

Supplementary Materials for

**Self-Induced Patched Receptor Down-Regulation Modulates Cell Sensitivity to the Hedgehog Morphogen Gradient**

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**The PDF file includes:**

Fig. S1. In situ hybridization to detect *GFP*-positive transcripts.

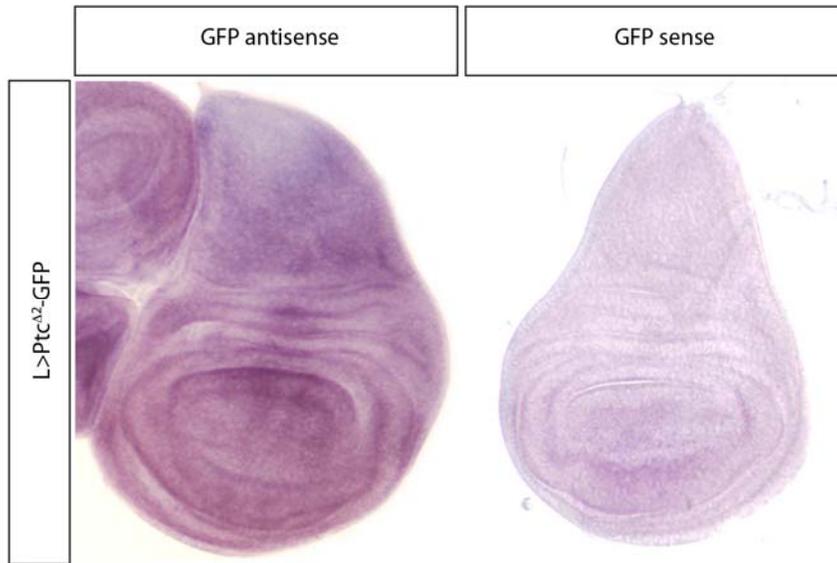
Fig. S2. Ptc<sup>Δ2</sup>-GFP (from the L>Ptc<sup>Δ2</sup>-GFP transgene) is down-regulated in leg and haltere discs.

Fig. S3. Ptc<sup>LDL</sup> is endocytosed and sequesters Hh, but does not repress Smo.

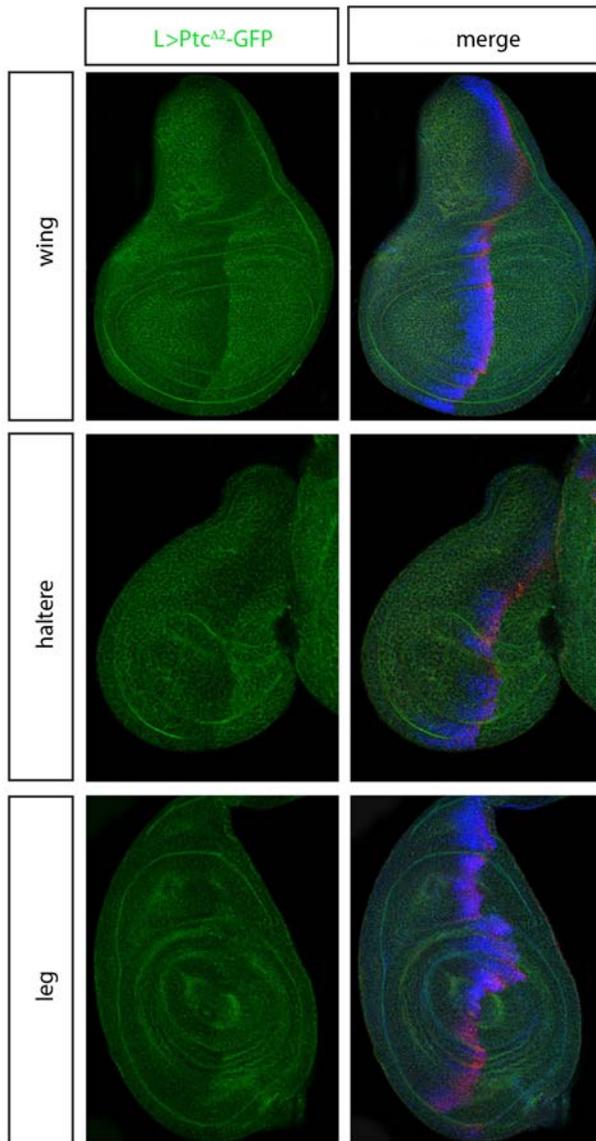
Fig. S4. Overexpression of Dispatched (Disp) does not induce degradation of Ptc-GFP (from the L>Ptc-GFP transgene).

Fig. S5. Ptc<sup>AAAA</sup>-GFP regulates Smo activity in the absence of endogenous Ptc.

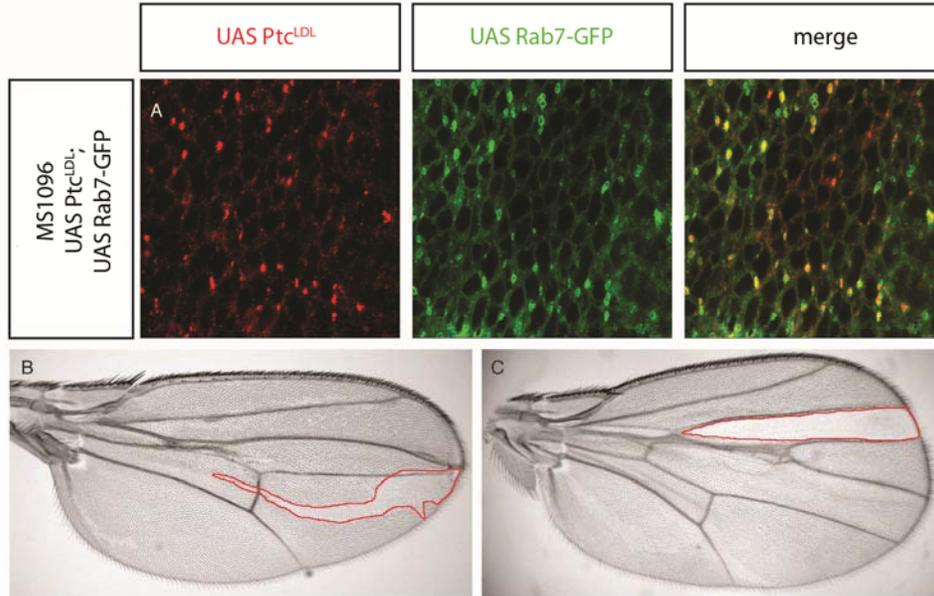
Fig. S6. The GFP tag does not affect the ability of Ptc<sup>Δ2</sup> to interact with endogenous Ptc.



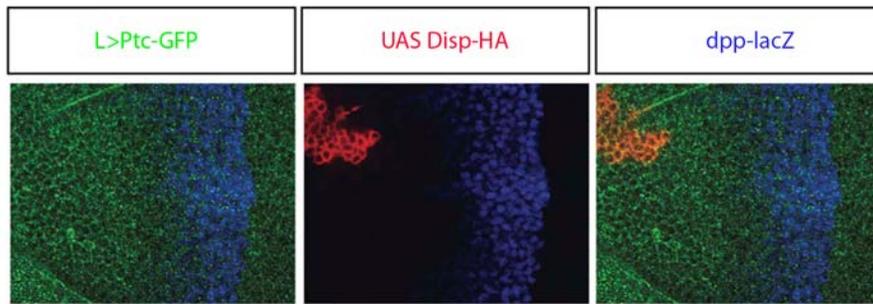
**Fig. S1.** In situ hybridization to detect *GFP*-positive transcripts. L>Ptc<sup>Δ2</sup>-GFP third instar larva wing imaginal discs were used to analyze transcription of the transgene encoding Ptc<sup>Δ2</sup>-GFP. Antisense *GFP* probe showed ubiquitous transcription; whereas the sense *GFP* probe showed limited signal. [Methods. L>Ptc<sup>Δ2</sup>-GFP flies were submitted to several heat shocks to induce Flp-mediated recombination to excise in vivo the >CD2,y<sup>+</sup>> Flp-out cassette, rendering viable flies. GFP cloned into pBluescriptSK+ (Stratagene) was used as a template to generate the sense and antisense probes with T7 and T3 polymerases, respectively. Probe generation and in situ hybridization were performed with standard protocols.]



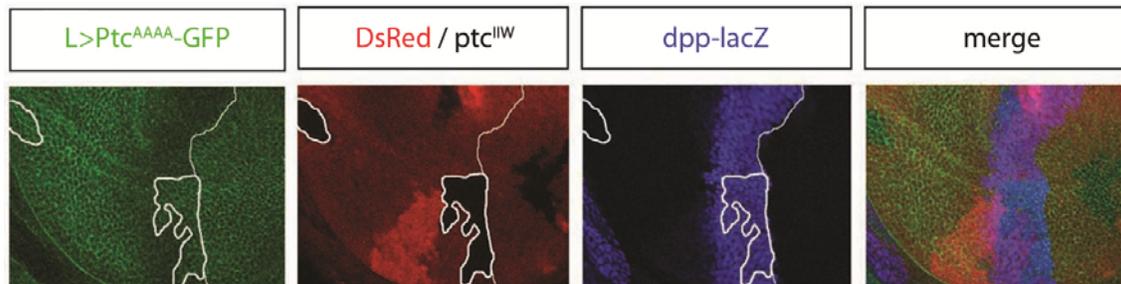
**Fig. S2.** Ptc<sup>Δ2</sup>-GFP (from the L>Ptc<sup>Δ2</sup>-GFP transgene) is down-regulated in leg and haltere discs. Complete Flp-out L>Ptc<sup>Δ2</sup>-GFP third instar larva discs showed GFP down-regulation at the AP boundary (green), marked by the presence of dpp-lacZ (blue) and endogenous Ptc (red).



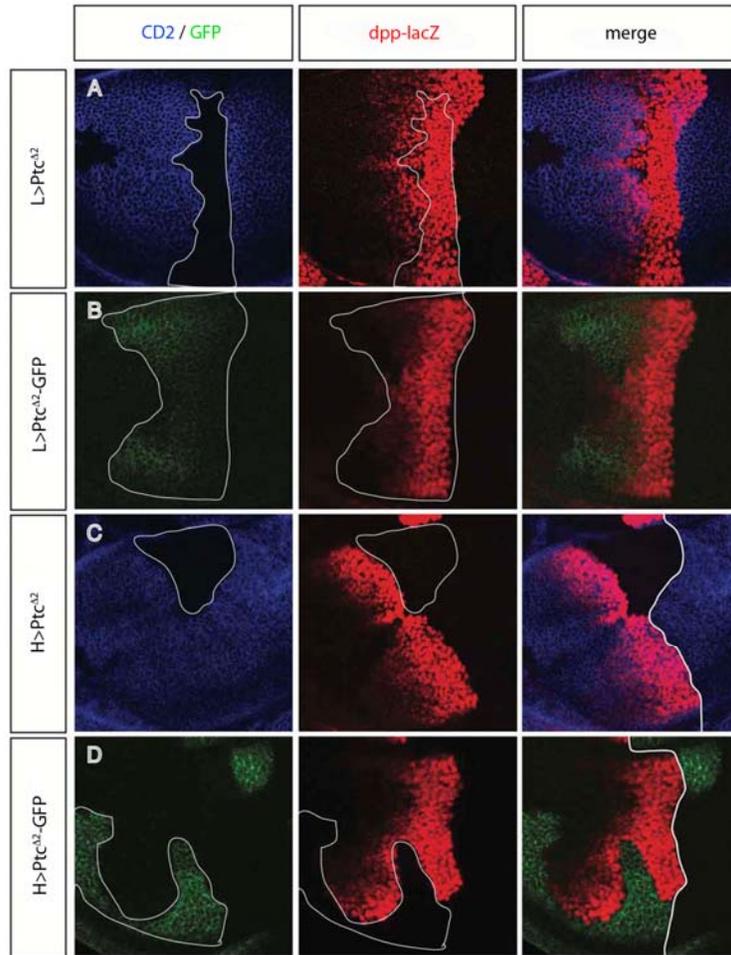
**Fig. S3.** Ptc<sup>LDL</sup> is endocytosed and sequesters Hh, but does not repress Smo. **(A)** Wing discs coexpressing UAS Ptc<sup>LDL</sup> and UAS Rab7-GFP (from Marcos González-Gaitán) driven by the MS1096 Gal4 line. Many vesicles are positive for both Ptc<sup>LDL</sup> (stained with an antibody against Ptc, red) and Rab7-GFP (green). **(B)** Posterior wild-type clone overexpressing Ptc<sup>LDL</sup>, marked by shavenoid, which removes hairs (outlined by a red line). Notice the non-autonomous effect of the clone in the anterior compartment, where the intervein domain between veins 3 and 4 is reduced. This phenotype shows that Hh is sequestered by Ptc<sup>LDL</sup> and cannot activate the pathway target genes in anterior cells abutting the AP boundary. **(C)** Anterior *ptc*<sup>-</sup> clone, also marked by shavenoid, which overexpresses Ptc<sup>LDL</sup>. Ptc<sup>LDL</sup> fails to block Smo activity inside the clone, as it can be seen by the overgrowth and vein 3 duplication. This contrasts with repression-competent Ptc molecules, like Ptc-GFP, that can repress Hh signalling in *ptc*- clones (see Fig. 2A). **[Methods.** The following genotypes were used: In panel A, *MS1096; UAS Ptc<sup>LDL</sup>/+; UAS Rab7-GFP/+*; in panel B, *y w hsp70-flp; FRT42D sha/FRT42D Tubα1>Gal80, CD10y<sup>+</sup>; UAS Ptc<sup>LDL</sup>/Tubα1>Gal4*; and in panel C, *y w hsp70-flp; FRT42D ptc<sup>HW</sup> sha/FRT42D Tubα1>Gal80, CD10y<sup>+</sup>; UAS Ptc<sup>LDL</sup>/Tubα1>Gal4*. Mitotic clones were induced by 1h heat shocks at 37°C to 24-48h larvae.]



**Fig. S4.** Overexpression of Dispatched (Disp) does not induce degradation of Ptc-GFP (from the L>Ptc-GFP transgene). The abundance of Ptc-GFP (green) is not reduced in an anterior flp-out clone overexpressing UAS Disp-HA [red, detected with an antibody against the HA tag (Clone 3F10, Roche).] AP boundary is marked by the presence of dpp-lacZ (blue). [Methods. The following genotype was used: *y w hsp70-flp; dpp-lacZ<sup>10628</sup> Tubα1>Gal80, y<sup>+</sup>>Gal4/UAS Disp-HA; rp49>Ptc-GFP -hsp70 3'UTR/+*. Flp-out clones were induced by 1h heat shocks at 37°C to 24-48h larvae.]



**Fig. S5.** Ptc<sup>AAAA</sup>-GFP regulates Smo activity in the absence of endogenous Ptc. Ubiquitous expression of Ptc<sup>AAAA</sup>-GFP (green; from the L>Ptc<sup>AAAA</sup>-GFP transgene) in a wing disc repressed Smo activity in *ptc<sup>-</sup>* clones (marked by the absence of DsRed) located far from the AP boundary (left clone), as it can be seen by the absence of *dpp-lacZ* expression (blue). In clones located next to the AP boundary (center clone), the presence of Hh blocked the repressive activity of Ptc<sup>AAAA</sup>-GFP and, therefore, Smo was free to signal, as it can be seen by the presence of *dpp-lacZ*. Notice that Ptc<sup>AAAA</sup>-GFP was stable in absence of endogenous Ptc. The horizontal thin white line marks the AP boundary. [Methods. the following genotype was used: *y w hsp70-flp; FRT42D ptc<sup>IIW</sup>/FRT42D Tubα1>DsRed; rp49> Ptc<sup>AAAA</sup>-GFP -hsp70 3' UTR /+*. Mitotic clones were induced by 1h heat shocks at 37°C to 24-48h larvae.]



**Fig. S6.** The GFP tag does not affect the ability of Ptc<sup>Δ2</sup> to interact with endogenous Ptc. (A) Clones expressing low amounts of Ptc<sup>Δ2</sup> (from the L>Ptc<sup>Δ2</sup> transgene), marked by the absence of CD2 (blue) fail to repress *dpp-lacZ* expression (red). (B) Similarly, clones expressing low amounts of Ptc<sup>Δ2</sup>-GFP (from the L>Ptc<sup>Δ2</sup>-GFP transgene, Table 1), marked by the presence of GFP (green), fail to repress *dpp-lacZ* expression. (C) Clones expressing high amounts of Ptc<sup>Δ2</sup> (from the H>Ptc<sup>Δ2</sup> transgene), marked by the absence of CD2 (blue) repress *dpp-lacZ* expression (red). (D) Similarly, clones expressing high amounts of Ptc<sup>Δ2</sup>-GFP (from the H>Ptc<sup>Δ2</sup>-GFP transgene, Table 1), marked by the presence of GFP (green), repress *dpp-lacZ* expression. In all panels, the Flp-out clones are marked by a thin white line. In panels C and D, the AP boundary is marked by a thick white line. [Methods. The following genotypes were used: *y w hsp70-flp; dpp-lacZ<sup>10628</sup> Tubal>Gal80, y<sup>+</sup>>Gal4/+; rp49>Ptc<sup>Δ2</sup> - hsp70 3'UTR (or rp49>Ptc<sup>Δ2</sup>-GFP -hsp70 3'UTR or Tubal>Ptc<sup>Δ2</sup> - Tubal 3'UTR or Tubal>Ptc<sup>Δ2</sup>-GFP - Tubal 3'UTR) /+.* Mitotic clones were induced by 1h heat shocks at 37°C to 24-48h larvae.]