SUPPORTING INFORMATION

A New Colitis Therapy Strategy by Using the Target Colonization of Magnetic Nanoparticle Internalized *Roseburia intestinalis*

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EXPERIMENTAL METHODS

Microscopy of Bacteria. For optical microscope, 20 μl of bacteria suspension was smeared, fixed, and stained with Gram Stain Kit (Solarbio G1060) or Crystal Violet Stain solution (Solarbio G1062) according to the manufacturer's instructions, respectively. The slides were subsequently examined under Olympus light microscope (DP 72) with an oil immersion lens. For SEM of whole cells, to be suitable for analysis, bacteria have to be fixed in shape and dehydrolysed prior to observation. In detail, MIONs-bacteria sediments were first washed with PBS for three times and then re-suspended in PBS containing 2% glutaraldehyde to fix their shape. Fixation took place about 1 h at room temperature, and the samples were then washed with PBS and serially dehydrolysed by rinsing with 30%, 50%, 70%, 80%, 100% ethanol (10 mins for each rinse). Then, the bacteria dispersions were deposited onto silica wafers, allowing the solvent to evaporate at room temperature followed by sputter-coated with a layer of gold (Au) and finally observed using a scanning electron microscope (ZEISS/Sigma 300). For TEM of whole cells, MIONs-bacteria sediments were first collected and incubated in 2.5% glutardialdehyde containing phosphate buffer (PBS, pH 6.0) overnight at 4 °C. Afterward, samples were rinsed several times in phosphate buffer and postfixed at room temperature for 1 h with 1% osmium tetroxide in phosphate buffer. After three washing steps in phosphate buffer, dehydration was performed with a graded acetone (50%, 70%, 90%, 100%, 100%, 10 mins for each rinse). Samples were then infiltrated in embedding solution with equivalent acetone for 12 hours at 37 °C and in embedding solution for 12 hours at 37 °C. Solidification was performed at 37 °C

overnight and 60 °C for 12 hours sequentially. After embedding the samples, 100 nm-ultrathin sections were cut with a diamond knife and mounted onto uncoated copper grids. TEM were taken by the TECNAI G2 F20 described above. Each experiment was repeated more than three times, and representative images are shown in the figures. Image rescaling and cropping were performed with Photoshop 9.0 software.

Supplementary Table 1

Composition of Medium

Component	Quantity
Processed Water	40 ml
Soybean-Casein Digest Broth	2.75% w/v
Yeast Extract	0.2% w/v
Animal Tissue Digest	0.05% w/v
Dextrose	0.2% w/v
Hemin	0.0005% w/v
Menadione	0.00005% w/v
Sodium Citrate	0.02% w/v
Thiols	0.1% w/v
Sodium Pyruvate	0.1% w/v
Saponin	0.26% w/v
Antifoaming Agent	0.01% w/v
Sodium Polyanetholsulfonate (SPS)	0.035% w/v

Viability of Bacteria. The viability of bacteria cells was investigated by staining with a combination dye method (LIVE/DEAD® *Bac*Light™ Bacterial Viability Kits, Molecular Probes, L13152). Briefly, the bacteria were cultured to logarithmic phase (~ 10^8 bacteria/mL) and centrifuged at 4,000 g/min at room temperature for 5 min (the setting was used throughout the entire protocol) to harvest the suspension cells. Then, the cell pellets were resuspended in 0.85% NaCl twice. 0.85% NaCl (for live bacteria) or 70% isopropyl alcohol (for killed bacteria and experimental control) was added to the pellets and incubated for 10 mins at room temperature. After that, pellet both samples and resuspend the pellets in 0.85% NaCl. Combine the samples with reagent (The final concentration of each dye will be 2 µM SYTO 9 stain and 18 µM propidium iodide), mix thoroughly, and incubate at room temperature in the dark for 15 minutes. The viable and nonviable cells can be distinguished under the fluorescence microscope and flow cytometry since the viable cells appear green while non-viable or membrane compromised cells appear red. All experiments were performed in triplicates.

Proliferation of Bacteria. Proliferation at different time point of the bacterial cells was assessed using MTT assay. Briefly, 100 µl cultured bacteria cells were diluted with 900 µl 0.85% NaCl. Then, 200 µl of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) solution (Sigma-Aldrich, 5 mg/mL) was added and cultivated at 37 °C for 1 hour. After incubation, the supernatant in each well was pipetted out, and 1 mL of dimethylsulfoxide (Sigma-Aldrich) was added to dissolve the purple/blue formazan. After gently shaken for 20 min in darkness, the absorbance was recorded

with a Microplate Reader (Biotek) at 510 nm. Three readings were accomplished for each specimen in each group.

Intracellular ROS Levels. For bacteria, the intracellular ROS levels were determined by Intracellular ROS Assay Kit (Beyotime, China) following the manufacturer's instruction. Briefly, the bacteria were cultured to logarithmic phase (~ 10^8 bacteria/mL) and centrifuged at 4,000 g/min at room temperature for 5 min (the setting was used throughout the entire protocol) to harvest the suspension cells. Then, the cell pellets were resuspended in DCFH-DA (10 μM), followed by 20 min of incubation at 37 ºC. After three washing steps in physiological saline, bacteria were detected by flow cytometry. For NCM460 cells, similar protocol was performed by Reactive Oxygen Species (ROS) Detection Assay Kit (BioVision).

Xylanase Activity Measurements. Cell-associated xylanase activity was determined by growing *R. intestinalis* cells in the medium containing 0.5% (w/v) xylan (Solarbio, X8163) for 15 h. Cells and wash fractions were harvested (4,000 g for 5 min at room temperature), resuspended in PBS to OD_{600}= 0.3 and xylanase activity was assayed using the 3,5-dinitrosalicylic acid (DNS) assay by NEX kit (Solarbio, BC2590). Next, samples were incubated for 5 min at 90 °C followed by absorbance at 540 nm (OD_{540}) measurement. Initial hydrolysis rates were determined by removing 1 ml aliquots every minute and quenching the reaction in 300 μl DNS reagent (50 °C, PH 6.0). Xylose was used as a standard.

Transwell Migration Assay. A transwell system (Corning, 3415) was applied to assess the migration of the bacterial cells. Briefly, the top and bottom chamber containing regular medium between which there are 3.0 μm pore filters, while bacterial cells were seeded into the bottom chamber. Naturally, settlement of the bacterial cells would be observed due to the gravity. In the experimental group, a magnetic field was settled above the top chamber. MTT assay was performed every 2 hours in the top chamber to quantity the number of bacteria.

Cytotoxicity Assays In Vitro. *In vitro* cytotoxicity assays were performed using the human colon epithelial cell line NCM460, which was obtained from the Cancer Research Institute of Central South University (Changsha, China). NCM460 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37 ˚C with 5% CO_2. NCM460 cells were cultivated in 96-well plates (0.7×10^3 cells/well) for 24 h. After incubation, the medium was removed and cells were incubated with fresh medium (control), 50 ug/ml of nanosuspension, or MIONs extract liquid (because the toxicity of free ions may be important). After the incubation periods, cells were washed and 100 μl of fresh culture medium, followed by 20 μl of MTT solution, was added to each well. After incubating with MTT solution for 3 h, 200 μl of dimethylsulfoxide was added to each well to dissolve the resulting formazan crystals. Each well was then repeatedly pipetted to completely dissolve formazan crystals, and the absorbance was measured at 540 nm using a 96-well plate

reader. Trypan blue (Solarbio, C0040) was also used to assess cell viability. Briefly, cells were centrifuged and resuspend the pellet in PBS. Then, mix 1 part of 0.4% trypan blue with 1 part of cell suspension and incubate ~3 min at room temperature. Finally, count the unstained (viable) and stained (nonviable) cells separately.

Finite Element Analysis of The NbFeB Magnet. The magnetic field simulation was performed using ansoft maxwell 16. Finite element analysis was performed on the NbFeB magnet (Changhong, China; diameter = 10 mm; height = 2 mm) to be used in subsequent rat studies. A density plot of the field lines was produced to show the shape of the field. The field magnitude along the z-axis directly above the magnet was calculated as well to show the decay of the field with distance.

Supplementary Table 2

Disease Activity Index

Weight loss (%)	stool consistency	Haemoccult	Score
0	normal	normal	0
5-10	soft	OB positive	1
10-15	very soft	red	2
15-20	diarrhea	dark red	3
>20	gross diarrhoea	gross bleeding	4

OB, occult blood.

Supplementary Table 3

Macroscopic scoring of Colitis

Macroscopic injury	Score
Normal appearance	0
Focal hyperaemia, no ulcers	1
Linear ulcers without hyperaemia and bowel wall thickening	2
Linear ulcers with inflammation at one site	3
Two or more sites of ulceration and inflammation	4
Major sites of damage extending >1 cm along the length of the colon	5
Major sites of damage extending >2 cm along the length of the colon (The score was increased by 1 for each additional cm of involvement)	6-10

Histology. For optical microscope examination, tissue samples from the distal colon of each animal were fixed in 4% buffered paraformaldehyde, dehydrated in the cold with shaking by means of a set of alcohols of increasing concentration, from percent 1 to absolute alcohol. At the end, the tissues were introduced successively in xylol, xylol/paraffin 50%, finishing in paraffin at 60 °C. Blocks were formed and then sectioned with a Leica Ultracut (Leica Microsystems, Wetzlar, Germany), hydrated and stained with hematoxylin–eosin and subsequently examined under Olympus light microscope (DP 72). Inflammation grading was carried out by two independent blinded observers, and lesions were analyzed using histological scoring criteria

(**Supplementary table 4**). Image rescaling and cropping were performed with Photoshop 9.0 software.

Supplementary Table 4

Histological scoring of colitis

Parameter	Score
Percentage of area involved	0=normal
	1=<10%
Crypt loss	2=10%
	3=10–15%
	4=15–50%
Erosions	0=intact epithelium
	1=involvement of the lamina propria
	2=involvement of the submucosa
	3=transmural ulceration
Number of follicle aggregates	0=absent
Oedema	1=weak
Infiltration of mononuclear and	2=moderate
polymorphonuclear cells	3=severe

Measurement of MPO. Neutrophil infiltration *in vivo* was assessed by measuring the granulocyte-specific enzyme Myeloperoxidase (MPO) in tissue. Intestinal MPO

activity was determined using MPO Detection Kit (Nanjing Jiancheng Bioengineering Institute, China). Briefly, the tissue was thawed, weighed, homogenized in 1 ml of 50 mmol/L potassium PBS containing 0.5% hexadecyltrimethylammonium hydroxide and centrifuged at 12,000 r/min at 4 °C for 20 min. MPO activity was assessed by measuring the H_2O_2-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine. One unit of enzyme activity was defined as the amount of MPO that caused a change in absorbance of 1.0/min at 460 nm and 37 ˚C. MPO activity was expressed as units per gram of total tissue (unit/g tissue).

Western Blotting Analysis. In Western Blot analysis, snap-frozen colon tissue sample were lysed in RIPA buffer (65 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) with proteinase inhibitors cocktail (Roche, IN). After incubated for 30 min at 4 °C with gentle agitation, the mixture was centrifuged for 10 min at 12,000 r/min. Protein concentration was measured using the Pierce BCA Protein Assay Kit (Thermo Scientific). Equal amounts of protein (50 μg) were heated at 95 °C for 5 min and separated in 10% Bis-Tris NuPage gels for SDS–PAGE. Thereafter, proteins were transferred onto PVDF membranes (Millipore), Membranes were blocked in 5% skim milk in TBS-T (25 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% Tween-20) for at least 1 hour and then incubated overnight with the appropriate antibodies : *Il-10* (Abcam, ab9969, 1:2,500), ZO-1 (Proteintech, 21773-1-AP, 1:500), Occludin (Bioss, bs-10011R, 1:500), Claudin-1 (Bioss, bs-1428R, 1:500), *Il-1β* (Bioss, bs-0812R, 1:500), *Il-6* (Proteintech, 21865-1-

AP, 1:500), *Tnf-α* (ABclonal, A11534, 1:1000), GAPDH (Proteintech, 60004-1-Ig, 1:5000). Membranes were then washed three times with TBS-T, incubated with the appropriate secondary antibody (1:5,000 dilution, proteintech) for 1 h at room temperature, and washed three times with TBS-T as above. Immunoreacting bands were visualized using a chemoluminescent method (ECL, Merck Millipore, Germany). Protein expression levels were quantified using Image Lab software and normalized to the levels of the GAPDH, which was used as a loading control. The lysates were run in parallel on multiple gels and probed for proteins of interest on parallel blots.

Rt-qpcr. For rt-qPCR assay, tissue sample of the distal 8 cm colon in rats was collected and stored in cold storage freezer (Forma™ 900) with tissue stabilizer (vazymeTM) after sacrificed. RNA was extracted from tissue samples with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocols and then be quantified by NanoDrop 2000. Complementary DNA (cDNA) was prepared by the protocols of Reverse Transcription kit (Thermo Fisher Scientific). In brief, 2 μg of total colon RNA was added into a sterile, nuclease-free tube on ice, with 1 μl Oligo (dT)18 primer, and deionized water total to 12 μl. After incubated at 65 °C for 5 min, 4 μl 5x Reaction Buffer, 1 μl RiboLock RNase inhibitor (20 U/μl), 2 μl dNTP Mix (10mM), and 1μl RevertAid M-MuLV RT (200 U/μl) were added into the reaction system. Then the 20μl mixture was incubated at 42 °C for 60 minutes, followed by heating at 70 °C for 5 min to finish the cDNA synthesis. Quantitative PCR was performed with a SYBR Green qPCR kit (Takara, Otsu, Japan) and an iCycler system (Bio-Rad) with the sets of

primers described in Supplementary Table 5. The rt-qPCR assay was performed in the presence of 10.0 µl 2 x ChamQ Universal SYBR qPCR Master Mix, 0.4 µl each forward and reverse primers (10 µM), 0.4 µl Complementary DNA (cDNA), and 8.8 µl deionized water in total volume of 20 µl. The PCR conditions were as follows: one cycle of 95 °C for 30 sec, followed by 40 two-temperature cycles of 95 °C for 5 sec and 60 °C for 30 sec. The primer sequences used in this study are shown in **Supplementary Table 5**. All reactions were performed in triplicate according to the manufacturer's recommended thermocycling conditions. The relative expression of target gene mRNA was calculated by 2-ΔΔCt method with normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Supplementary Table 5

Nucleotide sequences of primers used in RT-qPCR and qPCR

Gene	(5' –3')	Reverse (5'-3')
Il-1β	TGCTGATGTACCAGTTGGGG	CTCCATGAGCTTTGTACAAG
Il-6	CTGCTCTGGTCTTCTGGAGT	AGAGCATTGGAAGTTGGGGT
Il-10	TGGACAACATACTGCTGACAG	GGTAAAACTTGATCATTTCTGACAAG
Tnf-α	AAATGGGCTCCCTCTCATCAGTTC	TCTGCTTGGTGGTTTGCTACGAC
Ri	GCGGTRCGGCAAGTCTGA	CCTCCGACACTCTAGTMCGAC
16srRNA	AGAGTTTGATCCTGGCTCAG	TACGGCTACCTTGTTACGACTT

GAPDH	ACAGCAACAGGGTGGTGGAC	TTTGAGGGTGCAGCGAACTT

Cytokine Measurement. Blood was centrifuged for 10 min at 3,000 g at 4 °C after collected and the serum stored at -80 °C. *Tnf-α* (CRE0003), interleukin (*Il*) 6 *Il-1β* (CRE0006), *Il-6* (CRE0005), *Il-10* (CRE0007) was measured using a standard enzyme-linked immunosorbent assay (Beijing 4A Biotech Co., Ltd) according to manufacturer recommendations.

Immunohistochemistry. For immunochemistry, 4-μm-thick sections were cut from paraffin-embedded blocks. The sections were deparaffinized, dewaxed, and rehydrated. Antigen retrieval was performed in 10 mM sodium citrate buffer (pH 6.0) and blocking with protein block serum-free solution. Slides were then incubated overnight in primary antibody diluted in antibody diluent (*Il-1β* (Bioss, bs-0812R), *Il-6* (proteintech, 21865-1-AP), *Il-10* (Abcam, ab9969), *Tnf-α* (ABclonal, A11534), ZO-1 (proteintech, 21773-1-AP), Occludin (Bioss, bs-10011R), Claudin-1 (Bioss, bs-1428R)). Endogenous peroxidase was blocked with 3% hydrogen peroxide. Polymer-based secondary antibodies (Goodbio Technology, Wuhan, China) were used for detection with 3,3'-diaminobenzidine (DAB; Mai New Biotechnology Development Company, Fuzhou, China). Images were captured using ImageScope software.

Quantification of Intestinal Permeability. A 5-cm-long segment of colon was excised in rats *via* a midline laparotomy incision under deep isoflurane anaesthesia. The colonic

segment was immediately gently washed with PBS (PH 6.0), and one side of intestine was ligated. Next, 200 μl of fluorescein isothiocyanate (FITC)–dextran (FD4, 40mg/ml; average mol wt 3,000-5,000; Sigma-Aldrich) was applied to intestinal lumen, and another side was ligated. The intestinal pouch was shaking gently in 20 ml of PBS at 37°C for 60 min. Permeability of intestinal wall was evaluated ex vivo by measuring the leaked amount of FITC-dextran outside of the intestinal pouch.

Fluorescence In Situ Hybridization. FAM(488)-tagged 5'-TCAGACTTGCCGYACCGC-3' was used to probe the presence of *R. intestinalis*, and the FISH was performed as previously described. Briefly, Tissue sections 5 μm thick were cut at −20 °C and mounted on charged slides. The glass slides were placed in a warm oven at 62 °C for 120 min to help the section adhere to the slide. Then, deparaffinize the slides by running them through xylene to alcohol shortly before hybridization: 2× 15-min incubations in xylene, 2× 5-min incubations in absolute ethanol, 1× 5-min incubations in 85% ethanol, 1× 5-min incubations in 75% ethanol, and the slides were washed by deionized water; all steps can be carried out at RT. We changed the ethanol and xylene solutions every 40 slices containing intestinal contents to prevent fecal bacteria floating in the fluid and being found on the slices. Thereafter, the slices were heated once more in a warm oven at 50 °C for 25 min, then we demarked the area of the hybridization with a PAP-PEN and air-dry. Protease K (20 μg/ml) was added to incubate the slices for 25 minutes at 37 °C. We then washed the slices with PBS for 3×5 min and incubated the slices with prehybridization buffer (50 (v/v)

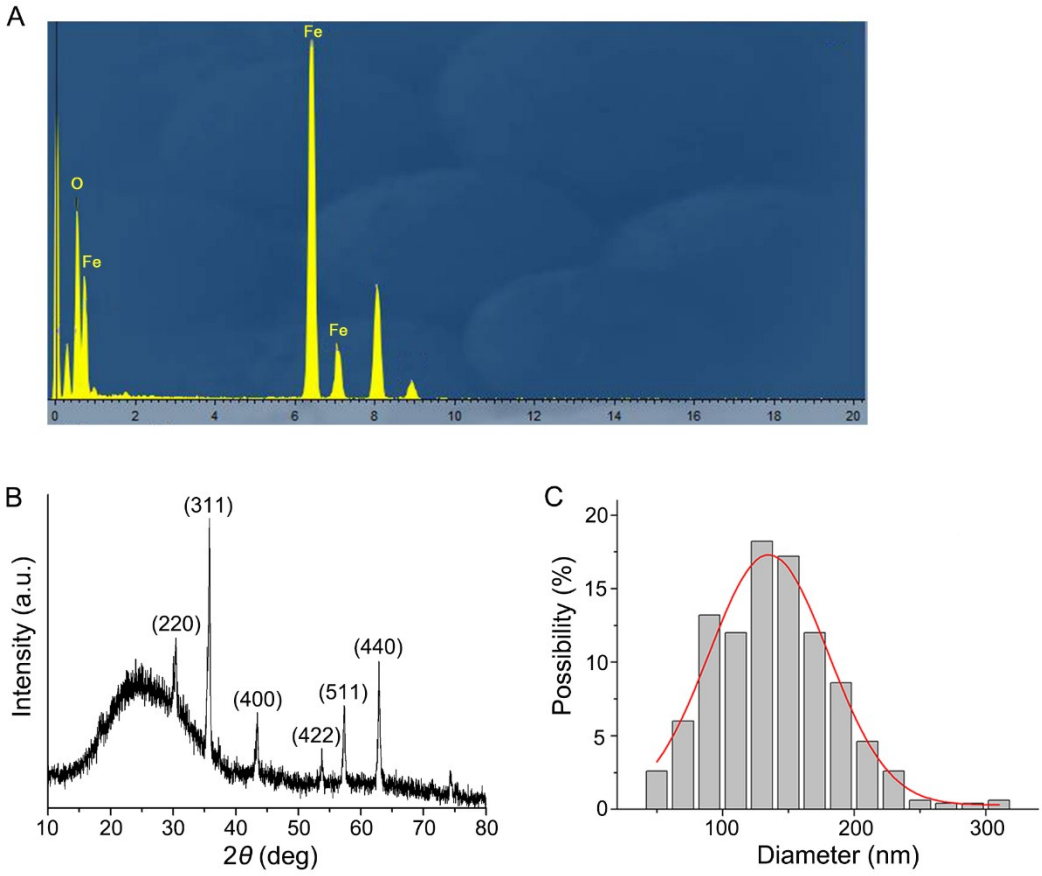
formamide, 0.1% (v/v) tween, 1× SSC, PH 6.0) for 1h at 37 °C. Subsequently, the slices were hybridized overnight at 37 °C in hybridization chambers with the oligonucleotide probe (final concentration of 8 ng/μl FISH probe solution of 30% formamide, 0.9 mol/L sodium chloride, 20 mmol/L Tris, pH 7.5, and 0.01% sodium dodecyl sulfate). Tissue sections were then washed for 10 min at 37 °C in wash buffer (2× SSC, 10% formamide), 2× 5min at 37 °C in wash buffer (1× SSC, 10% formamide), and 10 min at RT in wash buffer (0.5× SSC, 10% formamide). After that, the slices were dried at 46 °C, mounted with DAPI mount media for 8 minutes, and imaged using a NIKON DS-U3 Spectral Confocal Laser Scanning Microscope System by NIKON ECLIPSE CI. Acquired images were analyzed using Caseviewer and photoshop.

Qpcr. For qPCR analysis of *R. intestinalis* 16S rRNA (Supplementary Table 5), the genomic DNA of fecal or colon tissues was isolated by Trelief ™ Plant Genomic DNA Kit (TSINGKE, Beijing). In brief, identical tissue homogenization was undertaken prior to DNA extraction. Then, 400 μl of mixture was incubated at 65 °C for 30 min followed by centrifuged at 4 °C for 5 min at 12,000 × g. The supernatant was collected, blended with ethanol, and the extracted DNA was dissolved in 100 μl of TE (Tris-EDTA) buffer by silica column. DNA was quantified by NanoDrop 2000 and dilution to 400 ng/μl with deionized water. qPCR was carried out with the same system as above. The absolute abundance of *R. intestinalis* was measured by a standard curve surrounding the number of *R. intestinalis* cells and CT of *R. intestinalis cluster (16 S)* based on our unpublished data after normalization, that is, Quantity = 10^ ((CT –

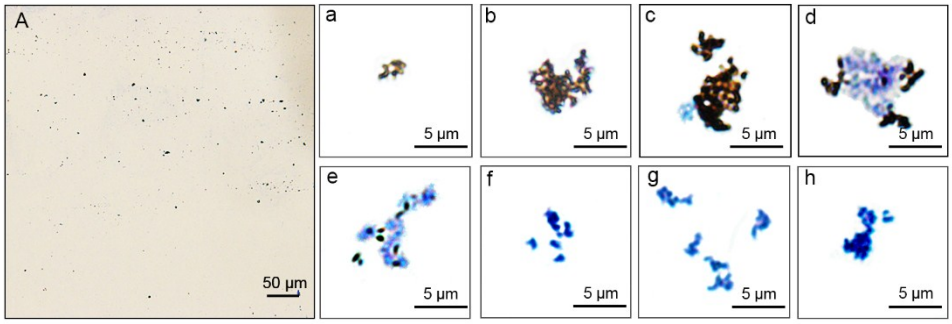
42.614) / -2.978).

Fecal Microbiome Analysis. Fecal samples were collected for 16S sequencing. DNA extraction, amplification of the V4 region of the 16S ribosomal RNA gene, and 2 x 300 bp sequencing on an Illumina MiSeq platform according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database. Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.9.1). Sequence depth ranged from 414,685 to 717,724. Alpha diversity metrics (i.e. bacterial diversity within a sample) and beta diversity (differences in composition across samples) were calculated using data rarefied to 414,685 sequences. Alpha diversity metrics included Rank-abundance, Chao1, and Shannon index. Beta diversity was calculated using unweighted UniFrac and visualized by principal coordinates analysis. Operational Taxonomic Units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU123)16S rRNA database using confidence threshold of 70%. The taxonomy of each ITS gene sequence was analyzed by Unite Classifier (https://unite.ut.ee/index.php).

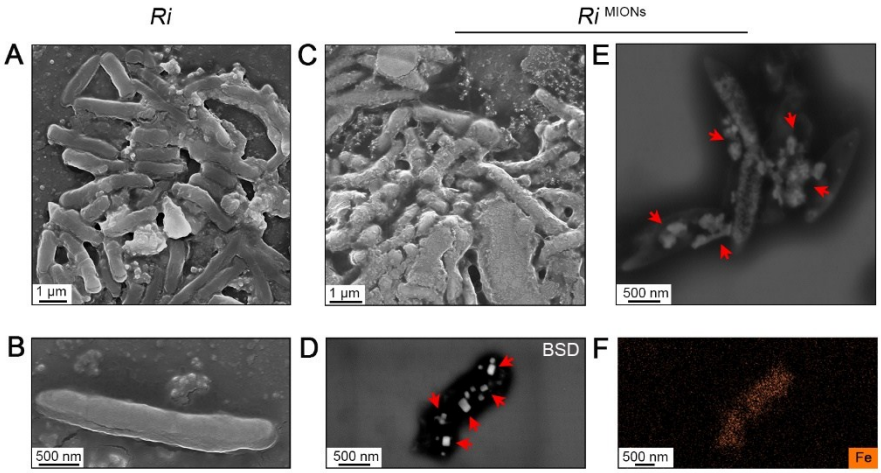
SUPPLEMENTARY FIGURE



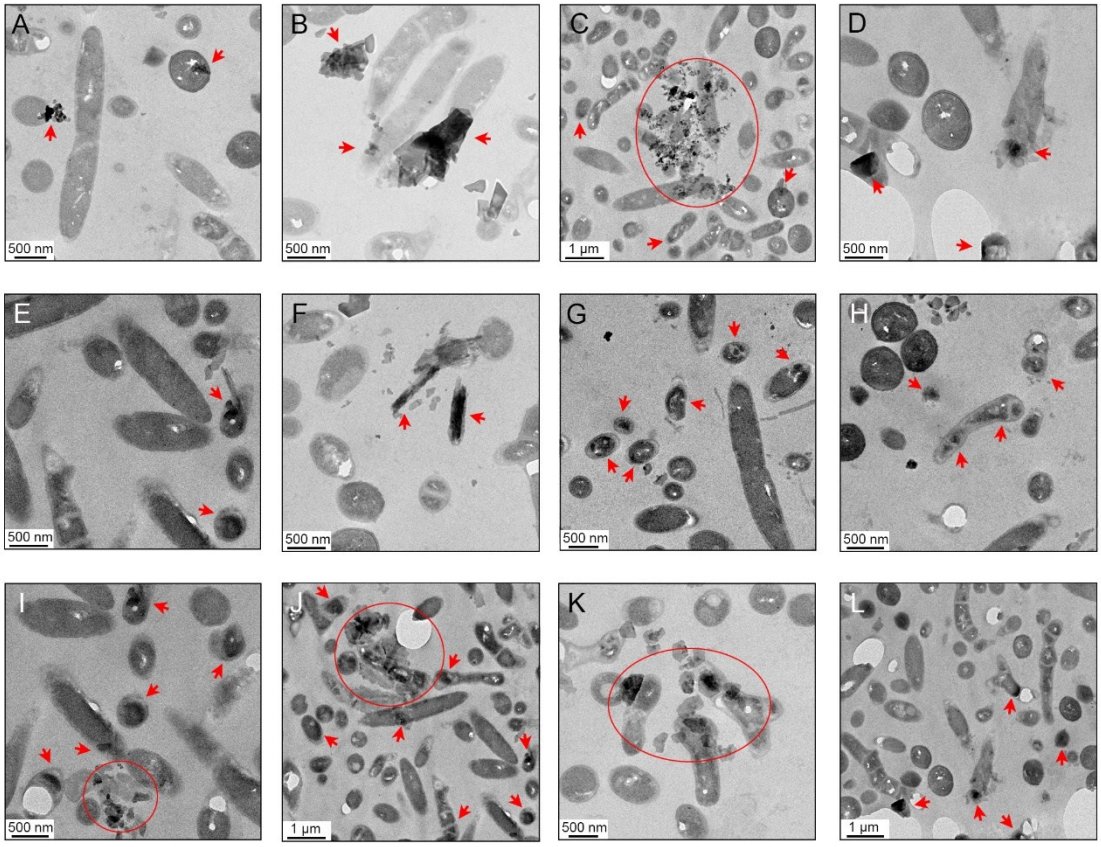
Supplementary Figure 1. Characterization of MIONs. (A, B) Energy-dispersive X-ray spectroscopy (A) and X-ray diffraction (B) confirm the nature of the clusters. (C) Size distributions of the samples.



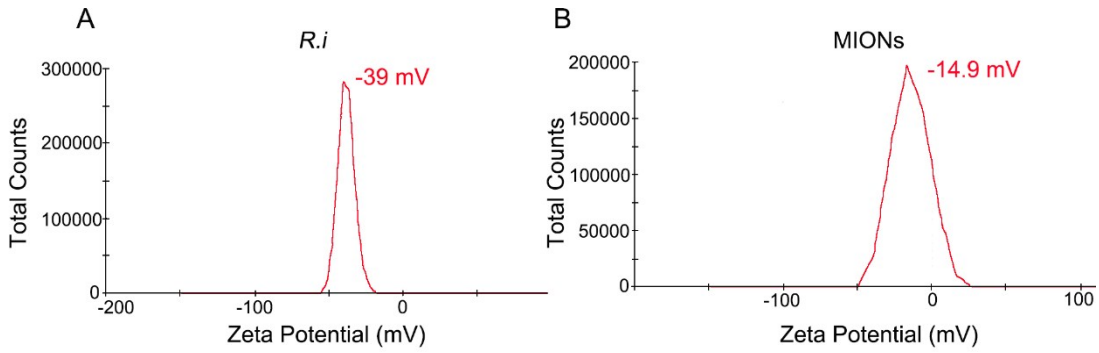
Supplementary Figure 2. Microscopic observation of MIONs. Representative microscopic observation of MIONs after crystal violet staining.



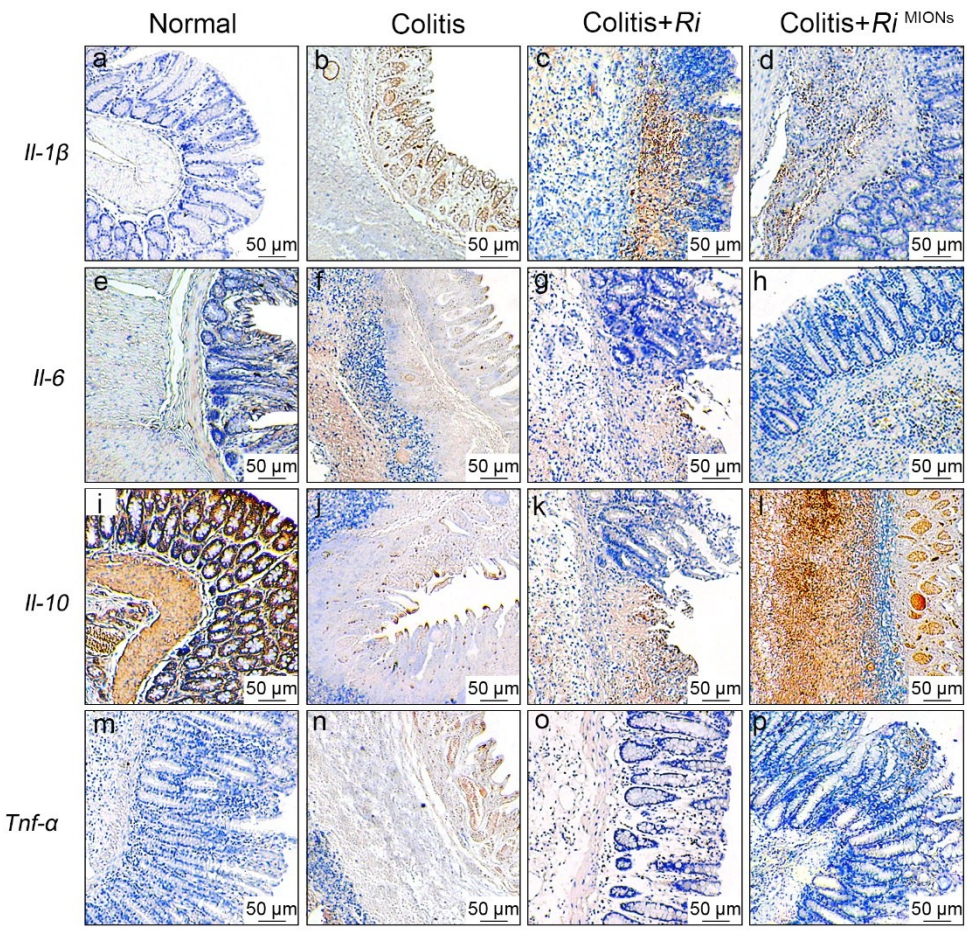
Supplementary Figure 3: SEM observation of *R. intestinalis* and *R. intestinalis*^{MIONs}. (A-E) Representative observation of *R. intestinalis* (A-B) and *R. intestinalis*^{MIONs} (C-E) Demonstrated by SEM images. (F) Single-element mapping of iron. Red arrows indicate MIONs attached to or internalized by *R. intestinalis*. SEM, scanning electron microscope.



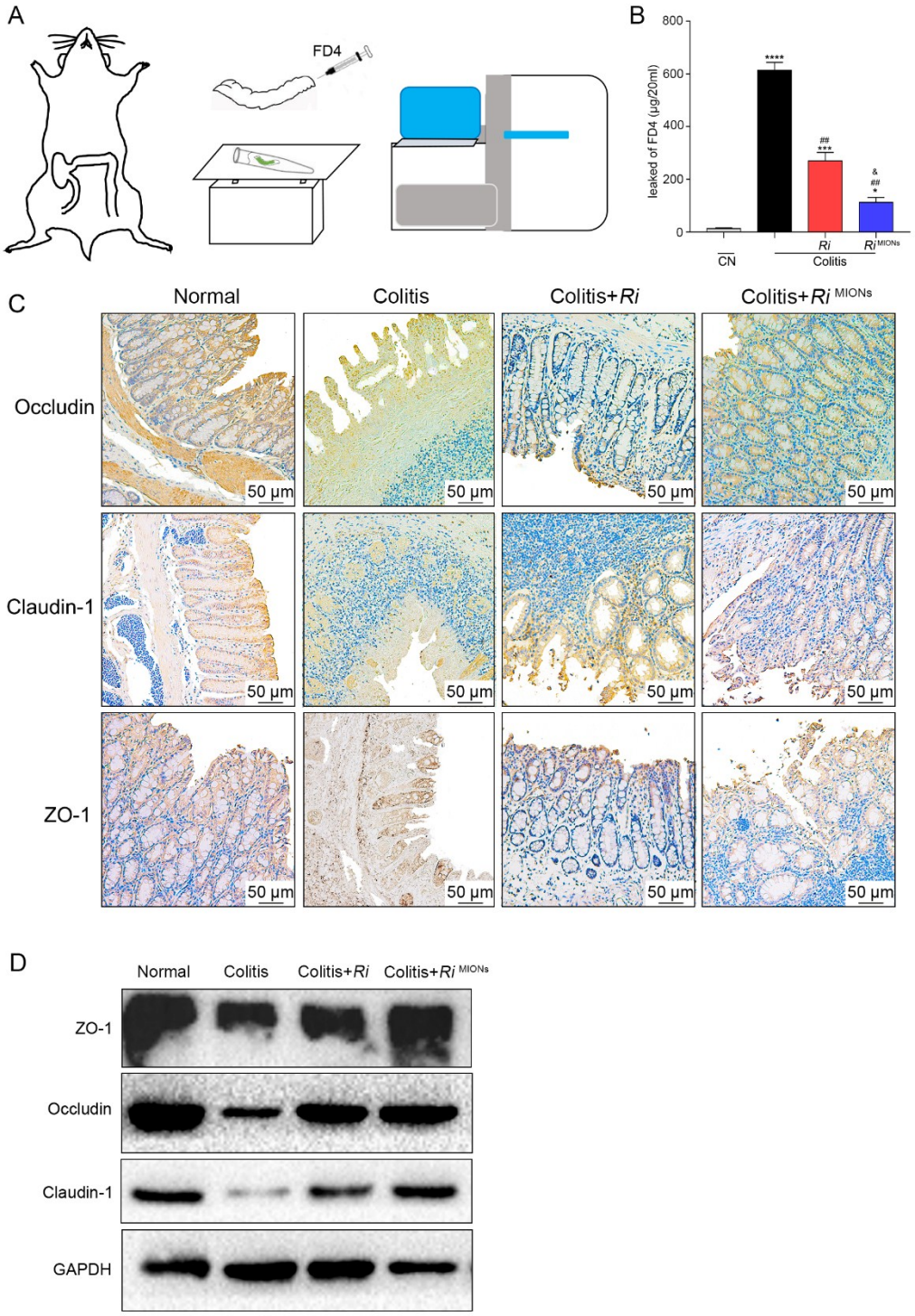
Supplementary Figure 4. TEM observation of *R. intestinalis*^{MIONs}. Representative observation of *R. intestinalis*^{MIONs} (A-L) demonstrated by TEM images. Red arrows or circles indicate MIONs attached to or internalized by *R. intestinalis*. TEM, transmission electron microscopy.



Supplementary Figure 5. Zeta potential results from *R. intestinalis* (A) and MIONs (B). The medium of *R. intestinalis* is H_2O, while the medium of MIONs is ethanol. The data showed in the figure are the mean value of three independent analysis.

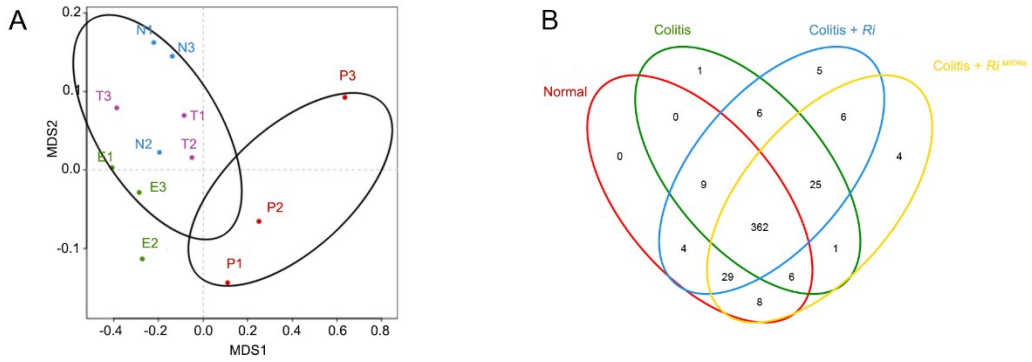


Supplementary Figure 6: *R. intestinalis*^{MIONs} regulated cytokines expression in colon tissues. Representative images of IHC (brown) staining of colon tissue from each indicated condition and were stained with *Il-1β* (a-d), *Il-6* (e-h), *Il-10* (i-l), and *Tnf-α* (m-p) antibody. IHC, immunohistochemistry; *Il*, interleukin.



Supplementary Figure 7. *R. intestinalis*^{MIONs} enhanced epithelial permeability of the colon tissues. (A, B) *In vivo* assessment of colonic epithelial permeability, as detailed in materials and methods, after 4 kDa FITC-dextran injection to the isolated intestinal cavity (n=3). (C, D) Representative images of IHC (brown) staining (C) of tight

junction comprises of proteins including Occludin, Claudin-1, and ZO-1 in colonic biopsy samples from each indicated condition and western blot (D) and. Data are representative of 3 independent experiments and shown in averages ± SEM. Statistical analysis was performed with one-way analysis of variance (ANOVA), followed by Tukey multiple comparison test. $^{*}P < .05$, $^{***}P < .001$, $^{****}P < .0001$ versus normal control; $^{\#\#}P < .01$ versus colitis control; $^{\&}P < .05$ versus *R. intestinalis* administrated group. FITC, fluorescein isothiocyanate; IHC, immunohistochemistry; ZO-1, Zonula occludens-1.



Supplementary Figure 8. *R. intestinalis*^{MIONs} supplementation rescues the composition of gut microbiota. (A) Nonmetric multidimensional scaling analysis on OTUs (n = 3 rats per group). Each symbol represents a single sample (from 1 rat) and is colored according to group. N1-3 represent normal group; P1-3 represent positive control group, that is, colitis group; E1-3 represent experimental group (colitis + *R. intestinalis*); and T1-3 represent treatment group (colitis + *R. intestinalis*^{MIONs}). (B) The Venn diagram shows that OTUs were overlapped in each group. OTUs, Operational taxonomic units.