Supplementary material. Yan et al., 2013

Table S1: Number of and elongation rates of actin filaments.

Reaction conditions	Number of filaments		Fast-growing filaments			Elongation rate in subunits/s	
	N	S.D.	P (in %)	S.D.		v	S.D.
1.3 µM actin+ 2.6 µM Profilin	11.5	6.66	0	0		11.71	1.77
+ 1.3 µM Cip4ΔBar	6.66	3.51	0	0		10.75	0.82
+ 20 nM DiaC	136.67	37.90	81.53	6.66	fast-growing	138.78	23.09
					slow-growing	11.07	1.32
+ 20 nM DiaC	22.8	12.56	6.45	7.00	fast-growing	91.99	7.62
+ 1.3 µM Cip4ΔBAR					slow-growing	11.15	0.56
+ 20 nM DiaC	65	20.74	6.16	1.69	fast-growing	122.04	19.99
+ 1.3 µM Cip4ΔBarΔSH3					slow-growing	10.05	1.26

Fig. S1. Endocytosis of extracellular cargo does not depend on *dia*. (A) To visualize endocytosis, fluorescently labelled wheat germ agglutinin (WGA-Alexa555) was injected into the extracellular perivitteline space. (B) Time-lapse recording 20 min after onset of.cellularization with a frame rate of 1 in 3 s. Arrow heads indicate endocytic events in wild type and *dia* embryos. (C) Photographs of WGA555 injected wild type and *dia[SY5]* embryos (frame rate of 1 in 5 min). Fluorescent particles in basal cytoplasm were marked in red. (D) Quantification of basal particles in a given section in each three wild type and *dia[SY5]* embryos. (E) WGA555 (red) injected wild type and *dia[SY5]* embryos fixed and stained for the FC marker Slam (green). Section in large magnification. Scale bar 10 µm. (D).

Fig. S2. Cip4-GFPΔSH3 does not induced cellularization defects. Embryos expressing Cip4-GFPΔSH3 were fixed and stained for (A) GFP (white/green), F-actin (white/red) and DAPI (blue) or (B) Dlg (white/green), Slam (white/red) and DAPI (blue). (A) Surface view and cross section of the same embryo.

Fig. S3. Constructs used in this study. Tags are labelled in grey (ZZ, GST), blue (myc) and green (GFP, Cherry). DiaN and DiaC contained a His6 tag at the C-terminus. The domain structure of Dia (FH3+RBD, FH1, FH2, DAD) is indicated in red, of Cip4 (F-BAR, HR1, SH3), in yellow. Numbers in parentheses indicated the amino acid residues contained in the construct. The Delta sign indicates the amino acid residues lacking in the construct.

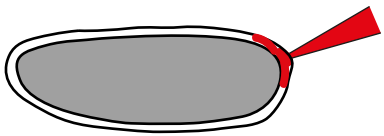
Figure S4. Proteins used in this study. Samples of the purified proteins as designated were analysed by SDS polyacrylamide electrophoresis and stained by Coomassie.

Figure S5. *dia* locus and alleles. Exons and coding sequence of *dia* are indicated by boxes and blue shading. Domain structure of Dia protein is indicated by boxes. *dia*[1] is a transposon insertion in the first exon (Castrillon1994). *dia*[5] is a derivative isolated after mobilisation of *dia*[1], which has not been molecularly characterised (Afshar2000). *dia*[SY*n*] alleles were induced by chemical mutagenesis. Point mutations were determined by sequencing of the isogenic chromosomes. Resulting alteration in the sequence of amino acid residues are indicated. *dia*[SY4] and *dia*[SY6] are clonal.

Figure S6. Actin filament elongation rates in the absence of presence of DiaC or Cip4. Analyses of actin assembly by single filament TIRF microscopy. 1.3 µM actin (23% Atto488 labelled) in the presence or absence of the Dia and Cip4 constructs at the concentrations indicated were used. Error bars indicate S.E.M. More than 20 filaments of at least three independent assays were analyzed for each condition.

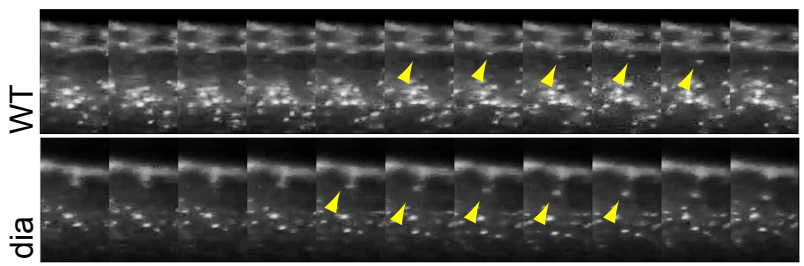
Supplemental material. Figure S1: Yan et al

A



Injection of labelled WGA into the perivitteline space

B



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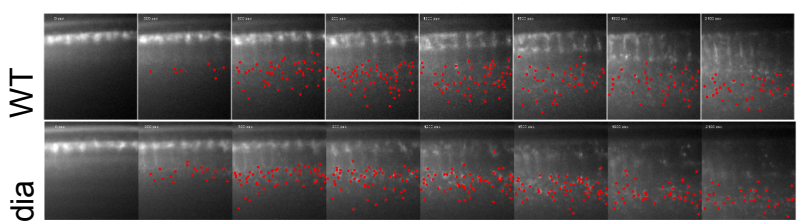

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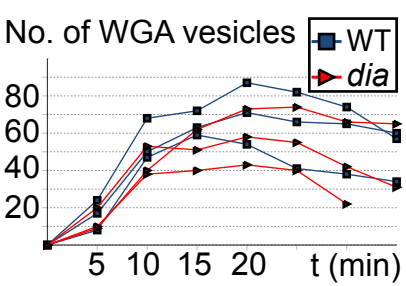

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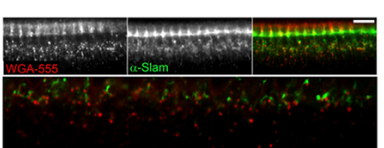
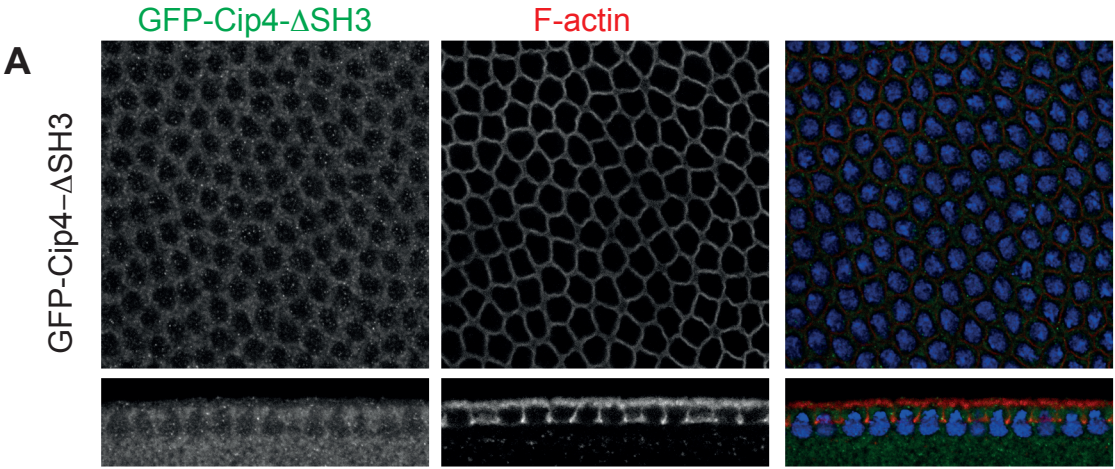


Supplemental material. Figure S2: Yan et al



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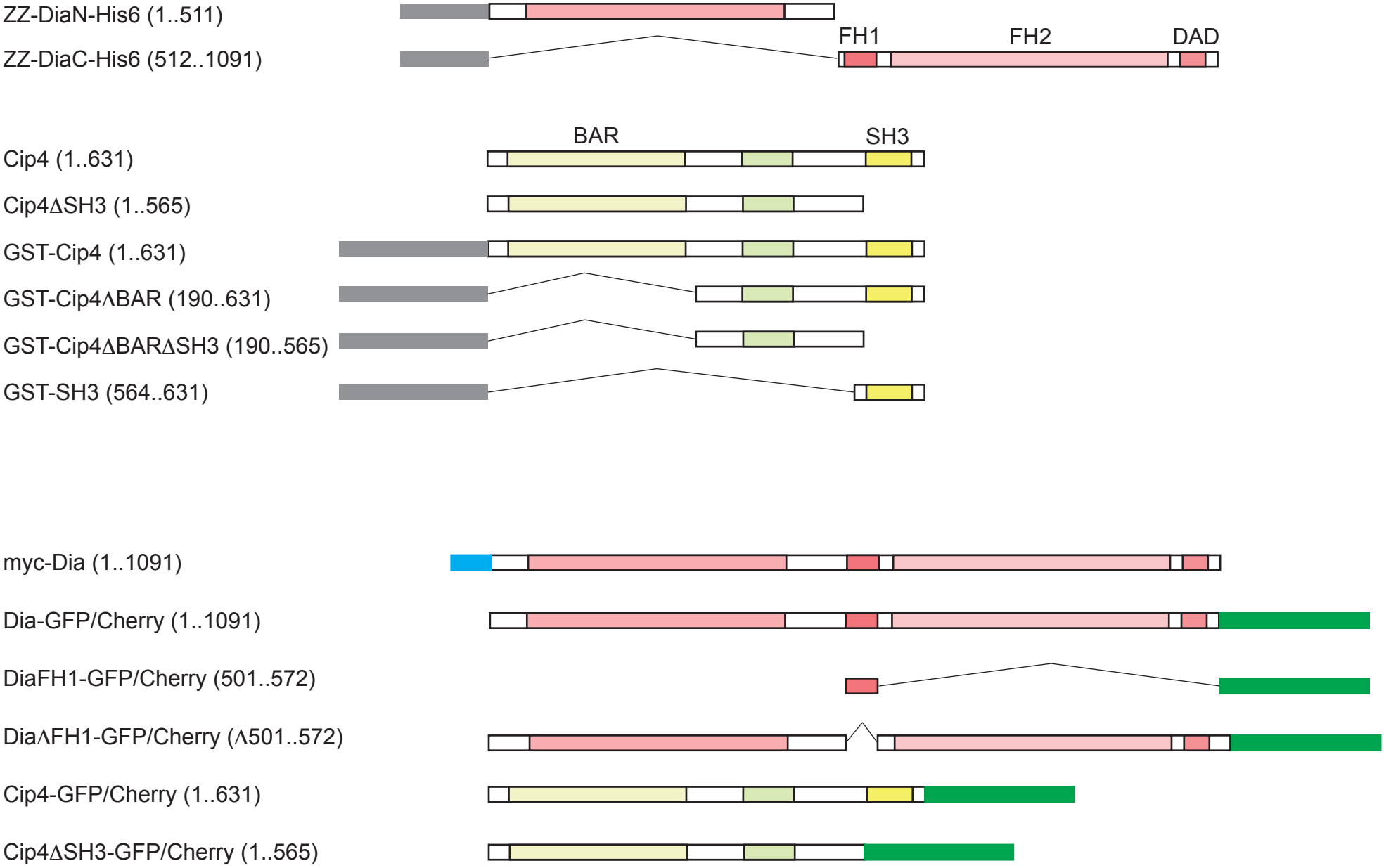

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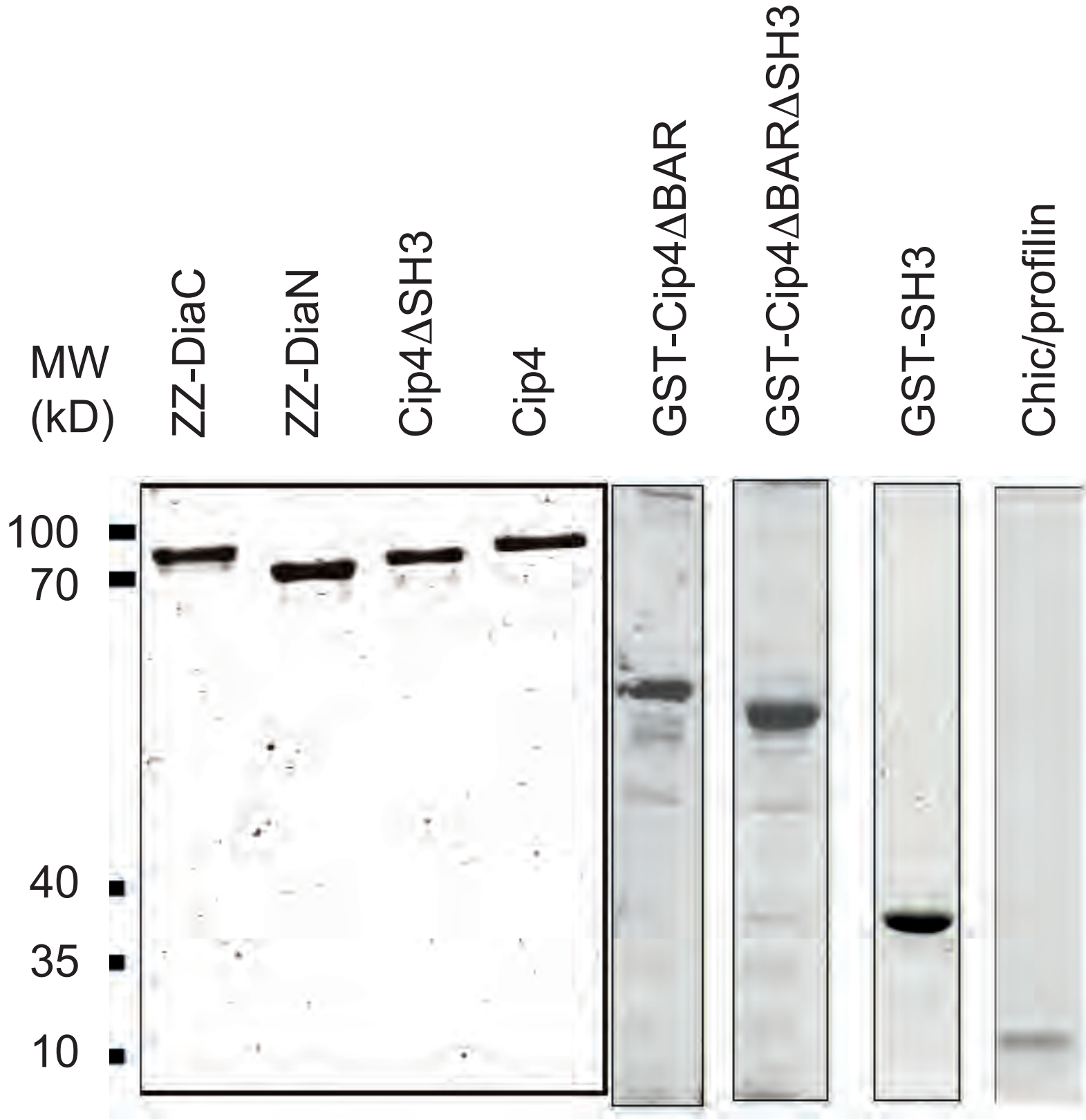
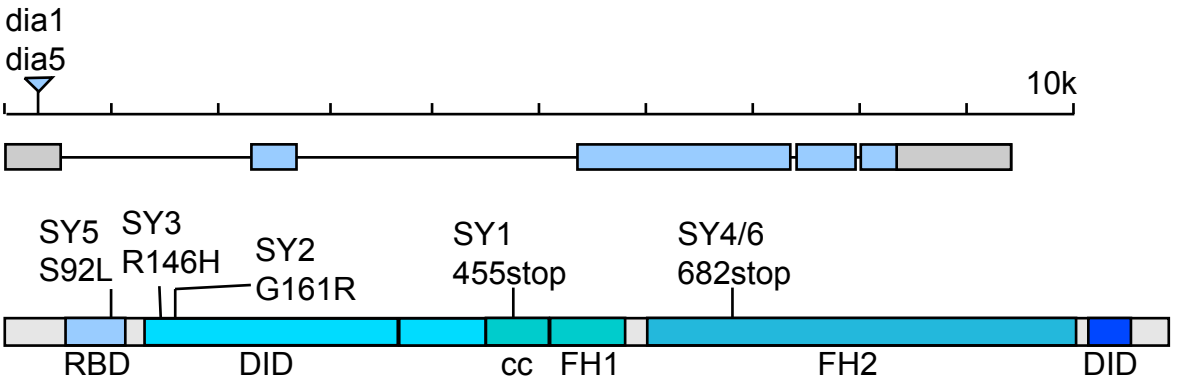
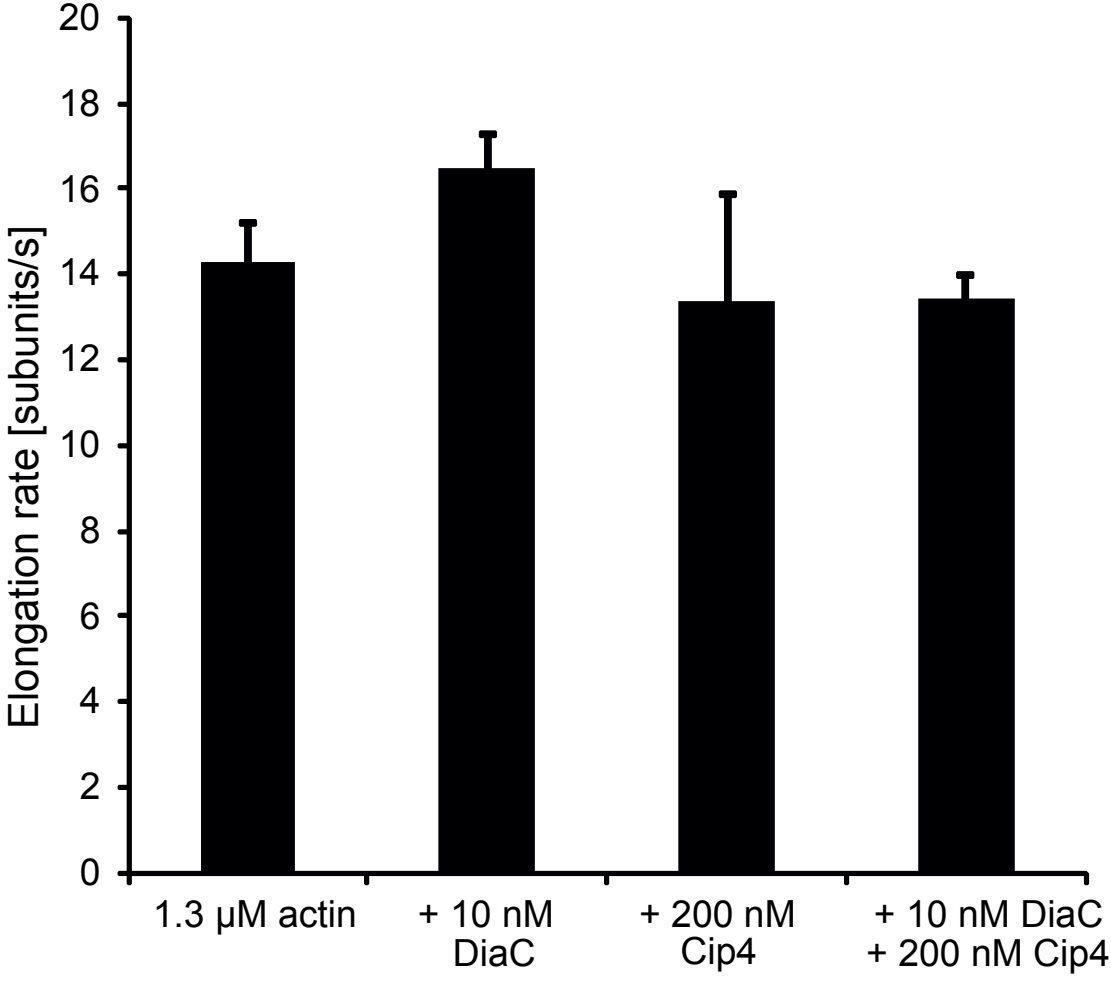


Supplemental material. Figure S5. Yan et al



Supplemental material. Figure S6



Movie 1. GFPslam in wild type embryo. GFPslam labels the basal domain of the metaphase furrow in mitosis and the furrow canal in interphase 14. Dynamical membrane extensions are observed in mitosis and initial interphase 14. In telophase 13 and progressively in interphase 14 the extensions disappear. Frame rate 1/5s, pixel size 130 nm, focal depth 50-60 µm. The time lapse recording shows the dynamics of GFPslam from interphase 13 to interphase 14.

Movie 2. GFPslam in *dia*[SY5] embryo. In *dia* embryos the metaphase furrows do not form properly and the furrow canals that form are often severely dilated. The dynamical membrane extensions remain visible throughout cellularization. GFPslam is expressed maternally by a tubulinVP16 GAL4 driver line. Frame rate 1/5s, pixel size 130 nm, focal depth 50-60 µm. The time lapse recording shows the dynamics of GFPslam from interphase 13 to interphase 14.

Movie 3. WGA endocytosis in wild type embryo. Frame rat 1/3s. The time lapse recordings show the incorporation of fluorescent protein (wheat germ agglutinin-Alexa555) injected into the extracellular perivitteline space. Note that endocytic particles appear all along the furrow.

Movie 4. WGA endocytosis in *dia[SY5]* embryo. Frame rat 1/3s. The time lapse recordings show the incorporation of fluorescent protein (wheat germ agglutinin-Alexa555) injected into the extracellular perivitteline space. Note that endocytic particles appear all along the furrow.

Movie 5. Wild type wing imaginal disc, GFP-tubulin, Histone2Av-RFP.

Movie 6. Wing imaginal disc expressing Cip4[myt], GFP-tubulin, Histone2Av-RFP.

Movie 7. Wing imaginal disc expressing *dia* RNAi, GFP-tubulin, Histone2Av-RFP. The time lapse recordings show the mitotic cells in wing imaginal discs recognizable by the chromosome dynamics labelled by Histone2Av-RFP. During cytokinesis the separation of the daughter cells is marked by the labeling of microtubules. In cell lacking cytokinesis two nuclei share a common arrangement of microtubules.

Movie 8. S2 cells expressing Dia-GFP, DiaΔFH1-GFP, or DiaFH1-GFP.

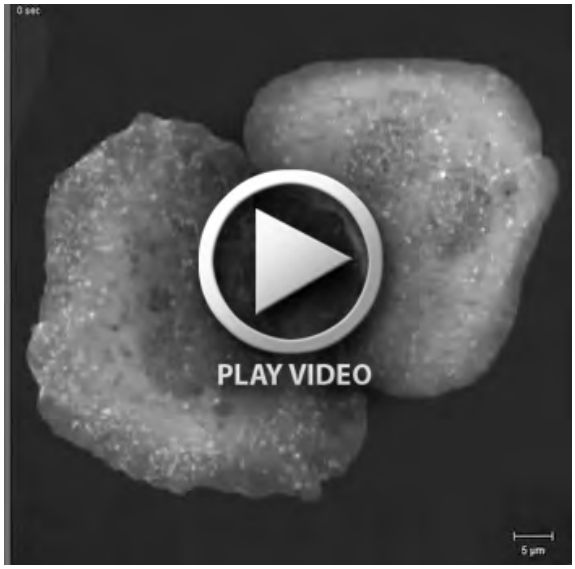
Movie 9. S2 cells expressing full length Dia-GFP and Cip4-Cherry.

Movie 10. S2 cells expressing full length dia-Cherry (green) and Cip4ΔSH3-GFP (red).

Movie 11. S2 cells expressing DiaΔFH1-GFP and Cip4-Cherry.

Movie 12. S2 cells expressing DiaFH1-GFP and Cip4-Cherry.

Movie 13. S2 cells expressing DiaΔDAD-GFP and Cip4-Cherry. S2 cells expressing differentially tagged Dia and Cip4 proteins. Cip4 expression induces tubular invaginations at the plasma membrane and labels intracellular vesicles. The time lapse recordings show the dynamics of the Dia and Cip4 proteins in relation to each other in interphase cells.



Movie 14. S2 cells expressing Cip4-Cherry treated with *dia* RNAi. S2 cells stably expressing Cip4-GFP. Expression of Cip4-GFP was induced 3 days after treatment with *dia* RNAi.

Movie 15. Analysis of actin assembly by single filament TIRF microscopy. Dynamics of actin filaments visualized by fluorescently labelled actin monomers in the presence profilin, DiaC, Cip4ΔBAR, Cip4ΔBARΔSH3 as indicated.