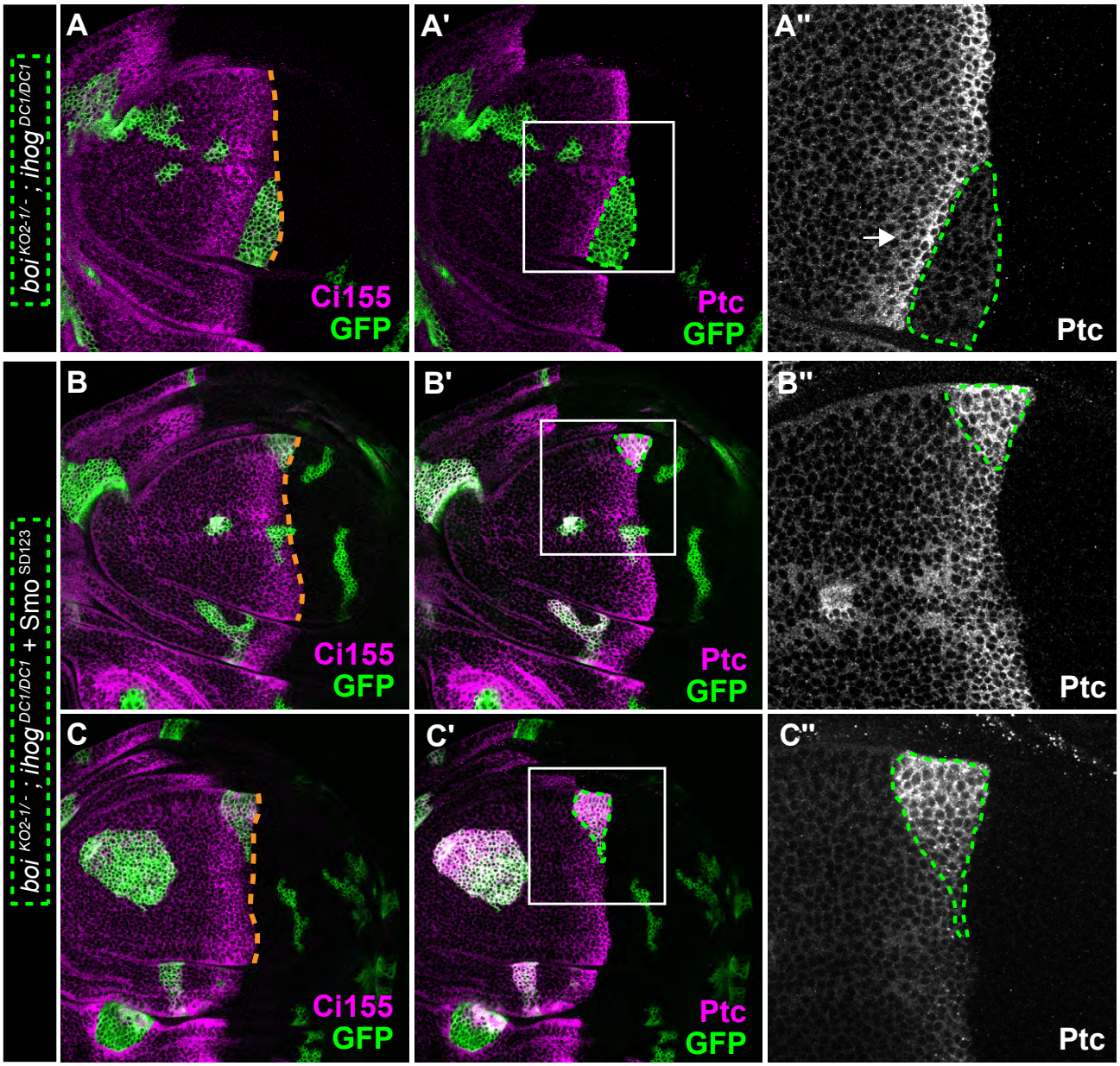

Figure S1. Confirmation with *boi*$^{KO2-1}$ that Ihog and Boi are dispensable for Hh sequestration.

(A-A") A control *ihog*$^{DC1/DC1}$ clone (green outlines in (A') and (A'')) in a *boi*$^{KO2-1/-}$ mutant. (A'') Higher-power view of boxed area in (A'). As expected, *boi*$^{KO2-1/-}$; *ihog*$^{DC1/DC1}$ double mutant cells failed to sequester Hh, as indicated by high levels of Ptc in cells immediately anterior to the clone (arrow in (A'')). Also as expected, they did not upregulate Ptc (GFP-positive, green outline), but were identifiable as anterior in origin since they express basal levels of Ci155 and Ptc. **(B-C")** Two examples of *boi*$^{KO2-1/-}$ *ihog*$^{DC1/DC1}$ double mutant clones expressing *UAS-smo*^{SD123} (green outline in (B' and C') and (B'' and C'')). (B'' and C'') Higher-power view of boxed area respectively in (B' and C'). Each example is adjacent to the anterior-posterior compartment boundary. Expression of *smo*^{SD123} in cells lacking *boi* and *ihog* rescues pathway activation (Ptc expression within clone (B'' and C'')) and is sufficient to sequester Hh as shown by the absence of high levels of Ptc anterior to the clone. Orange dashed lines in (A), (B) and (C) mark the normal position of the anterior-posterior boundary.

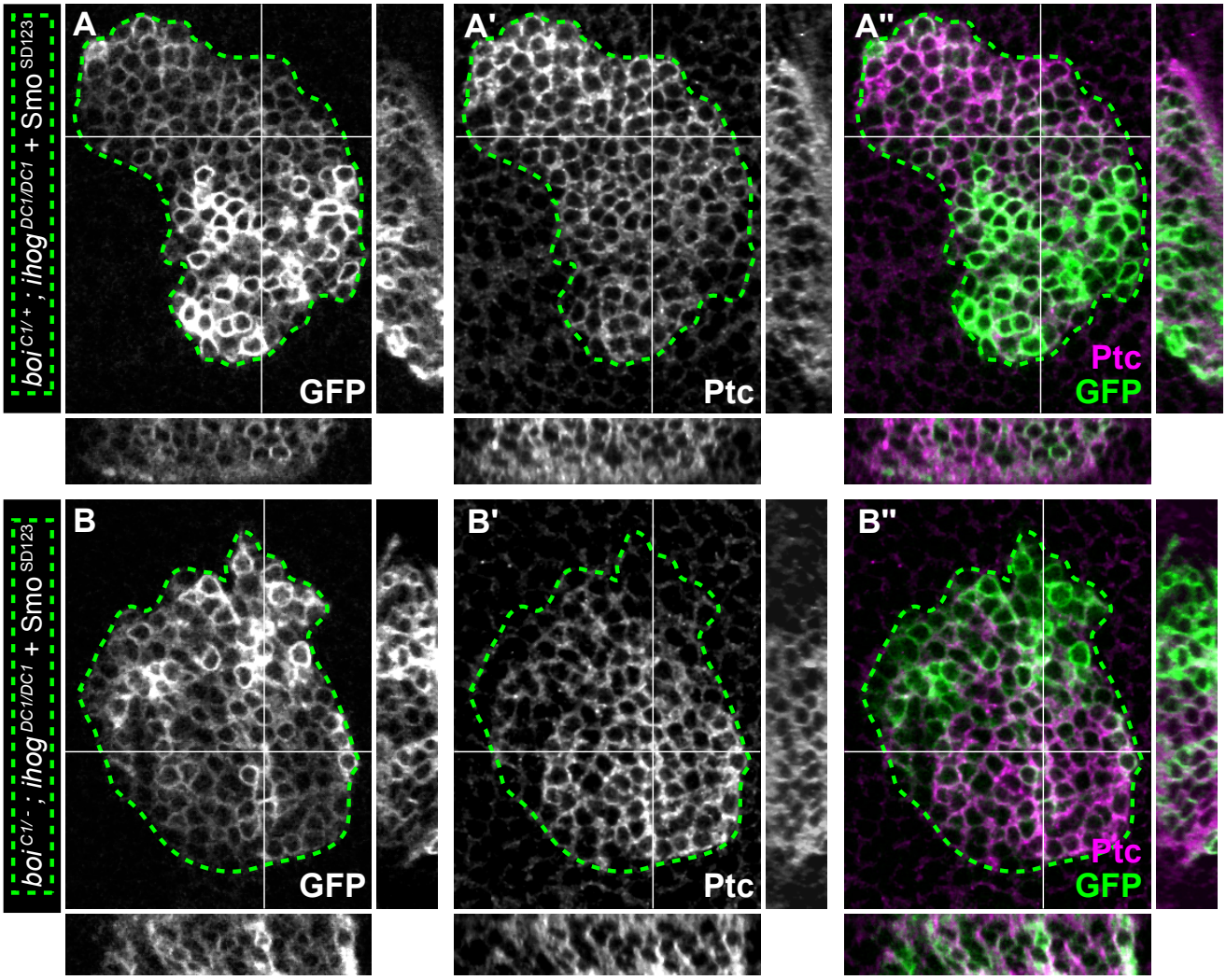

Figure S2. Ptc distribution appears unaffected in cells lacking Ihog and Boi.

(A-A") A control *boi*$^{C1/+}$, *ihog*$^{DC1/DC1}$ clone expressing the membrane-targeted reporter mCD8-GFP (green outline) and *UAS-smo*^{SD123}, which elicits pathway activation and thereby ensures adequate expression of Ptc in these controls and in double mutant clones in B-B''). The distribution of Ptc correlated well with that of mCD8-GFP. **(B-B")** In comparison, neither the degree of Ptc upregulation nor its subcellular distribution were altered when *smo*^{SD123} was expressed in *boi*$^{C1/-}$, *ihog*$^{DC1/DC1}$ double mutant clones (green outlines). The clones displayed were located in the anterior compartment of wing discs. White lines in A-B'' indicate the focal plane at which the representative confocal cross-sections were taken.

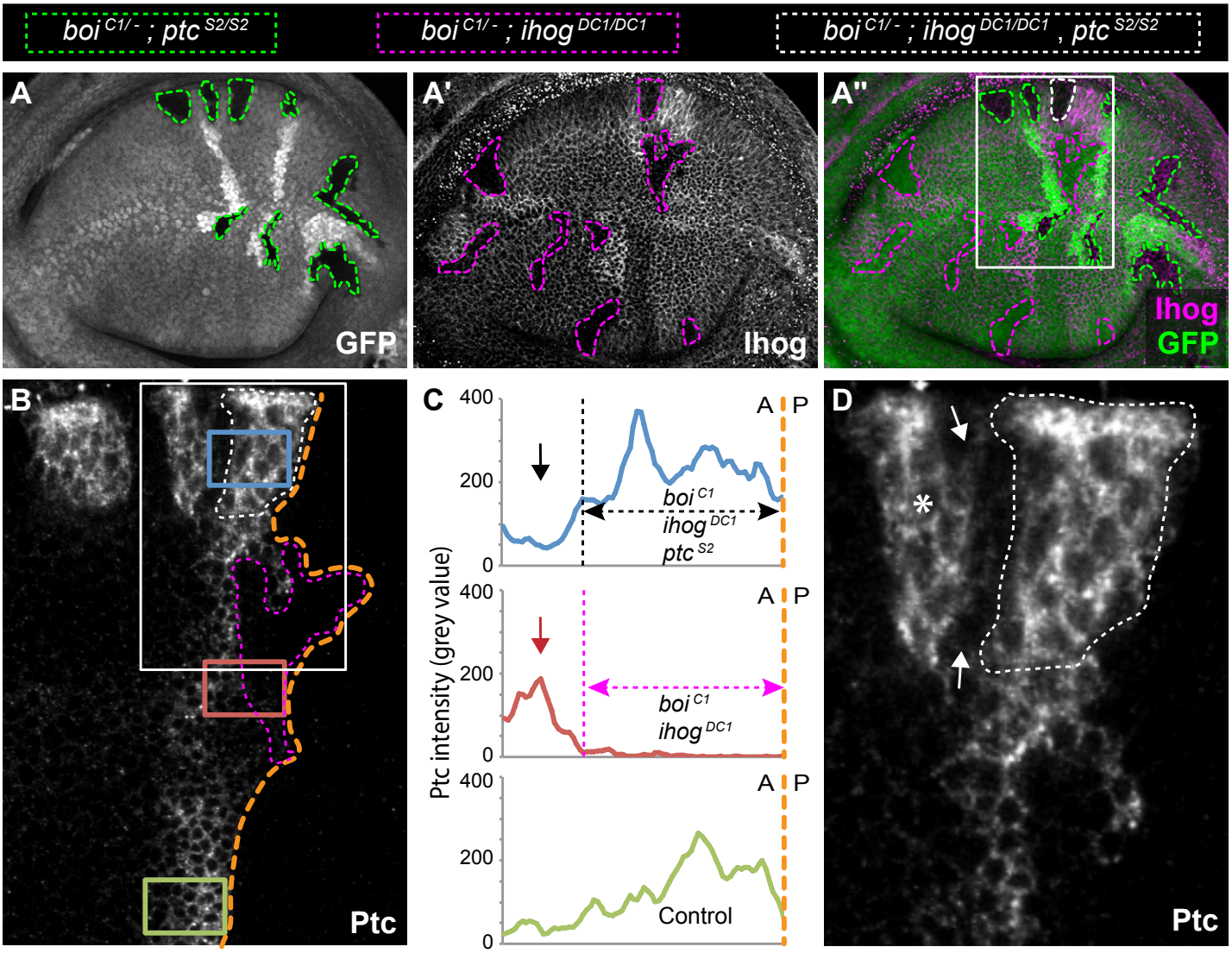

Figure S3. Hh is sequestered by triple mutant cells for Ihog, Boi and Ptc^{S2}.

(A-A") Wing disc clones generated in a *boi*$^{C1/-}$ larva, including *ptc*$^{S2/S2}$ clones (GFP-negative marked by green outlines in (A) and (A")) and *ihog*$^{DC1/DC1}$ clones (Ihog-negative marked by magenta outlines in (A') and (A")). A clone mutant for both *ptc* and *ihog* is indicated by white outlines in (A"). This is triple mutant because it occurs in a *boi*$^{C1/-}$ wing disc. **(B)** Higher-power view of boxed area in (A") showing a *boi*$^{C1/-}$; *ihog*$^{DC1/DC1}$; *ptc*$^{S2/S2}$ triple mutant clone (white outline) and a *boi*$^{C1/-}$; *ihog*$^{DC1/DC1}$ double mutant clone (magenta outline) generated adjacent to anterior-posterior boundary. Colored boxes: region of interest including five rows of cells starting from the anterior-posterior boundary (orange dashed line). Green box: control area containing no clones. Blue and red boxes: first three rows of cells located within a clone followed by 2 rows of cells just anterior to it. **(C)** Average Ptc intensity within corresponding colored boxed areas in (B). Top: Ptc intensity is elevated in within triple mutant clones (marked black double-headed arrow) but not just anterior (black arrow), indicating Hh sequestration. Middle: Hh is not sequestered by cells lacking Ihog and Boi (magenta double-headed arrow), and instead there is elevated Ptc expression (marking pathway activation) just anterior to the clone (red arrow). **(D)** Higher-power view of white boxed area in (B). A triple mutant clone (white outline) near the compartment boundary can sequester Hh, as indicated by low levels of Ptc just anterior to the clone (region between white arrows). More anteriorly, elevated Ptc staining (white asterisk) was the result of ectopic Ptc expression induced within another, separate *ihog*$^{DC1/DC1}$, *ptc*$^{S2/S2}$ clone.