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Capecitabine Combined with Fecal Microbiota Transplantation Prevents Colorectal Cancer Progression Through Correction of Microbial Dysbiosis and Immune Regulation

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Abstract

The significant economic burden of colorectal cancer (CRC) necessitates the development of innovative therapeutic approaches. Interest in the gut microbiota's role in CRC has increased. Capecitabine, as a chemotherapy, may disrupt the balance of the intestinal microbiota. This study investigated the anticancer effects of capecitabine combined with fecal microbiota transplantation (FMT) in a CRC mouse model caused by azoxymethane and dextran sodium sulfate. FMT was achieved with fecal microbiota from healthy mice through enema. Capecitabine decreased the number and diameter of cancer foci in CRC mice, while FMT supplementation had a more noticeable impact, indicated by increased body weight and survival rate. Capecitabine significantly reduced the abundance of pathogenic bacteria in mice with CRC, such as *Bacteroides*, *Enterorhabdus*, *Monoglobus*, *Rodentibacter*, uncultured_rumen_bacterium, *Turicibacter*, and *Streptococcus*. The supplementation of FMT more effectively reversed the gut microbiota dysbiosis in CRC mice, as demonstrated by the ACE and Chao 1 indices, PCoA analysis, and enhanced normal biological pathways. Microbial dysbiosis induced immunological dysfunction in CRC mice, indicated by abnormal immune cell recruitment and excessive cytokine production. Capecitabine treatment reduced immune cell infiltration, including CD3$^+$ T cells, CD4$^+$ T cells, and CD49b$^+$ NK cells, as chemotherapy often suppresses the immune system. The supplement of FMT increased the proportion of CD4$^+$ T cells, CD49b$^+$ NK cells, CD8$^+$ T cells, and LY6G$^+$ neutrophils, indicating improved immune responses against CRC. Moreover, capecitabine therapy alone reduced the overexpression of IL1a, IL6, IL12a, IL12b, IL17, IL22, FOXP3, STAT3, IFN-γ, TNF-α, TGF-β, GZMA, CXCR4, OPN, PD-1 and PD-L1. FMT supplementation resulted in a higher immune response to CRC, as it had a greater inhibitory effect on the overexpression of inflammatory cytokines and enhanced the production of IL10, IFN-γ, and CXCR4. These cytokines were positively correlated with *Azospirillum_sp._47_25*, *Romboutsia*, *Lactococcus,* Rikenella_sp._Marseille_P3215 and *Turicibacter* and negatively correlated with *Parabacteroids*, unclassified_Oscillospiraceae, *Marvinbryantia*, unclassified_Clostridia_vadinBB60_group, unclassified_Erysipelatoclostridiaceae, *A2*, *Roseburia*, Rikenellaceae_RC9_gut_group, *Acetatifactor* and unclassified_Clostridia. The combination of capecitabine and FMT is more effective at preventing CRC than capecitabine alone, as it reverses gut microbial abnormalities and boosts immune responses to CRC.

Keywords: colorectal cancer, dysbiosis, capecitabine, fecal microbiota transplantation, immune cells, cytokines.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer globally and the second cause of cancer-related mortality[1]. High incidences are observed in Australia, New Zealand, Europe, East Asia, and North America[2]. Risk factors for CRC include age, family history, smoking, drinking, and dietary behaviors[3]. Additionally, prolonged inflammation increases the risk of CRC[4]. Capecitabine is a fluorouracil anti-cancer medication administered orally that inhibits cancer progression by disrupting cellular DNA synthesis[5, 6]. However, as a chemotherapy drug, capecitabine may cause dysbiosis of the gut microbiota. It has also been linked to adverse effects on the gastrointestinal system, such as dyspepsia, stomatitis, enterocolitis, and abdominal pain[7].

The human gut contains more than 1,500 species of bacteria belonging to 50 different phyla, weighing approximately 1.5 kg on average. The gut microbiota influences colorectal carcinogenesis via several pathways, including inflammation, immune response modulation, and altered dietary component metabolism. These pathways can also result in the generation of toxic byproducts originating from microbes, such as metabolites or genotoxins[8]. *Fusobacterium nucleatum* (*F. nucleatum*) may infiltrate a variety of host cells, including fibroblasts, monocytes, epithelial cells, and endothelial cells, triggering a cascade of inflammation and the release of proinflammatory chemokines, such as IL6 and IL8[9]. *Bacteroides fragilis* (*B. fragilis*) may stimulate spermine oxidase expression in colonic epithelial cells, leading to increased reactive oxygen species, and thereby promoting the secretion of proinflammatory cytokines[10]. *Helicobacter pylori* (*H. pylori*) infection can cause persistent active inflammation and several DNA damage, leading to genomic instability[11]. *Streptococcus bovis* (*S. bovis*) recruits myeloid cells, particularly CD11b+TLR-4^+ cells, which may boost pro-tumor immunity in the tumor microenvironment[12]. *Ligilactobacillus* positively correlates with the anti-apoptotic marker Bcl-2 and tumor growth factors, such as NF-κB and β-catenin[13].

However, numerous beneficial microorganisms have been shown to play a protective role against CRC. External *lactobacilli* polysaccharides induce the onset of apoptosis in CRC cells by downregulating the expression of the SURVIVIN and BCL-2 genes and upregulating the expression of the Cas3, Cas9, and BAX genes[14]. *Bifidobacterium* interacts with proteins regulating the cell cycle, inhibiting the proliferation of cancer cells through activating of pro-caspases, downregulating the anti-apoptotic Bcl-2, and upregulating the pro-apoptotic Bax proteins[15]. Roseburia intestinalis produces short-chain fatty acids (SCFA) butyrate, which protects against CRC and potentially improves anti-PD-1 effectiveness by generating functional $CD8^+$ T cells[16]. *Phascolarctobacterium* generates SCFA, such as propionate, which increases host serotonin production in the colon and influences gut motility and hemostasis[17]. Probiotics *Lactobacillus* rhamnosus and *Lactobacillus bulgaricus* promote beneficial bacteria like *Akkermansia, Blautia*, and Ruminococcaceae while inhibiting pathogenic bacteria like *Escherichia coli* (*E. coli*) and *Klebsiella* in the gut[18]. Studies indicate that colibactin-producing *E. coli* may decrease $CD8^+$ T cell infiltration in the CRC mouse model[19]. *Klebsiella pneumoniae* induces precancerous lesions in germ-free mice and causes gut barrier dysfunction via increasing colon macrophage-mediated MMP-2/-9 activity[20].

Dysbiosis in the gut microbiota has a deleterious impact on immunological response, drug metabolism, intestinal mucosal barrier integrity, and pathogen defense[21]. FMT, which transfers healthy gut microbiota into the patient's gut by enema, colonoscopy, or capsule, is effective in treating gut microbiota dysbiosis[22]. FMT has been authorized by the FDA for the treatment of recurrent Clostridioides difficile infections[23]. In this investigation, a chemically induced CRC mouse model was employed to explore the combined effect of capecitabine and FMT on the development of CRC. Compared to capecitabine alone, combination treatment demonstrated greater anti-cancer potential. FMT reversed gut microbial dysbiosis induced by CRC and capecitabine. The combination of capecitabine and FMT enhances the immune responses against CRC by recruiting anti-cancer immune cells and regulating the expression of cytokines within the microenvironment.

2. Materials and methods

2.1. The CRC mouse model

Eight-week-old male Balb/c mice were obtained from Beijing Vital River Lab and housed without any intervention for a week. The mice were administered intraperitoneally the carcinogen azoxymethane (AOM, Sigma) at a dose of 10 mg/kg in the first week. Subsequently, the mice were provided with water containing the proinflammatory agent dextran sodium sulfate[24] (DSS, 2.5%, MP Biomedicals) in the second week. During weeks three and four, the mice were given drinking water without DSS. The treatment from weeks 2 to 4 was repeated for two more cycles. The mice were randomly and equally divided into four groups, normal control mice, CRC mice, capecitabine-treated CRC mice, and CRC mice treated with both capecitabine and FMT. The mice in each group were randomly allocated to four cages, with five mice per cage. All animal protocols of this study were reviewed and approved by the ethical guidelines of the Institutional Animal Care and Use Committee of Harbin Medical University (ethics code: IRB3079724) under the ARRIVE guidelines. Capecitabine (700 mg/kg, Roche) was mixed in 40 mM citrate buffer (pH 6.0) with gum arabic and then given orally once a day for 14 days, followed by a week of rest; this cycle was repeated three times[25]. The severity of CRC was evaluated using the disease activity index (DAI), a multi-parameter scoring system on a scale of 0 to 4, which included the number of cancer foci, the diameter of the cancer foci (mm), the length of the colon (cm), the percentage of body weight loss, the survival rate, the consistency of the stool, fecal blood, and the histology of intestinal tissue (Table 1).

Table 1. Criteria of disease activity index.

Score	**Cancer Foci Number**	**Cancer Foci Diameter (mm)**	**Colon Length (cm)**	**Body Weight Loss (%)**	**Survival Rate (%)**	**Stool Consistency**	**Hematochezia**	**HE Staining**
0	Normal colon	None	>15	0%	100%	Normal	None	Normal mucosa
1	1–2 colonic polyps present	<2 mm	13–15	1-5%	75–99%	Soft	Blood steak	Mild, <10%
2	3−5 colonic polyps present	2–3 mm	11–13	5-10%	50–74%	Semi-loose	Blood Clot	Mild to moderate, 10−25%
3	5−10 colonic polyps present	4–5 mm	10–11	10–15%	25–49%	Loose stool	Visible bloody stool	Moderate, 26−50%
4	>10 colonic polyps present	>5 mm	<10	>15%	0–24%	Diarrhea	Gross bleeding	Severe, >51%

2.2. Fecal microbiota transplantation

Fresh feces from normal control mice were collected, mixed with PBS at a concentration of 200 mg/ml, and centrifuged at 1,500 rpm for 1 min to obtain microbial supernatant. Each recipient received 1 ml of fecal slurry by enema once every 3 days for 9 weeks.

2.3. HE staining

Colorectal tissues from mice were fixed in 10% neutral formaldehyde for 24 hours before being embedded in paraffin. Paraffin slides of 3 mm colorectal tissues were stained with hematoxylin for 2 min and eosin for 1 min. After dehydration and hyalinization with ethanol and xylene, the tissues were sealed with neutral balsam. Finally, the prepared slides were imaged using a microscope (Olympus). The study evaluated colon inflammation using criteria for detecting inflammatory cell infiltration.

2.4. High-throughput sequencing of 16S rDNA

High-throughput 16S rDNA sequencing was utilized to assess the quantity, diversity, and similarity of gut bacteria. Before collecting fecal samples, the mice were kept for one week without any treatment to avoid the adverse effects of residual fecal bacteria. Subsequently, mice were moved to a sterile cage for feces sample collection. Two tubes of feces were collected from each cage and used for high-throughput sequencing of the intestinal microbiota (BioMarker, Beijing, China). Data were submitted to NCBI (PRJNA1063875), which contained six replicates in each experimental group. A standard OTU sequence with 97% similarity was employed for microbial diversity analysis.

2.5. Immunohistochemical detection of immune cells

Paraffin sections were dewaxed in xylene and rehydrated in ethanol before performing antigen retrieval in a microwave at high power for 2 min, followed by low power for another 10 min. Endogenous peroxidase activity was suppressed using 3% H2O2 over 10 min. Tissues were blocked using goat serum for 1 h at room temperature. Tissue sections were incubated overnight at 4°C with primary antibodies such as cleaved caspase-3 (9661, CST), Ki67 (A2094, Abclonal), CD3 (ARG52744, Arigo), CD4 (ab183685, Abcam), CD8 (ab217344, Abcam), FOXP3 (GB11093, Servicebio), F4/80 (ARG22476, Arigo), CD49b (AGR57601, Arigo) and LY6G (MAB1037100, R&D). The tissues were then incubated with secondary antibodies: HRP goat anti-rat IgG (H+L) (AS028, Abclonal), HRP goat anti-rabbit IgG (H+L) (AS014, Abclonal), and HRP goat anti-mouse IgG (H+L) (bs-0296G-HRP, Bioss) for 1 h at 37 °C. Subsequently, the tissues were stained using the DAB Peroxidase Substrate Kit (zli-9019, ORIGENE). After being dried in ethanol and xylene, the slides were sealed with neutral balsam. The distribution and staining intensity of positive markers were captured by the microscope (Olympus) and analyzed by ImageJ.

2.6. Western blot

Tissue proteins from samples were denatured in 5×SDS-PAGE loading buffer and electrophoresed on tris-glycine agarose gels. The separated proteins were then transferred to a nitrocellulose membrane, followed by a two-hour blocking step at room temperature using 5% non-fat milk. The membrane was then incubated at 4°C overnight with primary antibodies such as IL1a (A2170, Abclonal), IL6 (A11115, Abclonal), IL12a (A20383, Abclonal), IL12b (A13024, Abclonal), IL17 (A12454, Abclonal), IL10 (JES-2A5, Arigo), IL22 (A6216, Abclonal), FOXP3 (A5706, Abclonal), STAT3 (ARG-54150, Arigo), IFN-γ (A12450, Abclonal), TNF-α (BS1857, Pbclonal), TGF-β (A18692, Abclonal), GZMA (A6231, Abclonal), CXCR4 (A1303, Abclonal), OPN (A1499, Abclonal), PD-1 (A11973, Abclonal), PD-L1 (A11273, Abclonal), Tubulin (AC008, Abclonal) and GAPDH (A19056, Abclonal). Subsequently, the membrane was incubated for 2 hours at room temperature with the secondary antibody HRP goat anti-rabbit IgG (H+L) (AS014, Abclonal) or HRP goat anti-mouse IgG (H+L) (AS003, Abclonal). Band intensity was measured using Tanon-5200 and Tanon MP.

2.7. Statistical analysis

The Student-Newman-Keuls (SNK) test and log-rank test were used for statistical analysis. For analysis measuring the degree of association between sequenced OTU abundances of bacterial species with cytokines variations, Spearman's rank correlation was performed. GraphPad Prism 9.5 was used for statistical comparisons. Data was expressed as the mean±SD, and a two-tailed value of $p<0.05$ was taken to indicate statistical significance. Experiments were conducted at least three times to ensure accuracy.

3. Results

3.1. FMT combined with capecitabine inhibited CRC development more effectively than capecitabine alone

The schedule for establishing the CRC mouse model over a period of 10 weeks and treatment with capecitabine and FMT is shown in Figure 1A. In CRC mice, the average number of cancer foci was 13.3 with a diameter of 3.3 mm, whereas in capecitabine-treated mice, the average number of cancer foci was 8.8 with a diameter of 2.1 mm. Notably, the CAP-FMT treatment group displayed a mean of 4.3 neoplastic lesions (1.5 mm in diameter), accompanied by a statistically significant decrease in histopathological scoring. In CRC mice, there was a noticeable reduction in colon length due to epithelial overgrowth and inflammatory adhesion. When combined with FMT, capecitabine reversed the shortening and lengthened the colon, but capecitabine by itself was ineffective on its own (Figure 1B-E). As shown in Figure 1G, the CRC mice lost significantly more weight than the normal mice. Combined therapy with capecitabine and FMT reduced weight loss, although capecitabine did not. Additionally, the survival rate increased dramatically in CAP-FMT-treated mice, going from 55% (11/20) to 80% (16/20), while the proportion of mice treated with capecitabine only increased to 70% (14/20) (Figure 1H). The calculated DAI scores were significantly increased in the model group mice. Capecitabine treatment significantly reduced the DAI scores, and the addition of FMT led to a further decrease (Figure 1F). HE staining indicated that the CRC mice displayed evident colonic tissue damage, largely shown by the disrupted crypt structure and colonic mucosa, and increased inflammatory cell infiltration, leading to a thicker and weaker intestinal wall. Treatment with capecitabine did not improve colon structure but increased cancer cell apoptosis and decreased Ki67 expression. Nevertheless, the alleviated colon tissue damage was observed in mice receiving CAP-FMT treatment (Figure 1I-K).

3.2. FMT reversed gut microbial dysbiosis induced by CRC and capecitabine

The Illumina Novaseq platform was used to detect 16S rDNA of the gut microbiota. Each sample yielded an average of 89,665 clean reads after quality filtering. The gut microbiota was classified into 31 phyla, 58 classes, 169 orders, 326 families, 617 genera, and 722 species. The dominant bacteria in normal control mice were genera of Muribaculaceae (20.40%), *Alloprevotella* (16.52%), Lachnospiraceae (14.27%), *Alistipes* (8.99%), Rikenellaceae_RC9_gut_group (1.18%), unclassified_Clostridia_vadinBB60_group (0.87%), *Colidextribacter* (0.82%), Lachnospiraceae_UCG_006 (0.57%), *Roseburia* (0.55%), unclassified_Ruminococcaceae (0.49%), *Muribaculum* (0.35%), *Oscillibacter* (0.34%) and Candidatus_Udaeobacter (0.18%). However, the relative abundance of the gut microbiota in CRC mice was significantly altered at the phylum, family, genus, and species levels compared to normal control mice (Figure 2A-D). The dominant proportions of bacteria were *Bacteroides* (9.44%), *Ligilactobacillus* (1.73%), *Enterorhabdus* (0.90%), *Monoglobus* (0.45%), *Rodentibacter* (0.34%), uncultured_rumen_bacterium (0.29%), unclassified_RF39 (0.24%), *Turicibacter* (0.14%) and *Streptococcus* (0.05%) in CRC mice. Capecitabine reduced the most prominent bacteria: *Bacteroides*, *Enterorhabdus*, *Monoglobus*, *Rodentibacter*, uncultured_rumen_bacterium, *Turicibacter*, and *Streptococcus*. However, in capecitabine-treated mice, some gut microbiota with decreased abundance in CRC mice were less abundant, such as unclassified_Lachnospiraceae (4.1%), unclassified_Clostridia_vadinBB60_group (0.4%), *Colidextribacter* (0.17%) and Candidatus_Udaeobacter (0.001%). In this study, FMT was used to reverse the microbial dysbiosis induced by CRC and capecitabine.

FMT significantly increased the richness of the microbial community as represented by the ACE and Chao1 index (Figure 3A, B) and promoted the diversity of the gut microbiota as indicated by the PCoA analysis (Figure 3C). In Figure 3D, the most abundant gut bacteria in CRC mice showed complex positive or negative correlations with other gut bacteria, including *Bacteroides*, Prevotellaceae_UCG_001, *Candidatus saccharimonas*, *Ligilactobacillus*, *Lactobacillus,* and uncultured_rumen_bacterium, implying that variations in the number of microbes both influence and are influenced by the abundance of other microorganisms. A balanced gut microbiota is necessary to preserve a healthy gut microenvironment. Although the microbial dysbiosis of CRC mice was largely reversed by FMT, some gut bacteria were still difficult to colonize, such as unclassified_Alloprevotella, unclassified_Lachnospiraceae and *Rhodanobacter*, as revealed by LEfSe analysis (Figure 3E). To increase the possibility of colonization, these bacteria should be isolated and cultured for multiple transplantations.

The gut is generally dominated by anaerobes. Gram-positive anaerobic bacteria such as Clostridiales, Lachnospiraceae, and *Ruminococcus* are negatively associated with CRC progression. Clostridiales strains, which are reduced in CRC patients, are involved in a strong anti-cancer response via CD8$^+$ T cells[26]. Lachnospiraceae and *Ruminococcus* are prominent taxonomic groups in the human gut microbiota that degrades complex polysaccharides into SCFA such as acetate, butyrate, and propionate, which the host may utilize for energy[27, 28]. A reduction in these beneficial microorganisms is strongly attributed to the development of CRC. Capecitabine, a chemotherapy drug, further unbalances these microorganisms. Gram-negative anaerobic bacteria such as *Bacteroides*, *F16*, *Prevotella,* and *S24-7* were positively associated with CRC progression. By altering the DNA structure of intestinal epithelial cells and triggering the Wnt/β-catenin and EGFR signaling pathways, *Bacteroides* exacerbate the risk of CRC[29, 30]. *Prevotella* induces IL8, IL6, and CCL20 production in epithelial cells, which in turn triggers mucosal Th17 immune responses and neutrophil recruitment, ultimately resulting in systemic inflammation[31]. The supplement of the FMT reversed the microbial dysbiosis and inhibited CRC progression (Figure 3F-H).

The shift in microbial composition influences the function of the intestine. We subsequently performed KEGG pathway prediction[32] based on the 16S metagenomic data and found numerous enhanced metabolic pathways in CRC mice, such as the renin-angiotensin system[33], Epstein-Barr virus infection[34], hepatitis C[35] and mTOR signaling pathway[36], which have been reported to be positively associated with CRC progression. Additionally, some biological pathways were decreased such as cAMP signaling[37], bile secretion[38], p53 signaling[39], and the cGMP-PKG signaling[40] pathway that prevents inflammation and lowers the risk of CRC. These pathways were regulated by the combined treatment of capecitabine and FMT (Figure 3I).

3.3. FMT enhanced the immune responses against CRC by recruiting anti-cancer immune cells in the gut

Identification and removal of aberrant cells is a major function of the immune system. Microbial dysbiosis in the colon, can trigger aberrant immunological responses, such as abnormal immune cell recruitment and enhanced production of pro-inflammatory cytokines in the intestine. Robust immunological responses were indicated by a considerable increase in T cell infiltration in CRC mice, particularly CD3$^+$ T cells, CD4$^+$ T cells, and FOXP3$^+$ Treg cells. F4/80$^+$ macrophages and CD49b$^+$ NK cells were also involved in this strong immune response. However, cytotoxic CD8$^+$ T cells and LY6G$^+$ neutrophils were reduced in CRC mice. In capecitabine-treated mice, the infiltration of most immune cells was reduced, including CD3$^+$ T cells, CD4$^+$ T cells, CD49b$^+$ NK cells, FOXP3$^+$ Treg, cells, and F4/80$^+$ macrophages, as chemotherapy typically suppresses and destroys the immune system[41]. In contrast, FMT recruited many more CD4$^+$ T cells, CD8$^+$ T cells, CD49b$^+$ NK cells, and LY6G$^+$ neutrophils in the intestine while selectively reducing the accumulation of CD3$^+$ T cells, FOXP3$^+$ Treg cells, and F4/80$^+$ macrophages, indicating enhanced immune responses against CRC (Figure 4).

3.4. *The combination of capecitabine and FMT enhanced the immune responses against CRC by regulating the expression of cytokines within the microenvironment*

The disruption of intestinal homeostasis caused by microbial dysbiosis in CRC mice leads to aberrant cytokine production within the gut. This study identified several overexpressed cytokines in the intestines of CRC mice, such as the proinflammatory cytokines IL1a, IL6, IL12a, IL12b, IL17, and IL22; transcription factors FOXP3 and STAT3; immunostimulatory cytokines IFN-γ and TNF-α; immunosuppressive cytokine TGF-β; enzymatic modulator GZMA; chemokine receptor CXCR4; integrin factor OPN and immune checkpoints PD-1 and PD-L1 (Figure 5A).

IL1a increases the nuclear localization of the NF-κB p65 subunit, which protects CRC cells from 5-fluorouracil-induced cell death and enhances their proliferation[42]. By upregulating VEGF, MMP9, and bFGF in tumor-associated endothelial cells, tumor-associated macrophages, and MDSCs, the IL-6/STAT3 axis promotes angiogenesis in several malignancies[43]. IL12 a potent proinflammatory cytokine, stimulates Th1 cell development, resulting in the generation of nitric oxide and IFN-γ, boosting inflammatory reactions and NK cell cytotoxicity[44]. IL17 promotes carcinogenesis by altering the cellular stromal phenotype, activating inflammatory mesenchymal stem cells, and mobilizing myeloid cells[45]. IL22 stimulates the processes of epithelial-to-mesenchymal passage[46]. Interestingly, capecitabine therapy decreased the overexpressed proteins for IL1a, IL6, IL12a, IL12b, IL17 and IL22. When capecitabine and FMT were given to CRC mice, pro-inflammatory factors IL1a, IL17, and IL22 were more down-regulated, while the anti-inflammatory cytokine IL10 was up-regulated.

Forkhead box protein 3 (FOXP3) is essential for establishing an immunosuppressive tumor microenvironment. It controls the proliferation and activity of regulatory T-cells, particularly the $CD4^{+}CD25^{+}$ fraction[47]. The signal transducer and activator of transcription 3 (STAT3) are highly expressed in the inflammatory tumor environment, where it causes the release of reactive oxygen species (ROS), leading to DNA damage and oncogene activation[48]. Protein kinase 1, a serine-threonine kinase, is activated by interferon-gamma (IFN-γ), thereby promoting necrosis and programmed cell death[49]. TNF-α, a key inflammatory cytokine, may enhance colon cancer invasion by upregulating TROP-2 expression via the ERK1/2 signaling pathway[50]. Selective suppression of TGF-β could be a promising strategy to reduce colon cell growth, as it is considered a biomarker for CRC[51]. Granzyme (GZMA) activity may stimulate the production of autoantigens, ECM fragments, and proinflammatory cytokines, which may worsen the inflammatory response[52]. Capecitabine therapy decreased the expression of FOXP3, STAT3, TNF-α, and GZMA, as well as IFN-γ and TGF-β. The combination of capecitabine and FMT enhanced IFN-γ expression while downregulating TGF-β.

Endothelial cells express C-X-C chemokine receptor type 4 (CXCR4) and release CXCL12, which aids in the movement of immune cells between the bloodstream and the adjacent tissues[53]. Osteoponin (OPN) is the primary phosphoprotein released by malignant cells in individuals with advanced metastatic cancer[54]. Programmed death-1 (PD-1) is a T cell checkpoint that regulates T cell exhaustion. Its binding to its ligand, programmed death-ligand 1 (PD-L1), activates downstream signaling pathways and inhibits T cell activation[55]. Interestingly, treatment with capecitabine alone showed a decrease in the expression of CXCR4, OPN, PD-1, and PD-L1. The FMT supplement increased CXCR4 and more downregulated PD-L1 in CRC mice.

The expression of IL1a, IL17, IL22, IFN-γ, TGF-β, CXCR4 and PD-L1 showed negative correlations with *Parabacteroids*, unclassified_Oscillospiraceae, *Marvinbryantia*, unclassified_Clostridia_vadinBB60_group, unclassified_Erysipelatoclostridiaceae, *A2*, *Roseburia*, Rikenellaceae_RC9_gut_group, *Acetatifactor* and unclassified_Clostridia (Figure 5B, C). These gut microorganisms may play a critical role in CRC treatment and could even be considered biological agents for CRC therapy.

4. Discussion

Growing evidence indicates that CRC development is significantly influenced by the gut microbiota, with reduced beneficial microbes, increased pathogenic microbes, and lower overall diversity. Microbial dysbiosis alters host gene expression, metabolic processes, and immune responses, thereby facilitating carcinogenesis[56]. Capecitabine, which is the first-line drug for locally advanced and metastatic CRC[57], reduces cancer progression by disrupting DNA synthesis and inhibiting the RANK/RANKL pathway[58, 59]. Instead of aggravating the disturbance of intestinal flora in CRC mice as expected, capecitabine showed a complex regulatory impact on intestinal flora. The microbial dysbiosis brought on by capecitabine and CRC was successfully reversed by FMT. Interestingly, as compared to capecitabine alone, the combination treatment of capecitabine and FMT demonstrated a synergistic anti-cancer immunological impact.

Dysbiosis of the gut microbiota is associated with chronic inflammatory diseases and an increased risk of CRC. Our research identified an elevation of gut bacteria, including *Bacteroides*, *Ligilactobacillus*, *Enterorhabdus*, *Monoglobus*, *Rodentibacter*, uncultured_rumen_bacterium, *Turicibacter* and *Streptococcus*, in CRC mice, suggesting a potential role in the progression of CRC. The digestive tract-colonizing bacteria *B. fragilis* forms biofilms and its toxin triggers inflammatory responses that result in tissue damage, persistent inflammation, and even CRC[60]. *Turicibacter* presence is linked to reduced CD8$^+$ T cell numbers in the colonic mucosa of mice[61]. The surface protein T. pallidum membrane protein C of *Streptococcus angionosus* directly interacts with the gastric epithelial cell receptor Annexin A2 to facilitate colonization in the gastric mucosa. This, in turn, mediates the activation of the mitogen-activated protein kinases signaling pathway, thereby expediting the development of gastric carcinogenesis[62]. Capecitabine decreased the most dominant bacteria such as *Bacteroides*, *Enterorhabdus*, *Monoglobus*, *Rodentibacter*, uncultured_rumen_bacterium, *Turicibacter,* and *Streptococcus*. Certain pathogenic bacteria, including *Candidatus arthromitus* and *Ligilactobacillus*, did not decrease in quantity when exposed to capecitabine. However, all these gut bacteria in CRC mice were dramatically reduced when the therapy was supplemented with FMT.

The normal gut microbiota has a specialized role in immunomodulation, host nutrition metabolism, defense against infections, xenobiotic and drug metabolism, and structural integrity of the gut mucosa maintenance[63]. Muribaculaceae, *Alloprevotella*, Lachnospiraceae, *Alistipes*, Rikenellaceae_RC9_gut_group, *Colidextribacter*, Lachnospiraceae_UCG_006, *Roseburia*, Ruminococcaceae, *Oscillibacter*, *Muribaculum*, and *Candidatus_Udaeobacter* were the most common bacteria in normal control mice. Muribaculaceae is thought to be a viable "next-generation probiotic" since they can create SCFA, regulate the immune system, and improve the function of the intestinal barrier[64]. By means of 7α-dehydroxylation, gut Lachnospiraceae transform main bile acids into secondary bile acids, which inhibit enteric pathogens and regulate mucosal immunity[65]. *Colidextribacter* increases the levels of SCFA in the gut, especially butyric acid[66]. TLR5 expression in response to Roseburia intestinalis stimulation upregulates TSLP and IL-10, resulting in dendritic cell-mediated anti-inflammatory responses[67]. *Roseburia* enteris reduces gut inflammation by decreasing the expression of IL17[68]. Ruminococcaceae produces acetate, which is converted to butyrate by *Roseburia*, thereby exerting anti-inflammatory effects[69]. SCFAs benefit host health by improving gut barrier function, reducing oxidative stress, and providing anti-inflammatory and anti-carcinogenic effects[70, 71]. Ruminococcus gnavus can degrade extracellular lyso-glycerophospholipids, facilitate the immune surveillance function of CD8$^+$ T cells, and inhibit the development of CRC[72]. *Oscillibacter* has been demonstrated to diminish the number of migratory Th17 cells in tumors, which are thought to migrate from the intestines and peripheral circulation and release the pro-angiogenic cytokine IL17[73]. Nearly all of these beneficial bacteria were reduced in CRC mice. Capecitabine increased the number of some beneficial bacteria such as *Alistipes*, Rikenellaceae_RC9_gut_group, unclassified_Clostridia_vadinBB60_group, *Colidextribacter*, *Roseburia* and *Muribaculum*. The genera of unclassified_Lachnospiraceae, Lachnospiraceae_UCG_006, unclassified_Ruminococcaceae, *Oscillibacter*, and *Candidatus_Udaeobacter*, which were not recovered by capecitabine alone, nevertheless dramatically increased when combined with FMT. This indicates that FMT acts as a more effective therapy in restoring microbial dysbiosis.

In this study, essential metabolic processes such as cAMP signaling, p53 signaling, bile secretion, and cGMP-PKG signaling were reduced in CRC mice due to microbial dysbiosis. Activating PKA with cAMP or analogs can inhibit glioblastoma cell growth, increase differentiation, and cause apoptosis[37]. The gut microbiota controls bile acid secretion, which activates anti-cancer immune cells such as NK cells[38]. By transactivating target genes linked to the induction of cell cycle arrest and/or apoptosis, activated p53 encourages cell cycle arrest to allow DNA repair and/or apoptosis to stop the spread of cells with significant DNA damage[39]. The loss of cGMP signaling in colon cancer cells enhances the formation of actin-based motility organelles and invasion organelles, which play a role in tumor cell migration[40]. These pathways did not reactivate in mice treated with capecitabine, while FMT boosted their activity. In contrast, some abnormal pathways were activated in CRC mice, such as the renin-angiotensin system, the Epstein-Barr virus infection, hepatitis-C, and mTOR signaling. In a mouse model, inhibiting the renin-angiotensin system via angiotensin I converting enzyme reduces the liver metastasis of CRC[33]. By interacting with host genes, the Epstein-Barr virus plays a major role in driving the cell cycle and oncogenesis of Epstein-Barr virus-positive neoplasms[34]. Hepatitis-C virus infection is strongly associated with the development of lung, colorectal, pancreatic, and breast cancers[35]. Various types of cancer frequently exhibit aberrant mTOR signaling. When mTOR signaling is hyperactivated, it encourages cell division and metabolism, contributing to the development and spread of tumors[36]. Capecitabine did not influence these pathways, but FMT made them less active.

The gut microbiota plays an important role in maintaining gut health[74]. Microbial dysbiosis causes gut inflammation and the recruitment of abnormal immune cells[75]. *Salmonella enterica* induces long-lasting intracellular infections and induces a strong CD4$^+$ T cell response that produces IFN-γ[76]. Colibactin-positive *E. coli* is reported to be related to a reduction in CD8$^+$ T cell infiltration in a mouse model of CRC[19]. CD49b$^+$ overexpression is linked to poor survival rates in several cancers, including prostate, stomach, and colon cancer[77]. *B. fragilis* drives the formation of FOXP3$^+$ Tregs with a distinct "inducible" genetic signature. Monocolonization of germ-free mice with *B. fragilis* enhances Treg suppressive power and stimulates anti-inflammatory cytokine production solely from FOXP3$^+$ T cells in the gut[78]. Intratumoral tumor-associated macrophages may increase the aggressiveness of cancer cells by upregulating their MMP-2 and MMP-9 levels[79]. In this study, immune cells, including CD3$^+$ T cells, CD4$^+$ T cells, CD49b$^+$ NK cells, FOXP3$^+$ Treg cells, and F4/80$^+$ macrophages, were abnormally recruited by microbial dysbiosis, inducing immunological dysfunction. However, capecitabine therapy decreased the infiltration of these immune cells. The combination of capecitabine and FMT significantly further reduced the recruitment of CD3$^+$ T cells, FOXP3$^+$ Treg cells, and F4/80$^+$ macrophages in the intestine while recruiting more CD4$^+$ T cells, CD8$^+$ T cells, CD49b$^+$ NK cells, and LY6G$^+$ neutrophils indicating enhanced immune responses against CRC.

By activating macrophages in the tumor microenvironment, CD4$^+$ T cells may be able to mediate death that is not constrained by MHC I or II tumor expression, resulting in direct tumor killing of MHC-II-expressing tumors as well as indirect tumor killing of MHC-II-negative tumors[80]. CD4$^+$ T cells also promote antitumor immunity through secreting effector cytokines such as IFN-γ and TNF-α[81]. Cytotoxic CD8$^+$ T cells play a critical role in recognizing antigens on MHC-I molecules, which allows the immune system to detect and kill cancer cells[82]. *Bifidobacterium* enhances the activation of CD8$^+$ T cells and dendritic cells within the tumor microenvironment, resulting in a combined antitumor effect with PD-L1[83]. *Lactobacillus plantarum-L168* and its metabolite, indole-3-lactic acid, reduce colorectal carcinogenesis by enhancing the anti-tumor immunity of CD8$^+$ T cells[84]. CD49b$^+$ NK cells eliminate cancer cells by activating death receptors such as TRAIL-R1/R2 or Fas and releasing cytotoxic granules, including PRF1, GZMs, and granulysins[85]. The Fap2 protein of *F. nucleatum* interacts directly with T cell immunoglobulin and the ITIM domain, inhibiting the cytotoxicity of NK cells targeting cancer cells[86]. CD4$^+$ T cells effectively eliminate MHC-deficient tumors, enhancing cancer immunotherapies by complementing the cytolytic activity of CD8$^+$ T cells and NK cells[87]. *Lactobacillus plantarum* promotes antitumor immunity and delays tumor formation by enhancing CD8$^+$ T cell and NK cell infiltration, up-regulating IFN-γ production, and promoting Th1-type CD4$^+$ T differentiation[88]. Through the infusion of calcium ions mediated by the transient receptor potential cation channel, hydrogen peroxide secreted by neutrophils induces tumor cells to undergo apoptosis[89]. Notably, neutrophil elastase released by neutrophils can hydrolyze and release the death domain of CD95, killing cancer cells selectively while reducing damage to non-cancerous cells[90]. This study suggested that altering the gut microbiota toward a more anti-inflammatory and anti-carcinogenic state is beneficial for the inhibition of CRC progression.

An imbalance in the gut microbiota causes abnormal immune responses, leading to increased production of inflammatory cytokines[91]. Inflammatory cytokines and chemokines released by cancerous cells draw pro-inflammatory T cells and myeloid cells, creating a pro-tumorigenic milieu that in turn promotes the proliferation of cancer cells[92]. *Mycobacterium tuberculosis* exposure increases the production of IL1a in epithelial cells[93]. IL1a is a major driver of colon cancer that is linked to chronic inflammation in the colon[94]. IL6 and TNF-α, two inflammatory cytokines produced in response to *S. bovis*, result in the release of free radicals and nitric oxide, which ultimately alter cellular DNA and induce carcinogenesis[95]. IL12 triggers an immune response against tumor cells by increasing the polarization of M1 macrophages and the production of IFN-γ by Th1 cells. This then increases the activity of anti-tumor CD8$^+$ and NK cells[96]. Chronic inflammation, triggered by pro-inflammatory cytokines IL17 and TNF-α, may stimulate glycolysis and epidermal growth factor in CRC cells, promoting tumorigenesis[97]. Colon tumorigenesis caused by enterotoxigenic *B. fragilis* is linked to an IL17-driven myeloid signature that is characterized by the subversion of steady-state myelopoiesis in favor of the production of protumoral monocytic myeloid-derived suppressor cells[98]. IL22, a pro-inflammatory protein that is probably released by Th17 cells, causes the production of Cxcl1 and Cxcl5 by epithelial cells and encourages the migration of neutrophils to the intestinal lamina propria[99]. In CRC, FOXP3 stimulates protumoral cytokines IL-12p40 and IL-23p19, which lead to immune escape and tumor progression[100]. STAT3 promotes the synthesis of cyclnd1, c-myc, and survivin, which accelerates cell cycle progression in colon cancer[101]. An infection with *H. pylori* causes STAT3 to become activated and up-regulates several tumorigenic genes, including FGFR1 and BRUNOL4, which aid in the initiation and progression of gastric cancer[102]. TGF-β released by macrophages causes tumorigenic development through matrix metalloproteinase-dependent processes[103]. Overexpression of GZMA in ulcerative colitis resulted in the generation of IL6, IL8, and TNF-α, which aggravated and prolonged inflammation[104]. GZMA has a significant negative correlation with *Roseburia sp. NSJ-9* and *Roseburia sp. AM51-8*[105]. OPN is generated by a variety of cells in the tumor microenvironment, including fibroblast, immunological, endothelial, and tumor cells[106]. OPN may interact with CD44v6 to increase CRC cell proliferation and survival, possibly through the JNK pathway[107]. The PD-1/PD-L1 interactions inhibit T cell activation, increase immunological tolerance, and polarize immune cells into tumor-promoting phenotypes, allowing cancer development and immune escape[108]. Treatment of CRC cells with *Clostridium butyricum* metabolites, likely butyrate, boosts proteasome-mediated ubiquitination, resulting in MYC degradation, and improves anti-PD-1 therapeutic effectiveness by increasing CD8/CD4$^+$ cell infiltration and downregulating PD-L1 expression on tumor cells[109]. In this study, capecitabine attenuated elevated cytokine levels in CRC mice, with FMT further reducing IL1a, IL17, IL22, TGF-β, PD-L1 and elevating IL10, IFN-γ, and CXCR4. *Parabacteroides distasonis* minimizes intestinal inflammation by generating the anti-inflammatory cytokine IL10[110]. IFN-γ produced by CD4$^+$ Th1 cells acts as a cytotoxic cytokine and induces apoptosis in tumor cells[111]. *F. nucleatum* was found to activate the stimulator of interferon genes (STING) signaling pathway and accumulate IFN-γ$^+$CD8+TILs[112]. In an anti-tumor immunological response, CXCR4/CXCL12 co-internalizes with CD47 and cross-signals via T and B cell receptors, enabling tumor cell phagocytosis by macrophages[113].

Conclusion

Capecitabine reduces the risk of CRC without enhancing the dysbiosis of gut flora. The combination of capecitabine and FMT has synergistic effects that more effectively inhibit the development of CRC by reversing microbial dysbiosis and enhancing immune responses by recruiting anti-cancer immune cells and regulating the expression of inflammatory cytokines within the CRC microenvironment. These findings hold significant translational potential and could contribute to advancing therapeutic strategies for colorectal cancer.

Ethical approval

All animal procedures were conducted in accordance with the ethical guidelines of the Institutional Animal Care and Use Committee of Harbin Medical University (protocol number IRB3079724) and were performed in compliance with the ARRIVE guidelines.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

Muhammad Arshad.: Investigation, editing, sampling, analyzing, methodology, reporting the results, and writing–original draft. Chong-Yuan Zhang.: Analyzing, and methodology. Zhan-Kui Gao.: Investigation, methodology. Dan-Qi Xu.: Investigation. Chao-Yuan Fan.: Investigation. Bo-Wen Zhang.: Investigation. Jia-Xin Geng.: Investigation. Hui Sun.: Methodology. Yang Li.: Methodology. Aleksandr Kotusov.: Analyzing. Ning Zhang.: Funding acquisition. Shu-Lin Liu.: Supervision, and funding acquisition. Xiao-Qin Mu.:

Conceptualization, review and editing, supervision, and funding acquisition. All authors contributed to the article and approved the submitted version.

Data availability

The data for intestinal microbiota 16s rDNA are accessible at the National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) under the accession number PRJNA1063875. The correspondence author will provide the raw data used to support this article's conclusion.

Clinical trial number

Not applicable

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49.
2. Yang Y, Han Z, Li X, Huang A, Shi J, Gu J. Epidemiology and risk factors of colorectal cancer in China. Chin J Cancer Res. 2020;32(6):729-41.
3. Rebecca LS, Kimberly DM, Ahmedin J. Cancer statistics, 2020. CA: A Cancer Journal for Clinicians. 2020.
4. Mohsen M, Jalil M, Nourouz D, Abbas A. Chronic Inflammation and Its Role in Colorectal Cancer Development. Oncogen. 2022.
5. Kolinsky K, Shen BQ, Zhang YE, Kohles J, Dugan U, Zioncheck TF, Heimbrook D, Packman K, Higgins B. In vivo activity of novel capecitabine regimens alone and with bevacizumab and oxaliplatin in colorectal cancer xenograft models. Mol Cancer Ther. 2009;8(1):75-82.
6. Ishikawa T, Utoh M, Sawada N, Nishida M, Fukase Y, Sekiguchi F, Ishitsuka H. Tumor selective delivery of 5-fluorouracil by capecitabine, a new oral fluoropyrimidine carbamate, in human cancer xenografts. Biochem Pharmacol. 1998;55(7):1091-7.
7. Khan M, Kleyner R, Abbasi S, Haider A. Capecitabine-Induced Enterocolitis. Cureus. 2022;14(3):e22855.
8. Montalban-Arques A, Scharl M. Intestinal microbiota and colorectal carcinoma: Implications for pathogenesis, diagnosis, and therapy. EBioMedicine. 2019;48:648-55.
9. Han YW, Shi W, Huang GT, Kinder Haake S, Park NH, Kuramitsu H, Genco RJ. Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. Infect Immun. 2000;68(6):3140-6.
10. Goodwin AC, Destefano Shields CE, Wu S, Huso DL, Wu X, Murray-Stewart TR, Hacker-Prietz A, Rabizadeh S, Woster PM, Sears CL, Casero RA, Jr. Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. Proc Natl Acad Sci U S A. 2011;108(37):15354-9.
11. Kontizas E, Tastsoglou S, Karamitros T, Karayiannis Y, Kollia P, Hatzigeorgiou AG, Sgouras DN. Impact of Helicobacter pylori Infection and Its Major Virulence Factor CagA on DNA Damage Repair. Microorganisms. 2020;8(12).
12. Deng Q, Wang C, Yu K, Wang Y, Yang Q, Zhang J, Xu X. Streptococcus bovis Contributes to the Development of Colorectal Cancer via Recruiting CD11b^{+}TLR-4^{+} Cells. Med Sci Monit. 2020;26:e921886.
13. Zhou T, Wu J, Tang H, Liu D, Jeon BH, Jin W, Wang Y, Zheng Y, Khan A, Han H, Li X. Enhancing tumor-specific recognition of programmable synthetic bacterial consortium for precision therapy of colorectal cancer. NPJ Biofilms Microbiomes. 2024;10(1):6.
14. Tukenmez U, Aktas B, Aslim B, Yavuz S. The relationship between the structural characteristics of lactobacilli-EPS and its ability to induce apoptosis in colon cancer cells in vitro. Sci Rep. 2019;9(1):8268.
15. Nowak A, Paliwoda A, Błasiak J. Anti-proliferative, pro-apoptotic and anti-oxidative activity of Lactobacillus and Bifidobacterium strains: A review of mechanisms and therapeutic perspectives. Crit Rev Food Sci Nutr. 2019;59(21):3456-67.
16. Kang X, Liu C, Ding Y, Ni Y, Ji F, Lau HCH, Jiang L, Sung JJ, Wong SH, Yu J. Roseburia intestinalis generated butyrate boosts anti-PD-1 efficacy in colorectal cancer by activating cytotoxic CD8(+) T cells. Gut. 2023;72(11):2112-22.

17. Zhu L, Suhr Van Haute MJ, Hassenstab HR, Smith C, Rose DJ, Mustoe AC, Benson AK, French JA. Fecal Short-Chain Fatty Acid Concentrations Increase in Newly Paired Male Marmosets (Callithrix jacchus). mSphere. 2020;5(5).
18. Li C, Niu Z, Zou M, Liu S, Wang M, Gu X, Lu H, Tian H, Jha R. Probiotics, prebiotics, and synbiotics regulate the intestinal microbiota differentially and restore the relative abundance of specific gut microorganisms. J Dairy Sci. 2020;103(7):5816-29.
19. Lopès A, Billard E, Casse AH, Villéger R, Veziant J, Roche G, Carrier G, Sauvanet P, Briat A, Pagès F, Naimi S, Pezet D, Barnich N, Dumas B, Bonnet M. Colibactin-positive Escherichia coli induce a procarcinogenic immune environment leading to immunotherapy resistance in colorectal cancer. Int J Cancer. 2020;146(11):3147-59.
20. Wang X, Fang Y, Liang W, Cai Y, Wong CC, Wang J, Wang N, Lau HC, Jiao Y, Zhou X, Ye L, Mo M, Yang T, Fan M, Song L, Zhou H, Zhao Q, Chu ES, Liang M, Liu W, Liu X, Zhang S, Shang H, Wei H, Li X, Xu L, Liao B, Sung JJY, Kuang M, Yu J. Gut-liver translocation of pathogen Klebsiella pneumoniae promotes hepatocellular carcinoma in mice. Nat Microbiol. 2025;10(1):169-84.
21. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. World J Gastroenterol. 2015;21(29):8787-803.
22. Kaźmierczak-Siedlecka K, Daca A, Fic M, van de Wetering T, Folwarski M, Makarewicz W. Therapeutic methods of gut microbiota modification in colorectal cancer management - fecal microbiota transplantation, prebiotics, probiotics, and synbiotics. Gut Microbes. 2020;11(6):1518-30.
23. The U.S. Food and Drug Administration. FDA-approved fecal transplant therapy: FDA; 2023 [Available from: https://www.nebraskamed.com/health/conditions-and-services/.
24. Neufert C, Becker C, Neurath MF. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. Nature Protocols. 2007;2(8):1998-2004.
25. Traina TA, Dugan U, Higgins B, Kolinsky K, Theodoulou M, Hudis CA, Norton L. Optimizing chemotherapy dose and schedule by Norton-Simon mathematical modeling. Breast Dis. 2010;31(1):7-18.
26. Montalban-Arques A, Katkeviciute E, Busenhart P, Bircher A, Wirbel J, Zeller G, Morsy Y, Borsig L, Glaus Garzon JF, Müller A, Arnold IC, Artola-Boran M, Krauthammer M, Sintsova A, Zamboni N, Leventhal GE, Berchtold L, de Wouters T, Rogler G, Baebler K, Schwarzfischer M, Hering L, Olivares-Rivas I, Atrott K, Gottier C, Lang S, Boyman O, Fritsch R, Manz MG, Spalinger MR, Scharl M. Commensal Clostridiales strains mediate effective anti-cancer immune response against solid tumors. Cell Host Microbe. 2021;29(10):1573-88.e7.
27. Biddle A, Stewart L, Blanchard J, Leschine S. Untangling the Genetic Basis of Fibrolytic Specialization by Lachnospiraceae and Ruminococcaceae in Diverse Gut Communities. Diversity. 2013;5(3):627-40.
28. Maniar K SV, Kumar D, Moideen A, Bhattacharyya R, Banerjee D. Metformin: A candidate drug to control the epidemic of diabetes and obesity by way of gut microbiome modification. In: Faintuch J, Faintuch S, editors. Microbiome and Metabolome in Diagnosis, Therapy, and other Strategic Applications. Cambridge, MA: Academic Press (2019). p. 401–8.
29. Cao H, Luo S, Xu M, Zhang Y, Song S, Wang S, Kong X, He N, Cao X, Yan F, Wang B. The secondary bile acid, deoxycholate accelerates intestinal adenoma-adenocarcinoma sequence in Apc (min/+) mice through enhancing Wnt signaling. Fam Cancer. 2014;13(4):563-71.
30. Dossa AY, Escobar O, Golden J, Frey MR, Ford HR, Gayer CP. Bile acids regulate intestinal cell proliferation by modulating EGFR and FXR signaling. Am J Physiol Gastrointest Liver Physiol. 2016;310(2):G81-92.
31. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology. 2017;151(4):363-74.
32. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. Nucleic Acids Res. 2023;51(D1):D587-d92.
33. Neo JH, Ager EI, Angus PW, Zhu J, Herath CB, Christophi C. Changes in the renin angiotensin system during the development of colorectal cancer liver metastases. BMC Cancer. 2010;10:134.
34. Yin H, Qu J, Peng Q, Gan R. Molecular mechanisms of EBV-driven cell cycle progression and oncogenesis. Med Microbiol Immunol. 2019;208(5):573-83.
35. Mohamed HI, Abdelrahim EM, Elsayed AM, Shaaban SM, Eldahrouty HA. Relationship between hepatitis C virus infection and extrahepatic malignancies. Clin Exp Hepatol. 2023;9(3):202-9.
36. Tian T, Li X, Zhang J. mTOR Signaling in Cancer and mTOR Inhibitors in Solid Tumor Targeting Therapy. Int J Mol Sci. 2019;20(3).
37. Chen TC, Hinton DR, Zidovetzki R, Hofman FM. Up-regulation of the cAMP/PKA pathway inhibits proliferation, induces differentiation, and leads to apoptosis in malignant gliomas. Lab Invest. 1998;78(2):165-74.
38. Sipe LM, Chaib M, Pingili AK, Pierre JF, Makowski L. Microbiome, bile acids, and obesity: How microbially modified metabolites shape anti-tumor immunity. Immunol Rev. 2020;295(1):220-39.
39. Ozaki T, Nakagawara A. Role of p53 in Cell Death and Human Cancers. Cancers (Basel). 2011;3(1):994-1013.
40. Zuzga DS, Pelta-Heller J, Li P, Bombonati A, Waldman SA, Pitari GM. Phosphorylation of vasodilator-stimulated phosphoprotein Ser239 suppresses filopodia and invadopodia in colon cancer. Int J Cancer. 2012;130(11):2539-48.
41. Zhang S, Wang Z, Fan S, Liu T, Yoshida S, Yang S, Liu L, Hou W, Cao L, Wang J, Song Z, Li S, Zhang S, Wang H, Li J, Zheng H, Shen Z. Capecitabine Can Induce T Cell Apoptosis: A Potential Immunosuppressive Agent With Anti-Cancer Effect. Front Immunol. 2021;12:737849.
42. Kwon MJ, Hong E, Choi Y, Kang DH, Oh ES. Interleukin-1α promotes extracellular shedding of syndecan-2 via induction of matrix metalloproteinase-7 expression. Biochem Biophys Res Commun. 2014;446(2):487-92.
43. Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H, Yu H. Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. J Clin Invest. 2008;118(10):3367-77.

44. Trinchieri G. Interleukin-12 and interferon-gamma. Do they always go together? Am J Pathol. 1995;147(6):1534-8.
45. Huangfu L, Li R, Huang Y, Wang S. The IL-17 family in diseases: from bench to bedside. Signal Transduct Target Ther. 2023;8(1):402.
46. Lim C, Savan R. The role of the IL-22/IL-22R1 axis in cancer. Cytokine Growth Factor Rev. 2014;25(3):257-71.
47. Lal G, Bromberg JS. Epigenetic mechanisms of regulation of Foxp3 expression. Blood. 2009;114(18):3727-35.
48. Blaylock RL. Cancer microenvironment, inflammation and cancer stem cells: A hypothesis for a paradigm change and new targets in cancer control. Surg Neurol Int. 2015;6:92.
49. Thapa RJ, Basagoudanavar SH, Nogusa S, Irrinki K, Mallilankaraman K, Slifker MJ, Beg AA, Madesh M, Balachandran S. NF-kappaB protects cells from gamma interferon-induced RIP1-dependent necroptosis. Mol Cell Biol. 2011;31(14):2934-46.
50. Zhao P, Zhang Z. TNF-α promotes colon cancer cell migration and invasion by upregulating TROP-2. Oncol Lett. 2018;15(3):3820-7.
51. Gulley JL, Schlom J, Barcellos-Hoff MH, Wang XJ, Seoane J, Audhuy F, Lan Y, Dussault I, Moustakas A. Dual inhibition of TGF-β and PD-L1: a novel approach to cancer treatment. Mol Oncol. 2022;16(11):2117-34.
52. Hendel A, Hiebert PR, Boivin WA, Williams SJ, Granville DJ. Granzymes in age-related cardiovascular and pulmonary diseases. Cell Death Differ. 2010;17(4):596-606.
53. Herbert SP, Stainier DY. Molecular control of endothelial cell behaviour during blood vessel morphogenesis. Nat Rev Mol Cell Biol. 2011;12(9):551-64.
54. Mi Z, Guo H, Wai PY, Gao C, Kuo PC. Integrin-linked kinase regulates osteopontin-dependent MMP-2 and uPA expression to convey metastatic function in murine mammary epithelial cancer cells. Carcinogenesis. 2006;27(6):1134-45.
55. Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in cancer immunotherapy: clinical implications and future considerations. Hum Vaccin Immunother. 2019;15(5):1111-22.
56. Liwei W, Hao R, Bangmao W, Yuan Q, Weilong Z. Microbiota regulation in constipation and colorectal cancer. World Journal of Gastrointestinal Oncology. 2023.
57. Koukourakis GV, Zacharias G, Tsalafoutas J, Theodoridis D, Kouloulias V. Capecitabine for locally advanced and metastatic colorectal cancer: A review. World J Gastrointest Oncol. 2010;2(8):311-21.
58. Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei D, Groshen S, Lenz HJ. Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. Int J Colorectal Dis. 2002;17(1):46-9.
59. Shao M, Jiang C, Yu C, Jia H, Wang Y, Mao X. Capecitabine inhibits epithelial-to-mesenchymal transition and proliferation of colorectal cancer cells by mediating the RANK/RANKL pathway. Oncol Lett. 2022;23(3):96.
60. Cheng WT, Kantilal HK, Davamani F. The Mechanism of Bacteroides fragilis Toxin Contributes to Colon Cancer Formation. Malays J Med Sci. 2020;27(4):9-21.
61. Presley LL, Wei B, Braun J, Borneman J. Bacteria associated with immunoregulatory cells in mice. Appl Environ Microbiol. 2010;76(3):936-41.
62. Fu K, Cheung AHK, Wong CC, Liu W, Zhou Y, Wang F, Huang P, Yuan K, Coker OO, Pan Y, Chen D, Lam NM, Gao M, Zhang X, Huang H, To KF, Sung JJY, Yu J. Streptococcus anginosus promotes gastric inflammation, atrophy, and tumorigenesis in mice. Cell. 2024;187(4):882-96.e17.
63. Sasso JM, Ammar RM, Tenchov R, Lemmel S, Kelber O, Grieswelle M, Zhou QA. Gut Microbiome-Brain Alliance: A Landscape View into Mental and Gastrointestinal Health and Disorders. ACS Chem Neurosci. 2023;14(10):1717-63.
64. Ormerod KL, Wood DL, Lachner N, Gellatly SL, Daly JN, Parsons JD, Dal'Molin CG, Palfreyman RW, Nielsen LK, Cooper MA, Morrison M, Hansbro PM, Hugenholtz P. Genomic characterization of the uncultured Bacteroidales family S24-7 inhabiting the guts of homeothermic animals. Microbiome. 2016;4(1):36.
65. Jin WB, Li TT, Huo D, Qu S, Li XV, Arifuzzaman M, Lima SF, Shi HQ, Wang A, Putzel GG, Longman RS, Artis D, Guo CJ. Genetic manipulation of gut microbes enables single-gene interrogation in a complex microbiome. Cell. 2022;185(3):547-62.e22.
66. Liu X, Zhang Y, Li W, Zhang B, Yin J, Liuqi S, Wang J, Peng B, Wang S. Fucoidan Ameliorated Dextran Sulfate Sodium-Induced Ulcerative Colitis by Modulating Gut Microbiota and Bile Acid Metabolism. J Agric Food Chem. 2022;70(47):14864-76.
67. Shen Z, Luo W, Tan B, Nie K, Deng M, Wu S, Xiao M, Wu X, Meng X, Tong T, Zhang C, Ma K, Liao Y, Xu J, Wang X. Roseburia intestinalis stimulates TLR5-dependent intestinal immunity against Crohn's disease. EBioMedicine. 2022;85:104285.
68. Zhu C, Song K, Shen Z, Quan Y, Tan B, Luo W, Wu S, Tang K, Yang Z, Wang X. Roseburia intestinalis inhibits interleukin-17 excretion and promotes regulatory T cells differentiation in colitis. Mol Med Rep. 2018;17(6):7567-74.
69. Nogal A, Louca P, Zhang X, Wells PM, Steves CJ, Spector TD, Falchi M, Valdes AM, Menni C. Circulating Levels of the Short-Chain Fatty Acid Acetate Mediate the Effect of the Gut Microbiome on Visceral Fat. Front Microbiol. 2021;12:711359.
70. Sun Y, O'Riordan MX. Regulation of bacterial pathogenesis by intestinal short-chain Fatty acids. Adv Appl Microbiol. 2013;85:93-118.
71. van der Beek CM, Dejong CHC, Troost FJ, Masclee AAM, Lenaerts K. Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. Nutr Rev. 2017;75(4):286-305.
72. Zhang X, Yu D, Wu D, Gao X, Shao F, Zhao M, Wang J, Ma J, Wang W, Qin X, Chen Y, Xia P, Wang S. Tissue-resident Lachnospiraceae family bacteria protect against colorectal carcinogenesis by promoting tumor immune surveillance. Cell Host Microbe. 2023;31(3):418-32.e8.
73. Tajiri K, Shimizu Y. Gut bacteria may control development of hepatocellular carcinoma. Hepatobiliary Surg Nutr. 2017;6(6):417-9.

74. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012;336(6086):1268-73.
75. Akhtar M, Chen Y, Ma Z, Zhang X, Shi D, Khan JA, Liu H. Gut microbiota-derived short chain fatty acids are potential mediators in gut inflammation. Anim Nutr. 2022;8:350-60.
76. Goldberg MF, Roeske EK, Ward LN, Pengo T, Dileepan T, Kotov DI, Jenkins MK. Salmonella Persist in Activated Macrophages in T Cell-Sparse Granulomas but Are Contained by Surrounding CXCR3 Ligand-Positioned Th1 Cells. Immunity. 2018;49(6):1090-102.e7.
77. Ferraro A, Boni T, Pintzas A. EZH2 regulates cofilin activity and colon cancer cell migration by targeting ITGA2 gene. PLoS One. 2014;9(12):e115276.
78. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010;107(27):12204-9.
79. Kang JC, Chen JS, Lee CH, Chang JJ, Shieh YS. Intratumoral macrophage counts correlate with tumor progression in colorectal cancer. J Surg Oncol. 2010;102(3):242-8.
80. Kravtsov DS, Erbe AK, Sondel PM, Rakhmilevich AL. Roles of CD4+ T cells as mediators of antitumor immunity. Front Immunol. 2022;13:972021.
81. Tay RE, Richardson EK, Toh HC. Revisiting the role of CD4(+) T cells in cancer immunotherapy-new insights into old paradigms. Cancer Gene Ther. 2021;28(1-2):5-17.
82. Harty JT, Tvinnereim AR, White DW. CD8+ T cell effector mechanisms in resistance to infection. Annu Rev Immunol. 2000;18:275-308.
83. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre ML, Chang EB, Gajewski TF. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015;350(6264):1084-9.
84. Zhang Q, Zhao Q, Li T, Lu L, Wang F, Zhang H, Liu Z, Ma H, Zhu Q, Wang J, Zhang X, Pei Y, Liu Q, Xu Y, Qie J, Luan X, Hu Z, Liu X. Lactobacillus plantarum-derived indole-3-lactic acid ameliorates colorectal tumorigenesis via epigenetic regulation of CD8(+) T cell immunity. Cell Metab. 2023;35(6):943-60.e9.
85. Sordo-Bahamonde C, Lorenzo-Herrero S, Payer Á R, Gonzalez S, López-Soto A. Mechanisms of Apoptosis Resistance to NK Cell-Mediated Cytotoxicity in Cancer. Int J Mol Sci. 2020;21(10).
86. Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, Enk J, Bar-On Y, Stanietsky-Kaynan N, Coppenhagen-Glazer S, Shussman N, Almogy G, Cuapio A, Hofer E, Mevorach D, Tabib A, Ortenberg R, Markel G, Miklić K, Jonjic S, Brennan CA, Garrett WS, Bachrach G, Mandelboim O. Binding of the Fap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. Immunity. 2015;42(2):344-55.
87. Kruse B, Buzzai AC, Shridhar N, Braun AD, Gellert S, Knauth K, Pozniak J, Peters J, Dittmann P, Mengoni M, van der Sluis TC, Höhn S, Antoranz A, Krone A, Fu Y, Yu D, Essand M, Geffers R, Mougiakakos D, Kahlfuß S, Kashkar H, Gaffal E, Bosisio FM, Bechter O, Rambow F, Marine JC, Kastenmüller W, Müller AJ, Tüting T. CD4(+) T cell-induced inflammatory cell death controls immune-evasive tumours. Nature. 2023;618(7967):1033-40.
88. Yang X, Guo Y, Chen C, Shao B, Zhao L, Zhou Q, Liu J, Wang G, Yuan W, Sun Z. Interaction between intestinal microbiota and tumour immunity in the tumour microenvironment. Immunology. 2021;164(3):476-93.
89. Gershkovitz M, Caspi Y, Fainsod-Levi T, Katz B, Michaeli J, Khawaled S, Lev S, Polyansky L, Shaul ME, Sionov RV, Cohen-Daniel L, Aqeilan RI, Shaul YD, Mori Y, Karni R, Fridlender ZG, Binshtok AM, Granot Z. TRPM2 Mediates Neutrophil Killing of Disseminated Tumor Cells. Cancer Res. 2018;78(10):2680-90.
90. Cui C, Chakraborty K, Tang XA, Zhou G, Schoenfelt KQ, Becker KM, Hoffman A, Chang YF, Blank A, Reardon CA, Kenny HA, Vaisar T, Lengyel E, Greene G, Becker L. Neutrophil elastase selectively kills cancer cells and attenuates tumorigenesis. Cell. 2021;184(12):3163-77.e21.
91. Schirmer M, Smeekens SP, Vlamakis H, Jaeger M, Oosting M, Franzosa EA, Ter Horst R, Jansen T, Jacobs L, Bonder MJ, Kurilshikov A, Fu J, Joosten LAB, Zhernakova A, Huttenhower C, Wijmenga C, Netea MG, Xavier RJ. Linking the Human Gut Microbiome to Inflammatory Cytokine Production Capacity. Cell. 2016;167(4):1125-36.e8.
92. Francescone R, Hou V, Grivennikov SI. Microbiome, inflammation, and cancer. Cancer J. 2014;20(3):181-9.
93. Guler R, Parihar SP, Spohn G, Johansen P, Brombacher F, Bachmann MF. Blocking IL-1α but not IL-1β increases susceptibility to chronic Mycobacterium tuberculosis infection in mice. Vaccine. 2011;29(6):1339-46.
94. Malik A, Sharma D, Zhu Q, Karki R, Guy CS, Vogel P, Kanneganti TD. IL-33 regulates the IgA-microbiota axis to restrain IL-1α-dependent colitis and tumorigenesis. J Clin Invest. 2016;126(12):4469-81.
95. Ellmerich S, Djouder N, Schöller M, Klein JP. Production of cytokines by monocytes, epithelial and endothelial cells activated by Streptococcus bovis. Cytokine. 2000;12(1):26-31.
96. Mirlekar B, Pylayeva-Gupta Y. IL-12 Family Cytokines in Cancer and Immunotherapy. Cancers (Basel). 2021;13(2).
97. Straus DS. TNFα and IL-17 cooperatively stimulate glucose metabolism and growth factor production in human colorectal cancer cells. Mol Cancer. 2013;12:78.
98. Thiele Orberg E, Fan H, Tam AJ, Dejea CM, Destefano Shields CE, Wu S, Chung L, Finard BB, Wu X, Fathi P, Ganguly S, Fu J, Pardoll DM, Sears CL, Housseau F. The myeloid immune signature of enterotoxigenic Bacteroides fragilis-induced murine colon tumorigenesis. Mucosal Immunol. 2017;10(2):421-33.
99. Bernshtein B, Curato C, Ioannou M, Thaiss CA, Gross-Vered M, Kolesnikov M, Wang Q, David E, Chappell-Maor L, Harmelin A, Elinav E, Thakker P, Papayannopoulos V, Jung S. IL-23-producing IL-10Rα-deficient gut macrophages elicit an IL-22-driven proinflammatory epithelial cell response. Sci Immunol. 2019;4(36).
100. Stanilov N, Miteva L, Mintchev N, Stanilova S. High expression of Foxp3, IL-23p19 and survivin mRNA in colorectal carcinoma. Int J Colorectal Dis. 2009;24(2):151-7.

101. Lin L, Liu A, Peng Z, Lin HJ, Li PK, Li C, Lin J. STAT3 is necessary for proliferation and survival in colon cancer-initiating cells. Cancer Res. 2011;71(23):7226-37.
102. Zhao J, Dong Y, Kang W, Go MY, Tong JH, Ng EK, Chiu PW, Cheng AS, To KF, Sung JJ, Yu J. Helicobacter pylori-induced STAT3 activation and signalling network in gastric cancer. Oncoscience. 2014;1(6):468-75.
103. Mook OR, Frederiks WM, Van Noorden CJ. The role of gelatinases in colorectal cancer progression and metastasis. Biochim Biophys Acta. 2004;1705(2):69-89.
104. Hirayasu H, Yoshikawa Y, Tsuzuki S, Fushiki T. A role of a lymphocyte tryptase, granzyme A, in experimental ulcerative colitis. Biosci Biotechnol Biochem. 2007;71(1):234-7.
105. Yu H, Wang Q, Tang J, Dong L, Dai G, Zhang T, Zhang G, Xie K, Wang H, Zhao Z. Comprehensive analysis of gut microbiome and host transcriptome in chickens after Eimeria tenella infection. Front Cell Infect Microbiol. 2023;13:1191939.
106. Kariya Y, Kariya Y. Osteopontin in Cancer: Mechanisms and Therapeutic Targets. International Journal of Translational Medicine. 2022;2(3):419-47.
107. Rao G, Wang H, Li B, Huang L, Xue D, Wang X, Jin H, Wang J, Zhu Y, Lu Y, Du L, Chen Q. Reciprocal interactions between tumor-associated macrophages and CD44-positive cancer cells via osteopontin/CD44 promote tumorigenicity in colorectal cancer. Clin Cancer Res. 2013;19(4):785-97.
108. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010;236:219-42.
109. Xu H, Luo H, Zhang J, Li K, Lee MH. Therapeutic potential of Clostridium butyricum anticancer effects in colorectal cancer. Gut Microbes. 2023;15(1):2186114.
110. Kverka M, Zakostelska Z, Klimesova K, Sokol D, Hudcovic T, Hrncir T, Rossmann P, Mrazek J, Kopecny J, Verdu EF, Tlaskalova-Hogenova H. Oral administration of Parabacteroides distasonis antigens attenuates experimental murine colitis through modulation of immunity and microbiota composition. Clin Exp Immunol. 2011;163(2):250-9.
111. Markman JL, Shiao SL. Impact of the immune system and immunotherapy in colorectal cancer. J Gastrointest Oncol. 2015;6(2):208-23.
112. Gao Y, Bi D, Xie R, Li M, Guo J, Liu H, Guo X, Fang J, Ding T, Zhu H, Cao Y, Xing M, Zheng J, Xu Q, Xu Q, Wei Q, Qin H. Fusobacterium nucleatum enhances the efficacy of PD-L1 blockade in colorectal cancer. Signal Transduct Target Ther. 2021;6(1):398.
113. Mezzapelle R, Leo M, Caprioglio F, Colley LS, Lamarca A, Sabatino L, Colantuoni V, Crippa MP, Bianchi ME. CXCR4/CXCL12 Activities in the Tumor Microenvironment and Implications for Tumor Immunotherapy. Cancers (Basel). 2022;14(9).

Figure 1. FMT combined with capecitabine inhibited the development of CRC. Figure 1. FMT combined with capecitabine inhibited the development of CRC. (A) Timeline of the experimental procedure. (B) Images of intestinal tissue from each group, including normal control mice (Ctl), colorectal cancer mice (CRC), CRC mice treated with capecitabine (CAP), and CRC mice receiving capecitabine and FMT (CAP-FMT). (C-E) Average number and diameter of cancer foci, average length of colon tissue, $*p<0.05$, $**p<0.01$, n=6. (F) Clinical scores of disease activity index, $*p<0.05$, $**p<0.01$. (G) Average body weight of mice in each week. (H) Survival rate of mice, $*p<0.05$, $**p<0.01$. (I) HE staining of intestinal tissue. (J-K) Immunohistochemical staining of cleaved caspase-3 and Ki67 $*p<0.05$, $**p<0.01$, n=6.

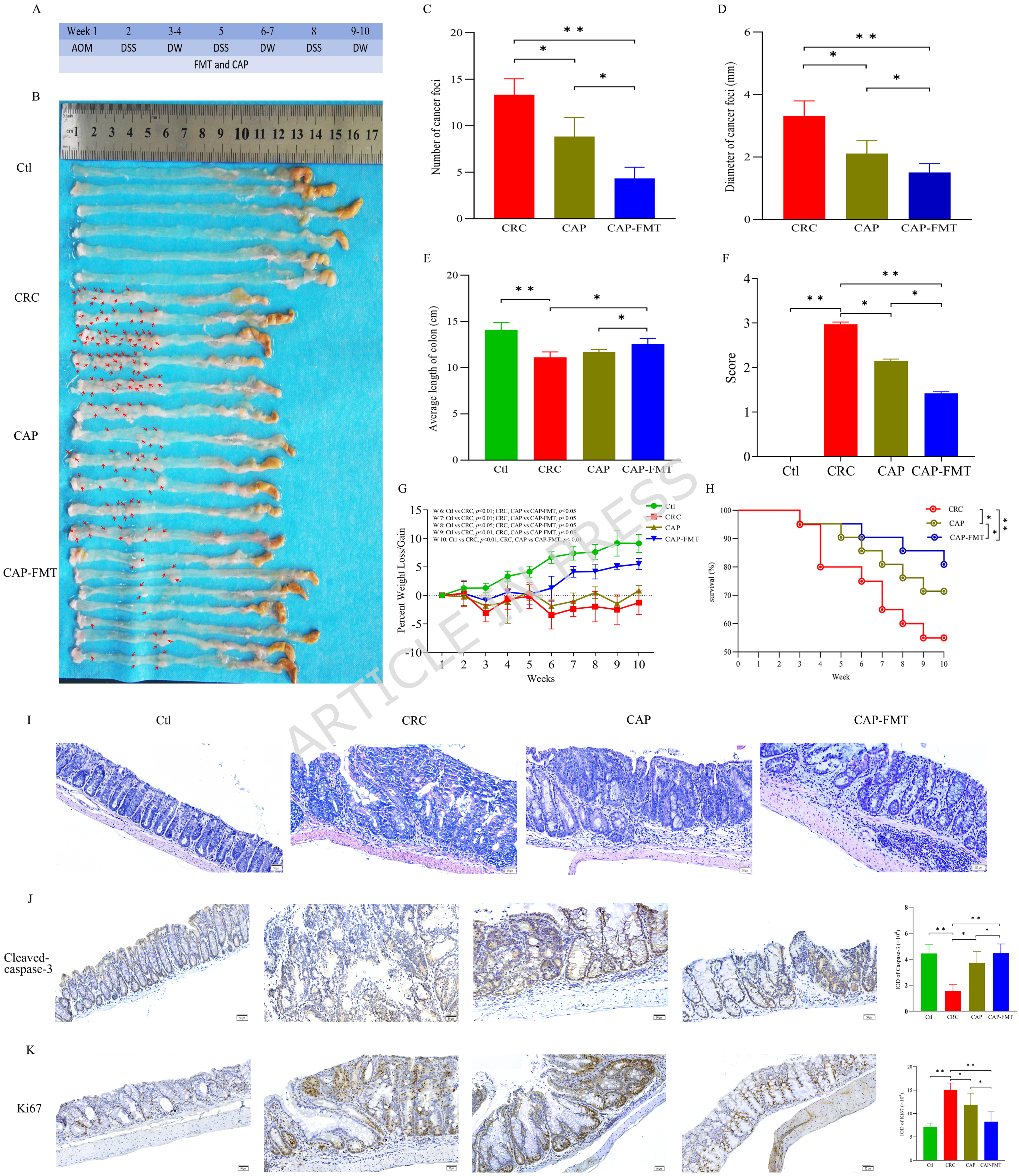
Figure 2. The distribution of intestinal microbiota in different classifications, including phylum, family, genus, and species levels (A-D)**.**

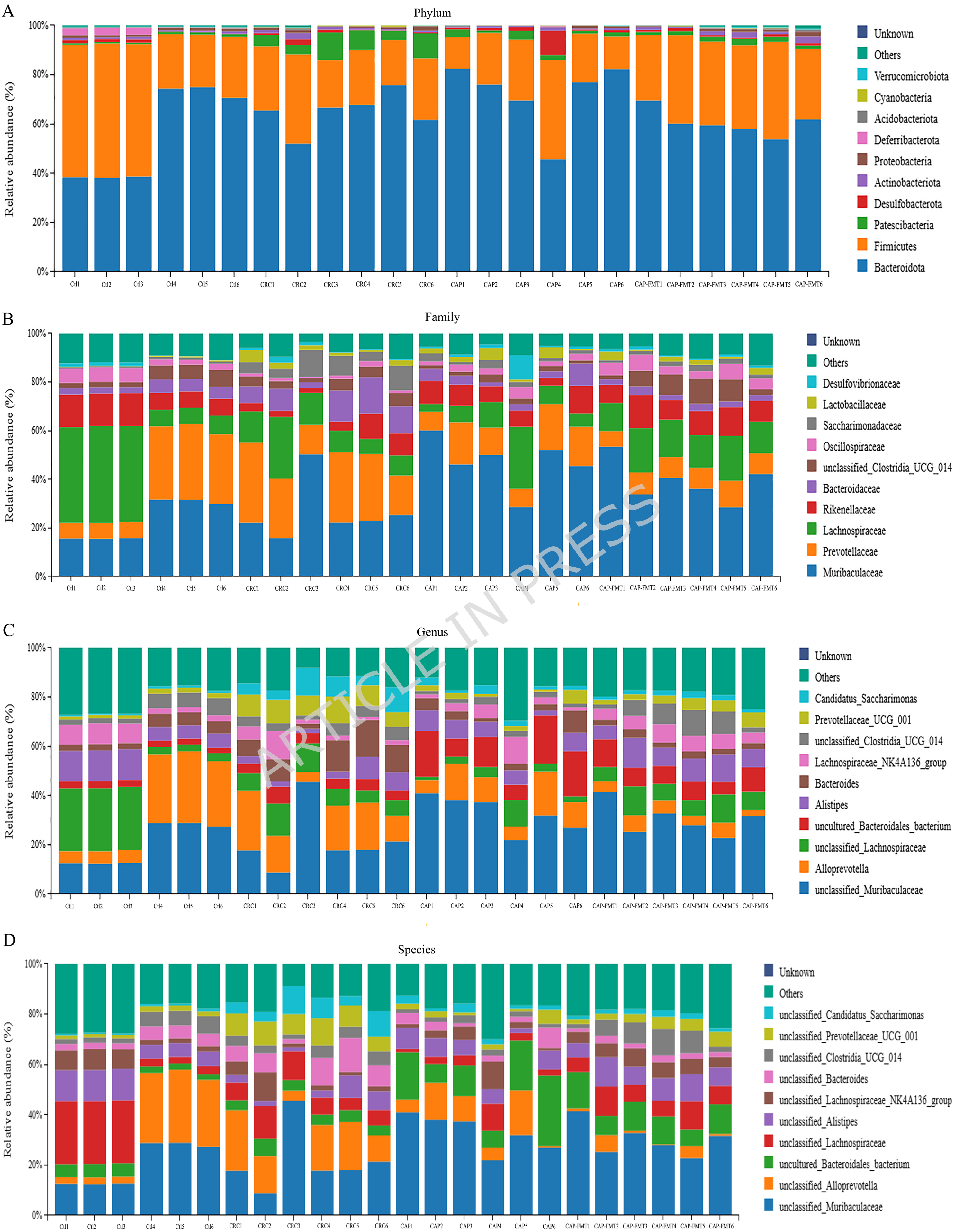
Figure 3. FMT corrected the microbial dysbiosis brought on by CRC and capecitabine. (A, B) Alpha diversity indicated by ACE and Chao 1 index, $*p<0.05$, $**p<0.01$, n = 6. (C) β diversity indicated by PCoA analysis. (D) The commensal network displays the gut bacteria that show positive and negative

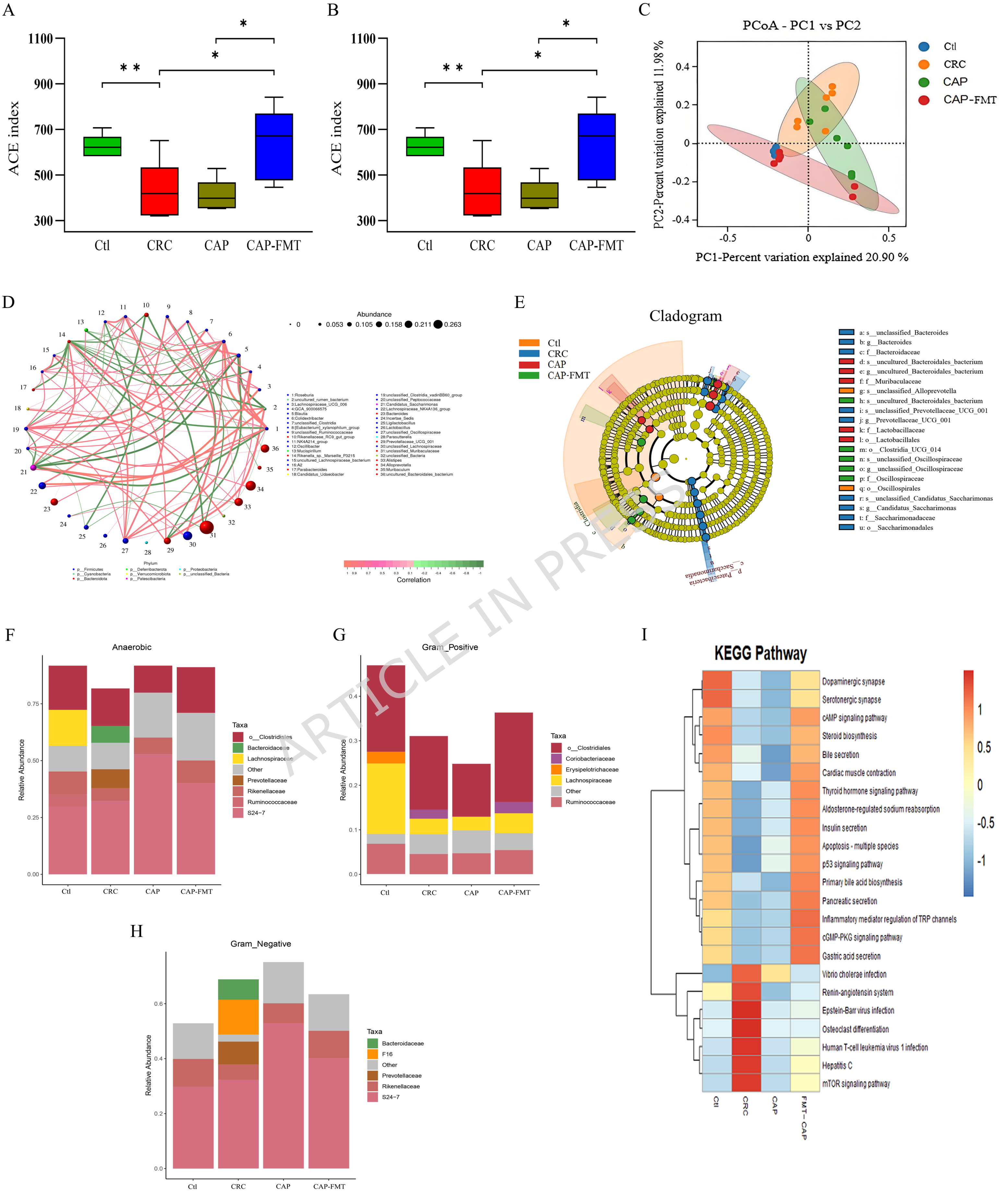
correlations in CRC progression. (E) LEfSe analysis identifies representative bacteria in each group. (F-H) Phenotypic distribution of anaerobic, gram-positive, and gram-negative bacteria. (I) KEGG metabolic pathways associated with CRC progression (Ref: 260475).

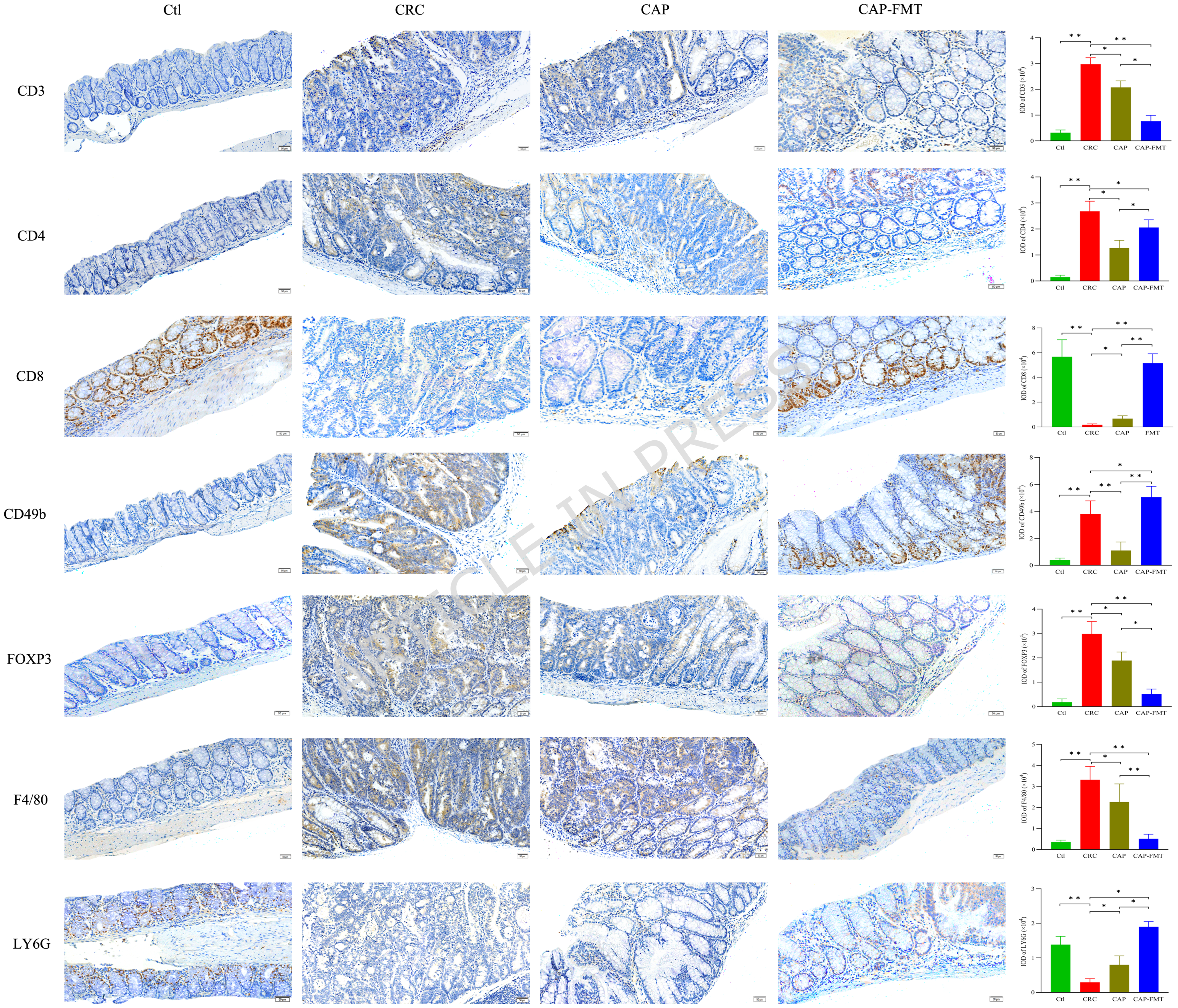
Figure 4. FMT improved immune responses against CRC by mobilizing anti-cancer immune cells in the gut. Brown staining was seen in the cytoplasm or cytomembrane of positive cells. The intestines from the Ctl, CRC, capecitabine, and CAP-FMT groups were imaged using a scanning system for microscopic photography, respectively. The ImageJ system was used to examine the average optical density of all immunohistochemically stained specimens, $^{*}p<0.05$, $^{**}p<0.01$, n = 6.

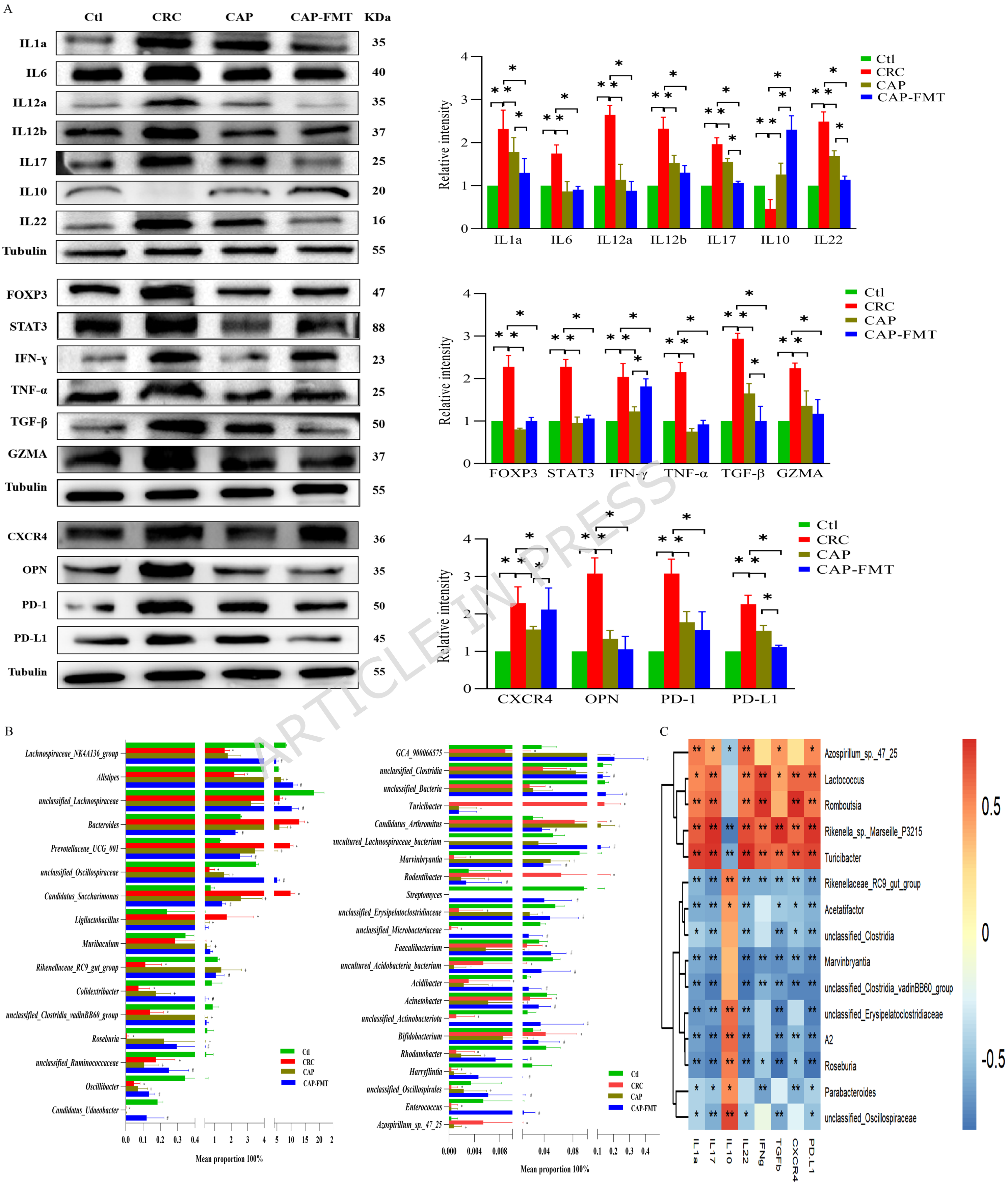
Figure 5. The combination of capecitabine and FMT regulated cytokine expression in the CRC microenvironment. (A) Differentially expressed cytokines were detected by western blot, $^{*}p<0.05$, n = 3. (B) The abundance of intestinal microbes associated with inflammatory cytokines, $^{*}p<0.05$, Ctl vs. CRC, $^{+}p<0.05$, CRC vs. CAP, $^{\#}p<0.05$, CAP vs. CAP-FMT, n = 6. (C) Spearman correlation analysis between intestinal microbes and cytokines $^{*}p<0.05$, $^{**}p<0.01$.


A
Week 1
2
3-4
5
6-7
8
9-10
AOM
DSS
DW
DSS
DW
DSS
DW
FMT and CAP
B
cm 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ctl
CRC
CAP
CAP-FMT
C
Number of cancer foci
0
5
10
15
20
CRC
CAP
CAP-FMT
* *
*
*
D
Diameter of cancer foci (mm)
0
2
4
6
CRC
CAP
CAP-FMT
* *
*
*
E
Average length of colon (cm)
0
5
10
15
20
Ctl
CRC
CAP
CAP-FMT
* *
*
*
F
Score
0
1
2
3
4
Ctl
CRC
CAP
CAP-FMT
* *
* *
*
*
G
W 6: Ctl vs CRC, p<0.01; CRC, CAP vs CAP-FMT, p<0.05
W 7: Ctl vs CRC, p<0.01; CRC, CAP vs CAP-FMT, p<0.05
W 8: Ctl vs CRC, p<0.05; CRC, CAP vs CAP-FMT, p<0.05
W 9: Ctl vs CRC, p<0.01, CRC, CAP vs CAP-FMT, p<0.05
W 10: Ct1 vs CRC, p<0.01, CRC, CAP vs CAP-FMT, p< 0.01
Ctl
CRC
CAP
CAP-FMT
Percent Weight Loss/Gain
15
10
5
0
-5
-10
1 2 3 4 5 6 7 8 9 10
Weeks
H
CRC
CAP
CAP-FMT
*
*
* *
survival (%)
100
90
80
70
60
50
0 1 2 3 4 5 6 7 8 9 10
Week
I
Ctl
CRC
CAP
CAP-FMT
J
Cleaved-caspase-3
IOD of Caspase-3 (×10⁴)
0
2
4
6
8
Ctl
CRC
CAP
CAP-FMT
* *
* *
*
*
K
Ki67
IOD of Ki67 (×10⁴)
0
5
10
15
20
Ctl
CRC
CAP
CAP-FMT
* *
* *
*
*
50 μm


A
Phylum
Relative abundance (%)
100%
80%
60%
40%
20%
0%
Ctl1
Ctl2
Ctl3
Ctl4
Ctl5
Ctl6
CRC1
CRC2
CRC3
CRC4
CRC5
CRC6
CAP1
CAP2
CAP3
CAP4
CAP5
CAP6
CAP-FMT1
CAP-FMT2
CAP-FMT3
CAP-FMT4
CAP-FMT5
CAP-FMT6
Unknown
Others
Verrucomicrobiota
Cyanobacteria
Acidobacteriota
Deferribacterota
Proteobacteria
Actinobacteriota
Desulfobacterota
Patescibacteria
Firmicutes
Bacteroidota
B
Family
Unknown
Others
Desulfovibrionaceae
Lactobacillaceae
Saccharimonadaceae
Oscillospiraceae
unclassified_Clostridia_UCG_014
Bacteroidaceae
Rikenellaceae
Lachnospiraceae
Prevotellaceae
Muribaculaceae
C
Genus
Unknown
Others
Candidatus_Saccharimonas
Prevotellaceae_UCG_001
unclassified_Clostridia_UCG_014
Lachnospiraceae_NK4A136_group
Bacteroides
Alistipes
uncultured_Bacteroidales_bacterium
unclassified_Lachnospiraceae
Alloprevotella
unclassified_Muribaculaceae
D
Species
Unknown
Others
unclassified_Candidatus_Saccharimonas
unclassified_Prevotellaceae_UCG_001
unclassified_Clostridia_UCG_014
unclassified_Bacteroides
unclassified_Lachnospiraceae_NK4A136_group
unclassified_Alistipes
unclassified_Lachnospiraceae
uncultured_Bacteroidales_bacterium
unclassified_Alloprevotella
unclassified_Muribaculaceae


A
ACE index
1100
900
700
500
300
Ctl
CRC
CAP
CAP-FMT
* *
*
*
B
ACE index
1100
900
700
500
300
Ctl
CRC
CAP
CAP-FMT
* *
*
*
C
PCoA - PC1 vs PC2
PC2-Percent variation explained 11.98 %
PC1-Percent variation explained 20.90 %
0.4
0.2
0
-0.2
-0.4
-0.5
0
0.5
Ctl
CRC
CAP
CAP-FMT
D
Abundance
0
0.053
0.105
0.158
0.211
0.263
1:Roseburia
2:uncultured_rumen_bacterium
3:Lachnospiraceae_UCG_006
4:GCA_900066575
5:Blautia
6:Colidextribacter
7:unclassified_Clostridia
8:[Eubacterium]_xylanophilum_group
9:unclassified_Ruminococcaceae
10:Rikenellaceae_RC9_gut_group
11:NK4A214_group
12:Oscillibacter
13:Mucispirillum
14:Rikenella_sp._Marseille_P3215
15:uncultured_Lachnospiraceae_bacterium
16:A2
17:Parabacteroides
18:Candidatus_Udaeobacter
19:unclassified_Clostridia_vadinBB60_group
20:unclassified_Peptococcaceae
21:Candidatus_Saccharimonas
22:Lachnospiraceae_NK4A136_group
23:Bacteroides
24:Incertae_Sedis
25:Ligilactobacillus
26:Lactobacillus
27:unclassified_Oscillospiraceae
28:Parasutterella
29:Prevotellaceae_UCG_001
30:unclassified_Lachnospiraceae
31:unclassified_Muribaculaceae
32:unclassified_Bacteria
33:Alistipes
34:Alloprevotella
35:Muribaculum
36:uncultured_Bacteroidales_bacterium
Phylum
p__Firmicutes
p__Cyanobacteria
p__Bacteroidota
p__Deferribacterota
p__Verrucomicrobiota
p__Patescibacteria
p__Proteobacteria
p__unclassified_Bacteria
Correlation
1
0.9
0.7
0.5
0.3
0.1
-0.2
-0.4
-0.6
-0.8
-1
E
Cladogram
Ctl
CRC
CAP
CAP-FMT
c__Clostridia
p__Patescibacteria
c__Saccharimonadia
a: s__unclassified_Bacteroides
b: g__Bacteroides
c: f__Bacteroidaceae
d: s__uncultured_Bacteroidales_bacterium
e: g__uncultured_Bacteroidales_bacterium
f: f__Muribaculaceae
g: s__unclassified_Alloprevotella
h: s__uncultured_Bacteroidales_bacterium
i: s__unclassified_Prevotellaceae_UCG_001
j: g__Prevotellaceae_UCG_001
k: f__Lactobacillaceae
l: o__Lactobacillales
m: o__Clostridia_UCG_014
n: s__unclassified_Oscillospiraceae
o: g__unclassified_Oscillospiraceae
p: f__Oscillospiraceae
q: o__Oscillospirales
r: s__unclassified_Candidatus_Saccharimonas
s: g__Candidatus_Saccharimonas
t: f__Saccharimonadaceae
u: o__Saccharimonadales
F
Anaerobic
Relative Abundance
0.00
0.25
0.50
0.75
Ctl
CRC
CAP
CAP-FMT
Taxa
o__Clostridiales
Bacteroidaceae
Lachnospiraceae
Other
Prevotellaceae
Rikenellaceae
Ruminococcaceae
S24-7
G
Gram_Positive
Relative Abundance
0.0
0.1
0.2
0.3
0.4
Ctl
CRC
CAP
CAP-FMT
Taxa
o__Clostridiales
Coriobacteriaceae
Erysipelotrichaceae
Lachnospiraceae
Other
Ruminococcaceae
H
Gram_Negative
Relative Abundance
0.0
0.2
0.4
0.6
Ctl
CRC
CAP
CAP-FMT
Taxa
Bacteroidaceae
F16
Other
Prevotellaceae
Rikenellaceae
S24-7
I
KEGG Pathway
Dopaminergic synapse
Serotonergic synapse
cAMP signaling pathway
Steroid biosynthesis
Bile secretion
Cardiac muscle contraction
Thyroid hormone signaling pathway
Aldosterone-regulated sodium reabsorption
Insulin secretion
Apoptosis - multiple species
p53 signaling pathway
Primary bile acid biosynthesis
Pancreatic secretion
Inflammatory mediator regulation of TRP channels
cGMP-PKG signaling pathway
Gastric acid secretion
Vibrio cholerae infection
Renin-angiotensin system
Epstein-Barr virus infection
Osteoclast differentiation
Human T-cell leukemia virus 1 infection
Hepatitis C
mTOR signaling pathway
Ctl
CRC
CAP
FMT-CAP
1
0.5
0
-0.5
-1


Ctl
CRC
CAP
CAP-FMT
CD3
CD4
CD8
CD49b
FOXP3
F4/80
LY6G
50 μm
IOD of CD3 (×10^4)
IOD of CD4 (×10^4)
IOD of CD8 (×10^4)
IOD of CD49b (×10^4)
IOD of FOXP3 (×10^4)
IOD of F4/80 (×10^4)
IOD of LY6G (×10^4)
0
1
2
3
4
5
6
8
Ctl
CRC
CAP
CAP-FMT
FMT
* *
*


A
Ctl
CRC
CAP
CAP-FMT
KDa
IL1a 35
IL6 40
IL12a 35
IL12b 37
IL17 25
IL10 20
IL22 16
Tubulin 55
FOXP3 47
STAT3 88
IFN-γ 23
TNF-α 25
TGF-β 50
GZMA 37
Tubulin 55
CXCR4 36
OPN 35
PD-1 50
PD-L1 45
Tubulin 55
Relative intensity
0
1
2
3
4
IL1a
IL6
IL12a
IL12b
IL17
IL10
IL22
FOXP3
STAT3
IFN-γ
TNF-α
TGF-β
GZMA
CXCR4
OPN
PD-1
PD-L1
*
B
Lachnospiraceae_NK4A136_group
Alistipes
unclassified_Lachnospiraceae
Bacteroides
Prevotellaceae_UCG_001
unclassified_Oscillospiraceae
Candidatus_Saccharimonas
Ligilactobacillus
Muribaculum
Rikenellaceae_RC9_gut_group
Colidextribacter
unclassified_Clostridia_vadinBB60_group
Roseburia
unclassified_Ruminococcaceae
Oscillibacter
Candidatus_Udaeobacter
0.0 0.1 0.2 0.3 0.4
1 2 3 4
5 10 15 20 2
Mean proportion 100%
GCA_900066575
unclassified_Clostridia
unclassified_Bacteria
Turicibacter
Candidatus_Arthromitus
uncultured_Lachnospiraceae_bacterium
Marvinbryantia
Rodentibacter
Streptomyces
unclassified_Erysipelatoclostridiaceae
unclassified_Microbacteriaceae
Faecalibacterium
uncultured_Acidobacteria_bacterium
Acidibacter
Acinetobacter
unclassified_Actinobacteriota
Bifidobacterium
Rhodanobacter
Harryflintia
unclassified_Oscillospirales
Enterococcus
Azospirillum_sp._47_25
0.000 0.004 0.008
0.04 0.08
0.1 0.2 0.3 0.4
Mean proportion 100%
*
#
C
Azospirillum_sp._47_25
Lactococcus
Romboutsia
Rikenella_sp._Marseille_P3215
Turicibacter
Rikenellaceae_RC9_gut_group
Acetatifactor
unclassified_Clostridia
Marvinbryantia
unclassified_Clostridia_vadinBB60_group
unclassified_Erysipelatoclostridiaceae
A2
Roseburia
Parabacteroides
unclassified_Oscillospiraceae
IL1a
IL17
IL10
IL22
IFNg
TGFb
CXCR4
PD.L1
0.5
0
-0.5
*
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