



The development of CRISPR-based medicines for the treatment of ocular diseases

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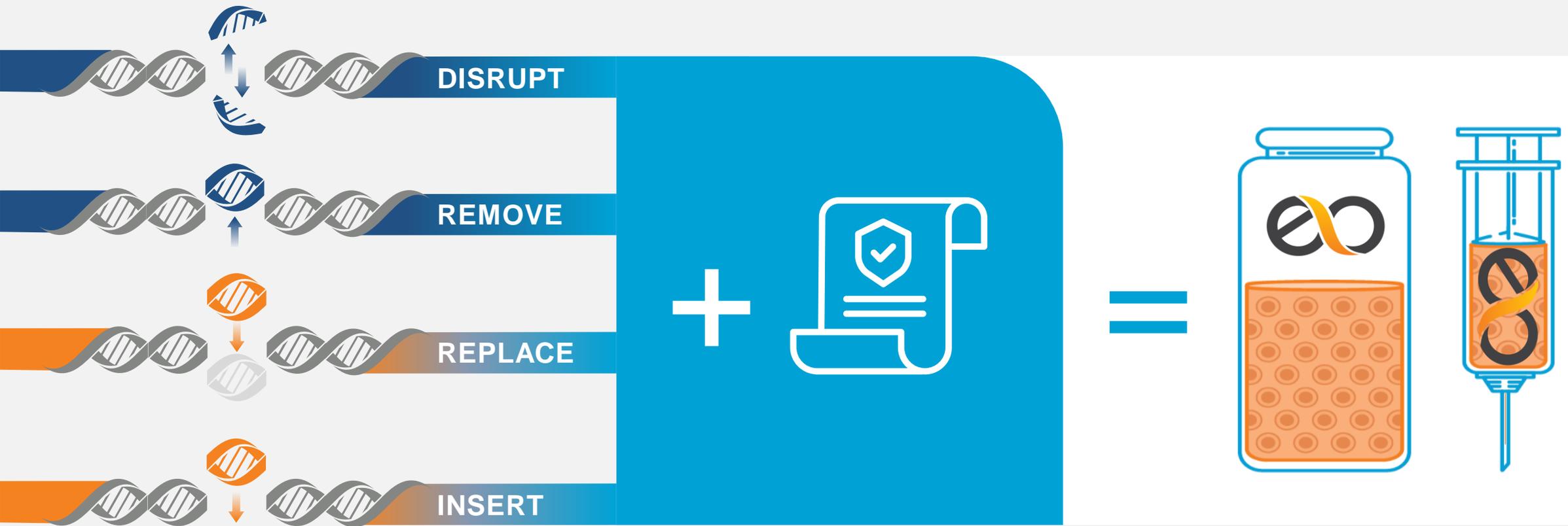
ASGCT 2021

Moving Genome Editing to the Clinic: From Technology to Therapeutics

May 10, 2021



Editas Medicine's powerful engine



Differentiated platform:
the *only* company with multiple
proprietary CRISPR editing systems

Unparalleled IP:
broadest and deepest
CRISPR IP portfolio

**Ability to develop widest range
of transformational genomic
medicines for serious diseases**

Preclinical development of gene editing experimental medicine EDIT-101



Introduction: Leber congenital amaurosis type 10 (LCA10) disease



Guide RNA selection: Editing with lead guide RNA combination



Specificity assessment: On-target and off-target editing in relevant tissues



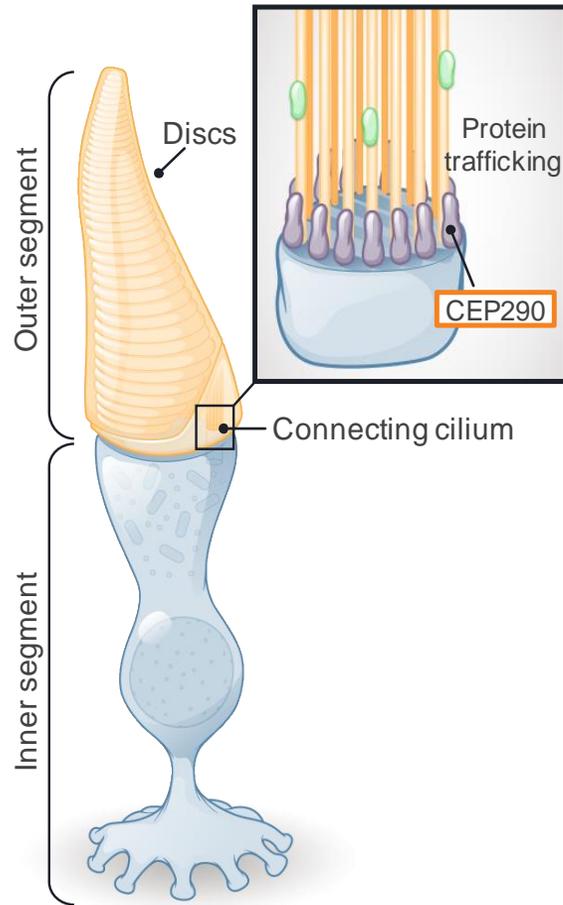
Safety and tolerability: Mouse and NHP study to evaluate efficacy, safety, and tolerability of EDIT-101



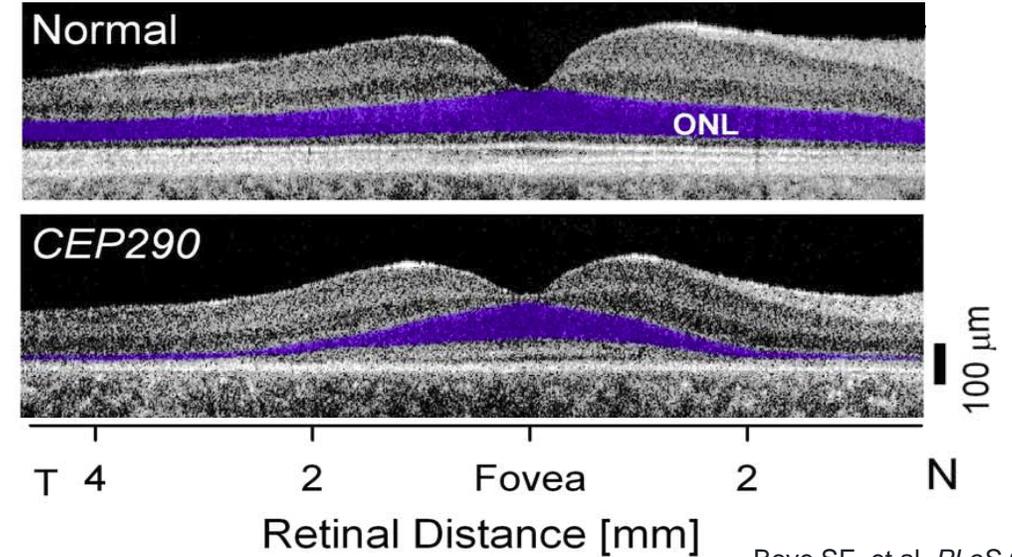
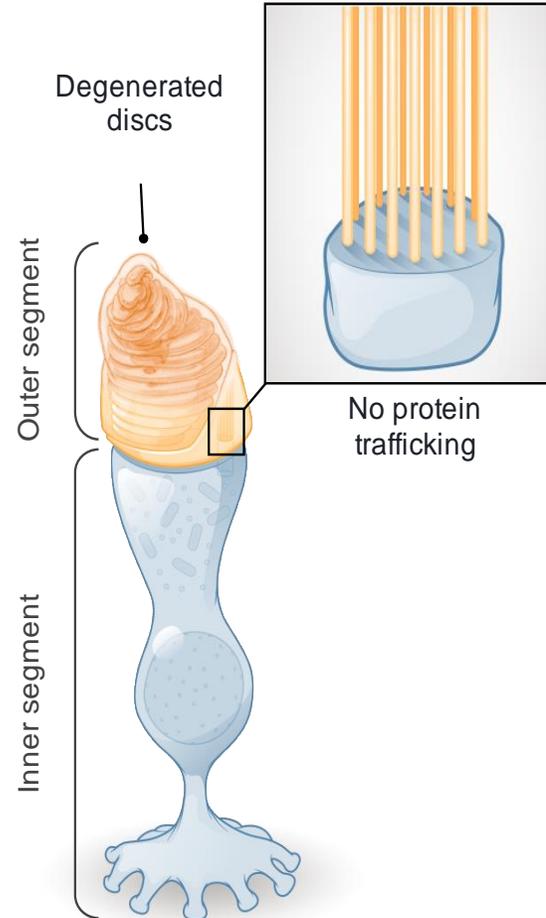
Conclusion: Clinical development of EDIT-101

LCA10 is caused by loss-of-function mutations in the CEP290 protein

Healthy photoreceptor



LCA10 photoreceptor

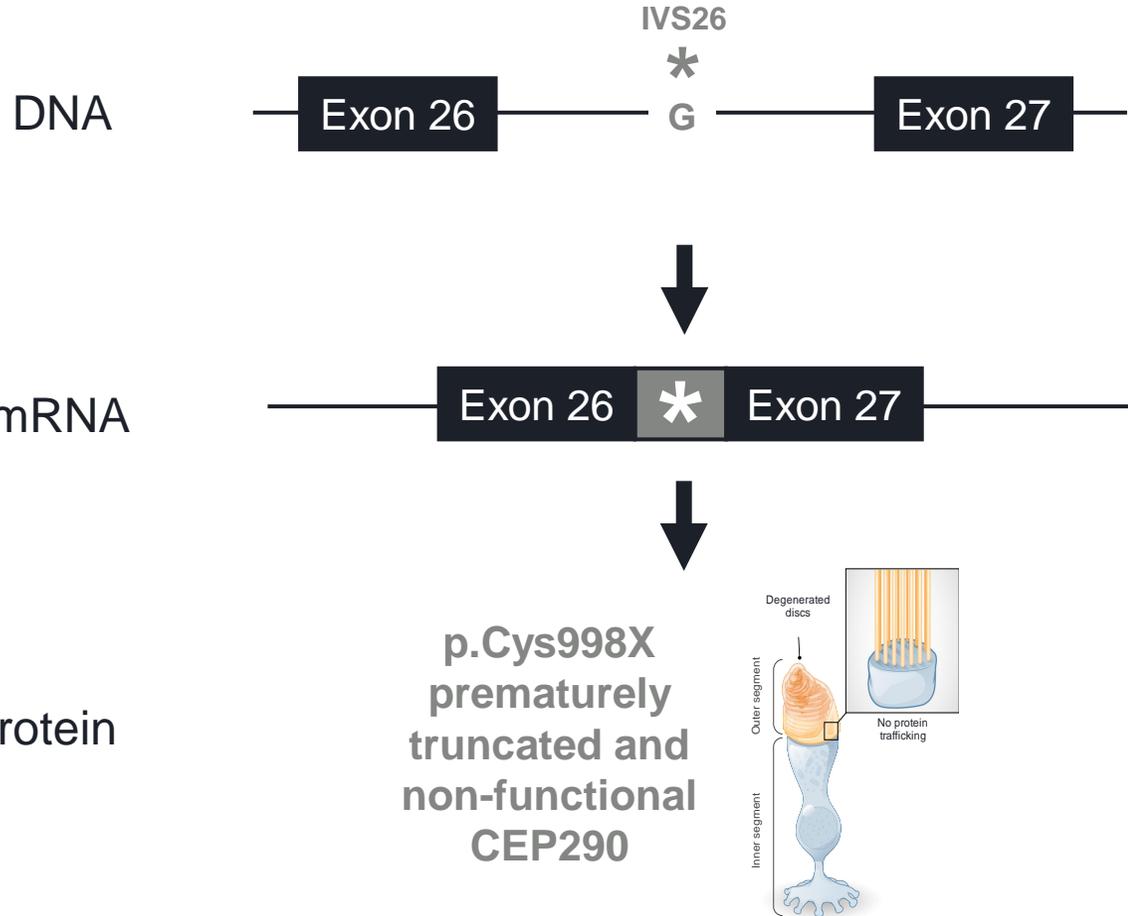


Boye SE, et al. *PLoS One* 2014

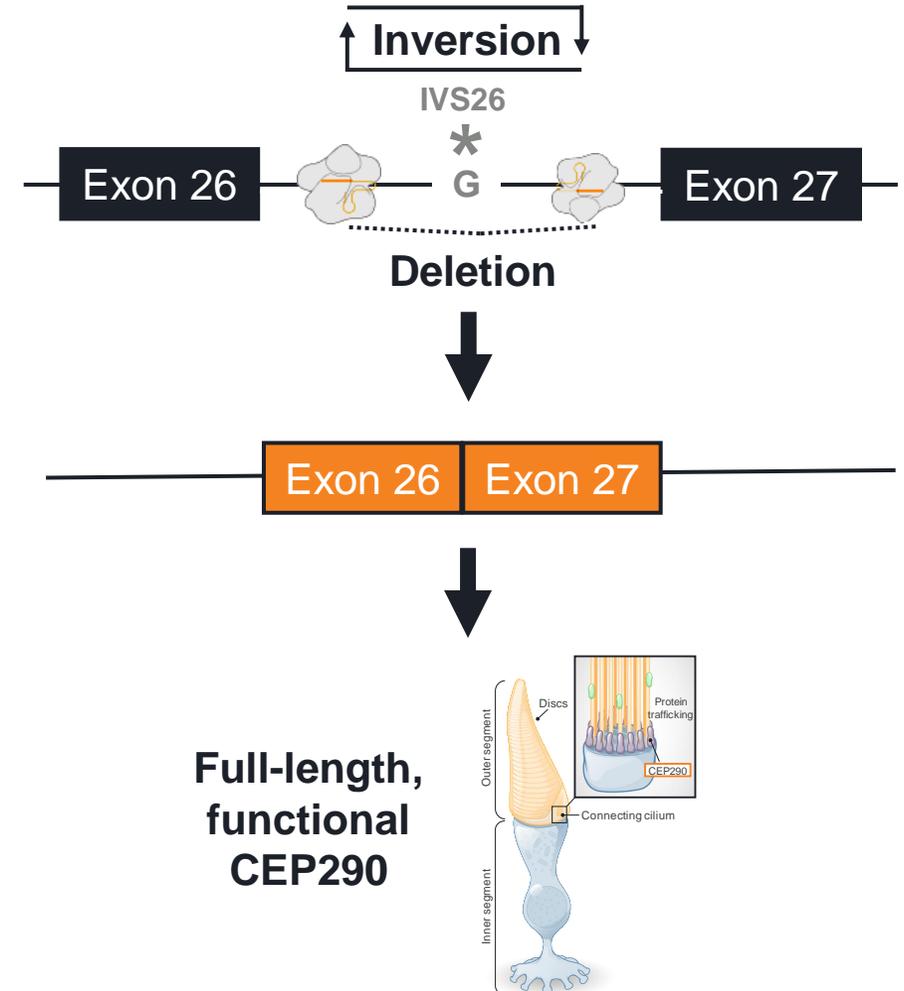
- The CEP290 gene encodes a 2479 amino acid, 290 kDa protein that localizes to the photoreceptor connecting cilium¹
- Required for the protein trafficking critical to outer segment regeneration and phototransduction²
- Restoring CEP290 protein expression in surviving foveal photoreceptors may improve vision in patients with LCA10

Gene repairs at c.2991+1655A>G mutation of CEP290 to full functional protein

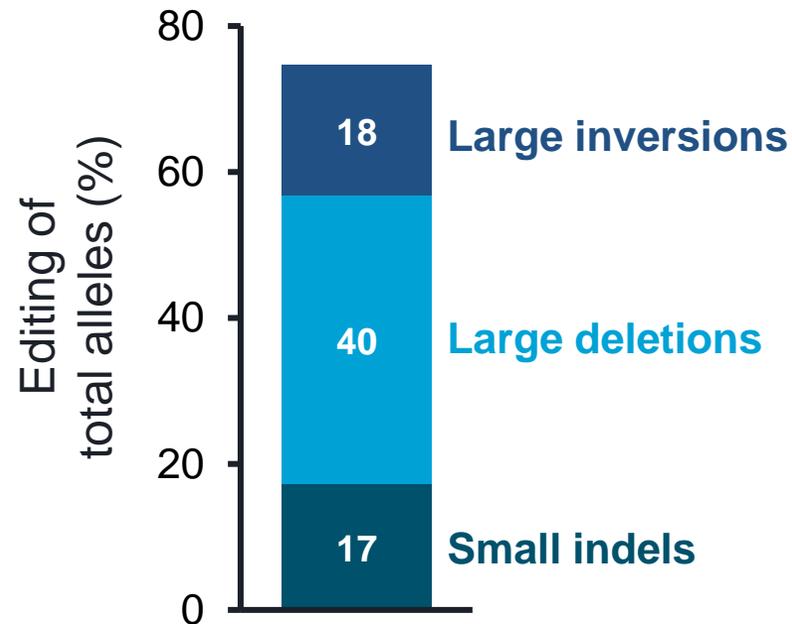
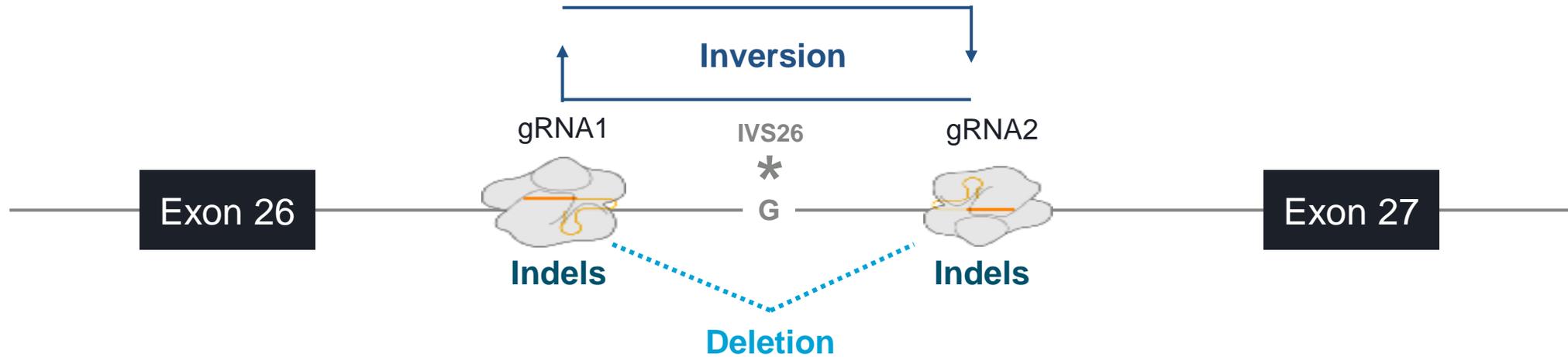
LCA10 disease



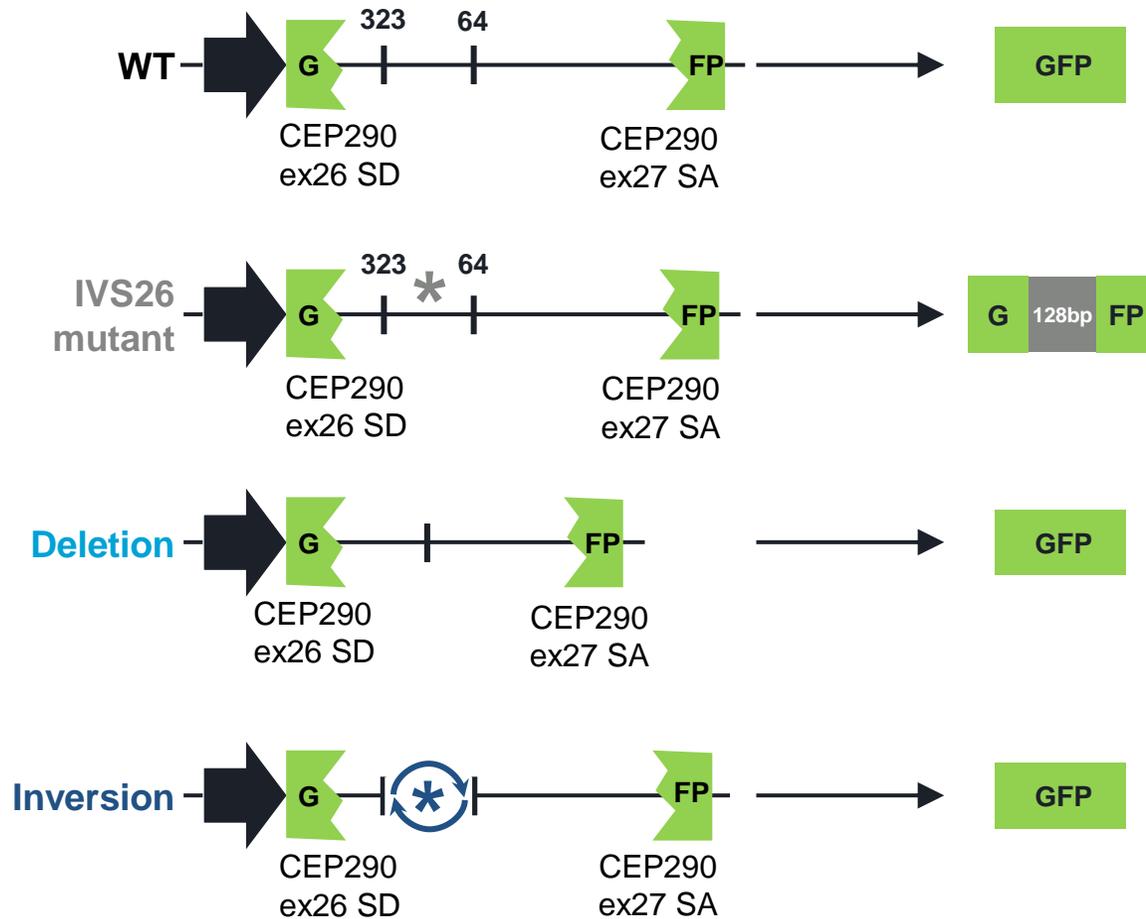
Gene editing therapeutic concept



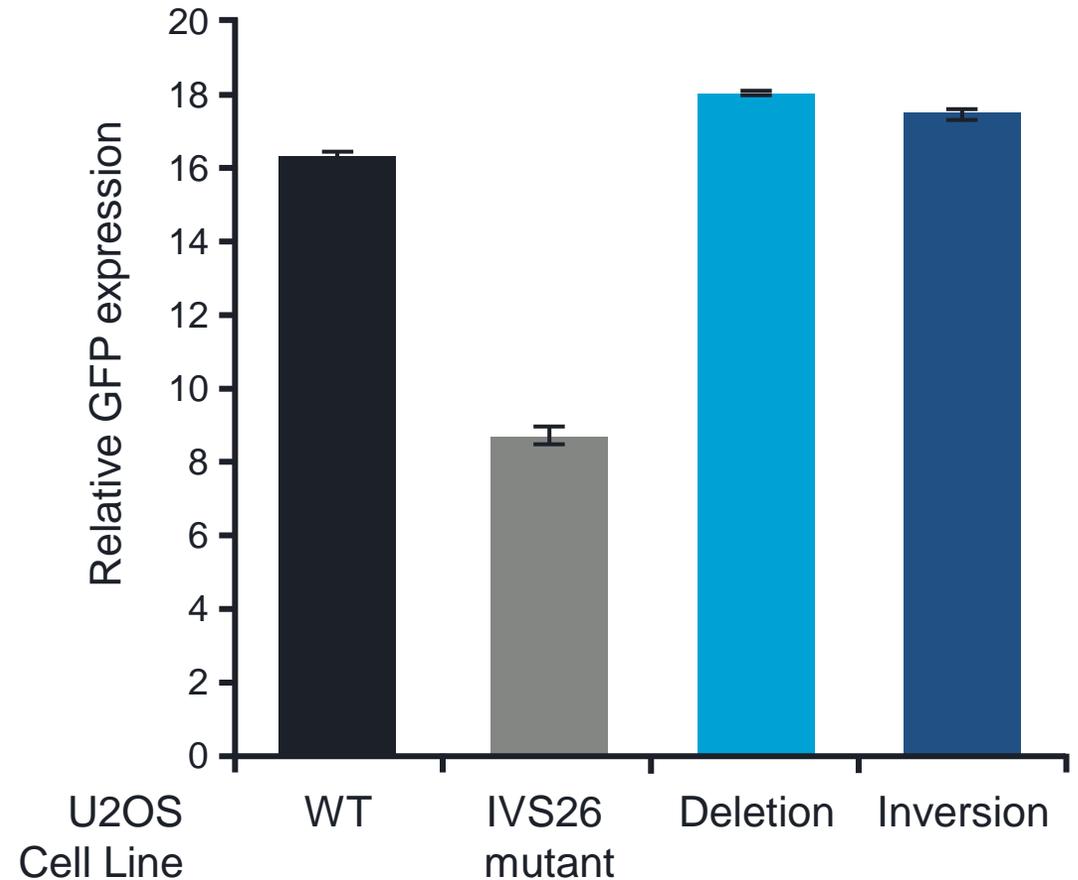
Editing causes inversions, deletions, and indels



Targeted deletions and inversions correct splicing

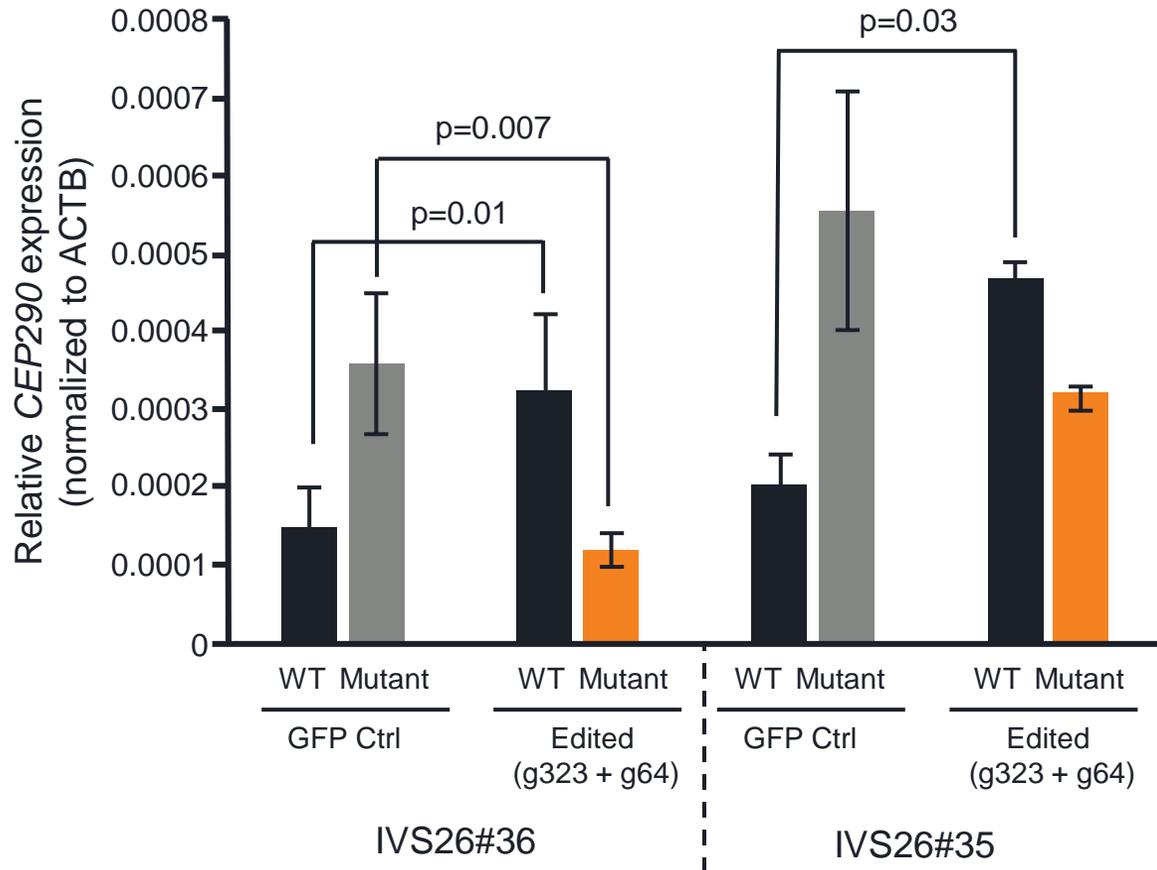


Correct splicing as determined by GFP expression

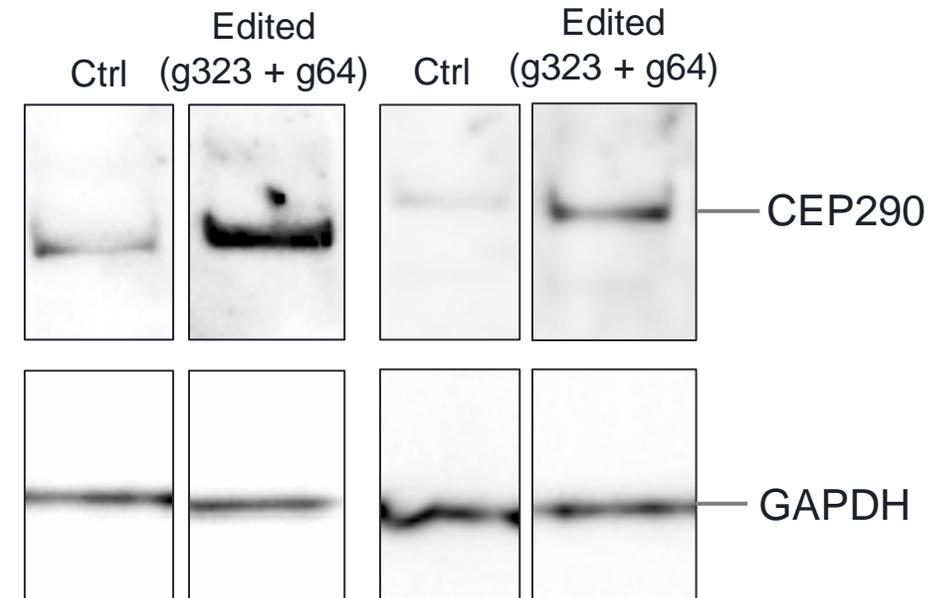


Editing corrects CEP290 splicing thereby restoring mRNA and protein expression

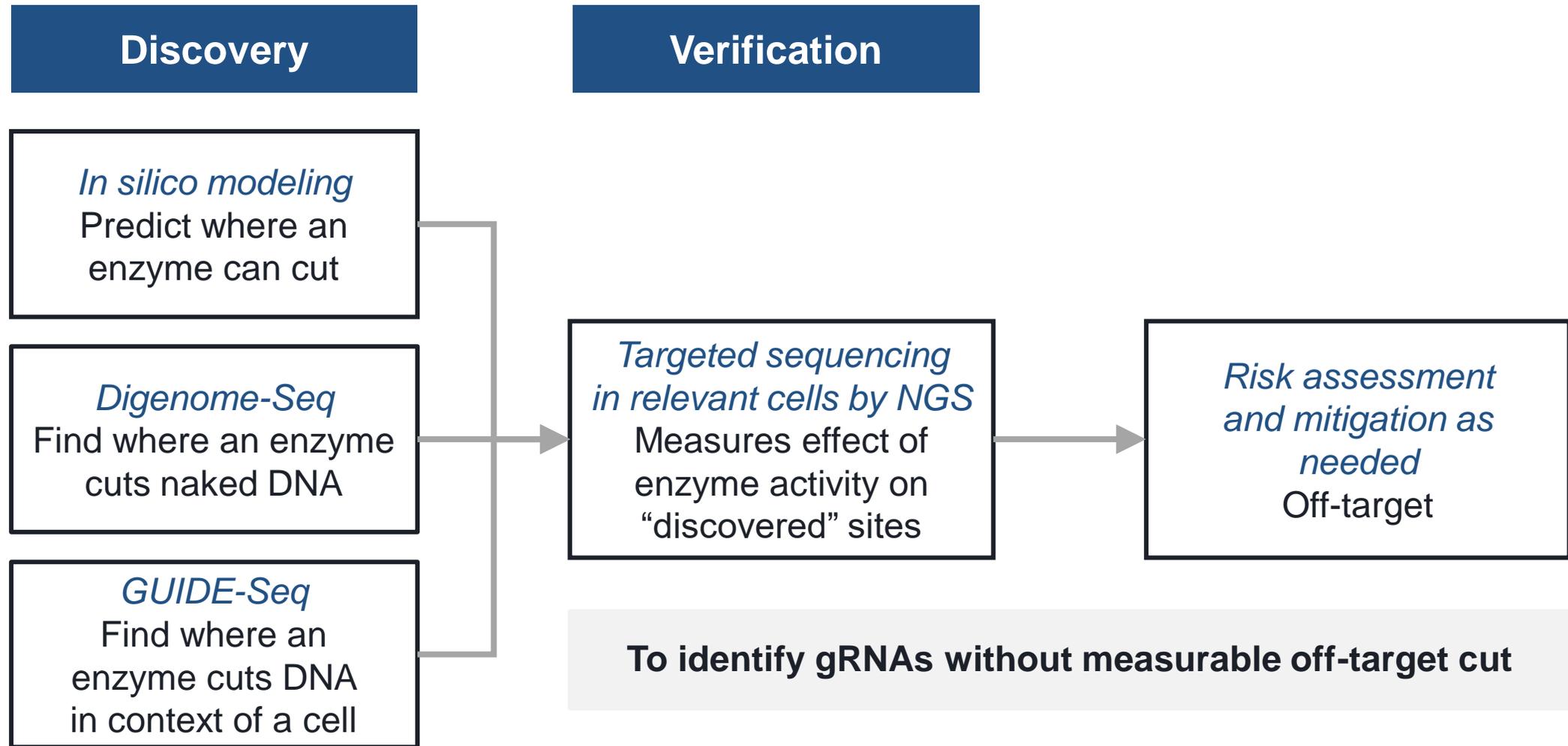
CEP290 mRNA expression



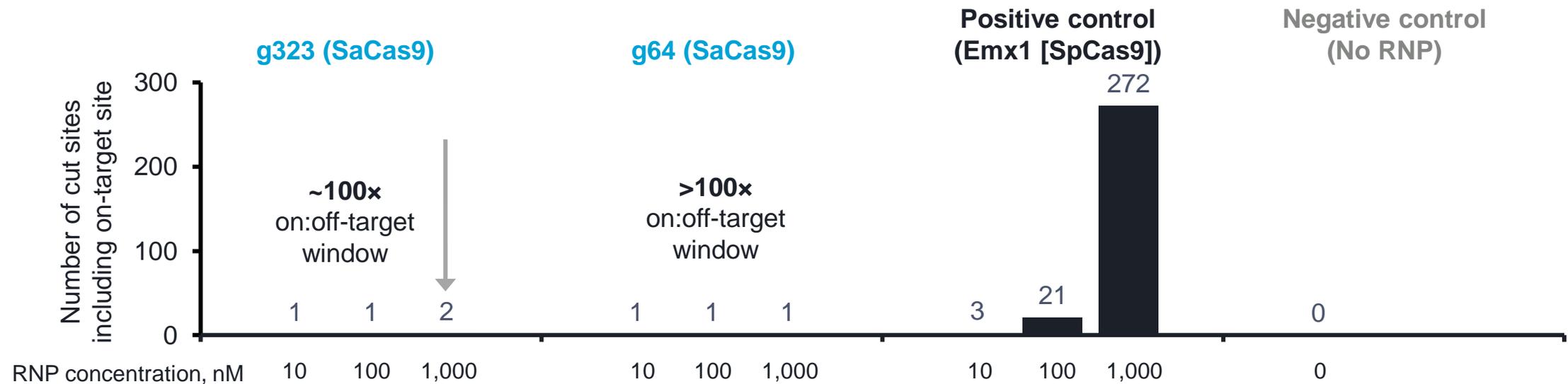
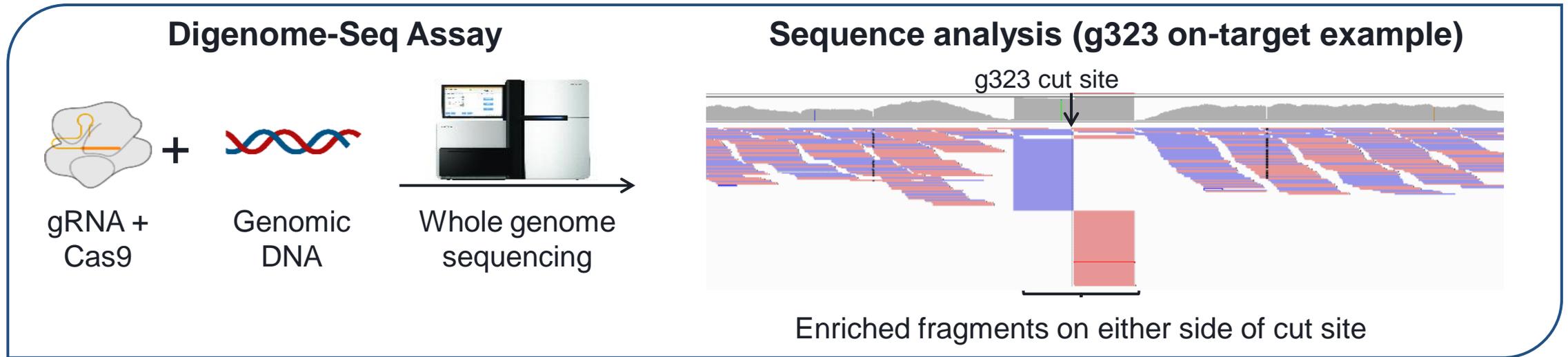
CEP290 protein expression



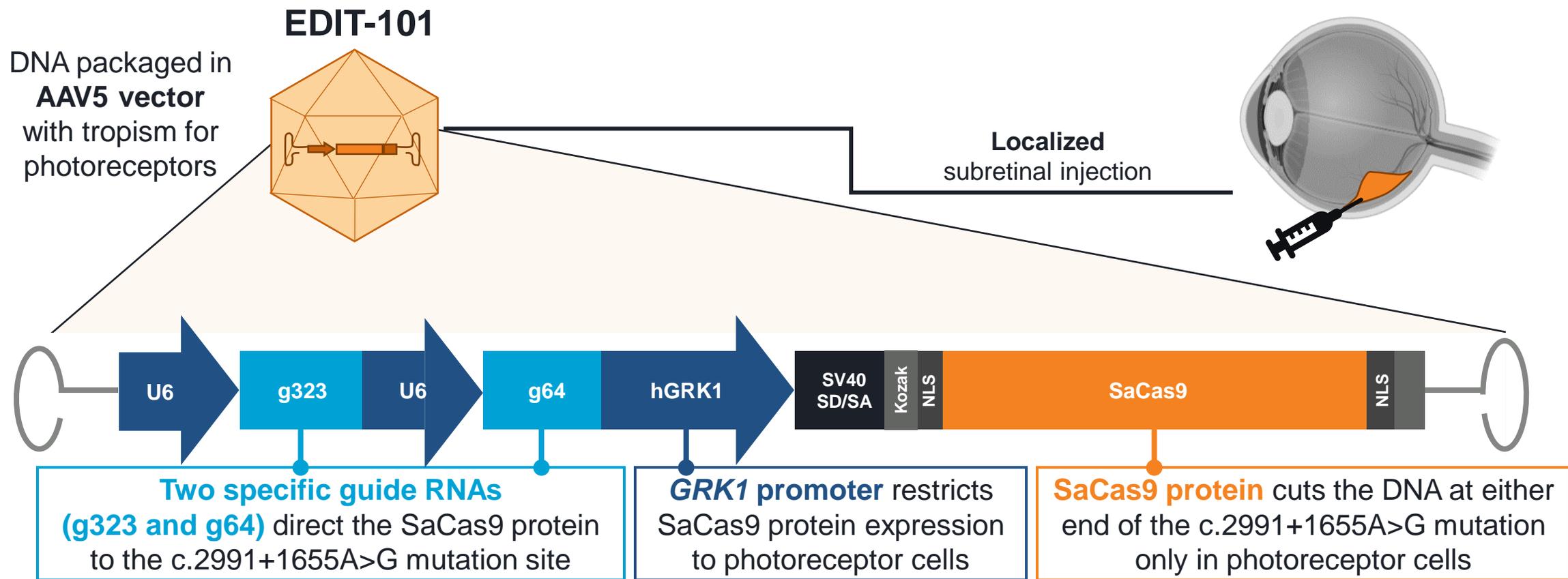
Comprehensive specificity assessment



Guide RNAs g323 and g64 demonstrated highly specific on-target cutting in a biochemical assay

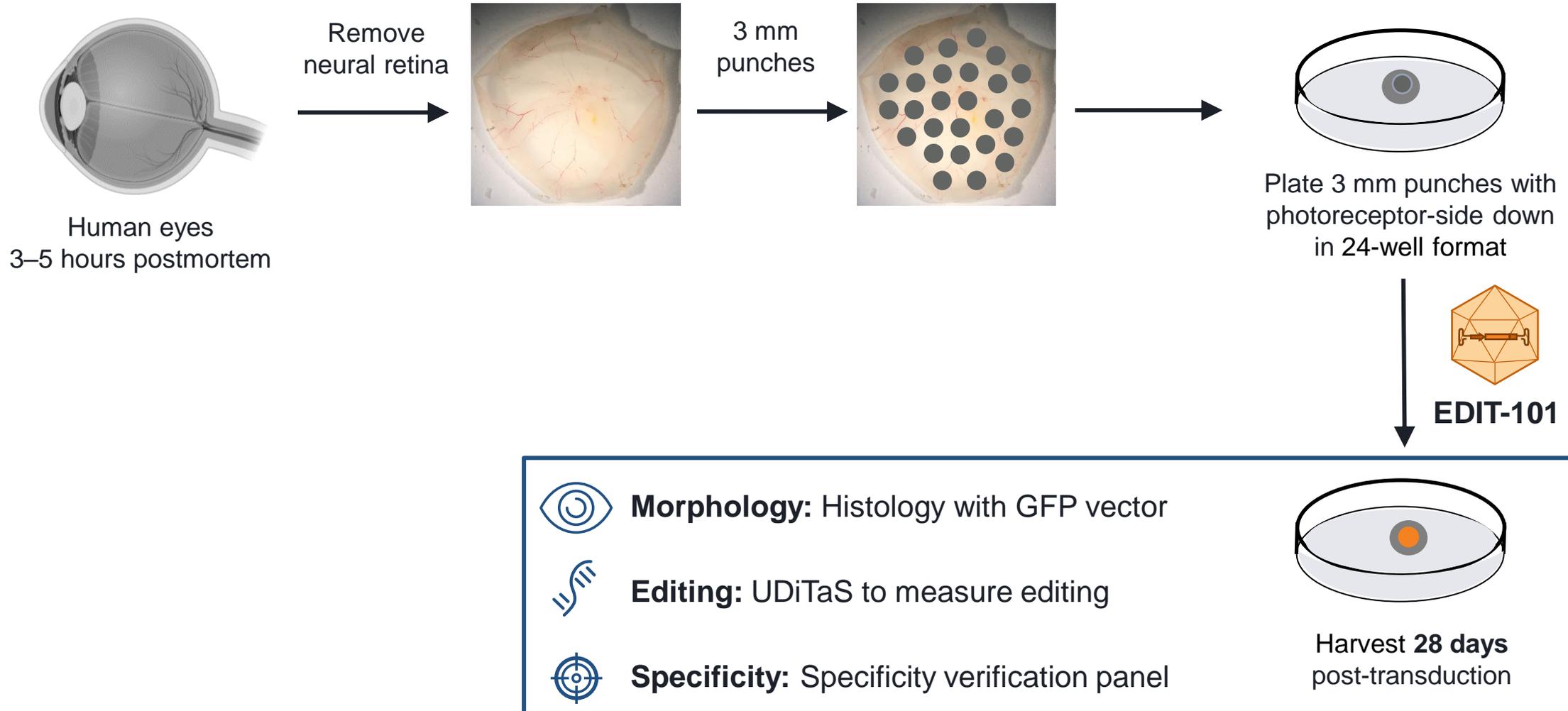


EDIT-101: an AAV5 vector with two gRNAs and DNA encoding Cas9 injected sub-retinally



Localized delivery, AAV5 tropism, specific guide RNAs, and restricted Cas9 expression facilitate targeted editing by EDIT-101 in photoreceptor cells

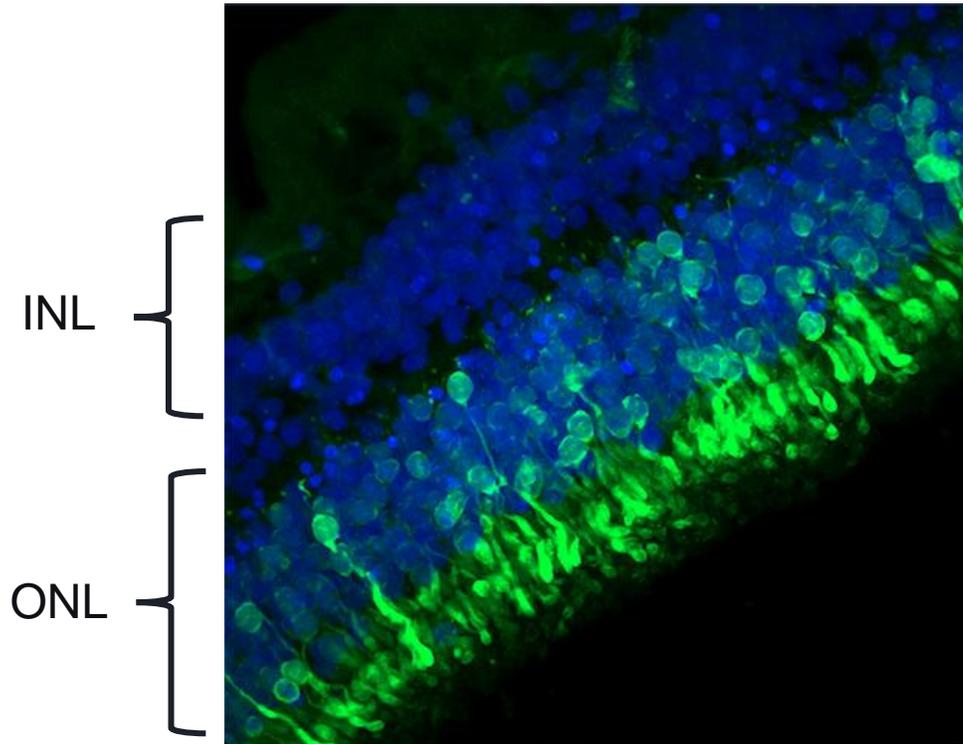
Editing and specificity analysis in human retinal explant model



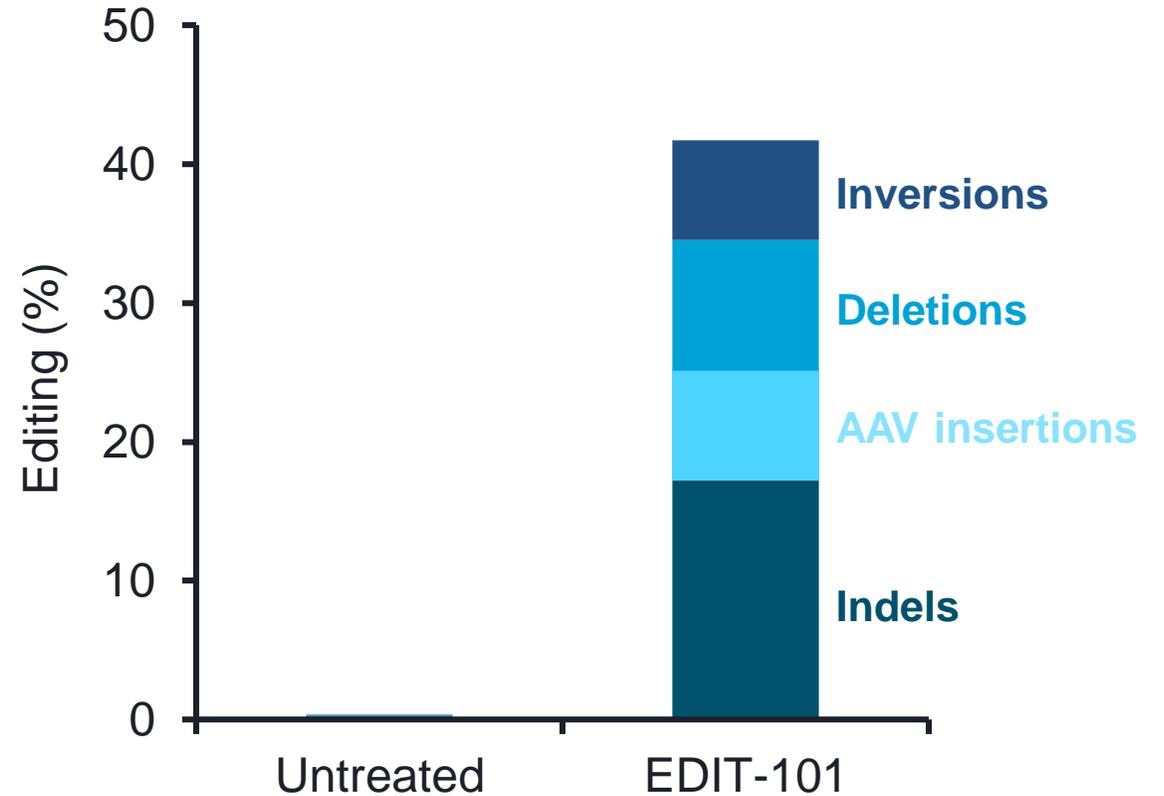
Confirmation of editing and quantitation in human retinal explant model

28 days post-transduction

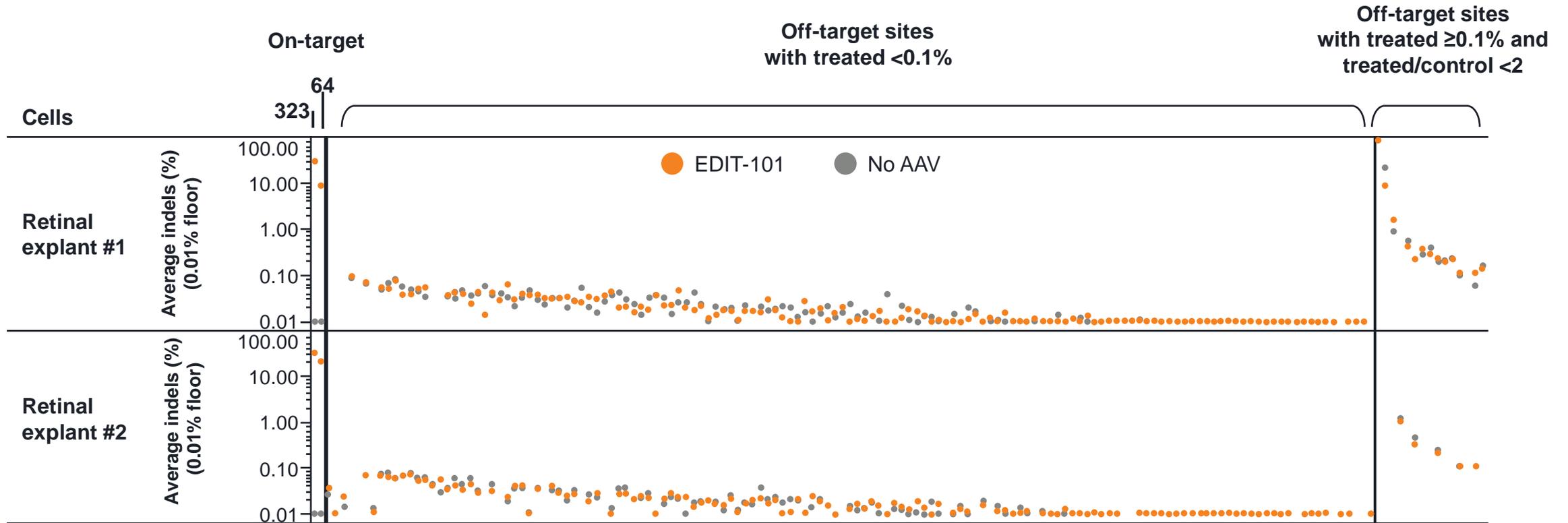
AAV5-GRK1-GFP (5e11 vg)



EDIT-101 (5e11 vg)



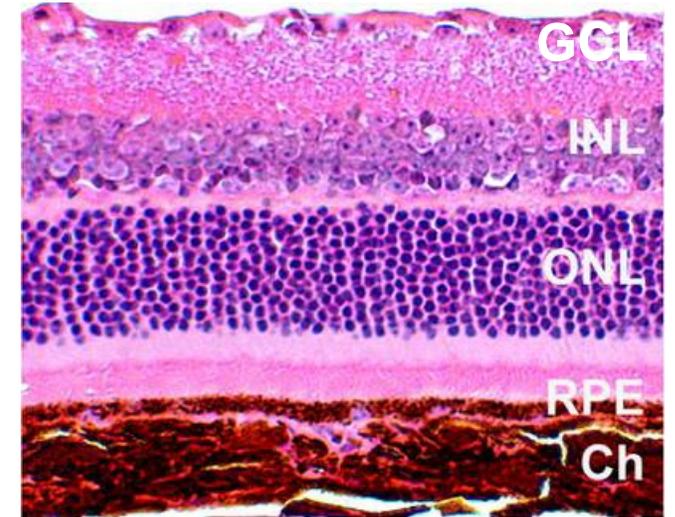
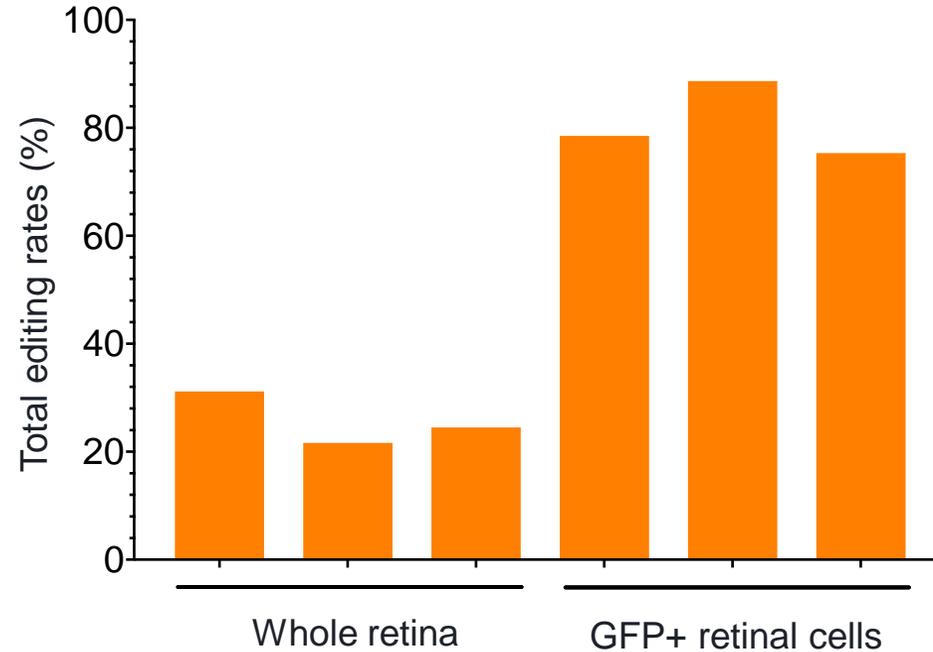
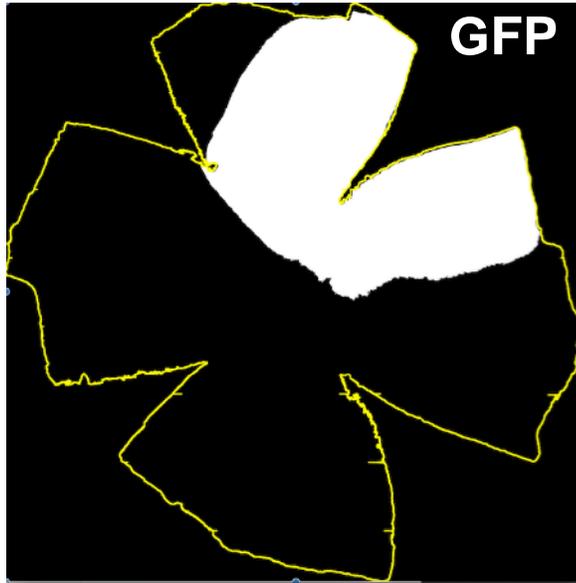
Specificity verification in retinal explants confirmed no off-target sites



The presence of on-target sites together with the absence of off-target sites confirmed that the guide RNAs were highly active and specific to the human CEP290 target sequence

Efficient transduction and editing of mouse retina by subretinal delivery of EDIT-101

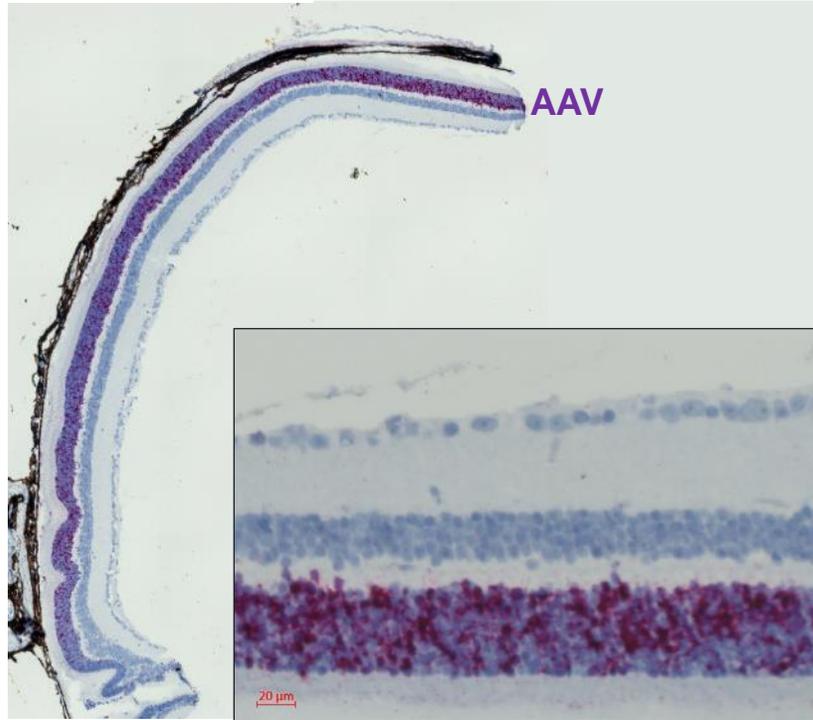
HuCEP290-IVS26 KI mice



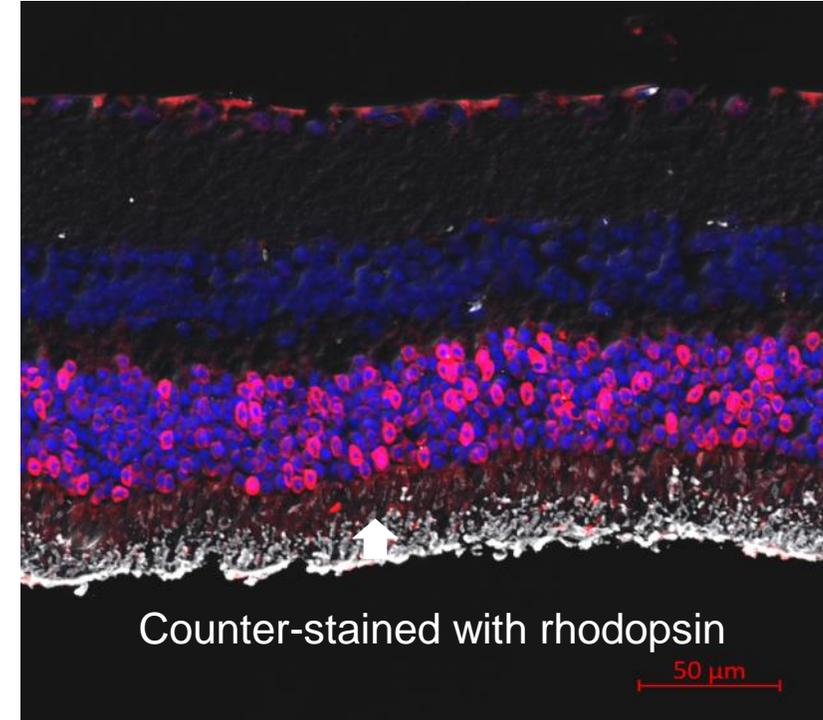
Over 80% of productive editing was achieved in the transduced photoreceptors

Efficient transduction of photoreceptor cells with EDIT-101 in HuCEP290-IVS26 KI mice

ISH of AAV vector genome



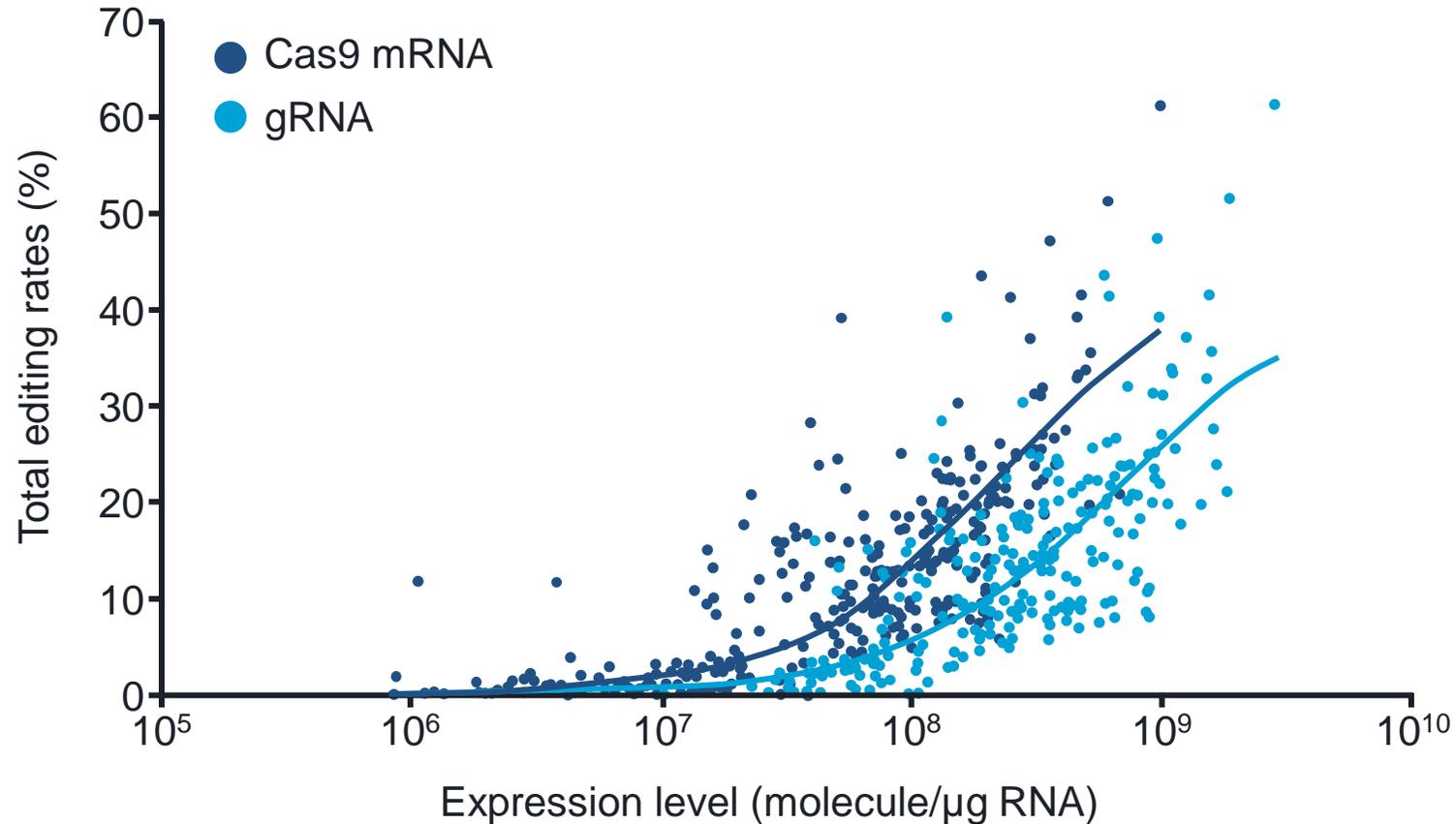
IHC of Cas9 protein



IHC of Cas9 in the area of AAV ISH showed essentially all photoreceptors in the bleb region were transduced

Both gRNA and Cas9 mRNA were highly expressed in the mouse retina

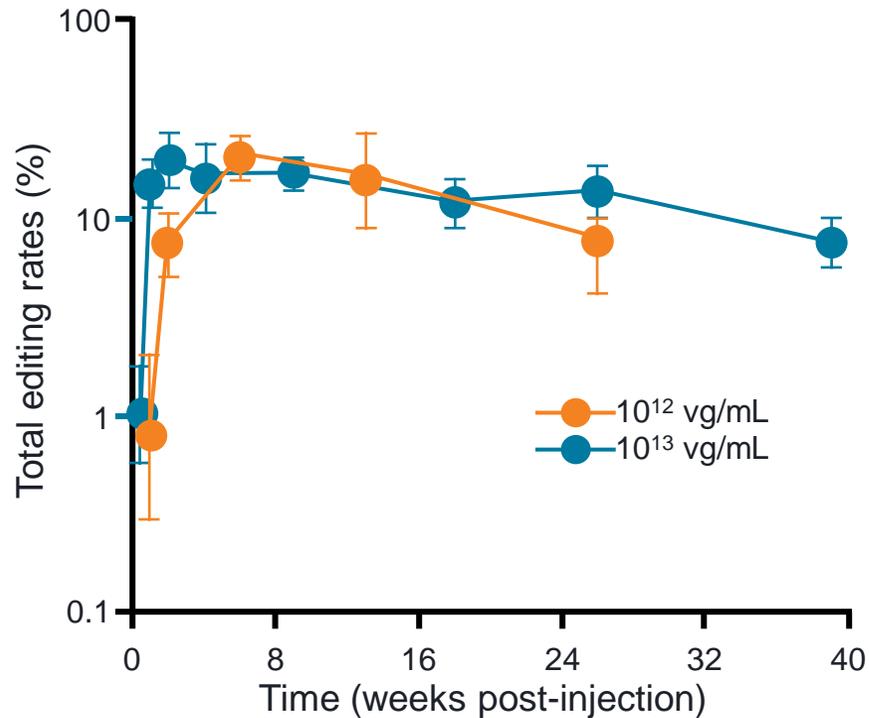
Relationship of editing-to-editing machinery



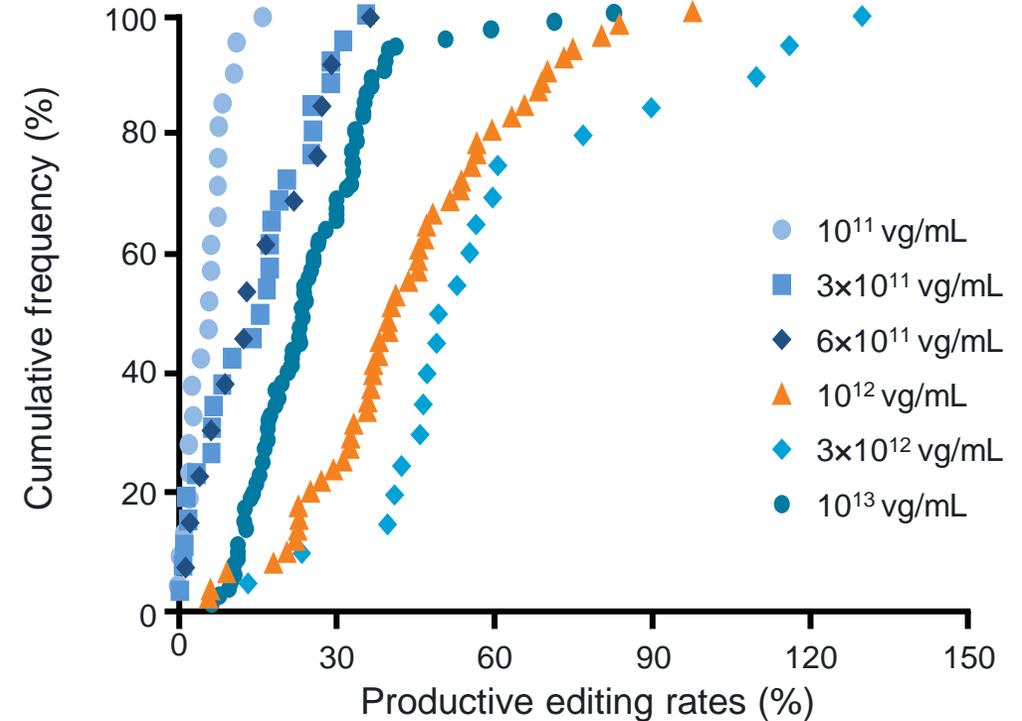
Adapted from Nature Medicine, Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10, 25, 2019;229–233, Maeder ML, et al, © The Author(s), under exclusive license to Springer Nature America, Inc., with permission of Springer. From: Fig. 2 | In vivo editing in HuCEP290 IVS26 knock-in mice

EDIT-101 demonstrated rapid and stable gene editing in HuCEP290-IVS26 KI mice

Total editing rates with EDIT-101 were maintained over 40 weeks post-injection

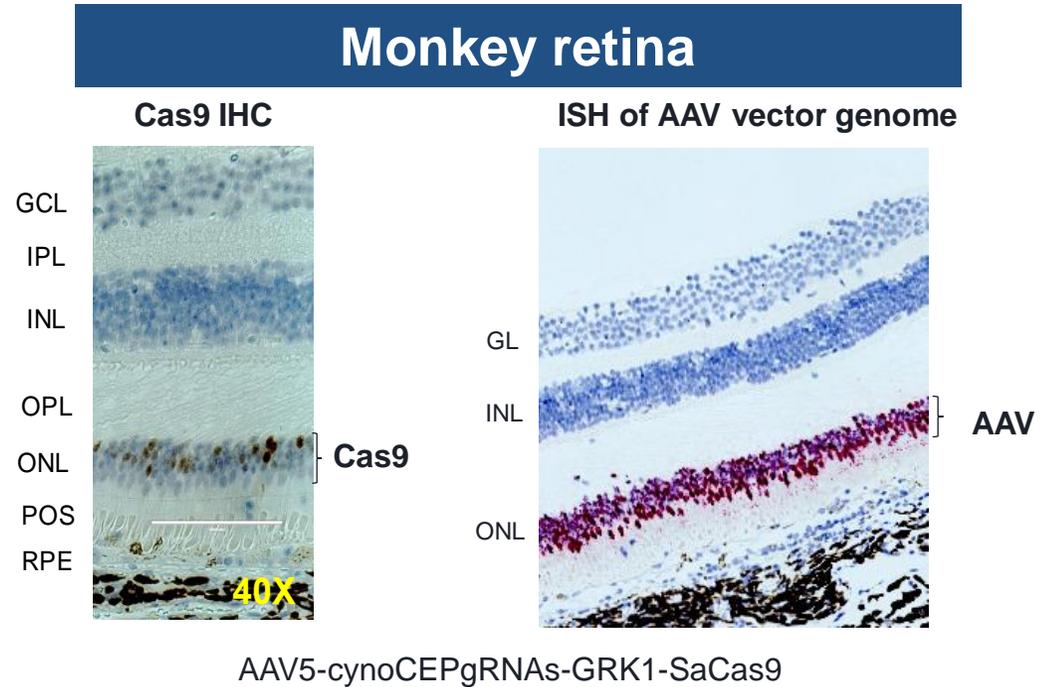


The optimal dose of EDIT-101 in the LCA10 mouse model appeared to be ~10¹² vg/mL

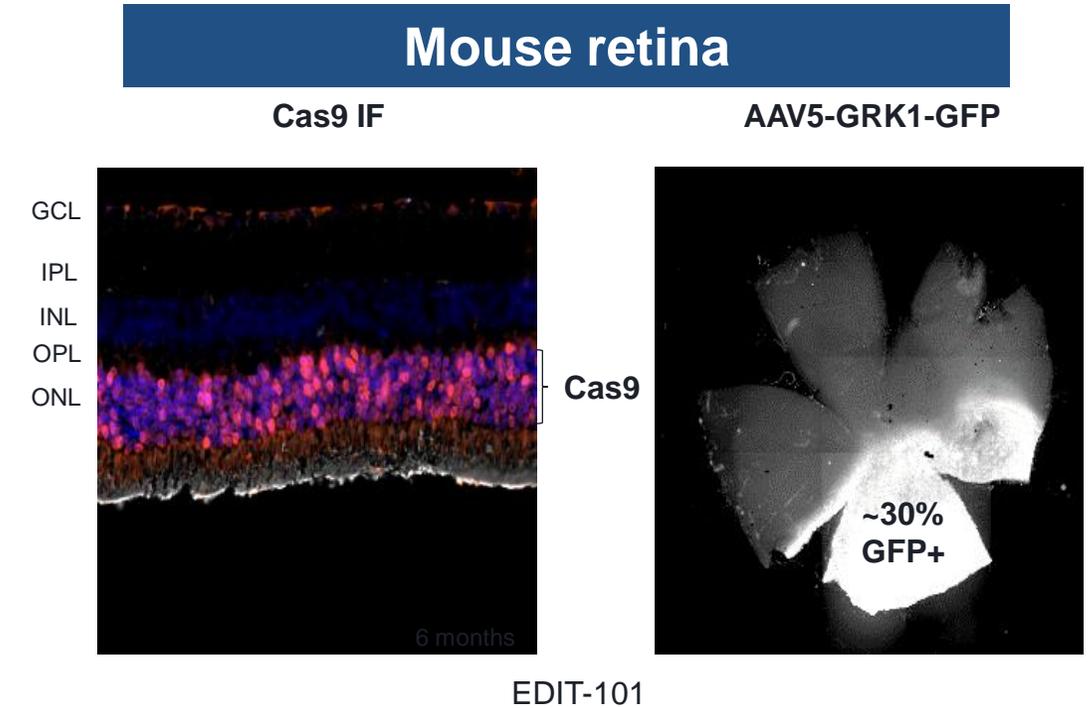


EDIT-101 achieved productive editing rates in a dose-dependent manner in an LCA10 mouse model

Retinal structural differences between mice and NHPs

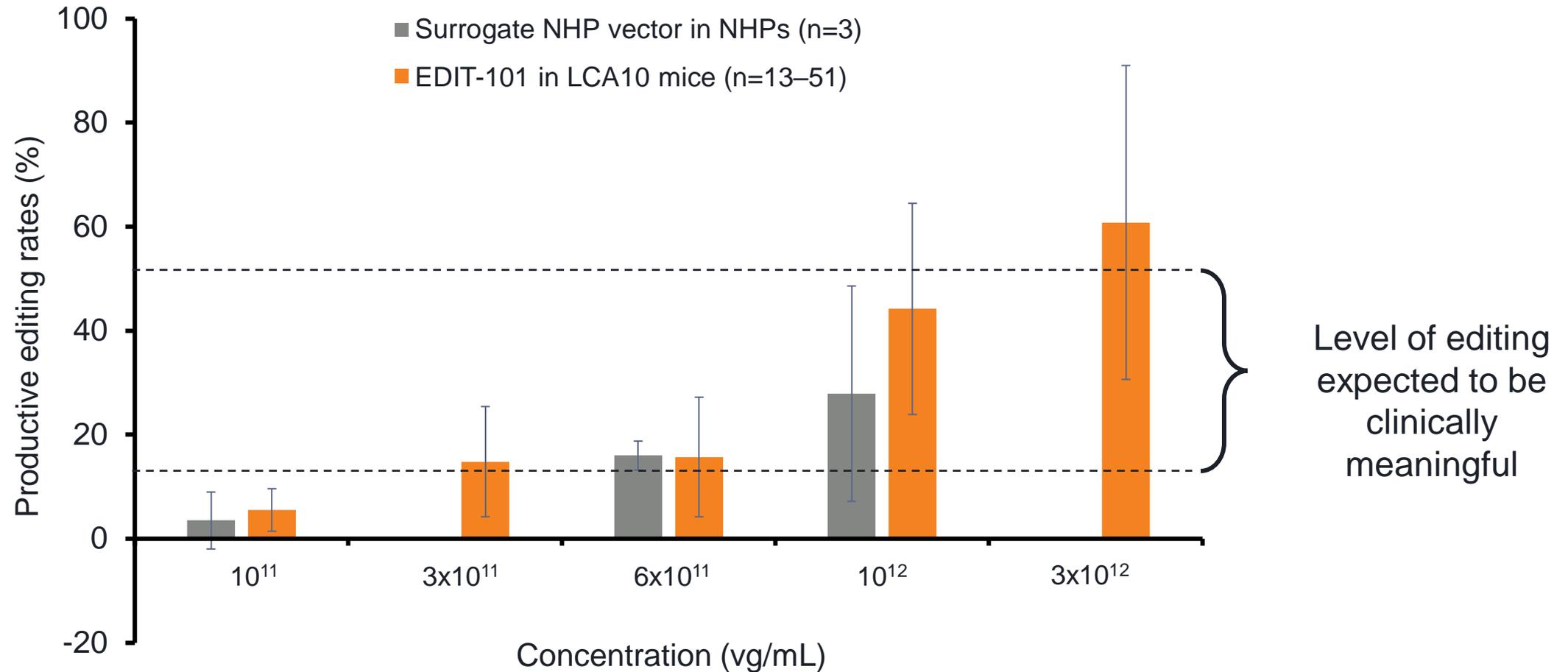


- Macula
- Photoreceptors: 25–30% of cells
- Most foveal photoreceptors are cones
- 8 mm retinal punch covering most of the bleb used for analysis but only photoreceptors (25–30%) express GRK1



- No macula
- Photoreceptors: 85–90% of cells
- 97% of photoreceptors are rods
- Entire retina collected for analysis but only 30% of neural retina transduced with 1 μ L AAV5

EDIT-101 had a similar dose response in mice and NHPs



At maximally tolerated doses, >50% editing is observed
EDIT-101 was well tolerated in NHPs and was not distinguishable from placebo

BRILLIANCE: A Phase 1/2 open-label study of EDIT-101 in adult and pediatric patients



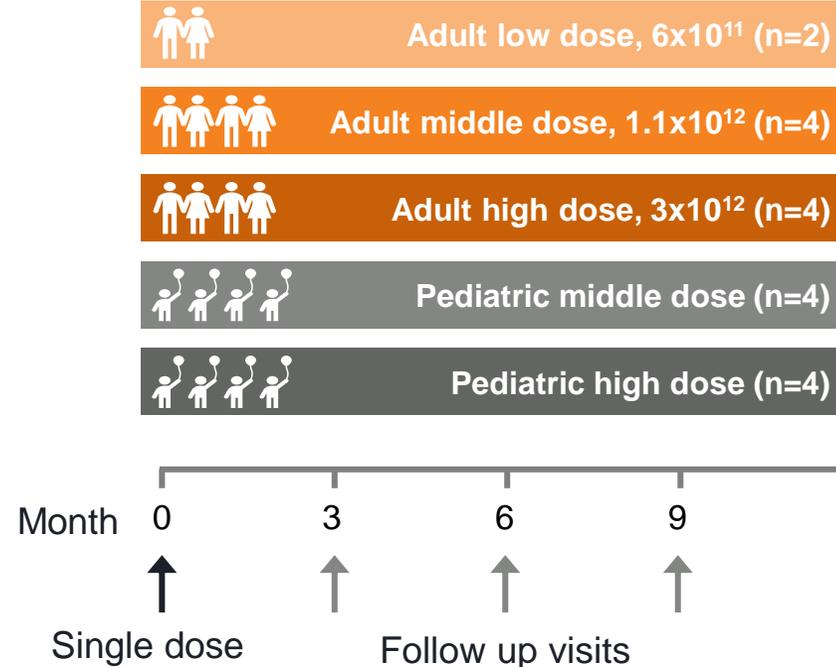
Objective:

To evaluate the safety, tolerability, and efficacy of EDIT-101 in patients with LCA10

Inclusion criteria

- Adult (≥ 18 years) or pediatric (3–17 years) patients
- LCA10 caused by c.2991+1655A>G mutation in the CEP290 gene
- BCVA 0.4 logMAR (20/50 Snellen equivalent)
- Failed mobility course at maximum level of difficulty

Phase 1/2, open-label, single ascending dose study (NCT03872479)



Primary outcomes: Safety

- Adverse events
- Dose-limiting toxicities

Key secondary outcomes:

- Maximum tolerated dose
- Visual navigation (Δ Mobility course score)
- BCVA (Δ LogMAR)
- Δ Macula thickness
- Pupillometry and microperimetry
- Light and contrast sensitivity
- Δ Color vision score
- Quality of life

Presented by Pierce E, et al. Retinal Cell and Gene Therapy Symposium (April 26, 2019).
"Progress Toward a Clinical Trial of CRISPR/Cas9-Mediated Genome Editing for CEP290-Associated Retinal Degeneration"

Acknowledgments



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