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Supplementary Materials for

A single intravenous injection of cyclosporin A–loaded lipid nanocapsules prevents retinopathy of prematurity

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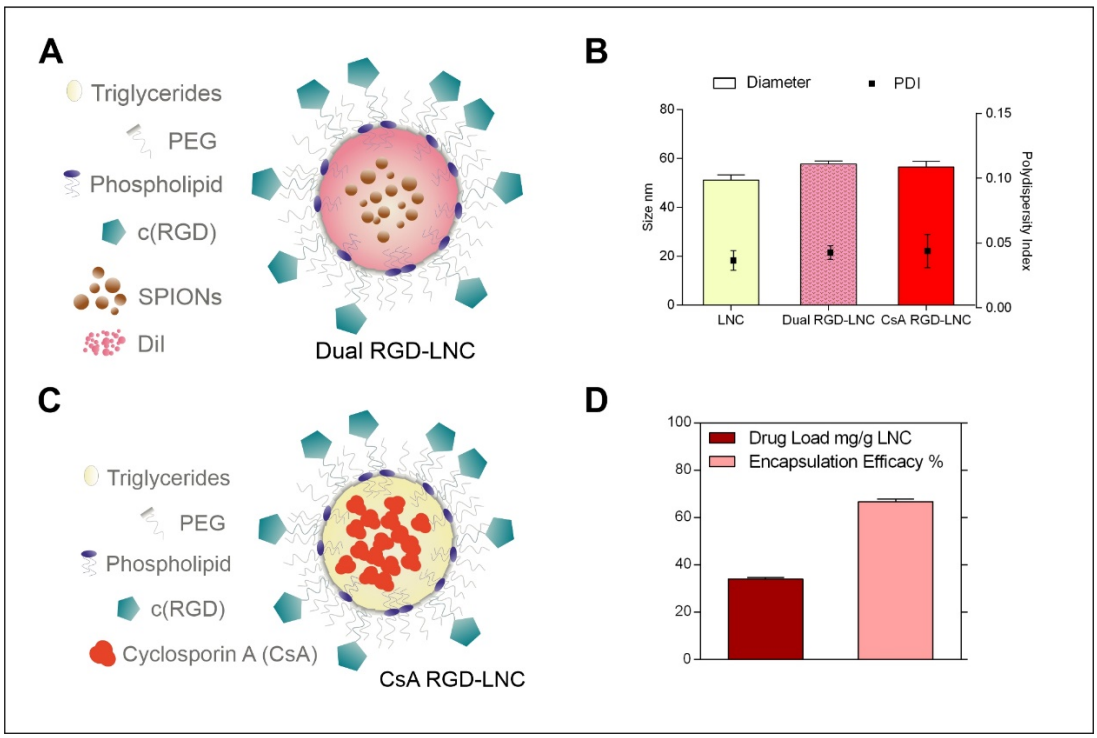

Fig. S1. Dual RGD-LNC and CsA RGD-LNC illustration and characterization. (A) Schematic illustration of RGD-LNCs simultaneously loaded with fluorescent dye (DiI) and electron dense super-paramagnetic iron oxide nanoparticles (SPIONs), referred to as Dual RGD-LNCs. (B) Hydrodynamic diameter and polydispersity index (PDI) of different LNC formulations. SPION/DiI and CsA loading does not alter size or PDI of LNCs. (C) Schematic illustration of CsA loaded RGD-LNCs, referred to as CsA RGD-LNCs. (D) Absolute drug-load in mg CsA per g LNC dispersion (34 mg/g LNC) and percentage encapsulation efficacy (experimental drug payload/theoretical drug payload). Results are presented as mean ± SD of n = 10 (B) and n = 3 (D) independent experiments.

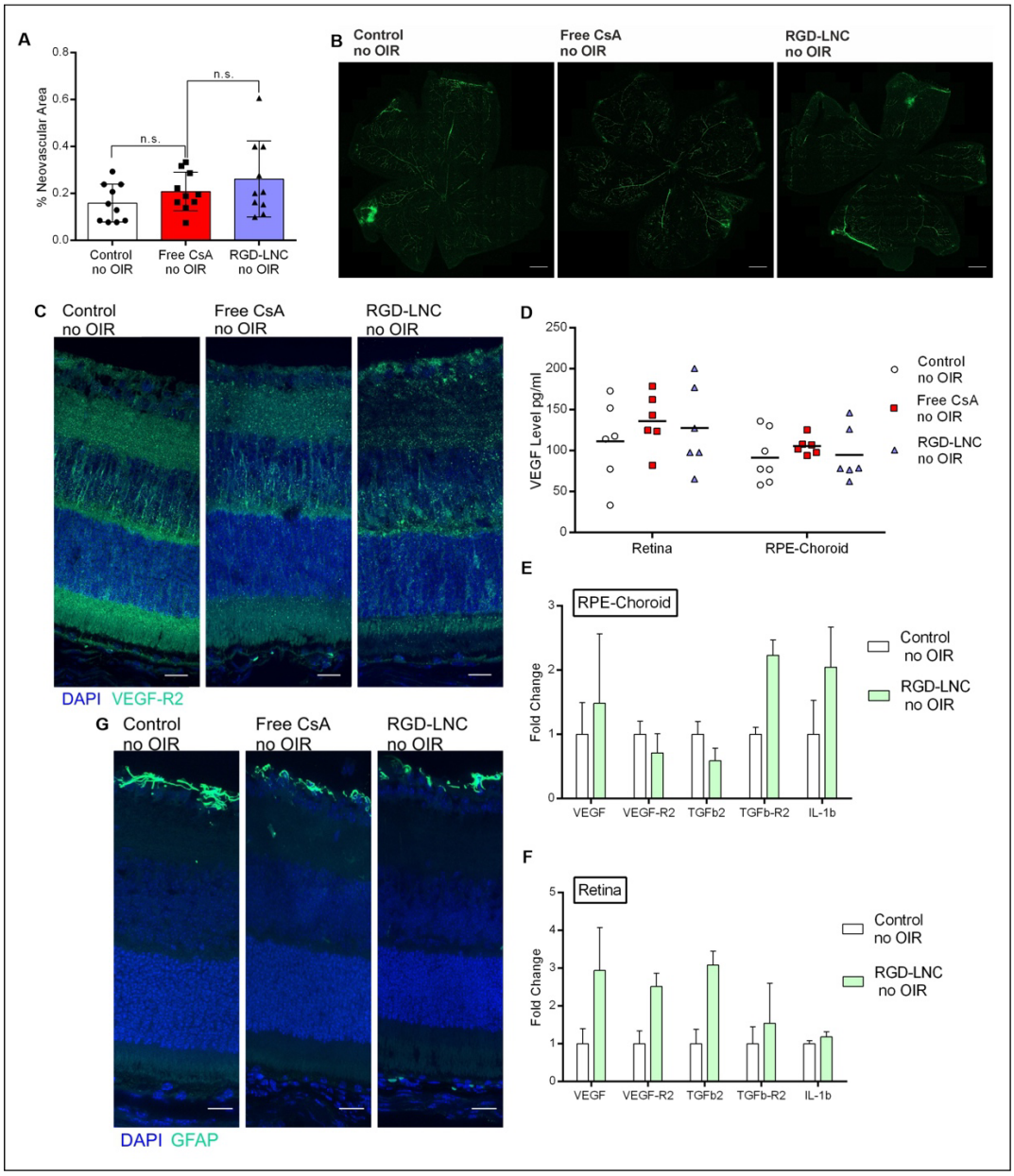

Fig. S2. Free CsA and RGD-LNCs treatment did not affect the retina of healthy mice. (A) Quantification of the relative neovascular area of mice without OIR at P17. (B) Representative images of retina whole mounts showing the retinal vasculature at P17. Green: FITC-Dextran. Scale bars: 500 µm. (C) Imaging of VEGF-R2 expression in the posterior eye. Scale bars: 20 µm. Blue: DAPI staining of cell nuclei; green: VEGF-R2. (D) Assessment of VEGF protein levels in the retina and RPE-choroid complex. (E and F) Representation of mRNA level fold change of VEGF, VEGF-R2, TGFb2, TGFb-R2 and IL-1β in the neural retina and RPE-choroid complex. (G) Imaging of GFAP expression in the posterior eye. Scale bars: 20 µm. Blue: DAPI staining of cell nuclei; green: GFAP. Results are presented as mean ± SD of at least n = 9 (A), n = 6 (D) and n = 5 (E and F) mice per treatment group. Levels of statistical significance are indicated as * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$ and n.s.: non-significant.

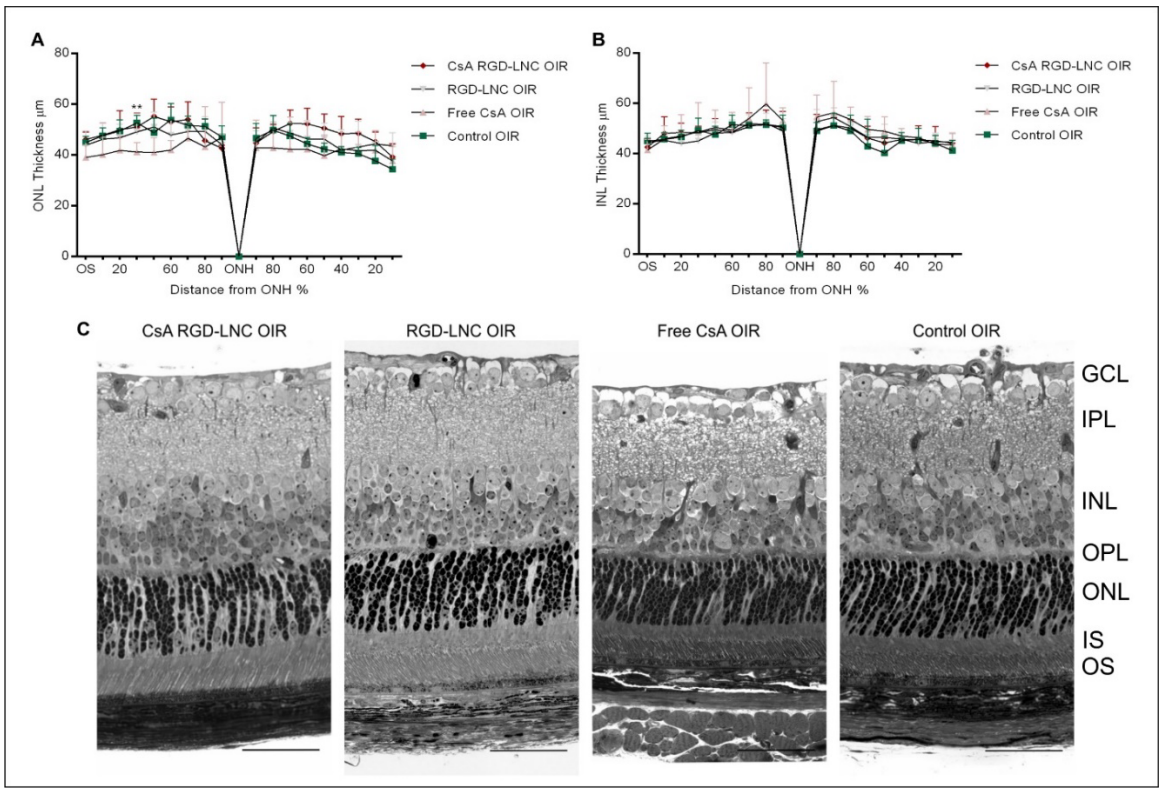

Fig. S3. Therapeutic intervention causes no morphological changes. Retinal topography of mice at P17. OS: photoreceptor outer segments; IS: photoreceptor inner segments; ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer. ONH: optic nerve head. (A) ONL thickness measurements. ** Comparison of Control OIR and free CsA OIR. Treatment with CsA solution reduces retinal thickness significantly in one of 10 measurement points, while CsA RGD-LNCs therapy had no effect on ONL thickness. (B) INL thickness measurements. (C) Representative images of the morphologic appearance of photoreceptors and RPE in the central retina of mice at P17 with and without OIR treated with CsA RGD-LNCs, RGD-LNCs or free CsA at P12 compared to control. Scale bars: 500 µm. Level of statistical significance is indicated as ** $P \leq 0.01$.