

UC Berkeley

UC Berkeley Electronic Theses and Dissertations

Title

Müller Glia as a Source and Target of Lipoxin B4: Neurodegenerative and Neuroprotective Roles of Retinal Glia in Glaucoma

Permalink

<https://escholarship.org/uc/item/7dt2x21z>

ISBN

9798189272110

Author

Kumar, Matangi Nandha

Publication Date

2026-05-01

Peer reviewed|Thesis/dissertation

Müller Glia as a Source and Target of Lipoxin B₄: Neurodegenerative and Neuroprotective
Roles of Retinal Glia in Glaucoma

by

Matangi Nandha Kumar

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Vision Science

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Karsten Gronert, Chair

Professor John G. Flanagan

Professor John G. Flannery

Professor William Rowland Taylor

Spring 2026

Müller Glia as a Source and Target of Lipoxin B₄: Neurodegenerative and Neuroprotective
Roles of Retinal Glia in Glaucoma

©2026

By Matangi Nandha Kumar

Abstract

Müller Glia as a Source and Target of Lipoxin B₄: Neurodegenerative and Neuroprotective Roles of Retinal Glia in Glaucoma

by

Matangi Nandha Kumar

Doctor of Philosophy in Vision Science

University of California, Berkeley

Karsten Gronert, Chair

Glaucoma is a chronic neurodegenerative disease characterized by progressive retinal ganglion cell loss and irreversible vision decline. Although elevated intraocular pressure is a major risk factor, pressure-lowering therapies do not fully prevent disease progression, indicating that additional mechanisms contribute to retinal injury. Among these, Müller glia are increasingly recognized as central regulators of retinal homeostasis whose responses to mechanical, metabolic, and inflammatory stress can determine whether the retina adapts to injury or progresses toward chronic dysfunction. This dissertation investigates Müller glia as both mediators and therapeutic targets in glaucomatous neuroinflammation, with particular emphasis on the endogenous neuroprotective lipid mediator Lipoxin B₄ (LXB₄).

Chapter 1 presents the biological framework for the dissertation by reviewing Müller glia in health and disease. It highlights their essential roles in structural support, neurotransmitter recycling, ion and water homeostasis, and neurovascular regulation, and examines how these functions become disrupted under sustained stress. Particular emphasis is placed on glaucoma as a disease in which biomechanical strain, inflammatory signaling, and neuronal vulnerability converge to drive maladaptive glial reactivity.

Chapter 2 examines lipid mediator signaling in the eye and positions lipoxins, particularly LXB₄, as endogenous regulators of inflammatory control and tissue protection. This chapter synthesizes evidence that protective lipid pathways operate across ocular tissues and that LXB₄ is an emerging neuroprotective mediator in the retina. Prior studies identifying lipoxin signaling in retinal astrocytes, microglia, and retinal ganglion cells support a multicellular model of retinal protection, while also revealing a key unresolved question: whether Müller glia directly participate in LXB₄ biosynthesis and signaling.

Chapter 3 addresses this question experimentally and identifies Müller glia as both a source and target of LXB₄ signaling during ocular hypertension. Using primary Müller

glia, immortalized Müller glia, lipidomics, transcriptomic analyses, and mouse models of ocular hypertension, this work demonstrates that Müller glia express a functional lipoxin biosynthetic pathway and endogenously generate LXB₄. Ocular hypertension induced Müller glia reactivity, increased TRPV4 expression, and activated inflammatory pathways including IL-6/STAT3 signaling. Pharmacologic activation of TRPV4 recapitulated these reactive changes in vitro, whereas LXB₄ suppressed TRPV4-associated gliosis, reduced inflammatory gene expression, decreased STAT3 phosphorylation, and limited TRPV4 upregulation in vivo. These findings identify a previously unrecognized Müller glia–LXB₄ regulatory circuit linking mechanosensitive stress signaling to endogenous retinal protection.

Chapter 4 extends this framework to Müller glia structure by developing an FFT-based workflow to quantify directional organization of RLBP1-labeled Müller glia stalks in retinal sections. This approach demonstrates that ocular hypertension disrupts Müller glia-associated structural organization, particularly in ganglion cell layer-associated regions, and that LXB₄ partially preserves this organization in acute ocular hypertension. These findings establish structural remodeling as a quantifiable readout of glia state and provide a practical method for measuring Müller glial-associated tissue disorganization in disease.

Taken together, this dissertation identifies LXB₄ as a central protective pathway in glaucomatous retinal stress and establishes Müller glia as both contributors to disease progression and targets for therapeutic modulation. More broadly, these studies support a model in which effective neuroprotection in glaucoma may require not only control of intraocular pressure, but also restoration of endogenous lipid-mediated pathways that limit chronic gliosis and preserve retinal homeostasis.

Contents i

Contents	i
----------	---

List of Figures	ii
-----------------	----

List of Tables	iii
----------------	-----

1 Müller glia: In Health and Disease	1
1.1 Introduction	1
1.2 Müller glia structure and function	2
1.3 Müller glia and the inner blood-retinal-barrier	4
1.4 Mechanisms of Müller glia activation	8
1.5 Müller glia dysfunction in retinal disease	13
1.6 Dissertation overview	17
2 Lipid Mediator Signaling and Protective Therapeutic Strategies in Glaucoma	19
2.1 Introduction	19
2.2 Lipid mediator signaling and inflammatory regulation in the retina	19
2.3 Lipoxin biosynthesis and signaling	22
2.4 Protective lipid mediators in the eye	24
2.5 Lipoxins as neuroprotective mediators in the retina	25
2.6 Summary and implications for glaucoma	29
3 Lipoxin B₄ Mitigates TRPV4-Activated Müller Cell Gliosis During Ocular Hypertension	32
3.1 Introduction	32
3.2 Materials and methods	34
3.3 Results	41
3.4 Discussion	47
4 FFT-Based Quantification of RLBP1-Labeled Müller Glia Stalk Organization Reveals Disruption During Ocular Hypertension	55
4.1 Introduction	55
4.2 Methods	57
4.3 Results	59
4.4 Discussion	66
5 Concluding Remarks	70
Bibliography	73

List of Figures

1.1	Development, retinal architecture, and core homeostatic functions of Müller glia.	5
1.2	Müller glia and the retinal neurovascular unit and blood-retinal barrier.	9
1.3	JAK/STAT3 signaling as a regulatory pathway controlling Müller glia reactivity.	14
2.1	Arachidonic acid-derived lipid mediator pathways with emphasis on lipoxin biosynthesis.	24
2.2	Ocular distribution and functions of protective lipid mediator pathways.	26
2.3	LXB ₄ protects the inner retina and reduces macroglia reactivity in ocular hypertension.	30
3.1	Functional expression of the lipoxin pathway in Müller glia.	42
3.2	Ocular hypertension (OHT) induces GFAP and TRPV4 expression.	44
3.3	Activation of TRPV4 induces Müller glia reactivity and enhances LXB ₄ formation.	45
3.4	LXB ₄ Regulates JAK-STATs in Homeostatic Müller Glia In Vitro and In Vivo .	46
3.5	LXB ₄ Suppresses the IL6-STAT Pathway In Vitro	48
3.6	LXB ₄ Treatment Suppresses Proinflammatory Markers and TRPV4 Expression In Vivo	49
3.7	Model Scheme for the Müller Glia TRPV4-LXB ₄ Circuit. Proposed scheme for the Müller Glia LXB ₄ pathway.	50
S1	Validation of Rat Immortalized Müller glia (rMC-1)	53
S2	LC Chromatograms and Retention Times	54
4.1	RLBP1 labels Müller glia in the neural retina and reveals altered organization during ocular hypertension.	61
4.2	Workflow for FFT-based quantification of directional organization of RLBP1-labeled Müller glia stalks.	64
4.3	Chronic ocular hypertension progressively disrupts directional organization of RLBP1-labeled Müller glia stalks.	65
4.4	LXB ₄ partially preserves RLBP1-associated directional organization in acute ocular hypertension.	67

List of Tables

1.1	Representative inputs, sensing pathways, and response programs involved in Müller glia activation.	12
1.2	Shared and disease-weighted features of Müller glia dysfunction in glaucoma, diabetic retinopathy, and AMD.	18
3.1	Sequence of Mouse Forward and Reverse Primers	35
3.2	Sequence of Rat Forward and Reverse Primers	35

Acknowledgments

First and foremost, I have to thank Karsten and John for their mentorship and for giving me the opportunity to grow as a researcher and scientist. When I came to Berkeley, I knew I wanted to study the retina and glaucoma. When I toured the Gronert lab during our first-year lab survey course, I learned all about the exciting projects studying glaucomatous neuroinflammation in the retina and neuroprotective lipoxins. I was so excited to pursue a project in this space, and with Karsten's suggestion to explore the relatively unexplored Müller glia, I went on a five-year deep dive and fell in love with this glial cell in the retina. Even though a PhD is quite an unstructured environment, I am especially appreciative of Karsten's more structured mentorship style through the first couple years of the program, with both him and John as constant sounding boards for all my questions, and the growing freedom and independence Karsten gave me towards the later years, to really grow and explore on my own.

I could not have learned all that I did without the guidance of my postdoctoral mentors, now scientists, Shruthi and Shubham, from the Gronert and Flanagan labs. When I joined the lab, I knew only a handful of molecular techniques, and from the very beginning, both of them were committed to making sure I learned the methods I needed to succeed and build a strong PhD foundation. I will always carry with me the advice Shubham gave me while I was studying for my qualifying exam: "Learn the why behind every step of a method for your QE, and then you'll know how to troubleshoot when something goes wrong." I now pass that advice on to every new student who joins the lab. Thank you, Shubham, for your friendship, being my office mate, for answering my endless questions, and for making me a better scientist. Shruthi, thank you for being both a mentor and a friend. I will always appreciate your life and career advice, and I have become a better, more well-rounded scientist because of you. I also want to thank all the members of the Gronert and Flanagan labs, past and present. You made this experience so meaningful, and through all of you I learned not only how to be a researcher, but also how to be a better mentor and teacher.

Thank you to my thesis committee members, John Flannery and Rowland, for being such supportive figures during my qualifying exam preparation, and for challenging me in ways that made me better at thinking through and answering difficult questions. I am also deeply grateful to the Vision Science community and faculty for creating such a supportive and inclusive environment, one where I always felt I could contribute and be heard. Teresa, thank you for your mentorship and training during my rotation, and for your guidance while I was a GSI. It has always been inspiring to hear you speak about your research with such clarity and passion, and I am so glad to have had you as a mentor during this process, as well as a neighbor on the fifth floor. Special thanks to our collaborators in the Sivak lab for being part of the broader community that made this research possible. I will always value our monthly discussions and our reunions at ARVO.

I would not have made it through five years of this PhD without the friends I found in my Vision Science cohort and the program as a whole. To our group, Aur Naur: Galen, Josie, and Sonali — from meeting on the first day of orientation to all our adventures, wine

nights, late-night hangouts, grueling QE study days, and writing sessions, you have been with me through it all. Poorna, I had such a great time when you rotated with us. I loved all the chit-chats we had while doing experiments, laughing in the office, and pacing the hallways back and forth between labs. I have found lifelong friendships in all of you, and I am so grateful to have grown alongside you and to have celebrated so many big life moments together, including watching you all travel across the world for my wedding.

To my rocks and best friends: Inara, thank you for being my Bay Area bestie and for being someone I could confide in, relax with, and see week after week. You brought so much joy to a program whose finish line often felt so far away. Shritha, my partner in crime and my other brain cell, thank you for loaning it to me so I could get through the past 5 years. I will give mine back to you now, since it is your turn to be a grad student. Rosh, thank you for being a light, just like your name, always grateful for our chitty-chat sessions. I cherish you all so deeply. Thank you for believing in me and reminding me that I could do this. And to every friend who has visited me in Berkeley: I love you, and thank you being a part of my journey.

My parents — I do not even know where to start. Thank you for giving me a space where curiosity was always encouraged, and where I was allowed to ask and explore any question I wanted. Even when it may have been annoying, that freedom fostered in me a desire to become someone who asks questions and works to find answers. My research is deeply personal, and I feel fortunate that I was able to study this disease for us. I know a PhD is not the most traditional or structured path, so thank you for supporting me through all the years of answering, “I don’t know when I’ll finish.” Now I can finally answer that question: yes, I finished this PhD. To my brother, even though we were often far from each other, I am grateful for all the time we did share. Thank you for bringing fun and light into this process.

Barath, my husband, we took a chance by starting PhDs at two different schools, and although long distance was grueling and one of the hardest things I have ever done, thank you for being the person I could lean on through all of it. I am so appreciative of your hard work and always in awe of your dedication to science. You have been such a great sounding board for my ideas, my biggest source of support, and one of the people who made this journey possible. I am so proud of us for making it through and for finally putting an end to long distance.

Finally, thank you to everyone whose path crossed mine during these five years. You have all played a role in helping me through this journey, and I will always appreciate the fun memories, support, and community I found here.

Chapter 1

Müller glia: In Health and Disease

1.1 Introduction

The retina is a highly specialized neural tissue of the central nervous system (CNS) that converts light into neural signals (Nguyen et al. 2021). While more than fifty distinct neuronal subtypes contribute to visual processing in the human retina, increasing evidence has highlighted the essential role of retinal glia in maintaining tissue homeostasis, coordinating responses to injury, and shaping neuroinflammatory signaling (García-Bermúdez et al. 2021).

The vertebrate retina contains three principal glia subtypes: astrocytes, microglia, and Müller glia (Tworig and Feller 2021). Among these, Müller glia represent the predominant macroglia population, comprising approximately ninety percent of retinal glia and uniquely originating from retinal progenitor cells (Tworig and Feller 2021; MacDonald, Charlton-Perkins, and Harris 2017). Spanning the full thickness of the retina, Müller glia provide structural support, regulate ion and water balance, recycle neurotransmitters, and contribute to blood–retinal barrier function. Through extensive interactions with neurons and vasculature, Müller glia maintain structural stability, metabolic support, and vascular integrity across retinal layers (Li, Liu, et al. 2019; Tout et al. 1993).

Müller glia are therefore uniquely positioned to support neuronal activity, metabolic demand, vascular function, and inflammatory signaling. In response to stress, they undergo transitions from homeostatic support to reactive states that can initially preserve tissue integrity but, when sustained, contribute to chronic inflammation and neurodegeneration. Defining the mechanisms that govern these state transitions is critical for understanding disease progression and identifying therapeutic opportunities.

In this chapter, I establish the biological framework for the dissertation by outlining the structural and physiological roles of Müller glia in retinal homeostasis, the major stressors that drive their activation, and the consequences of sustained reactivity in disease. Particular emphasis is placed on how mechanical, metabolic, and inflammatory stress reshape Müller glia function in ways relevant to glaucomatous neuroinflammation. This framework provides

the basis for subsequent chapters examining lipid mediator pathways, especially lipoxins and Lipoxin B4, as therapeutic regulators of Müller glia state.

1.2 Müller glia structure and function

Development of Müller glia

In contrast to retinal astrocytes, which enter the retina through the optic nerve during development, and microglia, which originate outside the neuroepithelium and later colonize the tissue, Müller glia arise from multipotent retinal progenitor cells and share lineage with retinal neurons (Tworig and Feller 2021; Paisley and Kay 2021; Li, Jiang, and Samuel 2019). Their differentiation occurs late in retinogenesis, alongside rod photoreceptors and bipolar cells, following the establishment of major synaptic layers. In rodents, Müller glial differentiation begins around embryonic day 18 and continues into the early postnatal period (Tworig and Feller 2021; Jadhav, Roesch, and Cepko 2009).

This late developmental timing is functionally significant. Unlike cortical radial glia, which guide neuronal migration during early neurogenesis, Müller glia mature within an already organized retinal circuit (Tworig and Feller 2021; Jadhav, Roesch, and Cepko 2009). This timing positions them to stabilize established neuronal architecture at a stage when synaptic activity and metabolic demand increase. As Müller glia mature, they assume key roles in maintaining extracellular homeostasis and supporting reliable neurotransmission.

Müller glia morphology and architecture

Müller glia are radially oriented cells that span the full thickness of the retina, and this depth-spanning geometry underlies their physiological roles. By extending through synaptic layers and terminating in specialized endfeet, Müller glia physically couple sites of neurotransmitter release, ionic flux, and metabolic demand with vascular and vitreous interfaces (García-Bermúdez et al. 2021; Kolb 1995; Grimes et al. 2024). This organization enables coordinated regulation across retinal depth rather than localized support within a single layer.

Across retinal layers, Müller glia adopt distinct morphological specializations that reflect local functional demands. In the outer retina, dense microvillar processes interface closely with photoreceptor inner segments and contribute to the outer limiting membrane through junctional complexes (MacDonald, Charlton-Perkins, and Harris 2017; Wang, O’Sullivan, et al. 2017; Jubran et al. 2025). Within the outer nuclear and outer plexiform layers, Müller glia processes extend between photoreceptors, bipolar cells, and horizontal cells, positioning them to regulate extracellular signaling within synaptic regions (Wang, O’Sullivan, et al. 2017; Grimes et al. 2025). Their cell bodies reside in the inner nuclear layer, while fine processes extend into the inner plexiform layer to interact with amacrine and bipolar cell synapses. At the vitreal surface, Müller glia endfeet form a dense network that ensheathes

neurons and retinal vessels, providing an anatomical basis for neurovascular signaling and contributions to blood–retinal barrier organization (Tout et al. 1993; Grimes et al. 2025).

Müller glia share a conserved radial organization across the retina, but their morphology is not identical across all retinal layers or regions. Large-scale ultrastructural reconstructions and layer-specific analyses reveal sublaminal specialization, particularly within the inner plexiform layer (Grimes et al. 2025). Variations in arbor density across functional sublayers suggest that Müller glia architecture may be tuned to the activity patterns of specific neuronal circuits (Wang, O’Sullivan, et al. 2017). Regional differences are also evident. In primate retina, Müller glia density and molecular profiles differ between foveal and peripheral regions, consistent with adaptation to the high metabolic and signaling demands of the fovea (Kar et al. 2024; Masri et al. 2025). In contrast, rodent retinas display a more uniform spatial distribution, although molecular heterogeneity is still observed (Wang, O’Sullivan, et al. 2017). These findings indicate that Müller glia structure is functionally specialized and responsive to local energetic and synaptic demands. This architectural organization provides an essential framework for interpreting structural remodeling in disease. Because Müller glia processes define physical boundaries between neurons, vessels, and extracellular space, even subtle morphological changes may have amplified effects on synaptic stability and vascular signaling. This depth-spanning architecture also positions Müller glia to regulate the extracellular environment at synapses, making neurotransmitter handling a central homeostatic function.

Neurotransmitter homeostasis

Müller glia are essential regulators of neurotransmitter homeostasis in the retina, where they help maintain the extracellular environment required for synaptic signaling. One of their central functions is the clearance of extracellular glutamate through excitatory amino acid transporters, particularly GLAST/EAAT1, which limits glutamate accumulation and protects retinal neurons from excitotoxic injury (Reichenbach, Wurm, et al. 2007; Peña and Vazquez 2022). After uptake, glutamate is converted to glutamine by glutamine synthetase, an enzyme highly enriched in Müller glia. Glutamine is then returned to neurons for neurotransmitter resynthesis, supporting continued synaptic activity while preventing excessive extracellular glutamate signaling (**Bringmann2009**; Reichenbach, Wurm, et al. 2007).

Müller glia also contribute to inhibitory neurotransmitter regulation through uptake systems for GABA, including GABA transporters expressed on Müller glial membranes. By participating in both glutamate and GABA handling, Müller glia help balance excitatory and inhibitory signaling across retinal circuits. Disruption of these pathways can impair neurotransmitter recycling, increase excitotoxic stress, and contribute to neuronal dysfunction in retinal disease. Thus, neurotransmitter homeostasis represents a key mechanism through which Müller glia support retinal signaling and protect neurons under physiological conditions.

Ion and water homeostasis

In addition to neurotransmitter regulation, Müller glia maintain extracellular ion and water balance across the retina. Neuronal activity is accompanied by changes in extracellular potassium, and Müller glia buffer these fluctuations through inwardly rectifying potassium channels, particularly Kir4.1, which are enriched in specialized membrane domains (Peña and Vazquez 2022; Han et al. 2024). This spatial potassium buffering helps stabilize neuronal excitability and supports normal retinal signaling. Experimental disruption of Kir4.1 alters potassium handling and changes electroretinogram responses, including reductions in b-wave components and altered slow potentials that reflect glial contributions to retinal physiology (Han et al. 2024; Reichenbach, Wurm, et al. 2007; Gao et al. 2021).

Water movement is closely coupled to ion transport and is mediated in part by aquaporin-4 (AQP4), which is enriched in Müller glial endfeet and other membrane regions associated with fluid exchange. Dysregulation of AQP4 and Kir4.1 has been linked to retinal edema, altered osmotic balance, and impaired retinal function in stress models (Han et al. 2024; Abhyankar et al. 2026; Reichenbach and Bringmann 2020). Together, Kir4.1- and AQP4-dependent mechanisms allow Müller glia to stabilize the ionic and osmotic environment of the retina. When these pathways are disrupted, impaired ion and water homeostasis can contribute to edema, altered neuronal signaling, and increased vulnerability to injury.

Together, these homeostatic roles position Müller glia as key regulators of the retinal microenvironment rather than passive structural support cells. However, the same properties that make them essential for neuronal and vascular support also position them to respond dynamically to retinal stress. When exposed to persistent mechanical, metabolic, or inflammatory stress, these homeostatic functions become vulnerable to dysregulation, shifting Müller glia toward reactive states that can reshape the retinal microenvironment.

1.3 Müller glia and the inner blood-retinal-barrier

Structural organization of the retinal neurovascular unit

Beyond regulating synaptic and ionic homeostasis, Müller glia also help coordinate vascular stability and metabolic exchange within the retinal neurovascular unit. The retina is among the most metabolically active tissues in the body, with exceptionally high oxygen and glucose demand compared with many other tissues, including average brain tissue (O’Leary and Campbell 2023). This high metabolic demand makes retinal function especially dependent on glia mechanisms that couple neuronal activity to vascular supply. As a result, retinal function depends on tightly regulated blood flow, and even modest reductions in perfusion increase susceptibility to oxidative and ischemic stress that manifests as visual dysfunction. This vulnerability is further shaped by a key anatomical constraint: much of the retina must remain optically transparent to preserve light transmission to photoreceptors, limiting vascularization within the outer retina. The retina therefore relies on a dual circulation

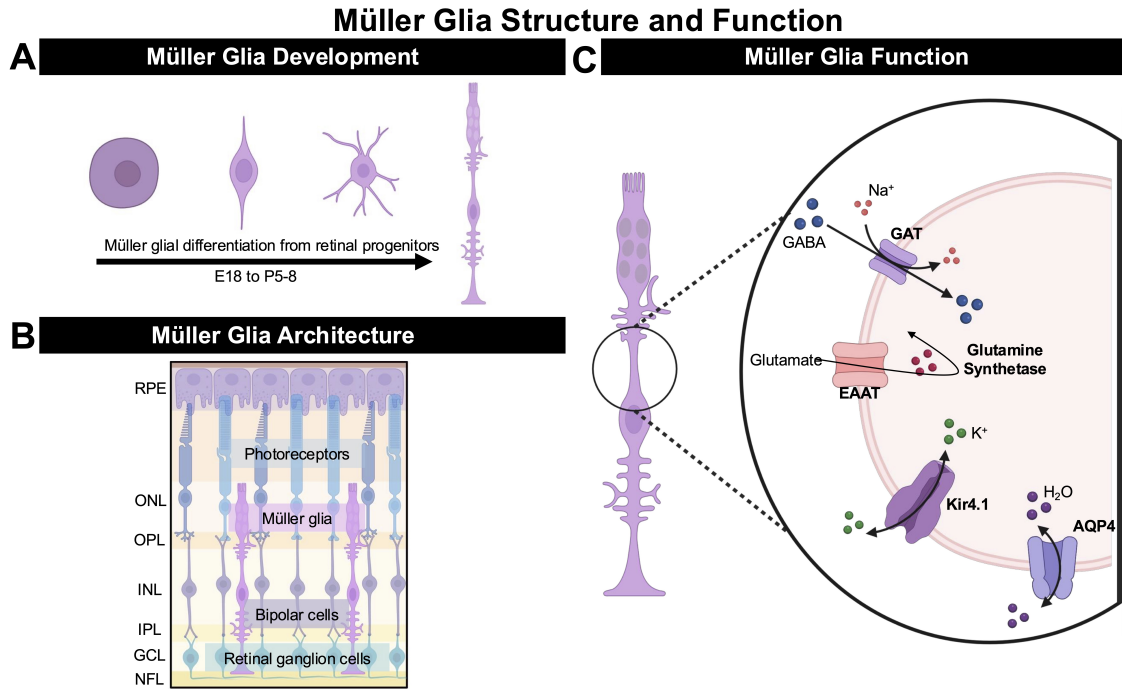


Figure 1.1: (A) Schematic of Müller glia development in the mouse retina. Müller glia arise from late-stage retinal progenitor cells and differentiate during late embryonic and early postnatal development, beginning around embryonic day 18 (E18) and continuing through approximately postnatal day 5 to 8 (P5–P8). (B) Schematic of Müller glia architecture within the layered retina. Müller glia extend radially across the neural retina, spanning from the outer to inner retinal compartments and interacting closely with retinal neurons throughout multiple laminae. Abbreviations: RPE, retinal pigment epithelium; PL, photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, nerve fiber layer. (C) Simplified schematic of representative Müller glia functions. These include neurotransmitter handling through GABA uptake and glutamate clearance, glutamine synthesis, potassium buffering mediated in part by Kir4.1, and water homeostasis associated with AQP4 (García-Bermúdez et al. 2021; Tworig and Feller 2021; Han et al. 2024).

system and specialized barrier properties collectively referred to as the blood–retinal barrier (BRB) (O’Leary and Campbell 2023; Bora et al. 2023; Runkle and Antonetti 2011). The outer retina is largely avascular and receives metabolic support primarily from the choroidal circulation. The outer BRB is formed by the retinal pigment epithelium (RPE) (O’Leary and Campbell 2023; Runkle and Antonetti 2011). In contrast, the inner retina is supplied by the retinal circulation arising from the central retinal artery. The inner BRB is established by tight junctions between retinal vascular endothelial cells and is supported by the retinal neurovascular unit, which includes pericytes and surrounding glia and immune cells that coordinate barrier maintenance and blood flow responses to local metabolic demand (Runkle and Antonetti 2011; Coorey, Shen, Chung, et al. 2012; Grimes et al. 2025).

Glia–vascular interactions within this unit are layer-specific. Astrocytes are primarily associated with vessels in the superficial vascular plexus, whereas Müller glia provide the dominant glial contribution to vascular interfaces in deeper retinal layers. Müller glial endfeet and processes closely appose capillaries and synaptic regions, positioning them to influence vascular permeability, blood flow, and neuron–vascular communication (Bora et al. 2023; Claude 1963; Coorey, Shen, Chung, et al. 2012). Ultrastructural studies show that Müller glial coverage of retinal vessels varies across the superficial, intermediate, and deep vascular plexuses, suggesting that regulation of the inner blood–retinal barrier is locally organized rather than uniform (Coorey, Shen, Chung, et al. 2012; Grimes et al. 2025). This layer-dependent organization may help align vascular stability with the metabolic and synaptic demands of specific retinal regions. During disease, reactive Müller glia can disrupt this balance by promoting permeability and inflammatory signaling, which may weaken barrier integrity, alter extracellular homeostasis, and impair neuron–vascular communication.

Regulation of the inner blood–retinal barrier

Under homeostatic conditions, Müller glia contribute to vascular stability by secreting anti-angiogenic factors and maintaining regulatory control over the neurovascular unit (Chen, Xia, et al. 2022). In the inner retina, barrier function is primarily established by tight junctions between endothelial cells, while surrounding cells modulate endothelial stability and permeability through signaling and structural support. Müller glia and endothelial cells also communicate through gap junction networks and connexin expression patterns that support intercellular coupling and coordinated responses to stress (Medina-Arellano et al. 2024). Connexin 43 is highly expressed in retinal glia, including Müller glia, and is also present in retinal vascular cells under specific conditions, supporting a role for gap junction communication in vascular regulation (Peña and Vazquez 2022).

A major pathway through which Müller glia influence vascular behavior is VEGF signaling. Müller glia, together with astrocytes and other retinal cells, produce VEGF under both developmental and stress conditions (Chen, Xia, et al. 2022; Le 2017). glia-derived VEGF contributes to retinal vascular growth and maintenance (Medina-Arellano et al. 2024; Le 2017). At the same time, Müller glia produce anti-angiogenic factors such as PEDF and thrombospondins, and shifts in the balance between pro- and anti-angiogenic signaling alter

vascular stability and promote neovascularization in disease (Medina-Arellano et al. 2024; Le 2017).

Because VEGF signaling is context dependent, shifts in ligand–receptor balance can alter outcomes toward vessel stabilization, permeability, or pathological angiogenesis. This makes Müller glia regulation of VEGF pathways an important determinant of neurovascular homeostasis and disease (Le 2017).

Neurovascular dysfunction and barrier breakdown

Angiogenesis is essential during retinal development, but vascular stability in the mature retina depends on maintaining a tightly regulated balance between pro-angiogenic and anti-angiogenic signals. When this balance is disrupted by hypoxia, ischemia, oxidative stress, or chronic inflammatory conditions, the neurovascular unit shifts toward barrier compromise, vascular leakage, and, in severe cases, pathological neovascularization. Müller glia are central to this transition because they respond rapidly to metabolic stress and alter the local profile of angiogenic and inflammatory mediators (Peña and Vazquez 2022; Vecino et al. 2016).

Under hypoxic or ischemic stress, Müller glia upregulate the release of pro-angiogenic and pro-inflammatory factors, including increased VEGF expression, which promotes vascular permeability and contributes to inflammatory vascular pathology (Yu et al. 2010; Medina-Arellano et al. 2024; Le 2017; Kumari, Barman, and Ganesan 2025). Conditional genetic studies demonstrate that Müller glia-derived VEGF is a major driver of vascular leakage and inflammatory lesions in retinal vascular disease models, supporting a causal role for Müller glia in barrier breakdown rather than a purely secondary response (Carpi-Santos et al. 2022). Müller glia also release additional mediators that influence endothelial behavior and remodeling, including FGF2 and cytokines such as $\text{TNF}\alpha$ which together amplify permeability and angiogenic signaling during disease (Balzamino et al. 2024).

Reactive and proliferative glia responses can further destabilize the blood–retinal barrier by altering Müller glia interactions with vascular and synaptic connections. EGFR signaling has been implicated in Müller glia proliferative responses in injury models, and growth factor signaling pathways that promote gliosis can intersect with vascular outcomes by shifting Müller glia toward states that favor permeability and inflammatory amplification (Peña and Vazquez 2022; Balzamino et al. 2024; Zhou et al. 2025). These findings support a model in which neurovascular dysfunction reflects not only endothelial pathology, but also stress-driven Müller glia state changes that reshape the balance of angiogenic and barrier-stabilizing signals at the inner blood–retinal barrier.

Müller glia–retinal ganglion cell metabolic coupling

Müller glia support retinal function not only by maintaining vascular stability but also by sustaining neuronal survival under metabolic stress. Because they span the full thickness of the retina and interface with both synaptic and vascular compartments, their metabolic

support extends across multiple neuronal classes, including retinal ganglion cells (RGCs), which are particularly vulnerable to metabolic disruption (Zhou et al. 2025).

A defining feature of Müller glia is their capacity to couple neuronal activity to metabolic and osmotic homeostasis. By controlling extracellular potassium and water balance, recycling neurotransmitters, and providing metabolic substrates, Müller glia stabilize the extracellular environment required for sustained RGC signaling (Vecino et al. 2016; Kumari, Barman, and Ganesan 2025; Zhou et al. 2025). This glia–neuron metabolic coupling becomes especially important during energetic challenge, when RGCs experience reduced substrate availability and increased oxidative stress. Under ischemic conditions, neurons release activity-dependent signals that engage Müller glia and shift glia physiology toward stress-responsive states, altering glia–neuron communication and potentially reshaping circuit output (Peña and Vazquez 2022; Zhou et al. 2025; Rombaut et al. 2023).

These observations support the view that Müller glia act as central metabolic intermediaries linking retinal activity with vascular supply. Although the mechanisms governing Müller glia–RGC coupling, and how they are altered during stress, remain incompletely defined, clarifying these pathways will be important for understanding why RGCs are particularly vulnerable in neurodegenerative retinal disease. Because these neurovascular and metabolic support functions are highly sensitive to mechanical, inflammatory, and oxidative stress, they provide a useful framework for understanding the inputs that drive Müller glia activation. Because Müller glia occupy this interface between neuronal demand, vascular function, and inflammatory signaling, they are well positioned to influence whether retinal stress resolves or progresses toward chronic dysfunction. The key question that follows is how sustained Müller glia reactivity changes homeostatic functions across disease contexts.

1.4 Mechanisms of Müller glia activation

Following retinal stress or injury, Müller glia are among the earliest responders and rapidly shift from homeostatic support toward reactive states that initially aim to preserve tissue integrity (Graca, Hippert, and Pearson 2018). This response, broadly termed gliosis, can be adaptive in early stages by stabilizing the extracellular environment, limiting damage spread, and supporting neuronal survival. However, when reactive programs are sustained or excessive, gliosis becomes maladaptive and contributes to chronic neuroinflammation, synaptic dysfunction, and progressive neurodegeneration (Graca, Hippert, and Pearson 2018; Seitz, Ohlmann, and Tamm 2013).

Müller glia activation is triggered by diverse inputs, including mechanical strain, metabolic imbalance, inflammatory cytokines, and disrupted neuronal activity. These inputs converge on shared transcriptional and signaling programs that generate graded reactive states. A commonly used hallmark of Müller glia reactivity is the induction of intermediate filament proteins, particularly glial fibrillary acidic protein (GFAP), vimentin, and nestin (Graca, Hippert, and Pearson 2018; Bringmann and Wiedemann 2011). These proteins

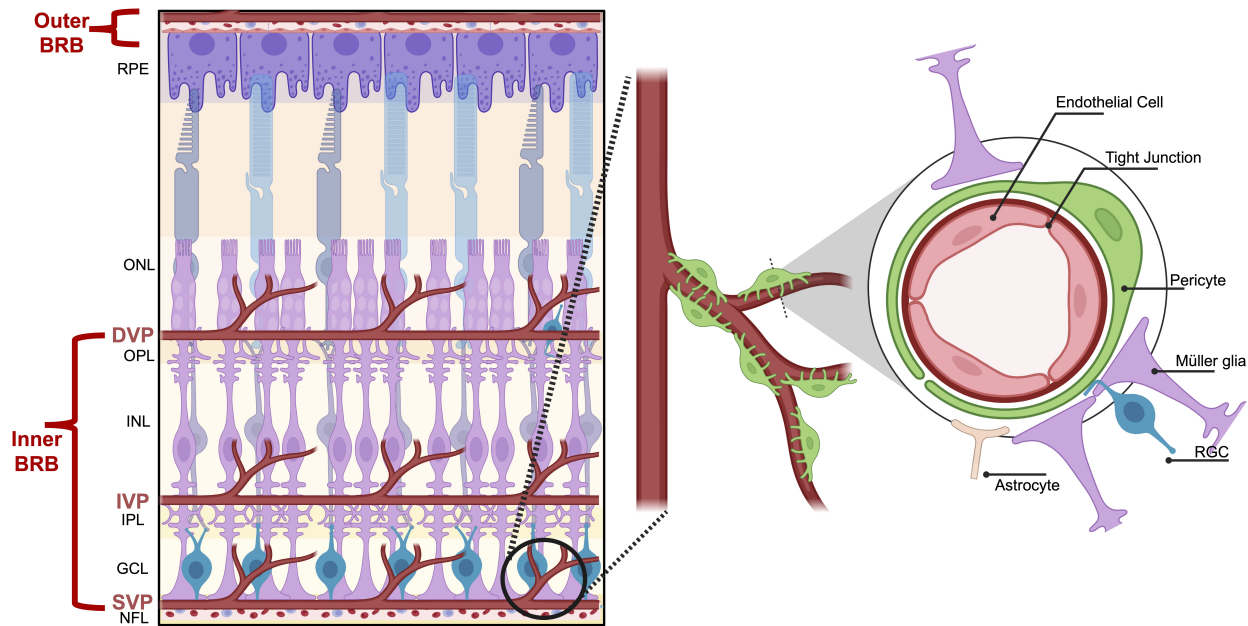


Figure 1.2: Schematic illustrating the position of Müller glia within the layered retina and their association with the outer and inner blood-retinal barriers. The outer blood-retinal barrier is formed by the retinal pigment epithelium (RPE), whereas the inner blood-retinal barrier is established by retinal vascular endothelial cells linked by tight junctions and supported by pericytes and glia cells. Müller glia endfeet contact vessels within the superficial (SVP), intermediate (IVP), and deep vascular plexuses (DVP). The magnified inset highlights the inner retinal neurovascular unit, in which Müller glia endfeet closely appose endothelial cells and pericytes, together with astrocytes and adjacent retinal ganglion cells (RGCs). Abbreviations: BRB, blood-retinal barrier; RPE, retinal pigment epithelium; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, nerve fiber layer; RGC, retinal ganglion cell (Tout et al. 1993; O’Leary and Campbell 2023; Runkle and Antonetti 2011).

are not only markers of reactivity but also components of the cytoskeletal remodeling that accompanies glial stress responses, supporting changes in process organization, mechanical stability, and interactions with damaged retinal tissue. In more severe or persistent conditions, these changes are accompanied by remodeling of retinal architecture, hypertrophy of glia processes, and, in some contexts, formation of glia scars that alter neuronal and vascular interactions.

In the following sections, I outline major classes of activating inputs, including mechanical stress and mechanotransduction, metabolic and oxidative stress, and inflammatory signaling, before highlighting convergent transcriptional programs that underlie gliosis across retinal disease contexts.

Mechanical stress and mechanotransduction

Because Müller glia anchor at the inner and outer limiting membranes, they are well positioned to detect forces acting across retinal depth. Unlike much of the central nervous system, which is mechanically buffered by surrounding structures and cerebrospinal fluid, the neural retina is a thin laminated tissue exposed to intraocular pressure and the vitreous interface, making it particularly sensitive to deformation, traction, and shear (Lundkvist et al. 2004). Key mechanical inputs include intraocular pressure-related strain, vitreoretinal traction associated with eye movements, osmotic swelling, and changes in extracellular matrix stiffness that accompany aging and disease (Lundkvist et al. 2004; Prieto-López, Pereiro, and Vecino 2024; Lindqvist et al. 2010). These forces are not purely structural, as they can reshape synaptic and vascular microenvironments and represent upstream drivers of Müller glia state transitions.

Müller glia respond to mechanical deformation through mechanotransduction pathways involving mechanosensitive ion channels, cytoskeletal remodeling, and adhesion signaling. Transient receptor potential (TRP) channels, particularly TRPV4, with reported contributions from TRPC1 in some contexts, as well as Piezo channels, have been implicated in encoding membrane tension and mechanical load. In parallel, integrin-mediated adhesions and cytoskeletal dynamics couple extracellular forces to intracellular signaling pathways (Jo, Ryskamp, et al. 2015; MacDonald, Randlett, et al. 2015). In intact tissue, Müller glia responses are also influenced by signals from mechanically stressed neurons and vascular cells making it important to distinguish direct mechanosensing from secondary signaling in experimental models (Prieto-López, Pereiro, and Vecino 2024; MacDonald, Randlett, et al. 2015).

A common early output of mechanical activation is an increase in intracellular calcium, which alters volume regulation and engages kinase and transcriptional programs associated with gliosis (Lindqvist et al. 2010; MacDonald, Randlett, et al. 2015; Cullimore et al. 2023). Downstream consequences include induction of reactivity markers, structural remodeling of glia processes, and increased release of inflammatory and vasoactive mediators that influence barrier permeability and neurovascular signaling. Because mechanical strain often coincides

with energetic challenge and oxidative stress in retinal disease, these pathways are closely linked to metabolic stress responses.

Inflammatory and immune signaling

Pro-inflammatory signaling is a well-established trigger of Müller glia activation and arises from cytokines and growth factors released by neurons, vascular-associated cells, and other glia, particularly microglia (Kirsch et al. 2010; Wang, Ma, et al. 2011). Müller glia are highly responsive to cytokines including $\text{TNF}\alpha$ and IL-6 family ligands, which activate pathways such as $\text{NF-}\kappa\text{B}$ and JAK/STAT signaling through gp130 (Zhao, Wan, et al. 2014). These inputs provide a direct route by which immune activation and tissue stress initiate Müller glia reactivity (Kirsch et al. 2010; Wang, Ma, et al. 2011; Ji et al. 2022; Coorey, Shen, Zhu, et al. 2015).

Microglia–Müller glia crosstalk represents a central axis of this response. Evidence from in vivo and in vitro models suggests that microglia activation often precedes or coincides with Müller glia activation, with microglia-derived factors such as $\text{TNF}\alpha$ and other cytokines acting as signals that engage $\text{NF-}\kappa\text{B}$ - and STAT-dependent programs in Müller glia (Wang, Ma, et al. 2011; Ji et al. 2022). This interaction is bidirectional: microglia release signals that activate Müller glia, while activated Müller glia, in turn, release cytokines and other mediators that propagate inflammatory signaling across retinal layers and further influence microglia and the retinal vasculature (Wang, Ma, et al. 2011; Ji et al. 2022). This coupling helps explain how localized immune activation can drive widespread Müller glia reactivity.

Although these stress inputs are often discussed separately, they converge in disease. Mechanical strain, oxidative stress, and inflammatory cytokine signaling interact to reinforce reactive Müller glia states and disrupt core homeostatic functions. This convergence suggests that effective therapy may require not only suppression of individual downstream pathways, but active engagement of endogenous programs that limit inflammation and restore homeostasis.

Regulatory pathways controlling müller glia reactivity

Beyond identifying activating inputs, it is critical to define the intracellular pathways that regulate Müller glia reactivity. Alongside $\text{NF-}\kappa\text{B}$, AP-1, and other stress-response pathways, one of the best-characterized signaling axes is the gp130–JAK–STAT3 pathway. Cytokines of the IL-6 family, including CNTF and LIF, signal through receptor complexes that recruit gp130, leading to activation of JAK kinases and phosphorylation of STAT3 (Hagemann et al. 2023; Kirsch et al. 2010). Phosphorylated STAT3 translocates to the nucleus and binds STAT-responsive elements within regulatory regions of target genes, including GFAP, promoting induction of intermediate filament proteins associated with gliosis (Coorey, Shen, Chung, et al. 2012; Brenner and Messing 2021).

Across multiple retinal injury models, increased LIF expression and STAT3 activation correlate with Müller glia reactivity, and genetic or pharmacological suppression of

Input Category	Key Signals and Sensors	Müller Glia Response
Mechanical	Elevated IOP, tissue stretch, cell swelling, shear stress; sensed through TRP channels, Piezo channels, and integrin–ECM signaling	Calcium influx, MAPK/ERK activation, reactive gliosis, cytoskeletal and extracellular matrix remodeling, and release of inflammatory mediators and growth factors
Oxidative and Metabolic	Hypoxia, ROS, NADPH oxidase activity, mitochondrial dysfunction, excitotoxic or metabolic stress, neurotoxins	Reactive gliosis, Nrf2 activation and glutathione-associated antioxidant responses, metabolic reprogramming with increased glycolytic dependence, impaired glutamate homeostasis, cytokine release, and in severe states, dedifferentiation or dysfunction
Inflammatory and Immune	Cytokines, chemokines, NF- κ B and JAK/STAT signaling, VEGF, inflammatory lipids, complement, and microglial activation	Reactive gliosis, chemokine and cytokine release, immune cell recruitment and glia–immune crosstalk, ROS/NO production, structural remodeling, vascular/barrier dysfunction, and proliferation

Table 1.1: Müller glia respond to diverse retinal stressors that can be broadly grouped into mechanical, oxidative/metabolic, and inflammatory/immune categories. This table highlights representative mediators and sensors associated with each class of input, together with common downstream Müller glia responses. These include reactive gliosis, calcium-dependent signaling, antioxidant and metabolic reprogramming, cytokine and chemokine release, structural remodeling, and altered vascular or barrier support. While shown separately for clarity, these pathways are highly interconnected in retinal disease and often act in combination to shape the magnitude and outcome of Müller glia reactivity (Chen, Xia, et al. 2022; Rombaut et al. 2023; Jo, Lakk, et al. 2022; Toft-Kehler et al. 2016).

gp130–JAK–STAT signaling reduces hallmark features of gliosis, including GFAP upregulation (Fischer and Bongini 2010). In regenerative systems such as zebrafish, this pathway is also engaged during injury responses and is linked to Müller glia reprogramming and progenitor-like states, highlighting that STAT3 can support both protective and plastic responses depending on context (Fischer and Bongini 2010).

Importantly, Müller glia reactivity is not uniformly detrimental; early activation can be neuroprotective. Therapeutic strategies targeting this pathway will therefore likely need to modulate the magnitude and duration of signaling rather than eliminate it entirely. Together, these pathways suggest that Müller glial reactivity is best understood not as a single fixed endpoint, but as a range of stress-associated states. Mechanical, metabolic, and inflammatory insults can converge on overlapping inflammatory, metabolic, and cytoskeletal programs, while the specific outcome depends on the initiating stressor, disease context, and duration of injury.

1.5 Müller glia dysfunction in retinal disease

After defining the inputs that trigger Müller glia activation and recognizing that early gliosis may be neuroprotective, it is equally important to consider the consequences of sustained activation in chronic retinal and neurodegenerative disease. Prolonged Müller glia reactivity is associated with structural remodeling and persistent inflammatory signaling that impair core homeostatic functions, including neurotransmitter clearance, ion and water regulation, metabolic support, and neurovascular stability. Over time, these changes contribute to neuronal dysfunction and accelerate degeneration through mechanisms including increased pro-inflammatory mediator release, heightened vulnerability to excitotoxic stress, and sustained crosstalk with activated microglia. Many of these disease-associated changes reflect sustained engagement of the activation pathways described above. Although Müller glia dysfunction contributes to multiple retinal diseases, the remainder of this dissertation focuses particularly on glaucoma, where biomechanical stress, inflammatory signaling, and neuronal vulnerability converge in ways that make Müller glia attractive therapeutic targets.

Age is an additional modifier of these responses. Evidence suggests that both the magnitude and persistence of gliosis, including GFAP induction, increase with age, potentially lowering the threshold for maladaptive reactivity after injury (Graca, Hippert, and Pearson 2018). In this section, we discuss how Müller glia functions are altered across major retinal degenerative and neurodegenerative conditions that disproportionately affect aging populations, with emphasis on how chronic gliosis reshapes Müller glia support of retinal neurons and the neurovascular unit.

Glaucoma: mechanical stress and RGC vulnerability

Glaucoma is a group of chronic optic neuropathies characterized by progressive retinal ganglion cell (RGC) dysfunction and loss, leading to irreversible vision loss. Elevated

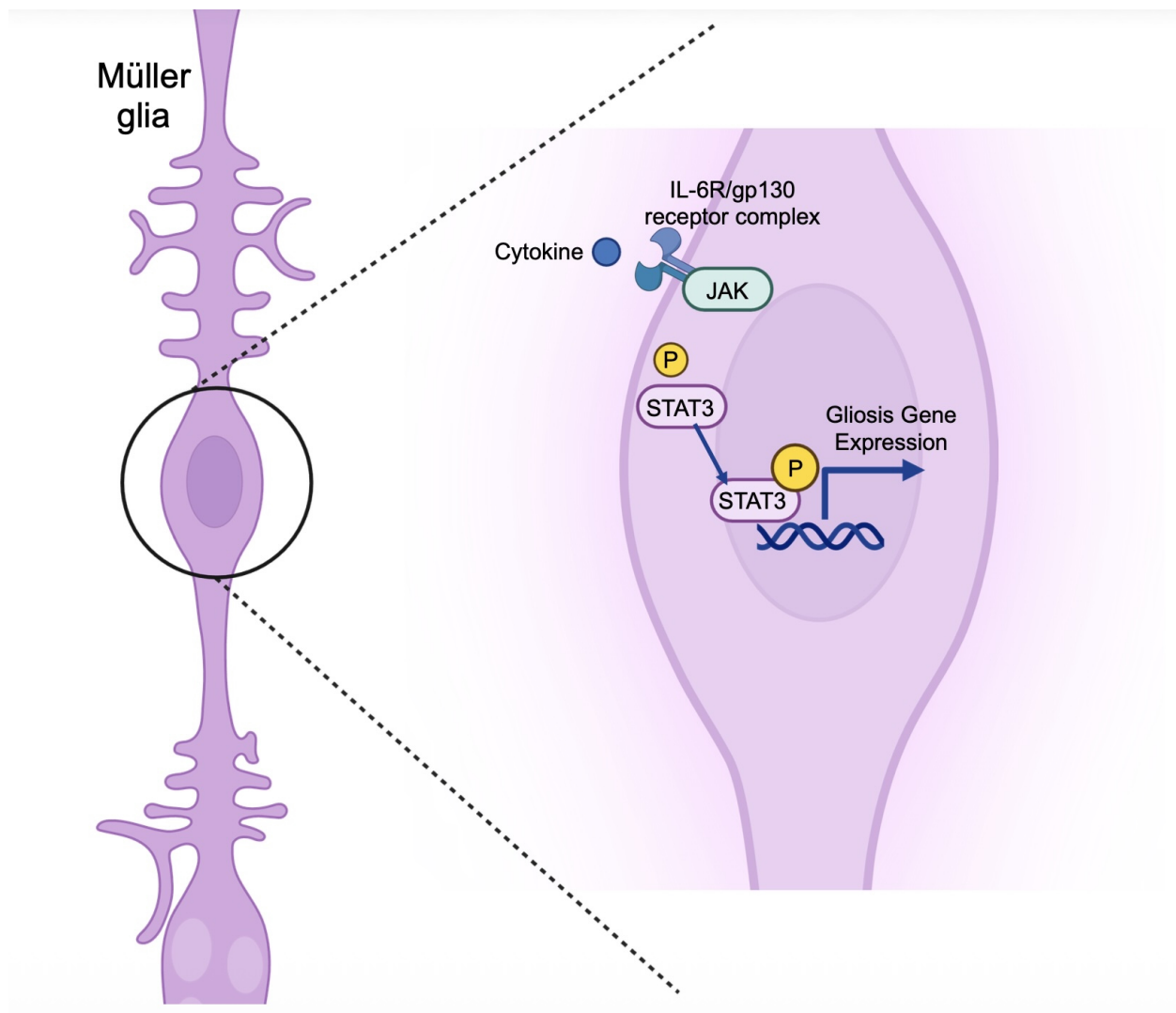


Figure 1.3: Schematic illustrating cytokine-driven activation of the JAK/STAT3 pathway in Müller glia. Binding of extracellular cytokines to the gp130/IL-6 receptor complex activates Janus kinase (JAK), leading to phosphorylation of signal transducer and activator of transcription 3 (STAT3). Phosphorylated STAT3 then translocates to the nucleus, where it promotes transcription of gliosis-associated genes, for example glial fibrillary acidic protein (GFAP) (Hagemann et al. 2023; Brenner and Messing 2021).

intraocular pressure (IOP) is a major modifiable risk factor, particularly in primary open-angle glaucoma, but disease progression reflects a broader neurodegenerative process shaped by mechanical strain, metabolic stress, and neuroinflammation (Graca, Hippert, and Pearson 2018; Inman and Horner 2007). Current clinical therapies primarily lower IOP, yet many patients continue to exhibit ongoing glia reactivity and RGC degeneration despite pressure reduction (García-Bermúdez et al. 2021). This highlights a central limitation in treatment: controlling the various biomechanical stressors does not resolve the cellular programs that sustain chronic neurodegeneration.

Müller glia are well positioned to sense IOP-related strain through mechanosensitive pathways and translate these cues into changes in extracellular homeostasis and inflammatory signaling (Jo, Lakk, et al. 2022). Across experimental glaucoma models, Müller glia gliosis, commonly indexed by increased GFAP expression, occurs early and may persist even after IOP normalizes, suggesting that transient stress can induce long-lasting glia state changes. The magnitude and pattern of GFAP induction vary across models, but in DBA/2J glaucoma, increased Müller glia GFAP correlates with disease severity and neuronal injury in advanced stages (Caldwell et al. 2023).

Mechanistically, Müller glia dysfunction directly increases RGC vulnerability by impairing core homeostatic roles, including glutamate clearance and potassium buffering. Dysregulation of glutamate transport and Kir channels elevates extracellular glutamate and potassium, increasing excitability and excitotoxic stress within retinal circuits (Seitz, Ohlmann, and Tamm 2013; Shinozaki and Koizumi 2021). In parallel, metabolic stress and mitochondrial dysfunction compromise energy-dependent glia support functions, further destabilizing the RGC microenvironment. Purinergic signaling may also contribute, as extracellular ATP release and activation of P2X receptors amplify calcium dysregulation and inflammatory signaling in glia and neurons, reinforcing degenerative cascades (García-Bermúdez et al. 2021; Seitz, Ohlmann, and Tamm 2013; Shinozaki and Koizumi 2021).

An unresolved question is whether Müller glia reactivity is a driver of RGC injury or a response to early neuronal stress. Evidence supports bidirectional communication: stressed RGCs release cytokines and injury signals that engage Müller glia, while Müller glia state changes reshape synaptic and metabolic conditions that influence RGC survival. Framing glaucoma as a feedback loop between retinal ganglion cell stress and glial reactivity may help explain why degeneration can persist even when IOP is reduced. In this model, biomechanical stress can initiate injury, while sustained glial reactivity may maintain inflammatory, metabolic, and extracellular changes that further compromise retinal ganglion cell survival. Effective neuroprotection may therefore require approaches that reduce IOP-related stress while also limiting maladaptive glial responses that perpetuate the degenerative environment. These features make glaucoma a particularly informative context in which to test whether redirecting Müller glial responses away from persistent reactivity can preserve retinal homeostasis.

Diabetic retinopathy and retinal ischemia

Diabetic retinopathy (DR) is a common neurovascular complication of chronic hyperglycemia and a major cause of vision impairment (Carpi-Santos et al. 2022; Vujosevic and Midena 2013; Fu et al. 2015). Although historically considered a vascular disease, substantial evidence supports early and progressive neuronal dysfunction alongside vascular pathology (Fu et al. 2015). Müller glia are central to this progression because they couple retinal neurons and vasculature and maintain key homeostatic functions required for blood–retinal barrier integrity, extracellular ion and water balance, and neurotransmitter clearance (Carpi-Santos et al. 2022; Yang, Qi, and Wang 2022; Coughlin, Feenstra, and Mohr 2017).

A major Müller glia pathway driving vascular leakage in DR is VEGF signaling. Müller glia represent a major retinal source of VEGF in diabetic conditions, and Müller glia–derived VEGF plays a causal role in diabetes-induced retinal inflammation and vascular leakage. Conditional Müller glia VEGF knockout models show reduced leukostasis, inflammatory marker expression, tight junction loss, acellular capillaries, and vascular leakage compared to diabetic controls (Yang, Qi, and Wang 2022; Le 2017; Arevalo et al. 2017). While these findings illustrate the broader relevance of Müller glia dysfunction across retinal disease, they also emphasize that the dominant pathological consequences of gliosis depend on disease context.

Age-related macular degeneration

Age-related macular degeneration (AMD) is a leading cause of vision loss in aging populations and represents a multifactorial neurodegenerative disease of the outer retina characterized by progressive photoreceptor dysfunction and degeneration, alongside structural and functional changes in the retinal pigment epithelium (RPE) (Navneet, Wilson, and Rohrer 2024; Leinonen, Bull, and Fu 2023; Zhao, Hao, et al. 2024). AMD is commonly classified into dry and wet forms, which differ in the presence of choroidal neovascularization, but both involve disruption of the macular microenvironment (Navneet, Wilson, and Rohrer 2024; Leinonen, Bull, and Fu 2023). Müller glia influence AMD pathogenesis through their structural and metabolic roles across the retinal column and their specialized organization in the macula (Navneet, Wilson, and Rohrer 2024).

In this context, Müller glia dysfunction may contribute less through direct ganglion cell vulnerability and more through disruption of metabolic, structural, and inflammatory support within the outer retinal microenvironment. Together with diabetic retinopathy, AMD highlights that Müller glia dysfunction is a shared feature of retinal disease, even though the most vulnerable neuronal and vascular compartments differ across conditions.

Shared features of Müller glia dysfunction across diseases

Considered together, these disease contexts reveal both conserved and disease-specific consequences of Müller glia dysfunction. Early in disease, these responses can be protective,

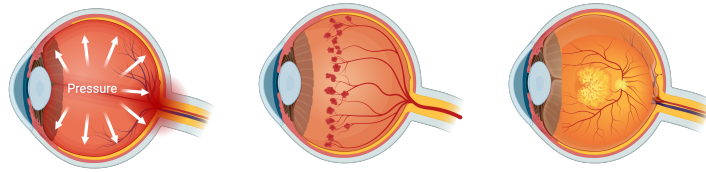
preserving retinal structure and homeostasis through trophic, anti-inflammatory, and anti-angiogenic support, as well as metabolic and structural regulation. With chronic stress, however, these adaptive responses transition into sustained gliosis and progressive disruption of core homeostatic functions, ultimately contributing to retinal dysfunction.

Despite these shared features, the consequences of Müller glia dysfunction are not uniform across disease contexts. Similar hallmarks of reactivity are observed across disorders, yet dominant pathological outcomes diverge, including photoreceptor degeneration in AMD and inherited retinal diseases, RGC loss in glaucoma, and vascular dysfunction in diabetic retinopathy. This divergence suggests that Müller glia reactivity is not a single stereotyped process but is shaped by the nature, location, and duration of the primary insult. Comparative analyses of inherited retinal degeneration models further support this view, showing broad induction of classical gliosis markers such as GFAP and vimentin, alongside differences in the spatial persistence of gliosis and molecular programs associated with extracellular and cell surface remodeling (Hippert et al. 2015).

These observations support a model in which Müller glia responses are spatially compartmentalized across retinal layers rather than uniformly distributed. Distinct Müller glia domains may respond preferentially to local signals from injured neurons, vascular stress, or interactions with activated microglia, giving rise to context-dependent structural and functional states. Under this framework, the identity and location of the initiating pathology shape Müller glia responses and, in turn, disease progression. This spatial perspective may help explain why diseases driven primarily by photoreceptor or vascular pathology, such as AMD and diabetic retinopathy, rarely culminate in substantial RGC loss, whereas glaucoma predominantly affects ganglion cells with relatively limited photoreceptor degeneration. Defining how these disease-specific Müller glia states emerge will be critical for determining whether Müller glia preserve retinal resilience or actively drive degeneration. This framework motivates the therapeutic question addressed in the following chapters: whether shifting Müller glia away from persistent stress-associated states and toward pro-resolving programs can improve retinal resilience in glaucoma.

1.6 Dissertation overview

Müller glia are central regulators of retinal homeostasis whose structural, metabolic, and neurovascular functions are essential for maintaining retinal integrity. As discussed throughout this chapter, these same features also make Müller glia highly responsive to mechanical, metabolic, and inflammatory stress, such that persistent disruption can shift them from supportive homeostatic cells toward reactive states that contribute to neuroinflammation, neuronal dysfunction, and tissue remodeling. Defining the mechanisms that govern these state transitions is therefore critical for understanding how glia responses influence retinal disease progression and for identifying therapeutic strategies that restore homeostasis rather than broadly suppressing glia function. This framework provides the basis for the remainder of this dissertation. Chapter 2 examines specialized pro-resolving lipid



	Glaucoma	Diabetic Retinopathy	AMD
Reactive Gliosis	Prominent	Prominent	Prominent
Glutamate Dysregulation	Strong	Moderate	Context Dependent
Ion and Water Imbalance	Moderate	Strong	Moderate
Metabolic and Redox stress	Strong	Strong	Strong
Vascular Barrier Dysfunction	Moderate	Strong	Moderate to strong, depending on subtype
Inflammation	Strong	Strong	Strong

Table 1.2: This table provides a comparative summary of Müller glia dysfunction across three common retinal diseases. Across glaucoma, diabetic retinopathy, and age-related macular degeneration (AMD), Müller glia exhibit overlapping patterns of reactive gliosis, inflammatory activation, metabolic and redox stress, and disruption of neurotransmitter, ion, water, and vascular homeostasis. At the same time, the relative prominence of these features varies by disease context, reflecting differences in initiating stressors, vascular involvement, and tissue compartment affected (García-Bermúdez et al. 2021; Rombaut et al. 2023; Coughlin, Feenstra, and Mohr 2017; Navneet, Wilson, and Rohrer 2024).

mediator pathways, with a focus on lipoxins and Lipoxin B4, as endogenous mechanisms that may restore retinal homeostasis and limit chronic Müller glia reactivity in glaucoma. Building on this background, Chapter 3 investigates how Lipoxin B4 modulates TRPV4-associated Müller glia reactivity during ocular hypertension, with particular emphasis on inflammatory signaling and gliosis-related pathways. Chapter 4 then extends these questions to Müller glia morphology, examining structural features of these cells as readouts of glia state and retinal remodeling in disease. Together, these chapters test the broader hypothesis that Müller glia are not only responders to retinal stress, but also tractable targets for therapeutic modulation in glaucomatous neuroinflammation.

Chapter 2

Lipid Mediator Signaling and Protective Therapeutic Strategies in Glaucoma

2.1 Introduction

Chapter 1 summarizes Müller glia as central regulators of retinal homeostasis, with essential roles in maintaining the structural, metabolic, and neurovascular integrity of the retina. It also highlighted how, under persistent stress, these cells can adopt reactive states that contribute to chronic inflammation and neuronal dysfunction. Together, these observations underscore the need for therapeutic strategies that do more than broadly suppress glia responses, but instead limit maladaptive inflammatory signaling while preserving tissue homeostasis. Among the pathways of interest are lipid mediators, a diverse class of bioactive molecules that regulate inflammatory signaling, cellular stress responses, and tissue protection. In this chapter, I review lipid mediator signaling in the eye, with particular emphasis on lipoxin biosynthesis and bioaction, the role of protective lipid mediator pathways in ocular tissues, and the emerging neuroprotective relevance of Lipoxin B₄ (LXB₄) in the retina and glaucoma.

2.2 Lipid mediator signaling and inflammatory regulation in the retina

The eye is a specialized immune-privileged sensory organ that must preserve tissue homeostasis and neural function while limiting damaging inflammation. This immune-regulatory environment is maintained through physical barriers, active immune tolerance, and endogenous mechanisms that help limit damaging inflammatory responses. The eye must continuously balance immune protection with preservation of tissue

homeostasis and neural function (Wei and Gronert 2017; Licican and Gronert 2010). Even relatively mild inflammation can alter neuronal signaling and compromise vision, making tight regulation of immune activity essential across ocular tissues.

This immune privilege is supported by both structural organization and cellular specialization across the eye. The cornea, aqueous humor, vitreous, and fovea are avascular, a feature that is essential for maintaining efficient transmission and processing of light. In the posterior segment, immune regulation is further reinforced by the blood-retinal barrier, which consists of an inner barrier formed by tight junctions between retinal vascular endothelial cells and an outer barrier formed by tight junctions between retinal pigment epithelium (RPE) cells (Wei and Gronert 2017; Flitter et al. 2021). Together, these barriers limit uncontrolled exchange between neural tissue and the systemic circulation while helping maintain the highly regulated retinal microenvironment required for phototransduction and neuronal function. This need for protection is especially important given the functional importance of vision, with a substantial portion of cerebral cortex devoted to visual processing and perception (Wei and Gronert 2017; Licican and Gronert 2010; Flitter et al. 2021).

The same principle of immune control extends across the ocular surface, where compartmentalized epithelial, secretory, and immune cell populations support tissue integrity despite constant environmental exposure. The cornea and conjunctiva, together with the tear-producing glands, are organized into distinct but integrated compartments that support barrier function, lubrication, antimicrobial defense, and wound healing (Bazan, Calandria, and Gordon 2013; Stahl et al. 2011; Fliesler 2010). Dysregulation of these cellular responses can lead to prolonged ocular surface inflammation, recruitment of adaptive immune responses, and ultimately loss of visual function. Because the cornea is continuously exposed to physical, chemical, and microbial insults, mechanisms that limit inflammation while permitting repair are particularly important in this tissue (Wei and Gronert 2017; Lee et al. 2015; English et al. 2017). This is clinically evident in conditions such as corneal abrasion and impaired wound healing, which remain significant problems in the settings of diabetic neuropathy, refractive surgery, and corneal transplantation.

Although the ocular surface has provided many of the foundational insights into endogenous inflammatory control in the eye, the retina presents a distinct and equally important challenge. The neural retina is composed of densely packed photoreceptors, interneurons, and retinal ganglion cells that convert light into electrical signals for transmission to the visual cortex. Unlike the corneal epithelium, the retina has little regenerative capacity, and injury to retinal neurons can therefore result in irreversible vision loss. The RPE also plays a central role in preserving retinal health by phagocytosing photoreceptor outer segments, supporting the visual cycle, maintaining outer blood-retinal barrier integrity, and expressing immunomodulatory factors. Disruption of RPE homeostatic function contributes directly to the pathogenesis of multiple retinopathies and further illustrates how tightly coordinated immune and metabolic control are required for retinal survival (Wei and Gronert 2017; Kaur and Singh 2023; Whitcup et al. 2013).

Despite its relative isolation behind a well-developed blood-tissue barrier, the retina remains vulnerable to oxidative stress, circulating inflammatory mediators, blood-borne

pathogens, and systemic immune dysregulation. Retinal immune responses are therefore organized to preserve local homeostasis while minimizing bystander damage to neural tissue. During injury or early disease, a range of cellular stressors activate pro-inflammatory genes and initiate innate immune responses that are normally counterbalanced by anti-inflammatory programs. These responses include activation of tissue-resident macroglia, recruitment or transformation of microglia, and the local production of cytokines, chemokines, and other inflammatory mediators. When tightly regulated, these mechanisms can serve adaptive or protective functions. However, when inflammatory signaling becomes prolonged or maladaptive, the resulting imbalance contributes to chronic retinal dysfunction and progressive neurodegeneration (Kaur and Singh 2023; Whitcup et al. 2013). Persistent inflammation or neuroinflammation is increasingly recognized as a common feature of many retinal diseases, including inherited retinal degenerations, retinal neovascular disorders, diabetic retinopathy, age-related macular degeneration, and glaucoma. Although each disease is initiated by distinct cellular and molecular insults, accumulating evidence suggests that chronic, low-grade inflammation contributes to disease progression across these conditions. In this context, sustained activation of macroglia and microglia, dysregulated cytokine signaling, oxidative stress, and barrier dysfunction can amplify neuronal vulnerability and promote tissue remodeling (Kaur and Singh 2023; Whitcup et al. 2013; Maurya, Lin, et al. 2024; Livne-Bar, Wei, et al. 2017). Thus, retinal disease is not shaped solely by the initiating insult, but also by the extent to which endogenous mechanisms can restrain inflammatory amplification and preserve tissue homeostasis over time.

Among the endogenous pathways that regulate these responses are lipid mediators, a diverse group of bioactive signaling molecules derived from polyunsaturated fatty acids (PUFAs) that play central roles in inflammation, immune regulation, and tissue homeostasis (Wei and Gronert 2017; Wei and Gronert 2019). Produced locally in response to cellular activation, lipid mediators include prostaglandins, leukotrienes, and other protective lipid mediators, and together these pathways shape both the initiation and limitation of inflammatory responses. Within this network, lipid mediators help determine the magnitude, timing, and character of the immune response in a highly tissue- and context-dependent manner (Wei and Gronert 2017; Wei and Gronert 2019). More broadly, work in the retina and optic nerve underscores that lipid mediator signaling is highly compartment- and receptor-dependent, such that closely related mediators can exert distinct protective or pathogenic effects depending on cell type and disease context (Kim et al. 2020).

Although inflammation is essential for protection against infection and injury, its magnitude and duration must be tightly controlled to avoid chronic tissue damage. This principle is particularly important in the retina, where inflammatory injury can directly compromise irreplaceable neural cells (Wei and Gronert 2017; Kaur and Singh 2023; Whitcup et al. 2013). Lipid mediator pathways are therefore of particular interest in retinal disease because they do not simply participate in host defense, but also shape whether inflammation remains adaptive or becomes pathologic. In the eye, endogenously produced bioactive lipid mediators at the ocular surface and within the retina contribute to the maintenance of tissue health, and disruption of these pathways has emerged as an important feature of

inflammatory and neurodegenerative disease. Within this broader framework, lipoxins are of particular interest as endogenous arachidonic acid-derived mediators that can suppress inflammatory activation while promoting neuroprotection, making them especially relevant to retinal stress and glaucomatous injury.

2.3 Lipoxin biosynthesis and signaling

Within this broader lipid signaling network, lipoxins are arachidonic acid-derived eicosanoids that act as endogenous regulators of inflammatory responses and tissue protection. Lipoxins were the first identified lipid mediators with anti-inflammatory and homeostatic actions and remain an important model for understanding how lipid pathways can limit excessive inflammation without broadly suppressing host defense (Wei and Gronert 2017; Licican and Gronert 2010). In contrast to lipid mediators that primarily amplify inflammatory signaling, lipoxins function locally within injured or inflamed tissues to counter leukocyte activation, dampen cytokine and chemokine production, and help preserve tissue integrity.

Lipoxins are generated through lipoxygenase (LOX)-dependent metabolism of arachidonic acid (AA), a polyunsaturated fatty acid esterified within membrane phospholipids and released upon cellular activation. Their biosynthesis typically occurs through sequential enzymatic steps involving 5-LOX, 12-LOX, and 15-LOX, which are expressed across leukocytes, platelets, epithelial cells, endothelial cells, and other tissue-resident cell types (Flitter et al. 2021; Gronert 2005; Levy, Gronert, and Clish 1998). Because these enzymes are often distributed across different cell populations, lipoxin production commonly occurs through transcellular biosynthesis, in which intermediates generated by one cell type are transferred to neighboring cells for further conversion (Levy, Gronert, and Clish 1998; Serhan, Hamberg, and Samuelsson 1984). This arrangement highlights an important feature of lipid mediator biology: inflammatory signaling is shaped not only by the activity of individual cells, but also by biochemical cooperation between interacting cell populations within the local tissue environment. This framework is particularly relevant to the retina, where close interactions between glia, neurons, vasculature, and infiltrating immune cells shape tissue responses during chronic stress.

Two major routes of lipoxin biosynthesis have been described. In one pathway, 15-LOX-mediated oxygenation of arachidonic acid in eosinophils, monocytes, macrophages, or epithelial cells generates intermediates that are then converted by 5-LOX in leukocytes to lipoxin A₄ (LXA₄) and Lipoxin B₄ (LXB₄) (Wei and Gronert 2017; Levy, Gronert, and Clish 1998; Serhan, Hamberg, and Samuelsson 1984). In a second pathway, 5-LOX-dependent conversion of AA to leukotriene A₄ (LTA₄) in leukocytes is followed by transfer of LTA₄ to adherent platelets, where 12-LOX catalyzes its further transformation into lipoxins (Levy, Gronert, and Clish 1998). These biosynthetic relationships underscore the close coupling between leukotriene and lipoxin pathways. Both are derived from shared substrates and intermediates, yet they often exert opposing biological effects, such that the balance between leukotriene and lipoxin production can influence the intensity and duration of inflammatory

responses (Fig. 2.1).

An important consideration in interpreting this literature is species-specific variation in LOX biology. In humans and non-human primates, lipoxin biosynthesis involves a true 15-LOX, whereas many rodent species instead express a 12/15-LOX with different positional specificity (Gronert et al. 2005; Snodgrass and Brüne 2019). This distinction is particularly relevant in experimental models of retinal and ocular disease, where murine systems are widely used to study endogenous lipid mediator pathways. Human 15-LOX is also of broader interest because it is highly expressed in mucosal and corneal epithelial tissues and is considered a major inducible enzyme in monocytes. In these tissues, 15-LOX contributes not only to lipoxin generation but also to the production of other protective lipid mediators, including DHA-derived resolvins and protectins. Together, these observations place 15-LOX at a central point in endogenous lipid pathways that limit inflammatory injury and support tissue homeostasis (Gronert et al. 2005; Snodgrass and Brüne 2019; Heydeck et al. 2023; Adel et al. 2016).

Among the endogenous lipoxins, LXA₄ was the first identified and remains the most extensively characterized. Many of its bioactions are mediated through the ALX/FPR2 receptor, through which it suppresses leukocyte recruitment, inflammatory mediator production, and other features of excessive immune activation. In contrast, the receptor mechanisms underlying LXB₄ signaling remain less well defined (Wei and Gronert 2017; Wei and Gronert 2019). Despite this, growing evidence indicates that LXB₄ exerts potent anti-inflammatory and neuroprotective effects in multiple tissues, including the retina, and may engage signaling programs that are distinct from those classically associated with LXA₄. This distinction is particularly relevant to the present work, as recent studies increasingly point to LXB₄ as an important regulator of retinal glia activation and neuronal survival in glaucoma.

Lipoxygenase activity has been reported across several ocular tissues, including the cornea, lens, and retina, as reflected by the formation of species-specific hydroxyeicosatetraenoic acids such as 12-HETE, 15-HETE, and 5-HETE, as well as leukotrienes that are attenuated by LOX inhibition (Wei and Gronert 2017; Gronert 2005). In addition to generating leukotrienes and LOX-derived arachidonic acid metabolites, the retina also produces DHA-derived protective mediators, reinforcing the idea that retinal tissue is equipped with endogenous lipid signaling systems that influence inflammatory tone, neuronal survival, and tissue homeostasis (Gronert 2005; Levy, Gronert, and Clish 1998). Within this broader framework, lipoxins are of particular interest because they link arachidonic acid metabolism to anti-inflammatory and neuroprotective signaling in a tissue where even modest inflammatory dysregulation can have lasting functional consequences in chronic retinal disease.

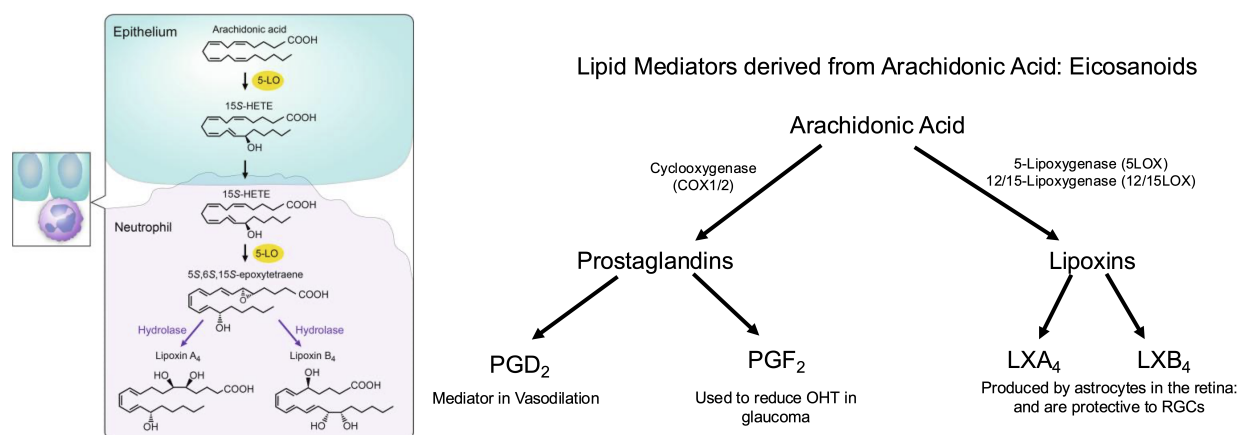


Figure 2.1: Schematic overview of arachidonic acid metabolism. The left panel shows the transcellular biosynthesis of lipoxins in epithelial cells and neutrophils. The right panel summarizes the broader arachidonic acid cascade, in which arachidonic acid can be metabolized through cyclooxygenase (COX-1/2) pathways to generate prostaglandins or through lipoxygenase (LOX) pathways, including 5-LOX and 12/15-LOX, to generate lipoxins (Levy and Serhan 2014).

2.4 Protective lipid mediators in the eye

In this context, protective lipid mediators, including lipoxin A₄ (LXA₄) and neuroprotectin D1 (NPD1), were first identified in the cornea, providing early evidence that the eye contains endogenous pathways that limit inflammatory damage and support tissue protection. Subsequent work has shown that these pathways help restrain leukocyte-driven inflammation, support wound healing, and preserve tissue integrity across multiple ocular tissues (Wei and Gronert 2017; Bazan, Calandria, and Gordon 2013).

Ocular tissues are also well equipped for lipid mediator biosynthesis. The corneal epithelium highly expresses 15-LOX and supports lipoxin generation, while the retina and retinal pigment epithelium, in addition to arachidonic acid, are enriched in omega-3 polyunsaturated fatty acid substrates, particularly docosahexaenoic acid (DHA), which is concentrated in photoreceptor outer segments and serves as a precursor for NPD1 (Wei and Gronert 2017; Lee et al. 2015; Chang et al. 2005; Biteman et al. 2007). Tear fluid and aqueous humor likewise contain measurable lipid mediators and their precursors, and advances in LC-MS/MS-based lipidomics have shown that ocular disease alters these profiles in both anterior- and posterior-segment fluids. Together, these observations support the concept that the eye contains endogenous lipid mediator circuits whose balance shifts with tissue stress and disease state (English et al. 2017; Walter et al. 2016).

Functionally, these mediators have been linked to multiple protective responses in the eye. LXA₄ promotes corneal wound healing, and lipoxin signaling through ALX/FPR2 regulates conjunctival goblet cell secretion, supporting ocular-surface homeostasis (Wei and Gronert 2017). Resolvin E1 improves tear production, preserves corneal epithelial integrity, and reduces inflammatory cell infiltration in experimental dry eye, while NPD1 promotes corneal nerve regeneration and protects retinal pigment epithelial cells from oxidative stress-induced apoptosis. These findings show that protective lipid mediators are not limited to suppressing inflammation, but also contribute to tissue repair, epithelial function, and cell survival across ocular compartments (Lee et al. 2015; English et al. 2017; Chang et al. 2005; Biteman et al. 2007; Walter et al. 2016). These endogenous pathways are also therapeutically relevant because ocular inflammation is accompanied by shifts in lipid mediator composition in tear fluid and aqueous humor (English et al. 2017). Earlier studies were limited to a small number of analytes, but broader lipidomic approaches now show that ocular disease is associated with widespread remodeling of inflammatory and protective lipid mediator pathways (Fig. 2.2). This has strengthened interest in pharmacologically amplifying endogenous protective lipid circuits rather than simply suppressing inflammation (Wei and Gronert 2017; Maurya, Kumar, et al. 2025). For the present dissertation, lipoxins are of particular interest because they are endogenous arachidonic acid-derived mediators with established roles in ocular immune regulation and growing evidence for direct actions on retinal ganglion cells and glia responses in glaucoma. The next section therefore focuses specifically on lipoxins in the retina, where their roles in retinal ganglion cell survival, glia regulation, and glaucomatous injury are becoming increasingly clear (Fig. 2.2).

2.5 Lipoxins as neuroprotective mediators in the retina

Lipoxins have emerged as important endogenous lipid mediators in the retina, where they act not only to suppress inflammatory activation but also to support neuronal survival under stress. Among the endogenous lipoxins, LXA₄ has been more extensively characterized, whereas the biology of LXB₄ remains less well defined despite increasing evidence that its actions are distinct and more potent (Livne-Bar, Wei, et al. 2017). In the retina, this distinction has become especially relevant because lipoxin signaling has now been linked to retinal ganglion cell (RGC) survival, regulation of glia reactivity, and protection in experimental models of glaucomatous injury (Livne-Bar, Wei, et al. 2017; Liu, Cullen, et al. 2024). Together, these studies support the view that lipoxins are integral components of endogenous protective signaling within the inner retina.

Astrocyte-derived lipoxins and retinal ganglion cell protection

Initial evidence for lipoxin-mediated neuroprotection in the retina emerged from studies of retinal astrocytes, which showed strong protective effects on neurons within the ganglion

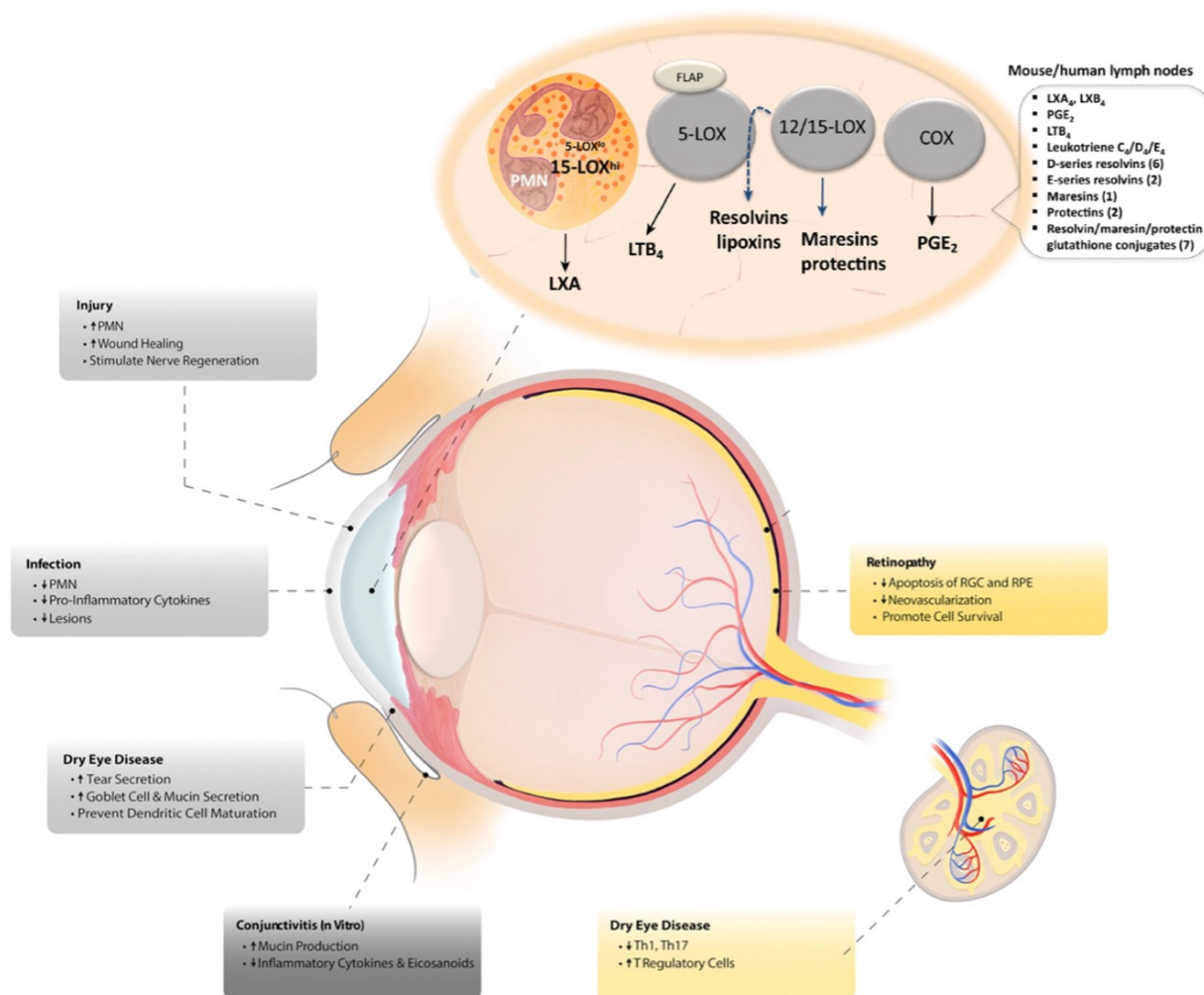


Figure 2.2: Schematic showing the biosynthesis and tissue-specific functions of endogenous lipid mediators across the eye. The top inset summarizes major enzymatic pathways involving 5-LOX, 12/15-LOX, and COX, which generate lipoxins, resolvins, maresins, protectins, leukotrienes, and prostaglandins. The eye diagram highlights reported actions of these mediators across ocular compartments, including reduced pathogens, irritants, and neutrophils (PMN) infiltration, enhanced wound healing and nerve regeneration after corneal injury, modulation of infection-associated inflammation, support of tear secretion and goblet cell function in dry eye and conjunctival models, and protection against retinal ganglion cell and retinal pigment epithelium apoptosis in retinopathy. These pathways also influence neovascularization and immune-cell responses, illustrating that endogenous lipid mediator signaling contributes broadly to ocular homeostasis, tissue protection, and inflammatory regulation (Wei and Gronert 2017; Kaur and Singh 2023).

cell layer. To determine whether this protection was mediated by soluble factors, astrocyte-conditioned medium was tested in excitotoxic retinal injury paradigms. These experiments demonstrated significant preservation of RGC survival, prompting lipidomic analysis of astrocyte-conditioned medium to identify candidate protective mediators. Among the lipid mediators detected, LXA₄ and LXB₄ were enriched in astrocyte-conditioned medium. These findings established astrocytes as a key source of endogenous lipoxins in the inner retina and implicated lipoxins as candidate mediators of astrocyte-derived neuroprotection (Fig. 2.3A) (Livne-Bar, Wei, et al. 2017).

Subsequent experiments showed that both LXA₄ and LXB₄ promote RGC survival after acute retinal injury (Fig. 2.3B), whereas inhibition of lipoxin pathway components worsens damage. Importantly, LXB₄ consistently showed greater potency than LXA₄ in several acute assays. This difference became even more notable in chronic glaucomatous injury, where delayed administration of LXB₄ in a circumlimbal suture model preserved RGC function, reduced retinal nerve fiber layer thinning, and improved RGC survival. These findings provided some of the first evidence that lipoxins can influence not only acute injury responses, but also chronic inner retinal neurodegeneration. They also suggested that LXB₄ may engage signaling mechanisms that are not fully explained by established LXA₄ receptor pathways (Livne-Bar, Wei, et al. 2017).

These early studies further indicated that lipoxin-mediated protection extends beyond direct effects on neurons alone. In ocular hypertension, both LXA₄ and LXB₄ reduced GFAP upregulation in macroglia, including Müller glia, suggesting that lipoxins influence the reactive retinal environment in parallel with preserving RGC survival (Fig. 2.3C). This finding shifted the interpretation of lipoxin action from a neuron-centered model toward one in which retinal glia actively participate in tissue protection (Livne-Bar, Wei, et al. 2017; Liu, Cullen, et al. 2024). Supporting this broader framework, lipoxin pathway components were identified in the retina, optic nerve, retinal astrocytes, and non-human primate optic nerve, and primary human astrocytes likewise retained the capacity to engage in lipoxin signaling, indicating that this pathway is conserved across species.

Dysregulation of the lipoxin pathway during ocular hypertension

More recent work has expanded this framework by showing that endogenous lipoxin pathways are not static during retinal stress, but become actively dysregulated in ocular hypertension. In the mouse retina and optic nerve, ocular hypertension was associated with decreased arachidonic acid and reduced 15-LOX pathway output, alongside increased 5-LOX pathway activity (Karnam et al. 2024). These changes indicate that the balance of lipoxin biosynthesis is altered during glaucomatous stress, potentially shifting the retinal environment away from homeostatic lipid signaling and toward persistent inflammatory activation. Rather than a simple loss of protection, ocular hypertension appears to remodel the lipoxin network itself.

This dysregulation has been examined in particular detail in astrocytes. In cytokine-challenged astrocytes, LXB₄ suppressed expression of reactive astrocyte markers and reduced amplified 5-LOX activity, suggesting that it can directly restrain inflammatory

reactivity while partially restoring a more balanced lipoxygenase profile. In vivo, LXB₄ similarly reduced expression of astrocyte-associated reactivity markers and increased expression of GLAST, consistent with a shift away from maladaptive macroglia activation (Karnam et al. 2024). Together, these findings identify astrocytes not only as a source of endogenous lipoxins, but also as a direct cellular target of LXB₄ during retinal stress. These findings also raise the possibility that dysregulation of lipoxin signaling is not confined to astrocytes, but may extend more broadly across macroglia populations during glaucomatous stress.

These observations are particularly relevant to glaucoma because they suggest that lipoxin signaling influences the retinal injury environment at the level of glia state control. In this context, LXB₄ does not appear to function solely through canonical LXA₄ receptor pathways. Experimental evidence indicates that inhibition of ALX/FPR2 is insufficient to block LXB₄-mediated protection, supporting the view that LXB₄ engages distinct signaling programs (Livne-Bar, Wei, et al. 2017; Liu, Cullen, et al. 2024; Mathew, Maurya, et al. 2026). Although its receptor mechanisms remain incompletely defined, the available data suggest that LXB₄ acts through pathways that suppress inflammatory amplification while preserving neuronal function, a profile that is especially relevant in chronic retinal disease.

LXB₄ regulation of retinal and optic nerve microglia responses

Beyond astrocytes, studies have also shown that resident microglia in the retina and distal myelinated optic nerve are cellular targets of LXB₄. Single-cell transcriptomic analysis demonstrated that LXB₄ regulates microglia gene expression in the healthy retina, including downregulation of pathways related to antigen processing and presentation as well as genes associated with environmental sensing and inflammatory transition. These findings were notable because they suggested that LXB₄ influences microglia programming even in the absence of overt injury, indicating a broader role in maintaining retinal immune balance (Maurya, Lin, et al. 2024).

In ocular hypertension, LXB₄ shifted microglia responses in the distal optic nerve away from disease-associated states and toward a phenotype more closely resembling homeostatic microglia. This included downregulation of key disease-associated microglia markers and correlation with reduced PI3K pathway activity, a pathway linked to pro-inflammatory cytokine production and neurodegeneration. Morphologic analyses further supported this interpretation, showing that microglia populations in both retina and optic nerve undergo dynamic state transitions during sustained ocular hypertension, and that LXB₄ treatment biases these responses toward a less reactive phenotype (Maurya, Lin, et al. 2024). Together, these studies extend lipoxin biology beyond astrocytes and RGCs and support a broader role for LXB₄ in limiting maladaptive neuroimmune remodeling in glaucoma.

Lipoxins as multicellular regulators of retinal stress and glaucomatous injury

Taken together, these studies support a multicellular model of lipoxin action in the retina. Astrocytes can generate lipoxins and contribute to neuronal protection, RGCs are preserved by exogenous lipoxin treatment in both acute and chronic injury models, and microglia in the retina and optic nerve respond directly to LXB₄ with shifts away from reactive or disease-associated states. These findings suggest that lipoxins do not act on a single retinal target, but instead influence the broader cellular network that shapes inflammatory tone, glial reactivity, and neuronal survival during stress.

Within this framework, LXB₄ is of particular interest because its protective actions appear to extend beyond canonical LXA₄ signaling and because its efficacy has been repeatedly linked to regulation of glial state in addition to direct neuroprotection. At the same time, an important gap remains. Compared with astrocytes and microglia, the contribution of Müller glia to endogenous lipoxin biosynthesis and lipoxin-responsive signaling has remained much less defined. This gap is particularly relevant in glaucoma, where Müller glia are central regulators of retinal homeostasis and prominent contributors to reactive gliosis.

Müller glia are the principal macroglia of the retina and support metabolic regulation, neurotransmitter recycling, ion and water balance, and neurovascular function. These roles place them in a position to strongly influence how the retina responds to stress. Because Müller glia share several protective and homeostatic features with astrocytes, they may also participate in endogenous lipoxin biosynthesis or serve as direct cellular targets of lipoxin signaling. However, despite evidence that lipoxin treatment can reduce GFAP expression and reactive markers within Müller glial processes, the specific role of Müller glia in lipoxin biology remains less defined (Liu, Cullen, et al. 2024).

Thus, a key unresolved question is whether Müller glia respond directly to exogenous LXB₄ and whether they contribute to endogenous lipoxin signaling within the stressed retina. This question is especially important in ocular hypertension, where Müller glia reactivity is closely linked to inflammatory signaling, TRPV4 upregulation, and chronic retinal stress. Addressing this question is essential for defining how broadly lipoxin-mediated protection extends across retinal cell types and whether Müller glia represent a previously underappreciated component of this pathway. This rationale forms the basis for the next chapter, which examines LXB₄ as a regulator of Müller glia reactivity and inflammatory signaling in the context of ocular hypertension and TRPV4-associated gliosis.

2.6 Summary and implications for glaucoma

Chapter 1 reviewed Müller glia as central regulators of retinal homeostasis whose structural, metabolic, and neurovascular support functions become increasingly vulnerable under persistent stress. It also emphasized that chronic retinal disease is shaped not only by

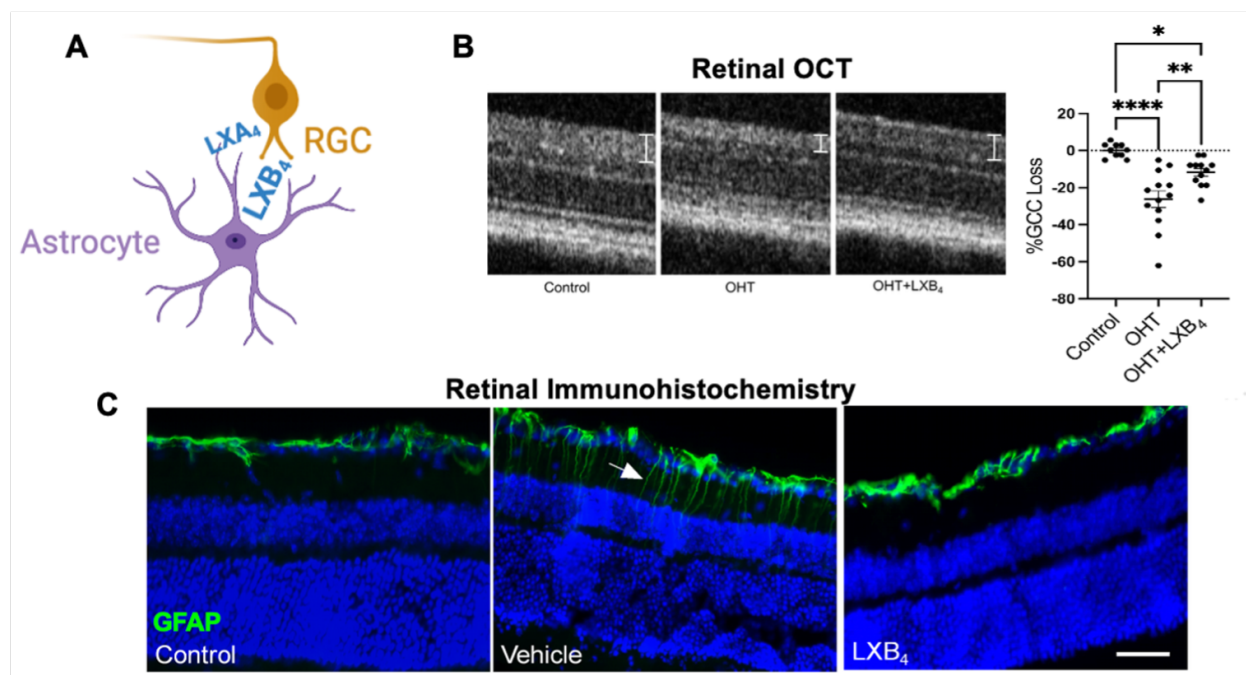


Figure 2.3: (A) Schematic of astrocyte-derived LXA₄ and LXB₄ signaling to retinal ganglion cells (RGCs). (B) Representative retinal OCT images and quantification of percent RGC loss in control, OHT, and OHT + LXB₄ eyes. LXB₄ treatment attenuates OHT-induced inner retinal degeneration. (C) Representative GFAP immunolabeling (green) with DAPI (blue) in control, vehicle-treated OHT, and LXB₄-treated OHT retinas (Maurya, Lin, et al. 2024; Liu, Cullen, et al. 2024; Karnam et al. 2024)

activation of inflammatory and gliotic pathways, but by failure to preserve homeostasis once these responses are engaged. Building on this framework, this chapter has examined lipid mediators as endogenous regulators of inflammatory signaling in the eye, with particular emphasis on lipoxins and other protective lipid pathways relevant to retinal stress. Together, the studies reviewed here support the view that endogenous lipid-mediated control of inflammatory activation is an important component of retinal tissue protection.

Within this broader framework, lipoxins, and particularly Lipoxin B₄ (LXB₄), have emerged as compelling candidates for retinal neuroprotection. Existing work shows that lipoxin signaling is present in the inner retina, becomes altered during ocular hypertension, and can influence both neuronal survival and glia reactivity across multiple retinal cell types. These findings suggest that glaucomatous injury may involve not only induction of reactive stress pathways, but also disruption of endogenous lipid pathways that normally restrain chronic inflammatory and glia activation. At the same time, important questions remain regarding how these pathways are regulated in Müller glia, whether Müller glia

contribute directly to endogenous LXB₄ biosynthesis, and how exogenous LXB₄ modulates the signaling programs that underlie reactive gliosis. These unresolved questions provide the rationale for the following chapters. Chapter 3 examines whether LXB₄ directly regulates Müller glia reactivity and inflammatory signaling in the context of ocular hypertension and TRPV4-associated gliosis, whereas Chapter 4 extends this framework by examining Müller glia morphology and structural remodeling as readouts of glia state in glaucomatous stress.

Chapter 3

Lipoxin B₄ Mitigates TRPV4-Activated Müller Cell Gliosis During Ocular Hypertension

3.1 Introduction

Glaucoma, a group of optic neuropathies, is characterized by the progressive degeneration of retinal ganglion cells (RGCs) and their axons. Elevated intraocular pressure (IOP), a major risk factor, disrupts retinal homeostasis and activates inflammatory pathways that accelerate RGC loss (Križaj 2018; Weinreb et al. 2016). While IOP-lowering therapies slow disease progression, they do not fully prevent RGC degeneration, underscoring the need for neuroprotective strategies that target underlying mechanisms. Emerging evidence suggests that glia–neuron interactions, including those involving Müller glia, play a critical role in glaucomatous neurodegeneration (Bringmann and Wiedemann 2011). However, the molecular mechanisms underlying the contributions of glia to RGC loss remain unclear. Elucidating these pathways is essential for developing therapies that preserve RGCs and slow disease progression.

Müller glia are the principal macroglia cells of the retina and extend from the inner limiting membrane to the outer limiting membrane (Bringmann and Wiedemann 2011; Wang, O’Sullivan, et al. 2017). This unique architecture enables them to detect and respond to mechanical, metabolic, and inflammatory stress across all retinal layers (Reichenbach and Bringmann 2010). Under physiological conditions, Müller glia regulate osmotic balance, support neuronal metabolism, and maintain ion and water homeostasis (Bringmann and Wiedemann 2011; Roesch et al. 2008). Following retinal injury, these cells become reactive, characterized by glial fibrillary acidic protein (GFAP) upregulation (Bringmann and Wiedemann 2011). Chronic gliosis can disrupt tissue repair, promote inflammation, and accelerate RGC degeneration (Seitz, Ohlmann, and Tamm 2013; Reichenbach and Bringmann 2010).

A key pathway driving Müller glia reactivity involves the nonselective, mechanosensitive cation channel transient receptor potential vanilloid 4 (TRPV4) (Jo, Ryskamp, et al. 2015; Li, Cheng, et al. 2021). TRPV4 is expressed in both RGCs and Müller glia and responds to mechanical insults, such as those caused by elevated IOP, by facilitating calcium influx and triggering downstream inflammatory signaling (Guarino, Paruchuri, and Thodeti 2020; Becker et al. 2005). Sustained TRPV4 activation in Müller glia induces a reactive phenotype characterized by proinflammatory cytokine release and upregulated GFAP expression (Jo, Ryskamp, et al. 2015; Li, Cheng, et al. 2021; Jo, Lakk, et al. 2022). This process involves Janus Kinases (JAK) and Signal Transducers and Activators of Transcription (STAT) signaling, including IL-6/gp130 activation, and direct STAT3 binding to the GFAP promoter, ultimately contributing to RGC apoptosis in glaucoma (Dodington, Desai, and Woo 2018; Coorey, Shen, Zhu, et al. 2015; Brenner and Messing 2021). Pharmacological activation of TRPV4 in Müller glia recapitulates key features of IOP-induced insult, making it a robust *in vitro* model for glaucomatous neuroinflammation (Jo, Ryskamp, et al. 2015; Li, Cheng, et al. 2021; Jo, Lakk, et al. 2022).

Lipoxins (LXA₄ and LXB₄) are endogenous eicosanoids whose canonical role is to counter-regulate chronic inflammation. They are synthesized through cellular interactions between selective cell types expressing 5-lipoxygenase (5-LOX) and 15-LOX (Wei and Gronert 2017; Mathew and Sivak 2024; Snodgrass and Brüne 2019). The anti-inflammatory actions are well established for LXA₄ and LXA₄ stable analogs and include inhibiting leukocyte recruitment, JAK/STAT signaling, and NF- κ B-driven cytokine production (Jaén et al. 2021; Zhang, Li, Fan, et al. 2022; Kim et al. 2020). LXB₄'s mechanism of action is distinct from LXA₄, not mediated by the LXA₄ receptor and remains to be fully defined. We recently identified lipoxin B₄ (LXB₄) as an endogenous lipid mediator in the healthy retina and as a promising neuroprotective target to prevent RGC death in glaucoma models (Livne-Bar, Wei, et al. 2017; Karnam et al. 2024). We've established that LXB₄ treatment in rat and mouse models of glaucomatous injury is neuroprotective in the retina stopping death and preserving RGC function and astrocyte homeostatic function, and in the optic nerve maintaining a microglia functional homeostatic phenotype (Karnam et al. 2024; Maurya, Lin, et al. 2024; Mathew, Maurya, et al. 2026). In the retina, astrocytes generate LXB₄ as part of their homeostatic function (Karnam et al. 2024). Our recent findings also revealed that treatment with LXB₄ reduces glia reactivity in both astrocytes and Müller glia following induced ocular hypertension (OHT) (Maurya, Lin, et al. 2024). However, whether Müller glia can endogenously produce LXB₄ or if LXB₄ modulates TRPV4-induced reactivity remains unclear. Given Müller glia's shared homeostatic roles with astrocytes and their abundance (accounting for 90% of all retinal macroglia), it is plausible that Müller glia serve as a major source of retinal LXB₄ and/or their metabolic precursors (Pereiro, Fernández, et al. 2020).

In this study, we investigated whether Müller glia express lipoxygenases required for LXB₄ biosynthesis (5-LOX and 15-LOX) and whether they generate LXB₄. We also examined whether LXB₄ treatment counter regulates TRPV4-induced Müller glia reactivity in both *in vitro* (TRPV4 agonist-treated Müller glia) and *in vivo* (mouse OHT) models. This study defines a novel glia-lipoxin regulatory mechanism and provides new insight into potential

neuroprotective strategies for glaucoma.

3.2 Materials and methods

Mice

All procedures were conducted according to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Berkeley. The mice were housed under a 12-h light/12-h dark cycle with ad libitum access to food and water.

Mouse model of ocular hypertension

Silicone oil-induced OHT was induced in 8-wk-old mice via an established protocol (Zhang, Fang, et al. 2019; Zhang, Li, Huang, et al. 2019; Zhang and Hu 2020). The mice were anesthetized by intraperitoneal (i.p.) ketamine (100 mg/kg) and xylazine (10 mg/kg), and topical ocular anesthesia was achieved with 0.5% proparacaine hydrochloride (Sandoz, Princeton, NJ). Under a surgical microscope, pharmaceutical-grade silicone oil (Alcon, Fort Worth, TX) was injected into the anterior chambers of both eyes using a 33 G Hamilton glass syringe (Reno, NV). Chronic mild OHT was induced by injecting 1.2 μ L of silicone oil. Acute severe OHT was induced by injecting 1.8 μ L of silicone oil. Sham-injected control mice received a corneal incision created with a sterile 31 G paracentesis needle without silicone oil. After each procedure, 0.3% tobramycin ophthalmic solution (Tobrex; Alcon, Fort Worth, TX) was applied topically. The IOP was measured via a rebound tonometer, as described previously (Karnam et al. 2024; Maurya, Lin, et al. 2024). OHT was sustained for up to 8 weeks, as previously validated (Zhang, Li, Huang, et al. 2019; Zhang and Hu 2020; Fang et al. 2021). In prior studies, IOP was measured once every two weeks using a rebound tonometer, confirming persistent IOP elevation throughout the experimental period. In the present study, repeated IOP measurements were not performed because pupil dilation required for tonometry can disrupt silicone oil-induced anterior chamber blockage and alter OHT severity. Instead, silicone oil droplet size was monitored as an established indicator of sustained OHT. Consistent with prior validation demonstrating a direct correlation between droplet size and IOP elevation, droplet size was used as an inclusion/exclusion criterion to ensure stable, non-leaking oil droplets (Zhang, Li, Huang, et al. 2019; Zhang and Hu 2020; Fang et al. 2021). Tissues from the chronic mild OHT group were collected at 2-, 4-, 6-, and 8-weeks post-injection. Eyes were enucleated and fixed in 4% paraformaldehyde (PFA) for immunohistochemistry (IHC). For the acute severe OHT group, mice were euthanized at 1 wk postinjection, and eyes were enucleated and fixed in 4% PFA for IHC.

Quantitative PCR

Total RNA was isolated from mouse retinas and Müller glia cultures via TRIzol reagent (Invitrogen, Waltham, MA) according to the manufacturer's instructions and quantified using our previously published protocol (Maurya, Lin, et al. 2024). cDNA was synthesized from 1 µg of RNA via iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA). The transcripts for Il6, Stat3, TNF α , Alox5, and Alox15 were quantified via GoTaq PCR master mix (Promega, Madison, WI) in a OneStep Plus qPCR system (Applied Biosystems, Waltham, MA). Gapdh served as the control for normalization. The mouse primers used in this study are listed in Table 3.1. Primers for rat genes used in this study are listed in Table 3.2.

Table 3.1: Sequence of Mouse Forward and Reverse Primers

Primer	Sequence (5' → 3')
Alox5 Forward	ACA GCT TAT CTG CGA GTA TGG
Alox5 Reverse	GGG AAA CAC AGG GAG GAA TAG
Alox15 Forward	TGG GTT CTC TGC CTT AGT GG
Alox15 Reverse	CAC TCA GGG TTG TCA CCT CA
Il6 Forward	CCC CAA TTT CCA ATG CTC TCC A
Il6 Reverse	CGC ACT AGG TTT GCC GAG TA
TNF α Forward	TGA TCG GTC CCC AAA GGG AT
TNF α Reverse	TGT CTT TGA GAT CCA TGC CGT
Stat3 Forward	ACC AAC GAC CTG CAG CAA TA
Stat3 Reverse	TCC ATG TCA AAC GTG AGC GA

Table 3.2: Sequence of Rat Forward and Reverse Primers

Primer	Sequence (5' → 3')
Alox5 Forward	AGA GTC AAG AAT CTG GTG GGC
Alox5 Reverse	GGT GAC AGT GTA GGA AGG CA
Alox15 Forward	GGG ACT CGG AAG CAG AAT TCA A
Alox15 Reverse	GCC CTG AAC CCA TCG GTA A
Il6 Forward	TCC TAC CCC AAC TTC CAA TGC TC
Il6 Reverse	TTG GAT GGT CTT GGT CCT TAG CC

Bulk RNA sequencing

Retinal dissociation and Müller glia enrichment

Three weeks after silicone oil-induced OHT, the mice were euthanized, and neural retinas (n = 4 per condition with each n consisting of 4 pooled retinas) were dissected free of retinal

pigment epithelium. Retinal tissue was dissociated into single cells via a papain dissociation system (Worthington, Columbus, OH), passed through a 40 μ m cell strainer, and washed with PBS containing 0.5% bovine serum albumin (BSA). Dead cells and debris were depleted with the Dead Cell Removal Kit (Miltenyi Biotech, Bergisch Gladbach, Germany). The remaining cell suspension was incubated on ice for 10 min with anti-GLAST (ACSA-1)-APC antibody at a 1:50 dilution (Miltenyi Biotech) protected from light. After washing and centrifugation, cells were incubated with anti-APC microbeads (Miltenyi Biotech) at 4°C for 15 min. Following a wash, the cells were passed through an LS column (Miltenyi Biotech) in a QuadroMACS separator. Unlabeled (GLAST) cells were collected as flow-through; the column was washed 3x with 3 mL of MACS buffer. GLAST Müller glia were eluted with 5 mL of MACS buffer via a plunger. Müller glia purity was validated by flow-cytometric quantification of GLAST cells (88% purity) following MACS isolation, and further confirmed by bulk RNA-seq analysis showing distinct PCA clustering and enrichment of canonical Müller glia marker genes (*Slc1a3*, *Gfap*, *Rlbpl1*, *ApoE*, *Ccl2*) relative to whole-retina controls.

RNA isolation and library preparation

Total RNA from enriched Müller glia was extracted via the Arcturus™ PicoPure RNA Isolation Kit (Thermo Fisher, Waltham, MA). RNA quality was assessed via an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), and samples with an RNA integrity number (RIN) of 7.0 were used for library construction. cDNA was synthesized and amplified via the SMARTer v4 Ultra-Low Input RNA Kit (Clontech, Mountain View, CA). cDNA was fragmented on a Diagenode Bioruptor Pico (Diagenode, Denville, NJ), and sequencing libraries were prepared with the KAPA HyperPrep DNA Kit (Roche, Basel, Switzerland). Libraries were pooled and sequenced on an Illumina NovaSeq 6000 S4 flow cell (QB3 Genomics Core, UC Berkeley; RRID: SCR_022170) to generate 150 bp paired end reads.

Bioinformatic analysis

The raw base call files were demultiplexed via Illumina bcl2fastq2 (v2.20). Adapter trimming and quality filtering were performed with Trim Galore (v0.6.6). Quality metrics were assessed with FastQC (v0.11.9). Cleaned reads were aligned to the mouse genome (mm39, UCSC) via STAR (v2.7.1a). Gene counts were obtained via featureCounts (version 1.5.3). Downstream analysis was conducted via RStudio (version 4.2.0). Transcript abundance was expressed as transcripts per million (TPM) using the `normalizeTPM()` function, which normalizes for both gene length and sequencing depth. Differential gene expression was determined with DESeq2 ($p_{\text{adj}} < 0.05$, $|\text{Log}_2\text{FC}| > 1$). Gene set enrichment analysis (GSEA) was performed with fgsea ($p_{\text{adj}} < 0.05$, $|\text{Log}_2\text{FC}| > 1$), and the results were visualized in ggplot2. Bulk RNA-sequencing datasets were deposited in GEO (GSE306785, RNAseq).

Cell culture

Primary Müller glia

Primary mouse Müller glia were isolated from postnatal day 8–10 C57BL/6J pups (Liu, Tang, and Liu 2017; Chen, Zhang, et al. 2021; Pereiro, Ruzafa, et al. 2020). Retinas were dissociated with papain (Worthington, Columbus, OH) into a single-cell suspension. The cells were plated on 6-well plates coated with 0.1% gelatin in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Waltham, MA) supplemented with 10% fetal bovine serum (FBS) (Avantor Seradigm; Radnor, PA) and 1% penicillin–streptomycin (Gibco, Waltham, MA). Cultures were maintained at 37°C in a humidified 5% CO₂ incubator. Müller glia identity was confirmed after 2 passages by immunofluorescence staining for Vimentin, Glutamine Synthetase, and Sox9. All the assays were conducted using cells after passage 3.

Immortalized rat Müller glia (rMC-1).

The immortalized rat Müller glia cell line (rMC-1; T0576) was obtained from the UC Berkeley Cell Culture Facility. The cells were cultured in DMEM supplemented with 10% FBS, 1% MEM NEAA (Gibco, Waltham, MA), 1% sodium pyruvate (Gibco, Waltham, MA), and 1% penicillin–streptomycin (Gibco, Waltham, MA). Immortalized cells were used in place of primary cells for experiments where abundant protein and RNA were needed. All experiments were performed using cells at passages 2–10.

In vitro treatments

Cells (both primary and immortalized) were treated with a specific TRPV4 agonist at a concentration of 10 μ M GSK1016790A (Millipore Sigma, G0798) or with an equivalent volume of vehicle (DMSO, < 0.02%) in culture medium. GSK1016790A has been extensively validated as a highly selective and potent TRPV4 agonist, with its effects validated by TRPV4 antagonists or genetic deletion in prior studies (Baratchi et al. 2019; Jin, Zheng, and Xia 2011). For LXB₄ treatment, cells were incubated in 1 μ M LXB₄ (Cayman Chemical, Ann Arbor, MI) or the corresponding vehicle (ethanol < 0.01%). For cotreatment experiments, the cells were pretreated with LXB₄ for 20 min before the addition of GSK1016790A.

Immunofluorescence staining

Enucleated eyes were fixed overnight in 4% PFA at 4°C and then dehydrated in a sucrose gradient (10%, 20%, and 30% sucrose in PBS) until the tissues sank. Eyes were embedded in optimal cutting temperature (OCT) medium and frozen at 80°C for immunohistochemistry (IHC). Ten-micron sections were cut on a Leica CM1900 cryostat (Leica Microsystems, Wetzlar, Germany) and mounted on Superfrost Plus slides (Thermo Fisher, Waltham, MA). For immunocytochemistry (ICC), primary and immortalized Müller glia were grown on glass coverslips, washed with PBS, and fixed in 4% PFA for 15 min at room temperature. The

sections and coverslips were permeabilized and blocked for 1 h in PBS containing 10% donkey serum and 0.25% Triton X-100 at RT. Primary antibodies against GFAP (1:1000; Abcam ab53554, Cambridge, UK), vimentin (1:500; Abcam ab8978, Cambridge, UK), Sox9 (1:200; Abcam ab185230, Cambridge, UK), Iba-1 (1:200; Cell Signaling E4O4W, Danvers, MA), and TRPV4 (1:400; Invitrogen PA5-41066, Waltham, MA) were diluted 1:1 (blocking buffer: PBS) and applied overnight at 4°C. The slides and coverslips were washed 3x in PBS (5 min each) and then incubated for 1 h at RT (in the dark) with Alexa Fluor 488- and 594-conjugated secondary antibodies (Invitrogen; 1:1000 dilution). Nuclei were counterstained with DAPI (1:3000), and samples were mounted with FluorSave™ mounting media (Sigma Aldrich, St. Louis, MO). Retinal sections and cell coverslips were imaged via a Nikon Ti-Eclipse fluorescence microscope (Nikon Instruments, Melville, NY) with a 20x objective. Both central and peripheral retina regions were captured for quantitative analysis.

Image analysis

The fluorescence images were analyzed via ImageJ (v1.53c; NIH). Regions of interest (ROIs) were defined based on DAPI staining. Binary masks of each ROI were generated, and the mean fluorescence intensity was measured in the corresponding channels. Negative control sections were used to define the background signal which was subtracted from all measurements.

Liquid chromatography–tandem mass spectrometry

Primary and immortalized Müller glia were collected following 24 h of incubation with or without the TRPV4 agonist. Müller glia cultures and conditioned media were collected in LC-MS-grade methanol for analysis and quantification of the eicosanoid and PUFA pathways via LC–MS/MS via our previously published protocol (Moltke et al. 2012). The supernatants containing lipids were extracted via C18 solid-phase columns and analyzed via a triple-quadrupole linear ion-trap LC–MS/MS system (AB SCIEX 4500 QTRAP) equipped with a Kinetex C18 mini-bore column. The mobile phase was a linear gradient of A (water, acetonitrile, and acetic acid (72:28:0.01 by volume)) and B (isopropanol and acetonitrile (60:40 by volume)), with a flow rate of 450 μ L/min. MS/MS analysis was performed in negative ion mode. PUFAs and lipoxygenase pathway metabolites were identified and quantified via scheduled multiple reaction monitoring (MRM) using 4–6 specific transitions for each analyte. Established diagnostic fragment ions were used for quantification: arachidonic acid (AA; 303→259 m/z), 5-hydroxyeicosatetraenoic acid (5-HETE; 319→115 m/z), 15-HETE (319→175 m/z), 12-HETE (319→179 m/z), LXA₄ (351→115 m/z), LXB₄ (351→221 m/z), and DHA (327→283 m/z) EPA (301→257). Peaks were identified based on the integration criterion of a signal-to-noise ratio of at least 5:1. Class-specific deuterated internal standards were used to validate the retention times of the analytes in each sample. Quantification was based on analyte-specific calibration curves

prepared from synthetic standards (Cayman Chemical, Ann Arbor, MI) (Hassan and Gronert 2009). The results were normalized to the number of cells (1×10^6 cells/sample).

Flow cytometry

Primary Müller glia were treated with a TRPV4 agonist and/or LXB₄ for 24 h and then harvested with TrypLE Express (Gibco, Waltham, MA). The cells were washed in ice-cold PBS and fixed/permeabilized. Fixed cells were incubated overnight at 4°C. The cells were washed in wash buffer and then stained with primary antibody for 1 h at RT in the dark, followed by washing with cell staining wash buffer. The cells were incubated with secondary antibody for 1 h at RT, washed, and resuspended in wash buffer for analysis via flow cytometry. The cells were analyzed via an Attune Acoustic Focusing Flow Cytometer (Thermo Fisher, Waltham, MA). GFAP cells were identified by comparison to isotype controls. The data were analyzed with FlowJo v10 (BD, Ashland, OR). The fraction of GFAP-positive cells was used for quantitative comparisons.

Phospho-kinase assay

For phospho-kinase profiling, the Proteome Profiler Human Phospho-Kinase Array (R&D Systems, Minneapolis, MN) was used. rMC-1 cells were treated with or without 1 μ M LXB₄ for 30 min and assay was performed according to the manufacturer's instructions. Protein concentrations were determined using a BCA assay (Pierce, Thermo Fisher), and 200 μ g of total protein per sample was used for each assay. Membranes imaged on a LI-COR Odyssey system. Spot intensities were quantified using LI-COR software after background subtraction and were normalized to the array's positive control signals.

LXB₄ treatment

For single-cell transcriptomic analysis, the mice were treated i.p. with LXB₄ methyl ester (Cayman Chemical, Ann Arbor, MI) (1 μ g in 100 μ L of PBS) once daily and topically (1 μ g in 5 μ L of PBS per eye) 3x daily for 3 days. Vehicle (ethanol) for LXB₄ methyl ester was removed under a stream of nitrogen, and LXB₄ methyl ester was resuspended in sterile PBS immediately prior to injection or topical treatment. On day 4, the mice were euthanized for retinal dissociation and single-cell library preparation. For IHC analysis, the mice received daily LXB₄ methyl ester (1 μ g in 100 μ L of PBS) via i.p. injection and topical ocular administration for 1 wk. The control mice received equivalent volumes of sterile PBS. LXB₄ was administered 15 min prior to OHT induction.

Single-cell transcriptomics

Single-cell transcriptomic data were reanalyzed for the Müller glia population from a previously published dataset from our laboratory (Maurya, Lin, et al. 2024). Retinas

were dissociated into single-cell suspensions via a papain dissociation kit (Worthington, Columbus, OH), followed by rod photoreceptor depletion in a magnetic column via CD133-biotin labeling and anti-biotin magnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany). Single cells were loaded on the 10x Chromium Single Cell 3 v3.1 platform (10x Genomics, Pleasanton, CA) to generate barcoded gel-bead emulsions, and captured RNA was reverse-transcribed and amplified into cDNA libraries, which were quality-checked on an Agilent Bioanalyzer. Libraries were sequenced on an Illumina NovaSeq S1 100SR flow cell, and raw BCL files were demultiplexed via Illumina bcl2fastq2 at the QB3 Genomics Core, UC Berkeley, Berkeley, CA (RRID: SCR_022170). The sequencing reads were aligned to the mm10 (mouse) genome with Cell Ranger software (10x Genomics, Pleasanton, CA). Downstream analyses were performed in Seurat (v4.1.0) as detailed in our recent publications (Maurya, Lin, et al. 2024; Hao et al. 2021). Retinal cell types were annotated by identifying cluster-specific markers with FindAllMarkers() and comparing them to known markers (Hao et al. 2021). Differential gene expression in Müller glia was determined via FindMarkers() and visualized with VlnPlot() and DotPlot(). The scRNA-seq data were deposited in GEO (GSE251716, scRNA-seq.)

Western blot

Treated Müller glia were lysed in RIPA buffer (Thermo Fisher, Waltham, MA) containing a protease and phosphatase inhibitor cocktail (Thermo Fisher, Waltham, MA) at a 1:100 dilution. Protein concentrations were determined via a BCA assay (Pierce, Thermo Fisher). Equal amounts of protein (20 µg) were denatured, separated on 10% SDS-PAGE gels, and then transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked in Intercept Blocking Buffer (LiCOR, NE) for 1 h at RT and then probed overnight at 4°C with the following antibodies: anti-phospho-STAT3 (Ser727; rabbit monoclonal; 1:1000; Abcam ab32143, Cambridge, MA), total anti-STAT3 (rabbit monoclonal; 1:2000; Abcam ab109085, Cambridge, MA), and anti-GAPDH (mouse monoclonal; 1:10,000; Cell Signaling Technology, Dancars, MA). After washing in TBS-T, the membranes were incubated with IRDye 800CW donkey anti-rabbit IgG and IRDye 680RD donkey anti-mouse IgG (1:10,000; LI-COR Biosciences, Lincoln, NE) for 1 h at RT in the dark. The blots were imaged on a LI-COR Odyssey system. Band intensities were quantified with ImageJ (NIH). Phospho-STAT3 signals were normalized to total STAT3 and GAPDH signals and normalized to those of untreated controls for fold-change calculations.

Statistical analysis

All the data are presented as the means $\pm$ standard errors of the means (SEMs). Biological replicates (n) represent the number of animals (for in vivo experiments) or independent cell culture experiments (for in vitro assays). Statistical analyses were conducted in GraphPad Prism 9.0 (GraphPad Software, San Diego, CA). For two-group comparisons, an unpaired Student's t-test was used. For multiple-group comparisons, one-way analysis of variance

(ANOVA) followed by Tukey's post hoc test was performed, with the assumption of a normal distribution of variance. Statistical significance was defined as $p < 0.05$.

3.3 Results

Müller glia express a functional lipoxin biosynthetic pathway

Our previous studies established that astrocytes generate lipoxins and that LXB₄ treatment reduces Müller glia reactivity (Karnam et al. 2024; Zhang, Li, Huang, et al. 2019). We next asked whether Müller glia themselves synthesize lipoxins. Primary mouse Müller glia were isolated and validated by ICC for canonical markers, including vimentin, glutamine synthetase, and SOX9, and were confirmed to be negative for GFAP and IBA1 (Fig. 3.1A). Immortalized rMC-1 cells were validated via the same markers and expressed all the same positive and negative markers (Fig. S1A). Bulk RNA-seq analyses of cultured Müller glia revealed robust expression of Alox5, along with significant expression of Alox5ap, Alox12 and Alox15 (Fig. S1B), indicating the presence of key lipoxygenase pathway enzymes for lipoxin generation. Targeted lipidomic profiling revealed the presence of arachidonic acid (AA) and its lipoxygenase metabolites 5-HETE, 12-HETE, and 15-HETE in both cell lysates and conditioned media from both primary (Fig. 3.1C) and rMC-1 cells (Fig. S1B). qPCR analysis established robust expression of the Alox5 (Ct: 27.18) and Alox15 (Ct: 28.05) genes in primary Müller glia, confirming the RNA-seq data (Fig. 3.1D). More importantly, Müller glia (both primary and immortalized) generated significant amounts of 5-HETE (1210 ng/mL) and 15-HETE (1502 ng/mL) and endogenous LXB₄ (114 pg/mL) (Fig. 3.1C, Fig. S1B, Fig. S2A). No LXB₄ was detected in media with serum and no cells which served as a control for cell specific formation. Together, these findings establish that primary Müller glia functionally express the LXB₄ biosynthetic pathway (Alox15 and Alox5), revealing a previously unrecognized glia lipid mediator pathway.

Ocular hypertension induces a reactive Müller glia phenotype and increases TRPV4 expression

To model sustained mechanical insult, we induced chronic OHT in C57BL/6J mice via anterior-chamber silicone-oil injection (Karnam et al. 2024; Maurya, Lin, et al. 2024; Zhang, Li, Huang, et al. 2019). Mice were euthanized, and the retinas were collected at 2, 6, and 8 wk post-injection to assess Müller glia reactivity by IHC with GFAP as an established marker (Fig. 3.2A). Imaging revealed an 80% increase in GFAP signal intensity in the ganglion cell layer (GCL) and inner nuclear layer (INL), specifically in astrocytes and Müller glia, at 2 wk and 6 wk (Fig. 3.2B). In parallel, the TRPV4 signal intensity increased significantly at every time point examined (Fig. 3.2C), with a 114.6% increase in TRPV4 expression relative to that in age-matched normotensive controls at 6 wk (Fig. 3.2D). qPCR analysis at 4 wk OHT revealed the upregulation of Il-6 (6.2-fold), Tnf α (20-fold), and Stat3 (25.5-fold)

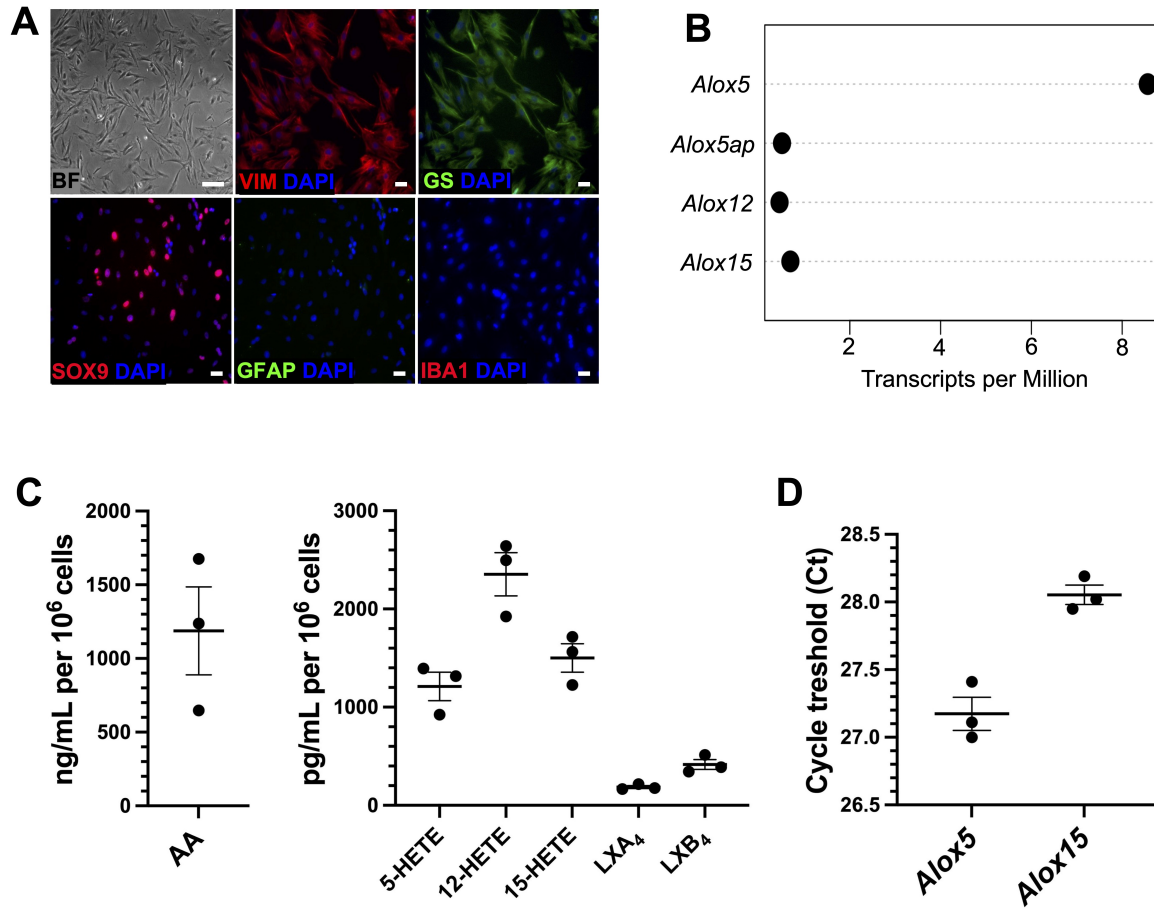


Figure 3.1: (A) Primary mouse Müller glia were stained for canonical markers (vimentin [VIM], glutamine synthetase [GS], and SOX9) and negative control markers (GFAP and IBA1). (B) Bulk RNA sequencing of primary Müller glia for lipoxygenase pathway genes ($n = 3$ biological replicates). (C) LC-MS/MS quantification of released eicosanoid substrate (AA) and corresponding lipoxygenase products in primary Müller glia ($n = 3$ biological replicates). (D) qPCR analysis of *Alox5* and *Alox15* expression in primary Müller glia ($n = 3$ biological replicates).

when compared with controls (Fig. 3.2E), which was consistent with proinflammatory activation. Gene set enrichment analysis (GSEA) of RNA-seq data from MACS-isolated Müller glia supported these findings, revealing significant upregulation of cytokine-related pathways, including Il-6 and Tnf α production. In addition, pathways related to visual perception and the response of sensory stimuli to light stimuli were also downregulated for the primary supportive cell of neuronal cells, indicating possible negative changes in Müller glia supportive functional pathways (Fig. 3.2F). These data demonstrate that chronic OHT induces gliosis-associated molecular changes, TRPV4 upregulation, and activation of inflammatory signaling pathways, highlighting the role of Müller glia as central responders to mechanical insult.

TRPV4 activation drives Müller glia reactivity and amplifies LXB₄ production

To isolate the specific contribution of TRPV4 signaling, primary and immortalized Müller glia were treated for 24 h with the selective TRPV4 agonist GSK. Cells from control (ctrl) and treated conditions were analyzed by flow cytometry, and GFAP+ cells were selectively gated (Fig. 3.3A). Quantification revealed that TRPV4 activation caused a 118% increase in GFAP-positive cells compared with vehicle-treated controls (DMSO) (Fig. 3.3B). ICC further confirmed the increased expression of GFAP and vimentin along Müller glia filaments following TRPV4 activation (Fig. 3.3C). Lipidomic analyses revealed that TRPV4 activation triggered a marked increase in the release of the lipoxygenase substrate arachidonic acid (AA) and 12-HETE a downstream signature 12/15-LOX metabolite. Notably, reactive Müller glia increased LXB₄ production by 2.3-fold relative to controls (Fig. 3.3D). Thus, TRPV4 activation is sufficient to drive Müller glia reactivity and activate lipoxygenase pathways to amplify intrinsic LXB₄ production.

LXB₄ suppresses JAK-STAT signaling in homeostatic Müller glia

Because IL-6/JAK/STAT is a central driver of reactive gliosis and was upregulated in our OHT model (Fig. 3.2E), we next investigated whether exogenous LXB₄ modulates this pathway. When immortalized Müller glia (rMC-1) were treated with LXB₄, a phosphokinase array revealed that LXB₄ selectively reduced the phosphorylation of STAT2-Y690 (18%), STAT5a/b-Y699 (16.8%), and STAT3-S727 (19%) (Fig. 3.4A). To assess the transcriptional consequences of LXB₄ signaling in vivo, retinas from LXB₄ treated mice were analyzed by single-cell RNA-seq. UMAP analysis revealed a distinct Müller–glia cluster (Fig. 3.4B), within which Stat1 and Stat3 transcript levels were reduced in LXB₄-treated mice (Fig. 3.4C). Differential gene expression analysis from single-seq data further revealed downregulation of Jak2, Il6st, Stat5b, and Stat2 (Fig. 3.4D), indicating broad suppression of the inflammatory JAK-STAT network. Collectively, these findings demonstrate that LXB₄ broadly attenuates JAK-STAT signaling at both the protein and transcriptional levels in homeostatic Müller glia.

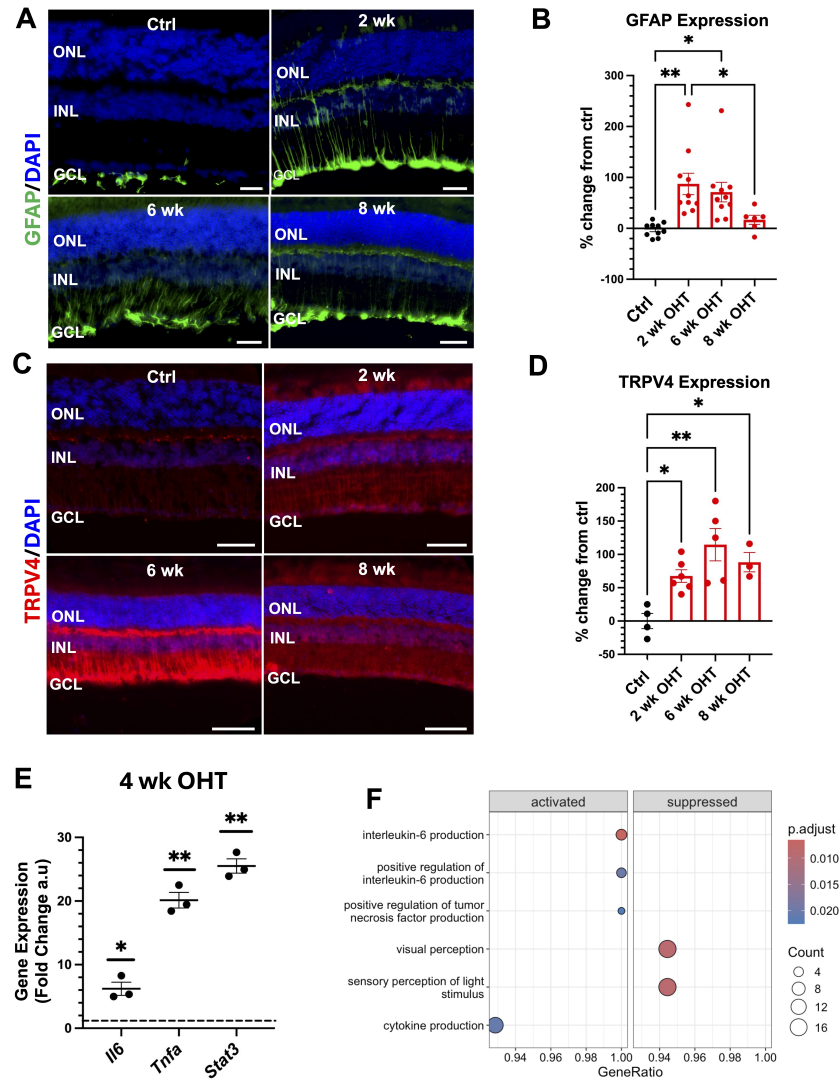


Figure 3.2: (A) Representative IHC images of retinal sections from mice with sustained OHT at 2, 6, and 8 wk post-injection and age-matched normotensive controls stained for GFAP and DAPI. (B) Quantification of GFAP immunoreactivity, expressed as the percent change from the control (n = 5-10). (C) Representative immunofluorescence images from mice with (OHT) and normotensive controls stained for TRPV4 and DAPI. (D) Quantification of TRPV4 immunoreactivity, expressed as the percent change from control (n = 3-7). (E) qPCR analysis of *Il6*, *Tnfa*, and *Stat3* in whole retinas collected 4 wk post-OHT (n = 3). Data were normalized to *Gapdh* and matched with normotensive controls. (F) Gene set enrichment analysis (GSEA) of MACS-isolated Müller glia from chronic OHT conditions. Images acquired at 20x magnification; scale bar = 50 μ m. GCL = ganglion cell layer, INL = inner nuclear layer, ONL = outer nuclear layer. Statistical analysis: one-way ANOVA with Tukey's post hoc test (*p < 0.05, **p < 0.01, ***p < 0.001) or unpaired Student's t-test (*p < 0.05, **p < 0.01).

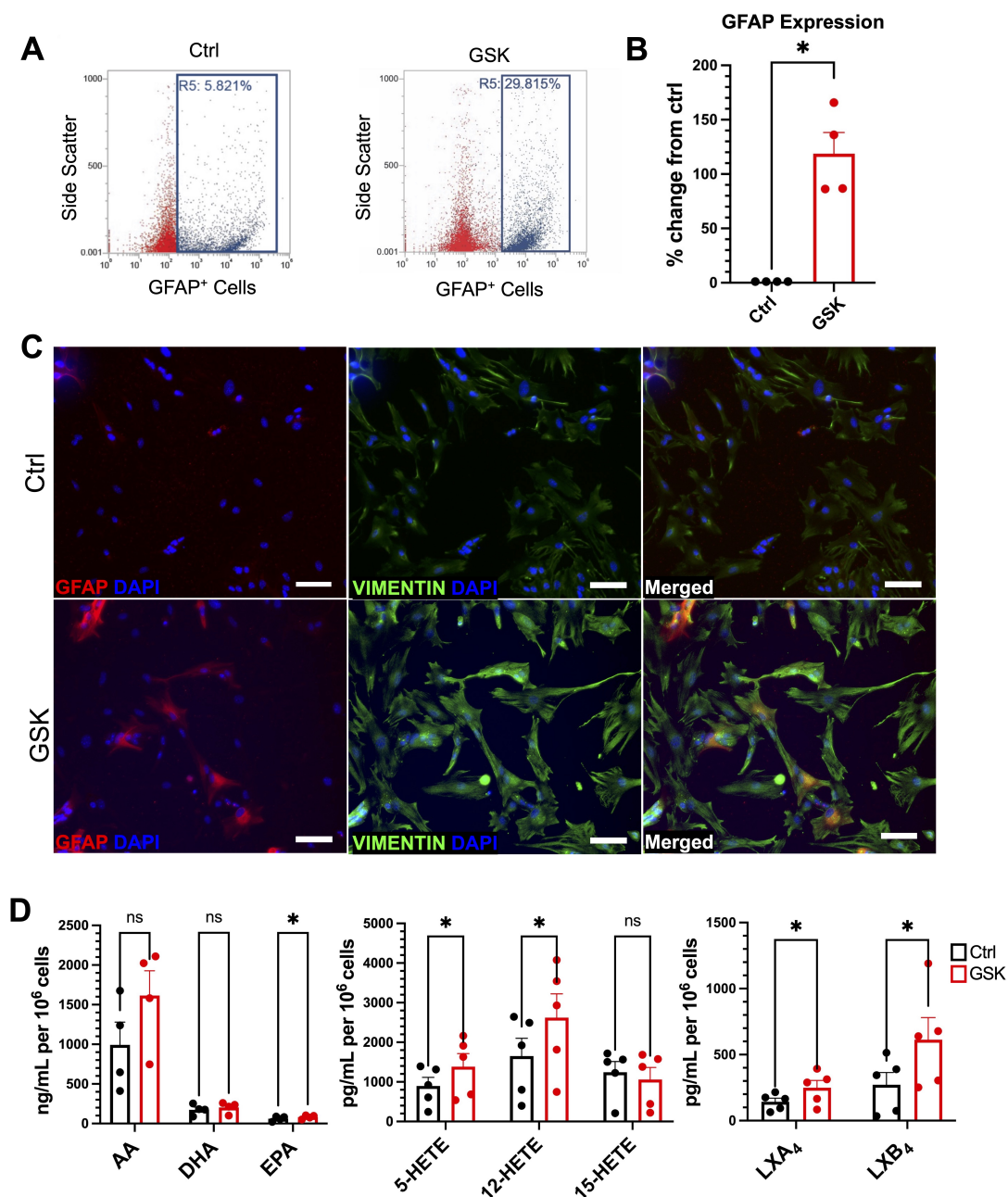


Figure 3.3: (A-B) Flow cytometry gating and quantification of GFAP cells following TRPV4 agonist (GSK) vs vehicle (DMSO, Ctrl) treatment ($n = 4$ biological replicates). (C) Representative ICC images of GFAP and vimentin in GSK- or vehicle-treated (Ctrl) Müller glia. (D) LC-MS/MS quantification of released PUFAs, 5-LOX, 12/15-LOX metabolites, and lipoxins (LXA₄, LXB₄) by Müller glia following GSK treatment ($n = 5$ biological replicates). Images acquired at 20x magnification; scale bar = 50 μ m. Statistical analysis: unpaired Student's t test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

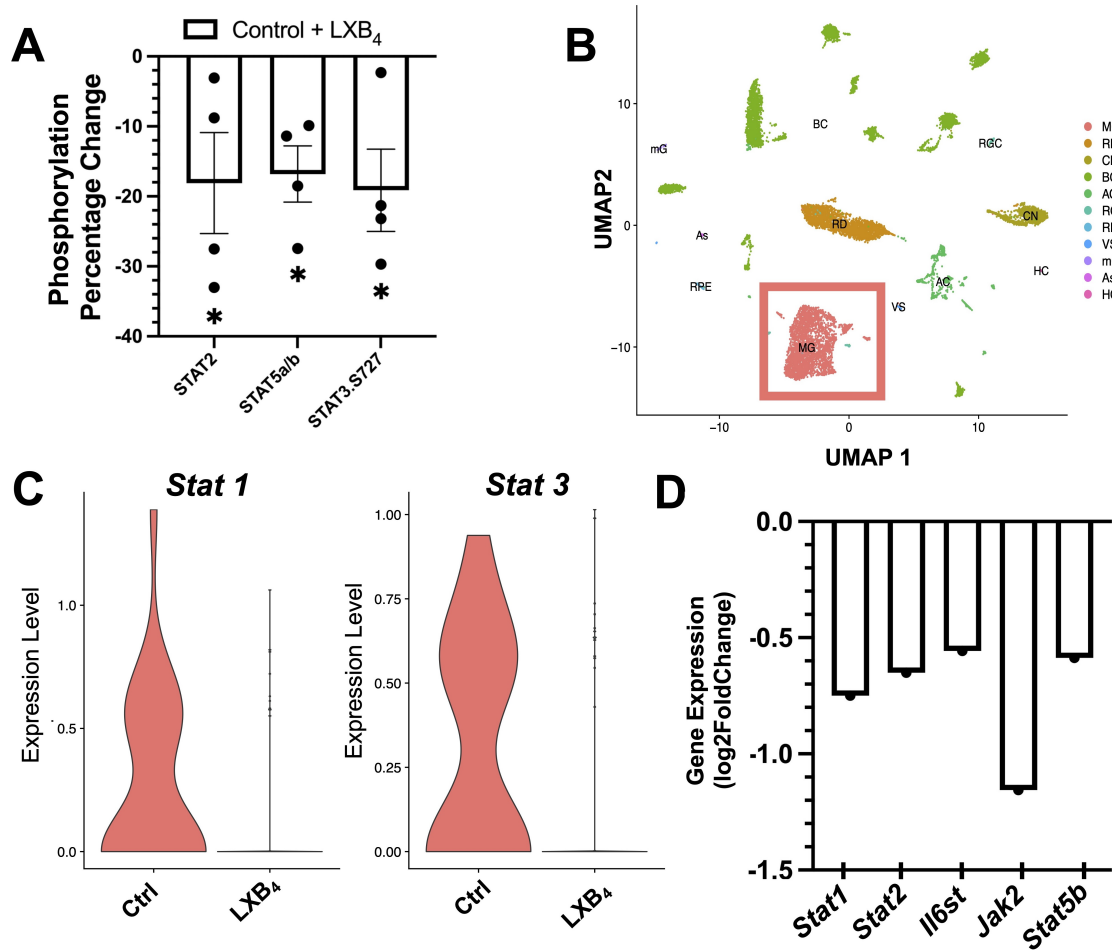


Figure 3.4: (A) Phospho-kinase array of immortalized Müller glia (rMC-1) treated with or without LXB₄ (n = 4). (B) UMAP projection of single-cell RNA sequencing data from retinas treated with or without LXB₄, highlighting the Müller glia (MG) populations. (C) Violin plots comparing Stat1 and Stat3 expression in MG populations between groups. (D) Differential gene expression of Jak2, Stat2, Stat5b, Il6st, and Stat1 in MGs from the scRNA-seq dataset. Statistical analysis: unpaired Student's t test (*p < 0.05).

LXB₄ inhibits TRPV4-induced Müller glia reactivity via IL-6/STAT3 suppression

To determine whether LXB₄ can prevent TRPV4-induced Müller glia reactivity, we pretreated primary Müller glia with LXB₄ prior to stimulation with the TRPV4 agonist GSK. After 24 h, flow cytometry analysis revealed that LXB₄ pretreatment significantly reduced the number of GFAP-positive cells by 84.6% compared with that in the GSK-treated controls (Fig. 3.5A-B). ICC further confirmed that the GFAP and vimentin filament intensities significantly decreased, returning to near-baseline levels (Fig. 3.5C). qPCR analysis revealed a significant decrease in Il6 expression in LXB₄-treated primary Müller glia (Fig. 3.5D). This reduction in Il6 (1.6-fold) was accompanied by decreased STAT3 phosphorylation (Ser727) by 75%, as determined by Western blotting and normalized to total STAT3 and GAPDH (Fig. 3.5D), which is consistent with Il6 acting upstream of STAT3 activation. Together, these results demonstrate that LXB₄ effectively suppresses TRPV4-induced inflammatory activation in immortalized Müller glia by downregulating Il6 expression, inhibiting STAT3 phosphorylation, and preventing the upregulation of cytoskeletal reactivity markers.

LXB₄ inhibits inflammatory gene expression and TRPV4 upregulation in chronic and acute OHT models

Finally, we evaluated the therapeutic potential of LXB₄ in both chronic and acute models of OHT (Karnam et al. 2024; Maurya, Lin, et al. 2024). In the chronic model, qPCR analysis of whole retina RNA from mice treated with LXB₄ after 4 wk of OHT revealed marked reductions in Stat3 (1.95-fold), Il6 (3.7-fold), and Tnf α (3.6-fold) expression when directly compared to vehicle-treated mice with OHT (Fig. 3.6A). In a separate acute OHT model (1 wk post-induction), IHC demonstrated that LXB₄ treatment prevented the OHT-induced increase in TRPV4 expression, particularly in the GCL and INL (Fig. 3.6B). TRPV4 signal intensity was reduced by 63% in LXB₄-treated mice when directly compared to vehicle-treated mice with OHT (Fig. 3.6C). These in vivo results reinforce the therapeutic neuroprotective potential of LXB₄, as it suppresses inflammation and TRPV4 upregulation in both acute and chronic OHT models of glaucomatous neurodegeneration.

3.4 Discussion

The role of Müller glia in glaucoma pathogenesis is complex and multifaceted, transitioning from an initially protective response to a chronic, maladaptive phenotype that accelerates RGC loss (Hu et al. 2021). Building on prior work showing that astrocyte-derived LXB₄ protects RGCs, we extended this paradigm to Müller glia (Karnam et al. 2024; Livne-Bar, Wei, et al. 2017). We show that these cells are both sensors of biomechanical insult via TRPV4 and active producers of an intrinsic, lipoxin-based anti-inflammatory circuit. These

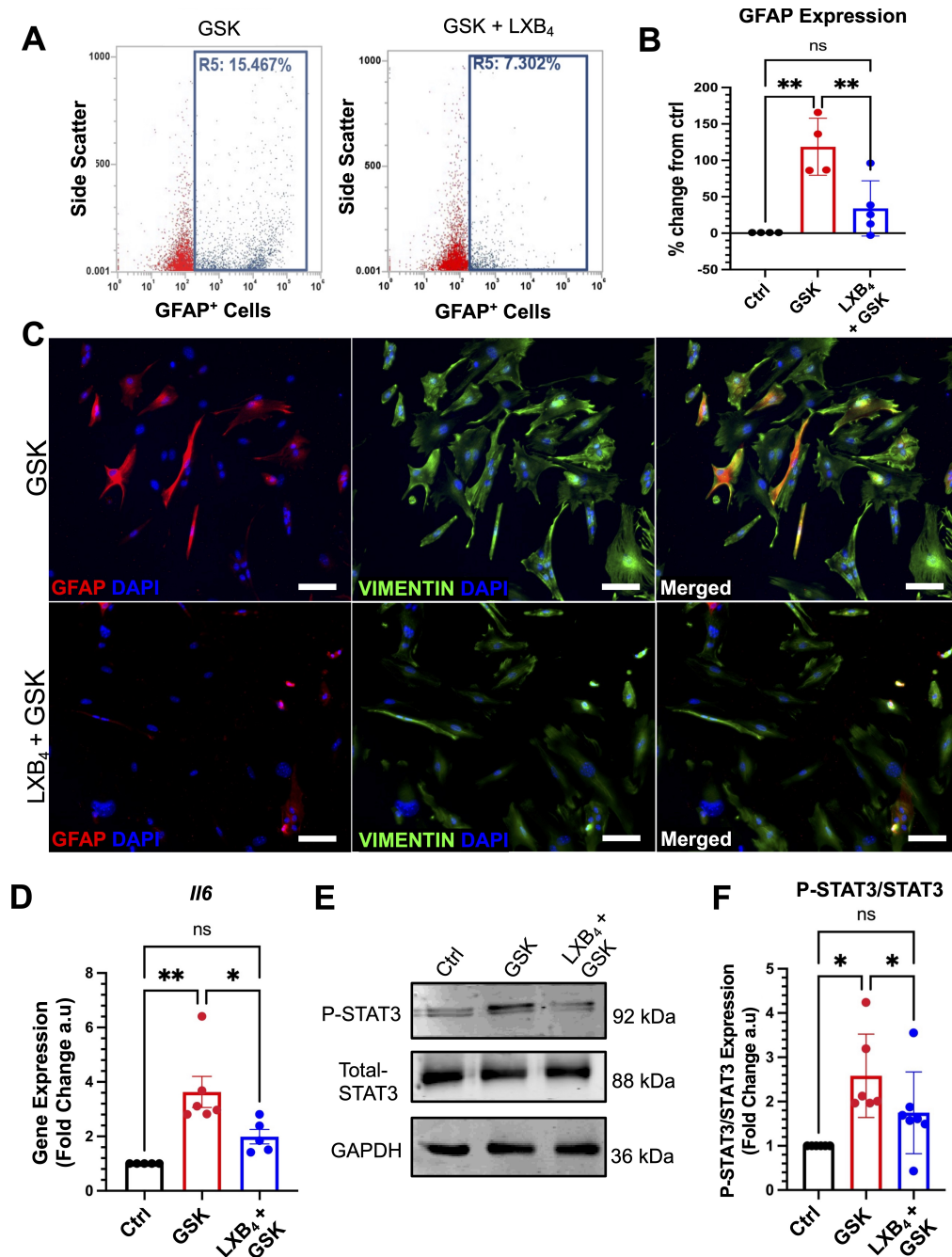


Figure 3.5: (A-B) Flow cytometry of GFAP cells in control (vehicle), GSK, or GSK + LXB₄ treated primary Müller glia (n = 4 biological replicates). (C) ICC images of GFAP and vimentin in the GSK, or GSK+LXB₄ treated cells. (D) qPCR results for *Il6* expression in control, GSK, or LXB₄ + GSK treated Müller glia (n = 5). (E-F) Western blot analysis of phospho-STAT3 (S727) and total STAT3. Representative blots are shown; GAPDH was used as a loading control (n = 5). Images acquired at 20x magnification; scale bar = 50 μ m. Statistical analysis: one-way ANOVA with Tukey's post hoc test (*p<0.05, **p<0.01).

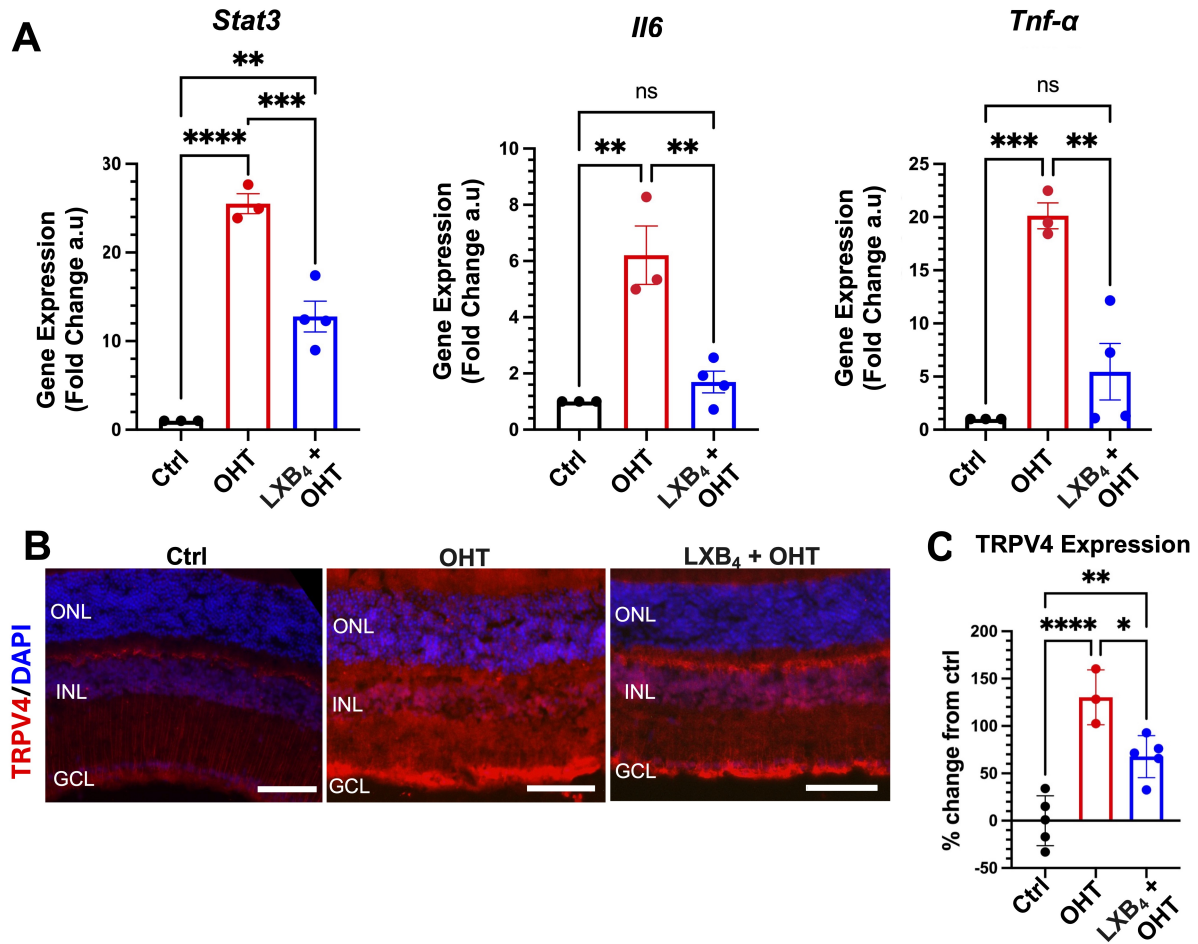


Figure 3.6: (A) qPCR analyses of *Stat3*, *Il6*, and *Tnf-α* expression in whole retinas of normotensive eyes and at 4 wk of chronic OHT with or without LXB₄ treatment (n = 4 biological replicates). (B) Representative IHC images of TRPV4 IHC in normotensive controls and those subjected to acute OHT for 1 wk with or without LXB₄ treatment. (C) Quantification of TRPV4 protein expression in control retinas and OHT-treated retinas with or without LXB₄ treatment (n = 3–5 biological replicates). Images acquired at 20x magnification; scale bar = 50 μm. Statistical analysis: one-way ANOVA with Tukey's post hoc test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

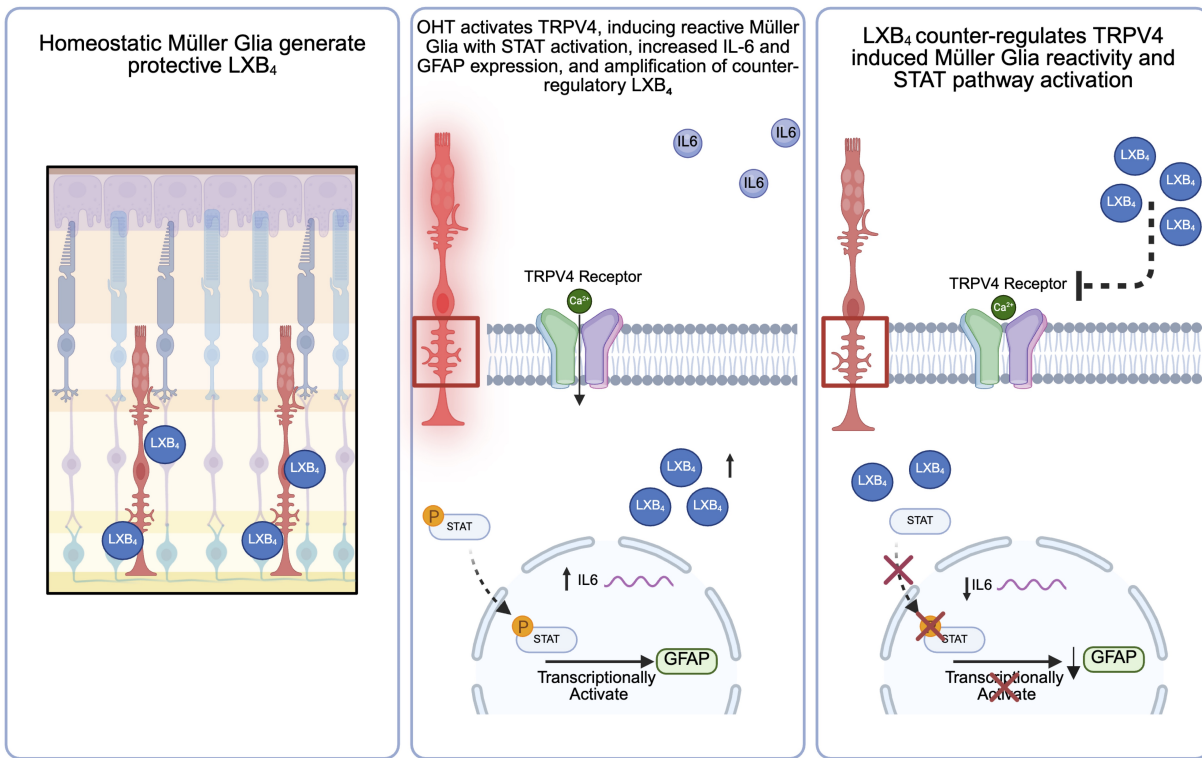


Figure 3.7: Homeostatic Müller glia express the full LXB₄ biosynthetic pathway and generate neuroprotective LXB₄. OHT leads to activation of mechanosensitive TRPV4 and upregulation of the ion channel in Müller Glia. This chronic and amplified activation induces an inflammatory reactive Müller Glia phenotype but in parallel amplifies neuroprotective LXB₄ as a counter-regulatory pathway. LXB₄ treatment downregulates TRPV4 and counter regulates TRPV4 signaling by inhibiting the STAT pathway and expression of IL6 and GFAP.

findings suggest that Müller glia not only contribute to neuroinflammation but also possess a self-regulatory mechanism that could be amplified to suppress gliosis and protect RGCs.

We discovered that Müller glia express a functional lipoxin biosynthetic circuit characterized by 5-LOX and 15-LOX expression and the endogenous production of LXB₄. While previous studies have identified retinal astrocytes as a source of lipoxins (Karnam et al. 2024), our findings extend this paradigm by demonstrating that Müller glia, the most abundant macroglia cell type in the retina, also is a key contributor to this neuroprotective retinal pathway. LC-MS/MS-based lipidomics confirmed that these cells not only express the necessary enzymes but also actively produce key lipoxin precursors (5-, 12-, and 15-HETE) and release neuroprotective LXB₄ under homeostatic conditions, revealing a previously unrecognized biosynthetic capacity.

In vivo, chronic OHT robustly upregulated TRPV4 in Müller glia, leading to the activation of canonical gliosis hallmarks, including IL-6/STAT3 signaling and GFAP expression. These findings align with those of previous studies in other central nervous system (CNS) models, which demonstrated that TRPV4 activation leads to Ca^{2+} -dependent p-STAT3 induction and that inhibiting STAT3 mitigates neurodegeneration (Herrmann et al. 2008; Lupien, Calkins, and Horner 2010; Gavino et al. 2016). Our in vitro data further support this link, showing that pharmacological TRPV4 activation in Müller glia recapitulates gliotic changes while increasing lipoxin production. These findings suggest a direct functional relationship between the biomechanical stress sensor TRPV4 and the gliotic response, highlighting a key regulatory point for Müller glia function.

TRPV4-activated Müller glia reactivity induced both inflammatory (IL-6 and $\text{TNF}\alpha$) and neuroprotective (LXB_4) pathways. The lipoxygenase pathways (5-LOX and 12/15-LOX) that were amplified, in addition to generating protective lipoxins, can also generate pro-inflammatory lipid mediators (Wei and Gronert 2017; Gronert 2010). 5-LOX, a rate limiting enzyme for lipoxin generation, can also generate pro-inflammatory leukotrienes. However, in vitro primary and immortalized Müller glia did not generate any detectable amounts of leukotrienes under any conditions. Consistent with our previous lipidomic analysis of healthy and glaucomatous retinas (Karnam et al. 2024; Livne-Bar, Wei, et al. 2017), these findings are consistent with our previous in vivo studies that detected no leukotrienes in the retina or optic nerve in healthy eyes or eyes with OHT (Karnam et al. 2024; Livne-Bar, Wei, et al. 2017). 12-HETE, a primary product of murine 12/15-LOX (Alox15), was increased in reactive rodent Müller glia; in contrast, human ALOX15 primarily generates 15-HETE (Moustafa et al. 2024). 12-HETE has been reported in patients with diabetic retinopathy and is known to activate and upregulate inflammatory markers, including IL-6 and $\text{TNF}\alpha$, in Müller glia (Moustafa et al. 2024; Chen, Liu, et al. 2024). These findings indicate that TRPV4-induced gliosis triggers activation of downstream lipoxygenase pathways which potentially amplifies LXB_4 neuroprotective signaling but may also initiate an inflammatory pathway (i.e., 12-HETE). Hence, TRPV4 activation triggered by OHT-induced biomechanical stress triggers gliosis but can also increase lipoxin biosynthesis in Müller glia, which indicates a potential self-regulating mechanism where TRPV4-driven gliosis is counterbalanced by amplified endogenous lipoxin signaling.

LXB_4 treatment disrupted the inflammatory circuit and restored homeostatic balance under chronic OHT. Our experiments revealed that LXB_4 treatment suppresses STAT3 phosphorylation, downregulates TRPV4 expression, and reverses gliosis (Figs. 3.4–3.6), effects that parallel those observed with pharmacological TRPV4 inhibition (Li, Cheng, et al. 2021). Our findings align with studies that have shown treatment with omega-3 pro-resolving lipid mediators, such as Maresin-1, has overlapping anti-inflammatory actions by decreasing TRPV4 expression in other CNS models (Pathak et al. 2023). Lipoxins, unlike Maresins, are an endogenous protective lipid mediator pathway in the retina. Hence, therapeutic amplification of LXB_4 signaling selectively targets the resident lipid mediator pathway in the retina. Stable analogs of lipoxin have been developed and especially the protective action of LXA_4 mimetics are well characterized in multiple models of CNS dysfunction (Martini et al.

2016). In models of posterior uveitis, a form of retinal inflammation, both LXA₄ and LXB₄ reduce macroglia reactivity, microglia activation, and macrophage infiltration (Livne-Bar, Maurya, et al. 2024). However, LXB₄'s neuroprotective actions in OHT and neurotoxic models of glaucomatous injury are more potent than those of LXA₄ (Livne-Bar, Wei, et al. 2017). While prior studies have focused on lipoxin activity in modulating astrocyte and microglia inflammatory responses, our current findings are the first to identify Müller glia as both a source and a target of lipoxin signaling, establishing a unique self-regulatory inflammatory circuit specific to the retina (Karnam et al. 2024; Maurya, Lin, et al. 2024; Liu, Cullen, et al. 2024; Livne-Bar, Wei, et al. 2017).

The identification of a counter-regulatory lipoxin circuit in Müller glia has potential translational implications for glaucoma treatment. Since Müller glia are the major glia cell type in the retina and originate from the same progenitor cells as RGCs do, they represent an ideal target for therapeutic intervention (Reichenbach and Bringmann 2010; Seitz, Ohlmann, and Tamm 2013). Therefore, strategies that augment this intrinsic protective circuit could be a targeted approach to prevent the maladaptive gliosis that precedes RGC degeneration. For example, this could be achieved pharmacologically with established stable LXB₄ analogs or genetically via macroglia specific AAV-mediated amplification of the lipoxin pathway. This targeted approach is particularly promising, as it leverages the retina's own neuroprotective mechanisms to restore homeostatic balance.

This study has several limitations. While our findings provide mechanistic insight into Müller glia reactivity, longer-term studies are needed to evaluate the impact of LXB₄ treatment on the homeostatic function of Müller glia. Although we focused on TRPV4 and STAT3 signaling, other inflammatory pathways, such as the NF- κ B or MAPK pathways, likely contribute to the gliotic response and deserve further exploration (Palazzo et al. 2022; Prieto et al. 2010). Additionally, much of our mechanistic work was performed in primary and immortalized Müller glia cultures. While these systems are invaluable for dissecting signaling pathways in a controlled environment, they inevitably simplify the cellular complexity of the retina. Important interactions between glia, neurons, and the vasculature are not fully understood *in vitro*. Future work using conditional knockout models and live imaging will help clarify the temporal dynamics of this lipid signaling axis *in vivo*.

In summary, we identified the intrinsic neuroprotective and anti-inflammatory lipoxin circuit in Müller glia, which are the primary and most abundant supportive glia in the retina. We established that chronic activation of the mechanosensitive TRPV4 ion channel in Müller glia drives sustained gliosis and IL-6/STAT3 pathway activation. This TRPV4-induced gliosis is counter-regulated by the amplification of Müller glia LXB₄ formation. LXB₄ targets both OHT-induced TRPV4 overexpression and TRPV4-induced STAT3 phosphorylation, thereby reversing the inflammatory reactivity of Müller glia. Hence, targeting the Müller glia lipoxin pathway could provide new therapeutic opportunities for glaucoma and other retinal neurodegenerative diseases associated with chronic activation of TRPV4 and gliosis of Müller cells (Fig. 3.7).

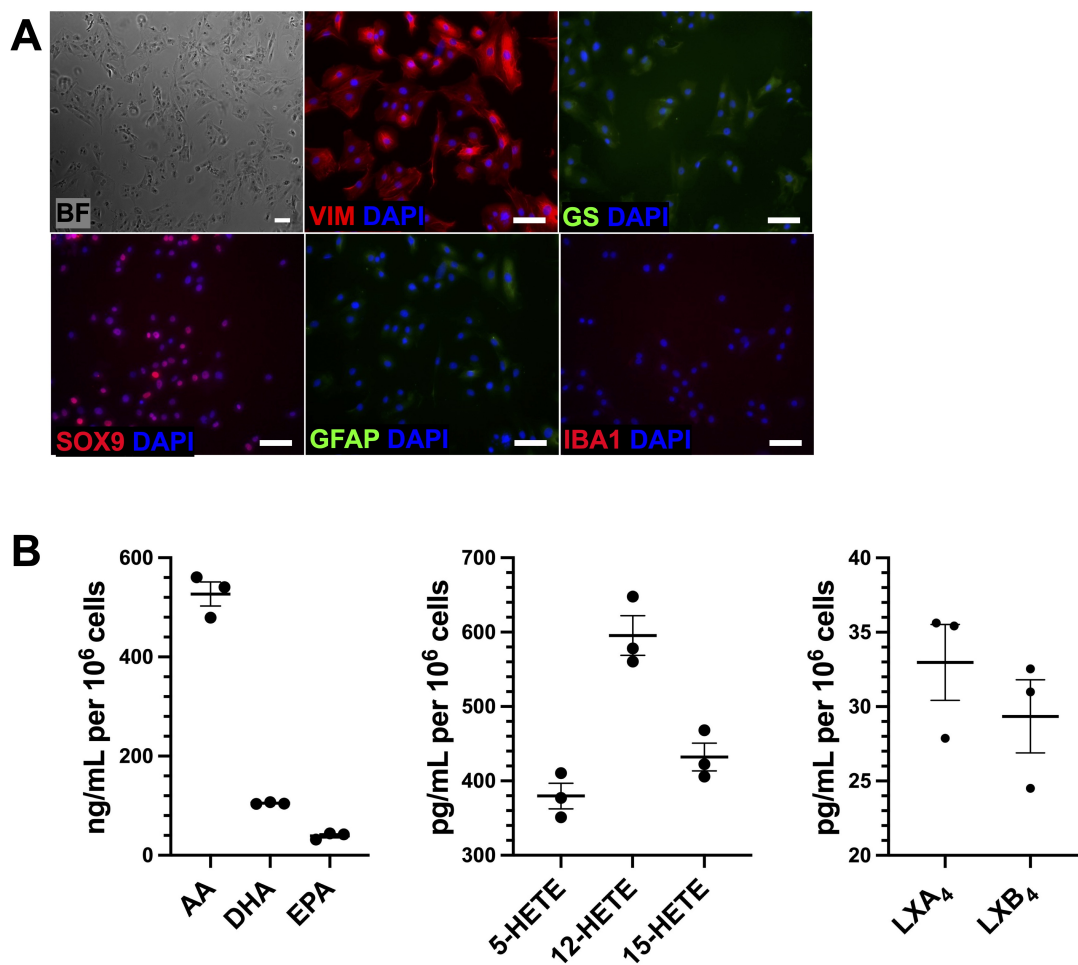


Figure S1: (A) Rat Immortalized Müller glia cultures (rMC-1) stained for canonical markers (vimentin [VIM], glutamine synthetase [GS], and SOX9) and negative markers (GFAP and IBA1). (B) LC-MS/MS analysis of polyunsaturated fatty acids (PUFAs) and lipoxigenase products released by immortalized Müller glia. Images acquired at 20x magnification; scale bar = 50 μ m

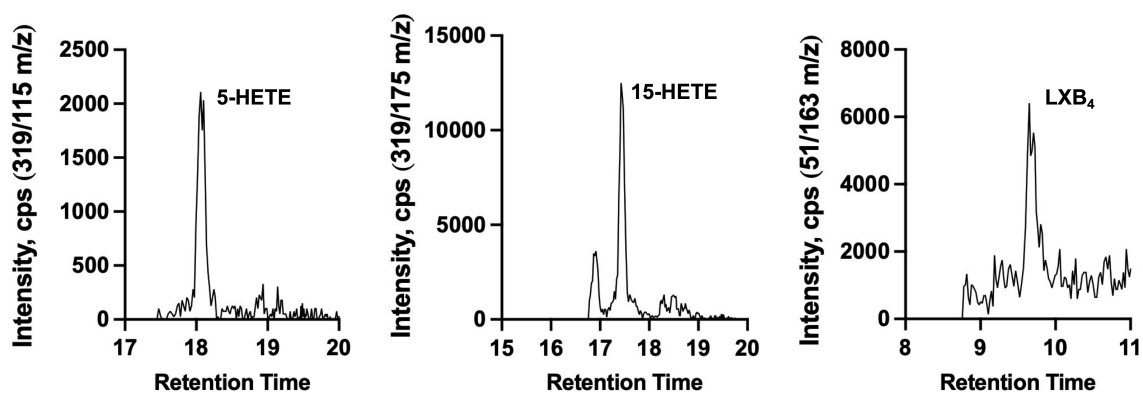


Figure S2: Representative diagnostic raw chromatograms and LC retention times for Primary Müller glia-generated 5-HETE, 15-HETE, and LXB₄.

Chapter 4

FFT-Based Quantification of RLBP1-Labeled Müller Glia Stalk Organization Reveals Disruption During Ocular Hypertension

4.1 Introduction

Müller glia are the principal macroglia of the retina and are distinguished by a highly polarized morphology that spans the full thickness of the neural retina (1,2). Their apical processes extend to the outer limiting membrane, where microvilli interface with photoreceptor inner segments, whereas their basal endfeet terminate at the inner retinal surface and closely associate with retinal ganglion cells, retinal vasculature, and other cells in the inner retina (Wang, O’Sullivan, et al. 2017; Chan-Ling 1991). Because they extend across retinal layers and maintain intimate contact with neuronal and vascular elements, Müller glia are positioned to sense and respond to local changes in retinal homeostasis across distinct microenvironments (Reichenbach and Bringmann 2010).

Müller glia are also among the earliest responders to retinal stress and injury. In an acute setting, reactive changes can support tissue stability and preserve retinal architecture. However, when gliosis is prolonged, these responses can become maladaptive and are associated with cytoskeletal remodeling, disruption of homeostatic support functions, formation of glia scars or membranes, and broader structural disorganization of retinal tissue (Reichenbach and Bringmann 2010; Hippert et al. 2015; Bringmann and Wiedemann 2011). Because Müller glia function is tightly linked to their radial architecture and layer-specific interactions, methods that quantify structural remodeling are important for understanding how reactive changes relate to neuronal and vascular dysfunction in disease (Wang, O’Sullivan, et al. 2017; Grimes et al. 2024; O’Leary and Campbell 2023; Coorey, Shen, Chung, et al. 2012).

Despite their importance, quantitative analysis of Müller glia structure remains challenging. In contrast to microglia and astrocytes, whose reactive states are often captured by changes in branching complexity, arborization, or process length, Müller glia retain a highly stereotyped radial organization that is less amenable to conventional morphology metrics (Colombo et al. 2022; Baldwin, Murai, and Khakh 2024; Maurya, Lin, et al. 2024). Recent advances, including the Fiji-based GliaMorph toolkit, have improved three-dimensional analysis of Müller glia architecture and texture, but these approaches depend heavily on image quality, segmentation performance, and well-resolved labeling, which can be difficult to achieve reproducibly in antibody-labeled tissue sections from mouse disease models (Kugler et al. 2023). These limitations create a need for simpler and more accessible analytical strategies that can capture biologically meaningful changes in Müller glia-associated structural organization across healthy and diseased tissue.

In the mouse retina, Müller glia stalks normally exhibit a strongly ordered radial arrangement across retinal layers. In ocular disease, this organization can become disrupted, resulting in reduced directional coherence and increased structural irregularity. Fourier-based image analysis is well suited to quantify such changes because line-like or fibrillar features with a preferred orientation generate directionally biased power distributions in the frequency domain, whereas disorganized structures produce broader, less directionally biased spectra. In related work, FFT-based directional coherence analysis has also been used to quantify neurite tortuosity in dense ocular nerve networks, supporting the broader utility of Fourier-domain metrics for assessing structural order in filamentous biological images (Smith et al. 2021). Accordingly, fast Fourier transform (FFT)-based analysis can be used to convert spatial information from Müller glia images into an angular power distribution that reflects the degree of alignment and orientation bias within a region of interest.

Here, we describe a simple FFT-based workflow for quantifying the directional organization of RLBP1-labeled Müller glia stalks in retinal sections. Because this approach is designed to measure directional organization, a marker that preserves continuous radial morphology is required. RLBP1 provided consistent labeling of Müller glia processes across retinal layers and was therefore used as the structural marker for analysis. By leveraging the intrinsic radial alignment of Müller glia, this method distinguishes organized from disrupted stalk patterning without requiring transgenic labeling, complex three-dimensional reconstruction, or highly specialized computational pipelines. Importantly, the assay quantifies marker-defined structural organization rather than full Müller glia morphology, providing an accessible framework for detecting Müller glia-associated remodeling in health and disease.

4.2 Methods

Mice

All animal studies were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and with approval from the Institutional Animal Care and Use Committee at the University of California, Berkeley. C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained under a 12-hour light/12-hour dark cycle with *ad libitum* access to food and water.

Mouse model of ocular hypertension

Silicone oil-induced ocular hypertension (OHT) was established in 8-week-old mice using a previously described protocol (Zhang, Li, Huang, et al. 2019; Zhang and Hu 2020; Fang et al. 2021). Mice were anesthetized with intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg), and topical ocular anesthesia was achieved using 0.5% proparacaine hydrochloride (Sandoz, Princeton, NJ). Under a surgical microscope, pharmaceutical-grade silicone oil (Alcon, Fort Worth, TX) was injected into the anterior chamber of both eyes using a 33 G Hamilton glass syringe (Reno, NV). To induce chronic mild OHT, 1.2 μ L of silicone oil was injected. To induce acute severe OHT, 1.8 μ L of silicone oil was injected. Sham-injected control mice underwent corneal incision with a sterile 31 G paracentesis needle without silicone oil injection. After each procedure, 0.3% tobramycin ophthalmic solution (Tobrex; Alcon, Fort Worth, TX) was applied topically.

IOP in this model has been previously characterized by rebound tonometry, as described (Karnam et al. 2024; Maurya, Lin, et al. 2024). OHT produced by silicone oil injection is sustained for up to 8 weeks, as previously validated. In prior studies, IOP was measured once every 2 weeks using a rebound tonometer, confirming persistent elevation throughout the experimental period. In the present study, repeated IOP measurements were not performed because pupil dilation required for tonometry can disrupt silicone oil-induced anterior chamber blockage and alter OHT severity. Instead, silicone oil droplet size was routinely monitored as an established indicator of sustained OHT. Consistent with prior validation demonstrating a direct correlation between droplet size and IOP elevation, droplet size was used as an inclusion/exclusion criterion to confirm stable, non-leaking oil droplets.

For the chronic mild OHT group, tissues were collected at 2-, 4-, 6-, and 8- weeks post-injection. For the acute severe OHT group, mice were euthanized 1-week post-injection. Eyes were enucleated and fixed in 4% paraformaldehyde for immunohistochemistry.

LXB₄ treatment

Mice in the acute severe OHT group received LXB₄ methyl ester (1 μ g in 100 μ L PBS) by intraperitoneal injection and topical ocular administration once daily for 1 week. The initial

dose was administered 15 minutes prior to OHT induction. Control mice received equivalent volumes of sterile PBS on the same schedule.

Tissue processing

Eyes were enucleated and a small incision was made at the corneoscleral junction using a 32 G needle. Whole eyes were fixed in 4% paraformaldehyde at room temperature for 1 hour to allow fixative to perfuse gradually into the globe through the incision site. Following initial fixation, the cornea, iris, and lens were carefully removed to generate scleral eyecups with the retina left undisturbed. Eyecups were then post-fixed in 4% paraformaldehyde at room temperature for an additional 2 hours. Following fixation, eyecups were washed once in 1× PBS and cryoprotected in a sucrose gradient of 10%, 20%, and 30% sucrose in PBS at 4°C. Tissues were advanced to the next sucrose concentration after sinking in the previous solution. Once in 30% sucrose, eyecups were maintained in solution until embedding. Eyecups were embedded in optimal cutting temperature (OCT) compound in sagittal orientation, with the eyecup opening positioned laterally, and frozen at 80°C for immunohistochemistry. Ten-micrometer sections were cut using a Leica CM1900 cryostat (Leica Microsystems, Wetzlar, Germany) and mounted onto Superfrost Plus slides (Thermo Fisher Scientific, Waltham, MA).

Immunostaining

Retinal cryosections were permeabilized and blocked for 1 hour at room temperature in a humidified chamber using blocking buffer containing 10% donkey serum and 0.25% Triton X-100 in 1X PBS. Primary antibodies against GFAP (1:1000; Abcam, ab53554, Cambridge, UK) and RLBP1 (1:400; Abcam, ab243664, Cambridge, UK) were diluted in a 1:1 mixture of blocking buffer and PBS and applied overnight at 4°C. Sections were then washed 3 times in PBS for 5 minutes each and incubated for 1 hour at room temperature in the dark with Alexa Fluor 488- and 594-conjugated secondary antibodies (Invitrogen; 1:1000). Nuclei were counterstained with DAPI (1:3000). Slides were mounted using FluorSave mounting medium (Sigma-Aldrich, St. Louis, MO) and covered with No. 1 coverslips (VWR, Radnor, PA).

Image acquisition

Retinal sections were imaged using an inverted Nikon Ti-Eclipse fluorescence microscope (Nikon Instruments, Melville, NY) with 20× and 40× objectives. Images were acquired as z-stacks. For quantitative analysis, both central and peripheral retinal regions were captured. Peripheral regions were defined as the tapered retinal edge, whereas central regions were defined as areas proximal to the optic nerve head. Image acquisition settings were held constant across experimental groups.

Image processing and analysis

Image processing was performed in ImageJ/Fiji (v1.53c; National Institutes of Health) using custom macros. Z-stack images were converted to maximum-intensity projections, after which RLBP1-labeled Müller glia processes were reduced to simplified centerline masks for downstream analysis. This step minimized variation introduced by signal thickness while preserving the overall spatial arrangement of labeled stalks.

Directional organization was then quantified by FFT-based analysis using the Canny Edge plugin. For each image, a standardized 296×296 pixel region of interest (ROI) was selected from the centerline mask and transformed into frequency space. The resulting power spectrum was sampled across angular sectors spanning 360° , and analysis was restricted to an annular region of the spectrum to exclude very low and very high spatial frequencies. Polar plots were generated from the angular power distribution to visualize directional bias within each ROI. An ellipse was then fit to each polar plot, and the ellipse aspect ratio was used as an index of isotropy. Because aspect ratio approached 0 for more elongated ellipses and 1 for more circular ellipses, this metric was interpreted as an index of isotropy, with higher values indicating reduced directional organization of RLBP1-labeled Müller glia stalks.

For each retina, 6 to 8 distinct ROIs of identical size were analyzed in either the outer nuclear layer defined as (ONL-INL) or ganglion cell layer (INL-GCL). ROI-level measurements from each animal were averaged, and this mean value was treated as one biological replicate (n). Batch processing was used throughout to ensure consistent analysis parameters across images. ROI placement and image-processing parameters were standardized and applied uniformly during batch analysis. ImageJ macros are available here: <https://github.com/matanginkumar/FFT-Based-Quantification-of-M-ller-glia-.git>.

Statistical analysis

All data are presented as mean $\pm$ SEM. For in vivo experiments, biological replicates (n) correspond to the number of animals. Statistical analyses were performed in GraphPad Prism 9.0 (GraphPad Software, San Diego, CA). Two-group comparisons were analyzed using an unpaired Student's t-test, whereas comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was defined as $P < 0.05$.

4.3 Results

RLBP1 distinguishes Müller glia stalk architecture from GFAP-positive astrocytic structures

To identify a marker suitable for visualizing Müller glia-associated structural organization, we first examined RLBP1 and GFAP labeling in sham-injected control retinas. In control

sections, RLBP1 labeled the characteristic radial morphology of Müller glia, with processes extending across retinal layers from the inner nuclear layer toward the ganglion cell layer and outer nuclear layer (Fig. 4.1A). By contrast, GFAP labeling was restricted primarily to astrocyte-associated structures in the ganglion cell layer and did not substantially overlap with radial RLBP1-positive Müller glia stalks under homeostatic conditions. The separated channels further emphasize the distinct architecture of these two macroglia populations and indicate that RLBP1 resolves Müller glia stalks independently of GFAP-positive astrocytic labeling.

To further assess whether RLBP1 distinguished Müller glia from astrocytes, we examined the optic nerve head and optic nerve region, which are enriched in GFAP-positive astrocytic structures but do not contain retinal Müller glia. In these regions, robust GFAP staining was observed within the optic nerve head and optic nerve, whereas RLBP1 signal was confined to the adjacent retina and was not detected within the optic nerve head or optic nerve itself (Fig. 4.1B). At the retinal margin approaching the optic nerve head, RLBP1-positive Müller glia processes remained visible within the retina but terminated before entering the optic nerve region. This spatial separation further supports the use of RLBP1 as a marker of retinal Müller glia stalk architecture rather than astrocytic structures.

We next evaluated this distinction using publicly available single-cell retinal datasets. Consistent with the immunostaining data, canonical macroglia markers such as GLUL and VIM were detected in both astrocytes and Müller glia, whereas RLBP1 was enriched in Müller glia and GFAP was associated primarily with astrocytes under nonreactive conditions (Fig. 4.1C) (Jadhav, Roesch, and Cepko 2009). Together, these findings support the use of RLBP1 as a practical Müller glia-enriched marker for quantifying stalk-associated structural organization in retinal sections.

Having established RLBP1 as a suitable marker for Müller glia stalk architecture, we next asked whether its labeling pattern was altered during ocular hypertension. In control retina, RLBP1-positive stalks appeared relatively uniform and radially aligned across the inner retina. In contrast, retinas subjected to 6 weeks of OHT showed a visibly altered RLBP1 staining pattern, with stalks appearing less uniformly aligned and more structurally heterogeneous (Fig. 4.1D). This disruption was particularly evident in the inner retina and motivated development of a quantitative pipeline to measure directional organization of RLBP1-labeled Müller glia stalks in subsequent analyses.

An FFT-based workflow quantifies directional organization of RLBP1-labeled Müller glia stalks

Maximum-intensity projection and centerline extraction of Müller glia stalks

To quantify directional organization of Müller glia stalks, 40× fluorescence z-stack images were first converted into maximum-intensity projections to capture the full extent of labeled stalks within each sampled field (Fig. 4.2A). Projected images were then processed using a custom ImageJ macro that reduced the labeled structures into simplified centerline masks.

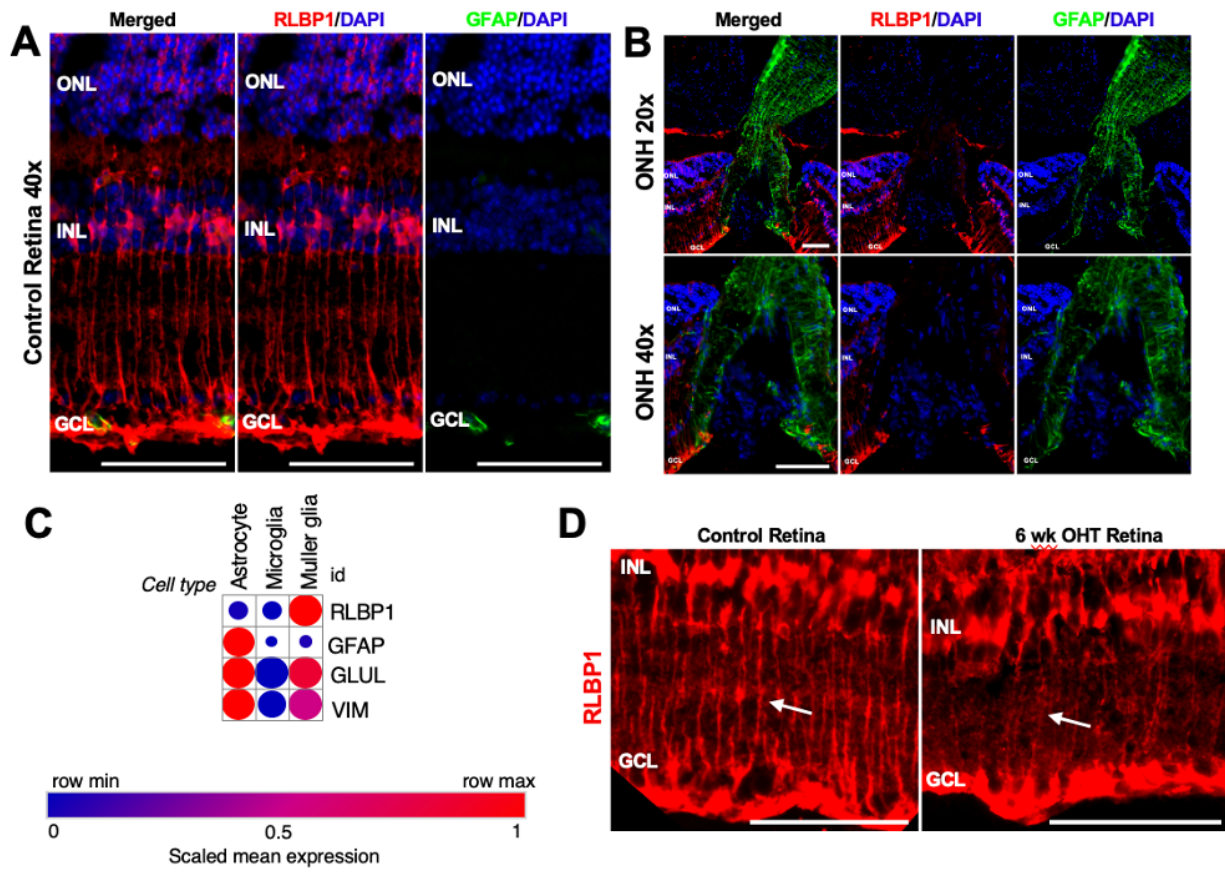


Figure 4.1: (A) Representative immunofluorescence images (40x magnification) of control retina stained for RLBP1 and GFAP with DAPI nuclear counterstain. Separated channels highlight the distinct distribution of RLBP1-positive Müller glia stalks and GFAP-positive astrocytic labeling. (B) Representative images of the optic nerve head (ONH) and adjacent optic nerve region stained for RLBP1 and GFAP (20x magnification above, 40x magnification below). (C) Publicly available single-cell retinal datasets showing expression of canonical macroglia markers and glia subtype-associated genes (20). (D) Representative RLBP1-labeled retinal sections from sham-injected control and 6-week ocular hypertension (OHT) retina (40x magnification). ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer; scale bar = 50 μ m

This approach identified local intensity maxima across image columns and rows to generate a two-dimensional representation of stalk organization. By minimizing variation introduced by signal thickness while preserving the overall spatial arrangement of Müller glia stalks, this step generated a standardized image representation suitable for downstream analysis.

FFT-based quantification of Müller glia stalk organization

To quantify stalk alignment, a standardized 296×296 pixel region of interest was selected from each centerline mask and analyzed by discrete Fourier transform (FFT) (Fig. 4.2A). Transformation of the image into frequency space enabled assessment of the overall directional distribution of Müller glia stalks within each sampled region. In this framework, regions containing more uniformly aligned stalks produced a more directionally biased Fourier power spectrum, whereas regions with less organized stalks produced a broader and more isotropic distribution. Thus, FFT provided an objective measure of directional organization that complemented visual inspection of the original images.

Polar plot visualization of FFT power spectra

To visualize directional bias within the Fourier output, the power spectrum was sampled across angular sectors spanning 360° and used to generate a polar plot (Fig. 4.2A). Analysis was restricted to an annular region of the spectrum to exclude very low and very high spatial frequencies and thereby focus on structural features most relevant to stalk organization. In this representation, elongated polar plots reflected a stronger directional preference and greater alignment of Müller glia stalks, whereas more circular plots reflected reduced directional bias and a more heterogeneous structural arrangement.

Ellipse aspect ratio provides an index of isotropy

To quantify directional organization, an ellipse was fit to each polar plot and the ellipse aspect ratio was measured as an index of isotropy (Fig. 4.2A). Because aspect ratio approached 0 for more elongated ellipses and 1 for more circular ellipses, higher values indicated a more isotropic distribution and reduced directional organization of RLBP1-labeled Müller glia stalks. Conversely, lower aspect ratio values reflected a more elongated ellipse and stronger directional coherence. In addition, the dominant angle of the fitted ellipse provided a measure of orientation bias within the sampled region. Together, these outputs enabled quantification of Müller glia stalk organization in terms of both directional coherence and orientation preference

Regional sampling across the retina and retinal layers

To determine whether Müller glia organization varied by retinal location, images were collected from both central and peripheral retina (Fig. 4.2B). Central regions were defined as areas closer to the optic nerve head, whereas peripheral regions were defined as areas toward

the outer edge of the retina. Because glaucoma primarily affects structures associated with the ganglion cell layer (GCL), analyses were also performed separately in ONL-associated and GCL-associated regions. This sampling strategy enabled us to test whether changes in Müller glia stalk organization were concentrated in inner retinal regions adjacent to the GCL or extended more broadly across the apicobasal axis of the stalk.

Chronic OHT progressively disrupts directional organization of RLBP1-labeled Müller glia stalks

To determine whether the FFT workflow could detect changes in Müller glia-associated structural organization during chronic OHT, we applied this analysis to RLBP1-labeled retinas collected at 2, 6, and 8 weeks after OHT induction and compared them with sham-injected controls. Analyses were performed in both central and peripheral retina and were further separated into ONL-associated and GCL-associated regions to assess whether changes in RLBP1 organization varied by retinal location and depth. Qualitatively, the projected images, centerline masks, and corresponding polar plots suggested a progressive loss of directional organization in the RLBP1-associated signal with increasing duration of OHT (Fig. 4.3A–D). In sham-injected controls, the polar plots were more elongated, consistent with stronger directional bias in RLBP1-labeled stalk patterning (Fig. 4.3A). By contrast, retinas from the 6- and 8-week OHT groups showed broader, more isotropic polar plot profiles, indicating reduced uniformity in RLBP1-associated structural organization (Fig. 4.3C,D). These differences were most apparent in GCL-associated regions of both central and peripheral retina.

Quantitative analysis supported these observations. In the central retina, ellipse aspect ratio was significantly increased by 327% at 6 weeks and 337% increase at 8 weeks in the GCL-associated region relative to control, consistent with progressive loss of directional organization during chronic OHT (Fig. 4.3E). A similar pattern was observed in the peripheral GCL-associated region, where ellipse aspect ratio was also significantly increased at later time points (Fig. 4.3F). In contrast, changes in ONL-associated regions were less pronounced. In the central ONL-associated region, variability between samples was greater, and only modest differences were detected across groups, with significance observed primarily between 2 and 8 weeks (Fig. 4.3G). Together, these findings indicate that chronic OHT is associated with progressive disruption of RLBP1-associated directional organization, with the strongest effects occurring in inner retinal regions adjacent to the GCL.

LXB₄ partially preserves RLBP1-associated directional organization in acute OHT

Because chronic OHT was associated with altered RLBP1-associated structural organization, we next asked whether LXB₄ treatment could preserve this pattern in an acute model of OHT. Acute OHT was induced for 1 week, with or without LXB₄ pretreatment, and

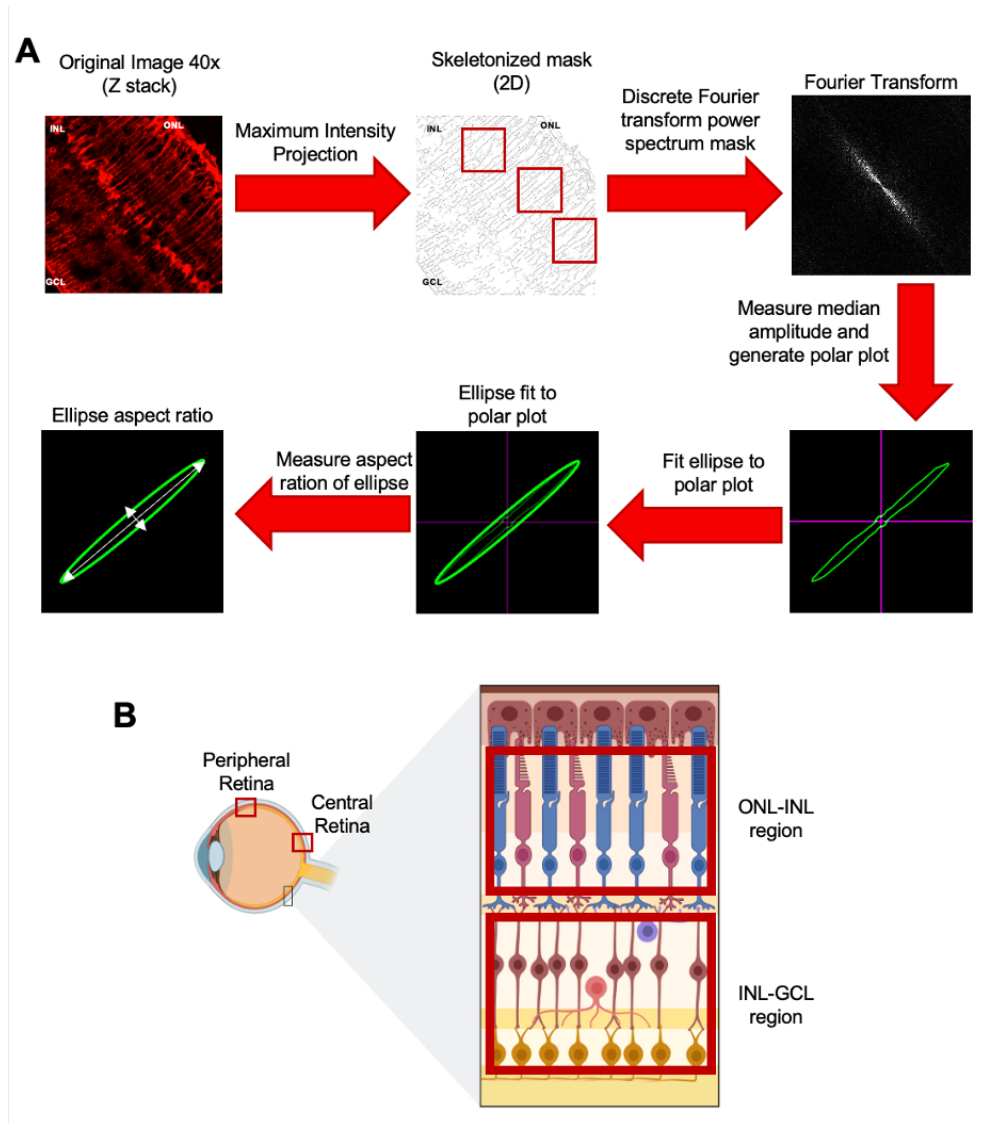


Figure 4.2: (A) Schematic of the image-analysis workflow. Fluorescence z-stack images (40x magnification) of RLBP1-labeled Müller glia stalks were converted to maximum-intensity projections and then reduced to simplified centerline masks by a custom ImageJ/Fiji macro. Standardized ROI were analyzed by discrete Fourier transform (FFT). Power spectra were sampled across angular sectors to generate polar plots. An ellipse was fit to each polar plot, and ellipse aspect ratio is calculated. (B) Schematic of regional sampling strategy. Images were collected from both central and peripheral retina and analyzed separately in ONL-associated and GCL-associated regions. ONL, outer nuclear layer; GCL, ganglion cell layer.

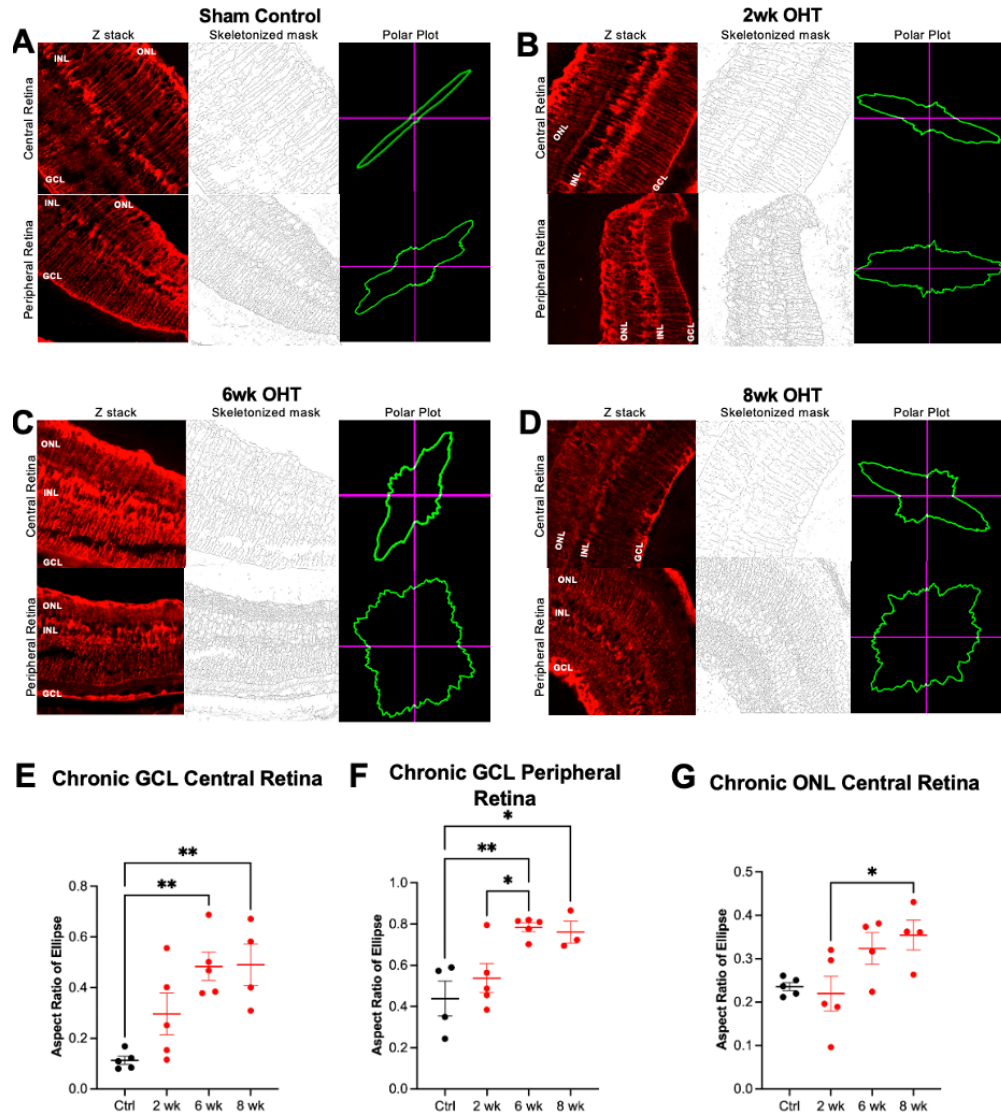


Figure 4.3: (A–D) Representative z-stack projections (40x magnification), corresponding centerline masks, and polar plots from sham-injected control retina and chronic mild OHT retina collected at 2, 6, and 8 weeks after induction. Representative images are shown for central and peripheral retina. (E) Quantification of ellipse aspect ratio in the INL-GCL-associated region of the central retina. (F) Quantification of ellipse aspect ratio in the INL-GCL-associated region of the peripheral retina. (G) Quantification of ellipse aspect ratio in the ONL-INL-associated region of the central retina. Data are presented as mean $\pm$ SEM; (n=5 biological replicates). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$).

RLBP1-labeled retinas were analyzed using the same FFT-based workflow applied in the chronic model. Analyses were performed in central and peripheral retina and were further subdivided into GCL-associated and ONL-associated regions to determine whether LXB₄-mediated effects varied by retinal location and layer.

Representative images revealed marked disruption of RLBP1-associated patterning in the acute OHT retina relative to sham controls. Compared with control retinas, acute OHT produced a less uniform RLBP1 signal in the z-projected images and corresponding centerline masks, accompanied by a marked shift in the polar plots toward a more isotropic profile (Fig. 4.4A,B). Qualitatively, these changes appeared more pronounced than those observed at earlier time points in the chronic model, indicating that the acute severe OHT paradigm produced substantial disruption in directional organization of the RLBP1-associated signal. In LXB₄-treated retinas, however, this pattern was partially preserved, with representative images and polar plots showing a more elongated and directionally biased profile than OHT alone (Fig. 4.4C).

Quantitative FFT analysis supported these observations. In the central GCL-associated region, acute OHT significantly increased ellipse aspect ratio by 453% relative to control, consistent with reduced directional organization of the RLBP1-associated signal. LXB₄ treatment significantly shifted this measure toward control values with 194% reduction compared to acute OHT group, indicating partial preservation of RLBP1-associated directional organization in this region (Fig. 4.4D).

Taken together, these findings indicate that the FFT workflow is sensitive to injury-associated disruption of RLBP1-labeled Müller glia stalk organization and can also detect partial preservation of this organization following LXB₄ treatment. Within this framework, the assay provides a practical quantitative readout of RLBP1-associated structural organization rather than a full reconstruction of individual Müller glia morphology.

4.4 Discussion

This study establishes an accessible FFT-based workflow for quantifying the directional organization of RLBP1-labeled Müller glia stalks in retinal sections. Müller glia are central regulators of retinal structure and homeostasis, and recent high-resolution imaging studies have further emphasized that their architecture is highly compartmentalized across retinal layers and vascular interfaces (Wang, O’Sullivan, et al. 2017; Grimes et al. 2024; Jadhav, Roesch, and Cepko 2009). At the same time, this structural complexity has made quantitative analysis challenging in routine antibody-labeled tissue. Existing approaches, including GliaMorph and related high-resolution reconstruction-based workflows, have substantially advanced Müller glia morphometric analysis by enabling three-dimensional segmentation, apicobasal texture analysis, and detailed structural quantification (Kugler et al. 2023). However, these methods depend heavily on labeling quality, segmentation performance, and imaging conditions, and are often best suited to datasets with strong signal quality and high-resolution acquisition. The present workflow was developed to address

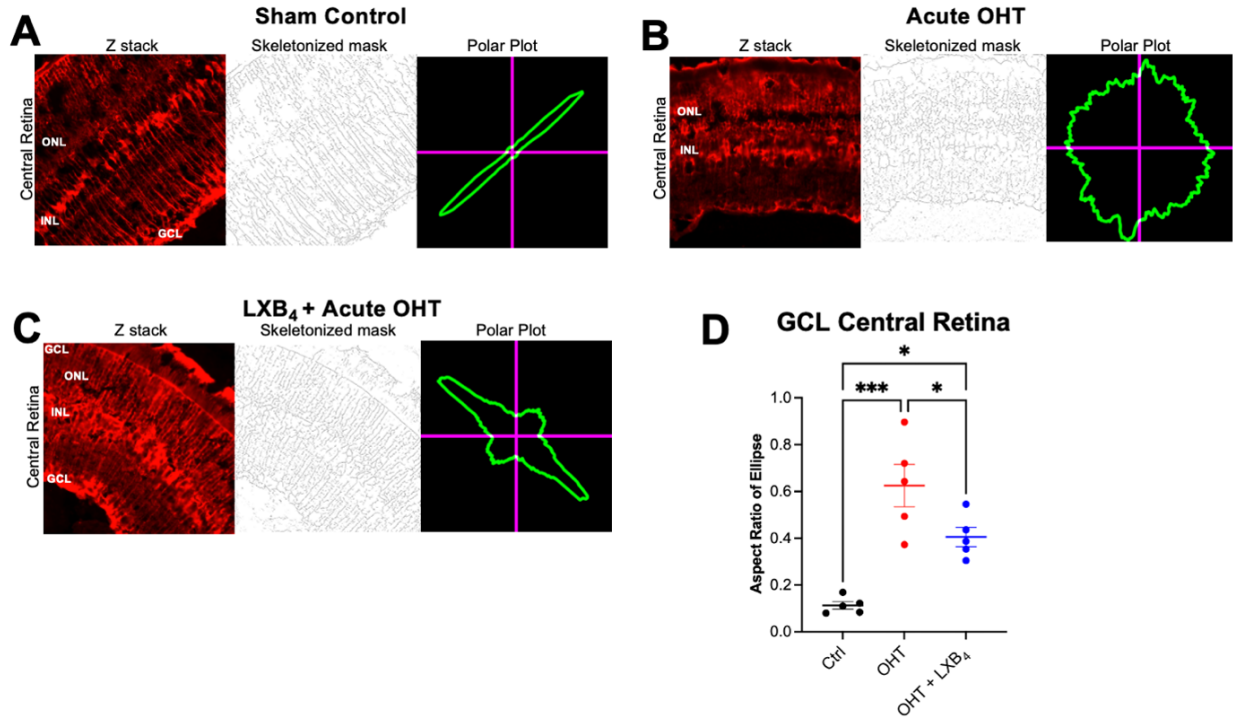


Figure 4.4: (A–C) Representative z-stack projections (40x magnification), corresponding centerline masks, and polar plots from sham-injected control retina, acute severe OHT retina, and acute severe OHT retina treated with LXB₄. Representative images are shown for central and peripheral retina. (D) Quantification of ellipse aspect ratio in the INL-GCL-associated region of the central retina. Data are presented as mean $\pm$ SEM; (n=5 biological replicates). Statistical comparisons were performed using one-way ANOVA followed by Tukey’s post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

a complementary need by providing a simpler and more accessible method for measuring directional organization of Müller glia stalks in standard retinal sections using a marker that preserves radial morphology. Within this framework, we show that chronic ocular hypertension progressively disrupts directional organization of RLBP1-labeled Müller glia stalks, particularly in GCL-associated regions, and that this pattern is partially preserved by LXB₄ treatment in acute OHT.

This workflow takes advantage of a defining structural feature of Müller glia: the ordered radial alignment of their stalks across retinal layers. By converting this spatial organization into a Fourier-domain representation and summarizing the resulting angular distribution through ellipse aspect ratio, the workflow captures changes in directional coherence that are readily visible in projected images but otherwise difficult to quantify objectively (Fig.

4.2A). More broadly, FFT-based analysis has been used to provide rapid and objective quantification of oriented microscopic structures by transforming spatial image information into frequency space, where alignment and orientation bias can be measured more directly than by intensity-based approaches alone (Taylor et al. 2013; Radotić, Braeken, and Kovačić 2017; Tonar et al. 2003). Fourier-based metrics have also been applied to biological images ranging from aligned cellular and tissue structures to dense ocular neurite networks, including cases in which full segmentation is difficult or impractical (Smith et al. 2021; Radotić, Braeken, and Kovačić 2017; Tonar et al. 2003; Hippert et al. 2015). Within this framework, the elongated polar profiles and lower aspect ratio values observed in control retina are consistent with strong directional order, whereas OHT shifts this organization toward a more isotropic pattern (Fig. 4.3A-C). Notably, the strongest changes were detected in GCL-associated regions, consistent with the inner retinal vulnerability of glaucoma and suggesting that this workflow is particularly sensitive to structural disruption in regions most closely associated with retinal ganglion cell stress (Fig. 4.3E). These features make the workflow well suited for comparative analysis of glia-associated structural organization across retinal injury models and treatment conditions.

The biological relevance of this assay is further supported by prior studies showing that Müller glia undergo structural remodeling across multiple retinal disease contexts. In GliaMorph, glaucoma-like degeneration in DBA/2J mice was associated with altered apicobasal distributions of Rlbpl, consistent with changes in Müller glia morphology and subcellular organization in a glaucoma-relevant setting (Kugler et al. 2023). In inherited retinal degeneration models, Müller glia likewise exhibit disease-dependent redistribution of gliosis-associated markers, including increased GFAP and vimentin labeling together with thickening of basal and apical processes in some models, further supporting the view that reactive changes are accompanied by alterations in glia structural organization rather than marker induction alone (Lindqvist et al. 2010). More broadly, Müller glia are thought to be particularly sensitive to retinal deformation because their long radial processes span the full thickness of the retina, placing them in a position to sense and respond to mechanical strain (Wang, Xu, et al. 2013; Vázquez-Chona, Clark, and Levine 2009). Consistent with this, mechanical stretch has been shown to induce remodeling-associated responses in Müller cells, including increased matrix metalloproteinase-2 (MMP-2) expression, supporting a role for Müller glia in structural adaptation of the injured retina (Vázquez-Chona, Clark, and Levine 2009). Within this context, the present findings that chronic OHT progressively disrupts directional organization of RLBP1-labeled stalks, and that acute OHT induces an even more pronounced shift toward isotropy, fit well with the broader literature indicating that Müller glia undergo disease-associated structural reorganization under glaucomatous and mechanically stressful conditions. The ability of the same workflow to detect partial preservation of directional organization following LXB₄ treatment further suggests that this assay can capture not only injury-associated disorganization, but also treatment-associated preservation of glia-associated directional organization.

At the same time, the interpretive limits of this method should be clearly recognized. This assay does not reconstruct the full morphology of individual Müller glia and should

not be interpreted as a one-to-one measure of whole-cell structural remodeling. Rather, it quantifies the directional organization of RLBP1-labeled processes in retinal sections. Because the analysis depends on marker-defined signal, changes in the measured output may reflect altered process arrangement, altered RLBP1 labeling pattern, or both. This distinction is particularly important because RLBP1 is a robust Müller glia-associated marker and regulatory element, but it is not itself a complete morphological readout of the cell. RLBP1 is highly expressed in Müller glia and has been widely used for Müller glia targeting, supporting its utility for visualizing radial stalk organization, although it is also expressed in other retinal cell populations such as the retinal pigment epithelium (Pellissier et al. 2014). In addition, the use of maximum-intensity projections and regional sampling provides a practical two-dimensional readout of local structural organization but does not capture the full three-dimensional complexity of Müller glia architecture. For these reasons, the present method is best viewed as a scalable assay of RLBP1-associated structural organization rather than a replacement for higher-resolution reconstruction-based morphometric approaches.

Despite these limitations, the workflow has several practical advantages that make it well suited for future studies. It does not require transgenic reporter lines, full single-cell segmentation, or highly specialized computational infrastructure, and it can be implemented using standard antibody labeling and Fiji/ImageJ-based analysis. This accessibility should facilitate its application across a range of retinal injury models in which Müller glia-associated structural organization may be altered, including glaucoma, ischemic injury, retinal detachment, and retinal degeneration. Future work could further refine the method by comparing multiple Müller glia markers, integrating it with three-dimensional segmentation or reporter-based imaging, and correlating directional organization with gliosis markers, neuronal loss, and functional outcomes. In glaucoma-related contexts, the assay may also prove useful for examining how retinal ganglion cell loss and Müller glia-associated structural reorganization evolve together during disease progression. More broadly, this workflow provides a foundation for incorporating quantitative structural analysis into studies of Müller glia reactivity, allowing glia remodeling to be assessed not only through marker intensity but also through changes in tissue-level spatial organization.

Chapter 5

Concluding Remarks

The main goal of this dissertation, as presented in Chapter 3, was to test whether Müller glia function as both a source and a target of LXB₄ signaling during ocular hypertension. Specifically, this work examined whether Müller glia contribute to endogenous LXB₄ production, whether ocular hypertension disrupts this pathway, and whether exogenous LXB₄ treatment can limit Müller glial reactivity and support a more homeostatic retinal environment. Across the chapters, this dissertation develops this question from biological framework to mechanistic testing.

Chapter 1 reviewed current knowledge showing that Müller glia are essential for maintaining retinal structure and homeostasis, while also emphasizing that these same functions become vulnerable under sustained mechanical, inflammatory, and metabolic stress. Within this framework, glaucoma is presented not only as a disease of retinal ganglion cell loss, but also as a condition that reshapes the glia environment that normally supports neuronal survival.

Chapter 2 extended this framework by examining lipid mediator signaling as an endogenous system that can limit inflammatory amplification while preserving tissue protection in the eye. In particular, this chapter highlighted lipoxins as arachidonic acid-derived mediators with growing relevance to retinal biology and glaucoma. The literature reviewed supports a multicellular model in which lipoxin signaling influences neurons, astrocytes, microglia, and macroglia responses across the injured retina. Within this broader context, LXB₄ emerged as especially compelling because it showed protective effects beyond canonical LXA₄ pathways and appeared closely linked to regulation of glia state during ocular hypertension. At the same time, the review identified an important gap: although Müller glia are the principal macroglia of the retina and major contributors to reactive gliosis, their role as a source and target of lipoxin signaling had remained poorly defined.

Chapter 3 addressed this gap directly and represents the central mechanistic advance of the dissertation. These studies show that Müller glia express a functional lipoxin biosynthetic pathway and generate endogenous LXB₄, identifying them as an intrinsic component of retinal lipid mediator signaling rather than only downstream responders to signals from

other cell types. The data further show that ocular hypertension induces Müller glia reactivity together with increased TRPV4 expression and inflammatory signaling, while pharmacologic activation of TRPV4 is sufficient to drive gliosis-associated changes and amplify LXB₄ formation. These observations support a model in which biomechanical stress engages a TRPV4-dependent reactive program in Müller glia, while also eliciting an intrinsic lipid-based counter-regulatory response. Most importantly, exogenous LXB₄ suppressed JAK/STAT signaling, reduced IL-6/STAT3-associated reactivity, and inhibited TRPV4 upregulation in both in vitro and in vivo models. Together, these findings define a previously unrecognized Müller glia–TRPV4–LXB₄ signaling axis and identify LXB₄ as a potential therapeutic modulator of maladaptive gliosis in glaucoma.

Chapter 4 extended this work from inflammatory signaling to structural remodeling. Because Müller glia function is closely linked to their radial architecture, reactive changes cannot be understood fully through marker induction alone. The FFT-based workflow developed here provides a practical method for quantifying directional organization of RLBP1-labeled Müller glia stalks in retinal sections and shows that ocular hypertension progressively disrupts this organization, particularly in ganglion cell layer-associated regions. The finding that acute OHT produced marked structural disorganization that was partially preserved by LXB₄ treatment is especially important because it links lipid-mediated glia regulation to tissue-level architecture. In this way, Chapter 4 complements the mechanistic findings of Chapter 3 by showing that LXB₄-associated protection is reflected not only in reduced inflammatory signaling, but also in preservation of Müller glia-associated structural organization within the retina.

Taken together, these chapters support a broader view of glaucoma pathogenesis in which neuronal injury, glia reactivity, inflammatory signaling, and structural disorganization are closely linked. Within this setting, Müller glia occupy a central position. Their architecture and homeostatic functions make them highly effective support cells in the healthy retina, but also make them sensitive responders to biomechanical and inflammatory stress in disease. The findings presented here suggest that glaucomatous injury involves not only activation of maladaptive pathways such as TRPV4- and STAT3-associated gliosis, but also disruption of endogenous lipid mediator pathways that may normally help limit chronic damage. In this context, LXB₄ emerges not as a nonspecific anti-inflammatory factor, but as a regulator of glia state with the potential to preserve retinal homeostasis at multiple levels.

Several important questions remain. The receptor mechanisms and downstream signaling pathways that mediate LXB₄ action in Müller glia are still being defined, and further work will be needed to determine how this pathway interacts with other inflammatory and mechanosensitive networks in the stressed retina. It will also be important to determine whether the structural changes captured by RLBP1-based FFT analysis correspond to specific shifts in Müller glia functional state, and whether preservation of Müller glia organization directly contributes to improved neuronal and visual outcomes. More broadly, future studies should address how Müller glia lipid signaling interacts with astrocytes, microglia, and retinal ganglion cells across different stages of glaucoma progression. These questions will help determine whether therapeutic amplification of endogenous lipoxin

pathways can move beyond proof-of-concept toward durable neuroprotective strategies.

In conclusion, this dissertation identifies Müller glia as both a source and a target of LXB₄ signaling in the retina and supports a model in which endogenous lipid mediator pathways help limit stress-induced glia dysfunction during glaucoma. By linking Müller glia homeostatic biology, lipoxin signaling, TRPV4-associated inflammatory reactivity, and structural remodeling, this work provides an integrated view of how macroglia shape retinal vulnerability and resilience. These findings deepen our understanding of Müller glia biology in glaucomatous injury and point toward therapeutic strategies aimed at preserving retinal homeostasis by modulating glia state rather than targeting neuronal loss alone.

Bibliography

- Abhyankar, SD, Y Xiao, N Mahajan, Q Luo, TR Cummins, AL Oblak, BT Lamb, TW Corson, and AD Bhatwadekar (2026). Müller Glial Kir4.1 Channel Dysfunction in APOE4-KI Model of Alzheimer's Disease. *GLIA* 74 (3), e70119.
- Adel, S, F Karst, À González-Lafont, M Pekárová, P Saura, L Masgrau, JM Lluch, S Stehling, T Horn, H Kuhn, et al. (2016). Evolutionary alteration of ALOX15 specificity optimizes the biosynthesis of antiinflammatory and proresolving lipoxins. *Proceedings of the National Academy of Sciences of the United States of America* 113, E4266–E4275.
- Arevalo, JF, AF Lasave, L Wu, D Acon, ME Farah, R Gallego-Pinazo, AA Alezzandrini, V Fortuna, H Quiroz-Mercado, G Salcedo-Villanueva, M Maia, M Serrano, and S Rojas (2017). VEGF production and signaling in Müller glia are critical to modulating vascular function and neuronal integrity in diabetic retinopathy and hypoxic retinal vascular diseases. *Vision Research* 139 (12), pp. 108–114.
- Baldwin, KT, KK Murai, and BS Khakh (2024). Astrocyte morphology. *Trends in cell biology* 34.7, pp. 547–565.
- Balzamino, BO, A Cacciamani, L Dinice, M Cecere, FR Pesci, G Ripandelli, and A Micera (2024). Retinal Inflammation and Reactive Müller Cells: Neurotrophins' Release and Neuroprotective Strategies. *Biology* 2024, Vol. 13, Page 1030 13 (12), p. 1030.
- Baratchi, S, P Keov, WG Darby, A Lai, K Khoshmanesh, P Thurgood, et al. (2019). The TRPV4 agonist GSK1016790A regulates the membrane expression of TRPV4 channels. *Frontiers in Pharmacology* 9, p. 432797.
- Bazan, NG, JM Calandria, and WC Gordon (2013). Docosahexaenoic acid and its derivative neuroprotectin D1 display neuroprotective properties in the retina, brain and central nervous system. *Nestle Nutrition Institute Workshop Series* 77, pp. 121–131.
- Becker, D, C Blase, J Bereiter-Hahn, and M Jendrach (2005). TRPV4 exhibits a functional role in cell-volume regulation. *Journal of Cell Science* 118, pp. 2435–2440.
- Biteman, B, IR Hassan, E Walker, AJ Leedom, M Dunn, F Seta, M Laniado-Schwartzman, and K Gronert (2007). Interdependence of lipoxin A₄ and heme-oxygenase in counter-regulating inflammation during corneal wound healing. *The FASEB Journal* 21 (9), pp. 2257–2266.
- Bora, K, N Kushwah, M Maurya, MC Pavlovich, Z Wang, and J Chen (2023). Assessment of Inner Blood–Retinal Barrier: Animal Models and Methods. *Cells* 2023, Vol. 12, Page 2443 12 (20), p. 2443.

- Brenner, M and A Messing (2021). Regulation of GFAP Expression. *ASN Neuro* 13.
- Bringmann, A and P Wiedemann (2011). Müller Glial Cells in Retinal Disease. *Ophthalmologica* 227 (1), pp. 1–19.
- Caldwell, RB, A Fouda, SAH Zaidi, A Abdelrahman, R Amato, M Cammalleri, A Melecchi, P Bagnoli, and V Porciatti (2023). Natural History of Glaucoma Progression in the DBA/2J Model: Early Contribution of Müller Cell Gliosis. *Cells* 2023, Vol. 12, Page 1272 12 (9), p. 1272.
- Carpi-Santos, R, RA de Melo Reis, FCA Gomes, and KC Calaza (2022). Contribution of Müller Cells in the Diabetic Retinopathy Development: Focus on Oxidative Stress and Inflammation. *Antioxidants* 2022, Vol. 11, Page 617 11 (4), p. 617.
- Chan-Ling, T (1991). Structure of the macroglia of the retina: Sharing and division of labour between astrocytes and Müller cells. *Journal of Comparative Neurology*.
- Chang, MS, C Schneider, RL Roberts, SB Shappell, FR Haselton, WE Boeglin, and AR Brash (2005). Detection and subcellular localization of two 15S-lipoxygenases in human cornea. *Investigative Ophthalmology & Visual Science* 46, pp. 849–856.
- Chen, Y, T Zhang, S Zeng, M Yam, M Gillies, and L Zhu (2021). Isolation, Culture, and Identification of Primary Müller Cells from Human Retina. *Bio-protocol* 11.
- Chen, Y, Q Xia, Y Zeng, Y Zhang, and M Zhang (2022). Regulations of Retinal Inflammation: Focusing on Müller Glia. *Frontiers in Cell and Developmental Biology* 10, p. 898652.
- Chen, Z, B Liu, D Zhou, M Lei, J Yang, Z Hu, and W Duan (2024). AQP4 regulates ferroptosis and oxidative stress of Muller cells in diabetic retinopathy by regulating TRPV4. *Experimental Cell Research* 439 (1), p. 114087.
- Claude, A (1963). The ultrastructure of the retinal vessels: III. Vascular-glial relationships. *Journal of Ultrastructure Research* 9 (1-2), pp. 47–64.
- Colombo, G, RJA Cubero, L Kanari, A Venturino, R Schulz, M Scolamiero, J Agerberg, H Mathys, LH Tsai, W Chachólski, et al. (2022). A tool for mapping microglial morphology, morphOMiCs, reveals brain-region and sex-dependent phenotypes. *Nature neuroscience* 25.10, pp. 1379–1393.
- Coorey, NJ, W Shen, SH Chung, L Zhu, and MC Gillies (2012). The role of glia in retinal vascular disease. *Clinical and Experimental Optometry* 95 (3), pp. 266–281.
- Coorey, NJ, W Shen, L Zhu, and MC Gillies (2015). Differential Expression of IL-6/gp130 Cytokines, Jak-STAT Signaling and Neuroprotection After Müller Cell Ablation in a Transgenic Mouse Model. *Investigative Ophthalmology & Visual Science* 56 (4), pp. 2151–2161.
- Coughlin, BA, DJ Feenstra, and S Mohr (2017). Müller cells and diabetic retinopathy. *Vision Research* 139 (9), pp. 93–100.
- Cullimore, B, J Baumann, CN Rudzitis, AO Jo, D Kirdajova, and D Križaj (2023). Mechanotransduction mechanisms in central nervous system glia. *Neural Regeneration Research* 18 (5), pp. 1031–1032.
- Dodington, DW, HR Desai, and M Woo (2018). JAK/STAT – Emerging Players in Metabolism. *Trends in Endocrinology and Metabolism* 29, pp. 55–65.

- English, JT, PC Norris, RR Hodges, DA Dartt, and CN Serhan (2017). Identification and profiling of specialized pro-resolving mediators in human tears by lipid mediator metabolomics. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 117, pp. 17–27.
- Fang, F, J Zhang, P Zhuang, P Liu, L Li, H Huang, et al. (2021). Chronic mild and acute severe glaucomatous neurodegeneration derived from silicone oil-induced ocular hypertension. *Scientific Reports* 11, p. 9052.
- Fischer, AJ and R Bongini (2010). Turning Müller Glia into Neural Progenitors in the Retina. *Molecular Neurobiology* 2010 42:3 42 (3), pp. 199–209.
- Fliesler, SJ (2010). Lipids and lipid metabolism in the eye. *Journal of Lipid Research* 51, pp. 1–3.
- Flitter, BA, X Fang, MA Matthay, and K Gronert (2021). The potential of lipid mediator networks as ocular surface therapeutics and biomarkers. *The Ocular Surface* 19, pp. 104–114.
- Fu, S, S Dong, M Zhu, DM Sherry, C Wang, Z You, JJ Haigh, and YZ Le (2015). Müller Glia Are a Major Cellular Source of Survival Signals for Retinal Neurons in Diabetes. *Diabetes* 64 (10), pp. 3554–3563.
- Gao, H, L A, X Huang, X Chen, and H Xu (2021). Müller glia-mediated retinal regeneration. *Mol Neurobiol* 58 (5), pp. 2342–2361.
- García-Bermúdez, MY, KK Freude, ZA Mouhammad, P van Wijngaarden, KK Martin, and M Kolko (2021). Glial Cells in Glaucoma: Friends, Foes, and Potential Therapeutic Targets. *Frontiers in neurology* 12, p. 624983.
- Gavino, AC, K Nahmod, U Bharadwaj, G Makedonas, and DJ Tweardy (2016). STAT3 inhibition prevents lung inflammation, remodeling, and accumulation of Th2 and Th17 cells in a murine asthma model. *Allergy* 71, pp. 1684–1692.
- Graca, AB, C Hippert, and RA Pearson (2018). Müller Glia Reactivity and Development of Gliosis in Response to Pathological Conditions. *Advances in Experimental Medicine and Biology* 1074, pp. 303–308.
- Grimes, WN, DM Berson, A Sabnis, M Hoon, R Sinha, H Tian, and JS Diamond (2024). The retina’s neurovascular unit: Müller glial sheaths and neuronal contacts. *bioRxiv*, p. 2024.04.30.591885.
- Grimes, WN, DM Berson, A Sabnis, M Hoon, R Sinha, H Tian, and JS Diamond (2025). Layer-specific anatomical and physiological features of the retina’s neurovascular unit. *Current Biology* 35 (1), 109–120.e4.
- Gronert, K (2005). Lipoxins in the eye and their role in wound healing. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 73, pp. 221–229.
- Gronert, K (2010). Resolution, the grail for healthy ocular inflammation. *Experimental Eye Research* 91, pp. 478–485.
- Gronert, K, N Maheshwari, N Khan, IR Hassan, M Dunn, and ML Schwartzman (2005). A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. *Journal of Biological Chemistry* 280, pp. 15267–15278.
- Guarino, BD, S Paruchuri, and CK Thodeti (2020). The role of TRPV4 channels in ocular function and pathologies. *Experimental Eye Research* 201, p. 108257.

- Hagemann, TL, S Coyne, A Levin, L Wang, MB Feany, and A Messing (2023). STAT3 Drives GFAP Accumulation and Astrocyte Pathology in a Mouse Model of Alexander Disease. *Cells* 2023, Vol. 12, Page 978 12 (7), p. 978.
- Han, C, Y Li, X Zheng, X Zhang, G Li, L Zhao, Z Chen, Y Yang, and W Zhang (2024). AQP4- and Kir4.1-Mediated Müller Cell Oedema Is Involved in Retinal Injury Induced By Hypobaric Hypoxia. *Molecular Neurobiology* 2024 62:2 62 (2), pp. 2012–2022.
- Hao, Y, S Hao, E Andersen-Nissen, WM Mauck, S Zheng, A Butler, et al. (2021). Integrated analysis of multimodal single-cell data. *Cell* 184, 3573–3587.e29.
- Hassan, IR and K Gronert (2009). Acute changes in dietary omega-3 and omega-6 polyunsaturated fatty acids have a pronounced impact on survival following ischemic renal injury and formation of renoprotective docosahexaenoic acid-derived protectin D1. *Journal of Immunology* 182, pp. 3223–3232.
- Herrmann, JE, T Imura, B Song, J Qi, Y Ao, TK Nguyen, et al. (2008). STAT3 is a Critical Regulator of Astrogliosis and Scar Formation after Spinal Cord Injury. *Journal of Neuroscience* 28, p. 7231.
- Heydeck, D, C Ufer, KR Kakularam, M Rothe, T Liehr, P Poulain, and H Kuhn (2023). Functional Characterization of Transgenic Mice Overexpressing Human 15-Lipoxygenase-1 (ALOX15) under the Control of the aP2 Promoter. *International Journal of Molecular Sciences* 24, p. 4815.
- Hippert, C, AB Graca, AC Barber, EL West, AJ Smith, RR Ali, and RA Pearson (2015). Müller Glia Activation in Response to Inherited Retinal Degeneration Is Highly Varied and Disease-Specific. *PLOS ONE* 10 (3), e0120415.
- Hu, X, GL Zhao, MX Xu, H Zhou, F Li, Y Miao, et al. (2021). Interplay between Müller cells and microglia aggravates retinal inflammatory response in experimental glaucoma. *Journal of Neuroinflammation* 18.
- Inman, DM and PJ Horner (2007). Reactive nonproliferative gliosis predominates in a chronic mouse model of glaucoma. *GLIA* 55 (9), pp. 942–953.
- Jadhav, AP, K Roesch, and CL Cepko (2009). Development and neurogenic potential of Müller glial cells in the vertebrate retina. *Progress in Retinal and Eye Research* 28 (4), pp. 249–262.
- Jaén, RI, S Sánchez-García, M Fernández-Velasco, L Boscá, and P Prieto (2021). Resolution-Based Therapies: The Potential of Lipoxins to Treat Human Diseases. *Frontiers in Immunology* 12.
- Ji, M, Q Sun, G Zhang, Z Huang, Y Zhang, Q Shen, and H Guan (2022). Microglia-derived TNF- α mediates Müller cell activation by activating the TNFR1-NF-B pathway. *Experimental Eye Research* 214 (1), p. 108852.
- Jin, K, X Zheng, and Y Xia (2011). Gene Expression Profiling via Multigene Concatemers. *PLoS ONE* 6, e15711.
- Jo, AO, M Lakk, CN Rudzitis, and D Križaj (2022). TRPV4 and TRPC1 channels mediate the response to tensile strain in mouse Müller cells. *Cell calcium* 104, p. 102588.

- Jo, AO, DA Ryskamp, TT Phuong, AS Verkman, O Yarishkin, N Macaulay, and D Križaj (2015). TRPV4 and AQP4 Channels Synergistically Regulate Cell Volume and Calcium Homeostasis in Retinal Müller Glia. *Journal of Neuroscience* 35 (39), pp. 13525–13537.
- Jubran, L, R Wannemacher, A Ulianytska, I Gerhauser, W Baumgärtner, and E Leitzen (2025). Novel insights into pathomechanisms of retinal neuronal degeneration and reactive gliosis in a murine model of GM1-gangliosidosis. *Scientific Reports* 2025 15:1 15 (1), pp. 29674–.
- Kar, D, R Singireddy, YJ Kim, O Packer, R Schalek, D Cao, KR Sloan, A Pollreisz, DM Dacey, and CA Curcio (2024). Unusual morphology of foveal Müller glia in an adult human born pre-term. *Frontiers in Cellular Neuroscience* 18, p. 1409405.
- Karnam, S, S Maurya, E Ng, A Choudhary, A Thobani, JG Flanagan, and K Gronert (2024). Dysregulation of neuroprotective lipoxin pathway in astrocytes in response to cytokines and ocular hypertension. *Acta Neuropathologica Communications* 2024 12:1 12 (1), pp. 58–.
- Kaur, G and N Singh (2023). Inflammation and retinal degenerative diseases. *Neural Regeneration Research* 18, pp. 513–518.
- Kim, C, I Livne-Bar, K Gronert, and JM Sivak (2020). Fair-Weather Friends: Evidence of Lipoxin Dysregulation in Neurodegeneration. *Molecular Nutrition & Food Research* 64.
- Kirsch, M, N Trautmann, M Ernst, and HD Hofmann (2010). Involvement of gp130-associated cytokine signaling in müller cell activation following optic nerve lesion. *GLIA* 58 (7), pp. 768–779.
- Kolb, H (1995). Glial cells of the Retina by Helga Kolb. *Webvision: The Organization of the Retina and Visual System*.
- Križaj, D (2018). “What is Glaucoma?” *The Organization of the Retina and the Visual System*. Ed. by H Kolb, R Nelson, E Fernandez, and B Jones.
- Kugler, E, I Bravo, X Durmishi, S Marcotti, S Beqiri, A Carrington, B Stramer, P Mattar, and RB MacDonald (2023). GliaMorph: a modular image analysis toolkit to quantify Müller glial cell morphology. *Development (Cambridge, England)* 150 (3), dev201008.
- Kumari, N, S Barman, and SK Ganesan (2025). Crosstalk Between Neuronal and Glial Cells in Diabetic Retinopathy: Mechanisms and Implications for Neurodegeneration. *Molecular Neurobiology* 2025 63:1 63 (1), pp. 173–.
- Le, YZ (2017). VEGF production and signaling in Müller glia are critical to modulating vascular function and neuronal integrity in diabetic retinopathy and hypoxic retinal vascular diseases. *Vision Research* 139 (12), pp. 108–114.
- Lee, JE, Y Sun, P Gjørstrup, and E Pearlman (2015). Inhibition of corneal inflammation by the resolvin E1. *Investigative Ophthalmology & Visual Science* 56, pp. 2728–2736.
- Leinonen, HO, E Bull, and Z Fu (2023). Neural and Müller glial adaptation of the retina to photoreceptor degeneration. *Neural Regeneration Research* 18 (4), pp. 701–707.
- Levy, BD, K Gronert, and CB Clish (1998). “Lipoxin Biosynthesis”. *Lipid Second Messengers*. Vol. 1, p. 83.
- Levy, BD and CN Serhan (2014). “Resolution and Regulation of Inflammation”. *Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms*, pp. 332–348.

- Li, F, D Jiang, and MA Samuel (2019). Microglia in the developing retina. *Neural Development* 2019 14:1 14 (1), pp. 12–.
- Li, Q, Y Cheng, S Zhang, X Sun, and J Wu (2021). TRPV4-induced Müller cell gliosis and TNF- α elevation-mediated retinal ganglion cell apoptosis in glaucomatous rats via JAK2/STAT3/NF- κ B pathway. *Journal of Neuroinflammation* 18, p. 271.
- Li, X, J Liu, J Hoh, and J Liu (2019). Müller cells in pathological retinal angiogenesis. *Translational Research* 207, pp. 96–106.
- Licican, EL and K Gronert (2010). Molecular circuits of resolution in the eye. *TheScientificWorldJournal* 10, pp. 1029–1047.
- Lindqvist, N, Q Liu, J Zajadacz, K Franze, and A Reichenbach (2010). Retinal Glial (Müller) Cells: Sensing and Responding to Tissue Stretch. *Investigative Ophthalmology & Visual Science* 51 (3), pp. 1683–1690.
- Liu, HH, PF Cullen, JM Sivak, K Gronert, and JG Flanagan (2024). Protective Effects of Lipoxin A4 and B4 Signaling on the Inner Retina in a Mouse Model of Experimental Glaucoma. *bioRxiv*, p. 2024.01.17.575414.
- Liu, X, L Tang, and Y Liu (2017). Mouse Muller Cell Isolation and Culture. *Bio-protocol* 7.
- Livne-Bar, I, J Wei, HH Liu, S Alqawlaq, GJ Won, A Tuccitto, K Gronert, JG Flanagan, and JM Sivak (2017). Astrocyte-derived lipoxins A4 and B4 promote neuroprotection from acute and chronic injury. *Journal of Clinical Investigation* 127, pp. 4403–4414.
- Livne-Bar, I, S Maurya, K Gronert, and JM Sivak (2024). Lipoxins A4 and B4 inhibit glial cell activation via CXCR3 signaling in acute retinal neuroinflammation. *Journal of Neuroinflammation* 2024 21:1 21 (1), pp. 18–.
- Lundkvist, A, A Reichenbach, C Betsholtz, P Carmeliet, H Wolburg, and M Pekny (2004). Under stress, the absence of intermediate filaments from Müller cells in the retina has structural and functional consequences. *Journal of cell science* 117 (Pt 16), pp. 3481–3488.
- Lupien, C, DJ Calkins, and PJ Horner (2010). Decreasing Gliosis by Inhibition of the Stat3 Pathway in a Microbead Mouse Model of Glaucoma. *Investigative Ophthalmology & Visual Science* 51, pp. 3192–3192.
- MacDonald, RB, M Charlton-Perkins, and WA Harris (2017). Mechanisms of Müller glial cell morphogenesis. *Current Opinion in Neurobiology* 47 (9), pp. 31–37.
- MacDonald, RB, O Randlett, J Oswald, T Yoshimatsu, K Franze, and WA Harris (2015). Müller glia provide essential tensile strength to the developing retina. *Journal of Cell Biology* 210 (7), pp. 1075–1083.
- Martini, AC, T Berta, S Forner, G Chen, AF Bento, RR Ji, et al. (2016). Lipoxin A4 inhibits microglial activation and reduces neuroinflammation and neuropathic pain after spinal cord hemisection. *Journal of Neuroinflammation* 13, pp. 1–11.
- Masri, RA, U Greferath, EL Fletcher, PR Martin, and U Grunert (2025). Immunohistochemistry and Spatial Density of Müller Cells in the Human Fovea. *Investigative Ophthalmology & Visual Science* 66 (2), pp. 46–46.

- Mathew, DJ and JM Sivak (2024). Lipid mediators in glaucoma: Unraveling their diverse roles and untapped therapeutic potential. *Prostaglandins, Other Lipid Mediators* 171, p. 106815.
- Mathew, DJ, S Maurya, J Ho, I Livne-Bar, D Chan, JW Zhang, YM Buys, M Sit, G Trope, DM Peters, et al. (2026). Lipidomic analysis reveals drug-induced lipoxin synthesis in glaucoma treatment. *JCI insight* 11.7.
- Maurya, S, M Kumar, B Bajoua, C Liu, P Sharma, J Han, A Choudhary, SC Adcock, S Karnam, JG Flanagan, and K Gronert (2025). Macrogliia-specific 15-Lipoxygenase Regulates OHT-induced Neurodegeneration and Microglia-mediated Neuroinflammation in the Optic Nerve. *Investigative Ophthalmology & Visual Science* 66 (8), pp. 4008–4008.
- Maurya, S, M Lin, S Karnam, T Singh, M Kumar, E Ward, J Sivak, JG Flanagan, and K Gronert (2024). Regulation of disease-associated microglia in the optic nerve by lipoxin B4 and ocular hypertension. *Molecular Neurodegeneration* 2024 19:1 19 (1), pp. 86–.
- Medina-Arellano, AE, JS Albert-Garay, T Medina-Sánchez, KH Fonseca, M Ruiz-Cruz, and LOd la Paz (2024). Müller cells and retinal angiogenesis: critical regulators in health and disease. *Frontiers in Cellular Neuroscience* 18, p. 1513686.
- Moltke, J von, NJ Trinidad, M Moayeri, AF Kintzer, SB Wang, N Van Rooijen, et al. (2012). Rapid induction of inflammatory lipid mediators by the inflammasome in vivo. *Nature* 490, pp. 107–111.
- Moustafa, M, A Khalil, NHE Darwish, DQ Zhang, A Tawfik, and M Al-Shabrawey (2024). 12-HETE activates Müller glial cells: The potential role of GPR31 and miR-29. *Prostaglandins, Other Lipid Mediators* 171, p. 106805.
- Navneet, S, K Wilson, and B Rohrer (2024). Müller Glial Cells in the Macula: Their Activation and Cell-Cell Interactions in Age-Related Macular Degeneration. *Investigative Ophthalmology & Visual Science* 65 (2), pp. 42–42.
- Nguyen, CTO, ML Acosta, SD Angelantonio, and TE Salt (2021). Editorial: Seeing Beyond the Eye: The Brain Connection. *Frontiers in Neuroscience* 15.
- O’Leary, F and M Campbell (2023). The blood–retina barrier in health and disease. *FEBS Journal* 290 (4), pp. 878–891.
- Paisley, CE and JN Kay (2021). Seeing stars: Development and function of retinal astrocytes. *Developmental biology* 478, p. 144.
- Palazzo, I, LJ Todd, TV Hoang, TA Reh, S Blackshaw, and AJ Fischer (2022). NF κ B-signaling promotes glial reactivity and suppresses Müller glia-mediated neuron regeneration in the mammalian retina. *Glia* 70, pp. 1380–1401.
- Pathak, Z, T Jadav, A Roy, M Chopra, N Singh, P Sengupta, et al. (2023). Maresin-1 prevents blood-spinal cord barrier disruption associated with TRPV4 elevation in the experimental model of spinal cord injury. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 1868, p. 159395.
- Pellissier, LP, RM Hoek, RM Vos, WM Aartsen, RR Klimczak, SA Hoyng, JG Flannery, and J Wijnholds (2014). Specific tools for targeting and expression in Müller glial cells. *Molecular Therapy Methods and Clinical Development* 1, p. 14009.

- Peña, JS and M Vazquez (2022). Harnessing the Neuroprotective Behaviors of Müller Glia for Retinal Repair. *Frontiers in bioscience (Landmark edition)* 27 (6), p. 169.
- Pereiro, X, R Fernández, G Barreda-Gómez, N Ruzafa, A Acera, J Araiz, et al. (2020). Comparative lipidomic analysis of mammalian retinal ganglion cells and Müller glia in situ and in vitro using High-Resolution Imaging Mass Spectrometry. *Scientific Reports* 10, p. 20053.
- Pereiro, X, N Ruzafa, A Acera, A Urcola, and E Vecino (2020). Optimization of a Method to Isolate and Culture Adult Porcine, Rats and Mice Müller Glia in Order to Study Retinal Diseases. *Frontiers in Cellular Neuroscience* 14.
- Prieto, P, J Cuenca, PG Través, M Fernández-Velasco, P Martín-Sanz, and L Boscá (2010). Lipoxin A4 impairment of apoptotic signaling in macrophages: implication of the PI3K/Akt and the ERK/Nrf-2 defense pathways. *Cell Death and Differentiation* 17, pp. 1179–1188.
- Prieto-López, L, X Pereiro, and E Vecino (2024). The mechanics of the retina: Müller glia role on retinal extracellular matrix and modelling. *Frontiers in Medicine* 11, p. 1393057.
- Radotić, V, D Braeken, and D Kovačić (2017). Microelectrode array-induced neuronal alignment directs neurite outgrowth: analysis using a fast Fourier transform (FFT). *European Biophysics Journal* 46, pp. 719–727.
- Reichenbach, A and A Bringmann (2010). *Müller Cells in the Healthy and Diseased Retina*, pp. 1–417.
- Reichenbach, A and A Bringmann (2020). Glia of the human retina. *Glia* 68 (4), pp. 768–796.
- Reichenbach, A, A Wurm, T Pannicke, I Iandiev, P Wiedemann, and A Bringmann (2007). Müller cells as players in retinal degeneration and edema. *Graefe's Arch Clin Exp Ophthalmol = Albrecht Graefes Archiv Klin Exp Ophthalmol* 245 (5), pp. 627–636.
- Roesch, K, AP Jadhav, JM Trimarchi, MB Stadler, B Roska, BB Sun, et al. (2008). The transcriptome of retinal Müller glial cells. *Journal of Comparative Neurology* 509, pp. 225–238.
- Rombaut, A, R Brautaset, PA Williams, and JR Tribble (2023). Glial metabolic alterations during glaucoma pathogenesis. *Frontiers in Ophthalmology* 3, p. 1290465.
- Runkle, EA and DA Antonetti (2011). The Blood-Retinal Barrier: Structure and Functional Significance. *Methods in Molecular Biology* 686, pp. 133–148.
- Seitz, R, A Ohlmann, and ER Tamm (2013). The role of Müller glia and microglia in glaucoma. *Cell and Tissue Research* 2013 353:2 353 (2), pp. 339–345.
- Serhan, CN, M Hamberg, and B Samuelsson (1984). Lipoxins: Novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proceedings of the National Academy of Sciences of the United States of America* 81, pp. 5335–5339.
- Shinozaki, Y and S Koizumi (2021). Potential roles of astrocytes and Müller cells in the pathogenesis of glaucoma. *Journal of Pharmacological Sciences* 145 (3), pp. 262–267.
- Smith, B, A Datta, J Lee, D Evans, and S Fleiszig (2021). Quantification of relative neurite tortuosity using Fourier transforms. *Journal of neuroscience methods* 361, p. 109266.
- Snodgrass, RG and B Brüne (2019). Regulation and Functions of 15-Lipoxygenases in Human Macrophages. *Frontiers in Pharmacology* 10.

- Stahl, A, TU Krohne, P Sapieha, J Chen, A Hellstrom, E Chew, FG Holz, and LEH Smith (2011). Lipid metabolites in the pathogenesis and treatment of neovascular eye disease. *British Journal of Ophthalmology* 95, pp. 1496–1501.
- Taylor, SE, T Cao, PM Talaulliker, and J Lifshitz (2013). Objective Morphological Quantification of Microscopic Images Using a Fast Fourier Transform (FFT) Analysis. *Current Protocols Essential Laboratory Techniques* 95, p. 9.5.1.
- Toft-Kehler, AK, IS Gurubaran, C Desler, LJ Rasmussen, DM Skytt, and M Kolko (2016). Oxidative Stress-Induced Dysfunction of Müller Cells During Starvation. *Investigative Ophthalmology & Visual Science* 57 (6), pp. 2721–2728.
- Tonar, Z, S Němeček, R Holota, J Kočová, V Třeška, J Moláček, T Kohoutek, and Š Hadravská (2003). Microscopic image analysis of elastin network in samples of normal, atherosclerotic and aneurysmatic abdominal aorta and its biomechanical implications. *Journal of Applied Biomedicine* 1, pp. 149–159.
- Tout, S, T Chan-Ling, H Holländer, and J Stone (1993). The role of müller cells in the formation of the blood-retinal barrier. *Neuroscience* 55 (1), pp. 291–301.
- Tworig, JM and MB Feller (2021). Müller Glia in Retinal Development: From Specification to Circuit Integration. *Frontiers in neural circuits* 15, p. 815923.
- Vázquez-Chona, F, AM Clark, and EM Levine (2009). Rlbp1 Promoter Drives Robust Müller Glial GFP Expression in Transgenic Mice. *Investigative Ophthalmology & Visual Science* 50, pp. 3996–4003.
- Vecino, E, FD Rodriguez, N Ruzafa, X Pereiro, and SC Sharma (2016). Glia-neuron interactions in the mammalian retina. *Progress in Retinal and Eye Research* 51, pp. 1–40.
- Vujosevic, S and E Midena (2013). Retinal Layers Changes in Human Preclinical and Early Clinical Diabetic Retinopathy Support Early Retinal Neuronal and Müller Cells Alterations. *Journal of Diabetes Research* 2013 (1), p. 905058.
- Walter, SD, K Gronert, AL McClellan, RC Levitt, KD Sarantopoulos, and A Galor (2016). ω -3 Tear Film Lipids Correlate With Clinical Measures of Dry Eye. *Investigative Ophthalmology & Visual Science* 57, pp. 2472–2478.
- Wang, J, ML O’Sullivan, D Mukherjee, VM Puñal, S Farsiu, and JN Kay (2017). Anatomy and spatial organization of Müller glia in mouse retina. *Journal of Comparative Neurology* 525 (8), pp. 1759–1777.
- Wang, M, W Ma, L Zhao, RN Fariss, and WT Wong (2011). Adaptive Müller cell responses to microglial activation mediate neuroprotection and coordinate inflammation in the retina. *Journal of Neuroinflammation* 2011 8:1 8 (1), pp. 173–.
- Wang, X, G Xu, J Fan, and M Zhang (2013). Mechanical stretching induces matrix metalloproteinase-2 expression in rat retinal glial (Müller) cells. *NeuroReport* 24, pp. 224–228.
- Wei, J and K Gronert (2017). The role of pro-resolving lipid mediators in ocular diseases. *Molecular Aspects of Medicine* 58, pp. 37–43.
- Wei, J and K Gronert (2019). Eicosanoid and Specialized Proresolving Mediator Regulation of Lymphoid Cells. *Trends in Biochemical Sciences* 44 (3), pp. 214–225.

- Weinreb, RN, CKS Leung, JG Crowston, FA Medeiros, DS Friedman, JL Wiggs, et al. (2016). Primary open-angle glaucoma. *Nature Reviews Disease Primers* 2, p. 16067.
- Whitcup, SM, RB Nussenblatt, SL Lightman, and DA Hollander (2013). Inflammation in Retinal Disease. *International Journal of Inflammation* 2013, p. 724648.
- Yang, S, S Qi, and C Wang (2022). The role of retinal Müller cells in diabetic retinopathy and related therapeutic advances. *Frontiers in Cell and Developmental Biology* 10, p. 1047487.
- Yu, PK, C Balaratnasingam, WH Morgan, SJ Cringle, IL McAllister, and DY Yu (2010). The Structural Relationship between the Microvasculature, Neurons, and Glia in the Human Retina. *Investigative Ophthalmology & Visual Science* 51 (1), pp. 447–458.
- Zhang, J, F Fang, L Li, H Huang, HC Webber, Y Sun, et al. (2019). A Reversible Silicone Oil-Induced Ocular Hypertension Model in Mice. *Journal of Visualized Experiments*.
- Zhang, J and Y Hu (2020). Comparing silicone oil-induced ocular hypertension with other inducible glaucoma models in mice. *Neural Regeneration Research* 15, p. 1652.
- Zhang, J, L Li, H Huang, F Fang, HC Webber, P Zhuang, et al. (2019). Silicone oil-induced ocular hypertension and glaucomatous neurodegeneration in mouse. *eLife* 8.
- Zhang, J, Z Li, M Fan, and W Jin (2022). Lipoxins in the Nervous System: Brighter Prospects for Neuroprotection. *Frontiers in Pharmacology* 13.
- Zhao, N, XN Hao, JM Huang, ZM Song, and Y Tao (2024). Crosstalk Between Microglia and Müller Glia in the Age-Related Macular Degeneration: Role and Therapeutic Value of Neuroinflammation. *Aging and Disease* 15 (3), p. 1132.
- Zhao, XF, J Wan, C Powell, R Ramachandran, MG Myers, and D Goldman (2014). Leptin and IL-6 family cytokines synergize to stimulate Müller Glia reprogramming and retina regeneration. *Cell Reports* 9 (1), pp. 272–284.
- Zhou, LY, ZG Liu, YQ Sun, YZ Li, ZQ Teng, and CM Liu (2025). Preserving blood-retinal barrier integrity: a path to retinal ganglion cell protection in glaucoma and traumatic optic neuropathy. *Cell Regeneration* 2025 14:1 14 (1), pp. 13–.