

Appendix

miR-181a/b downregulation: a mutation-independent therapeutic approach for Inherited Retinal Diseases

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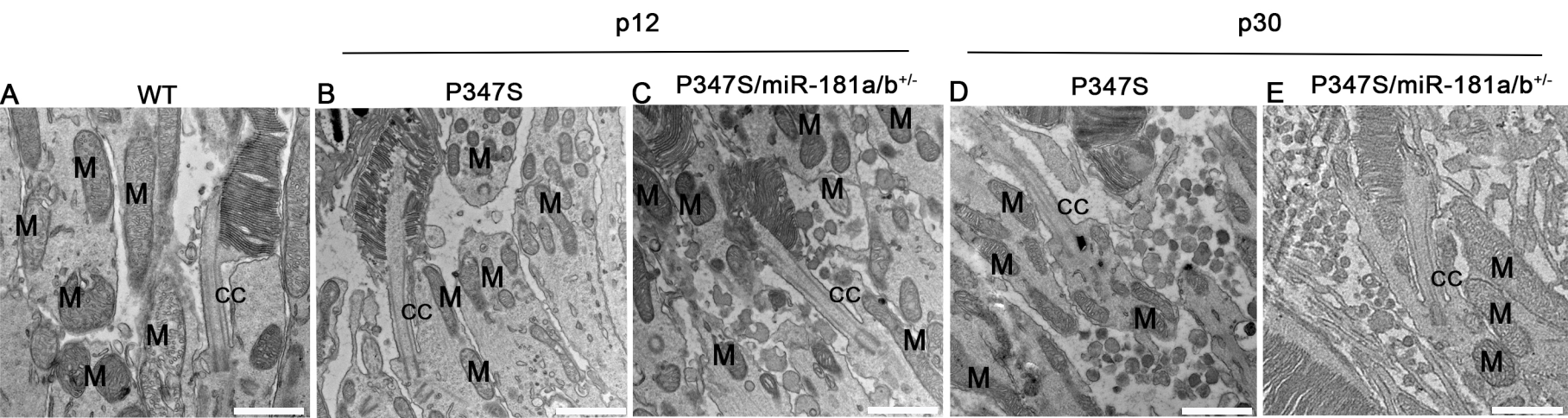
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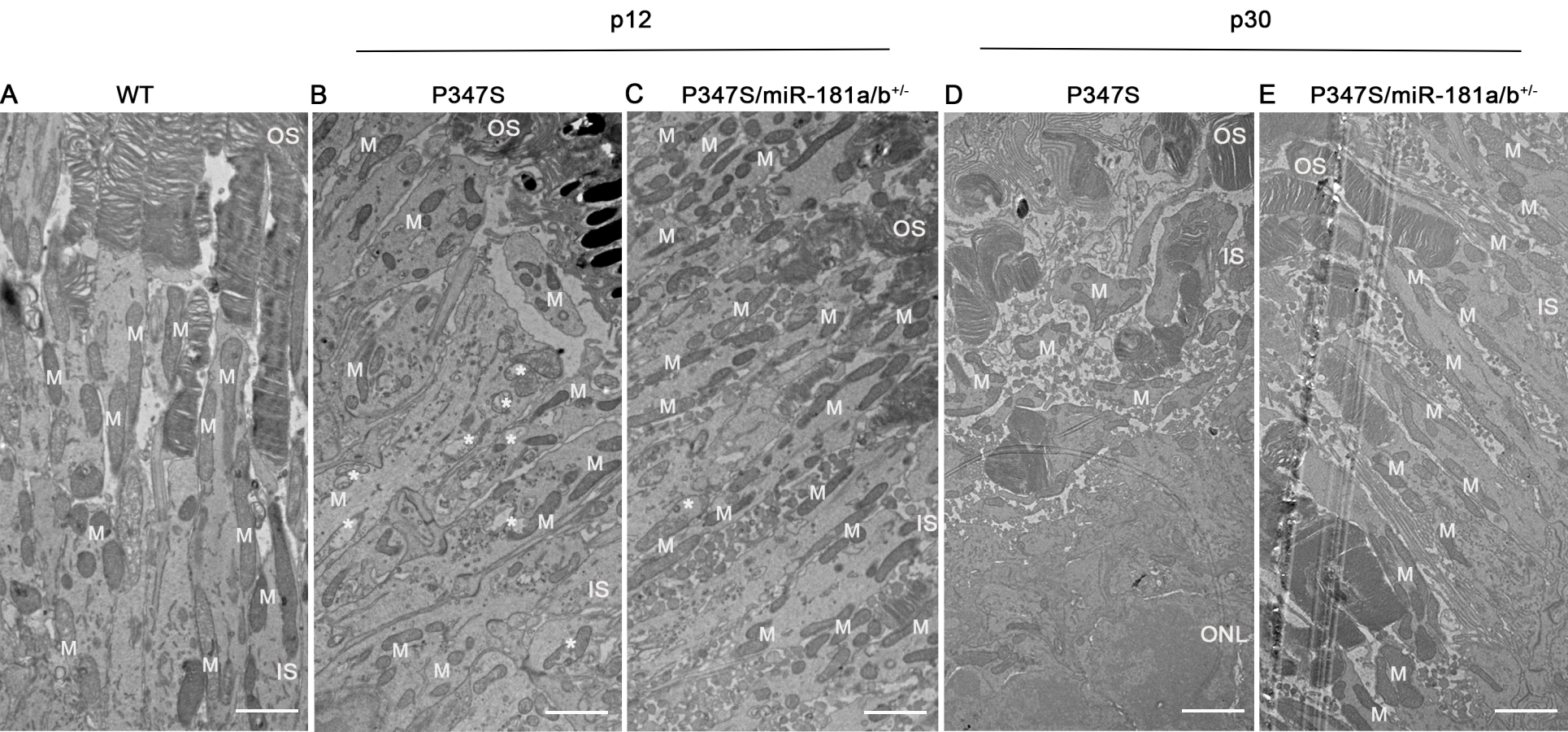
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The Electron Microscopy analysis of WT, P347S and P347S/miR-181a/b^{+/-} retinas at p12 and p30 was carried out on images where the sagittal orientation of the connecting cilium (cc) was visible (N≥2 animals/genotype/time point).
M: mitochondria; CC: Connecting Cilium

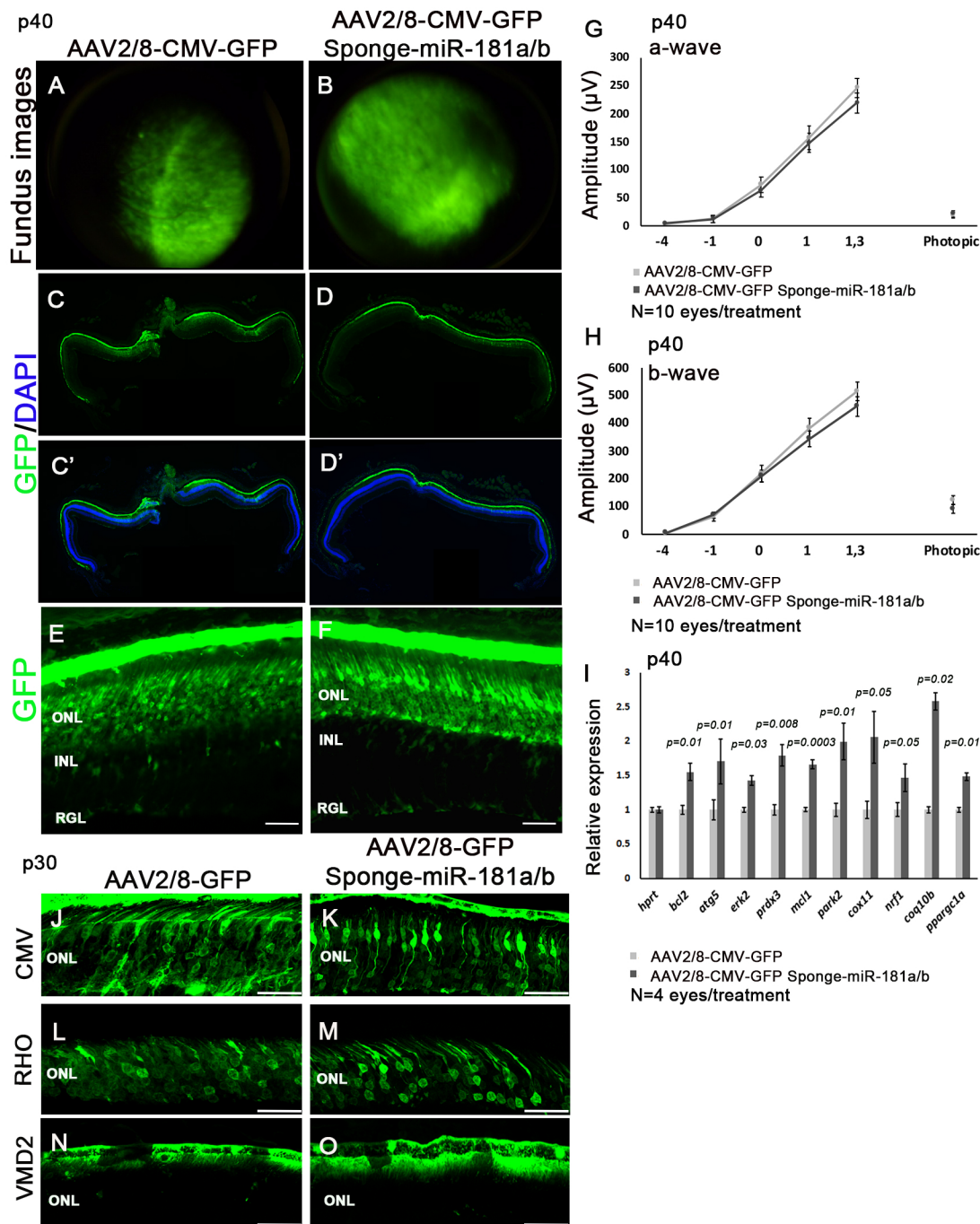
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The mitochondrial characterization was performed on Electron Microscopy images of WT, P347S and P347S/miR-181a/b^{+/-} retinas at p12 and p30 for all mitochondria located within the entire inner segment (IS), from the outer limiting membrane to the outer segment (OS) nascent disks.

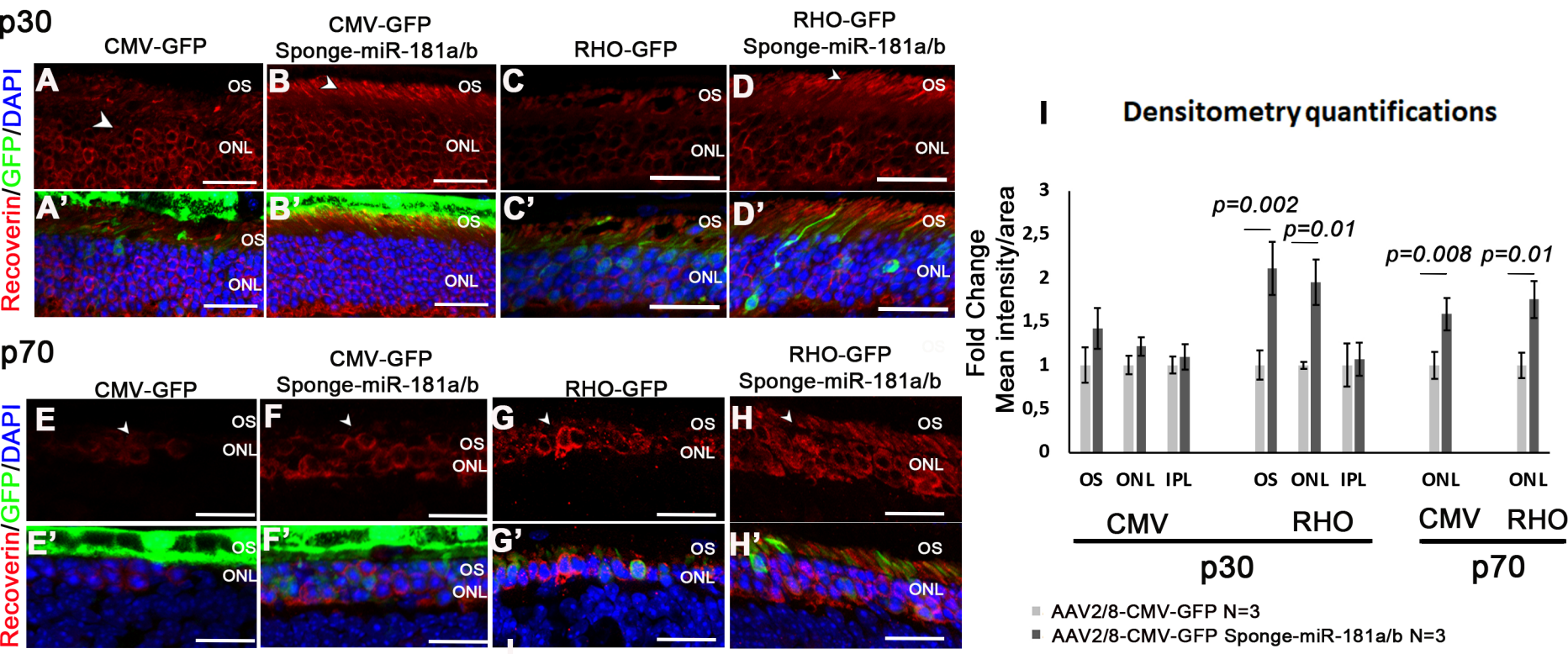
M: mitochondria; OS: Outer segment; IS: Inner Segment; *: swollen mitochondria (scale bars are 2 μm)

Figure Appendix S3



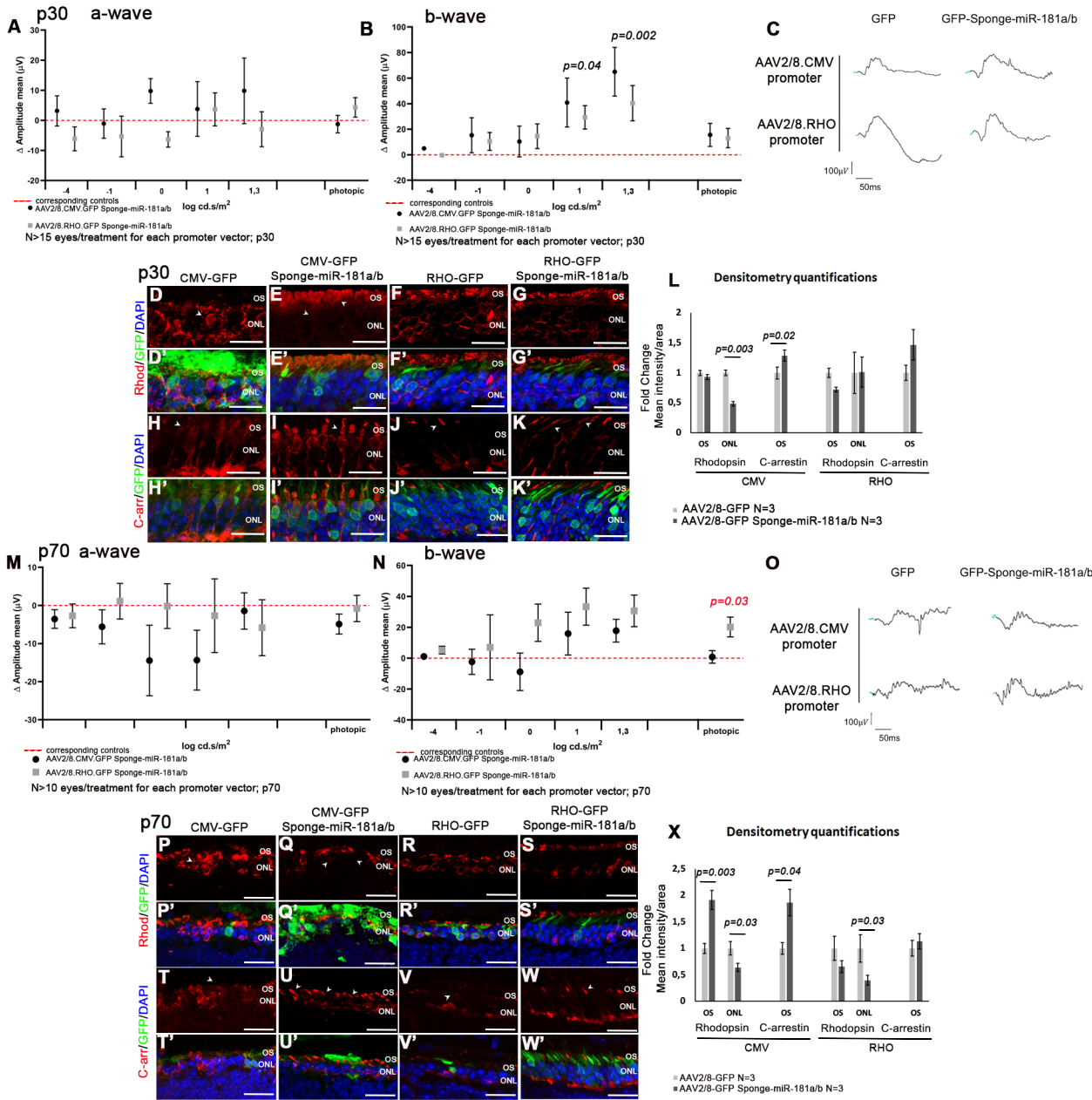
AAV2/8-Sponge-miR-181a/b delivery effectively downregulates miR-181a/b activity. A-F) Analysis of GFP expression in sub-retinally injected eyes. **A, B)** Analysis of eye fundi in AAV2/8.CMV.GFP (A) and AAV2/8.CMV.GFP-Sponge-miR-181a/b (B) sub-retinally-injected eyes. **C-D')** WT retinal sections of AAV2/8.CMV.GFP (C, C') and AAV2/8.CMV.GFP-Sponge-miR-181a/b (D, D') sub-retinally-injected eyes acquired using the TILE-SCAN tool with a Leica DM-6000 microscope. **E, F)** WT retinal sections of AAV2/8.CMV.GFP (E) and AAV2/8.CMV.GFP-Sponge-miR-181a/b (F) sub-retinally-injected eyes acquired using a Zeiss LSM700 confocal microscope. **G, H)** a-wave (G) and b-wave (H) ERG responses at p30 of AAV2/8.CMV.GFP and AAV2/8.CMV.GFP-Sponge-miR-181a/b of WT eyes sub-retinally-injected at p4. N=10 eyes/treatment. Data are presented as mean $\pm$ SD. Two-way ANOVA test. **I)** qRT-PCR analysis of miR-181a/b target transcripts in AAV.GFP-Sponge-miR-181a/b- vs AAV2/8.GFP-injected WT eyes. N=4 eyes/treatment. Data are presented as mean of Fold Change $\pm$ SEM. Student's t-test, paired. **J-O)** P347S retinal section of AAV2/8.GFP-Sponge-miR-181a/b- vs AAV2/8.GFP-injected eyes under the control of CMV promoter (J, K), RHO promoter (L, M) and VMD2 promoter (N, O). Scale bars are 50 μ m

Figure Appendix S4



Immunofluorescence analysis of Recoverin staining (p30 A-D', p70 E-H'; white arrowheads) in Sponge-miR-181a/b-injected eyes vs the corresponding controls. Scale bars are 25 μ m. Fluorescence densitometry quantification of Recoverin staining is reported in I, N=3 eye/treatment for each staining. Data are presented as mean of Fold Change $\pm$ SEM. Student's t-test, paired.

Figure Appendix S5



AAV2/8-Sponge-miR-181a/b delivery at p12/14 ameliorates the retinal phenotype of P347S mice. A-C) ERG response (a-wave in A and b-wave in B), reported as Delta (Δ) amplitude in P347S animals injected at p12/14 with AAV2/8.CMV.GFP-Sponge-miR-181a/b and AAV2/8.CMV.RHO-Sponge-miR-181a/b with respect to the corresponding control vectors (AAV2/8.CMV.GFP and AAV2/8.RHO.GFP; red dotted line)) at p30 ($N \geq 15$ eyes/treatment, for each promoter vector). P30 representative curves at 20 candles are reported in (C). **D-L)** Immunofluorescence analysis showed amelioration of Rhodopsin localization (p30 D-G') and C-arrestin expression with amelioration of the OS structure (p30 H-K', white arrowheads) in Sponge-miR-181a/b-injected eyes. Scale bars are 25 μ m. Fluorescence densitometry quantification of each staining at p30 is reported in L, $N=3$ eye/treatment for each staining. **M-O)** ERG response (a-wave in M and b-wave in N), reported as Delta (Δ) amplitude in P347S animals injected at p12/14 with AAV2/8.CMV.GFP-Sponge-miR-181a/b and AAV2/8.CMV.RHO-Sponge-miR-181a/b with respect to the corresponding control vectors (AAV2/8.CMV.GFP and AAV2/8.RHO.GFP; red dotted line) at p70 ($N \geq 10$ eyes/treatment, for each promoter vector). P70 representative curves at 20 candles are reported in (O). **P-X)** Immunofluorescence analysis showed amelioration of Rhodopsin localization (p70 P-S') and C-arrestin expression with amelioration of the OS structure (p70 T-W', white arrowheads) in Sponge-miR-181a/b-injected eyes. Scale bars are 25 μ m. Fluorescence densitometry quantification of each staining at p70 is reported in X, $N=3$ eye/treatment for each staining. AAV2/8.CMV.RHO-Sponge-miR-181a/b vs corresponding control (AAV2/8.CMV.GFP) p values are reported in red, AAV2/8.CMV.GFP-Sponge-miR-181a/b vs corresponding control (AAV2/8.CMV.GFP) p values are reported in black. Data are presented as mean of Delta (Δ) amplitude $\pm$ SD in A, B and M, N. Two-way ANOVA test. Data are presented as mean of Fold Change $\pm$ SEM in L and X. Student's t-test, paired.

Appendix Table S1: Primer sequences for qRT-PCR analysis.

Transcript	Forward	Reverse
<i>mmu-Hprt</i>	AGCTTGCTGGTGAAAAGGAC	GTCAAGGGCATATCCACAAC
<i>mmu-Gapdh</i>	GGTGCTGAGTATGTCGTGGA	CTAAGCAGTTGGTGGTGCAG
<i>mmu-Bcl2</i>	ACAACATCGCCCTGTGGATG	GTTTGTGCGACCTCACTTGTG
<i>mmu-Mcl1</i>	GCTTCATCGAACCATTAGCAG	CCAGCAGCACATTTCTGATG
<i>mmu-Xiap</i>	GGTCCTGATTGCAGATCTTG	GTCAACTGCTTCTGCACAC
<i>mmu-Atg5</i>	GCCTATATGTACTGCTTCATC	CAACGTCAAATAGCTGACTC
<i>mmu-Erk2</i>	CACCAACCTCTCGTACATCG	GTGCCCCGATGATGTCATTG
<i>mmu-Park2</i>	CCATCAAGAAGACCACCAAG	CAAGTGACATCTCTCTCTAC
<i>mmu-Nrf1</i>	CTTACTGGAGTCCAAGATGC	GGAGCCAACAGAATCCTTTC
<i>mmu-Cox11</i>	GAATCCTACTGACAAACCAG	GAGGTCGACATTCACCATTC
<i>mmu-Coq10</i>	GATGATCATGGCAGCTCGGA	CTCGCACAGATCTCTTTAGG
<i>mmu-Prdx3</i>	GGAGTATTTCTGCCTCAACAG	CTCTCCATTGACAACAGCAG
<i>mmu-Ppargc1a</i>	GGAATGCACCGTAAAATCTGC	TTCTCAAGAGCAGCGAAAGC
<i>mmu-Jak2</i>	CTACCAGATGGAAACTGTGC	GCCTCTGTAATGTTGGTGAG
<i>mmu-Stat3</i>	CAACCAGTCTGTGACCAGAC	GCCAGCTCTTCATCAGTCAG
<i>mmu-Drp1</i>	CCCGCATCCATGAGATCAAG	CGTCACTGCTGCAAATACAG
<i>mmu-Irf1</i>	CTTCGTCGAGGTAGGACGTG	CTTTGCTGCAGGAGCGATTC
<i>mmu-BclXl</i>	CACCTATCTGAATGACCACC	CCACACCAGCCACAGTCATG
<i>mmu-Pim</i>	CATGGTCTGCGGAGATATTC	CCGGATTTCTTCAAAGGAGG
<i>mmu-Birc3(cIAP)</i>	GCTGAGGTGCTGGGAATCTG	CATCTTCTGGGGAGTCTGAC
<i>mmu-Birc5(Survivin)</i>	GAACGAGCCTGATTTGGCCC	CTTGACAGTGAGGAAGGCGC
<i>mmu-Fos</i>	GAGAAACGGAGAATCCGAAG	GCAATCTCAGTCTGCAACGC
<i>mmu-Fgf2</i>	CTATCAAGGGAGTGTGTGCC	CTGCCCAGTTCGTTTCAGTG
<i>mmu-Gap43</i>	GGAGCCTAAACAAGCCGATG	CTGTCGGGCACTTTCCTTAG