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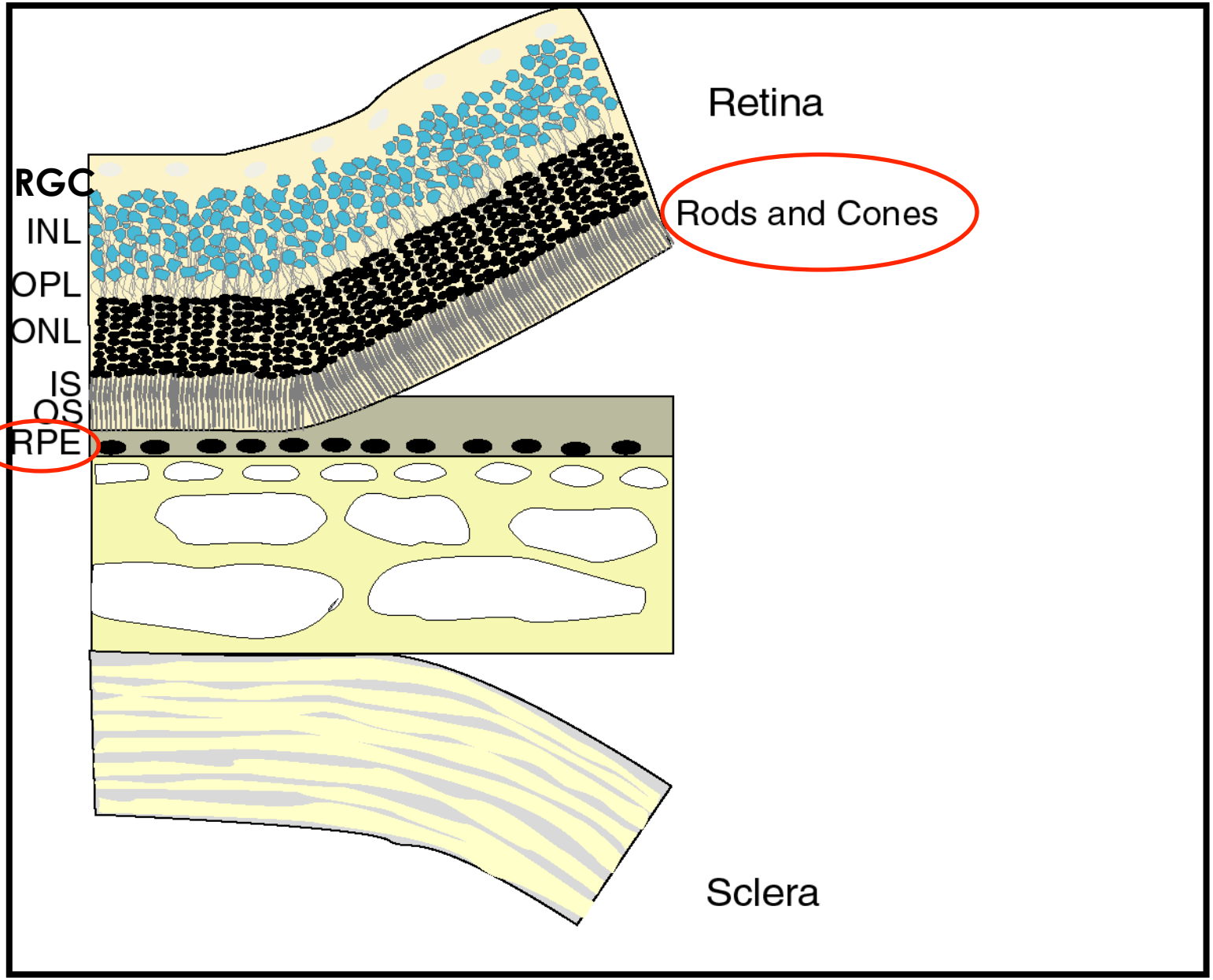
In the retina, this gene is expressed in both the rod and cone photoreceptor cells and the retinal pigment epithelium (RPE) that feeds rods and cones.

(SLIDE 1)

SLIDE 1



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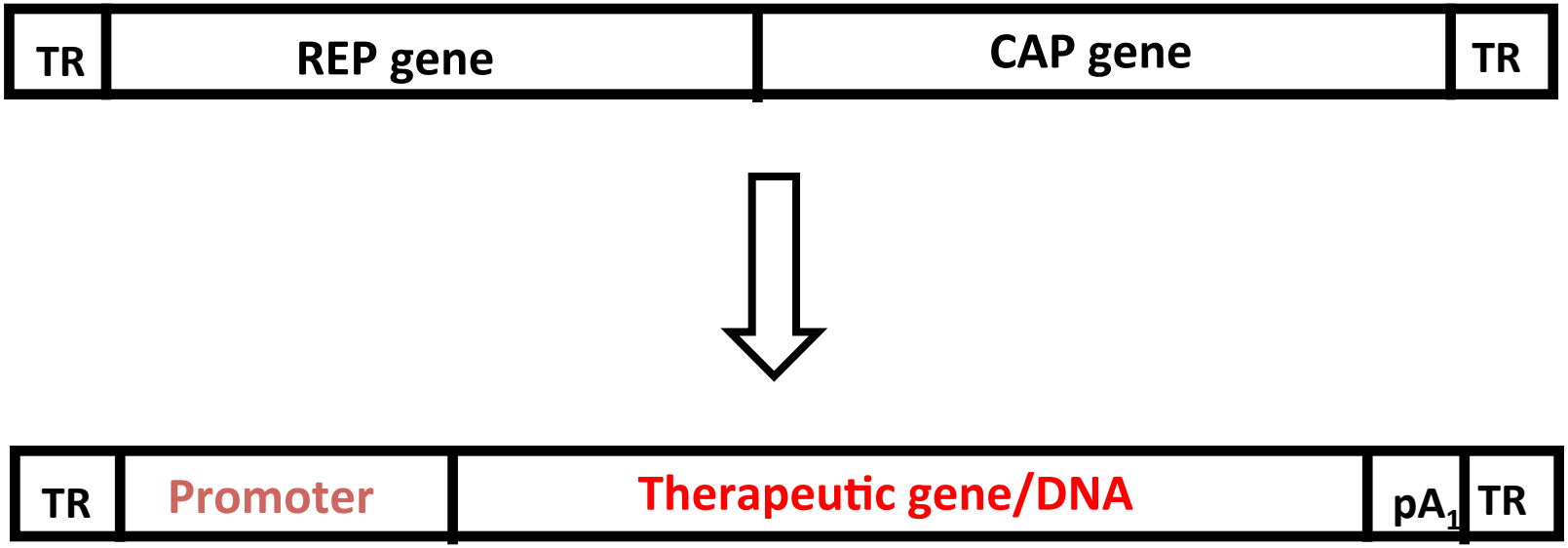
How do we do this?

We remove the two viral genes in the natural virus and replace them with the gene of interest.

(SLIDE 2)

Adeno-associated virus -- AAV

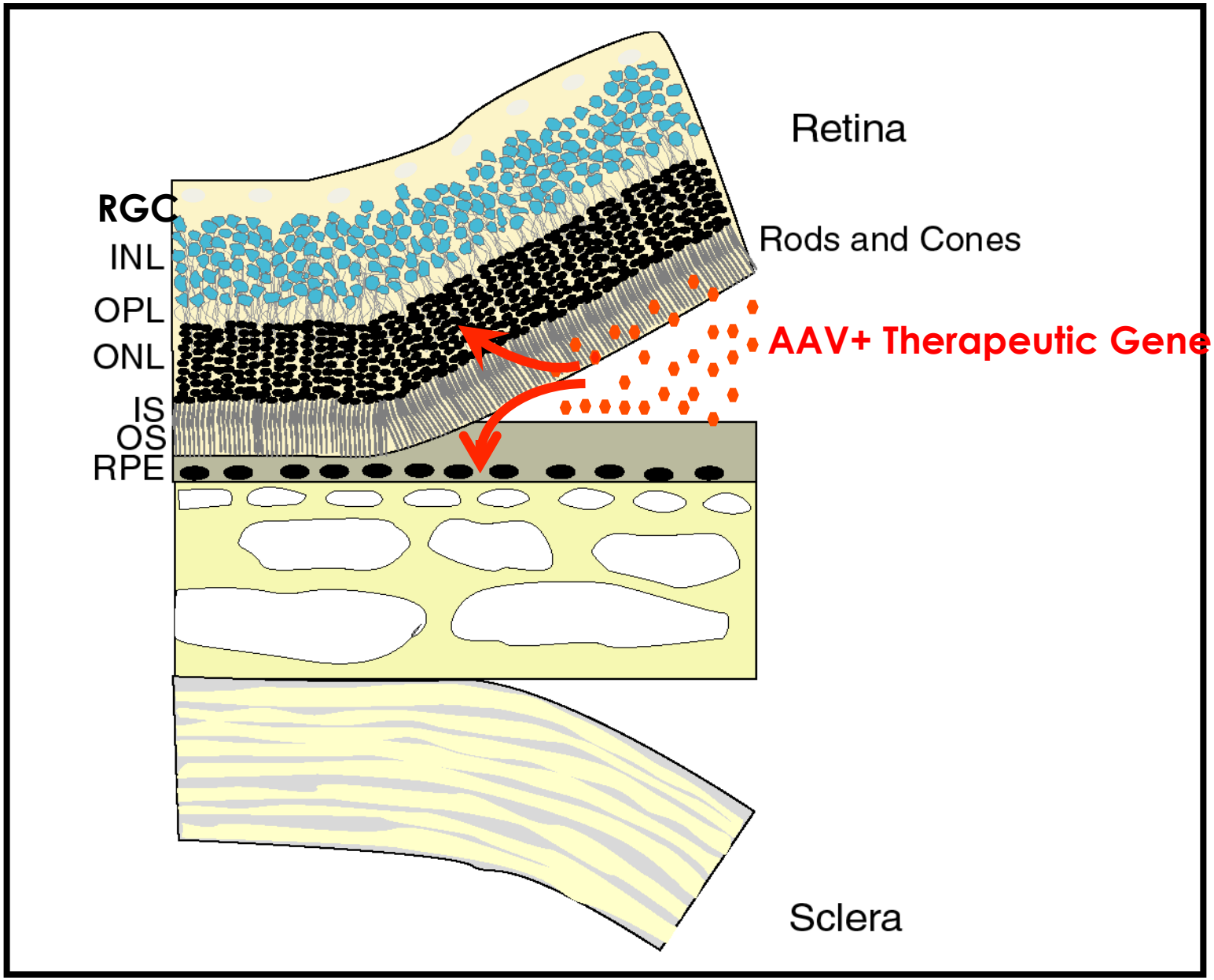


AAV Vector

For delivering genes to rods & cones and the RPE we inject vector into the subretinal space.

(SLIDE 3)

SLIDE 3


Retina
RGC
INL
OPL
ONL
IS
OS
RPE
Rods and Cones
AAV+ Therapeutic Gene
Sclera

Are AAV vectors safe in the retina?

We have treated over 15 forms of inherited blindness in mouse models and we find:

- **there is no loss of retinal function or structure**
- **with the proper design we can control precisely what retinal cells can use the gene**
- **most of the targeted retinal cells can be treated with a single vector injection**
- **the gene remains active for the lifetime of a mouse (~2 years), more than 10 years in dogs and 7 years and counting in humans**

(SLIDE 4)

AAV Vectors in the Retina

- Nonpathogenic
- Promoters control which retinal cells express the gene
- Up to 95% treatment of the targeted cell type
- AAV delivered genes last for life in rodents, >10yrs in dogs, 7yrs and counting in humans after a single treatment.

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7 years after the single vector treatment patients still maintain significant vision improvement without any safety issues.

So AAV vectors in the human retina are safe.

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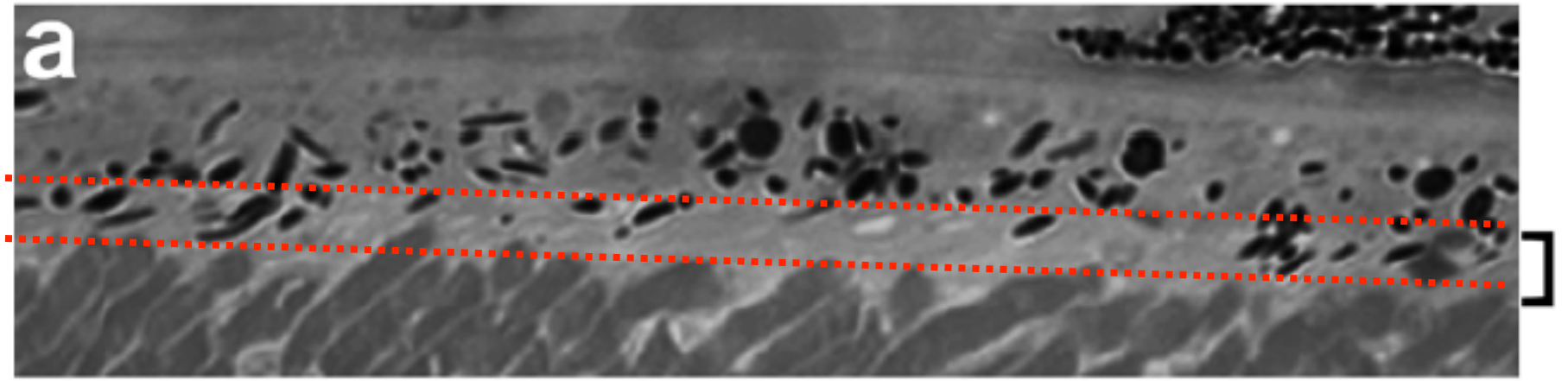
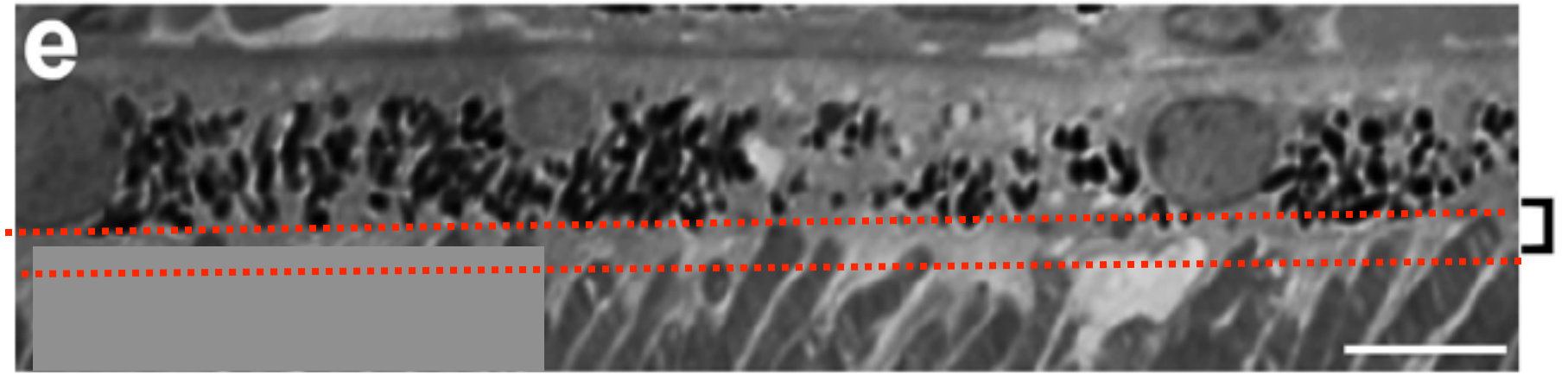
- **Myo7a promotes the presence of pigment granules at the bottom end of RPE cells. In Myo7a mouse these granules are missing. (SLIDE 5)**

Normal Mouse



Myo7a Mouse



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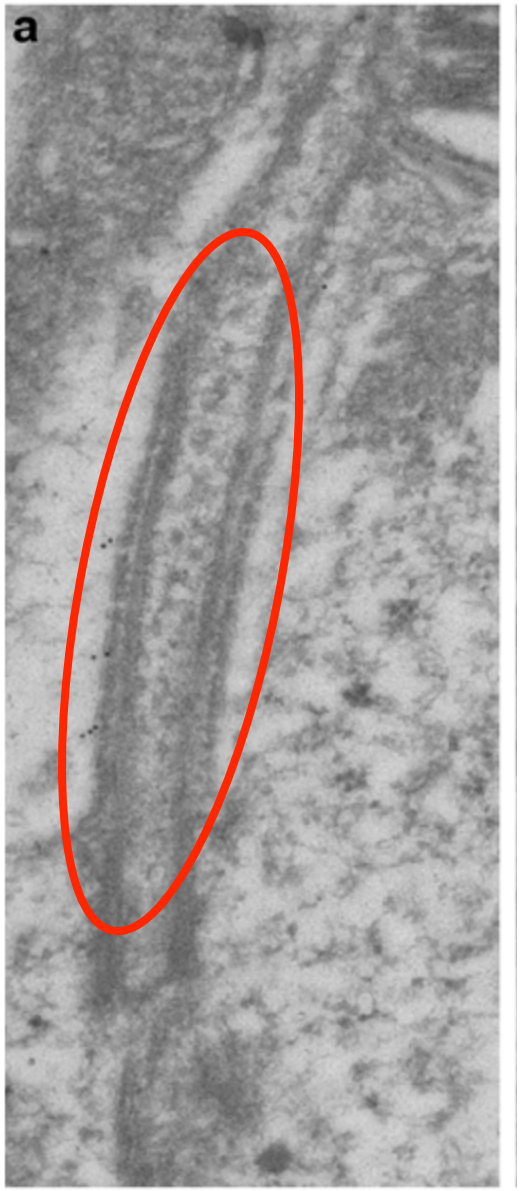
SLIDE 6

Myo7a mouse

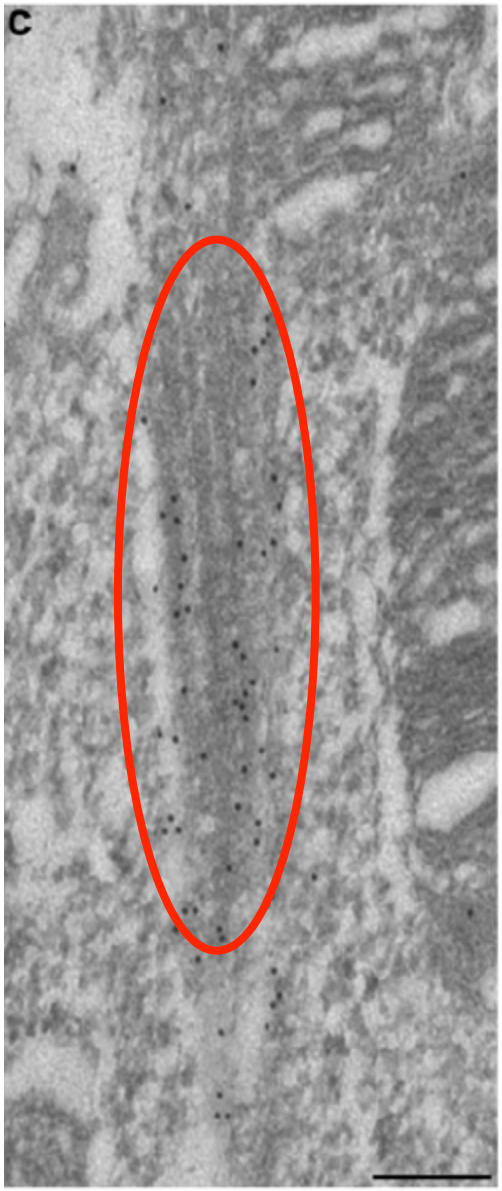


Outer Segment

Connecting Cilium

Inner Segment

Normal Mouse



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So, it is these two aspects of the Myo7a mouse retina that we can follow after AAV vector treatment.

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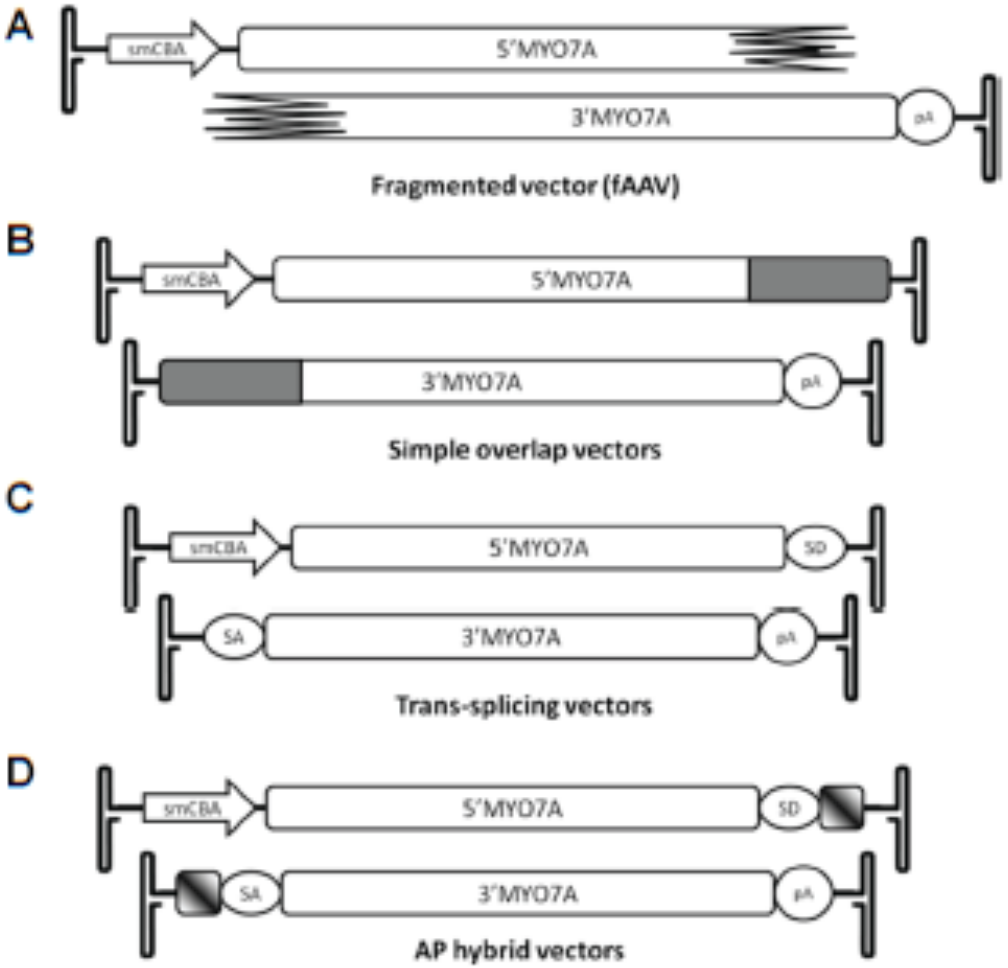
To solve this problem we developed a two vector system for making full length Myo7a:

- **Left part of the gene in one vector and the right part in a second vector.**

(SLIDE 7)

SLIDE 7


A
smCBA
5'MYO7A
3'MYO7A
pA
Fragmented vector (fAAV)
B
smCBA
5'MYO7A
3'MYO7A
pA
Simple overlap vectors
C
smCBA
5'MYO7A
SD
SA
3'MYO7A
pA
Trans-splicing vectors
D
smCBA
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AP hybrid vectors

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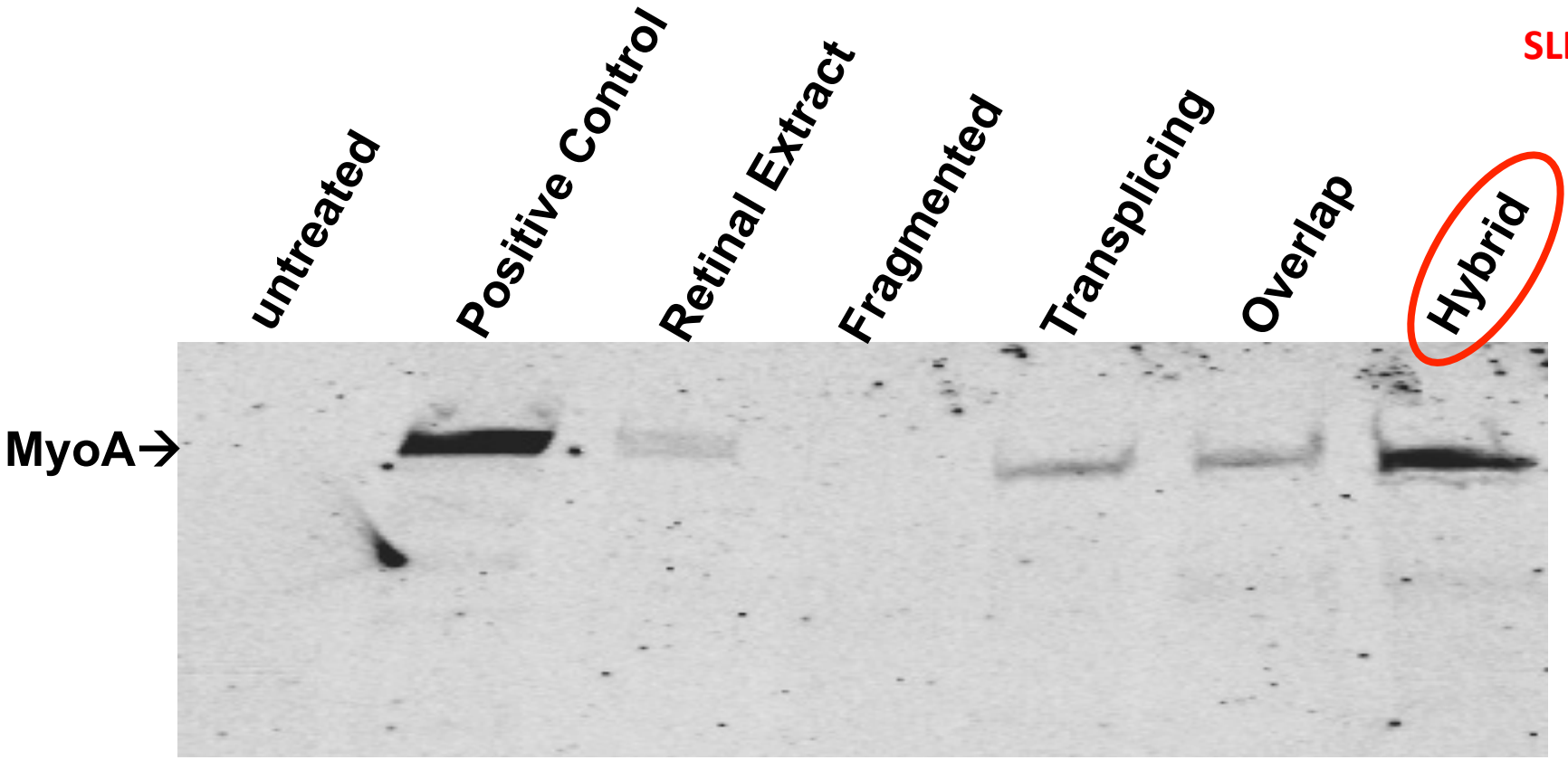
- **Left part of the gene in one vector and the right part in a second vector. (SLIDE 7)**
- **We then mixed left and right vectors together and first treated cells in a dish.**
- **After trying four different left-right pairs we found one pair that we call the "hybrid" vector that worked the best to give us the full length Myo7a. (SLIDE 8)**

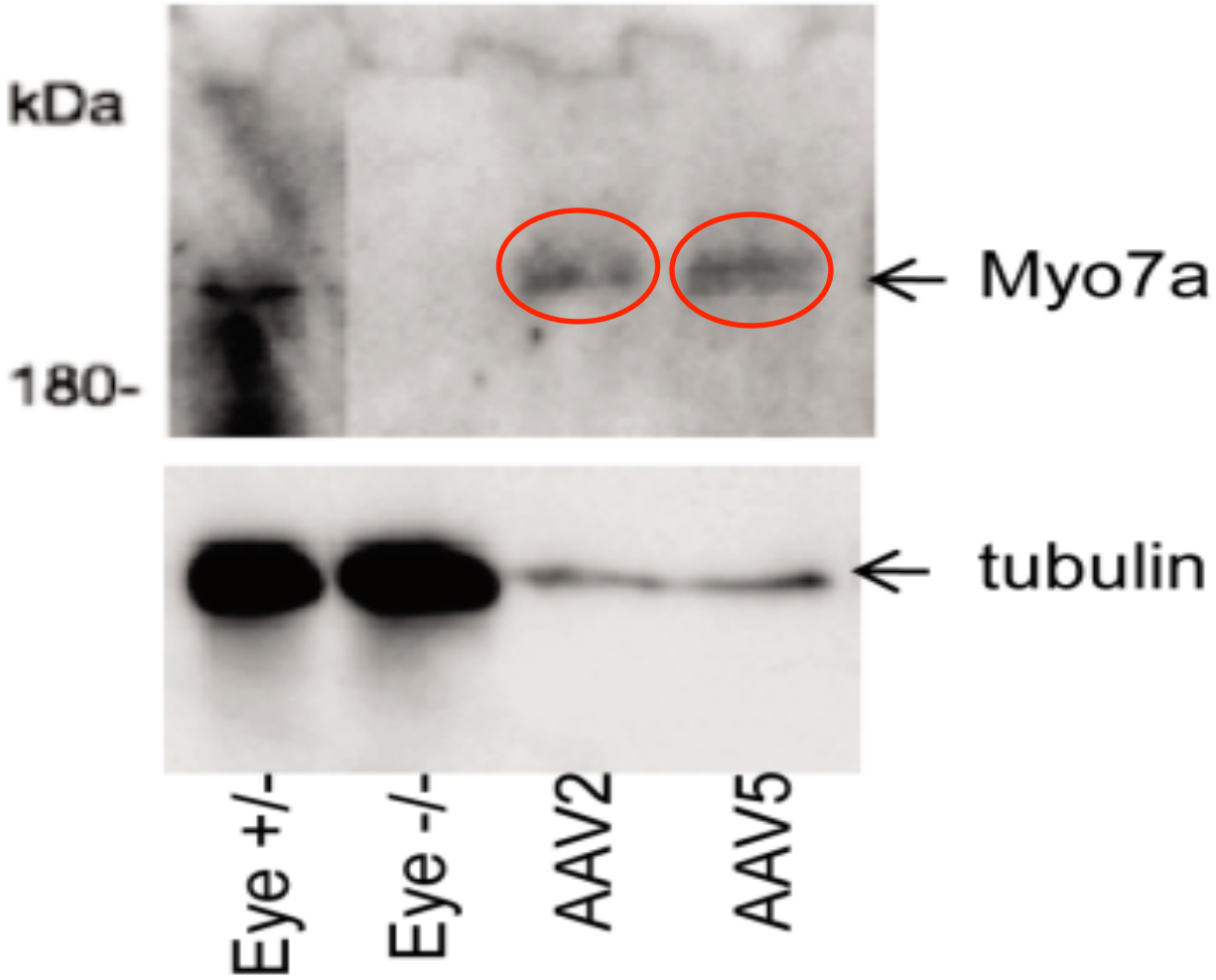
SLIDE 8


untreated
Positive Control
Retinal Extract
Fragmented
Transplicing
Overlap
Hybrid
MyoA→

We then tested the hybrid vector pair in the Myo7a mouse and found it also made full length Myo7a.

(SLIDE 9)

SLIDE 9



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We therefore isolated the Myo7a gene from retinas treated with the hybrid vector pair in 10 separate experiments and found that all 10 had the normal, full length DNA sequence.

(SLIDE 10)

Myo7a size problem solved!

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

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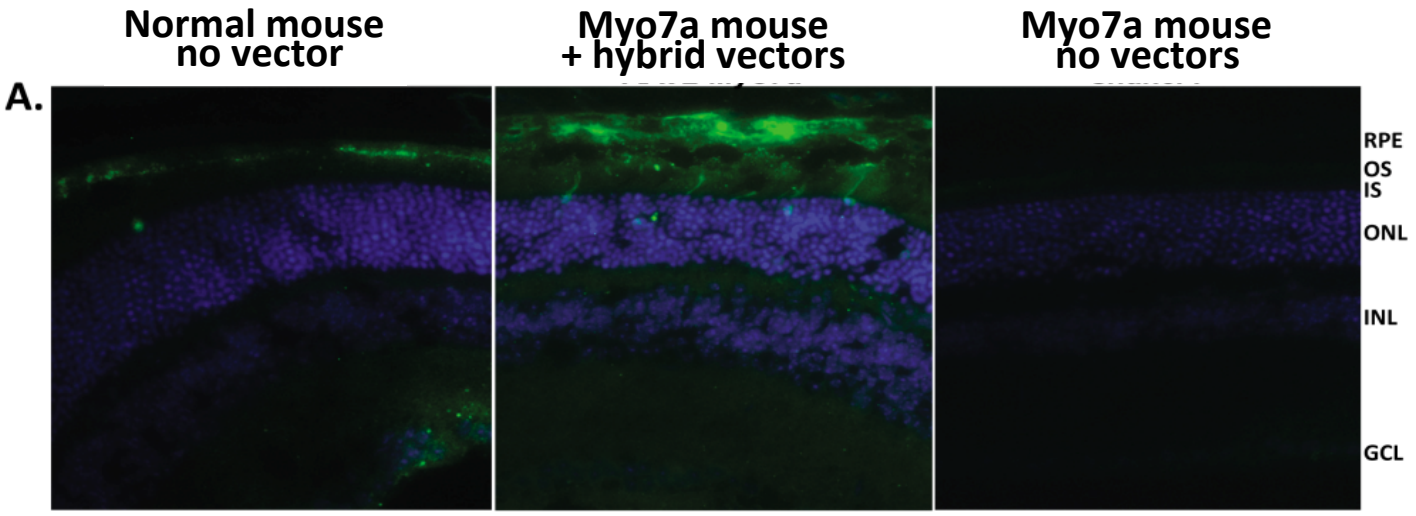
- **Restored Myo7a to the retina in the correct positions.**

(SLIDE 11)



Myo7a is Green

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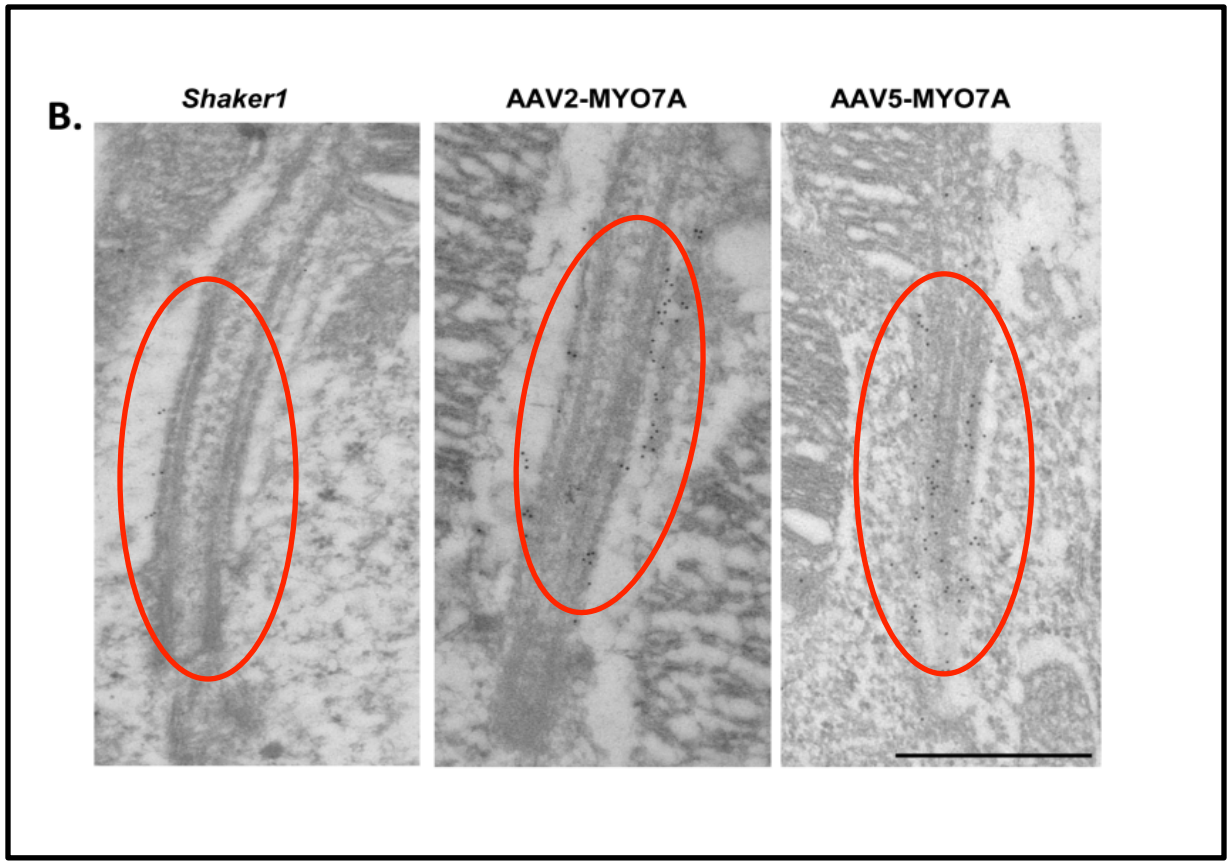
(SLIDE 11)

- **Restored movement of rhodopsin from the inner segment to the outer segment.**

(SLIDE 12)

SLIDE 12



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(SLIDE 11)

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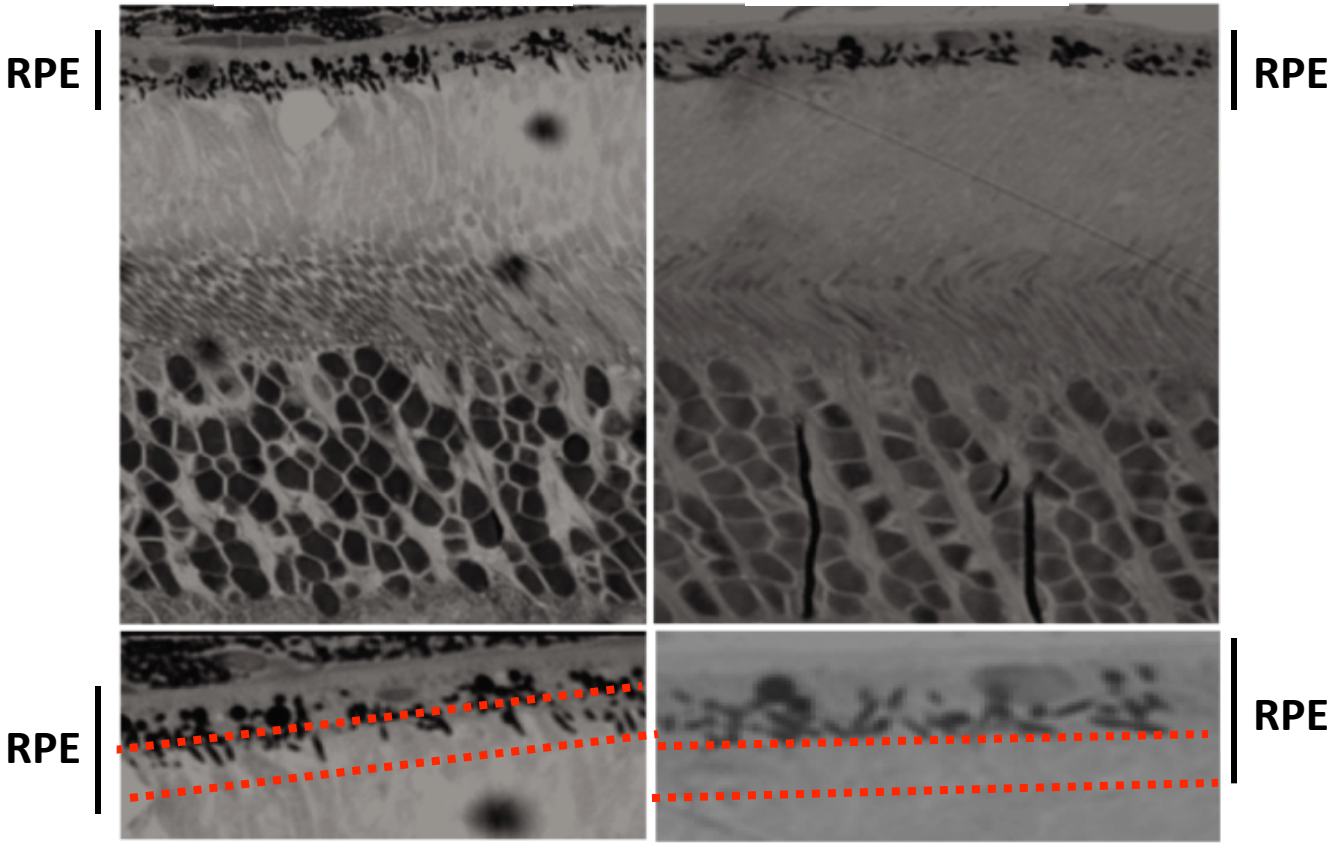
(SLIDE 12)

- **Restored pigment granules to the apical side of the RPE.**

(SLIDE 13)

Myo7a mouse
+ hybrid vectors

Myo7a mouse
no vectors



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Thank You