Supplementary information

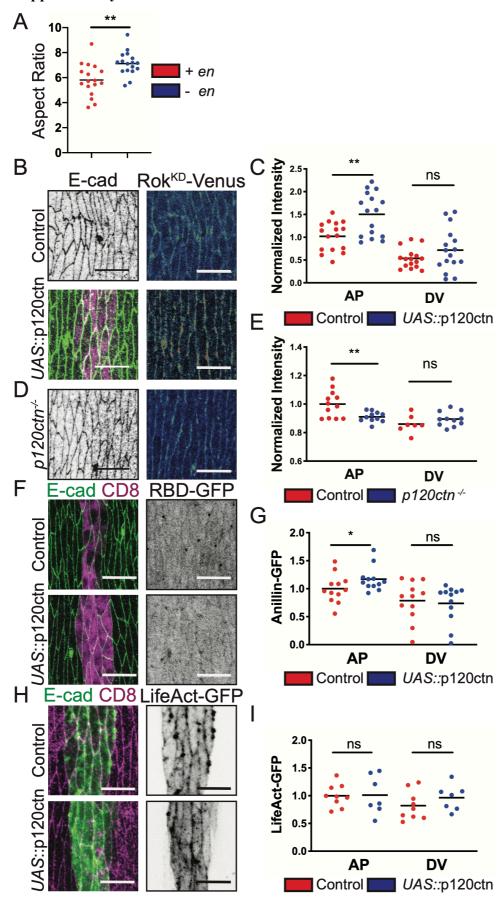


Figure S1. Morphological differences between compartments in embryonic epidermis and effects of p120ctn levels on Rok^{KD}-Venus, RDB-GFP and LifeAct-GFP.

(A) Aspect ratio of cells in the *engrailed*-positive (+*en*) and -negative (-*en*) compartments in embryonic epidermis. (**B-E**) Levels of Rok^{KD}-Venus following p120ctn level changes. (**B**) Representative examples (**B**, **D**) and levels (**C**, **E**) of Rok^{KD}-Venus in cells overexpressing *UAS*::p120ctn-GFP (**B-C**) or *p120ctn*-/- mutants (**D-E**). Cell outlines visualized with E-cad antibody (grey or green, left), Rok^{KD}-Venus visualized using rainbow intensity spectrum (right). (**F-G**) Representative images (**F**) and levels (**G**) of RBD-GFP in the epidermal cells of control and *UAS*::p120ctn-GFP expressing embryos. Cell borders were visualized with antibody against E-cad (green in **F**). (**H-I**) Representative images (**H**) and levels (**I**) of cortical actin visualized using *UAS*::LifeAct-GFP (green, left; grey, right). Cell outlines are visualized with E-cad antibody (magenta, left) Scale bars – 10 μm. Statistical analysis between cell borders measured by two-way ANOVA. *, p < 0.05, **, p < 0.01. Each dot represents an individual embryo, n number was 10-20 embryos per genotype with a minimum of 9 cells imaged per embryo.

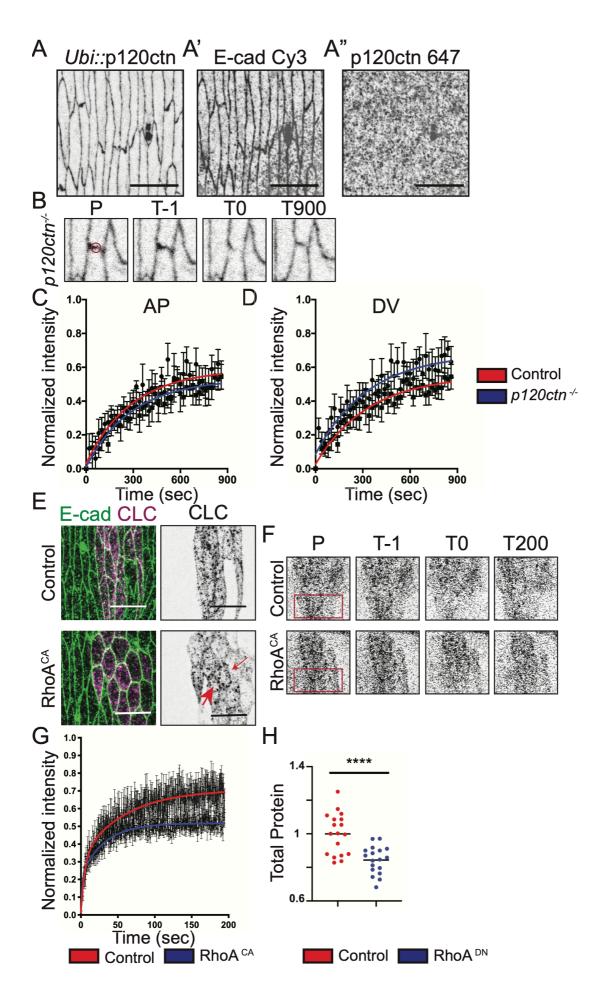


Figure S2. p120ctn antibody staining fails to replicate localisation of GFP tagged variant, while p120ctn loss leads to increase of immobile fraction of endogenously tagged E-cad-GFP and Rho regulatory construct effects the dynamics of CLC. (A) Apical view of epidermal cells of *ubi*::p120ctn-GFP expressing embryos. The localisation of the p120ctn-GFP (left) compared to the localisation of E-cad stained with an antibody (middle), and the staining pattern of the anti-p120ctn antibody in the same cells (right). (B-D) Representative examples (B) and quantification (C-D) of E-cad-GFP FRAP in p120ctn^{-/-} mutant cells. Panels in **B** show the DV cell border region bleached (Position P, red circle) at the prebleach (Time T-1), bleach (Time T0), and the end (Time T900) time points. Time is in seconds. Average recovery curves (mean \pm s.e.m.) and the best-fit curves (solid lines) are shown. All best-fit and membrane intensity data are in Table S1. (E-G). Localisation (E) and FRAP (F-G) of clathrin (UAS::CLC-GFP, grey, right; magenta, left) in cells co-expressing RhoA^{CA} (bottom) and control co-expressing UAS::CD8-Cherry (top). Cell borders are visualized by anti-E-cad antibody (green). Panels in F show the region bleached (Position P, red box) at the prebleach (Time T-1), bleach (Time T0), and the end (Time T400) time points. Time is in seconds. Average recovery curves (mean \pm s.e.m.) and the best-fit curves (solid lines) are shown in G. (H) The total levels of E-cad-GFP on the plasma membrane of control and RhoA^{DN} expressing cells. Scale bars – 10 μm. Statistical analysis was done using a two-tailed students t-test with Welch's correction. ****, p < 0.0001. Each

dot represents an individual embryo, n number was 10-20 embryos per genotype with a

minimum of 26 cells imaged per embryo. For FRAP 8-10 embryos were used.

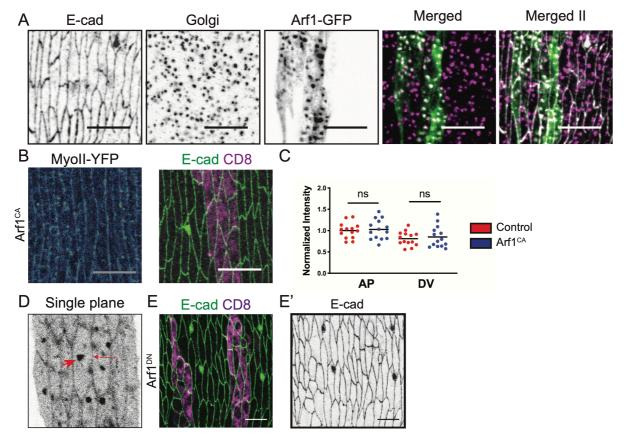
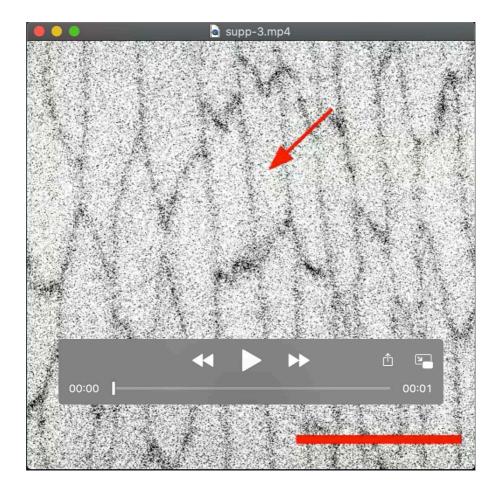


Figure S3. Arf1-GFP localization to the Golgi apparatus and plasma membrane, and effects of Arf^{CA} on MyoII-YFP, and Arf1^{DN} on cell shape.

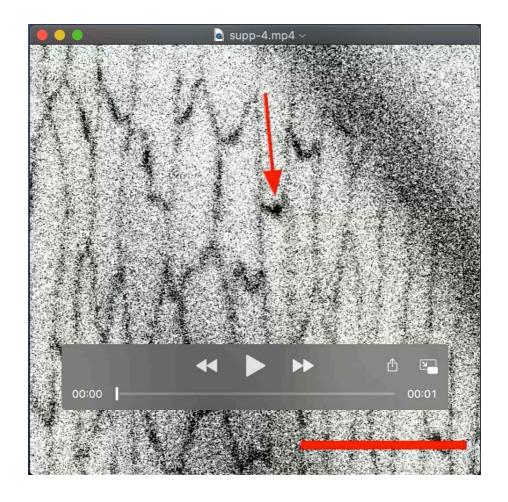
A. Apical view of the dorsolateral epidermis of *UAS*::Arf1-GFP expressing control embryo with cells borders are marked by antibody staining for E-cad (black, left; white, right), Golgi marked by immunostaining of Trans-Golgi (black, second left; magenta, two right-most), and Arf1-GFP localisation in the engrailed expressing cells (black, middle; green, two right-most). The same region is shown in main text (see Fig. 5). (**B-C**) Representative images (**B**) and levels (**C**) of MyoII-YFP (rainbow, left) in the cells expressing a constitutively active Arf1 (Arf1^{CA}). Cell borders are visualized with anti-E-cad antibody (green, right). (**D**) Single plane image of Arf1-GFP in the middle of the AJ. Large arrow indicates Golgi population and small arrow indicates plasma membrane resident population. (**I**) Low magnification image of epidermis expressing Arf1^{DN}. Scale bar is 10 μm. Statistical analysis between cell borders measured by two-way ANOVA. Each dot represents an individual embryo, n number was 10-20 embryos per genotype with a minimum of 17 cells imaged per embryo.

Table S1. Numerical values for each experiment presented in paper.

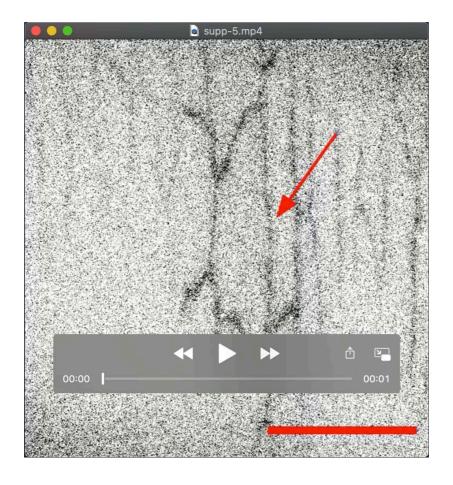
Click here to Download Table S1



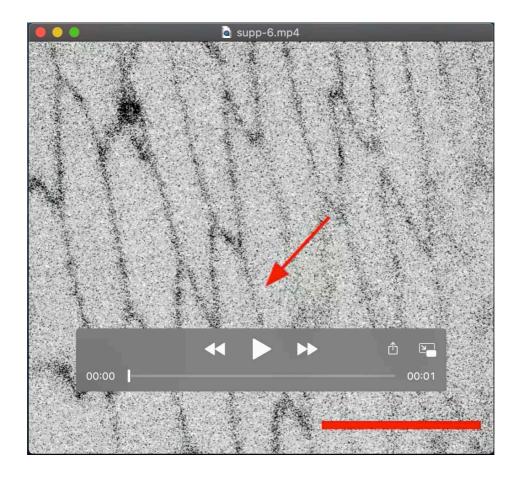
Movie 1. Laser ablation of AP cell border in control embryos. Arrow indicates the ablated junction. Scale bar $-10~\mu m$.



Movie 2. Laser ablation of DV cell border in control embryos. . Arrow indicates the ablated junction. Scale bar - 10 μm_{\odot}



Movie 3. Laser ablation of AP cell border in $\it UAS$::p120ctn expressing embryos. . Arrow indicates the ablated junction. Scale bar – 10 μm .



Movie 4. Laser ablation of AP cell border in $p120ctn^{-/-}$ mutant embryos. . Arrow indicates the ablated junction. Scale bar $-10~\mu m$.