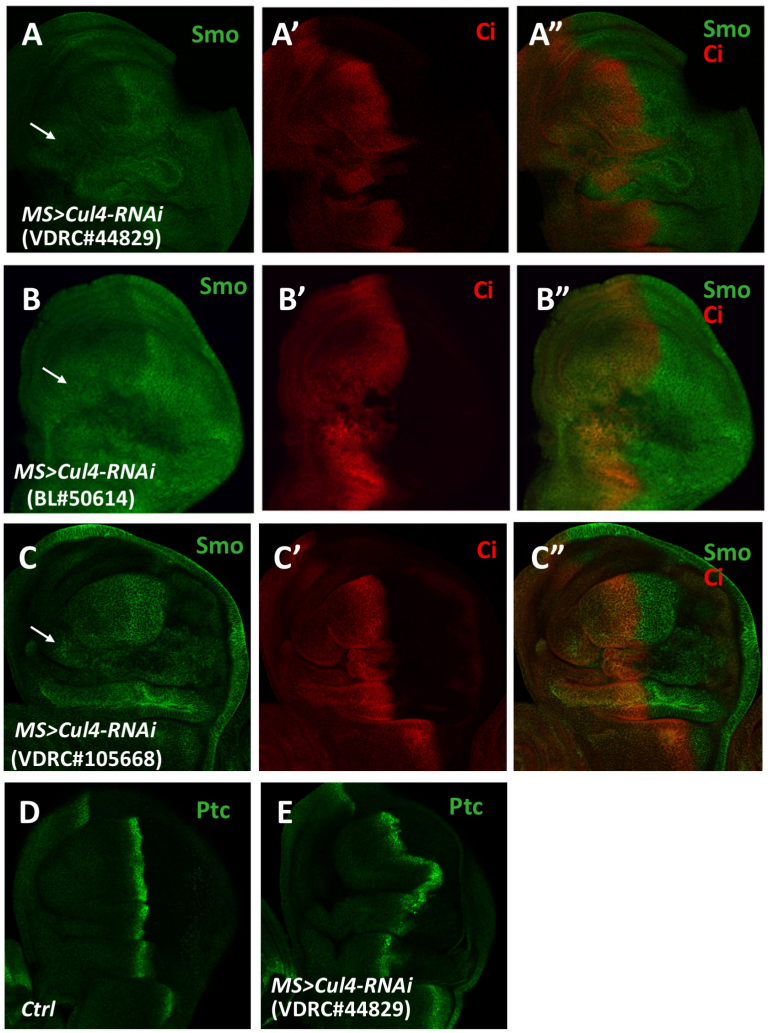

Fig. S1. Cul4 depletion resulted in Smo accumulation in A-compartment cells of wing imaginal discs

(A-C") Late third wing discs expressing the indicated *UAS-Cul4-RNAi* transgenes under the control of the *MS1096* Gal4 driver were immunostained for Smo (green) and Ci (Red). Ci expression marks the anterior compartment. Smo was ectopically accumulated in A-compartment cells distant from the A/P boundary (arrows) when Cul4 was knocked down. (D-E) Late third control (D) and Cul4 RNAi wing discs were immunostained for Ptc expression.

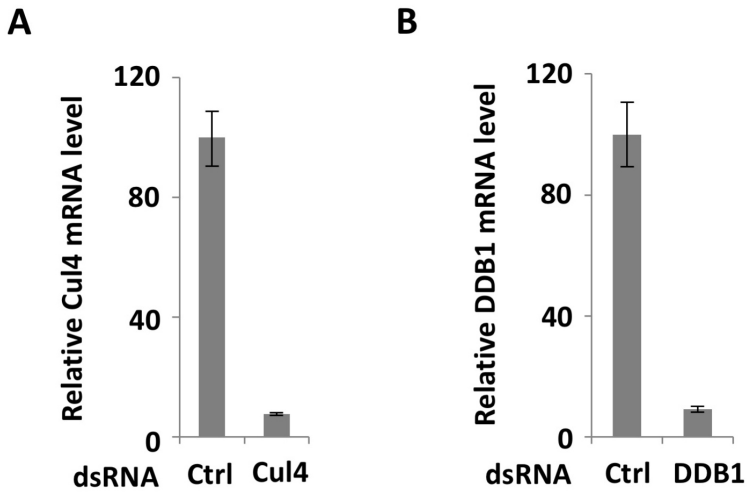

Fig. S2. dsRNA targeting Cul4 or DDB1 diminished their mRNA abundance

(A-B) S2 cells treated with dsRNA targeting Luc (Ctrl), Cul4, or DDB1 were subjected to qRT-PCR analysis to measure the mRNA level of Cul4 (A) or DDB1(B). Data are mean ± SD from three independent experiments.

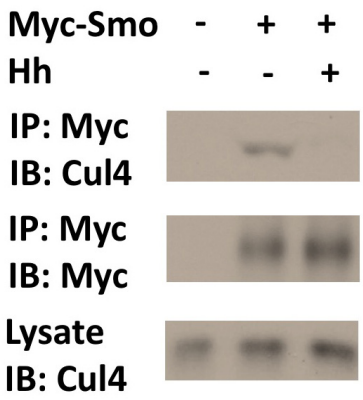

Fig. S3. Hh inhibits the association of Myc-Smo with endogenous Cul4

Immunoblot analysis of CoIP experiments. Myc-Smo transfected into S2 cells pulled down endogenous Cul4, which was inhibited by treating cells with Hh-conditioned medium.

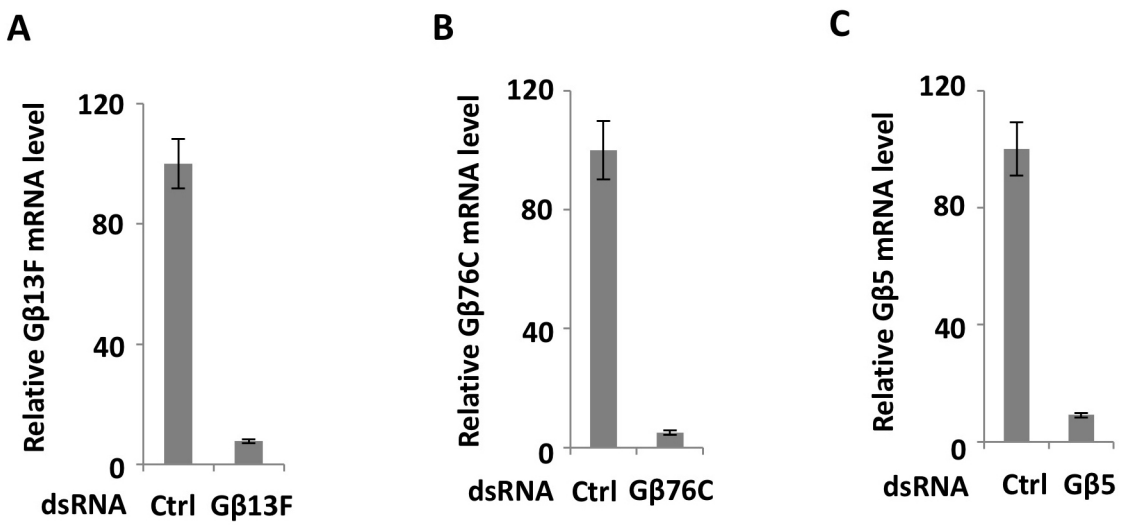

Fig. S4. dsRNA targeting Gβ13F, Fg-Gβ76C, or Gβ5 diminished their mRNA abundance

(A-C) S2 cells treated with dsRNA targeting Luc (Ctrl), Gβ13F, Fg-Gβ76C, or Gβ5 were subjected to qRT-PCR analysis to measure the mRNA level of Gβ13F (A), Fg-Gβ76C (B), or Gβ5 (C). Data are mean ± SD from three independent experiments.

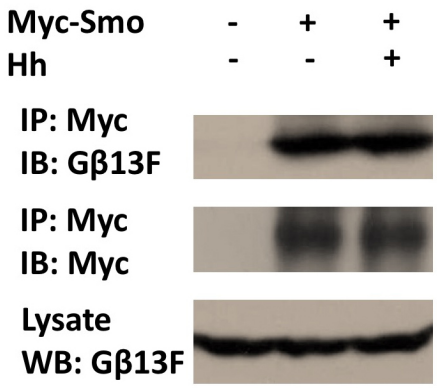

Fig. S5. Hh does not affect the association of endogenous Gβ13F to Myc-Smo

S2 cells stably expressing Myc-Smo were treated with control or Hh-conditioned medium. Cell extracts were immunoblotted with an anti-Gβ13F antibody or immunoprecipitated with an anti-Myc antibody, followed by immunoblotting with the anti-Myc antibody or the anti-Gβ13F antibody. Gβ13F was associated with Smo regardless the presence or absence of Hh.

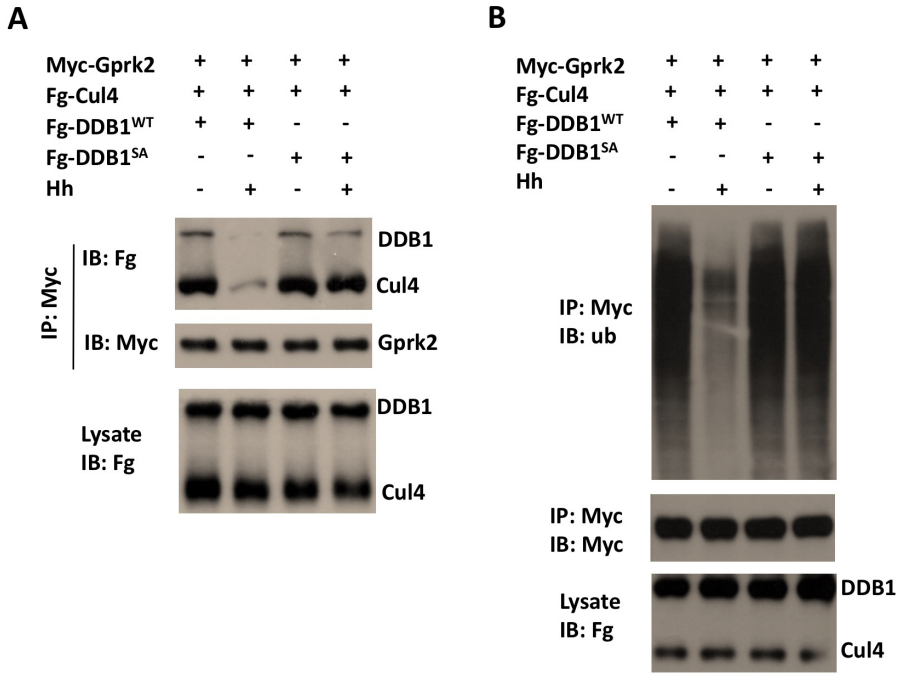

Fig. S6. PKA-mediated phosphorylation of DDB1 regulates the binding of Cul4-DDB1 to and Cul4-mediated ubiquitination of Gprk2

(A) Hh promoted the dissociation of Cul4-DDB1 but not Cul4-DDB1^{SA} from Gprk2. (B) Hh blocked ubiquitination of Gprk2 by Cul4-DDB1 but failed to inhibit ubiquitination of Gprk2 by Cul4-DDB1^{SA}.

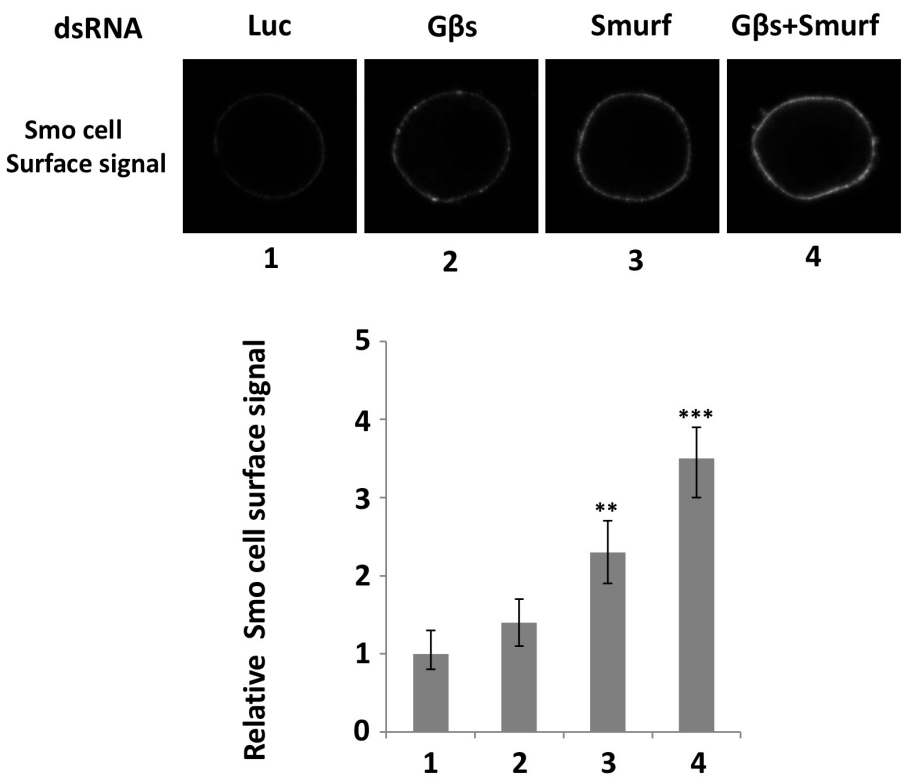

Fig. S7. Gβ acts in parallel with Smurf to regulate Smo cell surface expression

Immunostaining with an anti-Myc antibody prior to membrane permeabilization (top) and quantification (bottom) of cell surface Myc signals in S2 cells stably expressing Myc-Smo and treated with dsRNA for Luciferase (1), Gβ13F and Gβ76C (2), Smurf (3), or Gβ13F, Gβ76C and Smurf (4). Data are mean ± SD from two independent experiments. N=10 cells for each experimental condition. **, P<0.01, ***, P<0.001 (student's t-test).