

cis-Aconitic Acid Derived from *Clostridium sporogenes* Attenuates Colitis via Targeting of Neutrophil TREM1

Ye Chen

yechen@smu.edu.cn

Nanfang Hospital, Southern Medical University

Jiaxin Li

Shenzhen Hospital, Southern Medical University, Shenzhen

Weihao Tan

Nanfang Hospital, Southern Medical University <https://orcid.org/0009-0000-4765-865X>

Xiaoru Wei

Nanfang Hospital, Southern Medical University

Maijiayi Ye

Nanfang Hospital, Southern Medical University

Linjie Li

Nanfang Hospital, Southern Medical University

Pu Wang

Nanfang Hospital, Southern Medical University

Dekai Zheng

Department of Infectious Diseases, Nanfang Hospital, Southern Medical University

Guanyi Lin

Nanfang Hospital, Southern Medical University

Ying Wang

Shenzhen Hospital, Southern Medical University, Shenzhen

Yunying Gong

Nanfang Hospital, Southern Medical University

Qiujuan Ye

Nanfang Hospital, Southern Medical University

Yuxuan Zhu

Nanfang Hospital, Southern Medical University

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2 ***sporogenes* Attenuates Colitis via Targeting of**
3 **Neutrophil TREM1**

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5 Weihao Tan^{1#}, Xiaoru Wei^{1#}, Maijiayi Ye¹, Linjie Li¹, Pu Wang¹, Dekai Zheng³, Guanyi Lin¹,
6 Ying Wang², Yunying Gong¹, Qiujuan Ye¹, Yuxuan Zhu¹, Jiaxin Li^{1,2*}, Ye Chen^{1,2*}

7

8 **1.Department of Gastroenterology, State Key Laboratory of Organ Failure Research,**
9 **Guangdong Provincial Key Laboratory of Gastroenterology, Nanfang Hospital,**
10 **Southern Medical University, Guangzhou, China.**

11 **2.Department of Gastroenterology, Integrative Microecology Clinical Center,**
12 **Shenzhen Key Laboratory of Gastrointestinal Microbiota and Disease, Shenzhen**
13 **Clinical Research Center for Digestive Disease, Shenzhen Hospital, Southern**
14 **Medical University, Shenzhen, China.**

15 **3.Guangdong Provincial Key Laboratory for Prevention and Control of Major Liver**
16 **Diseases;Key Laboratory of Infectious Diseases Research in South China, Ministry**
17 **of Education; Department of Infectious Diseases, Nanfang Hospital, Southern**
18 **Medical University, Guangzhou, China.**

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20 #Co-First Authorship: Weihao Tan and Xiaoru Wei contributed equally to this work.

21 *Correspondence: yechen@smu.edu.cn (Ye Chen) and cocoljx2020@163.com (Jiaxin Li).

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Abstract

Inflammatory bowel disease (IBD) remains a significant therapeutic challenge, with current agents plagued by suboptimal efficacy and safety issues. While gut microbiota dysbiosis is increasingly acknowledged as a key driver of IBD pathogenesis, the identification of functionally characterized, translationally applicable microbial-metabolic axes remains an unmet need. Here, we demonstrate that *Clostridium sporogenes* (*C. sporogenes*) is significantly decreased in both IBD patients and experimental colitis models. Notably, supplementation with this commensal bacterium markedly alleviates disease severity in both DSS-induced chemical colitis and *I10^{-/-}* spontaneous genetic colitis, thereby validating its therapeutic potential. Critically, we reveal that the protective effects of *C. sporogenes* are mediated by its secretion of the bioactive metabolite *cis*-aconitic acid (CAA). We further establish that aconitate hydratase A (AcnA) is essential for CAA biosynthesis: an *acnA*-knockout *C. sporogenes* strain fails to produce CAA and loses therapeutic efficacy, whereas engineered *Escherichia coli* overexpressing *acnA* recapitulates both CAA production and colitis protection. Mechanistically, CAA directly binds to the neutrophil receptor triggering receptor expressed on myeloid cells-1 (TREM1), thereby inhibiting neutrophil release of pro-inflammatory mediators and NETosis—pathological processes central to IBD progression. Finally, the therapeutic effect of CAA is completely abolished in neutrophil-specific *Trem1* knockout (*Trem1^{NeutKO}*) mice, confirming TREM1 as the critical target of CAA. This work establishes the *C. sporogenes*-CAA-TREM1 pathway as a promising therapeutic strategy for IBD.

Keyword: Inflammatory bowel disease, *Clostridium sporogenes*, TREM1, *cis*-aconitic acid

Main

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic relapsing inflammatory disorder of the gastrointestinal tract characterized by abdominal pain, diarrhea, and weight loss [1-4]. The global incidence of IBD has risen progressively, imposing a substantial healthcare burden worldwide [5-8]. The pathogenesis of IBD involves complex interactions among genetic predisposition, environmental triggers, dietary factors, immune dysregulation, and gut microbiota alterations [9-14]. This multifactorial complexity and pronounced inter-individual heterogeneity pose significant challenges for contemporary therapeutic strategies, underscoring the urgent need to decipher disease-specific immunological mechanisms and develop precision medicine approaches.

Gut microbiome dysbiosis is intrinsically linked to IBD development [9]. Pathobionts including adherent-invasive *Escherichia coli* (AIEC) and *Fusobacterium nucleatum* exacerbate barrier disruption and inflammation [15,16], whereas commensals such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, *Lactobacillus johnsonii* and *Bifidobacterium adolescentis* demonstrate protective potential [17-20]. Our prior work identified *Clostridiales* abundance as a microbiome biomarker of IBD severity [21]. Among these, *C. sporogenes* stands as an important probiotic species within this order, having demonstrated therapeutic effects such as promoting neuronal regeneration [22] and ameliorating cardiac dysfunction in preclinical models [23]. However, the role of *Clostridium* in IBD remains to be fully elucidated.

Neutrophils play a pivotal role in the pathogenesis of IBD. Upon intestinal inflammation, neutrophils are rapidly recruited to the gut, where they contribute to tissue damage through multiple mechanisms [24, 25]. These include the release of pro-inflammatory mediators such as *Il-6*, *Tnf- α* , *Ifng*, and the neutrophil-chemoattractant *Cxcl1*, which collectively sustain inflammatory signaling and amplify mucosal damage. Activated neutrophils also release specific granule proteins including *S100a* (a component of calprotectin) and lipocalin-2 (*Lcn2*), which have been established as sensitive biomarkers reflecting neutrophil infiltration and disease activity in IBD [26]. In addition, neutrophils exacerbate intestinal injury through the formation of neutrophil extracellular traps (NETs). Elevated NETs markers have been detected in colonic tissues from IBD patients, and their presence is associated with disease severity [27-30]. Importantly, interventions targeting neutrophil infiltration or function have been shown to ameliorate experimental colitis, underscoring the therapeutic potential of modulating neutrophil activity in IBD [24,26,31,32].

Triggering receptor expressed on myeloid cells-1 (TREM1) serves as a critical amplifier of innate immunity in immune cells [33,34]. In the context of IBD, TREM1 activation has been shown to significantly contribute to disease pathogenesis through multiple synergistic mechanisms. Upon ligand engagement, TREM1 initiates intracellular signaling through several key pathways, including PI3K-Akt and MAPK cascades (MEK, ERK, p38),

which converge on the activation of transcriptional factors such as NF- κ B [35]. This signaling network drives the production of potent pro-inflammatory mediators, including *Il-6*, *Tnf- α* , and *Cxcl1*, thereby establishing a self-perpetuating inflammatory microenvironment within the intestinal mucosa. Recent evidence further indicates that TREM1 modulates key inflammatory mediators including *Ifng* and *Cxcl1*, as observed in experimental models where TREM1 deficiency significantly reduced these factors [36]. A particularly important mechanism through which TREM1 exacerbates intestinal inflammation is by enhancing the formation of NETs. Experimental evidence indicates that TREM1 activation promotes NET generation in both human and murine neutrophils, whereas its genetic or pharmacological inhibition effectively attenuates NETosis and associated tissue damage [37]. The critical role of TREM1 in IBD pathogenesis is further substantiated by in vivo studies demonstrating that TREM1 blockade produces significant therapeutic benefits. These include attenuation of clinical disease severity, improvement in histopathological scores, reduction of colon shortening, and promotion of mucosal healing [35]. Collectively, these findings position TREM1 as a promising therapeutic target for modulating the dysregulated immune response in IBD through its coordinated effects on inflammatory signaling, neutrophil activation, and tissue homeostasis.

Here, we show that *C. sporogenes* is significantly diminished in the gut of IBD patients and murine models, while its supplementation markedly ameliorates disease severity. Our data further identify that the protective effect of *C. sporogenes* is mediated by the bioactive metabolite *cis*-aconitic acid (CAA), produced via the aconitate hydratase A (AcnA) enzyme. CAA directly binds to TREM1 on neutrophils, inhibits the downstream PI3K-AKT pathway, and thereby suppresses pro-inflammatory cytokine release and NETs formation. This work suggests an interesting *C. sporogenes*-CAA-TREM1 axis in IBD pathogenesis and presents CAA as a promising small-molecule candidate for IBD therapy.

Results

Intestinal *C. sporogenes* is reduced in IBD patients and colitis mice

To investigate the association between *C. sporogenes* and IBD, we first examined the abundance of *C. sporogenes* in fecal samples obtained from 35 healthy controls and 50 patients diagnosed with IBD (relevant clinical information is presented in Supplemental Table1). qPCR analysis revealed a statistically significant reduction in *C. sporogenes* abundance in the IBD patient cohort compared to the healthy controls (Fig.1a). To further explore the clinical relevance of this observation, we assessed disease severity using established laboratory biomarkers, including procalcitonin (ProCT), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and plasma albumin levels. Notably, the abundance of fecal *C. sporogenes* exhibited a positive correlation with plasma albumin levels, while exhibiting negative correlations with ProCT, CRP, and ESR levels, respectively (Fig.1b). Consistent with the findings in human subjects, mice with dextran sulfate sodium (DSS)-induced colitis displayed a marked decrease in fecal *C. sporogenes* abundance relative to control mice (Fig.1c). Thus, these findings suggest that diminished colonization of *C. sporogenes* may contribute to progression of IBD.

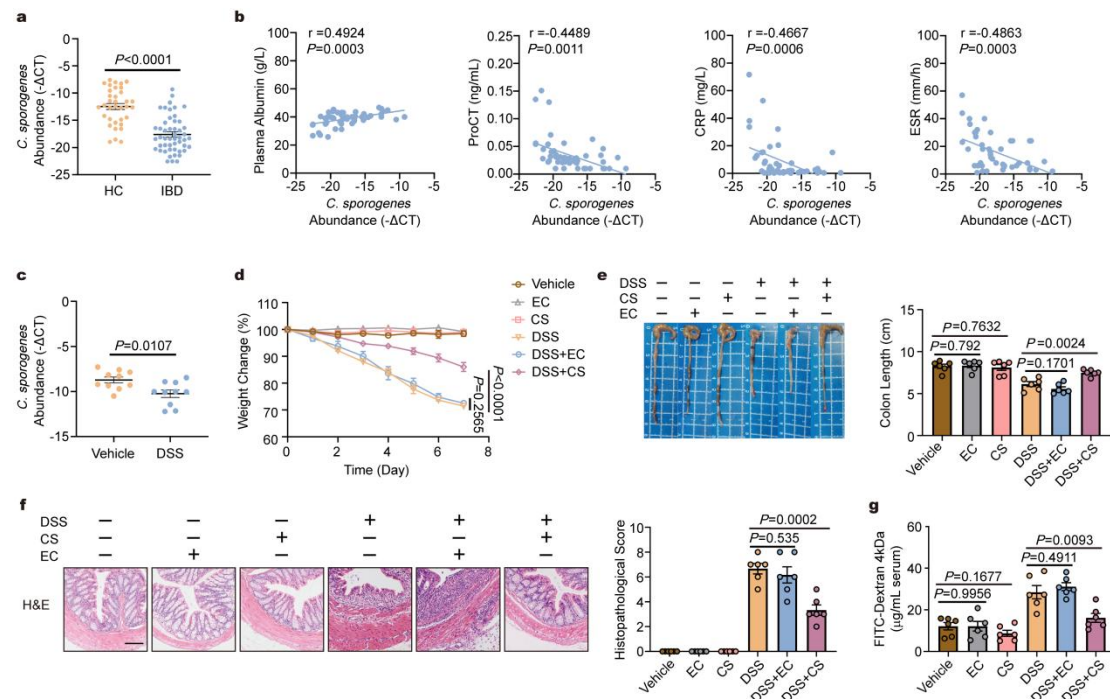


Fig.1: Intestinal commensal *C. sporogenes* ameliorate DSS-induced colitis in mice.

a-c, the abundance of *C. sporogenes* was tested by qPCR and expressed as $-\Delta Ct$, where higher $-\Delta Ct$ indicates higher bacterial load.

a, Bacteria genomic DNA was extracted from the human feces of the healthy control ($n = 35$) and inflammatory bowel disease (IBD) patients ($n = 50$).

b, The relationship of fecal *C. sporogenes* abundance with ProCT, CRP, ESR, and plasma albumin levels in IBD patients ($n = 50$).

c, Bacteria genomic DNA was extracted from the feces of the vehicle mice ($n = 10$) and mice treated with 3% DSS in drinking water for 7 consecutive days ($n = 10$).

d-g, Male C57BL/6J mice ($n = 6/\text{group}$) received 3% DSS in drinking water accompanied by daily oral gavage with either live *C. sporogenes* (CS, 10^8 CFU/mL, $150\mu L$) or live *E.coli* (EC, 10^8 CFU/mL, $150\mu L$) control for 7 days.

d, Longitudinal monitoring of body weight changes expressed as percentage baseline ($n = 6/\text{group}$).

e, f, Colon tissues were isolated on the last day of the experiment.

e, Representative macroscopic images of colon tissue from each group is provided, and the colon length was recorded ($n = 6/\text{group}$).

f, Histopathological evaluation of colon sections by H&E staining (scale bar = $100\mu m$). Histopathological scores of the DSS-induced colitis were evaluated ($n = 6/\text{group}$).

g, Intestinal permeability assessment via FITC-dextran (4 kDa) concentration (ng/mL) in mice serum ($n = 6/\text{group}$).

Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined by two-sided unpaired t-test (a, c, d, e, f and g), two-sided Spearman's correlation (b).

C. sporogenes ameliorates colitis in murine models

To explore the therapeutic role of *C. sporogenes* in colitis, we subjected mice to acute colitis induced by 3% DSS, with or without daily oral gavage of live *C. sporogenes*.

Administration of live *C. sporogenes* significantly attenuated DSS-driven body weight loss compared to the control (DSS+Vehicle) group (Fig.1d). Consistent with this observation, supplementation with live *C. sporogenes* markedly ameliorated pathological colon shortening (Fig.1e). Histopathological assessment further demonstrated reduced destruction of the intestinal epithelium and limited infiltration of inflammatory cells in mice treated with live *C. sporogenes* (Fig.1f). Notably, live *C. sporogenes* restored the integrity of the intestinal barrier, as evidenced by a reduction in serum fluorescein isothiocyanate (FITC)-dextran leakage (Fig.1g). By contrast, live *Escherichia coli* MG1655 (*E. coli*, EC) failed to replicate these protective effects (Fig.1d-g).

To further validate therapeutic efficacy of *C. sporogenes* in colitis, we employed interleukin-10 knockout (*Il10^{-/-}*) mice (Extended Data Fig.1a), a well-established model of spontaneous colitis [38]. As expected, oral administration of live *C. sporogenes* effectively ameliorated colitis, as demonstrated by attenuated body weight loss and prevention of colonic shortening (Extended Data Fig.1b, c). Additionally, histological assessment revealed a marked reduction in intestinal epithelial destruction and diminished goblet cell loss following treatment with live *C. sporogenes* (Extended Data Fig.1d, e). Meanwhile, treatment with live *C. sporogenes* significantly restored intestinal barrier in *Il10^{-/-}* mice, as evidenced by lower serum FITC-dextran levels compared to the untreated control group (Extended Data Fig.1f). Furthermore, immunofluorescence staining of tight junction proteins, including claudin-1, occludin, mucin 2 (MUC2), and zonula occludens-1 (ZO-1) proteins showed that *C. sporogenes* treatment significantly preserved the fluorescence signals of these proteins in the intestinal epithelium of *Il10^{-/-}* mice, relative to untreated controls (Extended Data Fig.1g). In addition, Western blot analysis further quantified significantly higher expression levels of these tight junction proteins in the live *C. sporogenes*-treated group compared to the untreated controls in *Il10^{-/-}* mice (Extended Data Fig.1h).

Collectively, these results indicate that *C. sporogenes* acts as a therapeutically relevant probiotic for alleviating colitis.

C. sporogenes generates *cis*-aconitic acid to ameliorate colitis

To determine whether the protective effects of *C. sporogenes* originate from its cellular components, we evaluated the efficacy of heat-killed *C. sporogenes* in ameliorating colitis in mice. However, the heat-killed *C. sporogenes* failed to improve intestinal inflammation in DSS-treated mice. By contrast, supplementation with the culture supernatant of live *C. sporogenes* significantly alleviated DSS-induced intestinal damage, indicating that the protective role of *C. sporogenes* in colitis is primarily mediated by its secreted bioactive metabolites (Extended Data Fig.2a-d).

To identify the protective metabolites secreted by live *C. sporogenes*, we conducted non-targeted metabolomic profiling of three sample groups: live *C. sporogenes* culture supernatant (CSCS), live *E. coli* culture supernatant (ECCS), and sterile Reinforced

Clostridial Medium (RCM). Heatmap visualization identified *cis*-aconitic acid (CAA) as one of the most differentially enriched metabolites in CSCS relative to both RCM and ECCS (Fig.2a). Subsequent liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantification confirmed that CAA levels in CSCS were significantly higher than those in both ECCS and RCM (Fig.2b). To further verify that *C. sporogenes* produces CAA after intestinal colonization, we colonized germ-free (GF) mice with *C. sporogenes* and detected fecal CAA levels. The baseline CAA levels and *C. sporogenes* abundance in GF mice were lower than those in specific pathogen-free (SPF) control mice. However, monocolonization with live *C. sporogenes* significantly increased CAA levels in feces, and this increase correlated with elevated fecal *C. sporogenes* abundance (Fig.2c-d).

To investigate the correlation between CAA and IBD, we analyzed clinical samples from healthy donors and IBD patients. Strikingly, plasma CAA concentrations in IBD patients were significantly lower than those in healthy controls, with a parallel reduction in fecal (Fig.2e, f, relevant clinical information is presented in Supplemental Table1). This finding indicates that CAA reduction may be associated with IBD progression. We next investigated whether CAA administration protects against colitis in mice. CAA supplementation markedly attenuated DSS-induced colitis, as evidenced by limited weight loss, restored colon length, mitigated intestinal epithelial damage, reduced goblet cell depletion, and decreased intestinal permeability (Fig.3a-d). Meanwhile, immunofluorescence staining of barrier-related proteins (claudin-1, MUC2, and ZO-1) showed that CAA treatment significantly maintained the fluorescence signals of these proteins in the intestinal epithelium of colitis mice relative to untreated controls (Fig.3e). Furthermore, Western blot quantification confirmed higher expression levels of these barrier-related proteins in the CAA-treated group compared to the untreated controls in mice with colitis (Fig.3f). In addition, these findings have also been validated in *Il10^{-/-}* mice (Extended Data Fig.1b-h).

Collectively, these results demonstrate that CAA generated by *C. sporogenes* is an important protective metabolite for ameliorating colitis progression.

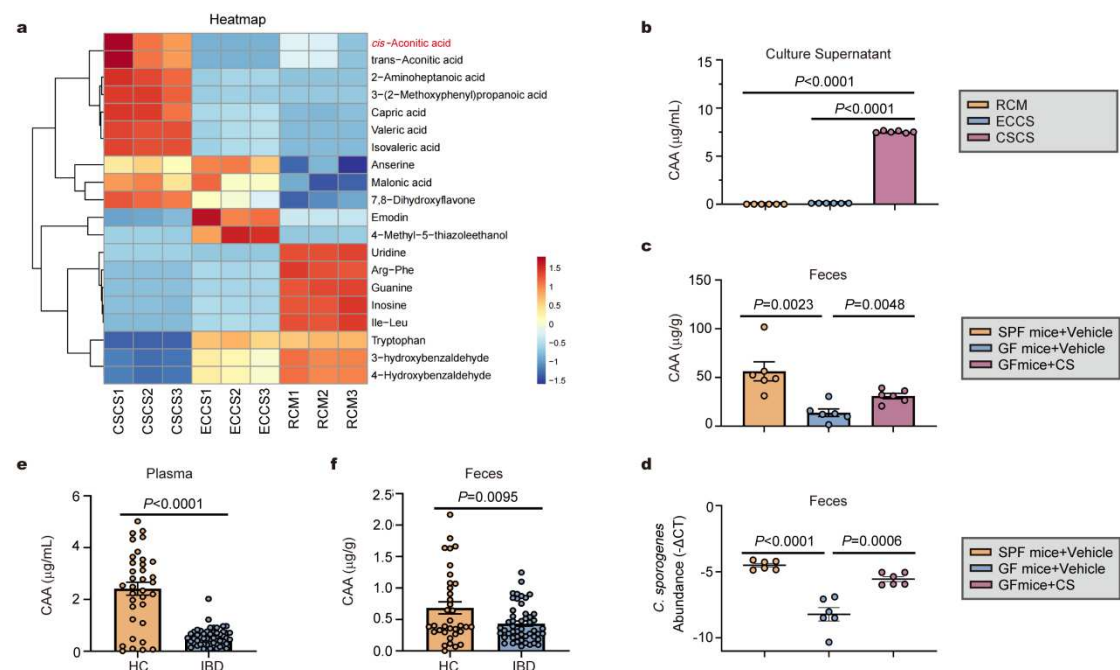


Fig.2: *C. sporogenes*-derived *cis*-aconitic acid (CAA) is depleted in IBD.

a, Heatmap visualization of non-targeted LC-MS metabolite profiles from live *C. sporogenes* culture supernatant (CSCS), live *E. coli* culture supernatant (ECCS), and Clostridia Reinforce Medium (RCM) fraction (n = 3). Metabolite abundance is color-coded: red (high), blue (low). CAA was identified as a candidate molecule participating in IBD development.

b, Targeted LC-MS/MS analysis of supernatant CAA levels in CSCS, ECCE and RCM (n = 6).

c, d, Germ-free (GF) mice (n = 6/group) received daily oral gavage with either live *C. sporogenes* (CS, 10^8 CFU/mL, 150 μL) or vehicle. Specific pathogen-free (SPF) mice (n = 6/group) received daily oral gavage with vehicle.

c, CAA concentration in feces from SPF mice and GF mice gavaged with live *C. sporogenes* or vehicle (n = 6).

d, Bacteria genomic DNA was extracted from the feces of the SPF mice and GF mice (n = 6), then the abundance of live *C. sporogenes* was tested by qPCR and expressed as $-\Delta Ct$, where higher $-\Delta Ct$ indicates higher bacterial load.

e, CAA concentration in plasma from IBD patients (n = 50) and healthy donors (n = 35).

f, CAA concentration in feces from IBD patients (n = 50) and healthy donors (n = 35).

Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined by two-sided unpaired t-test.

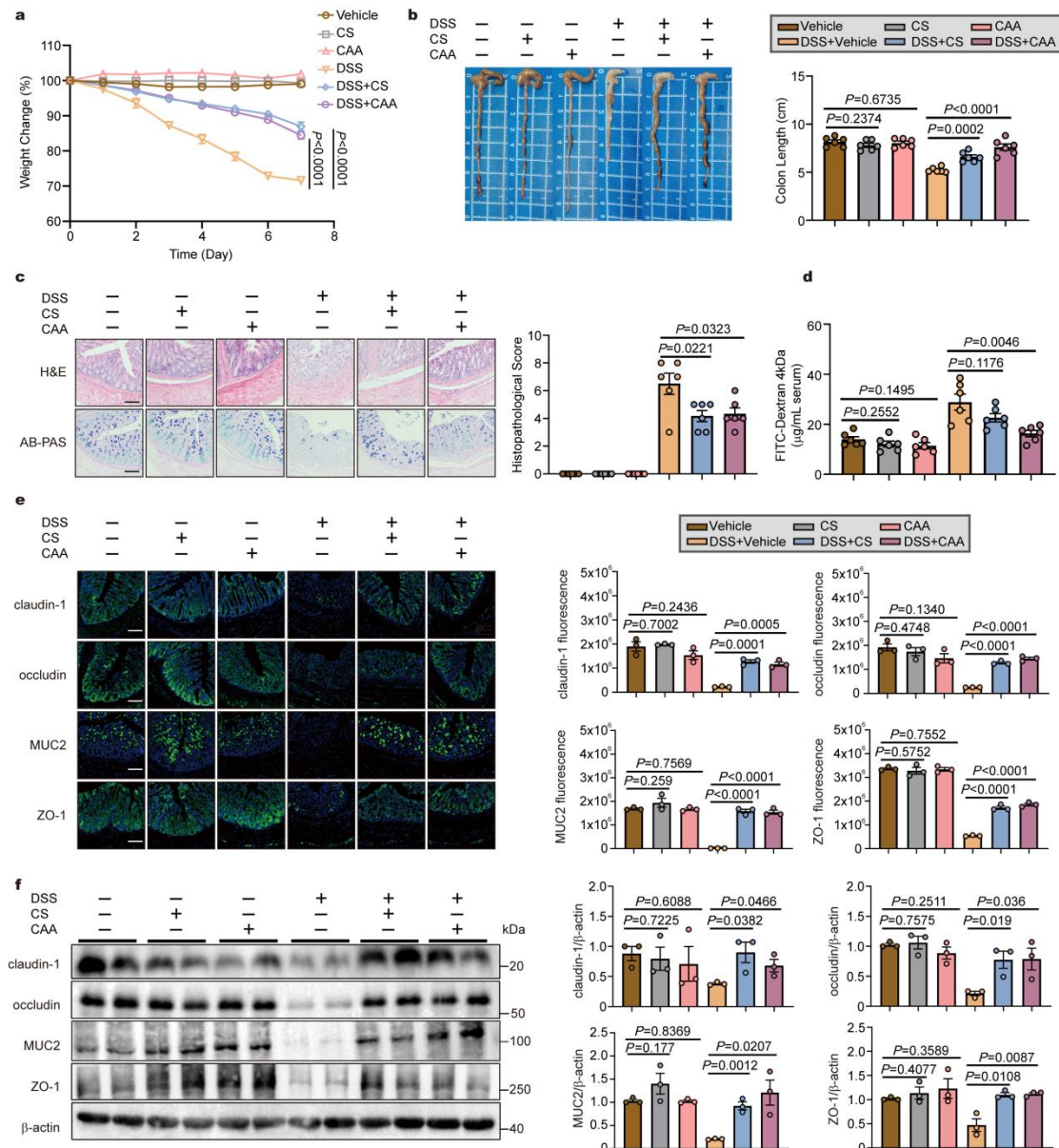


Fig.3:Both *C. sporogenes* and its metabolite CAA can ameliorate DSS-induced colitis.

a-f, Male C57BL/6J mice (n = 6/group) received 3% DSS in drinking water accompanied by daily oral gavage with either live *C. sporogenes* (CS, 10^8 CFU/mL, 150 μ L) or CAA (20mg/kg) for 7 days.

a, Longitudinal monitoring of body weight changes expressed as percentage baseline (n = 6/group).

b, c, e, f, Colon tissues were isolated on the last day of the experiment.

b, Representative macroscopic images of colon tissue from each group are provided, and the colon length was recorded (n = 6/group).

c, Histopathological evaluation of colon sections by H&E and AB-PAS staining (scale bar = 100 μ m). Histopathological scores of the DSS-induced colitis were evaluated (n = 6/group).

d, Intestinal permeability assessment via FITC-dextran (4 kDa) concentration (μ g/mL) in

mice serum (n = 6/group).

e, The localization of claudin-1, occludin, mucin 2 (MUC2), and zonula occludens-1 (ZO-1) in colon of mice was determined on the last day of the experiment by immunofluorescence staining. Scale bar = 100 μ m (n = 3).

f, The level of claudin-1, occludin, MUC2 and ZO-1 in the colon of mice was assessed by western blot analysis (n = 3). β -actin was used as a loading control.

Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined by two-sided unpaired t-test.

C. sporogenes generates *cis*-aconitic acid via aconitate hydratase A

Next, we aimed to explore how *C. sporogenes* generates CAA. Previous research has reported that CAA is produced through the dehydration of citric acid, a reaction catalyzed by the metabolic enzyme aconitate hydratase [39-41]. To identify the aconitate hydratase homolog in *C. sporogenes*, we searched the AlphaFold Protein Structural Database and identified CLOSPO_01101, an annotated as aconitate hydratase A (*acnA*) in the genome of *C. sporogenes* ATCC15579 as the putative CAA-synthesizing enzyme. To functionally validate the role of *acnA* in CAA production, we constructed an *acnA* knockout *C. sporogenes* strain (CSKO, Fig.4a,b). In vivo colonization assays confirmed that CSKO exhibited mucosal adhesion capacity comparable to that of the wild-type strain (CSWT, Fig.4c), ruling out the possibility that differences in colonization efficiency affect subsequent CAA-related results. Next, stable isotope tracing experiments with ^{13}C -labeled citric acid showed that CSWT co-culture resulted in robust enrichment of ^{13}C -labeled CAA, whereas CSKO produced significantly lower levels of ^{13}C -labeled CAA (Fig.4d). This result directly demonstrates that *acnA* is an important gene responsible for the dehydration of citric acid to CAA. Functionally, compared with CSWT, administration of CSKO failed to protect against DSS-induced colitis, as evidenced by sustained body weight loss, severe colon morphological damage, and impaired intestinal barrier function (Fig.4e-h), confirming that CAA is the key bioactive metabolite underlying *C. sporogenes*-mediated colonic protection.

To further confirm the conserved role of *acnA* in bacterial CAA biosynthesis, we engineered an *E. coli* MG1655 strain overexpressing *acnA* (OEEC, Fig.4i). Stable isotope tracing with ^{13}C -labeled citric acid revealed that OEEC exhibited a significantly higher metabolic flux toward ^{13}C -labeled CAA than the wild-type *E. coli* (Fig.4j). Importantly, OEEC administration recapitulated the protective effects of *C. sporogenes* against DSS-induced colitis, including mitigated weight loss, preserved colon length, and reduced histopathological scores (Fig.4k-n).

In conclusion, these data establish that *acnA* is one of the genes driving CAA biosynthesis in *C. sporogenes* and confirm CAA as the critical bioactive metabolite that mediates the colitis-ameliorating effect of *C. sporogenes*.

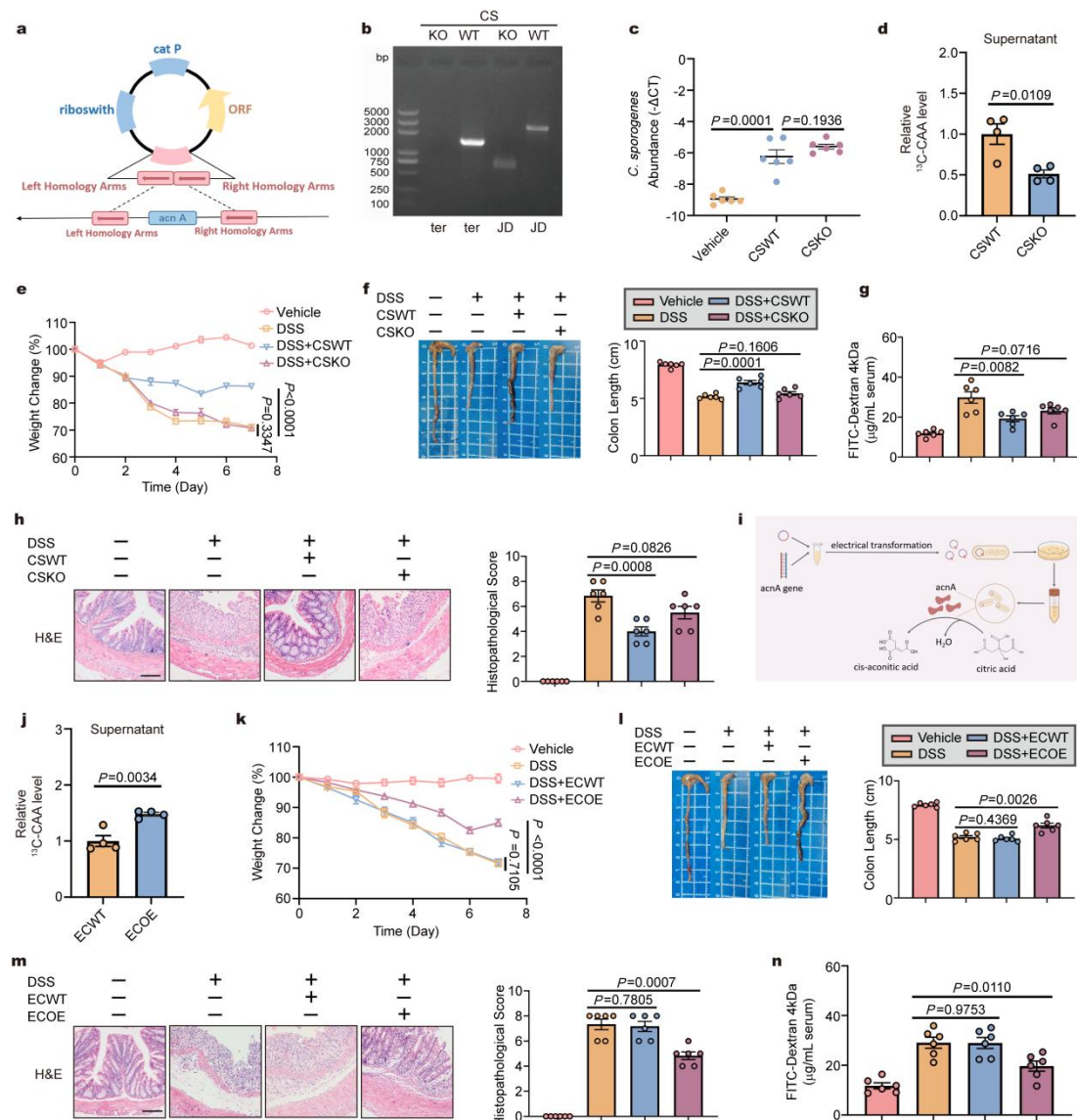


Fig.4:Genetic validation of *acnA*-dependent CAA production and its therapeutic role in colitis.

a, Schematic diagram of the *acnA*-knockout *C. sporogenes* (CSKO) via homologous recombination.

b, PCR validation of successful CSKO construction. Ter: target gene primer. JD: homology arm genotyping primers.

c, The fecal microbiota abundance of *C. sporogenes* following oral gavage with CSWT or CSKO in mice (n = 6/group), then the abundance of *C. sporogenes* was tested by qPCR and expressed as $-\Delta Ct$, where higher $-\Delta Ct$ indicates higher bacterial load.

d, Stable isotope tracing of ^{13}C -labeled CAA level in wild-type CSWT and CSKO supernatant (n = 4).

e-h, Male C57BL/6J mice (n = 6/group) received 3% DSS in drinking water accompanied by daily oral gavage with either CSKO or CSWT (10^8 CFU/mL, 150μL) for 7 days.

e, Longitudinal monitoring of body weight changes expressed as percentage baseline (n = 6/group).

f, h, Colon tissues were isolated on the last day of the experiment.

f, Representative macroscopic images of colon tissue from each group are provided, and the colon length was recorded (n = 6/group).

g, Intestinal permeability assessment via FITC-dextran (4 kDa) concentration (µg/mL) in mice serum (n = 6/group).

h, Histopathological evaluation of colon sections by H&E staining (scale bar = 100 µm). Histopathological scores of the DSS-induced colitis were evaluated (n = 6/group).

i, Schematic process illustrating the workflow for *acnA*-overexpression in *E. coli* (ECOE).

j, Stable isotope tracing of ¹³C-labeled CAA level in ECOE or ECWT culture supernatant (n = 4/group).

k-n, Male C57BL/6J mice (n = 6/group) received 3% DSS in drinking water accompanied by daily oral gavage with either ECOE or ECWT (10⁸CFU/mL, 150µL) for 7 days.

k, Longitudinal monitoring of body weight changes expressed as percentage baseline.

l, m, Colon tissues were isolated on the last day of the experiment (n = 6/group).

l, Representative macroscopic images of colon tissue from each group are provided, and the colon length was recorded (n = 6/group).

m, Histopathological evaluation of colon sections by H&E staining (scale bar = 100 µm). Histopathological scores of the DSS-induced colitis were evaluated (n = 6/group).

n, Intestinal permeability assessment via FITC-dextran (4 kDa) concentration (µg/mL) in mice serum (n = 6/group).

Data are presented as the mean ± s.e.m. Statistical significance was determined by two-sided unpaired t-test.

CAA ameliorates colitis by suppressing neutrophil function

Neutrophils are pivotal in the pathogenesis of IBD, serving as key effector cells that drive intestinal inflammation and contribute to barrier disruption [24]. Thus, we assessed the clinical relevance of circulating CAA levels to peripheral neutrophil percentages in a cohort of IBD patients (detailed clinical information is provided in Supplemental Table1). Notably, we observed an inverse correlation between plasma CAA levels and peripheral neutrophil percentages (Fig.5a), suggesting a potential immunomodulatory function of CAA.

Based on this clinical finding, we hypothesized that CAA modulates neutrophil-related pathology in colitis. We first evaluated whether CAA influences neutrophil infiltration into the intestinal lamina propria. Using flow cytometry in a DSS-induced colitis mice model, we found that both colonization with live *C. sporogenes* and direct CAA supplementation significantly reduced neutrophil infiltration (Fig.5b, c, Extended Data Fig.3a). In contrast, mice treated with CAA-nonproducing *E. coli* showed persistent neutrophil infiltration (Fig.5b, c), aligning with their failure to mitigate colitis and underscoring the specificity of CAA in controlling neutrophil infiltration.

We next investigated whether CAA impairs neutrophil activation and effector functions. Analysis of inflammatory mediator expression in isolated colonic neutrophils revealed that CAA treatment broadly downregulated cytokines (*Il-6*, *Tnf-α*, *Ifng*), chemokines (*Cxcl1*), and inflammation-related markers (*S100a9*, *Lcn2*) at the transcriptional level (Fig.5d-j), indicating suppression of neutrophil-derived inflammatory signals. Furthermore, we

390 examined the effect of CAA on neutrophil extracellular traps (NETs) formation, a
391 pathogenic process in IBD [27,28,42]. In colitis mice, CAA intervention significantly
392 reduced serum levels of cell-free DNA (a biomarker of NETosis) compared to untreated
393 controls (Fig.5k). Western blot and immunofluorescence analyses further demonstrated a
394 pronounced attenuation of citrullinated histone H3 (CitH3) expression in colonic tissues
395 following CAA administration, which is indicative of suppressed NETs formation during
396 colitis progression (Fig.5l,m). Complementary in vitro studies confirmed that CAA directly
397 suppresses PMA-induced NETs formation in human neutrophils (Fig.5n, o, Extended Data
398 Fig.3b).

399
400 To determine whether neutrophils are required for CAA-mediated protection, we
401 performed neutrophil depletion using an anti-Ly6G antibody. The therapeutic effect of CAA
402 on colitis was abolished upon neutrophil depletion, as evidenced by the fact that the DSS
403 + CAA + anti-Ly6G group failed to show any significant improvement in body weight
404 recovery, colon length, histological scores, or intestinal barrier integrity over the DSS +
405 anti-Ly6G group. (Fig.5p-s, Extended Data Fig.4a). By contrast, in a separate
406 macrophage depletion model using liposomal clodronate (Lipo), CAA still significantly
407 ameliorated colitis compared to the macrophage-depleted control group (DSS + Lipo)
408 (Extended Data Fig.4b-f). These findings establish that CAA alleviates colitis through a
409 mechanism that is fundamentally dependent on neutrophils but is independent of
410 macrophages.

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412 Together, these findings demonstrate that CAA alleviates colitis by suppressing gut
413 neutrophil infiltration, pro-inflammatory activation, and NETs release, highlighting an
414 important role for CAA in neutrophil regulation.

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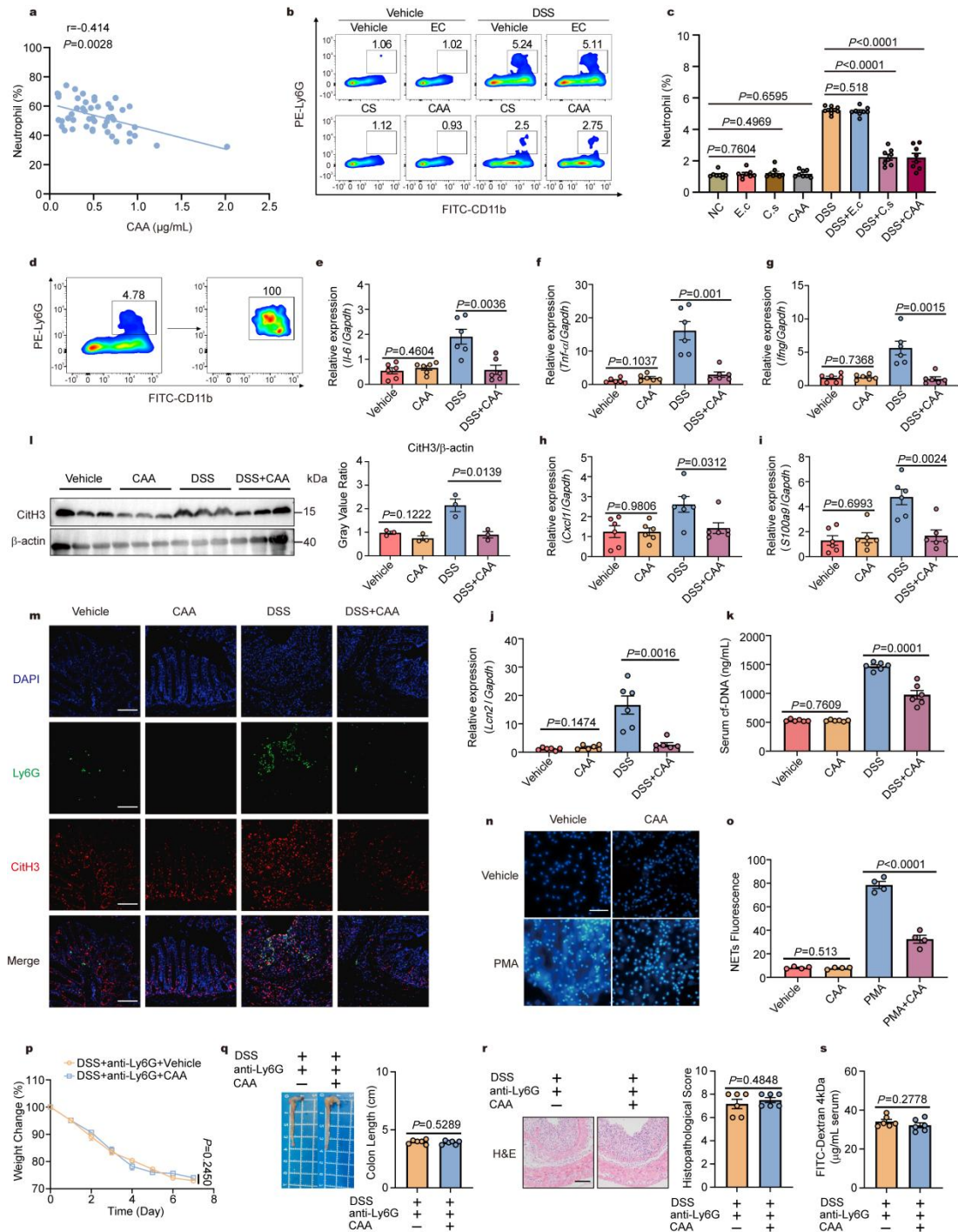


Fig.5:CAA suppresses neutrophil activation and NETosis to ameliorate colitis.

a, Spearman correlation analysis of serum CAA levels and neutrophil frequency in IBD patients (n = 50).

b-m, Male C57BL/6J mice received 3.0% DSS in drinking water accompanied by daily oral gavage with either live *C. sporogenes* (CS, 10^8 CFU/mL), live *E. coli* (EC, 10^8 CFU/mL) or CAA (20 mg/kg) for 7 days.

b, c, Flow cytometric analysis of neutrophil (CD11b⁺Ly6G⁺) infiltration in colonic lamina propria (n = 8/group).

d-j, mRNA expression of inflammatory mediators (*Il-6*, *Tnf-α*, *Ifng*, *Cxcl1*, *S100a9* and

Lcn2) in sorted colonic lamina propria cells (n=6).

k, Serum cell-free DNA (cf-DNA, NETs marker) quantification in different groups (n = 6).

l, Western blot analysis of citrullinated histone H3 (CitH3) in colon lysates. β -actin was used as a loading control (n = 3).

m, Immunofluorescence imaging of Ly6G⁺ neutrophils and CitH3 in colon sections (scale bars: 100 μ m).

n, o, In vitro NETosis assay: Peripheral neutrophils (5×10^5) isolated from healthy controls (HC, n=4) were pre-incubated with or without CAA (100 μ M) for 30 min and then stimulated with Phorbol 12-myristate 13-acetate (PMA, 100 ng/mL) for 4 h to induce NETs (scale bars: 200 μ m). DMSO, dimethyl sulfoxide.

p-s, Male C57BL/6J mice received 3.0% DSS in drinking water for 7 days. To deplete neutrophils, mice were intravenously administered 200 μ g of anti-mouse Ly6G antibody (anti-Ly6G) one day before and on days 1, 3, and 5 of DSS treatment. During the 7-day period, mice were divided into two groups: one group received only the neutrophil depletion treatments (DSS + anti-Ly6G), while the other group additionally received daily oral gavage with CAA (20 mg/kg) (DSS + anti-Ly6G + CAA).

p, Longitudinal monitoring of body weight changes expressed as percentage baseline (n = 6/group).

q, r, Colon tissues were isolated on the last day of the experiment.

q, Representative macroscopic images of colon tissue from each group are provided, and the colon length was recorded (n = 6/group).

r, Histopathological evaluation of colon sections by H&E staining (scale bar = 100 μ m). Histopathological scores of the DSS-induced colitis were evaluated (n = 6/group).

s, Intestinal permeability assessment via FITC-dextran (4 kDa) concentration (μ g/mL) in mice serum (n = 6/group).

Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined by two-sided Spearman's correlation (a) and two-sided unpaired t-test (b, c, e-k, m, n).

CAA alleviates colitis by targeting the TREM1-PI3K-Akt axis in neutrophils

Triggering receptor expressed on myeloid cells-1 (TREM1) is a critical amplifier of inflammatory responses in neutrophils, known to promote their infiltration into sites of inflammation, enhance pro-inflammatory cytokine release, and facilitate NETs formation [33, 35, 37]. Thus, we hypothesized that CAA might exert its anti-inflammatory effects by directly binding to and inhibiting TREM1. To test this hypothesis, we first employed molecular docking analysis, which predicted that CAA efficiently docks into the conserved ligand-binding pocket of TREM1, with the optimal conformation displaying a binding energy of -4.4 kcal/mol (Fig. 6a). Surface plasmon resonance (SPR) assays further confirmed the concentration-dependent binding between CAA and recombinant TREM1 protein (Fig. 6b). To validate direct CAA-TREM1 interaction in a cellular context, we employed cellular thermal shift assay (CETSA) and drug affinity-responsive target stability (DARTS). In CETSA, we observed that CAA treatment significantly increased the melting temperature of TREM1 in both HL-60 cells and mice primary bone marrow-derived neutrophils (BMDNs) (Extended Data Fig. 3c) compared to vehicle controls (Fig. 6c, f). In

DARTS, we found that CAA protected TREM1 from degradation in both HL-60 cells and BMDNs (Fig.6d, g). In addition, pull-down assays revealed a specific interaction between CAA and TREM1 in both HL-60 cells and BMDNs lysates (Fig.6e, h). Collectively, these results demonstrate that CAA directly binds to TREM1 in neutrophils.

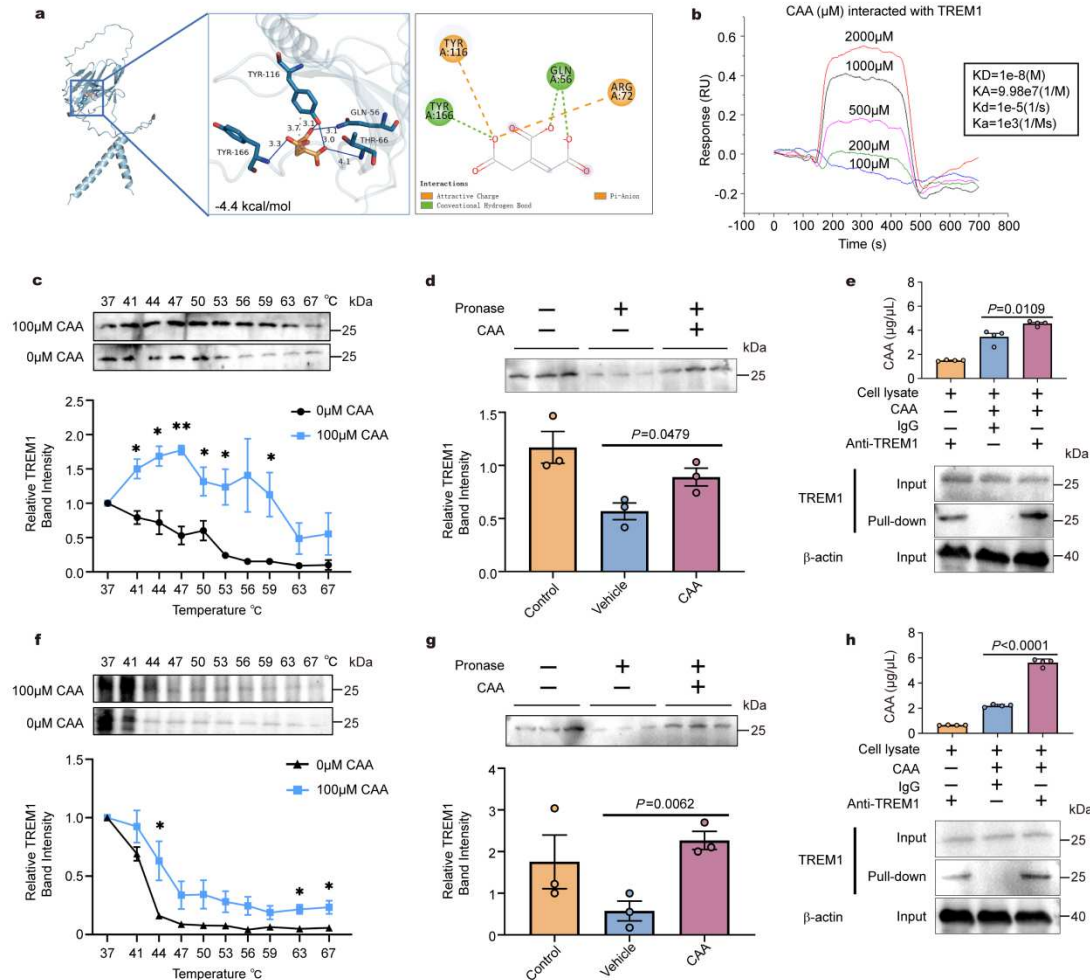


Fig.6:CAA directly targets TREM1 as validated by multi-method approaches.

a, Molecular docking analysis of CAA with the TREM1 (left), and the potential interaction binding site (right).

b, Surface plasmon resonance (SPR) sensorgrams showing dose-dependent binding kinetics between immobilized TREM1 and serially diluted CAA (100-2000 μ M).

c, Cellular thermal shift assay (CETSA) demonstrating CAA (100 μ M)-induced thermostabilization of TREM1 in HL-60 cell lysates (n = 3).

d, Drug affinity responsive target stability (DARTS) assay showing pronase resistance of TREM1 in CAA-treated HL-60 cell lysates (100 μ M, 1h, n = 3).

e, Pull-down assays using anti-TREM1 or rabbit IgG control in HL-60 cell lysates $\pm$ CAA (100 μ M). Eluates were analyzed by LC-MS/MS (top) and western blot (bottom). β -actin was used as a loading control (n = 4).

f, CETSA demonstrating CAA (100 μ M)-induced thermostabilization of TREM1 in mice primary bone marrow-derived neutrophil (BMDN) lysates (n = 3).

g, DARTS assay showing pronase resistance of TREM1 in CAA-treated BMDN lysates (100 μ M, 1h, n = 3).

h, Pull-down assays using anti-TREM1 or rabbit IgG control in BMDN lysates $\pm$ CAA (100 μ M). Eluates were analyzed by LC-MS/MS (top) and western blot (bottom). β -actin was used as a loading control (n = 4).

Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined by two-sided unpaired t-test. * P < 0.05, ** P < 0.01.

To definitively confirm that TREM1 mediates CAA's regulatory effects on neutrophils, we generated tamoxifen-inducible neutrophil-specific *Trem1* knockout mice (*Trem1*^{NeutKO}) via Cre-loxP recombination (Extended Data Fig.5a, b). Western blot analysis confirmed efficient TREM1 deletion in BMDNs from *Trem1*^{NeutKO} mice compared to *Trem1*^{fl/fl} littermates (Extended Data Fig.5c). We then induced colitis with DSS in both genotypes and administered CAA. Flow cytometric analysis of colonic lamina propria cells showed that CAA significantly reduced the frequency of neutrophils in *Trem1*^{fl/fl} mice, whereas no such reduction was observed in *Trem1*^{NeutKO} mice. This demonstrates that TREM1 is required for CAA-mediated inhibition of neutrophil infiltration (Fig.7a, b). Additionally, qPCR analysis of sorted colonic neutrophils revealed that CAA treatment downregulated the mRNA levels of multiple pro-inflammatory mediators (*Il-6*, *Tnf- α* , *Ifng*, *Cxcl1*, *S100a9*, *Lcn2*) in *Trem1*^{fl/fl} mice, but not in *Trem1*^{NeutKO} mice (Fig.7c–i). Furthermore, quantification of cf-DNA showed that CAA treatment reduced cf-DNA levels in *Trem1*^{fl/fl} mice but not in *Trem1*^{NeutKO} mice (Fig.7j). Western blot analysis further showed that CAA reduced Cith3 protein expression in neutrophils from *Trem1*^{fl/fl} mice, whereas no such reduction was observed in *Trem1*^{NeutKO} mice (Fig.7k). These findings collectively indicate that TREM1 is essential for CAA to modulate neutrophil infiltration, pro-inflammatory mediators production and NETosis. We next evaluated whether the loss of neutrophil TREM1 would abolish the therapeutic efficacy of CAA in colitis. In *Trem1*^{fl/fl} mice, CAA significantly ameliorated overall disease severity, as evidenced by reduced weight loss, preserved colon length, improved histopathological scores, and enhanced intestinal barrier integrity. By contrast, these protective effects were completely abrogated in *Trem1*^{NeutKO} mice (Fig. 7l–o), demonstrating that neutrophil-specific TREM1 is indispensable for the therapeutic action of CAA.

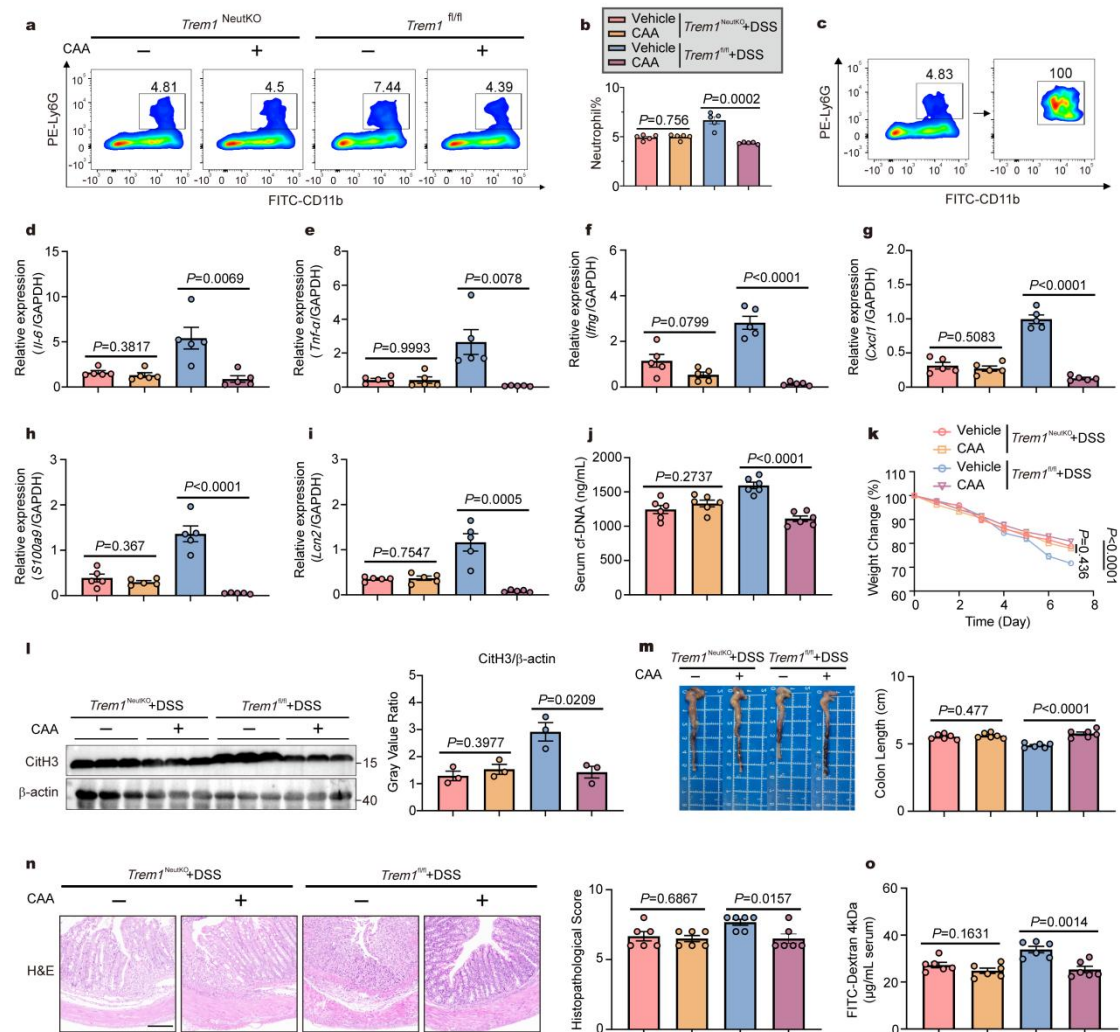


Fig.7:TREM1 mediates the therapeutic effects of CAA in colitis by regulating neutrophil recruitment and function.

a-o, *Trem1*^{NeutKO} mice or *Trem1*^{fl/fl} control mice (male, n = 6/group) received 3% DSS in drinking water for 7 days. This was followed by daily oral gavage with or without CAA (20mg/kg).

a, b, Flow cytometric analysis of neutrophil (CD11b⁺Ly6G⁺) infiltration in colonic lamina propria (n = 5/group).

c-i, mRNA expression of inflammatory mediators (*Il-6*, *Tnf-α*, *Ifng*, *Cxcl1*, *S100a9* and *Lcn2*) in sorted colonic lamina propria cells (n=5).

j, Serum cell-free DNA (cf-DNA, NETs marker) quantification in different groups (n = 6).

k, Longitudinal monitoring of body weight changes expressed as percentage baseline (n = 6/group).

l, Western blot analysis of citrullinated histone H3 (Cith3) in colon lysates. β-actin was used as a loading control (n = 3).

m, n, Colon tissues were isolated on the last day of the experiment.

m, Representative macroscopic images of colon tissue from each group are provided, and the colon length was recorded (n = 6/group).

n, Histopathological evaluation of colon sections by H&E staining (scale bar = 100 μ m).
Histopathological scores of the DSS-induced colitis were evaluated (n = 6/group).
o, Intestinal permeability assessment via FITC-dextran (4 kDa) concentration (μ g/mL) in mice serum (n = 6/group).
Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined by two-sided unpaired t-test.

To determine how CAA binding to TREM1 modulates intracellular signaling, we analyzed major inflammatory pathways downstream of TREM1, namely PI3K-Akt, MAPK, and NF- κ B [43]. In both HL-60 cells and BMDNs, CAA treatment broadly suppressed the phosphorylation of key components within these pathways, including PI3K-Akt, MAPK pathway components (including ERK, MEK, p38), and NF- κ B (p65) (Extended Data Fig.6). These findings demonstrate that CAA binding to TREM1 attenuates its downstream pro-inflammatory signaling network.

To pinpoint the pathway most critically involved in the therapeutic effect, we integrated the protein phosphorylation analysis with transcriptomic data. Gene set enrichment analysis (GSEA) of colonic RNA sequencing data specifically highlighted a significant downregulation of the PI3K-Akt pathway upon CAA treatment (Fig. 8a). Based on this convergent evidence from both protein phosphorylation and transcriptomic data, we specifically investigated the functional contribution of the PI3K-Akt pathway using a selective PI3K inhibitor (LY294002) in DSS-induced colitis mice. Notably, the combined administration of LY294002 and CAA did not enhance the protective effects compared to CAA monotherapy. Both regimens similarly improved body weight recovery, reduced colon shortening, lowered histological scores, and enhanced intestinal barrier integrity (Fig. 8b-g), suggesting that CAA alleviates colonic injury primarily through inhibiting the PI3K-Akt signaling axis. This lack of additive effect was consistently observed across neutrophil-related pathological processes: CAA monotherapy significantly reduced neutrophil infiltration into the colonic lamina propria, whereas the combination with LY294002 yielded no further reduction (Fig. 8h, i). Similarly, CAA monotherapy suppressed NETosis, as evidenced by non-significantly reduced CitH3 expression and a trend of decreasing serum cf-DNA. The addition of LY294002 did not enhance this suppression (Fig. 8j- l). Analysis of neutrophils isolated from the colonic lamina propria confirmed that CAA downregulated key inflammatory mediators (*Il-6*, *Tnf- α* , *Ifng*, *Cxcl1*, *S100a9*, *Lcn2*), with no additive effect from LY294002 (Fig. 8m-s). Taken together, our findings establish that suppression of the PI3K-Akt pathway is likely to be a central mechanism through which CAA ameliorates colitis and restrains neutrophil-driven pathology.

In conclusion, our results demonstrate that CAA alleviates colitis primarily through targeting the TREM1-PI3K-Akt axis in neutrophils, thereby restraining their infiltration, pro-inflammatory activation, and NETosis.

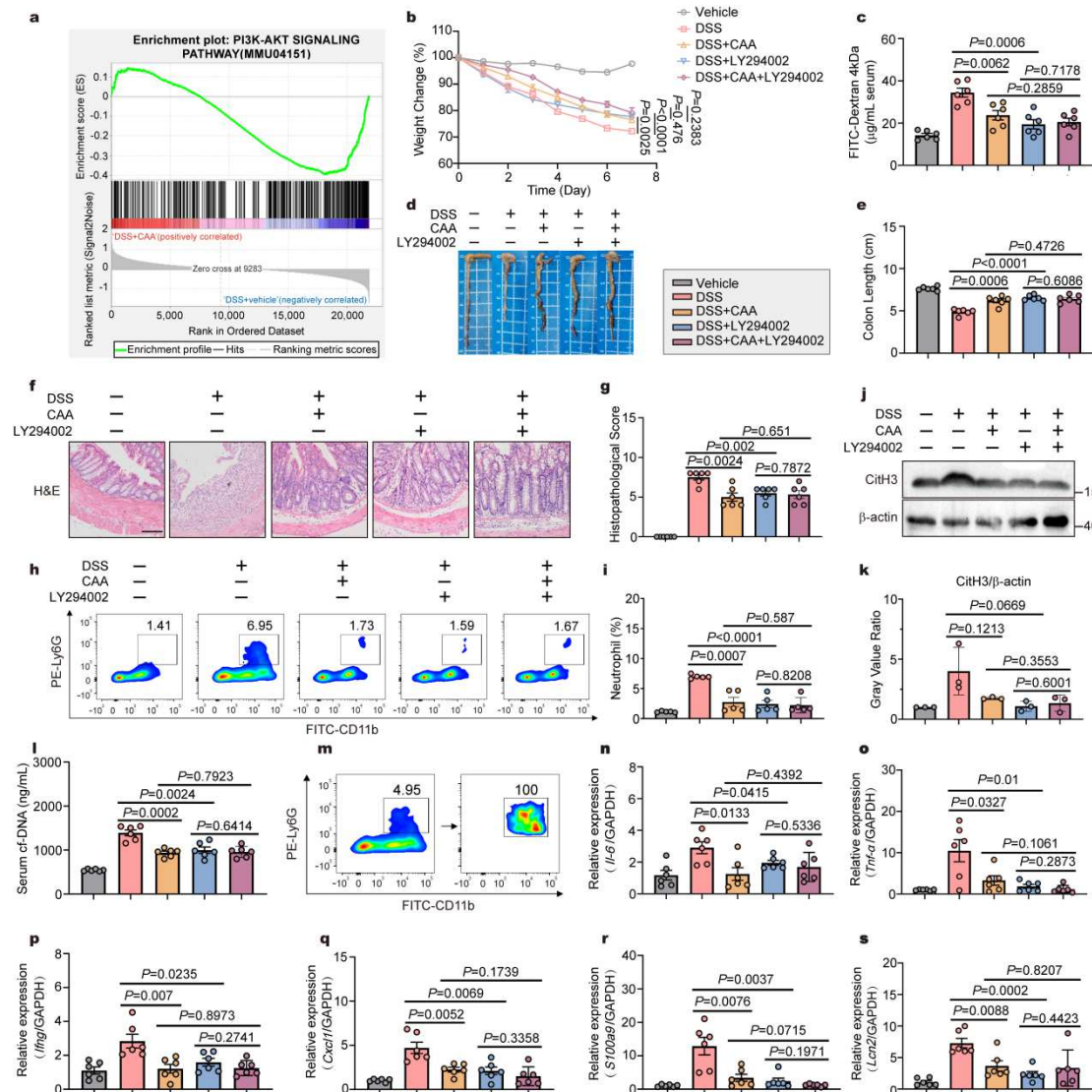


Fig.8:CAA ameliorates colitis by suppressing the PI3K-Akt signaling pathway to restrain neutrophil recruitment and activation.

a, Gene set enrichment analysis (GSEA) plot of PI3K-Akt signaling pathway (KEGG: mmu04151) in colonic tissues from DSS-induced colitis mice treated with or without CAA (20mg/kg).

b-s, Male C57BL/6J Mice (n = 6/group) received 3% DSS in drinking water for 7 days with the following interventions: 1) Vehicle; 2) DSS; 3) DSS + CAA (20 mg/kg/day); 4) DSS + LY294002 (50 mg/kg, twice weekly); 5) DSS + CAA + LY294002.

b, Longitudinal monitoring of body weight changes expressed as percentage baseline (n = 6/group).

c, Intestinal permeability assessment via FITC-dextran (4 kDa) concentration (μg/mL) in mice serum (n = 6/group).

d-g, Colon tissues were isolated on the last day of the experiment.

d, **e**, Representative macroscopic images of colon tissue from each group are provided, and the colon length was recorded (n = 6/group).

f, **g**, Histopathological evaluation of colon sections by H&E staining (scale bar = 100 μm). Histopathological scores of the DSS-induced colitis were evaluated (n = 6/group).

h, i, Flow cytometric analysis of neutrophil (CD11b⁺Ly6G⁺) infiltration in colonic lamina propria (n = 5/group).

j, k, Western blot analysis of citrullinated histone H3 (CitH3) in colon lysates. β -actin was used as a loading control (n = 3).

l, Serum cell-free DNA (cf-DNA, NETs marker) quantification in different groups(n = 6).

m-s, mRNA expression of inflammatory mediators (*Il-6*, *Tnf- α* , *Ifng*, *Cxcl1*, *S100a9* and *Lcn2*) in sorted colonic lamina propria cells (n=6).

Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined by two-sided unpaired t-test.

Safety evaluation of short-term supplementation with CAA and *C. sporogenes*
Finally, to assess the translational potential of *C. sporogenes* and CAA for colitis therapy, we conducted a comprehensive toxicological evaluation in mice exposed to short-term administration of either live *C. sporogenes* or CAA. Histopathological examination of major organs (heart, liver, lung, and kidney) revealed preserved tissue architecture with no signs of necrosis, inflammatory infiltration, or degenerative changes in either treatment group compared to controls (Extended Data Fig.7a). This indicates that neither *C. sporogenes* colonization nor CAA supplementation causes structural damage to major organs.

We further assessed serum biochemical markers to evaluate the functional integrity of vital organs: (1) Renal function markers: blood urea nitrogen (UREA) and creatinine (CREA) levels showed no significant variations, ruling out renal impairment; (2) Hepatic function markers: alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TBIL), and total protein (TP) levels were all within the normal reference range for mice and did not differ among groups; (3) Systemic inflammation markers: C-reactive protein (CRP) and procalcitonin (ProCT) levels were comparable to those of the control group, confirming the absence of systemic inflammatory responses (Extended Data Fig.7b–i).

Collectively, these data confirm that short-term oral administration of *C. sporogenes* or CAA at the therapeutically effective doses for colitis does not induce acute toxicity in mice, including no organ structural damage, no impairment of hepatic/renal function, and no systemic inflammation. These findings provide critical preclinical safety evidence to support the further development of *C. sporogenes* (probiotic) and CAA (postbiotic) as safe candidate interventions for IBD.

DISCUSSION

The intestinal microbiome has emerged as a rich source of diagnostic biomarkers and therapeutic targets for IBD. Here, we demonstrate that *C. sporogenes* is significantly depleted in both IBD patients and experimental colitis models, while supplementation with

this bacterium markedly alleviates colitis severity in two mechanistically distinct murine models—chemically induced (DSS) and genetic (*Il10^{-/-}*) colitis. Mechanistically, our findings reveal that *C. sporogenes* exerts its protective effects via the secretion of the bioactive metabolite CAA in an *AcnA* enzyme-dependent manner. Specifically, CAA inhibits the neutrophil TREM1-PI3K-Akt signaling axis, thereby suppressing pro-inflammatory cytokine release and NETs formation by neutrophils, which ultimately mitigates colonic inflammation in mice. Collectively, these results define a *C. sporogenes*-CAA-TREM1 axis that is essential for colitis mitigation. (Extended Data Fig. 8).

Beyond reported roles of *C. sporogenes* in neuronal regeneration [22] and cardiac repair [23], recent studies reveal how strain-specific ecological origins dictate therapeutic functions. The gas gangrene-derived strain *C. sporogenes* ATCC 19404 ameliorates colitis primarily through tryptophan metabolism and indole-3-propionic acid (IPA) production [44]. In contrast, our study establishes the human gut-adapted strain *C. sporogenes* ATCC 15579 as a commensal bacterium whose depletion correlates with IBD severity across clinical cohorts. Crucially, live *C. sporogenes* ATCC 15579 significantly attenuated DSS-induced colitis. This protective effect was not observed with treatment using heat-killed bacteria or the non-protective *E. coli* MG1655 strain, indicating that live metabolic activity is necessary. We identified *cis*-aconitic acid (CAA) as the core protective metabolite of this strain, distinct from ATCC 19404's IPA-dependent mechanism. This CAA-mediated efficacy extended to both chemically-induced (DSS) and spontaneous (*Il10^{-/-}*) colitis, with preliminary safety data supporting translational feasibility given its human fecal origin and non-pathogenic characteristics.

While *Flavonifractor plautii*-derived CAA has been implicated in vascular protection [45], our work extends the physiological relevance of CAA to intestinal barrier integrity in IBD. We demonstrated that both live *C. sporogenes* ATCC15579 and exogenous CAA were able to effectively maintain the production of tight junction proteins (claudin-1, occludin, ZO-1) and mucins, thereby counteracting colitis-specific paracellular permeability and ultimately alleviating colitis [46]. Genetic manipulation is a powerful tool for dissecting microbiota-host interactions at a molecular level [47,48]. Applying this approach, we demonstrated that the *acnA* gene is essential for CAA biosynthesis in *C. sporogenes*. The loss of colitis protection in *acnA*-KO strains (CSKO), despite intact gut colonization, further underscores that CAA production, not mere microbial presence, drives therapeutic efficacy. Notably, *acnA* is not unique to *C. sporogenes* but conserved across diverse gut bacteria [45]. This suggests that intestinal CAA levels are likely co-regulated by an *acnA*-expressing bacterial consortium, though current studies remain limited to single-strain characterizations. Our successful heterologous expression of *acnA* to *E. coli* (ECOE), which rescued CAA production and therapeutic activity, confirms that the enzyme's function is conserved across species and highlights the potential of engineered metabolite delivery systems. Future work should map the broader *acnA⁺* microbiome network regulating gut CAA homeostasis, particularly in dysbiotic states where native

microbial engraftment fails. Such efforts may unlock synergistic strategies targeting microbial consortia rather than single species.

Emerging evidence has established TREM1 as a critical amplifier of inflammatory responses in colitis, driving neutrophil activation, pro-inflammatory cytokine release, and disease progression in IBD [49-51]. Prior studies demonstrate that genetic ablation of TREM1 in mice significantly attenuates intestinal inflammation, positioning it as a promising therapeutic target for IBD [51]. Our work identifies that the gut microbiota-derived metabolite CAA from *C. sporogenes* functions as a TREM1 inhibitor. By targeting TREM1 on neutrophils, CAA suppresses pro-inflammatory signaling and NETs formation, thereby preserving mucosal integrity and dampening colitis severity. Although molecular docking analyses suggest that CAA binds TREM1 via hydrogen bonds to residues TYR116 (3.0 Å, 3.1 Å), GLN56 (3.1 Å, 3.3 Å), TYR166 (4.1 Å), and hydrophobic interactions with TYR116 (3.7 Å), we have not yet experimentally validated the precise binding mode or conformational dynamics of this interaction. Future studies employing site-directed mutagenesis of TREM1 (e.g., TYR116A, GLN56L) are essential to map the exact binding interface and quantify the affinity of CAA for TREM1. Such insights could guide the rational design of CAA-inspired synthetic analogs or other advanced therapeutic modalities with optimized TREM1-inhibitory potency.

While our study robustly established the therapeutic efficacy of live *C. sporogenes* and CAA in defined murine colitis models (DSS-induced and *Il10*^{-/-} spontaneous colitis), two translational aspects merit further investigation. First, given the profound heterogeneity of human gut microbiota in IBD patients, validating the intervention efficacy in models harboring complex human-derived microbial communities, such as humanized microbiome mice, is essential to predict clinical relevance. Second, advancing these discoveries toward clinical application requires optimizing delivery strategies for enhanced compound stability, defining long-term dosing regimens, and comprehensively assessing safety profiles in chronic disease contexts.

In conclusion, our study delineates *C. sporogenes*-derived CAA mitigates intestinal inflammation by directly targeting TREM1 on neutrophils. Beyond advancing our understanding of microbiome-immune crosstalk in IBD pathogenesis, these findings highlight CAA as a promising therapeutic agent, particularly for patients with IBD characterized by dysregulated neutrophil activation.

Method

Participants

The procedures involving patients were approved by the Medical Ethics Committee of Nanfang Hospital (approval number NFEC-2024-465; Guangzhou, China). IBD diagnoses were assessed by a standard combination of endoscopic, clinical and histological criteria. The demographic characteristics are shown in Supplemental Table1. Fecal samples and peripheral blood were collected from 50 IBD patients (male, n = 30; female, n = 20), and

35 healthy controls (male, n = 17; female n = 18). Informed consent was given by all subjects prior to sample collection.

Mice and treatments

Male C57BL/6J mice (8 weeks old, 22–25 g) were purchased from Cyagen Biosciences. Mice were maintained under SPF conditions. Male *Il10*^{-/-} (Il10-deficient) C57BL/6J mice (12 weeks old, 22–25 g) were obtained from the Cyagen Biosciences (Extended Data Fig. 1a).

Both *Trem1*^{fl/fl} mice and Ly6G-CreERT2 mice with a C57BL/6J background were purchased from Shulaibao Biotechnology Co., Ltd. *Trem1*^{fl/fl} (C57BL/6J) mice were crossed with Ly6G-CreERT2 mice to generate tamoxifen-inducible Neut-specific TREM1-deficient mice (*Trem1*^{NeutKO}). The DNA of mice was extracted using a commercial reagent kit (Vazyme, PD101) for *Trem1*^{fl/fl} mice and Ly6G-CreERT2 mice genotyping. Then DNA was amplified by PCR (primer information: *Trem1*-flox primers: F:CTCTCCCATTATGCACTGGTCTC; R:GAATCCAGTTCTTTCTGATGCCCT; Ly6G-2A-CreERT2 WT primers: F: GAGACAAGTCTGGGCCATCC; R:CGCGCGCCTGAAGATATAGA. Ly6G-2A-CreERT2 WT Mutant primers: F: TGCTTCTGTTTCAGCCTGGTT; R:CCTCCATTTC AATAGGTAG). Then PCR products were subjected to DNA electrophoresis and the results were analyzed. Both *Trem1*^{NeutKO} and *Trem1*^{fl/fl} mice of 8 weeks of age were injected intraperitoneally with tamoxifen every 24 h for five consecutive days.

Chemically-induced colitis was established by providing the mice with 3.0% (wt/vol) DSS (MP Biomedicals, 216011080, molecular weight of 36,000–50,000) dissolved in drinking water for 7 days. Starting from the first day of the DSS challenge, live *C. sporogenes*, live *E. coli*, live *C. sporogenes* culture supernatant, heat-killed *C. sporogenes*, CSKO, CSWT, ECOE or ECWT was administered by gavage at the indicated concentration (1×10⁸CFU/mL, 150μL) per day. CAA was administered by gavage at concentration of 20 mg/kg per day. Treatments were continued for 7 consecutive days.

Il10^{-/-} mice were administered with live *C. sporogenes* or CAA in the same concentration for 30 consecutive days. LY294002 was administered by intraperitoneal injection with dosage of 50 mg/kg twice in seven days[52].

To deplete neutrophils, mice were administered intravenously with 200 μg of anti-mouse Ly6G antibody (anti-Ly6G, BioLegend, 127632) or an equal dose of Ultra-LEAF™ Purified Rat IgG2a, κ Isotype Ctrl antibody (Con, BioLegend, 400544) as control. For macrophage depletion, mice received an intraperitoneal injection of 150 μL of clodronate-loaded liposomes (Lipo, FormuMax, F70101C-AC-2) or the same volume of control liposomes (Con, FormuMax, F70101C-AC-2) from the same combo kit as control. All treatments were administered 24 hours before and on days 1, 3, and 5 of the 3% DSS treatment.

The safety of *C. sporogenes* and CAA was evaluated in mice. In brief, 18 mice were randomly divided into three groups, including *C. sporogenes* group, CAA group and vehicle group. Mice in *C. sporogenes* group or CAA group were gavaged with CAA (20mg/kg) or *C. sporogenes* (10^8 CFU/mL, 150 μ L) every 24h for seven consecutive days. Then mice were sacrificed for the detection of organ damage. All kits for testing serum biochemical indices of mice were listed in Supplemental Table2.

All mice in vehicle groups were gavaged with PBS (150 μ L), except vehicle group in Extended Data Fig.2 were gavaged with sterile Reinforced Medium for Clostridia (RCM). All experiments began after 1 week of adaptive feeding. All mice were fed on a 12:12 h light:dark cycle, temperature of 22–24 °C, humidity at 50 $\pm$ 15% with free access to water and food at the Animal Institute of South China Agricultural University. The experimental animals were randomly assigned to a treatment after genotype identification. All animal experiments in this study were approved by the Welfare and Ethical Committee for Experimental Animal Care of South China Agricultural University.

Germ-free mice experiment

Germ-free (GF) mice were purchased from Cyagen Biosciences (Suzhou, China). In brief, 12 male C57BL/6J GF mice aged 8 weeks old were randomly assigned to the *C. sporogenes*-treated group and the vehicle-treated group. Experiments began after 1 week of adaptive feeding. Mice were gavaged with *C. sporogenes* (1×10^8 CFU/mL, 150 μ L) or PBS (150 μ L) as negative control. Seven days after the treatment, mouse plasma and feces were gathered for quantitative detection of CAA and *C. sporogenes* abundance.

Bacterial culture

Clostridium sporogenes (*C. sporogenes*, ATCC 15579) was used in the current study. *C. sporogenes* was cultured under strictly anaerobic conditions at 37 °C in RCM. *Escherichia coli* (*E. coli*, MG1655) was used as bacterial control and cultured under aerobic conditions at 37°C in a LB Broth. One day after culture, bacterial pellets were harvested by centrifugation and bacterial supernatant was collected through a 0.22 μ m filter. Bacterial pellets were resuspended in PBS. The concentration of bacteria was detected by measuring optical density (OD) at 600 nm wavelength. Bacteria supernatant was collected through a 0.22 μ m filter. Heat-killed *C. sporogenes* pasteurisation for 30 min at 70°C.

Microbial gene-editing strategies

The aconitate hydratase A (*acnA*, Gene ID: CLOSPO_01101) knockout *C. sporogenes* (CSKO) was performed using the homologous recombination technique. Briefly, we amplified a construct containing the 1011-bp upstream flanking regions upstream-*acnA*, thiamphenicol-resistant (*catP*) gene, theophylline-dependent riboswitch and a 1019-bp downstream flanking regions down-*acnA*, and the plasmid pJB94 was constructed by seamless cloning. The purified plasmids were transformed into competent cells of *E. coli* CA434, which were used as donor cells for subsequent matings. Plasmid-harboring *E. coli* CA434 cells were cultivated at 37°C in LB medium containing chloramphenicol as the selection marker. *C. sporogenes* cells were grown under anaerobic conditions at 37°C in

RCM and were then used as recipients for the conjugal mating with donor cells. Donor cells (*E. coli* at OD₆₀₀ 0.8) and recipient cells (*C. sporogenes* at OD₆₀₀ 1.0) were mixed at cell ratios of 1:2 in 1500 µL of mating medium. After mixing, the cells were centrifuged, and the pellet was resuspended. The suspension was then plated onto RCM plates and incubated anaerobically at 37 °C for 48 hours. Monoclones were selected and confirmed by PCR using primers (acnA-JD-F/R:CTGGTGAAGTACATCACCGACGA, acnA-JD-R:ATTCTATTAGACTCTCGTTTGGA) in the upstream and downstream flanking regions. The transformed positive clones were first inoculated into RCM solid medium supplemented with 10 mM theophylline and cultured at 37 °C. The following day, clones were transferred to fresh medium and incubated overnight at 37 °C. Subsequently, the grown clones were streaked onto two types of RCM agar plates: one with and one without thiamphenicol (5 µg/mL). Clones that failed to grow on the thiamphenicol-containing plates were selected as potential *acnA* knockout candidates. These candidates were finally confirmed by PCR using the acnA-ter and pJB-JD primer sets, yielding the CSKO strain.

The primer sequences of target genes used are listed below:

acnA-ter: Forward: 5'- GGATTCTACAGGAAGTATGGC-3'; Reverse: 5'- ATTTGTTCTGCGACTATTAT-3'

pJB-JD: Forward: 5'- CTGGTGAAGTACATCACCGACGA-3'; Reverse: 5'- ATTCTATTAGACTCTCGTTTGGA-3'

E. coli mutant strain was constructed by Forhigh Biotech (Hangzhou, China). In brief, *C. sporogenes acnA* sequence (*acnA*, Gene ID: CLOSP0_01101) was amplified by PCR using acnA-attλ-F/R primers. Plasmid backbone was reverse-amplified from attλ-up-tac-sfgfp-down-pUXT using attλ-his-F/acnA-R primers, and the plasmid was constructed by seamless cloning. Fusion products were cloned into *E. coli* DH5a, positive transformants were selected with ampicillin and verified by PCR using M13-F(pUC)/M13-R(pUC) primers. The constructed plasmid was used as template for PCR amplification of *acnA* flanked by attλ homologous arms using attλ-up-F/attλ-down-R primers. CRISPR-Cas9 system was applied for subsequent genome engineering. To knockin *acnA*, pCas9 plasmid (provided by Forhigh Biotech) was introduced into competent *E. coli* MG1655 and selected with kanamycin. The pCRISPR-MG1655-attλ sgRNA was designed with target site sequence 5'-TCAAGTTAGTATAAAAAAGC-3'. Next, *acnA* flanked by attλ homologous arms and pCRISPR-MG1655-attλ sgRNA were co-transformed into competent *E. coli* MG1655 by electroporation, followed by recovery on agar plates containing both kanamycin and spectinomycin. pCRISPR-MG1655-attλ sgRNA and pCas9 were cured by culturing at 42°C respectively. Integration of *acnA* into *E. coli* MG1655 (OE strain) was validated by PCR using primers attλ-up-F/R.

The primer sequences used are listed below:

M13-F(pUC): Forward: 5'- CCCAGTCACGACGTTGTAAAACG-3'; Reverse: 5'- AGCGGATAACAATTTACACAGG-3'

attλ: Forward: 5'- CACCCGGAGCGGCAGGGACATAT-3'; Reverse: 5'- CTGCTACGACCCGGATGCGCCAA-3'.

Preparation of human peripheral neutrophils

After density gradient centrifugation, neutrophils were collected from peripheral anticoagulated blood samples. In short, peripheral blood samples were firstly diluted with an equal volume phosphate-buffered solution and then carefully layered it on Ficoll Paque PLUS (Univ, 17144002) followed with gradient centrifugation at 2000 rpm for 30 min at 20°C. The bottom layer was collected to obtain neutrophils after hypotonic lysis of red blood cells with Lysing Buffer (BD Biosciences, 555899) according to manufacturer's instructions. Finally, neutrophils were resuspended in RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% FBS for next use. The purity of neutrophils (CD66b⁺, ≥90%) was confirmed by flow cytometric analysis before any assays (Extended data Fig. 3b).

Induction of NETs

Round coverslips were pre-coated with polyL-lysine (0.5 mg/mL) to promote the adhesion of neutrophils. The experiment used a 24-well sterile coverslips coated with poly-L-lysine. Neutrophils were pre-incubated with or without CAA (100 µM) for 30 min and then stimulated with Phorbol 12-myristate 13-acetate (PMA, 100 ng/mL) for 4 h to induce NETs. Finally, adherent neutrophils were fixed in 4% PFA and incubated with Hoechst 33342 (1:1000) to stain decondensed chromatin networks. Photographs of all specimens were observed under fluorescence microscope (Leica). The NETs fluorescence intensity of each specimen was analysed by image J (v.1.50b) followed by statistical mapping using GraphPad Prism (v.9.5.0).

Quantification of cell-free DNA

Quantification of cell-free DNA (cf-DNA) from murine plasma was performed with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, P7581) as per the manufacturer's instructions.

HL-60 culture

The human promyelocytic leukemia cell line HL-60 was also procured from Procell Life Science & Technology Co., Ltd. (Wuhan, China) and cultured in IMDM (Iscove's modified Dulbecco's medium) in a humidified 5 % CO₂ incubator at 37 °C. An HL-60 cell density of 10⁶ cells/mL underwent differentiation into neutrophil-like cells using 1.3 % DMSO for up to six days.

Bone marrow-derived neutrophil (BMDN) culture

Bone marrow cells were flushed with PBS from mouse femur and tibia using 25-gauge needles. The cell suspension was gently disaggregated and passed through 40µm cell strainer to produce single cell suspension. Red blood cells were removed by RBC lysis buffer. BMDN were purified with MojoSort Mouse Ly6G Selection Kit (BioLegend, 480124) as the BMDNs, which were used for subsequent applications. BMDNs purity and viability were assessed by flow cytometry (Extended data Fig. 3c).

Histopathology and Immunofluorescence (IF)

Colon tissue samples were fixated in 4% paraformaldehyde and were embedded in paraffin. Paraffin-embedded sections were deparaffinized and rehydrated, followed by staining with Hematoxylin and Eosin (H&E). For pathological assessment, the H&E stained sections were evaluated by a blinded pathologist following the criteria of histological score previously reported [53]. The colitis score (maximum = 8) was the sum of the following two features: Epithelium (0: Normal morphology; 1: Loss of goblet cells, 2: Loss of goblet cells in large areas; 3: Loss of crypts; 4: Loss of crypts in large areas), and infiltration (0: No infiltrate; 1: Infiltrate around crypt basis; 2: Infiltrate reaching to lamina muscularis mucosae; 3: Extensive infiltration reaching the lamina muscularis mucosae and thickening of the mucosa with abundant edema; 4: Infiltration of the lamina submucosa).

For immunofluorescence analyse of mouse samples, colonic tissues were sectioned at a thickness of 3 μ m, and the slides were treated with Tris-EDTA to retrieve antigens. Then, the tissue sections or fixed cells were incubated with primary antibodies overnight at 4 °C after being blocked with 5 % BSA in PBS for 60 min at room temperature. The next day, the tissues or cells were incubated with the Goat anti-Rabbit IgG (H+L) Alexa Fluor™ 488 (Invitrogen, A-11008) at room temperature for 1 h. Nuclei were mounted with DAPI. Images were acquired on a fluorescence microscope. Antibodies used in this study were listed in reporting summary. The immunofluorescence intensity of each slide was analysed by image J followed by statistical mapping using GraphPad Prism.

Alcian Blue/Periodic Acid–Schiff staining

Paraformaldehyde-fixed colonic tissue segments were stained using an Alcian Blue/Periodic Acid–Schiff (AB-PAS) kit (Solarbio, G1285) according to the manufacturer's provided protocol. Next, the images of the underlined tissue segments were taken via microscope (Olympus).

Isolation of lamina propria cells from mice colon

The colons were opened longitudinally, rinsed with PBS (Gibco, 10010023) to eliminate fecal matter, cut into 1 cm long strips, followed by stirring in Hanks' Balanced Salt Solution (HBSS, Gibco, 14025-076) containing 1 mM DTT (Fudebio, FD0011) and 2 mM EDTA (Gibco, 17892) at 37 °C for 30 min. The isolation of the cells was performed from the intestinal lamina propria following a previously described method. In brief, the intestinal tissue was digested using 1 mg/mL of collagenase (type IV, MCE, 9001-12-1), and 5% FBS (Gibco, A5670701) in RPMI 1640 medium (Gibco, 11875093) at 37 °C for 30 min with stirring. Every 30 minutes, we centrifuged the released cells and stored them in the culture medium. Fresh collagenase was added to replace the mucosal pieces at least 2 times. Finally, cells were collected for flow cytometry.

Flow cytometry

Cells were washed twice with PBS and treated with an Fc receptor-blocking antibody. Then single-cell suspensions were stained with antibodies labeled with different fluorescein according to the panel (incubating at 4 °C in the dark for 30 min). After surface

protein staining, cells were fixed with fixation. Flow cytometry was performed on the Fortessa (BD). Neutrophils (Ly6G⁺CD11b⁺) were isolated from the mouse colonic lamina propria by flow cytometric sorting using a FACS Aria III (BD Biosciences). Post-acquisition data analysis was performed using FlowJo software (v10.8.1). The gating strategy is detailed in Extended Data Fig. 3a. Antibodies used in this study were listed in reporting summary.

Metabolomics analysis

For non-targeted metabolomics, transfer 100 μ L of sample (bacterial culture supernatant or medium) to an EP tube and add 400 μ L of extraction solvent (methanol: acetonitrile = 1:1 (v/v)) containing isotope-labeled internal standards. Vortex the mixture for 30 s and sonicate it for 10 min in an ice-water bath. Then, incubate the mixture at -40 °C for 1 h. Thereafter, centrifuge the samples at 12,000 rpm (13,800 $\times$ g, rotor radius 8.6 cm) for 15 min at 4 °C. Finally, transfer the supernatant to an injection vial for instrumental analysis. For sample detection, LC-MS analyses were performed by Biotree (Shanghai Biotree Biotech Co. Ltd.) based on an UHPLC system. Two mobile phases were used: mobile phase A (5 mmol/L ammonium acetate and 5 mmol/L acetic acid in water) and mobile phase B (acetonitrile). An Orbitrap Exploris 120 mass spectrometer was used to acquire high-resolution MS/MS spectra. More detailed information is available on MetaB (<https://metab-platform.github.io/>). All samples were analyzed in a single batch, and run order was assigned stochastically to each sample. Nineteen quality control (QC) samples were distributed evenly throughout the sample set to monitor the stability of the instrument during the course of data acquisition. For data pre-processing and QC, an in-house program was used for peak detection and alignment. Then, an in-house MS2 database (BiotreeDB) and online databases (KEGG and Human Metabolome Database (HMDB)) were applied to match metabolites based on the exact mass data (m/z). To reduce the impact of systematic errors on the results and highlight biological variations, features that were detected in less than half of samples were removed as previously reported [54, 55]. For the remaining peaks, the missing values were extrapolated with one-half of the lowest observed value of all detected samples. QC samples were used to evaluate the stability of instrument and normalize the variations during the run. For dimensionality reduction, normalized data were used to perform dimensionality reduction analysis. R package `49` and `Rtsne50` were used in the dimensionality reduction analysis. For identification of differential metabolites and metabolite set enrichment analysis, Student's t-test was used for the identification of differential metabolites. Metabolite set enrichment analysis was conducted using the R package `MetaboAnalystR51`.

The LC-MS/MS analysis was performed by Verygenome Technology Co., Ltd. (Guangzhou, China). Specifically, for the analysis of CAA in human plasma, human feces, mice plasma, mice feces, bacterial culture supernatant or medium, 100 μ g of samples were extracted by 500 μ L methanol. 10 μ L internal standard mixed solution (250 ng/mL) was added into the extract as internal standards (IS) for the quantification. Then the extract was vortexed for 3 min, then kept in a refrigerator at -20 °C for 30 min, centrifuge at 12000 r/min for 10 min at 4 °C, 150 μ L of the supernatant was collected. The supernatant was

again centrifuged at 12000 r/min for 5 min at 4 °C, 100 µL of the supernatant was transferred for further LC-MS analysis. Then, a 10 µL of supernatant was injected into an Waters ACQUITY UPLC HSS T3 C18 column (100 mm×2.1 mm i.d. 1.8 µm) and samples were analyzed by the LC-MS/MS system using a ExionLCAD HPLC system coupled to an QTRAP® 6500+ triple quadrupole mass spectrometer System. Mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The samples were eluted by gradients as follows: 10% B, 1min 95% B, 8min; 95% B, 9.5min; 10% B, 12min. The ESI source operation parameters were as follows: ion source, ESI+/-; source temperature 550 °C; ion spray voltage (IS) 5500 V (Positive) , -4500 V (Negative); curtain gas (CUR) was set at 35 psi, respectively. CAA was analyzed using scheduled multiple reaction monitoring (MRM).

For ¹³C metabolic flux analysis, *C. sporogenes* were incubated with citric acid (Cambridge Isotope Laboratories, CLM-9021-0.01) for 1h at the initial OD600 was approximately 0.4, then the supernatant was collected for detected ¹³C-labeled CAA. For quantification of ¹³C-labeled CAA, 50µL extracted samples were injected into an Hypersil Gold C18 column (Thermo, Part No. 25002-102130) and samples were analyzed by the LC-MS/MS system using a thermo UPLC system (Vanquish) coupled to an HRMS (Obritrapp fusion). Mobile phase A was water and mobile phase B was methanol. The samples were eluted by gradients as follows: 5% B, 5min; 20% B, 5min; 95% B, 8min; 95% B, 10min. HRMS after UPLC (UPLC-HRMS) used negative ESI mode (ion source voltage: negative, -2500 V) in the mass range 100-500 Da in full-scan mode with a resolution of 120000. Ion transfer tube temperature was 320°C. Vaporizer Temperature was 350°C and collision energy was in the range 15, 30, 45 eV. The MS analysis was conducted using a negative electrospray ionization ion source. The optimized instrument parameters were source temperature (500°C), ion spray voltage (2500 V), curtain gas (15 psi), ion source gas 1 (55psi), ion source gas 2 (55 psi) and dwell time (100ms). Quantification was performed in multiple reaction monitoring mode at m/z. For data processing, the peak area was integrated by use of MultiQuant 1.2 software (AB SCIEX).

RNA sequencing

Total RNA of mice colon was isolated using the Trizol Reagent (Invitrogen Life Technologies, 15596018), after which the concentration, quality and integrity were determined using a NanoDrop spectrophotometer (Thermo Scientific). Three micrograms of RNA were used as input material for the RNA sample preparations. Sequencing libraries were generated according to the following steps. Firstly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in an Illumina proprietary fragmentation buffer. First strand cDNA was synthesized using random oligonucleotides and Super Script II. Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and the enzymes were removed. After adenylation of the 3' ends of the DNA fragments, Illumina PE adapter oligonucleotides were ligated to prepare for hybridization. To select cDNA fragments of the preferred 400-500 bp in length,

the library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, CA, USA). DNA fragments with ligated adaptor molecules on both ends were selectively enriched using Illumina PCR Primer Cocktail in a 15 cycle PCR reaction. Products were purified (AMPure XP system) and quantified using the Agilent high sensitivity DNA assay on a Bioanalyzer 2100 system (Agilent). The sequencing library was then sequenced on NovaSeq 6000 platform (Illumina) Shanghai Personal Biotechnology Co. Ltd. For KEGG enrichment analysis, p-value <0.05 was used as a threshold to determine significant enrichment for gene sets with the cluster Profiler R package.

RNA isolation and quantitative PCR (qPCR) analysis

Tissues were disrupted using lysis beads in a homogenizer unit (Precellys). RNA of tissues and cultured cells was isolated using the TRIzol reagent (Vazyme, R401) followed by reverse transcription using reverse transcriptase kits (Vazyme, R222). qPCR was performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Q711) on the Roche LightCycler 480 System (Roche). Relative expression levels were normalized to GAPDH and calculated as $2^{-\Delta\Delta C_t}$. The primer sequences of target genes are listed in Supplementary Table 3.

Fecal DNA extraction and the abundance of *C. sporogenes* quantification

Bacterial DNA from human or mouse feces was extracted using the M5 Stool Genomic Plus DNA Kit (Mei5bio, MF114-plus-01). qPCR was performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Q711) on the Roche LightCycler 480 System (Roche). The relative abundance of *C. sporogenes* was determined by qPCR and normalized to the all bacterial load (represented by the 16S rRNA gene abundance amplified using universal primers for all bacteria). Relative abundance was calculated by $-\Delta C_t$ method [20].

The primer sequences used are listed below:

All bacteria (16S rRNA): Forward: 5'- TGSTGCAYGGYTGTCGTCA-3'; Reverse: 5'- ACGTCRTCCMCACCTTCCTC-3'

Clostridium sporogenes (ATCC15579): Forward (Csp_qPCR_F): 5'- TTGGCTCTGCACCGGGAATC -3'; Reverse (Csp_qPCR_R): 5'- CTGCAAACGCCGTCCCTCTT -3'.

Western blotting

RIPA lysis buffer (Beyotime, P0013B) with 1 mM PMSF and protease /phosphatase inhibitor cocktail (Beyotime, ST506) was used to isolate proteins from colon tissues and cells. The lysates were centrifuged (13000 rpm, 4 °C, 15 min) and the supernatants were collected and measured with BCA protein assay kit (CWBI, CW0014). After denaturation with 5x SDS-PAGE buffer, same amounts of proteins (20 µg) were separated on 10 % SDS-PAGE gels, and transferred onto the PVDF membranes. The samples were then blocked in 5 % skim milk for 90 min before incubating the blocked membranes with corresponding primary antibodies overnight at 4 °C. The membranes were then washed with PBST for three times, followed by incubation with secondary antibodies for 60 min at room temperature (RT). Protein bands were visualized using ECL

chemiluminescence detection reagent (Fudebio, FD8020) following membrane incubation. All protein bands were normalized to β -actin. Antibodies used in this study were listed in reporting summary. β -actin was used as internal control.

Molecular docking

The structure of TREM1 (ID: Q9NP99) was obtained from the AlphaFold Protein Structure Database(<https://alphafold.ebi.ac.uk/>), in addition, the structure of CAA (Compound CID: 643757) was downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Afterward, Autodock Vina (v.1.1.2) was utilized for performing molecular docking. PyMOL (v.2.3.0) and Discovery Studio were used to visualize the docking results and examine the interaction between TREM1 and CAA active site. Finally, binding energy was calculated and the comparison was carried out between TREM1 and CAA.

Surface Plasmon Resonance (SPR) experiment

The binding affinity between *cis*-aconitic (CAA) acid and recombinant human TREM1 was determined using the PlexArray HT System (PlexArray, USA) with a 3D Dextran sensor chip (Plexera Bioscience, PX201). The 3D Dextran chip surface was first activated with an equimolar mixture of 0.49 M 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 0.1 M N-hydroxysuccinimide (NHS). Subsequently, recombinant human TREM1 (rhTREM1, Biodragon, BA02067) was immobilized onto the activated chip. The CAA (dissolved in 0.1% double-distilled water) flowed at increasing concentrations (100 μ M, 200 μ M, 500 μ M, 1,000 μ M, and 2,000 μ M). Flow rate was set at 30 μ L/min. The reaction signal data was collected with Plexera Data Explorer and analyzed with BIA evaluation software (v.4.1). The sensorgrams were then plotted using OriginPro (v.9).

Cellular thermal shift assay (CETSA)

BMDNs and HL-60 cells were lysed by RIPA buffer (Beyotime, P0013B), the suspension was freeze thawed in liquid nitrogen for three cycles. Then the lysate mixture was centrifuged at 15000 rpm for 15min at 4 °C. Cell suspensions were collected and aliquoted into 2 fractions. 100 μ M CAA or an equal volume of PBS was added and incubated for 1 hour at room temperature. The samples of each group were divided into 10 tubes equally and heated individually at different temperatures (37, 41, 44, 47, 50, 53, 56, 59, 63, and 67 °C) for 3 min and then cooled for 3 min at room temperature. Then, the samples were centrifuged, and the obtained cells were analyzed by western blotting.

Drug affinity responsive target stability (DARTS) assay

BMDNs and HL-60 lysates were prepared and diluted to 5 mg/mL, then incubated with or without 100 μ M CAA at room temperature for 1 h. Pronase (Sigma-Aldrich, P8811) was added to the samples at a dilution of 1:333 (w/w) for 30 min, then protein stability was analyzed using western blotting.

Pull-down assay

BMDNs and HL-60 were lysed with the pull-down buffer (50 mM Tris-HCl pH 7.4, 200 mM NaCl and 0.05% Tween-20) containing protease inhibitors. The lysate was then incubated with either the anti-TREM1 antibody (Proteintech, 11791-1-AP) or rabbit IgG (Proteintech, 98136-1-RR) with or without 100 μ M CAA at 4°C overnight while gently rotating in the pull-down buffer. The mixture was then incubated with Dynabeads Protein G beads (Invitrogen) for 2 hours at room temperature. Following this, samples were thoroughly washed with pull-down buffer 5 times and pull-down buffer without Tween-20 4 times before being eluted with 50 mM glycine (pH 2.8). A small portion of the eluate was used for western blot analysis to confirm successful pull-down of TREM1 while the remainder was used for LC-MS/MS analysis to detect the level of CAA binding to TREM1.

Statistical analysis

Animals were randomly assigned to treatment groups and data collection was randomized. Although no statistical methods were used a priori to determine sample sizes, our group sizes are consistent with those reported in prior publications[56-60]. Each independent experiment included at least three biological replicates; no data points were excluded from the analyses. Data collection and analysis were performed blind to the conditions of the experiments. Data distribution was assumed to be normal but this was not formally tested. Statistical analysis was performed with GraphPad Prism (v.9.5.0), R (v.1.0.12) and ClusterProfiler (v.10.1.2) with packages described in individual experiments. Statistical analyses of the data were conducted with GraphPad Prism (v.9.5.0) software using two-tailed unpaired t-test (with P value presented) and two-sided Spearman's correlation (reported as *r* and two-sided P value). Significant was defined as *P* values < 0.05. Technical replicates were averaged prior to statistical analysis. Data are presented as mean $\pm$ s.e.m. in bar and scatter plots. Individual data points denote biological replicates (sample size *n* per group detailed in figure legends).

Data availability

Non-target metabolomics data generated in this study have been deposited in OMIX database at the China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences database under accession code OMIX010681. Data access can be requested through the OMIX public portal, but any queries can be directed to Dr. Ye Chen (yechen@edu.smu.cn). This study did not generate any unique codes. All other data can be made available from the authors on reasonable request. Source data are provided with this paper.

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Author information

These authors contributed equally: Weihao Tan, Xiaoru Wei.

Department of Gastroenterology, State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Gastroenterology, Nanfang Hospital, Southern Medical University, Guangzhou, PR China.

Weihao Tan, Xiaoru Wei, Maijiayi Ye, Linjie Li, Pu Wang, Guanyi Lin, Ying Wang, Yunying Gong, Qiujuan Ye, Yuxuan Zhu & Ye chen.

Department of Gastroenterology, Integrative Microecology Clinical Center, Shenzhen Key Laboratory of Gastrointestinal Microbiota and Disease, Shenzhen Clinical Research Center for Digestive Disease, Shenzhen Technology Research Center of Gut Microbiota Transplantation, Shenzhen Hospital, Southern Medical University, Shenzhen, PR China.

Ying Wang, Jiaxin Li & Ye chen.

Guangdong Provincial Key Laboratory for Prevention and Control of Major Liver Diseases;Key Laboratory of Infectious Diseases Research in South China, Ministry of Education; Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, PR China.

Dekai Zheng.

Contributions

1330 Y.C. and W.T. conceptualized the study. W.T., X.W., J.L., M.Y., L.L., P.W., D.Z., G.L., Y.W.,
1331 Y.G., Q.Y.and Y.Z. carried out the investigations. W.T. wrote the original draft. Y.C. and J.L.
1332 reviewed and edited the manuscript. Y.C. and J.L. supervised the study.

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1334 Corresponding author

1335 Correspondence to Jiaxin Li or Ye Chen.

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1338 **Ethics declarations**

1339 Competing interests

1340 The authors declare no competing interests.

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