

Introduction

- Glaucoma is the leading cause of irreversible blindness worldwide and affects over 1% of the world's population^[1, 2].
- Unlike humans, zebrafish can regenerate all retinal cells following injury from multipotent progenitor cells derived from Müller glia^[3].
- Zebrafish are a powerful model for uncovering mechanisms that may be leveraged to therapeutically induce endogenous neurogenesis from human Müller glia to treat neurodegenerative disease^[4].
- Despite extensive research, there is yet to be a well-characterized, retinal ganglion cell (RGC) specific injury model in zebrafish to study the Müller glia regenerative response to specifically RGC loss^[5]. This limits the field to make therapeutic leaps for diseases where RGCs are lost, such as glaucoma.
- Furthermore, it is also unknown the regenerative capacity of a single Müller glia.

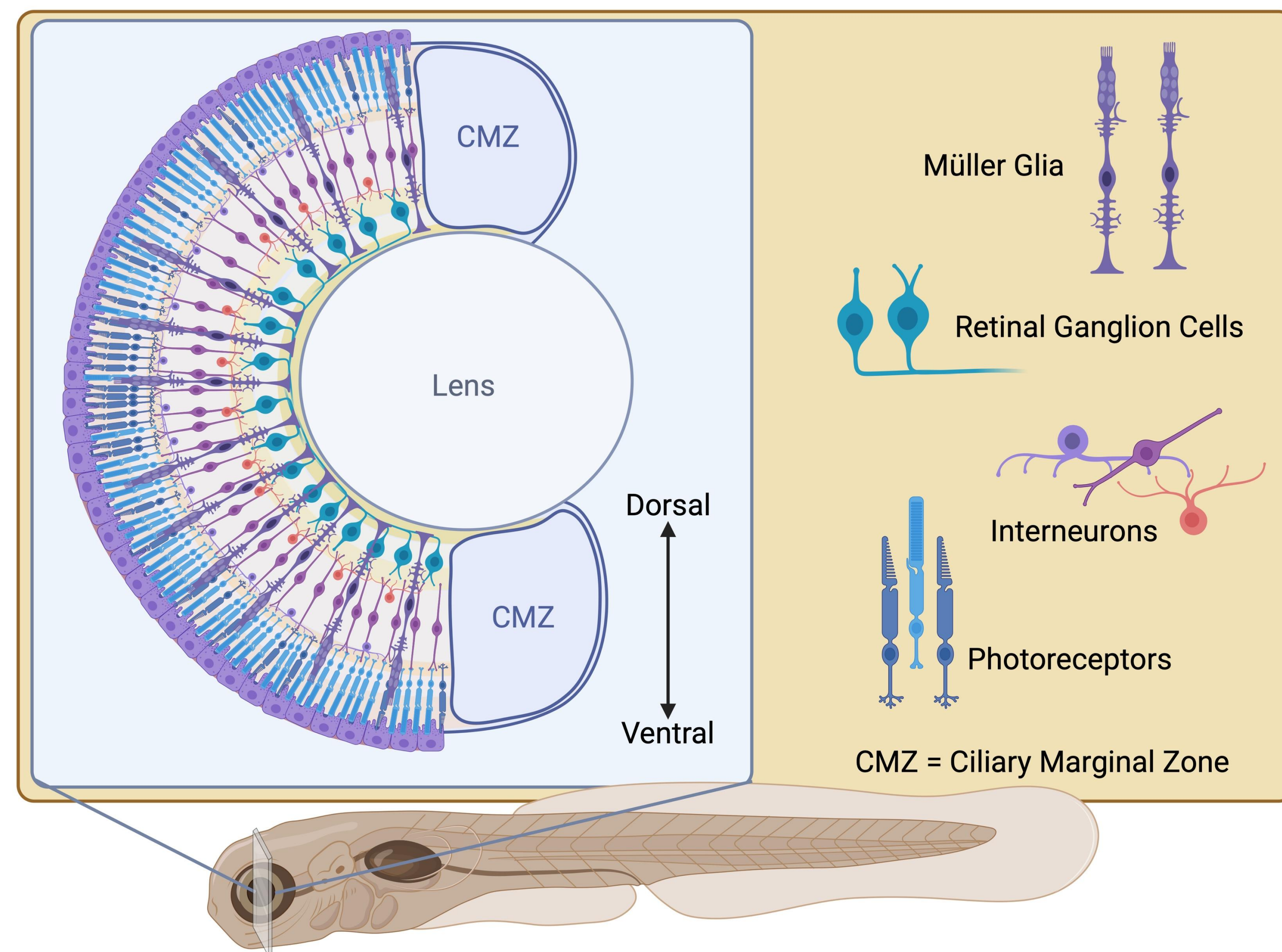


Figure 1. Zebrafish Müller glial-mediated retinal regeneration following retinal disease.

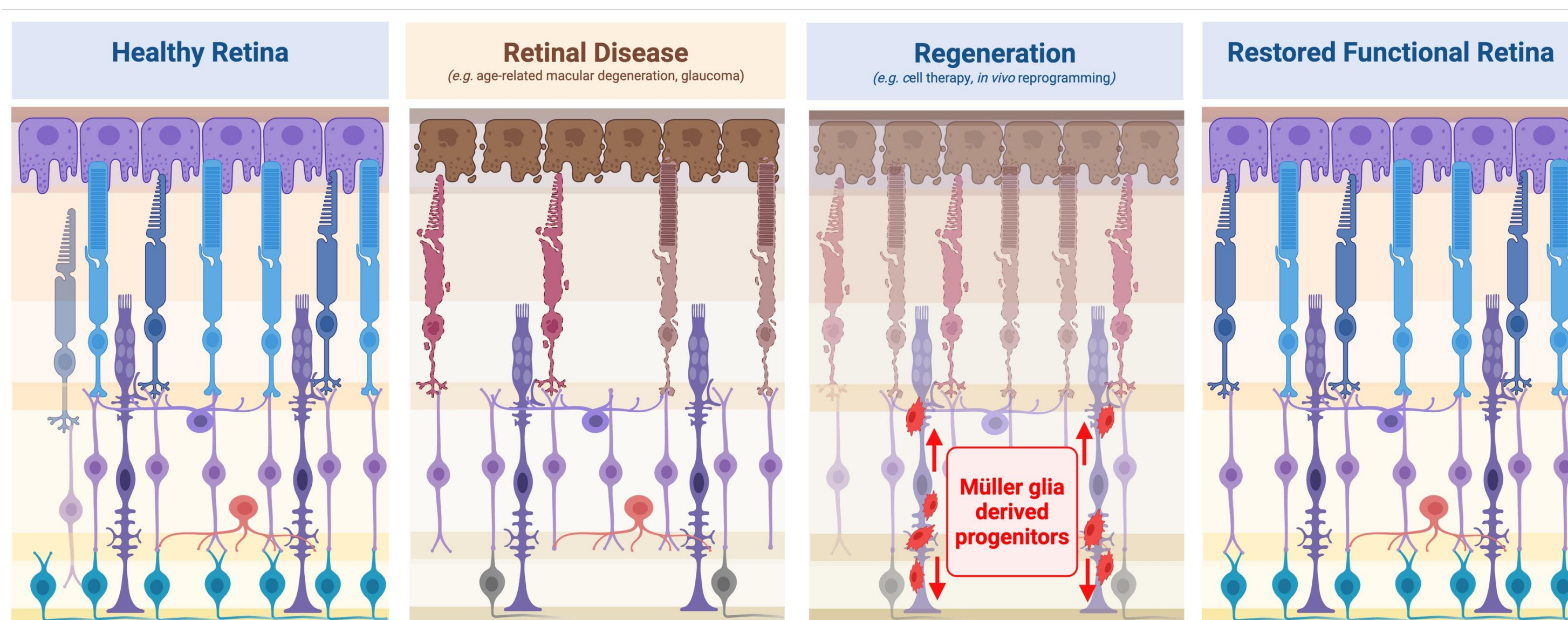
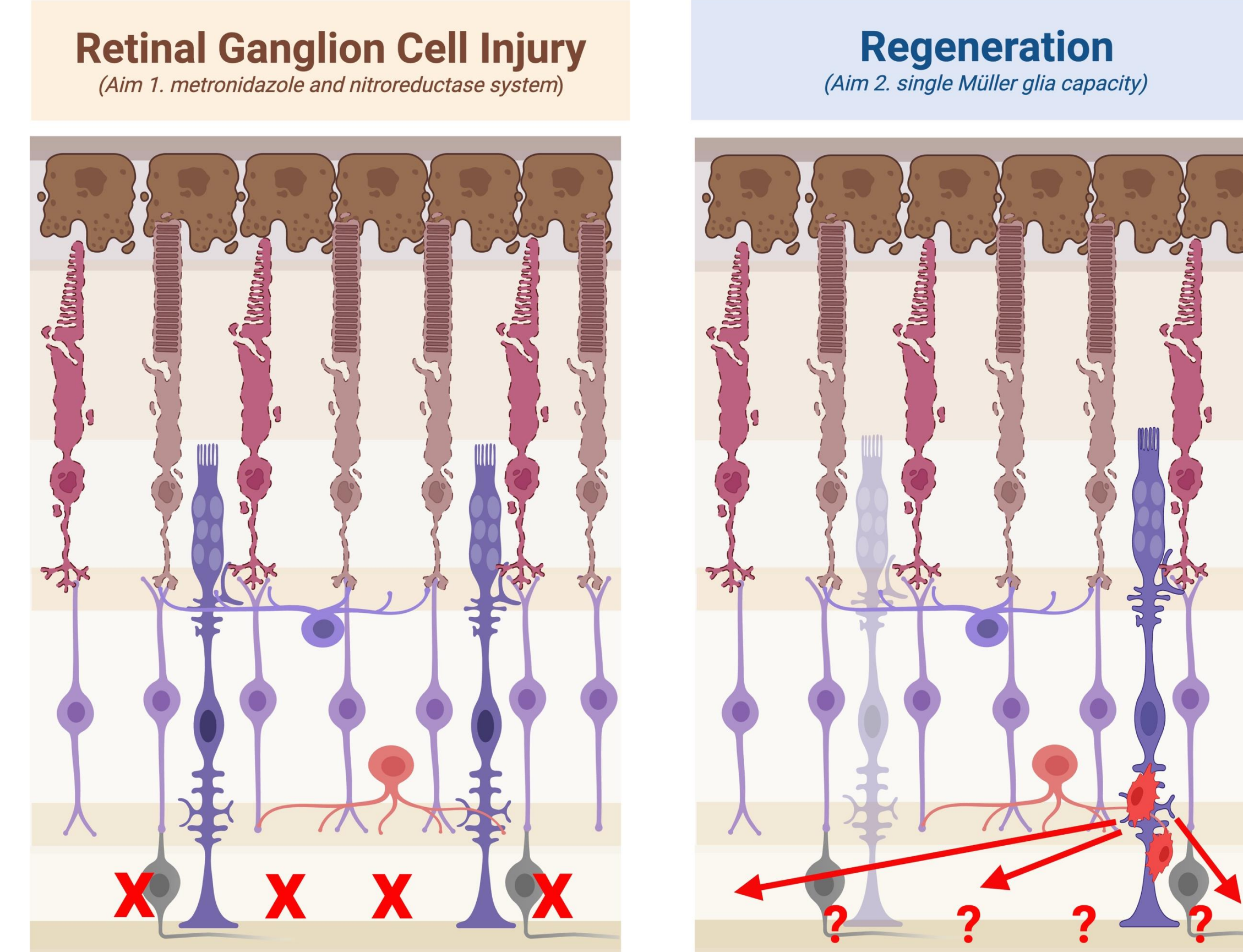


Figure 2. Zebrafish Müller glial-mediated retinal regeneration following retinal disease.

HYPOTHESIS

I hypothesize that following RGC-specific injury in zebrafish, Müller glia-derived progenitors will preferentially regenerate RGC during the regenerative response.

AIMS



- Aim 1. To characterize a recently generated RGC-specific injury model.
- Aim 2. To characterize regenerative response of a single Müller glia after RGC-specific injury.

Methodology

Aim 1

- Transgenically use NTR/MTZ system in retinal ganglion cells, Tg(*isl2b*:NTR-CFP), to cause RGC-specific injury.
- isl2b*, a gene transcribed in 60% of all RGC types, drives the expression of the bacterial enzyme nitroreductase (NTR).
- NTR converts the harmless prodrug metronidazole (MTZ) into cytotoxic metabolites that cause DNA cross-linking and cell death in a temporally dependent manner.

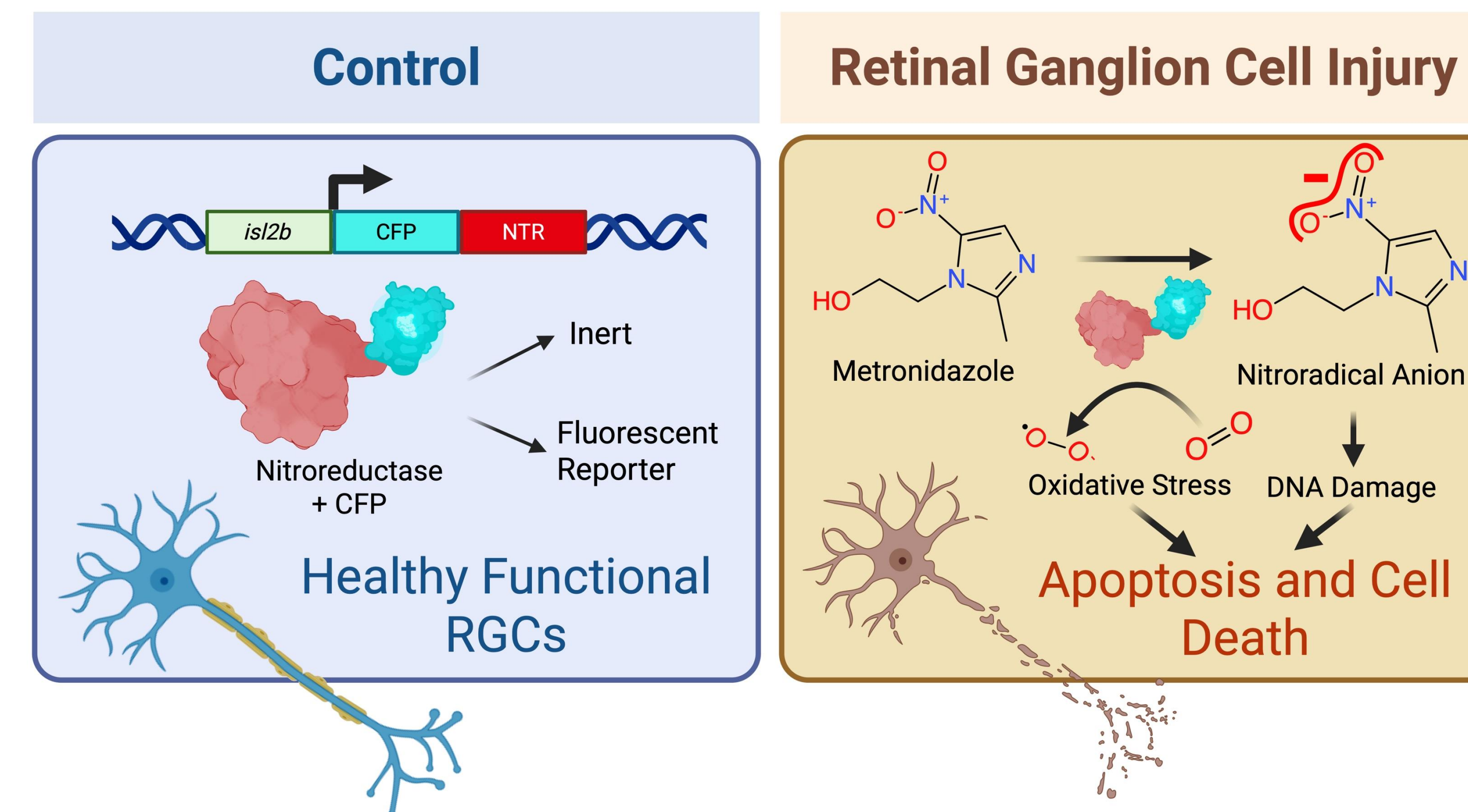


Figure 3. RGC-specific injury model using nitroreductase and metronidazole.

Aim 2

- Transgenically use photoconvertible fluorophore Kaede in Müller glia to track progenitors, Tg(*her12*:kaede, *isl2b*:NTR-CFP).
- her12* is a gene transcribed in active Müller glia responding to the RGC-specific injury.
- Kaede is a fluorophore that when translated is green and can be permanently converted to red when exposed to ultraviolet light. I will use a multiphoton microscope to convert individual Müller glia from green to red and track their red progenitors.

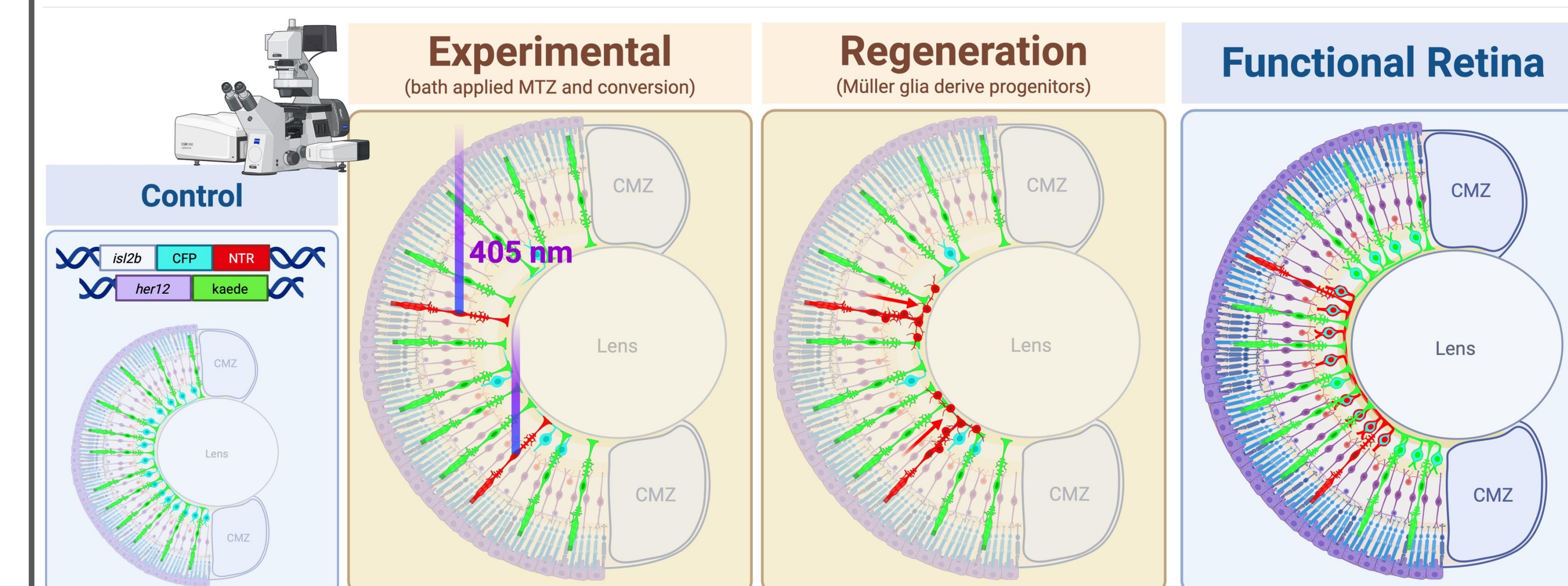


Figure 4. Conversion of Kaede within individual Müller glia following RGC-specific injury to track individual Müller glia-derived progenitors to quantify regenerative capacity.

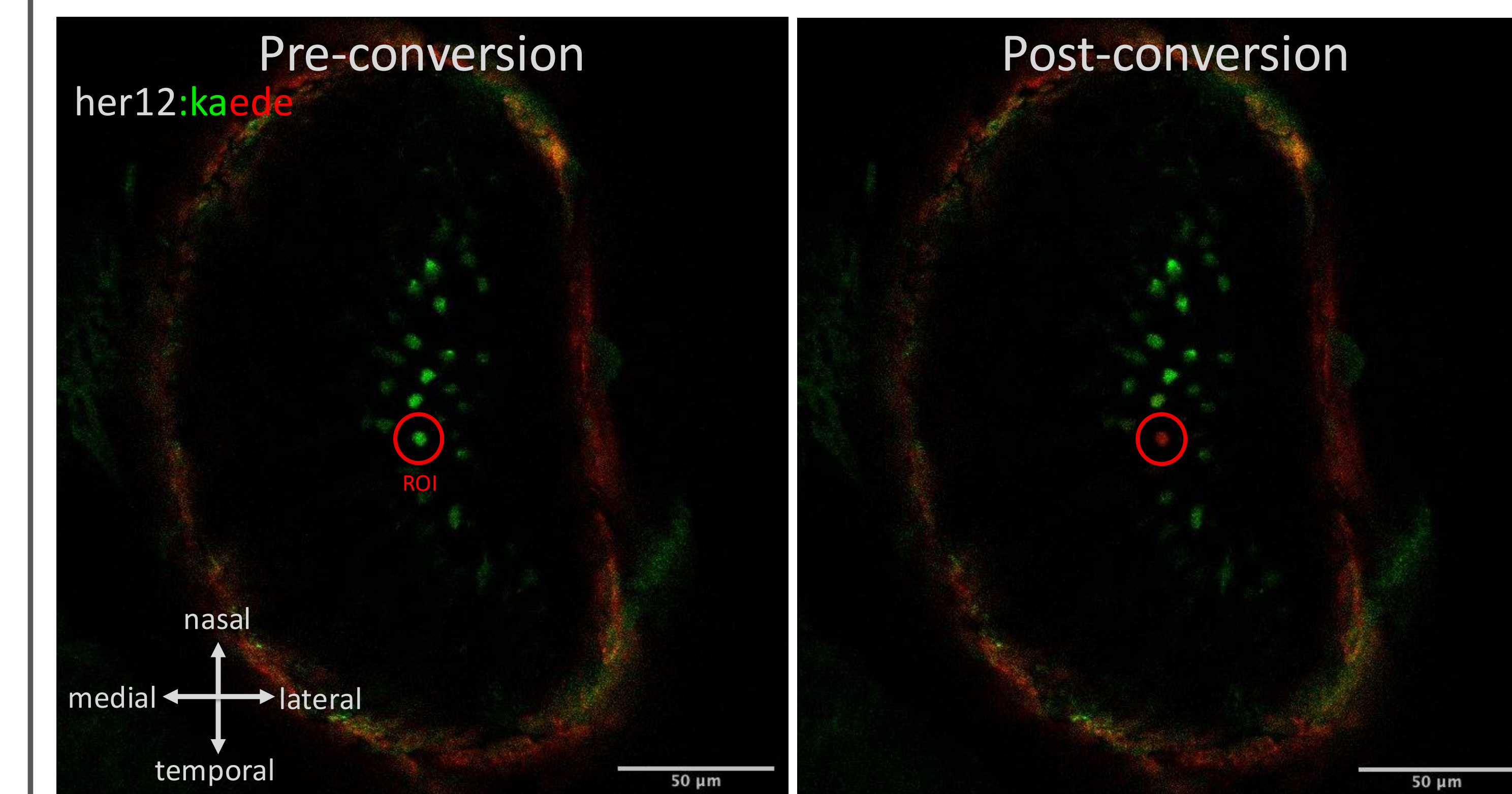


Figure 5. Kaede before and after conversion within an individual peripheral Müller glia in a Tg(*her12*:kaede) fish.

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