

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Drosophila strains. *puckered*^{E69} (*puclacZ* in the text, (Martin-Blanco et al., 1998)); *Ubi-Rab5-YFP* (Zhang et al., 2007); *Tub-Rab7-GFP* (Entchev et al., 2000); *Tub-Rab11-YFP* (Classen et al., 2005); UAS-ANF (Tsarouhas et al., 2007); *crag*^{GG43} and *crag*^{CJ101} contain premature stop codons at aa 161 and 367, respectively (Denef et al., 2008). UAS-*bsk*^{K53R} (Jasper et al., 2001); UAS-*hep*^{ACT} (Weber et al., 2000); UAS-*tkv*^{Q199D} (UAS-*tkv*^{ACT} in the text, (Hoodless et al., 1996)); *Ubx-Gal4* (Herranz et al., 2006); *dpp*¹⁰⁶³⁸ (*dpp-lacZ* in the text). *bsk*², *tkv*⁷, *EP-puckered* and other stocks are described in Flybase. To distinguish hemizygous or homozygous mutant embryos from their heterozygous siblings, we made use of the balancers *FM7,ftz-lacZ*, *CyO,wg-lacZ*, *CyO,twi-GFP* and *TM3,ftz-lacZ*.

Molecular localization of M13.M2. *scarface*^{M13.M2} is a *piggyBac-lacZ* insertion (kindly provided by Michalis Averof) localized in the first intron of the *scarface* locus. The 5' genomic sequence of the insertion is TAAAGTCTCTAGAATCTGGCATGGGATATCGATGG. The *piggyBac-lacZ* reporter plasmid was constructed as follows: using the primers CGGAATTCTCAGTAAAATACAAACACA and TCCCCCGGGGAAATGGTGGCGTAT, a 2.6 Kb fragment upstream of the *nubbin* gene (-2524bp to +82bp relative to the transcription start site) containing the endogenous DPE nubbin alpha promoter was amplified, digested with *EcoRI* and *SmaI* and subcloned in the reporter construct pSLhsp43-lacZ, thereby placing the *nubbin* promoter/enhancer upstream of *lacZ* and removing the basal *hsp43* promoter. Digesting this intermediate vector with *AscI*, the fragment containing the *nubbin* enhancer/promoter, the *lacZ* reporter and the SV40 polyA was isolated and cloned in the *AscI* site of the transformation vector pBac(3xP3-EGFPaf) (Horn et al., 2003). For the injections, phsp-pBac was used as a helper plasmid (Handler and Harrell, 1999).

Mobilization of the PiggyBac insertions. *scarface*^{M13.M2} and *scarface*^{PBss} are two semi-lethal PiggyBac insertions in the *scarface* locus (Fig. 1A). Surviving flies showed scars in the head. They were mobilized with the PiggyBac transposase stock *w;Sp/CyO*, *tubPBbase*, and revertants lacking either GFP (*scarface*^{PBss}) or RFP (*scarface*^{M13.M2}) expression in the eyes were selected and stocks generated. The semi-lethality and the adult phenotype were completely rescued in these flies.

Mobilization of the P-element insertion. *scarface*^{KG05129} is a viable P element insertion carrying the *white(+)* and *yellow(+)* markers and located in the third intron of *scarface* (Fig 1A). For the generation of P-element excisions, *yw;scarface*^{KG05129} males were crossed with *yw;Sp/CyO*; $\Delta(2-3)$,*Sb/TM6,Ubx* females

carrying the source of $\Delta(2-3)$ transposase on the third chromosome. Excisions of the P element were selected by the loss of the *white(+)* and/or *yellow(+)* markers in the F1 progeny. Individual revertants were crossed with TM3/TM6B flies and balanced. One lethal insertion (*scarf*^{A1.5}) that did not complement the two semi-lethal PiggyBac insertions *scarface*^{M13.M2} and *scarface*^{PBss} was isolated. PCR analysis was performed to molecularly characterize this allele. In the *scarf*^{A1.5} excision, which lacked the *white(+)* marker, the 3' P element end (primers: 5'-ACT CCT TGC TTC TTT ACG TAG T-3' and 5'-GAA CTG TGA TCC GGA AAC CAC C-3') and exons 2 and 3 were unaffected (primers exon 2: 5'-ACT TGA ATC TTG GGC AGT TGC T-3' and 5'-CTT GAG CCC CAG ATC CAG AGA A-3'; primers exon 3: 5'-ACT CCT TGC TTC TTT ACG TAG T-3' and 5'-GAA CTG TGA TCC GGA AAC CAC C-3') and the 5' P element end was lost (primers: 5'-CAC CCA AGG CTC TGC TCC CAC AAT-3 and 5'-GTA GGG AGG TGA GGA GCA AGA C-3'). A deletion of 404 bp of the nearby 5' genomic region was observed in *scarf*^{KG05129} flies. In order to molecularly map the deletion, a PCR was performed from genomic DNA (primers: 5'-CAC CCA AGG CTC TGC TCC CAC AAT-3 and 5'-GTA GGG AGG TGA GGA GCA AGA C-3'), the PCR product was cloned in pCR-XL-TOPO and two different clones were sequenced with the T7 primer. The 5' genomic sequence of the insertion is GTGATTACGAAGTGTGTGTGTGT. This deletion was maintained and not increased in *scarf*^{A1.5} embryos (primers: 5'-TGA CTG CAT TAA GGC GCC TCT-3' and 5'-TAG CTT TGT TTG CTG CTA ATC G -3').

Generation of transgenic flies. The *UAS-scarface* transgenic lines were generated as follows: the *scarface* cDNA was cut *EcoRI-XhoI* from *pOT2-scarface* cDNA (GH05918) and cloned into *pUASf*. This construct was injected in *w*¹¹⁸ embryos. *pUAS-scarface-C-Myc* was constructed as follows: a PCR fragment was generated encoding Scarface C-terminal from the *Sall* endogenous site to the last amino acid before the stop codon adding a 3' *EcoRI* site, the PCR product was then cut *Sall-EcoRI*. The Scarface N-terminal *EcoRI-Sall* was cut from *pOT2-scarface* cDNA. The two Scarface fragments were ligated at the *Sall* site and the final product *EcoRI-EcoRI* was cloned into the *EcoRI* site of *pUASf* containing a C-Myc epitope tag and checked for orientation. *pUAS-scarface-CD2-Myc* construct was generated as follows: fragments encoding Scarface C-terminal cDNA from *Sall* to the last amino acid before the stop codon and rat CD2 cDNA amino acids 2-334 were amplified by PCR using oligonucleotides that produce a 24-bp overlapping sequence at the fusion junction. The first two PCR products were used as template to amplify the full-length fusion that was cut *Sall-EcoRI* and cloned to the *pUAS-scarface-Myc* cut *Sall-EcoRI* partial. Details of constructs are available on request.

In situ hybridization and immuno-histochemistry. *In situ* hybridization was performed as described in (Azpiazu and Frasch, 1993), and embryos were mounted in Permount (Fisher Scientific). *scarface* and *dpp*

antisense Digoxigenin-labelled RNA probes were generated as described in (Tautz and Pfeifle, 1989) using the pOT2-*scarface* cDNA (GH05918, Berkeley *Drosophila* Genome Project), and a *dpp* cDNA (kindly provided by G. Morata). For immuno-stainings, embryos and discs were fixed and stained according to standard procedures. Signal amplification protocol of *scarf*^{PBss} – GFP embryos was mediated by a rabbit biotinylated secondary antibody (Amersham, 1/500) against GFP protein, followed by a blocking period and an incubation with Streptavidin-Horse radish peroxidase for 2h (Jackson Laboratories, 1/1000), and after several washes, a 6 min incubation with Tyramide Signal Amplification-Cy2 (1/75) (PerkinElmer).

Antibodies. Rabbit anti-pMAD (1/5000, kindly provided by Ginés Morata) and rabbit anti-Perlecan (1/500) was a gift from S. Baumgartner. Rabbit anti-GFP (Invitrogen, 1/600), mouse anti-GFP (Roche, 1/600), rabbit and mouse anti-βGal (Cappel, rabbit 1/600 and mouse 1/1000), mouse anti-Myc (Santa Cruz Biotechnology) and mouse and rabbit biotinylated anti-βGal (Amersham, 1/500). Guinea-pig anti-collagen IV (1/500, kindly provided by M. Ringuette). Rabbit anti-SPARC (1:500, (Martinek et al., 2002)). Secondary antibodies were from Molecular Probes (1/500). An antibody against an internal peptide of the Scarf protein containing 139 amino acids (from amino acid 372 to amino acid 510) was generated by cloning the coding DNA fragment from pOT2-*scarface* cDNA (digested with *NcoI* and *SallI*) to the pROEX expression plasmid. Protein expression was induced in BL-21 cells, and purified for injection. Antibody was generated in rats and rabbits.

Preparation of larval cuticles. Embryos were collected overnight and aged an additional 12 hours. First instar larvae were dechorionated in commercial bleach for 3 minutes, the vitelline membrane was removed in heptano-methanol 1:1 and after three gravity washes with methanol, larvae were washed with 0.1% Triton X-100 several times (10 min each). In some cases, the vitelline membrane was not removed and first instar larvae were dechorionated in commercial bleach for 3 minutes and directly collected in 0.1% Triton X-100. Larval cuticles were mounted in Hoyer's lactic acid (1:1) and allowed to clear at 65°C for at least 24 hours.

Germ line clones of *crag*. *crag*^{GG43} *FRT19A/FM7c* females were crossed with *yw ovo*^{D1} *FRT 19A hs-FLP/Y* males and allowed to lay eggs for one day in vials. Progeny was heat-shocked twice during second and third instar stages (72-96h) during 30 min at 37°C. Resulting *crag*^{GG43} *FRT19A/yw ovoD1 FRT19A*, *HS-Flp* females were crossed with wild type males, let them laying eggs in agar plates, and cuticles of this progeny were analyzed.

Time-lapse imaging and Image Handling. Stage 11 control *ubi-Cadherin-GFP* and mutant *scarf*^{Δ1.5} *ubi-Cadherin-GFP* embryos were collected in cages on grape agar plates and yeast paste, de-chorionated in

bleach for 2 minutes, washed in 0.1% Triton X-100, aligned on an agar plate, and picked up on a glue coated coverslip (glue was lab-made by dissolving tape in Heptane), and covered with a drop of Voltalef oil 10S (VWR International). A coverslip was stuck with Silicone high vacuum grease medium (Merck) to a Leica steel slide which provided a chamber so that aligned embryos were not covered by the slide, and embryos kept developing normally. All time-lapse images were obtained with a Leica SP5 scanning laser confocal microscope with the 40X immersion objective. Embryos were allowed to develop for 12 hours and high resolution electronic images were captured approximately every 5 minutes. Z-projections of every time stack were made afterwards with the Leica LAS AF software. Movies were processed with ImageJ (NIH) and Adobe Photoshop.

Embryo staging. *w¹¹¹⁸*, *mys¹/FM7,ftz-lacZ* and *scarf^{Δ1.5}/Cyo,wg-lacZ* flies were allowed to lay eggs during 3 hours at 25 °C and let them develop during 16 hours at 18°C. Embryos were fixed and stained for the expression of β-gal. *w¹¹¹⁸*, *mys¹* and *scarf^{Δ1.5}* embryos (lacking β-gal expression) were staged following morphological criteria (Campos-Ortega and Hartenstein, 1985).

Immunoprecipitation of Scarf in S2 cells. S2 cells were transfected in 6 well plates two times each with 1 µg pMT-Gal4-VP16 and 1 µg pUAS-Scarface-C-Myc, 1 µg pUAS-Scarface-CD2-Myc or pUAS empty vector as control, using 5 µl of CellFectin (Invitrogen, Carlsbad, CA, USA) per well. Cells were recovered for 8 h after transfection and induced for 2 days with 0.7mM CuSO₄. Supernatant and cells were separated by centrifugation and cells were lysed in 500 µl of 5mM Tris, 150mM NaCl and 1% Triton X-100 (pH 8). Supernatants and extracts from lysed cells were divided in two sets each. One set was immunoprecipitated with mouse anti-Myc antibody and the other set was immuno-precipitated with mouse anti-Actin antibody both overnight at 4°C. A 50 µl portion of protein G slurry was added to each tube for 30 min at 4°C. The beads were washed three times with PBS and 0.1% Triton X-100 and then boiled in 50 µl of 2x SDS-PAGE loading buffer, one-third was loaded on SDS-PAGE and blotted to Nitrocellulose membrane. Blot from IP: anti-Myc samples were probed with rat anti-Scarface antibody and blot from IP: anti-Actin samples were probed with mouse anti-Actin antibody. All buffers were supplemented with protease inhibitors (Boehringer Ingelheim GmbH, Ingelheim, Germany).

SUPPLEMENTARY FIGURES

Figure S1. (A) Lateral and ventral view of *Ubx-Gal4:UAS-lacZ* embryos at stages 12 (left panel) and 14 (right panel) labelled to visualize β -Gal protein (brown) expression. (B-D) Distribution of *scarf* transcripts in *Ubx-Gal4:UAS-Hep^{Act}* (B), *Ubx-Gal4:EP-puc* (C), and *Ubx-Gal4:UAS- Bsk^{DN}* (D) embryos at stages 13-14. Magnification of the boxed tissue is shown in the bottom panels. In all panels a lateral view of the embryos is shown. Note ectopic expression of *scarf* in the *Ubx* domain in (B), and loss of *scarf* expression in the *Ubx* domain in (C) and (D). (E) Lateral view of a *scarface^{PBss}/dpp-lacZ* embryo at stages 13 labelled to visualize GFP (green) and β -Gal protein expression (red) in LE cells. (F, G) Dorsal views of *tkv⁷/CyO,wg-lacZ* (F) and *tkv⁷* (G) embryos at stages 12-13 showing the distribution of *scarf* transcripts and labelled to visualize β -Gal (brown) protein expression. Note expression of *scarf* is not affected in the absence of *tkv* activity. (H) Distribution of *scarf* transcripts in *Ubx-Gal4:UAS-tkv^{Act}* in stages 12-13 embryos. Note expression of *scarf* is not affected. Bars = 25 μ m.

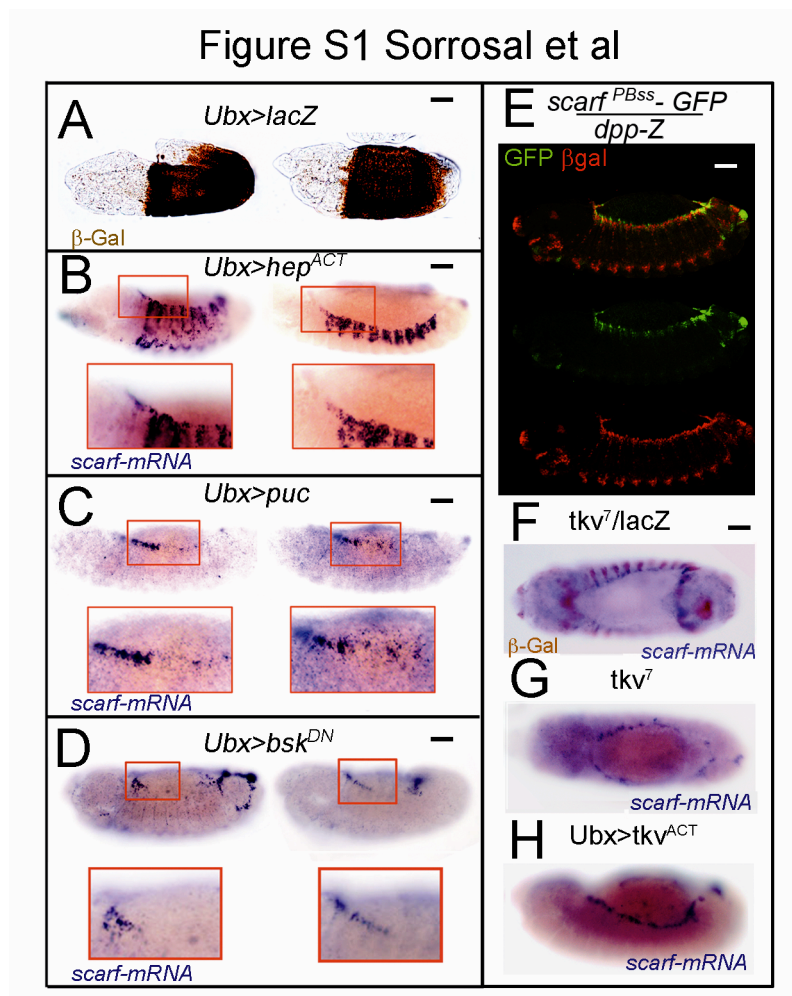


Figure S2. (A) Dorso-lateral view of a *scarf*^{Δ1.5}/*CyO,wg-lacZ* stage 13 embryo showing distribution of *scarf* transcripts (blue) in LE cells and anti β-Gal protein expression (brown). (B) Lateral view of a *scarf*^{Δ1.5} stage 14 embryo showing absence of *scarf* transcripts and absence of β-Gal protein expression (brown). Bar = 25 μm.

Figure S2

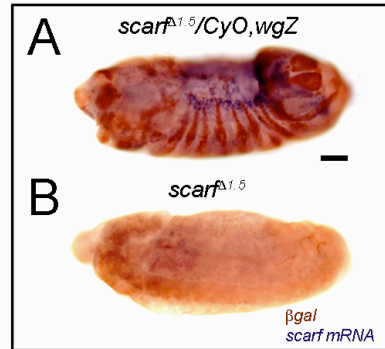


Figure S3. (A) Live image sequences of a developing *ubi-Cad-GFP scarf*^{Δ1.5} embryo during germ band retraction. Expression of Cad-GFP is shown in white. After 7 hours of development, the mutant embryo has not managed to retract while *wild-type* embryos retract in 2-3 hours approximately (see Fig. 3). Posterior tip of the embryo is marked by a red arrowhead. The movie is shown as supplementary material. Bar = 25 μm.

Figure S3 Sorrosal et al

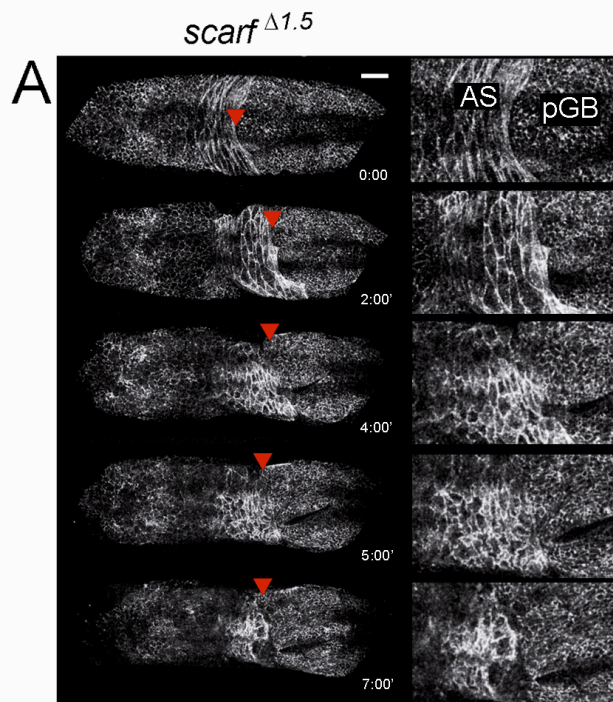


Figure S4. (A, B) Dorsal (A) and lateral (B) views of *Df(2R)nap14/CyO,wglacZ* (A) and *Df(2R)nap14* (B) embryos at stages 12-13 showing *dpp* mRNA distribution, also labeled with β -Gal (brown) to distinguish balanced embryos. (C) Dorsal view of a *Df(2R)nap14;puc^{E69}(lacZ)/+* mutant embryo labeled to visualize β -Gal (red) protein expression. Note that *dpp* and *puc-lacZ* expression levels at the LE were not affected in mutant embryos, indicating that the activity of JNK is not affected. (D, E) Lateral views of *wild type* (D) and *Df(2R)nap14* homozygous mutant embryos (E) labeled to visualize the phosphorylated form of Mad (pMAD, in red) to monitor Dpp signaling activity. Note that pMAD protein levels at the LE and throughout the lateral ectoderm (white arrows) were not affected in the absence of *scarf*. Bars = 25 μ m.

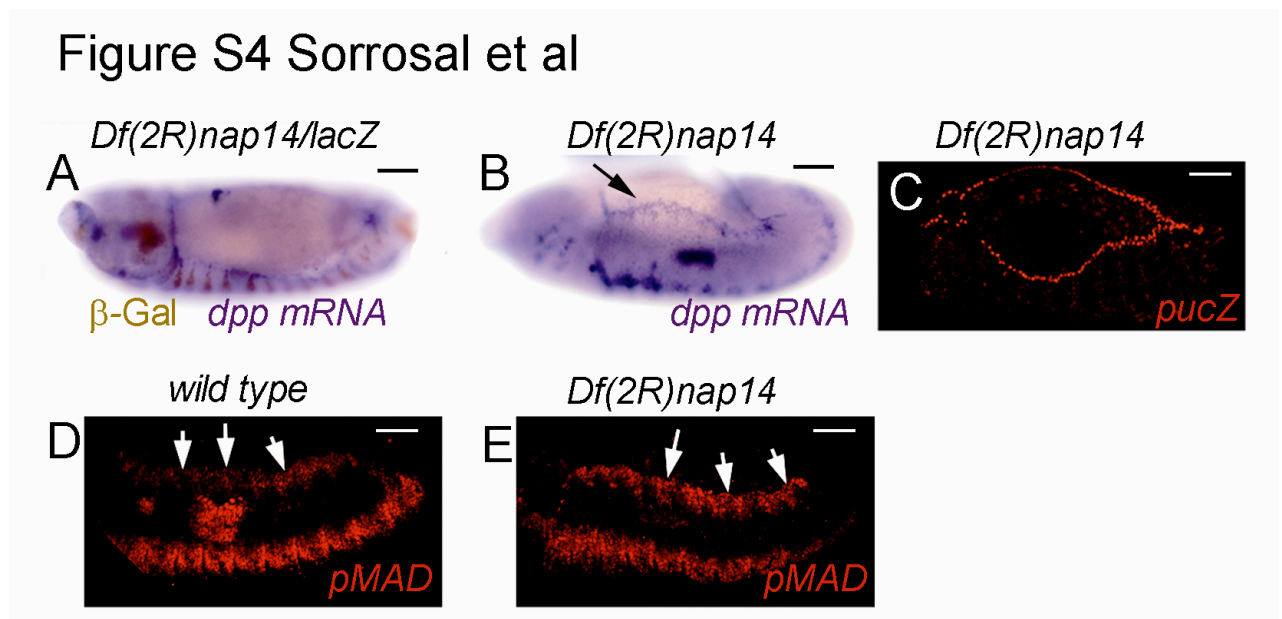


Figure S5 (A) Dorsal view of an *en-Gal4: UAS-GFP: UAS-Hep^{act}* embryo at stage 13, labelled to visualize GFP (green) and β PS Integrin (red) protein expression. Note that β PS is ectopically expressed in the *en* domain. (B) Dorsal view of a *wild type* embryo at stage 14, labelled to visualize β PS Integrin protein expression. (C) Dorsal view of an *en-Gal4: UAS-GFP: UAS-scarf* embryo at stage 14, labelled to visualize GFP (green), β PS (red) and Scarf (blue) protein expression. Note that Scarf is overexpressed in the *en* domain, but β PS is not ectopically induced. Magnifications can be observed in the right column. Bars = 25 μ m.

Figure S5 Sorrosal et al

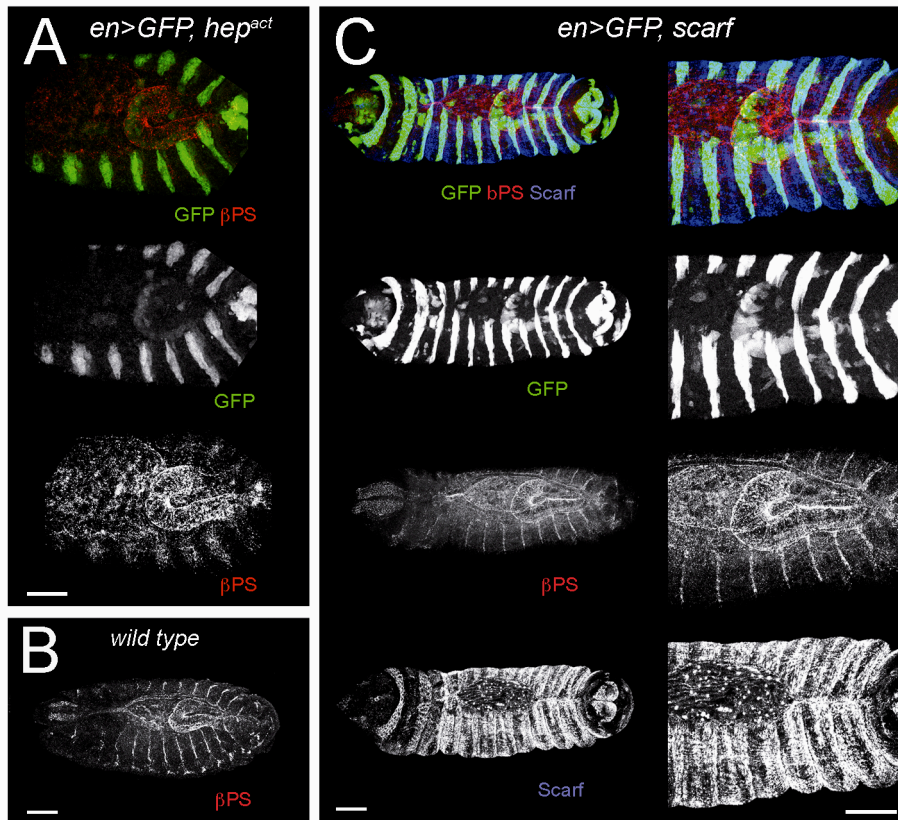


Figure S6 (A) Cross-sectional view of *scarf*^{PBss} embryo at stage 14 labeled to visualize E-cadherin (E-cad, blue), DAPI (red) and Laminin A (LanA, green) protein expression. Note LanA is mislocalized to the apical side of the AS and lateral ectoderm in both cases, and in addition, detachment between AS tissue and yolk cell can be observed. (B-E) Cross-sectional views of *wild type* (B), *scarf*^{Δ1.5} (C), *scarf*^{Δ1.5}; *arm-Gal4:UAS-scarf* (D) and *crag*^{GG43} (E) embryos at stages 12-14 showing localization of β-PS integrin (red), E-cadherin (E-cad, blue) or DAPI (blue, in C) and Laminin A (LanA, green) proteins in the lateral ectoderm (Lat-E) of the embryo. Note LanA is mislocalized to the apical side of the lateral ectoderm in (A) and (C), and it becomes well localized again to the basal side in the rescue experiment (D). The apical (ap) and basal (bs) sides of the lateral epithelium are marked in B. (F, G) Cross-sectional views of *scarf*^{Δ1.5} (F) and *scarf*^{Δ1.5}; *en-Gal4:UAS-scarf* (G) embryos at stages 12-14 showing localization of phospho-tyrosine (P-Tyr, red in F), β-PS integrin (red in G), DAPI (blue, in F), and Laminin A (LanA, green) proteins in the AS. Note in F that LanA is localized on the apical side of the AS, LanA protein levels are strongly reduced at the BM and the AS epithelium has lost its integrity. Note in G that LanA levels at the BM and epithelial integrity are largely rescued by expression of Scarf in the lateral ectoderm. Bars = 25 μm.

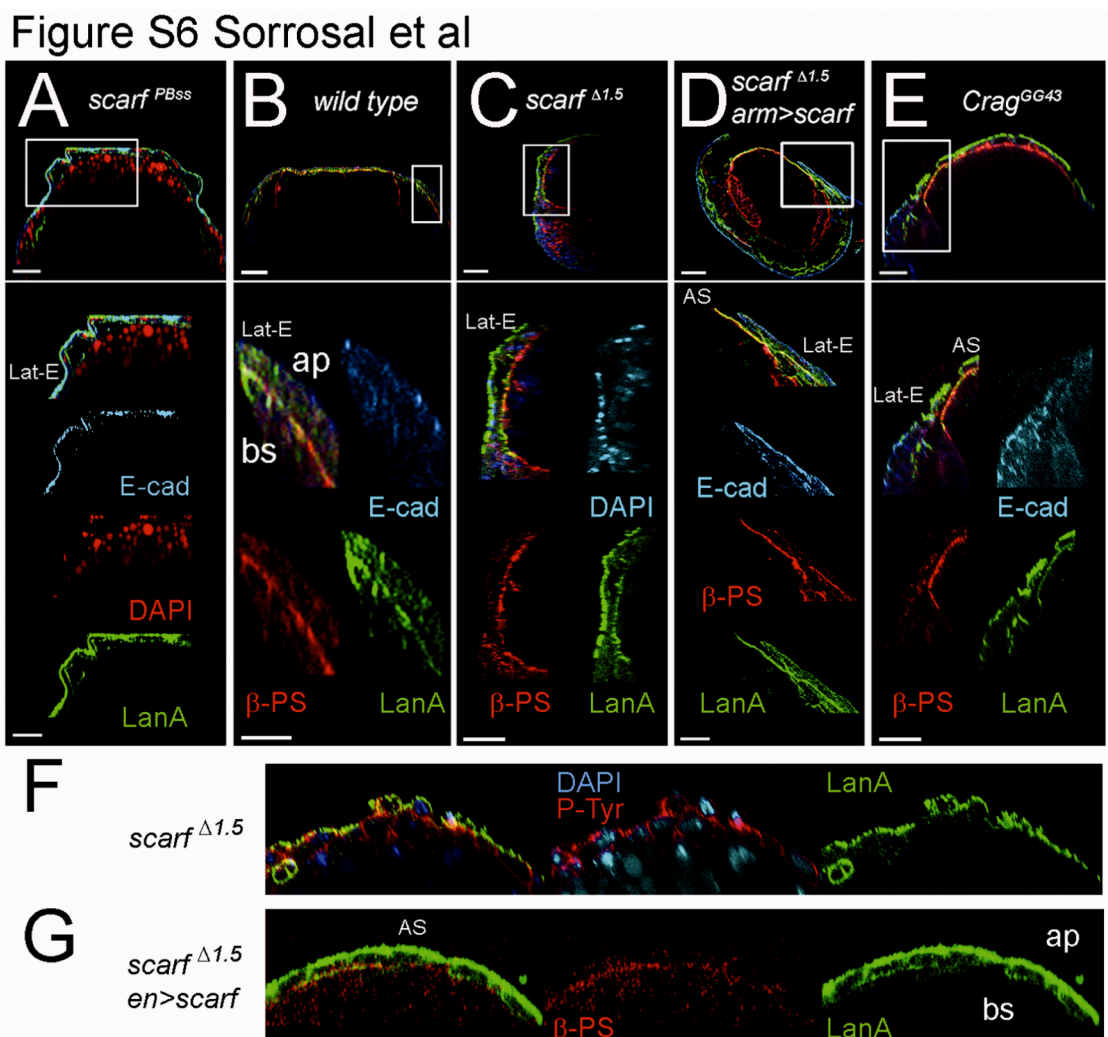
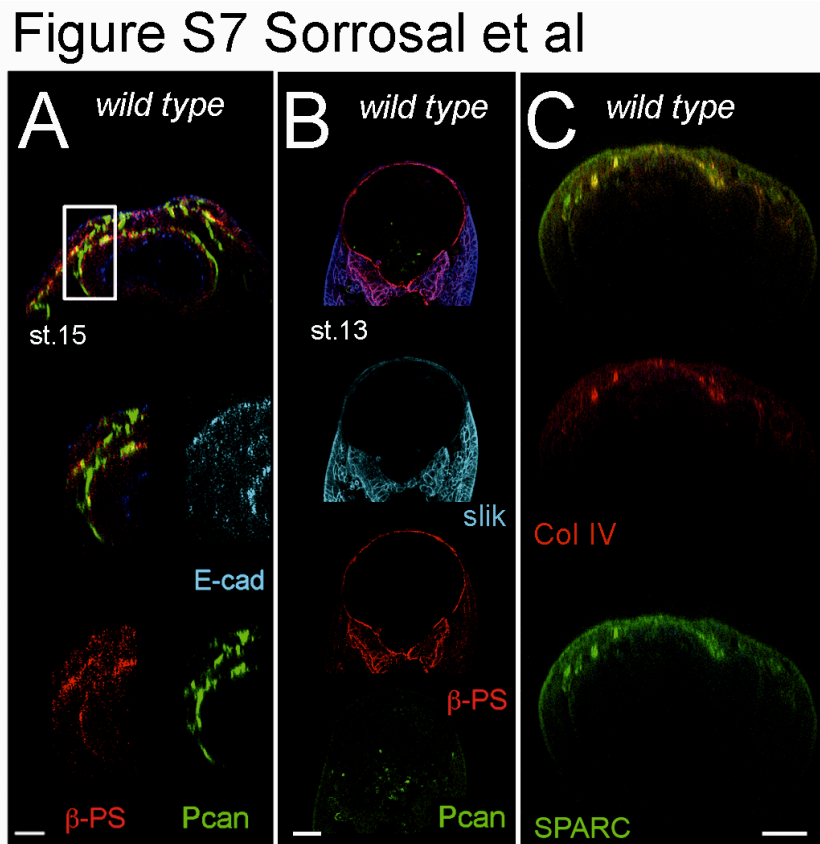


Figure S7 (A) Cross-sectional view of a *wild type* stage 15 embryo labeled to visualize Perlecan (Pcan, green), E-cadherin (Ecad, blue) and β -PS Integrin (β -PS, red) protein expression. (B,C) Cross-sectional views of wild type stage 13 embryos labeled to visualize Perlecan (Pcan, green, B), Slik (blue, B), β -PS Integrin (β -PS, red, B), Collagen IV (red, C) and SPARC (green, C). ap, apical; bs, basal. Bars = 25 μ m.



SUPPLEMENTARY MOVIES

Movie S1. Germ band retraction and dorsal closure of an *ubi-CadherinGFP* embryo. Acquisition time is shown at the lower right corner of this and all following movies. Time resolution: between 7 and 12 hours.

Movies S2-S5. Failures in germ band retraction and dorsal closure in *scarf ^{Δ 1.5} ubi-CadherinGFP* embryos. Embryos are not able to normally complete germ-band retraction (Movies S2 and S3), AS cells disappear before completion of germ-band retraction (Movie S3), the embryo retracts and manages to close but it reopens again (Movie S4), or AS cells detach from LE cells and also AS cells detach from each other (Movie S5). Time resolution: between 7 and 12 hours.

SUPPLEMENTARY REFERENCES

- Goldstein, R.E., Jiménez, G., Cook, O., Gur, D. and Paroush, Z. (1999) Hucklebein repressor activity in *Drosophila* terminal patterning is mediated by Groucho *Development* **126**, 3747-3755
- Azpiazú, N. and Frasch, M. (1993) tinman and bagpipe: two homeo box genes that determine cell fates in the dorsal mesoderm of *Drosophila*. *Genes Dev*, **7**, 1325-1340.
- Campos-Ortega, J.A. and Hartenstein, V. (1985) *The embryonic development of Drosophila melanogaster*. Springer Verlag, Berlin.
- Classen, A.K., Anderson, K.I., Marois, E. and Eaton, S. (2005) Hexagonal packing of *Drosophila* wing epithelial cells by the planar cell polarity pathway. *Dev Cell*, **9**, 805-817.
- Denef, N., Chen, Y., Weeks, S.D., Barcelo, G. and Schupbach, T. (2008) Crag regulates epithelial architecture and polarized deposition of basement membrane proteins in *Drosophila*. *Dev Cell*, **14**, 354-364.
- Entchev, E.V., Schwabedissen, A. and Gonzalez-Gaitan, M. (2000) Gradient formation of the TGF-beta homolog Dpp. *Cell*, **103**, 981-991.
- Handler, A.M. and Harrell, R.A., 2nd. (1999) Germline transformation of *Drosophila melanogaster* with the piggyBac transposon vector. *Insect Mol Biol*, **8**, 449-457.
- Herranz, H., Morata, G. and Milan, M. (2006) calderon encodes an organic cation transporter of the major facilitator superfamily required for cell growth and proliferation of *Drosophila* tissues. *Development*, **133**, 2617-2625.
- Hoodless, P.A., Haerry, T., Abdollah, S., Stapleton, M., O'Connor, M.B., Attisano, L. and Wrana, J.L. (1996) MADR1, a MAD-related protein that functions in BMP2 signaling pathways. *Cell*, **85**, 489-500.
- Horn, C., Offen, N., Nystedt, S., Hacker, U. and Wimmer, E.A. (2003) piggyBac-based insertional mutagenesis and enhancer detection as a tool for functional insect genomics. *Genetics*, **163**, 647-661.
- Jasper, H., Benes, V., Schwager, C., Sauer, S., Clauder-Munster, S., Ansorge, W. and Bohmann, D. (2001) The genomic response of the *Drosophila* embryo to JNK signaling. *Dev Cell*, **1**, 579-586.
- Martin-Blanco, E., Gampel, A., Ring, J., Virdee, K., Kirov, N., Tolkovsky, A.M. and Martinez-Arias, A. (1998) puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in *Drosophila*. *Genes Dev*, **12**, 557-570.
- Martinek, N., Zou, R., Berg, M., Sodek, J. and Ringuelette, M. (2002) Evolutionary conservation and association of SPARC with the basal lamina in *Drosophila*. *Dev Genes Evol*, **212**, 124-133.
- Tautz, D. and Pfeifle, C. (1989) A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene hunchback. *Chromosoma*, **98**, 81-85.
- Weber, U., Paricio, N. and Mlodzik, M. (2000) Jun mediates Frizzled-induced R3/R4 cell fate distinction and planar polarity determination in the *Drosophila* eye. *Development*, **127**, 3619-3629.
- Zhang, J., Schulze, K.L., Hiesinger, P.R., Suyama, K., Wang, S., Fish, M., Acar, M., Hoskins, R.A., Bellen, H.J. and Scott, M.P. (2007) Thirty-one flavors of *Drosophila* rab proteins. *Genetics*, **176**, 1307-1322.

Table S1. Penetrance (expressed in percentage) of cuticle phenotypes of different mutant conditions. Number of cuticles scored: $n(\text{scarf}^{\Delta 1.5}) = 728$; $n(\text{scarf}^{\Delta 1.5}, \text{arm} > \text{scarf}) = 148$; $n(\text{scarf}^{\Delta 1.5}, \text{en} > \text{scarf}) = 141$; $n(\text{scarf}^{\Delta 1.5}, \text{puc} > \text{scarf}) = 601$; $n(\text{Df}(2R)\text{nap}14) = 218$; $n(\text{mys}^1) = 707$; $n(\text{mys}^1; \text{scarf}^{\Delta 1.5}/+) = 345$; $n(\text{lanA}^{\text{MB01129}}) = 563$; $n(\text{mys}^1; \text{lanA}^{\text{MB01129}}/+) = 475$; $n(\text{mys}^1; \text{Df}(2L)\text{el81i1}(\text{wb})/+) = 523$; $n(\text{crag}^{\text{GG43}}) = 540$; $n(\text{crag}^{\text{GG43}}, \text{scarf}^{\Delta 1.5}/+) = 243$; $n(\text{crag}^{\text{CJ101}}) = 693$; $n(\text{crag}^{\text{CJ101}}, \text{scarf}^{\Delta 1.5}/+) = 270$.

	wild type	dorsally wrinkled	dorsal hole	germ band retraction defects	undifferentiated cuticle
<i>scarf</i>^{Δ1.5}	0	25,7	11,7	16,9	45,6
<i>scarf</i>^{Δ1.5}; <i>arm</i> > <i>scarf</i>	49,5	2	14,1	22,2	12,3
<i>scarf</i>^{Δ1.5}; <i>en</i> > <i>scarf</i>	45	4,2	10,5	21,3	19,2
<i>scarf</i>^{Δ1.5}; <i>puc</i> > <i>scarf</i>	22,2	19,8	19,2	16,8	22,1
<i>Df</i>(2R)<i>nap</i>¹⁴	0	57,5	10,3	6,4	25,7
<i>mys</i>¹	30,4	12,8	2,2	54,8	0
<i>mys</i>¹; <i>scarf</i>^{Δ1.5}/+	0	16,9	1,4	32,6	49
<i>lanA</i>^{MB01129}	95,5	4,2	0	0	0,54
<i>mys</i>¹; <i>lanA</i>^{MB01129}/+	0	27	3,4	71,58	0
<i>mys</i>¹; <i>Df</i>(2L)<i>el81i1</i>(wb)/+	6,4	21,6	0	72	0
<i>crag</i>^{GG43}	89,6	8,16	0	2,26	0
<i>crag</i>^{GG43}; <i>scarf</i>^{Δ1.5}/+	27,6	13,2	1,6	18	39,6
<i>crag</i>^{CJ101}	76,8	13,2	6,8	0,56	2,3
<i>crag</i>^{CJ101}; <i>scarf</i>^{Δ1.5}/+	0	9,2	2,3	4,5	84

Table S2. Developmental delay observed in *mys* and *scarf* mutant embryos. Percentage of embryos in the corresponding embryonic stages after 16 h of development at 18°C.

	Stage 10-11	Stage 12	Stage 13	Stage 14	Stage 15-16
<i>w¹¹¹⁸</i> (n=247)	18,4	20,5	28,9	7,1	25
<i>mys¹</i> (n= 138)	39,1	25	47,7	6,8	7,7
<i>scarf^{A1.5}</i> (n=120)	43,1	49,8	7,8	0	0