

Original Article

Fisetin regulates gut microbiota to decrease CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte count and IL-12 secretion to alleviate premature ovarian failure in mice

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Abstract: Currently, there are no studies reporting the efficacy of fisetin in premature ovarian failure (POF). In this study, using mouse and *Caenorhabditis elegans* models, we found that fisetin not only significantly reversed ovarian damage in POF mice, but also effectively increased *C. elegans* lifespan and fertility. Subsequently, we carried out 16S rRNA v3+v4 sequencing using fresh feces samples from each group of mice. Results showed that although there was no significant difference in the number of gut microbiomes between the different groups of mice, fisetin affected the diversity and distribution of gut microbiota in POF mice. Alpha and beta diversity analyses showed that in the gut of POF mice in the fisetin group, the bacterial count of uncultured_bacterium_f_Lachnospiraceae was significantly increased, while that of *Akkermansia* was significantly decreased. Finally, flow cytometry analysis showed that the numbers of CCR9⁺/CXCR3⁺/CD4⁺ T lymphocytes in the peripheral blood of POF mice in the fisetin group were significantly reduced, along with the number of CD4⁺/interleukin (IL)-12⁺ cells. Therefore, our data suggested that fisetin regulates the distribution and bacterial counts of *Akkermansia* and uncultured_bacterium_f_Lachnospiraceae in POF mice, and reduces peripheral blood CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte count and IL-12 secretion to regulate the ovarian microenvironment and reduce inflammation, thus exerting therapeutic effects against POF.

Keywords: Premature ovarian failure, fisetin, gut microbiota, CCR9⁺/CXCR3⁺/CD4⁺ T lymphocyte, interleukin-12

Introduction

Premature ovarian failure (POF) refers to amenorrhea caused by ovarian insufficiency before the age of 40 [1-3]. POF is characterized by primary or secondary amenorrhea, accompanied by elevated blood gonadotropin, reduced oestrogen, ovarian follicle atresia, and a persistent decrease in ovarian follicle reserve function [2, 4-6]. As a typical form of pathological senescence, POF severely affects pregnancy and quality of life in women [2, 4-6]. However, there are no effective therapies or drugs currently available for the treatment of POF.

Fisetin (3,7,3',4'-tetrahydroxyflavone) is a natural flavonol that is abundantly present in many fruits and vegetables and has classical antioxidant and anti-tumour properties [7-9]. Fis-

etin exhibits significant affinity with microtubule proteins and can bind to microtubules, which stabilizes its structure and function [7-9]. In our previous study, we investigated the epigenetic mechanism by which fisetin exerts an inhibitory function in renal cancer stem cells. Fisetin inhibited TET1 expression and reduced 5hmC modification in specific loci in the promoters of CCNY/CDK16 in renal cancer stem cells. This inhibition prevented gene transcription, causing cell cycle arrest and ultimately resulting in inhibition of renal cancer stem cell division [8]. However, there have been no reports so far on whether fisetin has therapeutic effects on POF in mice.

The interactions between gut microbiota and the human body have various effects, such as regulation of gut development and mucosal bar-

rier function, regulation of nutrient intake and metabolism, promotion of immune tissue maturation, and prevention of pathogen proliferation [10, 11]. Increasing numbers of studies have reported that gut microbiota (gut ecology) affects normal physiological and biochemical functions and is associated with various diseases in mammals. Guo et al. found that there were significant differences in the composition and distribution of gut microbiota between a letrozole-induced mouse model of polycystic ovary syndrome (PCOS) and healthy controls [12]. The levels of *Lactobacillus*, *Ruminococcus*, and *Clostridium* were lower, while the levels of *Prevotella* were significantly higher in the PCOS group than those in the control group [12]. Yuan et al. found significant differences between mice in the endometriosis and mock groups, where the reduction in *Bacteroidetes* levels was particularly significant [13]. In addition, an imbalance in gut ecology causes an abnormal increase in the blood oestrogen levels, stimulating the growth of endometriotic lesions and the pathology of cyclic bleeding [14, 15]. Hence, gut microbiota appears to be closely associated with the occurrence and outcome of gynaecological disorders [16].

In this study, we examined the hypothesis that fisetin regulates gut microbiota to alleviate POF in mice. Our results showed that fisetin regulated gut microbiota and decreased CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte count and interleukin (IL-12) secretion to alleviate POF in mice.

Materials and methods

Preparation of POF mouse model

POF mouse model was established based on the method used in our previous study [1-3]. Briefly, 10-week-old female C57BL/6 mice (n=30) were purchased from Shanghai Model Organisms Center (Shanghai, China). Mice were randomly divided into three groups of ten mice each. In the fisetin intervention group, 70 mg/kg of cyclophosphamide (CTX; Sigma-Aldrich, St. Louis, USA) was injected intraperitoneally, followed by subsequent intraperitoneal injections of 20 mg/kg CTX once every two days for four continuous weeks. In addition, 100 ng/kg fisetin (Sigma-Aldrich, St. Louis, USA) was administered once every two days from the start of model construction. In the control group, 70 mg/kg of CTX (Sigma-Aldrich, St.

Louis, USA) was injected intraperitoneally, followed by subsequent intraperitoneal injections of 20 mg/kg CTX once every two days for four continuous weeks. An equivalent dose of phosphate buffered saline (PBS) was administered once every two days from the start of model construction. A normal control group (WT) was also set up. This study was approved by the Ethics Committee of the Shanghai Geriatric Institute of Chinese Medicine (SH-AGESYDW2016018). All animal experiments conformed to the regulations of the Ministry of Science and Technology.

Immunohistochemical staining

Immunohistochemical staining was performed according to our previously published protocol [2, 3, 17]. Briefly, tissue sections were blocked with blocking solution (Beyotime Biotechnology Co., Ltd., Zhejiang, China) for 30 min at 37°C, followed by incubation with primary antibody for 45 min at 37°C. After washing, slides were mounted in immunofluorescence-grade blocking solution (Sigma-Aldrich, St. Louis, USA) containing DAPI.

Flow cytometry (FCM) analysis

Peripheral blood mononuclear cells (PBMCs) were obtained from each group, cultured at a concentration of 1×10^6 cells/mL, and stained with primary antibodies [CD199 (CCR9) monoclonal antibody (eBioCW-1.2 (CW-1.2))-PerCP, CD183 (CXCR3) monoclonal antibody (CXCR3-173)-PE, CD4 monoclonal antibody (GK1.5)-FITC, and IL-12 p35 monoclonal antibody (27537)-PE; Invitrogen, eBioscience™, Shanghai, China] in Dulbecco's PBS containing 10% bovine serum albumin on ice. Staining with an isotype control antibody (mouse IgG1-FITC, mouse IgG1-PE, mouse IgG1-PerCP, mouse IgG1-PE-Cyanine7 Invitrogen, eBioscience™, Shanghai, China) was performed to detect any non-specific binding. Evaluation of antibody staining by FCM was performed using FACSAria (Quanta SC, Beckman Coulter INC).

Gut microbiota analysis

Gut microbiota analysis was performed as described previously [18, 19]. In brief, fresh fecal samples were collected during the final 5 days for gut microbial analysis. Bacterial genomic DNA was extracted from frozen sam-

ples stored at -80°C . The V3 and V4 regions of the 16S rRNA gene were amplified by PCR using specific bacterial primers (forward: 5'-ACTCCTACGGGAGGAGCAGCA-3'; reverse: 5'-GGA-CTACHVGGGTWCTAAT-3'). High-throughput pyrosequencing of the PCR products was performed on an Illumina MiSeq platform at Biomarker Technologies Co. Ltd. (China). The raw paired-end reads from the original DNA fragments were merged using FLASH32 and assigned to each sample according to the unique barcodes. The UCLUST [21] in QIIME [20] (version 1.8.0) software was used to cluster sequences at 97% similarity. The tags were clustered into operational taxonomic units (OTUs). The alpha diversity index was evaluated using Mothur software (version, v.1.30). To compare the diversity index among samples, the number of sequences in each sample was standardized. Analysis treasure included OTU rank, rarefaction, and Shannon curves, and the Shannon, Chao1, Simpson, and ACE indexes were calculated. For beta diversity analysis, heatmaps of RDA-identified key OTUs, principal coordinate analysis (PcoA) [22], non-metric multi-dimensional scaling (NMDS) [23], and unweighted pair-group method with arithmetic mean (UPGMA) were performed using QIIME. Linear discriminant analysis (LDA) effect size (LEfSe) was used for the quantitative analysis of biomarkers in each group. Briefly, LEfSe analysis (at LDA threshold >4), the non-parametric factorial Kruskal-Wallis sum-rank test, and the unpaired Wilcoxon rank-sum test were performed to identify the most abundant taxa [24, 25].

Construction of Caenorhabditis elegans POF model and grouping

The N2 strain of *C. elegans* was a gift from Shanghai Model Organisms Center (Shanghai, China). Approximately 300 *C. elegans* specimens in the L4 stage were placed in 12 mL of NGM culture medium containing OP50 *Escherichia coli*. The worms were divided equally into three groups of 10 worms/4 mL. In the fisetin intervention group, 20 $\mu\text{g}/\text{mL}$ CTX and 10 ng/mL fisetin were added to the NGM culture medium. In the control group, 20 $\mu\text{g}/\text{mL}$ CTX and an equivalent dose of PBS were added to the NGM culture medium. In addition, a normal control group (WT) was set up.

Hematoxylin-eosin (HE) staining

Briefly, all fresh tissues were fixed in 4% paraformaldehyde at room temperature (Sigma-Aldrich, St. Louis, USA) for 30 min [1, 3]. Next, the tissues were subjected to gradient ethanol dehydration, paraffin-embedding, sectioning (thickness of 6 μm), and immersion in xylene for clearing. The tissue sections were stained with HE (Sigma-Aldrich, St. Louis, USA) before xylene (Sigma-Aldrich, St. Louis, USA) clearing and mounting with neutral resins (Sigma-Aldrich, St. Louis, USA).

C. elegans total RNA extraction and qPCR assay

TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) was used to extract total RNA from *C. elegans* in various groups, according to the manufacturer's instructions. After treatment with DNase I (Sigma-Aldrich, St. Louis, USA), total RNA was quantified, and reverse transcription was carried out using the ReverTra Ace- α First Strand cDNA Synthesis Kit (Toyobo). qRT-PCR was performed on the RealPlex4 Real-Time PCR Detection System (Eppendorf Co. Ltd., Hamburg, Germany). The SYBR Green Real-Time PCR Master Mix (Toyobo, Shanghai, China) was used as a fluorescent dye for nucleic acid amplification. A total of 40 amplification cycles were carried out as follows: denaturation at 95°C for 15 sec, annealing at 58°C for 30 sec, and extension at 72°C for 42 sec. The $2^{-\Delta\Delta\text{Ct}}$ method was used to calculate relative gene expression, where $\Delta\text{Ct} = \text{Ct}_{\text{genes}} - \text{Ct}_{\text{Act-1}}$; $\Delta\Delta\text{Ct} = \Delta\text{Ct}_{\text{all_groups}} - \Delta\text{Ct}_{\text{blank_control_group}}$. Target gene expression levels were normalized against the 18S rRNA expression levels. The primer sequences used were as follows: Egl-17, forward: 5'-CCATTGGCAACT-ATTCA-3', reverse: 5'-TTCCATCCCATTCTCC-3'; Daf-2, forward: 5'-ACCCGTGAGCAGTGTC-3', reverse: 5'-TTGCCAACGCATTTT-3'; Act-1, forward: 5'-CTCTTGCCCCATCAACCATG-3', reverse: 5'-CT-TGCTTGAGATCCACATC-3'.

Western blot analysis

The cells were lysed using a 2 \times loading lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China). The proteins were separated on 12% sodium dodecyl sulfate-polyacrylamide gels and transferred onto hybrid polyvinylidene difluoride membranes (Millipore, Bedford, MA,

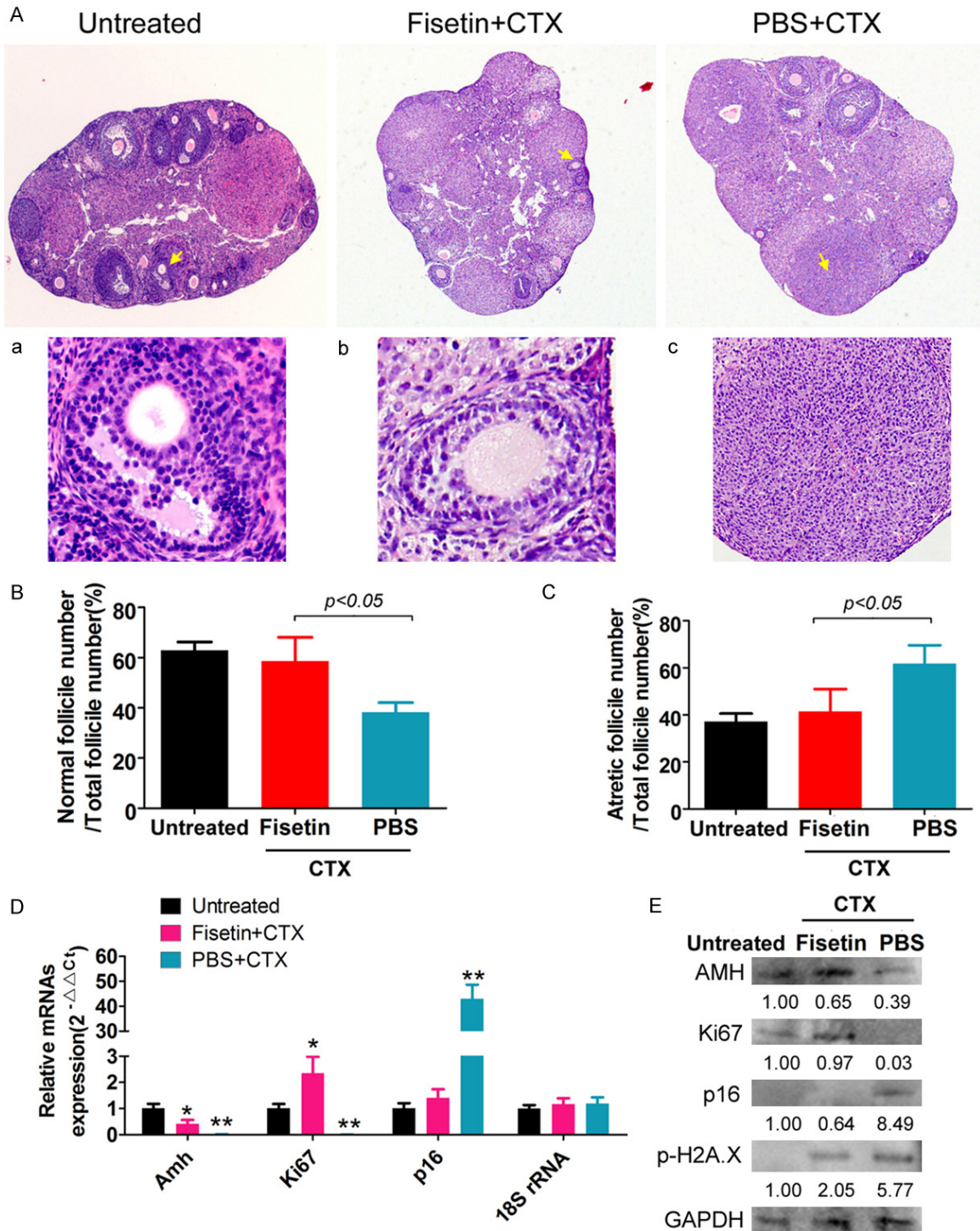


Figure 1. Fisetin effectively alleviates ovarian damage in POF mice. (A) HE staining results of the ovarian tissues in various groups of mice (magnification: 100×). (a-c) are the high magnification images (400×) of the sites indicated with yellow arrows. (B) The proportion of normal ovarian follicles in the different groups of mice. (C) The proportion of atretic ovarian follicles in the different groups of mice. Pathological testing by HE staining showed that the number of atretic ovarian follicles significantly increased, while the number of quality ovarian granulosa cells significantly decreased in the ovarian tissues of POF mice. Ovarian follicles at various stages of development appeared in the ovaries of POF mice in the fisetin group. (D) qPCR for quantitation of mRNA levels of genes related to ovarian granulosa cell proliferation and aging. **P<0.01 vs PBS+CTX group, *P<0.05 vs PBS+CTX group, as calculated by t-test. (E) Western blot analysis to detect the expression levels of proteins related to ovarian granulosa cell proliferation and aging. qPCR and western blotting results revealed that the expression levels of p16 and p-H2A.X in the ovaries of POF mice in the fisetin group were decreased significantly, while those of AMH and Ki67 were increased significantly.

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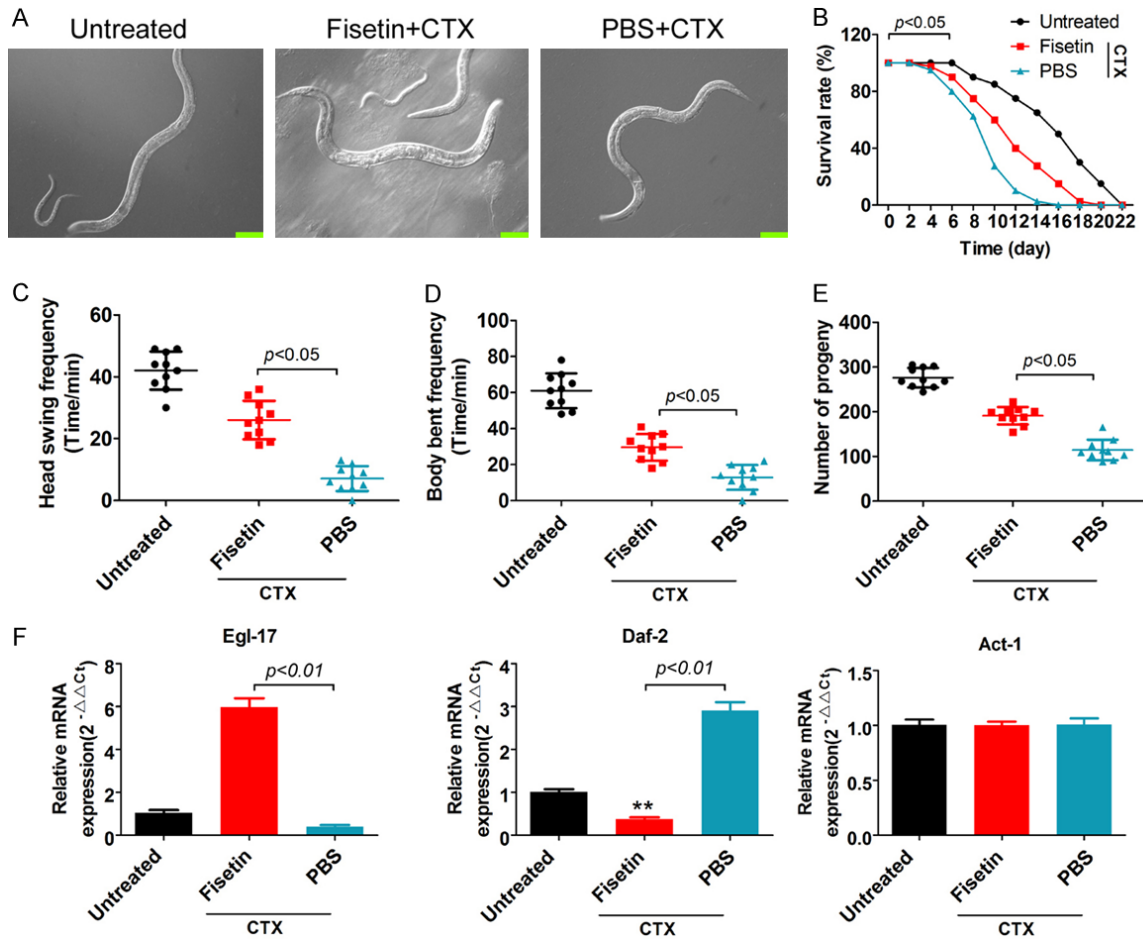


Figure 2. Fisetin effectively enhances *C. elegans* lifespan and fertility. A. Phenotypes of various groups of *C. elegans*. Scale bar: 100 μ m. B. Life span curve: the results showed that, compared with the PBS group, the life span of *C. elegans* was significantly increased in the fisetin group after CTX treatment. C. Frequency of head thrashes in various groups of *C. elegans*: Compared with PBS, fisetin significantly increased the frequency of head thrashes after CTX treatment. D. Frequency of body bends in various groups of *C. elegans*: Compared with PBS, fisetin significantly increased the frequency of body bends after CTX treatment. E. Number of pregnant *C. elegans* in the various groups: compared with PBS, fisetin significantly increased the number of pregnant *C. elegans* after CTX treatment. F. qPCR for quantitation of mRNA levels of genes related to the IIS signaling pathway. **P<0.01 vs PBS+CTX group, as calculated by t-test. qPCR results showed that, compared with PBS, fisetin significantly increased the mRNA expression of genes associated with the IIS signaling pathway in *C. elegans* after CTX treatment.

USA). After blocking with 5% (w/v) non-fat dried milk in Tris-buffered saline with Tween-20 (TBST; Beyotime Institute of Biotechnology), the membranes were washed four times (15 min each) with TBST at room temperature and incubated with primary antibodies, including rabbit anti-mouse Ki67 and AMH antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), rabbit anti-mouse p16, p-H2A.X, and GAPDH antibodies (Cell Signaling Technology, Beverly, MA, USA). After washing, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1:1,000; Santa Cruz Biotechnology) for 1 h.

Following washing four times (15 min each) with TBST at room temperature, the immunoreactive bands were visualized using an enhanced chemiluminescence kit (Perkin Elmer, Norwalk, CT, USA).

Evaluation of *C. elegans* behaviour and proliferation

Briefly, 40 *C. elegans* specimens at the L4 stage were collected from each group and placed in NGM culture medium containing OP50 *E. coli* [26-30]. The specimens were then observed under a microscope. The number of

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Table 1. OUTs

OTU_id	F	P	WT	taxonomy
OTU1	180722	327136	227626	k__Bacteria; p__Verrucomicrobia; c__Verrucomicrobiae; o__Verrucomicrobiales; f__Akkermansia; g__Akkermansia; s__uncultured_bacterium_g_Akkermansia
OTU10	10	11496	11	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Dubosiella; s__uncultured_bacterium_g_Dubosiella
OTU100	1095	1401	32	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU101	294	520	553	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU102	200	485	155	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus_1; s__uncultured_bacterium_g_Ruminococcus_1
OTU103	653	342	350	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU104	576	360	161	k__Bacteria; p__Fusobacteria; c__Fusobacteriia; o__Fusobacteriales; f__Fusobacteriaceae; g__Cetobacterium; s__uncultured_bacterium_g_Cetobacterium
OTU105	894	1198	1321	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU106	93	246	235	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Mollicutes_RF39; f__uncultured_bacterium_o_Mollicutes_RF39; g__uncultured_bacterium_o_Mollicutes_RF39; s__uncultured_bacterium_o_Mollicutes_RF39
OTU107	362	185	302	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Tyzzerella; s__uncultured_bacterium_g_Tyzzerella
OTU108	679	534	589	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU109	1147	169	553	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU11	1798	4875	4222	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotellaceae_NK3B31_group; s__uncultured_bacterium_g_Prevotellaceae_NK3B31_group
OTU110	893	446	981	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-006; s__uncultured_bacterium_g_Lachnospiraceae_UCG-006
OTU111	781	569	25	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU112	757	331	442	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU113	333	431	265	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_9; s__uncultured_bacterium_g_Ruminiclostridium_9
OTU114	830	294	711	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU115	88	370	395	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU116	164	230	411	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU117	7	339	185	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-010; s__uncultured_bacterium_g_Ruminococcaceae_UCG-010
OTU118	450	209	295	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU119	219	162	177	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU12	8070	3798	4800	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU120	431	163	399	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-001; s__uncultured_bacterium_g_Lachnospiraceae_UCG-001
OTU121	103	260	189	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-010; s__uncultured_bacterium_g_Ruminococcaceae_UCG-010

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OTU122	158	257	237	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Family_XIII; g__[Eubacterium]_nodatum_group; s__uncultured_bacterium_g_[Eubacterium]_nodatum_group
OTU123	237	145	158	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Negativibacillus; s__uncultured_bacterium_g_Negativibacillus
OTU124	869	491	567	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU125	517	105	411	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU126	266	261	167	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU127	322	230	370	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptococcaceae; g__uncultured_bacterium_f_Peptococcaceae; s__uncultured_bacterium_f_Peptococcaceae
OTU128	342	113	480	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__[Eubacterium]_coprostanoligenes_group; s__uncultured_bacterium_g_[Eubacterium]_coprostanoligenes_group
OTU129	597	303	217	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__ASF356; s__uncultured_bacterium_g_ASF356
OTU13	2040	6821	4164	k__Bacteria; p__Patescibacteria; c__Saccharimonadia; o__Saccharimonadales; f__Saccharimonadaceae; g__Candidatus_Saccharimonas; s__uncultured_bacterium_g_Candidatus_Saccharimonas
OTU130	161	156	159	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Eggerthellaceae; g__Enterorhabdus; s__uncultured_bacterium_g_Enterorhabdus
OTU131	211	269	246	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Shuttleworthia; s__uncultured_bacterium_g_Shuttleworthia
OTU132	501	176	308	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium; s__uncultured_bacterium_g_Ruminiclostridium
OTU133	304	400	348	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU134	437	2	65	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-010; s__uncultured_bacterium_g_Ruminococcaceae_UCG-010
OTU135	190	218	312	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU136	264	160	174	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__uncultured_bacterium_g_Blautia
OTU137	368	201	207	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Butyricoccus; s__uncultured_bacterium_g_Butyricoccus
OTU138	121	232	185	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU139	442	160	205	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__A2; s__uncultured_bacterium_g_A2
OTU14	3189	4246	5318	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU140	462	197	565	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU141	456	304	291	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU142	288	157	153	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__UBA1819; s__uncultured_bacterium_g_UBA1819
OTU143	25	283	77	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Mollicutes_RF39; f__uncultured_bacterium_o_Mollicutes_RF39; g__uncultured_bacterium_o_Mollicutes_RF39; s__uncultured_bacterium_o_Mollicutes_RF39
OTU144	579	154	92	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU145	436	102	194	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU146	131	249	387	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Betaproteobacteriales; f__Burkholderiaceae; g__Parasutterella; s__uncultured_bacterium_g_Parasutterella

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OTU147	55	246	178	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU148	204	164	100	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__uncultured_bacterium_f_Desulfovibrionaceae; s__uncultured_bacterium_f_Desulfovibrionaceae
OTU149	535	14	6	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU15	4172	4640	7762	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__uncultured_bacterium_g_Lactobacillus
OTU150	73	163	130	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU151	448	263	69	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU152	423	301	306	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_FCS020_group; s__uncultured_bacterium_g_Lachnospiraceae_FCS020_group
OTU153	299	227	342	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU154	155	114	146	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU155	147	165	456	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU156	554	232	442	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU157	504	261	334	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU158	734	235	478	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU159	114	201	74	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Mollicutes_RF39; f__uncultured_bacterium_o_Mollicutes_RF39; g__uncultured_bacterium_o_Mollicutes_RF39; s__uncultured_bacterium_o_Mollicutes_RF39
OTU16	7059	7257	6025	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU160	105	60	178	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium; s__uncultured_bacterium_g_Ruminiclostridium
OTU161	146	172	60	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU162	971	395	384	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-008; s__uncultured_bacterium_g_Lachnospiraceae_UCG-008
OTU163	320	75	217	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU164	522	123	190	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU165	149	196	259	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Marinifilaceae; g__Butyricimonas; s__uncultured_bacterium_g_Butyricimonas
OTU166	1031	1603	505	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU167	218	50	167	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU168	221	22	279	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU169	83	68	251	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uncultured_bacterium_g_Bacteroides
OTU17	2267	4446	916	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus_1; s__uncultured_bacterium_g_Ruminococcus_1

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OTU170	244	138	90	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__uncultured_bacterium_g_Blautia
OTU171	428	324	210	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU172	302	184	314	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU173	65	201	25	k__Bacteria; p__Cyanobacteria; c__Melainabacteria; o__Gastranaerophilales; f__Blattella_germanica_German_cockroach; g__uncultured_bacterium_f_Blattella_germanica_German_cockroach; s__uncultured_bacterium_f_Blattella_germanica_German_cockroach
OTU174	257	176	233	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU175	145	74	95	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Anaerotruncus; s__uncultured_bacterium_g_Anaerotruncus
OTU176	516	259	313	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU177	42	99	89	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Eggerthellaceae; g__uncultured_bacterium_f_Eggerthellaceae; s__uncultured_bacterium_f_Eggerthellaceae
OTU178	260	89	198	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus_1; s__uncultured_bacterium_g_Ruminococcus_1
OTU179	92	129	65	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_9; s__uncultured_bacterium_g_Ruminiclostridium_9
OTU18	4823	3857	3416	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU180	298	147	99	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU181	80	65	58	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__uncultured_bacterium_g_Alistipes
OTU182	129	91	135	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillibacter; s__uncultured_bacterium_g_Oscillibacter
OTU183	142	67	82	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU184	96	89	70	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__uncultured_bacterium_g_Streptococcus
OTU185	64	99	81	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__uncultured_bacterium_f_Erysipelotrichaceae; s__uncultured_bacterium_f_Erysipelotrichaceae
OTU186	70	107	219	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Erysipelatoclostridium; s__uncultured_bacterium_g_Erysipelatoclostridium
OTU187	356	76	236	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU188	29	2	326	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU189	64	81	61	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Mollicutes_RF39; f__uncultured_bacterium_o_Mollicutes_RF39; g__uncultured_bacterium_o_Mollicutes_RF39; s__uncultured_bacterium_o_Mollicutes_RF39
OTU19	1274	2689	3505	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotellaceae_UCG-001; s__uncultured_bacterium_g_Prevotellaceae_UCG-001
OTU190	140	113	50	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Eubacterium]_ventriosum_group; s__uncultured_bacterium_g_[Eubacterium]_ventriosum_group
OTU191	153	87	155	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU192	189	131	130	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU193	143	86	134	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU194	239	41	61	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae

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OTU195	21	69	92	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiales_vadinBB60_group; g__uncultured_bacterium_f_Clostridiales_vadinBB60_group; s__uncultured_bacterium_f_Clostridiales_vadinBB60_group
OTU196	289	143	248	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_5; s__uncultured_bacterium_g_Ruminiclostridium_5
OTU197	170	36	55	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU198	182	104	233	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU199	84	72	101	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU2	45952	51222	98973	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__uncultured_bacterium_g_Lactobacillus
OTU20	699	6055	675	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Turicibacter; s__uncultured_bacterium_g_Turicibacter
OTU200	406	164	147	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_5; s__uncultured_bacterium_g_Ruminiclostridium_5
OTU201	47	73	153	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU202	59	16	142	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU203	115	61	24	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Corynebacteriales; f__Nocardiaceae; g__Rhodococcus; s__bacterium_GC452011
OTU204	455	254	304	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU205	252	14	15	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU206	101	44	35	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-005; s__uncultured_bacterium_g_Ruminococcaceae_UCG-005
OTU207	189	70	108	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU208	79	55	91	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Family_XIII; g__[Eubacterium]_brachy_group; s__uncultured_bacterium_g_[Eubacterium]_brachy_group
OTU209	115	66	88	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU21	6617	9125	4045	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU210	31	38	90	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Eggerthellaceae; g__Enterorhabdus; s__uncultured_bacterium_g_Enterorhabdus
OTU211	11	46	138	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU212	43	26	94	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU213	66	39	44	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU214	39	31	121	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU215	118	63	125	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU216	32	67	79	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Family_XIII; g__Family_XIII_AD3011_group; s__uncultured_bacterium_g_Family_XIII_AD3011_group
OTU217	51	11	91	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU218	161	135	229	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae

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OTU219	52	50	87	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Family_XIII; g__Anaerovorax; s__uncultured_bacterium_g_Anaerovorax
OTU22	10088	6335	7824	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU220	301	0	0	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU221	27	49	66	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__Catabacter; s__Christensenella_timonensis
OTU222	180	43	72	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU223	96	181	189	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Tannerellaceae; g__Parabacteroides; s__uncultured_bacterium_g_Parabacteroides
OTU224	98	26	39	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU225	163	70	146	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU226	66	132	150	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__A2; s__uncultured_bacterium_g_A2
OTU228	82	60	32	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Eggerthellaceae; g__Enterorhabdus; s__uncultured_bacterium_g_Enterorhabdus
OTU229	28	66	49	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-005; s__uncultured_bacterium_g_Ruminococcaceae_UCG-005
OTU23	469	4097	1749	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Faecalibaculum; s__uncultured_bacterium_g_Faecalibaculum
OTU231	1640	1691	2775	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU232	181	304	185	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_9; s__uncultured_bacterium_g_Ruminiclostridium_9
OTU234	41	59	35	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Marinifilaceae; g__Butyricimonas; s__uncultured_bacterium_g_Butyricimonas
OTU235	112	15	45	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU237	32	59	43	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Candidatus_Soleaferrea; s__uncultured_bacterium_g_Candidatus_Soleaferrea
OTU239	231	54	88	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU24	5428	1844	2492	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU240	228	58	86	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU241	49	29	67	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-013; s__uncultured_bacterium_g_Ruminococcaceae_UCG-013
OTU242	53	54	27	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__uncultured_bacterium_g_Alistipes
OTU244	795	2641	3030	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU245	55	98	91	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU248	94	57	24	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__uncultured_bacterium_f_Erysipelotrichaceae; s__uncultured_bacterium_f_Erysipelotrichaceae
OTU25	3508	3405	3626	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU250	63	35	69	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae

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OTU251	101	78	64	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU253	145	64	13	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU255	38	13	95	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU256	238	490	481	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU258	46	24	139	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU259	92	64	29	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Aeromonadales; f__Aeromonadaceae; g__Aeromonas; s__uncultured_bacterium_g_Aeromonas
OTU26	5129	4177	3659	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU260	276	152	122	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillibacter; s__uncultured_bacterium_g_Oscillibacter
OTU262	173	28	104	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU267	715	215	686	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium; s__uncultured_bacterium_g_Ruminiclostridium
OTU27	1923	3489	3732	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU270	296	50	10	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-001; s__uncultured_bacterium_g_Lachnospiraceae_UCG-001
OTU272	27	62	55	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__Christensenellaceae_R-7_group; s__uncultured_bacterium_g_Christensenellaceae_R-7_group
OTU273	90	28	97	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU28	5810	3221	3670	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU284	76	60	33	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU288	45	65	36	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__uncultured_bacterium_g_Alistipes
OTU29	1413	2503	1632	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU291	15	51	79	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU292	156	28	109	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU293	58	38	41	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae_1; g__Candidatus_Arthromitus; s__uncultured_bacterium_g_Candidatus_Arthromitus
OTU294	77	68	61	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Marvinbryantia; s__uncultured_bacterium_g_Marvinbryantia
OTU297	107	25	259	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU3	11346	11026	13283	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Alloprevotella; s__uncultured_bacterium_g_Alloprevotella
OTU30	2362	3698	2913	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU304	140	44	111	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU31	2004	2032	202	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae

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OTU314	22	107	99	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Mollicutes_RF39; f__uncultured_bacterium_o__Mollicutes_RF39; g__uncultured_bacterium_o__Mollicutes_RF39; s__uncultured_bacterium_o__Mollicutes_RF39
OTU32	1637	544	1214	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Bilophila; s__uncultured_bacterium_g__Bilophila
OTU323	1094	1115	3245	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU33	2570	3593	3250	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Eubacterium]_ruminantium_group; s__uncultured_bacterium_g__[Eubacterium]_ruminantium_group
OTU337	278	157	207	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__GCA-900066575; s__uncultured_bacterium_g__GCA-900066575
OTU34	4113	1624	3205	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU35	3134	661	1991	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f__Lachnospiraceae; s__uncultured_bacterium_f__Lachnospiraceae
OTU357	480	492	270	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__uncultured_bacterium_g__Alistipes
OTU36	1478	1328	1176	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f__Muribaculaceae; s__uncultured_bacterium_f__Muribaculaceae
OTU361	2649	1057	261	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU364	179	358	492	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU366	757	104	219	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f__Lachnospiraceae; s__uncultured_bacterium_f__Lachnospiraceae
OTU37	6238	822	1038	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU371	417	67	164	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-001; s__uncultured_bacterium_g__Lachnospiraceae_UCG-001
OTU373	90	70	45	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Alicyclobacillaceae; g__Effusibacillus; s__Effusibacillus_pohliae
OTU378	194	79	286	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillibacter; s__uncultured_bacterium_g__Oscillibacter
OTU38	974	934	893	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__uncultured_bacterium_g__Alistipes
OTU39	1788	2909	2564	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uncultured_bacterium_g__Bacteroides
OTU399	184	112	13	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__GCA-900066575; s__uncultured_bacterium_g__GCA-900066575
OTU4	77346	1580	10	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Dubosiella; s__uncultured_bacterium_g__Dubosiella
OTU40	1580	252	786	k__Bacteria; p__Deferribacteres; c__Deferribacteres; o__Deferribacterales; f__Deferribacteraceae; g__Mucispirillum; s__Mucispirillum_schaedleri_ASF457
OTU400	423	164	363	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4B4_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4B4_group
OTU408	114	162	110	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f__Muribaculaceae; s__uncultured_bacterium_f__Muribaculaceae
OTU41	1384	2332	904	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae_1; g__Clostridium_sensu_stricto_1; s__uncultured_bacterium_g__Clostridium_sensu_stricto_1
OTU413	78	22	97	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f__Lachnospiraceae; s__uncultured_bacterium_f__Lachnospiraceae
OTU415	1174	92	212	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f__Lachnospiraceae; s__uncultured_bacterium_f__Lachnospiraceae
OTU42	5662	812	1352	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Eubacterium]_xylanophilum_group; s__uncultured_bacterium_g__[Eubacterium]_xylanophilum_group
OTU420	6	73	58	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Faecalibaculum; s__uncultured_bacterium_g__Faecalibaculum

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OTU423	39	159	197	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotellaceae_NK3B31_group; s__uncultured_bacterium_g__Prevotellaceae_NK3B31_group
OTU426	54	50	98	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-006; s__uncultured_bacterium_g__Lachnospiraceae_UCG-006
OTU427	151	115	123	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Acetatifactor; s__uncultured_bacterium_g__Acetatifactor
OTU428	115	69	68	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f__Lachnospiraceae; s__uncultured_bacterium_f__Lachnospiraceae
OTU43	1583	2309	1937	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f__Muribaculaceae; s__uncultured_bacterium_f__Muribaculaceae
OTU432	194	122	319	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f__Lachnospiraceae; s__uncultured_bacterium_f__Lachnospiraceae
OTU435	243	340	272	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g__Ruminococcaceae_UCG-014
OTU436	37	20	756	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU438	150	158	232	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f__Muribaculaceae; s__uncultured_bacterium_f__Muribaculaceae
OTU44	1671	1919	1780	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-006; s__uncultured_bacterium_g__Lachnospiraceae_UCG-006
OTU449	356	261	601	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f__Muribaculaceae; s__uncultured_bacterium_f__Muribaculaceae
OTU45	1220	880	2068	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f__Lachnospiraceae; s__uncultured_bacterium_f__Lachnospiraceae
OTU453	275	83	184	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-003; s__uncultured_bacterium_g__Ruminococcaceae_UCG-003
OTU46	873	1464	1535	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Eubacterium]_fissicatena_group; s__uncultured_bacterium_g__[Eubacterium]_fissicatena_group
OTU461	269	124	212	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_9; s__uncultured_bacterium_g__Ruminiclostridium_9
OTU463	84	15	261	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium; s__uncultured_bacterium_g__Ruminiclostridium
OTU466	178	180	292	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU47	1112	3497	3989	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g__Ruminococcaceae_UCG-014
OTU470	232	60	298	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU478	385	158	280	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Intestinimonas; s__uncultured_bacterium_g__Intestinimonas
OTU48	639	1193	927	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus_1; s__uncultured_bacterium_g__Ruminococcus_1
OTU485	155	9	57	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g__Roseburia
OTU486	948	861	350	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g__Ruminococcaceae_UCG-014
OTU487	218	211	332	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU489	220	209	358	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g__Lachnospiraceae_NK4A136_group
OTU49	1092	560	603	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__uncultured_bacterium_g__Alistipes

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OTU492	115	98	127	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU493	445	207	98	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnoclostridium; s__uncultured_bacterium_g_Lachnoclostridium
OTU495	252	64	178	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Eubacterium]_oxidoreducens_group; s__uncultured_bacterium_g_[Eubacterium]_oxidoreducens_group
OTU496	118	57	15	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnoclostridium; s__uncultured_bacterium_g_Lachnoclostridium
OTU5	10914	15909	16481	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU50	1484	1124	916	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU500	98	86	62	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU502	207	129	137	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU503	179	68	72	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU505	303	134	189	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Intestinimonas; s__uncultured_bacterium_g_Intestinimonas
OTU508	114	99	90	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU509	80	47	47	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU51	2189	1402	1899	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU510	217	190	339	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__A2; s__uncultured_bacterium_g_A2
OTU52	597	1074	330	k__Bacteria; p__Cyanobacteria; c__Melainabacteria; o__Gastranaerophilales; f__uncultured_bacterium_o_Gastranaerophilales; g__uncultured_bacterium_o_Gastranaerophilales; s__uncultured_bacterium_o_Gastranaerophilales
OTU520	274	158	247	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU521	245	364	292	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU522	77	34	95	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU524	90	132	92	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU528	98	143	126	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU53	722	513	818	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uncultured_bacterium_g_Bacteroides
OTU531	299	0	29	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU534	121	155	143	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU536	79	43	52	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_9; s__uncultured_bacterium_g_Ruminiclostridium_9
OTU54	2028	2218	1971	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__Muribaculum; s__Parabacteroides_sp_YL27
OTU544	61	65	50	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU545	524	711	679	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae

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OTU546	224	194	532	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU547	38	72	92	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__uncultured_bacterium_g_Roseburia
OTU55	530	1109	1238	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae; g__Romboutsia; s__uncultured_bacterium_g_Romboutsia
OTU552	180	275	417	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU56	1780	3053	2962	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU563	688	507	607	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU565	124	135	147	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Eggerthellaceae; g__Enterorhabdus; s__uncultured_bacterium_g_Enterorhabdus
OTU568	26	18	92	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__UBA1819; s__uncultured_bacterium_g_UBA1819
OTU57	1177	1160	1408	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU579	146	892	1006	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__Lactobacillus_gasseri
OTU58	984	509	674	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus_1; s__uncultured_bacterium_g_Ruminococcus_1
OTU581	103	327	242	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU584	101	97	87	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU59	2908	1923	3017	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU593	61	109	31	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU596	392	52	64	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Eubacterium]_xylanophilum_group; s__uncultured_bacterium_g_[Eubacterium]_xylanophilum_group
OTU599	80	97	31	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU6	15510	17930	6442	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU60	10229	12249	10054	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU600	216	120	235	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU602	51	44	64	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU609	70	50	91	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU61	2219	30	788	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__uncultured_bacterium_g_Blautia
OTU614	161	50	18	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU618	102	102	2	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU62	894	335	814	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium; s__uncultured_bacterium_g_Ruminiclostridium
OTU620	67	38	59	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnoclostridium; s__uncultured_bacterium_g_Lachnoclostridium

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OTU626	198	94	71	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU627	133	193	378	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU63	680	698	830	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU635	88	59	65	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_5; s__uncultured_bacterium_g_Ruminiclostridium_5
OTU636	98	102	55	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Rikenellaceae_RC9_gut_group; s__uncultured_bacterium_g_Rikenellaceae_RC9_gut_group
OTU64	272	1328	17	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uncultured_bacterium_g_Bacteroides
OTU640	638	698	1140	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU644	3945	113	0	k__Bacteria; p__Firmicutes; c__Erysipelotrichia; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Dubosiella; s__uncultured_bacterium_g_Dubosiella
OTU65	131	1061	353	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__uncultured_bacterium_g_Bifidobacterium
OTU652	259	323	590	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU658	243	289	708	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__uncultured_bacterium_g_Lactobacillus
OTU66	571	1195	642	k__Bacteria; p__Cyanobacteria; c__Melainabacteria; o__Gastranaerophilales; f__Candidatus_Gastranaerophilales_bacterium_Zag_111; g__uncultured_bacterium_f_Candidatus_Gastranaerophilales_bacterium_Zag_111; s__uncultured_bacterium_f_Candidatus_Gastranaerophilales_bacterium_Zag_111
OTU665	139	103	67	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU668	68	103	116	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uncultured_bacterium_g_Bacteroides
OTU669	36	53	55	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU67	1431	1281	883	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU675	238	41	81	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU679	57	51	48	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU68	1663	782	723	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__GCA-900066575; s__uncultured_bacterium_g_GCA-900066575
OTU680	255	151	155	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU682	107	119	91	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU69	776	406	394	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-009; s__uncultured_bacterium_g_Ruminococcaceae_UCG-009
OTU691	42	27	70	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU693	180	236	146	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU694	74	24	74	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-006; s__uncultured_bacterium_g_Lachnospiraceae_UCG-006
OTU697	39	41	102	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU698	55	64	113	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae

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OTU7	1698	10259	8220	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__uncultured_bacterium_g_Lactobacillus
OTU70	1877	1138	1819	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU702	44	40	93	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU706	45	37	58	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU709	168	48	127	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium; s__uncultured_bacterium_g_Ruminiclostridium
OTU71	1624	740	744	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU710	107	25	90	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU72	970	544	1118	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU721	96	67	238	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU723	544	366	773	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU73	379	684	176	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU733	58	79	86	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU736	83	96	94	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU739	104	128	93	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU74	955	721	724	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU741	200	134	195	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU748	228	439	507	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU75	806	592	554	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium; s__uncultured_bacterium_g_Ruminiclostridium
OTU751	99	19	47	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-001; s__uncultured_bacterium_g_Lachnospiraceae_UCG-001
OTU753	18	6	391	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU757	51	49	57	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU758	45	54	192	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU759	15	360	531	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU76	1076	1311	2973	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU761	236	45	340	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group

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OTU763	94	17	94	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_UCG-001; s__uncultured_bacterium_g_Lachnospiraceae_UCG-001
OTU77	110	819	509	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU78	436	377	1862	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU79	607	1287	1485	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Betaproteobacteriales; f__Burkholderiaceae; g__Parasutterella; s__Burkholderiales_bacterium_YL45
OTU8	9147	8334	13390	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU80	786	207	766	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s__uncultured_bacterium_g_Lachnospiraceae_NK4A136_group
OTU81	1274	259	442	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__uncultured_bacterium_f_Lachnospiraceae; s__uncultured_bacterium_f_Lachnospiraceae
OTU82	279	492	330	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminiclostridium_6; s__uncultured_bacterium_g_Ruminiclostridium_6
OTU83	2023	1199	1182	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Intestinimonas; s__uncultured_bacterium_g_Intestinimonas
OTU84	121	263	413	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__uncultured_bacterium_o_Rhodospirillales; g__uncultured_bacterium_o_Rhodospirillales; s__uncultured_bacterium_o_Rhodospirillales
OTU85	537	1525	545	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_UCG-014; s__uncultured_bacterium_g_Ruminococcaceae_UCG-014
OTU86	772	548	236	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__Rickettsiaceae; g__Candidatus_Hemipteriphilus; s__secondary_symbiont_of_Sitobion_miscanthi
OTU87	852	682	1203	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU88	893	300	473	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillibacter; s__uncultured_bacterium_g_Oscillibacter
OTU89	632	656	1021	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU9	3579	3390	2680	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Rikenellaceae_RC9_gut_group; s__uncultured_bacterium_g_Rikenellaceae_RC9_gut_group
OTU90	1003	980	1085	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU91	996	471	1283	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Marvinbryantia; s__uncultured_bacterium_g_Marvinbryantia
OTU92	59	576	115	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Atopobiaceae; g__Coriobacteriaceae_UCG-002; s__uncultured_bacterium_g_Coriobacteriaceae_UCG-002
OTU93	247	196	642	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__uncultured_bacterium_g_Lactobacillus
OTU94	352	555	436	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__Alistipes; s__uncultured_bacterium_g_Alistipes
OTU95	1126	442	798	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__uncultured_bacterium_f_Ruminococcaceae; s__uncultured_bacterium_f_Ruminococcaceae
OTU96	5136	5810	15918	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Muribaculaceae; g__uncultured_bacterium_f_Muribaculaceae; s__uncultured_bacterium_f_Muribaculaceae
OTU97	248	304	257	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Eggerthellaceae; g__Adlercreutzia; s__uncultured_bacterium_g_Adlercreutzia
OTU98	224	205	198	k__Bacteria; p__Cyanobacteria; c__Melainabacteria; o__Gastranaerophilales; f__uncultured_bacterium_o_Gastranaerophilales; g__uncultured_bacterium_o_Gastranaerophilales; s__uncultured_bacterium_o_Gastranaerophilales
OTU99	204	272	294	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcaceae_NK4A214_group; s__uncultured_bacterium_g_Ruminococcaceae_NK4A214_group

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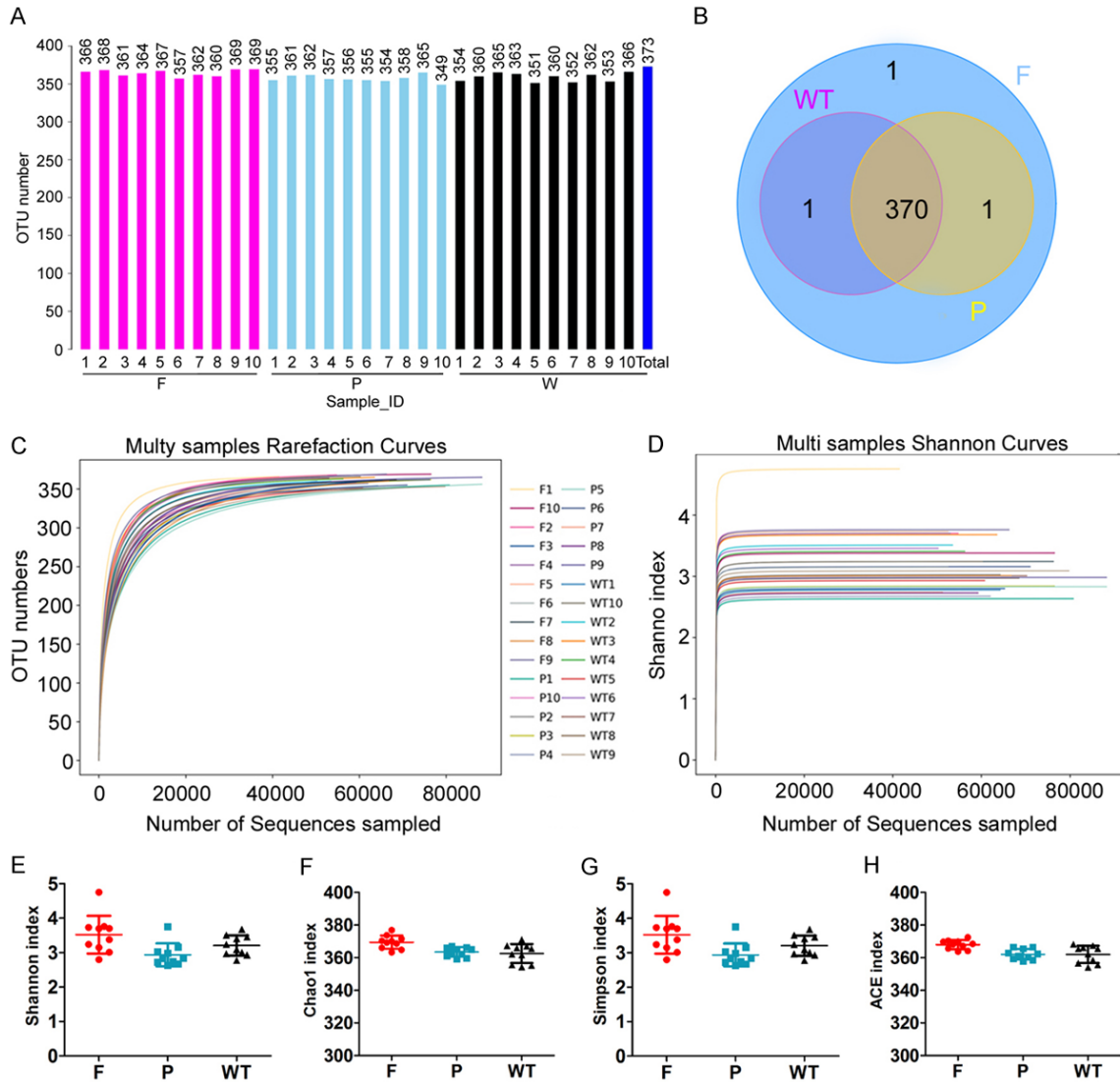


Figure 3. Alpha diversity analysis of the 16S rRNA v3+v4 sequencing results of the gut microbiota in the feces of mice in various groups. A. OTU statistical results of samples from various groups. B. OTU-Venn comparison results of samples from various groups. C. OTU rarefaction curve analysis results of samples from various groups. D. OTU Shannon index analysis results of samples from various groups. There were no significant differences in the number of OTUs between the three groups. E. Alpha diversity Shannon index statistics of OTUs in various groups. F. Alpha diversity Chao1 index statistics of OTUs in various groups. G. Alpha diversity Simpson index statistics of OTUs in various groups. H. Alpha diversity ACE index statistics of OTUs in various groups. The preliminary tests showed that fisetin intervention did not alter gut microbial counts in POF mice.

head thrashes and body bends were recorded for 1 min to calculate their frequency in *C. elegans* progeny. L4 stage *C. elegans* were randomly selected, and individual worms were added to the NGM culture medium of the experimental and control groups. The specimens were cultured at 20°C. The plates were rotated once every 12 h, and the worms were moved to NGM culture medium containing CTX until the end of the spawning period. The number of progenies in NGM culture medium from each group was calculated.

Statistical analysis

Each experiment was performed as least thrice, and GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Data from follicular count experiment, qPCR, *C. elegans* study, and FCM, are expressed as mean \pm standard error. Differences between groups were evaluated with Student's *t*-test. A *p* value less than 0.05 was considered statistically significant. For gut microbiota analysis, the LDA-effect size (LEfSe) method was used

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Table 2. Alpha diversity

Sample ID	OTU	ACE	Chao1	Simpson	Shannon	Coverage
F1	366	368.239	370	0.0176	4.7523	0.9998
F10	369	369.668	369.3333	0.1438	3.3786	1
F2	368	369.776	370.3333	0.0935	3.6994	0.9999
F3	361	368.485	373.6667	0.1884	2.7987	0.9997
F4	364	365.605	364.7895	0.0992	3.7008	0.9999
F5	367	370.371	371.5833	0.1003	3.7301	0.9998
F6	357	363.741	363.3333	0.139	3.1478	0.9996
F7	362	364.106	366	0.1605	3.2399	0.9999
F8	360	367.17	368.6364	0.1338	3.0117	0.9997
F9	369	372.423	376.8571	0.1209	3.759	0.9998
P1	355	360.549	363	0.2933	2.6332	0.9998
P10	349	358.482	367.0714	0.098	3.758	0.9995
P2	361	365.537	365.3333	0.2274	3.0253	0.9998
P3	362	366.436	366.3333	0.2428	2.8388	0.9998
P4	357	360.964	359.64	0.264	2.673	0.9998
P5	356	362.862	365	0.2592	2.8294	0.9998
P6	355	359.329	361	0.1832	3.1559	0.9998
P7	354	357.766	359.2	0.2854	2.739	0.9997
P8	358	362.32	362.1364	0.2907	2.7281	0.9998
P9	365	366.481	366.1538	0.2325	2.9807	0.9999
WT1	354	358.488	361.5	0.2225	2.7758	0.9998
WT10	366	367.391	367.25	0.1674	3.2391	0.9999
WT2	360	362.213	362	0.1198	3.5081	0.9999
WT3	365	368.208	371.1111	0.1165	3.6781	0.9998
WT4	363	365.394	367	0.1338	3.4047	0.9998
WT5	351	355.638	355.3333	0.2165	2.93	0.9998
WT6	360	366.101	365.913	0.0982	3.4549	0.9997
WT7	352	354.142	354.3333	0.2106	3.0075	0.9999
WT8	362	365.453	365.4375	0.2009	2.9697	0.9998
WT9	353	356.592	355.5	0.2009	3.088	0.9999

for the quantitative analysis of biomarkers in each group. LEfSe analysis (at LDA threshold >4), the non-parametric factorial Kruskal-Wallis sum-rank test, and the unpaired Wilcoxon rank-sum test were performed to identify the most abundant taxa.

Results

Fisetin effectively alleviates ovarian damage in POF mice and increases the number of normal ovarian follicles

A POF model was established by intraperitoneal injection of CTX in fisetin- or PBS-treated mice. Pathological testing by HE staining showed that mature ovarian follicles disappeared,

the number of atretic ovarian follicles significantly increased, and the number of quality ovarian granulosa cells significantly decreased in the ovarian tissues of POF mice in the PBS group (**Figure 1A**). Ovarian follicles at various stages of development were observed in the ovaries of POF mice in the fisetin group. Simultaneously, the proportion of normal ovarian follicles significantly increased while the proportion of atretic ovarian follicles significantly decreased (**Figure 1B and 1C**). qPCR and western blotting were used to detect the expression of proliferation- and aging-related biomarkers in each group. Results showed that the expression levels of AMH (an ovarian granulosa cell marker) and Ki67 (a cell proliferation marker) in the ovarian tissues of POF mice in the PBS group were lower significantly than those of mice in the untreated group (**Figure 1D**). However, the expression levels of p16 and phosphorylated H2A.X (pho-H2A.X) proteins (cell ageing markers) in the ovarian tissues of POF mice in the PBS group were significantly higher

than those of mice in the untreated group (**Figure 1D and 1E**). However, the expression levels of p16 and pho-H2A.X in the ovaries of POF mice in the fisetin group were significantly decreased, while those of AMH and Ki67 were significantly increased (**Figure 1D and 1E**). These data prove that fisetin effectively alleviates ovarian damage in POF mice and increases the number of normal ovarian follicles.

Fisetin significantly enhances C. elegans lifespan and fertility

Experimental results showed that compared with PBS, fisetin significantly increased the life span of *C. elegans* (**Figure 2A and 2B**), the frequency of head thrashes and body bends

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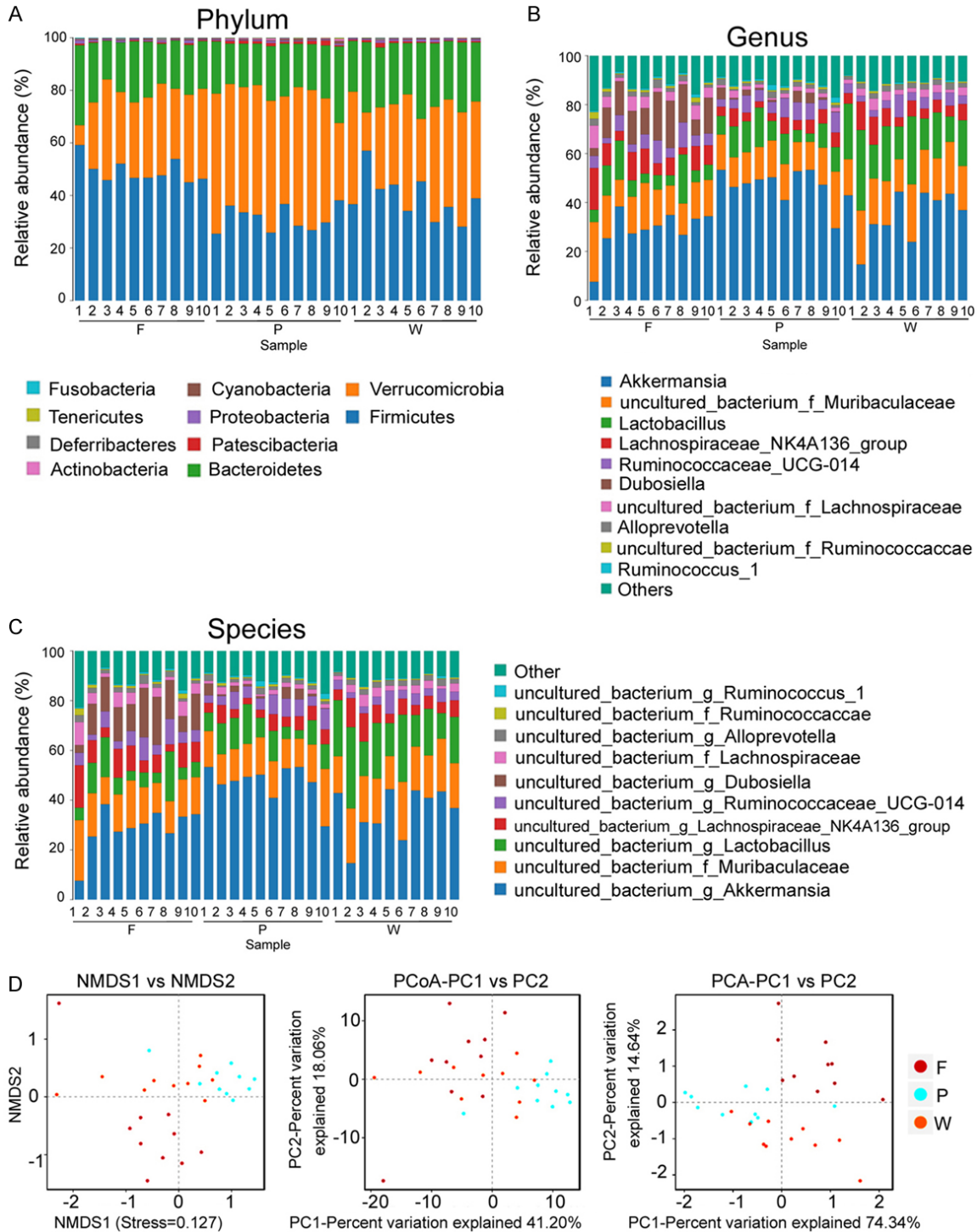


Figure 4. Beta diversity analysis of the 16S rRNA v3+v4 sequencing results of the gut microbiota of feces from various groups of mice. A. Community clustering analysis of OTUs in samples from various groups at the phylum level: analysis at the phylum level showed that the relative abundance of *Firmicutes* was significantly increased in the gut of group F compared with that in group P, while the relative abundance of *Verrucomicrobia* was significantly decreased compared with group F. B. Community clustering analysis of OTUs in samples from various groups at the genus level: analysis at the genus level showed that the relative abundance of *uncultured_bacterium_f_Lachnospiraceae* was significantly increased, while that of *Akkermansia* was significantly decreased in group F compared with that in group P. C. Community clustering analysis of OTUs in samples from various groups at the species level: analysis at the species level showed that the relative abundance of *uncultured_bacterium_f_Lachnospiraceae*

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was significantly increased, while that of uncultured_bacterium_g_Akkermans was significantly decreased in group F compared with that in group P. D. Beta diversity analysis of OTUs in samples from various groups based on unweighted UniFrac. Results revealed significant differences in community distribution between the microbial communities of the three groups.

Table 3. Classify

Sample	Kindom	Phylum	Class	Order	Family	Genus	Species
F1	1	10	15	18	33	89	91
F2	1	10	15	19	34	91	93
F3	1	10	15	18	34	90	92
F4	1	10	15	19	35	92	94
F5	1	10	15	19	34	91	93
F6	1	10	15	18	34	89	91
F7	1	10	15	18	32	89	91
F8	1	10	15	18	34	91	93
F9	1	10	15	18	34	91	93
F10	1	10	15	19	34	91	93
P1	1	10	15	19	34	90	92
P2	1	10	15	19	34	90	92
P3	1	10	15	18	34	91	93
P4	1	10	15	18	34	91	93
P5	1	10	15	18	34	90	92
P6	1	10	15	18	34	91	93
P7	1	10	15	18	34	88	90
P8	1	10	15	19	34	90	92
P9	1	10	15	18	34	91	93
P10	1	10	15	19	35	90	92
WT1	1	10	15	18	33	90	92
WT2	1	10	15	18	33	89	91
WT3	1	10	15	18	33	90	92
WT4	1	10	15	18	33	90	92
WT5	1	10	15	17	33	90	92
WT6	1	10	15	18	34	91	93
WT7	1	10	15	18	33	90	92
WT8	1	10	15	19	35	91	93
WT9	1	10	15	18	34	90	92
WT10	1	10	15	18	34	91	93
Total	1	10	15	19	35	92	94

(**Figure 2C, 2D**, [Supplementary Videos 1, 2, 3](#)), and the number of pregnant *C. elegans* after CTX treatment (**Figure 2E**). In addition, qPCR results showed that compared with that in the PBS group, the mRNA expression of genes associated with insulin/IGF-like signaling (IIS) in *C. elegans* was significantly increased in the fisetin group after CTX treatment (**Figure 2F**). Hence, our results showed that fisetin effectively increased *C. elegans* lifespan and fertility via stimulating the IIS signaling pathway.

Fisetin does not alter gut microbial counts in POF mice

After treating the feces of mice in the normal group (W), POF mice in the fisetin group (F) and PBS group (P), bacterial 16S rRNA v3+v4 sequencing was carried out to evaluate the composition of the gut microbiota and the distribution of bacterial communities. Thirty samples were sequenced to obtain 3,255,567 reads. After the splicing of paired-end reads and filtering, a total of 2,765,085 clean tags were generated. There were at least 56,084 clean tags per sample, and 92,170 clean tags were generated on an average (**Table 1**). The UCLUST in QIIME (version 1.8.0) software was used to cluster tags into OTUs based on 97% sequence similarity. There were no significant differences in OTU quantity between the three groups (**Figure 3A-E; Table 1**). Next, we generated Venn diagrams for OTUs, OTU rank, rarefaction curves, Shannon index curves, Chao1 curves, Simpson curves, and ACE curves (**Figure 3E-H; Table 2**). Results showed consistent numbers of OTUs. Preliminary tests showed that fisetin intervention did not alter gut microbial counts in POF mice.

Fisetin significantly alters the distribution and diversity of gut microbiota in POF mice

By comparing the representative sequences and microbial reference data of OTUs, every OTU can be classified into a species. Analysis at the phylum level showed that the relative abundance of *Firmicutes* in group F was significantly increased compared with that in group P, while the relative abundance of *Verucomicrobia* was significantly decreased compared with that in group F (**Figure 4A; Table 3**). Analysis at the genus level showed that the rel-

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Table 4. The result of Genus ANOVA

Genus	P (Mean)	P (Sd)	WT (Mean)	WT (Sd)	F (Mean)	F (Sd)	multiGroup (p)	multiGroup (p-corrected)	P:WT (p)	P:F (p)	WT:F (p)
A2	0.00071	0.00059	0.00112	0.00037	0.00127	0.00103	0.21318	0.26866			
ASF356	0.00049	0.00034	0.00035	0.00016	0.00098	0.00078	0.02296	0.06036			
Acetatifactor	0.00018	8.3E-05	0.00021	0.00013	0.00026	0.00021	0.45876	0.5147			
Adlercreutzia	0.00044	0.00027	0.0004	0.00013	0.00041	0.00015	0.91877	0.91877			
Aeromonas	9.4E-05	3.8E-05	4.7E-05	2.9E-05	0.00016	7.2E-05	0.0002	0.002	≥ 0.1	< 0.05	< 0.001
Akkermansia	0.47068	0.07268	0.3527	0.10065	0.28707	0.08508	0.00025	0.00229	< 0.02	< 0.001	≥ 0.1
Alistipes	0.00415	0.0024	0.00374	0.00096	0.00517	0.00149	0.17816	0.24104			
Alloprevotella	0.0154	0.00641	0.02097	0.00379	0.01955	0.00723	0.11644	0.18156			
Anaerotruncus	0.00011	8.6E-05	0.00015	8.1E-05	0.00024	0.00014	0.03213	0.0758			
Anaerovorax	8.1E-05	0.00006	0.00014	5.9E-05	9.2E-05	9.3E-05	0.20167	0.26132			
Bacteroides	0.00731	0.00344	0.00592	0.00232	0.00497	0.00174	0.14757	0.20886			
Bifidobacterium	0.0016	0.00158	0.00056	0.00028	0.00021	0.00012	0.00662	0.02647	< 0.05	< 0.01	≥ 0.1
Bilophila	0.00082	0.00034	0.00197	0.00147	0.00286	0.00274	0.05624	0.11009			
Blautia	0.0005	0.0002	0.00172	0.00139	0.0048	0.0046	0.00546	0.02284	≥ 0.1	< 0.01	< 0.05
Butyricoccus	0.00029	0.00021	0.00034	0.00022	0.00064	0.00058	0.10623	0.1685			
Butyricimonas	0.00039	0.0002	0.00045	0.00021	0.00031	0.00013	0.21607	0.26863			
Candidatus_Arthromitus	5.3E-05	4.4E-05	6.5E-05	1.4E-05	9.8E-05	4.9E-05	0.04071	0.09362			
Candidatus_Hemipteriphilus	0.00077	0.00058	0.00039	0.00033	0.00128	0.00074	0.00663	0.02539	≥ 0.1	≥ 0.1	< 0.01
Candidatus_Saccharimonas	0.00953	0.00442	0.00663	0.00408	0.00341	0.00192	0.00342	0.01745	≥ 0.1	< 0.01	≥ 0.1
Candidatus_Soleiferrea	8.5E-05	3.5E-05	6.7E-05	3.9E-05	5.9E-05	4.7E-05	0.36272	0.41713			
Catabacter	6.7E-05	0.00006	0.0001	3.5E-05	4.3E-05	3.9E-05	0.02009	0.05961			
Cetobacterium	0.00052	0.00023	0.00026	6.8E-05	0.00099	0.0003	0	7E-06	< 0.05	< 0.001	< 0.001
Christensenellaceae_R-7_group	9.7E-05	8.2E-05	8.4E-05	5.2E-05	4.3E-05	2.5E-05	0.1167	0.17895			
Clostridium_sensu_stricto_1	0.00352	0.00223	0.00146	0.00067	0.00224	0.00074	0.01032	0.03515	< 0.01	≥ 0.1	≥ 0.1
Coriobacteriaceae_UCG-002	0.00084	0.00042	0.00018	0.00011	9.8E-05	4.4E-05	1E-06	1.2E-05	< 0.001	< 0.001	≥ 0.1
Dubosiella	0.0195	0.01883	3.4E-05	2.4E-05	0.12983	0.05201	0	0	≥ 0.1	< 0.001	< 0.001
Effusibacillus	0.0001	0.00018	6.5E-05	0.00013	0.00017	0.00035	0.62335	0.65168			
Enterorhabdus	0.00057	0.00028	0.00068	0.00027	0.00065	0.0005	0.79595	0.81363			
Erysipelatoclostridium	0.00015	0.0001	0.00033	0.0004	0.00012	6.8E-05	0.1185	0.17872			
Faecalibaculum	0.00636	0.00523	0.0029	0.00155	0.00077	0.0003	0.00187	0.01231	< 0.1	< 0.01	≥ 0.1
Family_XIII_AD3011_group	0.00011	9.1E-05	0.00013	6.9E-05	5.6E-05	2.6E-05	0.07641	0.13783			
GCA-900066575	0.00152	0.00076	0.00156	0.00092	0.00384	0.00324	0.02141	0.05968			
Intestinimonas	0.00231	0.0009	0.00269	0.00164	0.00478	0.00345	0.04559	0.09986			
Lachnoclostridium	0.00045	0.00029	0.00027	0.0001	0.0011	0.0007	0.00063	0.00443	≥ 0.1	< 0.01	< 0.001
Lachnospiraceae_FCS020_group	0.00045	0.00036	0.00049	0.00013	0.00073	0.00052	0.19378	0.25468			
Lachnospiraceae_NK4A136_group	0.04873	0.01641	0.06535	0.029	0.08475	0.04163	0.0471	0.10077			
Lachnospiraceae_NK4B4_group	0.00023	0.00019	0.00062	0.0006	0.00067	0.00064	0.13038	0.19347			
Lachnospiraceae_UCG-001	0.00047	0.00029	0.00114	0.00068	0.00224	0.00176	0.00458	0.02219	≥ 0.1	< 0.01	< 0.1
Lachnospiraceae_UCG-006	0.00366	0.00189	0.00467	0.00208	0.00468	0.00314	0.56423	0.61796			

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Lachnospiraceae_UCG-008	0.00058	0.00049	0.00063	0.00045	0.00168	0.00103	0.0022	0.01263	≥ 0.1	< 0.01	< 0.01
Lactobacillus	0.09756	0.03877	0.19357	0.07214	0.08491	0.05631	0.00037	0.00313	< 0.01	≥ 0.1	< 0.001
Marvinbryantia	0.00076	0.00048	0.00209	0.00112	0.00193	0.0012	0.01055	0.03466	< 0.02	< 0.05	≥ 0.1
Mucispirillum	0.00039	0.00047	0.00125	0.00209	0.00255	0.00273	0.06836	0.12834			
Muribaculum	0.00328	0.00134	0.00313	0.00039	0.00342	0.00073	0.7791	0.80536			
Negativibacillus	0.00024	0.00024	0.00026	0.00015	0.00043	0.0003	0.16971	0.23304			
Oscillibacter	0.00094	0.0003	0.00167	0.00117	0.00257	0.00171	0.01923	0.05898			
Parabacteroides	0.00027	0.00013	0.00029	0.00024	0.00016	7.8E-05	0.1797	0.23959			
Parasutterella	0.00224	0.00108	0.00297	0.00169	0.00123	0.00038	0.00995	0.03522	≥ 0.1	≥ 0.1	< 0.01
Prevotellaceae_NK3B31_group	0.00743	0.00291	0.00713	0.0027	0.00305	0.00135	0.00049	0.00379	≥ 0.1	< 0.01	< 0.01
Prevotellaceae_UCG-001	0.00391	0.0015	0.00558	0.00206	0.00213	0.00063	0.00011	0.00148	< 0.1	< 0.05	< 0.001
Rhodococcus	8.4E-05	7.7E-05	3.8E-05	3.3E-05	0.00019	8.9E-05	0.00013	0.00153	≥ 0.1	< 0.01	< 0.001
Rikenellaceae_RC9_gut_group	0.00514	0.00196	0.00428	0.0018	0.00626	0.00218	0.10066	0.16247			
Romboutsia	0.00169	0.00115	0.00202	0.00114	0.00084	0.00038	0.0305	0.07584			
Roseburia	0.00395	0.00288	0.00323	0.00112	0.00732	0.00476	0.02154	0.05829			
Ruminiclostridium	0.00213	0.0008	0.0048	0.00343	0.00577	0.00466	0.06001	0.11502			
Ruminiclostridium_5	0.00055	0.00028	0.00073	0.00033	0.00135	0.00086	0.00833	0.03066	≥ 0.1	< 0.01	< 0.05
Ruminiclostridium_6	0.00071	0.0002	0.00055	0.00065	0.00046	0.00029	0.4272	0.48522			
Ruminiclostridium_9	0.00157	0.00082	0.00125	0.0006	0.00166	0.0009	0.46975	0.52068			
Ruminococcaceae_NK4A214_group	0.0004	0.00021	0.00045	0.00039	0.00033	0.00016	0.61725	0.65272			
Ruminococcaceae_UCG-003	0.00013	9.8E-05	0.0003	0.00025	0.00048	0.00046	0.05337	0.10911			
Ruminococcaceae_UCG-005	0.00016	7.8E-05	0.00013	8.6E-05	0.00022	0.00011	0.07868	0.13919			
Ruminococcaceae_UCG-009	0.0006	0.0002	0.00062	0.00041	0.00135	0.00079	0.0046	0.02117	≥ 0.1	< 0.01	< 0.02
Ruminococcaceae_UCG-010	0.00086	0.00068	0.00066	0.00048	0.00088	0.0004	0.60973	0.65227			
Ruminococcaceae_UCG-013	0.00004	2.5E-05	0.0001	8.6E-05	7.8E-05	4.6E-05	0.07933	0.13516			
Ruminococcaceae_UCG-014	0.05712	0.01945	0.04127	0.02049	0.05517	0.02555	0.2317	0.28422			
Ruminococcus_1	0.00981	0.00642	0.00443	0.00208	0.00706	0.0026	0.02674	0.06832			
Shuttleworthia	0.00043	0.00059	0.00038	0.00026	0.00035	0.00032	0.91742	0.9275			
Streptococcus	0.00013	6.7E-05	0.00011	5.1E-05	0.00016	7.3E-05	0.30318	0.35306			
Turicibacter	0.00856	0.00358	0.00107	0.00058	0.00111	0.00063	0	0	< 0.001	< 0.001	≥ 0.1
Tyzzrella	0.00029	0.00018	0.00049	0.00027	0.00063	0.0005	0.09768	0.16047			
UBA1819	0.00028	0.00016	0.0004	0.00022	0.00056	0.00045	0.13449	0.19639			
[Eubacterium]_brachy_group	8.3E-05	0.00006	0.00015	9.4E-05	0.00014	0.00012	0.30182	0.35599			
[Eubacterium]_coprostanoligenes_group	0.00018	0.00016	0.00072	0.0008	0.0006	0.00039	0.06971	0.12826			
[Eubacterium]_fissicatena_group	0.00211	0.0013	0.00235	0.0015	0.00145	0.00061	0.23596	0.28563			
[Eubacterium]_nodatum_group	0.00039	0.0003	0.00038	0.00015	0.00026	0.0001	0.26487	0.31647			
[Eubacterium]_oxidoreducens_group	9.8E-05	4.6E-05	0.00029	0.00025	0.00045	0.00038	0.02115	0.0608			
[Eubacterium]_ruminantium_group	0.00516	0.00145	0.00506	0.00308	0.00424	0.00144	0.5765	0.62398			
[Eubacterium]_ventriosum_group	0.00016	9.4E-05	7.5E-05	9.8E-05	0.00024	7.9E-05	0.00208	0.01276	≥ 0.1	≥ 0.1	< 0.01
[Eubacterium]_xylanophilum_group	0.00126	0.00044	0.00229	0.00103	0.01075	0.00775	9.7E-05	0.00149	≥ 0.1	< 0.001	< 0.001
uncultured_bacterium_f_Blattella_germanica_German_cockroach	0.0003	0.00014	4.1E-05	7.1E-05	0.00011	8.7E-05	1.6E-05	0.0003	< 0.001	< 0.001	≥ 0.1

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uncultured_bacterium_f_Candidatus_Gastranaerophilales_bacterium_Zag_111	0.00187	0.00119	0.00102	0.00054	0.00095	0.00083	0.05321	0.11126			
uncultured_bacterium_f_Clostridiales_vadinBB60_group	0.0001	0.00011	0.00014	0.00012	3.4E-05	6.1E-05	0.07895	0.13705			
uncultured_bacterium_f_Desulfovibrionaceae	0.00024	0.00011	0.00016	0.00011	0.00036	0.00029	0.08237	0.13778			
uncultured_bacterium_f_Eggerthellaceae	0.00014	8.1E-05	0.00014	5.6E-05	7.3E-05	4.9E-05	0.03097	0.07497			
uncultured_bacterium_f_Erysipelotrichaceae	0.00024	0.00017	0.00017	5.6E-05	0.00027	0.00015	0.20597	0.26319			
uncultured_bacterium_f_Lachnospiraceae	0.01666	0.00578	0.02775	0.01115	0.04241	0.02453	0.00467	0.02046	≥ 0.1	< 0.01	≥ 0.1
uncultured_bacterium_f_Muribaculaceae	0.14418	0.03356	0.18449	0.03143	0.15664	0.03905	0.0441	0.09895			
uncultured_bacterium_f_Peptococcaceae	0.00036	0.00018	0.0006	0.00025	0.00053	0.00037	0.1512	0.21077			
uncultured_bacterium_f_Ruminococcaceae	0.00707	0.0025	0.00508	0.00227	0.01045	0.00753	0.05467	0.10934			
uncultured_bacterium_o_Gastranaerophilales	0.00205	0.00135	0.00084	0.00034	0.00139	0.00053	0.015	0.0476	< 0.02	≥ 0.1	≥ 0.1
uncultured_bacterium_o_Mollicutes_RF39	0.00144	0.00082	0.00088	0.00035	0.00054	0.00019	0.00255	0.01378	< 0.1	< 0.01	≥ 0.1
uncultured_bacterium_o_Rhodospirillales	0.0004	0.00028	0.00063	0.00074	0.0002	0.00011	0.13943	0.20044			

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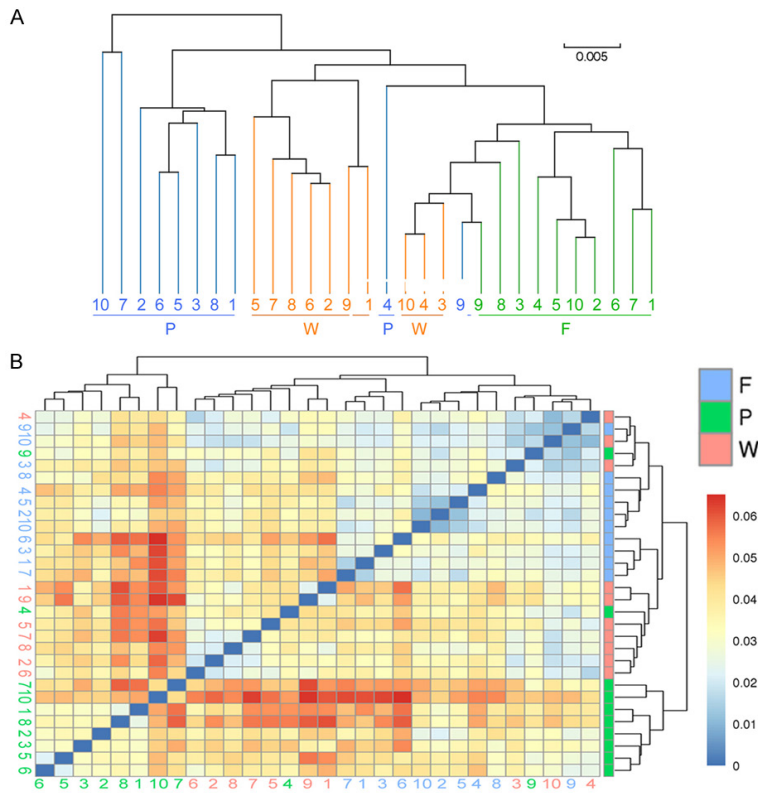


Figure 5. Hierarchical clustering analysis of the 16S rRNA v3+v4 sequencing results of the gut microbiota of feces from various groups of mice. A. Results of UPGMA analysis showed that the gut microbiota of mice in groups F and WT exhibited high homology and similar genetic backgrounds. B. Heatmap analysis of samples from various groups based on the distance algorithm (unweighted).

ativeabundanceofuncultured_bacterium_f_Lachnospiraceae was significantly increased, while that of *Akkermansia* was significantly decreased in group F compared with that in group P (Figure 4B; Table 3). Analysis at the species level showed that the relative abundance of uncultured_bacterium_f_Lachnospiraceae was significantly increased, while that of uncultured_bacterium_g_Akkermans was significantly decreased in group F compared with that in group P (Figure 4C; Table 3). The Bray-Curtis algorithm analysis revealed significant differences in distribution between the microbial communities of the three groups (Figure 4D; Table 4). Hierarchical clustering analysis by UPGMA showed that the gut microbiota of mice in groups F and WT exhibited high homology and similar genetic backgrounds (Figure 5A and 5B; Table 5). In addition, integrated analysis showed that, in group P, *Akkermansia* was the dominant bacteria, while the levels of uncultured_bacterium_f_Lachnospiraceae were relatively low; however, opposite microbial

distribution results were obtained for group F (Figure 6A; Tables 6, 7). This result was consistent with the species abundance result generated using QIIME software. LEfSe was used to identify high-dimensional biomarkers in the gut microbiota of various groups. The LDA score was set at 4.0, and species with LDA scores greater than the set value were considered to be important biomarkers (Figure 6B). As shown by cladogram and LDA analyses, the microbial count of uncultured_bacterium_f_Lachnospiraceae was significantly increased, while that of *Akkermansia* microorganisms was drastically reduced in the gut of group F; however, the distribution of these microbial communities was reversed in group P (Figure 7; Tables 6, 7).

Fisetin alters the expression of 16S functional genes and metabolic signaling pathways in the gut microorganisms in POF mice

Differential analysis of KEGG metabolic pathways was used to analyze the differences and changes in the functional genes of microbial communities in the metabolic pathways between different groups, thereby evaluating metabolic changes in different gut samples in response to environmental changes. Results showed that, compared with that in group P, the gut microbiota in group F showed increased carbohydrate and nucleotide metabolism, but decreased lipid and amino acid metabolism, thereby promoting membrane transport activation (environmental information processing), cell motility (cellular processes), and translation (genetic information processing) (Figure 8A; Table 8).

Through clusters of orthologous groups of proteins (COG) analysis, the distribution and abundance of homologous protein families in microorganisms were analyzed. Results showed that, compared with that in group P, the abundance of proteins associated with transcription, trans-

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Table 5. The result of Species ANOVA

Species	P (Mean)	P (Sd)	WT (Mean)	WT (Sd)	F (Mean)	F (Sd)	multiGroup (p)	multiGroup (p-corrected)	P::WT (p)	P::F (p)	WT::F (p)
Burkholderiales_bacterium_YL45	0.00186	0.00086	0.00236	0.00137	0.00102	0.00034	0.01401	0.04542	≥ 0.1	≥ 0.1	< 0.02
Christensenella_timonensis	6.7E-05	0.00006	0.0001	3.5E-05	4.3E-05	3.9E-05	0.02009	0.05901			
Effusibacillus_pohliae	0.0001	0.00018	6.5E-05	0.00013	0.00017	0.00035	0.62335	0.65105			
Lactobacillus_gasseri	0.00128	0.00063	0.00176	0.00226	0.00024	0.00017	0.05513	0.10797			
Mucispirillum_schaedleri_ASF457	0.00039	0.00047	0.00125	0.00209	0.00255	0.00273	0.06836	0.12599			
Parabacteroides_sp_YL27	0.00328	0.00134	0.00313	0.00039	0.00342	0.00073	0.7791	0.80479			
bacterium_GC452011	8.4E-05	7.7E-05	3.8E-05	3.3E-05	0.00019	8.9E-05	0.00013	0.00156	≥ 0.1	< 0.01	< 0.001
secondary_symbiont_of_Sitobion_miscanthi	0.00077	0.00058	0.00039	0.00033	0.00128	0.00074	0.00663	0.02595	≥ 0.1	≥ 0.1	< 0.01
uncultured_bacterium_f_Blattella_germanica_German_cockroach	0.0003	0.00014	4.1E-05	7.1E-05	0.00011	8.7E-05	1.6E-05	0.0003	< 0.001	< 0.001	≥ 0.1
uncultured_bacterium_f_Candidatus_Gastranaerophilales_bacterium_Zag_111	0.00187	0.00119	0.00102	0.00054	0.00095	0.00083	0.05321	0.11116			
uncultured_bacterium_f_Clostridiales_vadinBB60_group	0.0001	0.00011	0.00014	0.00012	3.4E-05	6.1E-05	0.07895	0.13493			
uncultured_bacterium_f_Desulfovibrionaceae	0.00024	0.00011	0.00016	0.00011	0.00036	0.00029	0.08237	0.13583			
uncultured_bacterium_f_Eggerthellaceae	0.00014	8.1E-05	0.00014	5.6E-05	7.3E-05	4.9E-05	0.03097	0.07464			
uncultured_bacterium_f_Erysipelotrichaceae	0.00024	0.00017	0.00017	5.6E-05	0.00027	0.00015	0.20597	0.26164			
uncultured_bacterium_f_Lachnospiraceae	0.01666	0.00578	0.02775	0.01115	0.04241	0.02453	0.00467	0.0209	≥ 0.1	< 0.01	≥ 0.1
uncultured_bacterium_f_Muribaculaceae	0.14418	0.03356	0.18449	0.03143	0.15664	0.03905	0.0441	0.0987			
uncultured_bacterium_f_Peptococcaceae	0.00036	0.00018	0.0006	0.00025	0.00053	0.00037	0.1512	0.20901			
uncultured_bacterium_f_Ruminococcaceae	0.00707	0.0025	0.00508	0.00227	0.01045	0.00753	0.05467	0.10934			
uncultured_bacterium_g_A2	0.00071	0.00059	0.00112	0.00037	0.00127	0.00103	0.21318	0.26718			
uncultured_bacterium_g_ASF356	0.00049	0.00034	0.00035	0.00016	0.00098	0.00078	0.02296	0.05996			
uncultured_bacterium_g_Acetatifactor	0.00018	8.3E-05	0.00021	0.00013	0.00026	0.00021	0.45876	0.51337			
uncultured_bacterium_g_Adlercreutzia	0.00044	0.00027	0.0004	0.00013	0.00041	0.00015	0.91877	0.91877			
uncultured_bacterium_g_Aeromonas	9.4E-05	3.8E-05	4.7E-05	2.9E-05	0.00016	7.2E-05	0.0002	0.00204	≥ 0.1	< 0.05	< 0.001
uncultured_bacterium_g_Akkermansia	0.47068	0.07268	0.3527	0.10065	0.28707	0.08508	0.00025	0.00234	< 0.02	< 0.001	≥ 0.1
uncultured_bacterium_g_Alistipes	0.00415	0.0024	0.00374	0.00096	0.00517	0.00149	0.17816	0.23925			
uncultured_bacterium_g_Alloprevotella	0.0154	0.00641	0.02097	0.00379	0.01955	0.00723	0.11644	0.17943			
uncultured_bacterium_g_Anaerotruncus	0.00011	8.6E-05	0.00015	8.1E-05	0.00024	0.00014	0.03213	0.07551			
uncultured_bacterium_g_Anaerovorax	8.1E-05	0.00006	0.00014	5.9E-05	9.2E-05	9.3E-05	0.20167	0.25968			
uncultured_bacterium_g_Bacteroides	0.00731	0.00344	0.00592	0.00232	0.00497	0.00174	0.14757	0.20703			
uncultured_bacterium_g_Bifidobacterium	0.0016	0.00158	0.00056	0.00028	0.00021	0.00012	0.00662	0.02705	< 0.05	< 0.01	≥ 0.1
uncultured_bacterium_g_Bilophila	0.00082	0.00034	0.00197	0.00147	0.00286	0.00274	0.05624	0.10789			
uncultured_bacterium_g_Blautia	0.0005	0.0002	0.00172	0.00139	0.0048	0.0046	0.00546	0.02334	≥ 0.1	< 0.01	< 0.05
uncultured_bacterium_g_Butyricoccus	0.00029	0.00021	0.00034	0.00022	0.00064	0.00058	0.10623	0.16643			
uncultured_bacterium_g_Butyricimonas	0.00039	0.0002	0.00045	0.00021	0.00031	0.00013	0.21607	0.26725			
uncultured_bacterium_g_Candidatus_Arthromitus	5.3E-05	4.4E-05	6.5E-05	1.4E-05	9.8E-05	4.9E-05	0.04071	0.09333			
uncultured_bacterium_g_Candidatus_Saccharimonas	0.00953	0.00442	0.00663	0.00408	0.00341	0.00192	0.00342	0.01783	≥ 0.1	< 0.01	≥ 0.1
uncultured_bacterium_g_Candidatus_Soleaferrea	8.5E-05	3.5E-05	6.7E-05	3.9E-05	5.9E-05	4.7E-05	0.36272	0.4158			
uncultured_bacterium_g_Cetobacterium	0.00052	0.00023	0.00026	6.8E-05	0.00099	0.0003	0	7E-06	< 0.05	< 0.001	< 0.001

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uncultured_bacterium_g_Christensenellaceae_R-7_group	9.7E-05	8.2E-05	8.4E-05	5.2E-05	4.3E-05	2.5E-05	0.1167	0.17694			
uncultured_bacterium_g_Clostridium_sensu_stricto_1	0.00352	0.00223	0.00146	0.00067	0.00224	0.00074	0.01032	0.03591	< 0.01	≥ 0.1	≥ 0.1
uncultured_bacterium_g_Coriobacteriaceae_UCG-002	0.00084	0.00042	0.00018	0.00011	9.8E-05	4.4E-05	1E-06	1.2E-05	< 0.001	< 0.001	≥ 0.1
uncultured_bacterium_g_Dubosiella	0.0195	0.01883	3.4E-05	2.4E-05	0.12983	0.05201	0	0	≥ 0.1	< 0.001	< 0.001
uncultured_bacterium_g_Enterorhabdus	0.00057	0.00028	0.00068	0.00027	0.00065	0.0005	0.79595	0.81325			
uncultured_bacterium_g_Erysipelatoclostridium	0.00015	0.0001	0.00033	0.0004	0.00012	6.8E-05	0.1185	0.17681			
uncultured_bacterium_g_Faecalibaculum	0.00636	0.00523	0.0029	0.00155	0.00077	0.0003	0.00187	0.01258	< 0.1	< 0.01	≥ 0.1
uncultured_bacterium_g_Family_XIII_AD3011_group	0.00011	9.1E-05	0.00013	6.9E-05	5.6E-05	2.6E-05	0.07641	0.13552			
uncultured_bacterium_g_GCA-900066575	0.00152	0.00076	0.00156	0.00092	0.00384	0.00324	0.02141	0.05919			
uncultured_bacterium_g_Intestinimonas	0.00231	0.0009	0.00269	0.00164	0.00478	0.00345	0.04559	0.09965			
uncultured_bacterium_g_Lachnoclostridium	0.00045	0.00029	0.00027	0.0001	0.0011	0.0007	0.00063	0.00452	≥ 0.1	< 0.01	< 0.001
uncultured_bacterium_g_Lachnospiraceae_FCS020_group	0.00045	0.00036	0.00049	0.00013	0.00073	0.00052	0.19378	0.25299			
uncultured_bacterium_g_Lachnospiraceae_NK4A136_group	0.04873	0.01641	0.06535	0.029	0.08475	0.04163	0.0471	0.10062			
uncultured_bacterium_g_Lachnospiraceae_NK4B4_group	0.00023	0.00019	0.00062	0.0006	0.00067	0.00064	0.13038	0.1915			
uncultured_bacterium_g_Lachnospiraceae_UCG-001	0.00047	0.00029	0.00114	0.00068	0.00224	0.00176	0.00458	0.02267	≥ 0.1	< 0.01	< 0.1
uncultured_bacterium_g_Lachnospiraceae_UCG-006	0.00366	0.00189	0.00467	0.00208	0.00468	0.00314	0.56423	0.61671			
uncultured_bacterium_g_Lachnospiraceae_UCG-008	0.00058	0.00049	0.00063	0.00045	0.00168	0.00103	0.0022	0.01291	≥ 0.1	< 0.01	< 0.01
uncultured_bacterium_g_Lactobacillus	0.09627	0.03827	0.19182	0.07082	0.08467	0.05625	0.00037	0.00314	< 0.01	≥ 0.1	< 0.001
uncultured_bacterium_g_Marvinbryantia	0.00076	0.00048	0.00209	0.00112	0.00193	0.0012	0.01055	0.03541	< 0.02	< 0.05	≥ 0.1
uncultured_bacterium_g_Negativibacillus	0.00024	0.00024	0.00026	0.00015	0.00043	0.0003	0.16971	0.2312			
uncultured_bacterium_g_Oscillibacter	0.00094	0.0003	0.00167	0.00117	0.00257	0.00171	0.01923	0.05831			
uncultured_bacterium_g_Parabacteroides	0.00027	0.00013	0.00029	0.00024	0.00016	7.8E-05	0.1797	0.23791			
uncultured_bacterium_g_Parasutterella	0.00038	0.00028	0.00062	0.00037	0.00022	6.9E-05	0.00975	0.03526	≥ 0.1	≥ 0.1	< 0.01
uncultured_bacterium_g_Prevotellaceae_NK3B31_group	0.00743	0.00291	0.00713	0.0027	0.00305	0.00135	0.00049	0.00387	≥ 0.1	< 0.01	< 0.01
uncultured_bacterium_g_Prevotellaceae_UCG-001	0.00391	0.0015	0.00558	0.00206	0.00213	0.00063	0.00011	0.00151	< 0.1	< 0.05	< 0.001
uncultured_bacterium_g_Rikenellaceae_RC9_gut_group	0.00514	0.00196	0.00428	0.0018	0.00626	0.00218	0.10066	0.16037			
uncultured_bacterium_g_Romboutsia	0.00169	0.00115	0.00202	0.00114	0.00084	0.00038	0.0305	0.07545			
uncultured_bacterium_g_Roseburia	0.00395	0.00288	0.00323	0.00112	0.00732	0.00476	0.02154	0.05786			
uncultured_bacterium_g_Ruminiclostridium	0.00213	0.0008	0.0048	0.00343	0.00577	0.00466	0.06001	0.11282			
uncultured_bacterium_g_Ruminiclostridium_5	0.00055	0.00028	0.00073	0.00033	0.00135	0.00086	0.00833	0.03133	≥ 0.1	< 0.01	< 0.05
uncultured_bacterium_g_Ruminiclostridium_6	0.00071	0.0002	0.00055	0.00065	0.00046	0.00029	0.4272	0.48382			
uncultured_bacterium_g_Ruminiclostridium_9	0.00157	0.00082	0.00125	0.0006	0.00166	0.0009	0.46975	0.51948			
uncultured_bacterium_g_Ruminococcaceae_NK4A214_group	0.0004	0.00021	0.00045	0.00039	0.00033	0.00016	0.61725	0.65192			
uncultured_bacterium_g_Ruminococcaceae_UCG-003	0.00013	9.8E-05	0.0003	0.00025	0.00048	0.00046	0.05337	0.10906			
uncultured_bacterium_g_Ruminococcaceae_UCG-005	0.00016	7.8E-05	0.00013	8.6E-05	0.00022	0.00011	0.07868	0.13695			
uncultured_bacterium_g_Ruminococcaceae_UCG-009	0.0006	0.0002	0.00062	0.00041	0.00135	0.00079	0.0046	0.02163	≥ 0.1	< 0.01	< 0.02
uncultured_bacterium_g_Ruminococcaceae_UCG-010	0.00086	0.00068	0.00066	0.00048	0.00088	0.0004	0.60973	0.65131			
uncultured_bacterium_g_Ruminococcaceae_UCG-013	0.00004	2.5E-05	0.0001	8.6E-05	7.8E-05	4.6E-05	0.07933	0.13316			
uncultured_bacterium_g_Ruminococcaceae_UCG-014	0.05712	0.01945	0.04127	0.02049	0.05517	0.02555	0.2317	0.28286			
uncultured_bacterium_g_Ruminococcus_1	0.00981	0.00642	0.00443	0.00208	0.00706	0.0026	0.02674	0.06792			
uncultured_bacterium_g_Shuttleworthia	0.00043	0.00059	0.00038	0.00026	0.00035	0.00032	0.91742	0.92729			

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uncultured_bacterium_g_Streptococcus	0.00013	6.7E-05	0.00011	5.1E-05	0.00016	7.3E-05	0.30318	0.35183			
uncultured_bacterium_g_Turicibacter	0.00856	0.00358	0.00107	0.00058	0.00111	0.00063	0	0	< 0.001	< 0.001	≥ 0.1
uncultured_bacterium_g_Tyzerella	0.00029	0.00018	0.00049	0.00027	0.00063	0.0005	0.09768	0.15831			
uncultured_bacterium_g_UBA1819	0.00028	0.00016	0.0004	0.00022	0.00056	0.00045	0.13449	0.19449			
uncultured_bacterium_g_[Eubacterium]_brachy_group	8.3E-05	0.00006	0.00015	9.4E-05	0.00014	0.00012	0.30182	0.35464			
uncultured_bacterium_g_[Eubacterium]_coprostanoligenes_group	0.00018	0.00016	0.00072	0.0008	0.0006	0.00039	0.06971	0.12601			
uncultured_bacterium_g_[Eubacterium]_fissicatena_group	0.00211	0.0013	0.00235	0.0015	0.00145	0.00061	0.23596	0.28436			
uncultured_bacterium_g_[Eubacterium]_nodatum_group	0.00039	0.0003	0.00038	0.00015	0.00026	0.0001	0.26487	0.31516			
uncultured_bacterium_g_[Eubacterium]_oxidoreducens_group	9.8E-05	4.6E-05	0.00029	0.00025	0.00045	0.00038	0.02115	0.06024			
uncultured_bacterium_g_[Eubacterium]_ruminantium_group	0.00516	0.00145	0.00506	0.00308	0.00424	0.00144	0.5765	0.62288			
uncultured_bacterium_g_[Eubacterium]_ventriosum_group	0.00016	9.4E-05	7.5E-05	9.8E-05	0.00024	7.9E-05	0.00208	0.01304	≥ 0.1	≥ 0.1	< 0.01
uncultured_bacterium_g_[Eubacterium]_xylanophilum_group	0.00126	0.00044	0.00229	0.00103	0.01075	0.00775	9.7E-05	0.00153	≥ 0.1	< 0.001	< 0.001
uncultured_bacterium_o_Gastranaerophilales	0.00205	0.00135	0.00084	0.00034	0.00139	0.00053	0.015	0.04701	< 0.02	≥ 0.1	≥ 0.1
uncultured_bacterium_o_Mollicutes_RF39	0.00144	0.00082	0.00088	0.00035	0.00054	0.00019	0.00255	0.01408	< 0.1	< 0.01	≥ 0.1
uncultured_bacterium_o_Rhodospirillales	0.0004	0.00028	0.00063	0.00074	0.0002	0.00011	0.13943	0.19859			

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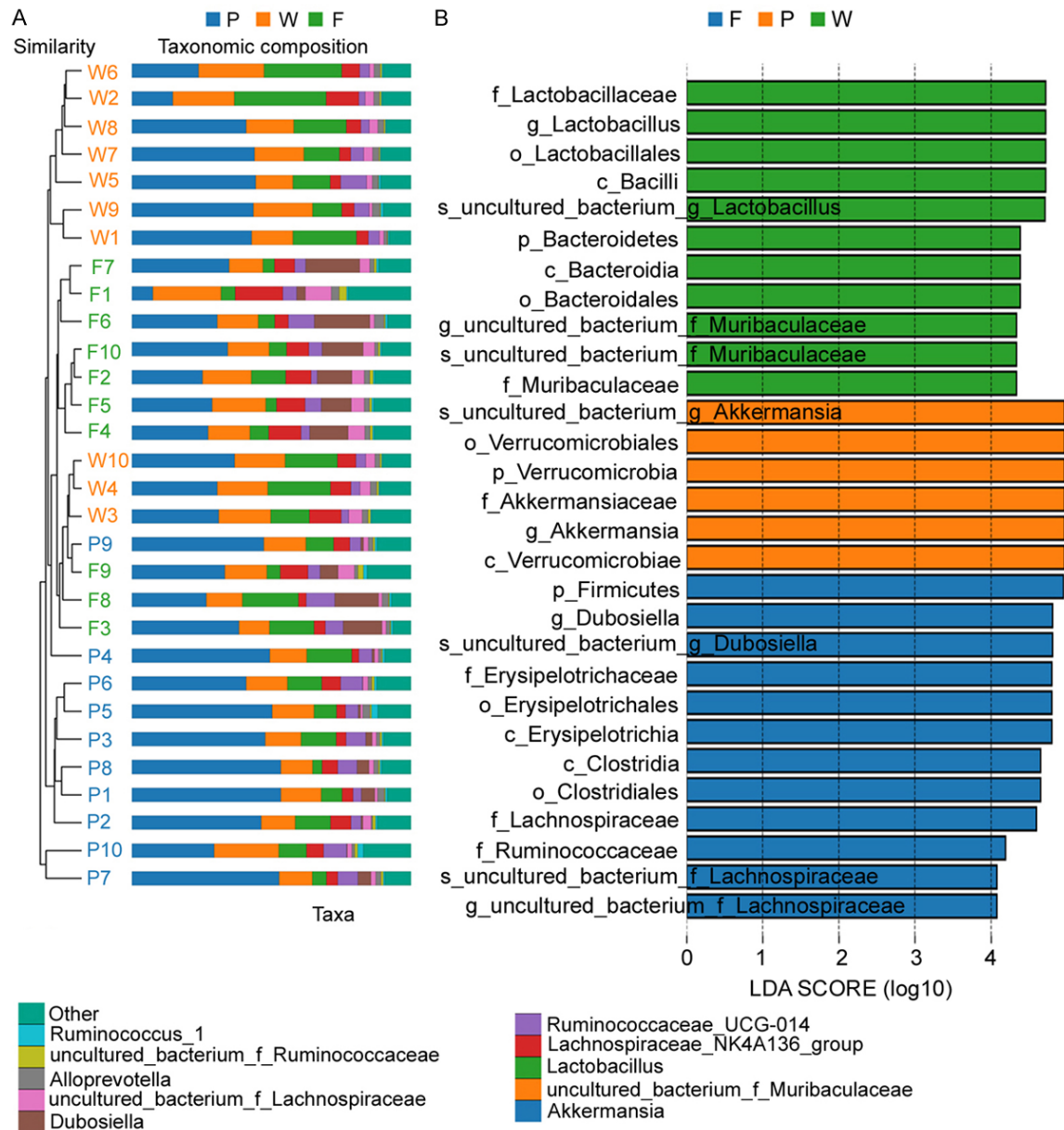


Figure 6. Significance analysis of differences in the gut microbiota of feces from various groups of mice. A. Combined UPGMA cluster tree and histogram analysis: integrated analysis showed that *Akkermansia* was the dominant bacteria in group P, while the levels of uncultured_bacterium_f_Lachnospiraceae were relatively low. However, opposite microbial distribution results were obtained for group F. B. LefSe analysis of samples from various groups: the microbial count of uncultured_bacterium_f_Lachnospiraceae was significantly increased, while that of *Akkermansia* microorganisms was drastically reduced in the gut microbiota of group F.

lation, ribosomal structure and biogenesis, and carbohydrate and nucleotide transport and metabolism was significantly increased in group F. Meanwhile, the abundance of proteins associated with cell wall/membrane/envelope biogenesis in cellular processes and signaling was significantly decreased (Figure 8B; Table 9). Thus, our results suggested that fisetin significantly affects the metabolic pathways of the gut microbiota in POF mice.

Fisetin significantly decreases CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte counts and IL-12 levels in POF mice

Immunofluorescence staining and FCM analysis showed that the peripheral blood CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte counts (Figure 9A and 9C) as well as the IL-12⁺/CD4⁺ T-lymphocyte counts were significantly lower in the fisetin group compared to those in the PBS group

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Table 6. Differential genus (F vs P)

Genus	Mean (F)	Variance (F)	Std.err (F)	Mean (P)	Variance (P)	Std.err (P)	P value	Q value
Blautia	4.80E-03	2.12E-05	1.45E-03	4.99E-04	3.80E-08	6.17E-05	9.99E-04	7.66E-03
[Eubacterium]_xylanophilum_group	1.07E-02	6.01E-05	2.45E-03	1.26E-03	1.92E-07	1.39E-04	9.99E-04	7.66E-03
Dubosiella	1.30E-01	2.71E-03	1.64E-02	1.95E-02	3.55E-04	5.95E-03	9.99E-04	7.66E-03
Mucispirillum	2.55E-03	7.45E-06	8.63E-04	3.85E-04	2.23E-07	1.49E-04	1.90E-02	5.29E-02
Lachnospiraceae_UCG-001	2.24E-03	3.10E-06	5.57E-04	4.66E-04	8.49E-08	9.22E-05	3.00E-03	1.72E-02
[Eubacterium]_oxidoreducens_group	4.50E-04	1.45E-07	1.20E-04	9.79E-05	2.16E-09	1.47E-05	9.99E-04	7.66E-03
Ruminococcaceae_UCG-003	4.81E-04	2.12E-07	1.46E-04	1.29E-04	9.57E-09	3.09E-05	1.40E-02	4.44E-02
Bilophila	2.86E-03	7.51E-06	8.67E-04	8.17E-04	1.17E-07	1.08E-04	1.20E-02	3.94E-02
[Eubacterium]_coprostanoligenes_group	5.99E-04	1.54E-07	1.24E-04	1.77E-04	2.59E-08	5.09E-05	4.00E-03	1.93E-02
Lachnospiraceae_NK4B4_group	6.73E-04	4.14E-07	2.04E-04	2.25E-04	3.74E-08	6.11E-05	3.50E-02	7.66E-02
Lachnospiraceae_UCG-008	1.68E-03	1.06E-06	3.26E-04	5.80E-04	2.35E-07	1.53E-04	7.99E-03	3.20E-02
Oscillibacter	2.57E-03	2.94E-06	5.42E-04	9.35E-04	8.82E-08	9.39E-05	4.00E-03	1.93E-02
Ruminiclostridium	5.77E-03	2.17E-05	1.47E-03	2.13E-03	6.46E-07	2.54E-04	9.99E-03	3.83E-02
Marvinbryantia	1.93E-03	1.43E-06	3.78E-04	7.58E-04	2.34E-07	1.53E-04	4.00E-03	1.93E-02
uncultured_bacterium_f_Lachnospiraceae	4.24E-02	6.02E-04	7.76E-03	1.67E-02	3.34E-05	1.83E-03	3.00E-03	1.72E-02
GCA-900066575	3.84E-03	1.05E-05	1.02E-03	1.52E-03	5.72E-07	2.39E-04	2.30E-02	6.04E-02
Ruminiclostridium_5	1.35E-03	7.38E-07	2.72E-04	5.51E-04	7.81E-08	8.84E-05	1.20E-02	3.94E-02
Lachnoclostridium	1.10E-03	4.84E-07	2.20E-04	4.51E-04	8.65E-08	9.30E-05	1.20E-02	3.94E-02
Rhodococcus	1.92E-04	7.90E-09	2.81E-05	8.44E-05	5.93E-09	2.43E-05	1.10E-02	3.94E-02
Ruminococcaceae_UCG-009	1.35E-03	6.19E-07	2.49E-04	5.97E-04	4.10E-08	6.41E-05	7.99E-03	3.20E-02
Tyzzereella	6.33E-04	2.54E-07	1.59E-04	2.86E-04	3.38E-08	5.82E-05	2.80E-02	6.77E-02
Anaerotruncus	2.40E-04	1.95E-08	4.42E-05	1.10E-04	7.47E-09	2.73E-05	1.50E-02	4.45E-02
Butyricicoccus	6.36E-04	3.35E-07	1.83E-04	2.94E-04	4.56E-08	6.75E-05	9.79E-02	1.70E-01
Intestinimonas	4.78E-03	1.19E-05	1.09E-03	2.31E-03	8.04E-07	2.84E-04	2.10E-02	5.68E-02
UBA1819	5.57E-04	1.99E-07	1.41E-04	2.77E-04	2.71E-08	5.20E-05	7.19E-02	1.37E-01
ASF356	9.80E-04	6.11E-07	2.47E-04	4.88E-04	1.13E-07	1.06E-04	7.49E-02	1.37E-01
Ruminococcaceae_UCG-013	7.84E-05	2.13E-09	1.46E-05	4.00E-05	6.16E-10	7.85E-06	2.70E-02	6.71E-02
Cetobacterium	9.91E-04	8.88E-08	9.42E-05	5.19E-04	5.12E-08	7.15E-05	5.00E-03	2.30E-02
Candidatus_Arthromitus	9.83E-05	2.39E-09	1.55E-05	5.30E-05	1.97E-09	1.41E-05	4.00E-02	8.36E-02
Roseburia	7.32E-03	2.27E-05	1.51E-03	3.95E-03	8.31E-06	9.12E-04	7.39E-02	1.37E-01
A2	1.27E-03	1.07E-06	3.27E-04	7.08E-04	3.44E-07	1.85E-04	1.60E-01	2.48E-01
Negativibacillus	4.25E-04	9.07E-08	9.52E-05	2.37E-04	5.67E-08	7.53E-05	1.35E-01	2.14E-01
Lachnospiraceae_NK4A136_group	8.48E-02	1.73E-03	1.32E-02	4.87E-02	2.69E-04	5.19E-03	1.50E-02	4.45E-02
Candidatus_Hemipteriphilus	1.28E-03	5.40E-07	2.32E-04	7.68E-04	3.33E-07	1.83E-04	9.99E-02	1.70E-01
Aeromonas	1.55E-04	5.17E-09	2.27E-05	9.40E-05	1.44E-09	1.20E-05	1.80E-02	5.17E-02
Lachnospiraceae_FCS020_group	7.34E-04	2.69E-07	1.64E-04	4.46E-04	1.29E-07	1.13E-04	1.79E-01	2.61E-01

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[Eubacterium]_brachy_group	1.35E-04	1.49E-08	3.85E-05	8.26E-05	3.57E-09	1.89E-05	2.42E-01	3.18E-01
Effusibacillus	1.69E-04	1.21E-07	1.10E-04	1.04E-04	3.39E-08	5.82E-05	6.79E-01	7.44E-01
Acetatifactor	2.60E-04	4.40E-08	6.63E-05	1.75E-04	6.88E-09	2.62E-05	2.68E-01	3.47E-01
uncultured_bacterium_f_Ruminococcaceae	1.05E-02	5.68E-05	2.38E-03	7.07E-03	6.25E-06	7.91E-04	2.21E-01	2.99E-01
uncultured_bacterium_f_Peptococcaceae	5.27E-04	1.35E-07	1.16E-04	3.55E-04	3.26E-08	5.71E-05	2.15E-01	2.95E-01
uncultured_bacterium_f_Desulfovibrionaceae	3.56E-04	8.20E-08	9.05E-05	2.42E-04	1.19E-08	3.46E-05	2.80E-01	3.53E-01
[Eubacterium]_ventriosum_group	2.36E-04	6.30E-09	2.51E-05	1.61E-04	8.92E-09	2.99E-05	5.79E-02	1.13E-01
Ruminococcaceae_UCG-005	2.21E-04	1.13E-08	3.36E-05	1.56E-04	6.14E-09	2.48E-05	1.34E-01	2.14E-01
Lachnospiraceae_UCG-006	4.68E-03	9.83E-06	9.91E-04	3.66E-03	3.56E-06	5.97E-04	3.86E-01	4.60E-01
Alloprevotella	1.96E-02	5.23E-05	2.29E-03	1.54E-02	4.11E-05	2.03E-03	1.90E-01	2.73E-01
Alistipes	5.17E-03	2.22E-06	4.71E-04	4.15E-03	5.74E-06	7.58E-04	2.88E-01	3.58E-01
Rikenellaceae_RC9_gut_group	6.26E-03	4.76E-06	6.90E-04	5.14E-03	3.82E-06	6.18E-04	2.42E-01	3.18E-01
Streptococcus	1.55E-04	5.29E-09	2.30E-05	1.30E-04	4.45E-09	2.11E-05	4.02E-01	4.68E-01
Anaerovorax	9.20E-05	8.72E-09	2.95E-05	8.06E-05	3.60E-09	1.90E-05	7.52E-01	7.95E-01
Enterorhabdus	6.51E-04	2.46E-07	1.57E-04	5.73E-04	7.95E-08	8.92E-05	6.50E-01	7.21E-01
uncultured_bacterium_f_Erysipelotrichaceae	2.71E-04	2.16E-08	4.64E-05	2.43E-04	2.94E-08	5.43E-05	7.05E-01	7.61E-01
uncultured_bacterium_f_Muribaculaceae	1.57E-01	1.53E-03	1.23E-02	1.44E-01	1.13E-03	1.06E-02	4.56E-01	5.17E-01
Ruminiclostridium_9	1.66E-03	8.04E-07	2.84E-04	1.57E-03	6.64E-07	2.58E-04	7.80E-01	8.08E-01
Muribaculum	3.42E-03	5.29E-07	2.30E-04	3.28E-03	1.80E-06	4.24E-04	7.81E-01	8.08E-01
Ruminococcaceae_UCG-010	8.77E-04	1.60E-07	1.27E-04	8.61E-04	4.60E-07	2.15E-04	9.57E-01	9.57E-01
Ruminococcaceae_UCG-014	5.52E-02	6.53E-04	8.08E-03	5.71E-02	3.78E-04	6.15E-03	8.35E-01	8.44E-01
Adlercreutzia	4.12E-04	2.24E-08	4.74E-05	4.38E-04	7.06E-08	8.40E-05	8.01E-01	8.19E-01
Lactobacillus	8.49E-02	3.17E-03	1.78E-02	9.76E-02	1.50E-03	1.23E-02	5.63E-01	6.32E-01
Ruminococcaceae_NK4A214_group	3.28E-04	2.52E-08	5.02E-05	3.96E-04	4.43E-08	6.66E-05	4.32E-01	4.96E-01
[Eubacterium]_ruminantium_group	4.24E-03	2.08E-06	4.56E-04	5.16E-03	2.11E-06	4.59E-04	1.93E-01	2.73E-01
Shuttleworthia	3.50E-04	1.05E-07	1.03E-04	4.27E-04	3.52E-07	1.88E-04	7.11E-01	7.61E-01
Butyricimonas	3.05E-04	1.55E-08	3.94E-05	3.86E-04	3.95E-08	6.28E-05	3.16E-01	3.87E-01
Erysipelatoclostridium	1.15E-04	4.60E-09	2.14E-05	1.51E-04	1.03E-08	3.21E-05	3.90E-01	4.60E-01
Ruminococcus_1	7.06E-03	6.78E-06	8.23E-04	9.81E-03	4.12E-05	2.03E-03	2.79E-01	3.53E-01
Candidatus_Soleaferrea	5.87E-05	2.25E-09	1.50E-05	8.46E-05	1.26E-09	1.12E-05	2.06E-01	2.87E-01
[Eubacterium]_fissicatena_group	1.44E-03	3.73E-07	1.93E-04	2.11E-03	1.69E-06	4.11E-04	1.62E-01	2.48E-01
Bacteroides	4.97E-03	3.03E-06	5.51E-04	7.31E-03	1.18E-05	1.09E-03	7.59E-02	1.37E-01
uncultured_bacterium_o_Gastranaerophilales	1.39E-03	2.85E-07	1.69E-04	2.05E-03	1.81E-06	4.26E-04	1.71E-01	2.53E-01
[Eubacterium]_nodatum_group	2.56E-04	1.08E-08	3.28E-05	3.93E-04	8.90E-08	9.43E-05	1.70E-01	2.53E-01
Ruminiclostridium_6	4.59E-04	8.34E-08	9.13E-05	7.09E-04	4.17E-08	6.46E-05	3.40E-02	7.66E-02
Catabacter	4.27E-05	1.55E-09	1.24E-05	6.66E-05	3.63E-09	1.90E-05	3.20E-01	3.87E-01
Clostridium_sensu_stricto_1	2.24E-03	5.42E-07	2.33E-04	3.51E-03	4.96E-06	7.04E-04	1.03E-01	1.72E-01
Akkermansia	2.87E-01	7.24E-03	2.69E-02	4.71E-01	5.28E-03	2.30E-02	9.99E-04	7.66E-03

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Parabacteroides	1.59E-04	6.02E-09	2.45E-05	2.67E-04	1.72E-08	4.14E-05	3.90E-02	8.34E-02
Parasutterella	1.23E-03	1.40E-07	1.19E-04	2.24E-03	1.17E-06	3.42E-04	6.99E-03	3.06E-02
Prevotellaceae_UCG-001	2.13E-03	3.98E-07	1.99E-04	3.91E-03	2.25E-06	4.74E-04	3.00E-03	1.72E-02
Family_XIII_AD3011_group	5.64E-05	6.94E-10	8.33E-06	1.06E-04	8.34E-09	2.89E-05	8.99E-02	1.59E-01
uncultured_bacterium_f_Candidatus_Gastranaerophilales_bacterium_Zag_111	9.53E-04	6.91E-07	2.63E-04	1.87E-03	1.42E-06	3.77E-04	5.79E-02	1.13E-01
uncultured_bacterium_f_Eggerthellaceae	7.27E-05	2.41E-09	1.55E-05	1.43E-04	6.61E-09	2.57E-05	3.40E-02	7.66E-02
uncultured_bacterium_o_Rhodospirillales	2.01E-04	1.21E-08	3.48E-05	4.04E-04	7.89E-08	8.88E-05	4.10E-02	8.37E-02
Romboutsia	8.38E-04	1.45E-07	1.20E-04	1.69E-03	1.33E-06	3.65E-04	2.70E-02	6.71E-02
Christensenellaceae_R-7_group	4.34E-05	6.21E-10	7.88E-06	9.67E-05	6.66E-09	2.58E-05	3.50E-02	7.66E-02
Prevotellaceae_NK3B31_group	3.05E-03	1.81E-06	4.25E-04	7.43E-03	8.47E-06	9.21E-04	9.99E-04	7.66E-03
uncultured_bacterium_o_Mollicutes_RF39	5.43E-04	3.60E-08	6.00E-05	1.44E-03	6.71E-07	2.59E-04	9.99E-04	7.66E-03
Candidatus_Saccharimonas	3.41E-03	3.67E-06	6.06E-04	9.53E-03	1.95E-05	1.40E-03	9.99E-04	7.66E-03
uncultured_bacterium_f_Blattella_germanica_German_cockroach	1.06E-04	7.62E-09	2.76E-05	3.03E-04	2.01E-08	4.48E-05	2.00E-03	1.41E-02
uncultured_bacterium_f_Clostridiales_vadinBB60_group	3.44E-05	3.73E-09	1.93E-05	1.03E-04	1.24E-08	3.51E-05	1.11E-01	1.82E-01
Bifidobacterium	2.11E-04	1.33E-08	3.65E-05	1.60E-03	2.50E-06	5.00E-04	9.99E-04	7.66E-03
Turicibacter	1.11E-03	3.92E-07	1.98E-04	8.56E-03	1.28E-05	1.13E-03	9.99E-04	7.66E-03
Faecalibaculum	7.69E-04	8.79E-08	9.37E-05	6.36E-03	2.74E-05	1.66E-03	9.99E-04	7.66E-03
Coriobacteriaceae_UCG-002	9.76E-05	1.91E-09	1.38E-05	8.39E-04	1.77E-07	1.33E-04	9.99E-04	7.66E-03

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Table 7. Differential species (F vs P)

Species	Mean (F)	Variance (F)	Std.err (F)	Mean (P)	Variance (P)	Std.err (P)	P value	Q value
uncultured_bacterium_g_Blautia	4.80E-03	2.12E-05	1.45E-03	4.99E-04	3.80E-08	6.17E-05	9.99E-04	1.04E-02
uncultured_bacterium_g_[Eubacterium]_xylanophilum_group	1.07E-02	6.01E-05	2.45E-03	1.26E-03	1.92E-07	1.39E-04	9.99E-04	1.04E-02
uncultured_bacterium_g_Dubosiella	1.30E-01	2.71E-03	1.64E-02	1.95E-02	3.55E-04	5.95E-03	2.00E-03	1.17E-02
Mucispirillum_schaedleri_ASF457	2.55E-03	7.45E-06	8.63E-04	3.85E-04	2.23E-07	1.49E-04	1.10E-02	3.56E-02
uncultured_bacterium_g_Lachnospiraceae_UCG-001	2.24E-03	3.10E-06	5.57E-04	4.66E-04	8.49E-08	9.22E-05	2.00E-03	1.17E-02
uncultured_bacterium_g_[Eubacterium]_oxidoreducens_group	4.50E-04	1.45E-07	1.20E-04	9.79E-05	2.16E-09	1.47E-05	9.99E-04	1.04E-02
uncultured_bacterium_g_Ruminococcaceae_UCG-003	4.81E-04	2.12E-07	1.46E-04	1.29E-04	9.57E-09	3.09E-05	1.20E-02	3.76E-02
uncultured_bacterium_g_Bifidobacterium	2.86E-03	7.51E-06	8.67E-04	8.17E-04	1.17E-07	1.08E-04	8.99E-03	3.13E-02
uncultured_bacterium_g_[Eubacterium]_coprostanoligenes_group	5.99E-04	1.54E-07	1.24E-04	1.77E-04	2.59E-08	5.09E-05	5.00E-03	2.35E-02
uncultured_bacterium_g_Lachnospiraceae_NK4B4_group	6.73E-04	4.14E-07	2.04E-04	2.25E-04	3.74E-08	6.11E-05	3.60E-02	7.86E-02
uncultured_bacterium_g_Lachnospiraceae_UCG-008	1.68E-03	1.06E-06	3.26E-04	5.80E-04	2.35E-07	1.53E-04	6.99E-03	2.74E-02
uncultured_bacterium_g_Oscillibacter	2.57E-03	2.94E-06	5.42E-04	9.35E-04	8.82E-08	9.39E-05	2.00E-03	1.17E-02
uncultured_bacterium_g_Ruminiclostridium	5.77E-03	2.17E-05	1.47E-03	2.13E-03	6.46E-07	2.54E-04	8.99E-03	3.13E-02
uncultured_bacterium_g_Marvinbryantia	1.93E-03	1.43E-06	3.78E-04	7.58E-04	2.34E-07	1.53E-04	5.99E-03	2.56E-02
uncultured_bacterium_f_Lachnospiraceae	4.24E-02	6.02E-04	7.76E-03	1.67E-02	3.34E-05	1.83E-03	2.00E-03	1.17E-02
uncultured_bacterium_g_GCA-900066575	3.84E-03	1.05E-05	1.02E-03	1.52E-03	5.72E-07	2.39E-04	3.20E-02	7.33E-02
uncultured_bacterium_g_Ruminiclostridium_5	1.35E-03	7.38E-07	2.72E-04	5.51E-04	7.81E-08	8.84E-05	1.30E-02	3.94E-02
uncultured_bacterium_g_Lachnoclostridium	1.10E-03	4.84E-07	2.20E-04	4.51E-04	8.65E-08	9.30E-05	9.99E-03	3.35E-02
bacterium_GC452011	1.92E-04	7.90E-09	2.81E-05	8.44E-05	5.93E-09	2.43E-05	7.99E-03	3.00E-02
uncultured_bacterium_g_Ruminococcaceae_UCG-009	1.35E-03	6.19E-07	2.49E-04	5.97E-04	4.10E-08	6.41E-05	6.99E-03	2.74E-02
uncultured_bacterium_g_Tyzzarella	6.33E-04	2.54E-07	1.59E-04	2.86E-04	3.38E-08	5.82E-05	2.80E-02	6.92E-02
uncultured_bacterium_g_Anaerotruncus	2.40E-04	1.95E-08	4.42E-05	1.10E-04	7.47E-09	2.73E-05	2.10E-02	5.63E-02
uncultured_bacterium_g_Butyricoccus	6.36E-04	3.35E-07	1.83E-04	2.94E-04	4.56E-08	6.75E-05	9.79E-02	1.64E-01
uncultured_bacterium_g_Intestinimonas	4.78E-03	1.19E-05	1.09E-03	2.31E-03	8.04E-07	2.84E-04	2.60E-02	6.60E-02
uncultured_bacterium_g_UBA1819	5.57E-04	1.99E-07	1.41E-04	2.77E-04	2.71E-08	5.20E-05	6.99E-02	1.29E-01
uncultured_bacterium_g_ASF356	9.80E-04	6.11E-07	2.47E-04	4.88E-04	1.13E-07	1.06E-04	8.99E-02	1.57E-01
uncultured_bacterium_g_Ruminococcaceae_UCG-013	7.84E-05	2.13E-09	1.46E-05	4.00E-05	6.16E-10	7.85E-06	3.10E-02	7.28E-02
uncultured_bacterium_g_Cetobacterium	9.91E-04	8.88E-08	9.42E-05	5.19E-04	5.12E-08	7.15E-05	3.00E-03	1.66E-02
uncultured_bacterium_g_Candidatus_Arthromitus	9.83E-05	2.39E-09	1.55E-05	5.30E-05	1.97E-09	1.41E-05	5.00E-02	1.04E-01
uncultured_bacterium_g_Roseburia	7.32E-03	2.27E-05	1.51E-03	3.95E-03	8.31E-06	9.12E-04	6.89E-02	1.29E-01
uncultured_bacterium_g_A2	1.27E-03	1.07E-06	3.27E-04	7.08E-04	3.44E-07	1.85E-04	1.42E-01	2.22E-01
uncultured_bacterium_g_Negativibacillus	4.25E-04	9.07E-08	9.52E-05	2.37E-04	5.67E-08	7.53E-05	1.56E-01	2.40E-01
uncultured_bacterium_g_Lachnospiraceae_NK4A136_group	8.48E-02	1.73E-03	1.32E-02	4.87E-02	2.69E-04	5.19E-03	1.50E-02	4.27E-02
secondary_symbiont_of_Sitobion_miscanthi	1.28E-03	5.40E-07	2.32E-04	7.68E-04	3.33E-07	1.83E-04	1.10E-01	1.78E-01
uncultured_bacterium_g_Aeromonas	1.55E-04	5.17E-09	2.27E-05	9.40E-05	1.44E-09	1.20E-05	1.70E-02	4.70E-02
uncultured_bacterium_g_Lachnospiraceae_FCS020_group	7.34E-04	2.69E-07	1.64E-04	4.46E-04	1.29E-07	1.13E-04	1.60E-01	2.40E-01
uncultured_bacterium_g_[Eubacterium]_brachy_group	1.35E-04	1.49E-08	3.85E-05	8.26E-05	3.57E-09	1.89E-05	2.37E-01	3.10E-01
Effusibacillus_pohliae	1.69E-04	1.21E-07	1.10E-04	1.04E-04	3.39E-08	5.82E-05	6.71E-01	7.34E-01
uncultured_bacterium_g_Acetatifactor	2.60E-04	4.40E-08	6.63E-05	1.75E-04	6.88E-09	2.62E-05	2.43E-01	3.13E-01

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uncultured_bacterium_f_Ruminococcaceae	1.05E-02	5.68E-05	2.38E-03	7.07E-03	6.25E-06	7.91E-04	2.20E-01	2.95E-01
uncultured_bacterium_f_Peptococcaceae	5.27E-04	1.35E-07	1.16E-04	3.55E-04	3.26E-08	5.71E-05	2.00E-01	2.72E-01
uncultured_bacterium_f_Desulfovibrionaceae	3.56E-04	8.20E-08	9.05E-05	2.42E-04	1.19E-08	3.46E-05	2.57E-01	3.22E-01
uncultured_bacterium_g_[Eubacterium]_ventriosum_group	2.36E-04	6.30E-09	2.51E-05	1.61E-04	8.92E-09	2.99E-05	6.99E-02	1.29E-01
uncultured_bacterium_g_Ruminococcaceae_UCG-005	2.21E-04	1.13E-08	3.36E-05	1.56E-04	6.14E-09	2.48E-05	1.38E-01	2.20E-01
uncultured_bacterium_g_Lachnospiraceae_UCG-006	4.68E-03	9.83E-06	9.91E-04	3.66E-03	3.56E-06	5.97E-04	3.92E-01	4.60E-01
uncultured_bacterium_g_Alloprevotella	1.96E-02	5.23E-05	2.29E-03	1.54E-02	4.11E-05	2.03E-03	1.80E-01	2.52E-01
uncultured_bacterium_g_Alistipes	5.17E-03	2.22E-06	4.71E-04	4.15E-03	5.74E-06	7.58E-04	2.91E-01	3.55E-01
uncultured_bacterium_g_Rikenellaceae_RC9_gut_group	6.26E-03	4.76E-06	6.90E-04	5.14E-03	3.82E-06	6.18E-04	2.38E-01	3.10E-01
uncultured_bacterium_g_Streptococcus	1.55E-04	5.29E-09	2.30E-05	1.30E-04	4.45E-09	2.11E-05	4.27E-01	4.94E-01
uncultured_bacterium_g_Anaerovorax	9.20E-05	8.72E-09	2.95E-05	8.06E-05	3.60E-09	1.90E-05	7.53E-01	7.96E-01
uncultured_bacterium_g_Enterohabdus	6.51E-04	2.46E-07	1.57E-04	5.73E-04	7.95E-08	8.92E-05	6.64E-01	7.34E-01
uncultured_bacterium_f_Erysipelotrichaceae	2.71E-04	2.16E-08	4.64E-05	2.43E-04	2.94E-08	5.43E-05	7.11E-01	7.69E-01
uncultured_bacterium_f_Muribaculaceae	1.57E-01	1.53E-03	1.23E-02	1.44E-01	1.13E-03	1.06E-02	4.53E-01	5.13E-01
uncultured_bacterium_g_Ruminiclostridium_9	1.66E-03	8.04E-07	2.84E-04	1.57E-03	6.64E-07	2.58E-04	7.95E-01	8.17E-01
Parabacteroides_sp_YL27	3.42E-03	5.29E-07	2.30E-04	3.28E-03	1.80E-06	4.24E-04	7.77E-01	8.12E-01
uncultured_bacterium_g_Ruminococcaceae_UCG-010	8.77E-04	1.60E-07	1.27E-04	8.61E-04	4.60E-07	2.15E-04	9.53E-01	9.53E-01
uncultured_bacterium_g_Ruminococcaceae_UCG-014	5.52E-02	6.53E-04	8.08E-03	5.71E-02	3.78E-04	6.15E-03	8.67E-01	8.76E-01
uncultured_bacterium_g_Adlercreutzia	4.12E-04	2.24E-08	4.74E-05	4.38E-04	7.06E-08	8.40E-05	7.99E-01	8.17E-01
uncultured_bacterium_g_Lactobacillus	8.47E-02	3.16E-03	1.78E-02	9.63E-02	1.46E-03	1.21E-02	6.13E-01	6.86E-01
uncultured_bacterium_g_Ruminococcaceae_NK4A214_group	3.28E-04	2.52E-08	5.02E-05	3.96E-04	4.43E-08	6.66E-05	4.31E-01	4.94E-01
uncultured_bacterium_g_[Eubacterium]_ruminantium_group	4.24E-03	2.08E-06	4.56E-04	5.16E-03	2.11E-06	4.59E-04	1.69E-01	2.40E-01
uncultured_bacterium_g_Shuttleworthia	3.50E-04	1.05E-07	1.03E-04	4.27E-04	3.52E-07	1.88E-04	7.23E-01	7.73E-01
uncultured_bacterium_g_Butyricimonas	3.05E-04	1.55E-08	3.94E-05	3.86E-04	3.95E-08	6.28E-05	2.78E-01	3.43E-01
uncultured_bacterium_g_Erysipelatoclostridium	1.15E-04	4.60E-09	2.14E-05	1.51E-04	1.03E-08	3.21E-05	3.76E-01	4.47E-01
uncultured_bacterium_g_Ruminococcus_1	7.06E-03	6.78E-06	8.23E-04	9.81E-03	4.12E-05	2.03E-03	2.56E-01	3.22E-01
uncultured_bacterium_g_Candidatus_Soleaferrea	5.87E-05	2.25E-09	1.50E-05	8.46E-05	1.26E-09	1.12E-05	1.92E-01	2.65E-01
uncultured_bacterium_g_[Eubacterium]_fissicatena_group	1.44E-03	3.73E-07	1.93E-04	2.11E-03	1.69E-06	4.11E-04	1.63E-01	2.40E-01
uncultured_bacterium_g_Bacteroides	4.97E-03	3.03E-06	5.51E-04	7.31E-03	1.18E-05	1.09E-03	8.59E-02	1.52E-01
uncultured_bacterium_o_Gastranaerophilales	1.39E-03	2.85E-07	1.69E-04	2.05E-03	1.81E-06	4.26E-04	1.66E-01	2.40E-01
uncultured_bacterium_g_[Eubacterium]_nodatum_group	2.56E-04	1.08E-08	3.28E-05	3.93E-04	8.90E-08	9.43E-05	1.68E-01	2.40E-01
uncultured_bacterium_g_Ruminiclostridium_6	4.59E-04	8.34E-08	9.13E-05	7.09E-04	4.17E-08	6.46E-05	5.09E-02	1.04E-01
Christensenella_timonensis	4.27E-05	1.55E-09	1.24E-05	6.66E-05	3.63E-09	1.90E-05	3.33E-01	4.01E-01
uncultured_bacterium_g_Clostridium_sensu_stricto_1	2.24E-03	5.42E-07	2.33E-04	3.51E-03	4.96E-06	7.04E-04	9.19E-02	1.57E-01
uncultured_bacterium_g_Akkermansia	2.87E-01	7.24E-03	2.69E-02	4.71E-01	5.28E-03	2.30E-02	9.99E-04	1.04E-02
uncultured_bacterium_g_Parabacteroides	1.59E-04	6.02E-09	2.45E-05	2.67E-04	1.72E-08	4.14E-05	5.39E-02	1.08E-01
uncultured_bacterium_g_Parasutterella	2.17E-04	4.75E-09	2.18E-05	3.82E-04	7.63E-08	8.74E-05	2.60E-02	6.60E-02
Burkholderiales_bacterium_YL45	1.02E-03	1.17E-07	1.08E-04	1.86E-03	7.43E-07	2.73E-04	5.99E-03	2.56E-02
uncultured_bacterium_g_Prevotellaceae_UCG-001	2.13E-03	3.98E-07	1.99E-04	3.91E-03	2.25E-06	4.74E-04	4.00E-03	1.98E-02
uncultured_bacterium_g_Family_XIII_AD3011_group	5.64E-05	6.94E-10	8.33E-06	1.06E-04	8.34E-09	2.89E-05	8.49E-02	1.52E-01
uncultured_bacterium_f_Candidatus_Gastranaerophilales_bacterium_Zag_111	9.53E-04	6.91E-07	2.63E-04	1.87E-03	1.42E-06	3.77E-04	5.99E-02	1.17E-01

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uncultured_bacterium_f_Eggerthellaceae	7.27E-05	2.41E-09	1.55E-05	1.43E-04	6.61E-09	2.57E-05	1.50E-02	4.27E-02
uncultured_bacterium_o_Rhodospirillales	2.01E-04	1.21E-08	3.48E-05	4.04E-04	7.89E-08	8.88E-05	3.70E-02	7.90E-02
uncultured_bacterium_g_Romboutsia	8.38E-04	1.45E-07	1.20E-04	1.69E-03	1.33E-06	3.65E-04	2.90E-02	6.98E-02
uncultured_bacterium_g_Christensenellaceae_R-7_group	4.34E-05	6.21E-10	7.88E-06	9.67E-05	6.66E-09	2.58E-05	3.30E-02	7.38E-02
uncultured_bacterium_g_Prevotellaceae_NK3B31_group	3.05E-03	1.81E-06	4.25E-04	7.43E-03	8.47E-06	9.21E-04	2.00E-03	1.17E-02
uncultured_bacterium_o_Mollicutes_RF39	5.43E-04	3.60E-08	6.00E-05	1.44E-03	6.71E-07	2.59E-04	9.99E-04	1.04E-02
uncultured_bacterium_g_Candidatus_Saccharimonas	3.41E-03	3.67E-06	6.06E-04	9.53E-03	1.95E-05	1.40E-03	2.00E-03	1.17E-02
uncultured_bacterium_f_Blattella_germanica_German_cockroach	1.06E-04	7.62E-09	2.76E-05	3.03E-04	2.01E-08	4.48E-05	4.00E-03	1.98E-02
uncultured_bacterium_f_Clostridiales_vadinBB60_group	3.44E-05	3.73E-09	1.93E-05	1.03E-04	1.24E-08	3.51E-05	1.07E-01	1.76E-01
Lactobacillus_gasseri	2.44E-04	2.99E-08	5.46E-05	1.28E-03	3.96E-07	1.99E-04	2.00E-03	1.17E-02
uncultured_bacterium_g_Bifidobacterium	2.11E-04	1.33E-08	3.65E-05	1.60E-03	2.50E-06	5.00E-04	9.99E-04	1.04E-02
uncultured_bacterium_g_Turcibacter	1.11E-03	3.92E-07	1.98E-04	8.56E-03	1.28E-05	1.13E-03	9.99E-04	1.04E-02
uncultured_bacterium_g_Faecalibaculum	7.69E-04	8.79E-08	9.37E-05	6.36E-03	2.74E-05	1.66E-03	9.99E-04	1.04E-02
uncultured_bacterium_g_Coriobacteriaceae_UCG-002	9.76E-05	1.91E-09	1.38E-05	8.39E-04	1.77E-07	1.33E-04	9.99E-04	1.04E-02

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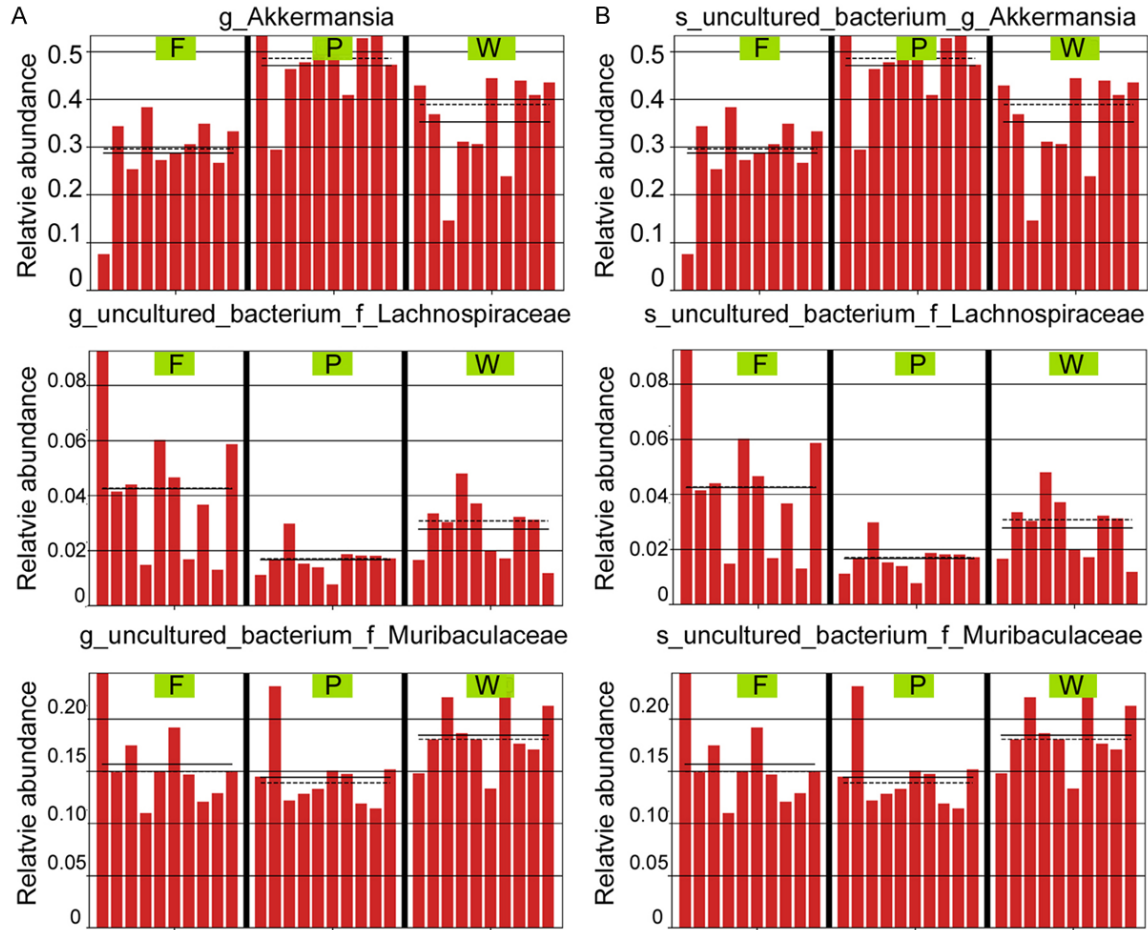
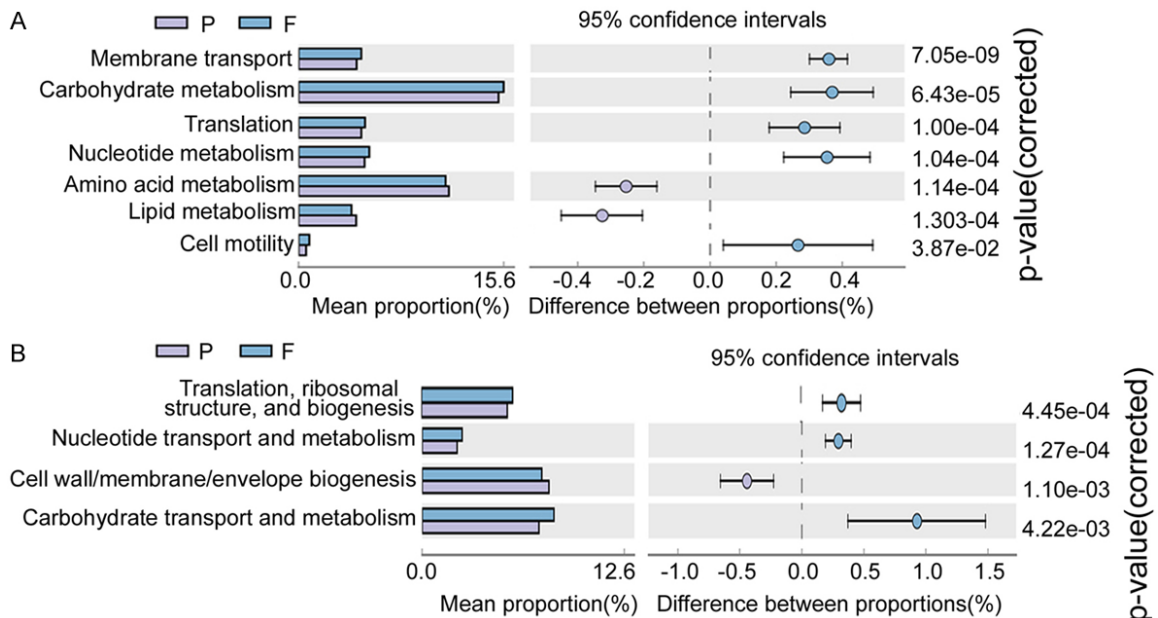


Figure 7. Significance analysis of differential gut microbial communities in the feces from various groups of mice. As shown by cladogram and LDA analyses, the microbial count of uncultured_bacterium_f_Lachnospiraceae was significantly increased, while that of *Akkermansia* microorganisms was drastically reduced in the gut microbiota of mice in Group F.



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Figure 8. 16S functional gene prediction analysis of gut microbiota in various groups of mice. A. KEGG functional prediction analysis showed that, compared with that in Group P, the gut microbiota in Group F could increase both carbohydrate and nucleotide metabolism, while decreasing both lipid and amino acid metabolism. B. COG functional prediction analysis showed that compared with that in Group P, the abundance of proteins associated with transcription, translation, ribosomal structure and biogenesis, carbohydrate transport and metabolism, and nucleotide transport and metabolism was significantly increased in Group F.

(**Figure 9B** and **9D**). Hence, our results suggested that fisetin significantly decreased CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte count and IL-12 secretion in POF mice.

Discussion

Currently, an increasing number of studies have found that POF is a multi-factorial disease [1-4, 6, 31]. Several factors, such as genetic factors, infection and inflammation, mental health condition, and hormone levels contribute towards the development of POF [1-4, 6]. Ovum maturation is an extremely complex process and requires the participation of hormones, growth factors, plasmin, and other substances [1-4, 6, 31]. The ovaries, which provide a natural microenvironment for ovum maturation, consist of granulosa, stromal, ovarian epithelial, and vascular endothelial cells [1-4, 6, 31]. The ovaries require large amounts of nutrients to function properly [1-4, 6, 31]. In the presence of endocrine and metabolic abnormalities in the body, the ovarian microenvironment is affected [1-4, 6, 31]. Therefore, maintaining an overall healthy endocrine and metabolic state is extremely important for the maintenance of a healthy ovarian microenvironment. Some studies have reported that fisetin, as a flavonol present in fruits and vegetables, possesses anti-oxidant, anti-inflammatory, and anti-aging effects in addition to its anti-cancer properties [7-9]. However, the exact mechanisms by which it exerts its effects are currently unknown. In this study, we investigated whether orally administered fisetin undergoes digestion and absorption in the gastrointestinal tract, and whether the gut microbiota carries out metabolic-biochemical reactions using fisetin as a raw material. However, whether fisetin affects the composition and metabolic pathways of the gut microbiota remains unknown.

In this study, we aimed to investigate: 1) whether fisetin exhibits therapeutic efficacy against POF, 2) whether fisetin exerts its effect in POF mice via regulating the gut microbiota, and 3) the mechanism by which fisetin regulates the

gut microbiota to alleviate POF. To achieve this, we first established POF mouse and *C. elegans* models by injection of CTX, followed by fisetin treatment. Our previous studies have confirmed that POF is typically characterized by pathological aging of the ovarian tissue [1, 17, 32]. *C. elegans* is a suitable animal model to study aging [32]. Therefore, in this study, we still selected *C. elegans* to evaluate the anti-aging function of fisetin. Results of pathological and molecular analyses suggested that fisetin could reverse ovarian damage and atresia in the ovarian follicles of mice and prevent premature senescence and reduction of ovarian function in *C. elegans*. Subsequently, we carried out 16S rRNA v3+v4 sequencing of fresh feces samples of mice in various groups to assess the composition of gut microbiota and differences in the distribution of specific flora. The results showed that fisetin treatment did not alter gut microbial counts in POF mice, but affected the composition and distribution of gut microbiota. Alpha and beta diversity analyses showed that the bacterial count of uncultured_bacterium_f_Lachnospiraceae was significantly increased in the gut microbiota of POF mice in the fisetin group, but that of *Akkermansia* was significantly reduced. Ruty and Zitvogel previously reported that the efficacy of PD-1 treatment was higher by 25-40% in some cancer patients containing *Akkermansia* in their gut compared to those lacking these gut microorganisms [33, 34]. Their results suggested that *Akkermansia* microorganisms may help mediate the immunoregulatory function of PD-1. Further, an in-depth study showed that when experimental mice were orally administered *Akkermansia* microorganisms, the recruitment of CCR9⁺/CXCR3⁺/CD4⁺ T lymphocytes increased, which suppressed IL-12-dependent PD-1 blockade, thereby increasing the cytotoxic effects of PD-1 antagonists [33, 34]. Hence, it suggested that *Akkermansia* microorganisms could effectively increase the activity of immune cells. In POF, certain immune cells, such as CD8⁺ T cells and M1 macrophages are in an overactivated state. In our study, we found that when fisetin allevi-

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Table 8. KEGG (P vs F)

Class 1	Class 2	P: mean rel.freq.(%)	P: std.dev.(%)	F: mean rel.freq.(%)	F: std.dev.(%)	p-values	p-values (corrected)	Difference between means	95.0% lower CI	95.0% upper CI
Metabolism	Carbohydrate metabolism	15.2158	0.098465	15.5841	0.1462	1.20E-05	6.43E-05	-0.368309	-0.493	-0.243608866
Metabolism	Lipid metabolism	4.37768	0.092	4.05142	0.146	4.24E-05	0.00013	0.326259	0.20377	0.448746455
Metabolism	Metabolism of cofactors and vitamins	6.40804	0.025488	6.24375	0.04993	6.28E-07	6.75E-06	0.164293	0.12404	0.204544501
Metabolism	Energy metabolism	7.0241	0.041369	6.89513	0.08907	0.001766	0.003302	0.128965	0.05808	0.199851964
Metabolism	Nucleotide metabolism	5.03781	0.109697	5.39101	0.14845	2.65E-05	0.000104	-0.3532	-0.4833	-0.223129628
Metabolism	Biosynthesis of other secondary metabolites	1.40308	0.008529	1.38291	0.01267	0.001143	0.002457	0.020177	0.00937	0.030980301
Metabolism	Amino acid metabolism	11.4485	0.069283	11.1952	0.11124	3.43E-05	0.000114	0.253385	0.16031	0.346457558
Metabolism	Metabolism of terpenoids and polyketides	2.09511	0.029918	2.02984	0.0807	0.043072	0.063866	0.065272	0.00241	0.128130574
Metabolism	Xenobiotics biodegradation and metabolism	2.36127	0.067271	2.17755	0.09547	0.000225	0.00057	0.183717	0.10126	0.266173915
Metabolism	Metabolism of other amino acids	2.05794	0.01307	2.06663	0.01359	0.183913	0.22595	-0.008688	-0.0219	0.004520898
Metabolism	Glycan biosynthesis and metabolism	3.10912	0.040491	2.91094	0.06041	4.72E-07	6.76E-06	0.198184	0.14672	0.249643975
Genetic Information Processing	Translation	4.76588	0.096159	5.05097	0.11761	2.80E-05	0.0001	-0.285099	-0.3918	-0.17841167
Metabolism	Global and overview maps	13.5316	0.056769	13.5306	0.151	0.984286	1.085239	0.001082	-0.1166	0.118814514
Human Diseases	Drug resistance	0.93973	0.015243	0.88032	0.04027	0.001492	0.003056	0.059412	0.028	0.090828372
Environmental Information Processing	Membrane transport	4.44104	0.051621	4.79906	0.06255	1.64E-10	7.05E-09	-0.358021	-0.415	-0.301081011
Environmental Information Processing	Signal transduction	3.44014	0.094492	3.2234	0.10309	0.000203	0.000546	0.216738	0.11875	0.31472534
Cellular Processes	Cell motility	0.5768	0.098952	0.84214	0.29143	0.025219	0.038729	-0.265346	-0.491	-0.039666622
Genetic Information Processing	Folding, sorting and degradation	2.28493	0.022624	2.21118	0.05424	0.002683	0.004438	0.073744	0.03108	0.116409292
Genetic Information Processing	Transcription	0.22556	0.00285	0.23609	0.00715	0.001518	0.002967	-0.010534	-0.0161	-0.004929328
Genetic Information Processing	Replication and repair	4.20832	0.071461	4.44943	0.09408	1.19E-05	7.31E-05	-0.241111	-0.3243	-0.157943165
Organismal Systems	Endocrine system	0.50868	0.005102	0.49429	0.00524	1.39E-05	6.65E-05	0.014387	0.00926	0.019511202
Environmental Information Processing	Signaling molecules and interaction	0	0	0	0	1	1.075	0	0	0
Cellular Processes	Cell growth and death	0.71639	0.007562	0.72397	0.01043	0.095905	0.128873	-0.007584	-0.0167	0.001498704
Cellular Processes	Transport and catabolism	0.64595	0.010187	0.5868	0.01182	1.53E-09	3.29E-08	0.059149	0.0482	0.070095597
Organismal Systems	Circulatory system	0.00053	0.000248	0.00075	0.00035	0.129507	0.168751	-0.000227	-0.0005	7.38E-05
Organismal Systems	Development	0	0	0	0	1	1.04878	0	0	0
Cellular Processes	Cellular community	0	0	0	0	1	1.02381	0	0	0
Organismal Systems	Immune system	0.11342	0.002376	0.12145	0.00272	3.25E-06	2.33E-05	-0.008032	-0.0106	-0.005497639
Organismal Systems	Environmental adaptation	0.16438	0.002798	0.17464	0.00853	0.005696	0.009071	-0.010257	-0.0168	-0.003666729
Organismal Systems	Nervous system	0.38693	0.007892	0.37309	0.01956	0.072761	0.100927	0.013842	-0.0015	0.02917844
Organismal Systems	Sensory system	0	0	0	0	1	1	0	0	0
Human Diseases	Endocrine and metabolic diseases	0.12607	0.004953	0.13412	0.00453	0.00208	0.003578	-0.008049	-0.0128	-0.003345654
Organismal Systems	Excretory system	0.11442	0.008281	0.08404	0.01532	0.000132	0.000377	0.030383	0.01792	0.042849674
Organismal Systems	Digestive system	0.01355	0.002889	0.01664	0.0038	0.069293	0.099319	-0.003087	-0.0064	0.000272377
Human Diseases	Neurodegenerative diseases	0.20071	0.002501	0.18878	0.00417	2.58E-06	2.22E-05	0.011936	0.00848	0.015393269
Human Diseases	Substance dependence	0.10587	0.010866	0.07204	0.019	0.000363	0.00082	0.038384	0.01822	0.049448215
Human Diseases	Infectious diseases: Bacterial	0.74233	0.002251	0.74307	0.00606	0.737421	0.834451	-0.000741	-0.0055	0.003982918
Human Diseases	Infectious diseases: Parasitic	0.10874	0.010226	0.07877	0.01618	0.000277	0.000661	0.029962	0.01638	0.04354289

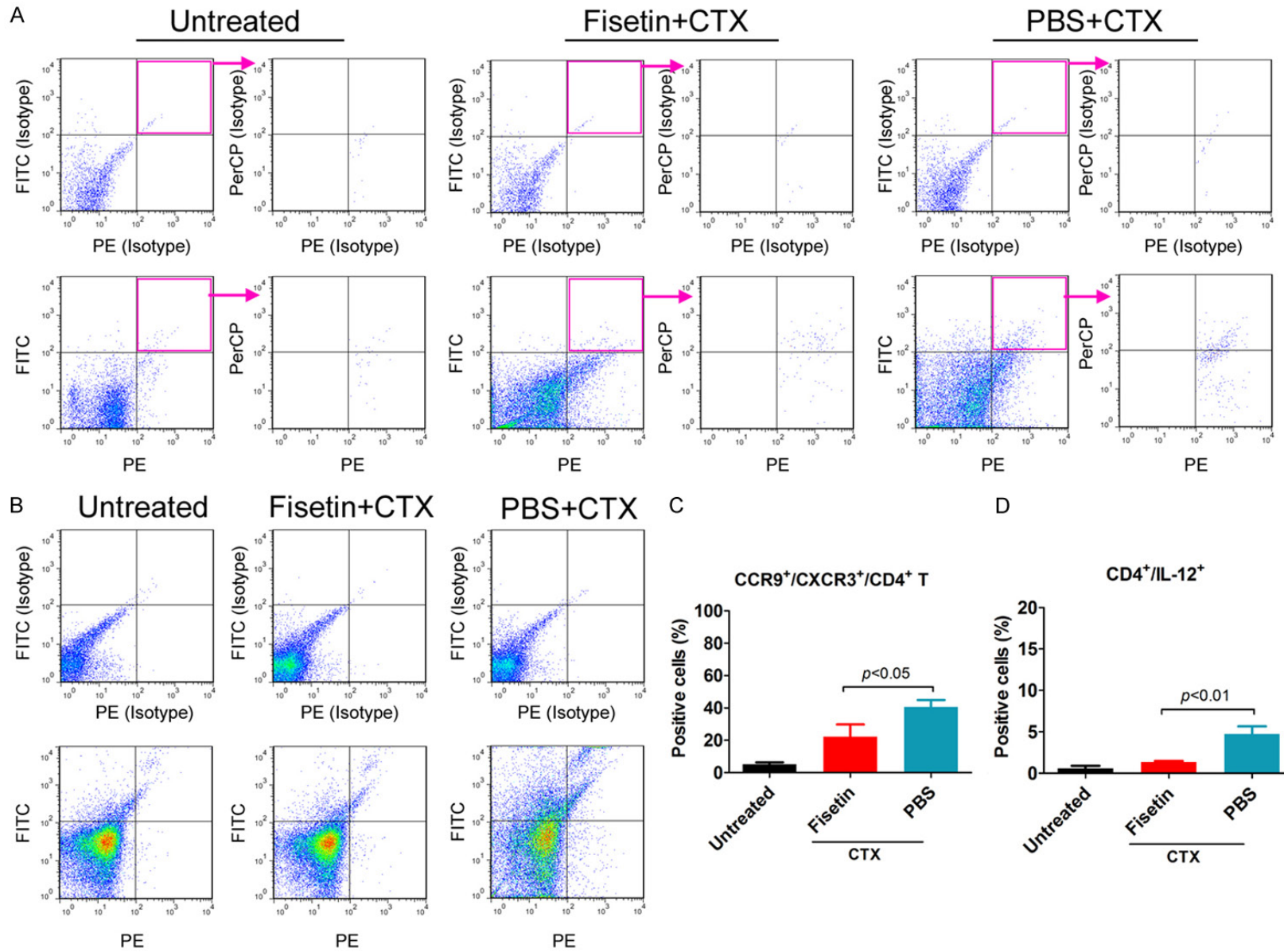
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Human Diseases	Infectious diseases: Viral	0.00122	0.000353	0.00103	0.00036	0.250759	0.299517	0.000198	-0.0002	0.000549562
Human Diseases	Cancers: Overview	0.94709	0.012065	0.8997	0.03345	0.001984	0.003554	0.047393	0.02139	0.073396642
Human Diseases	Cancers: Specific types	0.0935	0.003377	0.09216	0.00775	0.641648	0.745699	0.001345	-0.0048	0.007470511
Human Diseases	Immune diseases	0.05753	0.001452	0.06283	0.00216	1.59E-05	6.82E-05	-0.005301	-0.0071	-0.003461283
Human Diseases	Cardiovascular diseases	0.00017	8.21E-05	0.00025	0.00012	0.136062	0.172079	-7.41E-05	-0.0002	2.59E-05

Table 9. COG (P vs F)

Class 1	Class 2	P: mean rel.freq. (%)	P: std. dev.(%)	F: mean rel.freq. (%)	F: std. dev.(%)	p-values	p-values (corrected)	Difference between means	95.0% lower CI	95.0% upper CI
INFORMATION STORAGE AND PROCESSING	RNA processing and modification	0.04424	0.00378	0.0325	0.00631	0.00025419	0.000489	0.01174	0.0065	0.016973591
INFORMATION STORAGE AND PROCESSING	Chromatin structure and dynamics	0.04419	0.00408	0.03105	0.0071	0.00025742	0.00046	0.01313	0.00729	0.018970949
METABOLISM	Energy production and conversion	4.57045	0.08251	4.64817	0.11357	0.11569553	0.131472	-0.0777	-0.1767	0.02126776
CELLULAR PROCESSES AND SIGNALING	Cell cycle control, cell division, chromosome partitioning	0.96205	0.03834	1.09534	0.05938	4.13E-05	0.000172	-0.1333	-0.1834	-0.083179818
METABOLISM	Amino acid transport and metabolism	7.15645	0.03858	7.28732	0.04532	3.87E-06	4.83E-05	-0.1309	-0.1726	-0.089104438
METABOLISM	Nucleotide transport and metabolism	2.19879	0.08924	2.49334	0.11736	1.53E-05	0.000127	-0.2946	-0.3983	-0.190771936
METABOLISM	Carbohydrate transport and metabolism	7.28615	0.28753	8.21234	0.70532	0.00337815	0.004223	-0.9262	-1.4798	-0.372551875
METABOLISM	Coenzyme transport and metabolism	3.84124	0.0295	3.74354	0.0239	5.37E-07	1.34E-05	0.09769	0.07102	0.124367872
METABOLISM	Lipid transport and metabolism	2.81747	0.04787	2.58496	0.10658	5.52E-05	0.000197	0.23252	0.14803	0.317007595
INFORMATION STORAGE AND PROCESSING	Translation, ribosomal structure and biogenesis	5.39996	0.14566	5.72952	0.16261	0.00026715	0.000445	-0.3296	-0.4826	-0.176553788
INFORMATION STORAGE AND PROCESSING	Transcription	7.58899	0.12905	8.07005	0.35014	0.00245464	0.003409	-0.4811	-0.7537	-0.208450554
INFORMATION STORAGE AND PROCESSING	Replication, recombination and repair	6.10104	0.06951	6.32243	0.1059	8.84E-05	0.000246	-0.2214	-0.3111	-0.131655
CELLULAR PROCESSES AND SIGNALING	Cell wall/membrane/envelope biogenesis	7.8961	0.11425	7.45362	0.27131	0.00070138	0.001096	0.44248	0.22886	0.656102178
CELLULAR PROCESSES AND SIGNALING	Cell motility	1.57647	0.05978	1.42295	0.0702	0.00010096	0.000229	0.15352	0.08883	0.218210194
CELLULAR PROCESSES AND SIGNALING	Posttranslational modification, protein turnover, chaperones	3.4028	0.04029	3.23856	0.10484	0.00095795	0.001409	0.16425	0.08237	0.246128913
METABOLISM	Inorganic ion transport and metabolism	5.25735	0.08135	4.88141	0.15717	2.05E-05	0.000128	0.37595	0.24898	0.502910122
METABOLISM	Secondary metabolites biosynthesis, transport and catabolism	1.47389	0.05604	1.28654	0.09765	0.00018346	0.000382	0.18735	0.10704	0.267664261
POORLY CHARACTERIZED	General function prediction only	12.5919	0.14023	12.321	0.28875	0.02503819	0.029807	0.27085	0.03973	0.501972433
POORLY CHARACTERIZED	Function unknown	8.85463	0.24438	8.23404	0.452	0.00281298	0.003701	0.62059	0.25285	0.988323449
CELLULAR PROCESSES AND SIGNALING	Signal transduction mechanisms	5.65728	0.08178	5.71257	0.21303	0.48169017	0.523576	-0.0553	-0.2217	0.111075864
CELLULAR PROCESSES AND SIGNALING	Intracellular trafficking, secretion, and vesicular transport	2.86423	0.10006	2.4761	0.18932	9.53E-05	0.000238	0.38813	0.23469	0.541571855
CELLULAR PROCESSES AND SIGNALING	Defense mechanisms	2.39035	0.07462	2.70361	0.14814	7.10E-05	0.000222	-0.3133	-0.4324	-0.194074878
CELLULAR PROCESSES AND SIGNALING	Extracellular structures	0	0	0	0	1	1.041667	0	0	0
CELLULAR PROCESSES AND SIGNALING	Nuclear structure	0	0	0	0	1	1	0	0	0
CELLULAR PROCESSES AND SIGNALING	Cytoskeleton	0.02398	0.0013	0.019	0.0021	2.25E-05	0.000112	0.00498	0.00322	0.006738383

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Figure 9. Analysis of peripheral blood CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte counts and IL-12 levels in various groups of mice. A and C. FCM analysis showed that the peripheral blood CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte count of mice in the fisetin group was significantly lower than that of mice in the control group; B and D. FCM analysis showed that the peripheral blood CD4⁺/IL-12⁺ cell count of mice in the fisetin group was significantly lower than that of mice in the control group.

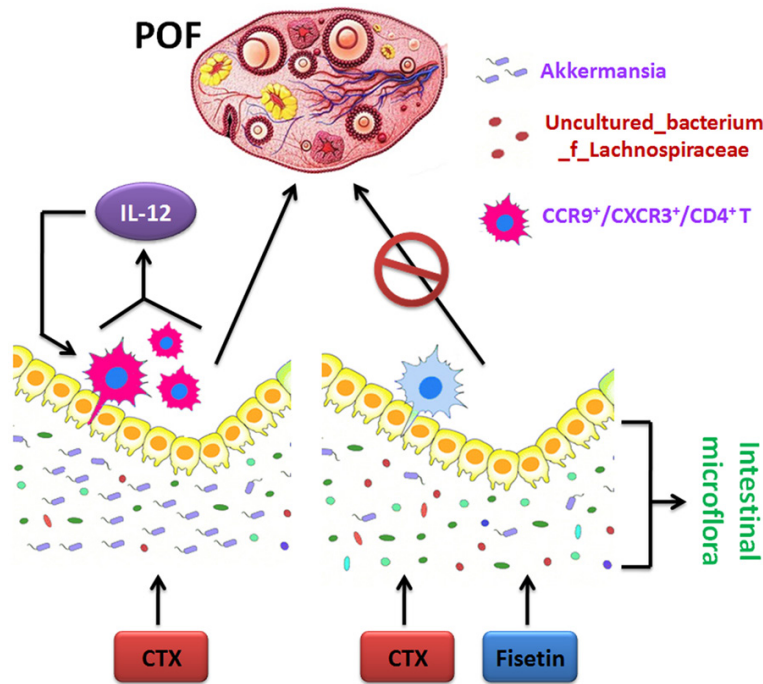


Figure 10. Fisetin alleviates POF in mice by regulating gut microbiota via decreasing CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte count and IL-12 secretion.

ates POF symptoms in POF mice, it also simultaneously decreases the number of *Akkermansia* microorganisms in the gut of mice. These results indicate that fisetin may decrease *Akkermansia* counts to reduce the counts and activity of CCR9⁺/CXCR3⁺/CD4⁺ T lymphocytes in POF mice. Subsequently, the counts of CCR9⁺/CXCR3⁺/CD4⁺ T lymphocytes and CD4⁺/IL-12⁺ cells in the PBMCs of various groups of mice were measured. Interestingly, CCR9⁺/CXCR3⁺/CD4⁺ T-lymphocyte and CD4⁺/IL-12⁺ counts were significantly decreased in POF mice in the fisetin group (**Figure 10**), which are consistent with the findings of Routy and Zitvogel [33, 34].

In summary, in this study, we showed that fisetin alleviates POF through novel pharmacological mechanisms, including regulating the distribution and counts of *Akkermansia* and uncultured_bacterium_f_Lachnospiraceae in POF mice to reduce peripheral blood CCR9⁺/

CXCR3⁺/CD4⁺ T-lymphocyte counts and IL-12 secretion. This in turn results in the regulation of ovarian microenvironment and reduction of inflammation, which have therapeutic effects on POF.

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Disclosure of conflict of interest

None.

Abbreviations

POF, Premature ovarian failure; Fisetin, 3,7,3',4'-tetrahydroxyflavone; FCM, Flow cytometry; PBMCs, Peripheral blood mononuclear cells; DPBS, Dulbecco's phosphate-buffered saline; LefSe, LDA-effect size; PCOA, Principal coordinates analysis; PCA, Principal component analysis; NMDS, Non-metric multi-dimensional scaling; UPGMA, Unweighted pair-group method with arithmetic mean; LDA, Line discriminant analysis; COG, Clusters of orthologous groups of proteins.

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