Supplemental material

Supplementary Figure legends

Figure S1. Network pharmacology results of MFH. (A) "Herb-Ingredient-Disease-Gene" network diagram of MFH. The herbs, ingredients, disease, genes and connection between them were colored. (B) The top 10 genes sorted by degree value. (C) KEGG enrichment analysis results of MFH. (D) The top 5 genes included in KEGG pathways (Sort by the number of KEGG pathways related to gene. The greater the degree of overlap of genes, the more the same pathways involved). Lines with different colors indicate different gene-pathway relationships.

Figure S2. GO enrichment analysis results. The top 10 biological processes (A), cell components (C) and molecular functions (E) and the top 5 genes correspond to three GO terms (B, D, F). Genes were sorted by the number of GO terms related to gene. Lines with different colors indicate different gene-term relationships.

Figure S3. Characterization of the gut microbiota in different group mice. (A) Relative abundance of top 9 Phylum in different treatment groups. (B) Observed features in different groups. (C) Shannon index in different groups. (D) The level of *Firmicutes* in the feces. (E) The level of *Bacteroidetes* in the feces. (F) The *Firmicutes/Bacteroidetes* ratio in the feces. (G) The level of *Proteobacteria* in the feces. The datas were determined by one-way ANOVA. (*P<0.05, **p<0.01, ***p<0.001; ns, nonsignificant).

Figure S4. LEfSe analysis for differential abundant taxa after CLP from different group. Mice underwent severe CLP were co-treated with MFH (1500mg/kg), ABX (500mg/mice) or MFH (1500mg/kg) plus ABX (500mg/mice) and were sacrificed 24h later, total fecal DNA was isolated and 16S rRNA genes were sequenced by 16S PacBio SMRT sequencing platform. (A) LEfSe analysis between ABX+CLP and ABX+MFH+CLP group. Threshold parameters were set as p=0.05 for the Mann-Whitney test and multi-class analysis=all against all. LDA score >2.0.

Figure S5. LEfSe analysis for differential abundant taxa after CLP from different group. Mice underwent severe CLP were co-treated with MFH (1500mg/kg), ABX (500mg/mice) or MFH (1500mg/kg) plus ABX (500mg/mice) and were sacrificed 24h later, total fecal DNA was isolated and 16S rRNA genes were sequenced by 16S PacBio SMRT sequencing platform. (A) LEfSe analysis between ABX+CLP and CLP alone group. (B) LEfSe analysis between ABX+MFH+CLP and CLP alone group. (C) LEfSe analysis between MFH+CLP and ABX+MFH+CLP group. (D) LEfSe analysis between MFH+CLP and ABX+CLP group. Threshold parameters were set as p=0.05 for the Mann-Whitney test and multi-class analysis=all against all. LDA score >2.0.

Figure S6. ERIC-PCR for differential therapy groups after CLP. Mice underwent severe CLP were co-treated with MFH (1500mg/kg), ABX (500mg/mice) or MFH (1500mg/kg) plus ABX (500mg/mice) and were sacrificed 24h late, total fecal DNA was isolated and 16S rRNA were detected by ERIC-PCR. (A) and (B) Representative

ERIC-PCR DNA fingerprints of the fecal microbiota of individual in different therapy groups. (C-I) Scores plot of OPLS-DA model processing for ABX+CLP vs. ABX+MFH+CLP (D), Sham vs. CLP (E), ABX+MFH+CLP vs. CLP (F), ABX+CLP vs. CLP (G), MFH+CLP vs. CLP (H), and ABX+CLP vs. MFH+CLP (I) respectively. S, sham; V, CLP; A, ABX+CLP; M, MFH+CLP; C, ABX+MFH+CLP.

Figure S7. MFH feces prolongs the survival of polymicrobial sepsis mice. (A) FMT experimental design: mice received vancomycin (100 mg/kg), neomycin sulfate (200 mg/kg), metronidazole (200 mg/kg) and ampicillin (200 mg/kg) intragastrically once daily for five days to deplete the gut microbiota, after which they received feces resuspended in PBS from CLP and MFH mice for three days. Severe CLP was performed, and the mice were sacrificed 24 h after CLP. (B) Body weight post ABX cocktail treatment. (C) Survival rate. (D) Principal coordinates analysis in MFH feces+CLP group and CLP feces+CLP group. (E) Relative abundance of top 6 phylum in different treatment groups. (F) Cladogram analysis between MFH feces+CLP and CLP feces+CLP group. *P<0.05, **p<0.01, ***p<0.001. The results are expressed as the mean ± SEM. Body weight curves were assessed by two-way ANOVA. Log-rank (Mantel-Cox) tests were performed for survival data.

Figure S8. Improvement in CLP injury by *L. johnsonii* depends on the participation of macrophages. (A) PMs, spleen and pPBMC were analyzed by flow cytometry for (F4/80+CD11b+CD45+) cells in mice (n=5). (B) Serum IL-10 level. (C) H&E staining in the ileum, liver, lung, and kidney (400x). Scale bar=50 μm. (D-G)

Representative quantitation on the right. (H) and (I) The relative mRNA levels of ZO-1 and occludin in the colon were measured. n=5. The results are expressed as the mean± SEM and were determined by one-way ANOVA and Log-Rank test (B). (*P<0.05, **p<0.01, ***p<0.001; ns, nonsignificant).

Figure S9. *L. johnsonii* promotes the release of IL-10 from M2 macrophages to alleviate CLP injury. (A) The proportion of IL-10^+ M2 macrophages were analyzed by flow cytometry in PMs, spleen and pPBMC of mice with sham group, CLP alone group, *L. johnsonii*+CLP group, anti-IL-10R mAb+CLP group and *L. johnsonii*+anti-IL-10R mAb+CLP group. (B) and (C) The relative protein and mRNA levels of IL-10. (D) and (f) HE staining in the ileum, liver (100x) and the quantication analysis. Scale bar=50 μm. (G) and (H) The relative mRNA levels of ZO-1 and occludin in the colon. (I) and (J) TNF-α, IL-1β, IL-6, CCL2, CCL3, CCL7, CXCL1, CXCL10 mRNA levels of liver and PLF. n=5. The results are expressed as the mean±SEM and were determined by one-way ANOVA or two-way ANOVA. (*P<0.05, **p<0.01, ***p<0.001; ns, nonsignificant).

Figure S10. Trp biosynthesis and metabolism contributes most among these metabolites in mice serum examined by UPLC/MS. (A) and (D) Partial least squares discriminant analysis (PLS-DA) scores plot of serum from mice showing segregation of metabolites between MFH+CLP group and ABX+CLP group (A), MFH+ABX+CLP group and ABX+CLP group (D). (B, E) Each dot represents an individual sample. Plot showing the variables selected by the PLS-DA model for a

given component component between MFH+CLP group and ABX+CLP group (B), MFH+ABX+CLP group and ABX+CLP group (E). (C, F) The variables are ranked by the absolute values of their VIP scores. Bubble diagram representing the significantly differed pathways between MFH+CLP group and ABX+CLP group (C), MFH+ABX+CLP group and ABX+CLP group (F).

Figure S11. Metabolites in mice serum examined by UPLC/MS. (A, C, E) Orthogonal partial least squares discriminant analysis (OPLS-DA) scores plot of serum from mice showing segregation of metabolites between ABX+CLP mice and CLP alone mice (A), ABX+MFH+CLP mice and CLP alone mice (C), ABX+MFH+CLP mice and MFH+CLP mice (E). (B, D, F) Each dot represents an individual sample. Plot showing the variables selected by the OPLS-DA model for a given component between ABX+CLP mice and CLP alone mice (B), ABX+MFH+CLP mice and CLP alone mice (D), ABX+MFH+CLP mice and MFH+CLP mice (F). Each dot represents an individual sample. The variables are ranked by the absolute values of their VIP scores.

Figure S12. PCs and BCAAs abundance increase and LPC decreases after MFH treatment. Lipid and amino acids metabolism in the serum were detected using UPLC-MS. The box and whisker plots summarize the normalized values of 16 significant metabolites ($P < 0.05$). (A) Normalized peak intensity of metabolites of fatty acids, including PC (16:0/22:6), PC (16:0/24:3), PC (36:5), PC (36:4), LPE (16:0), C16:1LPC and palmitoyl-L-carnitine, in MFH+CLP mice and CLP mice. (B)

Normalized peak intensity of amino acids, including L-Tryptophan, serine, phenylalanine, spermidine, isoleucine, L-Leucine, alanine, creatine, and asparagine. Data were analysed by Student's t-test.

Figure S13. Experimental design. (A) MFH pretreatment experiment. (B) *E. faecalis* and *L. johnsonii* pretreatment experiment. (C) Macrophage depletion experiment. (D) IL-10 depletion experiment. To explore Lactobacillus johnsonii promotes the release of IL-10 from macrophages to alleviate polymicrobial sepsis injury.