Title:High-sensitivity vision restoration via ectopic expression

of chimeric rhodopsin in mice

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One Sentence Summary:

Optogenetic therapy with Gloeobacter and human chimeric rhodopsin resulted in highly

sensitive visual restoration and protection effects.

Abstract

Photoreception requires amplification by mammalian rhodopsin through G protein

activation, which requires a visual cycle. To achieve this in retinal gene therapy, we

incorporated human rhodopsin cytoplasmic loops into *Gloeobacter* rhodopsin, thereby

generating *Gloeobacter* and human chimeric rhodopsin (GHCR). In a murine model of

inherited retinal degeneration, we induced retinal GHCR expression by intravitreal

injection of a recombinant adeno-associated virus vector. Retinal explant and visual

thalamus electrophysiological recordings, behavioral tests, and histological analysis

showed that GHCR restored dim-environment vision and prevented the progression of

retinal degeneration. Thus, GHCR may be a potent clinical tool for the treatment of retinal

disorders.

INTRODUCTION

Inherited retinal degeneration (IRD) is a major cause of vision loss. More than 2 million

people worldwide are blind due to IRD*(1)*, and few effective treatments exist. For retinitis

pigmentosa (RP), one of the most common forms of IRD, previous studies have reported

vision restoration in animal models using various molecules as optogenetic actuators*(2–9)*.

In addition, clinical trials are under way to investigate the effects of introducing

channelrhodopsin 2 (RST-001, ClinicalTrials.gov Identifier: NCT01648452) and

ChrimsonR (GS-030, ClinicalTrials.gov Identifier: NCT03326336) into retinal ganglion

cells (RGCs) via gene transduction achieved by intravitreal injection of recombinant

adeno-associated virus (rAAV). The first clinical case report on optogenetic therapy was

recently reported*(10)*. However, microbial opsins, such as channelrhodopsin 2, require

high light intensity, such as outdoor light intensity levels, to function*(11–13)*. They cannot

restore vision in dimly lit environments, such as indoors or at night, and strong light

irradiation can promote retinal degeneration*(14, 15)*. Physiological photoreception

mediated by mammalian rhodopsin, however, relies on amplification through G protein

activation. Although the introduction of vertebrate opsin improved photosensitivity in

mice*(9, 16)*, it is unclear how the chromophore retinal is metabolized in the retina where

the visual cycle is broken. Animal rhodopsin also causes toxicity if all-trans retinal is not

properly metabolized*(17, 18)*, and is, thus, hampered by safety and stability concerns in

terms of clinical application.

Because of the above limitations of animal visual opsins, one attempt to circumvent them

is the chimeric rhodopsin of melanopsin and G protein-coupled receptor (GPCR)*(8, 19)*.

Melanopsin is a non-visual opsin, and despite being an animal opsin, it is not easily

photobleached. However, it has a "bistable" photo-cycle and requires different

wavelengths of light for conformational change, which may result in unnatural

appearance*(20, 21)*.

Therefore, a chimeric rhodopsin of microbial opsin and GPCR*(22–24)*, is not

photo-bleached and is a monostable pigment like visual opsin, but may be able to achieve

highly sensitive visual restoration via G protein stimulation.

In this study, to achieve light sensitivity, stability, and safety, we attempted to restore

vision in mice using *Gloeobacter* and human chimeric rhodopsin (GHCR)*(23, 24)*.

RESULTS

Design of GHCR

Although there is no sequence identity between microbial and animal opsin, both possess

similar chromophore (retinal) and protein (seven-transmembrane helix) structures. As we

previously reported*(24)*, to generate GHCR, we replaced the second and third intracellular

loops of *Gloeobacter* rhodopsin with human sequences and introduced the E132Q

mutation (**Figure S1**). Previous work has shown that GHCR induces G protein activation

in vitro(24).

Restoring light-evoked activity in the retina with GHCR

We injected a viral vector (rAAV-DJ or rAAV-2) containing the GHCR coding sequence

under the control of the hybrid promoter comprising the CMV immediate-early enhancer,

CBA promoter, and CBA intron 1/exon 1, known as the CAGGS promoter,

(CAGGS-GHCR; **Figure 1a**) into the vitreous humor of 10-week-old *rd1* mice. We

adopted the rAAV-DJ vector to achieve more efficient, widespread gene transfer*(25, 26)*,

and used rAAV-2 as a benchmark, as it has already been used in the clinic*(27)*. The retinas

were harvested 2–4 months later. Enhanced green fluorescent protein (EGFP) reporter

gene expression was observed in the retina and in both the ganglion cell layer and the

inner nuclear layer (**Figure S2a, b**). To evaluate the function of ectopically expressed

GHCR in the mouse retina, we performed multi-electrode array (MEA) recording to

record the extracellular potential of RGCs (**Figure S2c**). As a result of photoreceptor

degeneration, the untreated control retina showed no RGC response as detected by MEA

(**Figure 1b**). In contrast, the treated retinas showed obvious light-induced responses down

to 10^{14} photons/cm^2/s of white light-emitting diode (LED) irradiation (**Figure 1c**).

Next, to create a stable vector for human gene therapy, we designed a codon-optimized

version of GHCR (coGHCR) and fused the ER2 endoplasmic reticulum (ER) export signal

to its C-terminus to increase gene expression levels. Immunolabelling revealed expression

across the whole retina, including in the bipolar cells, of treated *rd1* mice (**Figures 1d**). As

a result, the firing rate increased significantly, and a photoresponse was confirmed down

to 10^{13} photons/cm^2/s, which had not observed before optimization (**Figure 1e, f**). The

retinas of WT mice were highly responsive to all light stimulus levels under dark-adapted

conditions, but under light-adapted conditions, the firing rate was also modulated in

response to light stimulus intensity, and coGHCR response was similar to the

light-adapted conditions in WT mice (**Figure S2e**). No photoresponse to any light stimulus

level was obtained from control untreated mice. Moreover, the number of firing cells per

unit area also increased significantly (**Figure 1g**). Since rhodopsin shows selectivity for

Gi/o class G proteins upon heterologous expression*(28–31)*, we measured Gi/o activation

with a homogeneous time-resolved fluorescence (HTRF) cyclic adenosine monophosphate

(cAMP) assay. We observed a 5-fold increase in activation in coGHCR-treated compared

with GHCR-treated mice (**Figure 1h**). The maximum spectral sensitivity of retinas treated

with coGHCR was around 500 nm, and a photoresponse was obtained even upon

stimulation with light with a wavelength >600 nm (**Figure 1i**).

Restoration of visual cortex responses by GHCR

To investigate whether retinal light responses were transmitted to the visual cortex, we

then examined visual evoked potentials (VEPs) generated by the visual cortex (**Figure 2a**).

The output from the RGCs is sent through their axons (optic nerve) to the lateral

geniculate nucleus (LGN) of the thalamus, which is a region of the diencephalon, then

from the LGN to the primary visual cortex in the occipital lobe of the cerebral cortex. For

these experiments, we used *rd1* mice in which both eyes had been treated with the

AAV-DJ-CAGGS-GHCR, AAV-DJ-CAGGS-coGHCR, or control EGFP

(AAV-DJ-CAGGS-EGFP) vectors. Significant VEPs were not detected in the control or

GHCR-treated mice. In contrast, VEPs were observed in coGHCR-treated mice (**Figure**

2b). In response to 3 cd·s/m^2 light stimulation, the average VEP amplitude in

coGHCR-treated mice was significantly higher (56.4 µV; n = 6) than those in

GHCR-treated mice (22.1 µV; n = 8) and control mice (17.9 µV; n = 6) (**Figure 2c**).

Based on this result, all subsequent experiments were performed using coGHCR.

Characterization of the in vivo responses restored by GHCR transduction

Next, light-dark transition (LDT) testing was performed to investigate whether ectopic

expression of coGHCR in degenerating retinas led to behavioral changes due to vision

restoration (**Figure 3a**). Rodents with intact vision tend to stay in dark places as they are

nocturnal and feel uneasy in bright environments, whereas blind rodents spend roughly

half of their time in bright places. The coGHCR-treated mice spent significantly less time

in the bright area compared with the untreated *rd1* mutant mice (**Figure 3b**), thereby

confirming vision restoration via behavioral analysis. And the visual restoration effect was

still maintained after two years (**Figure 3c**). Furthermore, in order to directly compare the

effects of coGHCR with genes in clinical trials, we treated *rd1* mice with chimeric

rhodopsin (AAV-6-CAGGS-coGHCR), microbial opsin (AAV-6-CAGGS-ChrimsonR*(32)*),

animal rhodopsin (AAV-6-CAGGS-human rhodopsin), or the control EGFP

(AAV-6-CAGGS-EGFP) vector. At an illuminance of 3,000 lux, a significant reduction in

the time spent in the bright half of the observation area was noted for coGHCR-treated

mice (0.32; n = 6) compared with control mice (0.50; n = 8) (**Figure 3d**). A similar

tendency was observed in ChrimsonR-treated mice (0.36; n = 6). However, no obvious

change was observed in human rhodopsin-treated mice (0.48; n = 6). When the experiment

was carried out at an illumination of 10 lux, human rhodopsin-treated mice showed a

significant change in the time spent in the bright area (0.40; n = 6), whereas

ChrimsonR-treated mice did not show an obvious change (0.55; n = 6) (**Figure 3e**). The

coGHCR-treated mice again spent significantly less time in the bright area illuminated at

10 lux (0.40; n = 6).

Restored object recognition function upon GHCR gene therapy

LDT testing measures only light and dark discrimination. Visual recognition testing

(VRT) was performed to evaluate whether the mice could recognize an object with the

restored level of vision. Mice use vision for their cognitive functions, and are attracted to

fighting videos*(33–35)*. We examined mice in a place preference apparatus with a tablet

showing a fighting video (**Figure 3f**). The ratio of the time spent in the area with the

fighting compared with the time spent in the control area (showing a video of an empty

cage with the same illuminance) over 15 minutes was measured. The coGHCR-treated

(AAV-DJ-CAGGS-coGHCR) mice spent significantly more time in the fighting video half

of the apparatus (0.55, n = 33) than the untreated *rd1* mice (0.50, n = 30). On the other

hand, microbial opsin-treated (AAV-DJ-CAGGS-C1V1*(36)*) mice spent roughly

equivalent time in each half (0.49, n = 20) (**Figure 3g**).

GHCR protective effects against retinal degeneration

We employed another mouse model of retinal degeneration using mice with the P23H

RHO mutation, referred to as P23H mice*(37)*. P23H mice were selected to evaluate the

protective effect because they have slower retinal degeneration than *rd1* mice. We

subretinally delivered AAV DJ-CAGGS-coGHCR and the control (AAV

DJ-CAGGS-EGFP) vector into postnatal day (PND) 0–1 mouse retinas, targeting the outer

retina, and quantified the protective effects of the vector via morphological and

electrophysiological examination. Subretinal injection of AAV-DJ efficiently induced gene

expression in the murine outer retina (**Figure 4a**). Optical coherence tomography (OCT)

showed that the outer retinal thickness (ORT), which is the thickness from the outer

nuclear layer (ONL) to the rod outer segment (ROS), of coGHCR-treated mice (50.0 μm;

n = 13) was significantly greater than that of the control mice (42.7 μm; n = 10) at PND 30

(**Figure 4b, c**). The ORT of the treated mice remained significantly greater than that of

control mice until PND 50 (**Figure S3**).

Electroretinography (ERG) revealed that the treated mice had larger rod, mixed, and cone

response amplitudes (141.2 μV, 271.4 μV, and 159.0 μV, respectively; n = 9) than the

control mice (70.4 μV, 158.7 μV, and 99.1 μV, respectively; n = 14) at PND 30 (**Figure 4d,**

e). All amplitudes in the control mice gradually decreased, whereas all amplitudes in the

coGHCR-treated mice continued to increase until PND 42 (**Figure S4a, b c**). Thereafter,

the amplitudes in the treated mice also gradually decreased, although they remained

significantly higher than those in the control mice until PND 66.

We also performed terminal deoxynucleotidyl transferase dUTP nick end labeling

(TUNEL) to detect apoptosis in the retinas. The number of TUNEL-positive cells in the

coGHCR-treated mouse ONL (289.7 cells; n = 3) was significantly lower than that in the

control mouse ONL (67.3 cells; n = 3) at PND 31 (**Figure 5a–c**).

To expand these observations, we obtained transmission electron microscopy (TEM)

images of transverse sections from PND 31 mice. Consistent with the OCT results, the

ONL (**Figure 5d**) and ROS (**Figure 5e**) of coGHCR-treated mice were relatively intact

compared with those of controls, and the ROS structure was less disorganized (**Figure 5f**).

In addition, coGHCR-treated mice had less swelling of their ER, a feature that is

indicative of ER stress (**Figure 5g**).

We also performed western blotting to investigate ER stress. Expression of the ER stress

marker ATF4 was significantly lower, and expression of BiP, PERK, ATF6, and pIRE1

tended to be lower in treated mice than in control mice at PND 14 (**Figure S5a, b**).

Since retinoid levels are known to affect ER stress and retinal degeneration, retinoid

analysis of the treated eyes was performed. The amount of retinal was measured by HPLC

using the retinal oxime method after 10 minutes of exposure to 1000 lux, a fluorescent

lighting level assuming a normal indoor environment. The results showed that 11-cis

retinal oximes was significantly elevated in the treated eyes (54.1 ± 18.2 pmol/ 2 retinas; n

= 9) versus controls (39.5 ± 6.5 pmol/2 retinas; n = 9) (**Figure 5h, i**). No obvious changes

in the amount of all-trans-retinal oxime were observed (**Figure 5j**).

DISCUSSION

Because the phenotype of retinal degeneration is common across cases of retinitis

pigmentosa, regardless of genotype, the strategy of optogenetic therapy has great potential

as a universal therapeutic approach. It aims to target non-photoreceptive surviving neurons

in the retina, such as retinal ganglion cells and bipolar cells, and convert them to

photoreceptive.

In this study, we demonstrated that ectopic expression of coGHCR is an effective method

of optogenetic vision restoration in mice with retinal degeneration. MEA revealed that

photoresponses were maintained for retinal irradiance levels as low as 10^{13} photons/cm^2/s.

This is consistent with the response of the treated mice to 10 lux illumination in the

behavioral test, and represents a significant improvement in sensitivity compared with that

observed in previous studies of vision restoration with microbial opsins (threshold: 10^{14} to

10^{17} photons/cm^2/s)*(2–7)*, LiGluR/MAG photoswitches (threshold: 10^{15}–10^{16}

photons/cm^2/s)*(38, 39)*, or photoactivated ligands (AAQ threshold: 10^{15} photons/cm^2/s*(40)*

and DENAQ threshold: 4×10^{13} photons/cm^2/s*(41)*). Although some vectors restored

greater sensitivity, such as human rhodopsin*(9)*, cone opsin*(16)* and Opto-mgluR6 (10^{12}

photons/cm^2/s)*(8)*, our LDT results at 3,000 lux (similar to a cloudy outdoor environment)

suggest that photobleaching of rhodopsin like these does not work in bright environments.

coGHCR is adaptable to a light environment ranging from at least 10 lux (similar to a

night light levels with streetlights) to 3,000 lux, and is, thus, a suitable single-opsin vision

restoration tool.

Furthermore, the typical channelrhodopsins have a spectrum limited to blue light*(42)*,

which limits their use as a visual restoration tool. On the other hand, GHCR has a

spectrum peak around 500 nm and facilitates responses to red light. Irradiation of

high-energy light such as blue light can cause phototoxicity and cell death due to

generation of free radicals*(43)*. Therefore, there are concerns about phototoxicity in

optogenetic tools that operate under blue light, such as channelrhodopsin, and long

wavelength-shifted opsins have been developed*(44)*. In this regard, the GHCR has the

advantage of being highly sensitive and having a peak at intermediate (green) wavelengths,

making it responsive to short and long wavelengths and less likely to exceed safe limits of

light intensity*(45)*. In addition, behavioral tests showed that coGHCR enabled responses to

both sustained and transient stimulation lasting 10 ms. These findings suggested that

coGHCR gene therapy can restore sensitivity to multiple light environments encountered

in daily life.

The ERG amplitudes in coGHCR-treated mice continued to increase until PND 42, likely

because the coGHCR-mediated signal was additive with the innate amplitude. This is

consistent with the fact that gene expression of the AAV-DJ vector peaks at approximately

1.5 months after administration*(25)*. We observed no apparent changes in the shapes of the

ERG waveforms in the coGHCR-treated mice. The visual restoration effect was also

maintained for two years, which shows promise for long-term pharmacological effects and

safety.

coGHCR has Gt activity derived from rhodopsin*(24)*. Gt is also known to be cross-linked

with Gi/o*(46)*, and this was confirmed (**Figure 1h**). Although this study used a ubiquitous

promoter, which cannot be fully confirmed, Gi/o is generally expressed specifically in

ON-type bipolar cells*(47, 48)*, where the light-responsive signal is likely to have been

generated. When coGHCR is expressed ectopically in ON bipolar cells, it is expected to

inhibit responses. However, the restored responses observed by MEA were all ON

responses. In addition, the electrophysiological and behavioral results were similar to

physiological responses, and no reversal reaction observed. In *rd1* mice, photoreceptors

are mostly lost by 4 weeks after birth and no optical response is obtained after 7 weeks at

the latest*(49, 50)*. Therefore, responses from residual photoreceptors are unlikely in this

study. A similar phenomenon has been confirmed in previous studies; the excitatory

response is hypothesized to result from disinhibition of inhibitory amacrine cells*(6, 8, 9)*.

The safety of ectopic expression of opsins, such as channelrhodopsin 2, has been

previously reported*(3, 51, 52)*. To our knowledge, this is the first report of their protective

effects against retinal degeneration. In vitro studies have shown that the P23H opsin is

misfolded and retained in the ER*(53)*. ER retention of P23H opsin can induce the unfolded

protein response, leading to apoptosis*(54–57)*. Our results suggest that expression of

coGHCR in the retinal outer layer suppressed ER stress and photoreceptor apoptosis,

which led to protection against degeneration. The lack of 11-cis-retinal induces

cytotoxicity during the development of ROS in P23H mice*(58)*. In fact, the amount of

cis-retinal in the retina was significantly elevated after coGHCR treatment. Since

coGHCR uses all-trans retinal as a chromophore, like microbial opsin, it does not consume

cis-retinal and is free from photobleaching. Therefore, the expressed coGHCR may

suppress cis-retinal consumption via photoreceptor substitution. If this hypothesis is

correct, the protection effect of coGHCR may not be applicable to patients with all IRD

genotypes. However, there are more than 140 known RP-linked rhodopsin mutations, and

those that result in protein misfolding and retention in the ER are the most prevalent*(59,*

60).

In summary, the coGHCR vector has the advantages of both animal and microbial opsin

as a vision regeneration tool. It restores sensitivity and an action spectrum that enables

vision in lighting ranging from levels found outdoors to those in dimly lit indoor

environments via G protein stimulation without the risk of bleaching; it can also be

expected to protect against the progression of retinal degeneration in the majority of IRD

patients. These results suggest that coGHCR is worthy of consideration for clinical

application as a gene therapy for IRD.

MATERIALS AND METHODS

Study approval: All of the animal experiments were conducted in accordance with

protocols approved by Institutional Animal Care and Use Committee of Keio University

School of Medicine (#2808).

Please see supplemental information for detail.

Author Contribution

Y.K. and T.K. designed the research, wrote the manuscript. Y.K. performed the retinal

histology, MEA, ERG and VEP recordings, and WB, TEM, LDT and VRT experiments.

N.S. performed HPLC. K.Y. performed plasmid construction. K.K. performed AAV

production. Y.K. performed data processing and analysis. K.N., H.K., H.O and T.K. made

critical revisions of the manuscript. T.K. supervised the research.

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Declaration of Interests

Y.K., H.K., K.T., and T.K. are inventors on pending patents (PCT/JP2017/031579、

PCT/JP2019/ 1565) related to this work. Y.K., K.T., and T.K. are equity holders in Restore

Vision, Inc.

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 Figure legends

Figure 1. Ectopic GHCR expression restores light responses in the *rd1* mouse retina

(a) DNA expression cassette schematic. The GHCR coding sequence is driven by the

CAGGS promoter, flanked by inverted terminal repeats (ITR), and stabilized by a

polyadenylation signal sequence (pA) and a woodchuck hepatitis posttranscriptional

regulatory element (WPRE). (b, c, e) Raster plots and peri-stimulus time histograms for

light stimulation of control (AAV-DJ-CAGGS-EGFP) (b), GHCR-treated

(AAV-DJ-CAGGS-GHCR) (c), and coGHCR-treated (AAV-DJ-CAGGS-coGHCR) mice

(e). Responses to exposure to a white LED with varying light intensity for 1.0 s. Gray

shading around the averaged traces represents the standard error of the mean (SEM). (d)

Confocal image of a transverse *rd1* mouse retina section 2 months after

AAV-DJ-CAGGS-coGHCR intravitreal injection. Green, FLAG tag antibody signal

(vector); red, PKCα signal (bipolar cells); blue, 4′,6-diamidino-2-phenylindolenuclear

(DAPI) counterstaining. Scale bar, 50 μm. (f) Quantitation of the firing rates of RGCs

transduced with GHCR or coGHCR at the indicated light intensity. (g) Histogram showing

the number of RGCs that responded to light per unit area (2.6 mm^2) of the retinas of

GHCR- or coGHCR-treated mice (n = 3 each). (h) Changes in cAMP consumption in

response to Gi/o-coupled G-protein-coupled receptor activation in HEK293T cells

transfected with GHCR and coGHCR (n = 3 each). (i) Spectral sensitivity induced by

coGHCR (n = 23 cells each). Error bars represent the SEM. Data were analyzed with

Student's two-tailed t-test in (f, g) and one-way analysis of variance (ANOVA) and

Tukey's multiple comparison test in (h); * represents $p \leq 0.05$, ** represents $p \leq 0.01$, and

*** represents $p \leq 0.001$. GCL, ganglion cell layer; INL, inner nuclear layer.

Figure 2. coGHCR restored vision in *rd1* mice through the primary visual cortex

(a) Schematic view of the VEP recording strategy. (b) Representative VEP traces from

GHCR-treated, coGHCR-treated, and control mice. (c) The average amplitude of the

VEPs in the control (AAV-DJ-CAGGS-EGFP, n = 6), GHCR-treated

(AAV-DJ-CAGGS-GHCR, n = 8), and coGHCR-treated (AAV-DJ-CAGGS-coGHCR, n

= 6) mice. The stimulus was a white LED flash (3 cd·s/m^2). Signals were low-pass filtered

at 300 Hz and averaged over 60 trials. Error bars represent the SEM. Data were analyzed

with one-way ANOVA and Tukey's multiple comparison test; * represents p ≤ 0.05. V1,

visual cortex.

Figure 3. coGHCR-treated mouse behavior indicated vision restoration

(a) LDT testing schematic. Mice were tested in a $30 \times 45 \times 30$-cm box with equally sized

bright and dark chambers connected by a 5×5-cm opening, across which the mice could

move freely. (b, c) Percentage of time spent in the bright area (total, 10 min) by wild type

(n = 4), and control (AAV-DJ-CAGGS-EGFP) (n = 7 in (b) and n = 4 in (c)) and

coGHCR-treated (AAV-DJ-CAGGS-coGHCR) *rd1* mice (n = 6). LDT test at 3 months (b)

and 2 years (c) after treatment, 10 lux illumination. (d, e) The percentage of time spent in

the bright area (total, 10 min) by wild type (n = 6), and control (AAV-6-CAGGS-EGFP)

(n = 8), coGHCR-treated (AAV-6-CAGGS-coGHCR) (n = 6), ChrimsonR-treated

(AAV-6-CAGGS-ChrimsonR) (n = 6), and human rhodopsin-treated

(AAV-6-CAGGS-human-rhodopsin) *rd1* mice (n = 6). LDT test with 3,000 lux (d) and 10

lux (e) illumination. (f) VRT setup. Time spent in areas showing a video of mice fighting

(object half, blue) or an empty cage (control half, red) was measured. (g) Distribution of

time spent in the object half by wild type (n = 14), and control (no treatment) (n = 23),

AAV-2-coGHCR-treated (AAV-2-CAGGS-coGHCR) (n = 30),

AAV-DJ-coGHCR-treated (AAV-DJ-CAGGS- coGHCR) (n = 33), and

AAV-DJ-C1V1-treated (AAV-DJ-CAGGS-C1V1) *rd1* mice (n = 20). LDT test with 10

lux (d) and 3,000 lux (e) illumination. Black line, average value. Error bars represent the

SEM. Data were analyzed with one-way ANOVA and Tukey's multiple comparison test;

* represents $p \leq 0.05$.

Figure 4. Ectopic coGHCR expression protects against photoreceptor degeneration

(a) Confocal image of a transverse section through the P23H retina 2 months after

AAV-DJ-CAGGS-coGHCR subretinal injection. Green, FLAG tag fused to the

C-terminus of coGHCR; blue, DAPI nuclear counterstaining. Scale bar, 100 μm. (b) OCT

retinal image sections from coGHCR-treated and control (AAV-DJ-CAGGS-EGFP

subretinally injected) mice at PND 30. The white arrow indicates the measured ORT (from

ONL to cone outer segment). Scale bar, 20 μm. (c) Histogram of the measured ORT of the

coGHCR-treated (n = 13) and control mice (n = 10) at PND 30. (d, e) Representative ERG

waveforms (rod response, mixed response, and cone response) of coGHCR-treated (n =

14) and control mice (n = 9) (d). Histograms of the average ERG amplitudes from panel d

at PND 30 (e). Error bars represent SEM. Data were analyzed with the unpaired t-test; ***

represents $p \leq 0.001$. GCL, ganglion cell layer; INL, inner nuclear layer.

Figure 5. coGHCR treatment suppressed retinal apoptosis and ER stress

(a, b) TUNEL-stained transverse sections (a) and enlarged images of the white squares (b)

of coGHCR-treated and control (AAV-DJ-CAGGS-EGFP subretinally injected) mouse

retinas at PND 31. Red, TUNEL-positive cells; blue, DAPI nuclear counterstaining. Scale

bar, 1,000 μm in (a) and 100 μm in (b). (c) Histogram of the number of TUNEL-positive

cells in the ONLs of coGHCR-treated (n = 3) and control mice (n = 3) at PND 31. (d)

TEM images of transverse sections from coGHCR-treated and control mice at PND 31,

showing the outer retinal layer (d), the outer segment at low magnification (e) and high

magnification (f), and the inner segment (g). The arrowhead indicates swollen ER. Scale

bar, 20 μm in (d), 5 μm in (e), 1 μm in (f), and 500 nm in (g). (h) Chromatograms of

retinal in mouse retina analyzed by HPLC. 15 h dark adapted mice were exposed to light

of 1000 lux for 10 min and each retina was processed and retinal oximes extracted under

dim red light. Peak identification was determined using retinal standard reagents as

follows: 1, syn-11-cis-retinal oxime; 2, syn-all-trans-retinal oxime; 3, anti-11-cis-retinal

oxime; 4, anti-all-trans-retinal oxime. (i, j) Histogram quantifying the amount of retinal

oximes from coGHCR-treated (n = 9) and control mice (n = 9) obtained from HPLC. Error

bars represent SEM. Data were analyzed with the unpaired t-test; * represents $p \leq 0.05$, **

represents $p \leq 0.01$.