Supporting Information

Materials and Methods

Tissue culture
Caco-2 and HEK 293T cells were obtained from the American Tissue Culture Company and cultured according to their guidelines. DMEM, penicillin/streptomycin (P/S), 0.05% Trypsin, and DPBS were obtained from Corning, and Seradigm Premium Grade Fetal Bovine Serum (FBS) and Transfectagro were purchased from VWR. Cell culture Transwell inserts (transparent PET membrane, 12-well, 0.4 μm pore size) and Falcon 12-well companion plates were obtained from BD Falcon. Polybrene and puromycin were purchased from EMD Millipore Sigma. Lipofectamine 2000 was purchased from Thermo Fisher Scientific. Recombinant human TNFα was purchased from R&D Systems, and GNF351 was a kind gift from Avery August.

Metabolites
L-Tryptophan (Trp) was purchased from Chem-Impex International, Inc. Indole-3-aldehyde (I3A) and indole-3-pyruvate (IPyA) were obtained from Biosynth. Tryptophol (IEt) was obtained from Alfa Aesar, and kynurenine (Kyn) was purchased from Cayman Chemical Company. Indole (IND), indole-3-acetamide (IAM), DL-indole-3-lactate (ILA), 5-hydroxytryptamine (5HT), and indole-3-acetic acid (IAA) were purchased from Sigma Aldrich. Tryptamine hydrochloride (TrA) was obtained from TCI America, and indole-3-propionate (IPA) was obtained from Alfa Aesar. Indole-3-acrylate (IA) was purchased from Santa Cruz Biotechnology.

Histology
Hematoxylin was purchased from VWR, and eosin Y was obtained from Acros Organics. Canada Balsam was obtained from Ward's Science, and xylenes was obtained from Macron Fine Chemicals.

Antibiotics
Ampicillin and vancomycin hydrochloride were purchased from Sigma Aldrich. Neomycin sulfate hydrate and metronidazole were obtained from Alfa Aesar.

Western blotting
DC Protein Assay kit and Clarity Western ECL substrate were purchased from BioRad. SuperSignal West Pico Chemiluminescent Substrate was purchased from Thermo Fisher Scientific. Bovine serum albumin (BSA) was purchased from VWR, and non-fat dry milk was purchased from Laboratory Product Sales. Protease inhibitor (cOmplete) tablets were obtained from Roche. Sodium β-glycerophosphate was obtained from Alfa Aesar, and sodium orthovanadate was purchased from MP Biomedicals. Sodium fluoride was purchased from Chem-Impex International, Inc., and sodium pyrophosphate decahydrate was purchased from Fisher Scientific.

Immunofluorescence
Paraformaldehyde (PFA) 32% solution, EM grade, was purchased from Electron Microscopy Sciences, and DAPI Prolong Diamond was purchased from Thermo Fisher Scientific. Fisher HealthCare Tissue Plus OCT Compound was purchased from Fisher Scientific.

Transfection and plasmids
PEI was obtained from Polyplus Transfection. pGudluc6.1 and pRLTK were obtained from Gary Perdew (Pennsylvania State University), and pB2X and pRenilla were obtained from Ruslan Medzhitov (Yale University). pCMV-VSV-G, wildtype ezrin, T567A ezrin, and T567E ezrin mutants (C-terminal FLAG tagged) in pQCXIP expression vectors were a kind gift of Anthony Bretscher (Cornell University).

LC-MS
LC-MS grade methanol, water, acetonitrile, and formic acid (FA) were obtained from Fisher Scientific.

qPCR
RNABee was obtained from Tel-Test, Inc. Diethylpyrocarbonate (DEPC) and chloroform were purchased from Sigma Aldrich. Isopropanol and ethanol were purchased from VWR. Glycogen (Roche) was purchased from Krackeler Scientific. OligodT was purchased from Integrated DNA Technologies (IDT). dNTP was purchased from BioBasic. MMLV reverse transcriptase was purchased from Clontech, and PerfeCta SYBR Green SuperMix, Low ROX, were obtained from Quanta Biosciences.

Antibodies
Primary antibodies for ZO1 (clone 1A12, 33-9100) and occludin (clone 3F10, 33-1500) were purchased from Thermo Fisher Scientific. Anti-E-cadherin (clone 67A4, 562869) and anti-β-catenin (clone 14, 610154) antibodies were purchased from BD Biosciences. Anti-MLCK (clone K36, M7905) and anti-α-tubulin (clone B512, T5168) antibodies were purchased from EMD Millipore Sigma. Primary antibodies for MLC (clone E-4, sc-28329) was purchased from Santa Cruz Biotechnology and p-MLC (Ser19) (3675) was purchased from Cell Signaling Technology. Anti-ezrin antibody (CPTC-ezrin-1 supernatant) was purchased from the University of Iowa Developmental Studies Hybridoma Bank, and the p-ezrin (T567) rabbit antisera was generously provided by Anthony Bretscher (Cornell University). Anti-mouse horseradish peroxidase (HRP) (170-5947) and anti-rabbit HRP (170-6515) secondary antibodies for Western blot were purchased from BioRad. Donkey anti-mouse Alexa Fluor 594 (A21293), donkey anti-rabbit Alexa Fluor 594 (R37119), and Alexa Fluor 647 Phalloidin (A22287) were purchased from Thermo Fisher Scientific.

Primers
Human *Ahr* forward (TGGGTCCAGTCTAATGCACG) and reverse (TGCTCTGTTCCTTCCTCATCT), *Cyp1a1* forward (GTGATCCCAGGCTCCAAGAG) and reverse (AGAAGAAACTCCGTGGCCG), *Cyp1a2* forward (GCTGAATGGCTTCTACATCCCC) and reverse (GCGGTGAGGAACCGCTC), *Cyp1b1* forward (AACGTACCGGCCACTATCAC) and reverse (GCACTCGAGTCTGCACATCA), *Ahrr* forward (GCAGCGGAGATGAAAATGAGG) and reverse (TTCCGATTCGCACAGACTGG), *Zo1* forward (GACGTTTCCCCACTCTGAAA) and reverse (AGAGCACAGCAATGGAGGAA), and *Rpl13a* forward (TCCTCCTTTTCCAAGCGGC) and reverse (GGCCCAGCAGTACCTGTTT) primers were purchased from IDT. Mouse *Il10r* forward (AGTCTTCAGTTCTCAGGACGC) and reverse (GCAATGAATTCTAGGCTCAGGC), *Tnfr1* forward (GCTGTTGCCCCTGGTTATCT) and reverse (ATGGAGTAGACTTCGGGCCT), and *Rpl13a* forward (GCTGCCGAAGATGGCGGAGG) and reverse (ACCACCACCTTCCGGCCCA) were also purchased from IDT.

Mice
C57Bl/6 mice were acquired from Jackson Laboratories. *Ahr*+/- and *Ahr*-/- mice were obtained from Gary Perdew (Pennsylvania State University). All mice were subsequently bred and maintained at the animal facility of Cornell University and used at 8-12 weeks of age in accordance with the guidelines of the Institutional Animal Care and Use Committee and the Cornell Center for Animal Resources and Education (Protocol number 2015-0069). Mice were co-housed for 7 d prior to use and fed Envigo Teklad global irradiated 18% protein rodent diet meal 2918 as standard chow. Mice were anesthetized with Butler Schein Animal Health Isothesia Isoflurane, USP, 3% vol/vol. Dextran sodium sulfate (DSS) was obtained from Chem-Impex International, Inc., and fluorescein isothiocyanate (FITC)-dextran (FD4, average molecular weight 3,000 – 5,000) was obtained from Sigma Aldrich.

In vivo experiments
Antibiotic treatment
Mice were administered an antibiotic cocktail of ampicillin (9 mg/kg), metronidazole (9 mg/kg), neomycin (9 mg/kg), and vancomycin (4.5 mg/kg) via oral gavage every 12 h. Mice were pre-treated with antibiotics for 7 d

prior to receiving high tryptophan diet or metabolites and were continued to be administered the antibiotic cocktail for the remainder of the experiment.

High tryptophan diet
Standard chow (Envigo Teklad global irradiated 18% protein rodent diet meal 2918) or high tryptophan (Trp) diet was provided to mice *ad libitum* in feeding jars. The high tryptophan diet was prepared by supplementing standard chow (2 g Trp/kg diet) with an additional 40 g Trp/kg diet. Mice received high tryptophan diet for 7 d prior to DSS treatment and then for the remainder of the experiment.

Metabolite treatment
Mice were administered I3A (1000 mg/kg), IPyA (2900 mg/kg), or IEt (600 mg/kg). All metabolites were dissolved in dimethylsulfoxide (DMSO) and administered via oral gavage every 12 h. Control mice received equivalent volumes of DMSO via gavage. Mice were pre-treated with metabolite gavage for 2 d prior to DSS treatment and then for the remainder of the experiment.

DSS colitis
Mice were treated with 3% DSS (wt/vol) in their drinking water (*ad libitum*) for 7 d. The amounts of water consumed by each treatment group were monitored and compared to control groups.

Weight loss
Mice were weighed daily at the same time each day. Each mouse weight was normalized to itself and control mice on day 0.

4 kDa FITC-dextran intestinal permeability assay
On the last day of the experiment, mice were fasted for 4 h prior to gavage with FD4 (900 mg/kg) in PBS. Prior to euthanasia and 4 h post-gavage, 100 µl of blood was collected via retro-orbital bleed and centrifuged to obtain at least 50 µl of serum. Concentration of FD4 in the serum was determined using a SpectraMax Gemini EM Microplate Reader and a standard curve from a serial dilution of FD4 in PBS.

Colon length
Entire colons were resected upon euthanasia and measured.

Disease activity index
Stool consistency (0 = normal, 1 = mildly soft, 2 = soft, 3 = very soft, 4 = diarrhea) and blood in the colon (0 = none, 1 = mildly red, 2 = red, 3 = dark red, 4 = black) were assessed and added together to obtain a disease activity index (DAI) score between 0 and 8.

Histopathology
A portion of the distal colon was flushed with PBS, excised and fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned (5 µm), and stained with Harris hematoxylin and eosin Y. Samples were blinded, imaged using an Olympus CX41RF microscope, and given a score between 0 and 4, where 0 = normal pathology, 1 = mild, multifocal individual crypt epithelial cell attenuation and goblet cell depletion without inflammatory cell infiltrate, 2 = mild, multifocal crypt epithelial cell loss without inflammatory cell infiltrate, 3 = moderate, multifocal epithelial cell loss with mild lamina propria lymphocytic and neutrophilic infiltrate, and 4 = severe, diffuse surface epithelial cell erosion with extensive crypt epithelial cell necrosis and loss, mild lamina propria lymphocytic and rare neutrophilic infiltrate, and moderate submucosal edema.

Cryosection preparation for immunofluorescence
A portion of the distal colon was flushed with PBS, excised, and frozen in OCT. OCT blocks were sectioned (5 μm) on a Thermo Scientific Microm HM 525 cryostat, adhered to a glass slide, washed with PBS, and fixed with 4% PFA prior to staining.

Western blot lysates
A portion of the distal colon was flushed with PBS. Epithelial cells were scraped off using a pipette tip and lysed in 1X RIPA lysis buffer (150 mM sodium chloride, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 25mM Tris, 1 mM EDTA) with 1X protease inhibitor (cOmplete tablets) and sodium β-glycerophosphate (17.5 mM), sodium orthovanadate (1 mM), sodium fluoride (20 mM), and sodium pyrophosphate decahydrate (5 mM) added immediately prior to use. Samples from biological replicates were pooled before quantification by the DC Protein assay kit.

RNA isolation and qPCR analysis
Intestinal epithelial cells were collected from the distal colon, and RNA was purified using RNABee (300 μl) according to the manufacturer's instructions and quantified using a GE Nanovue. Using a BioRad C1000 Touch Thermal Cycler, RNA was reverse transcribed using an oligo(dT) primer and MMLV reverse transcriptase. cDNA samples were analyzed using PerfeCta SYBR Green SuperMix, Low ROX, and a BioRad CFX96 Real-Time PCR Detection System. PCR amplification conditions were as follows: 95 °C (3 min) and 40 cycles of 95 °C (15 s) and 60 °C (45 s). Relative expression of transcripts was normalized to the ribosomal protein L13a (*Rpl13a*). Data are represented as the fold induction over control samples.

LC-MS
Feces contents were collected fresh from mice and immediately flash frozen in liquid nitrogen. Frozen samples were dried on a VirTis Benchtop K Series Manifold Freeze Dryer. Dried samples were crushed and resolubilized in methanol (10x the volume of the dry weight of the samples) and rocked at room temperature for 1 h before collecting the supernatant, which was then dried down. Immediately prior to LC-MS analysis, the samples were resuspended in methanol (10x the volume of the dry weight of the samples) and filtered. LC-MS analysis was performed on an Agilent 6230 electrospray ionization–time-of-flight (ESI–TOF) MS coupled to an Agilent 1260 HPLC equipped with an Agilent Poroshell 120 ECC18 reverse phase column (3 x 50 mm, 2.7 μm) using a flow rate of 0.5 ml/min. The gradient was ramped from 90% water and 0.1% FA (Solvent A) and 10% acetonitrile and 0.1% FA (Solvent B) to 50% A and 50% B for 0.5 min. The gradient was then ramped to 35% A and 65% B for an additional 0.5 min, then to 15% A and 85% B for 4.5 min, followed by 0% A and 100% B for 0.75 min. The gradient was then held constant at 0% A and 100% B for an additional minute. For detection, the MS was equipped with a dual ESI source operating in positive or negative mode, acquiring in extended dynamic range from m/z 100–3200 at one spectrum per s; gas temperature: 325 °C; drying gas 10 L/min; nebulizer: 20 psi; fragmentor: 80 V. Quantification of metabolites was determined by integrating the extracted ion count of the exact masses of the metabolites, which were determined using commercial standards. Standard curves in which known amounts of metabolite were utilized to determine the amount of each metabolite in each sample, which was normalized to the dry weight of the fecal samples.

Caco-2 monolayers
Cell culture
Caco-2 cells were seeded at 15,000 cells/insert in Transwell inserts in 12-well companion plates. Monolayers were grown in DMEM supplemented with 10% FBS and P/S at 37 °C and 5% CO_2. Media was replaced every 2-3 days.

Treatment
On day 18, metabolites were added to the insert (1 mM unless otherwise specified) in DMSO. Equivalent volumes of DMSO were applied to control cells. On day 20, media and metabolites were replaced, and human

TNFα (hTNFα, 20 ng/ml) was added to the basolateral chamber. For monolayers treated with GNF351 (1 μM), this inhibitor was added initially to the media on day 17 and replenished on day 18 and 20.

Transepithelial electrical resistance
Transepithelial electrical resistance (TEER) was measured using a World Precision Instruments EVOM2 Epithelial Voltohmmeter. TEER was normalized to the surface area of the insert, each insert itself, and control inserts on day 0.

4 kDa FITC-dextran permeability assay
Monolayers were washed, and media was replaced with Hank's Balanced Salt Solution (HBSS) 22 h after addition of hTNFα. FD4 was added to the insert at 1 mg/ml in HBSS. After 2 h, 200 μl of HBSS was removed from each well. Concentration of FD4 in the well was determined using a SpectraMax Gemini EM Microplate Reader and a standard curve from a serial dilution of FD4 in HBSS.

Monolayer immunofluorescence
Monolayers were washed with PBS 24 h after addition of hTNFα and fixed with 4% PFA prior to staining.

Linearity index analysis
In Caco-2 cells stained for tight junction proteins ZO1 or occludin, cells were traced from vertex to vertex using either a freehand or straight line in FIJI ImageJ. The linearity index is the average ratio of the length of the freehand line to the length of the straight line for each vertex-to-vertex segment traced from 10 cells from each of the three replicate inserts per treatment.

WB lysate preparation
Monolayers were washed with PBS 24 h after addition of hTNFα, and cells were lysed with 100 μl 1X RIPA lysis buffer with protease and phosphatase inhibitors as above. Samples from biological replicates were pooled prior to quantification as above.

RNA isolation and qPCR analysis
Monolayers were washed with PBS 24 h after addition of hTNFα, and cells were lysed with 1 ml RNABee per insert prior to RNA purification and qPCR as above.

Ezrin transduction of Caco-2 monolayers
HEK 293T cells were seeded at 10^5 cells/well in 6-well plates to be 90-95% confluent the next day. The next day, cells were washed and media was replaced with Transfectagro and 5 μl PEI (1 mg/ml), 0.33 μg of pCMV-VSV-G, and 1 μg of pQCXIP vector, or wildtype ezrin, T567A or T567E FLAG-tagged mutants in the pQCXIP expression vector were added. The cells were incubated at 37 °C for 4 h, after which media was replaced, and the cells were incubated for an additional 2 d. Afterwards, polybrene was added at 4 μg/ml, and HEK 293T cell supernatant was collected and syringe filtered using a 0.45 μm filter. Filtered supernatant was added drop by drop to Caco-2 cells, and the cells were incubated for 2 d at 37 °C. Transduced cells were then split 1:2 into media containing puromycin (1 μg/ml). After selection, successful transduction was confirmed by anti-FLAG Western blotting, and cells were utilized to set up monolayers as previously described above.

Immunofluorescence
Fixed samples were permeabilized with 0.5% Triton X-100 in PBS at room temperature for 15 min, blocked with 5% BSA in PBS at room temperature for 1 h, and then incubated with the appropriate antibodies in 5% BSA in PBS at room temperature for 2 h. Antibodies against ZO1, occludin, E-cadherin, β-catenin, MLC, MLCK, and p-MLC were each diluted 1:1 in glycerol and used at a dilution of 1:100. The ezrin antibody and p-ezrin antisera were also diluted 1:1 in glycerol and used at a dilution of 1:200. Samples were incubated with appropriate species-specific Alexa Fluor 594 antibodies and Alexa Fluor 647 Phalloidin at a dilution of 1:500 in

5% BSA in PBS in the dark at room temperature for 1 h, then mounted with DAPI Prolong Diamond overnight. Samples were imaged with a Zeiss LSM 800 confocal laser scanning microscope equipped with 20X 0.8 NA and 40X 1.4 NA Plan Apochromat objectives, 405, 488, 561, and 640 nm solid-state lasers, and two GaAsP PMT detectors. Images shown are maximum intensity z-stack projections. Relative brightness of stained cells was quantified using FIJI ImageJ and normalized to control cells. For images of Caco-2 cells co-stained for ZO1 and actin, Pearson's correlation coefficient (R^2) was determined using FIJI ImageJ to quantify co-localization.

Western blot

Lysates were sonicated using a Heat Systems Ultrasonic Processer XL sonicator. Protein concentrations were determined using the DC Protein Assay kit and BioTek PowerWave XS2 plate reader. Protein concentrations were normalized using 6X Laemmli buffer and ddH_2O. Lysates were resolved on polyacrylamide gels and transferred to nitrocellulose. Membranes were blocked with 5% BSA in 25 mM Tris, 150 mM sodium chloride, and 0.1% Tween-20 solution (TBS-T) rocking at room temperature for 1 h, then probed with the appropriate antibodies in 5% milk or BSA in TBS-T with 0.05% sodium azide with rocking at 4 °C overnight. Occludin, β-catenin, and MLC primary antibodies were each diluted 1:1 with glycerol, then used at a 1:500 dilution in 5% BSA in TBS-T for Western blotting. E-cadherin, MLCK, and p-MLC primary antibodies were each diluted 1:1 with glycerol, then used at a 1:500 dilution in 5% milk in TBS-T. The ezrin antibody and p-ezrin antisera were diluted 1:1 in glycerol and used at 1:2,000 and 1:1,000, respectively, in 5% BSA in TBS-T. The antibodies for α-tubulin and GAPDH were used at 1:10,000 and 1:2,500, respectively, in 5% BSA in TBS-T. Overnight incubation in primary antibody was followed by washing and incubation with appropriate species-specific HRP antibody diluted at 1:10,000 in 5% milk in TBS-T with rocking at room temperature for 1 h. Western blots were developed using chemiluminescence detection reagents on a BioRad ChemiDoc MP. Occludin, E-cadherin, β-catenin, MLC, MLCK, ezrin, p-ezrin, α-tubulin, and GAPDH Western blots were developed using BioRad Clarity Western ECL substrate, and p-MLC Western blots were developed using SuperSignal West Pico Chemiluminescent Substrate. Densitometry was performed using FIJI ImageJ and normalized to the housekeeping protein (e.g., GAPDH) and control lysate bands.

AhR and NF-kB luciferase activity assays

Passive lysis buffer (5X PLB) was prepared with 125 mM Tris, pH 7.8, 10 mM 1,2-diaminocyclohexane tetraacetic acid (CDTA), 10 mM DTT, 5 mg/mL BSA, 5% (vol/vol) Triton X-100, and 50% (vol/vol) glycerol in ddH_2O. An aqueous solution of 1X firefly luciferase substrate was prepared containing 75 mM HEPES, pH 8.0, 4 mM $MgSO_4$, 20 mM DTT, 0.1 mM EDTA, 0.53 mM ATP, 0.27 mM coenzyme A, and 0.47 mM D-luciferin (firefly) in ddH_2O. An aqueous solution of 1X Renilla luciferase buffer was prepared containing 7.5 mM sodium acetate, pH 5.0, 400 mM sodium sulfate, 10 mM CDTA, 15 mM sodium pyrophosphate, and 0.025 mM 2-(4-aminophenyl)-6-methylbenzothiazole. A 100X Renilla luciferase substrate was prepared by diluting coelenterazine to 0.55 mM in anhydrous methanol and added to 1X Renilla luciferase buffer immediately prior to the assay.

AhR activity assay

HEK 293T cells were plated at 10^5 cells/well in a 24-well plate. At 50-60% confluency, the cells were transfected overnight with 1 µg of pGudluc6.1 and 100 ng of pRLTK per well. The next day, media was replaced, and cells were treated for 24 h with I3A, IPyA, or IEt (1 mM) or with an equivalent volume of DMSO. After 24 h, cells were washed with PBS, lysed with 1X PLB, and 20 µl of lysate was added to a 96-well white opaque plate. Afterwards, 50 µl of 1X firefly luciferase substrate was added to each well, and luminescence was measured for 10 min using a Turner BioSystems Veritas Microplate Luminometer. Immediately after, 50 µl of the 1X Renilla substrate was added to each well, and luminescence was measured for 10 min. Luciferase activity was determined by subtracting a blank and calculating the ratio of the firefly luciferase signal to the Renilla luciferase signal.

NF-kB activity assay

HEK 293T cells were plated at 10^5 cells/well in a 12-well plate. At 80-90% confluency, the cells were transfected overnight with 1 µg of pB2X and 0.1 µg of pRenilla per well. The next day, transfected cells were re-plated at 10^4 cells/well into a 96-well plate and incubated overnight. The next morning, cells were treated for 3 h with I3A, IPyA, or IEt (1 mM) or with an equivalent volume of DMSO. After 3 h, TNFα (10 ng/ml) was added to appropriate wells for 3 h. After 3 h, cells were washed with PBS, lysed with 1X PLB, and 20 µl of lysate was added to a 96-well white opaque plate. Afterwards, 50 µl of 1X firefly luciferase substrate was added to each well, and luminescence was measured for 10 min using a Turner BioSystems Veritas Microplate Luminometer. Immediately after, 50 µl of the 1X Renilla substrate was added to each well, and luminescence was measured for 10 min. Luciferase activity was determined by subtracting a blank and calculating the ratio of the firefly luciferase signal to the Renilla luciferase signal.

Statistical analysis

Experiments were completed at least three independent times. Error bars signify standard deviation from the mean. Statistical significance was determined using one-way ANOVA followed by post-hoc Tukey's test.

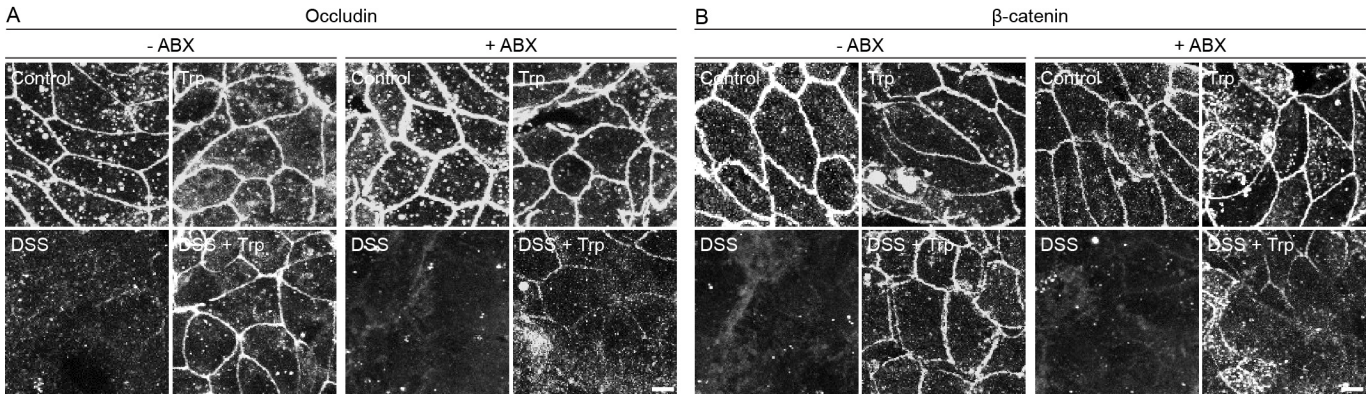


Figure S1. Microbial tryptophan (Trp) metabolites reduce disassembly of tight (TJ) and adherens junction (AJ) proteins within the intestines using a mouse model of colitis. C57Bl/6 mice were pre-treated with antibiotics (ABX: ampicillin (9 mg/kg), metronidazole (9 mg/kg), neomycin (9 mg/kg), and vancomycin (4.5 mg/kg), i.g.) for 7 d, then fed a Trp-rich diet (42 g Trp/kg diet) or standard chow (2 g Trp/kg diet) for 7 d, followed by administration of dextran sodium sulfate (DSS, 3%, wt/vol) or vehicle for 7 d (*ad libitum*) with continued antibiotic treatment and Trp feeding. Colon sections were stained for (A) occludin and (B) β-catenin and imaged by confocal microscopy. Data are representative of at least 3 independent experiments, n = 5 mice per group.

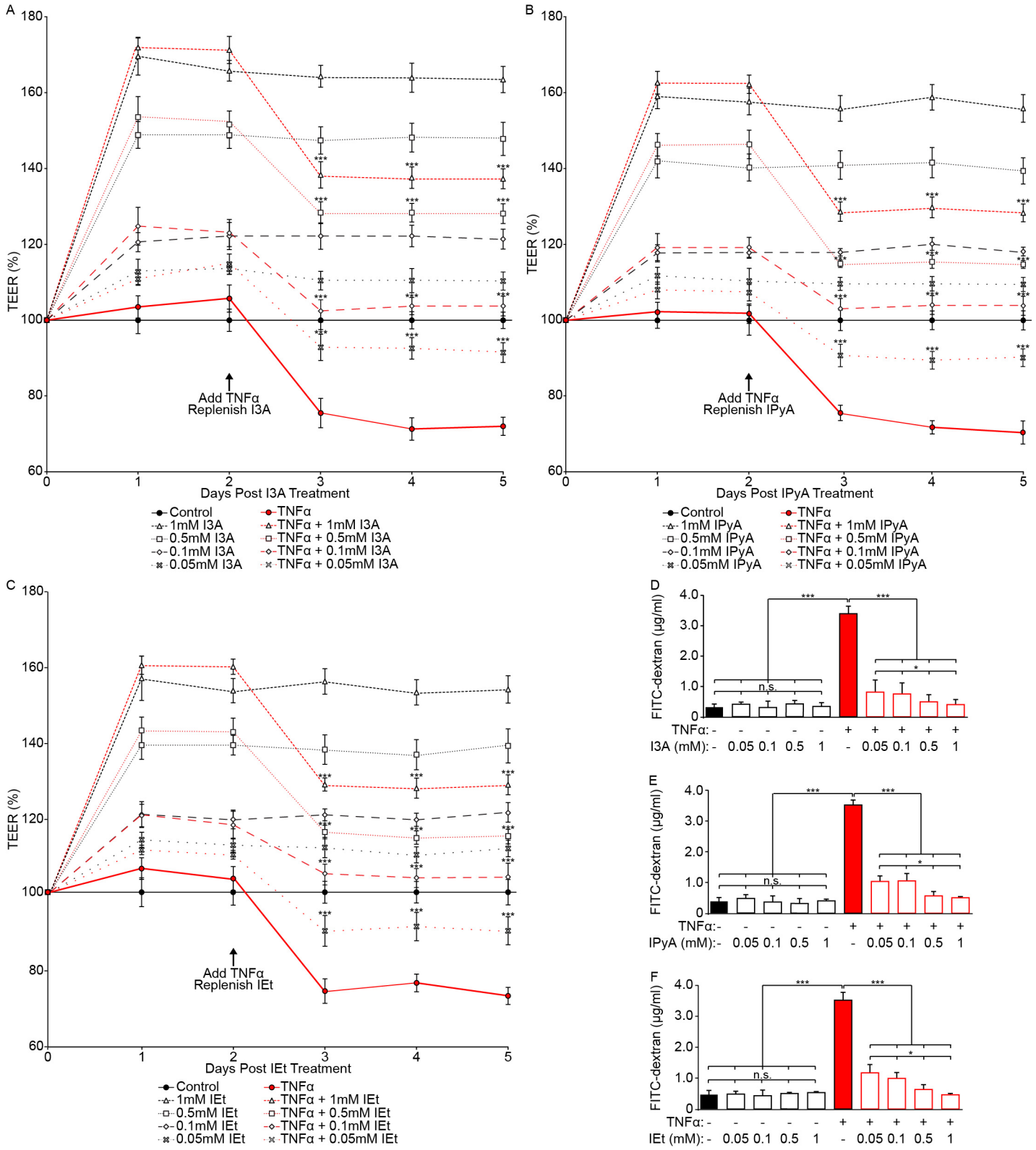


Figure S2. Tryptophan metabolites indole-3-aldehyde (I3A), indole-3-pyruvate (IPyA), and indole-3-ethanol (IEt) decrease epithelial permeability. Polarized Caco-2 monolayers were pre-treated with metabolite for 2 d at the indicated concentrations of metabolite or vehicle (0.1% DMSO) and then stimulated with TNFα (20 ng/ml) at the indicated times. Epithelial permeability was measured by (A-C) transepithelial electrical resistance (TEER) or (D-F) FITC-dextran paracellular transport from the apical to basolateral compartment. Data are

representative of at least 3 independent experiments. One-way ANOVA followed by post-hoc Tukey's test: n = 3, *$p<0.05$, ***$p<0.001$, n.s. = not significant.

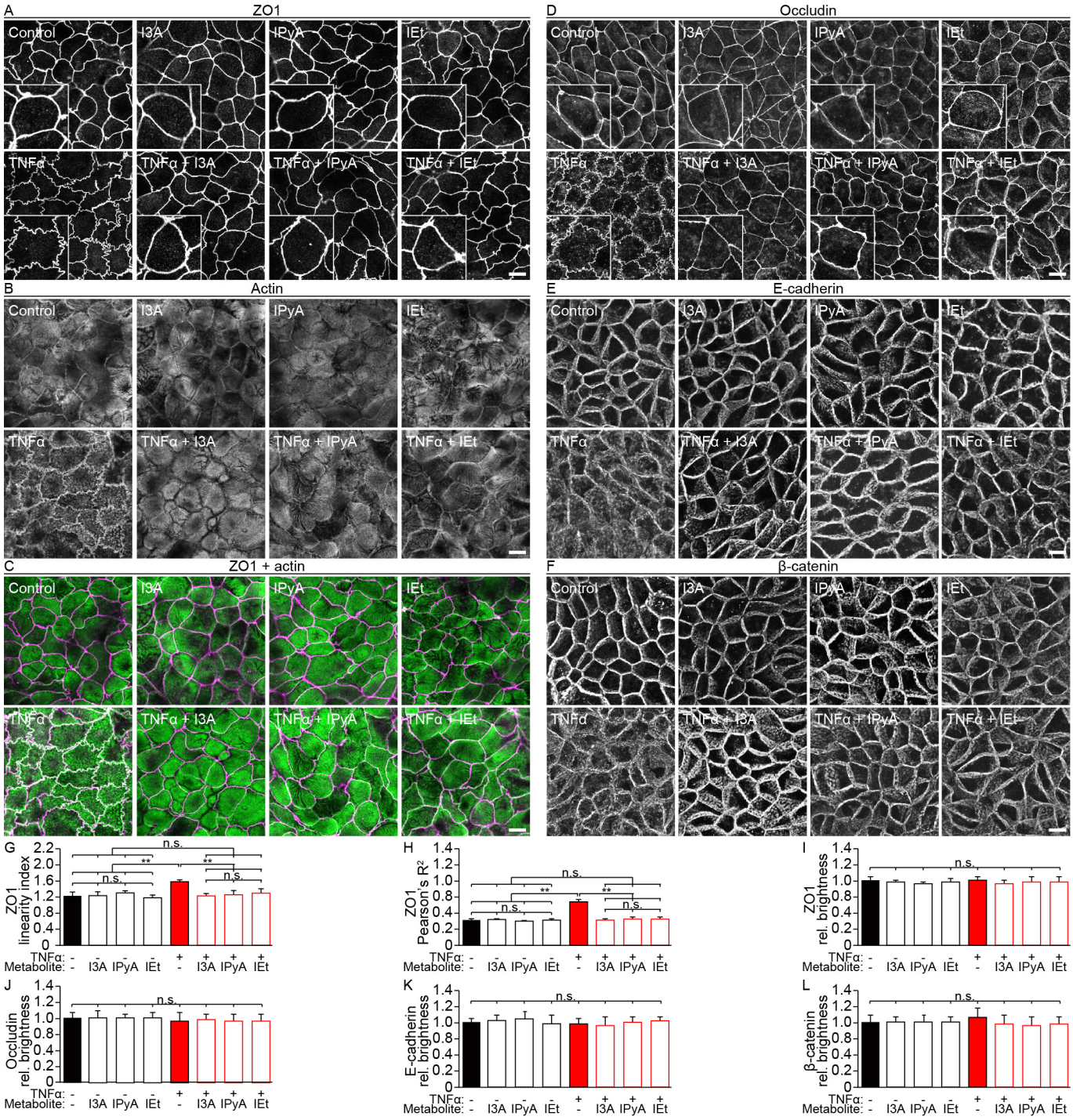


Figure S3. Tryptophan metabolites indole-3-aldehyde (I3A), indole-3-pyruvate (IPyA), and indole-3-ethanol (IEt) decrease epithelial permeability. (A-L) Polarized Caco-2 monolayers were pre-treated with metabolite (1 mM) for 2 d and then stimulated with TNFα (20 ng/ml) for 24 h, after which the cells were stained with the indicated antibodies and imaged by confocal microscopy. (C) Magenta = ZO1, green = actin, white = merged signal. Insets: 2.5x magnification. Scale bars = 20 µm. (G) Linearity index for ZO-1 was calculated (n = 30). (H) Pearson's correlation coefficient (R^2) was calculated for ZO1 and actin. (I-L) Relative (rel.) brightness of images with error as standard deviation from the mean was calculated (n = 15). Data are representative of at least 3 independent experiments. One-way ANOVA followed by post-hoc Tukey's test: n = 3, **p<0.01, n.s. = not significant.

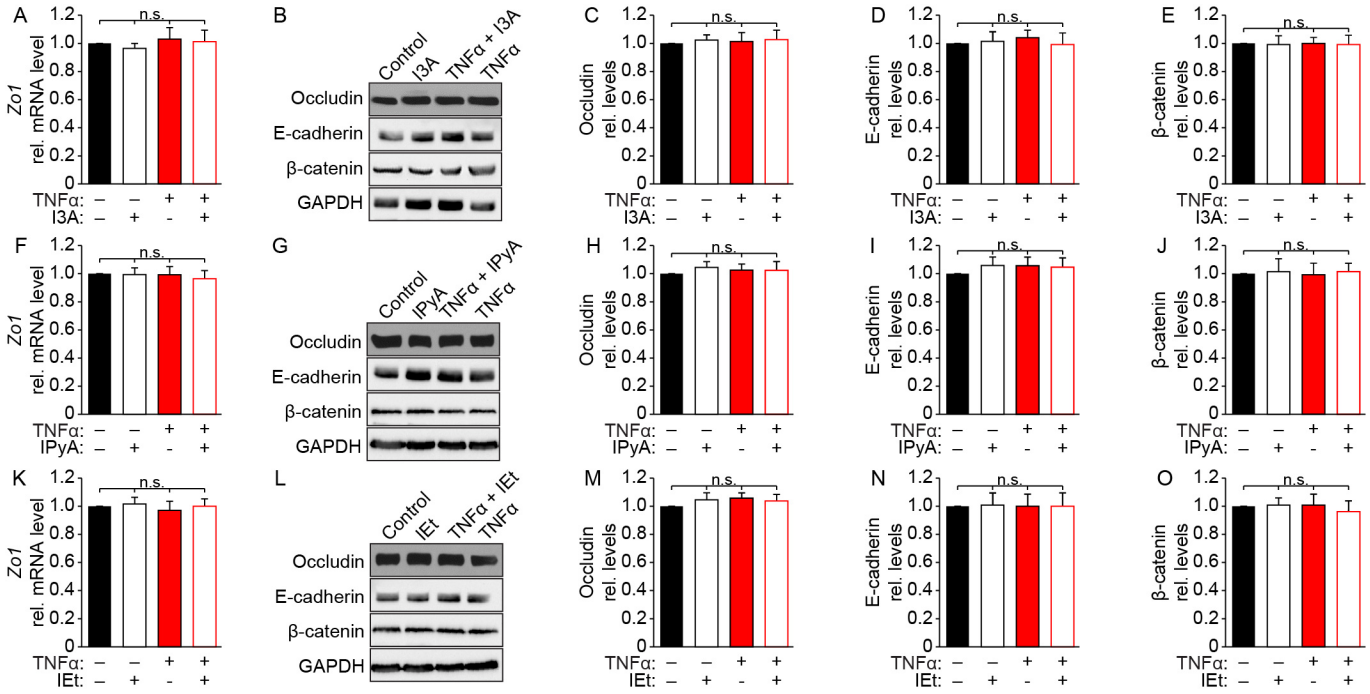


Figure S4. Tryptophan metabolites indole-3-aldehyde (I3A), indole-3-pyruvate (IPyA), and indole-3-ethanol (IEt) do not change levels of mRNA transcripts or protein expression of TJ and AJ proteins. Polarized Caco-2 monolayers were pre-treated with metabolite (1 mM) for 2 d and then stimulated with TNFα (20 ng/ml) for 24 h. (A,F,K) mRNA was isolated and analyzed by qPCR for *Zo1*. (B-E, G-J, L-O) Protein lysates were generated and analyzed by Western blotting for the indicated proteins and quantified by densitometry. Data are representative of at least 3 independent experiments. One-way ANOVA followed by post-hoc Tukey's test: n = 3, n.s. = not significant.

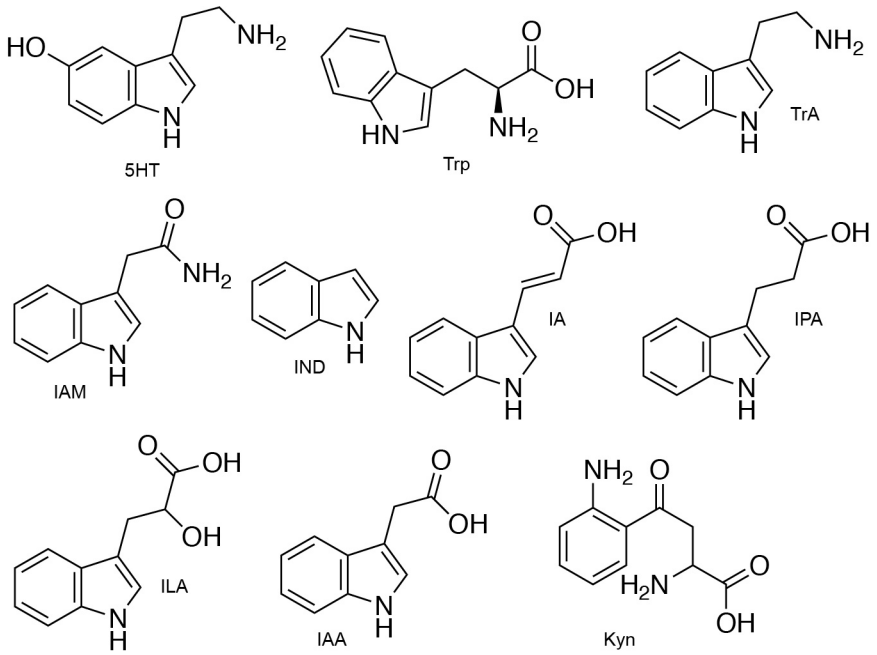


Figure S5. Chemical structures of tryptophan (Trp) metabolites that were tested and did not affect epithelial permeability in this study. Abbreviations: tryptophan (Trp), indole (IND), kynurenine (Kyn), indole-3-acetamide (IAM), indole-3-lactate (ILA), tryptamine (TrA), indole-3-acetic acid (IAA), indole-3-propionate (IPA), indole-3-acrylate (IA), 5-hydroxytryptamine (5HT).

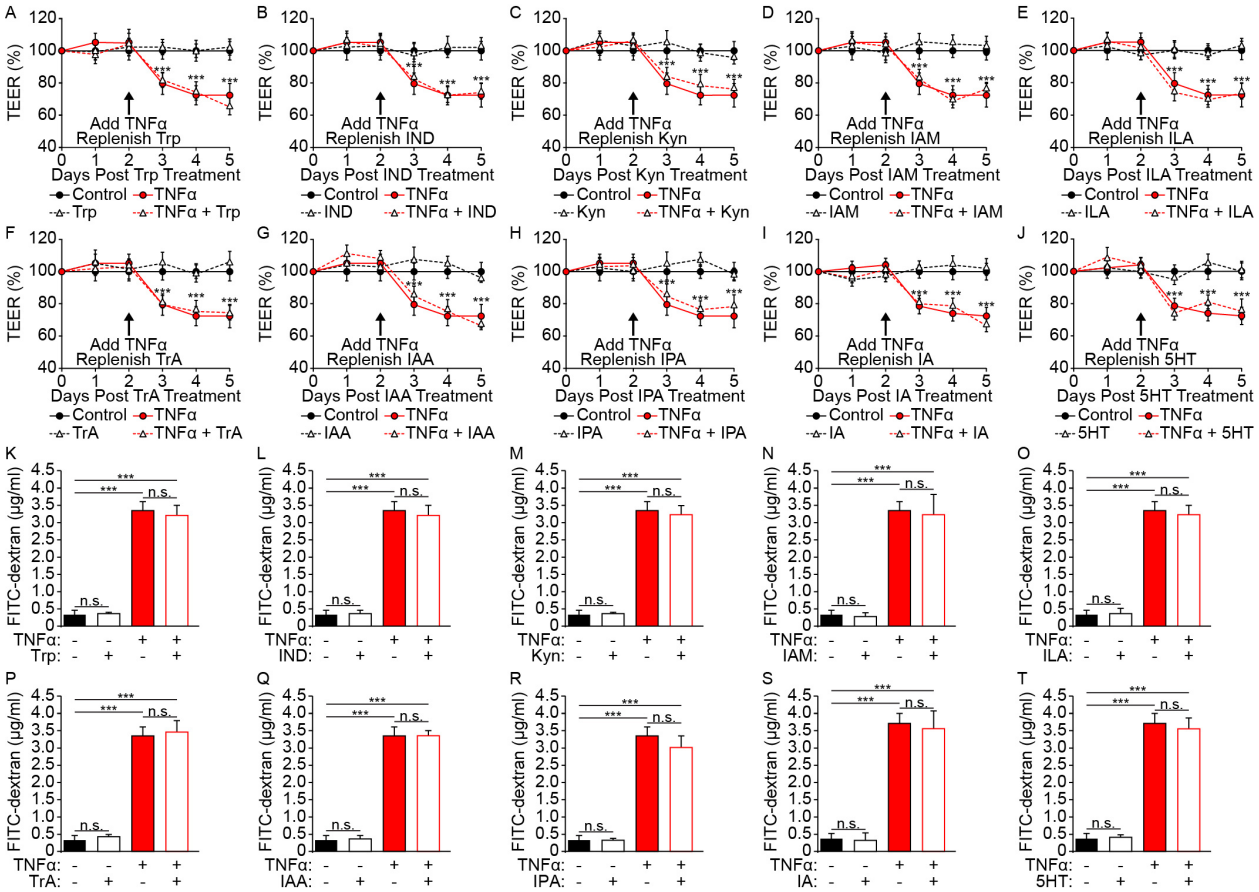
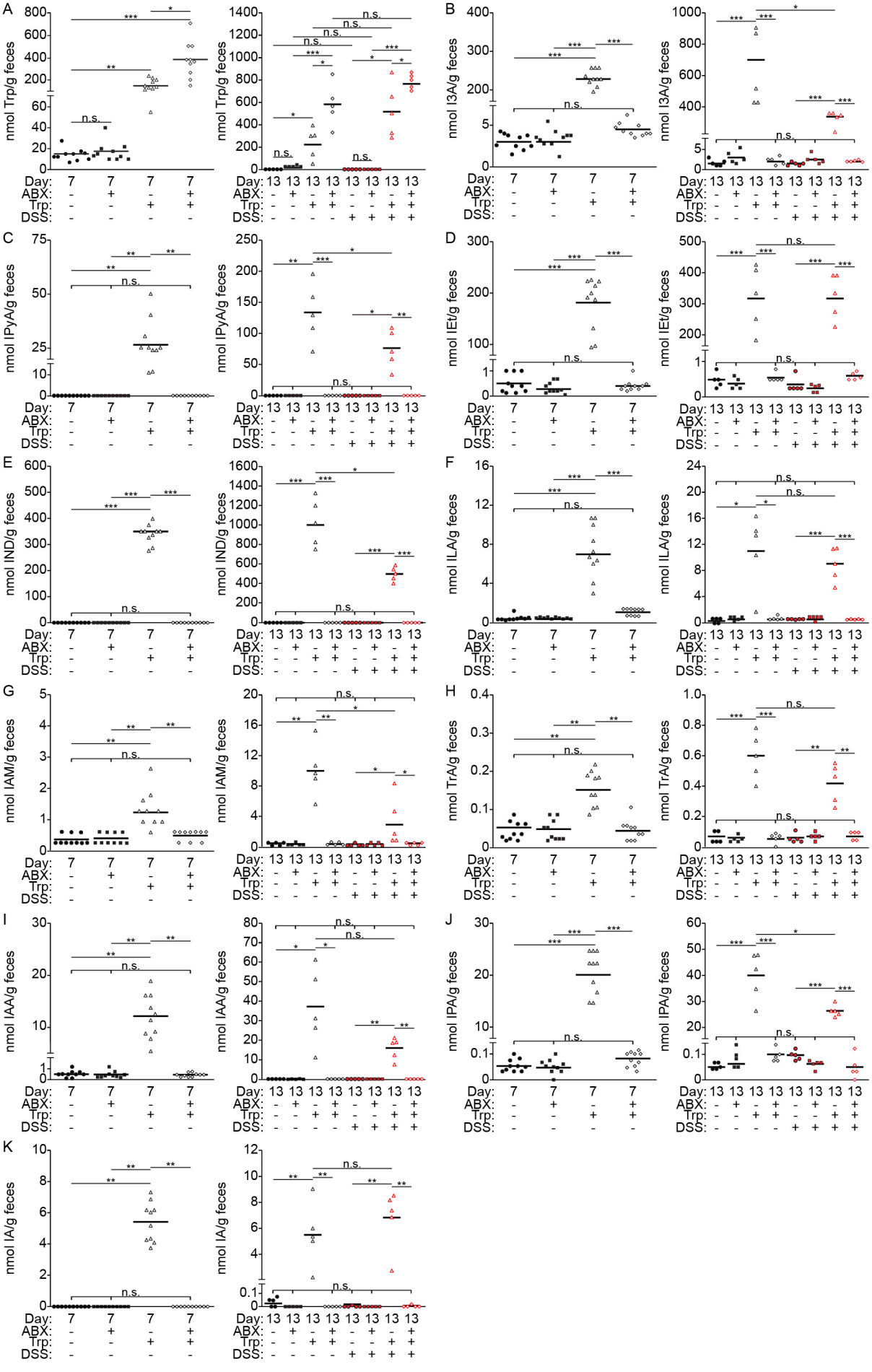


Figure S6. Additional tryptophan metabolites do not affect epithelial permeability. Polarized Caco-2 monolayers were pre-treated with indicated metabolite (1 mM) for 2 d and then stimulated with TNFα (20 ng/ml). Epithelial permeability was measured by (A-J) TEER at the indicated times or (K-T) FITC-dextran paracellular transport from the apical to basolateral compartment after 4 h. Abbreviations: tryptophan (Trp), indole (IND), kynurenine (Kyn), indole-3-acetamide (IAM), indole-3-lactate (ILA), tryptamine (TrA), indole-3-acetic acid (IAA), indole-3-propionate (IPA), indole-3-acrylate (IA), 5-hydroxytryptamine (5HT). Data are representative of at least 3 independent experiments. One-way ANOVA followed by post-hoc Tukey's test: n = 3, ***p<0.001, n.s. = not significant.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DSS:
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Figure S7. LC-MS quantification of tryptophan (Trp) metabolites during Trp-rich diet in mouse model of colitis. C57Bl/6 mice were pre-treated with antibiotics (ABX: ampicillin (9 mg/kg), metronidazole (9 mg/kg), neomycin (9 mg/kg), and vancomycin (4.5 mg/kg), i.g.) for 7 d, then fed a Trp-rich diet (42 g Trp/kg diet) or standard chow (2 g Trp/kg diet) for 7 d, followed by administration of dextran sodium sulfate (DSS, 3%, wt/vol) or vehicle for 7 d (*ad libitum*) with continued antibiotic treatment and Trp feeding. Metabolites from the fecal colonic contents were measured using mass spectrometry using commercial standards. Data are representative of at least 3 independent experiments, n = 5-10 mice per group. One-way ANOVA followed by post-hoc Tukey's test: *$p<0.05$, **$p<0.01$, ***$p<0.001$, n.s. = not significant.

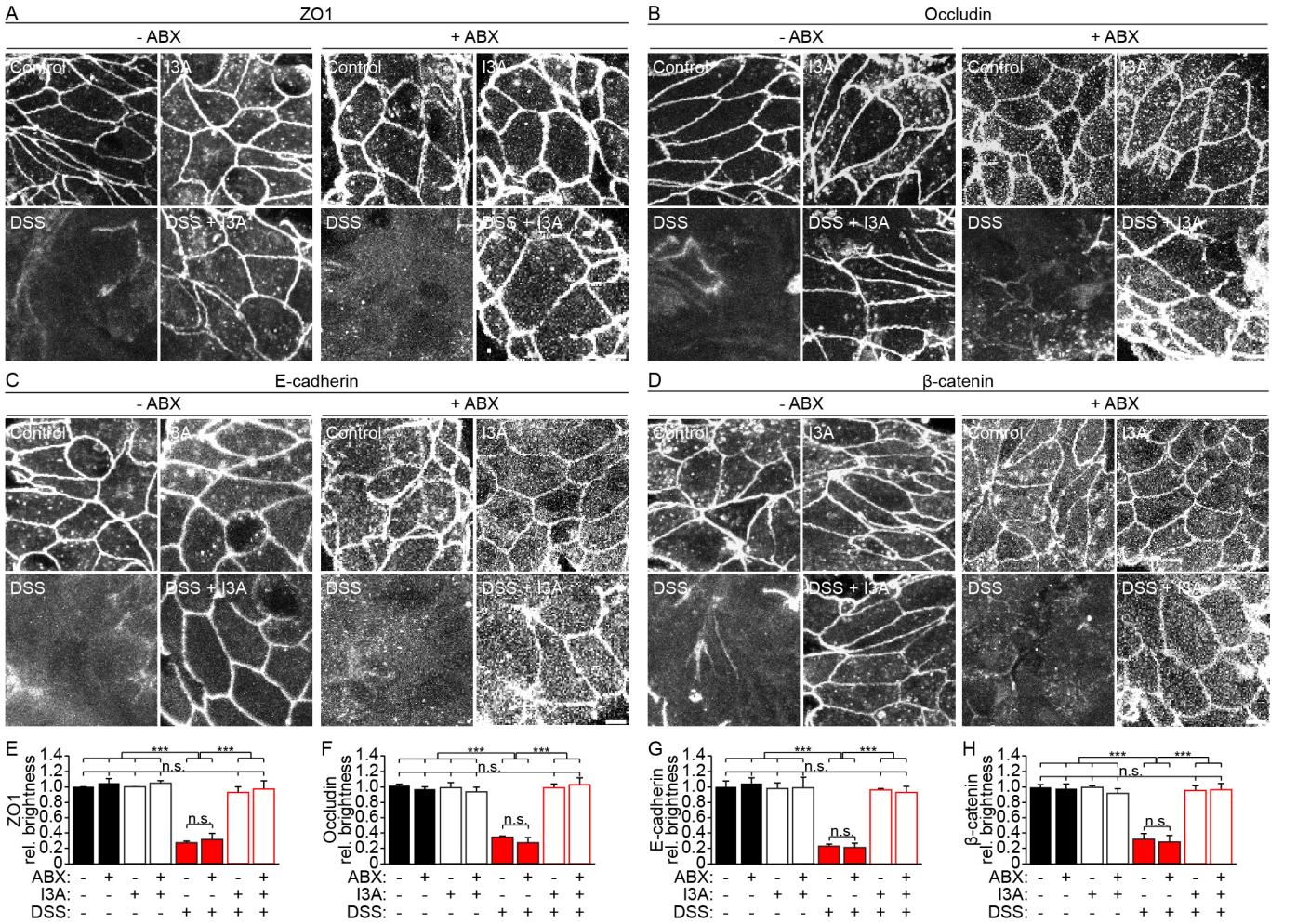

Figure S8. Tryptophan metabolite indole-3-aldehyde (I3A) prevents disruption of TJ and AJ proteins in a mouse model of colitis. C57Bl/6 mice were pre-treated with antibiotics (ABX: ampicillin (9 mg/kg), metronidazole (9 mg/kg), neomycin (9 mg/kg), and vancomycin (4.5 mg/kg), i.g.) for 7 d, followed by I3A (1000 mg/kg) for 2 d, and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued antibiotic and metabolite treatment. (A-H) Colon sections were stained for TJ and AJ proteins and imaged by confocal microscopy. Scale bars = 20 μm. (E-H) Relative (rel.) brightness of images with error as standard deviation from the mean was calculated (n = 15). Data are representative of at least 3 independent experiments, n = 5 mice per group. One-way ANOVA followed by post-hoc Tukey's test: ***p<0.001, n.s. = not significant.

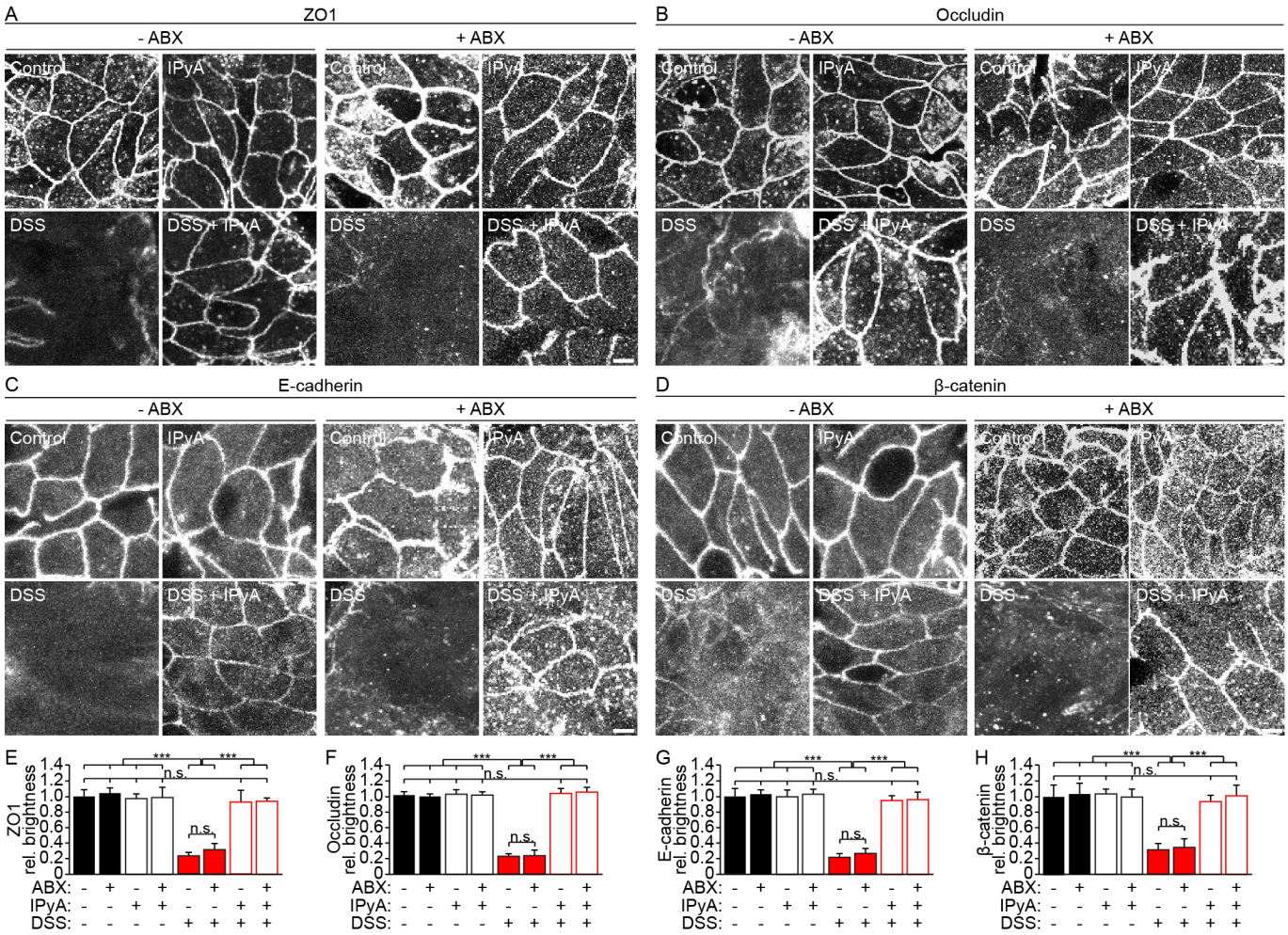

Figure S9. Tryptophan metabolite indole-3-pyruvate (IPyA) prevents disruption of TJ and AJ proteins in a mouse model of colitis. C57Bl/6 mice were pre-treated with antibiotics (ABX: ampicillin (9 mg/kg), metronidazole (9 mg/kg), neomycin (9 mg/kg), and vancomycin (4.5 mg/kg), i.g.) for 7 d, followed by IPyA (2900 mg/kg) for 2 d, and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued antibiotic and metabolite treatment. (A-H) Colon sections were stained for TJ and AJ proteins and imaged by confocal microscopy. Scale bars = 20 μm. (E-H) Relative (rel.) brightness of images with error as standard deviation from the mean was calculated (n = 15). Data are representative of at least 3 independent experiments, n = 5 mice per group. One-way ANOVA followed by post-hoc Tukey's test: ***$p<0.001$, n.s. = not significant.

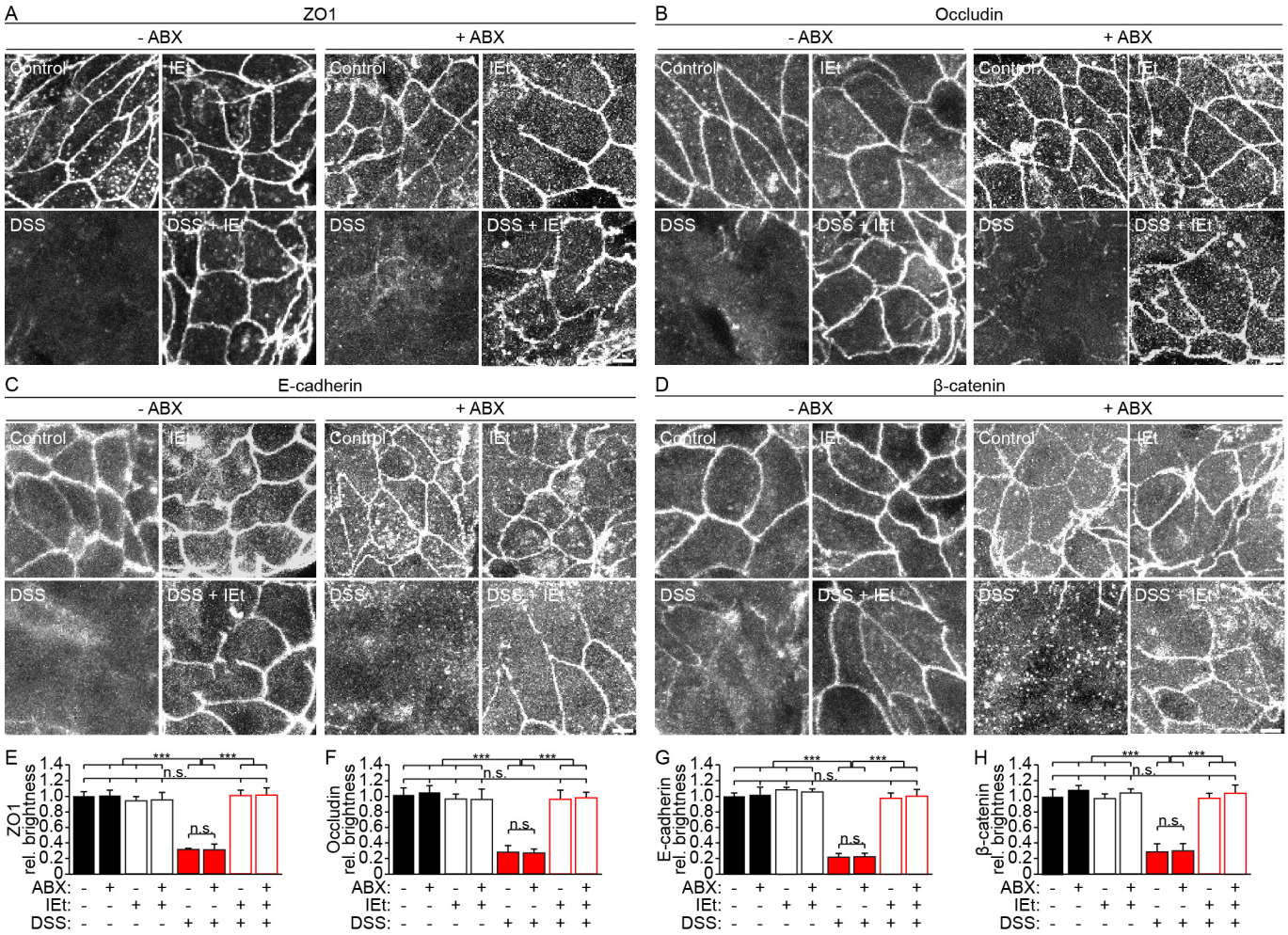

Figure S10. Tryptophan metabolite indole-3-ethanol (IEt) prevents disruption of TJ and AJ proteins in a mouse model of colitis. C57Bl/6 mice were pre-treated with antibiotics (ABX: ampicillin (9 mg/kg), metronidazole (9 mg/kg), neomycin (9 mg/kg), and vancomycin (4.5 mg/kg), i.g.) for 7 d, followed by IEt (600 mg/kg) for 2 d and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued antibiotic and metabolite treatment. (A-H) Colon sections were stained for TJ and AJ proteins and imaged by confocal microscopy. Scale bars = 20 μm. (E-H) Relative (rel.) brightness of images with error as standard deviation from the mean was calculated (n = 15). Data are representative of at least 3 independent experiments, n = 5 mice per group. One-way ANOVA followed by post-hoc Tukey's test: ***$p<0.001$, n.s. = not significant.

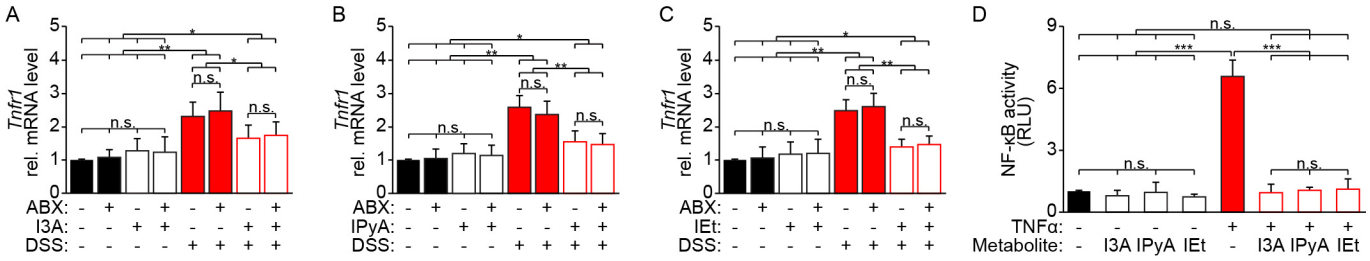

Figure S11. Tryptophan metabolites indole-3-aldehyde (I3A), indole-3-pyruvate (IPyA), and indole-3-ethanol (IEt) inhibit TNFR1 signaling. (A-C) C57Bl/6 mice were pre-treated with antibiotics (ABX: ampicillin (9 mg/kg), metronidazole (9 mg/kg), neomycin (9 mg/kg), and vancomycin (4.5 mg/kg), i.g.) for 7 d, followed by IEt (600 mg/kg), IPyA (2900 mg/kg), or I3A (1000 mg/kg) for 2 d and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued antibiotic and metabolite treatment. RNA from intestinal epithelial cells (IECs) was harvested and analyzed for *Tnfr1* levels by qPCR. (D) HEK 293T cells expressing luciferase downstream of NF-kB binding site were pre-treated with indicated metabolite (1 mM) for 3 h and then stimulated with TNFα (10 ng/ml) for an additional 3 h before luminescence was measured. Data are representative of at least 3 independent experiments, (A-C) n = 5 mice per group, (D) n = 3. One-way ANOVA followed by post-hoc Tukey's test: *p<0.05, **p<0.01, ***p<0.001, n.s. = not significant.

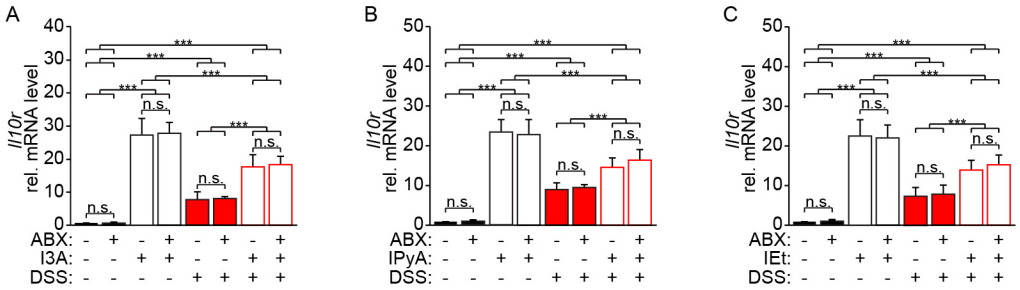

Figure S12. Tryptophan metabolites indole-3-aldehyde (I3A), indole-3-pyruvate (IPyA), and indole-3-ethanol (IEt) induce IL-10R in DSS colitis. C57Bl/6 mice were pre-treated with antibiotics (ABX: ampicillin (9 mg/kg), metronidazole (9 mg/kg), neomycin (9 mg/kg), and vancomycin (4.5 mg/kg), i.g.) for 7 d, followed by (A) I3A (1000 mg/kg), (B) IPyA (2900 mg/kg), or (C) IEt (600 mg/kg) for 2 d and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued antibiotic and metabolite treatment. RNA from IECs was harvested and analyzed for *Il10r* levels by qPCR. Data are representative of at least 3 independent experiments, n = 5 mice per group. One-way ANOVA followed by post-hoc Tukey's test: ***p<0.001, n.s. = not significant.

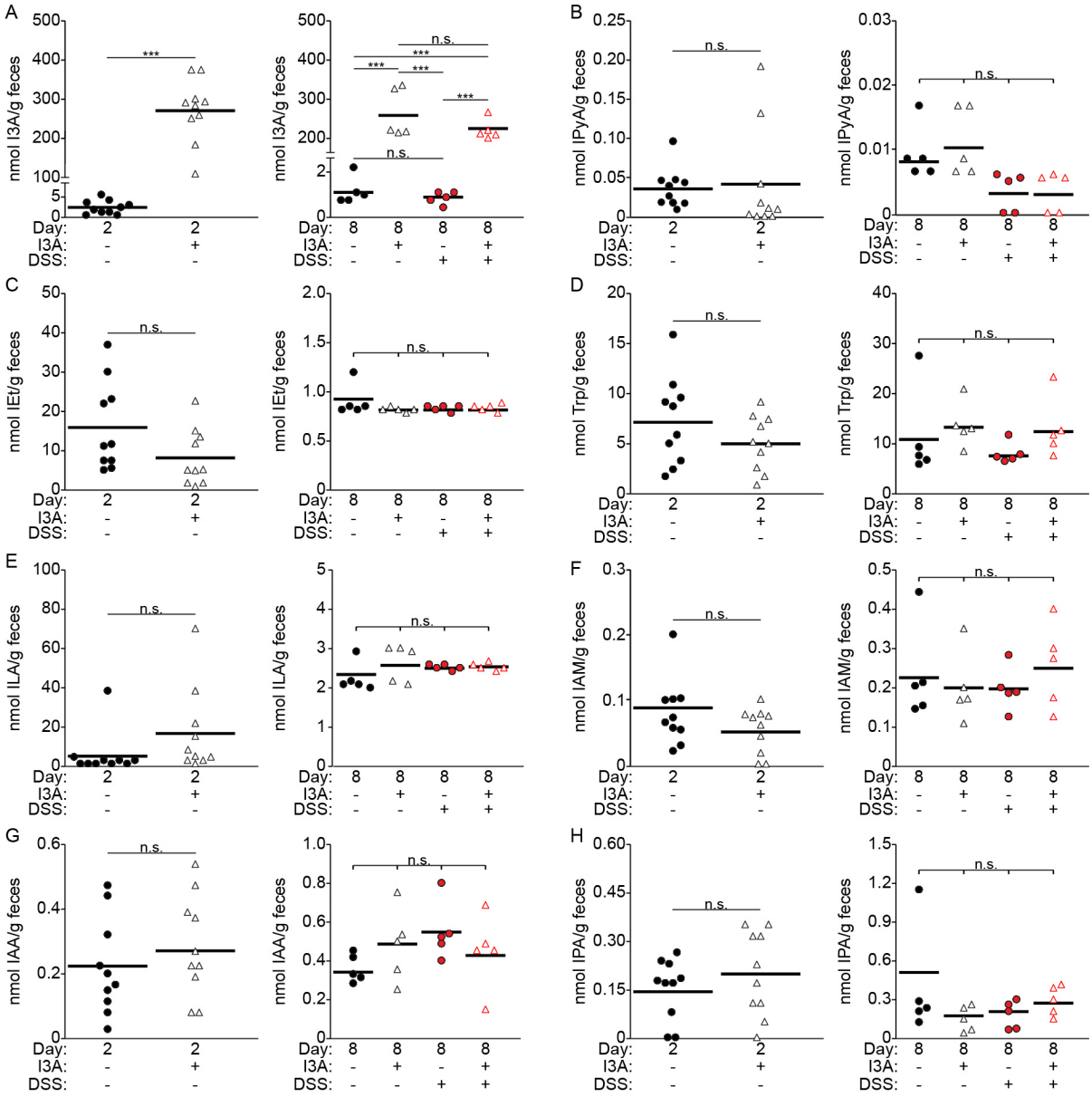

Figure S13. LC-MS quantification of Trp metabolites after oral gavage of I3A during mouse model of colitis. C57Bl/6 mice were pre-treated with I3A (1000 mg/kg) for 2 d and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued metabolite treatment. Metabolites from the fecal colonic contents were measured using mass spectrometry using commercial standards. Data are representative of at least 3 independent experiments, n = 5-10 mice per group. One-way ANOVA followed by post-hoc Tukey's test: ***p<0.001, n.s. = not significant.

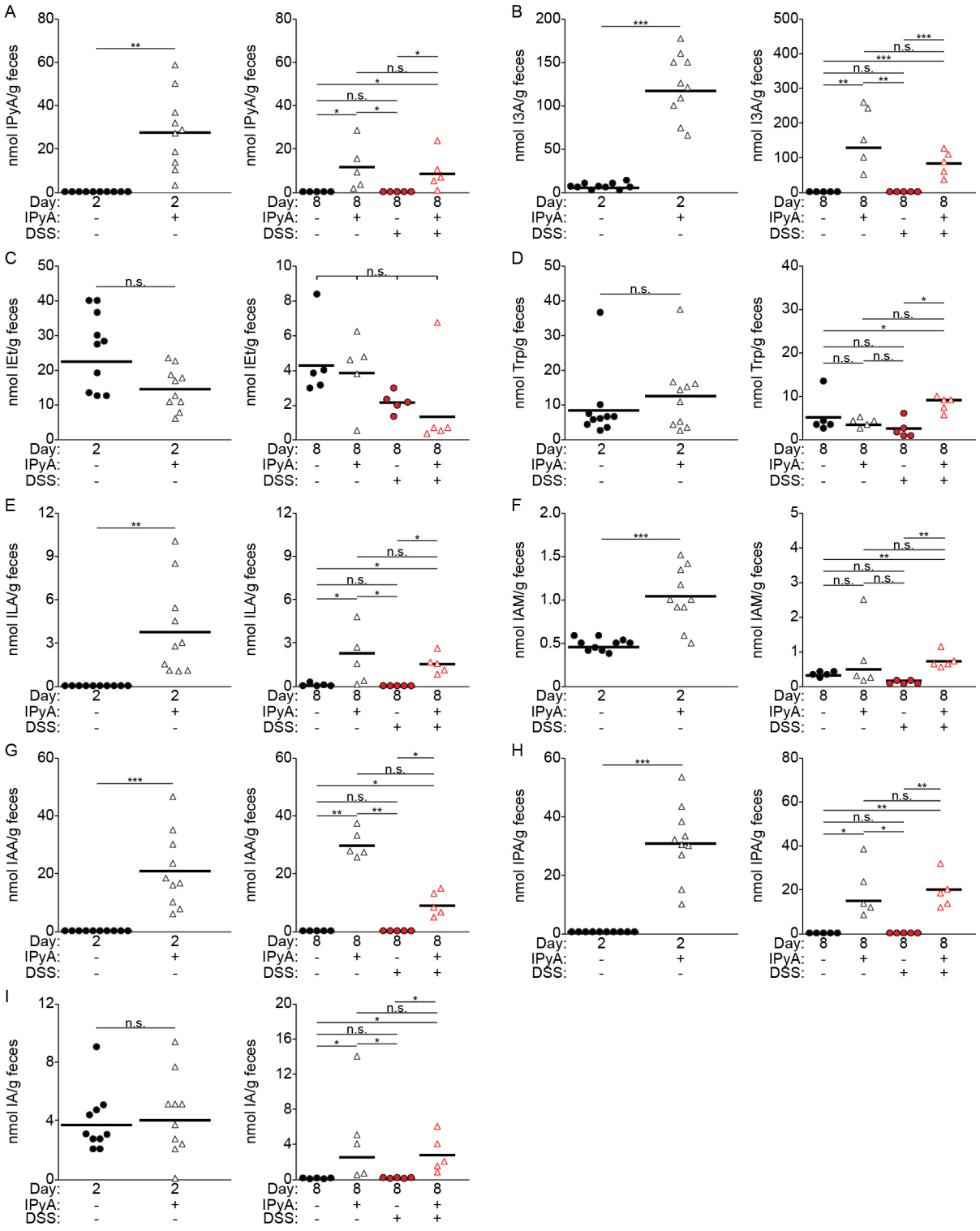

Figure S14. LC-MS quantification of Trp metabolites after oral gavage of IPyA during mouse model of colitis. C57Bl/6 mice were pre-treated with IPyA (2900 mg/kg) for 2 d and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued metabolite treatment. Metabolites from the fecal colonic contents were measured using mass spectrometry using commercial standards. Data are representative of at least 3 independent experiments, n = 5-10 mice per group. One-way ANOVA followed by post-hoc Tukey's test: *p<0.05, **p<0.01, ***p<0.001, n.s. = not significant.

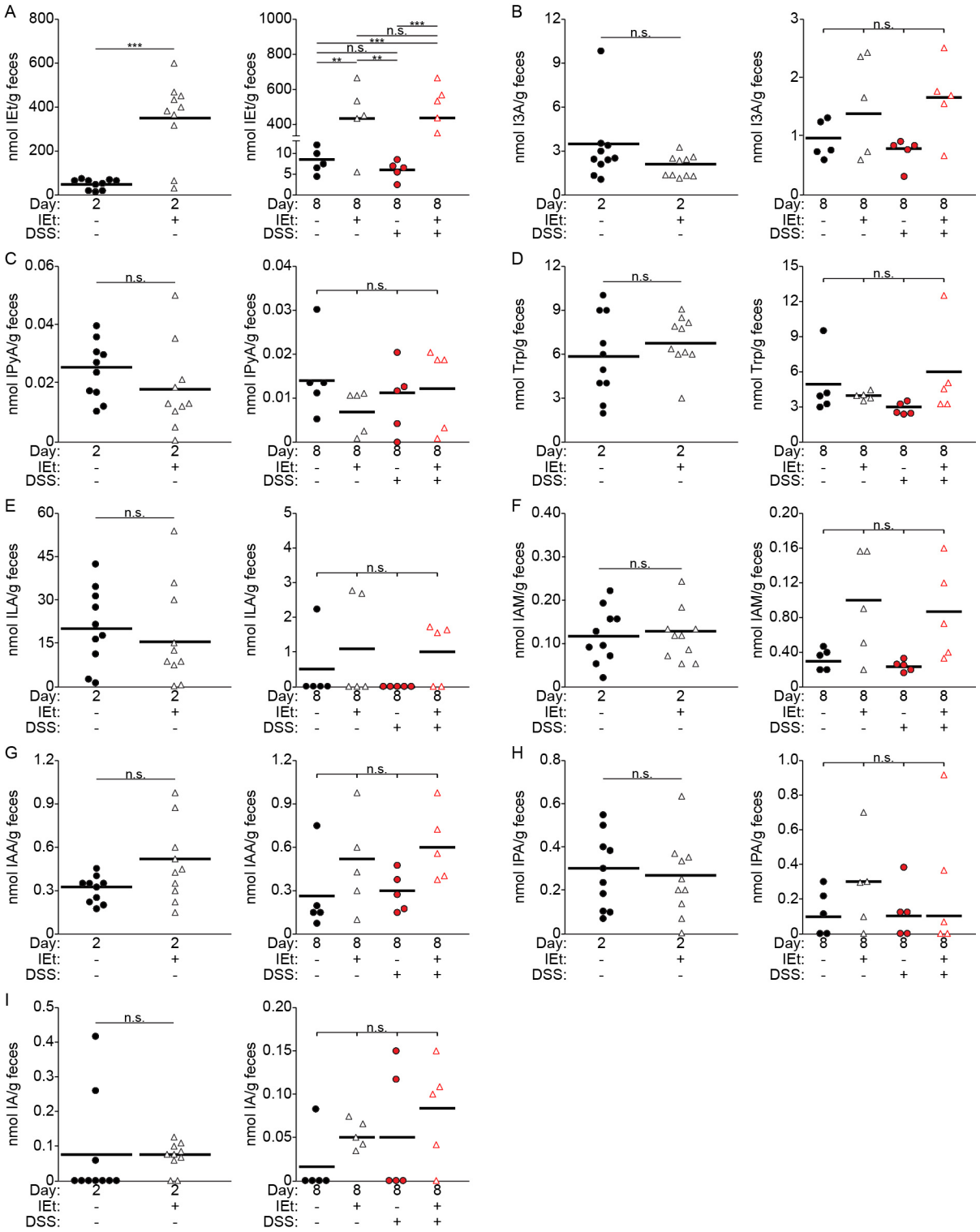

Figure S15. LC-MS quantification of Trp metabolites after oral gavage of IEt during mouse model of colitis. C57Bl/6 mice were pre-treated with IEt (600 mg/kg) for 2 d and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued metabolite treatment. Metabolites from the fecal colonic contents were measured using mass spectrometry using commercial standards. Data are representative of at least 3 independent experiments, n = 5-10 mice per group. One-way ANOVA followed by post-hoc Tukey's test: **p<0.01, ***p<0.001, n.s. = not significant.

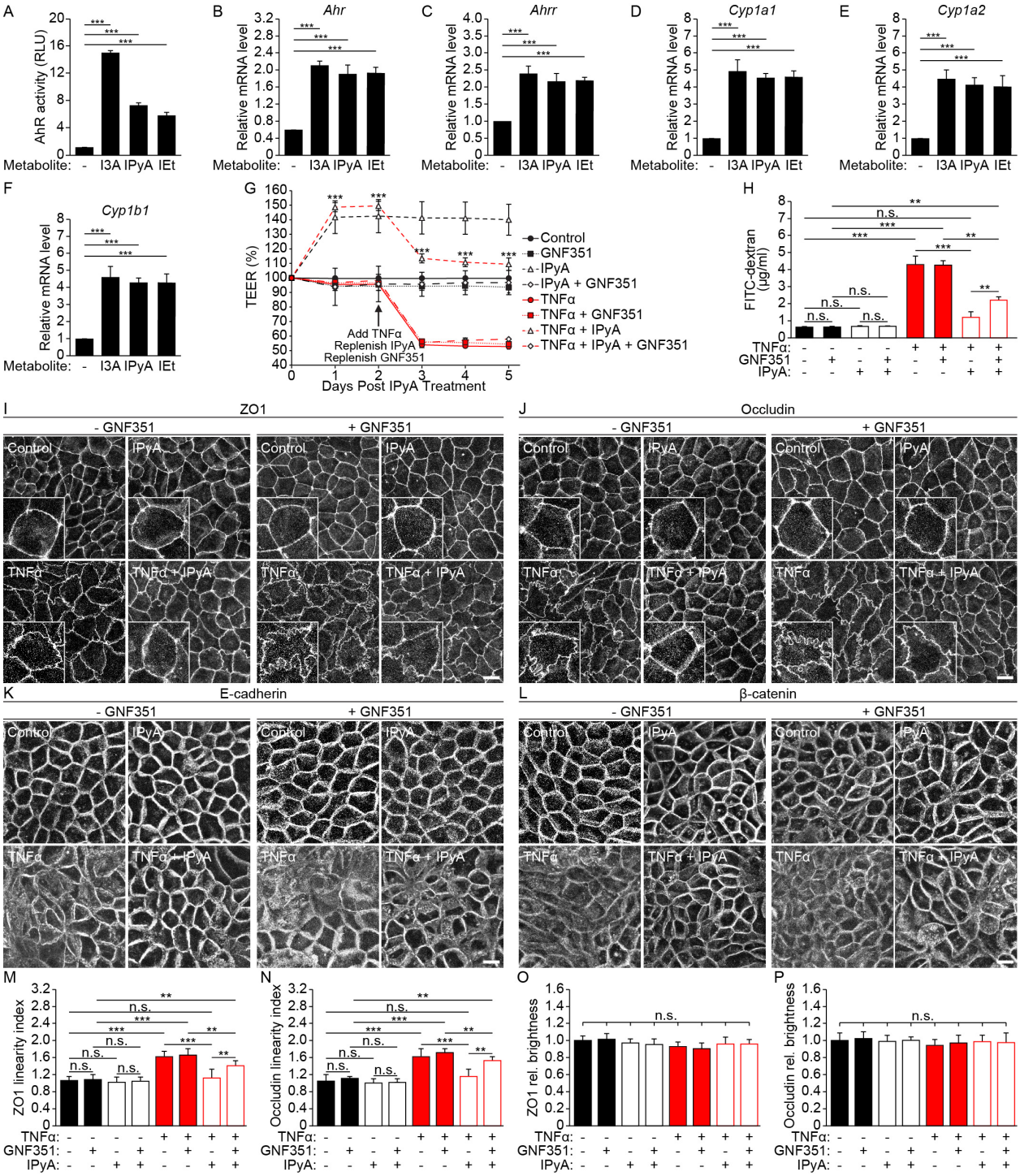

Figure S16. Tryptophan metabolites I3A, IPyA, and IEt are aryl hydrocarbon receptor (AhR) ligands, and the activity of IPyA can be blocked with an AhR inhibitor. (A) AhR luciferase reporter cell line was treated with each metabolite for 24 h, and luminescence was measured. RLU = relative luminescence units. (B-F) Polarized Caco-2 monolayers were treated with I3A, IPyA, and IEt (1 mM) for 8 h, and mRNA levels of downstream AhR target genes (indicated) were analyzed by qPCR. (G-H) Polarized Caco-2 monolayers were pre-treated with the AhR antagonist GNF351 for 24 h. Next, IPyA was added 2 d prior to stimulation with TNFα (20 ng/ml). Epithelial permeability was measured by (G) TEER and (H) FITC-dextran flux. (I-P) Samples were stained for TJ and AJ proteins indicated. Scale bars = 20 μm. (M-N) Linearity indices for ZO1 and occludin were calculated (n = 30). (O-P) Relative (rel.) brightness of images with error as standard deviation from the

mean was calculated (n = 15). Data are representative of at least 3 independent experiments. One-way ANOVA followed by post-hoc Tukey's test: n = 3, **p<0.01, ***p<0.001, n.s. = not significant.

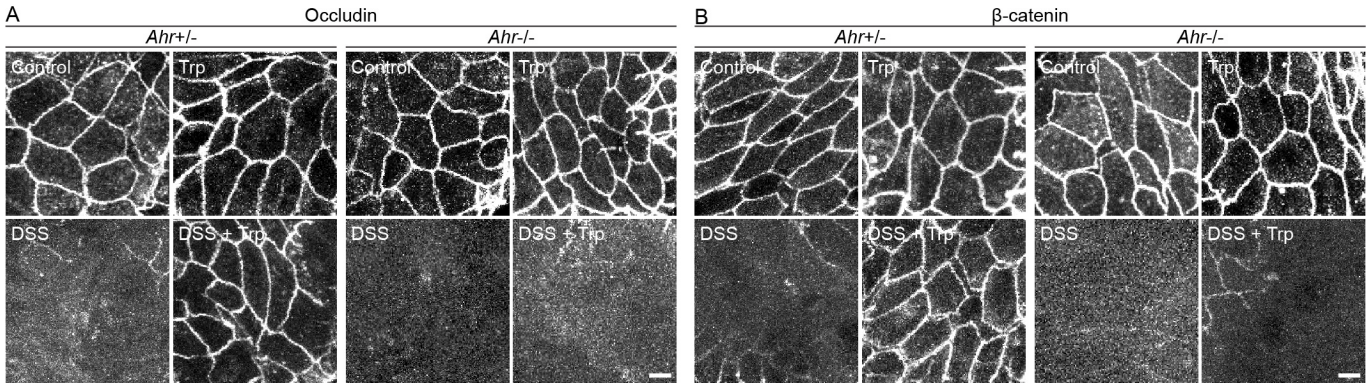

Figure S17. Effect of high tryptophan (Trp) diet in decreasing TJ and AJ disassembly in a mouse model of colitis is dependent on the aryl hydrocarbon receptor (AhR). *Ahr*+/- or *Ahr*-/- mice were fed a Trp-rich diet (42 g Trp/kg diet) or standard chow (2 g Trp/kg diet) for 7 d, followed by administration of dextran sodium sulfate (DSS, 3%, wt/vol) or vehicle for 7 d (*ad libitum*) with continued Trp feeding. Colon sections were stained for (A) occludin and (B) β-catenin and imaged by confocal microscopy. Scale bars = 20 μm. Data are representative of at least 3 independent experiments, n = 5 mice per group.

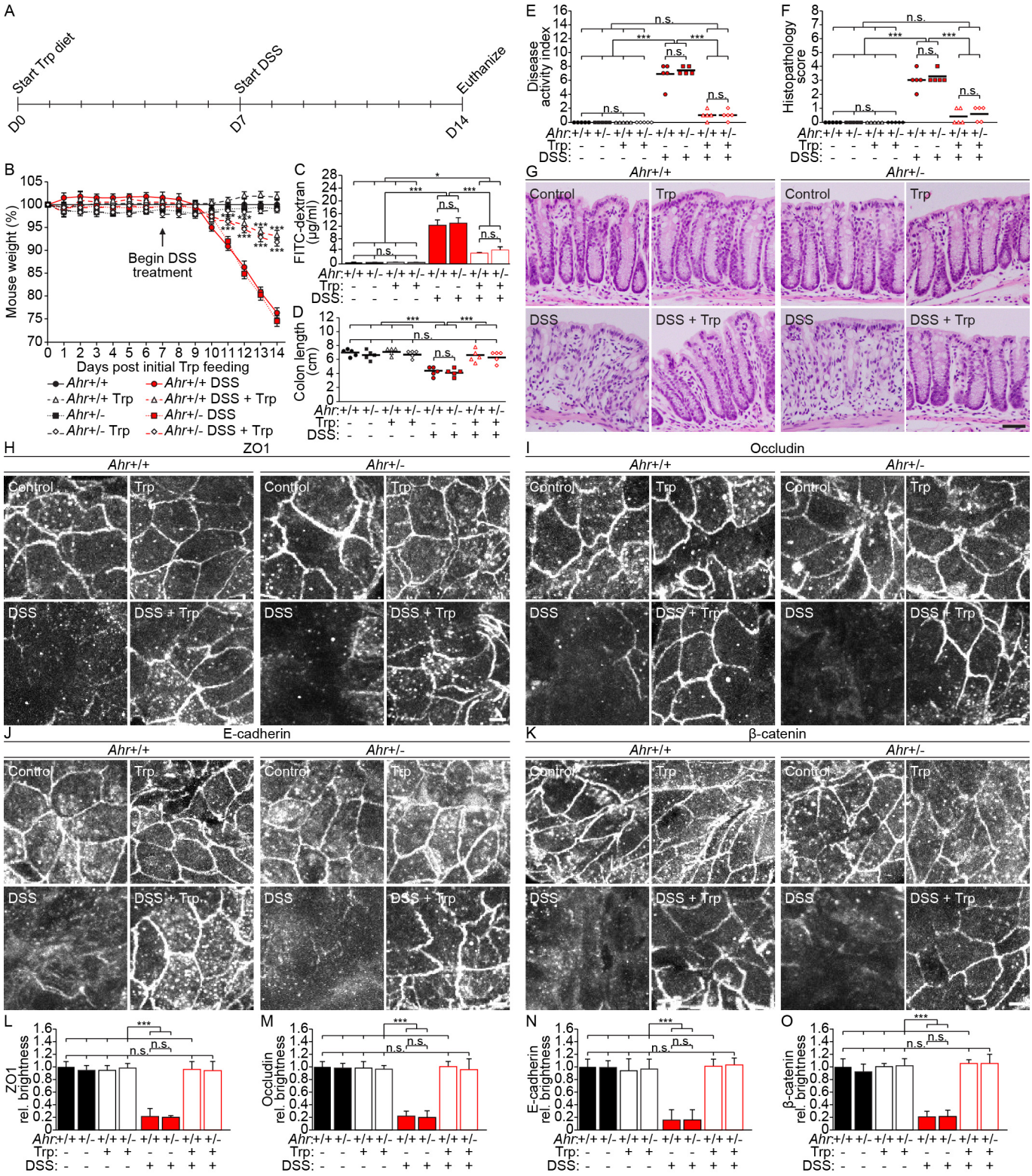

Figure S18. Effect of high tryptophan (Trp) diet in mouse model of colitis does not differ in AhR wildtype versus heterozygote mice. (A) *Ahr*+/+ or *Ahr*+/- mice were fed a Trp-rich diet (42 g Trp/kg diet) or standard chow (2 g Trp/kg diet) for 7 d, followed by administration of dextran sodium sulfate (DSS, 3%, wt/vol) or vehicle for 7 d (*ad libitum*) with continued Trp feeding. (B) The mice were weighed daily. (C) Mice were orally gavaged with FITC-dextran (900 mg/kg) on day 14, and serum levels of FITC-dextran were measured 4 h later. (D) On day 14, the mice were sacrificed, and colon lengths were measured, (E) disease activity index was

measured, and (F-G) the distal colon was stained with H&E and blindly scored (0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe) for epithelial damage, mononuclear and polymorphonuclear infiltrate, and submucosal edema. Scale bar = 50 μm. (H-O) Colon sections were stained for TJ and AJ proteins (indicated) and imaged by confocal microscopy. Scale bars = 20 μm. (L-O) Relative (rel.) brightness of images with error as standard deviation from the mean was calculated (n = 15). (H,L) ZO1; (I,M) Occludin; (J,N) E-cadherin; (K,O) β-catenin. Data are representative of at least 3 independent experiments, n = 5 mice per group. One-way ANOVA followed by post-hoc Tukey's test: *$p<0.05$, ***$p<0.001$, n.s. = not significant.

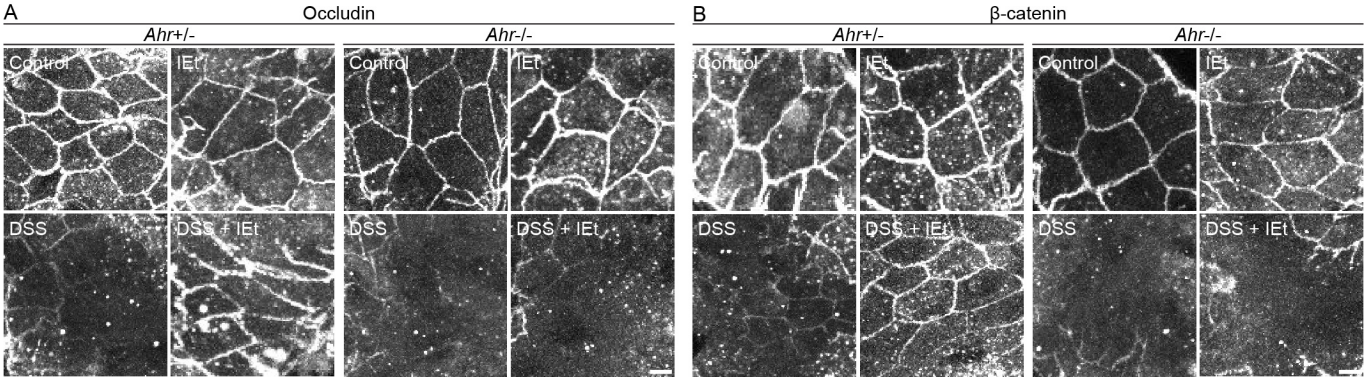

Figure S19. Effect of tryptophan metabolite IEt in decreasing TJ and AJ disassembly in mouse model of colitis is dependent on AhR. *Ahr*+/- or *Ahr*-/- mice were pre-treated with IEt (600 mg/kg) for 2 d and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued metabolite treatment. Colon sections were stained for (A) occludin and (B) β-catenin and imaged by confocal microscopy. Scale bars = 20 μm. Data are representative of at least 3 independent experiments, n = 5 mice per group.

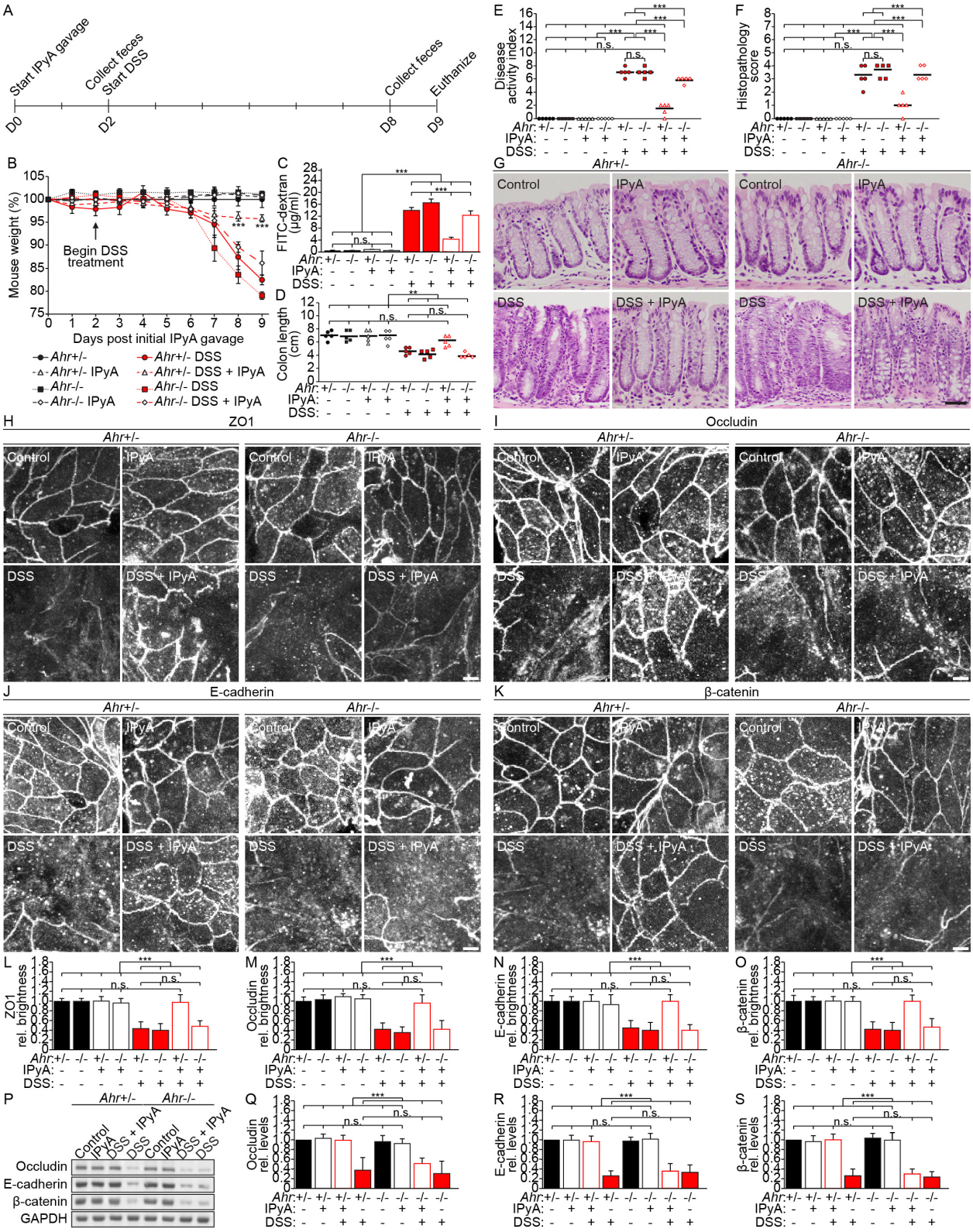

Figure S20. Effect of tryptophan metabolite IPyA in mouse model of colitis is dependent on AhR. (A) *Ahr*+/- or *Ahr*-/- mice were pre-treated with IPyA (2900 mg/kg) for 2 d and then administered DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued metabolite treatment. (B) The mice were weighed daily. (C) Mice were orally gavaged with FITC-dextran (900 mg/kg) on day 9, and serum levels of FITC-dextran were measured 4 h later. (D) On day 9, the mice were sacrificed, and colon lengths were measured, (E) disease activity index was measured, and (F-G) the distal colon was stained with H&E and blindly scored (0 = none, 1 = very mild, 2 =

mild, 3 = moderate, 4 = severe) for epithelial damage, mononuclear and polymorphonuclear infiltrate, and submucosal edema. Scale bar = 50 μm. (H-O) Colon sections were stained for TJ and AJ proteins and imaged by confocal microscopy. Scale bars = 20 μm. (L-O) Relative (rel.) brightness of images with error as standard deviation from the mean was calculated (n = 15). (H,L) ZO1; (I,M) Occludin; (J,N) E-cadherin; (K,O) β-catenin. (P-S) TJ and AJ protein levels were determined by Western blotting with the indicated antibodies and (Q-S) quantified by densitometry (n = 3). Data are representative of at least 3 independent experiments, n = 5 mice per group. One-way ANOVA followed by post-hoc Tukey's test: **$p<0.01$, ***$p<0.001$, n.s. = not significant.

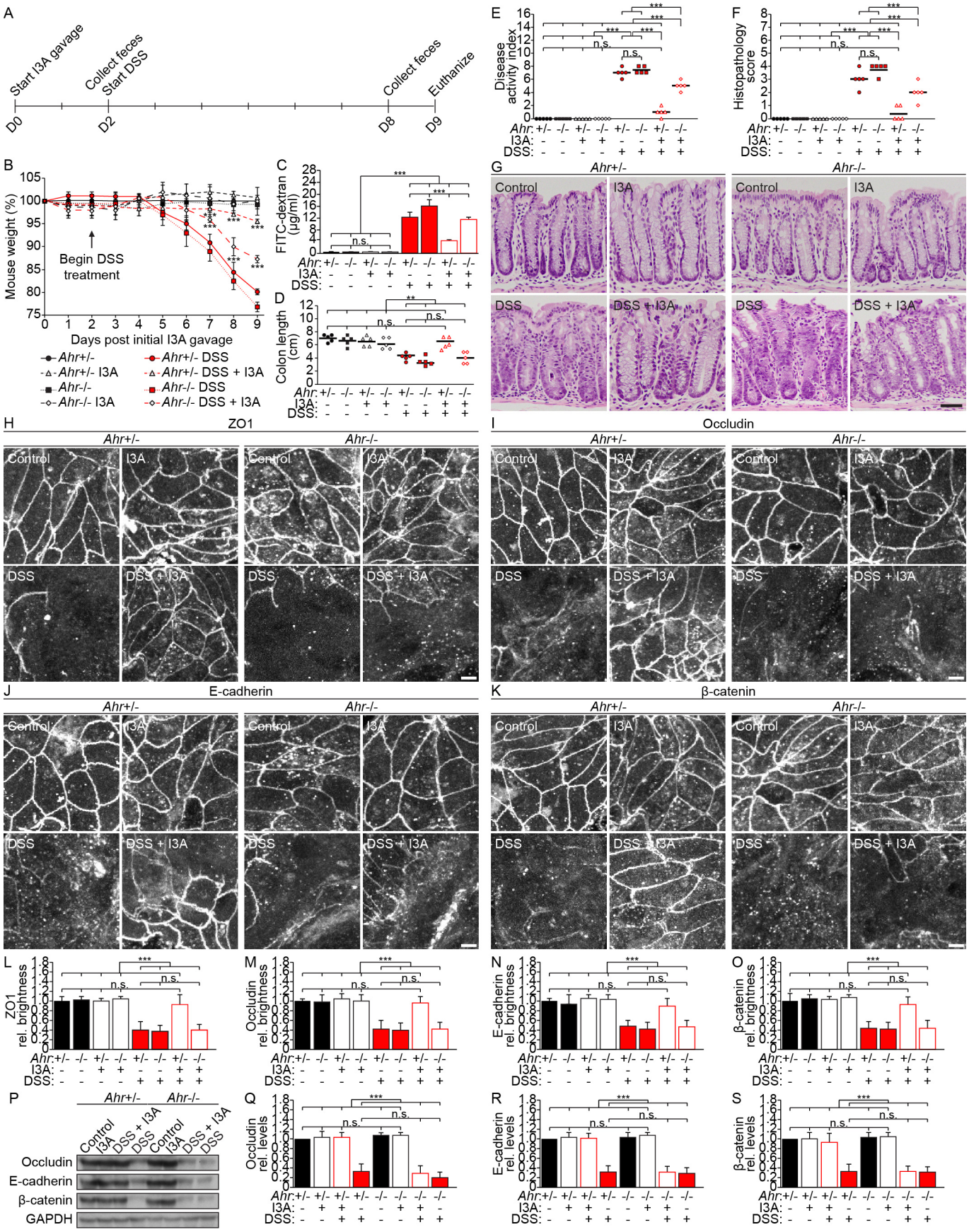

Figure S21. Effect of tryptophan metabolite I3A in mouse model of colitis is dependent on the aryl hydrocarbon receptor (AhR). (A) *Ahr*+/- or *Ahr*-/- mice were pre-treated with I3A (1000 mg/kg) for 2 d and then administered

DSS (3%, wt/vol) for 7 d (*ad libitum*) with continued metabolite treatment. (B) The mice were weighed daily. (C) Mice were orally gavaged with FITC-dextran (900 mg/kg) on day 9, and serum levels of FITC-dextran were measured 4 h later. (D) On day 9, the mice were sacrificed, and colon lengths were measured, (E) disease activity index was measured, and (F-G) the distal colon was stained with H&E and blindly scored (0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe) for epithelial damage, mononuclear and polymorphonuclear infiltrate, and submucosal edema. Scale bar = 50 μm. (H-O) Colon sections were stained for TJ and AJ proteins and imaged by confocal microscopy. Scale bars = 20 μm. (L-O) Relative (rel.) brightness of images with error as standard deviation from the mean was calculated (n = 15). (H,L) ZO1; (I,M) Occludin; (J,N) E-cadherin; (K,O) β-catenin. (P-S) TJ and AJ protein levels were determined by Western blotting with the indicated antibodies and (Q-S) quantified by densitometry (n = 3). Data are representative of at least 3 independent experiments, n = 5 mice per group. One-way ANOVA followed by post-hoc Tukey's test: **p<0.01, ***p<0.001, n.s. = not significant.

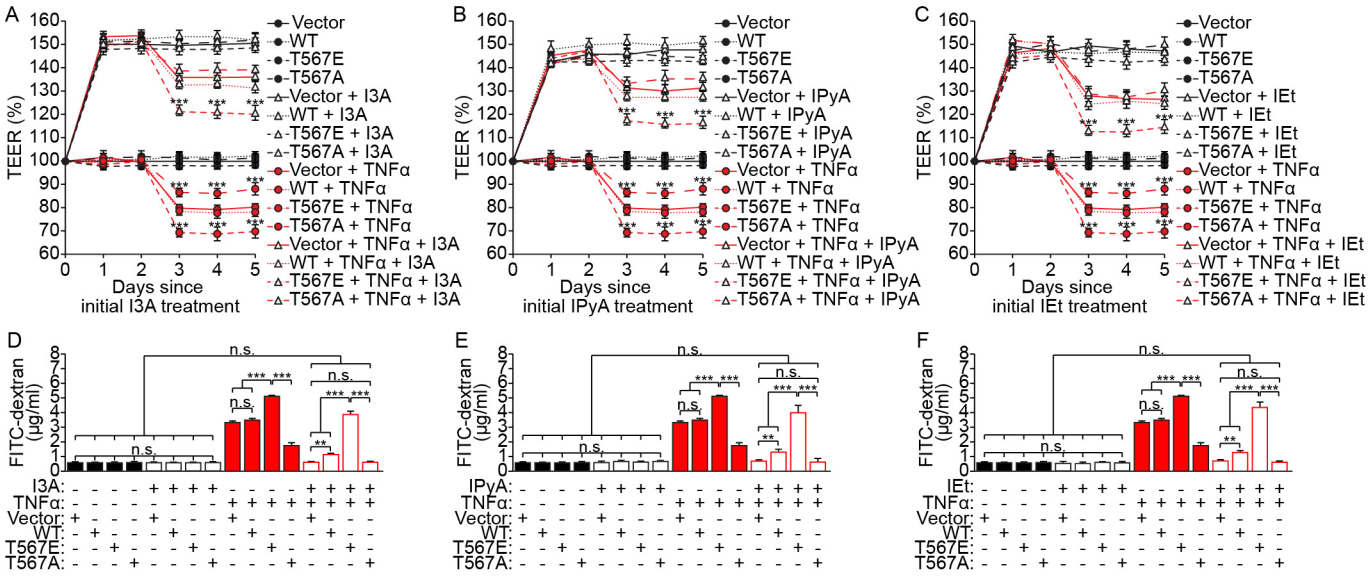

Figure S22. Activation of the actin regulatory protein ezrin increases epithelial permeability caused by TNFα, and metabolites prevent increased permeability. Polarized Caco-2 monolayers expressing the indicated ezrin mutants (phosphodeficient, T567A; phosphomimetic, T567E) versus wildtype (WT) ezrin were pre-treated with I3A, IPyA, and IEt (1 mM), followed by TNFα (20 ng/ml) for 24 h. (A-C) TEER and (D-F) FITC-dextran flux were measured. Data are representative of at least 3 independent experiments. One-way ANOVA followed by post-hoc Tukey's test: **p<0.01, ***p<0.001, n.s. = not significant.

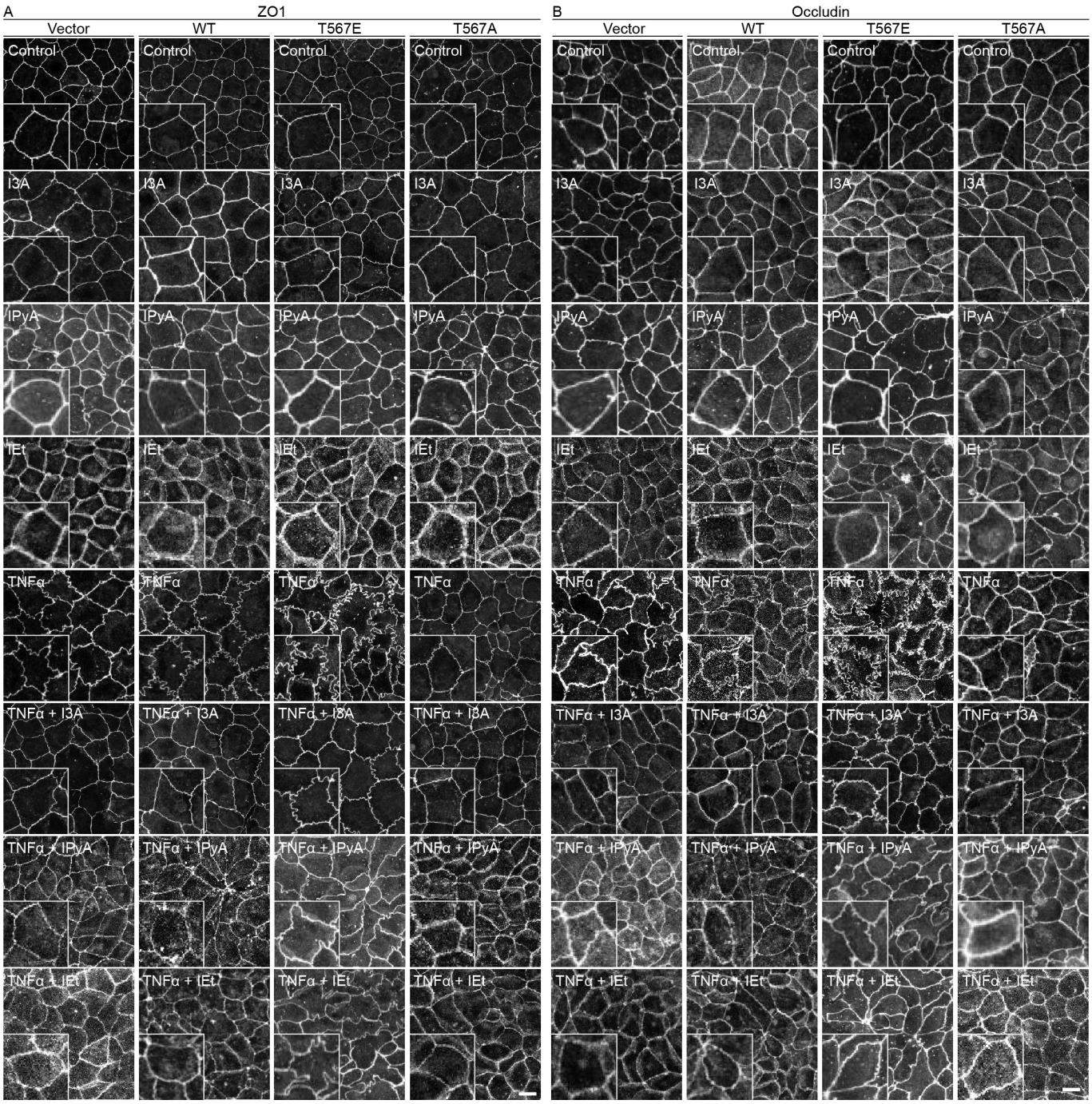

Figure S23. Activation of the actin regulatory protein ezrin increases sinuous phenotype caused by TNFα, which is prevented by Trp metabolites. Polarized Caco-2 monolayers expressing the indicated ezrin mutants (phosphodeficient, T567A; phosphomimetic, T567E) versus wildtype (WT) ezrin were pre-treated with indicated metabolites (1 mM), followed by TNFα (20 ng/ml) for 24 h. Cells were fixed, permeabilized, and stained for TJ proteins, followed by confocal microscopy. Insets: 2.5x magnification. Scale bars = 20 μm. Data are representative of at least 3 independent experiments.

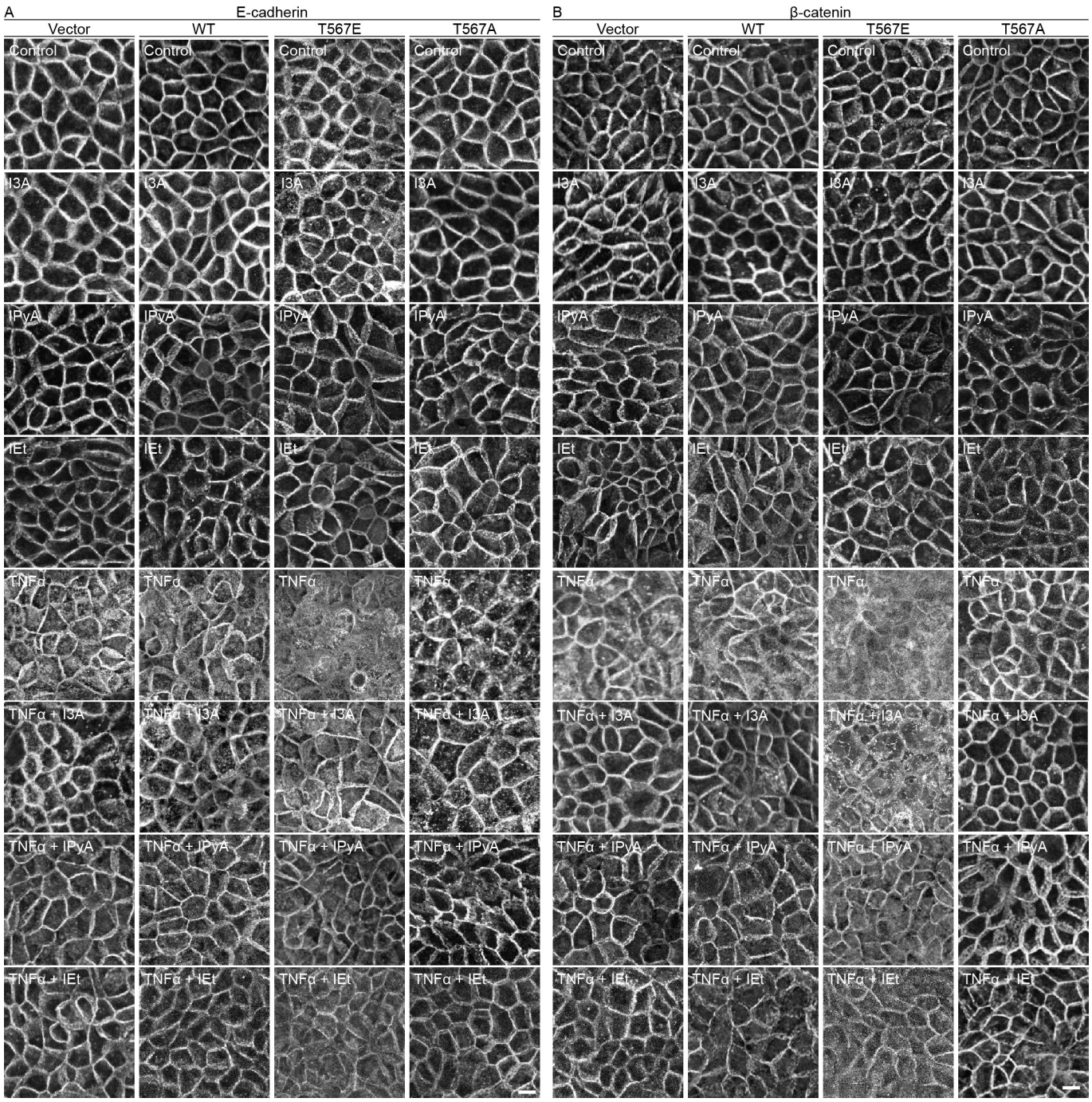

Figure S24. Activation of the actin regulatory protein ezrin increases mislocalization of AJ proteins caused by TNFα, which is prevented by Trp metabolites. Polarized Caco-2 monolayers expressing the indicated ezrin mutants (phosphodeficient, T567A; phosphomimetic, T567E) versus wildtype (WT) ezrin were pre-treated with indicated metabolites (1 mM), followed by TNFα (20 ng/ml) for 24 h. Cells were fixed, permeabilized, and stained for AJ proteins, followed by confocal microscopy. Insets: 2.5x magnification. Scale bars = 20 μm. Data are representative of at least 3 independent experiments.

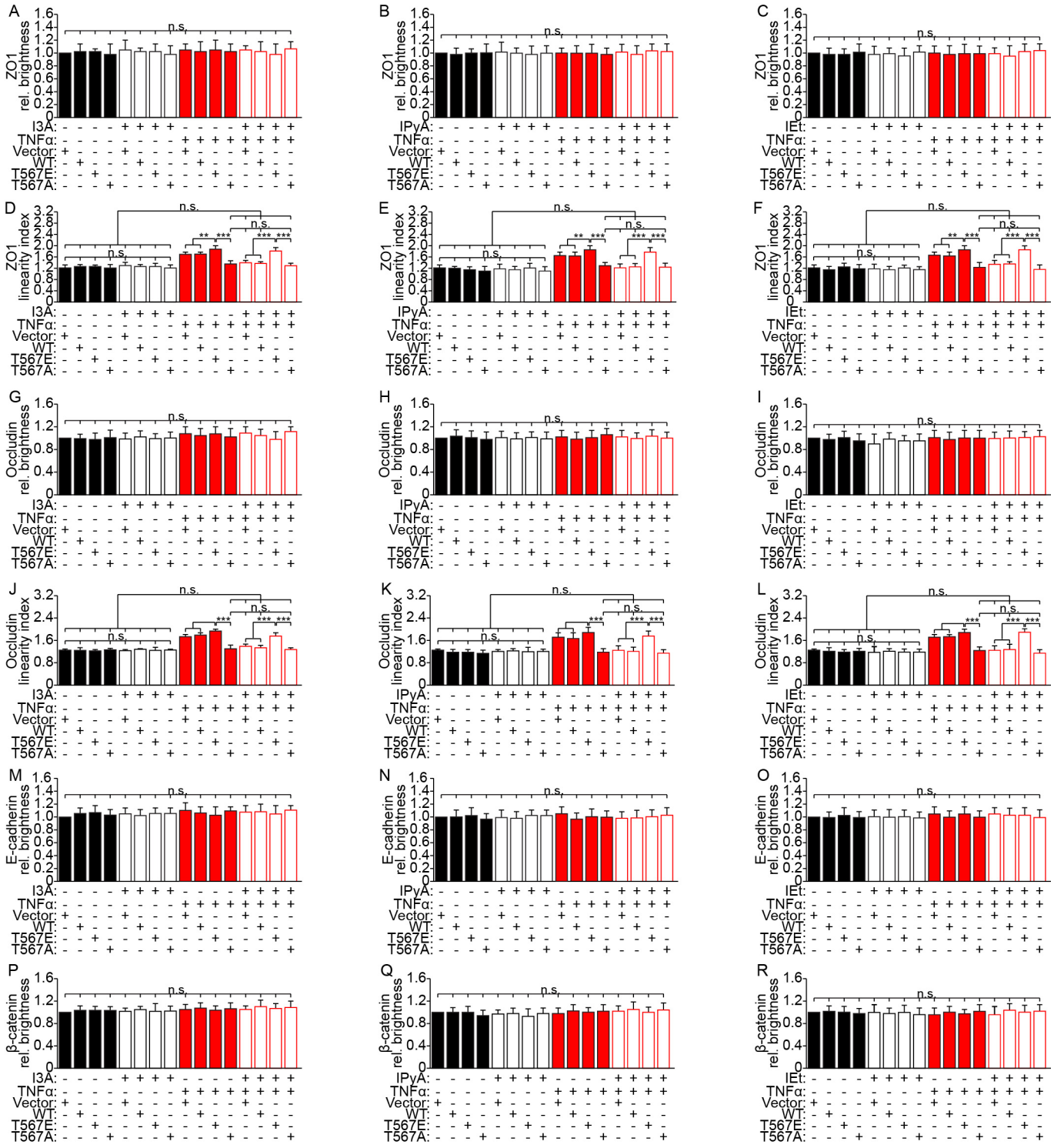

Figure S25. Activation of the actin regulatory protein ezrin increases sinuous phenotype and mislocalization of TJ and AJ proteins caused by TNFα, which is prevented by Trp metabolites. Polarized Caco-2 monolayers expressing the indicated ezrin mutants (phosphodeficient, T567A; phosphomimetic, T567E) versus wildtype (WT) ezrin were pre-treated with indicated metabolites (1 mM), followed by TNFα (20 ng/ml) for 24 h. Cells were fixed, permeabilized, and stained for TJ and AJ proteins, followed by confocal microscopy. (A-C, G-I, M-R) Relative (rel.) brightness of images with error as standard deviation from the mean was calculated (n = 15). (D-F, J-L) Linearity indices were calculated (n= 30). Data are representative of at least 3 independent

experiments. One-way ANOVA followed by post-hoc Tukey's test: n = 3, **p<0.01, ***p<0.001, n.s. = not significant.