Supplementary figure legends

Figure S1. Col is cell autonomously up-regulated in *Dsulf1*$^{\Delta P1}$ clonal anterior cells

(A,A') Expression pattern of Col (red) in wing disc carrying a *Dsulf1*$^{\Delta P1}$ clone located in the dorsal part of the A compartment, close to the AP boundary as visualized by the absence of GFP staining (green in A). Note the anterior extension of the Col-expressing domain in dorsal mutant cells compared to ventral wt cells (green). (B) Quantification of fluorescence intensity along the AP axis performed at the level of the *Dsulf1*$^{\Delta P1}$ clone (*Ant*, red) or wt cells (WT, green) in the A compartment. The areas used for quantification are indicated by yellow boxes in A'.

Figure S2. Removal of DSulf1 activity in the Hh receiving compartment results in Ptc up-regulation.

(A,A') Immunostaining of Ptc (red in A) in wing disc carrying *Dsulf1*$^{\Delta P1}$ clone located in the dorsal part of the A compartment, as visualized by the absence of GFP staining (green in A). (B,B') Quantification of fluorescence intensity at the level of dorsal mutant clonal cells (red, *Ant*) and ventral wt cells (WT, green in B'). The areas used for quantification are indicated by yellow boxes in B. Note the enlargement of Ptc-expressing domain as well as Ptc up-regulation in mutant clonal cells compared to wt (A,A' and B,B'). (C,D) Immunostaining of Ptc showing an enlargement of the Ptc-expressing domain in *Dsulf1*$^{\Delta P1}$ (D) mutant disc compared to wt (C). (E) Counting of Ptc-expressing cells in wt and *Dsulf1*$^{\Delta P1}$ wing discs and in wing disc carrying *Dsulf1*$^{\Delta P1}$ clone in the dorsal part of the A compartment (*Ant*). Error bars represent the standard deviation (***=$P<0.0005$ using a *t*-test). D = dorsal, V = ventral

Figure S3. Schematic representations of DSulf1 function in regulating Hh signalling in the wing imaginal disc.

In each panel, the blue curve indicates the level of Hh activity. **a.** In late 3rd instar wt wing disc, *col* (red) and *dpp* (orange) are activated at specific Hh activity thresholds (dotted lines). We propose that, in the P compartment, DSulf1 lowers Hh/HSPGs binding affinity at the apical surface of Hh-producing cells. This, by favouring the release of Hh from P cells (large purple arrow), contributes to enhance Hh signalling activity in the A receiving field. In the A compartment, DSulf1, acting similarly, decreases Hh signalling activity, thus limiting the anterior extension of Hh target gene expression domains. **b.** The absence of DSulf1 activity only in the A compartment results in an increased Hh/HSPGs interaction in A cells while Hh

release from P cells is not modified. This leads to a forward shift of Hh target gene expression domains. **c.** In absence of DSulf1 activity in the P compartment, Hh release from P cells decreases (small purple arrow), leading to a lowering of Hh signalling activity and to a backward shift of Hh target gene expression domains. **d.** In the complete absence of DSulf1 activity in the wing disc, Hh release from P cells decreases leading to a reduction of Hh signalling activity in A receiving cells. However, this results in a mild enlargement *col* and *dpp* expression domains. The timely regulated expression of *Dsulf1*, restricted to the A compartment at the onset of Hh target gene up-regulation, allows to reconcile these phenotypes. Indeed, in these mutants, the absence of Dsulf1 negative regulatory activity at early 3rd instar larval stage can explain the slight anterior extension of Hh target gene domains of expression but the lack of further DSulf1 positive regulatory activity starting, normally from mid 3rd instar larval, can account for the mild phenotype compared to disc carrying *Dsulf1* mutant cells only in the A compartment (b).