DOI: 10.1038/ncb2802

nature cell biology

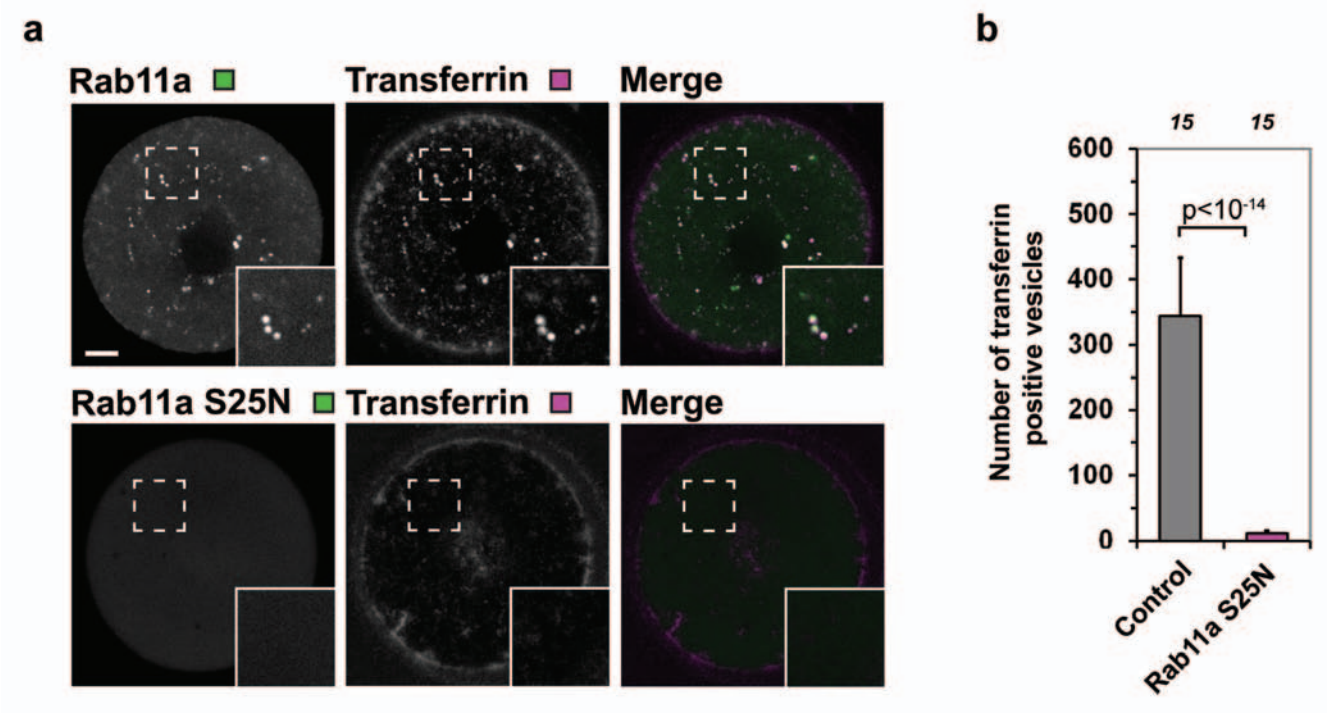

Figure S1 Loss of Rab11a-positive vesicles leads to mislocalization of the vesicles' cargo transferrin. (a) Transferrin localization in oocytes expressing mEGFP-Rab11a and oocytes expressing dominant-negative Rab11a (Rab11a S25N) (z-projection, 3 sections, every 0.66 µm). Boxed regions are magnified in inset. Scale bar, 10 µm. The number of transferrin-positive vesicles in control and dominant-negative Rab11a expressing oocytes is shown. Vesicles were identified and counted with the spot detection function of Imaris in an oocyte segment of 20x20x20 μm^3. (b) The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Data are mean, with error bars displaying s.d.. *P* values were calculated with Student's t-test.

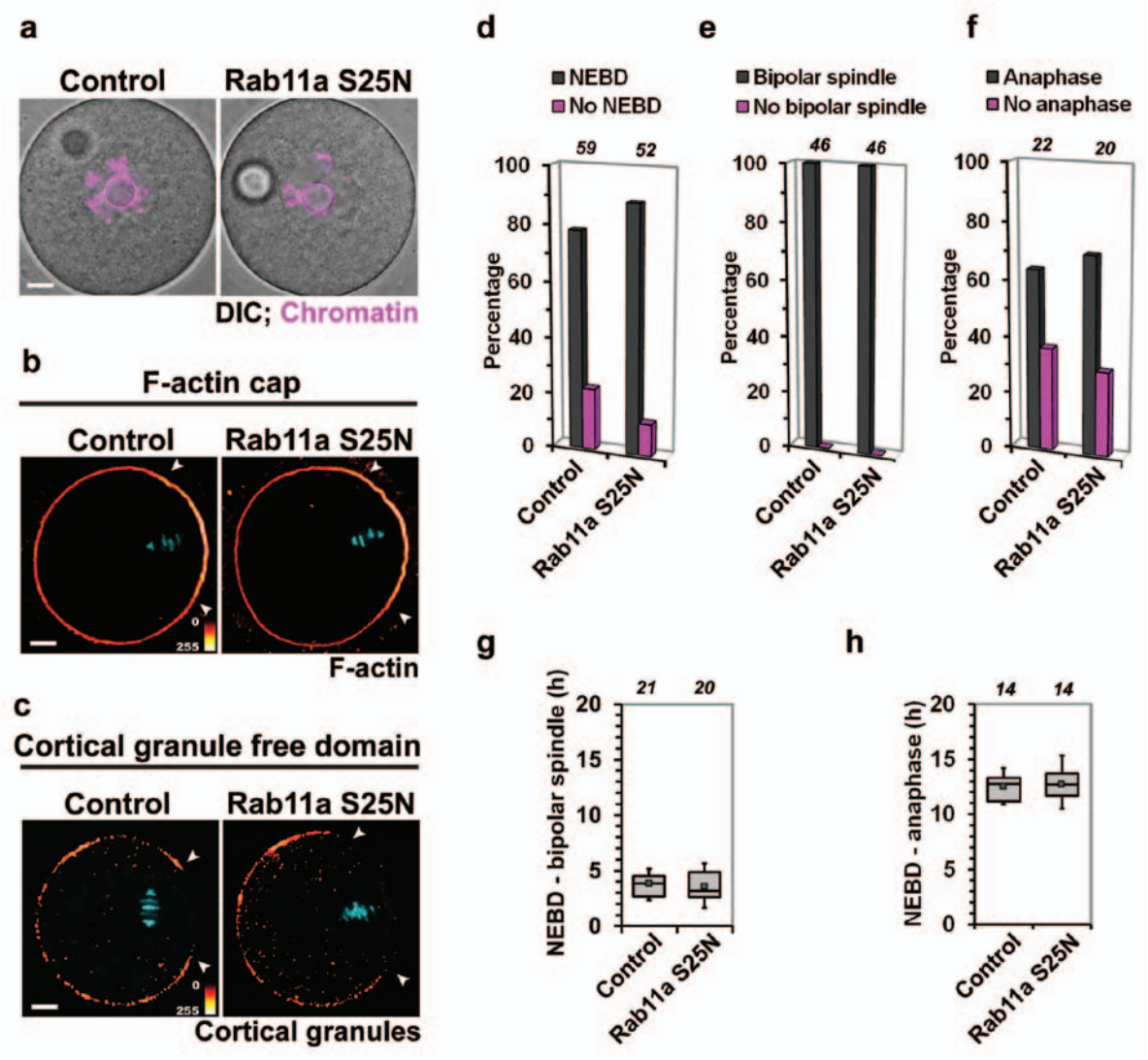

Figure S2 Rab11a-positive vesicles are dispensable for oocyte viability and progression through meiosis. (a) Live oocytes expressing H2B-mRFP (magenta, chromosomes, merged with DIC) and mEGFP-Rab11a (control) or mEGFP-Rab11a S25N (Rab11a S25N). Representative examples from 2 independent experiments (>10 oocytes total for each condition). Scale bar, 10 µm. (b) Control and mCherry-Rab11a S25N expressing oocytes from (C57BL x CBA) F1 females were fixed and stained with Hoechst (chromosomes, cyan) and fluorescent phalloidin (F-actin, pseudocoloured). The arrow heads highlight the cortical enrichment of actin in proximity of the chromosomes, which is independent of the presence of Rab11a-positive vesicles. Representative examples from 2 independent experiments (>10 oocytes total for each condition). Scale bar, 10 µm. (c) Control and mCherry-Rab11a S25N expressing oocytes from (C57BL x CBA) F1 females were fixed and stained with Hoechst (chromosomes, cyan) and fluorescent *Lens culinaris* agglutinin to label cortical granules (pseudocoloured). The arrow heads highlight the cortical granule free domain in proximity of the chromosomes, which is independent of the presence of Rab11a-positive vesicles. Representative examples from one experiment (>5 oocytes total for each condition). Scale bar, 10 µm. (d) The percentage of oocytes undergoing NEBD was quantified by live cell microscopy of oocytes expressing mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). The number of analysed oocytes is specified in italics (aggregation over 4 independent experiments). (e) The percentage of oocytes that form a bipolar spindle was quantified by live cell microscopy of oocytes expressing mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). The number of analysed oocytes is specified in italics (aggregation over 4 independent experiments). (f) The percentage of oocytes that progress into anaphase was quantified by live cell microscopy of oocytes expressing EGFP-MAP4 to label the spindle together with mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). (g) The time between NEBD and bipolar spindle assembly was measured in live oocytes expressing EGFP-MAP4 to label the spindle together with mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). Box plot as in Figure 1e. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). (h) The time between NEBD and anaphase onset was measured in live oocytes expressing mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). Box plot as in Figure 1e. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments).

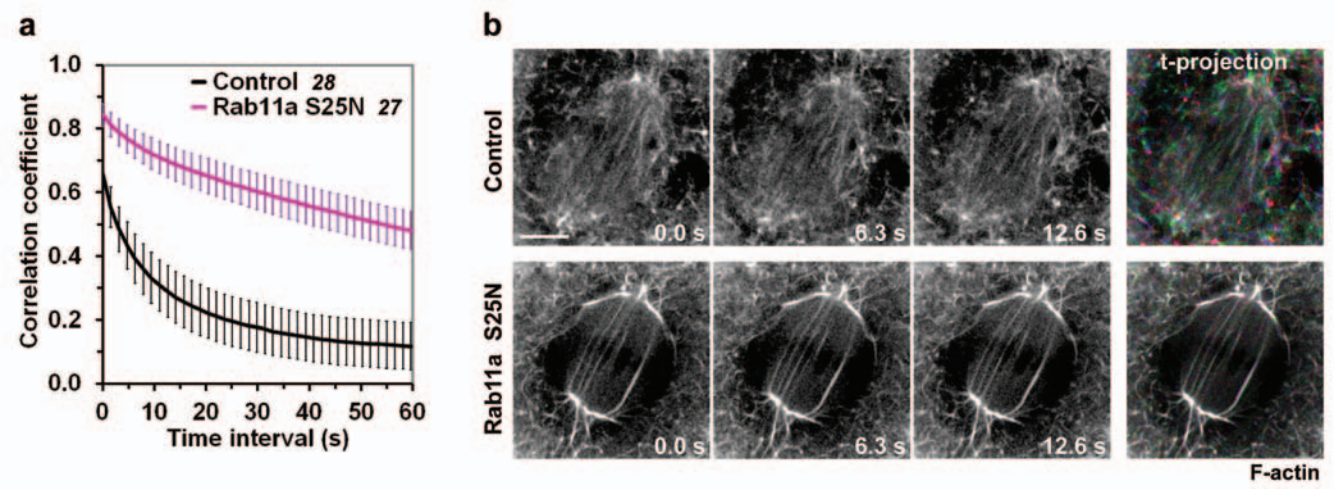

Figure S3 The spindle is trapped in a static actin network if the function of Rab11a-positive vesicles is blocked. (a) Image correlation analysis of actin network dynamics in live oocytes expressing EGFP-UtrCH to label F-actin together with mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). Each image in a time series (time interval 1.57 s) was correlated with the first image. For details see Experimental Procedures. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Data are mean, with error bars displaying s.d. (b) Live oocytes expressing EGFP-UtrCH to label F-actin together with mCherry-Rab11a (Control) or mCherry-Rab11a S25N (Rab11a S25N). Projections are time-coloured in RGB. Scale bar, 5 μm.

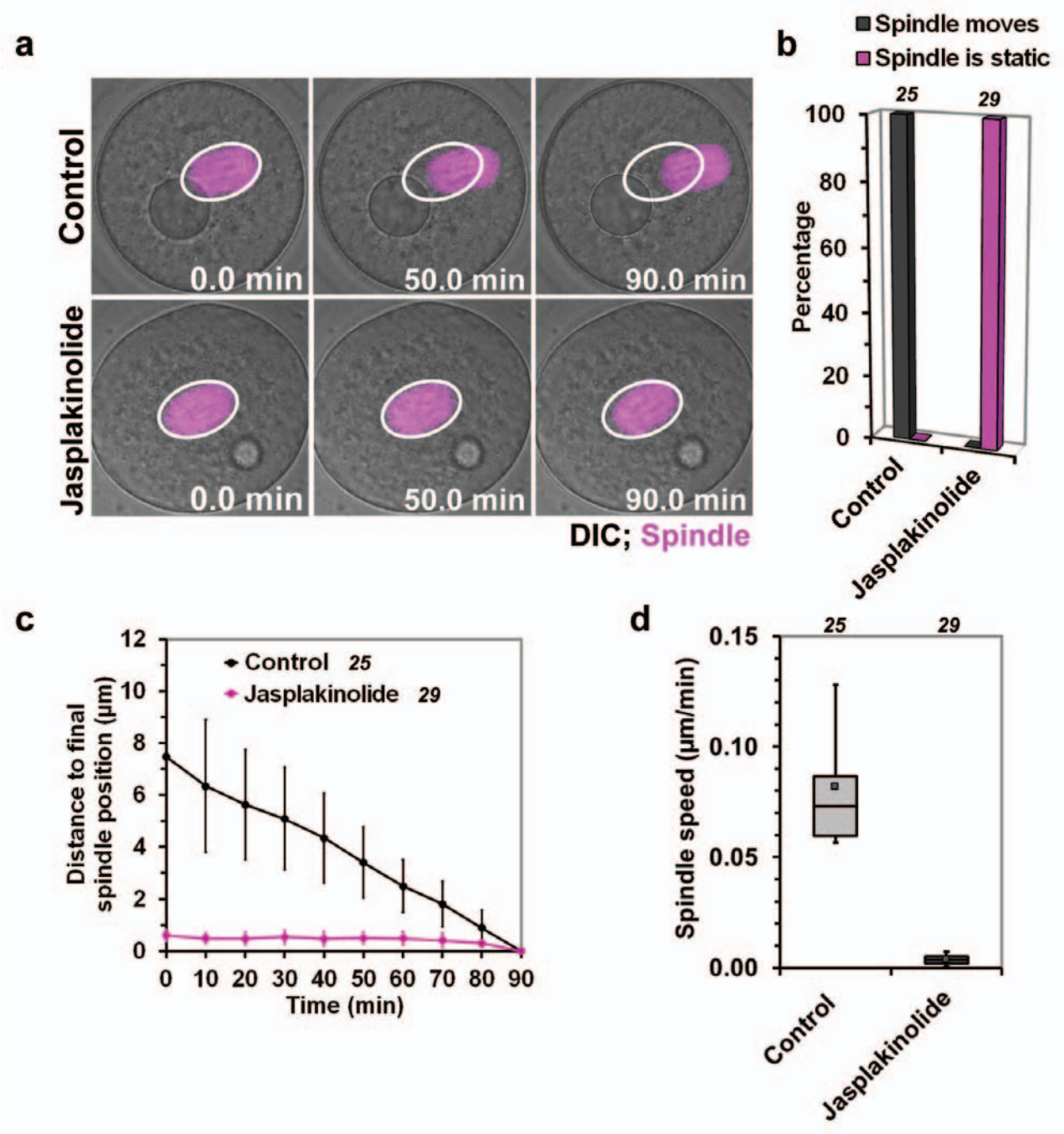

Figure S4 Network stabilization with jasplakinolide prevents asymmetric spindle positioning. (a) Spindle movements (magenta, EGFP-MAP4; microtubules, merged with differential interference contrast [DIC]) in live oocytes treated with DMSO (Control) or oocytes treated with 50 nM jasplakinolide (Jasplakinolide). White ovals mark initial spindle positions. Scale bar, 10 µm. (b) The efficiency of asymmetric spindle positioning in control and jasplakinolide treated oocytes is shown. The number of analysed oocytes is specified in italics (aggregation over 2-5 independent experiments). (c) The spindle was tracked in oocytes in 3D data sets (11 sections, every 8 µm) as shown in (a) and spindle movements were plotted. The number of analysed oocytes is specified in italics (aggregation over 2-5 independent experiments). Data are mean, with error bars displaying s.d. (d) The spindle speeds were determined from the plots in (c). The number of analysed oocytes is specified in italics (aggregation over 2-5 independent experiments). Box plot as in Figure 1e.

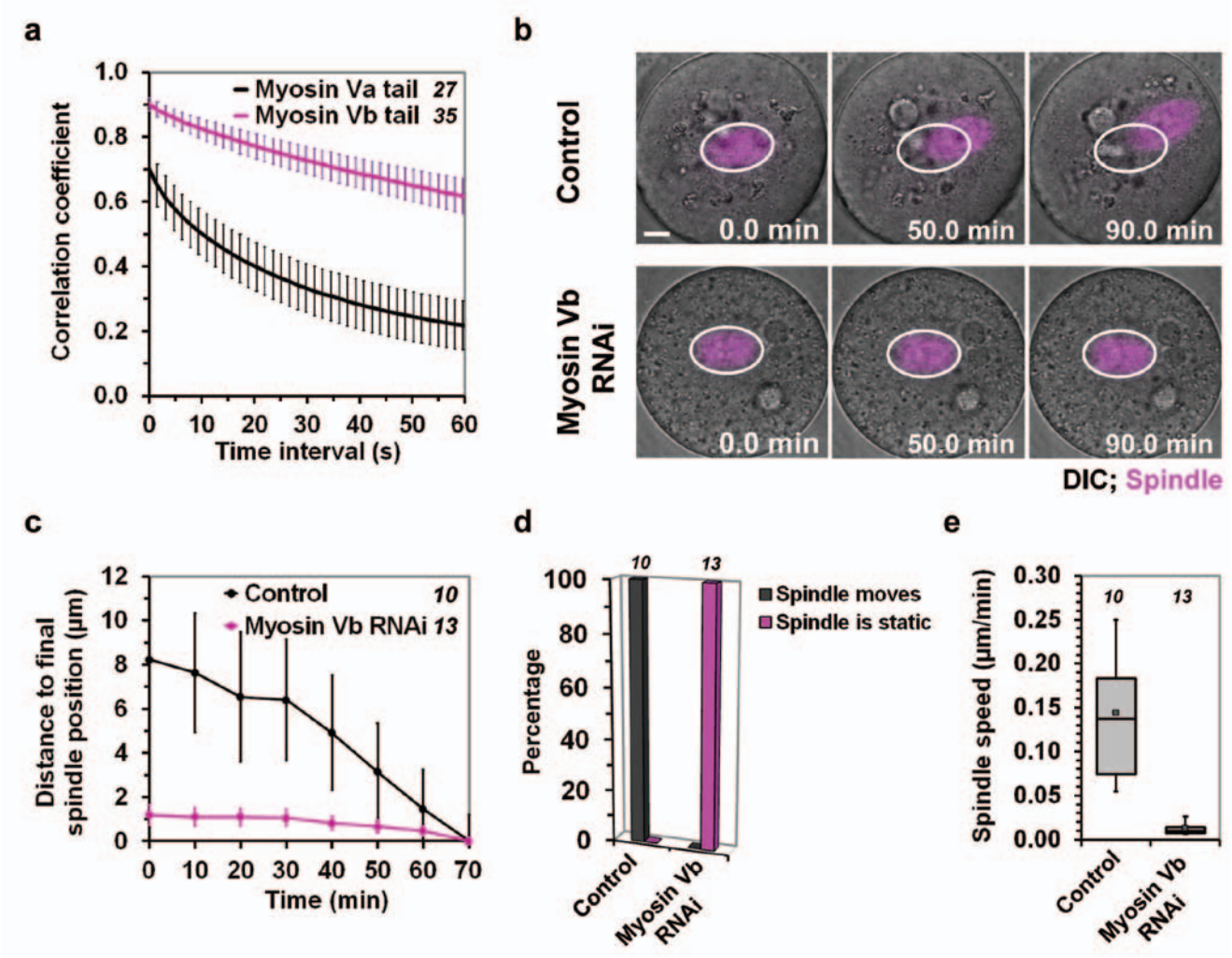

Figure S5 Myosin Vb mediates the actin network dynamics and asymmetric spindle positioning. (a) Image correlation analysis of actin network dynamics in live oocytes expressing EGFP-UtrCH to label F-actin together with mCherry-myosin-Va-tail or mCherry-myosin-Vb-tail. Each image in a time series (time interval 1.57 s) was correlated with the first image. For details see Experimental Procedures. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Data are mean, with error bars displaying s.d. (b) Spindle movements (magenta, EGFP-MAP4; microtubules, merged with differential interference contrast [DIC]) in live oocytes injected with control siRNA (Control) or with siRNAs targeting myosin Vb (Myosin Vb RNAi). White ovals mark initial spindle positions. Scale bar, 10 µm. (c) The spindle was tracked in oocytes in 3D data sets (11 sections, every 8 µm) as shown in (b) and spindle movements were plotted. The number of analysed oocytes is specified in italics (aggregation over 3-5 independent experiments). Data are mean, with error bars displaying s.d. (d) The efficiency of asymmetric spindle positioning in oocytes injected with control siRNA (Control) or with siRNAs targeting myosin Vb (Myosin Vb RNAi) is shown. The number of analysed oocytes is specified in italics (aggregation over 3-5 independent experiments). (e) The spindle speeds were determined from the plots in (c). The number of analysed oocytes is specified in italics (aggregation over 3-5 independent experiments). Box plot as in Figure 1e.

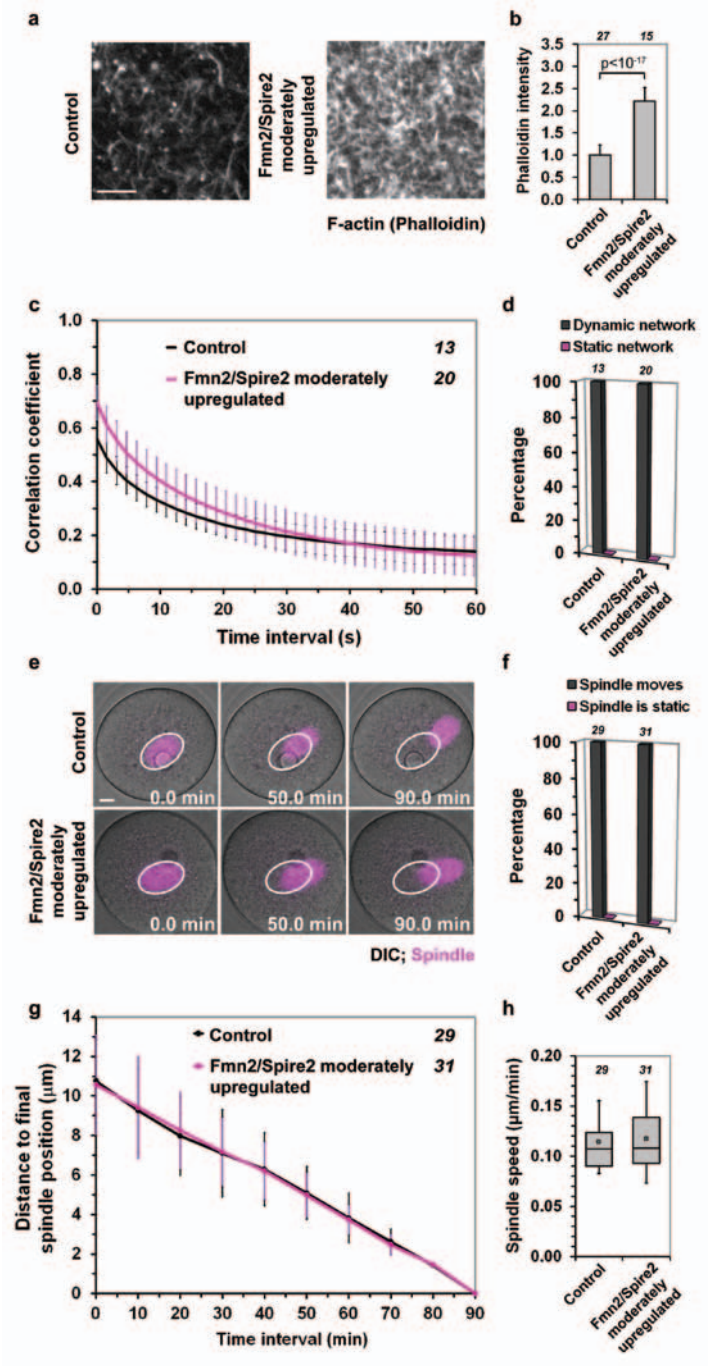

Figure S6 A moderate increase of network density does not block network dynamics or asymmetric spindle positioning. (a) Control oocytes (Control) and oocytes in which the actin nucleators Fmn2 and Spire2 were moderately overexpressed (Fmn2/Spire2 moderately upregulated) were fixed and stained for F-actin. Scale bar, 5 µm. (b) The mean intensity of F-actin in the cytoplasm (labelled by fluorescent phalloidin) was measured in oocytes as shown in (a). Error bars display s.d.. The number of analysed oocytes is specified in italics . *P* values were calculated with Student's t-test. (c) Image correlation analysis of actin network dynamics in live oocytes expressing EGFP-UtrCH in control oocytes (Control) and in oocytes moderately overexpressing Fmn2-mCherry and mCherry-Spire2 (Fmn2/Spire2 moderately upregulated). Each image in a time series (time interval 1.57 s) was correlated with the first image. For details see Experimental Procedures. The number of analysed oocytes is specified in italics. Data are mean, with error bars displaying s.d. (d) The percentage of oocytes with a dynamic or static actin network is shown. An image correlation coefficient below 0.5 after 30 s was scored as dynamic and above 0.5 as static. The number of analysed oocytes is specified in italics. (e) Spindle movements (magenta, EGFP-MAP4; microtubules, merged with differential interference contrast [DIC]) in control live oocytes (Control) and oocytes moderately overexpressing Fmn2-mCherry and mCherry-Spire2. White ovals mark initial spindle positions. Scale bar, 10 µm. (f) The efficiency of asymmetric spindle positioning in control oocytes (Control) and in oocytes moderately overexpressing Fmn2-mCherry and mCherry-Spire2 (Fmn2/Spire2 moderately upregulated) is shown. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). (g) The spindle was tracked in oocytes in 3D data sets (11 sections, every 8 µm) as shown in (e) and spindle movements were plotted. The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Data are mean, with error bars displaying s.d. (h) The spindle speeds were determined from the plots in (g). The number of analysed oocytes is specified in italics (aggregation over 2 independent experiments). Box plot as in Figure 1e.

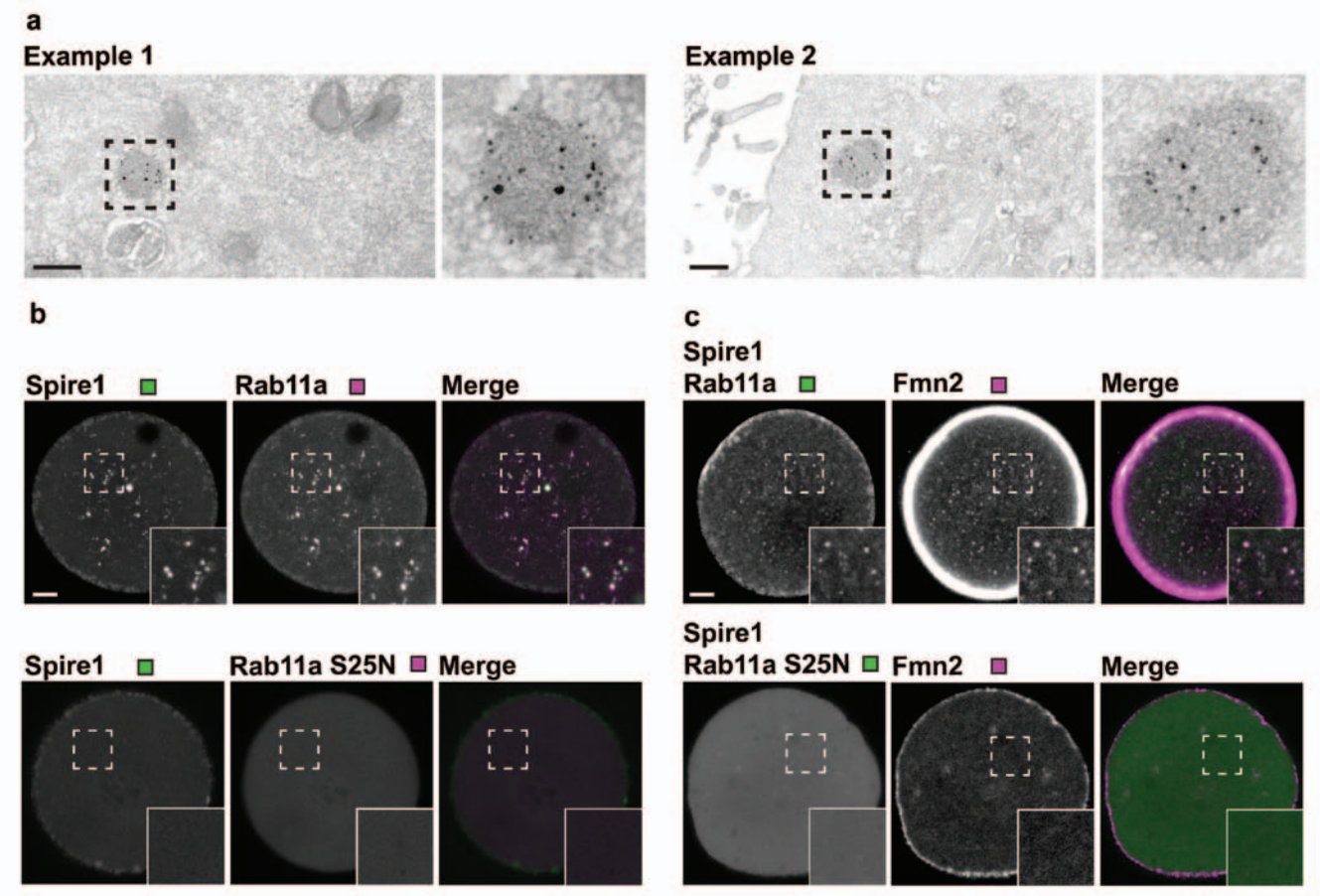

Figure S7 Localization of the network's actin nucleators in the presence and absence of Rab11-a positive vesicles. (a) Two additional examples of mEGFP-Spire2 localization in anti-GFP immunogold labelled oocytes. Boxed regions are magnified next to overview. Representative examples from 5 independent experiments (23 oocytes total). Scale bar, 200 nm. (b) Z-projection (5 sections, every 0.66 µm) of live oocytes expressing mEGFP-Spire1 with either mCherry-Rab11a (top panel) or mCherry-Rab11a S25N (bottom panel). Boxed regions are magnified in inset. Representative examples from 2 independent experiments (>15 oocytes total for each condition). Scale bar, 10 µm. (c) Z-projection (5 sections, every 0.66 µm) of live oocytes expressing Fmn2-mCherry together with mEGFP-Spire1 and mEGFP-Rab11a (top panel) or mEGFP-Spire1 and mEGFP-Rab11a S25N (bottom panel). Representative examples from 3 independent experiments (>30 oocytes total for each condition). Scale bar, 10 µm.

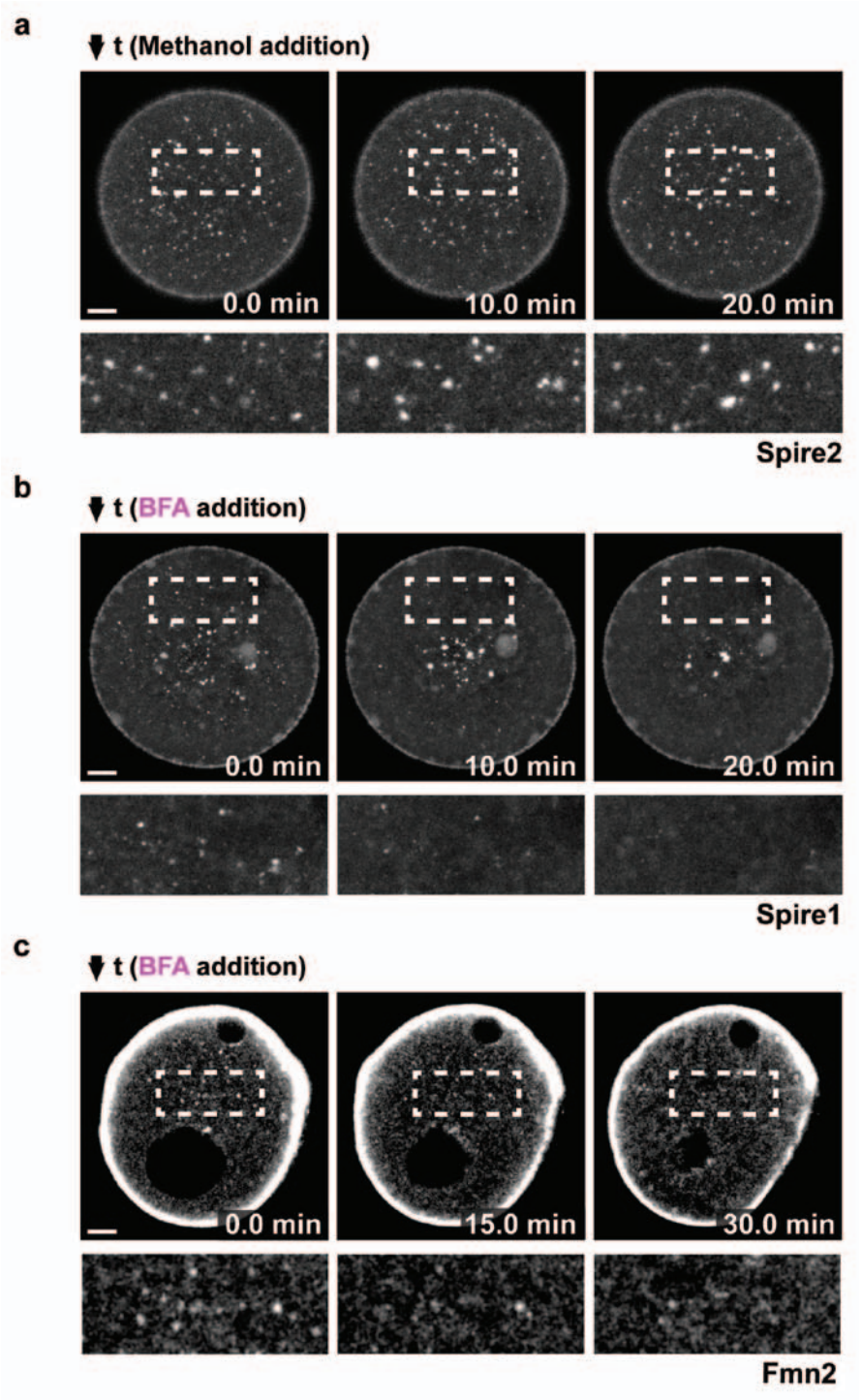

Figure S8 BFA addition releases the network's actin nucleators from vesicles. (a) Control for BFA addition experiment in Fig. 5i. mEGFP-Spire2 localization upon addition of methanol. (z-projection, 5 sections, every 0.66 µm). Scale bar, 10 µm. Boxed regions are magnified below. (b) mEGFP-Spire1 localization in live oocytes upon addition of 10 µM BFA (z-projection, 5 sections, every 0.66 µm). Representative example from 4 independent experiments (17 oocytes total). Scale bar, 10 µm. Boxed region is magnified below. (c) Fmn2-mCherry localization in live oocytes co-overexpressing mEGFP-Spire1 upon addition of 10 µM BFA. Only a single section is shown. Representative example from 2 independent experiments (6 oocytes total). Scale bar, 10 µm. Boxed region is magnified below.

Supplementary Video Legends

Supplementary Video S1. Rab11a-positive vesicles driving actin network dynamics.
Time-lapse imaging (single confocal section; time interval: 3.1 s) of the cytoplasmic actin network during asymmetric spindle positioning in oocytes expressing EGFP-UtrCH (F-actin; magenta) together with either mCherry-Rab11a (vesicles; green) or mCherry- Rab11a S25N. Please note that vesicles appear white in merge.

Supplementary Video S2. Rab11a-positive vesicles temporarily associate with the spindle during asymmetric spindle positioning.
Time-lapse imaging (z-projection of three sections, every 1.5 µm; time interval: 6.35 s) of an oocyte expressing mEGFP-Rab11a (vesicles; green) and mCherry-MAP4 (microtubules; magenta) during asymmetric spindle positioning.

Supplementary Video S3. The spindle is trapped in a static actin network if the function of Rab11a-positive vesicles is blocked.
Time-lapse imaging (single confocal section; time interval: 3.6 s) of the spindle area in oocytes expressing EGFP-UtrCH (F-actin) together with either mCherry-Rab11a or mCherry-Rab11a S25N.

Supplementary Video S4. Asymmetric spindle positioning requires Rab11a-positive vesicles.
Time-lapse imaging of F-actin (EGFP-UtrCH, white; single confocal section; time interval: 10 min) and chromosomes (Hoechst 33342, magenta, z-projection of three confocal sections, every 1.2 µm) in control or mCherry-Rab11a S25N expressing oocytes. Same oocyte as shown in Fig. 2d

Supplementary Video S5. 3D volume reconstruction of the trapped spindle shown in Supplementary Video S3.
Rotating 3D volume reconstruction of the trapped spindle in oocytes expressing EGFP-UtrCH (F-actin) together with mCherry-Rab11a S25N.

Supplementary Video S6. Myosin Vb drives the actin network dynamics.
Time-lapse imaging (one confocal section; time interval: 1.57 s) of the cytoplasmic actin network during asymmetric spindle positioning in oocytes expressing EGFP-UtrCH (F-actin) together with either mCherry-myosin-Va-tail or mCherry-myosin-Vb-tail.

Supplementary Video S7. mEGFP-Spire2 is released from vesicles and the plasma membrane into the cytoplasm upon BFA addition.
Time-lapse imaging of mEGFP-Spire2 (z-projection of five sections, every 2 µm; time interval: 13 s) in live oocyte. Time-lapse series starts directly after addition of BFA or corresponding amounts of methanol (Control).