USH1B Update

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Shout out to Miss Lia Porcano!

Usher Syndrome

- The most common condition affecting both vision and hearing
  - Recessively inherited
  - Prevalence: 7-14 in 100,000 people

- USH symptoms include:
  - Retinitis Pigmentosa
  - Bilateral Deafness/Hearing Loss
  - Vestibular/Balance problem

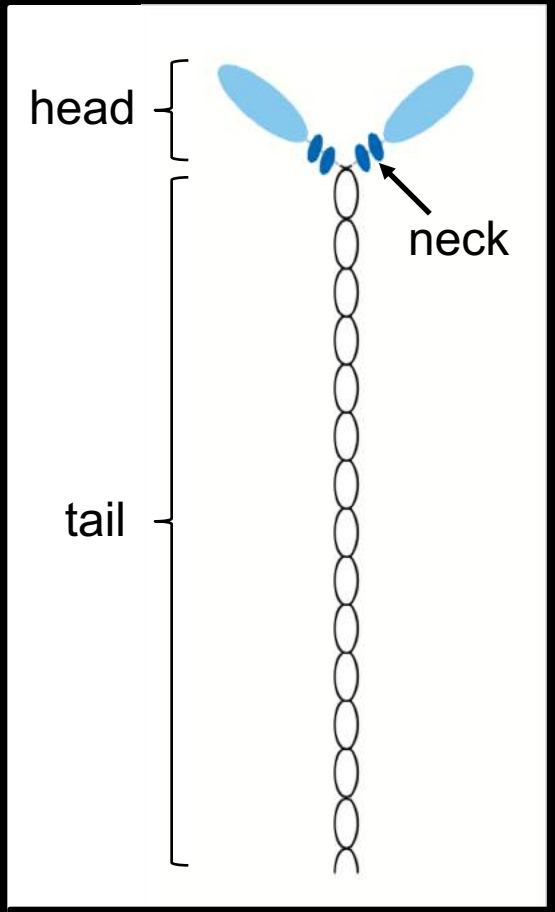
- USH Type 1B (USH1B) is the most severe subtype
  - Accounts for majority of USH1 cases
  - caused by mutations in *Myosin VIIA (MYO7A)*

http://www.perkins.org/history/legacy/deafblind

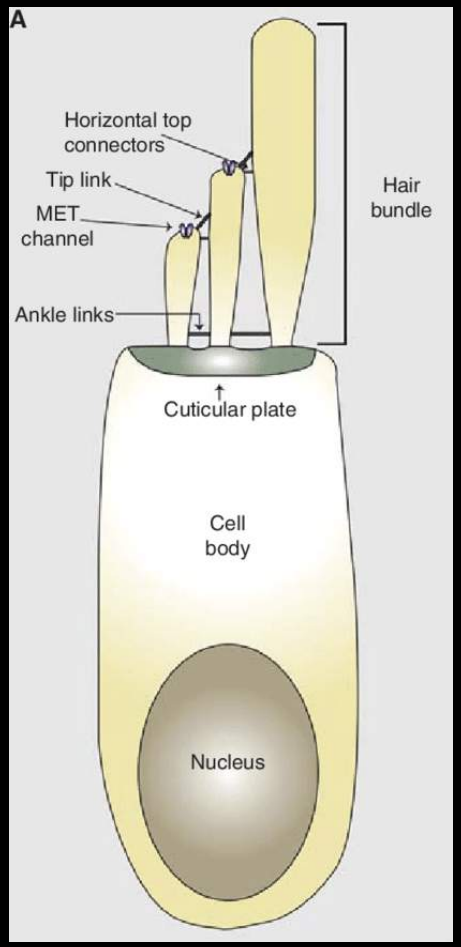
https://dbcitizens.org/equinox-usher-syndrome-awareness-day/

MYO7A

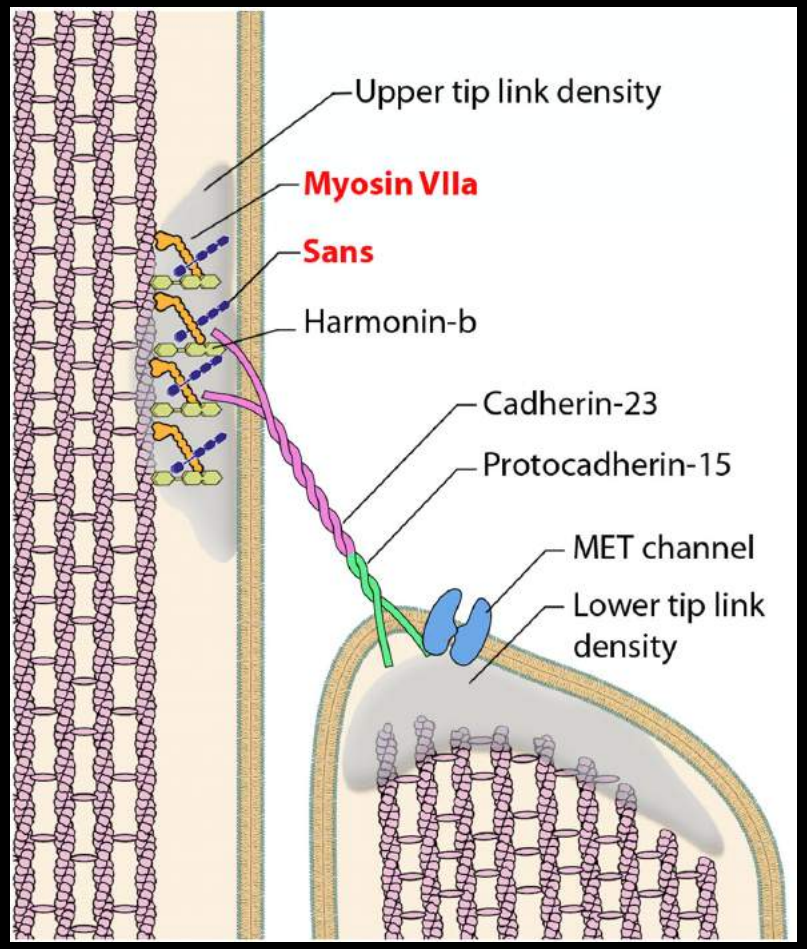
- Actin-based molecular motor
- N-terminal (head) domain contains actin- binding site and ATP-binding site
- 5IQ (neck) is stabilized by calmodulin
- Single a-helix (SAH) acts as lever
- C-terminal (tail) domain determines functional specificity
- FERM domains thought to be responsible for protein attachment to plasma membrane. Also shown to couple actin and microtubules
- Expressed in retina and hair cells of the inner ear



MYO7A Expression in the Inner Ear

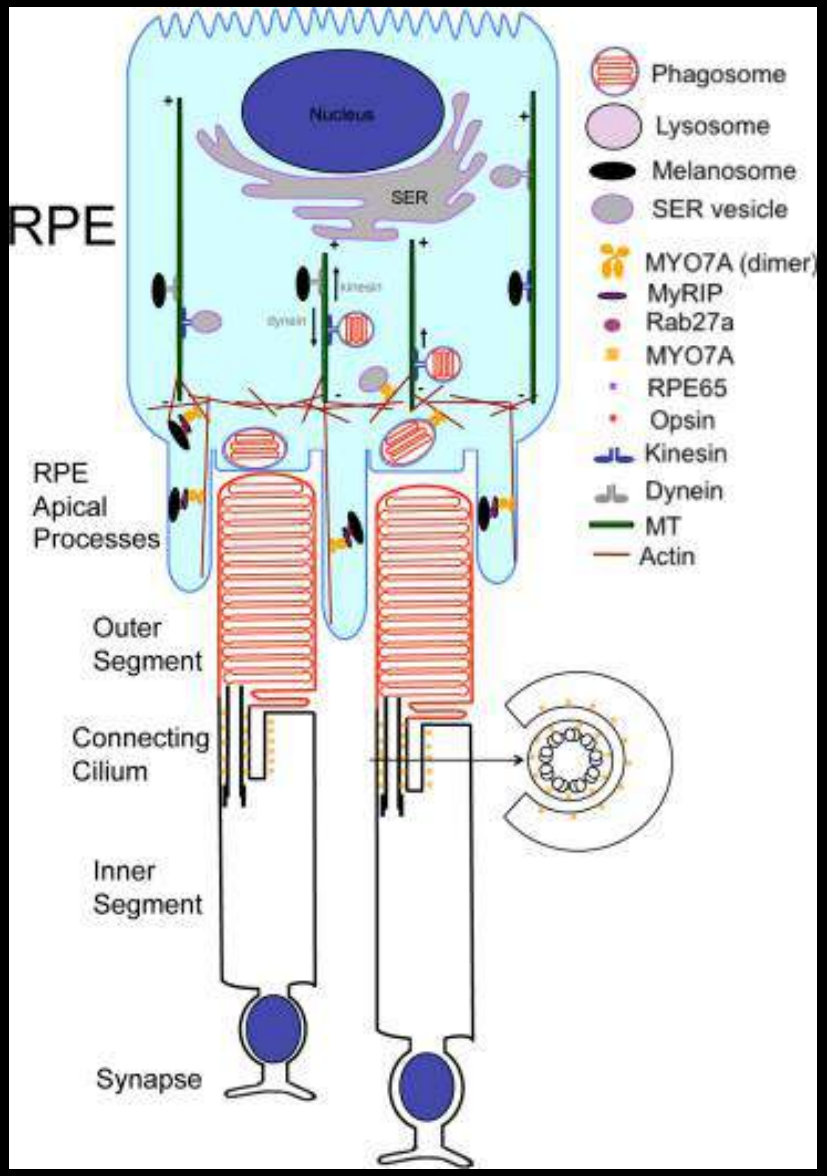


Cunningham & Muller, 2019



Grati & Kachar, 2011

MYO7A Expression in the Eye



Williams & Lopes, 2013

<u>In photoreceptors:</u>

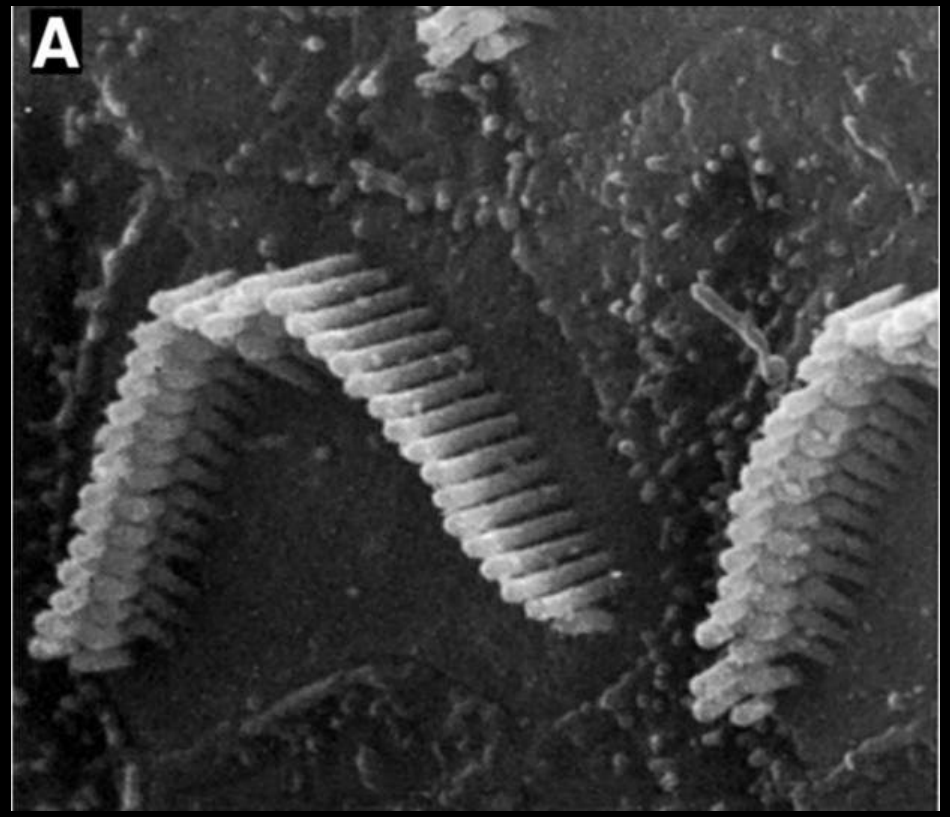
- Transport of rhodopsin from inner segment to outer segment

<u>In RPE:</u>

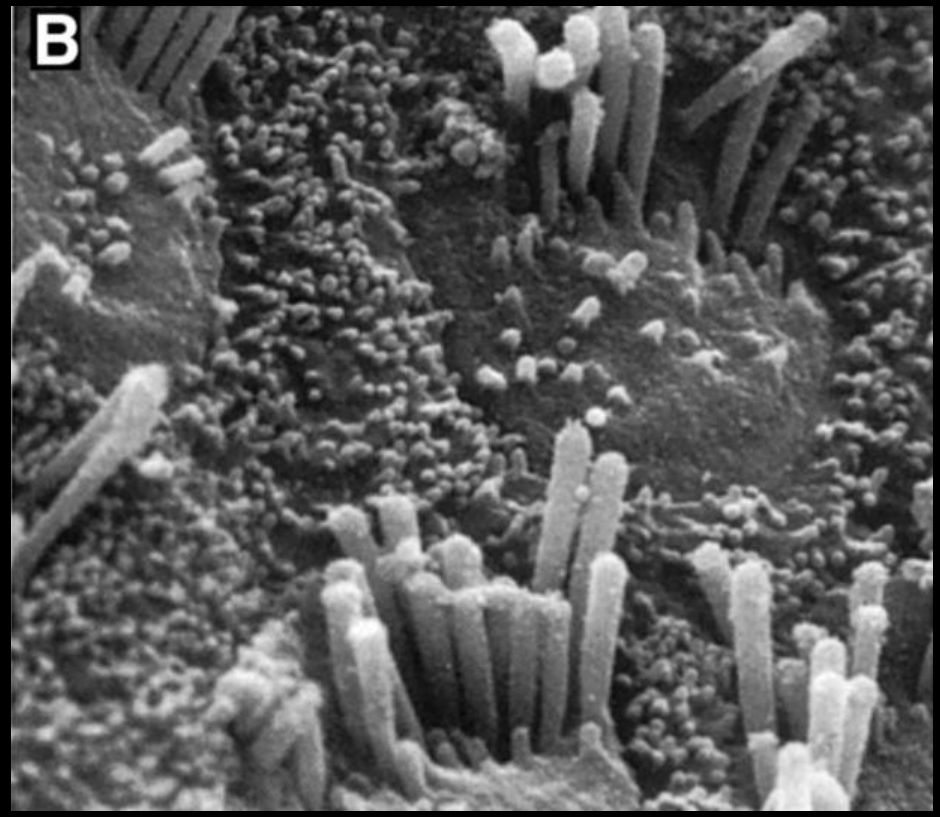
- Transport of melanosome to apical RPE
- Transport of phagosomes from apical RPE
- Transport of vesicles/RPE65 following light exposure

Why is hearing affected first?

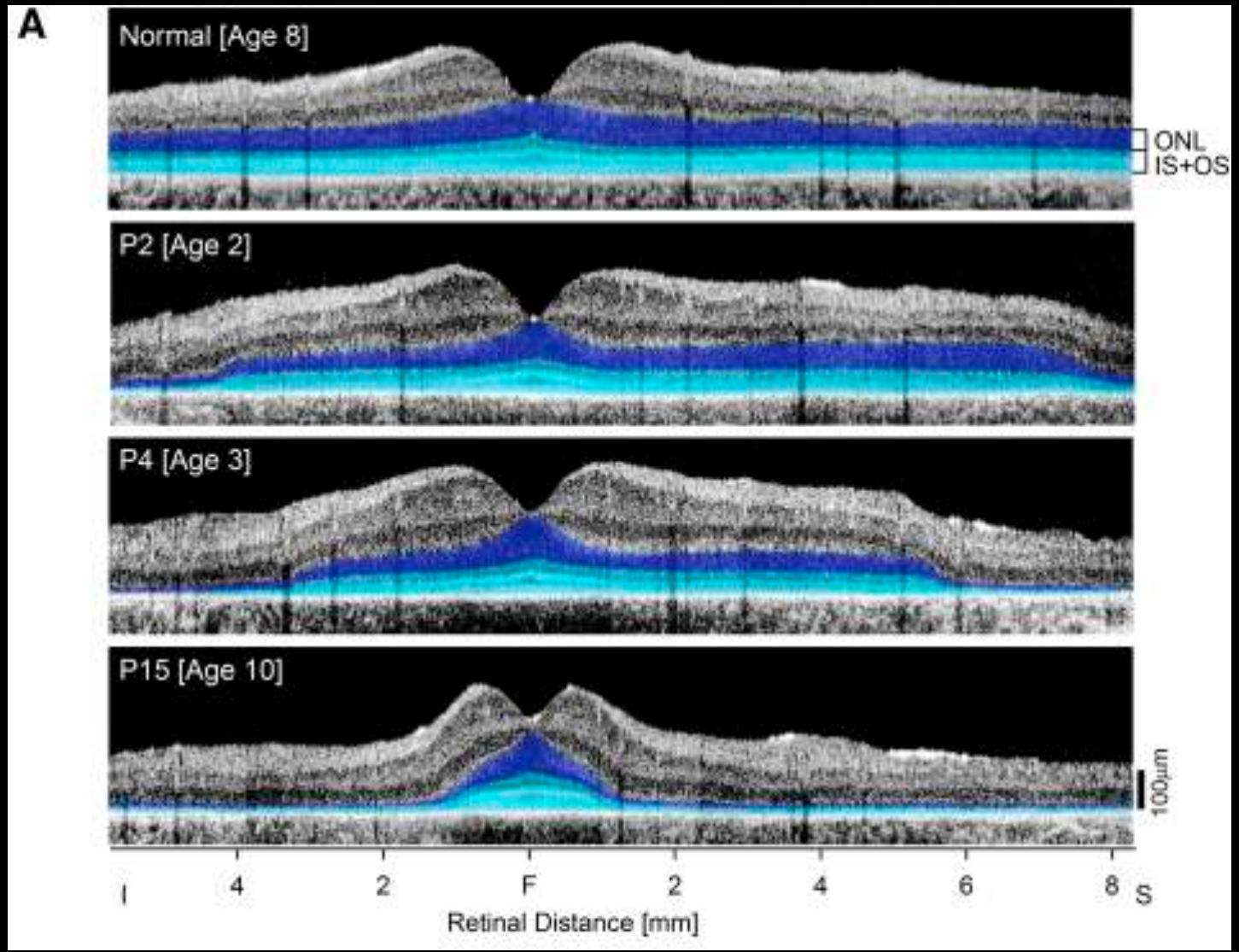
Wild Type



shaker-1 mouse (*Myo7a* mutant)



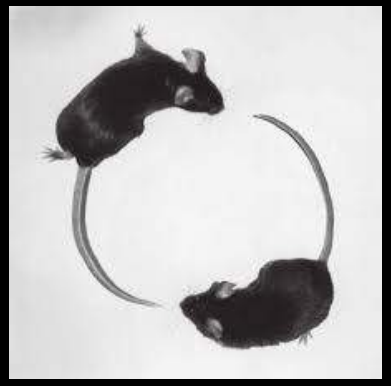
Ophthalmological Findings



- 33 patients
- Severe bilateral hearing impairment in early childhood
- Visual acuity in 1st two decades- 20/63, by 6th decade, less than 20/200
- Rod mediated vision lost in the first two decades
- Cone vision more slowly declines (ranges from normal to reduced in first 4 decades but becomes severely abnormal thereafter)
- Photoreceptor abnormalities antedate RPE changes
- Mutation is more important than age

Jacobson et al., IOVS, 2011

Developing a gene therapy strategy for USH1B



Lee et al. 2001

Shaker1 mouse

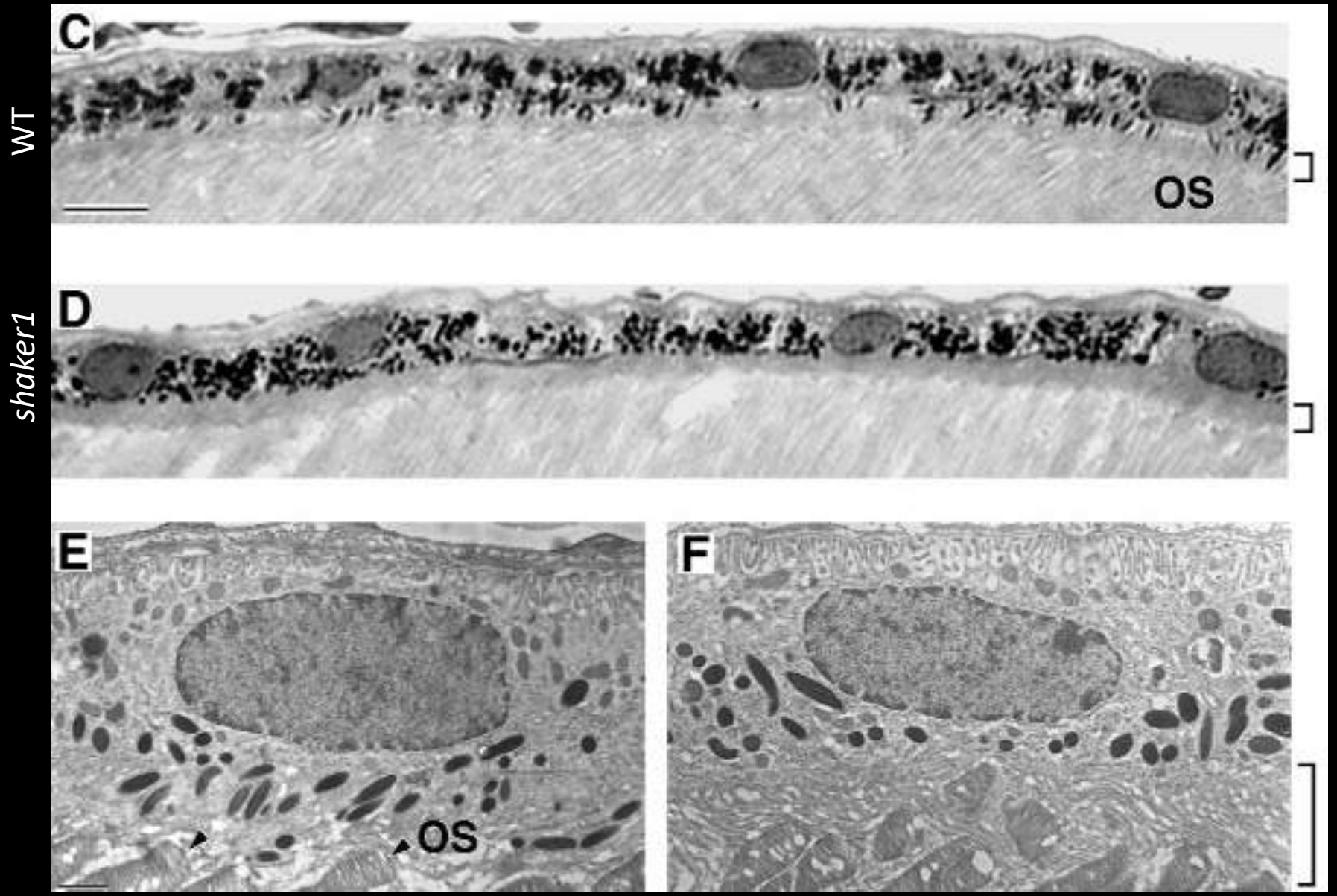
USH1B patient

- 4626SB allele
- null
- homozygous: hyperactive, head-tossing, circling behavior (vestibular dysfunction), mothers incapable of rearing, deaf
- heterozygous: normal
- No retinal degeneration
- No ERG phenotype*

- Mutations found throughout *MYO7A*
- Congenital deafness
- heterozygous: normal
- retinal degeneration
- Abnormal/absent ERG

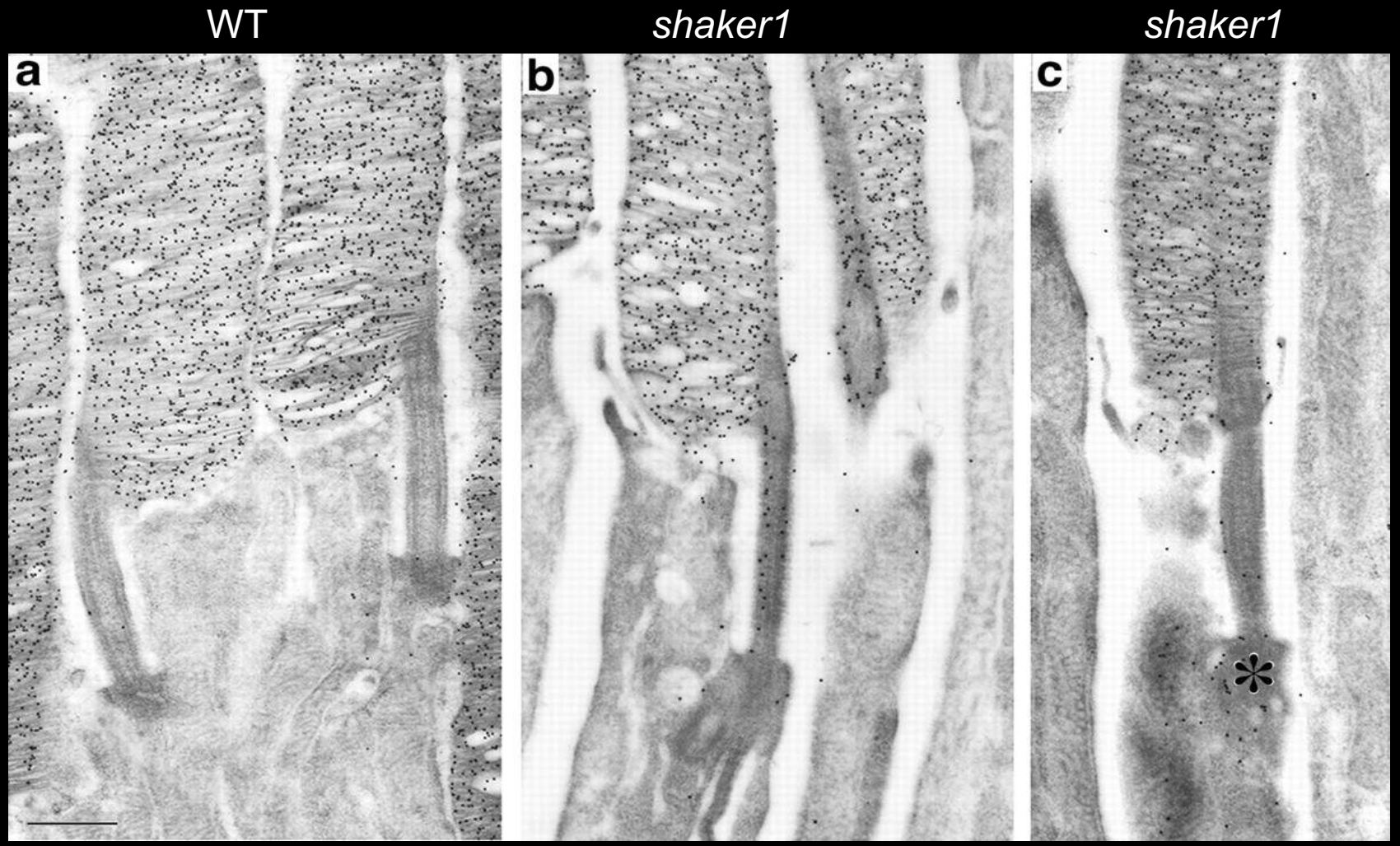
Are there any phenotypes in *shaker1* mice that may be used as outcome measures??

Apical migration of RPE melanosomes impaired in *shaker1* mice



David S. Williams, Nature Genetics, 1998

Opsin transport through the connecting cilium is disrupted in *shaker1* mice

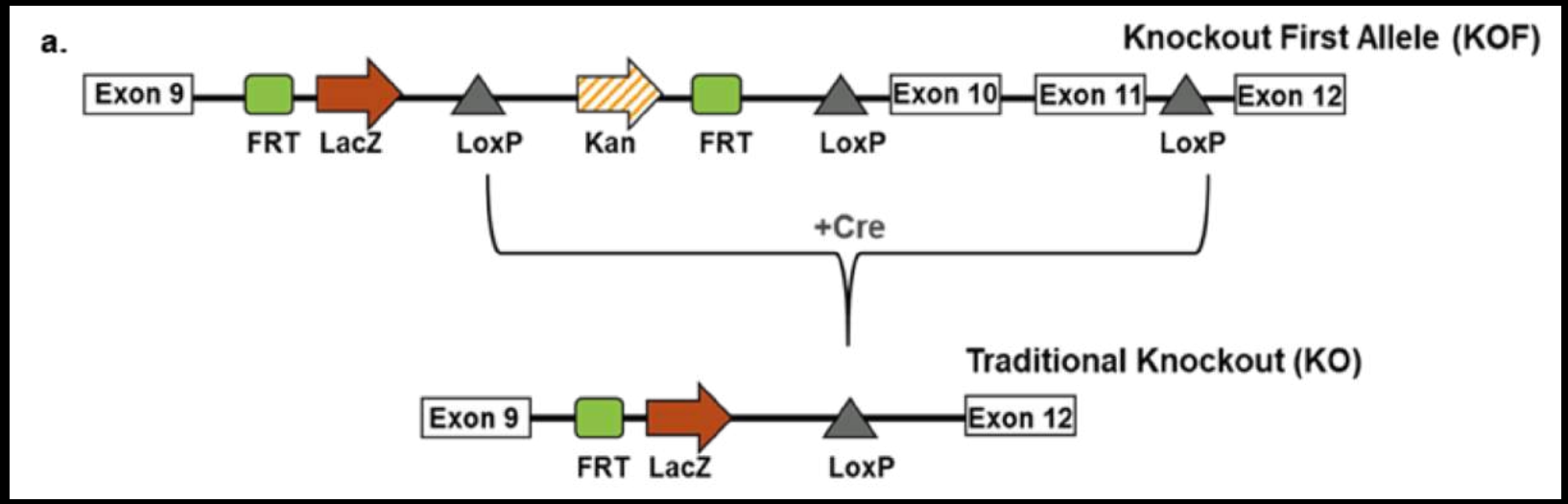


David S. Williams, J. Neuroscience, 1999

Traditional Myo7a KO Mice

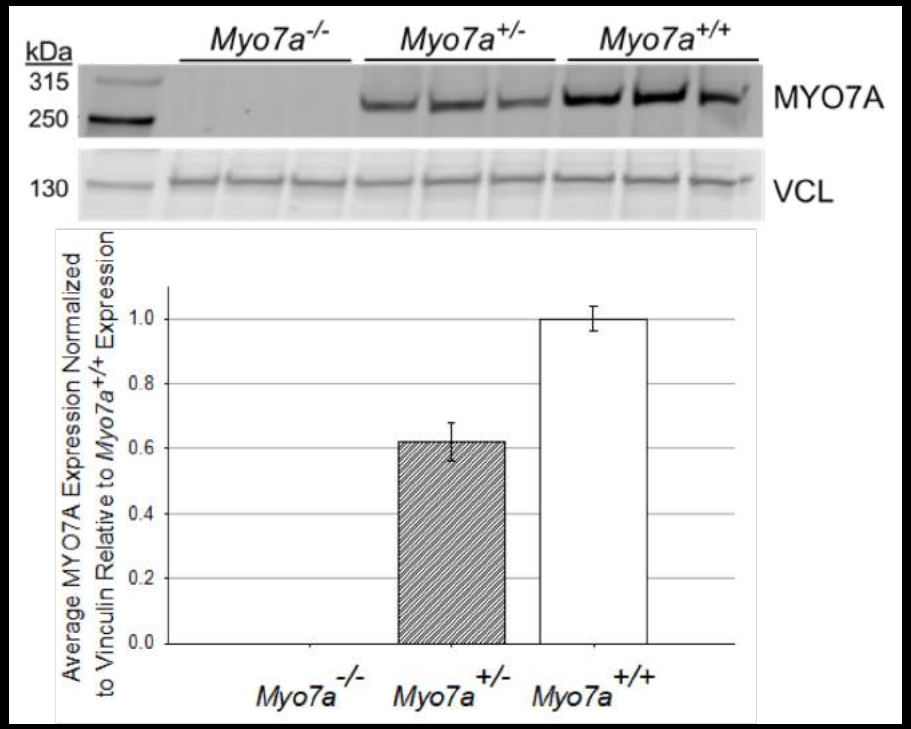
- Due to the availability *shaker1* mice, a knock-out mouse has never been created until now.

- We created *Myo7a KO* mice as a model that is easier to work with:
    - Simplified genotyping protocol
    - Known mutant MYO7A expression

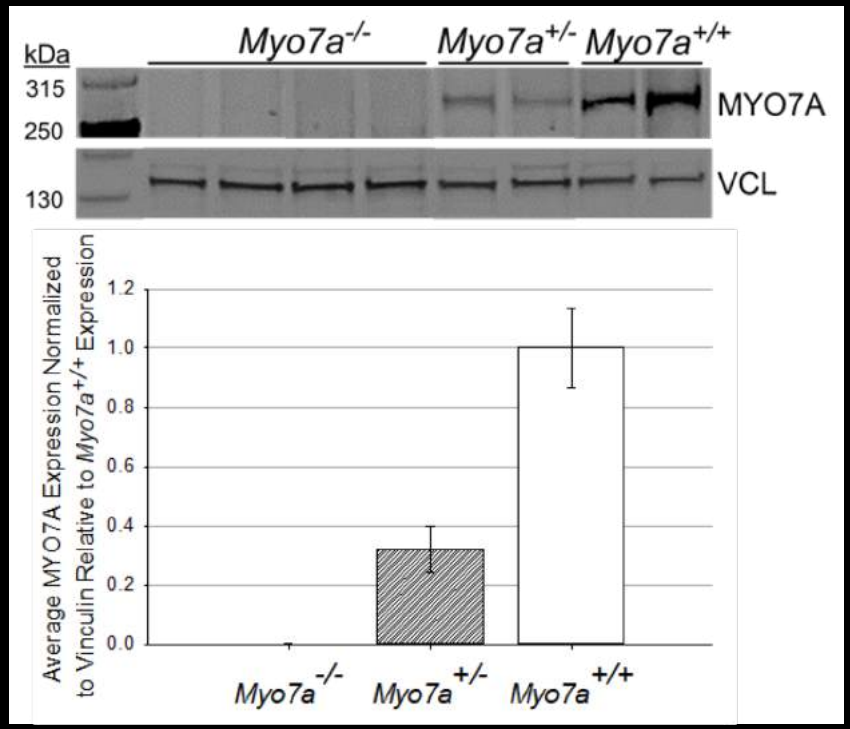


Confirmation of MYO7A Knock-out

Whole Eyes



Cochlea



Confirmation that *Myo7a*$^{-/-}$ mice do not express mutant protein

Characterization of *Myo7a*$^{-/-}$ Mice Summary

- *Myo7a*$^{-/-}$ mice do not express MYO7A (in both eyes & cochlea)
- *Myo7a*$^{-/-}$ mice are profoundly deaf. These mice also have highly disorganized hair cells.
- Vestibular hair cells appear well-maintained
- Retinal dysfunction in *Myo7a*$^{-/-}$ mice is mild
- There are no changes in outer nuclear layer thickness among the genotypes over time.

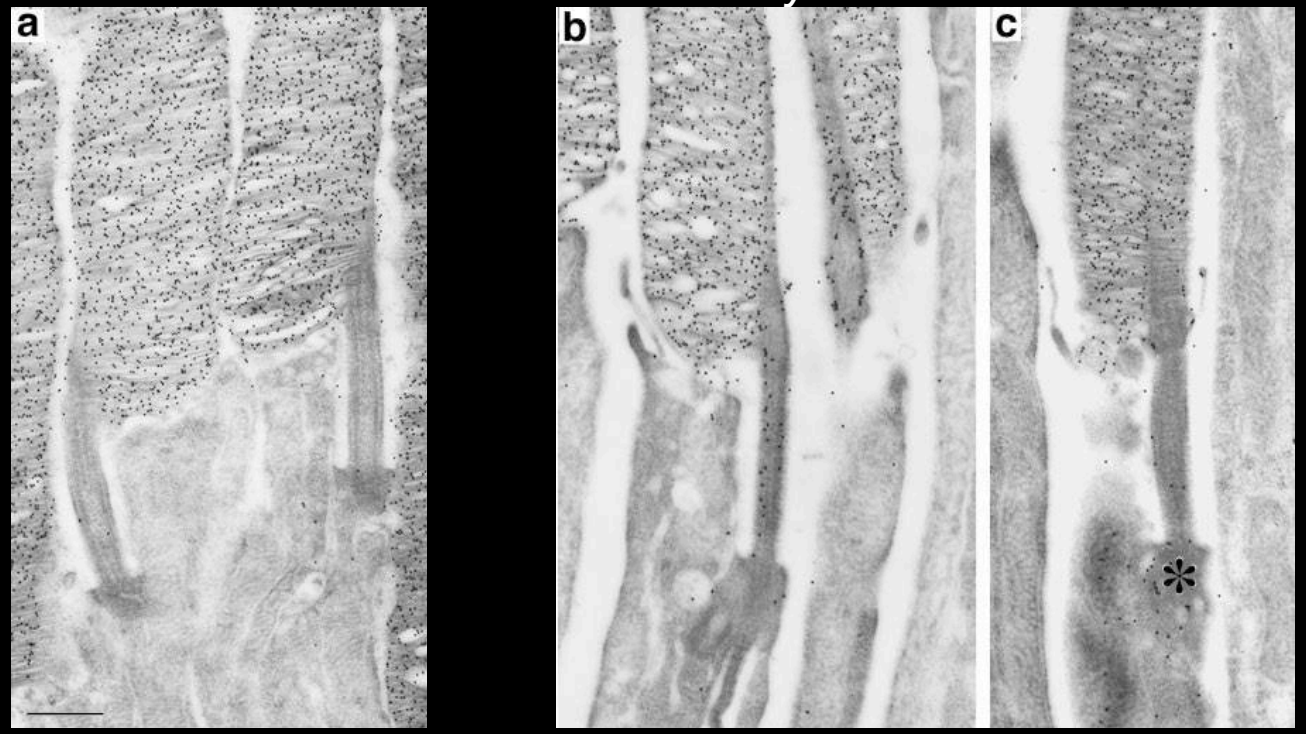
very reminiscent of the *shaker1* mouse model

Is there a better way to evaluate whether MYO7A is important in mouse photoreceptors?

- MYO7A has been reported to be involved in rhodopsin transport in rod photoreceptors
  - Aids in the transport of rhodopsin to rod outer segments
  - *Myo7a*$^{4626SB/4626SB}$ may present with impaired transport of rhodopsin & accumulation in connecting cilium

Control

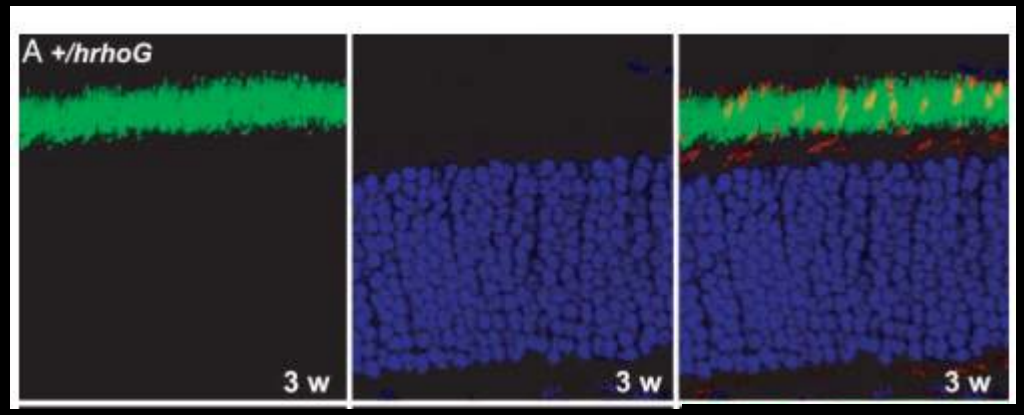
Myo7a$^{4626SB/4626}$



Liu et al, 1999

Myo7a:RHOGFP Model

X

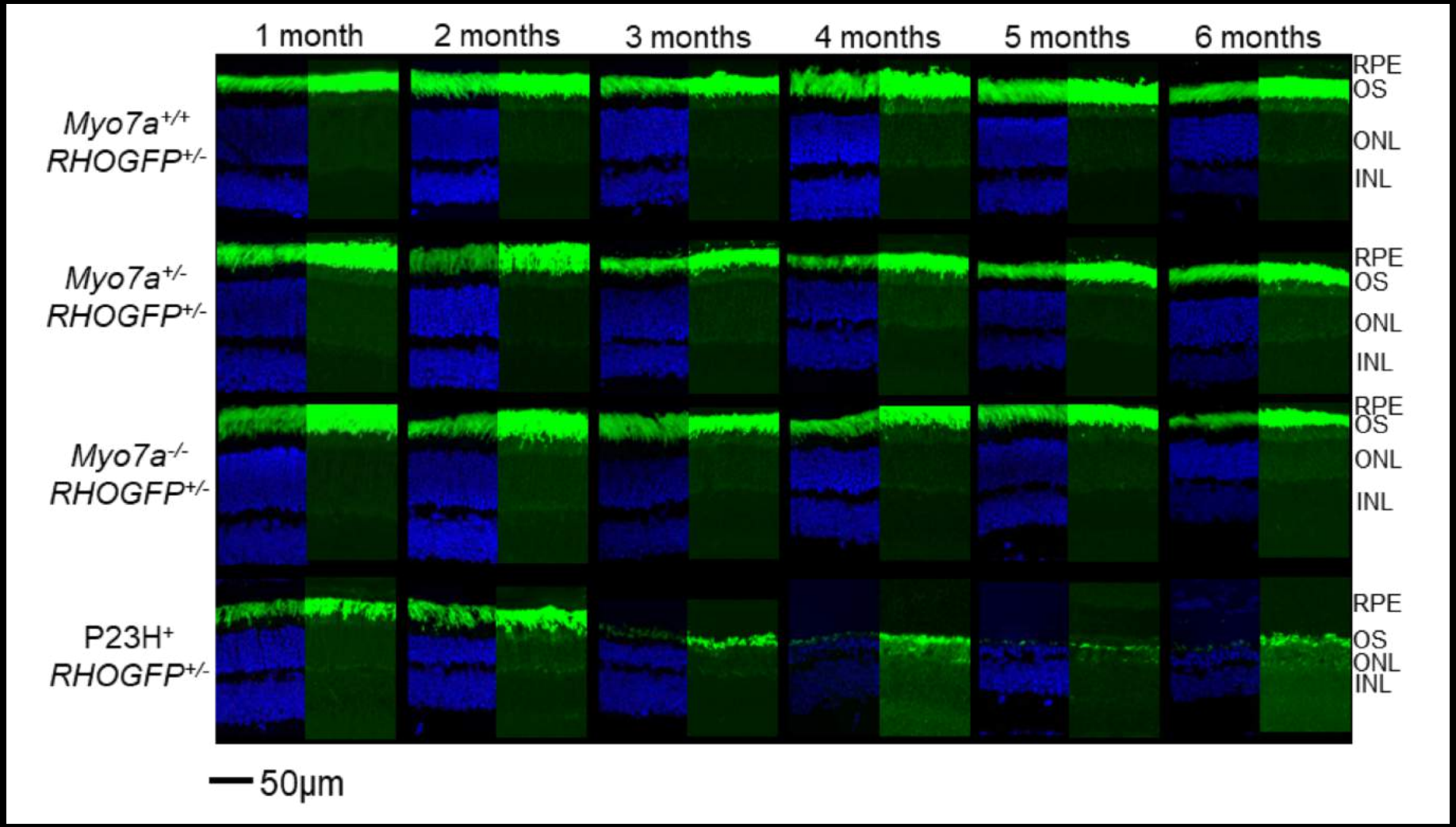


Chan et al, 2004

*P23H-RhoGFP mice generated as control

- RHOGFP fusion would exacerbate rhodopsin trafficking phenotype-> more easily tested
  - Accumulation of RHOGFP in photoreceptor inner segments
  - Increased rate of degeneration/thinning

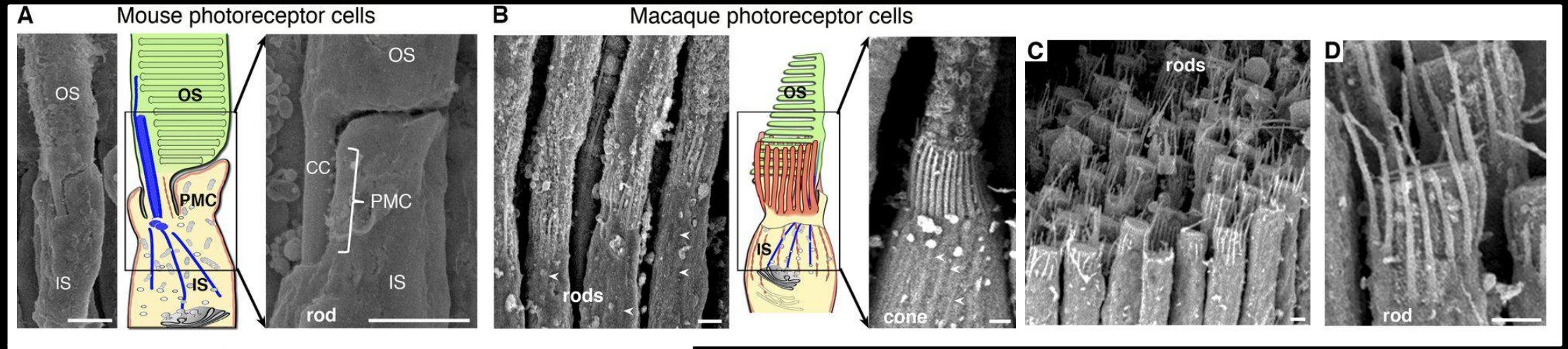
Myo7a:RhoGFP Rhodopsin Transport

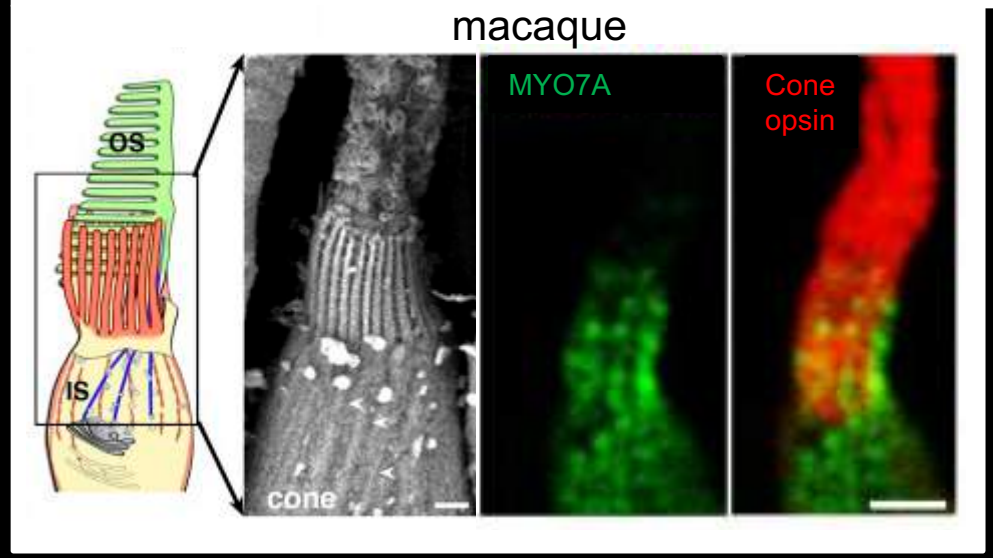


MYO7A doesn't seem to play a role in RHO transport in photoreceptors

Why don't shaker1 or Myo7a-/- mice have loss of retinal structure/function??

- Whether MYO7A is actually expressed in mouse photoreceptors remains controversial
- No question- MYO7A is definitely expressed in NHP and human photoreceptors

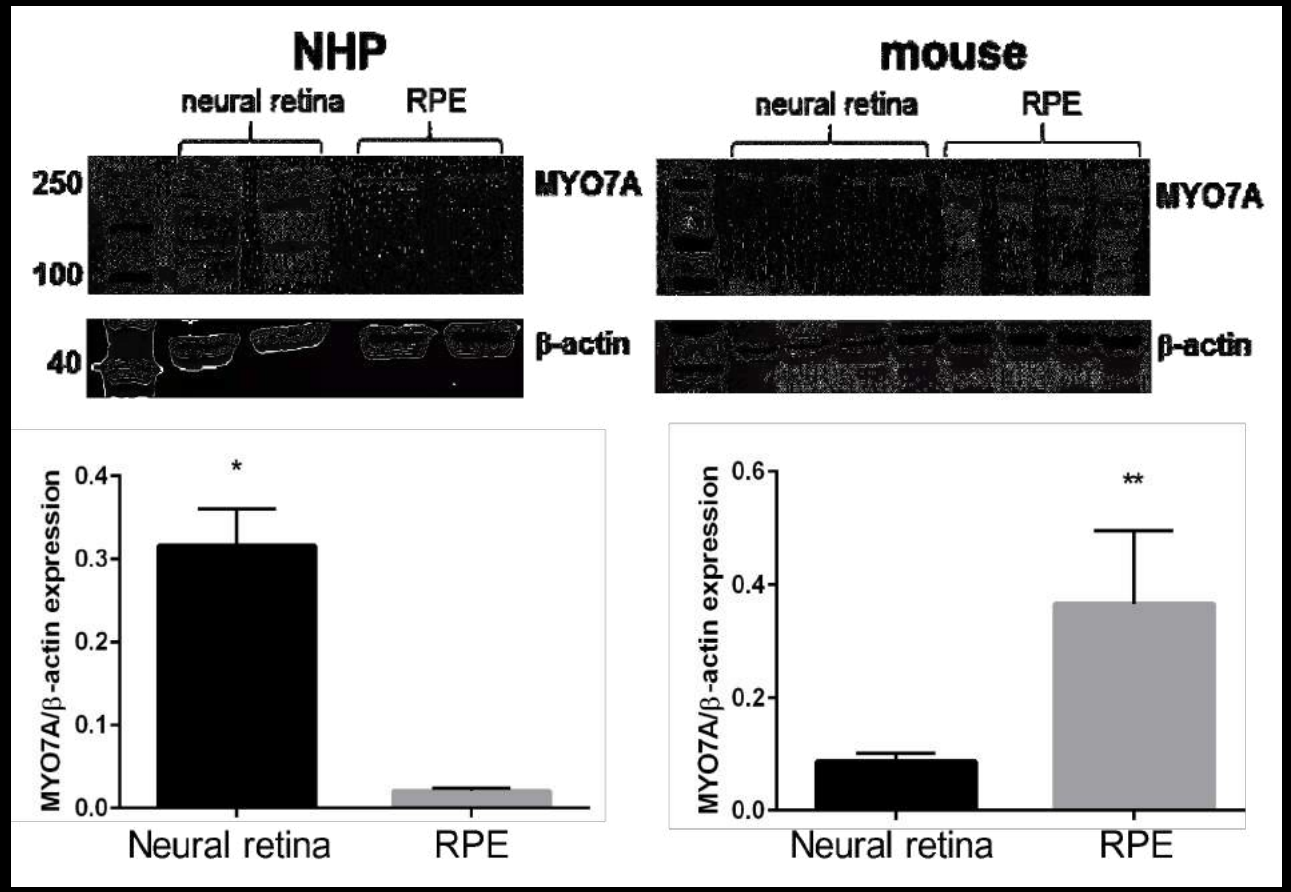




Primates have calycal processes
Mice do not

Adapted from Sahly et al., 2012

MYO7A is differentially expressed in mouse and non-human primate (NHP)

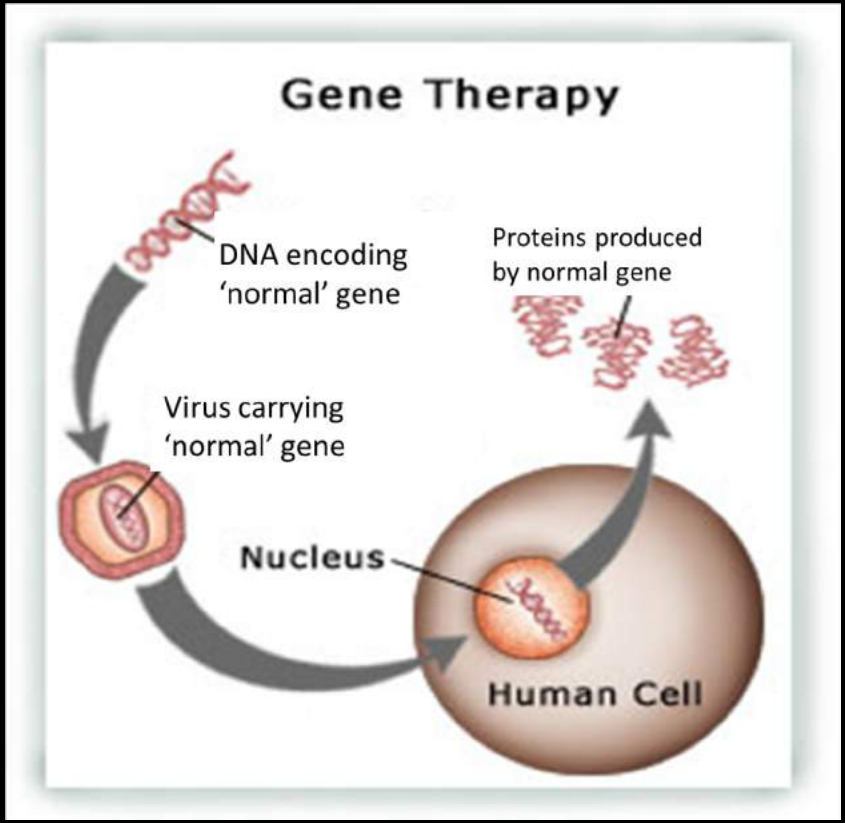


Mouse Characterization Conclusions

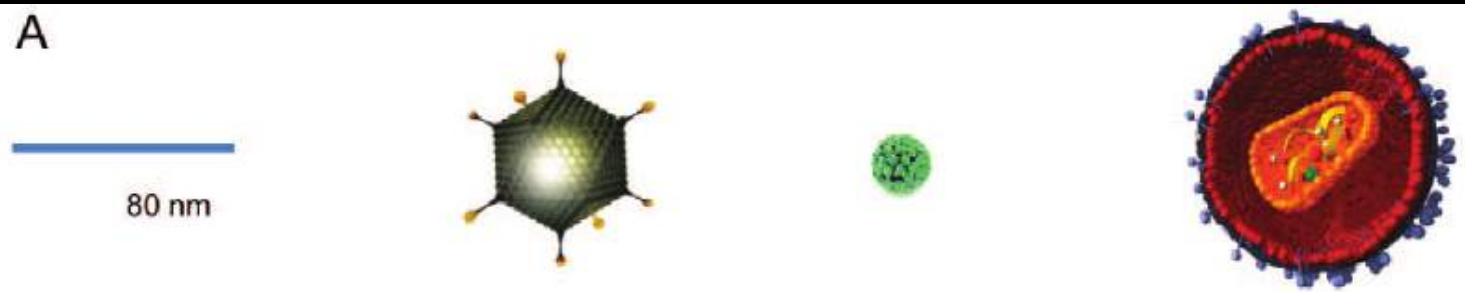
- Due to difference in structure and expression, these mice will not be useful for better understanding USH1B disease mechanisms and testing therapeutic efficacy

- These mice will be useful for testing and optimizing new vectors for their ability to drive expression of full length MYO7A
  - Easy genotyping
  - Null MYO7A expression

How will we deliver *MYO7A* cDNA (~6.7kb) to the retina?



A



B

	Adenovirus	AAV	Lentivirus
Viral Genome	dsDNA	ss or ds DNA	RNA
Cloning capacity	7.9kb	<5.0 kb	8.0 kb
Vector genome	episomal	~90% episomal & ~10 intergrated	Integrated
Major area of application	Short term gene expression & proof-of-principle studies	Long term gene expression of small genes	Long term gene expression of small to large genes & ex vivo modification of stem cells

AAV is the gold standard viral vector for delivering genes to retina, but capsid is too small to accommodate the entire MYO7A cDNA

Lentivirus and Adenovirus can accommodate cDNA, but both have drawbacks (inefficient transduction of post-mitotic retina and safety concerns

Lentivirus (cont.)

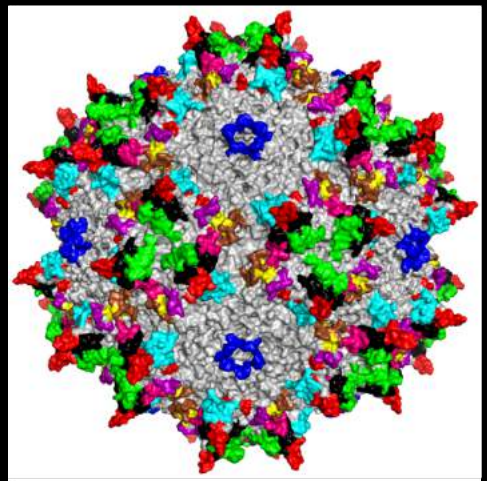
- Lenti-*MYO7A* corrects RPE phenotype (melanosome migration) of *shaker1* mice
- Lenti-*MYO7A* corrects opsin trafficking defect of shaker1 mice, although results were “spotty”

Clinical Trials

- Sanofi acquires SAR 421869 (aka “USHstat”) from Oxford Biomedica
- EIAV-based lentiviral approach
- Safe, well-tolerated
- To date- no evidence of biological activity
- Sanofi drops “USHstat” in 2019
- It goes back to Oxford Biomedica in June, 2020

Adeno-Associated Virus (AAV)

- Family: *Parvoviridae*
- Genus: *Dependoparvovirus*

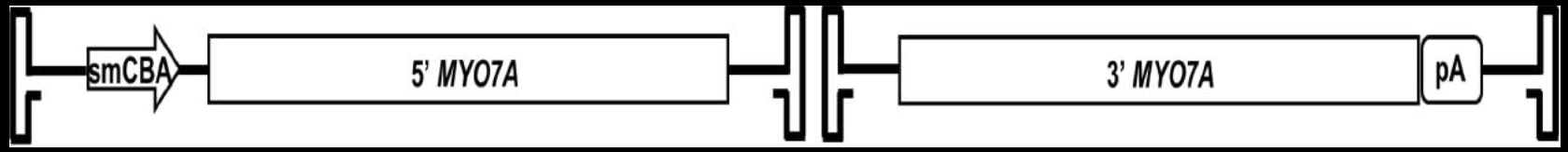

Surface rendering of AAV2 (PDB code 1lp3, Xie et al. 2002)

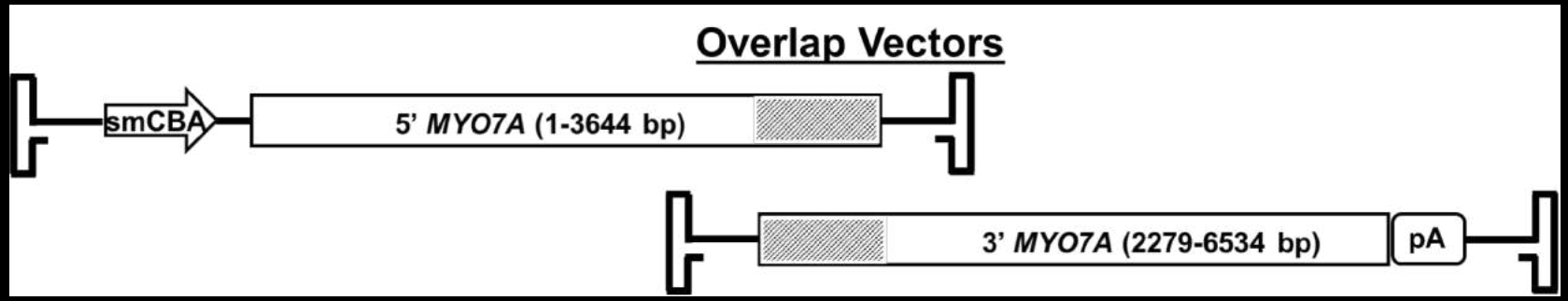
- Small, non-enveloped virus
- Ideal for gene therapy:
  - Non-pathogenic
  - Low Immunogenicity
  - Promotes persistent transgene expression
  - Numerous variants available

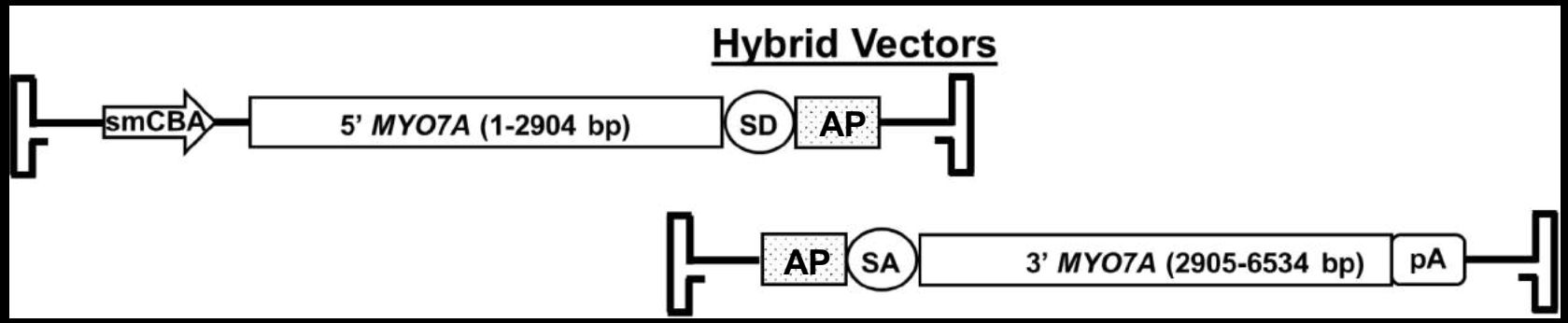
FDA NEWS RELEASE

FDA approves novel gene therapy to treat patients with a rare form of inherited vision loss

AAV Dual Vector Platforms

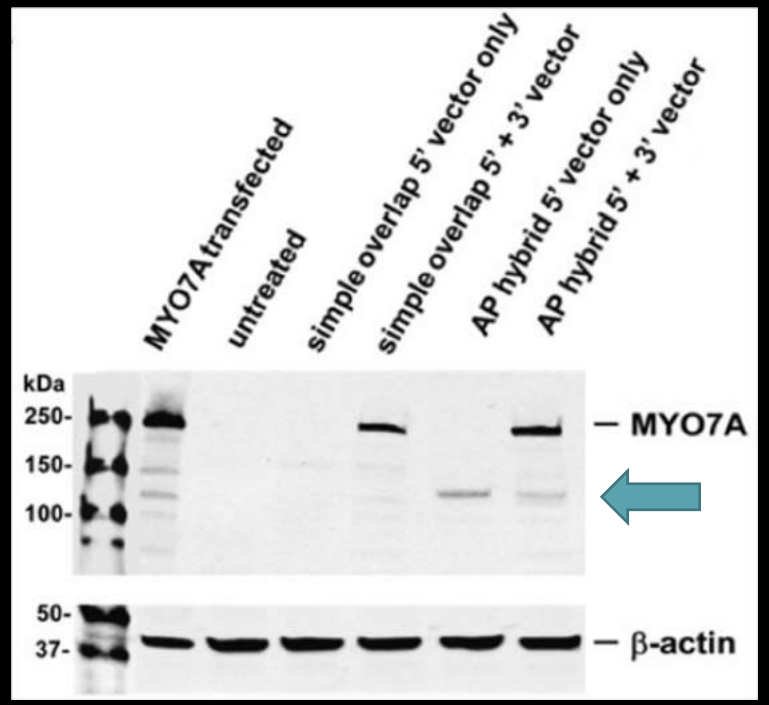






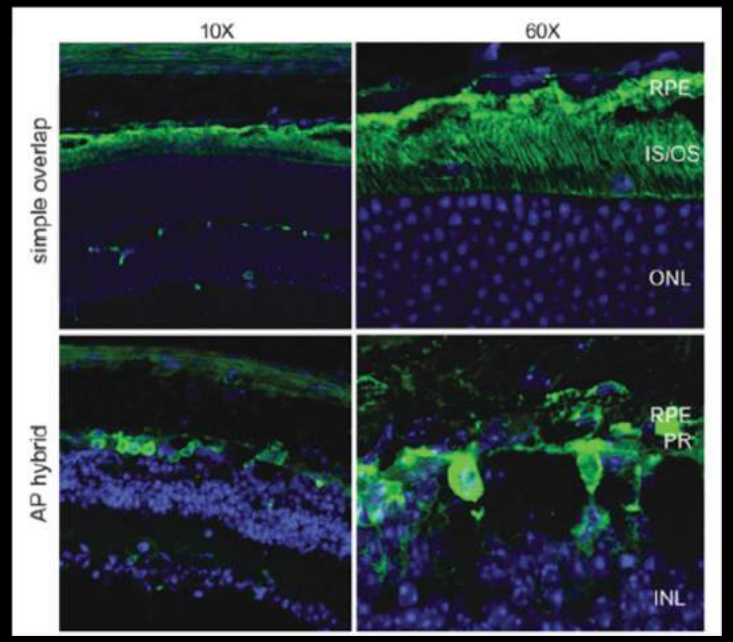
Previous work with Dual AAV-*MYO7A*

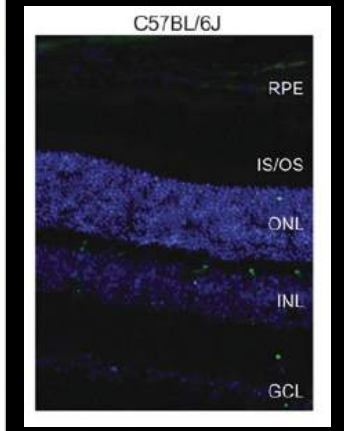
HEK293



2.0 E10 vg total virus
(1E10 vg front/back)

C57BL/6J α-HA



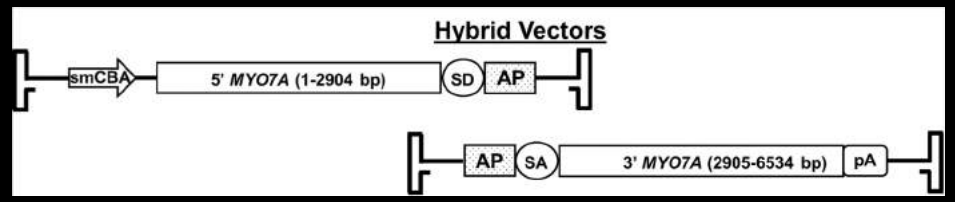


Adapted from Dyka et al. 2014

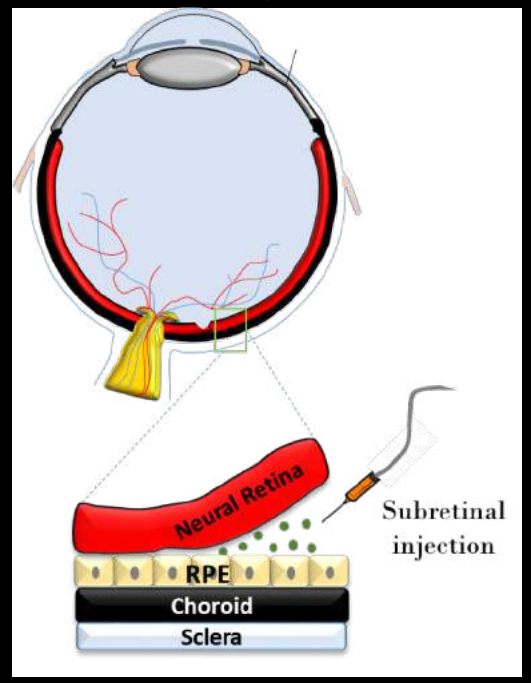
Improving *MYO7A* Dual Vectors

- Can dual vectors express MYO7A at a level comparable to endogenous expression in mice?
- What is causing functional decreases?
- Can we reduce/eliminate the production of truncated product and related toxicity?
- Can dual vectors express full-length MYO7A in a clinically relevant model (NHP)?

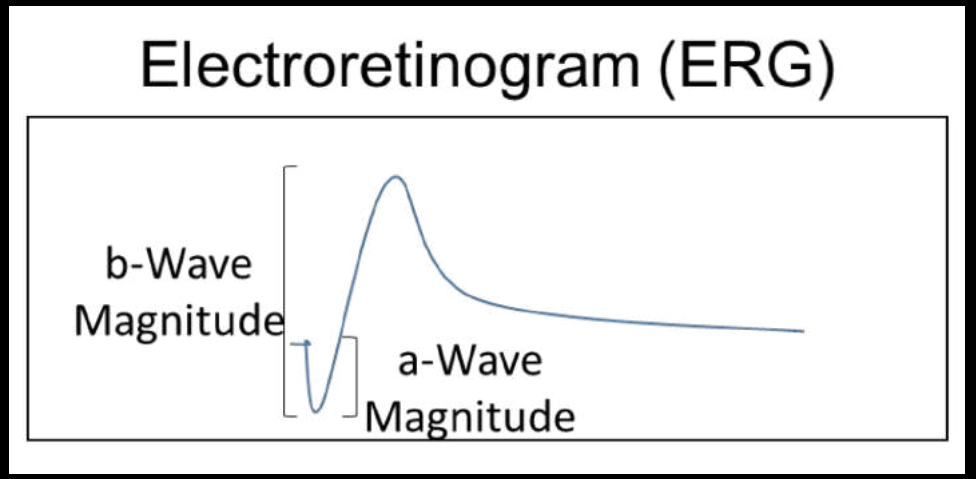
Experimental Design

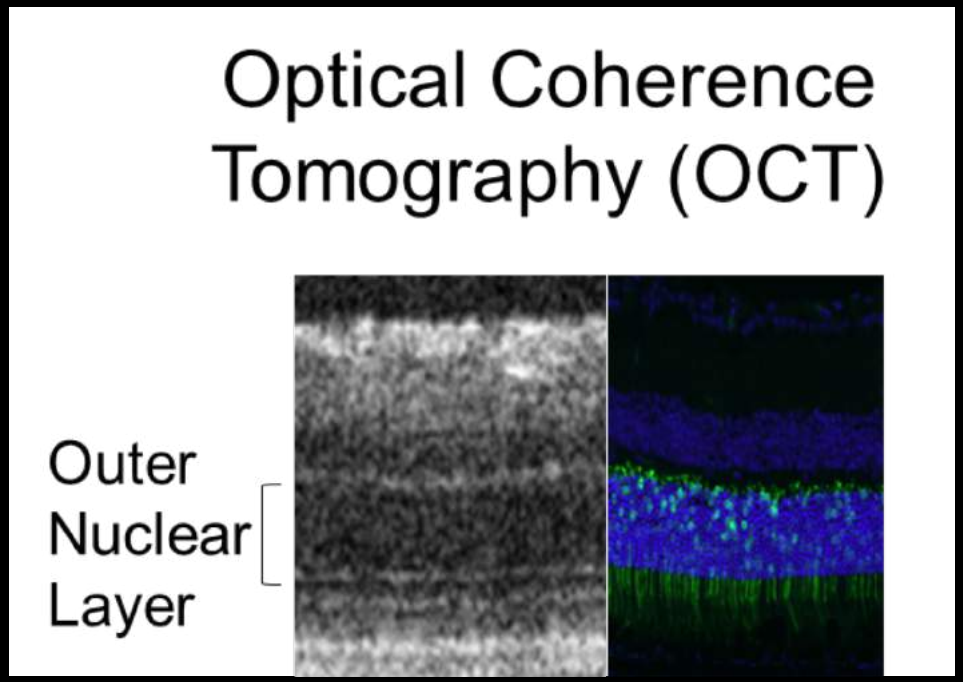


Titer: 5.0E8 vg total
(2.5 E8 vg each)

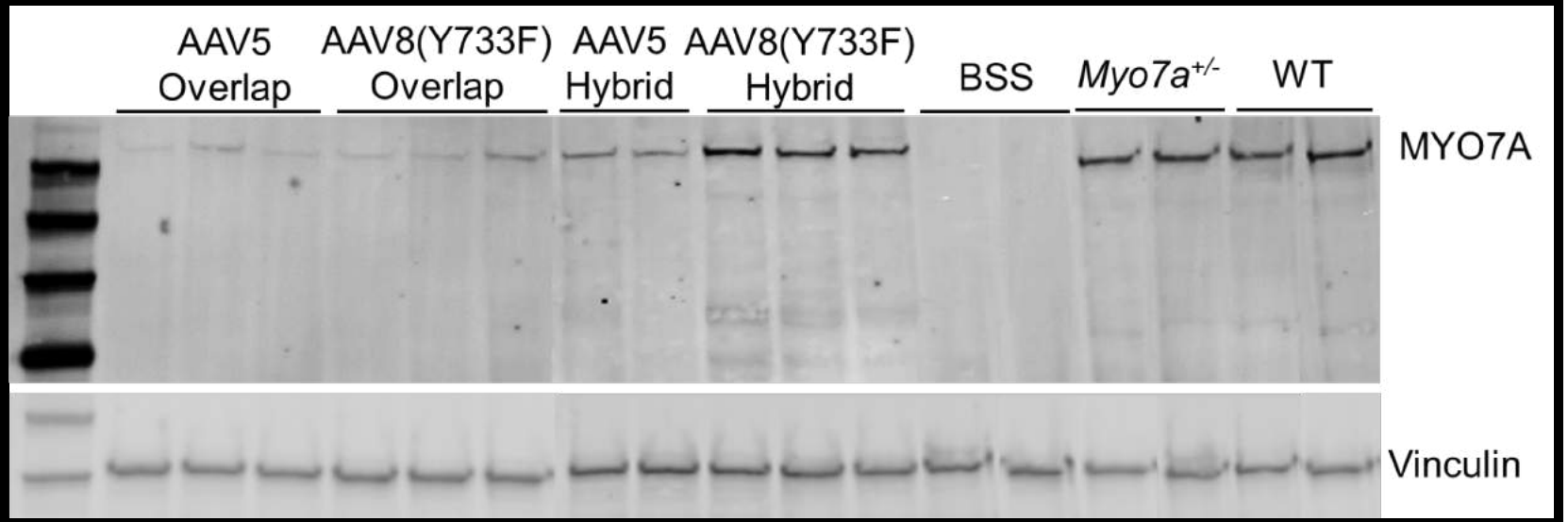


6 weeks post-injection

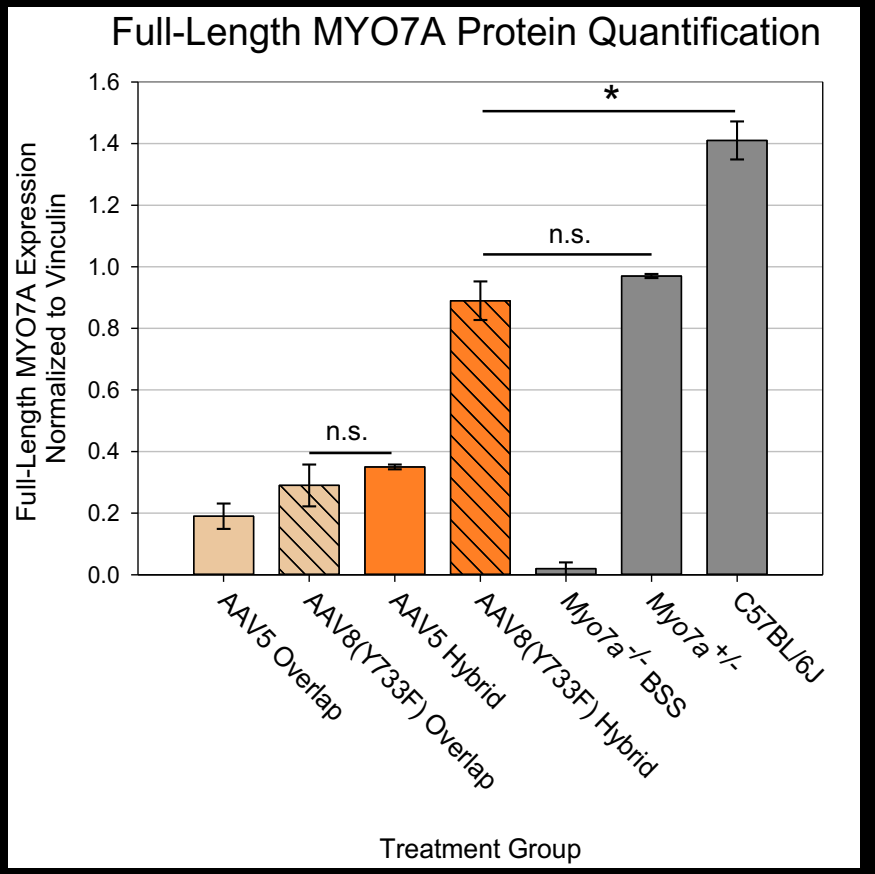




Hybrid Dual Vectors Result in the Highest Expression of MYO7A



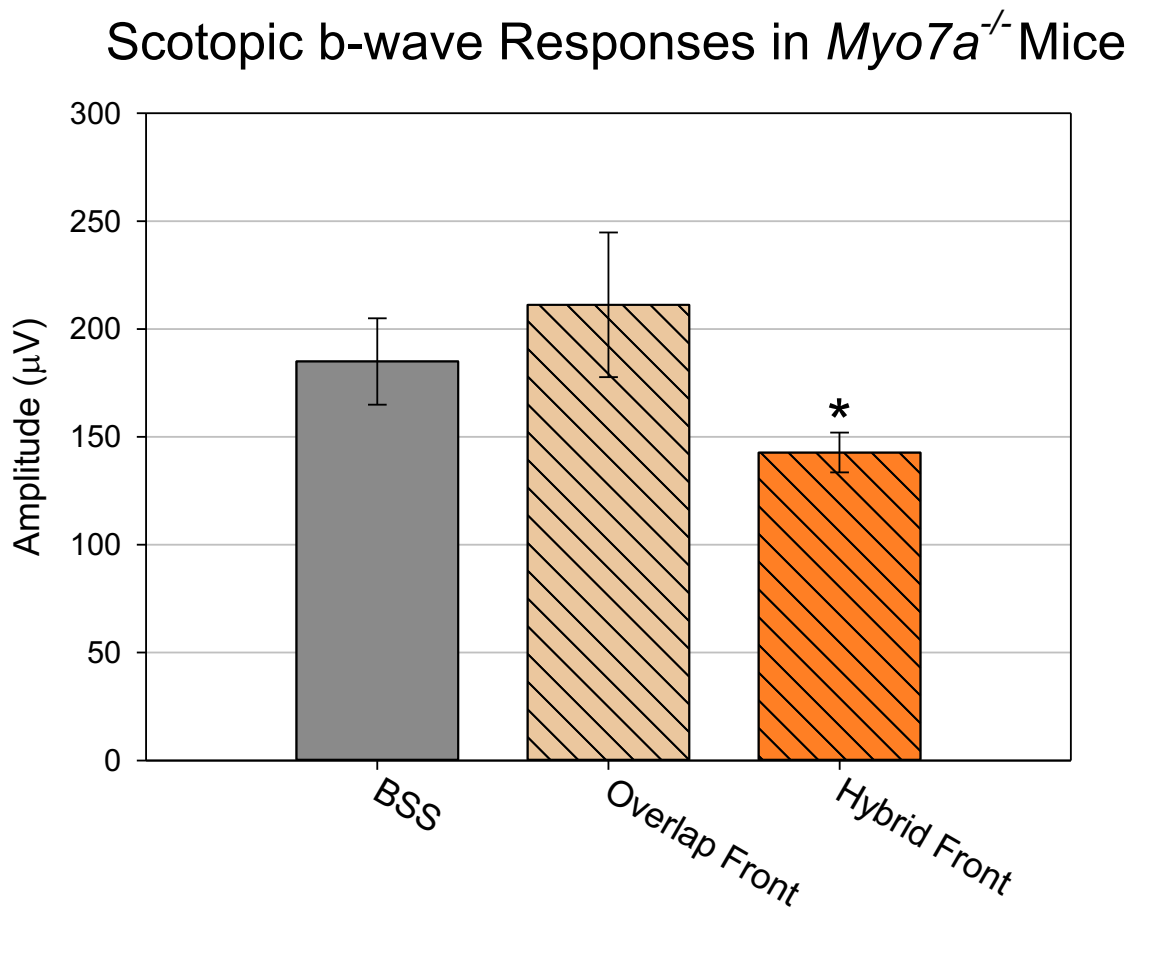
α-MYO7A

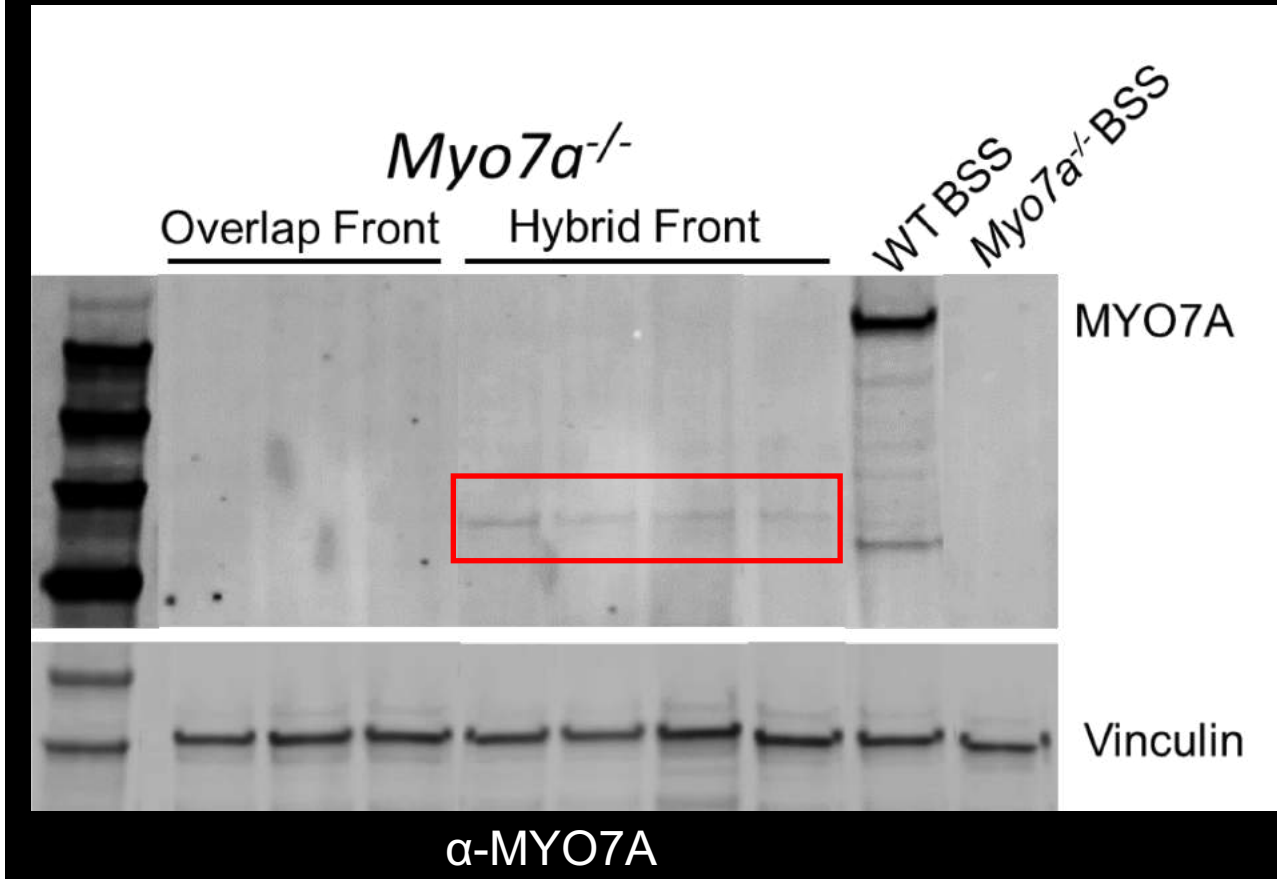


6 weeks post-injection

Injection Titer: 5.0E8 vg total (2.5 E8 vg each)

The Hybrid Front Half Vector Produces a Truncated Protein that Results in Loss of Retinal Function



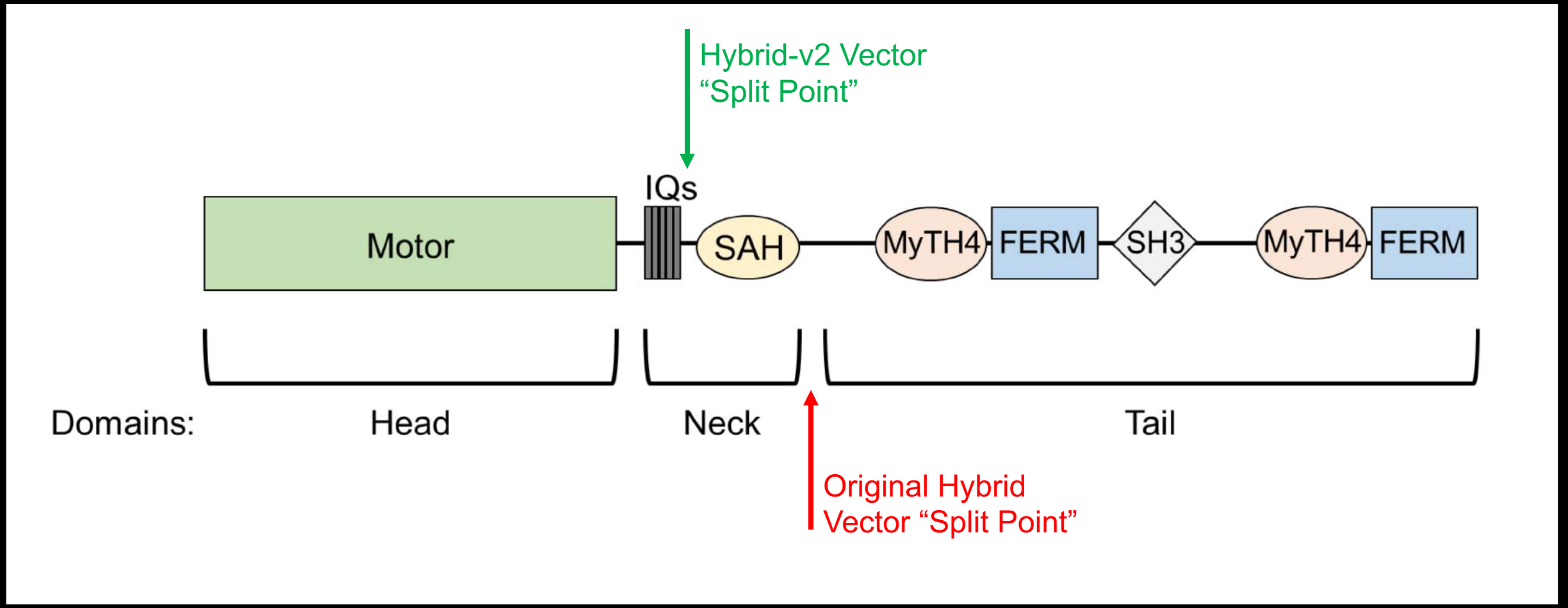


Packaged in: AAV8(Y733F)

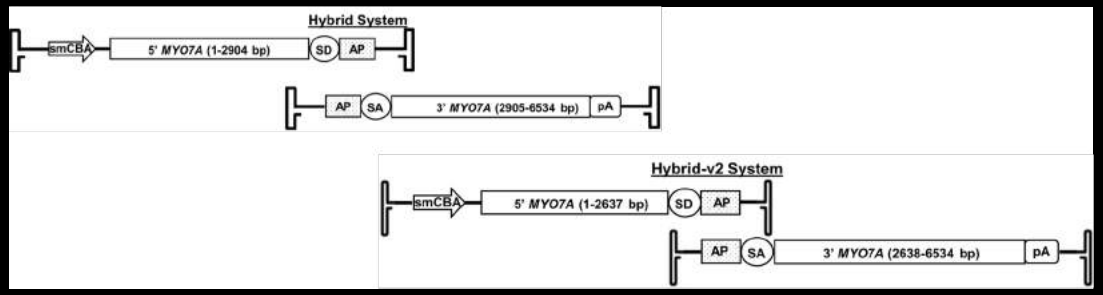
Injection Titer: 5.0E8 vg

6 weeks post-injection

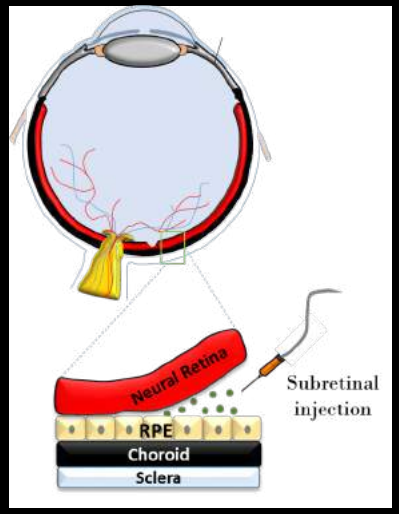
MYO7A Protein Structure as Explanation of Functional Decrease caused by Truncated Protein



Experimental Design



Titer: 5.0E8 vg total
(2.5 E8 vg each)



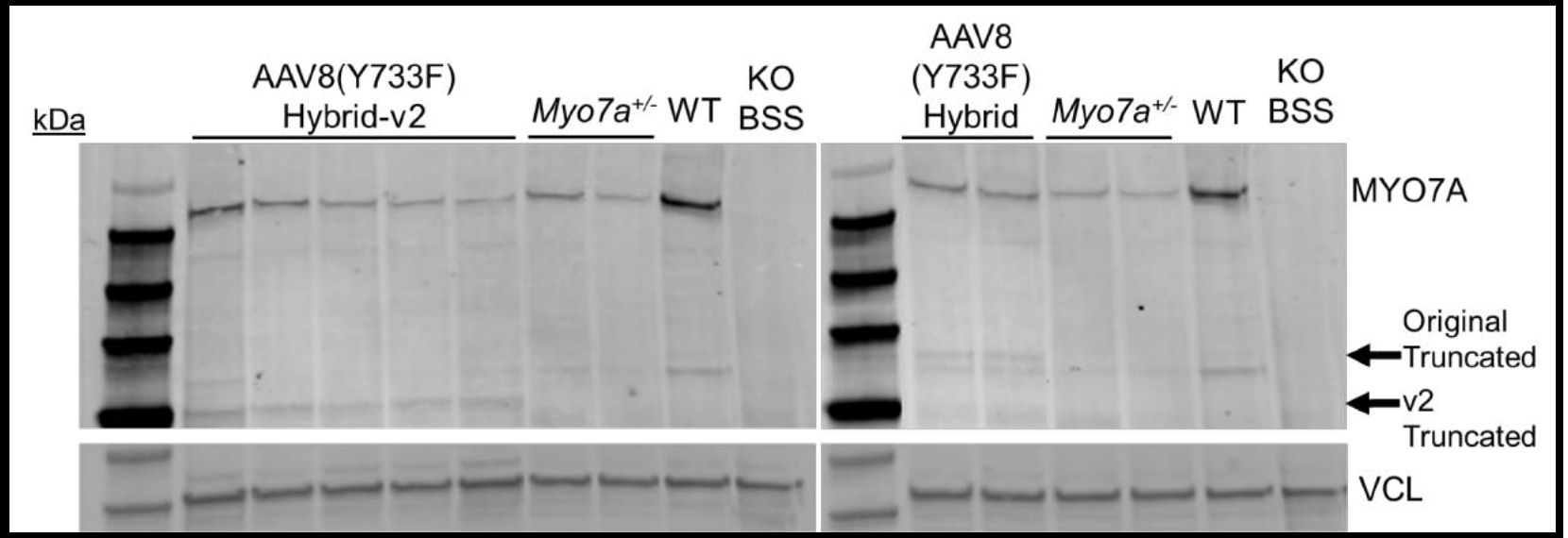
Myo7a$^{-/-}$ mice

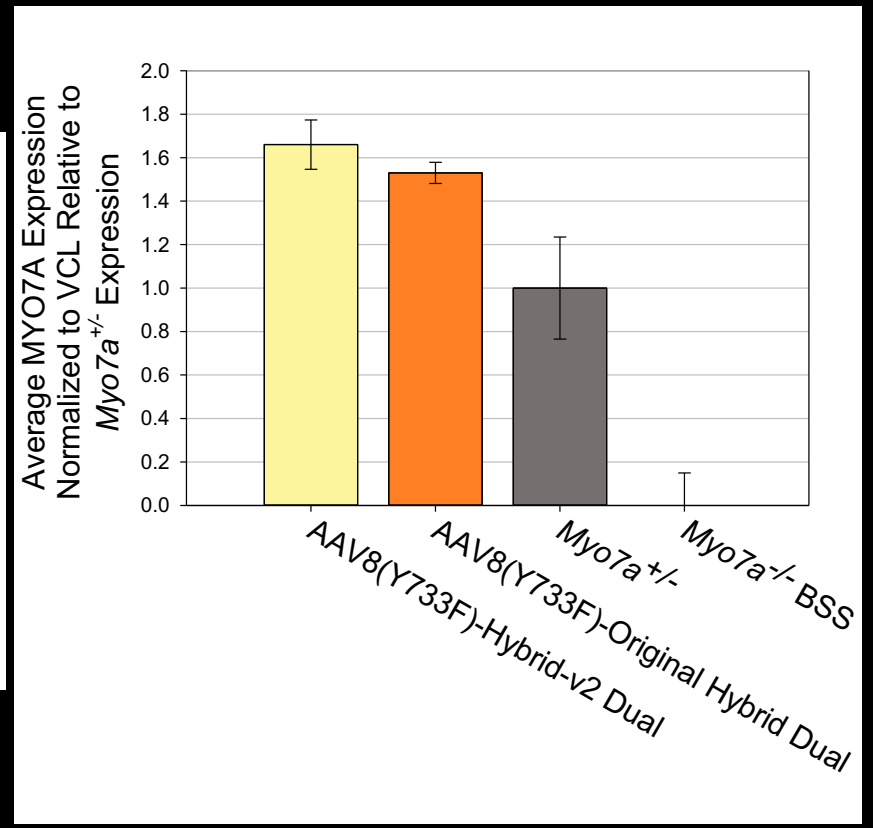
6 weeks post-injection

- Will the hybrid-v2 vectors express full-length MYO7A at the same level as originals?
- Will the hybrid-v2 front half vector produce truncated protein?
- Will hybrid-v2 vectors cause decreases in ERG amplitude?

ERG
OCT
Western Blot

Hybrid-v2 Dual Vectors Express MYO7A at a Level Comparable to Original Hybrid Vectors



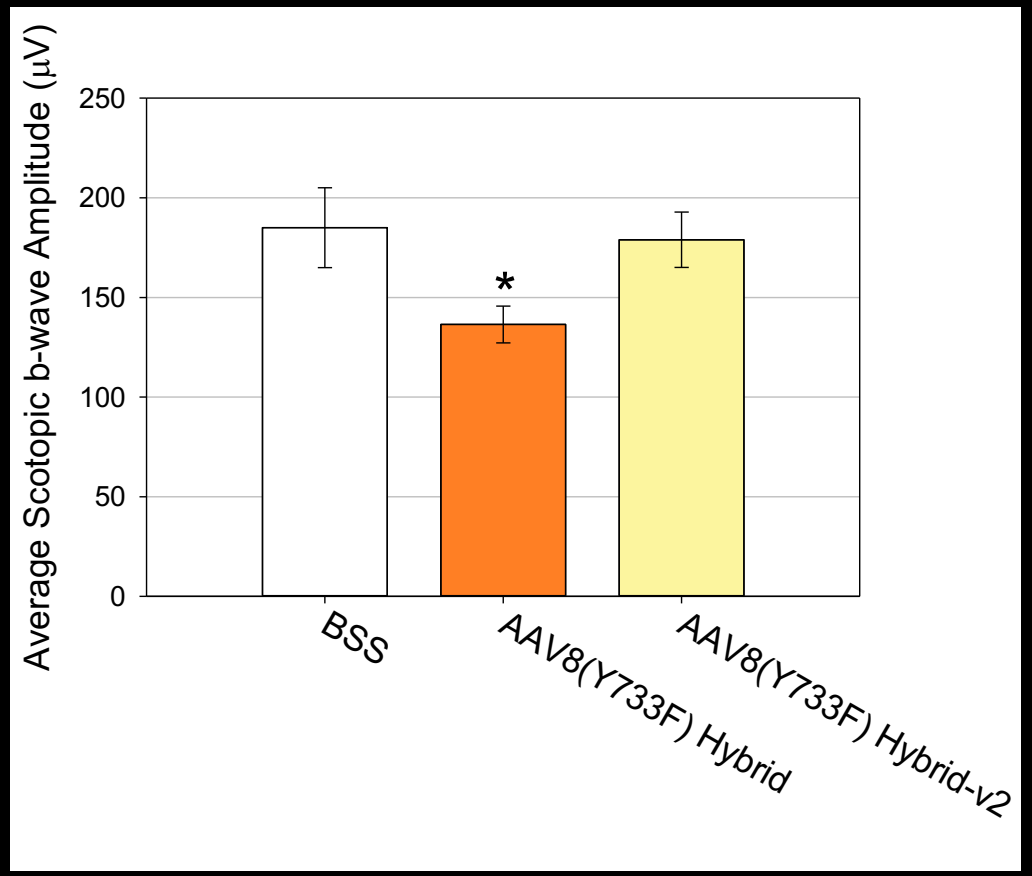


6 weeks post-injection

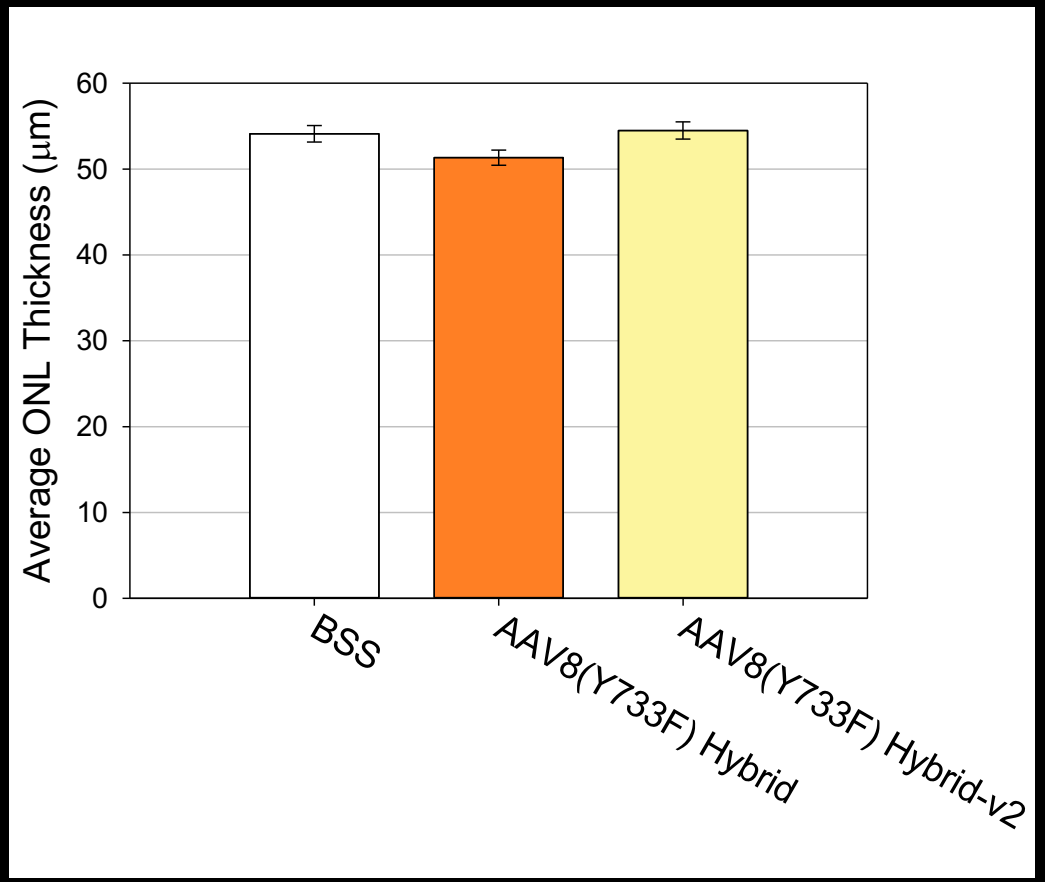
Injection Titer: 5.0E8 vg total (2.5 E8 vg each)

Hybrid-v2 Dual Vectors Do Not Cause Functional or Structural Decreases in Injected Mice

Scotopic b-wave Responses in *Myo7a*$^{-/-}$ Mice



Average ONL Thickness in *Myo7a*$^{-/-}$ Mice

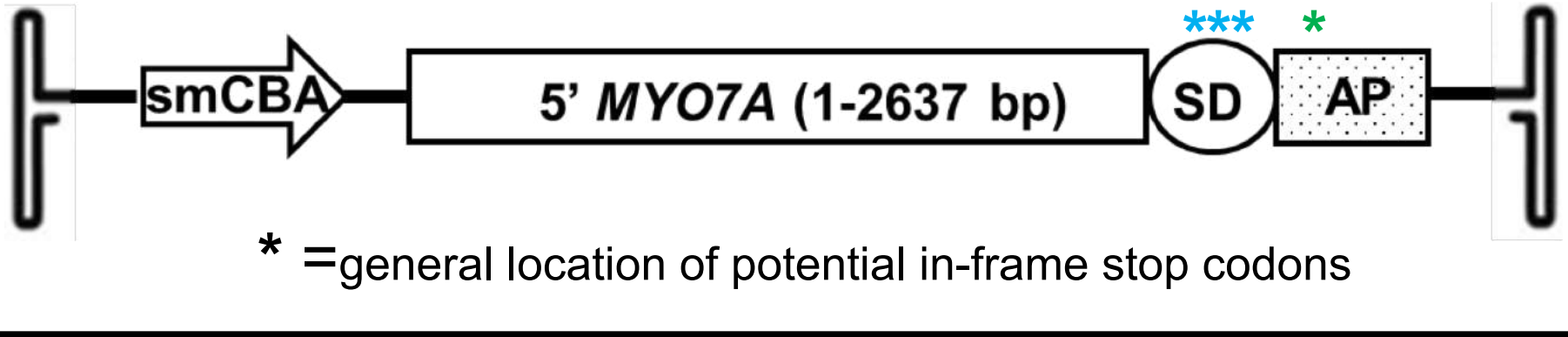


6 weeks post-injection

Injection Titer: 5.0E8 vg total (2.5 E8 vg each)

Can the Production of Truncated Protein be Reduced/Eliminated?

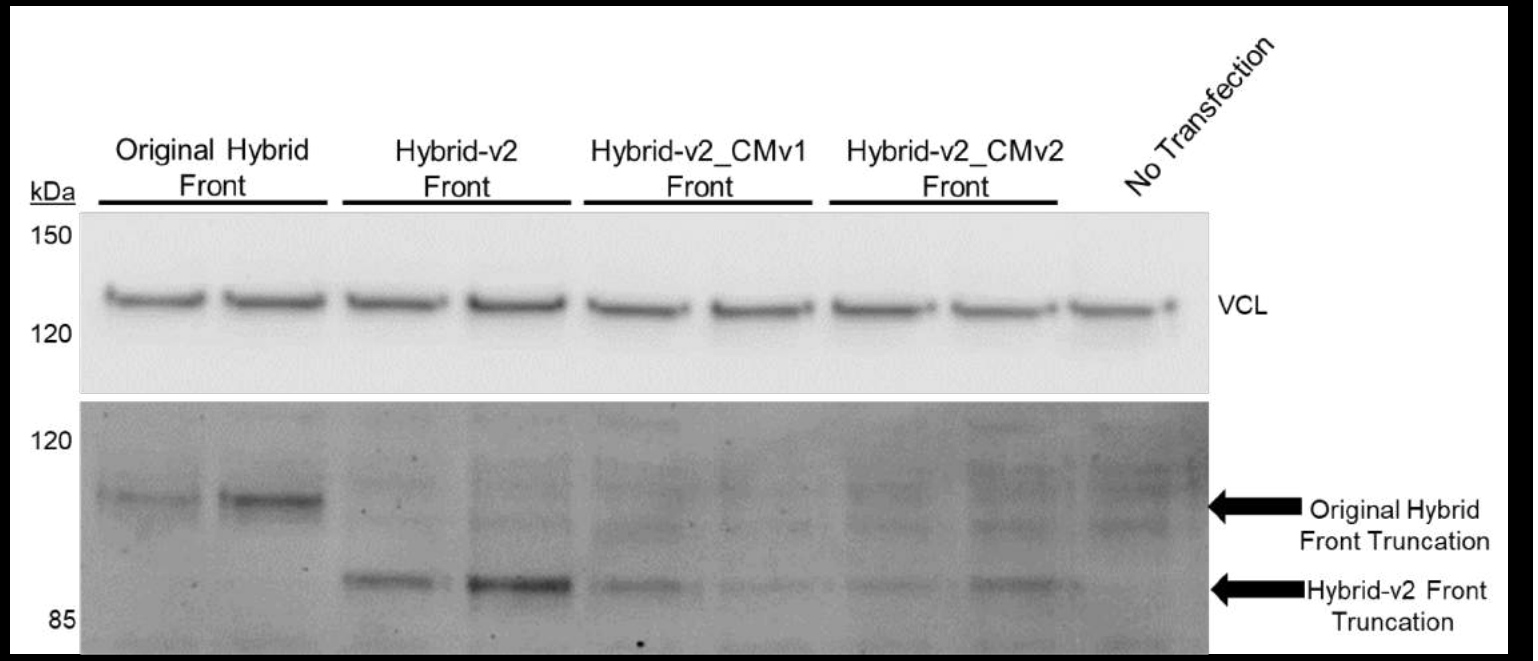
- Hypothesis: The exon-intron junction and AP sequence, in conjunction with the AAV ITR facilitates stabilization of the mRNA
  - Presence of potential in-frame stop codons provides further stabilization

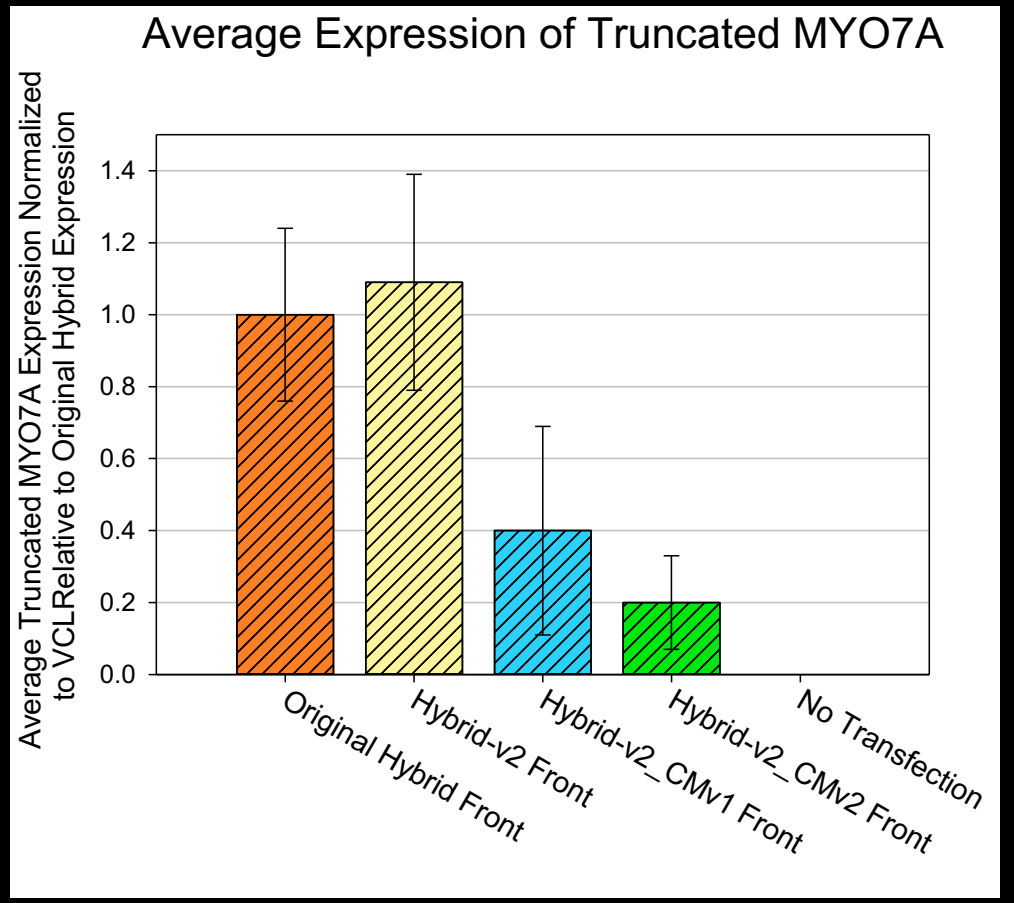


Hybrid-v2_CMv1: modification of 3 potential in-frame stop codons in AP intron

Hybrid-v2_CMv2: modification of 3 potential in-frame stop codons in AP intron + 1 potential in-frame stop codon in AP head

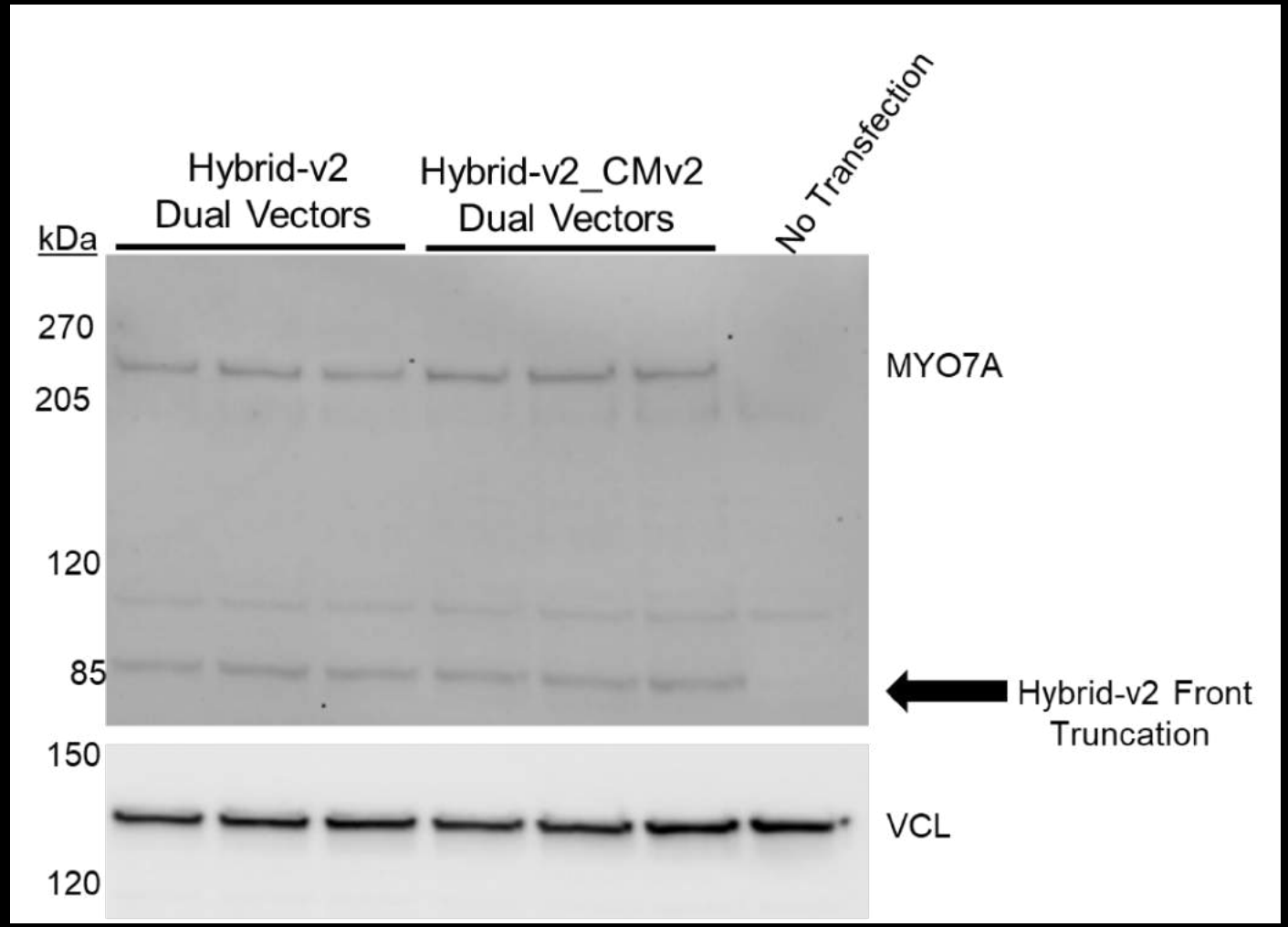
Codon Modification of the Hybrid-v2 Front Half Vector Significantly Reduces Production of Truncated Protein





Transfected HEK293 cells

Hybrid-v2_CMv2 Dual Vectors Produce Full-Length MYO7A *in vitro*

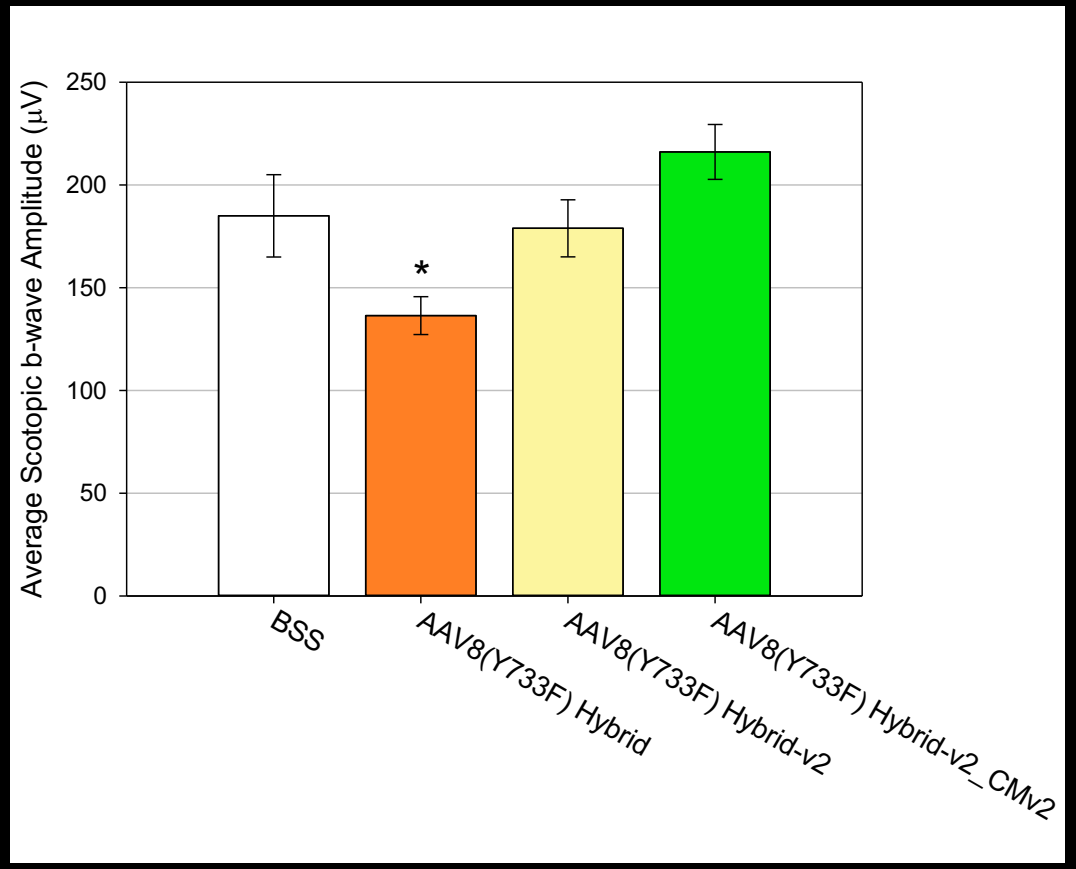


HEK293 Cells

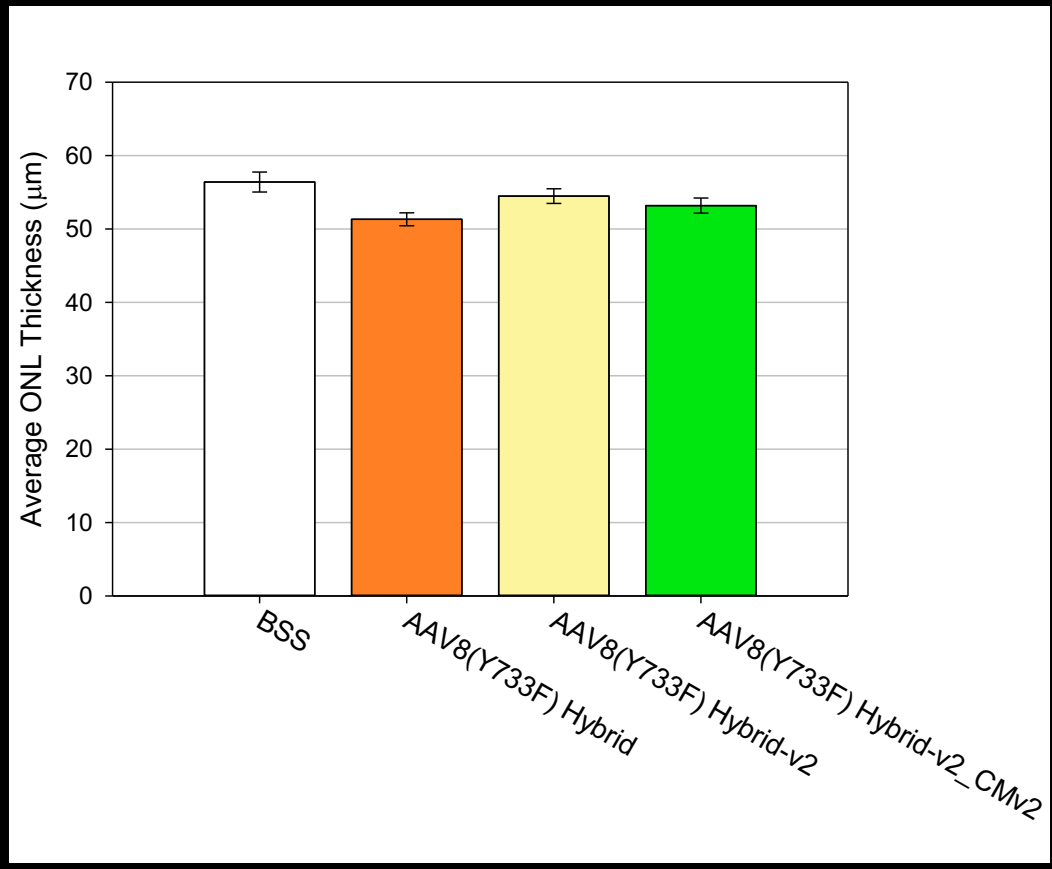
Packaged in AAV2
MOI 1:10,000 each virus

Hybrid-v2_CMv2 Dual Vectors Do Not Cause Functional or Structural Decrease in Injected Mice

Scotopic b-wave Responses in *Myo7a*$^{-/-}$ Mice



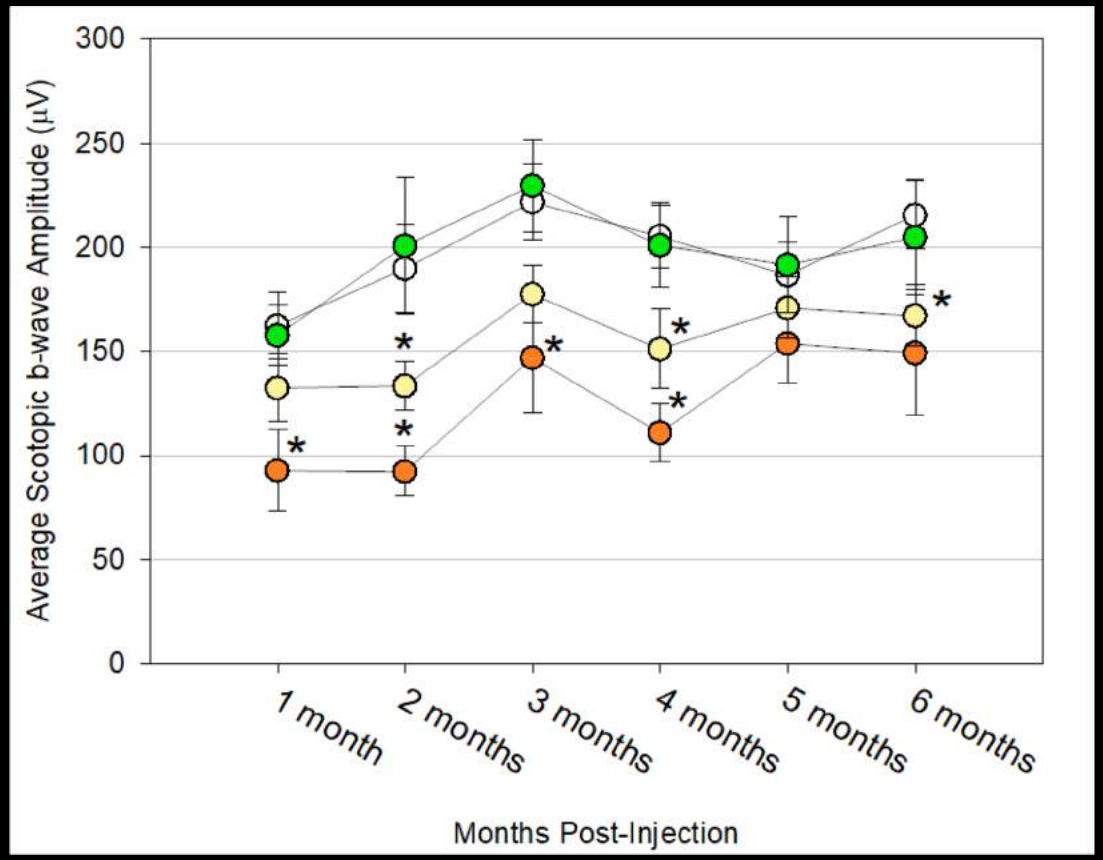
Average ONL Thickness in *Myo7a*$^{-/-}$ Mice



6 weeks post-injection

Packaged in AAV8(Y733F)
Injection Titer: 5.0E8 vg total (2.5 E8 vg each)

Long-term Analysis of *MYO7A* Hybrid-v2_CMv2 Dual Vectors Show Lack of Functional Decreases Over Time



BSS
Original Hybrid Dual Vectors
Hybrid-v2 Dual Vectors
Hybrid-v2_CMv2 Dual Vectors

Mice monitored monthly with ERG

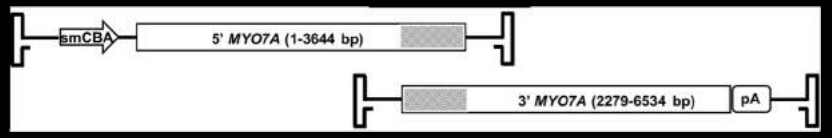
Packaged in AAV8(Y733F)

Injection Titer: 5.0E8 vg total (2.5 E8 vg each)

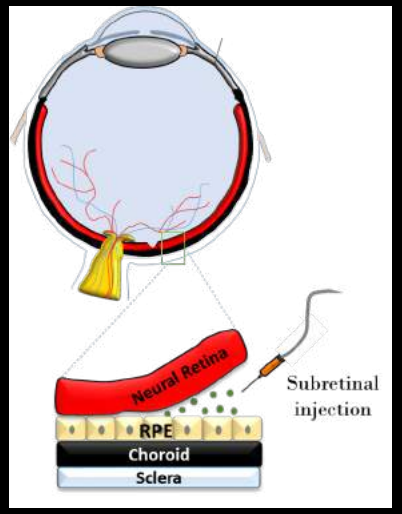
- For USH1B gene therapy to be successful, vectors must be designed to recapitulate the expression pattern of MYO7A in primate retina.
- Have tested simple overlap dual AAV vectors in non-human primate

Experimental Design

AAV5-*MYO7A* (Overlap Vectors ONLY)
containing smCBA or GRK1 promoter



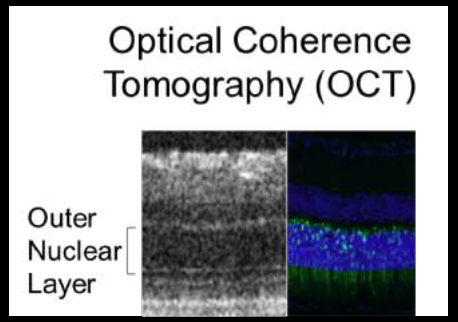
Titer: 4.0E8 vg total (2 E8 vg each)

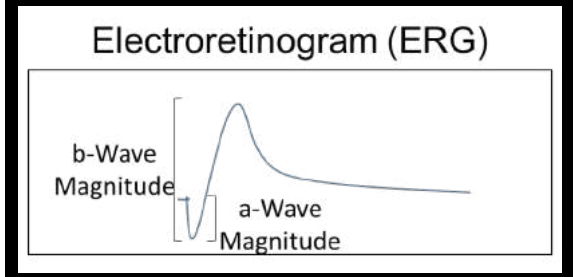


2 adult
Macaque

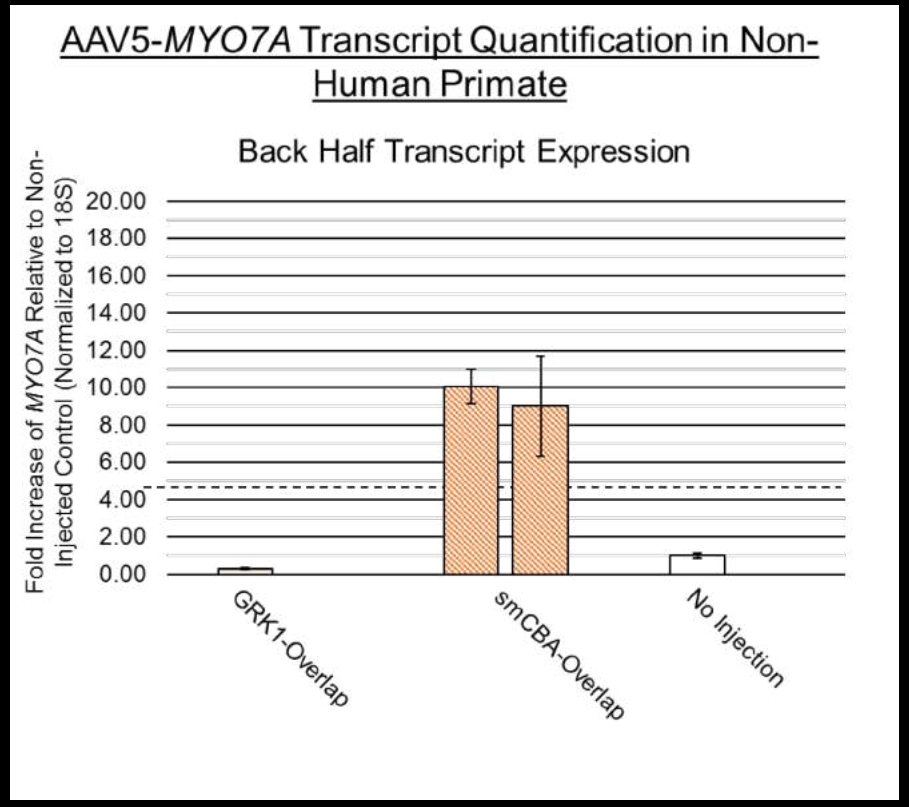
1 & 2 months
post-injection

Western Blot
DNA/RNA Analysis

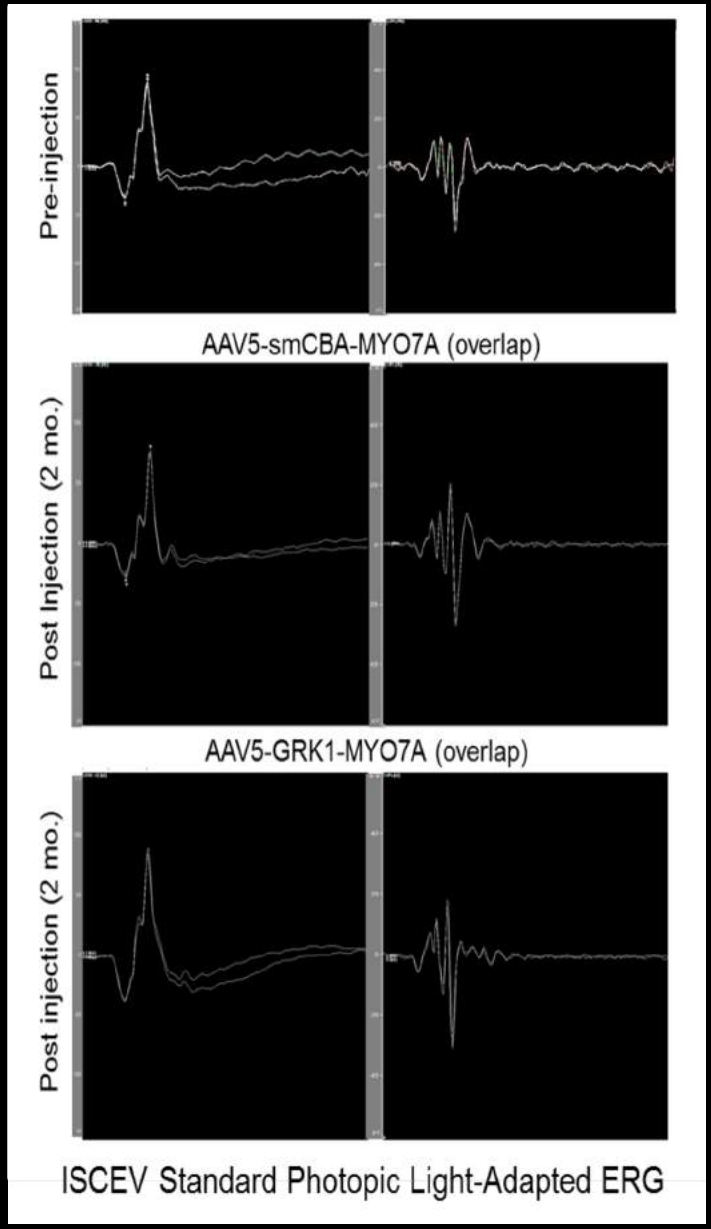


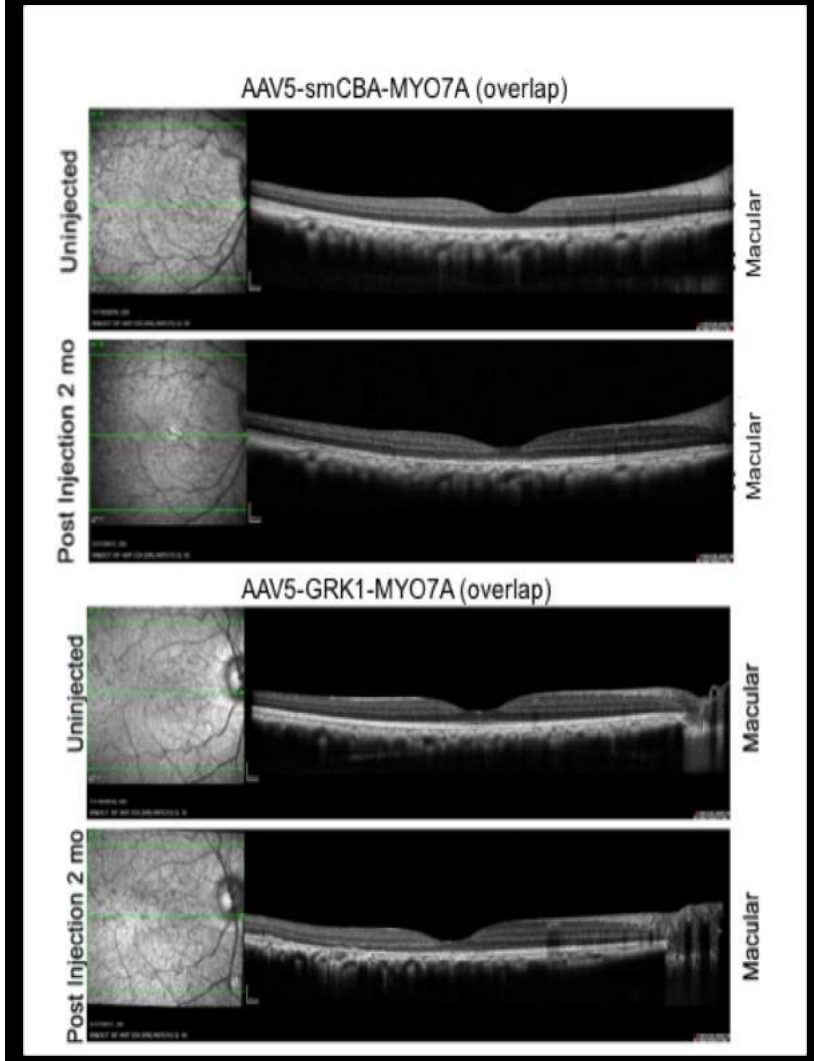


Can AAV Dual Vectors produce full-length *MYO7A* in Macaque?



Injection Titer: 4.0E8 vg total (2.0E8 vg each)



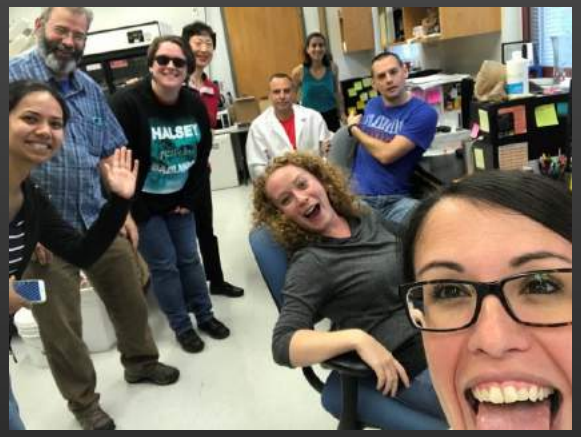


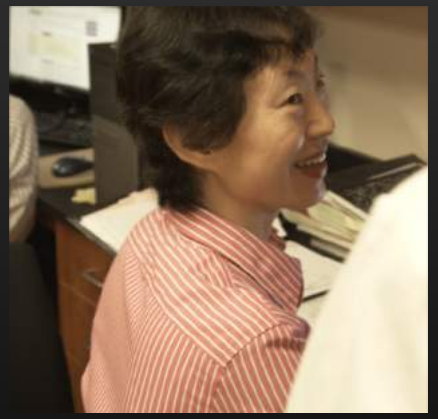
Conclusions

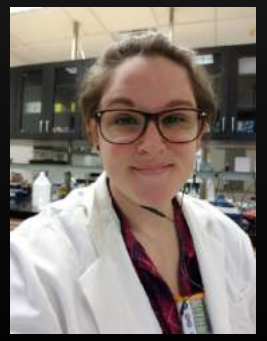
- All Dual AAV vectors are capable of expressing full-length MYO7A
  - AAV8(Y733F) Hybrid vectors produce full-length MYO7A at levels comparable to *Myo7a*$^{+/-}$
- Front half vectors produce a transcript
  - Only Hybrid Front vectors produce truncated protein
  - Truncated protein from AAV8(Y733F) promotes slight decrease in retinal function but no loss of retinal structure
- Back half vectors do not produce transcript/truncated protein
- The ex21/22 hybrid modifications eliminate the toxicity caused by the hybrid vectors
- Codon Optimization of the ex21 Front Half Hybrid vectors eliminates the production of truncated protein
- Dual AAV5 overlap vectors produce full-length *MYO7A* and are well tolerated in macaque


ATSENA
THERAPEUTICS

Acknowledgments







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Funding:

Foundation Fighting Blindness