

Erbin deficiency exacerbates sepsis-induced liver injury through mitochondrial ROS/NLRP3-mediated inflammatory responses

JING ZUO^{1*}, XING WANG^{2*}, GUOQING JING², XIAOJING WU¹ and XUEMIN SONG^{2,3}

¹Department of Anesthesiology, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, P.R. China;

²Research Centre of Anesthesiology and Critical Care Medicine, Zhongnan Hospital Affiliated with Wuhan University, Wuhan, Hubei 430071, P.R. China; ³Department of Anesthesiology, Hainan General Hospital, Haikou, Hainan 570311, P.R. China

Received May 22, 2026; Accepted September 29, 2026

DOI: 10.3892/mmr.2026.14045

Abstract. Sepsis-induced liver injury (SLI) is a life-threatening complication of sepsis characterized by microcirculatory dysfunction, oxidative stress, excessive inflammation, and immune dysregulation. However, the role of ErbB2 interacting protein (Erbin) in SLI remains unclear. In the present study, the functional role and underlying mechanism of Erbin were investigated in a cecal ligation and puncture (CLP)-induced mouse model of SLI using wild-type and Erbin-knockout (Erbin^{-/-}) mice, together with lipopolysaccharide (LPS)/adenosine triphosphate (ATP)-stimulated bone marrow-derived macrophages (BMDMs). Liver histopathology, mitochondrial ultrastructure, oxidative stress, inflammatory cytokine production, and pyroptosis-related signaling were assessed using H&E staining, transmission electron microscopy, biochemical assays, ELISA, quantitative PCR, immunohistochemistry, and western blotting. Erbin

deficiency markedly aggravated CLP-induced liver injury, as evidenced by more severe histopathological damage, mitochondrial disruption, oxidative stress, NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome activation, and pro-inflammatory cytokine production. Mechanistically, Erbin deficiency promoted mitochondrial ROS accumulation, enhanced thioredoxin-interacting protein (TXNIP)-NLRP3 interactions, and intensified inflammatory activation and pyroptotic responses. Mitochondrial ROS scavenging with MitoQ substantially attenuated the pro-pyroptotic and pro-inflammatory effects of Erbin deficiency in LPS/ATP-stimulated BMDMs. Collectively, these findings identify Erbin as an endogenous protective regulator that mitigates SLI by suppressing mitochondrial ROS-driven TXNIP/NLRP3 inflammasome activation and inflammatory responses.

Introduction

Sepsis is a life-threatening condition caused by a dysregulated host response to infection that triggers systemic inflammation and frequently culminates in multiorgan dysfunction and death (1,2). In this pathophysiological context, the liver serves as both a major metabolic center and an immune organ, which is essential for maintaining metabolic-immune homeostasis (3-5). Clinical evidence increasingly identifies sepsis-induced liver injury (SLI) as a notable determinant of prognosis, as the extent of hepatic damage is associated with disease severity and clinical outcomes (6,7). SLI arises from complex interactions among multiple hepatic cell populations, including hepatocytes and hepatic macrophages (8). Hepatic macrophages comprise tissue-resident Kupffer cells and recruited monocyte-derived macrophages, which have distinct functions during liver injury (9,10). Emerging evidence indicates that monocyte-derived macrophages shape the inflammatory microenvironment in SLI by acquiring a pro-inflammatory phenotype characterized by increased cytokine production and inflammasome activation, thereby amplifying inflammation and tissue injury (11). Elucidating the molecular mechanisms that regulate macrophage activation and inflammatory responses is therefore essential for developing effective treatments for SLI.

Correspondence to: Professor Xiaojing Wu, Department of Anesthesiology, Renmin Hospital of Wuhan University, 238 Liberation Road, Wuchang, Wuhan, Hubei 430060, P.R. China
E-mail: rm000851@whu.edu.cn

Professor Xuemin Song, Department of Anesthesiology, Hainan General Hospital, 19 Xiuhua Road, Xiuying, Haikou, Hainan 570311, P.R. China
E-mail: xueminson@whu.edu.cn

*Contributed equally

Abbreviations: Erbin, ErbB2 interacting protein; SLI, sepsis-induced liver injury; CLP, cecal ligation and puncture; LPS, lipopolysaccharide; ATP, adenosine triphosphate; BMDMs, bone marrow-derived macrophages; ROS, reactive oxygen species; mtROS, mitochondrial ROS; NLRP3, NOD-like receptor family pyrin domain containing 3; TXNIP, thioredoxin-interacting protein; IL-1 β , interleukin-1 β ; IL-18, interleukin-18; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; GSDMD, gasdermin D; qPCR, quantitative PCR; WT, wild type

Key words: erbin, sepsis-induced liver injury, inflammation, oxidative stress, NLRP3 inflammasome

Excessive reactive oxygen species (ROS) production contributes substantially to sepsis-induced multiorgan dysfunction and is closely associated with NLRP3 inflammasome activation and subsequent pyroptotic cell death (12-14). Pyroptosis is characterized by inflammasome assembly, proteolytic activation of inflammatory caspases, particularly caspase-1 and cleavage of gasdermin family proteins, culminating in membrane pore formation and the release of cellular contents and pro-inflammatory mediators, including IL-1 β and IL-18 (15-17). The NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome is a cytoplasmic multiprotein complex comprising NLRP3, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase-1 (18). Chen *et al* (19) reported that administration of the caspase-1 inhibitor Ac-YVAD-CMK inhibited hepatocyte pyroptosis and alleviated CLP-induced SLI.

ErbB2 interacting protein (Erbin) is a PDZ domain-containing adaptor protein that interacts with ERBB2/HER2 receptors and contains 16 N-terminal leucine-rich repeats and a C-terminal PDZ domain (20). Beyond its role in epithelial signaling, Erbin regulates inflammation by binding to NOD2 and modulating NOD2-dependent NF- κ B activation and cytokine production (21). Consistently, Erbin-deficient mice exhibit increased susceptibility to experimental colitis and more severe intestinal barrier impairment (22). Xie *et al* (23) further demonstrated that Erbin degradation activates the SAG-dependent RAS-RAF pathway, leading to ROS accumulation, autophagy and cellular senescence. NLRP3 inflammasome activators can also increase ROS production, whereas ROS inhibition suppresses caspase-1 activation and IL-1 β release in alveolar macrophages (24). These findings support a broader role for Erbin in oxidative and inflammatory signaling. Nevertheless, its contribution to sepsis-induced hepatic dysfunction remains unclear.

The aim of the present study was to determine the role of Erbin in SLI and to investigate whether Erbin regulates mitochondrial ROS (mtROS) accumulation and NLRP3 inflammasome activation, with a specific focus on macrophage-intrinsic inflammatory mechanisms. Given the complexity of hepatic cellular interactions, bone marrow-derived macrophages (BMDMs) were employed to examine macrophage-intrinsic inflammatory mechanisms. The findings indicate that Erbin deficiency exacerbates mtROS-dependent NLRP3 activation and inflammatory responses, thereby contributing to SLI progression.

Materials and methods

Ethics approval. All animal experiments were approved by the Animal Experimentation Committee of Wuhan University (Wuhan, China; approval no. WQ20210298). All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (25) and the ARRIVE 2.0 guidelines (26).

Animal experiments. Male C57BL/6 mice aged 6-8 weeks were housed under specific pathogen-free conditions with a 12-h light/dark cycle and ad libitum access to food and water. Experimental and control mice underwent the same fasting

protocol before cecal ligation and puncture (CLP) surgery. A total of two genetically modified mouse models were used for distinct experimental purposes. Global Erbin-knockout (Erbin^{-/-}) mice, obtained from Wuhan Xianran Biological Technology Co., Ltd., were used exclusively in the *in vivo* CLP model to evaluate the overall effect of Erbin deficiency on SLI. Macrophage-specific Erbin conditional-knockout mice (Erbin^{fl/fl/Lyz2-Cre}) were generated by crossing Erbin^{fl/fl} mice with Lyz2-Cre mice (both Cyagen Biosciences, Inc.). BMDMs isolated from Erbin^{fl/fl/Lyz2-Cre} mice and Erbin^{fl/fl} littermate controls were used exclusively for *in vitro* mechanistic experiments examining the macrophage-intrinsic role of Erbin in inflammatory signaling. Primers used to confirm the genotype can be found in Table I. A schematic of how the mice were used across experiments is presented in Fig. S1.

Sepsis model. General anesthesia was induced by intraperitoneal administration of pentobarbital sodium (50 mg/kg). The mice were placed in the supine position, and a 1-cm midline abdominal incision was made to expose the cecum. The cecum was ligated with 3-0 silk and punctured with a 21-gauge needle. It was then returned to the abdominal cavity, and the incision was closed. Subsequently, 1 ml of prewarmed 0.9% saline was administered subcutaneously for fluid resuscitation. Sham-operated mice underwent the same surgical procedure without cecal ligation or puncture (27). Following sepsis induction, the mice were monitored at least twice daily. Humane endpoints included >20% loss of body weight, inability to eat or drink, persistent recumbency or markedly reduced responsiveness, severe respiratory distress or cyanosis, severe dehydration, seizures, paralysis, coma or other signs of a moribund condition. Animals reaching any of these endpoints were immediately euthanized.

Histological analysis. Mice were euthanized by CO₂ inhalation in accordance with GB/T 39760-2021. CO₂ was delivered at a flow rate of 9 l/min, which corresponds to a volume displacement rate of 30% per minute for the 30-L euthanasia chamber, until respiration ceased, followed by cervical dislocation as a secondary method. Death was confirmed by the absence of respiration, heartbeat and pedal withdrawal reflex before tissue collection. Hepatic tissues were collected 24 h after CLP for histological evaluation. The right liver lobe was rinsed with PBS, fixed in 4% paraformaldehyde at 4°C for 24 h, embedded in paraffin, sectioned at 4 μ m. The sections were then stained with hematoxylin at room temperature for 4 min and eosin at room temperature for 1 min. Histopathological changes were examined by light microscopy and digitally imaged. Liver injury was scored semi-quantitatively according to six parameters: Cytoplasmic pallor, vacuolization, nuclear condensation, nuclear fragmentation, chromatin dissolution and sinusoidal erythrocyte accumulation. Each parameter was graded from 0 to 3, yielding a total score of 0-18 per microscopic field (28).

Survival analysis. After CLP surgery, mice were monitored every 12 h for 7 days, and survival was recorded throughout the observation period. Mice that failed to respond to external stimuli were considered dead. The sample size was comparable to those commonly used in survival analyses of CLP-induced

Table I. Primers used for mouse genotyping.

Mouse model	Primer	Sequence (5'-3')	Expected amplicon
Erbin floxed allele	Erbin-F1	ACACTGATGCTGTGATTGTAGTGT	Floxed allele: 226 bp; WT allele: 158 bp
	Erbin-R1	CATCTCTCTTTTACAGGCCTCCAG	
Lyz2-Cre transgene	Cre-F	CATATTGGCAGAACGAAAACGC	Cre-positive allele: 400 bp
	Cre-R	CCTGTTTCACTATCCAGGTTACGG	

WT, wild type.

sepsis models (29,30). Survival curves were generated using the Kaplan-Meier method and compared using the log-rank (Mantel-Cox) test in GraphPad Prism (version 10.6.1; Dotmatics).

Serum biochemical analysis. Commercial assay kits were used to measure serum alanine aminotransferase (ALT; cat. no. C009-2-1; Nanjing Jiancheng Bioengineering Institute), aspartate aminotransferase (AST; cat. no. C010-2-1; Nanjing Jiancheng Bioengineering Institute) and lactate dehydrogenase (LDH; cat. no. A020-2-2; Nanjing Jiancheng Bioengineering Institute) levels according to the manufacturer's instructions.

Immunohistochemistry. Liver tissues were fixed in 10% neutral buffered formalin (NBF) at 4°C for 24 h, embedded in paraffin, sectioned at 4 μ m thickness and then incubated overnight at 4°C with an anti-NLRP3 antibody (1:200; cat. no. GB114320-50; Wuhan Servicebio Technology Co., Ltd.). After three 5-min washes with PBS, the sections were incubated with the appropriate secondary antibody (1:200; cat. no. G1312; Wuhan Servicebio Technology Co., Ltd.) at 37°C for 30 min and developed using 3,3'-diaminobenzidine. The sections were counterstained with hematoxylin at room temperature for 4 min, dehydrated, mounted and examined by light microscopy. NLRP3-positive areas were quantified using ImageJ 1.54j software (National Institutes of Health).

Transmission electron microscopy. Liver samples were fixed with 2.5% glutaraldehyde at 4°C for 1 h and then with 1% osmium tetroxide at room temperature for 2 h. After dehydration, the samples were infiltrated with epoxy resin at 37°C for 8 h and embedded in epoxy resin at 60°C for 48 h. Ultrathin sections (80 nm) were cut and stained with 2% uranyl acetate at room temperature for 10 min and then with lead citrate at room temperature for 5 min, followed by examination under a Hitachi H-600 transmission electron microscope (Hitachi, Ltd.). Abnormal mitochondria were identified as previously described (31).

Cell culture and treatments. Consistent with previous studies investigating macrophage-mediated inflammatory mechanisms in liver disease (32-34), BMDMs from macrophage-specific Erbin conditional-knockout mice (Erbin^{fl/fl}/Lyz2-Cre) and Erbin^{fl/fl} littermate controls were used to examine the macrophage-intrinsic effects of Erbin deficiency. Following euthanasia, the femurs and tibiae were dissected, cleared of soft tissue and immersed in 75% ethanol for 5 min.

Bone marrow was flushed with PBS, passed through a 70- μ m cell strainer, and centrifuged at 300 x g for 10 min. Erythrocytes were lysed using ACK buffer (cat. no. R1013; Beijing Solarbio Science & Technology Co., Ltd.). The remaining cells were cultured in high-glucose DMEM (cat. no. 11995; Beijing Solarbio Science & Technology Co., Ltd.) supplemented with 10% FBS (Procell Life Science & Technology Co., Ltd.) and 25 ng/ml macrophage colony-stimulating factor (M-CSF; MedChemExpress) at 37°C in 5% CO₂ for 7 days to generate mature adherent BMDMs. To activate the inflammasome, BMDMs were primed with lipopolysaccharide (LPS; 1 μ g/ml; MilliporeSigma) for 4 h and subsequently stimulated with ATP (5 mM; MedChemExpress) for 30 min at 37°C (35). For MitoQ experiments, BMDMs were pretreated with 1 μ M MitoQ (HY-100116A; MedChemExpress; dissolved in 0.02% DMSO) for 2 h at 37°C before LPS/ATP stimulation. A concentration of 0.02% DMSO was used as the vehicle control in the corresponding groups.

Cell death assays. For the cell counting kit (CCK)-8 assay, BMDMs were seeded in 96-well plates at 1x10⁴ cells/well and subjected to the indicated treatments. CCK-8 reagent (10 μ l; Biosharp Life Sciences) was then added to each well, followed by incubation at 37°C for 30 min, and cell viability was measured using a microplate reader.

For the lactate dehydrogenase (LDH) assay, culture supernatants were collected and centrifuged at 500 x g for 10 min at 4°C. LDH release was measured using a commercial kit (cat. no. A020-2-2; Beyotime Biotechnology) according to the manufacturer's instructions.

For Calcein-AM/propidium iodide (PI) staining, BMDMs were seeded in 24-well plates at 1x10⁵ cells/well and subjected to LPS/ATP stimulation with or without MitoQ. Cells were stained using a Calcein-AM/PI kit (cat. no. BB-4126; Bebo Biotech). Briefly, cells were incubated with Calcein-AM working solution at 37°C in the dark for 30 min, washed twice with PBS, and then incubated with PI working solution under the same conditions for an additional 30 min. Following PBS washes, fluorescence images were acquired by microscopy, and cell death was quantified using ImageJ software.

ROS measurement. At 24 h after CLP, liver sections were prepared for dihydroethidium (DHE) staining as previously described (36). Intracellular ROS in LPS/ATP-stimulated BMDMs was assessed using a commercial ROS assay kit (cat. no. BL3157A; Biosharp Life Sciences) according to the manufacturer's instructions. Briefly, cells were incubated with

10 μ M DCFH-DA at 37°C for 30 min in the dark. Fluorescence images were acquired by microscopy, and fluorescence intensity was quantified using ImageJ software.

Commercial biochemical assay kits were used to measure malondialdehyde (MDA; cat. no. A003-1-2; Nanjing Jiancheng Bioengineering Institute) levels and superoxide dismutase (SOD; cat. no. A001-1-2; Nanjing Jiancheng Bioengineering Institute) activity in liver and BMDM protein homogenates according to the manufacturer's instructions.

mtROS measurement. mtROS production was assessed using MitoSOX Red (40778ES50; Shanghai Yeasen Biotechnology Co., Ltd.). BMDMs were seeded in 12-well plates, subjected to the indicated treatments, and incubated with 500 nmol/l MitoSOX Red in HBSS containing calcium and magnesium at 37°C for 30 min in the dark. After two washes with prewarmed PBS, fluorescence images were acquired by microscopy and analyzed using ImageJ software.

Mitochondrial membrane potential measurement. Mitochondrial membrane potential was assessed using a JC-1 Mitochondrial Membrane Potential Assay Kit (C2006; Beyotime Biotechnology) according to the manufacturer's instructions. BMDMs were seeded in six-well plates and subjected to the indicated treatments, followed by incubation with JC-1 working solution for 20 min at 37°C. The cells were then collected and analyzed by flow cytometry (Beckman Coulter, Inc.).

Cytokine quantification. Liver tissue (100 mg) was rinsed with ice-cold PBS and homogenized on ice in 900 μ l PBS (pH 7.4) containing 1x protease inhibitor cocktail (Roche Applied Science). Homogenates were centrifuged at 12,000 x g for 15 min at 4°C, and the supernatants were aliquoted and stored at -80°C until analysis. Cytokine concentrations were normalized to total protein concentrations determined using a BCA assay. TNF- α (cat. no. RK00027), IL-18 (cat. no. RK00104), IL-1 β (cat. no. RK04878) and IL-6 (cat. no. RK00008) levels in liver homogenate supernatants (37) and BMDM culture supernatants were measured using ELISA kits (all from ABclonal Biotech Co., Ltd.) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (BioTek; Agilent Technologies, Inc.).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted using TRIzol reagent (Beijing Solarbio Science & Technology Co., Ltd.), and reverse transcription was performed using ABScript III RT Mix (ABclonal Biotech Co., Ltd.) under the following conditions: 37°C for 2 min, 55°C for 15 min, 85°C for 5 min and then held at 4°C. qPCR was performed using 2X Universal SYBR Green Fast qPCR Mix (ABclonal Biotech Co., Ltd.) with the following thermocycling conditions: Initial denaturation at 95°C for 3 min, followed by 42 cycles of 95°C for 5 sec and 60°C for 30 sec. Relative gene expression was calculated using the $2^{-\Delta\Delta C_q}$ method (38), with β -actin as the internal control. Primer sequences are listed in Table II.

Co-immunoprecipitation (Co-IP). Whole-cell lysates were prepared in NP-40 lysis buffer (Beijing Solarbio Science &

Technology Co., Ltd.) supplemented with freshly prepared protease inhibitor cocktail and centrifuged at 14,000 x g for 10 min at 4°C to remove insoluble material. For each sample 1 ml supernatant was collected, of which 100 μ l was retained as the input and the remaining 900 μ l was used for co-immunoprecipitation. For each co-immunoprecipitation reaction, 900 μ l of lysate containing ~3 mg total protein was incubated overnight at 4°C with 4.5 μ g anti-NLRP3 antibody (1.5 μ g/mg protein; cat. no. A5652; ABclonal Biotech Co., Ltd.) or 4.5 μ g species-matched normal IgG (1.5 μ g/mg protein; cat. no. 30000-0-AP; Proteintech Group, Inc.) under gentle agitation. A volume of 30 μ l Protein A/G agarose beads (MedChemExpress) were then added and incubated for an additional 2-4 h with rotation at 4°C. The beads were extensively washed three times with ice-cold immunoprecipitation buffer, and the immunoprecipitated proteins were eluted by heating in SDS-PAGE loading buffer at 95-100°C for 5-10 min. Eluted proteins were separated by SDS-PAGE and analyzed by western blotting. Normal IgG was used as the negative immunoprecipitation control.

Western blotting. Total proteins were extracted from liver tissues and BMDMs using RIPA lysis buffer (Beyotime Biotechnology) supplemented with protease inhibitors (BioSharp Life Sciences). Protein concentrations were determined using a BCA assay kit (Beyotime Biotechnology). A total of 20 μ g protein per lane was separated on 8-12% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes (MilliporeSigma). Membranes were blocked with 5% non-fat milk and incubated overnight at 4°C with primary antibodies against Erbin (1:1,000; cat. no. NBP2-56104; Novus Biologicals, LLC), thioredoxin-interacting protein (TXNIP; 1:1,000; 14715; Cell Signaling Technology, Inc.), NLRP3 (1:2,000; A5652; ABclonal Biotech Co., Ltd.), GSDMD (1:1,000; A10164; ABclonal Biotech Co., Ltd.), caspase-1 (1:500; AG-20B-0042; AdipoGen Life Sciences) and β -actin (1:10,000; AC006; ABclonal Biotech Co., Ltd.). Membranes were subsequently incubated with species-matched horseradish peroxidase-conjugated secondary antibodies (1:10,000; cat. nos. SA00001-1 and SA00001-2; Proteintech Group, Inc.). Protein bands were visualized using an enhanced chemiluminescence detection system (Tanon Science and Technology Co., Ltd.) and quantified using ImageJ software.

Bioinformatics analysis. Publicly available transcriptomic datasets GSE294059 (39), GSE228541 (40) and GSE28750 (41) were obtained from the Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/>) database and analyzed using R software (version 4.5.2; RStudio, Inc.). Samples were classified according to the phenotype information provided in GEO. For GSE294059, sepsis and non-septic control samples were included; for GSE228541, sepsis and healthy control samples were included; and for GSE28750, only sepsis and healthy control samples were retained, whereas post-surgical samples were excluded. Each dataset was analyzed independently without cross-dataset normalization or batch correction.

For the RNA-sequencing datasets GSE294059 and GSE228541, gene-level count matrices were imported into R and matched with the corresponding sample information using GEO accession identifiers. Gene identifiers were

Table II. Primer sequences for PCR analyses.

Primer name	Primer sequences
Mouse-TNF- α forward primer	GGCATGGATCTCAAAGACAAC
Mouse-TNF- α reverse primer	CAAATCGGCTGACGGTGTG
Mouse-IL-1 β forward primer	GAAATGCCACCTTTTGACAGTG
Mouse-IL-1 β reverse primer	TGGATGCTCTCATCAGGACAG
Mouse-IL-6 forward primer	CATGTTCTCTGGGAAAT.CGTGG
Mouse-IL-6 reverse primer	GTACTCCAGGTAGCTATGGTAC
Mouse-IL-18 forward primer	GACTCTTGCGTCAACTTCAAGG
Mouse-IL-18 reverse primer	CAGGCTGTCTTTTGTCACGA
β -actin forward primer	AGACCTTCAACACCCAG
β -actin reverse primer	CACGATTTCCTCTCAGC

converted to official gene symbols using the AnnotationDbi (<https://bioconductor.org/packages/AnnotationDbi/>) and org.Hs.eg.db (<https://bioconductor.org/packages/org.Hs.eg.db/>) packages. ERBIN was identified using the official symbol ERBIN, its previous symbol ERBB2IP, and Entrez Gene ID 55914, whereas NLRP3 was identified using its official symbol and Entrez Gene ID 114548. Library sizes were normalized using the trimmed mean of M-values method implemented in the edgeR package (<https://bioconductor.org/packages/edgeR/>), and normalized expression was expressed as log₂ counts per million (log₂-CPM; prior.count=1). For the microarray dataset GSE28750, the GEO-provided RMA-normalized expression matrix was used. Probe sets were annotated using the hgu133plus2.db package (<https://bioconductor.org/packages/hgu133plus2.db/>), and multiple probes mapping to the same target gene were averaged to obtain gene-level expression values. ERBIN expression was compared between sepsis and corresponding control groups using a two-sided Wilcoxon rank-sum test, with Welch's t-test additionally performed as a sensitivity analysis. Associations between ERBIN and NLRP3 expression were evaluated within the sepsis group using Spearman's rank correlation as the primary analysis, with Pearson's correlation additionally reported. A two-sided $P < 0.05$ was considered to indicate a statistically significant difference.

Statistical analysis. Statistical analyses were performed using GraphPad Prism version 8.0.1 (Dotmatics). Data are presented as the mean $\pm$ SD unless otherwise indicated. Normality was assessed using the Shapiro-Wilk test before analysis. Comparisons between two unpaired groups with normally distributed data were performed using an unpaired Student's t-test, whereas multiple-group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. For experiments involving genotype and treatment as two independent factors, statistical differences were analyzed using ordinary two-way ANOVA, followed by Šídák's multiple-comparisons test for prespecified biologically relevant comparisons. Non-normally distributed data were analyzed using the Mann-Whitney U test. Survival curves were generated using the Kaplan-Meier method and compared using the log-rank (Mantel-Cox) test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Erbin deficiency aggravates sepsis-induced liver injury. To investigate the role of Erbin in SLI, liver tissue and serum samples were collected 24 h after CLP. Histological analysis showed that CLP induced hepatocyte death, inflammatory cell infiltration, disruption of the hepatic vasculature and disorganization of hepatic cords. These pathological changes were more notable in Erbin^{-/-}+CLP mice than in WT+CLP mice (Fig. 1A and B). Consistently, serum AST, ALT and LDH levels were markedly higher in Erbin-deficient mice after CLP (Fig. 1C and D). The 7-day survival was lower in Erbin^{-/-}+CLP mice than in WT+CLP mice [20.0% (4/20) vs. 55.6% (10/18)], respectively; Fig. 1E). In addition, liver homogenates from Erbin^{-/-}+CLP mice contained significantly higher levels of the pro-inflammatory cytokines TNF- α , IL-1 β , IL-18, and IL-6 than those from WT+CLP mice (Fig. 1F). Collectively, these findings indicate that Erbin deficiency exacerbates sepsis-induced liver injury.

Erbin deficiency enhances NLRP3 inflammasome activation during sepsis-induced liver injury. To determine whether Erbin regulates pyroptosis-associated signaling during SLI, NLRP3, caspase-1 p20 and GSDMD-N expression was examined by western blotting. CLP markedly activated the NLRP3 inflammasome pathway, and this activation was further enhanced in Erbin^{-/-} mice (Fig. 2C and D). Consistently, immunohistochemical analysis showed increased hepatic NLRP3 expression after CLP, with a greater increase in Erbin-deficient mice (Fig. 2A and B). These findings indicate that Erbin deficiency promotes NLRP3 inflammasome activation during SLI.

Erbin deficiency enhances pyroptosis and inflammation in BMDMs. Macrophage-hepatocyte crosstalk is increasingly recognized as a notable contributor to SLI progression (42). To investigate the macrophage-intrinsic role of Erbin, BMDMs were stimulated with LPS and ATP to activate the NLRP3 inflammasome and induce pyroptosis (43,44). Erbin deficiency markedly enhanced LPS/ATP-induced expression of NLRP3, caspase-1 p20, and GSDMD-N (Fig. 3A and B). CCK-8 assays, LDH release measurements and Calcein-AM/PI staining further showed reduced cell viability and increased cell

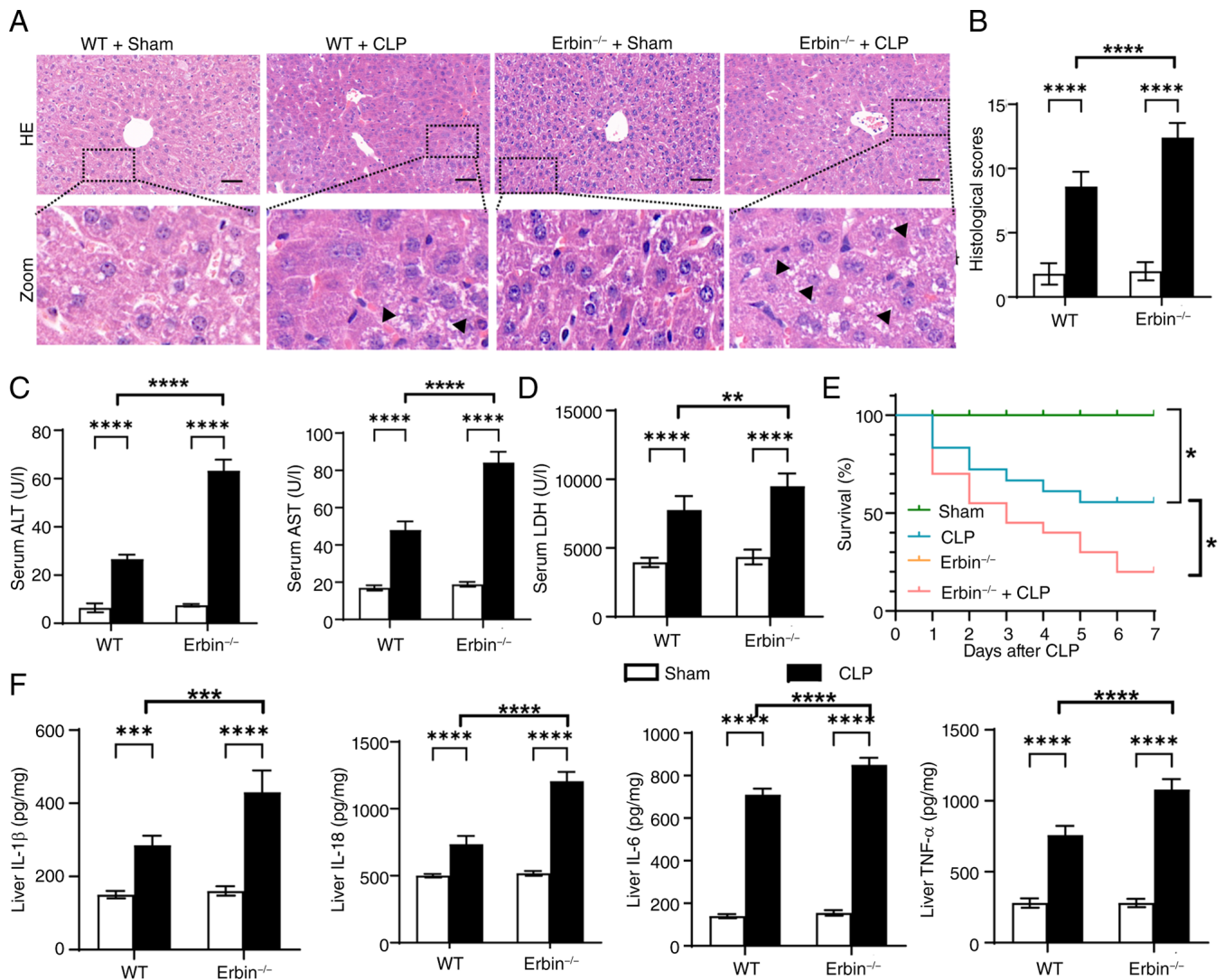


Figure 1. Erbin deficiency aggravates sepsis-induced liver injury. (A) Representative H&E-stained liver sections from WT and Erbin^{-/-} mice subjected to sham or CLP surgery. Black arrowheads indicate swollen hepatocytes. Scale bar, 50 μ m. (B) Liver injury scores (n=5). (C and D) Serum AST, ALT and LDH levels (n=6). (E) 7-day survival after CLP (WT+Sham, n=10; WT+CLP, n=18; Erbin^{-/-}+Sham, n=10; Erbin^{-/-}+CLP, n=20). (F) TNF- α , IL-6, IL-1 β and IL-18 levels in liver homogenate supernatants (n=4). Data are presented as the mean $\pm$ SD. **P<0.01, ***P<0.001 and ****P<0.0001. WT, wild type; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CLP, cecal ligation and puncture.

death in Erbin-deficient BMDMs after LPS/ATP stimulation (Fig. 3C-F). In addition, LPS/ATP increased IL-6, IL-18, TNF- α and IL-1 β mRNA expression, and these increases were further amplified by Erbin deficiency (Fig. 3G). Collectively, these findings indicate that Erbin negatively regulates pyroptotic and inflammatory responses in macrophages.

Erbin deficiency aggravates oxidative stress in vivo and in vitro. To evaluate the role of Erbin in mitochondrial injury and oxidative stress during SLI, mitochondrial ultrastructure and ROS production were examined *in vivo* and *in vitro*. Transmission electron microscopy revealed that CLP-induced mitochondrial abnormalities, including swelling, loss of cristae and matrix clearing, were more severe in Erbin^{-/-} mice than in WT mice (Fig. 4A and C). This mitochondrial damage was accompanied by increased hepatic ROS production, as indicated by DHE staining (Fig. 4B and D). Biochemical assays further showed higher MDA levels and lower SOD activity in Erbin^{-/-}+CLP mice than in WT+CLP mice (Fig. 4E and F).

Consistent results were obtained *in vitro*, where Erbin-deficient BMDMs exhibited greater intracellular ROS accumulation after LPS/ATP stimulation (Fig. 4G and H), together with higher MDA levels and lower SOD activity (Fig. 4I and J).

Erbin deficiency promotes TXNIP/NLRP3 pathway activation in vivo and in vitro. Excessive ROS promotes the interaction of TXNIP with NLRP3 and thereby activates the inflammasome (45). Because Erbin deficiency exacerbated sepsis-induced oxidative stress, TXNIP expression and its interaction with NLRP3 were examined. TXNIP and NLRP3 protein levels were increased after CLP and were further elevated in Erbin^{-/-} mice (Fig. 5A and B). Similarly, LPS/ATP increased TXNIP and NLRP3 expression in BMDMs, with greater increases observed in Erbin-deficient cells (Fig. 5C and D). Co-immunoprecipitation further showed that LPS/ATP enhanced the TXNIP-NLRP3 interaction and that Erbin deficiency further strengthened this association (Fig. 5E).

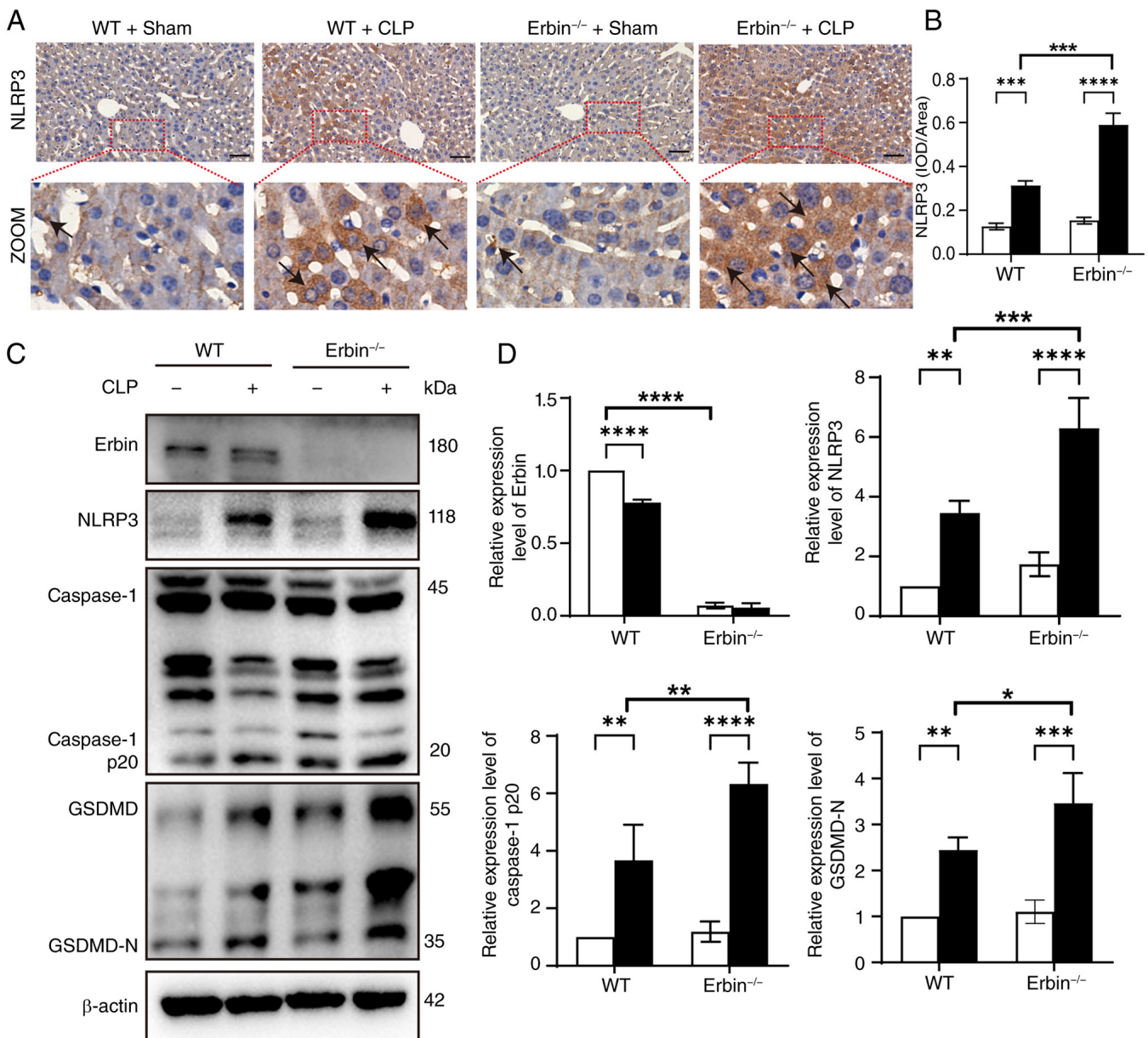


Figure 2. Erbin deficiency enhances NLRP3 inflammasome activation during sepsis-induced liver injury. (A) Representative immunohistochemical staining of hepatic NLRP3. Black arrows indicate NLRP3-positive staining. Scale bar, 50 μ m. (B) Quantification of NLRP3-positive areas (n=5). (C and D) Western blot analysis and quantification of Erbin, NLRP3, caspase-1 and GSDMD in liver tissues (n=3). Data are presented as the mean $\pm$ SD. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. WT, wild type; CLP, cecal ligation and puncture.

Erbin regulates pyroptosis through the mitochondrial ROS/TXNIP axis. To determine whether mtROS mediates the enhanced pyroptotic response associated with Erbin deficiency, MitoQ, a mitochondria-targeted antioxidant, was used to reduce mtROS. LPS/ATP stimulation increased mtROS levels in BMDMs, and this increase was further amplified by Erbin deficiency; MitoQ pretreatment markedly reduced mtROS accumulation in Erbin-deficient BMDMs (Fig. 6A and B). Mitochondrial membrane potential was subsequently assessed using JC-1 staining. LPS/ATP induced mitochondrial depolarization, which was exacerbated by Erbin deficiency, whereas MitoQ partially restored mitochondrial membrane potential in Erbin-deficient BMDMs (Fig. 6C and D). MitoQ did not alter Erbin expression (Fig. 6E and G), indicating that it acted downstream of Erbin. However, MitoQ partially reduced

TXNIP, NLRP3 and GSDMD-N expression in Erbin-deficient BMDMs after LPS/ATP stimulation (Fig. 6F and H). Consistently, Calcein-AM/PI staining and cytokine assays showed that MitoQ attenuated LPS/ATP-induced cell death and inflammatory cytokine production (IL-1 β , TNF- α , and IL-18) (Fig. 6I-K). Collectively, these results support a role for mtROS in Erbin deficiency-associated TXNIP/NLRP3 inflammasome activation and pyroptosis.

Clinical relevance of Erbin expression in human sepsis. To further examine the clinical relevance of Erbin, three independent GEO datasets containing samples from patients with sepsis and healthy controls were analyzed. Erbin expression was significantly decreased in patients with sepsis in GSE294059, whereas no significant differences were

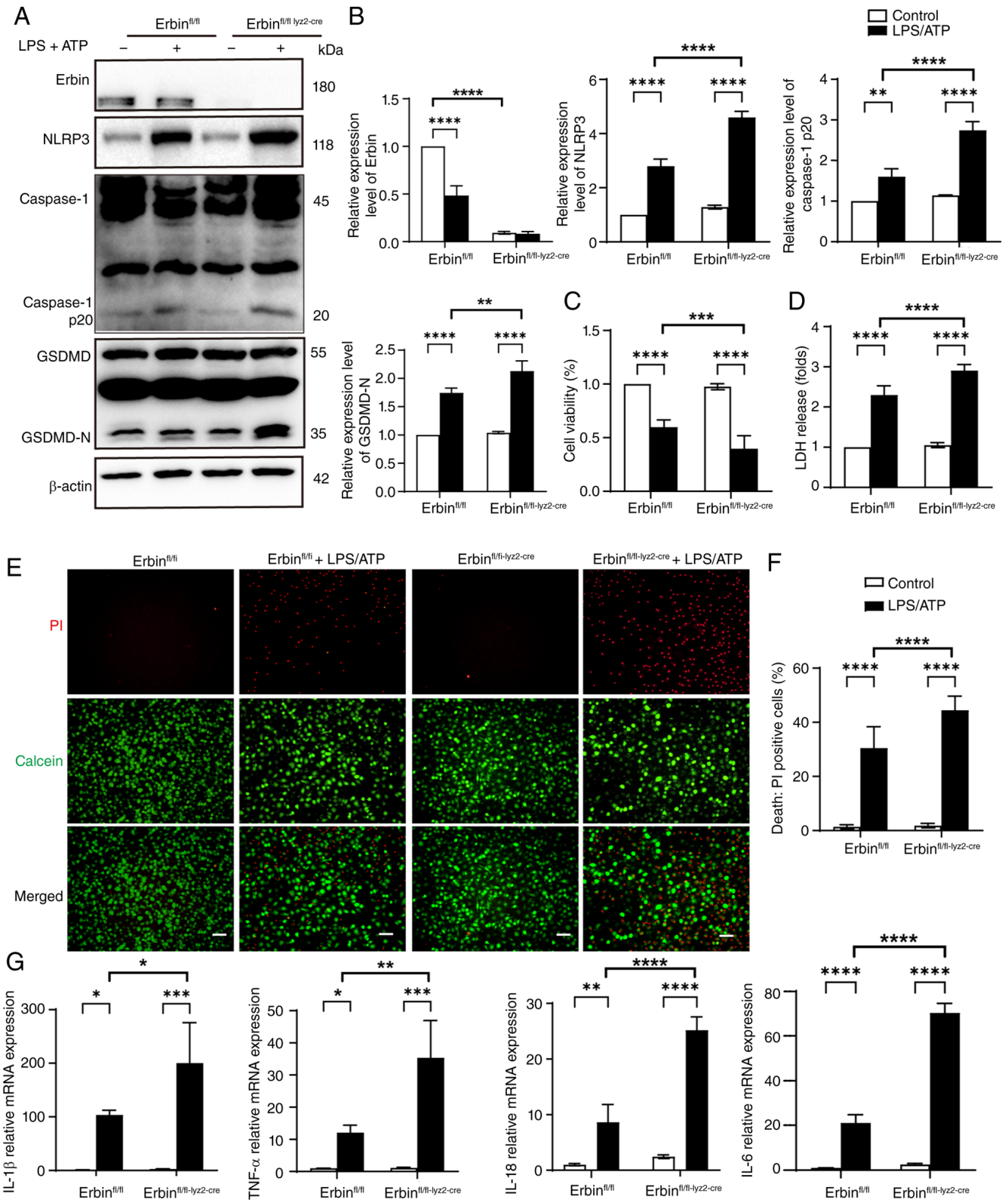


Figure 3. Erbin deficiency enhances pyroptosis and inflammation in BMDMs. (A and B) Western blot analysis and quantification of Erbin, NLRP3, caspase-1 and GSDMD in BMDMs (n=3). (C) Cell viability measured using the cell counting kit-8 assay (n=6). (D) Relative LDH release (n=5). (E and F) Representative Calcein-AM/PI staining and quantification of cell death (n=5). Calcein-AM and PI indicate live and dead cells, respectively. Scale bar, 100 μ m. (G) Relative mRNA levels of IL-6, IL-18, TNF- α and IL-1 β measured by RT-qPCR (n=3). Data are presented as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. BMDMs, bone marrow-derived macrophages; LDH, lactate dehydrogenase.

observed in GSE228541 or GSE28750 (Fig. S2A, C and E). Next, the correlation between Erbin and NLRP3 expression in sepsis samples was examined. No significant correlation

was detected in GSE294059 (Pearson $r=0.499$, $P=0.392$; Spearman $\rho=0.400$, $P=0.505$), GSE228541 (Spearman $\rho=0.332$, $P=0.246$) or GSE28750 (Pearson $r=-0.431$, $P=0.213$;

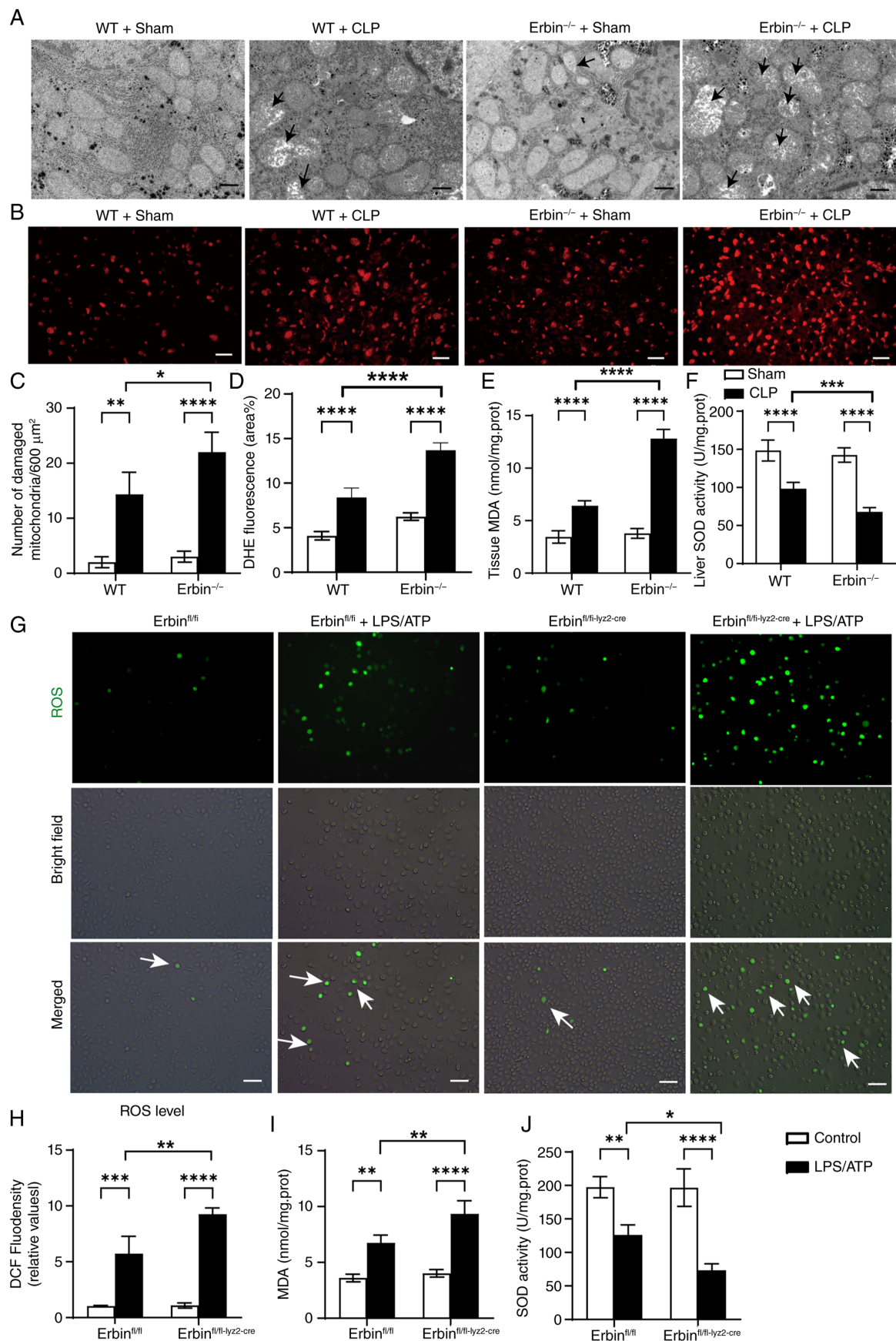


Figure 4. Erbin deficiency aggravates oxidative injury *in vivo* and *in vitro*. (A and C) Representative transmission electron microscopy images of liver mitochondria and quantification of damaged mitochondria (n=3). Arrows indicate damaged mitochondria. Scale bar, 1 μm. (B and D) Representative DHE staining of liver sections and fluorescence quantification (n=5). Scale bar, 50 μm. (E) Hepatic MDA levels and (F) SOD activity (n=5). (G and H) Representative DCFH-DA staining of BMDMs and fluorescence quantification (n=5). White arrows indicate ROS-positive cells. Scale bar, 50 μm. (I and J) MDA levels and SOD activity in BMDMs (n=5). Data are presented as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. MDA, malondialdehyde; SOD, superoxide dismutase; WT, wild type; CLP, cecal ligation and puncture; ROS, reactive oxygen species; BMDMs, bone marrow-derived macrophages; LPS, lipopolysaccharide.

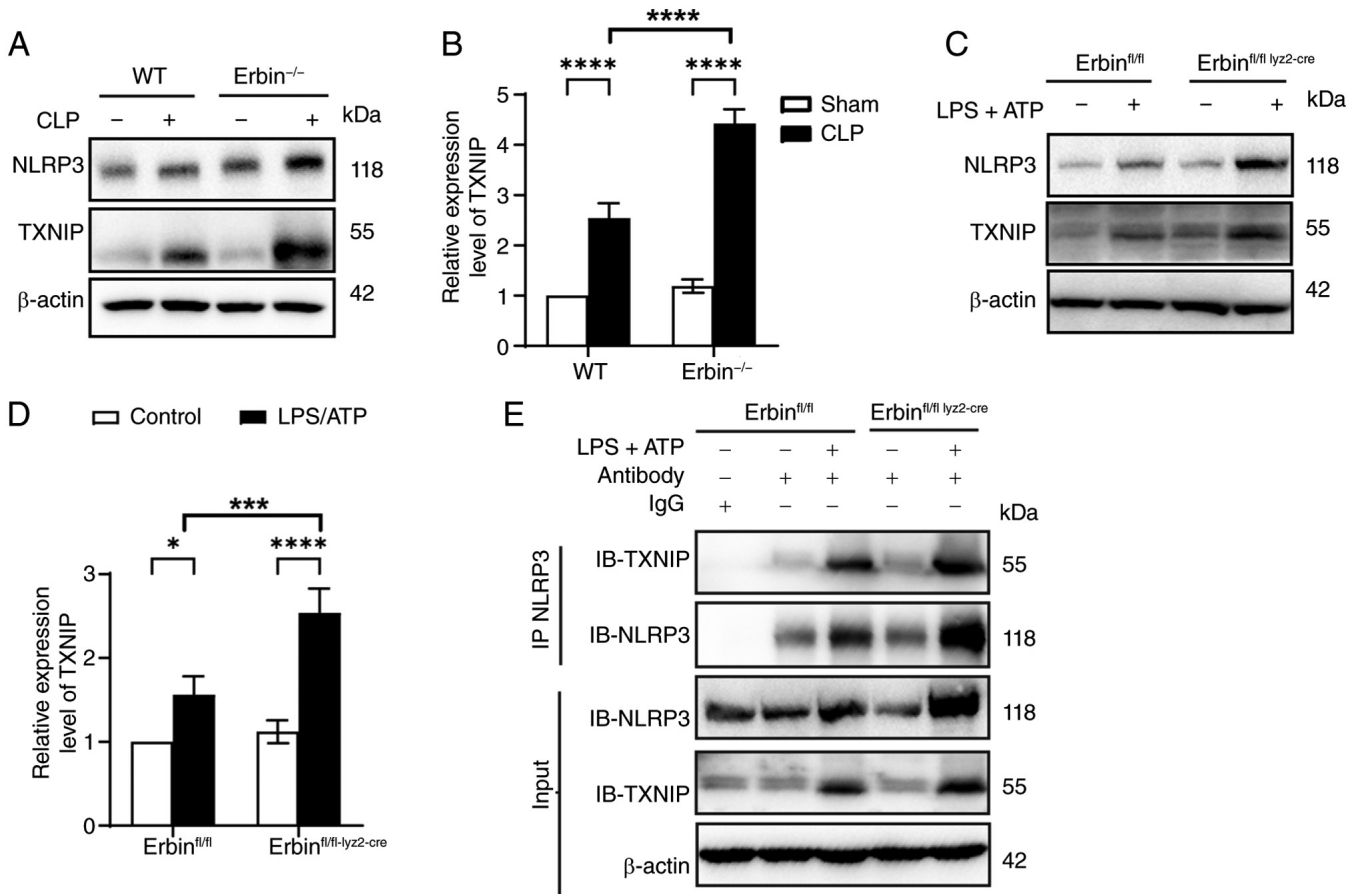


Figure 5. Erbin deficiency activates the TXNIP/NLRP3 inflammasome axis. (A and B) Western blot analysis and quantification of Erbin, TXNIP and NLRP3 in liver tissues (n=3). (C and D) Western blot analysis and quantification of Erbin, TXNIP and NLRP3 in BMDMs (n=3). (E) Co-immunoprecipitation analysis of the association between TXNIP and NLRP3 in BMDMs under the indicated conditions. Species-matched normal IgG was used as a representative negative IP control, and the corresponding input lysates are shown. The results support an association between TXNIP and NLRP3 under the experimental conditions examined. Data are presented as the mean $\pm$ SD. * $P < 0.05$, *** $P < 0.001$ and **** $P < 0.001$. WT, wild type; LPS, lipopolysaccharide; CLP, cecal ligation puncture; IB, immunoblotting; IP, immunoprecipitation.

Spearman $\rho = -0.358$, $P = 0.310$) (Fig. S2B, D and F). These data indicate that Erbin expression is altered in at least one human sepsis cohort, although this change and its association with NLRP3 expression were not consistent across datasets.

Discussion

Sepsis is a life-threatening syndrome characterized by systemic inflammation and organ dysfunction. Liver dysfunction is notably associated with poor outcomes in patients with sepsis (46), yet effective therapeutic strategies remain limited. In the present study, a murine CLP model was used to investigate the role of Erbin in SLI (19). Our previous work identified Erbin as a regulator of pyroptosis in sepsis-induced acute kidney injury and LPS-stimulated HK-2 cells, although the underlying mechanism remained unclear (47). Extending these observations, the present findings demonstrate that Erbin deficiency aggravates CLP-induced liver injury and enhances NLRP3 inflammasome activation. Notably, the present study extends previous observations by examining the contribution of Erbin to macrophage inflammatory responses during SLI. Specifically, the present findings show that Erbin deficiency aggravates sepsis-induced liver injury and enhances mitochondrial ROS/NLRP3-associated inflammatory responses,

while the BMDM experiments further support a macrophage-intrinsic contribution to this process.

During sepsis, pattern-recognition receptors detect pathogen-associated and damage-associated molecular patterns, promoting NLRP3 inflammasome assembly. Activated NLRP3 recruits ASC and pro-caspase-1, leading to caspase-1 activation and cleavage of pro-IL-1 β , pro-IL-18 and GSDMD, thereby inducing cytokine release and pyroptosis (48). NLRP3 inflammasome activation has a notable role in SLI. BATF-mediated transcriptional activation of GBP5 aggravates SLI by promoting NLRP3 inflammasome activation (49), whereas maresin 1 alleviates SLI by suppressing NLRP3-mediated macrophage pyroptosis and inflammation (50). Consistent with these studies, CLP activated hepatic pyroptosis in the present study, and Erbin deficiency further enhanced inflammasome activation and inflammatory responses. Excessive pro-inflammatory cytokine production by activated hepatic macrophages, including Kupffer cells and infiltrating monocyte-derived macrophages, contributes to sepsis-induced hepatic injury (51). Therefore, BMDMs were used in the present study as an *in vitro* model to investigate the macrophage-intrinsic role of Erbin under inflammatory stimulation. The *in vitro* studies with BMDMs consistently showed that Erbin depletion

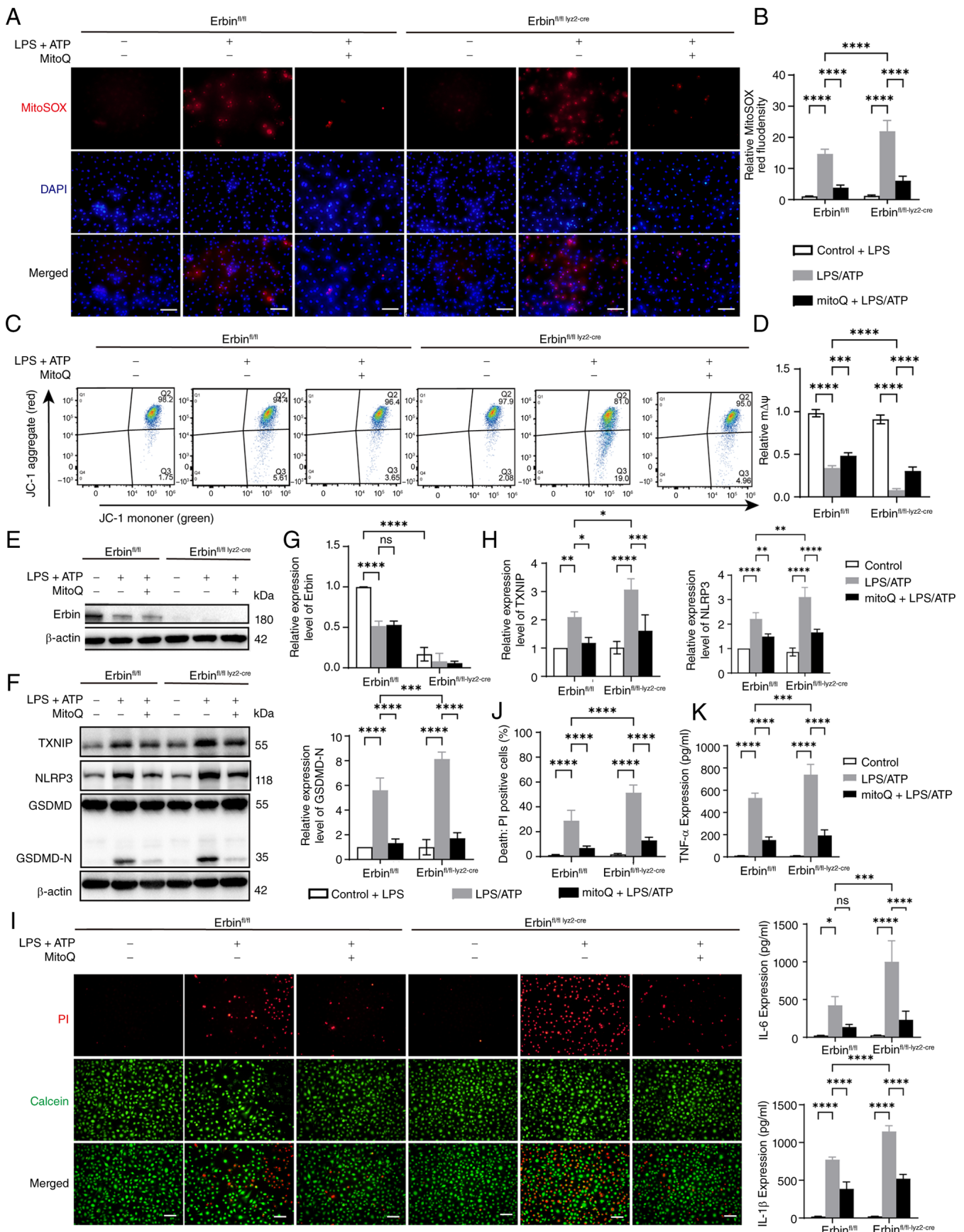


Figure 6. Erbin regulates pyroptosis through the mitochondrial ROS/TXNIP axis. (A and B) Representative MitoSOX Red staining and quantification of mitochondrial reactive oxygen species in BMDMs (n=4). Scale bar, 50 μ m. (C) Representative flow cytometry plots of JC-1 staining. (D) Quantification of the JC-1 red/green fluorescence ratio (n=4). (E and G) Western blot analysis and quantification of Erbin in BMDMs (n=3). (F and H) Western blot analysis and quantification of TXNIP, NLRP3 and GSDMD in BMDMs (n=3). (I and J) Representative Calcein-AM/PI staining and quantification of cell death (n=5). Calcein-AM and PI indicate live and dead cells, respectively. Scale bar, 100 μ m. (K) TNF- α , IL-6 and IL-1 β levels in BMDM culture supernatants (n=4). Data are presented as the mean $\pm$ SD. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. BMDMs, bone marrow-derived macrophages; LPS, lipopolysaccharide; ns, not significant.

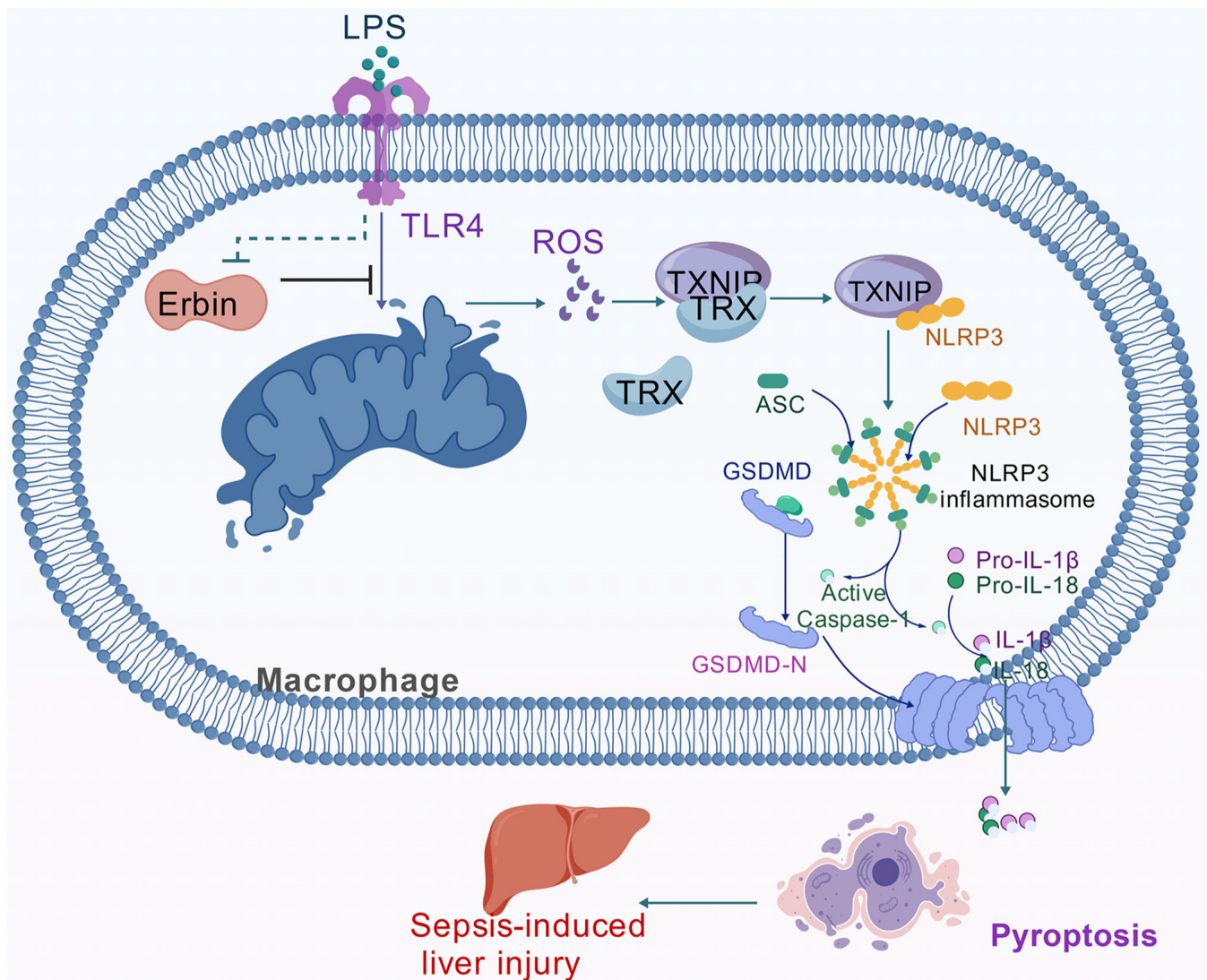


Figure 7. Proposed mechanism by which Erbin negatively regulates the mitochondrial ROS/TXNIP/NLRP3 signaling pathway during sepsis-induced liver injury. ROS, reactive oxygen species; GSDMD, gasdermin D; LPS, lipopolysaccharide.

enhanced LPS/ATP-induced inflammation and pyroptosis activation. These findings support a negative regulatory role of Erbin in macrophage pyroptotic responses.

Oxidative stress is a major contributor to sepsis-induced organ dysfunction (52,53). Accumulated ROS can activate the NLRP3 inflammasome and thereby amplify inflammatory injury (54). Erbin, an endogenous inhibitor of RAS-RAF signaling, has previously been implicated in ROS regulation (23). Consistent with the present study, Erbin deficiency increased ROS production and promoted TXNIP/NLRP3 inflammasome activation in both CLP-challenged mice and LPS/ATP-stimulated BMDMs. The ROS/TXNIP/NLRP3 axis is an established mechanism of inflammatory injury, and inhibition of ROS accumulation can suppress this pathway activation and reduce tissue damage (55,56). In line with this concept, loss of Erbin disrupted redox homeostasis and enhanced TXNIP/NLRP3 inflammasome activation and pyroptotic responses. Furthermore, Co-IP analysis showed that Erbin deficiency increased the interaction between TXNIP and NLRP3, a key event that promotes caspase-1 activation and IL-1 β maturation (57).

To further examine the causal contribution of mtROS, BMDMs were treated with MitoQ, a mitochondria-targeted antioxidant previously shown to suppress mtROS accumulation and NLRP3 inflammasome activation (58). MitoQ reduced mtROS accumulation and partially restored mitochondrial membrane potential in LPS/ATP-stimulated Erbin-deficient BMDMs. These changes were accompanied by reduced TXNIP expression and decreased NLRP3 and GSDMD-N levels. Because TXNIP links oxidative stress to NLRP3 inflammasome activation (56,59), these findings support mtROS as an upstream mediator of TXNIP/NLRP3 signaling in Erbin-deficient macrophages. MitoQ also attenuated cell death and inflammatory cytokine production, consistent with its reported anti-inflammatory effects (60). Notably, MitoQ did not alter Erbin expression, indicating that its protective effects occurred downstream of Erbin; however, the absence of a MitoQ-only control group limits assessment of its basal effects. Collectively, the present findings support a model in which Erbin deficiency aggravates SLI, at least in part, by enhancing mitochondrial ROS/TXNIP/NLRP3 signaling, while MitoQ attenuates this inflammatory response (Fig. 7).

Analysis of three independent human sepsis datasets showed significantly reduced Erbin expression in GSE294059, whereas no significant difference was observed in GSE228541 or GSE28750. In addition, Erbin expression was not significantly correlated with NLRP3 expression in the analyzed sepsis samples. The variability among clinical cohorts may be related to differences in disease stage, patient characteristics, treatment and sample composition. Moreover, NLRP3 inflammasome activation is regulated at both transcriptional and post-transcriptional levels and therefore may not be directly reflected by the correlation between Erbin and NLRP3 mRNA abundance. Further validation in well-characterized clinical cohorts, particularly measurements of circulating Erbin together with IL-1 β and IL-18, will be necessary to determine the clinical relevance of this pathway.

Several limitations should be considered. First, the use of global Erbin-knockout mice *in vivo* and BMDMs derived from macrophage-specific conditional-knockout mice *in vitro* limits cell type-specific interpretation. Although the *in vivo* findings demonstrate that Erbin deficiency aggravates SLI, the relative contributions of hepatocytes, Kupffer cells, recruited macrophages and other hepatic cell populations cannot be distinguished. Furthermore, BMDMs do not fully reproduce the heterogeneity and tissue-specific functions of hepatic macrophages, particularly resident Kupffer cells. Cell type-specific Erbin-knockout models and primary hepatic macrophages will therefore be required to define the contributions of individual hepatic cell populations. Second, although the survival difference was statistically significant, the relatively small sample size warrants validation in larger cohorts. Third, analysis of three independent human sepsis datasets showed significantly decreased Erbin expression in GSE294059, whereas no significant difference was observed in GSE228541 or GSE28750. In addition, Erbin expression was not significantly correlated with NLRP3 expression in the analyzed sepsis samples. These findings suggest that alterations in Erbin expression may occur in human sepsis, but they are not consistent across different clinical cohorts. The present study also lacks direct validation using clinical samples. Serum Erbin levels and their associations with inflammatory markers and disease severity remain to be investigated in future clinical cohorts.

In summary, the present findings support a protective role of Erbin in experimental SLI, associated with suppression of mitochondrial ROS accumulation and TXNIP/NLRP3 inflammasome activation. Further studies using cell type-specific models and clinical samples are needed to establish the translational relevance of Erbin and to determine whether it may represent a therapeutic target for SLI.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant nos. 82371370, 82172144 and 82372156).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JZ wrote the original draft, conceived the study, performed the animal and cell experiments, including establishment of the CLP model, isolation and culture of BMDMs, and biochemical and molecular experiments and organized the experimental data and performed the statistical analyses. XWa reviewed and edited the manuscript and participated in the design and implementation of the animal, cell and molecular biology experiments. GJ supervised the study and contributed to the study design and conceptualization. XWu reviewed and edited the manuscript, supervised the study, acquired funding and contributed to the conceptualization of the study. XS wrote the original draft, participated in the study design and acquired funding. JZ and XS confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Animal Experimentation Committee of Wuhan University (Wuhan, China; approval no. WQ20210298) and were conducted in accordance with the ARRIVE guidelines.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Selim SA, El-Baset SAA, Kattaia AAA, Askar EM and Elkader EA: Bone marrow-derived mesenchymal stem cells ameliorate liver injury in a rat model of sepsis by activating Nrf2 signaling. *Histochem Cell Biol* 151: 249-262, 2019.
- Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, *et al*: The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA* 315: 801-810, 2016.
- Yan J, Li S and Li S: The role of the liver in sepsis. *Int Rev Immunol* 33: 498-510, 2014.
- Kikuchi S, Piraino G, O'Connor M, Wolfe V, Ridings K, Lahni P and Zingarelli B: Hepatocyte-specific deletion of AMPK α 1 results in worse outcomes in mice subjected to sepsis in a sex-specific manner. *Front Immunol* 11: 210, 2020.
- Kim TS and Choi DH: Liver dysfunction in sepsis. *Korean J Gastroenterol* 75: 182-187, 2020.
- Liang H, Song H, Zhang X, Song G, Wang Y, Ding X, Duan X, Li L, Sun T and Kan Q: Metformin attenuated sepsis-related liver injury by modulating gut microbiota. *Emerg Microbes Infect* 11: 815-828, 2022.
- Savio LEB, de Andrade Mello P, Figliuolo VR, de Avelar Almeida TF, Santana PT, Oliveira SDS, Silva CLM, Feldbrügge L, Csizmadia E, Minshall RD, *et al*: CD39 limits P2X7 receptor inflammatory signaling and attenuates sepsis-induced liver injury. *J Hepatol* 67: 716-726, 2017.

8. Guo Y, Guo W, Chen H, Sun J and Yin Y: Mechanisms of sepsis-induced acute liver injury: A comprehensive review. *Front Cell Infect Microbiol* 15: 1504223, 2025.
9. Heymann F, Hammerich L, Storch D, Bartneck M, Huss S, Rüsseler V, Gassler N, Lira SA, Luedde T, Trautwein C and Tacke F: Hepatic macrophage migration and differentiation critical for liver fibrosis is mediated by the chemokine receptor C-C motif chemokine receptor 8 in mice. *Hepatology* 55: 898-909, 2012.
10. Nakamoto N, Ebinuma H, Kanai T, Chu PS, Ono Y, Mikami Y, Ojio K, Lipp M, Love PE, Saito H and Hibi T: CCR9+ macrophages are required for acute liver inflammation in mouse models of hepatitis. *Gastroenterology* 142: 366-376, 2012.
11. Xu W, Hou H, Yang W, Tang W and Sun L: Immunologic role of macrophages in sepsis-induced acute liver injury. *Int Immunopharmacol* 143: 113492, 2024.
12. Jia Y, Li Z, Feng Y, Cui R, Dong Y, Zhang X, Xiang X, Qu K, Liu C and Zhang J: Methane-rich saline ameliorates sepsis-induced acute kidney injury through anti-inflammation, antioxidative, and antiapoptosis effects by regulating endoplasmic reticulum stress. *Oxid Med Cell Longev* 2018: 4756846, 2018.
13. Zou Z, Liu B, Zeng L, Yang X, Huang R, Wu C, Zhu H, Gao Y, Yuan D and Yu J: Cx43 inhibition attenuates sepsis-induced intestinal injury via downregulating ROS transfer and the activation of the JNK1/Sirt1/FoxO3a signaling pathway. *Mediators Inflamm* 2019: 7854389, 2019.
14. Zhang X, Zhang JH, Chen XY, Hu QH, Wang MX, Jin R, Zhang QY, Wang W, Wang R, Kang LL, *et al*: Reactive oxygen species-induced TXNIP drives fructose-mediated hepatic inflammation and lipid accumulation through NLRP3 inflammasome activation. *Antioxid Redox Signal* 22: 848-870, 2015.
15. Kovacs SB and Miao EA: Gasdermins: Effectors of pyroptosis. *Trends Cell Biol* 27: 673-684, 2017.
16. Coll RC, Schroder K and Pelegrín P: NLRP3 and pyroptosis blockers for treating inflammatory diseases. *Trends Pharmacol Sci* 43: 653-668, 2022.
17. Vasudevan SO, Behl B and Rathinam VA: Pyroptosis-induced inflammation and tissue damage. *Semin Immunol* 69: 101781, 2023.
18. He H, Jiang H, Chen Y, Ye J, Wang A, Wang C, Liu Q, Liang G, Deng X, Jiang W and Zhou R: Oridonin is a covalent NLRP3 inhibitor with strong anti-inflammasome activity. *Nat Commun* 9: 2550, 2018.
19. Chen YL, Xu G, Liang X, Wei J, Luo J, Chen GN, Yan XD, Wen XP, Zhong M and Lv X: Inhibition of hepatic cells pyroptosis attenuates CLP-induced acute liver injury. *Am J Transl Res* 8: 5685-5695, 2016.
20. Birrane G, Chung J and Ladias JAA: Novel mode of ligand recognition by the Erbin PDZ domain. *J Biol Chem* 278: 1399-1402, 2003.
21. McDonald C, Chen FF, Ollendorff V, Ogura Y, Marchetto S, Lécine P, Borg JP and Nuñez G: A role for Erbin in the regulation of Nod2-dependent NF-kappaB signaling. *J Biol Chem* 280: 40301-40309, 2005.
22. Shen T, Li S, Cai LD, Liu JL, Wang CY, Gan WJ, Li XM, Wang JR, Sun LN, Deng M, *et al*: Erbin exerts a protective effect against inflammatory bowel disease by suppressing autophagic cell death. *Oncotarget* 9: 12035-12049, 2018.
23. Xie CM, Wei D, Zhao L, Marchetto S, Mei L, Borg JP and Sun Y: Erbin is a novel substrate of the Sag-βTrCP E3 ligase that regulates KrasG12D-induced skin tumorigenesis. *J Cell Biol* 209: 721-737, 2015.
24. Abais JM, Xia M, Zhang Y, Boini KM and Li PL: Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? *Antioxid Redox Signal* 22: 1111-1129, 2015.
25. Hawkins P, Morton DB, Burman O, Dennison N, Honess P, Jennings M, Lane S, Middleton V, Roughan JV, Wells S, *et al*: A guide to defining and implementing protocols for the welfare assessment of laboratory animals: Eleventh report of the BVAAWF/FRAME/RSPCA/UFPAW Joint working group on refinement. *Lab Anim* 45: 1-13, 2011.
26. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, *et al*: The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* 18: e3000410, 2020.
27. Rittirsch D, Huber-Lang MS, Flierl MA and Ward PA: Immunodesign of experimental sepsis by cecal ligation and puncture. *Nat Protoc* 4: 31-36, 2009.
28. Bi J, Zhang J, Ren Y, Du Z, Li Q, Wang Y, Wei S, Yang L, Zhang J, Liu C, *et al*: Irisin alleviates liver ischemia-reperfusion injury by inhibiting excessive mitochondrial fission, promoting mitochondrial biogenesis and decreasing oxidative stress. *Redox Biol* 20: 296-306, 2019.
29. Tsuji N, Tsuji T, Yamashita T, Hayase N, Hu X, Yuen PS and Star RA: BAM15 treats mouse sepsis and kidney injury, linking mortality, mitochondrial DNA, tubule damage, and neutrophils. *J Clin Invest* 133: e152401, 2023.
30. Yin XY, Tang XH, Wang SX, Zhao YC, Jia M, Yang JJ, Ji MH and Shen JC: HMGB1 mediates synaptic loss and cognitive impairment in an animal model of sepsis-associated encephalopathy. *J Neuroinflammation* 20: 69, 2023.
31. Cho HI, Kim SJ, Choi JW and Lee SM: Genipin alleviates sepsis-induced liver injury by restoring autophagy. *Br J Pharmacol* 173: 980-991, 2016.
32. Zhu J, Jin Z, Wang J, Wu Z, Xu T, Tong G, Shen E, Fan J, Jiang C, Wang J, *et al*: FGF21 ameliorates septic liver injury by restraining proinflammatory macrophages activation through the autophagy/HIF-1α axis. *J Adv Res* 69: 477-494, 2025.
33. Zhan Y, Xu D, Tian Y, Qu X, Sheng M, Lin Y, Ke M, Jiang L, Xia Q, Kaldas FM, *et al*: Novel role of macrophage TXNIP-mediated CYLD-NRF2-OASL1 axis in stress-induced liver inflammation and cell death. *JHEP Rep* 4: 100532, 2022.
34. Wang Z, Chen X, Li J, Chen M, Zhu H, Li X, Yu W, Xia N, Zhang Y, Sun L, *et al*: Macrophages Atp6v0d2 regulates XBP1-mediated cholesterol metabolism to suppress metabolic dysfunction-associated steatohepatitis progression. *Int Immunopharmacol* 161: 115088, 2025.
35. Chiu LY, Huang DY and Lin WW: PARP-1 regulates inflammasome activity by poly-ADP-ribosylation of NLRP3 and interaction with TXNIP in primary macrophages. *Cell Mol Life Sci* 79: 108, 2022.
36. Feng Y, Cui R, Li Z, Zhang X, Jia Y, Zhang X, Shi J, Qu K, Liu C and Zhang J: Methane alleviates acetaminophen-induced liver injury by inhibiting inflammation, oxidative stress, endoplasmic reticulum stress, and apoptosis through the Nrf2/HO-1/NQO1 signaling pathway. *Oxid Med Cell Longev* 2019: 7067619, 2019.
37. Liao J, Cao Y, Zhao J, Yu B, Wang Y, Li W, Li H, Lv S, Wen W, Cui H and Chen Y: Aqueous extract of *Polygala japonica* Houtt. ameliorated nonalcoholic steatohepatitis in mice through restoring the gut microbiota disorders and affecting the metabolites in feces and liver. *Phytomedicine* 118: 154937, 2023.
38. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
39. Luo W, Xu W, Yin Q, Song J, Wang J, Guan Y, Huang J, Yu J, Zou K, Jin D, *et al*: DPEP2 suppresses hyperinflammation via metabolic reprogramming of macrophages in sepsis. *Nat Commun* 17: 3710, 2026.
40. Oda S, Matsumoto H, Togami Y, Yoshimura J, Ito H, Onishi S, Muratsu A, Mitsuyama Y, Okuzaki D, Ogura H, *et al*: mRNA-miRNA integration analysis of T-cell exhaustion in sepsis from community-acquired pneumonia. *Acute Med Surg* 12: e70054, 2025.
41. Sutherland A, Thomas M, Brandon RA, Brandon RB, Lipman J, Tang B, McLean A, Pascoe R, Price G, Nguyen T, *et al*: Development and validation of a novel molecular biomarker diagnostic test for the early detection of sepsis. *Crit Care* 15: R149, 2011.
42. Possamai LA, Thursz MR, Wendon JA and Antoniadou CG: Modulation of monocyte/macrophage function: A therapeutic strategy in the treatment of acute liver failure. *J Hepatol* 61: 439-445, 2014.
43. Biasizzo M, Trstenjak-Prebanda M, Dolinar K, Pirkmajer S, Završnik J, Turk B and Kopitar-Jerala N: Cystatin C deficiency increases LPS-induced sepsis and NLRP3 inflammasome activation in mice. *Cells* 10: 2071, 2021.
44. Liu YG, Chen JK, Zhang ZT, Ma XJ, Chen YC, Du XM, Liu H, Zong Y and Lu GC: NLRP3 inflammasome activation mediates radiation-induced pyroptosis in bone marrow-derived macrophages. *Cell Death Dis* 8: e2579, 2017.
45. Tschopp J and Schroder K: NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol* 10: 210-215, 2010.
46. Piano S, Bartoletti M, Tonon M, Baldassarre M, Chies G, Romano A, Viale P, Vettore E, Domenicali M, Stanco M, *et al*: Assessment of Sepsis-3 criteria and quick SOFA in patients with cirrhosis and bacterial infections. *Gut* 67: 1892-1899, 2018.

47. Liu Y, Fang Q, Ming T, Zuo J, Jing G and Song X: Knockout of Erbin promotes pyroptosis via regulating NLRP3/caspase-1/Gasdermin D pathway in sepsis-induced acute kidney injury. *Clin Exp Nephrol* 27: 781-790, 2023.
48. He W, Dong H, Wu C, Zhong Y and Li J: The role of NLRP3 inflammasome in sepsis: A potential therapeutic target. *Int Immunopharmacol* 115: 109697, 2023.
49. Guo H, Ni M, Xu J, Chen F, Yao Z, Yao Y, Liu C and Du Q: Transcriptional enhancement of GBP-5 by BATF aggravates sepsis-associated liver injury via NLRP3 inflammasome activation. *FASEB J* 35: e21672, 2021.
50. Yang W, Tao K, Zhang P, Chen X, Sun X and Li R: Maresin 1 protects against lipopolysaccharide/d-galactosamine-induced acute liver injury by inhibiting macrophage pyroptosis and inflammatory response. *Biochem Pharmacol* 195: 114863, 2022.
51. Gong S, Yan Z, Liu Z, Niu M, Fang H, Li N, Huang C, Li L, Chen G, Luo H, *et al*: Intestinal microbiota mediates the susceptibility to polymicrobial sepsis-induced liver injury by granisetron generation in mice. *Hepatology* 69: 1751-1767, 2019.
52. Li Z, Liu T, Feng Y, Tong Y, Jia Y, Wang C, Cui R, Qu K, Liu C and Zhang J: PPAR γ alleviates sepsis-induced liver injury by inhibiting hepatocyte pyroptosis via inhibition of the ROS/TXNIP/NLRP3 signaling pathway. *Oxid Med Cell Longev* 2022: 1269747, 2022.
53. Galley HF: Oxidative stress and mitochondrial dysfunction in sepsis. *Br J Anaesth* 107: 57-64, 2011.
54. Latz E, Xiao TS and Stutz A: Activation and regulation of the inflammasomes. *Nat Rev Immunol* 13: 397-411, 2013.
55. Liu T, Wang W, Liu M, Ma Y, Mu F, Feng X, Zhang Y, Guo C, Ding Y and Wen A: Z-Guggulsterone alleviated oxidative stress and inflammation through inhibiting the TXNIP/NLRP3 axis in ischemic stroke. *Int Immunopharmacol* 89: 107094, 2020.
56. Han Y, Xu X, Tang C, Gao P, Chen X, Xiong X, Yang M, Yang S, Zhu X, Yuan S, *et al*: Reactive oxygen species promote tubular injury in diabetic nephropathy: The role of the mitochondrial ros-txnip-nlrp3 biological axis. *Redox Biol* 16: 32-46, 2018.
57. Chen D, Dixon BJ, Doycheva DM, Li B, Zhang Y, Hu Q, He Y, Guo Z, Nowrangi D, Flores J, *et al*: IRE1 α inhibition decreased TXNIP/NLRP3 inflammasome activation through miR-17-5p after neonatal hypoxic-ischemic brain injury in rats. *J Neuroinflammation* 15: 32, 2018.
58. Chen W, Guo C, Huang S, Jia Z, Wang J, Zhong J, Ge H, Yuan J, Chen T, Liu X, *et al*: MitoQ attenuates brain damage by polarizing microglia towards the M2 phenotype through inhibition of the NLRP3 inflammasome after ICH. *Pharmacol Res* 161: 105122, 2020.
59. Zhou R, Tardivel A, Thorens B, Choi I and Tschopp J: Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat Immunol* 11: 136-140, 2010.
60. Cen M, Ouyang W, Zhang W, Yang L, Lin X, Dai M, Hu H, Tang H, Liu H, Xia J and Xu F: MitoQ protects against hyperpermeability of endothelium barrier in acute lung injury via a Nrf2-dependent mechanism. *Redox Biol* 41: 101936, 2021.



Copyright © 2026 Zuo et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.