

CDC25B exacerbates sepsis-associated intestinal injury by upregulating OLR1 and activating TNF- α signaling

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Abstract

Intestinal barrier dysfunction is a critical driver of sepsis progression and mortality, yet its molecular regulation remains unclear. CDC25B, a cell cycle regulator recently implicated in inflammatory responses, is largely unexplored in intestinal sepsis. In this study, CDC25B expression was analyzed by immunohistochemistry, qRT-PCR, and western blot. CDC25B was knocked down or overexpressed in lipopolysaccharide (LPS)-treated Caco-2 cells to assess ROS, inflammatory cytokines, and barrier proteins. RNA-seq was used to screen downstream pathways regulated by CDC25B, with key molecular alterations further verified by qRT-PCR, western blot, and immunofluorescence. We observed that CDC25B was markedly upregulated in septic tissues and LPS-treated Caco-2 cells. CDC25B knockdown attenuated ROS production, cytokine release, and barrier protein loss, whereas overexpression aggravated these effects. RNA-seq and validation experiments revealed that CDC25B knockdown downregulated OLR1 and TNF- α expression, suppressed IKK α / β phosphorylation, and inhibited p65 nuclear translocation; conversely, overexpression enhanced these pathway activities. These findings indicate that CDC25B promotes intestinal barrier dysfunction and inflammatory responses through the regulation of both OLR1 expression and TNF- α signaling pathway activity, suggesting that CDC25B may represent a potential therapeutic target for intestinal sepsis.

Introduction

Sepsis is a systemic inflammatory response syndrome (SIRS) triggered by host exposure to bacteria, viruses, and other infectious pathogens[1]. It is characterized by a dysregulated host response to infection that leads to organ dysfunction, and may progress through a systemic inflammatory cascade to septic shock and multiple organ dysfunction syndrome (MODS)[2]. The global burden of sepsis is substantial, affecting approximately 48.9 million individuals and contributing to 11 million deaths annually, representing nearly 20% of all deaths worldwide[3]. A meta-analysis showed that the incidence of sepsis treated in hospitals was 189 cases per 100,000 person-years, with a mortality rate exceeding 25%, while the estimated incidence of sepsis requiring intensive care unit (ICU) admission was 58 cases per 100,000 person-years[4]. Therefore, sepsis, characterized by elevated morbidity and mortality, has emerged as a critical global health challenge[5].

The disruption of intestinal barrier integrity is now widely acknowledged to play a central role in the pathophysiology of sepsis. The intestinal barrier is composed of a monolayer of epithelial cells and intercellular tight junction complexes (TJs). The assembly of TJs requires transmembrane proteins such as occludin, claudin-1, and ZO-1, and this structure restricts the passage of luminal pathogens, toxins, and antigens into the circulation[6]. However, during sepsis, the translocation of bacteria and endotoxins triggers mucosal immune activation, thereby increasing intestinal permeability through alterations in TJs[7, 8]. Furthermore, inflammation-induced epithelial apoptosis plays an important role in sepsis progression. Studies in mouse sepsis models and postmortem examinations of septic patients have shown a significant increase in intestinal epithelial/lymphocyte apoptosis compared with non-septic

deaths, which is associated with disruption of barrier function[9]. Therefore, it is crucial to address intestinal barrier dysfunction in septic patients.

Cell division cycle 25 (CDC25) is a dual-specificity protein phosphatase[10]. It exists as three homologous genes in the mammalian genome, namely CDC25A, CDC25B, and CDC25C, among which CDC25B plays a critical role in cell cycle regulation[11]. Studies have shown that the expression level of CDC25B in T cells correlates with disease activity in rheumatoid arthritis, and low levels of CDC25B in patients with early arthritis may promote the progression of autoimmune arthritis[12]. However, whether CDC25B plays a role in other inflammatory diseases such as intestinal sepsis remains unexplored.

Oxidized low-density lipoprotein receptor 1 (OLR1) is a receptor for oxidized low-density lipoprotein (ox-LDL). It was first studied in vascular endothelial cells and was subsequently found to be expressed in human intestinal cell lines, as well as in endothelial cells, macrophages, and smooth muscle cells[13–15]. The role of OLR1 has been investigated in metabolism, cardiovascular diseases, cancer, and inflammatory diseases[16, 17]. Inflammatory cytokines including IL-1 α , IL-1 β and TNF- α have been proven to elevate OLR1 expression in cultured smooth muscle cells[18]. Studies have also demonstrated that mice deficient in OLR1 expression exhibit improved survival in a cecal ligation and puncture (CLP) model of sepsis. However, whether OLR1 contributes to sepsis-associated intestinal injury and the underlying mechanisms have yet to be elucidated.

Thus, using molecular biology approaches together with RNA-seq, this study sought to elucidate the regulatory function of CDC25B in intestinal barrier integrity and inflammation, and to further dissect the mechanistic involvement of OLR1 in sepsis.

Materials and Methods

Hematoxylin and eosin (H&E) staining

All experimental protocols were approved by the Ethics Committee of Bengbu Medical University (No. 2025 – 537). All methods were performed in accordance with the relevant guidelines and regulations. Formalin-fixed, paraffin-embedded (FFPE) intestinal tissue sections were obtained from the Department of Pathology of the First Affiliated Hospital of Bengbu Medical University. A total of 20 specimens were included in this study. Based on histopathological evaluation, the samples were classified into two groups: the sepsis group (n = 10), comprising specimens exhibiting characteristic intestinal mucosal damage with inflammatory cell infiltration as identified by H&E staining, and the control group (n = 10), consisting of specimens showing normal intestinal histological architecture without evidence of mucosal injury or inflammation. Following deparaffinization in xylene and rehydration via a graded ethanol gradient, tissue sections were stained with hematoxylin for 5 minutes, rinsed with running water, differentiated in 1% acid alcohol, blued with running water, and counterstained with eosin for 3 minutes. After staining, sections were dehydrated through a graded ethanol series, cleared with xylene, and

mounted with neutral resin. The staining results were observed and images were captured under an optical microscope.

Immunohistochemistry (IHC) staining

Tissue sections were first deparaffinized and rehydrated. Antigen retrieval was then performed by heating in citrate buffer. Endogenous peroxidase activity was quenched with 0.3% H₂O₂ for 10 minutes. Subsequently, sections were blocked with goat serum for 20 minutes. Sections were incubated with primary antibody against CDC25B (Proteintech, 28109-1-AP, 1:200) overnight at 4°C. The next day, sections were incubated with the corresponding secondary antibody, washed, and then visualized using DAB substrate. The chromogenic reaction was terminated with deionized water, and the nuclei were counterstained with hematoxylin. Finally, images were captured under an optical microscope. All stained sections were independently assessed by two experienced pathologists. IHC staining was semiquantitatively evaluated using an H-score system (0-300), calculated as $(1 \times P1) + (2 \times P2) + (3 \times P3)$, where P1–P3 represent the percentages of cells stained at weak, moderate, and strong intensities, respectively.

Cell culture, transfection, and drug treatment

The human colorectal adenocarcinoma cell line Caco-2 (iCell, h032) was cultured in MEM medium supplemented with 20% fetal bovine serum (ExCell Bio, FSP500) and maintained in an incubator at 37°C with 5% CO₂. Before experimentation, Caco-2 cells were seeded in 6-well plates at a density of 3×10^5 cells per well and cultured until they reached 70–80% confluence. Subsequently, the cells were treated with medium containing 10 µg/mL lipopolysaccharide (LPS) to establish a cell injury model. After modeling, negative control siRNA or CDC25B siRNA was transfected using Lipo3000 transfection reagent to achieve CDC25B knockdown.

Real-time quantitative PCR (qRT-PCR) and western blot analysis

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, 10090991). Reverse transcription and qRT-PCR were performed using the HiScript III All-in-one Reverse Transcription SuperMix Kit and the PerfectStart Green qPCR SuperMix Kit, respectively. The primer sequences for each gene are listed in Supplementary Table S1 (Supplementary Data), and the relative mRNA expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method. Total protein was extracted from Caco-2 cells using RIPA lysis buffer (Solarbio, R0010) containing 1% phenylmethylsulfonyl fluoride (PMSF). Protein samples were mixed with 5 × loading buffer, denatured by heating at 100°C for 5 minutes, and separated by 10% SDS-PAGE. After electrophoresis, the proteins were transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk in TBST and then incubated with corresponding primary antibodies overnight at 4°C. The primary antibodies used included anti-CDC25B (HUABio, ET1701-68),

anti-ZO-1 (HUABio, HA722797), anti-Occludin (HUABio, ET1701-76), anti-Claudin 1 (HUABio, HA721999), anti-IKK α / β (Abmart, TA3014); and anti-p-IKK α / β (Abmart, T55660). The membranes were washed and then incubated with horseradish peroxidase-conjugated secondary antibodies (anti-mouse or anti-rabbit). Protein bands were subsequently visualized using enhanced chemiluminescence substrate.

Enzyme-linked immunosorbent assay (ELISA)

Cell culture supernatants were collected and the concentrations of IL-6 and TNF- α were determined using commercially available ELISA kits (IL-6: EM0004; TNF- α : EM0010; HUABio, China), according to the manufacturer's instructions. Following the addition of samples, incubation, and color development, the optical density was measured at 450 nm using a microplate reader.

Detection of intracellular reactive oxygen species (ROS)

Caco-2 cells were seeded into confocal culture dishes at a density of 2×10^5 cells per dish and cultured for 24 hours. Except for the control group, cells in the other groups were treated with medium containing 10 μ g/mL LPS to establish the injury model. The experimental groups included: control group, LPS group, LPS + si-NC group, and LPS + si-CDC25B group. Cells were cultured for a further 48 hours after transfection. The culture medium was then removed. Cells were incubated in the dark with serum-free medium containing 10 μ mol/L DCFH-DA probe at 37°C for 30 minutes. After washing, fluorescent images were captured under a fluorescence microscope.

Immunofluorescence (IF) staining

For IF staining, Caco-2 cells were seeded onto sterile glass coverslips placed in 6-well plates at a density of 2×10^5 cells per well. After treatments, the culture medium was discarded, and cells were washed three times with pre-chilled PBS, then fixed with 4% paraformaldehyde for 15 minutes at room temperature. After fixation, cells were washed with PBS three times for 5 minutes per wash and then permeabilized with 0.2% Triton X-100 for 10 minutes at room temperature. Following permeabilization, cells were washed three times with PBS and blocked with 5% bovine serum albumin (BSA) for 30 minutes at room temperature. The blocking solution was removed, and cells were incubated with primary antibody against NF- κ B P65 (HUABio, ET1603-12, 1:200) overnight at 4°C. The next day, the primary antibody was recovered, and cells were washed with PBS three times for 5 minutes per wash, then incubated with fluorescence-conjugated secondary antibody (Alexa Fluor 488-conjugated goat anti-rabbit IgG) for 1 hour at room temperature in the dark. After secondary antibody incubation, cells were washed with PBS and stained with DAPI solution for 5 minutes in the dark for nuclear counterstaining. Finally, cells were washed three times with PBS, mounted with an anti-fade mounting medium, and observed under a confocal microscope, and images were captured.

RNA-seq and data analysis

Total RNA was isolated from the LPS-treated Caco-2 cells and LPS-treated Caco-2 cells with CDC25B knockdown. RNA quality assessment, library construction, and sequencing were performed by Novogene (Beijing, China) on an Illumina NovaSeq platform. Gene expression was quantified as transcripts per million (TPM) to find differentially expressed genes (DEGs). A fold change > 2.0, and a *P* value < 0.05 were set as the cutoff for defining significantly DEGs.

Statistical analysis

All data are presented as mean ± SD. Statistical analysis was performed using GraphPad Prism 9.0. Comparisons between two groups were performed using Student's t-test or Mann–Whitney U test. Comparisons among multiple groups were performed using one-way ANOVA followed by Dunnett's post-hoc test. A *P*-value < 0.05 was considered statistically significant.

Results

CDC25B is upregulated in human intestinal septic tissue

To detect the function of CDC25B in human intestinal septic tissues and normal intestinal tissues, H&E staining and IHC staining were performed. The IHC staining results indicated that the protein expression level of CDC25B in septic intestinal tissues was higher than that in the control group (Fig. 1A, B).

LPS induces inflammatory response and barrier dysfunction in Caco-2 cells

To establish an *in vitro* cellular model of intestinal sepsis, Caco-2 cells were treated with different concentrations of LPS for 24 hours. The mRNA expression levels of the inflammatory cytokines IL-1 β , TNF- α , and IL-6 were then examined by qRT-PCR. The results demonstrated that the 10 μ g/mL LPS treatment group exhibited significantly higher cytokine levels than the control and 1 μ g/mL groups (Fig. 2A). Therefore, 10 μ g/mL LPS was selected for subsequent experiments. LPS treatment significantly increased the secretion levels of IL-6 and IL-1 β compared with the control group (Fig. 2B, C). qRT-PCR and western blot analyses showed that LPS treatment significantly upregulated CDC25B mRNA and protein expression, while simultaneously downregulating the mRNA and protein levels of the intestinal barrier-related proteins ZO-1, Occludin, and Claudin-1 (Fig. 2D, E), indicating that LPS treatment induced severe disruption of the intestinal mechanical barrier. Furthermore, ROS staining revealed that oxidative stress levels were markedly elevated following 10 μ g/mL LPS treatment (Fig. 2F). These results indicate the successful establishment of an *in vitro* model of intestinal sepsis.

CDC25B knockdown alleviates LPS-induced oxidative stress and intestinal barrier injury

To further investigate whether CDC25B influences the inflammatory progression of intestinal sepsis, siRNA-mediated knockdown of CDC25B was performed in Caco-2 cells. Western blot and qRT-PCR

analyses demonstrated that all three siRNA sequences achieved knockdown efficiencies exceeding 85% (Fig. 3A, B). Subsequently, ROS staining and CCK-8 assays revealed that CDC25B knockdown attenuated oxidative stress levels in Caco-2 cells following LPS modeling (Fig. 3C). Furthermore, the expression levels of intestinal barrier-related proteins ZO-1, Occludin, and Claudin-1 were examined at both the protein and mRNA levels. Western blot and qRT-PCR results showed that CDC25B knockdown resulted in higher expression of ZO-1, Occludin, and Claudin-1 compared with the LPS-alone group, although their levels remained significantly lower than those in the control group (Fig. 3D-G). Collectively, these findings suggest that CDC25B contributes to the promotion of inflammatory progression in intestinal sepsis.

Figure 3 CDC25B knockdown alleviates LPS-induced oxidative stress and intestinal barrier injury in Caco-2 cells. (A-B) qRT-PCR and western blot analysis of CDC25B knockdown efficiency in Caco-2 cells. **** $p < 0.0001$. (C) ROS staining for detection of oxidative damage in LPS-treated Caco-2 cells following CDC25B knockdown. Scale bar, 50 μm . (D) Western blot analysis of CDC25B, ZO-1, Occludin, and Claudin-1 protein expression levels in LPS-treated Caco-2 cells after CDC25B knockdown. (E-G) qRT-PCR analysis of ZO-1 (E), Occludin (F), and Claudin-1 (G) mRNA expression levels in LPS-treated Caco-2 cells following CDC25B knockdown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

CDC25B knockdown suppresses OLR1 expression and TNF- α /NF- κ B inflammatory signaling

To further elucidate the molecular mechanisms underlying the role of CDC25B in the inflammatory progression of intestinal sepsis, RNA-sequencing (RNA-seq) analysis was performed on LPS-treated Caco-2 cells and LPS-treated Caco-2 cells with CDC25B knockdown. Heatmap analysis of differentially expressed genes revealed a marked downregulation of the inflammation-associated gene OLR1 upon CDC25B silencing (Fig. 4A, B). This finding was subsequently validated in LPS-treated CDC25B-knockdown Caco-2 cells, where qRT-PCR and western blot demonstrated that CDC25B knockdown significantly reduced OLR1 mRNA and protein levels (Fig. 4C, D). KEGG pathway enrichment analysis identified significant enrichment of the TNF- α signaling pathway. Consistent with this, qRT-PCR and western blot results showed that CDC25B knockdown significantly decreased TNF- α mRNA and protein expression (Fig. 4E-G). Given that IKK phosphorylation is required for NF- κ B activation downstream of TNF- α signaling, we evaluated the phosphorylation levels of IKK α / β . Western blot analysis revealed that CDC25B knockdown markedly reduced IKK α / β phosphorylation levels (Fig. 4G). Moreover, the nuclear translocation of its downstream effector NF- κ B p65 was assessed, and the results showed that CDC25B knockdown attenuated p65 nuclear translocation (Fig. 4H). Collectively, these findings suggest that CDC25B may promote intestinal sepsis progression through the regulation of OLR1 expression and the mediation of TNF- α -mediated inflammatory signaling pathways.

CDC25B overexpression aggravates intestinal sepsis by upregulating OLR1 and activating TNF- α signaling

To further validate that CDC25B promotes LPS-induced intestinal barrier dysfunction and inflammatory responses through the regulation of OLR1 and the TNF- α signaling pathway, Caco-2 cells were first

subjected to CDC25B overexpression and subsequently treated with LPS for modeling. The overexpression efficiency of CDC25B in Caco-2 cells was confirmed by qRT-PCR and western blot analysis (Fig. 5A, B). ROS staining revealed that CDC25B overexpression exacerbated LPS-induced oxidative damage (Fig. 5C). Furthermore, the expression levels of intestinal barrier-related genes were examined. qRT-PCR and western blot results demonstrated that CDC25B overexpression resulted in lower mRNA and protein levels of ZO-1, Occludin, and Claudin-1 compared with the LPS-alone group (Fig. 5D-G). Concurrently, the expression of OLR1 and TNF- α signaling pathway-related genes was assessed. Western blot and qRT-PCR analyses showed that CDC25B overexpression significantly increased IKK phosphorylation levels, and the mRNA and protein levels of TNF- α and OLR1 were markedly higher in the CDC25B overexpression group than in the group treated with LPS alone (Fig. 5H-K). Additionally, the nuclear translocation of the downstream effector p65 was examined, and the results indicated that CDC25B overexpression enhanced p65 nuclear translocation (Fig. 5L). Collectively, these findings further corroborate the regulatory role of CDC25B in modulating OLR1 expression and the TNF- α -mediated inflammatory signaling pathway.

Discussion

This study is the first to reveal the pro-inflammatory function of CDC25B in intestinal sepsis and to elucidate the molecular mechanism by which it upregulates OLR1 expression, activates the TNF- α signaling pathway, and consequently disrupts intestinal tight junctions and exacerbates barrier dysfunction.

LPS is widely used as a classical model to mimic sepsis-associated inflammatory responses and has been shown to induce various pro-inflammatory mediators across different cell types and tissues[19]. CDC25B, a cell cycle phosphatase, has recently garnered attention for its non-canonical pro-inflammatory functions. The inhibitor BN82002 has been shown to attenuate LPS-induced NO and PGE₂ production via suppression of the AKT2/NF- κ B pathway and to improve survival in septic mice[20]. In psoriasis, CDC25B overexpression has been implicated in a pro-inflammatory and proliferative vicious cycle involving NF- κ B and TNF- α [21, 22]. Building on these observations, the present study proposes that LPS-induced CDC25B upregulation enhances TNF- α expression, concomitant with NF- κ B pathway activation. We confirmed NF- κ B activation by assessing p65 nuclear translocation and IKK α / β phosphorylation, anchoring the functional site of CDC25B upstream of the NF- κ B cascade.

TNF- α is a central driver of barrier dysfunction in intestinal sepsis. It upregulates MLCK via activation of the ERK1/2-Elk-1 pathway, promoting MLC phosphorylation and internalization of tight junction proteins such as claudins, thereby increasing paracellular permeability of the intestinal epithelium[23]. Sustained TNF- α stimulation, exemplified by 400 ng/mL treatment for 96 hours, is sufficient to severely compromise intestinal barrier integrity[24]. Our data show that CDC25B knockdown significantly reduces TNF- α levels and restores ZO-1, Occludin, and Claudin1 expression, whereas CDC25B overexpression

exacerbates the loss of these proteins, reinforcing the notion that CDC25B acts upstream of TNF- α -mediated barrier disruption.

OLR1, a scavenger receptor, not only recognizes oxidized low-density lipoprotein but also serves as a bacterial surface receptor and triggers inflammatory responses via the NF- κ B pathway[25, 26]; its expression is markedly induced by pro-inflammatory cytokines such as TNF- α , IFN- γ , and IL-6[27–30]. In the intestinal microcirculation, endotoxemia has been shown to upregulate OLR1, whereas OLR1 inhibition attenuates LPS-induced leukocyte adhesion in the intestinal submucosa[31]. Notably, RNA-seq analysis revealed that CDC25B knockdown significantly reduced OLR1 transcript levels in LPS-stimulated Caco-2 cells, which was further validated by independent experiments showing that CDC25B silencing attenuates LPS-induced OLR1 upregulation. Moreover, the concurrent upregulation of OLR1 and TNF- α upon CDC25B overexpression, and their downregulation upon CDC25B silencing, suggest a potential positive feedback loop that may amplify intestinal inflammation and barrier disruption.

Nevertheless, several questions remain to be addressed. First, whether CDC25B directly binds the OLR1 promoter or exerts its effect entirely indirectly via the AKT2/NF- κ B pathway requires validation by ChIP and luciferase reporter assays. Second, the present findings are primarily derived from in vitro Caco-2 models; in vivo validation using intestinal-specific CDC25B knockout mice in sepsis models is needed to confirm the pathophysiological relevance of this axis. Elucidating these mechanisms will reinforce the rationale for CDC25B-directed therapeutic strategies in sepsis-associated intestinal injury.

In conclusion, our findings demonstrate that CDC25B drives intestinal barrier dysfunction and inflammatory responses by upregulating OLR1 expression and activating TNF- α signaling. This study establishes a novel CDC25B–OLR1/TNF- α –NF- κ B–tight junction disruption axis and suggests that CDC25B and OLR1 may serve as potential therapeutic targets for intestinal sepsis.

Declarations

Competing Interests

The authors declare that they have no financial or non-financial competing interests.

Ethics approval

This study was approved by the Ethics Committee of Bengbu Medical University (No. 2025 – 537).

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Author Contribution

Conceptualization, H.X. and G.L.; methodology, H.X. and L.T.Z.; investigation, L.T.Z. and X.L.W.; sample collection, P.Y.P.; data curation, X.K.L.; formal analysis, H.X. and X.K.L.; writing—original draft preparation, H.X.; writing—review and editing, G.L.; funding acquisition, H.X. and X.L.W. All authors have read and agreed to the published version of the manuscript.

Data Availability

The datasets generated and/or analysed during the current study are available in the NCBI BioProject database under accession number PRJNA1530793 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1530793>). The data are publicly available.

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Figures

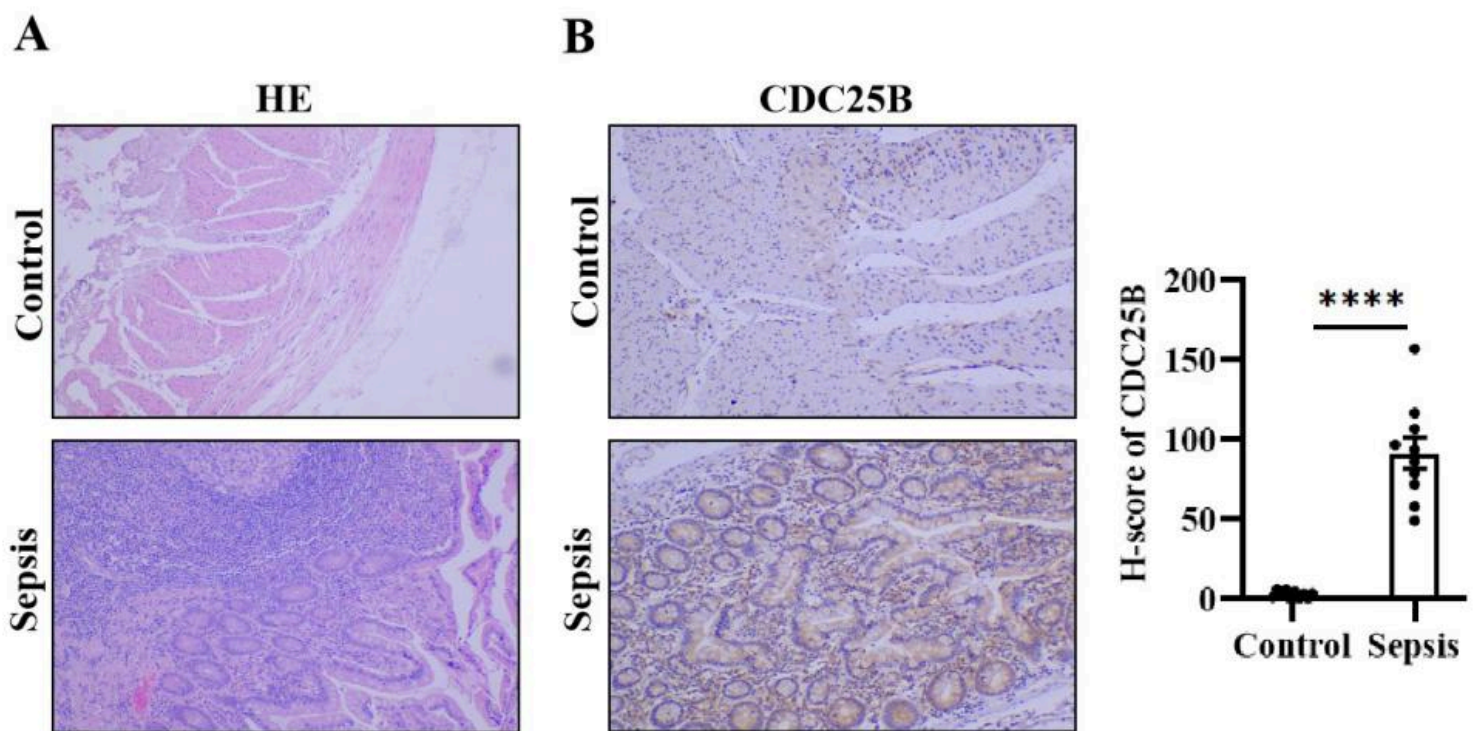


Figure 1

CDC25B is upregulated in human intestinal septic tissue. (A) H&E staining of human intestinal sepsis tissues and normal intestinal tissues. (B) IHC staining for CDC25B expression in human intestinal sepsis tissues and normal intestinal tissues, along with quantitative analysis of CDC25B protein expression. Original magnification, 10 $\times$. **** $p < 0.0001$.

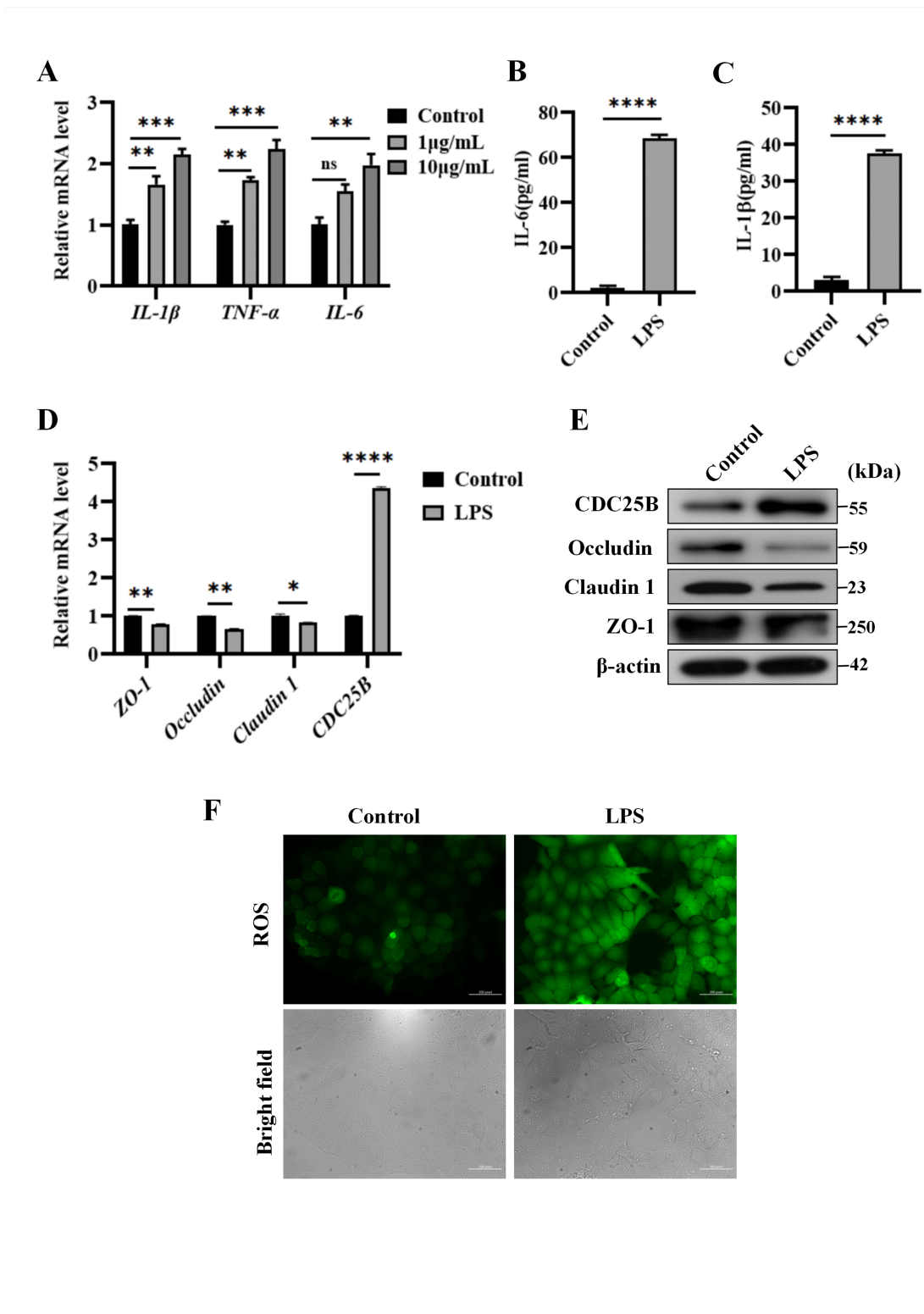


Figure 2

LPS induces inflammatory response and barrier dysfunction in Caco-2 cells. (A) qRT-PCR analysis of TNF- α , IL-6, and IL-1 β mRNA expression levels in Caco-2 cells treated with different concentrations of LPS. $**p < 0.01$, $***p < 0.001$. (B-C) ELISA quantification of IL-6 (B) and IL-1 β (C) concentrations in the supernatants of Caco-2 cells in the control and LPS-treated groups. $****p < 0.0001$. (D) qRT-PCR analysis of CDC25B, ZO-1, Occludin, and Claudin-1 mRNA expression levels in Caco-2 cells in the control and LPS-

treated groups. $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$. (E) Western blot analysis of CDC25B, ZO-1, Occludin, and Claudin-1 protein expression levels in Caco-2 cells in the control and LPS-treated groups. (F) ROS staining for the detection of oxidative damage in Caco-2 cells in the control and LPS-treated groups. Scale bar, 50 μm .

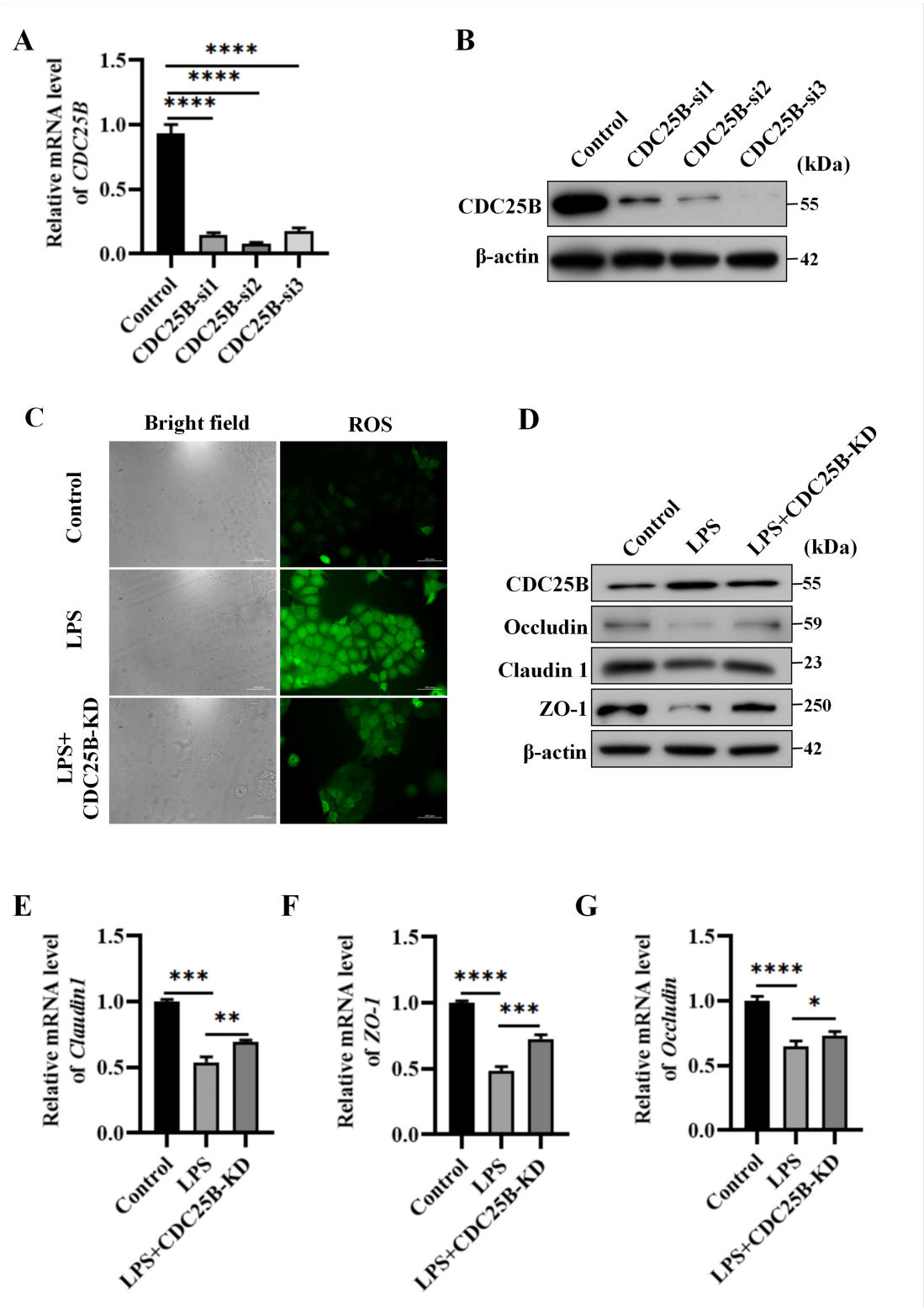


Figure 3

CDC25B knockdown alleviates LPS-induced oxidative stress and intestinal barrier injury in Caco-2 cells. (A-B) qRT-PCR and western blot analysis of CDC25B knockdown efficiency in Caco-2 cells. **** $p < 0.0001$. (C) ROS staining for detection of oxidative damage in LPS-treated Caco-2 cells following CDC25B knockdown. Scale bar, 50 μm . (D) Western blot analysis of CDC25B, ZO-1, Occludin, and Claudin-1 protein expression levels in LPS-treated Caco-2 cells after CDC25B knockdown. (E-G) qRT-PCR analysis of ZO-1 (E), Occludin (F), and Claudin-1 (G) mRNA expression levels in LPS-treated Caco-2 cells following CDC25B knockdown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

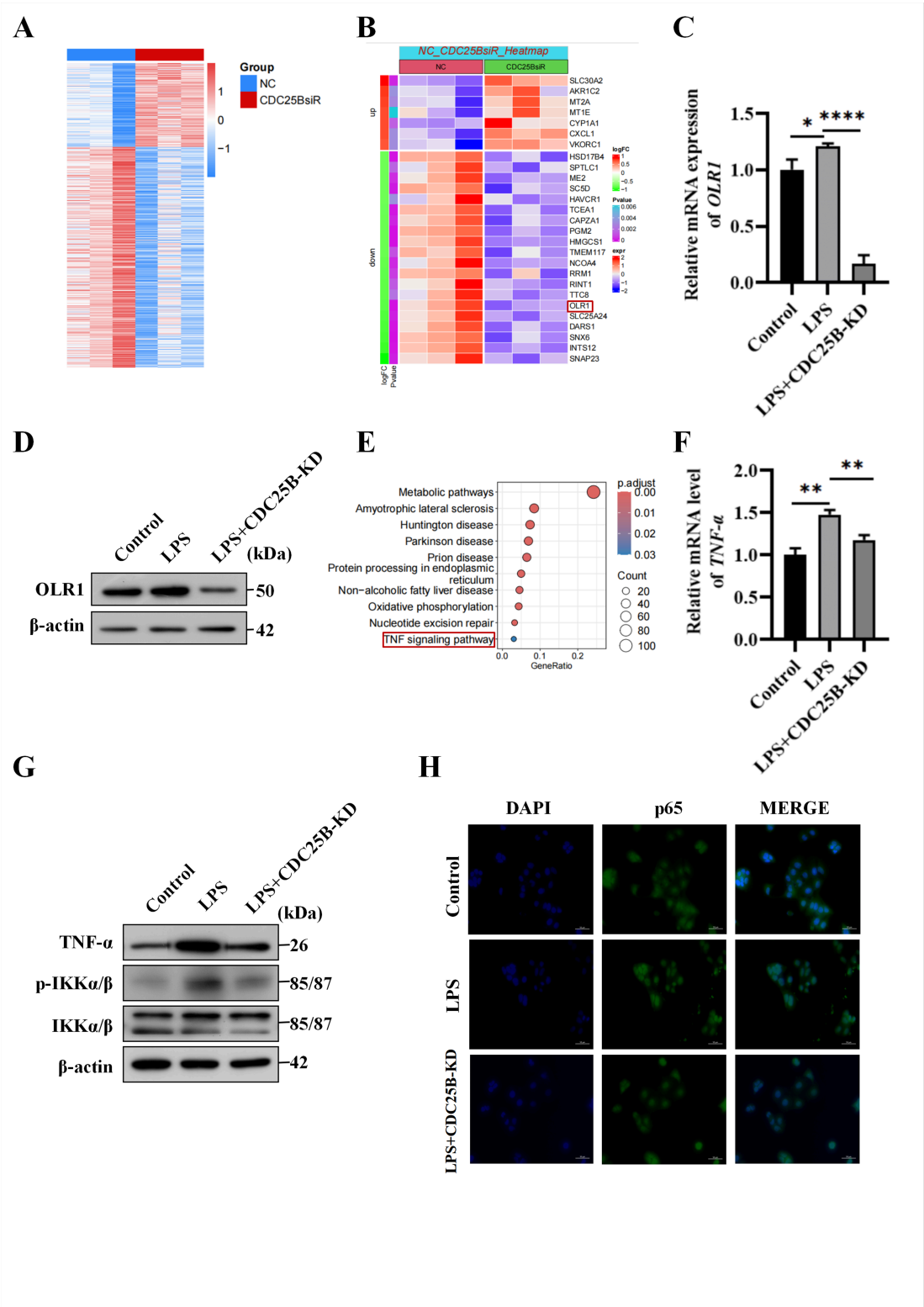


Figure 4

CDC25B knockdown suppresses OLR1 expression and TNF- α /NF- κ B inflammatory signaling. (A-B) Heatmaps showing differentially expressed genes identified by RNA-seq analysis following CDC25B knockdown in LPS-treated Caco-2 cells. (C-D) qRT-PCR and western blot analysis of OLR1 mRNA and protein expression levels in LPS-treated Caco-2 cells after CDC25B knockdown. $*p < 0.05$, $****p < 0.0001$. (E) Gene Ontology (GO) enrichment analysis showing the top 10 pathways with the smallest adjusted p -values. The adjusted p -values are presented on the y-axis, and gene ratios are shown on the x-axis. (F-G) qRT-PCR and western blot analysis of TNF- α mRNA and protein expression, as well as IKK α / β and phosphorylated IKK α / β (p-IKK α / β) protein levels, in LPS-treated Caco-2 cells following CDC25B knockdown. $**p < 0.01$. (H) Immunofluorescence staining showing the expression and subcellular localization of p65 protein in Caco-2 cells. Scale bar, 50 μ m.

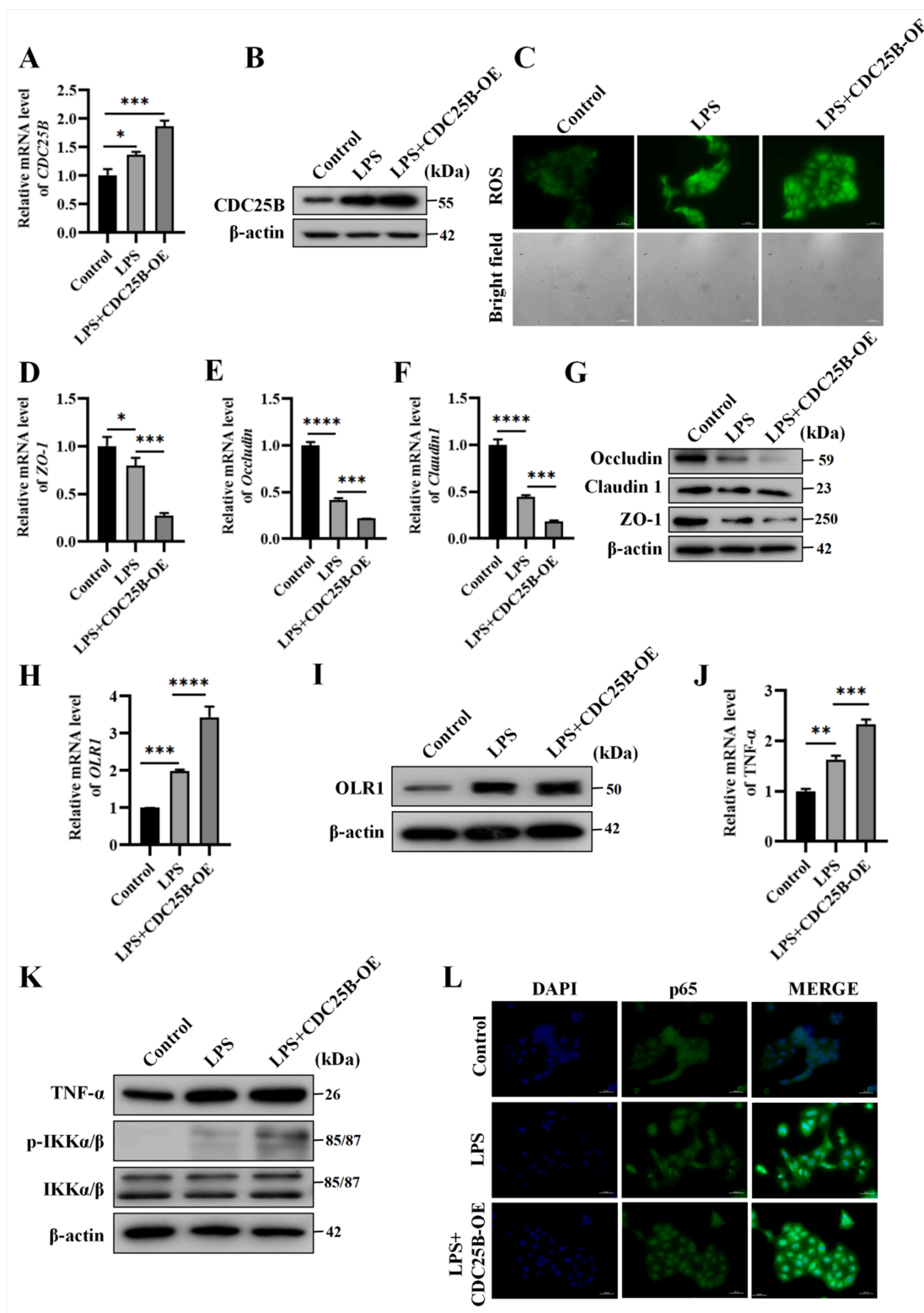


Figure 5

CDC25B overexpression aggravates intestinal sepsis by upregulating OLR1 and activating TNF- α signaling. (A-B) qRT-PCR and western blot analysis confirming CDC25B overexpression efficiency in Caco-2 cells. (C) ROS staining showing oxidative damage in LPS-treated Caco-2 cells following CDC25B overexpression. Scale bar, 50 μ m. (D-G) qRT-PCR and western blot analysis of ZO-1, Occludin, and Claudin-1 mRNA and protein expression levels in LPS-treated Caco-2 cells after CDC25B overexpression.

$*p < 0.05$, $***p < 0.001$, $****p < 0.0001$. (H-I) qRT-PCR and western blot analysis of OLR1 mRNA and protein expression levels in LPS-treated Caco-2 cells after CDC25B overexpression. $***p < 0.001$, $****p < 0.0001$. (J-K) qRT-PCR and western blot analysis of TNF- α mRNA expression, as well as IKK α / β and phosphorylated IKK α / β (p-IKK α / β) protein levels, in LPS-treated Caco-2 cells after CDC25B overexpression. $**p < 0.01$, $***p < 0.001$. (L) IF staining showing the expression and subcellular localization of p65 protein in Caco-2 cells. Scale bar, 50 μ m.

Supplementary Files

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

- [SupplementaryData.pdf](#)