Efficient *In Vivo* Gene Editing of Inherited Retinal Disease Genes in Mice and Non-Human Primates

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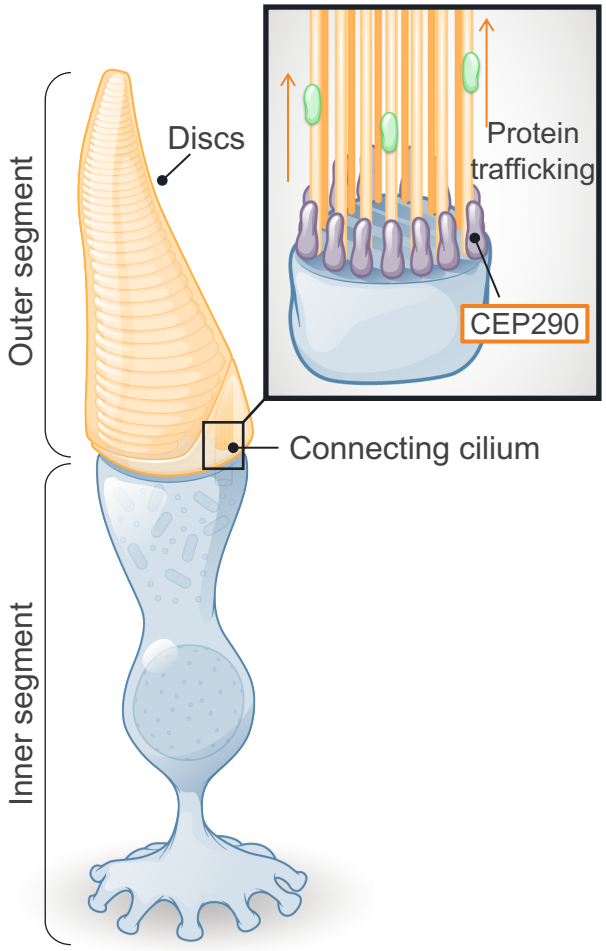
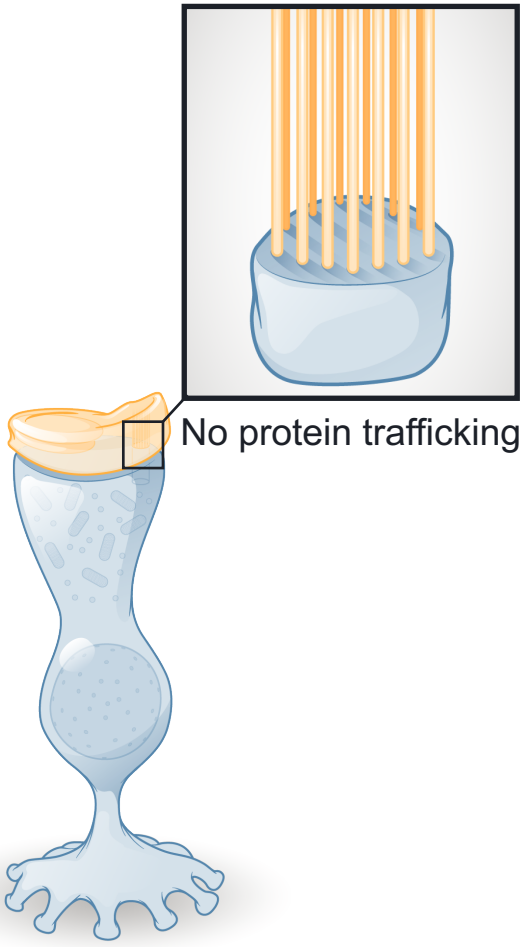
CEP290 in Ciliary Trafficking and Phototransduction

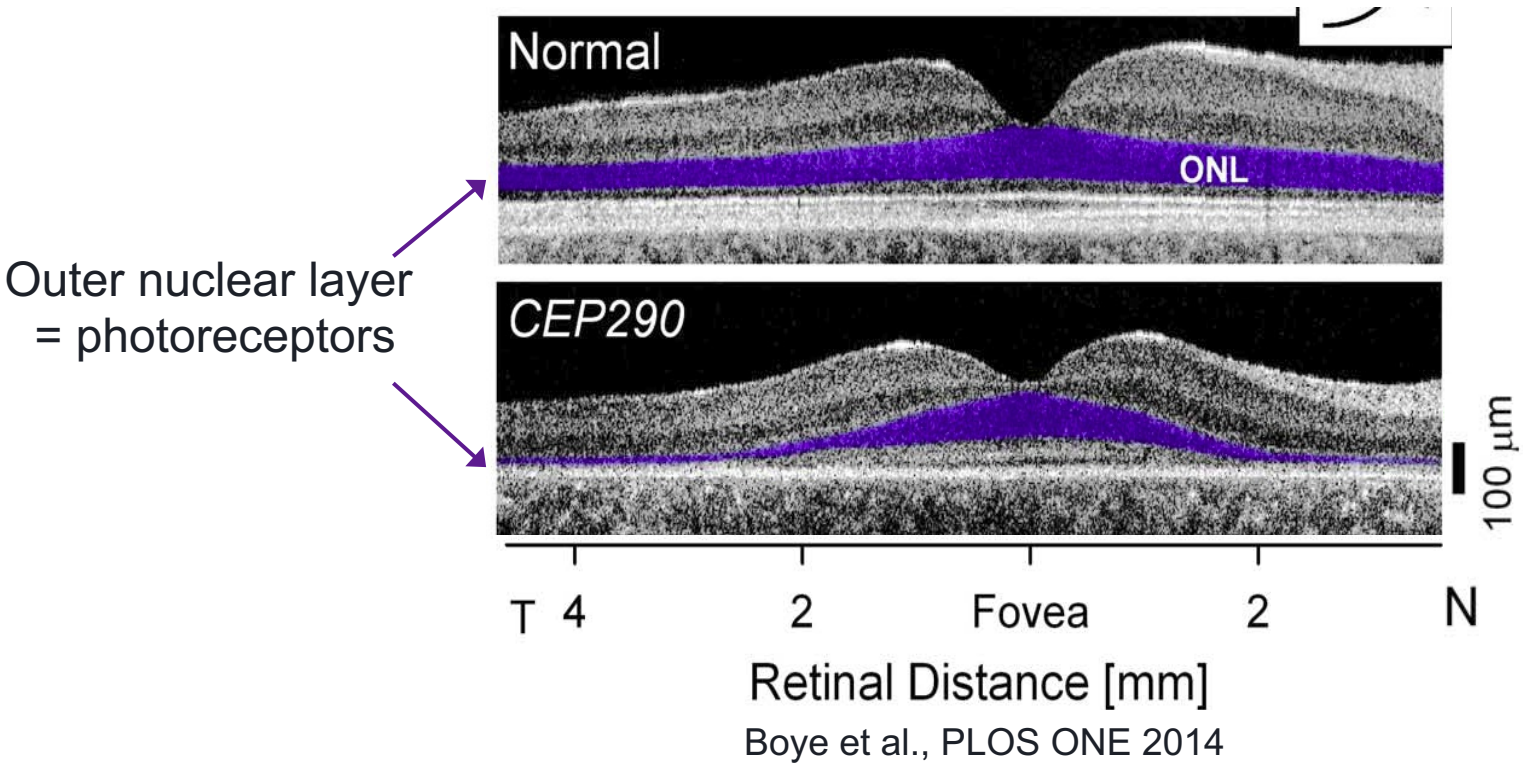
WT Photoreceptor



LCA10 Photoreceptor



Potential to Rescue Surviving Photoreceptors

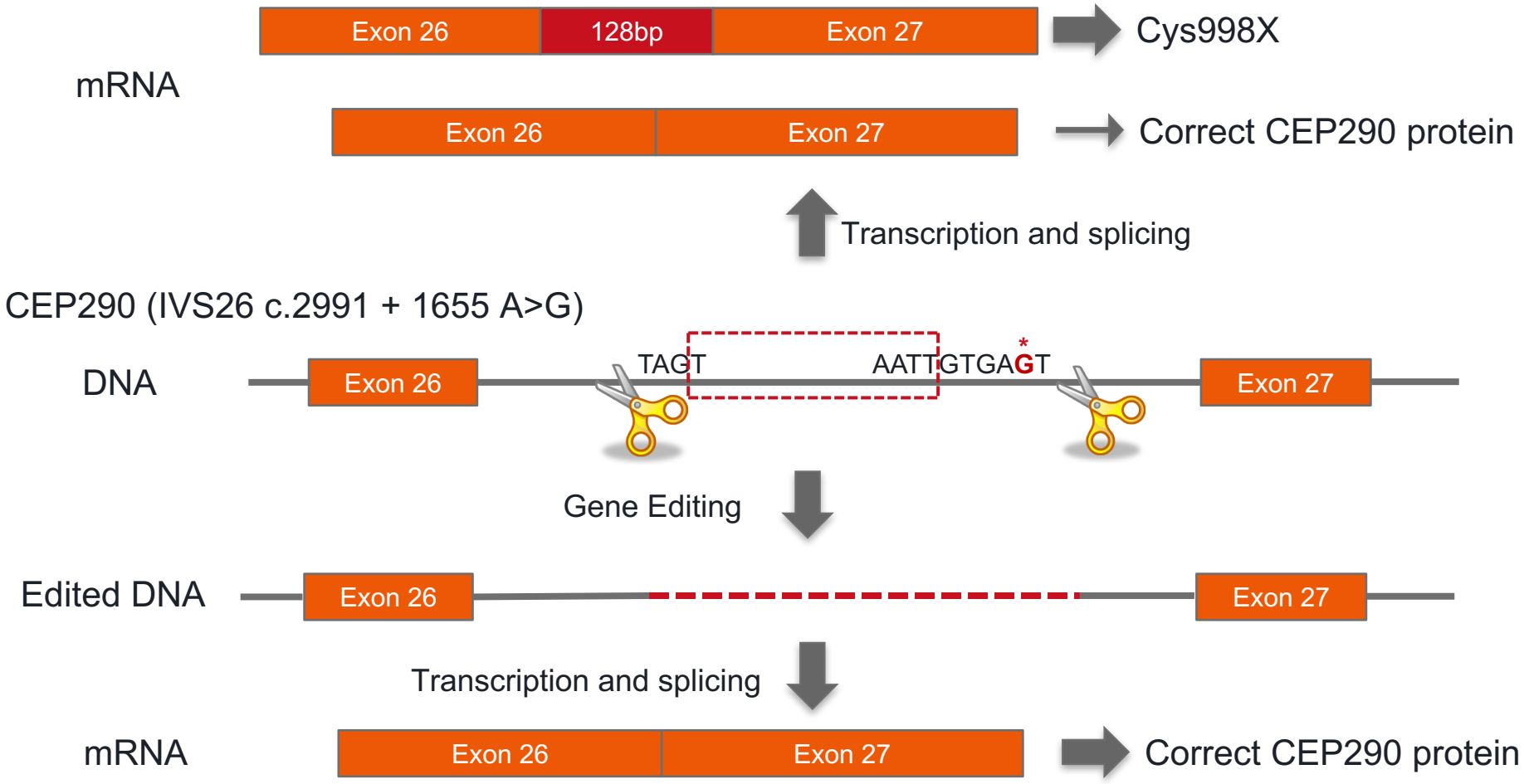


Boye et al., PLOS ONE 2014

- It is estimated that visual acuity can be achieved with ~10% of functioning photoreceptors[1,2]

1.Geller, Sieving and Green, *J. Opt. Soc. Am.*, 1992
2.Geller and Sieving, *Vision Res.*, 1993

Gene Editing to Repair *CEP290* Splicing Defect



Demonstration of *in vivo* Photoreceptor Editing in Mice

SCIENTIFIC REPORTS

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Targeted *RP9* ablation and mutagenesis in mouse photoreceptor cells by CRISPR-Cas9

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Research

Genome surgery using Cas9 ribonucleoproteins for the treatment of age-related macular degeneration

Kyoungmi Kim,[1,6] Sung Wook Park,[2,3,6] Jin Hyoung Kim,[3] Seung Hwan Lee,[1] Daesik Kim,[1,4] Taeyoung Koo,[1] Kwang-eun Kim,[1,4] Jeong Hun Kim,[2,3,5] and Jin-Soo Kim[1,4]

Genetics

AAV-Mediated CRISPR/Cas Gene Editing of Retinal Cells In Vivo

Sandy S. C. Hung,[1] Vicki Chrysostomou,[1] Fan Li,[1,2] Jeremiah K. H. Lim,[3] Jiang-Hui Wang,[1] Joseph E. Powell,[4,5] Leilei Tu,[1,6] Maciej Daniszewski,[1] Camden Lo,[7] Raymond C. Wong,[1] Jonathan G. Crowston,[1] Alice Pébay,[1] Anna E. King,[8] Bang V. Bui,[3] Guei-Sheung Liu,[1] and Alex W. Hewitt[1,2]

nature COMMUNICATIONS

ARTICLE

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Nrl knockdown by AAV-delivered CRISPR/Cas9 prevents retinal degeneration in mice

Wenhan Yu[1], Suddhasil Mookherjee[1], Vijender Chaitankar[2], Suja Hiriyanna[1], Jung-Woong Kim[2], Matthew Brooks[2], Yasaman Ataeijannati[1], Xun Sun[2], Lijin Dong[3], Tiansen Li[2], Anand Swaroop[2] & Zhijian Wu[1]

original article

CRISPR Repair Reveals Causative Mutation in a Preclinical Model of Retinitis Pigmentosa

Wen-Hsuan Wu[1,2,3], Yi-Ting Tsai[1,2,3], Sally Justus[1,2,3], Ting-Ting Lee[1,2,3], Lijuan Zhang[1,2,3,4], Chyuan-Sheng Lin[5], Alexander G Bassuk[6], Vinit B Mahajan[7,8] and Stephen H Tsang[1,2,3]

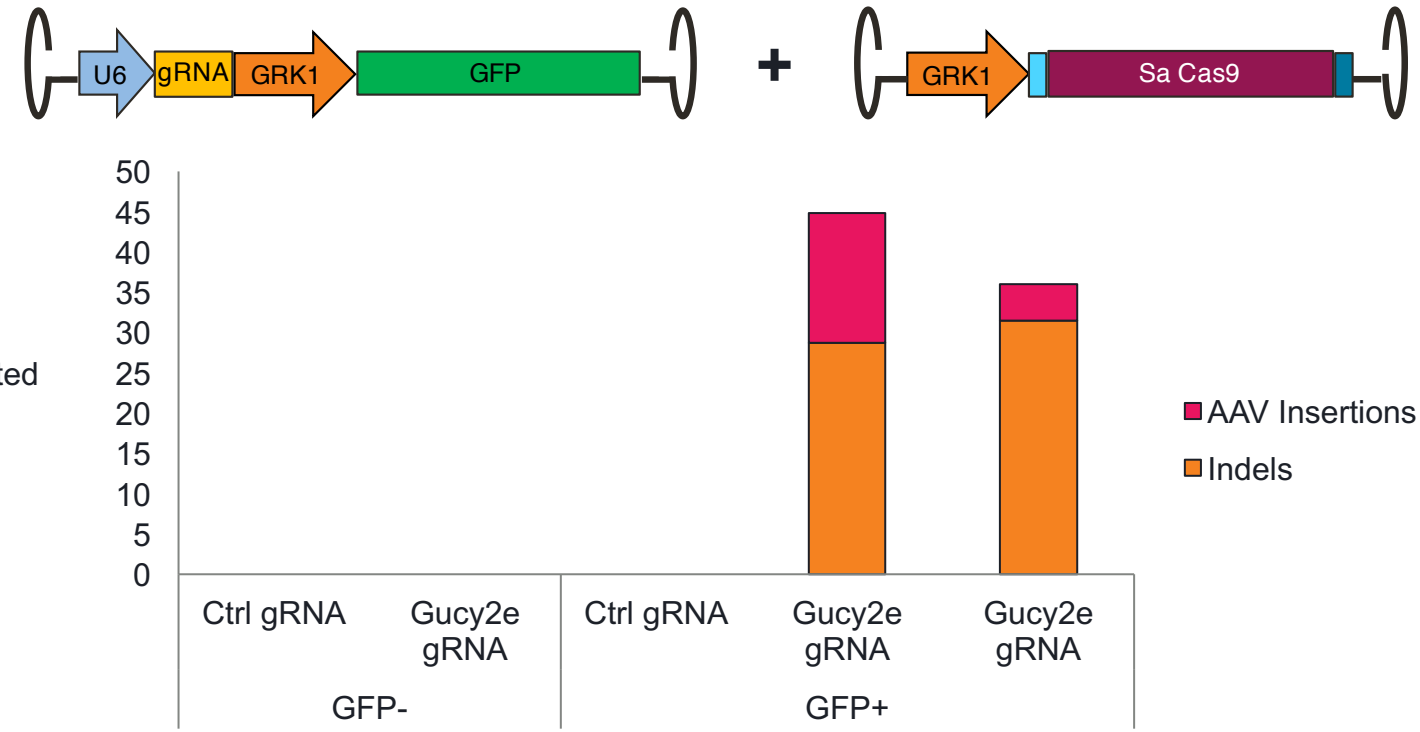
Citation: Molecular Therapy—Nucleic Acids (2016) 5, e389; doi:10.1038/mtna.2016.92
Official journal of the American Society of Gene & Cell Therapy
www.nature.com/mtna

In vivo Editing of the Human Mutant *Rhodopsin* Gene by Electroporation of Plasmid-based CRISPR/Cas9 in the Mouse Retina

Maria Carmela Latella[1], Maria Teresa Di Salvo[2], Fabienne Cocchiarella[1], Daniela Benati[1], Giulia Grisendi[3], Antonella Comitato[2], Valeria Marigo[2] and Alessandra Recchia[1]

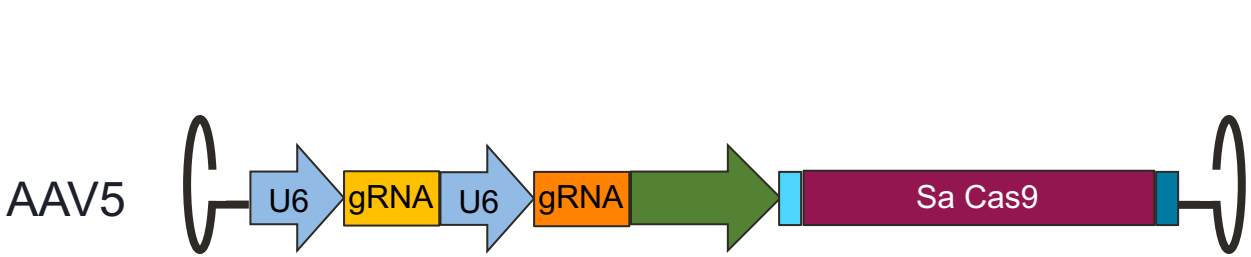
Efficient *in vivo* Editing of Photoreceptors in Mice

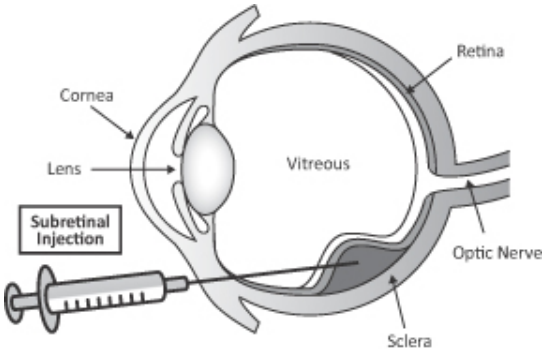
- Guanylate cyclase 1 encoded by *Gucy2e* gene in mouse, *GUCY2D* gene in primates
- Recessive mutations cause LCA1 and dominant mutations cause CORD6



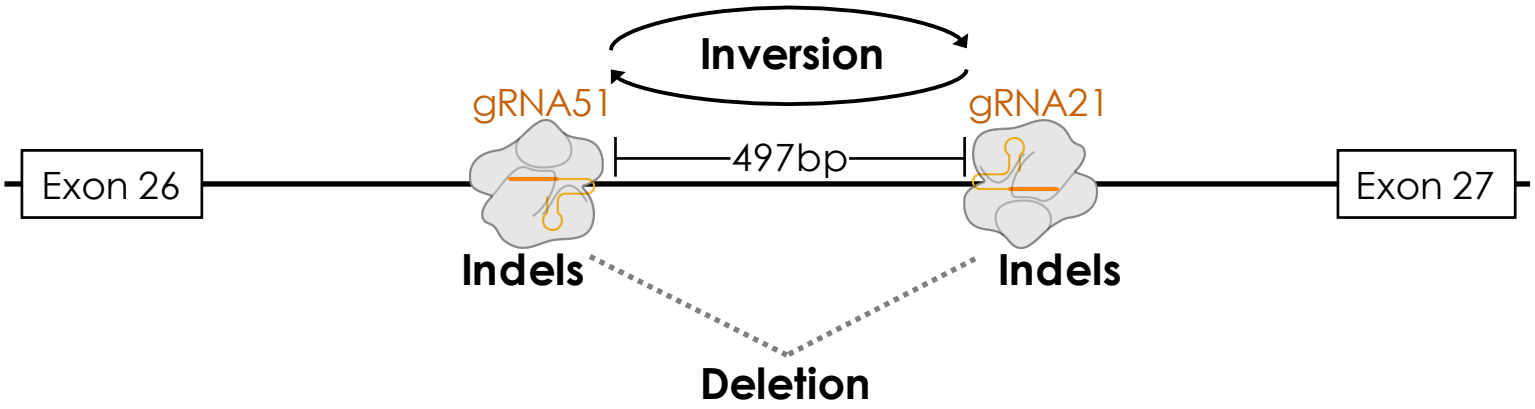
But would it work in primates?

Non-Human Primate Pilot Pharmacology Study

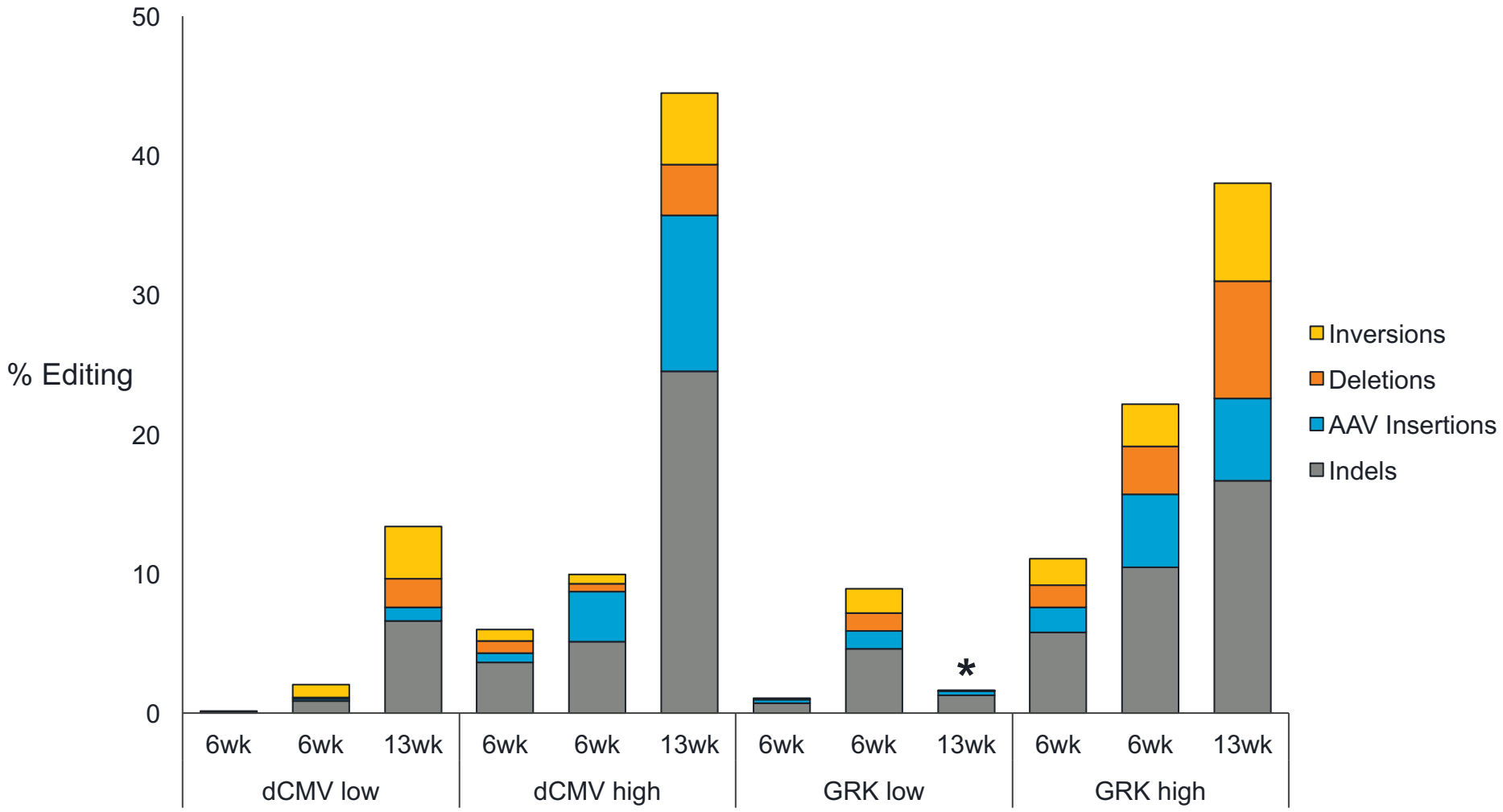




- Ubiquitous (dCMV) or photoreceptor-specific (GRK1) promoters
- 2 doses: 4E10 and 4E11 vg/eye
- 2 timepoints: 6 and 13 weeks

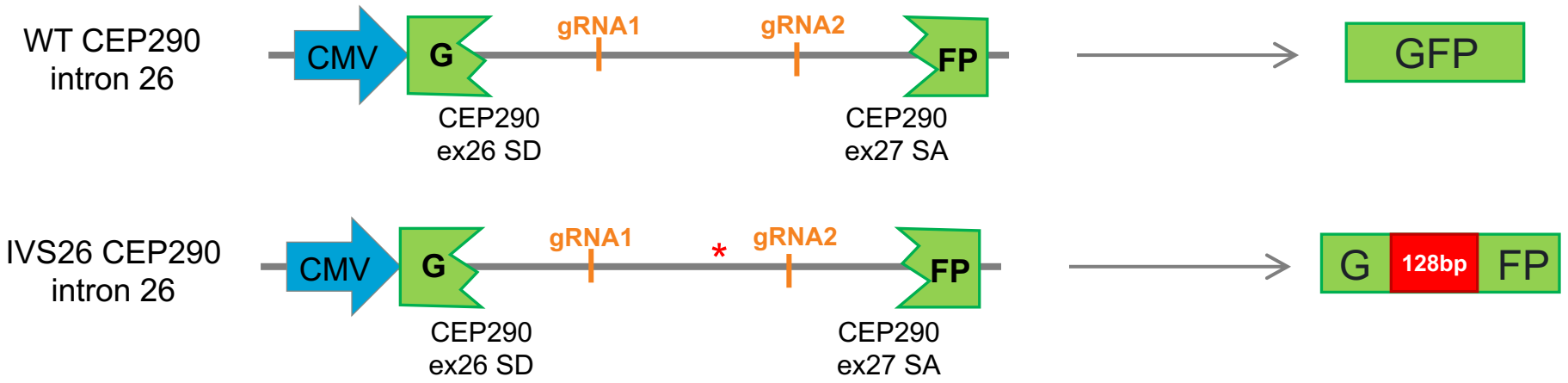


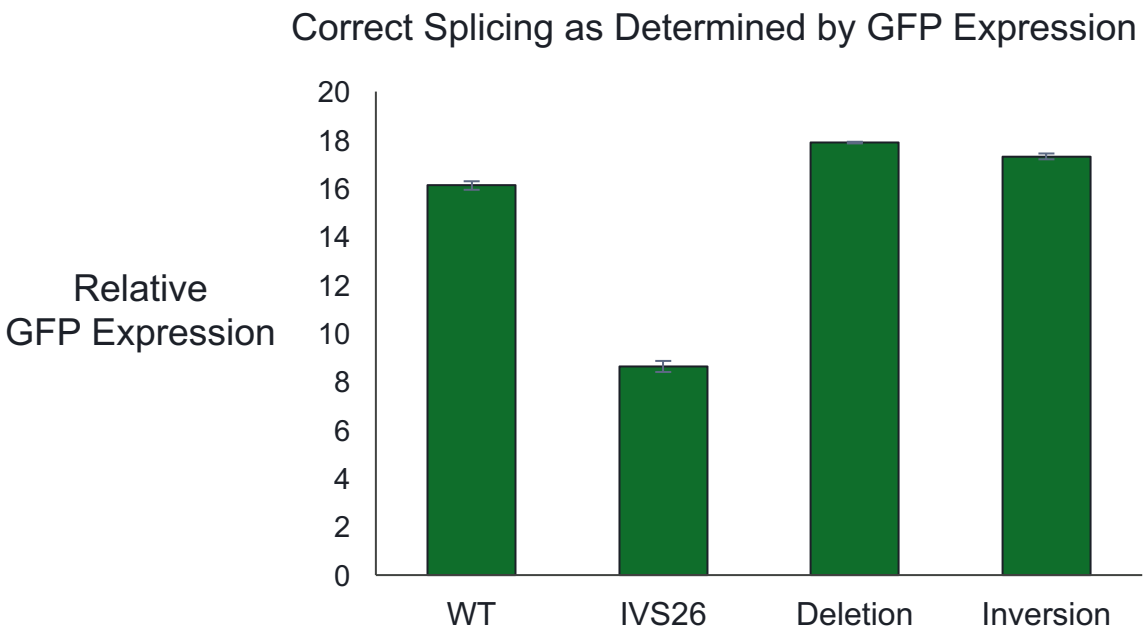
Targeted *CEP290* Editing in Primate Retina



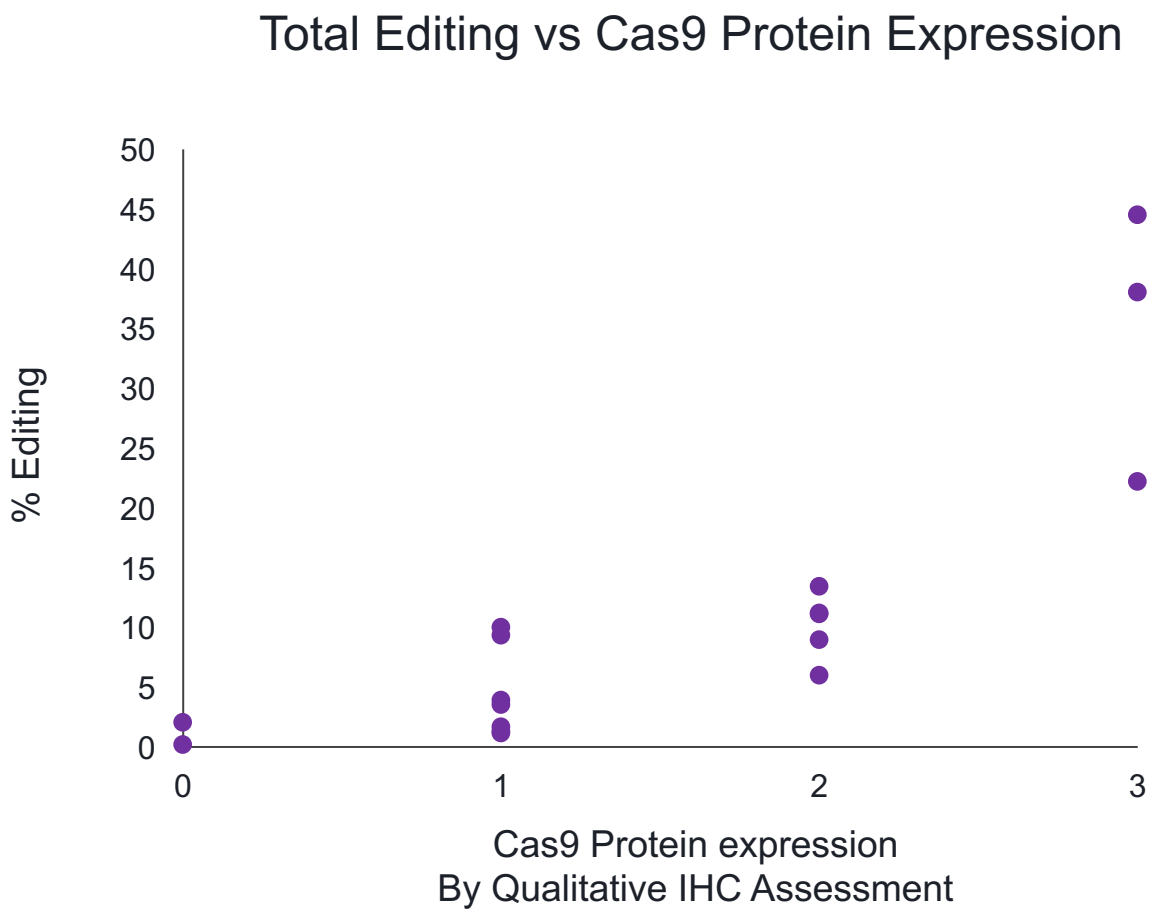
* Bleb not positively identified, punch taken at estimated site of injection

Targeted Deletions and Inversions Correct Splicing





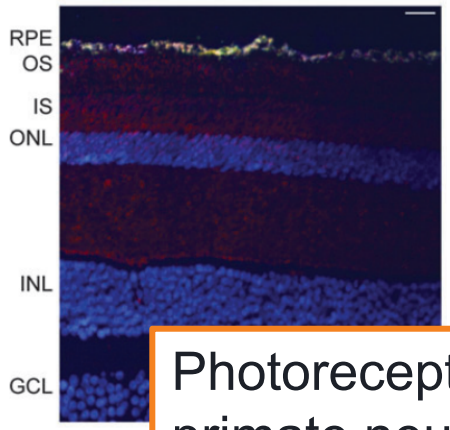
Editing Correlates with Cas9 Expression Level



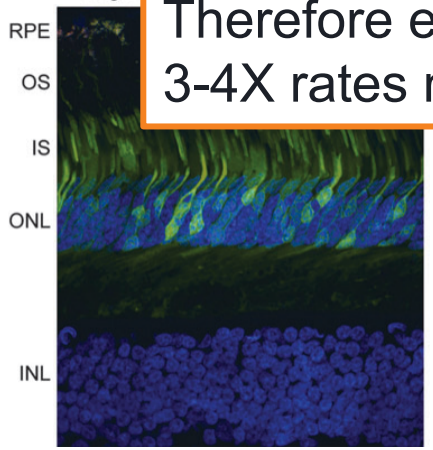
GRK1 Promoter in AAV5 Vector Confers Photoreceptor-Specific Expression

Rod and cone-specific expression of AAV5-GRK1-GFP demonstrated by Boye et al., HGT 2012

Untransduced (20X)

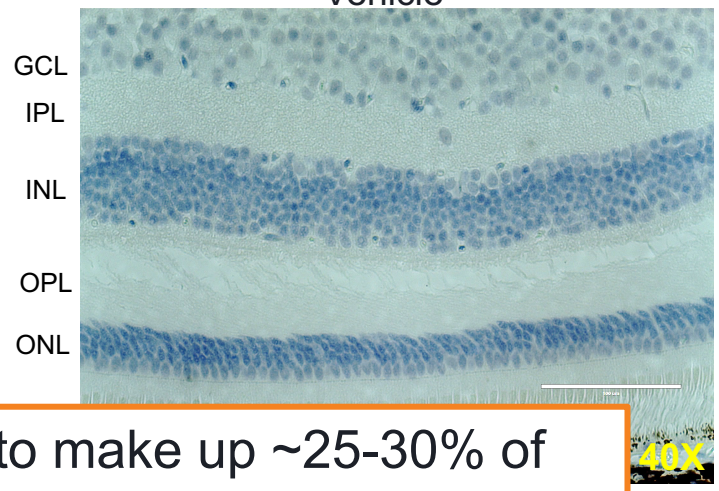


AAV5

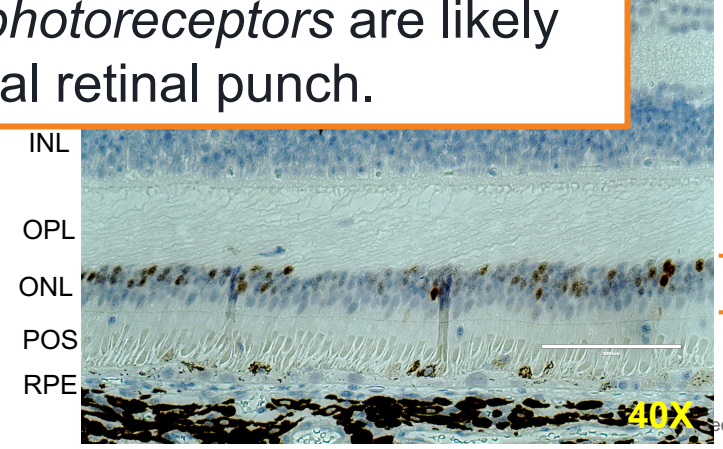


Cas9 expression detected only in ONL by IHC

Vehicle



Cas9 (high)



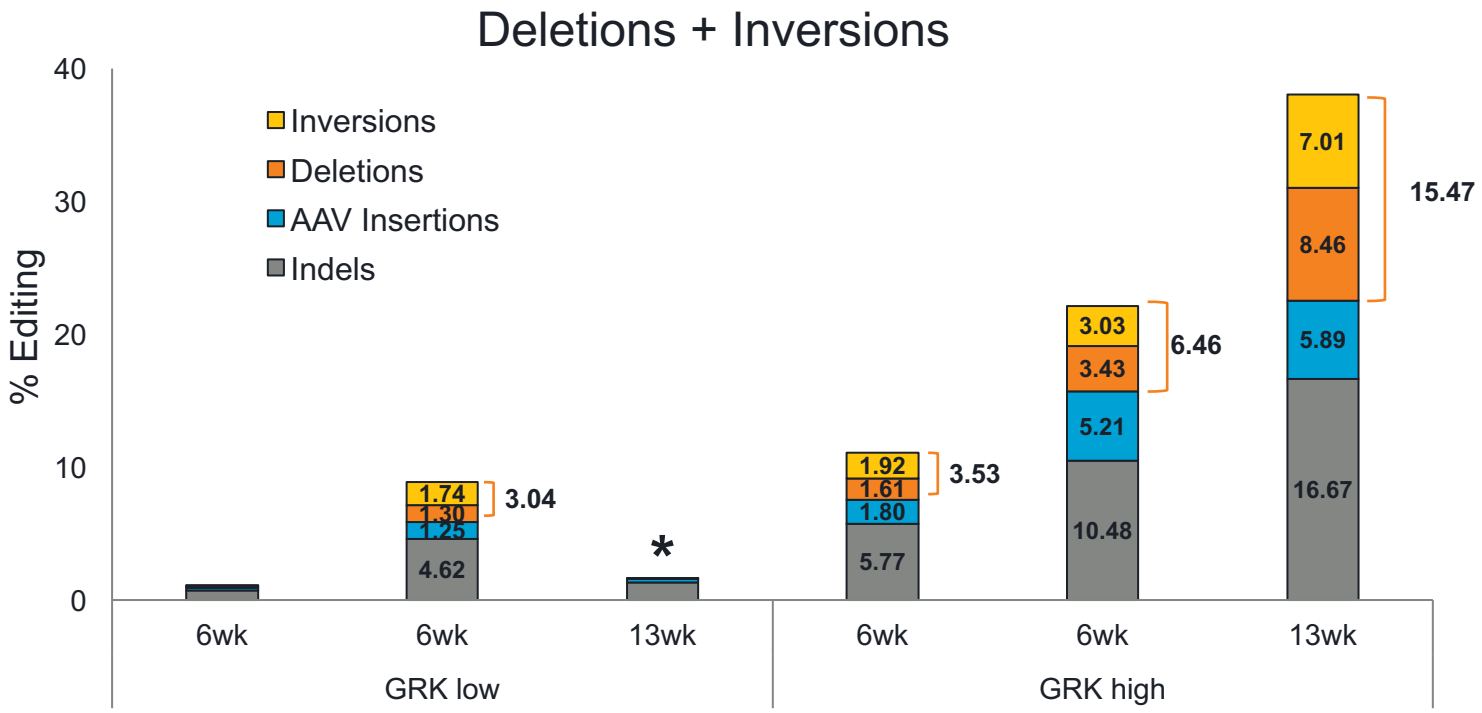
Cas9

Photoreceptors estimated to make up ~25-30% of primate neural retina.

Therefore editing rates *in photoreceptors* are likely 3-4X rates measured in total retinal punch.

Productive Editing Exceeds Therapeutic Threshold

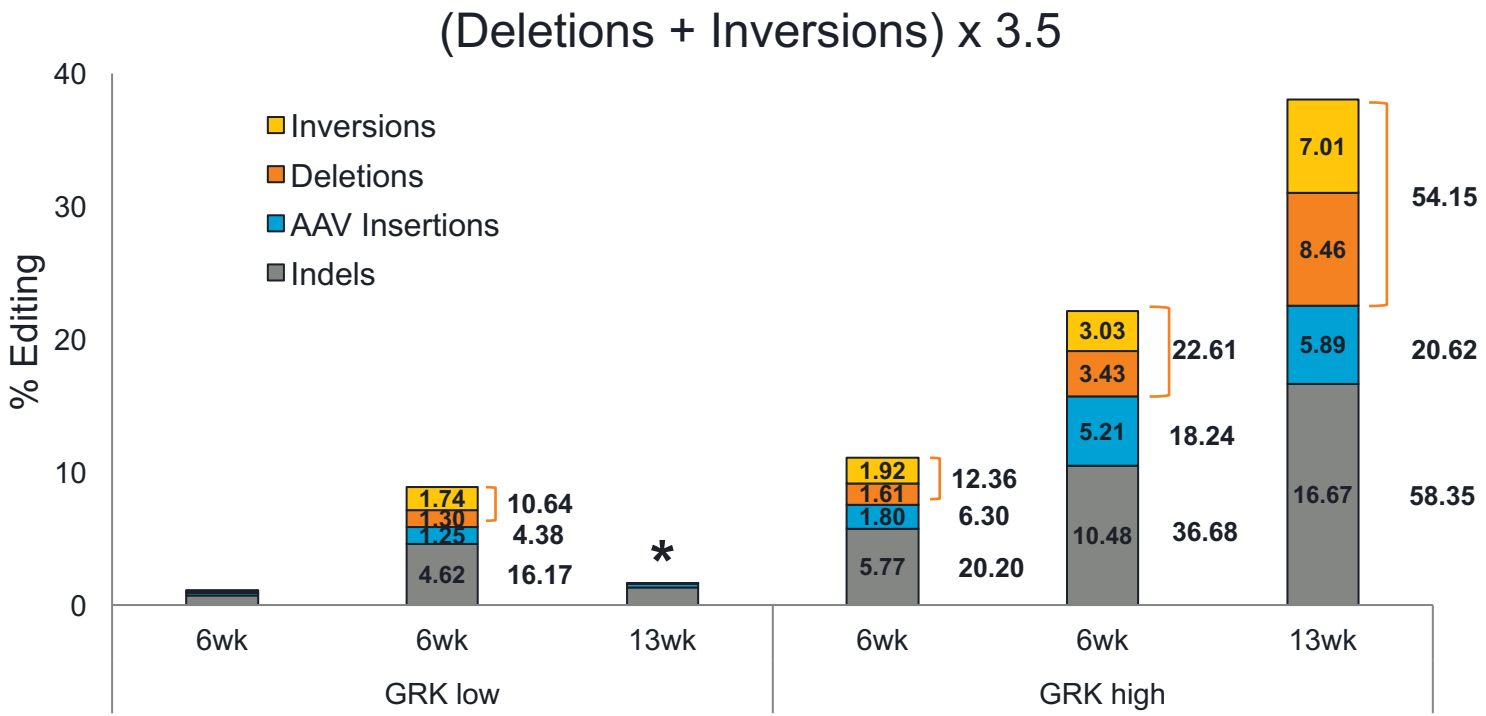
"Productive Editing" = Editing in photoreceptors that will restore wildtype CEP290 splicing



Productive editing of ~15% of all *CEP290* alleles in genomic DNA from all retinal cells within treated bleb region

Productive Editing Exceeds Therapeutic Threshold

"Productive Editing" = Editing in photoreceptors that will restore wildtype CEP290 splicing



Based on demonstration that Cas9 expression is limited to photoreceptors cells, level of productive editing in photoreceptors may be as high as 50%

Towards a Therapy for LCA10

- Productive editing of ~15% of alleles in total genomic DNA from treated non-human primate retinas
- Analysis demonstrates Cas9 expression is limited to photoreceptors – indicates productive editing in photoreceptor cells may be as high as 50% of all alleles
- This is well above the target editing rate needed to have a therapeutic effect in patients

CEP290 gene editing therapeutic has the potential to have a major impact on vision in LCA10 patients

Acknowledgements

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