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Supplemental Information

Filopodia-like Actin Cables Position Nuclei in Association with Perinuclear Actin in *Drosophila* Nurse Cells

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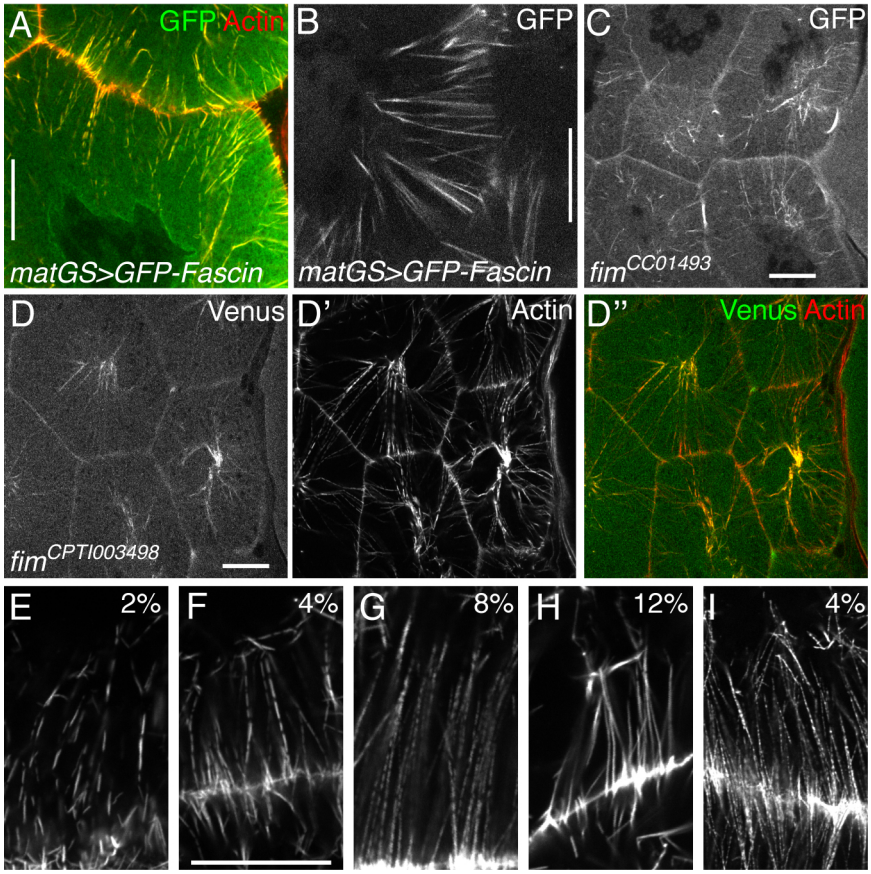

Figure S1, related to Figure 1: Localisation of actin bundling proteins and dependence of actin cable segmentation on fixation conditions.

(A) The expression of GFP-Fascin in a fixed sample (GFP-Fascin in green, actin in red) showing the segmentation of actin cables.

(B) GFP-Fascin in living tissue with continuous distribution along actin cables.

(C) The localisation pattern of *Drosophila* Fimbrin in nurse cells, labelled by a GFP gene trap.

(D) The localisation pattern of *Drosophila* Fimbrin in nurse cells, labelled by the Venus gene trap CPTI003498 (D Venus signal, D' actin, D'' overlay).

(E-I) The segmentation of actin cables depends on the fixation strength. (E) 2% formaldehyde for 10 min; (F) 4% formaldehyde for 10 min; (G) 8% formaldehyde for 10 min; note the irregularity of the segmentation along individual actin cables; (H) 12% formaldehyde for 10 min; (I) 4% formaldehyde for 30 min. All images are single z-sections with identical magnification, scale bar in F. Scale bars in all Figures 20μm.

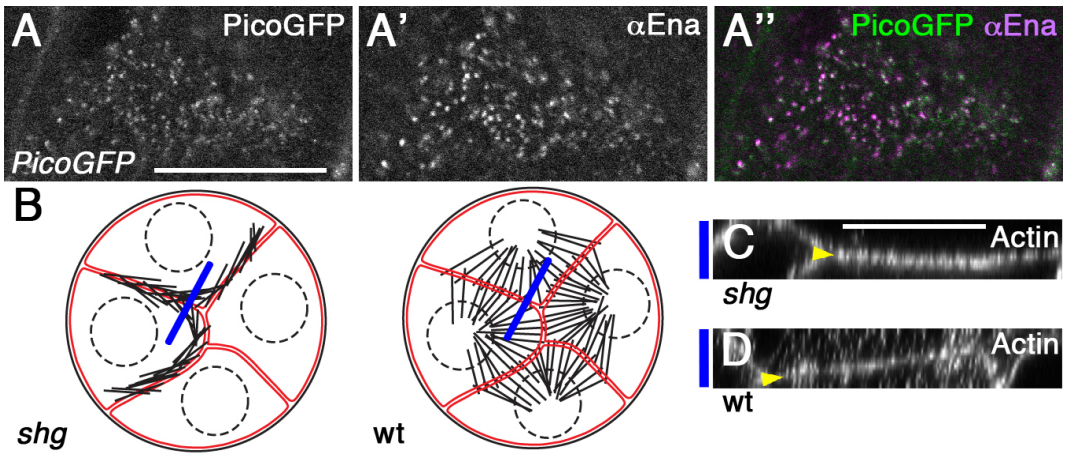

Figure S2, related to Figure 2: Pico and Enabled colocalisation and E-cadherin-dependent actin cable orientation.

(A) Single confocal section along the membrane between two nurse cells showing the co-localisation of PicoGFP and Enabled at the tip complexes (A PicoGFP, A' αEnabled, A'' overlay).

(B) Schematic drawing of a cross-section through *shg* mutant nurse cells showing actin cables lying on the membrane. For better visualisation the actin cables of only two nurse cells are depicted in the *shg* mutant egg chamber and nurse cell membranes are highlighted in red. Blue lines indicate locations of the orthogonal z-section of nurse cells shown in C and D. Model and lines are not to scale.

(C) Reconstructed section of the nurse cells in Figure 2D and G showing the lack of actin cables in the cytoplasm but actin cables lying on the membrane (actin in white).

(D) Reconstructed section of the nurse cells shown in Figure 2C showing actin cables in the cytoplasm between membrane and nucleus (actin in white). The yellow arrowheads in C and D mark the position of the cortical actin, the nuclei are not visible.

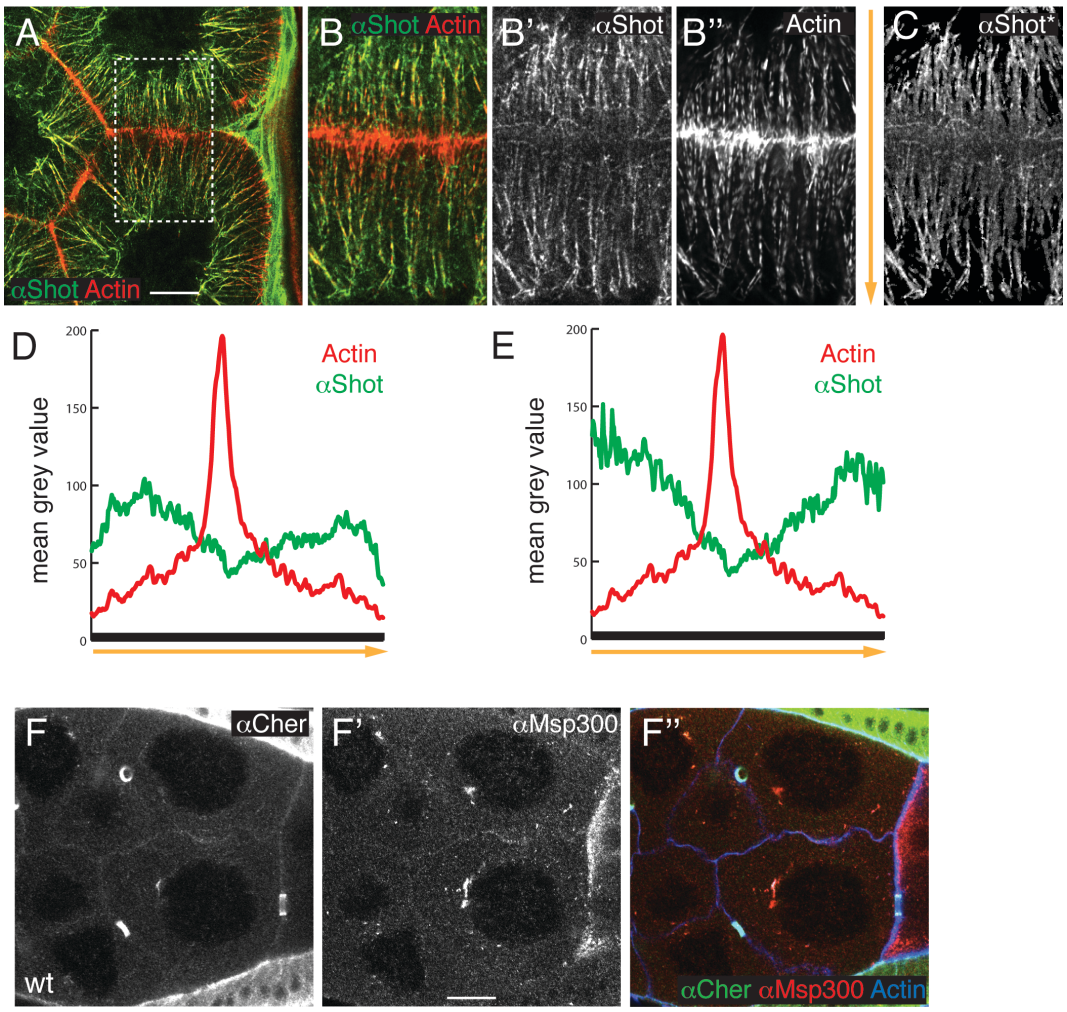

Figure S3, related to Figure 4: Quantification of Shot distribution along actin cables and localisation of Cheerio and Msp-300 prior to the formation of actin cables.

(A) Shot localisation along actin cables in nurse cells; the box indicates the area used for quantification (αShot in green, actin in red).

(B) Images used for quantification of Shot along actin cables (quantification in D). For each channel the mean grey value was calculated per line and plotted in the graph; the orange arrow marks the direction of line measurements. B' and B" show the separate channels (B' αShot, B" actin).

(C) αShot signal associated with actin cables (quantification in E). A mask generated from the actin channel was used to remove signal unrelated to actin cables from the Shot channel and then the remaining signal was quantified.

(D, E) Quantification of Shot localisation relative to actin localisation, without (D) or with (E) subtracting the actin cable unrelated αShot signal (αShot values in green, actin values in red).

(F) Localisation of Msp-300 and Cheerio during stage 9. Note the perinuclear patches of Cheerio colocalising with Msp-300 patches. (F αCheerio, F' αMsp-300, A" overlay).

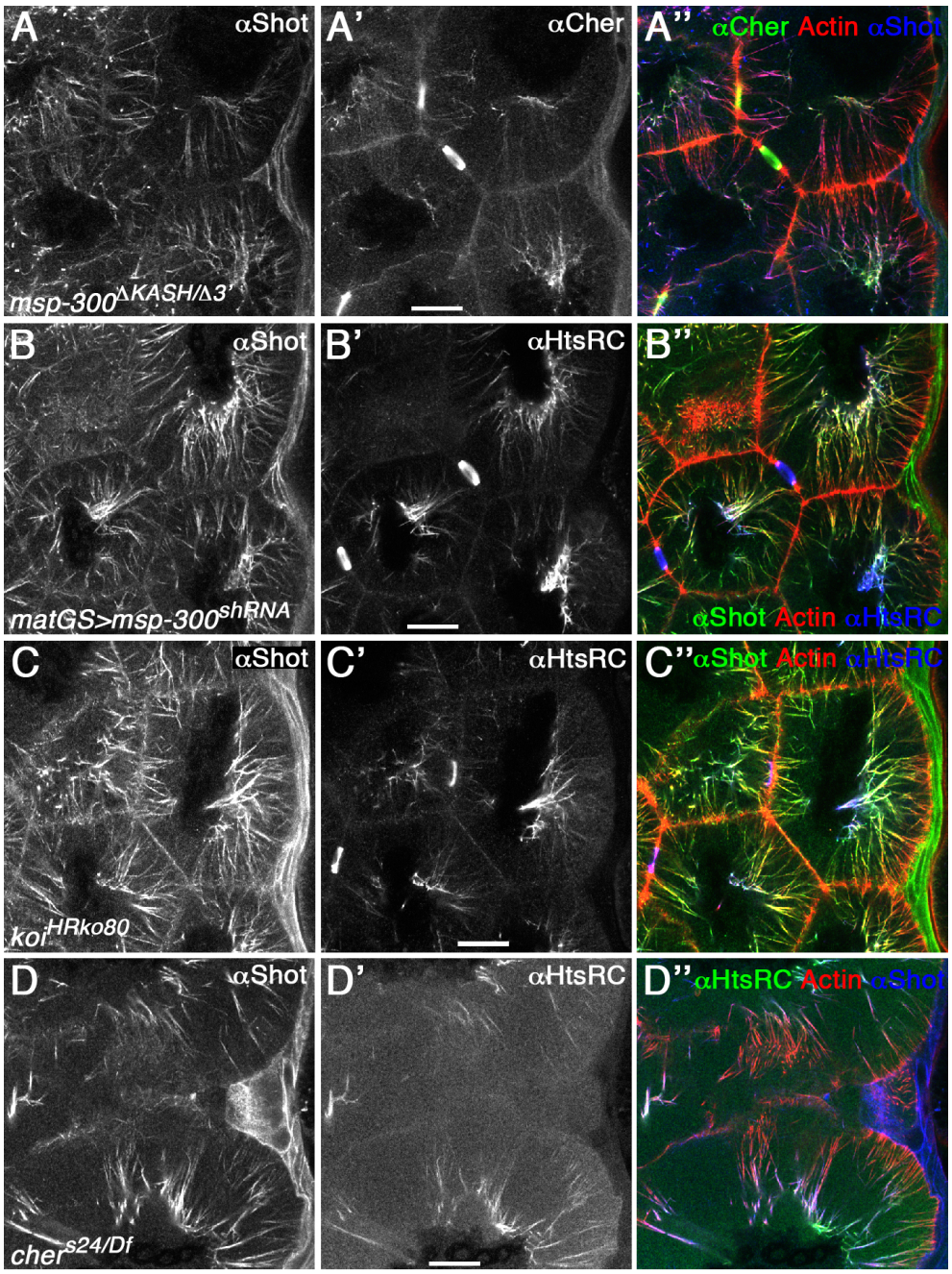

Figure S4, related to Figure 5: Shot and HtsRC localise to actin cables independently of Msp-300 and Cheerio.

(A) The deletion of the KASH domain of Msp-300 did not affect the localisation of Shot and Cheerio to actin cables.

(B) Knock-down of Msp-300 protein using RNAi did not affect the localisation of Shot and HtsRC to actin cables.

(C) Similarly, the deletion of the SUN protein Klaroid did not affect Shot or HtsRC localisation.

(D) Shot and HtsRC localise to actin cables in *cheerio* loss-of-function mutants.

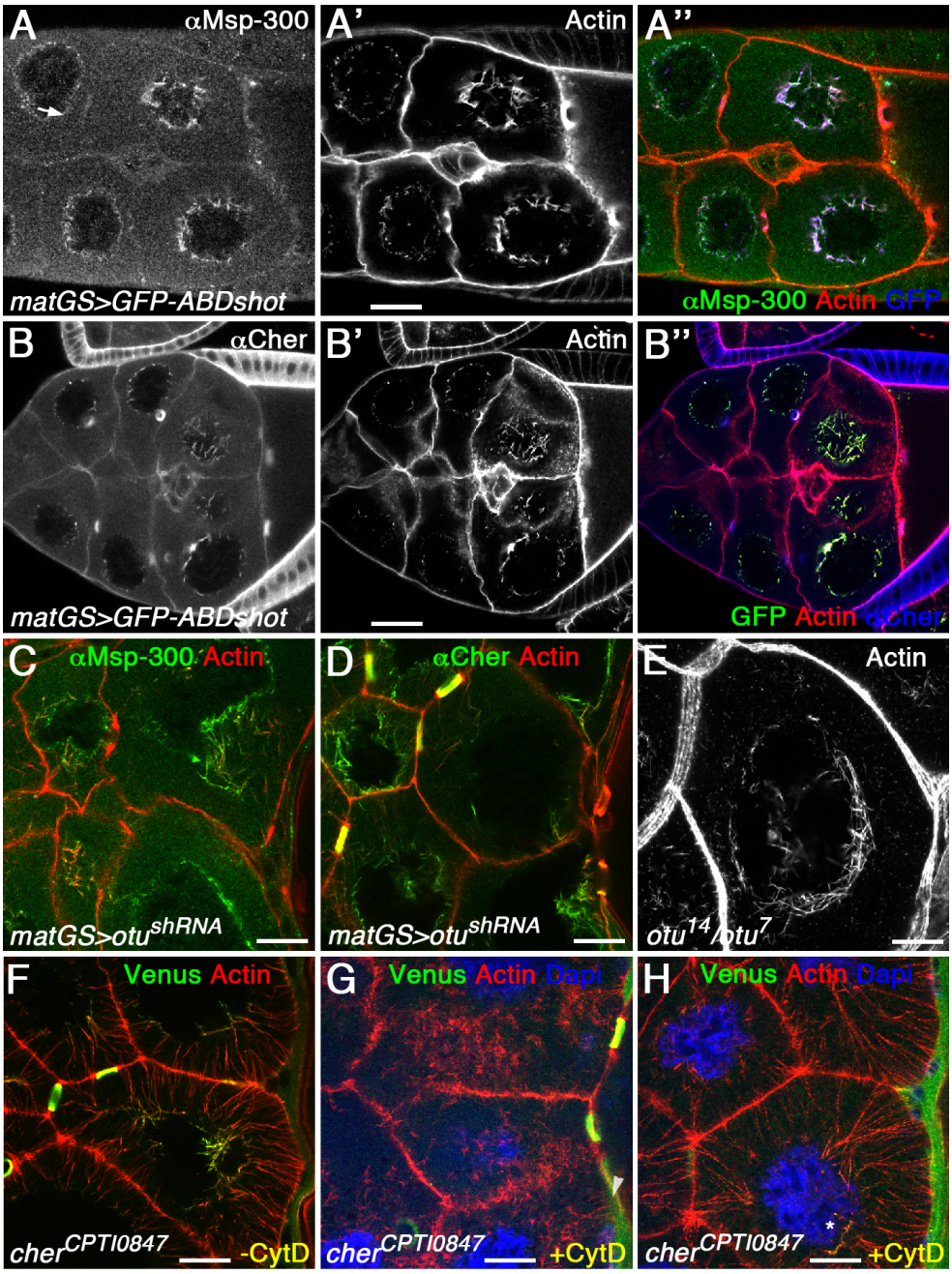

Figure S5, related to Figure 7: Stabilisation and disruption of the perinuclear actin meshwork in nurse cells.

(A, B) Nurse cells overexpressing the actin binding domain (ABD) of Shot showed an increase in perinuclear Msp300 (A), perinuclear Cheerio (B) and actin (A' and B'; A'' overlay of αMsp-300, actin and GFP-ABD-shot, B'' overlay of GFP-ABD-shot, actin and αCheerio).

(C, D) Knock-down of Ovarian tumor function resulted in an increase of perinuclear Msp300 (C, αMsp-300 in green, actin in red) and perinuclear Cheerio (D, αCheerio in green, actin in red) and a reduced formation of actin cables.

(E) Perinuclear actin was also increased in *ovarian tumor* mutations (projection of 4 z-sections).

(F-H) Disrupting the actin cytoskeleton using Cytochalasin D reduced Cheerio accumulation at the

(continued)

perinuclear ends of actin cables. (F) Control egg chamber showing the accumulation of Cheerio at the perinuclear ends of actin cables (Cheerio-Venus in green, actin in red). (G) An egg chamber with some actin cables treated with 20μg Cytochalasin D (CytD) was strongly affected, with actin patches throughout the cytoplasm, to which Cheerio-Venus did not localise (Cheerio-Venus in green, actin in red, nucleus in blue; arrowheads points to some actin cables). (H) A CytD-treated egg chamber with less cytoplasmic actin patches but well developed actin cables showed reduced accumulation of Cheerio at perinuclear ends of actin cables (Cheerio-Venus in green, actin in red, nucleus in blue; asterisk next to actin cables with reduced Cheerio-Venus accumulation).

Supplemental Movie Legends:

Movie S1, related to Figure 1: Actin cables are filopodia-like structures growing from the membrane towards the nucleus.

Time-lapse movie of bleached actin cables (see Figure 1 A, B) in nurse cells expressing GFP-Actin5C under the control of matGS-gal4. Actin cables extended continuously from the membrane to the nucleus (projection of 4 z-section spanning 3μm; recorded 1 frame/30s and playing with 10 frames/s, total length 22min; genotype: *mat-gal4.GS > UASp-GFP_actin5C*).

Movie S2, related to Figure 2: Actin cables in fixed wild type nurse cells.

Animated z-stack through fixed and stained wild type nurse cells showing actin cables and tip complexes (1μm between each section, covering a total of 21μm; αEnabled in green, actin in red; playing with 10 frames/s; genotype: w^{1118}).

Movie S3, related to Figure 2: Actin cables in nurse cells lacking E-cadherin.

Animated z-stack through fixed and stained shg^{1} mutant nurse cells showing actin cables and tip complexes lying on the membrane (1μm between each section, covering a total of 11μm; αEnabled in green, actin in red; playing with 10 frames/s; genotype: shg^{1}).

Movies S4a-c (concatenated), related to Figure 3:

Movie S4a (frames 1-132): Actin cables wrap around turning nuclei during nurse cell contraction.

Time-lapse movie illustrating how actin cables extended to the nucleus, positioned the nucleus and wrapped around turning nuclei during the contraction of nurse cells (z-projection of 5 sections spanning 8μm; recorded 1 frame/60s and playing with 10 frames/s, total length 120min; stage position readjusted part way through; genotype: *mat-gal4.GS > UASp-GFP_actin5C*). Due to the distance between projected sections, some actin cables falsely appear segmented. In addition, the entire length of some actin cables is not in the focal planes and so these cables incorrectly appear unattached from the membrane.

Movie S4b, see text (frames 134-233): Outgrowth of actin cables and nurse cell dumping.

Time-lapse movie highlighting the outgrowth of actin cables from the plasma membrane of nurse cells. The yellow arrow marks an actin cable that appeared to be pulled toward the nucleus (projection of 3 z-section spanning 2μm; recorded 1 frame/91.5s and playing with 10 frames/s, total length 128min; genotype: *mat-gal4.GS > UASp-GFP_actin5C*).

(continued)

Movie S4c (frames 235-303): Continued growth of actin cables leading to the turning of nuclei during nurse cell contraction.

Time-lapse movie of actin cables in nurse cell expressing GFP-Actin5C under the control of matGS-gal4. Turning of the nucleus is best observed in the marked nucleus (actin in grey and overlay of actin in green and Hoechst in magenta; single z-section; recorded 1 frame/62s and playing with 10 frames/s; genotype: *mat-gal4.GS > UASp-GFP_actin5C*).

Movies S5a-d (concatenated), related to Figure 7:

Movie S5a (frames 1-66): Latrunculin B leads to the disappearance of perinuclear actin.

Time-lapse movie of an egg chamber expressing GFP-Actin42A under the control of matGS-gal4 treated with 125μM Latrunculin B (added at frame "36 min"; actin in grey and overlay of actin in green and Hoechst in magenta; single z-section; recorded 1 frame/60.3s and playing with 10 frames/s; genotype: *mat-gal4.GS > UASp-GFP_actin42A*). Note that LatB treatment leads to an accumulation of actin-GFP in nurse cell nuclei.

Movie S5b (frames 68-114): Latrunculin B blocks dumping.

Time-lapse movie of an egg chamber expressing GFP-Actin5C under the control of matGS-gal4. 125μM Latrunculin B were added during dumping and led to a halt of dumping but not to the disassembly of present actin cables (added at frame "12 min"; actin in grey and overlay of actin in green and Hoechst in magenta; single z-section; recorded 1 frame/60.2s and playing with 10 frames/s; genotype: *mat-gal4.GS > UASp-GFP_actin5C*).

Movie S5c (frames 118-218): Latrunculin B blocks dumping.

Further time-lapse movie of the same egg chamber as in movie S5b. Dumping is completely blocked (overlay of actin in green and Hoechst in magenta; single z-section; recorded 1 frame/60.5s and playing with 10 frames/s; genotype: *mat-gal4.GS > UASp-GFP_actin5C*).

Movie S5d (frames 220-266): Cheerio in an egg chamber treated with Latrunculin B.

Time-lapse movie of an egg chamber expressing Cheerio-Venus treated with Latrunculin B (added at frame "33 min"; Cher-Venus in grey and overlay of Cher-Venus in green and Hoechst in magenta; single z-section; recorded 1 frame/118s and playing with 10 frames/s; genotype: $cher^{CPTI000847}$).