

Drp1 deficiency enhances macrophage efferocytosis and partially restores gut microbial dysbiosis in colitis

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Abstract

Dynamin-related protein 1 (Drp1) is a key regulator of mitochondrial fission, but its role in intestinal macrophages and gut microbial homeostasis during colitis remains unclear. Herein, Drp1 expression was increased in intestinal macrophages from patients with ulcerative colitis and mice with dextran sulfate sodium (DSS)-induced colitis, as demonstrated by transcriptomic, single-cell, and immunofluorescence analyses. To determine its functional role, colitis was induced in conditional Drp1 knockout mice and littermate controls. Macrophage Drp1 deficiency reduced disease activity, colon shortening, histopathological injury, and mucus barrier disruption. Colonic transcriptomic profiling further revealed reduced inflammatory signaling and enrichment of pathways related to macrophage differentiation, apoptotic-cell clearance, and efferocytosis. Consistently, Drp1-deficient macrophages exhibited enhanced apoptotic-cell uptake *in vitro*. Furthermore, 16S rRNA sequencing showed that Drp1 deficiency partially reversed colitis-associated alterations in gut microbial community structure, without significantly affecting overall alpha diversity. This microbial remodeling was characterized by increased *Akkermansia* and *Muribaculaceae*, while reduced *Escherichia-Shigella* and *Enterococcus*. Collectively, these findings indicate that Drp1 deficiency attenuates acute colitis by reshaping macrophage functional states and enhancing efferocytosis, while being associated with improved mucosal integrity and partial restoration of gut microbial dysbiosis.

Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic intestinal inflammation driven by complex interactions among host susceptibility, environmental factors, the gut microbiota, and dysregulated mucosal immunity (Cadwell and Loke 2025; Chang 2020). Despite major advances in biologic and small-molecule therapies, sustained mucosal healing remains difficult to achieve in many patients, suggesting that key mechanisms responsible for persistent inflammation and defective tissue recovery remain incompletely understood (Ho et al. 2020; Neurath and Vieth 2023). Macrophages are central to intestinal immune homeostasis. Under steady-state conditions, these cells help contain microbes, maintain immune tolerance, support epithelial integrity, and promote tissue repair (Hegarty et al. 2023). However, the monocyte-macrophage compartment is markedly remodeled during active IBD. Inflammatory macrophage populations accumulate in the mucosa and participate in cytokine production, immune-cell recruitment, and pathogenic cell-cell communication (Mitsialis et al. 2020; Smillie et al. 2019). Their functional state is therefore likely to influence both the magnitude and persistence of mucosal inflammation. Defining the intracellular pathways that govern macrophage responses may uncover new mechanisms contributing to IBD progression.

Mitochondria have emerged as key regulators of innate immune-cell function beyond their canonical role in energy production (Chan 2020). Dynamic remodeling of the mitochondrial network through coordinated fission and fusion influences cellular metabolism, reactive oxygen species generation, organelle quality control, and inflammatory signaling, thereby shaping macrophage responses to

environmental stress. Dynamin-related protein 1 (Drp1), encoded by DNM1L, is a key regulator of mitochondrial fission. In macrophages, lipopolysaccharide stimulation induces TLR4- and MyD88-dependent Drp1 activation and mitochondrial fragmentation (Kapetanovic et al. 2020). Disrupting Drp1-dependent fission alters inflammatory mediator production, mitochondrial respiration, and oxidative stress (Lin et al. 2022). Moreover, Drp1 can also regulate cytokine output through post-transcriptional mechanisms, indicating that mitochondrial dynamics intersect with inflammatory signaling at several levels (Gao et al. 2020). Thus, Drp1 may function not only as a structural regulator of mitochondria but also as a checkpoint linking inflammatory cues to macrophage functional responses.

Growing evidence implicates disturbed mitochondrial homeostasis and dynamics in intestinal inflammation (Haque et al. 2024; Mancini et al. 2020). In experimental colitis, Drp1 activation and other fission-associated changes are increased in the colon, while DSS induces mitochondrial fragmentation in both intestinal epithelial cells and macrophages. Systemic administration of P110, a peptide that disrupts the Drp1-Fis1 interaction, attenuates DSS- and DNBS-induced colitis (Mancini et al. 2020). Human data further support a link between macrophage mitochondrial homeostasis and UC. Loss of CLUH in human macrophages increases DRP1 availability, mitochondrial fragmentation, mitochondrial reactive oxygen species production, and inflammatory cytokine release (Khan et al. 2023). More recently, epithelial-specific Drp1 deficiency was shown to ameliorate DSS colitis by limiting mitochondrial fission, mitochondrial oxidative stress, and ZBP1-dependent PANoptosis (Ye et al. 2024). These studies provide strong evidence that excessive Drp1-dependent mitochondrial remodeling contributes to intestinal injury. However, they leave an important cell-specific question unresolved. Systemic pharmacological inhibition affects multiple intestinal cell populations, whereas epithelial-specific models define Drp1 function only within the epithelial compartment. Direct genetic evidence for a macrophage-intrinsic role of Drp1 in colitis remains poorly defined.

Given the central role of macrophages in intestinal inflammation and tissue homeostasis, we investigated whether macrophage Drp1 directly contributes to experimental colitis. Using conditional deletion of Drp1, we assessed disease severity and characterized the cellular and molecular consequences of Drp1 deficiency during intestinal inflammation. This approach allowed us to distinguish macrophage-intrinsic Drp1 activity from the broader effects of systemic inhibition or epithelial Drp1 signaling. Our findings identify macrophage Drp1 as a previously underexplored regulator of colitis and provide a cell-type-specific framework for understanding how mitochondrial dynamics shape intestinal inflammatory responses.

Methods

Clinical Sample Collection from Ulcerative Colitis Patients

This study enrolled patients with ulcerative colitis (UC) and healthy controls who underwent routine health examinations at the Department of Gastroenterology, the Second Affiliated Hospital of South China University of Technology (Guangzhou First People's Hospital), between January and May 2025.

Fecal samples and colonic tissue specimens were collected from all participants. UC diagnosis was confirmed based on clinical, endoscopic, and histopathological criteria, and disease activity was stratified according to the modified Mayo score system: patients with scores ranging from 6 to 12 were classified as having moderate-to-severe active disease, those with scores of 3 to 5 were defined as having mild active disease, and individuals with scores ≤ 2 and no subscore > 1 were considered to be in clinical remission. Healthy controls had no history of gastrointestinal disorders or related medication use. The study protocol was approved by the Ethics Committee of the Second Affiliated Hospital of South China University of Technology (Guangzhou First People's Hospital) and was conducted in accordance with the principles of the Declaration of Helsinki.

Data Collection and Processing of Public RNA-Seq and scRNA-Seq Datasets

The bulk RNA-seq dataset GSE76658 comprised 3 healthy control samples (WT_H₂O) and 3 DSS-induced colitis samples (WT_DSS); these 6 samples were selected for analysis. The single-cell transcriptomic dataset GSE264408 comprised 3 healthy control samples (Healthy), 3 DSS-induced acute colitis samples (Acute Colitis), and 4 DSS-induced chronic colitis samples (Chronic Colitis); these 10 samples were selected for analysis. Raw datasets were processed using R software (version 4.3.1). Probe IDs were converted to gene symbols by connecting to the ENSEMBL database; probes without gene symbols were removed, and average expression values were calculated for genes sharing the same symbol.

In this study, high-throughput sequencing data were normalized using the "DESeq2" package (version 1.42.0). Single-cell transcriptomic data were preprocessed using the Seurat R package (version 5.0.1). Cells were filtered according to the following quality control criteria: (1) cells with 200–6000 detected genes were retained; and (2) cells with a mitochondrial gene expression proportion below 15% were retained. The quality-controlled expression matrix was then normalized, subjected to highly variable gene selection and scaling, and linearly reduced in dimensionality using principal component analysis (PCA). Batch effects across samples were corrected using the "Harmony" R package (version 1.2.3), followed by two-dimensional visualization using Uniform Manifold Approximation and Projection (UMAP). Cell type annotation was performed based on the expression patterns of cluster-specific marker genes in conjunction with canonical cell marker genes reported in published literature. Dot plots were used to visualize the average expression level and the proportion of Dnm1l-expressing cells in the Healthy, Acute Colitis, and Chronic Colitis groups.

Generation and Genotyping of Drp1 cKO Mice

To generate myeloid-specific Drp1 conditional knockout mice, Drp1^{fl/+} heterozygous mice were crossed with Lyz2-Cre transgenic mice. Wild-type littermates were used as controls.

Genotyping was performed using two primer pairs: (a) Cre-F/Cre-R for detection of the Lyz2-Cre transgene, and (b) F2/R2 for detection of the Drp1 floxed allele. PCR products from tail genomic DNA were resolved by agarose gel electrophoresis. Mice carrying the Lyz2-Cre transgene displayed a specific amplicon of 388 bp, which was absent in wild-type mice. For the Drp1 floxed allele, homozygous mice exhibited a 240-bp band, wild-type mice showed a 200-bp band, and heterozygous mice presented both bands. After confirming the presence of the Cre transgene, individuals were further verified using the primer pair F2/R1. The resulting electrophoretic patterns confirmed the successful generation of Drp1 cKO mice.

DSS-Induced Acute Colitis Model

A total of 28 male mice were used in this study, including 14 wild-type C57BL/6 mice (6 weeks old, male, SPF grade) obtained from the Guangdong Laboratory Animal Center, and 14 Drp1 cKO mice purchased from Cyagen Biosciences (Suzhou, China). All animals were housed under specific pathogen-free conditions with a constant temperature of 25°C, a 12-h light/dark cycle, and ad libitum access to autoclaved water and standard chow. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of the Second Affiliated Hospital of South China University of Technology (Guangzhou First People's Hospital).

After one week of acclimatization, mice were randomly assigned to four experimental groups (n = 7 per group) according to body weight: (1) wild-type control group (WT_Control), (2) wild-type DSS-treated group (WT_DSS), (3) Drp1 cKO control group (Drp1 cKO_Control), and (4) Drp1 cKO DSS-treated group (Drp1 cKO_DSS).

To establish the acute colitis model, mice in the two DSS-treated groups (WT_DSS and Drp1 cKO _DSS) received 3% dextran sulfate sodium (DSS, molecular weight 36–50 kDa, MP Biomedicals) in their drinking water for 7 consecutive days. Meanwhile, mice in the two control groups (WT_Control and Drp1 cKO _Control) were given normal drinking water throughout the entire experimental period.

Disease Activity Index (DAI)

As outlined in Table 1, the Disease Activity Index (DAI) was evaluated based on a composite scoring system comprising three parameters: (1) percentage of body weight loss, (2) stool consistency, and (3) degree of fecal bleeding. Body weight was recorded daily at a fixed time, and stool characteristics were monitored and documented throughout the experimental period. To minimize subjective bias, all scores were independently assigned by two investigators, and the final DAI score for each mouse was calculated as the average of the two assessments.

Table 1
Disease Activity Index (DAI) scoring system.

Score	Weight loss (%)	Stool consistency	Fecal occult blood
0	0	Normal (well-formed pellets)	Negative
1	1–5	Soft (slightly loose)	Weakly positive (+)
2	5–10	Loose (without mucus)	Positive (++)
3	10–20	Diarrhea (with mucus)	Strongly positive (+++)
4	> 20	Watery diarrhea	Gross bleeding

H&E Staining

Colonic tissues were collected from deeply anesthetized mice, thoroughly rinsed with phosphate-buffered saline, and subsequently fixed in 4% paraformaldehyde. Following paraffin embedding and sectioning, tissue sections were subjected to hematoxylin and eosin staining. The severity of colitis was then evaluated based on histopathological scoring criteria, as detailed in Table 2.

Table 2
Histopathological Scoring Criteria for Colonic Tissue Damage.

Epithelial damage	Inflammatory cell infiltration	Score
No epithelial injury	Rare inflammatory cells in the lamina propria	0
Focal epithelial injury	Increased inflammatory cells with neutrophil infiltration in the lamina propria	1
Mucosal fusion and ulceration	Inflammatory cells extending into the submucosa	2
Full-thickness epithelial damage	Diffuse inflammatory cell infiltration throughout the submucosa	3

AB-PAS Staining

Colonic segments were fixed in Carnoy's solution (glacial acetic acid: absolute ethanol = 1:3). Paraffin-embedded tissue sections were then stained using the Alcian Blue-Periodic Acid-Schiff (AB-PAS) method. Following staining, goblet cells in the colonic sections were enumerated.

Immunofluorescence Staining of Tissue Sections

For single-color immunofluorescence, deparaffinized colonic tissue sections were incubated with primary antibodies against Drp1, MUC-2, Occludin, or Claudin-1, followed by incubation with Alexa Fluor™ 488-conjugated secondary antibodies. Nuclei were counterstained with DAPI.

For multiplex immunofluorescence, the expression of Drp1, CD68, CD86, CD206, and F4/80 in colonic tissues was detected using the TSA Plus Fluorescence Double/Triple Staining Kit according to the manufacturer's instructions. Images were acquired using a fluorescence slide scanner.

Western Blot Analysis

Colonic tissue samples were lysed in RIPA lysis buffer (Thermo Fisher Scientific, USA) supplemented with PMSF (Thermo Fisher Scientific, USA). Protein concentrations were determined using a BCA assay kit (Thermo Fisher Scientific, USA). Equal amounts of protein were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). The membranes were blocked with 5% non-fat milk and incubated overnight at 4°C with primary antibodies specific for Muc2 (Abcam, ab272692), ZO-1 (Abcam, ab96587), Occludin (Abcam, ab216327), Claudin-1 (Abcam, ab307692), CD206 (CST, 24595S), and CD80 (Abcam, ab254579), followed by incubation with HRP-conjugated secondary antibodies. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system.

Macrophage Isolation and Culture

Bone marrow-derived macrophages (BMDMs) were isolated from the femurs and tibiae of Drp1 cKO or wild-type mice and cultured in the presence of macrophage colony-stimulating factor (M-CSF). The human monocytic cell line THP-1, including both Drp1-knockout and wild-type cells (Cyagen Biosciences, Suzhou, China), as well as BMDMs, were maintained in DMEM supplemented with 20 ng/mL M-CSF, 20% heat-inactivated fetal bovine serum, and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO₂.

Apoptosis Induction and Fluorescent Labeling of Jurkat Cells

Jurkat cells (Procell Life Science & Technology Co., Ltd., Wuhan, China) were cultured in RPMI-1640 medium. Apoptosis was induced by treating the cells with 0.5 µg/mL staurosporine for 2.5 h at 37°C in a 5% CO₂ atmosphere. Following induction, cells were resuspended at a density of 2×10^7 cells/mL in RPMI-1640 medium and subjected to pHrodo-red staining for 25 min. The labeled cells were then collected, washed with RPMI-1640 basal medium, and resuspended in DMEM supplemented with 10% heat-inactivated fetal bovine serum at a final concentration of 1×10^7 cells/mL for subsequent co-culture experiments.

In Vitro Efferocytosis Assay

BMDMs, THP-1 cells, and apoptotic Jurkat cells were prepared as described above. To generate M2-polarized macrophages, BMDMs and THP-1 cells were stimulated with IL-4 in combination with IL-13 prior to the phagocytosis assay. Non-polarized cells (M0) were cultured in parallel with an equal volume of complete medium as controls. Following polarization, the culture medium was removed, and cells were incubated with pre-warmed MitoTracker Green staining solution at 37°C for 15–45 min. The staining solution was then discarded, and pHrodo-labeled apoptotic Jurkat cells were added to the

macrophages at a ratio of 5:1 (apoptotic cells: macrophages) in confocal dishes and co-cultured for 90 min. After co-culture, macrophages were washed and processed for immunofluorescence analysis by confocal microscopy.

RNA-Seq Analysis

Approximately 50 mg of distal colonic tissue was excised, rinsed with ice-cold PBS to remove intestinal contents, and immediately transferred to RNase-free microcentrifuge tubes before snap-freezing in liquid nitrogen. Total RNA was subsequently extracted using an RNA extraction kit for RNA-sequencing analysis. Library construction and sequencing were performed by Gene Denovo Biotechnology Co., Ltd. (Guangzhou, China). Raw sequencing data were processed and analyzed using the free online platform OmicShare Tools (<https://www.omicshare.com>).

Fecal 16S rRNA Gene Sequencing

Freshly collected cecal contents were immediately snap-frozen in liquid nitrogen and subsequently stored at -80°C until further processing. Genomic DNA was extracted from the samples using the QIAamp DNA Stool Mini Kit. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified by PCR using the universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GGACTACHVGGGTATCTAAT-3'). The resulting amplicons were purified and subjected to library preparation, followed by sequencing on the NovaSeq platform (Gene Denovo Biotechnology Co., Ltd., Guangzhou, China). All valid reads were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold. Subsequent bioinformatics analyses were performed using the free online platform OmicShare Tools (<https://www.omicshare.com>).

Statistical Analysis

Data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism 8.0 or R. Comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA). Statistical significance was defined as $p < 0.05$.

Results

Drp1 expression is elevated in intestinal macrophages from UC and experimental colitis

Given the critical role of macrophages in intestinal inflammation, we first investigated whether Drp1 expression is altered in intestinal macrophages during colitis. Analysis of the bulk RNA-seq dataset GSE76658 from the GEO database showed that the *Dnm1l*, the gene encoding dynamin-related protein 1 (Drp1), was significantly elevated in the lesioned colonic tissues of colitic mice compared with healthy controls (Fig. 1A). To further define the cellular distribution of Drp1 during colitis, we analyzed the single-cell RNA-seq dataset GSE264408. UMAP analysis identified the major intestinal cell populations, including macrophages and other immune and stromal subsets (Fig. 1B). Among mitochondrial

dynamics-related genes, Dnm1l exhibited a relatively higher expression in macrophages from the Acute Colitis group, whereas other fission-related genes displayed distinct expression patterns across disease states (Fig. 1C). Consistently, violin and differential dot plots revealed that, compared with the Healthy group, the distribution of Dnm1l expression in macrophages was significantly altered in the Acute Colitis group ($p = 0.023$); the difference between the Healthy and Chronic Colitis groups was also statistically significant ($p = 0.012$) (Fig. 1D-E).

We further validated these observations at the protein level in human and murine colonic tissues. Immunofluorescence staining showed significantly increased Drp1 fluorescence intensity was in macrophages (CD68⁺) within the colonic tissues of UC patients compared with healthy controls. Similarly, Drp1 expression was markedly increased in colonic macrophages (F4/80⁺) from DSS-induced murine colitis model (Fig. 1F). Together, these findings demonstrate that increased Drp1 expression is associated with intestinal macrophages in both human UC and experimental colitis.

Myeloid-specific Drp1 deficiency attenuates DSS-induced colitis

To determine the role of myeloid Drp1 in intestinal inflammation, WT and conditional Drp1 knockout (Drp1 cKO, Drp1^{fl/fl} Lyz2^{Cre}) were subjected to DSS-induced acute colitis. (Fig. 2A). Compared with the WT_DSS group, myeloid-specific Drp1 deficiency significantly attenuated DSS-triggered body-weight loss (Fig. 2B) and markedly reduced the disease activity index (DAI) score (Fig. 2C). Colon shortening was substantially alleviated in the Drp1 cKO_DSS group relative to the WT_DSS group (Fig. 2D, G). Consistently, H&E staining showed reduced mucosal damage and inflammatory cell infiltration following Drp1 deletion (Fig. 2E, H). AB-PAS staining further revealed preservation of goblet cells and the colonic mucus barrier in Drp1 cKO_DSS mice (Fig. 2F, I). Together, these findings indicate that conditional Drp1 deletion in the myeloid compartment attenuates acute DSS-induced colitis and preserves colonic mucosal integrity.

Conditional Drp1 deletion preserves intestinal barrier integrity in DSS-induced colitis

To further determine whether Drp1 deficiency protects the intestinal barrier during acute colitis, we examined mucus- and tight-junction-associated proteins in colonic tissues. Immunofluorescence staining revealed that the protein levels of Claudin-1, MUC2 and Occludin were markedly down-regulated in colonic tissues from WT_DSS colitis mice, whereas these reductions were substantially attenuated in Drp1 cKO_DSS mice (Fig. 3A). Consistently, western blot analysis demonstrated that Drp1 deficiency significantly up-regulated the protein abundance of MUC2, ZO-1 and Occludin. Claudin-1 expression also showed an increasing trend, although the difference did not reach statistical significance (Fig. 3B-C). These findings suggest that conditional Drp1 deletion helps preserve both the colonic mucus barrier and the epithelial tight-junction integrity.

Conditional Drp1 deletion suppresses inflammatory programs and enriches macrophage efferocytosis-related pathways during DSS-induced colitis

To explore the mechanism underlying the protective effects of Drp1 deficiency during colitis, RNA-seq analysis was performed on colonic tissues from mice in each group. Heatmap analysis revealed that multiple chemokines and pro-inflammatory cytokines were markedly down-regulated in the Drp1 cKO_DSS group compared with the WT_DSS group (Fig. 4A). Principal-component analysis (PCA) showed clear transcriptional separation between the WT_DSS and Drp1 cKO_DSS groups (Fig. 4B), indicating that myeloid-specific Drp1 deficiency profoundly reshapes the gene-expression profile of colonic tissues. Furthermore, differential expression analysis identified 59 up-regulated genes and 823 down-regulated genes in Drp1 cKO_DSS group relative to the WT_DSS group (Fig. 4C). KEGG pathway enrichment analysis showed that DEGs were mainly enriched in pathways related to inflammation and immunity, cell migration and adhesion, as well as intestinal mucosal immunity (Fig. 4D). These alterations were consistent with an overall attenuation of inflammatory signaling after Drp1 deletion. Gene Ontology (GO) functional enrichment analysis further confirmed that DEGs were mainly involved in the regulation of inflammatory responses and immune-cell functions across molecular function, biological process and cellular component categories (Fig. 4E). Consistently, inflammation-associated genes exhibited a decreased expression pattern following myeloid-specific Drp1 deletion, whereas intestinal-barrier-related genes were up-regulated (Fig. 4F).

We next examined whether Drp1 deletion was associated with broader changes in macrophage-related functional programs. GSEA revealed enrichment of gene sets associated with macrophage differentiation, apoptotic-cell clearance, phagocytosis and efferocytosis in the colonic tissues of Drp1 cKO_DSS mice compared to WT_DSS mice (Fig. 4G-J). These transcriptomic findings suggested that Drp1 deficiency may influence macrophage functional states beyond suppressing inflammatory signaling.

Conditional Drp1 deletion promotes an M2-like macrophage phenotype and enhances efferocytosis

Based on the transcriptomic enrichment of macrophage differentiation, apoptotic-cell clearance, and efferocytosis pathways, we next examined macrophage activation states and efferocytic function. Immunofluorescence co-staining showed markedly increased accumulation of F4/80⁺ macrophages in colonic tissues of the WT_DSS group. These cells exhibited stronger CD86 and weaker CD206 signals, consistent with a pro-inflammatory macrophage phenotype. In contrast, Drp1 cKO_DSS mice exhibited significantly fewer F4/80⁺CD86⁺ cells and substantially more F4/80⁺CD206⁺ cells than WT_DSS mice (Fig. 5A-B). Western blot results supported this shift, showing reduced CD80 and increased CD206 expression in the Drp1 cKO_DSS group (Fig. 5C-D). These data indicate that conditional Drp1 deletion favors an M2-like macrophage activation state during DSS-induced colitis.

To investigate the effect of myeloid-specific Drp1 deficiency on macrophage efferocytosis, we assessed the efferocytic capacity of BMDMs and THP-1-derived macrophages for pHrodo Red-labeled apoptotic Jurkat cells. Under IL-4/IL-13-induced M2-polarizing conditions, Drp1-deficient macrophages displayed greater uptake of apoptotic Jurkat cells than WT macrophages, as indicated by stronger intracellular pHrodo red signals. In contrast, no significant difference in apoptotic-cell uptake was observed between the two groups under M0-polarized conditions (Fig. 5E). Comparable results were obtained in both BMDMs and THP-1 cells. Collectively, these findings suggest that the enhanced efferocytic capacity of M2-like macrophages may contribute to the attenuated colitis phenotype observed after Drp1 deletion.

Conditional Drp1 deletion partially restores DSS-associated gut microbial dysbiosis.

To further investigate whether the conditional Drp1 deficiency was associated with alterations in the gut microbiota during colitis, 16S rRNA sequencing was performed on fecal samples from each experimental group. Venn-diagram analysis revealed distinct patterns of shared and unique OTUs between control and DSS-treated mice (Fig. 6A). Notably, Drp1 cKO_DSS mice harbored fewer unique OTUs than WT_DSS mice, indicating differences in microbial community membership between the two DSS-treated groups. However, alpha-diversity analysis using the shannon index showed no significant differences among the four groups (Fig. 6B), suggesting that overall microbial diversity was not markedly altered by either DSS treatment or conditional Drp1 deletion.

PCoA and PLS-DA analyses revealed clear shifts in microbial community structure following DSS-treatment (Fig. 6C and D). These differences were further supported by PLS-DA (Fig. 6E). Notably, the microbial composition of the Drp1cKO _DSS group clustered closer to that of the Drp1 cKO _Control group than was the WT_DSS profile to WT_Control mice. This finding indicate that Drp1 deficiency could partially reverse DSS-triggered shifts in microbial structure and restore gut microbial homeostasis (Fig. 6C-E). Stacked-bar plots further illustrated the distinct taxonomic composition at the phylum and family levels across the four groups (Fig. 6F-G). In general, DSS treatment reduced the relative abundance of beneficial families including Akkermansiaceae, Lactobacillaceae, Muribaculaceae and Lachnospiraceae, whereas increased the relative abundance of the pathogenic family Enterobacteriaceae. Compared with the WT_DSS group, the relative abundances of Akkermansiaceae and Muribaculaceae were markedly recovered in the Drp1 cKO _DSS group, indicating partial restoration of these DSS-sensitive taxa.

Indicator-species analysis further identified distinct bacterial signatures between WT_DSS and Drp1 cKO_DSS mice (Fig. 6H). *Akkermansia*, *Dubosiella* and *Eubacterium_brachy_group* were more abundant in Drp1 cKO_DSS mice, whereas pathogenic *Escherichia_Shigella* and *Enterococcus* were reduced. Consistently, the Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis revealed significant differences in abundant taxonomic taxa across experimental groups. Pathogenic taxa such as *Escherichia_Shigella* were enriched in the WT_DSS group, whereas beneficial taxa represented by *Akkermansia* dominated the Drp1 cKO _DSS group (Fig. 6I-J).

Targeted comparison of selected taxa further supported these differences. At the family level, DSS challenge reduced the relative abundance of the beneficial bacterium *Muribaculaceae*. At the genus level, DSS challenge reduced the relative abundance of the beneficial bacterium *Akkermansia*. At the species level, DSS challenge increased the relative abundance of *Bacteroides_sartorii*. Conditional Drp1 knockout reversed these changes and restored their relative abundances (Fig. 6K). Collectively, these results suggest that myeloid-specific Drp1 deficiency is associated with partial restoration of DSS-disrupted gut microbial composition, characterized by recovery of several commensal taxa and reduced abundance of inflammation-associated bacteria.

Discussion

In this study, we identified macrophage Drp1 as an important regulator of intestinal inflammation in experimental colitis. Through single-cell analysis, transcriptomic profiling, and immunofluorescence staining, increased Drp1 expression was identified in intestinal macrophages from patients with UC and DSS-treated mice. Conditional deletion of Drp1 markedly reduced disease severity and alleviated colonic histopathological damage. This protection was accompanied by improved mucus production and recovery of several mucosal barrier-related markers. Mechanistically, transcriptomic profiling further showed reduced inflammatory signaling and enrichment of macrophage differentiation, phagocytosis, apoptotic-cell clearance, and efferocytosis programs. Functional experiments confirmed that Drp1-deficient macrophages displayed enhanced efferocytic capacity under inflammatory conditions. In parallel, myeloid Drp1 deletion partially restored DSS-associated microbial dysbiosis. Together, these findings suggest that Drp1 influences colitis through coordinated effects on myeloid-cell function, mucosal repair, and intestinal homeostasis. Rather than acting only as a mitochondrial fission factor, Drp1 may therefore shape the inflammatory state of intestinal macrophages.

Our finding of increased macrophage Drp1 in colitis suggests that mitochondrial fission may contribute to macrophage dysfunction within the inflamed mucosa. Recent studies supported a functional link between Drp1 activity and macrophage inflammatory reprogramming. PGAM5-Drp1 signaling promotes inflammatory cytokine production through NF- κ B and MAPK pathways in macrophages (Bang et al. 2023). This pathway also enhances glycolytic and mitochondrial metabolic remodeling during inflammatory activation. In experimental autoimmune myocarditis, increased Drp1 activity was associated with mitochondrial fragmentation and inflammatory macrophage accumulation (Lin et al. 2025). Drp1 depletion reduced inflammatory macrophage activation and attenuated NF- κ B-associated responses in this model. Similar observations were recently reported during apical periodontitis. Inhibition of Drp1-dependent fission suppressed mitochondrial ROS, NLRP3 activation, and inflammatory macrophage responses (Yang et al. 2025). These findings support a role for excessive Drp1 activity in sustaining macrophage-mediated inflammation. However, this relationship appears to depend strongly on the tissue environment. Under ischemic stress, myeloid Drp1 deficiency instead promoted inflammatory metabolic reprogramming and impaired tissue repair (Yadav et al. 2025). These divergent findings highlight the context-dependent nature of Drp1 signaling in macrophages. Our results extend

this concept to intestinal inflammation. They suggest that sustained Drp1 activity favors a pathogenic macrophage state during colitis.

Transcriptomic profiling provided further insight into the macrophage changes induced by macrophage Drp1 deficiency during colitis. GSEA analysis showed enrichment of macrophage differentiation, phagosome, apoptotic-cell clearance, and efferocytosis programs after Drp1 deletion. These changes indicate a broader shift toward a phagocytic and pro-resolving macrophage state. Consistent with this interpretation, Drp1 deficiency increased markers associated with an M2-like phenotype. However, this phenotype likely represents only one component of the broader functional reprogramming. Efferocytosis is central to inflammation resolution because it prevents secondary necrosis and limits the release of intracellular danger signals(Doran et al. 2020; Schilperoort et al. 2023). It also reprograms macrophage metabolism and promotes IL-10 and other pro-resolving mediators(Sukka et al. 2024). In our study, Drp1-deficient macrophages showed greater apoptotic-cell uptake under both basal and IL-4/IL-13-conditioned states. This finding suggests that enhanced uptake is not simply secondary to alternative activation. Interestingly, Wang *et al.* reported that Drp1-mediated mitochondrial fission supports continued clearance of multiple apoptotic cells(Wang et al. 2017). Their study linked acute Drp1 activation to calcium handling and phagosome formation during serial efferocytosis. Our assay instead measured overall apoptotic-cell uptake without separating first and subsequent engulfment events. Drp1 may therefore exert stage-dependent effects during efferocytosis. Sustained Drp1 signaling can also promote mtROS generation, inflammatory signaling, and macrophage metabolic reprogramming(Bang et al. 2023). Limiting this state may increase basal phagocytic competence while preserving a pro-resolving phenotype. The context dependence of Drp1 is further supported by ischemic models, where myeloid Drp1 deficiency impaired reparative macrophage responses(Yadav et al. 2025). Thus, Drp1 is unlikely to act as a uniform regulator of efferocytosis. Its effects may depend on macrophage state, metabolic demand, tissue environment, and the stage of apoptotic-cell uptake.

Beyond its effects on macrophage function and mucosal repair, myeloid Drp1 deficiency also substantially reshaped the gut microbial community during colitis. Alpha diversity remained largely unchanged among the four groups. In contrast, beta-diversity analyses revealed marked differences in microbial community structure after DSS exposure. The microbiota of Drp1-deficient DSS mice shifted toward the corresponding control group. This pattern suggests partial ecological recovery rather than a generalized increase in microbial richness. One plausible mechanism involves restoration of the inflammatory and metabolic environment within the intestinal niche. Intestinal inflammation increases epithelial oxygenation and generates alternative respiratory electron acceptors in the lumen(Caballero-Flores et al. 2023; Schubert and Hardt 2026). These changes favor facultative anaerobes, particularly members of Enterobacteriaceae, over obligate anaerobic commensals. Therefore, the reduced abundance of *Escherichia-Shigella* and *Enterococcus* after Drp1 deletion may reflect diminished expansion of inflammation-associated taxa. Our findings on mucus and epithelial barrier recovery support this interpretation. Enhanced macrophage efferocytosis may further contribute by promoting inflammation resolution and mucosal repair(Wu et al. 2023). Efferocytic macrophages release pro-resolving mediators that support epithelial and stromal healing during experimental colitis(Martin-

Rodriguez et al. 2021). This repaired mucosal environment may restore ecological niches required by mucus-associated commensal bacteria. The recovery of *Akkermansia* and Muribaculaceae is particularly consistent with this possibility. *Akkermansia muciniphila* can strengthen epithelial barrier function and increase short-chain fatty acid production in DSS colitis(Zhang et al. 2024). Recent evidence also links *A. muciniphila* directly to reduced inflammatory macrophage activation through the HDAC5–DAB2 pathway(Miao et al. 2024). Muribaculaceae are prominent murine commensals that utilize complex carbohydrates and mucin-derived glycans and contribute to short-chain fatty acid production(Zhu et al. 2024). Their abundance is frequently reduced after disruption of the mucus barrier in experimental colitis. Thus, their recovery may represent restoration of mucus-associated microbial metabolism rather than direct bacterial selection by Drp1 deficiency. Collectively, these findings suggest a bidirectional interaction between myeloid inflammation and the intestinal microbiota. Drp1 deletion may initially improve the inflammatory and epithelial niche, thereby favoring recovery of commensal communities. These microbial changes could subsequently reinforce barrier integrity and pro-resolving immune responses. However, our 16S data establish association rather than microbiota-mediated causality. Fecal microbiota transplantation, cohousing, or microbiota depletion will be required to determine whether these microbial changes contribute directly to protection.

Taken together, our findings identify myeloid Drp1 as an important regulator linking macrophage mitochondrial dynamics to acute intestinal inflammation and mucosal injury during DSS-induced colitis. Beyond its canonical role in mitochondrial fission, Drp1 appears to influence a broader macrophage functional program involving inflammatory activation, apoptotic-cell clearance, and interactions with the intestinal microbial environment. These findings support a cell-specific role for Drp1 in shaping innate immune responses during colitis. Several limitations should be acknowledged. The acute DSS model mainly captures the early phase of apoptotic-cell clearance and is less suitable for evaluating later inflammation resolution and tissue repair. Moreover, enhanced efferocytosis and microbial restoration were associated with protection, but their causal contribution remains to be established.

Abbreviations

IBD
Inflammatory bowel disease
DNM1L
Dynamin-1-like protein gene
Drp1
Dynamin-related protein 1
DSS
Dextran sulfate sodium
WT
Wild-type
cKO
Conditional knockout

DAI
Disease Activity Index
H&E
Hematoxylin and eosin
AB-PAS
Alcian blue-periodic acid-Schiff
BMDMs
Bone marrow-derived macrophages
M-CSF
Macrophage colony-stimulating factor
THP-1
Human monocytic leukemia cell line
scRNA-Seq
Single-cell RNA sequencing
PCA
Principal component analysis
DAPI
4',6-diamidino-2-phenylindole

Declarations

Authors' contributions

C. H., Y. L., and Y.L.Z. conceived and designed the study and prepared the initial manuscript. C. H., Y. L., and Y. Z. conducted the animal experiments and were responsible for statistical analysis and data interpretation. X. G., Y. Z., and L.L.X. assisted with the animal experiments and monitored the general condition of the experimental animals. L.T.Z., Z. Y., and Y.C.Z. collected cell samples and performed the related assays. Y. L. collected the clinical samples. Y.J.Z. and Y.L.Z. contributed to data interpretation and critically revised the manuscript. All authors read and approved the final manuscript.

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Ethical approval and participant consent

The human research component (Grant No.: S-2022-060) received approval from the Ethics Committee of the Second Affiliated Hospital of South China University of Technology (Guangzhou First People's Hospital). Because of the retrospective nature of this study, the requirement for informed consent was waived. All animal procedures were authorized by the Animal Ethics Committee of the Second Affiliated Hospital of South China University of Technology (IACUC of Daoke Pharmaceutical Technology (Guangdong) Co., Ltd., approval No. IACUC-DK-2022-08-01-01) and carried out in strict compliance with the ARRIVE guidelines.

Data availability

The RNA-seq and 16S rRNA gene sequencing data generated and analyzed in this study have been deposited in the NCBI BioProject database and are available under accession number PRJNA1538226.

Competing interests

The authors declare no competing interests.

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Figures

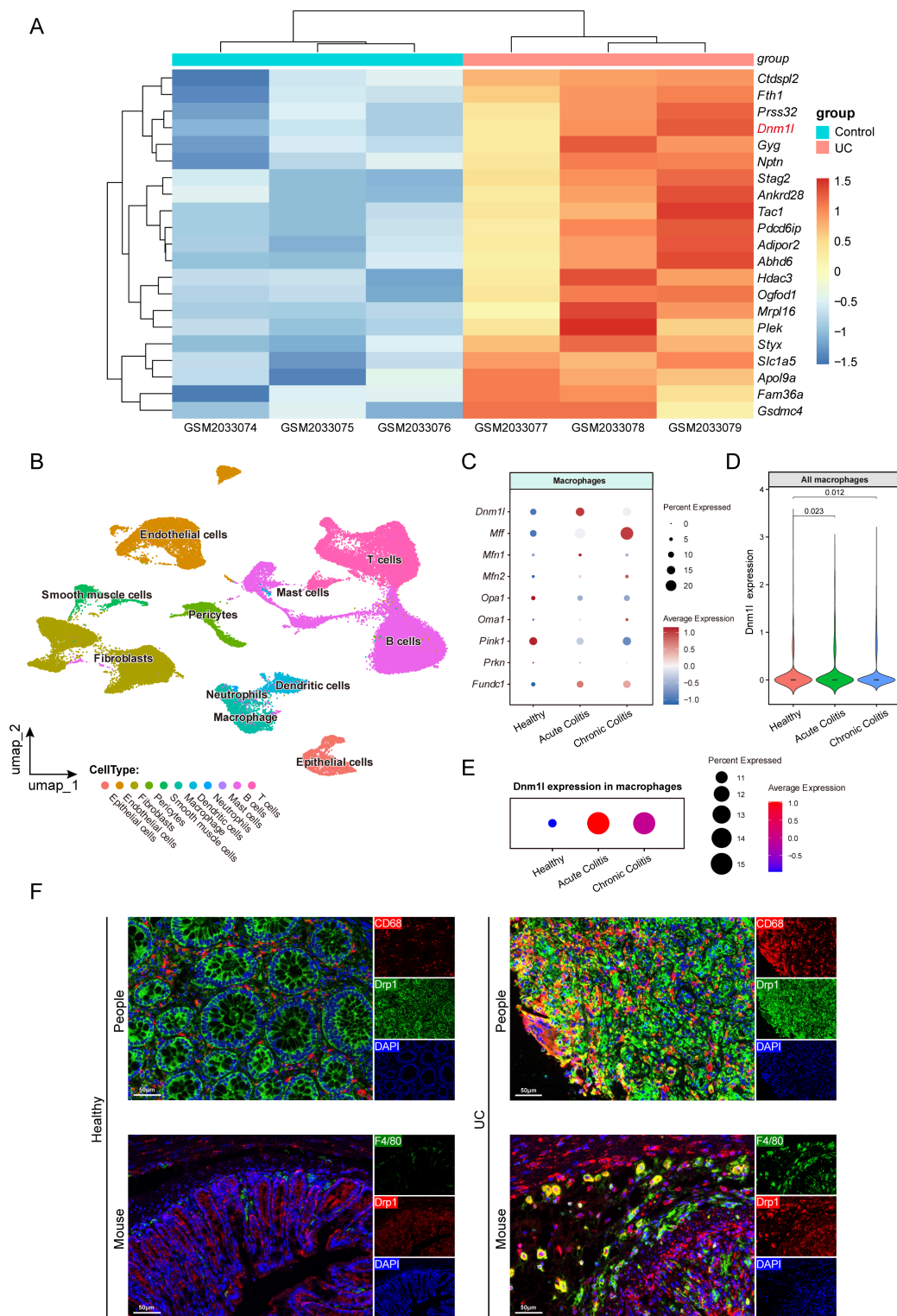


Figure 1

Drp1 expression is increased in intestinal macrophages in UC and experimental colitis. (A) Heatmap of differentially expressed genes in colonic tissues of colitic (n = 3) and healthy control mice (n = 3). (B) UMAP plot showing intestinal cell populations identified in the single-cell RNA-seq dataset GSE264408. (C) Dot plot showing the expression patterns of mitochondrial dynamics-related genes in macrophages from Healthy, Acute Colitis, and Chronic Colitis groups. (D) Violin plot showing the distribution of Dnm1l

expression in macrophages across the three groups. Statistical comparisons are indicated above the plots. (n = 3 for health, n = 3 for acute colitis, and n = 4 for chronic colitis). (E) Dot plot showing Dnm1l expression in macrophages across the three groups. (F) Representative immunofluorescence images showing colocalization of Drp1 with CD68 in human colonic tissues (left) and colocalization of Drp1 with F4/80 in DSS-treated mouse colonic tissues (right). Nuclei were counterstained with DAPI. Scale bars= 50µm. Between-group differences were analyzed using the two-tailed Wilcoxon rank-sum test.

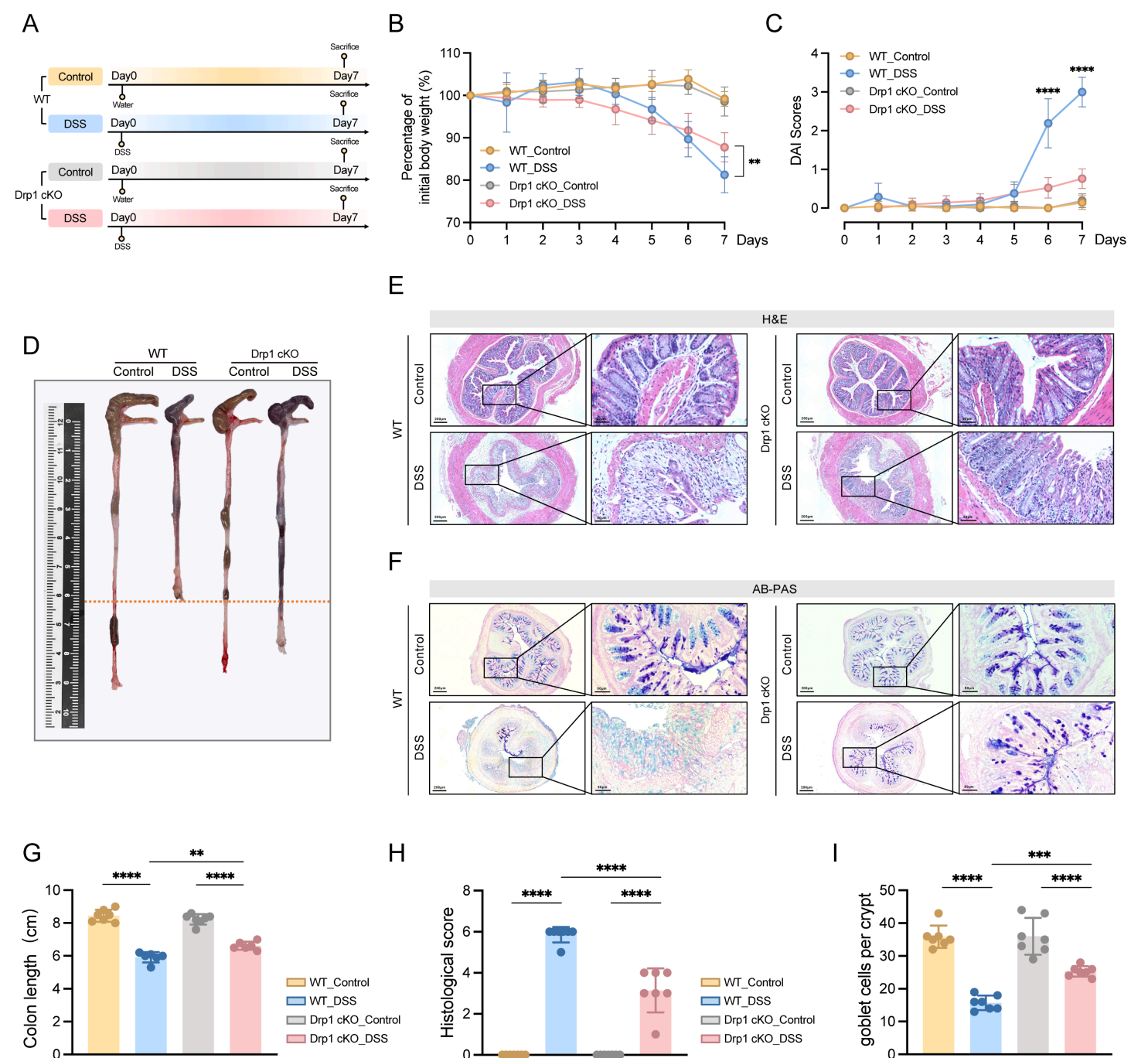


Figure 2

Myeloid-specific Drp1 deficiency attenuates DSS-induced colitis. (A) Schematic diagram of the experimental protocol for DSS-induced colitis in control and myeloid-specific Drp1-deficient mice (n=7 per group). (B) Daily body-weight changes during the 7-day experimental period. (C) Disease activity index (DAI) scores. (D) Representative photographs of colon tissues. (E) Representative hematoxylin and eosin (H&E) staining of colon sections. (F) Alcian blue-periodic acid-Schiff (AB-PAS) staining of colon sections. (G) Quantitative analysis of colon length. (H) Histopathological scores of colonic injury. (I) Quantification of goblet-cell numbers in the colon. Scale bars, 200 μ m and 50 μ m for left- and right-magnification images, respectively. Data are presented as mean $\pm$ SD. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, non-significance.

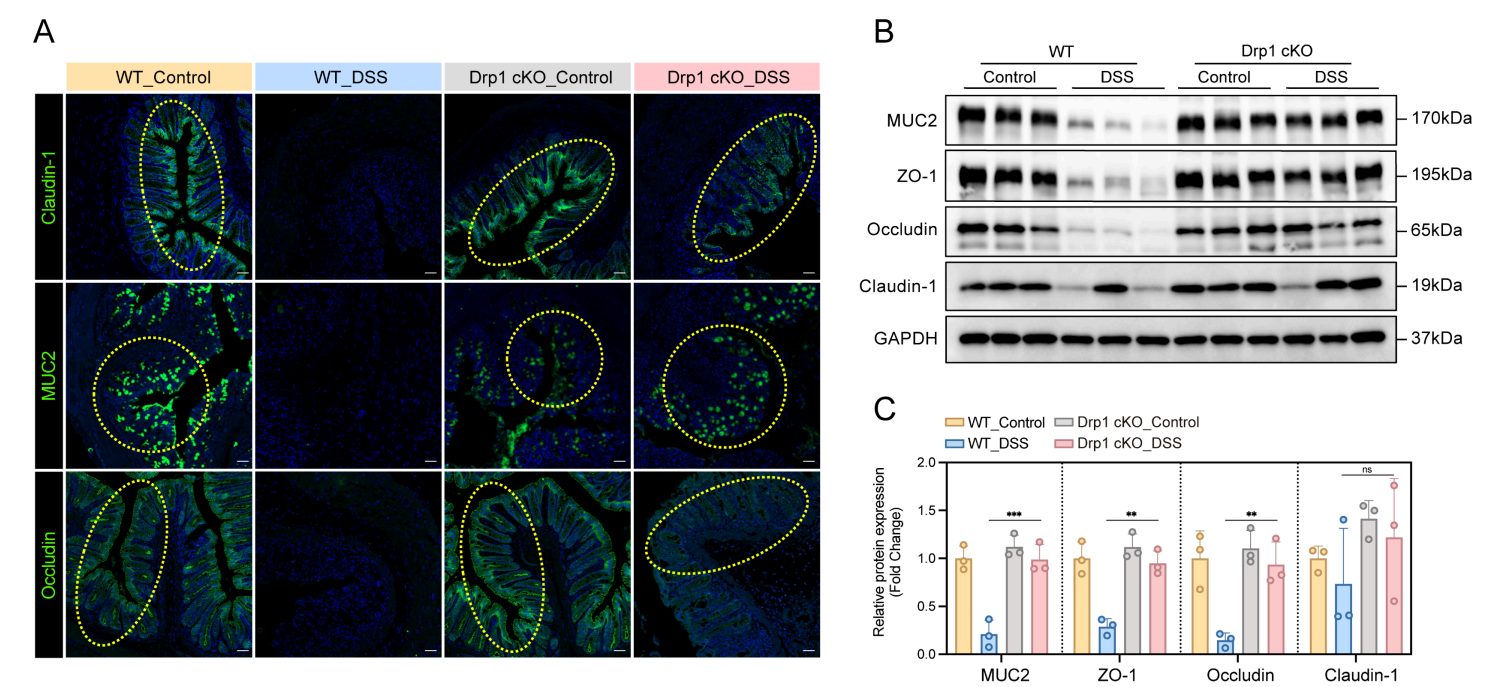


Figure 3

Conditional Drp1 deletion preserves intestinal barrier integrity during DSS-induced colitis. (A) Representative Immunofluorescence images of Claudin-1, MUC2 and Occludin in colonic tissues from the indicated groups. Nuclei were counterstained with DAPI. Scale bars=20 μ m. (B) Western blot analysis of MUC2, ZO-1, Occludin and Claudin-1. GAPDH was used as the loading control. (C) Densitometric quantification of MUC2, ZO-1, Occludin and Claudin-1 protein expression levels shown in (B), normalized to GAPDH and expressed relative to the WT_Control group. Data are presented as mean $\pm$ SD (n = 3 per group). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, non-significance.

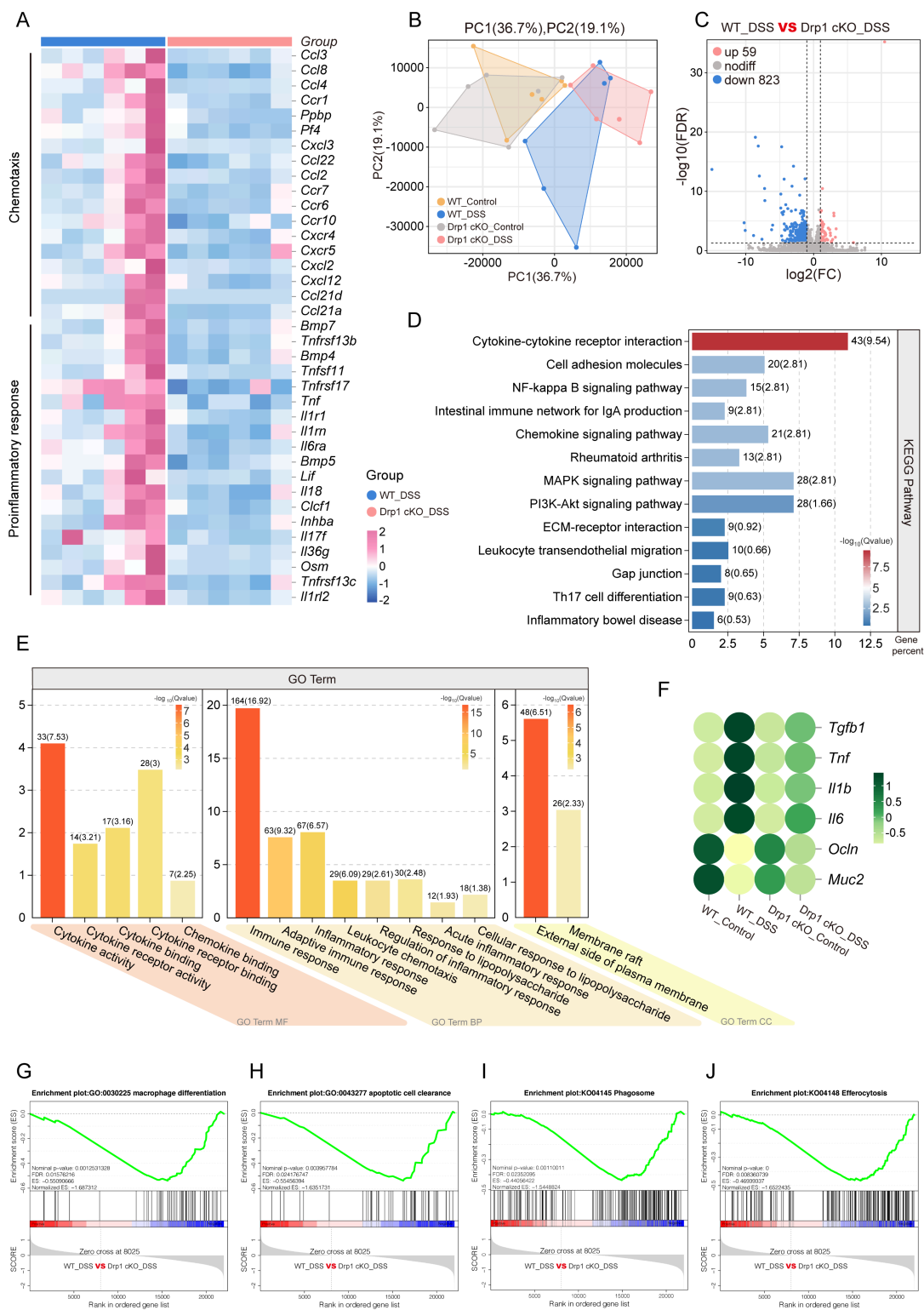


Figure 4

Transcriptomic profiling reveals altered inflammatory and macrophage-associated programs following conditional Drp1 deletion. (A) Heatmap showing expression of chemokine- and pro-inflammatory-response-related genes in colonic tissues from WT_DSS and Drp1 cKO_DSS mice. (B) Principal-component analysis (PCA) of colonic transcriptomes from the indicated groups. (C) Volcano plot of differentially expressed genes between colonic tissues of WT_DSS and Drp1 cKO_DSS mice. Pink

and blue dots indicate significantly upregulated and downregulated genes, respectively. (D) KEGG pathway enrichment analysis of differentially expressed genes between the WT_DSS and Drp1 cKO_DSS groups. (E) GO functional enrichment results associated with inflammation and macrophage polarization across Molecular Function, Biological Process and Cellular Component categories for differentially expressed genes between the WT_DSS and Drp1 cKO_DSS groups. (F) Dot plot showing the expression patterns of representative inflammatory genes (Tgfb1, Tnf, Il1b, and Il6) and intestinal barrier-related genes (Ocln and Muc2) across the four experimental groups. (G-J) GSEA enrichment plots demonstrating significantly up-regulated enrichment of gene sets related to macrophage differentiation (G), apoptotic-cell clearance (H), phagocytosis (I) and efferocytosis (J) in the Drp1 cKO_DSS group compared with the WT_DSS group.

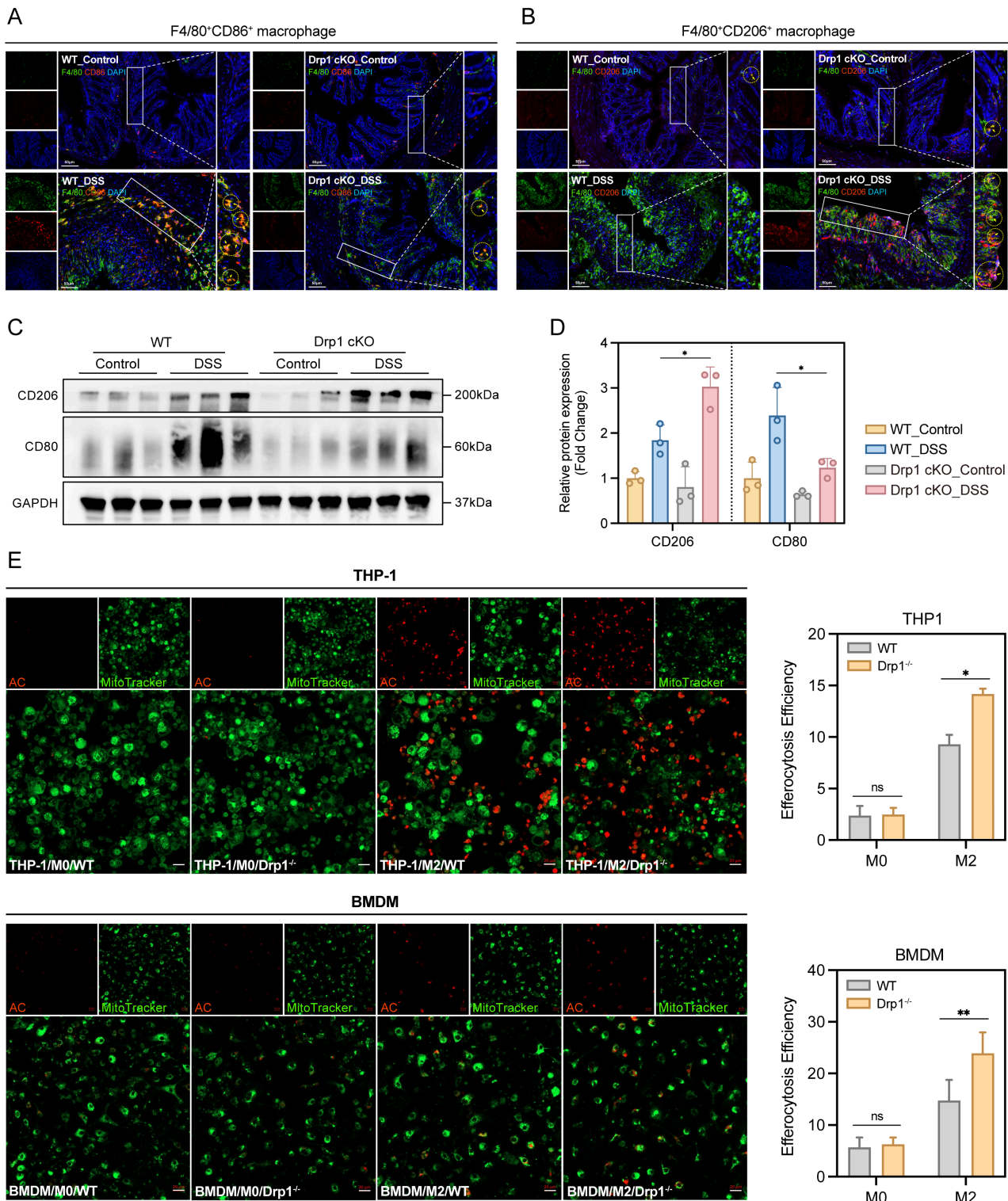


Figure 5

Conditional Drp1 deletion promotes an M2-like macrophage phenotype and enhances efferocytosis. (A) Representative immunofluorescence images showing co-staining and co-localization analysis of F4/80 and CD86 in colonic tissues from the indicated groups. Scale bar= 50 μ m. (B) Representative immunofluorescence images showing co-staining and co-localization analysis of F4/80 and CD206 in colonic tissues. Scale bar= 50 μ m. (C) Western blot analysis of CD206 and CD80 expression in colonic

tissues from WT and Drp1 cKO mice with or without DSS treatment. (D) Densitometric quantification of CD206 and CD80 protein expression levels shown in (C), normalized to GAPDH. (E) Representative confocal images showing apoptotic cell uptake by THP-1-derived macrophages and BMDMs. Apoptotic cells are shown in red, and macrophages were labeled with MitoTracker (green). Right panels: quantification of efferocytosis (percentage of MitoTracker⁺ macrophages containing apoptotic cells) from confocal images. Scale bar= 20 μ m. Data are presented as mean $\pm$ SD. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test (D) and the two-tailed Student's *t*-test (E). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, non-significance.

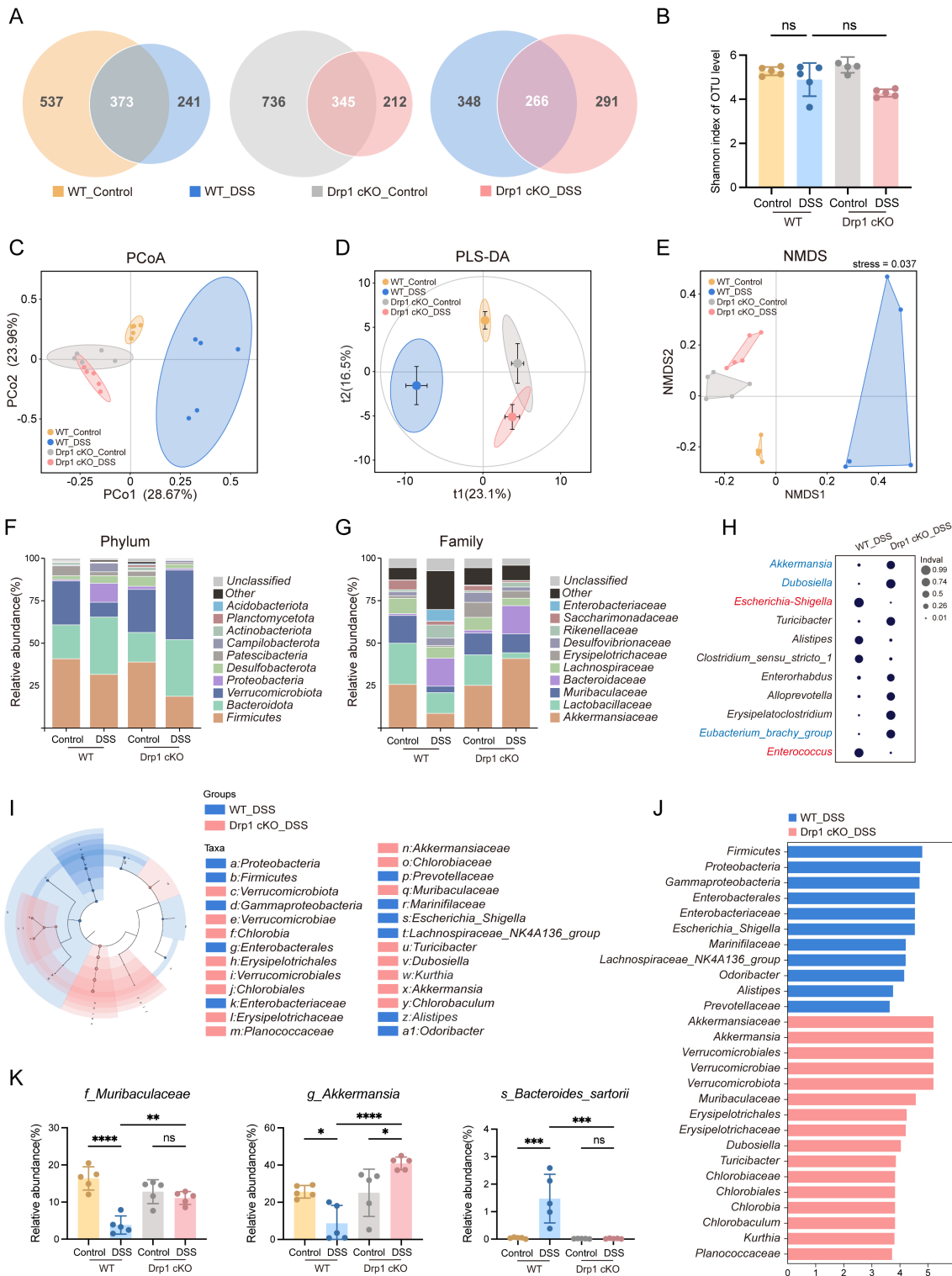


Figure 6

Conditional Drp1 deletion partially restores DSS-associated gut microbial dysbiosis. (A) Venn diagram illustrating shared and unique gut microbiota OTUs between the indicated experimental groups. (B) Alpha diversity analysis based on the Shannon index. (C) Principal coordinate analysis (PCoA). (D) Partial least-squares discriminant analysis (PLS-DA). (E) Non-metric multidimensional scaling (NMDS). (F and G) Stacked-bar plot showing relative abundance of taxa at the phylum and family level. (H)

Indicator-species analysis identifying bacterial genera with significant inter-group differences. (I-J) LEfSe (Linear Discriminant Analysis Effect Size) analysis identifying bacterial genera with significant inter-group differences. (K) Relative abundance of target bacteria at family, genus, and species taxonomic levels (n=5). Data are presented as mean $\pm$ SD. Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, non-significance.

Supplementary Files

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