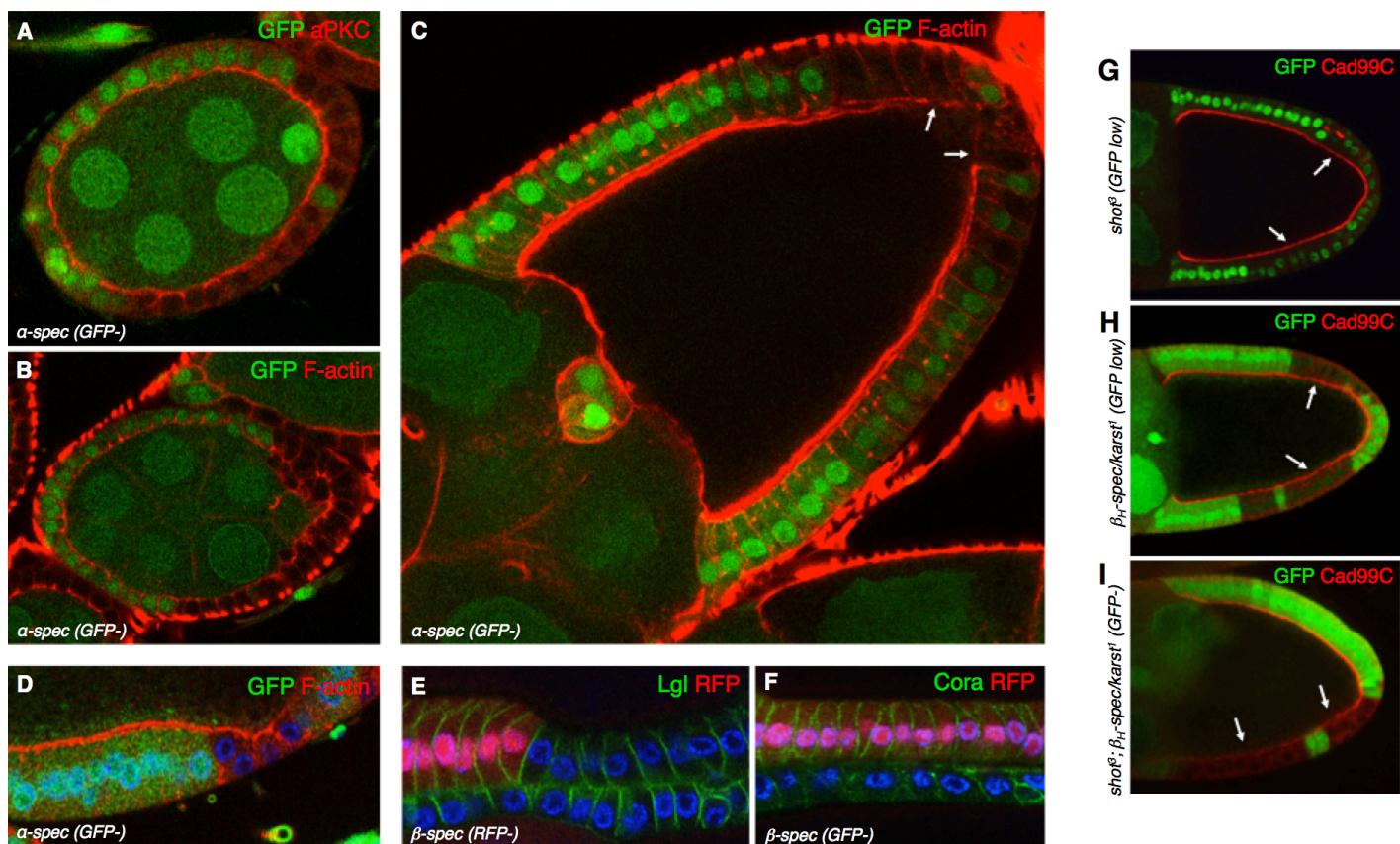


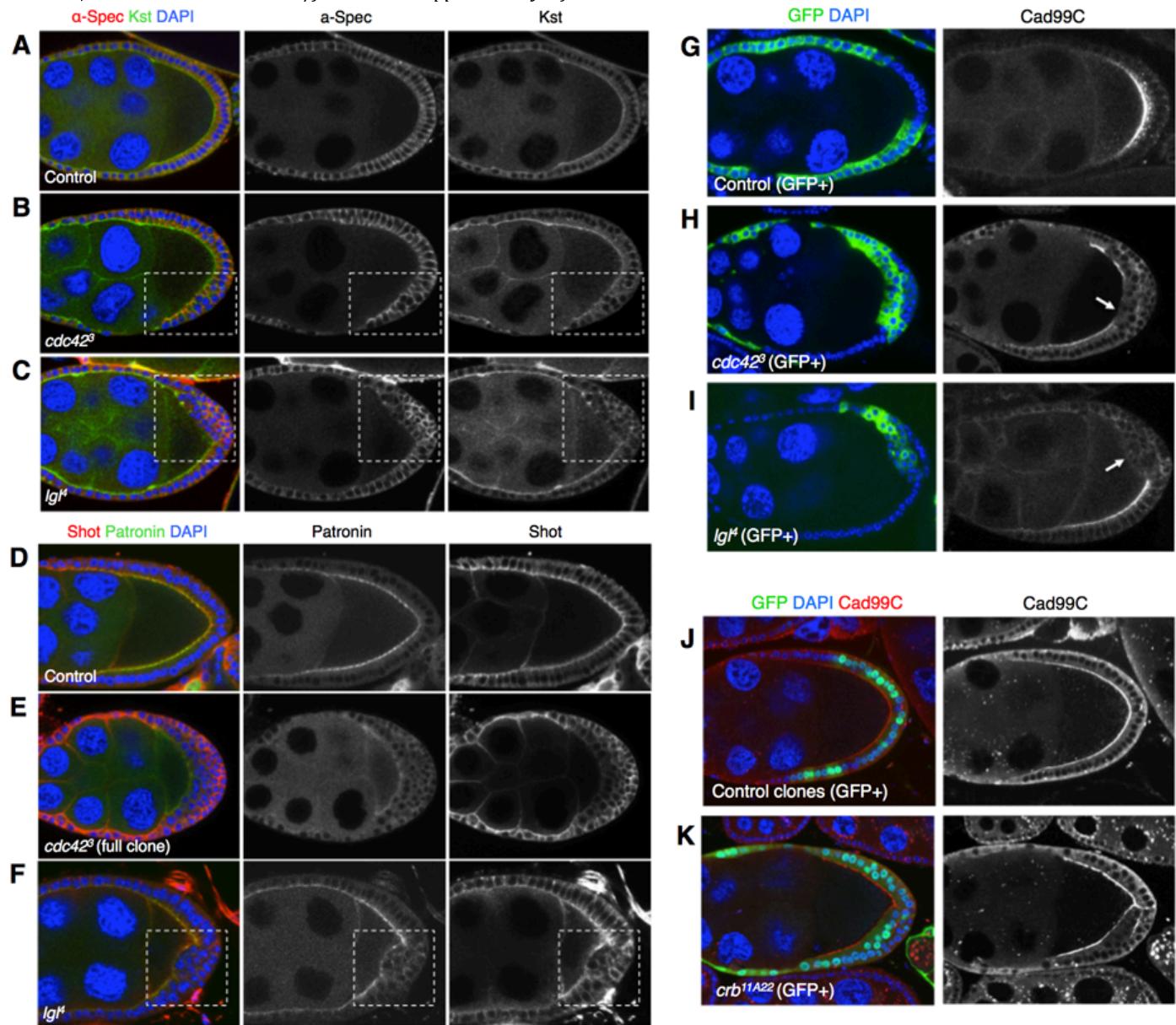
Supp Fig 1: Trafficking of Rab11 endosomes via Nuf and Dynein does not affect polarisation of aPKC and Dlg

(A) Top and middle panels: aPKC and Dlg localisation in control and *nuf* mutant (GFP positive clone) follicle cells; cell polarity is maintained in *nuf* mutants. Bottom panel: Rab11 RNAi (GFP positive clone) does not affect aPKC localisation but specifically affects Cad99C localisation at the apical membrane of follicle cells.(B) aPKC and Dlg localisation in control and Dynein RNAi follicle cells; polarity is not affected upon Dynein RNAi.



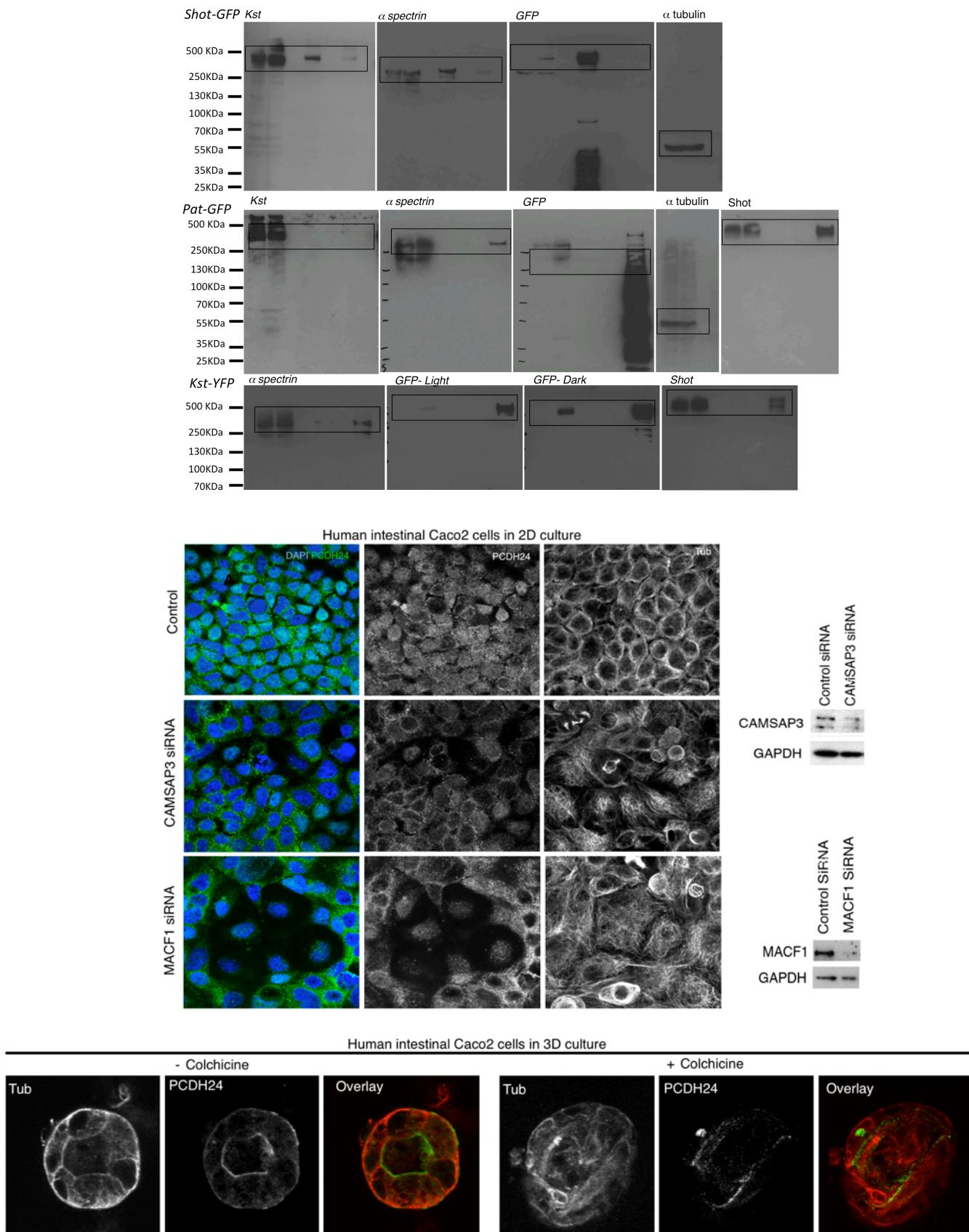
Supp Fig 2: The Spectrin cytoskeleton is not required to polarise aPKC and cortical F-actin, but is required for apical F-actin microvilli formation, columnar cell height, and Cad99C localisation

Loss of *a-spectrin* (GFP negative clone) does not affect aPKC localisation (A) or F-actin polarisation (B), however it does affect microvilli formation (arrows in C). Loss of *a-spectrin* (GFP negative clone) also causes a reduction in cell height, a phenotype not observed when microtubule polarisation is disrupted, suggesting that this is a separate function of the Spectrin cytoskeleton (D). In support of this notion, loss of *β-spectrin* (RFP negative clone) also causes a reduction in cell height and a reduction in the septate junction marker Coracle (Cora) (E,F). Single mutants of *shot* (G) or *β_H-spectrin/karst* (H) (clones marked with one copy of GFP) do not have an effect on Cad99C localisation or cell height, while double mutants of *shot* and *β_H-spectrin/karst* (GFP negative clone) exhibit loss of Cad99C from the apical membrane without affecting cell height (I).



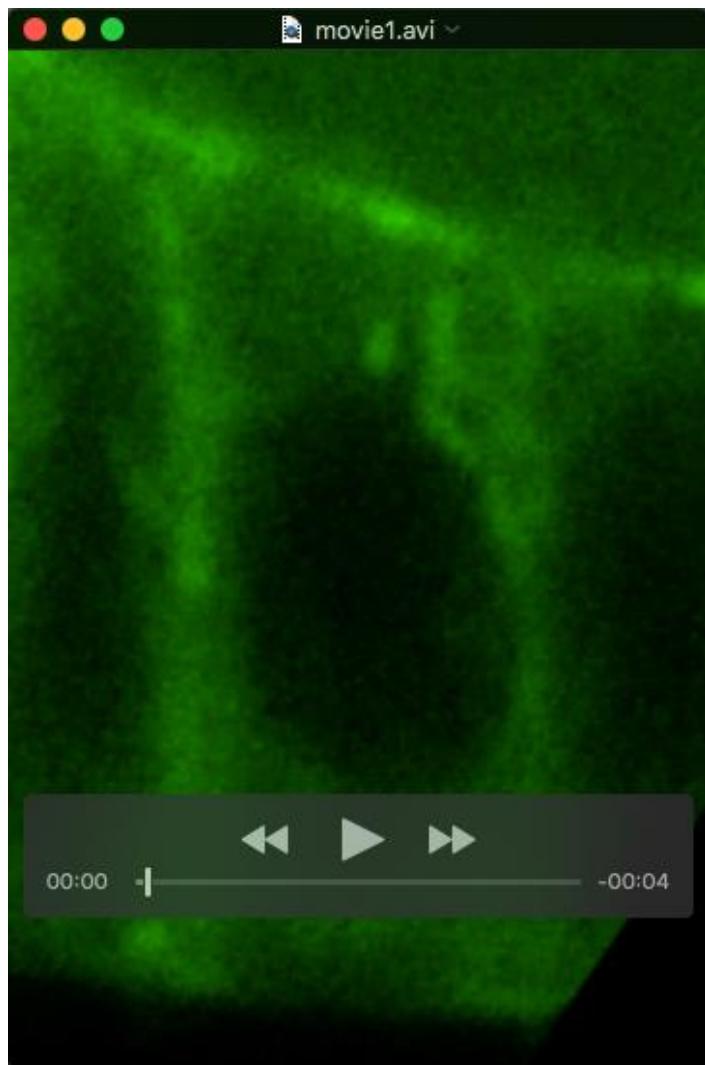
Supp Fig 3: Core apical-basal polarity determinants, but not Crumbs, are essential to polarise the Spectrin cytoskeleton, Patronin, Shot and Cad99C

α-Spectrin and β_H-Spectrin/Karst localisation in control (A), *cdc42* (B) or *IgM* (C) mutants (clones highlighted in box). Patronin and Shot localisation in control (D), *cdc42* mutant (full clone; E) or *IgM* mutant (clones highlighted in box; F). Cad99C localisation in control (G), *cdc42* (H) or *IgM* (I) mutants (GFP positive clone); the presence of apical domain in *IgM* mutant clones is not sufficient to polarise Cad99C to the apical membrane. Cad99C localisation is not affected in large null mutants for *crb* (GFP positive clone), consistent with the notion that Crb acts redundantly with Baz to organise apical-basal polarity in follicle cells (J, K).



Suppl Fig 4. Western blotting analysis of Shot-Patronin-Karst co-immunoprecipitations and analysis of microtubule polarisation and PCDH24 localisation in human Caco2 epithelial cells.

TOP: Western blots of co-IP analysis of Shot/Patronin/Karst interactions (related to Figure 3G). BOTTOM: Caco2 epithelial cells were cultured in 2D and subjected to siRNA knockdown of CAMSAP3 or MACF1, either of which disrupted microtubule organisation and cell shape, as well as the apical localisation of PCDH24. In 3D culture, PCDH24 localises to the lumen of cysts, and this localisation is impaired upon disruption of microtubules with Colchicine.



Movie 1. EB1-GFP in the follicle cell epithelium.

Table S1. Drosophila genotypes used in each figure

Fig. 1B w

Fig. 1C w

Fig. 1D w

Fig. 1E w

Fig. 1F w;; *Rab11-YFP*

Fig. 1G *yw hsflp/+; actin.FRT.CD2.FRT.Gal4/+; UAS.Rab11-IR/UAS.GFP*

Fig. 1H w

Fig. 1I *yw hsflp tub.Gal4 UAS.GFPnls/+;; FRT80B tubG80/FRT80B*

yw hsflp tub.Gal4 UAS.GFPnls/+;; FRT80B tubG80/nuf FRT80B

yw hsflp/+; UAS.CD8-GFP/+; GR1.Gal4/+

w;; GR1.Gal4/ UAS.dynein-IR

Fig. 2A *yw hsflp/+; UAS.CD8-GFP/+; GR1.Gal4/+*

Fig. 2B w;; *GR1.Gal4/ UAS.katanin60*

Fig. 2C w

Fig. 2D w

Fig. 2E w;; *GR1.Gal4/ ubi.patronin-GFP*

Fig. 2F w;; *GR1.Gal4/ UAS.shot-GFP*

Fig. 2G w; *UAS.patronin-IR/+; GR1.Gal4/+*

Fig. 2H *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT42B tubG80/shot³ FRT42B*

Fig. 2I *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT42B tubG80/shot³ FRT42B, UAS.patronin-IR*

Fig. 2J *yw hsflp/+; UAS.CD8-GFP/+; GR1.Gal4/+*

Fig. 2K *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT42B tubG80/shot³ FRT42B, UAS.patronin-IR*

Fig. 2L *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT42B tubG80/shot³ FRT42B, UAS.patronin-IR*

Fig. 3B *yw hsflp tub.Gal4 UAS.GFPnls/+;; FRT80B tubG80/FRT80B*

Fig. 3C *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT80B tubG80/α-spectrin^{e226} FRT80B*

Fig. 3D *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT80B tubG80/α-spectrin^{e226} FRT80B*

Fig. 3E *yw hsflp/+; α-spectrin^{e226} FRT80B/ubiGFPnls FRT80B*

Fig. 3F *yw hsflp/+; shot³ FRT42B/ubiGFPnls FRT42B; kst¹ FRT80B/ubiGFPnls FRT80B*

Fig. 3H *yw hsflp/+; shot³ FRT42B/ubiGFPnls FRT42B; kst¹ FRT80B/ubiGFPnls FRT80B*

Fig. 3I *yw hsflp/+; shot³ FRT42B/ubiGFPnls FRT42B; kst¹ FRT80B/ubiGFPnls FRT80B*

Fig. 3J *w*

w;; GR1.Gal4/ ubi.patronin-GFP

w;; GR1.Gal4/ UAS.shot-GFP

Fig. 4A *yw hsflp/+; UAS.CD8-GFP/Tj.Gal4*

Fig. 4B *yw hsflp/+; Tj.Gal4/+; UAS.dRip11-CT-GFP*

Fig. 4C *yw hsflp/+; Tj.Gal4/+; UAS.myoV-CT-GFP*

Fig. 4D *yw hsflp/+; Tj.Gal4/+; UAS.myoV-CT-GFP*

Fig. 4E *w;; GR1.Gal4/ UAS.dynein-IR*

Supplementary Figures

Fig. S1A *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT80B tubG80/FRT80B*

yw hsflp tub.Gal4 UAS.GFPnls/+; FRT80B tubG80/nuf FRT80B

yw hsflp/+; actin.FRT.CD2.FRT.Gal4/+; UAS.Rab11-IR/UAS.GFP

Fig. S1B *yw hsflp/+; UAS.CD8-GFP/+; GR1.Gal4/+*

w;; GR1.Gal4/ UAS.dynein-IR

Fig. S2A *yw hsflp/+; α-spectrin^{e226} FRT80B/ubiGFPnls FRT80B*

Fig. S2B *yw hsflp/+; α-spectrin^{e226} FRT80B/ubiGFPnls FRT80B*

Fig. S2C *yw hsflp/+; α-spectrin^{e226} FRT80B/ubiGFPnls FRT80B*

Fig. S2D *yw hsflp/+; α-spectrin^{e226} FRT80B/ubiGFPnls FRT80B*

Fig. S2E *β-spectrin^{G113} FRT19A/ubiRFPnls FRT19A; hsflp/+*

Fig. S2F *β-spectrin^{G113} FRT19A/ubiRFPnls FRT19A; hsflp/+*

Fig. S3A *yw hsflp/+; shot³ FRT42B/ubiGFPnls FRT42B; +/ubiGFPnls FRT80B*

Fig. S3B *yw hsflp/+; +/ubiGFPnls FRT42B; kst¹ FRT80B/ubiGFPnls FRT80B*

Fig. S3C *yw hsflp/+; shot³ FRT42B/ubiGFPnls FRT42B; kst¹ FRT80B/ubiGFPnls FRT80B*

Fig. S3D *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT80B tubG80/kst^{d1113} FRT80B*

Fig. S4A *yw hsflp FRT19A tubG80/FRT19A;; tub.Gal4 UAS.GFP/+*

Fig. S4B *yw hsflp FRT19A tubG80/cdc42³ FRT19A;; tub.Gal4 UAS.GFP/+*

Fig. S4C *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT40A tubG80/lgl⁴ FRT40A*

Fig. S4D *yw hsflp FRT19A tubG80/FRT19A; ubi.patronin-GFP/+; tub.Gal4/+*

Fig. S4E *yw hsflp FRT19A tubG80/cdc42³ FRT19A; ubi.patronin-GFP/+; tub.Gal4/+*

Fig. S4F *yw hsflp/+; FRT40A tubG80/lgl⁴ FRT40A; ubi.patronin-GFP/tub.Gal4*

Fig. S4G *yw hsflp FRT19A tubG80/FRT19A;; tub.Gal4 UAS.GFP/+*

Fig. S4H *yw hsflp FRT19A tubG80/cdc42³ FRT19A;; tub.Gal4 UAS.GFP/+*

Fig. S4I *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT40A tubG80/lgl⁴ FRT40A*

Fig. S4J *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT82B tubG80/ FRT82B*

Fig. S4K *yw hsflp tub.Gal4 UAS.GFPnls/+; FRT82B tubG80/ FRT82B crb^{11A22}*

Table S2. Primary antibodies for immunostaining

- rabbit anti-aPKC (C20, Santa Cruz) 1:100
- mouse anti-Dlg (4F3, DSHB) 1:500
- mouse anti- α -Spectrin (3A9, DSHB) 1:100
- rabbit anti- β H-Spectrin (Graham Thomas, PennState) 1:100
- rabbit anti-Cad99C (Christian Dahmann) 1: 500
- guinea pig anit-Cad99C (Dorothea Godt) 1:200
- guinea pig anti-Shot (Katja Roper) 1:100
- rabbit anti-Rab11 (Akira Nakamura, RIKEN, Kobe, Japan) 1:250
- mouse anti-Crumbs (CQ4, DSHB) 1:50
- mouse anti- α -tubulin (Sigma) 1:100
- rabbit anti-Nuf (Shigeo Hayashi, RIKEN, Kobe, Japan) 1:100
- Phalloidin-TRITC (Sigma) 1:100

Table S3. Primary antibodies for immunoblotting

mouse anti-GFP (Roche) 1:1000

guinea pig anti-Shot (Katja Roper, MRC-LMB, Cambridge, UK) 1:5000

rabbit anti-Patronin (Ron Vale, UCSF, San Francisco USA) 1:1000

mouse anti- α -Spectrin (3A9, DSHB) 1:1000

rabbit anti- β H-Spectrin (Graham Thomas, PennState, USA) 1:5000