# SUPPLEMENTARY INFORMATION

## SUPPLEMENTARY METHODS

**Plasmids.** *crb*$^{M11.M2}$ is a *piggyBac-lacZ* insertion. 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). *UAS-Ser*$^{DN}$ and *UAS-Dl*$^{DN}$ are truncated forms of Serrate and Delta, respectively, lacking the intracellular domains and behaving as dominant negative forms of the ligands (Sun and Artavanis-Tsakonas, 1996). Delta$^{DN}$ (aa #1 to #620: MHWIKC…VIAACVVFCM*) was constructed by PCR on Delta cDNA using the oligos: GGAATTCATGCATTGGATTAAATGT and GCTCTAGATCACATGCAGAAGACCACGCA. The PCR product was cut EcoRI-XbaI and cloned in pUASt. Ser$^{DN}$ (aa #1 to #1248: MYKMFR…FISLYWKQ*) was constructed by PCR on Ser cDNA using the following oligos: TATCTCTCCGTATACTGCTCTGAA and CCGCTCGAGTCACTGTTTCCAGTAAAGACT**.** The PCR product was cut AccI-XhoI and this product was ligated to a Ser cDNA fragment EcoRI-AccI (aa #1-972). The final product was ligated to pUASt EcoRI-XhoI.

**Genotypes of larvae used for genetic mosaic analysis:**

*hs-FLP (I); FRT82 crb*$^{11A22}$*/FRT82 arm-lacZ*.

*hs-FLP (I); FRT82 crb*$^{1}$*/FRT82 Ubi-GFP*

*hs-FLP (I)/Notch-lacZ; FRT82 crb*$^{11A22}$*/FRT82 Ubi-GFP*

*hs-FLP (I); wg-lacZ/+;FRT82 crb*$^{11A22}$*/FRT82 Ubi-GFP*

*hs-FLP (I); wg-lacZ/+;FRT82 crb*$^{1}$*/FRT82 Ubi-GFP*

*hs-FLP tub-Gal4 UAS-nucGFP (I);EP-mam*$^{DN}$*; FRT82 crb*$^{11A22}$*/FRT82 Gal80*

*hs-FLP tub-Gal4 UAS- nucGFP (I);UAS-Ser*$^{DN}$*; FRT82 crb*$^{11A22}$*/FRT82 Gal80*

*hs-FLP tub-Gal4 UAS- nucGFP (I);UAS-Dl$^{DN}$; FRT82 crb$^{11A22}$/FRT82 Gal80*

*hs-FLP tub-Gal4 UAS- nucGFP (I);UAS-N$^{ecd}$; FRT82 crb$^{11A22}$/FRT82 Gal80*

*hs-FLP tub-Gal4 UAS- nucGFP (I);UAS-crumbs$^{extra-TM-GFP}$; FRT82 crb$^{11A22}$/FRT82 Gal80*

*hs-FLP tub-Gal4 UAS- nucGFP (I);UAS-crumbs; FRT82 crb$^{11A22}$/FRT82 Gal80*

*f$^{36a}$hs-FLP (I); FRT82 crb$^{11A22}$/FRT82 P(f+)*

*sdt$^{XP96}$ FRT$^{9-2}$/Ubi-GFP FRT$^{9-2}$; hs-FLP (II)*

*N$^{co}$ FRT18/Ubi-GFP FRT18; hs-FLP (II)/+;crumbs-lacZ/+*

Clones were generated by giving an hour heat shock at 37 degrees during the second instar stage (48-72 h after egg laying). .

**LEGENDS TO SUPPLEMENTARY FIGURES**

**Supplementary Fig 1.** Different thresholds of Notch activity induce Cut, Wingless and Crumbs at the DV boundary. Crumbs (A, D and G), Cut (B, E and H) and Wingless (C, F and I) protein expression (white) in *Notch* $^{ts2}$ third instar wing imaginal discs raised at 18ºC (A-C), 25ºC (D-F) and 29ºC (G-I).

**Supplementary Fig 2.** Crumbs and Notch protein localization in the wing epithelium. (A, B) Wing discs with clones of cells lacking *crumbs* activity and marked by the absence of the β-Gal (green) marker. Armadillo (A) and E- Cadherin (B) are shown in red and localize to the Adherens Junctions. The lower panels show XY confocal sections of the wing pouch. The upper panels show XZ sections of the same wing discs at the level of the white lines. Ap, apical, Bs, basal side of the epithelium. Note Armadillo and E- Cadherin protein localization is not affected in mutant clones. (C) Subcellular localization of Notch (green) and Crumbs (red) in the wing disc. The upper and left panels show XZ and YZ sections of the same wing discs at the level of the white lines. Note Notch and Crumbs proteins colocalize at the Adherens Junctions. Notch protein was detected with an antibody against the intracellular domain. Similar results were obtained with an antibody against the extracellular domain of Notch. (D) Wing disc with clones of cells lacking *crumbs* activity and marked by the absence of the β-Gal (red) marker. The upper and right panels show XZ and YZ sections of the same wing discs at the level of the white lines. Notch is shown in green and is not affected in mutant clones.

**SUPPLEMENTARY REFERENCES**

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Sun, X. and Artavanis-Tsakonas, S. (1996) The intracellular deletions of Delta and Serrate define dominant negative forms of the Drosophila Notch ligands. *Development*, **122**, 2465-2474.