Butyrate ameliorates acute-on-chronic liver failure by regulating macrophage polarization

Jing Xiao[1#], Hang You[1#], Zongyi Liu[1], Yong Sun[1], Yu Zhang[1], Li Wei[1], Ning Ling[1], Hong Ren[1], Shiying Li[1*], Peng Hu[1*], Mingli Peng[1*]

[1]Department of Infectious Diseases, Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, the Second Affiliated Hospital, Chongqing Medical University



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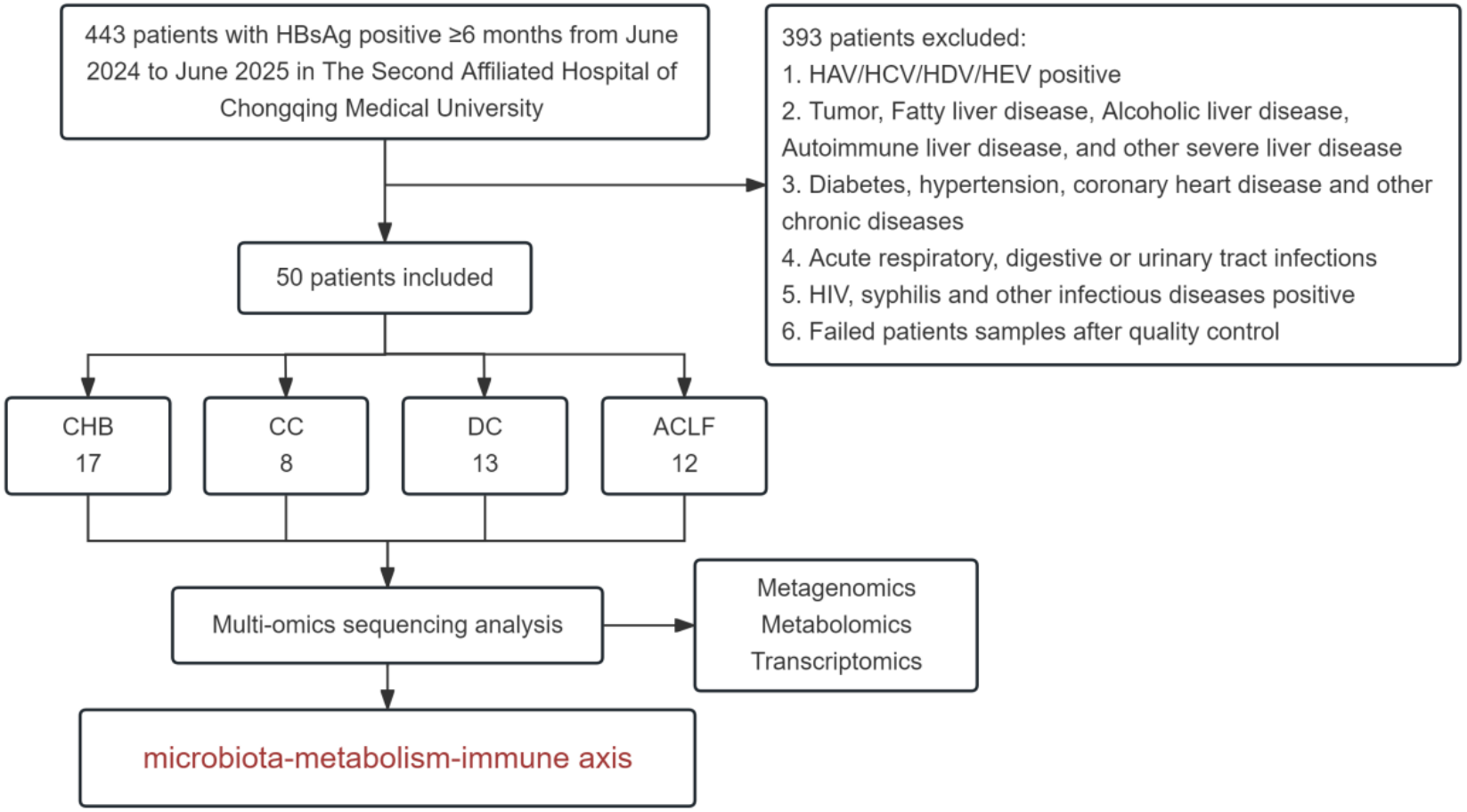
Background

- Acute-on-chronic liver failure (ACLF) refers to a syndrome of rapid deterioration of liver function in patients with relatively stable chronic liver diseases under the influence of various acute injury factors. It is a high-mortality and high-intensity systemic inflammatory response accompanied by organ/system failure, with a medium- and short-term mortality rate ranging from 23% to 77%. Treatment methods include targeting intestinal bacterial translocation and microbiome, liver inflammation, circulating immune cells, cell death and regeneration, and extracorporeal liver support, etc.
- The composition or metabolites of the intestinal microbiota interact with the liver through various mechanisms: the translocation of bacteria or their metabolites into the portal vein circulation increases; the circulating immune cells encountered by the translocated bacteria; the liver cells interacted with the bacteria after reaching the liver; the short-chain fatty acids (SCFAs) produced by the liver, such as acetate, propionate, and butyrate, or signaling molecules.
- SCFAs, mainly including acetic acid, propionic acid, and butyric acid, are mainly absorbed in the liver and periphery, while butyrate is mainly absorbed by the intestinal epithelium. SCFAs can regulate the physiological and biochemical functions of the host, including maintaining the natural intestinal barrier function at the colonic epithelium and mucosal level, intestinal motility, secretion of intestinal hormones, chromatin regulation, intestinal-liver axis, immune function, etc.

Objectives

- This study aims to clarify the regulatory relationship of microbiota - metabolism - immune in ACLF, specifically to clarify the role of SCFAs (butyric acid) in the occurrence and development of the disease, and to explore its possible mechanism of improving liver injury by regulating macrophage polarization and MAPK signaling, providing theoretical basis for the diagnosis and treatment of ACLF.

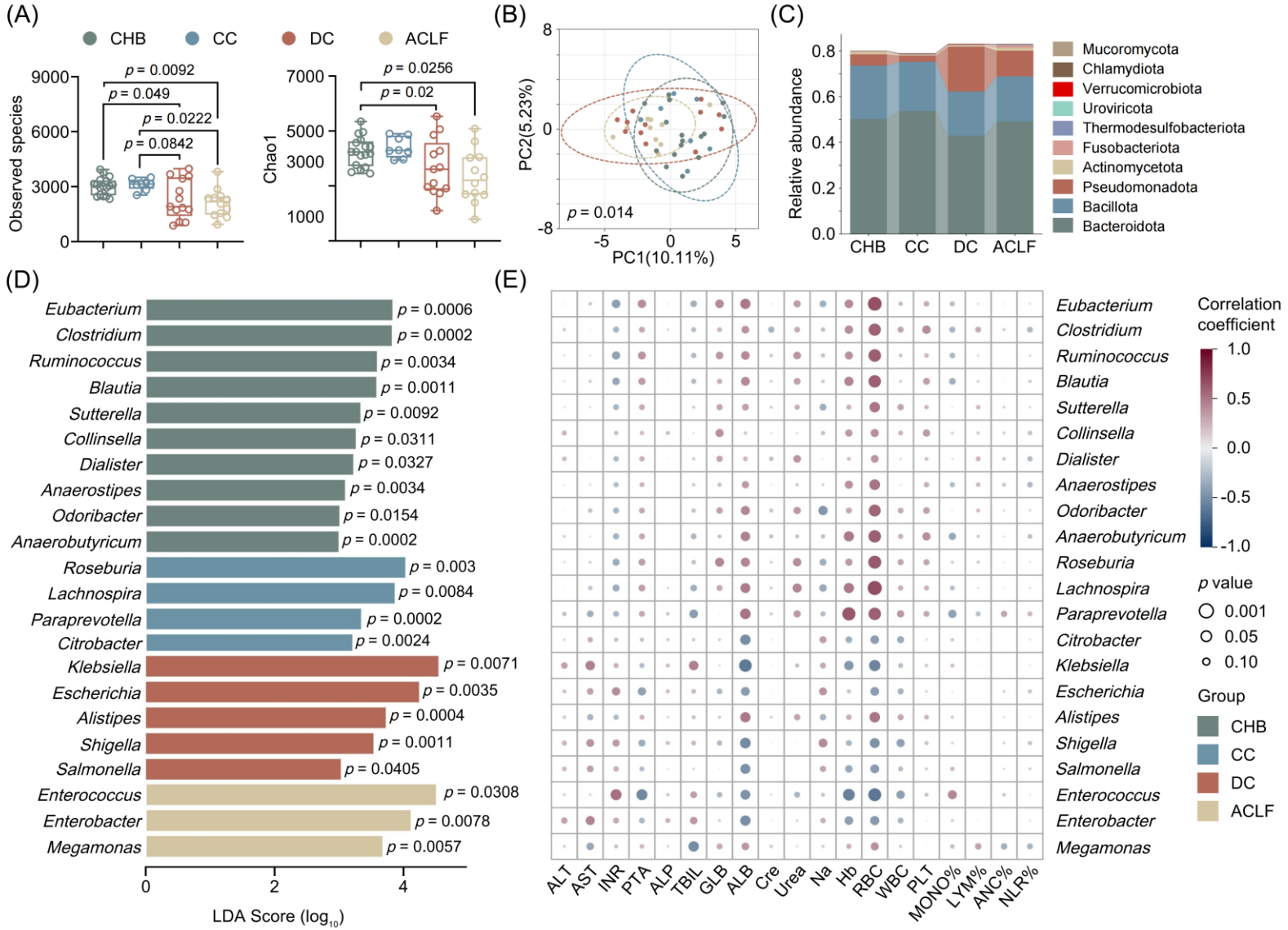
Study Design



A total of 50 patients were included in this study for metagenomics, metabolomics and transcriptomics sequencing.

Among them, 17 were patients with chronic hepatitis B, 8 were patients with compensated liver cirrhosis, 13 were patients with decompensated liver cirrhosis, and 12 were patients with acute-on-chronic liver failure.

1. Depletion of butyrate-producing bacteria in feces of HBV-ACLF patients

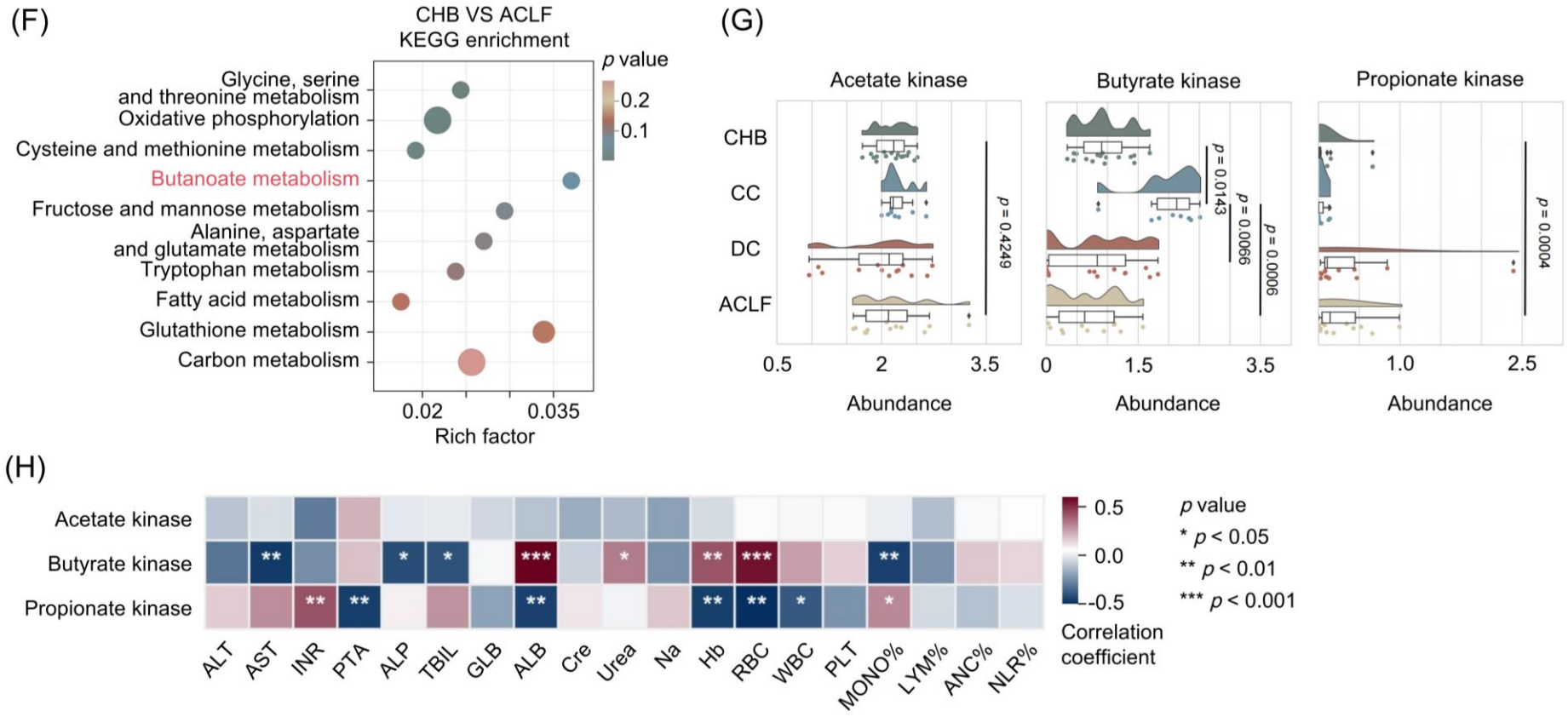


- There were significant differences in α diversity and β diversity among the four groups of patients.
- Each patient group exhibited a unique structure of the intestinal microbiota, but the direct correlation with clinical characteristics was limited.

Figure 1 Associations of the gut microbiota composition and functionality with ACLF.

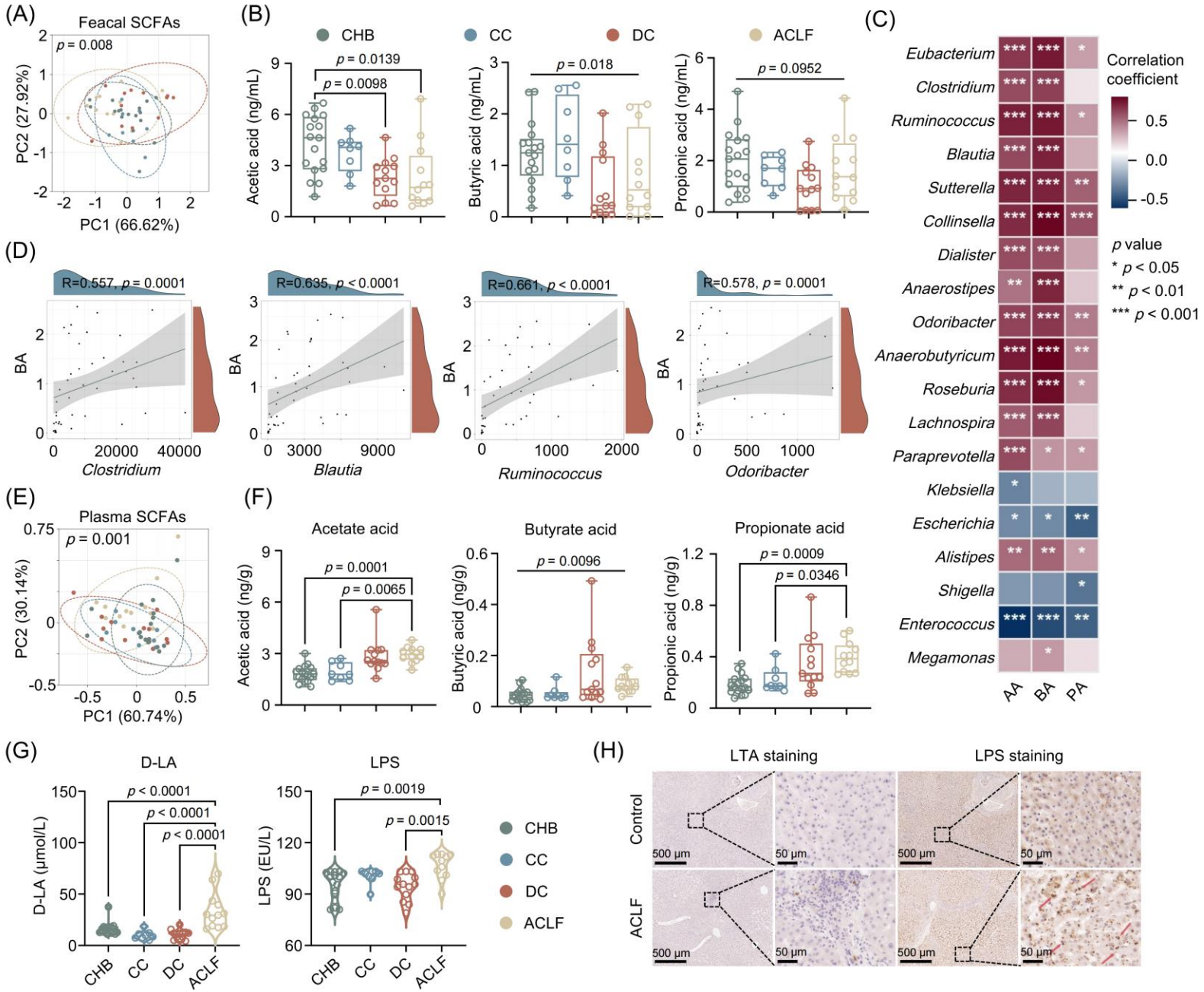
1. Depletion of butyrate-producing bacteria in feces of HBV-ACLF patients

Figure 1 Associations of the gut microbiota composition and functionality with ACLF.



- The KEGG analysis revealed that the butyrate metabolism pathway was significantly enriched.
- The butyrate kinase in ACLF patients was significantly reduced, and its level was negatively correlated with liver injury indicators and the percentage of peripheral blood monocytes.
- Butyrate metabolism may play a potential regulatory role in the progression of ACLF.

2. Butyrate metabolic dysregulation and intestinal barrier impairment in HBV-ACLF patients



- The fecal SCFA levels of patients with ACLF were decreased.
- The changes in SCFA levels were closely related to the microbial abundance.
- The contradictory results between fecal and plasma SCFA levels may be due to the impaired intestinal barrier function.
- The results of serum D-LA and LPS levels, as well as liver LTA and LPS, confirmed that bacterial translocation exists in ACLF patients.

Figure 2 SCFAs metabolic dysregulation in ACLF patients.

3. Immune function alterations in HBV-ACLF patients

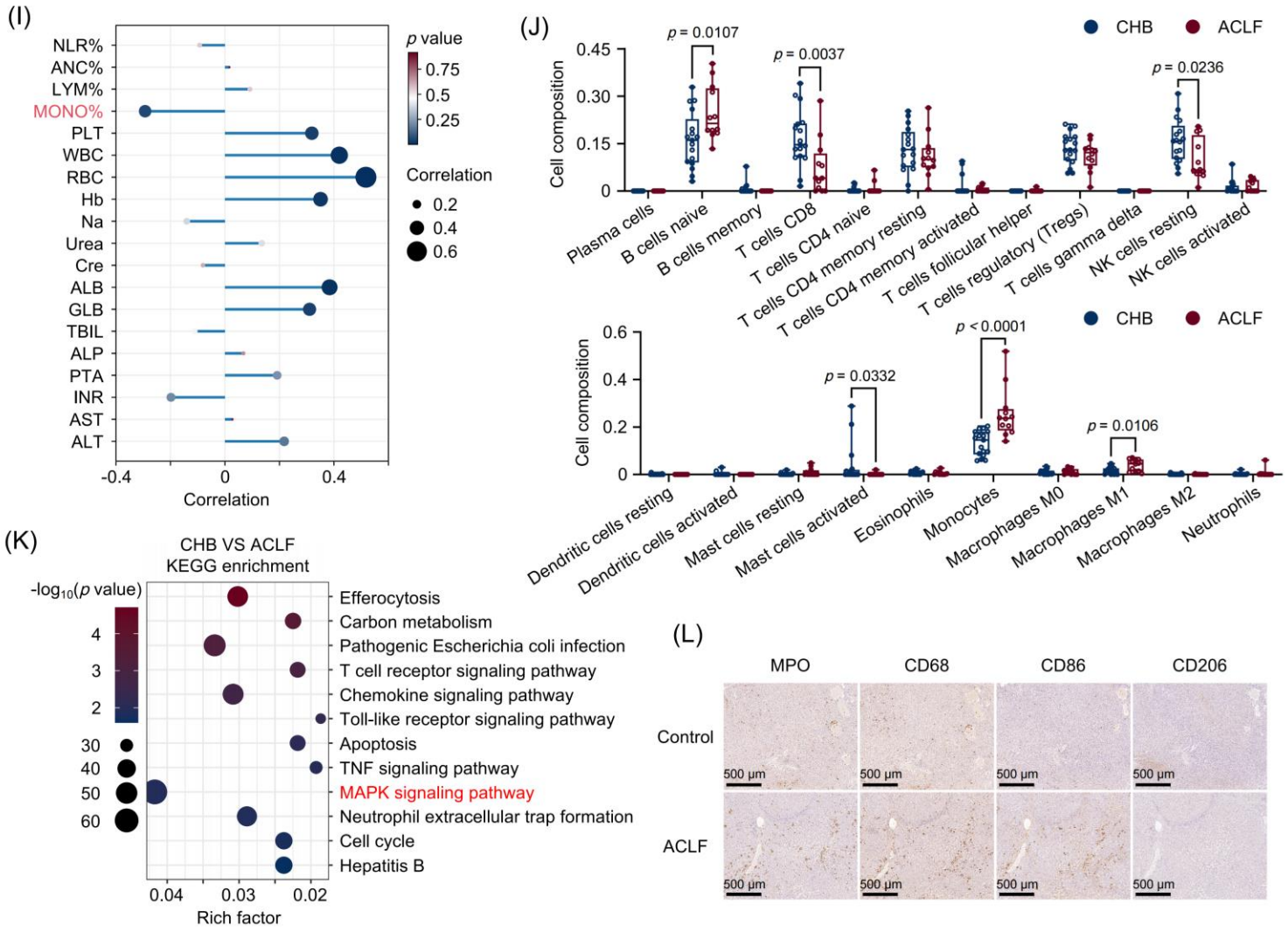
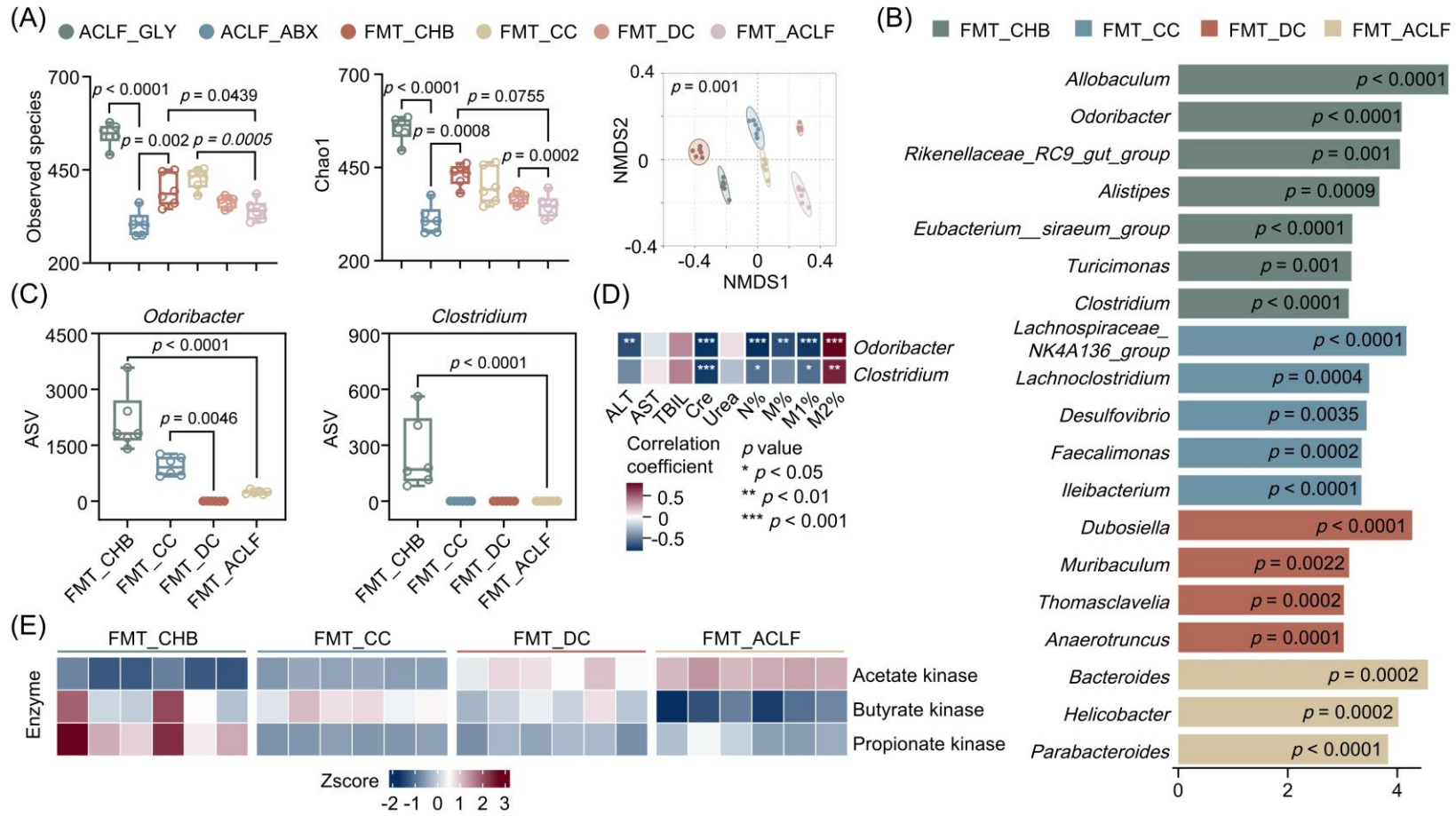


Figure 2 Immune dysfunction in ACLF patients.

- The level of BA was negatively correlated with the percentage of circulating monocytes.
- The transcriptome analysis results showed that patients with ACLF had adaptive immune suppression, but the innate immunity was activated (monocytes and M1 type macrophages) were enhanced.
- KEGG analysis revealed that the TNF, MAPK and NET pathways were significantly enriched.
- The inflammatory state of liver tissue in ACLF patients was obvious.
- In summary, the absence of BA-producing microbiota and the impaired intestinal barrier function jointly drived the systemic inflammatory response and liver immune infiltration in ACLF patients.

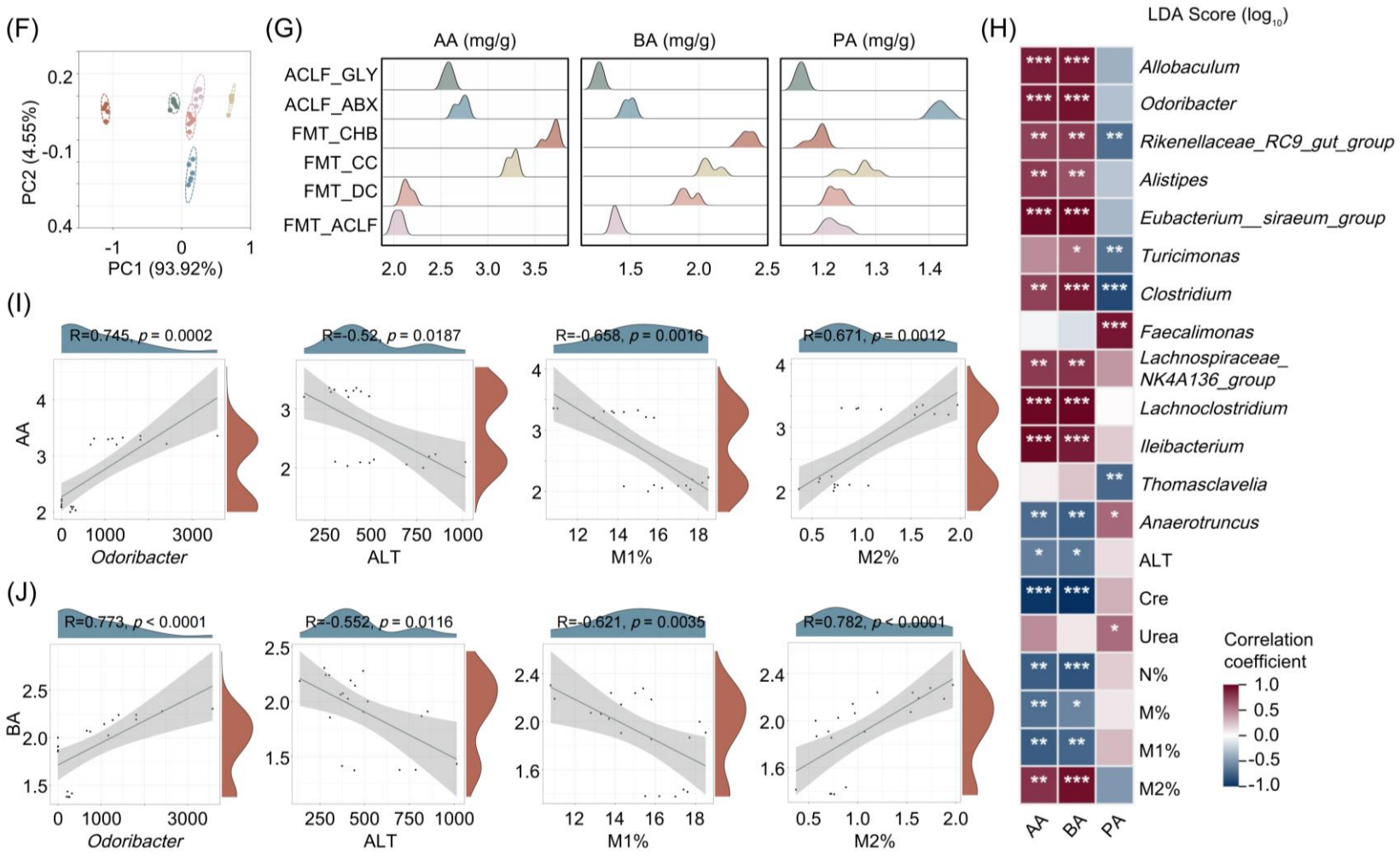
4. FMT recapitulates the microbiota and metabolic signatures observed in patients

Figure 3 The characteristics of microbiome in the cecum of FMT mice.



FMT reversed the decline in α diversity caused by antibiotics. The FMT_ACLF group still lacked key beneficial bacterial genera, such as the butyrate-producing bacteria *Odoribacter* and *Clostridium*. Functional prediction analysis showed that butyrate kinase was downregulated in the mice of the FMT_ACLF group.

5. FMT recapitulates the microbiota and metabolic signatures observed in patients



- Compared with the FMT_CHB group, the levels of AA and BA in the cecum of mice in the FMT_ACLF group were significantly lower, which was consistent with the results of patients.

- Both AA and BA were significantly positively correlated with the abundance of *Odoribacter* and M2-type macrophages in the liver, while they were significantly negatively correlated with ALT levels and M1-type macrophages in the liver.
- The dysbiosis associated with ACLF may promote liver inflammation and M1-type macrophage polarization by reducing the accumulation of BA.

Figure 3 The characteristics of SCFAs in the cecum of FMT mice.

6. Butyrate supplementation attenuates ACLF via the gut-liver axis

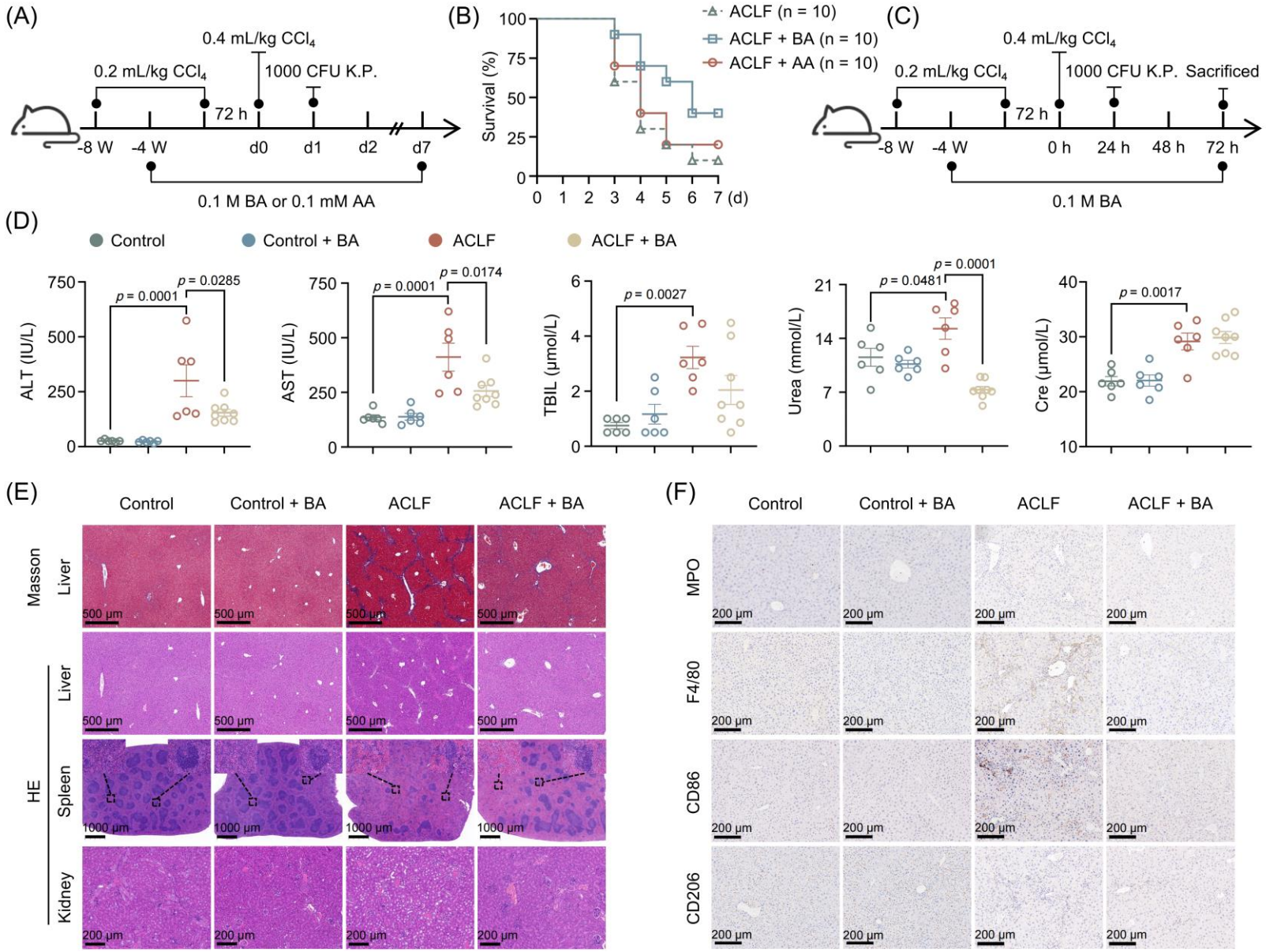
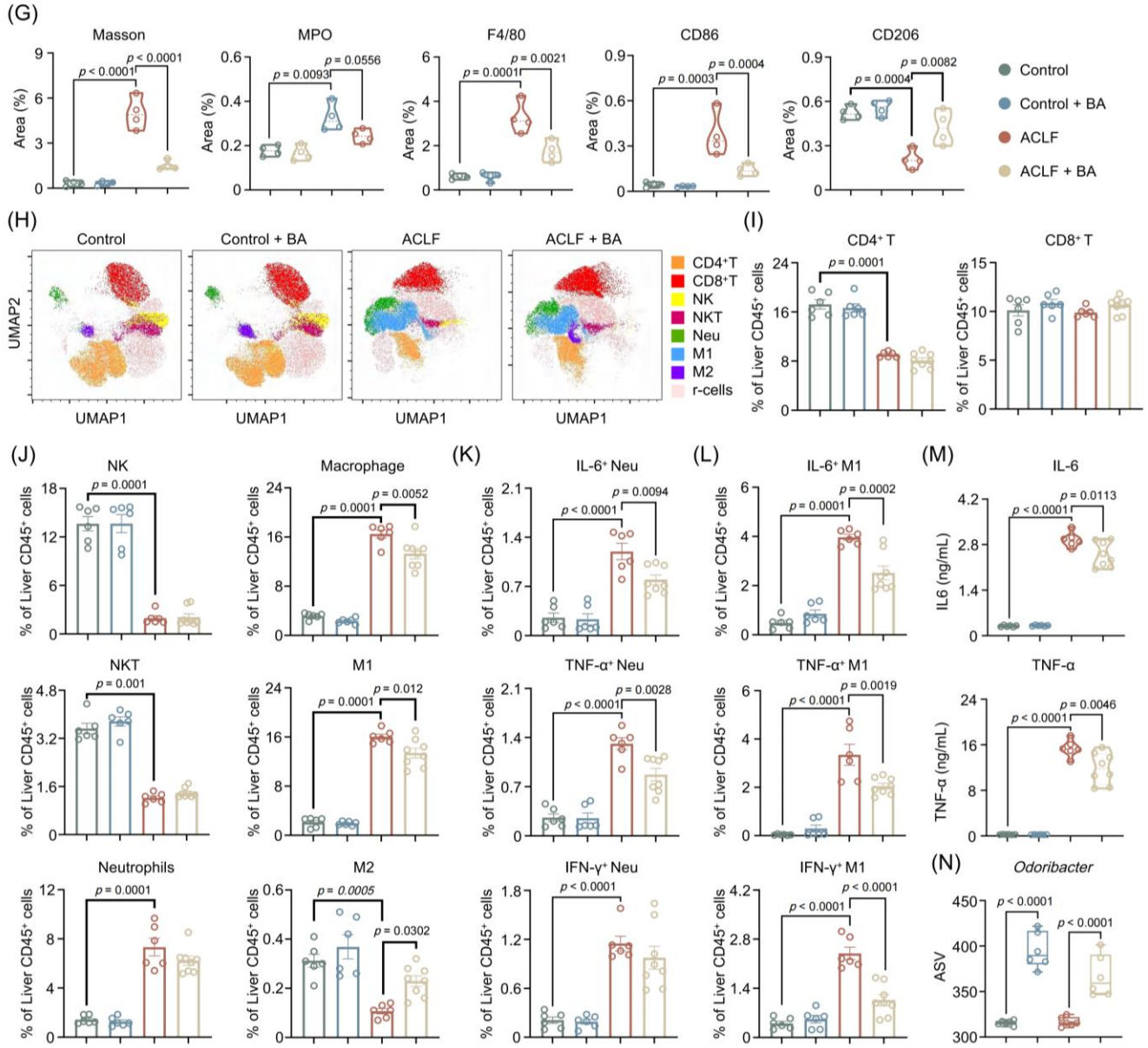


Figure 4 Butyrate alleviates the survival rate and hepatic inflammation in ACLF mice.

BA was administered to ACLF mice.

- Long-term treatment with BA significantly reduced the mortality rate of ACLF mice, alleviated liver damage, fibrosis and inflammatory infiltration.
- Mice treated with BA exhibited a significant reduction in neutrophil and macrophage infiltration in liver and spleen tissues.

6. Butyrate supplementation attenuates ACLF via the gut-liver axis

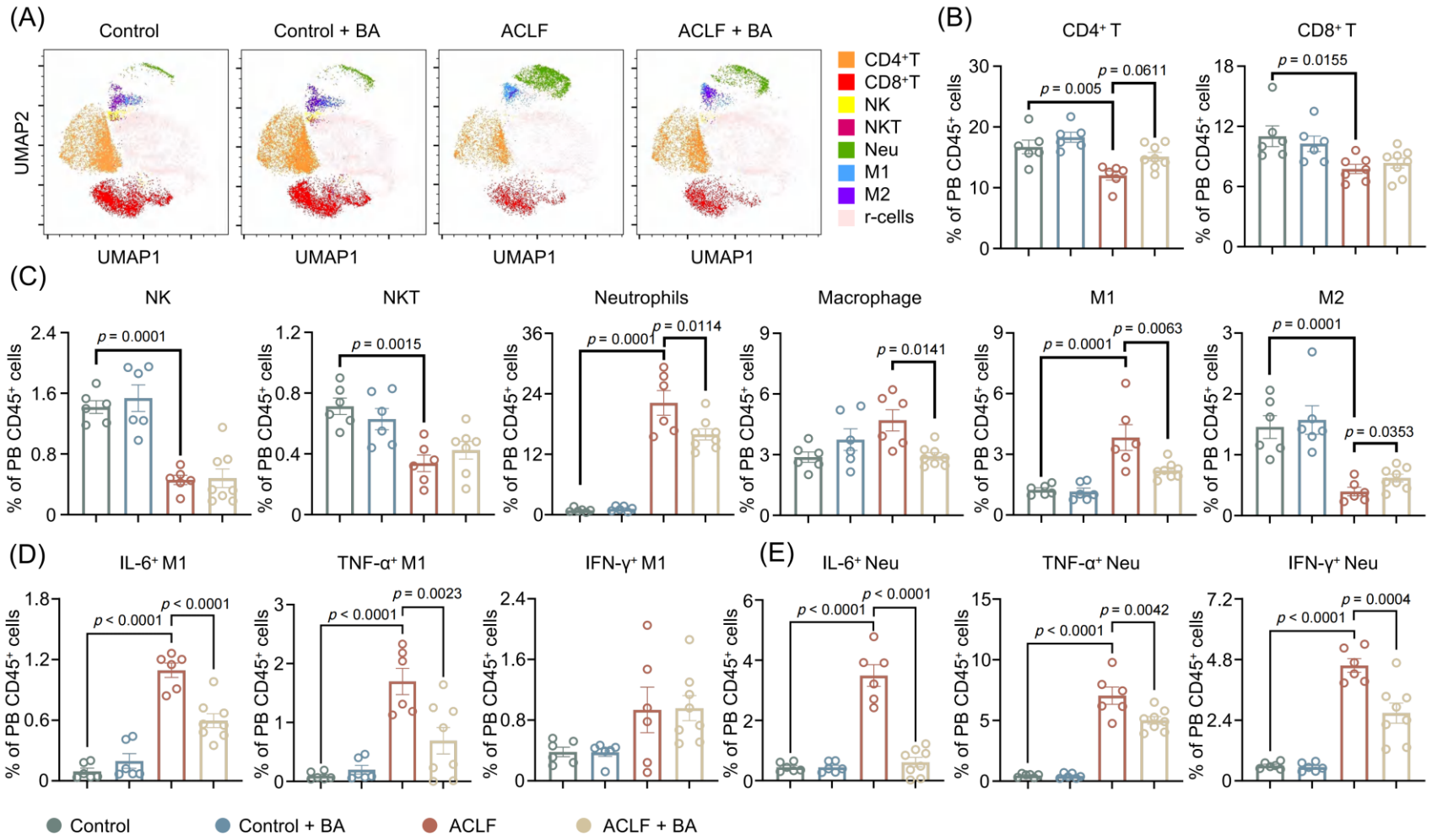


- Flow cytometry analysis showed that BA promoted the transformation of liver macrophages from the M1 type ($CD86^+$) to the M2 type ($CD206^+$).
- The levels of T cells, NK cells and NKT cells in the livers of ACLF mice were significantly reduced, which was consistent with the research results of ACLF patients.
- BA also inhibited the secretion of inflammatory cytokines IL-6 and TNF-α by pro-inflammatory cells.
- ELISA results showed that the levels of IL-6 and TNF-α in the liver decreased after BA treatment.
- BA treatment promoted the re-colonization of beneficial SCFAs-producing bacteria *Odoribacter*, establishing a positive feedback loop between metabolites and the microbial community.

Figure 4 Butyrate alleviates the survival rate and hepatic inflammation in ACLF mice.

6. Butyrate supplementation attenuates ACLF via the gut-liver axis

Figure 5 Butyrate improves peripheral inflammation in ACLF mice.



Apart from the liver, BA treatment also produced systemic effects:

- It reversed the increase in peripheral blood neutrophils and M1-type macrophages induced by ACLF, as well as the secretion of IL-6 and TNF-α, thus inhibited the progression of inflammation.

7. Butyrate supplementation restores gut barrier integrity in ACLF mice

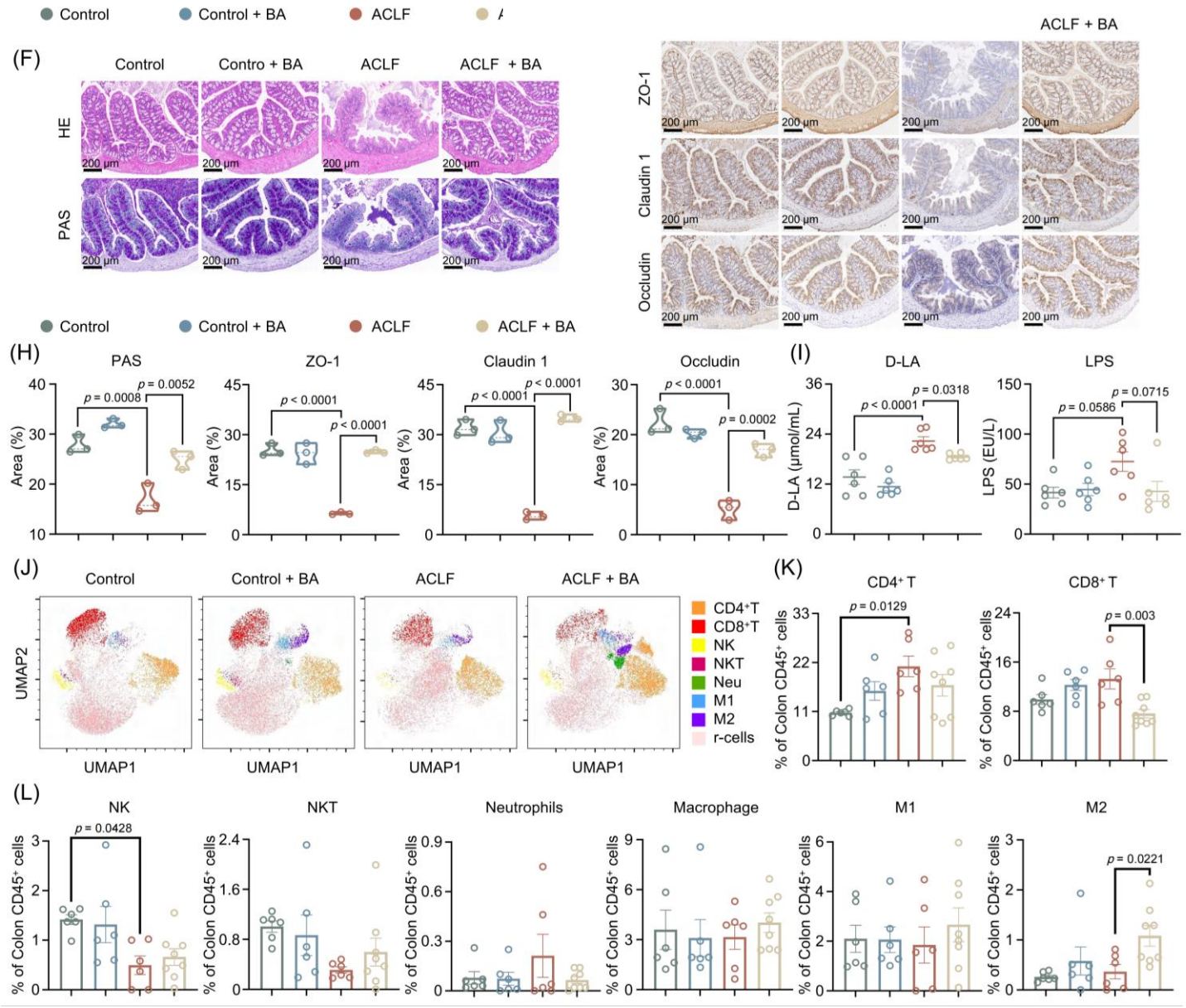


Figure 5 Butyrate improves intestinal barrier function in ACLF mice.

- BA restoreed the intestinal barrier damage caused by ACLF, and the expressions of colonic mucin and tight junction proteins (ZO-1, Claudin-1 and Occludin) recovered, while the levels of plasma D-LA and LPS decreased.
- BA increased the proportion of M2-type macrophages.
- The mechanism by which BA improved ACLF depended on the process of chronic remodeling of the gut-liver axis, that is, strengthening the intestinal barrier to prevent bacterial translocation and inhibiting the systemic inflammatory response.

8. Butyrate suppresses macrophage M1 polarization by inhibiting MAPK signaling

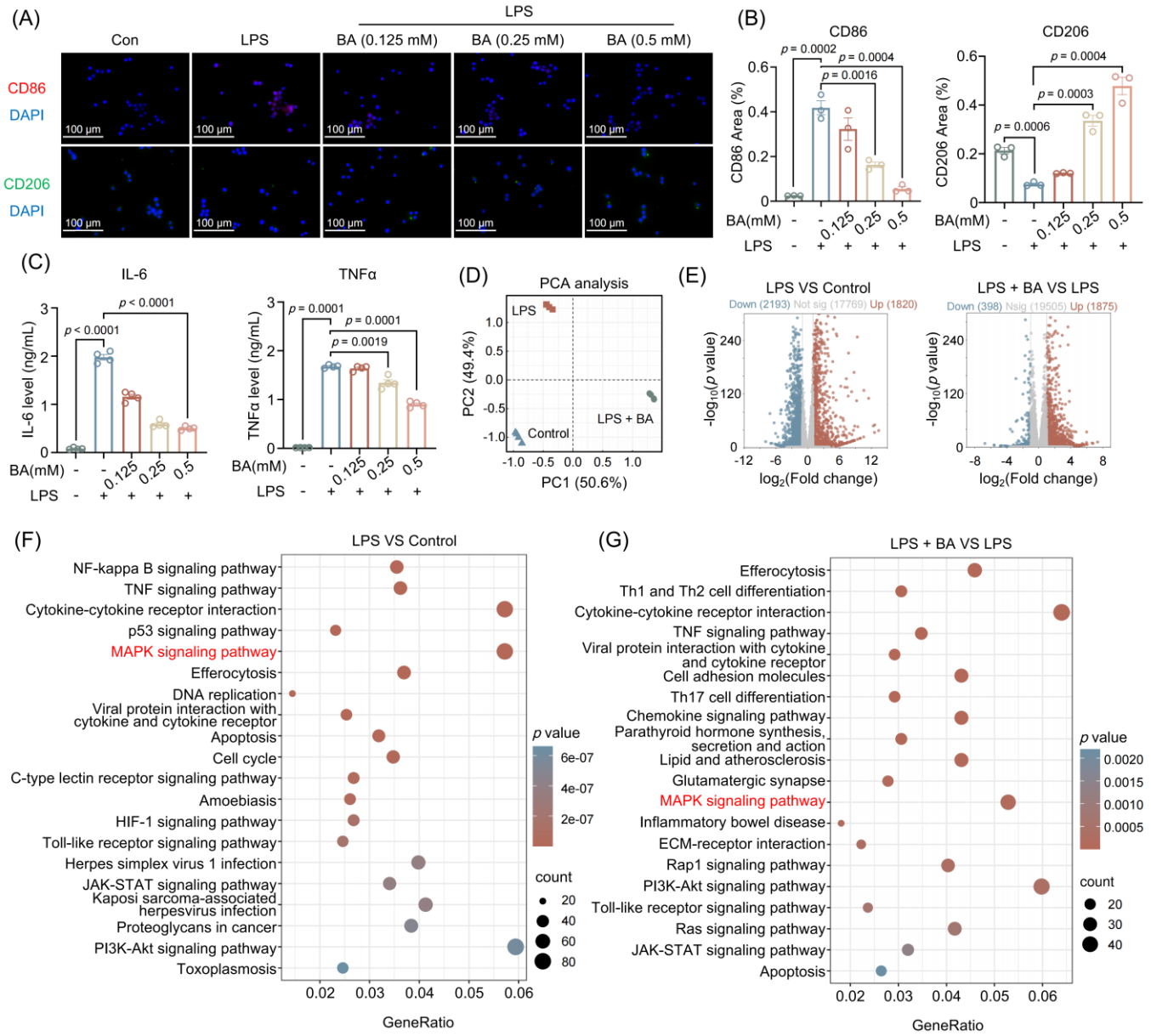


Figure 6 Butyrate inhibits the MAPK signaling pathway in macrophages.

Supplementing with BA can significantly reduce inflammatory responses in the liver, peripheral blood, and the intestinal tract, especially by inhibiting the polarization of pro-inflammatory M1 macrophages.

- Immunofluorescence results showed that BA treatment significantly inhibited M1 polarization induced by LPS, promoted M2 polarization, and inhibited the secretion of IL-6 and TNF-α.
- RNA sequencing revealed significant differences in the transcriptional profiles among the three groups of cells.
- KEGG indicated that BA significantly altered the gene expression involved in the TNF and MAPK signaling pathways, and these genes were highly enriched in LPS-stimulated cells and ACLF patients.

8. Butyrate suppresses macrophage M1 polarization by inhibiting MAPK signaling

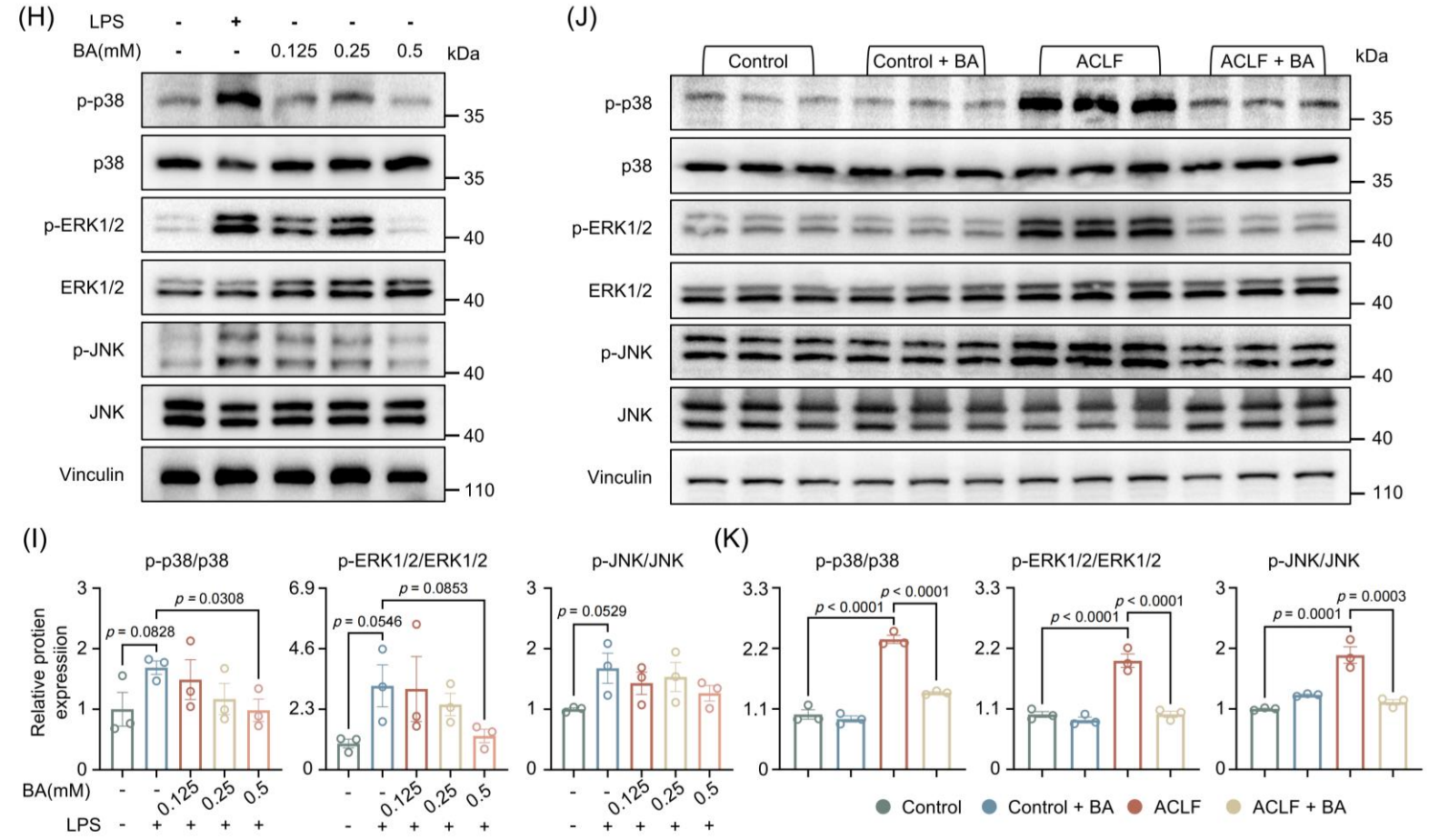


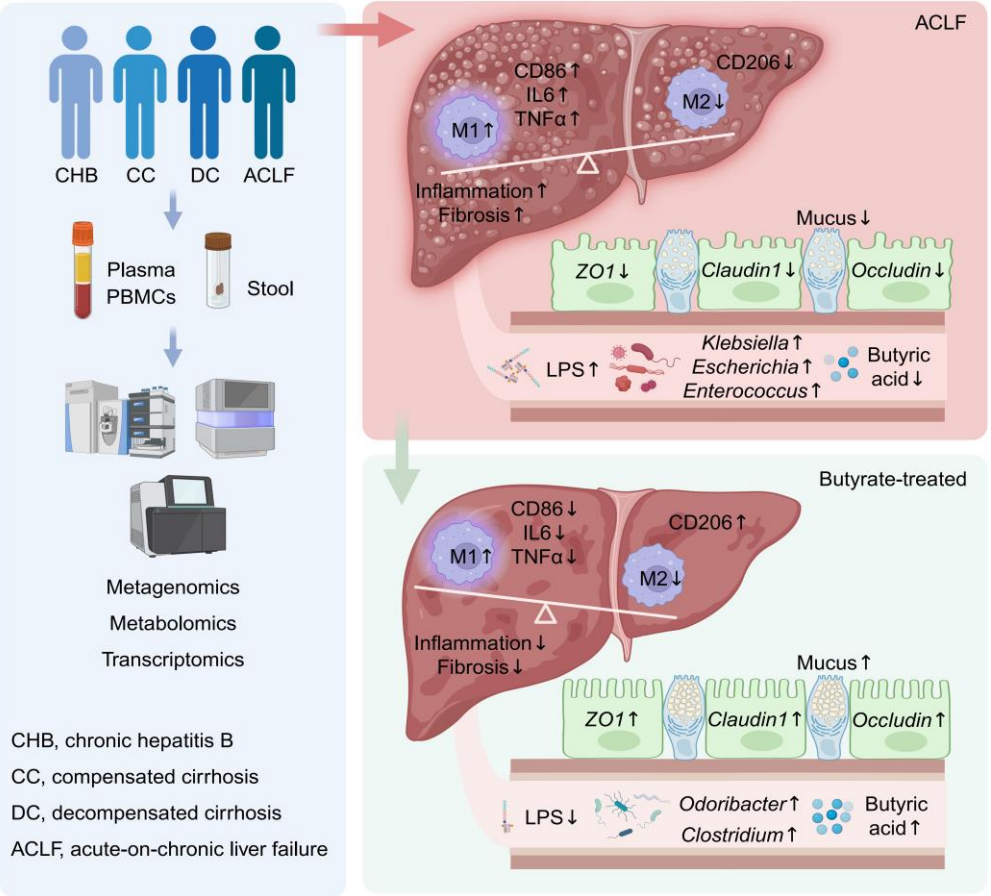
Figure 6 Butyrate inhibits the MAPK signaling pathway in macrophages.

Given the crucial role of MAPK in M1 polarization, we verified this target through WB.

- BA effectively inhibited the phosphorylation of p38, ERK1/2 and JNK in cells treated with LPS and liver tissues of ACLF mice.
- Overexpression of the p38 gene significantly weakened the ability of BA to inhibit M1 polarization, secretion of IL-6 and TNF-α.
- These data indicated that BA not only alleviated ACLF disease by improving intestinal barrier function, but also directly acted on macrophages to inhibit the MAPK signaling pathway and suppress M1 polarization.

Summary

Butyrate ameliorates HBV-ACLF by regulating macrophage polarization

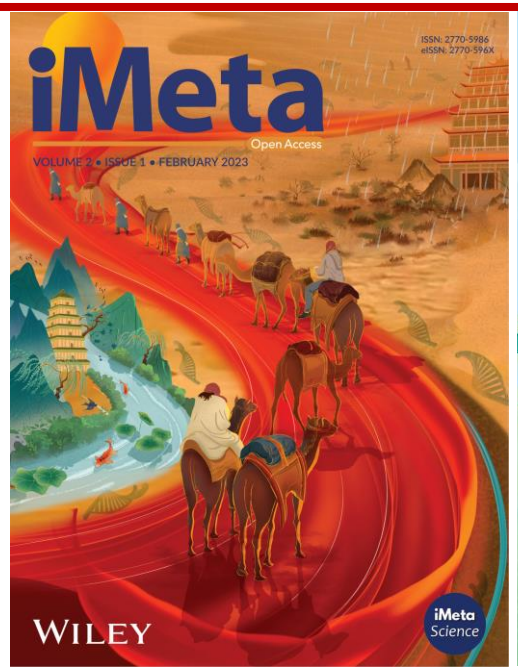


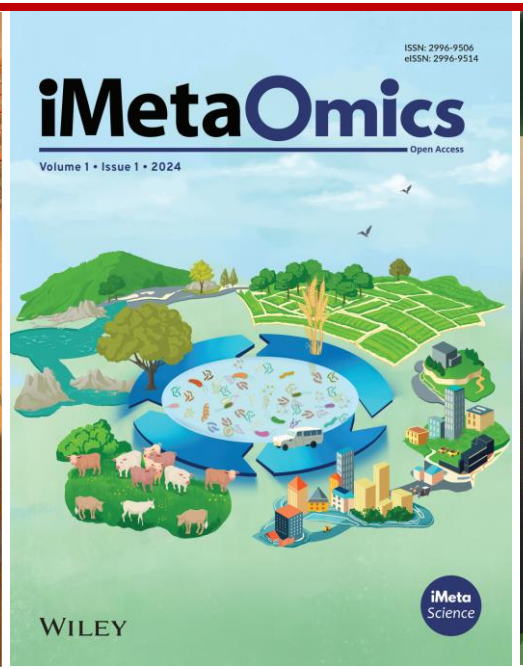
- Multi-omics profiling identifies progressive microbiota-metabolism-immunity axis dysfunction in ACLF, characterized by gut dysbiosis, reduced butyrate production, intestinal barrier disruption, microbial translocation and pro-inflammatory macrophage polarization, thereby exacerbating hepatic inflammation and fibrosis.
- Butyrate treatment coordinately reshapes the gut microbiota, increases beneficial butyrate-associated bacteria, restores mucus and tight-junction proteins, and reduces lipopolysaccharide level. These effects suppress pro-inflammatory macrophage activation and promote an anti-inflammatory phenotype, ultimately reducing hepatic inflammation and fibrosis and highlighting the microbiota-metabolism-immune axis as a potential therapeutic pathway in ACLF.

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