Supplemental Information

Bacterial indole-3-lactic acid affects epithelium-macrophage crosstalk to regulate intestinal homeostasis

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Supplementary methods

Fecal microbiota transplantation (FMT) in DSS-challenged mice. After collection, the fecal samples from people taking tinidazole or normal people were immediately stored at 4 °C under anaerobic conditions. Two grams of feces were homogenized in 5 mL of sterile Brain Heart Infusion containing 30% glycerine and 0.1% L-cysteine with vortex mixing and then centrifuged at 800 g for 5 min. The supernatants passed a 70-μm filter for the removal of large particles under anaerobic conditions. To remove intestinal microorganisms before FMT, mice were given a combination of the following antibiotics (AVMN) in drinking water: ampicillin (A610028-0025, 1 g/L), neomycin (A610366-0025, 1 g/L), metronidazole (A600633-0025, 1 g/L) and vancomycin (A600983-0001, 500 mg/L) (SangonBiotech, Shanghai, China). Mice were gavaged daily with 200 μl fresh fecal solution for fourteen days after six days of AVMN and two days of water treatment. After seven days of stool gavage, mice were given 3.5% DSS simultaneously to induce colitis for another 7 days.

The mouse model to test ILA in tinidazole-treated feces was shown in Fig. S7A. Two grams of stool was collected from healthy people and resuspended in 5 mL sterile anaerobic PBS and then centrifuged at 800×g for 5 min. The supernatant then passed through a 70-μm filter to remove large particulate. WT recipient mice were pretreated with AVMN for 7 days and then were intra-gastrically administered with 200 μL fresh fecal solution once for 10 days. Thereafter, mice were provided with the tinidazole (1 g/L, CM00694, Proteintech, Chicago, IL, USA) for 5 days. 200 mg of feces were collected from per mice on the last day of tinidazole treatment. Antibiotic-containing water was renewed every 3 to 4 days to maintain efficacy.

DNA extraction and 16S rRNA gene sequencing. Microbial genomic DNA in human and mouse feces samples was extracted using the Stool DNA Kit (D4015-02, Omega

Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The final DNA concentration and purification were determined by a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The DNA was evaluated by 1% (wt/vol) agarose gel electrophoresis.

16S rRNA gene sequencing was performed by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V3-V4 hypervariable regions of the 16S rRNA gene by a thermocycler PCR system (GeneAmp 9700, ABI, USA). The amplification program of the PCR was carried out as follows: 95 °C for 3 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, and a last extension at 72 °C for 10 min. The 20 μL PCR reaction system contained 4 μL 5×FastPfu buffer, 2 μL dNTPs (2.5 mM), 0.8 μL 338F primer (5 μM), 0.8 μL 806R primer (5 μM), 0.4 μL FastPfu polymerase, 0.2 μL BSA , and 10 ng template DNA. All samples were amplified in triplicate. The PCR product was isolated from a 2% agarose gel and purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified with the Quantus Fluorometer (Promega, USA). The purified amplicons were pooled in equimolar amounts. Paired-end sequencing was performed on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) in accordance with standard protocol by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Bioinformatic analysis of the fecal microbiota was performed with the Majorbio Cloud platform (https://cloud.majorbio.com). Raw data for bacterial sequence were deposited in the NCBI Sequence Read Archive (SRA) database under accession number PRJNA946039 and PRJNA945177, respectively.

Bacterial identification. The Bacterial Genomic DNA Extraction Kit (DP302-02,

TIANGEN, Beijing, China) was used to extract bacterial genomic DNA. Isolated strains were sequenced using 16S rRNA gene-specific primer pairs: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') in Sangon Biotech (Shanghai, China) to identify the bacterial species. The resulting sequences were compared with sequences in the EzBioCloud 16S rRNA databases (www.ezbiocloud.net).

Isolation of lymphocytes. Mesenteric lymph nodes were ground with RPMI 1640 medium containing 10% FBS on a 70-μm cell strainer to generate a single-cell suspension. After centrifugation, the cells were resuspended in RPMI 1640 medium for cell staining. The colon was opened longitudinally and washed by PBS, subsequently washed by PBS supplemented with 2 mM dithiothreitol (DTT, D1070, Solarbio, Beijing, China) for 10 min once and three times by PBS containing 5 mM EDTA for 30 min. The colonic tissue was then cut with scissors into small pieces and placed in 1 mL of RPMI 1640 medium containing 10% fetal bovine serum, 1 mg/mL collagenase IV (C5138, Sigma-Aldrich), 0.05 mg/mL hyaluronic acid, and 100 ng/mL DNaseI at 150 rpm for 30 minutes at 37 °C. After digesting, the cell solution was filtered through a 70-μm cell strainer (352350, BD Biosciences, San Jose, CA, USA) and re-suspended in 40% Percoll (Healthcare, Chicago, IL, USA), followed by centrifugation at 670 g for 30 min at 4 °C. Lamina propria lymphocytes were obtained in precipitation.

Flow cytometry. Single cell suspensions were surface stained with the following antibodies: anti-CD45 conjugated to PE-Cy7 (25-0451-82, eBioscience, San Diego, CA, USA), anti-CD11c conjugated to APC (117309, Biolegend, San Diego, CA, USA), anti-MHCII conjugated to PE (107607, Biolegend), anti-CD103 conjugated to PerCP/Cy5.5 (46-1031-82, eBioscience), anti-CD4 conjugated to APC-Cy7 (100414, Biolegend), anti-CD3 conjugated to FITC (100204, Biolegend), anti-CD4 conjugated to PE-Cy7

(25-0041-81, eBioscience), anti-CD25 conjugated to PE (102008, Biolegend), anti-CD45 conjugated to eFlour 506 (69-0451-82, Invitrogen), anti-CD11b conjugated to PE-Cy7 (25-0112-81, Invitrogen), anti-Ly6G conjugated to APC (127614, Biolegend), anti-F4/80 conjugated to FITC (11-4801-85, eBioscience), anti-Ly6C conjugated to PerCP/Cy5.5 (45-5932-80, Invitrogen), anti-MHCII conjugated to Alexa Fluor 700 (107622, Biolegend), anti-CCR2 conjugated to Brilliant Violet 421 (150605, Biolegend), anti-CCR5 conjugated to Brilliant Violet 506 (743697, eBioscience), anti-Lin conjugated to FITC (22-7770-72, Invitrogen), anti-CX3CR1 conjugated to PE/Dazzle (149013, Biolegend). For Treg and ILC3 cell analysis, after cell surface staining, cell suspensions were fixed, permeabilized using Foxp3/transcription factor staining buffer sets (00-5523-00, eBioscience) according to the manufacturer's instructions, and stained with anti-RORγt conjugated to APC (17-6981-82, eBioscience) and anti-Foxp3-APC (320014, Biolegend). For analysis of Th1, Th2, and Th17 cells, lymphocytes were stimulated for 5 h using a cell activation cocktail with Brefeldin A (423303, Biolegend). After being washed, cells were stained with dead cells using the Zombie NIRTM Fixable Viability Kit (423105, Biolegend), anti-CD3 conjugated to FITC (100204, Biolegend), and anti-CD4 conjugated to PE-Cy7 (25-0041-81, eBioscience). Then, cells were fixed for 30min with fixation buffer (420801, Biolegend), permeabilized for 10 min with intracellular staining permeabilization wash buffer (421002, Biolegend), and stained for 1h at 24 °C with anti-IL-17A conjugated to PE-Cy7 (25-7177-80, eBioscience) and anti-IFN-γ conjugated to PerCP/Cy5.5 (505821, Biolegend). These data were acquired on a FACS Canto II Flow Cytometer (BD Biosciences) and analyzed using the FlowJo software (FlowJo, Ashland, OR, USA).

RNA extraction and real-time PCR. BMDMs were stimulated with live bacteria (MOI = 0.2, 1, 5h) and LPS (0.5 μg/ml). In some experiments, SW480 cells were treated

with live bacteria (MOI = 1, 5, 10, 3h), heat-killed bacteria (MOI = 1, 5, 10, 3 h) or bacterial culture supernatant (bacterial culture supernatant: cell culture medium = 0.5:1, 1:1, 1.5:1, 3 h), bacterial culture supernatant fractions (bacterial culture supernatant: cell culture medium = 1.5:1, 3 h), LPS (6 μg/ml, L2880, Sigma-Aldrich), ILA (HY-113099, Med Chem Express, NJ, USA, 100 μM or 200 μM, 6h), HPLA (HY-113219, Med Chem Express, 10 μM or 50 μM, 6h), 5-MTP (M4001, Sigma-Aldrich, 10 μM or 50 μM, 6h), KynA (HY-100806, Med Chem Express, 10 μM or 50 μM, 6h), DCA (347795, Sigma-Aldrich, 10 mM, 16h), oligomycin (HY-16589, Med Chem Express, 5 nM, 3 h), AHR inhibitor CH-223191 (10 μM; HY-12684, Med Chem Express, 16 h), NF-κB inhibitor BAY 11-7082 (1 μM, HY-10257, Med Chem Express, NJ, USA, 16 h), NF-κB agonist PMA (100 nM, HY-18739, Med Chem Express, 16 h) , HIF inhibitor PX-478 (1 μM, HY-10231, Med Chem Express, 16 h), HIF agonist ML228 (10 nM, HY-10231, Med Chem Express, 3 h).

RNA from colonic tissues and cells was extracted using the Trizol reagent (Solarbio) in accordance with the manufacturer's directions. Subsequently, cDNA synthesis was performed using a HiFiScript cDNA Synthesis Kit (CW2569M, Cwbio). qRT-PCR was performed using SYBR Green qPCR Master mixes (CW0957M, Cwbio) on a thermocycler PCR system (LightCyler 96, Roche), and β-actin was used as endogenous control gene. $2^{-\Delta\Delta CT}$ method was used to calculate the transcript levels of the indicated genes.

Intestinal permeability assay. The mouse model was prepared as described in Fig. 2A. At day 6 after 3.5% DSS treatment, mice were gavaged orally with FITC-dextran (0.6 mg/g body weight, MW 3000–5000, Sigma-Aldrich, St. Louis, MO, USA). Mice receiving dextran-FITC were euthanized after 5 hours and blood was collected by cardiac puncture. The blood was centrifuged at 4 °C 2000g for 10 min. Plasma was

analyzed by measuring fluorescence intensity with Synergy HT Multi-Mode Microplate Reader (Bio-tek Winooski, Vermont, USA) at an excitation wavelength of 485 nm and emission wavelength of 525 nm.

Neutrophil depletion. The mouse model used to study the role of neutrophiles in the ILA treatment of DSS-induced colitis has been shown in Fig. S8K. From day -1, mice received IgG or anti-Ly-6G antibodies (0.2mg/per mouse, clone: 1A8, BioXCell) every 3 days during the course of the experiment. Mice were given 2.5% DSS for 8 days. From day 2, mice were daily gavaged with sterile water or 40 mg/kg ILA and sacrificed on day 6. Neutrophil depletion efficiency was determined by flow cytometry staining in cLP.

Macrophage transfer experiment. Splenocytes were isolated from WT mice and $CD45^+CD11b^+F4/80^+$ cells (macrophages) were sorted to high purity (>95%). 10^6 splenic macrophages were purified by flow cytometry and adoptively transferred into male $CCR2^{-/-}$ mice on day -1 and 4 via tail vein injection. Mice were given 2.5% DSS for 8 days. From day 2, mice were daily gavaged with sterile water or 40 mg/kg ILA and sacrificed on day 8 (Fig. S9A).

Analysis of bacteria-derived metabolites. The bacterial solution was centrifuged at 8000 g/min at 4 °C for 5 min to obtain supernatants, then filtered through a 0.22-mm membrane and separated using 100-, 50-, 30-, 10-, and 3-kDa molecular weight cutoff (MWCO) membranes (Merck Millipore, Billerica, MA, USA). The <3 kDa fraction of bacterial supernatants was frozen in a -80 °C refrigerator overnight, then freeze-dried into powder in a vacuum freeze dryer (Alpha 1–4 LD plus, CHRIST, Germany). 50 mg of powder was added to a 2 mL centrifuge tube, and a 6 mm diameter grinding bead was added. 400 μL of extraction solution (methanol:water = 4:1) containing 0.02 mg/mL internal standard (L-2-chlorophenylalanine) was used to extract the metabolites.

Samples were ground using the Wonbio-96c (Shanghai Wanbo Biotechnology Co., Ltd.) frozen tissue grinder for 6 min (-10 °C, 50 Hz), followed by ultrasonic extraction at a low temperature for 30 min (5 °C, 40 kHz). The samples were stored at -20 °C for 30 min, centrifuged for 15 min (4 °C, 13000 g), and the supernatant was pipetted into the sample vial for LC-MS/MS analysis. As a part of the system conditioning and quality control process, a pooled sample was prepared by combining equal volumes of each sample as a quality control (QC) control. The QC samples were disposed of and tested in the same manner as the analytic samples.

The LC-MS/MS analysis of the sample was performed on a Thermo UHPLC-Q Exactive HF-X system equipped with an ACQUITY HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm; Waters, USA) at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The mass spectrometric data were acquired using a Thermo UHPLC-Q Exactive HF-X Mass Spectrometer equipped with an electrospray ionization (ESI) source operating in positive mode and negative scanning mode.

The pretreatment of LC/MS raw data was performed by Progenesis QI (Waters Corporation, Milford, USA) software, and a three-dimensional data matrix in CSV format was exported. Internal standard peaks, as well as any known false positive peaks (including noise, column bleed, and derivatized reagent peaks), were removed from the data matrix, deredundant, and peak pooled. At the same time, the metabolites were identified by searching databases, and the main databases were the HMDB (http://www.hmdb.ca/), Metlin (https://metlin.scripps.edu/) and Majorbio Database. Uploaded to the Majorbio platform (https://cloud.majorbio.com) cloud, the data matrix obtained from the database search for data analysis. Differences in metabolites between the two groups were mapped into their biochemical pathways by means of metabolic enrichment and pathway analysis based on the KEGG database (http://www.

genome.jp/kegg/). Enrichment analysis was performed using the Python packages "scipy.stats" (https://docs.scipy.org/doc/scipy/) to obtain the most relevant biological pathways for experimental treatments.

RNA sequencing. SW480 cells were treated with PBS or LPS (6 μg/ml) in addition with DMSO or ILA (200 μM) for 6 h. Total RNA was extracted from the tissue using Trizol reagent according to the manufacturer's instructions (Invitrogen), and genomic DNA was removed using DNase I (TaKara). The quality of the RNA was determined using the 2100 Bioanalyser (Agilent Technologies) and quantified by the ND-2000 (NanoDrop Technologies). Only high-quality RNA samples (OD260/280=1.8~2.2, OD260/230≥2.0, RIN≥8.0, 28S:18S≥1.0, >1 μg) were used for sequencing library construction. Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China) performed RNA purification, reverse transcription, library construction and sequencing according to the manufacturer's instructions (Illumina, San Diego, CA). The transcriptome library was prepared following the TruSeq RNA sample preparation Kit from Illumina (San Diego, CA) using 1 μg of total RNA.

The expression level of each gene was calculated using the transcript TPM method to identify DEGs between two different groups. RSEM (http://deweylab.biostat.wisc.edu/rsem/) was used to quantify gene abundances. In addition, functional-enrichment analysis, including GO (Gene Ontology, http://www.geneontology.org) and KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/) was performed to identify which DEGs were significantly enriched in GO terms and metabolic pathways at P-adjust ≤ 0.05 compared to the whole-transcriptome background. Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do) performed GO functional enrichment and

KEGG pathway analysis. Analysis of differential gene expression, the KEGG pathway, and GSEA were performed at the Majorbio I-Sanger Cloud platform (https://cloud.majorbio.com). RNA-sequencing data have been deposited in NCBI under accession number PRJNA946043 and PRJNA946045.

Western blotting. Cells were lysed in RIPA lysis buffer (R0020, Solarbio, Beijing, China) with the addition of protease inhibitors (Roche, Basel, Switzerland) and phosphatase inhibitors (Roche). Protein lysates were boiled with 5 × SDS-buffer in 99 °C for 10 min. Samples were then separated on 10% SDS-PAGE gels and transferred to PVDF membrane. The PVDF membrane was blocked with 5% milk and incubated with anti-IκBαantibody (1:1000, ab32518, Abcam), anti-p-IκBαantibody (1:1000, Affinity Biosciences), anti-HIF1A antibody (1:1000, Affinity Biosciences) and anti-β-actin antibody (1:10000, ab6276, Abcam) overnight in 4 ℃, followed by incubation for 1 h with appropriate secondary antibodies. Immunoblots were visualized with Immobilon Western Chemiluminescent HRP Substrate (Merck Millipore, Billerica, MA, USA). The blot was scanned by Amersham Imager 600 with integrated analysis software (GE Healthcare Life Sciences, Buckinghamshire, United Kingdom). Immunoblots were quantified by using ImageJ software (National Institutes of Health, Bethesda, USA).

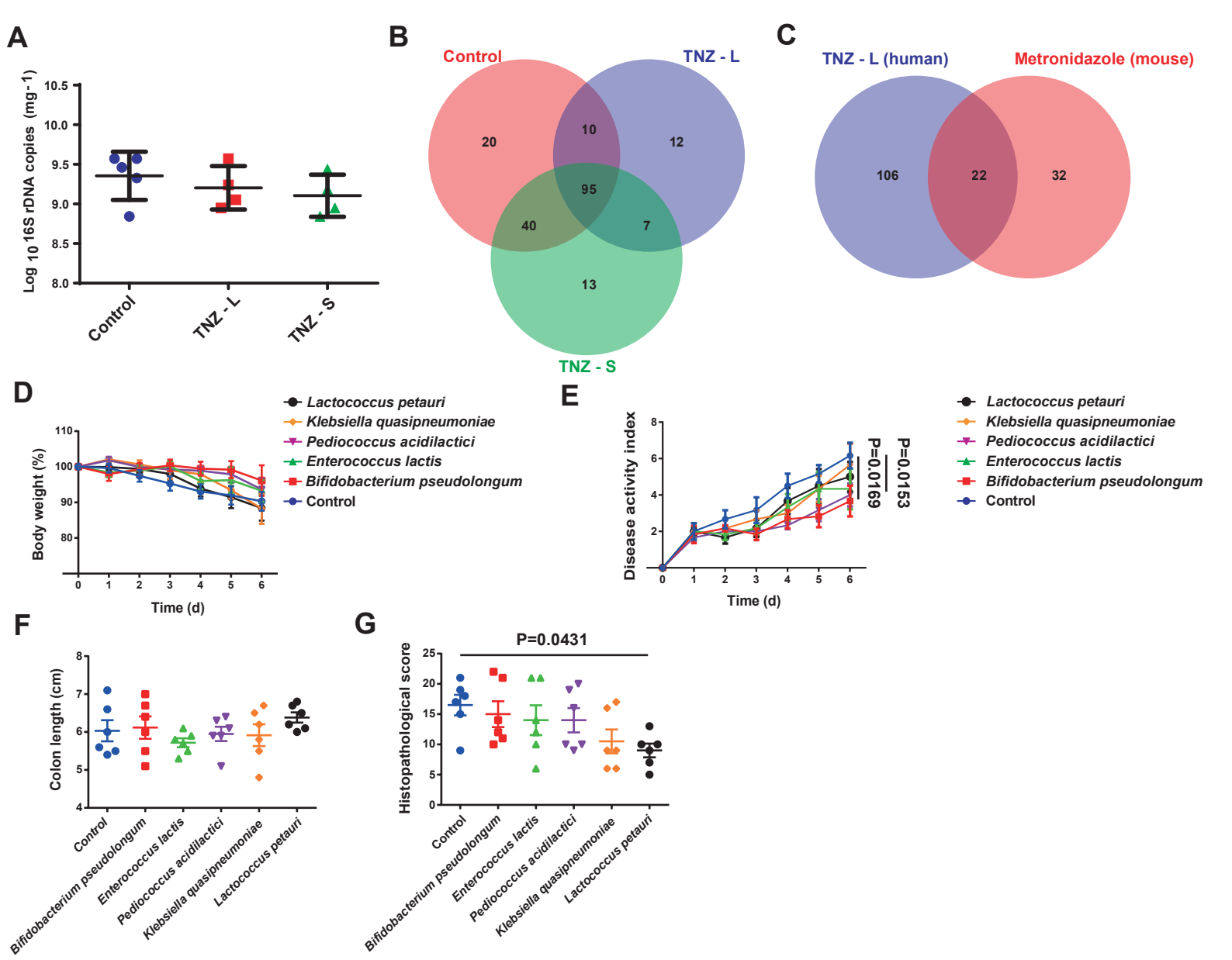
A
Log 10 16S rDNA copies (mg-1)
10.5
10.0
9.5
9.0
8.5
8.0
Control
TNZ - L
TNZ - S
B
Control
TNZ - L
TNZ - S
20
10
12
95
40
7
13
C
TNZ - L (human)
Metronidazole (mouse)
106
22
32
D
Body weight (%)
110
100
90
80
0 1 2 3 4 5 6
Time (d)
Lactococcus petauri
Klebsiella quasipneumoniae
Pediococcus acidilactici
Enterococcus lactis
Bifidobacterium pseudolongum
Control
E
Disease activity index
8
6
4
2
0 1 2 3 4 5 6
Time (d)
P=0.0153
P=0.0169
Lactococcus petauri
Klebsiella quasipneumoniae
Pediococcus acidilactici
Enterococcus lactis
Bifidobacterium pseudolongum
Control
F
Colon length (cm)
8
7
6
5
4
Control
Bifidobacterium pseudolongum
Enterococcus lactis
Pediococcus acidilactici
Klebsiella quasipneumoniae
Lactococcus petauri
G
Histopathological score
P=0.0431
25
20
15
10
5
0
Control
Bifidobacterium pseudolongum
Enterococcus lactis
Pediococcus acidilactici
Klebsiella quasipneumoniae
Lactococcus petauri

Figure S1. Effects of bacteria enriched in the gut microbiota of TNZ-L group on DSS-induced colitis in mice. (A-C) Stool samples from people taking tinidazole and normal people were collected and analyzed by 16S rRNA gene sequencing. (A) The amounts of bacterial DNA. (B) Comparison of different genus in the stool samples from the control, TNZ-L and TNZ-S groups through Venn diagrams. (C) Comparison of different genus in the stool samples from the TNZ-L group and mice treat with metronidazole and DSS in our previous data (SRA, PRJNA657382). (D-E) Schematic diagram is same to Figure 2A with different single bacteria administration. Analysis of body weight (D), disease activity index (E), colon length (F) and histopathological scores (G) in each group. n = 4 or 5 (A-C), n = 6 (D-G). Data are the mean ± SEM. One-way ANOVA (A, F-G), two-way ANOVA (D-E).

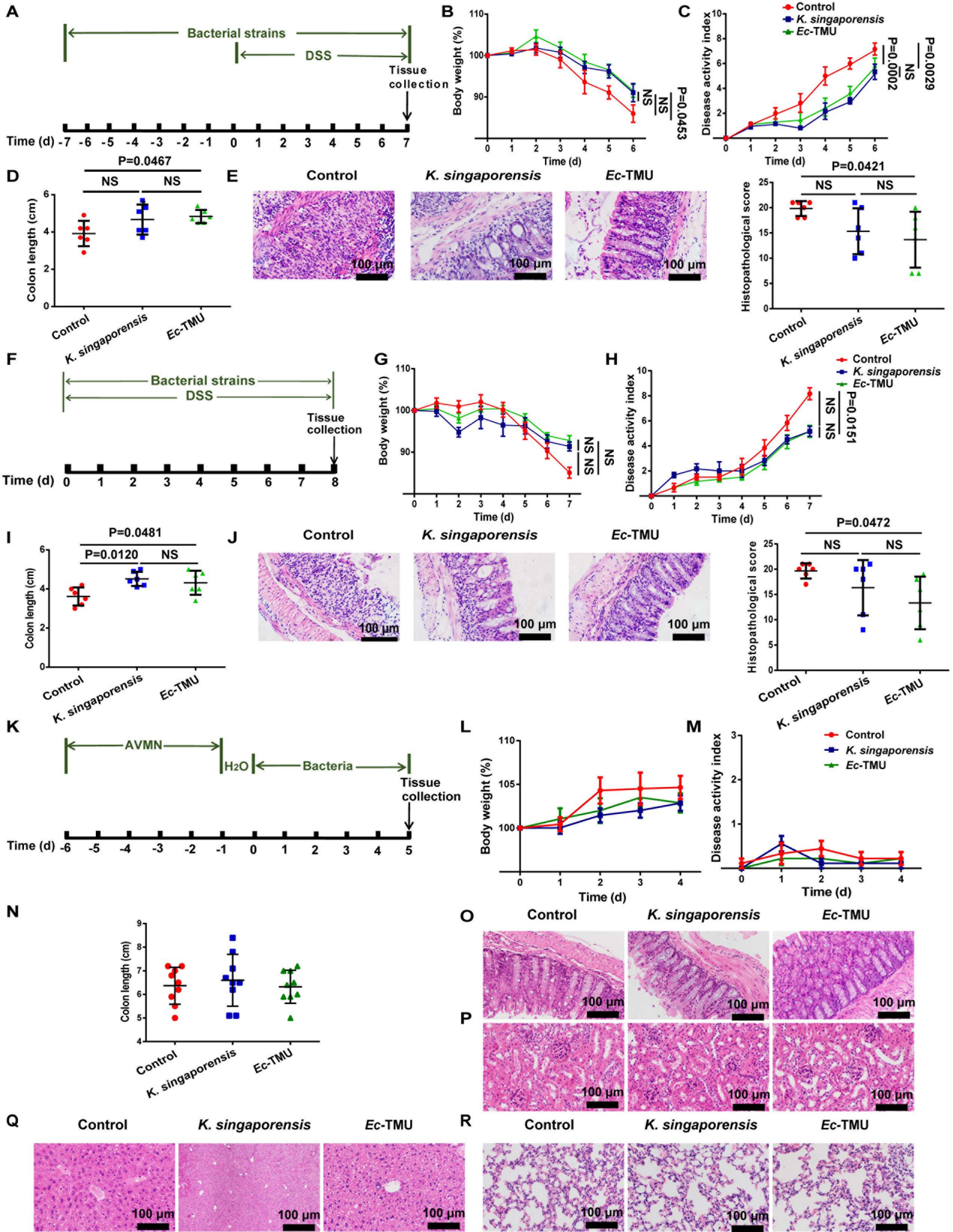
A
Bacterial strains
DSS
Tissue collection
Time (d) -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7
B
Body weight (%)
Time (d)
NS
NS
P=0.0453
C
Control
K. singaporensis
Ec-TMU
Disease activity index
Time (d)
P=0.0002
NS
P=0.0029
D
P=0.0467
NS
NS
Colon length (cm)
Control
K. singaporensis
Ec-TMU
E
Control
K. singaporensis
Ec-TMU
100 μm
P=0.0421
NS
NS
Histopathological score
F
Bacterial strains
DSS
Tissue collection
Time (d) 0 1 2 3 4 5 6 7 8
G
Body weight (%)
Time (d)
NS
NS
NS
H
Control
K. singaporensis
Ec-TMU
Disease activity index
Time (d)
NS
NS
P=0.0151
I
P=0.0481
P=0.0120
NS
Colon length (cm)
Control
K. singaporensis
Ec-TMU
J
Control
K. singaporensis
Ec-TMU
100 μm
P=0.0472
NS
NS
Histopathological score
K
AVMN
H2O
Bacteria
Tissue collection
Time (d) -6 -5 -4 -3 -2 -1 0 1 2 3 4 5
L
Body weight (%)
Time (d)
M
Control
K. singaporensis
Ec-TMU
Disease activity index
Time (d)
N
Colon length (cm)
Control
K. singaporensis
Ec-TMU
O
Control
K. singaporensis
Ec-TMU
100 μm
P
100 μm
Q
Control
K. singaporensis
Ec-TMU
100 μm
R
Control
K. singaporensis
Ec-TMU
100 μm

Figure S2. *Ec*-TMU protects against DSS-induced acute colitis in different models. (A) Schematic diagram for the mouse model to detect the prophylactic effect of single bacteria on DSS-induced colitis with the presence of gut microbiota. Mice were daily gavaged with 10^9 CFU of each strain for 7 days, and were subsequently exposed to the same strain or water plus 3% DSS for another 7 days, and mice were euthanized. (B-E) Analysis of body weight (B), disease activity index (C), colon length (D) and histopathological scores (E) in each group. (F) Schematic diagram for the mouse model to detect the therapeutic effect of single bacteria on DSS-induced colitis with the presence of gut microbiota. Mice were daily gavaged with 10^9 CFU of each strain or water in addition with 3% DSS exposing for 8 days, and mice were euthanized. (G-J) Analysis of body weight (G), disease activity index (H), colon length (I) and histopathological scores (J) in each group. (K) Schematic diagram for the mouse model to detect bacterial safety. Mice were gavaged daily with 10^9CFU of each strain or sterile water after five days of AVMN and one day of water treatment for 5 days, and mice were euthanized. (L-R) Analysis of body weight (L), disease activity index (M), colon length (N) and histopathological changes in colon (O), kidney (P), liver (Q), and lung (R). n = 6 (B-D, G-J); n = 9 (L-N). Data are the mean ±SEM. Two-way ANOVA (B-C, G-H, L-M) or one-way ANOVA (D-E, I-J, N). NS, not significant.

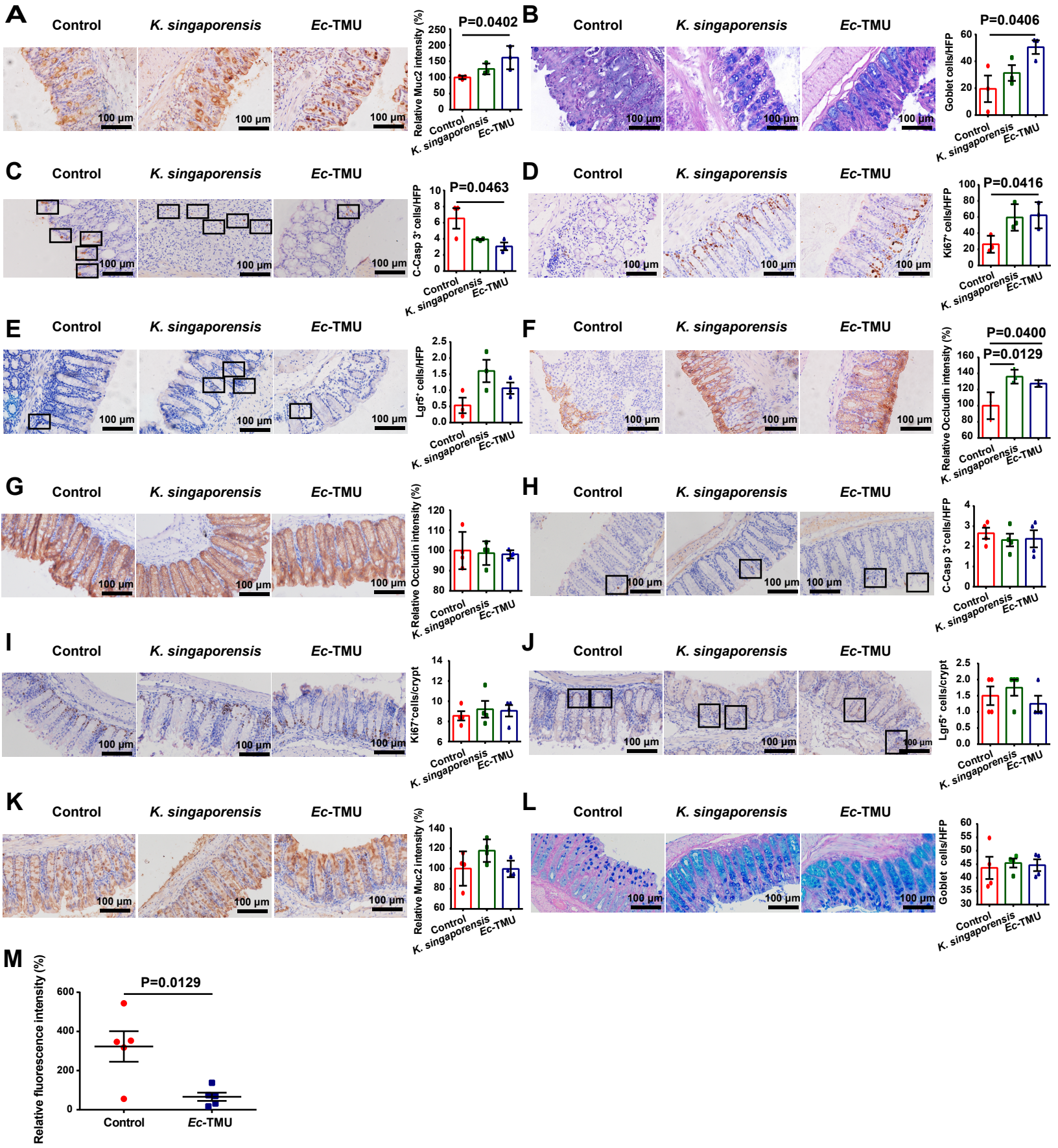
A
Control
K. singaporensis
Ec-TMU
100 μm
Relative Muc2 intensity (%)
P=0.0402
Control
K. singaporensis
Ec-TMU
B
Control
K. singaporensis
Ec-TMU
100 μm
Goblet cells/HFP
P=0.0406
Control
K. singaporensis
Ec-TMU
C
Control
K. singaporensis
Ec-TMU
100 μm
C-Casp 3+ cells/HFP
P=0.0463
Control
K. singaporensis
Ec-TMU
D
Control
K. singaporensis
Ec-TMU
100 μm
Ki67+ cells/HFP
P=0.0416
Control
K. singaporensis
Ec-TMU
E
Control
K. singaporensis
Ec-TMU
100 μm
Lgr5+ cells/HFP
Control
K. singaporensis
Ec-TMU
F
Control
K. singaporensis
Ec-TMU
100 μm
Relative Occludin intensity (%)
P=0.0400
P=0.0129
Control
K. singaporensis
Ec-TMU
G
Control
K. singaporensis
Ec-TMU
100 μm
Relative Occludin intensity (%)
Control
K. singaporensis
Ec-TMU
H
Control
K. singaporensis
Ec-TMU
100 μm
C-Casp 3+cells/HFP
Control
K. singaporensis
Ec-TMU
I
Control
K. singaporensis
Ec-TMU
100 μm
Ki67+cells/crypt
Control
K. singaporensis
Ec-TMU
J
Control
K. singaporensis
Ec-TMU
100 μm
Lgr5+ cells/crypt
Control
K. singaporensis
Ec-TMU
K
Control
K. singaporensis
Ec-TMU
100 μm
Relative Muc2 intensity (%)
Control
K. singaporensis
Ec-TMU
L
Control
K. singaporensis
Ec-TMU
100 μm
Goblet cells/HFP
Control
K. singaporensis
Ec-TMU
M
Relative fluorescence intensity (%)
P=0.0129
Control
Ec-TMU

Figure S3. Ec-TMU protects intestinal epithelial integrity in DSS-induced colitis. Schematic diagram is same to Figure 2A. (A-F) Representative Muc2 (A), goblet cells (B), cleaved caspase-3 (C), Ki67 (D), lgr5 (E) and Occludin (F) staining and quantitation in colon sections as indicated. Schematic diagram is same to Figure S3K. (G-L) Representative Occludin (G), cleaved caspase-3 (H), Ki67 (I), lgr5 (J), Muc2 (K) and goblet cells (L) staining and quantitation in colon sections as indicated. (M) Fluorescein isothiocyanate (FITC)-dextran measurement in serum of mice from models shown in Figure 2A. n = 3 (A to F). n = 4 (G to L). n = 5 (M). Data are the mean ±SEM. One-way ANOVA (A to L) or unpaired Student' s t test (M).

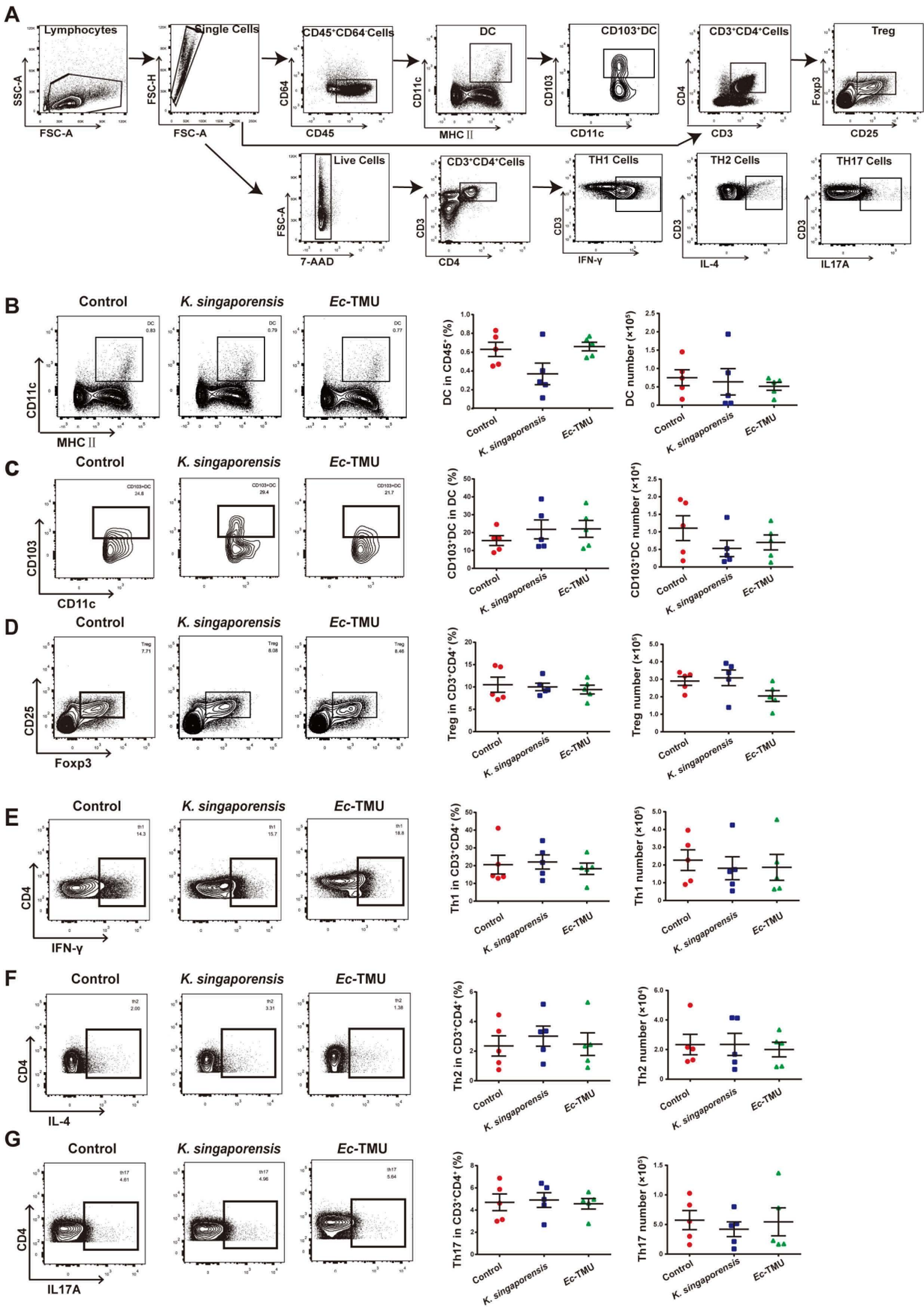
A
Lymphocytes
SSC-A
FSC-A
Single Cells
FSC-H
FSC-A
CD45+CD64-Cells
CD64
CD45
DC
CD11c
MHC Ⅱ
CD103+DC
CD103
CD11c
CD3+CD4+Cells
CD4
CD3
Treg
Foxp3
CD25
Live Cells
FSC-A
7-AAD
CD3+CD4+Cells
CD3
CD4
TH1 Cells
CD3
IFN-γ
TH2 Cells
CD3
IL-4
TH17 Cells
CD3
IL17A
B
Control
K. singaporensis
Ec-TMU
CD11c
MHC Ⅱ
DC in CD45+ (%)
DC number (×10⁵)
C
Control
K. singaporensis
Ec-TMU
CD103
CD11c
CD103+DC in DC (%)
CD103+DC number (×10⁴)
D
Control
K. singaporensis
Ec-TMU
CD25
Foxp3
Treg in CD3+CD4+ (%)
Treg number (×10⁵)
E
Control
K. singaporensis
Ec-TMU
CD4
IFN-γ
Th1 in CD3+CD4+ (%)
Th1 number (×10⁵)
F
Control
K. singaporensis
Ec-TMU
CD4
IL-4
Th2 in CD3+CD4+ (%)
Th2 number (×10⁴)
G
Control
K. singaporensis
Ec-TMU
CD4
IL17A
Th17 in CD3+CD4+ (%)
Th17 number (×10⁵)
Control
K. singaporensis
Ec-TMU

Figure S4. Effect of *Ec*-TMU on immune cells in MLN in DSS-induced acute colitis. MLN of mice (in the model of Figure 2A) from the *Ec*-TMU, *K. singaporensis* or control groups were collected, and lymphocytes were isolated, stained and analyzed by flow cytometry. (A) Gating strategy for flow cytometry analysis. (B) The percentage and number of DC. (C) The percentage and number of $CD103^{+}$DCs. (D) The percentage and number of $CD25^{+}Foxp3^{+}$ cells (Treg). (E) The percentage and number of IFN-$\gamma^{+}CD4^{+}$ cells (Th1). (F) The percentage of IL-$4^{+}CD4^{+}$ cells (Th2). (G) The percentage and number of IL-$17A^{+}CD4^{+}$ cells (Th17). n = 5. Data are the mean ± SEM. One-way ANOVA.

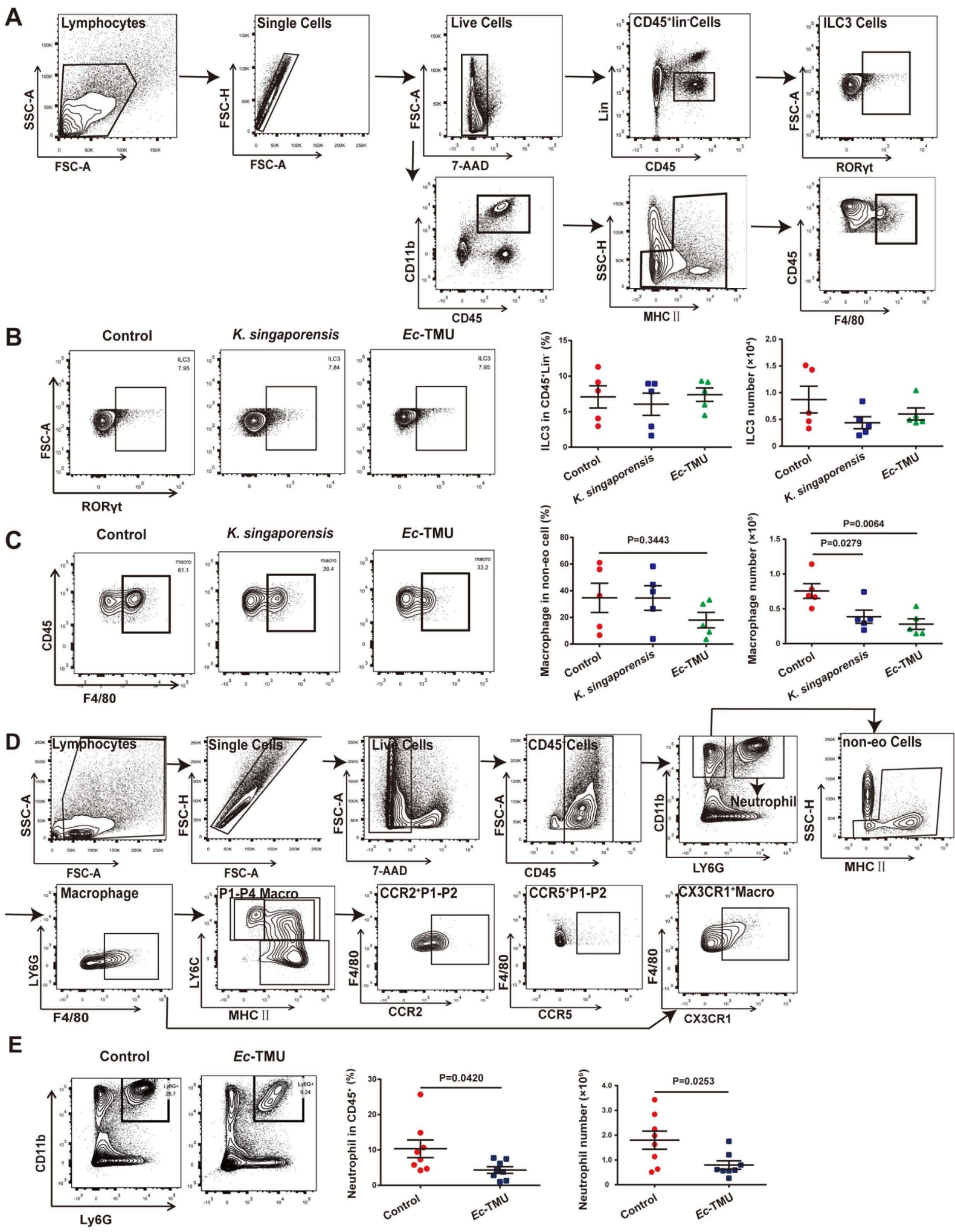
A
Lymphocytes
SSC-A
FSC-A
Single Cells
FSC-H
FSC-A
Live Cells
FSC-A
7-AAD
CD45+lin-Cells
Lin
CD45
ILC3 Cells
FSC-A
RORγt
CD11b
CD45
SSC-H
MHC Ⅱ
CD45
F4/80
B
Control
K. singaporensis
Ec-TMU
ILC3
7.95
7.84
7.95
FSC-A
RORγt
ILC3 in CD45+Lin- (%)
ILC3 number (×10⁴)
Control
K. singaporensis
Ec-TMU
C
Control
K. singaporensis
Ec-TMU
macro
61.1
39.4
33.2
CD45
F4/80
Macrophage in non-eo cell (%)
P=0.3443
Macrophage number (×10⁵)
P=0.0064
P=0.0279
Control
K. singaporensis
Ec-TMU
D
Lymphocytes
Single Cells
Live Cells
CD45+ Cells
Neutrophil
non-eo Cells
SSC-A
FSC-A
FSC-H
FSC-A
FSC-A
7-AAD
FSC-A
CD45
CD11b
LY6G
SSC-H
MHC Ⅱ
Macrophage
P1-P4 Macro
CCR2+P1-P2
CCR5+P1-P2
CX3CR1+Macro
LY6G
F4/80
LY6C
MHC Ⅱ
F4/80
CCR2
F4/80
CCR5
F4/80
CX3CR1
E
Control
Ec-TMU
Ly6G+
25.7
9.24
CD11b
Ly6G
Neutrophil in CD45+ (%)
P=0.0420
Neutrophil number (×10⁶)
P=0.0253
Control
Ec-TMU

Figure S5. Effect of *Ec*-TMU on immune cells in cLP in DSS-induced acute colitis. cLP of mice (in the model of Figure 2A) from the *Ec*-TMU, *K. singaporensis* or control groups were collected, and lymphocytes were isolated, stained and analyzed by flow cytometry. (A) Gating strategy for flow cytometry analysis. (B) The percentage and number of ILC3. (C) The percentage and number of macrophages. (D) Gating strategy for flow cytometry analysis of macrophage subsets. (E) The percentage and number of neutrophil cells. n = 5 (B-C), n = 8 (E). Data are the mean ± SEM. One-way ANOVA (B-C) or unpaired Student' s t test (E).

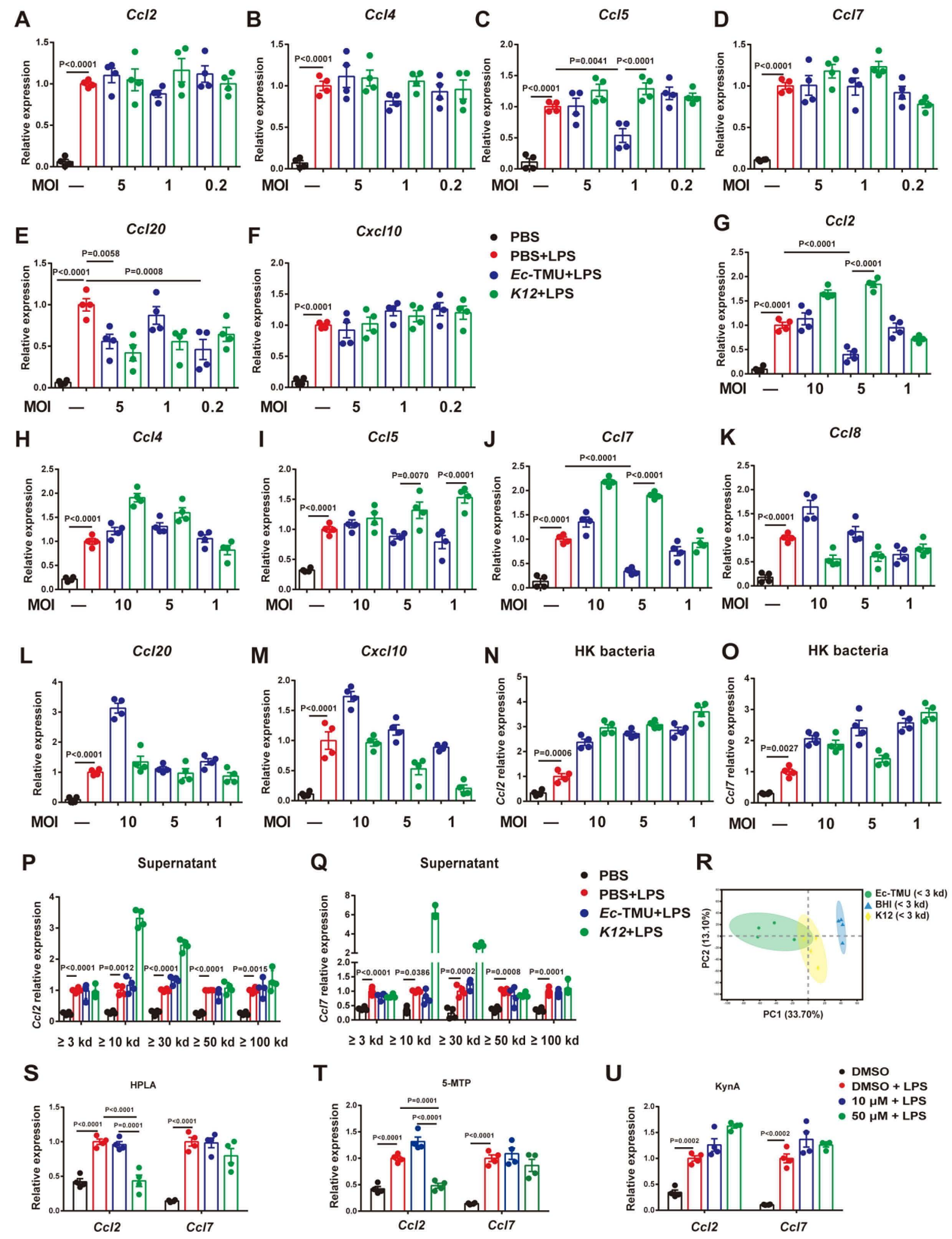
A
Ccl2
B
Ccl4
C
Ccl5
D
Ccl7
E
Ccl20
F
Cxcl10
G
Ccl2
H
Ccl4
I
Ccl5
J
Ccl7
K
Ccl8
L
Ccl20
M
Cxcl10
N
HK bacteria
O
HK bacteria
P
Supernatant
Q
Supernatant
R
S
HPLA
T
5-MTP
U
KynA
Relative expression
Ccl2 relative expression
Ccl7 relative expression
MOI
PBS
PBS+LPS
Ec-TMU+LPS
K12+LPS
≥ 3 kd
≥ 10 kd
≥ 30 kd
≥ 50 kd
≥ 100 kd
Ec-TMU (< 3 kd)
BHI (< 3 kd)
K12 (< 3 kd)
PC1 (33.70%)
PC2 (13.10%)
DMSO
DMSO + LPS
10 μM + LPS
50 μM + LPS

Figure S6. *Ec*-TMU directly reduced mRNA levels of *Ccl2* and *Ccl7* in epithelial cells. (A-F) BMDMs were stimulated with PBS, LPS (0.5 μg/ml), or LPS (0.5 μg/ml) in addition with *Ec*-TMU or K12 (MOI = 0.2, 1, and 5, 2 h). mRNA levels of *Ccl2* (A), *Ccl4* (B), *Ccl5* (C), *Ccl7* (D), *Ccl20* (E) and *Cxcl10* (F) were evaluated by qRT-PCR. (G-M) SW480 cells were stimulated with PBS, LPS (2.5 μg/ml), or LPS (2.5 μg/ml) in addition with *Ec*-TMU or K12 (MOI = 1, 5, 10，3 h). mRNA levels of *Ccl2* (G), *Ccl4* (H), *Ccl5* (I), *Ccl7* (J), *Ccl8* (K), *Ccl20* (L) and *Cxcl10* (M) were evaluated by qRT-PCR. (N-O) SW480 cells were stimulated with PBS, LPS (2.5 μg/ml), or LPS (2.5 μg/ml) in addition with HK bacteria (MOI = 1, 5, 10, 3 h). mRNA levels of *Ccl2* (N), *Ccl7* (O) were evaluated by qRT-PCR. (P-Q) SW480 cells were stimulated with PBS, LPS (10 μg/ml) in addition with different fractions separated from BHI, *Ec*-TMU, or *K12* culture supernatant. mRNA levels of *Ccl2* (P) and *Ccl7* (Q) in SW480 cells were evaluated by qRT-PCR. (R)Principal component analysis (PCA) score plots of metabolites (<3 kDa) from *Ec*-TMU culture supernatant, *K12* culture supernatant, or BHI. (S-U) SW480 cells were stimulated with LPS (6 μg/ml), in addition with DMSO, 10 μM or 50 μM HPLA (S), 5-MTP (T), HPLA (U) for 6 h, and mRNA levels of *Ccl2* and *Ccl7* were evaluated using qRT-PCR. n = 4 (A-Q, S-U). Data are the mean ±SEM. One-way ANOVA (A-Q, S-U).

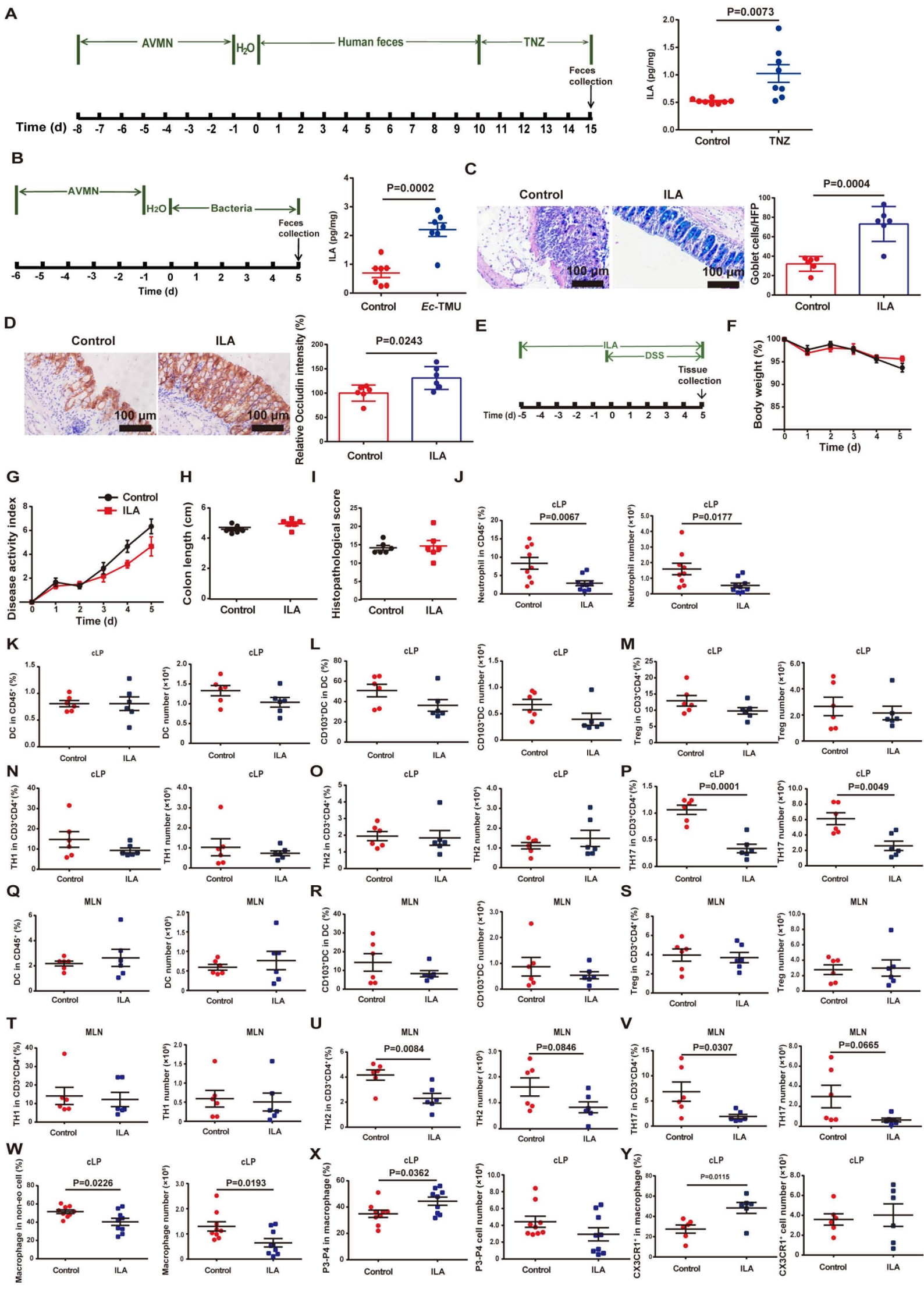
A
AVMN
H2O
Human feces
TNZ
Feces collection
Time (d) -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
P=0.0073
ILA (pg/mg)
Control
TNZ
B
AVMN
H2O
Bacteria
Feces collection
-6 -5 -4 -3 -2 -1 0 1 2 3 4 5
Time (d)
P=0.0002
ILA (pg/mg)
Control
Ec-TMU
C
Control
ILA
100 μm
100 μm
P=0.0004
Goblet cells/HFP
Control
ILA
D
Control
ILA
100 μm
100 μm
P=0.0243
Relative Occludin intensity (%)
Control
ILA
E
ILA
DSS
Tissue collection
Time (d) -5 -4 -3 -2 -1 0 1 2 3 4 5
F
Body weight (%)
Time (d)
G
Control
ILA
Disease activity index
Time (d)
H
Colon length (cm)
Control
ILA
I
Histopathological score
Control
ILA
J
cLP
P=0.0067
Neutrophil in CD45+ (%)
Control
ILA
cLP
P=0.0177
Neutrophil number (×10⁵)
Control
ILA
K
cLP
DC in CD45+ (%)
Control
ILA
cLP
DC number (×10⁴)
Control
ILA
L
cLP
CD103+DC in DC (%)
Control
ILA
cLP
CD103+DC number (×10⁴)
Control
ILA
M
cLP
Treg in CD3+CD4+ (%)
Control
ILA
cLP
Treg number (×10³)
Control
ILA
N
cLP
TH1 in CD3+CD4+ (%)
Control
ILA
cLP
TH1 number (×10⁵)
Control
ILA
O
cLP
TH2 in CD3+CD4+ (%)
Control
ILA
cLP
TH2 number (×10⁵)
Control
ILA
P
cLP
P=0.0001
TH17 in CD3+CD4+ (%)
Control
ILA
cLP
P=0.0049
TH17 number (×10⁴)
Control
ILA
Q
MLN
DC in CD45+ (%)
Control
ILA
MLN
DC number (×10⁵)
Control
ILA
R
MLN
CD103+DC in DC (%)
Control
ILA
MLN
CD103+DC number (×10⁴)
Control
ILA
S
MLN
Treg in CD3+CD4+ (%)
Control
ILA
MLN
Treg number (×10⁵)
Control
ILA
T
MLN
TH1 in CD3+CD4+ (%)
Control
ILA
MLN
TH1 number (×10⁵)
Control
ILA
U
MLN
P=0.0084
TH2 in CD3+CD4+ (%)
Control
ILA
MLN
P=0.0846
TH2 number (×10⁵)
Control
ILA
V
MLN
P=0.0307
TH17 in CD3+CD4+ (%)
Control
ILA
MLN
P=0.0665
TH17 number (×10⁵)
Control
ILA
W
cLP
P=0.0226
Macrophage in non-eo cell (%)
Control
ILA
cLP
P=0.0193
Macrophage number (×10⁵)
Control
ILA
X
cLP
P=0.0362
P3-P4 in macrophage (%)
Control
ILA
cLP
P3-P4 cell number (×10⁴)
Control
ILA
Y
cLP
P=0.0115
CX3CR1+ in macrophage (%)
Control
ILA
cLP
CX3CR1+ cell number (×10³)
Control
ILA

Figure S7. The effect of ILA on intestinal immune cells. (A) Schematic diagram for the mouse model to detect the effect of TNZ treatment on ILA content in mouse feces. ILA levels in the feces from mice treated with water or TNZ. (B) Schematic diagram for the mouse model to detect the effect of *Ec*-TMU treatment on ILA content in mouse feces. ILA levels in the feces from mice treated with PBS or *Ec*-TMU. (C-D) Representative goblet cells (C) and Occludin (D) staining and quantitation in colon sections. (E) Schematic diagram for the mouse model to detect the prophylactic effect of ILA on DSS-induced acute colitis. Mice were gavaged daily with ILA (40 mg/kg) or sterile water for five days. After five days of ILA administration, mice were given 2.5 % DSS simultaneously to induce colitis in addition with ILA administration for another 5 days, and mice were euthanized. (F-I) Analysis of body weight (F), disease activity index (G), colon length (H) and histopathological scores (I) in each group. (J-Y). MLN and cLP of mice (in the model of Figure 5I) from the ILA or control groups were collected, and lymphocytes were isolated, stained and analyzed by flow cytometry. (J) The percentage and number of neutrophils in the cLP. (K) The percentage and number of DCs in the cLP. (L) The percentage and number of $CD103^+$DCs in the cLP. (M) The percentage and number of Tregs in the cLP. (N) The percentage and number of IFN-γ^+CD4^+ cells (Th1) in the cLP. (O) The percentage and number of IL-4^+CD4^+ cells (Th2) in the cLP. (P) The percentage and number of IL-$17A^+CD4^+$ cells (Th17) in the cLP. (Q) The percentage and number of DCs in the MLN. (R) The percentage and number of $CD103^+$DCs in the MLN. (S) The percentage and number of Tregs in the MLN. (T) The percentage and number of IFN-γ^+CD4^+ cells (Th1) in the MLN. (U) The percentage and number of IL-4^+CD4^+ cells (Th2) in the MLN. (V) The percentage and number of IL-$17A^+CD4^+$ cells (Th17) in the MLN. (W) The percentage and number of macrophages. (X) The percentage and number of anti-inflammatory (P3-P4)

macrophages. (Y) The percentage and number of CX3CR1^{+} macrophages. n = 8 (A), n = 7 (B), n = 6 (C-D, F-I, K-V, Y) and n = 9 (J, W-X). Data are the mean ±SEM. Unpaired Student' s t test (A-D, H-Y) or two-way ANOVA (F-G).

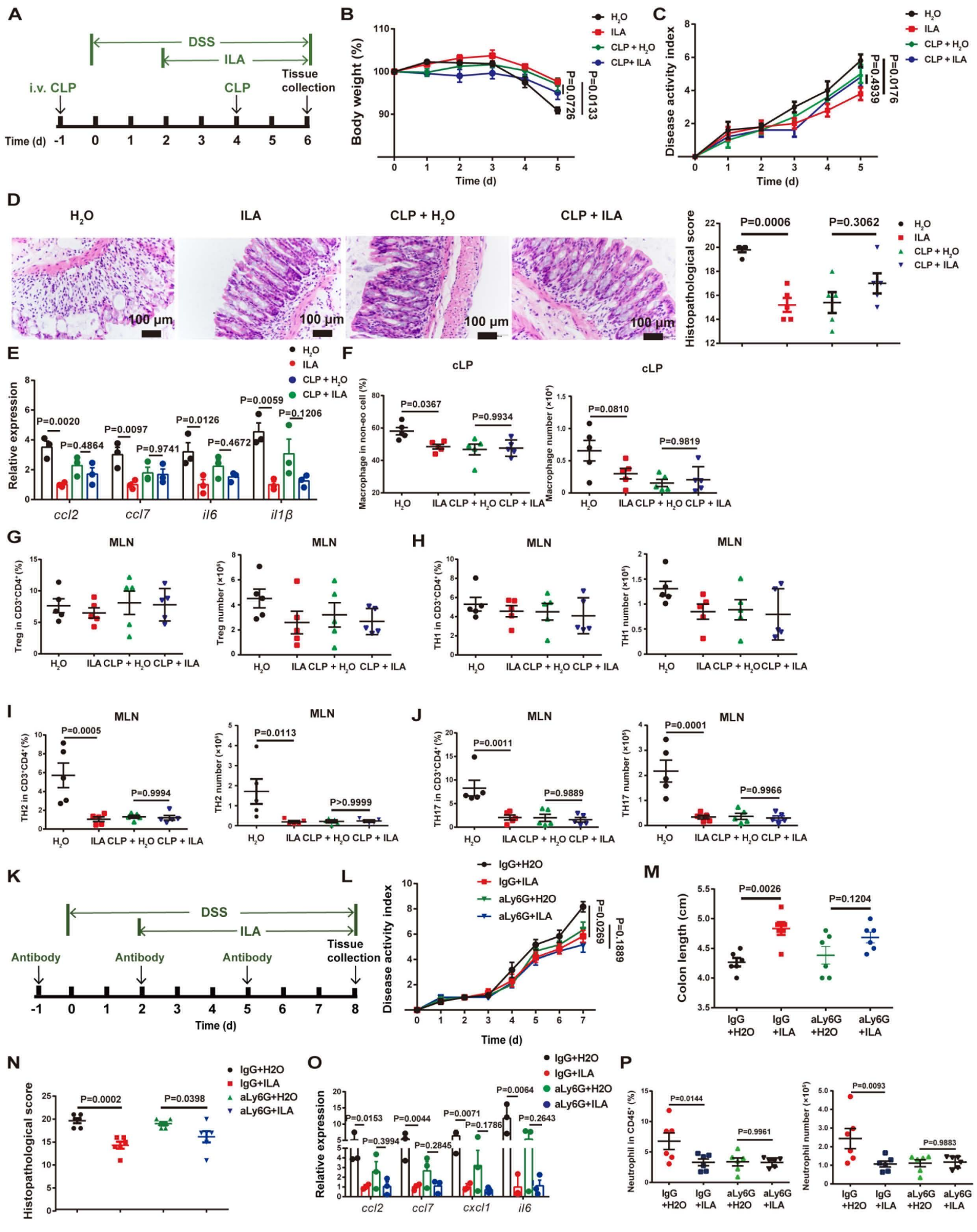
A
DSS
ILA
i.v. CLP
CLP
Tissue collection
Time (d) -1 0 1 2 3 4 5 6
B
Body weight (%)
H2O
ILA
CLP + H2O
CLP+ ILA
P=0.0726
P=0.0133
Time (d)
C
Disease activity index
P=0.4939
P=0.0176
Time (d)
D
H2O
ILA
CLP + H2O
CLP + ILA
100 μm
Histopathological score
P=0.0006
P=0.3062
E
Relative expression
P=0.0020
P=0.4864
P=0.0097
P=0.9741
P=0.0126
P=0.4672
P=0.0059
P=0.1206
ccl2
ccl7
il6
il1β
F
cLP
Macrophage in non-eo cell (%)
P=0.0367
P=0.9934
Macrophage number (×10⁴)
P=0.0810
P=0.9819
G
MLN
Treg in CD3+CD4+ (%)
Treg number (×10⁶)
H
TH1 in CD3+CD4+ (%)
TH1 number (×10⁵)
I
TH2 in CD3+CD4+ (%)
P=0.0005
P=0.9994
TH2 number (×10⁵)
P=0.0113
P>0.9999
J
TH17 in CD3+CD4+ (%)
P=0.0011
P=0.9889
TH17 number (×10⁵)
P=0.0001
P=0.9966
K
DSS
ILA
Antibody
Antibody
Antibody
Tissue collection
Time (d)
L
IgG+H2O
IgG+ILA
aLy6G+H2O
aLy6G+ILA
Disease activity index
P=0.0269
P=0.1889
Time (d)
M
Colon length (cm)
P=0.0026
P=0.1204
IgG +H2O
IgG +ILA
aLy6G +H2O
aLy6G +ILA
N
Histopathological score
P=0.0002
P=0.0398
O
Relative expression
P=0.0153
P=0.3994
P=0.0044
P=0.2845
P=0.0071
P=0.1786
P=0.0064
P=0.2643
ccl2
ccl7
cxcl1
il6
P
Neutrophil in CD45+ (%)
P=0.0144
P=0.9961
Neutrophil number (×10⁵)
P=0.0093
P=0.9883

Figure S8. The effect of ILA on DSS-colitis after macrophage or neutrophile deletion. (A) Schematic diagram for the mouse model of DSS-induced colitis combined with ILA and chlorophosphate liposomes (CLP) injection. After injected intravenously with chlorophosphate liposome or control liposome, mice were given 2.5% DSS for 6 days. After two days of DSS administration, mice were daily gavaged with sterile water or 40 mg/kg ILA. Mice were treated with CLP or PBS liposomes again on day 4. (B–D) Analysis of body weight (B), disease activity index (C), and histopathological scores (D) in each group. (E) mRNA expression levels of inflammatory cytokines in the colons. (F) The percentage and number of macrophages. (G) The percentage and number of Tregs. (H) The percentage of IFN-γ^+CD4$^+$ cells (Th1). (I) The percentage and number of IL-4$^+$CD4$^+$ cells (Th2). (J) The percentage and numbers of IL-17A$^+$CD4$^+$ cells (Th17). (K) Schematic diagram for the mouse model of DSS-induced colitis combined with ILA and Ly6G neutralizing antibody (aLy6G) injection. After injected intravenously with aLy6G or IgG, mice were given 2.5% DSS for 6 days. After two days of DSS administration, mice were daily gavaged with sterile water or 40 mg/kg ILA. Mice were treated with aLy6G or IgG again on day 2 and 5. (L–N) Analysis of disease activity index (L), colon length (M) and histopathological scores (N) in each group. (O) mRNA expression levels of inflammatory cytokines in the colons. (P) The percentage and number of neutrophils. n = 5 (B-D, F-J), n = 6 (L-N, P) and n = 3 (E, O). Data are the mean ± SEM, two-way ANOVA (B-C, L) or one-way ANOVA (D-J, M-P).

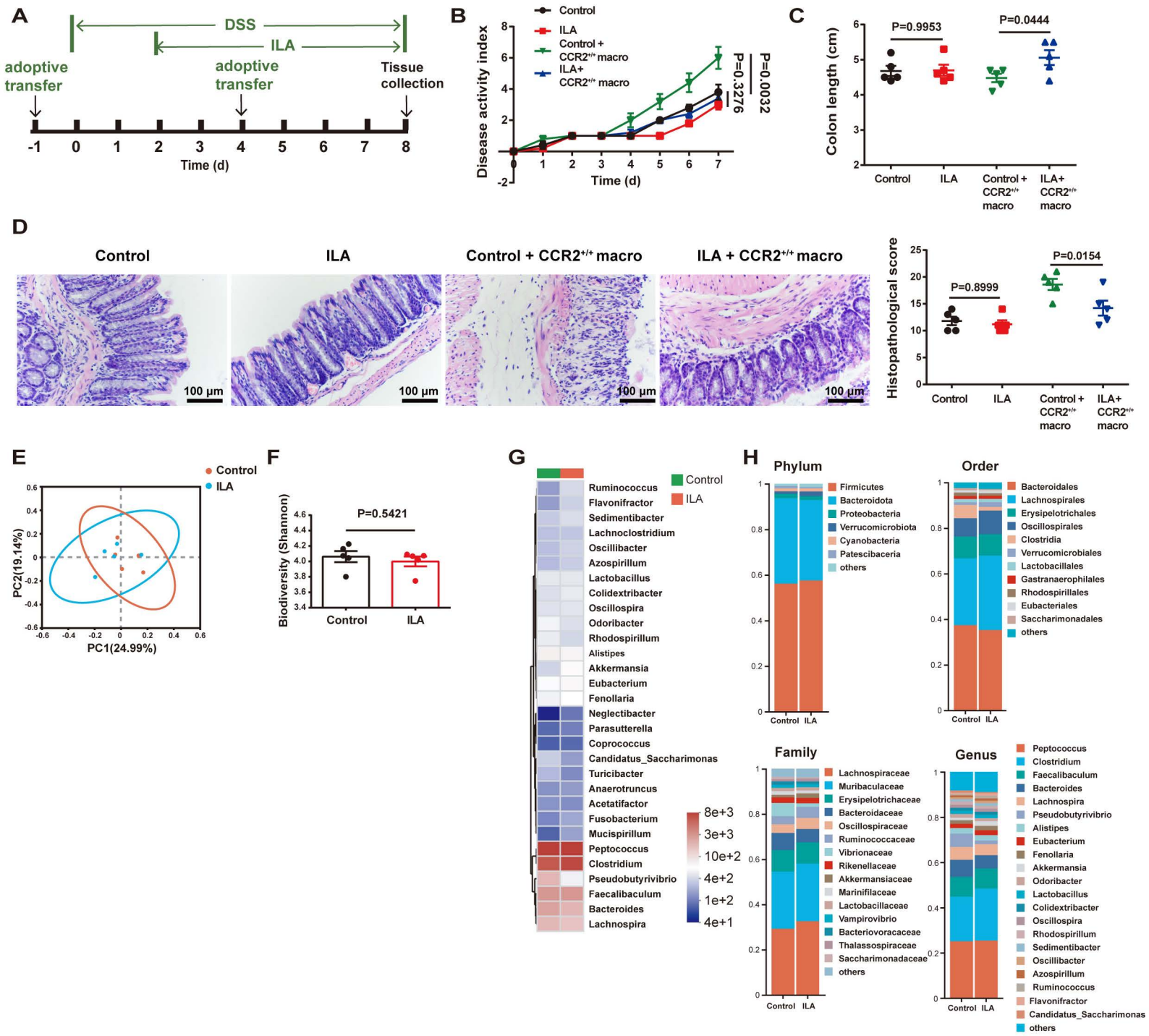
A
DSS
ILA
adoptive transfer
adoptive transfer
Tissue collection
-1 0 1 2 3 4 5 6 7 8
Time (d)
B
Control
ILA
Control + CCR2+/+ macro
ILA+ CCR2+/+ macro
Disease activity index
P=0.3276
P=0.0032
Time (d)
C
P=0.9953
P=0.0444
Colon length (cm)
Control
ILA
Control + CCR2+/+ macro
ILA+ CCR2+/+ macro
D
Control
ILA
Control + CCR2+/+ macro
ILA + CCR2+/+ macro
100 μm
P=0.8999
P=0.0154
Histopathological score
E
Control
ILA
PC2(19.14%)
PC1(24.99%)
F
P=0.5421
Biodiversity (Shannon)
G
Ruminococcus
Flavonifractor
Sedimentibacter
Lachnoclostridium
Oscillibacter
Azospirillum
Lactobacillus
Colidextribacter
Oscillospira
Odoribacter
Rhodospirillum
Alistipes
Akkermansia
Eubacterium
Fenollaria
Neglectibacter
Parasutterella
Coprococcus
Candidatus_Saccharimonas
Turicibacter
Anaerotruncus
Acetatifactor
Fusobacterium
Mucispirillum
Peptococcus
Clostridium
Pseudobutyrivibrio
Faecalibaculum
Bacteroides
Lachnospira
8e+3
3e+3
10e+2
4e+2
1e+2
4e+1
H
Phylum
Firmicutes
Bacteroidota
Proteobacteria
Verrucomicrobiota
Cyanobacteria
Patescibacteria
others
Order
Bacteroidales
Lachnospirales
Erysipelotrichales
Oscillospirales
Clostridia
Verrucomicrobiales
Lactobacillales
Gastranaerophilales
Rhodospirillales
Eubacteriales
Saccharimonadales
others
Family
Lachnospiraceae
Muribaculaceae
Erysipelotrichaceae
Bacteroidaceae
Oscillospiraceae
Ruminococcaceae
Vibrionaceae
Rikenellaceae
Akkermansiaceae
Marinifilaceae
Lactobacillaceae
Vampirovibrio
Bacteriovoracaceae
Thalassospiraceae
Saccharimonadaceae
others
Genus
Peptococcus
Clostridium
Faecalibaculum
Bacteroides
Lachnospira
Pseudobutyrivibrio
Alistipes
Eubacterium
Fenollaria
Akkermansia
Odoribacter
Lactobacillus
Colidextribacter
Oscillospira
Rhodospirillum
Sedimentibacter
Oscillibacter
Azospirillum
Ruminococcus
Flavonifractor
Candidatus_Saccharimonas
others
Control
ILA

Figure S9. The role of CCR2$^+$ macrophages in ILA's effect on DSS-colitis. (A) Schematic diagram for the mouse model to examine the role of CCR2$^+$ macrophages in ILA inhibition of colitis. (B-D) Analysis of disease activity index (B), colon length (C) and histopathological scores (D). (E-H) Stool samples from mice treated with sterile water or ILA (in the model of Fig. 5I) were collected and analyzed by 16S rRNA gene sequencing. (E) Shannon diversity index of the microbiota. (F) PCoA results based on unweighted UniFrac distances. (G) Relative abundance of bacteria by taxon-based analyses. (H) Heatmap to visualize the relative abundances of the 30 most predominant bacterial genera. n = 5 (B-D), n = 5 (E-H). Data are the mean ± SEM, two-way ANOVA (B), one-way ANOVA (C-D) or unpaired Student' s t test (E).

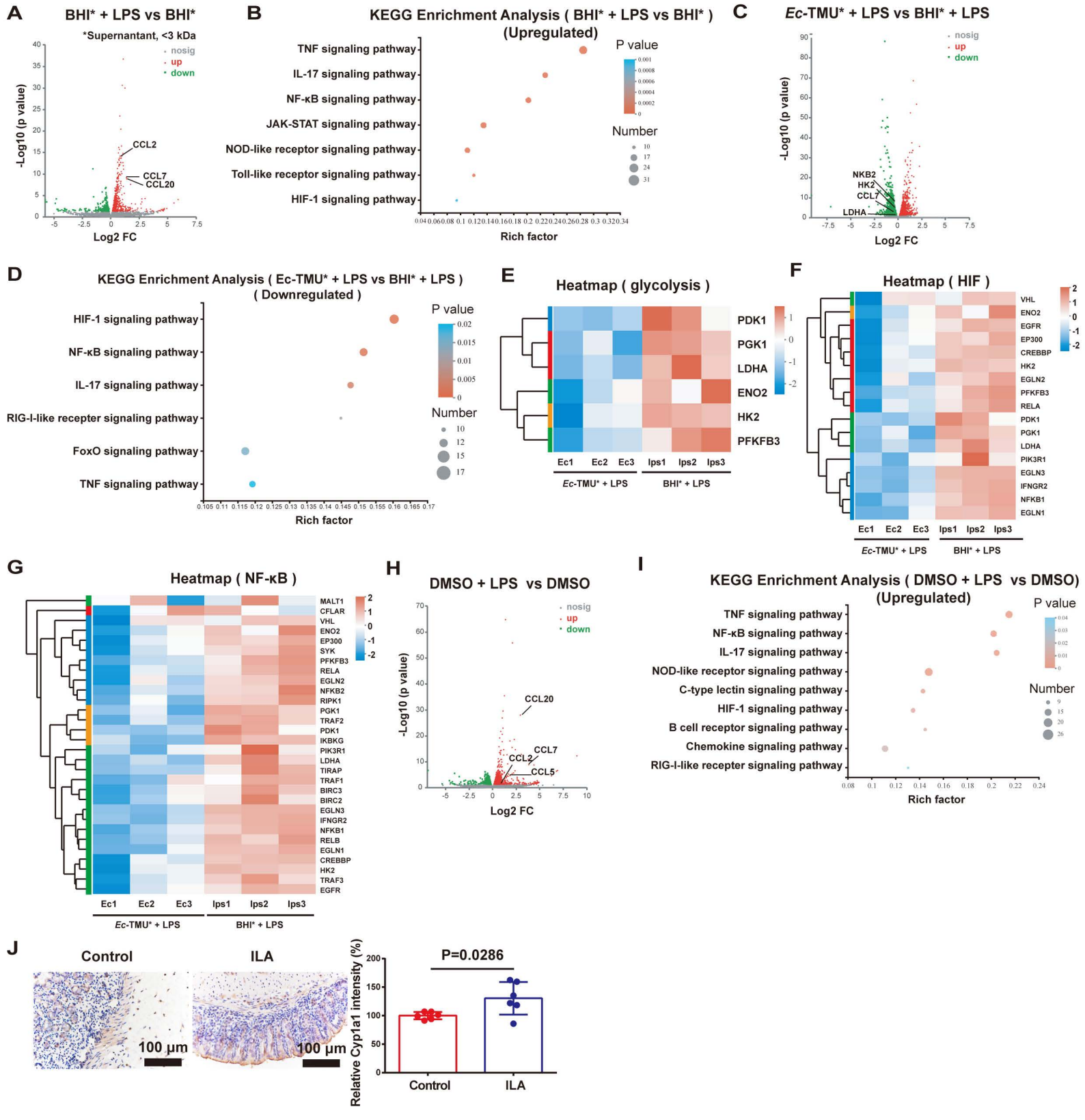
A
BHI* + LPS vs BHI*
*Supernantant, <3 kDa
nosig
up
down
-Log10 (p value)
Log2 FC
CCL2
CCL7
CCL20
B
KEGG Enrichment Analysis (BHI* + LPS vs BHI*)
(Upregulated)
TNF signaling pathway
IL-17 signaling pathway
NF-κB signaling pathway
JAK-STAT signaling pathway
NOD-like receptor signaling pathway
Toll-like receptor signaling pathway
HIF-1 signaling pathway
P value
Number
Rich factor
C
Ec-TMU* + LPS vs BHI* + LPS
nosig
up
down
-Log10 (p value)
Log2 FC
NKB2
HK2
CCL7
LDHA
D
KEGG Enrichment Analysis (Ec-TMU* + LPS vs BHI* + LPS)
(Downregulated)
HIF-1 signaling pathway
NF-κB signaling pathway
IL-17 signaling pathway
RIG-I-like recepter signaling pathway
FoxO signaling pathway
TNF signaling pathway
P value
Number
Rich factor
E
Heatmap (glycolysis)
PDK1
PGK1
LDHA
ENO2
HK2
PFKFB3
Ec1 Ec2 Ec3 Ips1 Ips2 Ips3
Ec-TMU* + LPS
BHI* + LPS
F
Heatmap (HIF)
VHL
ENO2
EGFR
EP300
CREBBP
HK2
EGLN2
PFKFB3
RELA
PDK1
PGK1
LDHA
PIK3R1
EGLN3
IFNGR2
NFKB1
EGLN1
Ec1 Ec2 Ec3 Ips1 Ips2 Ips3
Ec-TMU* + LPS
BHI* + LPS
G
Heatmap (NF-κB)
MALT1
CFLAR
VHL
ENO2
EP300
SYK
PFKFB3
RELA
EGLN2
NFKB2
RIPK1
PGK1
TRAF2
PDK1
IKBKG
PIK3R1
LDHA
TIRAP
TRAF1
BIRC3
BIRC2
EGLN3
IFNGR2
NFKB1
RELB
EGLN1
CREBBP
HK2
TRAF3
EGFR
Ec1 Ec2 Ec3 Ips1 Ips2 Ips3
Ec-TMU* + LPS
BHI* + LPS
H
DMSO + LPS vs DMSO
nosig
up
down
-Log10 (p value)
Log2 FC
CCL20
CCL7
CCL2
CCL5
I
KEGG Enrichment Analysis (DMSO + LPS vs DMSO)
(Upregulated)
TNF signaling pathway
NF-κB signaling pathway
IL-17 signaling pathway
NOD-like receptor signaling pathway
C-type lectin signaling pathway
HIF-1 signaling pathway
B cell receptor signaling pathway
Chemokine signaling pathway
RIG-I-like recepter signaling pathway
P value
Number
Rich factor
J
Control
ILA
100 μm
100 μm
P=0.0286
Relative Cyp1a1 intensity (%)
Control
ILA

Figure S10. *Ec*-TMU culture supernatant (<3 kDa) downregulates the HIF, NF-κB pathway and glycolysis in epithelial cells. (A-I) SW480 cells were stimulated with BHI, or LPS (10 μg/ml) in addition with BHI or *Ec*-TMU culture supernatant (750 μl, <3 kDa) for 3 h. (A and C) The volcano plot showing the upregulated and downregulated genes in SW480 cells treated with LPS, compared with BHI (A), or the upregulated and downregulated genes in SW480 cells treated with LPS plus *Ec*-TMU supernatant (<3 kDa), compared with LPS (C). (B and D) KEGG analyses of the upregulated pathways in SW480 cells treated with LPS, compared with BHI (B), or the upregulated pathways in SW480 cells treated with LPS plus *Ec*-TMU supernatant (<3 kDa), compared with LPS (D). (E-G) Heatmap depiction of gene expression involved in of glycolysis (E), HIF (F) and NF-κB (G) signaling pathway. (H) Volcano plot showing the upregulated and downregulated genes in SW480 cells treated with LPS, compared with PBS. (I) KEGG analysis showing the upregulated pathways in SW480 cells treated with LPS, compared with DMSO. (J) Representative CYP1A1 staining and quantitation in colon sections (in the model of Figure 5I). n=3 (A-I), n = 6 (J). Data are the mean ± SEM. Unpaired Student' s t test (J).

Table S1. Reagent and resource used in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-mouse CD45-PE-Cy7	eBioscience	Cat# 25-0451-82; RRID: AB_2734986
Anti-mouse CD11c-APC	Biolegend	Cat# 117309; RRID: AB_313778
Anti-mouse MHCII-PE	Biolegend	Cat# 107607 RRID: AB_313322
Anti-mouse CD103-PerCP/Cy5.5	eBioscience	Cat# 46-1031-82; RRID: AB_2573704
Anti-mouse CD4-APC-Cy7	Biolegend	Cat# 100414; RRID: AB_312699
Anti-mouse CD3-FITC	Biolegend)	Cat# 100204; RRID: AB_312661
Anti-mouse CD4-PE-Cy7	eBioscience	Cat# 25-0041-81; RRID: AB_469575
Anti-mouse CD25-PE	Biolegend	Cat# 102008; RRID: AB_312857
Anti-mouse CD45-eFlour 506	Invitrogen	Cat# 69-0451-82; RRID: AB_2637147
Anti-mouse CD11b-PE-Cy7	Invitrogen	Cat# 25-0112-81; RRID: AB_469587
Anti-mouse Ly6G-APC	Biolegend	Cat# 127614; RRID: AB_2227348
Anti-mouse F4/80-FITC	eBioscience	Cat# 11-4801-85; RRID: AB_2637192
Anti-mouse MHCII- Alexa Fluor 700	Biolegend	Cat# 107622; RRID: AB_493727
Anti-mouse CCR2-Brilliant Violet 421	Biolegend	Cat# 150605; RRID: AB_2571913
Anti-mouse CCR5- Brilliant Violet 506	eBioscience	Cat# 743697; RRID: AB_2741679
Anti-mouse Lin-FITC	Invitrogen	Cat# 22-7770-72; RRID: AB_2644066
Anti-mouse CX3CR1-PE/Dazzle	Biolegend	Cat# 149013; RRID: AB_2565697
Anti-mouse RORγt-APC	eBioscience	Cat# 17-6981-82; RRID: AB_2573254
Anti-mouse Foxp3-APC	Biolegend	Cat# 320014; RRID: AB_439750
Anti-mouse IL-17A-PE-Cy7	eBioscience	Cat# 25-7177-80; RRID: AB_10717952
Anti-mouse IFN-γ-PerCP/Cy5.5	Biolegend	Cat# 505821; RRID: AB_961361
CCL2 antibody	Proteintech	Cat# 66272-1-Ig
Alexa Fluor 594 - labeled second antibody	Proteintech	Cat# SA00013-4
Epcam antibody	Proteintech	Cat# 21050-1-AP
Alexa Fluor 488 - labeled second antibody	Proteintech	Cat# SA00013-2
CCL7 antibody	Bioss	Cat# bs-1987R
Muc2 antibody	Proteintech	Cat# 27675-1-AP
Ki67 antibody	Abcam	Cat# ab264429
Lgr5 antibody	Affinity Biosciences	Cat# DF2816
Caspase3 antibody	Cell Signaling Technology	Cat# 9664
Occludin antibody	Abcam	Cat# ab216327
p-IκBα antibody	Affinity Biosciences	Cat# AF2002
HIF1A antibody	Affinity Biosciences	Cat# AF1009
IκBα antibody	Abcam	Cat# ab32518
β -actin antibody	Abcam	Cat# ab6276
Chemiluminescent HRP Substrate	Merck Millipore	Cat# WBKLS

Cyp1A1 antibody	Affinity Biosciences	Cat# AF5312
IgG2a isotype control	BioXcell	Cat# BE0089
anti-mouse Ly6G	BioXcell	Cat# BE0075
HRP-labeled secondary antibody	Zsbio	Cat# PV-6001
Chemicals, Peptides, and Recombinant Proteins		
murine M-CSF	Peprotech	Cat# 315-02-50
Ampicillin	Sangon Biotech	Cat# A610028-0025
Metronidazole	Sangon Biotech	Cat# A600633-0025
Neomycin	Sangon Biotech	Cat# A610366-0025
Vancomycin	Sangon Biotech	Cat# A600983-0001
DSS	MP Biomedicals	Cat# 0216011090
tinidazole	Proteintech	Cat# CM00694
FITC-dextran	Sigma	Cat# 46944
PBS	Solarbio	Cat# P1020
LPS	Sigma	Cat# L2880
DMSO	Solarbio	Cat# D8372
DTT	Solarbio	Cat# D1070
Collagenase IV	Sigma	Cat# C5138
Percoll	GE	Cat# 17-0891-01
ILA	Med Chem Express	Cat# HY-113099
HPLA	Med Chem Express	Cat# HY-113219
5-MTP	Sigma-Aldrich	Cat# M4001
KynA	Med Chem Express	Cat# HY-100806
DCA	Sigma-Aldrich	Cat# 347795
CH-223191	Med Chem Express	Cat# HY-12684
BAY 11-7082	Med Chem Express	Cat# HY-10257
PX-478	Med Chem Express	Cat# HY-10231
oligomycin	Med Chem Express	Cat# HY-16589
Phorbol 12-myristate 13-acetate (PMA)	Med Chem Express	Cat# HY-18739
ML228	Med Chem Express	Cat# HY-10231

HiFiScript cDNA Synthesis Kit	CWBio	Cat# CW2569M
Ultra SYBR Mixture	CWBio	Cat# CW0957M
RIPA lysis buffer	Solarbio	Cat# R0020
Complete mini-EDTA-free protease inhibitor cocktail tablets	Roche	Cat# 11697498001
PhosSTOP™ Phosphatase inhibitors	Roche	Cat# 04906837001
OCT	Sakura	Cat# 4583
diaminobenzidine chromogenic substrate	Zsbio	Cat# ZLI-9017
Critical Commercial Assays		
E.Z.N.A.® soil DNA Kit	Omega Bio-tek	Cat# D4015-02
Bacterial Genomic DNA Extraction Kit	TIANGEN	Cat# DP302-02
ILA assay kit	Jiangsu Meimian Industrial	Cat# MM-46497M2
PBS and clodronate liposomes	Target Technology	Cat# CP-005-005
Foxp3/transcription factor staining buffer sets	eBioscience	Cat# 00-5523-00
Cell activation cocktail with Brefeldin A	Biolegend	Cat# 423303
Zombie NIRTM Fixable Viability Kit	Biolegend	Cat# 423105
fixation buffer	Biolegend	Cat# 420801
intracellular staining permeabilization wash buffer	Biolegend	Cat# 421002
Lactate assay kit	Sigma-Aldrich	Cat# MAK064
Experimental Models: Organisms/Strains		
C57BL/6J (WT)	Academy of Military Medical Science (China)	N/A
C57BL/6J (Germ-free)	GemPharmatech (China)	N/A
C57BL/6J (Ccr2-KO)	GemPharmatech (China)	N/A
E. coli K12	Beijing Dingguo Biotechnology Development Center (China)	N/A
SW480 cell line	ATCC	Cat# ccl-228
Oligonucleotides		
Mouse-*Ccl2* F: TTAAAAACCTGGATCGGAACCAA	Sangon Biotech	Custom made
Mouse-*Ccl2* R: GCATTAGCTTCAGATTTACGGGT	Sangon Biotech	Custom made
Mouse-*Ccl4* F: TTCCTGCTGTTTCTCTTACACCT	Sangon Biotech	Custom made
Mouse-*Ccl4* R:	Sangon Biotech	Custom made

CTGTCTGCCTCTTTTGGTCAG		
Mouse-*Ccl5* F:	Sangon Biotech	Custom made
GCTGCTTTGCCTACCTCTCC		
Mouse-*Ccl5* R:	Sangon Biotech	Custom made
TCGAGTGACAAACACGACTGC		
Mouse-*Ccl7* F:	Sangon Biotech	Custom made
GCTGCTTTCAGCATCCAAGTG		
Mouse-*Ccl7* R:	Sangon Biotech	Custom made
CCAGGGACACCGACTACTG		
Mouse-*Ccl20* F:	Sangon Biotech	Custom made
GCCTCTCGTACATACAGACGC		
Mouse-*Ccl20* R:	Sangon Biotech	Custom made
CCAGTTCTGCTTTGGATCAGC		
Mouse-*Cxcl10* F:	Sangon Biotech	Custom made
CCAAGTGCTGCCGTCATTTTC		
Mouse-*Cxcl10* R:	Sangon Biotech	Custom made
GGCTCGCAGGGATGATTTCAA		
Mouse-*Il6* F:	Sangon Biotech	Custom made
AGACAAAGCCAGAGTCCTTCAG		
Mouse-*Il6* R:	Sangon Biotech	Custom made
GAGCATTGGAAATTGGGGTAGG		
Mouse-*Il1β* F:	Sangon Biotech	Custom made
GGGCTGGACTGTTTCTAATGC		
Mouse--*Il1β* R:	Sangon Biotech	Custom made
CTTGTGACCCTGAGCGACC		
Mouse-*TNFα* F:	Sangon Biotech	Custom made
GATCGGTCCCCAAAGGGATG		
Mouse-*TNFα* R:	Sangon Biotech	Custom made
TTTGCTACGACGTGGGCTAC		
Mouse-*iNOS* F:	Sangon Biotech	Custom made
GTTCTCAGCCCAACAATACAAGA		
Mouse-*iNOS* R:	Sangon Biotech	Custom made
GTGGACGGGTCGATGTCAC		
Mouse-*Cxcl1* F:	Sangon Biotech	Custom made
TGGCTGGGATTCACCTCAAG		
Mouse-*Cxcl1* R:	Sangon Biotech	Custom made
CCGTTACTTGGGGACACCTT		
Mouse-*β-actin* F:	Sangon Biotech	Custom made
CACTGTCGAGTCGCGTCCA		
Mouse-*β-actin* R:	Sangon Biotech	Custom made
GACCCATTCCCACCATCACA		
Human-*Ccl2* F:	Sangon Biotech	Custom made
CAGCCAGATGCAATCAATGCC		
Human-*Ccl2* R:	Sangon Biotech	Custom made

TGGAATCCTGAACCCACTTCT		
Human-*Ccl4* F:	Sangon Biotech	Custom made
CTGTGCTGATCCCAGTGAATC		
Human-*Ccl4* R:	Sangon Biotech	Custom made
TCAGTTCAGTTCCAGGTCATACA		
Human-*Ccl5* F:	Sangon Biotech	Custom made
CCAGCAGTCGTCTTTGTCAC		
Human-*Ccl5* R:	Sangon Biotech	Custom made
CTCTGGGTTGGCACACACTT		
Human-*Ccl7* F:	Sangon Biotech	Custom made
ATGAAAGCCTCTGCAGCACT		
Human-*Ccl7* R:	Sangon Biotech	Custom made
GTCCTGGACCCACTTCTGTG		
Human-*Ccl8* F:	Sangon Biotech	Custom made
TGGAGAGCTACACAAGAATCACC		
Human-*Ccl8* R:	Sangon Biotech	Custom made
TGGTCCAGATGCTTCATGGAA		
Human-*Ccl20* F:	Sangon Biotech	Custom made
TGCTGTACCAAGAGTTTGCTC		
Human-*Ccl20* R:	Sangon Biotech	Custom made
CGCACACAGACAACTTTTTCTTT		
Human-*Cxcl10* F:	Sangon Biotech	Custom made
GTGGCATTCAAGGAGTACCTC		
Human- *Cxcl10* R:	Sangon Biotech	Custom made
TGATGGCCTTCGATTCTGGATT		
Human-*gapdh* F:	Sangon Biotech	Custom made
ACGGATTTGGTCGTATTGGG		
Human- *gapdh* R:	Sangon Biotech	Custom made
TGATTTTGGAGGGATCTCGC		
Human-*Hk1* F:	Sangon Biotech	Custom made
CGCAGCTCCTGGCCTATTAC		
Human-*Hk1* R:	Sangon Biotech	Custom made
CATGATTCACTTGCACCCGC		
Human-*Hk2* F:	Sangon Biotech	Custom made
CCTCGGTTTCCCAACTCTGC		
Human-*Hk2* R:	Sangon Biotech	Custom made
ACTGGTCAACCTTCTGCACT		
Human-*Pkm1* F:	Sangon Biotech	Custom made
AGAACTTGTGCGAGCCTCAA		
Human-*Pkm1* R:	Sangon Biotech	Custom made
GAGCAGACCTGCCAGACTC		
Human-*Ldha* F:	Sangon Biotech	Custom made
TTGTTGGGGTTGGTGCTGTT		
Human-*Ldha* R:	Sangon Biotech	Custom made

AGATATCCACTTTGCCAGAGACAA		
Human-*Eno2* F: AGCCTCTACGGGCATCTATGA	Sangon Biotech	Custom made
Human-*Eno2* R: TTCTCAGTCCCATCCAACTCC	Sangon Biotech	Custom made
Universal 16S rRNA F: GCCAGCAGCCGCGGTAA	Sangon Biotech	Custom made
Universal 16S rRNA R: AGGGTATCTAATCCT	Sangon Biotech	Custom made
Software and Algorithms		
FlowJo10.4	TreeStar	https://www.flowjo.com RRID: SCR_008520
Prism 6	GraphPad software	https://www.graphpad.com/ RRID: SCR_002798
Image Pro Plus	Media Cybernetics	http://www.mediacy.com/imageproplus/ RRID:SCR_007369
ImageJ	ImageJ	https://imagej.net/ RRID:SCR_003070
Deposited data		
16S rRNA gene sequencing	SRA	NCBI Sequence Read Archive (SRA): PRJNA946039 and PRJNA945177
RNA sequencing data	SRA	NCBI Sequence Read Archive (SRA): PRJNA946043 and PRJNA946045

Table S2. Characteristics of tinidazole users or patients with UC

Participants	Age (year)	Sex	ILA (ng/g)	Calprotein(μg/g)	CRP (mg/L)	Note
1	20	male	-	-	-	Healthy
2	23	female	-	-	-	Healthy
3	24	male	-	-	-	Healthy
4	21	female	-	-	-	Healthy
5	24	female	-	-	-	Healthy
6	25	female	-	-	-	Tinidazole, 1g/d, 3 d
7	25	male	-	-	-	Tinidazole, 1g/d, 3 d
8	21	female	-	-	-	Tinidazole, 1g/d, 3 d
9	20	female	-	-	-	Tinidazole, 1g/d, 3 d
10	23	male	-	-	-	Tinidazole, 1g/d, 3 d
11	26	female	-	-	-	Tinidazole, 1g/d, 3 d
12	21	male	-	-	-	Tinidazole, 1g/d, 3 d
13	24	female	-	-	-	Tinidazole, 1g/d, 3 d
14	13	female	88.6	576.2	65.4	UC
15	83	female	384	-	14.1	UC
16	22	male	58.2	585.7	24.7	UC
17	50	female	1100	-	1.8	UC
18	50	female	103	793.2	-	UC
19	52	male	73.7	294.2	-	UC
20	62	male	98.6	522.4	-	UC
21	40	male	608	69.4	2.9	UC
22	50	male	547	30	-	UC
23	65	female	2790	-	3.8	UC
24	70	male	2280	-	5.7	UC
25	64	female	1010	30	1.6	UC