The Effect of α-MSH on the Recovery of Ischemia/Reperfusion Retinal Muller Cells

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Introduction

- About 9.6 million people in the U.S. suffer from diabetic retinopathy
- Ischemia/Reperfusion injury in mice is a good model to study diabetic retinopathy.

Previous studies have found that the use of the neuropeptide α-Melanocyte-Stimulating Hormone(α-MSH) following I/R in mouse with diabetes I protects the retina from further damage[1]

Goal:
Investigate the effect of α-MSH on the recovery of retinal cells following Ischemia/Reperfusion (I/R injury).

Hypothesis:
α-MSH will improve recovery rates and decrease retinal damage following I/R.

Methods

Experiment:
To induce I/R in mice, intra-ocular pressure (IOP) of the mouse is raised to 90mmHg by cannulating the anterior chamber for 60 min using Phosphate Buffered Saline (PBS).
- α-MSH (or PBS) injection happens on day 1 and 5.
- 4 conditions
  - Untreated (Elevated IOP)
  - α-MSH with elevated IOP
  - Sham (similar procedure without elevated IOP)
  - Healthy (no injection)

The eyes were collected on day 7. Histology sectioning was done, and the sections of these samples are then immunostained and imaged.

Figure 1: Experimental procedure. PBS is injected into the anterior chamber at 90mmHg for 60 min.
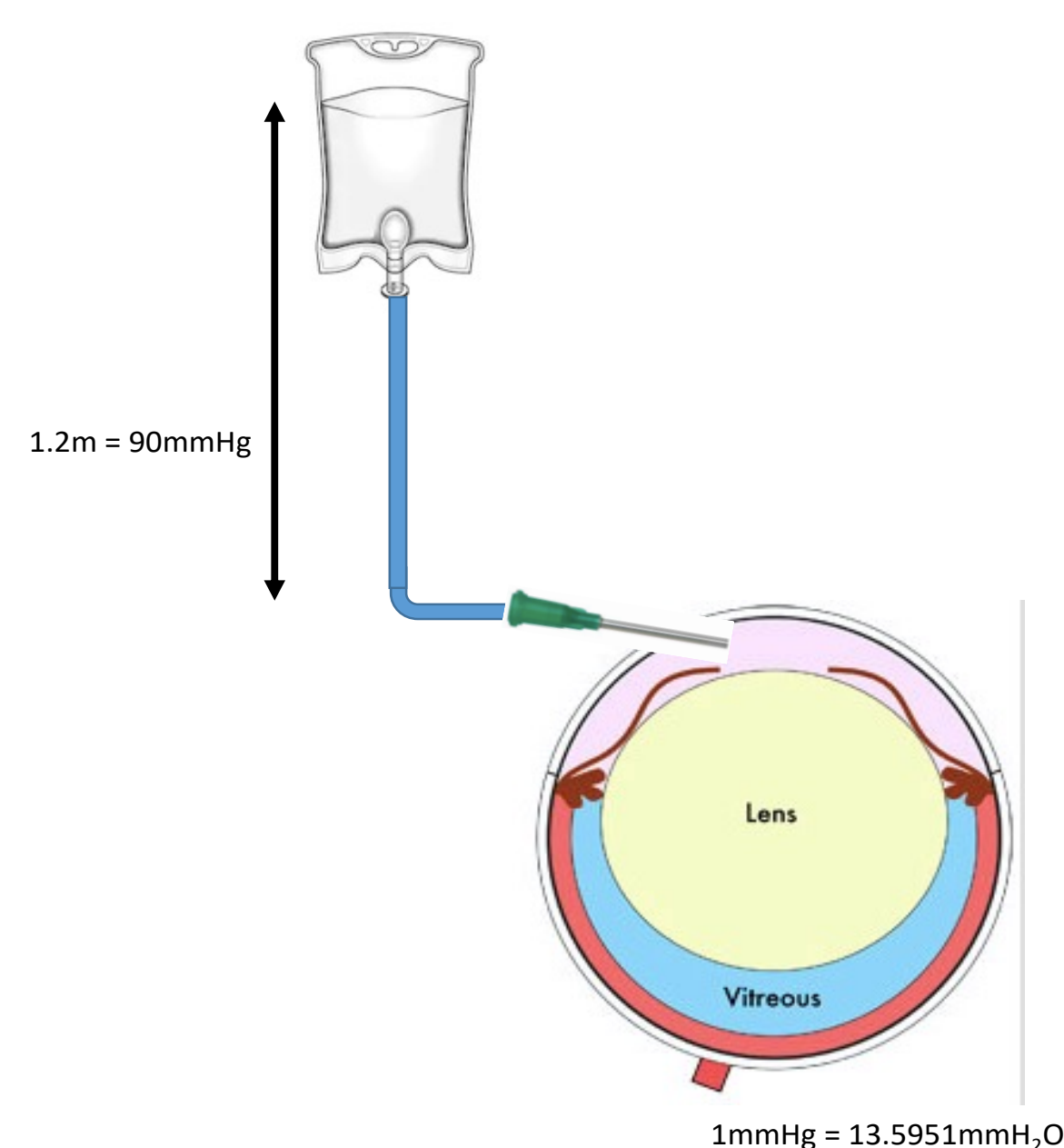
Immunostaining:
To evaluate damages on retinal muller cells, the expression of glial fibrillary acidic protein(GFAP) will be measured. Increase in GFAP expression by Muller Cells is proportional to the extent of retinal degeneration[2].
- Muller cells are stained with rabbit anti-glutamine synthetase IgG
- GFAP stained with mouse anti-GFAP antibody
- Cell nucleus is stained with DAPI

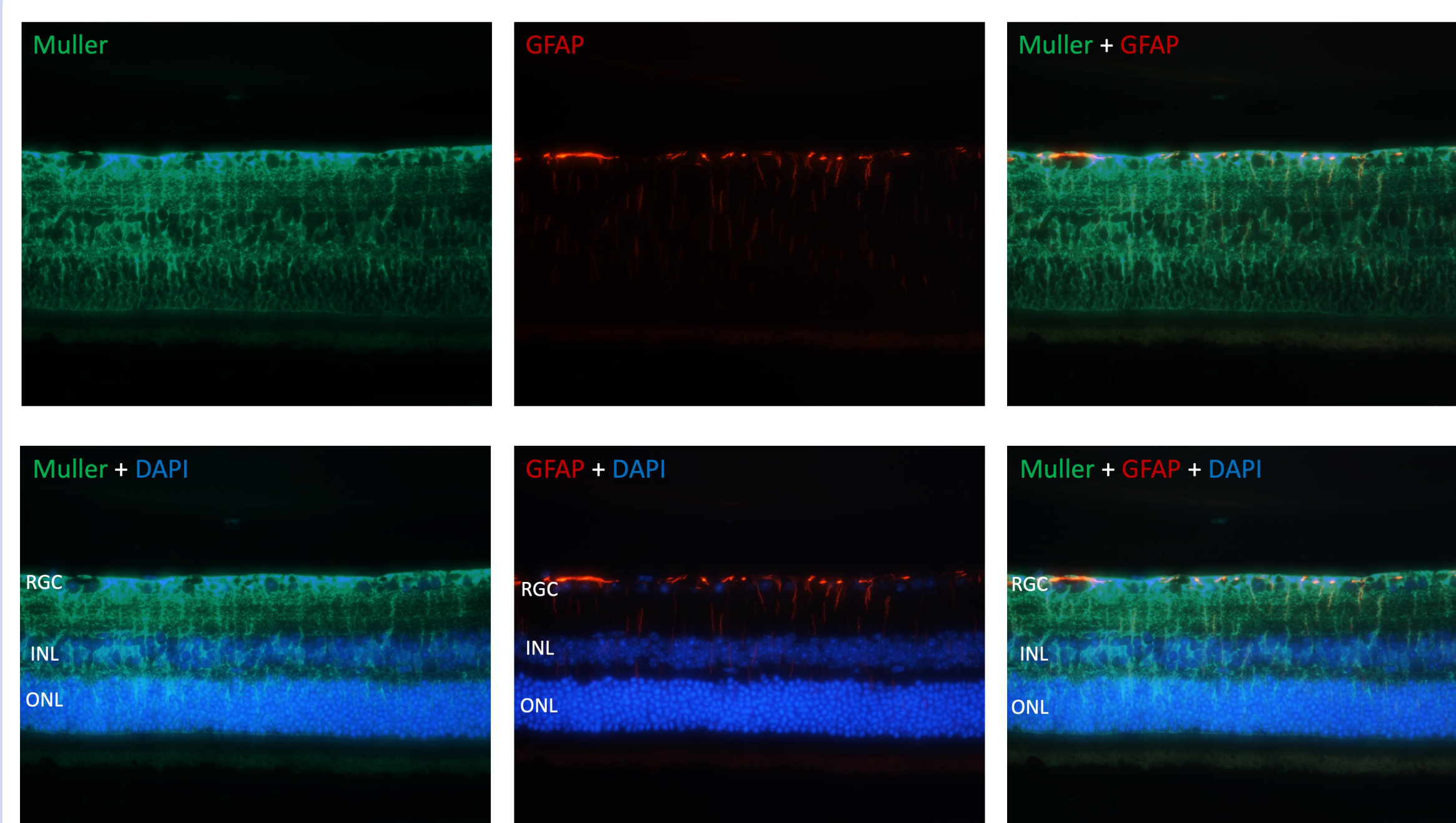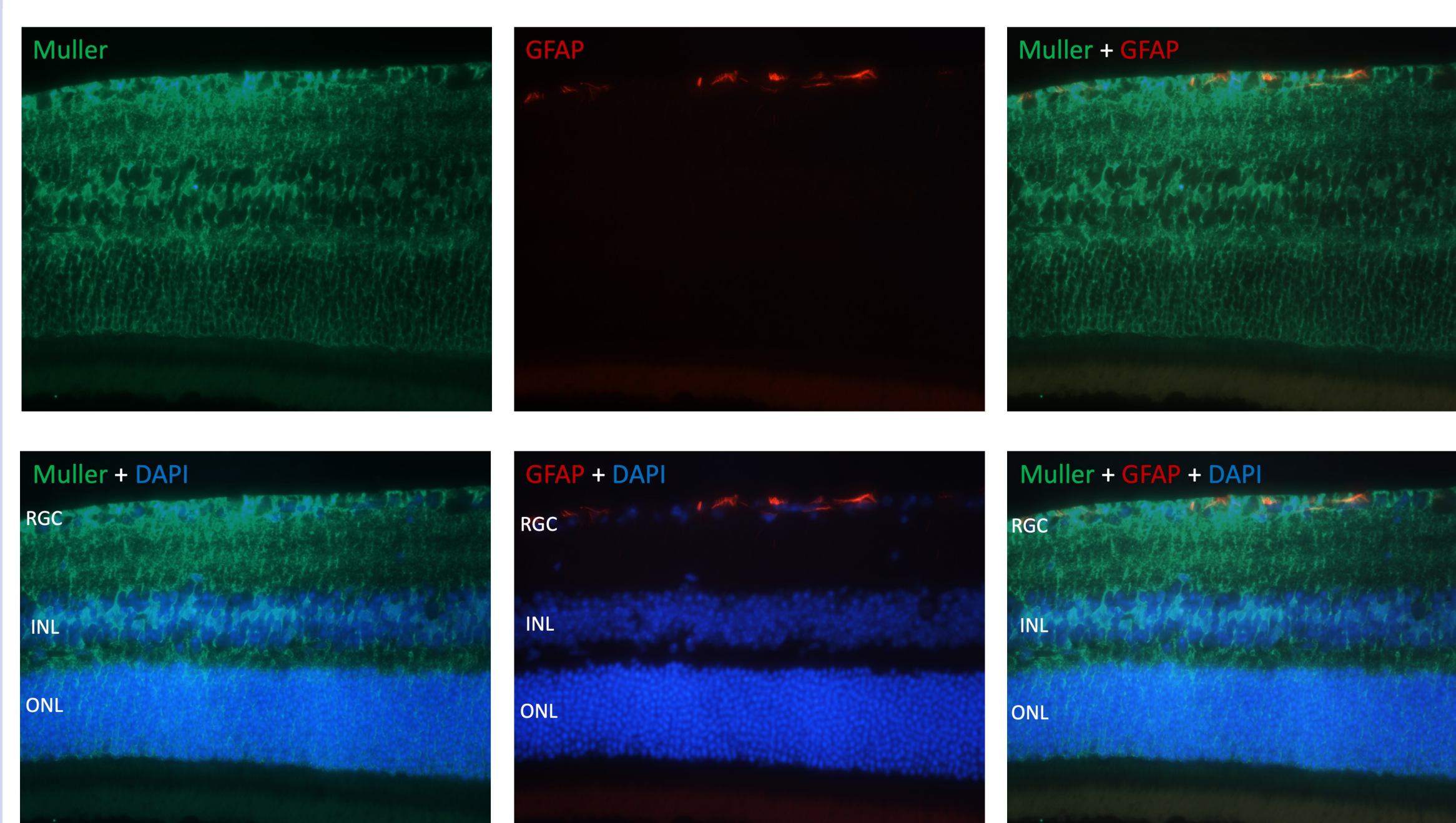The percentage of Muller cells expressing GFAP was measured by cell counting.

Results
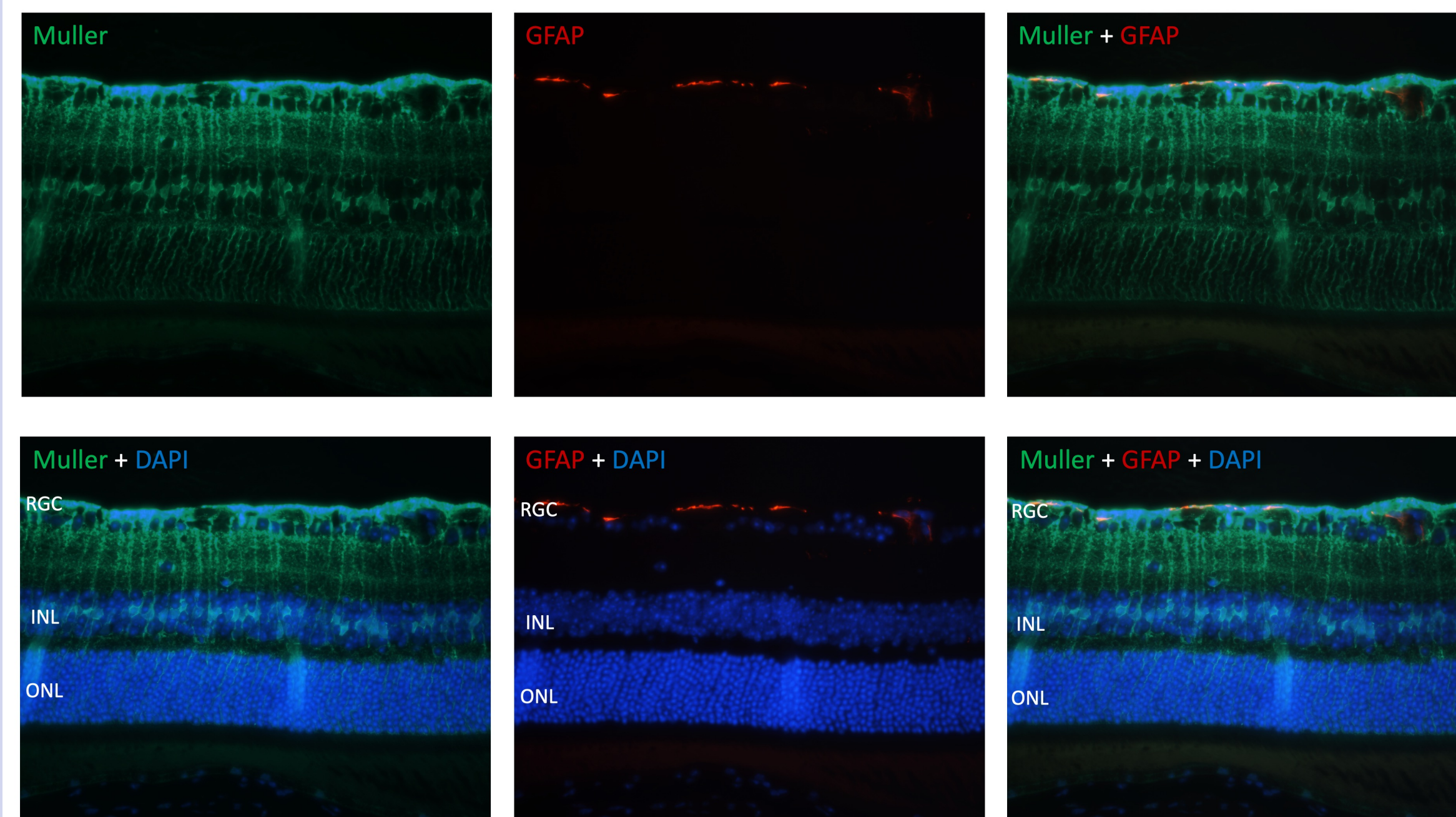
Figure 2: Naïve retina. GFAP is only expressed in in astrocytes
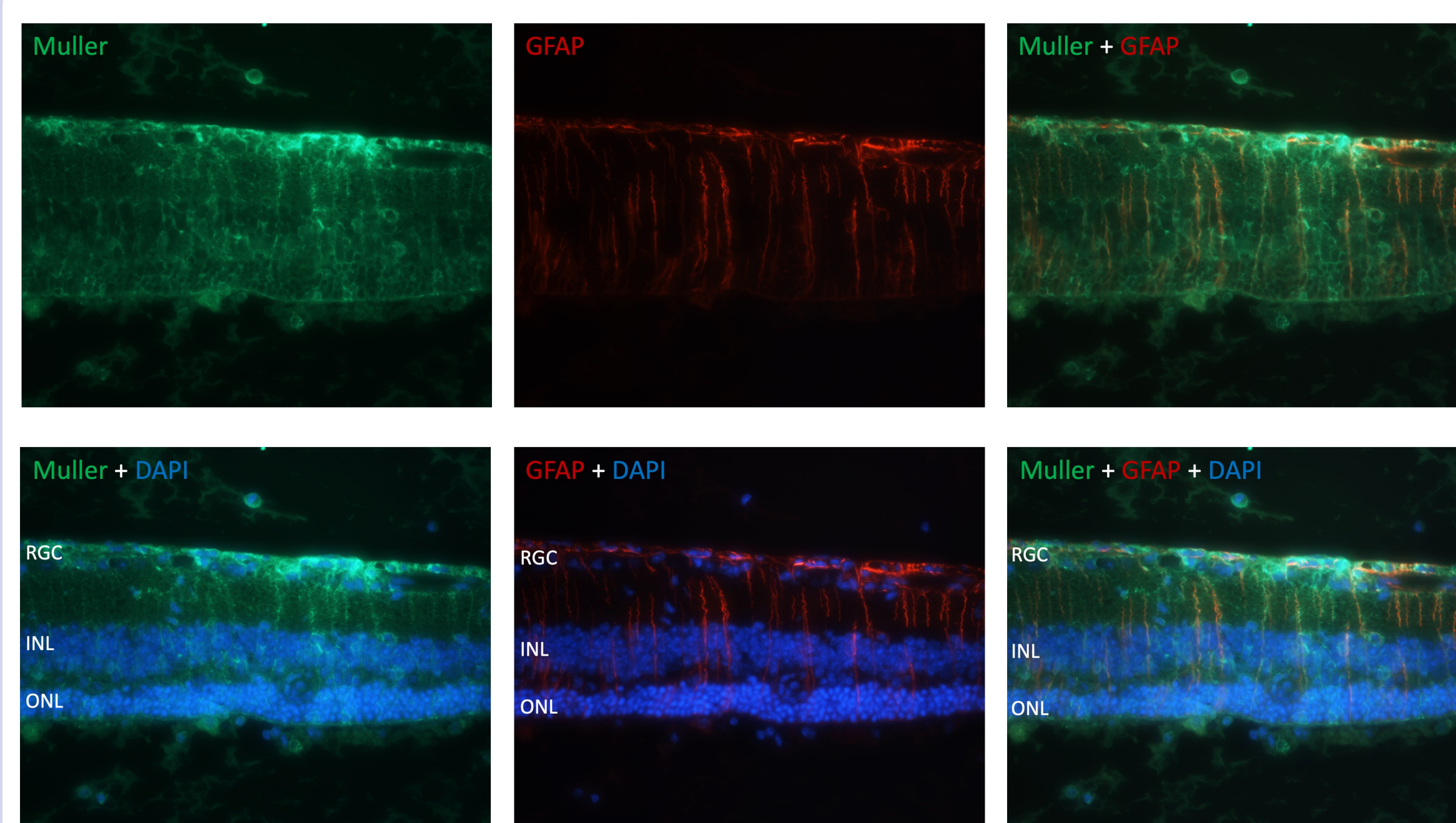
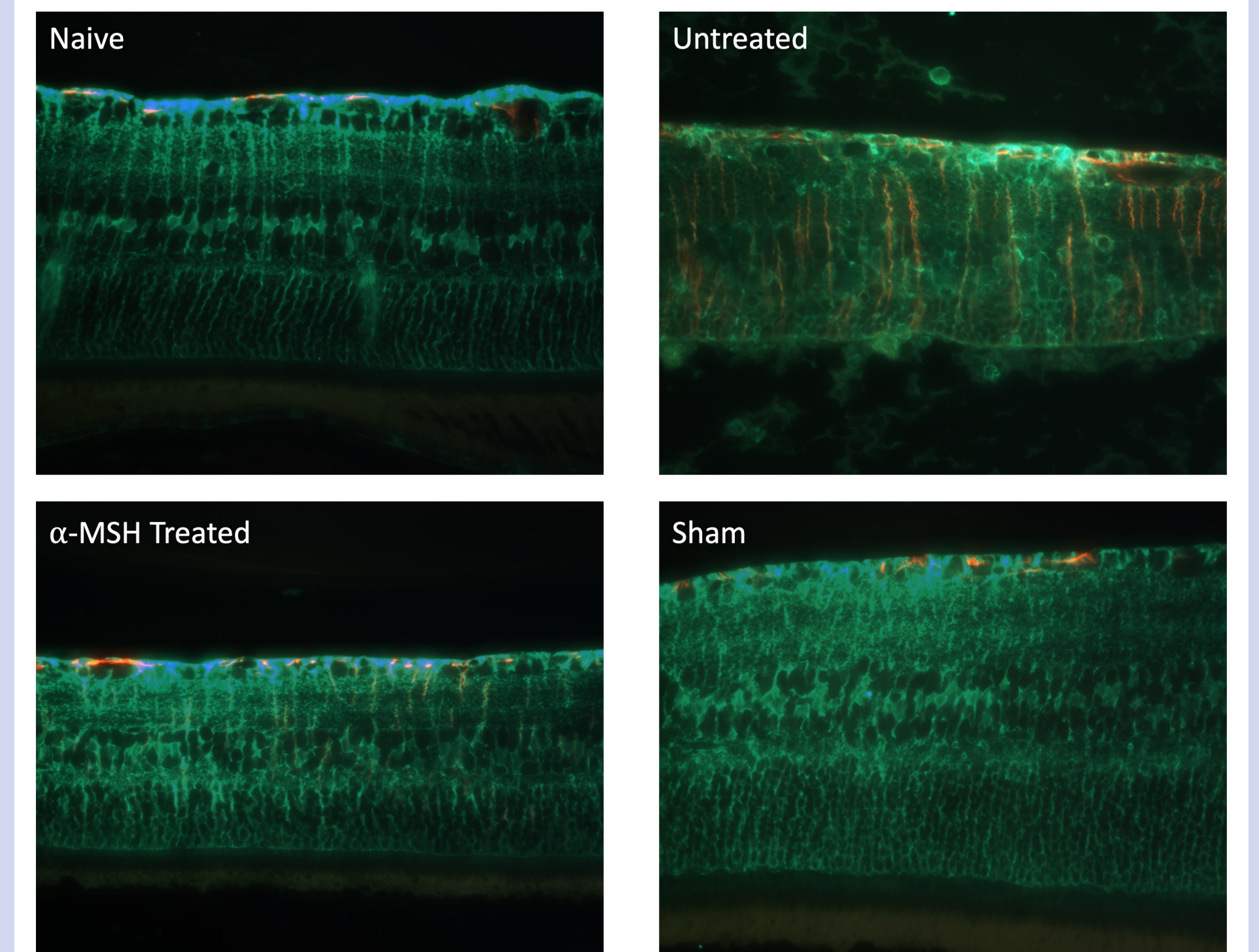**Figure 3:** Untreated retina. GFAP expression in muller cell fibers.

Figure 4: Retina treated with α-MSH. Significantly decreased GFAP expression in Muller cell fibers when compared to untreated.

Figure 5: Sham retina. Insignificant GFAP expression in Muller cell fibers when compared to Naïve retinas.

Results

Figure 6: Muller cell + GFAP images in all four retinas. Muller cells are shown in green and GFAP expression in red. Higher GFAP expression means higher retina degeneration

Percent of Retinal Muller Cells Expressing GFAP Following Ischemia/Reprofusion
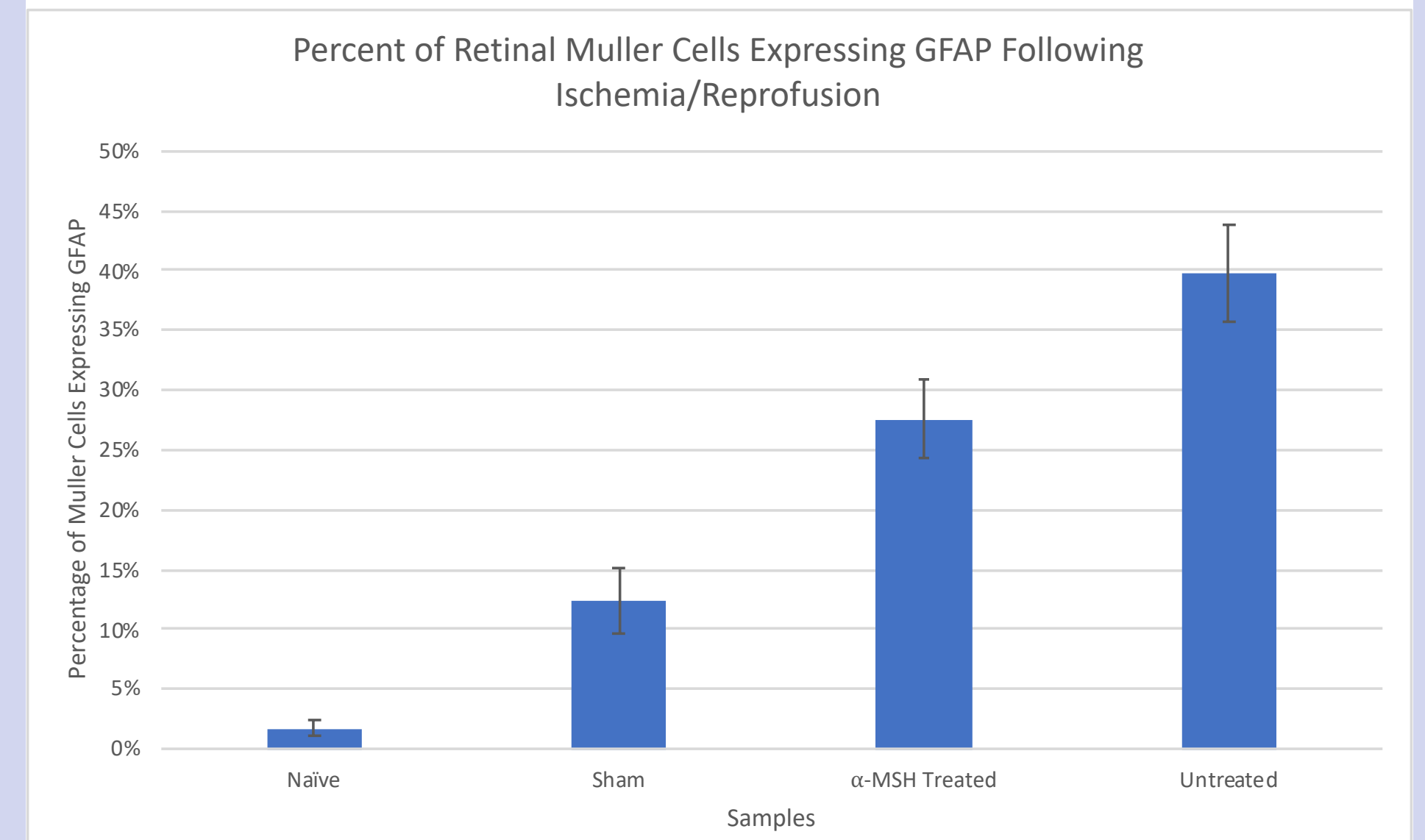
Figure 7: Percent of Muller cells expressing GFAP after cell counting.

Summary

About 1.7% of naïve muller cells express GFAP (Figure 2). The GFAP expression in untreated retinas were significantly higher at 39.7% (Figure 3). When compared to the untreated retinas, the α-MSH treated retinas (Figure 4) had significant lower GFAP expression rate of 27.6% ($p < 0.05$). In the sham retinas (Figure 5), the GFAP expression is insignificant compared to naïve, at 12.3%.

References

(1) Goit, R. K.; Taylor, A. W.; Lo, A. C. Y. Anti-Inflammatory α-Melanocyte-Stimulating Hormone Protects Retina after Ischemia/Reperfusion Injury in Type I Diabetes. Frontiers in Neuroscience 2022, 16. https://doi.org/10.3389/fnins.2022.799739.

(2) Eisenfeld, A. J.; Bunt-Milam, A. H.; Sarthy, P. V. Müller Cell Expression of Glial Fibrillary Acidic Protein after Genetic and Experimental Photoreceptor Degeneration in the Rat Retina. Investigative Ophthalmology & Visual Science 1984, 25 (11), 1321–1328.

Acknowledgements