Prime Editors efficiently and precisely correct pathological mutations causing rhodopsin associated autosomal dominant retinitis pigmentosa (adRP)

International Symposium on Retinal Degeneration

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Disclosures

Deepak Reyon declares he is a current employee of Prime Medicine, Inc. and owns equity in Prime Medicine.

Key Highlights

- **Prime Editing precisely corrects** predominant mutations that cause rhodopsin associated autosomal dominant retinitis pigmentosa (RHO adRP)
- **Prime Editing restores RHO p.P23H localization** in mutant cell-based assay
- Prime's novel dual AAV delivery platform demonstrates efficient delivery to **both human (retinal explants) and murine (*in vivo*) photoreceptors**
- **No off-target edits** were detected in photoreceptors

Prime Editing: A Gene Editing Technology That Is Programmable for Both *Search* and *Replace*

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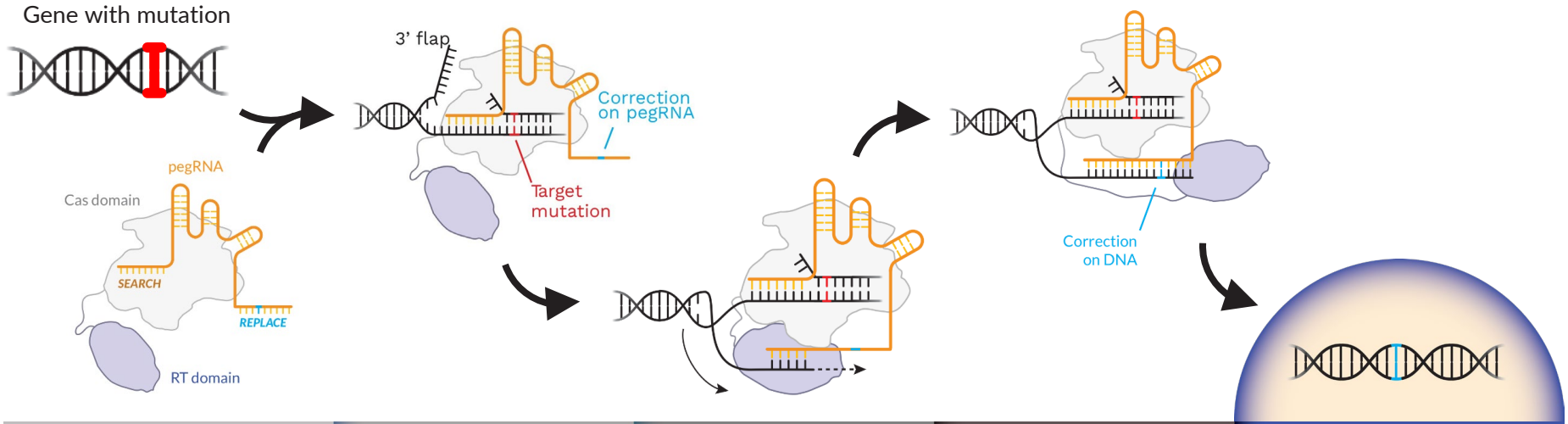


SEARCH

Prime editor complex initiates search for target DNA

FIND & NICK

Prime editor complex finds DNA with target mutation, nicks one strand

PRIME

Nicked DNA strand primes the RT domain for DNA synthesis

REPLACE

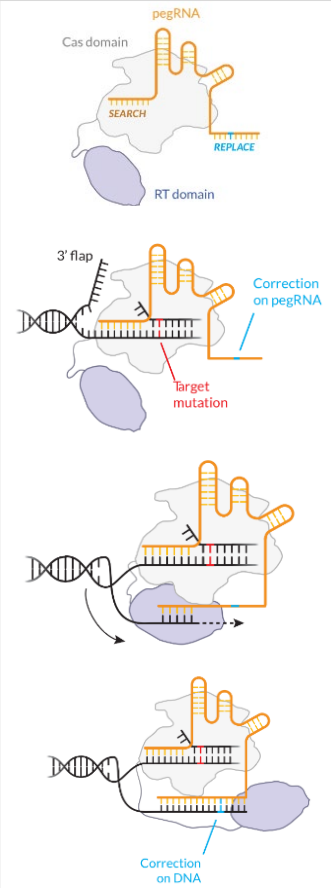
Prime editor complex copies in corrective DNA sequence

GENE CORRECTED

3' flap preferentially incorporated[1], excess flap repaired, gene fully corrected

[1] Completion of an edit requires 3 'edit checks,' or places where there has to be a match between the editor and the target DNA; pegRNA = prime editing guide RNA; RT = reverse transcriptase; Cas = CRISPR associated protein
Anzalone, et al (David R. Liu). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 2019.

Delivering the full promise of gene editing requires an extremely powerful technology



Prime Editing (PE) stands out as a best-in-class genetic medicine

Versatility: only gene editing technology with the capability to edit, correct, insert, and delete

- ✓ Performs and corrects insertions, deletions, and all twelve types of single base pair corrections
- ✓ Precisely targets to insert or delete kilobase-sized DNA
- ✓ Easily programmable to a unique target location and for a broad set of edits
- ✓ Restores gene function for multiple mutations with a single product (i.e., “hotspots”)

Precision: May be much safer with minimal, or no, off-target editing

- ✓ Does not create double stranded breaks: high specificity with low indels rate at targeted editing site
- ✓ Does not create double stranded breaks: minimal or no off-target activity
- ✓ Limited potential for “bystander editing” at target site

Efficiency: Durable and high-efficiency editing demonstrated across Prime Medicine portfolio

- ✓ Permanent edits that are passed along to daughter cells
- ✓ Corrects genes *in situ*, maintaining native gene control
- ✓ Single-dose, potentially curative correction to wild-type sequence

Breadth: Able to address ~90% of disease-causing mutations in multiple tissue types and cells

- ✓ Corrects mutations in dividing and non-dividing human cells
- ✓ 100’s of potential indications already available in Prime Editing’s toolbox

Prime Editing has potential to correct pathogenic mutations in *RHO* that cause autosomal dominant retinitis pigmentosa (RHO adRP)

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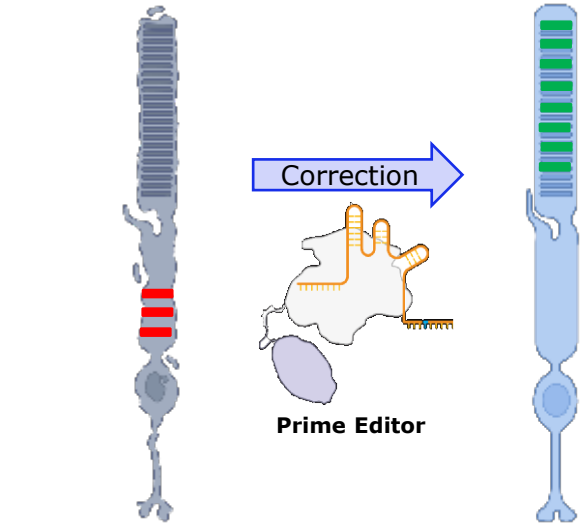
Rhodopsin associated autosomal dominant retinitis pigmentosa

- ***Description:***
  - Rare inherited retinal disease-causing progressive vision loss
  - Starts in early adolescence leading eventual blindness in adult life
- ***Human genetics and biology:***
  - Autosomal dominant disease caused by multiple mutations in the *RHO* gene
  - *RHO* encodes opsin which is critical for both the function and structure of rod photoreceptors
  - *RHO* p.P23H, p.V345L, and p.P347L mutations result in mislocalization of RHO leading to **photoreceptor degeneration**
  - These 3 mutations account for ~60% of RHO adRP patients
- ***Unmet need:***
  - No disease modifying approved therapies
- ***Prime Medicine's approach:***
  - Dual AAV based Prime Editing to correct the *RHO* p.P23H mutation or p.V345L/P347L hotspot mutations in rod photoreceptors to preserve retinal health and prevent vision loss



Mutant RHO mislocalization leads to photoreceptor degeneration

Wild Type RHO localizes to the photoreceptor outer segment

AAV: Adeno-associated virus

We are developing Prime Editors to correct multiple mutations that cause RHO adRP

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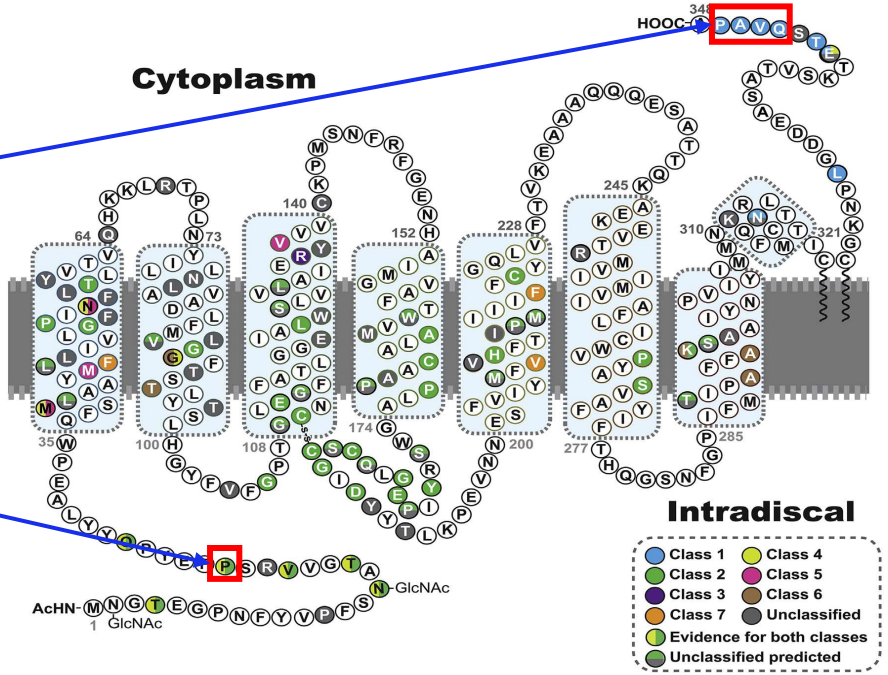
Prevalent pathogenic mutations are found at the N- and C- terminal regions of the RHO protein

18 pathogenic mutations in a mutational HOTSPOT, including **V345L** and **P347L,** at the C-terminal result in mis-localization of Rhodopsin

FAST disease progression:
(1st decade of life)

P23H mutation at the N-terminal results in sequestration of Rhodopsin within the ER

SLOWER disease progression:
(2nd decade of life)



Prime Medicine's strategy for development of precise editors for ocular indications

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Prime Editors are specific to human patient DNA sequence and designed for the correction of human mutations

***In vitro* testing**

Plasmid 1

N-term PE + ngRNA

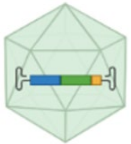
+

Plasmid 2

C-term PE + pegRNA

***In vivo* testing**

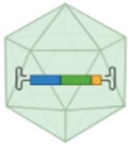
AAV 1



N-term Prime Editor

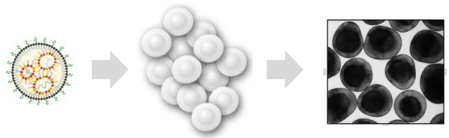
+

AAV 2



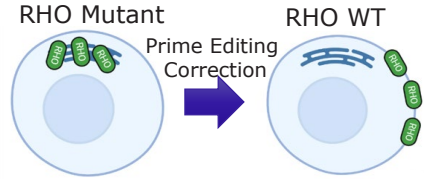
C-term Prime Editor

Generate retinal organoids from corrected patient cells



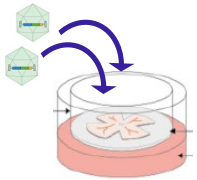
Status: Retinal organoid differentiation on going

Demonstrate phenotypic benefit of precise correction



Status: % membrane RHO correlates to % precise correction (Data in following slide)

Editing and off target analysis in Human Photoreceptors



Status: Demonstrated dual AAV mediated editing (Data in following slide)

***In vivo* correction following subretinal delivery**

Status: Precise correction in humanized mouse model (Data in following slide)

PE: Prime Editor; pegRNA: prime editor guide RNA; ngRNA: nick guide RNA

In vitro modeling of RHO P23H mislocalization phenotype

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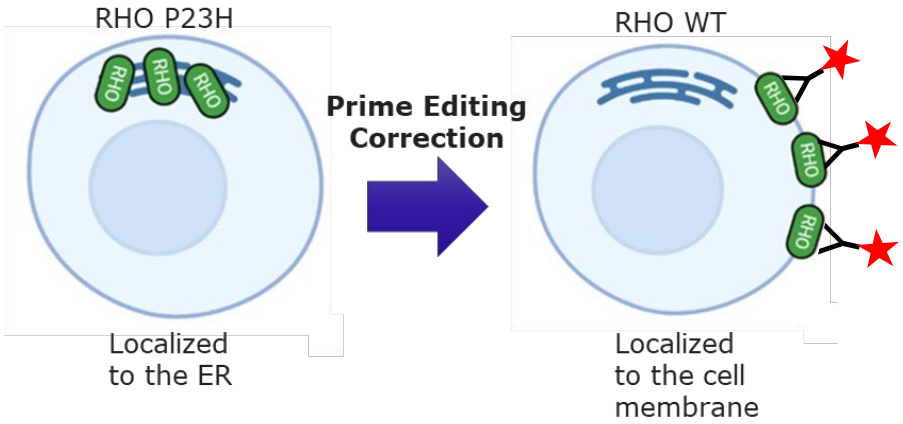
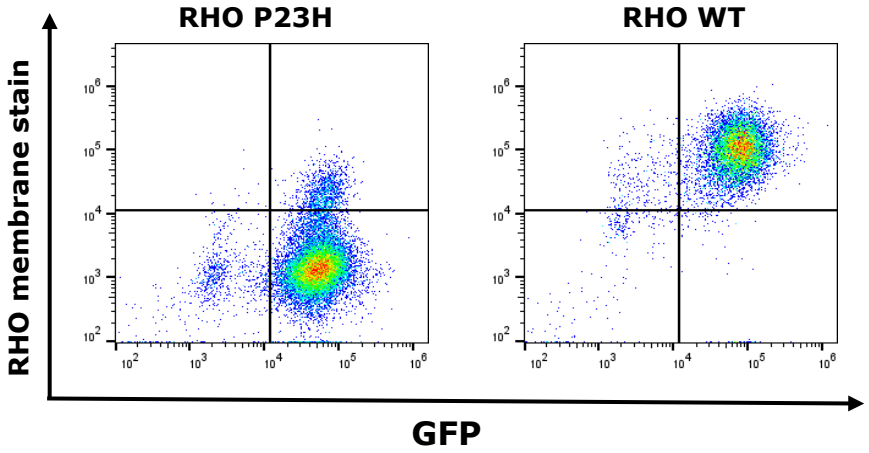
Flow cytometry-based assay to quantify membrane bound rhodopsin

General strategy for assessment of RHO localization



- Wildtype and mutant cell lines generated using lentiviral integration

Detection of membrane bound RHO by flow cytometry



- Antibody against 4D2 epitope (N-Term) used to quantify membrane bound RHO
- GFP in cassette used for cell line generation

Precise correction of RHO p.P23H correlates with RHO membrane localization *in vitro*

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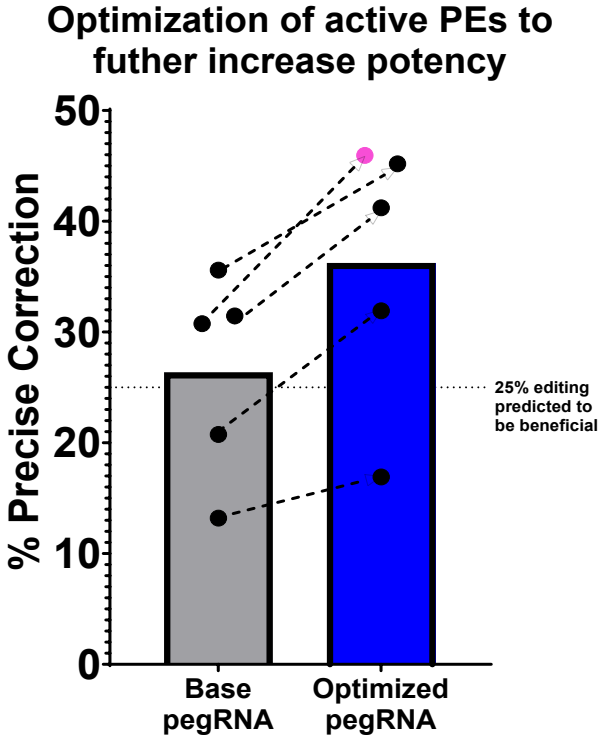
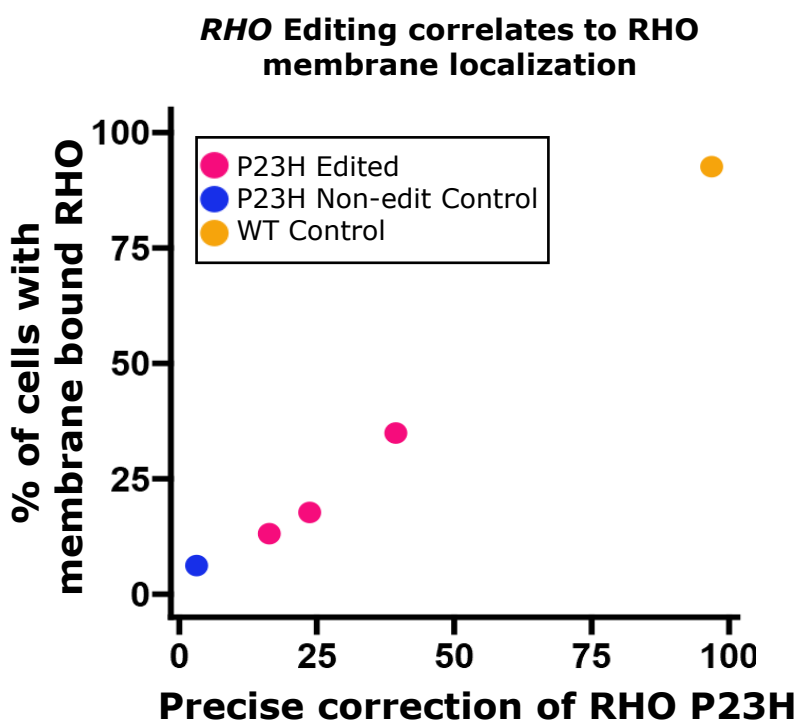
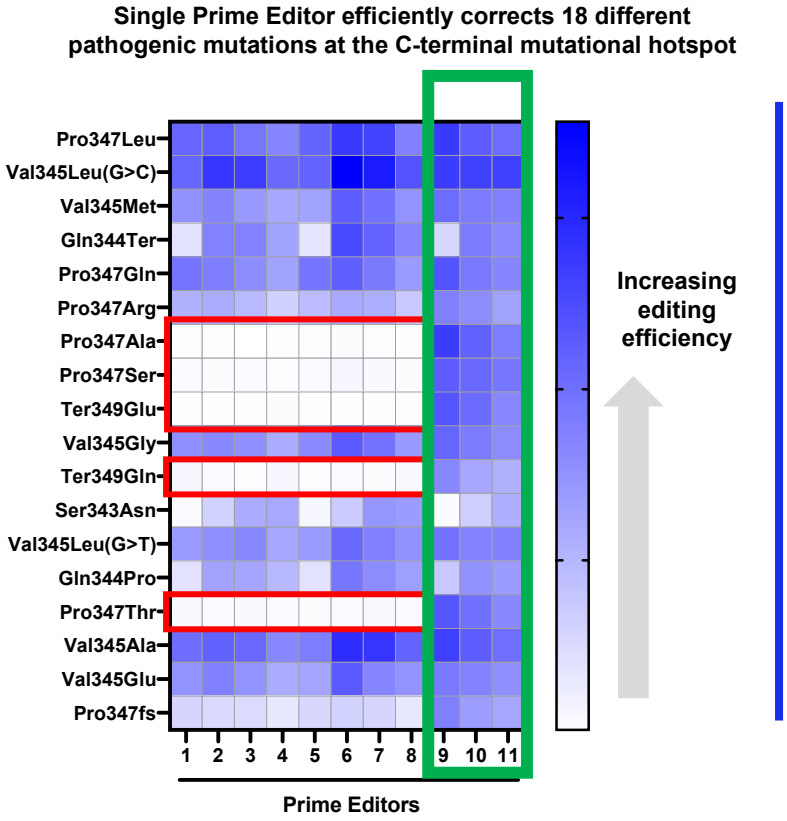
Over 1000 pegRNAs were screened using our HTS platform to identify highly active Prime Editors which were optimized

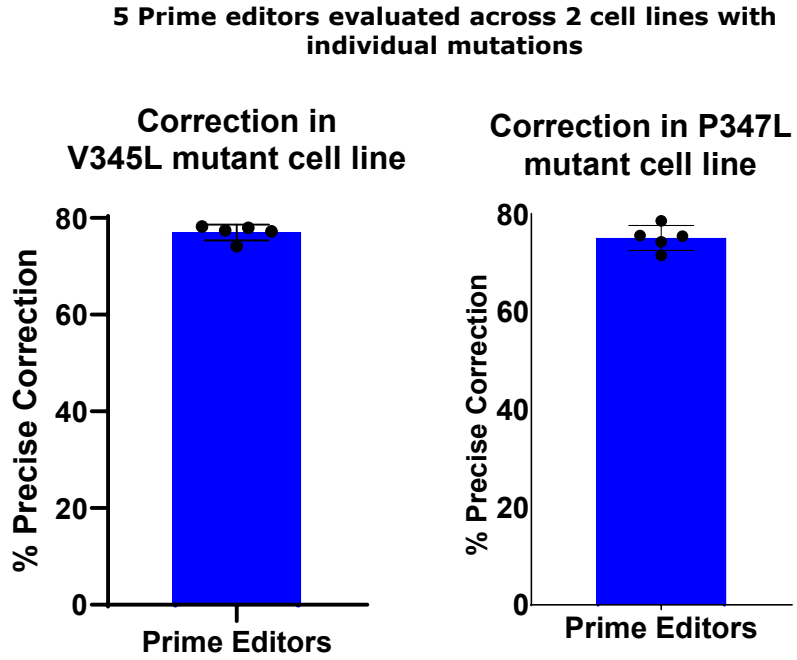




25% is predicted to be therapeutically beneficial based on Cideciyan et al., 1998. PNAS 95, 7103-7108.; PE: Prime Editor; pegRNA: Prime Editor guide RNA

Single Prime Editor corrects 18 different *RHO* mutations



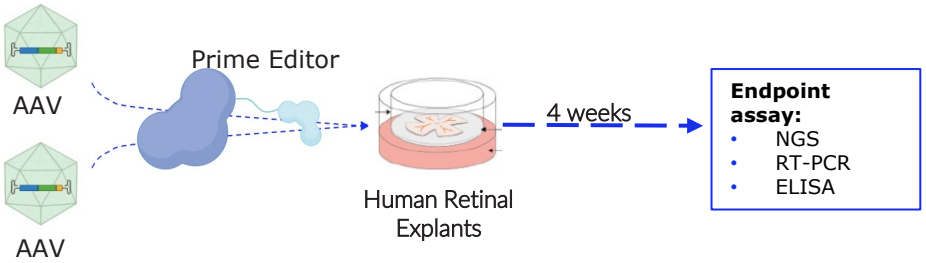


No activity due to PAM or spacer disruption

Editors targeting shared PAM/spacer across 18 mutations

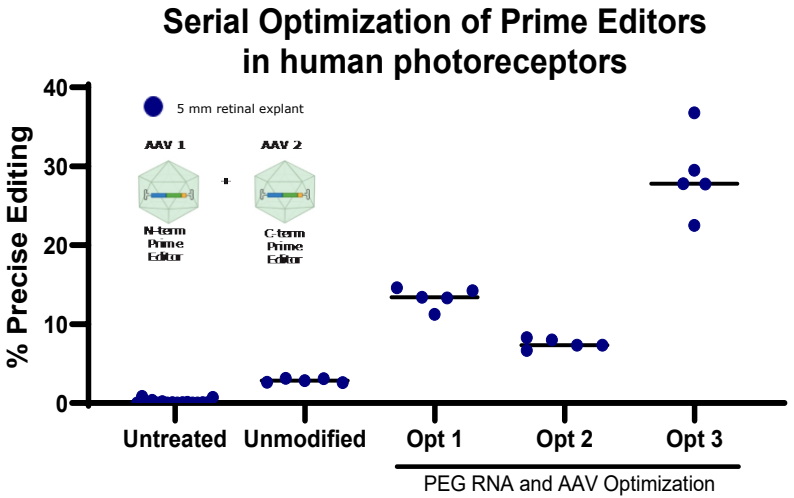
Efficient Prime Editing of *RHO* in human retinal explants

Prime Editors delivered using a dual AAV system



HUMAN RETINAL EXPLANT ASSAY

- Excellent supply chain from **eye banks**
- Retinal explants are dosed with **dual AAV system**
- Used to assess potential off target editing in the **photoreceptors**



PE: Prime Editor; Term: terminus; AAV: adeno-associated virus; NGS: next generation sequencing; RT-PCR: real-time polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay; mm: millimeter

Humanized mouse models have been developed by replacing the entire murine gene with the human gene

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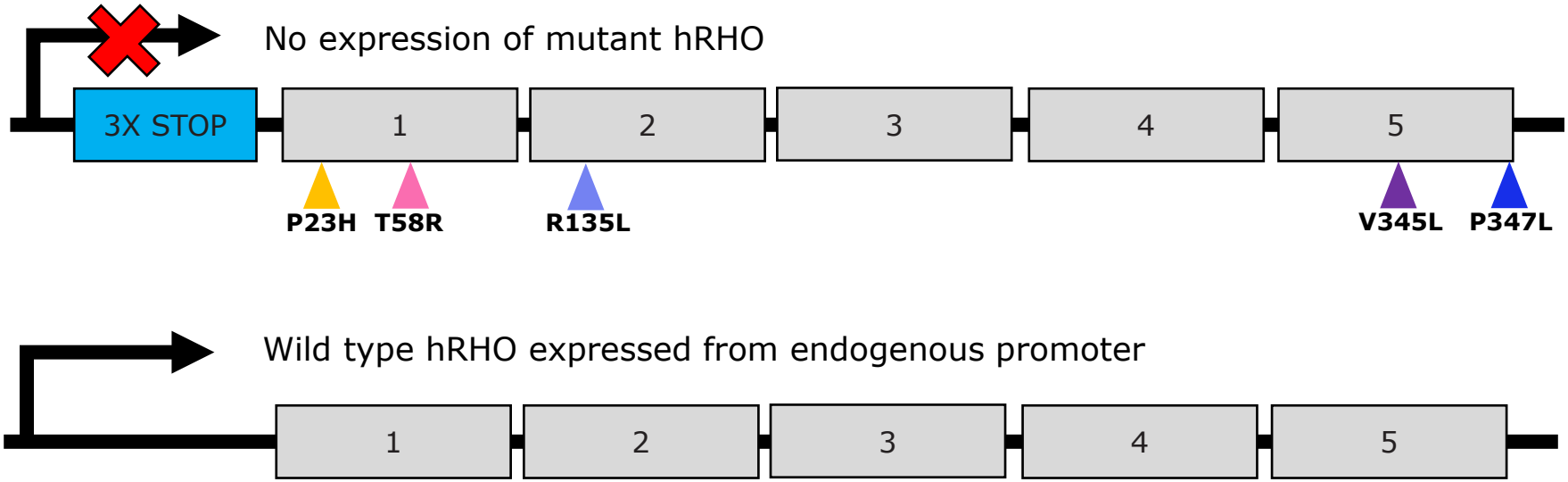
Prime Editors delivered to photoreceptors using a dual AAV system via subretinal injection



Precise correction in photoreceptors of humanized mice by Prime Editors

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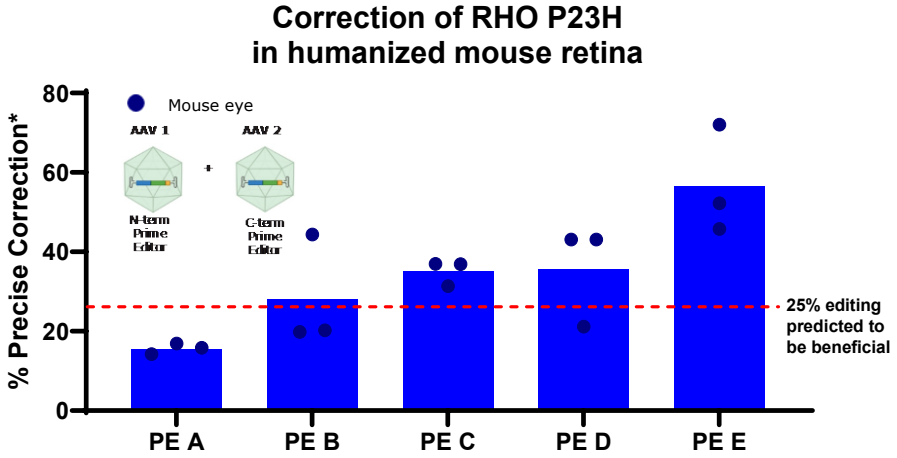
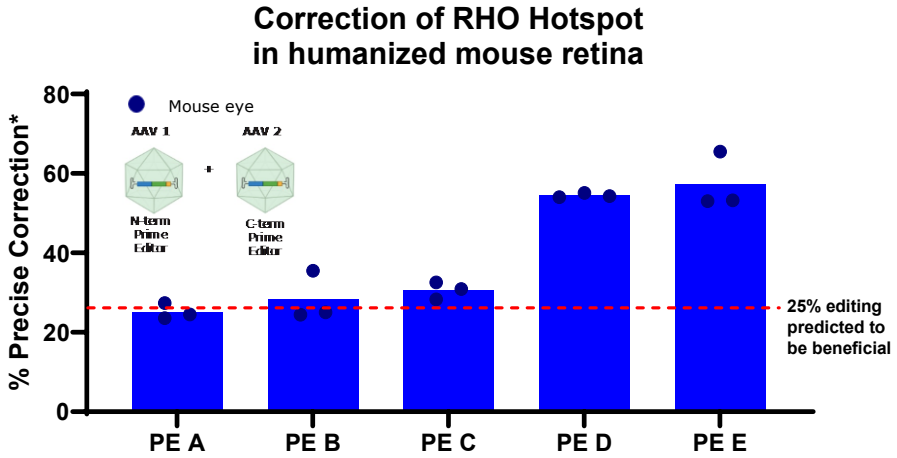
Up to 70% precise correction at RHO P23H and up to 65% at RHO hotspot with Prime Editors delivered subretinally using a dual AAV system

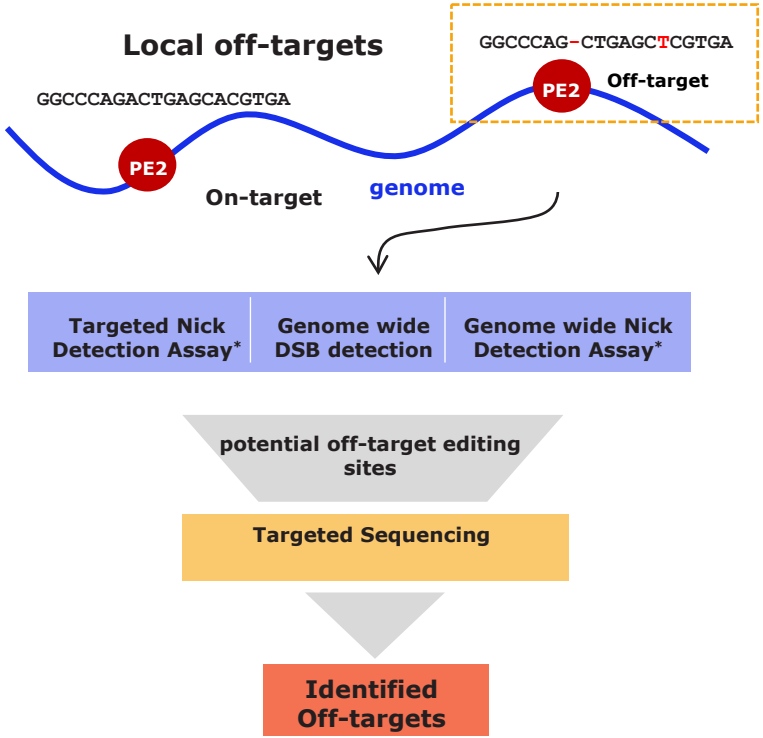


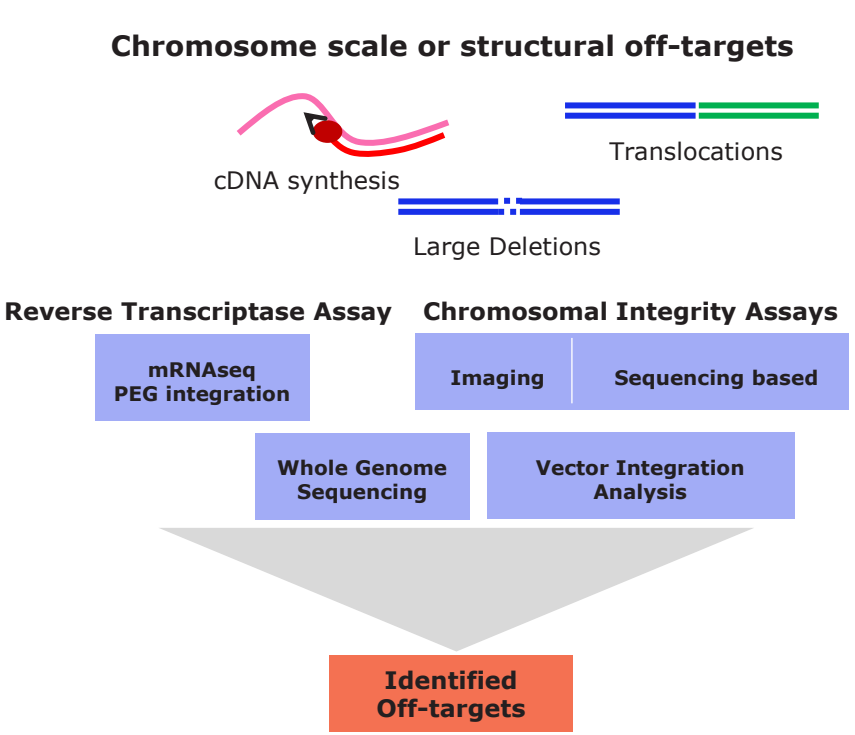


- Less than 0.5% on-target unintended edits were detected in photoreceptors
- Well tolerated with no detectable changes in retinal thickness or GFAP expression
- Prime Editors and AAVs are undergoing further optimizations

*Editing within transduced area of mouse retina; 25% is predicted to be therapeutically beneficial based on Cideciyan et al., 1998. PNAS 95, 7103-7108.

Safety: Prime's comprehensive suite of assays for off-target discovery





*Proprietary assay developed by Prime Medicine

New tools developed to identify potential Prime Editor off-target sites

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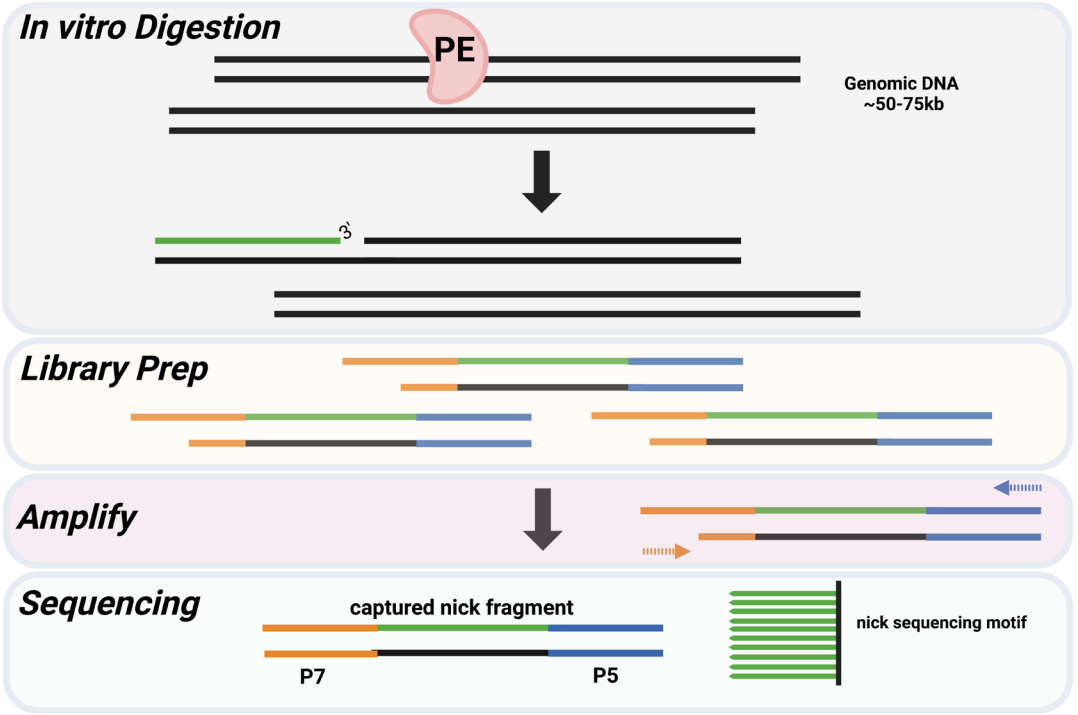
- Prime Editing generates single strand nicks in DNA, while most current off-target detection tools require double strand breaks
- PEG-seq is a genome wide approach that detects single strand nicks induced by gene editors and can identify potential off-targets of Prime Editors

3**P**rime **E**nd li**G**ation Sequencing (PEG-seq)



Preliminary analysis of potential sites does not detect off-target editing in human photoreceptors

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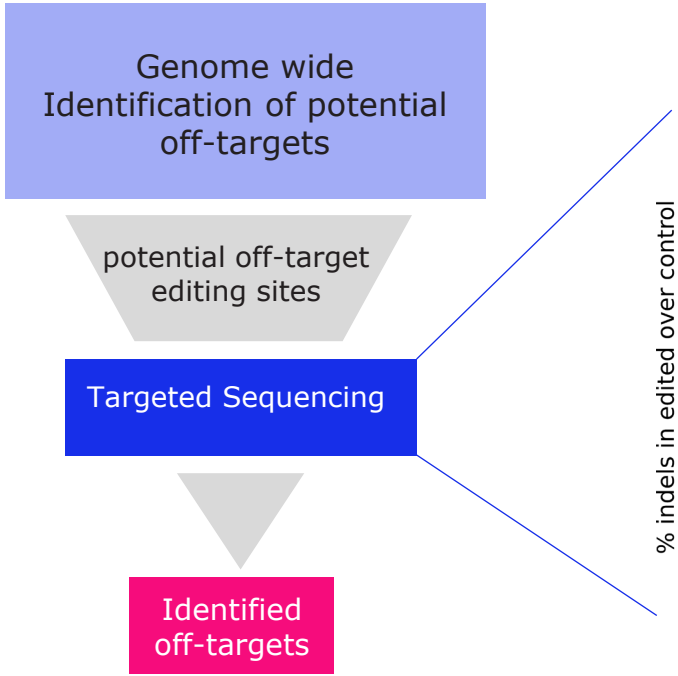
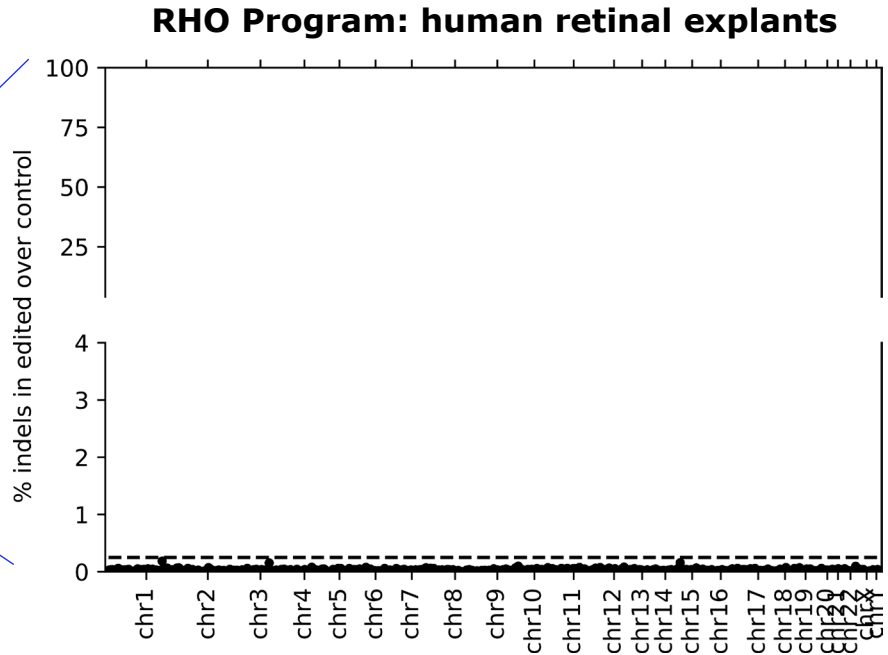
Performed using a lead RHO pegRNA and lead AAV delivery system





Analysis of AAV edited human retinal explant samples

Prime Editing does not result in measurable integration of AAV

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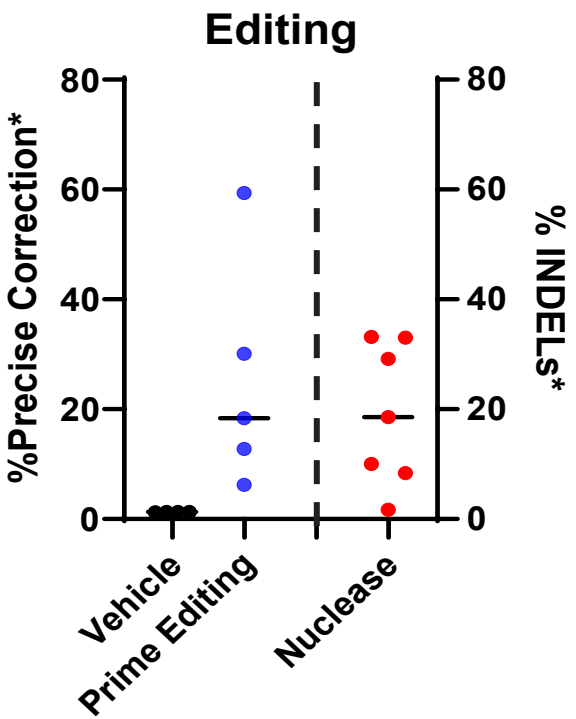
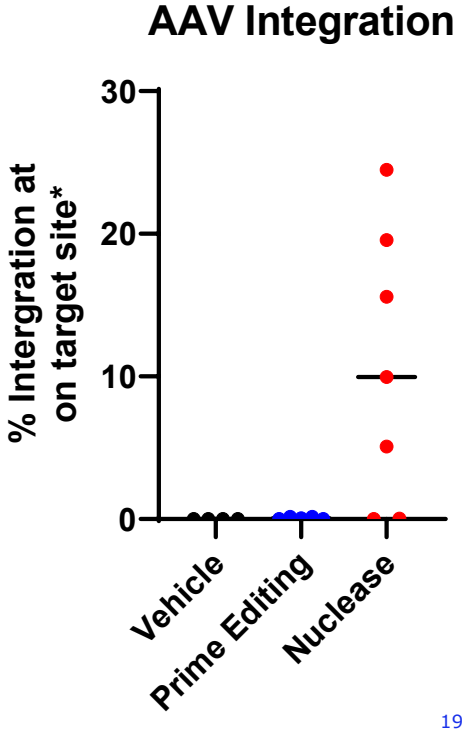
Prime Editing of mouse retina using dual AAV delivery does not result in detectable AAV genome integration

- Prime Editing of mouse retinas via subretinal delivery of AAV results in editing at a **non-RHO control genomic site** at similar rates to nuclease editors
- Prime Editing avoids measurable integration of the AAV vector at the edit site compared to AAV integration at the edit site in the presence of Cas9 nuclease**





*Editing/Integration within transduced area of mouse retina; **as measured by one-sided PCR

Humanized mouse models have been developed to temporally control expression of human mutant RHO

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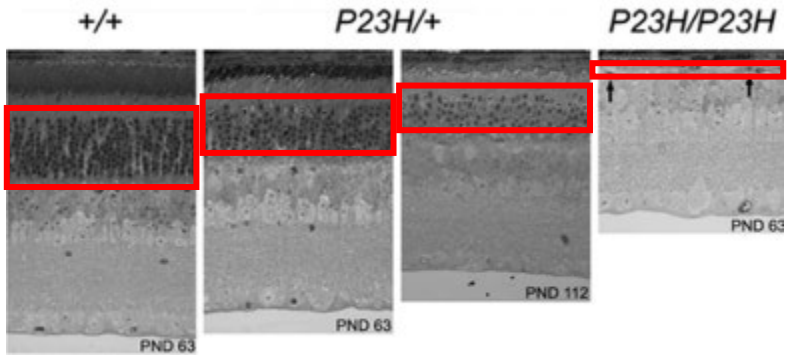
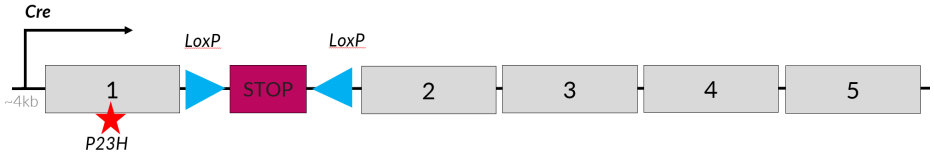
These models will be used to understand PK/PD relationships of Prime Editor leads or candidates

Rapid degeneration in heterozygous P23H mice



- Red boxes highlight the outer nuclear layer (ONL) which consists of photoreceptor nuclei
- As significant degeneration is expected postnatally in mice, we have developed a humanized mouse model with conditional expression RHO P23H

Prime has replaced the mouse *Rho* gene with the human *RHO* P23H gene, conditional upon Cre mediated excision of a stop codon



We are determining PK/PD relationships in response to Prime Editors in this model

Opportunity to conditionally activate human RHO P23H expression and study pharmacodynamic response to doses of Prime Editors

PND: Post natal day; PR: Photoreceptors; Sakami et al. THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 12, pp. 10551–10567, March 25, 2011

Conclusions

- Prime Editors **precisely correct predominant mutations including a mutational hotspot of 18 pathogenic mutations** in the *RHO* gene that cause RHO adRP
- Prime Editors **restore RHO protein localization** *in vitro*
- Prime's novel dual AAV delivery platform demonstrates efficient delivery to **both human (retinal explants) and murine (*in vivo*) photoreceptors**
- Prime has developed humanized mouse models understand **pharmacology of Prime Editors delivered** to the retina, target engagement and PK/PD relationships
- Developed new tools including **PEG-seq, a novel single strand break specific off-target method,** to identify potential Prime Editor safety risks
- Importantly, **no off-target edits and no AAV integrations** were detected in photoreceptors following Prime Editing

THANK YOU!

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