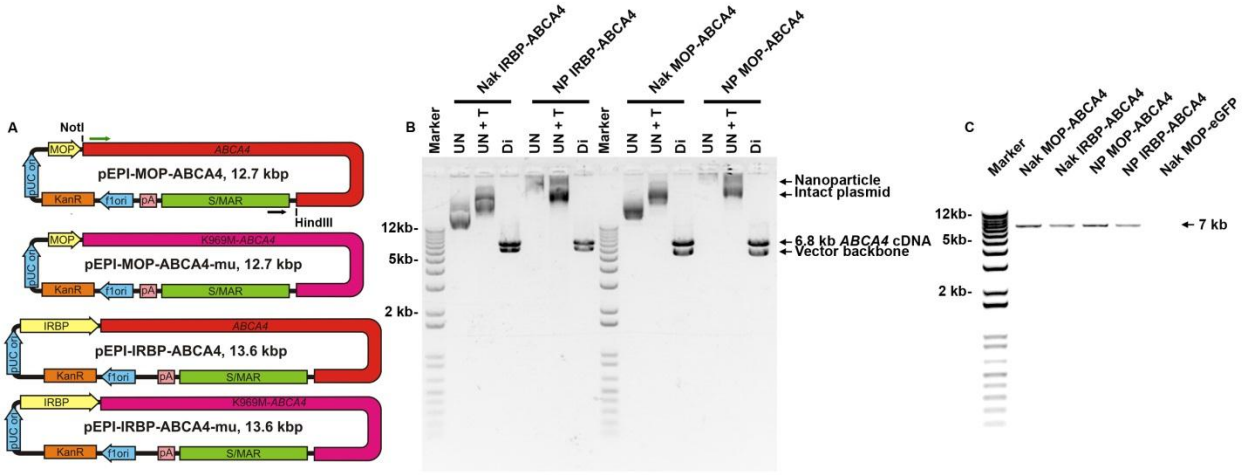
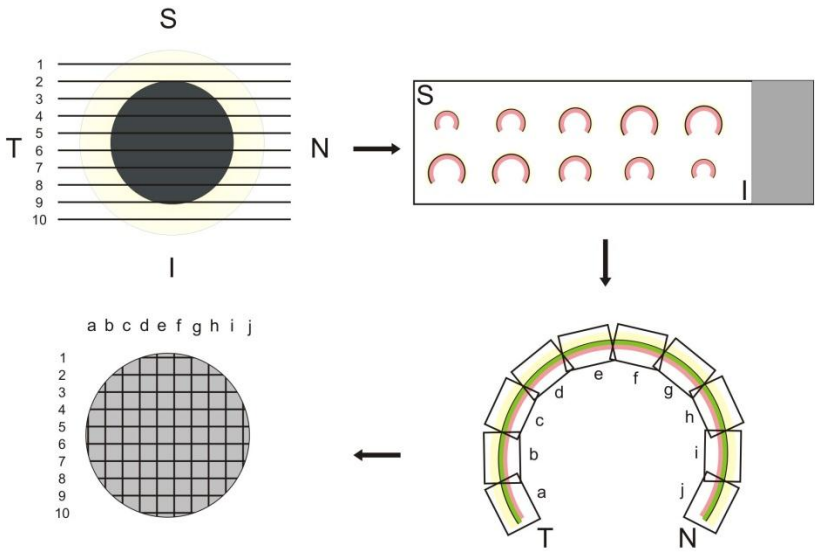
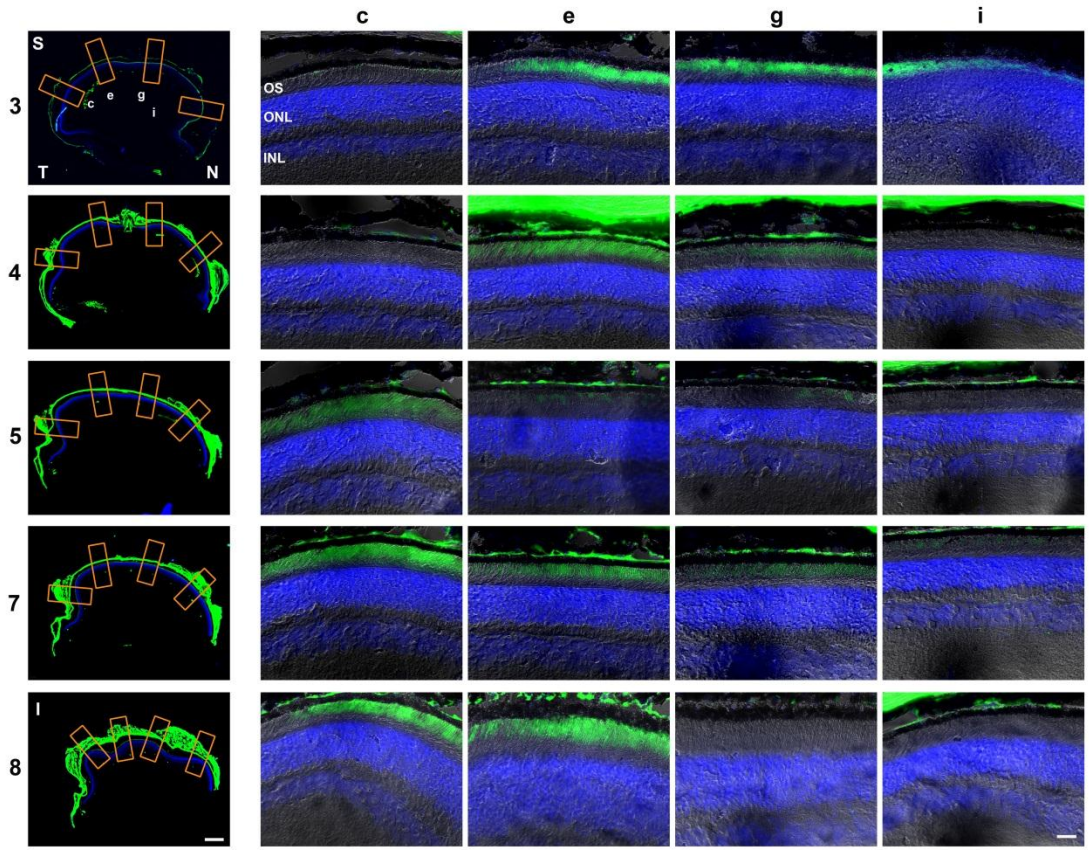
SUPPLEMENTAL FIGURES AND LEGENDS



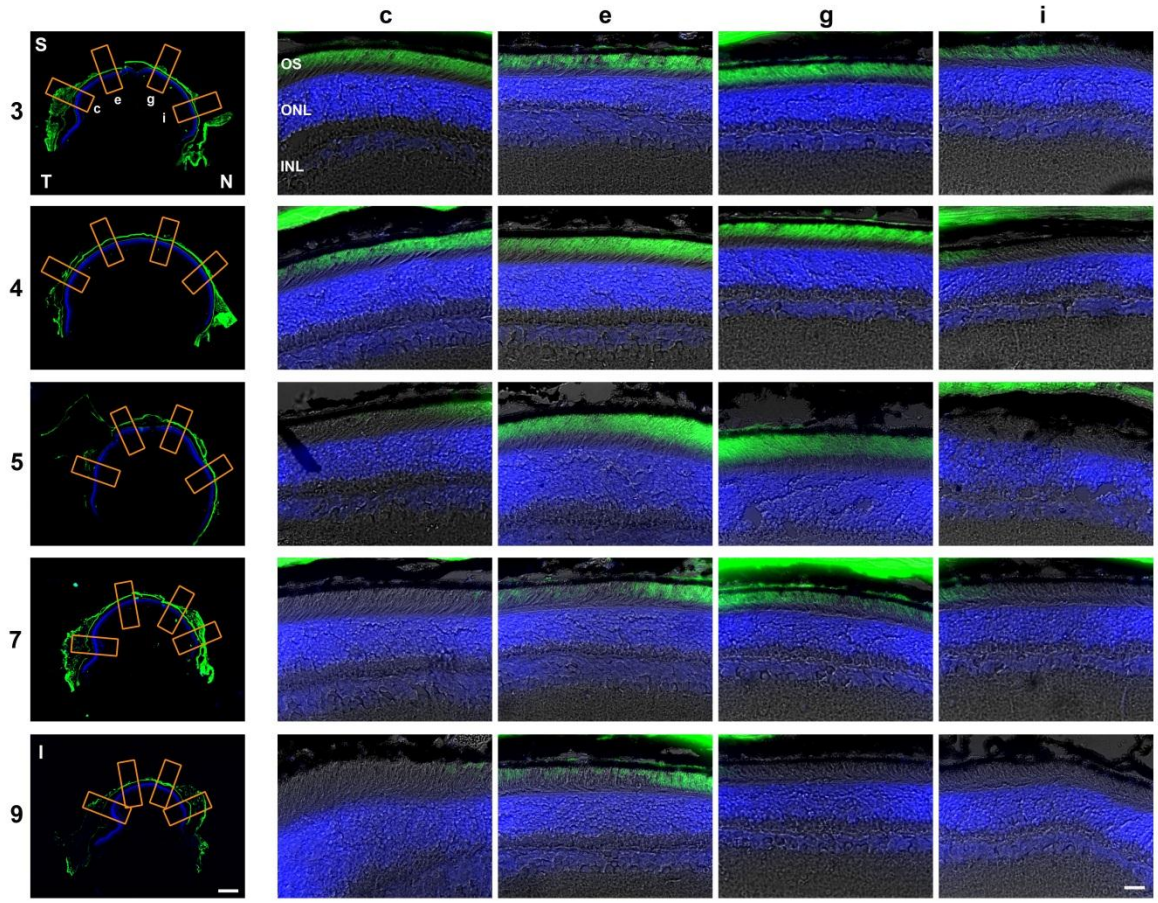
Supplemental Figure 1 Generation and testing of ABCA4 vectors. A. Shown are diagrams of the four different human ABCA4 expression vectors which were compacted into DNA NPs. The episomal pEPI-CMV-EGFP vector was generously provided by Dr. Hans J. Lipps (University Witten/Herdecke, Germany). The CMV promoter was replaced with either the IRBP (22) (1.3 kbp) or MOP (1) (0.5 kbp) promoter. WT or mutant (K969M) human *ABCA4* cDNA (6.8 kbp, kindly provided by Dr. Hui Sun at University of California, Los Angeles, USA) were then subcloned into the pEPI-IRBP/MOP constructs to yield pEPI-IRBP-ABCA4/pEPI-IRBP-ABCA4-mu (13,561 bp) and pEPI-MOP-ABCA4/pEPI-MOP-ABCA4-mu (12,693 bp). **B.** Naked DNA and NPs, after storage at 4ºC for 2.5 years were treated with 2.5% trypsin (**UN + T**) for 30 minutes at 37ºC to digest the lysine peptide used for compaction and recover the plasmid. The first lane in each set (**UN**) is a sample of the untreated material used for injections (naked or NP). As expected (3), undigested NPs do not migrate into the gel. To confirm identity of the plasmids, samples underwent restriction digest with NotI and HindIII (**Di**-see restriction site locations in **A**). Only bands corresponding to the excised *ABCA4* cDNA and the vector backbone are detected in digested samples. These results indicate that the NPs used for this study contain intact plasmid. **C.** To further confirm that the NPs (and naked DNA) were intact, DNA samples (after trypsin digest of NPs to release the DNA) underwent PCR amplification using a forward primer at the beginning of the *ABCA4* cDNA and a reverse primer in the S/MAR region (see small green arrows in A for location of primers. F: ATTCGCTTTGTGGTGGAACT; R: TGTCTCTCCGTTTCTGCATT). Only a single band of the appropriate size was detected. Naked MOP-eGFP (which has a binding site for the reverse primer but not the forward) was used as a negative control to confirm specificity of amplification. These data indicate that the NPs contain intact plasmid of the correct size.



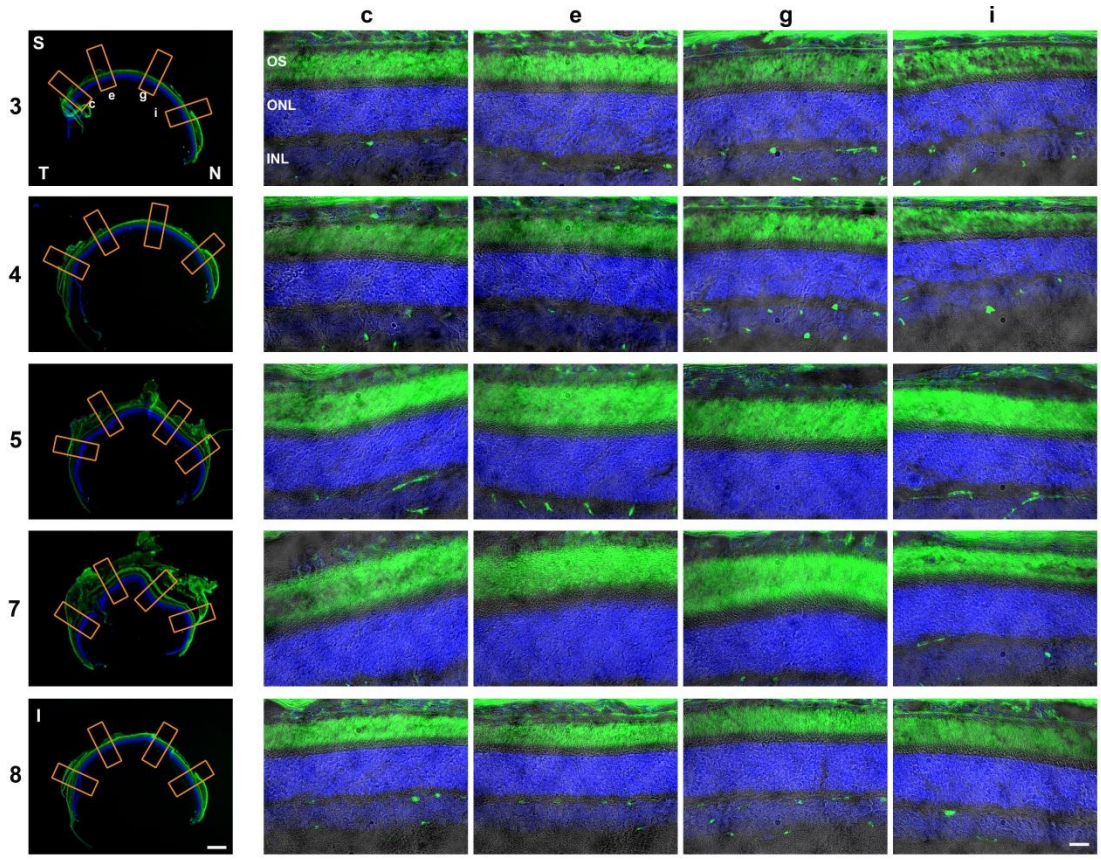
Supplemental Figure 2 Schematic of procedure for analyzing the distribution of ABCA4 expression. Whole eyes were cryoprotected and sectioned along the nasal-temporal plane from superior to inferior (top left). Sections every ~200 µm were collected (top right, 1-10) and labeled with antibodies against ABCA4. Images from adjacent frames from each section from temporal to nasal (bottom right, a-j) were then scored for ABCA4 expression by an observer blinded to treatment group. The scores were then plotted in the schematic shown on the bottom left with intensity of green color corresponding to intensity of expression (as in **Figure 2f**). S-superior, I-inferior, T-temporal, N-nasal.



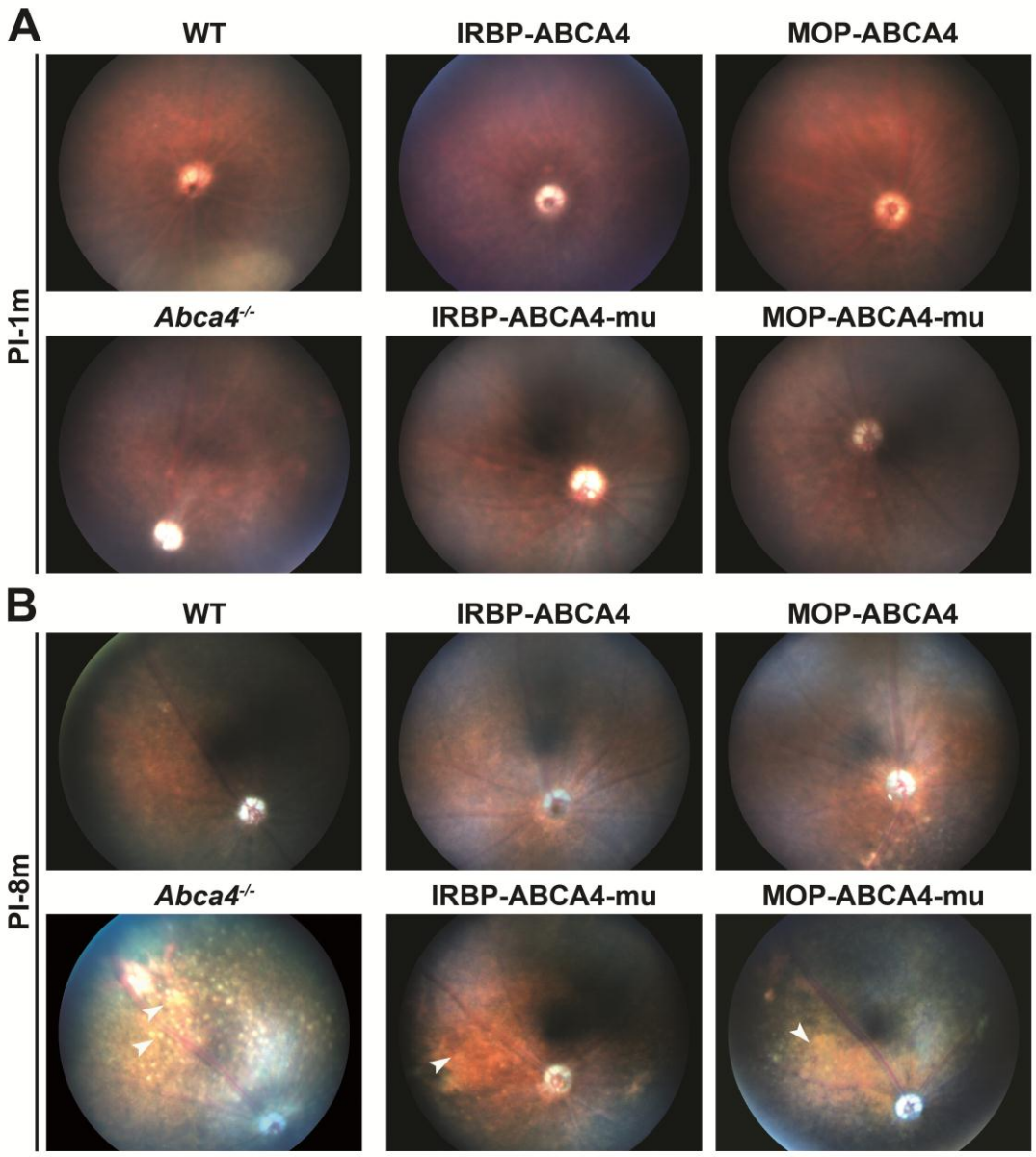
Supplemental Figure 3 Representative images showing distribution of ABCA4 after injection with NP-IRBP-ABCA4. Cyrosections (10 μm) throughout the retina of NP-IRBP-ABCA4 injected *Abca4*$^{-/-}$ eyes were immunostained with antibodies against ABCA4 (green) at PI-8 m. The left column shows lower magnification (2x, scale bar 400 μm) images of five representative retinal cryosections from the superior (top-S) to the inferior (bottom-I) of whole eye. Numbers correspond with approximate locations as per **Supplemental Figure 2.** The right columns show higher magnification (40x, scale bar 20 μm) images of four representative regions from the temporal to nasal (letters correspond with approximate locations as per **Supplemental Figure 2**). S-superior, I-inferior, T-temporal, N-nasal.



Supplemental Figure 4 Representative images showing distribution of ABCA4 after injection with NP-MOP-ABCA4. Cyrosections (10 μm) throughout the retina of NP-MOP-ABCA4 injected *Abca4*$^{-/-}$ eyes were immunostained with antibodies against ABCA4 (green) at PI-8 m. The left column shows lower magnification (2x, scale bar 400 μm) images of five representative retinal cryosections from the superior (top-S) to the inferior (bottom-I) of whole eye. Numbers correspond with approximate locations as per **Supplemental Figure 2.** The right columns show higher magnification (40x, scale bar 20 μm) images of four representative regions from the temporal to nasal (letters correspond with approximate locations as per **Supplemental Figure 2**). S-superior, I-inferior, T-temporal, N-nasal.



Supplemental Figure 5 Representative images showing distribution of ABCA4 in WT eyes. Cyrosections (10 µm) throughout the retina of WT eyes were immunostained with antibodies against ABCA4 (green) at PI-8 m. The left column shows lower magnification (2x, scale bar 400 µm) images of five representative retinal cryosections from the superior (top-S) to the inferior (bottom-I) of whole eye. Numbers correspond with approximate locations as per **Supplemental Figure 2.** The right columns show higher magnification (40x, scale bar 20 µm) images of four representative regions from the temporal to nasal (letters correspond with approximate locations as per **Supplemental Figure 2**). S-superior, I-inferior, T-temporal, N-nasal.



Supplemental Figure 6 NP-mediated ABCA4 gene delivery reduces retinal flecking in *Abca4*$^{-/-}$ mice. Shown are additional representative *in vivo* fundus images captured from treated mice at PI-1m (**a**) and PI-8m (**b**). Age matched WT and uninjected *Abca4*$^{-/-}$ mice were used as controls. Compared with the WT controls, signs of retinal flecking (white arrows) were observed in uninjected *Abca4*$^{-/-}$ and mutant NP injected animals at PI-8m but not in animals treated with MOP-ABCA4 or IRBP-ABCA4 NPs. No flecking was observed at PI-1 m in any group.

Supplemental Table 1: MOP-ABCA4 NP-treated animals exhibit complete recovery of dark adaptation after photobleach.

Supplemental Table 1: Summary of Recovery Characteristics			
Group	**Fraction Exhibiting 100% Recovery**	**Mean time to 100% Recovery (min.)**	**Mean Recovery at 50 minutes (%)**
WT	9/9	26.5 ± 3.2*	119.2 ± 6.3
Uninjected *Abca4*$^{-/-}$	3/9	50, 50, 40 remainder, >50**	87.1 ± 5.4
Saline	2/9	50, 40 remainder >50	84.1 ± 6.1
MOP-ABCA4	4/4	27.2 ± 7.7	110.2 ± 4.0
MOP-ABCA4-mu	0/4	>50	77.9 ± 4.3
IRBP-ABCA4	3/6	20, 40, 48, remainder, >50**	108 ± 14.2
IRBP-ABCA4-mu	1/6	30, remainder, >50	81.1 ± 9.7

* values are mean ± SEM. ** first row is the values (in minutes) for animals that exhibited 100% recovery; the remainder had recovery times that exceeded the duration of the experiment, i.e. greater than 50 minutes. As reported previously for WT mice (17), final recovery amplitudes in 4/4 MOP-ABCA4 animals exceeded pre-bleach levels (**Supplemental Table 1**). Differences between mean recovery time in WT vs. NP-MOP-ABCA4 treated animals were not significant by Student's t-test.