Supplemental Methods

Biodistribution

Following sacrifice, different, new forceps were used to enucleate treated and untreated eyes which retained approximately 0.5 cm of proximal optic nerve. Different, new dissection scissors were then used to cut the optic nerves away from the eyeballs after which they were snap frozen in liquid nitrogen and transferred to -80°C where they remained until the time of DNA extraction. Eyeballs were immersed in 4% paraformaldehyde (PAF) and processed for immunohistochemistry. Brains were removed and a stainless steel mouse coronal brain matrix (Harvard Apparatus, Holliston, MA) was used to isolate visual-specific regions. Right and left lateral geniculate nuclei were collected from one mouse per treatment group (at the latest time point), formalin fixed and saved in the event that vector genomes were recovered from brain and immunohistochemistry was necessary. Separate portions of right and left brain containing visual pathways were collected, snap frozen in liquid nitrogen and transferred to -80°C where they remained until the time of DNA extraction. Precautions were taken to avoid cross-contamination while harvesting tissues. Genomic DNA was extracted from tissues according to the manufacturer’s protocol (Qiagen DNeasy tissue kit). Resulting DNA concentrations were determined using an Eppendorf Biophotometer (Model 6131; Eppendorf, Hamburg, Germany). Quantitative PCRs were performed according to previously described methods with minor modifications (29). Primer pairs were designed to the SV40 poly-adenylation signal (SV40 polyA) region in each vector genome and standard curves established using known concentrations of plasmid DNA containing the same SV40 polyA target sequence. DNA samples were assayed in triplicate. In order to rule out false negatives due to inhibition of PCR, the third replicate was ‘spiked’ with plasmid DNA containing target (SV40 polyA) at a ratio of 100 copies/μg of genomic DNA. If > 40 copies of the spike-in DNA were detected, the sample was considered acceptable for reporting vector genome copies. In some cases samples failing ‘spike in’
were reanalyzed using less than 1 μg of genomic DNA in PCR reactions, thereby diluting out PCR inhibitors copurifying with DNA in the extracted tissue. Spike-in copy number was reduced proportionally to maintain the 100 copies/μg DNA ratio. Criteria for reporting vector genome copies were established according to previously described methods (29). Briefly, greater than 100 genome copies/μg was considered positive and the measured copy number/μg reported. Fewer than 100 copies/μg was considered negative.

**Tissue Preparation, Immunohistochemistry and Microscopy**

At sacrifice, concomitant with biodistribution studies performed at 7 months post- [AAV8(Y733F)-hGRK1-mGC1] and 10 months post- (AAV5-smCBA-mGC1 and AAV5-hGRK1-mGC1) injection, the limbus of treated GC1KO mice, age-matched, untreated GC1KO mice as well as age-matched congenic GC1+/+ mice were marked with a hot needle at the 12 o’clock position, facilitating orientation. Untreated GC1KO and GC1+/+ controls were age-matched to the AAV8(Y733F)-treated mice (8 months of age at the time of sacrifice). An additional GC1KO mouse treated with AAV5-hGRK1-mGC1 was sacrificed at 15 months post-injection and eyes prepared in the same manner. Eyes designated for cryosectioning and wholemount immunostaining were processed and immunostained according to previously described methods with minor modifications (3, 32). 10 micron retinal sections were incubated with antibodies directed against GC1 (rabbit polyclonal 1:200, sc-50512 Santa Cruz Biotechnology) or mouse cone arrestin (rabbit polyclonal “LUMIj”, 1:1000, generously provided by Dr. Cheryl Craft, Univ. of Southern California). Following primary incubation, IgG secondary antibodies Alexa-488 or Alexa-594, respectively, were applied for 1 hour at room temperature (1:500 in 1X PBS). Sections were counterstained with 4’, 6’- diamino-2-phenylindole (DAPI) for 5 minutes at room temperature. At 11 months post-injection, one GC1KO mouse that received treatment with AAV5-smCBA-mGC1 in one eye only was sacrificed and retinal wholemounts from treated and untreated eyes processed according to
previously described methods (32). Briefly, wholemounts were stained with LUMIj (1:1000) followed by 
IgG secondary Alexa-594 (1:500 in 1X PBS) and positioned on slides with the superior (dorsal) portion of 
the retina oriented at 12- o’clock. Retinal sections were analyzed by confocal microscopy (Leica TCS SP2 
AOBS Spectral Confocal Microscope equipped with LCS Version 2.61, Build 1537 software). Images were 
taken at identical exposure settings at 20X magnification. Retinal wholemounts were analyzed with a 
widefield fluorescent microscope (Zeiss Axioplan 2) equipped with QImaging Retiga 4000R Camera and 
QImaging QCapture Pro software. Quadrants of each wholemount were imaged at 10X under identical 
exposure settings and then merged together in Adobe Photoshop. Cone photoreceptor densities were 
analyzed in retinal wholemounts by counting cells labeled with secondary fluorophore directed against 
cone arrestin antibody in the inferior, central and superior retina as previously described with minor 
modifications (Boye et al., 2010). Briefly, four squares (100 µm²) were placed over identical areas in 
inferior, central or superior retinas of treated and untreated GC1KO retinas (total of 12 boxes per eye). 
One box per region is blown up for a higher resolution depiction of individual cone cells. Cones were 
counted in each box, values were averaged and standard deviations calculated. The standard t-test was 
used to calculate P-values between desired samples. Cone cell densities in each respective retinal region 
are reported as the average number of cells per square mm.

**mRNA quantification by real time-PCR**

Primer pairs for GCAP1, GNAT2, PDEα and GAPDH were identical to those used by Baehr et al (36). 
Primers for murine GC1 (forward primer: 5’-GACCCTTCTGCTGGTTCGATCCA, reverse primer: 5’-
CTGCATGTGTAGCAGCCTGTGCCTC) were designed to flank exon 5, the site of gene disruption in the 
GC1KO mouse (23) and generate an amplicon of 151 bps. Interestingly, a second GC1 primer pair 
targeted to exon 18 and 19 of GC1, well downstream of the gene disruption, produced a PCR product in 
the untreated GC1KO mouse sample and therefore these primers were not used (data not shown). PCR
produced appropriately sized amplicons in GC1 +/- and AAV-mGC1-treated GC1KO retina samples, but not in untreated GC1KO retina as expected (data not shown). Amplicon identity was verified by restriction digest with Stu I (NEB) which cleaves within the target sequence to yield fragments of 56 bps and 95 bps. rtPCR with GC1 and GAPDH primers on dilution series of reverse transcribed DNA (from both GC1 +/- and AAV-mGC1-treated GC1KO retina samples) resulted in similar slopes, indicating suitability of GC1 primers for quantifying both endogenous and vector mediated GC1 message (Figure S1).