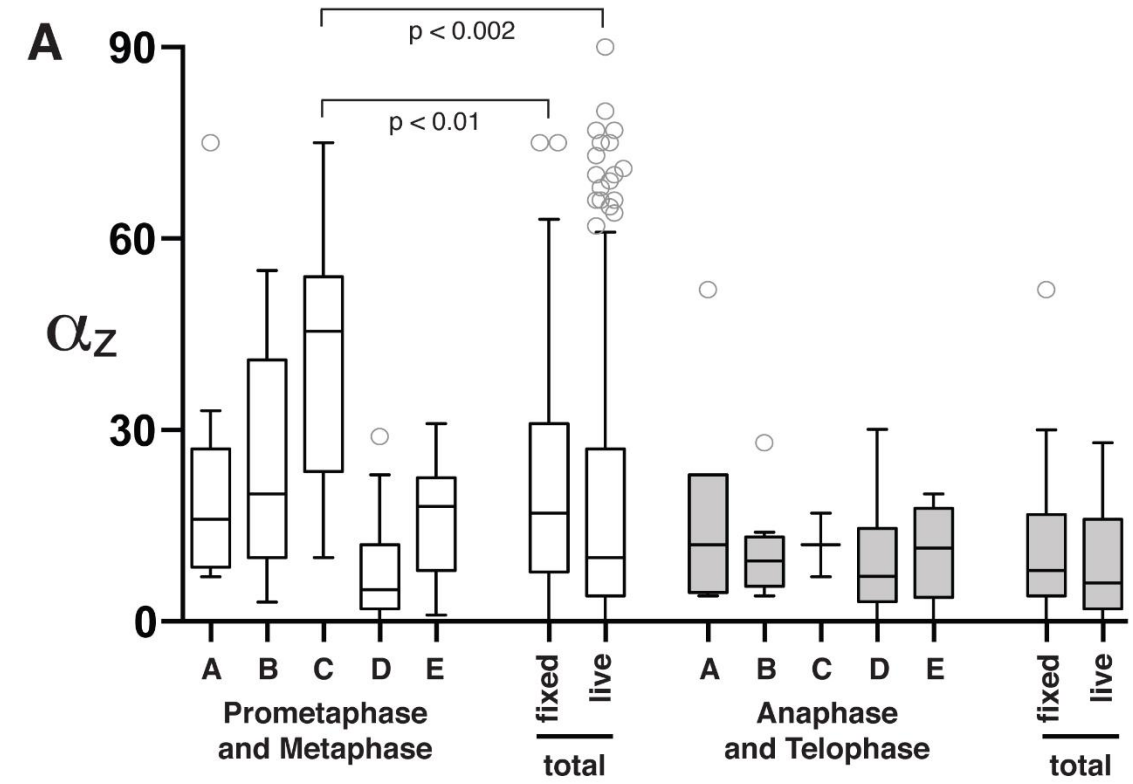
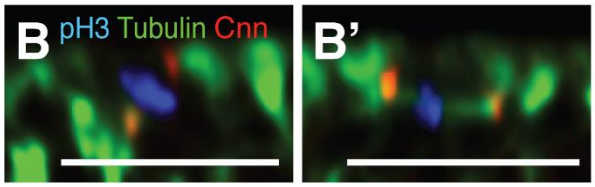
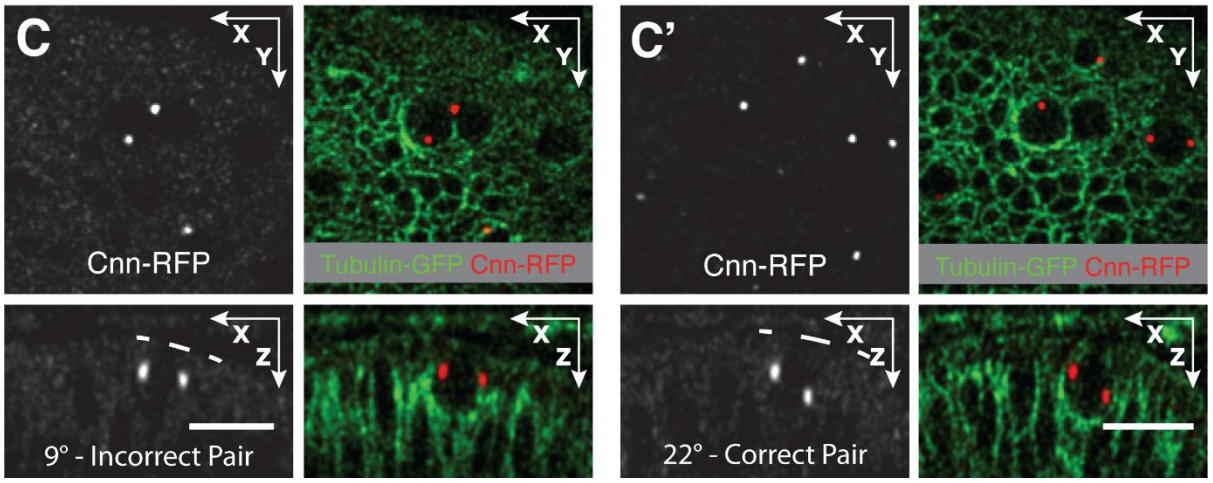
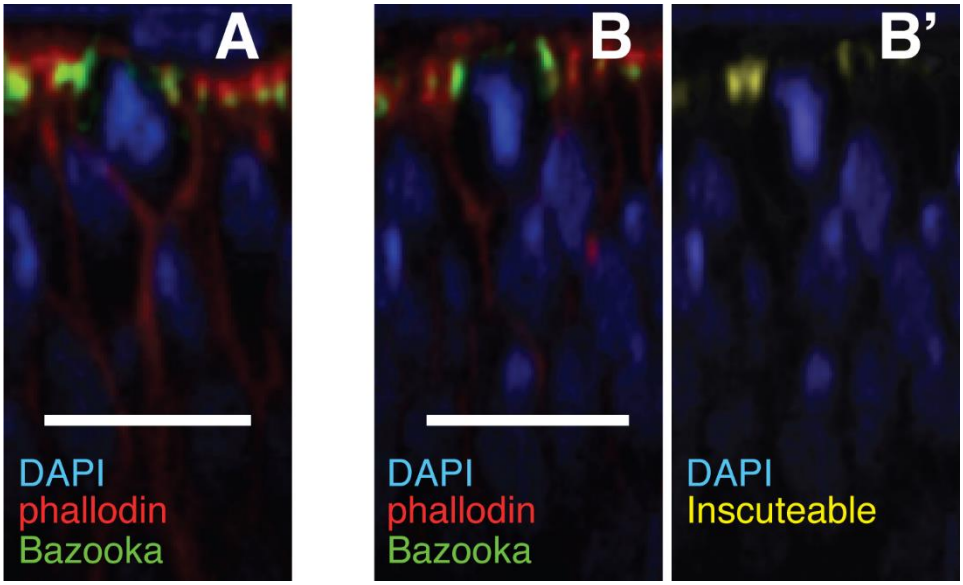






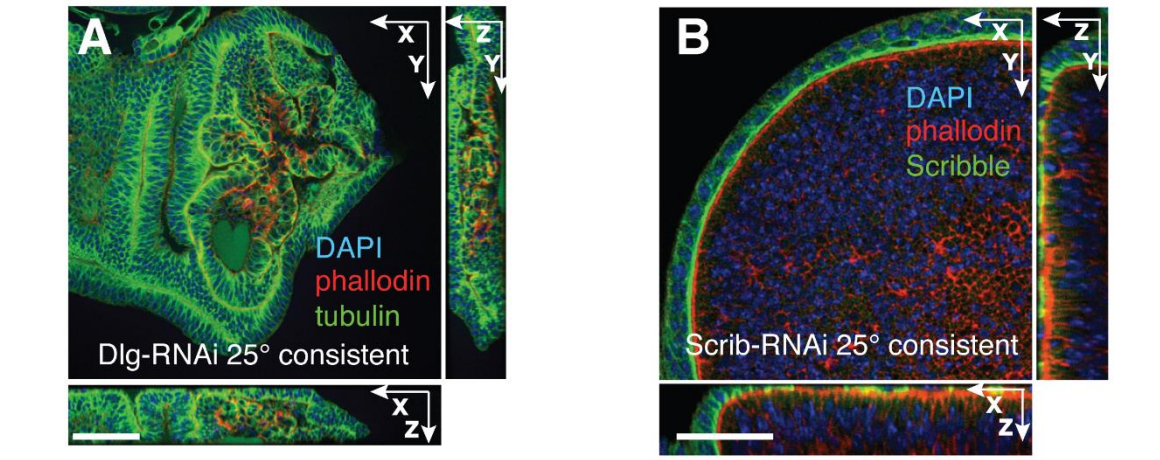
Supplementary Figure 1

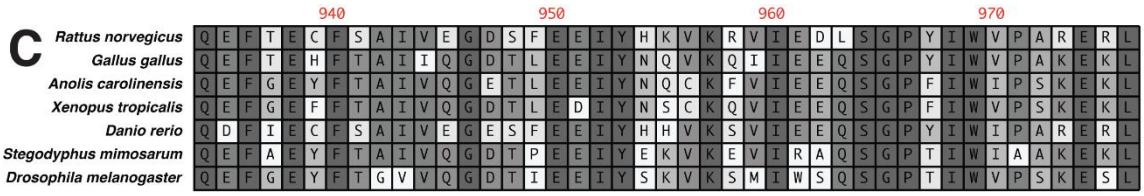
A) A comparison between fixed and live wing discs. Spindle and division angles were determined in five wing discs, labeled A-E, from three dissections of w^{1118} larvae. Pro-metaphase and metaphase are shown on the left (*n* = 13, 11, 14, 19, 9), and Anaphase and Telophase on the right (*n* = 9, 10, 2, 21, 6, 6). The total number of Prometaphase and Metaphase spindles is 66 in fixed tissue, 236 in live tissue. The total number of Anaphase and Telophase angles is 54 in fixed tissue, 59 in live tissue. Boxes represent the interquartile range (IQR), with a line marking the median. The whiskers extend from the lowest point within 1.5 IQR of the lower quartile to the highest point within 1.5 IQR of the upper quartile. **B and B')** Immunoreactivity to phospho-histone 3 (blue) is observed both prior to (B) and after spindle formation (B'). **C and C')** Centrosomes pairs can be mis-assigned in the absence of a marker for cell boundaries. In C, nearby centrosomes within the same XY plane appear to lie at opposite ends of a spindle with an angle of 9° in the Z-axis (relative to the plane of the tissue). The addition of the Tubulin marker (right – in green), which reveals the cell boundaries, shows that this pairing is incorrect. The correct centrosome pairing (C') has an angle of 22°. Scale bars in this figure represent 10 µm.

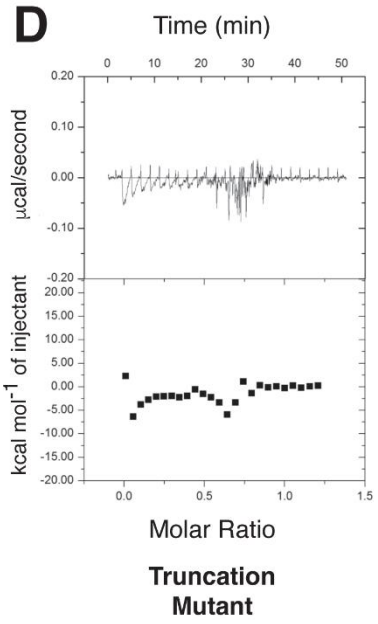


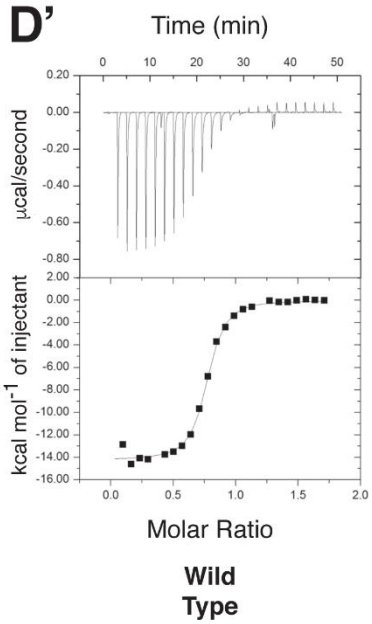
Supplementary Figure 2

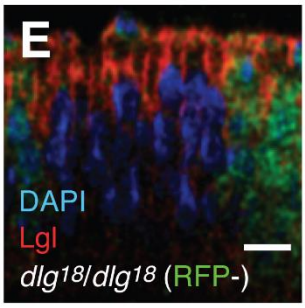
A) Bazooka localizes to the top (apical) of the lateral cortex in the wing disc. **B and B')** The localization of Bazooka (B) is unchanged by ectopic expression of Inscuteable (B'). These two images are of the same cell. Scale bars in this figure represent 10 μm.

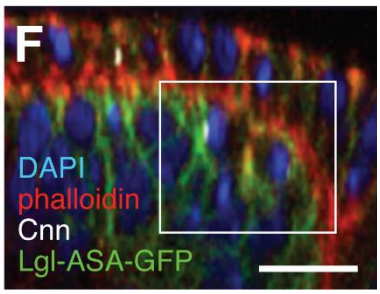

A
X
Y
Z
Y
DAPI
phallodin
tubulin
Dlg-RNAi 25° consistent
X
Z
B
X
Y
Z
Y
DAPI
phallodin
Scribble
Scrib-RNAi 25° consistent
X
Z


C
940
950
960
970
Rattus norvegicus
Gallus gallus
Anolis carolinensis
Xenopus tropicalis
Danio rerio
Stegodyphus mimosarum
Drosophila melanogaster


D
Time (min)
µcal/second
kcal mol-1 of injectant
Molar Ratio
Truncation
Mutant


D'
Time (min)
µcal/second
kcal mol-1 of injectant
Molar Ratio
Wild
Type

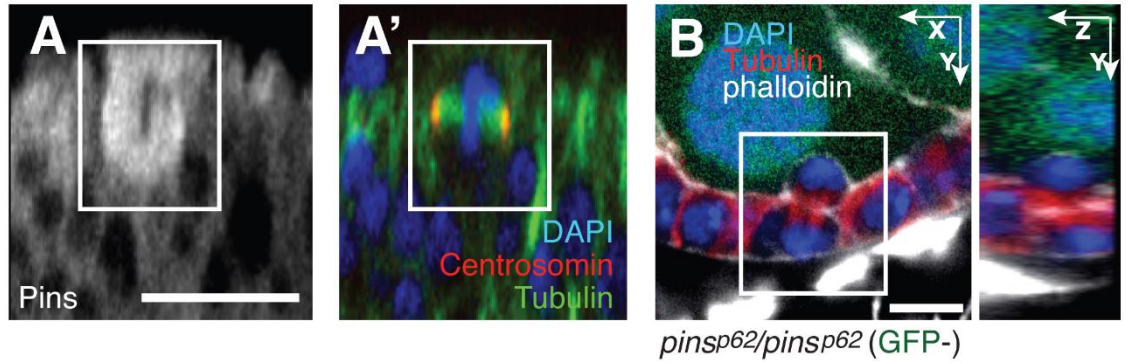

E
DAPI
Lgl
dlg18/dlg18 (RFP-)

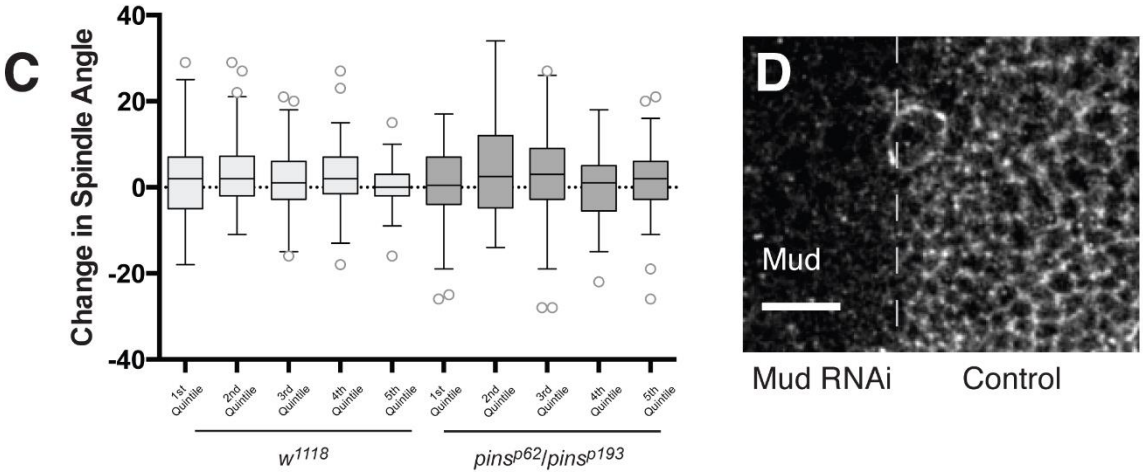

F
DAPI
phalloidin
Cnn
Lgl-ASA-GFP

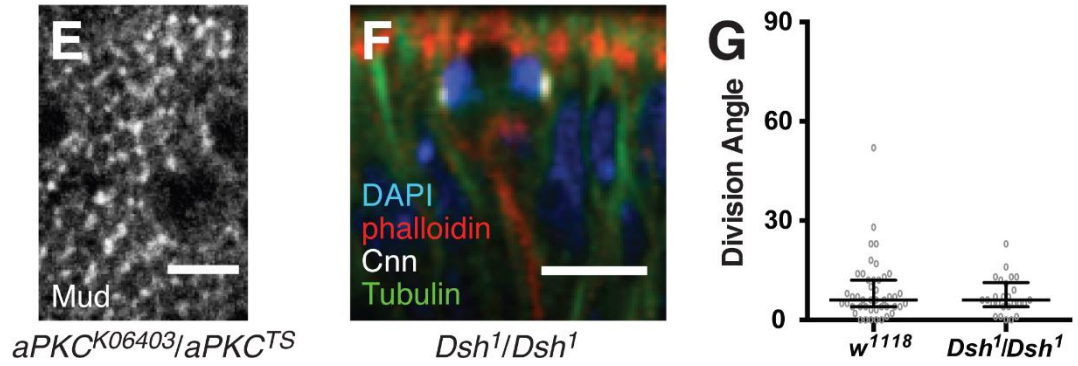
lgl4/lgl334

Supplementary Figure 3

A) A wing disc from a nubbin-Gal4 / UAS-Dlg-shRNAi larva that developed at 25° is highly disorganized. Scale bar = 100 µm. **B)** Scribble protein is not expressed in nubbin-Gal4 / UAS-Scribble-shRNAi larvae that developed at 25°. Scale bar = 100 µm. **C)** The last 43 amino acids of Discs Large (mammalian Dlg4) are conserved between arthropods and vertebrates. **D)** The truncation of 43AA from the Dlg4 GUK domain abrogates binding to phospho-Pins *in vitro.* Isothermal titration calorimetry failed to detect binding (indicated by the release of heat) between purified truncated Dlg4 and phospho-LGN. The titration was carried out at 2 min time intervals. The GUK domain is conserved between Dlg1 and Dlg4 in vertebrates, and the domains share a similar binding affinity to phospho-LGN (Zhu et al., 2011). Binding between full length GUK domain and phospho-LGN, shown in D', provides a positive control. **E)** Lgl localizes normally along the length of the lateral cortex in a dlg^{18} homozygous mutant clone. The mutant tissue is marked by the absence of RFP (here in green). The peripodial membrane is evident above the pouch. Scale bar = 10 µm. **F)** A normally-oriented division in an lgl^{4}/lgl^{334} wing disc rescued by UAS-Lgl-ASA-GFP driven by hedgehog-Gal4. Scale bar = 10 µm.







Supplementary Figure 4

A) Pins is cortically enriched in mitotic cells. Scale bar = 10 µm. **B)** Loss of Pins function causes misoriented cell divisions in the follicle epithelium. A division is shown in a $pins^{p62}$ mitotic clone, marked by the absence of RFP (in green). Scale bar = 10 µm. **C)** Side by side comparison of spindle orientation in control (light boxes – left) and $pins^{p62}/pins^{p193}$ mutant (dark boxes – right) wing discs over time. These data represent the per time point change in spindle angle, which is either towards (positive) or away from (negative) the plane of the tissue. No statistical difference was observed between any corresponding group. Boxes represent the interquartile range (IQR), with a center line at the median. The whiskers extend from the lowest point within 1.5 IQR of the lower quartile to the highest point within 1.5 IQR of the upper quartile. **D)** The anti-Mud antibody recognizes Mud in the wing disc. shRNA-mediated knockdown of Mud in the posterior compartment was driven by hedgehog-Gal4. The dashed line indicates the boundary of hh-Gal4 expression. Scale bar = 10 µm. **E)** Mud localization is normal in $aPKC^{K06403}/aPKC^{TS}$ transheterozygous mutant wing discs. Scale bar = 5 µm. **F)** A normally-oriented division in a Dsh^{1}/Dsh^{1} mitotic clone. Mutant tissue is marked by the absence of RFP (in green). **G)** Quantification of spindle angles in Dsh^{1}/Dsh^{1} mutant wing discs.

Movie 1

Spindle orientation over time. These are the same images shown as a timecourse in Figure 1A. Frames taken one minute apart. The frame rate is seven per second. Centrosomes were marked with Ubi-Cnn-RFP and tubulin with Ubi-α-Tub84B-GFP.