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In Vivo Genetic Eye Disease Correction Using Split AAV-Mediated Adenine Base Editing

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DISCLOSURE

- I am an employee and shareholder of Beam Therapeutics

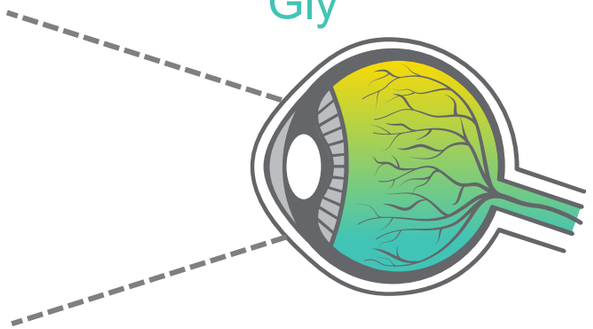
Base Editing For Stargardt Disease Correction

- Stargardt Disease (STGD) is an inherited blinding disorder that is characterized by progressive central vision loss
- Mutations in the ABCA4 (G1961E) gene cause the death of photoreceptors and retinal pigment epithelium (RPE)
- STGD affects ~100k people in developed countries[1]
  - The most prevalent mutation, **G1961E**, comprises 15% of all STGD patients[2]
- This G>A disease causing point mutation can be corrected via adenine base editing

Healthy Patient

GGA

Gly

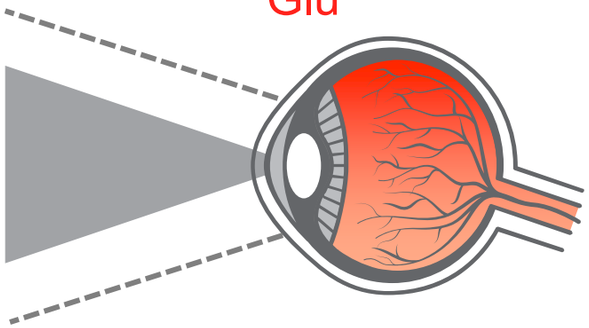


Clear Vision

STGD G1961E Patient

GAA

Glu



Impaired Central Vision

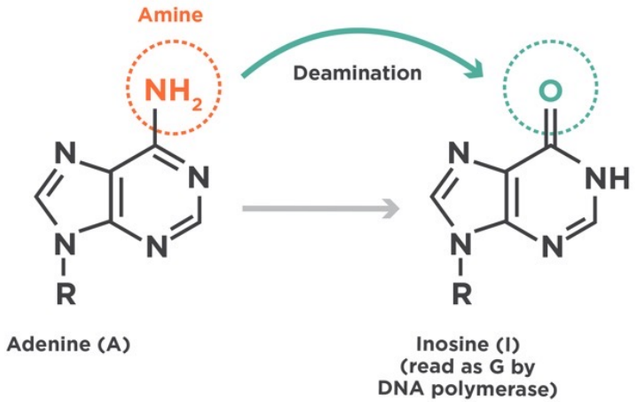
1. Stargardt Disease: Diagnosis, Causes & Treatment (clevelandclinic.org)
2. Fujinami, K. et al. Br J Ophthalmol 103, 390–397 (2019)

Base Editors Generate Permanent and Predictable Single Nucleotide Substitutions

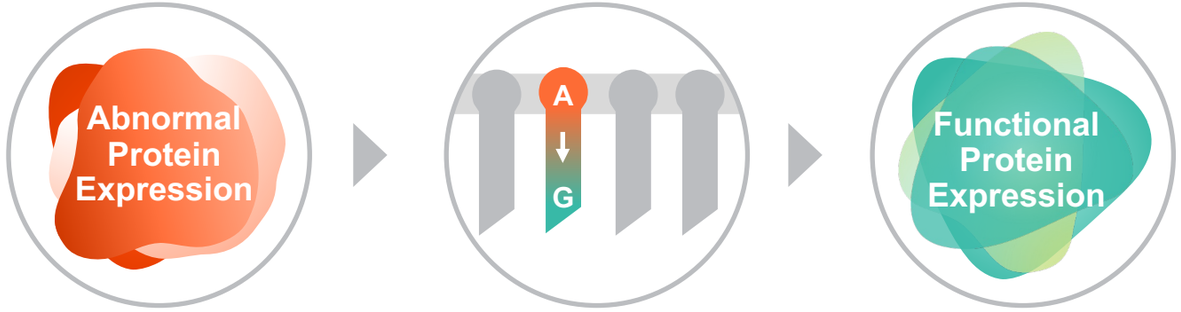
Base editor binds the target DNA and exposes a narrow editing window

A-to-G base editor ("ABE")

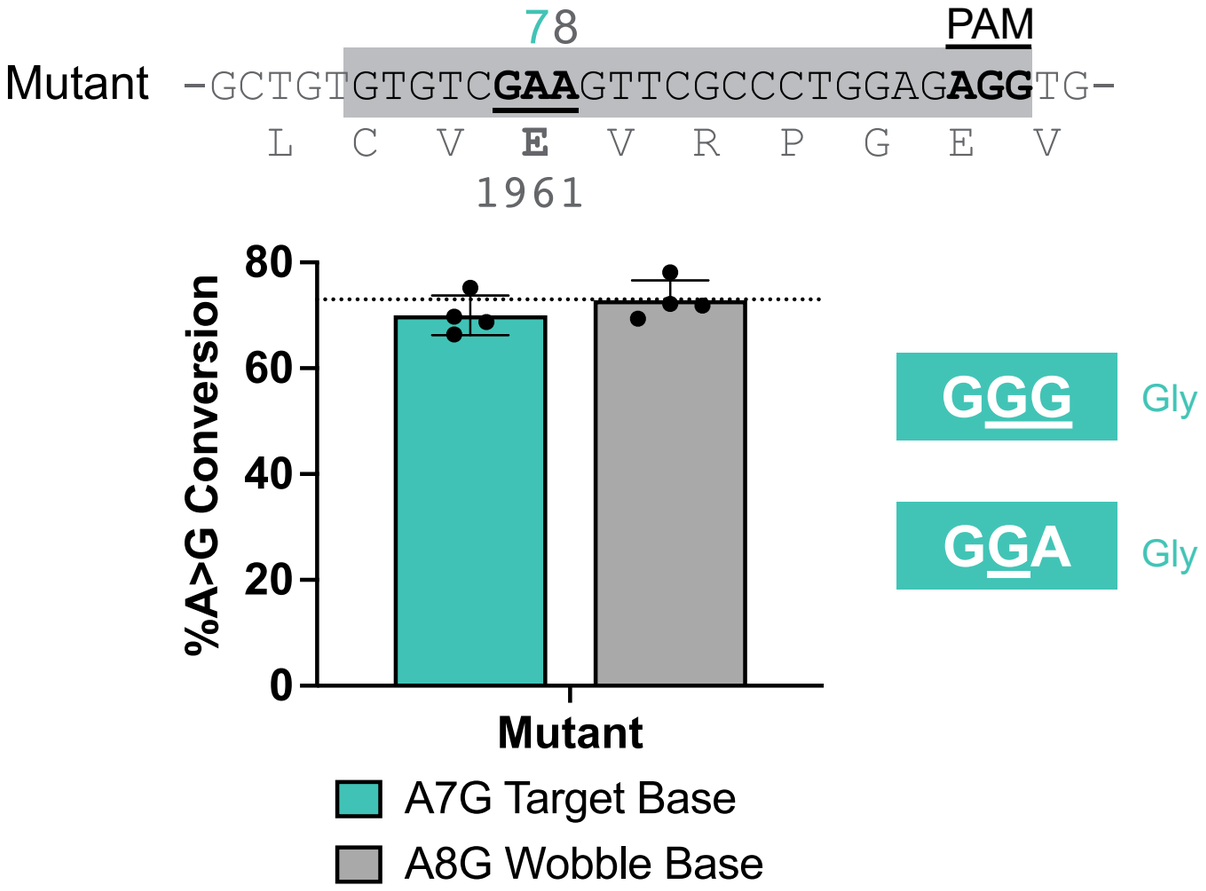
Deaminase chemically modifies target base, A>G edit made permanent by DNA repair/replication



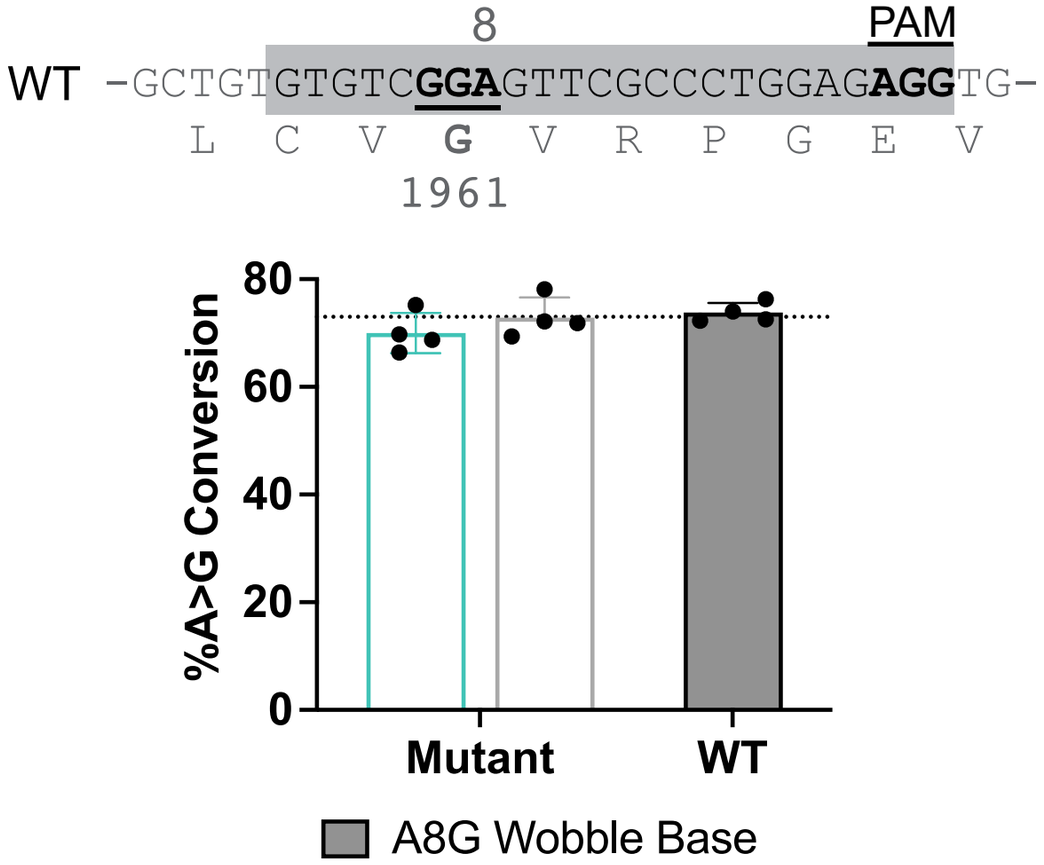
Gene Correction – Direct repair of point mutations to restore gene function



Dual Adenines In Codon 1961E Allows for Editing Assessment In Mutant and WT Tissue

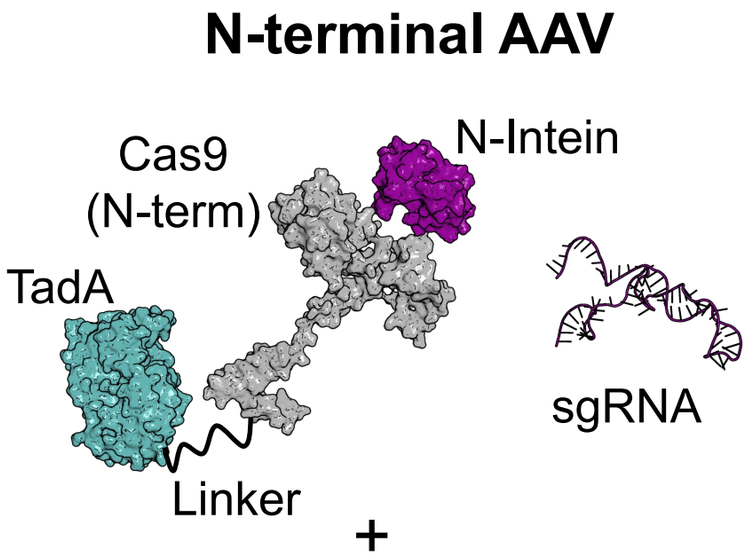


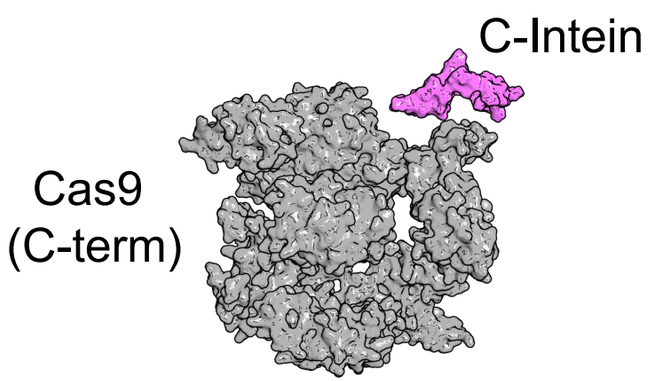
Editing of the target base alone or both the target base and wobble base leads to G1961E correction



A8G wobble base editing can be used as a surrogate for target base editing in WT models

Split Inteins Recombine Base Editors Post Translation And Maintain Editing Functionality





Split Inteins Recombine Base Editors Post Translation And Maintain Editing Functionality

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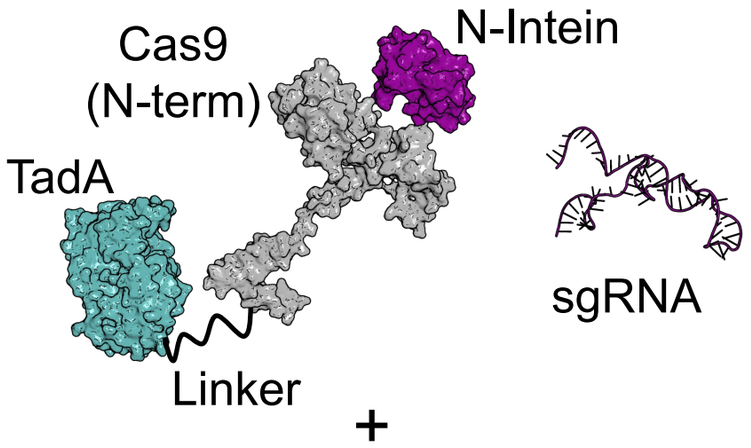
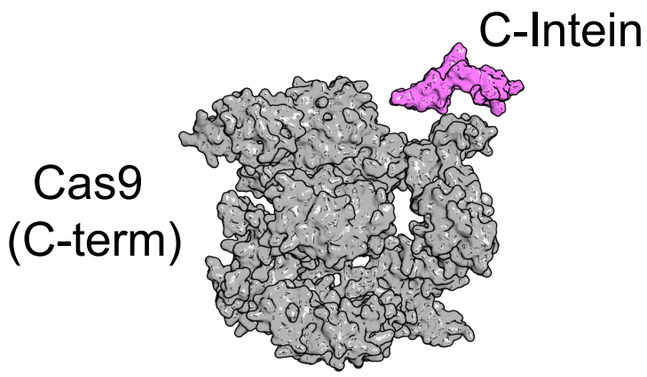
N-terminal AAV

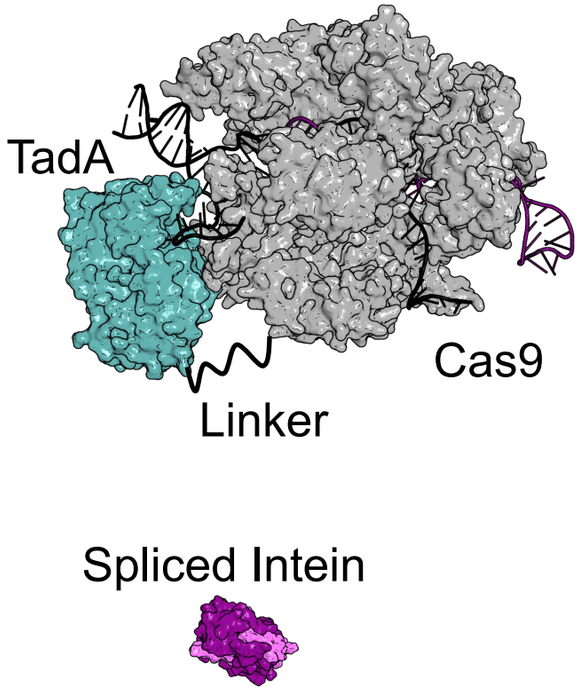


+

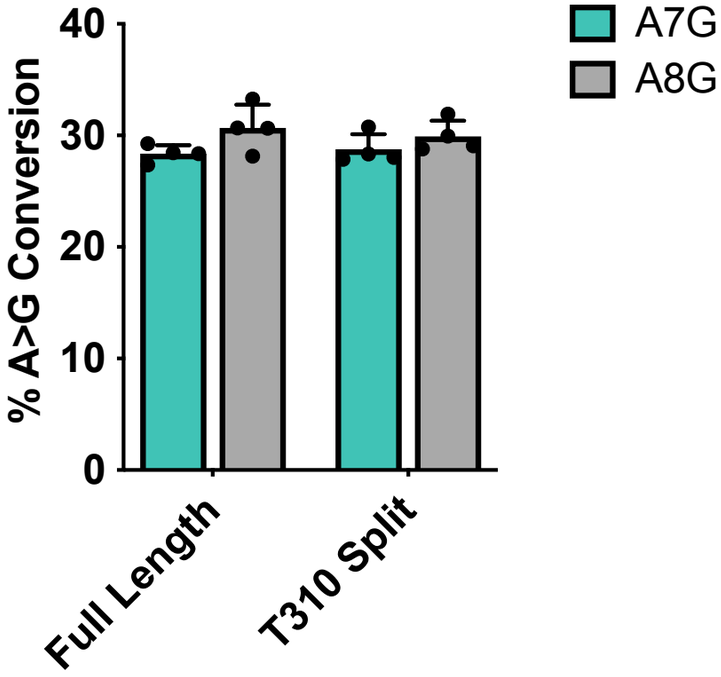
C-terminal AAV



ABE with dsDNA and sgRNA

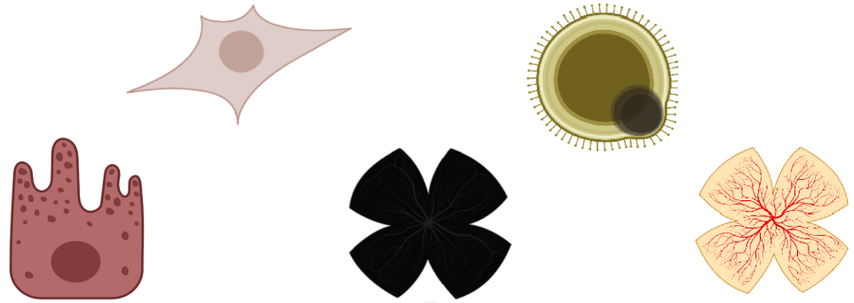
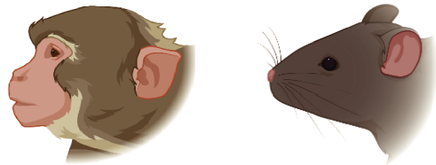
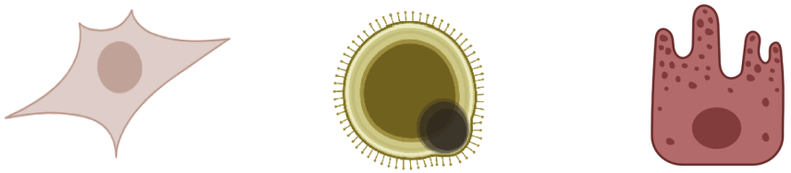
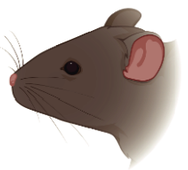


STGD 293T cells



Split base editors with inteins edit as efficiently as full-length editors by plasmid transfection

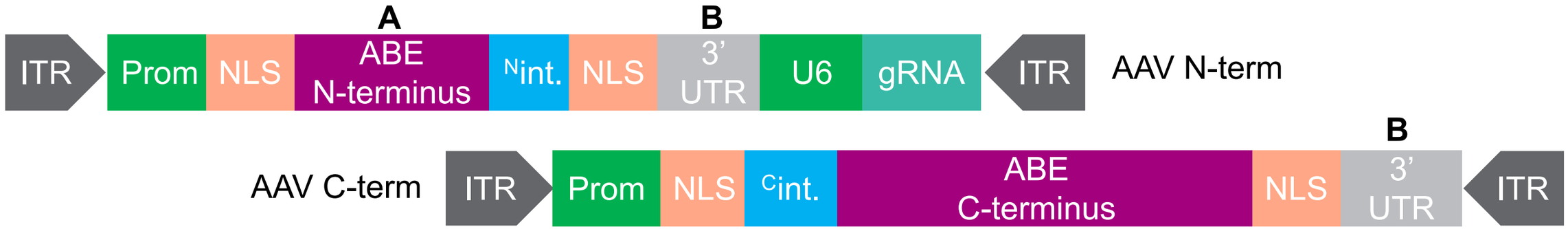
Multiple Model Systems Were Used to Optimize the Base Editor

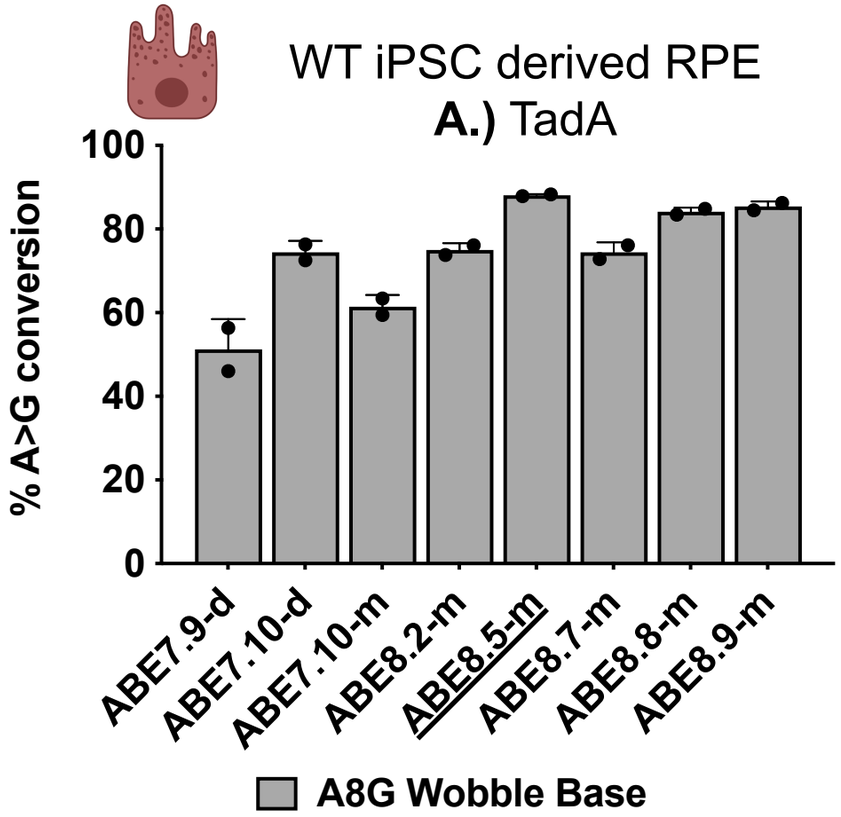
	In vitro	In vivo (SR Injection)
WT (A8G)		
Mutant (A7G)		

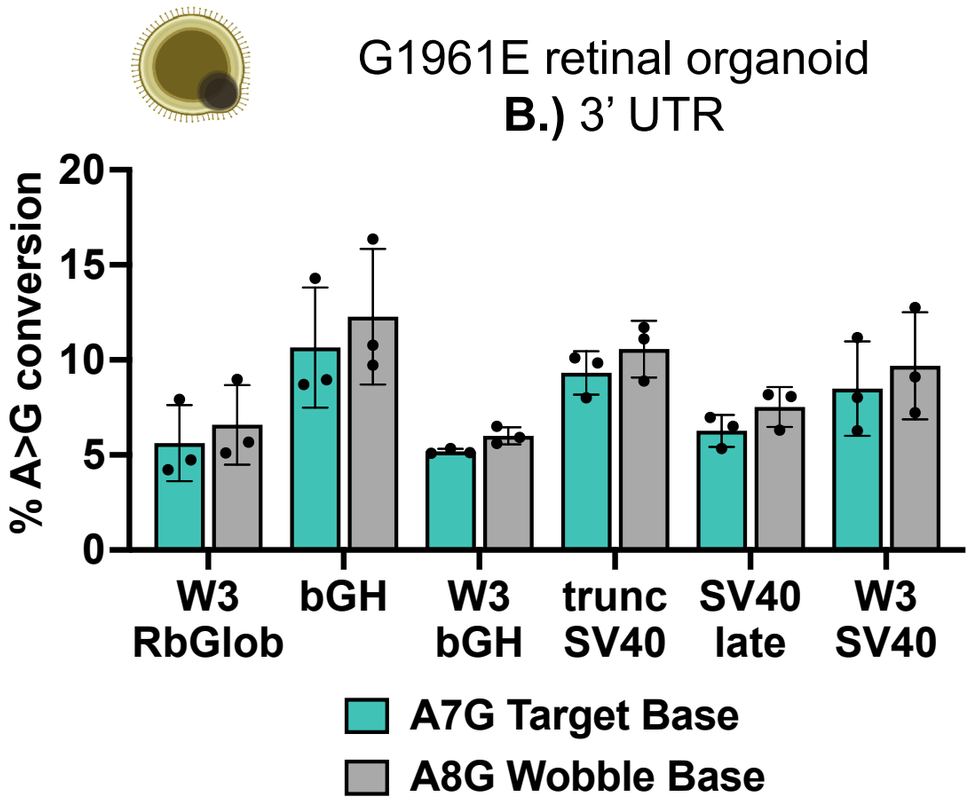
In vitro: 293T, human retinal organoids, iPSC derived RPE, human retinal explants, human RPE/choroid explants
In vivo: C57BL/6J mice (WT and *Abca4*^{huG1961E}), Non-human primates (cynomolgus macaques)

Made with BioRender

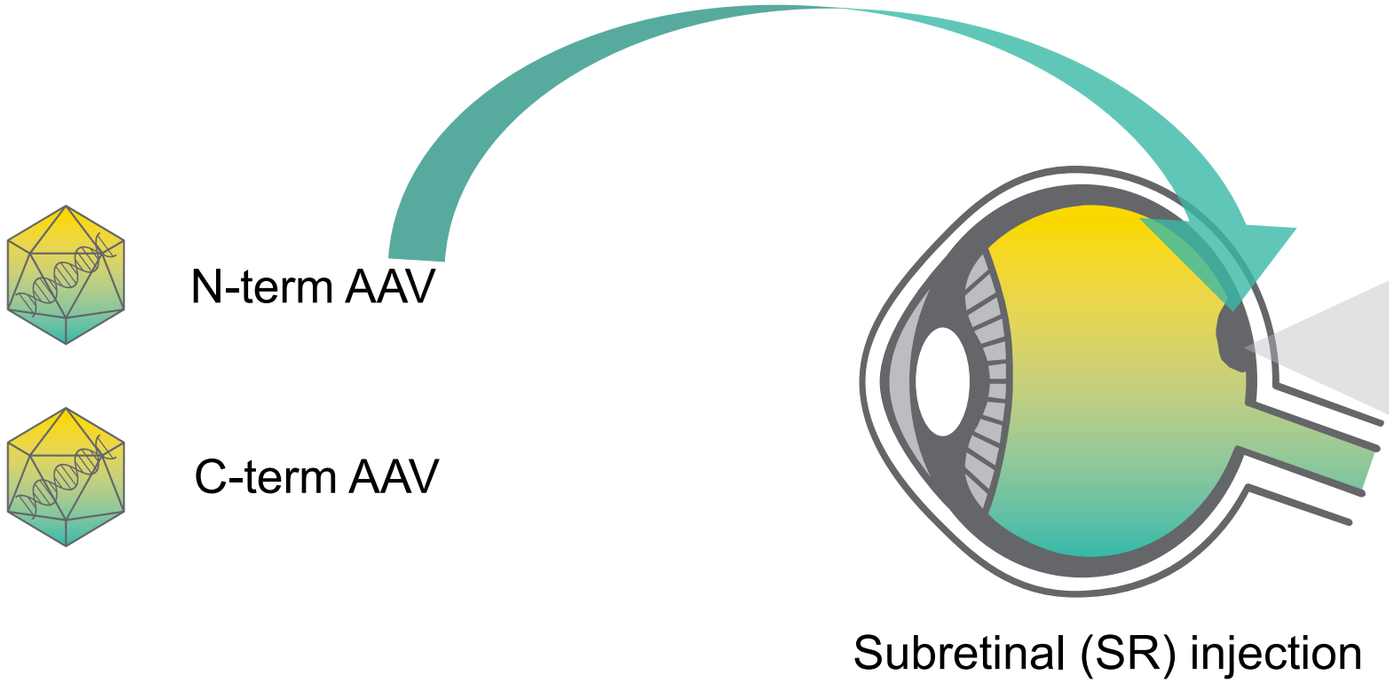
Editor Optimization in iPSC Derived RPE and Human Retinal Organoids



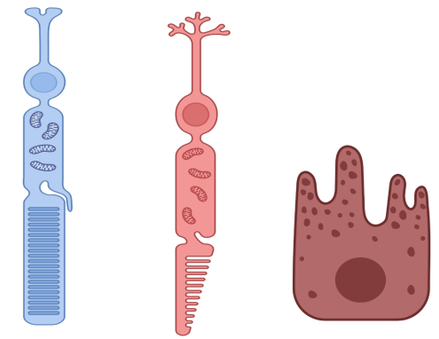




Photoreceptors and RPE Cells Can Be Base Edited Following Subretinal Injection of Split-AAV



- Subretinal injection is localized in a bleb between the RPE and photoreceptor outer segment, adjacent to the fovea
  - Fovea: cone rich region of the eye with highest visual acuity
- **Cone photoreceptors** and **RPE** are the primary cell types targeted for rescue in Stargardt Disease



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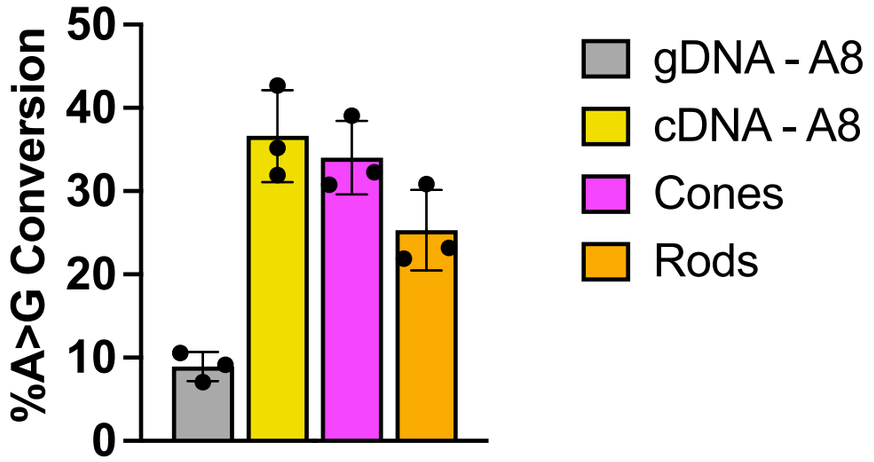
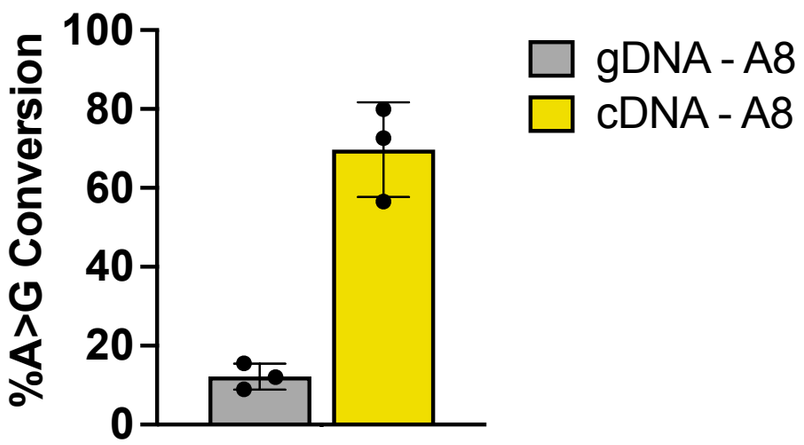
Optimized Editor And Capsid Delivery To Target Cells In Human Retinal Tissue

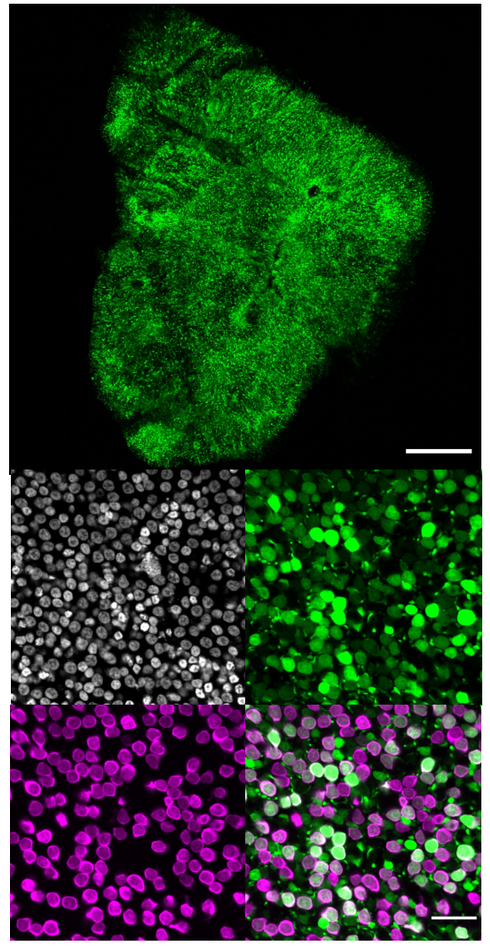
Human retinal explant



Human RPE/choroid explant



AAV5-CMV-eGFP
Hoescht, arrestin3, eGFP

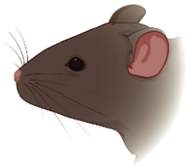


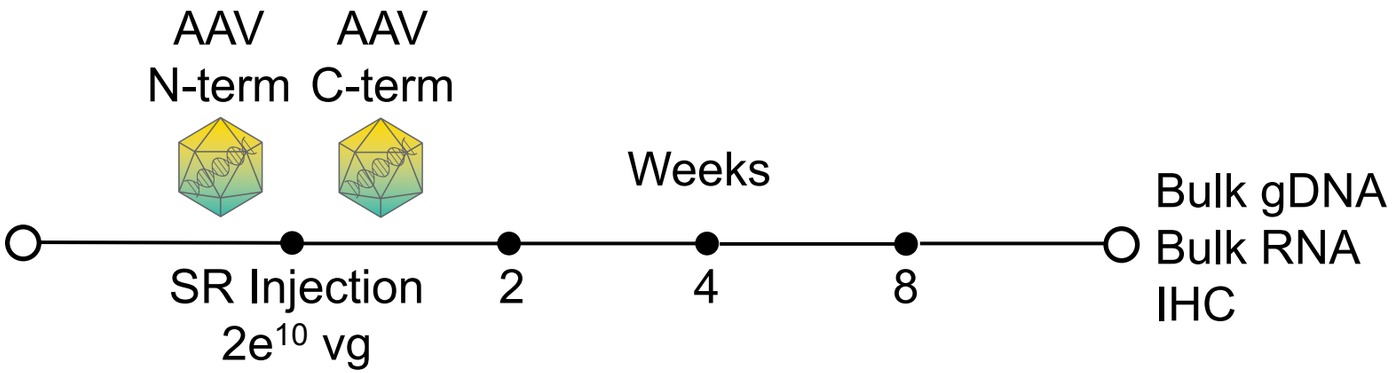
- Dual AAV5 transduction of human retina achieves >30% editing in cones
- RPE/choroid explants can reach up to 80% editing in the cDNA
  - ABCA4 is expressed in the RPE, but not the choroid
- Immunofluorescent imaging shows GFP delivery to cone cells by AAV5

Off-target analysis was done from edited human retina and RPE explants. There was no detectable off target editing seen across 418 sites tested.

Stargardt G1961E Humanized Mice Demonstrate In Vivo Allele Correction Using ABE

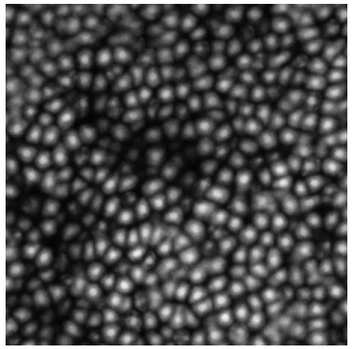
HuABCA4
G1961E KI



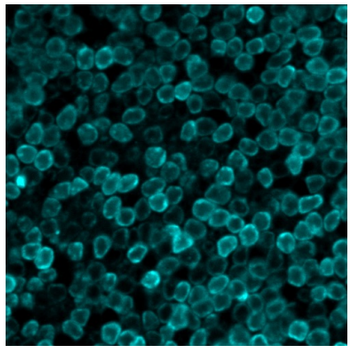


Retina

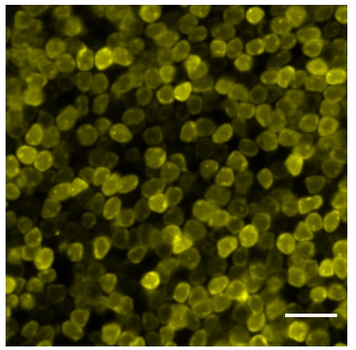
Hoechst

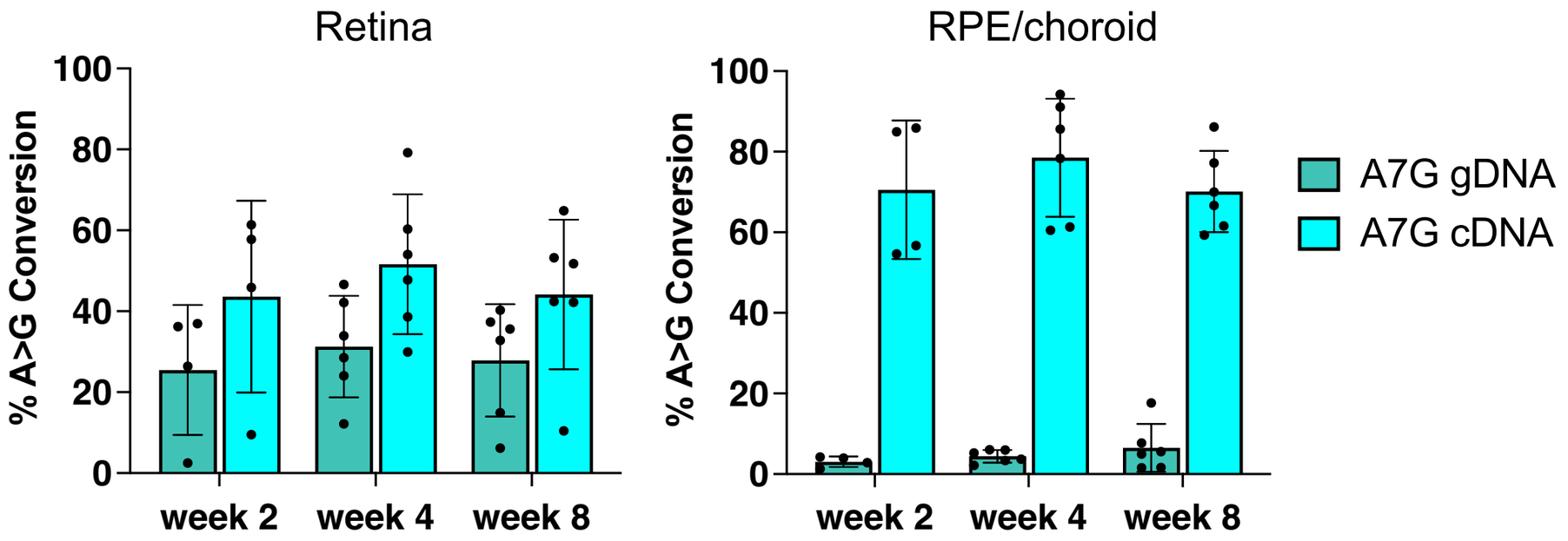


ABE(N)



ABE(C)





A7G gDNA
A7G cDNA

Subretinal Delivery to NHPs Achieves Therapeutically Relevant Levels of Base Editing in Cone and RPE Cells

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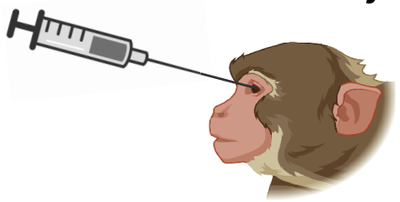
AAV5 SR Injection

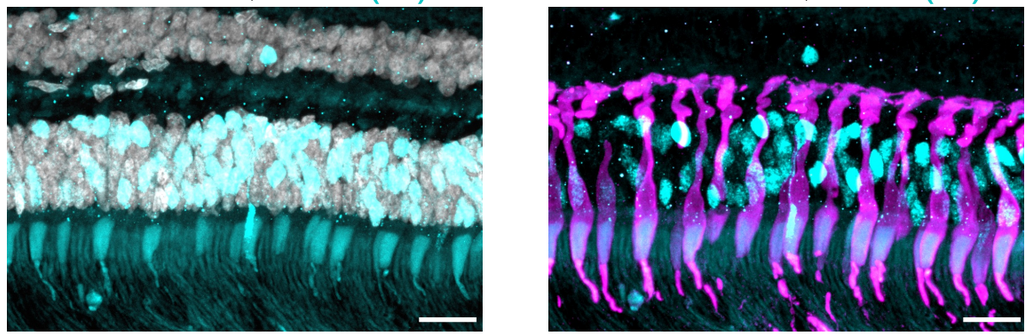


4-6 month incubation

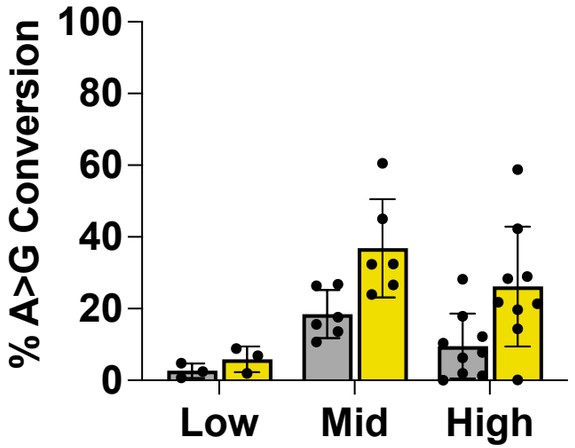
Low: $1e^{11}$ vg, Mid: $3e^{11}$ vg, High: $5e^{11}$ vg

Hoechst, ABE(N)

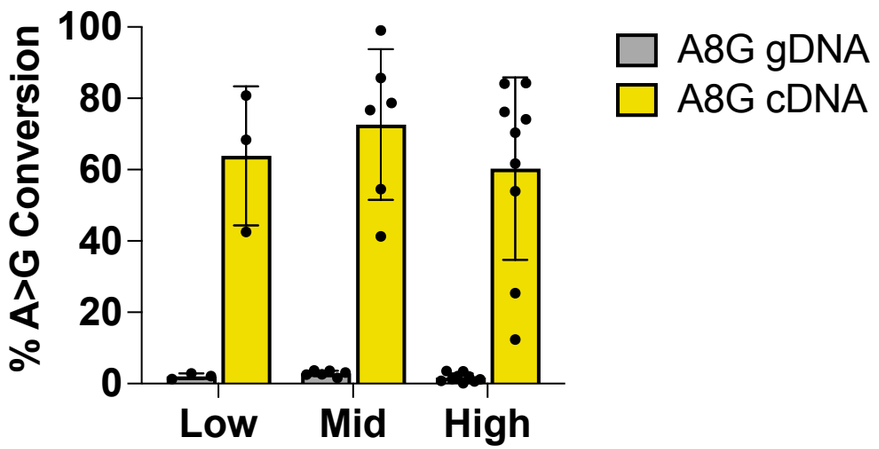
arrestin3, ABE(N)



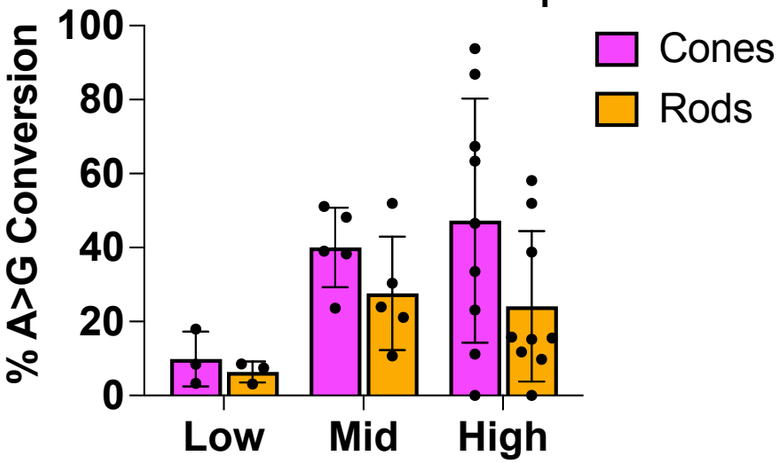
Bulk Retina



Bulk RPE/Choroid



Sorted Photoreceptors



- **Cone cells** are edited **>40%** at both the mid and high doses
- RPE cells are edited similarly across all doses

Clinically relevant levels of editing in cell types of interest of a mammal with a fovea

Conclusion: POC In Vivo Correction Of ABCA4 G1961E

- Editing optimization done in human retinal explants, iPSC derived RPE, and human retinal organoids can translate to in vivo data

- AAV5 delivers base editors to **photoreceptors** and **RPE** via subretinal delivery
  - **Base correction strategy allows for endogenous control of expression**
  - Corrected protein in desired location within cells types of interest

- Stargardt ABCA4 G1961E can be corrected in relevant cells of mutation-carrying mice

- A surrogate base can be efficiently base edited in NHPs and demonstrates feasibility in a mammalian model containing a fovea

- **It is estimated that 10-20% rescue of cones will be disease modifying[3]; we have achieved on average 40% editing of cones in NHP at therapeutically relevant doses**

3. Geller, A. *Vision Res*. Vol. 33, No. I I, pp. 1509-1524, 1993

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Thank You!

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BioRxiv publication
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