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Combined administration of BDNF and CNTF provides superior neuroprotection as compared to BDNF alone in a mouse optic nerve crush model

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Purpose

This study explores whether combining brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) provides enhanced neuroprotection in optic nerve crush (ONC) model, as compared to BDNF alone.

Methods

ONC was performed on C57BL/6JRj mice by crushing the right optic nerve for 3 seconds using self-closing forceps. BDNF alone or a combination of BDNF and CNTF were administrated as a single unilateral intravitreal (IVT) injection into the right eye immediately and 3 days after ONC. Spectral-domain optical coherence tomography (SD-OCT) and pattern electroretinography (PERG) were conducted at baseline and 7 days after ONC induction. Seven days post-ONC eyes were enucleated, and retinal flat-mounts were stained with RNA-binding protein with multiple splicing (RBPMS) to quantify the number of retinal galnglion cells (RGCs).
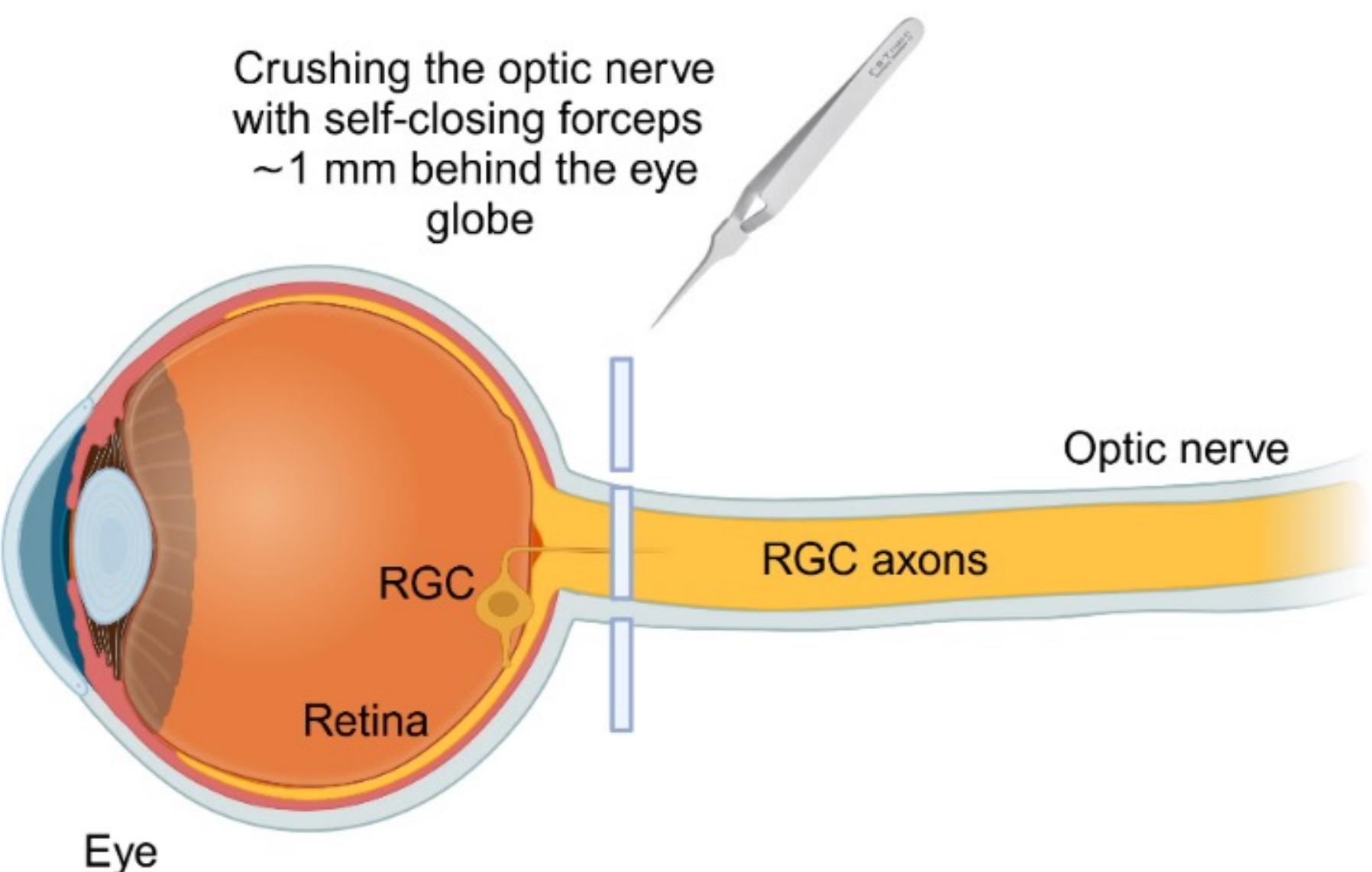

Crushing the optic nerve with self-closing forceps ~1 mm behind the eye globe

Optic nerve
RGC axons
RGC
Retina
Eye

Figure 1. ONC procedure illiustration.
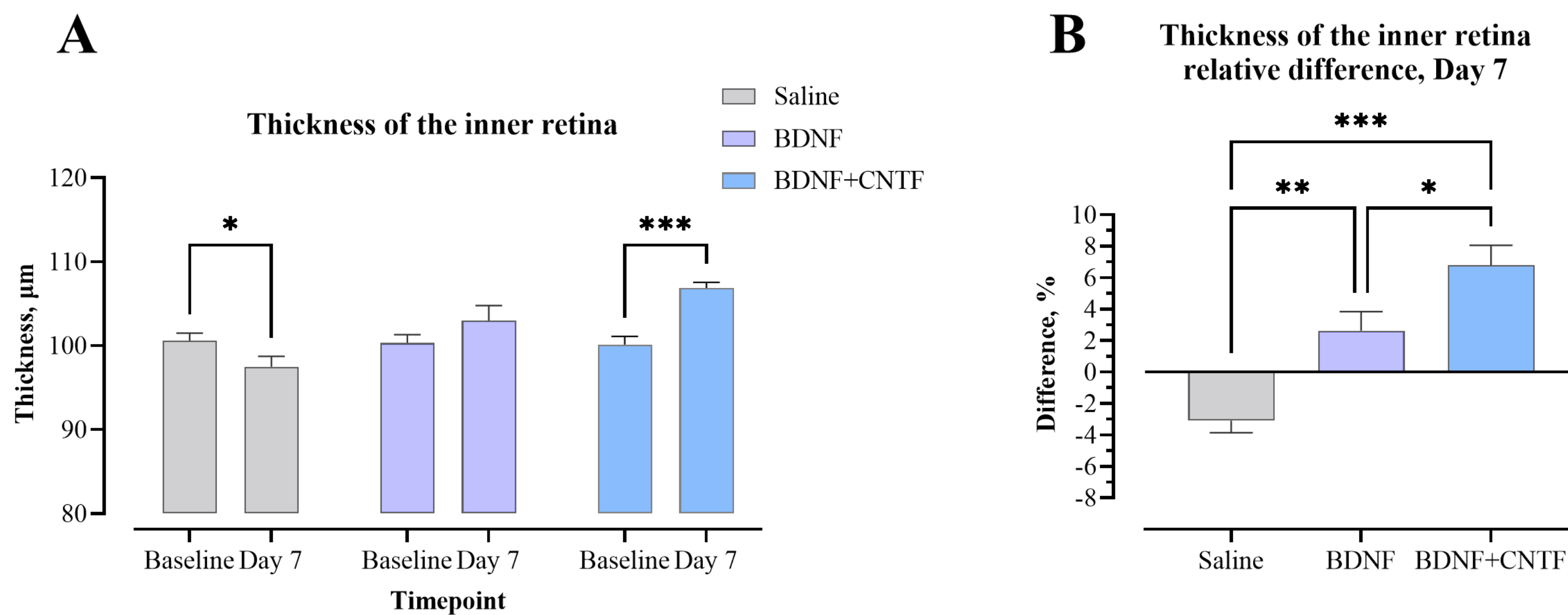
Results

Figure 2. A. Thickness of the inner retina at baseline and Day 7-post ONC (two-way ANOVA followed by Šídák's multiple comparisons test, *$P \leq 0.05$, ***$P < 0.001$). **B.** Thickness of the inner retina relative difference at Day 7 (one-way ANOVA followed by Tukey's multiple comparisons test, *$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$). Data are presented as mean±SEM. N=6-7 eyes per group.
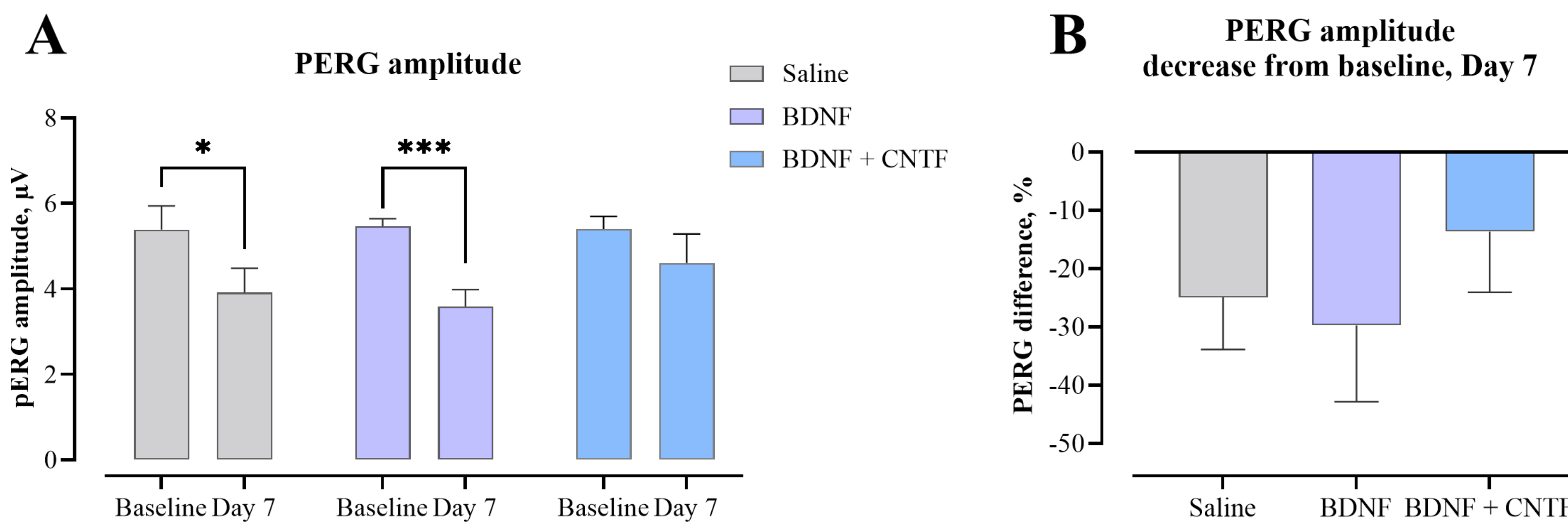

Figure 3. A. PERG amplitude at baseline and Day 7-post ONC (two-way ANOVA followed by Šídák's multiple comparisons test, *$P \leq 0.05$, ***$P < 0.001$). **B.** PERG amplitude decrease from baseline at Day 7 (one-way ANOVA, $P = 0.58$). Data are presented as mean±SEM. N=3-7 eyes per group.
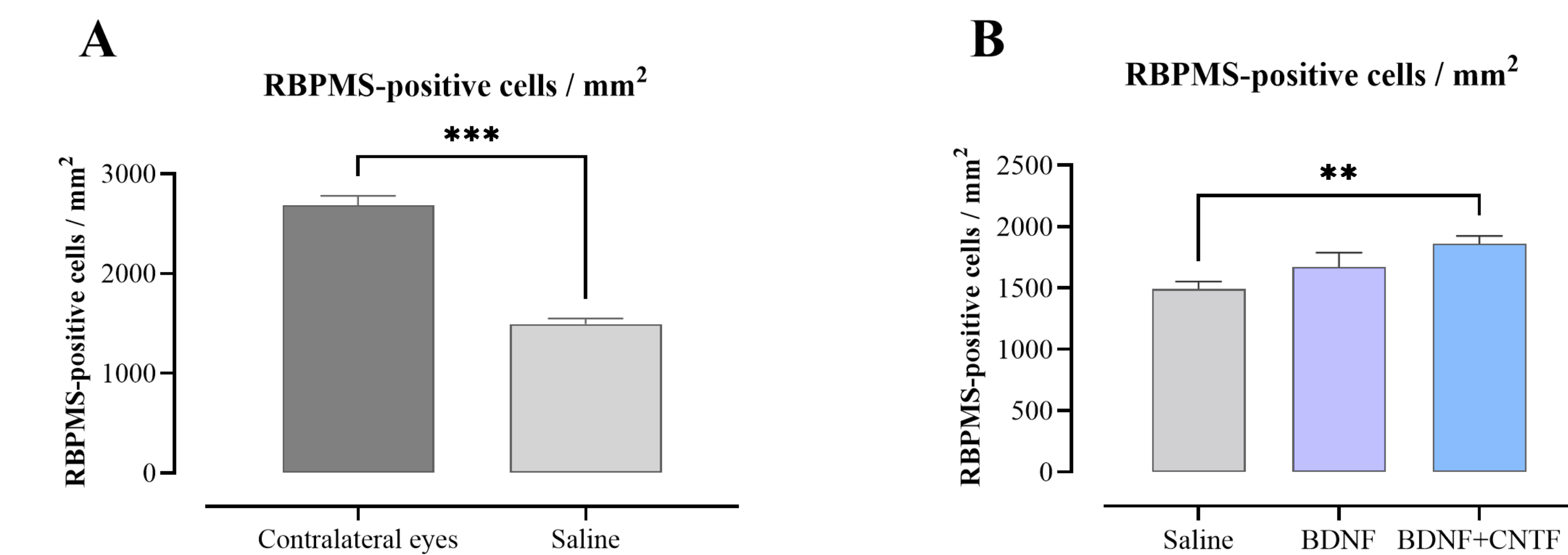

Figure 4. A. RGC density in contralateral and saline-treated eyes (unpaired t-test, ***$P < 0.001$). **B.** RBPMS-positive cell comparison between treatment groups (one-way ANOVA followed by Dunnett's multiple comparisons test, **$P < 0.001$). Data are presented as mean±SEM. N=6-7 retinal flat-mounts per group.

SD-OCT retina scan

Segmentation using AI-based algorithm

NFL/GCL
IPL
INL
OPL
ONL
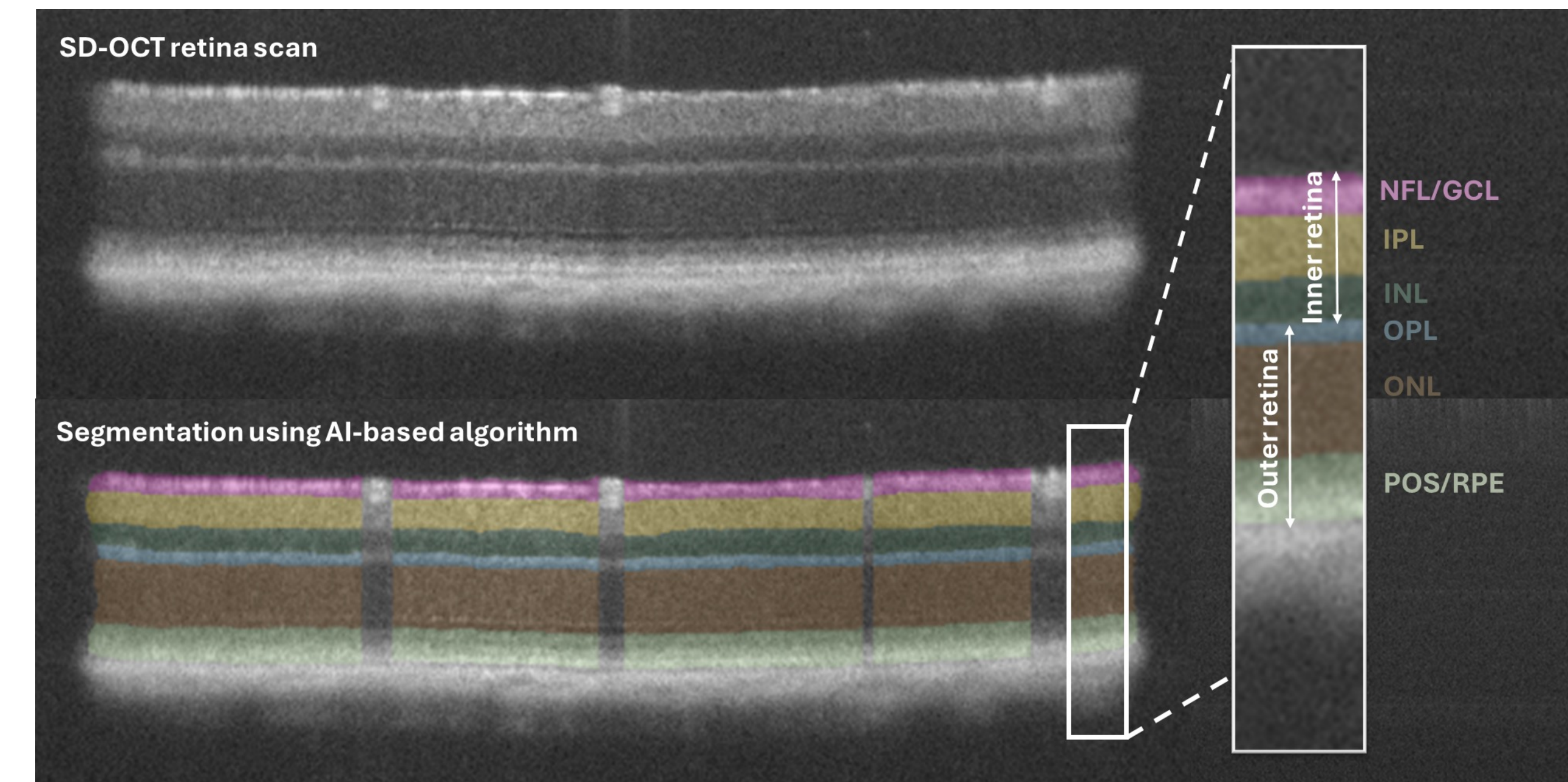POS/RPE
Inner retina
Outer retina

Figure 5. Representative image of AI-segmented retinal layers from the mouse SD-OCT scan (baseline) analyzed for retinal thickness measurements by a proprietary AI-based algorithm. NFL/GCL – nerve fiber and retinal ganglion cell layers (pink), IPL – inner plexiform layer (yellow), INL – inner nuclear layer (teal), OPL – outer plexiform layer (blue), ONL – outer nuclear layer (brown), POS/RPE – photoreceptor outer segments and retinal pigment epithelium (light green).
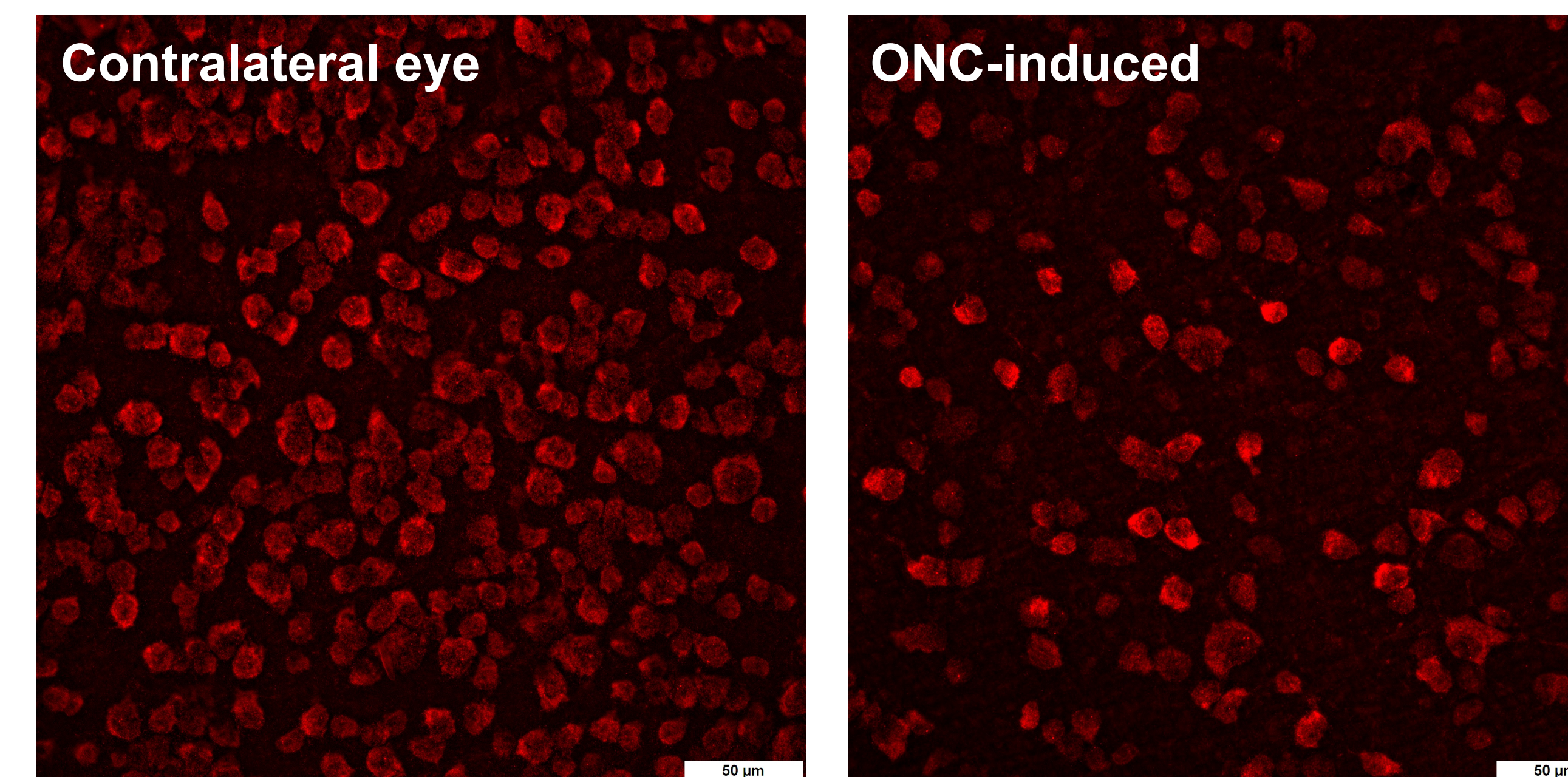

Contralateral eye
ONC-induced

Figure 6. Representative images of retinal flat-mount labeled with RBPMS antibodies of contralateral and ONC-induced eyes. RGC density was evaluated using stereology to obtain a statistically valid, accurate and unbiased analysis of sample. Scale bar = 50 μm.

Conclusion

Our findings suggest a superior efficacy of the combined BDNF and CNTF treatment in alleviating the thinning of inner retina, preserving visual function, and attenuating RGC loss in the mouse ONC model, as compared to BDNF-only treatment.

Disclosures
IL, TP, MD: none
SR: Experimentica Ltd. (I,S)
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