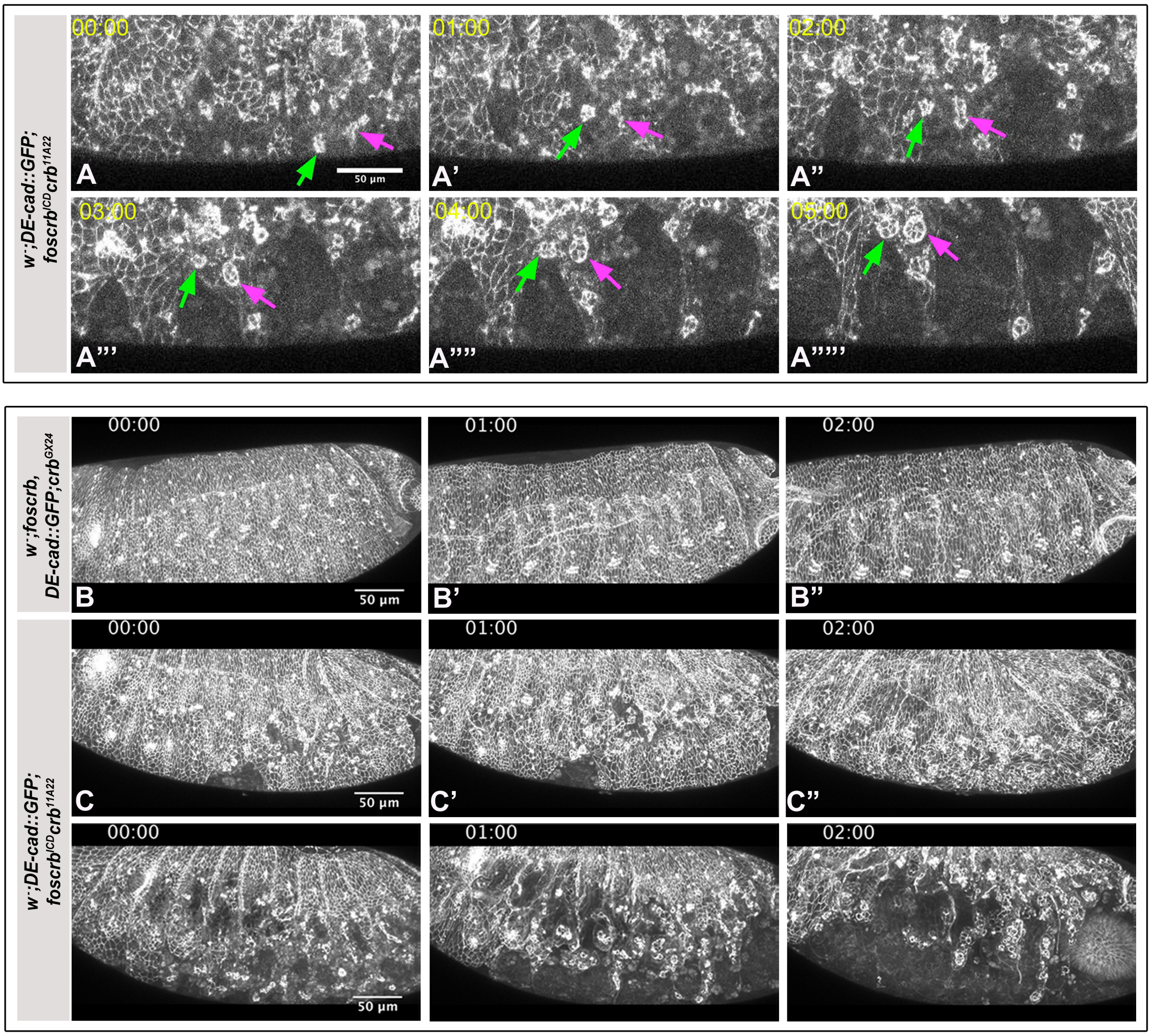
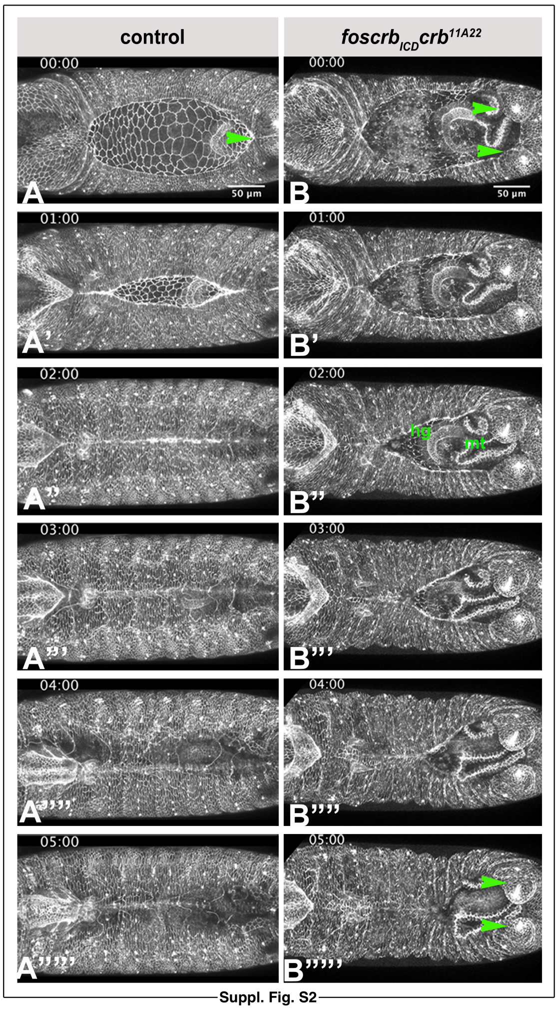
Supplementary figures and movies



Supplementary Figure S1. (For Fig.2)

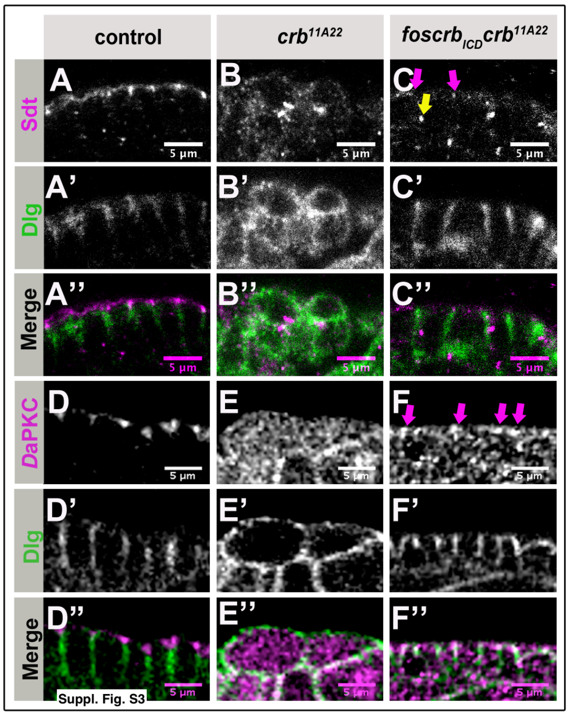
A-A''''': Higher magnifications of stills of time lapse movies shown in Fig. 3, to highlight the phenotypes in the ventral epidermis. Magenta and green arrows point to two different groups of cells that gradually assume a "cyst-like" structure. Scale bar = 50µm.

B-D''. Stills of time lapse movies showing expression of GFP-tagged *D*E-Cadherin in embryos after dorsal closure. Control: *foscrb, DE-cad;;GFP; crb*^{GX24}. C-C'' and D-D'' show two different embryos of the same genotype. Anterior is left, dorsal up. Scale bar = 50µm.



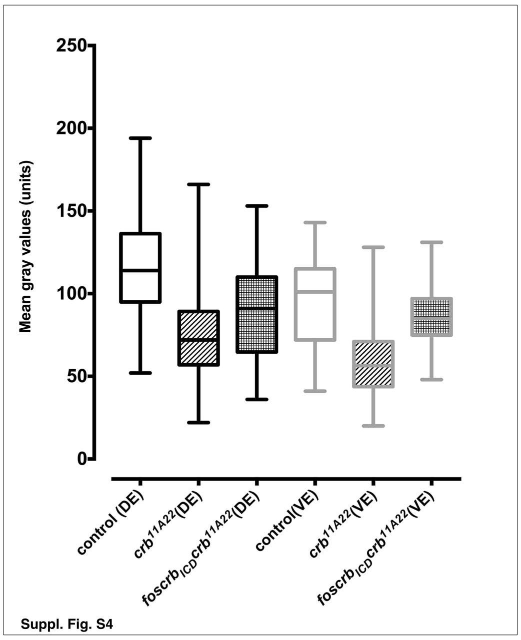
Supplementary Figure S2. (For Fig.2)

A-B”””’: Stills of time lapse movies showing expression of GFP-tagged *D*E-Cadherin in embryos during dorsal closure. Control: *foscrb, DE-cad;;GFP; crb^{GX24}*. Dorsal closure fails in some mutant embryos with hg and Mt exposed out (B”). The posterior zippering is not initiated due to failure in fusion of the posterior most abdominal segments (green arrow heads in B and B””’). Scale bar = 50µm.



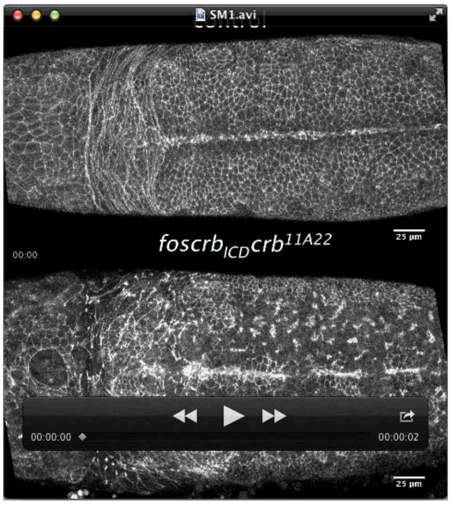
Supplementary Figure S3. (For Fig.4)

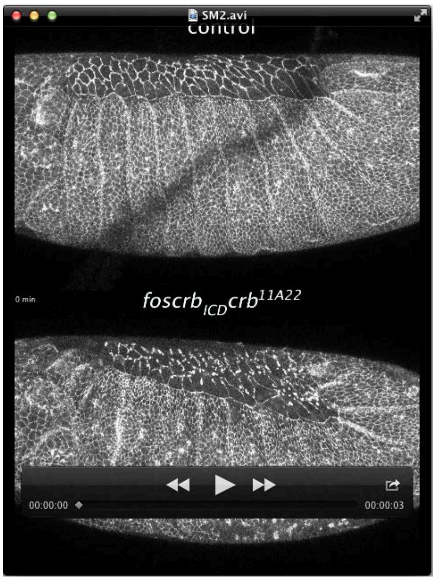
Stage 13 *foscrb; crb*^{GX24} control (A-A'', D-D''), *crb*^{11A22} (B-B'', E-E'') and *foscrb*$_{ICD}$ *crb*^{11A22} (C-C'', F-F'') embryos stained with either Sdt or *D*aPKC (apical) and Dlg as the (lateral). Magenta arrow in C: apical enrichment of Sdt; yellow arrow in C: punctae staining of Sdt inside cells; magenta arrow in F: Apical enrichment of *D*aPKC. Scale bar = 5 µm



Supplementary Figure S4. (For Fig.5)

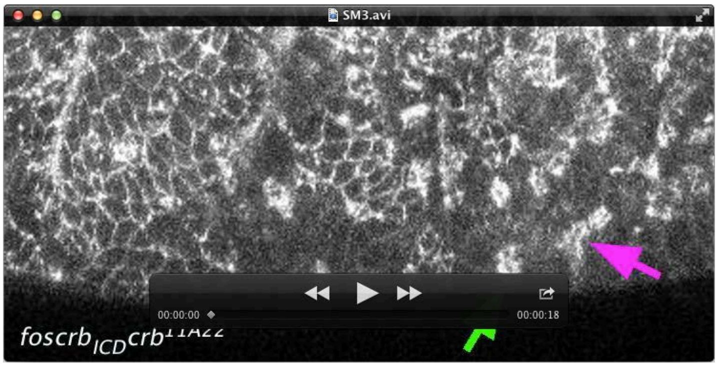
Quantification of Notch intra staining. For the imaging, a z-stack was imaged in similar areas for controls and mutants in the dorsal epidermis and ventral epidermis. The slice with apical Bazooka staining was used to quantify the intensities for Notch Intra staining ensuring mostly apical Notch enrichment was quantified.





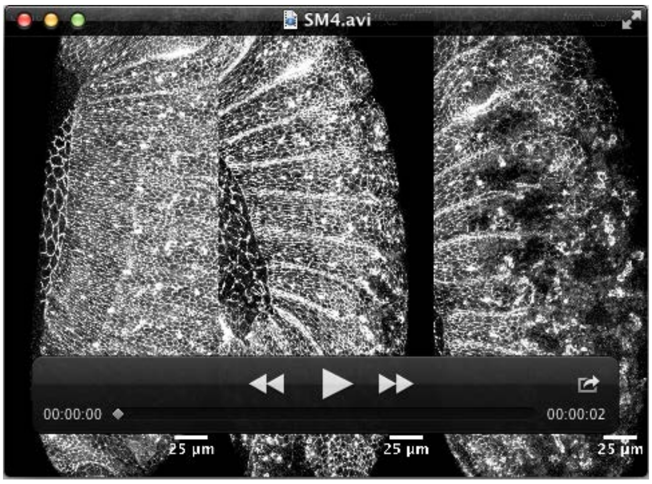
Supplementary Movie M1, M2 (For Fig.2)

Time lapse movies of endogenously tagged *D*E-Cadherin-GFP lines during germ band retraction (dorsal view) (M1) and dorsal closure (lateral view)(M2).



Supplementary Movie M3 (For Fig.2)

Close up of the disintegrated ventral epidermis of the *foscrb*$_{ICD}$ *crb*^{11A22} embryos from M2.



Supplementary Movie M4 (For Fig.2)

Two embryos of the genotype *foscrb*$_{ICD}$ *crb*^{11A22} tagged with *D*E-Cadherin-GFP showing different degrees of rescue.

Supplementary Material and Methods

Establishment of transgenic fly lines

The procedure is modified from that published previously (Bird et al., 2011)

Region of *crb* where homology arms were targeted:

A description of the different regions in the *crb* locus, where the forward and reverse homology arms were targeted for the *foscrb* variants is mentioned below. The introns are represented in small letters (in black) and the exons are represented in capital letters (in blue). The region of the homology arm is highlighted in green and the stop codon TAG is mentioned in bold letters. The corresponding exon no. is mentioned on the right.

a) *foscrb*$_{ICD}$:

Forward homology arm

tgcagATGTCGCCTCAGTGGCGGTGCCGACGAAGGAGGCGTACTTTAATGGCTCCA CTTACCTCCGCCTCACCACGCCGATGCCCATTTGGGATCACTCGGCGATTAGTTT CCGCTCGTGCCGCGGCGGCGAGATCCTCGCCCAGCAGTACAACAAGAACTCCAT TGTAATCTCAGTGCTCAATGACTTTCTGCAAATCTCACTGGCTGGACCCGCCGTC CATGGGCCCAACAACCGGCTGGATGTCAAGCTGCCCTACCAACTGCTGGACAAC CGCTGGCATACGCTGCAGTTCAAGTACGAGTACGGAAATCTCTACCTGCATGTG GATCGCGCGGCAAGCATATTTGgtaagtaaccatgccgttctggctgccccaaattggatttcaggctctatcgc ccactcattcgggtcag	Exon 2

Reverse homology arm

GTACCGATGTGCAAATGTAGTCTGGGATACACTGGTCGCCTGTGCGAGCAGGAC ATTAACGAGTGCGAATCGAATCCATGCCAGAACGGTGGTCAGTGTAAGGACCTC GTCGGCAGGTACGAGTGCGATTGCCAGGGCACCGGGTTCGAGGGCATTCGCTGT GAAAATGACATCGACGAGTGCAACATGGAGGGAGATTACTGCGGCGGATTGGG CCGGTGTTTCAACAAGCCCGGATCCTTCCAGTGCATCTGCCAGAAACCCTATTG CGGAGCCTACTGCAACTTTACGGATCCCTGCAACGCTACGGACCTCTGCTCAAA CGGCGGTCGCTGCGTAGAGTCCTGCGGCGCCAAACCGGACTACTACTGCGAGTG TCCGGAAGGTTTCGCGGGAAAGAATTGCACAGCACCGgtaagttttcaatgatacactacaaattc	Exon 12
agtgaaagaaaaacaatatattgatttcttcgctttttcagATTACGGCCAAGGAGGACGGGCCTTCGACC ACAGACATTGCCATCATTGTAATACCCGTAGTGGTGGTGCTGCTGCTGATCGGG GAGCCCTCCTGGGCACCTTCCTGGTGATGGCCAGGAACAAGCGAGCAACCAGG GGCACCTATAGCCCGAGCGCGCAAGAGTACTGCAACCCACGGCTGGAAATGGA CAACGTACTGAAGCCACCGCCGGAAGAGCGACTAATT**TAG…………**	Exon 13

b) *foscrb*$_{EGFP\text{-}ECD}$

Forward homology arm: The two sequences shaded in green were combined to generate the forward homology arm. This was done to make sure that the stop codon (bold TAG) is in frame.

gtaagttttcaaatgatacactacaaattcagtgaaagaaaaacaatatattgatttcttcgctttttcagATTACGGCCA AGGAGGACGGGCCTTCGACCACAGACATTGCCATCATTGTAATACCCGTAG TGGTGGTGCTGCTGCTGATCGCGGGAGCCCTCCTGGGCACCTTCCTGGTGAT GGCCAGGAACAAGCGAGCAACCAGGGGCACCTATAGCCCGAGCGCGCAAG AGTACTGCAACCCACGGCTGGAAATGGACAACGTACTGAAGCCACCGCCGG AAGAGCGACTAATT**TAG**TTTTGAGTTTTGAGCA……	Exon 13

Reverse homology arm

ATTACGGCCAAGGAGGACGGGCCTTCGACCACAGACATTGCCATCATTGTA ATACCCGTAGTGGTGGTGCTGCTGCTGATCGCGGGAGCCCTCCTGGGCACCT TCCTGGTGATGGCCAGGAACAAGCGAGCAACCAGGGGCACCTATAGCCCGA GCGCGCAAGAGTACTGCAACCCACGGCTGGAAATGGACAACGTACTGAAG CCACCGCCGGAAGAGCGACTAATT**TAG**TTTTGAGTTTTGAGCATGAACGAC GATTAGCAAAGCAAACAAAAGATATTTTTAAATCCGCCCATATACACCTAG CTGTAGGAGTAACTCAATGTTTTGTACTAAGTT……	Exon 13

<u>The homology arms used</u>

The sequences of the homology arms used to generate and amplify the rpsl-neo modification cassette are given below. The homology arms consist of a sequence corresponding to *crb* locus (in blue letters) followed by a sequence corresponding to the rpsl-neo casstte (in black letters).

foscrb variant	**Forward homology arm**	**Reverse homology arm**
foscrb$_{ICD}$	ATGTCGCCTCAGTGGCGGTG CCGACGAAGGAGGCGTACT TTAATGGCTCCGGCCTGGTG ATGATGGCGGGA	GTTTTTCTTTCACTGAATTTG TAGTGTATCATTTGAAAACT TACCGGTGCTCAGAAGAACT CGTCAAGAAGG
foscrb$_{EGFP\text{-}ECD}$	CCCTCCTGGGCACCTTCCTG GTGATGGCCAGGAACAAGC GAGCAACCAGGATTTAGGG CCTGGTGATGATGGCGGGAT CG	AATATCTTTTGTTTGCTTTGC TAATCGTCGTTCATGCTCAA AACTCAAAATCAGAAGAAC TCGTCAAGAAGG

Table 1 : Homology arms used for the first recombineering step

List of oligonucleotides used for the 2nd recombineering step

foscrb variant	**Lagging strand**	**Leading strand**
foscrb$_{ICD}$	A*T*GTCGCCTCAGTGGCGGTGCCGACGAAGGAGGCGTACTTTAATGGCTCCGCACCGGTAAGTTTTCAAATGATACACTACAAATTCAGTGAAAGAAAAAC	G*T*TTTTCTTTCACTGAATTTGTAGTGTATCATTTGAAAACTTACCGGTGCGGAGCCATTAAAGTACGCCTCCTTCGTCGGCACCGCCACTGAGGCGACAT
foscrb$_{EGFP-ECD}$	C*C*CTCCTGGGCACCTTCCTGGTGATGGCCAGGAACAAGCGAGCAACCAGGATTTAGTTTTGAGTTTTGAGCATGAACGACGATTAGCAAAGCAAACAAAAGATATT	A*A*TATCTTTTGTTTGCTTTGCTAATCGTCGTTCATGCTCAAAACTCAAAACTAAATCCTGGTTGCTCGCTTGTTCCTGGCCATCACCAGGAAGGTGCCCAGGAGGG

Table 2: Single stranded oligos used in the second recombineering step

List of primers used for PCR and sequencing

foscrb variant	**Forward primer**	**Reverse primer**
foscrb$_{ICD}$	GGCCTGGTGATGATGGCGGGATCG	TACGCTTAGATACTCTAGAGC
foscrb$_{EGFP-ECD}$	ACCTGCCAGAATGGATTCA	TCAGAAGAACTCGTCAAGAAGG

Table 3: Primers for checking correct integration of rpsl-neo cassette

foscrb variant	**Forward primer**	**Reverse primer**
foscrb$_{ICD}$	GGCAATCTTGATCTTCTCTTG	TACGCTTAGATACTCTAGAGC
foscrb$_{EGFP-ECD}$	GGTACGAGTGCGATTGCCAG	TACGCTTAGATACTCTAGAGC

Table 4: Primers for checking correct removal of rpsl-neo cassette

foscrb variant	**Forward primer**	**Reverse primer**
foscrb$_{ICD}$	GGCAATCTTGATCTTCTCTTG	TACGCTTAGATACTCTAGAGC
foscrb$_{EGFP-ECD}$	GGTACGAGTGCGATTGCCAG	TACGCTTAGATACTCTAGAGC

Table 5: Primers for sequencing generated variants

List of fly lines and antibodies used in this study

$w^{-};;crb^{11A22}/TTG$	*crb* null allele; BSC #3448
$w^{-};;crb^{GX24}/TTG$	*crb* null allele (Huang et al., 2009)
$w^{-}; foscrb;crb^{GX24}$	(Klose et al., 2013)
$w^{-};;foscrb_{ICD}crb^{11A22}/TTG$	This study
$w^{-};;foscrb_{ICD}crb^{GX24}/TTG$	This study
$w^{-};;foscrb_{EGPF\text{-}ECD}crb^{GX24}/TTG$	This study
$w^{-};;foscrb^{EGFP}crb^{GX24}$	This study
$w^{-};DE\text{-}cad::GFP$	*D*E-cadherin fused with GFP knock-in allele; homozygous viable (Huang et al., 2009)
$w^{-};UAS\text{-}flw\text{-}HA$	BSC #23703
w-;UAS – Notch[intraMHLS]/Cyo[wg-lacZ];MKRS/TM6	Gift from Thomas Klein (Klein and Arias, 1998)
UAS-DE-cad	Klebes, A. and Knust, E. (unpublished results)
TTG	BSC #6663

Table 6: Fly lines used in this study

	Animal	Dilution	Source
anti-Crb2.8	Rat	1:500	(Richard et al., 2006)
anti-Crb Intra	Rabbit	1:200	(Kumichel et al., 2015)
anti-Disc Large	Mouse	1:50	DSHB #4F3
anti-Bazooka	Rabbit	1:500	(Wodarz et al., 1999)
anti-*D*Patj	Rabbit	1:1000	(Richard et al., 2006)
anti-Stranded at Second	Rabbit	1:500	(Wodarz et al., 1995). Kindly provided by D.Cavener
anti-Hunchback	Guinea pig	1:500	(Mettler et al., 2006). Kindly provided by J.Urban
anti-Stardust	Rabbit	1:500	(Berger et al., 2007)
anti-Par6	Guinea pig	1:500	(Kim et al., 2009). Kindly provided by A.Wodarz
anti-aPKC	Rabbit	1:200	Santa Cruz C20
anti-Notch	Mouse	1:100	DSHB C17.9C6 Conc. Purified
anti-Neurotactin	Mouse	1:10	DSHB BP106
anti-Achaete	Mouse	1:10	(Skeath and Carroll, 1992). DSHB
anti-Deadpan	Guinea pig	1:500	(Levy and Larsen, 2013). Kindly provided by J. Skeath
anti-phospho tyrosine	Mouse	1:100	BD Biosciences

Table 7: List of primary antibodies used in this study

References

Berger, S., N.A. Bulgakova, F. Grawe, K. Johnson, and E. Knust. 2007. Unravelling the genetic complexity of *Drosophila stardust* during photoreceptor morphogenesis and prevention of light-induced degeneration. *Genetics*. 176:2189-2200.

Bird, A.W., A. Erler, J. Fu, J.K. Heriche, M. Maresca, Y. Zhang, A.A. Hyman, and A.F. Stewart. 2011. High-efficiency counterselection recombineering for site-directed mutagenesis in bacterial artificial chromosomes. *Nat Methods*. 9:103-109.

Huang, J., W. Zhou, W. Dong, A.M. Watson, and Y. Hong. 2009. Directed, efficient, and versatile modifications of the *Drosophila* genome by genomic engineering. *Proc Natl Acad Sci*. 106:8284-8289.

Kim, S., I. Gailite, B. Moussian, S. Luschnig, M. Goette, K. Fricke, M. Honemann-Capito, H. Grubmuller, and A. Wodarz. 2009. Kinase-activity-independent functions of atypical protein kinase C in Drosophila. *J Cell Sci*. 122:3759-3771.

Klein, T., and A.M. Arias. 1998. Interactions among Delta, Serrate and Fringe modulate Notch activity during Drosophila wing development. *Development*. 125:2951-2962.

Klose, S., D. Flores-Benitez, F. Riedel, and E. Knust. 2013. Fosmid-based structure-function analysis reveals functionally distinct domains in the cytoplasmic domain of *Drosophila* Crumbs. *G3 (Bethesda)*. 3:153-165.

Kumichel, A., K. Kapp, and E. Knust. 2015. A Conserved Di-Basic Motif of *Drosophila* Crumbs Contributes to Efficient ER Export. *Traffic*. 16:604-616.

Levy, P., and C. Larsen. 2013. Odd-skipped labels a group of distinct neurons associated with the mushroom body and optic lobe in the adult Drosophila brain. *J Comp Neurol*. 521:3716-3740.

Mettler, U., G. Vogler, and J. Urban. 2006. Timing of identity: spatiotemporal regulation of hunchback in neuroblast lineages of Drosophila by Seven-up and Prospero. *Development*. 133:429-437.

Richard, M., F. Grawe, and E. Knust. 2006. *D*PATJ plays a role in retinal morphogenesis and protects against light-dependent degeneration of photoreceptor cells in the *Drosophila* eye. *Dev Dyn*. 235:895-907.

Skeath, J.B., and S.B. Carroll. 1992. Regulation of proneural gene expression and cell fate during neuroblast segregation in the Drosophila embryo. *Development*. 114:939-946.

Wodarz, A., U. Hinz, M. Engelbert, and E. Knust. 1995. Expression of Crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell*. 82:67-76.

Wodarz, A., A. Ramrath, U. Kuchinke, and E. Knust. 1999. Bazooka provides an apical cue for Inscuteable localization in *Drosophila* neuroblasts. *Nature*. 402:544-547.