Supplemental Digital Content 1

TABLE. The baseline characteristics of all CD patients recruited

	HC (n = 20)	Active CD (n = 20)	*P*
Sex			0.74
Male	9	7	
Female	11	13	
Age, mean ± SD, yr	31.5 ± 5.5	29.3 ± 6.3	0.260
Duration, mean ± SD, yr	—	3.5± 1.4	—
Extent of disease*			—
L1	—	0	
L2	—	12	
L3	—	8	
L4	—	0	
Behavior*			—
B1		6	
B2		11	
B3		0	
Bx+P		3	
HBI, mean ± SD	—	13.9±5.7	—
Current smoker	2	1	—
Surgery	—	3	—
Family history of IBD	0	0	—
Extra-intestinal symptoms	—	4	—
Therapy			
5-ASA	—	6	—
Corticosteroid	—	12	—
Immunosuppressant(AZA, 6MP, MTX)	—	11	—
Infliximab	—	4	—
Biopsy sites			—
Ascending colon	4	8	—
Transverse colon	6	3	—
Descending colon	7	7	—
Sigmoid colon	3	2	—

*: According to Montreal Classification 2005.
HBI, Harvey-Bradshaw Index; AZA, azathioprine; 6MP, 6 mercaptopurine; MTX, methotrexate.

Polymerase Chain Reaction (PCR)

First, human colon biopsy samples, harvested Caco2 and T84 cells and mice colon samples (2×2 mm) were kept in RNALater (Qiagen, Hilden, Germany) for 24

hours at 4℃ and another 24 hours at -20℃ to prevent RNA degradation. Trizol (Thermo Fisher Scientific, Waltham, MA, USA) was added to the specimens to extract total RNA, according to the manufacturer's instruction.

Cell Culture

Cells were grown in 6-well plates (Corning, New York, NY, USA) for PCR assays, and in 12-well Costar Transwell plates (pore size: 0.4μm, cell growth area: 1.12 cm^2) (Corning, New York, NY, USA) for transepithelial electrical resistance (TEER) assays. Caco2 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Hyclone, Logan, UT, USA) with 20% fetal bovine serum (FBS) (Hyclone). T84 cells were grown in DMEM/F12 medium (Hyclone) with 10% FBS. Cells were kept at 37°C with 5% CO2 supplement in a humidified cell culture incubator (Thermo Fisher Scientific).

Transepithelial Electrical Resistance (TEER) Assay

TEER values of Caco2 and T84 cells in Transwell plates were determined with Millicell ERS-2 (Millipore, Billerica, MA, USA) following the manufacture's instruction. The electrical resistance of empty wells with only medium (Ro) and wells with medium and cells (Rt) were measured respectively. The units of Ro and Rt were Ω. TEER was calculated using the equation TEER = (Rt-Ro) ×1.12 cm^2, and its unit is $\Omega \cdot cm^2$.

Induction of Colitis in Mice

Colitis induction was performed and TNBS presensitization solution and TNBS/ethanol enema solution were prepared according to a standard protocol, with slight modifications by adding an additional TNBS/ethanol enema on day 5 to induce a more sustained and prolonged inflammation in murine colon. Briefly, on day -6, mice in TNBS group and anti-TNF group were sensitized with by application of 150 μL of TNBS presensitization solution to the shaved dorsal skin, while mice in NC group were treated with presensitization solution without TNBS. On day 0, mice were fasted to chow but had free access to water for 24 hours. On day 1 and 5, experimental colitis in TNBS group and anti-TNF group was induced by enema of 100μL of TNBS/ethanol enema solution, while mice in NC group were treated with saline enema. Mice were anesthetized by intraperitoneal injection of 0.15 mL of 50 g/L chloral hydrate and then administered TNBS/ethanol or saline slowly via a sterile 3.5 F catheter inserted 40 mm proximal to the anus. Mice were kept with the head down in a vertical position for additional 60 seconds to ensure sufficient distribution of TNBS/ethanol or saline in the colon.

Histological Evaluation

The colon was cut open with a surgical scissors longitudinally along the mesenteric border to examine its gross changes in the appearance of the mucosa. Then colon was cut into sections of 0.5 cm, fixed in 10% formalin, embedded in paraffin, sectioned, dewaxed and stained with hematoxylin and eosin. Stained sections were

examined for evidence of colitis using an established histological colitis scoring system (Table 3 Supplemental Digital Content 2). This score ranges from 0 to 14, including 4 parameters: inflammation severity (0-3), inflammation extent (0-3), crypt damage (0-4) and percent of colon involved (0-4).

Intestinal Permeability

In the present study, Fluorescein isothiocyanate (FITC)-Dextran 4000 (Sigma-Aldrich) test was employ to evaluate the colonic permeability in mice. Briefly, mice were anaesthetized and 100 μL of 25 mg/mL FITC-Dextran solution was delivered via rectal enema, quite similar to the induction of colitis with TNBS/ethanol. Mice were kept with the head down in a vertical position for additional 60 seconds to ensure sufficient distribution of FITC-Dextran in the colon as well. Then mice were sacrificed by cervical dislocation. Blood was harvested from their portal veins and light protected. 0.1 mL of blood was added into 1.9 mL 50mM Tris solution (pH 10.3, with 150mM NaCl) (Beyotime, Beijing, China) and then centrifuged (10 minutes, 4000 rpm, 4 ℃). The supernatant was collected and added into a 96-well plate (Corning, New York, NY, USA). The analysis for the FITC-Dextran concentration was carried out with the fluorescence spectrophotometer Multiskan GO (Thermo Fisher Scientific) at an excitation wave length of 480 nm and an emission wave length of 530 nm.