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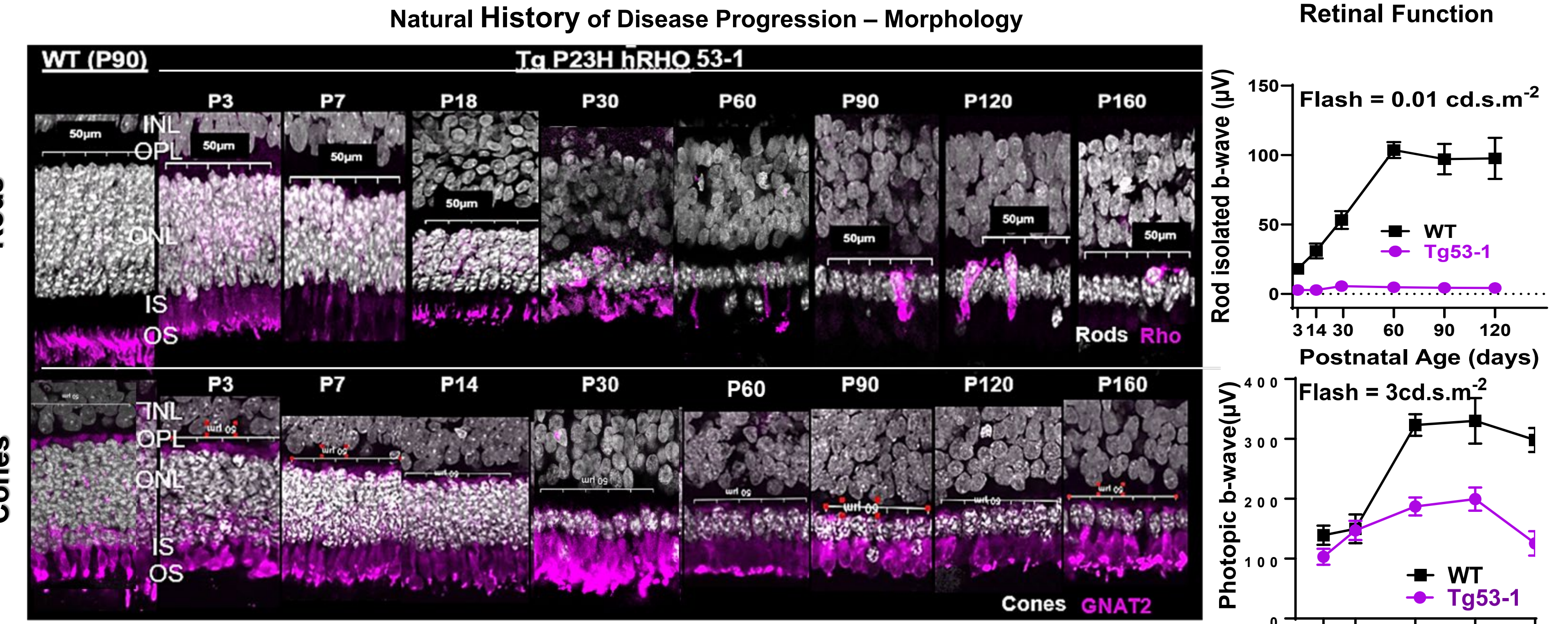


## PURPOSE

Evaluate the safety and efficacy of OPGx-RHO, a clinical-grade single vector consisting of adeno-associated virus serotype 5 (AAV5) consisting of an shRNA targeted knockdown RHO (mutant or WT) and a codon optimized human RHO replacement cDNA resistant to RNA interference in a Transgenic Proline-to-Histidine at codon 23 human rhodopsin (TgP23H hRHO) pig model of adRP.

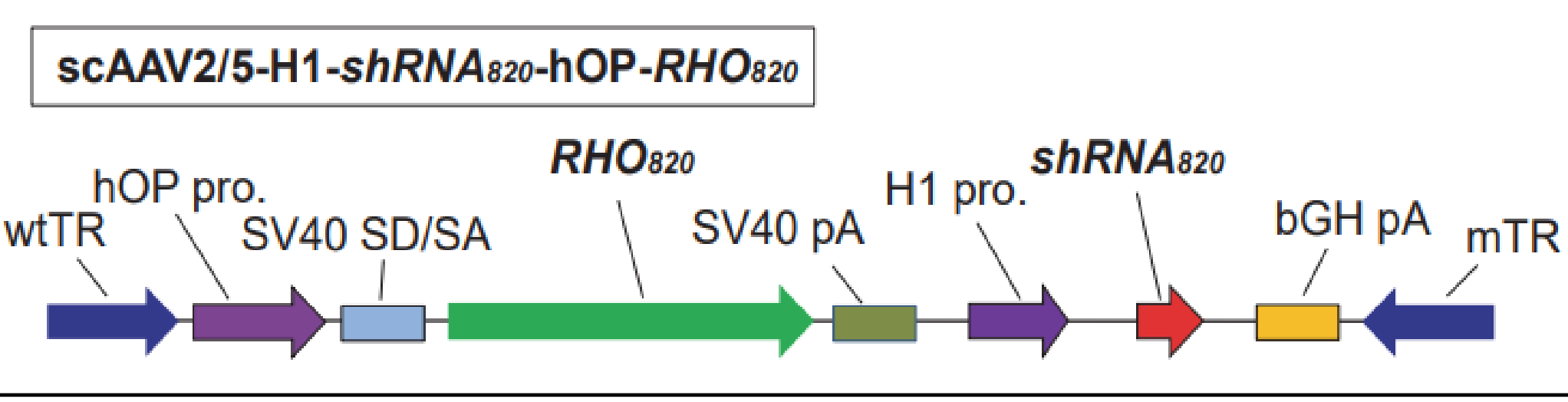
## BACKGROUND

### The Tg P23H hRHO pig model of adRP

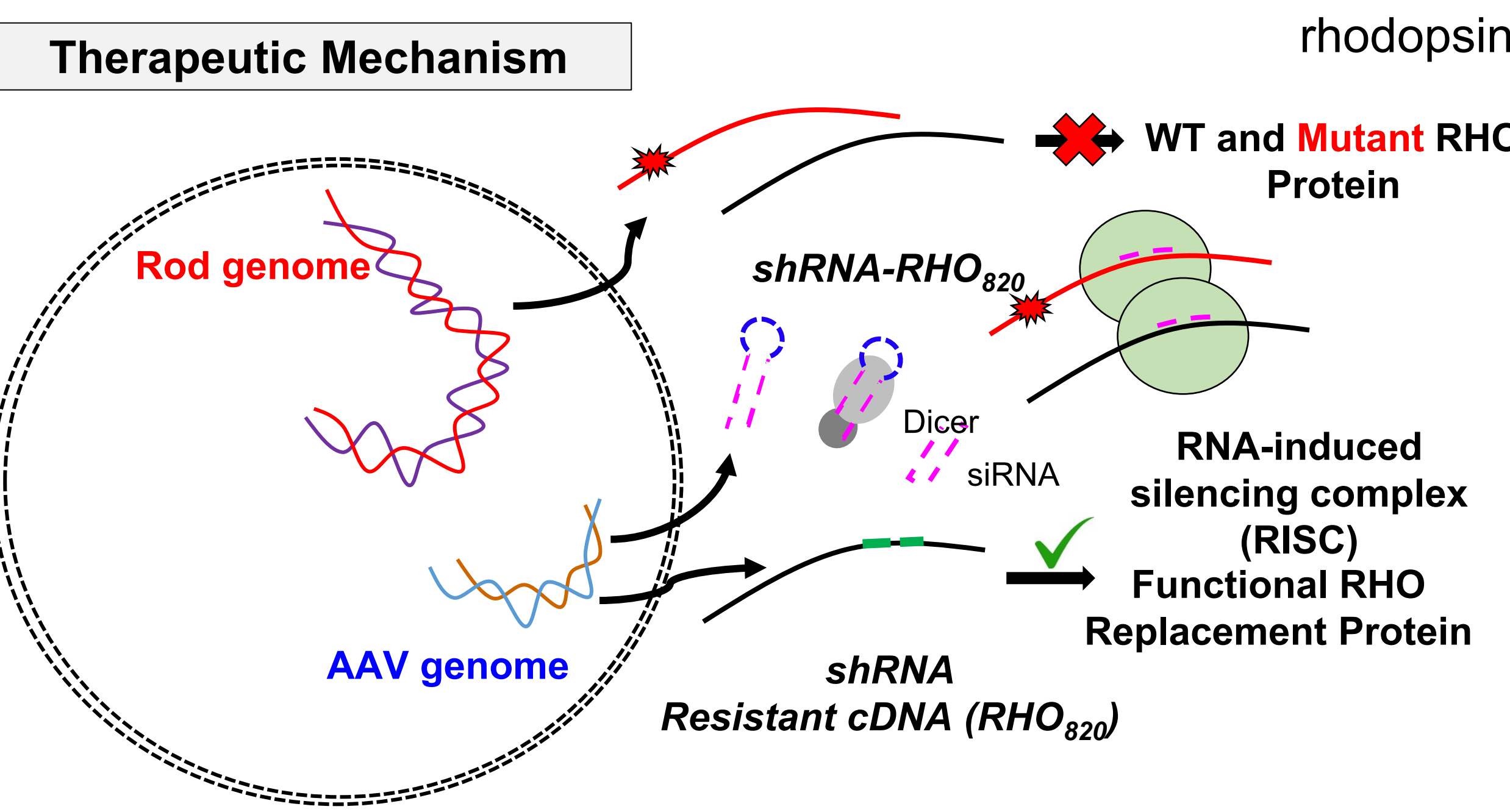


- SUMMARY of TgP23H Natural History:**
- No rod isolated ERG function from birth
  - Large numbers of rods in 1<sup>st</sup> postnatal week for therapeutic targets

### Mutation-independent knockdown and replace :OPGx-RHO

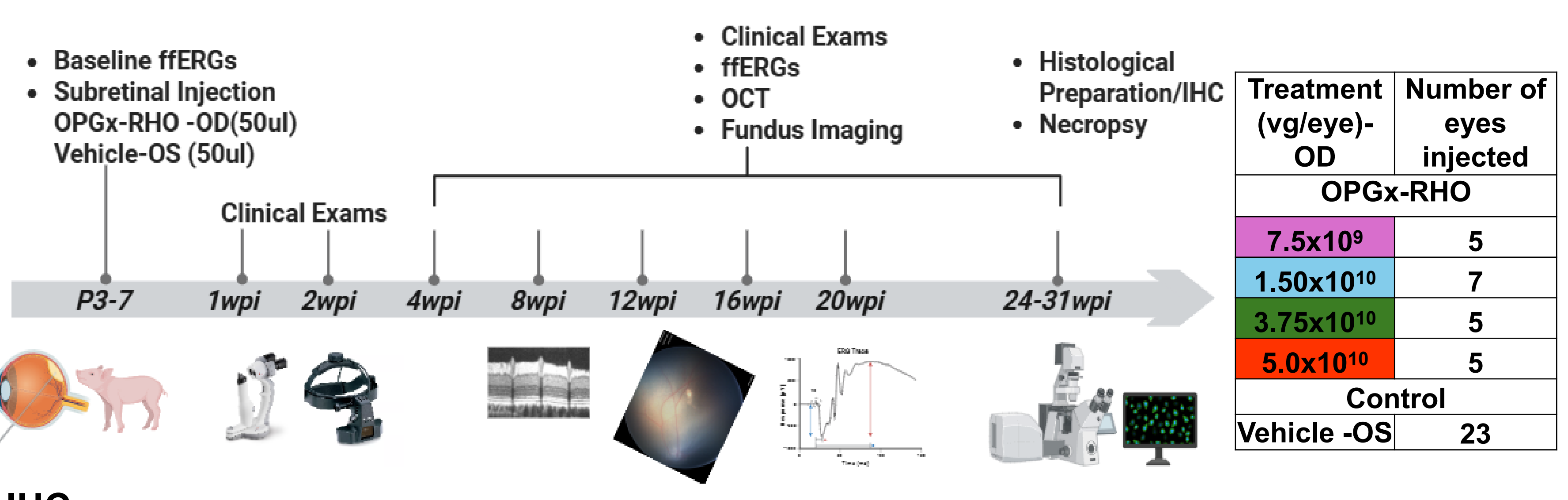


The therapeutic: a dual rAAV vector that combines a short hairpin RNA (*shRNA*<sub>820</sub>) to eliminate expression of mutant human and wildtype (WT) pig rhodopsin and an shRNA resistant RHO cassette (*RHO*<sub>820</sub>) to replace rhodopsin.



a single adeno-associated virus (AAV) vector expressed both an shRNA targeted to both mutant human RHO mRNA and WT (pig) RHO and a RHO gene containing nucleotide changes that prevented the binding of the siRNA product without changing the amino acid sequence of RHO.

## METHODS

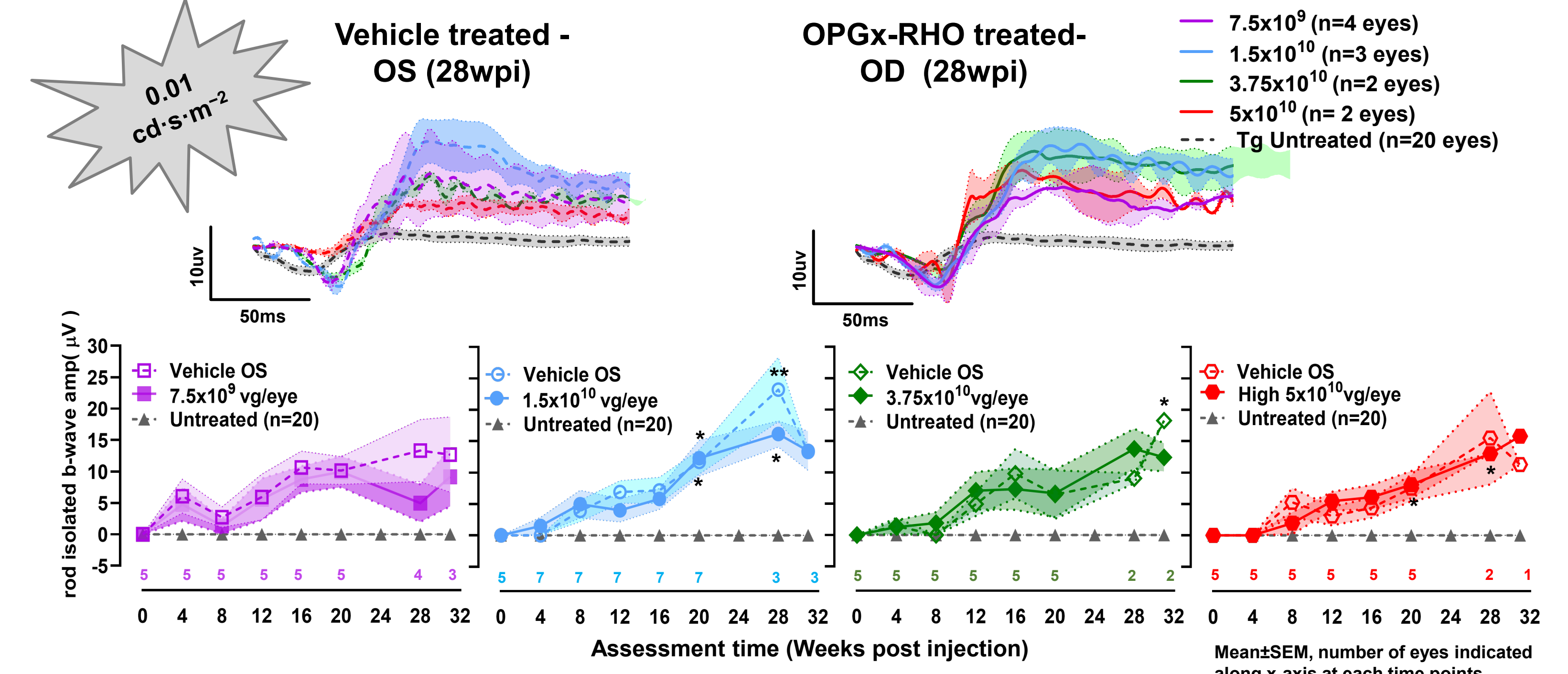


**IHC:**  
**Rod Morphology Antibodies:** Rhodopsin 1D4 (Mouse, ThermoFischer) 1:1000; REEP6 (Rabbit, Proteintech) 1:500  
**Cone Morphology Antibodies:** M-opsin (Chicken, Kerfast) 1:500; GNAT2 (Mouse, gifted by Jim Hurley) 1:500  
**Inflammation Marker:** GFAP (Goat, ThermoFischer) 1:1000

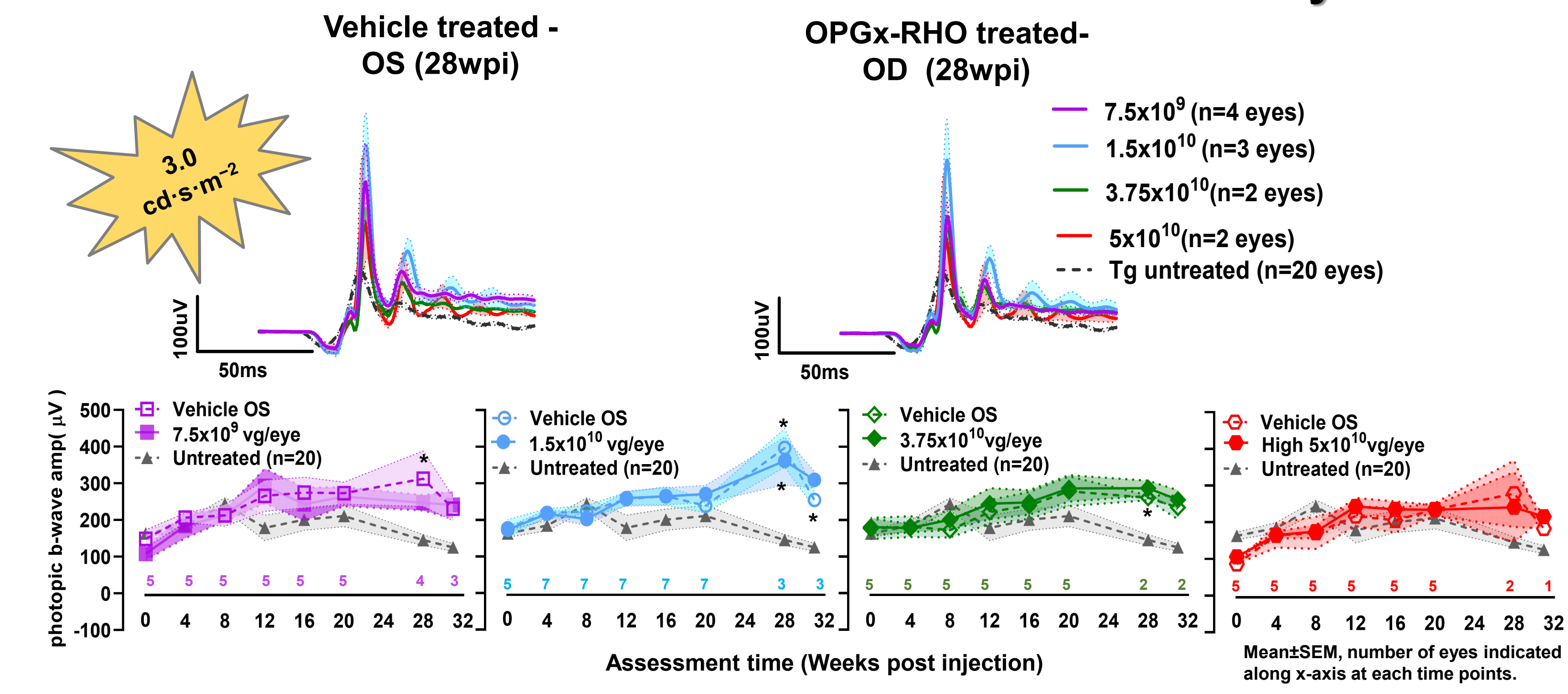
**Statistics:**  
 fERG - Kruskal Wallis Test; Dunn's multiple comparisons, q-PCR :Wilcoxon test  
 \* p<0.05; \*\* p<0.01

## RESULTS

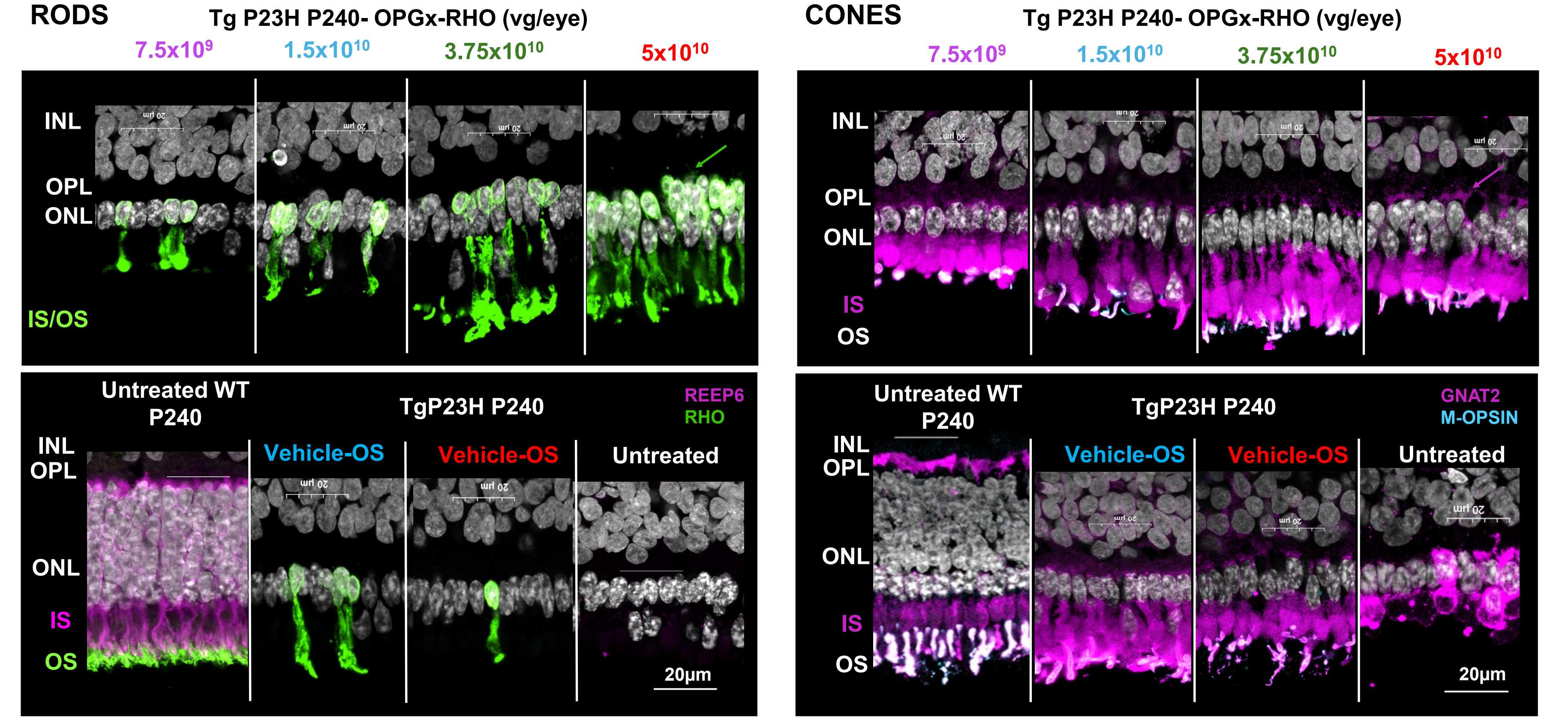
### OPGx-RHO generates a rod isolated fERG response in treated and vehicle-treated contralateral eyes.



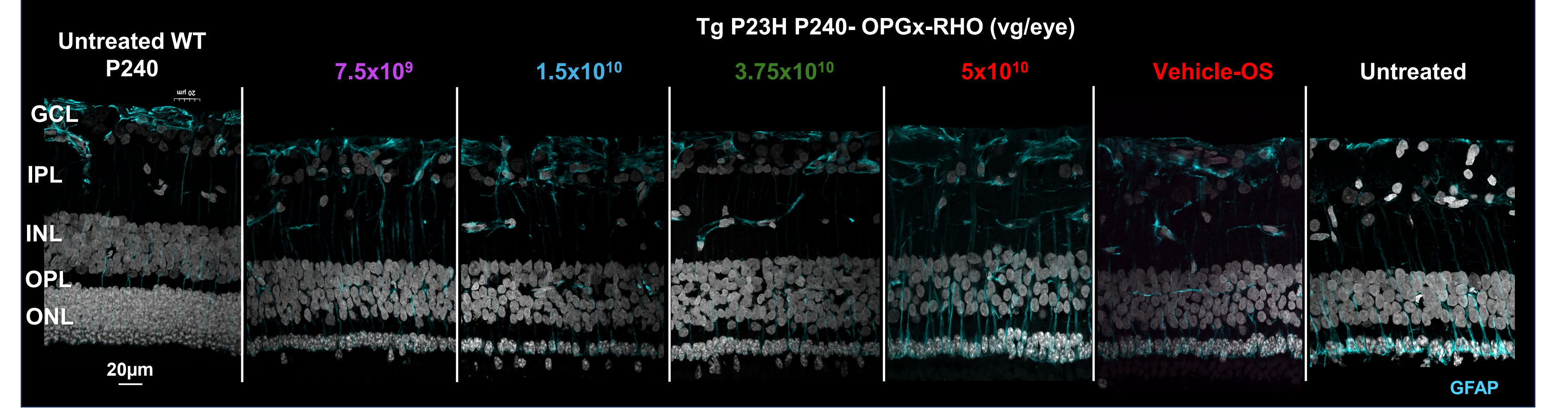
### OPGx-RHO maintains cone-mediated fERG responses in treated and vehicle-treated contralateral eyes



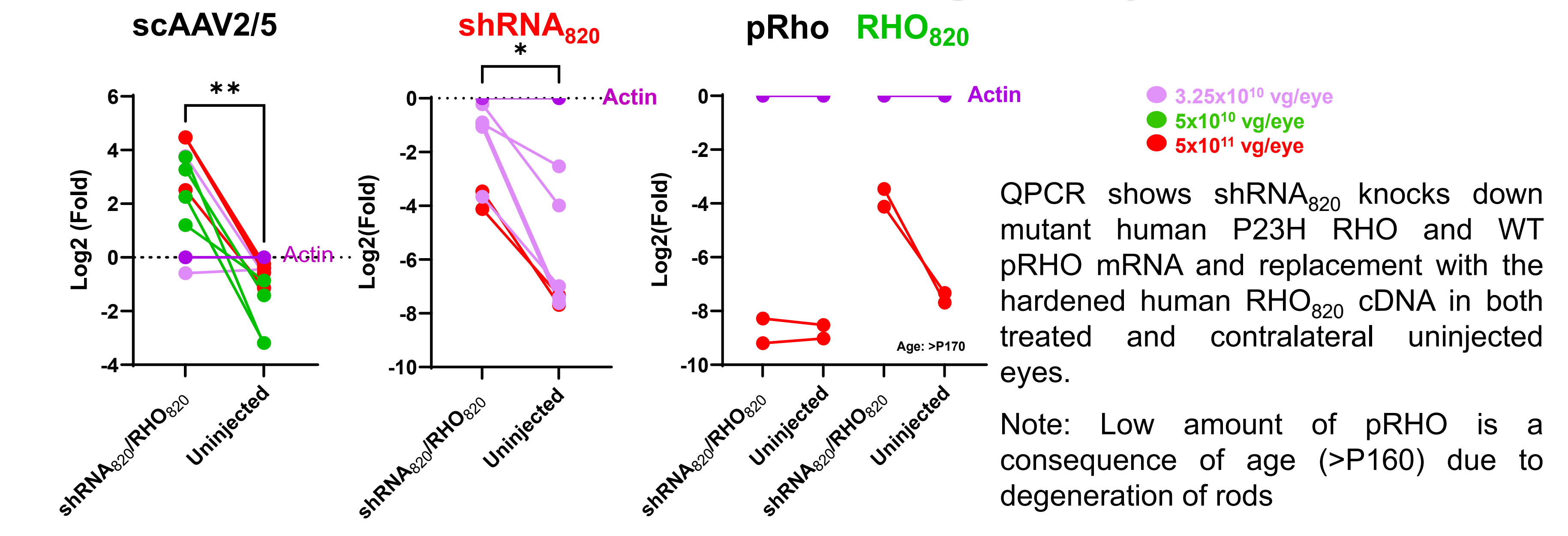
### OPGx-RHO maintains rod and cone morphology in treated and contralateral eyes compared untreated age-matched TgP23H retinas



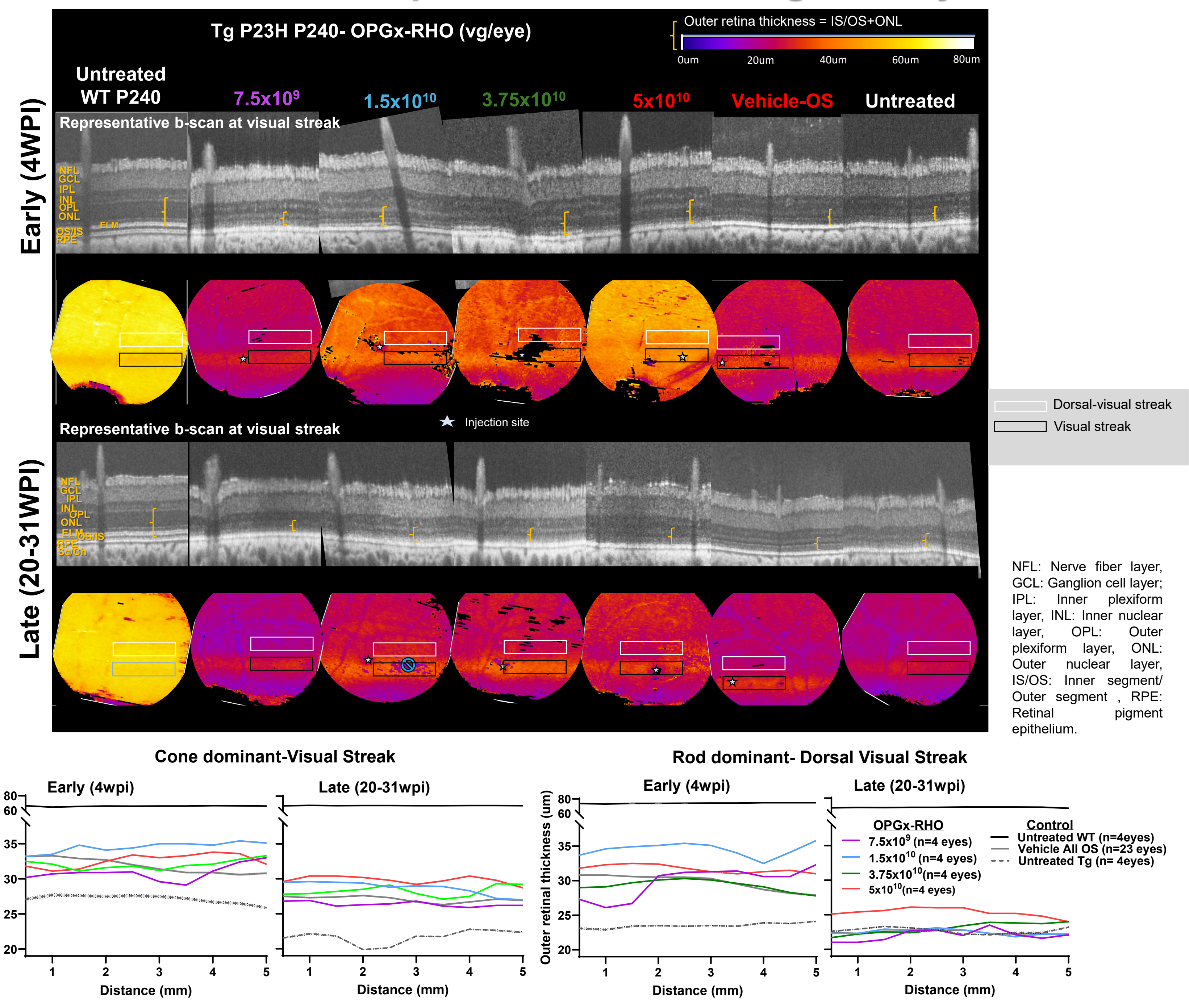
### 5x10<sup>10</sup> vg/eye OPGx-RHO induced inflammation in (2/5 eyes)-enhanced GFAP expression



### Research grade shRNA<sub>820</sub>/RHO<sub>820</sub> is transferred from treated to untreated, contralateral TgP23H eyes



### OPGx-RHO maintains laminar outer retinal integrity and thickness compared to untreated TgP23H eyes



Limited outer retinal thinning between early and late timepoints suggests cone maintenance  
 Only 5x10<sup>10</sup> vg/eye OPGx-RHO maintains outer retinal thickness suggests rod rescue.

## CONCLUSIONS

- In Vivo OPGx-RHO:**
- Induces rod-isolated responses
  - Maintains cone responses
  - Maintains laminar structure
  - Maintains rod and cone morphology
  - The minimal effective dose of OPGx-RHO is 1.5x10<sup>10</sup> vg/eye.
  - In 2/5 eyes treated with 5x10<sup>10</sup> vg/eye OPGx-RHO showed increased GFAP expression, suggesting an immune response.
  - These effects are also observed in the contralateral vehicle-treated eyes suggesting interocular transfer of OPGx-RHO, which was observed and published previously (Chulbul et al 2023, Vision Research).

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