

Expanded View Figures

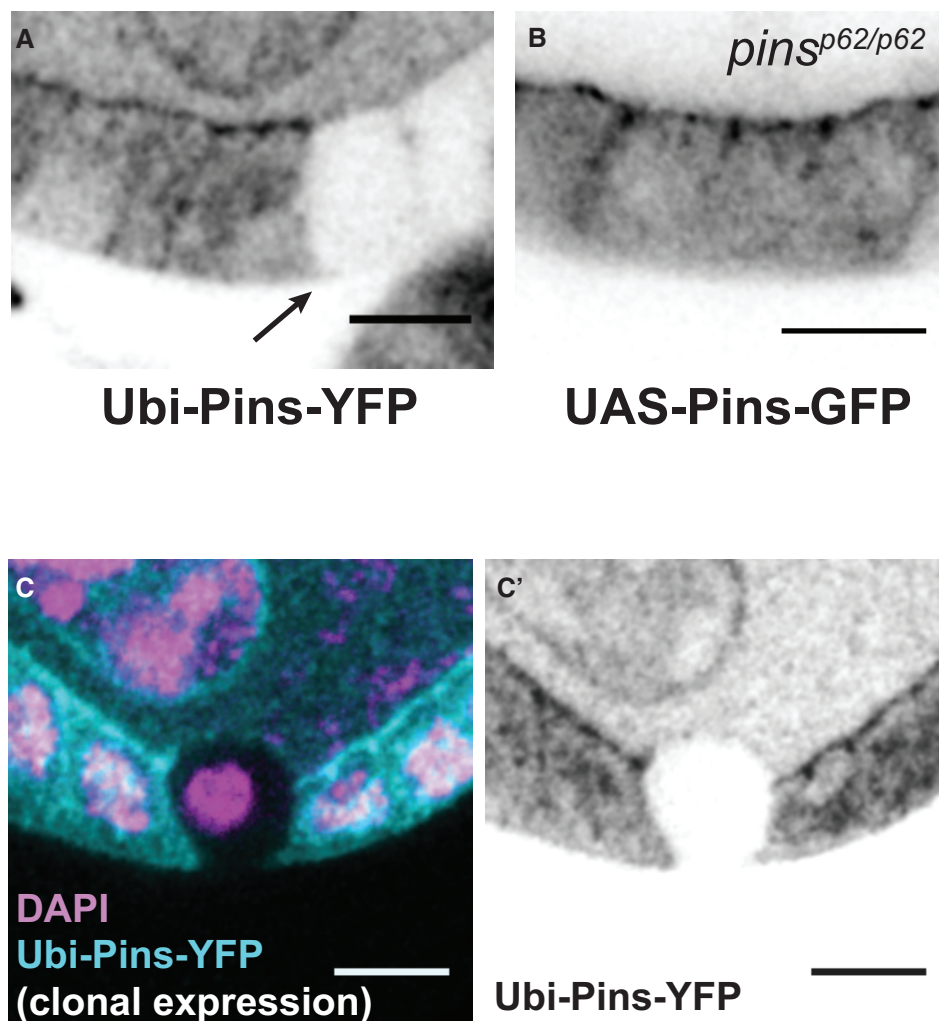


Figure EV1. Pins signal at the border between interior germline and exterior follicle cells reflects apical localization in follicle cells—Related to Fig 2.

- A Clonal expression of Ubi-Pins-YFP shows that most signal at the border between germline and follicle cells originates in the follicle epithelium. The arrow marks the clone border.
- B Pins-GFP appears at the apical surface when expressed only in the follicle epithelium.
- C Clonal expression of Ubi-Pins-YFP shows that signal at the border between the germline and a mitotic follicle cell originates primarily in the mitotic cell. In this field of view, only the mitotic cell lacks expression of Ubi-Pins-YFP.

Data information: scale bars = 5 microns.

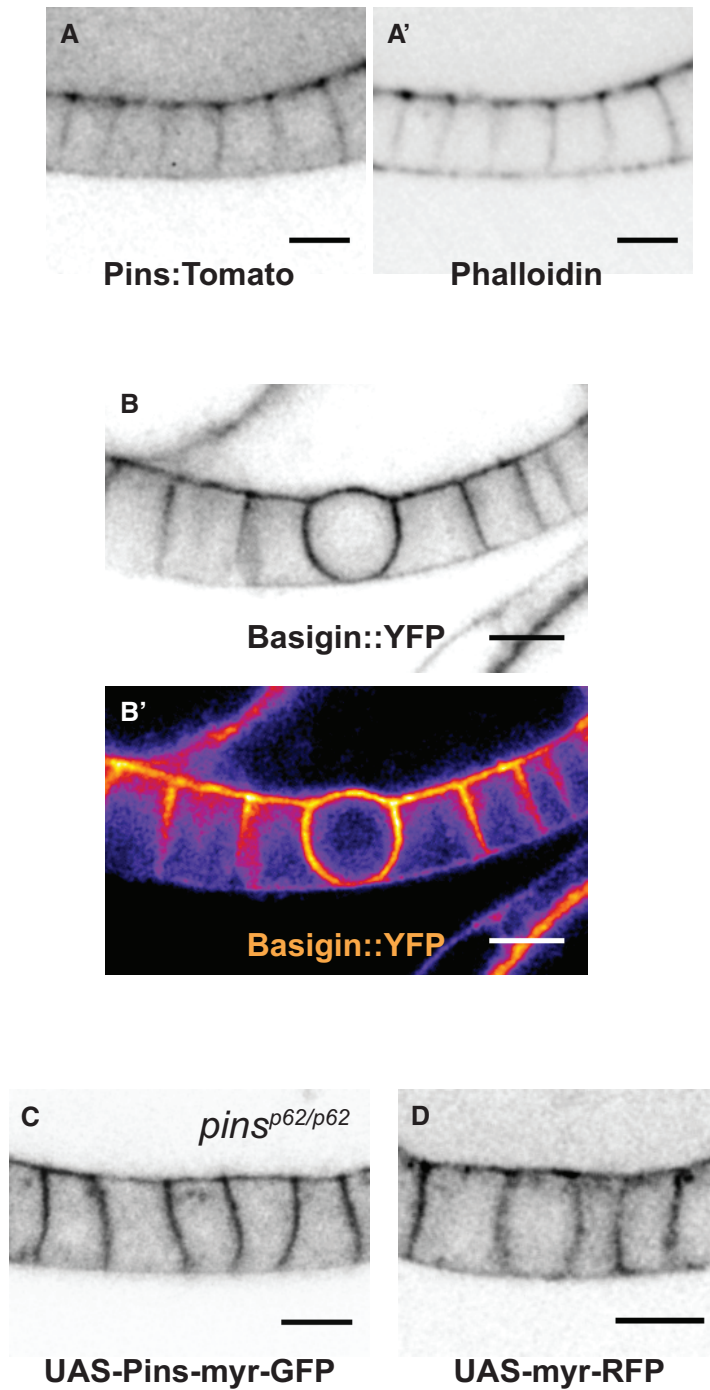


Figure EV2. Localization of cortical proteins in the follicular epithelium—Related to Fig 3.

- A** Pins:Tom and actin overlap at the apical and lateral cortex during interphase.
- B** Basigin::YFP is enriched in a mitotic cell. Both a b/w and heat map image are shown to highlight enrichment. The protein can be found at the borders of germline and follicle cells.
- C, D** UAS-myr-Pins-GFP (**C**) and UAS-myr-RFP localization (**D**) show similar localization in interphase follicle cells. Scale bars = 5 microns.

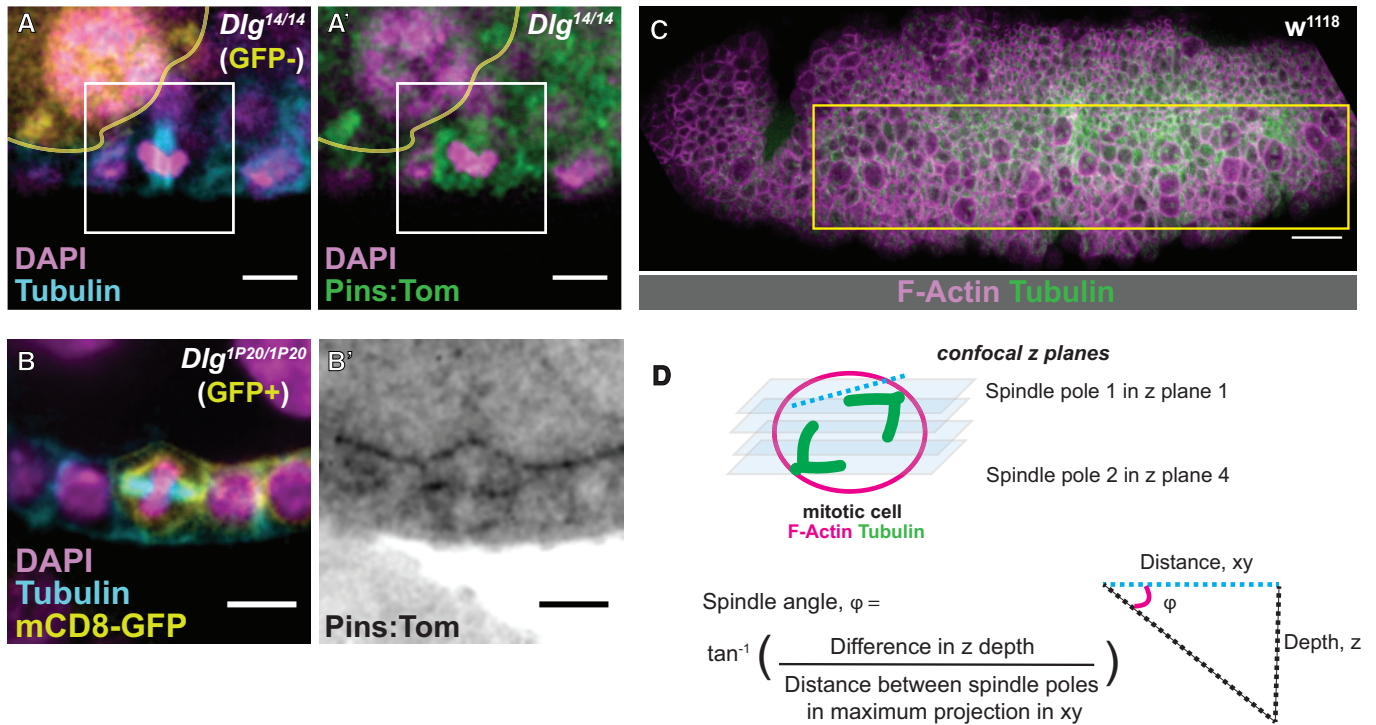


Figure EV3. Dlg is not required for cortical localization of Pins and explanation of the method of spindle angle measurements in the embryo—Related to Fig 4.

Pins:Tom is observed at the cell cortex in:

- A A mitotic *Dlg*¹⁴/*Dlg*¹⁴ cell and
 B A mitotic *Dlg*^{1P20}/*Dlg*^{1P20} cell. For the former, mutant tissue is marked by the absence of RFP. For the latter, mutant tissue is marked by the presence of mCD8-GFP.
 Scale bars = 5 microns (A, B).
 C The region of the ventrolateral embryonic ectoderm used to quantify spindle angles from a representative fixed and immunostained embryo. Scale bar = 20 microns.
 D A schematic showing the method to determine the spindle angle of mitotic cells in the early embryonic ectoderm, described in the Materials and Methods.

Figure EV4. Insc^A and Insc^B cells are distinguished by the pattern of Inscuteable localization—Related to Fig 6.

- A In Insc^A cells, Inscuteable is strongly enriched at the apical surface.
 B Insc^B cells show a stronger enrichment of Inscuteable at lateral surfaces. In an extreme example, Inscuteable is only lateral.
 C Mitotic spindle reorientation in a *Dlg*^{1P20}/*Dlg*^{1P20} cell expressing Inscuteable. Quantification in Fig 6F.
 D As a complementary visualization strategy to that provided in Fig 6B and D, we divided the relative signal intensity data for Mud, Pins, and Insc in Insc^A and Insc^B cells into bins of 15 degrees each. Box plot central bands = mean, whiskers = standard deviation. These data are presented here side-by-side for each protein in the two conditions. Only the intensity of Inscuteable showed a statistical difference (as determined by ANOVA) between Insc^A and Insc^B cells. Quantification from Insc^A: 5 cells, Insc^B: 7 cells (biological replicates).

Data information: scale bars = 5 microns (A–C).

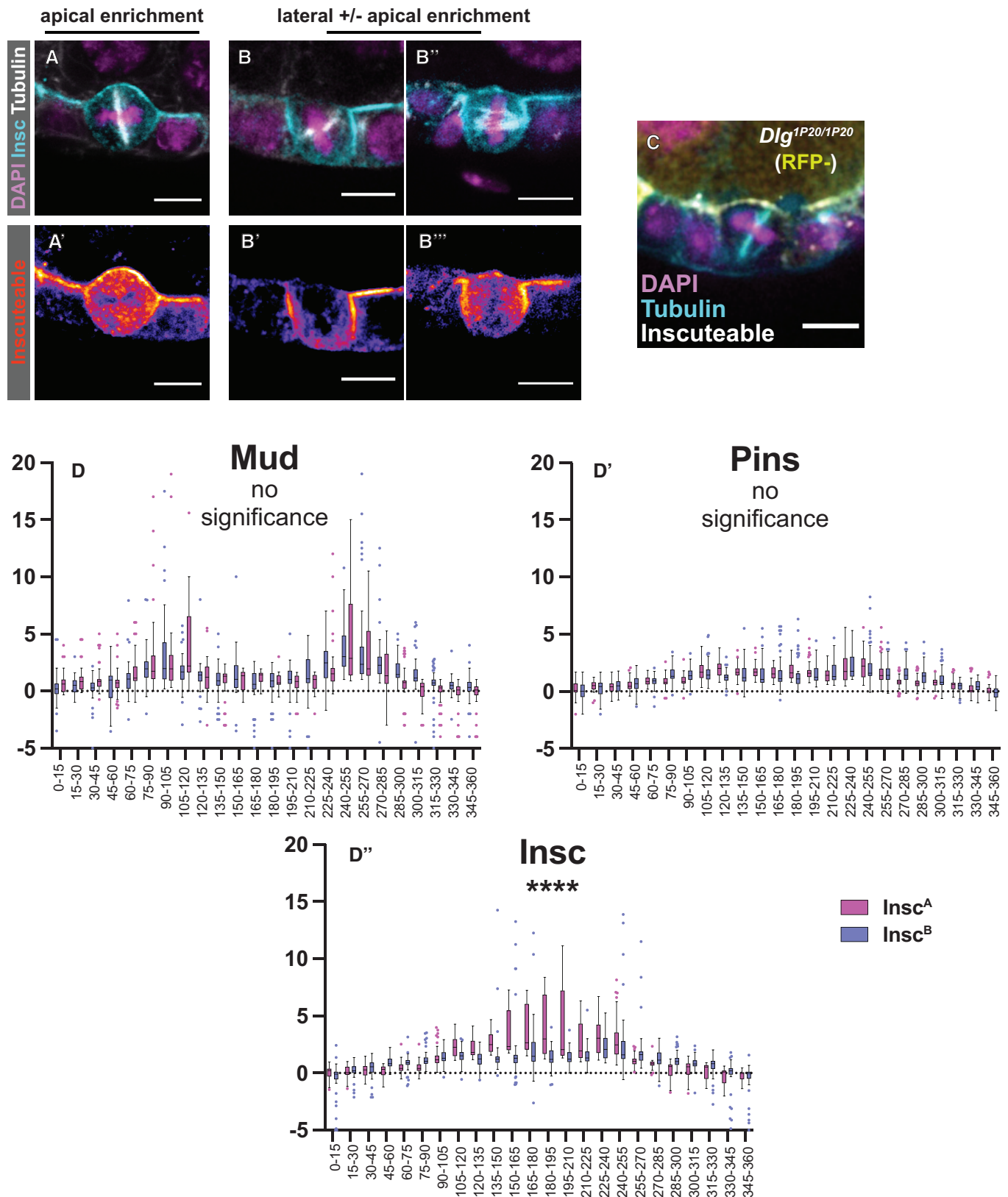


Figure EV4.