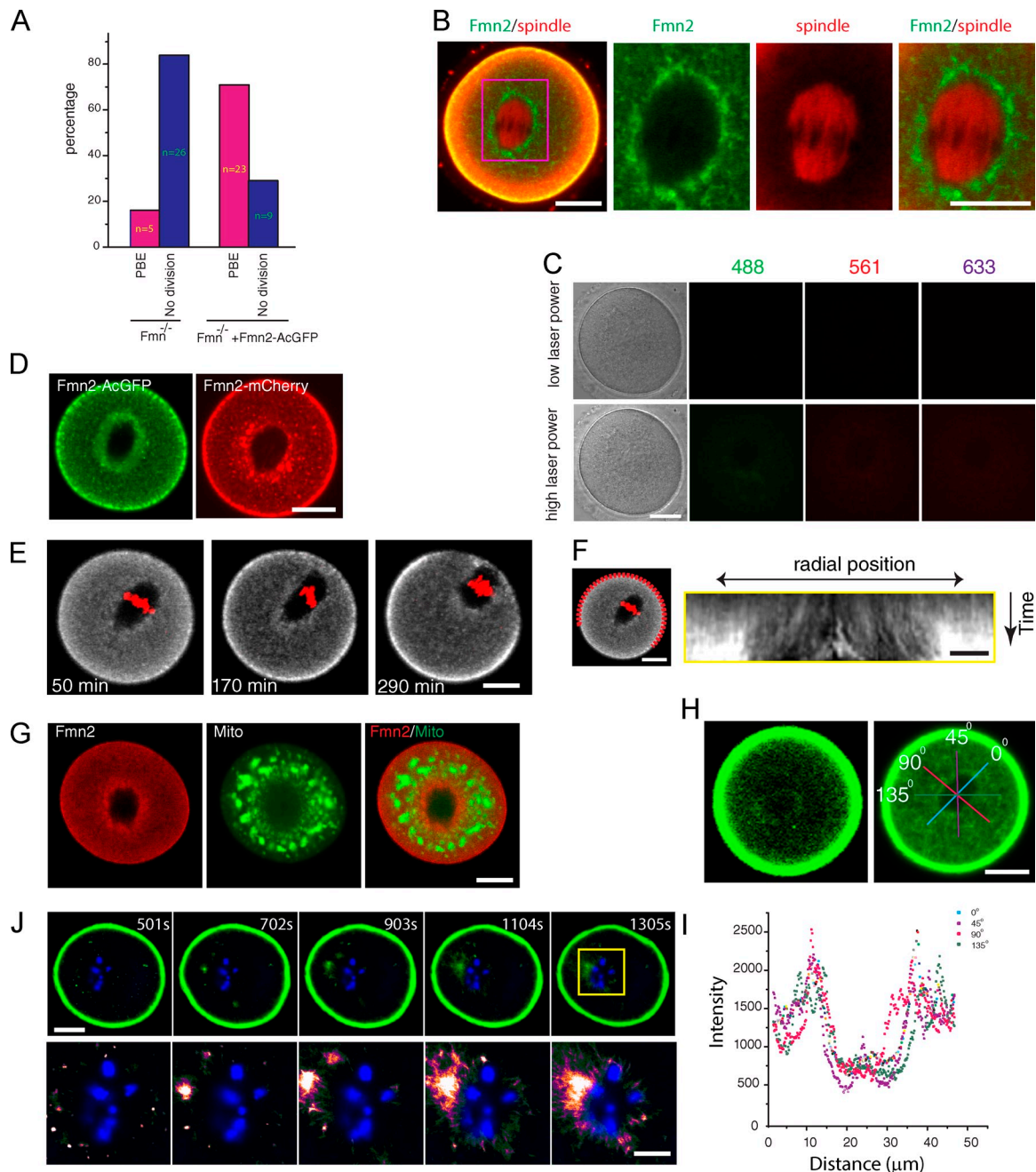
Yi et al., <http://www.jcb.org/cgi/content/full/jcb.201211068/DC1>

Figure S1. Fmn2 localization and actin organization in MI mouse oocytes. (A) Quantification of the polar body extrusion frequency of Fmn2-null oocytes after Fmn2-AcGFP mRNA microinjection, showing that the tagged construct rescues the null to a large extent. A total of 31 (for Fmn2 null) and 32 (for Fmn2 null + Fmn2-AcGFP) oocytes were analyzed. PBE, polar body extrusion. (B) Representative images showing Fmn2-AcGFP localization relative to the spindle. Fmn2-AcGFP localizes to the spindle periphery as well as to the cortex. A magnified view of the box region is shown in the right three images. (C) The spindle peripheral localization of Fmn2 is not caused by autofluorescence. Representative images of unlabeled mouse MI oocyte subjected to a low laser power or high laser power scan for laser lines of 488-, 561-, and 633-nm on a confocal microscope (LSM 510 Meta) using a Plan ApoChromat 40 \times , 1.2 NA water immersion objective. The detailed settings are low laser power 488 (26.8% laser power, detection gain 580), 561 (35.7% laser power, detection gain 580), and 633 (15.1% laser power, detection gain 580); and high laser power 488 (100% laser power, detection gain 780), 561 (100% laser power, detection gain 780), and 633 (100% laser power, detection gain 780). In all cases, the autofluorescence is minimal. (D) Images of Fmn2-AcGFP or Fmn2-mCherry in mouse oocytes, taken with lower laser power and even smaller detection gain. Both Fmn2 probes localize to the cortex and spindle periphery. (E) Time-lapse images showing Fmn2 became excluded from the cortical region in front of the approaching chromosomes. (F) Kymograph generated along the red dotted line on the left showing Fmn2 exclusion from the cortical cap. (G) Mitochondria (Mito), detected by using mito-EGFP, localize farther away from spindle compared with Fmn2. (H) Images of F-actin distribution labeled with UtrCH-GFP at low level (left) or high level (right) expression in the MI oocyte at migration stage. (I) Four lines in the right image of H were drawn at 0, 45, 90, and 135° relative to the long spindle axis, and the fluorescence intensity profiles were displayed in the respective colors, showing increased intensity (intensity peaks) around the spindle. (J) Full view of time-lapse images showing new F-actin growth in the vicinity of the chromosome after cytochalasin D washout. Bottom images show the magnified view of the heat map of the box region. Bars: [B–J [top]] 20 μ m; [J [bottom]] 10 μ m.

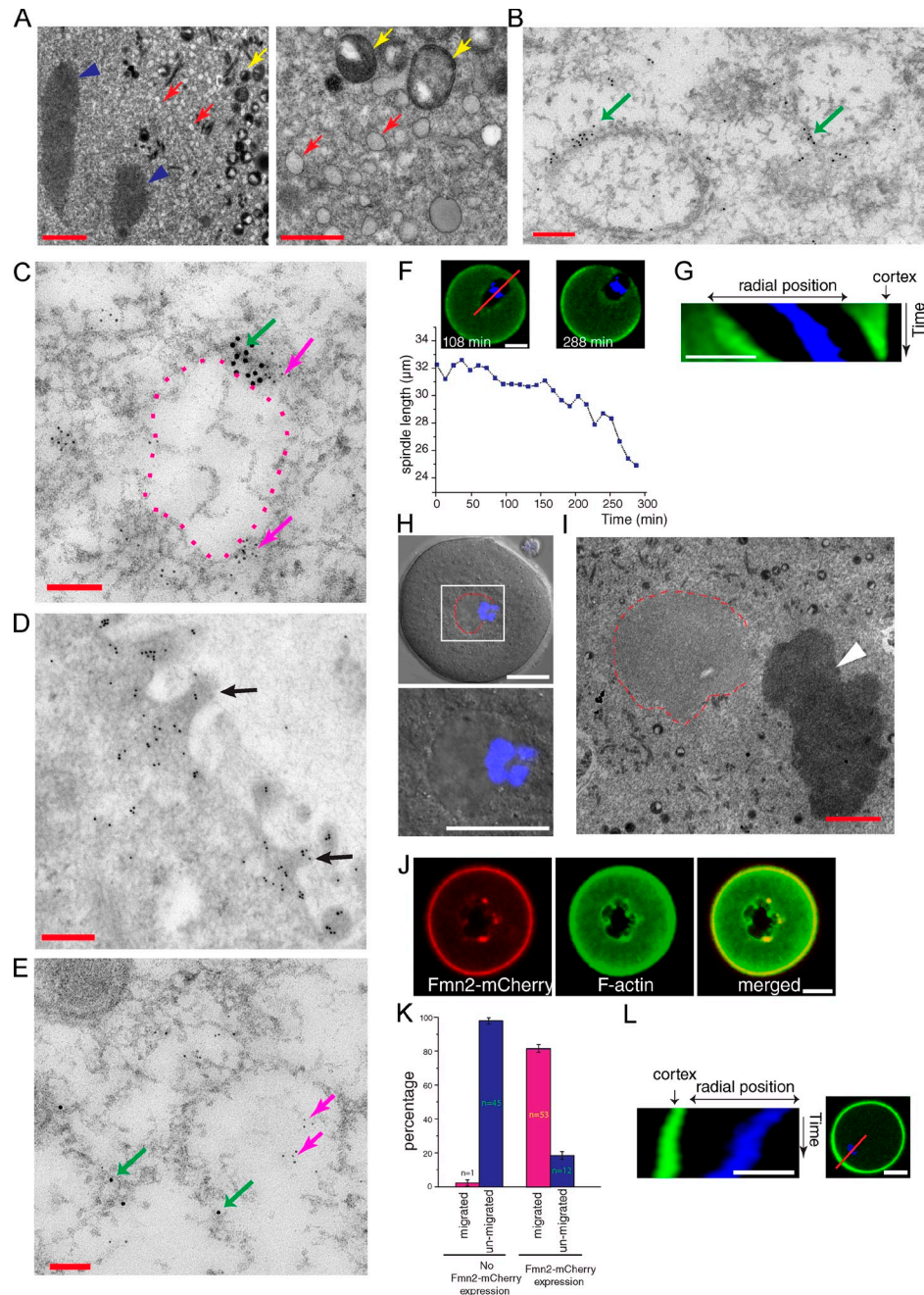


Figure S2. Immuno-EM of Fmn2 and additional evidence that the chromosomes/spindle experiences a pushing force during the migration to the cortex.

(A) Thin-sectioning EM of an oocyte in the metaphase of MI. ER organizes into vesicular structures surrounding the spindle/chromosomes (blue arrowheads). Red arrows show vesicular ER. Yellow arrows show mitochondria. Right image is a higher magnification view of vesicular ER and mitochondria in MI mouse oocyte. (B) Immunogold labeling of Fmn2-AcGFP in mouse oocyte at the spindle migration stage. Fmn2 localizes to the vesicular ER structures, as detected by the immunogold particles (green arrows). (C) Double immunogold labeling of Fmn2 (large gold particles, green arrow) and Sec61-β, an ER component (small gold particles, magenta arrows). The ER membrane is outlined with the magenta-colored dashed line. (D) Fmn2 (small gold particles) is also present on microvilli at the oocyte periphery, detected by immunogold labeling. Black arrows show microvilli. (E) Immunogold localization of Fmn2 with the anti-Fmn2 antibody. Endogenous Fmn2 (large gold particles, green arrows) is present at the ER membrane. Anti-KDEL was used to label ER structures (small gold particles, magenta arrows). Note that KDEL is an ER retention signal, and accordingly, anti-KDEL labels the proteins inside the membrane. (F and G) Spindle length compaction toward the end of the migration. (F) Images shown are frames from a time-lapse video of a spindle, apparent as the zone containing chromosomes (blue) roughly outlined by Fmn2-AcGFP fluorescence (see Fig. S1 B) at different time points before arrival at the cortex. Note the shortening of the long axis of the spindle in the right image compared with the one on the left. Quantification of the spindle length over time is shown below the images. The data shown are from a representative video ($n = 5$). (G) Kymograph generated along the red line shown in F, showing that spindle shortening occurred mostly by compaction from the side distal to the cortex, consistent with a pushing force at this side. (H and I) A zone (referred to as spindle remnant) in the cytoplasm of mouse oocytes with a distinct appearance under light microscopy and EM after this migration. (H) An example of spindle remnant (outlined with dashed red line) after colcemid treatment under light microscopy. The spindle remnant is attached to one side of the chromosomes (blue). The boxed region is magnified on the bottom. (I) The spindle remnant (outlined with the dashed red line) is of distinct appearance under thin-sectioning EM. The white arrowhead shows chromosomes. (J) Fmn2-mCherry expression induces GV deformation and GV migration. Fmn2-mCherry localizes to foci on the GV envelope, in an oocyte arrested at the GV stage, and nucleates actin filaments. Invaginations are observed in these sites, suggesting forces from the Fmn2-mediated actin assembly are being exerted on the GV envelope. (K) Quantification of the percentage of oocytes undergoing GV migration to the cortex with or without Fmn2-mCherry expression. Shown are means and SEM from three experiments. (L) Cortical cap protrudes rather than indents as the chromosomes/spindle migrates toward the cortex. The left shows the kymograph generated along the red line in the image on the right from a representative time-lapse video of spindle migration (cortex labeled with UtrCH-GFP). Bars: (A [left]) 1 μm; (A [right]) 0.5 μm; (B–E) 100 nm; (F–H, J, and L) 20 μm; (I) 2 μm.

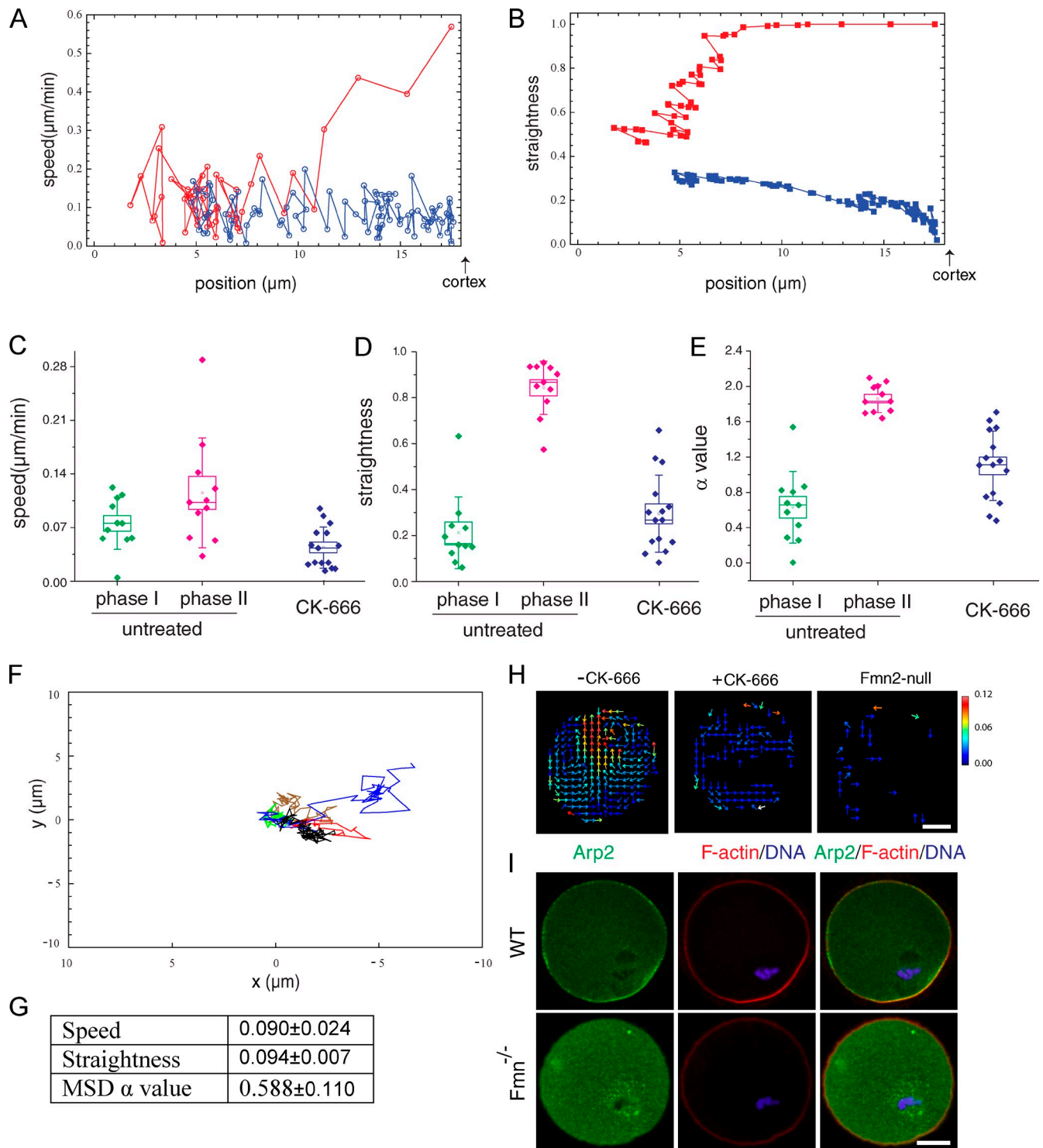
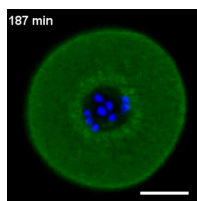
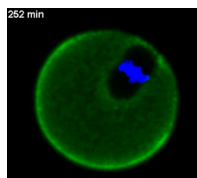


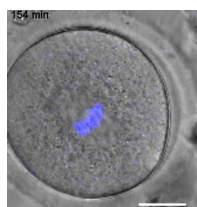
Figure S3. Biphasic movement of spindle/chromosomes and the Arp2/3-dependent cytoplasmic streaming. (A–E) Fast, directed second phase of spindle migration is abolished by Arp2/3 inhibition. (A and B) Speed (A) and straightness (B) analysis of spindle migration as a function of the displacement of the chromosomes from the cell center. Examples are from 26 oocytes analyzed. Red plots are from a representative untreated oocyte. Blue plots are from a representative CK-666-treated oocyte. (C–E) Quantification of speed (C), straightness (D), and MSD α value (E) of phase I and phase II of spindle migration. The same analyses were performed in CK-666-treated oocytes, but these oocytes do not have the second, fast phase of spindle movement. The box range represents SEM, the small square is the mean, whiskers show SDs, and the line inside the box is the median. (F) Trajectories of spindles that failed to reach the cortex after CK-666 treatment. Examples are from 17 oocytes analyzed. (G) Quantification of speed, straightness, and MSD α value of the spindle migration shown in F. (H) Cytoplasmic streaming analyzed by using STICS analysis. Vector map of control oocyte, CK-666-treated oocyte, and *Fmn2*^{-/-} oocyte generated by STICS analysis. The heat bar unit is given in micrometers per minute. (I) *Fmn2* is required for cortical localization of the Arp2/3 complex. *Fmn2*^{-/-} oocytes were fixed and stained with the rabbit anti-Arp2 antibody. Arp2 is concentrated at the cortical cap in wild-type oocyte but absent from the cortex of *Fmn2*-null oocytes. Bars, 20 μ m.



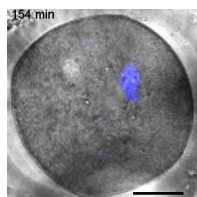
Video 1. **Dynamic localization of Fmn2 in an oocyte before spindle migration.** Fmn2-AcGFP (green) was expressed in the mouse oocyte. Blue shows Hoechst staining of chromosomes. Frames are 11 min apart, and video length is 550 min. Bar, 20 μ m.



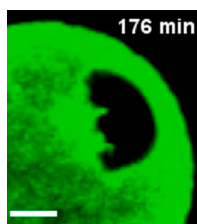
Video 2. **Exclusion of cortical Fmn2 and shortening of the spindle length toward the end of migration.** Fmn2-AcGFP (green) was expressed in the mouse MI oocyte. Blue shows Hoechst staining of chromosomes. Frames are 12 min apart, and video length is 288 min. Bar, 20 μ m.



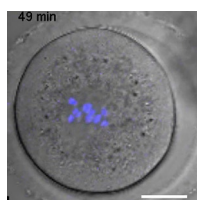
Video 3. **Biphasic chromosome movement.** Time-lapse imaging of an oocyte undergoing spindle migration. Blue shows Hoechst staining of chromosomes. Note that the slow, less directed spindle motion is followed by an abrupt, straight migration toward the cortex. Frames are 5.5 min apart, and video length is 275 min. Bar, 20 μ m.



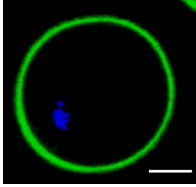
Video 4. **Chromosome migration after spindle disassembly with colcemid.** The video shows two examples. In the left image, the chromosome migrates from an initial position near the cell center, whereas in the right image, the migration started near the cortex. Note that the spindle remnant is trailing behind the chromosomes during the migration. Also note that the trajectory of the oocyte on the left shows a biphasic manner, whereas the trajectory of the oocyte on the right only showed highly directed movement. Furthermore, the cytoplasmic streaming occurred concurrent with the second phase in the oocyte on the left. It can also be observed from the image on the right that cytoplasmic streaming continues even after the chromosomes reached the cortex. Blue shows Hoechst staining of chromosomes. Frames are 5.5 min apart, and video length is 236 min. Bar, 20 μ m.



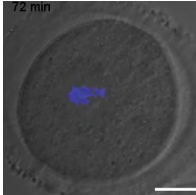
Video 5. **GV migration upon Fmn2-mCherry expression.** Fmn2-mCherry was not imaged in this video, and UtrCH-GFP (green) was expressed to show F-actin dynamics. Frames are 11 min apart, and video length is 176 min. Bar, 10 μ m.



Video 6. **An oocyte undergoing spindle migration followed by first polar body extrusion.** The video shows that cytoplasmic streaming continued to maintain the oocyte set of the chromosomes at the cortex after polar body extrusion. As such, the streaming in the MI oocyte is a continuation of the streaming that started during spindle migration in MI. Blue shows Hoechst staining of chromosomes. Frames are 5.5 min apart, and video length is 544 min. Bar, 20 μ m.



Video 7. **An oocyte undergoing spindle migration followed by first polar body extrusion and MII spindle positioning.** The UtrCH-GFP (green) was used to label cortical changes during spindle migration. Kymograph of the UtrCH-GFP was provided as in Fig. S2 L. The video again shows that cytoplasmic streaming continues to the MII arrest stage to maintain the oocyte set of chromosomes/MI I spindle in place close to the cortex. Frames are 11 min apart, and video length is 840 min. Bar, 20 μ m.



Video 8. **Absence of a second phase of straight and fast movement when Arp2/3 is inhibited.** Blue shows Hoechst staining of chromosomes. Frames are 5.5 min apart, and video length is 378 min. Bar, 20 μ m.

A zip file is also provided that contains custom plugins written for ImageJ.