Letter

Sirtuin 3 Attenuates Acute Lung Injury by Decreasing Ferroptosis and Inflammation through Inhibiting Aerobic Glycolysis

Kewei Qin[1,2,&], Qingqing Ji[1,&], Weijun Luo[1,&], Wenqian Li[3], Bingbing Hao[4], Haiyan Zheng[5], Chaofeng Han[4], Jian Lou[6,#], Liming Zhao[5,#], and Xingying He[1,#]

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is a severe clinical disorder characterized by widespread inflammation, diffuse alveolar damage, and pulmonary edema, often leading to respiratory failure and death. Despite significant advances in clinical care, ALI/ARDS remains the leading cause of death among intensive care unit patients. Sepsis is the primary risk factor for the development of ALI/ARDS, as excessive inflammatory responses contribute to organ injury and high mortality in critically ill patients. Mitochondrial dysfunction is closely associated with the severity of lung injury and mortality in sepsis-related ARDS, and mitochondrial functional enhancement alleviates sepsis severity in endotoxin-induced ALI, suggesting that its regulation is a potential therapeutic strategy for ALI/ARDS.

Sirtuin 3 (Sirt3), an NAD-dependent deacetylase involved in diverse physiological and pathological processes, facilitates mitochondrial metabolic adaptation to stress by activating cytoplasmic signaling pathways and nuclear gene expression, thereby modulating proliferation, differentiation, and cell death. Sirt3 has been shown to mitigate ALI by reducing oxidative stress and improving mitochondrial metabolism[1]. Ferroptosis, a recently identified form of regulated cell death in ALI, is characterized by iron-dependent lipid peroxidation, reactive oxygen species (ROS) overproduction, and disrupted cellular redox homeostasis. Mitochondria play a central role in ferroptosis, harboring both pro-ferroptotic and anti-ferroptotic machinery. While Sirt3 can suppress ferroptosis by maintaining mitochondrial redox homeostasis[2], the underlying mechanisms remain unclear. Given the close relationship between Sirt3 and mitochondrial metabolism, we hypothesize that Sirt3 activation regulates ferroptosis and inflammation in alveolar epithelial cells *via* reprogramming energy metabolism, thereby reducing lipopolysaccharide (LPS)-induced ALI.

To investigate the role of Sirt3 in ALI and its regulatory pathways, an LPS-induced ALI model was established in *Sirt3*$^{-/-}$ mice and their littermates (*Sirt3*$^{+/-}$ mice), followed by RNA-seq analysis. A total of 1,926 differentially expressed genes (DEGs) were identified (Supplementary Figure S1A). Sequencing data revealed that the upregulated genes were significantly enriched in inflammatory pathways (Supplementary Figure S1B), whereas the downregulated genes were prominently enriched in glutathione metabolism based on KEGG analysis (Supplementary Figure S1C). Reactome enrichment analysis further demonstrated that the upregulated genes were associated with NF-κB pathway activation and glycolysis regulation *via* fructose 2,6-bisphosphate metabolism (Supplementary Figure S1D), whereas the downregulated genes were associated with glutathione synthesis/recycling and glycogen synthesis (Supplementary Figure S1E). Protein-protein interaction (PPI) network analysis of ferroptosis-related DEGs in ALI highlighted the involvement of Sirt3, Gpx4, and RELA in ferroptosis regulation (Supplementary Figure S2), suggesting a

doi: 10.3967/bes2025.110

1. Department of Anesthesiology, Second Affiliated Hospital of Naval Medical University, Shanghai 200003, China; 2. Senior Department of Otolaryngology Head and Neck Surgery, The Sixth Medical Centre, Chinese PLA (People's Liberation Army) General Hospital, Beijing 100048, China; 3. Department of Anesthesiology, Zhongshan Hospital, Fudan University, Shanghai 200032, China; 4. Department of Histology and Embryology, National Key Laboratory of Immunity and Inflammation, Naval Medical University, Shanghai 200433, China; 5. Department of Emergency Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200120, China; 6. Key Laboratory of Respiratory Disease of Zhejiang Province, Department of Respiratory and Critical Care Medicine, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou 310009, Zhejiang, China

potential connection between Sirt3, the NF-κB pathway, and ferroptosis. Collectively, these findings indicated that Sirt3 may modulate ALI progression by regulating ferroptosis-associated glutathione metabolism and inflammatory responses.

To investigate the function of Sirt3 in inflammatory responses during LPS-induced ALI, we established an LPS-induced ALI mouse model. The production of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) in the bronchoalveolar lavage fluid (BALF) of *Sirt3*$^{-/-}$ mice was notably higher compared with their littermates (*Sirt3*$^{+/-}$) *in vivo* (Figure 1A). Total cell counts in the BALF of *Sirt3*$^{-/-}$ mice were dramatically increased compared with the control group (Figure 1B). The lung wet/dry (W/D) ratio and BALF protein concentration—two key indicators of pulmonary vascular permeability in ALI/ARDS—were significantly elevated in *Sirt3*$^{-/-}$ mice (Figure 1C–D). Histological analysis revealed more severe inflammatory infiltration in the lungs of *Sirt3*$^{-/-}$ mice compared with littermates (*Sirt3*$^{+/-}$) in the ALI model (Figure 1E). At the cellular level, mRNA expression of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) in *Sirt3*$^{-/-}$ alveolar epithelial type II cells (AEC II) was significantly higher than in *Sirt3*$^{+/-}$ AEC II after LPS stimulation (Figure 1F). Furthermore, cell viability of *Sirt3*$^{-/-}$ alveolar epithelial cells was markedly reduced compared with *Sirt3*$^{+/-}$ AEC II under LPS treatment (Figure 1G). Consistently, survival rates of *Sirt3*$^{-/-}$ mice were significantly lower than those of *Sirt3*$^{+/-}$ mice in LPS-induced ALI (Figure 1H). These results indicated that *Sirt3* deficiency exacerbates LPS-induced ALI by amplifying inflammatory responses and promoting alveolar epithelial cell death.

Receptor-interacting protein kinase 1 (RIP1) is a critical effector of inflammatory responses and cell death pathways. It regulates various forms of cell death, including apoptosis and necroptosis. NF-κB pathway activation induces the transcription of inflammation-related factors such as IL-1β, IL-6, and TNF-α. Moreover, numerous studies have demonstrated that NF-κB may activate antiapoptotic pathways while paradoxically participating in cell death regulation. Therefore, we investigated the activation of RIP1, NF-κB, and the expression of ferroptosis marker Gpx4. Compared with control (*Sirt3*$^{+/-}$) mice, *Sirt3*$^{-/-}$ mice exhibited increased phosphorylation of RIP1 (p-RIP1) and p65 (p-p65) along with reduced Gpx4 expression in lung tissue (Figure 2A–C). This pattern was recapitulated in alveolar epithelial cells: *Sirt3*$^{-/-}$ cells showed enhanced p-RIP1 and p-p65 activation but decreased Gpx4 expression compared to *Sirt3*$^{+/-}$ AEC II (Figure 2D). Importantly, Sirt3 overexpression in the alveolar epithelial cell line MLE12 significantly attenuated p-RIP1 and p-p65 activation, while restoring Gpx4 expression (Figure 2E). Given that Gpx4 inactivation induced by glutathione depletion drives ferroptosis and previous studies having established the association between NF-κB activation and Gpx4 suppression[3], our findings demonstrated that *Sirt3* deficiency activates NF-κB signaling to downregulate Gpx4 expression, thereby promoting ferroptosis in alveolar epithelial cells.

LPS stimulation induces ROS production in mitochondria. Although ROS play a major role in inflammatory processes, ferroptosis is primarily initiated through ROS-driven lipid peroxidation and iron overload. Notably, ROS interact with NF-κB signaling pathways through multiple mechanisms. The glutathione antioxidant system, which comprises glutathione (GSH), glutathione peroxidase (GPX), and related enzymes, serves as a key regulator of lipid peroxidation by preventing excessive ROS generation. To elucidate how *Sirt3* deficiency exacerbates ferroptosis in LPS-induced ALI, we analyzed cell membrane lipid peroxidation and ROS production. Notably, *Sirt3*$^{-/-}$ alveolar epithelial cells exhibited significantly enhanced lipid peroxidation compared to *Sirt3*$^{+/-}$ controls during LPS-induced ALI (Figure 2F). Intriguingly, LPS stimulation increased ROS production in both groups, with markedly higher potential observed in *Sirt3*$^{-/-}$ cells than in *Sirt3*$^{+/-}$ cells (Figure 2G). Collectively, these findings suggested that Sirt3 suppresses ferroptosis via ROS modulation. These observations are further supported by evidence that mitochondrial ROS overproduction disrupts endothelial barrier function and directly triggers ferroptosis[4]. Sirt3 is known to enhance ROS detoxification *via* SOD2 deacetylation[5], and its deficiency likely exacerbates LPS-induced ROS accumulation, leading to redox imbalance and ferroptosis[6].

Mitochondria play a pivotal role in regulating cellular metabolism, signal transduction, and cell death pathways. Dysfunctional mitochondria drive the shift from mitochondrial respiration to glycolysis in cancer cells. However, the relationship between mitochondrial dysfunction and glycolysis in ferroptosis remains unclear. To determine how Sirt3 modulates glycolytic metabolism and ROS dynamics during LPS stimulation, we assessed the activity of two key metabolic enzymes: Tricarboxylic acid cycle regulator pyruvate dehydrogenase A (PDHa) and glycolytic enzyme Lactate dehydrogenase A (LDHa). *In vivo* analyses revealed that *Sirt3*$^{-/-}$ mice exhibited

reduced p-PDHa activation but significantly elevated p-LDHa activation compared to *Sirt3*$^{+/-}$ controls, indicating enhanced aerobic glycolysis in *Sirt3*$^{-/-}$ lungs (Figure 2H–I). Consistently, *Sirt3*$^{-/-}$ alveolar epithelial cells recapitulated this metabolic pattern (Figure 2D). Conversely, Sirt3 overexpression *in vitro* increased p-PDHa expression, while suppressing p-LDHa expression (Figure 2E). These findings demonstrated that Sirt3 attenuates aerobic glycolysis and ferroptosis. The metabolic

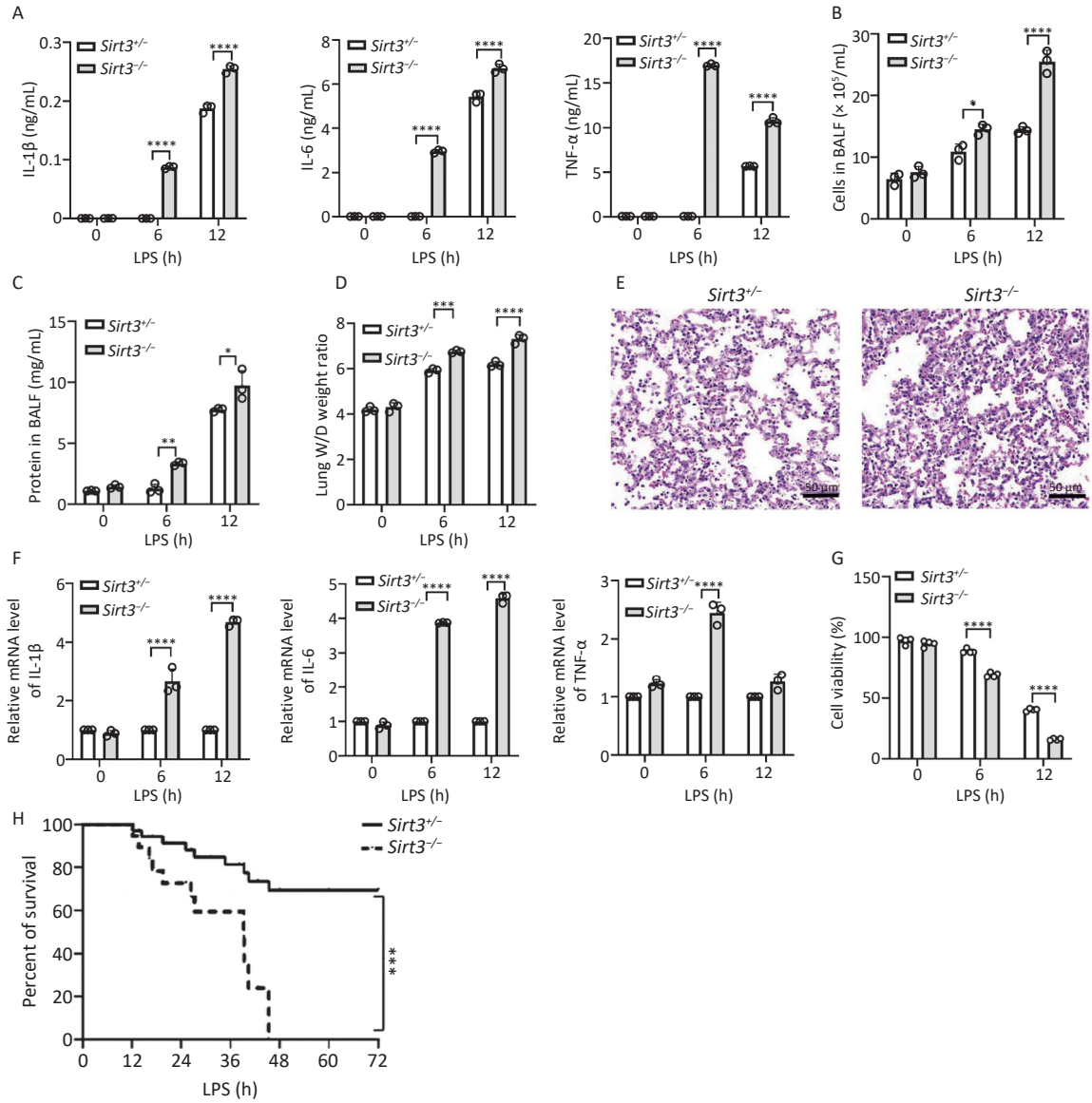

Figure 1. Sirt3 deficiency aggravates LPS- induced lung injury. (A) Pro-inflammatory cytokines (IL-6, IL-1β and TNF-α) in BALF were detected by ELISA in ALI mice (12 h for intratracheal infusion of LPS). (B) Total cells in BALF were counted by an automated cell counter in ALI mice (12 h for intratracheal infusion of LPS). (C) Protein concentrations in BALF were tested using BCA protein assay kit in ALI mice (12 h for intratracheal infusion of LPS). (D) Lung W/D ratio. (A–D, n = 3 mice per group) (E) Lung tissues in ALI mice were stained using HE staining. (F) IL-6, IL-1β and TNF-α *in vitro* were detected by qRT-PCR (6 h after LPS stimulating). (G) Viability of *Sirt3*$^{+/-}$, *Sirt3*$^{-/-}$ AEC II with LPS was detected by CCK8 Assay (6 h after LPS stimulating). Data were presented as mean ± SD of three independent experiments. (H) Survival rate in ALI mice was analyzed by Kaplan Meier on GraphPad Prism 10 (n = 6 mice per group). ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$).

reprogramming toward glycolysis under *Sirt3* deficiency aligns with studies showing NF-κB activation drives LDHA-dependent glycolysis[7]. Such shifts deplete antioxidants (e.g. GSH) and amplify ROS production, to create a permissive environment for ferroptosis[8,9]. Furthermore, it has also been

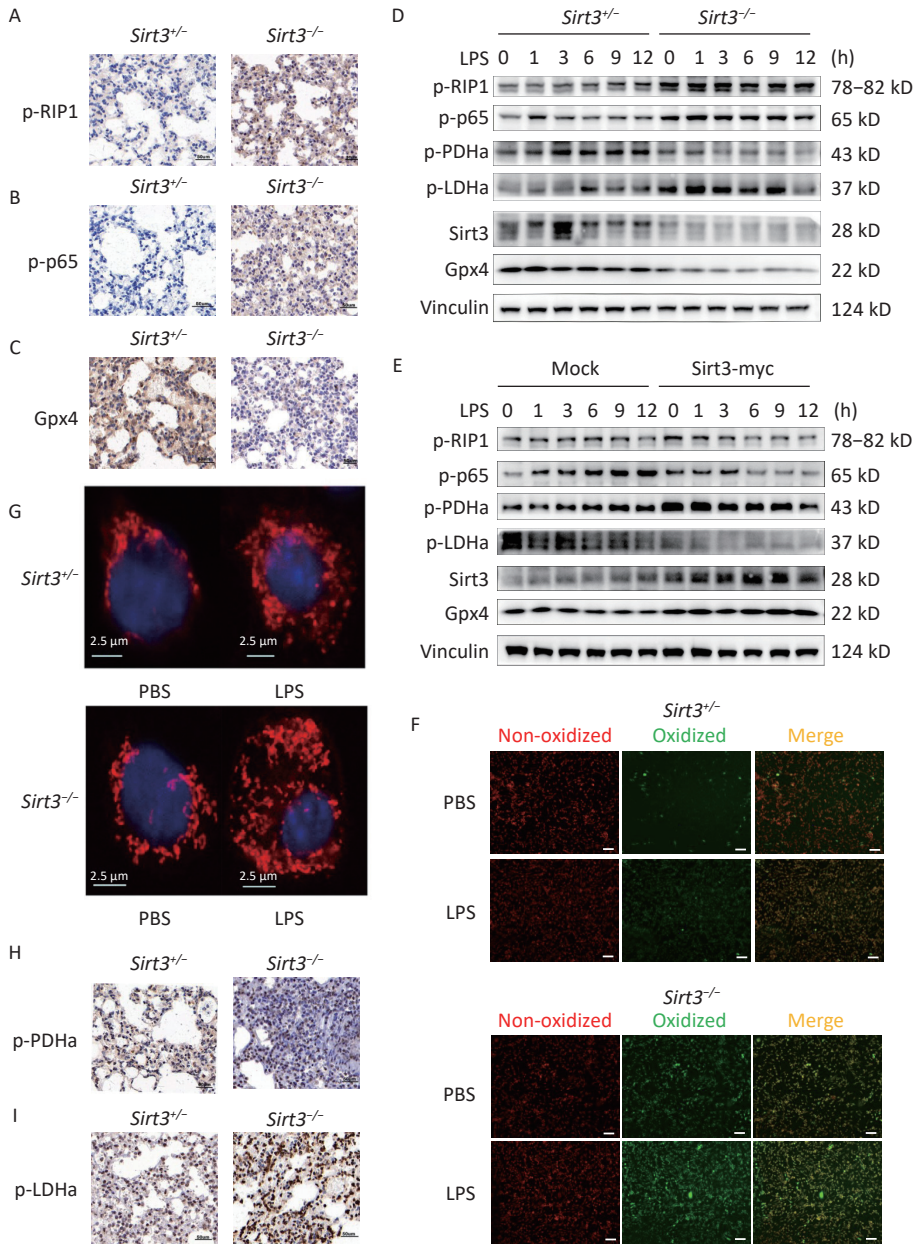

Figure 2. Sirt3 suppresses ferroptosis and inflammation *via* inhibition of aerobic glycolysis. (A–C) Immunohistochemical staining of p-RIP1, p-p65, Gpx4 in lung tissues of *Sirt3*$^{+/-}$ and *Sirt3*$^{-/-}$ littermates in ALI model. (D) Western blot for p-RIP1, p-p65, p-LDHa, p-PDHa, Gpx4 and Sirt3 in *Sirt3*$^{+/-}$ and *Sirt3*$^{-/-}$ AEC II. (E) Western blot for p-RIP1, p-p65, p-LDHa, p-PDHa, Gpx4 and Sirt3 expression in Sirt3 overexpressed MLE-12 cells. Data presented three independent experiments. (F) Lipid peroxidation levels detected by Lipid Peroxidation Kit in *Sirt3*$^{+/-}$ and *Sirt3*$^{-/-}$ AEC II (6 h after PBS or 1 μg/mL LPS stimulation). Images were photographed using a fluorescence microscope, Scale bar = 100 μm. (G) Mito Tracker Red CMXRos for *Sirt3*$^{+/-}$ and *Sirt3*$^{-/-}$ AEC II (6 h after PBS or 1 μg/mL LPS stimulation). Images were photographed using a laser confocal microscope. (H–I) Immunohistochemical staining of p-PDHa, p-LDHa in lung tissues of *Sirt3*$^{+/-}$ and *Sirt3*$^{-/-}$ littermates in ALI model.

reported that aerobic glycolysis can promote inflammatory responses[10], which corroborates our previous findings of synchronized increases in p-LDHa, p-RIP1, and p-p65 expression following *Sirt3* deficiency (Figure 2D). Collectively, these results (Figure 2) suggested that Sirt3 suppresses ferroptosis and inflammation by inhibiting aerobic glycolysis.

Sirt3 is widely expressed in both blood-derived cells, such as macrophages and neutrophils, and organ-derived cells, such as lung epithelial cells. To

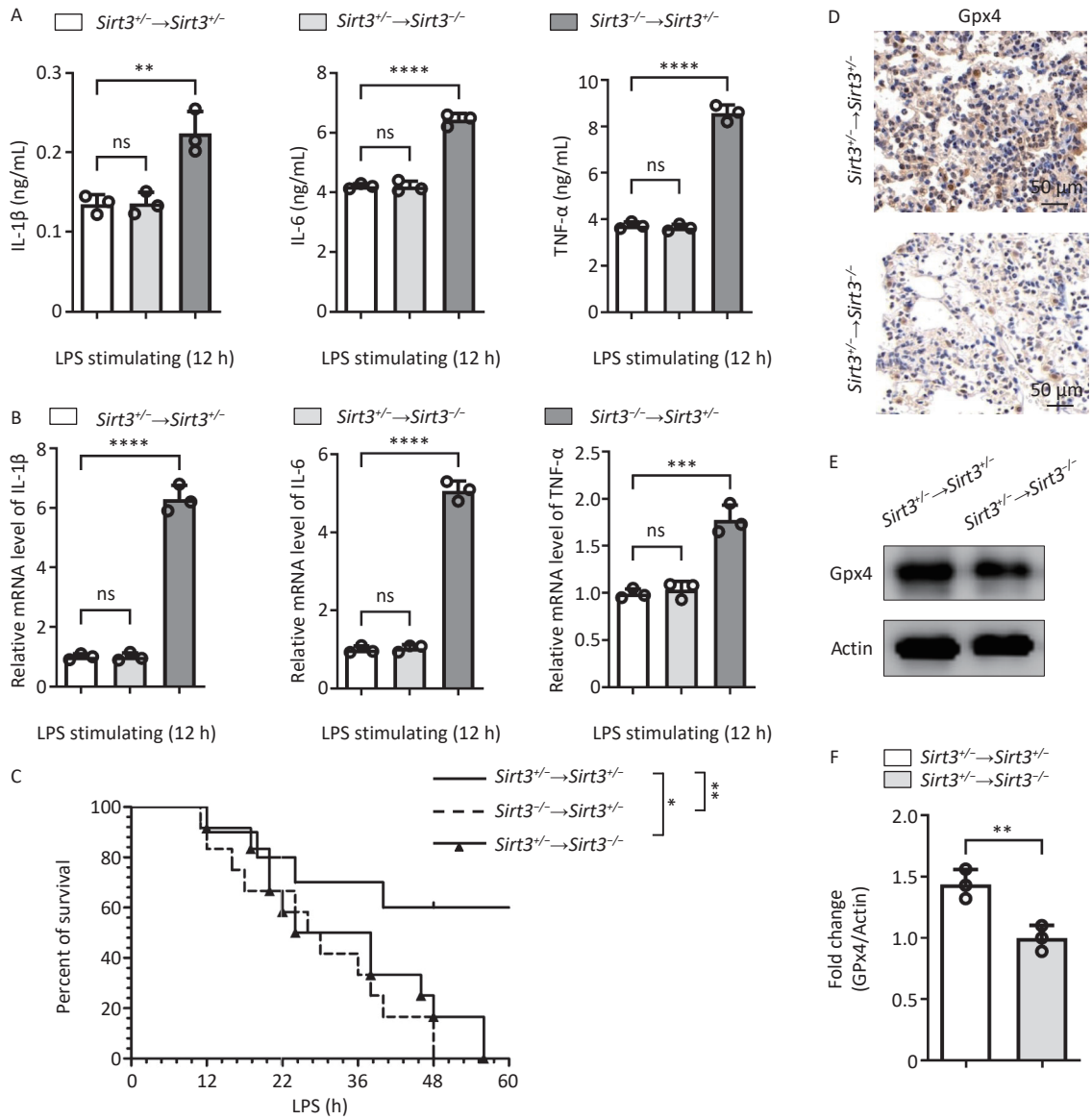

Figure 3. Sirt3 decreases inflammation in blood-derived cells and inhibits ferroptosis in lung epithelia cells. (A) Pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) in BALF were detected by ELISA in ALI mice (12 h for intratracheal infusion of LPS) after BMT. (B) IL-6, IL-1β, and TNF-α in lung tissue from ALI mice after BMT were detected by qRT-PCR. (A–B, n = 3 mice per group) Data were presented as mean ± SD of three independent experiments. (C) Survival rate in ALI mice was analyzed by Kaplan Meier on GraphPad Prism 10 (n = 10 mice per group). (D) Immunohistochemical staining of Gpx4 in lung tissues of ALI mice (12 h for intratracheal infusion of LPS) after BMT. (E) Western blot for Gpx4 in lung tissues of ALI mice (12 h for intratracheal infusion of LPS) after BMT. Data presented three independent experiments. (F) Subsequent semi-quantitative analysis of Gpx4 expression in lung tissues of ALI mice after BMT. Results are expressed as protein/Actin ratio. ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$).

explore the role of Sirt3 in different cell types, we reconstructed the bone marrow cells transplantation experiments. Therefore, we used an allogeneic bone marrow transplantation (BMT) mouse model to observe the changes in the protective effect of Sirt3. The *Sirt3*$^{-/-}$ and *Sirt3*$^{+/-}$ bone marrow cells were transplanted to *Sirt3*$^{+/-}$ mice irradiated with 8.5 Grey. LPS-induced pro-inflammatory cytokines (IL-6, IL-1β and TNF-α) were significantly higher in *Sirt3*$^{-/-}$ → *Sirt3*$^{+/-}$ group, compared with *Sirt3*$^{+/-}$ → *Sirt3*$^{+/-}$ group (Figure 3A). Quantitative PCR analysis of lung tissue showed similar results (Figure 3B). The survival rate *in vivo* of the *Sirt3*$^{-/-}$ → *Sirt3*$^{+/-}$ mice was lower than that of the *Sirt3*$^{+/-}$ → *Sirt3*$^{+/-}$ mice in LPS-induced ALI (Figure 3C). These results showed that Sirt3 inhibits inflammation in blood-derived cells. Then we transplanted bone marrow of *Sirt3*$^{+/-}$ to *Sirt3*$^{-/-}$ or *Sirt3*$^{+/-}$ mice to further determine the role of Sirt3 on ferroptosis in lung epithelial cells. The *Sirt3*$^{+/-}$ → *Sirt3*$^{-/-}$ group showed less Gpx4 expression than the *Sirt3*$^{+/-}$ → *Sirt3*$^{+/-}$ group, indicating a higher ferroptosis level (Figure 3D–F). The production of inflammatory cytokines (IL-6, IL- 1β and TNF-α) in the *Sirt3*$^{+/-}$ → *Sirt3*$^{-/-}$ group was not significantly higher than that in the *Sirt3*$^{+/-}$ → *Sirt3*$^{+/-}$ group (Figure 3A-B), which indicated that Sirt3 majorly regulates ferroptosis in lung epithelial cells. The *Sirt3*$^{+/-}$ → *Sirt3*$^{-/-}$ mice also showed lower survival rates than the *Sirt3*$^{+/-}$ → *Sirt3*$^{+/-}$ group and a later onset of death compared to the *Sirt3*$^{-/-}$ → *Sirt3*$^{+/-}$ group in LPS-induced ALI (Figure 3C). These results suggested that Sirt3 inhibits ferroptosis in lung epithelial cells and decreases inflammation in blood-derived cells.

This study revealed a novel mechanism by which Sirt3 alleviates LPS-induced ALI through the regulation of ferroptosis in alveolar epithelial cells. The main findings of this study were as follows: (1) *Sirt3* deficiency promotes inflammation in LPS-induced alveolar epithelial cells and lung parenchymal injury; (2) *Sirt3* deficiency leads to LPS-induced ferroptosis in alveolar epithelial cells through activation of the NF-κB signaling pathway; (3) *Sirt3* deficiency promotes LPS-induced ROS production, leading to ferroptosis in alveolar epithelial cells, accompanied by a reversal of cellular energy metabolism toward glycolysis; (4) Sirt3 functions in both blood- and organ-derived cells. In summary, Sirt3 regulates ferroptosis and inflammation in alveolar epithelial cells to reduce LPS-induced ALI *via* NF-κB signaling pathways and glycolytic metabolism. Our results provided new insights into the mechanism by which Sirt3 links glycolytic reprogramming, ferroptosis, and inflammation in LPS-induced ALI, outlining novel mechanisms and drug targets.

Funding This work was supported by National Natural Science Foundation of China (82471792, 82270004, 81870061, 82202382, 31770945); Beijing Municipal Natural Science Foundation (7242135) and Zhejiang Provincial Natural Science Foundation (LY20H010003).

Competing Interests The authors declare that they have no competing interests.

Ethics All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Scientific Investigation Board of the Naval Medical University. This study did not involve human participants.

Authors' Contributions Study concept and design: Chaofeng Han, Jian Lou, Liming Zhao, Xingying He. Experimental work: Kewei Qin, Qingqing Ji, Weijun Luo. Data analysis and manuscript drafting: Kewei Qin, Qingqing Ji, Weijun Luo. Data analysis, discussion, and manuscript revision: Wenqian Li, Bingbing Hao, Haiyan Zheng, Xingying He, Kewei Qin, Chaofeng Han.

Acknowledgements We thank the GEO network for its generous sharing of large amounts of data, and Tianliang Li, Zhixiao Liu, and Xinyue Deng for their help with this research.

Data Sharing The supplementary materials will be available in www.besjournal.com.

[&]These authors contributed equally to this work.

[#]Correspondence should be addressed to Xingying He, Tel: 86-21-63586818, E-mail: hxy_leon@hotmail.com; Liming Zhao, Tel: 86-21-38804518, E-mail: 1976zlm@163.com; Jian Lou, Tel: 86-571-87783730, E-mail: ljpumc@163.com

Biographical notes of the first authors: Kewei Qin, Associate Researcher, majoring in prevention and control of infectious disease, E-mail: qwfyqkw@126.com; Qingqing Ji, Resident Doctor, majoring in prevention and treatment of acute lung injury, E-mail: 1511653081@qq.com; Weijun Luo, Resident Doctor, majoring in perioperative organ protection, E-mail: 3298834486@qq.com

Received: February 16, 2025;
Accepted: July 28, 2025

REFERENCES

1. Ning L, Rui X, Guorui L, et al. A novel mechanism for the protection against acute lung injury by melatonin: mitochondrial quality control of lung epithelial cells is

preserved through SIRT3-dependent deacetylation of SOD2. Cell Mol Life Sci, 2022; 79, 610.
2. Li QW, Liao JZ, Chen WJ, et al. NAC alleviative ferroptosis in diabetic nephropathy via maintaining mitochondrial redox homeostasis through activating SIRT3-SOD2/Gpx4 pathway. Free Radic Biol Med, 2022; 187, 158−70.
3. Li SB, He YP, Chen KX, et al. RSL3 drives ferroptosis through NF-κB pathway activation and GPX4 depletion in glioblastoma. Oxid Med Cell Longev, 2021; 2021, 2915019.
4. Zheng DD, Liu J, Piao H, et al. ROS-triggered endothelial cell death mechanisms: focus on pyroptosis, parthanatos, and ferroptosis. Front Immunol, 2022; 13, 1039241.
5. Zeng ZH, Yang YT, Dai XG, et al. Polydatin ameliorates injury to the small intestine induced by hemorrhagic shock via SIRT3 activation-mediated mitochondrial protection. Expert Opin Ther Targets, 2016; 20, 645−52.
6. Wu J, Deng ZY, Sun MM, et al. Polydatin protects against lipopolysaccharide-induced endothelial barrier disruption via SIRT3 activation. Lab Invest, 2020; 100, 643−56.
7. Arra M, Swarnkar G, Ke K, et al. LDHA-mediated ROS generation in chondrocytes is a potential therapeutic target for osteoarthritis. Nat Commun, 2020; 11, 3427.
8. Yao XM, Li W, Fang D, et al. Emerging roles of energy metabolism in ferroptosis regulation of tumor cells. Adv Sci (Weinh), 2021; 8, 2100997.
9. Yang J, Ma SY, Xu R, et al. Smart biomimetic metal organic frameworks based on ROS-ferroptosis-glycolysis regulation for enhanced tumor chemo-immunotherapy. J Control Release, 2021; 334, 21−33.
10. Li C, Peng J, Xiao L, et al. Pseudomonas aeruginosa-derived extracellular vesicles enhance macrophage aerobic glycolysis that fuels inflammation. Front Microbiol, 2025; 16, 1619101.