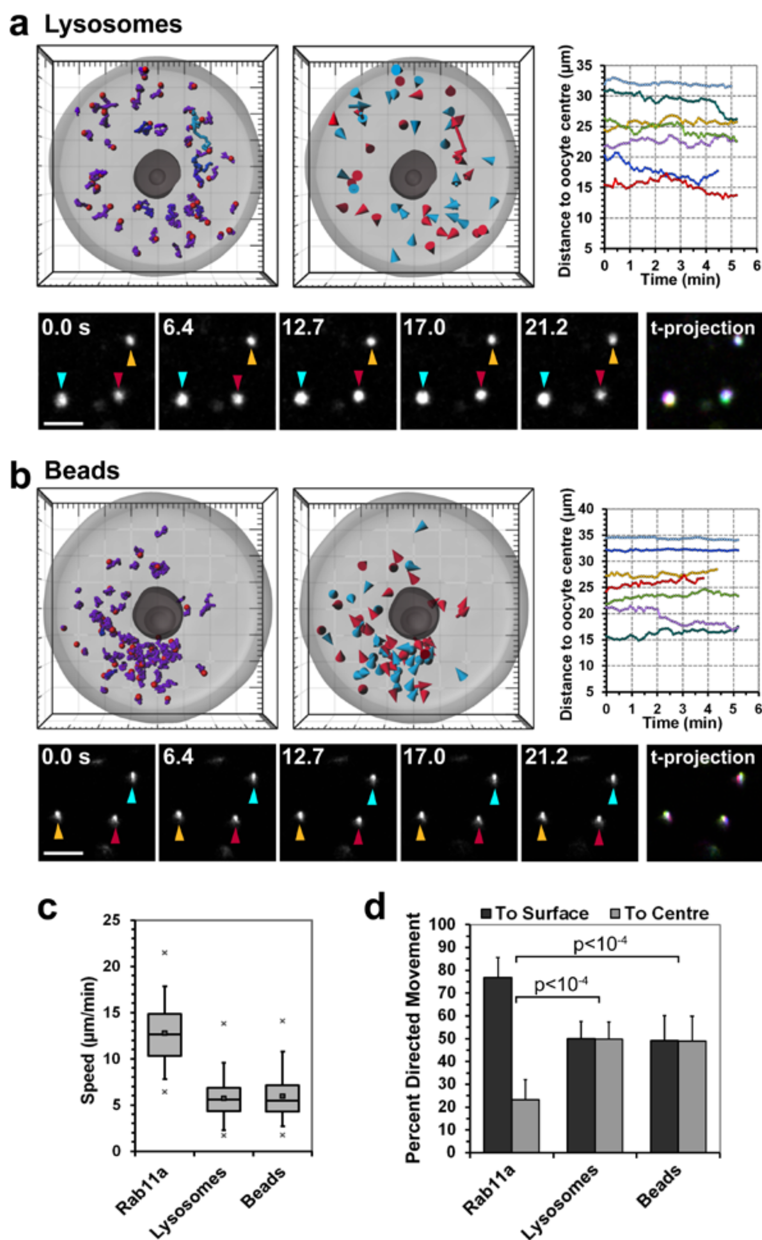
DOI: 10.1038/ncb2353

nature cell biology



Supplementary Fig. 1. Vesicle transport is not driven by cytoplasmic flows or Brownian motion

(a) 3D data sets of lysosomes (labelled with Lysotracker) in mouse oocytes were acquired and processed as described for Fig. 1c, d. The bottom row shows an example of lysosome movements. A time-coloured projection is shown (t-projection), with lysosomes looking coloured if mobile and white if stationary. Time: seconds; Scale bar: 5 µm.

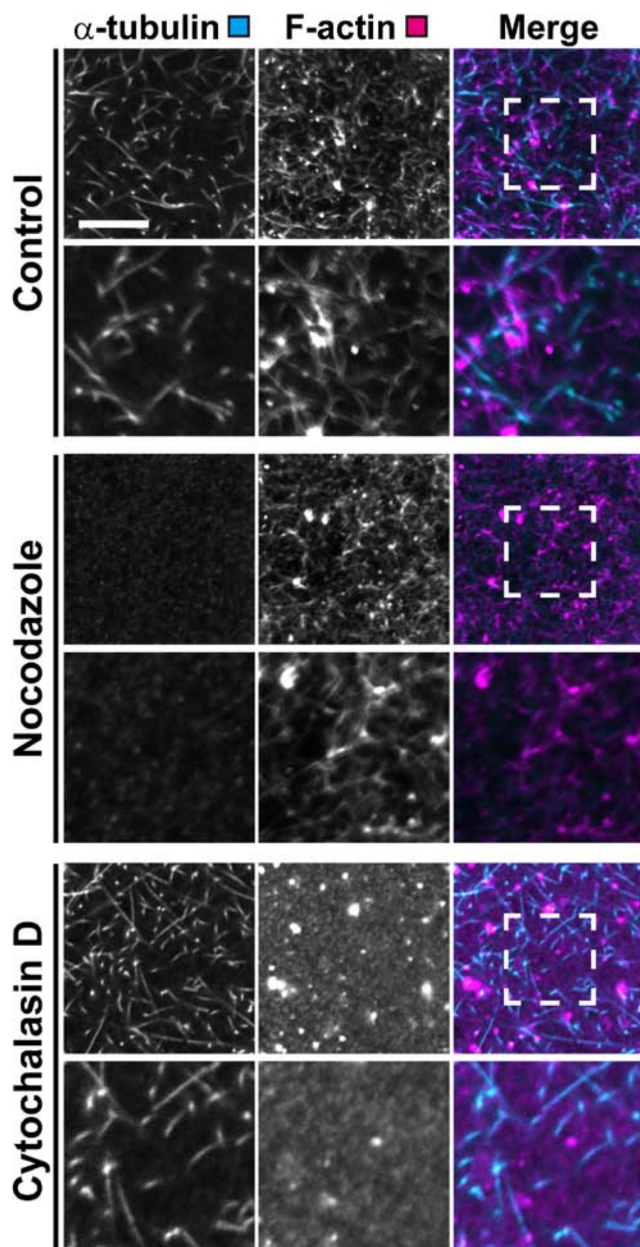
© 2011 Macmillan Publishers Limited. All rights reserved.

(b) 3D data sets of fluorescent beads (diameter: 0.5 μm) in mouse oocytes were acquired and processed as described for Figures 1c and 1d. The bottom row shows an example of bead movements. A time-coloured projection is shown (t-projection), with beads looking coloured if mobile and white if stationary. Time: seconds; Scale bar: 5 μm.

(c) Beads and lysosomes were tracked as in (a, b), and the speed of Rab11a labelled vesicles (599 tracks, 10 oocytes), lysosomes (534 tracks, 8 oocytes) and fluorescent beads (763 tracks, 6 oocytes) is shown. Box plot as in Fig. 2d.

(d) Beads and lysosomes were tracked as in (a, b), and it was determined whether they moved to the centre (To Centre; corresponding to blue arrows in (a, b)) or to the surface of the cell (To Surface; corresponding to red arrows in (a, b)). Mean data from 10 control cells, 6 cells injected with fluorescent beads and 8 cells with fluorescent lysosomes are shown, with error bars displaying s.d.. P-values were calculated with Student's t-test.

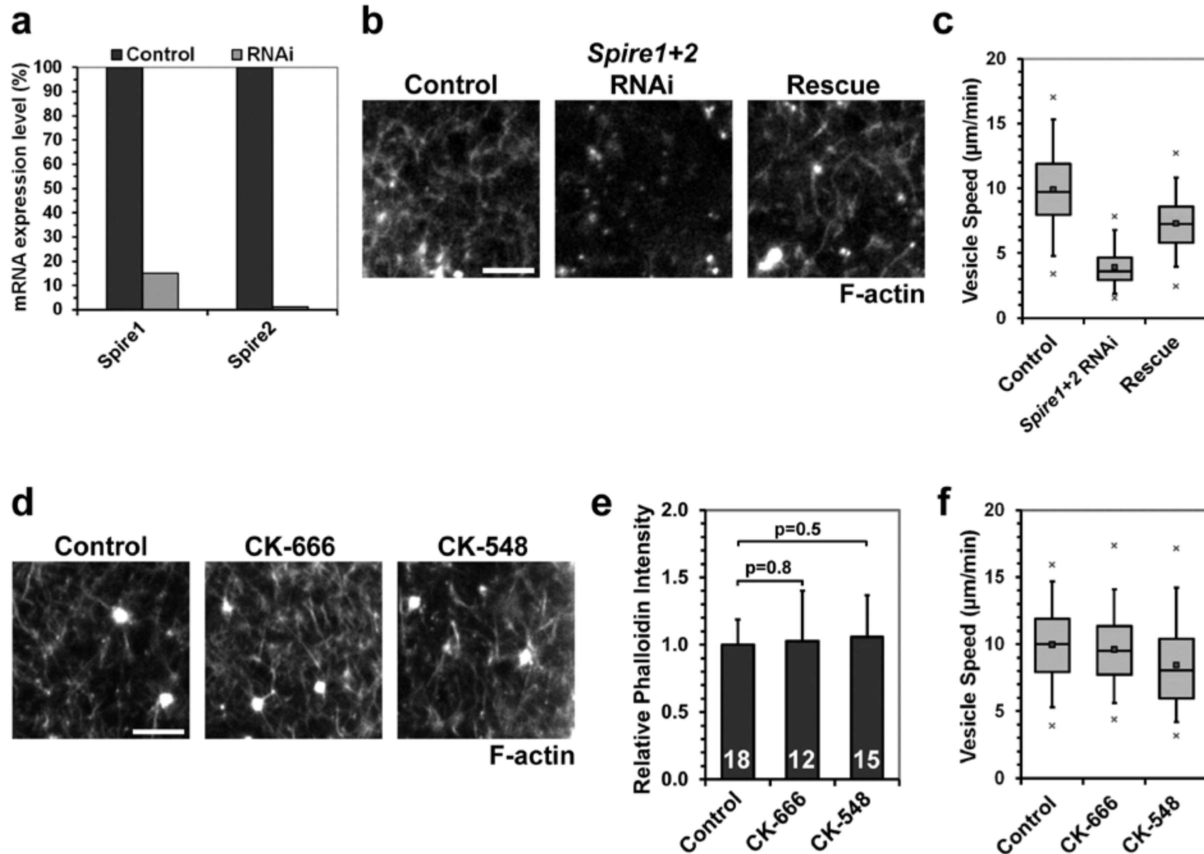
© 2011 Macmillan Publishers Limited. All rights reserved.



Supplementary Fig. 2. The actin and microtubule cytoskeleton are independent of each other.

Control (top), nocodazole (middle) or cytochalasin D (bottom) treated cells were fixed and stained for α-tubulin (cyan) and F-actin (magenta). Boxed regions are magnified in the bottom row of each panel. The images illustrate that actin filaments and microtubules do not coalign, and that depolymerisation of either cytoskeletal structure does not affect the other one. Scale bar: 10 μm.

© 2011 Macmillan Publishers Limited. All rights reserved.



Supplementary Fig. 3. Actin filaments required for vesicle transport specifically depend on Spire1 and Spire2.

(a) mRNA expression 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 (RNAi). mRNA levels were normalized to those in oocytes injected with control siRNA. Data are mean from two independent experiments.

(b) Oocytes were injected with control siRNA (Control), Spire1 and Spire2 siRNAs (*Spire1+2* RNAi) and Spire1 and Spire2 siRNAs together with human Spire1 and Spire2 mRNAs (Rescue). Oocytes were fixed and stained with Alexa Fluor 488 phalloidin to label F-actin. Scale bar: 5 µm. See also[1].

(c) Vesicles were tracked as in Fig. 1 and their speed in oocytes injected with scrambled negative control siRNA (Control; 300 tracks, 6 oocytes), Spire1 and Spire2 siRNAs (*Spire1+2* RNAi; 369 tracks, 7 oocytes) and Spire1 and Spire2 siRNAs together with human Spire1 and Spire2 mRNAs (Rescue; 1388 tracks, 11 oocytes) is shown. Box plot as in Fig. 2d. The

© 2011 Macmillan Publishers Limited. All rights reserved.

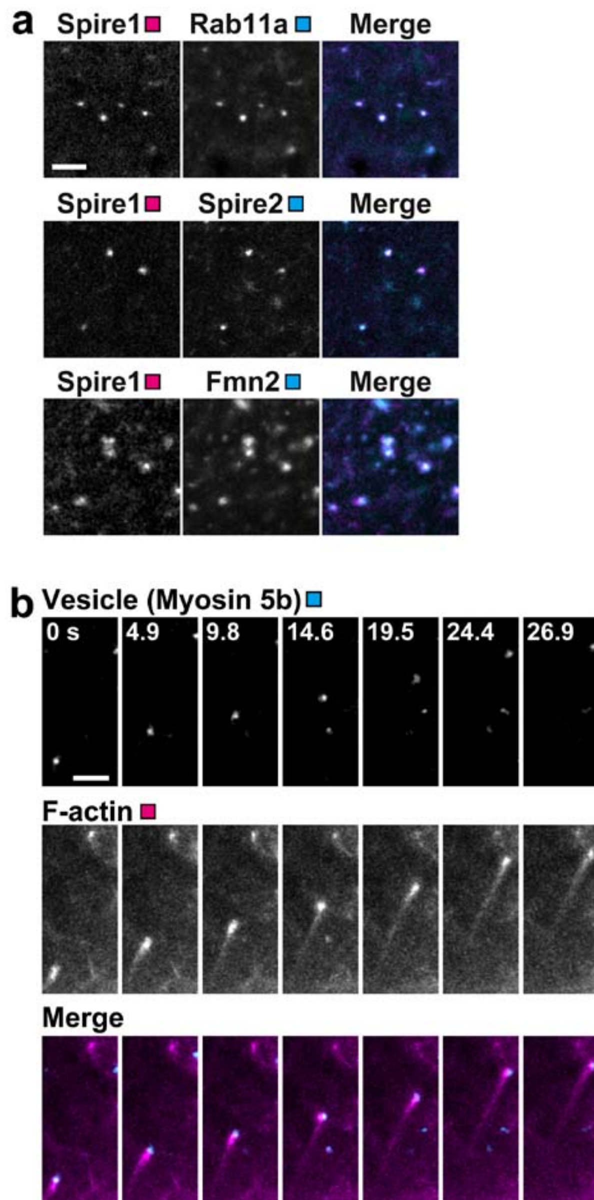
speed of vesicles in the rescue experiment is slightly slower than in control cells, which might be due to differences between the mouse and human Spire proteins and/or a slight deviation from physiological *Spire* expression levels in the rescue experiment.

(d) Oocytes that were treated for one hour with 80 μM CK-689 (Control; CK-689 is an inactive control for CK-666), 80 μM CK-666 or 100 μM CK-548 (CK-666 and CK-548 are inhibitors of the Arp2/3 complex) were fixed and stained with Alexa Fluor 488 phalloidin to label F-actin. Scale bar: 5 μm.

(e) The mean intensity of the phalloidin staining was measured in oocytes as shown in (d) that were treated with 80 μM CK-689 (Control), 80 μM CK-666 or 100 μM CK-548 for one hour. Data are mean, with error bars displaying s.d. Number of analysed oocytes is indicated on bars. p values were calculated with Student's t test.

(f) Vesicles were tracked as in Fig. 1 and their speed in oocytes treated with 80 μM CK-689 (Control; 406 tracks, 4 oocytes), 80 μM CK-666 (1124 tracks, 6 oocytes) or 100 μM CK-548 is shown (610 tracks, 4 oocytes). Duration of drug treatment was one hour before imaging. Box plot as in Fig. 2d.

© 2011 Macmillan Publishers Limited. All rights reserved.

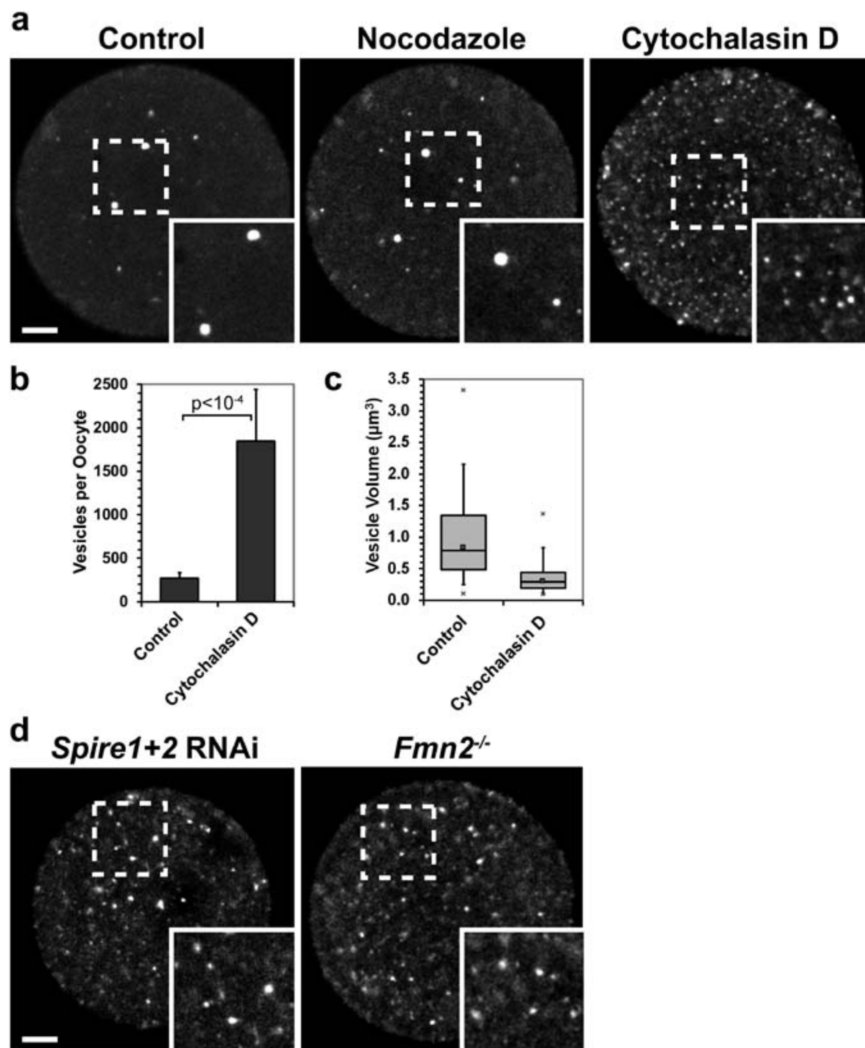


Supplementary Fig. 4. Vesicle movement upon actin nucleation from vesicle surface.

(a) Actin nucleators colocalise on vesicles. Live cells expressing Spire1-mCherry and Rab11a-mEGFP, Spire2-mEGFP or Fmn2-mEGFP. Fmn2 is likely recruited to vesicles by Spire1 and Spire2 since overexpressed Fmn2 requires slightly overexpressed Spire1 or Spire2 for its recruitment, which is consistent with published data[2]. Scale bar: 5 μm.

(b) Live cell expressing EGFP-UtrCH (F-actin) and mCherry-Myosin 5b (Vesicle). Time: seconds; Scale bar: 5 μm.

© 2011 Macmillan Publishers Limited. All rights reserved.



Supplementary Fig. 5. Vesicles are smaller and more numerous if the actin network is absent

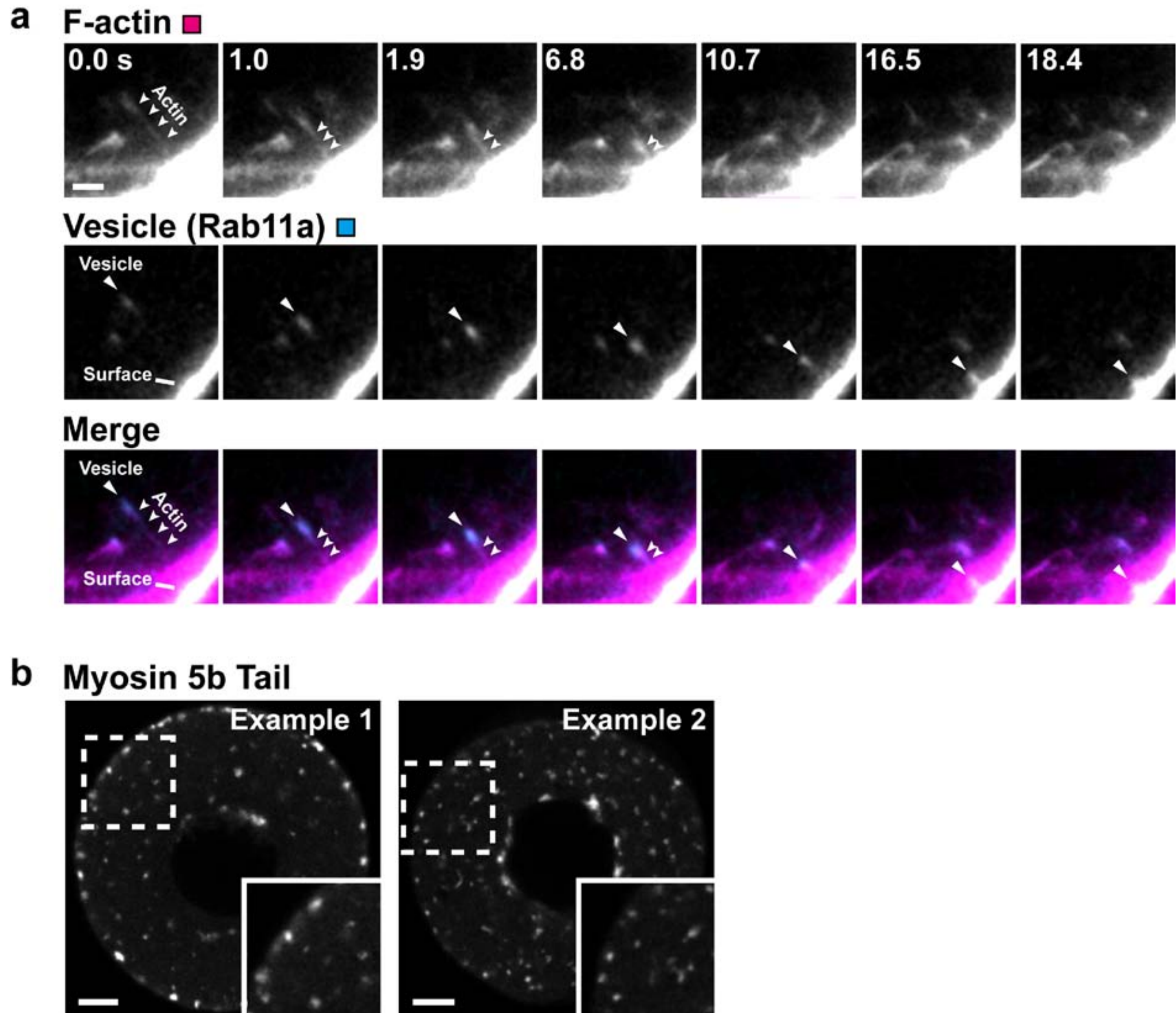
(a, d) Live control, nocodazole or cytochalasin D treated, *Spire1* and *Spire2* co-depleted and *Fmn2*$^{-/-}$ cells expressing Rab11a-EGFP. Boxed regions are magnified in inset. Scale bar: 10 µm.

(b) The number of vesicles in control (n=6) and cytochalasin D treated oocytes (n=6) is shown. Vesicles were identified and counted with the spot detection function of Imaris in an oocyte segment of ~80 pl. The precise volume of the oocyte segment was determined with the isosurface function in Imaris. The number of vesicles per oocytes was calculated based on a total oocyte volume of 220 pl. Data are mean, with error bars displaying s.d..

© 2011 Macmillan Publishers Limited. All rights reserved.

(c) The vesicle volume in control (6 oocytes; 615 vesicles) and cytochalasin D treated oocytes (6 oocytes; 4138 vesicles) is shown. Vesicles were identified with the spot detection function of Imaris. The vesicle volume was calculated from the vesicle diameter that was determined with the region growing function in Imaris assuming a spherical vesicle shape. Box plot as in Fig. 2d.

© 2011 Macmillan Publishers Limited. All rights reserved.



Supplementary Fig. 6. Mechanism of vesicle movement to cell surface.

(a) Vesicle moving along actin filament to cell surface. Live cell expressing EGFP-UtrCH (F-actin; magenta) and mCherry-Rab11a (Vesicle; cyan). Note that the actin cortex looks much thicker than it actually is because it had to be saturated to visualize intracellular actin filaments. Cross-talk of the actin cortex in the channel for detection of mCherry-Rab11a gives a more precise impression of the position of the surface. The high background in the actin channel in close proximity to the cell surface is due to out of focus light from the bright actin cortex. Time: seconds; Scale bar: 2 μm.

(b) Myosin 5b tail carrying vesicles are located underneath the cell surface and in the cytoplasm. Two examples of live cells expressing Myosin 5b tail fused to mCherry. Boxed region is magnified in insert. Scale bar: 10 μm.

© 2011 Macmillan Publishers Limited. All rights reserved.

Supplementary References

1 Pfender, S., Kuznetsov, V., Pleiser, S., Kerkhoff, E. & Schuh, M. Spire-Type Actin Nucleators Cooperate with Formin-2 to Drive Asymmetric Oocyte Division. *Current biology : CB*, doi:10.1016/j.cub.2011.04.029 (2011).

2 Kerkhoff, E. *et al.* The Spir actin organizers are involved in vesicle transport processes. *Curr Biol* **11**, 1963-1968, doi:S0960-9822(01)00602-9 [pii] (2001).

© 2011 Macmillan Publishers Limited. All rights reserved.