

Paeonol restrains microglial inflammation in retinal ischemia– reperfusion injury through HIF-1 α /MIF signaling

Chen Wang

Department of Ophthalmology, Yiyang Central Hospita

Guanfeng Wang

Department of Ophthalmology, The Third Affiliated Hospital of Guangzhou Medical University

Hong Wang

Department of Ophthalmology, Yiyang Central Hospita

Zhaohong Guo

Department of Ophthalmology, Yiyang Central Hospita

Qingqing Yi

ocularistye@163.com

Department of Ophthalmology, Yiyang Central Hospita

Article

Keywords: paeonol, retinal ischemia–reperfusion, microglia, single-cell RNA sequencing

Posted Date: October 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10959183/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Retinal ischemia–reperfusion (IR) injury causes neuronal damage and excessive inflammation, in which microglia play a critical role. Paeonol (PAE) exhibits anti-inflammatory and neuroprotective properties, but its protective mechanisms in retinal IR remain unclear. Here, we evaluated the effects of PAE in a mouse retinal IR model and integrated single-cell RNA sequencing analysis with an oxygen–glucose deprivation/reoxygenation (OGD/R) model in BV2 microglia to investigate the underlying mechanisms. PAE alleviated IR-induced retinal structural damage and cell death and reduced HIF-1 α and pro-inflammatory factor expression. Single-cell analysis revealed extensive remodeling of retinal intercellular communication following IR, with microglia prominently involved in inflammatory signaling and MIF–CD74/CD44 communication markedly enhanced. Microglia exhibited inflammatory and stress-associated transcriptional reprogramming, while regulatory network analysis suggested HIF-1 α as a potential upstream regulator of *Mif* and *Il1b*. In BV2 cells, PAE suppressed OGD/R-induced HIF-1 α , MIF, and IL-1 β expression and reduced IL-1 β , IL-6, and TNF- α release, whereas CoCl₂ attenuated these effects. Collectively, these findings suggest that PAE may alleviate retinal IR injury by suppressing HIF-1 α /MIF-associated microglial inflammation and potentially modulating MIF–CD74/CD44 signaling.

Introduction

Retinal ischemia–reperfusion (IR) injury is an important pathological process involved in glaucoma, diabetic retinopathy, retinal artery occlusion, and other ischemic retinal diseases, and can lead to irreversible retinal neuronal loss and visual dysfunction^{1–3}. During ischemia, interruption of oxygen and energy supply results in ATP depletion, disruption of ionic homeostasis, and mitochondrial dysfunction. Subsequent restoration of blood flow further exacerbates tissue injury through oxidative stress and immune activation^{4–7}. Although current clinical management primarily focuses on restoring retinal perfusion or controlling intraocular pressure, effective treatments targeting post-reperfusion neuroinflammation and secondary neurodegeneration remain limited. Therefore, identifying the key cellular populations and therapeutically targetable molecules that sustain inflammation after IR is essential for improving the outcomes of ischemic retinal diseases.

Microglia are the principal resident immune cells of the retina and play essential roles in tissue homeostasis, synaptic maintenance, and clearance of cellular debris⁸. Following retinal ischemia, microglia rapidly become activated and undergo migratory, transcriptional, and metabolic reprogramming, accompanied by the production of IL-1 β , IL-6, TNF- α , and various chemokines^{9–11}. While an appropriate microglial response facilitates damage containment and clearance of dying cells, persistent activation can amplify inflammation, disrupt the blood–retinal barrier, and exacerbate retinal ganglion cell loss¹². Recent single-cell studies have revealed substantial transcriptional remodeling of microglia following retinal IR, characterized by a shift from homeostatic toward inflammation-associated states^{13,14}. These findings further highlight microglia as key regulators of the inflammatory microenvironment following retinal IR injury.

Hypoxia-inducible factor-1 α (HIF-1 α) is a key transcription factor that enables cellular adaptation to hypoxia¹⁵. Under normoxic conditions, HIF-1 α is hydroxylated by prolyl hydroxylase domain proteins (PHDs), recognized by von Hippel–Lindau (VHL) protein, and rapidly degraded. Hypoxia or inflammatory stimulation inhibits this degradation, resulting in HIF-1 α accumulation and nuclear translocation, where it regulates genes involved in glycolysis, angiogenesis, cell survival, and immune-inflammatory responses^{16–19}. In myeloid cells, sustained HIF-1 α activation can promote a metabolic shift from oxidative phosphorylation toward glycolysis and enhance the expression of inflammatory mediators such as IL-1 β ²⁰. Increased retinal HIF-1 α expression has also been observed following retinal IR and has been implicated in microglial inflammatory and metabolic reprogramming^{21,22}. However, HIF-1 α can exert both adaptive and detrimental effects depending on the temporal and cellular context of ischemia. The mechanisms by which HIF-1 α links altered intercellular communication to inflammatory responses in microglia following retinal IR therefore remain incompletely understood.

Paeonol (PAE), a major phenolic bioactive compound derived from *Paeonia suffruticosa* and other medicinal plants, exhibits anti-inflammatory, antioxidant, and cytoprotective properties^{23–25}. Previous studies have largely attributed its anti-inflammatory effects to modulation of canonical signaling pathways, including TLR4/NF- κ B and PI3K/Akt^{26,27}. More recently, PAE was reported to suppress HIF1A-associated glycolytic and inflammatory responses in microglia. Molecular docking, molecular dynamics simulation, cellular thermal shift assay, and nuclear–cytoplasmic fractionation further suggested that PAE may affect HIF-1 α protein stability and nuclear translocation²⁸. However, these observations were primarily derived from LPS-induced septic encephalopathy and N9 microglial models. Whether PAE modulates HIF-1 α -dependent microglial inflammation under retinal IR and hypoxia/reoxygenation conditions remains unknown.

Based on these observations, we investigated whether PAE protects against retinal IR injury by modulating HIF-1 α -associated microglial inflammatory signaling. We first evaluated the effects of PAE on retinal structural injury, cell death, and inflammation in a mouse model of retinal IR. Single-cell transcriptomic analysis was then used to characterize IR-induced alterations in retinal intercellular communication and microglial transcriptional regulatory networks and to identify candidate inflammatory signaling pathways. Finally, an oxygen–glucose deprivation/reoxygenation (OGD/R) model in BV2 microglial cells, combined with pharmacological stabilization of HIF-1 α by CoCl₂, was used to investigate the relationship among PAE, HIF-1 α , MIF, and inflammatory responses.

Result

PAE attenuates retinal IR injury and inflammatory responses

To evaluate the protective effect of PAE against retinal IR injury, mice received intraperitoneal injections of PAE after IR induction, and retinal tissues were collected on day 3 after IR. H&E staining showed that the retinal layers in the Sham group exhibited clear structural organization with relatively regular cellular arrangement, whereas IR injury resulted in marked thinning of the inner retina accompanied by disruption

of retinal architecture. No significant difference was observed between the IR and IR + DMSO groups, whereas PAE treatment significantly alleviated IR-induced thinning of the inner retina, indicating that PAE attenuated retinal structural damage following IR injury (Fig. 1A, B). TUNEL staining showed that only a few TUNEL⁺ cells were detected in the retinas of the Sham group, whereas the numbers of TUNEL⁺ cells were markedly increased in the IR and IR + DMSO groups. Compared with the IR + DMSO group, PAE treatment significantly reduced the number of TUNEL⁺ cells in the retina, indicating that PAE attenuated IR-induced retinal cell death (Fig. 1C, D).

We next examined hypoxia-responsive and inflammation-related proteins in retinal tissues. Western blotting showed that IR injury significantly increased the protein levels of HIF-1 α , IL-1 β , IL-6, and TNF- α , whereas vehicle treatment did not significantly alter the levels of these proteins. PAE treatment significantly reduced the protein levels of HIF-1 α , IL-1 β , IL-6, and TNF- α , whereas no significant differences in these proteins were observed between the IR + DMSO and IR groups, indicating that PAE suppressed the hypoxic and inflammatory responses in the retina following IR injury (Fig. 1E, F).

Collectively, PAE alleviated IR-induced thinning of the inner retina, reduced the number of TUNEL⁺ cells, and decreased the expression of HIF-1 α and multiple pro-inflammatory cytokines in retinal tissues.

Single-cell RNA sequencing reveals the retinal cellular landscape following IR

To characterize changes in retinal cellular composition and intercellular communication following IR injury, we performed scRNA-seq analysis of three retinal samples from each of the Control and IR groups. After quality control and removal of low-quality cells, the distributions of detected genes, UMI counts, and mitochondrial gene percentages were comparable across samples, with no apparent sample-specific increase in mitochondrial transcript levels (Fig. 2A). Correlation analysis showed a strong positive correlation between nCount_RNA and nFeature_RNA, whereas nCount_RNA was only weakly correlated with percent.mt, supporting the overall quality and complexity of the dataset (Fig. 2B). PCA revealed extensive overlap among cells from the six samples, with partial group-related differences in their distribution across the principal component space (Fig. 2C). PCA and Harmony analyses further showed that the major cellular heterogeneity was well captured and that sample integration was stable, supporting the selection of the first 20 Harmony dimensions for subsequent analyses (Supplementary Fig. S1). Comparison of clustering stability across different resolutions supported a resolution of 0.4 for cell clustering (Supplementary Fig. S2). Further dimensionality reduction using t-SNE and UMAP identified multiple transcriptionally distinct cell clusters, with cells from all biological samples contributing to the major clusters (Fig. 2D, E).

Cell types were annotated according to cluster-specific differentially expressed genes and established retinal cell-type markers reported in previous studies (Fig. 3A, B). ACs specifically expressed *Gad1* and *Slc32a1*, BCs expressed *Vsx2* and *Scgn*, cones expressed *Opn1mw* and *Arr3*, RGCs expressed *Sncg* and *Slc17a6*, rods expressed *Pde6b* and *Nrl*, and RPE cells expressed *Rpe65* and *Tyr*. Among immune and glial populations, microglia (micro) expressed *P2ry12* and *Cx3cr1*, macrophages (MG) expressed *Ly6c2*

and *Ccr2*, neutrophils expressed *S100a8* and *S100a9*, and Macroglia (Macro) specifically expressed *Glul* and *Slc1a3* (Fig. 3C). Analysis of cellular composition showed that rods represented the most abundant cell type, accounting for 55.34% of all cells (Fig. 3D). We next compared the global patterns of cell–cell communication between the Control and IR groups. The number of inferred significant ligand–receptor interactions increased from 260 in the Control group to 589 in the IR group, representing an approximately 126.5% increase, whereas the overall communication strength increased from 11.824 to 12.531, corresponding to an approximately 6.0% increase (Fig. 3E).

These findings suggest that IR injury expand the repertoire of ligand–receptor interactions and communicating cell pairs, resulting in a broader and more complex retinal intercellular communication network.

IR reshapes the retinal cell–cell communication network

To systematically characterize changes in intercellular signaling following retinal IR injury, we constructed cell–cell communication networks for the Control and IR groups based on ligand and receptor expression patterns derived from the single-cell transcriptomic data (Fig. 4A). Compared with the Control group, the IR group exhibited substantially more communication links among cell types and a denser network, with increased interaction strengths among multiple cell populations. Notably, Micro were extensively involved in the communication network following IR injury, while marked alterations were also observed in the communication patterns of Macro, MG, VEC/Peri, RGCs, and other neuronal populations. These results indicate extensive remodeling of the retinal cell–cell communication network following IR injury. To further determine the direction of these communication changes, we compared the differences in the number of significant ligand–receptor interactions between individual cell pairs in the IR and Control groups (Fig. 4B). When microglia acted as either signal senders or receivers, the predicted numbers of interactions with Macro, DC, MG, Tc, Neu, and VEC/Peri, as well as predicted autocrine interactions within microglia, were increased following IR injury.

We next compared the overall signaling patterns across retinal cell types between the two groups (Fig. 4C). Cell–cell communication in the Control group predominantly involved NRXN, NCAM, NEGR, PTN, CADM, LAMININ, and NECTIN signaling, which are associated with neural adhesion, cell–cell interactions, and tissue homeostasis. In contrast, the IR group exhibited distinct immune-, inflammatory-, and injury-associated signaling, including MIF, APP, CCL, SPP1, ANNEXIN, TWEAK, COMPLEMENT, SEMA6, CD80, CD86, TNF, CSF3, and VEGF signaling. These changes suggest a shift in retinal intercellular communication from a homeostatic state toward immune-inflammatory and tissue-remodeling programs following IR injury. To further compare the relative communication activity of individual signaling pathways between the two groups, we analyzed the outgoing and incoming signaling patterns as well as the overall signaling strength across retinal cell types. Heatmaps of outgoing signaling (Supplementary Fig. 3A), incoming signaling (Supplementary Fig. 3B), and overall signaling strength (Fig. 4D) revealed marked alterations in the signaling roles of retinal cell populations following IR injury. Notably, microglia exhibited enhanced incoming signaling and participated in a broader range

of outgoing signaling pathways in the IR group, further highlighting their central role in the remodeled communication network. Moreover, the information flow of MIF and other IR-specific pathways was detected exclusively in the IR group, further indicating a shift toward injury-associated signaling following IR.

Collectively, IR injury induced systematic remodeling of the retinal cell–cell communication network in both interaction number and signaling composition, with microglia emerging as a central cell population characterized by enhanced incoming and outgoing signaling. Concomitantly, retinal intercellular communication shifted from neural–vascular homeostatic support toward immune inflammation, chemotaxis, complement activation, and tissue remodeling.

IR enhances microglial MIF–CD74/CD44 signaling

To identify candidate ligand–receptor signals associated with microglial responses following IR injury, we focused on macroglia, microglia, and retinal ganglion cells (RGCs) and mapped differential gene expression between the two groups onto the CellChat-inferred communication network. Among these interactions, Mif–(Cd74 + Cd44) signaling was markedly enhanced across multiple sender–receiver pairs involving microglia (Fig. 5A). In contrast, neural adhesion- and synaptic connectivity-related signals, including NRXN–NLGN and NCAM, were reduced in the IR group (Fig. 5B). Direct comparison of specific ligand–receptor interactions between the two groups further showed enhanced Mif–(Cd74 + Cd44) signaling in the Macro→Macro, Macro→Micro, Micro→Macro, Micro→Micro, RGC→Macro, and RGC→Micro directions in the IR group (Supplementary Fig. S4). These findings suggest that IR injury may enhance microglia-associated MIF–CD74/CD44 signaling.

We next examined ligand–receptor pairs with relatively high predicted communication probabilities in the IR group. Mif–(Cd74 + Cd44) exhibited high communication probabilities across multiple microglia-associated interactions, while App–Cd74 and CCL–CCR signaling also showed relatively high predicted communication probabilities in the IR group (Fig. 5C). In contrast, interactions such as Tgfb1–Tgfb1/2 in the Micro→Micro direction exhibited relatively low predicted communication probabilities in the IR group (Fig. 5D). Together, these results indicate that IR remodels the microglia-associated communication network, characterized by enhanced inflammatory and chemotactic signaling accompanied by attenuation of selected homeostasis-related interactions.

To explore potential links between candidate ligands and injury-responsive genes within the microglial population, we further performed NicheNet ligand–target regulatory potential analysis for Micro→Micro signaling. Candidate ligands including MIF, CCL2, CCL4, CCL5, SPP1, THBS1, and FN1 showed varying degrees of predicted regulatory potential for differentially expressed genes in microglia (Fig. 5E). Further quantitative comparison of changes in predicted communication probabilities between the two groups revealed that Mif–(Cd74 + Cd44) exhibited the largest increase in communication probability from the Control to the IR group (Fig. 5F).

Collectively, IR injury induced extensive remodeling of ligand–receptor communication among macroglia, microglia, and RGCs. MIF–CD74/CD44 signaling was enhanced across multiple communication directions involving microglia and showed the largest increase in predicted communication probability, suggesting that it may represent a key candidate signaling mechanism underlying microglial communication following IR injury.

HIF-1 α /MIF potentially regulates microglial inflammatory responses

Our cell–cell communication analysis identified MIF–CD74/CD44 as a candidate signaling axis preferentially enhanced in microglia following IR injury. To determine whether altered MIF-associated communication was accompanied by intrinsic transcriptional reprogramming in microglia and to explore its potential upstream regulators, we compared the gene expression profiles of microglia between the Control and IR groups.

Differential expression analysis identified *Saa3*, *Lgals3*, *S100a8*, *S100a9*, *Fabp5*, *Arg1*, *Slpi*, *Pf4*, and *Spp1* among the top upregulated genes ranked by log2FC (Fig. 6A). The marked upregulation of *Saa3*, *S100a8*, *S100a9*, and *Lgals3* indicated enhanced transcriptional programs associated with acute inflammation, injury responses, and myeloid immune activation in IR microglia. Concurrent upregulation of *Fabp5*, *Spp1*, and *Pf4* further suggested alterations in processes related to lipid metabolism, cell adhesion, extracellular matrix remodeling, and tissue repair. GSEA further revealed enrichment of acute inflammatory response, granulocyte chemotaxis, receptor ligand activity, IL-17 signaling, ECM–receptor interaction, complement response, interferon- γ response, and angiogenesis in IR microglia (Fig. 6B). Together, these findings indicate that IR induces a shift in microglia from a homeostatic state toward an injury-responsive state characterized by acute inflammation, chemotaxis, and stress responses.

To identify key molecules within the network of genes upregulated in IR microglia, we constructed a protein–protein interaction (PPI) network in which node size and color represented network degree (Fig. 6C). *IL1B* exhibited the highest degree and occupied a central topological position within the network, while *ACTB*, *FN1*, and *ANXA5* also showed relatively high connectivity. *HIF1A*, *CD44*, *LGALS3*, *HMOX1*, *APP*, *SOD2*, *ARG1*, *GPX1*, and *TXN1* were distributed within secondary core regions, indicating that the upregulated network involved multiple processes related to inflammation, hypoxic adaptation, oxidative stress, metabolic alterations, and extracellular matrix remodeling.

To investigate the potential upstream transcriptional regulation of these inflammation-associated genes, we constructed a TF–target network using high-confidence DoRothEA regulons at confidence levels A–C. Within this focused network, the *HIF1A* regulon showed relatively high inferred activity in the IR group and displayed predicted activating regulatory relationships with both *Mif* and *Il1b*, while also connecting to differentially expressed targets including *Lgals1*, *Abcb1b*, *Ppp1r10*, and *Trf* (Fig. 6D). These findings link *HIF1A* to the MIF-associated communication changes at the transcriptional regulatory level, suggesting that HIF-1 α may serve as a candidate upstream regulator of *Mif* expression following IR injury.

Collectively, IR induced extensive inflammatory, metabolic, and stress-associated transcriptional reprogramming in microglia, with IL1B emerging as a major topological hub of the upregulated PPI network and HIF-1 α potentially contributing to the upstream transcriptional regulation of MIF–CD74/CD44 signaling.

PAE regulates MIF and inflammatory factors in microglia by suppressing HIF-1 α

To determine whether HIF-1 α is involved in the regulation of MIF and inflammatory factor expression in microglia and to evaluate the regulatory effects of PAE, we established an OGD/R model in BV2 microglial cells. Western blotting showed that OGD/R significantly increased the protein levels of HIF-1 α , MIF, and IL-1 β compared with the Control group. PAE treatment markedly suppressed these increases, whereas co-treatment with CoCl₂ restored HIF-1 α , MIF, and IL-1 β expression to levels approaching those observed in the OGD/R group (Fig. 7A, B).

Immunofluorescence staining further confirmed these changes. OGD/R markedly increased the fluorescence intensities of HIF-1 α , MIF, and IL-1 β in BV2 cells, whereas PAE treatment significantly reduced their mean fluorescence intensities. Co-treatment with CoCl₂ attenuated the inhibitory effects of PAE, resulting in increased fluorescence intensities of all three proteins (Fig. 7C, D). ELISA showed that OGD/R significantly increased the concentrations of IL-1 β , IL-6, and TNF- α in the culture supernatants, indicating enhanced release of inflammatory factors from BV2 cells. PAE treatment significantly reduced the release of these inflammatory factors, whereas their concentrations increased again following co-treatment with CoCl₂ (Fig. 7E).

Together, these results demonstrate that PAE suppresses OGD/R-induced HIF-1 α and MIF expression as well as inflammatory factor production, whereas stabilization of HIF-1 α by CoCl₂ attenuates the anti-inflammatory effects of PAE. The coordinated changes in HIF-1 α and MIF further support the involvement of HIF-1 α -related signaling in the regulation of MIF expression and inflammatory responses in BV2 microglia. Combined with the preceding single-cell analysis, these findings suggest that PAE may attenuate the inflammatory response to retinal ischemia–reperfusion injury by suppressing HIF-1 α -mediated MIF–CD74/CD44 inflammatory signaling in microglia, thereby reducing the production of pro-inflammatory factors, including IL-1 β , IL-6, and TNF- α .

Disussion

Retinal IR injury involves transient ischemia followed by oxidative stress upon reperfusion, disruption of the blood–retinal barrier, immune cell activation, and multiple forms of cell death^{29–32}. The inner retina, particularly retinal ganglion cells and their associated synaptic structures, is highly vulnerable to ischemic injury. In the present study, marked thinning of the inner retina and increased numbers of TUNEL-positive cells were observed on day 3 after IR, accompanied by elevated expression of IL-1 β , IL-6, and TNF- α . These findings indicate the coexistence of structural injury and a robust inflammatory

response following retinal IR. PAE significantly ameliorated these alterations, suggesting that its protective effects involve not only suppression of inflammatory mediator expression but also attenuation of retinal cell death associated with inflammatory and hypoxic stress. Previous studies have similarly demonstrated that retinal IR induces microglial activation, peripheral myeloid cell infiltration, and production of inflammatory mediators such as TNF and IL-1 β ^{33–35}. Single-cell studies of retinal IR have further revealed myeloid cell activation, enhanced inflammatory programs, and injury across multiple retinal cell populations, consistent with our findings^{13,36–38}.

Notably, the role of HIF-1 α in ischemic tissues is highly dependent on the temporal and cellular context³⁹. Transient and moderate activation of HIF-1 α may facilitate cellular adaptation to hypoxia by promoting metabolic adaptation and vascular repair^{40,41}. However, under conditions of sustained inflammation or dysregulated immunometabolism, persistent HIF-1 α accumulation may promote glycolytic reprogramming of myeloid cells and enhance pro-inflammatory gene expression^{42,43}. The elevated HIF-1 α observed on day 3 after IR may reflect persistent HIF-1 α activation occurring in parallel with amplification of the inflammatory response. The concomitant reduction in HIF-1 α expression, inflammatory mediators, and retinal structural damage following PAE treatment suggests that suppression of excessive HIF-1 α -associated inflammatory signaling at this stage may contribute to the protective effects of PAE.

To further define the cellular sources and potential signaling networks underlying IR, we performed single-cell analysis of Control and IR retinas. IR markedly increased the number of inferred significant cell–cell interactions, whereas the increase in overall communication strength was relatively modest. This disproportionate change suggests that IR primarily expands the repertoire of communicating cell pairs and ligand–receptor interactions, thereby increasing the breadth and complexity of the retinal communication network, rather than uniformly strengthening all pre-existing signaling interactions. Signal pattern analysis further revealed a pronounced shift in the nature of retinal intercellular communication. Control retinas were predominantly characterized by signaling pathways associated with neural adhesion, cell–cell interactions, and tissue homeostasis, including NRXN, NCAM, NECTIN, LAMININ, and PTN. In contrast, IR retinas exhibited prominent immune-, inflammatory-, and injury-associated signaling, including MIF, CCL, SPP1, TNF, COMPLEMENT, and CD80/CD86 pathways, together with signals related to extracellular matrix remodeling. These findings suggest that retinal IR shifts the intercellular communication network from a neural–vascular homeostatic state toward an immune-inflammatory and tissue-remodeling state. Similar changes involving myeloid activation, enhanced microglia-associated communication, and disruption of neuronal homeostatic signaling have been reported in previous studies on retinal ischemia in mice and nonhuman primates^{30,44,45}.

Among the retinal cell populations, microglia exhibited extensive alterations in both incoming and outgoing signaling following IR. Notably, MIF–CD74/CD44 was among the ligand–receptor interactions showing prominent enhancement, accompanied by increased CCL–CCR, SPP1-related, and THBS1-related signaling. These changes suggest that microglia participate extensively in the inflammatory

communication network following IR and highlight MIF signaling as a potential contributor to this response. CD74 is not only an MHC class II-associated molecule but also functions as a noncanonical receptor for MIF, with CD44 acting as a co-receptor in MIF-mediated signaling^{46–48}. Previous studies in microglia have implicated MIF–CD74 signaling in the regulation of MAPK signaling and inflammatory mediator production^{49,50}, while CD74-positive microglia/macrophages have increasingly been associated with neuroinflammatory states in the central nervous system^{51,52}. Nevertheless, the biological effects of MIF–CD74 signaling are context dependent and may vary across inflammatory and disease settings^{28,53}. Therefore, our findings support MIF–CD74/CD44 as a candidate communication axis associated with microglial responses to retinal IR.

Differential expression and enrichment analyses provided further evidence of extensive transcriptional reprogramming in microglia following IR. Injury- and immune response-associated genes, including *Saa3*, *Lgals3*, *S100a8*, *S100a9*, *Fabp5*, and *Spp1*, were markedly upregulated, while GSEA revealed enrichment of gene sets associated with acute inflammatory responses, granulocyte chemotaxis, IL-17 signaling, complement responses, IFN- γ responses, and extracellular matrix interactions. These findings indicate that microglia undergo broad transcriptional remodeling involving chemotaxis, immune metabolism, extracellular matrix regulation, and innate immune responses rather than merely exhibiting increased expression of individual inflammatory mediators. PPI analysis identified IL1B as a central hub within the network of upregulated proteins, suggesting that it may represent an important effector of the inflammatory response in IR microglia. TF activity and TF–target network analyses further indicated increased inferred HIF1A activity and predicted regulatory relationships between HIF1A and both *Mif* and *Il1b*. These observations provide transcriptional regulatory evidence supporting the hypothesis that HIF-1 α may act upstream of MIF and IL-1 β .

The OGD/R experiments further bridged the gap between the computational predictions and experimental validation. OGD/R increased HIF-1 α , MIF, and IL-1 β expression in BV2 microglia and enhanced the release of IL-1 β , IL-6, and TNF- α , whereas PAE markedly suppressed these responses. Importantly, CoCl₂ co-treatment restored HIF-1 α and MIF expression and increased inflammatory factor production, thereby attenuating the inhibitory effects of PAE. These findings support the involvement of HIF-1 α -related signaling in the anti-inflammatory effects of PAE and further suggest a functional relationship between HIF-1 α and MIF expression under OGD/R conditions. Together with the single-cell communication results, these data support a model in which PAE may suppress microglial inflammatory responses by modulating HIF-1 α -associated MIF signaling. Recent studies have also found that paeonol can inhibit HIF1A-related glycolysis and inflammatory response in microglia, and can be partially reversed by CoCl₂, consistent with the observations in this study²⁸.

In this study, we investigated the protective effects of PAE against retinal ischemia–reperfusion (IR) injury and its potential anti-inflammatory mechanisms through in vivo evaluation, single-cell transcriptomic analysis, and in vitro validation. First, intraperitoneal administration of PAE significantly alleviated IR-induced thinning of the inner retina, reduced TUNEL-positive cells, and decreased retinal

expression of HIF-1 α , IL-1 β , IL-6, and TNF- α . Second, single-cell analysis revealed extensive remodeling of the retinal cell–cell communication network after IR, characterized by a marked increase in inferred significant ligand–receptor interactions and broad involvement of microglia in inflammatory and injury-responsive signaling. Third, differential expression, enrichment, PPI, and TF–target network analyses collectively suggested that HIF-1 α , MIF, and IL-1 β may constitute an interconnected inflammatory regulatory module in microglia, with MIF–CD74/CD44 communication markedly enhanced following IR. Finally, in the BV2 OGD/R model, PAE suppressed HIF-1 α , MIF, and IL-1 β expression and reduced the release of IL-1 β , IL-6, and TNF- α , whereas CoCl₂ attenuated these effects. Collectively, these findings suggest that PAE may protect against retinal IR injury, at least in part, by suppressing HIF-1 α -associated MIF and inflammatory signaling in microglia.

A strength of the present study is the integration of in vivo phenotypic evaluation, single-cell transcriptomic analysis, and in vitro mechanistic validation. We first demonstrated that PAE ameliorated retinal structural injury and inflammation following IR, then used single-cell data to identify IR-associated cellular communication networks and candidate signaling pathways, and finally evaluated the regulation of HIF-1 α , MIF, and inflammatory factors by PAE in a microglial OGD/R model. This strategy provides disease model-derived evidence for the proposed mechanism rather than relying solely on a predefined pathway based on previous studies. Several limitations should nevertheless be acknowledged. First, ligand–receptor communication and TF regulatory networks were inferred computationally from gene expression and database-derived relationships, and protein-level colocalization and interaction of MIF with CD74/CD44 in retinal tissues remain to be validated. Second, direct genetic or pharmacological manipulation of MIF, CD74, or CD44 was not performed; therefore, the causal contribution of the MIF–CD74/CD44 axis to the protective effects of PAE remains to be established. Finally, the present study primarily evaluated histological and inflammatory changes at day 3 after IR, and longer-term studies are required to determine whether the protective effects of PAE are sustained and translate into preservation of retinal function.

Conclusion

In conclusion, PAE attenuated retinal IR-induced structural damage and cell death and reduced the expression of HIF-1 α and multiple pro-inflammatory factors. Single-cell analysis revealed extensive remodeling of retinal cell–cell communication following IR, characterized by a shift from homeostatic signaling toward immune-inflammatory and tissue-remodeling programs, with microglia emerging as a major participant in the altered communication network. Integrated communication, transcriptional, and regulatory network analyses further identified HIF-1 α , MIF–CD74/CD44, and IL-1 β as a potential inflammatory regulatory network associated with microglial responses to IR. In vitro OGD/R experiments further demonstrated that PAE suppressed HIF-1 α , MIF, and inflammatory factor expression in microglia, whereas CoCl₂ attenuated these anti-inflammatory effects.

Collectively, these findings suggest that PAE may alleviate the inflammatory response to retinal IR injury by suppressing HIF-1 α -mediated MIF–CD74/CD44 inflammatory signaling in microglia, thereby reducing

the production of pro-inflammatory factors, including IL-1 β , IL-6, and TNF- α .

Materials and methods

1. Experimental animals and ethical approval

Eight-week-old male C57BL/6J mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Mice were housed at a constant temperature of 24°C under a 12-h light/dark cycle, with free access to food and water. All animal experiments were approved by the Institutional Animal Ethics Committee of Yiyang Central Hospital and were performed in accordance with relevant guidelines and regulations for the care and use of laboratory animals. The study was conducted in compliance with the ARRIVE guidelines. At the end of the experiments, mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium at a dose of 60 mg/kg and subsequently euthanized by cervical dislocation.

2. Retinal ischemia–reperfusion model and treatment

Mice were randomly divided into four groups: Sham, IR, IR + DMSO, and IR + PAE groups. Mice in the Sham group underwent anesthesia and anterior chamber puncture only. IR model was induced by acute elevation of intraocular pressure. Briefly, mice were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg), followed by pupil dilation with tropicamide. A 30-gauge needle connected to a saline reservoir was inserted into the anterior chamber, and the reservoir was elevated to maintain the intraocular pressure at 110 mmHg for 60 min. Retinal whitening and interruption of retinal blood flow were observed during the procedure to confirm retinal ischemia. After 60 min of ischemia, the needle was removed to restore intraocular pressure and allow retinal reperfusion. Mice in the IR + PAE group received intraperitoneal injections of PAE (MCE, USA) at 10 mg/kg on the day of IR induction and on days 1, 2, and 3 after IR, once daily for a total of four injections. Mice in the IR + DMSO group received an equal volume of vehicle at the same concentration and time points.

3. Hematoxylin and eosin staining and retinal thickness measurement

Mice were euthanized on day 3 after IR induction, and the eyeballs were collected and fixed in 4% paraformaldehyde for 24 h. After dehydration through a graded ethanol series, clearing in xylene, and paraffin embedding, serial sections were prepared along the optic nerve–corneal axis. Sections were deparaffinized, rehydrated, and subjected to H&E staining. Images were acquired using a confocal microscope (Nikon, Japan), and retinal thickness was measured using ImageJ.

4. TUNEL staining

Retinal cell apoptosis was assessed using a TUNEL apoptosis detection kit. Paraffin sections were deparaffinized, rehydrated, and treated with proteinase K, followed by incubation with TUNEL reaction solution according to the manufacturer's instructions (Beyotime, China) at 37°C for 60 min in the dark. Nuclei were counterstained with DAPI, and images were acquired using a confocal microscope (Nikon, Japan). The number of TUNEL-positive cells per field was quantified using ImageJ.

5. Cell culture and treatments

BV2 microglial cells were purchased from ATCC (USA) and cultured in high-glucose DMEM supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Cells were divided into four groups: Control, OGD/R, OGD/R + PAE, and OGD/R + PAE + CoCl₂. Cells in the Control group were cultured in complete medium under normoxic conditions. For OGD/R group, the culture medium was replaced with glucose- and serum-free DMEM, and cells were incubated under hypoxic conditions (1% O₂, 5% CO₂, and 94% N₂) for 3 h. The medium was then replaced with complete medium, and cells were reoxygenated under normoxic conditions for 24 h. In the OGD/R + PAE group, cells were pretreated with 10 µM paeonol for 0.5 h before OGD, and 10 µM paeonol was maintained throughout OGD and reoxygenation. In the OGD/R + PAE + CoCl₂ group, cells were pretreated with 10 µM paeonol and 100 µM CoCl₂ for 0.5 h before OGD, and the same concentrations were maintained throughout OGD and reoxygenation.

6. Western blotting

Retinal tissues or cell samples were homogenized in RIPA lysis buffer containing protease and phosphatase inhibitors and lysed on ice for 30 min. Lysates were centrifuged at 12,000 × g for 15 min at 4°C, and the supernatants were collected. Protein concentrations were determined using the BCA assay. Equal amounts of protein were separated by SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked with 5% non-fat milk for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies against HIF-1α (CST; 1:1,000), MIF (Abcam; 1:1,000), IL-1β (Abcam; 1:1,000), IL-6 (CST; 1:1,000), TNF-α (CST; 1:1,000), and GAPDH (Epizyme; 1:5,000). After washing with TBST, the membranes were incubated with the corresponding HRP-conjugated secondary antibodies (Epizyme; 1:5,000) for 1 h at room temperature. Protein bands were visualized by chemiluminescence (Bio-Rad, USA), and band intensities were quantified using ImageJ. The original blots are presented in Supplementary Figure S5 and S6.

7. Immunofluorescence staining

After treatment, cells were fixed with 4% paraformaldehyde for 15 min, washed with PBS, permeabilized with 0.1% Triton X-100 for 30 min, and blocked with 5% BSA for 1 h at room temperature. Cells were then incubated overnight at 4°C with primary antibodies against HIF-1α (CST; 1:500), MIF (Abcam; 1:500), and IL-1β (Abcam; 1:500). The following day, cells were incubated with FITC-conjugated secondary

antibodies (Beyotime; 1:500) for 1 h at room temperature in the dark, followed by nuclear counterstaining with DAPI. Images were acquired using a confocal microscope (Nikon, Japan), and mean fluorescence intensity was quantified using ImageJ.

8. Enzyme-linked immunosorbent assay

Cell culture supernatants from each group were collected and centrifuged at $1,000 \times g$ for 10 min at 4°C to remove cellular debris. The concentrations of IL-1 β , IL-6, and TNF- α in the culture supernatants were measured using mouse ELISA kits (Elabscience, China). Absorbance was measured at 450 nm with a reference wavelength of 570 nm using a microplate reader. Cytokine concentrations were calculated from the standard curves and expressed as pg/mL. All samples were assayed in duplicate.

9. Single-cell RNA-seq data processing and cell-type annotation

Publicly available mouse retinal scRNA-seq data were obtained from the Genome Sequence Archive (GSA) under accession number PRJCA008174³⁰ and reanalyzed in this study. The dataset included three control libraries (CON1–CON3) and three retinal ischemia–reperfusion (IR) libraries (IR1–IR3). The dataset included three control libraries (BLANK1–BLANK3; CRX330417–CRX330419) and three retinal ischemia–reperfusion (IR) libraries (IR1–IR3; CRX330420–CRX330422). Single-cell transcriptomic data were analyzed in R (version 4.5.3) using Seurat (version 5.4.0). Filtered feature-barcode matrices generated by the Cell Ranger pipeline were imported into Seurat, and cells with 200–2,500 detected genes and < 5% mitochondrial transcripts were retained for subsequent analysis. Gene expression matrices were normalized using the LogNormalize method, and 2,000 highly variable genes were identified and scaled before principal component analysis (PCA). Batch effects among libraries were corrected using Harmony (version 1.2.4), with sample identity specified as the batch variable. The first 20 Harmony dimensions were used for graph-based clustering and Uniform Manifold Approximation and Projection (UMAP) visualization. Cluster stability across different resolutions was evaluated using clustree (version 0.5.1). Cell clusters were annotated based on cluster-specific marker genes, established cell-type markers, and published retinal single-cell transcriptomic studies. The resulting annotations were used for downstream cell-type-specific analyses.

10. Differential expression and gene set enrichment analyses

Microglia were extracted from the integrated dataset, and differential gene expression between the IR and control groups was analyzed using the Wilcoxon rank-sum test implemented in Seurat. *P* values were adjusted for multiple comparisons using the Benjamini–Hochberg method. Genes with $FDR < 0.05$ and $\log_2FC > 0.25$ were defined as upregulated in the model group, whereas genes with $FDR < 0.05$ and $\log_2FC < -0.25$ were defined as downregulated in the model group. Genes with $\log_2FC > 1$ and $FDR < 0.01$ were further classified as strongly upregulated, whereas genes with $\log_2FC < -1$ and $FDR < 0.01$

were classified as strongly downregulated. Gene set enrichment analysis (GSEA) was performed using genes ranked according to the log₂ fold change between IR and control microglia. Gene sets from Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG)^{54,55}, MSigDB Hallmark, and Reactome were included, and pathways with FDR < 0.05 were considered significantly enriched.

11. Protein–protein interaction (PPI) network analysis

Significantly upregulated genes in IR microglia were submitted to the STRING database to construct a protein–protein interaction (PPI) network. Network topology was analyzed in Cytoscape, and hub genes were identified according to node degree.

12. Transcription factor activity and regulatory network analysis

Transcription factor (TF) activity in microglia was inferred using the mouse DoRothEA regulatory network and decoupleR/VIPER, retaining TF–target interactions with confidence levels A–C. Differential TF activity between IR and control microglia was calculated, and TFs showing the largest activity changes were selected for regulatory network construction. TF–target networks were generated by retaining predicted target genes that were also differentially expressed between IR and control microglia.

13. Cell–cell communication analysis

Cell–cell communication networks were inferred separately for the control and IR groups using CellChat and the CellChatDB.mouse ligand–receptor database. Overexpressed ligands and receptors were identified, and communication probabilities for ligand–receptor interactions and signaling pathways were calculated and aggregated to generate cell–cell communication networks. Differences in the number and strength of interactions, outgoing and incoming signaling patterns, and pathway-level information flow between the two conditions were subsequently compared. Ligand–receptor interactions among macrophages, microglia, and retinal ganglion cells were further examined to characterize condition-specific communication patterns.

14. NicheNet ligand–target analysis

NicheNet was used to evaluate the regulatory potential of candidate microglial ligands on IR-associated transcriptional changes in microglia. Candidate ligands were defined based on ligands expressed by microglia and those showing enhanced communication in the CellChat analysis, whereas differentially expressed genes between IR and control microglia were used as the target gene set. Ligand activities and ligand–target regulatory potentials were inferred using the prior ligand–target model implemented in NicheNet.

Statistical analyses

Quantitative data are presented as the mean $\pm$ standard deviation (SD). Comparisons between two groups were performed using *t*-test. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). *P* value < 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, USA).

Declarations

Competing interests

The authors state that there are no personal or commercial conflicts of interest based on this study

Funding

The funding for this study was provided by Yiyang Central Hospital Institutional Youth Scientific Research Project (grant number: 2026QN06).

Author Contribution

Chen Wang performed most of the experiments, analyzed the data, and drafted the manuscript. Guanfeng Wang contributed to the experiments and data analysis. Hong Wang and Zhaohong Guo assisted with the experiments and data collection. Qingqing Yi conceived and designed the study, supervised the research, and revised the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgement

The authors acknowledge Kanehisa Laboratories for providing the KEGG pathway database used in this study.

Data Availability

All data supporting the conclusions of this manuscript are provided in the text and figures. For further information, please contact the authors for data requests.

References

1. Hirooka, K. & Kiuchi, Y. The Retinal Renin-Angiotensin-Aldosterone System: Implications for Glaucoma. *Antioxid. (Basel)*. **11**, 610. <https://doi.org/10.3390/antiox11040610> (2022).
2. Antonetti, D. A. et al. Diabetes Renders Photoreceptors Susceptible to Retinal Ischemia-Reperfusion Injury. *Invest. Ophthalmol. Vis. Sci.* **65**, 46. <https://doi.org/10.1167/iovs.65.13.46> (2024).
3. You, M. et al. Injectable, antioxidative, and loaded with exosomes/Liproxstatin-1 hydrogel as a potential treatment for retinal ischemia–reperfusion by inhibiting ferroptosis and apoptosis. *Chem. Eng. J.* **497**, 154509. <https://doi.org/10.1016/j.cej.2024.154509> (2024).
4. Belenichev, I. et al. Targeting Mitochondrial Dysfunction in Cerebral Ischemia: Advances in Pharmacological Interventions. *Antioxidants (Basel)* **14**, (1). (2025). <https://doi.org/10.1016/10.3390/antiox14010108>
5. Cheng, Y., Zhai, Y. J., Wang, S. S. & Fan, Y. Y. Proton-Sensitive Receptors/Channels: Therapeutic Target for Ischemic Stroke, Focusing on Their Roles and Mechanisms. *Curr Neuroparmacol.* <https://doi.org/> (2025).
6. Syu, S. H., Chang, C. Y., Shih, H. J. & Huang, C. J. Pyrroloquinoline Quinone Mitigates Testicular Injury and Reduces Oxidative Stress, Mitochondrial Dysfunction, and Apoptosis in Rats with Testicular Ischemia-Reperfusion Injury. *Antioxidants (Basel)* **14**, (11). <https://doi.org/> (2025).
7. Ansari, F. J. et al. Engineered ATP-loaded extracellular vesicles: a dual-functional strategy for improving myocardial infarction therapy. *Drug Deliv Transl Res.* <https://doi.org/> (2026).
8. Huang, Y. et al. Dual extra-retinal origins of microglia in the model of retinal microglia repopulation. *Cell Discov* **4**, 9. <https://doi.org/> (2018).
9. Lin, Y. et al. Unraveling the role of microglial activation and immune regulation in glaucomatous neurodegeneration: insights into its dynamic interactions with retinal cells. *VISUAL NEUROSCIENCE* **42**, 0–0. <https://doi.org/> (2025).
10. Péntek, L. et al. & Kovács-Öller, T. Eyes Are the Windows to the Soul: Reviewing the Possible Use of the Retina to Indicate Traumatic Brain Injury. *Int J Mol Sci* **26**, 5171. <https://doi.org/> (2025).
11. Xu, Z. et al. Microglial phagocytosis involves hyperoxia-induced vessel regression in the neonatal retina. *VISUAL NEUROSCIENCE* **42**, 0–0. <https://doi.org/> (2025).
12. Zhou, L. et al. Suppression of inner blood-retinal barrier breakdown and pathogenic Müller glia activation in ischemia retinopathy by myeloid cell depletion. *J Neuroinflammation* **21**, 210. <https://doi.org/> (2024).
13. Huang, Y. et al. Integrating single-cell and spatial transcriptomics to reveal the spatiotemporal dynamics of retinal cells during ischemia-reperfusion injury progression in rats. *Cell Biosci* **16**, 59. <https://doi.org/> (2026).
14. Wen, Y. et al. Acarbose modulates microglial Pkm2 acetylation to reshape immunometabolism and preserve retinal neurons after ischemia-reperfusion. *J. Neuroinflammation.* **23**, 206. <https://doi.org/10.1186/s12974-026-03838-8> (2026).
15. Kang, J. S. et al. Baf155 regulates skeletal muscle metabolism via HIF-1a signaling. *PLoS Biol.* **21**, e3002192. <https://doi.org/10.1371/journal.pbio.3002192> (2023).

16. Arias, C., Sepúlveda, P., Castillo, R. L. & Salazar, L. A. Relationship between Hypoxic and Immune Pathways Activation in the Progression of Neuroinflammation: Role of HIF-1 α and Th17 Cells. *Int. J. Mol. Sci.* **24**, 3073. <https://doi.org/10.3390/ijms24043073> (2023).
17. Bakleh, M. Z. & Al Haj Zen, A. The Distinct Role of HIF-1 α and HIF-2 α in Hypoxia and Angiogenesis. *Cells* **14**, 673. (2025). <https://doi.org/10.3390/cells14090673>
18. Cheng, L. et al. Neuropeptide VF promotes hypoxia-inducible factor-1 α expression in age-related macular degeneration via the aldehyde dehydrogenase 2/D-glycerate axis. *Vis. Neurosci.* **42**, 0–0. <https://doi.org/10.48130/vns-0025-0023> (2025).
19. Ye, Y. et al. In vitro study: HIF-1 α -dependent glycolysis enhances NETosis in hypoxic conditions. *Front. Immunol.* **16**, 1583587. <https://doi.org/10.3389/fimmu.2025.1583587> (2025).
20. Miska, J. et al. HIF-1 α Is a Metabolic Switch between Glycolytic-Driven Migration and Oxidative Phosphorylation-Driven Immunosuppression of Tregs in Glioblastoma. *Cell. Rep.* **27**, 226–237e4. <https://doi.org/10.1016/j.celrep.2019.03.029> (2019).
21. Sung, M. S. et al. HDAC2 Regulates Glial Cell Activation in Ischemic Mouse Retina. *Int. J. Mol. Sci.* **20**, 5159. <https://doi.org/10.3390/ijms20205159> (2019).
22. Pei, W. B. et al. A novel photosensitizer berberine derivative B12 induces apoptosis and suppresses HIF-1 α expression in colorectal cancer cells via mitochondria-to-nucleus sequential-targeting. *Acta Pharmacol. Sin.* **47**, 1249–1269. <https://doi.org/10.1038/s41401-025-01728-y> (2027).
23. Yu, L. et al. Synthesis and evaluation of acetylferulic paeonol ester and ferulic paeonol ester as potential antioxidants to inhibit fish oil oxidation. *Food Chem.* **365**, 130384. <https://doi.org/10.1016/j.foodchem.2021.130384> (2021).
24. Huang, S. et al. Development of Paeonol Liposomes: Design, Optimization, in vitro and in vivo Evaluation. *Int. J. Nanomed.* **17**, 5027–5046. <https://doi.org/10.2147/IJN.S363135> (2022).
25. Gong, S., Lang, S., Jiang, X. & Li, X. Paeonol ameliorates ferroptosis and inflammation in chondrocytes through AMPK/Nrf2/GPX4 pathway. *Front. Pharmacol.* **16**, 1526623. <https://doi.org/10.3389/fphar.2025.1526623> (2025).
26. Morsy, M. A. et al. Paeonol Attenuates Hepatic Ischemia/Reperfusion Injury by Modulating the Nrf2/HO-1 and TLR4/MYD88/NF- κ B Signaling Pathways. *Antioxid. (Basel)*. **11** (9). <https://doi.org/10.3390/antiox11091687> (2022).
27. Liu, S. et al. Paeonol ameliorates endometrial hyperplasia in mice via inhibiting PI3K/AKT pathway-related ferroptosis. *Phytomedicine* **109**, 154593. <https://doi.org/10.1016/j.phymed.2022.154593> (2023).
28. Zhang, N. et al. Paeonol prevents sepsis-associated encephalopathy via regulating the HIF1A pathway in microglia. *Int. Immunopharmacol.* **143**, 113287. <https://doi.org/10.1016/j.intimp.2024.113287> (2024).
29. Huang, H. et al. Maintaining blood retinal barrier homeostasis to attenuate retinal ischemia-reperfusion injury by targeting the KEAP1/NRF2/ARE pathway with lycopene. *Cell. Signal.* **88**, 110153. <https://doi.org/10.1016/j.cellsig.2021.110153> (2021).

30. Li, Y. et al. Single-cell RNA sequencing reveals a landscape and targeted treatment of ferroptosis in retinal ischemia/reperfusion injury. *J. Neuroinflammation*. **19**, 261. <https://doi.org/10.1186/s12974-022-02621-9> (2022).
31. Huang, Y. et al. Retinal cell-targeted liposomal ginsenoside Rg3 attenuates retinal ischemia-reperfusion injury via alleviating oxidative stress and promoting microglia/macrophage M2 polarization. *Free Radic Biol. Med.* **206**, 162–179. <https://doi.org/10.1016/j.freeradbiomed.2023.06.024> (2023).
32. Chai, P., Zhao, F., Jia, R., Zhou, X. & Fan, X. Lactate/lactylation in ocular development and diseases. *Trends Mol. Med.* **31**, 1073–1076. <https://doi.org/10.1016/j.molmed.2024.07.001> (2025).
33. He, S., Liu, C., Ren, C., Zhao, H. & Zhang, X. Immunological Landscape of Retinal Ischemia-Reperfusion Injury: Insights into Resident and Peripheral Immune Cell Responses. *Aging Dis.* **16**, 115–136. <https://doi.org/10.14336/AD.2024.0129> (2024).
34. Hu, W. et al. miR-16-5p Protects RGCs Against Retinal Ischemia-Reperfusion Injury by Modulating Astrocyte-Mediated Neuroinflammation Through the Wip1/NF-κB Signaling Axis. *Mol. Neurobiol.* **63** <https://doi.org/10.1007/s12035-026-05944-3> (2026). 645 [pii].
35. Yuan, J. et al. Leonurine alleviates retinal ischemia-reperfusion injury by inhibiting microglia activation. *Toxicol. Appl. Pharmacol.* **506**, 117616. <https://doi.org/10.1016/j.taap.2025.117616> (2026).
36. Zeng, S., Du, L., Lu, G. & Xing, Y. C. R. E. G. Protects Retinal Ganglion Cells loss and Retinal Function Impairment Against ischemia-reperfusion Injury in mice via Akt Signaling Pathway. *Mol. Neurobiol.* **60**, 6018–6028. <https://doi.org/10.1007/s12035-023-03466-w> (2023).
37. Feng, J. et al. Resveratrol Ameliorates Retinal Ischemia-Reperfusion Injury by Modulating the NLRP3 Inflammasome and Keap1/Nrf2/HO-1 Signaling Pathway. *Mol. Neurobiol.* **61**, 8454–8466. <https://doi.org/10.1007/s12035-024-04105-8> (2024).
38. Jobling, A. I. et al. Microglial regulation of the retinal vasculature in health and during the pathology associated with diabetes. *Prog Retin Eye Res.* **101349** <https://doi.org/10.1016/j.preteyeres.2025.101349> (2025).
39. Hirayama, Y. & Koizumi, S. Hypoxia-independent mechanisms of HIF-1α expression in astrocytes after ischemic preconditioning. *Glia* **65**, 523–530. <https://doi.org/10.1002/glia.23109> (2017).
40. Belapurkar, R. et al. A transient increase of HIF-1α during the G1 phase (G1-HIF) ensures cell survival under nutritional stress. *Cell. Death Dis.* **14**, 477. <https://doi.org/10.1038/s41419-023-06012-7> (2023).
41. Su, Y. et al. Photocatalytic oxygen evolution and antibacterial biomimetic repair membrane for diabetes wound repair via HIF1-α pathway. *Mater. Today Bio.* **20**, 100616. <https://doi.org/10.1016/j.mtbio.2023.100616> (2023).
42. Kim, G. D., Ng, H. P., Chan, E. R. & Mahabeleshwar, G. H. Kruppel-like factor 6 promotes macrophage inflammatory and hypoxia response. *FASEB J.* **34**, 3209–3223. <https://doi.org/10.1096/fj.201902221R> (2020).

43. Wang, Z. et al. Polystyrene nanoplastics reprogrammed pulmonary metabolisms mediated by immune regulation of myeloid hypoxia-inducible factor 1 α . *Environ. Int.* **203**, 109777. <https://doi.org/10.1016/j.envint.2025.109777> (2025).
44. Gong, L. et al. Description of a Nonhuman Primate Model of Retinal Ischemia/Reperfusion Injury. *Transl Vis. Sci. Technol.* **12**, 14. <https://doi.org/10.1167/tvst.12.6.14> (2023).
45. Li, L. et al. Single-Cell Transcriptomic Sequencing Reveals Tissue Architecture and Deciphers Pathological Reprogramming During Retinal Ischemia in *Macaca fascicularis*. *Invest. Ophthalmol. Vis. Sci.* **65**, 27. <https://doi.org/10.1167/iovs.65.1.27> (2024).
46. Zhang, L. et al. CD74 is a functional MIF receptor on activated CD4(+) T cells. *Cell. Mol. Life Sci.* **81**, 296. <https://doi.org/10.1007/s00018-024-05338-5> (2024).
47. Bandola-Simon, J. & Roche, P. A. MHC Class II and Beyond: Complex Role of CD74 in Cancer. *Cells* **15**, 128. <https://doi.org/10.3390/cells15020128> (2026).
48. Doherty, E. et al. Pathogenic role of MIF receptor (CD74) expressing T cells in inflammatory arthritis. *Proc. Natl. Acad. Sci. U S A.* **123**, e2509156123. <https://doi.org/10.1073/pnas.2509156123> (2026).
49. Cao, C. et al. Targeting CD74 in microglia to modulate experimental cerebral ischemia and reperfusion injury: insights from Single-Cell and bulk transcriptomics. *Mol. Brain.* **18**, 46. <https://doi.org/10.1186/s13041-025-01197-8> (2025).
50. Zhang, F. et al. MIF regulates T helper 17 cell differentiation by activating the p38 MAPK signaling pathway to drive the pathogenesis of EAP. *Int. Immunopharmacol.* **159**, 114959. <https://doi.org/10.1016/j.intimp.2025.114959> (2025).
51. Cheng, W. et al. Single-cell RNA Sequencing Identifies a Novel Subtype of Microglia with High Cd74 Expression that Facilitates White Matter Inflammation During Chronic Cerebral Hypoperfusion. *Neurochem Res.* **49**, 2821–2841. <https://doi.org/10.1007/s11064-024-04206-9> (2024).
52. Wang, G. N., Liu, S. & Xu, W. Meis1-driven suppression of MIF/CD74 signaling promotes M2 macrophage polarization and protects against inflammatory damage in experimental acute lung injury. *Cell. Biol. Toxicol.* **42** (1). <https://doi.org/10.1007/s10565-026-10166-3> (2026).
53. Parkins, A., Skeens, E., McCallum, C. M., Lisi, G. P. & Pantouris, G. The N-terminus of MIF regulates the dynamic profile of residues involved in CD74 activation. *Biophys. J.* **120**, 3893–3900. <https://doi.org/10.1016/j.bpj.2021.08.025> (2021).
54. Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y. & Ishiguro-Watanabe, M. KEGG: biological systems database as a model of the real world. *Nucleic Acids Res.* **53** <https://doi.org/10.1093/nar/gkae909> (2024). D672-D677.
55. Kanehisa, M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci.* **28**, 1947–1951. <https://doi.org/10.1002/pro.3715> (2019).

Figures

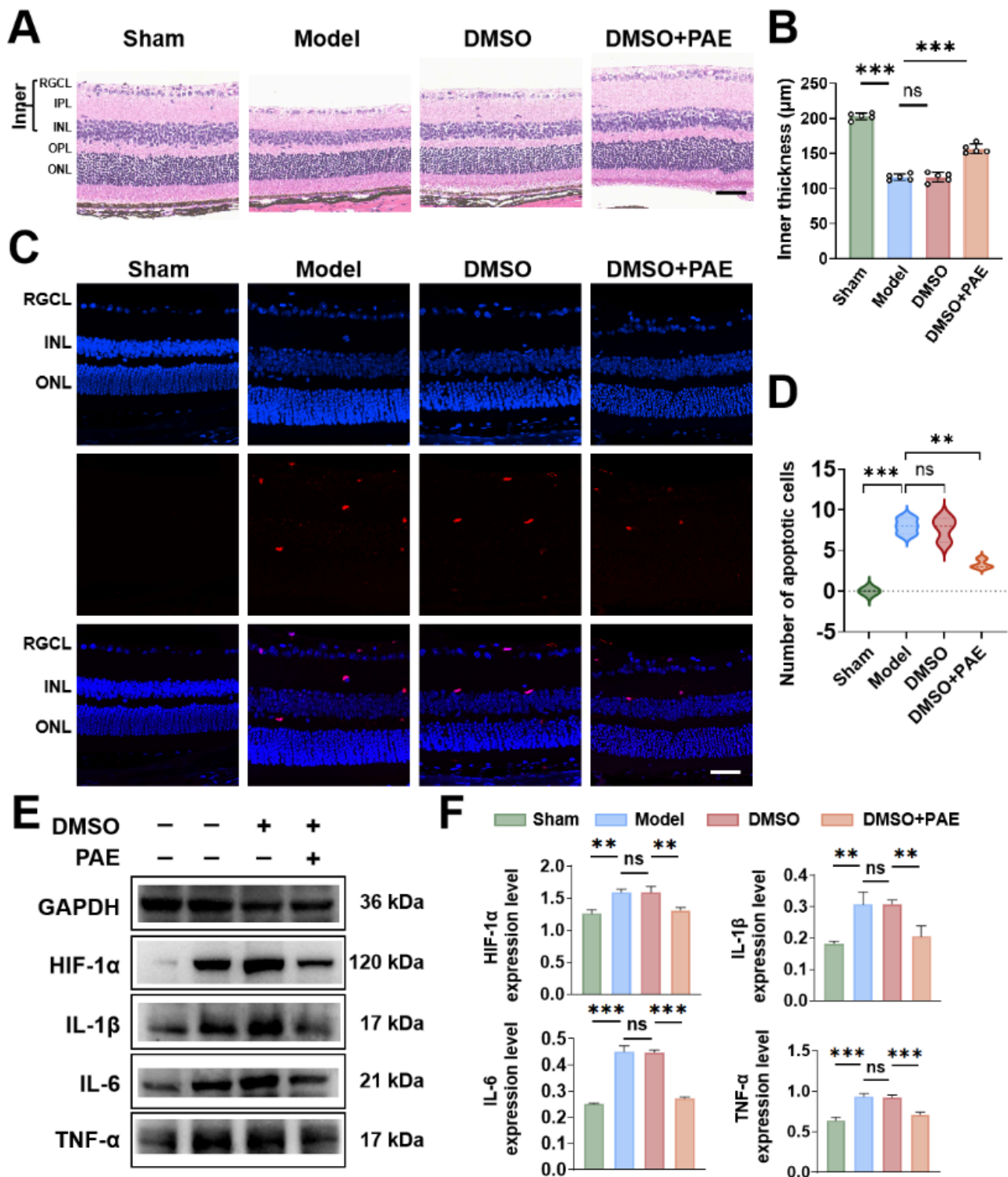


Figure 1

PAE attenuates retinal ischemia–reperfusion injury and inflammatory responses. A mouse model of retinal IR injury was established by acute elevation of intraocular pressure. PAE (10 mg/kg) was administered intraperitoneally once daily on the day of IR induction and for three consecutive days thereafter, and retinal tissues were collected on day 3 after IR. Mice were divided into the Sham, IR, DMSO, and DMSO + PAE groups. **(A)** Representative hematoxylin and eosin (H&E)-stained retinal

sections showing the retinal ganglion cell layer (RGCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), and outer nuclear layer (ONL). **(B)** Quantification of inner retinal thickness. **(C)** Representative TUNEL-stained retinal sections. Nuclei were counterstained with DAPI (blue), and TUNEL-positive signals are shown in red. **(D)** Quantification of the number of TUNEL-positive cells per field. **(E)** Western blot analysis of HIF-1 α , IL-1 β , IL-6, and TNF- α protein expression in retinal tissues, with GAPDH used as the loading control. **(F)**Quantification of the relative protein expression levels. **P < 0.01, ***P < 0.001; ns, not significant.

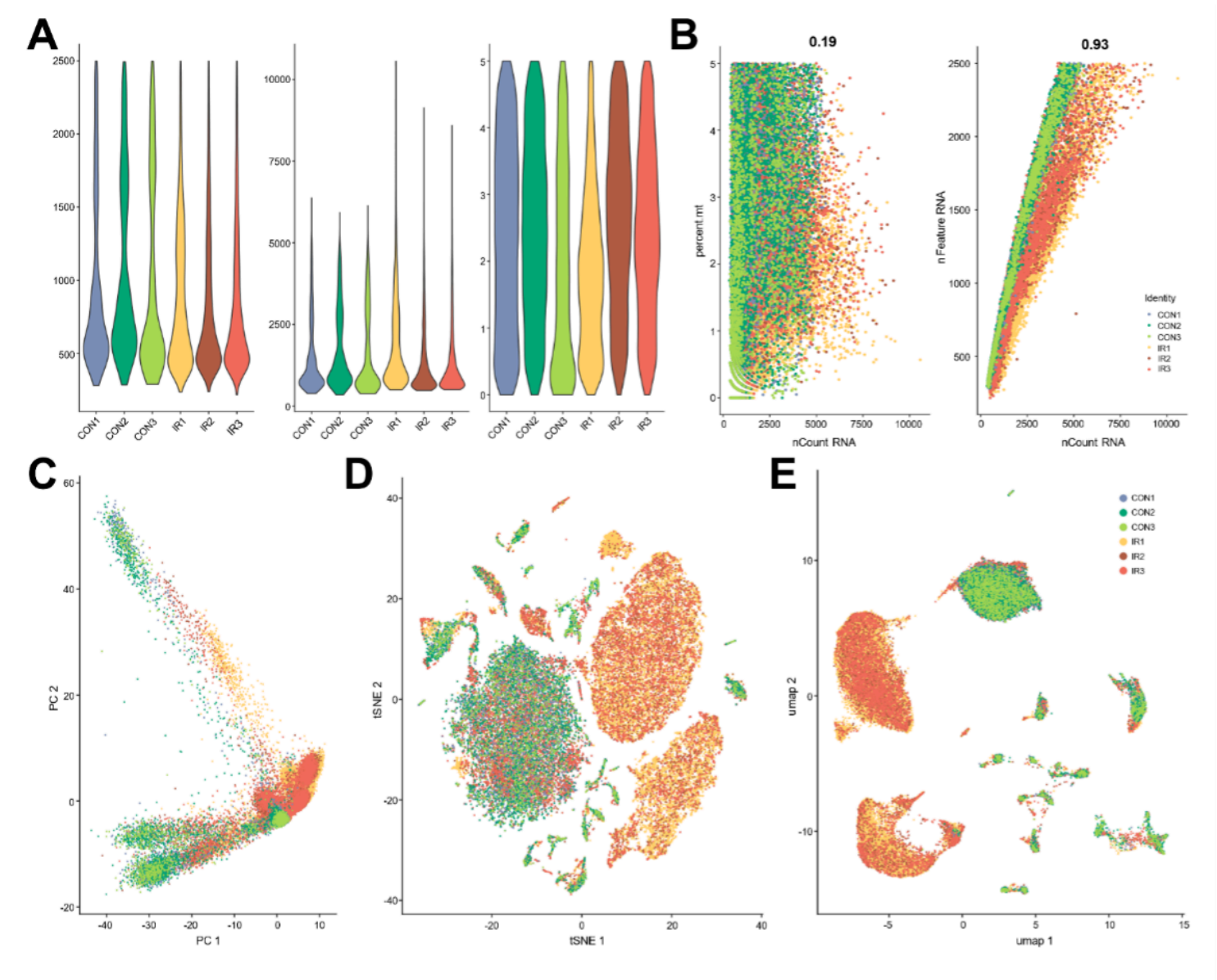


Figure 2

Quality assessment, principal component analysis, and nonlinear dimensionality reduction of retinal single-cell transcriptomic data. (A) Violin plots showing nFeature_RNA, nCount_RNA, and percent.mt in three Control samples and three IR samples. (B) Correlation analysis of nCount_RNA with percent.mt and nFeature_RNA. Values indicate correlation coefficients. (C) Cell-level PCA based on highly variable

genes, with colors representing individual biological samples. (D, E) t-SNE and UMAP representations of all cells after data integration, with colors indicating individual samples.

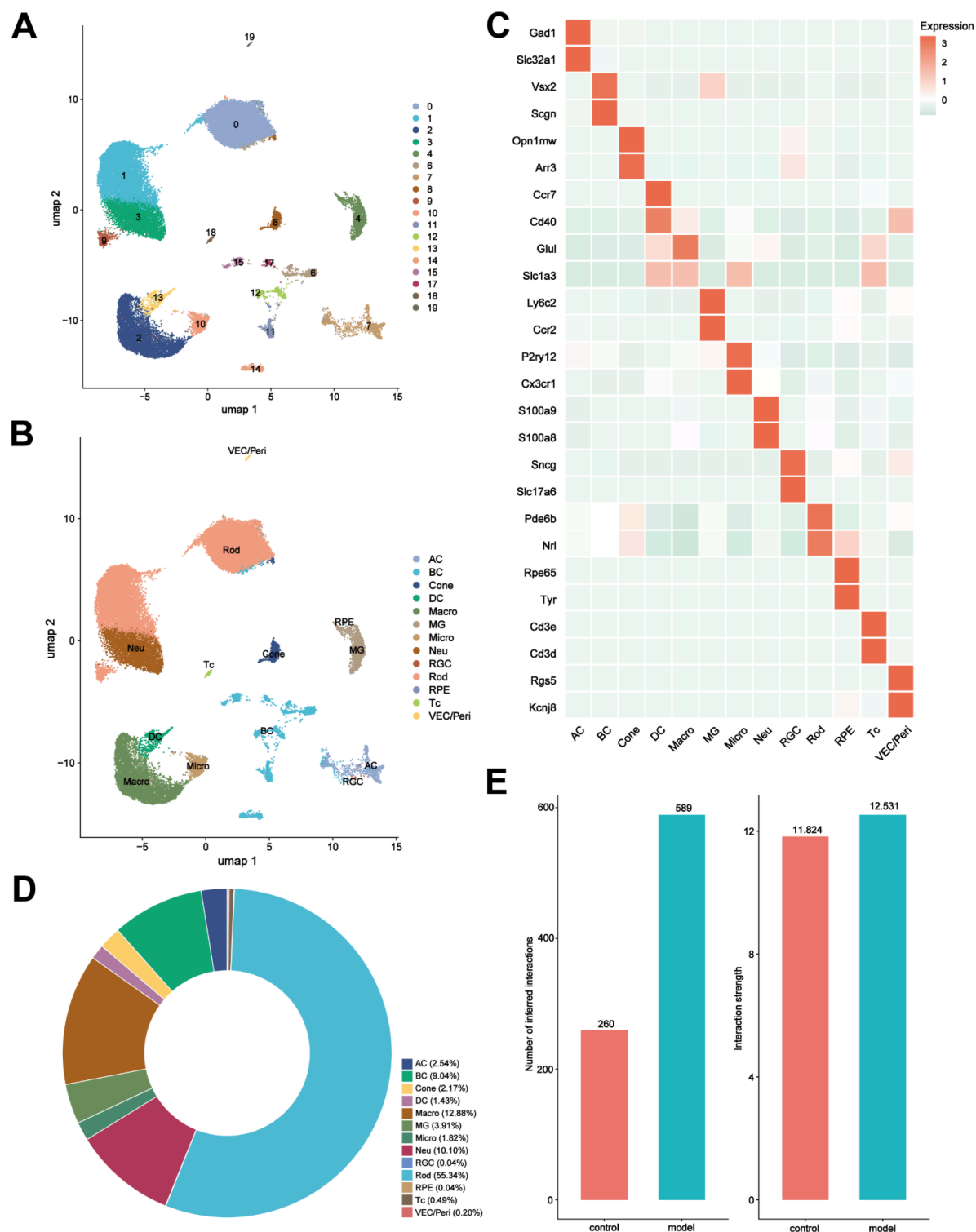


Figure 3

Identification of major retinal cell types and global changes in cell–cell communication following IR.

(A) UMAP visualization of unsupervised clustering of integrated retinal single-cell data, with colors and

numbers indicating individual cell clusters. (B) UMAP visualization of annotated retinal cell types, including amacrine cells (AC), bipolar cells (BC), cones (Cone), dendritic cells (DC), macroglia (Macro), macrophages (MG), microglia (Micro), neutrophils (Neu), retinal ganglion cells (RGC), rods (Rod), retinal pigment epithelial cells (RPE), T cells (Tc), and vascular endothelial cells/pericytes (VEC/Peri). (C) Heatmap showing the relative expression of representative marker genes across retinal cell types. (D) Overall proportions of different cell types in the integrated dataset. (E) Number of inferred significant ligand–receptor interactions and overall communication strength in the Control and IR groups.

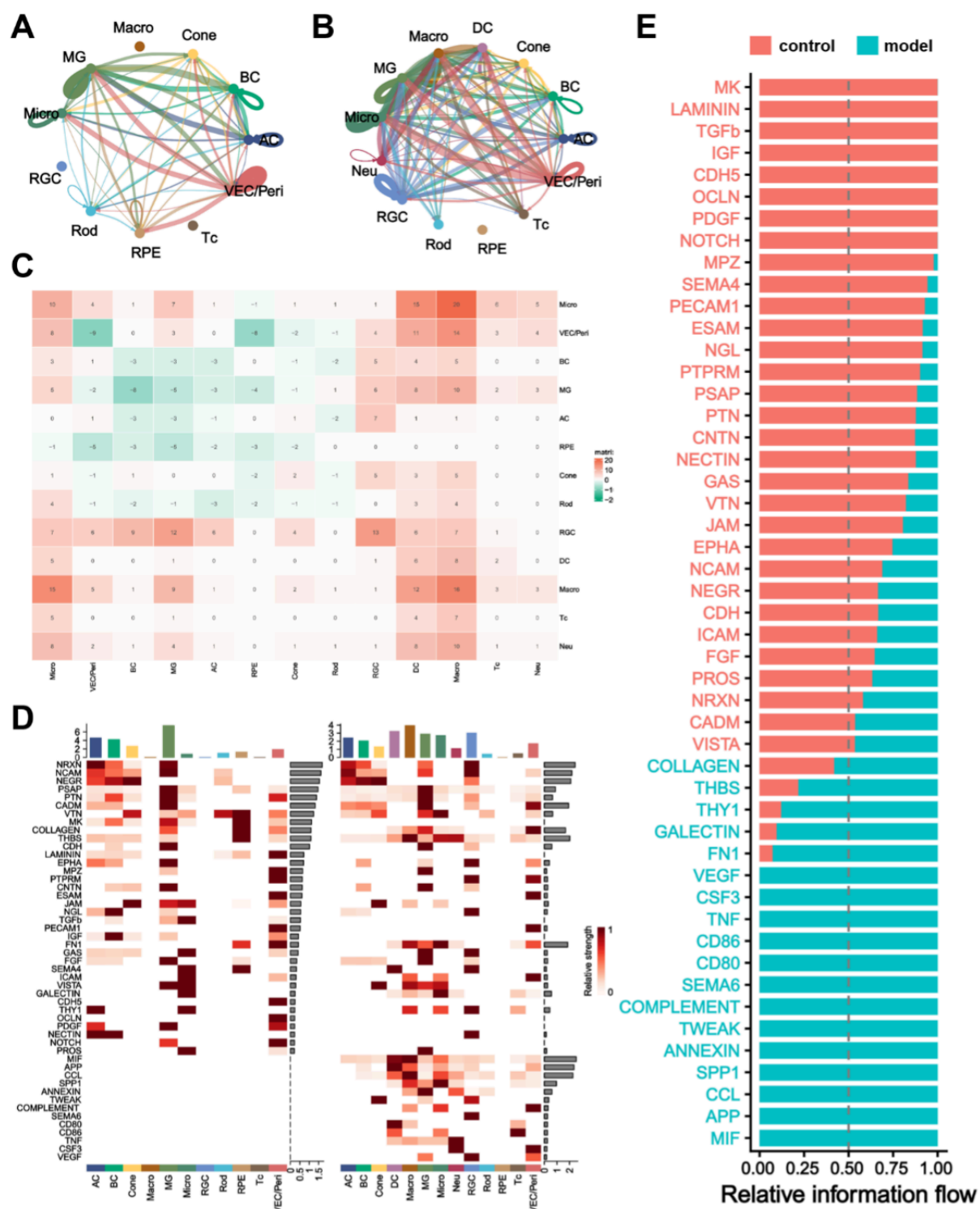


Figure 4

Remodeling of retinal cell–cell communication following IR injury. (A, B) Inferred cell–cell communication networks in the Control (A) and IR (B) groups. Edge width indicates the number of inferred significant ligand–receptor interactions. (C) Heatmap showing differences in the number of inferred interactions between the IR and Control groups (IR minus Control), with rows and columns representing sender and receiver cells, respectively. (D) Heatmaps showing overall signaling patterns across retinal cell types in the Control and IR groups. The top and right bar plots indicate the overall signaling activity of each cell type and the overall contribution of each signaling pathway, respectively. (E) Comparison of relative information flow across signaling pathways between the Control and IR groups. Information flow was calculated from the aggregated communication probabilities of all significant ligand–receptor interactions within each signaling pathway.

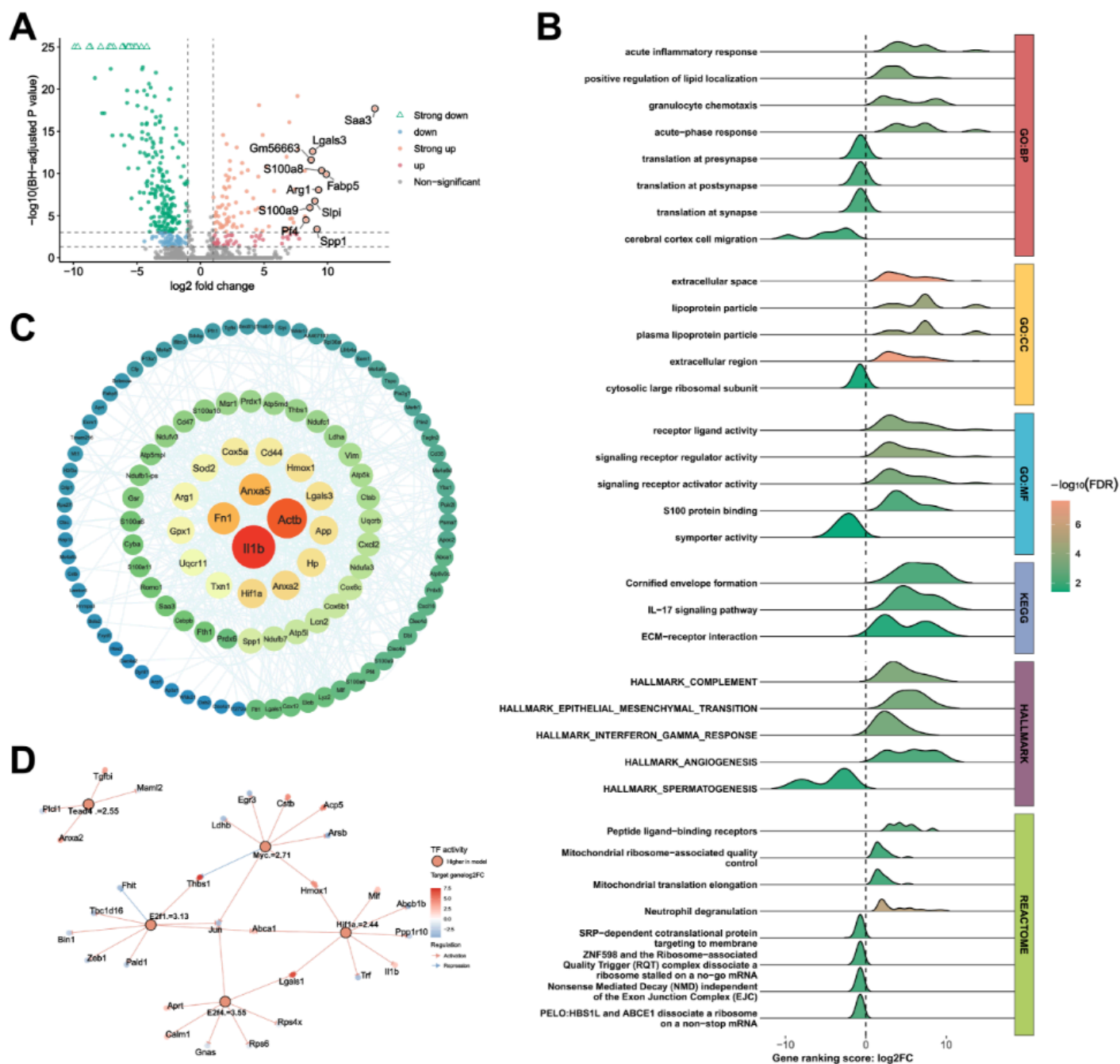


Figure 6

HIF-1α/MIF is associated with IL1β-centered inflammatory transcriptional reprogramming in microglia.

(A) Volcano plot of differentially expressed genes in microglia between the Control and IR groups. (B) GSEA based on genes ranked by log2 fold change between the IR and Control groups. (C) PPI network of genes upregulated in IR microglia. Node size and color represent degree, and edges indicate known or predicted protein associations. (D) TF–target regulatory network in microglia. Large nodes with black borders represent transcription factors with increased inferred activity in the IR group, and small nodes

represent differentially expressed target genes. Red and blue arrows indicate activating and inhibitory regulatory relationships, respectively, based on DoRothEA regulons with confidence levels A–C.

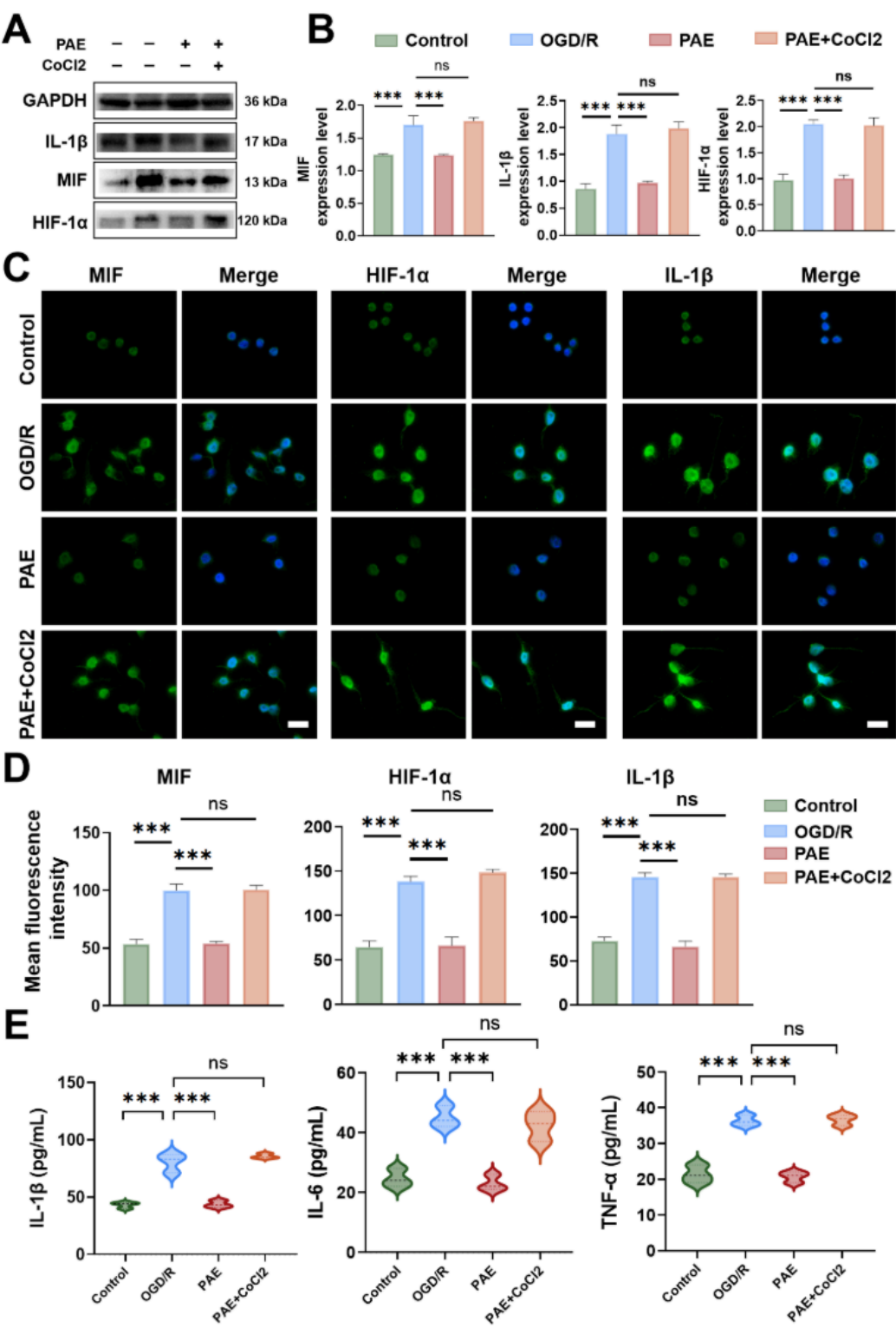


Figure 7

PAE suppresses HIF-1α and reduces MIF and inflammatory factor expression in BV2. BV2 cells were divided into Control, OGD/R, OGD/R + PAE, and OGD/R + PAE + CoCl₂ groups. OGD/R consisted of 3 h of

oxygen–glucose deprivation followed by 24 h of reoxygenation. Cells were pretreated with PAE (10 μ M) for 0.5 h before OGD and continuously exposed to PAE during OGD/R, with CoCl_2 (100 μ M) added where indicated. (A) Representative Western blots of HIF-1 α , MIF, and IL-1 β , with GAPDH as the loading control. (B) Quantification of relative protein expression. (C) Representative immunofluorescence images of HIF-1 α , MIF, and IL-1 β . Green, target proteins; blue, DAPI. Scale bar = 100 μ m. (D) Quantification of mean fluorescence intensity. (E) ELISA analysis of IL-1 β , IL-6, and TNF- α concentrations in culture supernatants. ***P < 0.001; ns, not significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure.V3.docx](#)