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Mechanistic Study of Mesenchymal Stem Cell-Derived Exosomes from Induced Pluripotent Stem Cells in Mitigating Ischemia-Reperfusion Injury in Mice Liver

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Research Article

Keywords: Hepatic ischemia-reperfusion injury, Exosomes, Mesenchymal Stem Cell, Induced Pluripotent Stem Cell, Mouse

Posted Date: March 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8700122/v1

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Additional Declarations: No competing interests reported.

Abstract

Background: Human induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) provide a unique, non-tissue-derived source of mesenchymal stem cells. The exosomes they secrete have been shown to limit tissue injury, modulate immune responses, and promote cellular self-repair following damage. However, the potential of iPSC-MSCs-Exo to improve hepatic ischemia-reperfusion injury, along with the specific mechanisms involved, remains unclear. This study aimed to establish a mouse model of hepatic ischemia-reperfusion injury to investigate the protective effects of exosomes derived from iPSC-MSCs and explore the underlying mechanisms.

Methods: iPSC-MSCs-Exo was prepared and extracted in vitro. Seventy-two mice with hepatic ischemia-reperfusion injury were randomly divided into four groups: Ham group, IR group, IR+PBS group, and IR+Exo group. The Exo group received iPSC-MSCs-Exo via inferior vena cava injection, while the R+PBS group received an equivalent dose of PBS. Postoperative effects of iPSC-MSCs-Exo on hepatic ischemia-reperfusion injury were evaluated at 6, 12, and 24 hours through serum transaminase levels, oxidative stress markers, HE staining of liver tissue, transmission electron microscopy, and Western blotting.

Results: IPSC-MSCs-Exo were successfully isolated, with sizes ranging from 45 to 120 nanometers. Following the injection of iPSC-MSCs-Exo into the hepatic ischemia-reperfusion injury model, peripheral blood levels of AST and ALT were significantly reduced compared to the control group. Hepatocyte necrosis and sinusoidal congestion were markedly alleviated, reflected by a significant decrease in Suzuki scores. The number of TUNEL-positive and caspase-3-positive cells also decreased significantly. Among oxidative stress markers, SOD levels increased while MDA and MPO levels decreased. Furthermore, the expression levels of apoptosis-related proteins Bax, HMGB1, and Bcl-2 significantly decreased, while Parkin expression increased.

Conclusion: IPSC-MSCs-Exo alleviate hepatic ischemia-reperfusion injury, potentially by reducing oxidative stress and inhibiting apoptosis.

Introduction

Hepatic ischemia-reperfusion injury is a common pathological process that occurs following hepatobiliary surgery, specifically during the restoration of blood flow in liver transplant donors or when hepatic pedicles are clamped during resection. This phenomenon represents a secondary injury that occurs when blood flow is restored to ischemic liver tissue [1–2]. Despite ongoing improvements in surgical techniques that have significantly decreased postoperative complication rates in hepatobiliary surgery, hepatic ischemia-reperfusion injury continues to be a significant contributor to postoperative hepatic dysfunction and even liver failure. Therefore, effectively mitigating the ischemia-reperfusion injury response after hepatobiliary surgery is crucial for enhancing patient perioperative outcomes.

The mechanisms underlying ischemia-reperfusion injury, such as oxidative stress, inflammatory responses, calcium dysregulation, and mitochondrial dysfunction, have been extensively researched [3–

6]. Prevention and treatment strategies primarily involve preoperative conditioning and postoperative pharmacological interventions, although their overall efficacy remains suboptimal. Recently, stem cell therapy has gained attention for its considerable potential in treating various diseases. Among different types of stem cells, mesenchymal stem cells (MSCs) have emerged as an ideal choice for organ repair due to their low immunogenicity, multipotent differentiation capabilities, and ability to secrete a variety of cytokines. Studies indicate [7–9] that MSCs can effectively prevent ischemia-reperfusion injury responses in the heart, kidneys, and liver, although the specific mechanisms involved require further investigation.

Recent studies [10–11] suggest that the therapeutic effects of MSCs primarily arise from their paracrine mechanisms, which provide benefits through the production of bioactive factors that influence neighboring cells. Among these factors, exosomes have emerged as particularly promising therapeutic components. Exosomes are membrane-bound vesicles formed from multivesicular bodies and released when these vesicles fuse with the plasma membrane. They contain various proteins, including adhesion molecules, heat shock proteins, cytoplasmic enzymes, and signaling molecules. Studies indicates [12] that MSC-derived exosomes can limit tissue damage, modulate immune responses, and promote cellular self-repair following injury.

Induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) offer a non-tissue-derived source of mesenchymal stem cells, exhibiting the characteristic morphology, antigen profile, and differentiation potential typical of MSCs [13]. However, whether iPSC-MSCs-Exo can also improve hepatic ischemia-reperfusion injury and the specific mechanisms involved remain unclear. Therefore, this study established a mouse model of hepatic ischemia-reperfusion injury to investigate the protective effects of exosomes derived from iPSC-MSCs on hepatic ischemia-reperfusion injury and their potential mechanisms of action.

Materials and methods

Preparation and Characterisation of iPSC-MSCs-Exo

The second-generation research-grade iPSC-MSCs (RC 01005, Shownin) were purchased from Shownin Biotechnologies Co. Exosomes were isolated using a combination of classical ultracentrifugation and ultrafiltration techniques. iPSC-MSCs were seeded into T75 cell culture flasks for expansion. Once the cell density reached 50–60%, the medium was aspirated, and the flasks were washed three times with PBS. Following this, 15 mL of serum-free MesenGro hMSC medium was added to each flask, and the flasks were placed in a cell culture incubator for continued culture for 48 hours (37°C, 5% CO_2). Collect the supernatant, centrifuge (40°C, 300g, 10 min) to remove cells and cell debris, and centrifuge the supernatant again (40°C, 2000g, 20 min) to remove cell debris. Filter the supernatant through a 0.22 μm filter and transfer to an ultrafiltration centrifuge tube (100,000 NWCO). Centrifuge (40°C, 4000g, 30–50 min) and concentrate the supernatant to 200–300 μl. Transfer this concentrate to an ultracentrifuge tube for the first ultracentrifugation (40°C, 10,000g, 30 min), followed by a second ultracentrifugation (40°C,

100,000g, 60 min). Carefully aspirate the supernatant, resuspend in PBS, and ultracentrifuge again. Repeat this process three times to remove residual proteins and purify the extracted exosomes. Resuspend in PBS, filter through a 0.22 μm filter, and transfer the filtrate to an ultrafiltration centrifuge tube. Centrifuge (40°C, 4000g, 30–50 min). Concentrate the filtrate to 200–300 μL, transfer to a sterile Eppendorf tube, and store at -80°C for subsequent experiments.

Observe the morphology of iPSC-MSCs-Exo using transmission electron microscopy. Analyze the diameter and concentration of exosomes with the Izon qNano nanoparticle analysis system. Determine the protein concentration of iPSC-MSCs-exosomes using the BCA assay. Characterize exosome markers by employing CD9 antibody (1:500; Abcam), CD81 antibody (1:1000; Abcam), Alix antibody (1:1000; Abcam), and Calnexin antibody (1:1000; Proteintech) to detect exosome marker proteins, with cell lysate samples serving as positive controls.

Animal experiments and grouping

The experimental subjects were SPF grade inbred male C57BL/6 mice, approximately 8 weeks old and weighing around 20g. These animals were purchased from Beijing Weitong Lihua Animal Experimental Co. Ltd., and were housed at the Medical Research Center of Beijing Chaoyang Hospital to ensure normal room temperature, a clean environment, standard feed, and a consistent 12-hour circadian rhythm.

After administering isoflurane inhalation anaesthesia, a midline abdominal incision (1–2 cm) was made to expose the liver. The hepatic hilum was carefully dissected, and the hepatic artery, common hepatic duct, and portal vein were occluded using non-traumatic vascular clamps. Partial hepatic ischemia was confirmed by observing a change in liver color. During the procedure, the incision was covered with a moist cotton pad to prevent fluid loss and maintain warmth. After 30 minutes, the vascular clamps were removed, and the return of blood flow was verified by the liver tissue turning a healthy red color. At this point, based on group assignment, an inferior vena cava injection was performed. Following the procedure, the abdominal cavity was sutured with sterile silk thread. All mice (including sham and model groups) were humanely euthanized via sodium pentobarbital overdose. Sodium pentobarbital solution was administered via intraperitoneal injection at a dose of 150 mg/kg. This dosage significantly exceeds the typical anesthetic dose (usually 40–50 mg/kg) to ensure rapid induction of deep anesthesia followed by respiratory depression and cardiac arrest, resulting in a painless and ethical endpoint.

After reperfusion periods of 6, 12, and 24 hours (with six mice per group euthanized at each time point), liver tissue and serum samples were collected for further testing. To obtain blood specimens, one eyeball was enucleated under anesthesia. The blood was centrifuged at 5000 rpm for 10 minutes at 4°C, and the serum was transferred for storage at -80°C. The abdomen was opened to harvest liver tissue, with a portion fixed in 4% paraformaldehyde tissue fixative, while the remaining ischemic liver segments were placed in EP tubes, rapidly frozen in liquid nitrogen, and stored at -80°C for subsequent experiments.

Animal experimental groups: A total of 72 mice were randomly divided into four groups: Sham group (n = 18): underwent abdominal incision and closure without further intervention; IR group (n = 18): experienced hepatic ischaemia-reperfusion injury without inferior vena cava injection; IR + PBS group (18 mice): received 100μl PBS solution via inferior vena cava injection following hepatic ischemia-reperfusion; IR + Exo group (18 mice): received 100μl Exo suspension (containing 100μg) via inferior vena cava injection after hepatic ischemia-reperfusion.

Cell experiments and grouping

Isolation of Primary Mouse Liver Cells: Intact mouse livers were obtained from experimental mice and immersed in pre-chilled DMEM medium at 4°C before being minced. The minced tissue was passed through a 70-μm metal sieve, followed by a 40-μm metal sieve. The cells were then resuspended in pre-chilled DMEM medium at 4°C to create a suspension of primary mouse hepatocytes. The suspension was centrifuged at 800 rpm for 5 minutes at 4°C to collect the cell pellet. The pellet was resuspended in DMEM medium at 4°C and washed twice with DMEM medium at 800 rpm. Live cell count was determined using the trypan blue staining method under a light microscope. The cell density was adjusted according to experimental requirements, and cells were seeded at appropriate concentrations into culture flasks or plates. The cultures were incubated in a cell culture incubator under standard conditions (37°C, 5% CO_2) for at least 24 hours, avoiding movement of culture flasks before cell adherence. The medium was subsequently changed every 2 days or experiments proceeded directly.

Cell experiment groups: Control group: underwent conventional culture for 24 hours; Exo group: cultured in medium supplemented with prepared exosomes (concentration 10 μg/ml), for 24 hours; Cocl2 group: cultured in medium supplemented with prepared Cocl2 solution (concentration 25 mmol/L), for 24 hours; Cocl2 + Exo group: cultured in medium supplemented with both Cocl2 (concentration 25 mmol/L) and exosome (concentration 10 μg/ml) solutions for 24 hours. Cell morphology in each group was observed using transmission electron microscopy.

Measurement of Liver Function and Oxidative Stress Markers

2mL of peripheral blood from each rat were placed at room temperature for 30 minutes, then separated by centrifugation (3500 g/min for 10 min) to obtain serum. ELISA was applied to measure serum levels of alanine aminotransferase (ALT, Jiancheng, China) and aspartate aminotransferase (AST, Jiancheng, China).

Retrieve liver tissue stored at -80°C, weigh it accurately, and place it into a homogenisation tube. Prepare an ice-cold PBS solution (ice-cold) at a ratio of (tissue: PBS) = 1 : 9. Homogenise tissue using an electric homogeniser (4500 rpm, 1 min). Subsequently, centrifuge the homogenate at 4°C (2500 rpm, 10 min), then collect the supernatant for subsequent use. After collecting the supernatant, determine the protein concentration using the BCA Protein Assay Kit (Xiheng Biotechnology). Following the manufacturer's

instructions, assess the activities of superoxide dismutase (SOD), malondialdehyde (MDA), and myeloperoxidase (MPO) in the liver tissue using their respective assay kits (Xiheng Biotechnology).

Histological analysis

Liver tissue samples were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned to produce 4-μm-thick slides. These slides were subjected to haematoxylin-eosin (H&E) staining, and histological analysis was conducted to evaluate the inflammatory response and the severity of tissue damage. The severity of hepatic injury was graded according to the Suzuki score (14), which categorises sinusoidal congestion, hepatocyte necrosis, and ballooning degeneration into grades 0–4. A score of 0 indicated no necrosis, congestion, or ballooning degeneration; a score of 1 indicated mild congestion, single-cell necrosis, or ballooning degeneration; a score of 2 indicated moderate congestion, ballooning degeneration, or lobular necrosis < 30% scored 2; Moderate congestion, ballooning, or lobular necrosis < 60% scored 3 points; Severe congestion, ballooning, or lobular necrosis > 60% scored 4 points. Three liver sections were examined per rat, with three high-power fields (×100) randomly selected for analysis from each section. The mean score per animal was calculated by summing all scores and dividing by 9.

TUNEL staining was performed on paraffin sections using an in-situ cell death detection kit (Roche). The sections were examined and photographed under a microscope (Leica). Following imaging, Image-Pro Plus 6.0 analysis software was used to measure the number of positive cells in three fields of view per section, along with the corresponding total cell count. The positivity rate (%) was calculated using the formula: Positivity rate (%) = (Number of positive cells / total number of cells) x 100.

Western blot analysis for the detection of apoptosis-related protein expression

Take liver tissue stored at -80°C and weigh approximately 100 mg using a balance before placing it into a grinding tube. Add RIPA lysis buffer at a tissue:RIPA ratio of 0.1 mg:1 ml, followed by 20 μl of 50 x protease inhibitor. Centrifuge the mixture at 4°C and 12,000 rpm for 15 minutes. Determine the protein concentration using the BCA assay kit. Conduct SDS-PAGE electrophoresis at 10% with 10 μL of protein, then cut the gel and perform a wet transfer at a constant current of 300 mA for 60 minutes. The membrane was blocked with a 5% BSA solution at room temperature for 90 minutes. Incubate with primary antibodies for Bax, HMGB1, Parkin, and Bcl-2 (1:1,000 dilution) overnight at 4°C. Wash the membrane three times with TBST for 10 minutes each. Incubate with a secondary antibody at room temperature for 2 hours, followed by three additional washes with TBST, each lasting 10 minutes. Apply an appropriate amount of developing solution evenly onto the surface of the PVDF membrane. Finally, place the membrane in a darkroom and develop it using an exposure unit.

Statistical analysis

All collected data were statistically analyzed using SPSS 19.0 software. Quantitative data that followed a normal distribution were expressed as mean ± standard deviation, whereas non-normally distributed data were presented as median (interquartile range). For comparisons of quantitative data among multiple groups, analysis of variance (ANOVA) was used for normally distributed data, while the rank sum test was employed for non-normally distributed data. For comparisons between two groups, the t-test was applied to normally distributed data, and the rank sum test was used for non-normally distributed data. Error plots were utilized to illustrate the observed indicators. A p-value < 0.05 was considered statistically significant.

Results

iPSCs were successfully induced into iPSC-MSCs and iPSC-MSCs-Exo exhibited exosome markers

Transmission electron microscopy (TEM) revealed that iPSC-MSCs-Exo displayed cup-shaped or spherical structures ranging in size from 45 to 120 nanometers (Fig. 1A-B). Characterization of iPSC-MSCs-Exo isolated by ultracentrifugation showed a peak diameter distribution at 71 nm in Nanosight particle size analysis (Fig. 1C). Protein detection confirmed the expression of exosome-positive markers CD81/CD9/Alix, with no expression of Calnexin, thereby verifying exosomal secretion (Fig. 1D).

iPSC-MSCs-Exo injection can reduce serum transaminase levels

ALT and AST levels in the blood of mice from each group were measured at 6-, 12-, and 24-hours post-hepatic ischemia-reperfusion injury (Fig. 2A-B). The results indicated that the IR + Exo group had lower transaminase levels than both the IR + PBS group and the IR group at all post-operative time points. These findings suggest that iPSC-MSCs-Exo injection effectively improves liver function indicators after hepatic ischemia-reperfusion injury.

iPSC-MSCs-Exo injection can ameliorate hepatic ischemia-reperfusion injury

Histological examination of liver tissue from mice in each group at 6, 12, and 24 hours post-hepatic ischemia-reperfusion injury, using hematoxylin and eosin (H&E) staining (Fig. 3A), revealed significant histological improvements in the IR + Exo group compared to the IR and IR + PBS groups. Liver samples from the IR and IR + PBS groups showed larger areas of necrosis, accompanied by sinusoidal congestion and cellular swelling, while the IR + Exo group exhibited markedly reduced hepatic cell necrosis and less severe sinusoidal congestion. Pathological evaluation using the Suzuki score indicated that the IR + Exo group had lower scores than both the IR and IR + PBS groups (Fig. 3B). TUNEL staining of liver tissues at 6, 12, and 24 hours post-ischemic reperfusion demonstrated a significant reduction in TUNEL-positive

and caspase-3-positive cells in the iPSC-MSCs-Exo group compared to the IR and IR + PBS groups (Fig. 3C). These results indicate that iPSC-MSCs-Exo injection effectively ameliorates hepatocyte necrosis following hepatic ischemia-reperfusion injury.

iPSC-MSCs-Exo injection ameliorates oxidative stress following hepatic ischemia-reperfusion injury

Oxidative stress markers in liver tissues were assessed at 6, 12, and 24 hours post-ischemic reperfusion in each group (Fig. 4). The IR+iPSC-MSCs-Exo group exhibited elevated SOD levels and reduced MDA and MPO levels compared to the IR and IR + PBS groups. These results indicate that iPSC-MSCs-Exo injection effectively mitigates oxidative stress responses following hepatic ischemia-reperfusion injury.

iPSC-MSCs-Exo injection may improve hepatic cell ischemia-reperfusion injury by suppressing or mitigating mitochondrial damage

Compared to the sham-operated group, mitochondria exhibited varying degrees of damage following hepatic ischemia-reperfusion injury. This damage was primarily characterized by mitochondrial matrix dissolution and extensive loss of cristae during the early phase of ischemia-reperfusion (Fig. 5A). As time progressed, the most severe damage was observed at 12 hours, marked by mitochondrial vacuolation and the appearance of autophagosomes and autophagolysosomes (Fig. 5B). By 24 hours, partial mitochondrial repair was noted (Fig. 5C). Following exosome infusion, mitochondria displayed preserved structures with a uniform matrix, intact cristae, and only isolated damage. The matrix appeared lighter, cristae were reduced, and autophagy was enhanced, evidenced by visible autophagosomes and autophagolysosomes (Fig. 5A-C).

At 6, 12, and 24 hours post-hepatic ischemia-reperfusion injury, apoptosis and autophagy-related protein markers were detected in liver tissues across all groups (Fig. 6). Compared to the IR and IR + PBS groups, injection of iPSC-MSCs-Exo significantly reduced the expression levels of Bax, HMGB1, and Bcl-2 proteins, while increasing Parkin protein expression associated with autophagy. These results indicate that iPSC-MSCs-Exo injection mitigates ischemia-induced liver injury by reducing apoptosis and activating autophagy.

Discussion

Ischemia-reperfusion injury is highly prevalent in hepatobiliary surgery, particularly during hepatic resection when the portal is occluded and in liver transplantation during blood flow occlusion. This injury poses a significant challenge in hepatobiliary surgery. The injury process triggers multiple immune cascade reactions, including excessive oxidative stress, overactivation of Kupffer cells, and massive neutrophil infiltration [15–18].

Ischemia activates Kupffer cells, which are the primary source of inflammatory mediators such as IL-6 and TNF-α. These mediators upregulate the expression of adhesion molecules and recruit neutrophils into the liver [19–20]. Subsequently, during reperfusion, infiltrating neutrophils induce excessive oxidative stress, depleting antioxidant defenses such as superoxide dismutase (SOD) and glutathione (GSH). This cascade exacerbates inflammation, ultimately leading to hepatocyte necrosis and apoptosis. Current strategies to mitigate hepatic ischemia-reperfusion injury primarily involve the use of antioxidant emergency drugs and ischemic preconditioning [21–22], but overall efficacy remains suboptimal.

Adipose-derived mesenchymal stem cells are a type of stem cell with the potential for multi-tissue differentiation. Research has confirmed that intravenous administration of adipose-derived mesenchymal stem cells can protect against hepatic ischemia-reperfusion injury. However, studies have also shown that less than 1% of intravenously administered adipose-derived mesenchymal stem cells actually migrate to target organs [23]. Furthermore, the low survival rate of mesenchymal stem cells within the host limits their efficacy at target sites.

Exosomes, nanoscale vesicles secreted by living cells, have become a significant focus of research in recent years, garnering considerable attention from scholars. These vesicles deliver specific intracellular proteins and nucleic acid molecules, exerting various biological effects. With no cellular structure, exosomes present no risk of embolism and can be easily stored and transported, effectively overcoming the limitations associated with cell therapy. Research indicates that exosomes exhibit biological functions similar to those of their parent cells [24–26].

Different cell type, including dendritic cells, T cells, tumor cells, and mesenchymal stem cells, secrete varying amounts of exosomes, with mesenchymal stem cells derived from human tissues yielding the highest quantities. The therapeutic potential of exosomes derived from mesenchymal stem cells has been validated in multiple disease models. For instance, exosomes purified from these cells have shown protective effects in mouse models of myocardial and renal ischemia-reperfusion injury [27–28]. Additionally, recent studies suggest that mesenchymal stem cell-derived exosomes confer hepatoprotective benefits in a carbon tetrachloride-induced mouse liver injury model [29–30].

In recent years, iPSCs have emerged as an ideal source for stem cell therapy. Their ability to generate cells from any human tissue, combined with unlimited proliferative capacity and the absence of ethical concerns, underscores their promise. In this study, we successfully induced iPSCs into iPSC-MSCs, which exhibited typical mesenchymal stem cell characteristics, and isolated their secreted exosomes.

We further evaluated the protective capacity of iPSC-MSCs-Exo in a mouse model of hepatic ischemia-reperfusion injury and explored its potential mechanisms. The results demonstrated that immediate administration of iPSC-MSCs-Exo after reperfusion provided hepatoprotective effects. This was evidenced by a suppression of ALT and AST release, a reduction in necrosis scores, decreased hepatic morphological damage, and alleviation of oxidative stress responses.

Damaged or dying hepatocytes actively release HMGB1, an inflammatory cytokine that plays a pivotal role in ischemia-reperfusion injury. Studies have confirmed HMGB1's ability to promote inflammatory responses and induce apoptosis [31–32]. Apoptosis can be activated through the mitochondrial pathway, where the anti-apoptotic protein Bcl-2 stabilizes the mitochondrial membrane and inhibits caspase-3 activation, while the pro-apoptotic protein Bax has the opposite effect. Consequently, the extent of apoptosis initiation primarily depends on the balance between Bcl-2 and Bax, which are the main anti-apoptotic and pro-apoptotic proteins within the Bcl-2 family, respectively. Monitoring the expression levels of these proteins reflects the cellular apoptosis status [33–35].

In this study, both in vivo and in vitro experiments demonstrated reduced apoptosis levels in liver cells following iPSC-MSCs-Exo treatment. This confirms that the protective effect of iPSC-MSCs-Exo against hepatic ischemia-reperfusion injury may be attributed to its anti-apoptotic action.

Although our experiments preliminarily suggest that anti-apoptosis is a potential mechanism for iPSC-MSCs-Exo's protective effect against hepatic ischemia-reperfusion injury, further mechanistic studies are needed. Future research should analyze the proteins and nucleic acid molecules carried by iPSC-MSCs-Exo to identify their active components and key cellular signaling pathways. Additionally, investigations should focus on changes in cellular autophagy levels, regulation of ion channel activity, and metabolism of reactive oxygen species or carbohydrates.

In summary, this study demonstrates that iPSC-MSCs-Exo therapy reduces inflammatory responses, alleviates oxidative stress, and inhibits apoptotic reactions in the liver following ischemia-reperfusion injury. These findings indicate that iPSC-MSC-derived exosomes have the potential to protect the liver from ischemia-reperfusion injury.

Declarations

Ethical approval

The authors are accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This experiment did not involve any clinical human trials. All animal experiments were approved by the Experimental Animal Ethics Committee of PLA General Hospital (approval number: 2025-x5-21). Clinical trial number: not applicable.

Final approval of manuscript

All authors.

Competing interests

The authors declare no conflict of interest.

Footnote

None.

Funding

This study was funded by the Independent Innovation Scientific Research Fund of the Fourth Medical Center Chinese PLA General Hospital(2024-4ZX-MS-01).

Author Contribution

Haida Shi: Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing –original draft, Writing – review & editing. Yang Chen: Formal analysis, Methodology, Validation. Xin Jin: Methodology, Software, Validation, Visualization, Writing – original draft, Writing-editing. Haofeng Cheng: Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft. Lin Zhou: Formal analysis, Visualization. Huanxian Ma: Formal analysis,Investigation, Validation. Ming-Gen Hu: Validation, Writing – review & editing.

Acknowledgement

None.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

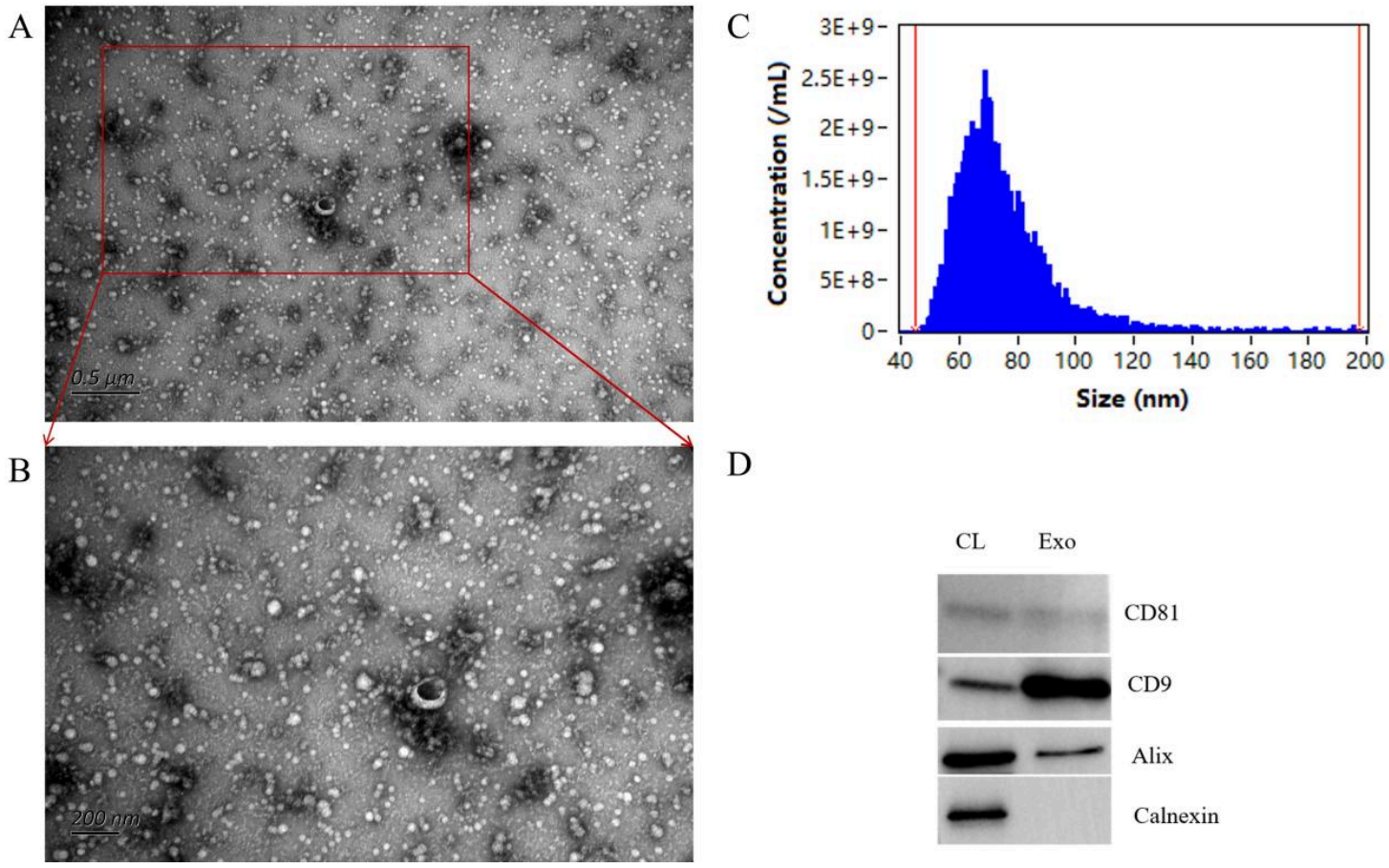


Figure 1

A. Transmission electron microscopy (TEM) morphology of iPSC-MSCs-Exo (0.5μm); B. TEM morphology of iPSC-MSCs-Exo (200nm); C. Nanosight particle size analysis results for iPSC-MSCs-Exo; D. Protein detection shows Exo protein expression as CD81(+), CD9(+), Alix(+), Calnexin(-).

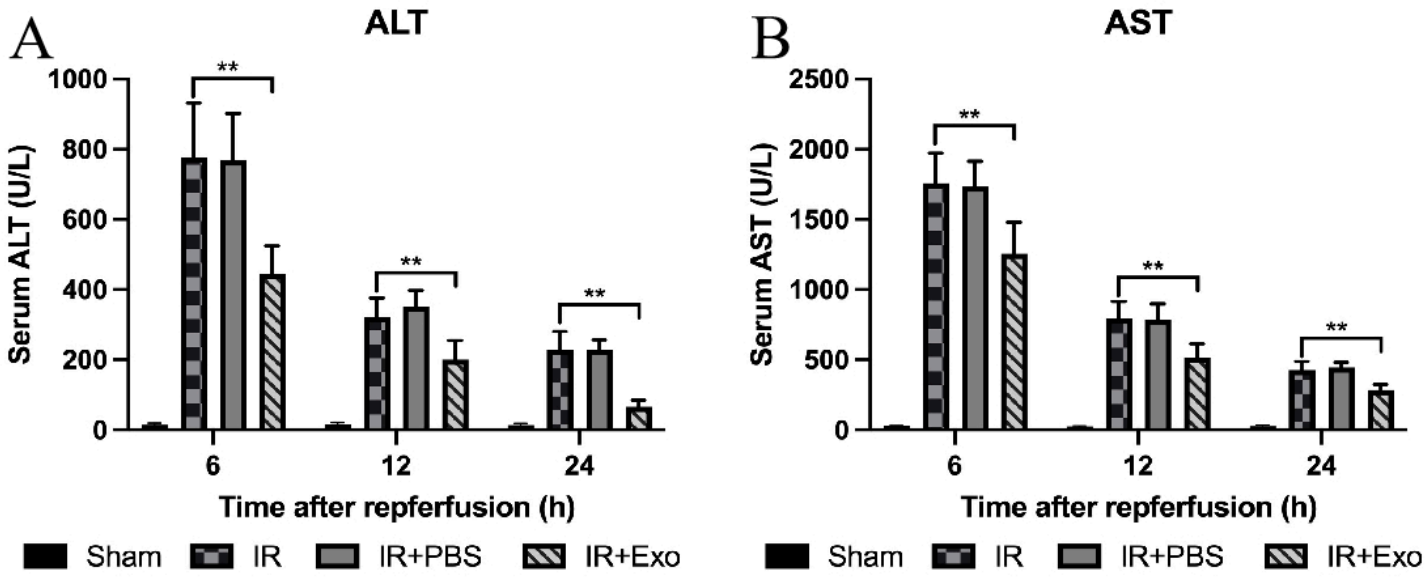


Figure 2

Effects of iPSC-MSCs-Exo on Serum Transaminase Levels: A. ALT levels at different time points in mice across groups (units: U/L). B. AST levels at different time points in mice across groups (units: U/L). *P < 0.05, **P < 0.01 (compared with IR group).

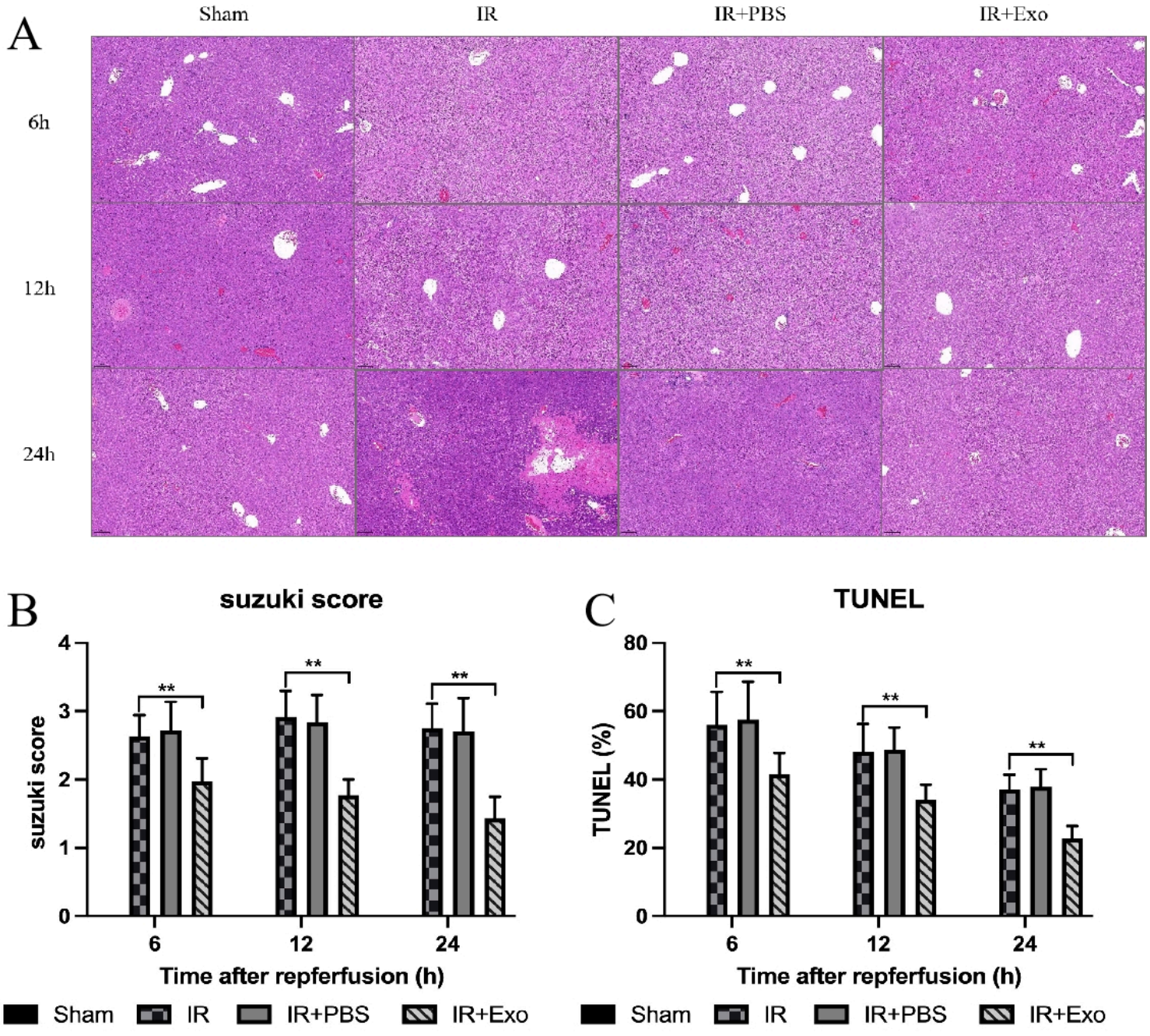


Figure 3

Effects of iPSC-MSCs-Exo on hepatic ischaemia-reperfusion injury: A. H&E staining images at different time points in mice from various groups (scale bar, 100 μm). B. Suzuki scores at different time points in mice from various groups. C. Quantitative analysis of TUNEL-positive cells at different time points in mice from various groups (unit: %). *P < 0.05, **P < 0.01 (compared with IR group)

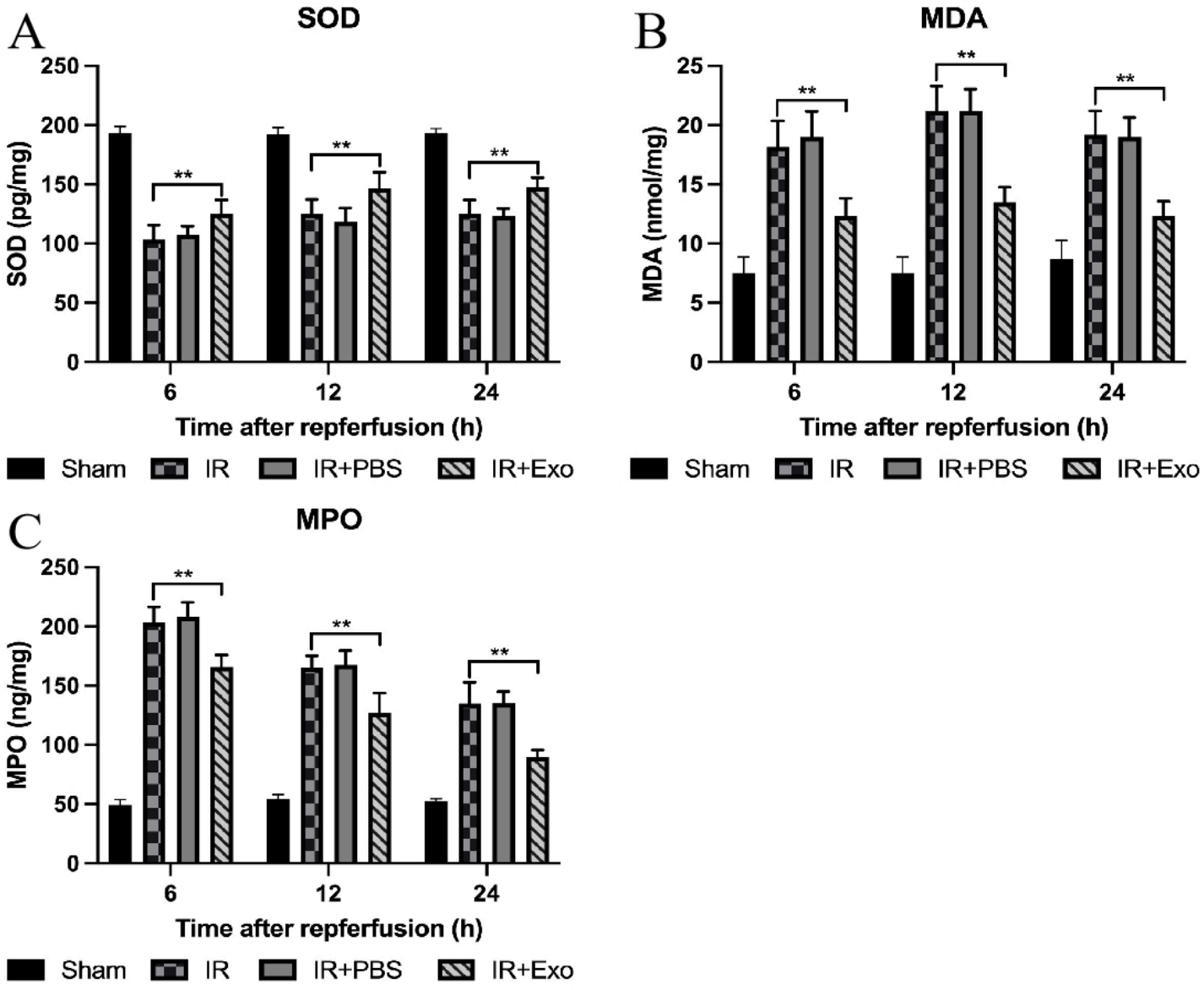


Figure 4

The effects of iPSC-MSCs-Exo on oxidative stress markers in hepatocytes: A. SOD levels at different time points in mice across groups (unit: pg/mg). B. Analysis of the MDA levels at different time points in the indicated groups (unit: nmol/mg). C. MPO levels at different time points in mice across groups (unit: ng/mg). *P < 0.05, **P < 0.01 (compared with the IR group)

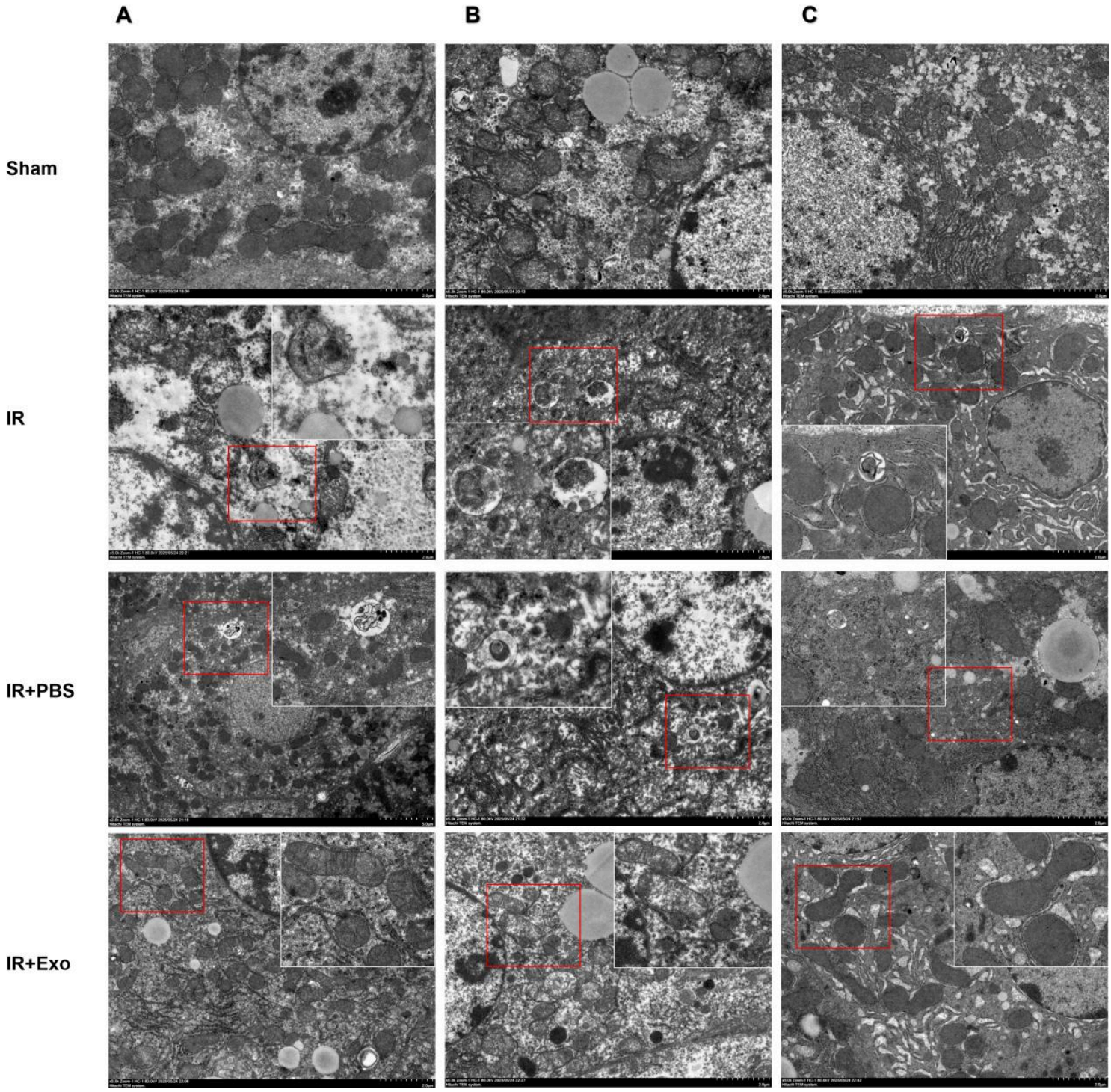


Figure 5

Mitochondrial damage levels in liver tissue at different ischemia-reperfusion injury time points, as observed under transmission electron microscopy. At 6 hours post-ischemic reperfusion injury, mitochondrial matrix dissolution and extensive cristae loss were observed in both IR and IR+PBS groups (A). By 12 hours, marked mitochondrial vacuolation accompanied by autophagosomes and autophagolysosomes was evident (B). Mitochondrial damage gradually showed signs of repair by 24 hours (C). Following exosome infusion, mitochondrial damage was markedly reduced. Enhanced

autophagy between 6 and 12 hours promoted repair, and by 24 hours, mitochondrial structures were clearly defined and largely restored to normal (Figure 5 A-C)

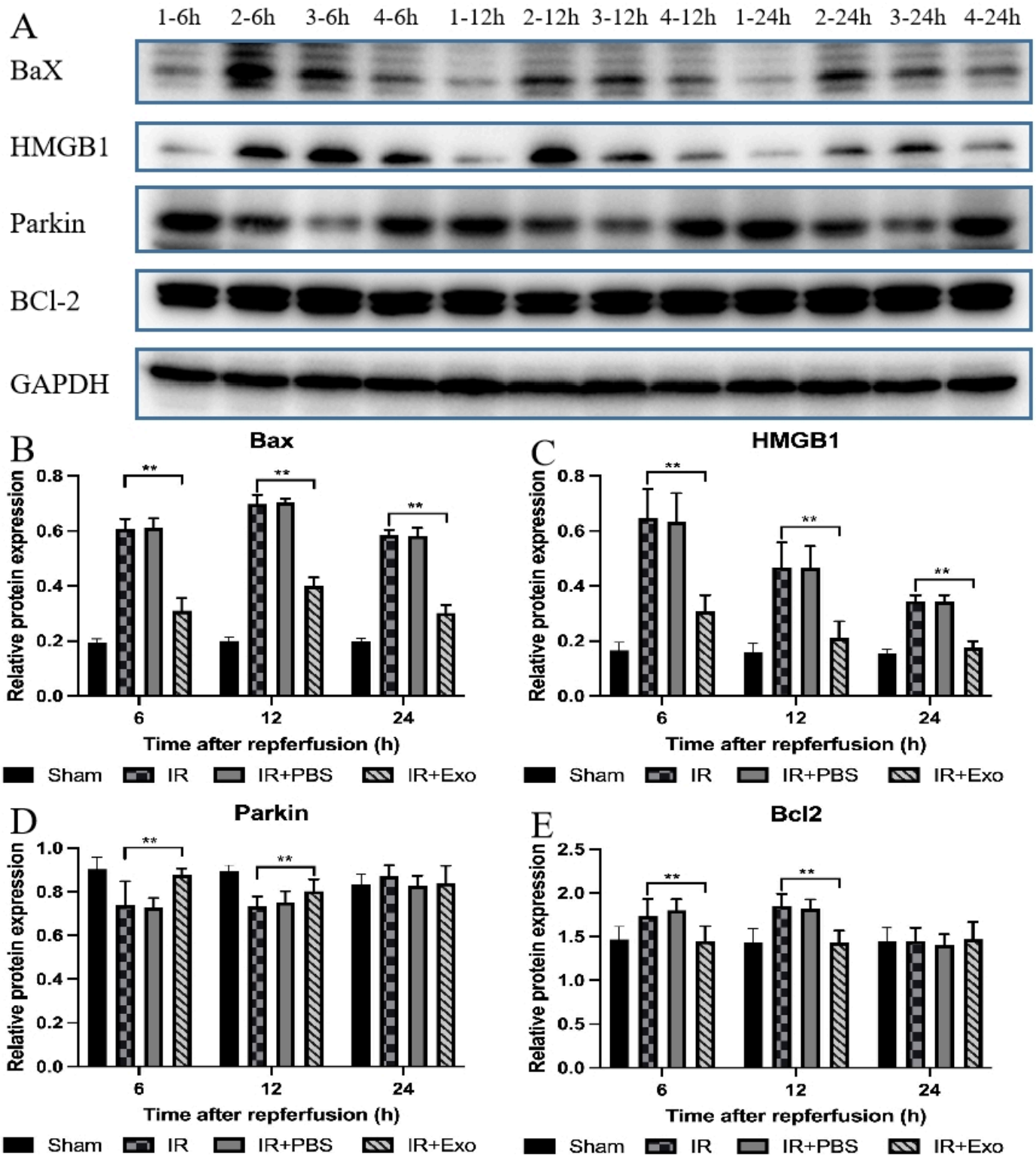


Figure 6

Effects of iPSC-MSCs-Exo on hepatic apoptosis-related protein markers: A. Expression levels of apoptosis-related proteins at different time points across mouse groups (1: Sham group; 2: IR group; 3: IR+PBS group; 4: IR+Exo group). B. Bax protein levels at different time points across mouse groups. C.

HMGB1 protein levels at different time points across mouse groups. D. Comparison of the Parkin protein levels at different time points in mice from the indicated groups. E. Bcl2 protein levels at different time points in the indicated groups. *P < 0.05, **P < 0.01 (compared with IR group)

Supplementary Files

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

- Figure6.AWBoriginaldata.zip
- Figure1D.WBrawdata.zip