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Supplemental Information

A Drug-Tunable Gene Therapy for Broad-Spectrum Protection against Retinal Degeneration

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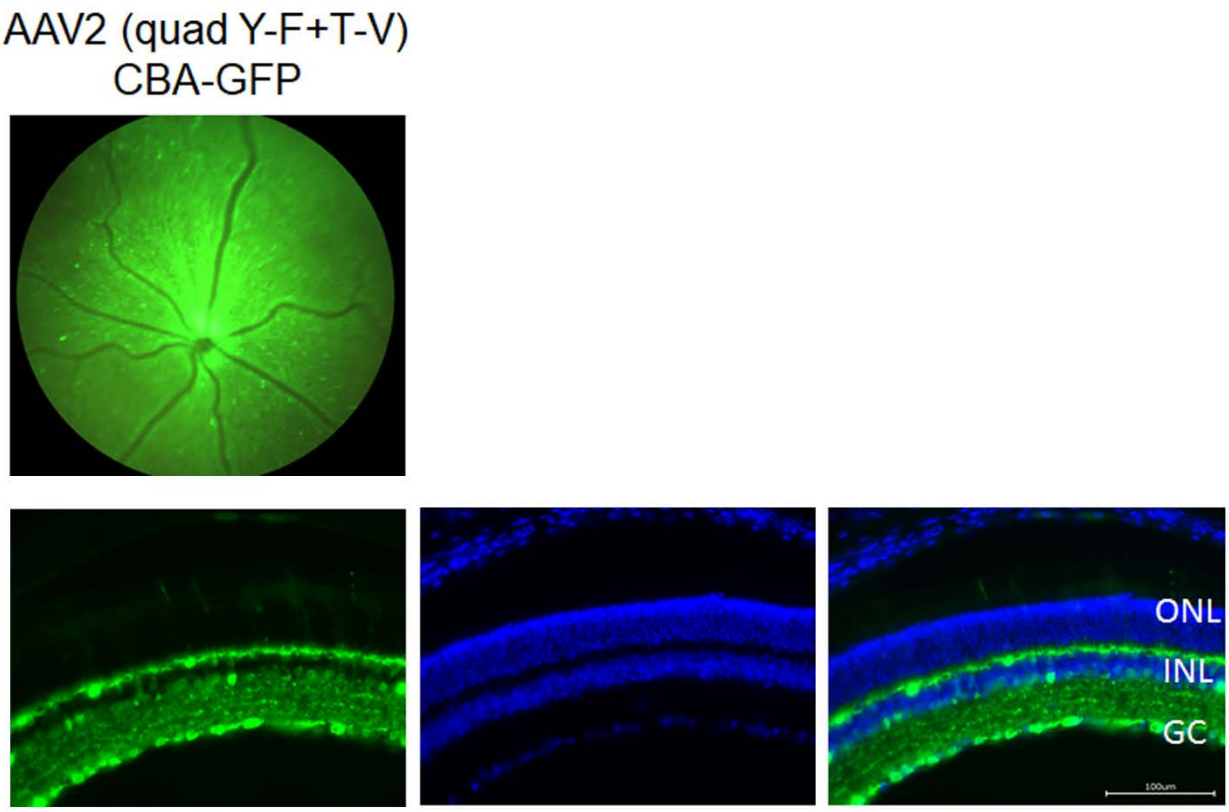
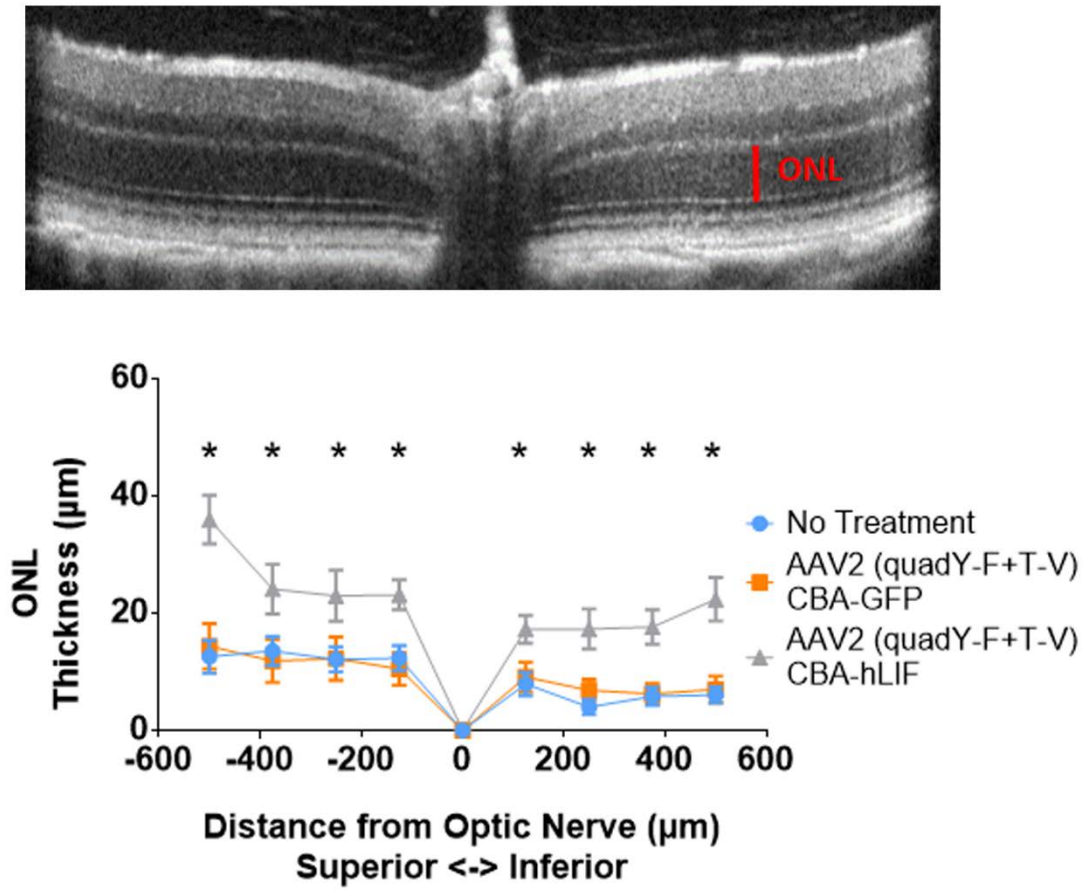
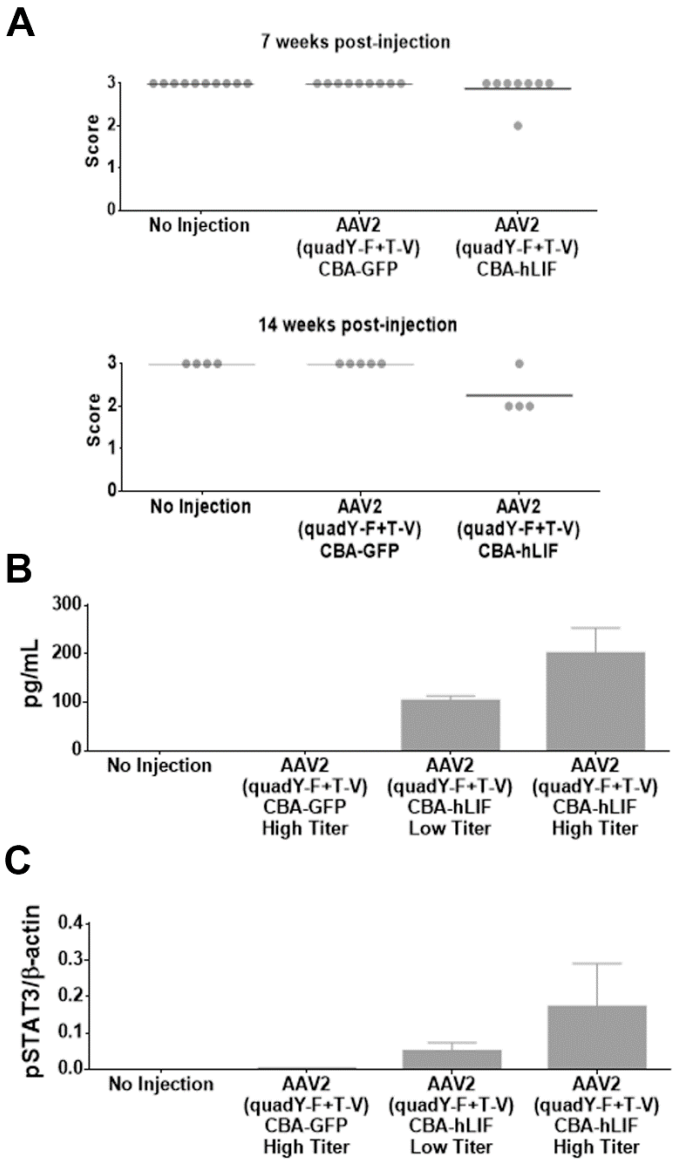


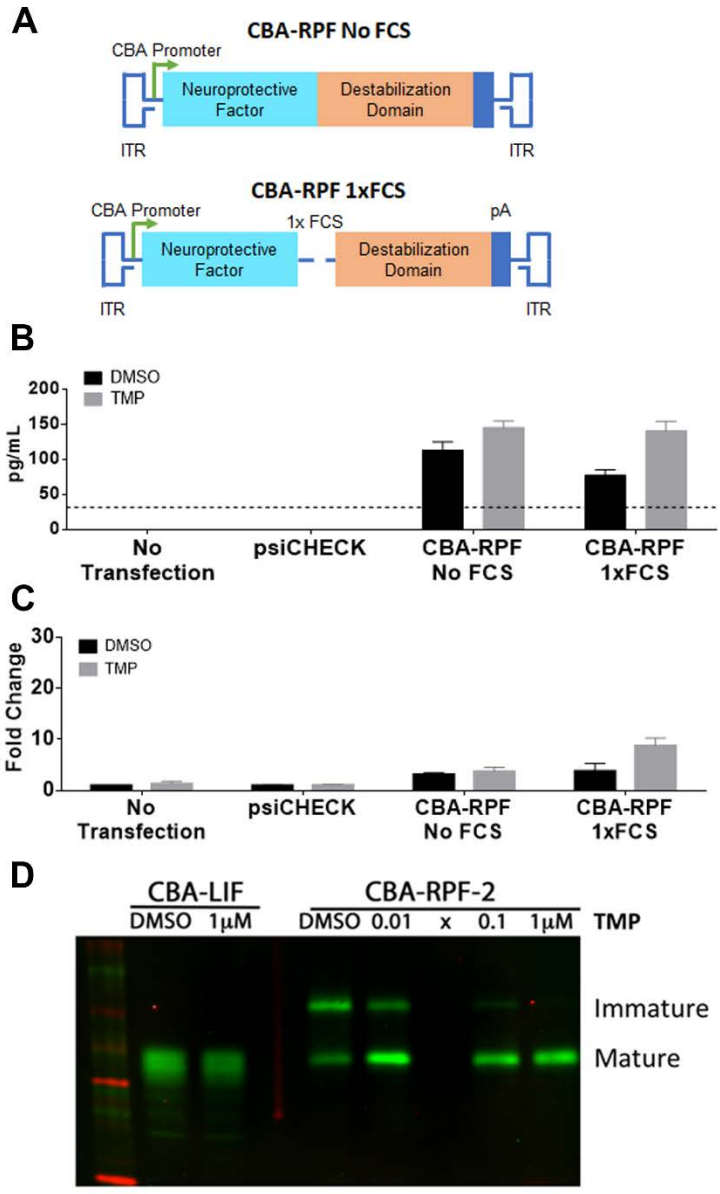
Supplemental Figure 1. Transduction profile of AAV2 (quadY-F+T-V) in the mouse retina. Top: Fundus images of the retina 7 weeks post-injected with AAV2 (quadY-F+T-V) CBA-GFP. The green channel shows the extent of GFP expression in the retina. Bottom: Immunohistochemistry cross section of the same retina showing GFP (green) expression and DAPI nuclei staining (blue). Majority of ganglion cells and some cell in the inner nuclear layer show GFP expression, while expression is only seen in a few photoreceptors in the outer nuclear layer. Images were taken at 40X magnification. White bar indicates 100μm. ONL = Outer nuclear layer, INL = Inner nuclear layer and GC = Ganglion cell layer.



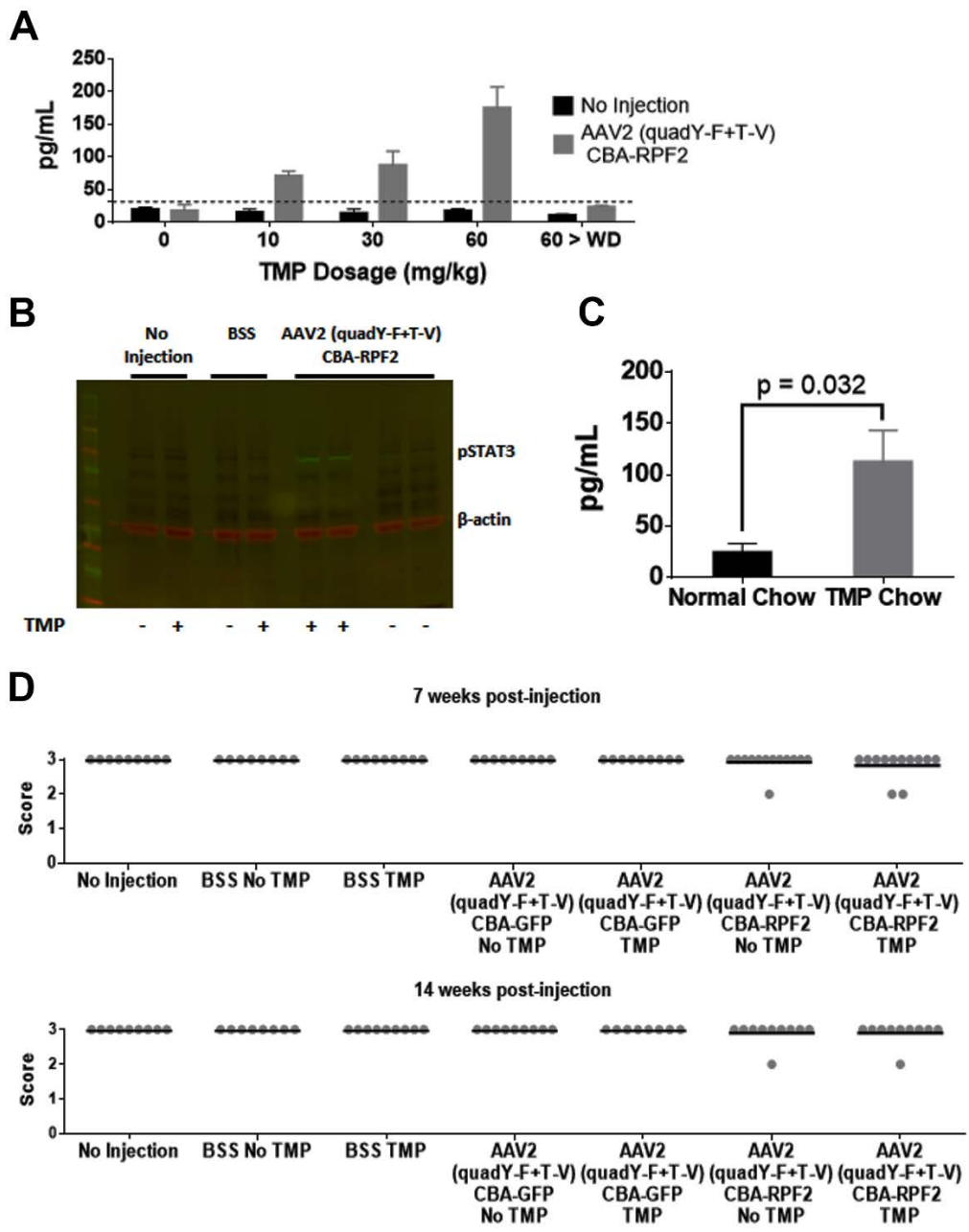
Supplemental Figure 2. AAV delivery of hLIF protects the retina from light damage. Top: Representative OCT image showing the ONL thickness quantification method. Bottom: Spider plot showing the quantification of the ONL thickness of light damaged retinas treated with low titer AAV2 (quad Y-F+T-V) CBA-hLIF. No treatment and AAV2 (quad Y-F+T-V) CBA-GFP injected were used as controls. Groups treated with vectors carrying CBA-hLIF show significant preservation of the ONL compared to controls. Two-way ANOVA with Sidak post-hoc test was performed. n = 8 biological replicates. * indicate $p < 0.05$. Error bars represent SD for each group.



Supplemental Figure 3. Characterization of AAV-hLIF delivery in the mouse retina. (A) Scoring of retinas injected with high titer AAV2 (quadY-F+T-V) CBA-hLIF 7 (top) and 14 (bottom) weeks post-injection. Each filled circle represents one animal (n = 3-10) and the black lines represent the mean for each group. Retinas constitutively expressing LIF had low retinal quality scores. **(B)** Quantification of hLIF in retinas injected with low titer or high titer AAV2 (quadY-F+T-V) CBA-hLIF as determined by ELISA. Higher viral titer delivery led to increased hLIF expression in the retina. **(C)** Immunoblot analysis of pSTAT3 Y705 levels in retinas treated with AAV2 (quadY-F+T-V) CBA-hLIF. Animals treated with the AAV2 (quadY-F+T-V) CBA-hLIF had increased activated STAT3 levels when compared to controls and were greater in high titer-treated groups. For B and C, n = 3-4 biological replicates per group. Error bars represent SD for each group.

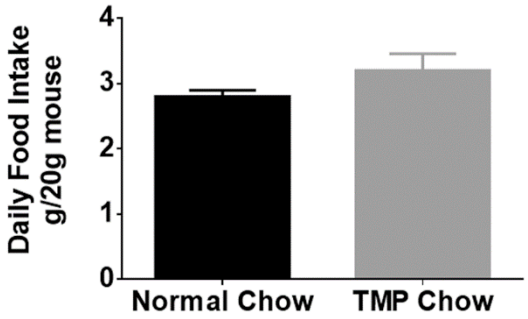


Supplemental Figure 4. Iterations of RPF2 development. (A) Schematics of CBA-RPF iterations with no or one furin cleavage site (FCS). **(B)** Levels of cytokine secreted by rMC-1 transfected cells into the conditioned media detected by ELISA. Dotted line indicates the limit of detection for the assay (32pg/ml). **(C)** Quantification of pSTAT3 in rMC-1 cells treated with conditioned media. The initial iterations of RPF2 were not efficiently regulated by TMP. Additionally, these iterations resulted in low STAT3 activation. For B and C, n = 3 biological replicates. Error bars represent SD for each group. **(D)** Dose-dependent stabilization and maturation of RPF2 by TMP in transfected rMC-1 cells by immunoblot. Increased stabilization and processing of RPF2 to its mature form (lower band) is observed at higher TMP concentration treatments compared to DMSO treatment.

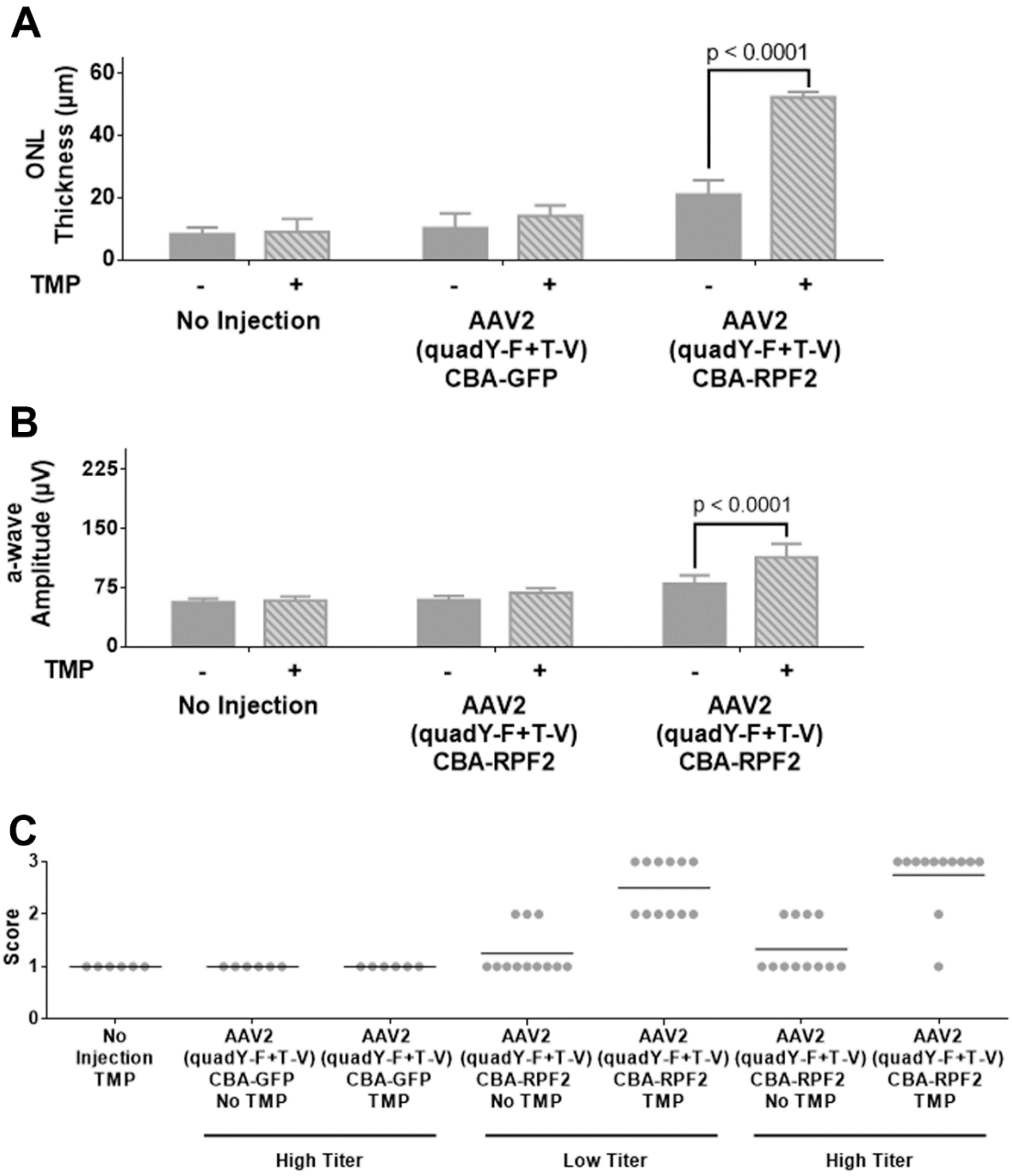


Supplemental Figure 5. Characterization of RPF2 *in vivo.* (A) ELISA levels of RPF2 in the retinal lysates of animals injected with high titer AAV2 (quadY-F+T-V) CBA-RPF2 at varying concentrations of TMP. RPF2 stabilization increased in a dose-dependent manner and was reversible after TMP withdrawal. These changes were not observed in non-injected mice. n = 2 biological replicates for the no injection group and 4 biological replicates for AAV2 (quadY-F+T-V) CBA-RPF2. Error bars represent SD for each group. **(B)** Immunoblot showing STAT3 activation in retinas treated with AAV2 (quadY-F+T-V) CBA-RPF2 with and without TMP. Activation of STAT3 is only observed in RPF2-treated retinas following TMP treatment. **(C)** Stabilization of RPF2 as detected by ELISA after one week of oral TMP administration. AAV2

(quadY-F+T-V) CBA-RPF2 injected animals fed the TMP diet for 7 days show significant stabilization of RPF2 in the retina. Student's t-test with Holm-Sidak post-hoc test was performed; n = 4 biological replicates and error bars represent SD for each group. **(D)** Scoring of retinas injected with high titer AAV2 (quadY-F+T-V) 7 (top) and 14 (bottom) weeks post-injection. Each filled circle represents one animal (n = 9-14) and the black lines represent the mean for each group. Animals injected with RPF2 did now show any significant long-term retinal adverse effects when compared to controls.



Supplemental Figure 6. TMP diet did not alter eating habits of the animal. Food intake and mouse weight was measured daily for 7 days for animals on normal and TMP supplemented chow. The TMP diet did not lead to any feeding behavior changes compared to controls. Error bars represent SD for each group. n = 4 biological replicates per group. Student's t-test was performed but no significance between the two groups was observed.



Supplemental Figure 7. High titer AAV2 (quadY-F+T-V) CBA-RPF2-injected retinas showed greater retinal preservation after bright light exposure as compared to low titer counterparts. (A) Averaged thickness of the retinal outer nuclear layer and **(B)** retinal function by ERG after RPF2 stabilization in high titer-treated animals. Two-way ANOVA with Sidak post-hoc test was performed for A-B; n = 6-12 biological replicates per group. Error bars represent SD for each group. **(C)** Retinal scoring of low titer and high titer AAV-RPF2-treated after light damage. Treated animals showed less signs of edema, rosette formation and infiltrating immune cells compared to the control groups. n = 6-12 biological replicates per group.

Plasmid Name	**Promoter**	**Protein expressed**	**Use**
pTR-UF11	chicken beta-actin	Green Fluorescent Protein	To create the AAV2 (quadY-F+T-V) CBA-GFP for animal studies
pTR-CBA-hLIF	chicken beta-actin	human Leukemia Inhibitory Factor	To overexpress hLIF in cell culture and create the AAV2 (quadY-F+T-V) CBA-hLIF for animal studies.
pTR-CBA-RPF2	chicken beta-actin	Retinal Protective Factor 2	To overexpress RPF2 in cell culture and create the AAV2 (quadY-F+T-V) CBA-RPF2 for animal studies.
psiCHECK (Promega Corporation)	SV40 early enhancer/promoter	*Renilla* luciferase	To overexpress luciferase in cell culture as a negative control

Supplemental Table 1. Plasmids used in this study

Name	Company	Catalog No.	Species raised	Use
Biotin hLIF	R&D Systems	BAF250	Goat	0.3µg/ml Detection for ELISA/1:500 dilution Immunoblots
hLIF	R&D Systems	AF-250-NA	Goat	3µg/ml Capture for ELISA
Streptavidin-HRP	R&D Systems	DY998		1:200 dilution Detection signal for ELISA
pSTAT3	Cell Signaling	9145L	Rabbit	1:2,000 dilution Immunoblots
beta actin	Abcam	ab6276	Mouse	1:10,000 dilution Immunoblots
Streptavidin-800CW	Li-Cor	926-32230		1:5,000 dilution Immunoblots
anti-mouse IRDye 680RD	Li-Cor	926-68170	Goat	1:10,000 dilution Immunoblots
anti-rabbit IRDye 800CW	Li-Cor	925-32211	Goat	1:10,000 dilution Immunoblots
s-opsin	Santa Cruz	sc-14363	Goat	1:300 dilution Immunohistochemistry
m-opsin	EMD Millipore	AB5405	Rabbit	1:300 dilution Immunohistochemistry
anti-goat Alexa Fluor 488	Life Technologies	A21206	Donkey	1:500 dilution Immunohistochemistry
anti-rabbit Alexa Fluor 488	Life Technologies	A11055	Donkey	1:500 dilution Immunohistochemistry

Supplemental Table 2. Antibodies used in this study