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Research article

Depletion of Polypyrimidine tract binding protein 1 (*ptbp1*) activates Müller glia-derived proliferation during zebrafish retina regeneration via modulation of the senescence secretome

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ABSTRACT

Polypyrimidine Tract Binding protein 1 (PTB) is an alternative splicing factor linked to neuronal induction and maturation. Previously, knockdown experiments supported a model in which PTB can function as a potent reprogramming factor, able to elicit direct glia-to-neuron conversion *in vivo*, in both the brain and retina. However, later lineage tracing and genetic knockouts of PTB did not support direct neuronal reprogramming. Nevertheless, consistent with the PTB depletion experiments, we show that antisense knockdown of PTB (*ptbp1a*) in the zebrafish retina can activate Müller glia-derived proliferation and that depletion of PTB can further enhance proliferation when combined with acute NMDA damage. The effects of PTB are consistent with a role in controlling key senescence and pro-inflammatory genes that are part of the senescence secretome that initiates retina regeneration.

1. Introduction

Degenerative eye diseases continue to be a leading cause of blindness, affecting millions of people worldwide. Conditions such as age-related macular degeneration (AMD) and retinitis pigmentosa target the retina, the innermost part of the eye composed of multiple layers of light-sensitive nervous tissue that project sensory information to the optic nerve. Damage or loss of one or more layers of the retina is irreversible in humans and other mammals. In contrast, zebrafish (*Danio rerio*) possess the ability to naturally regenerate lost neurons and cells of the retina after damage in a Müller glia-dependent manner (Bernardos et al., 2007; Nagashima et al., 2013; Raymond et al., 2006; Yurco and Cameron, 2005). In response to damage zebrafish, Müller glia undergo dedifferentiation and asymmetric division for self-replacement and the generation of a pool of proliferating neuronal progenitor cells that can replace all lost cell types (Fausett and Goldman, 2006; Raymond et al., 2006; Wan and Goldman, 2016; Yurco and Cameron, 2005). Extensive work is ongoing to identify genes and pathways that regulate zebrafish retina regeneration and determine why regeneration is blocked in humans (Lahne et al., 2020; Lyu et al., 2023; Nagashima and Hitchcock, 2021; Pavlou and Reh, 2023; Wan and Goldman, 2016). Immediately after damage, inflammation is activated, but as regeneration proceeds, there is a dynamic response that shifts from a pro-inflammatory state toward a pro-regenerative state (Bludau et al., 2024; Iribarne, 2021; Iribarne and Hyde, 2022b; Mitchell et al., 2018; Nagashima and Hitchcock, 2021; Silva et al., 2020). The inability to effect this switch leads to chronic inflammation, fibrosis and scarring (Iribarne and Hyde, 2022a; Mitchell et al., 2019; Palazzo et al., 2022; Todd et al., 2019, 2020).

In the retina, senescent cells play a key role in modulating inflammatory responses during regeneration through the release of factors that collectively make up the Senescence Associated Secretory Phenotype (SASP)(Coppé et al., 2010; He and Sharpless, 2017; Konar et al., 2024). Early after retina damage in zebrafish, a subset of macrophages and glial cells express markers of senescence and the release of SASP factors from these cells can modulate the microenvironment (Konar et al., 2024; Oubaha et al., 2016). As regeneration proceeds, senescent cells are cleared, coincident with a shift toward an anti-inflammatory, pro-regenerative response (Konar et al., 2024). Premature removal of senescent cells inhibits regeneration by altering the required dynamic

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changes in inflammation (Konar et al., 2024).

Recently, two different strategies were used to down-regulate the alternative splicing factor PTB (Van Nostrand et al., 2020) in the retina and the brain that suggested direct glia to neuron conversion with rescue of visual function after retina damage and replacement of dopaminergic neurons in a mouse model of Parkinson's disease (Qian et al., 2020; Zhou et al., 2020). However, follow up lineage tracing and genetic knockout experiments did not support a role for PTB in direct neuronal conversion (Hoang et al., 2023; Wang et al., 2021; Xie et al., 2022). The conflicting results could be due to different experimental strategies, but might also be due to differences between knockdowns, knockouts, and genetic compensation or transcriptional adaptation (El-Brolosy and Stainier, 2017; Fu and Mobley, 2023; Rossi et al., 2015). Despite the controversy, we show here that antisense knockdown of PTB in otherwise undamaged zebrafish retinas leads to the generation of proliferating cells and further enhances the number of Müller glia-derived proliferating cells when combined with NMDA damage. Consistent with a role for PTB in modulating senescence and regulating SASP expression (Georgilis et al., 2018; Hensel et al., 2022; Li et al., 2024; Yang et al., 2024), depletion of PTB in the retina causes an increase in the number of senescent cells and expression of senescent markers. Together, our data argue that depletion of PTB contributes to retina regeneration by modulating the retinal microenvironment that facilitates Müller glia-derived regeneration.

2. Methods

2.1. Zebrafish husbandry and maintenance

Experiments were performed using randomly selected male and female zebrafish, either wild-type AB or *Tg(1016tuba1a:GFP)* zebrafish. Zebrafish were kept at 28 °C on a 12:12 h light-dark cycle. Experiments were conducted in accordance with Vanderbilt University Institutional Animal Care and Use Committee (IACUC) approval #M1800200.

2.2. Antisense Oligonucleotide (ASO) Treatment

Antisense oligonucleotides (ASOs) were generated targeting *ptbp1a* using the following sequences:

Zebrafish PTBP1a ASO 2: 5′-TCTCTAAACGGCCGTCCATG-3'
Zebrafish PTBP1a ASO 3: 5′-GACGTAACTGGATCCCAGAGG-3'
Zebrafish PTBP1a ASO 4: 5′-GTGCAACCTGAAAGAGTGCG-3'

ASOs were synthesized by Millipore Sigma (Missouri, USA). All internucleotide linkages were phosphorothioates interspersed with phosphodiesters, and all cytosine residues were 5'-methylcytosines.

Adult zebrafish aged 5–10 months were anesthetized by immersion in a 0.16 % Tricaine solution (MESAB, Syndel, Washington USA). Using a sapphire blade, a small cut was made in the sclera of the left eye. 0.5 μL of 500 μM ASO were intravitreally injected into the incision site. An equal volume of 500 μM GFP ASO (5′- CAACAAGATGAAGAGCACCAA-3′) was used as a control, while another group was injected with an equal volume of 1X phosphate-buffered saline (PBS) as an injection control. Zebrafish were pretreated with PTB ASOs 1 day prior to NMDA damage.

2.3. NMDA damage model

N-methyl-D-aspartate (NMDA) is a commonly used damaging agent that induces cytotoxic damage to the ganglion and amacrine cells of the retina (Dvoriantchikova et al., 2023; Karl et al., 2008; Luo et al., 2019; Sucher et al., 1997). 24 h post ASO injection (hpi), zebrafish were anesthetized once again using Tricaine. An additional incision was made on the sclera of the left eye, and 0.5 μL of a 100 mM NMDA solution (Sigma Aldrich, Missouri, USA) was intravitreally injected. 1X PBS injections were used as an injection control for NMDA damage. Retinas were collected at 3dpi post NMDA damage.

2.4. Immunohistochemical staining

Fluorescent staining was performed as previously described (Konar et al., 2024). All samples were collected 3 days post injection (3dpi) for time matching. Upon collection, eyes were fixed in 4 % paraformaldehyde overnight at 4 °C. Eyes were then incubated in a 5 % sucrose solution with 4 changes and kept overnight in 30 % sucrose at 4 °C. After sucrose treatment, eyes were placed in a 2:1 Cryomatrix (Fisher Scientific, Texas, USA) and 30 % sucrose solution for 2 h at 4 °C and then fully embedded in Cryomatrix. Eyes were sectioned at 15 μm thickness using a Leica cryostat and placed on charged Histobond slides (Fisher Scientific, New Jersey, USA). Slides were dried on a heating block and stored at −80 °C. Prior to IHC staining, slides were placed on a heating block and then rehydrated in 1X PBS for 30 min. Slides were then subjected to antigen retrieval in 5 mM Sodium Citrate buffer (0.05 % Tween-20, pH 6.0) in a boiling water bath before being allowed to cool to room temperature (RT) (Konar et al., 2024). Slides were then placed in a blocking solution (3 % donkey serum, 0.1 % Triton X-100 in 1X PBS) for 2 h at room temperature (RT) then incubated at 4 °C in primary antibody solution overnight. Primary antibodies are listed in Table 1. Afterwards, slides were incubated in secondary antibody solutions with To-Pro-3 for 2 h at RT. Secondary antibodies included donkey anti-mouse 488 (1:500), donkey anti-mouse cy3 (1:500), and donkey anti-rabbit 488 (1:500) (all from Jackson Immuno, Pennsylvania, USA). Slides were then mounted with VectaShield Antifade Mounting Medium (Vector Labs, California, USA).

2.5. Senescence-associated β-galactosidase staining

Slides were warmed and rehydrated in 1X PBS. Sections were then stained in accordance with instructions from a SA-βGal kit from Cell Signaling Technology (Massachusetts, USA). Briefly, staining solution was warmed to 37 °C then adjusted to a pH 6.0. Slides were incubated with staining solution overnight at 37 °C. The staining solution was then washed off the slides, and slides were incubated in a 1:1000 dilution of To-Pro-3 (ThermoFisher, Pennsylvania, USA) in 1X PBS for nuclear staining. Slides were then mounted using VectaShield antifade mounting medium.

2.6. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

qRT-PCR was performed on retinas collected 3 days post ASO injection as described (Kent et al., 2021). Retinas were placed in TriZol (Invitrogen, California, USA) after dissection for total RNA purification. For cDNA synthesis, we used the Accuscript High-Fidelity 1st Strand cDNA Synthesis kit (Agilent, California, USA). PCR amplification of cDNA was performed using the SYBR Green master mix (Bio-Rad Laboratories, California, USA) with a Bio-Rad CFX96 real-time system. Amplification levels were normalized to 18S rRNA levels using the ΔΔCt method. 3 retinas were pooled per replicate. Table 2 lists the primer sets:

Table 1
List of antibodies used for IHC staining.

Name	Target	Concentration	Source
Proliferating cell nuclear antigen (PCNA)	Actively proliferating cells	1:500	Abcam
GFP	Dedifferentiated Müller glia in tg(1016*tuba*1a:gfp) zebrafish	1:500	Abcam
4c4	Macrophages/Microglia	1:1000	Hitchcock Lab (University of Michigan)

Table 2
Primer sequences used for qRT/PCR.

Name	Sequence (5'→3')
ptbp1a	F- TGTCTGATGGAACCCAAGCC
	R- AAGGCCTTAACCACAGCTCC
p21	F- CAGCGGGTTTACAGTTTCAGC
	R- TGAACGTAGGATCCGCTTGT
p16	F- GAGGATGAACTGACCACAGCA
	R- CAAGAGCCAAAGGTGCGTTAC
mmp9	F- TGATGTGCTTGGACCACGTAA
	R- ACAGGAGCACCTTGCCTTTTC
sema7a	F- GGTTTTTCTGAGGCCATTCC
	R- GGCACTCGTGACAAATGCTA
npm1a	F- CACCAGCGAAACCAAAGACC
	R- CGTGGTGTTTGTGGCGTTTT
il6	F- GACGTGGTATAAAGACAACTGGAAC
	R- AAGGATAGGGAAGTGCTGGATG
18S rRNA Control	F- TTACAGGGCCTCGAAAGAGA
	R- AAACGGCTACCACATCCAAG

2.7. Imaging and image processing

IHC stains were imaged using a META Zeiss LSM Meta 510 confocal microscope in the Vanderbilt Center for Imaging Shared Resource (CISR) Core. SA-BGal and ToPRo were imaged on a NIKON AZ100M Widefield microscope. Confocal images were processed using ZEN Blue version 3.1, with additional analyses performed using ImageJ. AZ100M images were initially processed via NIS-Elements Viewer 5.21 and further analyzed with ImageJ.

2.8. Quantification and statistical analysis

For experiments involving immunostaining and cell number quantification, sections were evaluated in an unbiased and blinded manner. Stained slides were evaluated in the central retina, and the dorsal and ventral regions were counted to include the entirety of the retinal region, spanning approximately ~300–400 μm from the optic nerve in either direction. We excluded regions of extreme structural damage or near the ciliary marginal zone. Counts were obtained from at least two independent central retina sections and averaged for every eye section. We calculated significance using a two-way ANOVA with Turkey's post hoc test for intergroup comparisons GraphPad Prism 10.0.1. Graphs demonstrate the average ± standard error of the mean. The number of fish used in each experiment are listed in the figure legends.

2.9. Single-cell RNA sequencing analysis

Previously processed single-cell RNA sequencing (scRNAseq) datasets representing undamaged zebrafish retinas were used to generate a Seurat (v. 5.1.0) object (Hao et al., 2024; Hoang et al., 2020). To visualize localization of *ptbp1a* expression to specific cell types, normalized expression data was overlaid on Uniform Manifold Approximation and Projection (UMAP) coordinates for the undamaged retina samples and the plot1cell package (v. 0.0.0.9000) was used to summarize cluster-level expression data (Healy and McInnes, 2024; McInnes et al., 2018; Wu et al., 2022).

3. Results

3.1. Depletion of PTB induces proliferation

Antisense oligonucleotides (ASOs) have been used to deplete PTB after transfection into cultured cells, lentiviral shRNA delivery into mouse astrocytes, addition to human cortical organoid media, and by simple injection into mouse cerebrospinal fluid (Juliano et al., 2008; McDowall et al., 2024; Rinaldi and Wood, 2018; Sazani and Kole, 2003). We generated ASOs containing phosphorothioate linkages and 5′-methylcytosine modifications (Maimon et al., 2021) that target transcripts encoded by the two zebrafish genomic copies of PTB (PTBP1a and PTBP1b) (Fig. 1A). We tested the effects of intravitreal injection of these ASOs on DNA replication, as marked by expression of Proliferating Cell Nuclear Antigen (PCNA). Compared to control ASOs targeting GFP in otherwise undamaged retinas, we found that targeting *ptbp1a* led to significant increases in PCNA + cells, whereas targeting *ptbp1b* had no effect (Supplemental Fig. 1). We therefore focused on *ptbp1a* and found that intravitreal injection of *ptbp1a* ASOs successfully reduced *ptbp1a* expression levels by ~70 % compared to PBS-injected controls at 3 days post injection (Fig. 1B and C). Control ASOs targeting GFP did not cause significant reduction in *ptbp1a* expression.

3.2. Müller glia-derived proliferation increases after ptbp1a knockdown

To determine whether the increase in proliferation observed after injection of ASOs targeting *ptbp1a* is a regeneration-associated response mediated by Müller glia, we used *Tg[1016;tuba1a;GFP]* transgenic zebrafish. In this line, GFP marks dedifferentiated Müller glia and Müller glia-derived progenitor cells (Fausett and Goldman, 2006). We also tested how injection of *ptbp1a* ASOs would affect proliferation when combined with retinal damage by injection of NMDA, which causes significant neuronal death in the ganglion cell layer (Dvoriantchikova et al., 2023). We thus pretreated *Tg[1016;tuba1a;GFP]* fish with an intravitreal injection of *ptbp1a* ASOs followed by a second injection after 24hr. of either PBS or NMDA (Fig. 2A). Retinas were then collected at 3 days post NMDA injection and immunostained for GFP and PCNA. In control retinas in the absence of NMDA damage, no proliferation was observed after injection of either PBS or ASOs targeting GFP (Fig. 2B and C). Injection of ASOs targeting *ptbp1a* in the absence of NMDA damage resulted in the detection of numerous PCNA + cells in the Inner Nuclear Layer (INL), some of which co-localized with GFP. When NMDA damage was combined with injection of *ptbp1a* ASOs, we observed a striking increase in the number of PCNA + cells in the INL and the majority of these cells co-localized and formed clusters with GFP + cells (Fig. 2B). The formation of Müller glia-derived clusters of proliferating cells (GFP+/PCNA+) in the INL is characteristic of bona fide regeneration (Rajaram et al., 2014).

3.3. ptbp1a knockdown induces a senescent, pro-inflammatory response

One possible mechanistic explanation to resolve the controversy over the role of PTB during retina regeneration is that depletion of PTB activates regeneration through control of secreted factors that affect the local microenvironment and induce a Müller glia response. Consistent with this, depletion of PTB has been shown to regulate the inflammatory secretome and induce cellular senescence (Georgilis et al., 2018; Hensel et al., 2022; Li et al., 2024; Yang et al., 2024). If reduced expression of PTB can induce or promote senescence in the retina, it would be consistent with our previous discovery of a key role for senescent cells during retina regeneration (Konar et al., 2024). To test this, we injected wildtype zebrafish retinas with ASOs targeting *ptbp1a* and quantified the impact on senescence markers and senescence gene expression. At 3dpi, we found that compared to ASOs targeting GFP, *ptbp1a* knockdown significantly increased the number of senescent cells as marked by expression of Senescence Associated β-galactosidase (SA-βgal^{+}), a traditional marker of senescent cells (Dimri et al., 1995; Itahana et al., 2007) (Fig. 3A and B). We also observed upregulation of two additional markers of senescence that regulate cell cycle arrest, *p16* and *p21*, via qRT/PCR (Dodig et al., 2019; Schmitt et al., 2002) (Fig. 3C–D). These data are consistent with depletion of PTB inducing a senescent response (Li et al., 2024; Yang et al., 2024).

The appearance of senescent cells after depletion of *ptbp1a* predicts that SASP factors should also be upregulated. We thus isolated RNA at 3dpi and performed qRT/PCR to analyze the expression of key SASP factors (Basisty et al., 2020). We observed a significant increase in the

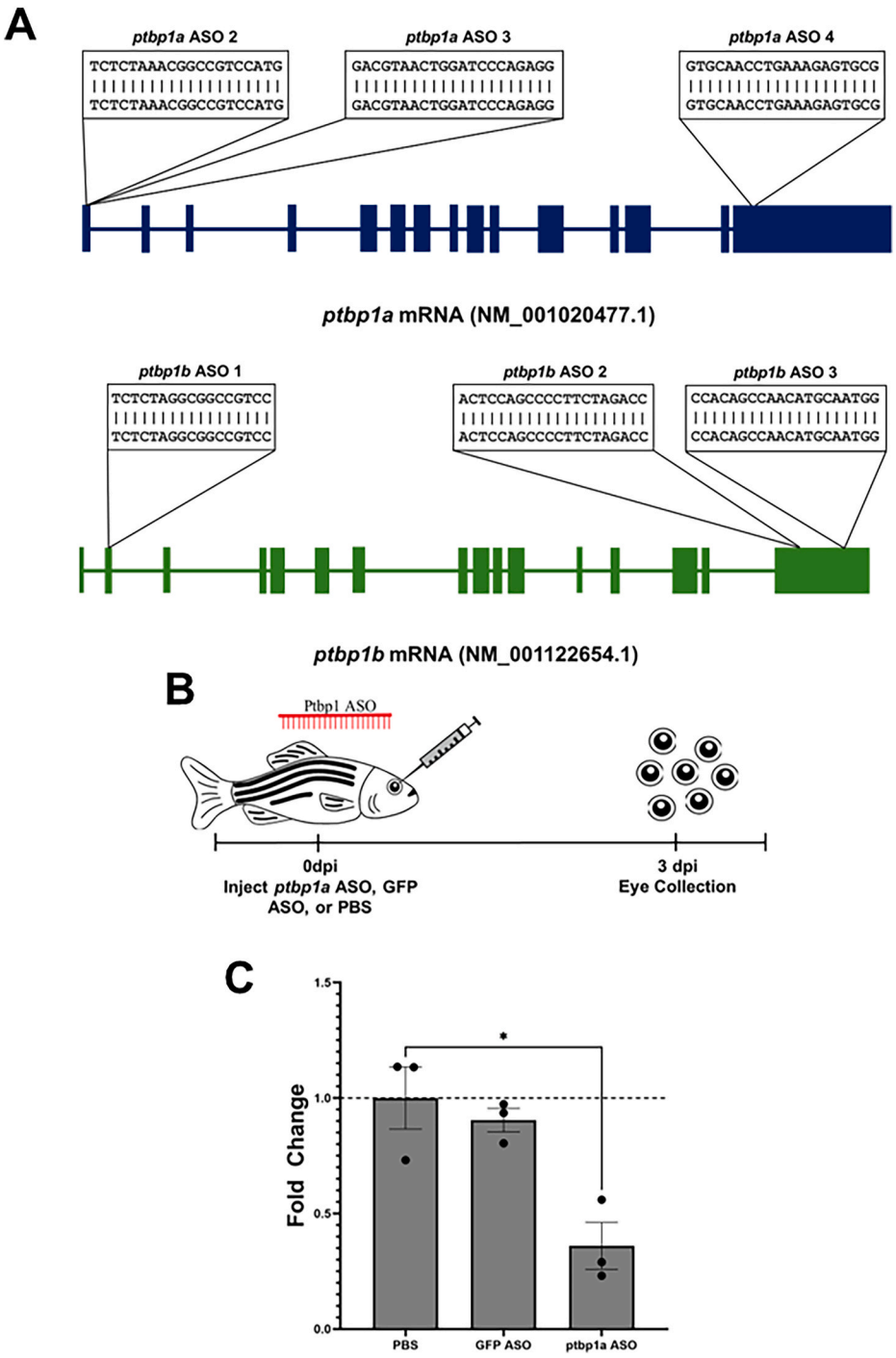

Fig. 1. Creation and knockdown confirmation with antisense oligonucleotides targeting ptbp1a and b. Adult zebrafish were injected with antisense oligonucleotides (ASOs) targeting *ptbp1a*. (A) Schematic highlighting targeting schemes for PTBP1a and PTB1b ASOs based off previous literature (Maimon et al., 2021). ASOs targeted near the start codon and 3′ UTR for optimal transcript coverage. (B) Injection and collection schematic for verification of *ptbp1a* ASO treatment. (C) ASO-mediated knockdown of *ptbp1a* was observed via qRT/PCR at 3dpi. (n = 3, * = $p < 0.05$).

expression of the SASP factors matrix metalloproteinase 9 (*mmp9*) and interleukin-6 (*il6*) (Fig. 3E–F). This is consistent with *ptbp1a* regulation of the pro-inflammatory SASP secretome (Georgilis et al., 2018). Additionally, we found that *ptbp1a* knockdown caused upregulation of the pro-regenerative factor nucleophosmin 1 (*npm1a*), which has been shown to regulate Müller glia-derived progenitor production through *chx10/vsx2* (Fig. 3G) (Basisty et al., 2020; Konar et al., 2025; Ouchi et al., 2012). Finally, we observed a decrease in the expression of semaphorin 7a (*sema7a*), which is a negative regulator of the immune system, further suggesting that *ptbp1a* knockdown influences inflammation and SASP expression (Czopik et al., 2006) (Fig. 3H). Combined, the data support the finding that depletion of *ptbp1a* induces senescence and is consistent with a role for SASP factors in the activation of regeneration.

3.4. *ptbp1a expression is localized to glial and endothelial cells in the retina*

We previously showed that the primary senescent cell type that is detectable after retina damage in zebrafish is derived from microglia/macrophages, as marked by immunostaining with 4c4 antibodies (Konar

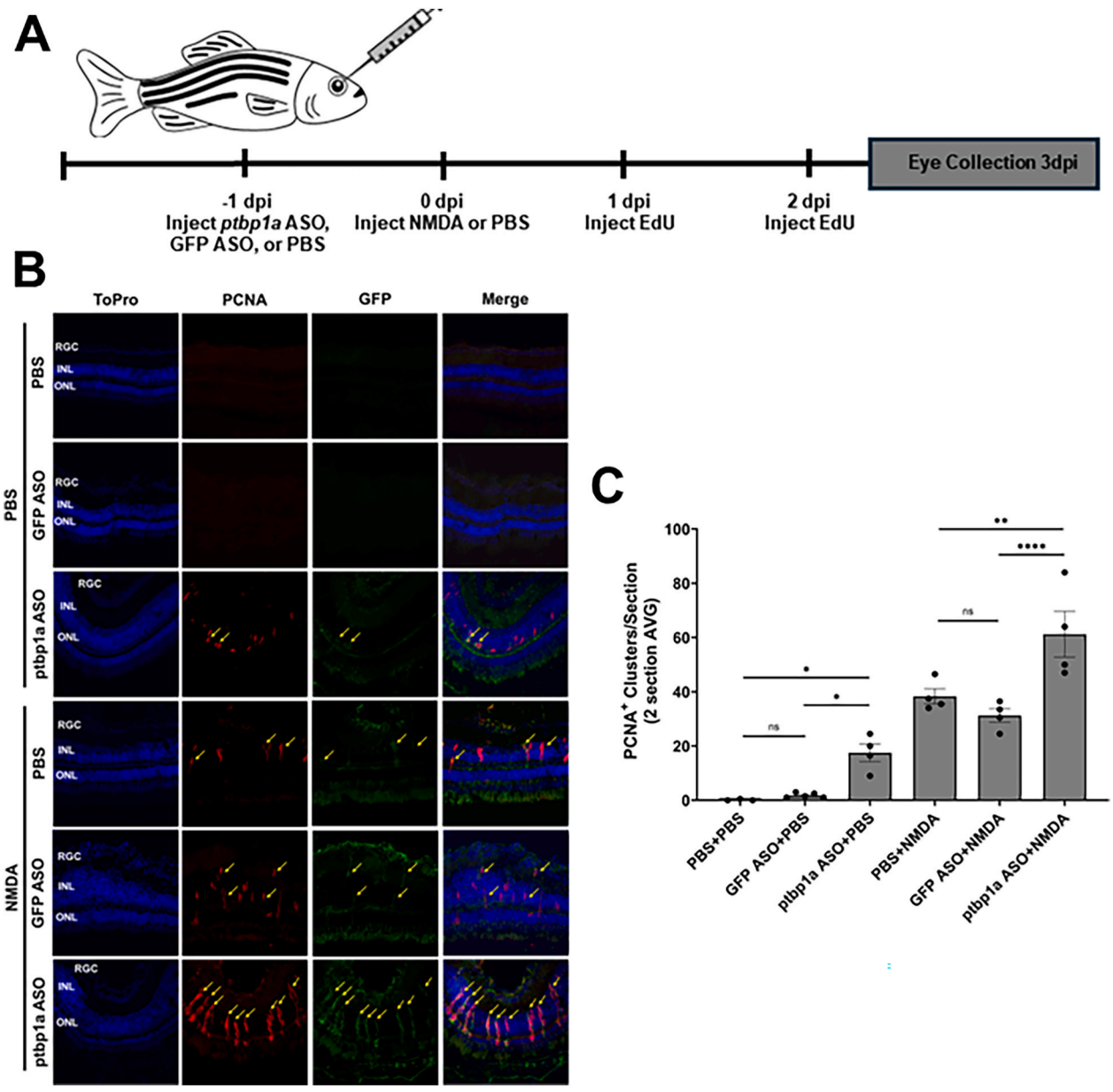

Fig. 2. *ptbp1a* knockdown is sufficient to induce a Müller glia-derived proliferative response. (A) Tg[1016;tuba1a;GFP] transgenic zebrafish were treated with ASOs targeting *ptbp1a* in the presence and absence of damage at 3dpi. (B) Staining for PCNA and *tuba1a*, a marker for Müller glia-derived proliferation, showed that the PCNA+ cells induced by *ptbp1a* knockdown were also *tuba1a*+. (C) In both the presence and absence of damage, *ptbp1a* knockdown induced a proliferative response in the retina. (n = 3–6, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = p,0.0001).

et al., 2024; Rovira et al., 2022). After intravitreal injection of *ptbp1a* ASOs, we observed an increase in the number of 4c4+ cells in the retina with a larger increase observed when combined with NMDA damage (Fig. 4A and B). This is consistent with localization of *ptbp1a* expression in microglia, as determined by scRNAseq analyses (Fig. 4C and D). Interestingly, *ptbp1a* expression in undamaged retinas was also detected in resting Müller glia, activated Müller glia, Müller glia-derived progenitor cells, and vascular endothelial cells (Fig. 4E). Analysis of scRNAseq data sets showed that these same cells express multiple SASP factors, one of which is PTB (Konar et al., 2025).

4. Discussion

Here, we show that knockdown of PTB (*ptbp1a*) in the zebrafish retina induces a senescent response with proliferation of Müller glia-derived progenitor cells. It is known that inflammation must be properly modulated for retina regeneration (Bludau et al., 2024; Iribarne and Hyde, 2022b; Leach et al., 2021; Mitchell et al., 2018; Nagashima and Hitchcock, 2021; Zhou et al., 2022) and our discovery that depletion of *ptbp1a* leads to upregulation of pro-inflammatory SASP factors such as *mmp9* and *Il6* is consistent with an early requirement for inflammation. Strikingly, we find that depletion of PTB can induce Müller glia-derived proliferation in undamaged retinas although the effects are much more robust in the presence of damage.

4.1. PTB and retina regeneration

Both shRNA knockdown and overexpression of *miR-124* have shown that decreased levels of PTB can induce neuronal differentiation, including direct conversion of fibroblasts to neurons (Makeyev et al., 2007; Xue et al., 2013). Those studies prompted experiments to package either PTBP1-targeting shRNA vectors or CRISPR/CasRx into adenovirus

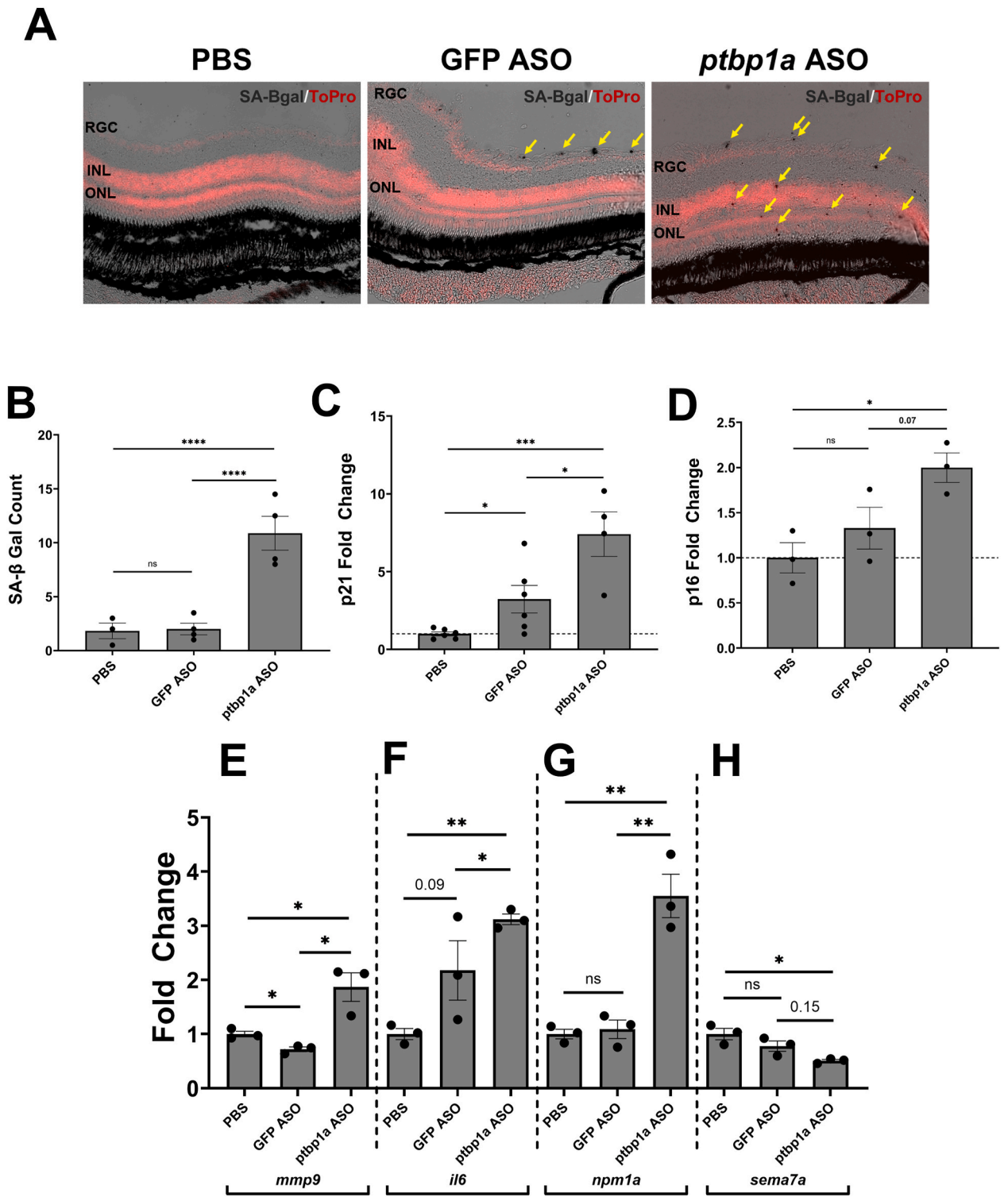

Fig. 3. Knockdown of *ptbp1a* induces expression of senescence markers and Senescence Associated Secretory Phenotype factors. ASO treated retinas were analyzed for markers and gene associated with senescence and the Senescence Associated Secretory Phenotype (SASP). (A–B) Knockdown of *ptbp1a* at 3dpi caused an increase in the number of SA-βgal+ cells in the retina, in the absence of damage. (C–D) *ptbp1a* knockdown also led to an upregulation in the expression of *p21* and *p16*, both are cell cycle regulators that are highly associated to senescence onset. (E–H) Knockdown also led to an increase in the pro-inflammatory SASP factors *mmp9* (E), IL-6 (F), the pro-regenerative SASP factor *npm1a* (G), and semaphorin 7a (*sema7a)* , which negatively regulates the immune system (H) . (n = 3–6, * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p,0.0001).

associated vectors for delivery to the brain and retina and resulted in the surprising finding that knockdown of *ptbp1* could induce direct glia-to-neuron conversion (Qian et al., 2020; Zhou et al., 2020). It was later found that the vectors used to deplete *ptbp1* were not specific for Müller glia and cell-lineage experiments and genetic knockout of PTB also did not support direct neural conversion (Hoang et al., 2023; Wang et al., 2021; Xie et al., 2022). Despite the controversy, multiple studies support the finding that knockdown of PTB induces gene expression

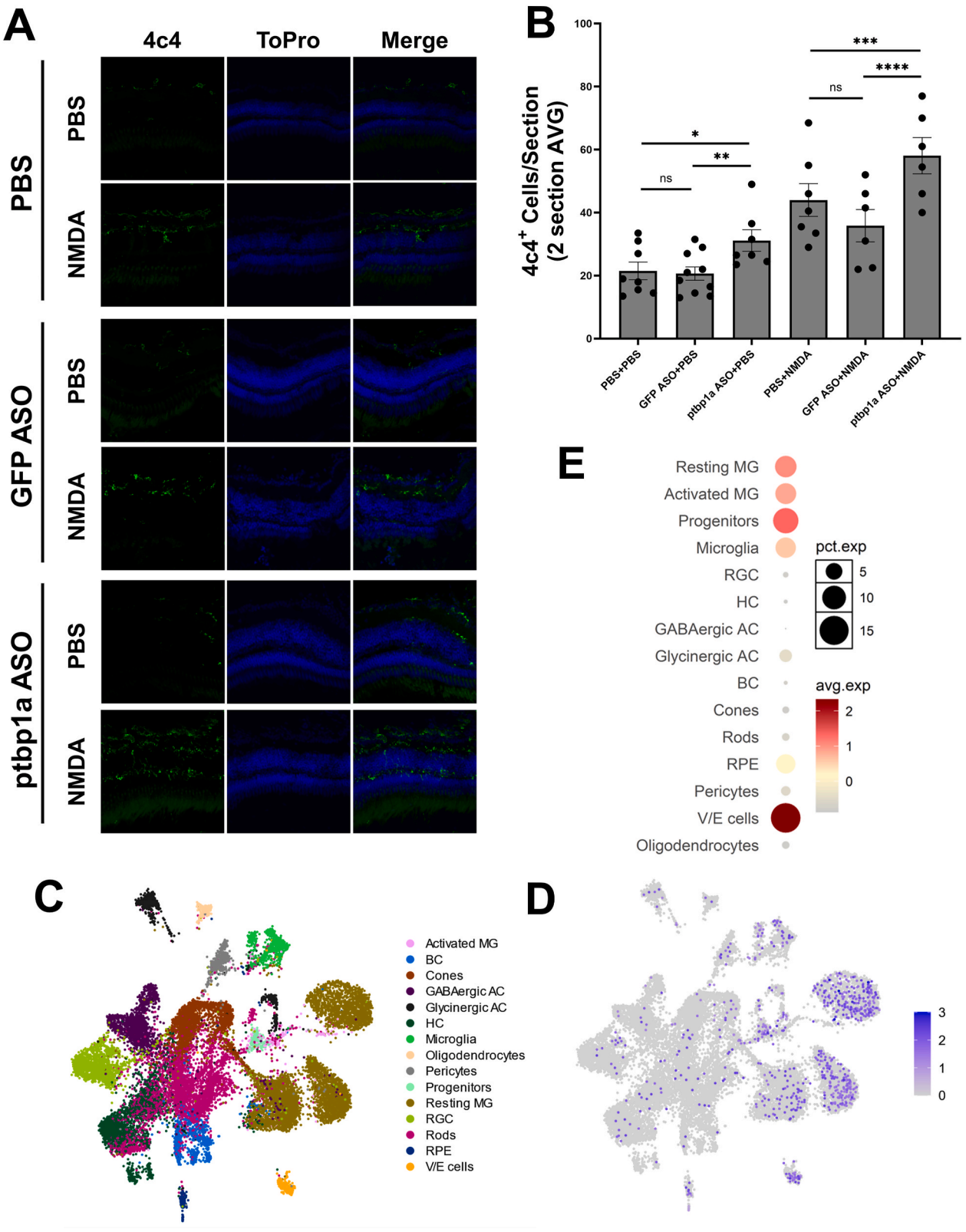

Fig. 4. Figure 4. ptbp1a is localized to glial cells and shows increased immune cell number after knockdown. Antisense oligonucleotides (ASOs) targeting *ptbp1a* were injected into wild type (AB) zebrafish in the presence and absence of NMDA damage and evaluated at 3dpi for 4c4+ immune cell count. (A–B) Both in the presence and absence of damage, *ptbp1a* knockdown resulted in an increase in the number of 4c4+ immune cells in the retina. (C–D) Uniform Manifold Approximation and Projection (UMAP) visualization of cell type expression of *ptbp1a* in control undamaged eyes. (E) Dot plot analysis of undamaged zebrafish retinas showed expression of *ptbp1a* primarily in glial cells and vascular/endothelial cells. (n = 7–9, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = p,0.0001).

changes by mechanisms that remain unclear but which are consistent with neuronal differentiation (Maimon et al., 2021; Makeyev et al., 2007; Xue et al., 2013). Genetic compensation or transcriptional adaptation might explain differences between the PTB knockout and knockdown studies (El-Brolosy and Stainier, 2017; Fu and Mobley, 2023; Rossi et al., 2015). This could be particularly relevant for PTB studies because neuronal induction and maturation are controlled by overlapping regulatory loops that involve *miR-124, miR-9*, and the REST and BRN2 transcription complexes (Hu et al., 2018). Any of the individual factors within these loops could conceivably be transcriptionally altered by knockouts (and knockdowns) of PTB (Fu and Mobley, 2023). Another non-mutually exclusive explanation could be that depletion of PTB from immune-derived cell populations, a subset of Müller glia, or vascular endothelial cells induces a senescent response and that subsequent changes in the SASP secretome provide pro-inflammatory signals within the microenvironment that initiate a proliferative, Müller glia-derived regenerative response. This is consistent with a role for depletion of PTB in inducing senescence and inflammation (Georgilis et al., 2018; Hensel et al., 2022; Li et al., 2024; Yang et al., 2024).

4.2. PTB, senescence, and inflammation

One major facet of PTB functionality is that it plays a role in both senescence regulation and inflammatory state modulation (Georgilis et al., 2018; Hensel et al., 2022; Li et al., 2024; Yang et al., 2024). Zebrafish are unique in that they can modulate their inflammatory status from highly pro-inflammatory at the beginning of regeneration, to a more anti-inflammatory and pro-regenerative environment during later stages of regeneration (Bludau et al., 2024; Iribarne and Hyde, 2022b; Nagashima and Hitchcock, 2021; Zhou et al., 2022). This is a consistent with the transient detection and clearance of senescent cells as regeneration proceeds (Konar et al., 2024). Knockdown of PTB in zebrafish induced an increase in immune cell numbers (4c4+ cells) (Fig. 4A and B) and also upregulated numerous genes associated with the pro-inflammatory state (Fig. 3E–F). This suggests that PTB is playing a role in modulating the inflammatory state of the retina. The pro-inflammatory stage is often correlated with Müller glia reactivity, consistent with the observed increase in Müller glia-derived proliferation (Fig. 2B and C) (Bludau et al., 2024; García-García et al., 2024; Nelson et al., 2013). Together, our data support a model whereby depletion of *ptbp1* induces a change in SASP factor expression in the retina and that such microenvironmental changes can initiate a Müller glia-derived proliferative response.

CRediT authorship contribution statement

Gregory J. Konar: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Audrey L. Lingan:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Kyle T. Vallone:** Writing – review & editing, Software, Formal analysis, Data curation. **Tu D. Nguyen:** Data curation. **Zachary R. Flickinger:** Data curation, Conceptualization. **James G. Patton:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exer.2025.110420.

Data availability

Data will be made available on request.

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