Segregation of ipsilateral retinal ganglion cell axons at the optic chiasm requires the Shh receptor Boc.

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Supplementary material

Supplementary Figure Legends

Figure S1. Cdon is evenly distributed in the developing retina. A-A”, Cdon expression as detected by β-gal histochemistry on horizontal sections of Cdon<sup>+/lacZ</sup> E15 mice. Cdon is expressed throughout the retina, both in the GCL and the NBL. Similar levels of expression are observed in the ventro-temporal crescent (A”) and the ventro-nasal crescent (A’). B-B”” Immunostaining of horizontal sections of E17 rat retina shows that Cdon (green) colocalizes with neurofilament (red) in RGC axons in the RAL. Nuclear staining (DAPI, blue) shows the absence of cell bodies in the RAL. VT, ventro-temporal retina; NBL, neuroblast layer; GCL, ganglion cell layer; RAL, RGC axon layer; NF, neurofilament. Scale bars: A, 100 µm; A’-A”, 50 µm; B-B””, 20 µm.

Figure S2. Boc and EphB1 colocalize in RGC axons. Confocal images show the colocalization of Boc (goat anti-Boc antibody) and EphB1 in RGC axons of E17 ventral rat retina (horizontal section). The arrow points to RGC axons that are positive for Boc and EphB1. NBL, neuroblast layer; GCL, ganglion cell layer. Scale bar, 10 µm.

Figure S3. Characterisation of the axons growing out of retinal explants. E17 rat retinal explants were cultured 24 hr in vitro, fixed and immunostained. A,B, Axons extending over long distances outside the explants are all positive for the RGC marker neurofilament (green). Nuclear staining (DAPI) shows the position of the explant. (blue, B). Retinal axons exiting the explants are also positive for Tag1, a cell
surface marker expressed by RGCs from the ventro-temporal crescent (C). D-F, Boc (goat antibody) and EphB1 colocalize in RGC axons and growth cones. Arrows indicates growth cones that are positive for Boc and EphB1. Scale bars: A,B, 25µm; C-F, 10 µm.

**Figure S4.** ShhN induces dose dependent RGC axon retraction. E17 rat retinal explants characterized in Fig. S3 were treated with different concentrations of the N-terminal form of Shh (ShhN). The relative retraction represents the number of axons retracting versus the number of axons growing.

**Figure S5.** Quantification of the collapse response shown in Fig. 3C-D. The bars represent the percentage of E17 rat RGC growth cones that have collapsed 20 min after the addition of 10 nM ShhN.
Figure S3
Figure S4
Figure S5