

Figure S1, related to Figure 3. Negligible coexpression of Tbr2 and Tph2^{Cre} in image-stabilizing RGCs

(A-D) Whole-mount retinas from Tph2^{Cre}-tdTomato (red)::Hoxd10-GFP (green) mice, immunostained for Tbr2 (blue). Arrowheads indicate Hoxd10-RGCs with Tbr2 and Tph2^{Cre} coexpression and dashed circles indicate Hoxd10-RGCs that lack Tph2^{Cre}. Scale bar, 25 μ m. (E) Quantification of % of Hoxd10-RGCs coexpressing Tbr2 and

Tph2^{Cre}. Data are represented as mean \pm SEM (n = 3 mice).

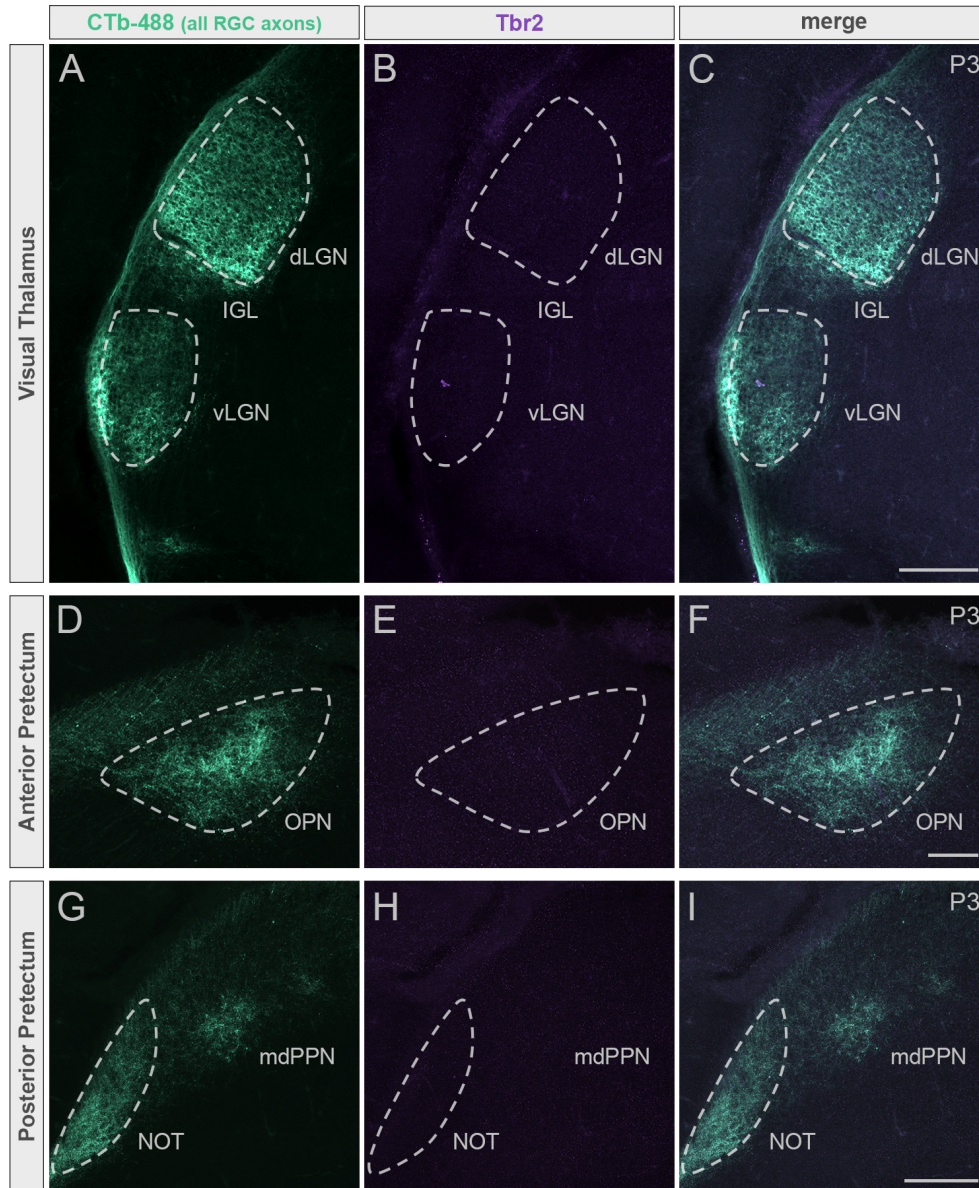


Figure S2, related to Figure 3. Tbr2 is not expressed in retinorecipient subcortical targets

(A-I) Coronal view of all RGC axons (CTb-488, green) and staining for Tbr2 (purple) in wildtype mice at P3. Scale bars, 100 μ m (D-F); 200 μ m (A-C and G-I). dLGN, dorsal lateral geniculate nucleus; IGL, intergeniculate nucleus; vLGN, ventral lateral geniculate nucleus; mdPPN, medial division of the posterior pretectal nucleus; NOT, nucleus of the optic tract; OPN, olivary pretectal nucleus.

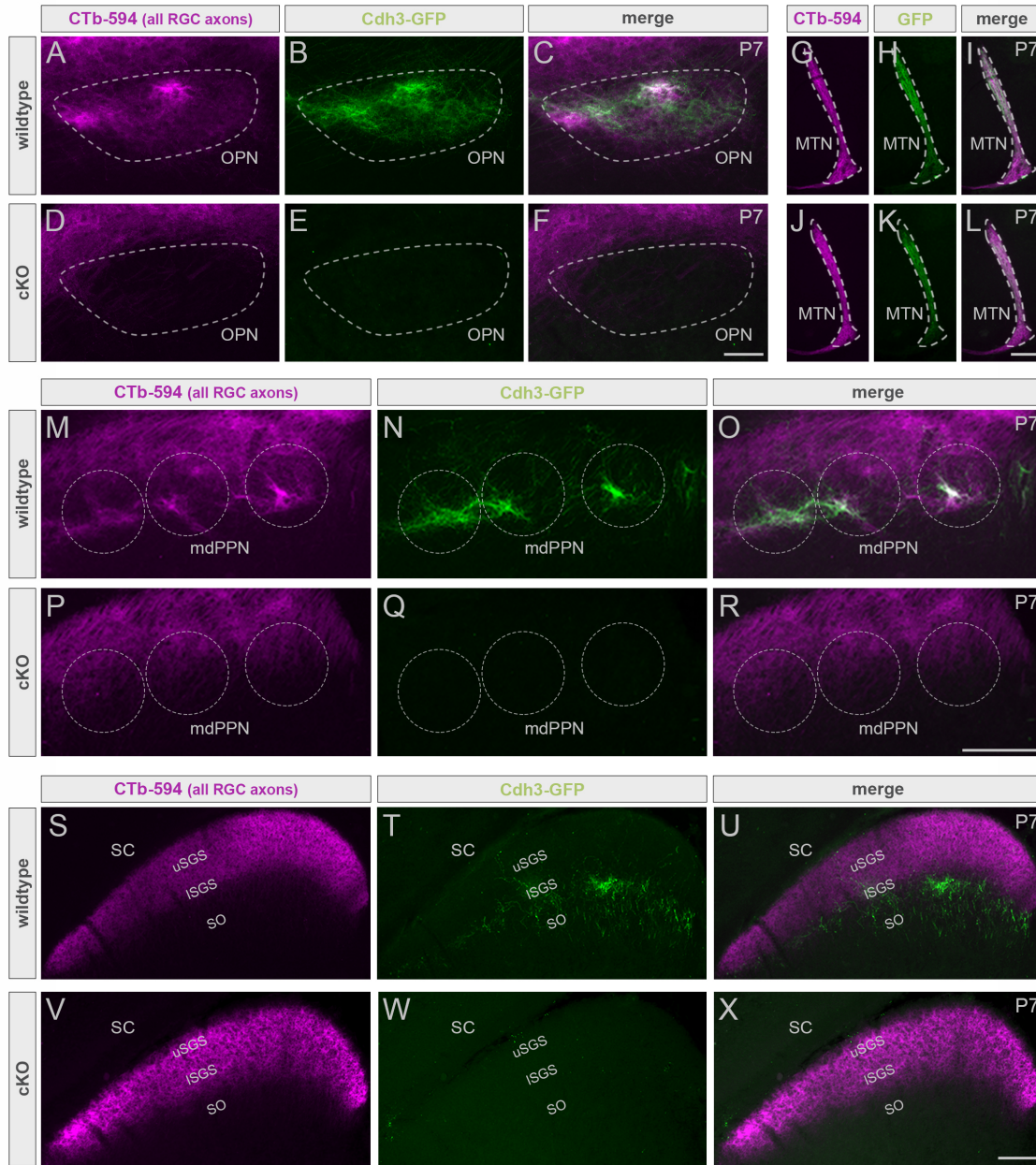


Figure S3, related to Figure 4. Ambient-luminance-sensing RGCs are absent from pretectum by the end of the first postnatal week

(A-X) All RGC (CTb-594, magenta) and ambient-luminance-sensing RGC (Cdh3-GFP, green) axons in wildtype (top panels) and cKO (bottom panels) mice at P7. Scale bars, 100 μ m (A-F); 200 μ m (G-X). mdPPN, medial division of the posterior pretectal nucleus; MTN, medial terminal nucleus; NOT, nucleus of the optic tract; OPN, olivary pretectal nucleus; SC, superior colliculus; uSGS, upper stratum griseum superficiale; lSGS, lower stratum griseum superficiale; SO, stratum opticum.

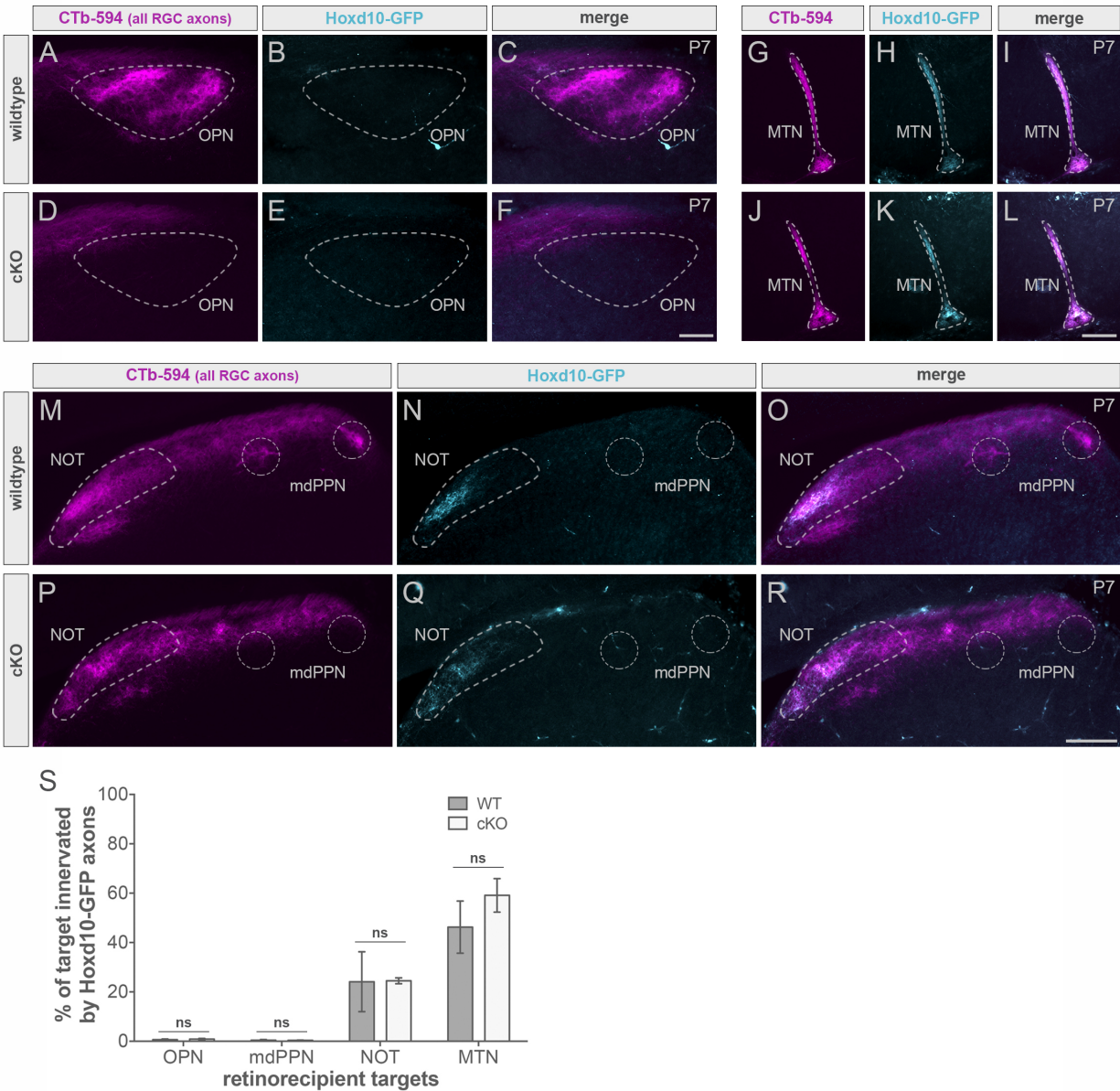


Figure S4, related to Figure 5. Image-stabilizing RGCs grow beyond the location of the OPN and mdPPN in the absence of ambient-luminance-sensing retinal ganglion cells

(A-R) All RGC (CTb-594, magenta) and image-stabilizing RGC (Hoxd10-GFP, cyan) axons in wildtype (top panels) and cKO (bottom panels) mice at P5-P8. Scale bars, 100 μ m (A-F); 200 μ m (G-R). mdPPN, medial division of the posterior pretectal nucleus; MTN, medial terminal nucleus; NOT, nucleus of the optic tract; OPN, olivary pretectal nucleus. (S) Quantification of % of target innervated by Hoxd10-GFP axons in wildtype (WT, dark gray) and cKO (light gray) mice. Data are represented as mean \pm SEM (n = 4 mice); Student's *t*-test.

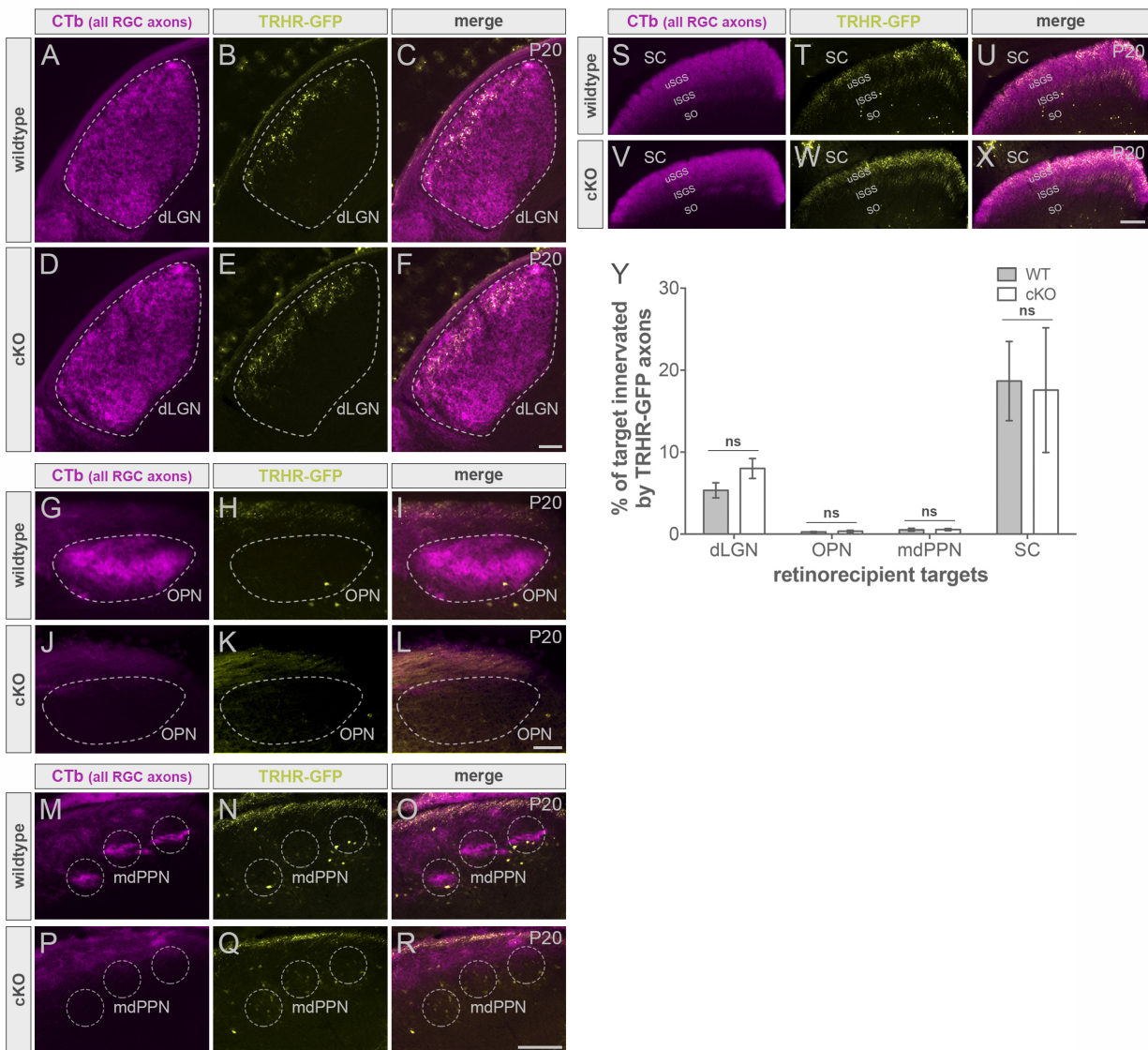


Figure S5, related to Figure 5. The retinofugal axonal innervation pattern of posterior-tuned On-Off DSGCs is not affected in the absence of ambient-luminance-sensing RGCs

(A-X) All RGC (CTb-594, magenta) and posterior-tuned On-Off DSGC (TRHR-GFP, yellow) axons in wildtype (top panels) and cKO (bottom panels) mice at P20. Scale bars, 100 μ m (A-L); 200 μ m (M-X). dLGN, dorsal lateral geniculate nucleus; mdPPN, medial division of the posterior pretectal nucleus; OPN, olivary pretectal nucleus; SC, superior colliculus; uSGS, upper stratum griseum superficiale; ISGS, lower stratum griseum superficiale; SO, stratum opticum. (Y) Quantification of % of target innervated by TRHR-GFP axons in wildtype (WT, dark gray) and cKO (light gray) mice. Data are represented as mean \pm SEM (n = 4-6 mice); Student's *t*-test.

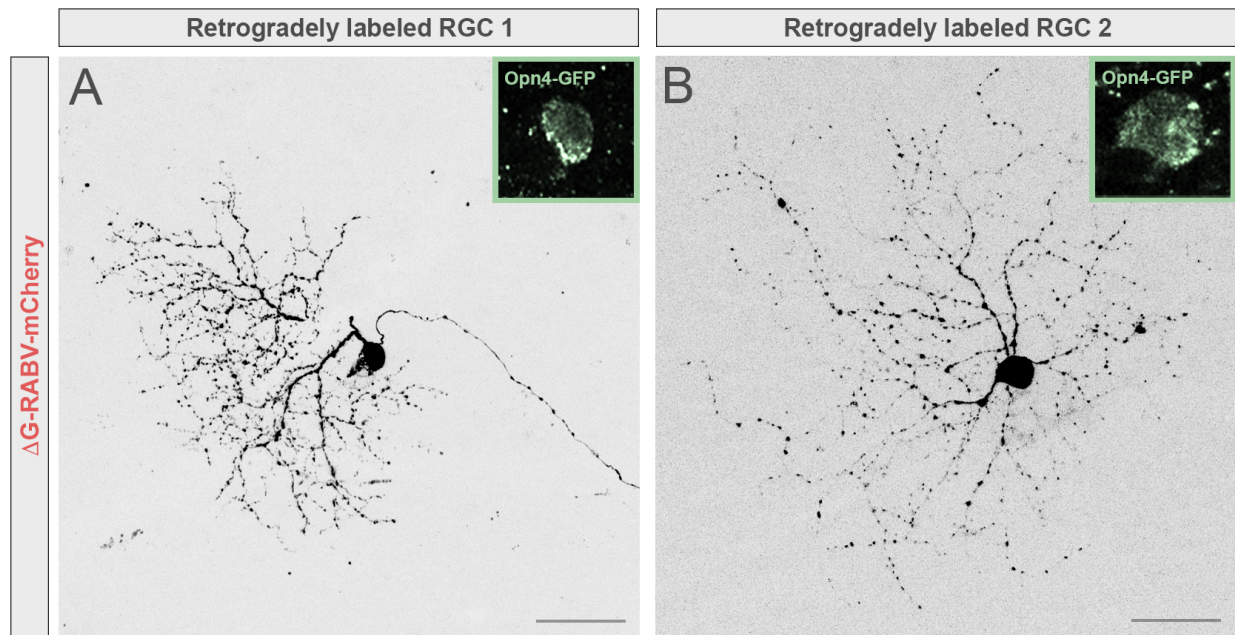


Figure S6, related to Figure 6. RGCs retrogradely labeled from the OPN of *Tbr2* cKOs are intrinsically photosensitive retinal ganglion cells (ipRGCs).

(A and B) Injection of Δ G-RABV-mCherry into the OPN of a *Tbr2*^{fl/fl}; *Tph2*^{Cre}; *Opn4*-GFP (cKO) mouse resulted in labeling of two RGCs, both of which expressed *Opn4*-GFP (also called melanopsin-GFP) (insets), indicating they are ipRGCs. Scale bars, 25 μ m

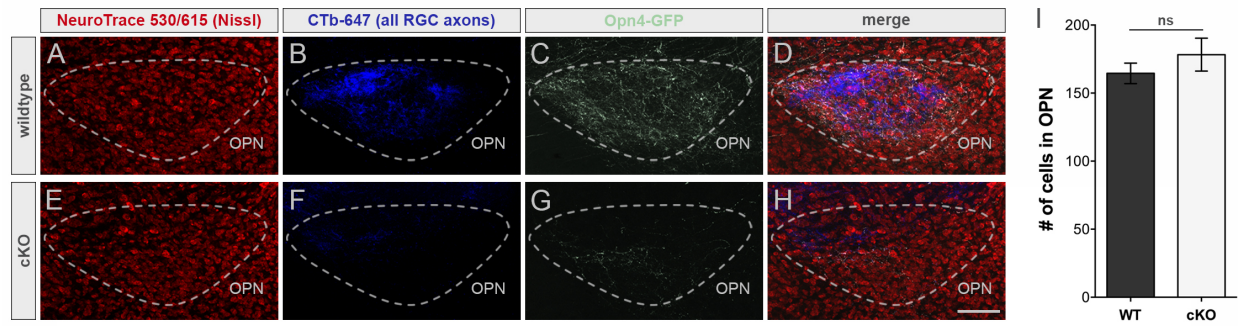


Figure S7, related to Figure 7. Diminished RGC axon input to the OPN caused by genetic deletion of *Tbr2* does not lead to cell loss in the OPN.

(A-H) Coronal view of Nissl stain (NeuroTrace 530/615, red), all RGC axons (CTb-647, blue) and ambient-luminance-sensing RGC axons (Opn4-GFP, light green) in wildtype (top panels) and cKO (bottom panels) mice. Scale bars, 100µm. OPN, olivary pretectal nucleus. (I) Quantification of the number of cells within a 200µm x 200µm area of the OPN in wildtype (WT, dark gray) and cKO (light gray) mice. Data are represented as mean ± SEM (n = 4 mice); Student's *t*-test.