

Expanded View Figures

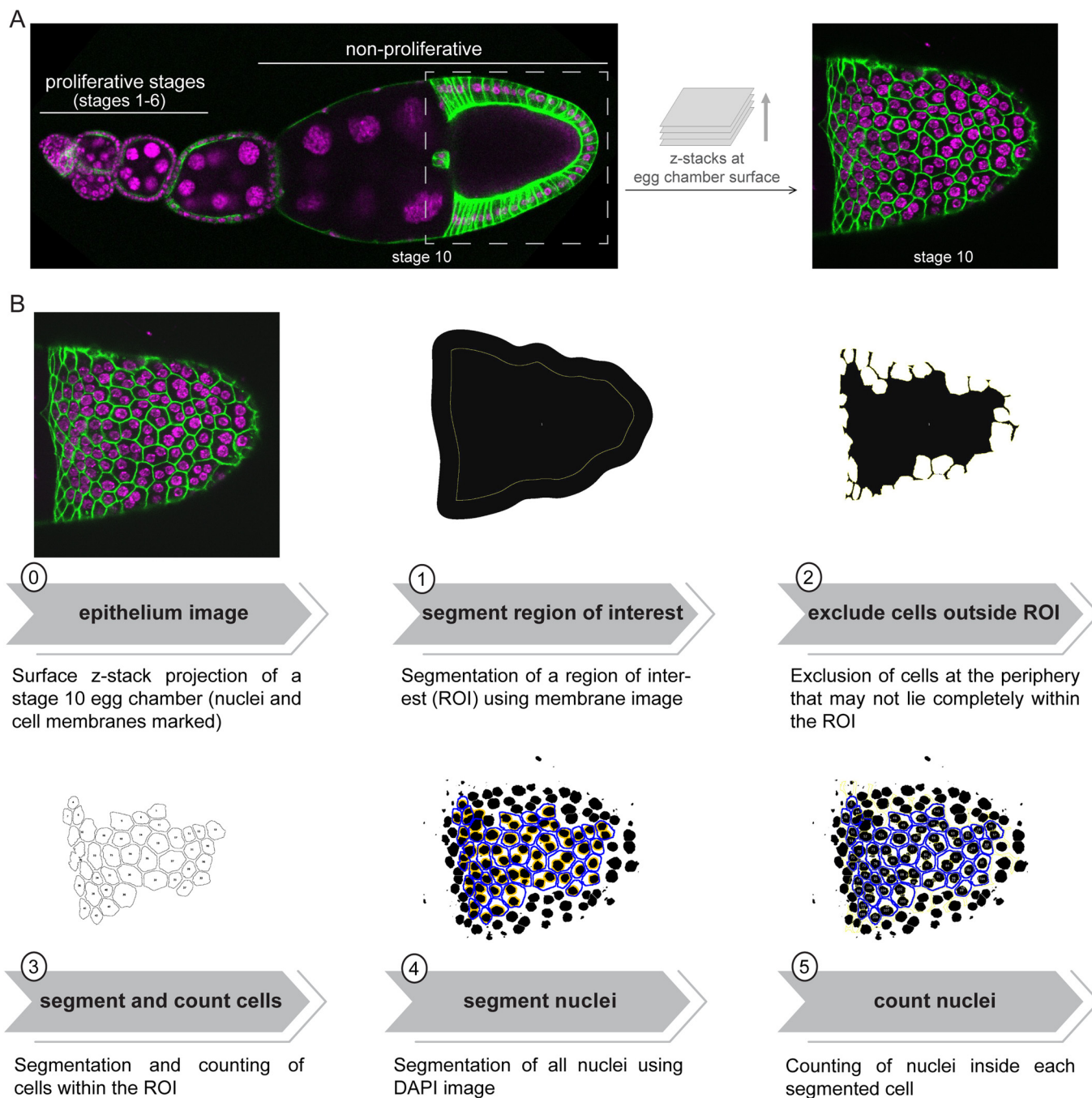
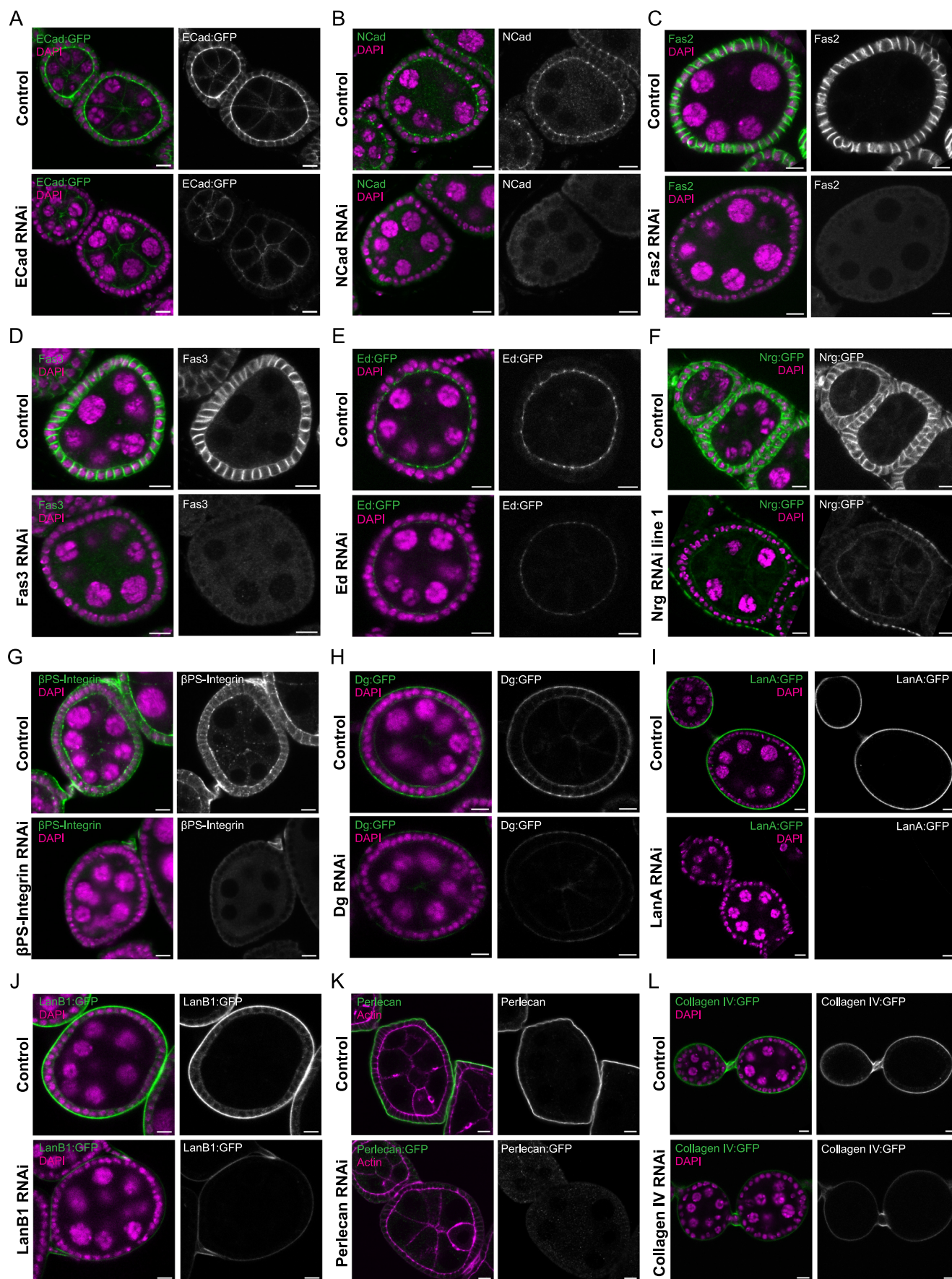


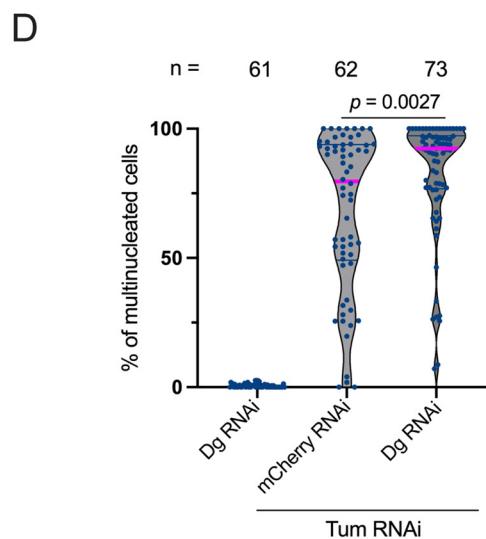
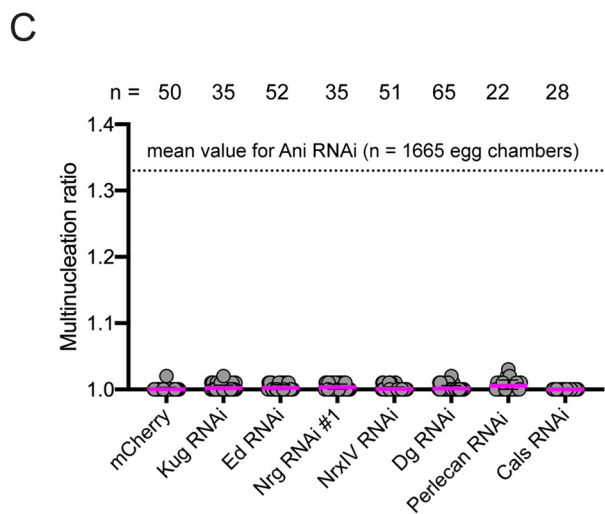
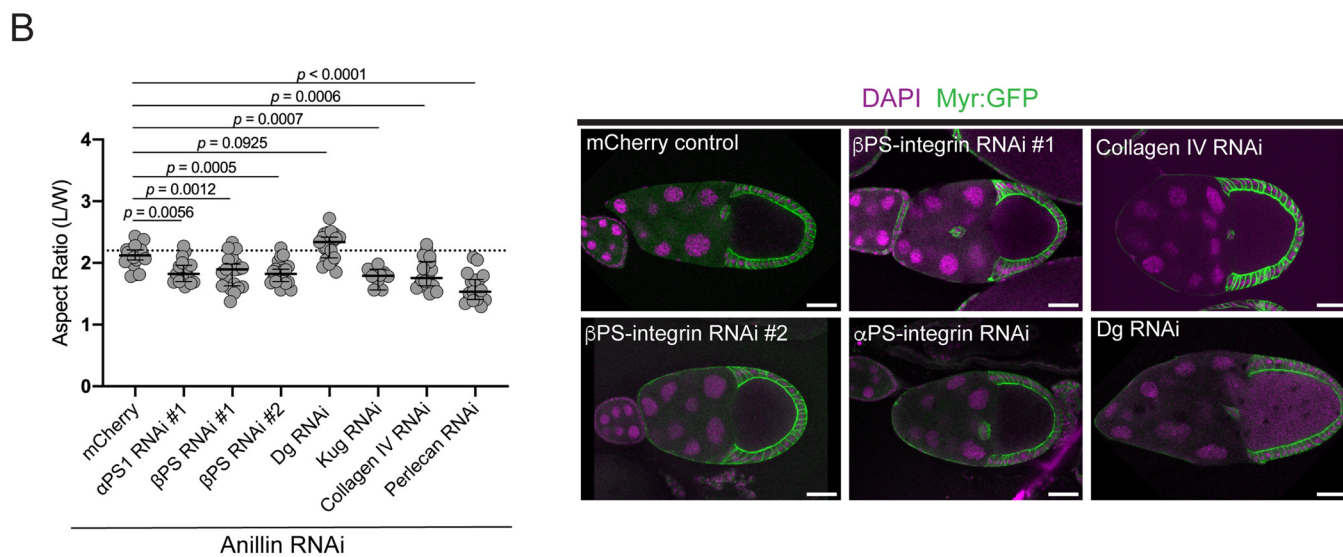
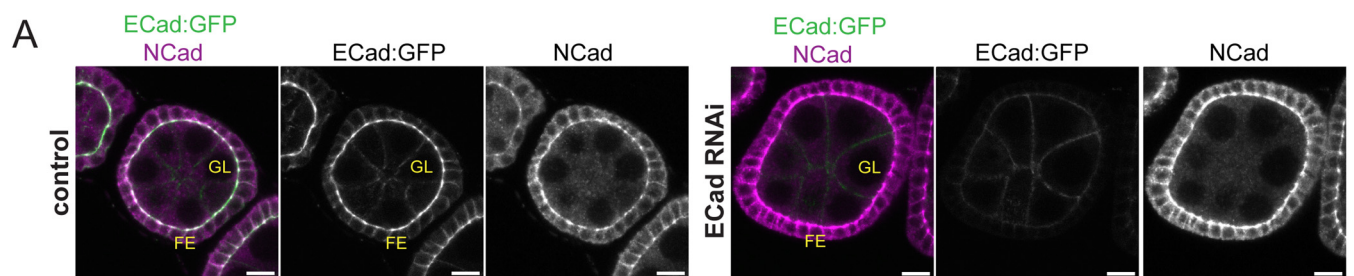
Figure EV1. Pipeline for the quantification of cytokinesis defects in the follicular epithelium of stage 10 egg chambers.

(A) Surface z-projections of non-proliferative stage 10 egg chambers were used to quantify the cytokinesis defects from the *in vivo* genetic modifier screen (nuclei marked with DAPI (magenta) and cell membranes endogenously expressing Myr:GFP (green)). At this stage of oogenesis, follicle cells are no longer undergoing mitosis, allowing us to directly correlate multinucleation with cytokinesis failure, and are larger than at younger stages, facilitating the automated segmentation of nuclei and cell membranes. (B) A macro for FIJI was developed for the automated segmentation and counting of nuclei and cells in two-channel images of the follicular epithelium (nuclei in magenta and cell membranes in green). Note that this was restricted to the central area of the egg chamber, to avoid misleading quantifications from nuclei/cells at the periphery of the egg chambers. After running the macro, a manual validation of the segmentation of both nuclei and cells was performed for all images. The obtained values were used to calculate the Multinucleation Ratio, as explained in detail in the Methods section.



◀ **Figure EV2. Validation of RNAi-mediated depletion of proteins from the genetic modifier screen.**

(A) Midsagittal images of control and ECad RNAi egg chambers endogenously expressing ECad:GFP (green) and DAPI (magenta). (B) Midsagittal images of control and NCad RNAi egg chambers, stained for NCad (green) and DAPI (magenta). (C) Midsagittal images of control and Fas2 RNAi egg chambers, stained for Fas2 (green) and DAPI (magenta). (D) Midsagittal images of control and Fas3 RNAi egg chambers, stained for Fas3 (green) and DAPI (magenta). (E) Midsagittal images of control and Ed RNAi egg chambers endogenously expressing Ed:GFP (green) and stained for DAPI (magenta). (F) Midsagittal images of control and Nrg RNAi (line 1) egg chambers endogenously expressing Nrg:GFP (green) and stained for DAPI (magenta). (G) Midsagittal images of control and β PS-integrin RNAi egg chambers, stained for β PS-integrin (green) and F-actin (magenta). (H) Midsagittal images of control and Dg RNAi egg chambers endogenously expressing Dg:GFP (green) and stained for DAPI (magenta). (I) Midsagittal images of control and LanA RNAi egg chambers endogenously expressing LanA:GFP (green) and stained for DAPI (magenta). (J) Midsagittal images of control and LanB1 RNAi egg chambers endogenously expressing LanB1:GFP (green) and stained for DAPI (magenta). (K) Midsagittal images of control and Perlecan RNAi egg chambers, stained for Perlecan (green) and F-actin (magenta). (L) Midsagittal images of control and Collagen IV RNAi egg chambers endogenously expressing Collagen IV:GFP (green) and stained for DAPI (magenta). (A–L) Scale bars: 10 μ m.



◀ **Figure EV3. Complementary analysis of RNAi-mediated depletion and their effects on cytokinesis.**

(A) Midsagittal images of control and ECad RNAi egg chambers endogenously expressing ECad:GFP (green) and stained for NCad (magenta). Expression of RNAi for ECad in the follicular epithelium (FE) induces efficient protein depletion. As anticipated, reduction of ECad:GFP fluorescence by ECad RNAi is restricted to the follicular epithelium (FE) and ECad levels are normal in the germline (GL). Scale bar: 10 μ m. (B) Midsagittal images and measurement of the aspect ratio in stage 10 egg chambers co-depleted for Anillin and the indicated basal proteins, in comparison with control (UAS-mCherry). Aspect ratio was calculated as the ratio between egg chamber length and width, as depicted in the bottom image (DAPI in magenta and cell membrane in green (Myr:GFP)). Each dot represents the aspect ratio of an analyzed egg chamber. The dashed line represents the anticipated aspect ratio value for this egg chamber stage, as calculated in (Jia et al, 2016). Median $\pm$ 95 Confidence Interval is shown. *P* value was calculated by an ordinary one-way ANOVA. Scale bar: 50 μ m. (C) Multinucleated ratio in egg chambers depleted for the regulators of cell-cell and cell-matrix interactions that enhanced multinucleation in the Anillin RNAi modifier screen (Fig. 1D-F), in comparison with control (UAS-mCherry). Depletion of these molecules on their own (in an unperturbed cytokinetic background) does not cause multinucleation. Each dot represents the Multinucleation Ratio of an analyzed egg chamber. Median is indicated. The dashed line represents the mean value of the multinucleation ratio for co-expression of Anillin RNAi with the control line (UAS-mCherry). (D) Frequency of multinucleated cells in egg chambers either co-expressing UAS-Dg RNAi with UAS-mCherry RNAi (UAS titulation control), UAS-Tum RNAi with UAS-mCherry RNAi or UAS-Tum RNAi with UAS-Dg RNAi. UAS-driven depletion of Tum causes strong multinucleation frequency in the follicular epithelium, which is significantly increased by the concomitant depletion of Dg. Violin plots and median (magenta) is indicated. Each dot represents an egg chamber. Sample size (*n*) is indicated on top. *P* value calculated by non-parametric unpaired Mann-Whitney test.

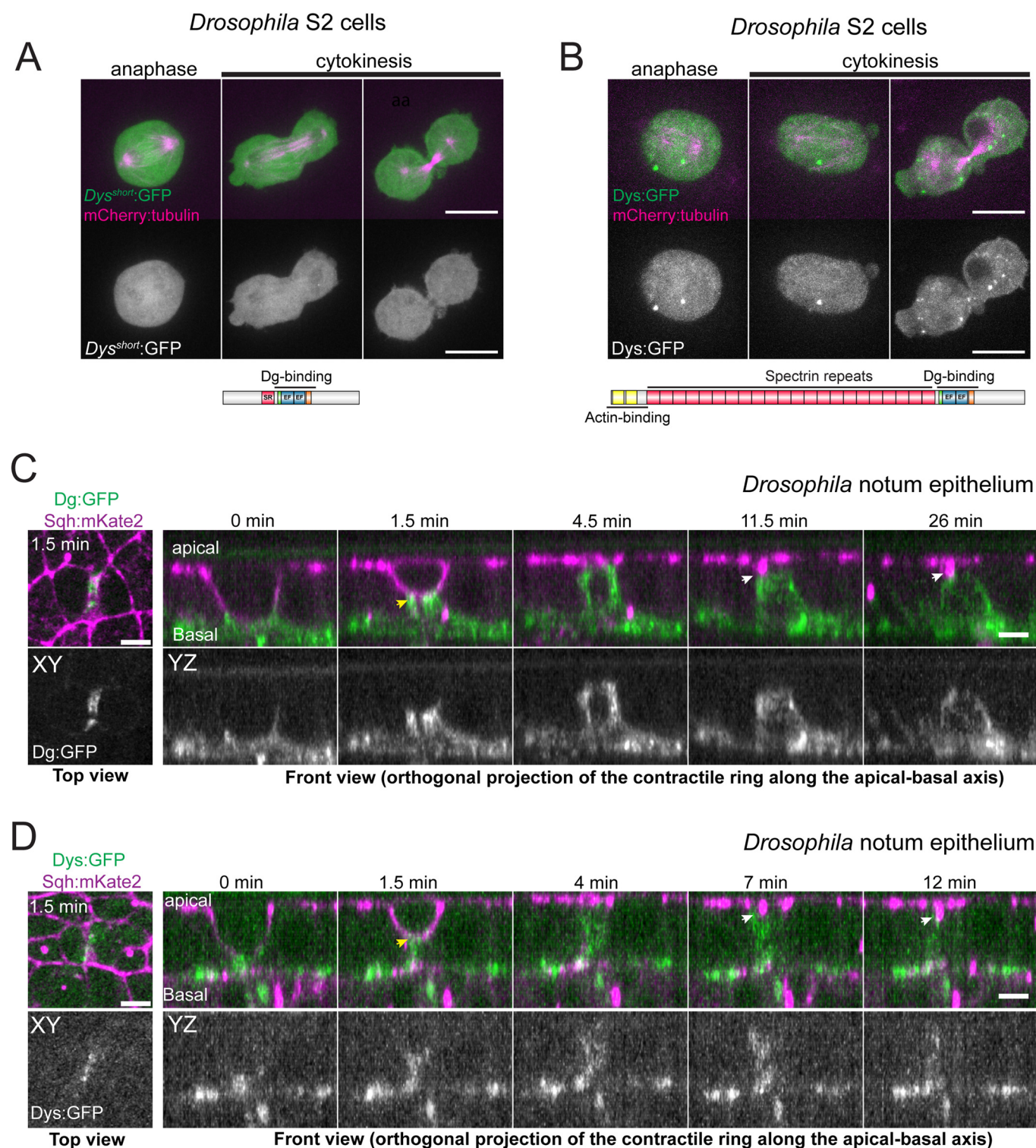


Figure EV4. Dynamic redistribution of the DAPC during epithelial cytokinesis in *Drosophila* S2 cells and pupal notum epithelium.

(A, B). The short Dys isoform E (A) and the long isoform H (B) do not show any local accumulation during cytokinesis in *Drosophila* S2 cells. Dys isoforms are GFP-tagged versions (green) and mCherry:Tubulin labels microtubules (magenta). Scale bars: 10 μ m. (C, D). Redistribution of endogenously tagged Dg:GFP (A) and Dys:GFP (B) during epithelial cytokinesis in the *Drosophila* pupal dorsal thorax (notum). During ring (labeled with Sqh:mKate2) constriction, Dg:GFP (A) and Dys:GFP (B) accumulate at the basal part of the ingressing membrane (yellow arrows). After ring closure, both proteins become enriched close to the midbody (white arrows). Scale bars: 5 μ m.

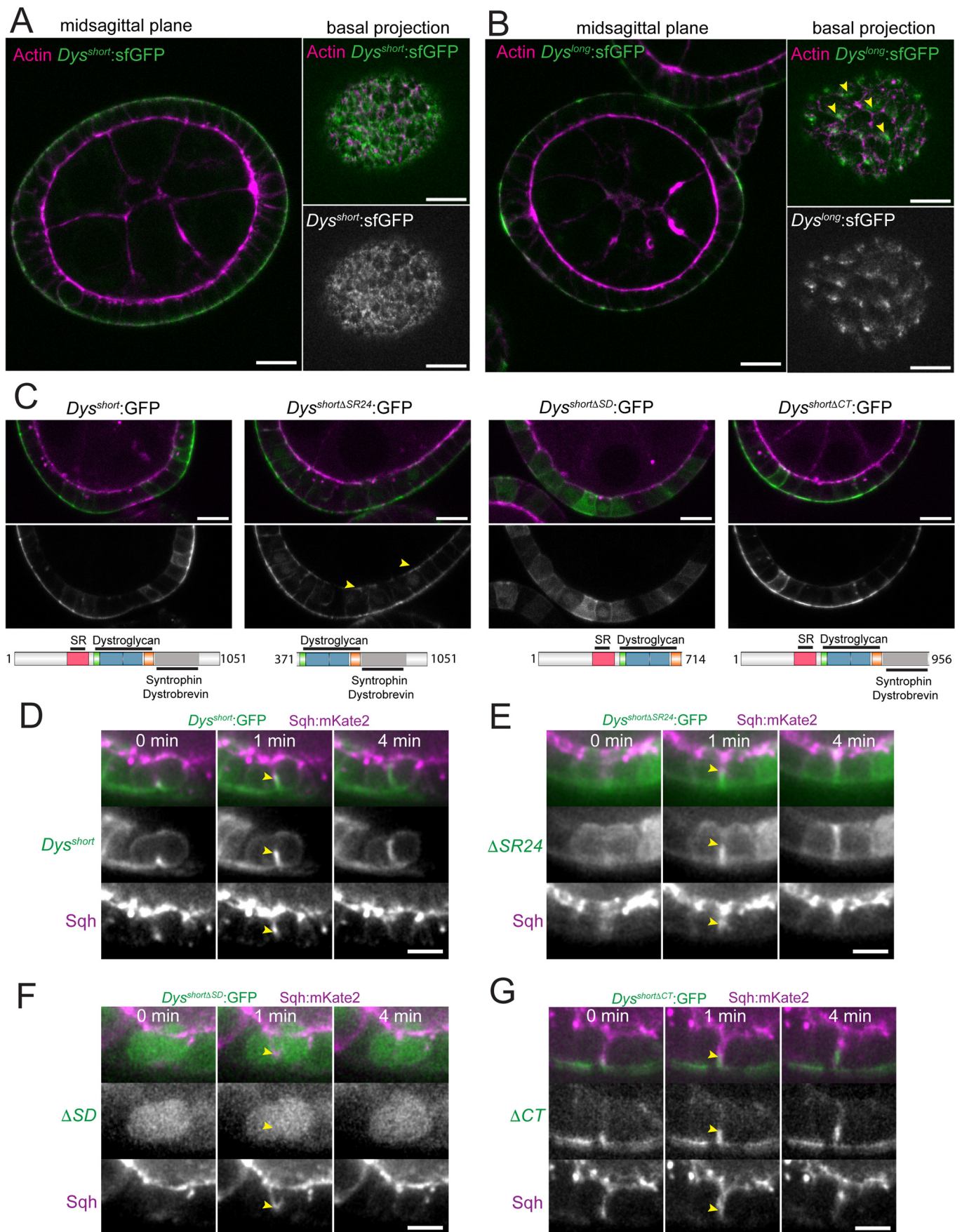




Figure EV5. Spatial distribution of Dystrophin isoforms and truncated versions.

(A) Midsagittal images (left) and basal projection (right) of the proliferative follicular epithelium endogenously expressing *Dys^{short}:sfGFP* (green) and stained for actin (magenta). *Dys^{short}* mainly localizes at the basal domain of epithelial cells. Scale bar: 10 μ m. (B) Midsagittal images (left) and basal projection (right) of the proliferative follicular epithelium endogenously expressing *Dys^{long}:sfGFP* (green) and stained for actin (magenta). *Dys^{long}* mainly localizes to the basal domain in a planar polarized manner. Scale bar: 10 μ m. (C) Midsagittal images of egg chambers expressing UAS-driven *Dys^{short}:GFP*, *Dys^{short} Δ SR24:GFP*, *Dys^{short} Δ SD:GFP* or *Dys^{short} Δ CT:GFP* and expressing Sqh:mKate2 (magenta). The short *Dys* isoform mainly localizes at the basal domain of epithelial cells, which is not altered by the deletion of the most downstream C-term region (*Dys^{short} Δ CT*). Partial mislocalization to the apical domain (yellow arrows) is observed in egg chambers expressing *Dys^{short} Δ SR24:GFP*. Deletion of syntrophins/Dystrobrevin interaction sites leads to *Dys* mislocalization and only weak basal enrichment (*Dys^{short} Δ SD*). Note that these UAS-driven constructs show mosaic expression levels in the follicular epithelium. Scale bar: 10 μ m. (D–G) Time-lapse images of follicle cells expressing UAS-driven *Dys^{short}:GFP*, *Dys^{short} Δ SR24:GFP*, *Dys^{short} Δ SD:GFP* or *Dys^{short} Δ CT:GFP*. *Dys^{short}* (A) becomes enriched at the ingressing membrane during ring constriction, which is labeled with Sqh:mKate2 (position of the basal part of the ring is marked with arrows). *Dys^{short} Δ SR24* (E) and *Dys^{short} Δ CT* (G) present a similar cytokinetic redistribution. In contrast, removal of the syntrophins/Dystrobrevin binding sites prevents *Dys* accumulation in the ingressing membrane during epithelial cytokinesis (F). Scale bar: 5 μ m.