

Inventory of Supplemental Information

Figure S1: This figure shows that inactivation of *Cdon* in *Boc*^{-/-} cerebella does not further decrease the proliferation of CGNPs in response to Shh, indicating that the residual Shh-dependent proliferation observed in *Boc*^{-/-} CGNPs (Fig. 2I) is not due to expression of *Cdon* at earlier stages of development. It supports Fig. 2.

Figure S2: (A, B) This figure presents the immunostaining of E18.5 *Gas1*^{-/-};*Boc*^{-/-} cerebella with anti-Lim1 and anti-Pax6 antibodies to show that CGNPs are specified and localize normally. This figure supports Fig. 4A by showing that the decrease in proliferative CGNPs is not due to a lack of specification of these cells. (C) This figure shows that inactivation of *Gas1* and *Boc* does not lead to a change in *Cdon* expression in the cerebellum; therefore, the small EGL remaining in *Gas1*^{-/-};*Boc*^{-/-} cerebella is not due to an ectopic expression of *Cdon*. This figure supports Fig. 4A. (D) This figure illustrates the methodology we used to measure the thickness of the EGL along the postero-anterior axis presented in Fig. 4B (E) This figure shows that treatment with higher doses of ShhN (60 and 90 nM) does not further increase the proliferation of E18.5 *Ctl* (*Gas1*^{+/+};*Boc*^{-/-} and *Gas1*^{+/-};*Boc*^{-/-}) and *Gas1*^{-/-};*Boc*^{-/-} CGNPs when compared to proliferation levels obtained at 30 nM ShhN. This figure is in support of Fig. 4F

Figure S3: (A, B, C) These figures present controls done to support immunoprecipitation experiments and cell surface binding assays shown in Fig. 5. We find that the Boc/Ptch1 and Gas1/Ptch1 interactions are specific, as both Disp1 and Smo, two multi-span transmembrane proteins implicated in Shh signaling, failed to interact with either Boc (A, B, C) or Gas1 (A, B).

Figure S4: This figure illustrates the methodology used for the double-immunoprecipitation assays presented in Figure 5F.

Figure S5: This figure shows that in mammalian cells neither Boc nor Gas1 appear to play a major role in Ptch1 relocalization to the cell surface of COS7 nor NIH 3T3 cells. We show this both in immunostaining experiments (A and B) and surface biotinylation (C) experiments. This figure supports Fig. 7.

Supplemental Table 1: This table is a summary of the dissociation constants (K_d) calculated for the Shh mutants used in this study. This table supports figure 6.