

Nuclear fragile X mental retardation-interacting protein 1-mediated ribophagy regulates immune function of dendritic cells in polymicrobial sepsis

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Abstract

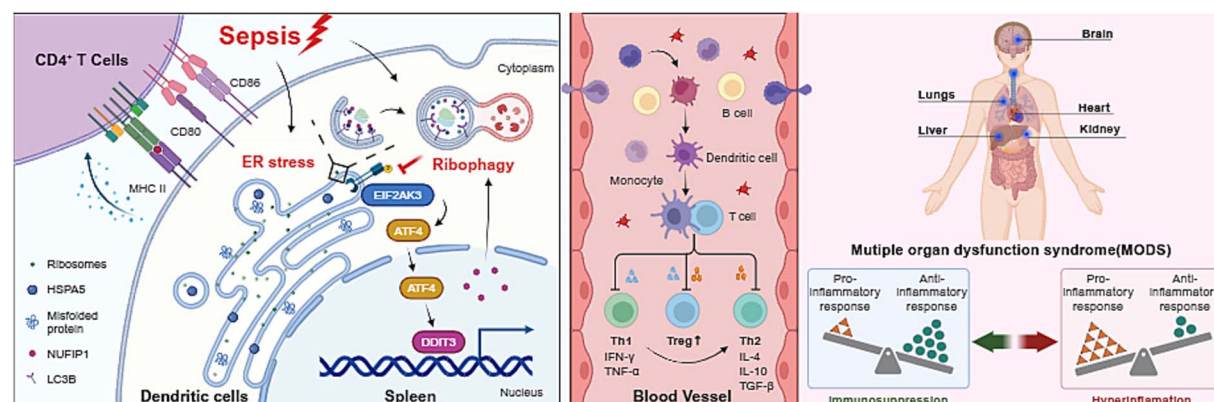
Background: Dendritic cells are crucial in the development of sepsis, yet the effect of ribophagy on dendritic cell activation remains unclear. This study aimed to investigate the potential role of nuclear fragile X mental retardation-interacting protein 1 (NUFIP1), a selective autophagy receptor, on sequestering ribosomes in autophagosomes to maintain dendritic cell function during early stages of sepsis.

Methods: Splenic dendritic cells were isolated using CD11c⁺ microbeads and treated with lipopolysaccharide. Sepsis models were generated using cecal ligation and puncture. Expression of dendritic cell surface molecules was detected using flow cytometry. Cytokine level was quantified using enzyme-linked immunosorbent assay kits. Laser scanning confocal microscopy was employed to observe ribophagy and endoplasmic reticulum (ER) morphology. Transmission electron microscopy was used to examine autophagosomes containing ribosomes. Western blotting was performed to determine the levels of ribophagy- and ER stress-associated proteins.

Results: The results showed that NUFIP1-mediated ribophagy was significantly activated under septic challenge and facilitated the functional activation of dendritic cells by mitigating excessive ER stress. Deletion of *Nufip1* resulted in reduced expression of surface molecules on dendritic cells, inhibited T-cell proliferation, exacerbated peripheral immunosuppression and severe multiple organ damage, and increased mortality. Salubrinal, a specific inhibitor of EIF2A dephosphorylation, rescued dendritic cell dysfunction in septic mice with *Nufip1* deficiency. Mechanistically, NUFIP1 interacted directly with ATF4 and regulated its nuclear translocation.

Conclusions: These findings suggest that NUFIP1 regulates ER stress through the EIF2AK3–ATF4–damage-inducible transcript 3 pathway, highlighting its critical regulatory role in sepsis. Thus, NUFIP1 represents a new target for sepsis therapy.

Graphical Abstract



Keywords: Sepsis; Dendritic cells; Nuclear fragile X mental retardation-interacting protein 1; Ribophagy; Immunosuppression

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Highlights

- Nuclear fragile X mental retardation-interacting protein 1 (NUFIP1)–mediated ribophagy is essential for maintaining dendritic cell function during the early stages of sepsis.
- NUFIP1 mitigates excessive endoplasmic reticulum stress *via* the EIF2AK3-ATF4–damage-inducible transcript 3 pathway.
- NUFIP1 directly interacts with ATF4 and regulates its nuclear translocation.
- NUFIP1 plays a critical regulatory role in sepsis, and represents a novel target for sepsis therapy.

Background

The Third International Consensus Definition for Sepsis and Septic Shock (Sepsis 3.0) describes sepsis as a life-threatening organ dysfunction caused by dysregulated host response to infection [1]. Sepsis is a major concern in the intensive care unit, and remains a leading cause of increased mortality among critically ill patients [2]. Recent epidemiological data show that approximately 48.9 million sepsis cases occur each year globally, resulting in over 11 million deaths [3].

Studies have shown that sepsis is associated with marked dysfunction of both innate and adaptive immunity, which contributes to a poor prognosis for patients [4, 5]. Dendritic cells serve as specialized antigen-presenting cells (APCs) that directly stimulate T cells, thereby linking the innate and adaptive immune systems [6]. During sepsis, dysfunction in dendritic cell numbers and functions disrupt immune networks and contribute to multiple organ dysfunction syndrome (MODS) [7–9]. Previous studies suggest that sestrin2 (SESN2) prevents the development and activation of dendritic cells by inhibiting ferroptosis in dendritic cells exposed to lipopolysaccharide (LPS) [10]. In our previous study, we found that TNF- α -induced protein 8-like 2 (TIPE2) suppressed autophagic activity of dendritic cells by inhibiting the TAK1/JNK signaling pathway and negatively regulated the immune function of dendritic cells during sepsis [11]. Therefore, dendritic cell dysfunction plays a crucial role in the emergence of immune suppression in sepsis, resulting in delayed mortality and susceptibility to nosocomial infections among patients with sepsis [12].

Ribosomes, the primary site of protein biosynthesis, are the most abundant intracellular organelles. Ribophagy, a key component of ribosome-associated quality control (RQC), involves the selective degradation of ribosomes in an autophagy-dependent manner [13]. In 2008, Kraft et al. [14] reported that mature ribosomes are degraded *via* selective autophagy under starvation conditions in yeast. In 2018, Wyant et al. [15] identified nuclear fragile X mental retardation-interacting protein 1 (NUFIP1) as the receptor involved in ribophagy in mammals. NUFIP1, a nucleoprotein, interacts with zinc finger HIT domain protein 3 (ZNHIT3) to form NUFIP1–ZNHIT3 ribonucleoprotein heterodimers. These complexes relocate from the nucleus to various organelles, such as autophagosomes, lysosomes, and autolysosomes, when nutrients are scarce or mechanistic target of rapamycin complex 1 (mTORC1) is inhibited. During this process, NUFIP1 directly binds to autophagosomes *via* light chain 3 (LC3)-interacting region (LIR), followed by the recruitment of ribosomes to lysosomes for degradation. Thus, the absence of NUFIP1 or alterations in its LIR sequence negatively impacts cell survival under nutrient deprivation. NUFIP1-mediated ribophagy plays a vital role in the maintenance of intracellular protein homeostasis and cell viability, thereby contributing to the pathogenesis and development of various human diseases. A previous study

demonstrated that cancer-associated fibroblasts (CAFs) utilize NUFIP1-mediated autophagy to release nucleosides, thereby promoting pancreatic tumor growth [16]. A recent study revealed that low-protein diet leads to intestinal inflammation by interfering with NUFIP1-DNA damage response (DDR) signaling, consequently triggering necroptosis [17]. Of note, our previous study revealed that NUFIP1 exerts a protective effect on the immune function of dendritic cells *via* ribophagic activation in the setting of sepsis. Thus, a comprehensive investigation of the molecular mechanism of ribophagy and its precise regulation for the maintenance of immune function homeostasis will pave the way for developing novel strategies to improve immune response of dendritic cells following sepsis.

Ribosomes and the endoplasmic reticulum (ER) have a close structural and functional relationship, and their interaction plays a vital role in maintaining intracellular protein homeostasis, and enabling cells to cope with both internal and external stress. Various physiopathological conditions, including infection and nutritional deficiencies, interfere with ER homeostasis and lead to disturbances in the dynamic balance of Ca²⁺ transportation and accumulation of unfolded or misfolded protein in the ER. This results in ER stress, and triggers the unfolded protein response (UPR) [18, 19]. The UPR can be triggered through three primary pathways: eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3), ER to nucleus signaling 1 (ERN1), and activating transcription factor 6 (ATF6) [20]. Specifically, the eukaryotic translation initiation factor 2A (EIF2A), which acts downstream of EIF2AK3, triggers a broad suppression of protein synthesis and upregulates the expression of transcriptional activator 4 (ATF4) and DNA damage-inducible transcript 3 (DDIT3), contributing to increased apoptosis and growth inhibition. NUFIP1-mediated ribophagy is reportedly associated with ER stress, which is involved in the pathogenesis of neonatal hypoxia/ischemia. Treatment with rapamycin results in colocalization of autophagosomes with ribosomal protein S6 (RPS6) and protects against hypoxic/ischemic brain injury, implying that increased ribophagy is critical in maintaining intracellular homeostasis by targeting ER stress [21].

Our previous studies showed increased ribophagic activity in T lymphocytes and dendritic cells at early stages of sepsis. A significant increase in apoptosis was noted in both T and dendritic cells following *Nufip1* deficiency [22, 23]. Additional studies verified that NUFIP1-driven ribophagy inhibited T-cell apoptosis *via* EIF2AK–ATF4–DDIT3 signaling [22], whereas NUFIP1 reduced dendritic cell apoptosis through the ERN1–XBP1 pathway [23]. However, the relationship between ribophagy and immune dysfunction in dendritic cells during sepsis has not been reported. The current study sought to examine how NUFIP1-mediated ribophagy influences the functional activation of dendritic cells upon septic challenge and identify the underlying regulatory pathway. Our results indicate that NUFIP1-mediated ribophagy is critically

involved in the functional activation of dendritic cells by attenuating overactivated ER stress under septic exposure. Mechanistically, NUFIP1 regulates EIF2AK-ATF4-DDIT3 signaling by directly binding to ATF4, thereby modulating its nuclear translocation. NUFIP1-mediated ribophagy plays a vital role in safeguarding against dysregulated immune response and multi-organ damage secondary to sepsis.

Methods

Animals

Male C57BL/6 J wild-type (WT) mice (6–8 weeks old, weighing 20–25 g) were sourced from the Institute of Laboratory Animal Science at Peking Union Medical College (Beijing, China). *Nufip1*^{+/-} mice on a C57BL/6 J genetic background were bred at the Shanghai Model Organisms Center (Shanghai, China). Conditional knockout mice targeting NUFIP1 in dendritic cells on a C57BL/6 J background (*Cd11c*^{cre}*Nufip1*^{fl/fl}) and their littermates (*Nufip1*^{fl/fl}) were generated by Cyagen Biosciences Inc. (Suzhou, China).

Cecal ligation and puncture-induced sepsis

Sepsis was induced by cecal ligation and puncture (CLP) following a 12 h fasting period. Mice were randomly assigned to the sham or CLP groups. Anesthesia was induced by intraperitoneal injection of 0.3% pentobarbital sodium solution (10 ml/kg). A 1-cm cut was created along the midline of the abdomen, just beneath the xiphoid process. The cecum was exteriorized, ligated 1 cm from the end, punctured once with a 22 G needle, and gently squeezed to expel feces before being returned to its original position. The abdominal incision was then sutured. Subsequently, 1 ml of 0.9% saline was subcutaneously injected into the back of the neck of the mouse. The control group underwent identical procedures excluding the CLP.

Isolation of splenic dendritic cells

The murine spleens were excised aseptically. Splenic dendritic cells were isolated using two distinct CD11c⁺ Dendritic Cell Isolation Kits from Miltenyi Biotec (Bergisch Gladbach, Germany) and STEMCELL Technologies (Vancouver, Canada) according to the manufacturer's guidelines.

Isolation of murine peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated using a density gradient centrifugation-based PBMC Isolation Kit (TBDsciences, Tianjin, China) according to the manufacturer's instructions. Briefly, blood was collected through retroorbital bleeding. The cell suspensions were placed over Lymphoprep™ and centrifuged at 500 × g for 30 min. The white cloudy intermediate layer with PBMCs was collected for further analyses.

Cell culture and stimulation

Dendritic and DC2.4 cells were cultured in Roswell Park memorial institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS), 100 U/ml of penicillin, and 100 μl/ml of streptomycin. Cells were cultured in a humidified incubator at 37°C with 5% CO₂. The cells were then exposed to 1 μg/ml LPS for 6, 12, 24, or 72 h following pretreatment with 20 μM salubrinol for 30 min. The cells were collected at designated intervals for additional testing.

Flow cytometric analysis

The cells were resuspended in 100 μl of phosphate-buffered saline (PBS) followed by the addition of 1 μl of antibody and then incubated for 30 min at ambient temperature. They were then fixed with 1% paraformaldehyde. Flow cytometric (FCM) was used to assess expression of dendritic cell surface markers and determine the cell proportions among the PBMCs. Dendritic cells were grown together with splenic CD4⁺ T lymphocytes. CD4⁺ T cells treated with 5 mg/ml concanavalin A (Con A) were pre-cultured for 24 h and then co-cultured with dendritic cells (20:1) for 68–72 h. The proliferative activity of T cells was measured using carboxyfluorescein succinimidyl ester (CFSE).

Western blotting

Cells from each group were lysed and the protein was quantified using a BCA Protein Quantification Kit. The proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to the polyvinylidene difluoride membrane using the wet transfer method. The membranes were incubated with the primary antibody (1:1000) overnight at 4°C, followed by incubation with the secondary antibody (1:5000) for 1 h at room temperature. Specific antibodies against the following proteins were used for western blotting (WB): NUFIP1 (Proteintech, Cat. No. 12515-1-AP), p62 (CST, #39749), LC3B (CST, #83506), HSPA5 (CST, #3177), phosphorylated EIF2AK3 (CST, #3179), EIF2AK3 (CST, #3192), ATF4 (CST, #11815), phosphorylated EIF2A (CST, #3398), EIF2A (CST, #5324), and DDIT3 (CST, #2895). A monoclonal antibody targeting β-actin served as a control to ensure equal protein loading.

Laser scanning confocal microscopy

Cells were collected from different groups and fixed with 4% paraformaldehyde. The membrane was permeabilized using 0.3% Triton X-100, and then the cells were incubated with anti-NUFIP1 (1:200) antibody in 1% bovine serum albumin (BSA) at 4°C overnight. The cells were then incubated with a fluorescence-labeled secondary antibody (1:200) in 1% BSA for 1 h at room temperature. Thereafter, the cells were incubated with LC3B (1:50) or lyso-tracker (1:1000) in 1% BSA overnight at 4°C. The cell suspension (35 μl) was placed on a slide and a sealing agent containing 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (5 μl) was added followed by application of a coverslip. The localization and expression of proteins were observed by laser scanning confocal microscopy (LSCM).

Transmission electron microscopy

Splenic dendritic cells were collected from the sham and CLP-24 h groups and fixed with 2.5% glutaraldehyde. The fixed cell clumps were sent to the Academy of Military Sciences (China) for subsequent preparation and analyses.

Enzyme-linked immunosorbent assay

The concentrations of cytokines, including interleukin (IL)-2, IL-4, IL-10, interferon (IFN)-γ, transforming growth factor (TGF)-β, and tumor necrosis factor (TNF)-α in plasma and culture supernatants were quantified using corresponding enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems according to the manufacturer's instructions.

Co-immunoprecipitation assay

Cell samples were collected from each group and processed as follows: (i) Cells were lysed by adding a pre-configured

lysis working solution. (ii) Cells in the IP group were treated with the corresponding primary antibody, whereas cells in the IgG group were treated with the IgG antibody. (iii) Immunoprecipitation was performed adding an appropriate amount of magnetic beads to the IP and IgG samples to form protein-antibody complexes. (iv) The magnetic beads were collected using a magnetic rack and the unbound proteins were removed by washing five times. (v) The bound proteins were eluted by adding 1× SDS-PAGE buffer and heating for 10 min. The supernatant containing the target protein was collected with a magnetic rack, and the magnetic beads were discarded. (vi) The eluted proteins were identified by WB.

Histological examination

Lungs, heart, liver, and kidneys from the different groups of mice were dissected, fixed in 4% paraformaldehyde, and subsequently embedded in paraffin. Tissue cryosections (4–5- μ m thick) were deparaffinized and stained with hematoxylin and eosin (HE) for histological evaluation. Histological features were examined and analyzed using microscopy. Histological scores for the organs were assessed using a four-point scale ranging from 0 (none) to 3 (severe), with each criterion receiving a score. At least three microscopic fields were examined for each specimen (Supplemental data S1) [24].

Molecular docking

Molecular docking was performed as follows: (i) Preparation of the structures: the protein sequences were downloaded from the Uniprot database (<https://www.uniprot.org/>), and the three-dimensional structures were retrieved from the AlphaFold protein structure database (<https://alphafold.com/>). (ii) Docking process: the Pymol and LigPlot+ software were used to conduct and visualize both three-dimensional and two-dimensional force analyses. (iii) Validation: the PROtein binDIng enerGY prediction (Prodigy) web server was used to predict protein binding energy (<https://rascar.science.uu.nl/prodigy/>) (UniProt protein sequences are provided in Supplemental data S2).

Plasmid transfection

DC2.4 cells were cultured until they reached the desired confluence. The plasmid DNA was purified using a plasmid purification kit and quantitated. An appropriate amount of plasmid DNA was diluted in a suitable transfection reagent (lipofectamine or PEI) according to the manufacturer's instructions. The DNA-transfection reagent mixture was incubated at room temperature for 15–30 min to allow complex formation. The DNA-reagent complex was then added to the cultured cells, mixed gently, and incubated for 4–6 h (or as recommended by the manufacturer) at 37°C in a CO₂ incubator. Post transfection, the medium was replaced with fresh growth medium and the cells were incubated under standard culture conditions. After 24–48 h, the transfection efficiency of the cells was analyzed using WB. Co-immunoprecipitation (Co-IP) was performed to detect the interaction between proteins (plasmid sequence and vector information are shown in Supplemental data S3).

Statistical analyses

Statistical analysis was conducted using the GraphPad Prism (version 9.3.0, San Diego, CA, USA) software. Results are shown as the mean $\pm$ standard deviation (SD) derived from

three separate experiments. Multiple comparisons were performed using one- or two-way analysis of variance (ANOVA), and differences between two groups were assessed using the Student's *t*-test. Kaplan–Meier survival curve and log-rank test were employed to evaluate the survival rate of mice with sepsis. Statistical significance was set at $p < 0.05$.

Results

LPS drives dendritic cell activation and NUFIP1-mediated ribophagy

To investigate the association between ribophagy and dendritic cell activation, dendritic cells were treated with LPS for varying time periods (6, 12, 24, and 72 h) to mimic septic conditions in a controlled environment. FCM analysis showed upregulation of CD80, CD86, and MHC-II expression in dendritic cells following LPS stimulation, that peaked at 24 h and decreased by 72 h post-stimulation (Figure 1a).

We then examined the effect of LPS on ribophagy. Ribophagy was observed in splenic dendritic cells at 6, 12, 24, and 72 h post-LPS stimulation, as evidenced by the increased NUFIP1 protein levels, enhanced conversion of LC3-II to LC3-I, and p62 degradation (Figure 1b). LSCM showed colocalization of NUFIP1 with autophagosomes (LC3B puncta) and lysosomes (lyso-tracker) in splenic dendritic cells (Figure 1c). Transmission electron microscopy revealed an increase in autophagosomes containing ribosomes at 24 h post-CLP surgery (Figure 1d). Following prolonged LPS exposure (72 h), the ribophagic activity in dendritic cells was significantly inhibited compared with that in the 24 h LPS group (Figure 1b–c). These findings demonstrate that LPS stimulation upregulates NUFIP1-mediated ribophagy, accompanied by dendritic cell activation.

NUFIP1 is essential for dendritic cell activation during endotoxemia

Organelle-specific autophagy plays an intricate role in modulating dendritic cell activation in response to septic challenge. To determine the role of NUFIP1-mediated ribophagy in dendritic cell activation, we isolated splenic dendritic cells from *Nufip1*^{+/-} and WT mice, following treatment with LPS for 24 h. The results showed a decrease in CD80, CD86, and MHC-II levels in dendritic cells from *Nufip1*^{+/-} mice relative to that from WT mice following LPS injection (Figure 2a). IL-12 p70 production also reduced in *Nufip1*^{+/-} dendritic cells following LPS challenge (Figure 2b). CD4⁺ T cells cultured alongside LPS-treated *Nufip1*^{+/-} dendritic cells showed a marked decrease in proliferation, as evidenced by a smaller fraction of dividing cells, implying that *Nufip1*-deficient dendritic cells have reduced T-cell priming capacity (Figure 2c). Additionally, following LPS stimulation, the IL-2 and IFN- γ concentrations in co-culture medium were significantly lower in *Nufip1*^{+/-} dendritic cells compared with that in WT dendritic cells, whereas IL-4 levels were higher, leading to a notable reduction in IFN- γ /IL-4 ratio (Figure 2d). These results indicate that *Nufip1* deficiency downregulates the functional activation of dendritic cells in response to LPS.

NUFIP1 mediates dendritic cell activation in polymicrobial sepsis

Next, we examined the effect of NUFIP1-dependent ribophagy on dendritic cell activation in CLP-induced sepsis. For

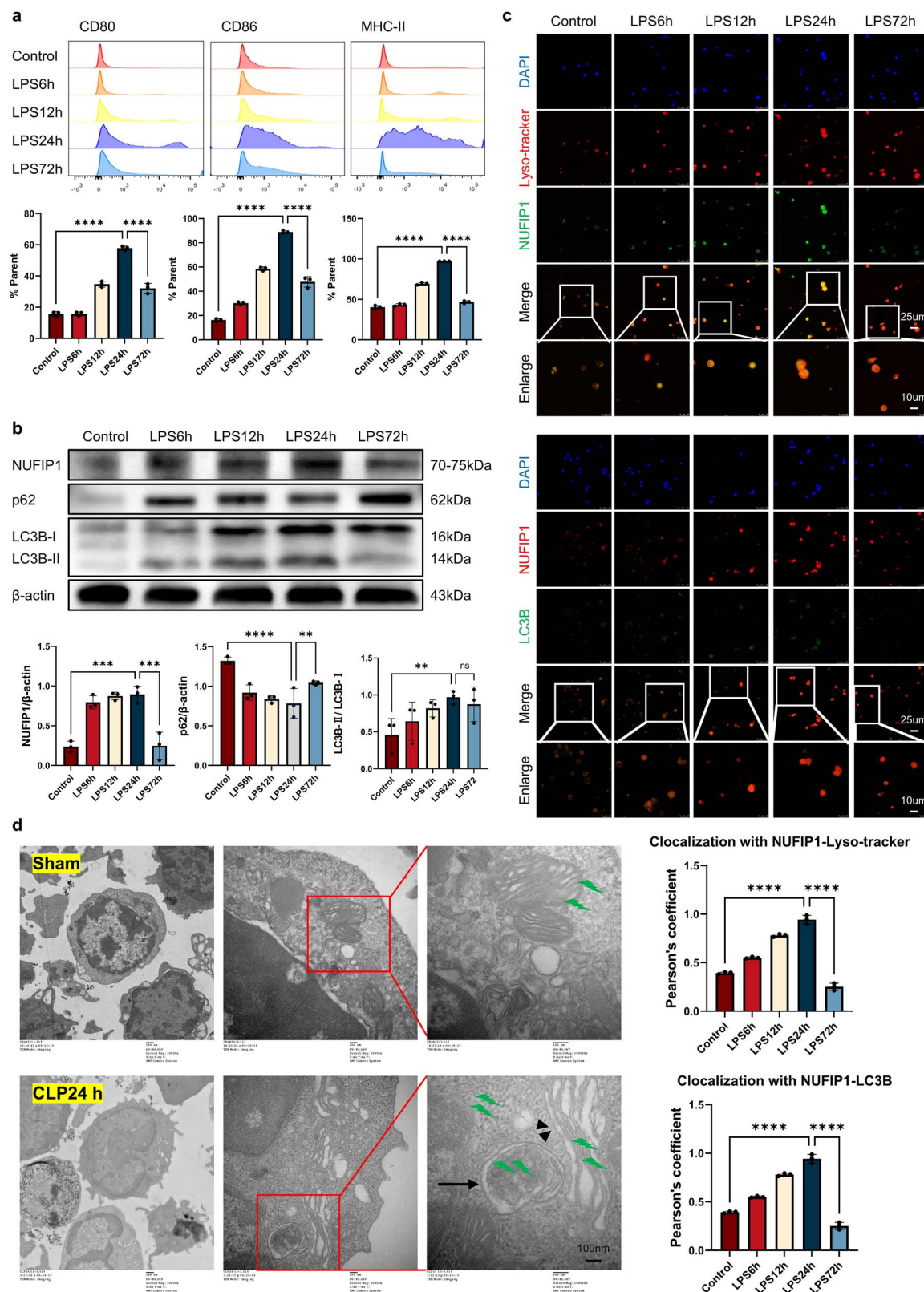


Figure 1. LPS drives dendritic cell activation and NUFIP1-mediated ribophagy. Splenic dendritic cells from WT mice were exposed to LPS for 0, 6, 12, 24, and 72 h prior to analyses. **(a)** Flow cytometric histograms and quantitative bar charts showed expression levels of CD80, CD86, and MHC-II. **(b)** Western blotting analysis of NUFIP1, p62, and LC3B protein levels at various time points. **(c)** Confocal immunofluorescence images illustrating the colocalization of NUFIP1 with lysosomes (Lyso-tracker) (left panel) and autophagosomes (LC3B puncta) (right panel), scale bar: 25 μ m & 10 μ m. **(d)** Transmission electron microscopic images showing autophagosomes containing ribosomes. Lightning icons indicate ribosomes, arrow indicates autophagosomes containing ribosomes, and triangle indicates swollen ER, scale bar: 100 nm. Data are presented as mean $\pm$ standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's *post hoc* test. Ns, not significant; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. LPS lipopolysaccharide, NUFIP1 nuclear fragile X mental retardation-interacting protein 1, WT wild-type, LC3B microtubule-associated protein 1 light chain 3 β , ER endoplasmic reticulum

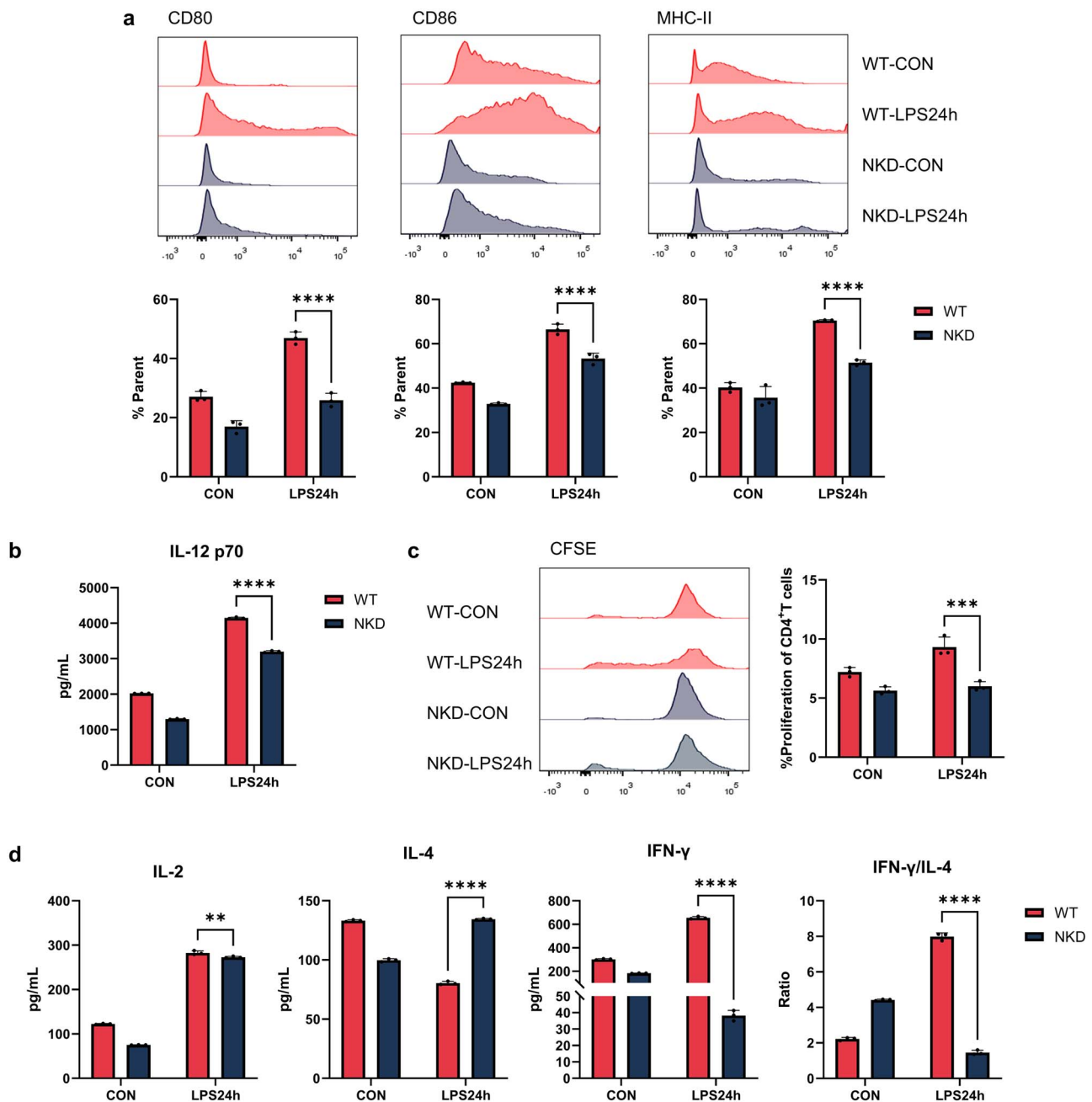


Figure 2. NUFIP1 is essential for dendritic cell activation during endotoxemia. (a-d) Splenic dendritic cells isolated from WT and *Nufip1*^{-/-} mice were exposed to either LPS or PBS for 24 h prior to various assays. (a) Flow cytometry was used to assess expression levels of CD80, CD86, and MHC-II. (b) IL-12 p70 concentration in the culture supernatants were measured using ELISA. (c) Cell division in CD4⁺ T cells was analyzed through flow cytometry following staining with CFSE dye. (d) Levels of IL-2, IL-4, and IFN-γ in co-culture supernatants were quantified by ELISA. Data are presented as mean ± standard deviation (SD). Statistical significance and multiple comparisons were determined by two-way ANOVA followed by Tukey's *post hoc* test. ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. NUFIP1 nuclear fragile X mental retardation-interacting protein 1, LPS lipopolysaccharide, PBS phosphate-buffered saline, IL interleukin, ELISA enzyme-linked immunosorbent assay, CFSE carboxyfluorescein succinimidyl ester, IFN interferon

this, we used conditional NUFIP1 knockout mice specifically targeting dendritic cells (*Cd11c*^{cre}*Nufip1*^{fl/fl}), and their littermates (*Nufip1*^{fl/fl}). By comparing the functional alterations in dendritic cells derived from septic *Nufip1*^{fl/fl} and *Cd11c*^{cre}*Nufip1*^{fl/fl} mice, we found that dendritic cells lacking NUFIP1 exhibited diminished activation upon septic exposure, as evidenced by levels of dendritic cell activation markers, cytokine secretion, and proliferative activity of CD4⁺ T-cell in co-culture assays (Figure 3a-c). We further investigated the effect of *Nufip1* depletion on sepsis-induced

immune suppression. *Nufip1* deficiency in dendritic cells aggravated immunosuppressive status, even at the early stage of sepsis, as evidenced by a substantial reduction in CD3⁺ T cells, CD4⁺ T cells, and a marked increase in CD4⁺Foxp3⁺ regulatory T cells (Tregs) (Figure 4a-c). Meanwhile, *Cd11c*^{cre}*Nufip1*^{fl/fl} mice exhibited increased secretion of IL-4, IL-10, and TGF-β, whereas IL-2 and IFN-γ production was reduced (Figure 4d). HE staining revealed more severe organ injuries in septic *Cd11c*^{cre}*Nufip1*^{fl/fl} mice compared to that in their littermates (Figure 4e).

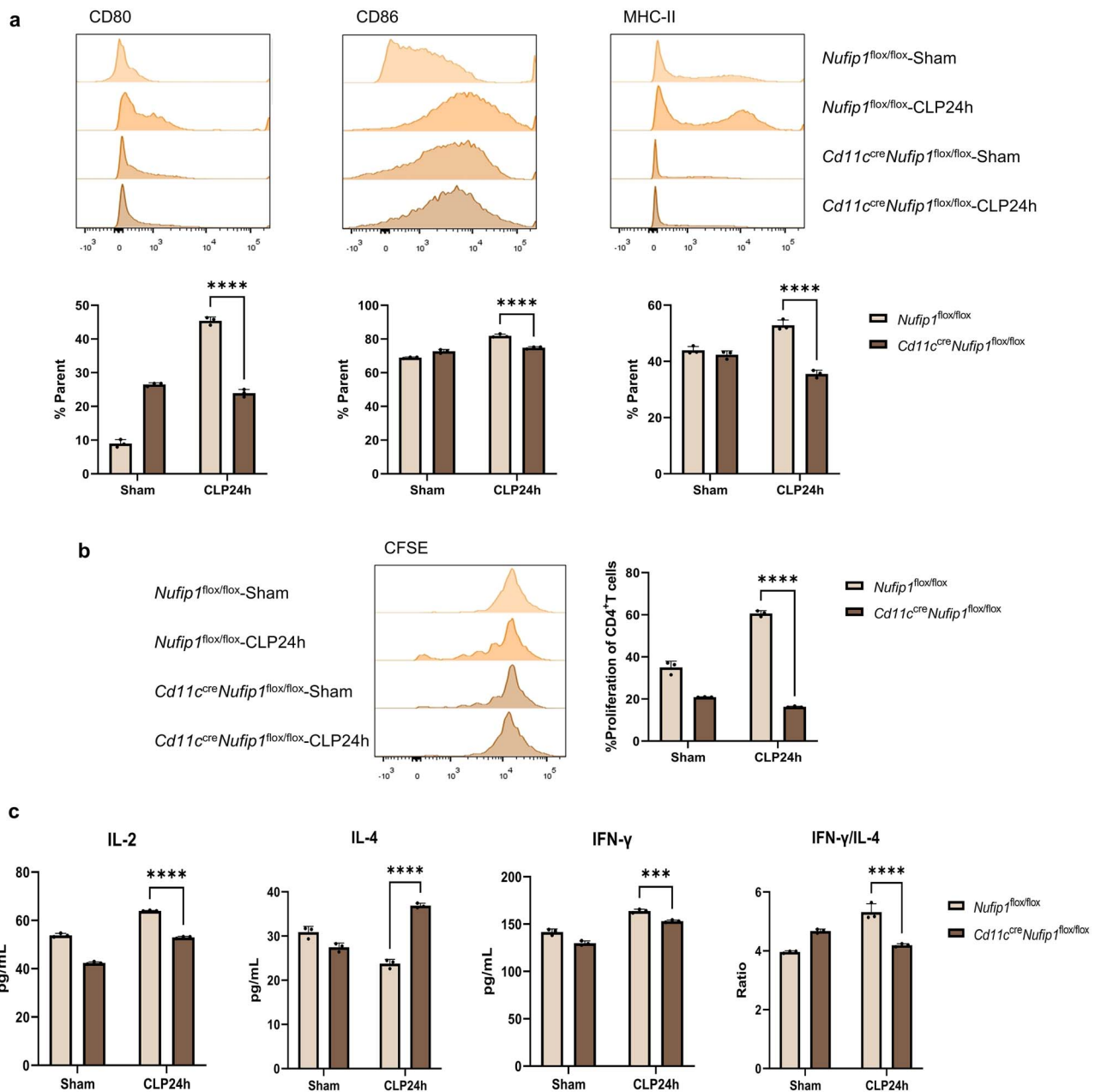


Figure 3. NUFIP1 mediates dendritic cell activation in polymicrobial sepsis. (a-c) Splenic dendritic cells were harvested from $Nufip1^{flox/flox}$ and $Cd11c^{cre}Nufip1^{flox/flox}$ mice that underwent either CLP or sham surgery for subsequent analyses. (a) Flow cytometry was used to assess expression levels of CD80, CD86, and MHC-II. (b) Cell division in CD4⁺ T cells was evaluated using flow cytometry. (c) ELISA was used to measure the levels of IL-2, IL-4, and IFN-γ. Data are presented as mean ± standard deviation (SD). Statistical significance was determined using two-way ANOVA followed by Tukey's *post hoc* test. *** $p < 0.001$, **** $p < 0.0001$. NUFIP1 nuclear fragile X mental retardation-interacting protein 1, CLP cecal ligation and puncture, ELISA enzyme-linked immunosorbent assay, IL interleukin, IFN interferon

Moreover, analysis of 7-day survival rates indicated that $Cd11c^{cre}Nufip1^{flox/flox}$ mice exhibited an earlier peak of septic death compared to $Nufip1^{flox/flox}$ littermates (Figure 4f). These findings confirm that impaired NUFIP1-mediated ribophagy exacerbates dendritic cell dysfunction in sepsis, leading to immune suppression and septic death.

Ribophagy attenuates excessive ER stress following septic insults

Our experiments revealed that moderate ER stress was conducive to dendritic cell activation (Figure S1). Previously, we reported that excessive ER stress hinders the maturation

and functional activation of dendritic cells [25, 26]. To identify the role of ribophagy in maintaining cellular homeostasis by mitigating ER stress overactivation and dysfunction [27–29], we examined whether NUFIP1 mediates the immune function of dendritic cells by modulating excessive ER stress. The relationship between the EIF2AK3–ATF4–DDIT3 pathway and NUFIP1-mediated ribophagy was evaluated using WT and $Nufip1^{+/-}$ dendritic cells treated with LPS. LPS-induced expression of heat shock protein family A member 5 (HSPA5), ATF4, DDIT3, p-EIF2AK3, and p-EIF2A in dendritic cells was further augmented by depletion of $Nufip1$ (Figure 5a). Immunofluorescence analysis showed

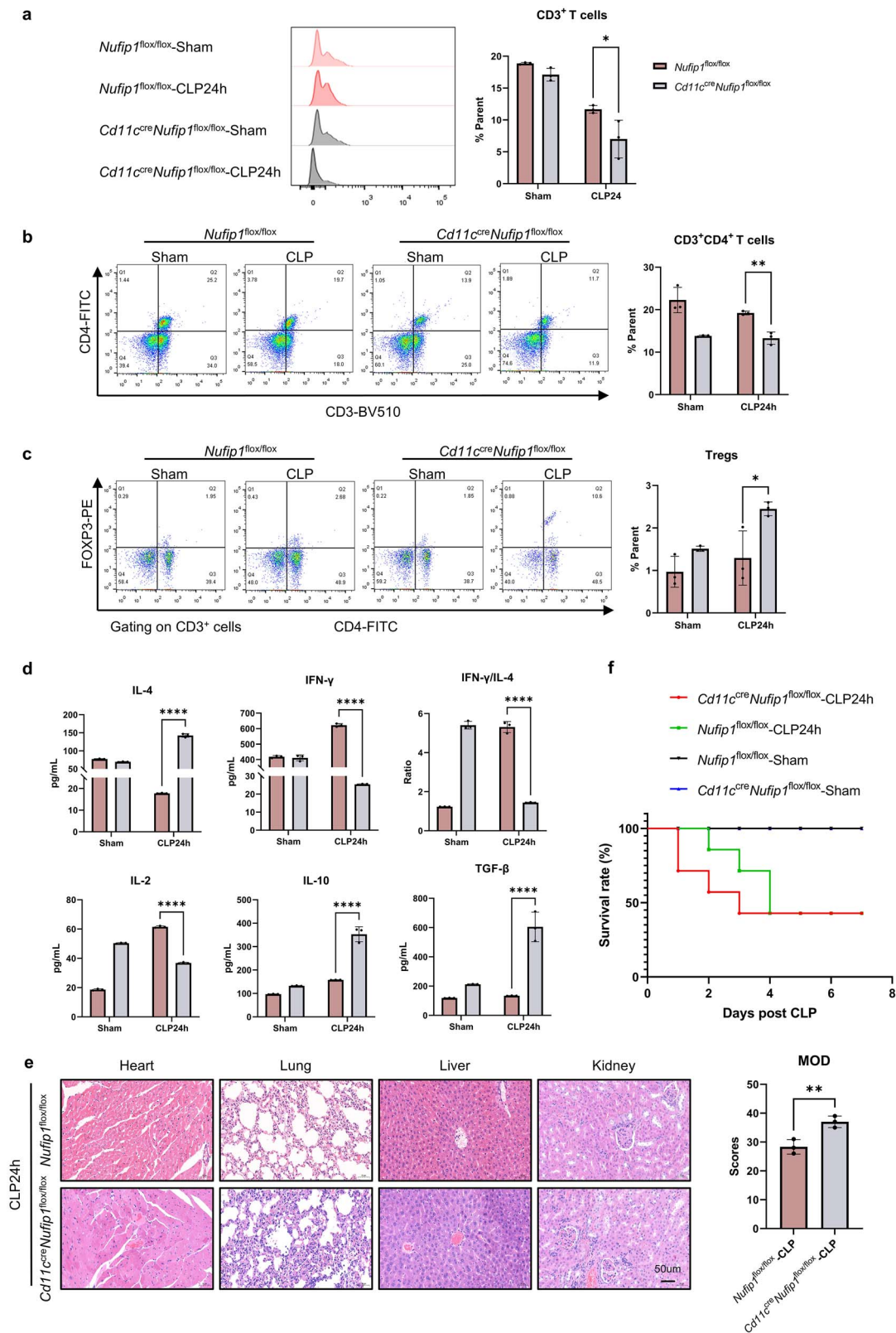


Figure 4. *Nufip1* deficiency exacerbates the immunosuppressive response in septic mice and increases sepsis-induced mortality. (a-c) PBMCs were isolated from *Nufip1^{fl/fl}* and *Cd11c^{cre}Nufip1^{fl/fl}* mice post-CLP surgery. (a) Flow cytometry was used to evaluate the percentage of CD3⁺ T cells. (b) The percentage of CD3⁺ CD4⁺ T cells were analyzed using flow cytometry. (c) The proportion of CD3⁺CD4⁺Foxp3⁺ Tregs was quantified using flow cytometry. (d) Circulating levels of cytokines including IL-2, IL-4, IL-10, IFN-γ, and TGF-β were determined using ELISA. (e) Pathological alterations in various organs, including the lungs, liver, kidneys, and heart were visualized by HE staining in mice that underwent either sham or CLP surgery, scale bar: 50 μm. (f) Survival rate of the septic mice were monitored and compared over a period of 7 d following CLP surgery. Results are presented as mean ± standard deviation (SD). Statistical analyses were performed with two-way ANOVA followed by Tukey's *post hoc* test (A-D) or by comparing survival curves using the log-rank test. **p* < 0.05, ***p* < 0.01, *****p* < 0.0001. PBMCs peripheral blood mononuclear cells, CLP cecal ligation and puncture, Tregs regulatory T cells, IL interleukin, IFN interferon, TNF tumor necrosis factor, TGF transforming growth factor, ELISA enzyme-linked immunosorbent assay

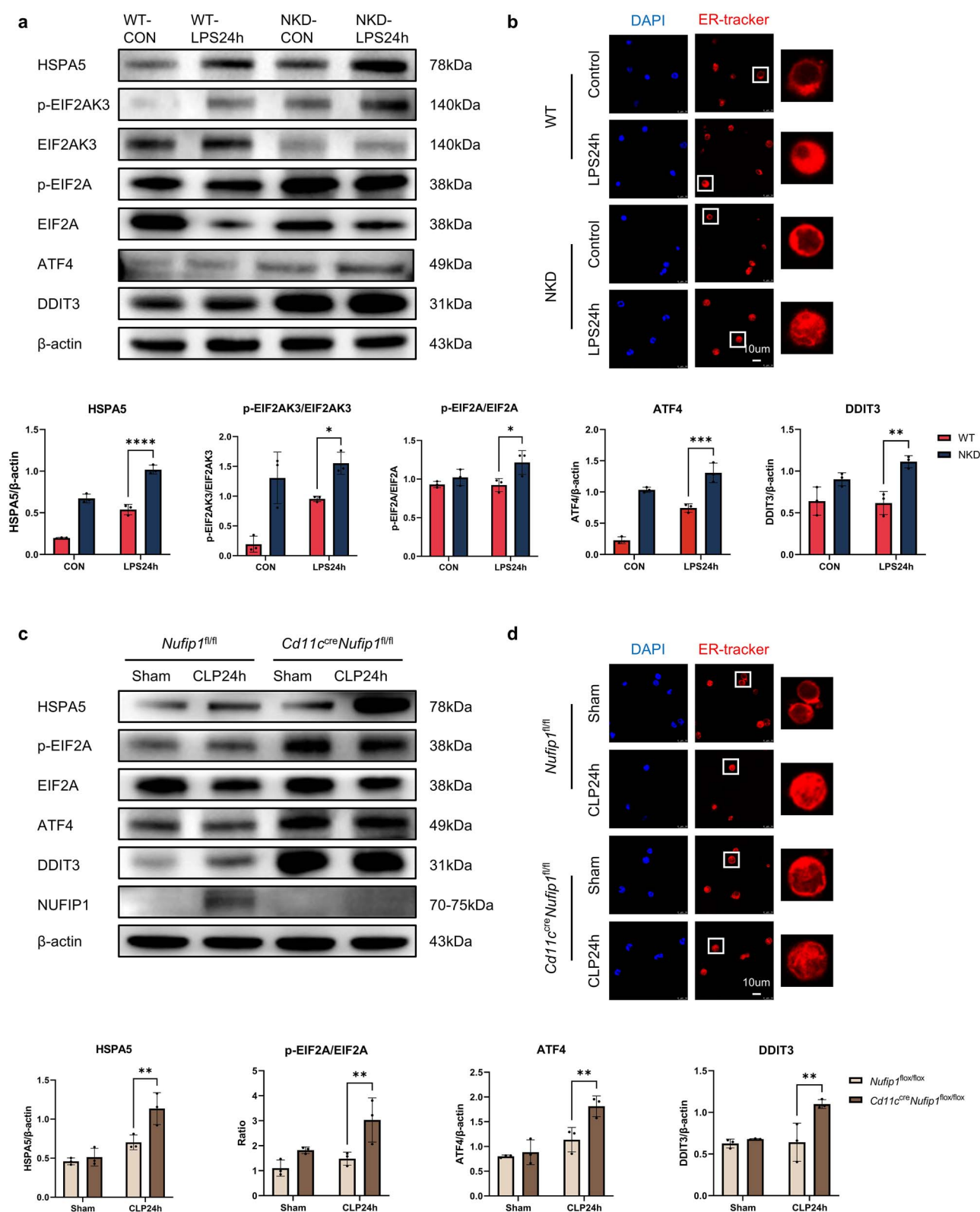


Figure 5. Septic exposure significantly upregulates UPR in dendritic cells, which is further enhanced by *Nufip1* deficiency. (a-b) Splenic dendritic cells from WT and *Nufip1*^{-/-} mice were treated with either LPS or PBS for 24 h. (a) Western blotting was used to analyze the expression levels of HSPA5, p-EIF2AK3, EIF2AK3, p-EIF2A, EIF2A, ATF4, and DDIT3. (b) Representative confocal immunofluorescence images showing the ER morphology, scale bar: 10 μm. (c-d) Splenic dendritic cells were harvested from *Nufip1*^{fl/fl} and *Cd11c*^{Cre}*Nufip1*^{fl/fl} mice following CLP or sham surgery. (c) Western blotting analysis was performed to determine expression levels of HSPA5, p-EIF2A, EIF2A, ATF4, and DDIT3. (d) Representative confocal immunofluorescence images showing ER morphology, scale bar: 10 μm. Results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using two-way ANOVA with Tukey's *post hoc* test. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. UPR unfolded protein response, WT wild-type, LPS lipopolysaccharide, PBS phosphate-buffered saline, HSPA5 shock protein family A member 5, EIF2AK3 eukaryotic translation initiation factor 2α kinase 3, EIF2A eukaryotic translation initiation factor 2A, ATF4 activating transcription factor 4, DDIT3 DNA damage-inducible transcript 3, ER endoplasmic reticulum, CLP cecal ligation and puncture

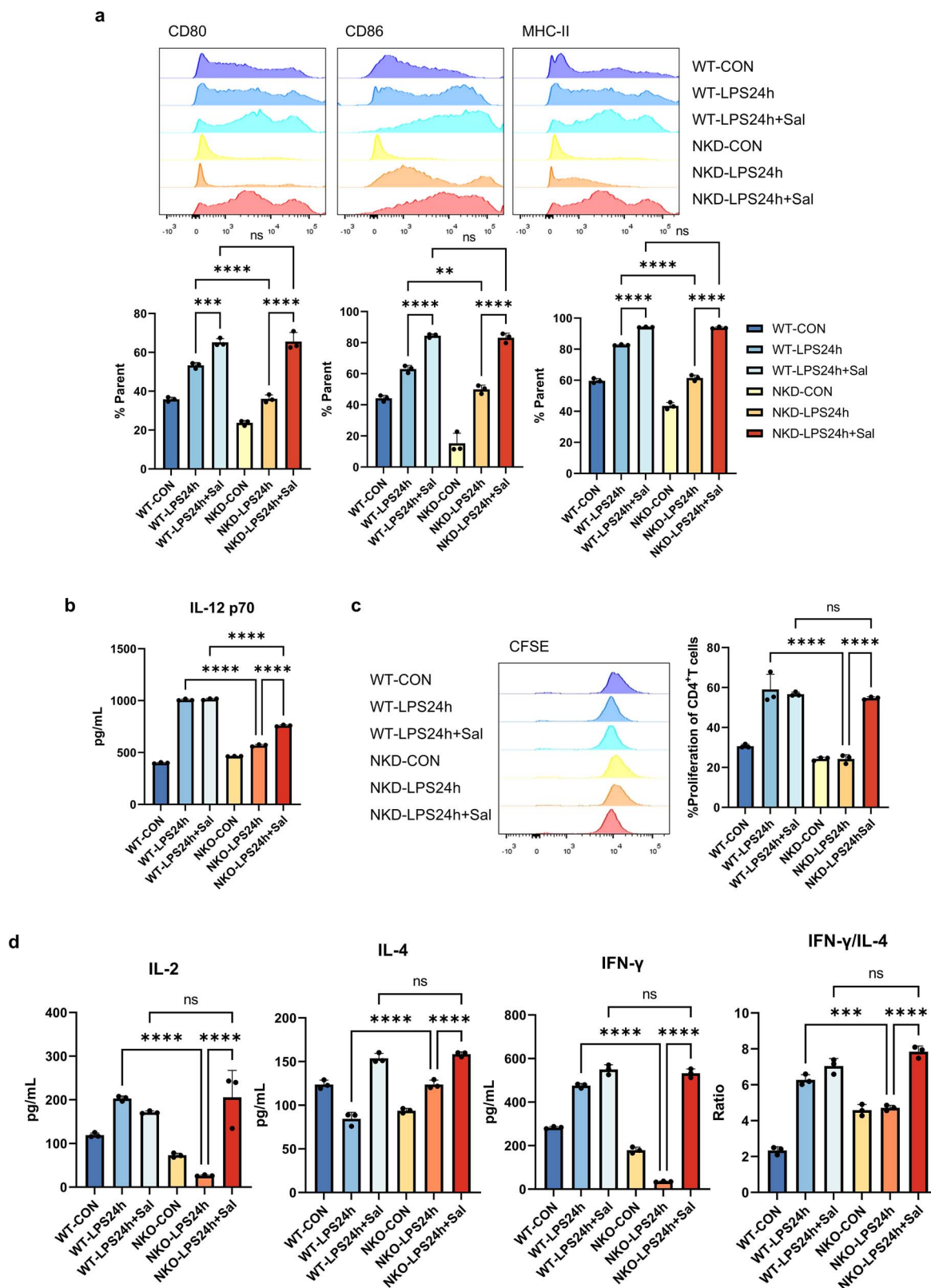


Figure 6. NUFIP1-mediated ribophagy alleviates overactivated UPR in an EIF2AK3–ATF4–DDIT3 dependent manner. **(a–d)** Splenic dendritic cells from WT and *Nufip1*^{+/−} mice were pre-incubated with or without salubrinal, an EIF2A dephosphorylation inhibitor, for 30 min before LPS stimulation. **(a)** Flow cytometry was used to assess the expression of CD80, CD86, and MHC-II. **(b)** IL-12 p70 concentration in culture supernatant was determined using ELISA. **(c)** The percentage of dividing CD4⁺ T cells, as indicated by CFSE dye staining, was evaluated by flow cytometry. **(d)** Levels of IL-2, IL-4, and IFN-γ in co-culture supernatants were measured by ELISA. Data are presented as mean ± standard deviation (SD). Statistical significance was analyzed using two-way ANOVA followed by Tukey's *post hoc* test. ns, not significant; ** *p* < 0.01, *** *p* < 0.001, **** *p* < 0.0001. *NUFIP1* nuclear fragile X mental retardation-interacting protein 1, *UPR* unfolded protein response, *EIF2AK3* eukaryotic translation initiation factor 2α kinase 3, *ATF4* activating transcription factor 4, *DDIT3* DNA damage-inducible transcript 3, *WT* wild-type, *LPS* lipopolysaccharide, *IL* interleukin, *ELISA* enzyme-linked immunosorbent assay, *CFSE* carboxyfluorescein succinimidyl ester, *IFN* interferon

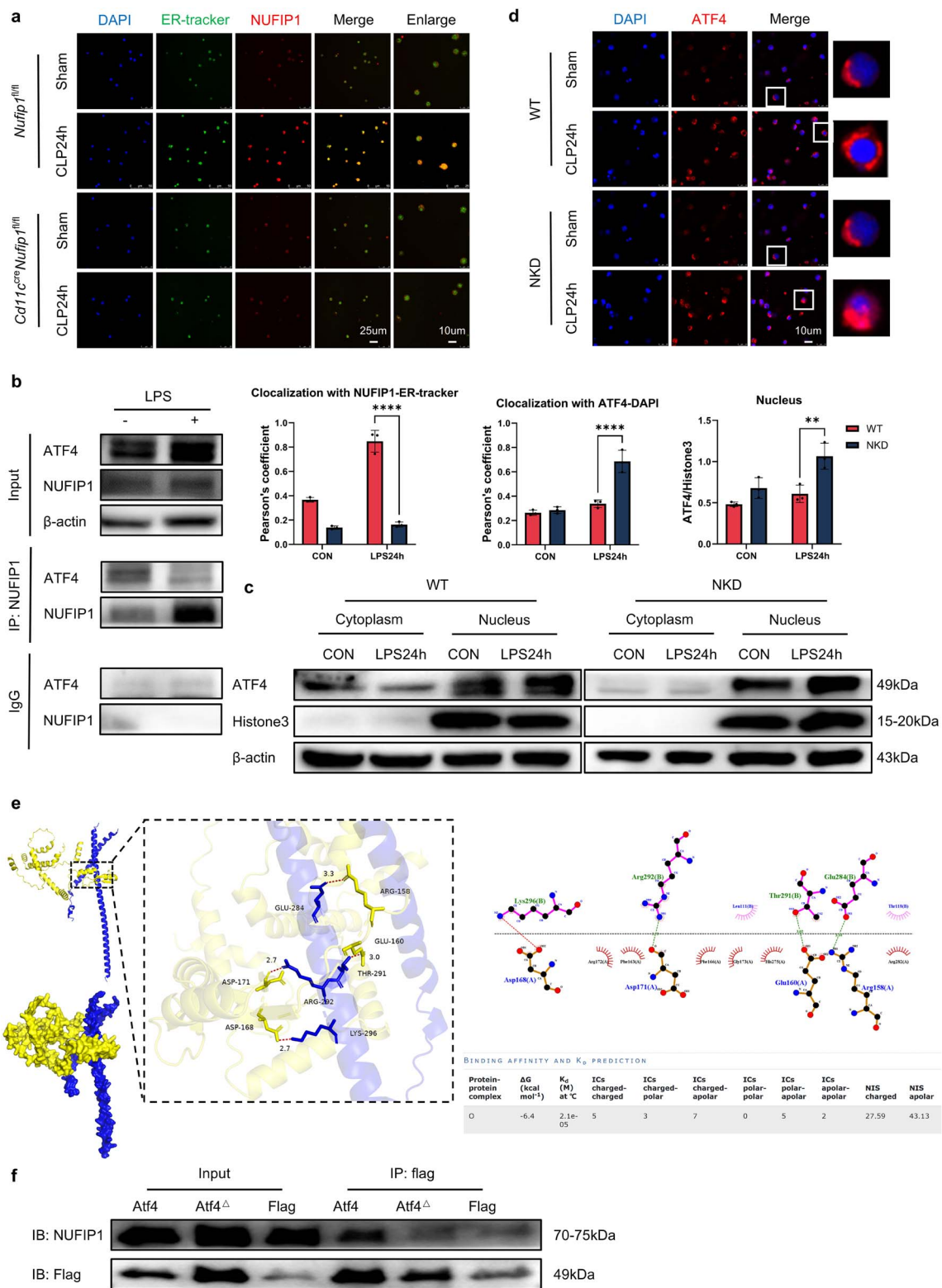


Figure 7. NUFIP1 modulates the EIF2AK3–ATF4–DDIT3 pathway by regulating ATF4 nuclear translocation. **(a)** Splenic dendritic cells were obtained from *Nufip1^{fl/fl}* and *Cd11c^{Cre}Nufip1^{fl/fl}* mice that underwent either CLP or sham surgery. Immunofluorescence imaging showing colocalization of NUFIP1 with the endoplasmic reticulum (ER-tracker), scale bar: 25 μ m & 10 μ m. **(b–d)** Splenic dendritic cells from WT and *Nufip1^{+/-}* mice were exposed to LPS or PBS for 24 h before additional analyses. **(b)** NUFIP1-binding proteins were extracted from total cell lysates by immunoprecipitation. **(c)** Immunoblotting of ATF4 levels in both the cytosol and nucleus, with β -actin and histone 3 serving as controls for cytosolic and nuclear proteins, respectively. **(d)** Laser confocal microscopy was used to evaluate ATF4 expression in dendritic cells, and the nuclei were stained using DAPI, scale bar: 10 μ m. **(e)** A structure-based analysis of the protein interaction interface between NUFIP1 and ATF4. The images illustrate the predicted NUFIP1-ATF4 complex structure with labeled interaction hotspot residues. **(f)** Flag-binding proteins were extracted from total cell lysates by immunoprecipitation. Results are presented as mean $\pm$ standard deviation (SD). Statistical analysis was conducted using two-way ANOVA followed by Tukey's *post hoc* test. ** $p < 0.01$, **** $p < 0.0001$. NUFIP1 nuclear fragile X mental retardation-interacting protein 1, EIF2AK3 eukaryotic translation initiation factor 2 α kinase 3, ATF4 activating transcription factor 4, DDIT3, DNA damage-inducible transcript 3, CLP cecal ligation and puncture, LPS, lipopolysaccharide, PBS phosphate-buffered saline, DAPI 4,6-diamidino-2-phenylindole, dihydrochloride

that *Nufip1* deficiency resulted in significant ER membrane fragmentation following LPS exposure (Figure 5b). Similarly, EIF2A phosphorylation, along with HSPA5, ATF4, and DDIT3 expression, were significantly higher in splenic dendritic cells from *Cd11c^{cre}Nufip1^{fl/fl}* mice that underwent CLP surgery compared to that from *Nufip1^{fl/fl}* septic mice (Figure 5c). LSCM confirmed notable structural alterations, including irregular distortion, swelling, and fragmentation of the ER, in dendritic cells lacking *Nufip1* (Figure 5d). We next assessed the protective effect of salubrinal, a specific inhibitor of EIF2A dephosphorylation, in reversing dendritic cell dysfunction caused by *Nufip1* deficiency. FCM analysis showed that treatment with salubrinal prior to LPS stimulation partially restored the functional deficits in splenic dendritic cells from *Nufip1^{+/-}* mice (Figure 6a). Compared with LPS-treated *Nufip1^{+/-}* dendritic cells, salubrinal-treated *Nufip1^{+/-}* dendritic cells exhibited enhanced CD4⁺ T-cell proliferative activity and differentiation propensity toward Th1 (Figure 6b-d). These data suggest that NUFIP1-mediated ribophagy may attenuate ER stress in an EIF2AK3–ATF4–DDIT3-dependent manner, thereby maintaining dendritic cell activation during sepsis.

NUFIP1 regulates the nuclear translocation of ATF4

ATF4 is a crucial transcription factor that connects ER stress with autophagy [27, 28]. ATF4 interacts with cell cycle progression protein 1 (CCPG1) to alleviate ER stress [29]. Therefore, we investigated whether NUFIP1 regulates ER stress by interacting with ATF4. NUFIP1 localized on ER in *Nufip1^{fl/fl}* dendritic cells, and its fluorescence intensity increased at 24 h post-CLP, but was absent in *Nufip1*-depleted cells (Figure 7a). We further performed Co-IP experiments using NUFIP1 antibody and identified direct interaction between NUFIP1 and ATF4 in splenic dendritic cells, which decreased following LPS treatment (Figure 7b). In addition, both WB and LCSM analyses showed that *Nufip1* deficiency resulted in enhanced translocation of ATF4 to the nucleus in LPS-treated dendritic cells (Figure 7c-d). To further examine the potential interaction between NUFIP1 and ATF4, we conducted and visualized both three-dimensional and two-dimensional force analyses. In the three-dimensional image in Figure 7e, the NUFIP1 protein is depicted as a yellow ribbon, whereas the ATF4 protein is illustrated as a blue ribbon. The potential interaction is indicated as red dashed lines. In the two-dimensional visualization, green dashed lines denote hydrogen bonds, whereas red dashed lines denote hydrophobic interactions. Our findings indicate a binding energy of -6.4 kcal/mol between NUFIP1 and ATF4. Generally, more negative binding energy between a ligand and its target protein indicates greater stability (Figure 7e). Further studies verified that NUFIP1 binds to residues 284–296 in ATF4 (Figure 7f). Collectively, these data indicate that NUFIP1 modulates the activity of EIF2AK3–ATF4–DDIT3 signaling by directly influencing ATF4 nuclear translocation.

Discussion

Sepsis occurs when infection propagates beyond its initial location, causing a dysregulated immune reaction that leads to multiple organ failure. The results of the present study suggest that NUFIP1 plays a key role in activating dendritic cells in sepsis by promoting the degradation of ribosomes *via*

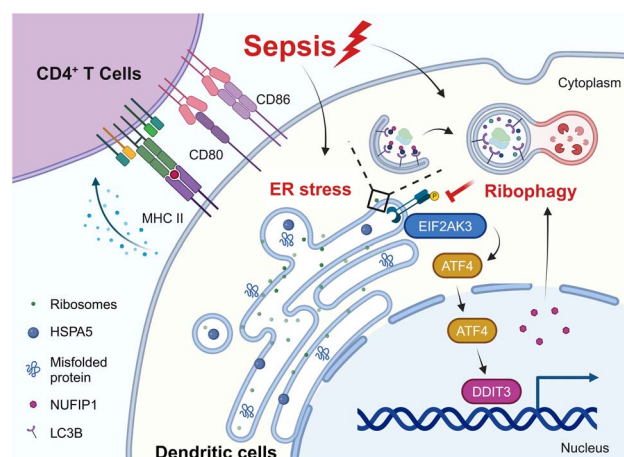


Figure 8. Dysregulated NUFIP1-mediated ribophagy impairs the immune function of dendritic cells in sepsis. NUFIP1-driven ribophagy alleviates the hyperactivated UPR *via* the EIF2AK3–ATF4–DDIT3 signaling pathway. Continuous septic exposure markedly impairs NUFIP1-mediated ribophagy, severely reducing dendritic cell function and resulting in notable immunosuppression following septic challenge. *NUFIP1* nuclear fragile X mental retardation-interacting protein 1, *UPR* unfolded protein response, *EIF2AK3* eukaryotic translation initiation factor 2 α kinase 3, *ATF4* activating transcription factor 4, *DDIT3* DNA damage-inducible transcript 3, *ER* endoplasmic reticulum, *HSPA5* shock protein family A member 5, *LC3B* microtubule-associated protein 1 light chain 3 β , *ER stress* endoplasmic reticulum stress

ribophagy. NUFIP1-associated ribophagy mitigates excessive ER stress by modulating the EIF2AK3–ATF4–DDIT3 pathway in dendritic cells. Thus, targeting NUFIP1 may offer a novel strategy for managing host immune suppression in the setting of sepsis (Figure 8).

The general consensus is that dysfunction of dendritic cells in sepsis contributes to worsening immunosuppression, which is strongly associated with a greater risk of secondary infections and higher fatal outcomes in individuals with sepsis. Hence, understanding the precise mechanisms underlying dendritic cell dysfunction holds the promise of reversing sepsis-related immune suppression [5, 7].

In our previous study, we proposed that quality control of multiple organelles (QCMO) may be essential for the maintenance of immune cell functions [30]. Organelle-selective autophagy or organellophagy is an intracellular process that eliminates damaged or redundant organelles *via* autophagic receptor-mediated mechanisms, including mitophagy, reticulophagy, ribophagy, pexophagy, nucleophagy, and lysophagy, which play pivotal roles in quality control of various organelles [31]. Emerging evidence suggests that reticulophagy regulator 1 (RETREG1)-mediated reticulophagy is crucial for maintaining ER homeostasis and regulating immune function [32, 33]. In a report on allergic rhinitis, single nucleotide polymorphisms (SNPs) in the upstream promoter region of *Retreg1* showed clear negative correlation with SNPs in the CD39 promoter [34]. CD39, an important marker on Tregs, inhibits airway inflammation by reducing extracellular ATP. ATP enhances RETREG1 expression in PBMCs, indicating that RETREG1 may be critically involved in managing local immune response [34, 35]. RETREG1-mediated reticulophagy has been shown to protect against sepsis-induced myocardial injury in mice by ameliorating inflammation and cardiomyocyte apoptosis [36]. Conversely, PTEN-induced putative kinase 1

(PINK1)-mediated mitophagy mitigates macrophage activation induced by LPS and IFN- γ . This counteraction results in immunosuppression, reduced bacterial clearance, and lower survival rates in mouse models of sepsis [37]. Therefore, the impact of organelle-specific autophagy on host immune response may be context-dependent. Recent findings suggest that the quality control of ribosomes is another critical way to maintain cell functions and survival under stressful states [13]. Prolonged exposure to the septic stimulus dysregulates ribosome numbers and functions, resulting in excessive production of aberrant proteins, which negatively impacts cell viability [23, 38]. Organelle-specific autophagy extensively interacts with various cellular processes. Studies have shown that agents typically used to induce selective autophagy also promote increased ribosome and cytosolic protein reporter flux. This is often referred to as "bystander" autophagy [39]. For instance, reticulophagy may eliminate the bound ribosomes while engulfing the ER. Conversely, ribophagy may influence reticulophagy *via* the connection of ER stress.

Ribophagy, an indispensable component in the quality control mechanism of ribosomes, specifically degrades damaged or excessive ribosomes [14, 15], and adjusts the ribosomal turnover ratios suitable for the internal environment and facilitates cellular survival during stressful conditions. In pancreatic ductal adenocarcinoma (PDAC), CAFs were found to exploit NUFIP1-dependent autophagy for ribosome secretion, favoring PDAC growth. Conversely, targeting NUFIP1 in the mesenchyme inhibited nucleoside secretion and inhibited tumor progression [16]. A recent study identified a new pathway in which amino acid deficiency impacts DDR by reducing the level of NUFIP1 protein, resulting in the accumulation of DNA damage in intestinal tissue of mice with inflammatory bowel disease (IBD), and exacerbates the enteritis phenotype [17]. Our previous study in septic mice showed that NUFIP1-mediated ribophagic activity was significantly activated in CD4⁺ T lymphocytes at early stages of sepsis. However, sepsis-induced T-cell apoptosis was markedly elevated following *Nufip1* depletion [22]. Thus, dysfunction of NUFIP1-mediated ribophagy is of great significance in the development of several human diseases. In the present study, both NUFIP1 expression and ribophagy activation revealed a similar pattern, corresponding with the immune functionality of dendritic cells. We further confirmed the critical involvement of NUFIP1-mediated ribophagy in the activation of dendritic cells upon septic challenge by constructing *Nufip1*^{+/−} and *Cd11c*^{cre}*Nufip1*^{fl/fl} mice. The *Nufip1*-deficient dendritic cells exhibited impaired activation compared with the WT dendritic cells. Of note, septic mice with *Nufip1* depletion in dendritic cells showed marked exacerbation of peripheral immune suppression and organ injuries, resulting in increased 7-day mortality post-CLP. Thus, this study indicates that impaired activation of NUFIP1-mediated ribophagy results in dampened dendritic cell activation and sepsis-induced immunosuppression. Nevertheless, other forms of organelle degradation may also exert a synergistic effect. Recently, it was reported that PINK1-driven mitophagy prevents the compromised immune activation of dendritic cells in sepsis by controlling mitochondrial division *via* dynamin-related protein 1 [40]. Taken together, it is plausible that modulating ribophagy through NUFIP1 could be a novel therapeutic approach for treating immunosuppression caused by sepsis.

Ribosomes are the main sites for intracellular protein synthesis, whereas the ER is responsible for the processing,

folding and modification of secretory and membrane-resident proteins post-translation, and both play indispensable yet synergistic roles in maintaining protein homeostasis. Any factor that disrupts ER homeostasis can finally lead to ER stress. A certain extent of ER stress activates the downstream UPR pathway, which facilitates ER load and restores proteostasis, thereby improving cellular ability to adapt to stressful conditions. When the stimulus is too strong or long-acting, UPR fails to reverse cytotoxic ER stress and restore ER homeostasis in time, thus causing proteotoxicity and even programmed cell death [10, 41, 42]. Our previous reports demonstrated that UPR and its key player XBP1 were crucial for the maturation and activation of dendritic cells triggered by high mobility group box-1 protein (HMGB1). Knockdown of *XBP1* led to defective maturation and activation of dendritic cells exposed to HMGB1 [26]. Salubrin administration markedly improved the development and activation of dendritic cells [25]. Likewise, enhanced ribophagic activity following LPS exposure significantly alleviated excessive ER stress in T cells, thereby facilitating cell survival under stressful states, which is consistent with our findings [21]. Therefore, we hypothesized that NUFIP1-mediated ribophagy exerts a protective effect on dendritic cell functions by inhibiting hyperactivated ER stress.

In the present study, NUFIP1 deficiency resulted in the overactivation of ER stress, as evidenced by marked upregulation of the EIF2AK3–ATF4–DDIT3 pathway and ER fragmentation. Meanwhile, blocking EIF2A partially rescued sepsis-induced dendritic cell dysfunction caused by *Nufip1* depletion, which is consistent with our previous observation that salubrin ameliorates functional deficits in dendritic cells in sepsis [10]. Other studies have reported that blocking EIF2AK3 signaling in dendritic cells restores effector CD4⁺ T-cell response and reduces airway inflammation following respiratory syncytial virus infection [43]. Triggering the EIF2AK3–ATF4 pathway in tumor-associated macrophages is strongly related to the anti-cancer immune reaction [44]. ATF4 is essential for loperamide-induced reticulophagy and autophagic cell death through RETREG1/testis-expressed sequence 264 protein in glioblastoma cells [27]. In ovarian granulosa cells, ATF4 interacts with CCPG1 through microtubule-associated protein 1 light chain 3 β (LC3B) to alleviate ER stress via induction of reticulophagy [29]. Thus, ATF4 plays an important role in mediating immune function and viability *via* its interactions with various organellophagy receptors. Our results suggest that NUFIP1 directly interacts with ATF4 and regulates its nuclear translocation, negatively modulating the EIF2AK3–ATF4–DDIT3 pathway.

Targeted molecular therapy for sepsis is a developing field, with various clinical cases and trials investigating different strategies to improve outcomes. Some notable examples include: anti-TNF therapy (Etanercept, Infliximab), IL-1 receptor antagonist (Anakinra), anti-C5a therapy (Eculizumab), Toll-like receptor 4 antagonists (Eritoran), and recombinant human activated protein C (Drotrecogin Alfa) [45]. Studies have found that NUFIP1-engineered exosomes derived from human bone marrow mesenchymal stem cells (hBM-MSCs) have the potential to regulate apoptosis and neurological injury induced by propofol in newborn rats, suggesting that targeting NUFIP1 may be of great significance in the prevention and treatment of human diseases [46]. Although there is limited direct clinical data on NUFIP1-targeted therapy for sepsis, theoretical possibilities exist based on its biological functions. The advantages

of NUFIP1-targeted therapy are as follows: (i) Specificity: NUFIP1-based therapies may potentially offer more precise targeting of molecular pathways involved in the immune response to sepsis. (ii) Regulation of protein synthesis to modulate stress response: by influencing ribosome biogenesis and function, NUFIP1 may help modulate the immune response and protein synthesis pathways that are dysregulated in sepsis. (iii) Potential for novel mechanisms: as a relatively unexplored target, NUFIP1 may open new avenues for therapeutic intervention distinct from traditional anti-inflammatory or anti-microbial approaches. The potential disadvantages of NUFIP1-based therapies are as follows: (i) Limited clinical evidence: direct clinical trials or case studies evaluating NUFIP1-targeted therapies in sepsis are lacking, making it difficult to predict its efficacy and potential outcomes. (ii) Complex regulation: NUFIP1 is involved in complex cellular processes; modulating its activity may have unforeseen consequences on cell function and overall patient health. (iii) Potential side effects: given its role in fundamental cellular processes such as protein synthesis, targeting NUFIP1 may influence physiological cellular function, potentially leading to adverse effects. (iv) Challenges in drug development: developing molecules that specifically and effectively target NUFIP1 within the complex environment in a patient with sepsis would be challenging.

Certain limitations should be considered when interpreting this study. Firstly, we demonstrated that the potential mechanism through which NUFIP1-mediated ribophagy regulates dendritic cell activation involves EIF2AK3–ATF4–DDIT3 signaling. Further in-depth investigations are needed to verify the specific pathways of ribophagy that regulate dendritic cell function. Secondly, the current study does not provide a direct and precise link between aggravated ER stress and impaired functional activation of dendritic cells. Thirdly, validating our findings in human sepsis is necessary for the development of therapeutic strategies and immune monitoring indicators. To address these gaps, future studies should focus on several approaches as discussed next. Conducting longitudinal studies would help in tracking the activation markers over time and correlating these parameters with clinical outcomes. Implementing more sophisticated functional assays would enable direct assessment of the immune responses elicited by dendritic cells with specific activation markers. Utilizing single-cell RNA sequencing and proteomics may provide a detailed map of the heterogeneity within dendritic cell populations.

Conclusions

Collectively, NUFIP1 expression modulates dendritic cell activation *via* ribophagy during sepsis development, and regulates ER stress through the EIF2AK3–ATF4–DDIT3 pathway. This study provides novel insights into the cellular and molecular mechanisms underlying sepsis-induced immunosuppression, paving the way for new immunomodulatory strategies for sepsis treatment.

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Author contributions

Li-yu Zheng (Investigation [lead], Writing—original draft [lead]), Peng-yi He (Investigation [equal]), Peng-yue Zhao (Writing—review &

editing [equal]), Yu Duan (Investigation [supporting]), Meng-yao Wu (Investigation [supporting]), Shu-ting Wei (Investigation [supporting]), Yao Wu (Project administration [lead]), Dong Ning (Data curation [lead], Methodology [lead], Software [lead]), Lei Zheng (Data curation [lead]), Renqi Yao (Conceptualization [equal], Writing—review & editing [lead]), Yongming Yao (Conceptualization [lead], Supervision [lead], Writing—review & editing [equal]).

Supplementary data

Supplementary data are available at *Burns & Trauma Journal* online.

Ethics approval and consent to participate

The experiments were carried out according to the NIH Guide for Care and Use of Laboratory Animals and approved by Scientific Investigation Committee of the Chinese PLA General Hospital (Approval No. SYXK2021–0016; Beijing, China).

Conflict of interest

The authors declare no conflict of interest.

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Data availability

The data and material are available from the corresponding authors upon reasonable request.

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M. *et al.* The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016;315:801–10. <https://doi.org/10.1001/jama.2016.0287>.
2. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. *Nat Rev Dis Primers* 2016;2:16045. <https://doi.org/10.1038/nrdp.2016.45>.
3. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR. *et al.* Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the global burden of disease study. *Lancet*. 2020;395:200–11. [https://doi.org/10.1016/S0140-6736\(19\)32989-7](https://doi.org/10.1016/S0140-6736(19)32989-7).
4. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat Rev Nephrol* 2018;14:121–37. <https://doi.org/10.1038/nrneph.2017.165>.
5. Torres LK, Pickkers P, van der Poll T. Sepsis-induced immunosuppression. *Annu Rev Physiol* 2022;84:157–81. <https://doi.org/10.1146/annurev-physiol-061121-040214>.
6. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. *Nat Rev Immunol* 2019;19:89–103. <https://doi.org/10.1038/s41577-018-0088-1>.
7. Yao RQ, Ren C, Zheng LY, Xia ZF, Yao YM. Advances in immune monitoring approaches for sepsis-induced immunosuppression. *Front Immunol* 2022b;13:891024. <https://doi.org/10.3389/fimmu.2022.891024>.
8. Zheng LY, Duan Y, He PY, Wu MY, Wei ST, Du XH. *et al.* Dysregulated dendritic cells in sepsis: functional impairment and

- regulated cell death. *Cell Mol Biol Lett* 2024;29:81. <https://doi.org/10.1186/s11658-024-00602-9>.
9. Ren C, Yao RQ, Wang LX, Li JC, Chen KW, Wu Y. *et al.* Antagonism of cerebral high mobility group box 1 ameliorates dendritic cell dysfunction in sepsis. *Front Pharmacol* 2021;12:665579. <https://doi.org/10.3389/fphar.2021.665579>.
 10. Li JY, Ren C, Wang LX, Yao RQ, Dong N, Wu Y. *et al.* Sestrin2 protects dendrite cells against ferroptosis induced by sepsis. *Cell Death Dis* 2021b;12:834. <https://doi.org/10.1038/s41419-021-04122-8>.
 11. Liu SQ, Ren C, Yao RQ, Wu Y, Luan YY, Dong N. *et al.* TNF- α -induced protein 8-like 2 negatively regulates the immune function of dendritic cells by suppressing autophagy via the TAK1/JNK pathway in septic mice. *Cell Death Dis* 2021;12:1032. <https://doi.org/10.1038/s41419-021-04327-x>.
 12. Bouras M, Asehnoune K, Roquilly A. Contribution of dendritic cell responses to sepsis-induced immunosuppression and to susceptibility to secondary pneumonia. *Front Immunol* 2018;9:2590.
 13. Zhao PY, Yao RQ, Zhang ZC, Zhu SY, Li YX, Ren C. *et al.* Eukaryotic ribosome quality control system: a potential therapeutic target for human diseases. *Int J Biol Sci* 2022;18:2497–514. <https://doi.org/10.7150/ijbs.70955>.
 14. Kraft C, Deplazes A, Sohrmann M, Peter M. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nat Cell Biol* 2008;10:602–10. <https://doi.org/10.1038/ncb1723>.
 15. Wyant GA, Abu-Remaileh M, Frenkel EM, Laqtom NN, Dharamdasani V, Lewis CA. *et al.* NUFIP1 is a ribosome receptor for starvation-induced ribophagy. *Science*. 2018;360:751–8. <https://doi.org/10.1126/science.aar2663>.
 16. Yuan M, Tu B, Li H, Pang H, Zhang N, Fan M. *et al.* Cancer-associated fibroblasts employ NUFIP1-dependent autophagy to secrete nucleosides and support pancreatic tumor growth. *Nat Cancer* 2022;3:945–60. <https://doi.org/10.1038/s43018-022-00426-6>.
 17. Ming H, Tan J, Cao SY, Yu CP, Qi YT, Wang C. *et al.* NUFIP1 integrates amino acid sensing and DNA damage response to maintain the intestinal homeostasis. *Nat Metab* 2025;7:120–36. <https://doi.org/10.1038/s42255-024-01179-5>.
 18. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. *Nat Rev Mol Cell Biol* 2020;21:421–38. <https://doi.org/10.1038/s41580-020-0250-z>.
 19. Khan MM, Yang WL, Wang P. Endoplasmic reticulum stress in sepsis. *Shock*. 2015;44:294–304. <https://doi.org/10.1097/SHK.0000000000000425>.
 20. Ghemrawi R, Khair M. Endoplasmic reticulum stress and unfolded protein response in neurodegenerative diseases. *Int J Mol Sci* 2020;21:6127. <https://doi.org/10.3390/ijms21176127>.
 21. Carloni S, Albertini MC, Galluzzi L, Buonocore G, Proietti F, Balduini W. Increased autophagy reduces endoplasmic reticulum stress after neonatal hypoxia-ischemia: role of protein synthesis and autophagic pathways. *Exp Neurol* 2014;255:103–12. <https://doi.org/10.1016/j.expneurol.2014.03.002>.
 22. Zhao PY, Yao RQ, Zheng LY, Wu Y, Li YX, Dong N. *et al.* Nuclear fragile X mental retardation-interacting protein 1-mediated ribophagy protects T lymphocytes against apoptosis in sepsis. *Burns Trauma* 2023;11:tkac055. <https://doi.org/10.1093/burnst/tkac055>.
 23. Zheng LY, Yao RQ, Dong N, Wu Y, Yao YM. The effect of ribophagy on lipopolysaccharide-induced apoptosis of dendritic cells. *Zhonghua Ji Zhen Yi Xue Za Zhi* 2022a;31:1334–40.
 24. Yao RQ, Li ZX, Wang LX, Li YX, Zheng LY, Dong N. *et al.* Single-cell transcriptome profiling of the immune space-time landscape reveals dendritic cell regulatory program in polymicrobial sepsis. *Theranostics*. 2022a;12:4606–28. <https://doi.org/10.7150/thno.72760>.
 25. Zhu XM, Dong N, Wang YB, Zhang QH, Yu Y, Yao YM. *et al.* The involvement of endoplasmic reticulum stress response in immune dysfunction of dendritic cells after severe thermal injury in mice. *Oncotarget*. 2017;8:9035–52. <https://doi.org/10.18632/oncotarget.14764>.
 26. Zhu XM, Yao FH, Yao YM, Dong N, Yu Y, Sheng ZY. Endoplasmic reticulum stress and its regulator XBP-1 contributes to dendritic cell maturation and activation induced by high mobility group box-1 protein. *Int J Biochem Cell Biol* 2012;44:1097–105. <https://doi.org/10.1016/j.biocel.2012.03.018>.
 27. Zielke S, Kardo S, Zein L. *et al.* ATF4 links ER stress with reticulophagy in glioblastoma cells. *Autophagy*. 2021;17:2432–48. <https://doi.org/10.1080/15548627.2020.1827780>.
 28. B'chir W, Maurin AC, Carraro V, Averous J, Jousse C, Muranishi Y. *et al.* The eIF2 α /ATF4 pathway is essential for stress-induced autophagy gene expression. *Nucleic Acids Res* 2013;41:7683–99. <https://doi.org/10.1093/nar/gkt563>.
 29. Li H, Jing Y, Qu X, Yang J, Pan P, Liu X. *et al.* The activation of reticulophagy by ER stress through the ATF4-MAP1LC3A-CCPG1 pathway in ovarian granulosa cells is linked to apoptosis and necroptosis. *Int J Mol Sci* 2023;24:2749. <https://doi.org/10.3390/ijms24032749>.
 30. Yao RQ, Ren C, Xia ZF, Yao YM. Organelle-specific autophagy in inflammatory diseases: a potential therapeutic target underlying the quality control of multiple organelles. *Autophagy*. 2021;17:385–401. <https://doi.org/10.1080/15548627.2020.1725377>.
 31. Duan Y, Yao RQ, Ling H, Zheng LY, Fan Q, Li Q. *et al.* Organelle-phagy regulates cell death: a potential therapeutic target for inflammatory diseases. *J Adv Res* 2025;70:371–91. <https://doi.org/10.1016/j.jare.2024.05.012>.
 32. Mo J, Chen J, Zhang B. Critical roles of FAM134B in ER-phagy and diseases. *Cell Death Dis* 2020;11:983.
 33. Duan Y, Yao RQ, Zheng LY, Dong N, Wu Y, Yao YM. *et al.* Influence of family with sequence similarity 134, member B-mediated reticulophagy on lipopolysaccharide-induced apoptosis of mouse dendritic cells. *Zhonghua Shao Shang Yu Chuang Mian Xiu Fu Za Zhi* 2023;39:857–66. <https://doi.org/10.3760/cma.j.cn501225-20230227-00063>.
 34. Melchiorri P, Puan KJ, Andiappan AK, Poh TY, Starke M, Zhuang L. *et al.* Genetic analysis of an allergic rhinitis cohort reveals an intercellular epistasis between FAM134B and CD39. *BMC Med Genet* 2014;15:73.
 35. Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MA, Muskens F. *et al.* Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 2007;13:913–9. <https://doi.org/10.1038/nm1617>.
 36. Li T, Chen Y, Li Y, Yao Z, Liu W. FAM134B-mediated endoplasmic reticulum autophagy protects against sepsis myocardial injury in mice. *Aging*. 2021a;13:13535–47. <https://doi.org/10.18632/aging.202786>.
 37. Patoli D, Mignotte F, Deckert V, Dusuel A, Dumont A, Rieu A. *et al.* Inhibition of mitophagy drives macrophage activation and antibacterial defense during sepsis. *J Clin Invest* 2020;130:5858–74. <https://doi.org/10.1172/JCI130996>.
 38. Zheng LY, Yao RQ, Yao YM. Update advances in ribosome-associated quality control and ribophagy. *Prog Biochem Biophys* 2022b;49:1648–57.
 39. An H, Harper JW. Systematic analysis of ribophagy in human cells reveals bystander flux during selective autophagy. *Nat Cell Biol* 2018;20:135–43. <https://doi.org/10.1038/s41556-017-0007-x>.
 40. Wu Y, Chen L, Qiu Z, Zhang X, Zhao G, Lu Z. PINK1 protects against dendritic cell dysfunction during sepsis through the regulation of mitochondrial quality control. *Mol Med* 2023;29:25. <https://doi.org/10.1186/s10020-023-00618-5>.
 41. Wang LX, Ren C, Yao RQ, Luo YN, Yin Y, Wu Y. *et al.* Sestrin2 protects against lethal sepsis by suppressing the pyroptosis of dendritic cells. *Cell Mol Life Sci* 2021;78:8209–27. <https://doi.org/10.1007/s00018-021-03970-z>.
 42. Wang LX, Zhu XM, Luo YN, Wu Y, Dong N, Tong YL. *et al.* Sestrin2 protects dendritic cells against endoplasmic reticulum

- stress-related apoptosis induced by high mobility group box-1 protein. *Cell Death Dis* 2020;**11**:125. <https://doi.org/10.1038/s41419-020-2324-4>.
43. Narayanan S, Elesela S, Rasky AJ, Morris SH, Kumar S, Lombard D. *et al.* ER stress protein PERK promotes inappropriate innate immune responses and pathogenesis during RSV infection. *J Leukoc Biol* 2022;**111**:379–89. <https://doi.org/10.1002/JLB.3A0520-322RR>.
 44. Raines LN, Zhao H, Wang Y, Chen HY, Gallart-Ayala H, Hsueh PC. *et al.* PERK is a critical metabolic hub for immunosuppressive function in macrophages. *Nat Immunol* 2022;**23**:431–45. <https://doi.org/10.1038/s41590-022-01145-x>.
 45. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. *BMJ*. 2016;**23**:i1585. <https://doi.org/10.1136/bmj.i1585>.
 46. Sun W, Zhao P, Hu S, Zhao Z, Liu B, Yang X. *et al.* NUFIP1-engineered exosomes derived from hUMSCs regulate apoptosis and neurological injury induced by propofol in newborn rats. *Neurotoxicology*. 2024;**102**:81–95. <https://doi.org/10.1016/j.neuro.2024.04.002>.