



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

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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

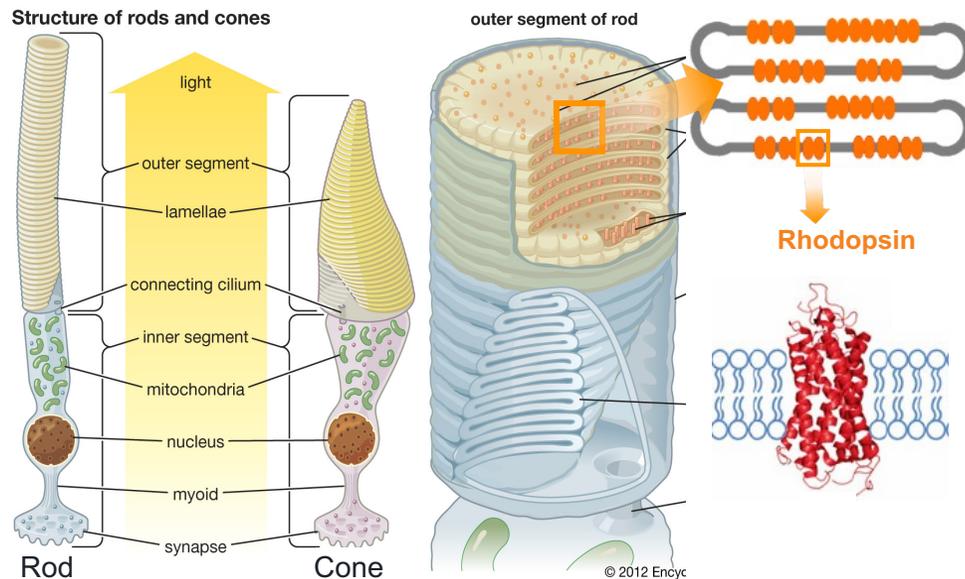


# Rhodopsin-Associated adRP (RHO-adRP)

## Rhodopsin (RHO)

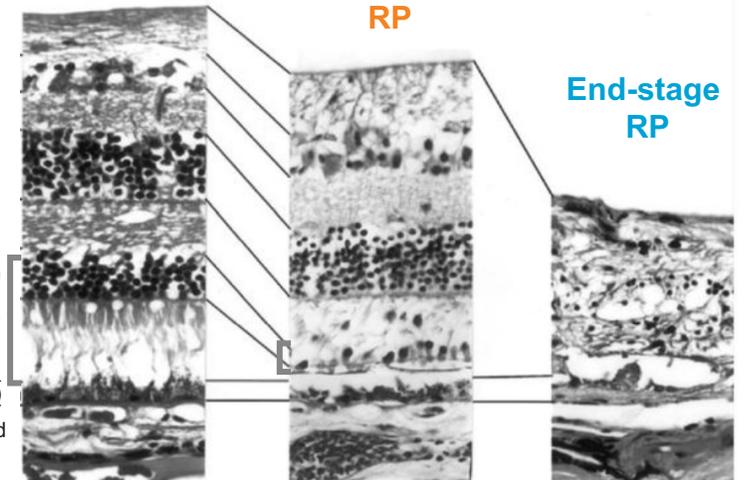
- A light-sensitive receptor protein involved in visual phototransduction in rods
- Located in the outer segments of rods
- Approximately 30% (US and UK) of adRP caused by *RHO* dominant mutations
- Prevalence: 7,500 patients in US and 12,100 patients in EU and UK
- >150 mutations identified in the *RHO*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

## Photoreceptor structure



## Normal

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



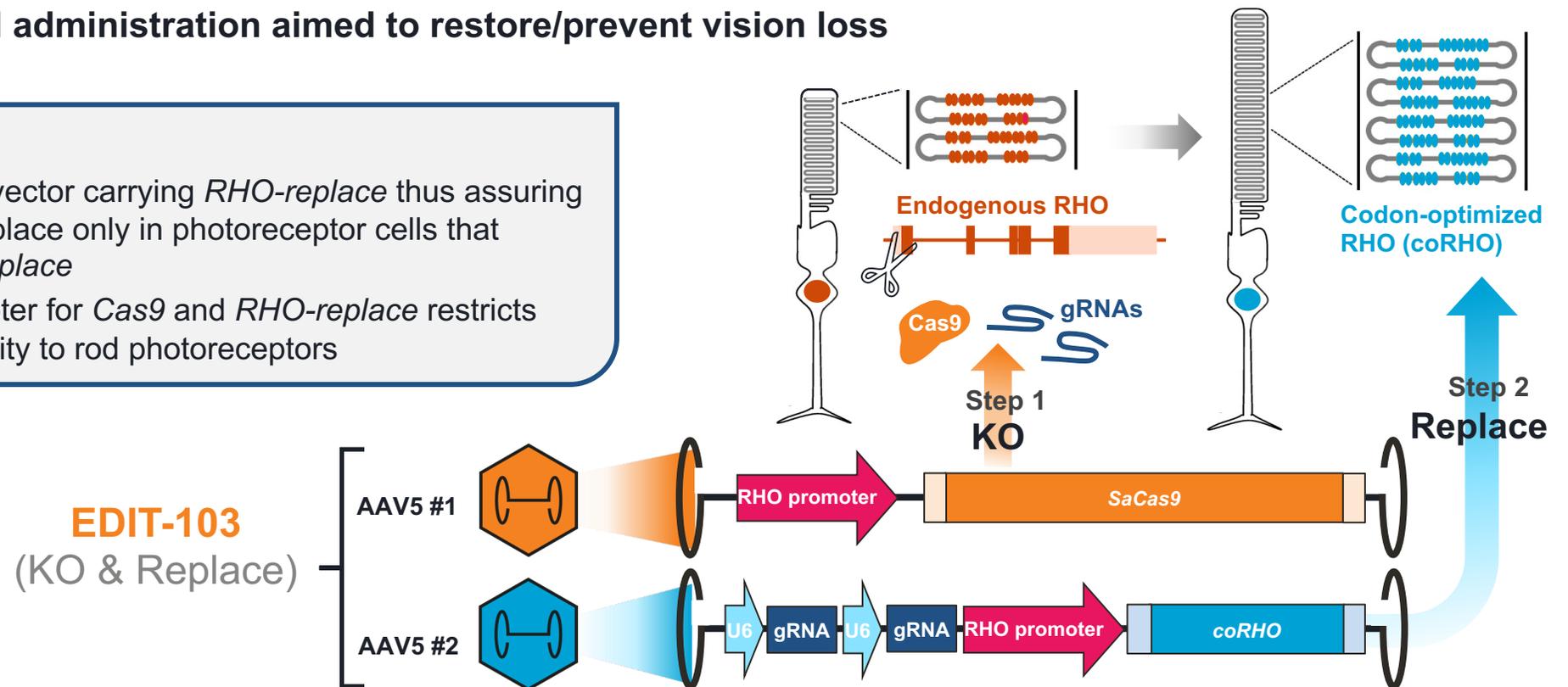
Valle D. et al. OMMBID 2014

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

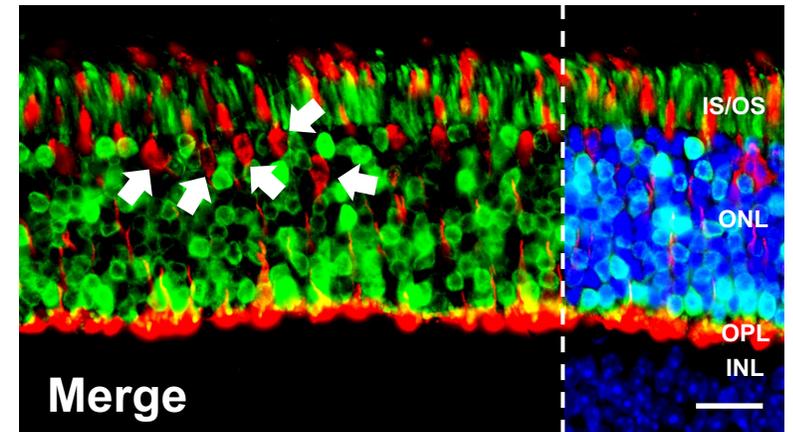
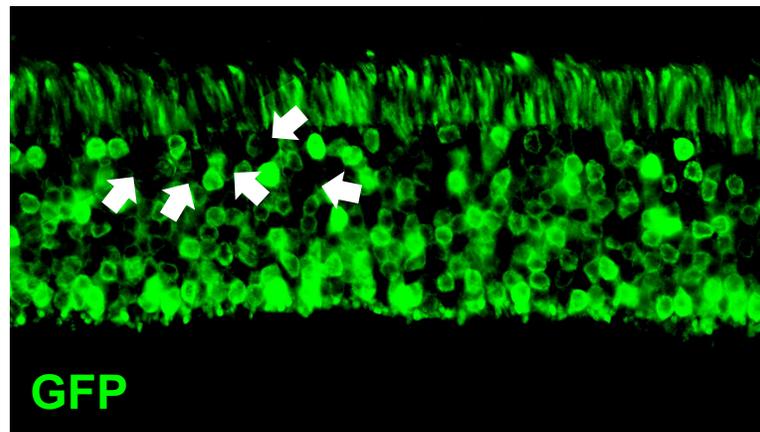
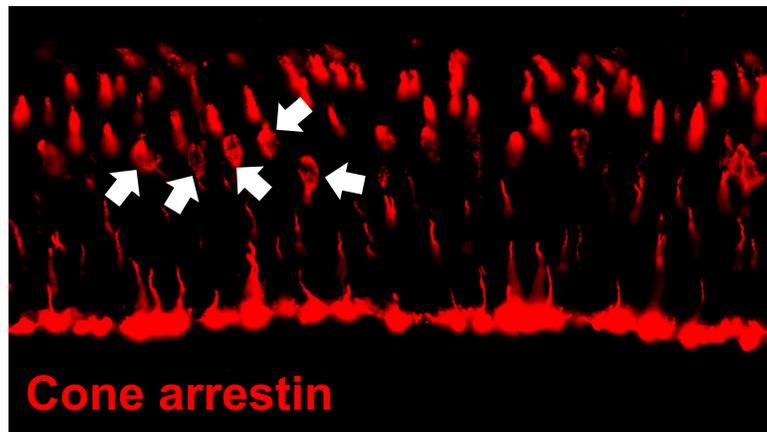
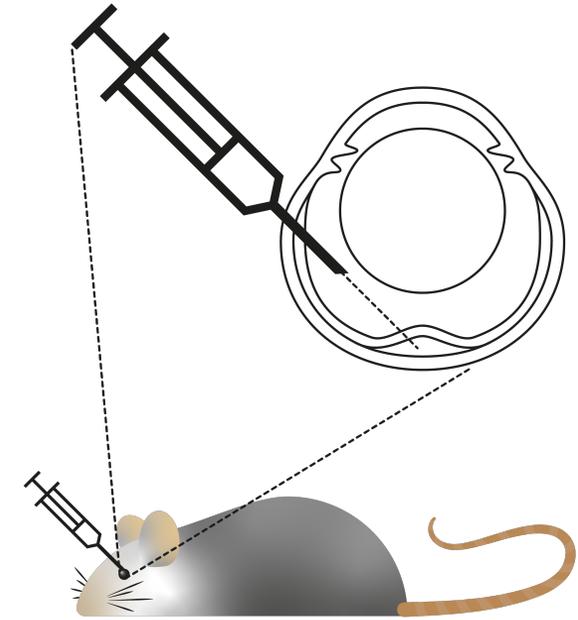
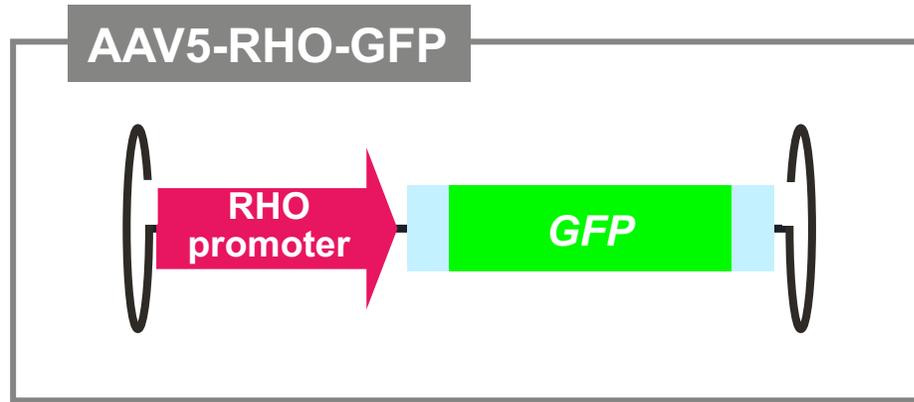
- Agnostic to any RHO mutation – thus will knockout any dominant gain-of-function rhodopsin mutant
- **Step 1:** Both mutant and normal endogenous *RHO* will be knocked out in the treated area
- **Step 2:** Exogenous normal *RHO* (resistant to editing) will replace endogenous *RHO*
- **One-time subretinal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



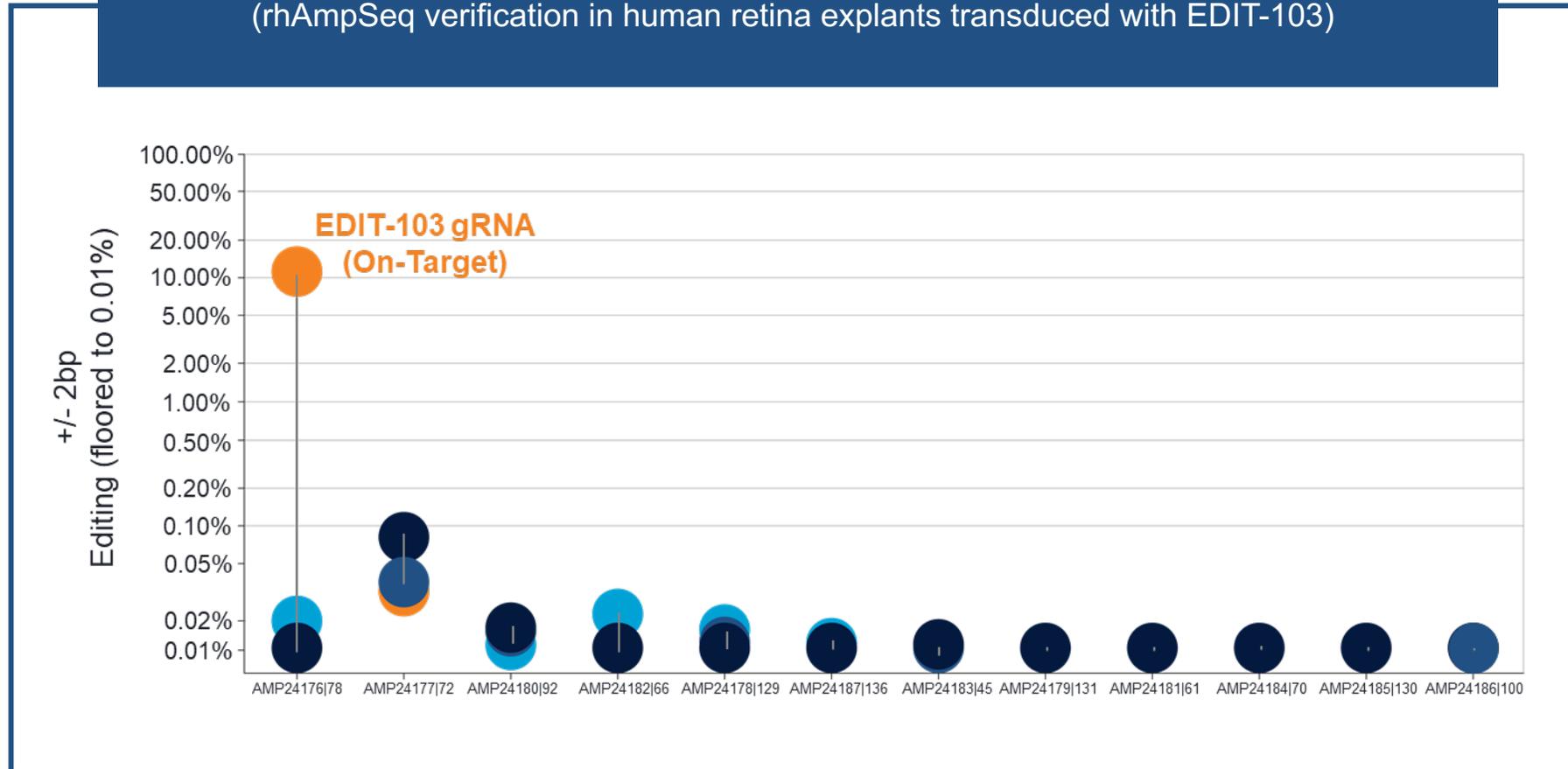
# The RHO Promoter Restricts Gene Expression to Rod Photoreceptors in the Mouse Eyes



Scale bar = 20  $\mu$ m

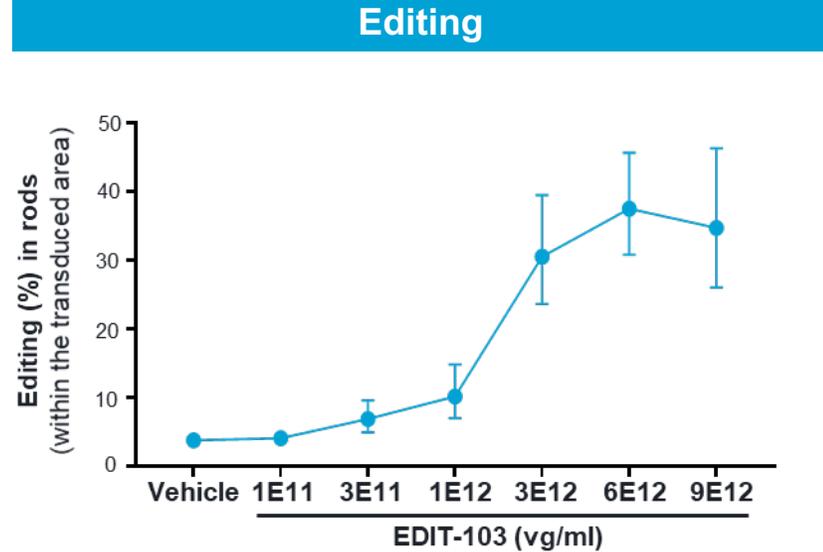
# EDIT-103 is Highly Specific: No Detectable Off-Target Editing

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

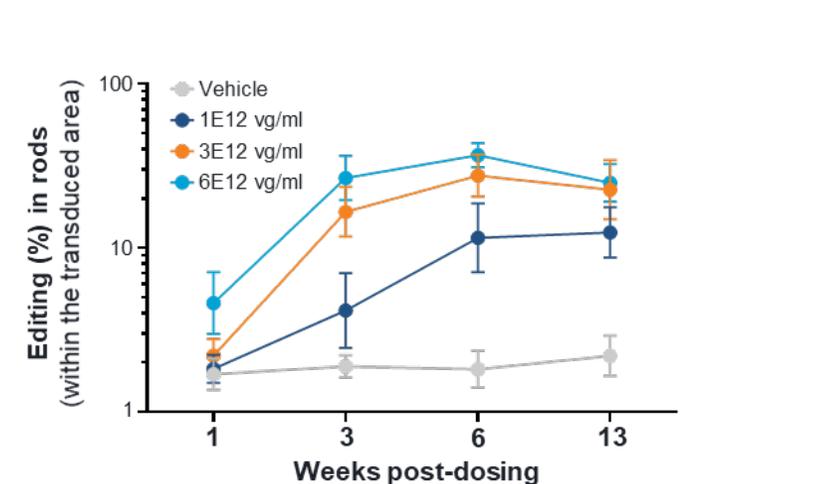


# EDIT-103 in Humanized $mRho^{hRHO/+}$ Mice: Demonstrates Rapid and Stable Gene Editing

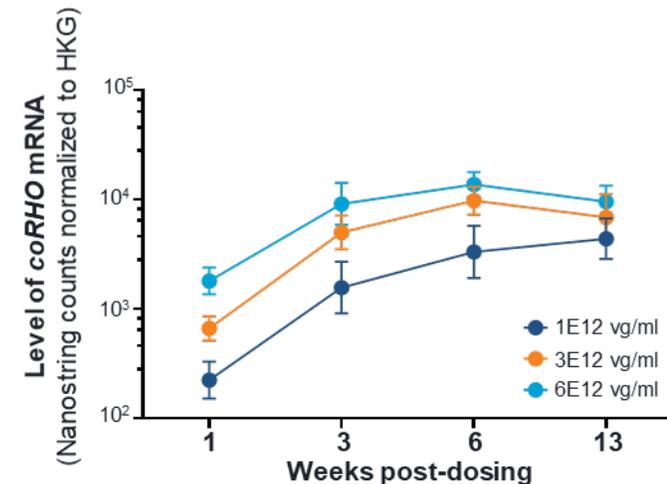
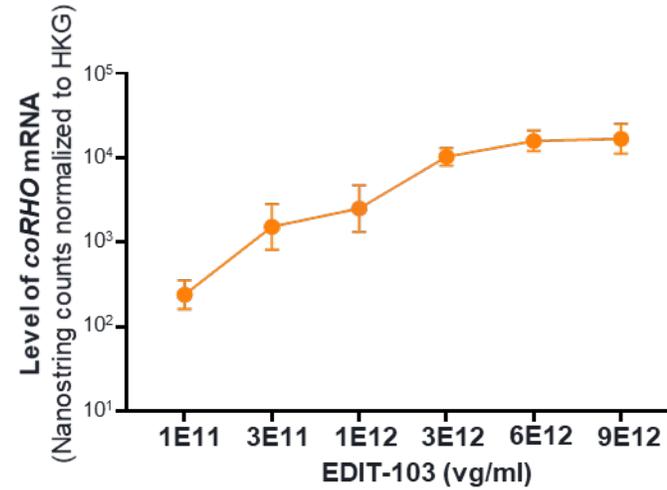
Dose escalation



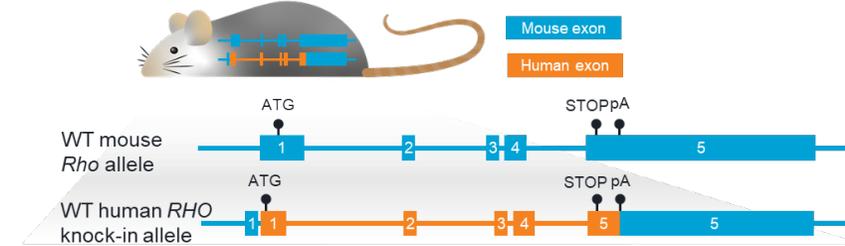
Time course



$hRHO$  mRNA replacement



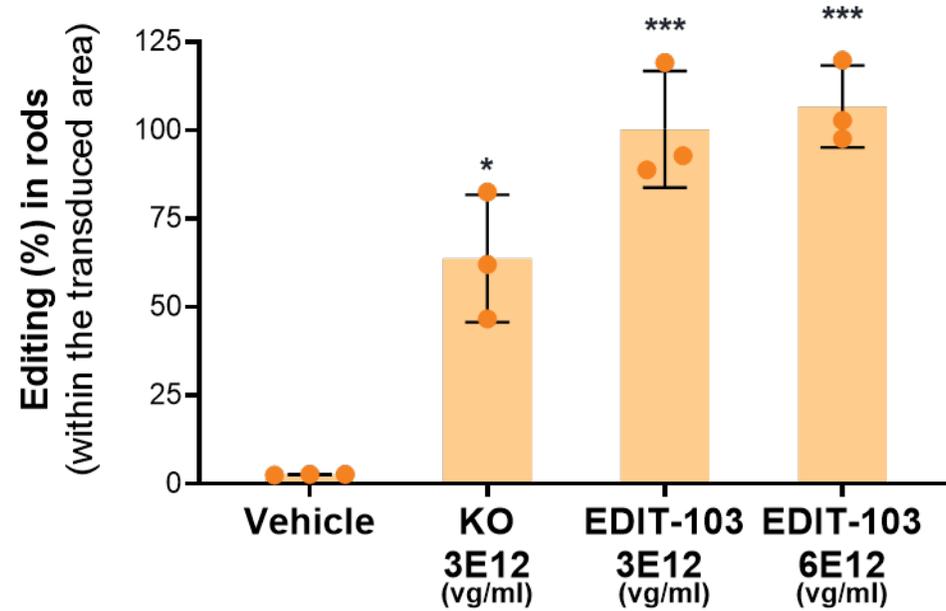
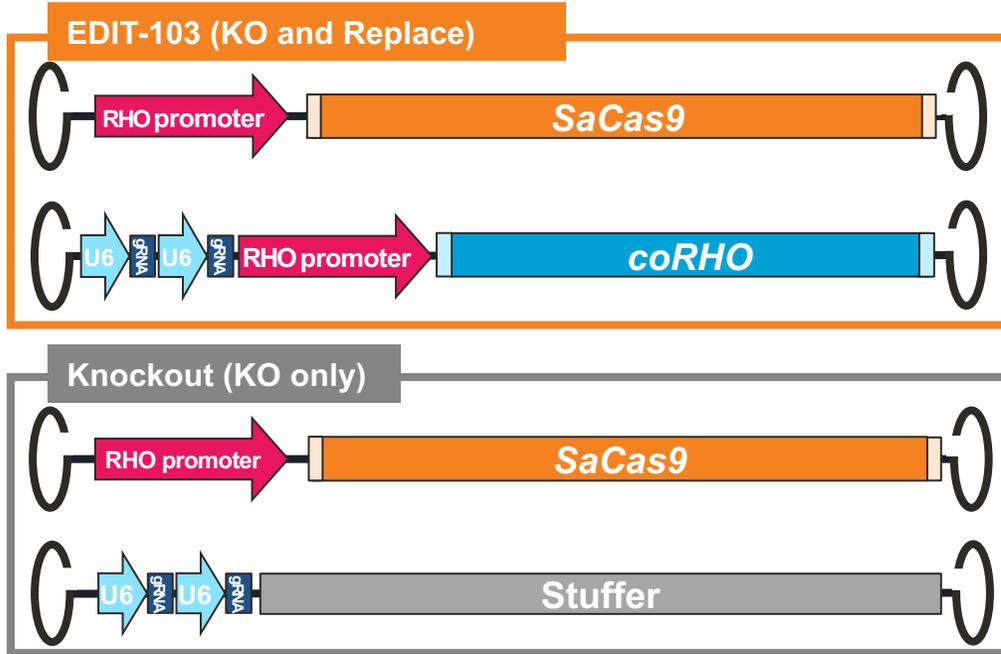
$mRho^{hRHO/+}$  mouse model



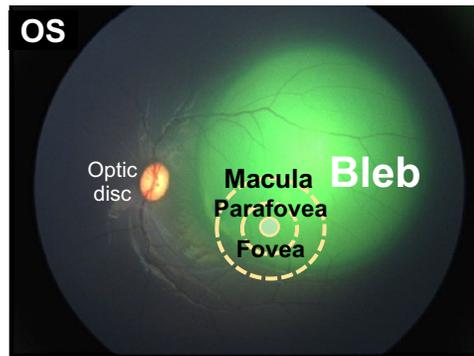
- >25% of rods are edited
- Editing rates increase in a dose-dependent manner
- Editing plateau at ~6 weeks post-injection at doses  $\geq 3E12$  vg/ml

AAV ratio: 1:1  
Volume: 1  $\mu$ l  
Geometric mean with  
95% confidence interval is presented

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



Volume: 100  $\mu$ l  
AAV ratio: 1:1  
Time point: 13 weeks  
Mean ( $\pm$ SD) is presented  
\* $p$ <0.05, \*\*\* $p$ <0.001



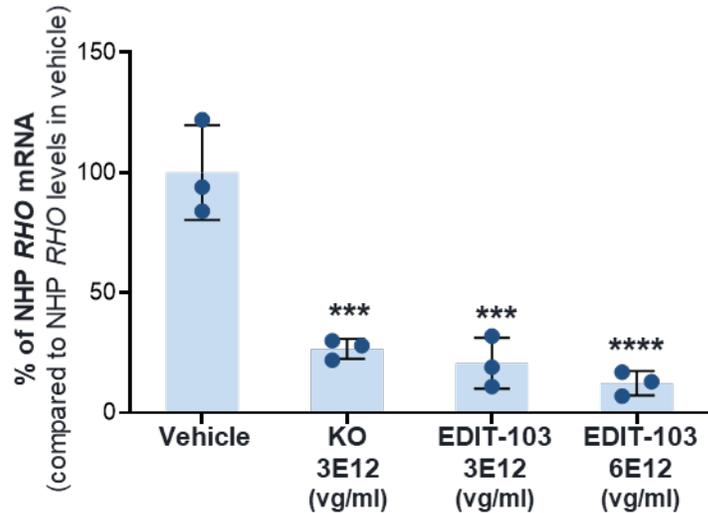
~100% editing (within the transduced area) in NHP

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

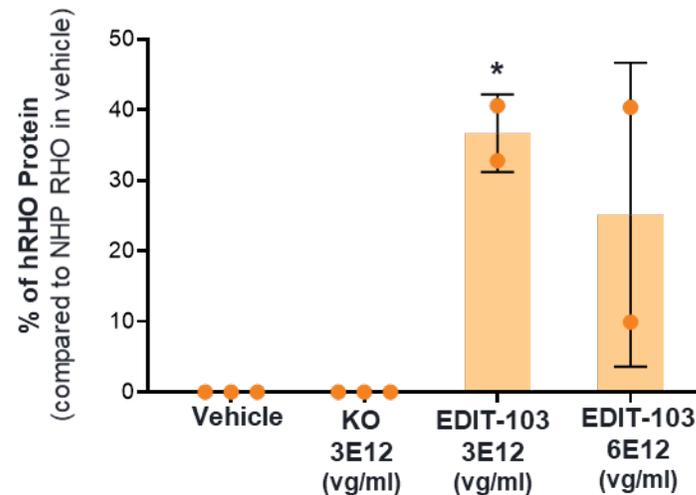
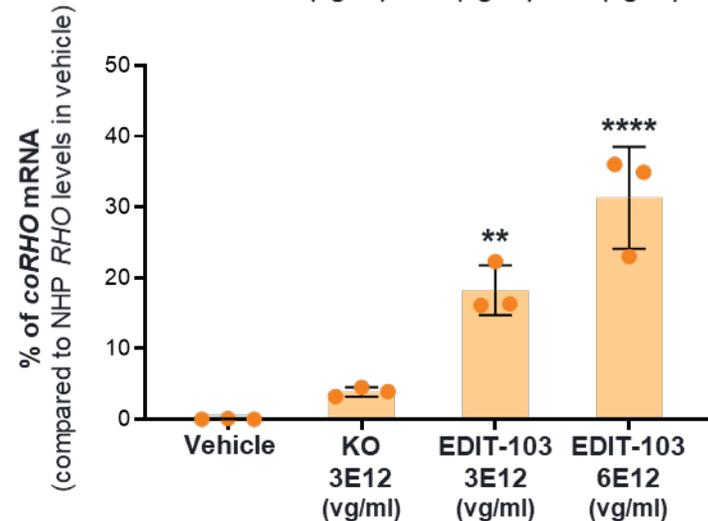
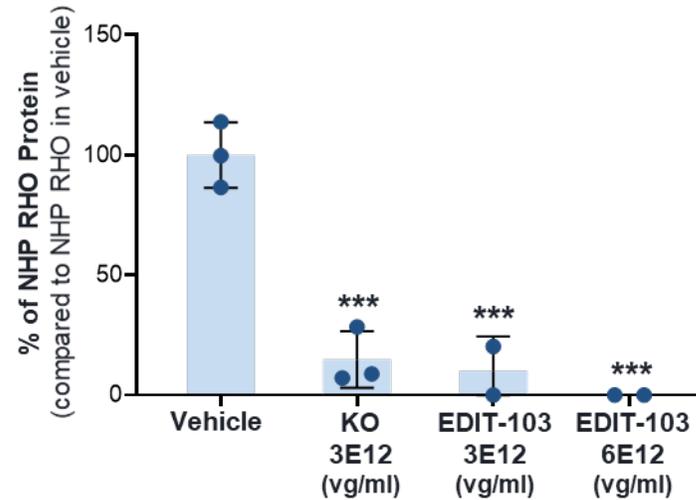
Endogenous NHP RHO

Human RHO Replacement

RHO mRNA



RHO Protein

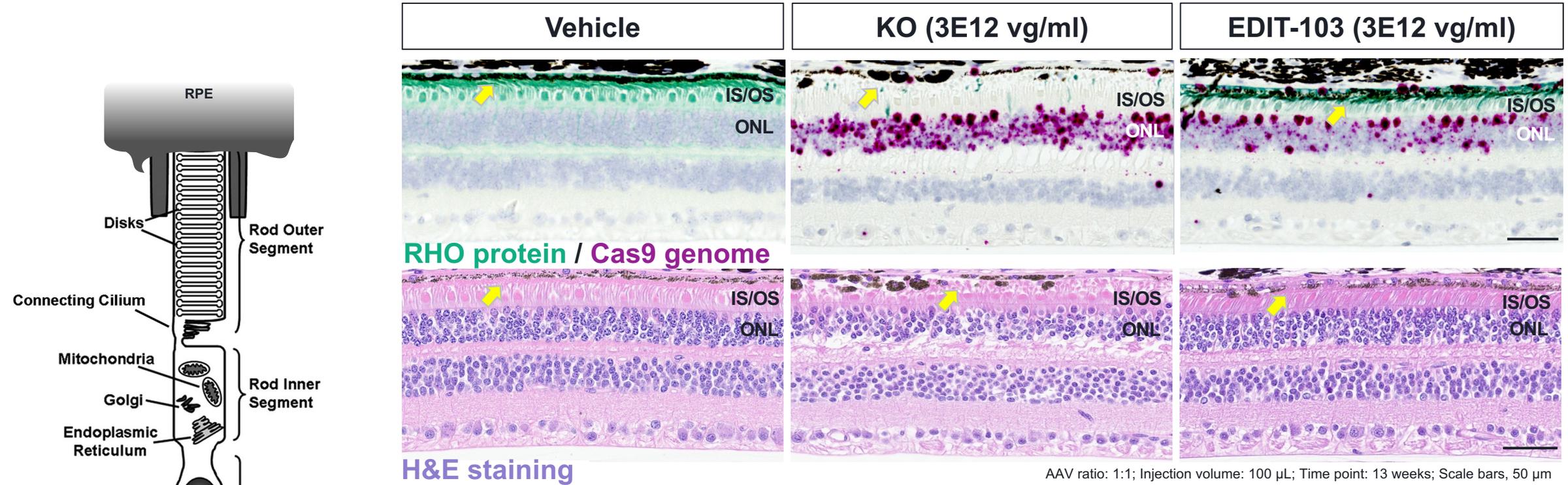


- 80% and 90% knockdown of endogenous NHP RHO mRNA levels were achieved at doses of 3E12 and 6E12 vg/ml, respectively. This resulted in 90% and 100% of RHO protein knockdown.
- RHO replacement mRNA levels increased with dose and resulted in >30% of RHO protein levels.

Volume: 100  $\mu$ 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

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

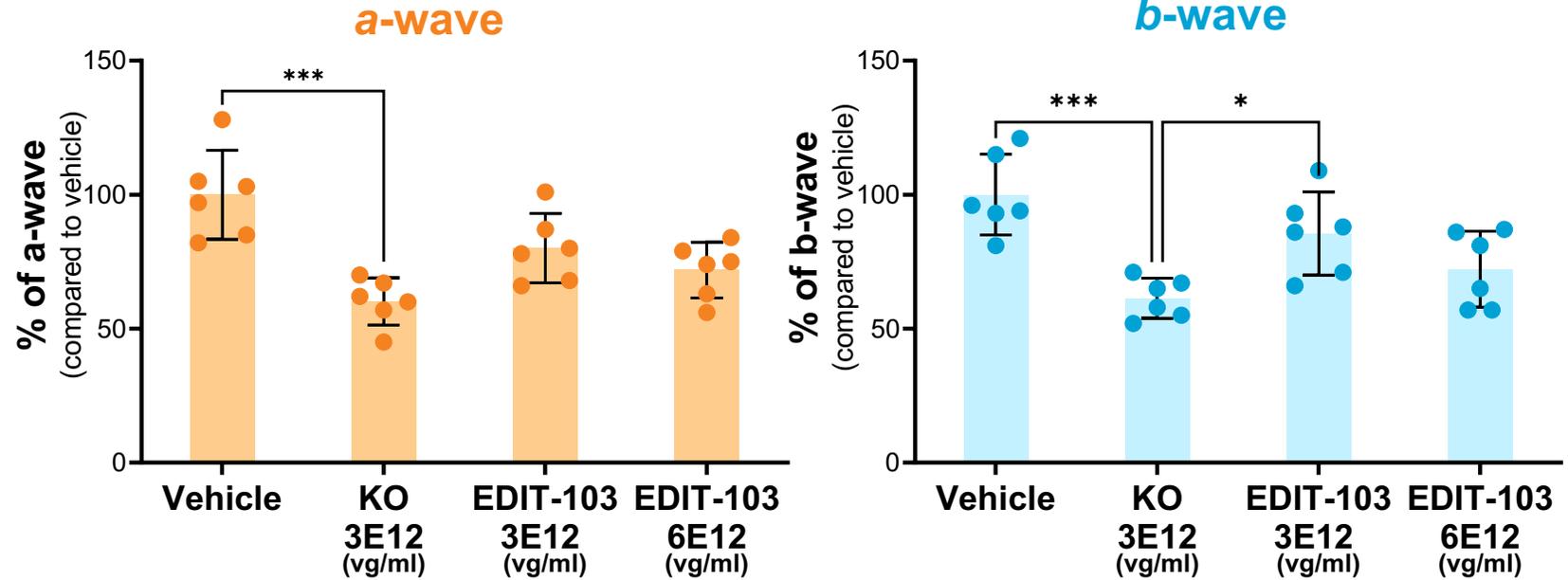
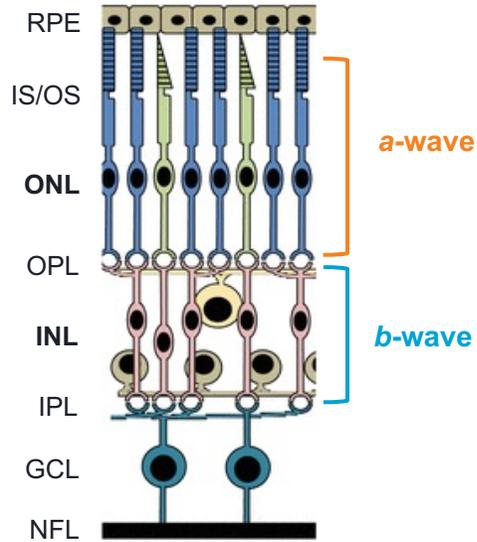
# RHO Protein Expression and Retina Morphology was Preserved in EDIT-103-Treated NHP Retinas Compared with KO-Treated Retinas



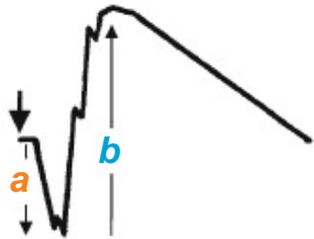
- AAV transduction in the treated groups reveals positive **Cas9 genome** staining
- Nearly absent **RHO protein** and lack of OS (yellow arrow) in the KO group
- Preservation of **RHO protein**, improved photoreceptor organization, and improved IS/OS morphology in the EDIT-103-treated groups

# 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  $\mu$ L; Time point: 13 weeks; Mean ( $\pm$ 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

# 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*<sup>hRHO/+</sup> 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  $\geq 3E12$  vg/ml
- NHP:
  - EDIT-103 achieved nearly **100%** editing
  - **>30%** RHO replacement protein levels
  - Morphological and functional photoreceptor preservation

# Acknowledgements

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Kate Zhang

\* Alphabetical order according to last names

Thank you

