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Targeting IDO1 Suppresses RPE Ferroptosis and NLRP3 Inflammasome Activation and Ameliorates NaIO₃-Induced dry age-related macular degeneration

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Abstract:

Background:

Dry age-related macular degeneration (AMD) is a common chronic degenerative ocular disease for which no effective disease-modifying therapeutic options to date. Indoleamine 2,3-dioxygenase 1 (IDO1) has been reported to reshape cellular redox and metabolic states through ferroptosis; however, its potential involvement in the pathogenesis of dry AMD remains largely unexplored.

Methods:

Bioinformatic analysis identified IDO1 as a differentially expressed gene associated with dry AMD. IDO1 expression in retinal pigment epithelial (RPE) cells was silenced using siRNA or inhibited by the selective IDO1 inhibitor Epacadostat to evaluate ferroptosis. Transcriptome sequencing was performed to identify downstream signaling pathways, and NLRP3 overexpression was used for rescue experiments to verify the causal relationship. In vivo, a NaIO₃-induced retinal degeneration model was established, and Epacadostat was intravitreally injected. Retinal morphology (H&E staining, OCT, fundus photography, and autofluorescence) and function (ERG) were quantitatively assessed.

Results:

Both genetic and pharmacological inhibition of IDO1 significantly alleviated ferroptotic phenotypes and suppressed NLRP3 inflammasome activation in RPE cells. Overexpression of NLRP3 partially reversed the protective effects of IDO1 inhibition, suggesting that NLRP3 acts downstream of IDO1-dependent ferroptosis. In the NaIO₃-induced mouse model, Epacadostat treatment preserved retinal structure and improved retinal function.

Conclusions:

Targeting IDO1 effectively suppresses ferroptosis and inflammasome activation in RPE cells, providing dual structural and functional protection to the retina. These findings highlight IDO1 as a potential therapeutic target for dry AMD.

Keywords: dry AMD; IDO1; ferroptosis; GPX4; NLRP3 inflammasome.

Introduction:

Age-related macular degeneration (AMD) is a chronic, progressive retinal disorder that predominantly affects older adults and primarily involves the macula, leading to gradual loss of central vision. Extracellular deposits (drusen) between the retinal pigment epithelium (RPE) and Bruch's membrane, progressive RPE and photoreceptor dysfunction, and—at advanced stages—geographic atrophy (GA) are key pathological hallmarks[1, 2]. Clinically, AMD is classified into neovascular (“wet”) and non-neovascular (“dry”) forms, with dry AMD accounting for ~85–90% of cases. In 2023, the U.S. Food and Drug Administration approved pegcetacoplan and avacincaptad pegol for GA; both inhibit the complement cascade and slow atrophic lesion enlargement, yet neither has demonstrated a statistically significant improvement in visual function[3-5]. These limitations underscore the need to delineate upstream pathogenic mechanisms and identify new therapeutic targets for dry AMD.

Iron is essential for vital cellular functions, including oxygen transport and DNA synthesis. Nevertheless, an excess of labile Fe^{2+} can catalyze the Fenton reaction, wherein Fe^{2+} interacts with hydrogen peroxide (H_2O_2) to produce highly reactive hydroxyl radicals. This reaction leads to the peroxidation of polyunsaturated fatty acids (PUFAs) and the subsequent accumulation of lipid hydroperoxides (PUFA-OOH). These processes culminate in a regulated form of cell death known as ferroptosis, which is distinct from apoptosis and necrosis. [6, 7]. Mounting evidence implicates ferroptosis as a driver of RPE dysfunction: oxidative stress perturbs iron homeostasis, accelerates ferritin degradation, and expands the labile iron pool, thereby amplifying lipid peroxidation. Once engaged, ferroptosis propagates a feed-forward loop in which lipid peroxides and mitochondrial damage further escalate reactive oxygen species (ROS). Compromise of antioxidant defenses—most notably glutathione peroxidase-4 (GPX4)—sensitizes RPE cells to ferroptotic injury. In turn, RPE failure disrupts photoreceptor trophic support, weakens Bruch's membrane barrier function, triggers local inflammation, and promotes extracellular matrix remodeling, collectively accelerating dry AMD pathology[8-10]. These observations position the oxidative stress–ferroptosis axis as a compelling target space for RPE-protective interventions.

Indoleamine 2,3-dioxygenase 1 (IDO1) is a heme-containing, rate-limiting enzyme of the kynurenine pathway whose expression is inducible by pro-inflammatory cues and often elevated in chronic inflammation, infection, cancer, and aging[11]. Beyond immune regulation, accumulating evidence indicates that IDO1 activity can reshape cellular redox and metabolic states in ways that may intersect with ferroptosis. Specifically, flux through the kynurenine pathway yields metabolites such as kynurenine (KYN) and quinolinic acid (QUIN) with reported effects on redox balance and iron handling; sustained IDO1 activation has also been associated with glutathione (GSH) depletion and reduced antioxidant capacity, as well as perturbations in NAD^+ metabolism and mitochondrial function that can increase reactive oxygen species (ROS) [12-15]. Within the chronically inflamed retina, these immunometabolic shifts could plausibly sensitize RPE cells to ferroptotic stress and exacerbate oxidative injury. While the extent and context-dependence of these interactions remain to be defined, the putative IDO1–ferroptosis link offers a testable framework for investigating new therapeutic targets in dry AMD.

Methods

1. Animal model

All animal procedures were carried out following the guidelines of the Animal Care and Use Committee of The First Affiliated Hospital of Gannan Medical University, and handled according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. C57BL/6 mice (aged 6–8 weeks, weight = 20 ± 2 g) were purchased from the Charles River (Weitong Lihua Co. Ltd.). Animals were randomly divided into three groups: Control, Sodium iodate (NaIO_3)-induced retinal denervation, NaIO_3 plus Epacadostat treatment. NaIO_3 was administered via bolus intraperitoneal injection at 35 mg/kg. Following anesthesia, Epacadostat was delivered by intravitreal injection to the right eye on days 1 and 3 after NaIO_3 exposure (1 μL stock solution per eye), with PBS-diluted DMSO used as the vehicle control.

2. Western blot (WB)

Homogenized mouse retina–choroid tissues or RPE cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (Beyotime) supplemented with a protease/phosphatase inhibitor cocktail (Selleck) at 4 °C on a rocking platform (100 rpm) for 10 min. Protein concentration was measured using the BCA assay (Thermo Fisher Scientific). The protein samples were separated by 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked with 5% non-fat milk and then incubated with primary antibody at 4°C overnight. The next day, the membranes were incubated with HRP-conjugated secondary antibodies and proteins were detected by chemiluminescence.

Primary antibody: IDO1 (#13268-1-AP, 1:1000, proteintech), GPX4(#T56959, 1:1000, Abmart), NLRP3 (#T55651, 1:1000, Abmart), caspase-1 (#P79884-2R, 1:1000, Abmart), IL-1 β (#TA5103, 1:1000, Abmart), GAPDH (#8884, 1:5000, CST). Bands of WB were quantified using Image J.

3. Quantitative Real-Time PCR(qRT-PCR)

Total RNA was extracted from tissues or cultured cells using the EASYspin Plus kit (Aidlab). First-strand complementary DNA (cDNA) was synthesized with a reverse transcription kit (Vazyme, Nanjing, China). Quantitative PCR was performed using SYBR Green qPCR Master Mix (Vazyme) on a Bioer real-time PCR system. Relative mRNA levels were calculated by the $2^{-\Delta\Delta\text{Ct}}$ method with GAPDH as the internal reference. Primer sequences are provided in Table S1 (Supporting Information).

4. Cell Culture

Human RPE cells (ARPE-19; ATCC) were cultured in DMEM/F-12 (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% 100 U/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin (Sigma-Aldrich) and maintained at 37°C in a 5% CO_2 atmosphere. Silibinin (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO).

5. Cell Counting Kit-8 (CCK-8) Assay

Cells were seeded in 96-well plates at a density of 10^4 cells per well. After exposure to H_2O_2 for 24 hr, cell viability was assessed using Cell Counting Kit-8 (CCK-8) (#ab228554, Abcam). Absorbance at 450 nm was measured using a Sunrise spectrophotometer (Tecan).

6. siRNA transfection

IDO1-specific siRNA was obtained from Haixing Biosciences (Shanghai, China). The siRNA construct and Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific) were combined in reduced-serum Opti-MEM medium (Thermo Fisher Scientific) according to the manufacturer's protocol. After 6 hr of incubation, the medium was replaced with fresh DMEM/F12 with 10 % FBS, and the cells were further cultured for 48 hr.

7. Plasmids and cell transfection

Nlrp3 overexpression plasmids (pcDNA 3.1- Nlrp3) were designed and constructed by Genechem Company (Shanghai, China). Empty pcDNA 3.1 plasmids were used as the negative control. When RPE reached approximately 70–80% confluence, the cells were transfected with different plasmids using RNAiMAX Transfection Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Transfection efficacy was assessed by WB and qRT-PCR.

8. Hoechst 33342/PI assay

Cells were incubated at room temperature for 25 min with a mixed solution of Hoechst 33342 and propidium iodide (# P0137, Beyotime) according to the manufacturer's instructions, and images were acquired using a fluorescence microscope.

9. Wound Healing Assay

ARPE-19 cells were seeded in 35 mm culture dishes. When the cell confluence reached approximately 80%, a scratch was created using a 200 μ L plastic pipette tip. Images of the wound area were captured at 0 and 24 hours using an optical microscope. The wound width was quantified using ImageJ software.

10. Malondialdehyde(MDA) Assay

According to the manufacturer's instructions, a lipid peroxidation MDA assay kit (#S0131S, Beyotime) was used to determine the MDA levels in retinal–choroidal homogenates and cultured cells.

11. ROS Detection

Cells were washed and incubated with 2',7'-dichlorodihydrofluorescein diacetate (2.5 μ M dissolved in phenol red-free medium) (#S0033S, Beyotime) at 37 °C in the dark for 30 min. Fluorescent images were captured using a BX53F microscope (Olympus, Tokyo, Japan); the mean fluorescence intensity was quantified using ImageJ software.

12. Transmission Electron Microscopy (TEM).

Cells were fixed in 2.5% glutaraldehyde (0.1 M cacodylate, pH 7.4; 45–60 min, RT), post-fixed in 1% OsO₄ (30–60 min, 4 °C), dehydrated in graded ethanol, transitioned via propylene oxide, and embedded in epoxy resin. Ultrathin sections (60–80 nm) were contrasted with uranyl acetate and lead citrate and imaged on a (hitachi, Japan) at 80–120 kV.

13. RNA Sequencing and Transcriptome Analysis

Total RNA was isolated with TRIzol (Invitrogen) and quantified on a NanoDrop ND-1000; integrity was confirmed on an Agilent 2100 Bioanalyzer (RIN > 7.0) and by denaturing agarose gel electrophoresis. From 1 μ g total RNA, poly(A)⁺ RNA was enriched with Dynabeads Oligo(dT) (two rounds) and fragmented at 94 °C for 5–7 min (Mg²⁺ fragmentation). First-strand cDNA was synthesized with SuperScript II, and U-labeled second strands were generated using E. coli DNA polymerase I and RNase H in the presence of dUTP. After end repair/A-tailing, indexed adapters carrying T-overhangs were ligated, fragments were size-selected with AMPure XP beads, UDG-treated, and PCR-amplified (95 °C 3 min; 8 cycles of 98 °C 15 s, 60 °C 15 s, 72 °C 30 s; final 72 °C 5 min). Libraries with an average insert size of $\sim 300 \pm 50$ bp were sequenced as 2 $\times$ 150 bp paired-end reads on an Illumina NovaSeq 6000. Raw reads were filtered with fastp to remove adapter contamination, low-quality bases, and undetermined nucleotides, and quality metrics were re-checked with fastp. Clean reads were aligned to the Homo sapiens GRCh38 reference genome using HISAT2. Per-sample assemblies were generated with StringTie, merged with gffcompare to obtain a unified transcriptome, and expression levels (FPKM) were estimated with StringTie. Differentially

expressed mRNAs were identified with edgeR using fold change > 2 or < 0.5 and $p < 0.05$.

14. Enzyme-Linked Immunosorbent Assay (ELISA)

The concentrations of IL-1 β (#PI305, Beyotime) and caspase-1 (#RK01035, Abclonal) in the culture medium were determined using ELISA kits according to the manufacturers' instructions. As described above, absorbance at 450 nm was measured using a Sunrise microplate reader.

15. Cell Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.1% Triton X-100 for 10 min, and blocked with 2% bovine serum albumin for 30 min. Samples were incubated with primary antibodies overnight at 4 °C, washed, and then incubated with an Alexa Fluor 594–conjugated goat anti-rabbit IgG secondary antibody (#SA00013-4, Proteintech) for 1 h. Nuclei were counterstained with DAPI (#62248, Thermo Fisher Scientific). Primary antibodies included NLRP3 (#T55651, 1:200, Abmart), and IDO1 (#13268-1-AP, 1:100, proteintech). Fluorescence microscopy was performed as described above, and images were analyzed using ImageJ.

16. Hematoxylin–Eosin (HE) Staining and Immunofluorescence

Fixed eye tissues were dehydrated, paraffin-embedded, and consecutively cut into sections of 4 μ m in thickness. The sections were subjected to routine HE staining. For immunofluorescence labeling of NLRP3, Caspase-1, IL-1 β and GPX4, deparaffinized and rehydrated sections were boiled in citrate buffer (pH 6.0) (#P0081, Beyotime) for 20 min to retrieve the antigen. The sections were blocked with 2 % bovine serum albumin for 1 hr, followed by incubation with NLRP3 (#T55651, 1:100, Abmart), caspase-1 (#P79884-2R, 1:100, Abmart), IL-1 β (#TA5103, 1:100, Abmart), and GPX4 (#T56959, 1:100, Abmart), at 4 °C overnight. After washing, the sections were treated with Alexa Fluor 594-conjugated goat anti-rabbit IgG at room temperature for 1 hr, and then counterstained with DAPI. The slides were examined and photographed using the Olympus BX53F fluorescence microscope. Quantification of the fluorescence signal was performed using ImageJ.

17. Clinical Study

In vivo retinal lesions in anesthetized mice were assessed by optical coherence tomography (OCT; RTVue XR Avanti, Optovue) and color fundus photography (Daytona, Optos). Mice were then dark-adapted overnight. Systemic anesthesia, pharmacologic mydriasis, and topical corneal anesthesia were performed as described above. Flash electroretinography (fERG) was conducted as previously reported. Briefly, mice were positioned on a heating pad maintained at 37 °C. A subcutaneous needle electrode in the tail served as ground, and a second needle electrode placed in the cheek served as the reference. A corneal contact electrode was gently positioned on the central cornea as the active electrode. Signals were recorded using a visual electrophysiology system (RetiMINER-C ERG; IRC, Chongqing, China).

18. Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). All data are presented as the mean $\pm$ standard error of the mean (SEM) from at least three independent experiments. Comparisons between two groups were conducted using Student's t-test, while one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied for multiple group comparisons. A P value of < 0.05 was considered statistically significant.

Results:

1. Identify and verify the key molecules of AMD-IDO1

To identify key molecules associated with dry age-related macular degeneration (AMD), we

performed differential analyses on the disease and control groups from the GSE135092 and GSE122270 datasets. Using a $|\log FC| > 0.585$ and a $p\text{-value} < 0.05$ as selection criteria, we identified 38 common genes at the intersection of the two datasets (Figure 1A-1B). We then conducted Mendelian randomization (MR) analysis on the top 15 genes with the largest expression variations, which revealed causal relationships between IDO1, NOV, PI3, and AMD (Figure 1C-1F). Specifically, inverse variance weighted (IVW) MR analysis showed that IDO1 was significantly associated with early AMD (OR = 1.108, 95% CI: 1.026–1.197, $p = 0.009$). We further investigated IDO1 through mRNA and western blot analyses, which demonstrated a significant increase in IDO1 expression levels in the disease group compared to the control group (Figures 1G-I). These results confirm IDO1 as a differentially expressed gene with a potential causal role in dryAMD.

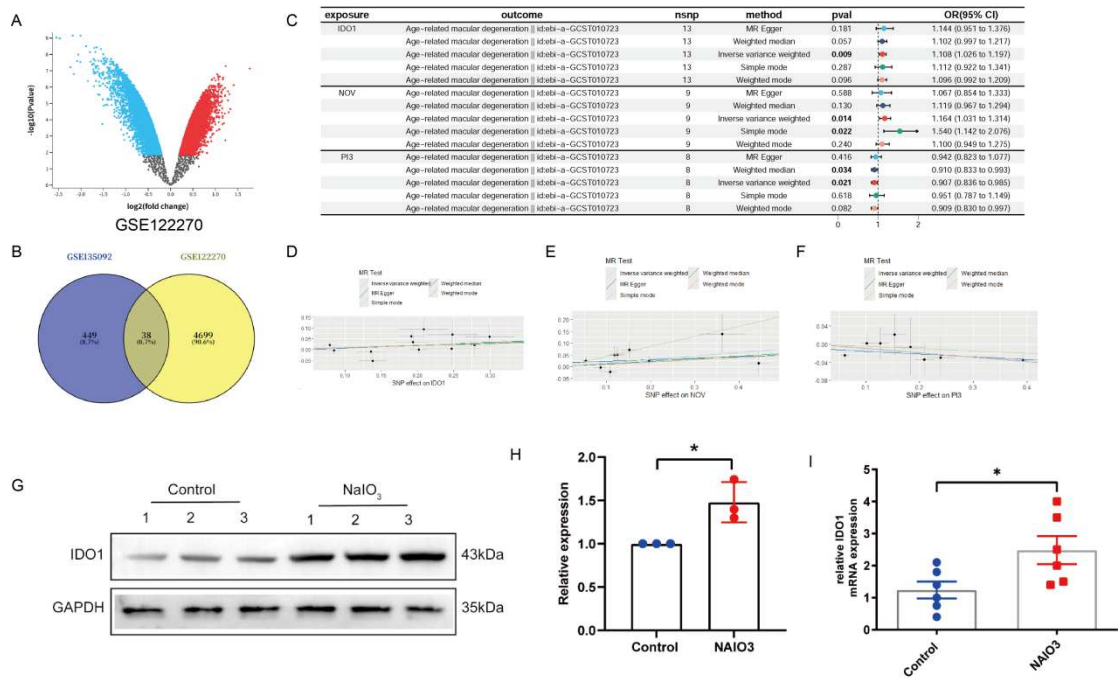


Figure 1. Bioinformatics analysis for identification of differentially expressed genes.

(A) The volcano chart of GSE122270 shows that red indicates an upward adjustment and blue indicates a downward adjustment. (B) The Venn plot shows the intersection genes of GSE122270 and GSE135092. (C) Mendelian randomization (MR) results for exposures IDO1, NOV, and PI3 with clinical AMD outcomes. The forest plot shows odds ratios (OR) and 95% confidence intervals for different MR methods. (D–F) Scatter plots showing MR analyses between SNP effects and outcomes for (D) IDO1, (E) NOV, and (F) PI3. (G) Western blot analysis of IDO1 protein expression. GAPDH served as a loading control. (H) Quantification of IDO1 protein expression from (G). (I) Relative mRNA expression of IDO1 measured by qRT-PCR in NaIO_3 -treated and control RPE cells. Data are shown as mean $\pm$ SD. * $p < 0.05$.

2. IDO1 Inhibition Alleviates Oxidative Stress-Induced Injury in RPE Cells

To establish a stable oxidative stress model, RPE cells were exposed to increasing concentrations of H_2O_2 (0–800 μM). Morphological observations revealed that, with increasing concentrations, cells exhibited progressive signs of damage, including reduced cell density, shrinkage, and detachment (Figure 2A). CCK-8 assays revealed a dose-dependent reduction in cell viability: at 400 μM H_2O_2 , cell viability decreased to ~50% of the control group (half-maximal inhibitory

concentration, IC_{50}), so this concentration was selected for subsequent experiments (Figure 2B). Next, we evaluated the effect of IDO1 inhibition on H_2O_2 -induced injury. WB analysis demonstrated that H_2O_2 exposure significantly increased IDO1 protein expression compared with the control group. Importantly, IDO1 knockdown (Si-IDO1) or pharmacological inhibition by Epacadostat (Epac) markedly reduced IDO1 expression, while the negative control siRNA (Si-NC) showed no effect (Figure 2C, D). Functional assays demonstrated that H_2O_2 significantly decreased cell viability, whereas Si-IDO1 and Epac partially restored viability (Figure 2E). ROS levels were evaluated by fluorescence quantification. H_2O_2 treatment significantly increased intracellular ROS, while IDO1 inhibition by either Si-IDO1 or Epac substantially decreased ROS accumulation (Figure 2F). PI staining was then used to evaluate cell death. H_2O_2 markedly increased the number of PI-positive nuclei, whereas both Si-IDO1 and Epac significantly reduced the proportion of dead cells, indicating that inhibition of IDO1 alleviates oxidative stress-induced cell death (Figure 2G). Finally, wound-healing assays revealed that H_2O_2 severely impaired the migration capacity of RPE cells. Both IDO1 knockdown and Epac treatment markedly promoted cell migration compared with H_2O_2 alone (Figure 2H, I). Collectively, these results demonstrate that oxidative stress upregulates IDO1 expression in RPE cells, leading to increased ROS production, reduced viability, enhanced cell death, and impaired migration. Targeting IDO1, either genetically or pharmacologically, significantly mitigates these deleterious effects.

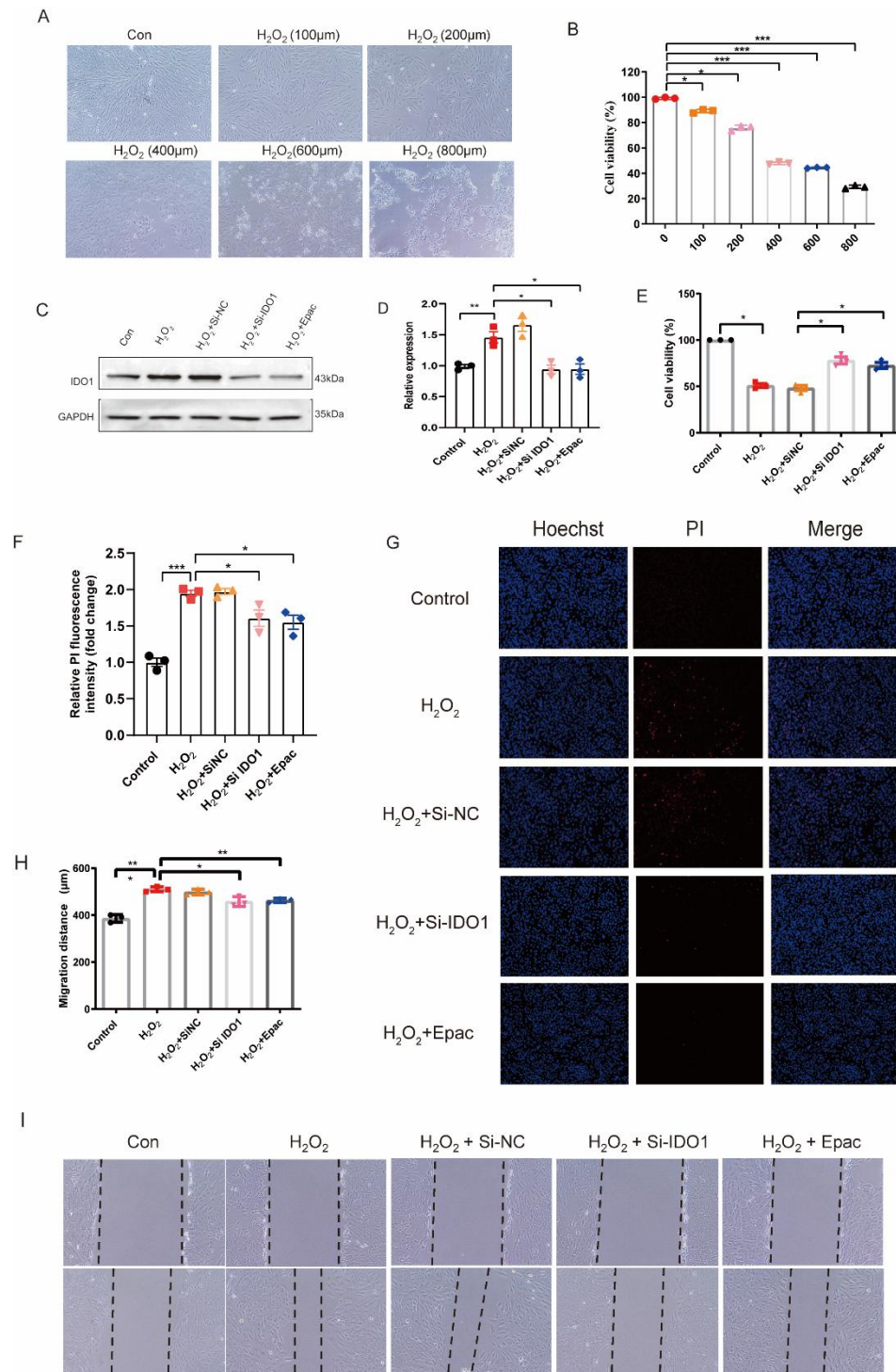


Figure 2. IDO1 inhibition alleviates H_2O_2 -induced oxidative stress injury and migration impairment in RPE cells.

(A) Representative phase-contrast images showing morphological changes of RPE cells after treatment with different concentrations of H_2O_2 (0–800 μ M). (B) CCK-8 assay showing a dose-dependent decrease in cell viability with increasing concentrations of H_2O_2 . (C) Western blot analysis of IDO1 protein expression. GAPDH served as a loading control. (D) Quantification of IDO1 protein expression across different treatment groups. (E) Effects of IDO1 knockdown or Epac treatment on cell viability under oxidative stress. (F) Quantification of intracellular ROS levels under different conditions. (G) PI/Hoechst staining showing dead cells (PI-positive nuclei,

red) and total nuclei (Hoechst, blue). (H) Quantitative analysis of cell migration distance measured by wound-healing assays. (I) Representative wound-healing images showing the effects of IDO1 inhibition on RPE cell migration. Data are presented as mean $\pm$ SEM, n = 3. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$, $P < 0.01$, $P < 0.001$.

3. IDO1 inhibition attenuates H₂O₂-induced ferroptosis in RPE cells

To investigate the role of IDO1 in ferroptosis induced by oxidative stress, we first examined the viability of RPE cells under different treatment conditions. As shown in Figure 3A, compared with the H₂O₂-treated group, the H₂O₂ + Erastin group exhibited a significant decrease in cell viability. Erastin, a well-known ferroptosis inducer, further exacerbated cell death, confirming that ferroptosis is involved in H₂O₂-induced cellular injury. In contrast, both IDO1 knockdown (Si-IDO1) and pharmacological inhibition by Epac significantly improved cell viability, indicating that IDO1 inhibition can protect RPE cells from oxidative stress-induced ferroptotic death. We then measured markers of ferroptosis: MDA (a lipid peroxidation byproduct) and GPX4 (a key ferroptosis suppressor). H₂O₂ exposure markedly increased MDA levels, while Si-IDO1 and Epac reduced MDA accumulation (Figure 3B). Intracellular ROS levels were then quantified as a hallmark of oxidative stress. H₂O₂ dramatically increased ROS production, whereas both Si-IDO1 and Epac substantially reduced ROS accumulation, indicating that inhibition of IDO1 mitigates oxidative damage (Figure 3C, D). WB analysis showed that glutathione peroxidase 4 (GPX4), a key negative regulator of ferroptosis, was significantly downregulated following H₂O₂ treatment. Notably, both genetic knockdown and pharmacological inhibition of IDO1 restored GPX4 expression levels (Figure 3E, F). Transmission electron microscopy (TEM) was used to observe ultrastructural changes typical of ferroptosis. In the H₂O₂ group, mitochondria were shrunken with condensed membranes and reduced cristae, which are characteristic features of ferroptotic cell death. These mitochondrial morphological changes were largely reversed by IDO1 knockdown or Epac treatment (Figure 3G). Together, these findings demonstrate that oxidative stress triggers ferroptosis in RPE cells through ROS accumulation and GPX4 downregulation, while inhibition of IDO1 effectively suppresses these changes, protecting RPE cells from ferroptotic damage.

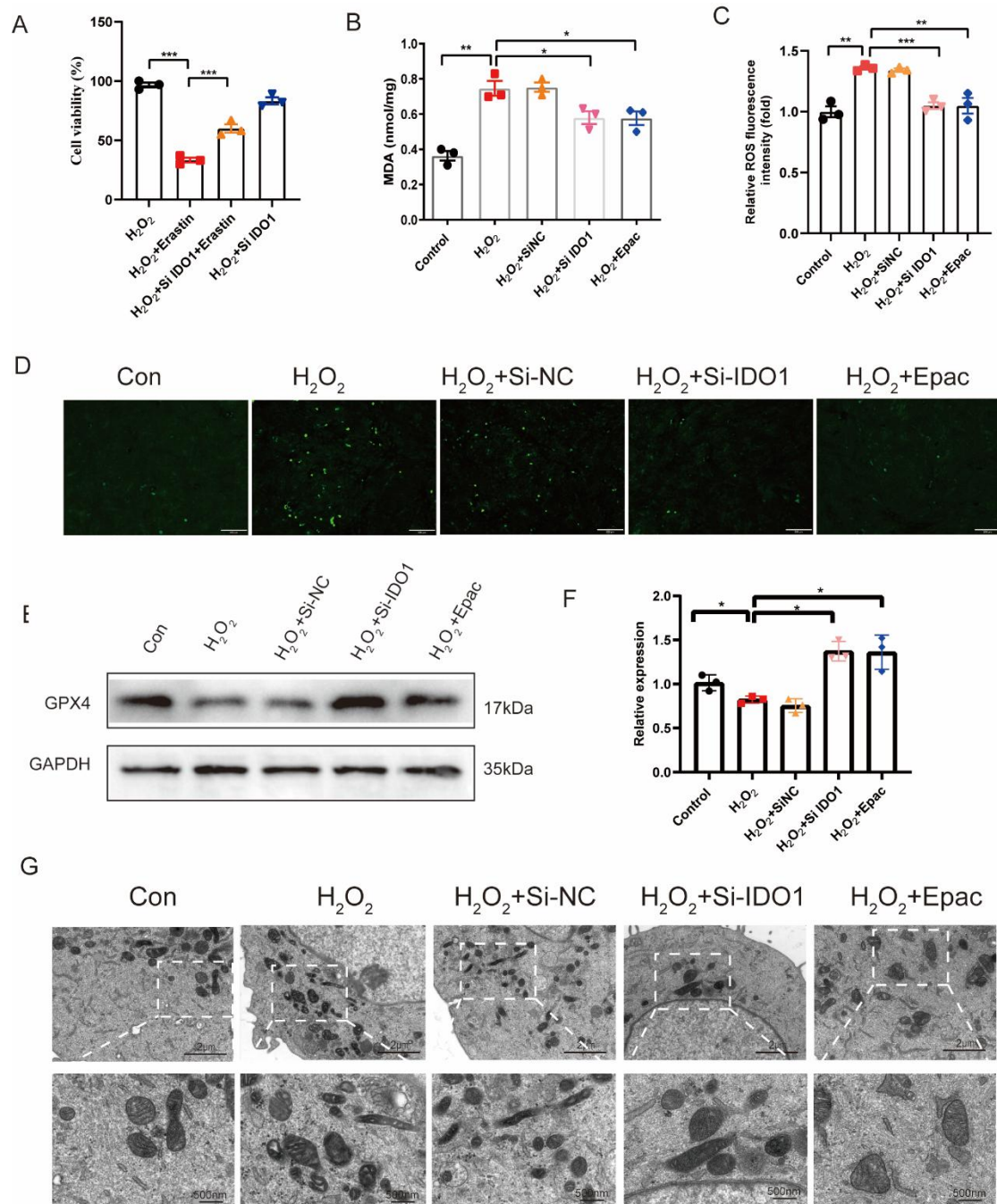


Figure 3. IDO1 inhibition attenuates H_2O_2 -induced ferroptosis in RPE cells.

(A) CCK-8 assay showing the effects of erastin, Si-IDO1, and Epac on cell viability under oxidative stress conditions. (B) Quantification of malondialdehyde (MDA) levels as a marker of lipid peroxidation. (C) Quantification of intracellular ROS levels in different treatment groups. (D) Representative ROS fluorescence images showing oxidative stress levels (scale bar = 200 μ m). (E) Western blot analysis of GPX4 expression, with GAPDH as the loading control. (F) Quantification of GPX4 expression normalized to GAPDH. (G) TEM images showing mitochondrial morphology. Upper panels represent low magnification (scale bar = 2 μ m), and lower panels show high magnification with detailed mitochondrial structures (scale bar = 500 nm). Data are presented as mean $\pm$ SEM, $n = 3$. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$, $P < 0.01$, $P < 0.001$.

4. IDO1 promotes oxidative stress-induced activation of the NLRP3 inflammasome pathway in RPE cells

To identify downstream pathways by which IDO1 contributes to oxidative injury in RPE cells, we performed RNA-seq on H₂O₂-treated cells with or without the IDO1 inhibitor Epac. Differential expression analysis (volcano plot) identified 28,766 transcripts in total, among which 1,153 were significantly upregulated and 147 were downregulated in the H₂O₂ group relative to the H₂O₂ + Epac group (Figure 4A). Notably, NLRP3 emerged among the most strongly induced transcripts under H₂O₂, prompting the hypothesis that inflammasome signaling may act downstream of IDO1 during oxidative stress. Consistent with this, gene set enrichment analysis (GSEA) revealed significant enrichment of “positive regulation of NLRP3 inflammasome complex assembly” in the H₂O₂ group (Figure 4B), providing pathway-level convergence on NLRP3 as a candidate effector. We next prioritized validation targets using three criteria—effect size and significance in RNA-seq ($|\log_2FC|$ and adjusted P), pathway relevance to oxidative injury and innate immune signaling, and directional concordance with Epac rescue. qRT-PCR was performed for 8 transcripts highlighted by these criteria (NME1-NME2, GLTPD2, ASB12, ADCY5, NLRP3, CDRT4, PFDN5, and AP4S1). Among them, NLRP3 mRNA showed the most pronounced and consistent reduction after Epac treatment across replicates, with GLTPD2, ADCY5, and CDRT4 also decreasing, whereas AP4S1 increased (Figure 4C). Taken together, the magnitude of change, enrichment of an NLRP3-centric gene set, and the known role of NLRP3 in linking oxidative stress to inflammatory cell death provided a coherent rationale to focus subsequent mechanistic experiments on NLRP3 as a putative downstream node of IDO1 in RPE cells. WB analysis revealed that H₂O₂ markedly increased the protein expression of NLRP3, caspase-1, and IL-1 β . Epac treatment significantly reduced NLRP3 expression, whereas caspase-1 and IL-1 β showed no significant change (Figure 4D, E). ELISA assays were performed to quantify the release of inflammasome-related cytokines. H₂O₂ exposure significantly increased the secretion of IL-1 β and caspase-1, while Epac treatment markedly reduced their release (Figure 4F, G). Immunofluorescence staining further confirmed these results, showing that H₂O₂ induced strong co-localization of IDO1 and NLRP3 in the cytoplasm. Silencing IDO1 markedly decreased NLRP3 fluorescence intensity, indicating that IDO1 positively regulates NLRP3 expression under oxidative stress conditions (Figure 4H). Collectively, these results indicate that IDO1 upregulation under oxidative stress activates the NLRP3 inflammasome and promotes inflammatory cytokine secretion in RPE cells.

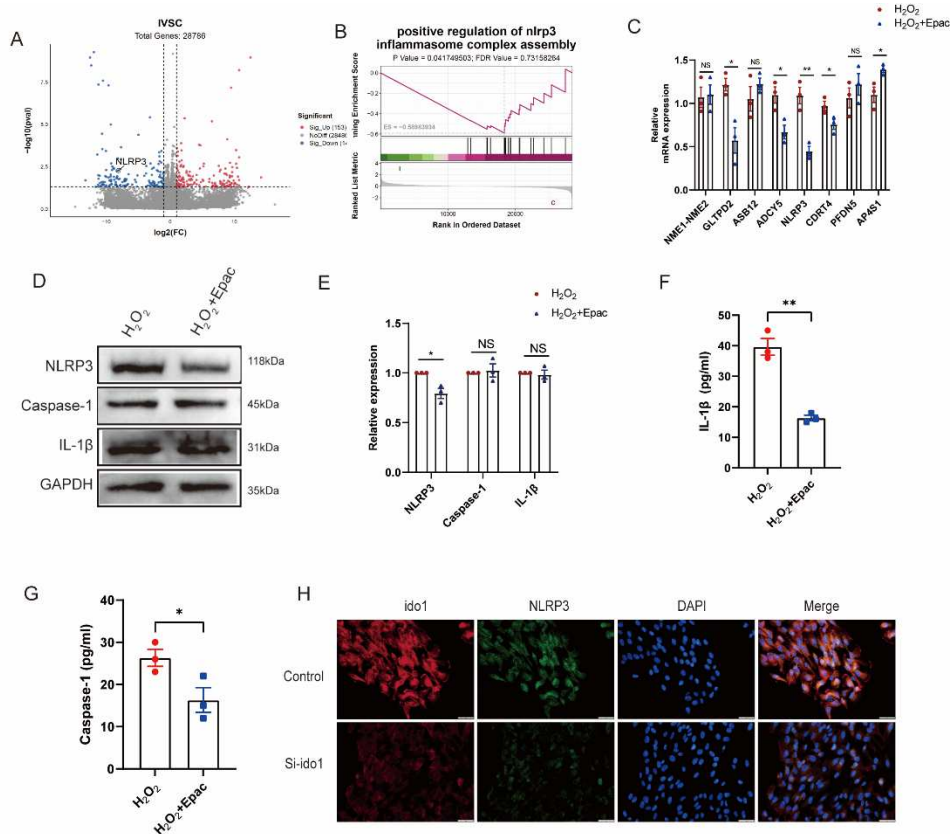


Figure 4. IDO1 promotes oxidative stress-induced activation of the NLRP3 inflammasome pathway in RPE cells.

(A) Volcano plot of differentially expressed genes between H_2O_2 and $H_2O_2 + Epac$ groups. Upregulated genes are shown in red, and downregulated genes are shown in blue. (B) GSEA showing enrichment of the “positive regulation of NLRP3 inflammasome complex assembly” pathway in the H_2O_2 group. (C) qRT-PCR validation of selected genes. (D) Western blot analysis of NLRP3, caspase-1, and IL-1 β expression, with GAPDH as a loading control. (E) Quantification of protein expression levels from (D). (F, G) ELISA assays showing IL-1 β and caspase-1 secretion levels. (H) Immunofluorescence staining showing co-localization of IDO1 (red) and NLRP3 (green). DAPI (blue) labels the nuclei. Data are presented as mean $\pm$ SEM, $n = 3$. Statistical analysis was performed using one-way ANOVA followed by Tukey’s post hoc test. $P < 0.05$, $P < 0.01$, NS: not significant.

5. Overexpression of NLRP3 reverses the protective effects of IDO1 inhibition against H_2O_2 -induced ferroptosis in RPE cells

To confirm whether NLRP3 acts as a downstream effector of IDO1 in regulating oxidative stress-induced ferroptosis, RPE cells were transfected with NLRP3 overexpression plasmids. qRT-PCR and WB confirmed successful overexpression of NLRP3 at both mRNA and protein levels (Figure 5A–C). WB analysis demonstrated that H_2O_2 exposure markedly reduced GPX4 expression, a negative regulator of ferroptosis. Treatment with Epac significantly restored GPX4 levels, indicating its protective effect. However, overexpression of NLRP3 partially abolished the ability of Epac to upregulate GPX4 (Figure 5D, E), suggesting that NLRP3 mediates IDO1-driven ferroptosis. Similarly, Epac reduced H_2O_2 -induced MDA accumulation and ROS production, but these effects were reversed by oe-NLRP3 (Figure 5F–H). These results confirm that NLRP3 acts downstream of IDO1 and mediates IDO1-driven ferroptosis in RPE cells.

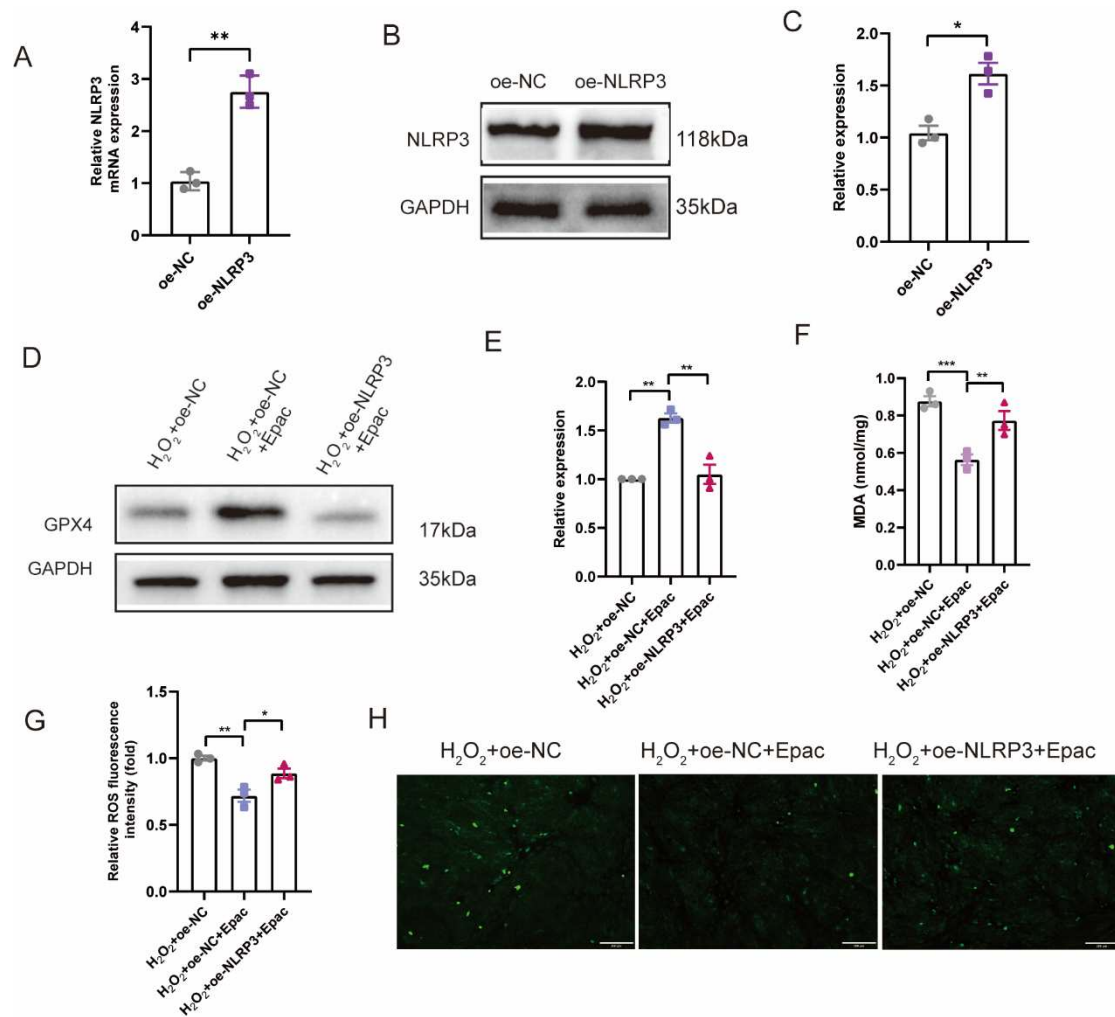


Figure 5. NLRP3 overexpression reverses the protective effects of IDO1 inhibition against H₂O₂-induced ferroptosis in RPE cells.

(A) qRT-PCR analysis confirming NLRP3 mRNA overexpression compared with the negative control (oe-NC). (B) Western blot showing increased NLRP3 protein expression after overexpression. (C) Quantification of NLRP3 protein levels from (B). (D) Western blot analysis of GPX4 expression in different treatment groups, with GAPDH as the loading control. (E) Quantification of GPX4 protein levels normalized to GAPDH. (F) MDA levels were measured to assess lipid peroxidation. (G) Quantification of intracellular ROS fluorescence intensity. (H) Representative ROS fluorescence images in each group (scale bar = 200 μ m). Data are presented as mean $\pm$ SEM, $n = 3$. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$, $P < 0.01$, $P < 0.001$.

6. IDO1 inhibition protects retinal structure and function in NaIO₃-induced dry AMD mouse model

To evaluate the protective effects of IDO1 inhibition *in vivo*, a NaIO₃-induced dry AMD mouse model was established. Epac (50 ng/ μ L) or PBS was intravitreally injected on days 1 and 3 after NaIO₃ administration, and the mice were sacrificed on day 7 for structural and functional assessments (Figure 6A). H&E staining revealed that NaIO₃ treatment caused significant thinning and disorganization of the outer nuclear layer (ONL)—a layer containing photoreceptor nuclei.

Epac treatment markedly alleviated these changes and preserved ONL thickness (Figure 6B,C). To evaluate retinal health, fundus photography, autofluorescence imaging, and OCT were conducted. In the NaIO₃ group, fundus images showed mottled pigmentation and disorganization, which improved with Epac treatment. Autofluorescence imaging indicated RPE dysfunction in NaIO₃-treated mice through abnormal spots, but these were significantly reduced with Epac. OCT scans revealed that NaIO₃ caused retinal thinning and structural disruption, while Epac maintained retinal thickness and organization (Figure 6E). Quantification confirmed that total retinal thickness was significantly decreased in NaIO₃ mice and significantly increased after Epac treatment (Figure 6D). ERG results further showed that NaIO₃ treatment caused a marked reduction in both a-wave and b-wave amplitudes, indicating severe retinal functional impairment, whereas Epac treatment significantly improved both parameters (Figure 6F, G). Collectively, These results demonstrate that IDO1 inhibition preserves retinal structure and function in a mouse model of dry AMD.

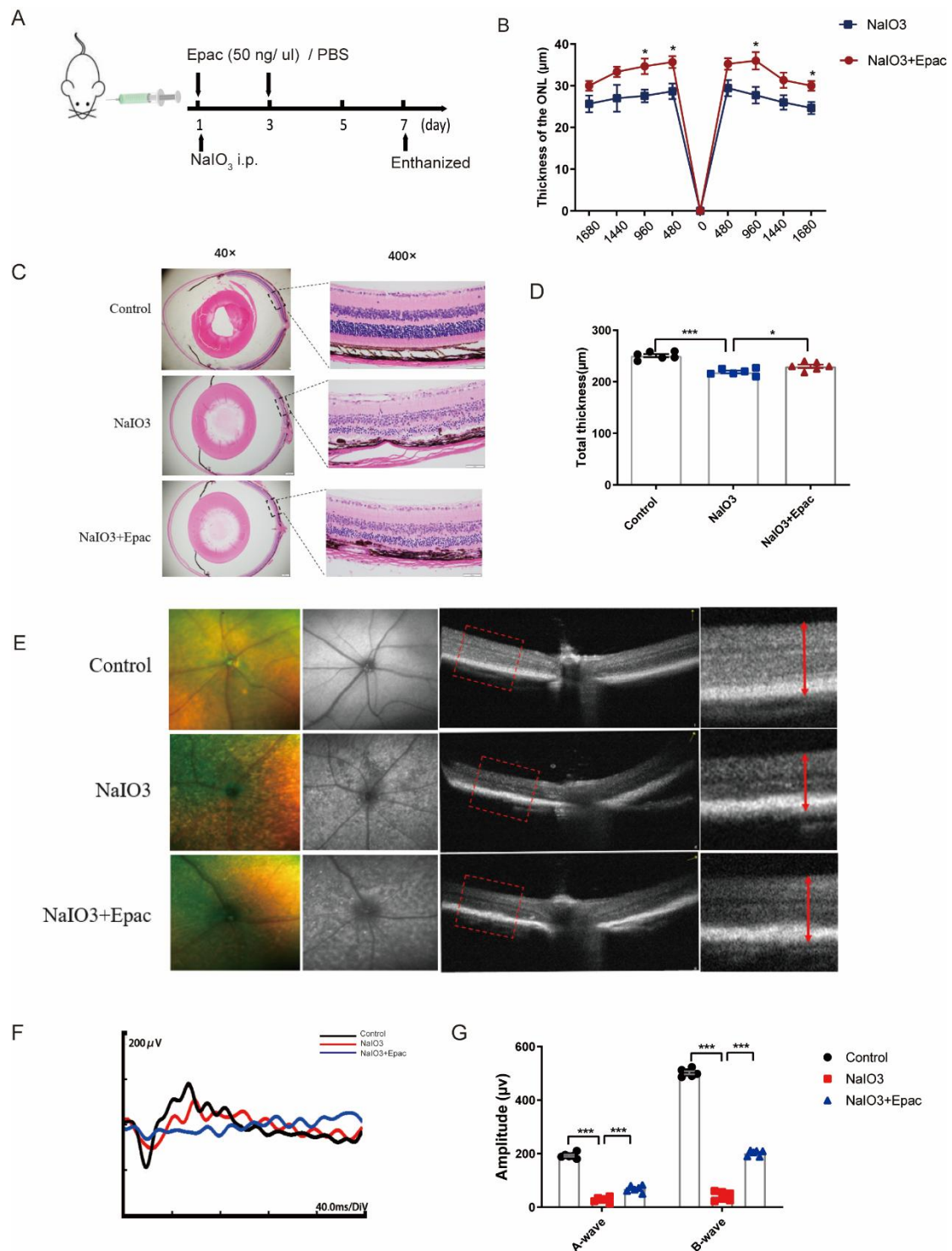


Figure 6. IDO1 inhibition protects retinal structure and function in NaIO₃-induced dry AMD mouse model.

(A) Experimental timeline showing NaIO₃ intraperitoneal injection and intravitreal administration of Epac or PBS. (B) Quantification of ONL thickness at various distances from the optic nerve head using H&E staining. (C) H&E staining showing retinal morphology at 40× and 400× magnifications. (D) Quantitative analysis of total retinal thickness across different groups. (E) Representative fundus images (left), OCT scans (middle), and magnified OCT images (right) of each group. Red arrows indicate ONL boundaries. (F) Representative ERG waveforms showing a-wave and b-wave responses in each group. (G) Quantification of ERG a-wave and b-wave amplitudes. Data are presented as mean ± SEM, n = 6 mice per group. Statistical analysis was performed using one-way

ANOVA followed by Tukey's post hoc test. $P < 0.05$, $P < 0.01$, $P < 0.001$.

7. Epacadostat alleviates NaIO₃-induced retinal degeneration by suppressing IDO1/NLRP3 inflammasome activation and restoring GPX4 expression

To further verify the downstream mechanism of IDO1 inhibition observed in RPE cells, we examined whether Epac could modulate ferroptosis- and inflammasome-related signaling in vivo using the NaIO₃-induced dry AMD mouse model. WB analysis showed that NaIO₃ administration markedly increased the protein levels of IDO1 and NLRP3 compared with the control group, whereas Epac treatment significantly reduced both expressions (Figure 7A–C, $P < 0.05$). Immunofluorescence analysis revealed that NaIO₃ exposure markedly reduced GPX4 expression while enhancing the fluorescence intensity of NLRP3, caspase-1, and IL-1 β , indicating concomitant activation of ferroptosis and inflammasome pathways in retinal degeneration. In contrast, Epac treatment restored GPX4 expression and significantly suppressed the upregulation of NLRP3 inflammasome-related molecules (Figure 7D–K, $P < 0.05$). These findings suggest that Epac mitigates NaIO₃-induced oxidative and inflammatory injury by inhibiting IDO1/NLRP3 inflammasome activation and restoring GPX4 expression, highlighting the crosstalk between ferroptosis and inflammation in the pathogenesis of dry AMD.

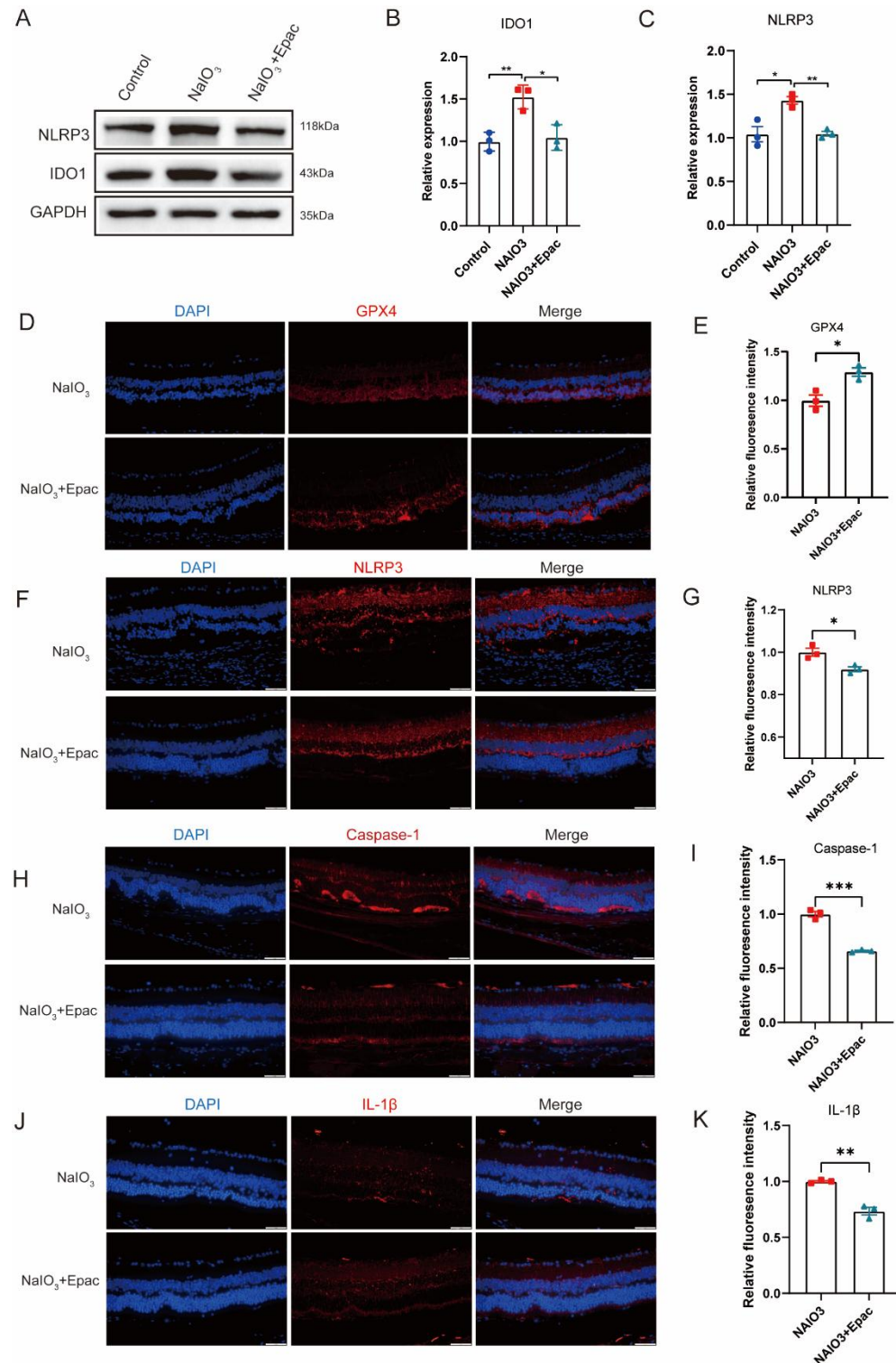


Figure 7. Epacadostat alleviates NaIO₃-induced retinal degeneration by suppressing IDO1/NLRP3 inflammasome activation and restoring GPX4 expression.

(A) Western blot analysis showing protein levels of IDO1 and NLRP3 in control, NaIO₃, and NaIO₃ + Epac groups. GAPDH was used as a loading control.

(B–C) Quantitative analysis of IDO1 and NLRP3 expression levels.

(D) Representative immunofluorescence images of GPX4 (red) and DAPI (blue) in retinal sections.

(E) Quantification of GPX4 fluorescence intensity.

(F–G) Immunofluorescence staining and quantification of NLRP3 (red) and DAPI (blue).

(H–I) Immunofluorescence staining and quantification of caspase-1 (red) and DAPI (blue).

(J–K) Immunofluorescence staining and quantification of IL-1 β (red) and DAPI (blue).

Scale bar = 50 μ m. Data are presented as mean $\pm$ SEM (n = 3). *P < 0.05; **P < 0.01; ***P < 0.001, ****P < 0.0001.

Discussion:

Age-related macular degeneration (AMD) is a leading cause of blindness in older adults, imposes a major socioeconomic burden, and still lacks approved disease-modifying therapies[16, 17]. The retinal pigment epithelium (RPE) is an early and vulnerable nexus where oxidative stress, iron metabolism, and innate immune signaling intersect [18]. Here, we identify a previously unrecognized role of indoleamine 2,3-dioxygenase 1 (IDO1) in dry AMD. In a sodium iodate (NaIO₃) mouse model, IDO1 expression was elevated, whereas pharmacologic IDO1 inhibition partially preserved retinal structure and function. In H₂O₂-challenged RPE cells, IDO1 was upregulated alongside GPX4 downregulation, increased ROS/MDA, and canonical ferroptotic ultrastructural changes; both IDO1 silencing and Epcadostat reversed these readouts. Transcriptomics and validation assays indicated activation of the NLRP3–caspase-1–IL-1 β axis, and forced NLRP3 expression partially restored injury phenotypes despite IDO1 blockade, positioning NLRP3 downstream of IDO1 as an amplification node.

Extensive evidence links oxidative stress to ferroptosis, conceptualized as an iron-dependent disequilibrium in lipid peroxidation. Under oxidative stress, antioxidant defenses (e.g., GSH/GPX4) are blunted while pro-oxidant factors (e.g., Fe²⁺, lipid peroxyl radicals) rise, disrupting redox homeostasis, driving lipid peroxidation, and triggering ferroptosis[19, 20]. Mitochondria are a major ROS source; their dysfunction escalates ROS generation and sensitizes membrane lipids—especially PUFA-PE—to oxidation, pushing cells toward the ferroptotic threshold[21]. Ferroptosis has increasingly been implicated in AMD pathogenesis[22, 23]. With aging, retinal iron accumulation progresses in parallel with declining antioxidant capacity (e.g., GSH/GPX4), intensifying Fenton chemistry and lipid peroxidation; RPE is affected first, followed by photoreceptors, making ferroptosis a key driver[24]. Preclinical studies suggest that iron chelators, lipophilic radical-trapping antioxidants, and interventions targeting the GPX4/FSP1–CoQ₁₀ lipid-repair axis can mitigate ferroptosis-associated retinal degeneration and merit translational evaluation[9, 25, 26]. Consistently, we observed in H₂O₂-exposed RPE cells IDO1 upregulation, higher ROS/MDA, GPX4 reduction, and loss of mitochondrial cristae—hallmarks of ferroptosis—while IDO1 downregulation alleviated these phenotypes and translated in vivo into improved retinal structure and function in NaIO₃-treated mice. We therefore propose a parsimonious mechanism: IDO1 activation increases membrane lipid-peroxidation burden while diminishing GPX4-mediated reductive clearance, heightening ferroptosis susceptibility; conversely, IDO1 inhibition raises the antioxidant threshold and reduces lipid peroxidation, aligning with attenuation of ferroptosis-related phenotypes.

Mechanistically, IDO1 downregulation dampened NLRP3-inflammasome signaling, reflected by reduced NLRP3 protein and decreased secretion of IL-1 β and caspase-1. The partial reversal of Epcadostat's protection by NLRP3 overexpression provides causal support that NLRP3 is not merely correlative but participates in IDO1-dependent RPE injury. Crosstalk between ferroptosis and inflammasomes has been reported[27]. GPX4, a central negative regulator of ferroptosis, also

suppresses caspase-11-dependent pyroptosis and IL-1 β release; in bone-marrow-derived macrophages, GPX4 loss does not affect inflammasome priming but enhances caspase-11 activation and IL-1 β maturation upon *Escherichia coli* infection or LPS electroporation, and myeloid-specific Gpx4 deletion increases susceptibility to polymicrobial infection in a GSDMD/caspase-11-dependent manner, indicating that GPX4 restrains NLRP3 activation[28]. In AMD, lipocalin-2 (LCN2) can shift toward a dimeric variant with diminished iron-chelating capacity, promoting RPE iron accumulation; in engineered mice, RPE iron dyshomeostasis activates NLRP3 via cGAS–STING1, and inflammasome activation in turn augments oxidative stress and cell death[8, 29]. Concordantly, RPE with elevated LCN2 and intracellular iron exhibit increased superoxide and upregulated SOD1. Collectively, iron loading and oxidative stress drive inflammatory amplification through cGAS–STING1–NLRP3 activation, while GPX4 decline releases the brake on NLRP3/pyroptotic signaling, creating a feed-forward loop between ferroptosis and inflammasome activation that exacerbates tissue injury. Our findings connect RPE oxidative damage to functional decline in vivo and place IDO1 upstream of both processes. Moreover, IDO1-mediated regulation of NLRP3 helps explain the structural and functional retinal protection observed after IDO1 inhibition. Notably, IL-1 β released from RPE can induce photoreceptor death[30, 31] and promote VEGF secretion [32], and caspase-1 can contribute to RPE death and retinal degeneration[33].

This study represents the first to identify IDO1 as a potential therapeutic target, thereby enhancing our understanding of its involvement in the pathogenesis of dry AMD. However, several limitations must be acknowledged. Firstly, the study's reliance on the NaIO₃ acute retinal degeneration model and the ARPE-19 two-dimensional (2D) cell line presents issues of limited model relevance and a lack of human validation. These models do not adequately capture the chronic, age-dependent, polygenic, and microenvironmental interactions characteristic of human dry AMD, a significant challenge in the field. Furthermore, due to the difficulty in obtaining clinical samples, we did not validate IDO1 expression or its correlation with clinical metrics in patient tissues. Future research should incorporate more clinically relevant models, such as chronic/aging or genetically susceptible animal models or organoids, to facilitate a more comprehensive exploration. Secondly, the absence of gene-edited mice for validation is a notable limitation. Lastly, our mechanistic investigations were confined to the cellular level, lacking comprehensive animal-level studies, which should be a focus of future research endeavors.

In summary, convergent in vitro and in vivo data indicate that IDO1 drives oxidative injury in the outer retina by jointly promoting RPE ferroptosis and NLRP3-inflammasome activation. IDO1 inhibition—exemplified by Epacadostat—attenuates cellular damage and preserves retinal structure and function, nominating IDO1 as a mechanistically anchored therapeutic target for dry AMD.

Declarations:

Ethics approval and consent to participate:

The animal experiments were conducted by the “Guiding Principles in the Care and Use of Animals” (China) and approved by the Laboratory Animal Ethics Committee of the First Affiliated Hospital of Gannan Medical University (the approval number: LLSC-2025302).

Consent for publication:

Not applicable.

Availability of data and materials:

Publicly available in a repository:

RNA-seq data were deposited into the Gene Expression Omnibus database under accession number GSE135092 and GSE122270 are available at the following URL:

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135092>;

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE122270>;

All the summary statistics used for the included MR analyses can be obtained from the GWAS database. (<https://opengwas.io/>)

Other analytical data can be obtained from the corresponding author.

Competing interests:

The authors declare no competing interests.

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Authors' contributions:

Lei Xu performed the experiments, analyzed the data, organized the results, and wrote the manuscript. Jianmin Hu conceived the study, designed the experiments, managed the data, and reviewed the manuscript. Yiping Jiang performed formal analysis and improved the methodology. Linlin Liu, Linying Xie conducted the experiments and collected the data. All authors have read and agreed to the published version of the manuscript.

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