

**Absence of Fas receptor signaling reduces photoreceptor cell death and improves retinal function in a mouse model of P23H autosomal dominant retinitis pigmentosa**

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- None related to this talk
- University of Michigan – employee, patents
- Mass Eye and Ear Infirmary - patent
- ONL Therapeutics, Inc. – equity, consultant, royalties

## Summary

- The Fas-receptor appears to be an important regulator of photoreceptor cell death in the P23H mouse model of autosomal dominant retinitis pigmentosa
- Functional knock-out of the Fas receptor, as occurs when we cross the P23H mouse with the Fas-lpr mouse, results in improved photoreceptor survival and function
- Reduced photoreceptor death associated with reduced activation of immune response in the retina
- The rescue of the photoreceptors was independent of the presence of the P23H rhodopsin – in other words, nothing was done to correct the P23H allele, meaning that the rescue of the photoreceptors was in a mutation-independent manner

# Introduction

- Autosomal dominant retinitis pigmentosa (adRP) is a common cause of inherited retinal degeneration
- The P23H variant of rhodopsin (P23H-RHO)– in which the 23<sup>rd</sup> amino acid, proline, is replaced by a histidine – is a common cause of adRP
- P23H-RHO results in rhodopsin misfolding, elevated ER stress and photoreceptor cell death
- As a dominant mutation, not easily amenable to gene therapy
- Significant unmet need for a method to prevent PR death in a mutation-independent manner

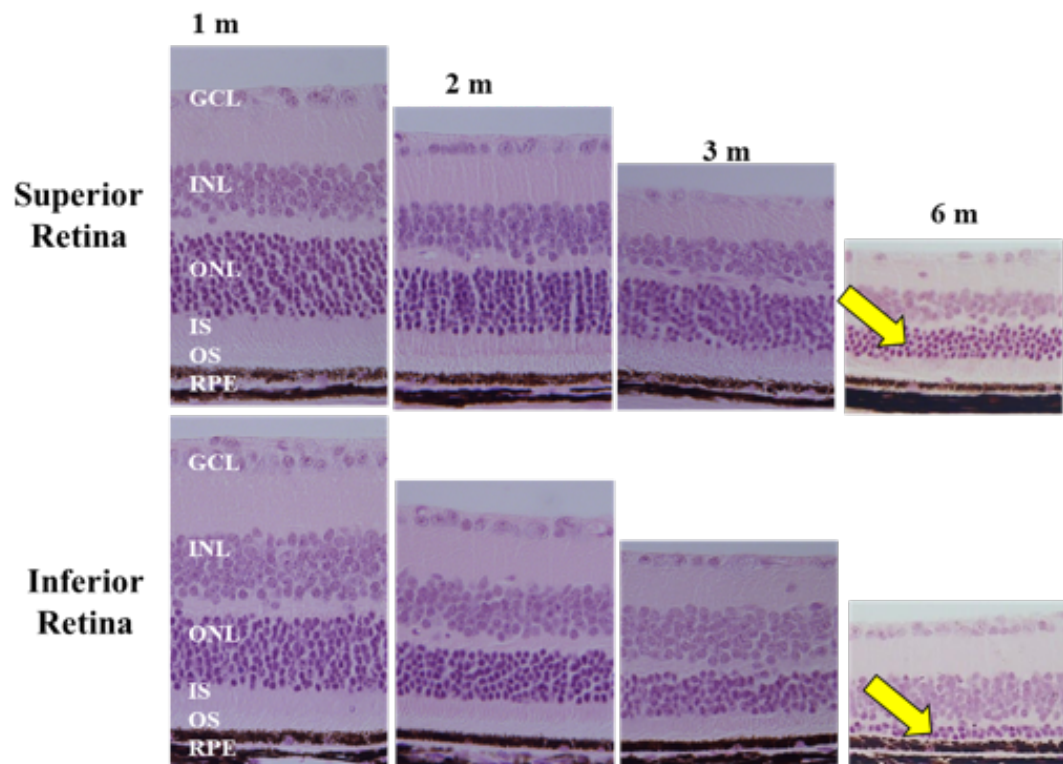
- The Fas receptor is a key regulator of cell death and associated inflammation in a number of animal models of retinal disease:
  - Retinal detachment
  - Age-related macular degeneration
  - Glaucoma
- Fas is an upstream activator of cell death, and blocking Fas in the above mentioned models results in reduced apoptosis and necroptosis
- Blocking Fas in these models also reduces activation of retinal microglia, and less production of inflammatory cytokines and chemokines

## Research Question

In this project we will test the hypothesis that defective Fas signaling will result in reduced photoreceptor cell death in the P23H mouse model of adRP

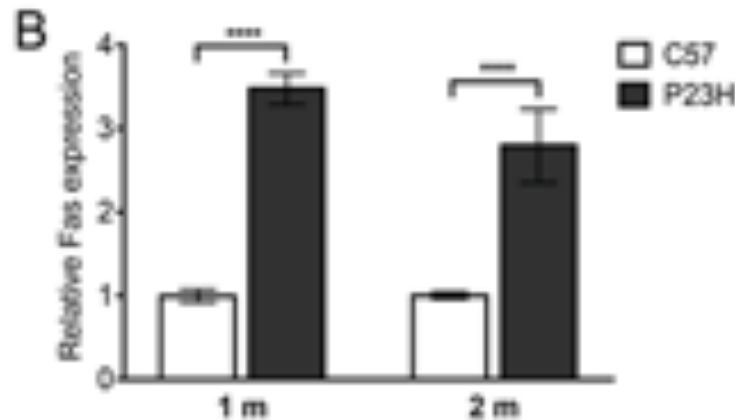
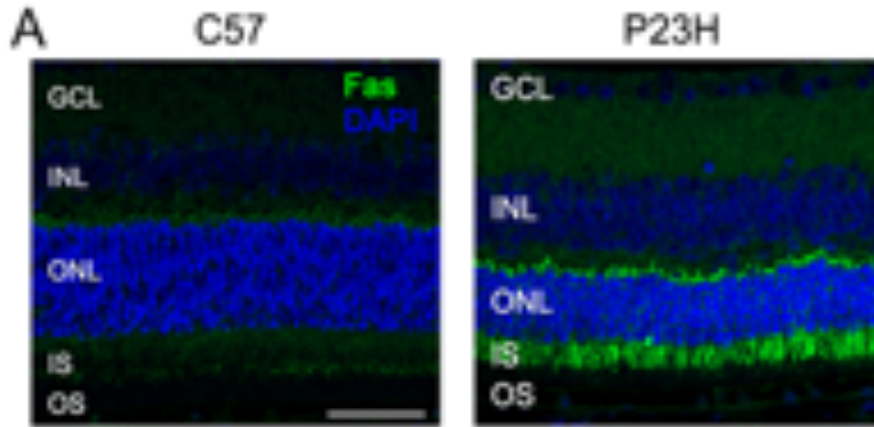
## Methods – Mouse Strains

Retinal Degeneration in the P23H Mouse



- P23H mouse model: developed by Palczewski lab (JBC. 2011;286:10551)
  - Mimics the human condition
  - Inferior retina degenerates more rapidly than the superior retina
- Fas-lpr mouse: developed by Watanabe et al (Nature. 1992;356:314)
  - contains a point mutation in the Fas gene rendering the Fas receptor inactive (a functional knock-out)

# Fas expression increased in the P23H retina

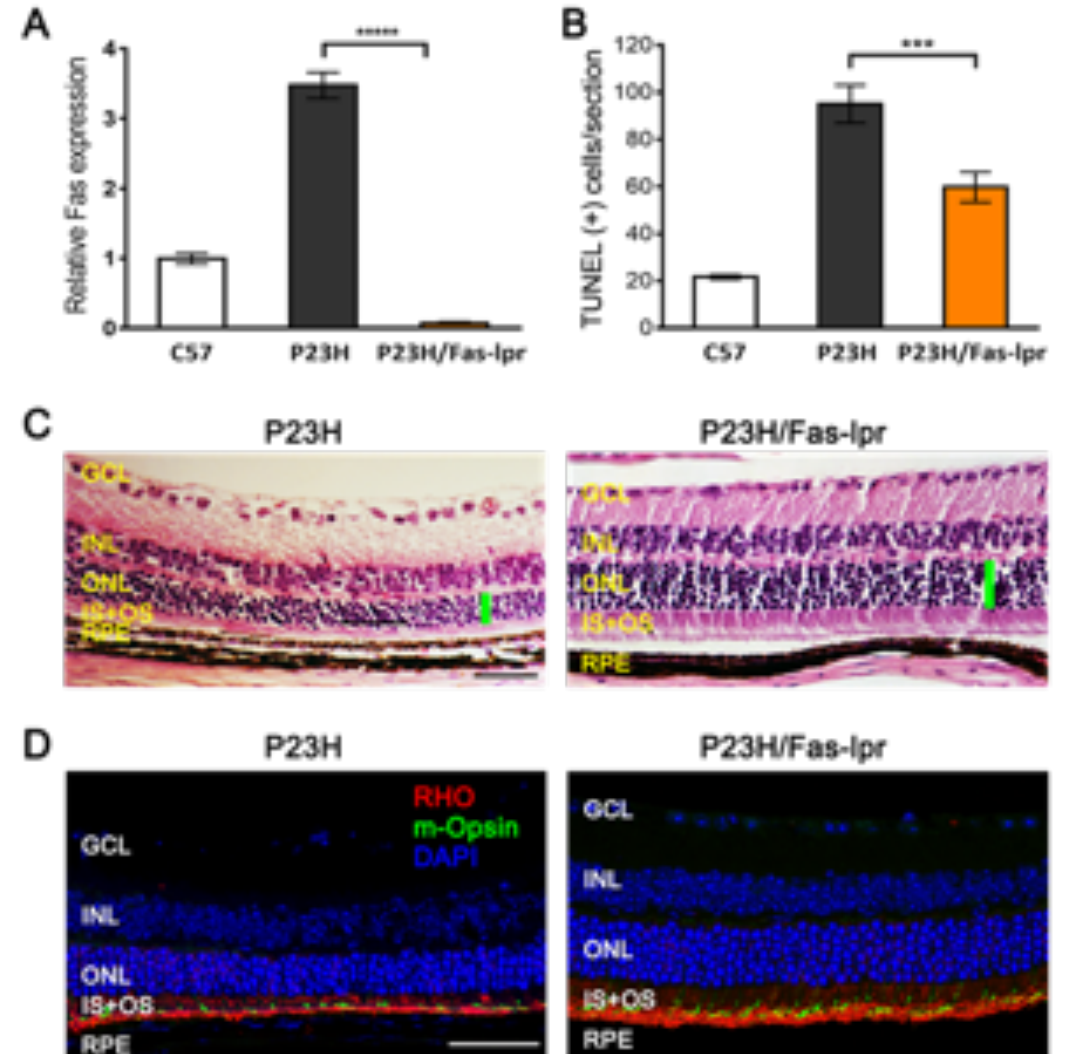


There is marked increase in the expression of the Fas receptor in the photoreceptors of the P23H mouse retina, as compared to the expression seen in the C57 (wild-type control) retina.

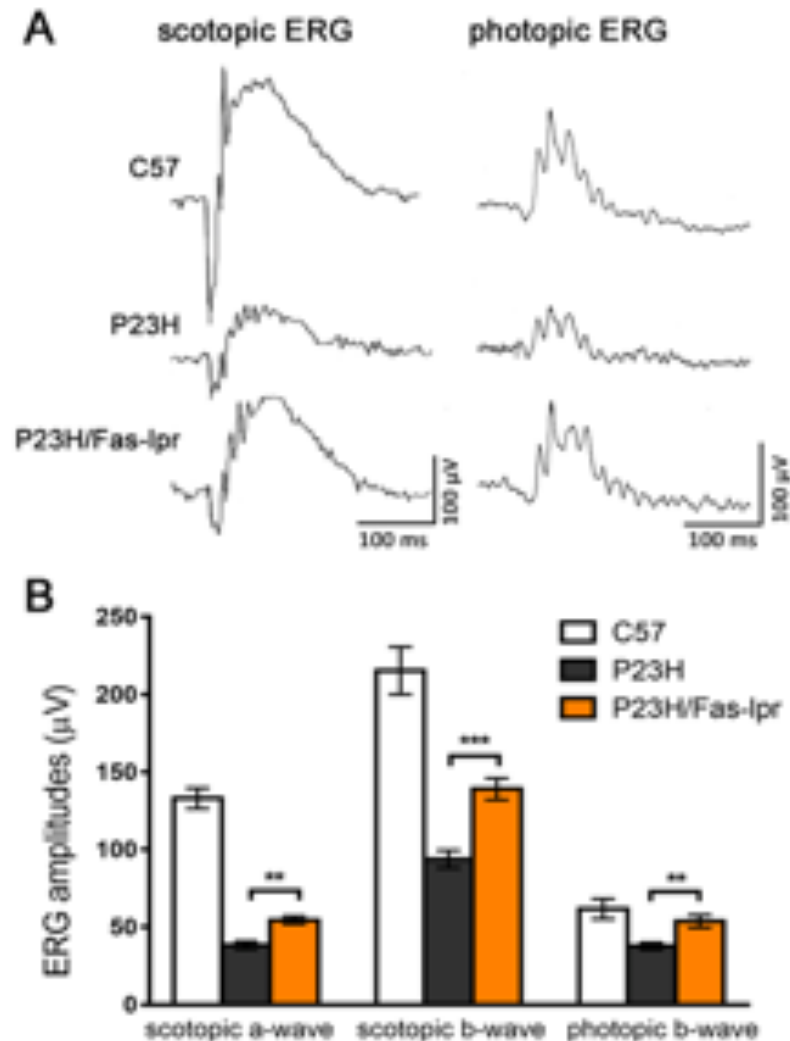


# Fas deficiency reduces retinal cell death

- Crossing the P23H mouse to the Fas-lpr mouse results in:
  - A. Less Fas expression in the retina
  - B. Less TUNEL staining of photoreceptors
  - C. Increased outer nuclear layer (ONL) thickness
  - D. Increased expression of rhodopsin and cone (m-) opsin

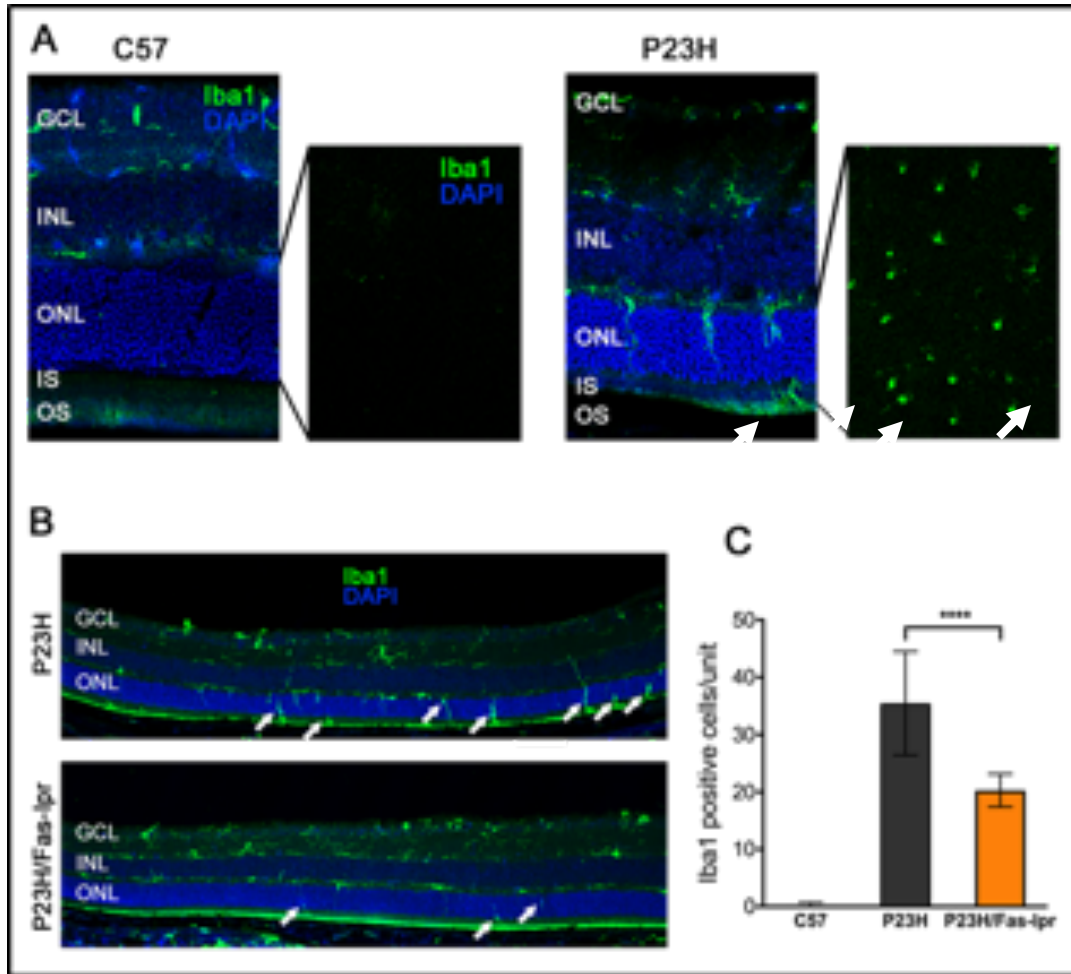


# Fas deficiency preserves retinal function

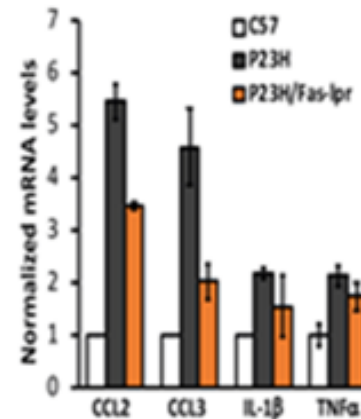


The increased number of photoreceptors seen in the P23H/Fas-lpr mouse results in improved electrical function of the retina (mice at 4 months of age).

# Fas deficiency prevents inflammatory response



Representative retina cross section and flat-mount images from C57 and P23H mice that show activation and migration of Iba1-positive cells in P23H mice at 1 month (white arrows). B) Representative images and C) quantification showing reduced migration of Iba1-positive cells to the ONL in Fas-deficient mice at 2 months (white arrows). n=4. p<0.01.



The reduction in inflammatory cells is associated with reduced expression of inflammatory markers such as CCL2, CCL3, IL-1 $\beta$ , and TNF $\alpha$ .

## Conclusions

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- Functional knock-out of the Fas receptor, as occurs when we cross the P23H mouse with the Fas-lpr mouse, results in improved photoreceptor survival and function
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- Note that the rescue of the photoreceptors was independent of the presence of the P23H rhodopsin – in other words, nothing was done to correct the P23H allele, meaning that the rescue of the photoreceptors was in a mutation-independent manner

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