

## **Supplementary Fig. 1**

### **Neither mutation of Pins nor overexpression of Inscuteable cause disorganization of the follicle epithelium**

**A)** Loss of Pins function does not affect the organization of the follicle cell monolayer. *pins*<sup>p62</sup> mutant clones are marked by the absence of GFP. This is one of 83 ovarioles imaged.

**B)** Inscuteable expression promotes reorientation of cell division. The circled division is in early telophase, with the two daughters connected by a thick, central midbody. This is one of seven completely reoriented divisions imaged.

**C)** UAS-GFP is a reliable marker for Inscuteable expression. For the experiment in Fig. 1E we used the FLPout system to express UAS-Inscuteable and UAS-GFP in large clonal populations. The sample in Fig. 1E was also stained for Inscuteable, which is shown here in red. GFP is not always a reliable marker in the follicle epithelium as it can leak between sister follicle cells through somatic ring canals. However, immunoreactivity with the anti-Inscuteable antibodies overlaps substantially with the expression of UAS-GFP. This result was confirmed in six ovarioles imaged. This result show that UAS-GFP is a reliable marker for Inscuteable expression in Fig. 1F and Supplementary Video 1.

**D)** Inscuteable expressing follicle cells do not express the neuroblast marker Deadpan. Traffic-Jam Gal4 was used to drive both UAS-Inscuteable and UAS-myr.RFP in the follicle epithelium. The Deadpan antisera gives a non-specific background signal (D') that is not due to Deadpan protein as it extends into the germline, is not nuclear, and does not correlate with myr.RFP intensity (D).

**E)** Neither product of a misoriented division is apoptotic. This is one image representative of 13. A supernumerary polar cell provides a positive control for caspase-3 immunoreactivity. This was shown previously<sup>27</sup>.

**F)** Co-expression of p35 with UAS-Inscuteable does not cause tumor formation. This image is representative of 23 Stage 4-6 egg chambers.

**G)** Expression of p35 in *pins<sup>p62</sup>* mutant clones (cells lacking GFP) does not cause tumor formation. This image is representative of 12 clones < 5 cells in Stage 4-6 egg chambers. Scale bars in this figure represent 10 $\mu$ M.

## **Supplementary Fig. 2**

### **Follicle cells move relative to the layer during division**

**A)** An early stage egg chamber, imaged live, has an uneven appearance. The plasma membrane is stained with Cell Mask. This unevenness was consistently observed in over two hundred live imaging experiments using a variety of markers. This particular staining (Cell Mask alone) was performed five times.

**B)** Mitotic cell rounding is accompanied by an increase in di-phosphorylated (active) myosin regulatory light chain (Spaghetti Squash). This is one image representative of 11.

**C)** Detachment of a new daughter cell from the basement membrane (in box) is confirmed by three dimensional imaging. The lateral marker Discs large extends fully around the basal cortex of the daughter cell. Images are 20 planes spaced .5  $\mu$ m apart, collapsed to show the full diameter of the cell in all dimensions. This is one image representative of 14.

Scale bars in this figure represent 10 $\mu$ M.

## **Supplementary Fig. 3**

### **Reintegration occurs in the embryo and optic lobe**

**A)** Reintegration in the neuroepithelium of the optic lobe. These data are also presented in Fig. 3C with false coloring to indicate division products. This is one of two complete reintegrations imaged.

**B)** Quantification of Domain 11 spindle angles in wild type (n = 33 cells) or embryos expressing Inscuteable (n = 34 cells). The distribution of angles differs significantly, with a *p* value of  $1.618 \times 10^{-12}$  as determined by the Kolmogorov-Smirnov test. Spindle angles are presented as a cumulative data plot.

**C)** A transverse image of an embryo at approximately Stage 8 of development shows exogenously expressed Inscuteable (in red) localized apically. A dividing epithelial cell at telophase (within the white outline) is oriented along the apical-basal axis. This is representative of previous work published by Kraut *et al*<sup>5</sup>.

**D)** The basal product of a misoriented division in the early embryo reintegrates apically into the layer. The cell is followed in both XY and YZ planes. The dashed grey line in the first image of the YZ plane (top left) indicates the plane of focus in the XY images below. The white arrows in the YZ planes (top) point to the basal daughter cell. The arrow in the XY plane at 3' points to the first appearance of the reintegrating cell in the plane of focus. This "hole" in the layer does not become obvious again until 6', when the nucleus of the reintegrating cell moves into the focal plane.

Scale bars in this figure represent 10µM.

#### **Supplementary Fig. 4**

##### **Cell reintegration does not depend on myosin or adherens junctions**

**A)** Zipper::YFP does not show localized cortical enrichment apically or basally in a dividing cell detached from the basement membrane. The arrow points to enrichment at the contractile ring. The egg chamber was imaged live. This is one of nine divisions imaged with these markers.

**B)** A reintegrating cell can demonstrate expansion at its apical cortex. The egg chamber was imaged live using Basigin::YFP to mark cell outlines. This is one of seven such expansions observed.

**C)** The apical daughter cell of an Inscuteable-induced perpendicular division inherits the adherens junction belt (large arrows). A new adherens junction is also made between sister cells (small arrow). Gal4 activity marked with UAS-myristoylated.RFP. This is one image representative of three.

**D)** Live imaging shows that an *arm*<sup>3</sup> mutant cell can reintegrate. Mutant cells are marked by the absence of GFP. Cell outlines were marked with CellMask. This is one of two complete reintegrations imaged.

Scale bars in this figure represent 10 $\mu$ M.

### **Supplementary Fig. 5**

#### **Neuroglian and Fas2 are required for reintegration but not polarity**

**A)** Neuroglian is expressed at lateral cell-cell contacting surfaces in the embryonic ectoderm at approximately Stage 8 of development. A' is a Z-reconstruction of images taken 0.5 $\mu$ M apart. This is one image representative of 5.

**B)** Neuroglian is expressed along the lateral cortex of cells in the developing neuroepithelium. This is one image representative of eight.

**C)** *Fas2*<sup>G0336</sup> clones are Fas2 protein null as measured by antibody staining. This is one image representative of nine.

**D and E)** Epithelial cell polarity is unaffected by the *Fas2* mutation (D) or Nrg-RNAi (E), as revealed by staining for the polarity markers aPKC (apical - red) and Discs large (lateral - green). Wild type cells in (D) are marked by the absence of RFP (in gray). These images represent one of seven (D) or five (E).

**F-I)** Loss of Nrg does not alter that expression of factors that influence adhesion at the level of adherens junctions. Actin-Gal4 drives clonal expression of GFP and Nrg shRNAi. Egg chambers were stained for F) DE-Cadherin, G) Armadillo, H) Par-6, and I) Bazooka. These images represent one of six (F), four (G), six (H), and five (I).

**J)** A series of still images showing the failure of a cell expressing both Nrg-shRNA and Inscuteable to reintegrate in follicle epithelium. The cell was observed for 80 minutes total. Cell outlines were marked with CellMask. This is one of three such failed reintegrations imaged.

Scale bars in this figure represent 10 $\mu$ M.

### **Supplementary Movie 1**

#### **Reintegration of a follicle cell after a misoriented division**

GFP marks cells expressing Inscuteable. Tubulin-RFP marks the mitotic spindle. Frames taken one minute apart. The frame rate is seven per second. Each frame is a merge of 4 planes spaced  $1\mu\text{M}$  apart. The scale bar represents  $10\mu\text{M}$ .

### **Supplementary Movie 2**

#### **Reintegration in wild type follicle epithelium**

Cells marked with Basigin::YFP to reveal cell outlines and Jupiter-Cherry to reveal the mitotic spindle. Each frame is a merge of four planes spaced  $1\mu\text{M}$  apart. Frames taken one minute apart. The frame rate is seven per second. The scale bar represents  $10\mu\text{M}$ .

### **Supplementary Movie 3**

#### **Reintegration in the neuroepithelium of the optic lobe**

Inscuteable expression was induced to misorient spindles and divisions. Cell outlines were marked with Basigin::YFP. Each frame represents a Z-projection of three slices spaced one  $\mu\text{m}$  apart. The apical division product is marked with a red asterisk. After reaching the bottom of the tissue the cell again moves up and divides. Frames are two minutes apart. The frame rate is five per second. The scale bar represents  $10\mu\text{M}$ .

### **Supplementary Movie 4**

#### **A wild type follicle cell expands its apical surface as it reintegrates into the monolayer**

The other division product is not visible in this plane of focus. Cell outlines marked with CellMask. Frames taken one minute apart. The frame rate is seven per second. The scale bar represents  $10\mu\text{M}$ .

### **Supplementary Movie 5**

#### **Myosin Regulatory Light Chain is not asymmetrically localized in a wild type reintegrating follicle cell**

Myosin is labelled with Sqh-RFP. Cell outlines are marked with Basigin-YFP. Frames taken one minute apart. The frame rate is seven per second. The scale bar represents 10 $\mu$ M.

### **Supplementary Movie 6**

#### **A transient adherens junction is made by the basal daughter cell of a misoriented division**

UAS-Inscuteable expression was driven with Traffic-Jam Gal4. Frames were taken one minute apart. The frame rate is seven per second. Note that the apical daughter cell moves backwards out of the plane of focus as it reintegrates. The scale bar represents 10 $\mu$ M.

### **Supplementary Movie 7**

#### **Nrg localizes along the cortex during reintegration**

Nrg-YFP remains localized along the cortex during reintegration. Frames taken one minute apart. The frame rate is seven per second. This reintegration takes place in wild type tissue. The scale bar represents 10 $\mu$ M.

### **Supplementary Movie 8**

#### **A *Fas2*<sup>G0336</sup> mutant follicle cell expressing Inscuteable fails to reintegrate**

Mutant cells are marked by the absence of RFP in green. Cell outlines marked with CellMask. UAS-Inscuteable expression was driven with Traffic-Jam Gal4. Frames taken one minute apart. The frame rate is seven per second. The scale bar represents 10 $\mu$ M.