Supplementary Figures

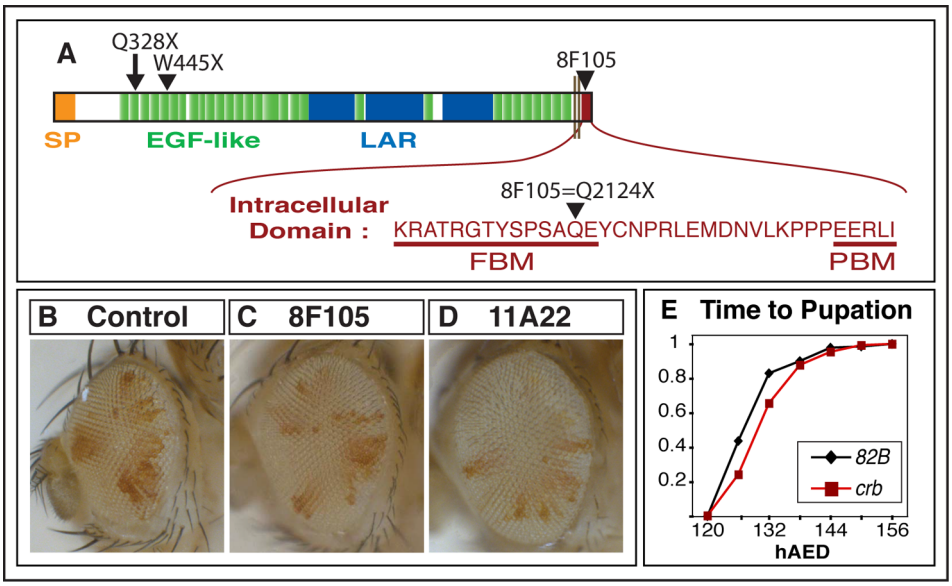

Figure S1 – Phenotypes and mutations responsible for various *crb* alleles

(A) Schematic representation of the Crb protein showing the location of the signal peptide (SP), EGF-like repeats, laminin A repeats (LAR), the transmembrane domain and the intracellular domain (ICD). The amino-acid sequence of the entire ICD is shown including the FERM domain-binding motif (FBM) and the PDZ-binding motif (PBM). Also indicated are changes resulting from two of the mutant alleles recovered in our screen, Q328X and W445X, as well as a previously published allele, 8F105. **(B-D)** show adult eye phenotypes of the 3R parental chromosome **(B)**, crb^{8F105} **(C),** and the reported null allele, crb^{11A22} **(D)** when flipped over a cell-lethal tester chromosome. **(E)** Quantification of time to 50% pupation of larvae with control or *crb* mosaic eye discs. Each time point is an average from 3 independent experiments.

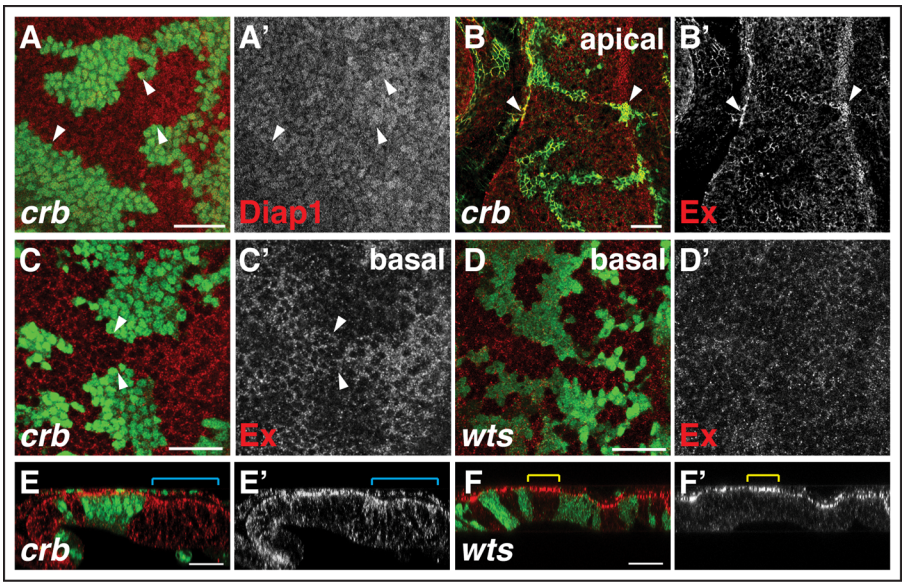

Figure S2 – Changes in HSW pathway activity in *crb* mutant clones

(A-F) Mosaic third-instar eye-imaginal discs. Mutant clones are GFP-negative. **(A)** Anti-DIAP1 (red) staining of *crb* mosaic disc. **(B)** Apical section of *crb* mosaic disc stained with anti-Crb (green) and anti-Ex (red) antibodies. **(C,D)** Basal section of *crb* mosaic disc **(C)** or *wts* mosaic disc **(D)** stained with Ex antibody (red). Ex is observed in these sections in *crb* clones (**C**) but not in *wts* clones (**D**). **(E, F)** XZ section of *crb* mosaic disc **(E)** or *wts* mosaic disc **(F)** stained with Ex antibody (red). Brackets denote *crb* (blue) or *wts* (yellow) mutant clones.
In **(E)** and **(F)** apical is up for the disc proper.
Scale bars: 20μm

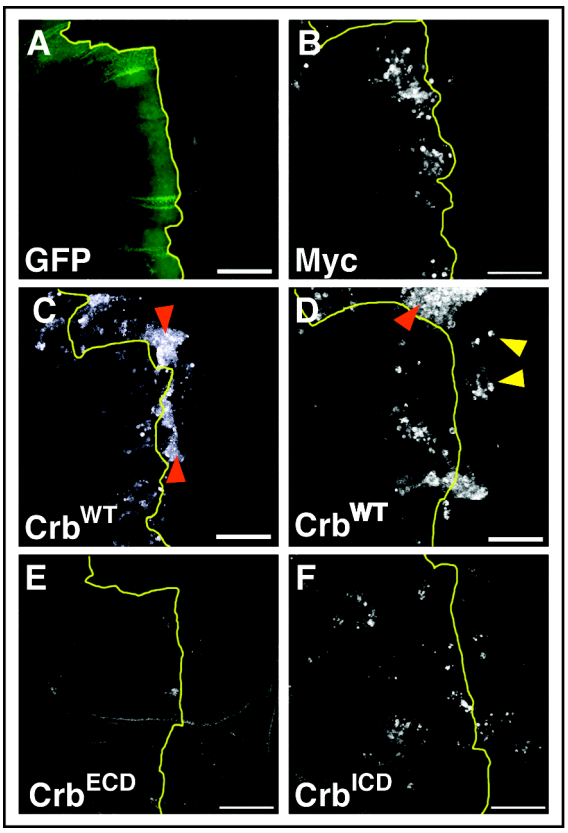

Figure S3 – Apoptosis resulting from *ptc*-GAL4-driven expression of trangenes encoding different Crb domains

(A-F) Z-stack projections from wing imaginal discs expressing various transgenes in under *ptc*-Gal4 control and stained with AC3. The A-P compartment boundary is indicated in each image by a yellow line. **(A)** GFP-expressing control. **(B)** High levels of AC3-positive cells are found on the anterior side of compartment boundary when Myc is overexpressed, presumably because of autonomous cell death. **(C, D)** AC3 positive cells are found on both sides of the compartment border upon expression of full-length Crb. **(C)** includes more apical planes than **(D)**. **(E)** No increase AC3 levels upon expression of Crb-ECD. **(I)** Expression of Crb-ICD also induces apoptosis on both sides of the compartment boundary, but AC3-positive cells are more dispersed. Anterior is left.

Scale bars: 50µm.

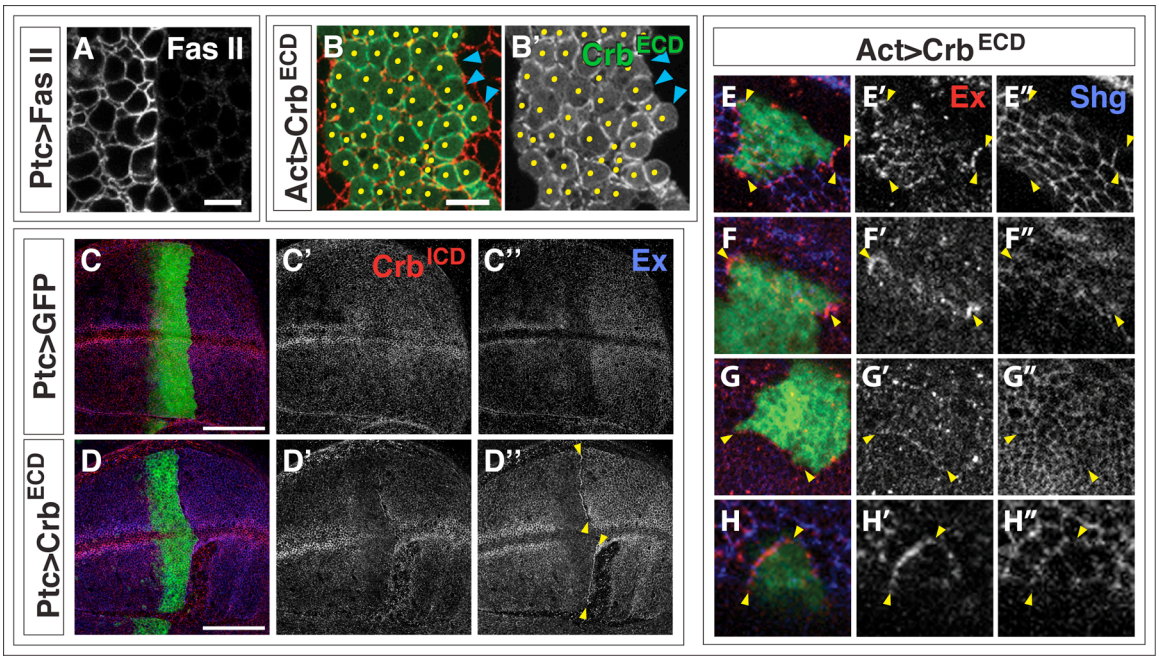

Figure S4 – Asymmetric protein localization at borders of differential Crb expression. **(A)** Border between the anterior compartment, overexpressing Fas II (stained with anti-Fas II antibody), and posterior compartment, containing wild-type cells in wing imaginal discs. **(B)** A flip-out clone overexpressing the Crb-ECD protein visualized by its GFP-tag (green). Green channel shown in **(B')**. Blue arrowheads denote membranes that abut wild-type cells, where GFP-tagged Crb-ECD is found at much lower levels. Phalloidin is shown in red. **(C,D)** Max projections of the apical slices from Z-stacks of wing discs expressing GFP **(C)** or GFP-tagged Crb-ECD **(D)** driven by the *ptc*-Gal4 driver and stained for Crb-ICD (Red) or Ex (Blue). **(E-H)** Clones overexpressing GFP-tagged Crb-ECD and stained for Ex (Red) and Shg (Blue). The four clones are in different regions of the same wing disc. For each clone, a max projection was made from 3-5 apical Z-sections the apical domain (the region where Ex was expressed above background levels). This was necessary because clone borders are not even along the Z-axis. Therefore the borders of clones were blurry in Max Projections of the entire Z-stack. Ex, but not Shg, is observed to accumulate at the clone boundary.

	Total number of AC3+ Cells	Number of cells that are AC3+ and GFP+		Number of cells that are AC3+ and GFP-	
		Total	Border	Total	Border
FRT82B	70	23	20	47	22
FRT82B crbQ328X	273	188	150	85	19

Supplementary Table 1 – Genotypes and locations of AC3-positive cells in *crb* mutant clones and control clones

Table showing the numbers of AC3-positive (AC3+) cells in mosaic eye imaginal discs from flies that were *eyFLP; 82B/82B GFP+ w+* ("FRT 82B") or *eyFLP; 82B crb / 82B GFP+ w+* ("FRT 82B crb"). 12 discs of each genotype were dissected and stained with AC3. The number of AC3-positive cells in one confocal section was counted and whether these AC3-positive cells were GFP-positive, GFP-negative, or within one cell diameter of a clone border was noted.

	Total Discs	Total Sections	# Convex Sections		# Concave Sections	
ptc>GFP	8	32	3	9%	3	9%
ptc>Crb-ICD	9	36	1	3%	12	33%
ptc>Crb-ECD	13	48	21	44%	2	4%

Supplementary Table 2 – Quantification of the morphological effects resulting from overexpression of different Crb proteins

Table showing the frequency of morphology phenotypes observed for GFP, Crb-ICD, and Crb-ECD overexpressing wing discs. Four sections were examined in each disc and the number of sections in which the region of highest ptc expression was convex (bent toward the apical surface) or concave (bent toward the basal surface) were noted. The remaining sections were flat. The morphology of discs overexpressing full-length Crb was so consistently convex that it did not warrant quantification.