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## Supplemental information

**Preclinical studies in support**

**of phase I/II clinical trials to treat**

***GUCY2D*-associated Leber congenital amaurosis**

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## Supplemental Text

### Detailed Description of Findings in NHP GLP Tox Study #1.

Clinical ophthalmic examination noted the subretinal injection site, when visible, to appear flat with pigment alteration (mottling) in all eyes (including control) following dosing. Subretinal injection of vehicle control article was well tolerated and resulted in a procedure-related anterior segment inflammatory response that resolved by Day 15 and a mild to moderate posterior segment inflammatory response throughout the entire 9-month post dose observation period. In contrast, subretinal injection with AAV5-hGRK1-GUCY2D (GMP lot) resulted in a dose-dependent, severe anterior and posterior segment inflammatory response that generally peaked between Days 15 and 29. The anterior segment inflammation began to reduce in severity by Day 29 and resolved by Week 26 -30 in eyes administered  $1.0 \times 10^{12}$  or  $4.0 \times 10^{12}$  vg/mL and persisted through Week 39 in eyes administered  $1.0 \times 10^{13}$  or  $4.9 \times 10^{13}$  vg/mL. Dose-related posterior segment inflammation persisted through Week 39 at all dose levels of AAV5-hGRK1-GUCY2D and was characterized by varying degrees of vitreous cell, vitreous haze, white perivascular sheathing around retinal blood vessels, and subretinal to choroidal inflammatory foci within the injection site. Mottling of pigment in the RPE outside of the original subretinal injection bleb was sporadically observed in eyes administered 1.0 or  $4.9 \times 10^{13}$  vg/mL and posterior synechia was observed in an eye administered  $4.9 \times 10^{13}$  vg/mL. Other than for an increased frequency of abnormally low IOP in eyes administered the viral vector, no clear and consistent difference was noted in IOP between groups.

OCT noted subretinal procedure- related findings of retinal detachment, accumulation of hyper-reflective material (HRM), and subretinal hyper-reflective material (SHRM) in the fovea, superiorly and at the edge of the bleb that persisted over time. While a variety of responses were noted within each dose level, the onset, incidence and severity of retinal degenerative changes were appreciated with increasing doses of AAV5-hGRK1-GUCY2D. Findings of an absent

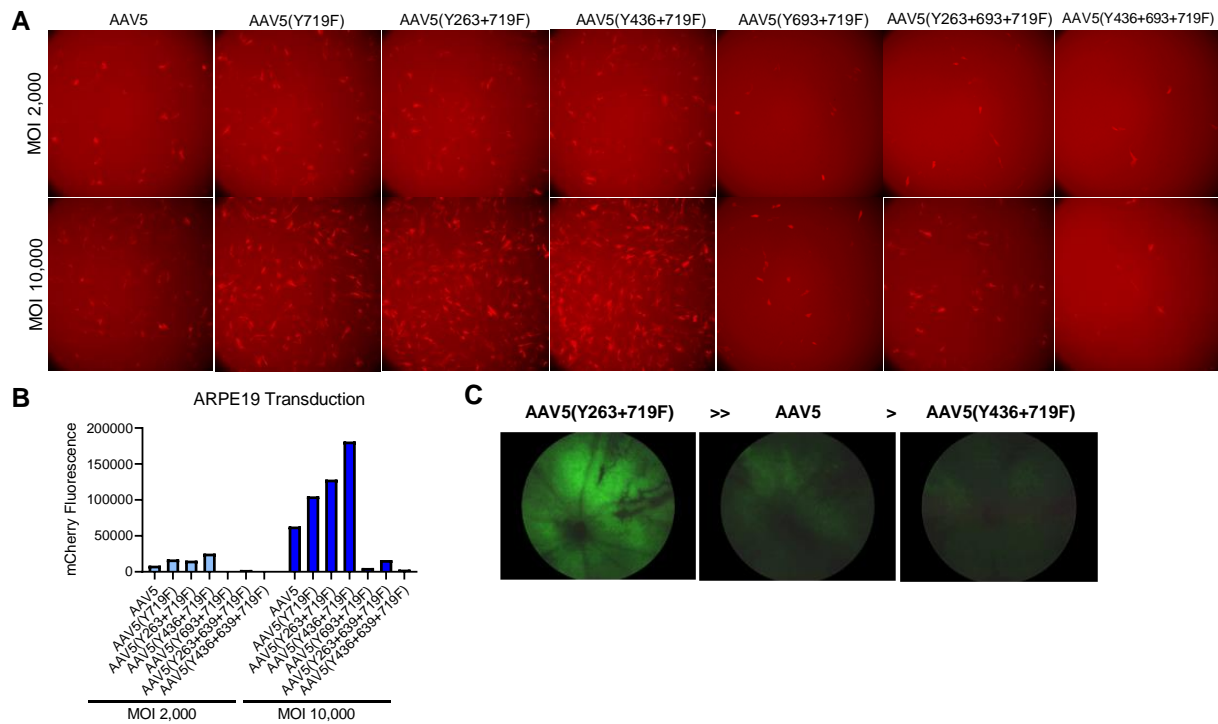
bacillary layer, thinned or absent outer nuclear layer (ONL), choroidal disorganization and hyper-reflective foci (HF) and chorioretinal atrophy appeared earlier and more often with increasing dose levels. Findings of perivascular sheathing and retinal nerve fiber layer (RNFL) thickening, which are often associated with an inflammatory response, also appeared earlier and more frequently with increasing dose levels. Persistent inflammation noted on clinical ophthalmic examinations was consistent with the OCT findings.

Administration of AAV5-hGRK1-GUCY2D resulted in significantly reduced macular function (as assessed by mfERG) in eyes administered  $1.0 \times 10^{13}$  or  $4.9 \times 10^{13}$  vg/mL at Week 4 of the dosing phase. The mfERG of eyes administered lower dose levels of  $1.0 \times 10^{12}$  or  $4.0 \times 10^{12}$  vg/mL was less consistently affected at Week 4, although individual animals were affected. No consistent evidence of recovery was noted in the mfERG when tested at Weeks 12, 25 or 38 and, by Week 38, the lower dose levels of  $1.0 \times 10^{12}$  or  $4.0 \times 10^{12}$  vg/mL had progressed to include notably reduced macular function. For the scotopic ffERG, at Week 11 there was minor or no ERG depression at a dose level of  $1.0 \times 10^{12}$  vg/mL, some decrease in ffERG amplitude at  $4.0 \times 10^{12}$  vg/mL, and a marked depression of ffERG at  $1.0 \times 10^{13}$  or  $4.9 \times 10^{13}$  vg/mL. There was no evidence of recovery in the ffERG by Week 37, although two high dose females administered  $4.9 \times 10^{13}$  vg/mL with mfERG findings did not show depressed ffERGs, suggesting lesser extra-macular involvement in these females compared with males or other dose groups. The depression of retinal function in the foveal location in most animals by Week 38 implies a significant decrease in central visual function occurred in eyes of all AAV5-hGRK1-GUCY2D dose levels by 38 weeks post dose.

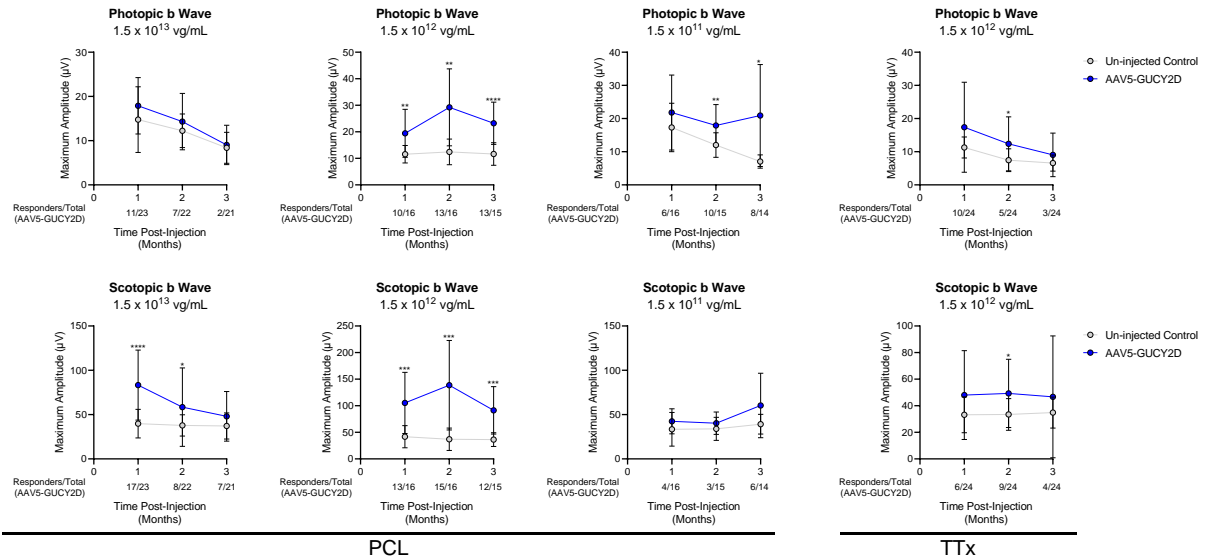
AAV5-hGRK1-GUCY2D-related microscopic observations were present at similar severity in all dose levels. Findings primarily affected the temporal posterior segment of the right eye in the area of the subretinal injection, and consisted of retinal degeneration/loss (disorganization, thinning, and/or loss of the photoreceptors, outer nuclear layer, outer plexiform layer, and

occasionally the inner nuclear layer), necrosis/loss of the retinal pigmented epithelium (RPE) cells, mononuclear cell inflammation, vitreous exudate, and decreased pigmentation in the remaining RPE cells. Minimal retinal vacuolation was also present in one male in each group administered AAV5-hGRK1-GUCY2D.

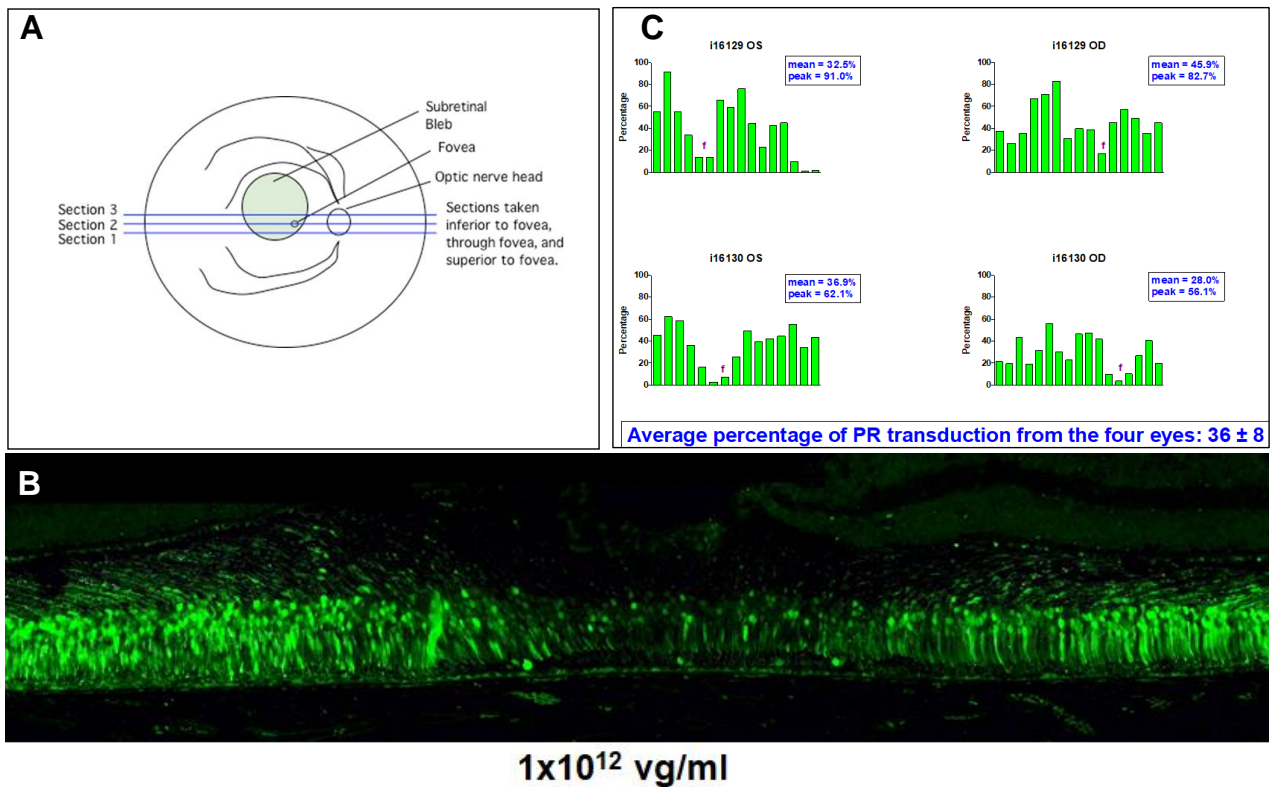
Adverse retinal findings seen via OCT included absent bacillary layer, thinned or absent outer nuclear layer, choroidal disorganization, persistent hyper-reflective foci, chorioretinal atrophy, perivascular sheathing and retinal nerve fiber layer thickening. The OCT retinal findings had adverse microscopic correlates of retinal degeneration/loss (disorganization, thinning, and/or loss of the photoreceptors, outer nuclear layer, outer plexiform layer, and occasionally the inner nuclear layer), necrosis/loss of the retinal pigmented epithelium cells, decreased pigmentation in the remaining RPE cells, mononuclear cell inflammation, and vitreous exudate. The OCT and microscopic retinal findings correlated with marked depression of retinal function by Week 38 as assessed by full-field and multi-focal electroretinography



**Figure S1.** AAV5-based vectors containing self-complementary smCBA-mCherry genomes were tested for their relative transduction efficiency in APRE19 cells at low (2,000) or high (10,000) MOI. mCherry expression was captured with a fluorescent microscope (A) and transduction efficiency was quantified by flow cytometry (B). AAV5 and two AAV5-based capsid mutants containing hGRK1-GFP were selected for characterization subretinally injected mice. Fluorescent fundus images were taken to evaluate GFP expression at 1 month post-injection with vector at  $1 \times 10^{12}$  vg/mL ( $1 \times 10^9$  vg/eye) (C).



**Figure S2.** AAV5-hGRK1-GUCY2D produced via Producer Cell Line (PCL) or Triple Transfection (TTx) significantly improves retinal function in subretinally injected GCDKO mice. Cone-mediated (photopic, top row) and rod-mediated (scotopic, bottom row) function were evaluated in GCDKO mice for 3 months post injection with PCL-made vector at low ( $1.5 \times 10^{11}$  vg/mL), mid ( $1.5 \times 10^{12}$  vg/mL) or high ( $1.5 \times 10^{13}$  vg/mL) concentrations, and with TTx-made vector at mid ( $1.5 \times 10^{12}$  vg/mL) concentration. These correspond to doses of  $1.5 \times 10^8$ ,  $1.5 \times 10^9$ , and  $1.5 \times 10^{10}$  vg/eye, respectively. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p > 0.0001$  as determined by multiple paired t tests with Holm-Sidak correction for multiple comparisons. The number of responders/total number of mice analyzed at each time point is reported on the X axis. Statistical analysis included all mice per cohort.



**Figure S3.** Schematic representation of how NHP sections were analyzed for GFP expression (A). Representative image of AAV5-hGRK1-mediated GFP expression in the foveal pit of cynomolgus macaque following subretinal injection at a concentration of  $1 \times 10^{12}$  vg/mL ( $1.2 \times 10^{11}$  vg/eye) (B). Percentage of photoreceptors transduced following a subretinal administration of AAV5-hGRK1-eGFP at  $3.3 \times 10^{11}$  vg/mL ( $4.0 \times 10^{10}$  vg/eye) in two NHPs, 4 eyes is shown to illustrate how quantification was performed (C).

**Table S1-** Study design for quantitatively evaluating photoreceptor mediated expression from AAV5-based capsid variants. AAV5 and two AAV5-based capsid variants containing either GFP or *Gucy2e* were subretinally delivered at low ( $1.0 \times 10^{11}$  vg/mL) or high ( $1.0 \times 10^{12}$  vg/mL) concentration. This corresponds to  $1.0 \times 10^8$  and  $1.0 \times 10^9$  vg/eye, respectively.

Group	Number of Animals Dosed	Test Article	Vector Dose		
			Volume	vg/mL	Total vg
1	12	AAV5-hGRK1-GFP	1 $\mu$ L	$1.0 \times 10^{11}$	$1.0 \times 10^8$
2	13	AAV5-hGRK1-GFP	1 $\mu$ L	$1.0 \times 10^{12}$	$1.0 \times 10^9$
3	13	AAV5(Y263+719F)-hGRK1-GFP	1 $\mu$ L	$1.0 \times 10^{11}$	$1.0 \times 10^8$
4	12	AAV5(Y263+719F)-hGRK1-GFP	1 $\mu$ L	$1.0 \times 10^{12}$	$1.0 \times 10^9$
5	12	AAV5(Y436+719F)-hGRK1-GFP	1 $\mu$ L	$1.0 \times 10^{11}$	$1.0 \times 10^8$
6	12	AAV5(Y436+719F)-hGRK1-GFP	1 $\mu$ L	$1.0 \times 10^{12}$	$1.0 \times 10^9$
7	20	AAV5-hGRK1- <i>Gucy2e</i>	1 $\mu$ L	$1.0 \times 10^{11}$	$1.0 \times 10^8$
8	20	AAV5-hGRK1- <i>Gucy2e</i>	1 $\mu$ L	$1.0 \times 10^{12}$	$1.0 \times 10^9$
9	20	AAV5(Y263+719F)-hGRK1- <i>Gucy2e</i>	1 $\mu$ L	$1.0 \times 10^{11}$	$1.0 \times 10^8$
10	21	AAV5(Y263+719F)-hGRK1- <i>Gucy2e</i>	1 $\mu$ L	$1.0 \times 10^{12}$	$1.0 \times 10^9$
11	19	AAV5(Y436+719F)-hGRK1- <i>Gucy2e</i>	1 $\mu$ L	$1.0 \times 10^{11}$	$1.0 \times 10^8$
12	20	AAV5(Y436+719F)-hGRK1- <i>Gucy2e</i>	1 $\mu$ L	$1.0 \times 10^{12}$	$1.0 \times 10^9$



**Table S2-** Quantification of titer in dose retains from the same vectors described in Table S1. TA= test article. Note that AAV5(Y263+719F)-hGRK1-GFP had a measured titer over 600 times greater than the expected titer. This discrepancy was considered when analyzing results.

Group	Test Article	Date of Test Article Preparation	Expected Titer (vg/mL)	Measured Titer (vg/mL)	% Recovery
1	AAV5-hGRK1-GFP	8-Sep-14	1.0x10 <sup>11</sup>	1.76x10 <sup>11</sup>	176
2	AAV5-hGRK1-GFP	25-Aug-14	1.0x10 <sup>12</sup>	1.30x10 <sup>12</sup>	130
2	AAV5-hGRK1-GFP	2-Sep-14	1.0x10 <sup>12</sup>	1.17x10 <sup>12</sup>	117
3	AAV5(Y263+719F)-hGRK1-GFP	30-Sep-14	1.0x10 <sup>11</sup>	1.67x10 <sup>11</sup>	169
4	AAV5(Y263+719F)-hGRK1-GFP	25-Aug-14	1.0x10 <sup>12</sup>	6.69x10 <sup>12</sup>	669
4	AAV5(Y263+719F)-hGRK1-GFP	2-Sep-14	1.0x10 <sup>12</sup>	6.68x10 <sup>12</sup>	668
5	AAV5(Y436+719F)-hGRK1-GFP	8-Sep-14	1.0x10 <sup>11</sup>	1.34x10 <sup>11</sup>	134
5	AAV5(Y436+719F)-hGRK1-GFP	11-Sep-14	1.0x10 <sup>11</sup>	1.51x10 <sup>11</sup>	151
6	AAV5(Y436+719F)-hGRK1-GFP	2-Sep-14	1.0x10 <sup>12</sup>	7.74x10 <sup>11</sup>	77.4
6	AAV5-hGRK1-Gucy2e	8-Sep-14	1.0x10 <sup>12</sup>	1.04x10 <sup>12</sup>	104
7	AAV5-hGRK1-Gucy2e	30-Sep-14	1.0x10 <sup>11</sup>	1.85x10 <sup>11</sup>	185
7	AAV5-hGRK1-Gucy2e	9-Oct-14	1.0x10 <sup>11</sup>	1.99x10 <sup>11</sup>	199
7	AAV5-hGRK1-Gucy2e	21-Oct-14	1.0x10 <sup>11</sup>	1.67x10 <sup>11</sup>	167
8	AAV5-hGRK1-Gucy2e	11-Jul-14	1.0x10 <sup>12</sup>	1.40x10 <sup>12</sup>	140
8	AAV5-hGRK1-Gucy2e	24-Jul-14	1.0x10 <sup>12</sup>	1.52x10 <sup>12</sup>	152
8	AAV5-hGRK1-Gucy2e	6-Aug-14	1.0x10 <sup>12</sup>	1.40x10 <sup>12</sup>	140
8	AAV5(Y263+719)-hGRK1-Gucy2e	20-Aug-14	1.0x10 <sup>12</sup>	1.58x10 <sup>12</sup>	158
9	AAV5(Y263+719)-hGRK1-Gucy2e	30-Sep-14	1.0x10 <sup>11</sup>	1.05x10 <sup>11</sup>	105
9	AAV5(Y263+719)-hGRK1-Gucy2e	9-Oct-14	1.0x10 <sup>11</sup>	6.08x10 <sup>11</sup>	60.8
9	AAV5(Y263+719)-hGRK1-Gucy2e	21-Oct-14	1.0x10 <sup>11</sup>	1.29x10 <sup>11</sup>	129
10	AAV5(Y263+719)-hGRK1-Gucy2e	6-Aug-14	1.0x10 <sup>12</sup>	1.30x10 <sup>11</sup>	130
10	AAV5(Y263+719)-hGRK1-Gucy2e	11-Jul-14	1.0x10 <sup>12</sup>	1.20x10 <sup>12</sup>	120
10	AAV5(Y263+719)-hGRK1-Gucy2e	20-Aug-14	1.0x10 <sup>12</sup>	1.30x10 <sup>12</sup>	130
11	AAV5(Y436+719F)-hGRK1-Gucy2e	9-Oct-14	1.0x10 <sup>11</sup>	1.80x10 <sup>12</sup>	180
11	AAV5(Y436+719F)-hGRK1-Gucy2e	21-Oct-14	1.0x10 <sup>11</sup>	2.00x10 <sup>11</sup>	200
12	AAV5(Y436+719F)-hGRK1-Gucy2e	20-Aug-14	1.0x10 <sup>12</sup>	1.58x10 <sup>12</sup>	158
12	AAV5(Y436+719F)-hGRK1-Gucy2e	25-Aug-14	1.0x10 <sup>12</sup>	1.48x10 <sup>12</sup>	148

**Table S3.** Study design for evaluating retinal function following subretinal delivery of AAV5-hGRK1-GUCY2D in GCDKO mice.

Group	Number of Animals Dosed (M/F)	Test Article	Vector Dose		
			Volume	vg/mL	Total vg
1	19 (13M/6F)	AAV5-hGRK1-GUCY2D	1 µL	1.5 x 10 <sup>12</sup>	1.5 x 10 <sup>9</sup>
2	22 (4M/18F)	AAV5-hGRK1-GUCY2D	1 µL	1.5 x 10 <sup>13</sup>	1.5 x 10 <sup>10</sup>

**Table S4.** Study design for evaluating retinal function following subretinal delivery of AAV5-GUCY2D, produced via triple transfection vs. producer cell line technology, in GCDKO mice. PCL = Producer Cell Line Manufactured, TTx = Triple Transfection Manufactured

Group	Number of Animals Dosed (M/F)	Test Article	Vector Dose		
			Volume	vg/mL	Total vg
1	25 (15M/10F)	AAV5-GUCY2D (PCL)	1 µL	1.5 x 10 <sup>13</sup>	1.5 x 10 <sup>10</sup>
2	16 (9M/7F)	AAV5-GUCY2D (PCL)	1 µL	1.5 x 10 <sup>12</sup>	1.5 x 10 <sup>9</sup>
3	16 (8M/8F)	AAV5-GUCY2D (PCL)	1 µL	1.5 x 10 <sup>11</sup>	1.5 x 10 <sup>8</sup>
4	34 (18M/16F)	AAV5-hGRK1-GUCY2D (TTx)	1 µL	1.5 x 10 <sup>12</sup>	1.5 x 10 <sup>9</sup>

**Table S5.** Hybrid study design for evaluating safety and efficacy following subretinal delivery of AAV5-GUCY2D to GC1KO mice. PCL = Producer Cell Line Manufactured

Group	Number of Animals Dosed (M/F)	Test Article	Vector Dose		
			Volume	vg/mL	Total vg
1	20 (14M/6F)	Vehicle	1 µL	N/A	N/A
2	36 (27M/9F)	AAV5-GUCY2D (PCL)	1 µL	3.3 x 10 <sup>11</sup>	3.3 x 10 <sup>8</sup>
3	41 (14M/27F)	AAV5-GUCY2D (PCL)	1 µL	1.5 x 10 <sup>11</sup>	1.5 x 10 <sup>8</sup>
4	17 (9M/8F)	AAV5-GUCY2D (PCL)	1 µL	3.3 x 10 <sup>10</sup>	3.3 x 10 <sup>7</sup>

**Table S6.** Study design for evaluating retinal function following subretinal delivery of AAV5-*Gucy2e* and AAV5-*GUCY2D* to GC1KO mice

Group	Number of Animals Dosed (M/F)	Test Article	Vector Dose		
			Volume	vg/mL	Total vg
1	21 (13M/8F)	AAV5- <i>GUCY2D</i>	1 $\mu$ L	$3.3 \times 10^{11}$	$3.3 \times 10^8$
2	23 (15M/8F)	AAV5- <i>GUCY2D</i>	1 $\mu$ L	$1.0 \times 10^{11}$	$1.0 \times 10^8$
3	18 (9M/9F)	AAV5- <i>GUCY2D</i>	1 $\mu$ L	$3.3 \times 10^{10}$	$3.3 \times 10^7$
4	15 (9M/6F)	AAV5- <i>Gucy2e</i>	1 $\mu$ L	$3.3 \times 10^{11}$	$3.3 \times 10^8$
5	22 (12M/10F)	AAV5- <i>Gucy2e</i>	1 $\mu$ L	$1.0 \times 10^{11}$	$1.0 \times 10^8$
6	19 (7M/12F)	AAV5- <i>Gucy2e</i>	1 $\mu$ L	$3.3 \times 10^{10}$	$3.3 \times 10^7$

**Table S7-** A statistical comparison of photopic (cone-mediated) function in GC1KO mice 1 month following subretinal injection of either AAV5-*GUCY2D* or AAV5-*Gucy2e* (study design in Table S6). Statistical comparisons across treatment groups were conducted using One-way Anova with Tukey's post-test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

Group		Statistical Significance							
		<i>GUCY2D</i>				<i>Gucy2e</i>			
		Un-injected	Hi	Med	Low	Un-injected	Hi	Med	Low
<i>GUCY2D</i>	Hi	****					n.s.	***	****
	Med	**	*				****	n.s.	*
	Low	n.s.	****	**			****	n.s.	n.s.
<i>Gucy2e</i>	Hi					****			
	Med					n.s.	****		
	Low					n.s.	****	n.s.	

**Table S8-** A statistical comparison of photopic (cone-mediated) function in GC1KO mice 2 months following subretinal injection of either AAV5-GUCY2D or AAV5-Gucy2e (study design in Table S6). Statistical comparisons across treatment groups were conducted using One-way Anova with Tukey's post-test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001

Group		Statistical Significance							
		<i>GUCY2D</i>				<i>Gucy2e</i>			
		Un-injected	Hi	Med	Low	Un-injected	Hi	Med	Low
<i>GUCY2D</i>	Hi	****					***	*	****
	Med	n.s.	n.s.				****	n.s.	n.s.
	Low	n.s.	****	n.s.			****	n.s.	n.s.
<i>Gucy2e</i>	Hi					****			
	Med					n.s.	****		
	Low					n.s.	****	n.s.	

**Table S9-** A statistical comparison of photopic (cone-mediated) function in GC1KO mice 3 months following subretinal injection of either AAV5-GUCY2D or AAV5-Gucy2e (study design in Table S6). Statistical comparisons across treatment groups were conducted using One-way Anova with Tukey's post-test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001

Group		Statistical Significance							
		<i>GUCY2D</i>				<i>Gucy2e</i>			
		Un-injected	Hi	Med	Low	Un-injected	Hi	Med	Low
<i>GUCY2D</i>	Hi	****					n.s.	*	****
	Med	n.s.	*				****	n.s.	n.s.
	Low	n.s.	****	n.s.			****	n.s.	n.s.
<i>Gucy2e</i>	Hi					****			
	Med					n.s.	****		
	Low					n.s.	****	n.s.	

**Table S10-** A statistical comparison of scotopic (rod-mediated) function in GC1KO mice 1 month following subretinal injection of either AAV5-GUCY2D or AAV5-Gucy2e (study design in Table S6). Statistical comparisons across treatment groups were conducted using One-way Anova with Tukey's post-test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001

Group		Statistical Significance							
		<i>GUCY2D</i>				<i>Gucy2e</i>			
		Un-injected	Hi	Med	Low	Un-injected	Hi	Med	Low
<i>GUCY2D</i>	Hi	n.s.					n.s.	**	n.s.
	Med	n.s.	n.s.				n.s.	**	n.s.
	Low	n.s.	n.s.	n.s.			n.s.	n.s.	n.s.
<i>Gucy2e</i>	Hi					n.s.			
	Med					n.s.	n.s.		
	Low					n.s.	n.s.	**	

**Table S11.** Summary of photoreceptor (PR) transduction in NHP following a single subretinal administration of AAV5-hGRK1-eGFP. Both the average PR transduction across all eyes evaluated as well as the peak PR transduction across all eyes are reported.

Dose Level (vg/eye)	Concentration (vg/mL)	Number of Eyes	Average PR Transduction Per Group	Peak PR Transduction Across Eyes
1.2 x 10 <sup>11</sup>	1.0 x 10 <sup>12</sup>	8	51%	94.0%
8.0 x 10 <sup>10</sup>	6.7 x 10 <sup>11</sup>	4	50%	80.9%
4.0 x 10 <sup>10</sup>	3.3 x 10 <sup>11</sup>	4	36%	91.0%
1.2 x 10 <sup>10</sup>	1.0 x 10 <sup>11</sup>	8	4%	21.6%
4.0 x 10 <sup>9</sup>	3.3 x 10 <sup>10</sup>	4	0.6%	5.2%
1.2 x 10 <sup>9</sup>	1.0 x 10 <sup>10</sup>	4	0.3%	3.7%

**Table S12.** GLP study design to evaluate biodistribution of subretinally injected AAV5-GUCY2D in rats

Group No.	Test Article	Dose Level (vg/eye) <sup>a</sup>	Dose Conc. (vg/mL)	Number of Animals							
				Day 4 Necropsy		Day 15 (Week 3) Necropsy		Day 29 (Week 5) Necropsy		Day 92 (Month 3) Necropsy	
				M	F	M	F	M	F	M	F
1	Vehicle	0	0	2	2	2	2	2	2	2	2
2	AAV5-GUCY2D	2.0 x 10 <sup>9</sup>	1.0 x 10 <sup>12</sup>	5	5	5	5	5	5	5	5
3	AAV5-GUCY2D	2.0 x 10 <sup>10</sup>	1.0 x 10 <sup>13</sup>	5	5	5	5	5	5	5	5

M: Male, F: female, Conc: concentration

<sup>a</sup>: Unilateral subretinal injection at a dose volume of 2 µL/right eye/animal. The left eye of each animal served as an untreated control

**Table S13.** Summary of AAV5-GUCY2D vector DNA concentrations in tissues and fluids from rats subretinally injected with the low dose ( $2.0 \times 10^9$  vg/eye; Group 2 in Table S12)

Summary AAV5-GUCY2D Vector DNA Concentrations in Group 2 Tissues and Fluids								
Sample Type	Day 4		Day 15		Day 29		Day 92	
	Conc	N	Conc	N	Conc	N	Conc	N
Blood	<LLOQ	0/10	<LLOQ	0/10	-	-	-	-
Brain-FB-Left	<LLOQ	0/10	168.57 to 193.01	2/10	43.44 to 422.17	5/10	45.14 to 53.36	2/10
Brain-FB-Right	<LLOQ	0/10	<LLOQ	0/10	-	-	-	-
Brain-NVC-Left	44.49 to 46.71	2/10	48.89 to 497.34	2/10	37.10 to 624.20	6/10	37.44 to 153.20	3/10
Brain-NVC-Right	<LLOQ	0/10	<LLOQ	0/10	-	-	-	-
Diaphragm	53.61 to 98.49	3/10	<LLOQ	0/10	<LLOQ	0/10	-	-
Eye-Untreated	<LLOQ	0/10	<LLOQ	0/10	-	-	-	-
Eye-Treated (right)	390941.39 to 1656975.66	10/10	197519.07 to 1478457.85	10/10	28776.31 to 2163739.12	10/10	10885.89 to 949049.61	10/10
Heart	<LLOQ	0/10	<LLOQ	0/10	-	-	-	-
Kidney	37.29 to 79.46	4/10	44.8	1/10	33.60	1/10	<LLOQ	0/10
LN Mandibular	47.19 to 3709.17	10/10	45.33 to 179.14	4/10	36.59 to 108.61	2/10	54.73 to 69.37	2/10
Liver	65.26 to 898.66	9/10	46.24 to 243.04	5/10	<LLOQ	0/10	<LLOQ	0/10
Lung	51.43 to 338.87	9/10	32.06 to 218.19	5/10	47.33 to 84.99	2/10	<LLOQ	0/10
Muscle	<LLOQ	0/10	<LLOQ	0/10	-	-	-	-
ONU*	<LLOQ	0/10	6320.87	1/10	1177.04	1/10	<LLOQ	0/10
ONT* (right)	705.53 to 2637.07	5/10	991.35 to 16360.66	4/10	4133.33 to 35015.16	4/10	1677.95 to 2923.20	4/10
Ovary	81.86 to 96.83	3/5	<LLOQ	0/5	<LLOQ	0/5	-	-
Spleen	90.37 to 1539.41	10/10	61.00 to 476.26	7/10	66.34 to 1055.39	2/10	49.46 to 160.13	2/10
Testes	68.33	1/5	<LLOQ	0/5	<LLOQ	0/5	-	-

LLOQ = Lower Limit of Quantitation: 25 copies/reaction; - = tissues not analysed as two consecutive negative postdose qPCR results were obtained

Conc. = Concentration of GUCY2D (copies/ $\mu$ g DNA); N = Number of Animals with Signal >LLOQ / Number of Animals Tested

FB = Forebrain; NVC = Near Visual Cortex; ONT = Optic Nerve from treated eye; ONU = Optic Nerve from untreated eye



**Table S14.** Summary of AAV5-GUCY2D vector DNA concentrations in tissues and fluids from rats subretinally injected with the high dose ( $2.0 \times 10^{10}$  vg/eye; Group 3 from Table S12)

Summary AAV5-GUCY2D Vector DNA Concentrations in Group 3 Tissues and Fluids								
Sample Type	Day 4		Day 15		Day 29		Day 92	
	Conc	N	Conc	N	Conc	N	Conc	N
Blood	550.00 to 3952.04	6/10	<LLOQ	0/10	< LLOQ	0/10	< LLOQ	0/10
Brain-FB-Left	62.81	1/10	137.79 to 1812.20	4/10	48.59 to 62.03	4/10	35.69	1/10
Brain-FB-Right	<LLOQ	0/10	62.1	1/10	< LLOQ	0/10	< LLOQ	0/10
Brain-NVC-Left	67.31 to 271.54	5/10	74.91 to 2727.79	8/10	153.36 to 361.51	5/10	< LLOQ	0/10
Brain-NVC-Right	<LLOQ	0/10	<LLOQ	0/10	-	-	-	-
Diaphragm	126.17 to 4587.77	6/10	288.81	1/10	< LLOQ	0/10	< LLOQ	0/10
Eye-Untreated	39.06 to 90.04	3/10	49.01	1/10	< LLOQ	0/10	< LLOQ	0/10
Eye-Treated (right)	961036.39 to 13556646.54	10/10	859882.40 to 9333341.91	10/10	145796.13 to 1292278.86	10/10	10042.24 to 246133.04	10/10
Heart	56.16 to 277.01	5/10	60.73	1/10	< LLOQ	0/10	< LLOQ	0/10
Kidney	38.64 to 1170.50	8/10	534.97	1/10	51.63 to 249.84	4/10	< LLOQ	0/10
LN Mandibular	153.84 to 5216.84	10/10	115.81 to 3170.21	10/10	49.76 to 1706.59	10/10	151.30 to 2304.06	6/10
Liver	378.11 to 34430.57	10/10	48.54 to 2629.84	9/10	37.11 to 110.31	3/10	< LLOQ	0/10
Lung	116.76 to 5189.30	10/10	50.96 to 1744.11	8/10	40.44 to 1114.01	8/10	38.46 to 50.70	2/10
Muscle	52.87 to 68.27	2/10	<LLOQ	0/10	< LLOQ	0/10	-	-
ONU*	<LLOQ	0/10	1341.05 to 2193.71	2/10	< LLOQ	0/10	< LLOQ	0/10
ONT* (right)	1828.63 to 75432.80	9/10	3288.33 to 121612.20	10/10	3141.86 to 13576.67	5/10	1646.22 to 2543.11	2/10
Ovary	62.47 to 1092.24	5/5	<LLOQ	0/5	41.14 to 53.01	2/5	< LLOQ	0/5
Spleen	514.36 to 28235.64	10/10	66.21 to 8562.81	10/10	45.66 to 1071.41	9/10	282.21	1/10
Testes	92.30 to 144.80	2/5	135.33	1/5	59.30	1/5	< LLOQ	0/5

LLOQ = Lower Limit of Quantitation: 25 copies/reaction; - = tissues not analysed as two consecutive negative postdose qPCR results were obtained

Conc. = Concentration of GUCY2D (copies/ $\mu$ g DNA); N = Number of Animals with Signal >LLOQ / Number of Animals Tested

FB = Forebrain; NVC = Near Visual Cortex; ONT = Optic Nerve from treated eye; ONU = Optic Nerve from untreated eye

**Table S15.** Quantification of biodistribution (presence of vector genomes) in retinas (within and outside the injection bleb) of NHPs dosed in GLP Tox Study #1 (Mean  $\text{Log}_{10}$  GUCY2D copies/ $\mu$ g RNA).

Tissue	Timepoint	Dose Level (vg/mL)				
		0	$1.0 \times 10^{12}$	$4.0 \times 10^{12}$	$1.0 \times 10^{13}$	$4.9 \times 10^{13}$
Retinal punch (bleb area)	Day 29	1.0 <sup>a</sup>	6.6	6.7	7.0 <sup>b</sup>	6.7
Retinal Punch (non bleb area)	Day 29	1.0 <sup>a</sup>	1.5	3.1	1.8 <sup>b</sup>	3.4

<sup>a</sup> Equivalent to all samples <LLOQ.

**Table S16.** Quantification of biodistribution (presence of vector genomes) in remaining tissues and blood of NHPs dosed in GLP Tox Study #1 (Group Mean Copies/ $\mu\text{g}$  DNA)

Tissue	Timepoint	Sex	Dose Level (vg/mL)				
			0	$1.0 \times 10^{12}$	$4.0 \times 10^{12}$	$1.0 \times 10^{13}$	$4.9 \times 10^{13}$
Blood- DNA	Predose 1	M	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
	Predose 2	M	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
	Week 2	M	<LLOQ	179.74	765.01	764.16	1367.47
		F	<LLOQ	481.64	585.09	1326.54	17437
	Week 5	M	<LLOQ	<LLOQ	55.84	58.77	205.53
		F	<LLOQ	<LLOQ	<LLOQ	70.24	969
	Week 9	M	NA	<LLOQ	<LLOQ	<LLOQ	151.64
		F	NA	<LLOQ	<LLOQ	<LLOQ	792.23
	Week 13	M	NA	NA	<LLOQ	<LLOQ	<LLOQ
		F	NA	NA	<LLOQ	<LLOQ	444.73
	Week 17	M	NA	NA	NA	NA	<LLOQ
		F	NA	NA	NA	NA	<LLOQ
	Week 21	M	NA	NA	NA	NA	<LLOQ
		F	NA	NA	NA	NA	<LLOQ
	Week 26	M	NA	NA	NA	NA	NA
		F	NA	NA	NA	NA	<LLOQ
Brain (Right Occipital Lobe)	Day 29	M	<LLOQ	2585.76	938.04	1322.69	971.72
		F	<LLOQ	1472.69	885.79	826.79	1996.13
Brain (Left Occipital Lobe)	Day 29	M	<LLOQ	510.04	776.41	185.29	462.88
		F	<LLOQ	631.56	223.69	176.37	791.74
Optic Nerve (Right)	Day 29	M	<LLOQ	48449.63	9423.44	20728.11	<LLOQ
		F	<LLOQ	<LLOQ	64037.9	<LLOQ	3393.5
Spleen	Day 29	M	<LLOQ	3487.01	209.54	2301.24	2346.26
		F	<LLOQ	71.94	972.26	54545.4	27021.98
Liver	Day 29	M	<LLOQ	273.33	<LLOQ	69.91	55.71
		F	<LLOQ	<LLOQ	67.04	3657.13	252.23

Note: LLOQ for blood samples = 36 copies/ $\mu\text{g}$ /DNA. For calculation purposes <LLOQ was assigned as LLOQ/2 (18 copies/ $\mu\text{g}$  DNA)

NA = Not analyzed

**Table S17.** Summary of Anti-AAV5 antibodies present in serum and aqueous humor of NHPs subretinally injected in GLP Tox Study #1. Concentrations of  $1.0 \times 10^{12}$ ,  $4.0 \times 10^{12}$ ,  $1.0 \times 10^{13}$ , and  $4.9 \times 10^{13}$  vg/mL were delivered in 150  $\mu$ L, corresponding to  $1.5 \times 10^{11}$ ,  $6.0 \times 10^{11}$ ,  $1.5 \times 10^{12}$ , and  $7.4 \times 10^{12}$  vg/eye, respectively.

Number of Animals with Serum Anti-AAV5 Antibodies					
Timepoint	Dose Level (vg/mL)				
	0	$1.0 \times 10^{12}$	$4.0 \times 10^{12}$	$1.0 \times 10^{13}$	$4.9 \times 10^{13}$
Predose 1	0 of 8	1 of 8	0 of 8	3 of 8	4 of 8
Predose 2	0 of 8	1 of 8	0 of 8	2 of 8	4 of 8
Week 2	0 of 8	1 of 8	4 of 8	7 of 8	8 of 8
Week 5	0 of 8	7 of 8	8 of 8	8 of 8	8 of 8
Week 9	0 of 4	4 of 4	4 of 4	4 of 4	4 of 4
Week 13	0 of 4	4 of 4	4 of 4	4 of 4	4 of 4
Week 17	0 of 4	4 of 4	4 of 4	4 of 4	4 of 4
Week 21	0 of 4	4 of 4	4 of 4	4 of 4	4 of 4
Week 26	1 of 4	4 of 4	4 of 4	4 of 4	4 of 4
Week 30	1 of 4	4 of 4	4 of 4	4 of 4	4 of 4
Week 34	1 of 4	4 of 4	4 of 4	4 of 4	4 of 4
Week 39	0 of 4	4 of 4	4 of 4	4 of 4	4 of 4

Number of Animals with Aqueous Anti-AAV5 Antibodies					
Timepoint	Dose Level (vg/mL)				
	0	$1.0 \times 10^{12}$	$4.0 \times 10^{12}$	$1.0 \times 10^{13}$	$4.9 \times 10^{13}$
Predose	0 of 8	0 of 8	0 of 8	0 of 8	0 of 8
Interim Necropsy	0 of 8	4 of 4	4 of 4	4 of 4	4 of 4
Terminal Necropsy	0 of 4	4 of 4	4 of 4	4 of 4	4 of 4

**Table S18.** Bridging study design for evaluating the effects of two lots of AAV5-GUCY2D (vector from the GLP Tox study vs. the GMP clinical candidate) on retinal function in subretinally injected GC1KO mice

Group	Number of Animals Dosed (M/F)	Test Article	Lot	Dose Volume	Dose Concentration (vg/mL)	Dose (vg/eye)
1	20 (9M/11F)	AAV5-GUCY2D	Tox Lot	1 $\mu$ L into one eye	1.5 x 10 <sup>11</sup>	1.5 x 10 <sup>8</sup>
2	20 (11M/9F)				3.3 x 10 <sup>10</sup>	3.3 x 10 <sup>7</sup>
3	20 (8M/12F)		GMP Lot		1.5 x 10 <sup>11</sup>	1.5 x 10 <sup>8</sup>
4	21 (12M/9F)				3.3 x 10 <sup>10</sup>	3.3 x 10 <sup>7</sup>

**Table S19.** Preclinical Data Summary informs dose selection in Phase I/II clinical trials. NOAEL= No observable adverse effect level, MED= minimum effective dose

Dose Concentration (vg/mL)	GCDKO/GC1KO Mouse Studies	NHP Pharmacology Studies (peak % transduction)	GLP Toxicology
1.0 x 10 <sup>13</sup>	Efficacy/ Toxicity ( $\downarrow$ ONL by OCT)	Not done	
1.0 x 10 <sup>12</sup>	Efficacy GCDKO	94% PR transduction	NOAEL
6.7 x 10 <sup>11</sup>	Not done	81% PR transduction	
3.3 x 10 <sup>11</sup>	Efficacy GC1KO	91% PR transduction	
1.0 x 10 <sup>11</sup>	Efficacy GCDKO/ Efficacy GC1KO	22% PR transduction	
3.3 x 10 <sup>10</sup>	Efficacy GC1KO	5% PR transduction	MED
1.0 x 10 <sup>10</sup>	Not done	4% PR transduction	

**Table S20.** Summary of *in vivo* experiments conducted in this study

Description of Study	Study Design Table	Animal model	Test/control article used	concentration (vg/mL)	dose (vg/eye)	# of animals dosed (M/F)	Statistics employed				
Compare PR transduction and ERG improvements following subretinal injection of AAV5-based vectors containing GFP or Gucy2e, respectively	Table S1	GCDKO mouse	AAV5-hGRK1-GFP	1 x 10 <sup>11</sup>	1 x 10 <sup>8</sup>	12	stats performed on ERG data (Figure 1) using one-way ANOVA with Tukey's post-test analysis				
			AAV5-hGRK1-Gucy2e			20					
			AAV5(Y263+719)-hGRK1-GFP			13					
			AAV5(Y263+719)-hGRK1-Gucy2e			20					
			AAV5(Y436+719)-hGRK1-GFP			12					
			AAV5(Y436+719)-hGRK1-Gucy2e			19					
			AAV5-hGRK1-GFP	1 x 10 <sup>12</sup>	1 x 10 <sup>9</sup>	13					
			AAV5-hGRK1-Gucy2e			20					
			AAV5(Y263+719)-hGRK1-GFP			12					
			AAV5(Y263+719)-hGRK1-Gucy2e			21					
			AAV5(Y436+719)-hGRK1-GFP			12					
			AAV5(Y436+719)-hGRK1-Gucy2e			20					
Evaluate efficacy of AAV5 containing human GUCY2D	Table S3	GCDKO mouse	AAV5-hGRK1-GUCY2D	1.5 x 10 <sup>12</sup>	1.5 x 10 <sup>9</sup>	19 (13M/6F)	stats performed on ERG data (Figure 2) using multiple paired t tests with Holm-Sidak correction for multiple comparisons.				
				1.5 x 10 <sup>13</sup>	1.5 x 10 <sup>10</sup>	22 (4M/18F)					
Compare efficacy of AAV5-hGRK1-GUCY2D vectors manufactured via producer cell line (PCL) vs. triple transfection (TTx) process	Table S4	GCDKO mouse	AAV5-hGRK1-GUCY2D (PCL)	1.5 x 10 <sup>11</sup>	1.5 x 10 <sup>8</sup>	16 (8M/8F)	stats performed on ERG data (Figure S2) using multiple paired t tests with Holm-Sidak correction for multiple comparisons. Stats performed on ERG data (Figure 3) using two-way ANOVA with Sidak's post-test analysis. Stats performed on OCT data (Figure 3) using two-way ANOVA with Tukey's post-test				
				1.5 x 10 <sup>12</sup>	1.5 x 10 <sup>9</sup>	16 (9M/7F)					
				1.5 x 10 <sup>13</sup>	1.5 x 10 <sup>10</sup>	25 (15M/10F)					
			AAV5-hGRK1-GUCY2D (TTx)	1.5 x 10 <sup>12</sup>	1.5 x 10 <sup>9</sup>	34 (18M/16F)					
Hybrid study evaluating AAV5-hGRK1-GUCY2D	Table S5	GC1KO mouse	AAV5-hGRK1-GUCY2D	3.3 x 10 <sup>10</sup>	3.3 x 10 <sup>7</sup>	17 (9M/8F)	stats performed on ERG and OCT data (Figure 4) using by two-way ANOVA with Tukey's post-test analysis.				
				1.5 x 10 <sup>11</sup>	1.5 x 10 <sup>8</sup>	41 (14M/27F)					
				3.3 x 10 <sup>11</sup>	3.3 x 10 <sup>8</sup>	36 (17M/9F)					
			Vehicle	N/A	N/A	20 (14M/6F)					
Compare therapeutic response to AAV5 vectors containing murine Gucy2e vs. human GUCY2D	Table S6	GC1KO mouse	AAV5-hGRK1-GUCY2D	3.3 x 10 <sup>10</sup>	3.3 x 10 <sup>7</sup>	18 (9M/9F)	stats performed on ERG data (Table S7, S8, S9, S10 and Figure 5) using (need this info from Dana). Stats performed on OCT data (Figure 5) using two-way ANOVA with Tukey's post-test analysis				
			AAV5-hGRK1-GUCY2D	1.0 x 10 <sup>11</sup>	1.0 x 10 <sup>8</sup>	23 (15M/8F)					
			AAV5-hGRK1-GUCY2D	3.3 x 10 <sup>11</sup>	3.3 x 10 <sup>8</sup>	21 (13M/8F)					
			AAV5-hGRK1-Gucy2e	3.3 x 10 <sup>10</sup>	3.3 x 10 <sup>7</sup>	19 (7M/12F)					
			AAV5-hGRK1-Gucy2e	1.0 x 10 <sup>11</sup>	1.0 x 10 <sup>8</sup>	22 (12M/10F)					
			AAV5-hGRK1-Gucy2e	3.3 x 10 <sup>11</sup>	3.3 x 10 <sup>8</sup>	15 (9M/6F)					
Evaluate photoreceptor transduction in subretinally injected NHPs	Table S11	cynomolgus macaque	AAV5-hGRK1-GFP	1.0 x 10 <sup>10</sup>	1.2 x 10 <sup>8</sup>	2	N/A				
				3.3 x 10 <sup>10</sup>	4.0 x 10 <sup>9</sup>	2					
				1.0 x 10 <sup>11</sup>	1.2 x 10 <sup>10</sup>	4					
				3.3 x 10 <sup>11</sup>	4.0 x 10 <sup>10</sup>	2					
				6.7 x 10 <sup>11</sup>	8.0 x 10 <sup>10</sup>	2					
				1.0 x 10 <sup>12</sup>	1.2 x 10 <sup>11</sup>	4					
GLP rat biodistribution study	Table S12	Long Evans rats	AAV5-hGRK1-GUCY2D	1.0 x 10 <sup>12</sup>	2.0 x 10 <sup>9</sup>	40 (20M/20F)	N/A				
				1.0 x 10 <sup>13</sup>	2.0 x 10 <sup>10</sup>	40 (20M/20F)					
			Vehicle	N/A	N/A	16 (8M/8F)					
GLP NHP Safety Study #1	Table 1	cynomolgus macaque	AAV5-hGRK1-GUCY2D	1.0 x 10 <sup>12</sup>	1.5 x 10 <sup>11</sup>	8 (4M/4F)	N/A				
				4.0 x 10 <sup>12</sup>	6.0 x 10 <sup>11</sup>	8 (4M/4F)					
				1.0 x 10 <sup>13</sup>	1.5 x 10 <sup>12</sup>	8 (3M/4F)					
				4.9 x 10 <sup>13</sup>	7.4 x 10 <sup>12</sup>	8 (4M/4F)					
			Vehicle	N/A	N/A	8 (4M/4F)					
GLP NHP Safety Study #2	Table 2	cynomolgus macaque	AAV5-hGRK1-GUCY2D <sup>a</sup>	1.0 x 10 <sup>11</sup>	1.5 x 10 <sup>10</sup>	3F	N/A				
				3.3 x 10 <sup>11</sup>	5.0 x 10 <sup>10</sup>	3F					
				1.0 x 10 <sup>12</sup>	1.5 x 10 <sup>11</sup>	3F					
			Vehicle <sup>a</sup>	N/A	N/A	3F					
			AAV5-hGRK1-GUCY2D <sup>b</sup>	1.0 x 10 <sup>11</sup>	1.5 x 10 <sup>10</sup>	3F					
				3.3 x 10 <sup>11</sup>	5.0 x 10 <sup>10</sup>	3F					
				1.0 x 10 <sup>12</sup>	1.5 x 10 <sup>11</sup>	3F					
			Vehicle <sup>b</sup>	N/A	N/A	3F					
			Evaluate comparability between Tox lot vs. GMP test articles	Table S18	GC1KO mice	AAV5-hGRK1-GUCY2D (Tox lot)		3.3 x 10 <sup>10</sup>	3.3 x 10 <sup>7</sup>	20 (11M/9F)	stats performed on ERG data (Figure 6) using by one-way ANOVA with Tukey's post-test analysis. Stats performed on OCT data (Figure 6) using two-way ANOVA with Tukey's post-test analysis
								3.3 x 10 <sup>11</sup>	3.3 x 10 <sup>8</sup>	20 (9M/11F)	
AAV5-hGRK1-GUCY2D (GMP lot)	3.3 x 10 <sup>10</sup>	3.3 x 10 <sup>7</sup>				21 (12M/9F)					
3.3 x 10 <sup>11</sup>	3.3 x 10 <sup>8</sup>	20 (8M/12F)									

<sup>a</sup>, mild steroid regimen, <sup>b</sup>, moderate steroid regimen