Gene Therapy for *MYO7A* USH1B

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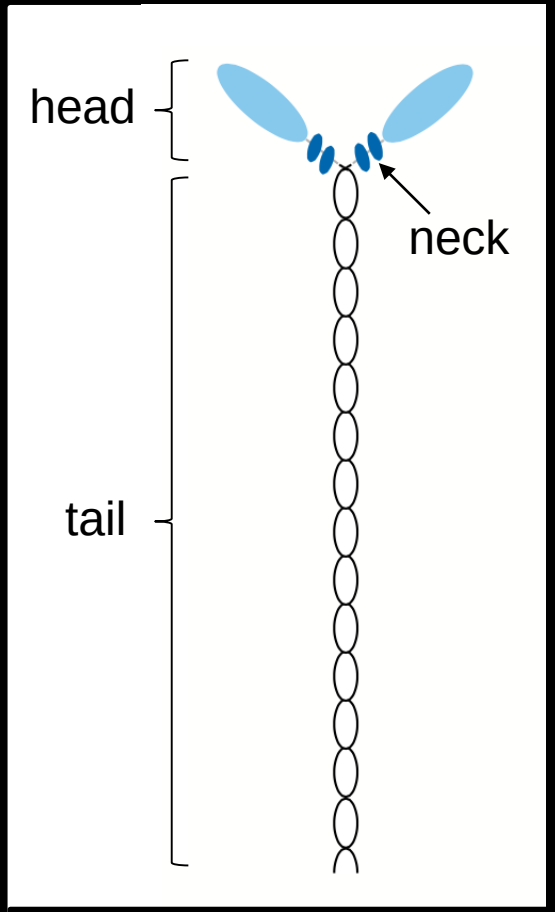
	Type 1	Type 2	Type 3
Hearing	Profound deafness In both ears from birth	Moderate to severe hearing loss from birth	Normal at birth; progressive loss in childhood or early teens
Vision	Decreased night vision before age 10	Decreased night vision begins in late childhood or teens	Varies in severity; Night vision problems Often begin in teens
Vestibular Function (balance)	Balance problems From birth	normal	Normal to near normal, chance of later problems
	70%	**26%**	**4%**

Molecular Definition:

- Type 1: *MYO7A, USH1C, CDH23, PCDH15, SANS*
- Type 2: *USH2A, VLGR1, WHRN*
- Type 3: *USH3A*

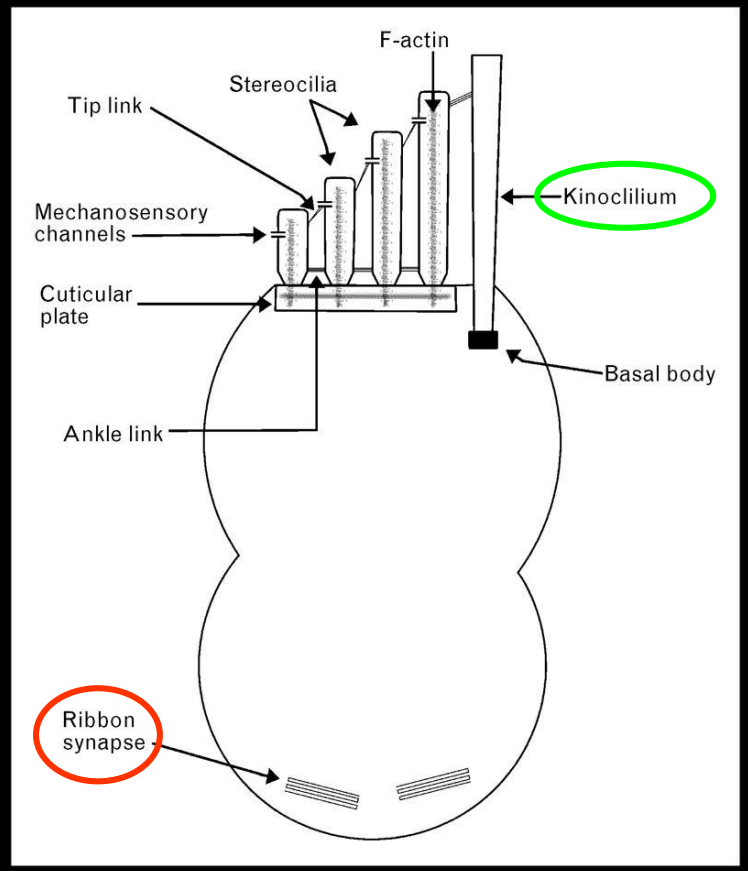
MYO7A

- 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

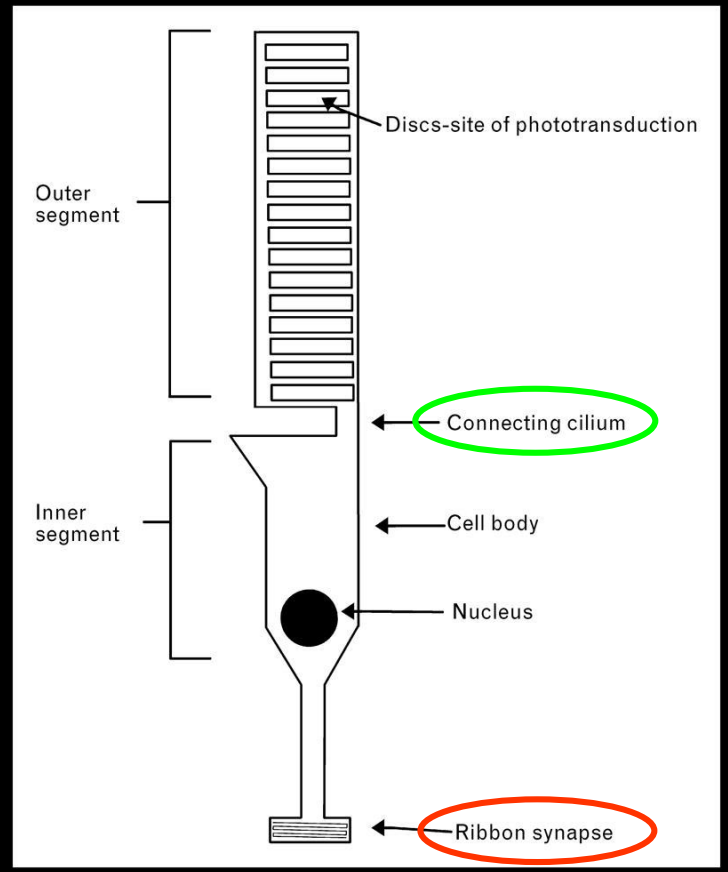


MYO7A mutations affect both hearing and vision

Schematic of a cochlear hair cell

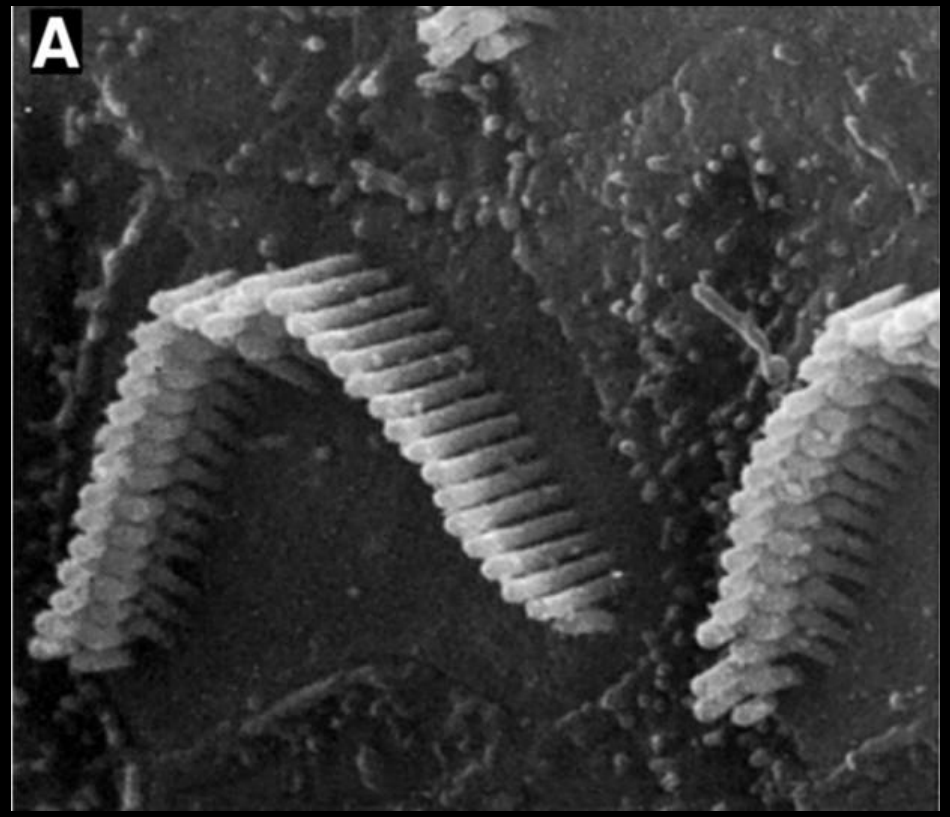


Schematic of a photoreceptor cell

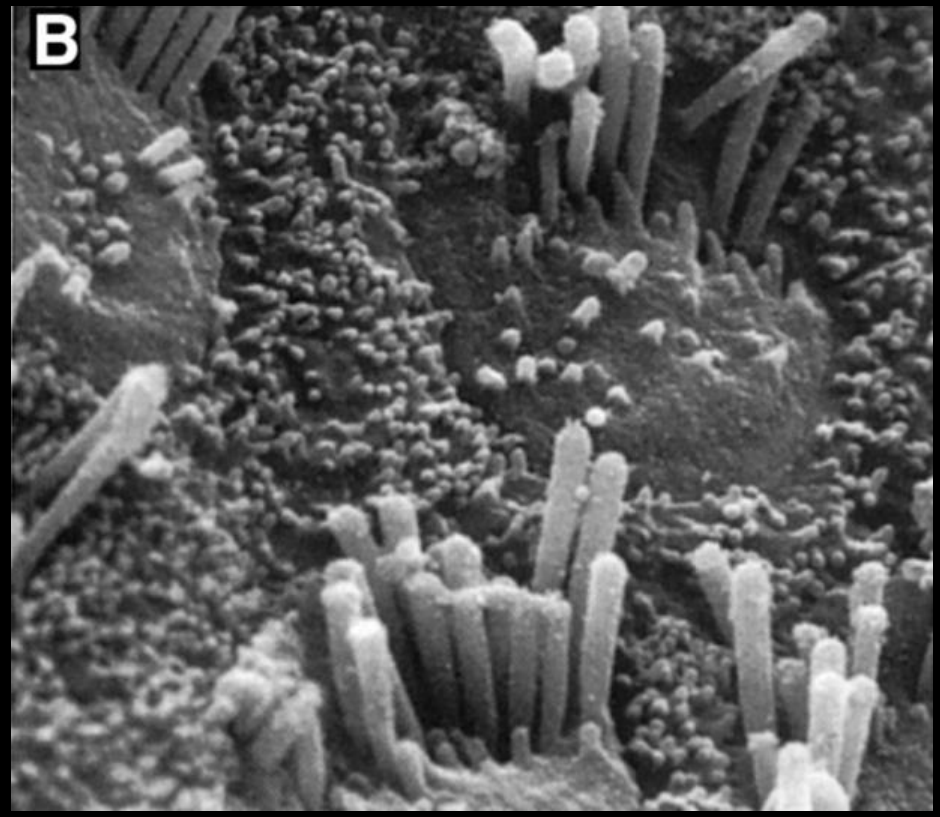


Why is hearing affected first?

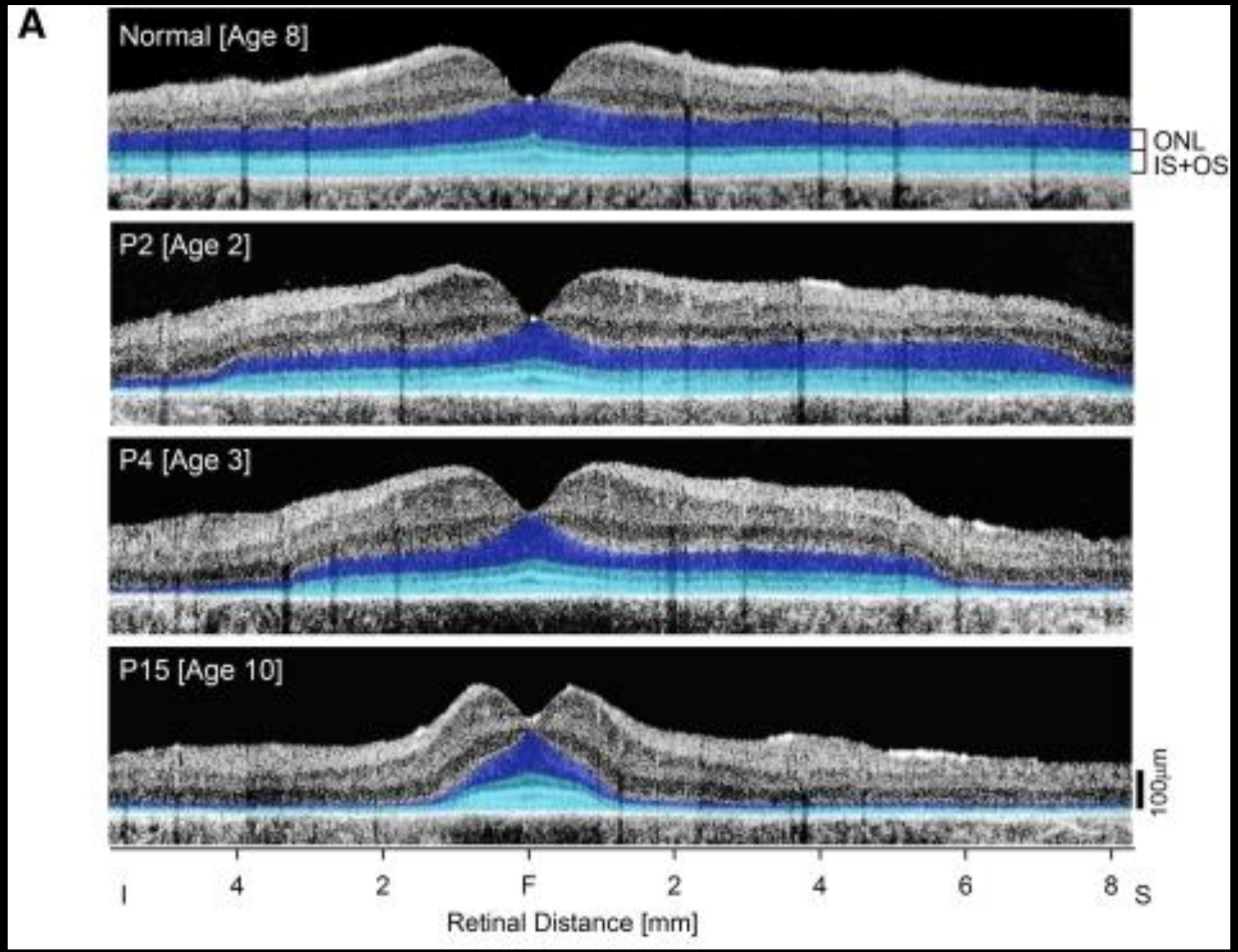
Wild Type



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

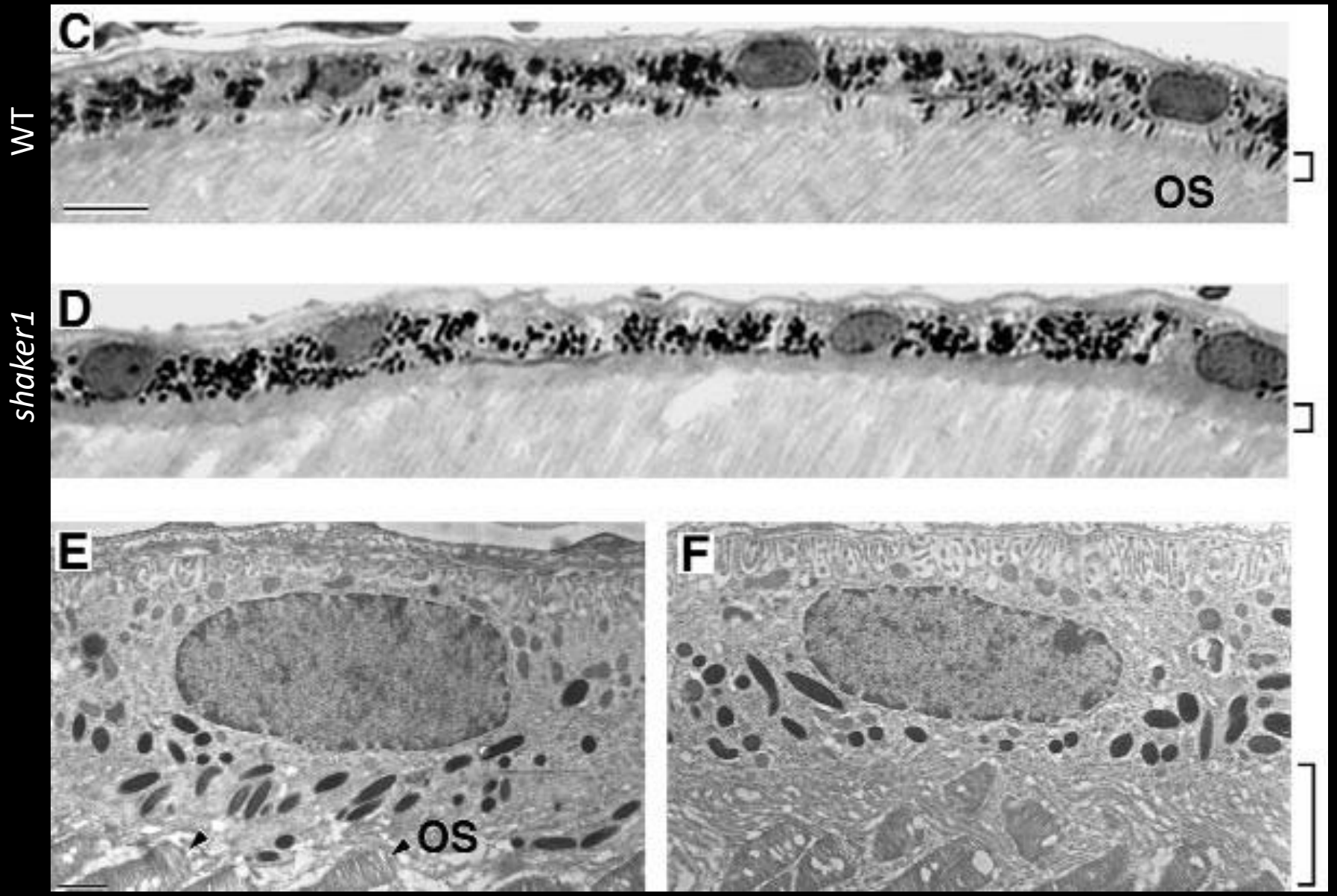
Shaker1 mouse

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

USH1B patient

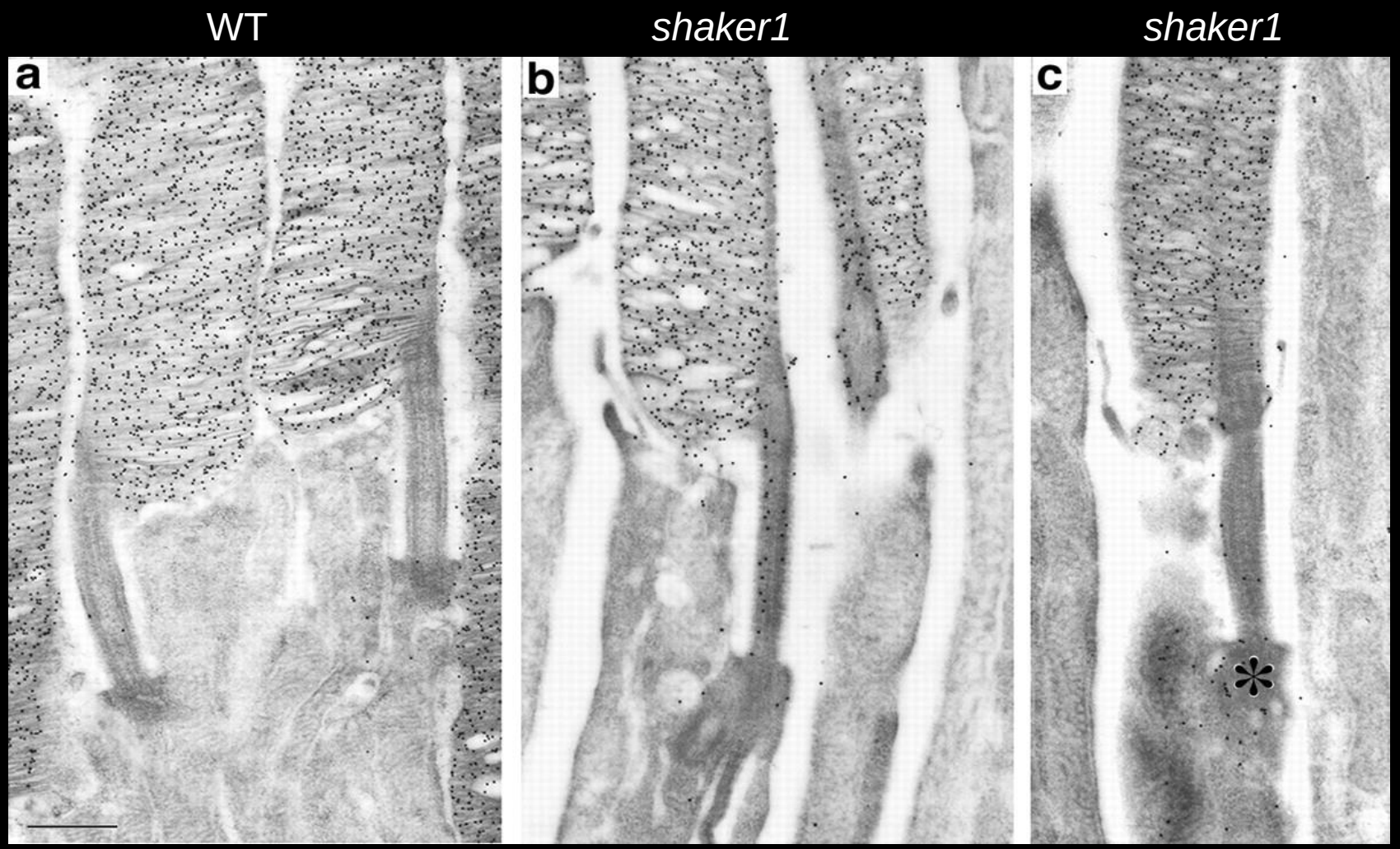
- Most mutations in head domain
- Congenital deafness
- heterozygous: normal
- retinal degeneration
- Abnormal/absent ERG

Apical migration of RPE melanosomes impaired in *shaker1* mice



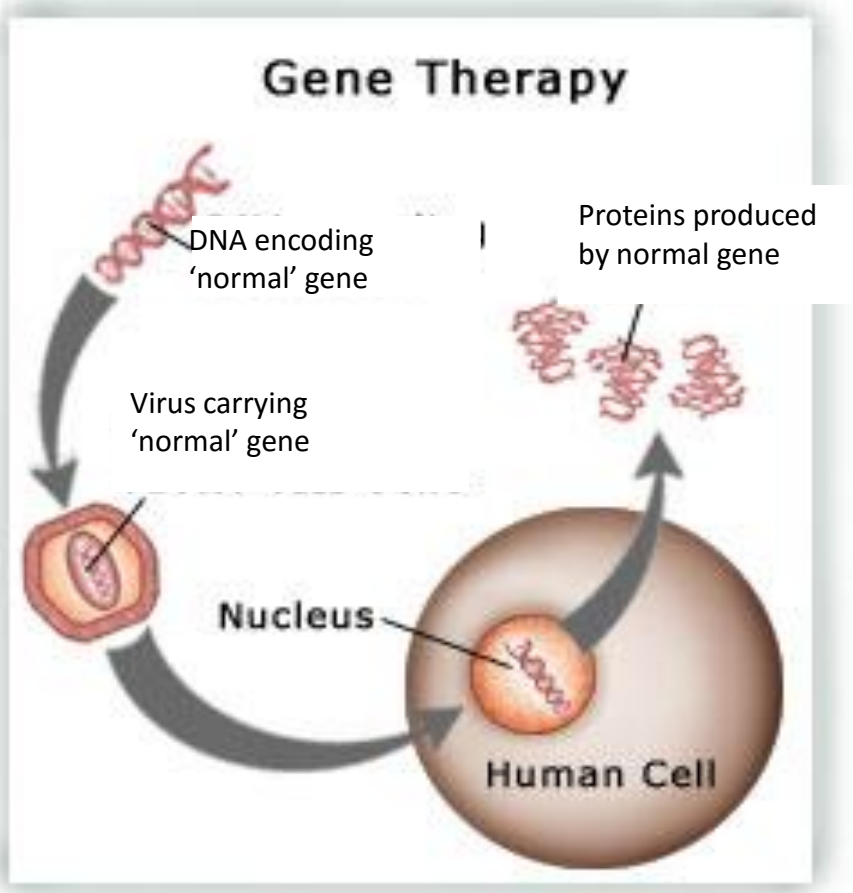
David S. Williams, Nature Genetics, 1998

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

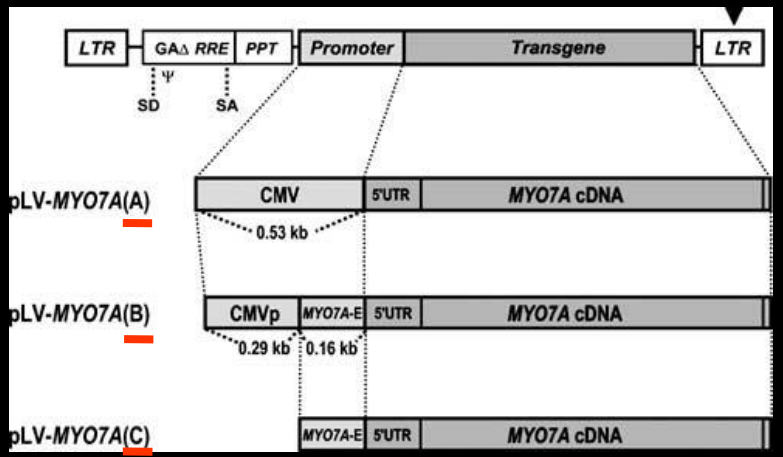


David S. Williams, J. Neuroscience, 1999

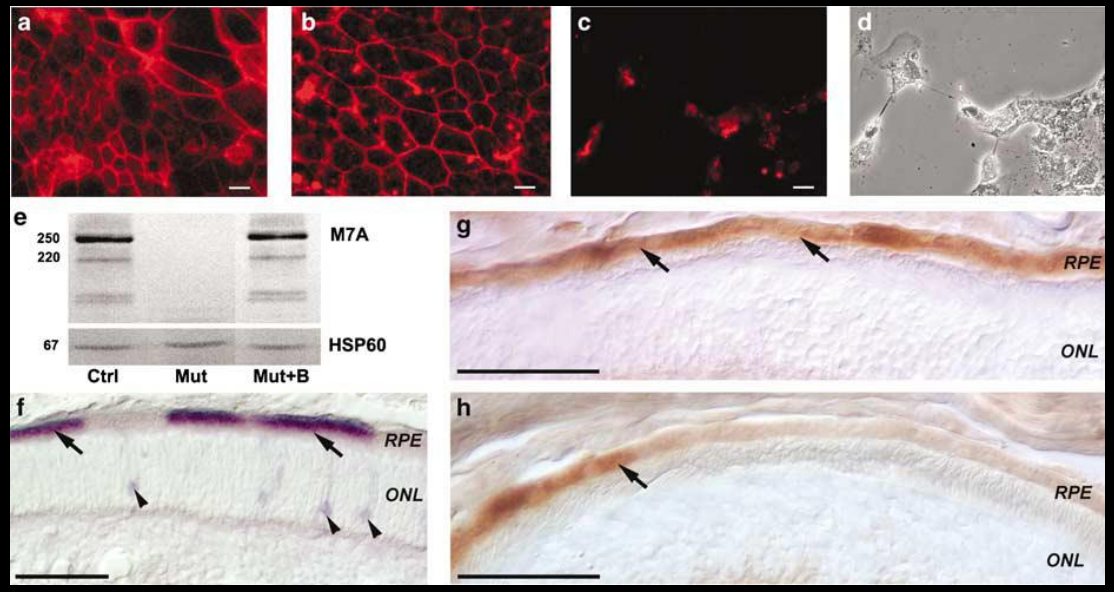
How does gene therapy work?



Size of Myo7a cDNA (~7kb) affects choice of viral vector
First attempt- Lentivirus



LV-Myo7a (B) infects RPE and
some photoreceptors of *shaker1* retina



a- Myo7a +/- RPE
b- Myo7a -/- cells infected with B
c- Myo7a -/- cells infected with A
d- phase contrast of c
e- Western blot
f- retina injected w/ LV-AP
g- shaker1 retina injected with LV-Myo7a (B) (central)
h- shaker1 retina injected with LV-Myo7a (B) (peripheral)

Hashimoto et al., 2007

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”

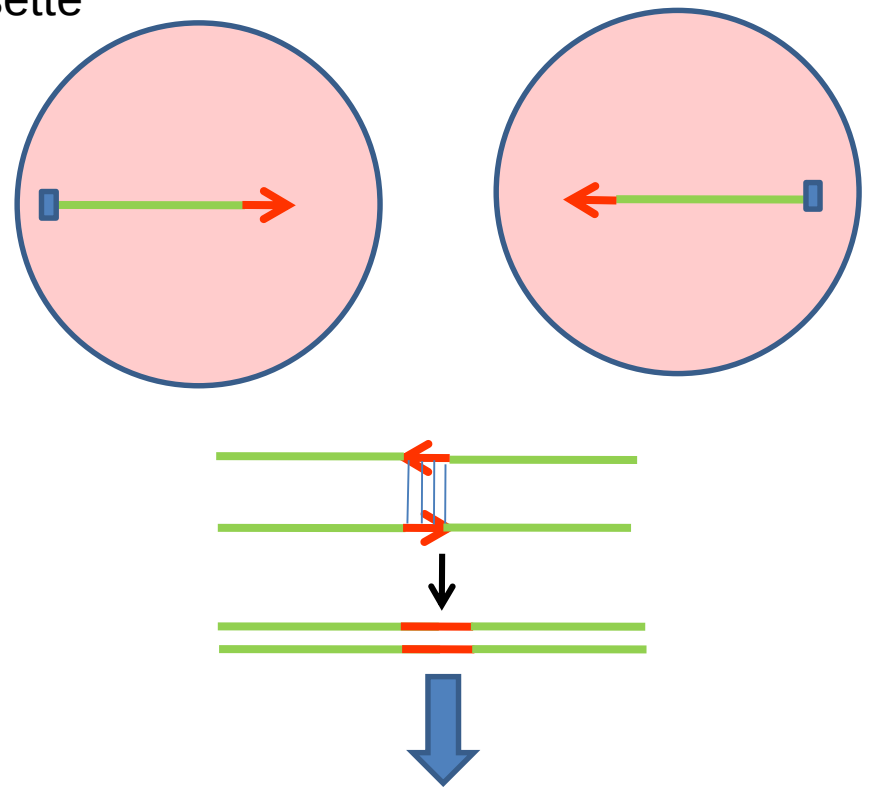
Ongoing Clinical Trials

- SAR 421869 (aka “USHstat”)
- EIAV-based lentiviral approach
- Safe, well-tolerated
- *To date- no evidence of biological activity*

“Heterogeneous” or “fragmented” AAV

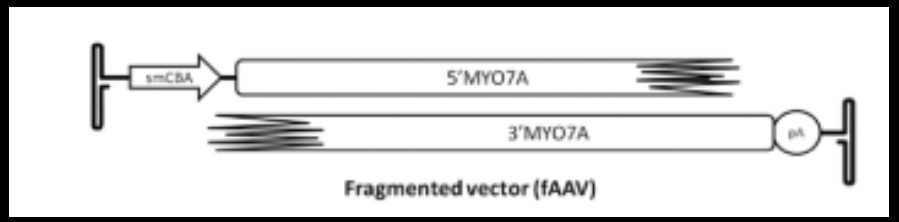
Each AAV capsid carries a “+” or “–” strand of the recombinant vector genome. With overstuffed vectors, these strands may be truncated (to ~ 5kb) yet likely still contain overlapping sequence (if the overall cassette is less than 10kb).

- These strands anneal in cells to reconstitute full length (double stranded) vector cassette



Complete protein

Using AAV to deliver large genes



Lopes + Boye et al., *Gene Therapy* 2013

Correction of mutant phenotypes is achieved following subretinal injection of fAAV-Myo7a

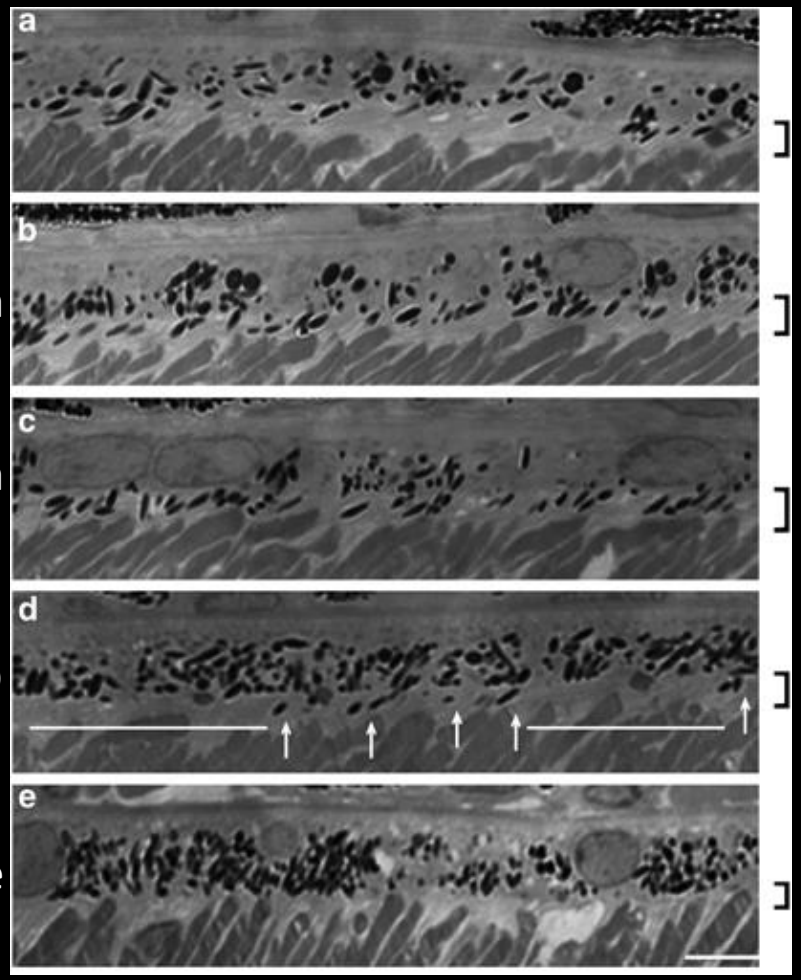
WT

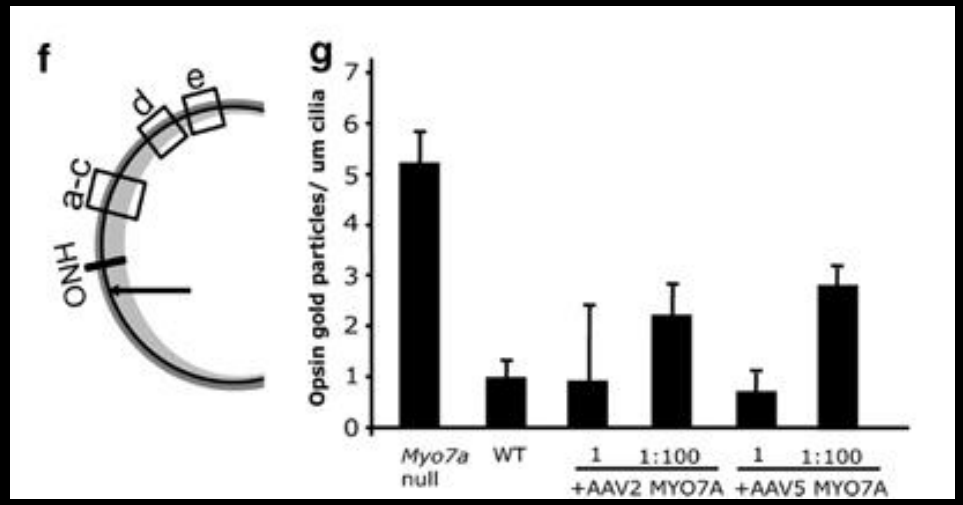
fAAV2-Myo7a

fAAV5-Myo7a

edge of injection bleb

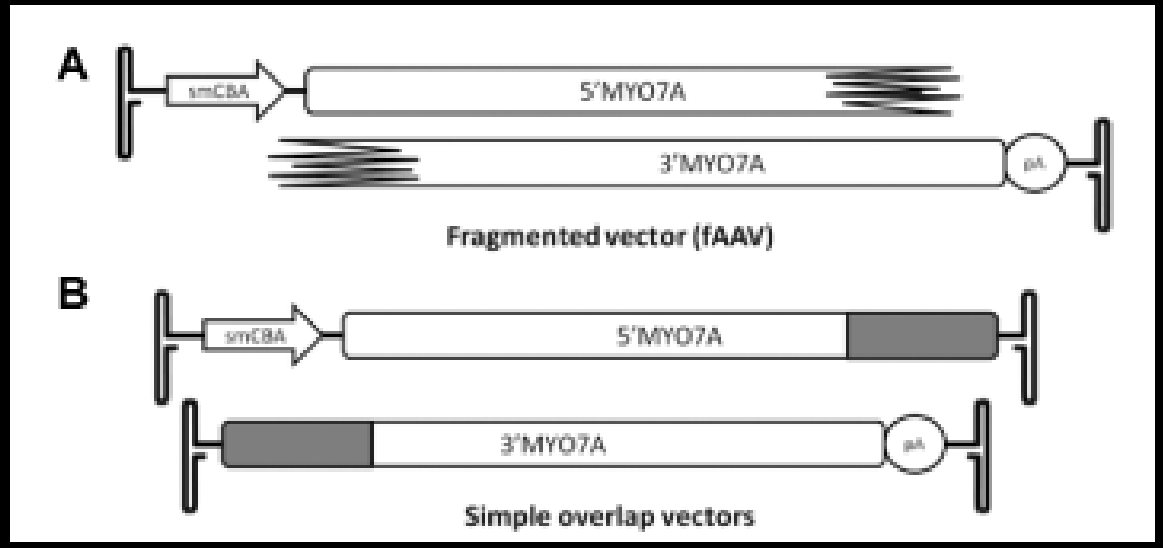
outside injection site

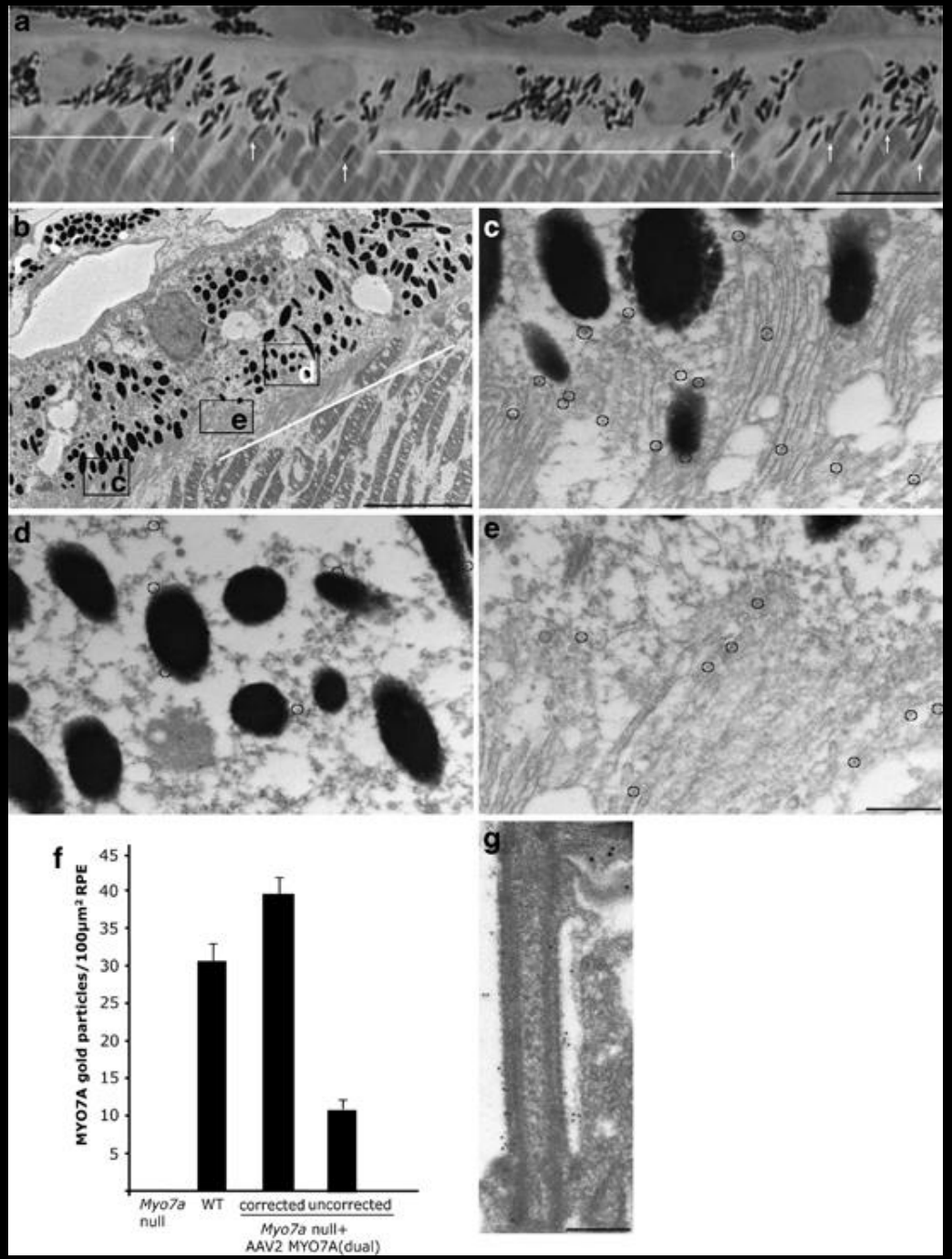




Lopes + Boye et al., *Gene Therapy* 2013

Design dual AAV-Myo7a vectors with defined genetic payloads



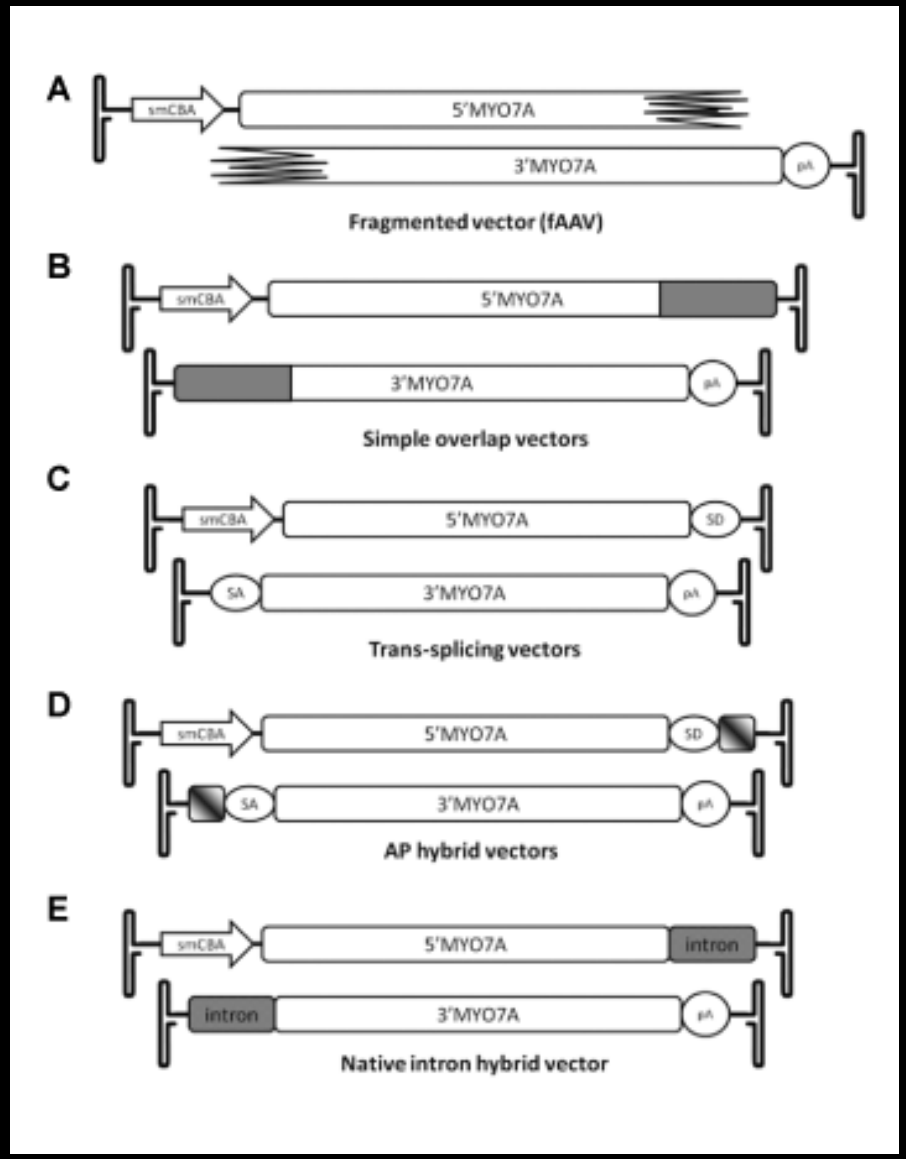


Simple overlap AAV2-Myo7a vectors correct both phenotypes in *shaker1* mice

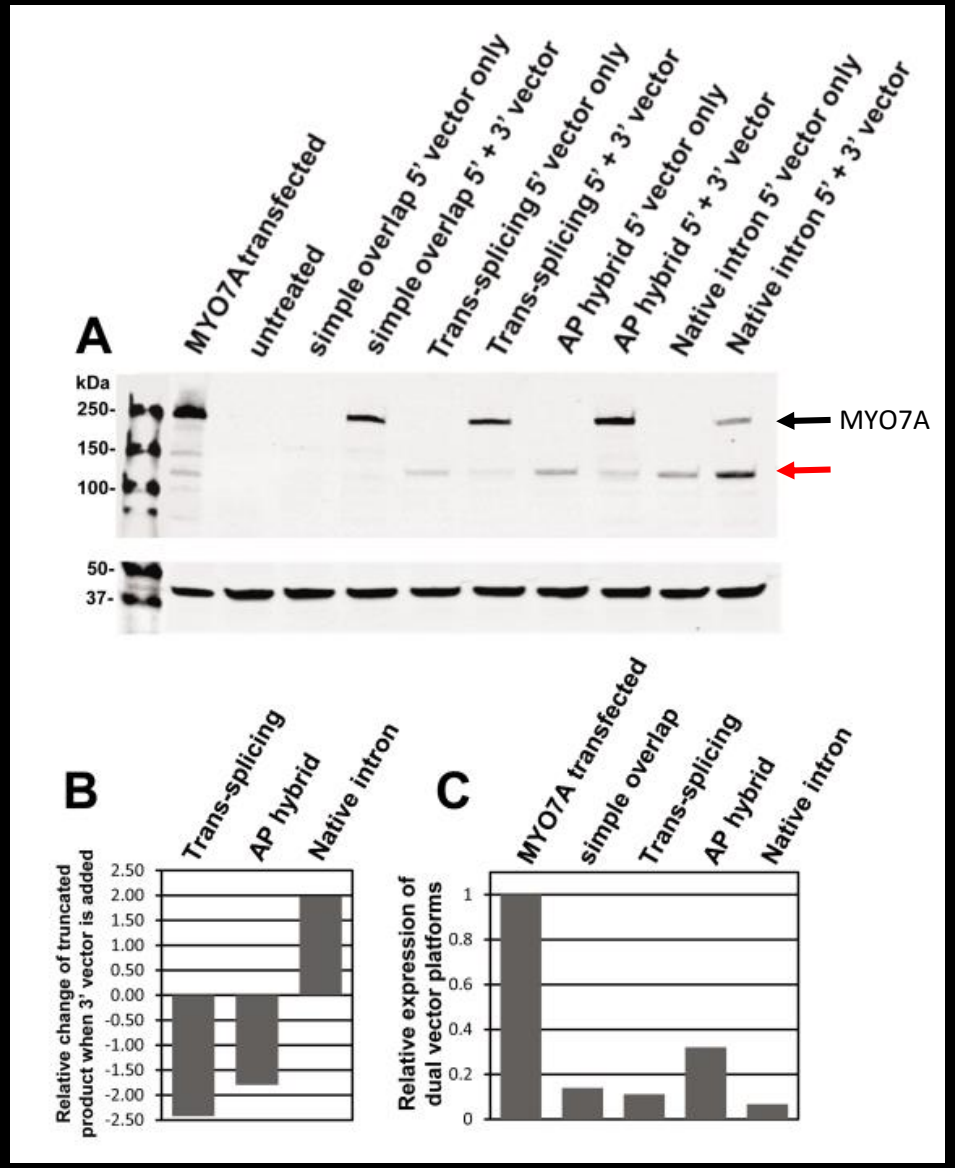
Rescue is "spotty"

Lopes + Boye et al., *Gene Therapy* 2013

Design more efficient dual AAV-MYO7A vectors

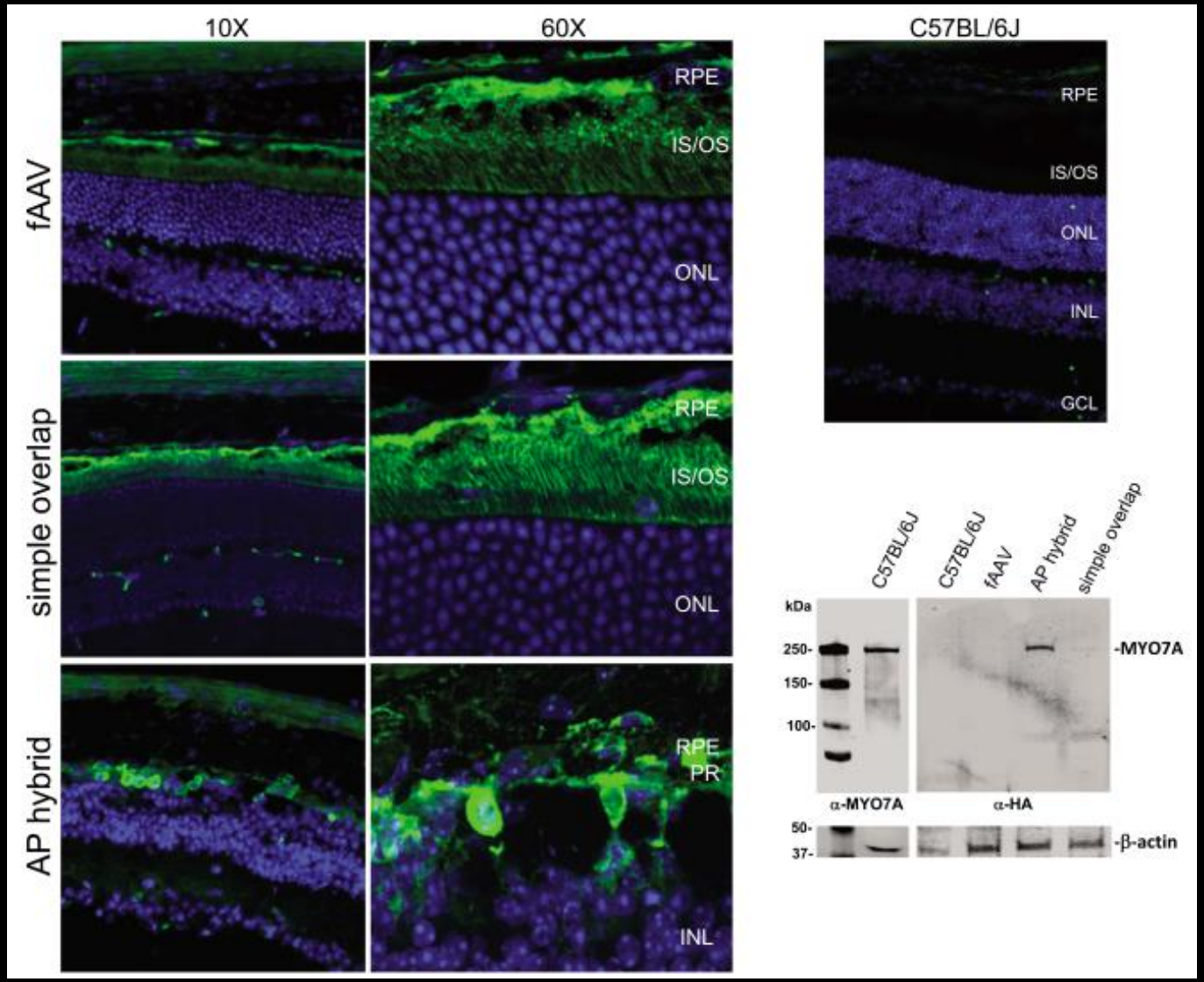


Relative efficiency of dual AAV-Myo7a vectors



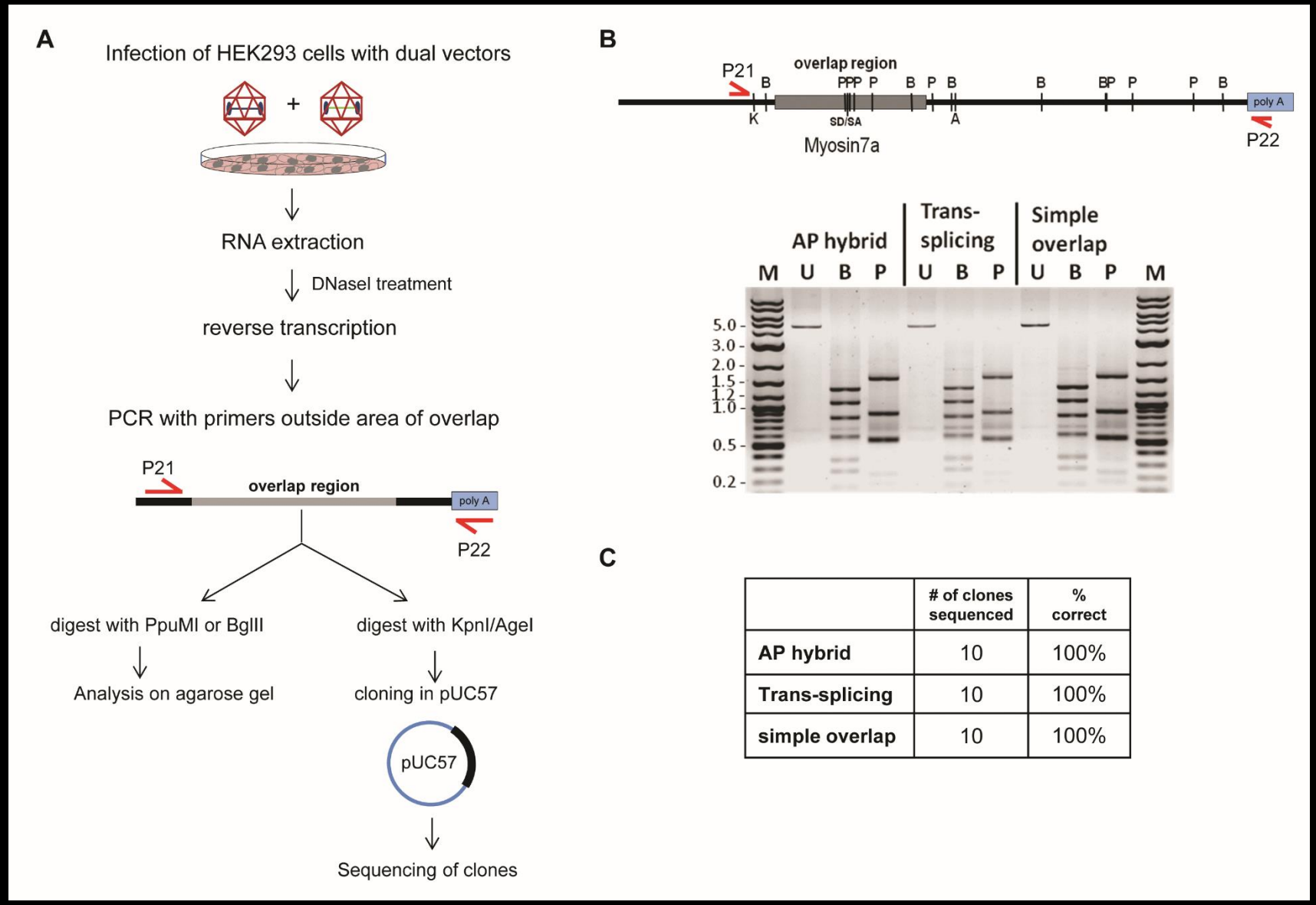
Dyka et al., *Human Gene Therapy* 2014

Dual AAV-mediated MYO7A expression *in vivo*



Dyka et al., *Human Gene Therapy* 2014

Transcript fidelity of dual AAV-mediated Myo7a



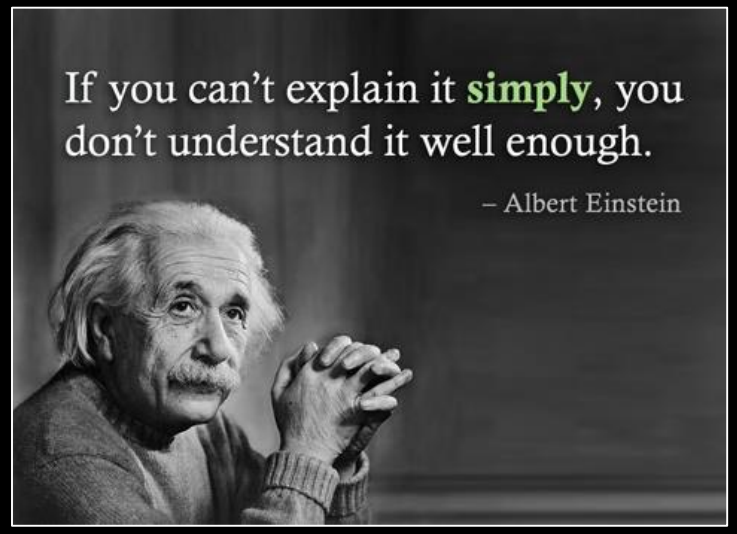
	# of clones sequenced	% correct
AP hybrid	10	100%
Trans-splicing	10	100%
simple overlap	10	100%

Dyka et al., *Human Gene Therapy* 2014

What's been accomplished so far:

- fAAV vectors drive full length MYO7A in *shaker1* retina/RPE
- fAAV vectors correct *shaker1* phenotypes
- Simple overlap dual AAV vectors with defined genetic payloads also correct *shaker1* phenotypes
- AP hybrid dual AAV vectors drive higher levels of MYO7A expression *in vitro* and *in vivo*
- Sequence of dual AAV-mediated Myo7a transcript has 100% fidelity to endogenous message

These results lay the groundwork for an AAV-based treatment for USH1B

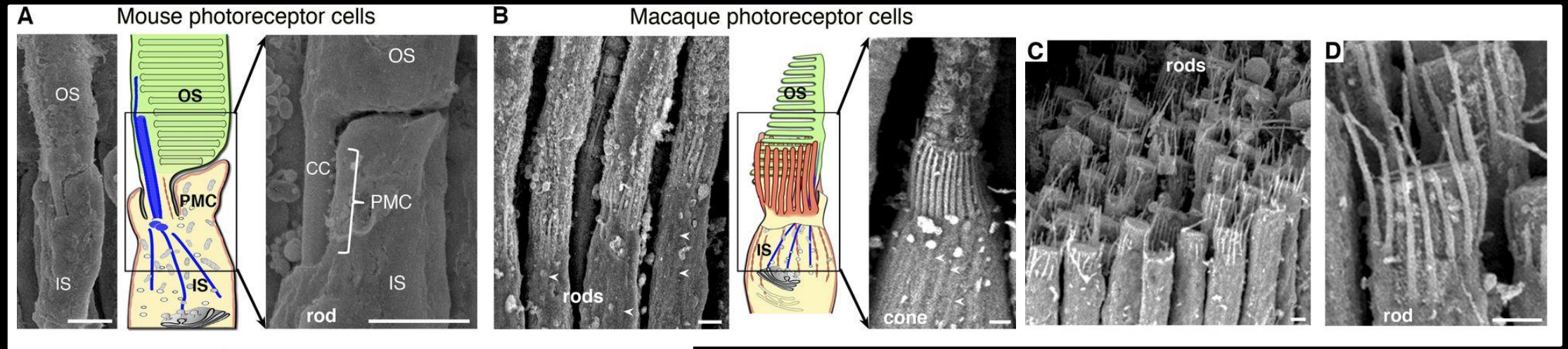


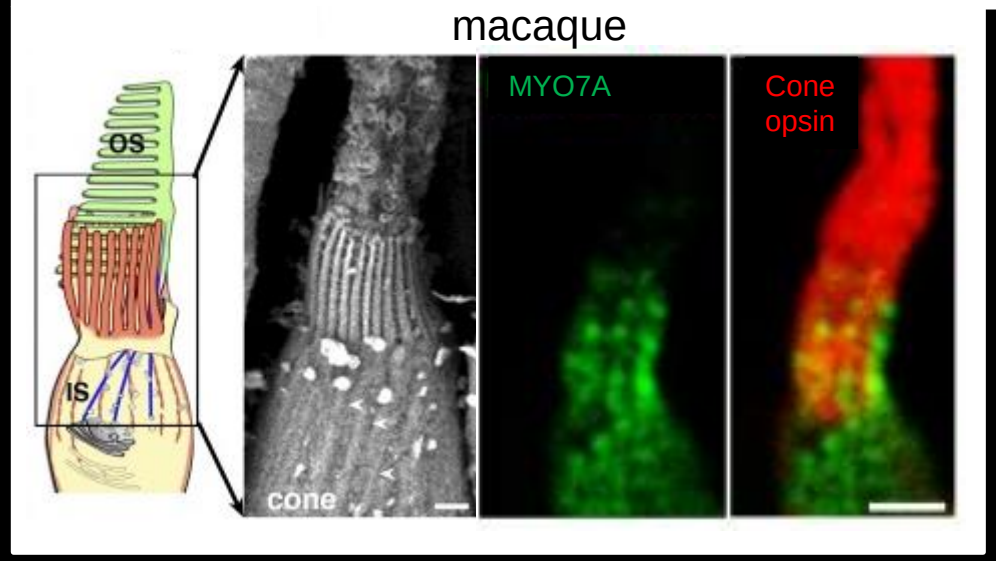
LINGERING QUESTIONS:

- *Why don't shaker1 mice have loss of retinal structure/function??*
- *Will truncated proteins have an impact?*

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

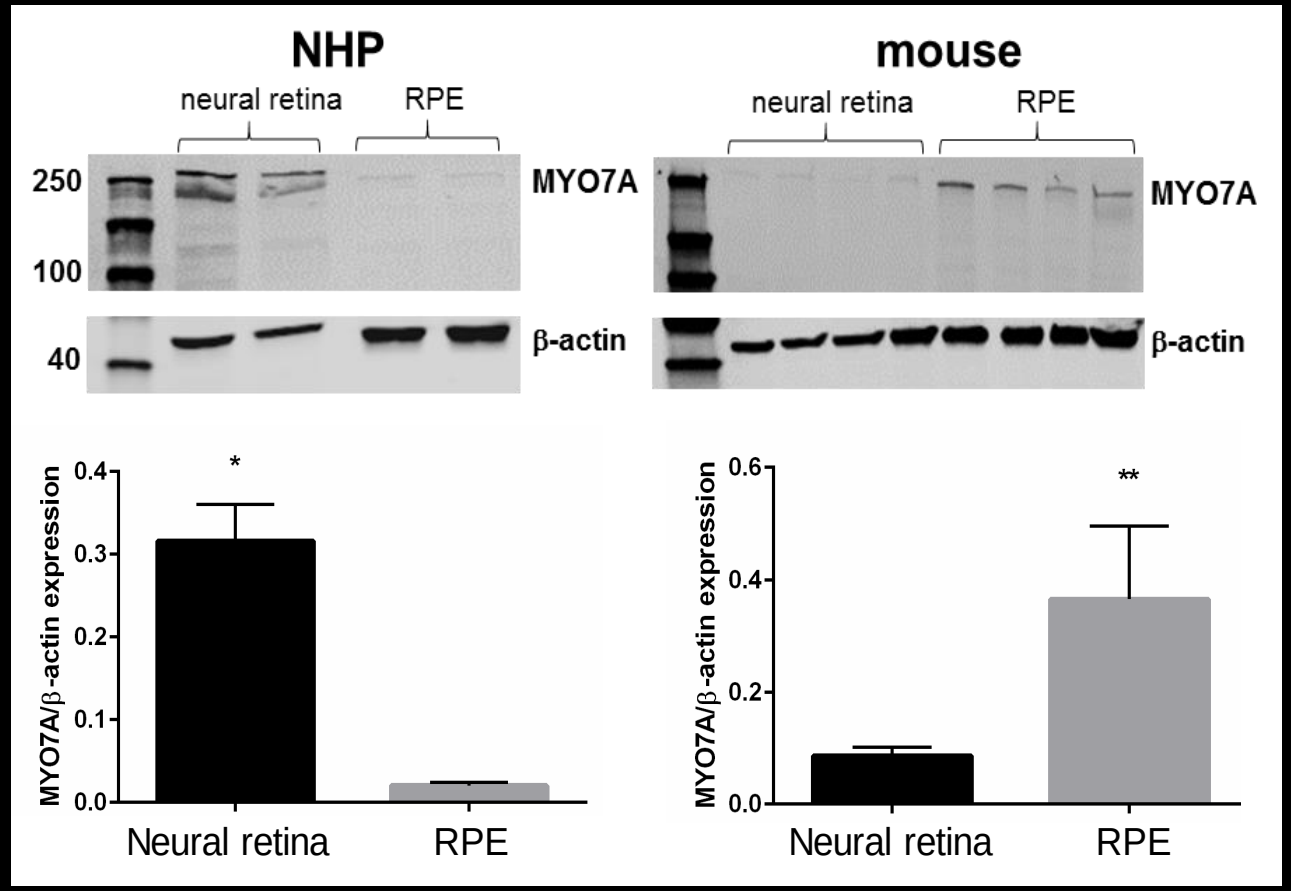
- Whether MYO7A is actually expressed in mouse photoreceptors remains controversial
- No question- MYO7A is definitely expressed in NHP and human photoreceptors



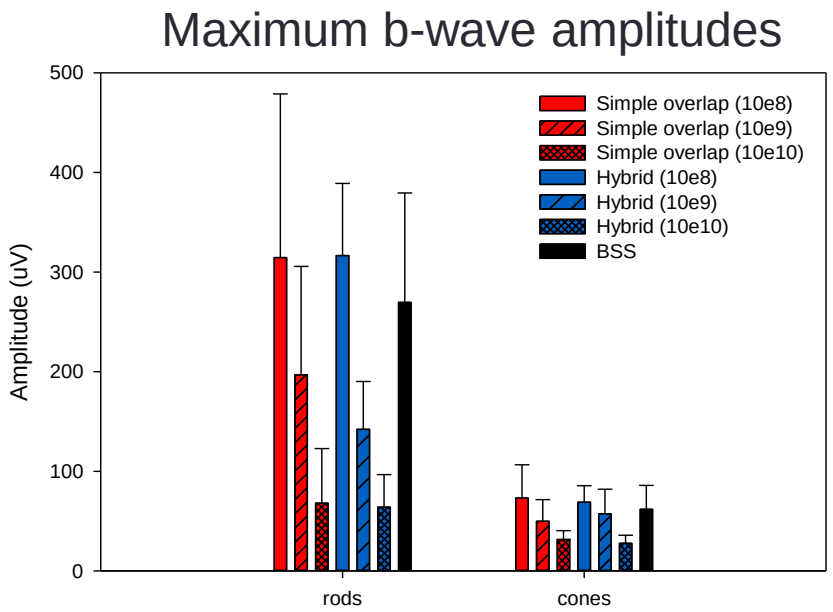


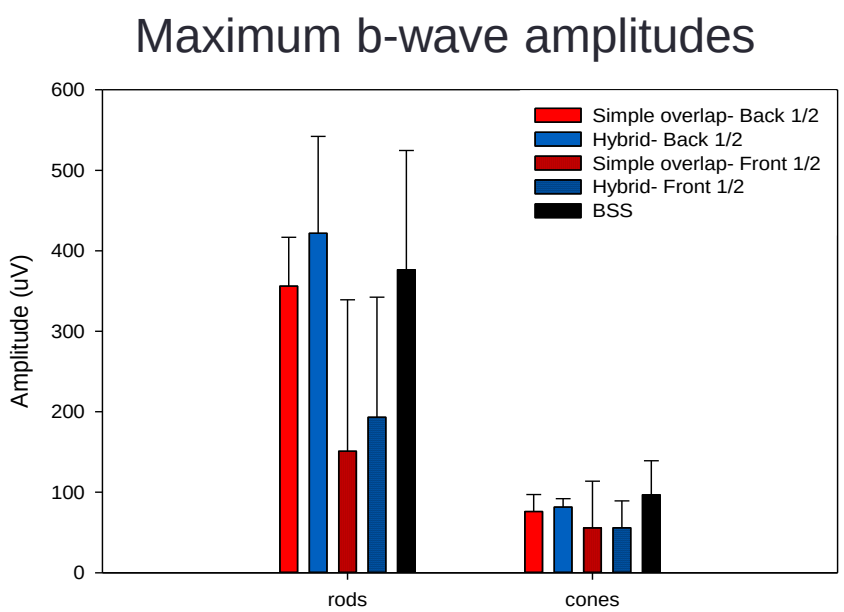
Primates have calyceal processes
Mice do not

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



Impact of dual, front half only, or back half only AAV vectors (at different doses) on retinal function of WT mice





- Clear dose response observed
- No significant difference between eyes injected with 10e8 vg vs. BSS
- Injection of front half vectors alone negatively affects ERG
- Injection of back half vectors alone has no impact

Summary

- Lentivirus-based gene therapy proving ineffective for correcting retinal phenotype in USH1B patients
- In USH1B patients, photoreceptors are the primary site of disease
- MYO7A is differentially expressed in mouse vs. primate
- Mice lack calyceal processes (site of MYO7A expression in primate photoreceptors)
- AAV is the gold standard for delivering genes to photoreceptors
- fAAV and dual AAV-Myo7a vectors correct melanosome migration in *shaker1* mice
- Dual AAV-mediated Myo7a transcript has 100% fidelity to WT MYO7A
- Dual AAV vectors and/or front half vectors alone lead to loss of retinal structure and function in mouse

Future Directions

- It is our belief that, for USH1B gene therapy to be successful, vectors must be designed to recapitulate the expression pattern of MYO7A in primate retina.
- We have begun testing dual AAV vectors in non-human primate
- If necessary, evaluate methods to ablate production of spurious truncation products and/or increase vector potency
- Recently received FFB/Gund Harrington Scholar Award to pursue this work!