



A Mutation-Independent CRISPR/Cas9-Based 'Knockout and Replace' Strategy to Treat Rhodopsin-Associated Autosomal Dominant Retinitis Pigmentosa (RHO-adRP)

*Chi-Hsiu Liu, Pavlina Wolf, Ruhong Dong, Yan Huang, Diana Tabbaa, Eugenio Marco, Brian Duke, Andrea Pinilla, Asha Pant, Racheal D'Souza, Judith Newmark, Georgia Giannoukos, Kate Zhang, Adrian Timmers, Mark S. Shearman and **Mariacarmela Allocca***

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Disclosure

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

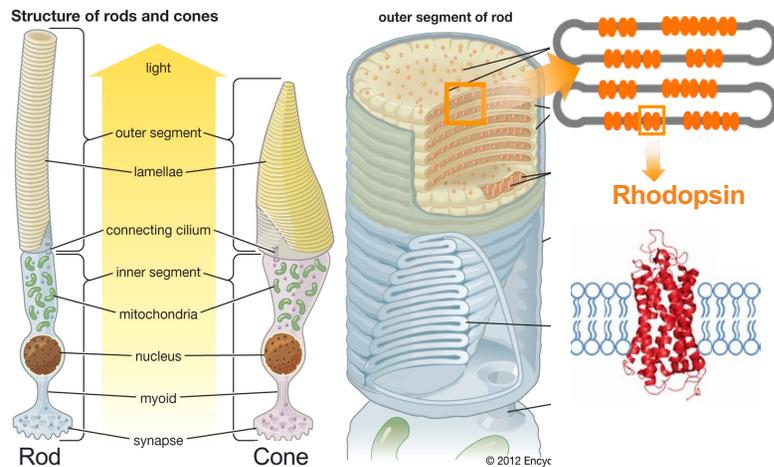


Rhodopsin-Associated adRP (RHO-adRP)

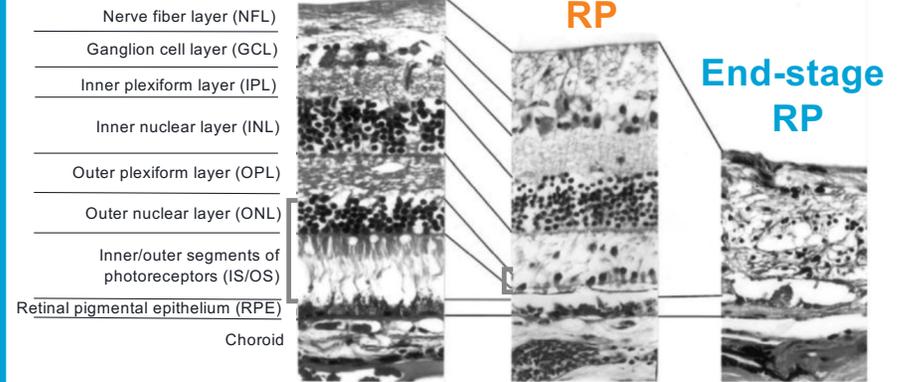
Rhodopsin (RHO)

- 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-adRP¹
- Dominant mutations in the *RHO* gene are toxic for the rods: progressive loss of rods followed by loss of cones

Photoreceptor structure



Normal



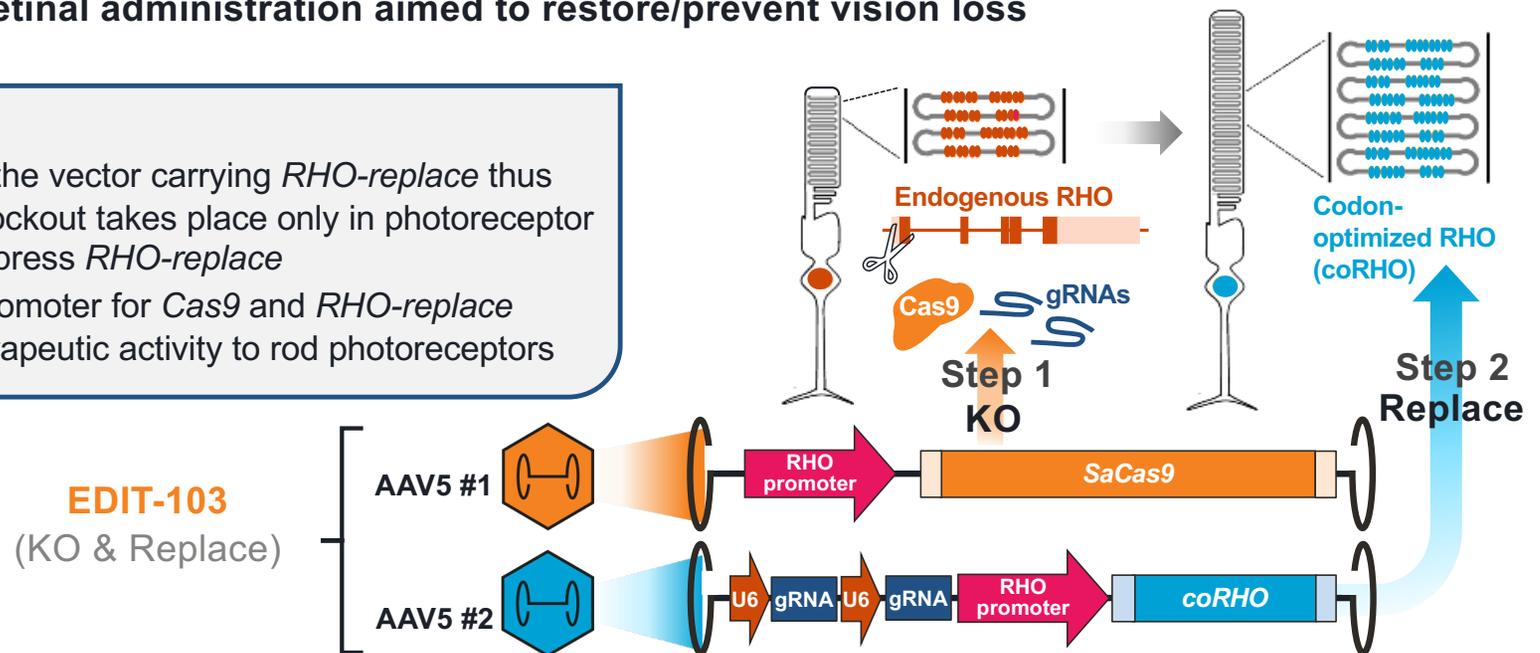
Valle D. et al. OMMBID 2014

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

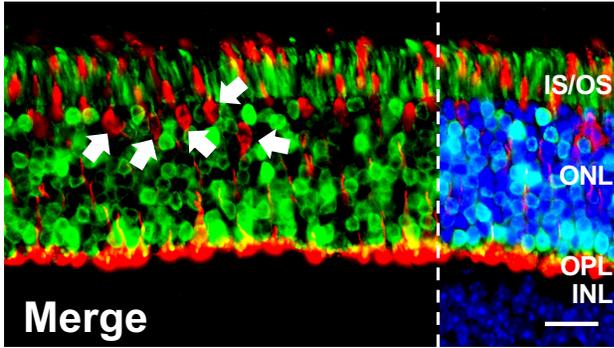
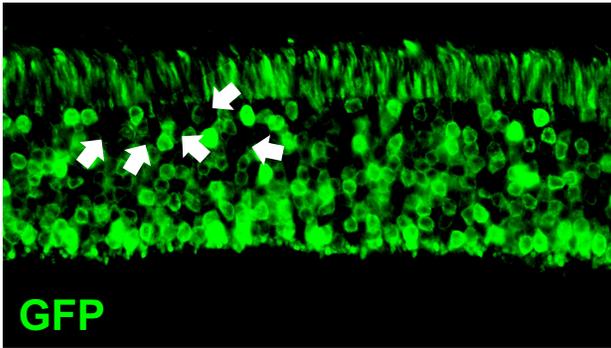
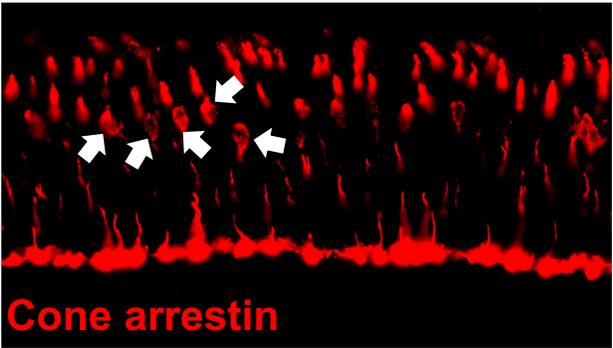
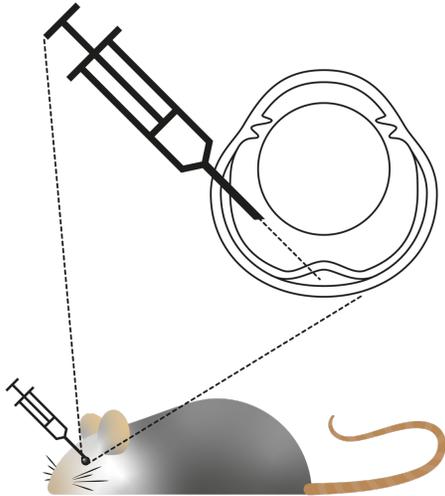
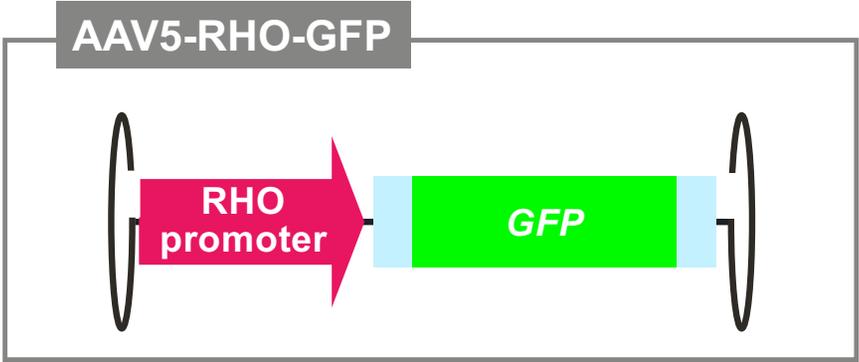
- Agnostic to any RHO mutation – thus will knockout any dominant gain-of-function rhodopsin mutant
- **Step 1:** Both mutant and normal endogenous *RHO* will be knocked out in the treated area
- **Step 2:** Exogenous normal *RHO* (resistant to editing) will replace endogenous *RHO*
- **One-time subretinal 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



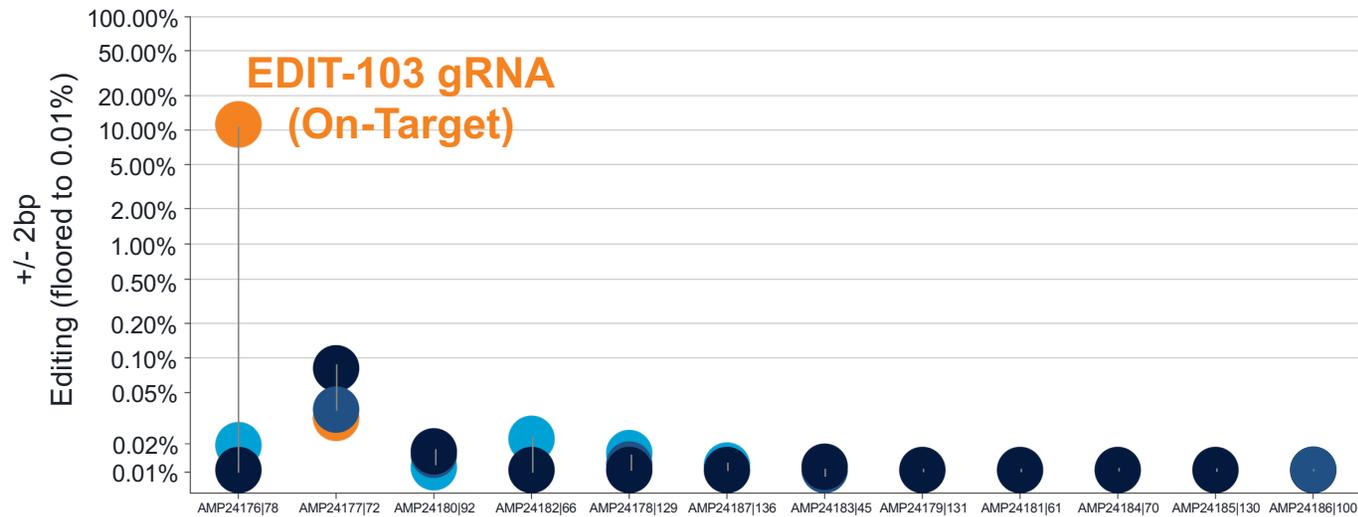
The RHO Promoter Restricts Gene Expression to Rod Photoreceptors in the Mouse Eyes



Scale bar = 20 μ m

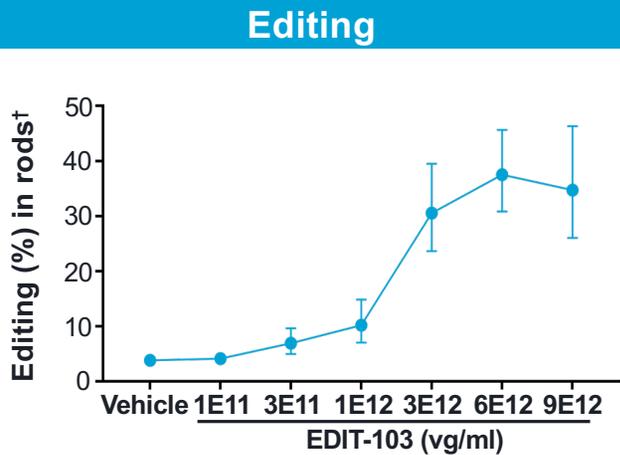
EDIT-103 is Highly Specific: No Detectable Off-Target Editing

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

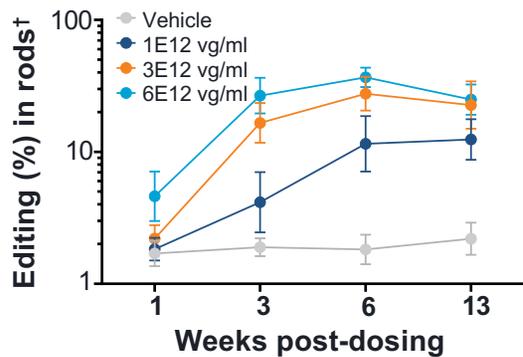


EDIT-103 in Humanized $mRho^{hRHO/+}$ Mice: Demonstrates Rapid and Stable Gene Editing

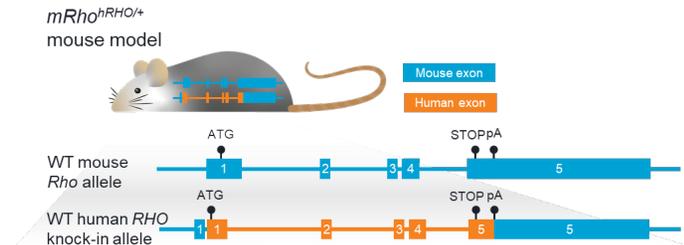
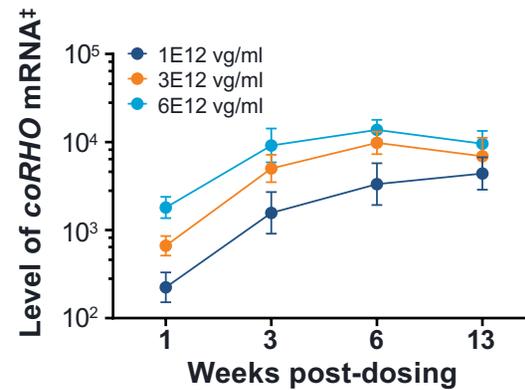
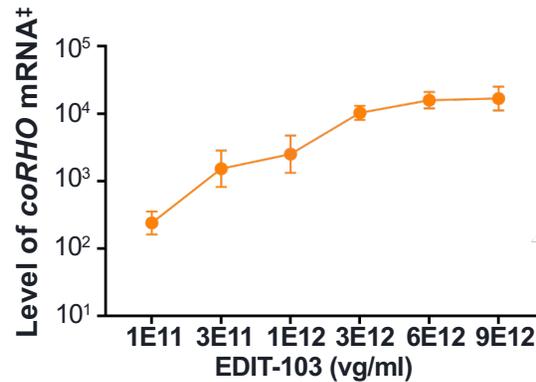
Dose escalation



Time course



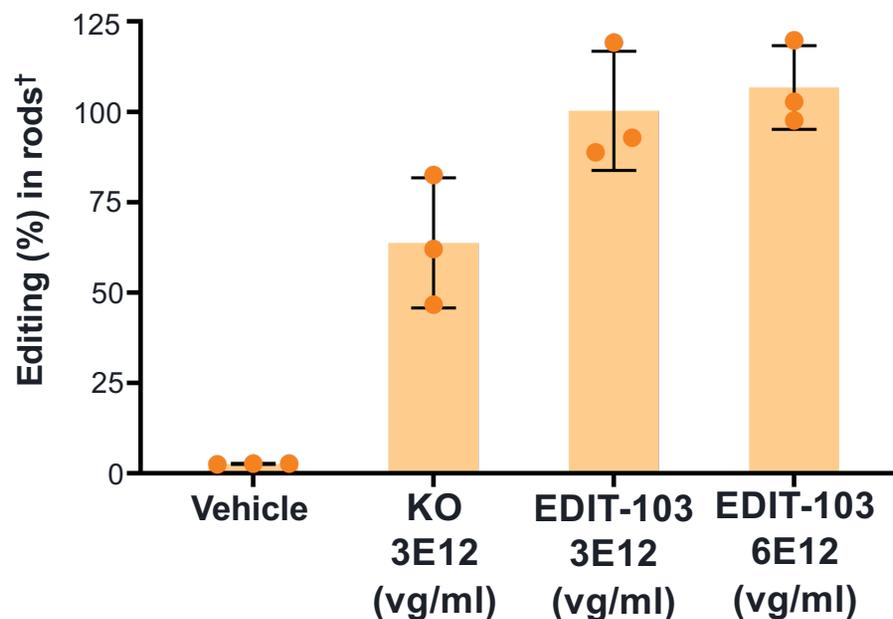
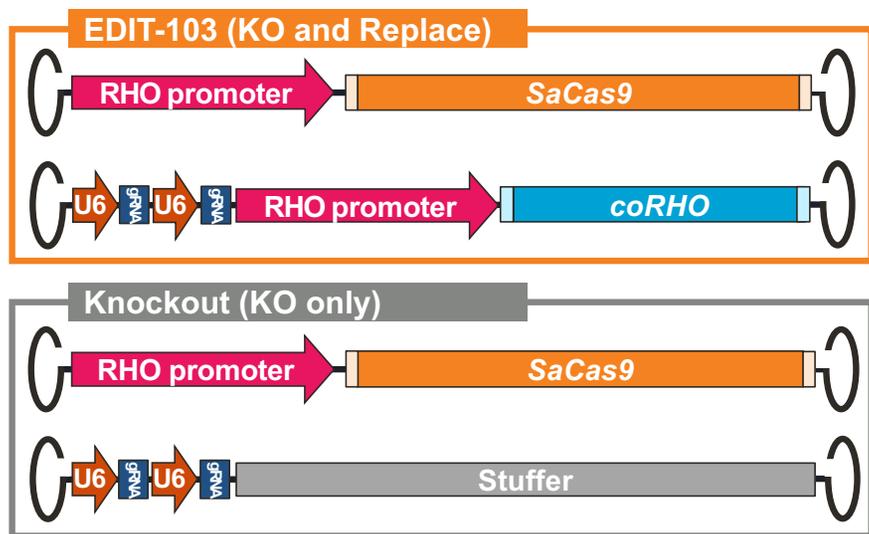
$hRHO$ mRNA replacement



- >25% of rods are edited
- Editing rates increase in a dose-dependent manner
- Editing plateau at ~6 weeks post-injection at doses $\geq 3E12$ vg/ml

AAV ratio: 1:1
 Volume: 1 μ l
 Geometric mean with 95% confidence interval is presented.
 †Within the transduced area.
 ‡Nanostring counts normalized to HKG.

EDIT-103 in Non-Human Primates (NHPs): Approximately 100% Editing in Transduced Photoreceptors



Volume: 100 μ l; AAV ratio: 1:1; Time point: 13 weeks; Mean (\pm SD) is presented; * p <0.05, *** p <0.001; [†]Within the transduced area.

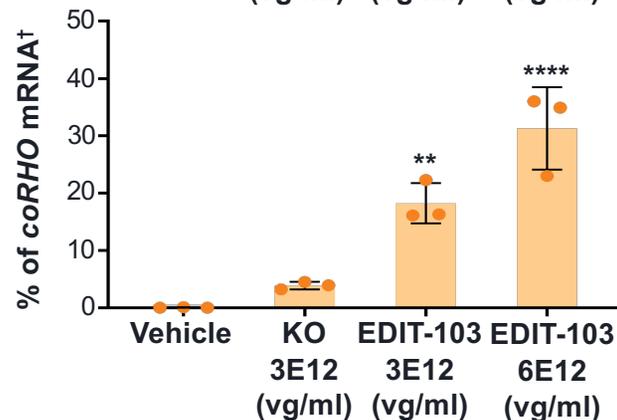
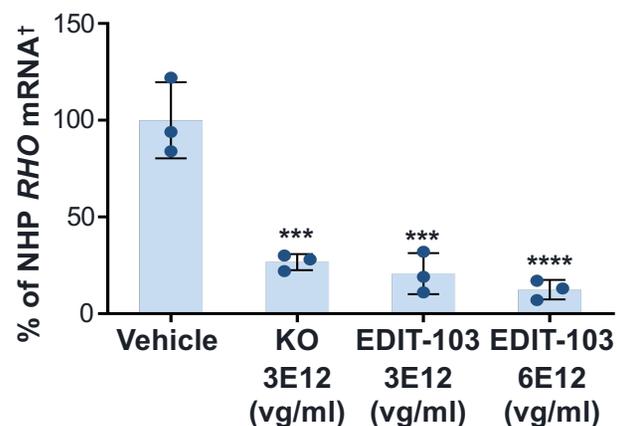
~100% editing (within the transduced area) in NHP

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

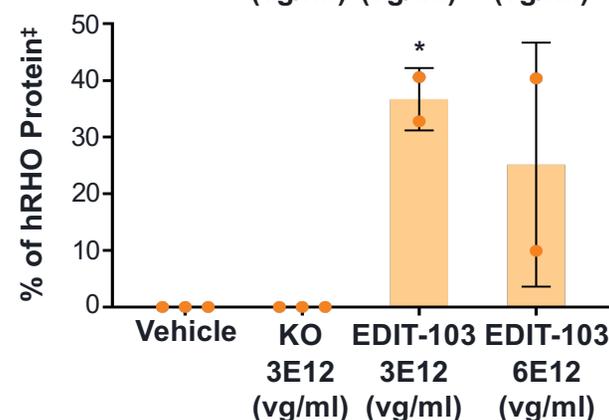
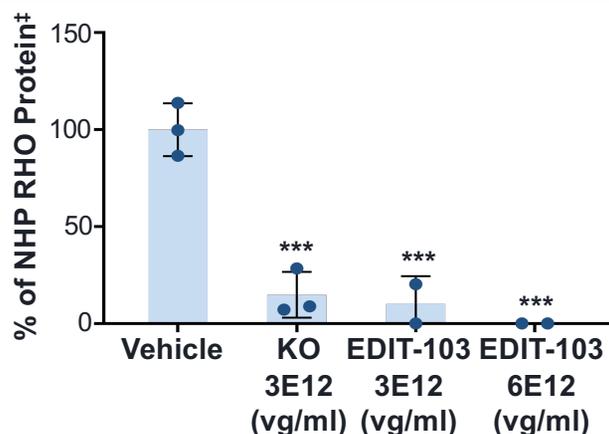
Endogenous NHP RHO

Human RHO Replacement

RHO mRNA



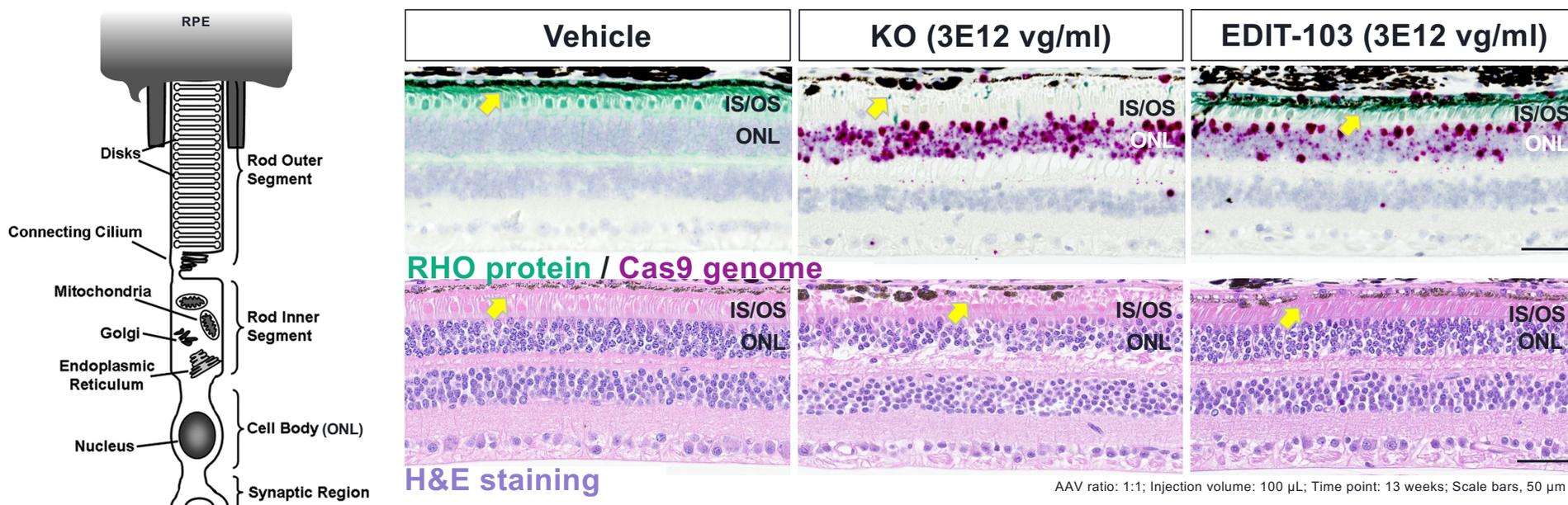
RHO Protein



- 80% and 90% knockdown of endogenous NHP RHO mRNA levels achieved at 3E12 and 6E12 vg/ml doses, resulting in 90% and 100% of RHO protein knockdown, respectively
- RHO replacement mRNA levels increased with dose and resulted in >30% of RHO protein levels

Volume: 100 μ l
 AAV ratio: 1:1
 Time point: 13 weeks
 Mean (\pm SD) is presented.
 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$
[†]Compared to NHP RHO levels in vehicle.
[‡]Compared to NHP RHO in vehicle.

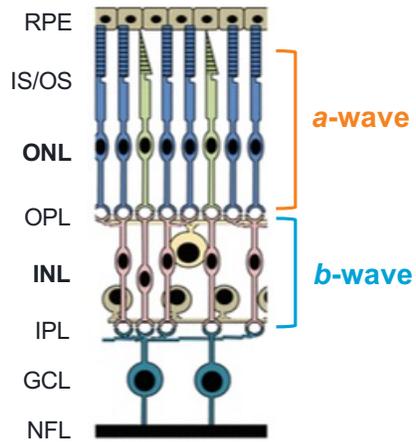
RHO Protein Expression and Retina Morphology was Preserved in EDIT-103-Treated NHP Retinas Compared with KO-Treated Retinas



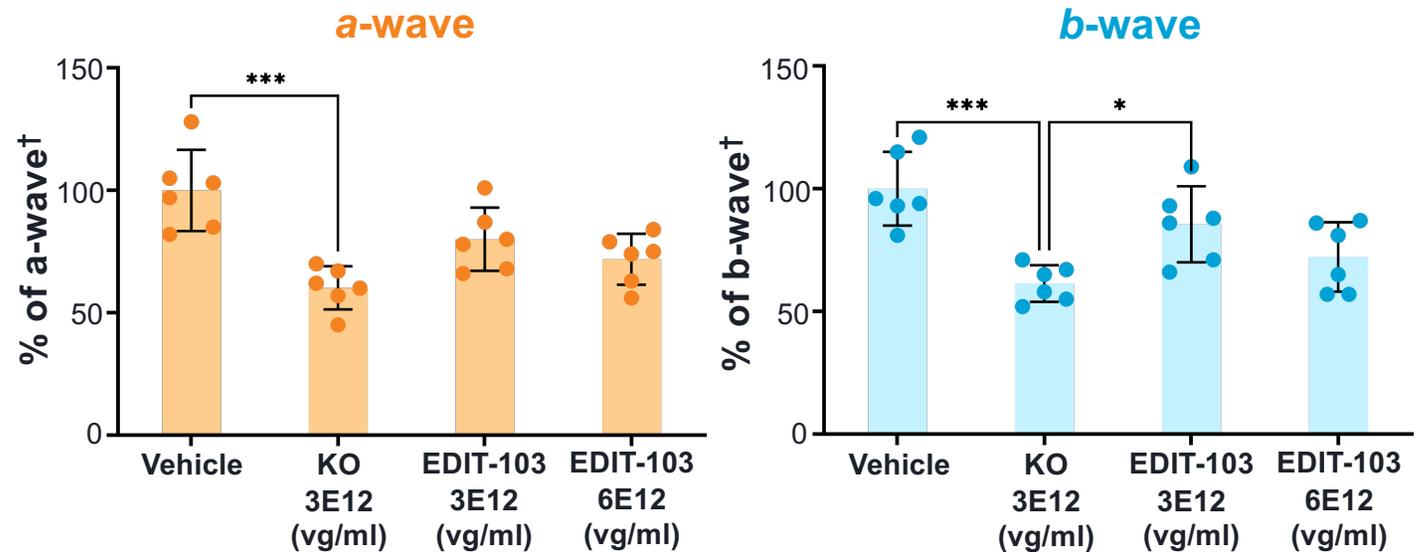
- AAV transduction in the treated groups reveals positive **Cas9 genome** staining
- Nearly absent **RHO protein** and lack of OS (yellow arrow) in the KO group
- Preservation of **RHO protein**, improved photoreceptor organization, and improved IS/OS morphology in the EDIT-103-treated groups

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

Retinal layers



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



AAV ratio: 1:1; Injection volume: 100 μ L; Time point: 13 weeks; Mean (\pm SD) is presented; * p <0.05, *** p <0.001; †Compared to vehicle.

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

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:
 - plateau at 6 weeks and is sustained until end of study (13 weeks)
 - increase in a dose-dependent manner
 - > 25% gene editing at doses $\geq 3E12$ vg/ml
- NHP:
 - EDIT-103 achieved nearly **100%** editing
 - **>30%** RHO replacement protein levels
 - Morphological and functional photoreceptor preservation

Acknowledgements

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* Alphabetical order according to last names

Thank you

