Spatial multi-omics unveils sphingolipid metabolic reprogramming within the retinal pathological niche

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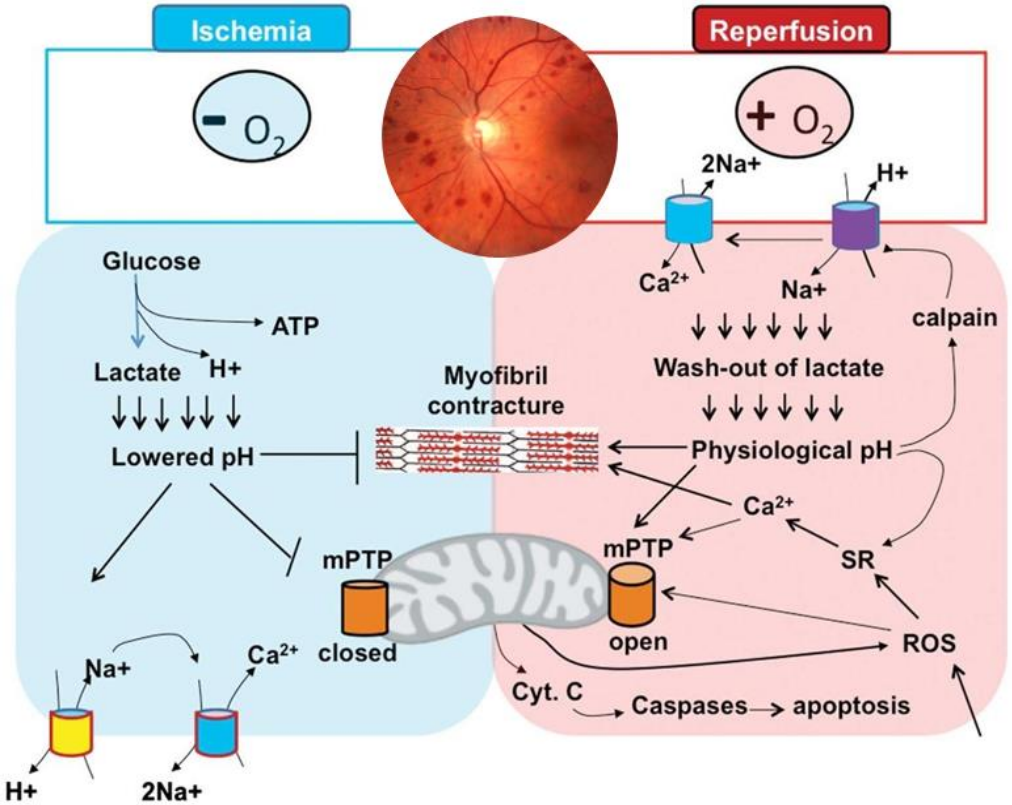
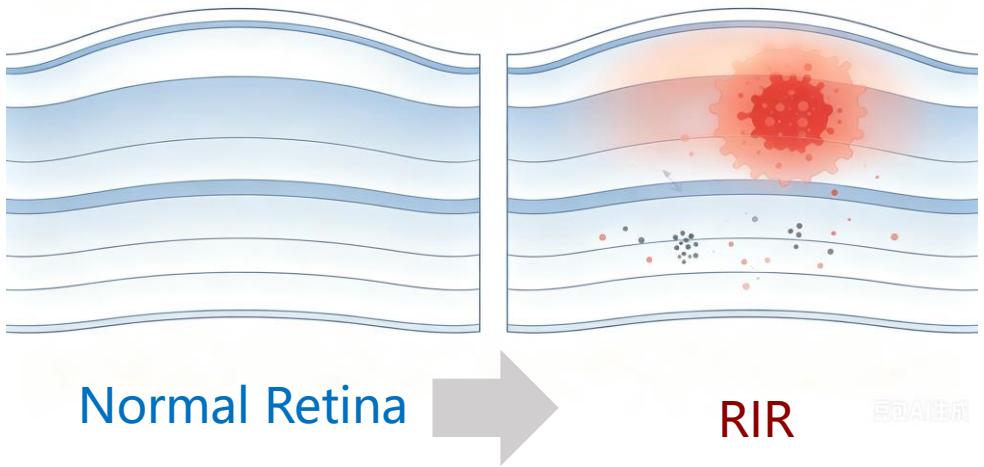
Introduction

Retinal ischemia-reperfusion (RIR) injury constitutes the core pathological mechanism triggering irreversible visual impairment in glaucoma and numerous other ocular diseases, and it causes far more severe tissue damage than simple retinal ischemia alone



Rosenbaum et al. Journal of neuroscience research, 2010

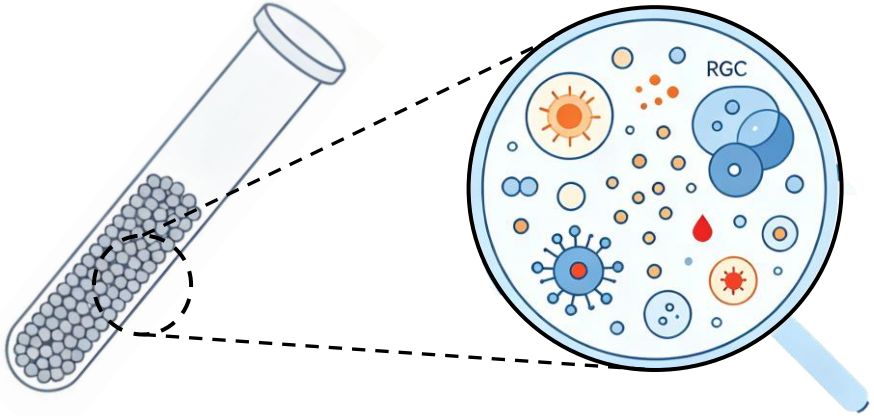
RIR injury **exhibits pronounced spatial heterogeneity across the retina**, with metabolic dysfunctions serving as its prominent manifestations, such as mitochondrial damage and excessive accumulation of reactive oxygen species (ROS).



Problems to Be Addressed

Question

Conventional bulk homogenate-based omics technologies **fail to resolve the spatial heterogeneity of metabolites** as well as cell-type-specific metabolic alterations across distinct cellular subsets.

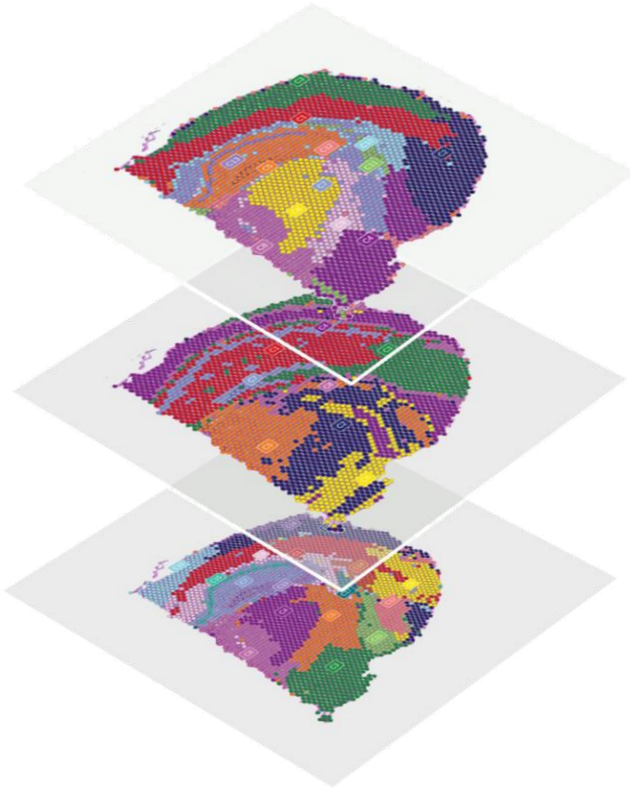


Current research has not clarified:
How metabolic stress is initiated within the local microenvironment surrounding retinal ganglion cells, nor the specific subcellular ecological niches of the retina where metabolic regulatory dysregulation arises.

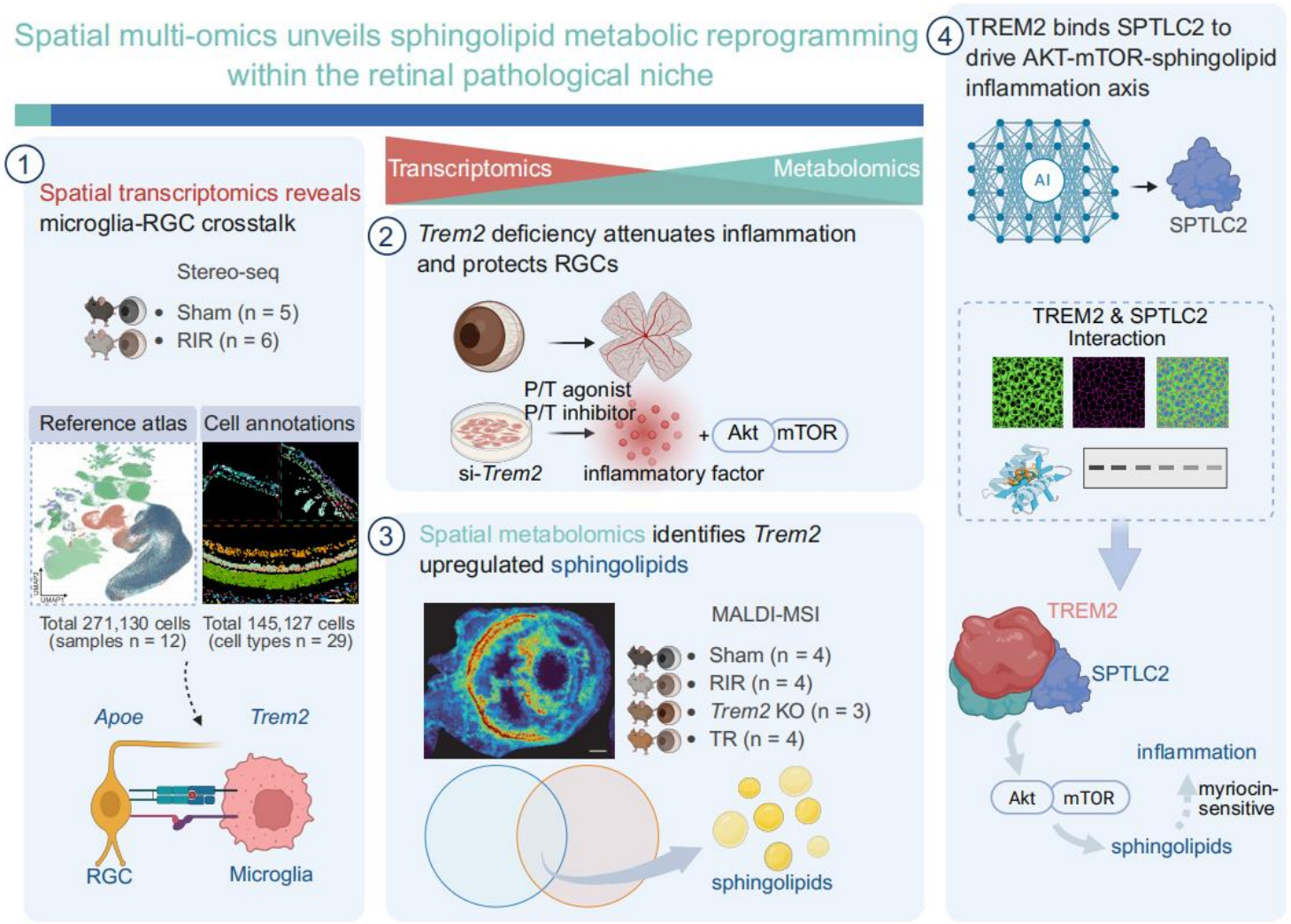
Moses L, Pachter L. Nature methods, 2022

Solutions

√ **Spatial transcriptomics and spatial metabolomics** enable in situ analysis retaining spatial positional information, which offers a promising approach to address the above questions.。



Highlights



- **Spatial multi-omics maps the microenvironment of retinal ischemia-reperfusion injury (RIR).**

The retinal system shifts from a neuron-centered phenotype to an immunometabolic phenotype.

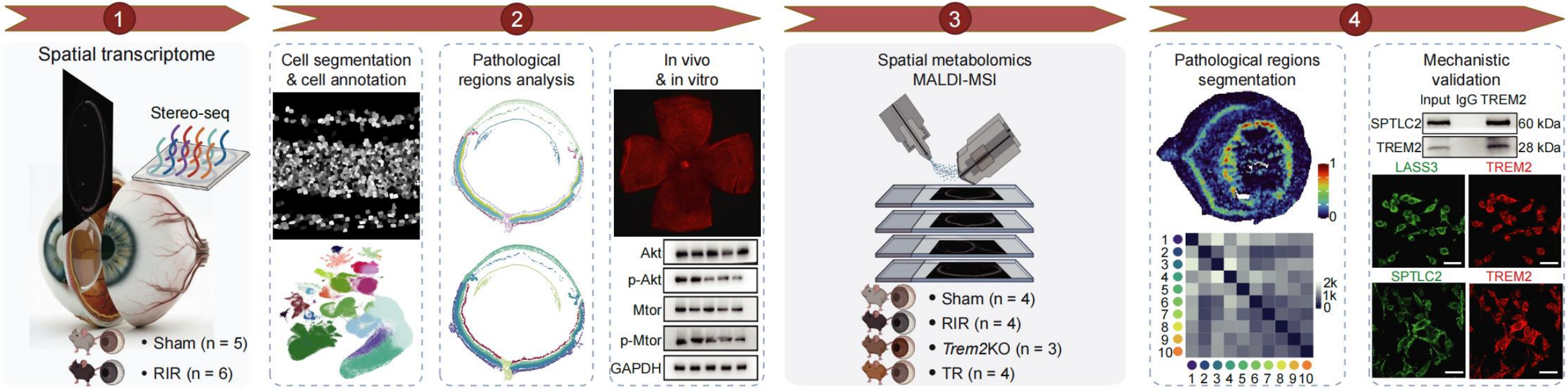
- **Trem2^{+} microglia accumulate within the ganglion cell layer (GCL).**
- These microglia co-localize with degenerating retinal ganglion cells (RGCs) in the pathological microenvironment.
- **TREM2 interacts with SPTLC2.**

The mechanism underlying this interaction is linked to sphingolipid metabolic reprogramming.

- **Targeting SPTLC2 confers therapeutic potential.**

Inhibition of sphingolipid synthesis relieves inflammation and protects (RGCs.

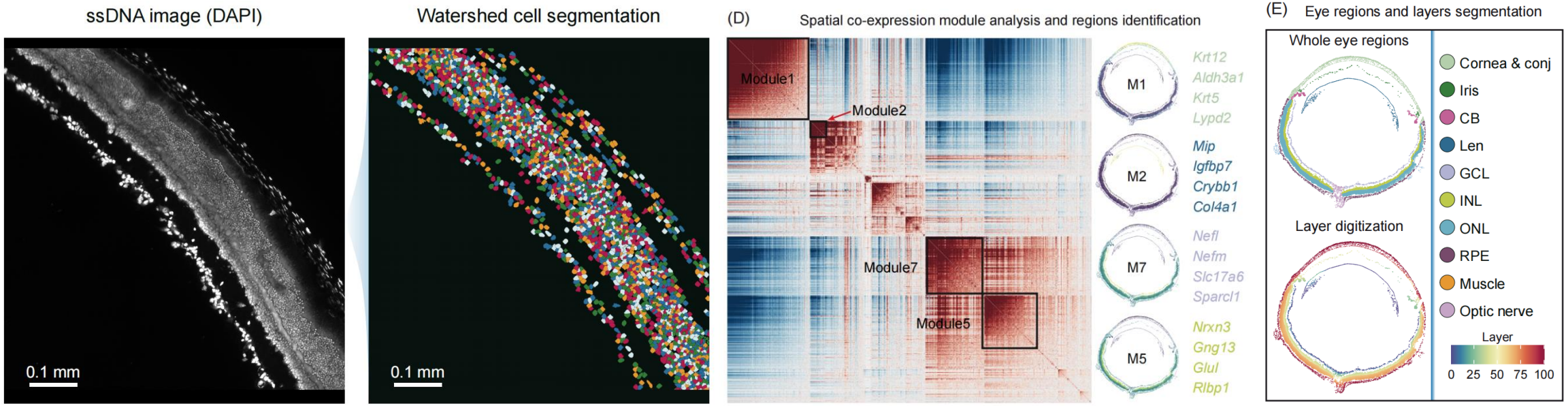
Method



Methods and Technical Route

- **Whole-eye spatial transcriptome construction:** Use Stereo-seq to obtain spatially resolved whole-eyeball gene expression profiles.
- **Single-cell annotation & pathological region analysis:** Segment cells via ssDNA nuclear staining; realize spatial cell typing and ocular regional partition.
- **Spatial metabolomics detection:** Acquire in situ metabolite distribution and characterize metabolic remodeling after RIR injury and Trem2 knockout.
- **In vivo and in vitro validation:** Identify the GCL-centered sphingolipid metabolic axis; verify TREM2-SPTLC2 interaction and druggable potential.

Result 1: High-resolution spatial transcriptomics maps the whole-eye molecular anatomical atlas

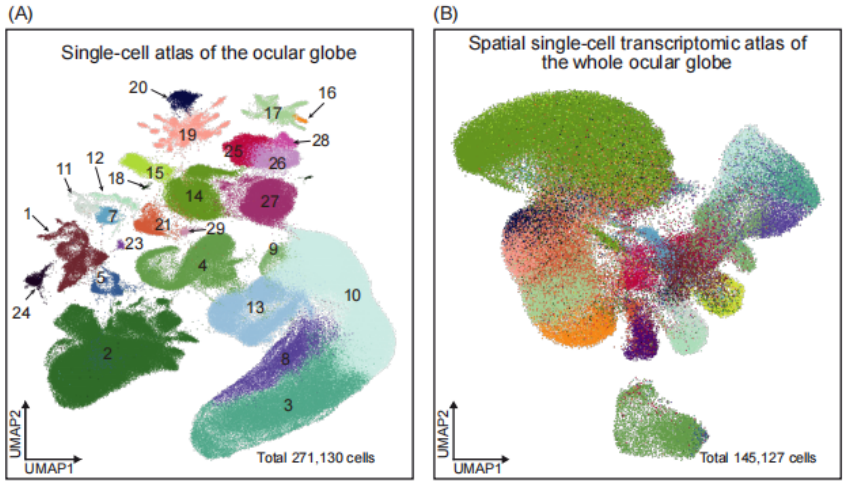


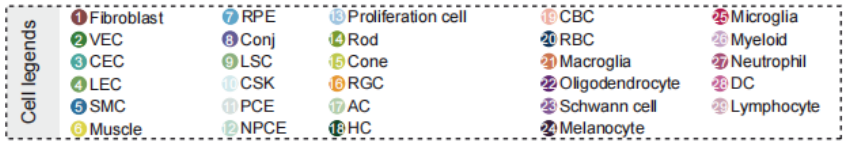
- **High-resolution spatial transcriptomics mapping:** Single-cell segmentation performed via ssDNA staining and watershed algorithm after Stereo-seq sequencing; high inter-sample correlation ensures reliable data.

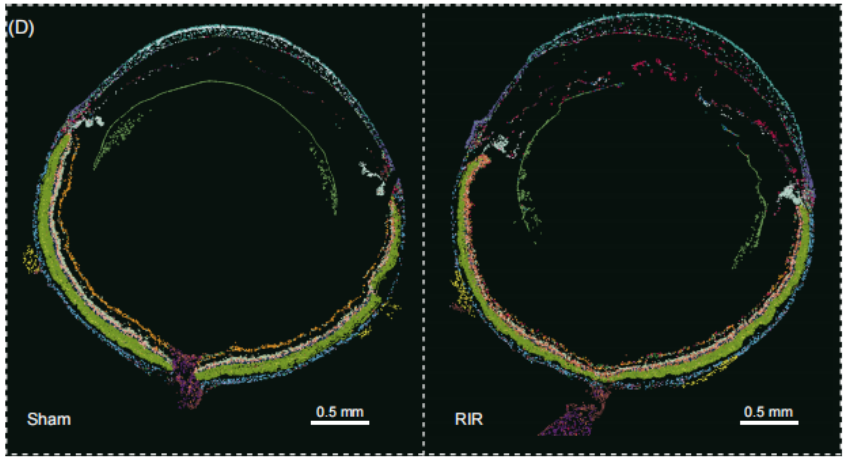
- **Regional definition by co-expression modules:** Spatial co-expression analysis identifies signature gene sets corresponding to ten anatomical structures, including cornea, iris, ciliary body, retinal GCL, INL and ONL.

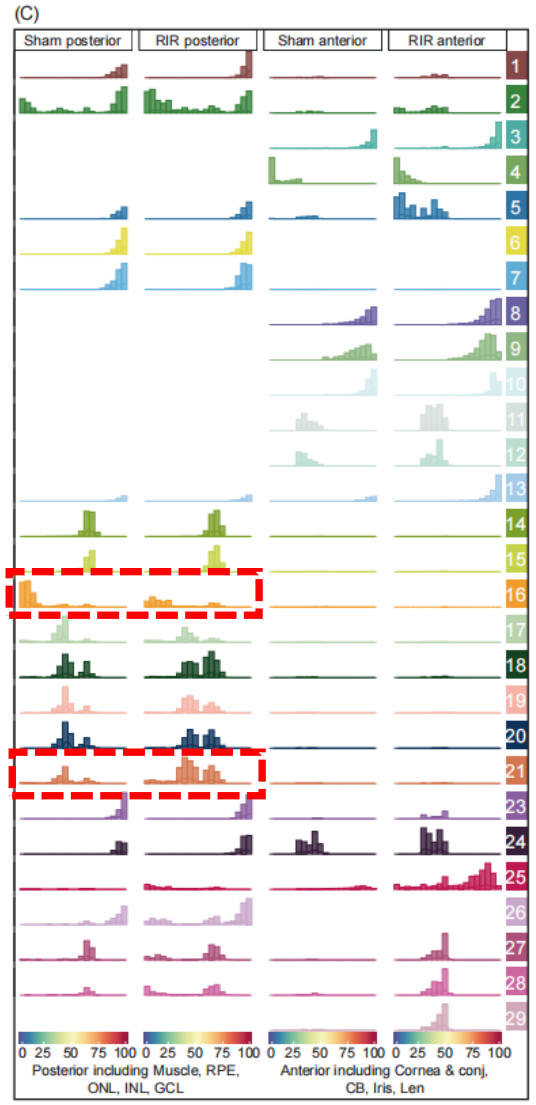
- **Zoning establishment and functional verification:** Two spatial zoning systems are constructed: anatomical partitioning and continuous digital partitioning. Canonical markers such as *Rho, Nefl* and *Krt12* match anatomical locations perfectly, and each retinal layer displays distinct functional specialization.

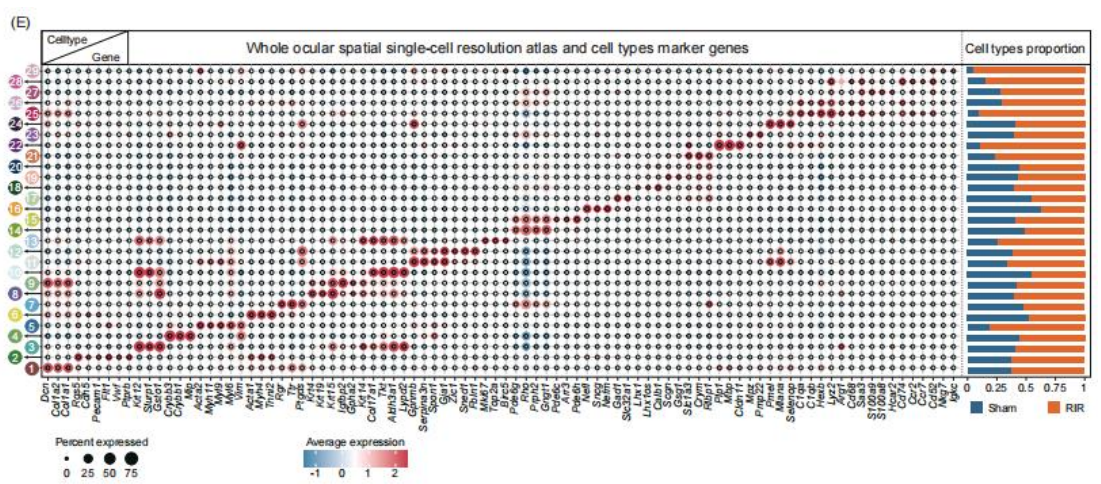
Result 2: RIR injury triggers spatial rearrangement of retinal RGCs and microglia





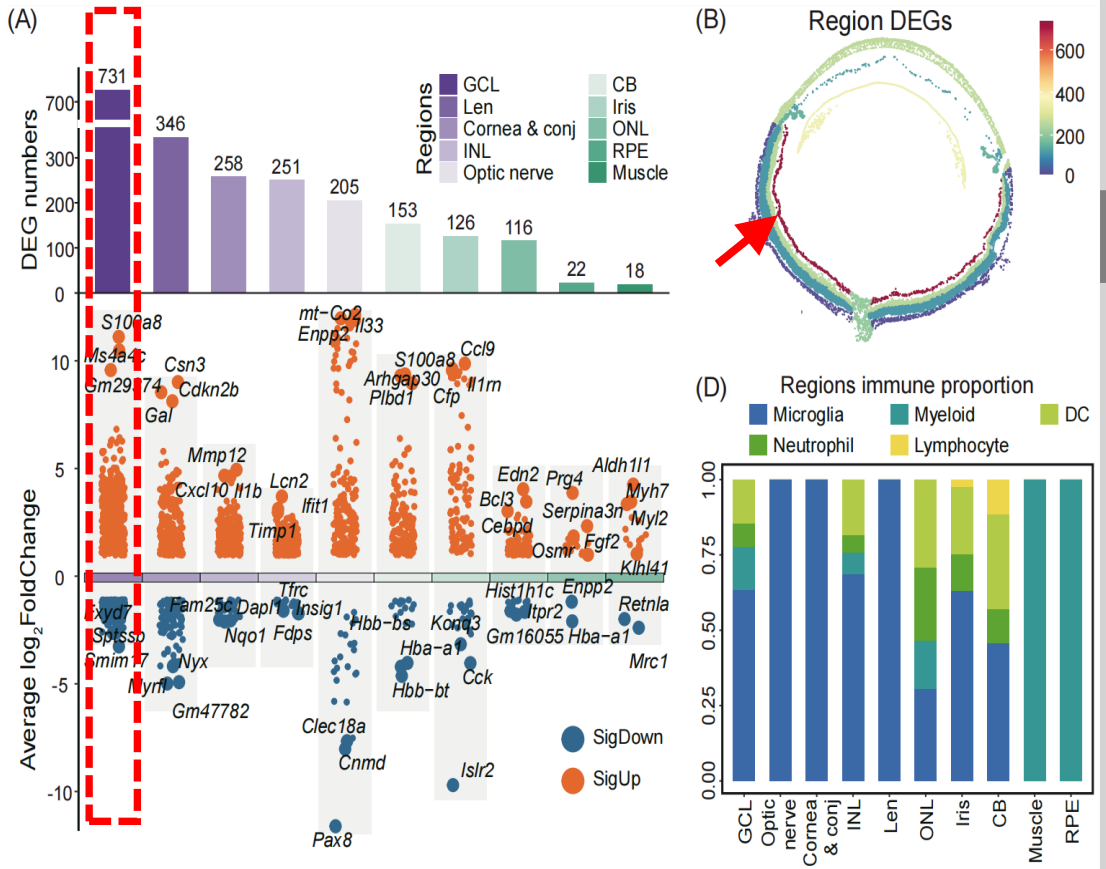




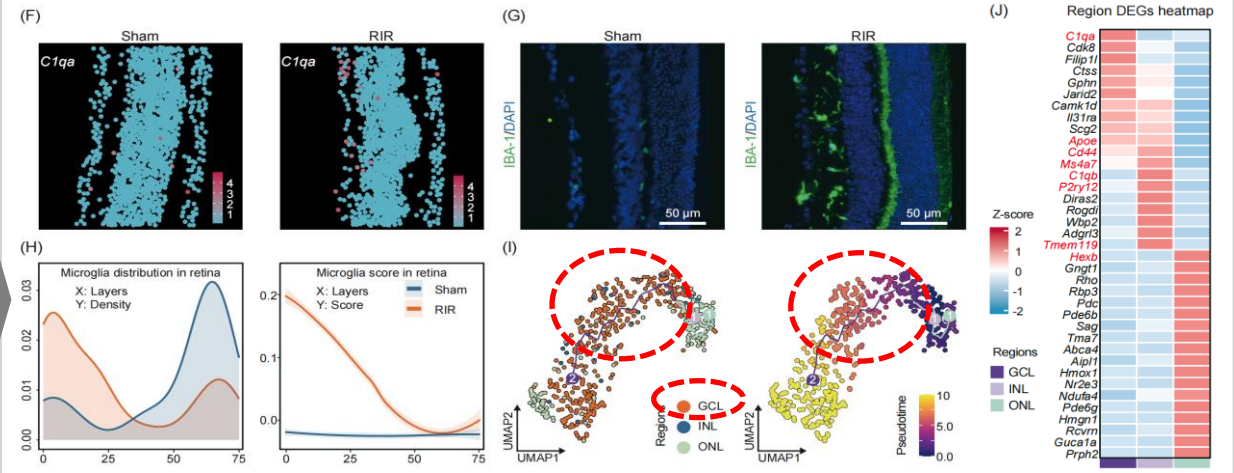


- **Cell annotation:** Based on public single-cell data, 29 ocular cell types were spatially annotated.
- **RGC damage:** Radial density quantification confirmed RGC loss within the GCL.
- **Immune recruitment:** Retinal resident microglia migrate from outer retina to injured GCL after RIR, alongside accumulation of myeloid and dendritic cells.

Result 3: Trem2–Apoe axis in GCL microglia remodels the pathological microenvironment

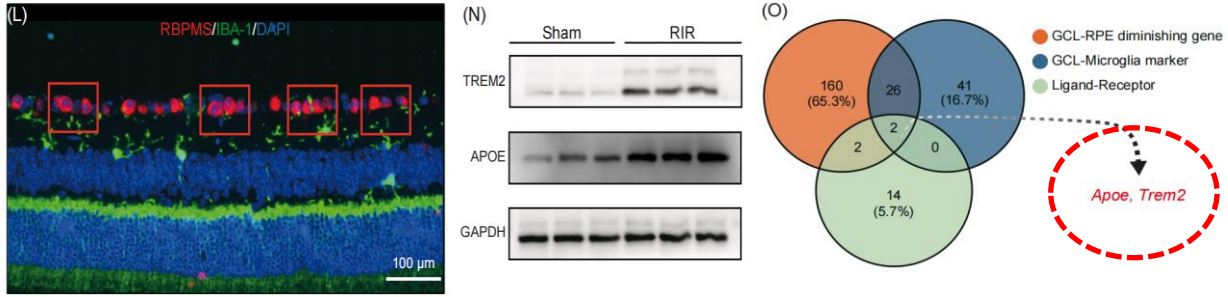


Regional specificity: RIR-induced transcriptomic alterations were predominantly restricted to the GCL, with suppressed visual function pathways and activated microglial inflammatory pathways.

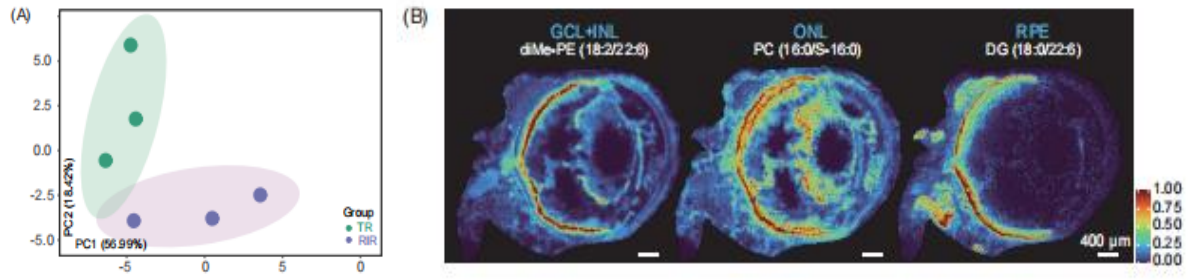


Layer-specific microglial activation: Microglial activation exhibited a GCL–INL–ONL gradient, wherein GCL microglia presented a pro-inflammatory phenotype

Core regulatory mechanism: Microglia dominated the immune population around injured RGCs, and the *Trem2–Apoe* axis was identified as the central regulatory pathway.



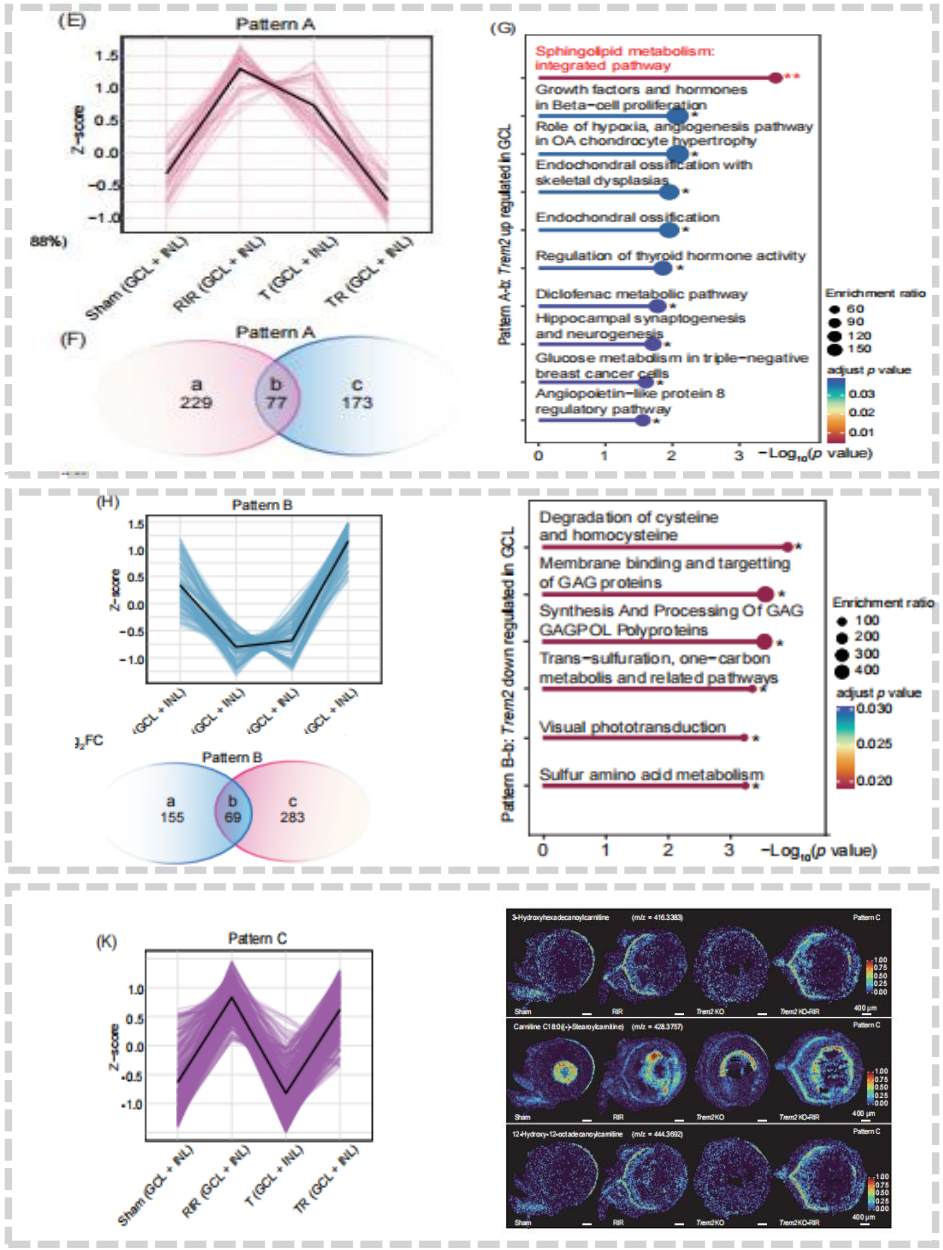
Result 4: Spatial metabolomics verifies Trem2-mediated sphingolipid reprogramming in the inner retina



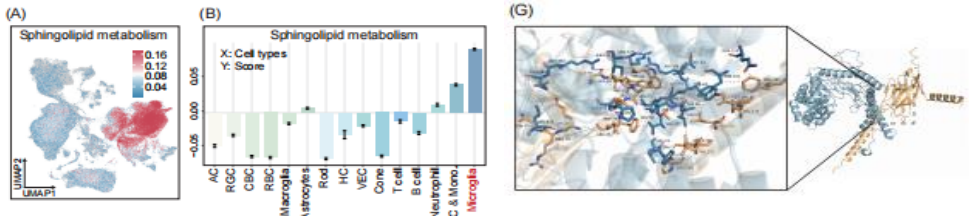
Overall metabolic disparities: *Trem2* knockout profoundly reshaped RIR-triggered retinal metabolism, with metabolic alterations mainly localized to the GCL and INL, consistent with spatial findings from transcriptomics.

3 distinct metabolic clusters were categorized

- **Pattern A:** Trem2-dependent upregulated cluster dominated by sphingolipid metabolism
- **Pattern B:** Trem2-dependent downregulated metabolites
- **Pattern C:** Trem2-independent metabolic alterations

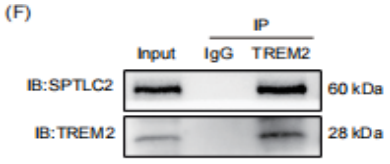


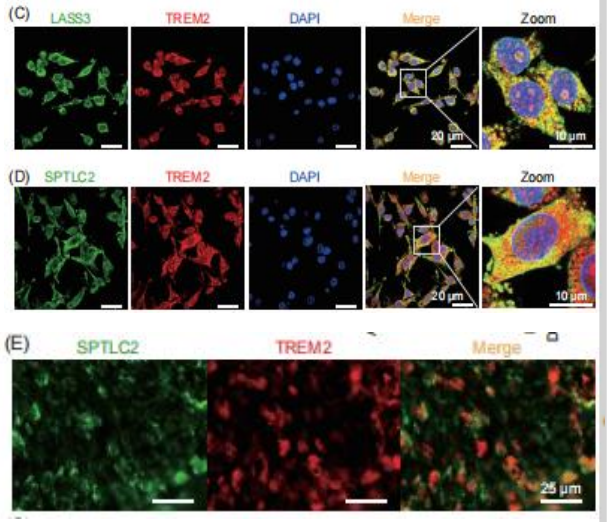
Result 5: TREM2 binds SPTLC2 to target sphingolipid synthesis and exerts neuroprotective effects



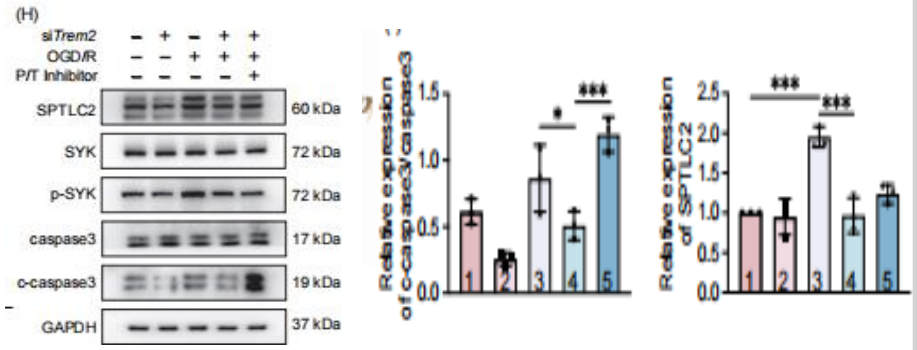
Target prediction: Single-cell data from multiple retinal disease models show sphingolipid metabolism is enriched in microglia; molecular docking predicts direct binding between TREM2 and SPTLC2

Interaction validation: Immunofluorescence co-localization and Co-IP confirm specific physical interaction of the two proteins.

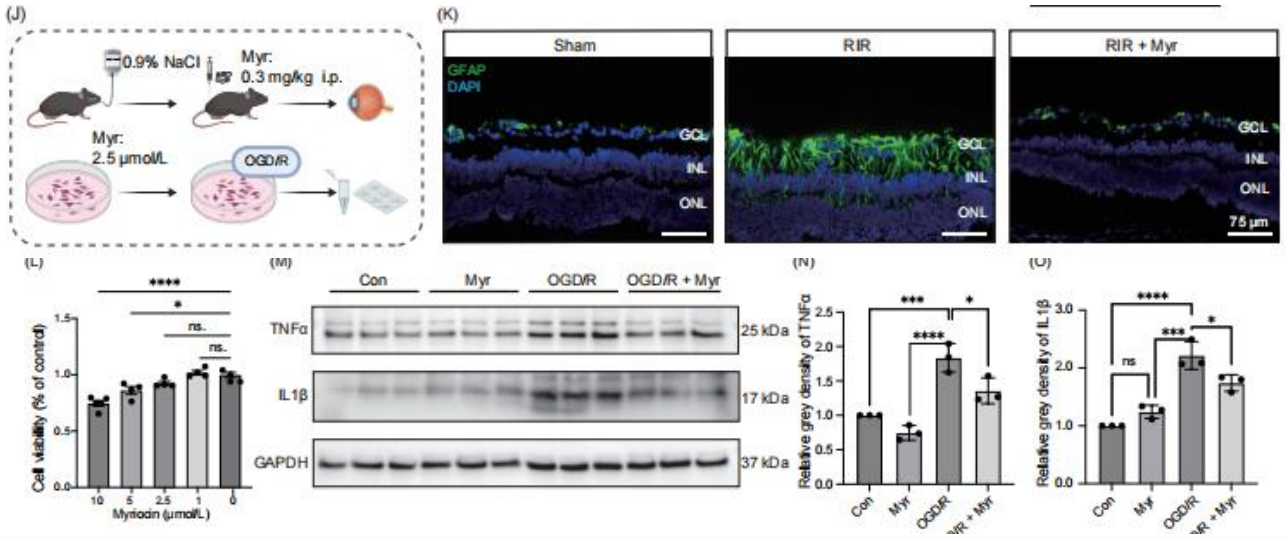




Mechanism: Trem2 upregulates SPTLC2 via AKT-mTOR pathway, independent of microglial phenotypic shifts.



Therapeutic verification: The sphingolipid synthesis inhibitor Myriocin alleviates glial activation and microglial inflammation, possessing anti-inflammatory and neuroprotective potential.



Conclusion

1. Spatial multi-omics analysis of retinal ischemia–reperfusion injury reveals an in situ phenotypic shift of the ganglion cell layer from a neuron-dominant network to an immunometabolic network.

2. Spatially, *Trem2*$^{+}$ microglial recruitment is tightly linked to RGC damage. The TREM2–SPTLC2 axis further mediates local sphingolipid reprogramming, uncovering a novel pathological mechanism and a promising therapeutic target.

Yunhong Shi, Jinpei Lin, Yi Wu, Shengsong Xu, Naiyuan Zhang,. 2026. Spatial multi-omics unveils sphingolipid metabolic reprogramming within the retinal pathological niche. ***iMeta*** 1: e175. https://doi.org/10.1002/imt2.175

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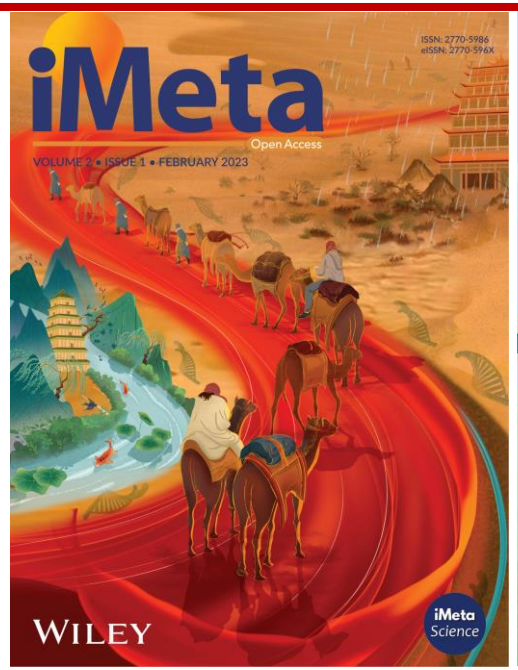

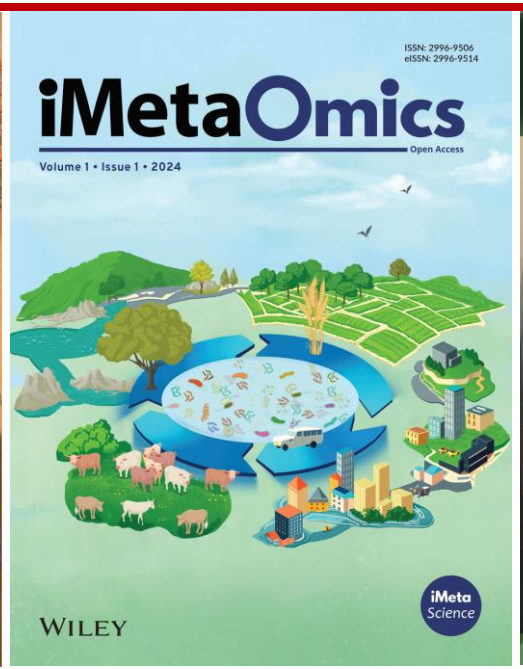

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