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Supplementary Information for

Intestinal barrier dysfunction orchestrates the onset of inflammatory host-microbiome cross-talk in a human gut inflammation-on-a-chip

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SI Materials and Methods

Microbial Cultures. The VSL#3 formulation (Sigma-Tau Pharmaceuticals, Inc.) that contains the microbial cells of *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, *L. delbrueckii subsp. bulgaricus*, *Bifidobacterium breve*, *B. longum*, *B. infantis*, and *Streptococcus thermophilus* was used for the probiotics study. The culture of VSL#3 microbial cells was performed in a mixture (1:1, vol/vol) of the autoclaved Lactobacilli MRS Broth (MRS; Difco) and Reinforced Clostridial Medium (RCM; Difco) in an anaerobic glove box conditioned in 5% CO_2, 5% H_2, and 90% N_2 without shaking for 12 h. Non-pathogenic green fluorescence protein (GFP)-labeled *E. coli* cells (D21f1) were pre-cultured in the Lysogeny broth (LB) medium (Difco) in a shaking incubator at 200 rpm for 12 h. The culture broth was centrifuged at 10,000×*g* for 1 min, then the cell pellet was resuspended with the antibiotic-free cell culture medium (final cell density, ~1.0×10^7 cfu/mL) and used for co-culture experiments.

To isolate the individual genus from the VSL#3 bacterial cultures, single colony isolation method was applied to the pre-cultured VSL#3 culture broth. After we prepare the pre-culture of VSL#3 cells, a single colony of Lactobacillus spp., Bifidobacterium spp., and Streptococcus sp. was isolated on the MRS agar plate in the anaerobic glove box. Isolated individual colonies were subcultured in both solid (MRS agar) and liquid medium (a mixture of MRS and RCM) anaerobically. The liquid culture of the individual isolated strains was used to visualize their single cell-level morphology via bacterial live/dead assay (live, SYTO 9; dead, propidium iodide). The growth profile of the total VSL#3 cells (Fig. S13*A*) or the individual genus of VSL#3 cells (Fig. S13*E*) was performed in the DMEM (20% FBS, no antibiotics) medium at various concentrations of DSS (0, 0.5, 1.0, 2.0, and 4.0%, wt/vol). The growth of isolated strains was monitored by measuring the optical density (OD) at 600 nm in a microplate reader (SpectraMax i3, Molecular Devices) for 18 h.

Assessment of Epithelial Barrier Function. TEER value was measured using Ag/AgCl electrodes connected to an ohm meter (87V Industrial Multimeter, Fluke Corporation). Normalized TEER value was calculated based on the equation as follows; normalized TEER = $(\Omega_t - \Omega_{blank})/(\Omega_0 - \Omega_{blank})$, where Ω_t is a resistance at the measured time point since the onset of experiment, Ω_{blank} is a resistance without the epithelium, and Ω_0 is a resistance at the onset time point. Apparent permeability was evaluated using a 20 kDa fluorescein isothiocyanate (FITC)-dextran (Sigma). Briefly, after microengineered intestinal villi were cultured for 5 days in a device, FITC-dextran (1 mg/mL) dissolved in the culture medium was perfused through the upper microchannel at 30 μL/h in the presence of mechanical deformations (10% in strain, 0.15 Hz in frequency) while the lower microchannel was flowed with the fresh culture medium at the same flow rate in a humidified CO_2 incubator at 37°C. Culture medium collected from the lower microchannel for up to 12 h was used to detect the fluorescence intensity (Excitation at 495 nm and emission at 519 nm) in a microplate reader (Molecular Devices). The absolute amount of the transported FITC-dextran was estimated by a standard curve (concentration vs. fluorescence intensity). The apparent permeability was calculated using an equation, P_{app} (cm/s) = $dQ/dt\times1/AC_0$, where dQ/dt is a flux when the

concentration reaches to steady state (μg/h), *A* is a surface area of the cell culture channel (m^2), and C_0 is an initial concentration of FITC-dextran ($\mu g/m^3$).

Cell Viability Analysis. To perform Live/Dead assay, we used the mixed solution of Calcein AM (2 μM) and ethidium homodimer-1 (4 μm; Thermo Scientific) dissolved in the serum-free, antibiotic-containing DMEM (i.e. L/D assay reagent). For the Caco-2 cells grown in a 96 well plate, we replaced the culture medium to 100 μL L/D assay reagent, incubated at 37°C for 30 min, washed the cell monolayer with PBS ($Ca^{2+}$and Mg^{2+}-free) twice, then monitored using a confocal microscopy. It is noted that this method involves the removal of detached cells during the washing step when the cells are challenged to DSS treatment (Fig. S4*B*). Alternatively, we directly added the concentrated L/D assay reagent (2-fold) into the well plate lined by the Caco-2 cells (w/ or w/o DSS), then imaged the cells after incubation at 37°C for 30 min (Fig. S5). To assess the viability of Caco-2 cells in the early period of DSS treatment (Fig. S7*B*), Caco-2 cells grown in a T75 flask were trypsinized, resuspended into single cells (1×10^6 cells per milliliter), dispensed into the 96-well plate (100 μL), pre-coated with the Matrigel (600 μg/mL), and attached on the well surface (37°C, 1 h). Caco-2 cells were then challenged to DSS (2%, wt/vol) for 1, 2, 3, and 6 h, respectively, and L/D assay was immediately performed. For the Caco-2 villi grown in the microfluidic device, we perfused the concentrated L/D reagent (2-fold) into the upper microchannel at 30 μL/h at 37 °C for 30 min, washed the villi with PBS (Ca^{2+}-Mg^{2+}-free), then took images via confocal microscopy.

Cytotoxicity Analysis. To measure the level of cytotoxicity of inflammatory cells, we performed the lactate dehydrogenase (LDH) assay following the manufacturer's instruction (CytoTox 96 Non-Radioactive Cytotoxicity Assay; Promega). Briefly, the culture effluent from both the apical (AP) and the basolateral (BL) microchannels were independently collected for 2 h. Collected effluents were centrifuged at 3,000×*g* for 5 min to remove detached cells or cell debris, then stored at -80°C until use. To quantitate the LDH level, the collected cell-free sample (50 μL) and the reaction substrate (50 μL) were mixed in a clear bottom black 96 well plate, then incubated in dark at room temperature for 30 min. The enzymatic reaction was terminated by adding the stop solution and absorbance of the reaction mixture was detected at 490 nm using a microplate reader (Molecular Devices). To measure the maximum LDH level as the positive control, Caco-2 cells were harvested from a Gut Chip, treated with 1% (wt/vol) sodium dodecyl sulfate (lysis reagent) at 37°C for 45 min, centrifuged at 300×*g* for 5 min, then the supernatant was used. The amount of LDH released (%) was calculated based on the equation; (percentage cytotoxicity) = 100 × (OD_{490} of experimental group) / (OD_{490} of maximum release).

Inflammation Study. To induce inflammatory response, we obtained deidentified human whole blood from two independent healthy donors and isolated peripheral blood mononuclear cells (PBMC) as an immune element. PBMCs were isolated by the density gradient centrifugation (400×*g* for 30min at room temperature; brake-off) method using a Histopaque-1077 (Sigma-Aldrich) and subsequent washing steps with PBS (200×*g* for 10 min; 3 times). After adjusting the cell density (4.0×10^6 cells per milliliter) in the cell culture medium, PBMC suspension was introduced into the vascular (basolateral) microchannel, then the entire device setup was flipped over and incubated in a humidified 37°C CO_2 incubator for 2 h without flow. After incubation, the setup was positioned back and resumed to flow culture medium at 50 μL/h with mechanical deformations (10%, 0.15 Hz).

To perform immune cell recruitment assay, PBMCs were stained with CellTracker Green CMFDA (final concentration at 10 μM; Thermo Fisher Scientific) by following the manufacturer's instruction. We washed the labeled PBMC with PBS (Ca^{2+}- and Mg^{2+}-free) twice, adjusted the cell density with cell culture medium (4.0×10^6 cells per milliliter), then introduced into the device. The number of recruited PBMCs was quantitated by counting the individual fluorescent PBMCs from the acquired images by ImageJ. At least 5 images per chip were used for the quantification.
To quantify the level of secreted cytokines, culture medium was collected from both upper and lower microchannels independently for 16 h, centrifuged at 3,000×*g* for 5 min to remove cells and cell debris, and immediately stored at -80°C until use. Quantification of inflammatory cytokines (IL-1β, IL-6, IL-10, and TNF-α) released by cells was performed using an OptEIA ELISA kit (BD Biosciences). Standard curves were prepared by serially diluting the provided recombinant proteins of IL-1β, IL-6, IL-10, and TNF-α in the assay diluent (Ca^{2+}-Mg^{2+}-free PBS containing 10% (vol/vol) FBS). Before the assay, capture antibody was coated to a 96-well plate at 4°C for overnight, following the blocking step using the assay diluent. Samples were incubated for 2 h, then biotin-conjugated detection antibody was treated for 1 h along with the streptavidin-conjugated horse radish peroxidase (HRP). Substrate solution was prepared by mixing equal volume of tetramethylbenzidine and hydrogen peroxide, provided in TMB Substrate Reagent Set (BD Biosciences) and 100 μL was applied to each well and incubated for 30 min, then 50 μL of stop solution (2 M, H_2SO_4) was added to each well. The amount of cytokines in each sample was estimated by detecting the absorbance of at 450 nm and calculating the concentration based on the standard curve. All the assays were performed at room temperature.

Effect of Direct Cell Contact on the ROS Generation. To investigate the effect of direct contact between DSS-treated intestinal epithelium and PBMC on the ROS generation, Transwell inserts that have different pore sizes (0.4 or 8.0 μm; 6.5 mm in diameter; 0.33 cm^2 in surface area; Corning) were used. After a Caco-2 monolayer formed on the Matrigel-coated (300 μg/mL) porous insert (target TEER, >800 $\Omega\cdot cm^2$) was challenged to DSS (2%, wt/vol) for 24 h, the Transwell insert was placed upside down, then the PBMC resuspended in DMEM (2×10^4 cells per insert) was loaded on an opposite side of the epithelial compartment (i.e. the BL side of the Transwell insert). The setup was moved inside a humidified 6" Petri dish with a cover to prevent evaporation and contamination, then incubated in a 37°C CO_2 incubator for 2 h. After incubation, the inserts were flipped over again, then placed into each well of a 24 well plate. The BL chamber was filled with 600 μL of DMEM cell culture medium, and 0.4 μL of ROS detection reagent (CellROX Orange; Thermo Scientific) was added into the AP chamber containing 100 μL of cell culture medium. The well plate was then incubated at 37°C for additional 30 min, washed with PBS (Ca^{2+}- and Mg^{2+}-free), and imaged by confocal microscopy.

Cytokine Production in PBMC-Probiotics Co-culture. To measure the amount of inflammatory cytokines released by the PBMC in response to the probiotic bacteria, VSL#3 cells were inoculated to the PBMCs that were contained in a 96 well plate (1.5×10^5 cells/well). Density of VSL#3 cells was adjusted at 2.5, 2.5×10^{-1}, 2.5×10^{-2}, 2.5×10^{-3}, 2.5×10^{-4}, 1.25×10^{-4}, and 6.25×10^{-5} of multiplicity of infection (MOI). MOI was determined based on the number of PBMCs in each well. The plate was incubated at 37°C for 24 h, then the cell-free supernatant in each well was harvested after centrifugation (3,000×*g* for 5 min). ELISA (BD Biosciences) was used to quantify the amount of IL-1β, IL-6, IL-10 and TNF-α. The PBS containing FBS 10% (vol/vol) was

used for the dilution of samples, if needed. It is noted that the MOI at 2.5 is the same MOI that was applied in Fig. 5.

Image Analysis. To perform in situ immunofluorescence visualization to the intestinal villi, epithelial cells cultured in a microfluidic device were fixed with paraformaldehyde (4% wt/vol; Electron Microscopy Sciences) for 10 min, permeabilized with Triton X-100 (0.3% vol/vol, Sigma) for 30 min, then blocked with bovine serum albumin (BSA, 2% wt/vol, Hyclone Laboratories, Inc.) for 1 h. All reagents were flowed into the upper microchannel at 30 μL/h at room temperature. Washing with PBS (Ca^{2+}- and Mg^{2+}-free) was carried out between each step at the same flow rate for 10 min. Primary or fluorophore-conjugated antibodies dissolved in the filtered BSA solution (2%, wt/vol) was subsequently filled or flowed into the upper or lower microchannel, respectively, at 30 μL/h at room temperature for 3 h, then further incubated at 4°C overnight. Next, secondary antibodies (anti-rabbit IgG H&L labeled with DyLight 488 or anti-mouse IgG H&L conjugated with Alexa Fluor 555; Abcam) prepared in BSA solution (2%, wt/vol) were introduced to the both upper and lower microchannels same as the steps of primary antibodies staining, protected from light for 3 h at room temperature. After washing with PBS, nuclei or F-actin staining was performed by introducing 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; 5 μg/mL; Thermo Scientific) or Alexa Fluor 647 Phalloidin (330 nM; Thermo Fisher Scientific), respectively, at room temperature for 30 min. F-actin staining was performed simultaneously with the nuclei staining, if needed. To visualize E-cadherin, ZO-1, and MUC2, monoclonal Alexa Fluor 488-conjugated anti-E-cadherin (BD Biosciences), polyclonal anti-ZO-1 (Thermo Fisher Scientific), and monoclonal anti-MUC2 (Santa Cruz Biotechnology) antibodies were used.

For the immune cell recruitment analysis, we first stained the plasma membrane of the villus epithelium with CellMask (Deep Red, Thermo Fisher Scientific; 500× dilution in cell culture medium) by flowing the staining reagent into the upper microchannel at 30 μL/h for 30 min. Next, we added the PBMCs stained with CellTracker Green CMFDA (10 μM; Thermo Fisher Scientific) into the lower microchannel.

For the mucus visualization, Wheat Germ Agglutinin (WGA) Alexa Fluor 633 conjugate (Thermo Fisher Scientific) that targets the sialic acid and N-acetylglucosamine in the epithelial mucus was used for the live cell imaging. Briefly, the culture medium containing WGA (25 μg/mL) was introduced to the upper microchannel (30 μL/h) in a 37°C CO_2 incubator for 30 min. After washing with PBS in the dark, the stained setup was used for the fluorescence imaging. As a counter-staining, we occasionally performed nuclei staining with 5 μg/mL Hoechst 33342. No stretching motions were applied during the staining procedures.

All the fluorescence image was acquired using a DMi8 microscope system (Leica) equipped with the TCS SPE confocal system with solid state excitation laser sources of 405 nm, 488 nm, 532 nm, and 635 nm and an Ultra high dynamic PMT detector. Phase contrast, differential interference contrast (DIC), or fluorescence imaging of a microfluidic device was performed using a 25× objective (NA 0.95; water immersion; Leica). Acquisition of images was conducted by the LAS X software. The 3D reconstruction of the z-stacked images was performed using a 3D module installed in the software.

Phase contrast images were obtained using a DMi1 inverted phase contrast microscope (Leica). Morphology of intestinal epithelium was taken using a 20× objective (NA 0.3; Leica). Images of bacterial colonies grown on an agar plate were recorded by a TL5000 Ergo stereomicroscope (Leica). Darkfield illumination method was used to analyze the bacterial colonies.

Quantification of the fluorescence intensity was performed using ImageJ software. In CellROX oxidative stress analysis performed in the gut inflammation-on-a-chip, average intensity of each image was used for the quantification, then blank value was subtracted from the absolute vales. In CellROX oxidative stress assessment performed in Transwell, cell contour was drawn on each image then average intensity was measured. At least five different images of each experimental group were used for the quantification. Quantification of WGA fluorescence intensity was also based on the average fluorescence intensity measured in ImageJ.

Gene Expression Analysis. Expression level of the Nrf2 gene was analyzed by the quantitative real-time polymerase chain reaction (qRT-PCR) method. After we harvested the cell mixture of PBMCs and epithelial cells from the co-cultured devices, we separated the PBMCs from this mixture using the human CD45+ microbeads via the magnetic-activated cell sorting (MACS; Miltenyi Biotec, Germany) system by following the manufacturer's instruction. Briefly, harvested cells were suspended in 80 μL of MACS buffer containing BSA (0.5%, wt/vol) and EDTA (2 mM) in PBS, then mixed with 20 μL of anti-CD45 antibody conjugated magnetic beads. The mixture was incubated on ice for 15 min. After the reaction, 1 mL MACS buffer was added into the tube, then centrifuged at 300×*g* for 10 min at 4°C. After the supernatant was completely removed, a cell pellet was resuspended with 500 μL MACS buffer on ice, which was then applied to a LS column for the separation of CD45+ and CD45- cells. Isolated cells were centrifuged (300×*g*, 5 min, 4°C) and supernatant was removed. Total RNA of isolated CD45+ cells, represent leukocyte, were extracted using TRIzol reagent (Thermo Fisher Scientific), a cell lysis reagent, and a cDNA library was prepared using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), following the manufacturer's instruction. Concentration of cDNA was measured using a NanoDrop microvolume UV-Vis spectrophotometer (Thermo Scientific). The sequence of primers (Integrated DNA Technologies) was designed as follows; GAPDH forward primer (FW), 5'-ACC CAC TCC TCC ACC TTT GAC-3', GAPDH backward primer (BW), 5'-CCA CCA CCC TGT TGC TGT AG-3'; Nrf2 FW, 5'-CAG CGA CGG AAA GAG TAT GA-3', Nrf2 BW, 5'-TGG GCA ACC TGG GAG TAG-3'. The cDNA (250 ng) and the forward and backward primers (10 pmol for each) were mixed with 2× SYBR Select Master Mix (Thermo Fisher Scientific), then PCR reaction was carried out using a Quantstudio 3 real-time PCR system (Applied Biosystems). Cycling condition for qRT-PCR followed the sequence of 50°C for 2 min, 95°C for 10 min, 50 cycles of 95°C for 15 s and 60°C for 1 min. GAPDH was used as a housekeeping gene. Relative mRNA expression level was determined by ΔΔCT value.

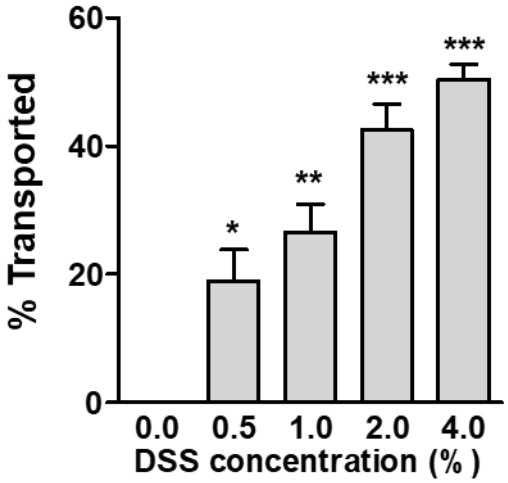

Fig. S1. DSS treatment increases the transport of a paracellular marker through the Caco-2 villi in a dose-dependent manner. Transport profile of the paracellular marker (FITC-dextran, 20 kDa; 1 mg/mL) through the Caco-2 monolayer grown in a Transwell as a function of DSS concentration. A Caco-2 cell monolayer grown in a Transwell (pore size, 0.4 μm) for 6 days was challenged to DSS at various concentrations (0, 0.5, 1.0, 2.0, and 4.0%, wt/vol). Percentage of the transported FITC-dextran was calculated by measuring the fluorescence level of culture medium collected from the apical and basolateral compartments (n = 5). The fluorescence intensity was measured using a microplate reader (Excitation at 495 nm/Emission at 519 nm). (% Transported) = (amount of FITC-Dextran in the basolateral side) / (amount of applied FITC-Dextran) × 100, *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$.

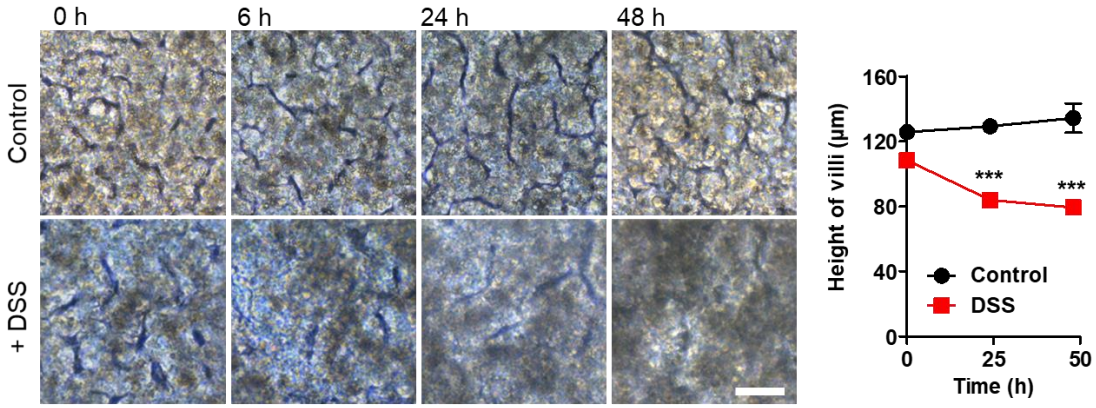

Fig. S2. DSS treatment significantly compromises the villus microarchitecture and the height of villi. A time course of the morphological changes (*Left*) and the height (*Right*) of the intestinal villi in response to the absence ("Control") or the presence of DSS (2%, wt/vol; "+DSS") were provided. Morphological change of microengineered intestinal epithelium on-chip was recorded using a phase contrast microscope. Villus height was quantitated (a right plot chart; n = 8) by tracking the Z-position between the surface of a porous membrane (i.e. on the basement membrane that epithelium bound) and the villus tip using a DIC microscope. The group without DSS treatment was used as Control. (Scale bar, 50 μm.) ***$P < 0.0001$.

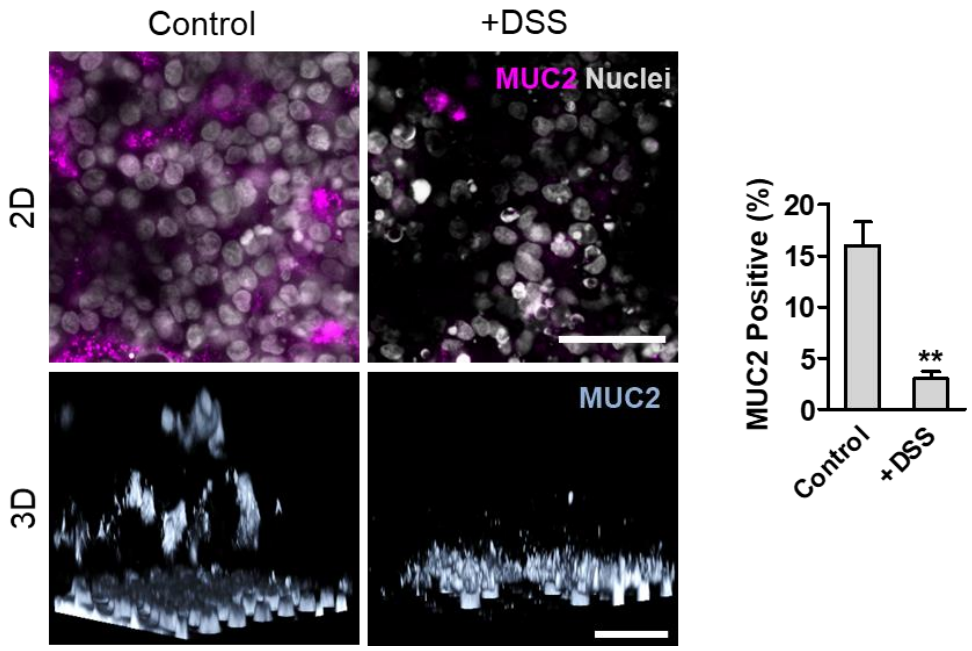

Fig. S3. Addition of DSS dramatically decreases the production of mucus and the number of MUC2-positive cells. Mucin 2 (MUC2) was highlighted using an immunofluorescence microscopy in the absence ("Control") or the presence of DSS treatment (2%, wt/vol; "+DSS") for 48 h. Z-stacked images acquired by a confocal microscope were used to present 2D top-down views or 3D reconstruction views to visualize the distribution of MUC2. The population of MUC2-positive cells was quantitated in the right column chart (n = 4). (Scale bars, 50 μm.) ***P* < 0.001.

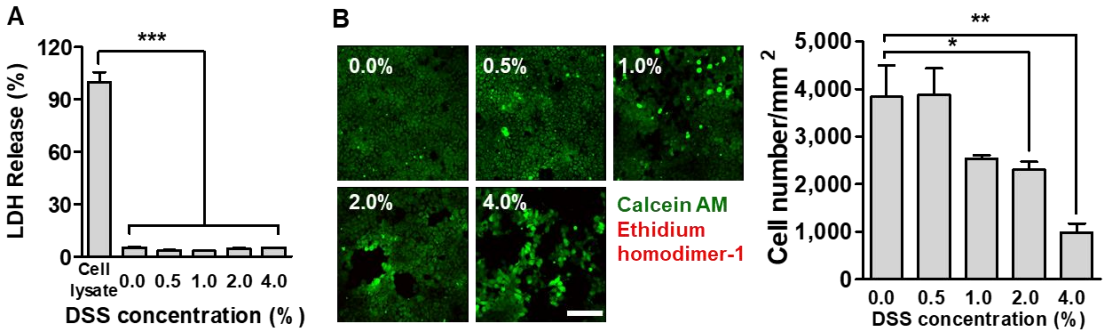

Fig. S4. DSS treatment induces the dissociation of Caco-2 cells from a monolayer without cytotoxic effect. (*A*) Quantification of LDH released from a Caco-2 monolayer cultured on the Transwell at various DSS concentrations (0, 0.5, 1.0, 2.0, and 4.0%, wt/vol). When the Caco-2 monolayer grown in a Transwell shows the TEER value higher than 800 Ω·cm^2, DSS was added to the apical side of the Transwell (n = 3). After 24 h of the DSS treatment, samples collected from the apical side were used to measure the level of LDH. The cell lysate harvested from one Transwell insert which is equivalent ~3×10^4 cells was used as a positive control. (*B*) A Live/Dead assay reveals that the Caco-2 monolayers grown in a 96 well plate for 72 h does not show any cell death regardless of the treatment of DSS at various concentrations for 48 h. A Live/Dead staining reagent containing Calcein AM (live) and ethidium homodimer-1 (dead) was added to each well, then fluorescent images were taken using a confocal microscope. It is notable that even detached cells did not show any dead signals. A column chart displays the number of live cells per unit area at various DSS concentrations (n = 4). (Scale bar, 50 μm.) *P < 0.05, **P < 0.001, ***P < 0.0001.

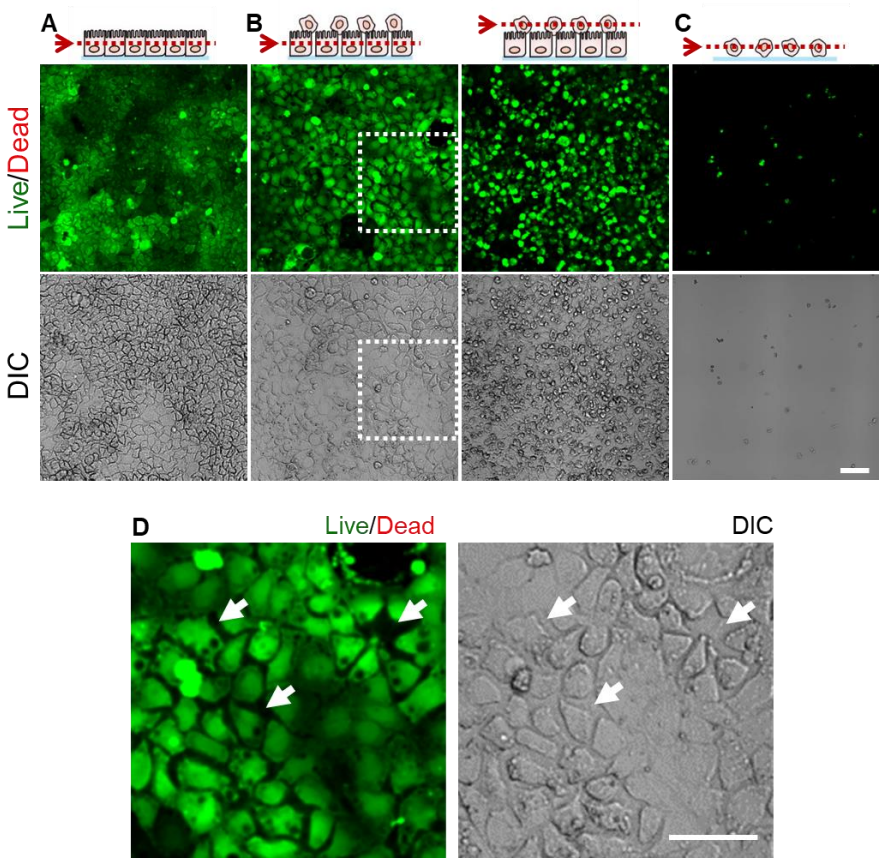

Fig. S5. DSS treatment causes the dissociation and detachment of Caco-2 cells from a monolayer without cell death. Caco-2 intestinal epithelial cells were cultured in a 96 well cell plate for 6 days until confluency and cultured for another 48 h in the absence or the presence of DSS (2%, wt/vol) in the culture medium. (*A*) Live/Dead analysis and a DIC image show an intact Caco-2 monolayer in the absence of DSS. (*B*) DSS treated epithelium was viable and no dead cells were found when images were taken with focal planes of both the cell layer (*Left*) and floating single cells (*Right*). (*C*) Floating single cells from a DSS treated well were collected and cell viability was tested. Collected cell suspension was dropped on a slide glass for the imaging. (*D*) Inset images from (*B*) were enlarged to show a severely dissociated epithelium in response to DSS treatment. Gaps between cells (white arrows) due to the DSS treatment and the subsequent cell detachment were observed. (Scale bars, 50 μm.)

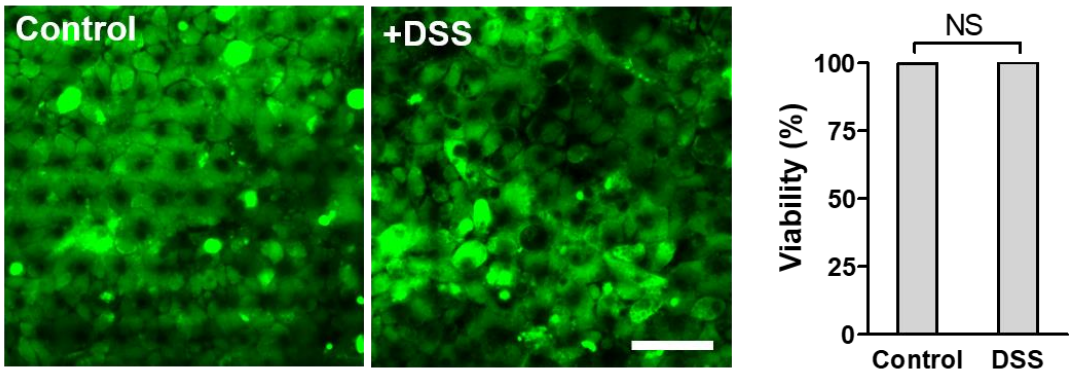

Fig. S6. DSS treatment does not decrease cell viability in the microengineered intestinal villi cultured in a gut-on-a-chip. Live/Dead assay was performed by adding a mixture containing Calcein AM (Live; green) and ethidium homodimer-1 (Dead; red) to the villi pre-conditioned with DSS (2%, wt/vol) for 48 h (+DSS). It is noted that no dead cells were observed. Quantification of the fluorescent images confirms that no significant difference of viability was detected (*Right*). (Scale bar, 50 μm.) NS, not significant.

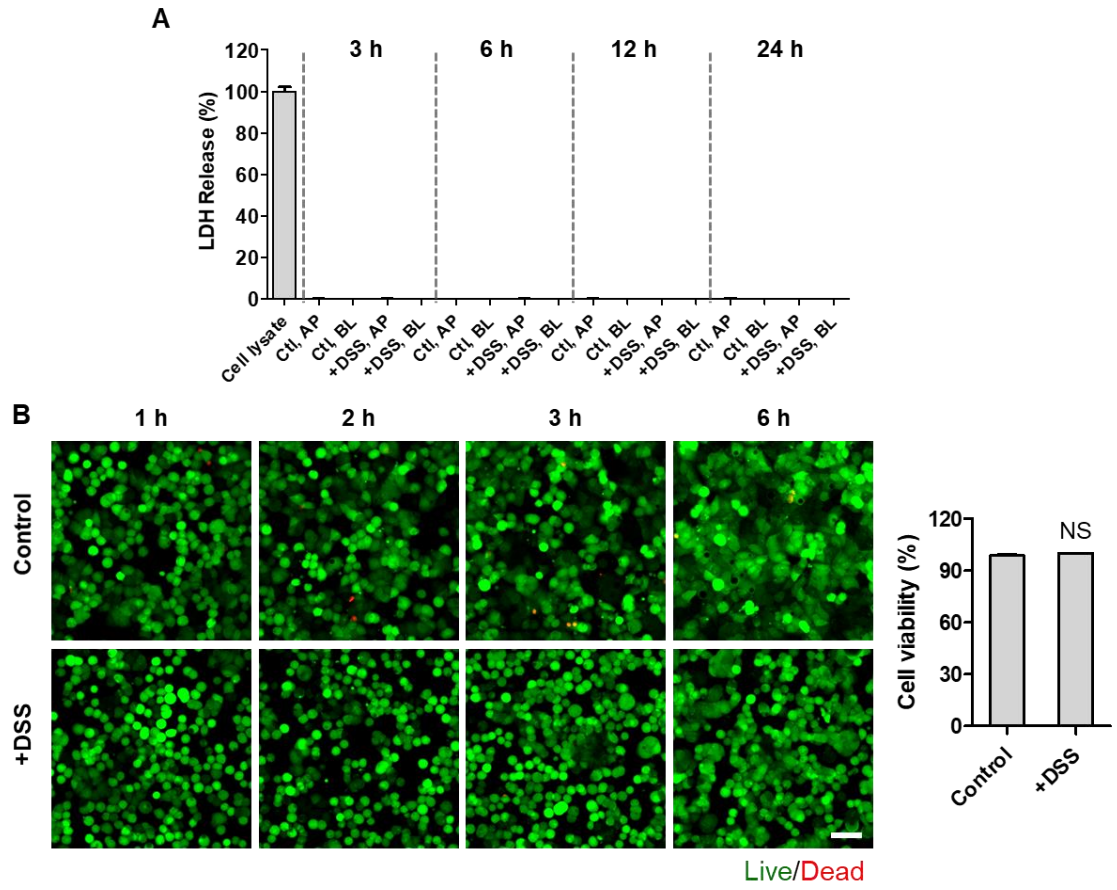

Fig. S7. Treatment of DSS does not induce cytotoxicity nor cell death at the early stage of cultures up to 24 h. (*A*) Cytotoxicity of the human intestinal epithelium grown on the gut inflammation-on-a-chip in the absence (Ctl) or the presence (+DSS) of DSS (2%, wt/vol) was assessed by measuring the LDH activity of the effluents of apical (AP) and basolateral (BL) microchannels of the human gut inflammation-on-a-chip. No detectable level of LDH activity was measured in both control and DSS treated epithelium at each time point. (*B*) Live/Dead cell viability analysis performed on a Matrigel-coated 96 well cell culture plate. Caco-2 cells were seeded (1×10^5 cells per well) on each well incubated for 1 h for an attachment of the cells. After the cell attachment, culture medium was changed into DSS ("+DSS", 2%, wt/vol) containing medium or normal DMEM medium ("Control"). Cell viability analysis was conducted at 1, 2, 3, and 6 h after the DSS treatment. No detectable dead cell signals were found in DSS treated Caco-2 cells. Cell viability was quantitated by counting the number of live cells per total number of cells in the images at 6 h of DSS treatment (*Right*, n = 4). (Scale bar, 50 μm.) NS, not significant.

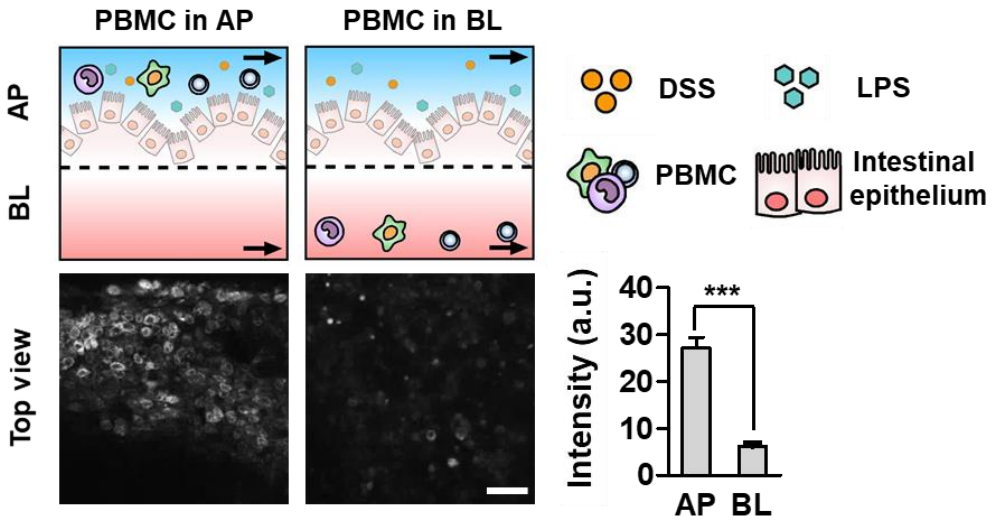

Fig. S8. The direct contact of the DSS-challenged epithelium with PBMC results in the increased oxidative stress. A schematic drawing (*Upper* panel) shows the location of PBMC (4.0×10^6 cells per milliliter) introduced to either the apical (AP) or the basolateral (BL) microchannel. DSS and LPS were co-administered to the AP side. After 24 h, CellROX reagent was used to visualize the level of cytoplasmic ROS (*Lower* panel, corresponding to each schematic). Images were taken using a confocal microscope. Intensity of fluorescence images was quantitated using ImageJ (a right chart; n = 36). (Scale bar, 50 μm.) ***$P < 0.0001$.

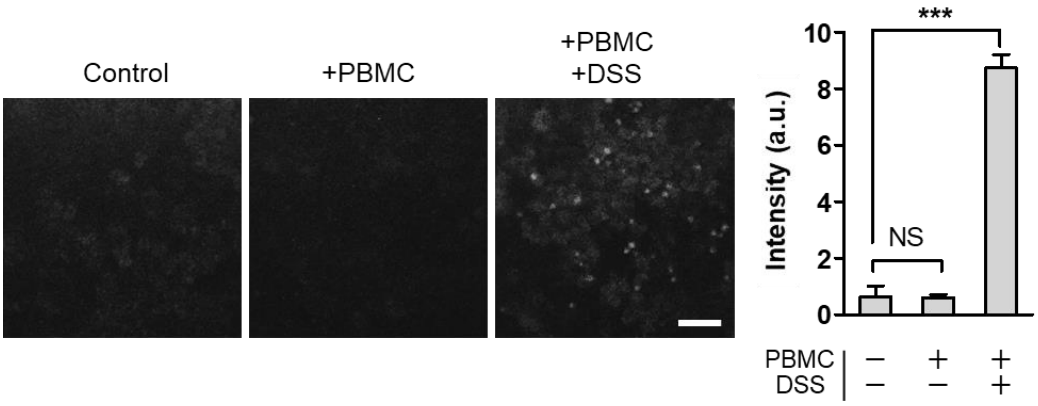

Fig. S9. Direct addition of PBMC to the Caco-2 monolayer grown on the Transwell exerts oxidative stress only in the presence of DSS. Caco-2 cells were grown in Transwell inserts until TEER reaches >800 Ω·cm. After DSS treatment in the AP side of the inserts for 48 h, PBMC (4×10^3 cells per well) was subsequently introduced to the AP side and incubated for 2 h. CellROX reagent was added to the AP side and imaged by a confocal microscope (*Left*). Fluorescence intensity was quantitated using ImageJ (*Right*). Cell contour was drawn to measure average intensity of each drawn area. More than 5 images per experimental condition were used and total number of read is 21 per each group. (Scale bar, 50 μm.) NS, not significant. ***$P < 0.0001$.

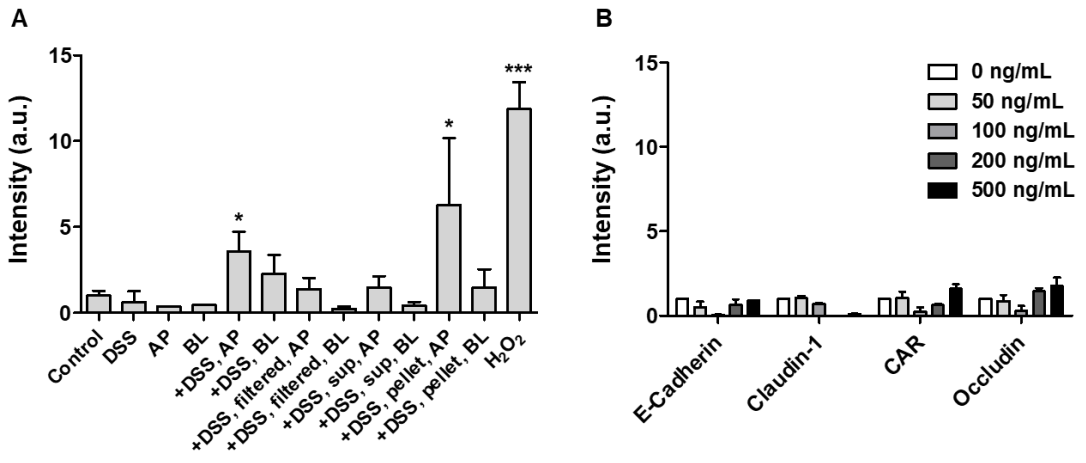

Fig. S10. Cytoplasmic ROS generation occurs when the PBMC elicits the direct contact with the detached DSS-challenged cells in the conditioned medium. (*A*) PBMC (4×10^4 cells) were used to detect the cytoplasmic ROS generation in response to the normal DMEM medium ("Control"), DSS-containing DMEM ("DSS"; 2%, wt/vol), conditioned medium collected from the apical ("AP") or the basolateral ("BL") compartment of a normal gut-on-a-chip, conditioned medium collected from the apical ("+DSS, AP") or the basolateral ("+DSS, BL") channel of a DSS-challenged gut-on-a-chip, filtrate (0.2 μm cut off) of the directionally collected DSS-challenged conditioned medium ("+DSS, filtered, AP" or "+DSS, filtered, BL"), the supernatant of the centrifuged DSS-challenged conditioned medium ("+DSS, sup, AP" or "+DSS, sup, BL"), cell pellet of the centrifuged DSS-challenged conditioned medium ("+DSS, pellet, AP" or "+DSS, pellet, BL"), or H_2O_2-containing DMEM (final concentration, 1 mM; Positive control) and incubated in each well of a 96 well cell culture plate for 4 h at 37°C (n = 3). Culture medium, 100 μL. (*B*) PBMC was resuspended in each junctional protein containing DMEM medium and incubated in each well of a 96 well cell culture plate for 4 h at 37°C (n = 2). CellROX Orange reagent was added to each well and fluorescence intensity was measured using a microplate reader for estimating the level of cytoplasmic ROS (Excitation at 545 nm, emission at 565 nm). **P* < 0.05, ***P* < 0.001, ****P* < 0.0001.

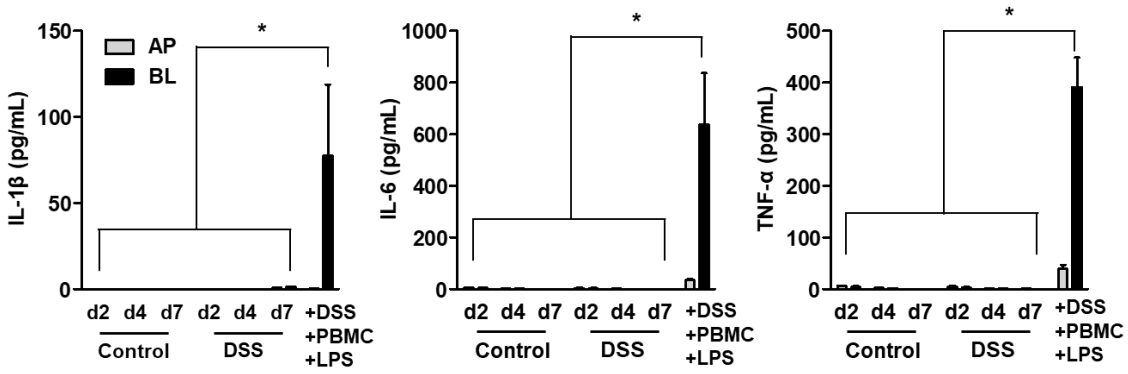

Fig. S11. Treatment of DSS for up to 7 days does not induce the secretion of proinflammatory cytokines. Treatment of DSS ("+DSS"; 2%, wt/vol) to the lumen side of villus epithelium in the device for 2, 4, or 7 days did not induce the production of IL-1β, IL-6, or TNF-α in the AP nor the BL side in the presence of PBMC (4×10^6 cells per milliliter) in the BL side. Control, no addition of DSS. Effluent collected from the AP and the BL microchannels was respectively collected, then used for measuring the level of secreted cytokines (n = 4). As a positive control, cytokine level of the setup that contains DSS (2%) and LPS (10 ng/mL) in the AP channel and PBMC (4×10^6 cells per milliliter) in the BL channel was measured ("+DSS, +PBMC, +LPS"). After 16 h incubation, the positive control resulted in the significant production of IL-1β, IL-6, and TNF-α only in the BL side. *$P < 0.05$.

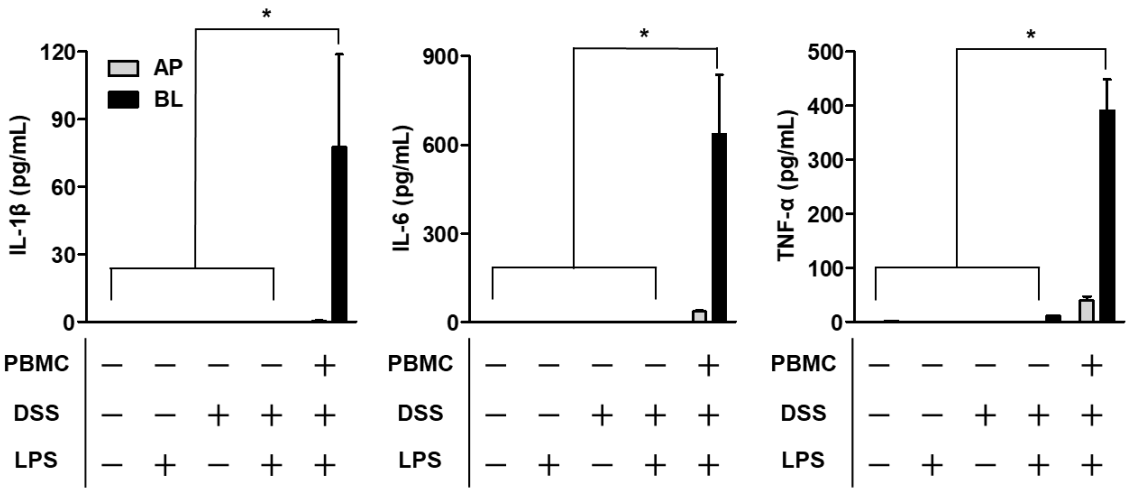

Fig. S12. Secretion of proinflammatory cytokines is only manifested in the presence of PBMC in the presence of both DSS and LPS in the Caco-2 cultures. After the DSS treatment (2%, wt/vol) for 48 h, culture medium collected from AP and BL microchannels of the gut-inflammation-on-a-chip was used for measuring secretory proinflammatory cytokines of IL-1β, IL-6, and TNF-α (n = 4). No detectable level of cytokines was produced in the absence of PBMC. As a positive control, cytokine level of the setup that contains DSS (2%) and LPS (10 ng/mL) in the AP channel and PBMC (4×10^6 cells per milliliter) in the BL channel was measured (+DSS, +PBMC, +LPS). *P < 0.05.

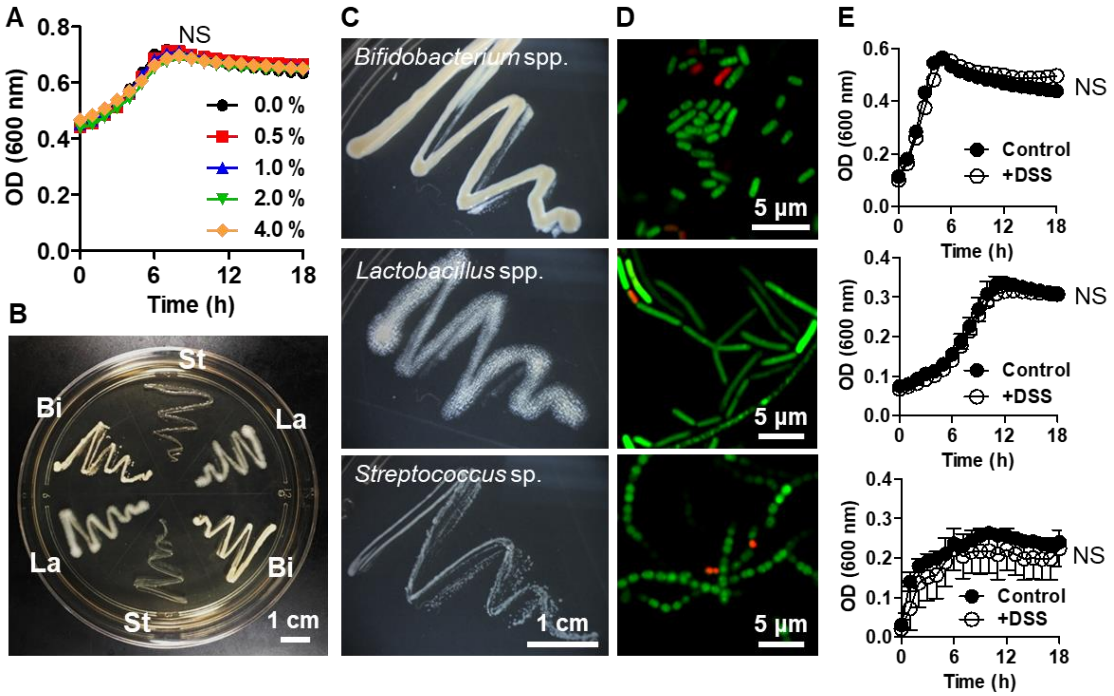

Fig. S13. DSS does not hamper the growth of VSL#3 bacterial cells. (*A*) VSL#3 bacterial cells were suspended in the antibiotics-free DMEM (initial cell density, 1.0×10^7 cfu/mL) and 100 μL of the cell suspension was inoculated in a 96 well plate, where DSS was added at 0, 0.5, 1.0, 2.0, and 4.0% (wt/vol) (n = 3). Optical density at 600 nm was read with 1 h interval for 16 h at 37°C without shaking. The plate was sealed with Parafilm to minimize evaporation of the cell culture medium. Specific growth rate of overall VSL#3 cell mixture did not show significant difference at various DSS concentrations (e.g. $\mu_{0.0\%}$ = 0.069 h^{-1}, $\mu_{0.5\%}$ = 0.075 h^{-1}, $\mu_{1.0\%}$ = 0.070 h^{-1}, $\mu_{2.0\%}$ = 0.068 h^{-1}, and $\mu_{4.0\%}$ = 0.059 h^{-1}), suggesting that the presence of DSS does not alter the growth profile of VSL#3 cells during the chip experiment. Specific growth rate was calculated based on the equation of $\mu = (1/X)(dt/dX)$, where the X is the cell mass and t is the time. (*B*) Major bacterial genera (*Bifidobacterium* spp., Bi; *Lactobacillus* spp., La; and *Streptococcus* sp., St) in the VSL#3 product were isolated and cultured on a MRS agar plate. (*C*) Zoomed-in images of the isolated bacterial cells grown on a MRS agar plate taken by a stereomicroscope. (*D*) Live/Dead assay of the isolated bacterial cells were performed by using a mixture of SYTO 9 (green) and propidium iodide (red) solutions. Bacterial cell suspension was mixed with the Live/Dead solution in equal volume and 10 μL of the mixture was dropped on a slide glass, covered with a cover glass, and then observed under a confocal microscope. (*E*) Growth profile of each genus, *Bifidobacterium* spp., *Lactobacillus* spp., and *Streptococcus* sp., suspended in antibiotics-free DMEM in the absence (closed) or the presence DSS (open; 2%, wt/vol) (n = 3). NS, not significant.

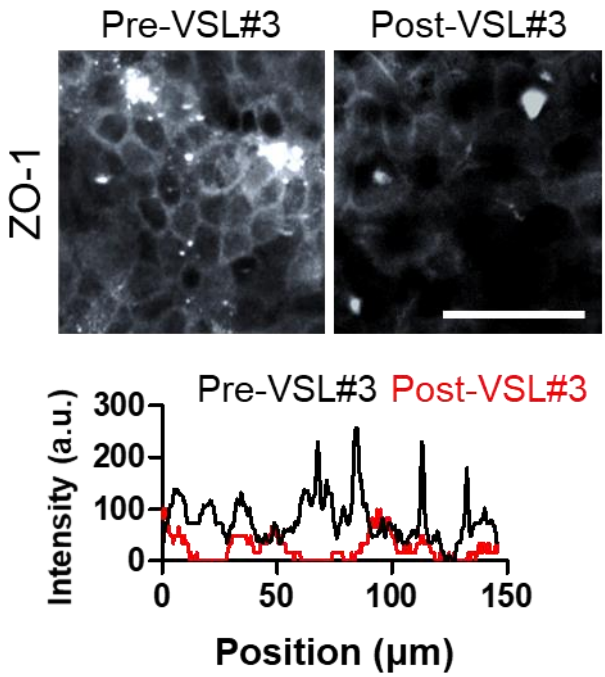

Fig. S14. Co-culture of VSL#3 prior to DSS treatment prevents the disruption of epithelial tight junction protein, ZO-1, in the gut inflammation-on-a-chip. Localization of ZO-1 in the intestinal epithelial cell was visualized when VSL#3 probiotic strains were co-cultured prior to the DSS treatment (Pre-VSL#3) or after the DSS treatment (Post-VSL#3). Immunofluorescence imaging method was used and imaging analysis was performed on a confocal microscope. A line scan of representative images of pre- and post-VSL#3 treatment in the presence of DSS was performed using ImageJ, at random positions. (Scale bar, 50 μm.)

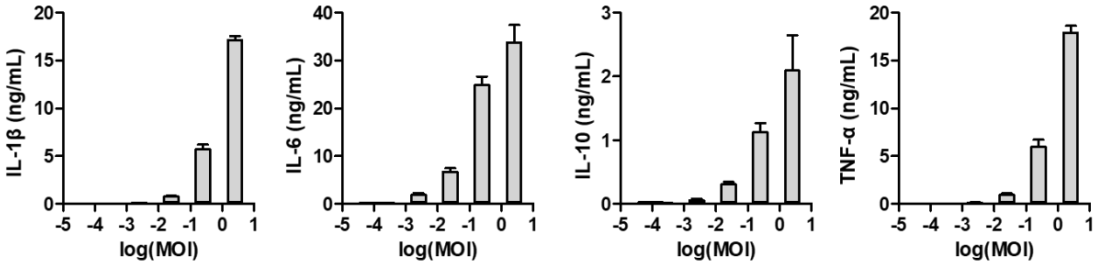

Fig. S15. PBMC produces inflammatory cytokines in response to VSL#3 cells as a function of multiplicity of infection (MOI). PBMCs (1.5×10^{5} cells per well) were challenged to the VSL#3 cells, where the MOI was adjusted at 2.5, 2.5×10^{-1}, 2.5×10^{-2}, 2.5×10^{-3}, 2.5×10^{-4}, 1.25×10^{-4}, and 6.25×10^{-5}. It is noted that the MOI at 2.5 is the same MOI that was applied in Fig. 5. All cultures were maintained in a 37°C CO_2 incubator for 24 h (n = 6). Quantification of the amount of secreted cytokines was carried out by ELISA.

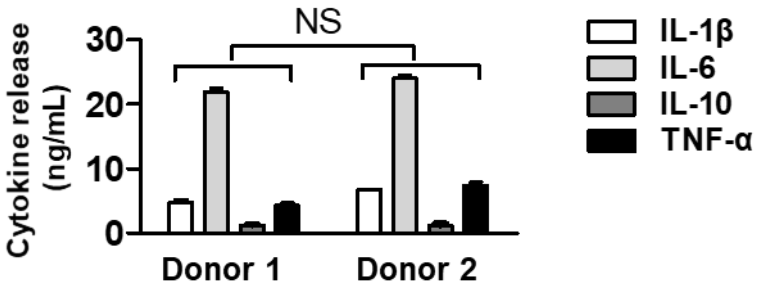

Fig. S16. Profiles of the production of inflammatory cytokines are similar between two independent PBMC samples isolated from different blood donors. PBMCs from two independent donors were dispensed in the 96-well plate (1.5×10^5 cell per well), then challenged to VSL#3 cells (MOI, 0.25) at 37°C for 24 h. After the incubation, cell-free culture broth was used to measure the level of cytokines (IL-1β, IL-6, IL-10, and TNF-α) by ELISA (n = 3). NS, not significant.