Supplemental Information

Spire-Type Actin Nucleators

Cooperate with Formin-2 to Drive

Asymmetric Oocyte Division

Sybille Pfender, Vitaliy Kuznetsov, Sandra Pleiser, Eugen Kerkhoff, and Melina Schuh
**Figure S1. Individual depletion of Spire1 or Spire2 does not block asymmetric spindle positioning, actin network formation or polar body extrusion**

(A-C) The relative expression of Spire1 (A), Spire2 (B) and Fmn2 (C) in different tissues and oocytes was measured by quantitative real-time PCR. mRNA levels were normalized to those in oocytes. Data are mean±s.d. from two independent experiments.

(D) mRNA levels of Spire1 were determined by quantitative real-time PCR in oocytes injected with scrambled negative control siRNA (Control) and Spire1 siRNAs (RNAi). mRNA levels were normalized to those in oocytes injected with scrambled negative control siRNA. Data are mean±SEM from two independent experiments.

(E) mRNA levels of Spire2 were determined by quantitative real-time PCR in oocytes injected with scrambled negative control siRNA (Control) and Spire2 siRNAs (RNAi). mRNA levels were normalized to those in oocytes injected with scrambled negative control siRNA. Data are mean±SEM from two independent experiments.

(F) mRNA levels of Spire1 and Spire2 were determined by quantitative real-time PCR in oocytes injected with scrambled negative control siRNA (Control) and Spire1 and Spire2 siRNAs. mRNA levels were normalized to those in oocytes injected with scrambled negative control siRNA. Data are mean±SEM from three independent experiments.

(G) Spindle movements in live oocytes expressing mCherry-α-tubulin (red, microtubules, merged with DIC). Oocytes were injected with scrambled negative control siRNA (Control), Spire1 siRNAs (Spire1 RNAi) and Spire2 siRNAs (Spire2 RNAi). White ovals mark initial spindle positions. Time: hr:min. Scale bar: 10 µm.

(H) The spindle was tracked in oocytes in 3D data sets (13 sections, every 7 µm) as shown in (G). Spindle movements in control (black; v=0.17±0.05 µm/min; n=10), Spire1 RNAi (red; v=0.13±0.05 µm/min; n=10; p=0.07) and Spire2 RNAi (grey; v=0.13±0.08 µm/min; n=12; p=0.2)

(I) Oocytes from control, Spire1 RNAi and Spire2 RNAi were fixed during asymmetric spindle positioning (4-5 hours after germinal vesicle breakdown) and stained with Alexa Fluor 488 phalloidin to label F-actin. Scale bar: 5 µm.

(J) The mean intensity of the cytoplasmic phalloidin staining was measured in oocytes as shown in (I) that were injected with control siRNA (Control; black), with Spire1 siRNAs (Spire1 RNAi; red) and Spire2 siRNAs (Spire2 RNAi; red). Data are mean±s.d.. P-values were calculated with Student’s t-test.

(K) Polar body extrusion in oocytes that were injected with scrambled negative control siRNA (Control), with Spire1 siRNAs (Spire1 RNAi) or Spire2 siRNAs (Spire2 RNAi). Scale bar: 10 µm.
Polar body extrusion efficiency was scored for oocytes that progressed into anaphase and were injected with scrambled negative control siRNA (Control), *Spire1* siRNAs (*Spire1 RNAi*) and *Spire2* siRNAs (*Spire2 RNAi*).
Figure S2. Individual overexpression of human Spire1 or Spire2 rescues asymmetric spindle positioning and polar body extrusion

(A) Spindle movements in live oocytes expressing EGFP-α-tubulin or mCherry-α-tubulin (red, microtubules, merged with DIC). Oocytes were injected with scrambled negative control siRNA (Control), Spire1 and Spire2 siRNAs (Spire1+2 RNAi) and Spire1 and Spire2 siRNAs together with human Spire1 (Rescue (Spire1)) or Spire2 mRNAs (Rescue (Spire2)). White ovals mark initial spindle positions. Time: hr:min. Scale bar: 10 µm.

(B) Spindle movement (µm) over time (min) for Control, Rescue (Spire1), Rescue (Spire2), and Spire1+2 RNAi.

(C) Polar body extrusion percentages for Control, Spire1+2 RNAi, Rescue (Spire1), and Rescue (Spire2).
(B) The spindle was tracked in oocytes in 3D data sets (13 sections, every 7 µm) as shown in (A). Spindle movements in control (black; v=0.12±0.05 µm/min; n=17), Spire1+2 RNAi (red; v=0.04±0.03 µm/min; n=13; p≤10⁻⁵) and rescue experiments with human Spire1 (blue; v=0.11±0.06 µm/min; n=7; p=0.6) or with human Spire2 (grey; v=0.12±0.06 µm/min; n=7; p=0.94).

(C) Representative metaphase II oocytes expressing EGFP-α-tubulin or mCherry-α-tubulin (red, microtubules, merged with DIC) that were injected with scrambled negative control siRNA (Control), with Spire1+2 siRNAs (Spire1+2 RNAi) and Spire1+2 siRNAs together with human Spire1 (Rescue (Spire1)) or human Spire2 mRNA (Rescue (Spire2)). Scale bar: 10 µm.

(D) Polar body extrusion efficiency was scored for oocytes that progressed into anaphase and were injected with scrambled negative control siRNA (Control), Spire1+2 siRNAs (Spire1+2 RNAi), Spire1+2 siRNAs together with human Spire1 (Rescue (Spire1)) or human Spire2 mRNAs (Rescue (Spire2)). Oocyte numbers are indicated in white on the columns.
Figure S3. Mouse oocytes divide symmetrically if asymmetric spindle positioning is blocked by artificially increasing the network density

(A) Wildtype oocytes (Wildtype) and oocytes highly overexpressing Spire1+2 and Fmn2 were fixed during asymmetric spindle positioning and stained with Alexa Fluor 488 phalloidin to label F-actin. Boxed regions are magnified in the right panel. Scale bars: 10 µm
(B) The mean intensity of the actin network was measured in oocytes as shown in (A) that were wildtype (black column) or overexpressing Spire1+2 and Fmn2 (red column). Data are means±s.d. P-values were calculated with Student’s t-test.

(C) Spindle movements in live oocytes expressing EGFP-MAP4 (red, microtubules, merged with DIC). Oocytes were wildtype (Wildtype) or overexpressing Spire1+2 and Fmn2 (Nucleator overexpression). White ovals mark initial spindle positions. Time: hr:min. Scale bar: 10 µm.

(D) The spindle was tracked in wildtype oocytes (Wildtype; black; v=0.08±0.03 µm/min; n=19) and oocytes overexpressing Spire1+2 and Fmn2 (Nucleator overexpression; red; v=0.05±0.02 µm/min; n=14; p≤10^{-3}; time points interpolated to 10 min intervals) in 3D data sets (13 sections, every 7 µm) as shown in (C).

(E) Anaphase in live oocytes expressing EGFP-α-tubulin (red, microtubules) merged with DIC. Asymmetric spindle positioning was blocked by severely overexpressing Spire1 and Spire2. Boxed regions are magnified without DIC below. Scale bars: 10 µm. Time: hr:min.

(F) Live oocytes were labelled with EGFP-α-tubulin and asymmetric spindle positioning was blocked by severely overexpressing Spire1+2/Fmn2. Oocytes were monitored by long term time-lapse microscopy and symmetric division or cytokinetic failure upon anaphase onset were scored.
Figure S4. Network assembly is not dependent on Rho GTPase activity, but is controlled by the expression level of Spire1/Spire2 and Fmn2

(A) Oocytes that were injected with 10 pl of 60 µg/ml BSA, 60 µg/ml Exoenzyme C3 (final c in oocyte: 120 nM), exposed to 3 µg/ml Toxin B or injected with mRNA encoding Rac1T17N or Cdc42T17N were fixed during asymmetric spindle positioning and stained with Alexa Fluor 488 phallodin to label F-actin. Different conditions are specified on the left side of the images. Scale bars: 10 µm.

(B) The mean intensity of the cytoplasmic phalloidin staining was measured in oocytes as shown in (A) that were injected with 10 pl of 60 µg/ml BSA, 60 µg/ml Exoenzyme C3 (final c in oocyte: 120 nM), exposed to 3 µg/ml Toxin B or injected with mRNA encoding Rac1T17N or Cdc42T17N. Data are mean±s.d.. P-values were calculated with Student’s t-test. Quantification of the density of the actin network revealed that Rho GTPases are not required to activate Spire1/Spire2 and Fmn2 dependent actin nucleation in mouse oocytes. Instead, inhibition of Cdc42 and Rac1 and treatment with Toxin B slightly increased the density of the actin network. This could be due to a direct inhibitory effect of Rho GTPases
on Spire1/Spire2 and Fmn2 dependent F-actin nucleation, or due to indirect effects because more monomeric actin is available for Spire1/Spire2 and Fmn2 when other actin nucleators that are Cdc42 and Rac1 dependent are inactive.

(C) Wildtype oocytes (Wildtype) and oocytes injected with different concentrations of mRNAs encoding Spire1-mCherry, Spire2-mCherry and Fmn2-mCherry (lower row; 1x, 3x and 10x) were fixed during asymmetric spindle positioning and stained with Alexa Fluor 488 phalloidin to label F-actin.

(D) The mean cytoplasmic phalloidin staining was measured in oocytes as shown in (C) that were injected with different concentrations of mRNAs encoding Spire1-mCherry, Spire2-mCherry and Fmn2-mCherry (red columns; 1x, 3x and 10x) and compared to the intensity in wildtype oocytes (black columns; 1x, 3x and 10x). Data are means±s.d. P-values were calculated with Student's t-test.
Supplemental Movies

Supplemental Experimental Procedures

**Measurement of the cytoplasmic network density**

The quantify the density of the cytoplasmic actin network, we measured the mean intensity of Alexa Fluor 488 phalloidin staining in the cytoplasm and in a region outside of the oocyte for background subtraction. Images in control and perturbed situations were acquired with identical imaging conditions and care was taken that images were not saturated during acquisition. To accurately measure comparable intensities in different oocytes, images for quantification were always acquired in the centre of the oocyte as determined by the maximum radius of the oocyte. Average, standard deviation, and statistical significance based on Student's t-test were calculated in Excel.

**Automated 3D tracking of the spindle**

To measure the kinetics of asymmetric spindle positioning, we injected oocytes with mRNA encoding fluorescently labelled α-tubulin or MAP4 to label microtubules. We then recorded z-stacks of the entire oocyte volume during asymmetric spindle positioning using Zeiss’ MultiTime Series macro. We corrected for drifts during image acquisition with the ‘correct drift’ function of Imaris (Bitplane) after segmenting the
oocyte volume by low thresholding on the soluble pool of the fluorescent reporter. Afterwards we segmented the spindle by applying a higher threshold and tracked the spindle’s centre of mass during asymmetric positioning using Imaris.

For averaging of asymmetric spindle positioning in different oocytes, we temporally aligned the different data sets to the time when the spindle slowed down due to arrival at the cortex. In Spire1 and Spire2 co-depleted oocytes or oocytes from Formin-2 knockout mice, where the spindle did not reach the cortex, the data sets were aligned to time points corresponding to the end of asymmetric spindle positioning in controls. The distance of the spindle to the alignment position was calculated for each time point by processing the spindle coordinates in Excel and plotted over time. To calculate average velocities of asymmetric spindle positioning, the spindle velocity in each oocyte was calculated by linear regression analysis of the displacement plots. Average, standard deviation, and statistical significance based on Student’s t-test were calculated in Excel.

qRT-PCR
For qRT-PCR results in Figures S1A-S1C, mRNA was extracted from tissues using TRIzol reagent (Invitrogen) and cDNA was generated using the QuantiTec Reverse Transcription Kit (Qiagen). Real-time PCR was performed with the Light Cycler 480 (Roche) using SYBR Green. β-microglobulin mRNA was used for normalization. The following primer sets were used: Spire1, forward, 5’ agc tct gct tct gtt gcc ga 3’, reverse, 5’ ctc gaa cag ctt tcc ccc c 3’; Spire2, forward, 5’ aaa tca agc agg agc gga gg 3’, reverse 5’ ggt ggg ggc ttt gag cag ga 3’ (194 bp fragment); Fmn1, forward, 5’ agc tgg tgt gta agg agt cc 3’, reverse, 5’ gct ggg ggt gac ctc ct 3’ (194 bp fragment); Fmn2, forward, 5’ gtg agg cgg aag ccg gta aa 3’, reverse, 5’ aca cct cct
tct cgc cga gt 3’ (201 bp fragment); \textit{\beta-microglobulin}, forward, 5’ atg gga agc cga acatc g 3’, reverse, 5’ cag tct cag tgg ggg tga at 3’.

For qRT-PCR results in Figure S1D-S1F, mRNA was extracted using an RNeasy Mini Kit (Qiagen) and cDNA was generated using the High Capacity RNA-to-cDNA Kit from (Applied Biosystems). Real-time PCR was performed with the 7900 HT Real-Time Fast PCR System (Applied Biosystems) using SYBR Green. \textit{GAPDH} mRNA was used for normalization. The following primer sets were used: \textit{Spire1}, forward, 5’ gacagcctctgctgaggagg 3’; \textit{Spire1}, reverse, 5’ gggcaagaattttgaggcttctc 3’; \textit{Spire2}, forward, taccaccagcagctcagaag 3’, \textit{Spire2}, reverse, 5’ tcgtgactgctttccagctgc 3’; \textit{GAPDH}, forward, 5’ agagctgacgggaagctcact 3’; \textit{GAPDH}, reverse, 5’ tgcctgctcaccagctttcttgat 3’.

\textbf{siRNA sequences}

The following siRNAs from Qiagen were used:

Mm-Spire1\_1 SI01431591 AAGGTAGAAAGTATAGAAATA
Mm-Spire1\_2 SI01431598 TGCATTGAAATTTATAGTTTA
Mm_Spire1\_3 SI01421605 CACCATCATTAAGATACAGTA
Mm-Spire1\_4 SI01431612 TTTGAGATAATTTTCTGACTAA
Mm_Spire2\_1 SI01431619 CAGGAATGATTAGCTGCGCAA
Mm_Spire2\_2 SI01431626 CAGAGGATGCAACTACAAAA
Mm_Spire2\_3 SI01431633 ACCCTTGCGCATGTACATATA
Mm_Spire2\_4 SI01431640 TTTCTGTAGCGTAAAGATGAA

For the data displayed in Figure 1F, the following siRNA sets were used:

Oligos A: Mm_Spire1\_1, Mm_Spire1\_3, Mm_Spire2\_1, Mm_Spire2\_4
Oligos B: Mm_Spire1\_2, Mm_Spire1\_4, Mm_Spire2\_2, Mm_Spire2\_3
Oligos C: Mm_Spire1\_1, Mm_Spire2\_4
Oligos A were used for all other co-depletion experiments and all rescue experiments. For individual depletion of *Spire1* and *Spire2* in Figures S1 and S2, siRNAs corresponding to Oligos A (Mm_Spire1_1, Mm_Spire1_3 and Mm_Spire2_1, Mm_Spire2_4) were used.

All control oocytes in RNAi experiments were injected with scrambled negative control siRNA.