Supplemental Information

Inventory of Supplementary Material

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Supplementary Movie M1 Junction cut in an early third instar disc, related to Fig. 1

Wing disc expressing E-cad:GFP, cut with a micropoint laser at 1.7s.

Supplementary Movie M2 Junction cut in a late third instar disc, related to Fig. 1

Wing disc expressing E-cad:GFP, cut with a micropoint laser at 2.3s.

Supplemental Figures

Supplementary Figure S1 Influence of altered myosin activity in wing discs, related to Figure 2

A-D) Examples of third instar wing discs expressing *en-Gal4* and A) control, B) *UAS-rok-RNAi,* C) *UAS-rok.CAT,* D) *UAS-sqh.EE,* stained for expression of Wg (red), with posterior cells marked by expression of GFP (green) and nuclei stained by Hoechst (blue). E) Quantitation of the relative areas of the A and P compartments of the wing pouch (defined by the inner ring of Wg expression (N=9 to 13 discs per genotype). F) Examples of third instar wing discs expressing *nub-Gal4* and *UAS-Dcr2,* stained for expression of Dcr2 (red), with nuclei stained by Hoechst (blue). G) Quantitation of relative Zip:GFP levels between P and A compartments in discs of the genotypes depicted in panels H to J (N=6 to 10 discs/genotype). H-J) Third instar wing discs expressing *en-Gal4* and H) *UAS-rok-RNAi,* I) *UAS-rok.CAT,* J) *UAS-sqh.EE,* and expressing Zip:GFP or Sqh:GFP (both show similar localization profiles), with posterior cells marked by expression of UAS-RFP or UAS-Dcr2 (blue).

Supplementary Figure S2 Cellular consequences of altered ROCK activity, related to Figure 3

A-D) Third instar wing imaginal discs expressing *en-Gal4 UAS-dcr2* and A,B) *UAS-rok-RNAi,* C) *UAS-rok.CAT,* D) *UAS-sqh.EE,* stained for *puc-lacZ* (green, marks JNK activation), cleaved caspase 3 (Cas3, red, marks apoptosis) and Dcr2 or En (blue). Panels marked prime show individual stains of discs to the left. E,F) Third instar wing discs expressing *en-Gal4 UAS-dcr2 UAS-GFP* or *UAS-RFP* (green) and E) *UAS-bsk.DN,* or F) *UAS-sqh.EE UAS-bsk.DN,* stained for ex-lacZ (magenta). Dashed yellow line marks A-P compartment boundary.

Supplementary Figure S3 *yki* interacts genetically with *rok* to modulate wing growth, related to Figure 4

A-C) Adult wings from flies expressing *nub-Gal4 UAS-dcr2* and A) yki^{b5}/+, B) *UAS-rok-RNAi,* C) *UAS-rok-RNAi* yki^{b5}/. D) Mean wing areas for the indicated genotypes (lettered according to genotypes displayed in figure panels above), calculated from 9 to 15 wings per genotype. Error bars indicate sem, statistical significance of selected pairwise combinations are indicated by colored lines (**** indicates P≤0.0001).

Supplementary Figure S4 Apical localization of Jub is promoted by cytoskeletal tension, related to Figure 5

Third instar wing imaginal discs expressing Jub:GFP (green). A-B) Early (A) and late (B) third instar wing discs, stained for expression of E-cad (red), with D-V (white) and A-P (yellow) compartment boundaries marked by arrows. Wg expression (blue) marks the D-V boundary and proximal wing. C) High magnification of *en-Gal4 UAS-dcr2 UAS-RFP* (blue) with *UAS-rok.CAT*. D) Quantitation of Jub:GFP levels in lysates of third instar wing discs from control (w-) and *nub-Gal4 UAS-sqh.EE*, normalized to ß-Tubulin levels (N= three biological replicates). E-H) *en-Gal4 UAS-dcr2 UAS-RFP* (blue), with E) *UAS-rok-RNAi*. Panels below show vertical sections. *ex-lacZ* is also shown in the vertical section, it is low in P cells here. G) *UAS-sqh.EE.* Panels below show vertical sections. *ex-lacZ* is also shown in the vertical section, it is high in P cells here. H) *UAS-ex-RNAi.* Panels below show vertical sections. I) *UAS-sqh.EE UAS-bsk-RNAi.* Panels below show vertical sections. Panels marked by prime symbols show individual stains of discs to the left, as indicated. Dashed yellow line marks the A-P compartment boundary.

Supplementary Figure S5 Influence of tension and α-catenin on apical localization of Jub, related to Figure 6

A,B,D,E) Third instar wing discs expressing Jub:GFP and E-cad:RFP, dissected and incubated for 90 min in WM1 media (control, A, D) or WM1 media + 1 mM Y-27632 (ROCK inhibitor, B,E) at 30°C. A,B show columnar epithelial cells, D,E show peripodial cells, with nuclei stained by Hoechst (blue). C) Quantitation of Jub:GFP and E-cad:RFP levels from discs cultured and imaged in parallel under identical conditions in the presence or absence of Y-27632, with the ratios normalized to the mean ratio in untreated samples (N=9). F,G) *en-Gal4 UAS-dcr2 UAS-*

RFP (blue) *UAS-α-cat-RNAi,* stained and imaged for expression of Jub:GFP (green) and α-cat or E-cad (red). Panels marked by prime symbols show individual stains of discs to the left, as indicated. Panels to the right in G (-z) show vertical sections. Yellow dashed lines mark the A-P compartment boundary. H) Co-immunoprecipitation of α-cat with Jub:GFP from third instar wing discs. Lysates were made from 200 discs with or without Jub:GFP, precipitated with anti-GFP, and blotted with anti-GFP and anti-α-cat. The upper two panels show the relative amounts in the lysates (Input), and the lower two panels show the amounts of Jub:GFP and α-cat precipitated with GFP-Trap_A beads. Some non-specific precipitation of α-cat is visible, but consistently lower than from animals expressing Jub:GFP. Quantitation is based on three biological replicates.

Supplementary Figure S6 In vivo localization of Wts, related to Fig 7

A) Wing disc expressing GFP:Wts (green), and stained for E-cad (red). B) Wing disc expressing Wts:V5 (green), and stained for E-cad (red). C) *en-Gal4 UAS-dcr2 UAS-RFP* (red) *UAS-wts-RNAi, GFP:Wts.* Depletion of GFP in posterior cells confirms the *wts*-specificity of the GFP pattern. D) Schematic of the *wts* genomic locus (http://flybase.org), with the GFP insertion site of GFP:Wts indicated by the green arrow, and the V5 insertion site of Wts:V5 indicated by the red arrow. E) Co-immunoprecipitation of Wts with Jub:GFP from third instar wing discs. Lysates were made from 200 discs with or without Jub:GFP, precipitated with anti-GFP, and blotted with anti-GFP and anti-Wts. The upper two panels show the relative amounts in the lysates (Input), and the lower two panels show the amounts of Jub:GFP and Wts precipitated with GFP-Trap_A beads. Some non-specific precipitation of Wts is visible, but consistently lower than from animals expressing Jub:GFP. Quantitation is based on three biological replicates.

F) Vertical section through wing disc expressing Jub:GFP (green), and stained for Ex (blue) and Wta:V5 (red), as indicated, white arrow points to location of a Jub-Wts puncta. G) Co-localization analysis of confocal stacks imaged for Jub:GFP, Wts:V5 and Ex, using Pearson's Correlation Coefficient with Costes thresholding (Barlow et al., 2010). Wts and Jub localization are highly correlated, whereas Wts and Ex are negatively correlated.

Supplemental Experimental Procedures

Drosophila genetics

Expression of UAS lines was achieved using *nub-Gal4* (Azpiazu and Morata, 2000) or *en-Gal4*. For analysis of wing growth, crosses were done at 25°C. Where multiple UAS lines were combined we also compared effects of adding irrelevant UAS transgenes. Female wings were mounted in Methyl salicylate:Canada Balsam and wings were photographed at the same magnification on a Zeiss Axioplan2. Wing sizes (adult wings and wing discs) were measured using ImageJ, and statistical analysis was performed using Graphpad Prism software (One-way Anova with Tukey-Kramer). For analysis of effects on gene expression and protein localization, most crosses were done at 29°C. Posterior cells were marked in *en-Gal4* experiments by expression of *UAS-GFP*, *UAS-mRFP*, *UAS-lacZ* or *UAS-dcr2*. RNAi was induced including *UAS-dcr2* (Dietzl et al., 2007), and using the following UAS-hairpin transgenes: *jub* RNAi (vdrc38442 and HMS00714), *wts* RNAi (vdrcKK106174 and GD9928), *rok* RNAi (vdrc104675 and vdrc3793), *ex RNAi* (vdrcGD22994), *α-cat* RNAi (vdrcKK107916), *bsk* RNAi (vdrcKK108156). Over-expression experiments used *UAS-bsk.DN[2]* (FBti0021048), *UAS-rok.CAT[3.1]*, *UAS-rok.CAT-KG[3]*, and *UAS-sqh.E20E21* (Winter et al., 2001) (Bertet et al.,

2009). Protein and gene localization and expression was monitored using previously characterized transgenes *ex-lacZ*, *puc-lacZ, Jub:GFP, zip:GFP, sqh:GFP, Ubi-Ecad:RFP* and *Ubi-Ecad:GFP* (Hamaratoglu et al., 2006) (Sabino et al., 2011). A genomic Myc:Wts:V5 construct (P[acman]Myc:Wts:V5, abbreviated Wts:V5) was created in a 35 kb BAC clone by recombineering and introduced into the attP2A site on the X chromosome using phiC31 integration (Feng, 2009). A genomic GFP tagged Wts was created using the MIMIC insertion MI05605 (BL#41426) (Venken et al., 2011), injected with plasmid DGRC#1298 by Rainbow transgenics. A UAS-tagBFP transgene was created by inserting tagBFP into pUAST and using P element transformation. Compartment boundaries were marked in live imaging by combining *ptc-Gal4* (BL#2017), *ap-Gal4*(BL#3041) and *UAS-tagBFP(III)*. Genetic interactions modulating wing size employed yki^{b5} or jub^{E1}, and *jub* mutant clones were induced by crossing *mRFP.nls hs-FLP FRT19A; wts:EGFP* to jub^{II} *FRT19A* (Thakur et al., 2010).

Live imaging and Laser ablation

For laser cutting of cell junctions (Farhadifar et al., 2007), wing discs were dissected from third instar larvae and cultured in WM1 (Zartman et al., 2013) in a 4-well chambered coverglass (Nunc™ Lab-Tek™ II) coated with poly-lysine. Discs were imaged every 0.2 s on a Perkin Elmer Ultraview spinning disc confocal microscope, and ablation of junctions was achieved using a Micropoint pulsed laser (Andor) at 365 nm (Fernandez-Gonzalez et al., 2009). For comparing tension in younger versus older wing discs, compartment boundaries were visualized using *ap-Gal4 ptc-Gal4 UAS-Tag:BFP*. Junctions at or near compartment boundaries are avoided and junctions perpendicular to the proximal distal axis, and at similar relative locations in older and younger discs, were compared.

To compare tensions in the posterior versus anterior compartment of wing discs with altered ROCK or Sqh activity in posterior cells, the posterior compartment was marked using *en-Gal4 UAS-RFP*. Pairs of junctions with corresponding proximal-distal locations and orientations in the posterior versus anterior compartments were ablated and the ratios of Posterior/Anterior velocities calculated.

Quantitative Image Analysis

Quantitation of in vivo expression data was performed on confocal stacks using a 3D image analysis program (Volocity). To control for alterations in tissue morphology, and cell size and shape, we used markers to define the volume to be quantified. For ex-lacZ and Yki, we used a DNA stain (Hoechst) to identify nuclei, as the ß-gal gene encoded by *ex-lacZ* has a nuclear localization signal. The mean intensity per nuclear volume within anterior-dorsal, anterior-ventral, posterior dorsal, and posterior ventral quadrants of the wing pouch was measured. These quandrants excluded nuclei at the dorsal-ventral compartment boundary (where ex-lacZ is never expressed), the A-P compartment boundary (where ex-lacZ is high even in wild-type) and the outermost edge of the wing pouch (where ex-lacZ is always high). Ratio's between mean anterior and posterior compartment staining were then determined for each disc. As dorsal and ventral staining intensities were similar, these were pooled. However, for experiments involving rok-RNAi, only intensities in the dorsal compartment were measured, because the ventral compartment appeared much smaller, and there was often JNK activation in remaining ventral cells. For Wts:GFP and Jub:GFP we used E-cadherin to define the junctional GFP expression to be quantified. Two different methods were employed, both gave similar results. In one, E-cad staining within a roughly

20 cells region of interest was used to define an object for quantitation of mean fluorescence intensities, and identically sized objects were assayed in anterior-dorsal, anterior-ventral, posterior-dorsal, and posterior-ventral compartments, and P/A ratios of staining intensity determined. Alternatively, 0.5 um radius lines were manually drawn along the visible center of E-cad staining, and line intensity profiles calculated for all fluorescent channels, and intensities along lines in different regions compared. Junctional F-actin intensity was determined using E-cad defined junctional objects as described for Jub:GFP, but as junctional myosin intensity does not overlap well with E-cad, especially under high tension, it was determined by using 0.5 um radius line intensities along the center of visible myosin:GFP. Co-localization was determined on 3D confocal stacks using Volocity software and automatic threshold settings based on Costes (Barlow et al., 2010).

Statistical tests

Statistical significance was calculated using Graphpad Prism softwear, with results generally indicated by **** ($P \leq 0.0001$), *** ($P \leq 0.001$), ** ($P \leq 0.01$), * ($P \leq 0.05$), ns ($P > 0.05$). For pairwise comparisons we used t tests and for comparisons amongst multiple samples we used one way Anova. For comparisons involving ratios, statistical tests were done on log transforms of the ratios.

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