Supplemental information

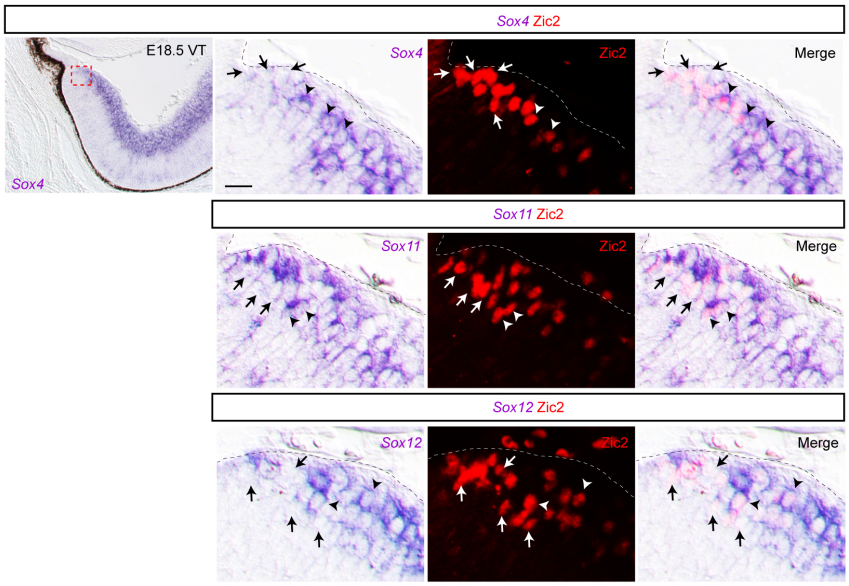

Figure S1. Related to Figure 1: Expression of SoxC mRNAs in the retina at E18.5. Expression of *Sox4*, *Sox11* and *Sox12* mRNAs in VT retina where Zic2^{+} reside. As seen in the same section, the majority of Zic2 cells in this section do not express SoxC TFs (arrow), but a few RGCs at the border of the zone where Zic2 expression ceases express SoxC TFs (arrowheads). VT, ventrotemporal. Scale bars: 20 μm.

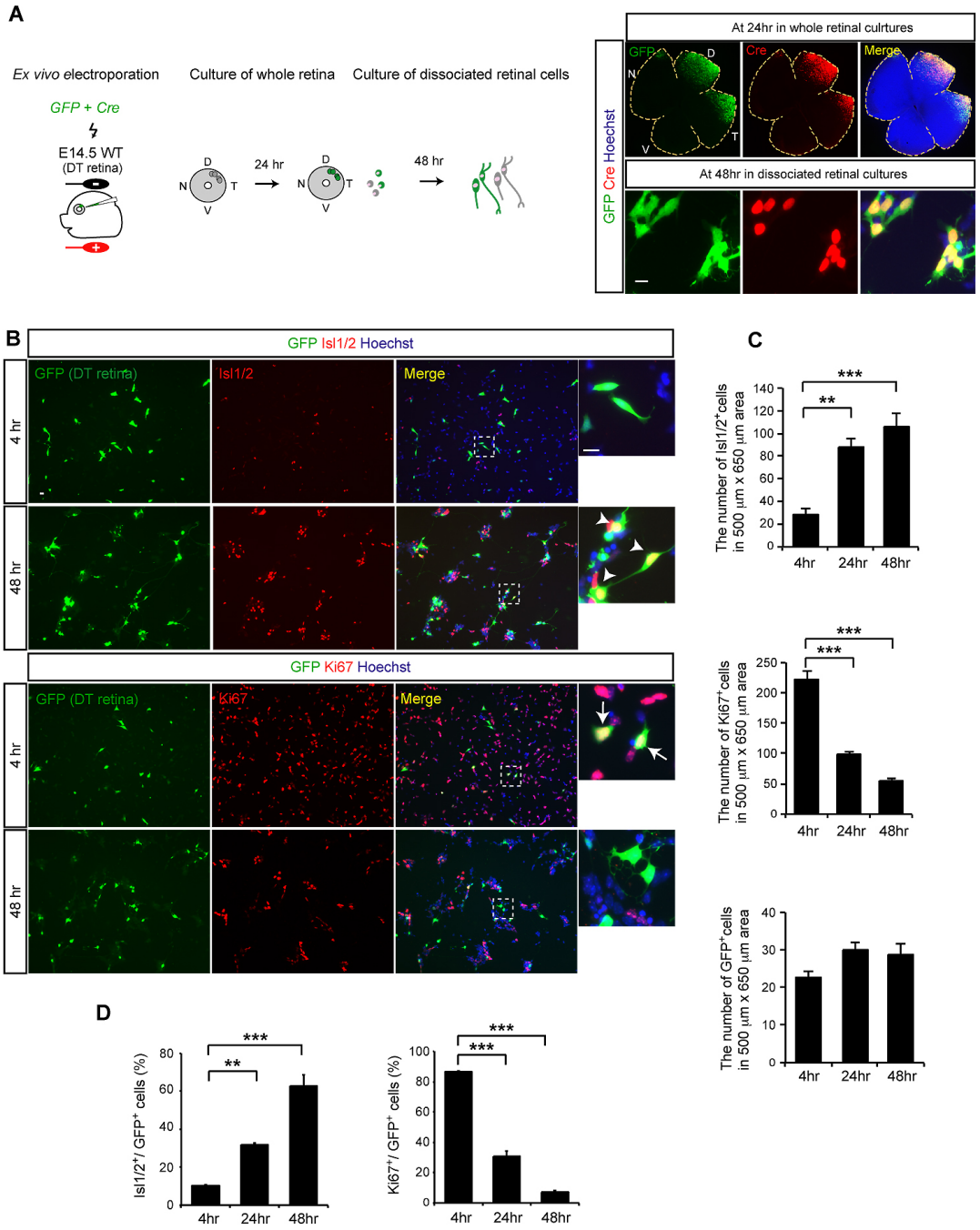

Figure S2. Related to Figure 2: Expression of GFP and Cre in electroporated DT retina and time course of maturation of GFP$^+$ DT dissociated retinal cell cultures.

(A) Schema of *ex vivo* electroporation of *CAG-GFP* and *CAG-Cre* plasmids into E14.5 WT DT retina and cell cultures. GFP$^+$/Cre$^+$ DT retinal cells were detected in eyecup

cultures after 24 hr and still detected in dissociated retinal cell culture. (B) After *ex vivo* electroporation of *CAG-GFP* into DT retina, and whole retinal culture, at 4 hr dissociated retinal cultures, most GFP^+ DT retinal cells express Ki67 (arrows), while after 48 hr most GFP^+ DT retinal cells express Islet1/2 (Isl1/2) (arrowheads). (C) Quantification of the number of $Isl1/2^+$, $KI67^+$ and GFP^+ cells per area = 500 μm x 650 μm: micrographs taken with a 20 X objective at 4, 24 and 48hr in dissociated retinal cultures. n =3, one-way ANOVA with repeated measures. (D) Quantification of $Isl1/2^+/GFP^+$ cells (%) and $Ki67^+/GFP^+$ cells (%) in WT DT retina (>290 of total GFP^+ cells counted for each condition, n = 3, one-way ANOVA with repeated measures). DT, dorsotemporal. Scale bars: 10 μm. ** $p<0.01$, *** $p<0.001$.

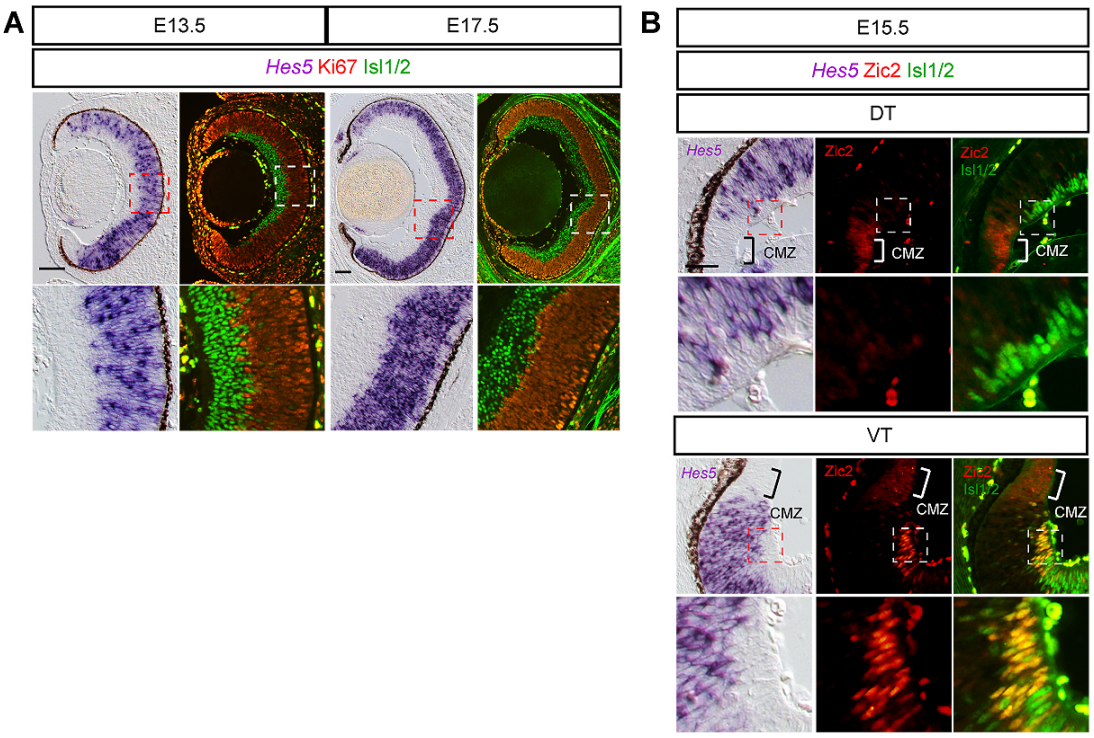

Figure S3. Related to Figure 3: Hes5 is expressed by progenitor cells in the retina. (A) *Hes5* mRNA is expressed in Ki67^{+} progenitors at E13.5 and 17.5 (same sections at each age). (B) *Hes5* mRNA is not expressed in Islet1/2^{+} RGCs in contralateral (DT and non-VT) and ipsilateral (VT) retina nor in the CMZ (brackets) at E15.5, when ipsilateral and contralateral RGCs are segregated in this region. DT, dorsotemporal; VT, ventrotemporal. Scale bars: 100 μm.

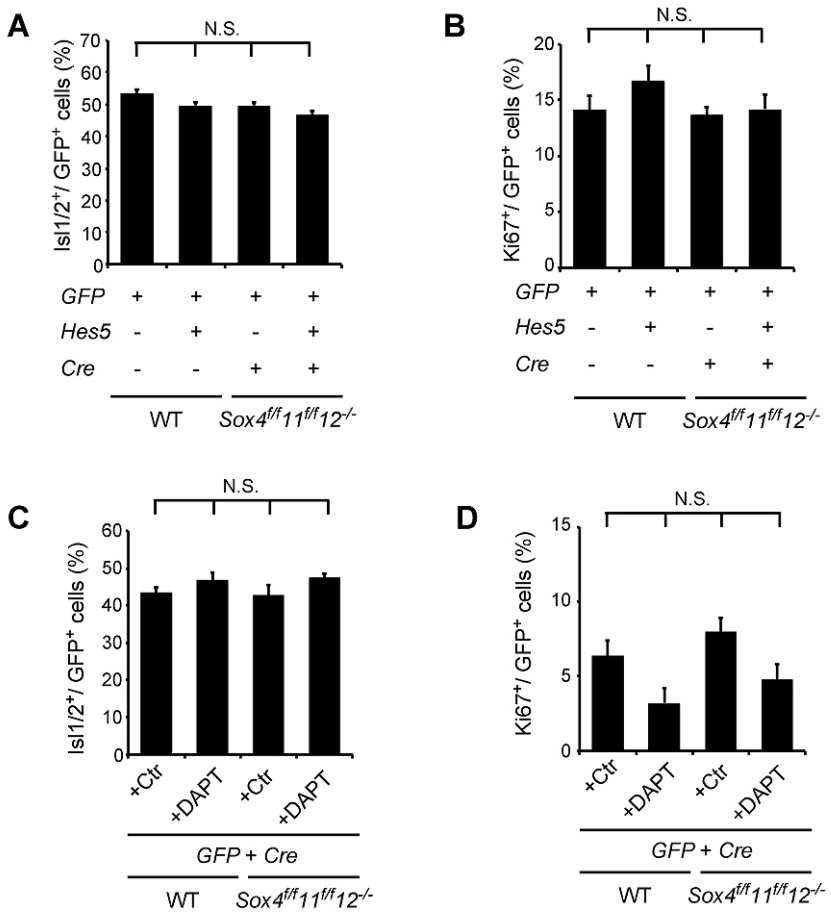

Figure S4. Related to Figure 3: RGC differentiation in VT retina is not affected by Notch-Hes5 signaling. (A, B) Quantification of Islet1/2^{+}/GFP^{+} cells (%) (A) and Ki67^{+}/GFP^{+} cells (%) (B) in WT or *Sox4*$^{-/-}$*Sox11*$^{-/-}$*Sox12*$^{-/-}$ VT retina after Hes5 is overexpressed at E14.5, but counted after 48 hours in dissociated culture (>370 of total GFP^{+} cells counted for each condition, n = 3, one-way ANOVA). (C, D) Quantification of Islet1/2^{+}/GFP^{+} cells (%) (C) and Ki67^{+}/GFP^{+} cells (%) (D) in WT or *Sox4*$^{-/-}$*Sox11*$^{-/-}$ *Sox12*$^{-/-}$ VT retinal cells cultured with DAPT or with DMSO alone (control) (>260 of total GFP^{+} cells counted for each condition, n = 4, one-way ANOVA.). N.S. not significant.

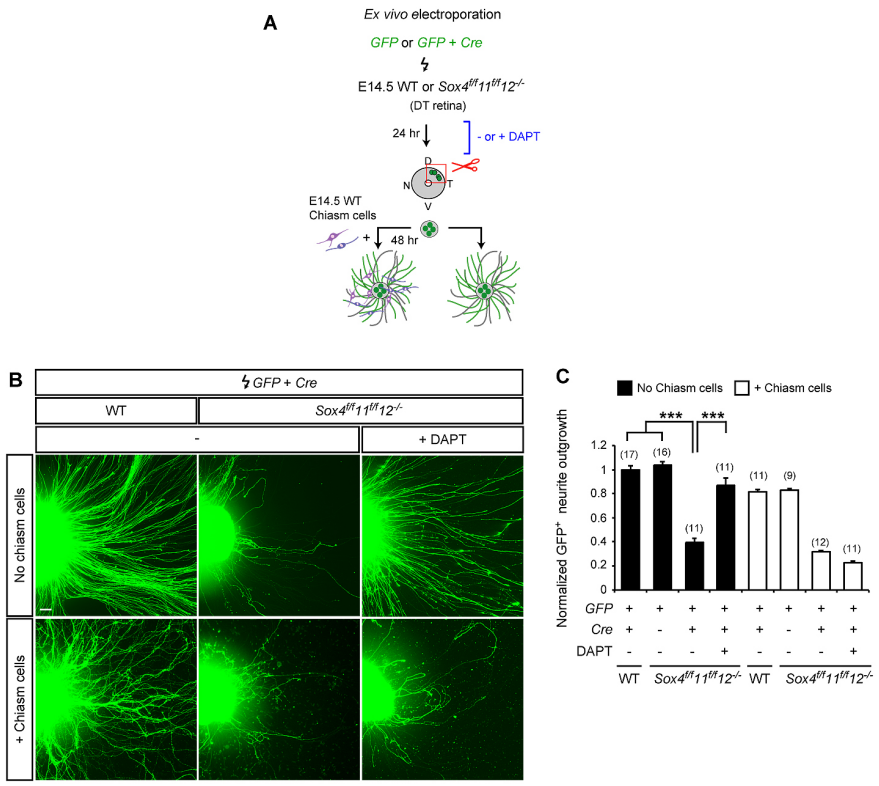

Figure S5. Related to Figure 5: Axon growth with and without DAPT to block Notch signaling

(A) Schema of *ex vivo* electroporation into E14.5 WT or *Sox4*$^{f/f}$*Sox11*$^{f/f}$*Sox12*$^{-/-}$ DT retina *ex vivo*, culture of the whole retina with or without DAPT, and analysis of GFP^{+} axon outgrowth after 48 hr in DT retinal explants with and without chiasm cells. (B) Representative images of WT or *Sox4*$^{-/-}$*Sox11*$^{-/-}$*Sox12*$^{-/-}$ GFP^{+} DT axon outgrowth in the presence or absence of chiasm cells, with or without DAPT treatment. DAPT rescues differentiation defects (neurite outgrowth) in SoxC mutant explants, but not when on chiasm cells. (C) Quantification of WT, *Sox12*$^{-/-}$, *Sox4*$^{-/-}$*Sox11*$^{-/-}$*Sox12*$^{-/-}$ GFP^{+} DT retinal outgrowth in the presence or absence of chiasm cells with or without DAPT. (n) = number of explants for each condition, two-way ANOVA. DT, dorsotemporal. Scale bars: 40 μm. *** $p<0.001$

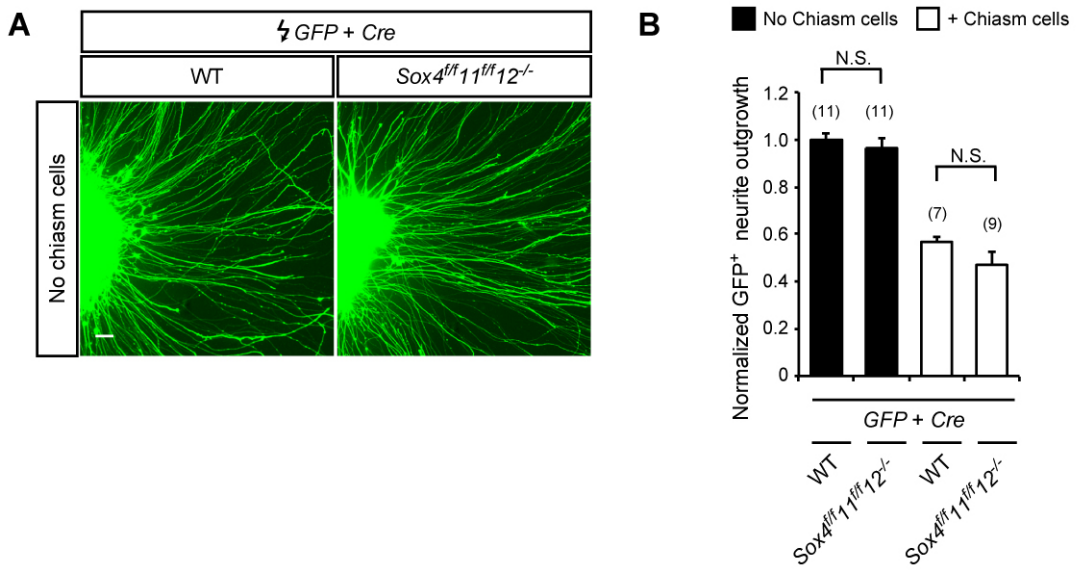

Figure S6. Related to Figure 5: SoxC transcription factors do not affect RGC axon outgrowth from VT retinal explants. (A) Representative images of WT or *Sox4*$^{-/-}$ *Sox11*$^{-/-}$*Sox12*$^{-/-}$ GFP^{+} VT axon outgrowth without chiasm cells. (B) Quantification of WT, *Sox12*$^{-/-}$, *Sox4*$^{-/-}$*Sox11*$^{-/-}$*Sox12*$^{-/-}$ GFP^{+} VT retinal outgrowth in the presence or absence of chiasm cells (n) = number of explants for each condition, two-way ANOVA. VT, ventrotemporal. Scale bar: 40 μm. N.S. not significant.

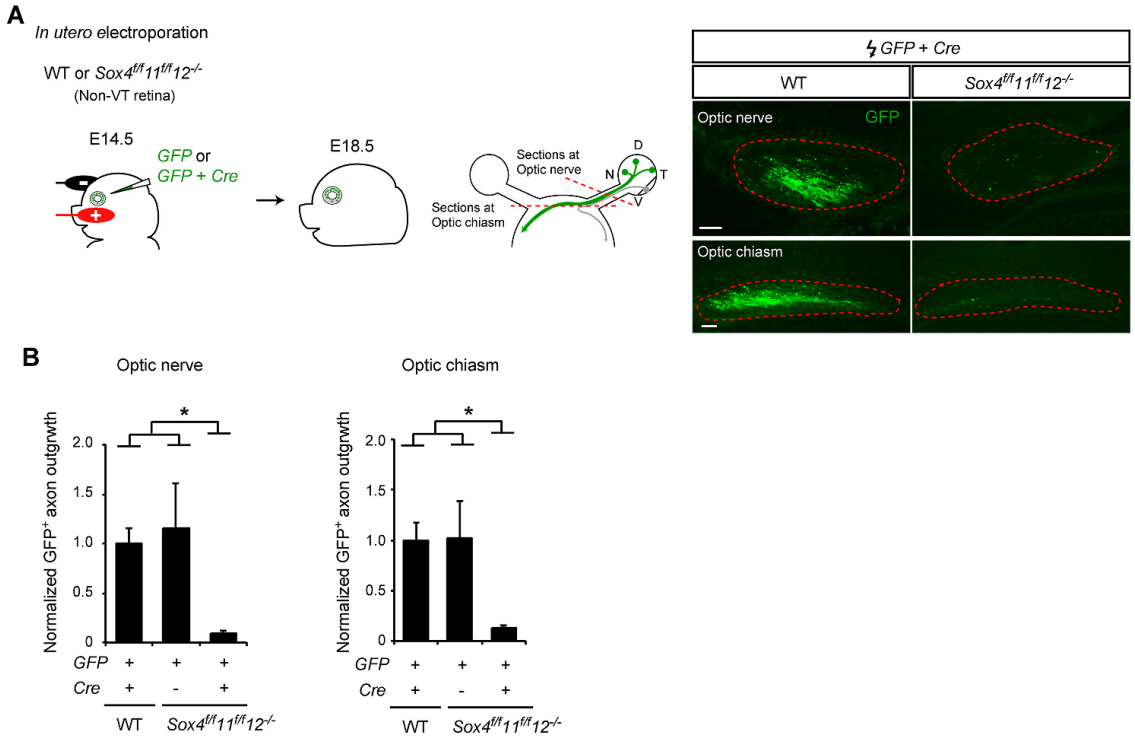

Figure S7. Related to Figure 6: Deletion of SoxC genes by *in utero* retinal electroporation of *Cre* leads to defective RGC axon outgrowth.

(A) *In utero* retinal electroporation of *CAG-GFP* alone or both *CAG-GFP* and *CAG-Cre* plasmids into E14.5 WT or *Sox4*$^{f/f}$*Sox11*$^{f/f}$*Sox12*$^{-/-}$ non-VT retina and analysis of RGC axon projection at E18.5. Axon outgrowth is impaired in *Sox4*$^{-/-}$*Sox11*$^{-/-}$*Sox12*$^{-/-}$ electroporated retina in the optic nerve and optic chiasm. (B) Quantification of GFP^{+} axon outgrowth (normalized to WT) of WT, *Sox12*$^{-/-}$ and *Sox4*$^{-/-}$*Sox11*$^{-/-}$*Sox12*$^{-/-}$ non-VT retina to optic nerve and the optic chiasm. n = 3 – 4, one-way ANOVA. Scale bar: 100 μm. * $p<0.05$.

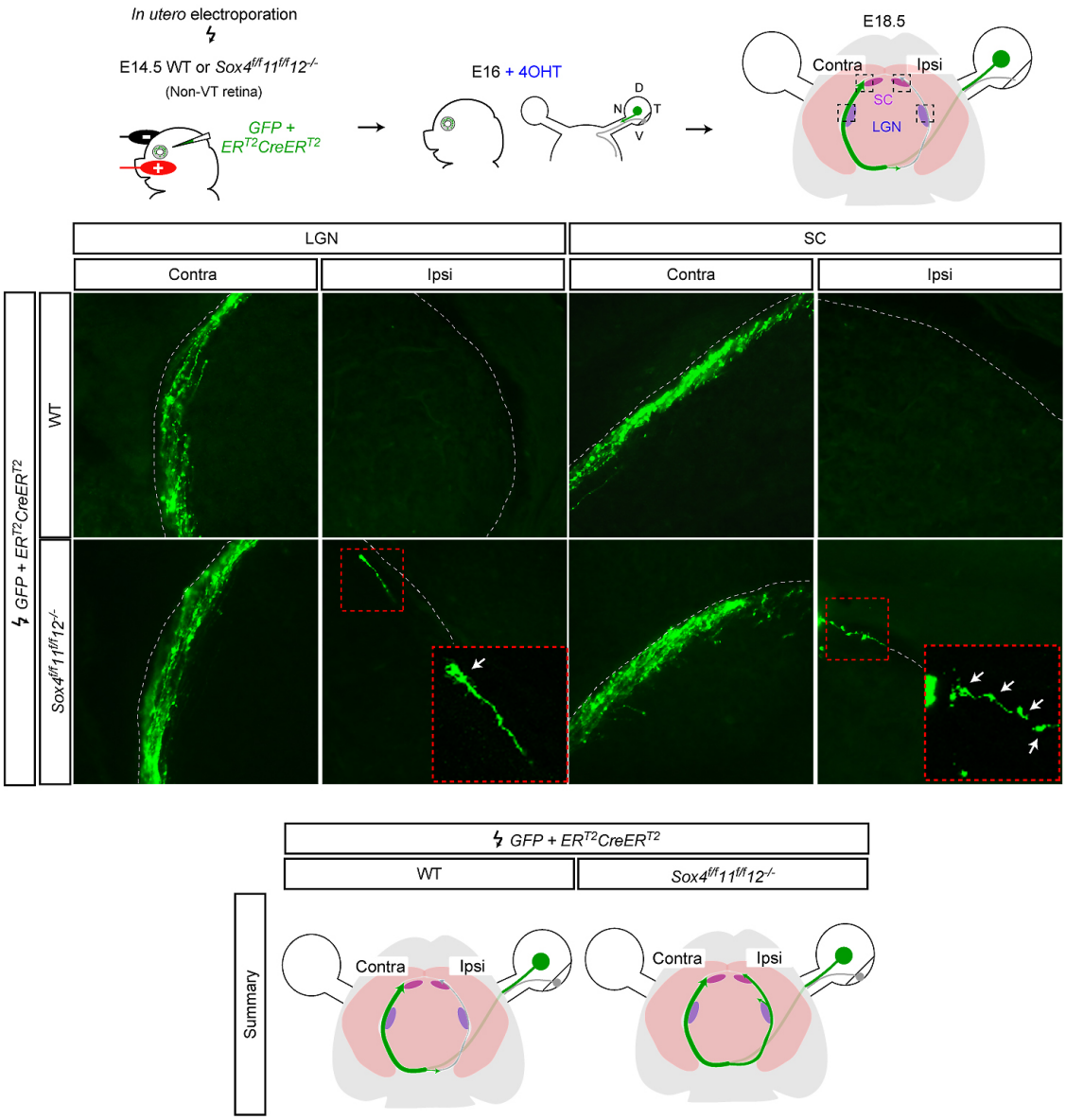

Figure S8. Related to Figure 6: RGC axon projection in postmitotic RGCs after deletion of SoxC genes after growth has commenced.

In utero retinal electroporation of *CAG-GFP* and *CAG-ER^{T2}CreER^{T2}* plasmids into E14.5 WT or *Sox4$^{f/f}$Sox11$^{f/f}$Sox12$^{-/-}$* central retina, followed by injection of 4OHT i.p. at E16, and analysis of contralateral and ipsilateral RGC retinal axon projections to the superior colliculus (SC) and lateral geniculate nucleus (LGN) at E18.5.