Disclosure

The speaker and the co-authors are employees and stockholders of Editas Medicine.

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MEDICINE

Autosomal Dominant Retinitis Pigmentosa (adRP)

- An inherited autosomal dominant retinal disease leading to blindness in later life
- Symptoms:
  - Decreased night vision (nyctalopia)
  - Loss of peripheral vision (tunnel vision), and eventually significant decline in central vision
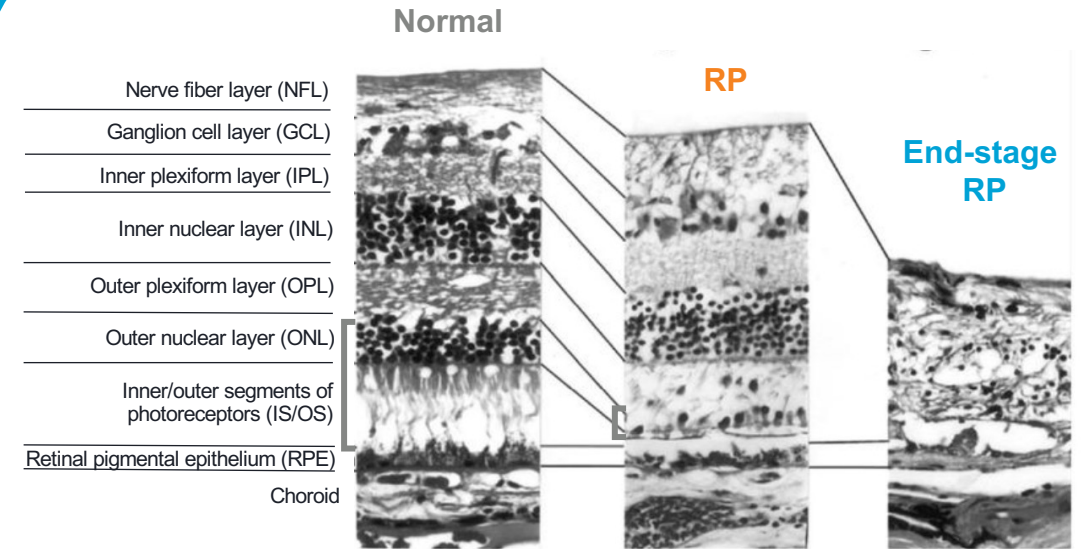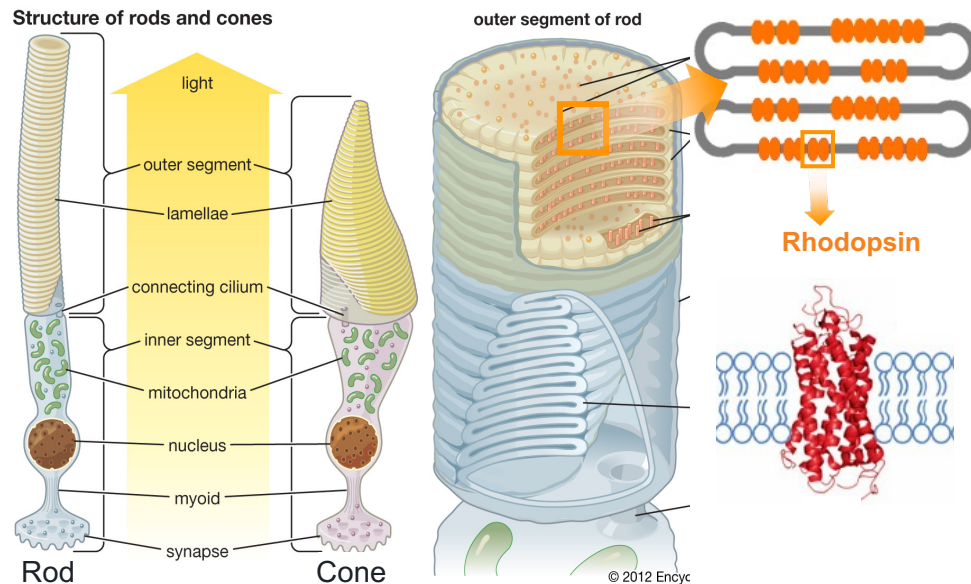- No approved treatments

Normal vision

Tunnel vision

Rhodopsin-Associated adRP (RHO-adRP)

Rhodopsin (RHO)
- A light-sensitive receptor protein involved in visual phototransduction in rods
- Located in the outer segments of rods
- Approximately 30% (US and UK) of adRP caused by *RHO* dominant mutations
- Prevalence: 7,500 patients in US and 12,100 patients in EU and UK
- >150 mutations identified in the *RHO* gene cause RHO-adRP[1]
- Dominant mutations in the *RHO* gene are toxic for the rods: progressive loss of rods followed by loss of cones

Photoreceptor structure

Structure of rods and cones

light

outer segment
lamellae
connecting cilium
inner segment
mitochondria
nucleus
myoid
synapse

Rod   Cone

outer segment of rod

Rhodopsin

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Normal   **RP**   **End-stage RP**

Nerve fiber layer (NFL)
Ganglion cell layer (GCL)
Inner plexiform layer (IPL)
Inner nuclear layer (INL)
Outer plexiform layer (OPL)
Outer nuclear layer (ONL)
Inner/outer segments of photoreceptors (IS/OS)
Retinal pigmental epithelium (RPE)
Choroid

Valle D. et al. OMMBID 2014

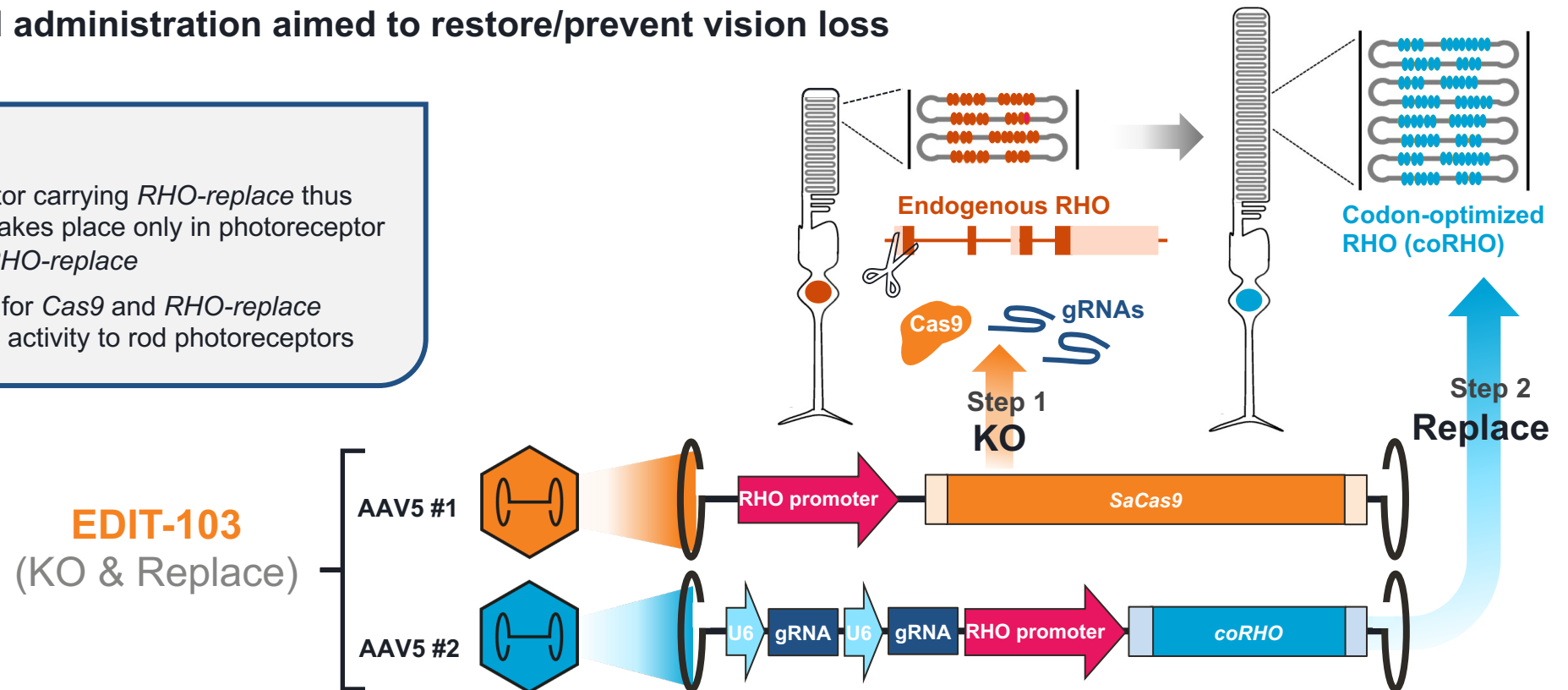1. Athanasiou, D. *et al*. *Prog Retin Eye Res* 2018; 62: 1–23.

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EDIT-103: Dual AAV-Based "Knockout and Replace" Therapeutic Strategy

- **Agnostic to any RHO mutation – thus will knockout any dominant gain-of-function rhodopsin mutant**

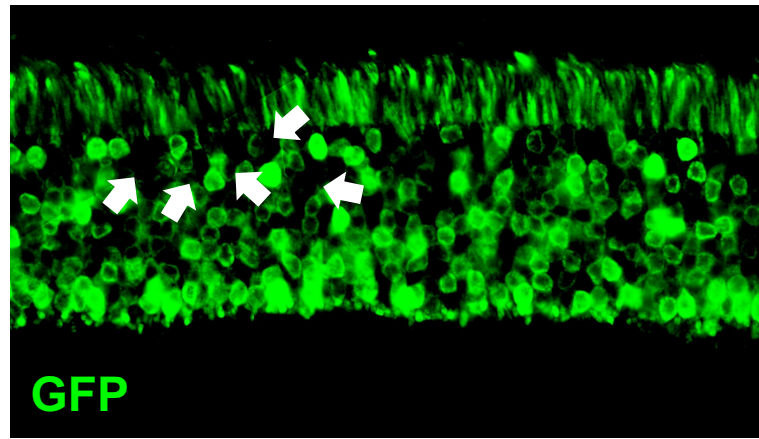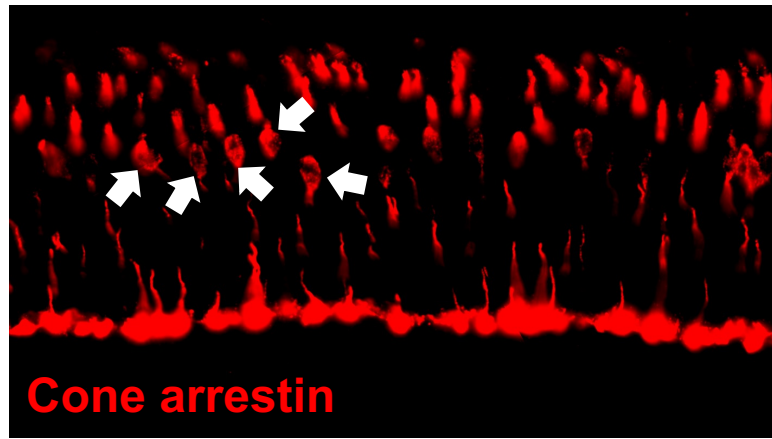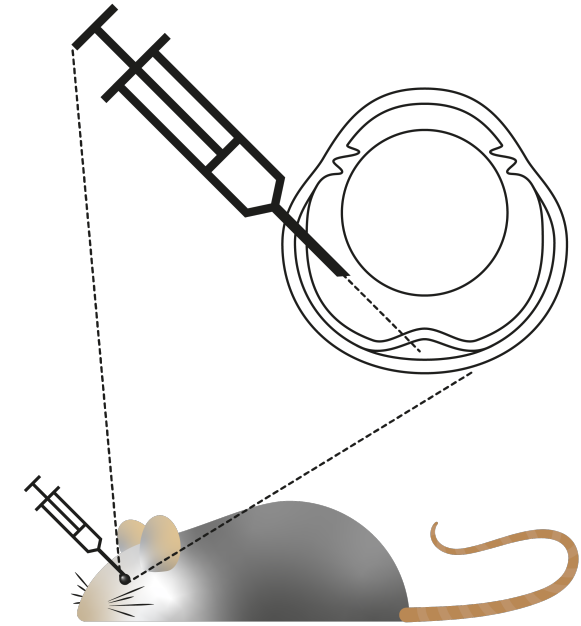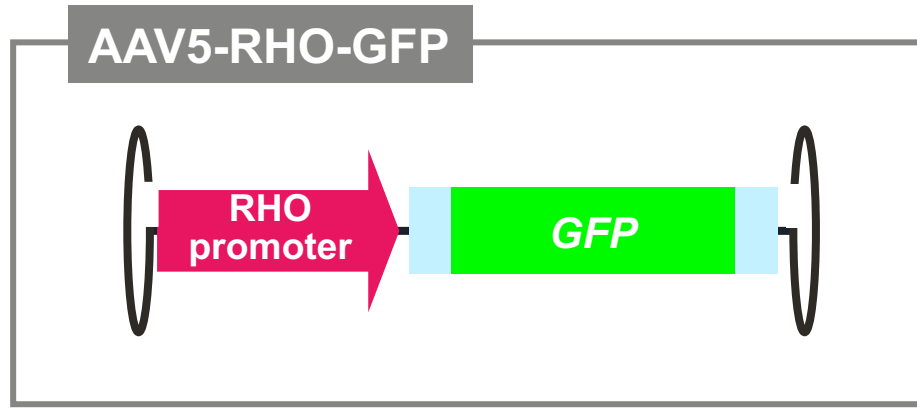- **Step 1:** Both mutant and normal endogenous *RHO* will be knocked out in the treated area

- **Step 2:** Exogenous normal *RHO* (resistant to editing) will replace endogenous *RHO*

- **One-time subretinal administration aimed to restore/prevent vision loss**

Details:

- gRNA is on the vector carrying *RHO-replace* thus assuring knockout takes place only in photoreceptor cells that express *RHO-replace*

- The *RHO* promoter for *Cas9* and *RHO-replace* restricts therapeutic activity to rod photoreceptors

Endogenous RHO

Codon-optimized RHO (coRHO)

Cas9   gRNAs

Step 1
KO

Step 2
Replace

EDIT-103
(KO & Replace)

AAV5 #1 — RHO promoter — SaCas9

AAV5 #2 — U6 — gRNA — U6 — gRNA — RHO promoter — coRHO

AAV: adeno-associated virus; Cas9: CRISPR-associated protein 9; coRHO: codon-optimized rhodopsin; gRNA: guide ribonucleic acid; KO: knockout; RHO: rhodopsin; SaCas9: *staphylococcus aureus* CRISPR-associated protein 9.

The RHO Promoter Restricts Gene Expression to Rod Photoreceptors in the Mouse Eyes

AAV5-RHO-GFP

RHO promoter → GFP

Cone arrestin

GFP

Merge

IS/OS

ONL

OPL

INL

Scale bar = 20 μm

AAV: adeno-associated virus; GFP: green fluorescent protein; INL: inner nuclear layer; IS/OS: inner/outer segments of photoreceptors; ONL: outer nuclear layer; OPL: outer plexiform layer; RHO: rhodopsin
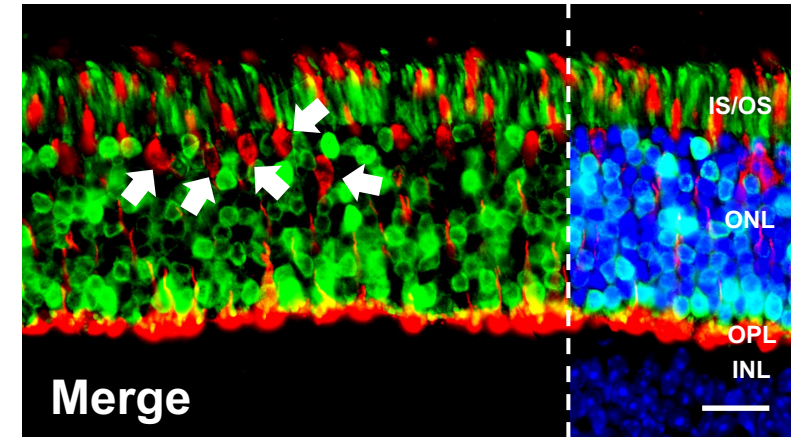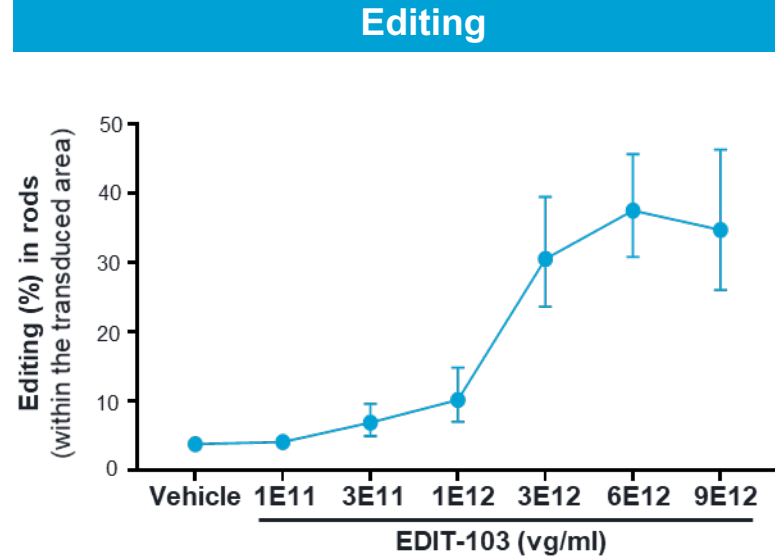
EDIT-103 is Highly Specific: No Detectable Off-Target Editing

NO OFF-TARGET EDITING AT OFF-TARGET CANDIDATES
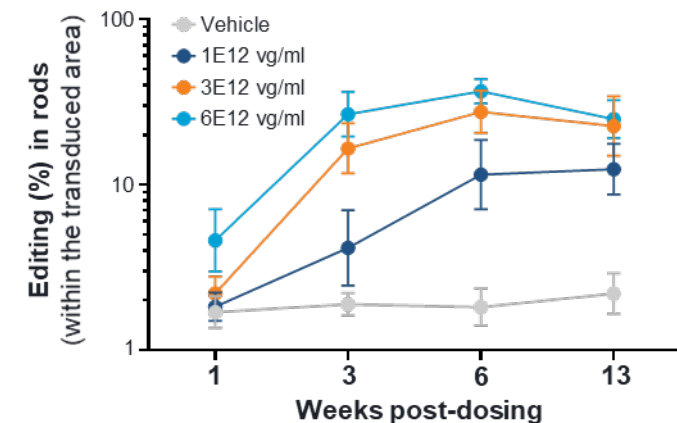(rhAmpSeq verification in human retina explants transduced with EDIT-103)

EDIT-103 gRNA (On-Target)

y-axis: Editing (floored to 0.01%) +/- 2bp

x-axis categories: AMP24176|78, AMP24177|72, AMP24180|92, AMP24182|66, AMP24178|129, AMP24187|136, AMP24183|45, AMP24179|131, AMP24181|61, AMP24184|70, AMP24185|130, AMP24186|100

AAV: adeno-associated virus; bp: base pair; gRNA: guide ribonucleic acid; rhAmpSeq: RNase H2 enzyme-dependent amplicon sequencing; RHO: rhodopsin.

EDIT-103 in Humanized *mRho*^{hRHO/+} Mice: Demonstrates Rapid and Stable Gene Editing
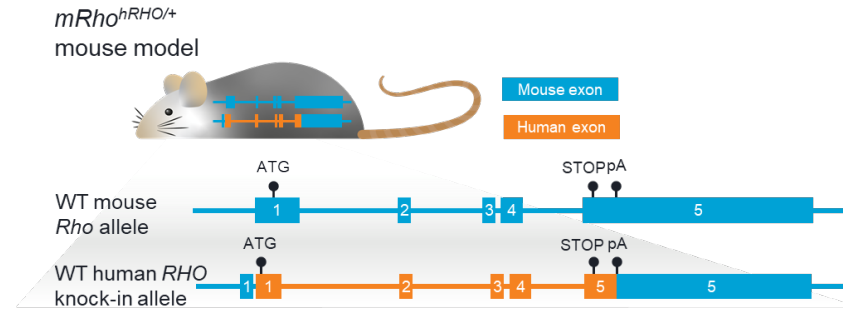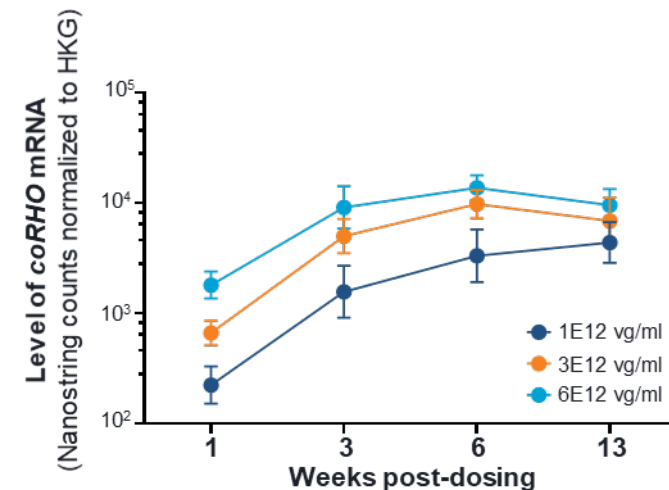
- >25% of rods are edited
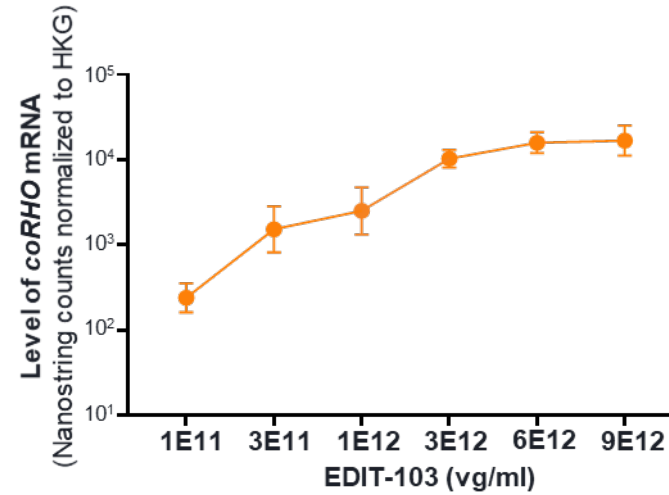- Editing rates increase in a dose-dependent manner
- Editing plateau at ~6 weeks post-injection at doses ≥ 3E12 vg/ml

AAV ratio: 1:1
Volume: 1 µl
Geometric mean with 95% confidence interval is presented

AAV: adeno-associated virus; coRHO: codon optimized rhodopsin; hRHO: human rhodopsin; HKG: house-keeping gene; mRNA: messenger ribonucleic acid; PR: photoreceptor; RHO: rhodopsin; WT: wildtype.

EDIT-103 in Non-Human Primates (NHPs): Approximately 100% Editing in Transduced Photoreceptors
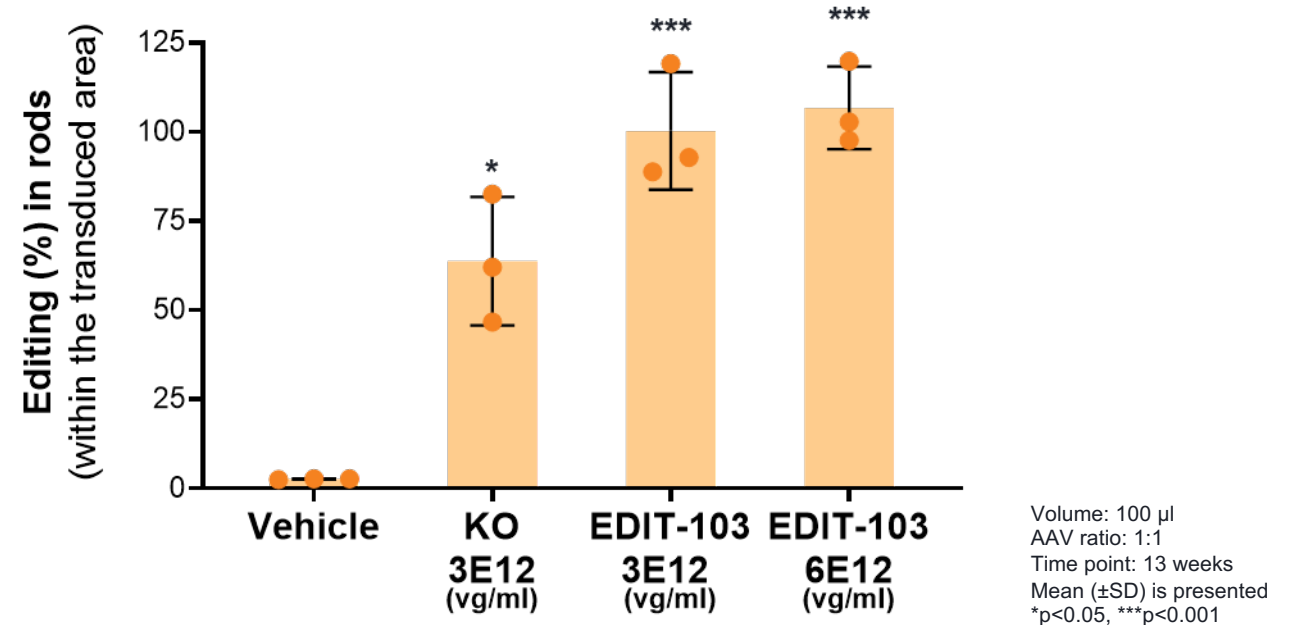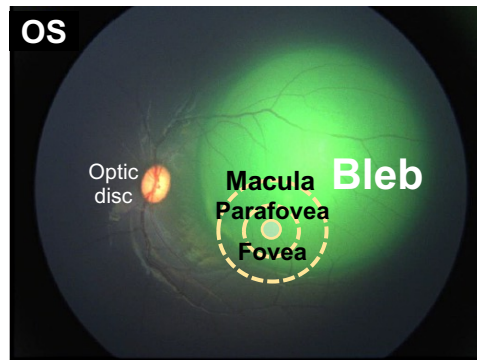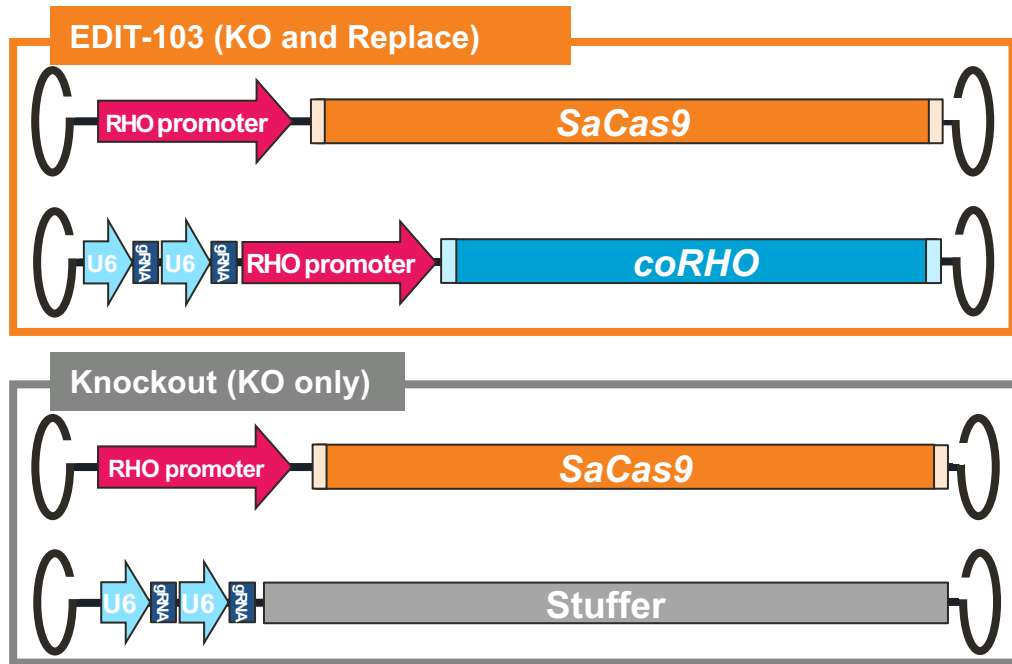
~100% editing (within the transduced area) in NHP

Volume: 100 µl
AAV ratio: 1:1
Time point: 13 weeks
Mean (±SD) is presented
*p<0.05, ***p<0.001

EDIT-103 in NHPs: Nearly Complete Knockdown of the Endogenous RHO and Over 30% RHO Protein Replacement

RHO mRNA

RHO Protein

Endogenous NHP RHO
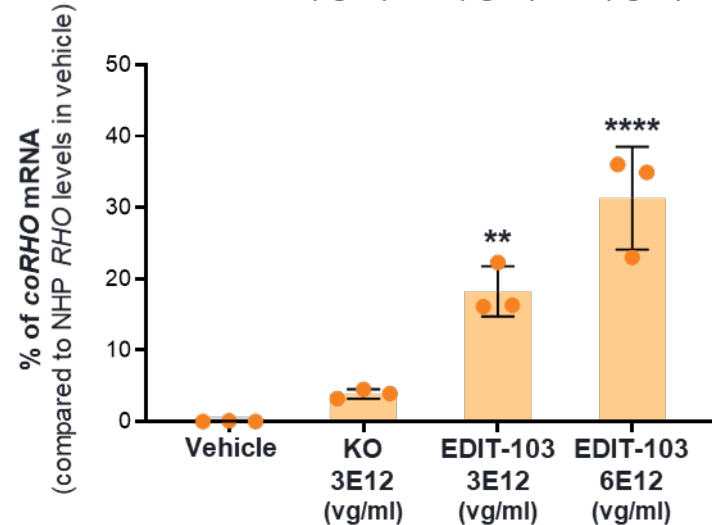
Human RHO Replacement
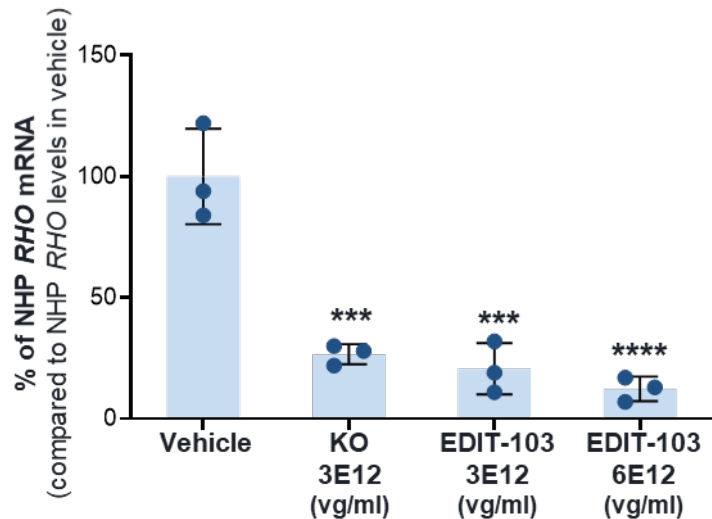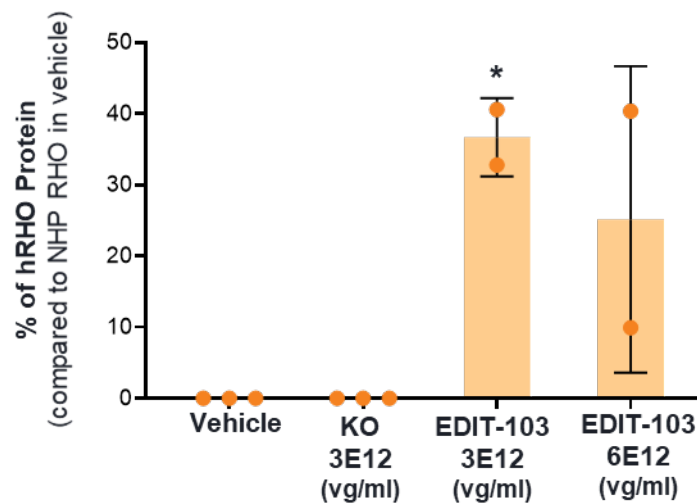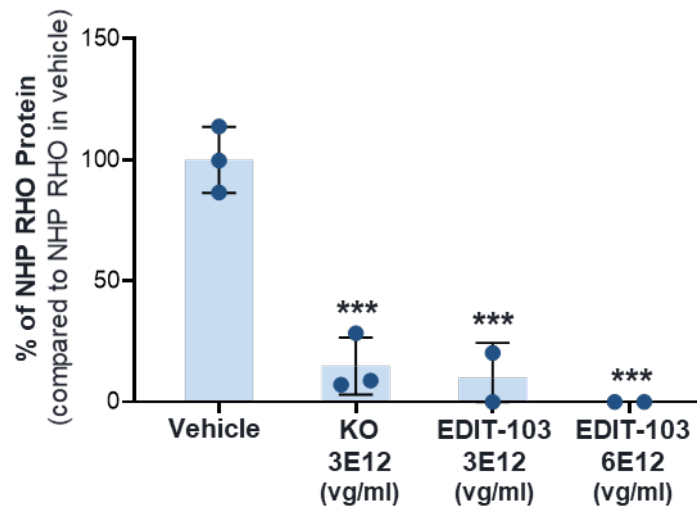
- 80% and 90% knockdown of endogenous NHP *RHO* mRNA levels were achieved at doses of 3E12 and 6E12 vg/ml, respectively. This resulted in 90% and 100% of RHO protein knockdown.

- *RHO* replacement mRNA levels increased with dose and resulted in >30% of RHO protein levels.

Volume: 100 µl
AAV ratio: 1:1
Time point: 13 weeks
Mean (±SD) is presented
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

AAV: adeno-associated virus; coRHO: codon-optimized rhodopsin; KO: knockout; mRNA: messenger ribonucleic acid; NHP: non-human primate; RHO: rhodopsin; SD: standard deviation.

RHO Protein Expression and Retina Morphology was Preserved in EDIT-103-Treated NHP Retinas Compared with KO-Treated Retinas

| Vehicle | KO (3E12 vg/ml) | EDIT-103 (3E12 vg/ml) |

RHO protein / Cas9 genome

H&E staining

AAV ratio: 1:1; Injection volume: 100 µL; Time point: 13 weeks; Scale bars, 50 µm

- AAV transduction in the treated groups reveals positive **Cas9 genome** staining
- Nearly absent **RHO protein** and lack of OS (yellow arrow) in the KO group
- Preservation of **RHO protein**, improved photoreceptor organization, and improved IS/OS morphology in the EDIT-103-treated groups

AAV: adeno-associated virus; H&E: Hematoxylin and Eosin; IS/OS: inner/outer segments of photoreceptors; KO: knockout; NHP: non-human primate; ONL: outer nuclear layer; RHO: rhodopsin

Retina Function Preserved in the EDIT-103-Treated NHP Eyes Compared to the KO Only Treated Eyes

Retinal layers
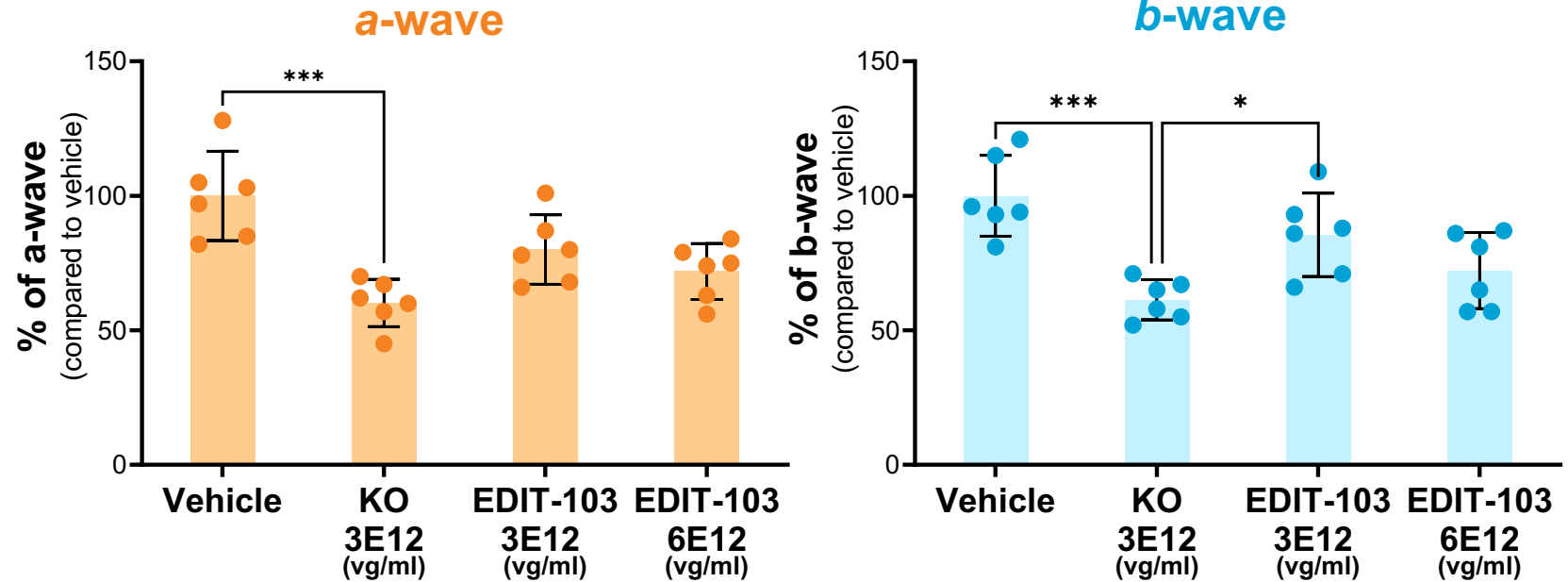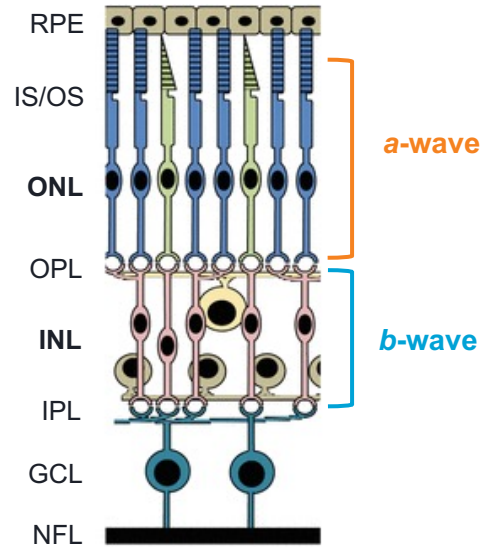
Retinal layer diagram showing (top to bottom): RPE, IS/OS, ONL, OPL, INL, IPL, GCL, NFL. The *a-wave* bracket spans RPE through ONL/OPL; the *b-wave* bracket spans OPL through IPL.

Dark-adapted 3.0 ERG (combined rod-cone response)

ERG trace with *a* and *b* wave components labeled.

a-wave — % of a-wave (compared to vehicle), y-axis 0–150. Groups: Vehicle, KO 3E12 (vg/ml), EDIT-103 3E12 (vg/ml), EDIT-103 6E12 (vg/ml). *** between Vehicle and KO 3E12.
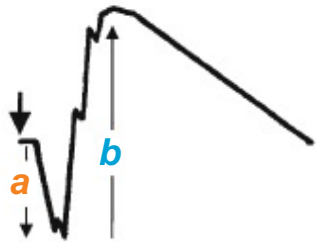

b-wave — % of b-wave (compared to vehicle), y-axis 0–150. Groups: Vehicle, KO 3E12 (vg/ml), EDIT-103 3E12 (vg/ml), EDIT-103 6E12 (vg/ml). *** between Vehicle and KO 3E12; * between KO 3E12 and EDIT-103 3E12.

AAV ratio: 1:1; Injection volume: 100 µL; Time point: 13 weeks; Mean (±SD) is presented; $*p<0.05$, $***p<0.001$

- KO of endogenous *RHO* significantly reduced *a*- and *b*-wave amplitudes
- EDIT-103 dosing preserved *a*- and *b*-wave amplitudes

AAV: adeno-associated virus; ERG: electroretinogram; GCL: ganglion cell layer; INL: inner nuclear layer; IPL: inner plexiform layer; IS/OS: inner/outer segments of photoreceptors; KO: knockout; NFL: nerve fiber layer; ONL: outer nuclear layer; OPL: outer plexiform layer; RHO: rhodopsin; RPE: retinal pigmental epithelium; SD: standard deviation.

Summary

EDIT-103 is a **one-time, high efficacy, mutation-agnostic gene medicine** to permanently suppress the toxic gain-of-function associated with RHO-adRP

Ex vivo: EDIT-103 shows high specificity in human retinal explants

In vivo:

- $mRho^{hRHO/+}$ mouse:
    - EDIT-103 achieved **rapid** and **stable** gene editing:
        - Editing plateau at 6 weeks and is sustained until end of study (13 weeks)
        - > 25% gene editing at doses ≥ 3E12 vg/ml
- NHP:
    - EDIT-103 achieved nearly **100%** editing
    - **>30%** RHO replacement protein levels
    - Morphological and functional photoreceptor preservation

NHP: non-human primate; RHO: rhodopsin.

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* Alphabetical order according to last names

Thank you