Supporting Information

Multifarious Biologic Loaded Liposomes that Stimulate the Mammalian Target of Rapamycin Signaling Pathway Show Retina Neuroprotection after Retina Damage

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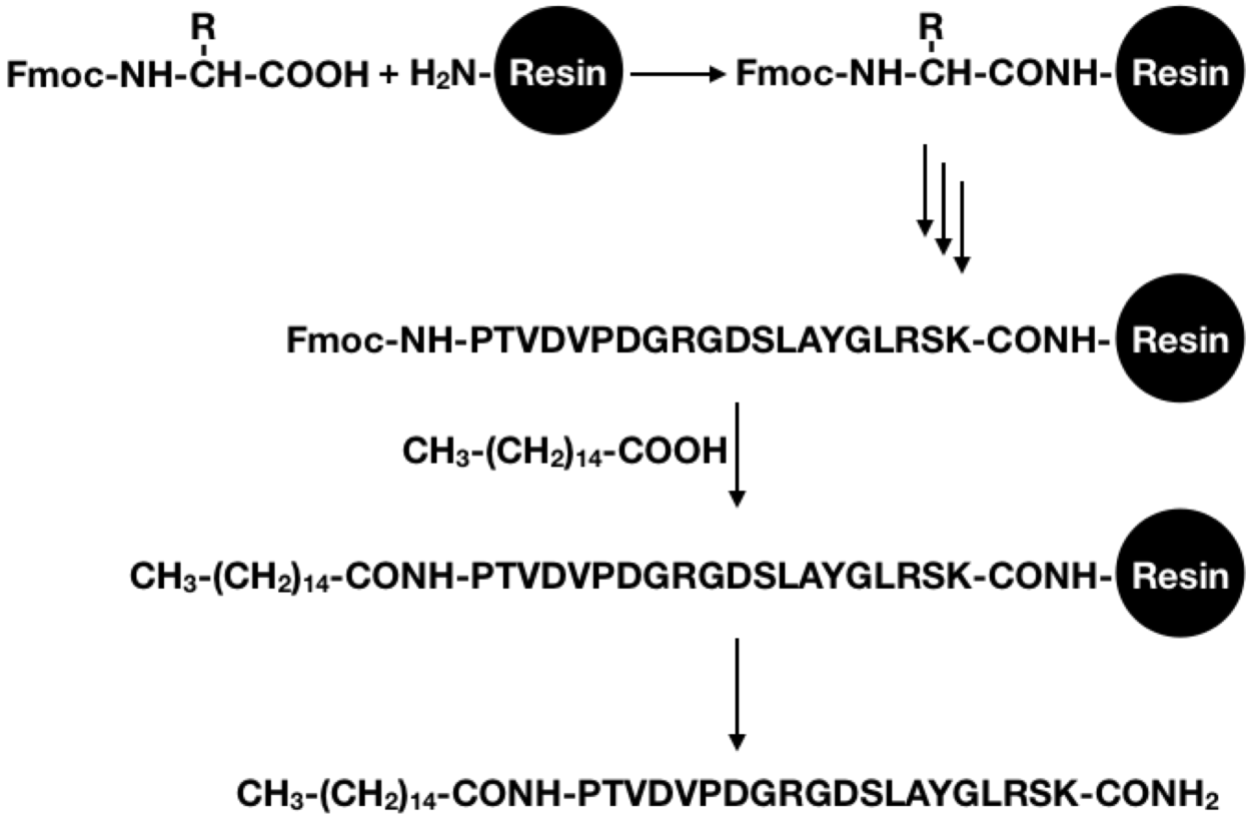


Figure S1. Lipopetide synthesis schematic using OPP as the example. Peptides were synthesized using solid phase strategies prior to lipidation at the N-terminal followed by decoupling from the TentaGel resin. See 'Methods' for further details.

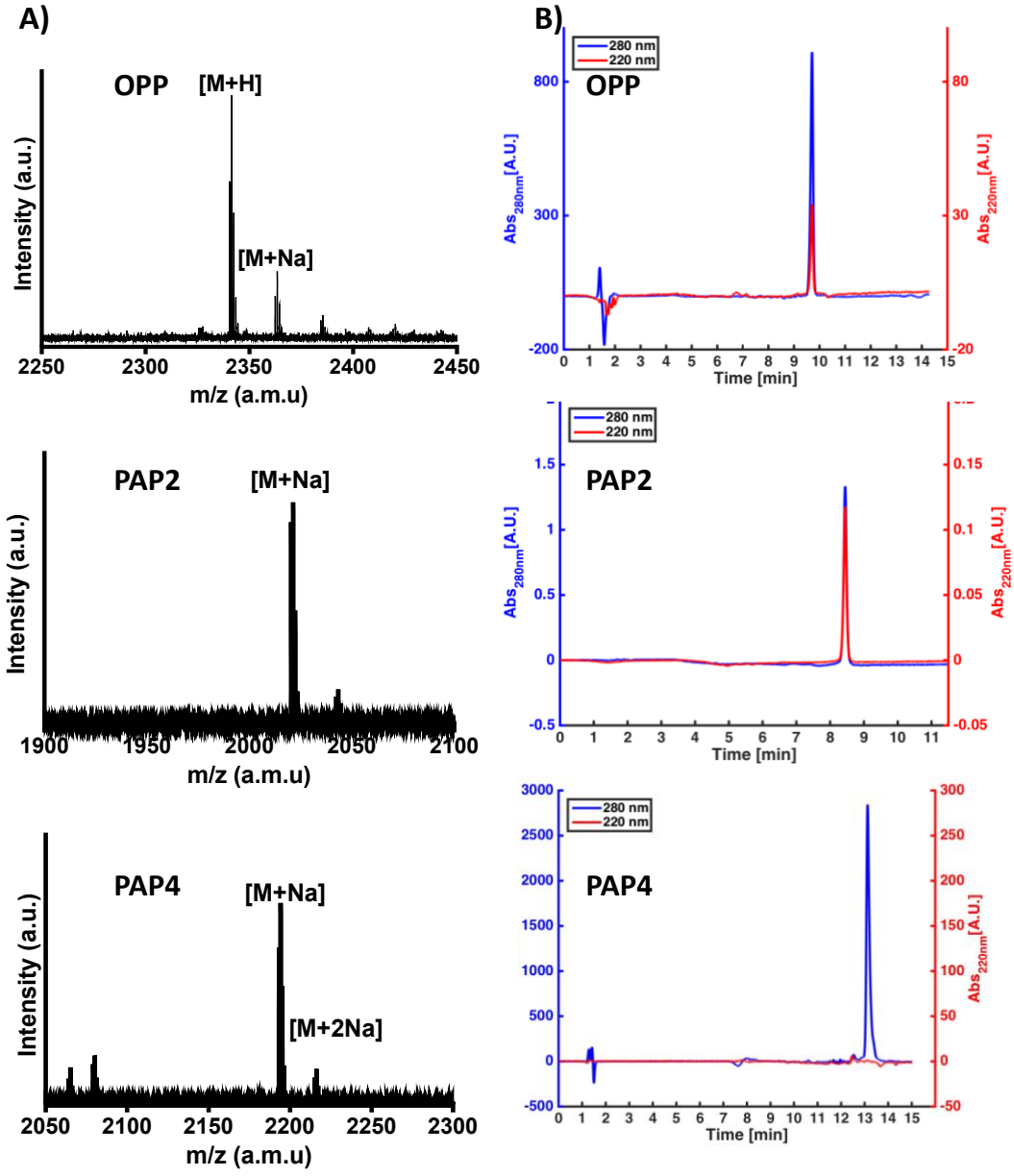


Figure S2. Lipopeptide characterization. **A)** MALDI-ToF mass spectrums showing parent ions of OPP (M m/z = 2341.8), PAP2 (M m/z = 1998.9) and PAP4 (M m/z = 2171.8); **B)** HPLC chromatograms of lipopeptides. All analytical HPLC was run on a Waters XTerra RP8 column, with a 15 minute linear gradient going from 0-100% eluent B. Eluent A = 5% acetonitrile, 0.1% TFA in water and eluent B = 0.1% TFA in acetonitrile.

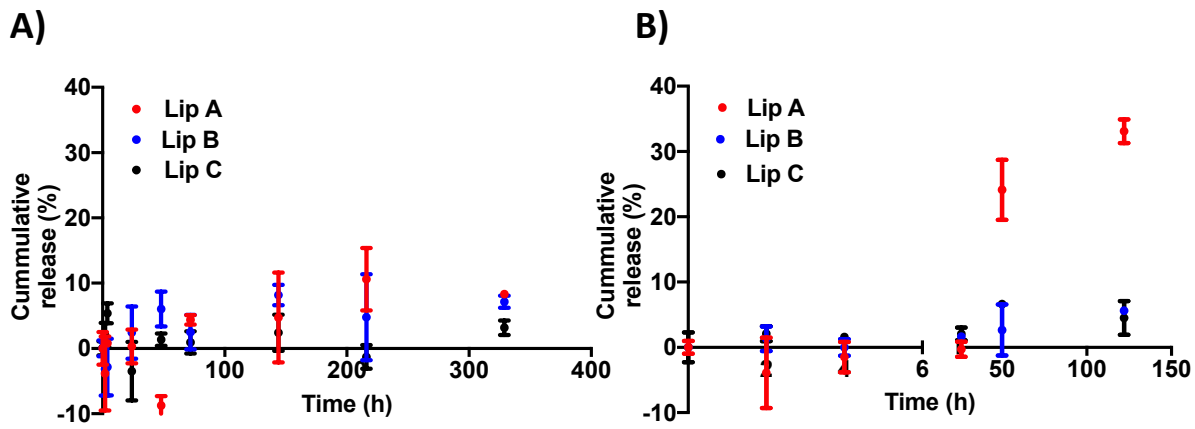


Figure S3. Calcein leakage from liposomes: **A)** Liposomes in HBS at 37°C; **B)** Liposomes in 10% FBS in HBS at 37°C.

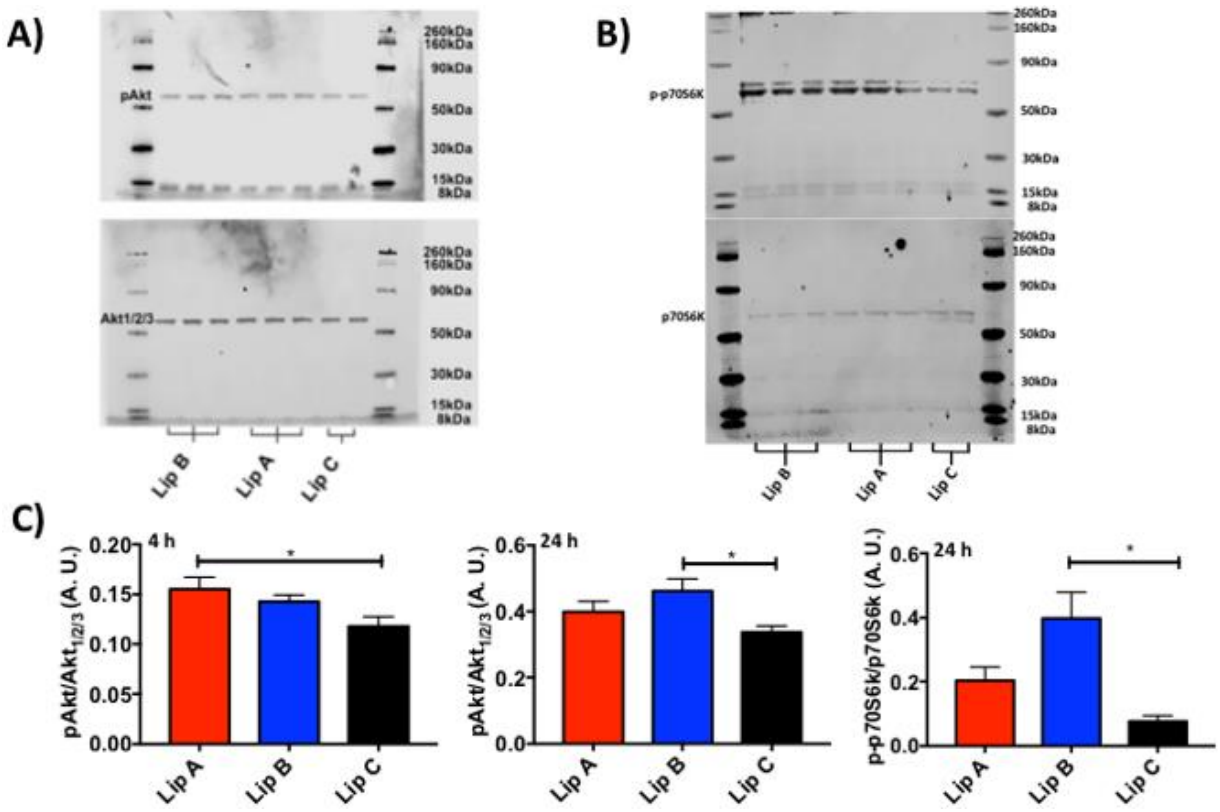


Figure S4. Liposome bioactivity: **A)** Example western blot stained for phosphorylated Akt and Total Akt from HEK293T cells incubated with liposomes for 24 h; B) Example western blot stained for phosphorylated p70S6 kinase and p70S6 kinase from HEK293T cells incubated with liposomes for 24 h. **C)** Akt and p70S6K activation as determined by optical densitometry. Akt data shown for 4 and 24 hours, p70S6K data shown for 24 hours. Densitometry was performed in Image Studio Lite using the built in add rectangle function. The histograms show the mean values with standard deviation (one-way ANOVA, *$P \leq 0.05$).

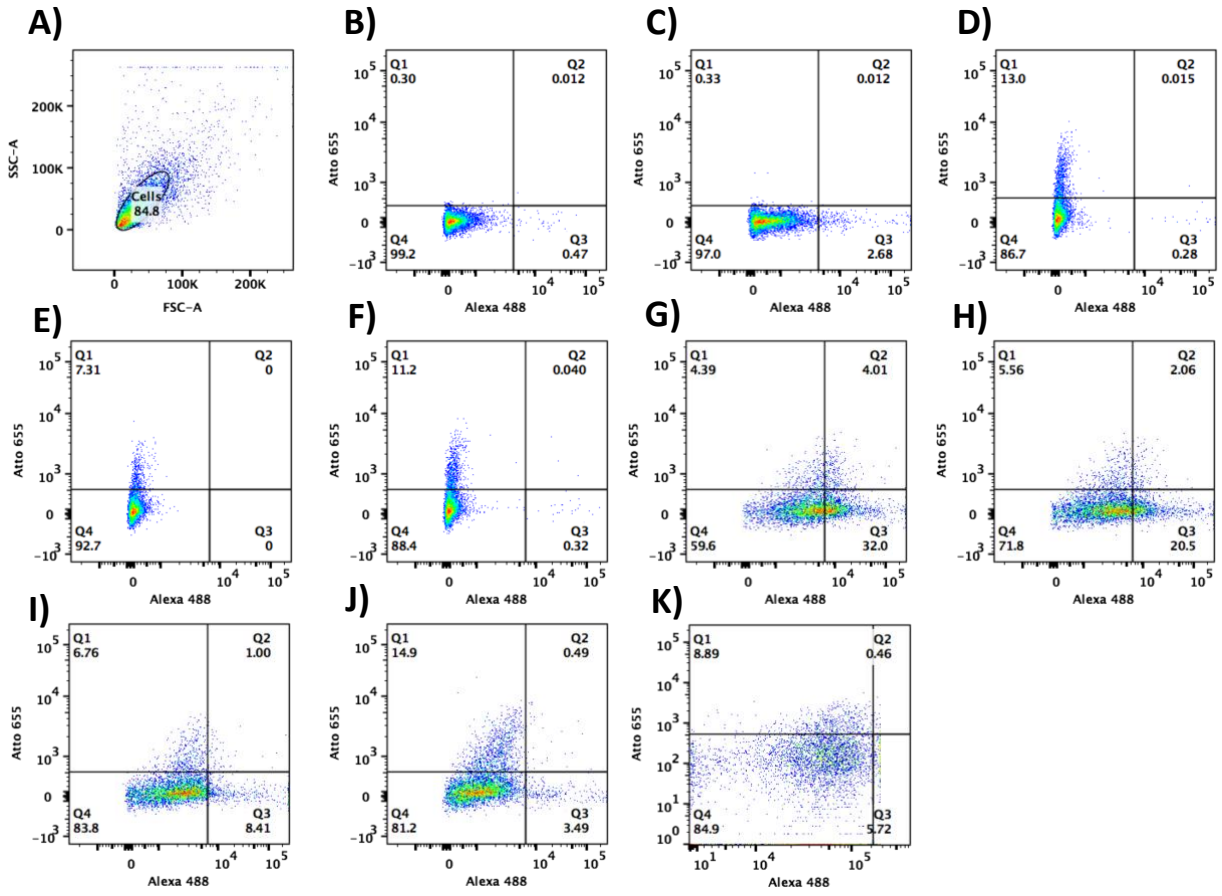


Figure S5. Example flow cytometry scatter plots: **A)** Cell gate; **B)** ISO-mouse negative for liposomes used to gate for cell stained and liposomes; **C)** ISO-Rabbit negative for liposomes used to gate for cell stained and liposomes; **D)** Lip A no cell stain; **E)** Lip B no cell stain; **F)** Lip C no cell stain; **G)** Anti-recoverin for Photoreceptor cells + Lip B; **H)** Anti-PCKα for bipolar cells + Lip B; **I)** Anti-RBPMS for RGCs + Lip B; **J)** Anti-calbindin for horizontal cells + Lip A; **K)** Anti-GS for Müller glia cells + Lip A. Liposomes were marked with Atto655, cell type stains were labelled with Alexa488. Organoids were incubated with liposomes for 12 h at 37 °C 5% CO_2. Data analysis was carried out in FlowJo software.

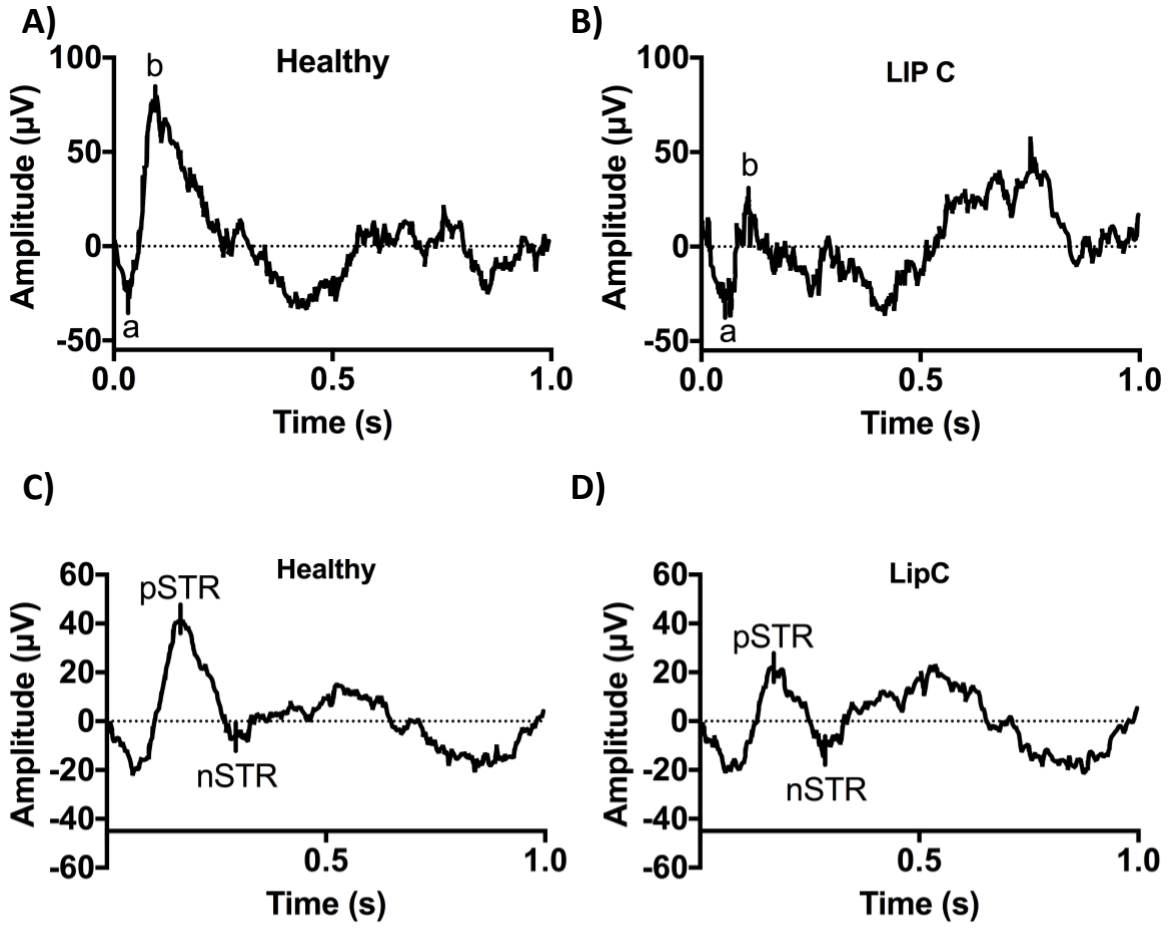


Figure S6. Example electroretinograms from dark adapted mice: **A)** An electroretinogram from a healthy eye (flash stimulation 0.1 cd-s/m^2). The a-wave and b-wave components are indicated with (a) and (b) respectively; **B)** Electroretinogram from a Lip C treated eye after NMDA exposure (flash stimulation 0.1 cd-s/m^2). The a-wave and b-wave components are indicated with (a) and (b) respectively; **C)** An electroretinogram showing STR from a Lip A treated eyes after NMDA exposure (flash stimulation 0.001 cd-s/m^2). The positive and negative component of the STR (pSTR and nSTR) have been indicated on the graphs; **D)** An electroretinogram showing STR from a Lip C treated eyes after NMDA exposure (flash stimulation 0.001 cd-s/m^2). The positive and negative component of the STR (pSTR and nSTR) have been indicated on the graphs.

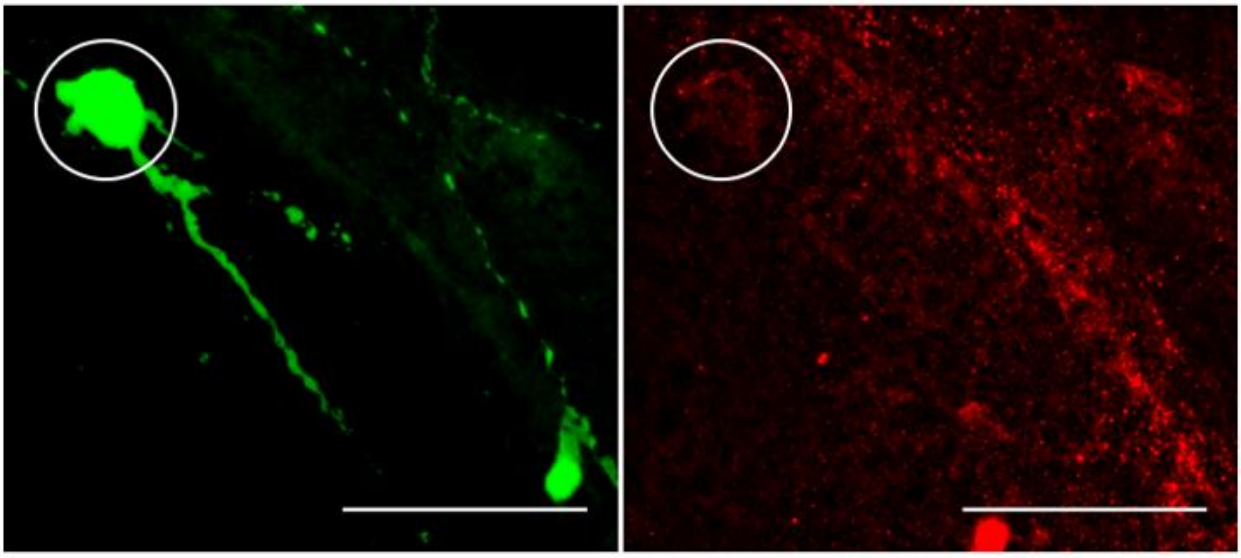


Figure S7. Example micrograph of a tRGC showing Thy1-GFP (left) and RBMPS (right) tRGCs showed high Thy1-GFP expression and lower RBMPS expression than host RGCs (Scale bars = 60 μm).

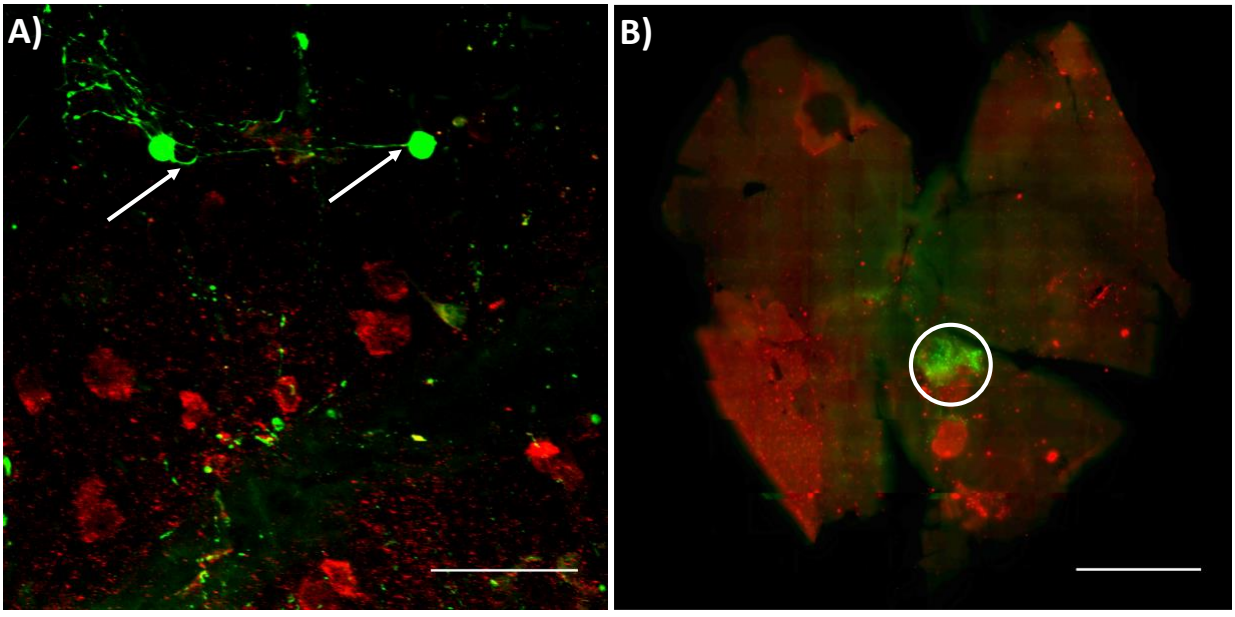


Figure S8. Host and transplant RGC survival: A) Example micrographs of Lip C + tRGCs group. White arrows indicate axons. tRGCs are in green, host RGCs in red (scale bar = 60 μm); B) Example tile scan of the whole retina from Lip C + tRGCs group. tRGCs are in green (highlighted by a white circle) and host RGCs are in red (scale bar = 1 mm).

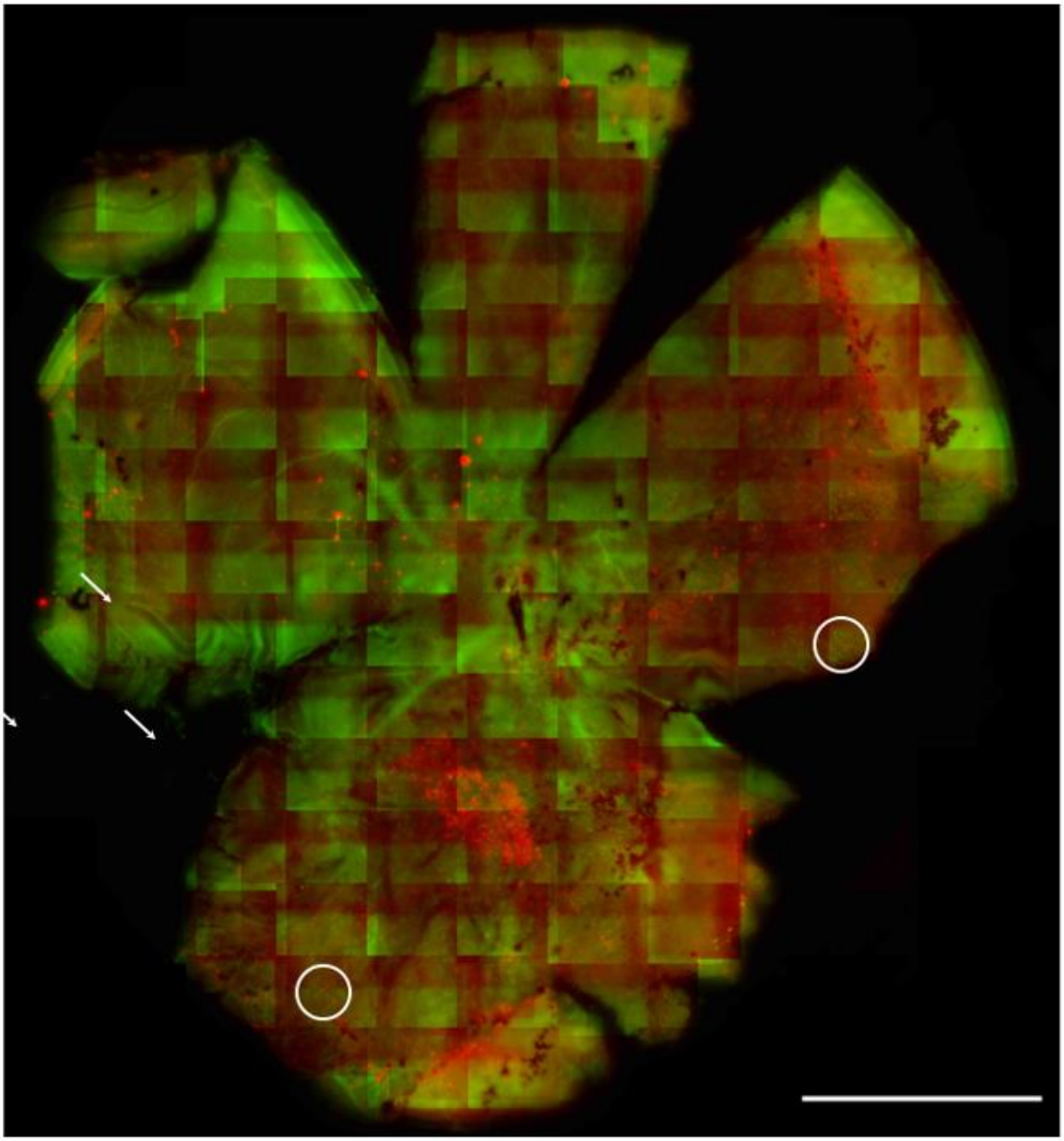
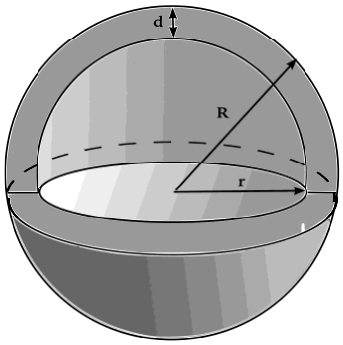


Figure S9. Example tile scan of the whole retina from Lip C + tRGCs group. tRGCs are in green (highlighted by a white circle) and host RGCs are in red (scale bar = 1 mm).

Calculations of proteins per liposome



1) Area of bilayer membrane

$A_{bilayer} = 4\pi \cdot R^2 + 4\pi \cdot (R - d)^2$

Number of lipids in one liposome

$$N_{Lipids} = \frac{A_{bilayer}}{(X_{Component\ 1} \cdot A_{Component\ 1}) + (X_{Component\ 2} \cdot A_{Component\ 2}) + \cdots}$$

X is the mole fraction of each component in the bilayer. A is the surface area of each component. Surface areas for molecules can be found from various sources (e.g. ee Edholm, O; Nagle, J. F.; Areas of molecules in membranes consisting of mixtures. *Biophysical journal* **2005**, *89*(3), 1827-1832 etc.)

2) Number of liposomes in a given concentration and volume

$n_{Lipids} = N_{Lipids} \cdot N_A$

Where n_{Lipids} is the number of lipids in 1 mole of liposomes and N_A is Avogadro's constant

$$N_{Liposomes} = \frac{(C_{Lipids} \cdot V)}{n_{lipids}}$$

Where $N_{Liposomes}$ is the number of liposomes in a given concentration and volume

3) Number of proteins per liposome ($N_{P/L}$)

$N_{Proteins} = C_{Proteins} \cdot V \cdot N_A$

Where $N_{Proteins}$ is the number of proteins in a given concentration and volume

$$N_{P/L} = \frac{N_{proteins}}{N_{Liposomes}}$$

Cell culture medias

Embryonic stem cell maintenance media (ES medium)

The ES medium components were as follows: 1:1 Dulbecco's modified eagle's medium (DMEM): Ham's F12 (F12) medium supplemented with 1% 100x Glutamax supplement (Gibco, Thermo Fisher), 1% 100x non-essential amino acids (Gibco, Thermo Fisher), 1% 100x sodium pyruvate (Gibco, Thermo Fisher), 1% 100x antibiotic-antimycotic solution (Gibco, Thermo Fisher), $0.7x10^{-3}$% 16 M β-Mercaptoethanol (Sigma-Aldrich), 8.5 ng/mL mouse leukemia inhibitory factor (Sigma-Aldrich) and 11% heat-inactivated fetal bovine serum (HI FBS) (Gibco, Thermo Fisher).

Optic vesicle medium (OV-medium)

The OV medium components were as follows: 1:1 DMEM:F12 supplemented with 1% 100x Glutamax supplement (Gibco, Thermo Fisher), 1% 100x non-essential amino acids (Gibco, Thermo Fisher), 1% 100x sodium pyruvate (Gibco, Thermo Fisher), 1% 100x antibiotic-antimiycotic solution (Gibco, Thermo Fisher), 1% 100x lipid concentrate (Gibco, Thermo Fisher), 0.17% insulin-transferrin-selenium-ethanolamine (ITS-X) supplement (Gibco, Thermo Fisher), $0.7x10^{-3}$% 16M β-Mercaptoethanol (Sigma-Aldrich), 1.3% HI FBS (Gibco, Thermo Fisher) and 0.5 mM N-Acetyl-L-Cysteine (NAC) (Sigma-Aldrich).

Optic cup medium (OC-medium)

The OC medium components were as follows: 1:1 DMEM:F12 supplemented with 1% 100x Glutamax supplement (Gibco, Thermo Fisher), 1% 100x non-essential amino acids (Gibco, Thermo Fisher), 1% 100x sodium pyruvate (Gibco, Thermo Fisher), 1% 100x lipid concentrate (Gibco, Thermo Fisher), 1% 100x antibiotic-antimycotic solution (Gibco, Thermo Fisher), $0.7x10^{-3}$% 16 M β-Mercaptoethanol (Sigma-Aldrich), 0.5 mM NAC (Sigma-Aldrich) and 1.9 % 50x NS21 supplement (R&D Systems).

RGC medium

The RGC medium components were as follows: 1:1 DMEM:F12 supplemented with 1% 100x Glutamax supplement (Gibco, Thermo Fisher), 1% 100x non-essential amino acids (Gibco, Thermo Fisher), 1% 100x sodium pyruvate (Gibco, Thermo Fisher), 1% 100x lipid concentrate (Gibco, Thermo Fisher), 1% 100x antibiotic-antimycotic solution (Gibco, Thermo Fisher), $0.7x10^{-3}$% 16 M β-Mercaptoethanol (Sigma-Aldrich), 0.5 mM NAC (Sigma-Aldrich), 1.9 % 50x NS21 supplement (R&D Systems), 20 ng/mL IGF-1 (Cell Guidance Systems), 20 ng/mL CTNF (Cell Guidance Systems), 10 ng/mL GDNF (Cell Guidance Systems), 20 μM forskolin (Sigma-Aldrich), 1:500 100x Insulin-transferrin-selenium (ITS) supplement (Gibco, Thermo Fisher).