

# Zebrafish Retinal Ganglion Cell Survival in the Context of Pro-Apoptotic Bax Signaling

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## INTRODUCTION

Loss of vision in glaucoma is the result of damage to the optic nerve and death of retinal ganglion cells (RGCs), the cells that connect the eye to the brain. In patients and mammalian models of the disease, RGCs die by BAX-dependent apoptosis. However, under similar disease-like conditions, zebrafish RGCs are resistant to apoptosis. Using the BAX protein as a starting point we are attempting to identify the mechanism behind zebrafish RGC survival. Our goal here is to develop a system for controlled induction of RGC apoptosis in zebrafish to visualize and measure BAX activity. Then we examine molecular differences between zebrafish and human BAX proteins in human cells and zebrafish. Zebrafish BAX homologs, *baxa* and *baxb*, are missing a phosphorylation site (S184) that is critical for anti-apoptotic activity in mammals. We test whether adding this site to the zebrafish BAX proteins, *baxa* and *baxb*, is sufficient to induce cytoplasmic localization in human cells. Finally, we create transgenic zebrafish expressing fluorescent BAX fusion proteins in RGCs to visualize BAX localization under glaucoma-like conditions.

## DESIGN & METHODS

- Develop a retinal explant assay to image BAX-dependent apoptosis in zebrafish RGCs using *isl2b:eGFP* transgenic fish.
  - Retina from adult zebrafish was dissected, mounted RGC side down on glass bottom dish, and imaged using the Keyence or Zeiss LSM980 (Figure 1)
  - Apoptosis was measured using TUNEL assay and Hoechst nuclear staining for pyknotic nuclei (Figure 2 and 3).
  - Apoptosis was blocked using iMAC drug or *baxa/b* antisense Morpholino (not shown).
- Determine if adding the homologous Bax S184 phosphorylation site residue to the zebrafish *baxa* and *baxb* proteins results in differential subcellular localization.
  - Mouse and zebrafish eGFP-Bax fusion proteins were expressed in HeLa cells using FuGENE HD transient transfection and time lapse imaged to observed subcellular localization and apoptosis rate.
  - Expression Plasmids
    - pCS2-eGFP-MmBAX
    - pCS2-eGFP-*baxa*
    - pCS2-eGFP-*baxb*
    - pCS2-eGFP-*baxaA186S*
    - pCS2-eGFP-*baxbG198S*
  - Mitochondria were stained using MitoTracker Red (Figure 4 and 5).
- Generation of transgenic zebrafish expressing eGFP-BAX fusion proteins specifically in RGCs for visualizing BAX subcellular localization under disease-like conditions. Transgenic lines created using Tol2-Gateway system. 2-3 independent lines created for each transgene (Figure 6).
  - elavl3:eGFP-MmBAX*
  - elavl3:eGFP-baxa*
  - elavl3:eGFP-baxb*
  - elavl3:eGFP-baxaA186S*
  - elavl3:eGFP-baxbG198S*

## RESULTS

Figure 1. *isl2b:eGFP* positive RGCs disappear over the first 24 hours in explant culture.

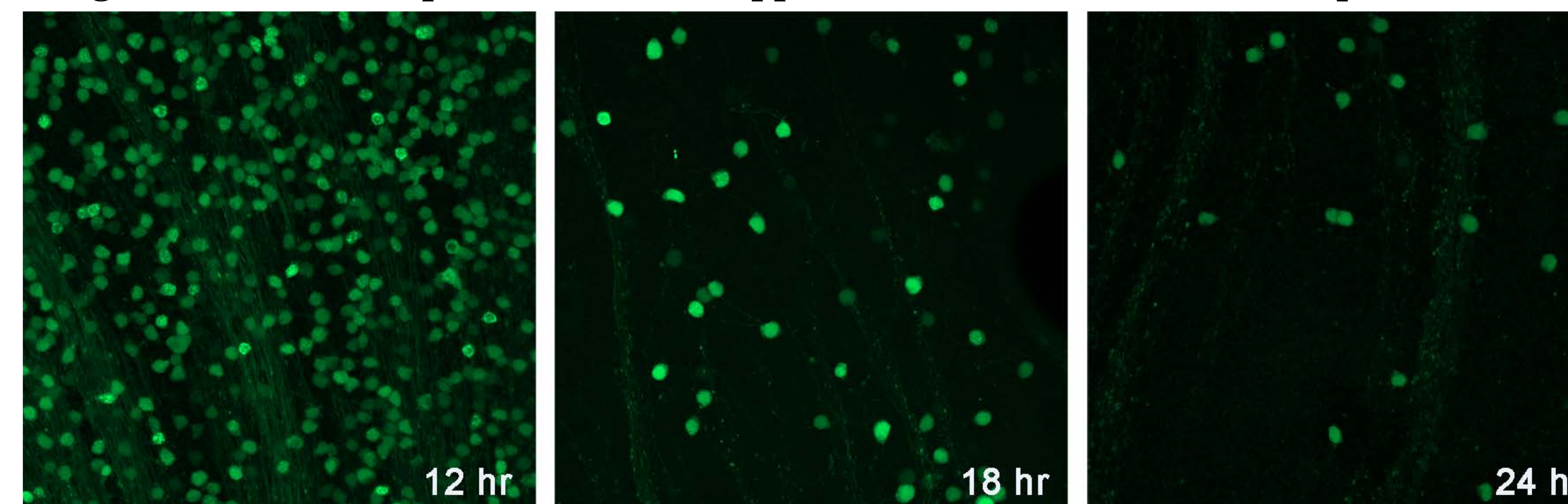


Figure 2. RGCs progressively exhibit pyknotic nuclei in explant culture.

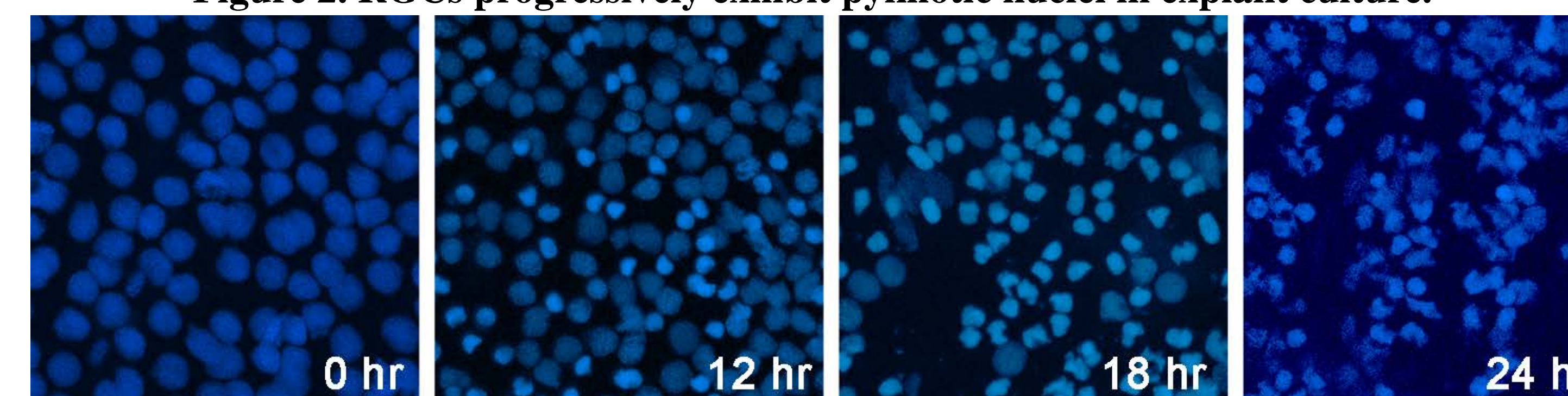


Figure 3. RGCs become TUNEL positive in explant culture.

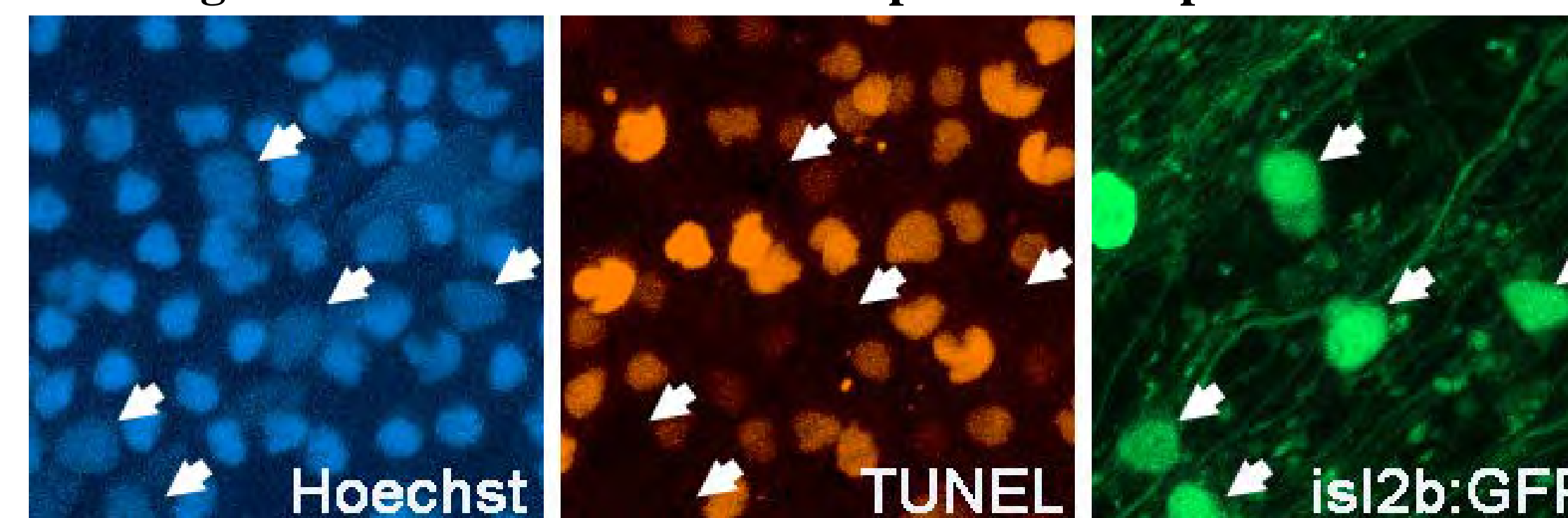
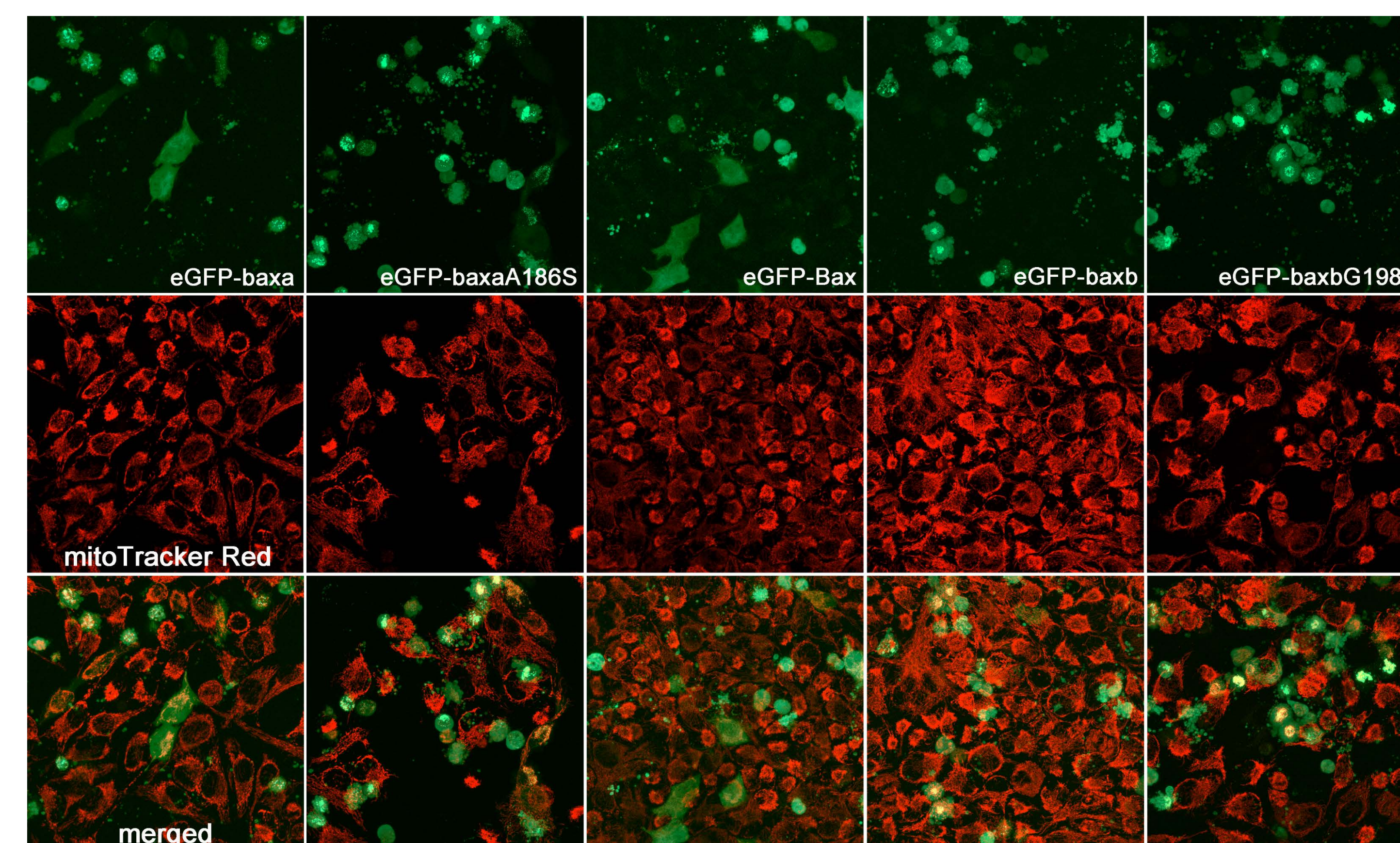


Figure 4. Addition of a C-terminal phosphorylation site does not decrease *baxa/b* toxicity in transiently transfected HeLa cells.



## RESULTS

Figure 5. Addition of phosphorylation site to zebrafish *baxa/b* does not decrease cell death.

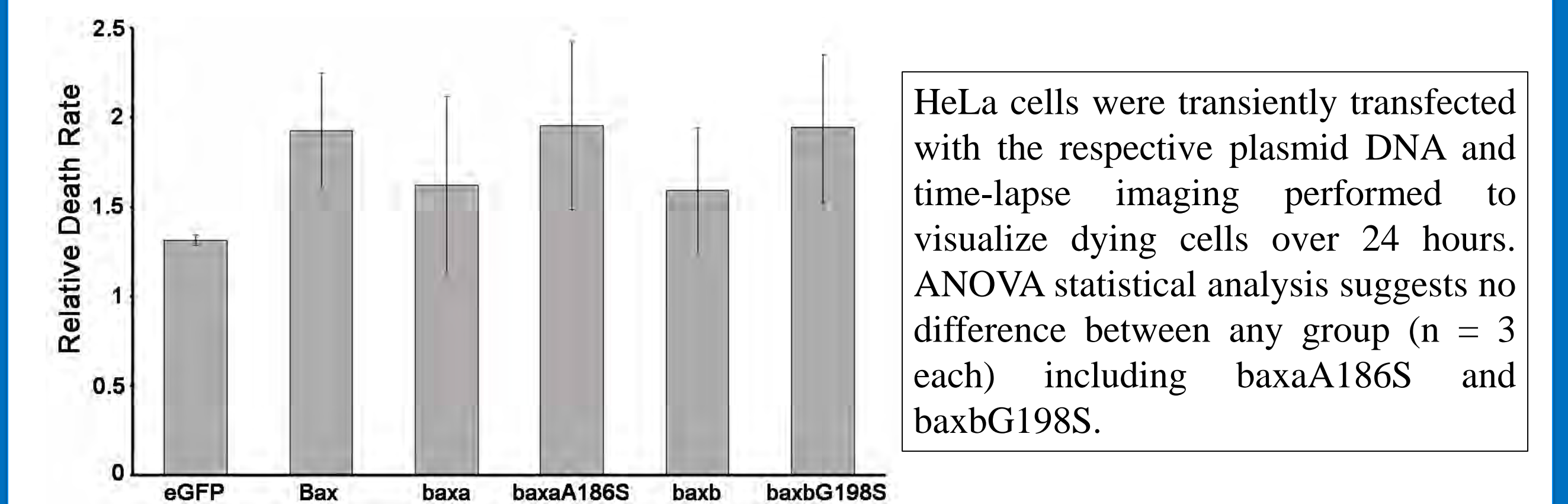
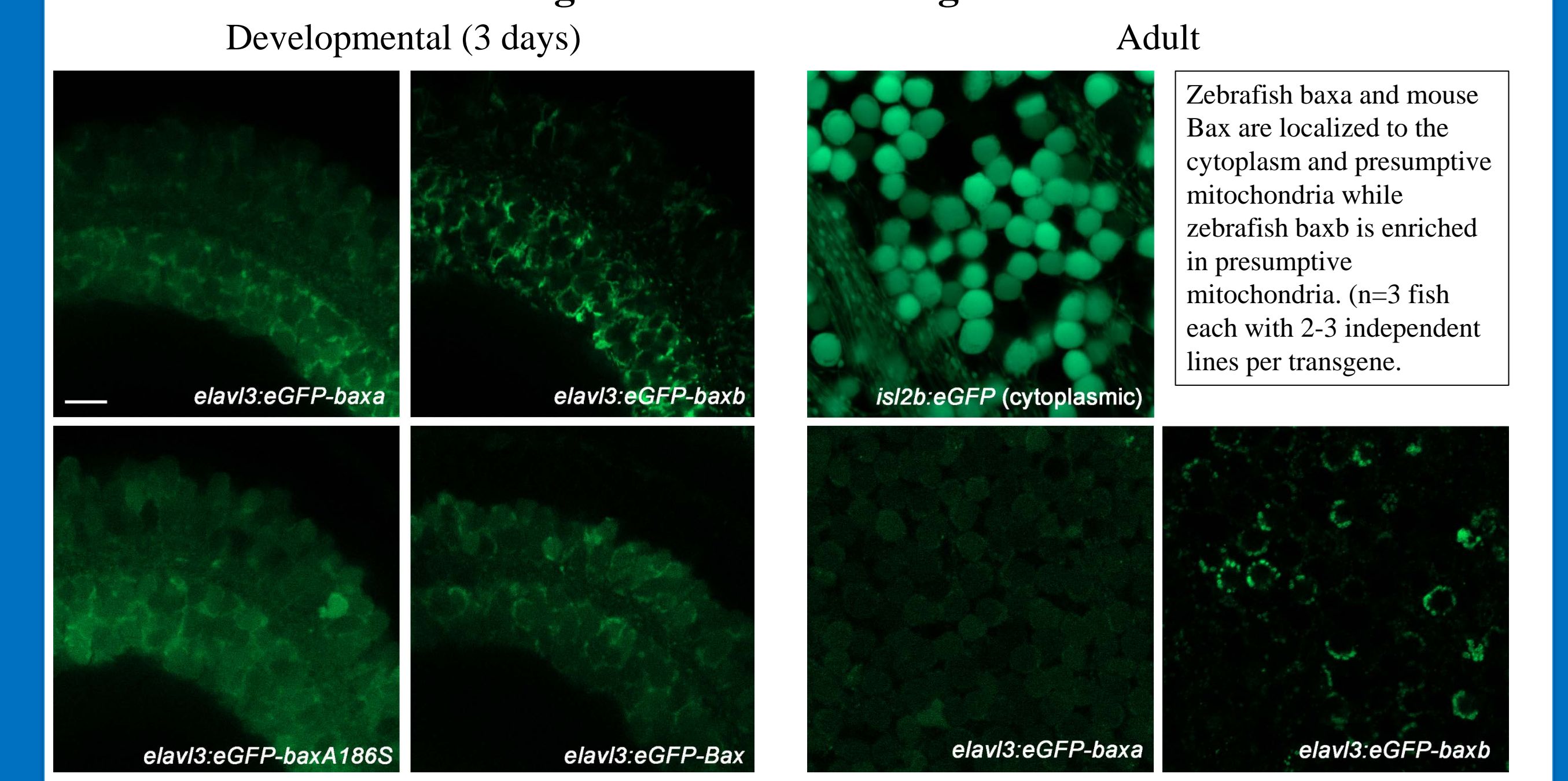


Figure 6. Expression of fluorescent mouse Bax and zebrafish *baxa/b* in transgenic zebrafish transgenic lines.



## CONCLUSIONS AND FUTURE DIRECTIONS

With the goal of visualizing BAX-dependent apoptosis in RGCs we have used a retinal explant assay and transgenic zebrafish lines to label BAX proteins. We successfully induce TUNEL positive pyknotic nuclei in explanted RGCs, however inhibition of BAX function did not prevent this suggesting an alternative mechanism. Zebrafish *baxa/b* is missing a critical phosphorylation site that exists in mammals for cytoplasmic sequestration. We found that this site was not necessary for cytoplasmic localization of *baxa* and that *baxb* was largely found at the mitochondria no matter the condition tested in mammalian cells or zebrafish RGCs. Finally, we generated transgenic zebrafish to visualize BAX localization in vivo. We are currently using these fish to examine BAX localization after elevated intraocular pressure or optic nerve injury, models of glaucoma-like conditions.

## ACKNOWLEDGEMENTS

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