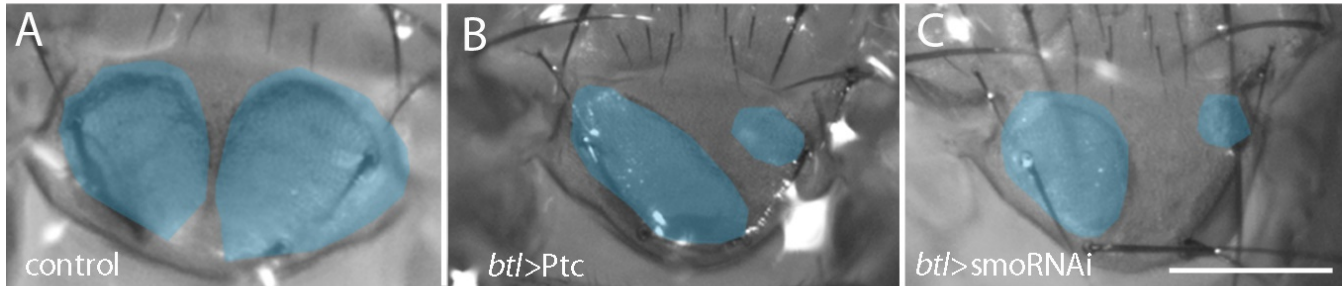


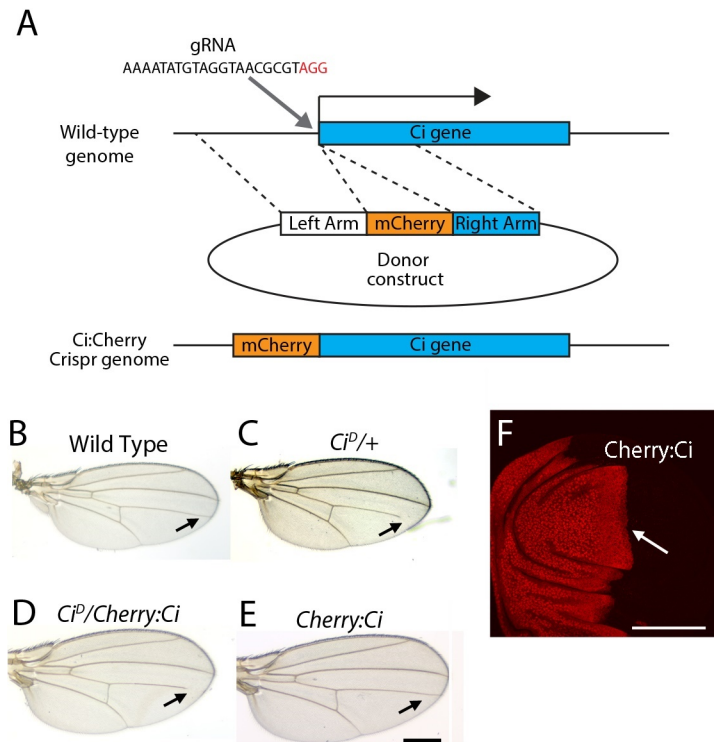
**Fig. S1. Hh signaling regulates cell division rate in ASP**

(A-D') ASPs stained with DAPI (gray) and  $\alpha$ -PH3 (red); control (A,A', *btl-Gal4*>+); Ptc expression (B,B', *btl-Gal4*>Ptc), *Ptc<sup>Δloop2</sup>* (C,C', *btl-Gal4*>*Ptc<sup>Δloop2</sup>*), *smoRNAi* (D-D', *btl-Gal4*>*smoRNAi*). Top and lower panels - upper and lower optical sections, as in Fig. 1A'. ASP outlined by dashed yellow lines; arrows, PH3 positive cells. (E) Number of ASP cells in control and indicated genotypes. (F) Percentage PH3 positive cells. (G-I') Same as (A-I') except stained with DAPI and  $\alpha$ -Caspase (green). (J) Percentage Caspase positive cells. Scale bars: 25 $\mu$ m.



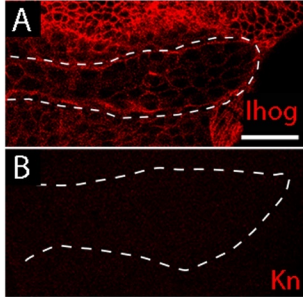
**Fig. S2. Adult dorsal air sac morphology sensitive to Hh signaling**

Abnormal morphology of adult air sacs (blue areas) in flies over-expressing Ptc (B, *btl-Gal4>Ptc*) or smoRNAi (C, *btl-Gal4>smoRNAi*) compared to control (A, *btl-Gal4>+*). Scale bar: 50µm.



**Fig. S3. Cherry:Cb Knock-in**

(A) Design of Cherry:Cb CRISPR knock-in. PAM sequence in gRNA, red. (B-E) Adult wings, arrows indicate portion of vein L4 missing in  $Cb^{P/+}$ . Scale bar: 1mm. (F,G) Frontal views showing fluorescence in wing disc expressing Cherry:Cb. Arrow indicates change in intracellular distribution. Scale bar: 50  $\mu$ m.

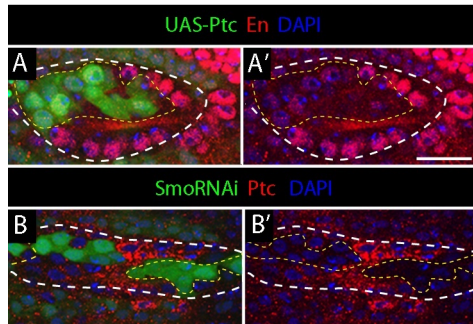


**Fig. S4. Ihog and Kn distributions in the ASP**

(A-B) ASPs (outlined by white dotted line) do not stain with (A)  $\alpha$ -Ihog or (B)  $\alpha$ -Kn antibodies.

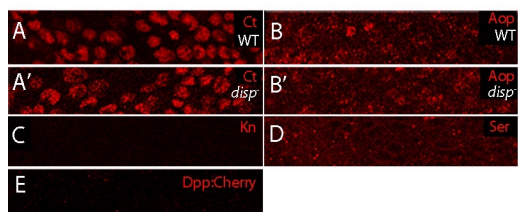
Scale bar: 20 $\mu$ m.





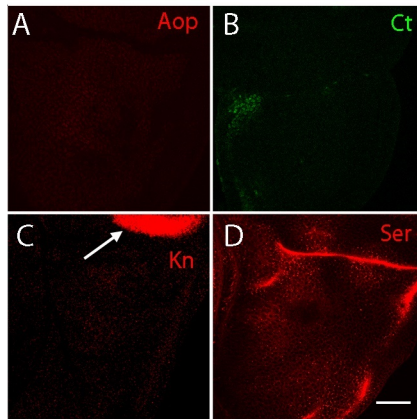
**Fig. S5. Ptc and En expression in the ASP is dependent on Hh signaling**

ASPs outlined with white dashed lines. (A,A') α-En staining (red) is reduced in cell clones (outlined with dashed yellow lines) expressing Ptc (green). (B,B') α-Ptc staining (red) is reduced in cell clones (outlined with dashed yellow lines) expressing smoRNAi (green). Scale bar: 20μm.



**Fig. S6. Myoblast distributions of Ct, Aop, Ser, Dpp, and Kn**

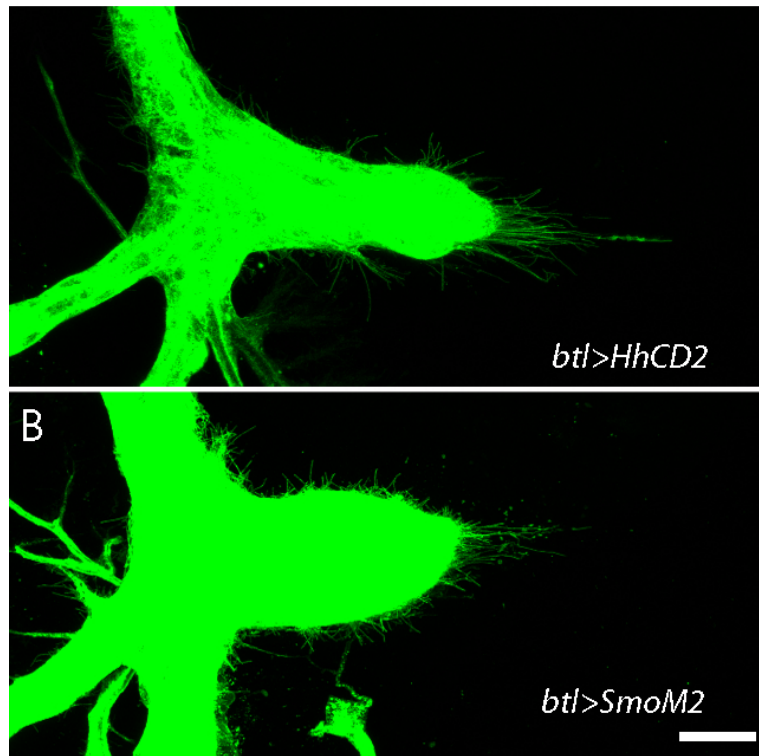
(A-B')  $\alpha$ -Ct and  $\alpha$ -Aop staining in myoblasts of wild type (A,B) and *disp* ASPs (A',B'). (C)  $\alpha$ -Kn antibody staining, (D)  $\alpha$ -Ser antibody staining, (E) Dpp:Cherry fluorescence. Scale bar: 20 $\mu$ m.



**Fig. S7. Notum distributions of Ct, Aop, Ser, and Kn**

Higher magnification images of notum region outlined by dashed yellow lines in Fig. 4A.

Staining by  $\alpha$ -Ct (A),  $\alpha$ -Ser (B),  $\alpha$ -Aop (C), and  $\alpha$ -Kn (D) antibodies. Note Kn staining in wing hinge region (arrow) but not notum. Scale bar: 50 $\mu$ m.



**Fig. S8. Constitutive expression of Hh:CD2 and SmoM2 causes defects in the ASP**  
(A-B) Morphologically abnormal ASPs in genotypes that expressed *HhCD2* (A, *btl-Gal4*, *UAS-mCD8: GFP/+*; *UAS-Hh:CD2/+*) or *SmoM2* (B, *btl-Gal4*, *UAS-mCD8:GFP/+*; *UAS-SmoM2/+*). Scale bar: 20 $\mu$ m.