

Gene Therapy for Ocular Diseases

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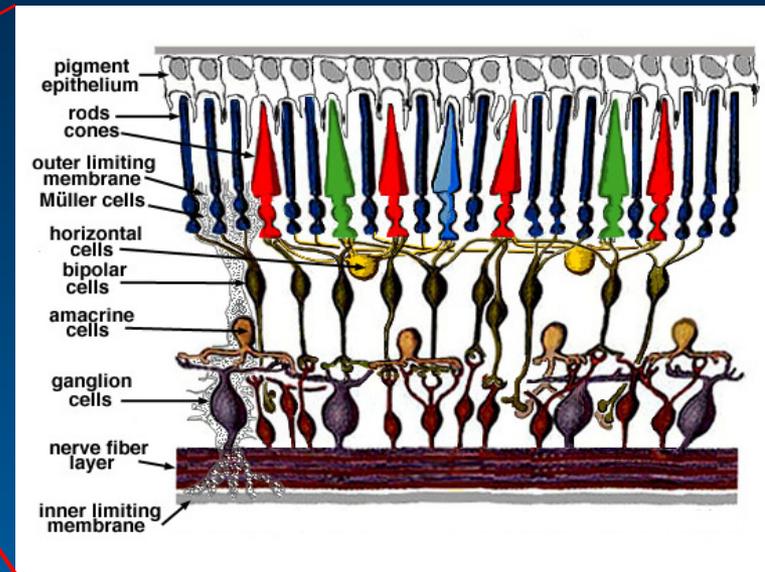
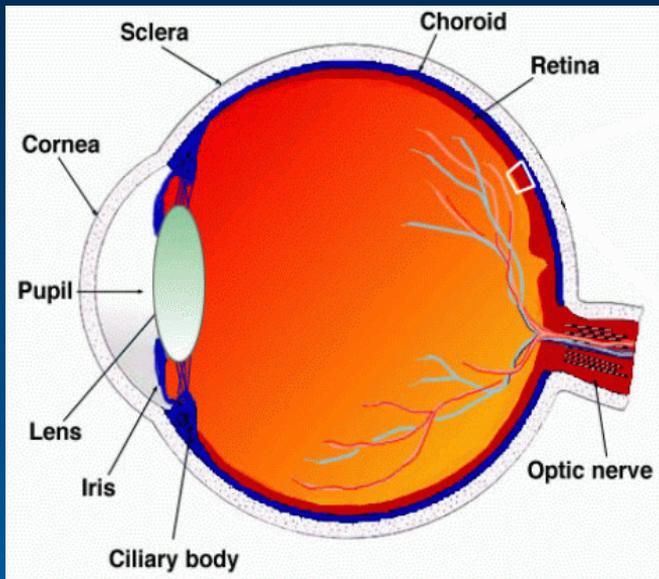
NIH Workshop
Gene Therapy: Charting a Future Course

Three critical research questions that need to be answered in this field over the next 10 years:

1. Can we develop gene therapies for photoreceptor-mediated retinal disease?
2. How can we more safely deliver genes to photoreceptors in fragile, degenerate retinas?
3. How can we treat inherited retinal diseases associated with mutations in large genes?

1. Can we develop gene therapies for photoreceptor-mediated retinal disease?

RPE65 Leber congenital amaurosis- LCA2



← RPE65

Leber Congenital Amaurosis



Photoreceptors

RPE

Other

* *GUCY2D*- LCA1

* *AIPL1*

* *LCA5*

* *RPGRIP1*

* *CRX*

* *CEP290*

* *IMPDH1*

* *RD3*

* *RDH12*

* *TULP1*

* *CRB1*

* *IQCB1*

* *SPATA7*

* *RPE65*- LCA2

* *MERTK*

* *LRAT*

* *KCNJ13*

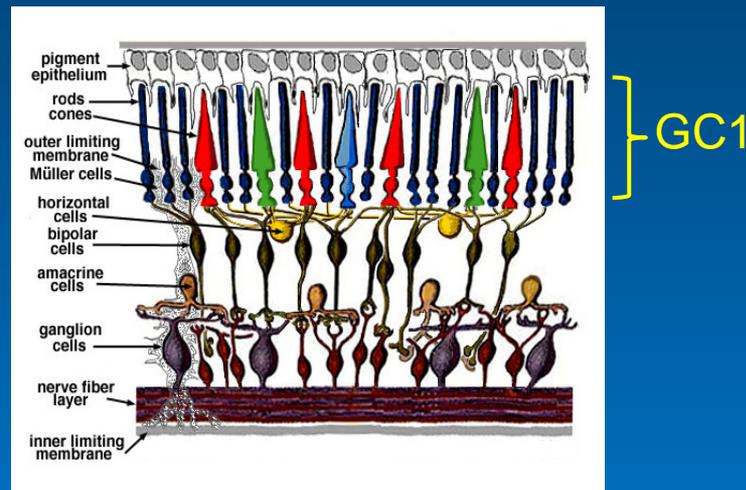
* *NMNAT1*

- Most severe form of pediatric retinal dystrophy
- Affects ~ 1 in 50,000 live births
- 5% of all inherited retinopathies
- Diagnosis at birth or within first few months of life
- Autosomal recessive

Clinically and genetically heterogeneous

GUCY2D Leber Congenital Amaurosis (LCA1)

- *GUCY2D*-LCA1 is one of the most common forms of LCA (~15% of cases)
- *GUCY2D* encodes a protein called “Guanylate Cyclase 1” (GC1) that is expressed in photoreceptor outer segments (predominantly cones)
- GC1 is a key enzyme in the phototransduction cascade





Photoreceptors are where vision begins

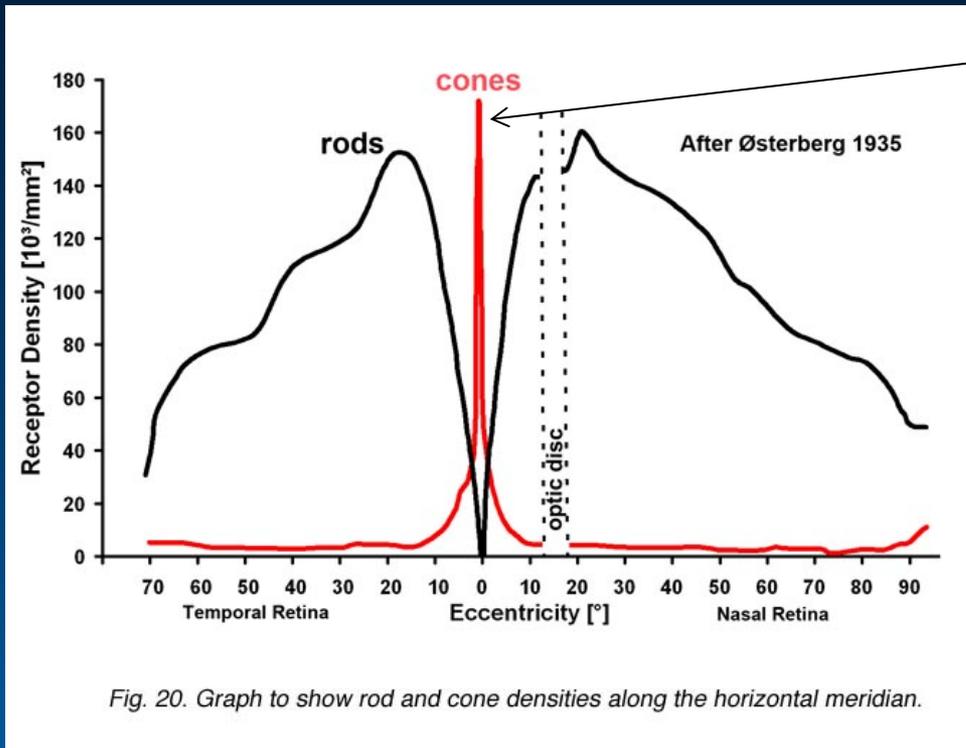
RODS



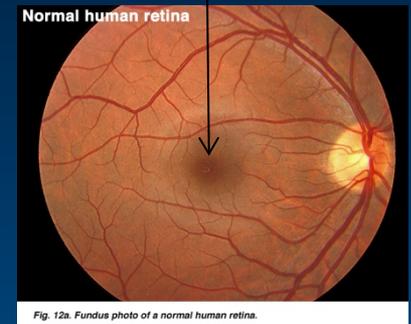
CONES



Spatial distribution of rods and cones



macula/fovea



LCA1 patients present with:

- Severely impaired vision
- Inability to 'fixate'
- Abnormal or absent electroretinogram (ERG)
- Normal fundus
- Pendular nystagmus
- Roving eye movements
- Absent ocular pursuit
- Eye poking



Models of GC1 deficiency which have been used in proof of concept studies

GUCY1*B chicken



- Naturally occurring, null
- Cone dominant retina
- Non recordable ERG
- Cones/rods degenerate

GC1KO mouse



- Engineered, null
- Rod dominant retina
- Cone ERG non recordable
- Rod ERGs partially (30-50%) retained and variable
- Only cones degenerate

**GC2- close relative of GC1
known to express in rods**

GC1/GC2 dko (Gcdko) mouse



- Engineered, null
- No GC activity
- Rod dominant retina
- Non recordable ERG
- Cones/rods degenerate

Long term conclusions from our animal studies:

1. AAV vectors drive mGC1 expression exclusively in photoreceptors
2. AAV vectors stably restore cone and rod-mediated retinal function (ERG)
3. AAV vectors restore cone- and rod- mediated visual behavior
4. AAV vectors prevent photoreceptor degeneration
5. Treatment restores normal levels of GC1 and GCAP1
6. The functional efficiency of AAV-delivered GC1 is normal

These results lay the ground work for the development of an AAV-based therapy for treatment of LCA1

- **Boye SE**, Boye SL, Pang J, Ryals R, Everhart D, Umino Y, Neeley AW, Besharse J, Barlow R, Hauswirth WW. Functional and behavioral restoration of vision by gene therapy in the guanylate cyclase-1 (GC1) knockout mouse. *PLoS One*. 2010 Jun 25;5(6):e11306.
- SL Boye, T Conlon, K Erger, R Ryals, A Neeley, T Cossette, J Pang, F Dyka, WW. Hauswirth, **SE Boye**. "Long term preservation of cone photoreceptors and restoration of cone function by gene therapy in the guanylate cyclase-1 knockout (GC1KO) mouse." *IOVS*, 2011
- SL Boye, IV Peshenko, WC Huang, SH Min, I McDoom, X Liu, FM Dyka, TC Foster, Y Umino, S Karan, SG Jacobson, W Baehr, A Dizhoor, WW Hauswirth and **SE Boye**. "AAV-mediated gene therapy in the guanylate cyclase (RetGC1/RetGC2) double knockout mouse model of Leber congenital amaurosis". *Human Gene Therapy* 2012 Dec 4. [Epub ahead of print]

A key component to moving forward is that patients need to be characterized!
Their natural history needs to be understood!

Our clinical colleague, Dr. Samuel Jacobson is funded via the FFB to thoroughly characterize LCA1 patients for enrollment in a clinical trial.
His findings were impressive.....

Preclinical characterization:

- 11 patients
- Ages 6 months-37 years
- Different mutations in *GUCY2D*
- nystagmus, visual impairment noted in 1st year of life
- Visual acuities ranged between 20/100, bare light perception, and no fix/follow
- Recordable rod ERGs detectable in 4/11 patients
- Cone ERGs unrecordable

LCA1 patient phenotype:

- Rods are preserved
- Cone outer segments (OS) lost or shortened
- FST reveals rods have variable sensitivity to light across patients
- ERG reveals rods have variable function across patients
- Mobility tests reveal that some patients behave normally in dim light (rod-mediated vision)
- Cones lack function and have no sensitivity

Implications for LCA1 clinical trial:

Cone function/OS renewal is the favored efficacy outcome measure

What now?

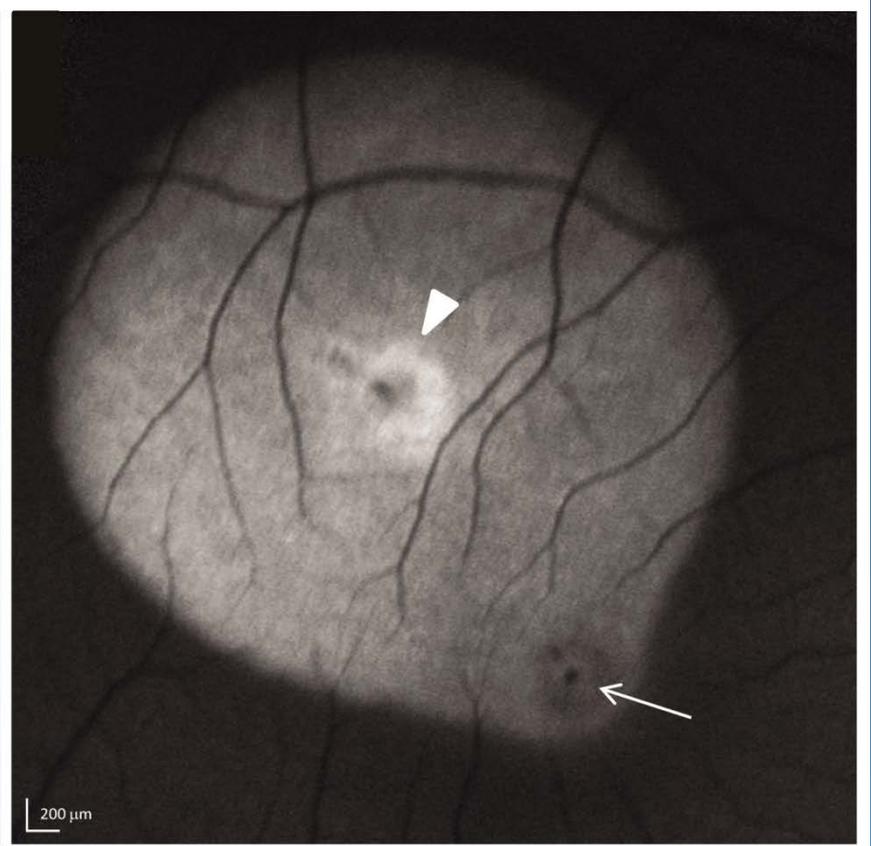
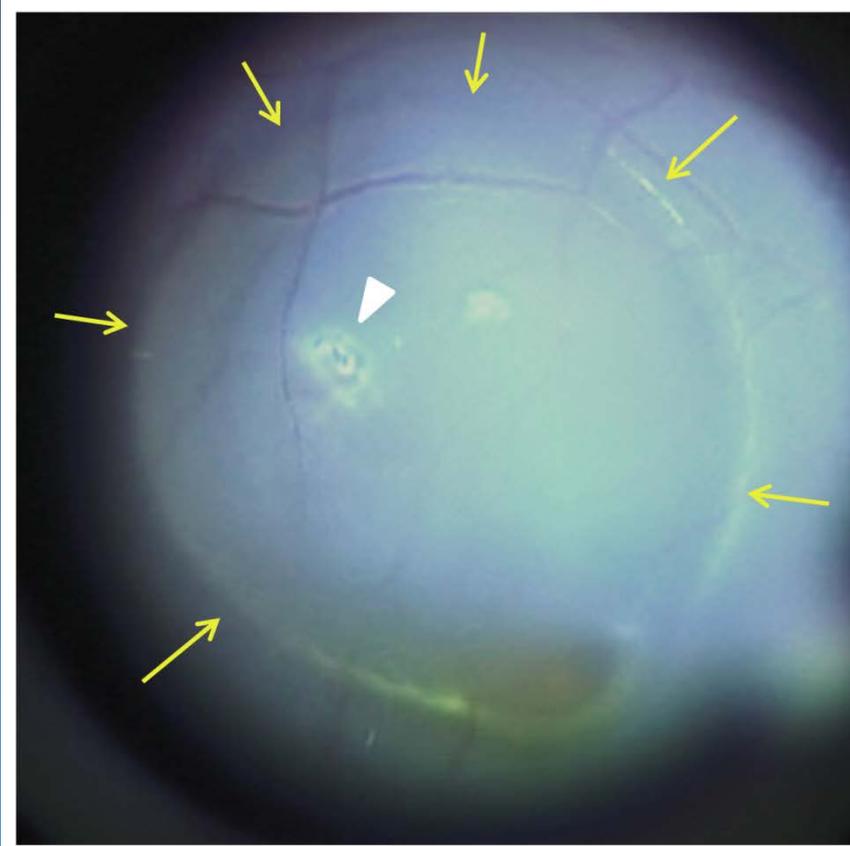
- Route of injection? - subretinal
- AAV serotype - AAV5 or AAV8?
- Promoter - hGRK1?

Is hGRK1 promoter active in both rods and cones of the primate retina?

AAV5-hGRK1-GFP

Still from injection video

Fluorescent (488) Spectralis image
5 weeks post-injection

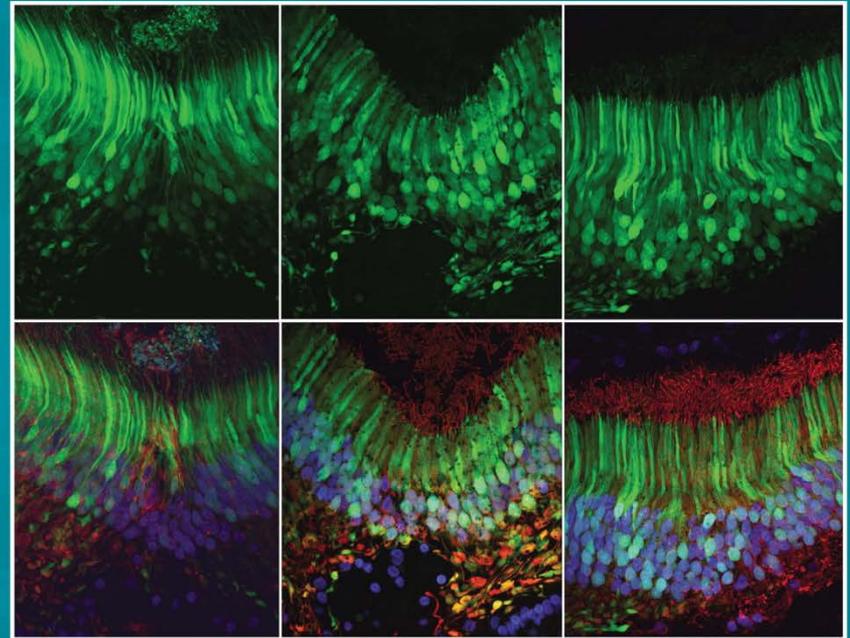
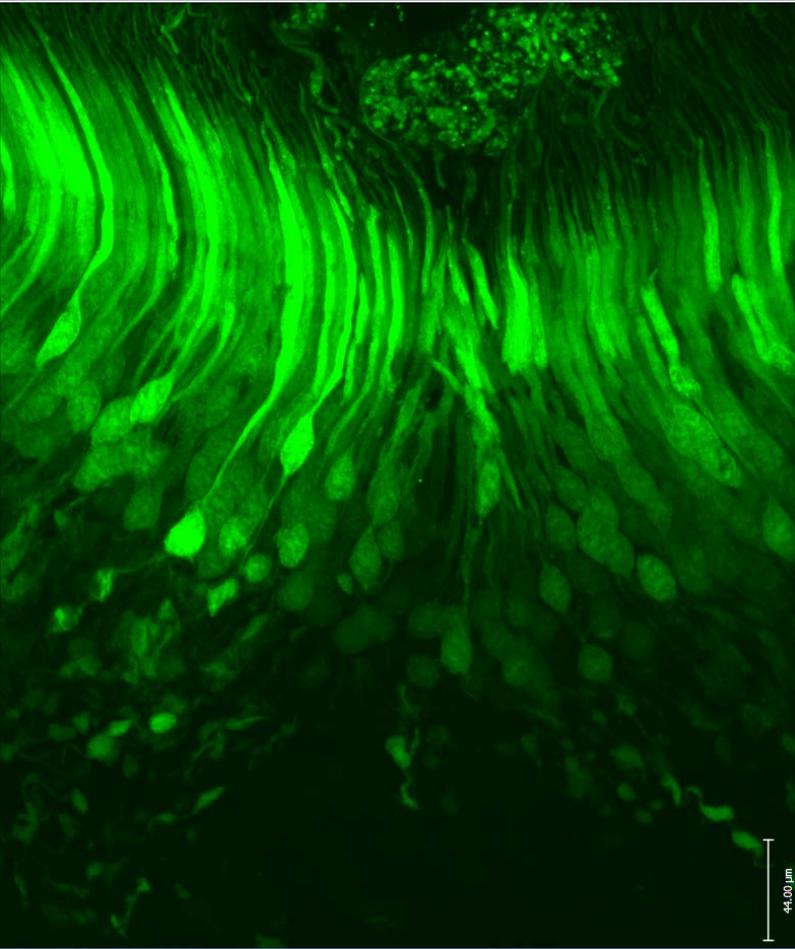


GFP expression is confined to the injection bleb which encompasses the fovea



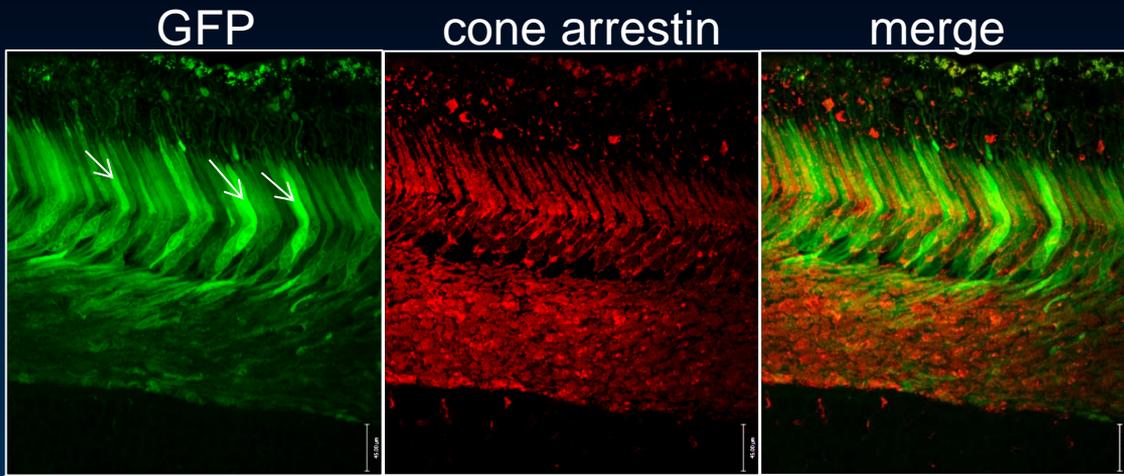
Human Gene Therapy

including DNA, RNA, and Cell Therapies

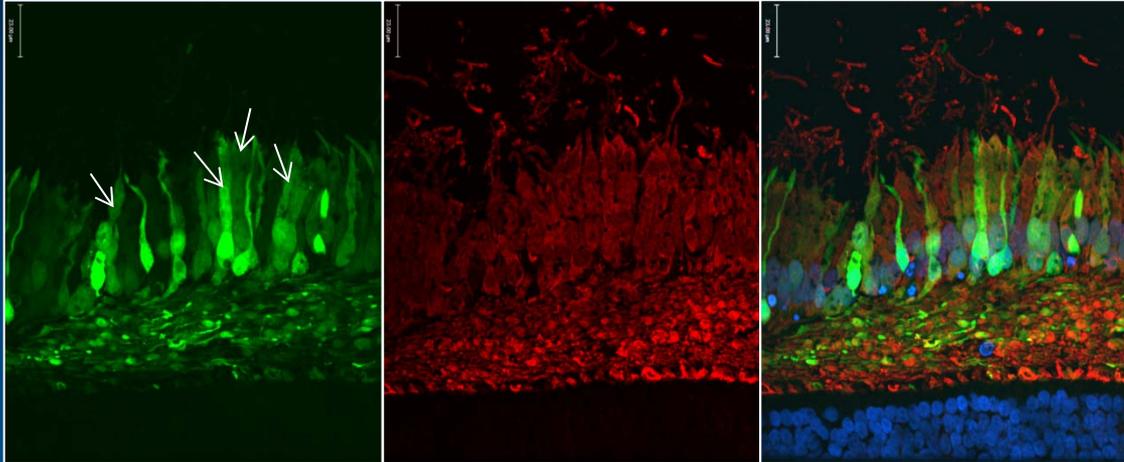


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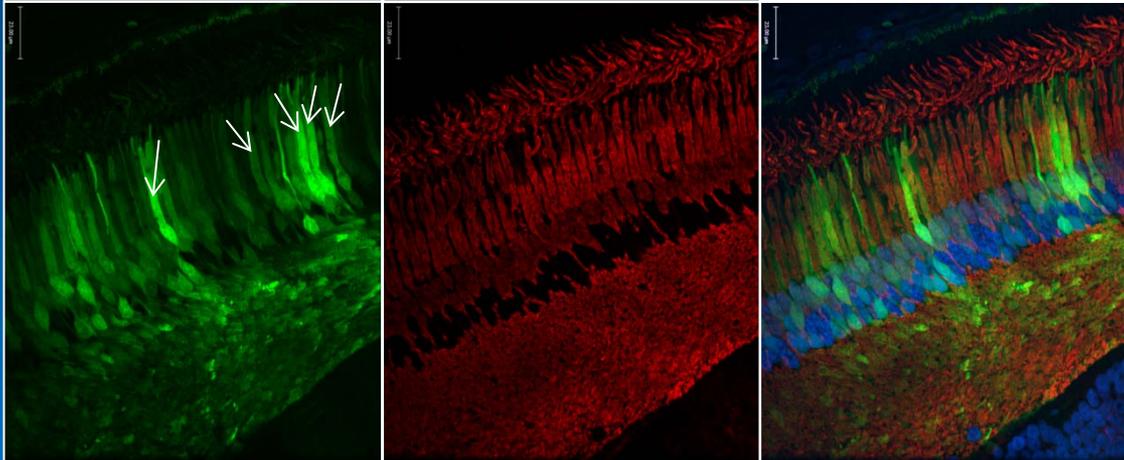
Monkey 1



Monkey 2



Monkey 3

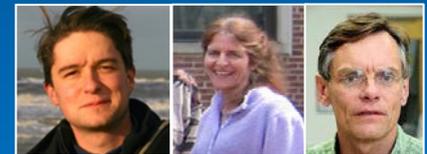


AAV5 transduces foveal and parafoveal cones

AAV8- “little to no parafoveal cone transduction”, “rod transduction better than cone transduction”

“Dosage thresholds for AAV2 and AAV8 Photoreceptor Gene Therapy in Monkey.”

Vandenberghe LH et al. Sci Transl Med. 2011.

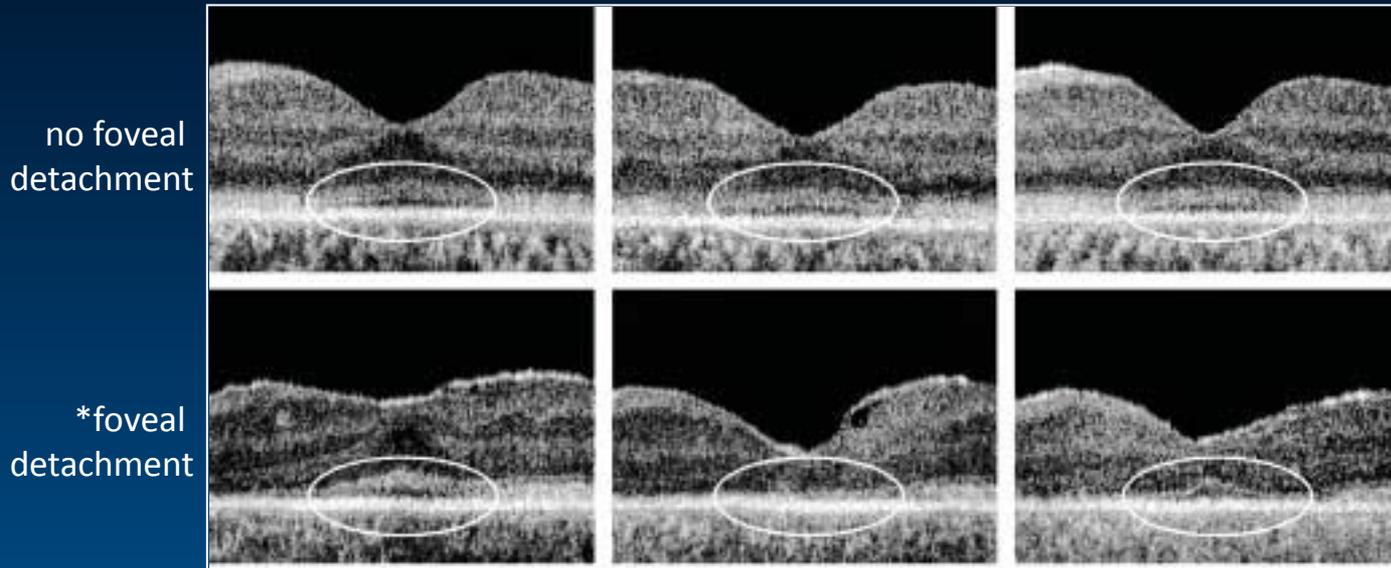


1. Can we develop gene therapies for photoreceptor-mediated retinal disease?

With support from the Foundation Fighting Blindness and pharma, we move towards clinical application of a photoreceptor-targeted gene therapy

subretinal injection of AAV5-hGRK1-GUCY2D

2. How can we more safely deliver genes to photoreceptors in fragile, degenerate retinas?



Subretinal injection under the fovea results in a loss of central retinal thickness

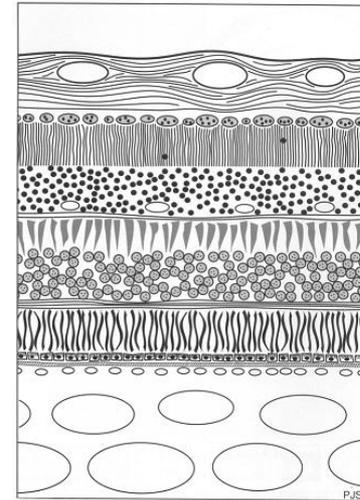
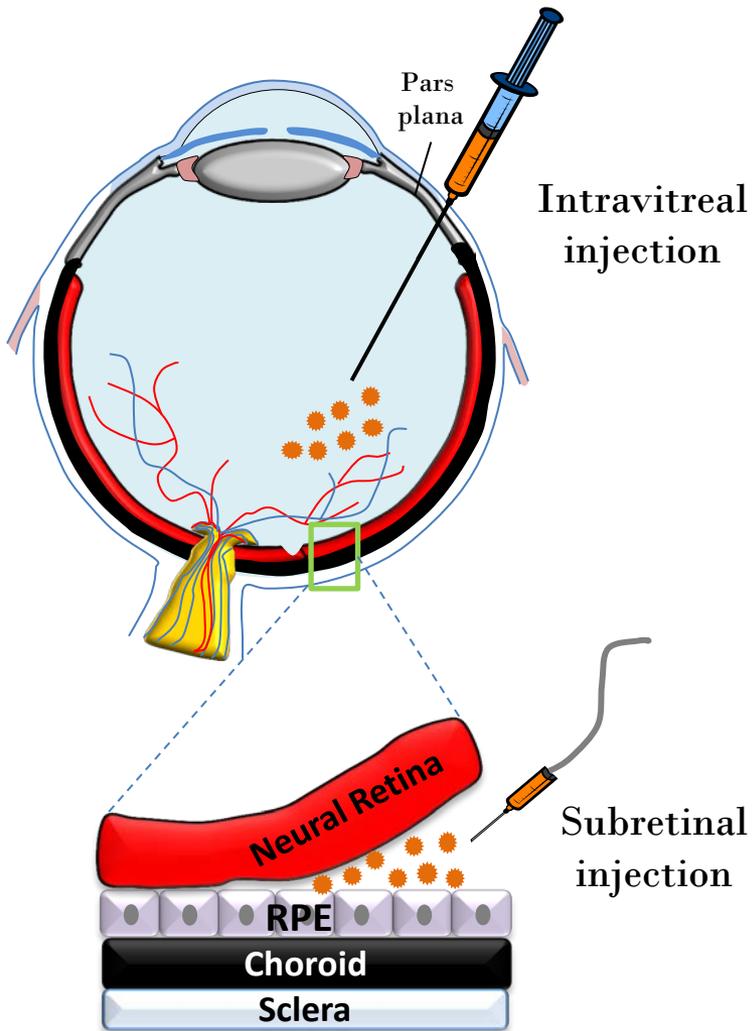
Some patients with foveal detachments exhibit a loss in central visual acuity

“Gene Therapy for Leber Congenital Amaurosis Caused by RPE65 Mutations. Safety and Efficacy in 15 Children and Adults Followed up to 3 Years”
Jacobson SG et al., Arch Ophthalmol (2012)



Can we target foveal cones via a safer/less-invasive intravitreal injection?

**Barrier #1:
INNER LIMITING MEMBRANE**



Lentivirus- 80-100 nm
Adenovirus- 90-100 nm
AAV- 20 nm

Barrier #2: Receptor binding

Evaluating the ability of standard AAV serotypes to bind ILM

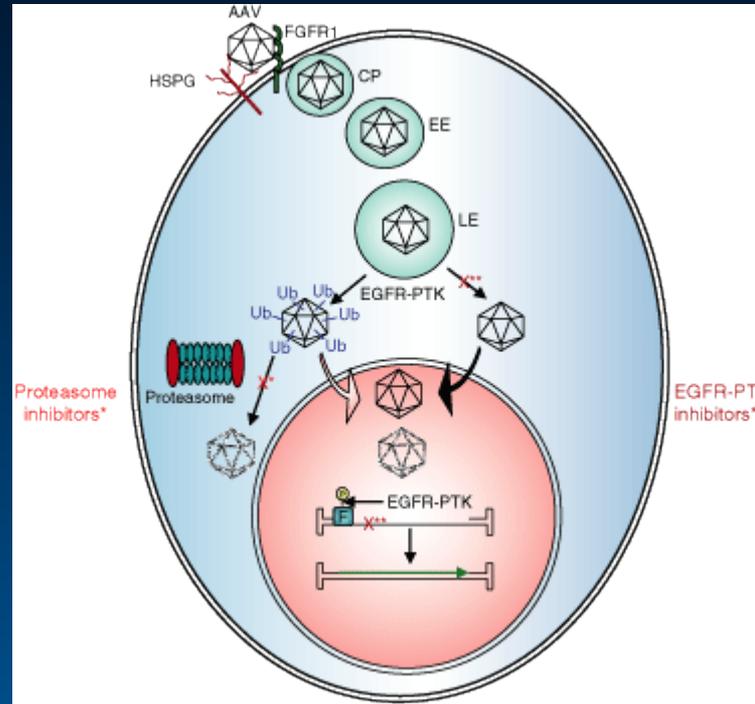
- Fluorescently labeled capsid surface of AAV1, AAV2, AAV5, AAV8, AAV9 and injected into the rat vitreous
- AAV2>AAV9>AAV8 accumulate at vitreoretinal junction
- AAV1, AAV5 show no accumulation
- Likely receptor-mediated
- AAV2 (HSPG)- abundant in ILM
- AAV2, AAV8, AAV9 (laminin)- abundant in ILM, Mueller cell endfeet and RGC
- AAV1, AAV5 (sialic acid)- absent at ILM



“Inner Limiting Membrane Barriers to AAV-mediated retinal transduction from the vitreous.” Dalkara D. et al., Molecular Therapy (2009)

Barrier #3: Intracellular trafficking

Strategies for improving transduction of outer retina from the vitreous



2008: Srivastava et al. find that inhibition of tyrosine phosphorylation via tyrosine kinase inhibitors increases transduction efficiency of AAV2 vectors

Provides rationale behind generation of tyrosine-phenylalanine (Y-F) capsid mutants

Other phosphorylated residues (threonine, serines) can be similarly mutated



Our hypothesis is that the AAV capsid can be simultaneously optimized to confer adhesion to the inner limiting membrane and traffic to the outer retina while maintaining or gaining photoreceptor tropism!

How can we measure this in vivo?



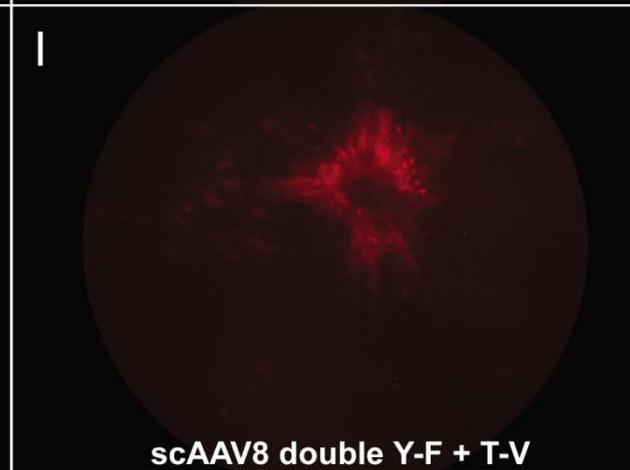
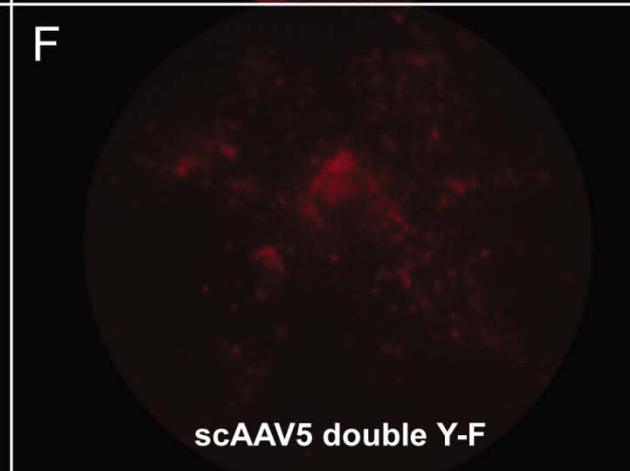
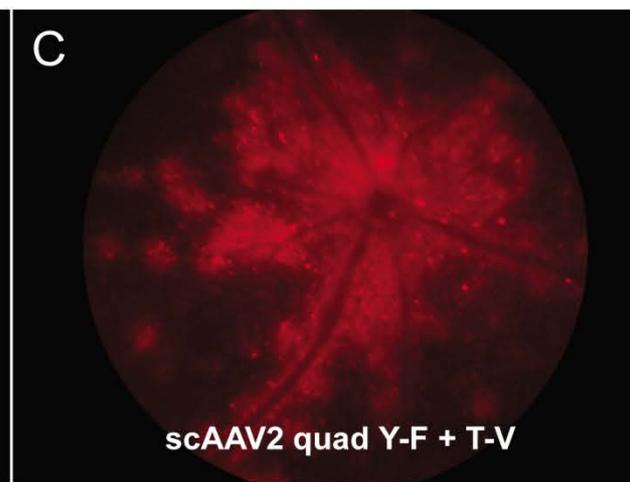
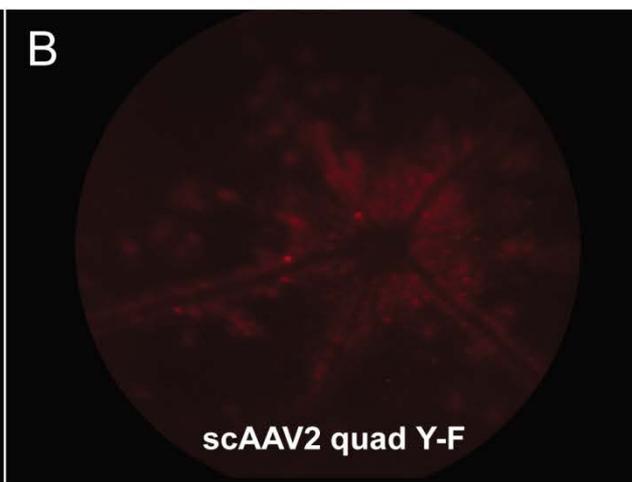
Rho-GFP mouse

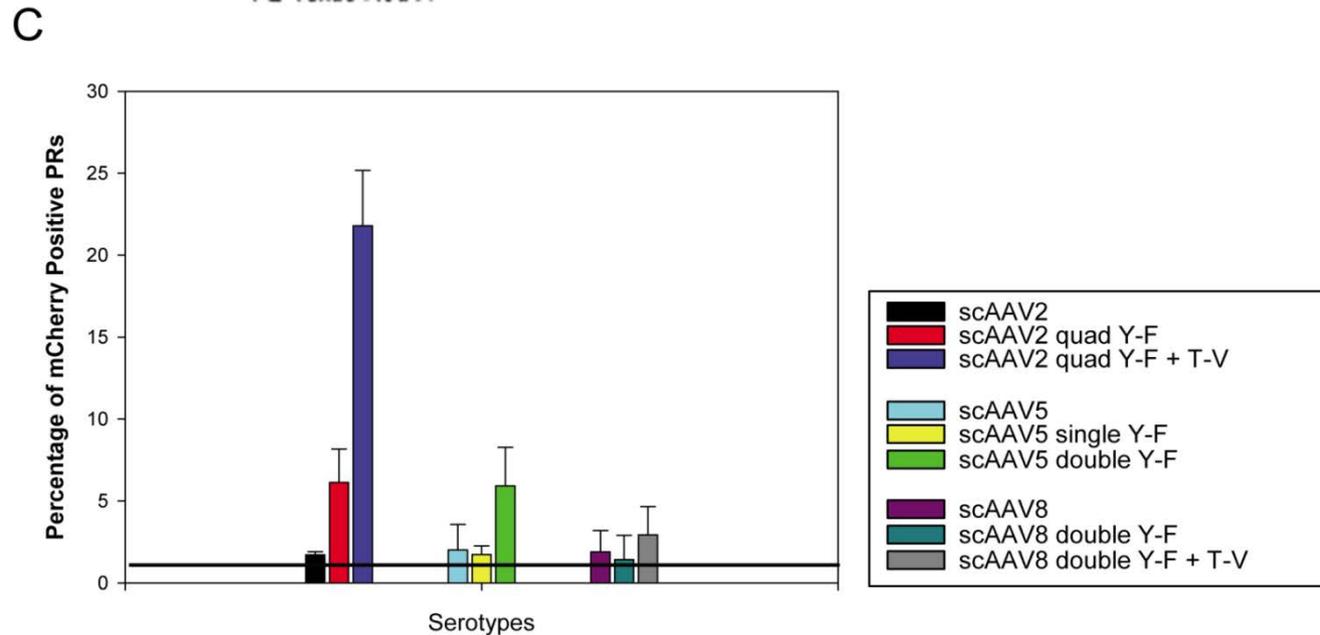
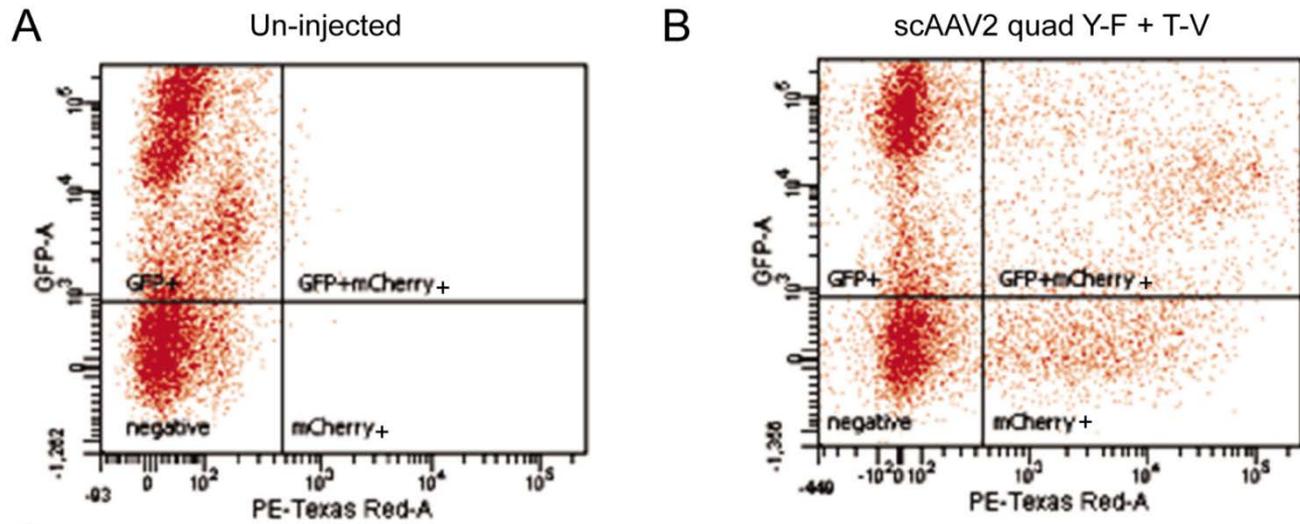
knock in of human rhodopsin fused to GFP

- faithfully mimics expression and distribution of WT rhodopsin in heterozygotes
- Allows sorting of photoreceptor (rods) cell population from rest of retina

In-vivo quantification

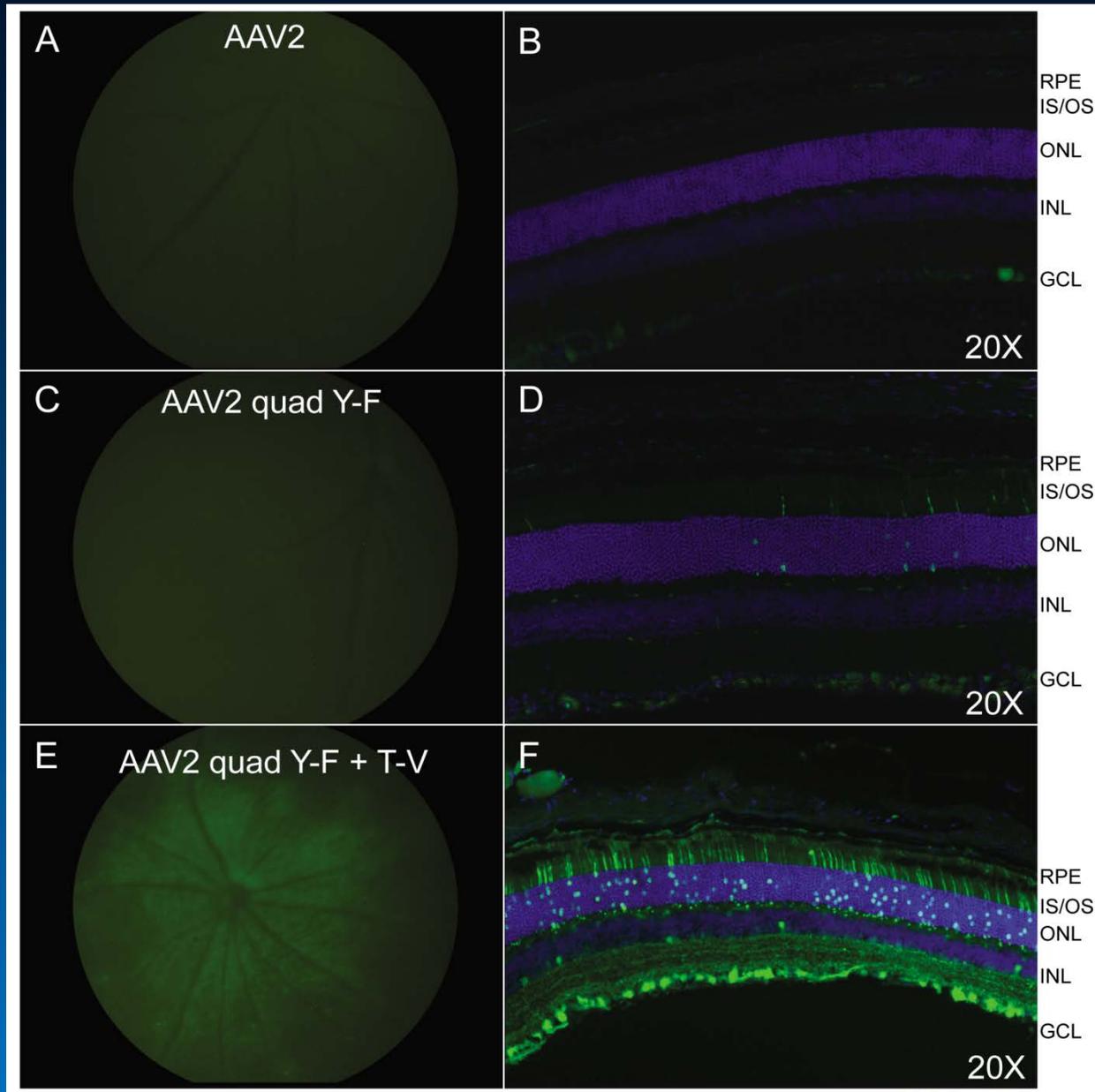
- Self-complementary smCBA-mCherry packaged in capsid variants
- Injected intravitreally into Rho-eGFP mice
- 4 weeks post injection fundus photographs recorded, retinas collected and dissociated for FACS
- GFP+ (photoreceptor) and mCherry+ (transduced) cell population is quantified





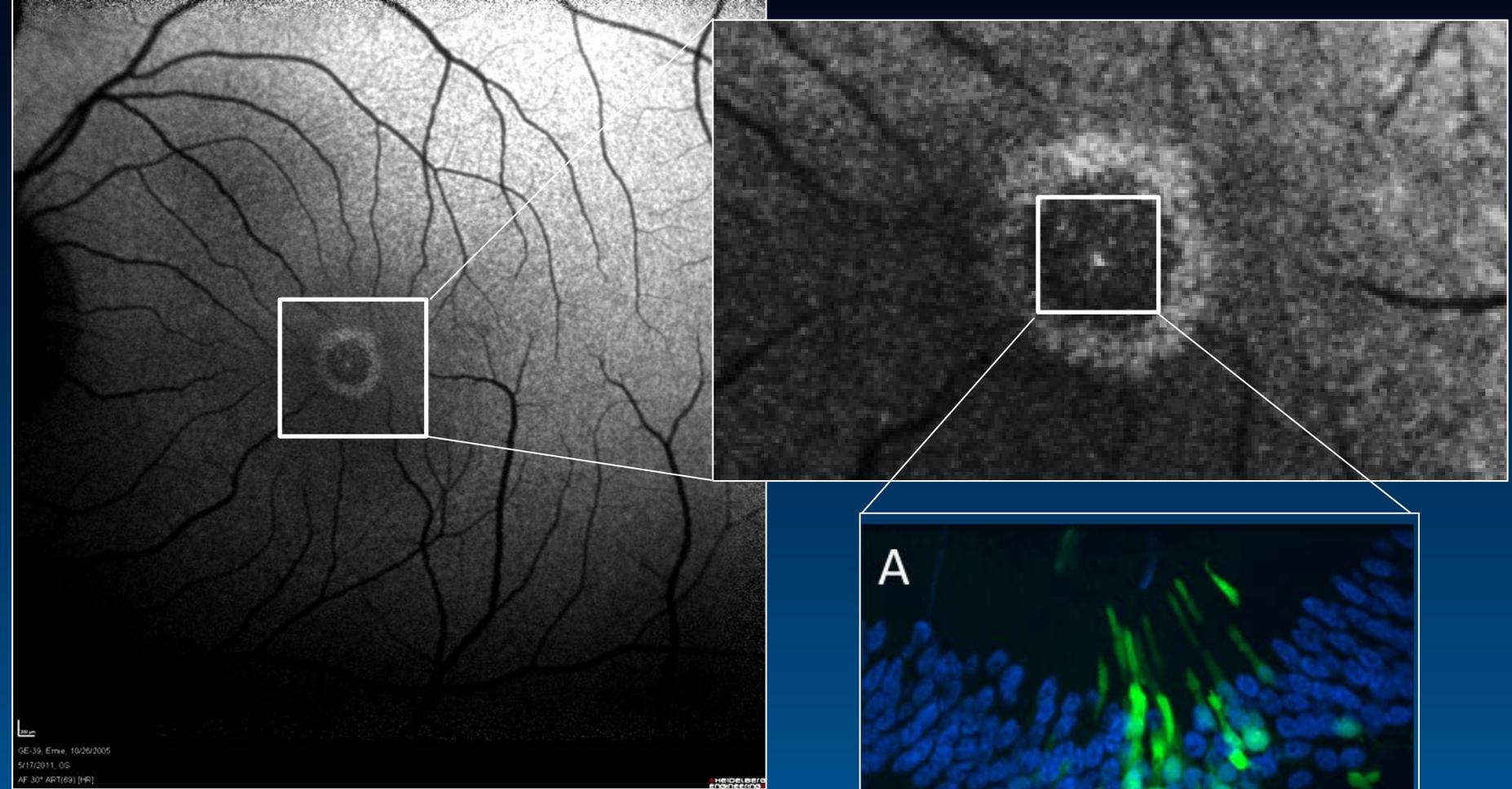
CN Kay, RC Ryals, GV Aslanidi, SH Min, Q Ruan, J Sun, FM Dyka, D Kasuga, AE Ayala, K Van Vliet, M Agbandje-McKenna, WW Hauswirth, SL Boye, and **SE Boye**. Targeting photoreceptors via intravitreal delivery using novel, capsid-mutated AAV vectors. PLoS One (in press).

Results when photoreceptor-specific promoter is incorporated into AAV2 variants driving GFP

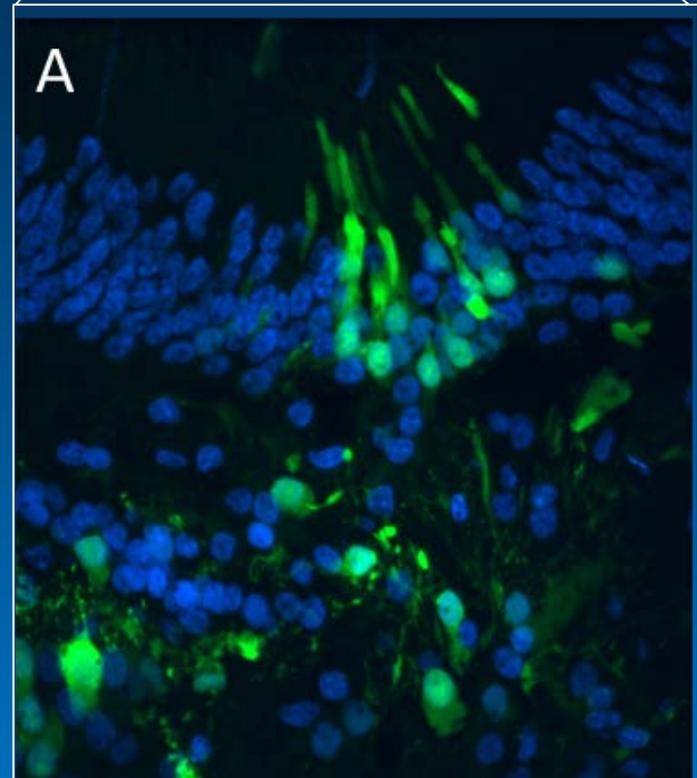


WHERE ARE WE NOW?

- Through rational design, we have isolated a serotype, AAV2(quad Y-F + T-V) that is capable of transducing 25% of photoreceptors following intravitreal injection in mouse!
- This, and others, are being screened in non-human primate
- We are also mining unique capsid motifs using directed evolution (results coming soon!)
- Rational design + directed evolution can be combined!



NHP injected intravitreally with
AAV2(triple)-CBA-GFP
(10^{11})



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3. How can we treat inherited retinal diseases associated with mutations in large genes?

Stargardt's disease, CEP290 LCA, MYO7A Usher syndrome

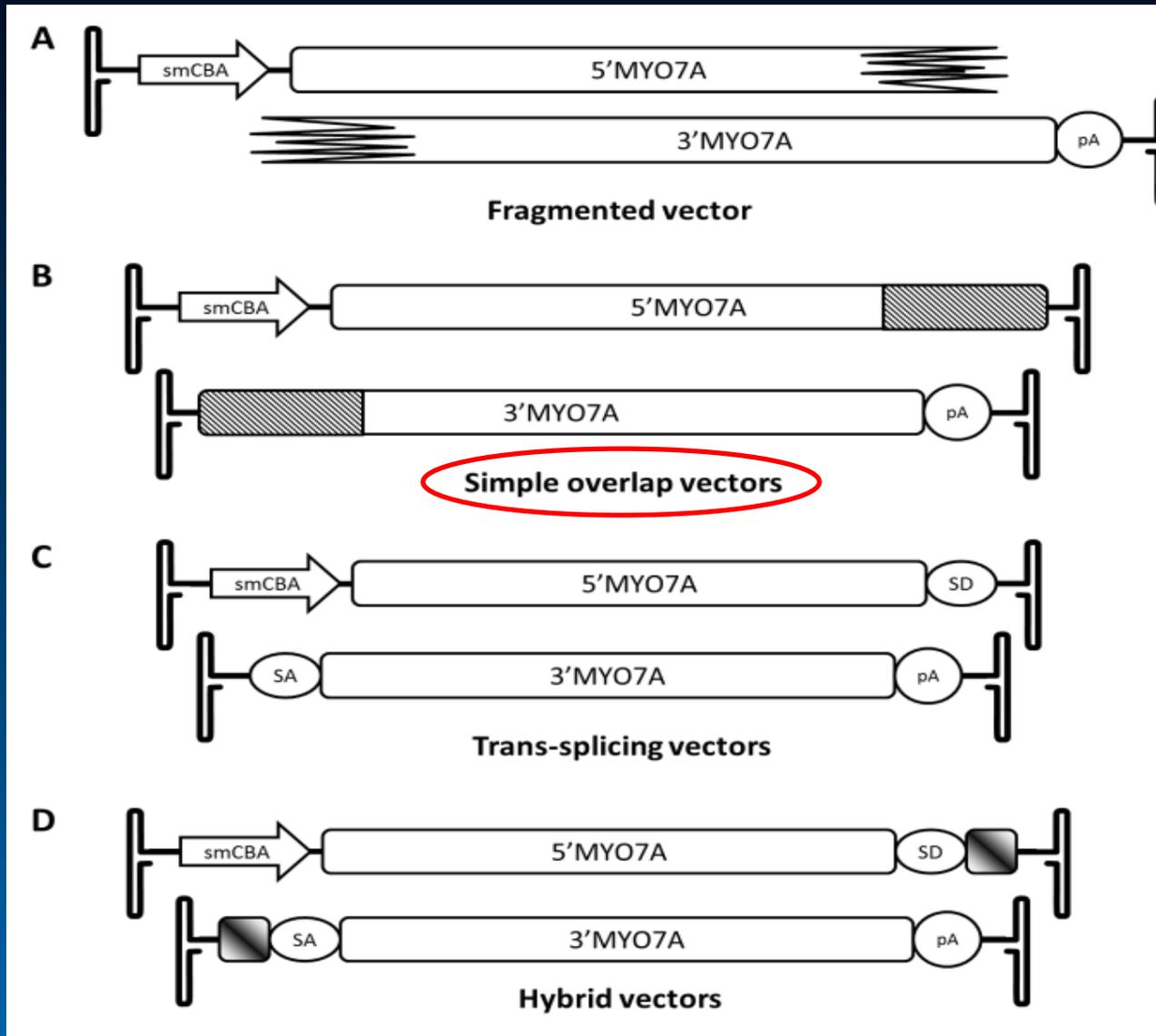
The carrying capacity of AAV is ~5kb

Can we divide large gene cDNAs in half and deliver each half in a separate capsid?

MyosinVIIa (~7kb cDNA)- Usher syndrome (USH1b)

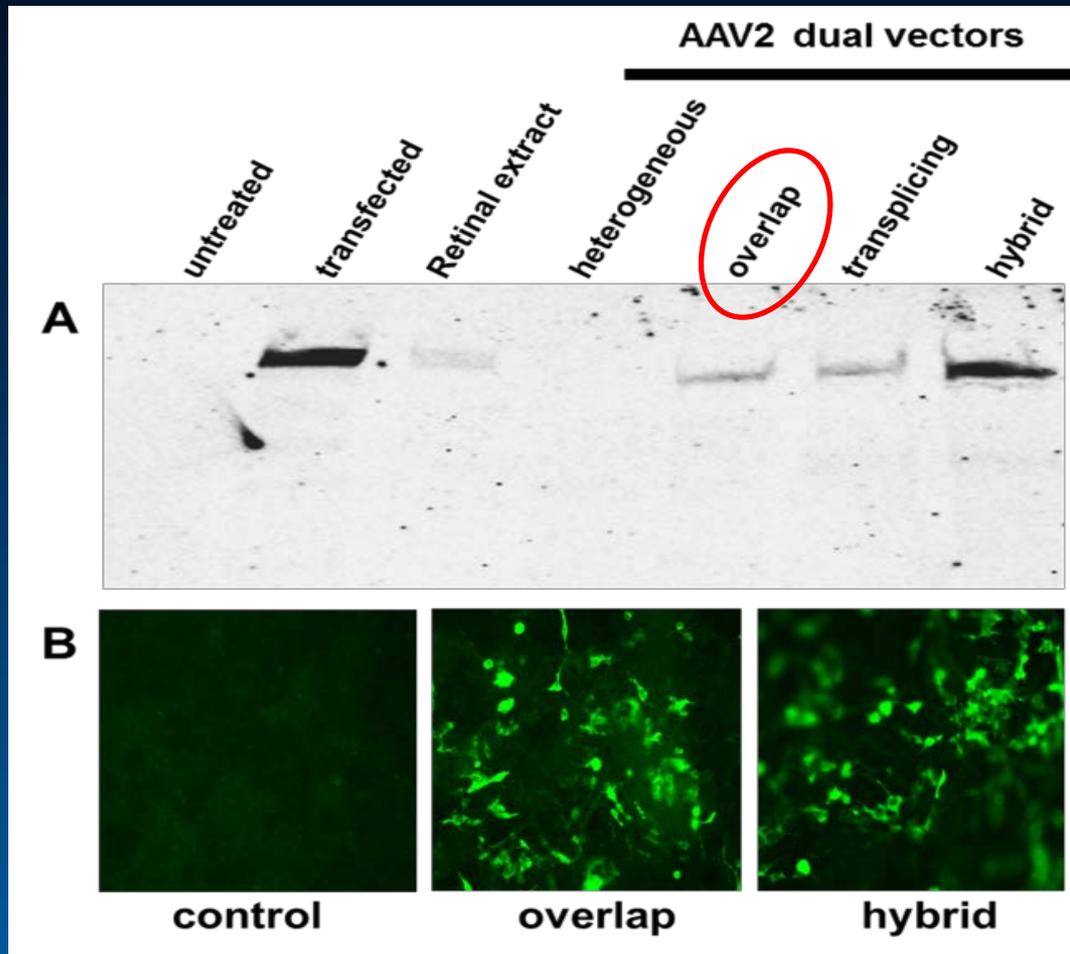
MYO7A expressed in photoreceptors and RPE with the eye and hair cells of the inner ear

We have evaluated several dual AAV vector platforms *in vitro* and *in vivo*



Vanda Lopes*, **Shannon E. Boye***, Carrie M. Louie, Sanford Boye, Frank Dyka, Vince Chiodo, William W. Hauswirth, D. S. Williams. "Retinal gene therapy with a large MYO7A cDNA using adeno-associated virus". *Gene Therapy* Jan 24. doi: 10.1038/gt.2013.3. [Epub ahead of print]

*equal contribution



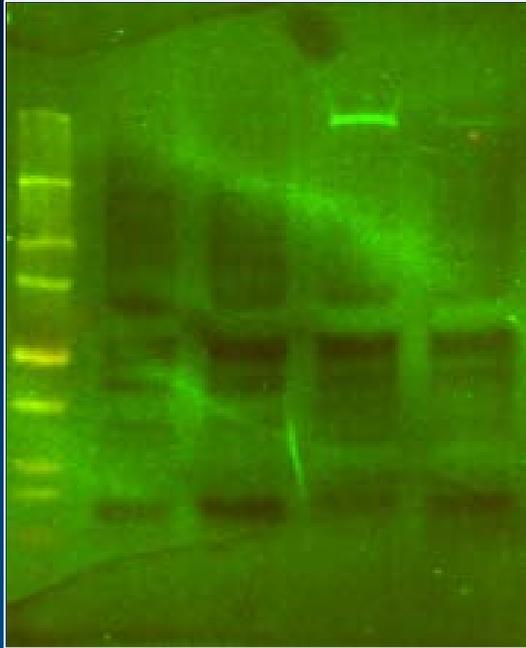
Vanda Lopes*, **Shannon E. Boye***, Carrie M. Louie, Sanford Boye, Frank Dyka, Vince Chiodo, William W. Hauswirth, D. S. Williams. "Retinal gene therapy with a large MYO7A cDNA using adeno-associated virus". *Gene Therapy* Jan 24. doi: 10.1038/gt.2013.3. [Epub ahead of print]

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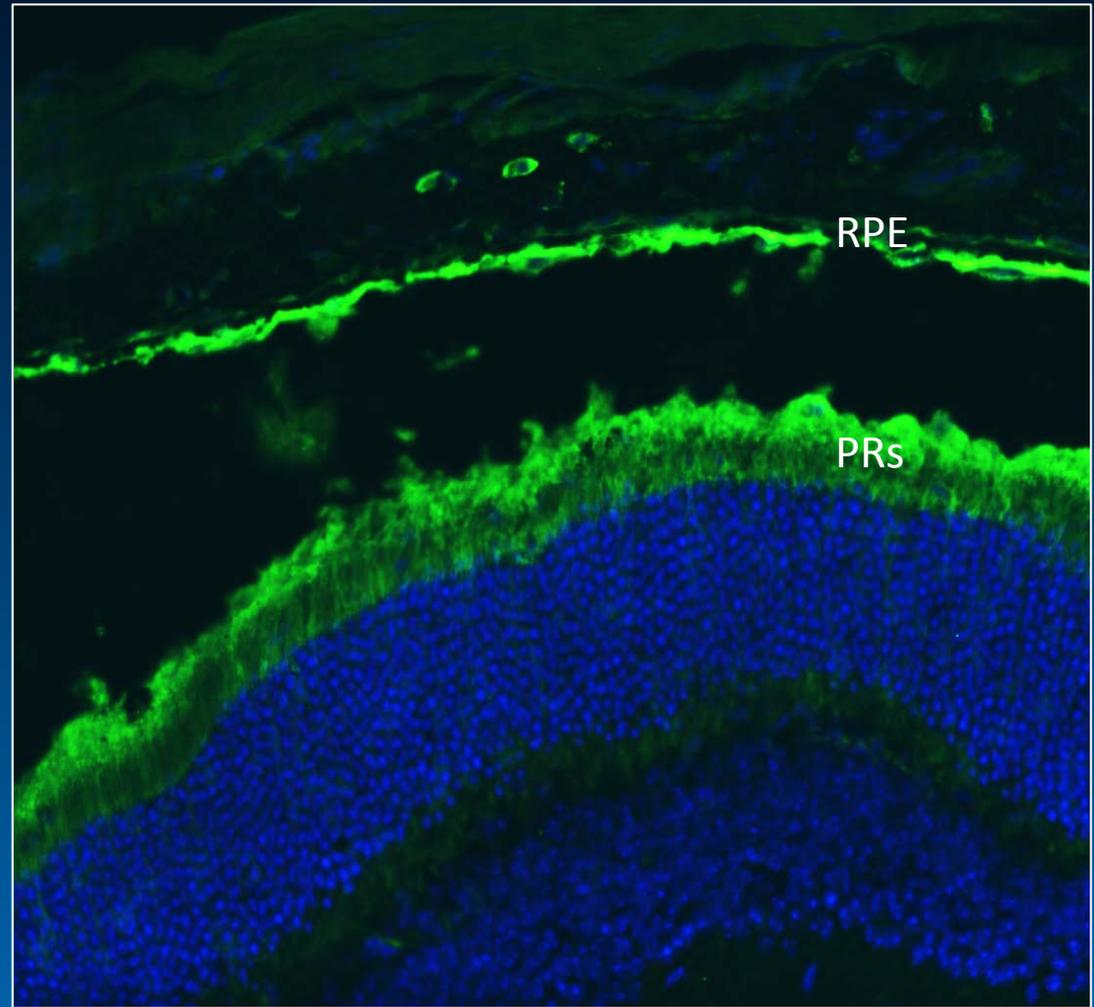
C57BL/6 mice injected with HA-tagged Myo7a dual vectors

Probed for HA

1 2 3 4



- 1: C57BL/6
- 2: fragmented
- 3: hybrid
- 4: simple overlap

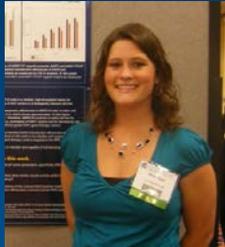


C57BL/6 mouse injected with AAV8(733)- based simple 'overlap' vectors

Thank you!!!

U. of Florida

Bill Hauswirth, PhD
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Mark Clark
Kristen Sandefer



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Igor Peshenko, PhD

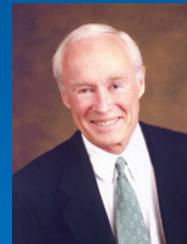


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