Role for Usher proteins in regulated protein trafficking in photoreceptors: potential mechanism for light-induced retinal degeneration in Usher syndrome.

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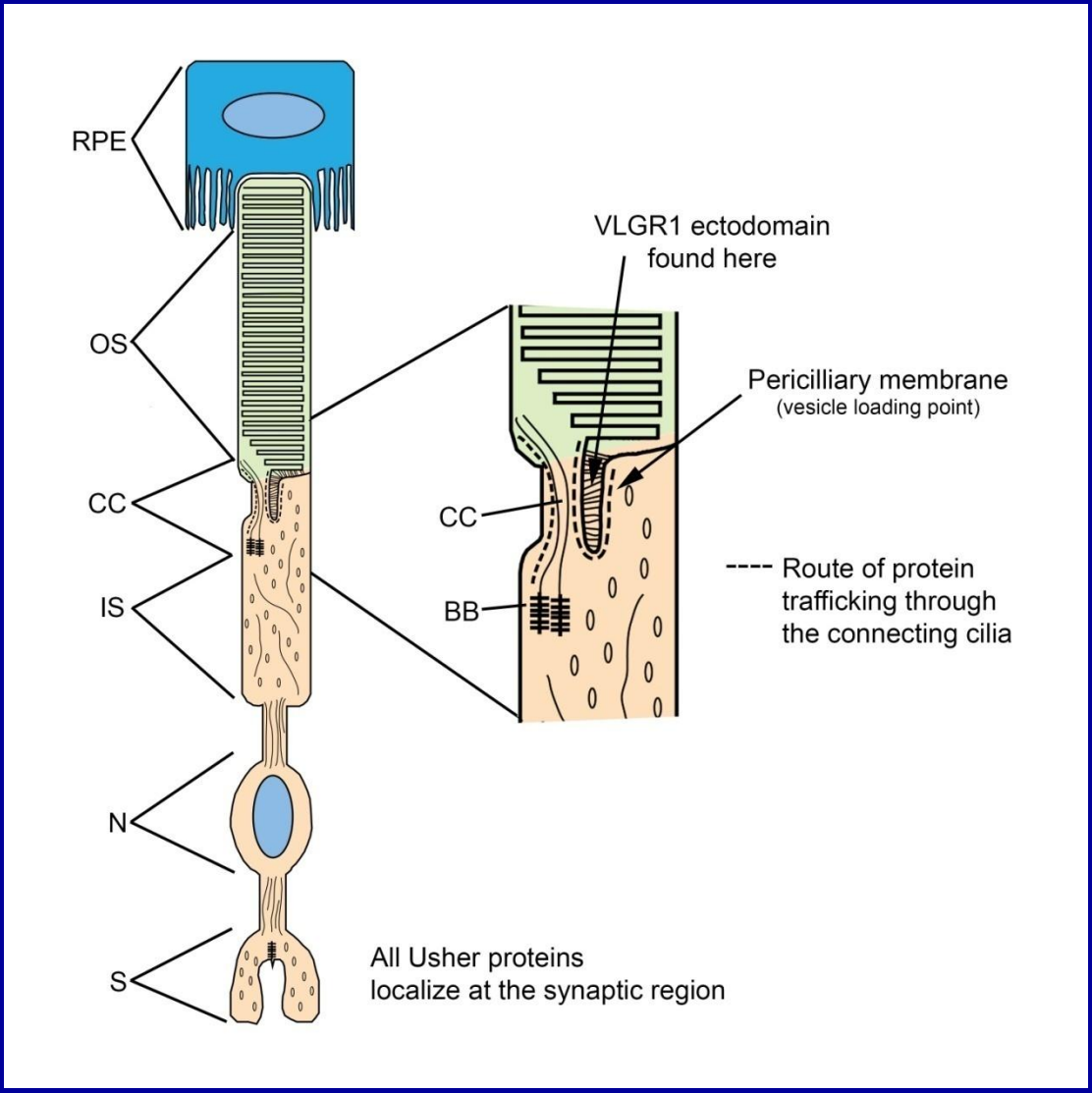
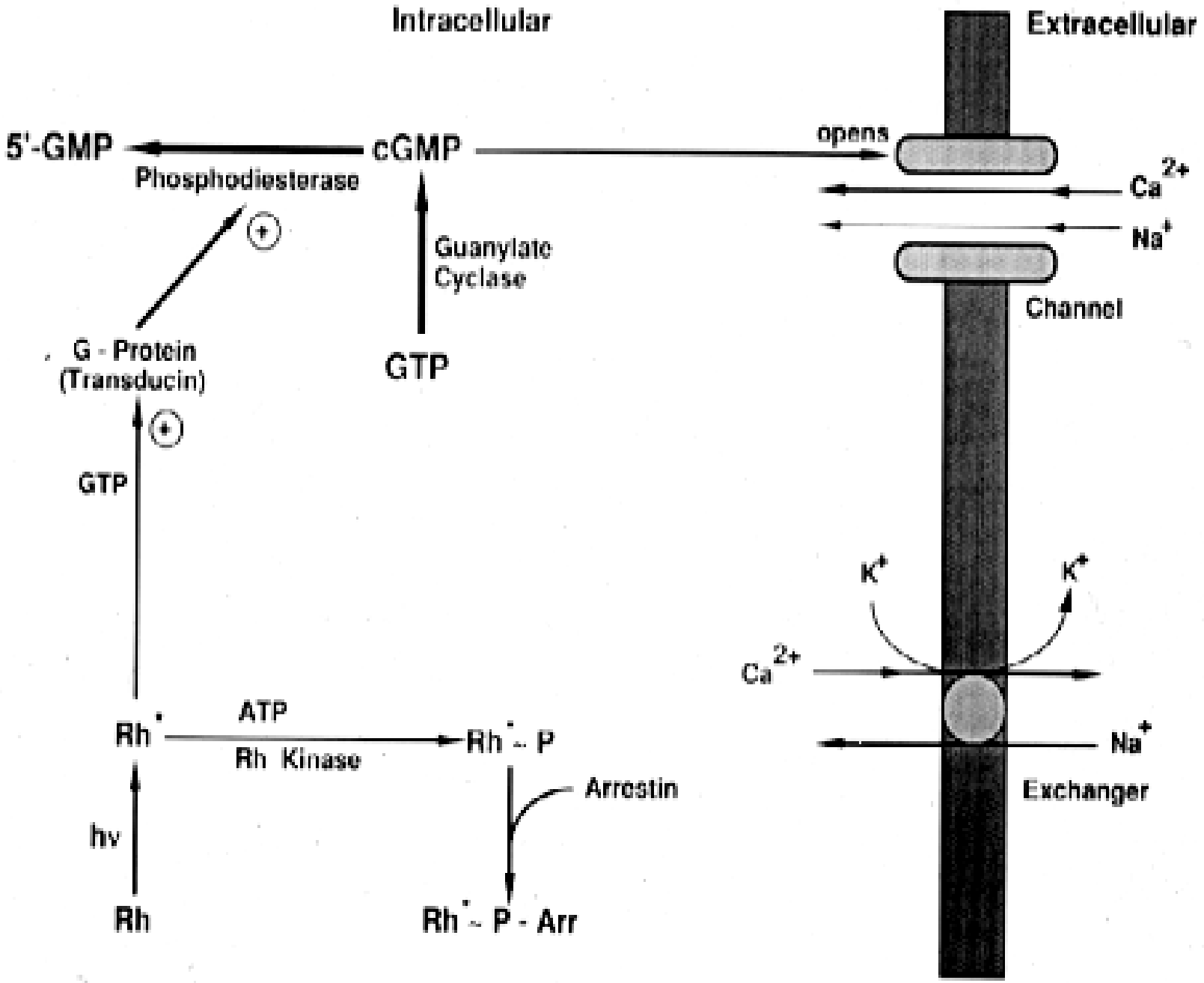
Usher syndrome sub-types

Clinical sub-types	Gene	Protein	Protein function	Symptoms		
				Hearing	Vestibular	*Retinintis pigmentosa*
1B	MYO7A	Myosin7A	Motor protein	Profound Congenital	Absent	First decade
1C	USH1C	Harmonin	Scaffold protein			
1D	CDH23	CDH23	Adhesion molecule	Mixed	Mixed	Varies
1F	PCDH15	PCDH15	Adhesion Molecule	Profound Congenital	Absent	First decade
1G	USH1G	SANS	Scaffold protein			
1J	USH1J	CIB2	Integrin binding protein			
2A	USH2A	Usherin	Adhesion Molecule	Sloping Congenital	Normal	Mild
2C	GPR98	VLGR1	G-protein receptor			
2D	DFNB31	Whirlin	Scaffold protein			
3A	CLRN1	Clarin-1		Progressive	Variable	?

The model for Usher protein function in stereocilia development and mechanotransduction machinery is attractive, however a synonymous function cannot be ascribed in photoreceptors.

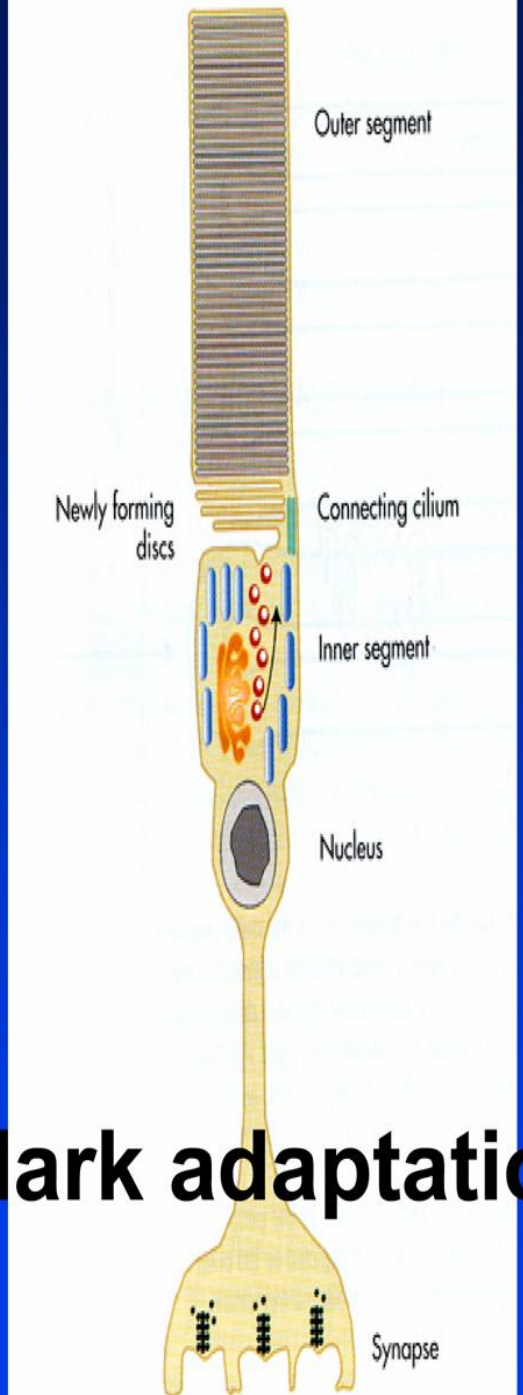
All Usher proteins have been localized to the periciliary/connecting cilia region and the synaptic region of photoreceptors.




Intracellular
Extracellular
5'-GMP
cGMP
Phosphodiesterase
Guanylate Cyclase
GTP
opens
Ca2+
Na+
Channel
G - Protein (Transducin)
GTP
K+
K+
Ca2+
Na+
Exchanger
Rh*
ATP
Rh Kinase
Rh*~ P
Arrestin
hv
Rh
Rh*~ P - Arr

Arrestin

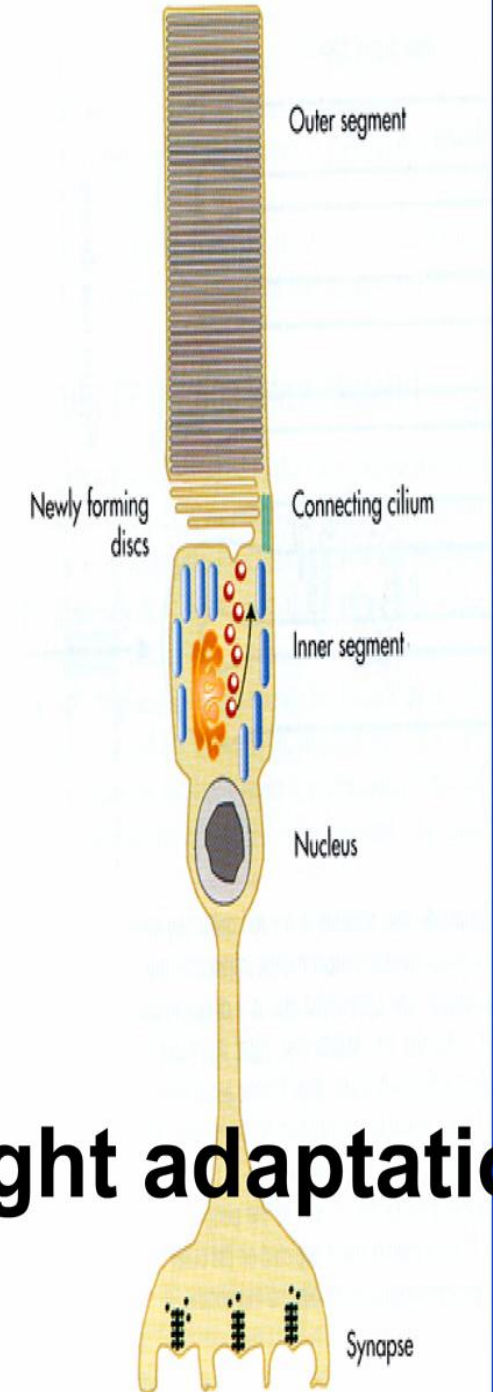
Transducin



Under dark adaptation

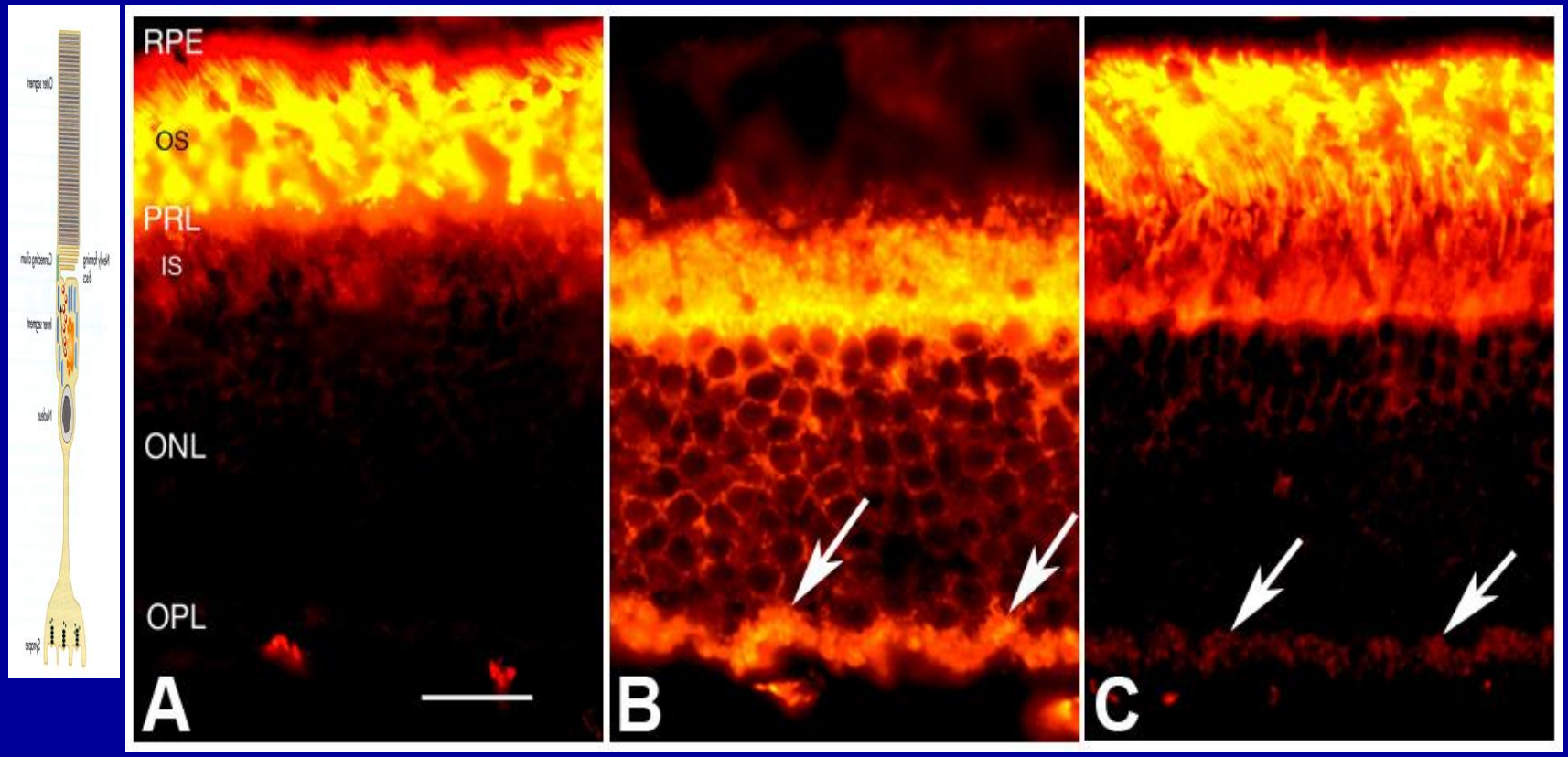
Transducin

Arrestin



Under light adaptation

Delay of transducin α translocation in Shaker1 rods

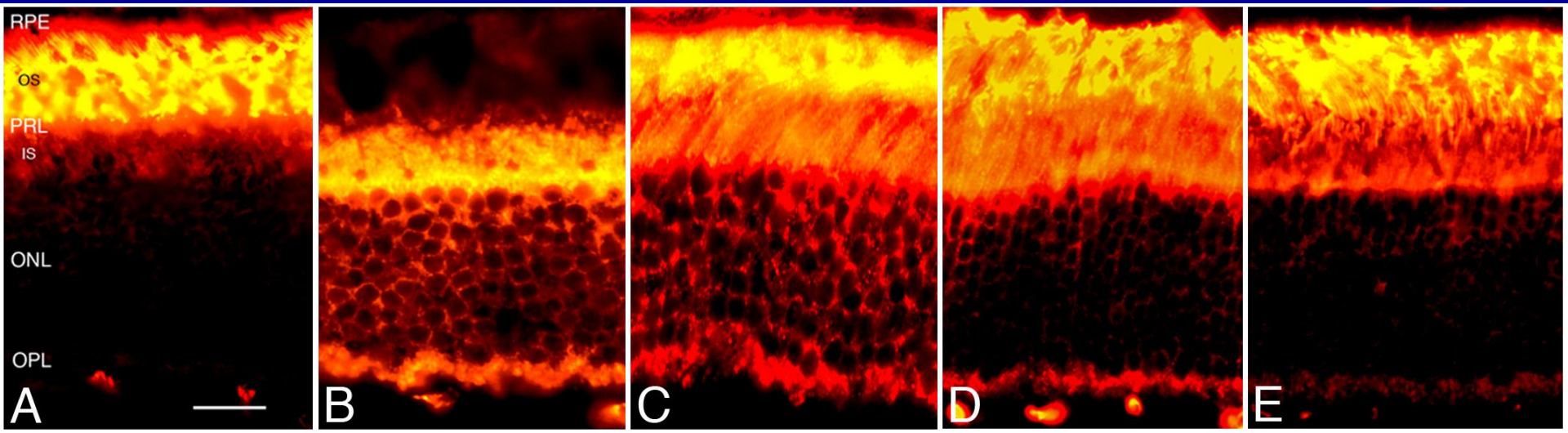


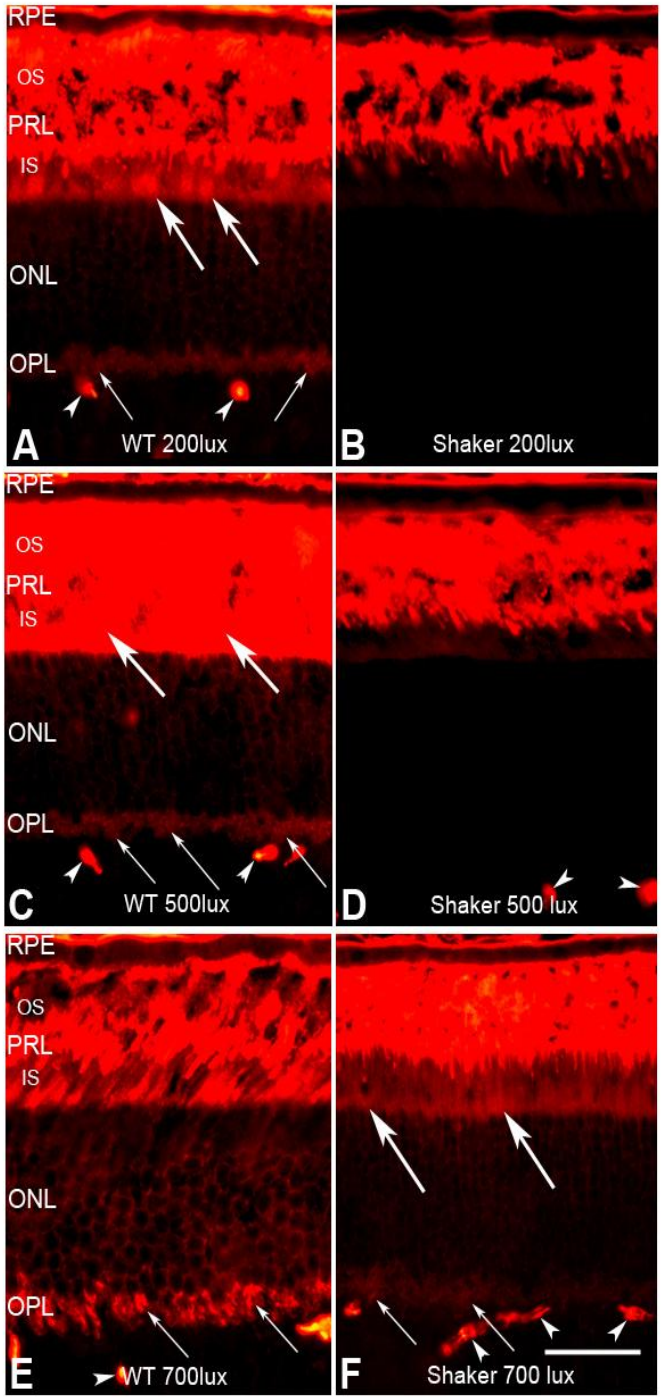
Dark-adapted WT

Light-adapted WT

Light-adapted Shaker1

Usherin hypomorph, Ames Waltzer, and shaker mice all show defects in light induced alpha-transducin protein translocation



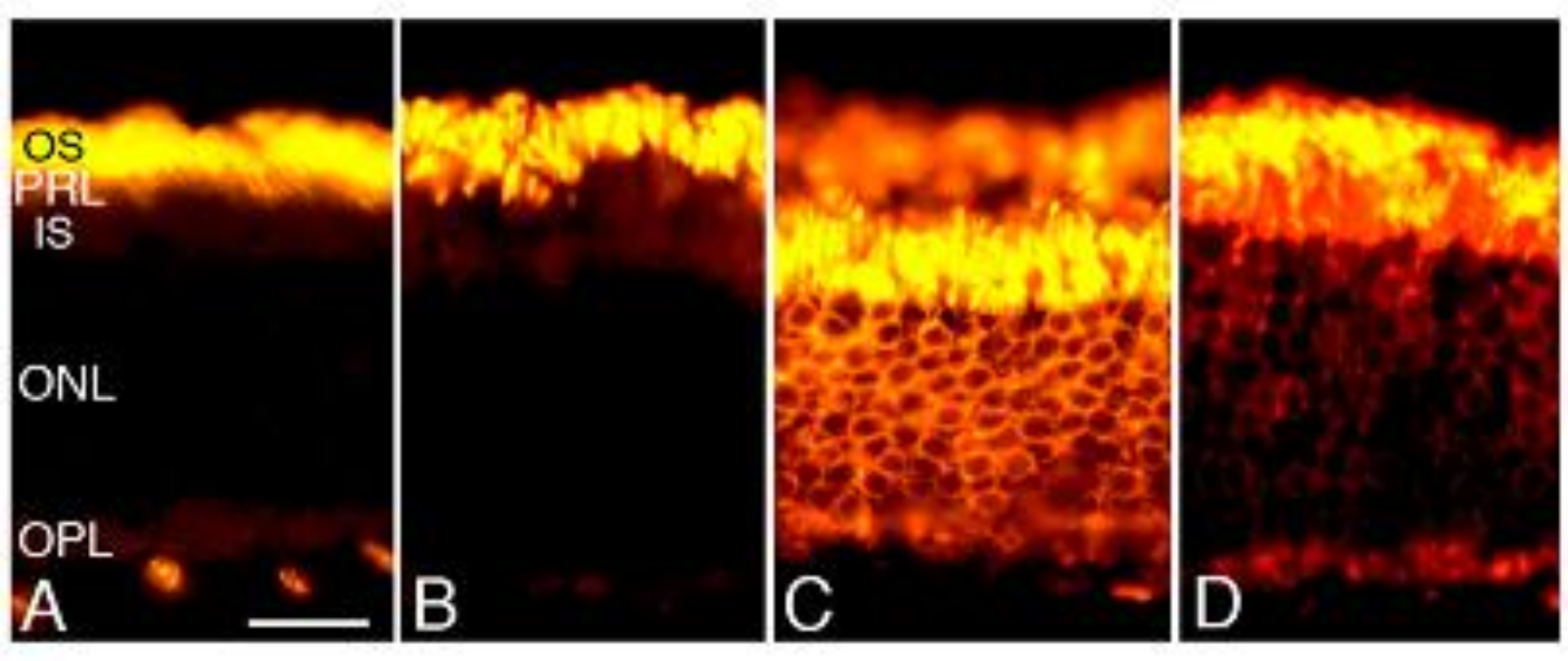


The threshold for light-activated transducin translocation in shaker1 mice has been shifted to a higher level

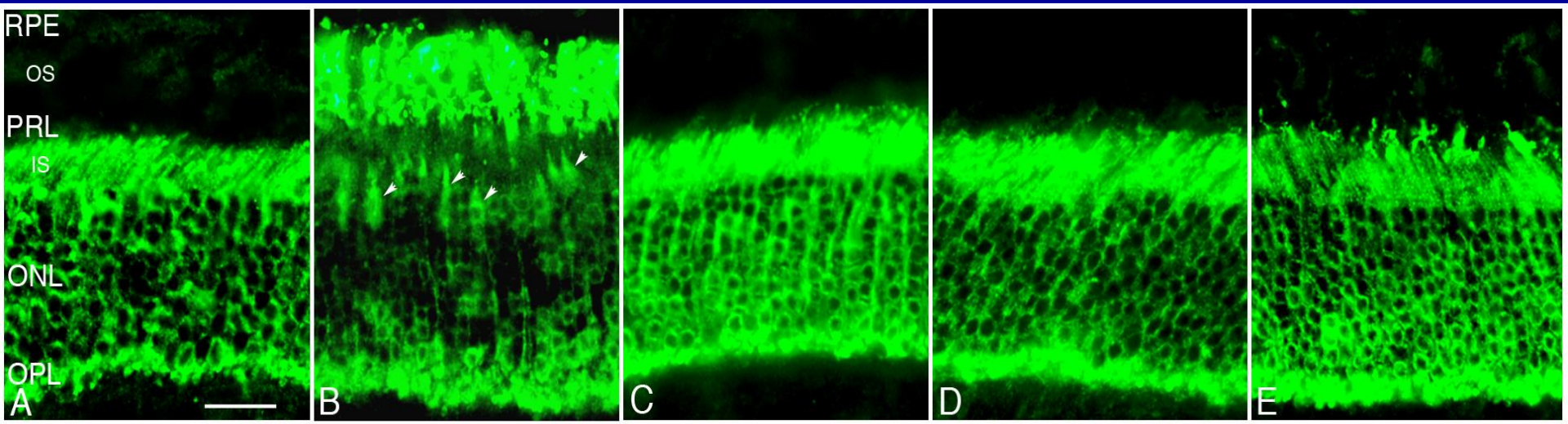
In WT mice, 200 lux light can activate transducin translocation in rod photoreceptors

In shaker1 mice, the threshold for light-activated transducin translocation in rods has been shifted to 700 lux

Light-induced translocation of the a-subunit of transducin is partially blocked by treatment of neural retinal explants with Brefeldin A

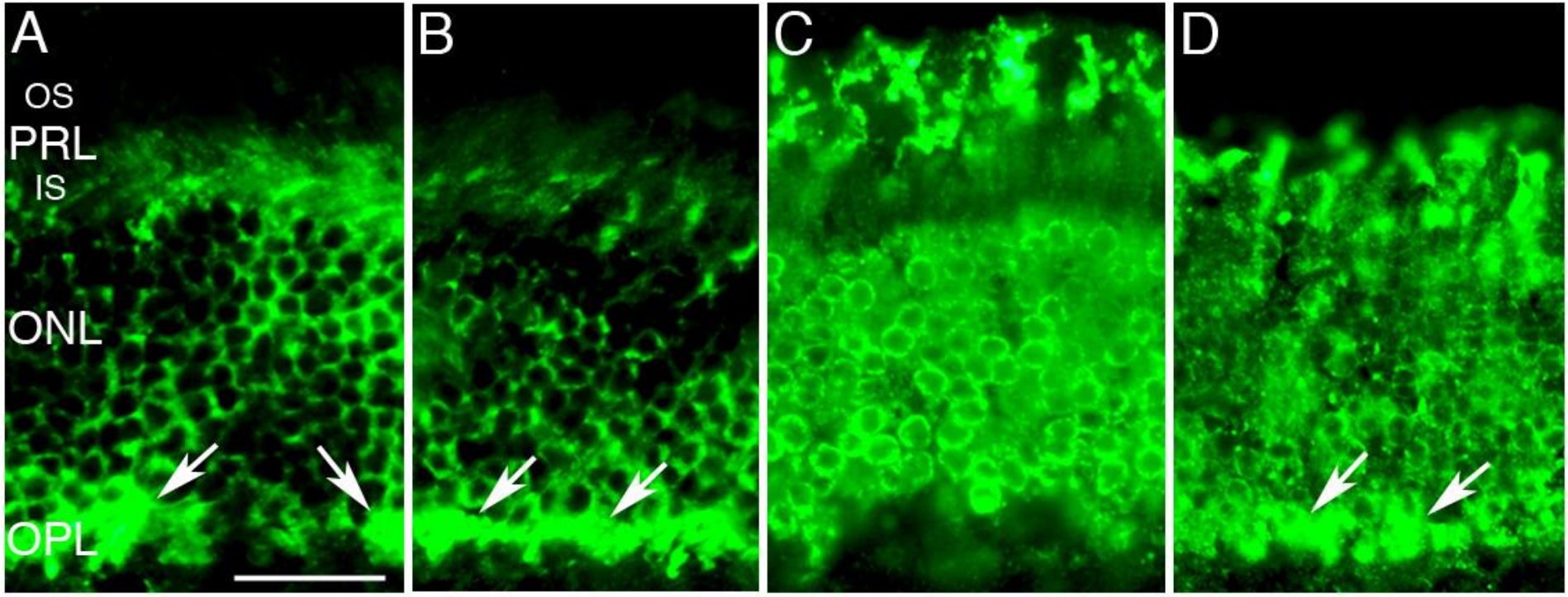


Usherin hypomorph, Aimes Waltzer, and shaker mice all show defects in light induced arrestin protein translocation

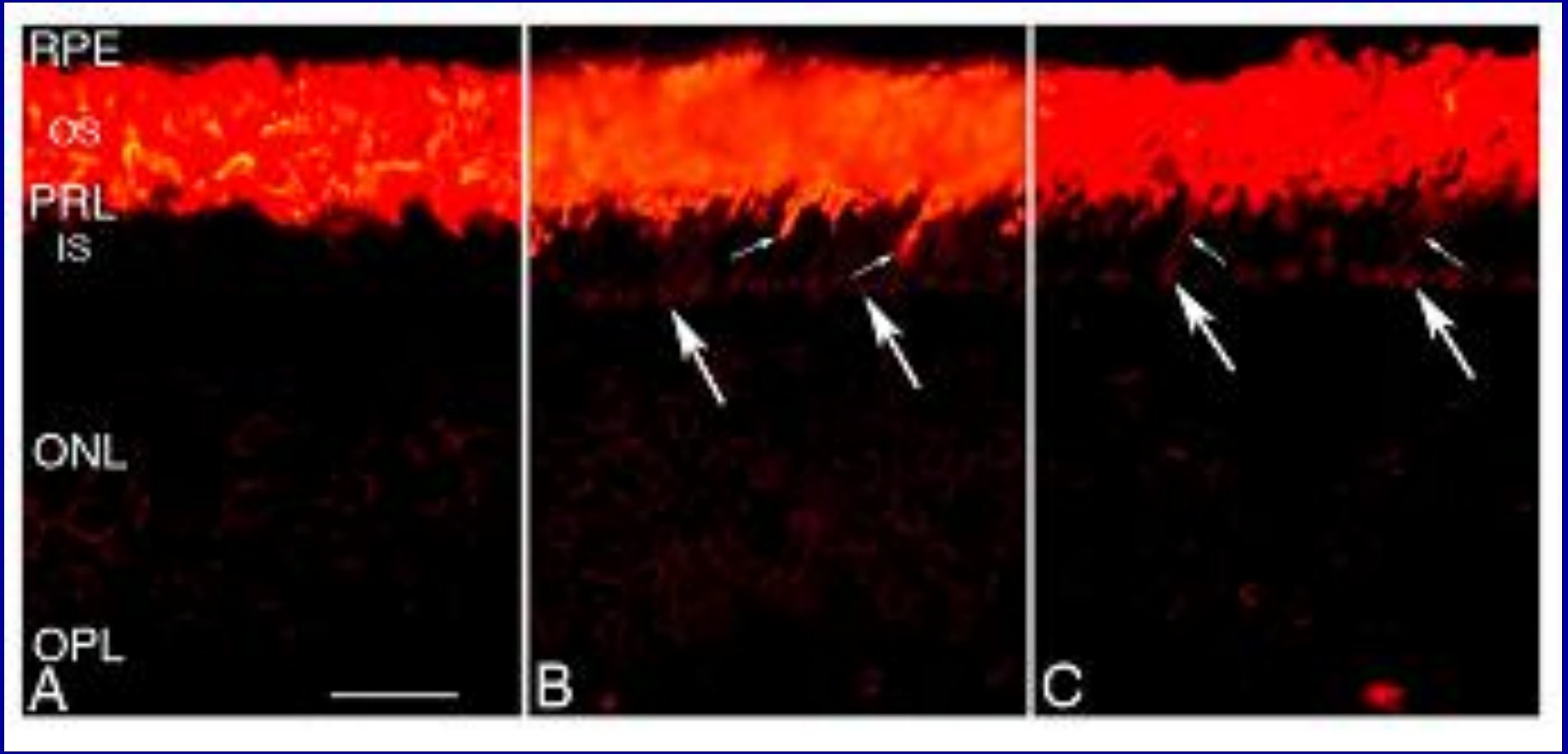


DA WT **LA WT** **LA USHHM** **LA Ames Waltzer** **LA Shaker**

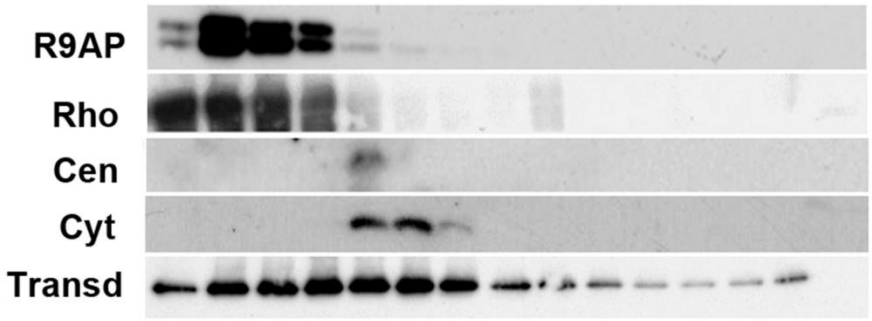
Brefeldin A partially blocks arrestin translocation in organotypic cultures of neural retina.

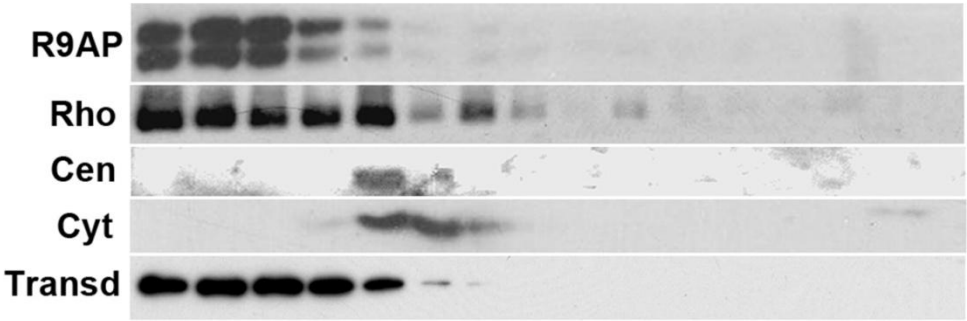


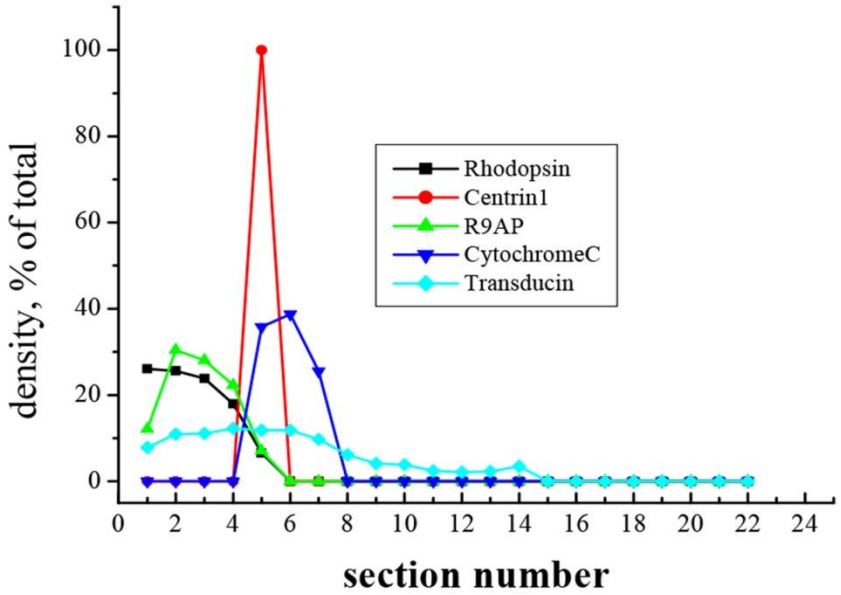
Rhodopsin is present in the connecting cilia of both *whirler* and *shaker1* photoreceptors.

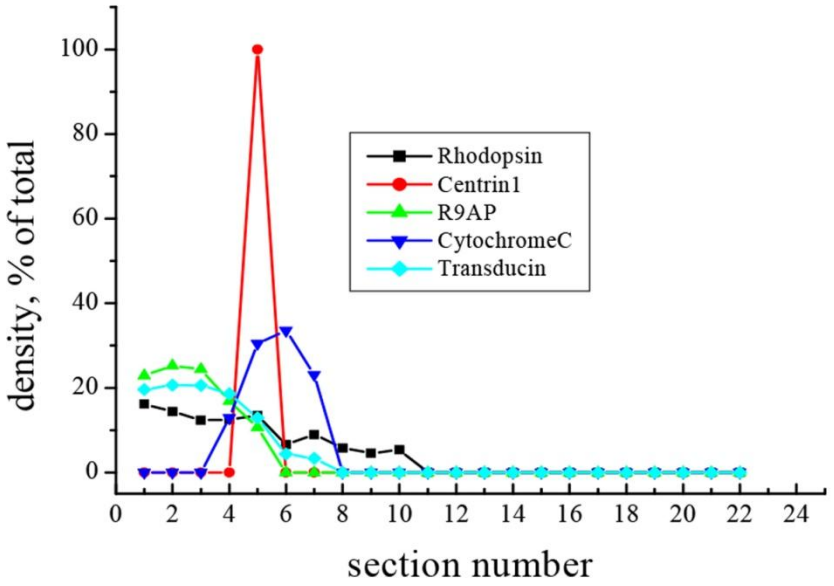


Serial tangential section immunoblotting confirms elevated light threshold for α-transducin translocation in whirler mice



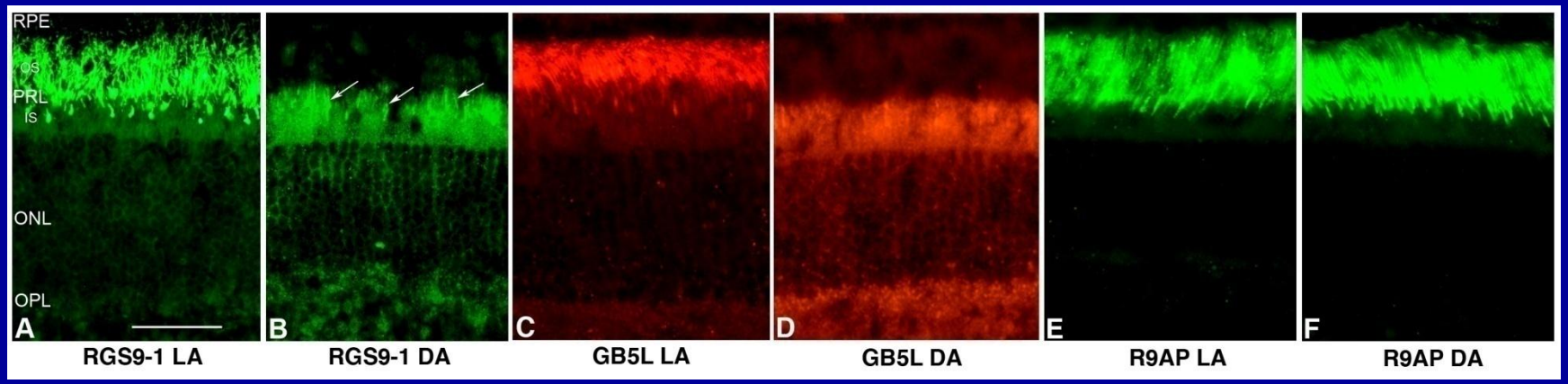




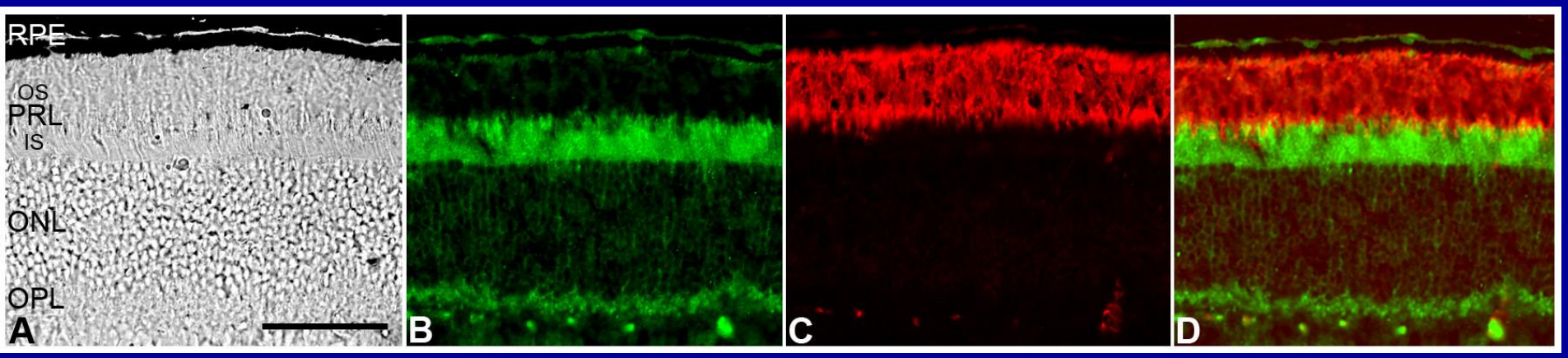


Our Team of investigators recently discovered that RGS9-1 and Gb5L translocate to the inner segments of photoreceptors in the dark. They relocate to the outer segments under extreme low light stimulus, which is why this phenomenon was not reported previously.

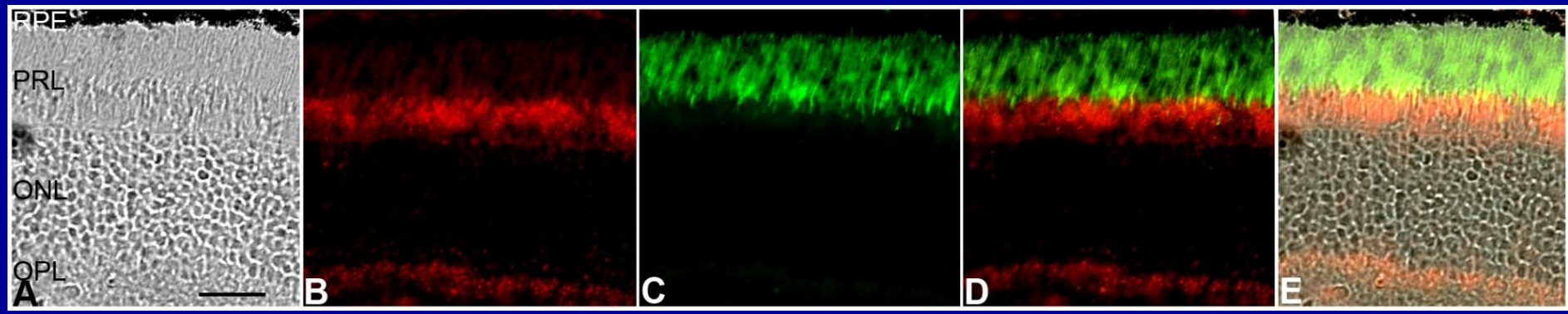
RGS9 and Gβ5L show different distribution in mouse rods following light (LA) and dark adaptation (DA).



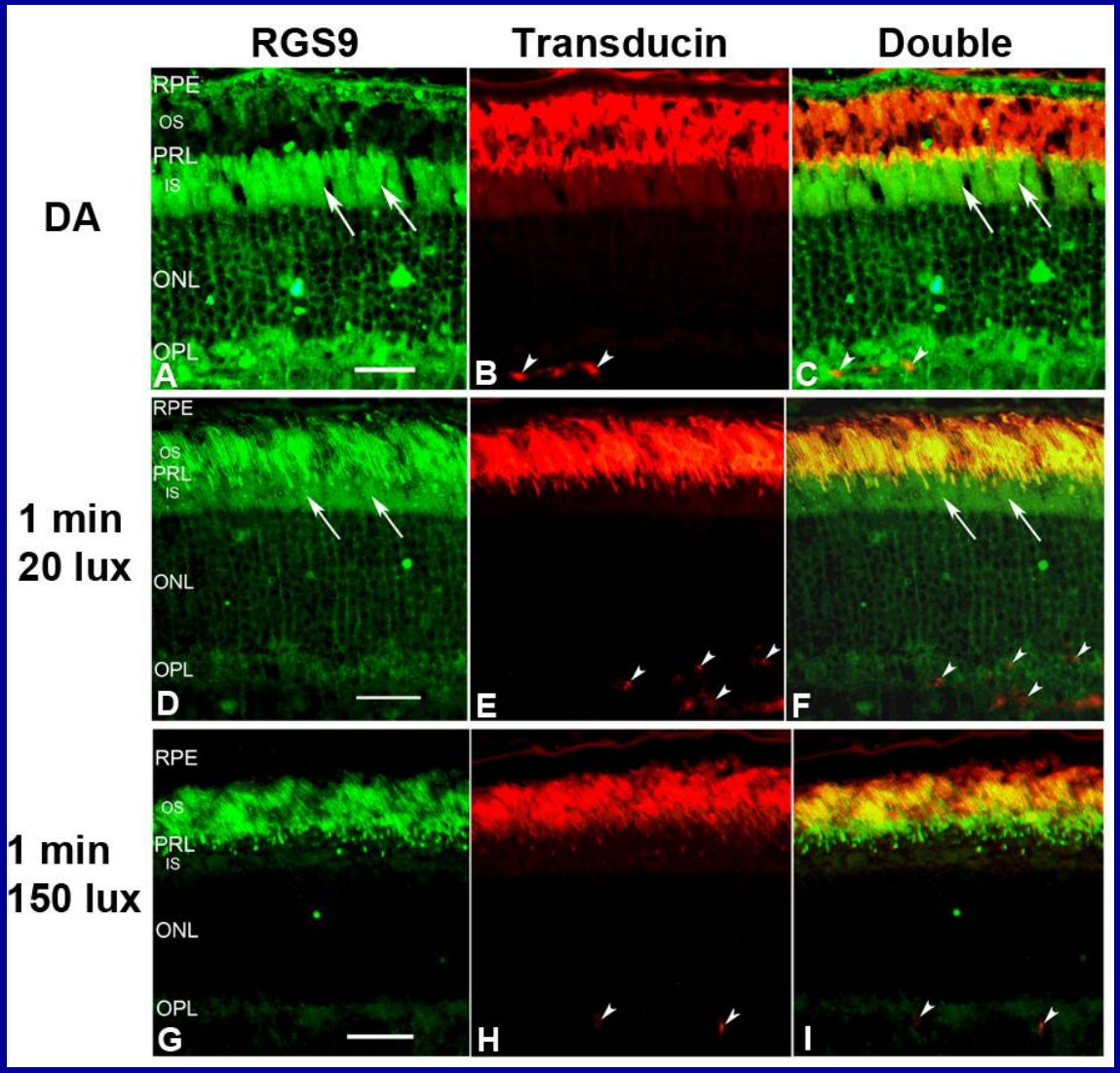
Double labeling studies using anti-RGS9 and anti-rhodopsin antibodies on dark adapted mouse retina isolated and processed in complete darkness

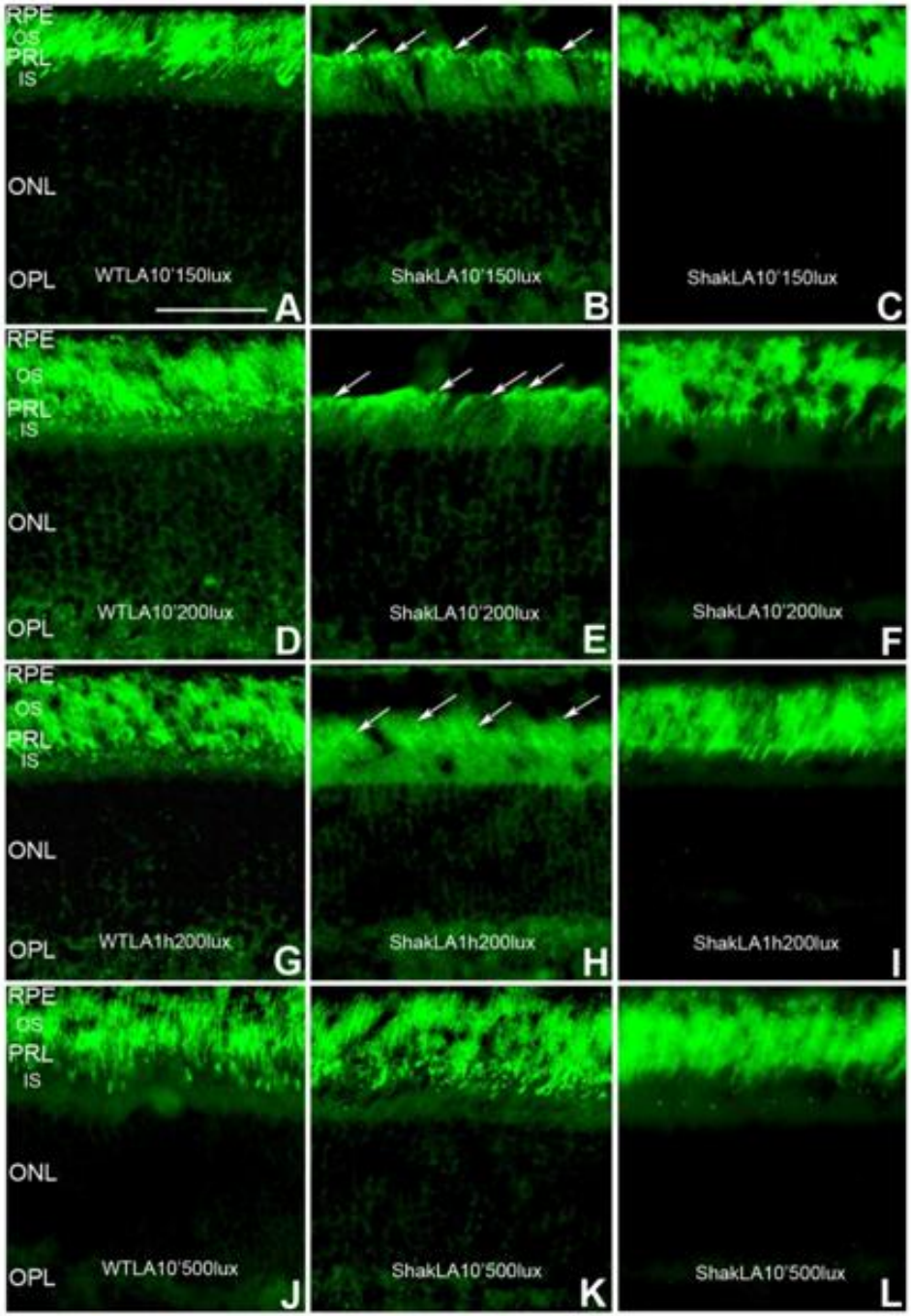


Double labeling studies using anti-Gβ5L and anti-R9AP antibodies on a dark adapted mouse retina isolated and processed in complete darkness

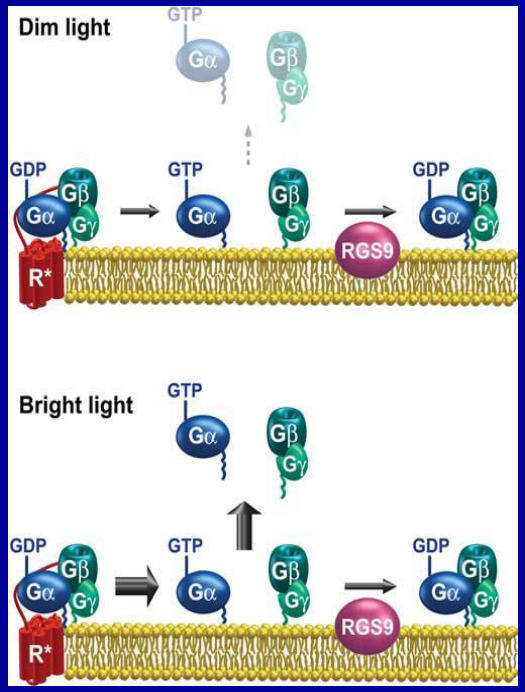


Double labeling studies using anti-RGS9 and anti-Transducin α antibodies confirm that RGS9 is translocated between rod outer segments and inner segments during light/dark adaptation





In *shaker1* mice, the light activation threshold for RGS9's distribution to rod outer segments is shifted to a higher level. R9AP is always located in rod outer segments regardless of light conditions



High light sensitive mode under dim light

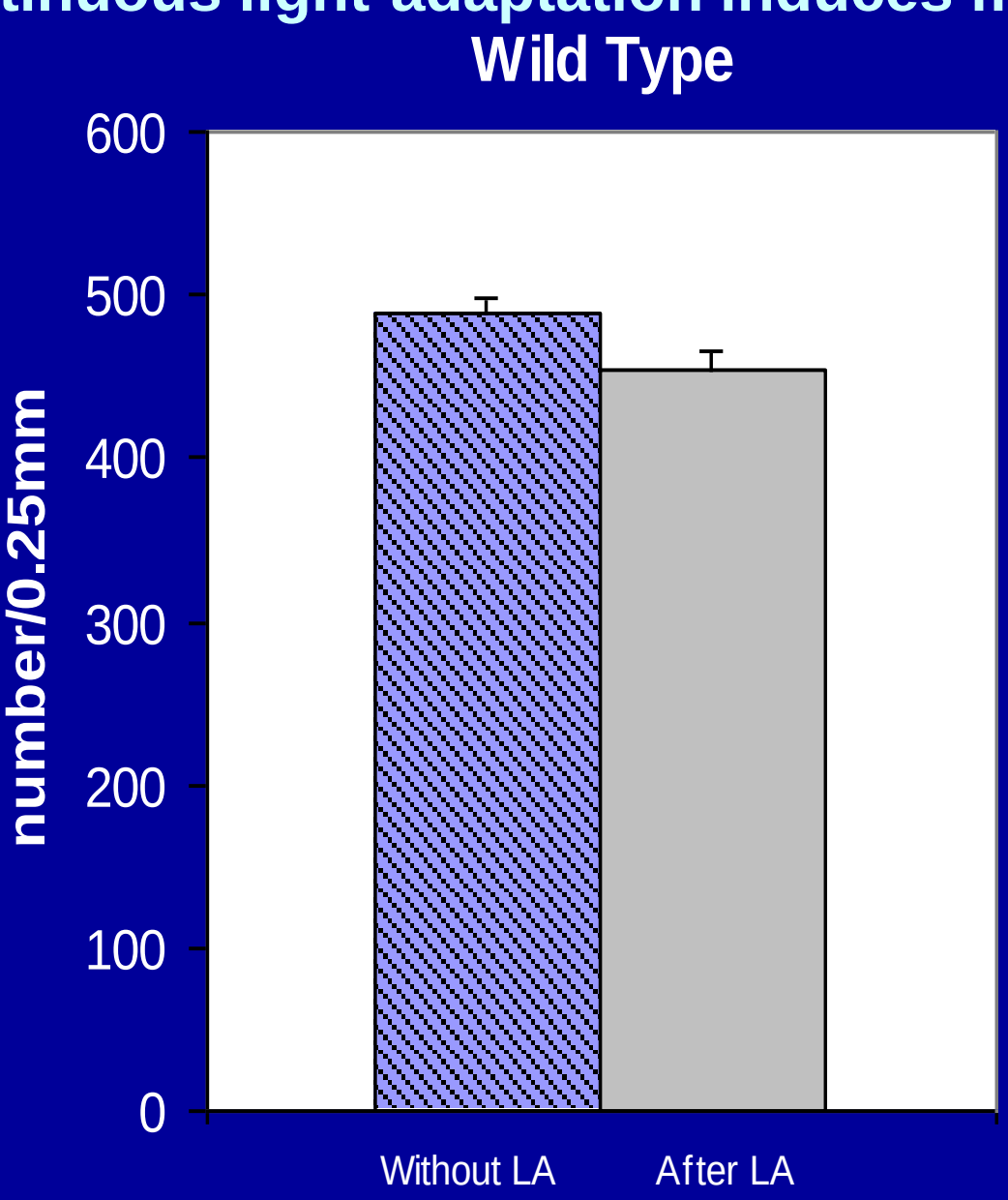
Low light sensitive mode under bright light

Elevated threshold of translocation means that phototransduction in the shaker1 rod outer segments will continue to work in high light sensitive mode under bright light. This will increase the metabolic and oxidative stress in the rods of shaker1 mice under bright light conditions and will increase the accumulation of superoxides. This makes the rods more vulnerable to light-induced degeneration.

Collectively this data predicts that Usher mutations result in impaired light-regulated protein transport in photoreceptors.

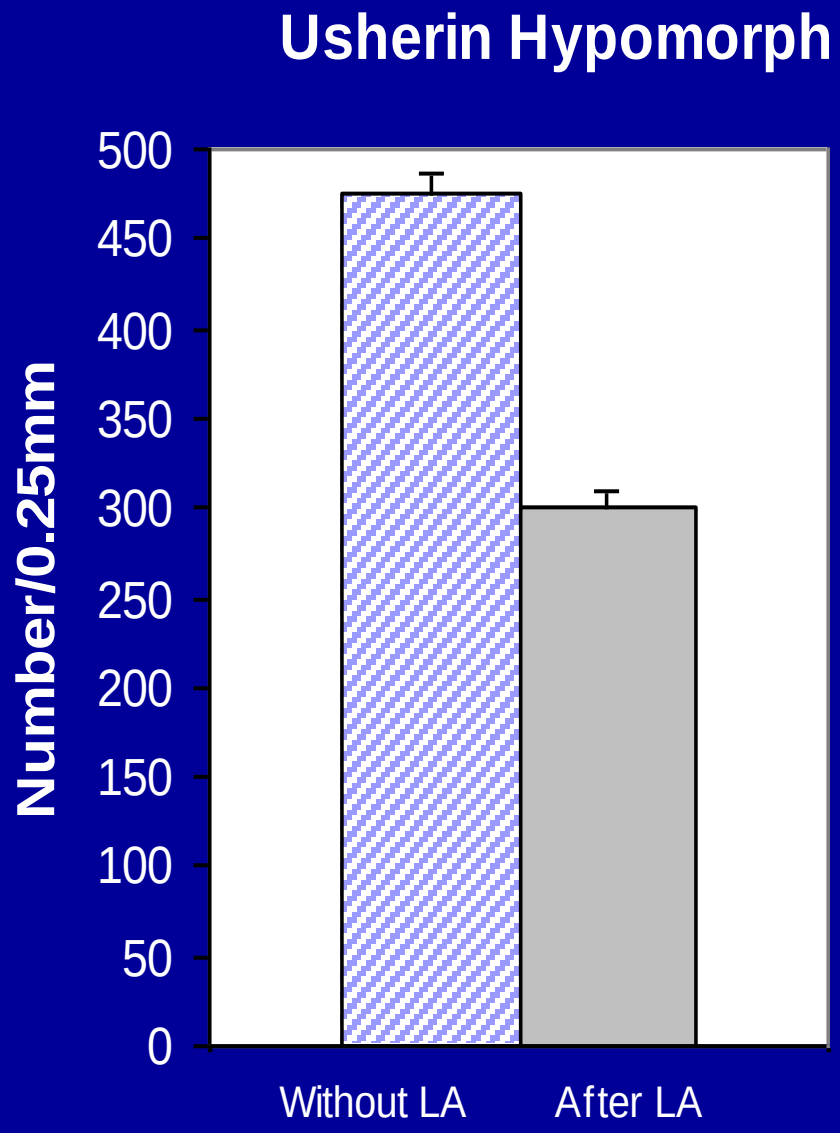
This functional model would predict that threshold shifts for RGS-9-1/Gβ5L and α-transducin in Usher mice would make them more susceptible to light induced photoreceptor degeneration.

Continuous light-adaptation induces mild rod degeneration.

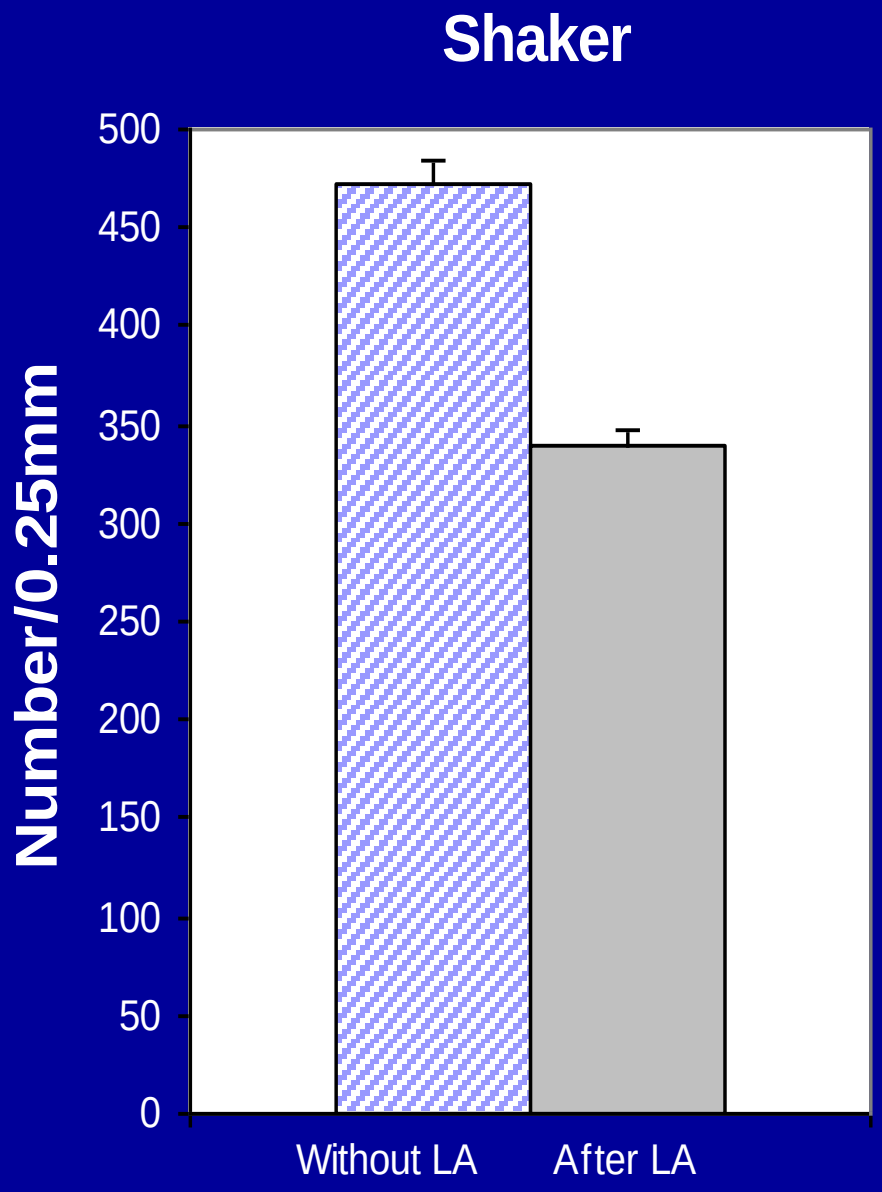


Under continuous high intensity (2500 lux) light-adaptation for 6 days, rod numbers in WT mouse retina are reduced 5-10%.

Continuous light-adaptation induces more severe rod degeneration in Usher mice.

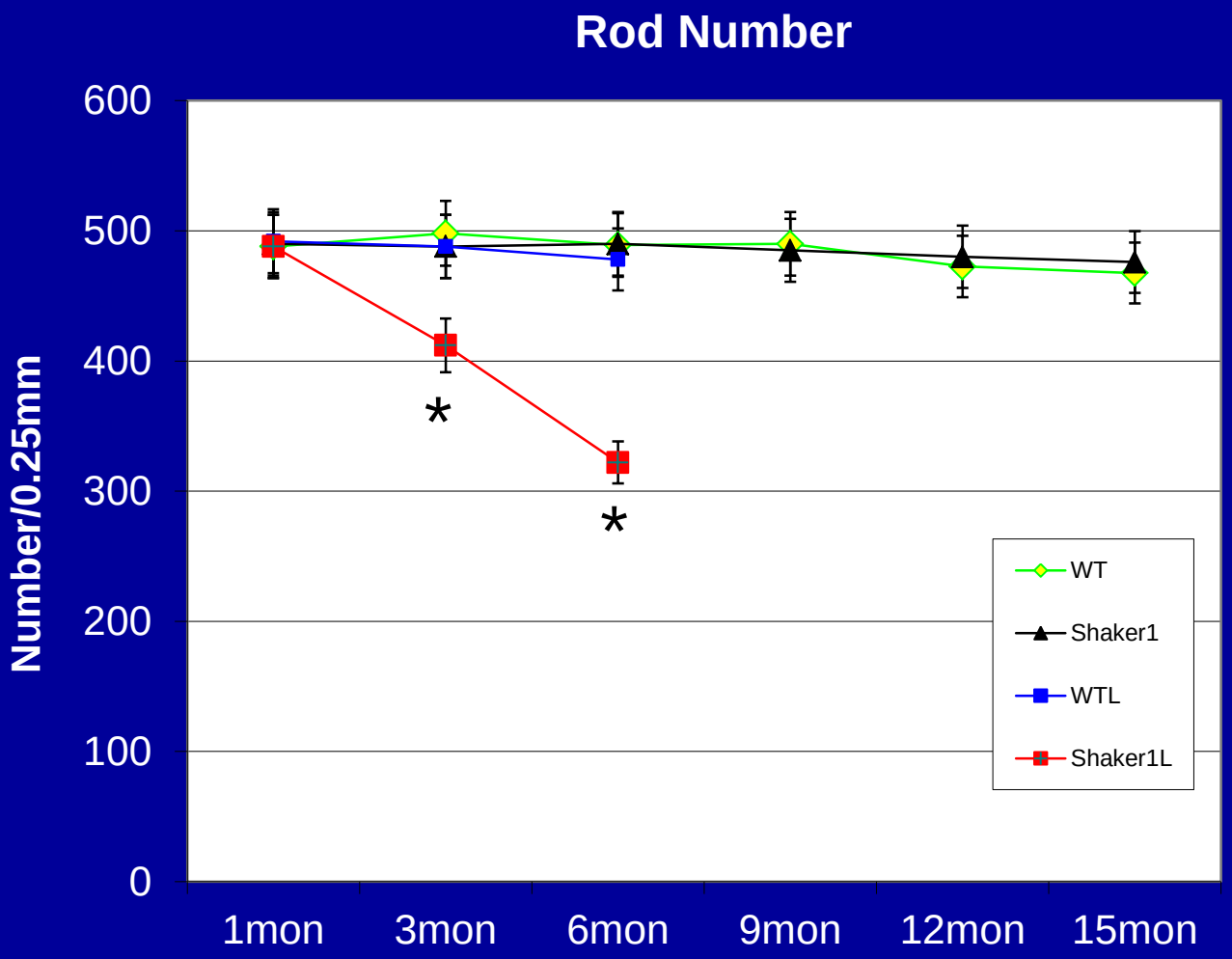


Under continuous high intensity (2500 lux) light-adaptation for 6 days, rod number in Usherin Hypomorph mouse retina could be reduced 30-35%.

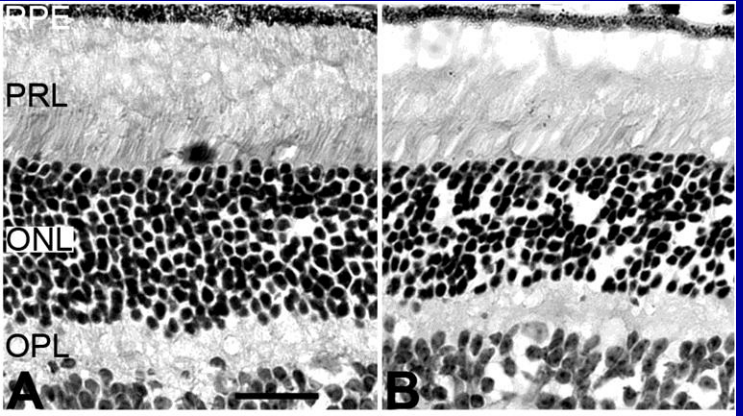


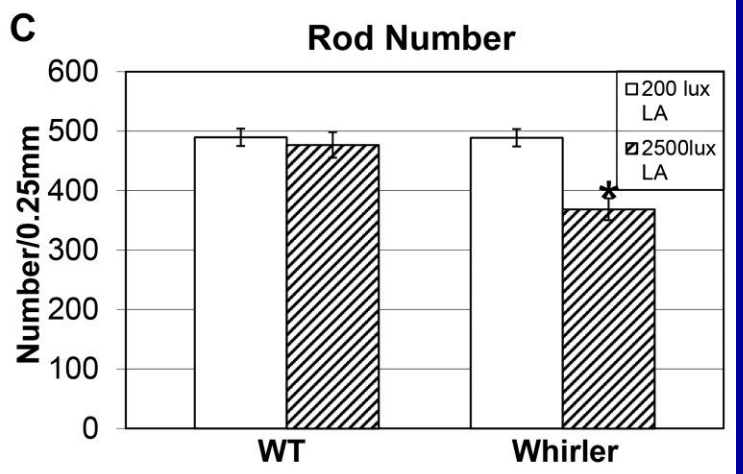
Under continuous high intensity (2500 lux) light-adaptation for 6 days, rod number in Shaker mouse retina could be reduced 25-30%.

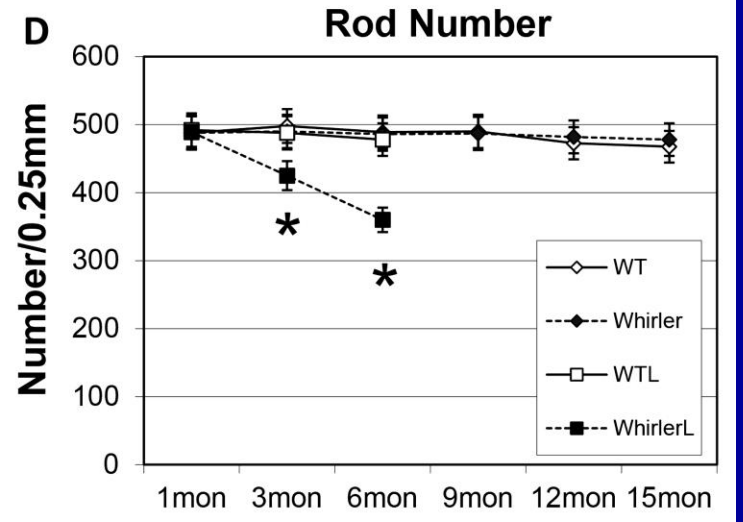
Reared under moderate light/dark cycle induced rod degeneration



Shaker1 mice reared under moderate light/dark cycle developed severe retinal degeneration in less than 6 months.





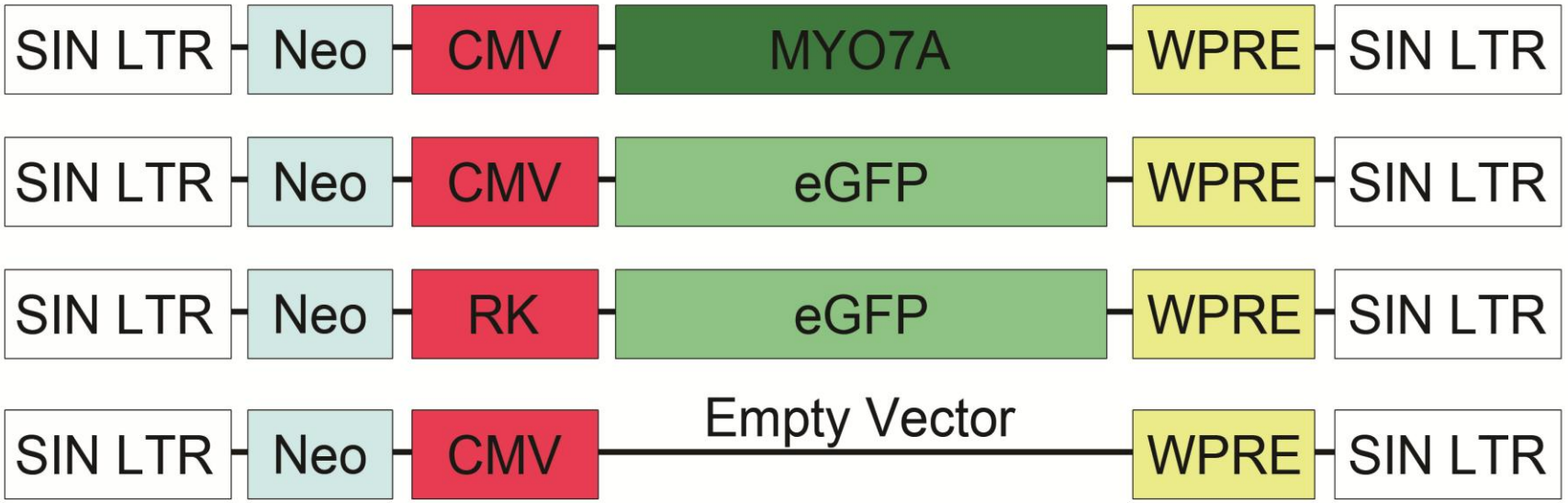


The Whirler mouse is also susceptable to light-induced retinal degeneration

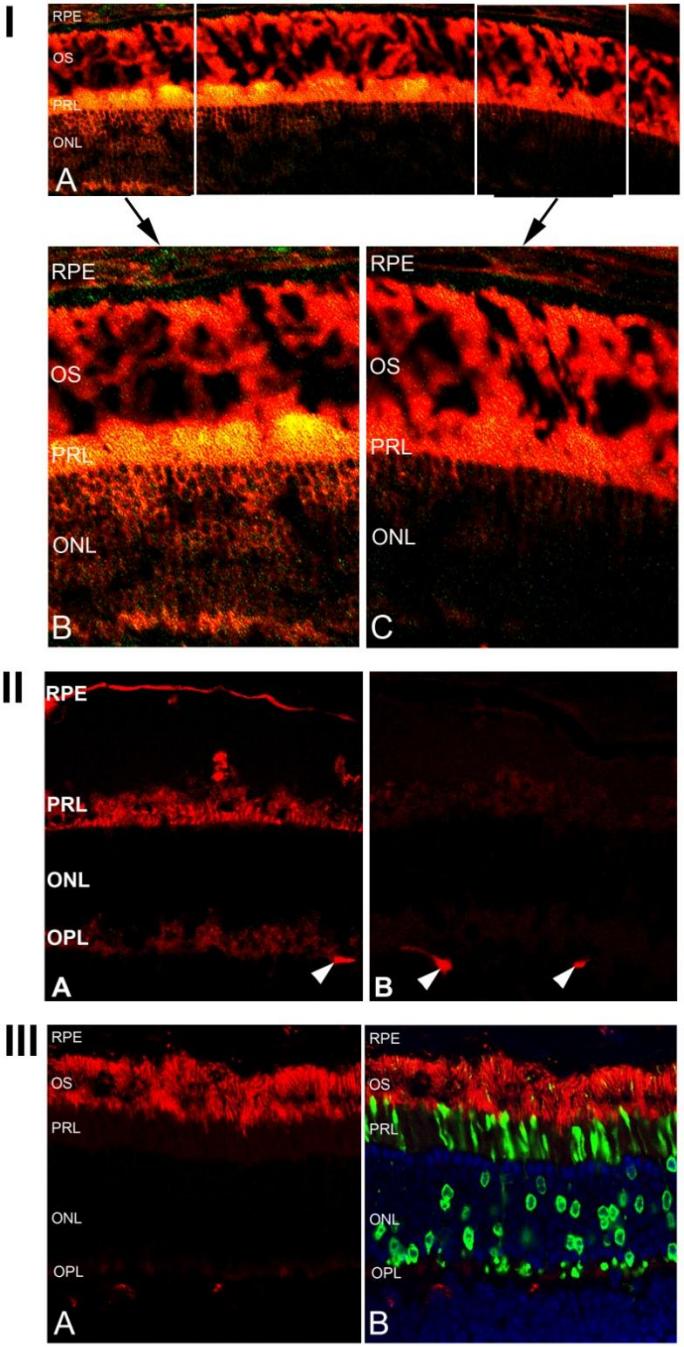
The argument can be made that these observations are not directly linked to myosin 7A dysfunction in *shaker1* mice.

To address this we attempted to rescue the phenotypes by gene therapy.

Schematic diagram showing the genetic structure of EIAV vectors used and the UshStat® vector genome (at top)

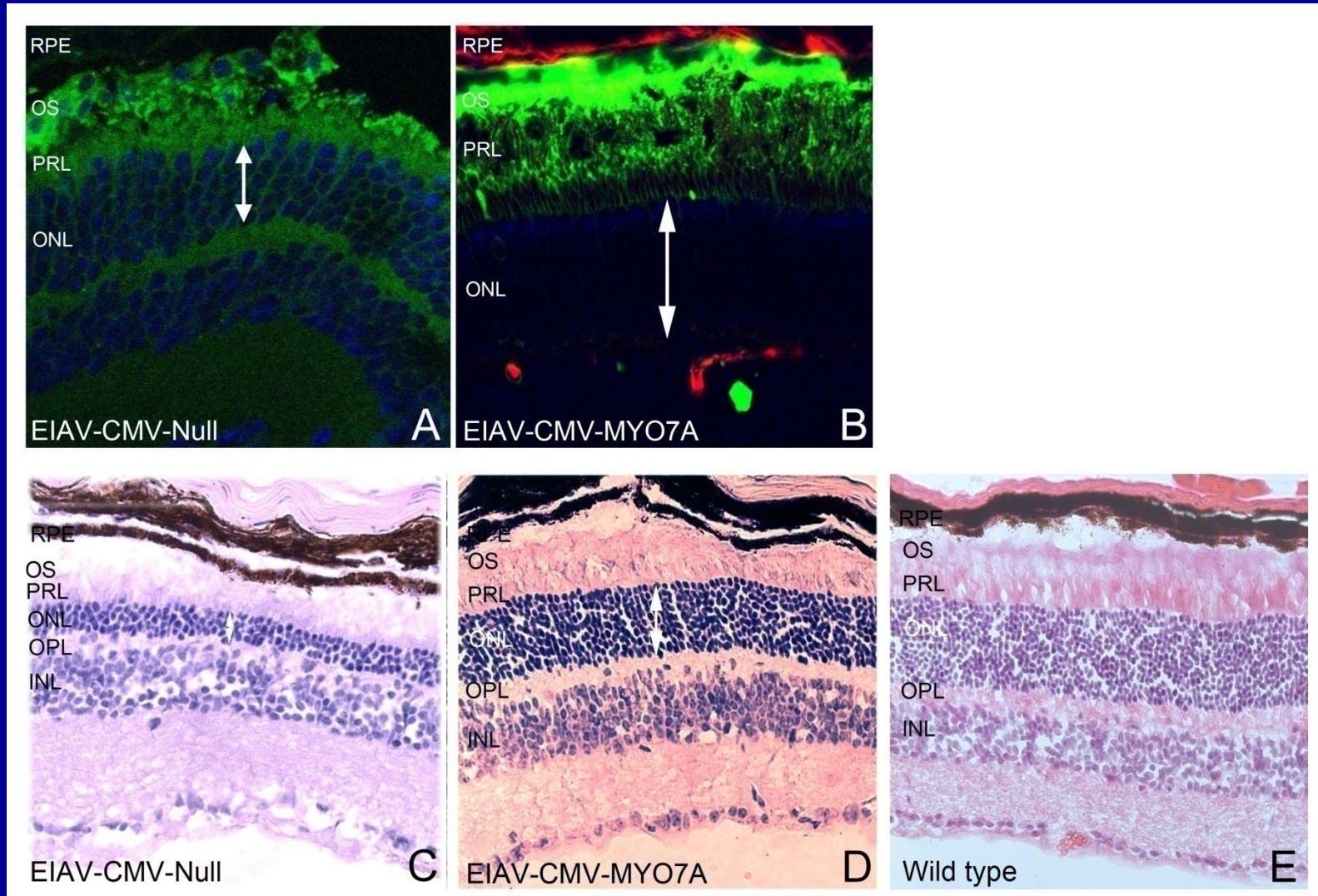


SIN LTR - Self inactivating long term repeat
Neo - Neomycin Open Reading Frame (ORF)
CMV - Cytomegalovirus promoter (constitutive)
RK- Rhodopsin Kinase promoter (photoreceptor specific)
eGFP - Green fluorescent protein ORF
MYO7A - Myosin VIIA ORF
WPRE - woodchuck hepatitis virus post-transcriptional regulatory element

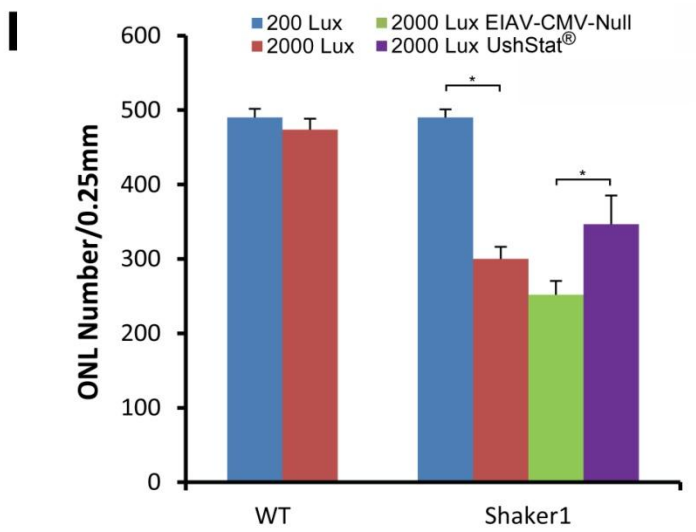


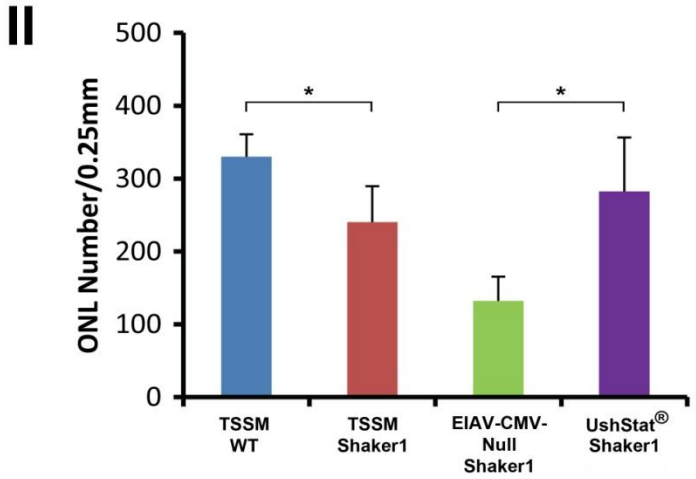
Expression of wild type myosin VIIa in *shaker1* retinas restores normal thresholds for light-induced α-transducin translocation

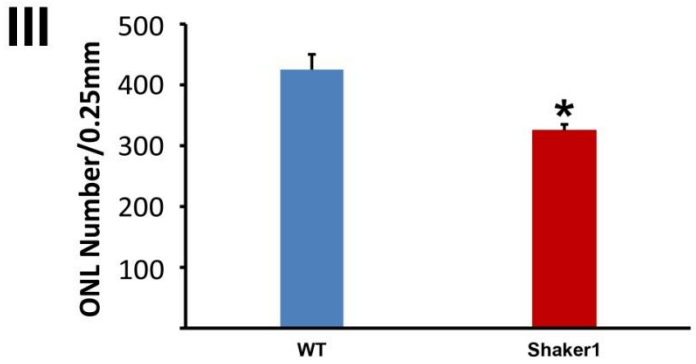
Subretinal injection of *shaker1* retinas with EIAV-CMV-MYO7A (UshStat®) rescues the light-induced photoreceptor degeneration phenotype



Quantitative analysis of EIAV-CMV-MYO7A (UshStat®)-mediated rescue of the photoreceptor degeneration phenotype in *shaker1* mice







Conclusions

- Defects in light-dependent photoreceptor phototransduction protein translocation are found in several Usher Syndrome mouse models.
- Moderate light-dependent rod degeneration was demonstrated in these mouse models, which is likely linked to the defects in protein translocation.

Acknowledgements

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- Yatish Lad
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- Peter Widdowson

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