OMTN, Volume *35*

Supplemental information

Single intravitreal administration of a tetravalent siRNA exhibits robust and efficient gene silencing in mouse and pig photoreceptors

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Supplemental Figures

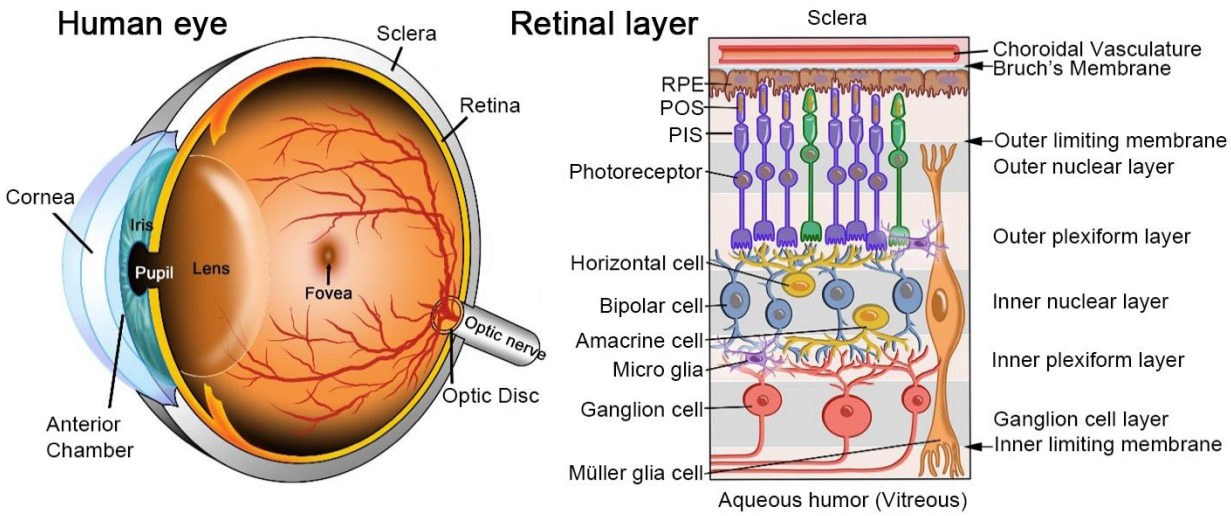

Figure S1. Schematic of eye and retina. Left panel: Schematic of a human eye showing the cornea, the anterior chamber, which is the space between the cornea and the iris, the pupil, the lens, the retina with the fovea, which is the area of high acuity vision in humans, the optic disc and the optic nerve, and the tissue holding the eye together, the sclera. Right panel: Schematic of a retinal cross-section showing the different cell layers and cell types. Width of individual layers are not to actual proportions. The Müller glia cells, span the retina, have the nucleus in the inner nuclear layer and form with their end-feet the inner and outer limiting membranes. Ganglion cells are in the ganglion cell layer and project their axons through the optic nerve to the brain. All interneurons are in the inner nuclear layer, including amacrine cells, horizontal cells, and bipolar cells. Amacrine and bipolar cells connect to ganglion cells in the inner plexiform layer while horizontal cells and bipolar cells connect to photoreceptors in the outer plexiform layer. Photoreceptors have their cell bodies in the outer nuclear layer. The photoreceptor inner segment (PIS) is on the outer side of the outer limiting membrane and contains all the major cytoplasmic components of the cell. The photoreceptor outer segment (POS) is connected to the PIS by the connecting cilium. The POS is the region where light photons are absorbed. POSs are surrounded by apical microvilli processes of the retinal pigmented epithelium (RPE). The RPE digests every day ~10% of each POS. RPE cells are attached to a basal membrane referred to as the Bruch's membrane. On the other side of the Bruch's membrane is the choroidal vasculature and the sclera. The siRNA that is injected intravitreally migrates across the inner limiting membrane and the retinal cell layers to the photoreceptors and the RPE cells. Very little remains in the vitreous or migrates to the lens and cornea.

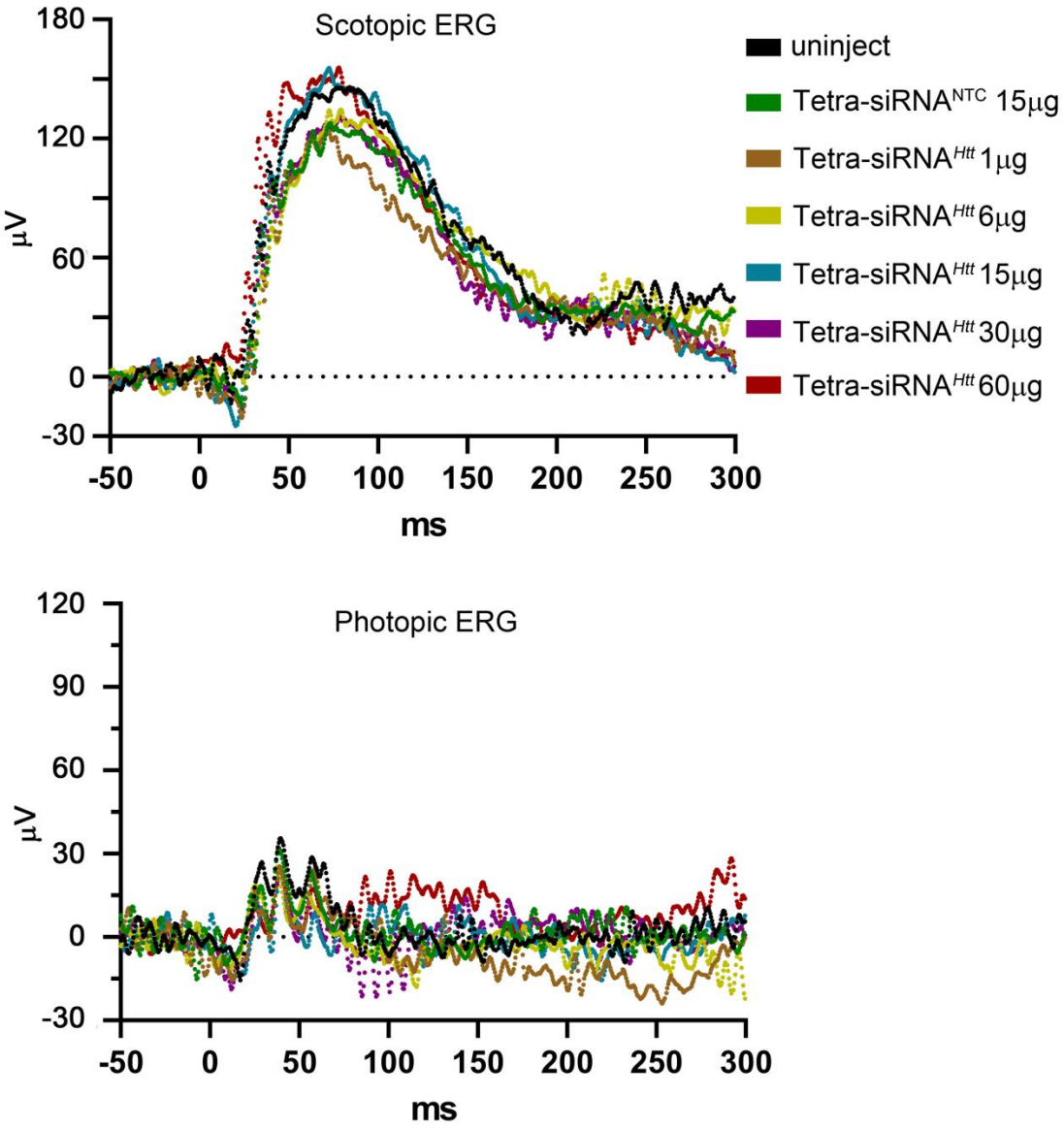

Figure S2. Electroretinogram wave forms. ERG wave forms for the scotopic (rod response, upper graph) and photopic (cone response, lower graph) ERG recordings performed 2 months post intravitreal injection with the different doses of the tetra-siRNA^{Htt}. Shown is one example per dose. The averages of the recordings are shown in Figure 3C. Scotopic recordings were performed at 1 cd.s/m^2. Photopic ERG recordings used a background intensity of 9cd.s/m^2 and a flash intensity of 10 cd.s/m^2.

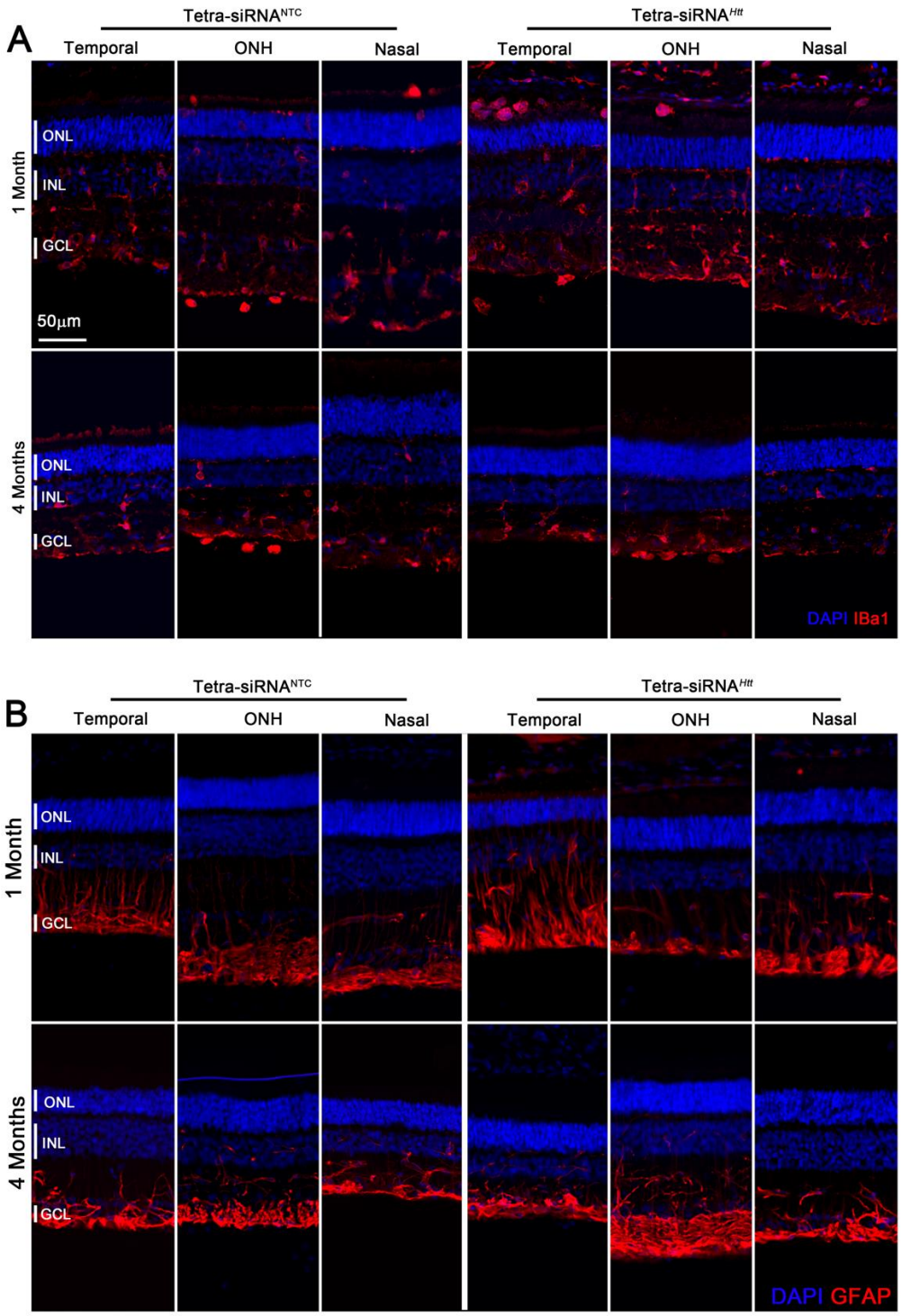

Figure S3. Safety of HTT silencing in porcine retina. (A and B) Retinal cross-sections stained for Iba1 (A: red signal) or GFAP (B: red signal) at 1-, and 4-months post intravitreal injection with 300µg of siRNA. The temporal side, where siRNA was injected shows slightly higher microglia activity and GFAP upregulation at 1-month post-injection. By 4-months post-injection microglia activity and GFAP expression appear normal again. Blue: nuclei marked with DAPI; red: Iba1 signal in (A) and GFAP signal in (B); ONL: outer nuclear layer; INL: inner nuclear layer; GCL: ganglion cell layer. Scale bar: 50µm. Images are representative images from 3 pigs per time point and siRNA.