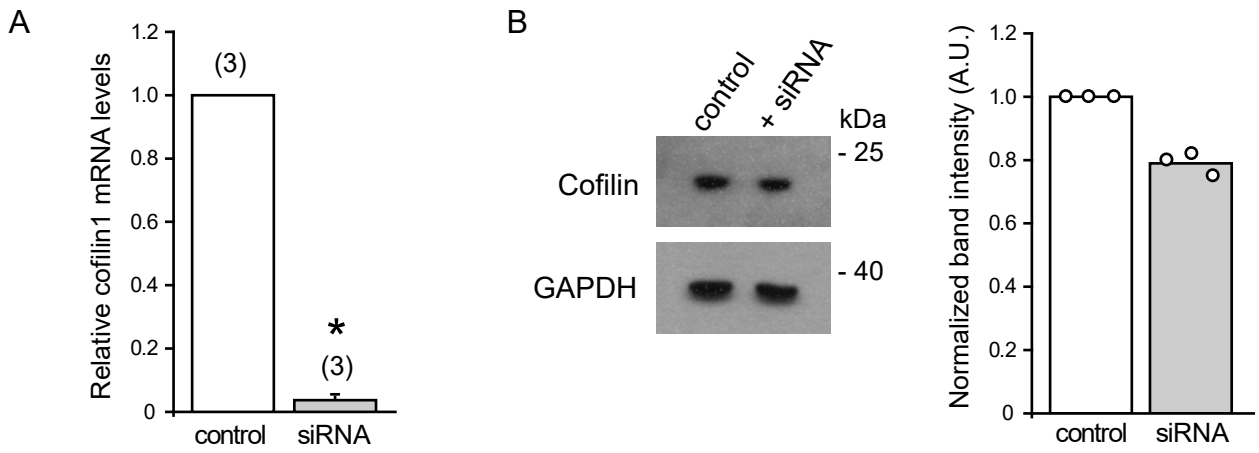


Fig. S1. Depletion of cofilin mRNA by RNA interference.

GV oocytes were injected with siRNA against cofilin1 and cultured for 22 h with milrinone to maintain prophase arrest. (A) Quantification of cofilin1 mRNA levels by quantitative real time PCR, using beta actin mRNA as an internal control for normalization. Data are means +/- SD of three independent experiments. * : $P<10^{-5}$. (B) Detection of total cofilin levels in GV oocyte lysates, by western blot. GAPDH detection was used as a loading control. The bar graph shows the quantification of band intensities after normalization to the GAPDH signal. The experiment was repeated three times.

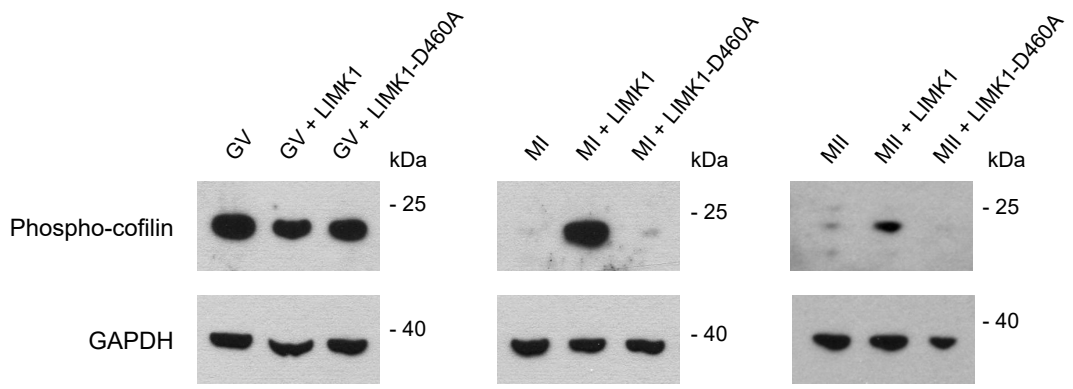


Fig. S2. LIMK1 overexpression increases phospho-cofilin levels.

Phospho-cofilin was detected in lysates from 70-80 GV (left panel), MI (middle panel) and MII (right panel) oocytes. Overexpression of LIMK1 (+LIMK1), or catalytically-inactive LIMK1-D460A (+LIMK1-D460A) was achieved by microinjection of the corresponding cRNA. For GV oocytes (left panel), injection was performed at the GV stage and oocytes were cultured in vitro for 3 h before lysis. For MI oocytes, injection was performed at the GV stage and oocytes were cultured in vitro until the MI (NEBD+6h) stage. For MII oocytes, injection was performed in freshly isolated MII oocytes, which were cultured in vitro for 3 h before lysis. Note that MI oocytes expressed LIMK1 for an extended period of time (7-8 h), which may explain the higher band intensity in comparison with MII oocytes. Detection of GAPDH was used as a loading control. Data are representative of at least 3 similar observations for LIMK1-overexpression and two similar observations for LIMK1-D460A overexpression.

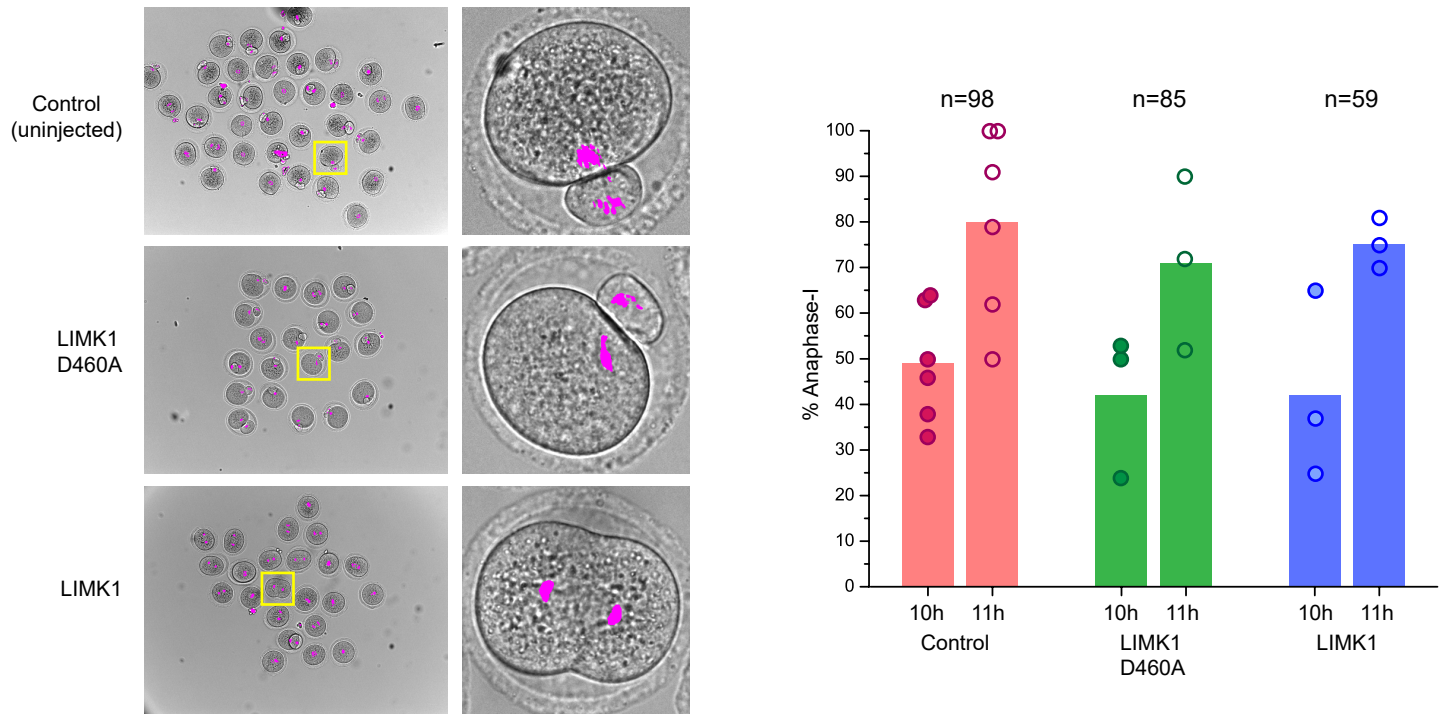


Fig. S3. LIMK1 overexpression does not affect the timing of anaphase-I.

Oocytes arrested at the GV stage with milrinone were injected with cRNA encoding LIMK1 or LIMK1^{D460A}, or were left uninjected (Control). After milrinone washout, oocytes were cultured in M16 medium until anaphase-I. Oocytes were scored for anaphase-I at 10 h (t=10h) and 11 h (t=11h) post-milrinone washout, using Hoechst-33342 staining to visualize chromosome configuration. The left panel shows representative images of all 3 populations of oocytes, taken at t=11h. Chromosomes are labeled with Hoechst 33342 (magenta). Magnified images of individual oocytes (as indicated by the yellow square) are shown. Note the absence of polar body protrusion in LIMK1-expressing oocytes. The right panel shows the rate of anaphase-I as observed at t=10h and t=11h, expressed as percentages of the total number of oocytes scored in each experiment. Data points represent individual experiments, and the bars represent the corresponding mean value. The total number of oocytes scored is indicated above each data sets.

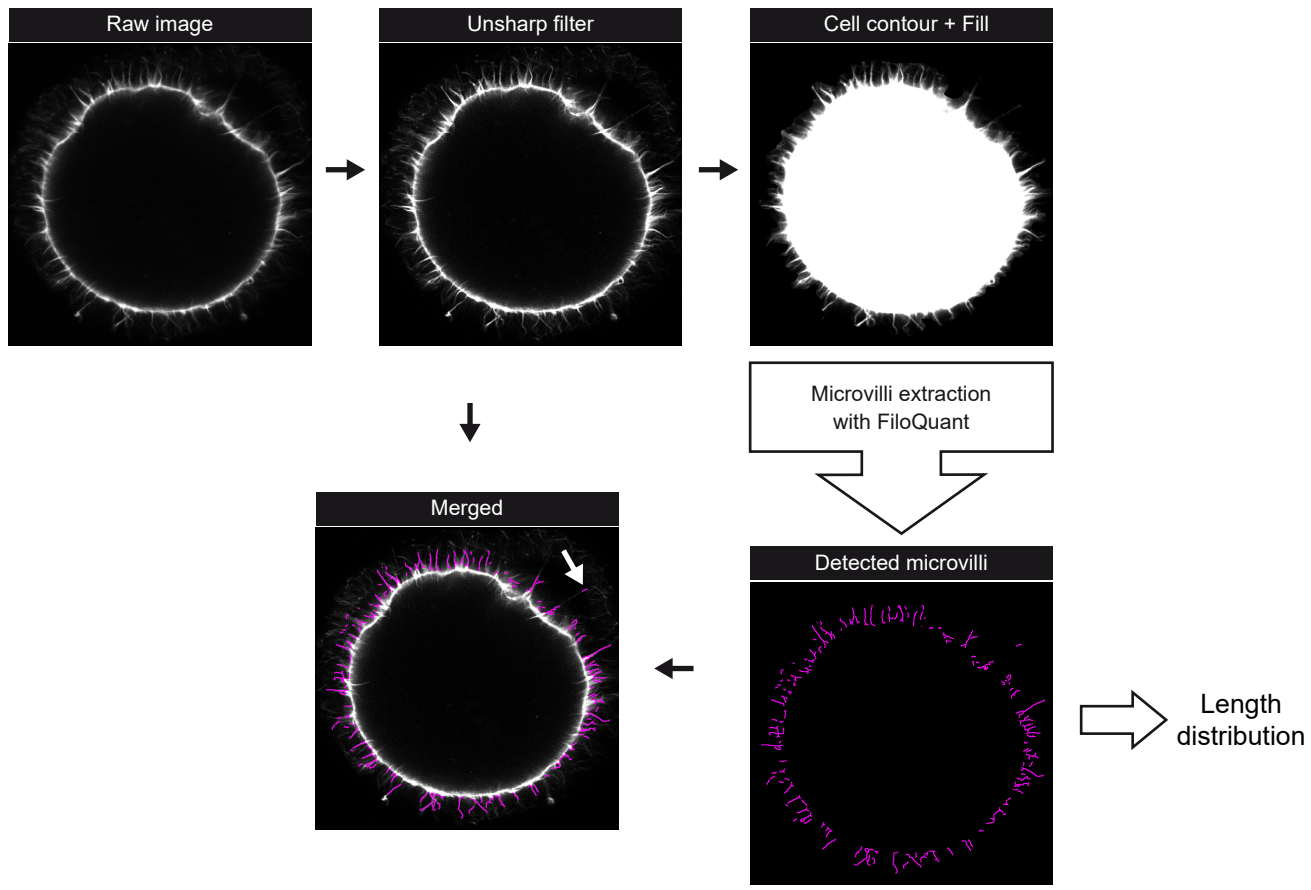


Fig. S4. Detection of elongated microvilli with Filoquant.

Filoquant (Jacquemet et al., 2017) was used to detect microvilli in fixed oocytes stained with Alexa Fluor 568-phalloidin. The image processing workflow is shown for a LIMK1-expressing MI oocyte treated with CK-666. Raw confocal images (thickness 1 µm) were first processed with Fiji to sharpen the fluorescence signal and delineate the cell contour. Microvilli (magenta) were resolved with Filoquant in the single image analysis mode, by iteratively adjusting the settings for cell edge detection and “filopodia” detection. In the merged image, the white arrow points to a “broken” microvillus fragment.

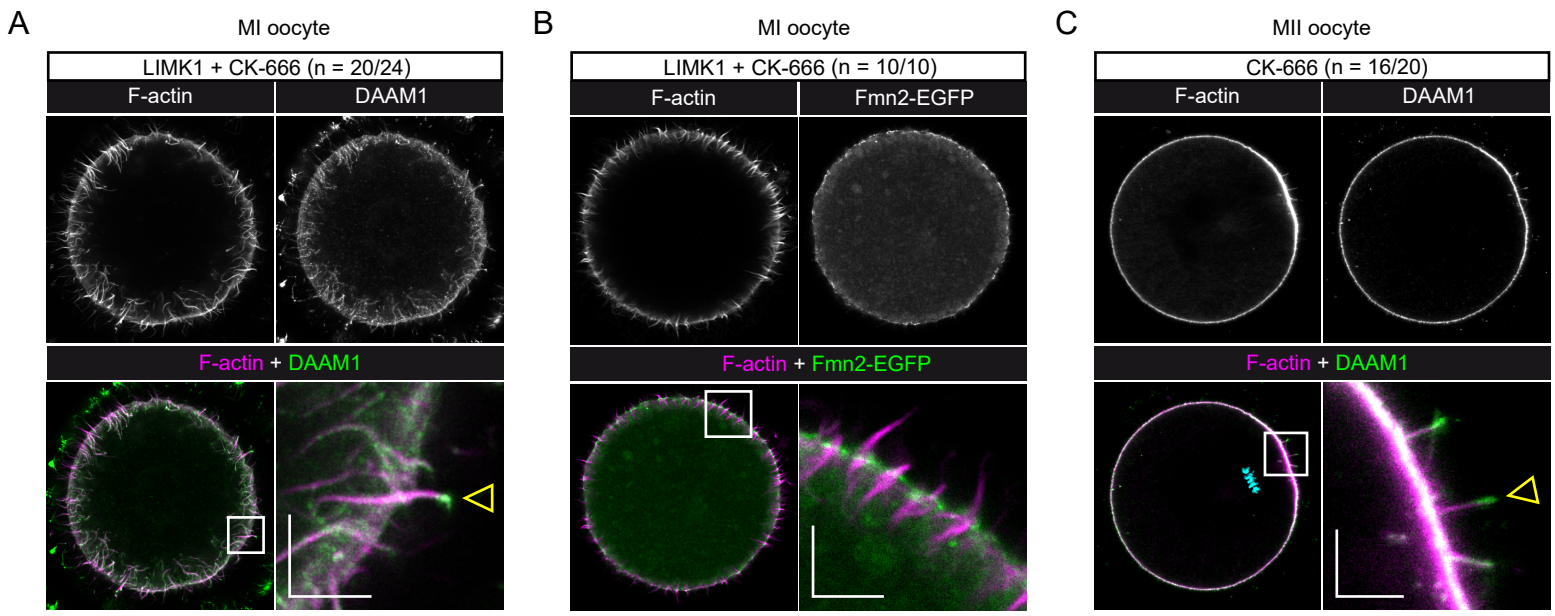


Fig. S5. DAAM1 decorates elongated microvilli and accumulates at the tips.

(A,B) LIMK1-expressing MI oocytes treated with CK-666 (100 μM) and fixed at MI (NEBD+6h). (C) MII oocyte treated with CK-666 (100 μM) for 3h before fixation. In (A) and (C), oocytes were immuno-stained for DAAM1 (green). In (B), oocytes were coinjected with cRNA encoding Fmn2-EGFP (green). F-actin was stained with Alexa Fluor 568-phalloidin (magenta). Chromosomes in (C) are labeled with TO-PRO-3 (cyan). Expanded views of cortical areas with elongated microvilli (white boxes) are shown. The number of oocytes examined showing a similar pattern is indicated in parentheses. Scale bars represent 5 μm. (D) A selection of individual DAAM1 fluorescence profiles. The fluorescence intensity of DAAM1 staining (in arbitrary units) along individual microvilli was plotted as a function of the distance (μm). Six distinct profiles are shown, from six different MI oocytes (as in panel A) and six different MII oocytes (as in panel C). Fluorescence profiles highlighted in green refer to the elongated microvilli marked by an arrowhead in (A) and (C). Note the increased intensity at microvillar tips, reflecting DAAM1 accumulation.

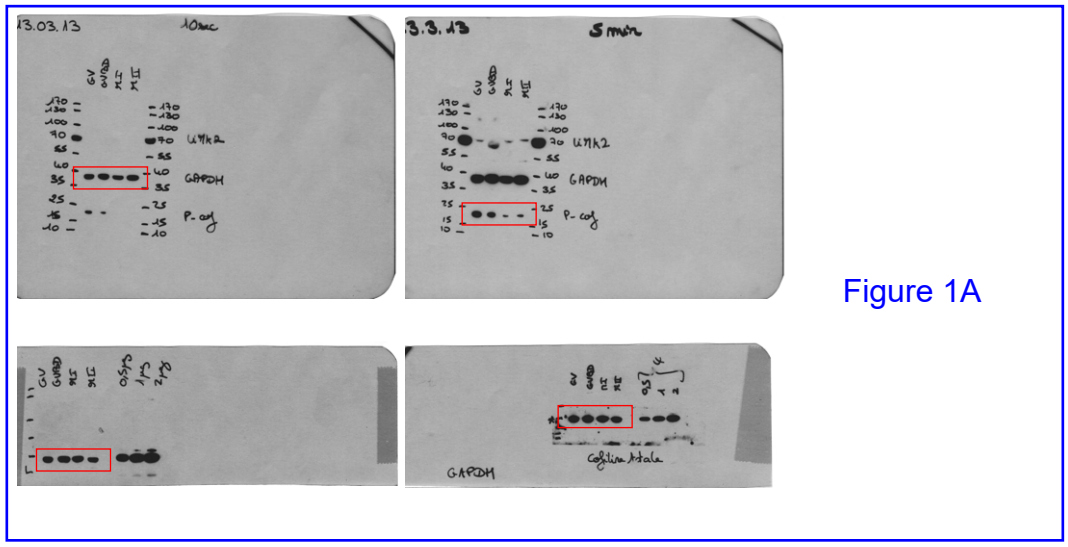


Figure 1A

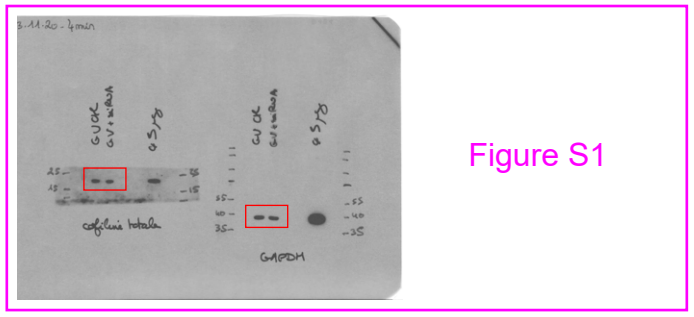


Figure S1

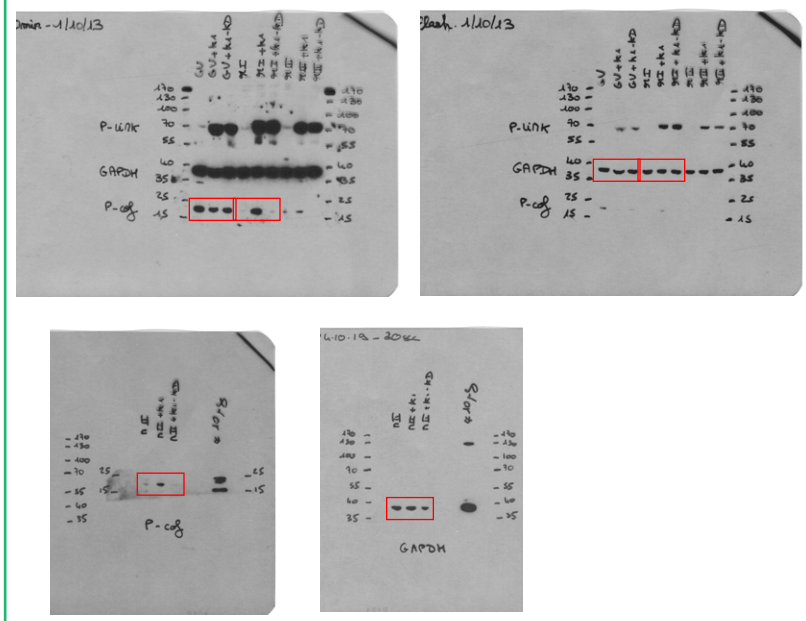
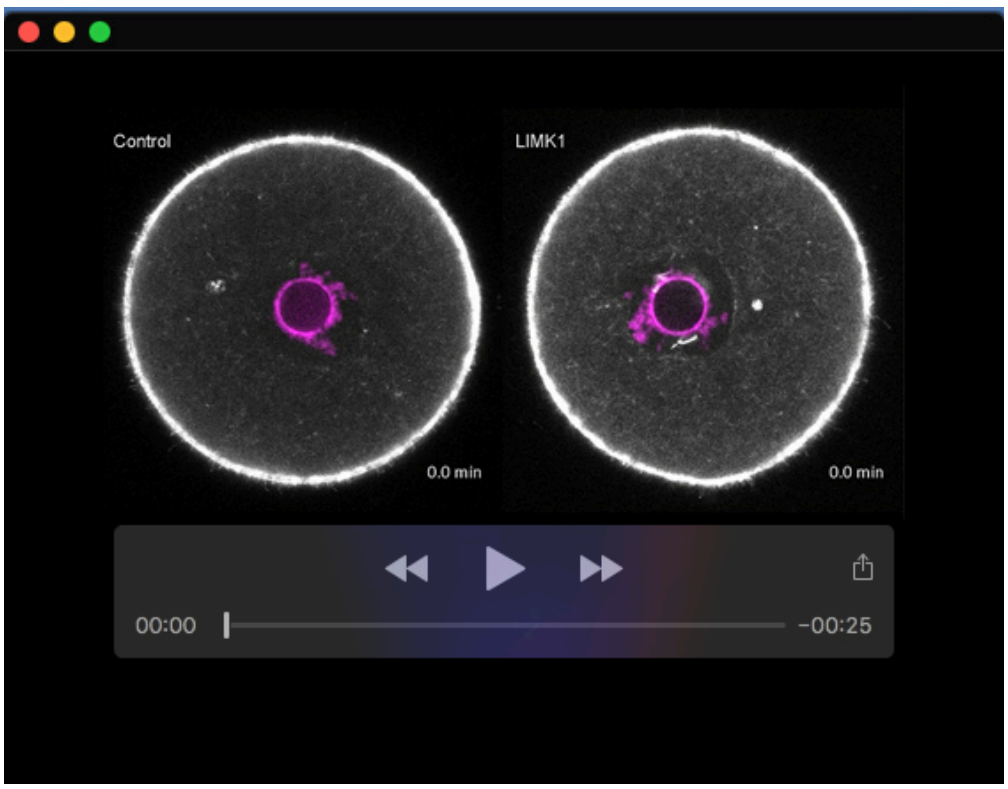
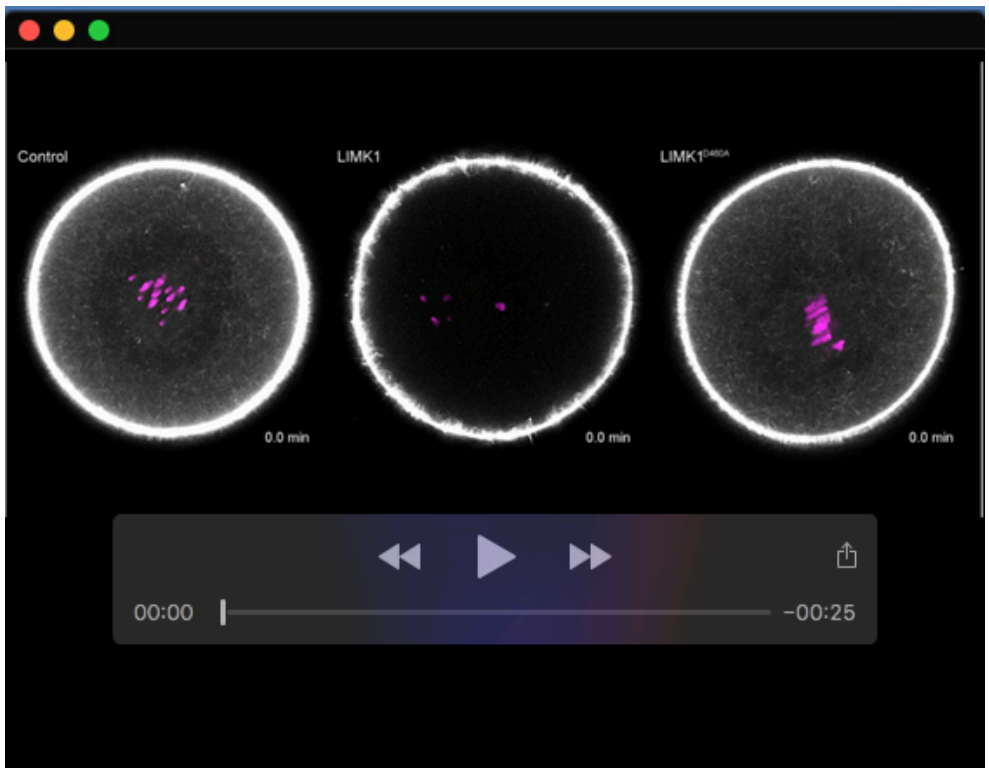


Figure S2

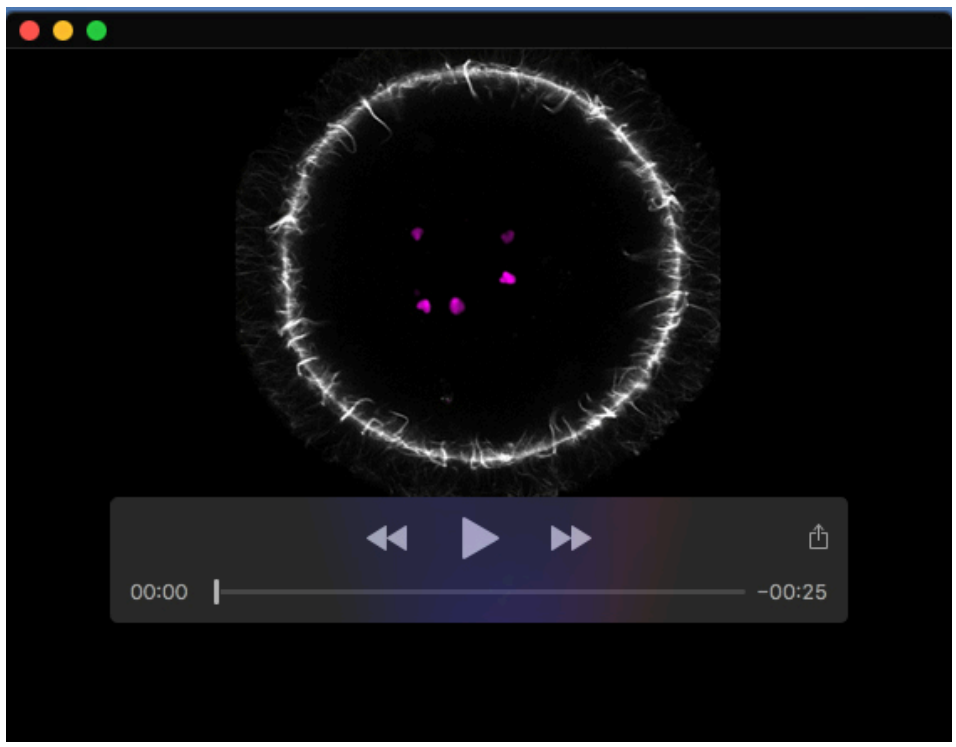
Fig. S6. Uncropped images of the Western blot data.



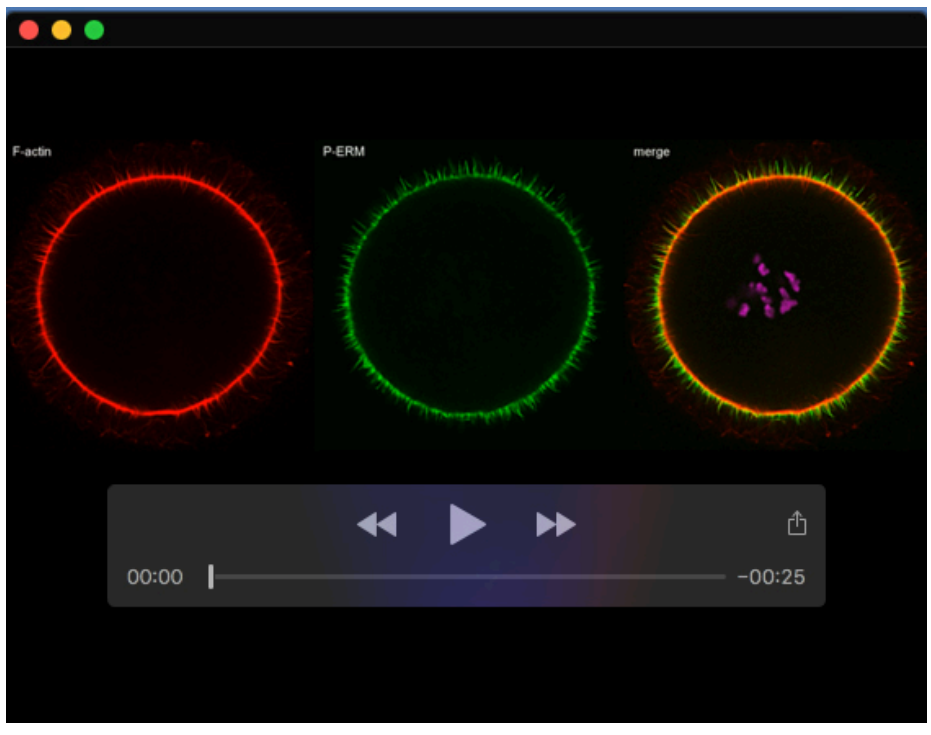
Movie 1. Cofilin is dispensable for cytoplasmic F-actin dynamics in GV oocytes and during NEBD. Time-lapse confocal imaging (one image every 30 sec) of F-actin networks in GV oocytes expressing EGFP-UtrCH together with catalytically-inactive LIMK1^{D460A} (Control, left), or LIMK1 (right). Spontaneous meiosis resumption was triggered by washing off milrinone, leading to nuclear envelope breakdown. Chromosomes are labeled with Sir-DNA (magenta). Confocal section thickness is 1 µm.



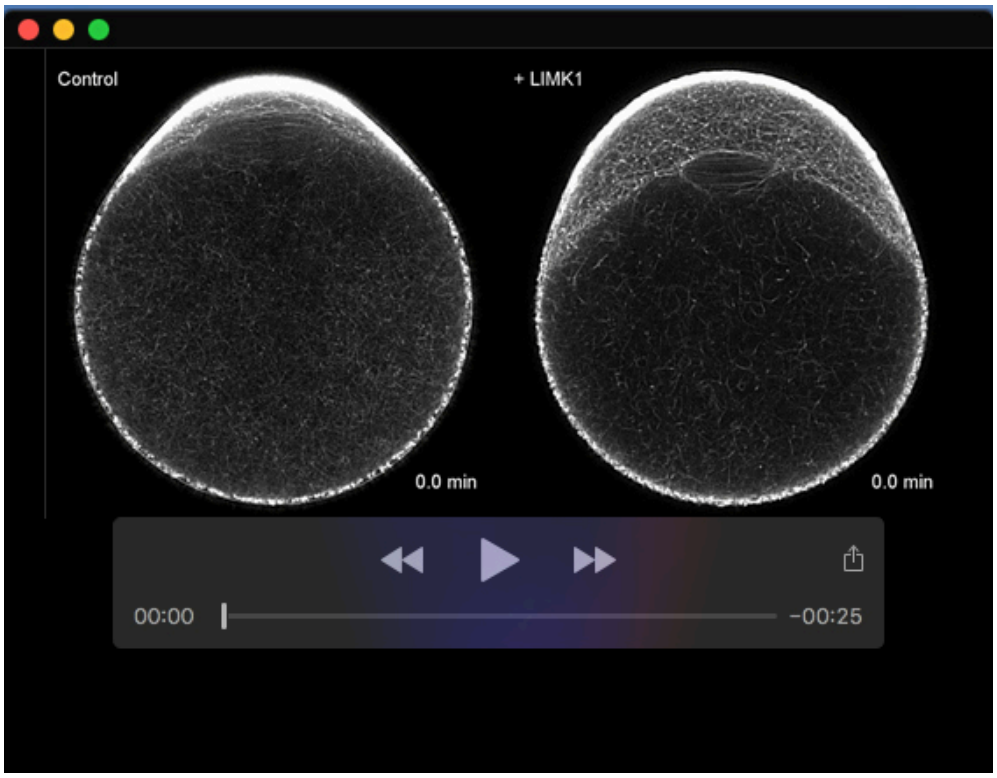
Movie 2. Cytoplasmic F-actin is absent in LIMK1-expressing MI oocytes. Time-lapse confocal imaging (one image every 30 sec) of F-actin networks in oocytes expressing EGFP-UtrCH. Left to right: uninjected MI oocyte (Control), LIMK1-expressing MI and LIMK1^{D460A}-expressing MI oocytes. Chromosomes are labeled with Sir-DNA (magenta). Confocal section thickness is 1 µm.



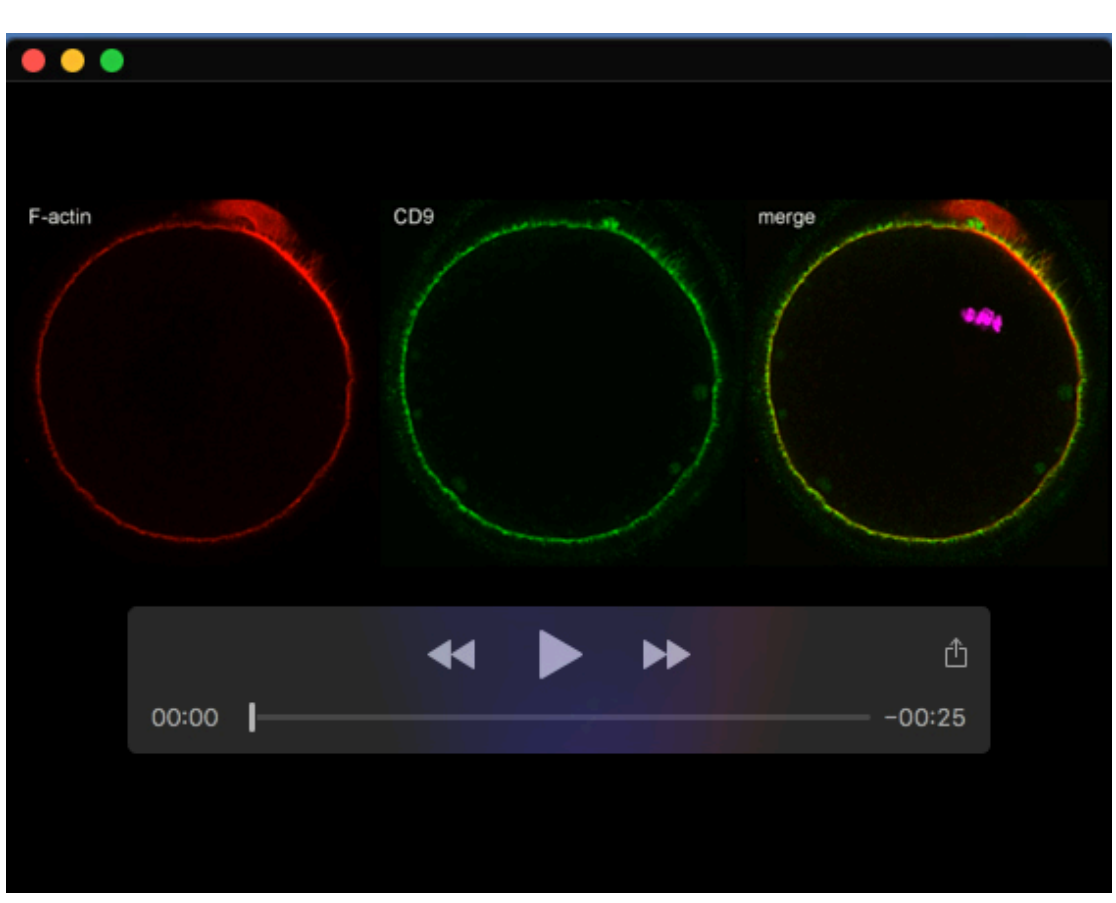
Movie 3. Arp2/3 inhibition exacerbates microvillar actin elongation. Confocal z-stack of a fixed LIMK1-expressing MI oocyte labeled for F-actin with Alexa Fluor 568-phalloidin. CK-666 (100 µM) was added to the culture medium at the onset of meiosis resumption (milrinone wash). Chromosomes are labeled with TO-PRO-3 (magenta). Confocal section thickness is 1 µm. The outermost layer shows a weak labeling of transzonal projections crossing the zona pellucida.



Movie 4. Elongated microvilli are enriched in P-ERM. Confocal z-stack of a LIMK1-expressing MI oocyte treated with CK-666 (100 µM) and labeled for F-actin (Alexa Fluor 568-phalloidin, red) and P-ERM (green). Chromosomes are labeled with TO-PRO-3 (magenta). Confocal section thickness is 1 µm.



Movie 5. F-actin dynamics in MII and LIMK1-expressing MII oocytes. Time-lapse confocal imaging (one image every 30 sec) of F-actin networks in a control MII oocyte expressing LIMK1-D460A (left) and an MII oocyte expressing LIMK1 (right). Oocytes also express EGFP-UtrCH to label actin filaments. Confocal section thickness is 1 µm.



Movie 6. Ectopic microvilli are enriched in CD9. Confocal z-stack of an MII oocyte treated with CK-666 (100 µM) for 3h, then fixed and labeled for F-actin (Alexa Fluor 568-phalloidin, red) and CD9 (green). Chromosomes are labeled with TO-PRO-3 (magenta). The red halo at the start of the series is due to the F-actin signal in the first polar body. Confocal section thickness is 1 µm.