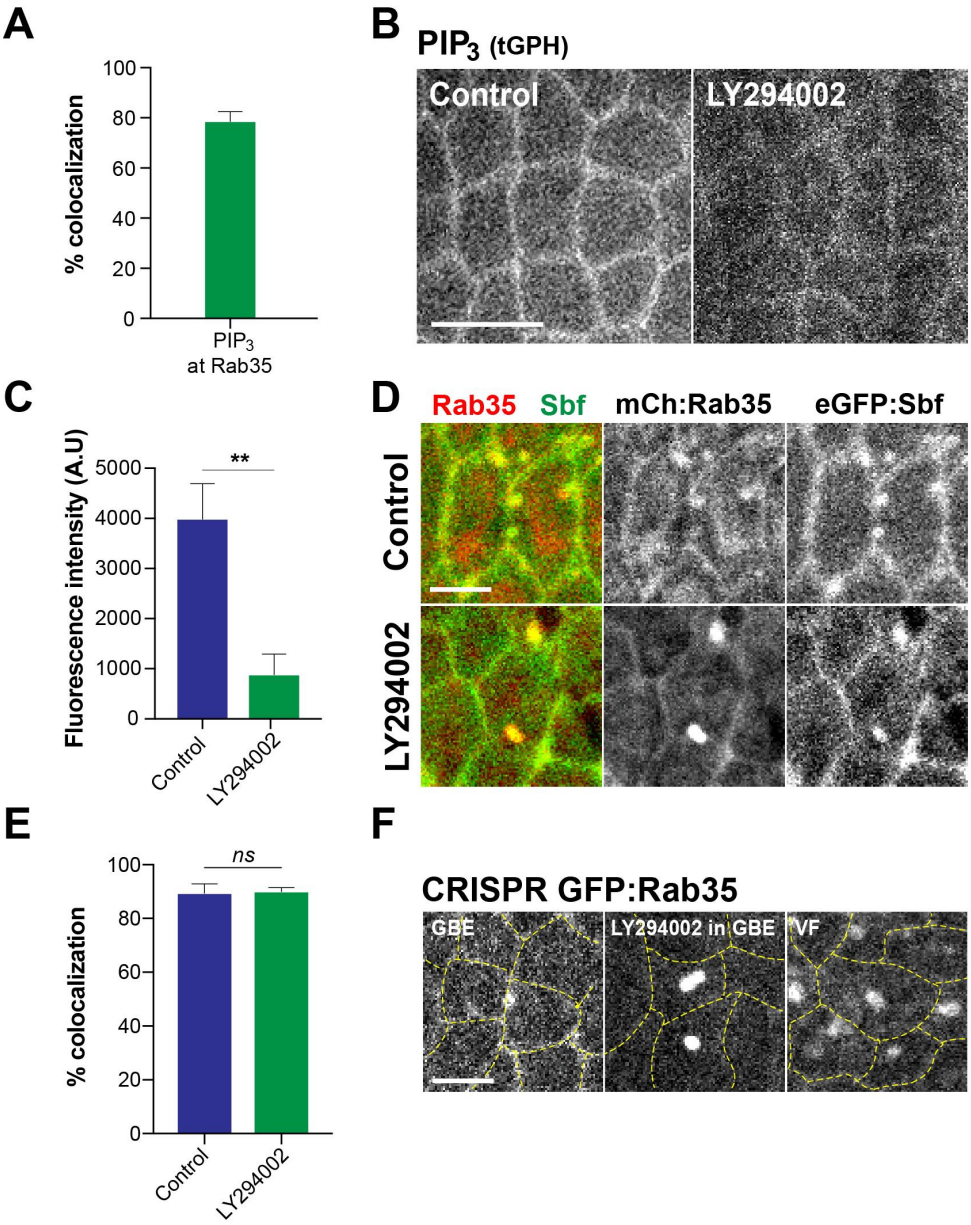
Supplemental figures and figure legends

Supplemental figure 1

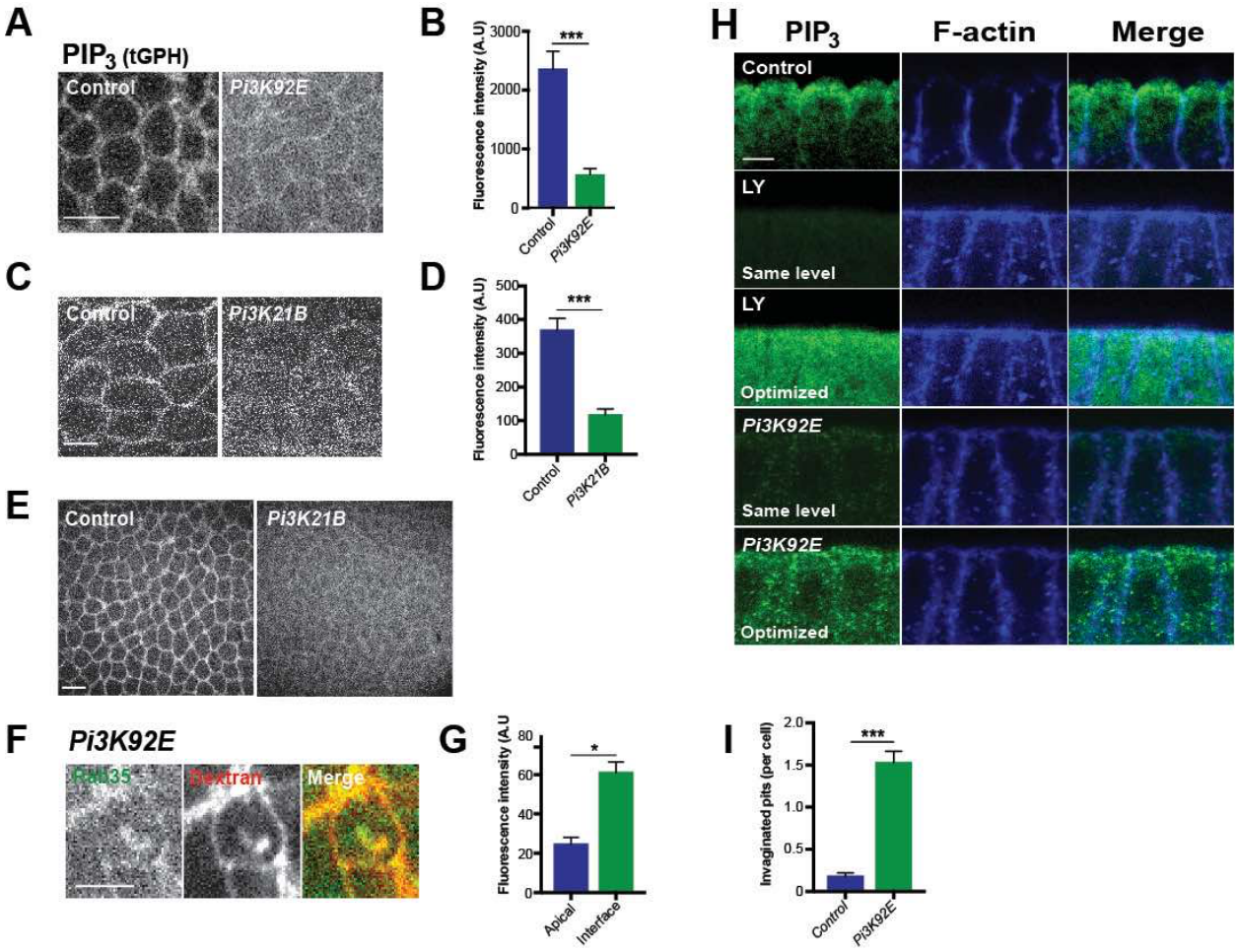


Supplemental figure 1. Disruption of *Pi3K* function using LY294002 engages Sbf/Rab35 network to apical surfaces in intercalating cells, related to Figure 1.

(A) Quantification of the percentage of Rab35 compartments which overlap with interface PIP3 compartments and vice versa. (B) Efficiency of LY294002 action on PIP_3 levels. PIP_3 GFP sensor levels (tGPH) in

control and LY294002 injected embryos. (C) Quantification of PIP_3 fluorescence intensities in control and LY294002 injected embryos. n= 134 cells from 4 embryos (control) and 112 cells from 4 embryos (LY294002). (D) Two-color live imaging of embryos expressing mCh:Rab35 and eGFP:Sbf in control and LY294002 injected embryos. (E) Percentage of colocalization between Rab35 and Sbf showed in (D). n=146 cells from 3 embryos (control) and 112 cells from 3 embryos (LY294002). (F) Comparison of apical/medial CRISPR GFP:Rab35 localization between intercalating cells injected with either control or LY294002 and constricting ventral furrow cells. Scale bars equal 5 μm. Error bars indicate measured standard error; statistical significance has been calculated using Student's t-test in (C) and (E). $^{**}P<0.005$.

Supplemental figure 2

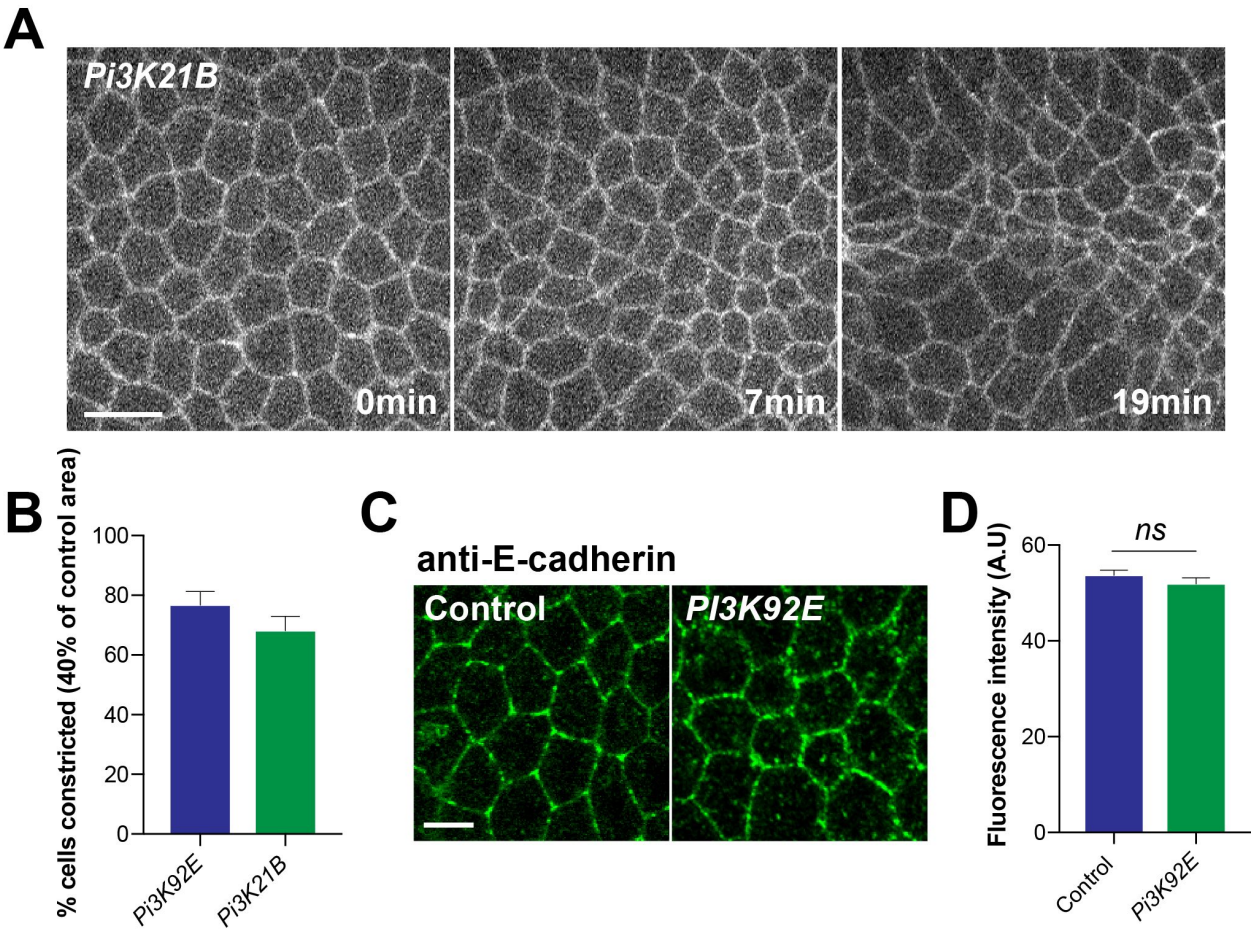


Supplemental figure 2. PIP_3 disruption levels in LY294002 injected, *Pi3K92E,* and *Pi3K21B* shRNA embryos, related to Figure 2.

(A-E) PIP_3 GFP sensor levels (tGPH) in control, *Pi3K92E,* and *Pi3K21B* shRNA embryos. (B,D) Quantification of the fluorescence intensity in control, *Pi3K92E,* and *Pi3K21B* shRNA. n= 4 embryos for each condition. (E) Lower magnification view showing uniformity of PIP_3 knockdown after *Pi3K21B* disruption. (F) Apical Rab35 compartments in *Pi3K92E* shRNA are filled with dextran immediately after dextran is injected into extracellular space. (G) Apical and junctional (the apical end of cell-cell interfaces) PIP_3 level measurements in control intercalating cells. Measurements were performed on immunostained embryos. n=48 cells from 5 embryos. (H) Immunostaining images of PIP_3 sensor, tGPH stained with anti-GFP antibody and phalloidin (F-actin) in apical-basal orientation in control, LY294002, and Pi3K92E disrupted

embryos. “Same level” images were leveled and imaged equivalently, while “optimized” images were optimized for visualization of the remaining low signal. (I) Quantification of pits number per cell in images imaged by scanning electron microscope. Scale bars in (A), (C), (E), (F), and (H) equal 5 μm. Error bars indicate measured standard error; statistical significance has been calculated using Student’s t-test. *P<0.05, ***P<0.0005.

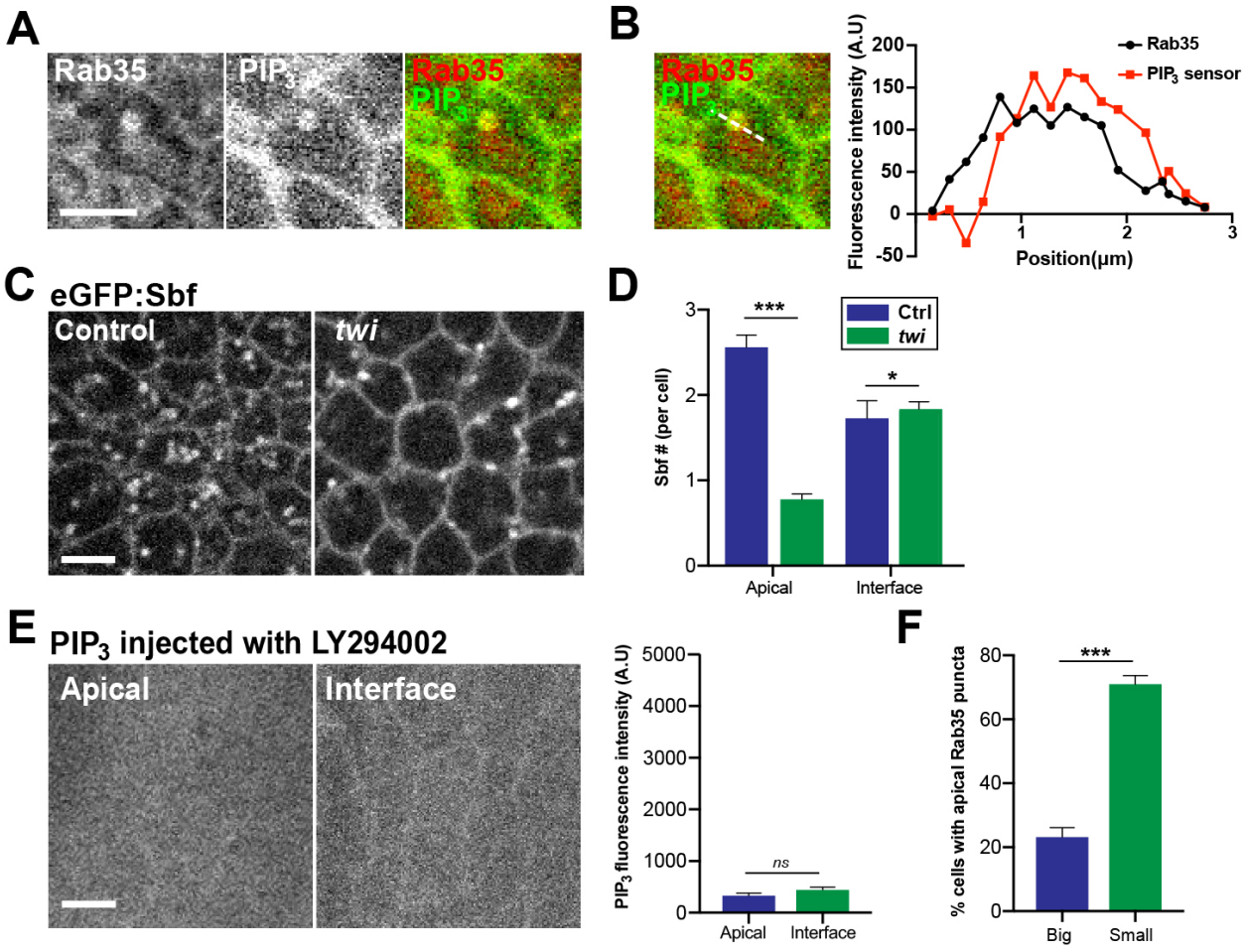
Supplemental figure 3



Supplemental figure 3. Disruption of *Pi3K21E* leads to ectopic constriction but normal E-cadherin localization, related to Figure 3.

(A) Time-lapse images of embryos expressing a cell outline marker (Spider:GFP) in the germband epithelium in *Pi3K21E* background. (B) Percentage of cells that can constrict to 40% of cell area in 13m. n= 205 cells from 3 embryos (*Pi3K92E*) and 1877 cells from 3 embryos (*Pi3K21B*). Scale bar in (A) equals 5 µm. (C) Control and *Pi3K92E* embryos fixed and stained with anti-E-cadherin. (D) Quantification of E-cad fluorescence intensity in control and *Pi3K92E* shRNA embryos. n= 101 cells from 3 embryos (control) and 99 cells from 3 embryos (*Pi3K92E*). Scale bar in (A) equals 5 µm, error bars indicate measured standard error.

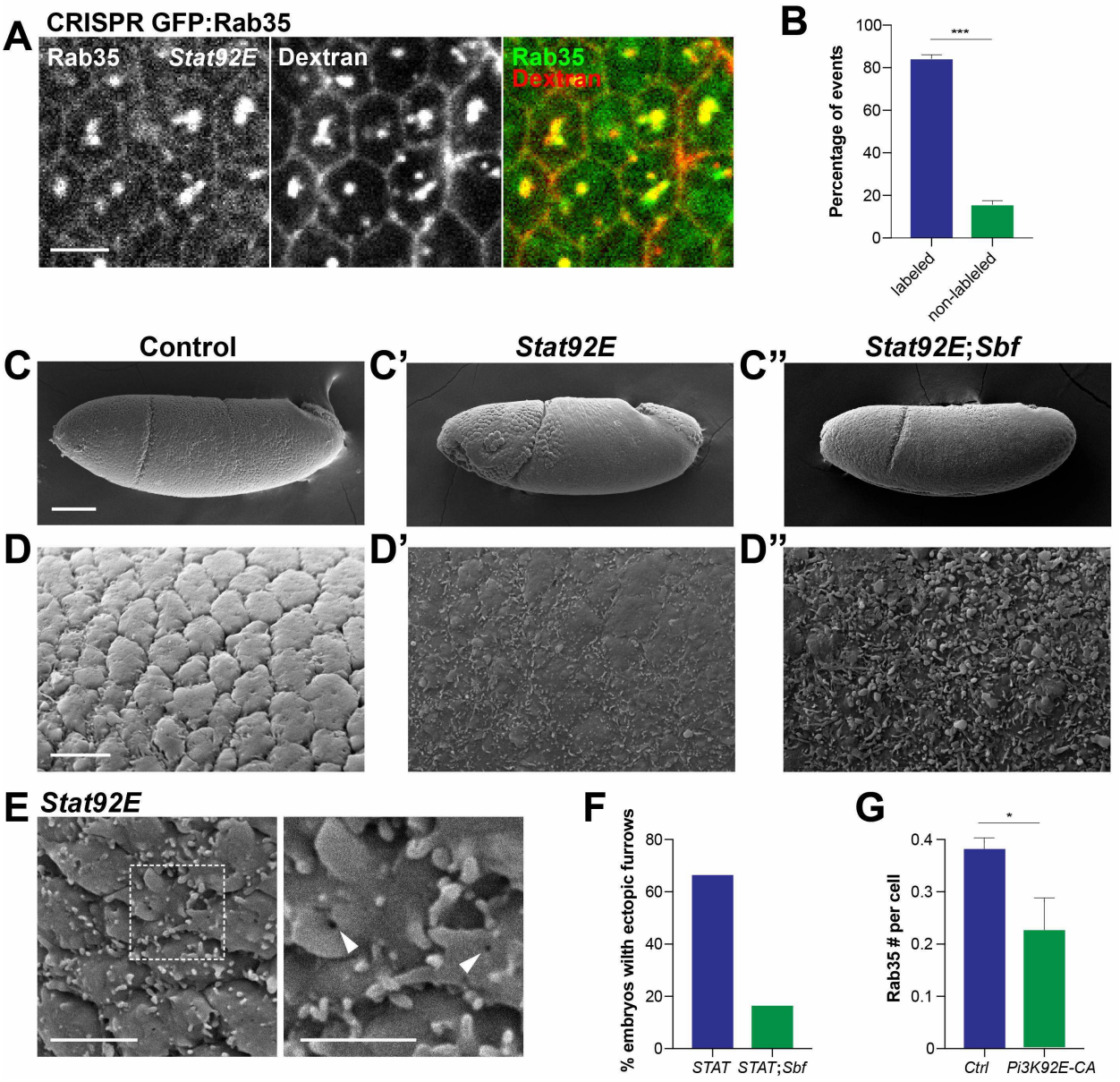
Supplemental figure 4



Supplemental figure 4. PIP_3 is enriched at Rab35 positive tubules extending from apical surfaces, related to Figure 5. (A) Two-color live imaging of embryos expressing mCh:Rab35 and tGPH during ventral furrow formation. (B) Colocalization between mCh:Rab35 and tGPH. Left panel: a representative image. Right panel: a line plot of Rab35 and PIP_3 fluorescence intensities. Dashed line represents the line along which the line-plot measurement was performed. (C) Live imaging of eGFP:Sbf in control and *twist* cells during ventral furrow formation. (D) Number of Sbf compartments at apical surfaces and cell junctions during ventral furrow formation. n=118 cells from 3 embryos (control) and 201 cells from 3 embryos (*twist*). (E) PIP_3 levels at apical surfaces and cell junctions in cells injected with LY294002. Left panel: still images of PIP_3 biosensor, tGPH. Right panel: quantitation of PIP_3 levels at apical surfaces and cells junctions. n=134 cells from 5 embryos. Scale bars equal 5 μm. (F) Percentage of "small" (area < 25 μm^2) and "big" (area >

50 μm^2) cells that contain apical Rab35 compartments in LY294002 injected embryos at 25 min. n= 101 cells from 3 embryos. Error bars indicate measured standard error; statistical significance has been calculated using Student's t-test in (E) and (F) and Mann-Whitney U-test in (D). *P<0.05, ***P<0.0005.

Supplemental figure 5

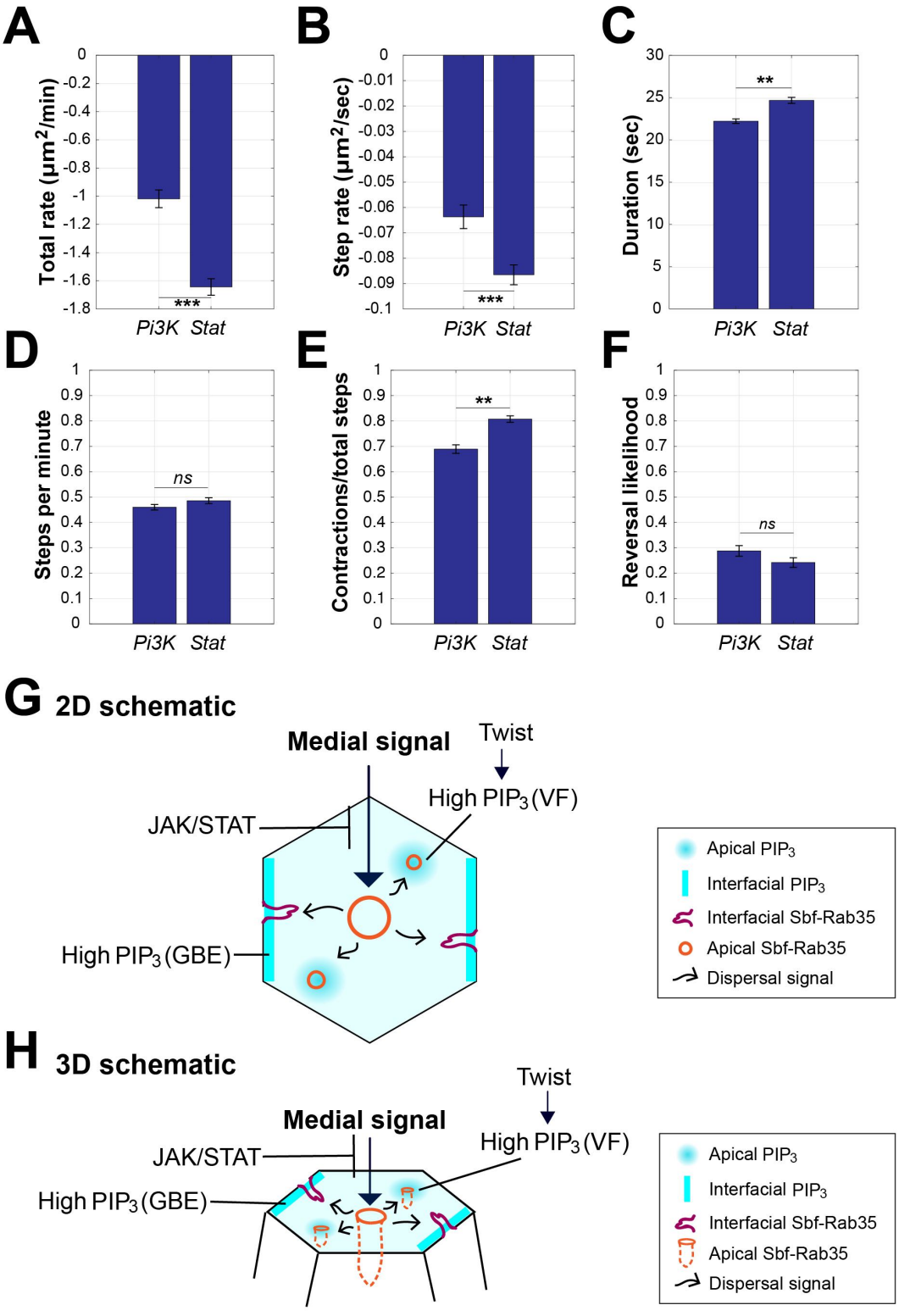


Supplemental figure 5. Ectopic furrow invagination triggered by JAK/STAT disruption requires the function of Sbf-Rab35 ratcheting pathway, related to Figure 6.

(A) Images of embryo expressing CRISPR:GFP:Rab35 in *Stat92E* shRNA injected with dextran into extra-cellular space. (B) Percentage of labeled Rab35 compartments in (A). n=108 cells. (C-D'') Representative scanning electron microscopy images of control (C,D), *Stat92E* shRNA (C',D') and *Stat92E* shRNA; *Sbf* shRNA (C'',D'') embryos in the germband epithelium. (E) SEM images of germband cells in embryos

expressing *Stat92E* shRNA. The white dashed boxes on the left panels mark the magnified regions shown on the right. Arrowheads mark invaginated pits. (F) *Sbf* disruption rescues the ectopic apical furrow induced by *Stat92E* shRNA. n= 12 embryos (*Stat*) and 7 embryos (*Stat*; *Sbf*). Scale bars in (A) and (D) equal 5 µm. (G) Number of Rab35 apical puncta in control and *Pi3K-CA* embryos (n= 20 cells each). **P*<0.05, ****P*<0.0005. Scale bar in (C) equals 50 µm. Scale bar in (E) left panel equals 5 µm and the scale bar in right panel equals 2.5 µm; error bars indicate measured standard error.

Supplemental figure 6



Supplemental figure 6. *PIP_3* disrupted cells possess different contractile stepping dynamics than those of *Jak/Stat* compromised cells, related to Figure 6.

(A) Ectopic apical constriction rates of *Pi3K92E* shRNA and *Stat92E* shRNA cells in 7.5 mins. (B,C and D) step rate (B), duration (C) and frequency (D) of MSD-defined steps in *Pi3K92E* shRNA and *Stat92E* shRNA cells. (E) Fraction of contractile steps in 7.5 mins. (F) Likelihood of contractile steps followed by the expansion steps in 7.5 mins. n= 537 cells from 3 embryos (*Pi3K*) and 397 cells from 3 embryos (*Stat*) in (A-F). (G,H) Two dimensional (G) and three dimensional (H) models of how PIP_3 and JAK/STAT pathways work as a dispersal switch to engage Sbf-Rab35 cell ratcheting at either apical or interface surfaces. A central, medial signal directs the recruitment of large, stable Sbf/Rab35 tubular compartments at the apical surface. In ventral cells, central Rab35 is dispersed to smaller, more dynamic tubules by high apical levels of PIP_3, which are regulated by the D-V patterning gene Twist. In intercalating cells, which lack Twist expression, higher levels of PIP_3 at interfaces leads to the relocalization of Sbf/Rab35 from apical-medial region to the cell junctions. JAK/STAT signaling is proposed to regulate Sbf/Rab35 membrane ratcheting activity through an inhibition of the medial signal and upregulation of contractile dynamics (not shown). Error bars indicate measured standard error; statistical significance has been calculated using Kolmogorov-Smirnov test. $^{**}P<0.005$, $^{***}P<0.0005$.

Supplemental Movie legends

Video 1. Defective Rab35 dynamics during cell intercalation when PIP_3 level is compromised, related to Figure 1. Live imaging of CRISPR GFP:Rab35; tdTomato:Gish (plasma membrane marker) in control and LY294002 injected embryos. Scale bar equals 5 μm.

Video 2. Defective Rab35 dynamics during ventral furrow formation when PIP_3 level is compromised, related to Figure 5. Live imaging of CRISPR GFP:Rab35; tdTomato:Gish (plasma membrane marker) in control and LY294002 injected embryos. Scale bar equals 5 μm.