Triggering receptor expressed on myeloid cell 1 activation attenuates colitis via modulating microbiome and enhancing barrier function

Dong Hyuk Seo

Department of Medical Science

The Graduate School, Yonsei University

Triggering receptor expressed on myeloid cell 1 activation attenuates colitis via modulating microbiome and enhancing barrier function

Directed by Professor Jae Hee Cheon

The Master's Thesis
submitted to the Department of Medical Science
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Dong Hyuk Seo

December 2017

This certifies that the Master's Thesis of

Dong Hyuk Seo is approved

Thesis supervisor : Jae Hee Cheon

Thesis Committee Member#1 : Jeon Soo Shin

Thesis Committee Member#2 : Seung Won Kim

The Graduate School

Yonsei University

December 2017

ACKNOWLEDGEMENTS

I would like to express the deepest gratitude to Professor Jae Hee Cheon who gave the opportunity to perform this project and under his guidance that I gained valuable advice and technology for this study.

I am also grateful to Dr. Seung Won Kim for providing guidance and presenting various clues to analyze results and solve problems to complete this thesis.

Lab members for their support and cooperation during my degree, thank you.

Finally, I would like to thank the members of my family who shared their support, morally, financially and physically.

2017. 12

Dong Hyuk Seo

TABLE OF CONTENTS

ABSTRACT 1

Ⅰ. INTRODUCTION 3

Ⅱ. MATERIALS AND METHODS 5

1. Animal model and assessment 5
2. Evaluation of disease activity index 6
3. Histology and immunohistochemistry 6
4. Cell culture and treatment 6
5. Flow cytometry 7
6. Neutrophil and monocyte depletion 7
7. Bone marrow-derived neutrophils 8
8. Bone marrow-derived macrophages 8
9. Immunostaining and flow cytometric analysis 9
10. Phagolysosomal acidification assay 10
11. Reactive oxygen species measurement 10
12. Enzyme-linked immunosorbent assay (ELISA) 10
13. Evaluation of microbiota changes by pyrosequencing 10
14. Salmonella strains and gentamicin protection assay 11

15. Statistical analysis 12

Ⅲ. RESULTS 13

1. TREM-1 agonist protects mice from colitis either intrarectal or intraperitoneal administration and controls gut microbiota 13
2. TLR4 signaling is required for the therapeutic effects of peritoneal administration of TREM-1 agonist on colitis 21
3. TREM-1 agonist decreases bacterial load and modulates neutrophil phenotype 31
4. Macrophages stimulated with TREM-1 agonist are required to boost neutrophils 41
5. TREM-1 inhibits apoptosis and prolongs survival of macrophages and intestinal epithelial cells and protects against colonic damage 50

Ⅳ. DISCUSSION 55

Ⅴ. CONCLUSION 63

Ⅵ. REFERENCES 65

Ⅶ. ABSTRACT (IN KOREAN) 72

LIST OF FIGURES

Figure 1. Either rectal or systemic administration of TREM-1 agonist prevents murine colitis and affect microbiota composition.....15

Figure 2. Therapeutic administration of TREM-1 agonist ameliorates colitis in wild-type but not in TLR4 knockout (KO) mice.....23

Figure 3. Gene expression profiles by therapeutic administration of TREM-1 agonist are altered by TLR4 and Myd88 knockout mice.....26

Figure 4. TREM-1 agonist-treated mice rescue colitis in TLR4 and MyD88 KO mice but not sufficiently.....27

Figure 5. TREM-1 agonist alters the function of neutrophil and macrophages in colitis.....33

Figure 6. TREM-1 agonist promotes bacteria handling of the neutrophil.....37

Figure 7. Macrophage is required to boost neutrophil function by TREM-1 agonist.....43

Figure 8. TREM-1 agonist confers wound healing with cooperation with neutrophil and macrophage.....51

ABSTRACT

Triggering receptor expressed on myeloid cell 1 activation attenuates colitis via modulating microbiome and enhancing barrier function

Dong Hyuk Seo

Department of Medical Science
The Graduate School of Yonsei University
(Directed by Professor Jae Hee Cheon)

Although stimulation of triggering receptor expressed on myeloid cell 1 (TREM-1) strongly enhances inflammatory responses to bacterial compounds, TREM-1 can also enhance bacterial clearance and promote resolution of infection. Furthermore, TREM-1 has been shown to be underexpressed in macrophages in the human intestines, which are constantly exposed to the gut bacteria, thus implying their capacity to control gut microbiome. In the present study, I sought to clarify the role and underlying mechanisms of TREM-1 agonist and explored the therapeutic potential of the TREM-1 agonist on experimental murine colitis.

Mouse colitis including toll-like receptor 4 (TLR4) and myeloid differentiation primary response 88 (MyD88) knockout mice was induced by either the administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) into the colon or dextran sulfate sodium (DSS) via oral route. Different TREM-1 agonists (anti-TREM-1 antibodies) were injected intrarectally or intraperitoneally, respectively. Body weight, colon length, and disease activity index were checked. I used bone marrow-derived macrophages (BMDMs) and neutrophils (BMDNs). I also did transplantation and depletion of neutrophils or macrophages from mice. I extracted peripheral monocyte-derived macrophages and neutrophils from the

human blood and then used these cell types. The histopathology, bacterial profile, and cytokine level were examined using immunohistochemistry, bacterial 16S rRNA analysis, and enzyme-linked immunosorbent assay, respectively. Gene expression was examined using quantitative reverse transcription polymerase chain reaction. Western blot, immunofluorescent stain, and wound healing assay were also performed.

Either intrarectal or intraperitoneal administration of TREM-1 agonist ameliorated mouse colitis by either TNBS or DSS. TREM-1 agonist enhanced bacterial clearance and resolved dysbiosis by cooperation between neutrophils and macrophages, in which TLR4 signaling was required. Likewise, the levels of inducible NOS (INOS), interleukin-22 (IL-22) were increased by the TREM-1 agonist, which leads to a restoration of barrier function in intestinal epithelial cells.

TREM-1 activated the functions of neutrophils and macrophages and protects colitis via rebalancing of microbiota and protection of tissue repair. These experiments highlight the importance of TREM-1 in the maintenance of intestinal homeostasis, which suggests that TREM-1 targeting might be a therapeutic strategy for intestinal bowel disease (IBD).

Key words: cd177, epithelial cell, inflammatory bowel disease, interleukin 1 beta, interleukin 22, macrophage, neutrophil, triggering receptor expressed on myeloid cell 1 agonist

Triggering receptor expressed on myeloid cell 1 activation attenuates colitis via modulating microbiome and enhancing barrier function

Dong Hyuk Seo

Department of Medical Science

The Graduate School of Yonsei University

(Directed by Professor Jae Hee Cheon)

Ⅰ. INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder influenced by dysregulated innate and adaptive immune responses.[1] Currently, there are no curative treatments for IBD, although the introduction of biological therapies targeting tumor necrosis factor (TNF) has improved the quality of treatment.[2] Although the overall safety profile of these agents is good, anti-TNF agents also increase the risk of opportunistic infections.[3] Recent studies have highlighted the importance of host innate immune responses to microbes in the pathogenesis of IBDs. IBD feature polymorphonuclear neutrophil (PMN) infiltration of intestinal mucosa and repeated epithelial injury. [4,5] Resident lamina propria mononuclear cells (LPMCs) are the sentinels and first-responders of the gut-associated lymphoid tissues (GALT) and possess unique attributes that shape the gastrointestinal tract as a largely tolerant environment while maintaining effective clearance of microbes. Importantly, LPMCs direct subsequent adaptive immune responses, thereby regulating local inflammation.

Host innate responses to bacterial infections are primarily mediated by cells of myeloid origin such as neutrophils, monocytes/macrophages, and dendritic cells (DCs),[6] which play a pivotal role in inflammatory disease, although excessive inflammation owing to bacterial infections can lead to tissue damage and septic shock.[7] Triggering receptor expressed on myeloid cells (TREM) is a 30-kDa immunoglobulin-like family whose members have a critical role in modulating infection-induced inflammation.[8,9] TREM-1 is expressed on monocytes and neutrophils, airway epithelial cells, hepatic endothelial cells, natural killer (NK) cells, DCs, B and T cells.[10] In contrast, TREM-1 has been shown to be underexpressed in the resident intestinal macrophages in the human intestines where are constantly exposed to the gut bacteria, implying their capacity to potentiate TLR induced responses possibly as a further adaptation to the antigen-rich microenvironment of the intestinal mucosa.[11] TREM-1 expression is dramatically increased in skin, biological fluids and tissues infected by bacteria and fungi.[8,12] TREM- 1 ligation with an agonist antibody amplifies host immune reactions to bacterial compounds, cytokine production, bactericidal activity, and phagocytosis in monocytes [13] and promotes degranulation and antimicrobial functions of neutrophils.[11] Although modulation of the TREM-1 pathway improves outcomes in several inflammatory animal models,[8,14,15] and blocking TREM-1 with antagonistic peptides reduces serum inflammatory cytokines and attenuates chemically induced colitis in mice,[16] recently it was reported that DAP12-deficient mice were more sensitive to endotoxin compared to matched, wild-type mice.

The role of TREM-1 in driving the chronic inflammation of human IBD is not known, although a putative pathogenic role of TREM-1 was reported in rheumatoid arthritis[17] and bacterial pneumonia in humans.[18] In this study, I want to show that TREM-1 activation would attenuate chemically induced colitis through regulating microbiome and strengthen barrier function by the interaction between macrophage and neutrophil, in vivo.

Ⅱ. MATERIALS AND METHODS

1. Animal Model and Assessment

I used 8-week-old male C57BL/6 (Orient, Seongnam, South Korea) mice. The mice were maintained on a 12:12-hour light: dark cycle under specific pathogen-free conditions. The mice had access to standard diet and water until they reached the desired age. The mice were randomly divided into three groups: Water controls (water+IgG: IgG-treated at 4 μg/mouse), 2,4,6-trinitrobenzene sulfonic acid (TNBS)- or dextran sodium sulfate (DSS)-groups (TNBS+IgG and TNBS+α-TREM-1: α-TREM-1 treated at 20 μg/mouse or DSS+IgG and DSS+α-TREM-1). Each group consisted of 3–4 mice. Mice were mildly anesthetized with an intraperitoneally (i.p.) of 2 mg/kg Zoletil (Virbac Laboratories, Carros, France) and 10 mg/kg xylazine (Rompun, Bayeranimalhealth Co., Suwon, Korea). TNBS-induced colitis was induced by the administration of 5% (w/v) TNBS solution (100 μl) in 50% ethanol into the colon of lightly anesthetized mice via a thin round-tip needle equipped with a 1-mL syringe inserted into the colon 4 cm proximal to the anus. The animals were kept in a head-down vertical position for 2 min to distribute the agents within the entire colon and caecum (day 0). Throughout the experiment, mice were monitored for body weight loss and overall mortality. In dextran sulfate sodium (DSS, MP Biomedicals, Solon, OH, USA) -induced colitis, mice were given 3% (weight/volume) DSS in their drinking water from day 0 for 6 consecutive days. The mice were daily monitor and colitis was evaluated. Before one day to be sacrificed (day 7), drinking water including DSS was changed to pure drinking water only. After 3 days from the day TNBS solution treated and after 7 days of DSS administration, the mice were euthanized and the entire colon was quickly removed from the cecum to the anus, opened longitudinally and gently cleared of the stool by phosphate buffer saline (PBS). The colon

length was measured between the ileocecal junction and the proximal rectum. The colon tissue was then used for mRNA, protein, and histological analyses.

2. Evaluation of Disease Activity Index (DAI)

Mice was examined daily for body weight, stool consistency, and the presence of gross blood in the stool or at the anus. A previously validated disease activity index (DAI) that ranged from 0 to 4 was calculated based on the following parameters: stool consistency (0, negative; 1 and 2, loose; 3 and 4, diarrhea), gross bleeding (0, absence; 2 and 3, blood tinged; 4, presence), and weight loss (0, none; 1, 1%–5%; 2, 5%–10%; 3, 10%–20%; 4, >20%) .[19] The calculated DAI = (weight loss + stool consistency + gross bleeding)/3. The severity of colitis was evaluated by an independent observer who was blinded to the treatment.

3. Histology and Immunohistochemistry

Samples of colonic tissue were fixed in 10% buffered formalin, embedded in paraffin and stained hematoxylin-eosin (H&E). Histological examination was performed on the distal colon for each animal. Paraffin blocks were sectioned and stained using the following antibodies and respective dilution: F4/80 (eBioscience; San Diego, CA, USA). High-temperature Ag retrieval was performed by the immersion of the slides in a water bath at 95–98°C in a 10 mM trisodium citrate buffer of pH 6.0 for 45 min. Nonspecific binding was blocked by incubating sections for 1 hr with goat normal serum diluted in PBS. Samples were blocked for endogenous peroxidase activity by using 1% H_2O_2. After overnight incubation at 4°C with primary Ab, the slides were washed with PBS and incubated with the secondary Ab. Images were obtained using a microscope (Olympus BX41; Olympus Optical, Tokyo, Japan).

4. Cell Culture and Treatment

Flat-bottomed microtiter plates were precoated with 5 g/mL of an anti-human TREM-1 monoclonal antibody (R&D Systems; Minneapolis, MN, USA) or an isotype-matched control antibody overnight at 4°C. Cells were incubated in these wells for 24 hours for TREM-1 stimulation.

5. Flow Cytometry

Cell suspensions were prepared in PBS containing 2% FBS. Cells (1×10^6) were blocked with mouse Fc blocking solution (anti-mouse CD16/CD32 mAb 2.4G2, BD Bioscience; Minneapolis, MN, USA) and stained for 30 minutes at 4 °C with the appropriate antibodies. The antibodies used included: human and mice anti-Ly6G (BD bioscience, 1:50, 551460) which is specific for Ly6G without Ly6C cross-reactivity, anti-IL-22 (ebioscience), (1:50, 22URTI), anti-F4/80 (ebioscience), (1:200, BM8), anti-CD11b (eBioscience), (1:50, ICRF4), anti-CD206 (BioLegend; San Diego, CA, USA) (1:50, C068C2), anti-TREM-1 (BD bioscience, 1:100, 193015), (ebioscience, 1:100, TR3MBL1). For intracellular staining, a Foxp3/Transcription factor staining buffer set was purchased from eBioscience. Data were acquired using a FACSVerse and BD LSR Fortessa flow cytometer (BD Bioscience) and analyzed using FlowJo software (Tree Star, San Carlos, CA, USA).

6. Neutrophil and Monocyte Depletion

Mice were injected i.p. with antibodies (Abs) on days 21 and +1 postinfection. Anti-Ly6G (clone 1A8) and IgG2a isotype control (clone 2A3) were injected at a dose of 300 mg. Anti-Gr1 (clone RB6-8C5) and IgG2b isotype control (clone LTF-2) were injected at a dose of 80 mg. All Abs were from Bio X Cell (West Lebanon, NH, USA). Alternatively, mice were injected intravenously (i.v.) with 200 mL PBS, PBS liposomes, or clodronate liposomes (clodronate liposomes.com, Amsterdam, The Netherlands) 1 or 3 d prior to

infection. To assess neutrophil and monocyte depletion, blood was collected from the tail vein immediately before TREM-1 treatment on day 0, and cell populations were enumerated by flow cytometry.

7. Bone Marrow–derived Neutrophils

Bone marrow was obtained from sacrificed female C57BL/6 mice by flushing the femurs and tibias with DMEM, using a syringe with a 22-gauge needle. Cells were released from clumps by drawing the suspension through a syringe with a 22-gauge needle, and cell suspensions were passed through a 70-mm-pore cell strainer (BD Falcon, San Diego, CA) to remove tissue debris. Red blood cells were lysed with RBC Lysis Buffer (Biolegend). Remained cells were resuspended with MACS buffer manually made and isolated by neutrophil isolation kit (Miltenyi biotec; Bergisch Gladbach, Germany). Isolated neutrophils were suspended in HBSS, without Ca^{2+} and Mg^{2+}, and held on ice prior to testing.

8. Bone Marrow–derived Macrophages (BMDMs) Culture

Bone marrow was obtained, and bone marrow cells were isolated as explained in upper section 7. The cells were plated in DMEM containing 10% FBS, 1% penicillin–streptomycin–L-glutamine (Life Technologies, Gaithersburg, MD, USA), and 16% L929–conditioned media and incubated at 37°C in 5% CO_2 air and fed on day 4 by replacing the medium supplemented with 16% L929 cell-conditioned medium. At day 7, the cells were harvested with a cell scraper and replated with fresh media in plates needed for each experiment. Afterwards, adherent cells were stimulated for 24 hr with lipopolysaccharides (LPS, 200 ng/mL) in the presence or absence of α-TREM-1 (800 ng/mL). After stimulation, the culture was centrifuged (1000 g, 3 min), and the cell-free supernatant was collected and stored at -70°C for cytokine determination.

9. Immunostaining and Flow Cytometric Analysis

HT-29 or RAW 264.7 cells were grown on 8-well chamber slides (Nunc, Rochester, NY, USA), and infected with constitutively GFP-expressing wild-type S. typhimurium or isogenic mutants as indicated at multiplicity of infection (MOI) of 10:1, or exposed to biotinylated LPS (100 ng/mL), and incubated for the specifically mentioned period. Discrimination of extra and intracellular bacteria was achieved using Salmonella strain carrying a GFP expression construct (green) in combination with a mixture of two mouse monoclonal anti-Salmonella O-antigen (anti-O4 and anti-O5) antibodies visualized with a Texas-Red conjugated anti-mouse secondary antibody (red) in the absence of cell permeabilization. Due to impaired penetration of the anti-Salmonella antibodies into the cells, intracellular bacteria appear green, whereas extracellular bacteria exhibit an orange (green plus red) color. Biotinylated LPS was detected using Texas-Red conjugated with Streptavidin (Jackson ImmunoResearch, PA, USA). For permeabilization of the eukaryotic cell membrane, saponin was adjusted to a final concentration of 0.5%. After the indicated time periods, cells were fixed in 5% PFA and counterstained with MFP488- or MFP647-phalloidin (MoBiTec, Go¨ttingen, Germany). Cells were subsequently mounted in DAPI containing Vecta shield (Vector Laboratories, CA, USA) and visualized using an ApoTome-equipped Axioplan 2 microscope connected to an AxioCam Mr digital Camera (Carl Zeiss MicroImaging, Inc. Jena, Germany). Flow cytometric detection of intracellular GFP-expressing bacteria in intact epithelial cells was carried out in Trypsin-EDTA 0.05% treated, fixed m-ICcl2 cells using a FACS Caliber apparatus (BD bioscience). In addition, flow cytometry was used to quantify the number of GFP-expressing bacteria in cell lysates. To standardize the volume examined, a defined quantity of Cy5-labelled particles was added to all samples and the data acquisition on GFP-positive bacteria (recorded in channel Fl-1) was limited until a simultaneously recorded number of 10.000 events in the far-red channel (Cy5, Fl-4) was reached.

10. Phagolysosomal Acidification Assay

Macrophages were incubated with LPS (200 ng/mL) or α-TREM-1 (100 g/mL) for 2 hr. Cells then were treated with LysoTracker Red DND-99 (Thermo Scientific, MA, USA) 100 nmol/L for 30 min, fixed in 4% paraformaldehyde, and stained with Hoechst 33342, 8.115 nmol/L (Thermo Scientific). Cells were visualized using confocal microscopy with a Carl Zeiss (Thornwood, NY, USA) LSM 700 laser scanning microscope. At least 10 high-powered fields and 100 cells were counted.

11. Reactive Oxygen Species Measurements

For the studies of reactive oxygen species (ROS) production, carboxy-H2DCFDA (Thermo Scientific) was used according to the manufacturer's protocol. To load the cells with the carboxy-H2DCFDA dye, the cells were resuspended in HBSS pH 7.4 with 1 mM Ca^{2+}, 1 mM Mg^{2+}, 1% (v/v) FCS, and 1 μM carboxy-H2DCFDA at 107 cell/mL; incubated for 20 min at 37°C; and washed twice in fresh HBSS pH 7.4, prior to stimulation and analysis.

12. Enzyme-linked Immunosorbent Assay (ELISA)

The concentrations of TNF-α in culture medium were determined using commercial ELISA kits (R&D systems) according to manufacturer instructions.

13. Evaluation of Microbiota Changes by Pyrosequencing

Colon tissue samples from mice were collected. Total DNA from these samples was then isolated with FastDNA™ SPIN Kit for Soil kit (MP Biomedicals) according to the manufacturer's recommendation. DNA was subsequently gel purified and PCR was performed in triplicate for each primer pair and pooled to minimize random PCR bias. The reaction mixture contained 1 mL of DNA (10 ng/mL), 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of dNTPs, 16 PCR buffer, 1 U platinum TAQ DNA polymerase (Thermo

Scientific) and 0.40 mmol/L of forward modified primer consisting of 454 adaptor A (59-CCATCTCATCCCTGCGTGTCTCCGACTCAG-39; Genome Sequencer FLX system, Roche, Basel, Switzerland), unique 10-base tag sequence (ATATCGCGAG, CGTGTCTCTA, CTCGCGTGTC, TAGTATCAGC, TCTCTATGCG) and universal broad-range bacterial primer 59-AYTGGGYDTAAAGNG and 0.40 mmol/L of reverse primer consisting of adaptor B (59-CCATCTCATCCCTGCGTGTCTCCGACTCAG- 39) and universal primer TACNVGGGTATCTAATCC. PCR conditions were as follows: 16: 95℃, 3 min; 356: 94℃, 50 sec; 40℃, 30 sec; 72℃, 60 sec; 16: 72℃, 5 min and a final hold at 4℃. The length of PCR product was checked on the agarose gel electrophoresis. PCR product was subsequently purified using magnetic beads (AMPure beads, Beckman Coulter Genomics, MA, USA). Concentration was measured on Qubit fluorometer (Thermo Scientific). Equimolar amounts of PCR product from each sample were used for unidirectional 454 FLX amplicon pyrosequencing using LIB-L emPCR kits following the manufacturer's protocols (Roche Diagnostics, Basel, Switzerland).

14. Salmonella Strains and Gentamicin Protection Assay

Salmonella enterica serovar typhimurium (*S. typhimurium*)-expressing GFP (NCTC 12023) were freshly grown in a unagitated microaerophilic or shaking (250 r.p.m) culture by inoculating in 10 mL Luria- Bertani broth then incubating overnight (approximately 16 hr) at 37°C, until mid-logarithmic growth was reached (OD_{600}: 0.5) as described previously.[20] HT-29 cells were seeded at 40,000 cells per well into 24-well tissue cultures plates (Falcon, Le Pont de Claix, France) in 1 mL of growth medium with antibiotics and cultured for 2 days until 70 to 80% confluence. Cells were washed with 1 mL of DMEM before the addition of bacteria. Cells were pretreated with inhibitors and subsequently stimulated for 2 hr before infection. Bacterial suspensions were added to the cell culture wells at MOI of 10:1 in the antibiotic-free medium by dilution. After 1 hr of coculture of

bacteria and macrophages in a 37°C/5% CO_2 incubator, cell cultures were washed twice with 1 mL DMEM and incubated 1 hr with growth medium supplemented with 50 mg/mL gentamicin (PAA, Pasching, Austria) to kill extracellular bacteria. Cell culture supernatants, as well as cell lysates, were collected after the indicated periods of time and stored at -20℃. One hundred microliters of each culture supernatant were plated onto tryptic soy agar (TSA) plates (Oxoid, Dardilly, France) to confirm the absence of living extracellular bacteria. At 3 hr post infection, cells cultures were washed with DMEM and subsequently lysed by a 10-min incubation period with 1% Triton X-100 in deionized water to determine the number of colony-forming units (CFU) recovered from the lysed macrophages. To quantify internalized bacteria, suspension dilutions of cell lysates were plated in duplicate on TSA plates followed by an overnight incubation at 37℃. For each donor, conditions were tested in three parallel infection experiments and results were normalized to individual mean CFU of the unstimulated or vehicle-treated condition.

15. Statistical Analysis

GraphPad Prism 5.0 Software (GraphPad Software, Inc, CA, USA) was used for statistical data analyses, with a two-tailed Student's t-test for comparisons of two datasets and analysis of variance (ANOVA) for multiple comparisons. Significance was accepted when P-values were less than 0.05. Experimental results were expressed as mean values and standard error of the mean (SEM).

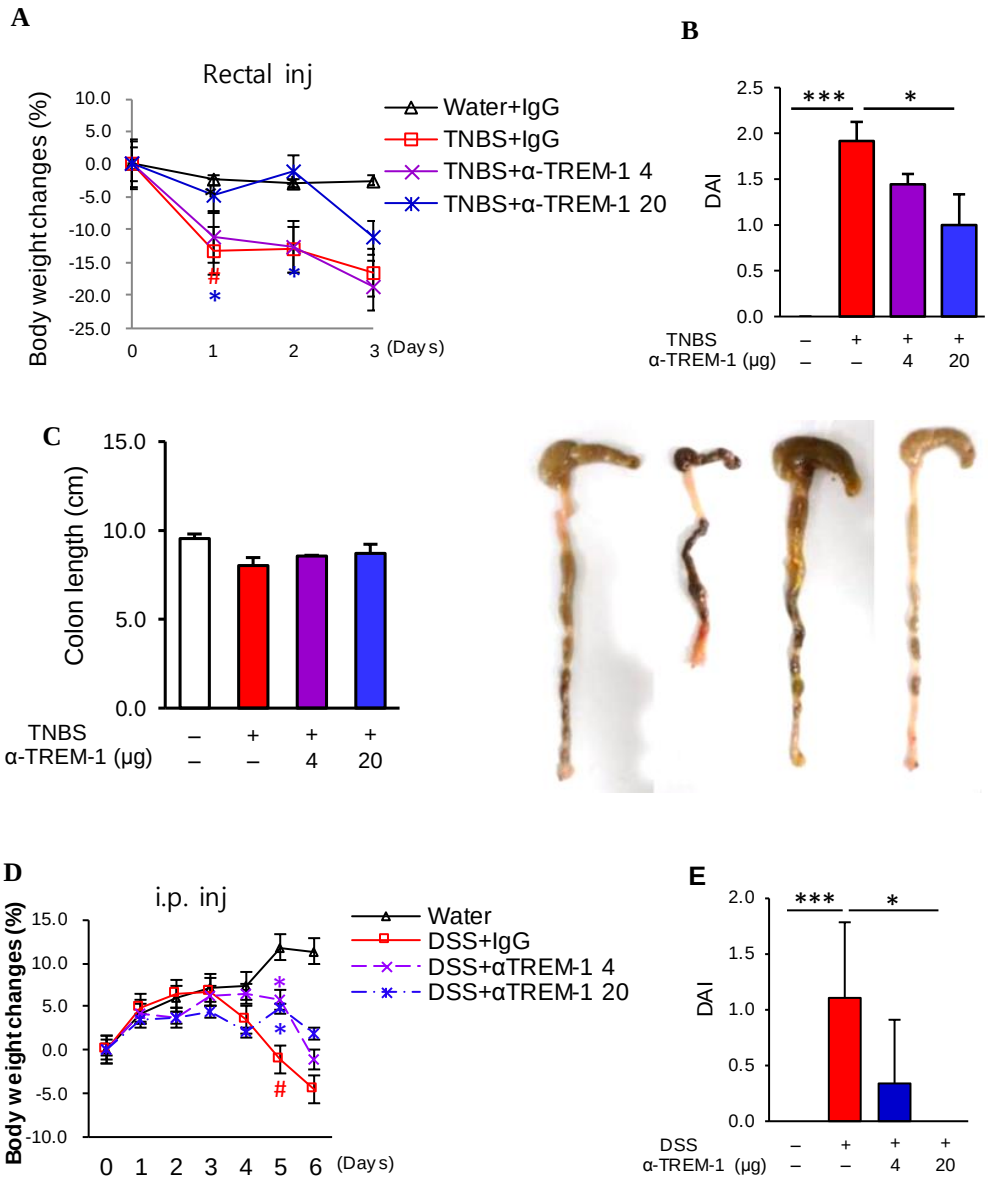
Ⅲ. RESULTS

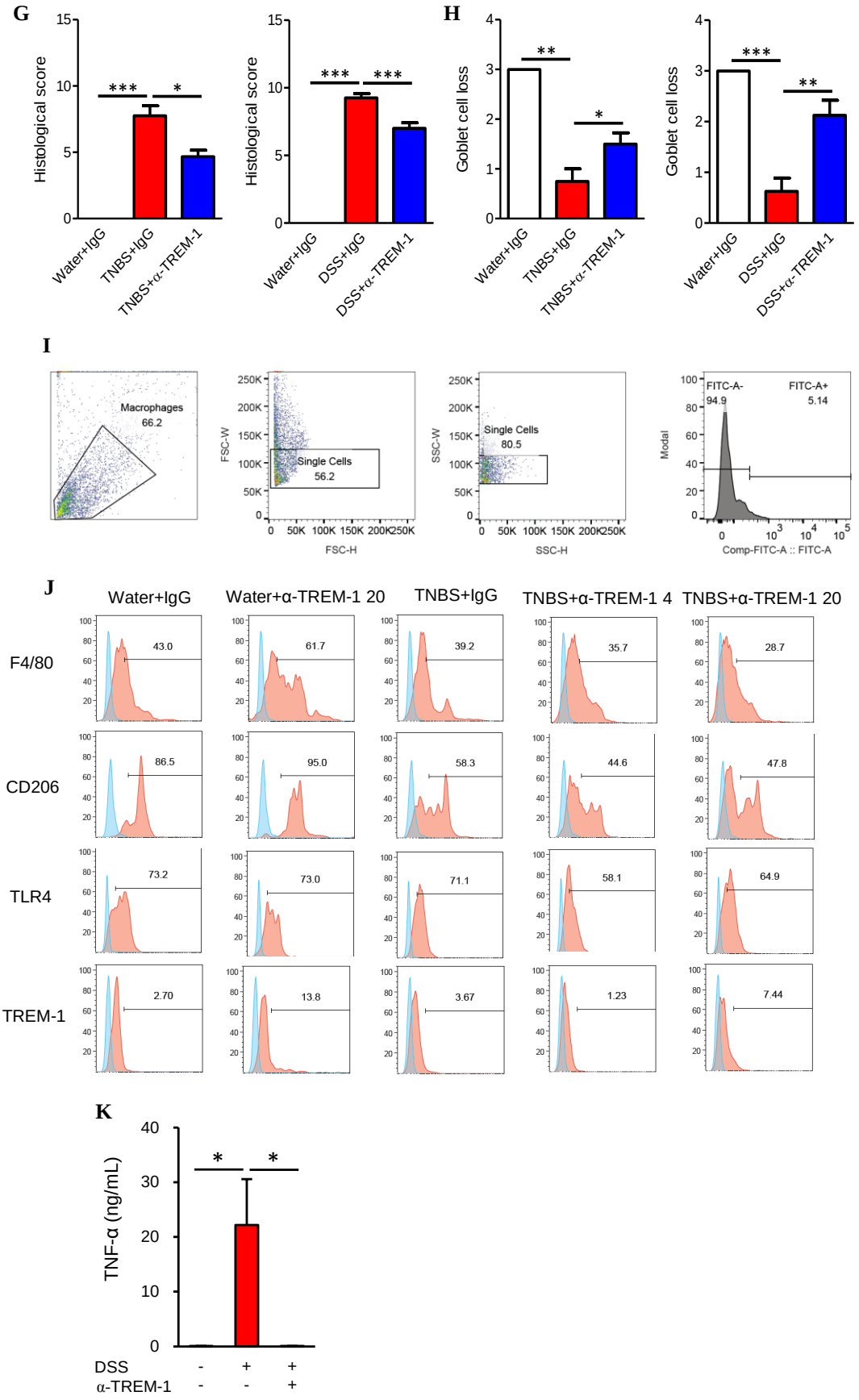
1. TREM-1 agonist administration protects mice from colitis and controls gut microbiota

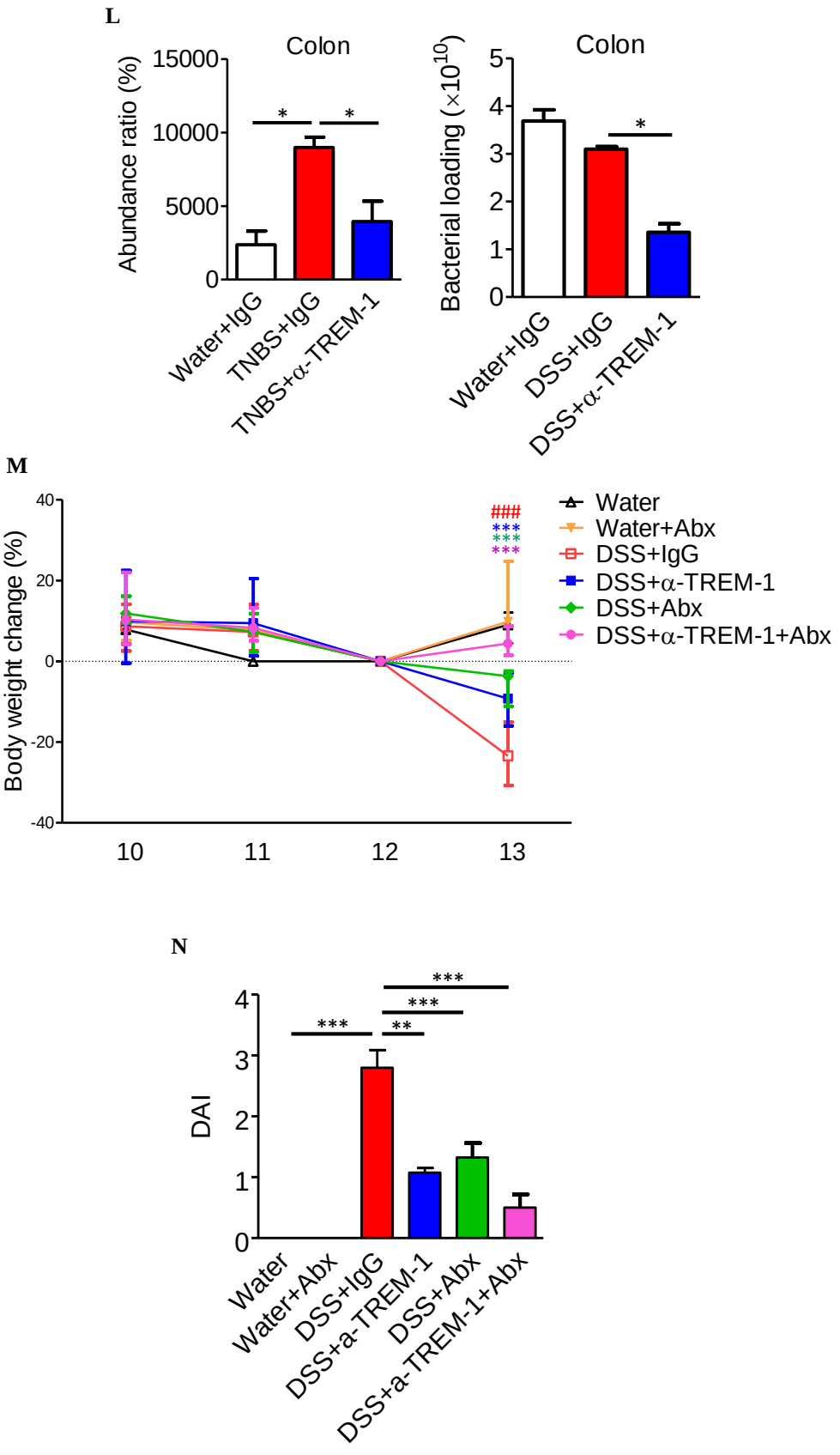
Because the ligands for TREM-1 have not been characterized yet, first, I examined the effect of a TREM-1 agonist on murine colitis induced by the rectal administration of TNBS. I directly stimulated TREM-1 by the rectal administration of an agonistic anti-TREM-1 antibody (α-TREM-1) together with TNBS. Administration of TNBS induced severe colitis, Th1-mediated CD-like colitis, in mice as assessed by weight loss and disease activity index (DAI) that incorporates body weight loss, stool consistency, and gross bleeding. Paradoxically, I found that α-TREM-1 administration remarkably displayed body weight gain, longer colon length, and lower DAI compared to isotype control (IgG)-treated control groups dose-dependently (**Figure 1A**, **1B**, and **Figure 1C**). In TNBS-induced colitis, H&E staining revealed a pronounced cell (neutrophil, mononuclear cell) infiltration into the lamina propria, goblet cell loss, and disintegrated crypts in colons of IgG-treated mice, whereas the histological evaluation of colons from α-TREM-1-treated mice revealed a prominent decrease in histological damage and goblet cell loss compared with colons of IgG-treated mice (**Figure 1F** upper, **1G** left, **1H** left). To investigate the effects of α-TREM-1 on the TREM-1 expression on macrophages, I determined the distribution of TREM-1^+ macrophages during colitis induction. TNBS increased TREM-1^+ cells in colon tissues, which was more induced by α-TREM-1 (**Figure 1I**, **1J**).

The effects of α-TREM-1 through rectal administration suggest that α-TREM-1 can affect directly IECs. In the second set of experiments, to confirm therapeutic potential of α-TREM-1 on colitis and test whether the effects could affect at other sites, I used DSS-induced colitis, a Th2-mediated UC-like model considered to be driven by macrophages, and i.p. administrated α-TREM-1.[21] Similar to the results of TNBS-colitis, α-TREM-1-treated DSS-groups displayed body weight gain, longer colon length, and lower DAI compared to vehicle-treated control groups (**Figure 1D**–**1E** and **Figure 1K**). The histological evaluation of colons from α-TREM-1-treated mice revealed a prominently decreased score for inflammation compared to colons of vehicle-treated mice (**Figure 1F** lower and **1G** right). Moreover, the goblet cells showed near complete restoration in

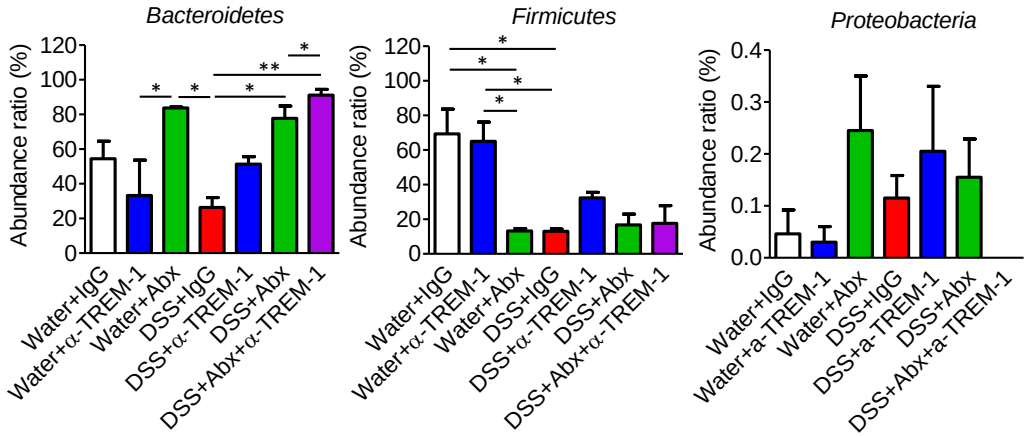
colon sections from the α-TREM-1-treated DSS groups when compared with IgG-treated groups (**Figure 1H** right). α-TREM-1-treated DSS-groups showed decreased serum TNF-α levels when compared with IgG-treated groups (**Figure 1K**). The effects of α-TREM-1 by i.p. administration, as well as intrarectal administration, suggested that treatment of α-TREM-1 influences systemic modulators, such as neutrophil and macrophages as well as mucus. Because TREM-1 has been well known to promote clearance of bacteria, I investigated bacterial burden in colon tissues. Colon tissues from α-TREM-1 rectally administrated mice with TNBS- or DSS-colitis showed decreased bacterial load compared to those from IgG-treated groups (**Figure 1L**). To further find out the involvement of bacterial modulation of TREM-1, I depleted the endogenous microbiota with antibiotics treatment for 2 days and then investigated the effects of antibiotics and α-TREM-1 on colitis and microbiota composition in C57BL/6 strains. Antibiotics- or α-TREM-1 treated or both treated groups significantly reduced bacterial burden, body weight changes, and DAI. And those groups also decreased colon length as compared to DSS+IgG-treated groups (**Figure 1M, 1N**). Notably, the effect of antibiotics was additively increased when treated with α-TREM-1. To investigate the effect of α-TREM-1 on microbiota composition, I performed metagenomic profiling using 16S rRNA sequencing of DNA of stool in the cecum and proximal colon tissues from DSS- and TNBS-treated mice. As shown in **Figure 1O**, **1P**, the relative abundance of *Bactroidetes* is drastically rescued in the stool of α-TREM-1- or antibiotics-treated groups compared to that of the DSS+IgG groups. The colon tissues revealed a taxa abundance with a significant decrease in the proportion of *Proteobacteria* in TREM-1- or antibiotics-treated groups relative to DSS+IgG-treated mice (**Figure 1Q** and **Figure 1R**). However, *Bacteroides* displayed different profiles between DSS and TNBS models (**Figure 1P, 1R**). Altogether these results indicate that TREM-1 agonist leads to the ecological alteration in bacterial profile as well as affects bacterial burden. Taken together, α-TREM-1-mediated attenuation on colitis in mice is associated with an inhibition of bacterial translocation and alteration of gut bacterial communities inclined to non-diseased compositions.



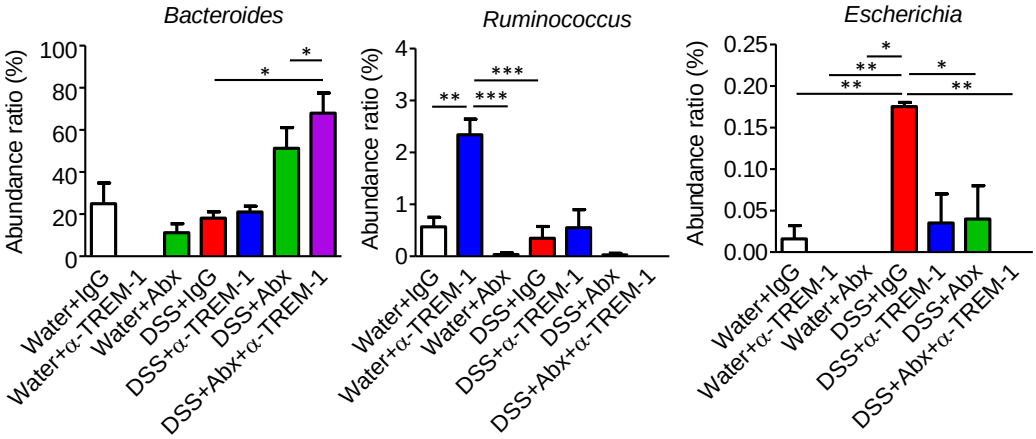




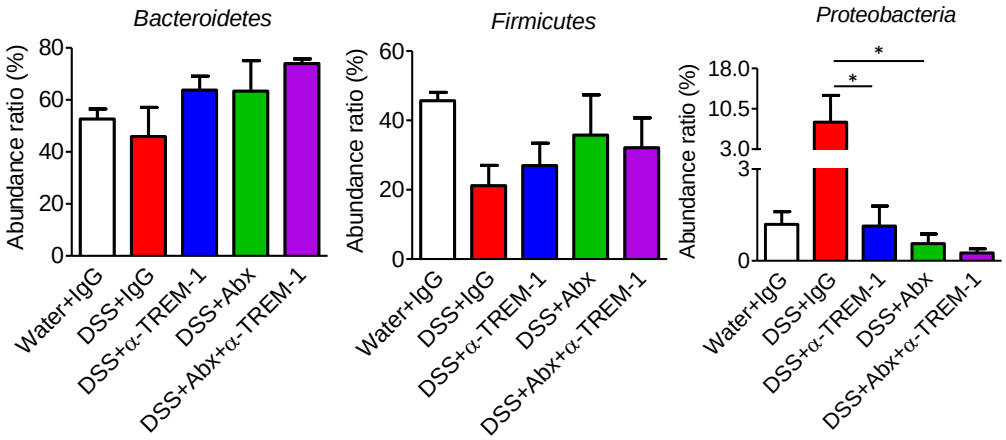
O Cecum



P Cecum



Q Colon



R Colon

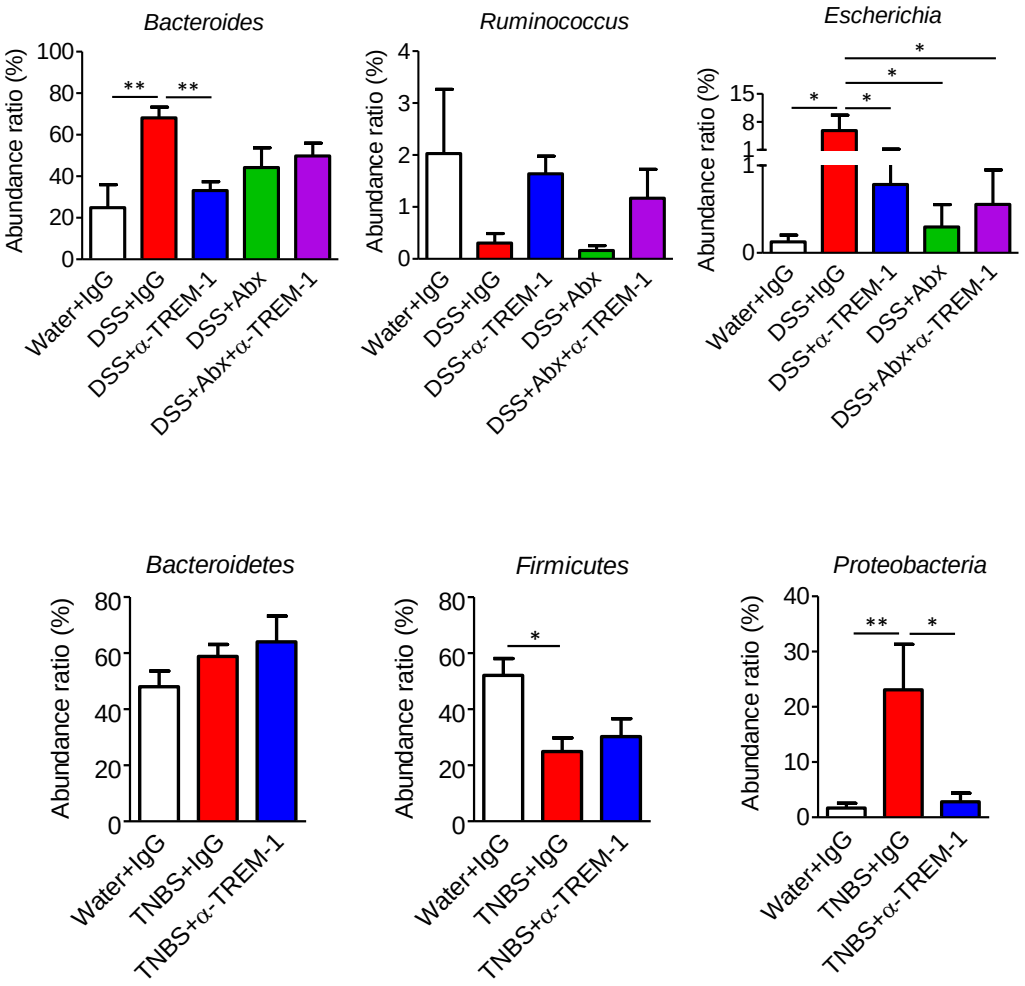


Figure 1. Both rectal and systemic administration of TREM-1 agonist prevent mouse colitis and affect microbiota. (A–C) Rectal administration of TREM-1 agonist in TNBS-treated mice. The colitis was evaluated after intrarectal injection of TNBS with IgG or α-TREM-1. (D–E) Systemic administration of TREM-1 agonist in DSS-treated mice. DSS was supplied with drinking water and α-TREM-1 was i.p injected into the mouse. Rectal administration of TNBS- or DSS-induced severe colitis in mice as assessed by weight loss and disease activity index (DAI) that incorporates body weight loss, stool consistency, and gross bleeding, while administration of TREM-1 agonist recovered body weight and colon length and reduced DAI dose-dependently. (A, D) Body weight changes (B, E). Disease activity index (DAI). (C) Colon length. DAI after the sacrifice was scored according to the criteria outlined in the Materials and Methods. (F–H) Histopathology. (F) Representative sections of hematoxylin and eosin stain (H&E). Original magnification: x200. Colon tissues of TNBS (upper)- and DSS (lower)-treated mice were stained with H&E. (G) Histological score. (H) Goblet cell score. (I–J) TNBS increased TREM-1^{+} cells in colon tissues. (I) Single cell gating. (J) Cell population expressing each target. (K) TNF-α protein level extracted from colon tissue, Measured by ELISA. (L) Bacterial load in colon tissue. The bacterial burden was evaluated PCR of 16s rRNA levels in colon tissues. (M–N) The synergetic effect of the TREM-1 agonist and antibiotics treatment. (M) body weight changes (N). DAI. (O–R) Microbiota profiles. (O, P) Phylum level changes in stool. (Q, R) Phylum level changes in the colon. Data are expressed as the mean ± S.E.M. (n = 4). Veh, treated with IgG; TNBS, injected with TNBS; Abx, treated with antibiotics. *p < 0.05 vs. TNBS+Veh, **p < 0.01 vs. TNBS+Veh, ***p < 0.005 vs. TNBS+Veh. Analyses were performed using one-way ANOVA with Tukey's posttest.

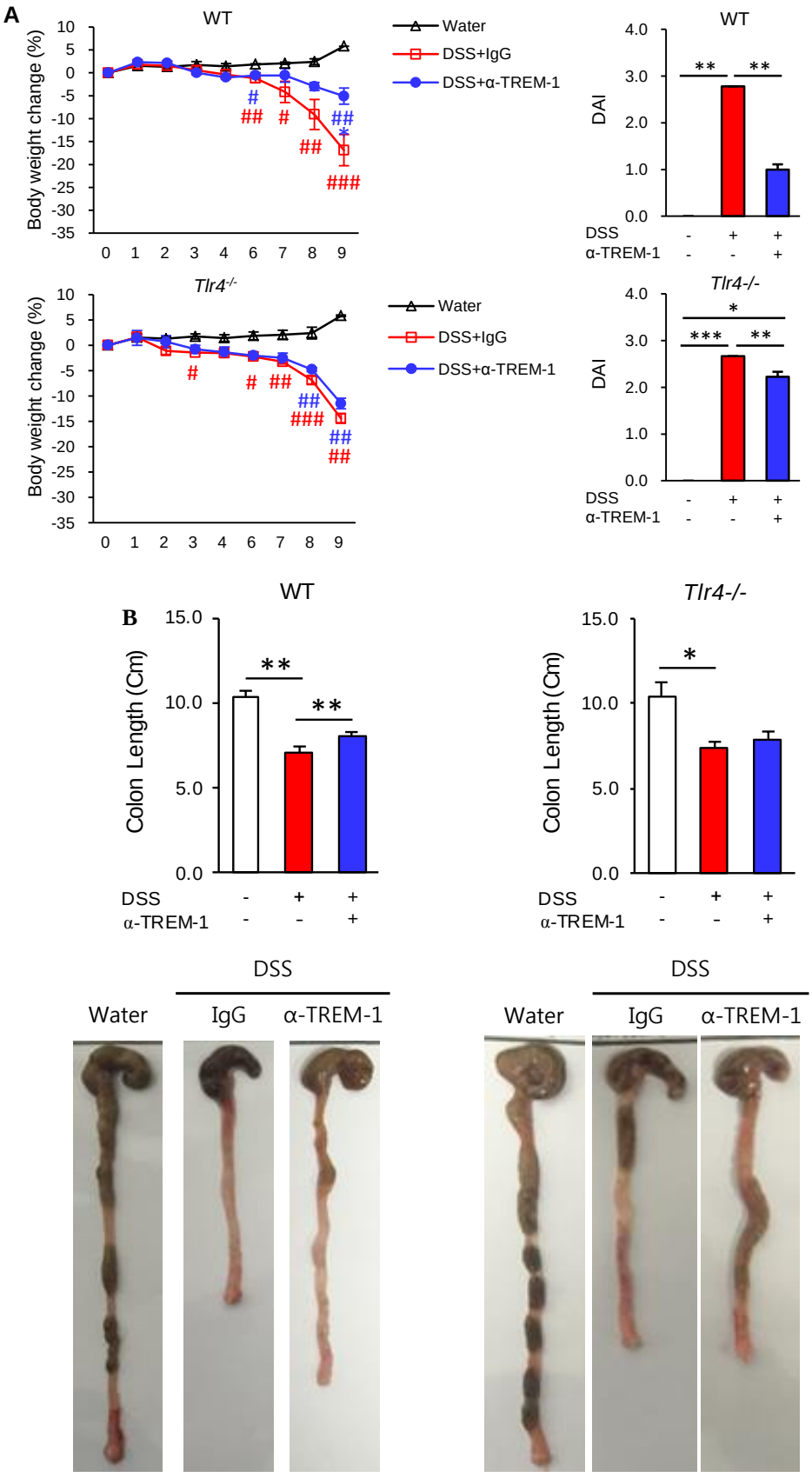
2. TLR4 signaling is required in therapeutic effects of peritoneal administration of TREM-1 agonist

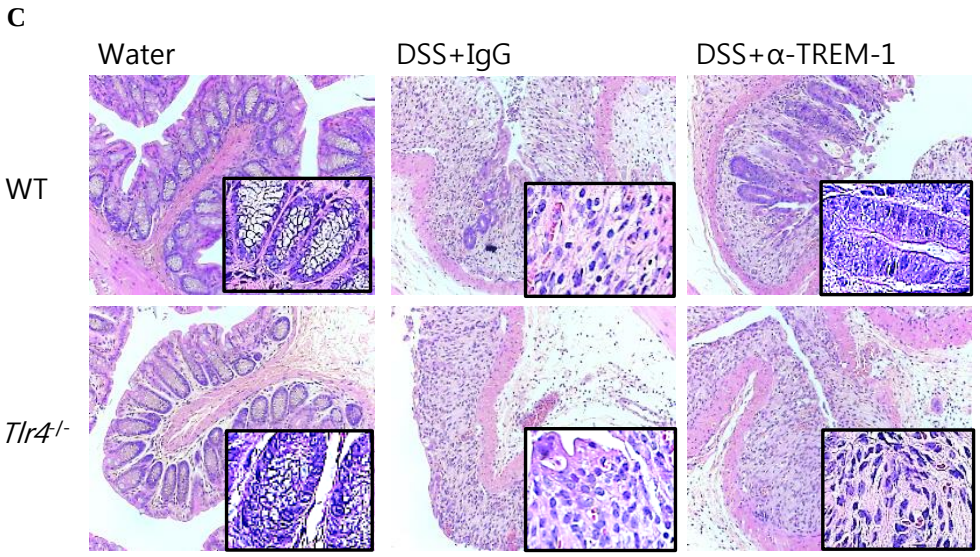
TREM-1 modulates the activity and availability of key proteins in the TLR4 signaling cascades[22] and TREM-1 induction occurs through TLR-mediated pathways.[23] Because the interaction between TREM-1 and TLR4 may be a crucial component of the antibacterial function of macrophages, I investigated the therapeutic effects of TREM-1 agonist in TLR4 knockout (KO) mouse by administration of α-TREM-1 for 2 days after DSS-treatment. In the same manner with C57BL/6 mouse strain, α-TREM-1 revealed a marked attenuation of colonic pathology with only mild inflammation in wild-type BALB/c mice, compared with IgG-treated mice: The reduction of body weight, after α-TREM-1-administration, was approximately 5%, while that in IgG-treated mice was about 20%. However, α-TREM-1-treated DSS-groups in TLR4 KO mice did not improve body weight gain, colon length, and DAI compared to vehicle-treated control groups (**Figure 2A, 2B**). To explore the effects of α-TREM-1 administration on histopathology and goblet cells, I performed periodic acid-Schiff (PAS) staining. The histological evaluation of colons from α-TREM-1-treated TLR4 KO mice did not improve colitis and goblet cell restoration compared to wild-type mice (**Figure 2C** upper and middle and **2D**), suggesting that the anti-inflammatory effects of α-TREM-1 are mediated by TLR4/MyD88 signaling. Colon tissues from α-TREM-1-treated wild-type mice with DSS-colitis showed decreased bacterial load compared to those from IgG-treated groups, whereas those from α-TREM-1-treated TLR4 KO mice indicated bacterial load similar to those from IgG-treated groups (**Figure 2E**).

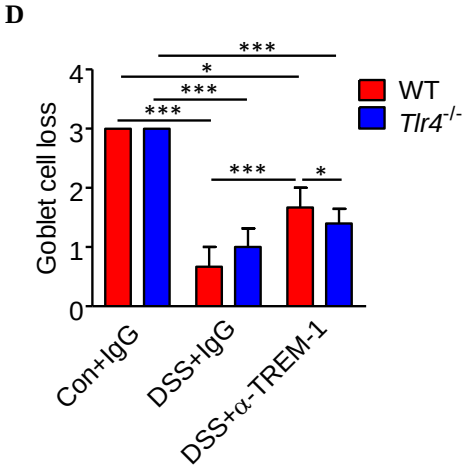
Tissue samples from identical distal parts of the colon from all mice were used to compare mRNA expression by quantitative RT-PCR. Inflammatory molecules, such as tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), and interleukin-1β (IL-1β) have been shown to be critical factors of the stimulation of macrophages. DSS+IgG-groups and α-TREM-1-treated groups showed significantly higher expression of *Tnfa*, *Inos*, and *Il1b* in the affected colon compared with water-groups (**Figure 3A**), in which α-TREM-1-treated groups showed dramatically increased *Inos* expression when compared with water-groups (**Figure 3A** middle). In contrast, α-TREM-1-treated groups showed significantly decreased expression of *Tnfa* and *Il1b* in the colon compared with

DSS+IgG-groups in TLR4 KO mice (**Figure 3B**) and did not further increase expression of *Inos* (**Figure 3B** middle). These results suggest that *Inos* expression may be correlated with the reduced bacterial load in the colon of α-TREM-1-treated groups with colitis.

Having observed that α-TREM-1 modulates microbiota in murine colitis model and that wild-type and TLR4 KO mice had different microbiota profiles, I sought to assess whether the abolished α-TREM-1 effect in TLR4 KO mouse was due to the gut microbiota. IgG- and α-TREM-1-treated mice were cohoused for 6 wk to exchange microbiota each other (**Figure 4A**). There was a reduction in body weight change until day 8 after DSS-treatment compared to IgG-treated mice but this effect disappeared at the end of the observation period (**Figure 4B**). Consistently, cohosing had no effect on DAI and histopathology (**Figure 4B–4E**). Thus, I postulated that the effect of the microbiota was temporary, and I tried to perform oral transplantation of fecal microbiota obtained from mice treated with α-TREM-1 for 6 days (**Figure 4F**). The treatment had little effect on restoration of histology and goblet cell in the colon (**Figure 4H**, **4I**, **4J**), although body weight changes, DAI, and M2 macrophages were similar between DSS-treated all groups. (**Figure 4G**, **4H**, **4K**, **4L**). In the same set, I transplanted fecal microbiota of wild-type mice treated with α-TREM-1 to DSS-treated MyD88 KO mice (**Figure 4M**). Myd 88 KO mice indicated a mild reduction of DAI and goblet cell loss, but there is no difference in body weight changes when the stool of α-TREM-1-treated mice transferred to DSS-treated mice (**Figure 4N**, **4O**, **4P**). At this point, I had no proof that microbiota per se has the sustained effect of α-TREM-1.







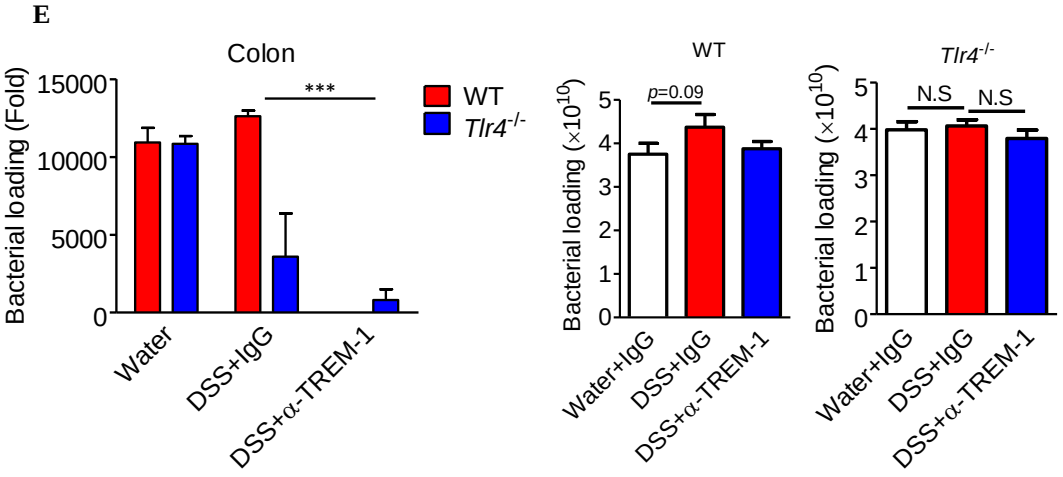


Figure 2. Therapeutic administration of TREM-1 agonist ameliorates colitis in wild-type but not in TLR4 knockout (KO) mice. (A) Body weight changes (left) and disease activity index (right) in wild-type and TLR4 KO mice. α-TREM-1 was once administrated on day 3 of 3% DSS-treatment. (B) Colon length. (C–D) Histopathology. (C) Representative images of periodic acid-Schiff (PAS) staining of the colonic mucosa for goblet cells in wild-type (upper), and TLR4 KO (lower) mice. Original magnification: x200. (D) Goblet cell score. (E) Bacterial load in colon tissue. Data are expressed as the mean ± S.E.M. (n = 4). IgG, treated with IgG; DSS, injected with dextran sulfate sodium; α-TREM-1, treated with α-TREM-1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$. Analyses were performed using one-way ANOVA with Tukey's posttest.

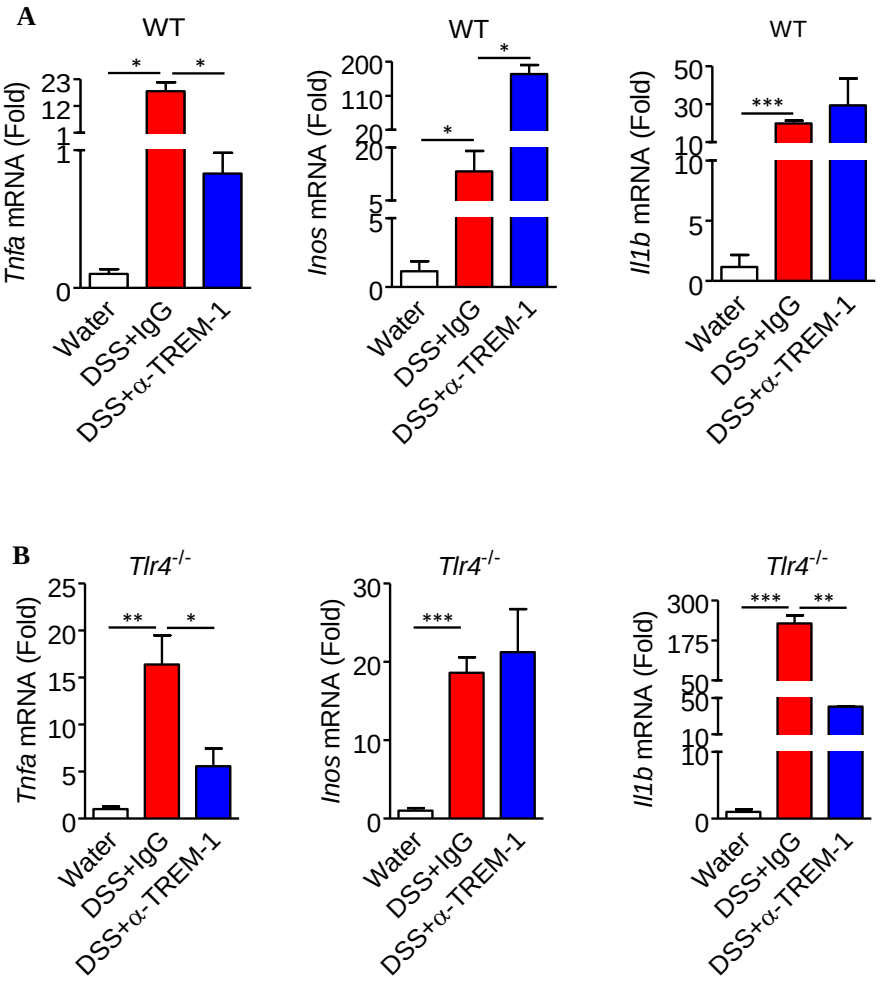
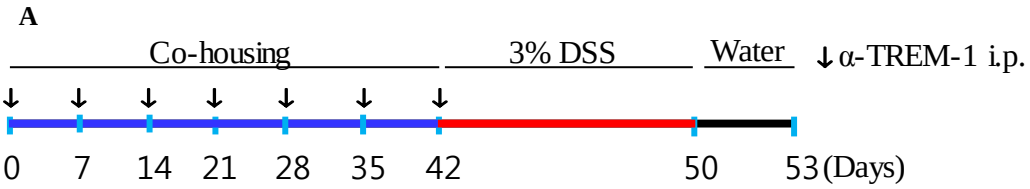
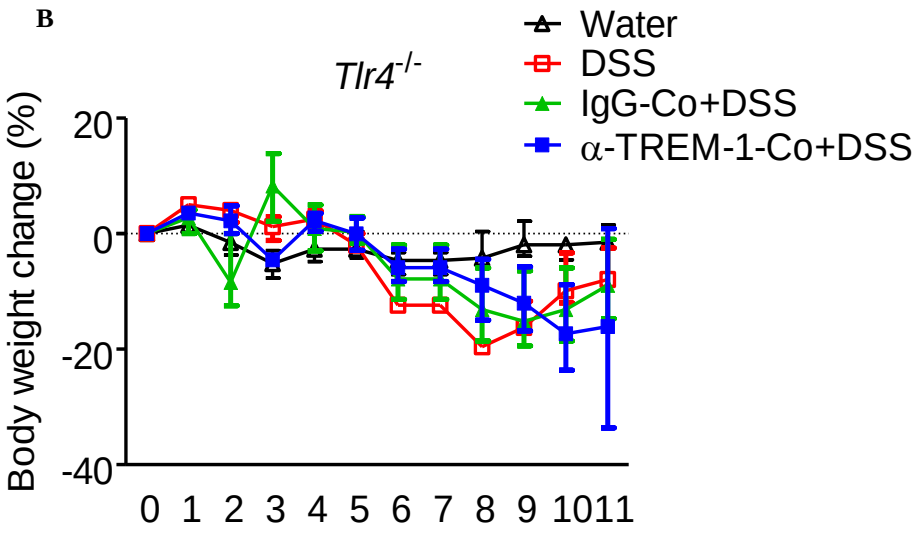
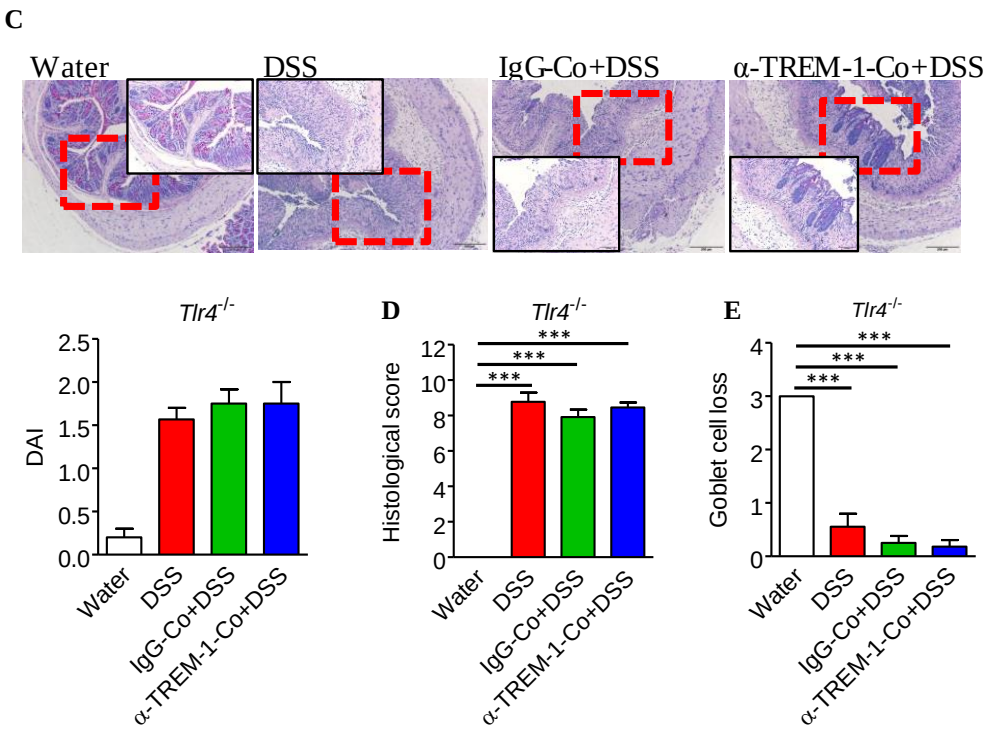
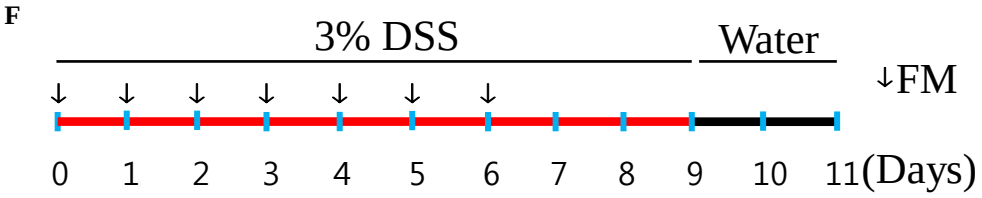


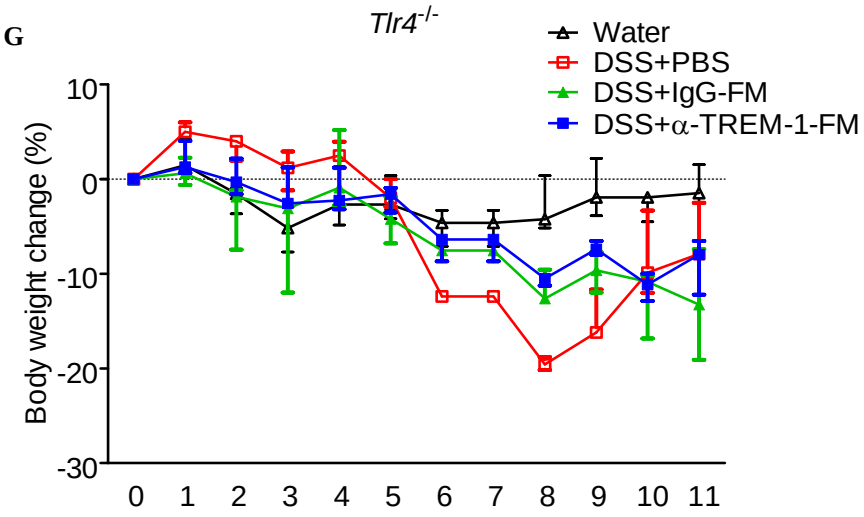
Figure 3. Gene expression profiles by therapeutic administration of TREM-1 agonist are altered by wild-type and TLR4 knockout. (A) Gene expression profile in wild-type mice. (B) Gene expression profiles in TLR4 KO mice. Quantitative RT-PCR was used to evaluate the expression profile of genes related to the proinflammatory cytokines, bacteria handling, and inflammasome (*Tnfa*, *iNos*, *Il1b*). Data represent means ± S.D. values of at least 4 independent experiments, each using duplicate wells. Water, treated with drinking water; DSS, injected with dextran sulfate sodium; α-TREM-1, treated with α-TREM-1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$. Analyses were performed using one-way ANOVA with Tukey's posttest.

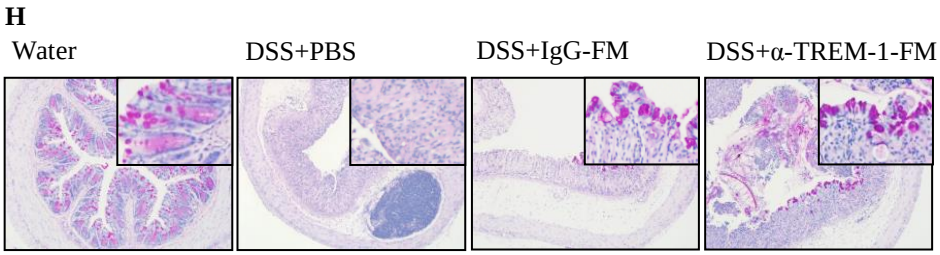


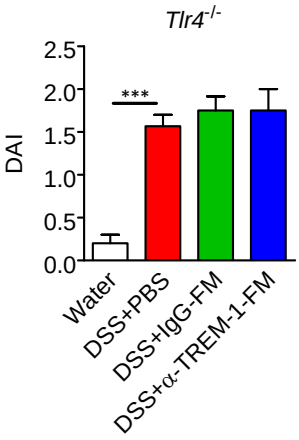


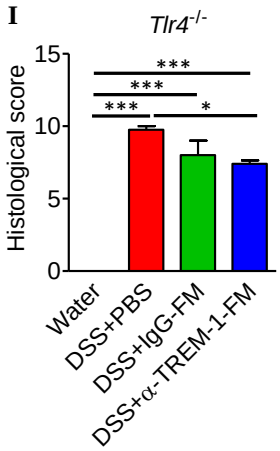


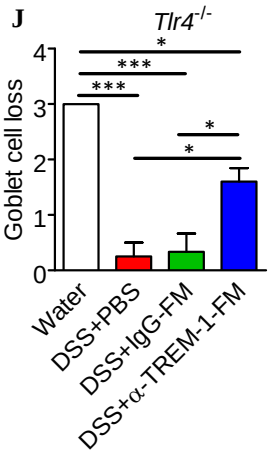


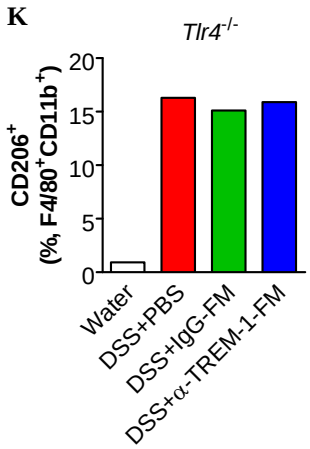


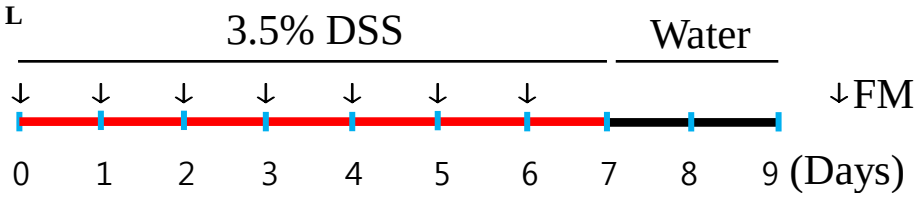


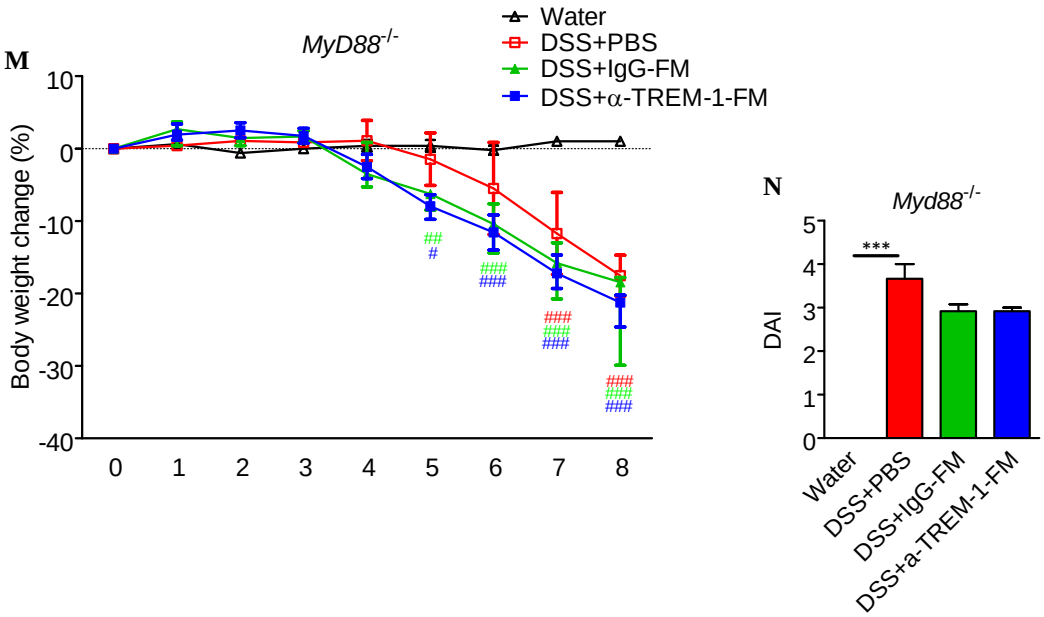












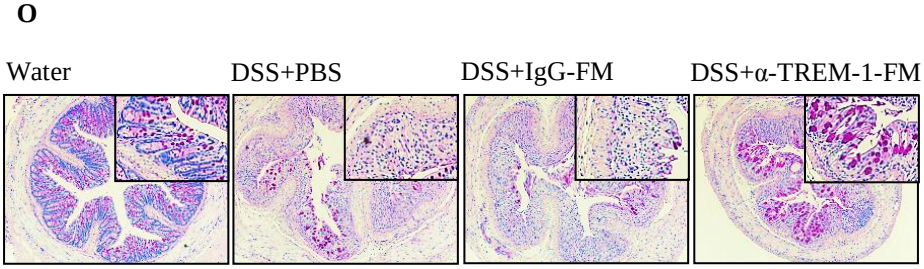
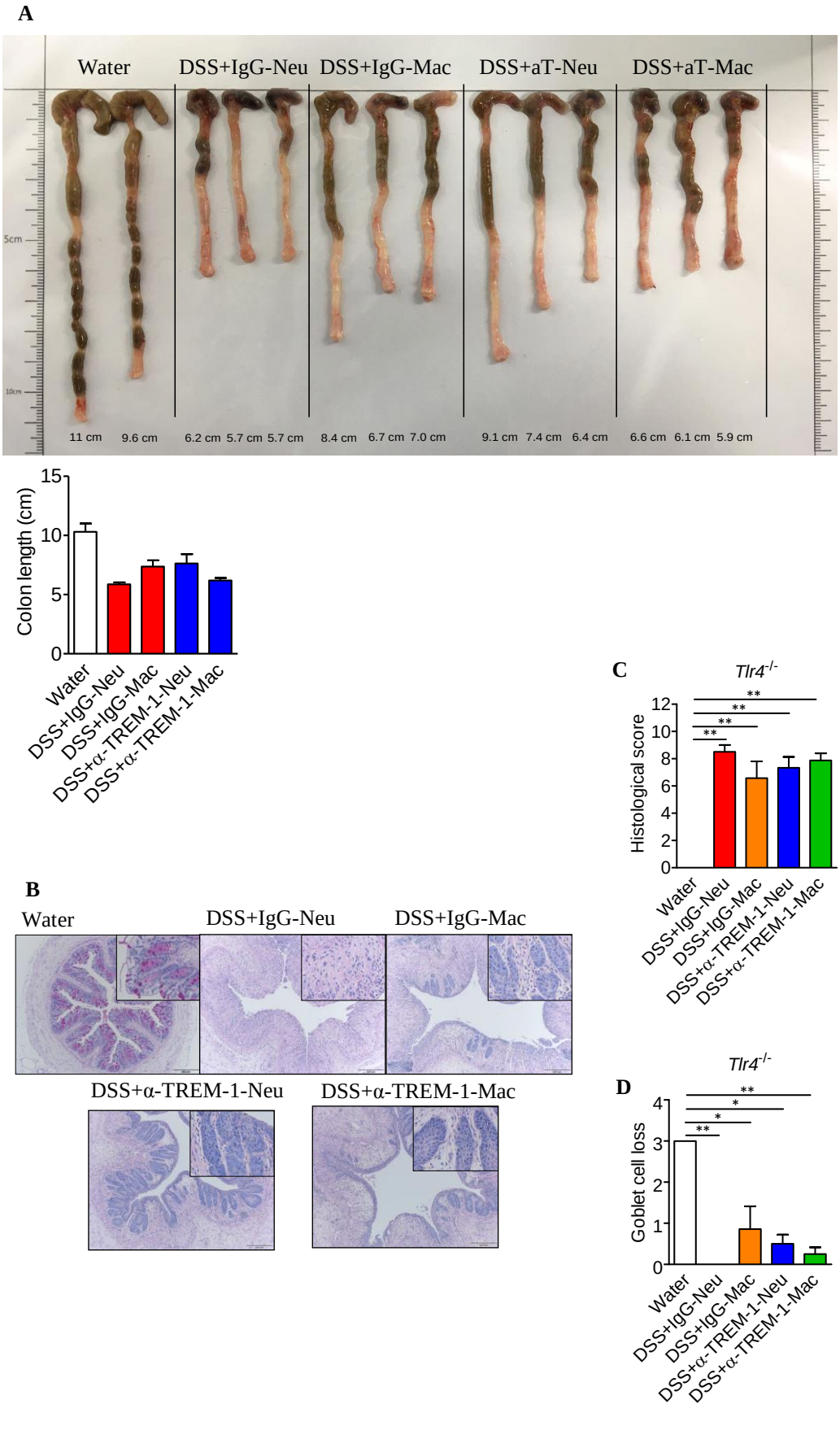


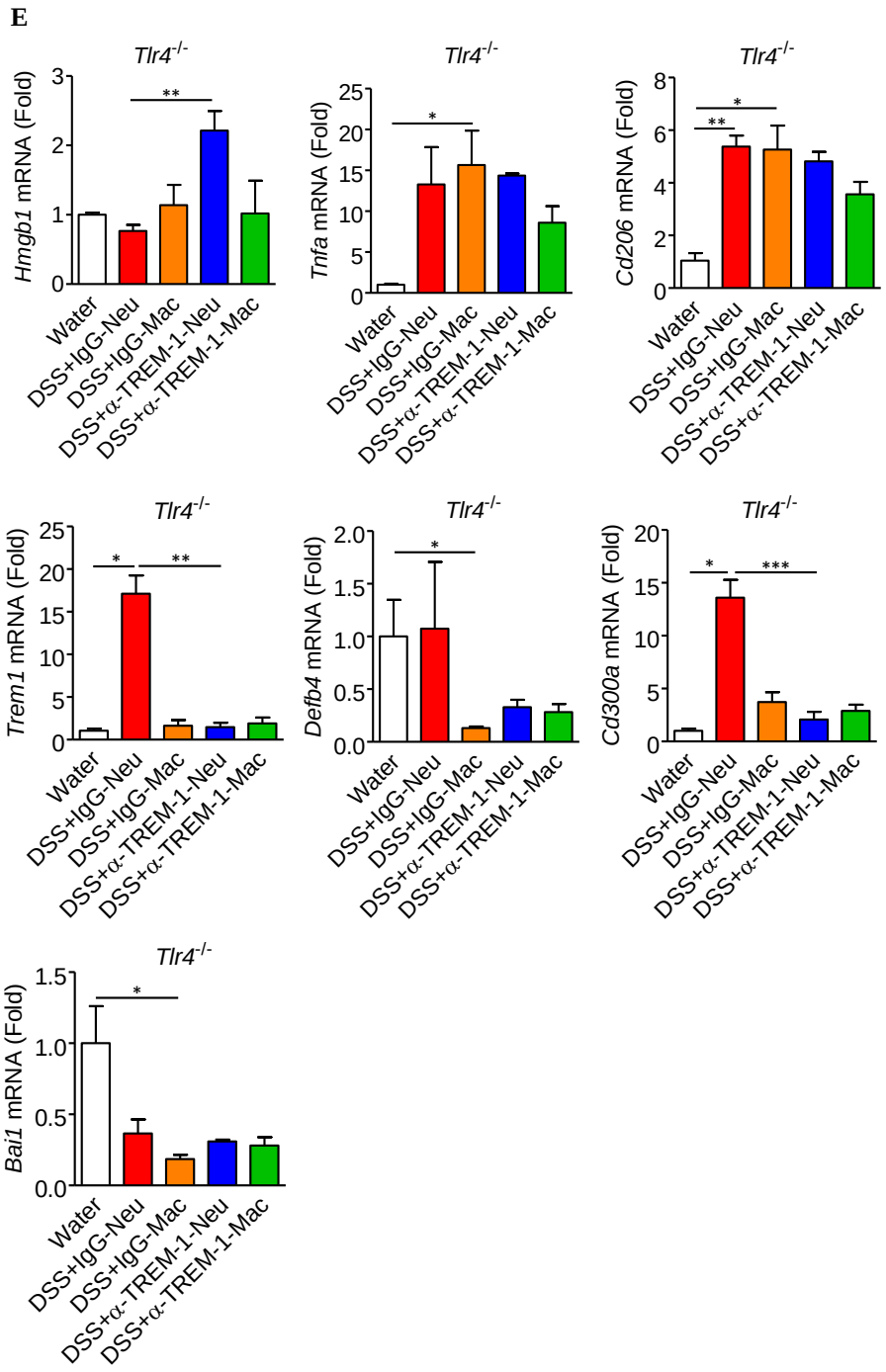
Figure 4. TREM-1 agonist-treated mice rescue colitis in TLR4 and MyD88 KO mice but not sufficiently. (A–E) Co-housing wild-type with TLR4 KO mice. (A) Timetable of co-housing and DSS administration in TLR4 KO mice. TLR4 KO Mice were co-housed with wild-type mice for 7 days, then DSS was treated for 8 days, and was exchanged with drinking water for 2 days until the endpoint of the experiment. (B) Body weight changes. (C) Histopathology and disease activity index. Representative images of periodic acid-Schiff (PAS) staining of the colon. Original magnification: x200. (D) Histological score. (E) Goblet cell score. (F–J) Transplantation of fecal microbiota into TLR4 KO mice. (F) Timetable of fecal microbiota and DSS administration in TLR4 KO mice. Mice were orally administrated with fecal microbiota daily for 7 days after DSS treatment and DSS was changed with drinking water for 2 days until the endpoint of the experiment. (G) Body weight changes. (H-J) Histopathology and disease activity index. (H) Disease activity index and representative images of periodic acid-Schiff (PAS) staining of the colon. Original magnification: x100. (I) Histological score. (J) Goblet cell score. (K) $F4/80^+$, $CD11b^+$, $CD206^+$ macrophage in PCCs. (L-N) Timetable of fecal microbiota transplantation and DSS administration in MyD88 KO mice. Mice were orally administrated with FM daily for 7 days after DSS treatment and DSS was changed with drinking water for 2 days until the endpoint of the experiment. (L) Timetable of fecal microbiota transplantation and DSS administration in MyD88 KO mice. (M) Body weight changes. (N) Disease activity index. (O) Representative images of PAS staining of the colon. Original magnification: x100. Data are expressed as the mean ± S.E.M. (n = 4). water, treated with drinking water; DSS, injected with dextran sulfate sodium; IgG- or α-TREM-1-Co, TLR4 KO mice co-housed with wild-type mice treated with IgG or α-TREM-1; IgG- or α-TREM-1-FM, fecal microbiota transplanted groups extracted from IgG or α-TREM-1 treated wild-type mice. $*p < 0.05$, $**p < 0.01$, $***p < 0.005$. Analyses were performed using one-way ANOVA with Tukey's posttest.

3. TREM-1 agonist decreases bacterial load and modulates neutrophil phenotype.

Colitis induced by DSS is characterized by epithelial damage and a neutrophilic infiltrate.[24] Next, to closely investigate the effects of α-TREM-1 on neutrophils and macrophages, I injected α-TREM-1 into the peritoneum of TLR4 KO mice and isolated neutrophils and macrophages following the treatment. The isolated cells were injected i.p. into DSS-treated mice. Although not statistically significant, DSS-induced colitis was unaffected by transplantation of IgG-treated neutrophils or macrophages. And, same phenomenon was also observed on the mice receiving α-TREM-1-treated neutrophils or macrophages. Nonetheless, there were little bit increased colon length in the mice receiving α-TREM-1-treated neutrophils compared to the mice transplanted with IgG-treated neutrophils (**Figure 5A**). The mice injected with α-TREM-1-stimulated macrophages and neutrophils had reduced histological score (**Figure 5B–5D**). The expression of *Hmgb1* was significantly higher in the groups receiving α-TREM-1-treated neutrophil than IgG-treated neutrophil. By contrast, the expression of *Trem1*, *Defb4*, and *Cd300a* in groups receiving α-TREM-1-treated neutrophil and macrophage was significantly reduced compared to IgG-treated neutrophil (**Figure 5E**). And, the expression of *Bai1* was not presented any significant differences between DSS-treated all groups. These distinct changes in several gene expressions of neutrophils suggest that the α-TREM-1-treatment may modulate the phenotype of these colitogenic neutrophils in addition to macrophages. To confirm neutrophil function in the anti-colitic effect by α-TREM-1, I tried to deplete neutrophil using Ly6G antibody in DSS-induced colitis. Observed any differences were in the colon with respect to the DAI between DSS+IgG and DSS+Ly6G+α-TREM-1groups (**Figure 5F**, **5G**). As shown in histopathological analysis, the anti-colitic effect of α-TREM-1 completely blocked in Ly6G-treated groups (**Figure 5H–5J**), indicating the protective effect of neutrophils in therapeutic effect of α-TREM-1. To investigate modulation of neutrophil by α-TREM-1 in vitro, I stimulated HL-60 cells with α-TREM-1 in an inflammatory condition. α-TREM-1-treated HL-60 cells showed dramatically increased neutrophil extracellular trap formation by LPS (**Figure 6A**). As lysosomes may increase in abundance independently of autophagy and microtubule-associated protein 1A/1B chain 3B (LC3), an ubiquitin-like protein,[25] is converted during autophagy to a lipid-bound form (LC3-II) associated with autophagosome membranes, I confirmed those results by measuring the endogenous processing of lysosomes and LC3 using anti-LC3-II antibody and western blotting. α-

TREM-1-treated HL-60 cells showed increased autophagy (**Figure 6B, 6C**). Interestingly, α-TREM-1-treated HL-60 cells increased IL-22 expression compared to controls. (**Figure 6D**). To further confirm these results, I isolated neutrophils from the bone marrow (**Figure 6E**) and stimulated bone marrow-derived neutrophils (BMDNs) with α-TREM-1 in an inflammatory condition. α-TREM-1-treated BMDNs showed dramatically increased NET formation by LPS (**Figure 6F** upper). I measured the endogenous processing of lysosome and LC3 using lysosome marker anti-LAMP-1 and anti-LC3-II antibodies. α-TREM-1-treated BMDNs showed increased autophagy (**Figure 6G** upper). I hypothesised that increased NET formation and autophagy would be affected by TREM-1 stimulation. Therefore, I knock down TREM-1 by using siRNA targeting TREM-1 gene. When TREM-1 was knocked down, NET formation was decreased, despite TREM-1 stimulation (**Figure 6F** lower and **Figure 6G** lower). α-TREM-1 treatment with LPS increased mRNA expression of *Il22* as well as *Reg3g* in BMDNs from wild-type mice but not in TLR4 KO mice (**Figure 6H**). Because neutrophils do function cooperatively with macrophages, I investigated the effects of α-TREM-1 on the interaction between neutrophils and macrophages.





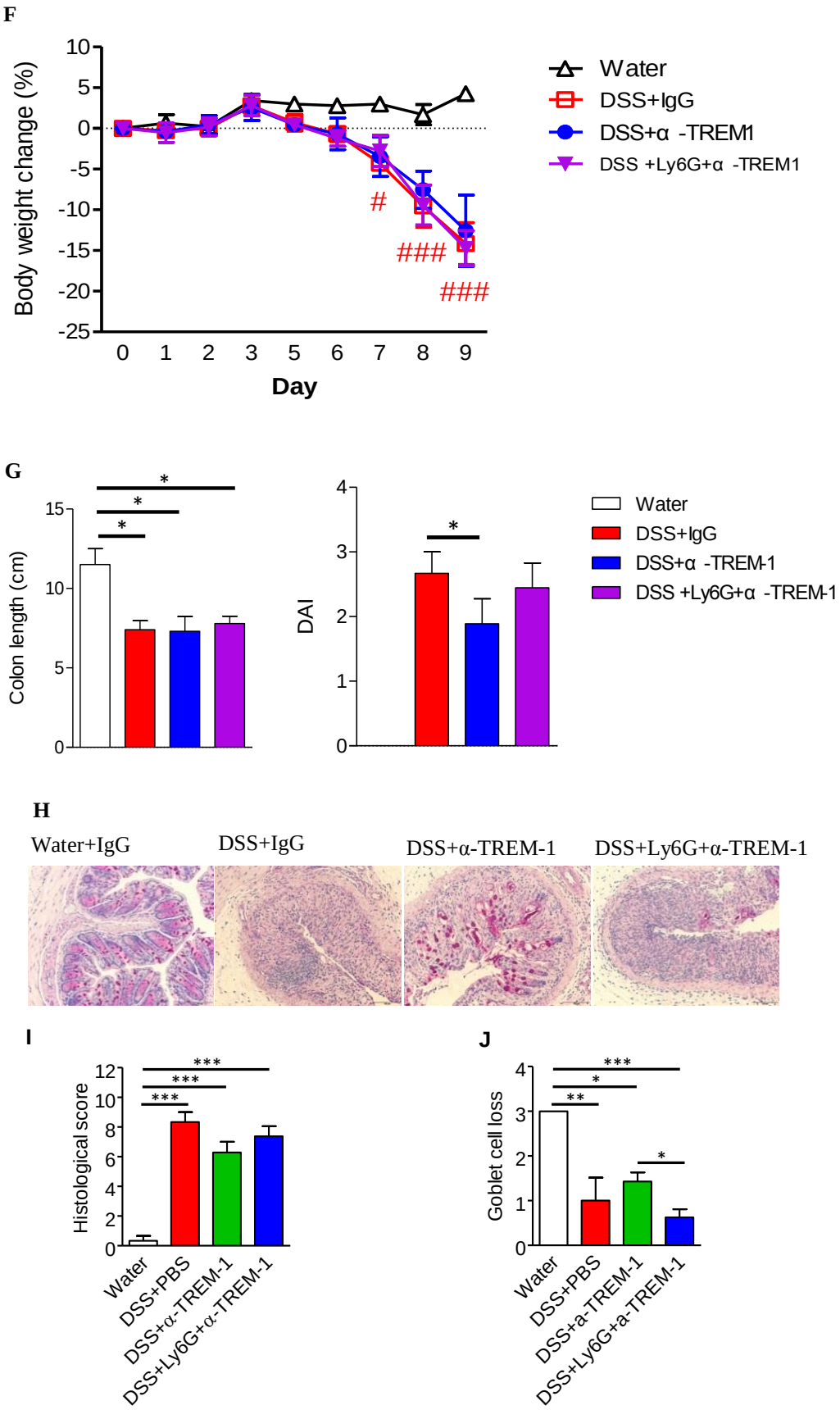
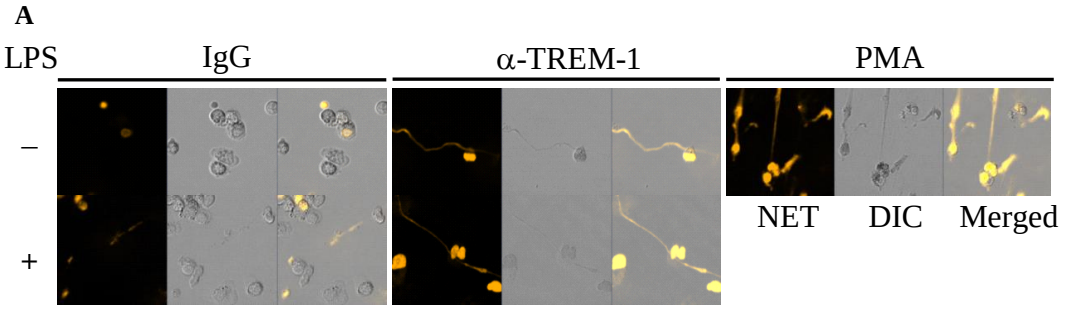
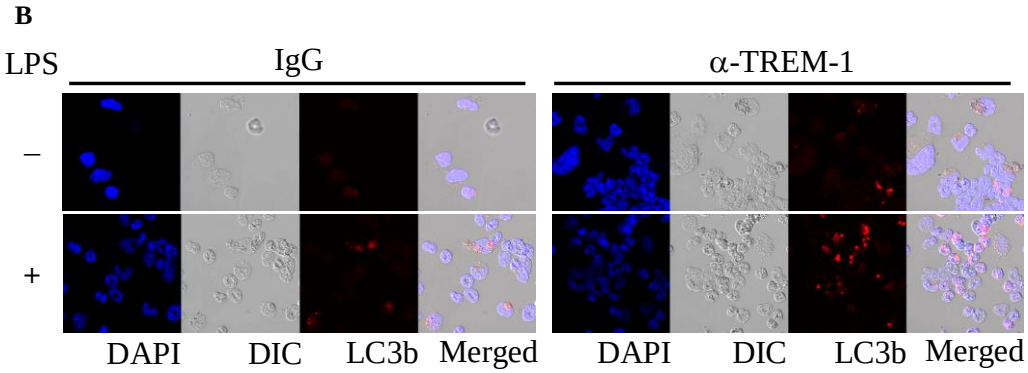
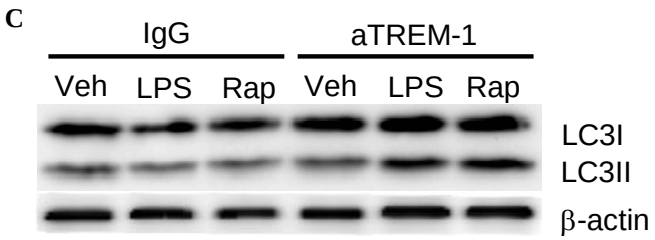
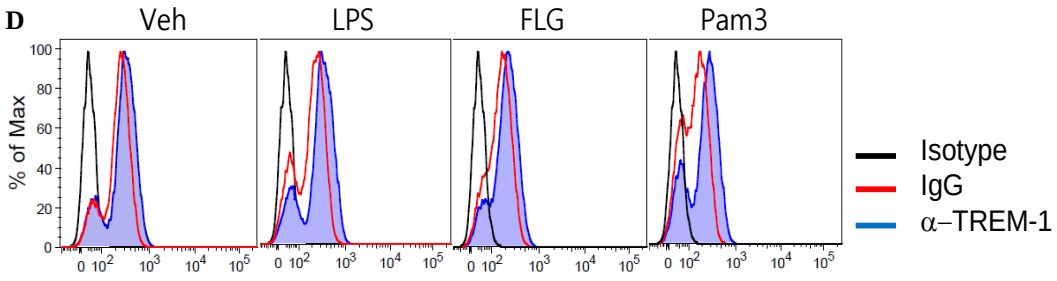


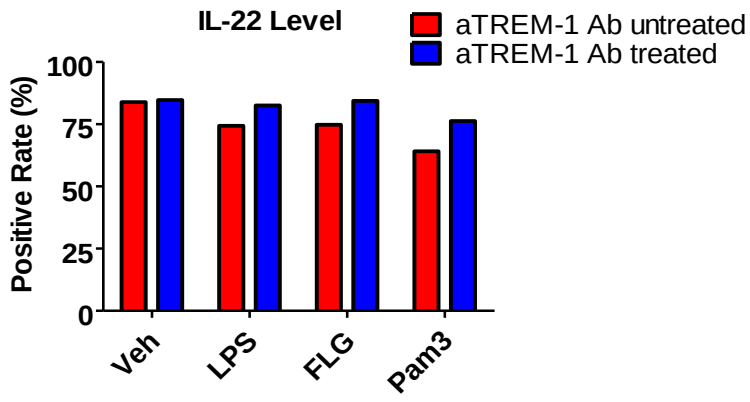
Figure 5. TREM-1 agonist alters the function of neutrophil and macrophages in colitis. (A–D) Transplantation of neutrophils and macrophages isolated from the peritoneal cavity of TREM-1 agonist or IgG-treated mice. (A) Colon length. (B–D) Histopathology. (B) Representative images of periodic acid-Schiff (PAS) staining of the colon. Original magnification: x200. (C) Histological score. (D) Goblet cell score. (E) The transcript level of *Tnfa, Hmgb1, Cd206, Trem1, Defb4, Cd300a, and Bai1* in the colon. (F–J) Neutrophil depletion affects TREM-1 agonist effect. (F) Body weight changes. (G) Colon length and disease activity index. (H–J) Histopathology. (H) Representative images of periodic acid-Schiff (PAS) staining of the colon. Original magnification: x200. (I) Histological score. (J) Goblet cell score. Data are expressed as the mean ± S.E.M. (n = 4). Water, treated with drinking water; DSS, injected with dextran sulfate sodium; IgG- or α-TREM-1-Neu, peritoneal neutrophil-transplanted-TLR4 KO mice groups extracted from IgG or α-TREM-1 treated wild-type mice; IgG- or α-TREM-1-MAC, peritoneal macrophage-transplanted-TLR4 KO mice groups extracted from IgG or α-TREM-1 treated wild-type mice; α-TREM-1, treated with α-TREM-1; Ly6G, neutrophil-depleted group. $*p < 0.05$, $**p < 0.01$, $***p < 0.005$. Analyses were performed using one-way ANOVA with Tukey's posttest.

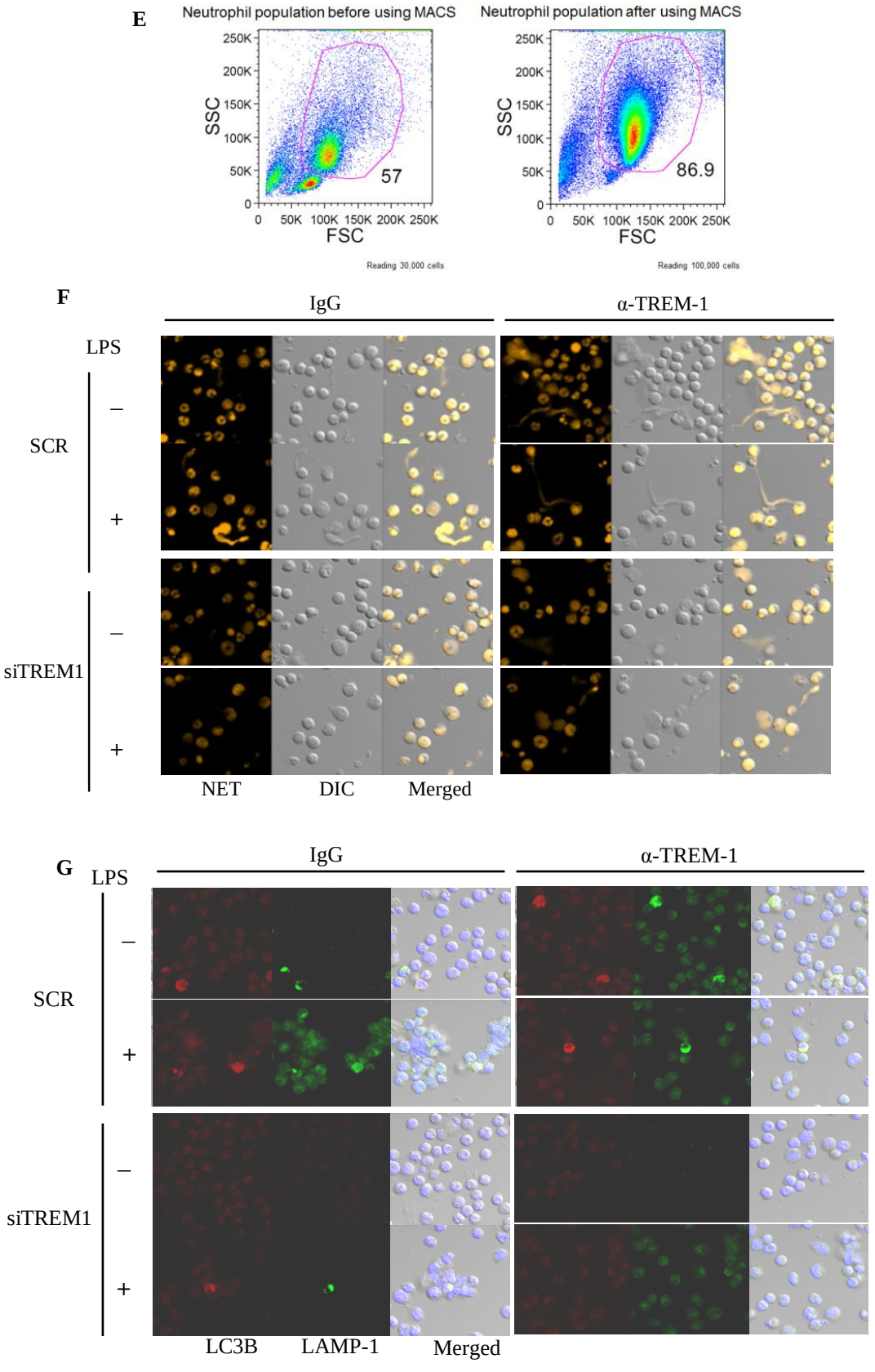












H

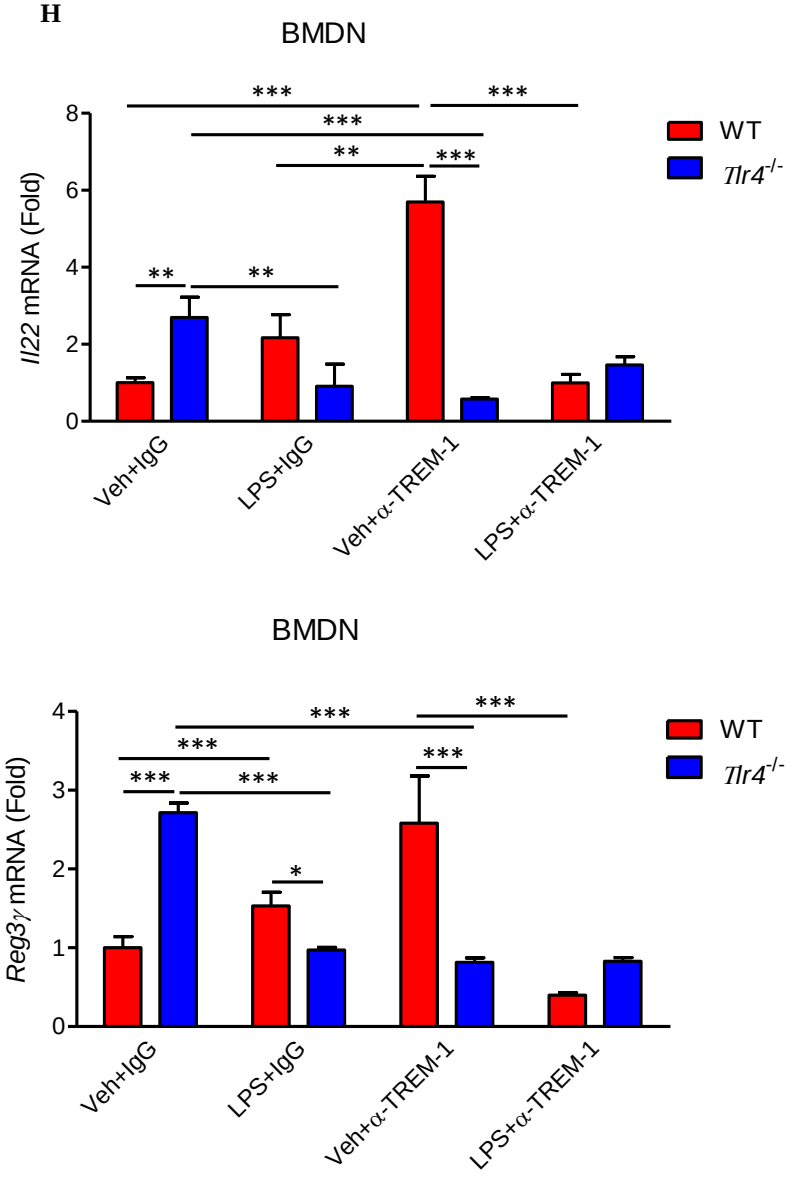


Figure 6. TREM-1 agonist promotes bacteria handling of the neutrophil. (A) Evaluation of neutrophil extracellular trap. The NET assay was performed. (B) Evaluation of autophagy. (C) Western blot of LC3II was carried out. (D) IL-22 expression in neutrophils. Flow cytometry analysis was used to evaluate the IL-22 expression in neutrophils ($F4/80^{-}Cd11b^{+}Ly6G^{+}$) populations. Numbers indicate frequencies of IL-22 fractions among neutrophils. (E) Neutrophil population by flow cytometry before and after MACS sorting. (F) Evaluation of NETosis. The NET assay was performed after TREM-1 knocked down. (G) Comparison of autophagy between TREM-1 knocked down cells by using siTREM-1 and those with scrambled siRNA. (H) Gene expression profiles. Transcript levels of *Il22*, *Reg3g* were evaluated by quantitative RT-PCR. Data are expressed as the mean ± S.E.M. (n = 4). PMA, treated with Phorbol-12-Myristate-13-Acetate; Rap, treated with rapamycin as the positive control of LC3-II; FLG, treated with flagellin; Pam3, treated with Pam (3) CSK (4). *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$. Analyses were performed using one-way ANOVA with Tukey's posttest.

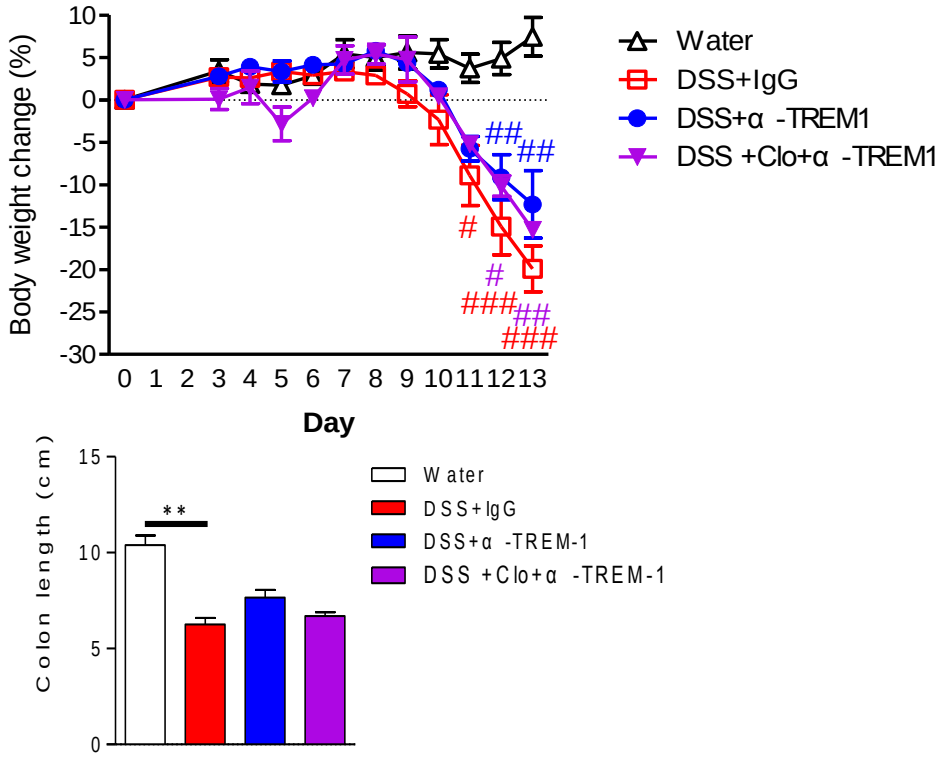
4. Macrophage stimulated with TREM-1 agonist is required to boost neutrophil function

Next, to investigate the role of macrophages in α-TREM-1, I depleted macrophages in mice using clodronate liposomes. The vehicle-treated mice pretreated with control (PBS) liposomes and α-TREM-1 had less increased colitis severity than α-TREM-1-treated mice pretreated with clodronate-encapsulated liposomes, indicating that systemic macrophage depletion deteriorates DSS-induced colitis in mice (**Figure 7A–7C**). Increases of *Inos* and *Il1b* expressions by α-TREM-1 were abolished in clodronate-treated groups, but *Tnfa* and *Tlr4* expressions were increased compared with DSS+α-TREM-1 groups (**Figure 7D**).

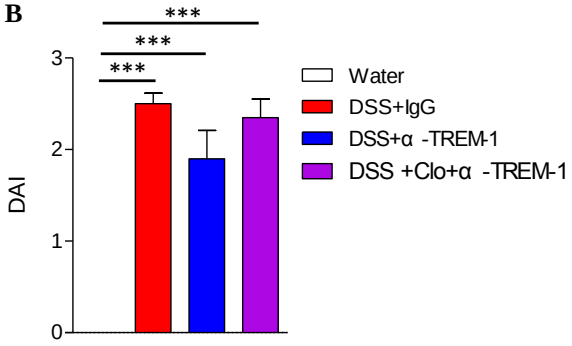
Phagocytosis and lysis of bacteria with potent bactericidal proteins and ROS are essential for clearance of bacteria.[26] TREM-1 expression on macrophages is well known to be upregulated in response to LPS and other inflammatory stimuli.[8,13,27] To confirm these effects and the synergetic effects α-TREM-1 on TLR4 signaling, I pretreated α-TREM-1 (800 ng/mL) for 30 min and then stimulated BMDM with LPS (200 ng/mL) and for 4 h to rule out any direct interactions with LPS as the cause of the inhibitory activity. α-TREM-1-stimulation with LPS increased M1 polarized macrophages (**Figure 7E**). Similar to those of animal experiments, α-TREM-1 further increased the *Inos* and *Il1b* expression by LPS treatment alone or LPS and MDP co-treatment (**Figure 7F**, **7G**). In addition, acidotropic staining intensity was steadily enhanced during LPS and MDP with TREM-1 agonist antibodies treatment. However, when TREM-1 gene was knocked down by siRNA targeting TREM-1, acidotropic staining intensity was vanished (**Figure 7H**). Of note, LPS stimulation with MDP showed all the more decrease in lysotracker stain, which was consistent upregulation of *Irakm*, a negative regulator of TLR signaling, and *Trem1* expression (**Figure 7I**). Consistent with RT-PCR results, western blotting showed increased showed further increased accumulation of LC3-II as well as iNOS by α-TREM-1 (**Figure 7J**). There are suggestions that peptidoglycans on the surface of gram-positive bacteria and endotoxins on gram-negative bacteria may be involved in the stimulation of TREM-1.[12,28,29] α-TREM-1 further increased the *Inos* expression by peptidoglycan (TLR2 ligand) or Pam3CSK4 (TLR1/2 synthetic agonist) treatment but further decreased *Cox2* expression (**Figure 7K**). To further evaluate this phenomenon using live bacteria, I infected RAW264.7 cells with GFP-expressing *S. typhimurium*. GFP and lysotracker signals indicating autolyososome were increased, indicating increased phagocytosis. Furthermore, I observed significantly higher GFP signal that localize to lysosomes in α-TREM-1-treated groups, indicating increased fusion of phagosome containing *S.*

typhimurium with lysosome that confirms decreased bacterial survival in α-TREM-1-treated groups (**Figure 7L)**. *Trem1*, *Il10*, *Cd206*, *Nlrp3*, and *Nlrc4* expressions on RAW264.7 cells were strongly upregulated by LPS stimulation and this effect was more amplified by α-TREM-1 (**Figure 7M**) as previously reported[8,13] in company with *Il10* and *Cd206* expression. To confirm these results, I conducted an experiment in human macrophages, THP-1 cells. α-TREM-1 stimulation in THP-1 cells, upregulated *NLRC4* but not *NLRP3* by TLR ligands and upregulated *NLRP3*, but down-regulated *NLRC4* by Pam3 (**Figure 7N**). To further investigate the involvement of TLR4 signaling in this phenomenon, I treated TLR4 inhibitor, CLI-095, in RAW264.7 cells. When I blocked TLR4 signaling, the strongly upregulated *Tnfa* and *Trem1* by LPS stimulation were down-regulated as well as *Il10* and *Cd206* (**Figure 7O**).

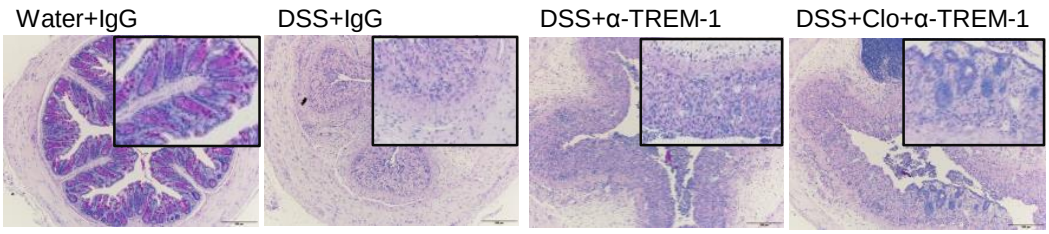
A

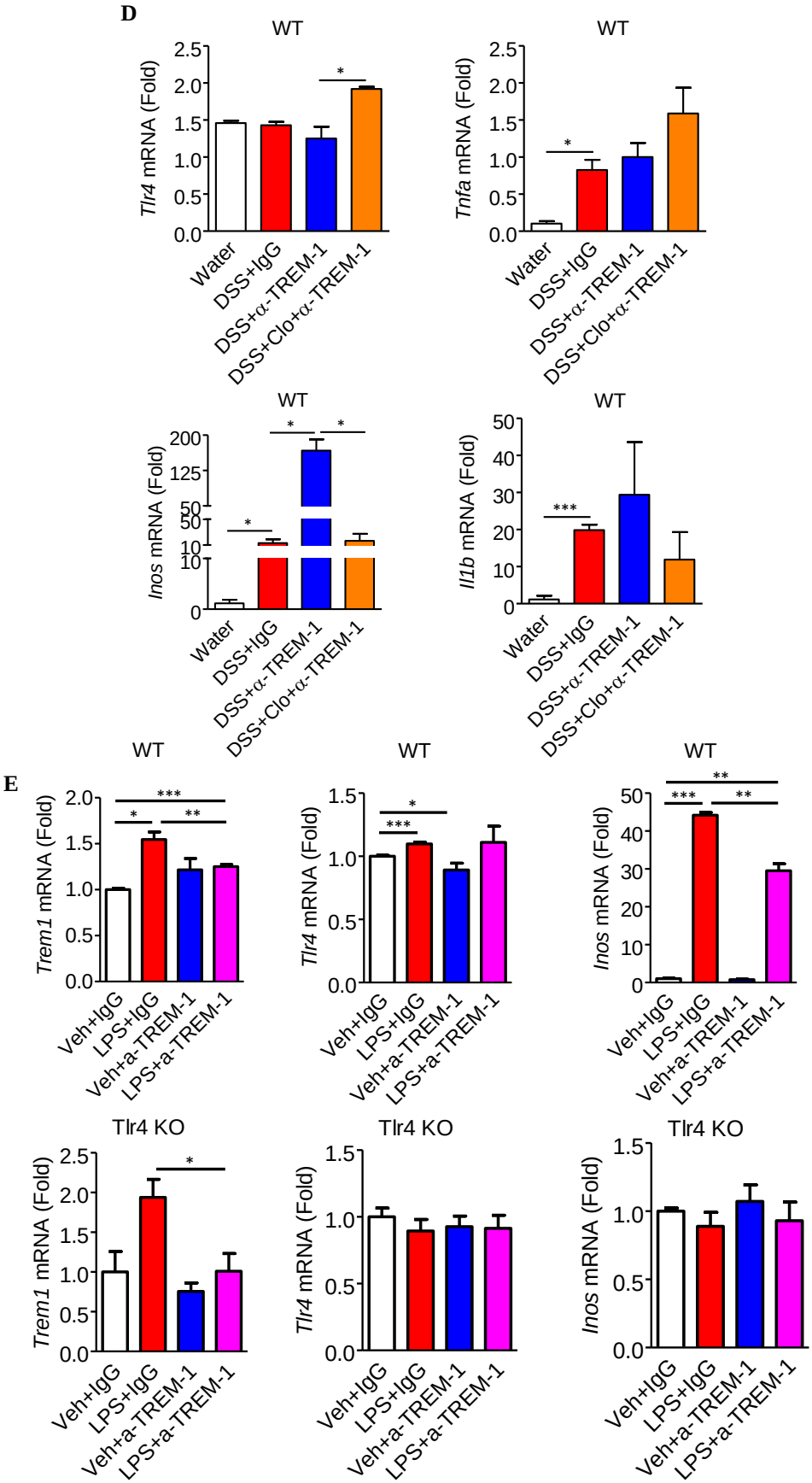


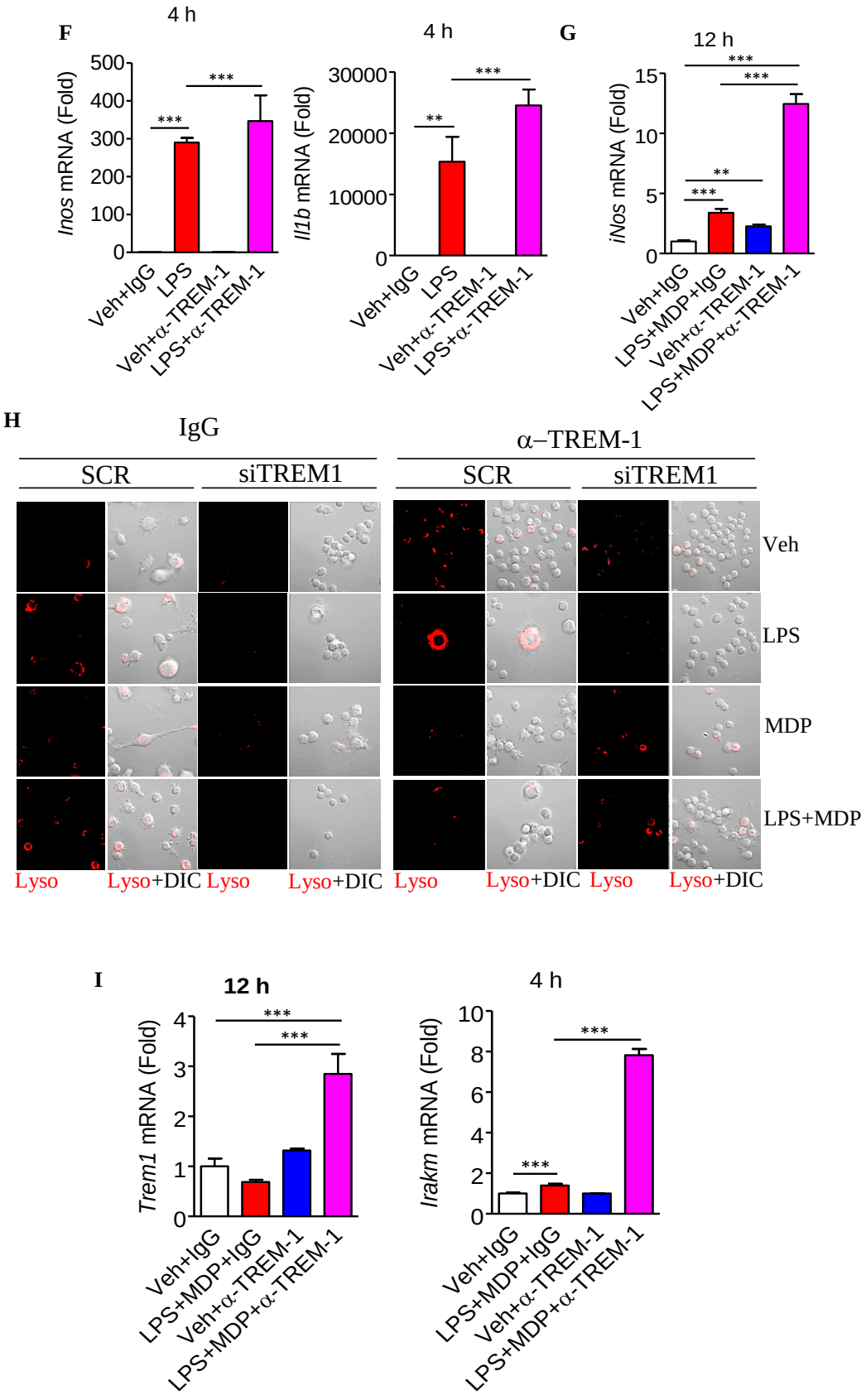
B

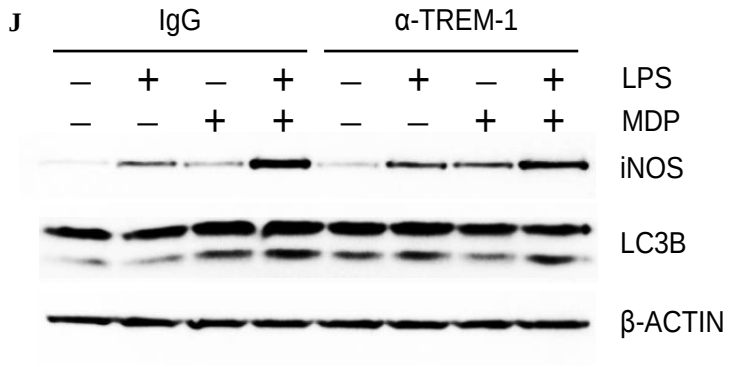


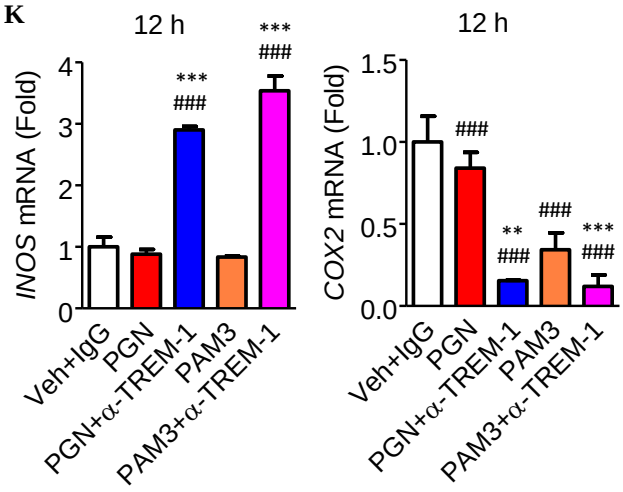
C

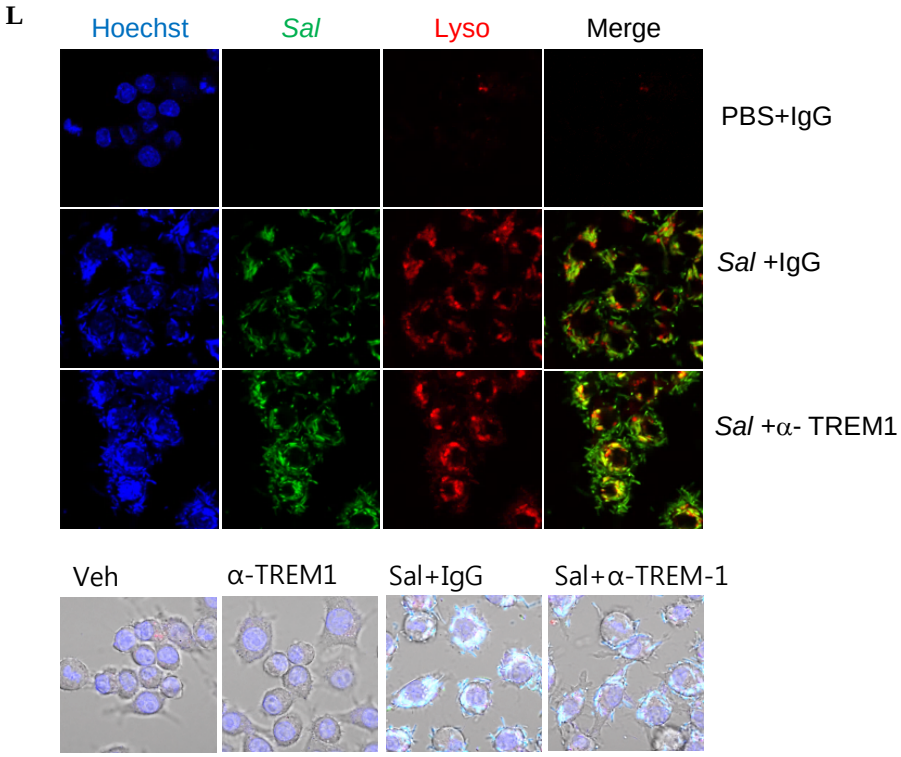




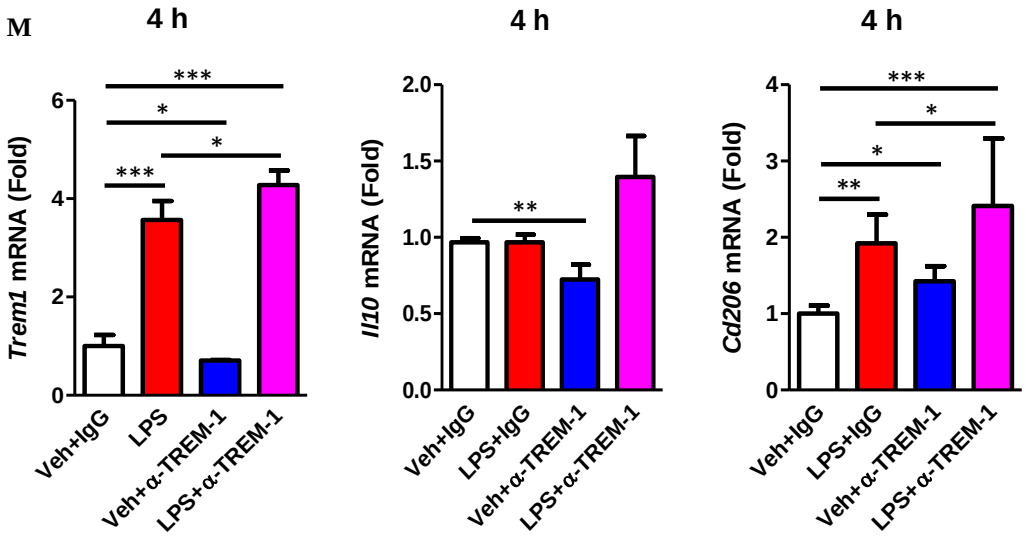


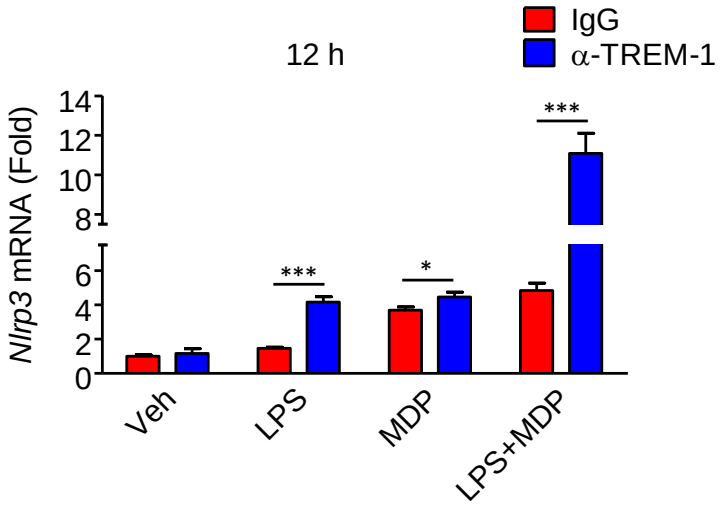


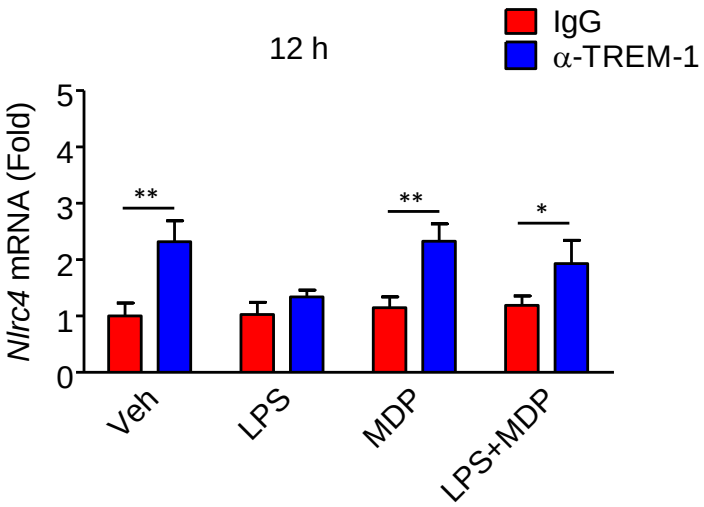




M







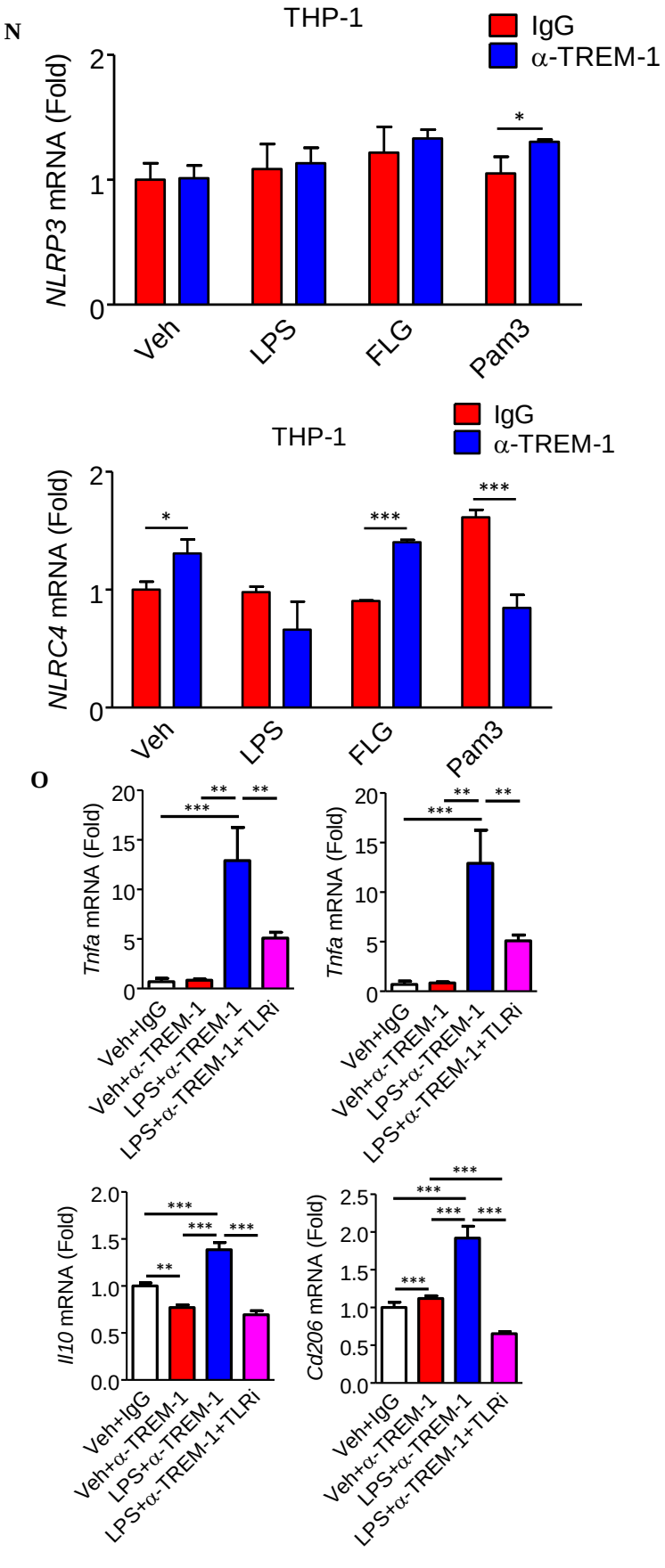


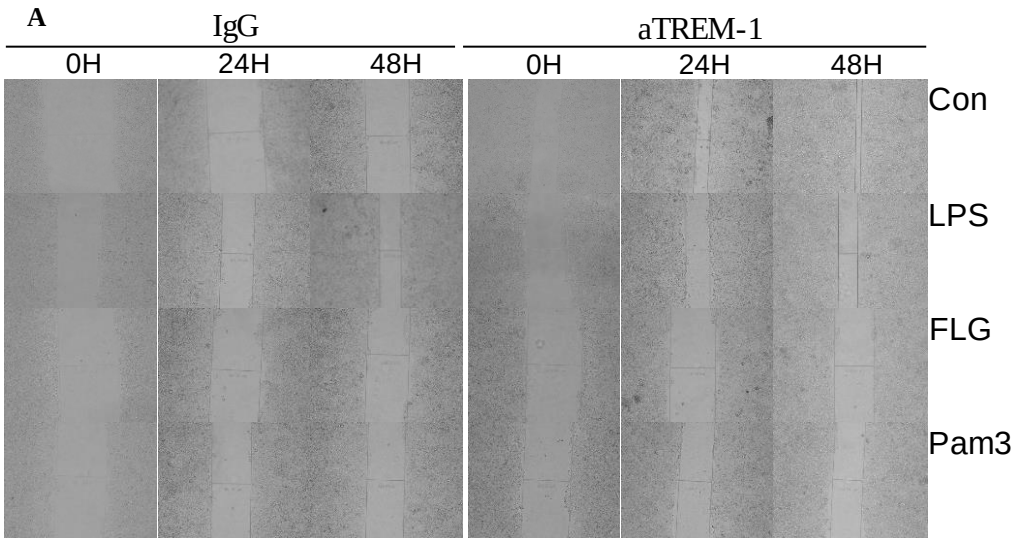
Figure 7. Macrophage is required to boost neutrophil function by TREM-1 agonist. (A–E) Macrophage depletion affects anti-colitic effects of the TREM-1 agonist. (A) Body weight changes. (B) Disease activity index. (C) Histopathology. Representative images of periodic acid-Schiff (PAS) staining of the colon. Original magnification: x200. (D) Expression profiles of proinflammatory genes including *Tnfa*, *Tlr4*, *Inos*, and, *Il1b* in colon tissue. Transcript levels were evaluated by quantitative RT-PCR. (E) Expression profiles of proinflammatory genes including *Tlr4, Trem1,* and, *Inos* in bone marrow-derived macrophages of wild-type and TLR4 knockout mice. Transcript levels were evaluated by quantitative RT-PCR. (F-G) Transcript levels of genes related to bacterial handling (*Inos*, *Il1b*, and *Inos*) in RAW264.7 cell after (F) 4h and (G) 12h from treatment. (H) Evaluation of lysosome activation in LPS stimulation whether treated siTREM-1 and siSCRAMBLE. (I) Transcript levels of genes related to inhibition of TLR signaling (*Irakm, Trem1*). (J) Immunoblotting for autophagy protein, LC3-II, and iNOS based on β-actin. (K) Expression profiles of pro-inflammatory genes (*Inos, Cox2*). (L) Evaluation of lysosome activation and clearance of *S. typhimurium.* (M) Expression profiles of anti-inflammatory genes in RAW264.7 cells (*Trem1, Il10,* and, *Cd206*) and inflammasome gene (*Nlrp3 and Nlrc4*). (N) Expression profiles of inflammasome genes in THP-1 cells (*NLRP3 and NLRC4*). (O) Expression profiles of pro- and anti-inflammatory genes in RAW264.7 cells treated with TLR4 inhibitor (*Tnfa, Trem1, Il10,* and, *Cd206*). Data are expressed as the mean ± S.E.M. (n = 4). Water, treated with drinking water; DSS, injected with dextran sulfate sodium; Clo, treated with clondronate-encapsulated liposomes; α-TREM-1, treated with α-TREM-1; MDP, treated with muramyl dipeptide; PGN, treated with peptidoglycan; Pam3, treated with Pam (3) CSK (4); LPS, treated with lipopolysaccharide. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$. Analyses were performed using one-way ANOVA with Tukey's posttest.

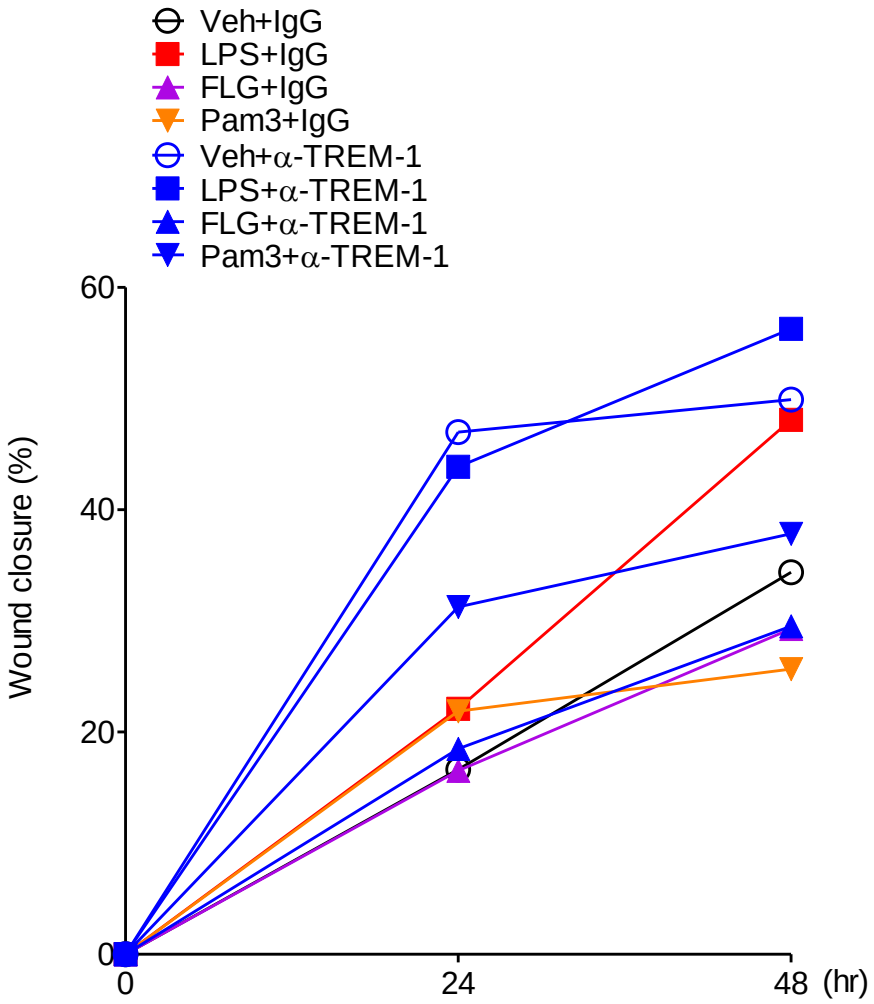
.

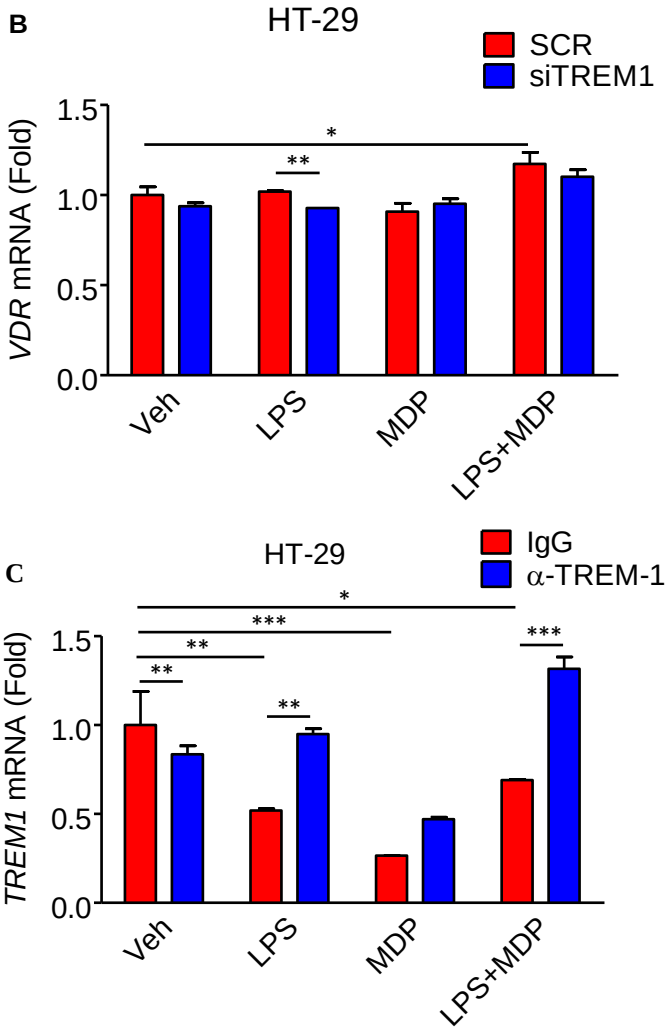
5. TREM-1 inhibits apoptosis and prolongs survival of macrophage and intestinal epithelial cell and protect against colonic damage

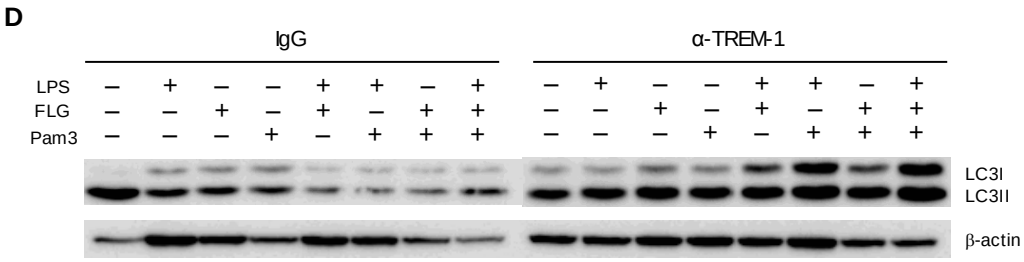
The effects of α-TREM-1 through rectal administration suggest that α-TREM-1 can affect directly intestinal epithelial cells. Next, I performed a wound healing assay to evaluate the effects of α-TREM-1-treated neutrophils on wound repair. Scraped HT-29 cells were cultured in culture media of IgG- or α-TREM-1-pretreated HL-60 cells with LPS, FLG, or Pam3. I found that culture media from α-TREM-1-alone- or α-TREM-1+LPS-groups mediated significantly greater wound closure in the epithelial cells compared with IgG-groups, whereas α-TREM-1+FLG- or α-TREM-1+Pam3-groups showed no difference compared with IgG-groups (**Figure 8A**). However, α-TREM-1-treated HT-29 cells in normal culture media showed no differences between groups in wound healing (data not shown). Vitamin D receptor expression was decreased in TREM-1 knock-down cells compared to control cells when only treated with LPS (**Figure 8B**). The *Trem1* expression is synergistically increased in LPS+MDP groups compared to LPS or MDP alone group when HT-29 cells were cultured with culture media from α-TREM-1-treated HL-60 cells (**Figure 8C**). Western blotting showed further increased accumulation of LC3-II in IEC by culture media from α-TREM-1-treated HL-60 cells, suggesting that a factor in neutrophils induced by α-TREM-1 confer steady accumulation of LC3-II (**Figure 8D**). Similar results were observed when HT-29 cells were cultured in culture media of IgG- or α-TREM-1-treated THP-1 cells with LPS, flagellin, or Pam3 (**Figure 8E**).

In an animal model, goblet cell restoration was observed in α-TREM-1-treated groups. To find the factors promoting goblet cell differentiation, I treated α-TREM-1 in HT-29 cells. No goblet cell differentiation was observed in α-TREM-1-treated HT-29 cells (data not shown). The TREM-1 expression in HT-29 cells promoted the production of antibacterial molecules TREM-1 receptor mRNA and protein were detectable in HT-29 cells. These results demonstrate that only myeloid cells but also gastric epithelial cells are able to express the TREM-1 receptor. Rakoff-Nahoum *et al.*[30] recently, showed increased epithelial injury in TLR4 KO and MyD88 KO mice in the setting of reduced epithelial proliferation. Since the effect of a-TREM1 on anal treatment was demonstrated in animal models, I speculate that TREM-1 can directly influence on IECs in terms of wound repair.









E

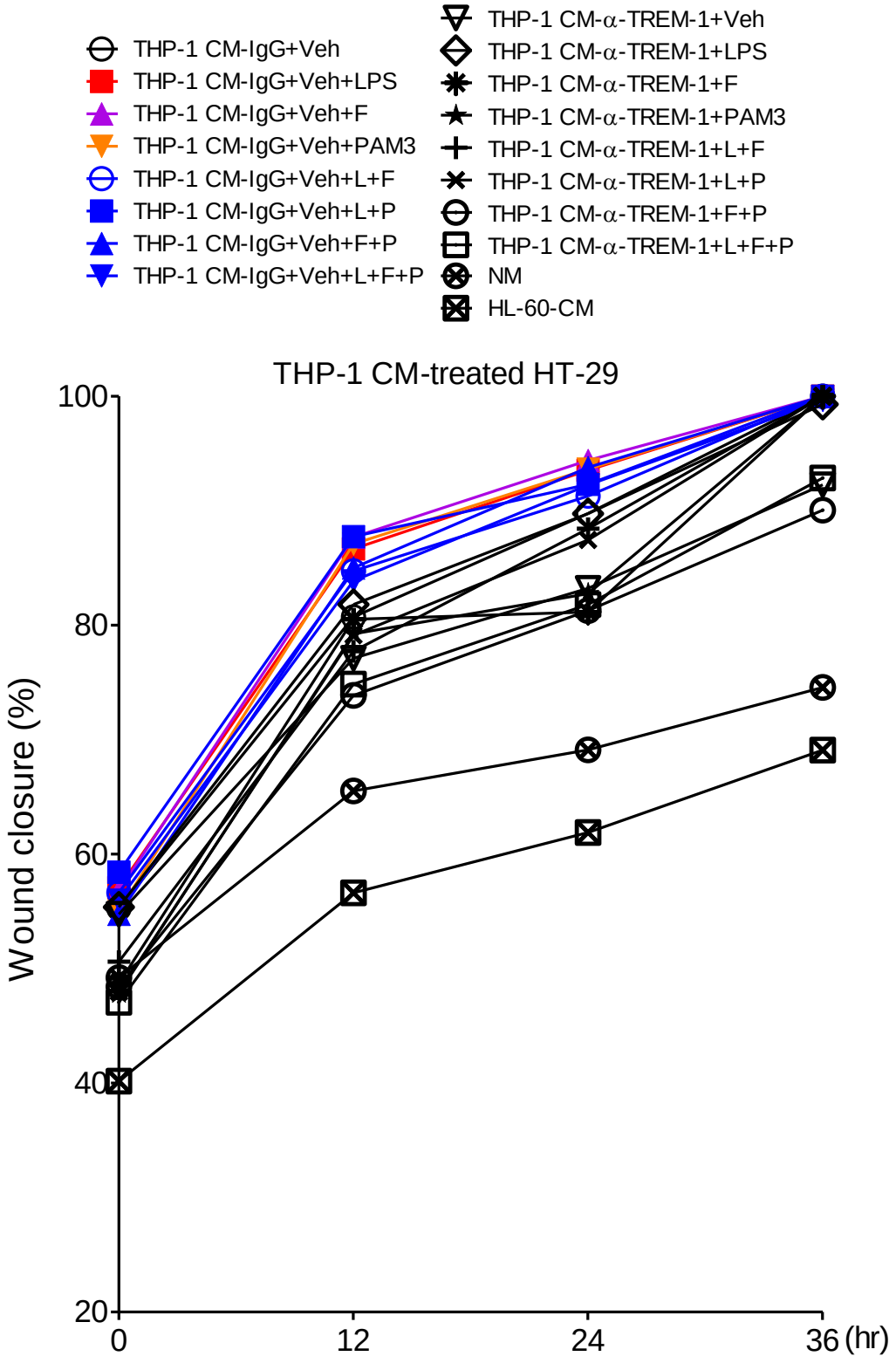


Figure 8. TREM-1 agonist confers wound healing with cooperation with neutrophil and macrophage. (A) Wound healing assay. HT-29 cells were cultured in conditioned media from HL-60 cells treated with TREM-1 agonist and wound healing assays were performed. (B) Comparison of *VDR* expression between TREM-1 knocked down cells by using siTREM-1 and those with scrambled siRNA. Transcript levels of *Vitamin D Receptor.* Transcript levels were evaluated by quantitative RT-PCR. (C) Transcript levels of *TREM1*. Transcript levels were evaluated by quantitative RT-PCR. (D) Evaluation of autophagy. Western blotting of LC3II was carried out. (E) Wound healing assay. HT-29 cells were cultured in conditioned media from THP-1 cells treated TREM-1 agonist and wound healing assays were performed. Data are expressed as the mean ± S.E.M. (n = 4). α-TREM-1, treated with α-TREM-1; MDP, treated with muramyl dipeptide; FLG, treated with flagellin; Pam3, treated with Pam (3) CSK (4); LPS, treated with lipopolysaccharide. $*p < 0.05$, $**p < 0.01$, $***p < 0.005$. Analyses were performed using one-way ANOVA with Tukey's posttest.

Ⅳ. DISCUSSION

In IBD, the intestinal flora can penetrate the intestinal mucosa.[31] Defects of antibacterial autophagy in Crohn's disease can lead to delayed clearance of intestinal microbiota penetrating the epithelial barrier, which might ultimately give rise to granuloma formation.[32] Along with this, single nucleotide polymorphisms (SNPs) in genes involving microbial sensing (*Nod2, Nfκb1, Card9* etc) and bacterial handling pathways (*Atg16l1, Irgm* etc), and integrating antimicrobial adaptive immune responses (*Il23r, Il10, Il12, Jak2, Stat3* etc) were associated with enhanced risk of IBD.[33,34] In the normal human intestine, TREM-1 mRNA was generally absent and it was consistently expressed at high levels in affected segments from CD and Ulcerative colitis patients with active disease, whereas in the macroscopically unaffected tissue samples obtained from sites adjacent to the affected area in patients with CD and in tissue samples with inactive disease from patients with UC, TREM-1 mRNA expression is substantially reduced and barely detectable.[16] In the previous study, TREM-1 has hardly detectable in non-microbial in non-microbial inflammations but is strongly upregulated by extracellular bacteria.[8] TREM-1/3–deficient neutrophils had a 50% reduction in ROS production in lung infection[35] and TREM-1 KO mouse diminished clearance of *K. pneumoniae* in the small intestine.[36] Neutrophils eliminate *P. aeruginosa* through a number of O_2-independent pathways.[37] LP-17, a 17-mer TREM-1 binding peptide for blocking TREM-1 signaling, may play a role in blocking neutrophil migration.

TREM-1 administrated mice display macroscopically enlarged ceca, phenotyping antibiotics-administrated mice. Treatment of mice with the anti-TREM-1 antibody enhanced clearance of bacteria and increased survival.[38] In the small intestine, TREM-1 stimulation can also enhance bacterial clearance and promote resolution of infection,[38] whereas and impaired bacterial clearance causes enhanced *K. pneumoniae* translocation.[36] TREM-1-mediated bacterial clearance in the small intestine is an important immune response against *K. pneumoniae*.[36] The early production of the proinflammatory cytokines TNF-α and IL-1 is crucial for bacterial clearance from the pulmonary compartment and, consequently, survival.[39]

To ensure specificity of the agonistic TREM-1 mAb, I used three different antibodies. I showed that α-TREM-1 promotes clearance of bacteria and alteration in bacterial profile. α-TREM-1 significantly decreased the proportion of *Bacteroides* that induce colitis.[40] The iNOS facilitates macrophage to generate NO, a highly reactive free radical with

antimycobacterial properties, in phagosome and clear infected bacteria.[41] In our data, restoration of dysbiosis may be due to the reduced bacterial burden via bacterial clearance by expressing *Inos* when the macrophages were activated by α-TREM-1.

Activation of TREM-1 stimulates the production of proinflammatory mediators such TNF-α, MCP-1, granulocyte-monocyte colony–stimulating factor, and M1 proinflammatory cytokines,[15, 26] which enables TREM-1 to contribute to innate immunity.[12,13] A common feature of TREM-1 downstream signaling is the link with the phosphorylated DNAX activation protein 12, growth receptor binding protein 2, and phosphatidylinositol-3 kinase to amplify TLRs.[24, 28, 42] Proinflammatory effects of TREM-1 may be further enhanced in the presence of TLR-mediated signaling.[23] Mammalian cells produce antimicrobial peptides such as cathelicidin in neutrophils and epithelia.[43]

Induction of autophagy rescues bacterial handling in the macrophages. It was reported that antibacterial autophagy initiated by the nucleotide-binding oligomerization domain (NOD2) - receptor-interacting serine/threonine-protein kinase 2 - X linked inhibitor of apoptosis protein pathway is a key defect in disorders presenting with granulomatous intestinal inflammation and dysregulated cytokine response is the consequence of incomplete bacterial clearance and pharmaceutical induction of autophagy can restore bacterial killing, suggesting its potential therapeutic strategy.[32] Here, I showed that TREM-1 activation affects autophagy. I observed that TREM-1 stimulation with LPS induces LPS-induced alternative autolysosome formation and resulted in normal bacterial degradation.

Neutrophils are short-lived effector cells in innate immune system and most abundant leucocyte population in the blood, which also typically the first leucocytes to be recruited into the inflammatory areas.[44] PMN migration across epithelial monolayers is often associated with barrier defects,[45] epithelial injury, and crypt abscesses formation.[46] However, as evident from recent work, PMNs may also play important temporal roles in the resolution of inflammation and healing processes. For example, PMNs secrete lipid mediators, including lipoxins, resolvins, and protectins that facilitate tissue healing.[47] Furthermore, PMN migration across lung epithelial cells triggers transcriptional activation of b-catenin and its target genes,[48] suggesting that PMNs, through interactions with IECs, can contribute to the regulation of epithelial cell proliferation. Specifically, the apically expressed epithelial proteins CD44v6 and CD55 have both been shown to regulate PMN transepithelial migration.[49] Intercellular adhesion molecule-1 (ICAM-1)

expression was found to facilitate PMN adhesion and retention at the apical epithelial membrane in inflamed intestines.[50] Furthermore, ligation of ICAM-1 by migrating PMNs has been shown to signal cytoskeletal reorganization in both endothelial and epithelial cells, leading to alterations in barrier function.[50] In IECs, Akt acts upstream of b-catenin to induce signaling events that play key roles in regulating epithelial cell proliferation.[51] Here I found that TREM-1 stimulates the proliferation and differentiation of the epithelial cells, which augments the wound repair in the intestine.

Accordingly, numerous clinical examples of impaired neutrophil function are linked to increased rates of infection and death, including chronic granulomatous disease, neutropenic fever, and lymphocyte adhesion deficiency. Neutrophil depletion exaggerated TNBS-induced colitis in rats.[52] TREM-1 stimulation induces a rapid neutrophil degranulation and oxidative burst[11] and TREM-1 is also required for neutrophil migration in lung inflammation.[35] Neutrophils, the most abundant circulating leukocyte, play a diverse role in response to infection using ROS intermediates, antimicrobial peptides, NETs, and numerous pattern recognition receptors (PRRs).[53] NET is a complex lattice of extracellular fibers, primarily composed of decondensed chromatin, with bound antimicrobial proteins.[54] This study showed that TREM-1 mediated NET DNA bactericidal effect in vitro as the previous study described.[36] Consistently, I found that TREM-1 stimulation increased myeloperoxidase (MPO), an enzyme present in NET and also a marker of neutrophil activity. $CD177^{+}$ neutrophils demonstrated enhanced bactericidal activity with producing high levels of ROS, MPO, NET and antimicrobial peptides and they also produce low levels of proinflammatory cytokines but high levels of IL-22.[55] Neutrophil interactions with epithelial-expressed ICAM-1 enhances intestinal mucosal wound healing.[56] In IECs, Akt acts upstream of b-catenin to induce signaling events that play key roles in regulating epithelial cell proliferation.[51] Expression of ICAM-1 in IEC was found to facilitate neutrophil adhesion in inflamed intestines.[50] Furthermore, ligation of ICAM-1 by migrating PMNs has been shown to lead to alterations in barrier function.[50] Neutrophils have also been demonstrated to express hepatocyte growth factor, which can aid in tissue regeneration.[57] Compatible with these results, our data indicate that neutrophil modulation by TREM-1 stimulation can attenuate colitis through bacterial clearance and barrier function. TREM-1 siRNA decreases bacterial clearance by impairing the neutrophil oxidative burst.[58] I showed that α-TREM-1 increases phagocytosis, decreases circulating bacterial counts, and ameliorates colitis. Macrophages differentiate from circulating monocytes in the peripheral blood and

migrate into the gut to become resident macrophages. Resident macrophages change their phenotypes and their physiology according to various stimuli.[59]

Because lamina propria macrophages are important in gut immune homeostasis and early control of bacterial infection is dependent on macrophages,[60] macrophage-mediated innate immune response functions as the first line of host defense against a pathogen. Macrophages in CD express a number of pattern recognition receptors suggesting that defective sensing of bacteria may be important.[61] Decreased macrophage cytokine release may lead to poor neutrophil chemotaxis to the sites of mucosal breaches and to inadequate bacterial clearance and chronic inflammation.[62] Indeed, the diminished macrophage pro-inflammatory cytokine release in CD leads to impaired neutrophil chemotaxis and consequently bacteria breach the bowel wall.[62] Macrophages can phagocytose and eliminate intracellular microbes by multiple bactericidal mechanisms, including acidification of the phagosomes and delivering phagosomes to the lysosomes for degradation, generating bactericidal free radicals, such as reactive oxygen and nitrogen species, activating programmed cell death. A decreased macrophage ROS production is observed after activation with Phorbol-12-Myristate-13-Acetate in CD.[63] TREM-1 was upregulated on BMDM after stimulation with MyD88-dependent TLR agonists, such as LPS.[64] TREM-1 agonist does not enhance TLR-induced antimicrobial activity in monocytes, and may actually inhibit it. Instead, TREM-1 activation only made a very modest increase in phagocytic activity.[23]

Bacterial killing in macrophages is MDP-dependent.[32] Interestingly, MDP stimulation with LPS showed all the more decrease lysotracker stain, which was consistent upregulation of *Irakm* and *Trem1* expression. Gene expression omnibus profile analysis indicates that LPS suppresses *Atg5*, *Atg7*, and *Nod2* genes, suggesting the presence of another negative regulatory mechanism in macrophages. Transfer of TREM-1-stimulated PCCs reduces colitis. Macrophage engulfment of apoptotic neutrophils is required for wound healing.[65] Macrophage depletion using clodronate-containing liposomes significantly attenuates DSS-induced colitis in WT mice as evaluated by clinical and histological scores. In contrast, Macrophage depletion reduced the effects of α-TREM-1. The absence of macrophages in the colon during DSS treatment led to an increased loss of body weight, higher DAI scores, greater shortening of the colon, increased inflammation, and worsened histopathology in colon tissue. These results are in contrast to those of Watanabe *et al*,[66] which showed that depletion of colonic macrophages by intrarectal injections of clodronate liposomes prevented chronic colitis in an IL-10 KO

model of colitis.[67]

TLR activation leads to important bactericidal activity through ROS generation, cytokine production, and increased survival, all of which can contribute to the pathogenesis of chronic inflammation when signaling become dysregulated in neutrophils and macrophages.[68] Although TLR4 ligation induces NF-κB activation, which may promote cell survival through the induction of antiapoptotic genes, recent evidence has demonstrated that TLR4 activation may also result in macrophage apoptosis.[69] NF-κB activation is critical not only for the induction of inflammation but also for macrophage survival because NF-κB regulates the expression of both proinflammatory and anti-apoptotic proteins.[70] Moreover, blocking TLR4 impairs mucosal healing during DSS-induced colitis. TLR signaling is an important link between luminal bacteria and intestinal inflammation. The presence of TLRs is necessary for the expression of TREM-1 in response to specific TLR ligands.[71,72] Surprisingly, TLR4-deficient ($TLR4^{-/-}$) mice develop more secure bleeding compared with wild-type mice after DSS exposure as the previous study reported.[73] Histological examination of the colon revealed that $TLR4^{-/-}$ mice have no neutrophilic infiltrate, whereas control mice have a dense neutrophilic infiltrate. An increase in gram-negative bacterial translocation to mesenteric lymph nodes was seen in $TLR4^{-/-}$ mice more frequently than wild-type littermates given DSS. Similar results were seen in $MyD88^{-/-}$ mice. The reason underlying the decrease in neutrophil recruitment is diminished chemokine production by lamina propria macrophages and a diminished chemotactic response by neutrophils. Although the luminal flora is polymicrobial, other TLRs do not substitute for this initial function of limiting bacterial translocation in the face of epithelial injury. BMDM from TLR4 and TLR2 KO mice failed to induce expression of TREM-1 message and protein in response to their specific ligands.[72]

Activation of TLRs and their shared adaptor protein, MyD88, was unexpectedly protective against DSS-induced damage in the gut. Our observation of perturbed mucosal barrier function in TLR4 and MyD88 KO mice may be directly or indirectly related to defective neutrophil recruitment. Commensal bacteria provide TLR-dependent signals, which are required for epithelial cell proliferation and the production of tissue-protective factors, suggesting that the activation of TLR by commensal bacteria is essential for intestinal homeostasis. Despite the many beneficial effects of commensal bacteria on host physiology, the lack of MyD88 enables microbiota to function as pathogenic bacteria that

cause mortality in these mice during DSS-induced colonic damage. The lack of a crucial component of the TLR, IL-1, and IL-18 signaling cascade changes the beneficial host-bacteria relationship into a highly pathogenic relationship, which causes the mortality of MyD88$^{-/-}$ mice after DSS treatment. The cooperation between TLR9 and caspase-1 plays a major role in protecting the host from commensal bacteria. Surprisingly, TLR2, TLR4, and TLR5 were dispensable for resistance to DSS-induced colonic damage. TLR4 activation induces the MyD88-dependent signaling pathway and activates the nuclear factor kappa-light-chain-enhancer of activated B cells and the production of proinflammatory cytokines.[74] In the present study, I have extended further on the reciprocal regulation between TREM-1 and TLRs by revealing that TREM-1 takes part into a regulatory loop that is specifically activated by TLR4. Results of this study showed that, in comparison with C57BL/6 wild-type mice, the anti-colitic effect of α-TREM-1 was attenuated by TLR4 ablation. Since α-TREM-1 functions as a positive regulator of inflammatory responses, present data uncover a striking mechanism through which macrophages function as a gatekeeper signaling in regulating the intestinal immune response to microbiota. Activation of TLR4/MyD88 signaling by LPS results in prolonged IL-6 secretion in TREM-1 knockdown macrophages. TLR signaling might have a greater effect on the stability and resilience of less complex microbial populations.[75] The interaction between TREM-1 and TLR4 appears to be crucial, where TREM-1 modulates the activity and availability of key proteins in the TLR4 signaling cascade.[22] TLR4 induces TREM-1 expression independent of MyD88.[72] The interaction between TREM-1 and TLR4 has been investigated in detail.[22] The production of IRAK-M, which interacts with TLR2, -4, and -9, and the induction of Toll-interacting protein, which interacts with TLR2 and -4, are down-regulated significantly after engagement of TREM-1.[38] TREM-1 also down-regulates the expression of single Ig IL-1-related receptor and ST2.[76] Down-regulation of these negative regulators causes upregulation of the TLR signaling cascade and increased cytokine production. TREM-1 has been shown to have synergistic effects on the proinflammatory cytokine production induced by recognition of peptidoglycans by NOD1 and NOD2.[77] However, treatment with α-TREM-1 did not improve a colitis in TLR4$^{-/-}$ mice, suggesting that the anti-inflammatory effects of α-TREM-1 are mediated by TLR signaling and TLR4/TREM-1 synergy may be an important component of the antibacterial function of macrophages by TREM-1. In addition to recognition of LPS, TLR4 recognizes heat shock proteins, which may be released by dead or dying IECs.[78] Negative regulators of PRR signaling include A20, IL-1

receptor-associated kinases m (IRAK-M), single Ig IL-1-related receptor, toll interacting protein, suppressor of cytokine signaling1, and peroxisome proliferator-activated receptor gamma. IRAK-M is a member of the IRAK family of adaptor molecules.[79] IRAK-M blocks the formation of IRAK-1/TNF receptor-associated factor 6 complexes, preventing dissociation of IRAK-1/IRAK-4 from the TLR receptor and thereby inhibiting downstream signaling and activation of nuclear factor kappa-light-chain-enhancer of activated B cells.[80] In the gut, IRAK-M is described as an important factor for the establishment of epithelial barrier integrity. The major anti-inflammatory effects of IRAK-M are seen in the early acute phase of colitis. IL-1 receptor-associated kinase m downregulates DSS-induced colitis.[81]

The intestinal epithelium, consisting of one single cell layer, act as a barrier to protect against invading foreign substances from the bacteria-rich intestinal lumen and contact with the highly diverse microbiota.[82] Epithelial cells continually undergo apoptosis and epithelial wound repair by increased cell migration and proliferation,[83] which are regulated by many signaling molecules, including various growth factors and cytokines,[84] In particular, b-catenin signaling have emerged as a key regulator of IEC proliferation and survival.[85] The enterocytes express a wide range of PRRs whose functions and engagement by Microbe-associated molecular patterns are essential for the homeostasis of the intestinal mucosa.[86] Thus, commensal bacteria can affect epithelial tissue homeostasis. The other way around, IECs are shown to modulate the microbiota through the secretion of antimicrobial peptides[87] and by facilitating the transport of secretory IgA.[88] Intrarectal administration of TREM-1 agonist suppresses immune-cell independent colitis. Krüppel-like factor 4 regulates the goblet cell differentiation and macrophage polarization toward M2 via sequestration of co-activators required for NF-κB activation.[89] Colonic goblet cells express TLRs and MyD88.[90] Epithelial cells of MyD88-deficient mice express diminished levels of the epidermal growth factor receptor ligands amphiregulin and epiregulin and MyD88 signaling in nonhematopoietic cells protects mice against induced colitis by regulating specific epidermal receptor ligands.[91] Moreover, targeted deletion of MyD88 in intestinal epithelial cells results in Mucin-2 and antibacterial peptides.[92] Another contributor to the decrease in proliferative response of the epithelium to damage may be more directly related to neutrophils. A recent study demonstrating the role of cathelicidin in regulation of TREM-1 in neutrophils,[93] suggests a complex network of innate immune responses that may also include those observed in epithelial cells as well.[94]

IL-22 regulates intestinal barrier immunity.[95] IL-22 has already been confirmed as a regulator of the expression of antimicrobial proteins such as the S100 family proteins, β-defensin family proteins, Reg family proteins and lipocalin-2,[96] which are important in the control of gut pathogens. IL-22 plays a protective role in the host inflammatory response to microbial infections or in promoting the release of inflammatory mediators. In addition to its antibacterial activity, IL-22 can enhance the survival and proliferation of epithelial cells for tissue differentiation and healing.[97] The mucus layers are vital for preventing colitis, but they must function cooperatively with other elements of the epithelial innate immune system. Deletion of either TLR-MyD88 signaling or the NLRP6 inflammasome renders the host susceptible to severe experimental colitis.[92,98] Mucus components, including Muc2, are produced by intestinal goblet cells.

Ⅴ. CONCLUSION

Several mechanisms appear to contribute to the protective effects of TREM-1 agonist. One is antimicrobial activity through the immune cell and antimicrobial materials by the TREM-1 agonist. Another unexplored mechanism that likely contributes to the protective effect of 5-HT4–receptor stimulation is secretion of mucus from goblet cells. The mucus layer serves as a protective barrier, and disruption of this barrier with mucolytic agents or deletion of the Mucin 2 gene results in colitis.[99] Goblet cells express the TREM-1, and TREM-1activation leads to degranulation. One mechanism by which TREM-1 stimulation is acting is through enhanced wound healing processes. TREM-1 stimulation increased both cell proliferation and epithelial cell migration in a TREM-1–antagonist–sensitive manner. Epithelial erosions, ulcers, and decreased epithelial barrier integrity all are common features of active colitis, and these conditions likely would be mitigated by enhanced epithelial proliferation and migration. The anti-inflammatory effects of TREM-1 activation also may involve resistance of the epithelium to the harmful effects of oxidative stress. Oxidative stress and resultant epithelial apoptosis are key features of inflammation and have been shown in both DSS and TNBS colitis. In this study, I present a new therapeutic agent through TREM-1 stimulation of colitis was demonstrated the importance of inflammation in the control signaling of the TREM-1, although more research is needed. In conclusion, our results suggest α-TREM-1 ameliorates colitis in mice via neutrophil and macrophage activation and wound healing. These experiments highlight the reciprocal interaction of TLR4 and TREM-1 in the maintenance of intestinal homeostasis through the augmented bacterial clearance. TREM-1 agonist may be useful for further evaluation as a potential anti-inflammatory reagent. The mechanism of anti-colitic effect of TREM-1 agonist in colitis is probably multi-factorial and requires further elucidation in future studies. Further insights into the mechanisms facilitating E. coli persistence within macrophages in CD and the role of this in pathogenesis may provide new avenues of therapy. Our findings reveal a regulatory role for TREM-1 stimulation in maintaining tissue homeostasis in colitis.

In this study, I show a vital role for TREM-1 as its activation accelerated the induction of the early host response to colitis, resulting in augmented bacterial elimination together with accelerated resolution of inflammation and ultimately improved colitis. This study revealed mechanisms through which α-TREM-1 affect bactericidal activity and wound healing via reciprocal activation of macrophages and neutrophils. These results suggest a novel mechanism of TREM-1 through boosting the activation of myeloid cells and the

wound healing and anti-colitic effect of TREM-1 agonist could be a new insight of therapeutic strategy to treat IBD. Most recently, autophagy induction was suggested as a therapeutic strategy for IBD.[100]

REFERENCES

1. Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. *Autoimmun Rev.* 2014;13(1):3-10.
2. Peyrin-Biroulet L. Anti-TNF therapy in inflammatory bowel diseases: a huge review. *Minerva Gastroenterol Dietol.* 2010;56(2):233-243.
3. Guerra I, Algaba A, Perez-Calle JL, Chaparro M, Marin-Jimenez I, Garcia-Castellanos R, et al. Induction of psoriasis with anti-TNF agents in patients with inflammatory bowel disease: a report of 21 cases. *J Crohns Colitis.* 2012;6(5):518-523.
4. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. *Gastroenterology.* 2011;140(6):1704-1712.
5. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature.* 2007;448(7152):427-434.
6. Medzhitov R, Janeway C, Jr. Innate immunity. *N Engl J Med.* 2000;343(5):338-344.
7. Glauser MP, Zanetti G, Baumgartner JD, Cohen J. Septic shock: pathogenesis. *Lancet.* 1991;338(8769):732-736.
8. Bouchon A, Facchetti F, Weigand MA, Colonna M. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. *Nature.* 2001;410(6832):1103-1107.
9. Klesney-Tait J, Turnbull IR, Colonna M. The TREM receptor family and signal integration. *Nat Immunol.* 2006;7(12):1266-1273.
10. Matesanz-Isabel J, Sintes J, Llinas L, de Salort J, Lazaro A, Engel P. New B-cell CD molecules. *Immunol Lett.* 2011;134(2):104-112.
11. Radsak MP, Salih HR, Rammensee HG, Schild H. Triggering receptor expressed on myeloid cells-1 in neutrophil inflammatory responses: differential regulation of activation and survival. *J Immunol.* 2004;172(8):4956-4963.
12. Colonna M, Facchetti F. TREM-1 (triggering receptor expressed on myeloid cells): a new player in acute inflammatory responses. *J Infect Dis.* 2003;187 Suppl 2:S397-401.
13. Bouchon A, Dietrich J, Colonna M. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. *J Immunol.* 2000;164(10):4991-4995.
14. Gibot S, Kolopp-Sarda MN, Bene MC, Bollaert PE, Lozniewski A, Mory F, et al. A soluble form of the triggering receptor expressed on myeloid cells-1 modulates the inflammatory response in murine sepsis. *J Exp Med.* 2004;200(11):1419-1426.
15. Hamerman JA, Tchao NK, Lowell CA, Lanier LL. Enhanced Toll-like receptor responses in the absence of signaling adaptor DAP12. *Nat Immunol.* 2005;6(6):579-586.
16. Schenk M, Bouchon A, Seibold F, Mueller C. TREM-1--expressing intestinal macrophages crucially amplify chronic inflammation in experimental colitis and inflammatory bowel diseases. *J Clin Invest.* 2007;117(10):3097-3106.

17. Kuai J, Gregory B, Hill A, Pittman DD, Feldman JL, Brown T, et al. TREM-1 expression is increased in the synovium of rheumatoid arthritis patients and induces the expression of pro-inflammatory cytokines. *Rheumatology (Oxford).* 2009;48(11):1352-1358.
18. Gibot S, Cravoisy A, Levy B, Bene MC, Faure G, Bollaert PE. Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. *N Engl J Med.* 2004;350(5):451-458.
19. Nam SY, Kim JS, Kim JM, Lee JY, Kim N, Jung HC, et al. DA-6034, a derivative of flavonoid, prevents and ameliorates dextran sulfate sodium-induced colitis and inhibits colon carcinogenesis. *Exp Biol Med (Maywood).* 2008;233(2):180-191.
20. McCormick BA, Colgan SP, Delp-Archer C, Miller SI, Madara JL. Salmonella typhimurium attachment to human intestinal epithelial monolayers: transcellular signalling to subepithelial neutrophils. *J Cell Biol.* 1993;123(4):895-907.
21. Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology.* 1994;107(6):1643-1652.
22. Arts RJ, Joosten LA, van der Meer JW, Netea MG. TREM-1: intracellular signaling pathways and interaction with pattern recognition receptors. *J Leukoc Biol.* 2013;93(2):209-215.
23. Bleharski JR, Kiessler V, Buonsanti C, Sieling PA, Stenger S, Colonna M, et al. A role for triggering receptor expressed on myeloid cells-1 in host defense during the early-induced and adaptive phases of the immune response. *J Immunol.* 2003;170(7):3812-3818.
24. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest.* 1993;69(2):238-249.
25. Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy.* 2012;8(4):445-544.
26. Kobayashi SD, Voyich JM, DeLeo FR. Regulation of the neutrophil-mediated inflammatory response to infection. *Microbes Infect.* 2003;5(14):1337-1344.
27. Liu CL, Hsieh WY, Wu CL, Kuo HT, Lu YT. Triggering receptor expressed on myeloid cells-1 in pleural effusions: a marker of inflammatory disease. *Respiratory medicine.* 2007;101(5):903-909.
28. Arts RJ, Joosten LA, Dinarello CA, Kullberg BJ, van der Meer JW, Netea MG. TREM-1 interaction with the LPS/TLR4 receptor complex. *Eur Cytokine Netw.* 2011;22(1):11-14.
29. Poukoulidou T, Spyridaki A, Mihailidou I, Kopterides P, Pistiki A, Alexiou Z, et al. TREM-1 expression on neutrophils and monocytes of septic patients: relation to the underlying infection and the implicated pathogen. *BMC Infect Dis.* 2011;11:309.
30. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell.* 2004;118(2):229-241.

31. Mitchell R, Kremer A, Westwood N, Younge L, Ghosh S. Talking about life and IBD: A paradigm for improving patient-physician communication. *J Crohns Colitis.* 2009;3(1):1-3.
32. Schwerd T, Pandey S, Yang HT, Bagola K, Jameson E, Jung J, et al. Impaired antibacterial autophagy links granulomatous intestinal inflammation in Niemann-Pick disease type C1 and XIAP deficiency with NOD2 variants in Crohn's disease. *Gut.* 2017;66(6):1060-1073.
33. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature.* 2012;491(7422):119-124.
34. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature.* 2011;474(7351):307-317.
35. Klesney-Tait J, Keck K, Li X, Gilfillan S, Otero K, Baruah S, et al. Transepithelial migration of neutrophils into the lung requires TREM-1. *J Clin Invest.* 2013;123(1):138-149.
36. Lin YT, Tseng KY, Yeh YC, Yang FC, Fung CP, Chen NJ. TREM-1 promotes survival during Klebsiella pneumoniae liver abscess in mice. *Infect Immun.* 2014;82(3):1335-1342.
37. Weiss J, Victor M, Stendhal O, Elsbach P. Killing of gram-negative bacteria by polymorphonuclear leukocytes: role of an O2-independent bactericidal system. *J Clin Invest.* 1982;69(4):959-970.
38. Lagler H, Sharif O, Haslinger I, Matt U, Stich K, Furtner T, et al. TREM-1 activation alters the dynamics of pulmonary IRAK-M expression in vivo and improves host defense during pneumococcal pneumonia. *J Immunol.* 2009;183(3):2027-2036.
39. Rijneveld AW, Florquin S, Branger J, Speelman P, Van Deventer SJ, van der Poll T. TNF-alpha compensates for the impaired host defense of IL-1 type I receptor-deficient mice during pneumococcal pneumonia. *J Immunol.* 2001;167(9):5240-5246.
40. Bloom SM, Bijanki VN, Nava GM, Sun L, Malvin NP, Donermeyer DL, et al. Commensal Bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. *Cell Host Microbe.* 2011;9(5):390-403.
41. Miller BH, Fratti RA, Poschet JF, Timmins GS, Master SS, Burgos M, et al. Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. *Infect Immun.* 2004;72(5):2872-2878.
42. Gingras MC, Lapillonne H, Margolin JF. TREM-1, MDL-1, and DAP12 expression is associated with a mature stage of myeloid development. *Mol Immunol.* 2002;38(11):817-824.
43. Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. *Nat Immunol.* 2005;6(6):551-557.
44. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol.* 2013;13(3):159-175.
45. Chin AC, Parkos CA. Neutrophil transepithelial migration and epithelial barrier function in IBD: potential targets for inhibiting neutrophil trafficking. *Ann N Y Acad Sci.* 2006;1072:276-287.
46. Weber DA, Sumagin R, McCall IC, Leoni G, Neumann PA, Andargachew

R, et al. Neutrophil-derived JAML inhibits repair of intestinal epithelial injury during acute inflammation. *Mucosal Immunol.* 2014;7(5):1221-1232.
47. Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LA, et al. Resolution of inflammation: state of the art, definitions and terms. *FASEB J.* 2007;21(2):325-332.
48. Zemans RL, Briones N, Campbell M, McClendon J, Young SK, Suzuki T, et al. Neutrophil transmigration triggers repair of the lung epithelium via beta-catenin signaling. *Proc Natl Acad Sci U S A.* 2011;108(38):15990-15995.
49. Brazil JC, Lee WY, Kolegraff KN, Nusrat A, Parkos CA, Louis NA. Neutrophil migration across intestinal epithelium: evidence for a role of CD44 in regulating detachment of migrating cells from the luminal surface. *J Immunol.* 2010;185(11):7026-7036.
50. Sumagin R, Robin AZ, Nusrat A, Parkos CA. Transmigrated neutrophils in the intestinal lumen engage ICAM-1 to regulate the epithelial barrier and neutrophil recruitment. *Mucosal Immunol.* 2014;7(4):905-915.
51. Nava P, Kamekura R, Quiros M, Medina-Contreras O, Hamilton RW, Kolegraff KN, et al. IFNgamma-induced suppression of beta-catenin signaling: evidence for roles of Akt and 14.3.3zeta. *Mol Biol Cell.* 2014;25(19):2894-2904.
52. Kuhl AA, Kakirman H, Janotta M, Dreher S, Cremer P, Pawlowski NN, et al. Aggravation of different types of experimental colitis by depletion or adhesion blockade of neutrophils. *Gastroenterology.* 2007;133(6):1882-1892.
53. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol.* 2011;11(8):519-531.
54. Tillack K, Breiden P, Martin R, Sospedra M. T lymphocyte priming by neutrophil extracellular traps links innate and adaptive immune responses. *J Immunol.* 2012;188(7):3150-3159.
55. Zhou G, Yu L, Fang L, Yang W, Yu T, Miao Y, et al. CD177+ neutrophils as functionally activated neutrophils negatively regulate IBD. *Gut.* 2017.
56. Sumagin R, Brazil JC, Nava P, Nishio H, Alam A, Luissint AC, et al. Neutrophil interactions with epithelial-expressed ICAM-1 enhances intestinal mucosal wound healing. *Mucosal Immunol.* 2016;9(5):1151-1162.
57. Grenier A, Chollet-Martin S, Crestani B, Delarche C, El Benna J, Boutten A, et al. Presence of a mobilizable intracellular pool of hepatocyte growth factor in human polymorphonuclear neutrophils. *Blood.* 2002;99(8):2997-3004.
58. Gibot S, Massin F, Marcou M, Taylor V, Stidwill R, Wilson P, et al. TREM-1 promotes survival during septic shock in mice. *Eur J Immunol.* 2007;37(2):456-466.
59. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity.* 2010;32(5):593-604.
60. Smith PD, Smythies LE, Shen R, Greenwell-Wild T, Gliozzi M, Wahl SM. Intestinal macrophages and response to microbial encroachment. *Mucosal*

Immunol. 2011;4(1):31-42.

61. Kamada N, Hisamatsu T, Okamoto S, Chinen H, Kobayashi T, Sato T, et al. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis. *J Clin Invest.* 2008;118(6):2269-2280.
62. Smith AM, Rahman FZ, Hayee B, Graham SJ, Marks DJ, Sewell GW, et al. Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. *J Exp Med.* 2009;206(9):1883-1897.
63. Palmer CD, Rahman FZ, Sewell GW, Ahmed A, Ashcroft M, Bloom SL, et al. Diminished macrophage apoptosis and reactive oxygen species generation after phorbol ester stimulation in Crohn's disease. *PLoS One.* 2009;4(11):e7787.
64. Zanzinger K, Schellack C, Nausch N, Cerwenka A. Regulation of triggering receptor expressed on myeloid cells 1 expression on mouse inflammatory monocytes. *Immunology.* 2009;128(2):185-195.
65. Sylvia CJ. The role of neutrophil apoptosis in influencing tissue repair. *J Wound Care.* 2003;12(1):13-16.
66. Watanabe N, Ikuta K, Okazaki K, Nakase H, Tabata Y, Matsuura M, et al. Elimination of local macrophages in intestine prevents chronic colitis in interleukin-10-deficient mice. *Dig Dis Sci.* 2003;48(2):408-414.
67. Qualls JE, Kaplan AM, van Rooijen N, Cohen DA. Suppression of experimental colitis by intestinal mononuclear phagocytes. *J Leukoc Biol.* 2006;80(4):802-815.
68. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, et al. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature.* 2011;472(7344):476-480.
69. Suzuki T, Kobayashi M, Isatsu K, Nishihara T, Aiuchi T, Nakaya K, et al. Mechanisms involved in apoptosis of human macrophages induced by lipopolysaccharide from Actinobacillus actinomycetemcomitans in the presence of cycloheximide. *Infect Immun.* 2004;72(4):1856-1865.
70. Karin M, Lin A. NF-kappaB at the crossroads of life and death. *Nat Immunol.* 2002;3(3):221-227.
71. Ornatowska M, Azim AC, Wang X, Christman JW, Xiao L, Joo M, et al. Functional genomics of silencing TREM-1 on TLR4 signaling in macrophages. *Am J Physiol Lung Cell Mol Physiol.* 2007;293(6):L1377-1384.
72. Zheng H, Heiderscheidt CA, Joo M, Gao X, Knezevic N, Mehta D, et al. MYD88-dependent and -independent activation of TREM-1 via specific TLR ligands. *Eur J Immunol.* 2010;40(1):162-171.
73. Fukata M, Michelsen KS, Eri R, Thomas LS, Hu B, Lukasek K, et al. Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis. *Am J Physiol Gastrointest Liver Physiol.* 2005;288(5):G1055-1065.
74. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol.* 2010;11(5):373-384.

75. Ubeda C, Lipuma L, Gobourne A, Viale A, Leiner I, Equinda M, et al. Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. *J Exp Med.* 2012;209(8):1445-1456.
76. Wu M, Peng A, Sun M, Deng Q, Hazlett LD, Yuan J, et al. TREM-1 amplifies corneal inflammation after Pseudomonas aeruginosa infection by modulating Toll-like receptor signaling and Th1/Th2-type immune responses. *Infect Immun.* 2011;79(7):2709-2716.
77. Netea MG, Azam T, Ferwerda G, Girardin SE, Kim SH, Dinarello CA. Triggering receptor expressed on myeloid cells-1 (TREM-1) amplifies the signals induced by the NACHT-LRR (NLR) pattern recognition receptors. *J Leukoc Biol.* 2006;80(6):1454-1461.
78. Vabulas RM, Wagner H, Schild H. Heat shock proteins as ligands of toll-like receptors. *Curr Top Microbiol Immunol.* 2002;270:169-184.
79. Kobayashi K, Hernandez LD, Galan JE, Janeway CA, Jr., Medzhitov R, Flavell RA. IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell.* 2002;110(2):191-202.
80. Shibolet O, Podolsky DK. TLRs in the Gut. IV. Negative regulation of Toll-like receptors and intestinal homeostasis: addition by subtraction. *Am J Physiol Gastrointest Liver Physiol.* 2007;292(6):G1469-1473.
81. Berglund M, Melgar S, Kobayashi KS, Flavell RA, Hornquist EH, Hultgren OH. IL-1 receptor-associated kinase M downregulates DSS-induced colitis. *Inflamm Bowel Dis.* 2010;16(10):1778-1786.
82. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell.* 2014;157(1):121-141.
83. Nakahashi-Oda C, Udayanga KG, Nakamura Y, Nakazawa Y, Totsuka N, Miki H, et al. Apoptotic epithelial cells control the abundance of Treg cells at barrier surfaces. *Nat Immunol.* 2016;17(4):441-450.
84. Nava P, Koch S, Laukoetter MG, Lee WY, Kolegraff K, Capaldo CT, et al. Interferon-gamma regulates intestinal epithelial homeostasis through converging beta-catenin signaling pathways. *Immunity.* 2010;32(3):392-402.
85. Nava P, Capaldo CT, Koch S, Kolegraff K, Rankin CR, Farkas AE, et al. JAM-A regulates epithelial proliferation through Akt/beta-catenin signalling. *EMBO Rep.* 2011;12(4):314-320.
86. Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. *Nat Rev Immunol.* 2010;10(2):131-144.
87. Salzman NH, Hung K, Haribhai D, Chu H, Karlsson-Sjoberg J, Amir E, et al. Enteric defensins are essential regulators of intestinal microbial ecology. *Nat Immunol.* 2010;11(1):76-83.
88. Peterson DA, McNulty NP, Guruge JL, Gordon JI. IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host Microbe.* 2007;2(5):328-339.
89. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, et al. Kruppel-like factor 4 regulates macrophage polarization. *J Clin Invest.* 2011;121(7):2736-2749.

90. Knoop KA, McDonald KG, McCrate S, McDole JR, Newberry RD. Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. *Mucosal Immunol.* 2015;8(1):198-210.
91. Brandl K, Sun L, Neppl C, Siggs OM, Le Gall SM, Tomisato W, et al. MyD88 signaling in nonhematopoietic cells protects mice against induced colitis by regulating specific EGF receptor ligands. *Proc Natl Acad Sci U S A.* 2010;107(46):19967-19972.
92. Frantz AL, Rogier EW, Weber CR, Shen L, Cohen DA, Fenton LA, et al. Targeted deletion of MyD88 in intestinal epithelial cells results in compromised antibacterial immunity associated with downregulation of polymeric immunoglobulin receptor, mucin-2, and antibacterial peptides. *Mucosal Immunol.* 2012;5(5):501-512.
93. Amatngalim GD, Nijnik A, Hiemstra PS, Hancock RE. Cathelicidin peptide LL-37 modulates TREM-1 expression and inflammatory responses to microbial compounds. *Inflammation.* 2011;34(5):412-425.
94. Diamond G, Legarda D, Ryan LK. The innate immune response of the respiratory epithelium. *Immunol Rev.* 2000;173:27-38.
95. Li LJ, Gong C, Zhao MH, Feng BS. Role of interleukin-22 in inflammatory bowel disease. *World J Gastroenterol.* 2014;20(48):18177-18188.
96. Sabat R, Wolk K. Research in practice: IL-22 and IL-20: significance for epithelial homeostasis and psoriasis pathogenesis. *J Dtsch Dermatol Ges.* 2011;9(7):518-523.
97. Ki SH, Park O, Zheng M, Morales-Ibanez O, Kolls JK, Bataller R, et al. Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. *Hepatology.* 2010;52(4):1291-1300.
98. Lamkanfi M, Dixit VM. Mechanisms and functions of inflammasomes. *Cell.* 2014;157(5):1013-1022.
99. Johansson ME, Gustafsson JK, Holmen-Larsson J, Jabbar KS, Xia L, Xu H, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut.* 2014;63(2):281-291.
100. Nys K, Agostinis P, Vermeire S. Autophagy: a new target or an old strategy for the treatment of Crohn's disease? *Nat Rev Gastroenterol Hepatol.* 2013;10(7):395-401.

ABSTRACT (IN KOREAN)

Triggering receptor expressed on myeloid cell 1 활성체의 장 내 미생물과 장벽 기능 조절을 통한 장 내 염증 완화

<지도교수 천 재 희>

연세대학교 대학원 의과학과

서 동 혁

Triggering receptor expressed on myeloid cell 1 (TREM-1)을 자극 할 경우 박테리아 물질에 대한 면역반응의 활성화가 일어나지만, TREM-1은 또한 박테리아 제거 및 감염을 해결하는데 도움을 줄 수 있다. 또한 TREM-1은 인간의 대장에 있는 대식세포에서는 지속적인 위장 박테리아와의 접촉에도 불구하고 발현이 억제 된 것으로 보고 되었는데, 이는 대식세포가 위장의 미생물을 조절하는 능력이 있다는 것을 시사한다. 본 논문에서는 TREM-1 활성의 근간에 있는 신호전달체계를 명확히 하고, TREM-1 활성체의 대장염증 완화 치료제로서의 가능성을 대장염증 실험모델에서 증명하기 위해 진행하였다.

먼저 toll like receptor 4 (TLR4)와 myeloid differentiation primary response 88 (MyD88) 유전자를 제거한 생쥐와 야생형 생쥐에 2,4,6-trinitrobenzene sulfonic acid (TNBS)와 dextran sulfate sodium (DSS)를 각각 항문과 구강을 통하여 주입 한 후 장염을 유도한 동물 모델을 만들었고, 이에 TREM-1 활성체를 각각 항문과 복강에 주입하였다. 이후 체중변화, 대장길이, 질병의 활성도를 확인하였다. 골수에서 유래된 대식세포와 호중구를 동물모델에서 제거 또는 이식하는 방법을 통하여 완화 효과 실험을

진행하였다. 조직학적 분석, 박테리아 프로필, 싸이토카인 수치 등을 면역화학염색, 박테리아 16S 리보좀 RNA, ELISA 등을 이용하여 확인하였고, qPCR, immunoblotting 등을 이용하여 유전자 발현 정도를 확인하였다.

TNBS와 DSS에 의한 장염 유도 동물 모델에서 항문 및 복강 내로 TREM-1 활성체를 주입했을 때 장염이 완화 되는 것을 확인하였다. TREM-1 활성체가 박테리아 제거를 유도하고, dysbiosis를 완화 하는 것을 확인하였고, 이 때 대식세포와 호중구가 관련 되어 있으나, TLR4 유전자가 제거된 생쥐에서는 호전양상이 없는 것으로 보아, TREM-1 활성체에 의한 대장염증 완화는 TLR4 신호 전달이 동반되어야 한다는 것을 확인하였다. 뿐만 아니라 TREM-1 활성체는 iNOS, IL-22의 발현 증가를 유도하고, 결론적으로 장내 상피세포의 장벽기능을 회복을 이끄는 것을 확인하였다.

이 연구는 TREM-1 활성이 호중구와 대식세포 기능을 활성화시키고, 조직 재생 유도, 장내 미생물 환경 복구를 통한 대장 염증을 완화시키고, TREM-1이 장 내 항상성 유지에 중요한 영향을 미친다는 것을 밝혀냈으며, 염증성 장질환 치료제로서의 가능성을 확인한 연구이다.

.

핵심되는 말: CD177, 상피세포, 염증성 장질환, 인터루킨 1 베타, 인터루킨 22, 대식세포, 호중구, Triggering receptor expressed on myeloid cell 1 agonist