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ORIGINAL RESEARCH

Inhibitors of KAT2A Alleviate the Progression of AKI by Alleviating Macrophage Ferritinophagy

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Background: Acute kidney injury (AKI) involves complex inflammatory responses in which macrophage dysfunction plays a central role. Although histone acetyltransferase KAT2A has been implicated in immune regulation, its role in macrophage ferritinophagy during AKI remains unclear.

Methods: Single-cell RNA sequencing analysis of mouse kidney tissue identified abnormal activation of ferritinophagy and upregulation of KAT2A in renal macrophage during AKI progression. Colocalization of FTH1 and LAMP1 and the increased fluorescence intensity of FTH1 and NCOA4 proteins, and KAT2A proteins in macrophage of kidney in AKI samples were detected via immunofluorescence staining. Functional impacts of KAT2A on macrophage ferritinophagy were assessed using KAT2A knockdown and overexpression plasmids in RAW264.7 cell lines. Butyrolactone 3 (MB-3), a specific KAT2A inhibitor, was administered via intraperitoneal injection 24 hours post-IR to assess the influence of MB-3 on renal pathological changes and the activity of macrophage ferritinophagy.

Results: In this study, using single-cell RNA sequencing and dual immunofluorescence, we observed aberrant ferritinophagy in renal macrophages, marked by increased colocalization of FTH1 with LAMP1 and NCOA4, alongside elevated CD68 expression. KAT2A was upregulated in macrophages from both human AKI biopsies and murine models. Genetic knockdown of KAT2A suppressed ferritinophagy, reduced NCOA4 and FTH1 expression, decreased FTH1-LAMP1 colocalization, and inhibited cGAS signaling. Conversely, KAT2A overexpression exacerbated these processes. Critically, NCOA4 silencing abolished KAT2A-driven ferritinophagy and cGAS-STING activation. Pharmacological inhibition of KAT2A with MB-3 significantly attenuated renal injury, macrophage infiltration, and ferritinophagy, and reduced colocalization of KAT2A or NCOA4 with F4/80.

Conclusion: These findings demonstrate that KAT2A promotes AKI progression via NCOA4-mediated ferritinophagy and cGAS-STING inflammatory signaling in macrophages, highlighting KAT2A inhibition as a promising therapeutic strategy for AKI.

Keywords: KAT2A, ferritinophagy, macrophages, cGAS-STING

Introduction

Acute Kidney Injury (AKI), also known as acute renal failure, is a syndrome marked by a sudden reduction in kidney function, resulting in azotemia, fluid and electrolyte disorders, acid–base imbalance, and systemic complications. It occurs in 10–15% of hospitalized patients and affects over 50% of ICU patients.[1] AKI is linked to high short-term mortality and serious long-term outcomes, such as progression to chronic kidney disease (CKD) and heightened risk of cardiovascular events. This dual health burden significantly contributes to global morbidity and imposes considerable socioeconomic costs on healthcare systems worldwide.[2,3] Despite improvements in supportive care, targeted treatments addressing the fundamental pathophysiology of AKI are still lacking. Thus, uncovering key molecular mechanisms and identifying novel therapeutic targets are crucial for advancing clinical management and outcomes in AKI.

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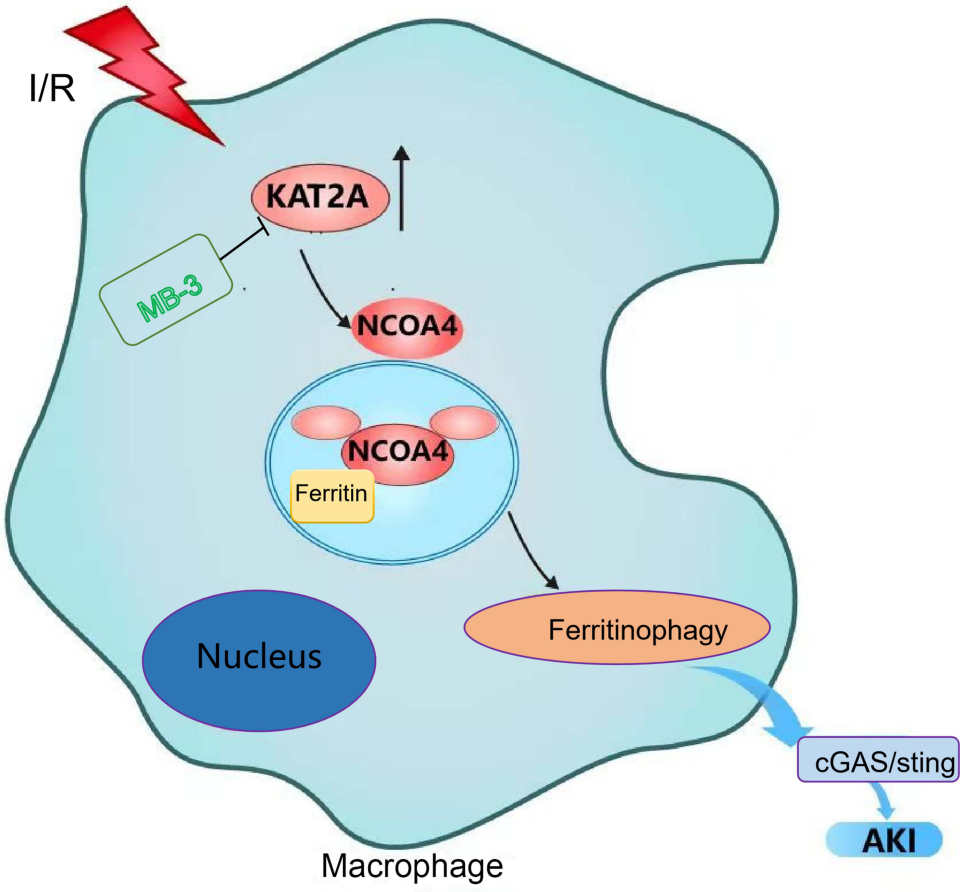
Graphical Abstract



Macrophages are highly plastic immune cells that undergo context-dependent polarization, acquiring distinct functional phenotypes in response to microenvironmental signals.[4,5] In acute kidney injury (AKI), macrophages play a pivotal role in disease progression.[6–8] Specifically, pro-inflammatory M1 macrophages are predominant in the initial phase of AKI. They contribute to tissue damage through the release of inflammatory cytokines such as IL-6 and TNF-α, recruitment of neutrophils and monocytes via chemokines including CCL2 and CXCL1, and production of cytotoxic reactive oxygen species (ROS). In contrast, M2 macrophages promote tissue repair in the resolution phase through efferocytosis, anti-inflammatory cytokine secretion, and pro-angiogenic activities.[9,10] Ferritinophagy is a selective form of autophagy that degrades ferritin heavy chain (FTH1) through the cargo receptor NCOA4, resulting in the release of labile iron and maintenance of cellular iron homeostasis.[11] Under physiological conditions, ferritinophagy supports essential cellular functions such as proliferation and differentiation. However, its pathological overactivation leads to iron overload, ferroptosis – a form of iron-dependent cell death driven by lipid peroxidation and promotes M1 macrophage polarization.[12,13] In AKI, ferroptosis has been identified as a key contributor to renal tubular epithelial cell injury.[14] Nonetheless, the specific role and regulation of macrophage ferritinophagy in AKI remain poorly understood.

Lysine acetyltransferase 2A (KAT2A, also known as GCN5), a highly conserved histone acetyltransferase, regulates diverse cellular processes including gene expression, metabolism, and inflammation through its acetyltransferase activity.[15,16] In AKI, KAT2A has been suggested to promote inflammatory responses and apoptosis via histone acetylation, contributing to disease progression.[17] Emerging evidence indicates that KAT2A also plays a role in macrophage polarization and immunometabolism. KAT2A modulates macrophage fatty acid oxidation and inflammatory signaling via

acetylation of key metabolic regulators.[16] Silencing KAT2A has been shown to suppress M1 polarization and NLRP3 activation in sepsis models, thereby attenuating myocardial injury.[18] KAT2A facilitates NLRP3 inflammasome activation in inflammatory macrophages through metabolic-epigenetic reprogramming in rheumatoid arthritis and Butyrolactone 3 (MB-3), a KAT2A histone acetyltransferase inhibitor, inhibits H3K9ac modification, enhance NRF2 binding to the promoters of *Il1b* and *Nlrp3*, and suppress their transcription.[19] Pharmacological inhibition of KAT2A by MB-3 refers to the process by which the small molecule compound MB-3 specifically targets the catalytic activity of the KAT2A enzyme, thereby blocking its biological functions. Therefore, KAT2A, by regulating macrophage inflammatory responses, represents a potential therapeutic target for inflammatory-related diseases. Nevertheless, it remains unknown whether KAT2A influences AKI by regulating macrophage ferritinophagy through epigenetic mechanisms.

In this study, we investigated the role of KAT2A in regulating macrophage ferritinophagy during AKI. Our results show that KAT2A expression is upregulated in macrophages from both AKI mice and human patients. We further demonstrate that overexpression of KAT2A promotes NCOA4-dependent ferritinophagy in macrophages. Importantly, treatment with the KAT2A inhibitor Butyrolactone 3 (MB-3) attenuated kidney injury and improved pathological outcomes in a mouse model of ischemia-reperfusion-induced AKI. These findings reveal a novel epigenetic mechanism through which KAT2A drives ferritinophagy in renal macrophages and provides preclinical support for targeting KAT2A as a therapeutic strategy in AKI.

Materials and Methods

Single-Cell RNA Sequencing Analysis

Single-cell RNA sequencing data were processed using Seurat V5.0 in R studio. Quality control included filtering out low-quality cells and genes below a minimum expression threshold. Normalization was performed using log (CPM + 1) to account for differences in sequencing depth. Highly variable genes (HVGs) were identified through variance-stabilizing transformation. Dimensionality reduction was carried out using PCA followed by nonlinear visualization with UMAP. Cell clusters were identified using graph-based (Louvain) clustering. Cluster identities were annotated based on established marker genes. Expression patterns were visualized through UMAP embeddings, violin plots, dot plots, and feature plots.

Human Clinical Samples

Human renal biopsy specimens were obtained from the Department of Nephrology, Affiliated Hospital of Guangdong Medical University. The cohort included patients with AKI and control individuals with minimal change disease (MCD). All AKI cases were pathologically diagnosed as acute tubular necrosis, while MCD controls showed no signs of tubular injury upon biopsy confirmation. Exclusion criteria consisted of pre-existing CKD, previous immunosuppressive treatment, and AKI secondary to other glomerular diseases such as crescentic glomerulonephritis, vasculitis, or IgA nephropathy. This study complies with the Declaration of Helsinki and approved by the Institutional Review Board of the hospital (Approval No. YS2022259), and informed consent was acquired from all participants for the use of kidney tissues in research.

Preparation of Renal I/R Injury Model

Male C57BL/6 mice (8 weeks old) were purchased from Guangzhou Yancheng Biotechnology Co., Ltd. and maintained under specific pathogen-free (SPF) conditions at 22–24°C with a 12-h light/dark cycle. Food and water were provided ad libitum. The mice were randomly assigned to the control group (Sham, n=6), MB-3 (MCE, Shanghai, China) group (n=6), I/R group (n=6) and I/R+ MB-3 (n=6) groups. Mice were intravenously injected in the presence or absence of MB-3 with the dose of 10 mg/kg prior to unilateral I/R. I/R injury model was performed according to our recent study.[20] In brief, mice were anesthetized, and their left renal pedicle were clamped for 30 to induce renal I/R injury. Sham-operated controls underwent identical procedures without clamping. Mice recovered with ad libitum access to food and water. To asses renal function, right nephrectomy was performed 24 h prior to sacrifice. At 3 days post-reperfusion, mice were euthanized and their left kidneys were harvested. Animal studies were performed following approval from the

Animal Experimental Ethics Committee at Guangdong Medical University Affiliated Hospital (AHGDMU-LAC-I(1)-2208-B019) and adhered to the NIH Guide for the Care and Use of Laboratory Animals.

Histological Assessment

Kidney tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at a thickness of 3 μm. Sections were stained with hematoxylin and eosin (H&E), Masson's trichrome, and periodic acid–Schiff (PAS), and examined by light microscopy. Tubular injury was evaluated based on morphological alterations including brush border loss, tubular dilation, epithelial disruption, flattening, and cellular sloughing. A semi-quantitative analysis of renal cortical damage was performed by scoring the extent of necrosis, brush border loss, cast formation, and tubular dilation. The injury severity was graded from 0 to 5 according to the percentage of affected cortical area: 0 (none), 1 (<10%), 2 (11–25%), 3 (26–45%), 4 (46–75%), and 5 (>76%) (22, 23).

Cell Culture and Transfection

RAW264.7 cells (ATCC® CRL-2190™) were cultured in DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (Gibco), and maintained at 37°C in a 5% CO_2 humidified incubator. For transient knockdown, cells were transfected with NCOA4-specific siRNA (*GCTAAGACACCCAAGACTA*) using Lipofectamine 2000 (Invitrogen, California, USA) according to the manufacturer's instructions. For transient transfection, RAW264.7 cells were transfected with either a KAT2A overexpression plasmid (pLV3-CMV-KAT2A(human)-3×FLAG, MiaoLing, P68079) or a KAT2A-specific shRNAplasmid (pLKO.1-U6-KAT2A(human)-shRNA6-PGK, MiaoLing, P48344) using Lipofectamine 2000, following the manufacturer's protocol. Cells were cultured for 24 hours post-transfection to allow for sufficient expression or knockdown before subsequent experiments. Transfection efficiency and alterations in KAT2A expression were confirmed by immunoblotting.

Western Blot Analysis

After treatment, cells were lysed using RIPA buffer containing protease inhibitors (Beyotime, Shanghai, China). The lysates were centrifuged at 13,800 × g for 10 minutes at 4°C to collect the supernatant. Protein concentration was determined with a BCA protein assay kit (Beyotime, Shanghai, China). Equal amounts of protein were separated by 12% SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, USA). The membranes were blocked with 5% non-fat milk in TBST for 1 hour at room temperature, followed by incubation overnight at 4°C with primary antibodies: anti-GPX4 (#sc-166437, Santa Cruz Biotechnology, Texas, USA; diluted 1:500), anti-NCOA4 (#DF4255-200, Affinity Biosciences, Jiangsu, China; diluted 1:2000), anti-FTH1 (#HY-P80670, MCE, Shanghai, China; diluted 1:1000), anti-cGAS (#DF4255-200, Affinity Biosciences, Jiangsu, China; diluted 1:1000), anti-p-Sting (#AWA53879, Abiowell, Chengdu, China; diluted 1:1000), anti-KAT2A anti-KAT2A (#3305S, Cell Signaling Technology, MA, USA; diluted 1:1000) and anti-β-Tubulin (#10094-1-AP, Proteintech, Wuhan, China; diluted 1:2000). After washing, the membranes were incubated with HRP-conjugated secondary antibodies (Proteintech, Wuhan, China; 1:5000 dilution) for 2 hours at room temperature. Protein bands were visualized using an ECL substrate (Millipore, Shanghai, China) and quantified with ImageJ software (NIH).

Immunofluorescence Staining

For paraffin-embedded tissue sections, 4% paraformaldehyde-fixed paraffin-embedded blocks sliced (4–7 μm), floated on 40°C water, mounted, dried at 60°C for 2 h. Tissue sections were then deparaffinized and rehydrated. After antigen retrieval, the samples were permeabilizated with 0.5% Triton X-100 in PBS for 20 minutes. For cells, post-transfected RAW264.7 cells were fixed with 4% paraformaldehyde in PBS for 10 minutes at room temperature and permeabilizated with 0.5% Triton X-100 in PBS for 10 minutes. Subsequently, both tissue and cell samples were subjected to blocking with 5% BSA for 1 hour and then incubated at 4°C overnight with primary antibodies: anti-KAT2A (#3305S, Cell Signaling Technology, MA, USA; diluted 1:500), anti-NCOA4 (#DF4255-200, Affinity Biosciences, Jiangsu, China; diluted 1:200), anti-LAMP1 (#sc-20011, Santa Cruz Biotechnology, Texas, USA), and anti-FTH1 (#HY-P80670, MCE, Shanghai, China; diluted 1:500), anti-CD68 (#66231-2-Ig, Proteintech, Wuhan, China; diluted 1:500) antibodies (1:200

in PBS). Following three washes with PBS, the samples were incubated with corresponding fluorescent secondary antibodies Alexa Fluor 488/594-conjugated secondaries (Abcam,1:500) for 1 hour at room temperature protected from light. Nuclei were counterstained with 1 μg/mL DAPI (Beyotime) for 5 min. Images were acquired using an Olympus FV3000 fluorescence microscope (Evident, Tokyo, Japan).

Statistical Analysis

Statistical significance was determined using SPSS 20.0. All quantitative data are expressed as mean ± standard deviation (SD). For comparisons between two groups, unpaired Student's *t*-test was applied to normally distributed data; for multi-group comparisons, one-way ANOVA followed by Tukey's post-hoc test was used. Significance was defined as $P < 0.05$.

Results

Single-Cell Sequencing Analysis Showed Abnormal Activation of Macrophage Ferritinophagy in AKI Progression

Single cell sequencing analysis of mouse kidney tissue showed that compared with the sham surgery control group, the increased macrophage population in the kidney tissue of mice in the I/R-AKI group was Damage_Macro cluster, and the decreased macrophage population was Normal_Macro cluster (Figure 1A and B). Furthermore, the differential gene expression analysis of single-cell sequencing showed that compared with other macrophage clusters, the expression of *Ncoa4, Fth1* and *Map1lc3b* genes was significantly upregulated in the Damage_Macro cluster (Figure 1C and D). In addition, the expression levels and colocalization of FTH1 and LAMP1 in the renal tubules of I/R-AKI mice are also detected by immunofluorescence staining. As shown in Figure 1E and F, the expression levels of FTH1 and LAMP1 were increased, and co-localization of the two proteins were also augmented.

Determination of Aberrantly Activated Macrophage Ferritinophagy in the Kidney of AKI Patients

Next, ferritinophagy-related proteins in kidney biopsy samples from AKI patients were measured by immunofluorescence staining. The results showed that the fluorescence intensity of FTH1 and LAMP1 in the kidney tissue of AKI patients was increased and the colocalization was enhanced (Figure 2A and B), suggesting abnormal accumulation of ferritin in tubular lysosomes. Subsequently, the macrophage marker CD68 with FTH1 and NCOA4, respectively, were co-stained in AKI renal biopsies using immunofluorescence assay. The results demonstrated that the levels of FTH1 and NCOA4 proteins in renal macrophages were upregulated (Figure 2C and D) and the colocalizations of FTH1 with CD68, and NCOA4 with CD68 in kidney (Figure 2E and F) are enhanced in AKI patients. These results revealed aberrant ferritinophagy induction in renal macrophages during AKI.

Single-Cell Sequencing Analysis Showed That the Expression of KAT2A in Renal Macrophages Was up-Regulated in the AKI State

In order to further explore the molecular mechanism of macrophage iron autophagy overactivation in I/R-AKI mice, the characteristics of damage macro cell clusters in single-cell transcriptome sequencing results were reanalyzed. The results showed that *Kat2a* was a highly expressed marker gene in the Damage_Macro clstuer (Figure 3A and B). Furthermore, the macrophage marker CD68 with KAT2A were co-stained by immunofluorescence assays to detect the expression levels of KAT2A in macrophages in kidney of IR-AKI mice and clinical AKI samples, respectively. The results showed that the expression level of KAT2A was significantly increased renal macrophages in IR-AKI mice and AKI patients (Figure 3C and F).

KAT2A Promotes Ferritinophagy in Macrophages in vitro

The regulatory effect of KAT2A on ferritinophagy in macrophages was further investigated at the cellular level. RAW264.7 cell lines were transfected with either KAT2A knockdown (KAT2A-KD) or overexpression (KAT2A-OE)

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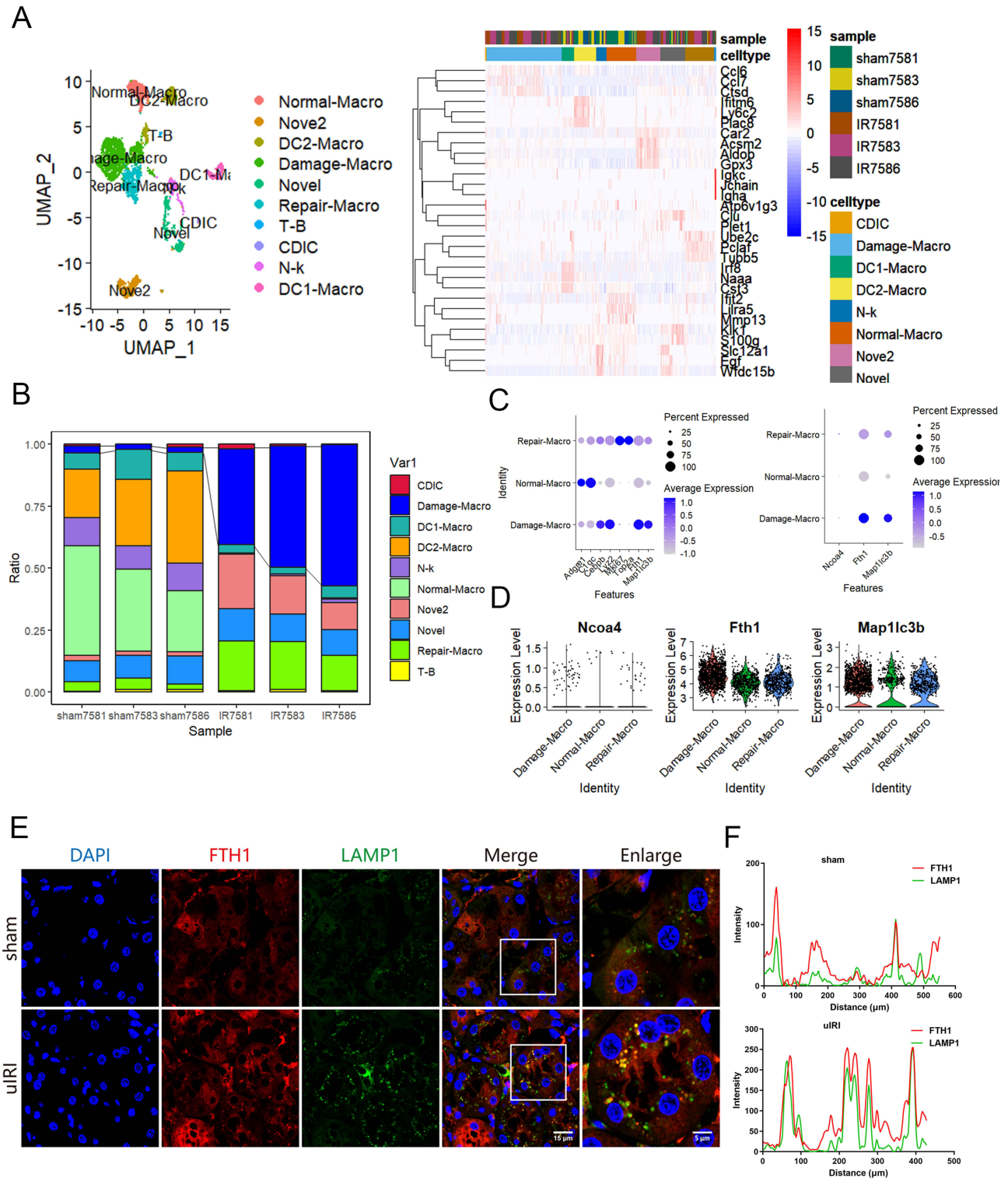

Figure 1 Abnormal activation of ferritinophagy in macrophage during AKI progression. (**A** and **B**) Single cell sequencing analysis of mouse kidney tissue showed that compared with the sham surgery control group, the increased macrophage population in the kidney tissue of mice in the I/R-AKI group was Damage_Macro, and the decreased macrophage population was Normal_Macro. (**C** and **D**) The differential gene expression analysis of single-cell sequencing showed that compared with other macrophage clusters, the expression of *Ncoa4*, *Fth1* and *Map1lc3b* genes was significantly upregulated in the Damage_Macro cluster. (**E**) Representative images of immunofluorescence staining of LAMP1 and FTH1 proteins in the renal cortex of C57BL/6 mice after sham surgery or I/R injury. (**F**) Quantitative analysis of the expression levels of LAMP1 and FTH1 proteins in (**E**).

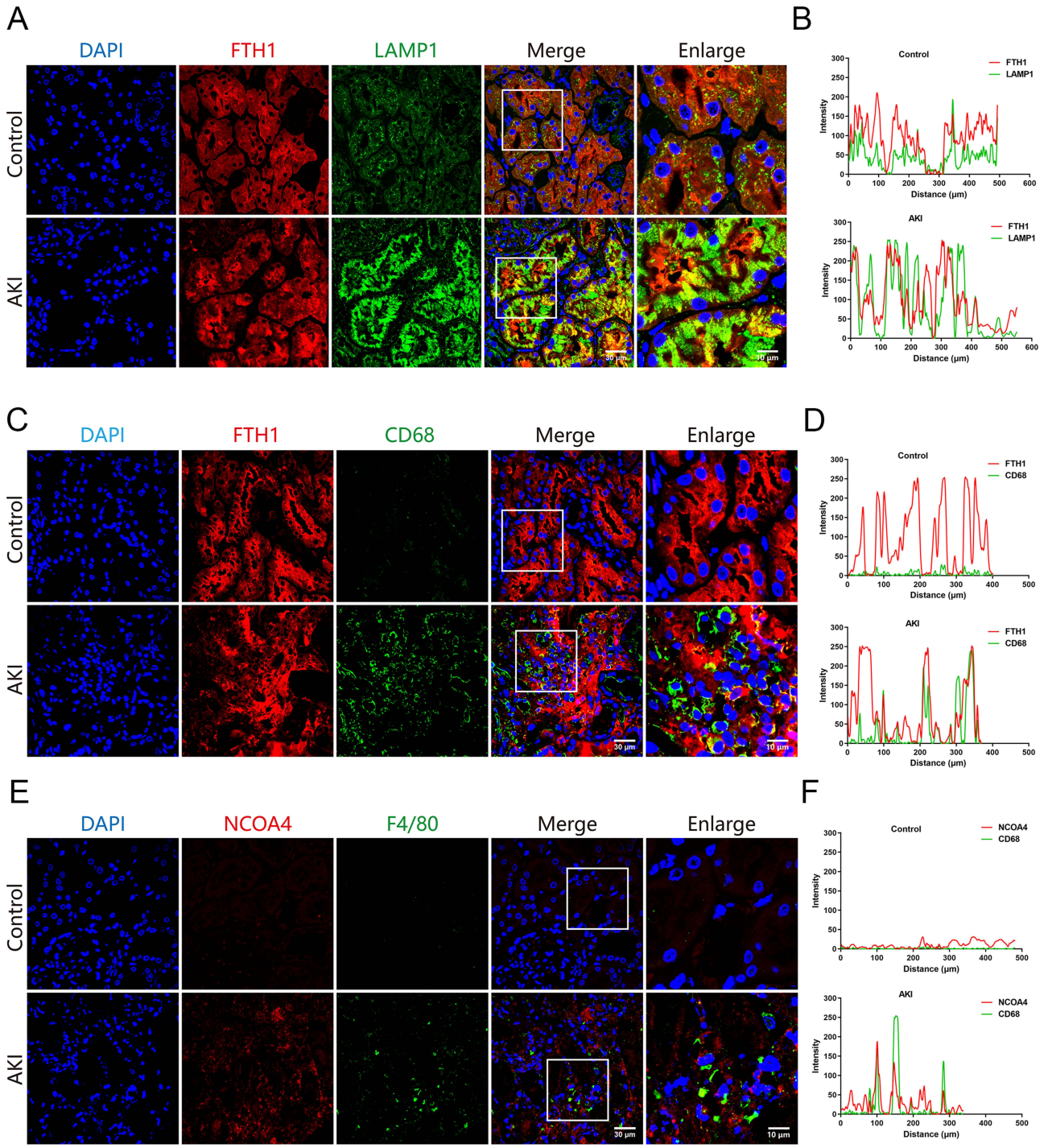

Figure 2 Macrophage ferritinophagy is abnormally activated in renal of AKI patients. (**A**) Representative pictures of LAMP1 and FTH1 proteins by immunofluorescence staining in kidney biopsy samples from AKI patients. (**B**) Quantitative analysis of the expression levels of LAMP1 and FTH1 proteins in (**A**). (**C**) Representative pictures of CD68 and FTH1 proteins by immunofluorescence staining in kidney biopsy samples from AKI patients. (**D**) Quantitative analysis of the expression levels of CD68 and FTH1 proteins in (**C**). (**E**) Representative pictures of CD68 and NCOA4 proteins by immunofluorescence staining in kidney biopsy samples from AKI patients. (**F**) Quantitative analysis of the expression levels of CD68 and NCOA4 proteins in (**E**).

plasmids transient transfection and the expression levels of KAT2A, ferritinophagy-related proteins such as FTH1, NCOA4, GPX4, and cGAS signaling-associated proteins such as cGAS, p-Sting and Sting in these cells were detected by Western blot. As shown in Figure 4A and B, the expression levels of KAT2A, NCOA4, cGAS, p-Sting/Sting in macrophages were down-regulated and FTH1 and GPX4 proteins were upregulated in KAT2A-KD RAW264.7 cells.

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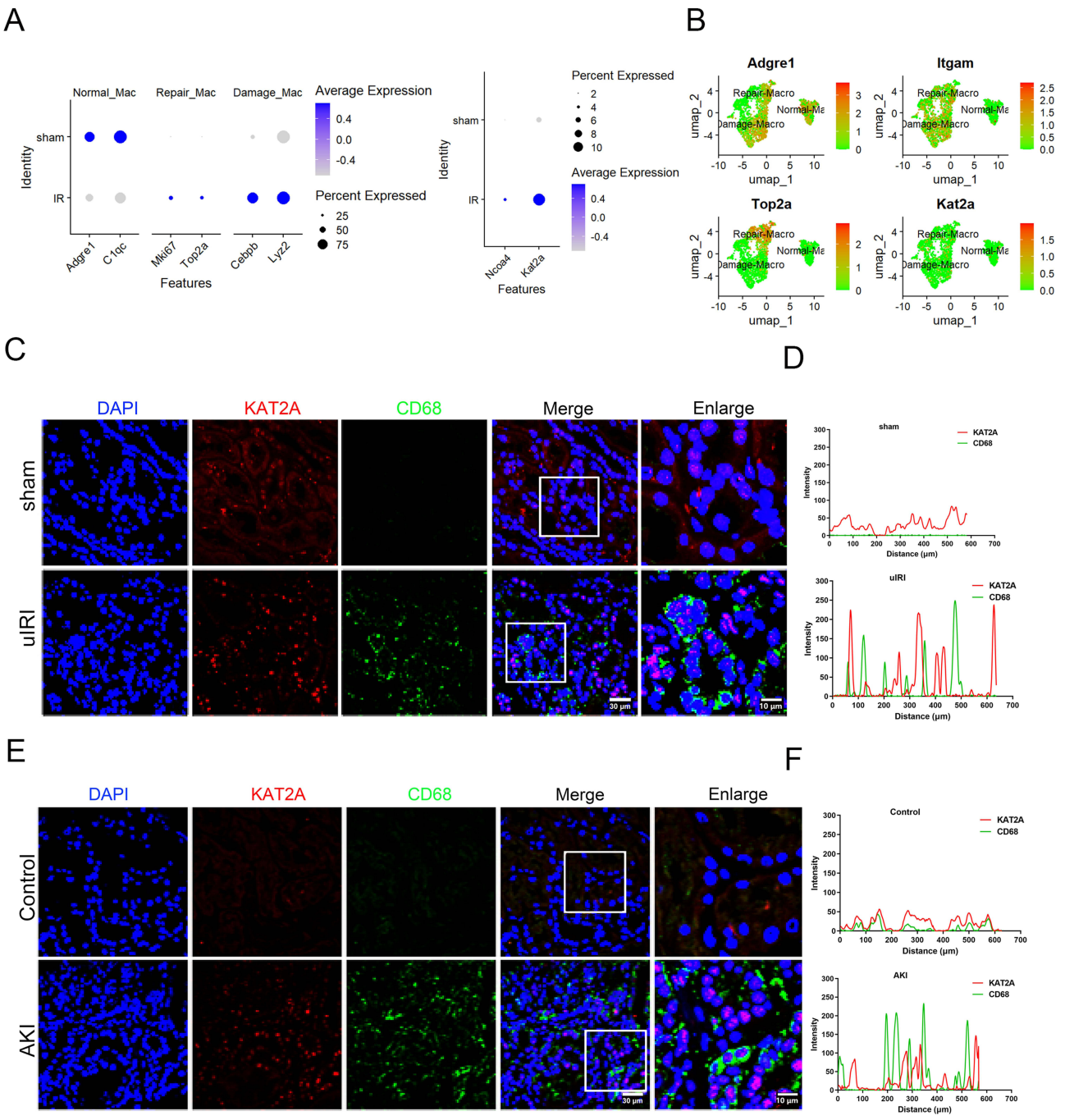

Figure 3 KAT2A is upregulated in renal macrophages in the AKI progression. (**A** and **B**) The single-cell transcriptome sequencing analysis showed that *Kat2a* is a highly expressed marker gene in the Damage_Macro clstuer. (Normal_Macro: Adgre1; Repair_Macro: Top2a). (**C**) Representative pictures of KAT2A and CD68 proteins by immunofluorescence staining in kidney of IR-AKI injured mice. (**D**) Quantitative analysis of the expression levels of KAT2A and CD6 proteins in (**C**). (**E**) Representative pictures of KAT2A and CD68 proteins by immunofluorescence staining in kidney biopsy samples from AKI patients. (**F**) Quantitative analysis of the expression levels of KAT2A and CD6 proteins in (**E**).

However, the opposite results were exhibited in KAT2A-OE RAW264.7 cells. Additionally, the colocalization of FTH1 and LAMP1 proteins were measured using immunofluorescence assay KAT2A-KD an KAT2A-OE RAW264.7 cells. The colocalization intensity of FTH1 with LAMP1 was attenuated in KAT2A-KD RAW264.7 cells but augmented in KAT2A-OE cells, consistent with the increased FTH1-LAMP1 co-localization induced by the positive control Bafilomycin A1 (Figure 4C–H). These results revealed that KAT2A overexpression induces activation of ferritinophagy in macrophages.

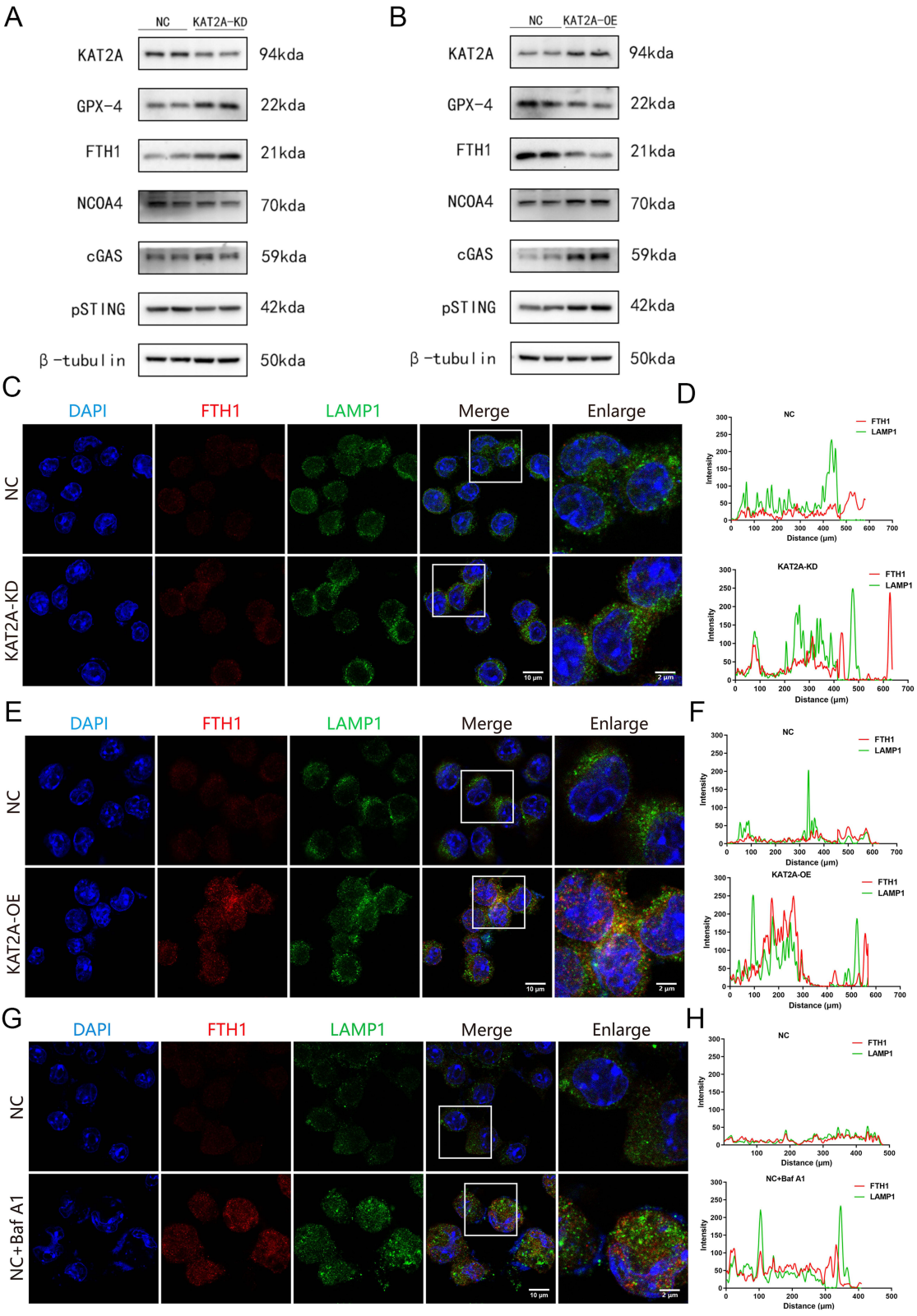

Figure 4 KAT2A induces ferritinophagy in macrophages in vitro. (**A** and **B**) The expression of KAT2A, GPX4, FTH1, NCOA4, cGAS, and p-Sting proteins in RAW264.7 cells after KAT2A knockdown and overexpression were measured by Western blot. (**C**) Representative pictures of KAT2A and CD68 proteins by immunofluorescence staining in FTH1 and LAMP1 in KAT2A downregulated RAW264.7 cells. (**D**) Quantitative analysis of the expression levels of KAT2A and CD6 proteins in (**C**). (**E**) Representative pictures of KAT2A and CD68 proteins by immunofluorescence staining in FTH1 and LAMP1 in KAT2A upregulated RAW264.7 cells. (**F**) Quantitative analysis of the expression levels of KAT2A and CD6 proteins in (**E**). (**G**) Representative pictures of KAT2A and CD68 proteins by immunofluorescence staining in FTH1 and LAMP1 in positive control Bafilomycin A1 treated RAW264.7 cells. (**H**) Quantitative analysis of the expression levels of KAT2A and CD6 proteins in (**G**).

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Knockdown of NCOA4 Inhibits the Pro-Inflammatory Effects of KAT2A on Macrophages

To further explore the specific molecular mechanism by which KAT2A regulates the inflammatory response in macrophages, we observed the effect of NCOA4 knockdown on the inflammatory response of RAW264.7 cells overexpressing KAT2A. Western blotting results showed that the expression levels of cGAS and p-STING protein in RAW264.7 cells overexpressed KAT2A were significantly inhibited after NCOA4 knockdown (Figure 5A). Furthermore, we explored the effect of NCOA4 knockdown on the colocalization of FTH1 and LAMP1 in RAW264.7 cells overexpressing KAT2A using immunofluorescence staining. The results showed that the colocalization signal of FTH1 and LAMP1 in RAW264.7 cells was significantly weakened after NCOA4

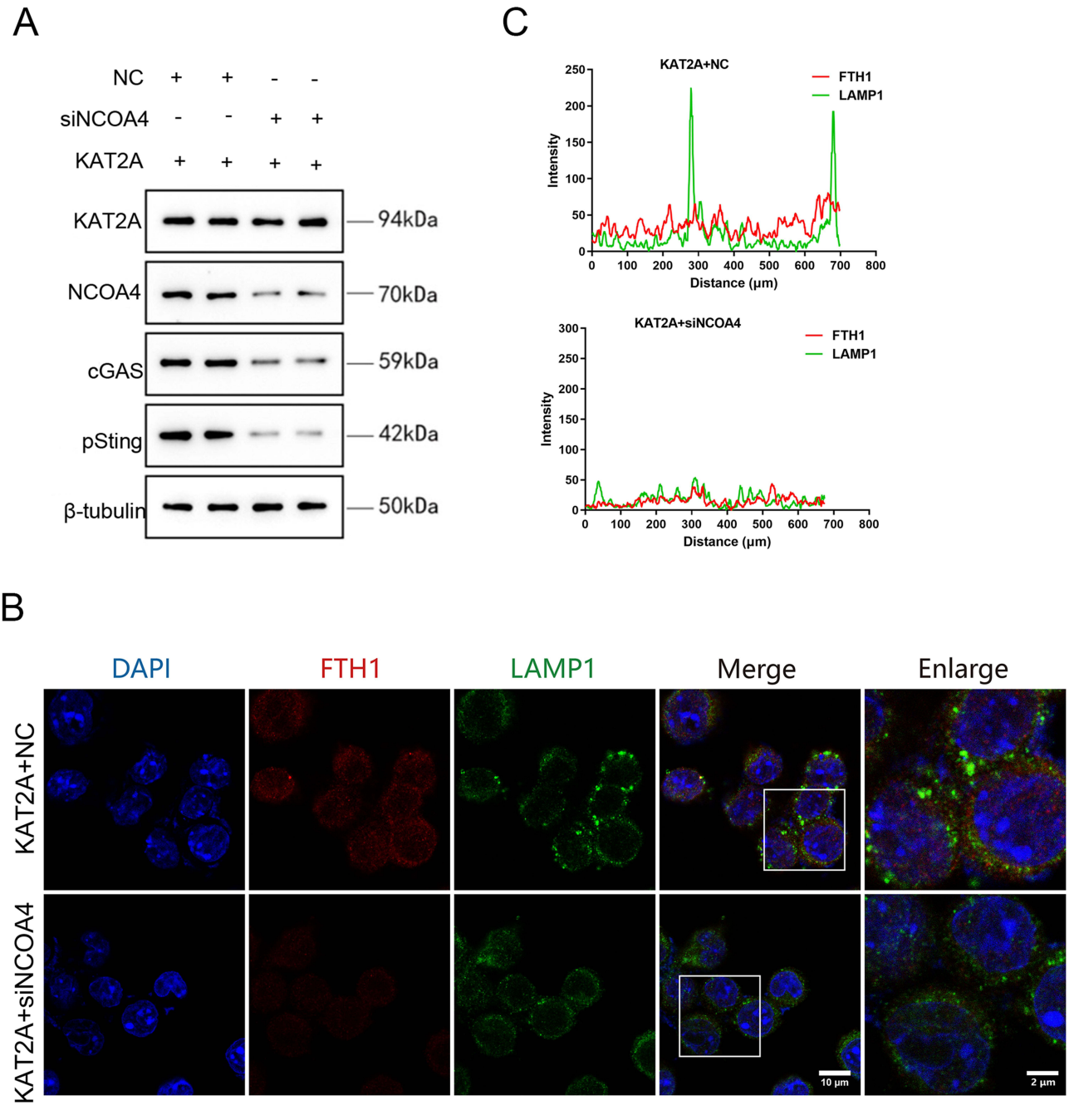

Figure 5 Knockdown of NCOA4 has inhibitory effects on KAT2A-induced pro-inflammatory macrophages. (**A**) The expression of KAT2A, NCOA4, cGAS and p-Sting proteins expression were detected by Western blot in RAW264.7 cells co-treated KAT2A upregulation with NCOA4 knockdown. (**B**) Representative pictures of FTH1 and LAMP1 proteins by immunofluorescence staining in KAT2A upregulated RAW264.7 cells in the presence or absence of NCOA4 siRNA. (**C**) Quantitative analysis of the expression levels of FTH1 and LAMP1 proteins in (**B**).

knockdown (Figure 5B and C). These results suggest that KAT2A is involved in the pro-inflammatory response by regulating NCOA4-mediated ferritinophagy in macrophages.

KAT2A Inhibitors Attenuate Pathological Changes of Renal in IR-AKI Mice

To further investigate whether Butyrolactone 3 (MB-3), a specific KAT2A inhibitor), ameliorates renal pathology in mice with ischemia-reperfusion injury (IR), MB-3 was administered via intraperitoneal injection 24 hours post-IR. Renal pathological changes were assessed using triple histological staining such as H&E (hematoxylin and eosin), PAS (periodic acid-Schiff) and Masson's trichrome. H&E staining results showed that uIRI resulted in severe tubular damage, including widespread epithelial cell edema, cytoplasmic vacuolization, and brush border loss and MB-3 treatment significantly attenuated these pathological changes (Figure 6A). PAS staining results demonstrated that uIRI induced focal glomerular basement membrane thickening and increased glycogen deposition in tubular epithelia/lumina MB-3 administration reduced glycogen accumulation and preserved basement membrane integrity (Figure 6B). Masson staining results showed that uIRI-treated mice developed mild mesangial collagen hyperplasia and interstitial fibrosis and the collagen deposition markedly decreased in MB-3-treated uIRI mice (Figure 6C). These results suggested that KAT2A inhibitors may have a potentially mitigating effect on AKI progression.

KAT2A Inhibitors Attenuate the Inflammatory Infiltration and Ferritinophagy of Renal Macrophages in IR-AKI Mice

We further explored the impact of KAT2A inhibitors on the inflammatory infiltration and ferritinophagy of renal macrophages in IR-AKI mice using immunofluorescence staining. The expression of KAT2A and F4/80 proteins in kidney tissue of IR-AKI mice was up-regulated by uIRI treatment, and significantly decreased after MB-3 treatment (Figure 7A and C). The expression level of NCOA4 protein in macrophages was higher in the uIRI co-treated with DMSO group compared to the control group, suggesting that overactivated ferritinophagy in renal macrophages under

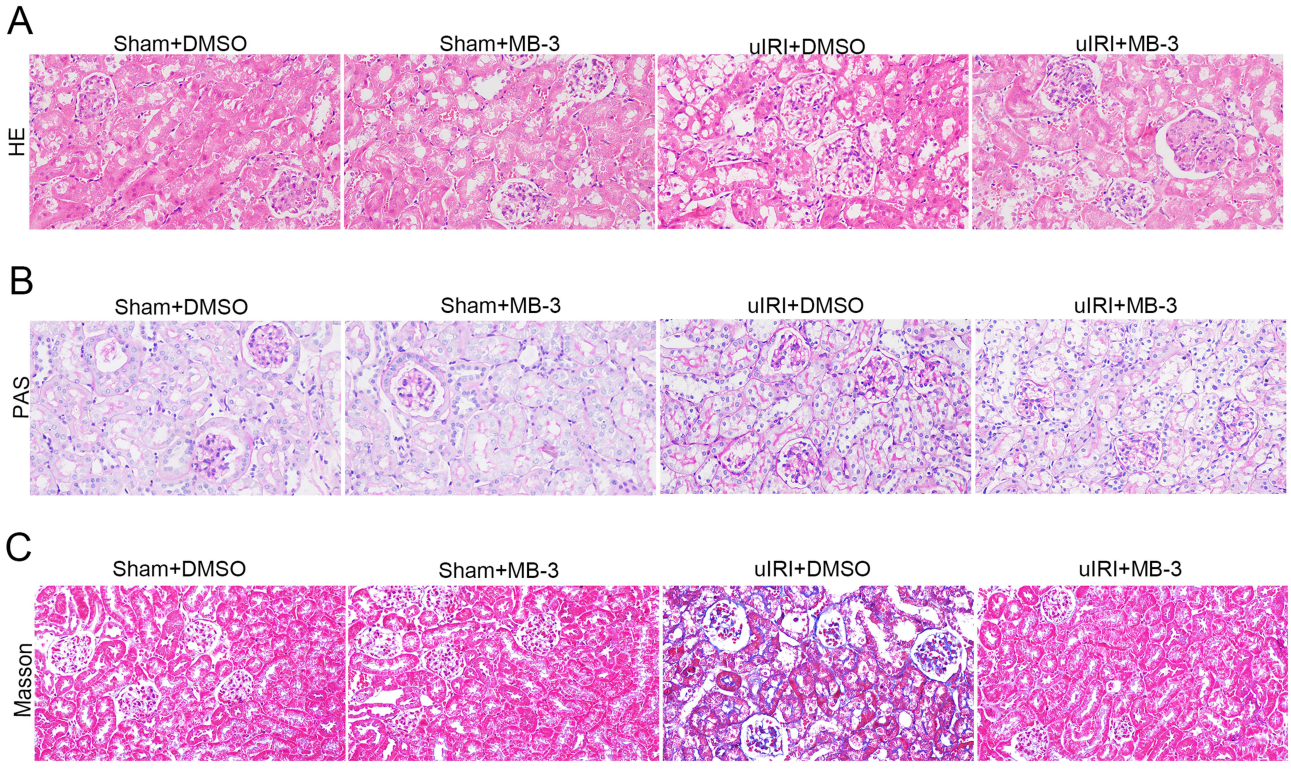

Figure 6 KAT2A inhibitors improves pathological changes of renal tissues in IR-AKI mice. (**A**) HE staining image of r renal tissues in sham-treated and I/R-treated C57BL/6 mice in the presence or absence of MB-3 (200 ×). (**B**) PAS staining image of kidney from sham-treated and I/R-treated C57BL/6 mice in the presence or absence of MB-3 (200 ×). (**C**) Masson's trichrome staining in the renal cortex from sham-treated and I/R-treated C57BL/6 mice in the presence or absence of MB-3 (200 ×).

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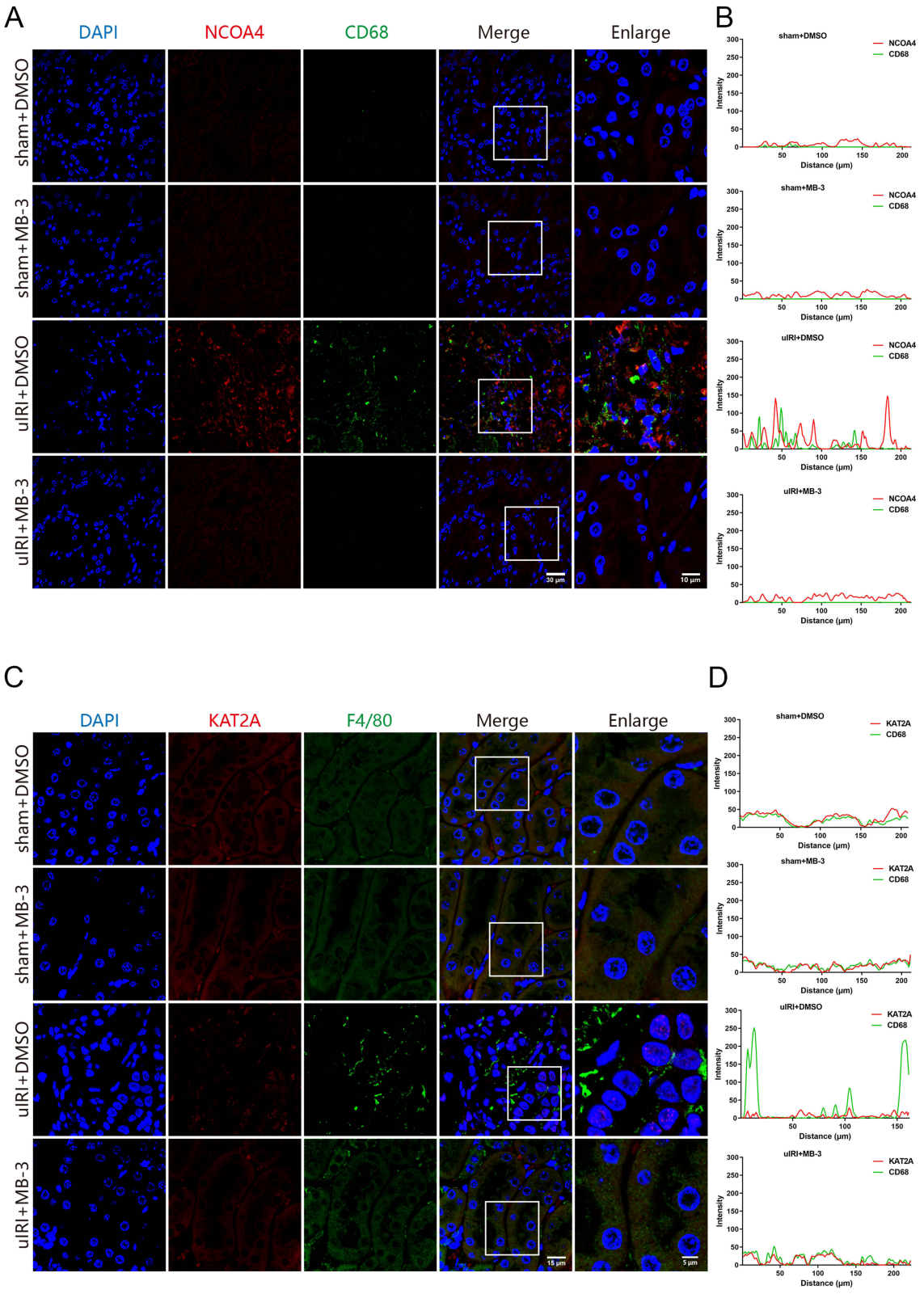

Figure 7 KAT2A inhibitors inhibits the inflammatory infiltration and macrophage ferritinophagy of renal in IR-AKI mice. (**A**) Representative pictures of NCOA4 and CD68 proteins by immunofluorescence staining in kidney of IR-AKI injured mice. (**B**) Quantitative analysis of the expression levels of NCOA4 and CD68 proteins in (**A**). (**C**) Representative pictures of KAT2A and CD68 proteins by immunofluorescence staining in kidney of IR-AKI injured mice. (**D**) Quantitative analysis of the expression levels of KAT2A and CD68 proteins in (**C**).

IR-AKI conditions. In contrast, the expression level of NCOA4 in macrophages was significantly reduced in the IR-AKI mice after treatment with the KAT2A inhibitor MB-3 (Figure 7B and D). These data indicated that KAT2A inhibitors can effectively block ferritinophagy activation in renal macrophages in IR-AKI progression.

Discussion

Our study identifies the KAT2A–NCOA4–ferritinophagy axis as a novel and critical mechanism driving macrophage-mediated renal inflammation in acute kidney injury (AKI). Through an integrated approach combining single-cell RNA sequencing of human samples, in vitro mechanistic studies, and interventional animal models, we demonstrate that: (1) ferritinophagy is markedly activated in renal macrophages during AKI; (2) KAT2A upregulation directly enhances ferritinophagic activity in macrophages; (3) the pro-inflammatory function of KAT2A depends on NCOA4-mediated ferritinophagy; and (4) pharmacological inhibition of KAT2A ameliorates renal injury by suppressing ferritinophagy and subsequent inflammatory responses in macrophages.

Iron is essential for fundamental biological processes including oxygen transport, ATP synthesis, and DNA metabolism (22). While iron deficiency is linked to anemia (23) and systemic dysfunction in tissues such as skeletal and cardiac muscle (24), iron overload exacerbates oxidative stress via iron-catalyzed free radical generation, contributing to pathologies such as hereditary hemochromatosis (25). Tight regulation of iron homeostasis is therefore critical for cellular and tissue function. Ferritinophagy, a selective autophagic process mediated by the cargo receptor NCOA4, degrades ferritin to release stored iron and maintain intracellular iron balance (26, 27). This process is dynamically regulated by cellular iron levels (11). Macrophages play a key role in systemic iron recycling by phagocytosing red blood cells, metabolizing hemoglobin, and releasing iron-elevating both local and systemic iron availability (13). Excess iron promotes M1 macrophage polarization and inflammatory responses (28), which contributes to renal damage during ischemia-reperjury (I/R) injury through monocyte infiltration and pro-inflammatory mediator release (29, 30).

Notably, emerging evidence implicates NCOA4-mediated ferritinophagy in the pathogenesis of AKI (21, 31, 32), though its macrophage-specific role remains unclear. In this study, single-cell sequencing and co-localization of ferritin with LAMP1 revealed enhanced lysosomal ferritin degradation in macrophages during early AKI, underscoring the importance of ferritinophagy in driving iron overload and inflammatory macrophage activation. Ferritinophagy is subject to post-translational regulation. Recent studies indicate that HERC2-mediated degradation of NCOA4 plays a key role in modulating ferritinophagy (33–35).

KAT2A, a lysine acetyltransferase involved in inflammatory and immune regulation, has been previously linked to macrophage-mediated inflammation (19, 36). In the present study, single-cell sequencing and immunofluorescence staining revealed upregulated KAT2A expression in renal macrophages within I/R-AKI models, suggesting its potential role in exacerbating inflammatory responses during AKI. Using both knockdown and overexpression approaches, we demonstrated that KAT2A promotes NCOA4-mediated ferritinophagy in macrophages. Furthermore, consistent with prior reports linking KAT2A to cGAS pathway activation through transcriptional and post-translational mechanisms in inflammatory contexts (38), our data also indicate that KAT2A positively regulates cGAS signaling. Importantly, silencing NCOA4 abolished the pro-inflammatory effects induced by KAT2A overexpression, establishing NCOA4 as a critical downstream effector. These findings collectively indicate that KAT2A drives inflammatory responses in AKI by enhancing ferritinophagy in macrophages. Moreover, as a histone acetyltransferase, KAT2A may transcriptionally facilitate ferritinophagy possibly through acetylation of H3K9 at the NCOA4 promoter, a hypothesis that warrants further investigation.

In this study, KAT2A was identified as a critical regulator of ferritinophagy hyperactivation and pro-inflammatory polarization in renal macrophages during AKI, particularly in the ischemia–reperfusion (I/R) model. To evaluate the therapeutic potential of targeting KAT2A, we investigated the effects of its selective inhibitor, MB-3, in I/R-induced AKI mice. MB-3 competitively binds to the acetyl-CoA site of KAT2A, inhibiting its acetyltransferase activity and subsequent transcriptional regulation (39, 40). Pathological staining and immunofluorescence analysis revealed that MB-3 treatment significantly attenuated renal injury, inflammatory infiltration, and macrophage ferritinophagy. The anti-inflammatory effect of MB-3 aligns with previous findings by Liu et al (18), supporting its role in protecting against AKI through suppression of the KAT2A-NCOA4-ferritinophagy axis in macrophages. While this study preliminarily demonstrates that pharmacological inhibition of KAT2A ameliorates AKI progression in an I/R model, several limitations should be noted.

The I/R model, though widely used, does not fully reflect the multifactorial etiology of human AKI. Future studies should incorporate additional AKI models—such as cisplatin-induced nephrotoxicity—and animals with metabolic comorbidities to improve clinical relevance. Moreover, as MB-3 inhibits KAT2A across cell types, the specific contribution of macrophage KAT2A remains to be delineated. Generation of macrophage-specific KAT2A knockout mice will help clarify the cell-autonomous mechanisms underlying its function.

Conclusion

This study establishes KAT2A as a key regulator of NCOA4-dependent macrophage ferritinophagy that promotes kidney injury following ischemia-reperfusion. Importantly, pharmacological inhibition of KAT2A exerted a protective effect, highlighting its therapeutic potential for AKI. This study provides novel mechanistic insight into AKI pathogenesis and highlights KAT2A as a promising target for future therapeutic development.

Ethics Approval and Consent to Participate

Approved by the Institutional Review Board and Animal Experimental Ethics Committee of Guangdong Medical University Affiliated Hospital. This study complies with the Declaration of Helsinki. Informed consent was obtained for all participants.

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Disclosure

The authors declare no competing interests.

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