SUPPLEMENTARY INFORMATION

Title: Microbiota-derived butyrate dynamically regulates intestinal homeostasis through regulation of actin-associated protein synaptopodin

Authors: Ruth X. Wang[a,b], J. Scott Lee[a], Eric L. Campbell[c], and Sean P. Colgan[a]

[a]Mucosal Inflammation Program, Department of Medicine, [b]Medical Scientist Training Program, University of Colorado School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045; [c]Centre for Experimental Medicine, Queens University Belfast, Northern Ireland

Corresponding Author:

Sean P. Colgan, PhD

12700 E. 19th Ave. B146

Aurora, CO 80045

303-724-7235

sean.colgan@cuanschutz.edu

This PDF contains:

Supplementary Materials and Methods (text)

Supplementary Tables S1-S5

Supplementary Figures S1-S10

Video Legends for Supplementary Videos S1-S2

SI References

Other supplementary materials for this manuscript include the following:

Videos S1-S2

www.pnas.org/cgi/doi/10.1073/pnas.1917597117

SUPPLEMENTAL MATERIALS AND METHODS

Animal Studies

Mice

C57BL/6 mice were purchased from Jackson Laboratories. 129S *Synpo*$^{-/-}$ mice were purchased from Jackson Laboratories as heterozygotes. These mice were bred into separate homozygous knockout mice (KO) and homozygous wild-type mice (WT). Animals were maintained and bred in standard housing conditions under 24 h/day, 7 days/week veterinary care available at the University of Colorado Anschutz Medical Campus (AMC) animal facility. Genotypes were confirmed with tail clippings through Transnetyx prior to 21 d of age. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at AMC. Germ free mice were ordered and used through the Gnotobiotic Core at AMC.

Antibiotic Microbiota Depletion and Tributyrin Supplementation

8-week-old female C57BL/6 mice were pre-administered an antibiotic cocktail consisting of 1 mg/mL ampicillin, gentamicin, and neomycin, and 0.5 mg/mL metronidazole and vancomycin (Sigma-Aldrich) for 5 d *ad libitum*. Then, 200 μL bolus of oral tributyrin gavage supplementation (Sigma-Aldrich) was given daily for 3 d.

Germ Free Tributyrin Supplementation

8-week-old male and female C57BL/6 germ free mice were administered 200 μL bolus of oral tributyrin gavage supplementation daily for 3 d. IECs were collected from mice colons through scrapings.

Dextran Sodium Sulfate (DSS) Colitis

Both female and male mice were analyzed at 7-9 weeks of age. Mice were switched to water bottles in their cages to become familiarized with this drinking system the day before the experiment began. On day 0, the DSS group received drinking water with 3% DSS (molecular weight 36,000–50,000; MP Biomedicals), and the H2O group received drinking water. Treatment was given for 5 d, and then mice were allowed to recover for 5 d on regular drinking water before sacrifice. A disease activity index (DAI) score was assessed daily to evaluate the development of colitis based on the parameters of weight loss compared to initial weight, stool consistency, and rectal bleeding. Scores were defined as weight loss: 0 (0%), 1 (1-5%), 2 (5-10%), 3 (11-20%), and 4 (>20%); stool consistency: 0 (well-formed pellets), 2 (pasty, semi-formed pellets), and 4 (liquid stools); and rectal bleeding: 0 (no blood), 2 (hemoccult positive), and 4 (gross bleeding). Maximum DAI possible was

12 (1). Colon lengths were measured at time of sacrifice, and tissue collected for histology, immunofluorescence, RNA, and protein analyses. For butyrate supplementation, mice were given 5mM butyrate in the drinking water for 7 d before beginning DSS. 5mM butyrate was maintained in drinking water during DSS and continued after DSS was pulled. HCl was used to pH match the water for the appropriate controls.

Colon Permeability

Mice were administered 100 μL of 100 mg/mL 70-kDa FITC-dextran (Sigma-Aldrich) by oral gavage, and blood and urine were collected after 2 h before sacrifice.

Histological Scoring

Distal colon samples were fixed in methacarn (methanol:chloroform:acetic acid, 60:30:10) before preparation for histological analysis and staining with hematoxylin and eosin. All histological quantitation was performed blinded and scored. Each sample was assessed for three independent parameters including severity of inflammation (0-3: none, slight, moderate, severe), depth of injury (0-3: none, mucosal, mucosal and submucosal, transmural), and amount of crypt damage (0-4: none, basal 1/3 damaged, basal 2/3 damaged, only surface epithelium intact, entire crypt and epithelium lost). Each independent parameter score was then multiplied by a factor reflecting the percentage of tissue involved (x1: 0-25%, x2: 26-50%, x3: 51-75%, x4: 76-100%) and then totaled. Maximum histological score possible was 40 (2).

Cell Line Analyses

Cell Culture

Cells were cultured in 95% air with 5% CO_2 at 37 °C in standard media made of DMEM:F-12 supplemented with 10% calf serum, 1% penicillin/streptomycin, and 1% GlutaMAX™ (ThermoFisher Scientific).

shRNA Knockdown

Lentiviral particles encoding shRNA directed against SYNPO (TRCN0000218429) or scramble short hairpin control (TRC2-SHC216, MISSION® TRC shRNA; Sigma-Aldrich) were transduced into T84 cells to derive SYNPO KD and SHC cell lines. Cells were incubated in 2 mL standard media solution with lentiviral particles and 8 μg/mL polybrene (Sigma-Aldrich) for 24 h. Cells were then incubated in standard media for 24 h before selection with 6 μg/mL puromycin (Sigma-Aldrich) media.

Enteroid Culture

Murine intestinal organoids/enteroids were isolated from wild-type C57BL/6 mice and cultured as previously described (3). Briefly, colonic tissues were minced and then enzymatically digested and dissociated in GentleMACS tubes (Milteny Biotec). The homogenized tissues were then filtered through a 70 μm cell strainer and resuspended in Matrigel (Corning). Cells were cultured in L-WRN conditioned media.

Quantitative PCR

TRIzol® reagent (ThermoFisher Scientific) was used to isolate total RNA from cells. cDNA was prepared using the iScript cDNA Synthesis Kit (Bio-Rad). Quantitative PCR analysis was performed using the Power SYBR™ Green master mix (Applied Biosystems) in a thermocycler. Fold change in expression of target mRNA relative to β-actin (*ACTB)* mRNA was calculated using the delta-delta Ct method. Primer sequences used are as follows: *SYNPO* - forward, 5′- ATGGAGGGGTACTCAGAGGAG-3′, reverse, 5′-CTCTCGGTTTTGGGACAGGTG′; *P21* - forward, 5′- TGTCCGTCAGAACCCATGC-3′, reverse, 5′-AAAGTCGAAGTTCCATCGCTC-3′; and *ACTB* - forward, 5′- CATGTACGTTGCTATCCAGGC-3′, reverse, 5′-CTCCTTAATGTCACGCACGAT-3′ (4).

Single-cell RNA Sequencing Analysis

Human T84 cells were used for evaluating the impact of butyrate at the single-cell level by RNA-sequencing. Following treatment of confluent cells with butyrate (5 mM, 8 h), cells were prepared as single-cell suspensions by trypsinization and gentle washing in PBS (ThermoFisher Scientific). Cells were stained with Hoechst 33324 (Thermofisher Scientific) and Propidium Iodide (ThermoFisher Scientific) and diluted to achieve a density of 1-2 cells per 50 nL in a final dispensing mix which contained a diluent, RNAsin (New England Biolab) and 0.35X PBS (without Ca^{2+} and Mg^{2+}, pH 7.4, ThermoFisher Scientific). Cells, positive controls, negative controls, and fiducial mix, were dispensed onto ICell8 Single Cell System (WaferGen Biosystems) for selection. Each well received 50 nL of either cell mix, positive control, negative control, or fiducial mix. Total RNA (~10 pg) was dispensed into selected nanowells for selection. From this analysis, 31 cells were prepped for sequencing that produced 22 libraries with mapped reads including 2 positive control K562 RNA samples. Cells were sequenced on two HISeq lanes with a total of 9.4 million reads recovered, from which 7.5 million mapped uniquely to the human genome hg38. The negative controls had few reads (~200), while the positive controls had almost three orders of magnitude more reads (110,000). The number of cells passing basic reads, genes, and % mitochondrial mapping filters was very high (87.5%). From this analysis, 958 genes were

differentially expressed (p<0.05) where 291 were down-regulated by butyrate and 667 were up-regulated by butyrate exposure. Table S3 lists the top 100 induced and repressed genes from this screen.

Transepithelial Resistance (TEER) Measurement and Barrier Flux Assay

Cells were plated on 0.33 cm^2 transwell inserts (Corning, 0.4 μm) at 58,000 cells/insert, and TEER readings were measured over 48 h using an epithelial volt-ohm meter (EVOM², World Precision Instruments). Where indicated, the cells were treated with butyrate, propionate, or acetate (Sigma-Aldrich). Inserts were then transported to a 37 °C work chamber and rinsed three times with Hanks' balanced salt solution (HBSS) buffer. Inserts were then placed into a 24-well plate with 1 mL of HBSS buffer on the basolateral side, and apically treated with 100 μL of 1 μg/μL 3-kDa FITC-Dextran (ThermoFisher Scientific). 100 μL of buffer from the basolateral chamber was collected at time 0 and then at 30 min intervals for 2 h while the plate with inserts was rotated and incubated at 37 °C. Fluorescence was detected for the various time points using a Promega™ GloMax® plate reader and a flux rate calculated.

Wound Healing Assay

Cells were plated at 35,000 cells/well on a 96-well ImageLock plate (Essen Bioscience Inc.) and incubated until a confluent cell monolayer formed (~24 h). Precise and reproducible wounds were made in all wells with a WoundMaker (Essen Bioscience Inc.). After wounding, media was aspirated from each well, and each well was gently washed with PBS before 100 μL of control media or media containing treatment (butyrate, acetate, propionate) was added. Initial images were taken immediately after wounding at 10X using the IncuCyte live - cell imaging (Essen Bioscience Inc.), and then every 2 h over the course of 38 h. Relative wound closure % was quantified for every image using the relative wound density metric, a measure of cell density in the wound area relative to the cell density outside of the wound area.

F/G Actin Quantification

Cells were plated on 1.9 cm^2 24-well plates (Corning) at 140,000 cells/well and allowed to adhere overnight. The cells were then washed with 1 mL of PBS before adding control media or treatment with cytochalasin D (Sigma-Aldrich) or butyrate and incubated for 6 h. Cells were then washed with 1 mL of ice-cold PBS before lysis with 200 μL actin stabilization buffer (0.1 M PIPES, pH 6.9, 30% glycerol, 5% DMSO, 1 mM $MgSO_4$, 1 mM EGTA, 1% TX -100, 1 mM ATP, Sigma-Aldrich) with protease inhibitors (ThermoFisher Scientific) on ice for 30 min followed by thorough, repeated pipetting to ensure lysis. A 100 μL aliquot was

transferred to a new tube and centrifuged at 4°C for 75 min at 16,000 g. The supernatant containing the globular (G)-actin was transferred to a new tube, and the remaining pellet containing filamentous (F)-actin was solubilized with 100 µL of actin depolymerization buffer (0.1 M PIPES, pH 6.9, 1 mM $MgSO_4$, 10 mM $CaCl_2$, and 5 µM cytochalasin D, Sigma-Aldrich). 20 µL of G-actin and F-actin fractions were separated by western blot (rabbit anti-β-actin, 1:15,000, ab8227, Abcam). Densitometry was determined using ImageJ and ratio of F-actin to total actin calculated (5).

Western Blot

Cell and tissue samples were rinsed with PBS and collected and lysed in 200µL of radioimmunoprecipitation assay (RIPA, 50 mM Tris-HCl pH 8.0, 1 mM EDTA, 1% Triton X-100, 10% SDS, 0.5% sodium deoxycholate, 150 mM NaCl, Sigma-Aldrich) buffer with protease inhibitors on ice. Samples were homogenized by sonication, and insoluble materials removed by centrifugation at 10,000 g for 5 min at 4°C. Protein quantity of the supernatant was determined using the Bradford reagent (Bio-Rad). Samples were then boiled in Laemmli sample buffer (Bio-Rad) for 5 min at 100 °C. Samples (20 µg) were run and separated on 8% SDS-PAGE gels (Bio-Rad) and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad). Membranes were blocked for 1 h at room temperature in Tris-buffered saline (TBS, Sigma-Aldrich) with 1% Tween-20 (TBS-T, Sigma-Aldrich) and 5% milk. Membranes were then incubated with primary antibodies in blocking buffer overnight at 4 °C (rabbit anti-SYNPO, 1:1000, ab224491, Abcam; rabbit anti-β-actin 1:15,000, ab8227, Abcam). Membranes were washed for four 10 min TBS-T washes before incubation with secondary antibodies in blocking buffer for 1 h at room temperature (peroxidase conjugated goat anti-rabbit IgG, 1:5000, ThermoFisher Scientific). After four 10 min TBT-washes, the membranes were incubated with chemiluminescence detection solution (ThermoFisher Scientific) and imaged on an imager (ChemiDoc™ MP, Bio-Rad).

Immunofluorescence

T84 cells were plated on collagen-coated coverslips (Thermofisher Scientific, Sigma-Aldrich) and allowed to adhere and grow over 48 h. Where designated, cells were treated with 5 mM butyrate for 22 h. Cells were fixed with 4% paraformaldehyde (PFA, Sigma-Aldrich) in phosphate buffered saline (PBS) for 15 min, permeabilized with 0.1% Triton X-100 for 15 min, and blocked in PBS containing 10% normal goat serum for 1 h at room temperature. Cells were incubated with primary antibodies in blocking buffer overnight at 4 °C (rabbit

anti-SYNPO, 1:100, ab224491, Abcam; mouse anti-ZO-1, 1:100, 33-9100, ThermoFisher Scientific; rhodamine phalloidin, 1:50, ThermoFisher Scientific), followed by incubation with secondary antibodies in blocking buffer for 1h at room temperature (Alexa Fluor 555-conjugated goat anti-mouse, Alexa Fluor 488 goat anti-rabbit, 1:1000, Invitrogen). Each step was followed with three 5 min PBS washes. Coverslips were mounted onto slides with ~20 µL ProLong Diamond Mountant with 4′,6-diamidino-2-phenylindole (DAPI, ThermoFisher Scientific).

Colon tissue slides were deparaffinized through a series of washes: xylene (3 min, 2x), 50% xylene/50% ethanol (3 min), ethanol (3 min, 2x), 95% ethanol (3 min), 70% ethanol (3 min), 50% ethanol (3 min), and cold tap water rinse. Deparaffinized slides were placed in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, 0.05% Tween 20, pH 9.0) for heat-induced epitope retrieval in a decloaking chamber (Biocare Medical) at 95 °C for 20 min and then equilibrated to room temperature. The slides were washed twice for 5 min in Tris buffered saline (TBS) with 0.025% Triton X-100 (TBS-X), and the tissues were permeabilized by immersion in TBS with 0.2% Triton X-100 for 8 min, then the slides were again washed twice for 5 min in TBS-X. The tissues were blocked using 10% normal goat serum and 1% bovine serum albumin (BSA) in TBS for 2 h at room temperature. Primary antibody in TBS containing 1% BSA (rabbit anti-SYNPO, 1:50, NBP2-39100, Novus Biologicals; rat anti-ZO-1, 1:50, MABT11, Millipore Sigma) was then added to the tissue and the slides incubated overnight at 4 °C. After two 5 min TBS-X washes, secondary antibody incubation in 1% BSA TBS was done for 1 h at room temperature (Alexa Fluor 555-conjugated goat anti-rat, Alexa Fluor 488 goat anti-rabbit, 1:500). Then after three 5 min TBS-X washes, the slides were mounted with a coverslip and ~50 µL of ProLong Diamond Mountant with DAPI.

Immunolabeling was visualized and fluorescent image taken with an AxioCam MRc5 attached to an AxioImager A1 microscope (Zeiss). Confocal fluorescence images were obtained using an Olympus FV1000 confocal laser scanning microscope.

Statistical Analysis

Data are expressed as mean values ± SEM. Statistical significance was evaluated with an unpaired two-tailed Student's t test for direct comparisons and 1-way or 2-way ANOVA with Fisher's Least Significant Different (LSD) test for multiple comparisons. A p value of less than 0.05 was considered significant (GraphPad Prism 8).

Table S1: Rate of TEER formation in T84 cells.

Condition	Rate (h^{-1})	P-Value (Compared to Control)
Control	k = 0.0511	N/A
1mM Butyrate	k = 0.2692	$p<0.0001$
1mM Acetate	k = 0.0557	ns
1mM Propionate	k = 0.0453	ns

Nonlinear regression analysis of rate of barrier formation in T84 cells treated with SCFAs.

Table S2: Rate of wound closure in T84 cells.

Condition	Rate (h^{-1})	P-Value (Compared to Control)
Control	k = 0.0207	N/A
5mM Butyrate	k = 0.1242	$p<0.0001$
5mM Acetate	k = 0.0188	ns
5mM Propionate	k = 0.0309	ns

Nonlinear regression analysis of rate of wound healing in T84 cells treated with SCFAs.

Table S3: Single-cell RNA sequencing summary.

Top induced genes

Rank	Gene	Mean	Fold Change	fcSE	pvalue
1	CTDSPL	24.908	21.482	2.0343	4.59E-26
2	SRSF1	36.004	21.38	1.7225	2.24E-35
3	SYNPO	1.5445	21.329	2.9677	6.62E-13
4	CGN	21.486	20.758	1.9895	1.74E-25
5	PCYT1A	18.012	20.711	1.98	1.31E-25
6	OR7E38P	8.7049	20.706	2.2776	9.79E-20
7	TGS1	10.469	20.696	2.6485	5.54E-15
8	STK10	1.4991	20.506	3.2135	1.76E-10
9	SPRED1	13.281	20.383	2.2766	3.45E-19
10	VDR	11.869	20.31	2.2972	9.44E-19
11	SEC24C	4.9807	20.304	2.9303	4.24E-12
12	ILF3-AS1	4.1792	20.28	2.7698	2.45E-13
13	TRAFD1	10.35	20.276	2.4135	4.43E-17
14	BIRC2	8.9476	20.177	2.6606	3.36E-14
15	COQ10B	8.6558	20.121	2.572	5.16E-15
16	KCNQ1OT1	21.929	20.057	2.1029	1.46E-21
17	ECI2	9.086	19.97	2.7856	7.56E-13
18	MYLIP	7.2264	19.969	3.0009	2.84E-11
19	EGLN1 (PHD2)	8.2282	19.94	2.2108	1.89E-19
20	EZH1	7.3934	19.93	2.1675	3.76E-20
21	KIF11	12.188	19.914	1.8748	2.36E-26
22	TSPAN31	12.846	19.903	3.4885	1.16E-08
23	PUS7L	10.048	19.866	2.2364	6.52E-19
24	ITSN2	10.445	19.846	2.664	9.36E-14
25	UBA5	9.828	19.846	2.6405	5.66E-14
26	SRSF8	11.206	19.834	2.4442	4.87E-16
27	GTF2IRD1	2.6921	19.793	3.7585	1.39E-07
28	ZNF24	16.539	19.785	2.0772	1.65E-21
29	LRIG2	10.47	19.778	2.5296	5.34E-15
30	NMD3	9.3978	19.772	1.9905	2.99E-23
31	TGFBR2	15.733	19.768	2.2594	2.14E-18
32	MYCBP2	4.9116	19.664	2.831	3.75E-12
33	PALLD	2.7204	19.662	6.2286	0.0015957
34	C7orf50	12.118	19.62	1.9936	7.48E-23
35	NDUFAF3	25.606	19.607	1.8579	4.91E-26
36	EIF4H	9.8862	19.577	1.9233	2.47E-24
37	TANC1	9.048	19.574	2.6348	1.09E-13
38	MZT1	6.2334	19.551	3.5882	5.08E-08
39	NDFIP2	12.197	19.55	2.2874	1.26E-17
40	SUPT6H	16.487	19.53	1.9335	5.48E-24

41	BMP4	3.0286	19.524	2.3795	2.31E-16
42	SREK1	21.39	19.517	1.7386	3.04E-29
43	CAT	18.871	19.516	1.8116	4.63E-27
44	GPATCH2L	10.289	19.484	3.3629	6.89E-09
45	EXOSC10	9.8136	19.444	2.6577	2.55E-13
46	NAB1	13.15	19.44	2.0333	1.16E-21
47	AAR2	11.677	19.433	2.3996	5.57E-16
48	TM2D1	9.7647	19.407	2.2149	1.92E-18
49	HERPUD1	10.54	19.368	1.926	8.63E-24
50	XPO5	4.3035	19.347	2.8543	1.22E-11
51	BTBD10	14.068	19.345	2.1256	8.94E-20
52	TNRC6A	12.589	19.324	2.2355	5.43E-18
53	KLC1	3.8372	19.322	3.7238	2.12E-07
54	CETN3	7.6149	19.314	2.4995	1.10E-14
55	BLCAP	14.943	19.285	1.8053	1.23E-26
56	NAA40	3.7429	19.262	3.2783	4.21E-09
57	MMAA	1.714	19.198	5.0651	0.00015047
58	SAPCD2	13.437	19.165	2.1257	1.96E-19
59	KDELC2	3.8012	19.115	3.247	3.93E-09
60	DECR1	15.469	19.044	2.2478	2.41E-17
61	NETO2	6.4505	18.981	2.4524	9.97E-15
62	MAPKAPK5	7.8239	18.979	2.2965	1.41E-16
63	STK17B	13.707	18.941	2.2643	6.01E-17
64	BAG5	11.735	18.931	2.1245	5.07E-19
65	SMG7	9.2344	18.924	2.1539	1.55E-18
66	MTPAP	7.3453	18.908	2.5277	7.42E-14
67	HN1L	27.964	18.897	1.6991	9.84E-29
68	ATP6V1A	9.4757	18.885	2.6958	2.46E-12
69	MYSM1	9.0831	18.884	2.2298	2.48E-17
70	GMEB1	7.0671	18.873	3.4068	3.03E-08
71	GNS	8.202	18.845	2.7531	7.64E-12
72	MRPL50	4.496	18.845	2.5691	2.21E-13
73	COX15	14.619	18.835	1.7942	8.86E-26
74	ALDH6A1	5.076	18.816	3.093	1.18E-09
75	PPM1B	7.7404	18.793	2.6537	1.42E-12
76	MRPL15	8.3283	18.781	2.2244	3.09E-17
77	SGK2	9.6462	18.768	2.3683	2.29E-15
78	CPSF6	16.288	18.72	2.0339	3.44E-20
79	DESI2	5.9056	18.715	2.3351	1.10E-15
80	CCNC	9.1205	18.682	2.0642	1.42E-19
81	LGR5	13.652	18.66	1.802	3.97E-25
82	MEF2C	9.5538	18.635	2.6292	1.37E-12
83	CPD	13.301	18.617	2.2337	7.77E-17
84	DHFR	10.944	18.562	2.2852	4.55E-16

85	CANT1	8.2011	18.544	2.6356	1.98E-12
86	UBXN7	8.4499	18.525	2.3426	2.62E-15
87	PHC2	7.8714	18.52	3.106	2.48E-09
88	EDC3	7.5804	18.497	2.171	1.60E-17
89	DOLPP1	1.3864	18.489	3.3487	3.37E-08
90	SPINK1	11.081	18.457	2.2972	9.42E-16
91	BTF3L4	4.8398	18.442	3.6209	3.52E-07
92	FBXO21	10.657	18.421	2.4907	1.41E-13
93	ARIH2	44.951	18.367	2.4082	2.40E-14
94	ARCN1	14.823	18.367	2.1066	2.82E-18
95	RBPJ	13.418	18.201	2.1943	1.09E-16
96	PPP1CA	15.55	18.17	1.7979	5.18E-24
97	IPMK	12.618	18.137	2.6355	5.90E-12
98	C12orf65	16.783	18.118	2.5569	1.38E-12
99	ELK1	9.9552	18.104	2.6807	1.44E-11
100	RNF5	3.0519	18.099	2.3972	4.35E-14
235	CLDN1	14.484	5.1227	2.5005	0.040495
312	CLDN3	22.318	2.7989	1.2138	0.02112

Top repressed genes

Rank	Gene	Mean	Fold Change	fcSE	pvalue
1	SLC10A3	2.6176	-29.682	4.1278	6.44E-13
2	CENPO	3.5107	-29.621	3.6379	3.88E-16
3	C1RL-AS1	7.8141	-29.518	3.7289	2.45E-15
4	HSPG2	6.5733	-24.784	3.9062	0
5	SPR	6.1029	-23.491	2.6199	3.07E-19
6	LSM10	24.197	-23.318	3.3818	5.39E-12
7	TMEM254	8.5631	-23.097	2.3634	1.47E-22
8	POLR2C	8.0814	-21.798	2.9728	2.26E-13
9	ANKRD28	5.3992	-21.546	3.3222	8.84E-11
10	PDHX	8.2804	-21.453	2.2703	3.40E-21
11	KLHL18	7.1614	-21.436	2.5402	3.21E-17
12	MED30	9.0949	-20.996	2.4172	3.76E-18
13	ELOF1	5.2532	-20.602	2.9941	5.95E-12
14	RFXANK	10.405	-20.584	2.0151	1.71E-24
15	HPCAL1	3.8643	-20.543	2.7713	1.24E-13
16	ZNF394	8.8703	-20.48	2.4607	8.61E-17
17	ATG101	5.7972	-19.509	3.3324	4.79E-09
18	TMEM101	10.619	-19.475	2.6316	1.36E-13
19	ATXN10	6.7772	-19.455	2.8031	3.90E-12
20	CERS2	5.5287	-19.291	2.6893	7.32E-13
21	CISD2	6.9998	-18.635	2.8492	6.14E-11
22	UNG	8.6312	-18.311	2.6381	3.89E-12
23	TNKS	13.731	-18.176	3.2304	1.84E-08
24	NBN	8.3992	-18.165	2.3014	2.95E-15
25	ZNF267	2.6548	-17.85	3.7222	1.62E-06
26	GMPR2	11.196	-17.446	2.6052	2.13E-11
27	TIMM10B	6.5687	-17.35	2.4612	1.80E-12
28	HPS6	3.6075	-17.213	4.8999	0.00044309
29	ZDHHC2	2.2494	-17.205	5.259	0.0010695
30	FAM46A	10.421	-17.116	2.075	1.60E-16
31	COBL	3.3801	-17.018	2.6038	6.32E-11
32	UBL7	3.8285	-16.93	3.5141	1.45E-06
33	ZNF280C	6.0818	-16.792	3.5097	1.71E-06
34	STX8	11.262	-16.728	2.591	1.07E-10
35	KIAA1147	8.6636	-16.673	2.3323	8.75E-13
36	ZNHIT3	11.525	-16.268	2.5657	2.29E-10
37	EXOSC9	4.0836	-16.251	3.8614	2.57E-05
38	HCG18	7.6174	-16.239	2.8295	9.52E-09
39	BCAT2	6.6507	-16.166	2.7111	2.48E-09
40	IL17RB	11.48	-16.122	2.4995	1.12E-10
41	WDR26	6.7404	-15.958	3.3003	1.33E-06
42	COIL	5.7107	-15.956	2.3995	2.94E-11

43	ZDHHC16	5.3751	-15.935	4.354	0.00025234
44	GPALPP1	7.0913	-15.882	3.8632	3.94E-05
45	NDC1	6.1367	-15.818	2.699	4.61E-09
46	MRPL45	4.4336	-15.771	2.9476	8.78E-08
47	YTHDF3	2.4786	-15.702	3.7667	3.06E-05
48	POLDIP3	5.6483	-15.552	2.9528	1.39E-07
49	BFAR	12.972	-15.513	2.434	1.85E-10
50	PROCR	2.3257	-15.503	2.403	1.11E-10
51	AASDHPPT	8.2649	-15.302	2.9804	2.83E-07
52	VAMP3	5.185	-15.249	3.3733	6.17E-06
53	POLR3C	4.8778	-15.201	2.6582	1.07E-08
54	AKR7A3	4.4793	-15.189	3.0194	4.89E-07
55	COX18	2.1627	-15.172	4.1481	0.00025448
56	MRPS22	5.2027	-15.155	2.9597	3.05E-07
57	C6orf89	8.7535	-14.945	2.8934	2.40E-07
58	GPATCH8	9.2792	-14.908	2.4532	1.22E-09
59	MTF2	4.2354	-14.752	2.6374	2.23E-08
60	GPRC5C	2.7817	-14.627	4.0975	0.00035747
61	SF3A3	17.486	-14.607	2.1387	8.50E-12
62	C4orf33	7.0972	-14.49	2.8085	2.48E-07
63	C6orf136	9.0043	-14.465	3.2338	7.72E-06
64	NUP205	9.6923	-14.269	2.4105	3.23E-09
65	PREB	5.191	-14.097	2.9951	2.52E-06
66	ZNF107	10.037	-14.089	2.724	2.31E-07
67	ANG	8.8173	-14.024	3.7381	0.00017569
68	ITGAE	7.417	-13.974	3.3192	2.55E-05
69	LANCL1	3.4359	-13.932	3.2161	1.48E-05
70	LYN	3.6837	-13.885	2.7821	6.02E-07
71	TMEM126B	6.2878	-13.679	3.1923	1.83E-05
72	MOV10	10.872	-13.464	2.9355	4.51E-06
73	ZNF701	8.1793	-13.161	2.6249	5.34E-07
74	REG4	6.5555	-13.049	4.071	0.0013485
75	MCM10	7.6352	-12.904	2.443	1.28E-07
76	CXCL16	6.7378	-12.871	3.1452	4.27E-05
77	TFIP11	5.2978	-12.659	2.9797	2.15E-05
78	NTPCR	6.8781	-12.547	3.1919	8.46E-05
79	ERBIN	41.837	-11.275	1.9851	1.35E-08
80	REST	41.618	-11.001	2.072	1.10E-07
81	POLR1B	13.374	-10.578	2.7947	0.00015377
82	CNOT10	15.668	-9.8955	2.1287	3.34E-06
83	PCGF5	24.057	-9.862	2.398	3.91E-05
84	AMPD3	17.629	-9.8242	3.1013	0.0015361
85	HAUS2	14.644	-9.7589	2.7827	0.00045328
86	CCDC25	24.189	-9.5528	1.9027	5.15E-07

87	IP6K2	63.062	-9.5428	1.8125	1.40E-07
88	ARHGDIB	25.621	-9.2823	1.7956	2.35E-07
89	NIT2	27.264	-9.2177	2.2745	5.07E-05
90	HAUS8	31.485	-9.1425	2.6449	0.000547
91	NAA25	13.81	-9.0206	2.6752	0.00074652
92	CIAO1	29.896	-8.9164	2.0152	9.66E-06
93	TMED9	12.511	-8.8466	1.8674	2.17E-06
94	NCAPH	32.465	-8.6893	1.9577	9.06E-06
95	SUCO	10.839	-8.5449	2.6297	0.0011565
96	MAGED1	21.689	-8.5259	1.8446	3.80E-06
97	CEP295	25.097	-8.5121	2.2985	0.00021274
98	FAR2	15.541	-8.4903	2.5971	0.0010787
99	DNTTIP1	13.227	-8.4677	2.583	0.0010444
100	TAOK2	9.1917	-8.3893	2.9219	0.0040894
115	CLDN2	0.212553806	-2.2341	2.4058	0.035307

Top 100 induced and repressed genes from single-cell RNA sequencing analysis. Cytoskeletal/barrier-related genes highlighted in red.

Table S4: Rate of TEER formation in SHC and SYNPO KD cells.

Cell Type/Condition	Rate (h^{-1})	P-Value (Compared to Control)
SHC/Control	k = 0.0385	N/A
SHC/1mM Butyrate	k = 0.1233	$p<0.0001$
SYNPO KD/Control	k = 0.0135	$p<0.05$
SYNPO KD/1mM Butyrate	k = 0.0199	$p<0.05$

ns

Nonlinear regression analysis of rate of barrier formation in SHC and SYNPO KD cells treated with 1mM butyrate.

Table S5: Rate of wound closure in SHC and SYNPO KD cells.

Cell Type/Condition	Rate (h^{-1})	P-Value (Compared to Control)
SHC/Control	k = 0.0885	N/A
SHC/1mM Butyrate	k = 0.2055	$p<0.0001$
SYNPO KD/Control	k = 0.0452	$p<0.001$
SYNPO KD/1mM Butyrate	k = 0.0536	$p<0.001$

ns

Nonlinear regression analysis of rate of wound closure in SHC and SYNPO KD cells treated with 1mM butyrate.

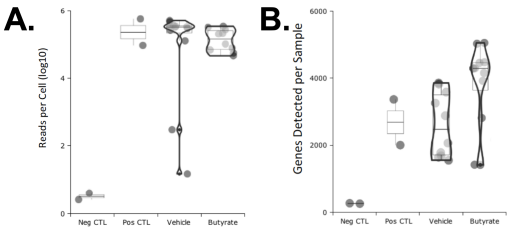

Figure S1: *Single-cell RNA sequencing analysis metrics*. (A) Reads per cell for vehicle and butyrate treatment groups (*n*=10, *ns* by 1-way ANOVA). (B) Genes detected per sample for vehicle and butyrate treatment groups *n*=10, *ns* by 1-way ANOVA).

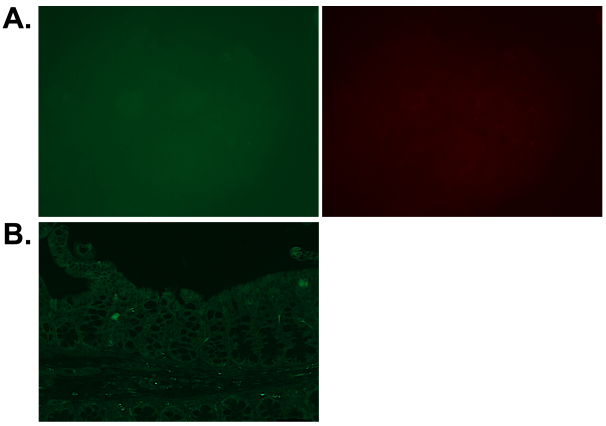

Figure S2: *Secondary antibody control for immunofluorescence.* (A) FITC and rhodamine secondary antibody only staining in T84 cells. (B) FITC secondary antibody only staining in mouse colonic tissue.

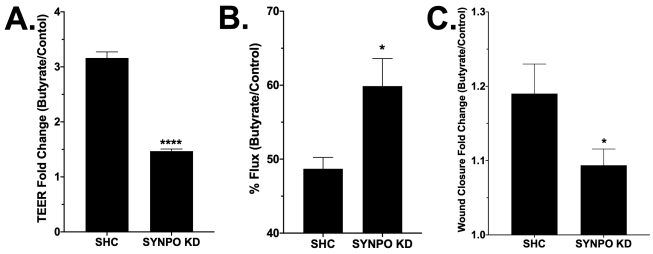

Figure S3*: TEER, flux, and wound closure fold change analysis for SHC and SYNPO KD cells.* (A) Fold change in final TEER reached after 48 h between butyrate treatment versus control in SHC and SYNPO KD cells (n=6, error bars: SEM, **** $p<0.0001$ by Student's t-test). (B) Percentage of remaining FITC-dextran flux in SHC and SYNPO KD cells after butyrate treatment (n=6, error bars: SEM, * $p<0.05$ by Student's t-test). (C) Fold change in wound closure reached after 38 h between butyrate treatment versus control in SHC and SYNPO KD cells (n=5, error bars: SEM, * $p<0.05$ by Student's t-test).

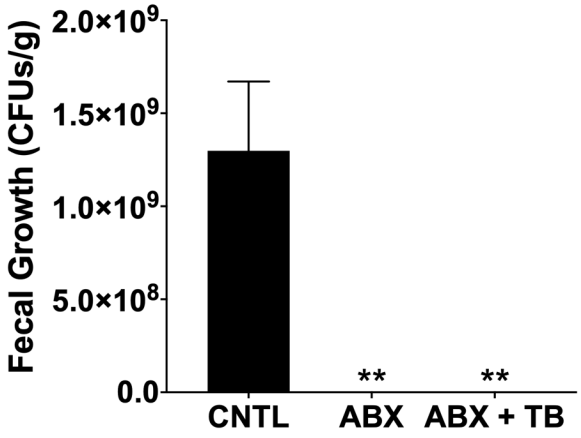

Figure S4: *Fecal bacteria content in mice treated with antibiotics combination.* Colony-forming units (CFUs) per gram of fecal sample in control (CNTL), antibiotics-treated microbiota-depleted (ABX), and butyrate add-back mice (ABX + TB) (*n*=4-5, error bars: SEM, ** $p<0.01$ by 1-way ANOVA, Fisher's multiple comparison).

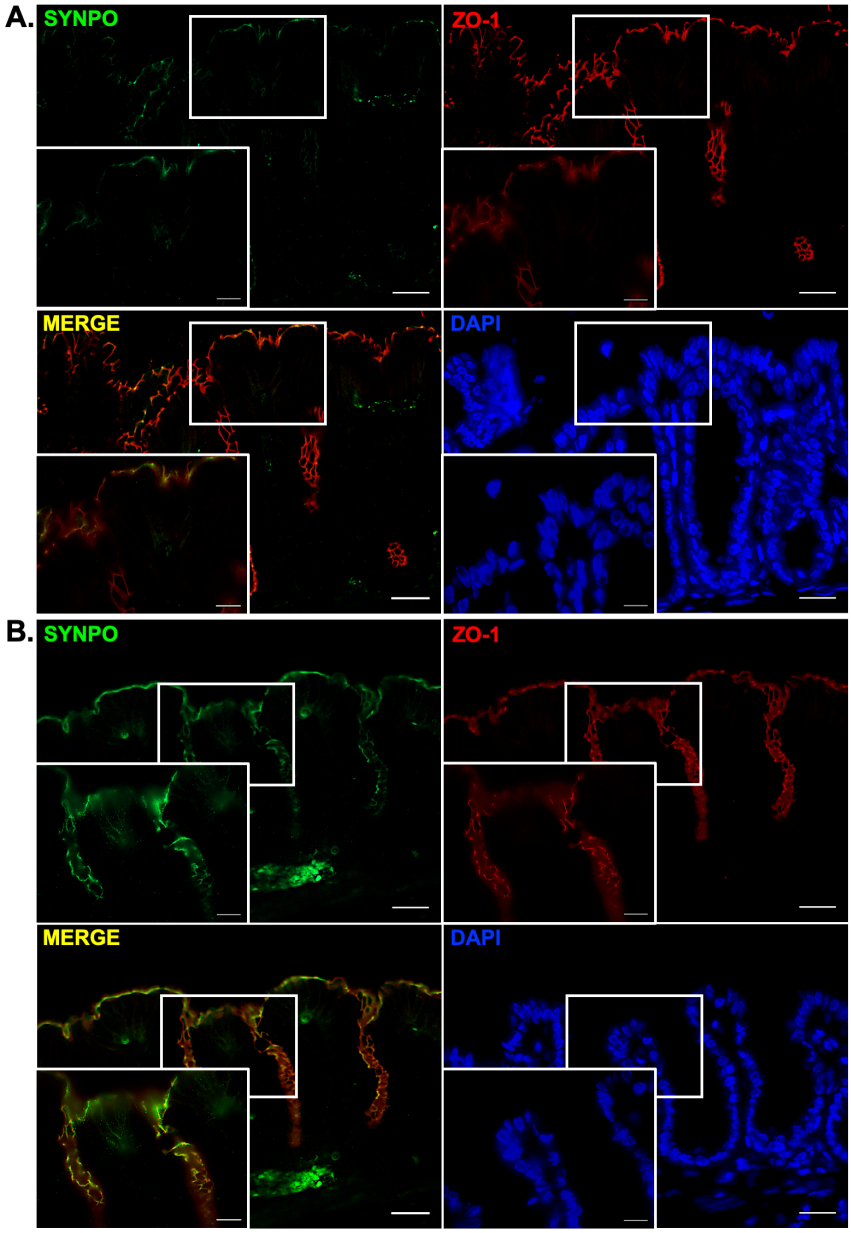

Figure S5: *SYNPO and ZO-1 immunofluorescence in germ free (GF) mice given tributyrin or glycerol control*. (A) SYNPO and ZO-1 staining in GF glycerol control mouse colonic tissue (scale bar: 20 μm, 400X, inset: 10 μm, 1000X). (B) SYNPO and ZO-1 staining in GF tributyrin (butyrate add-back) mouse colonic tissue (scale bar: 20 μm, 400X, inset: 10 μm, 1000X).

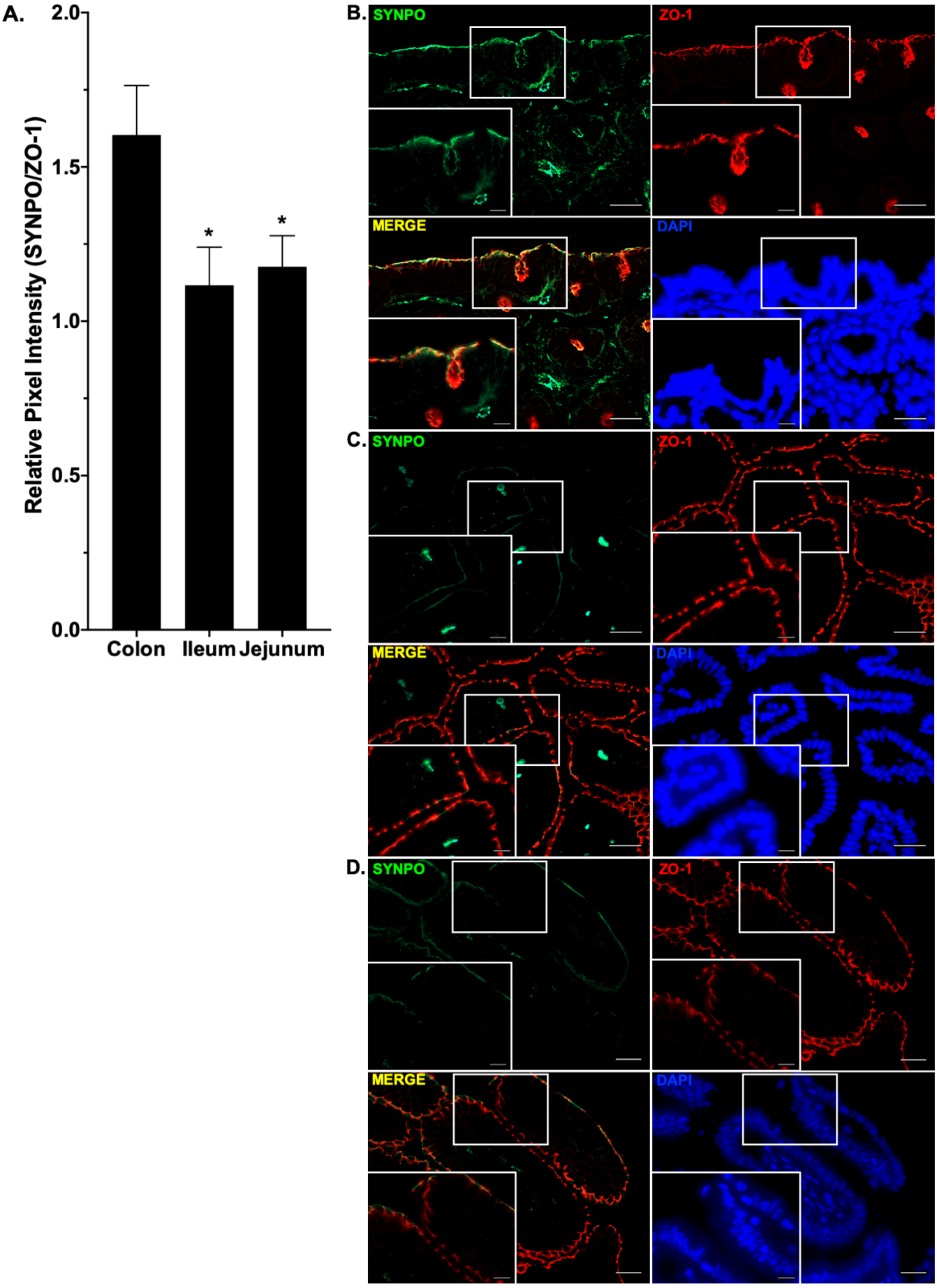
A.
Relative Pixel Intensity (SYNPO/ZO-1)
2.0
1.5
1.0
0.5
0.0
*
*
Colon
Ileum
Jejunum
B.
SYNPO
ZO-1
MERGE
DAPI
C.
SYNPO
ZO-1
MERGE
DAPI
D.
SYNPO
ZO-1
MERGE
DAPI

Figure S6: *SYNPO expression in mouse jejunum, ileum, and colon*. (A) Relative pixel intensity of SYNPO normalized to ZO-1 in mouse jejunum, ileum, and colon tissue (*n*=5, error bars: SEM, * $p<0.05$ by 1-way ANOVA, Fisher's multiple comparison). (B) Representative immunofluorescence staining in the colon (scale bar: 20 μm, 400X, inset: 10 μm, 1000X). (C) Representative immunofluorescence staining in the ileum (scale bar: 20 μm, 400X, inset: 10 μm, 1000X). (D) Representative immunofluorescence staining in the jejunum (scale bar: 20 μm, 400X, inset: 10 μm, 1000X).

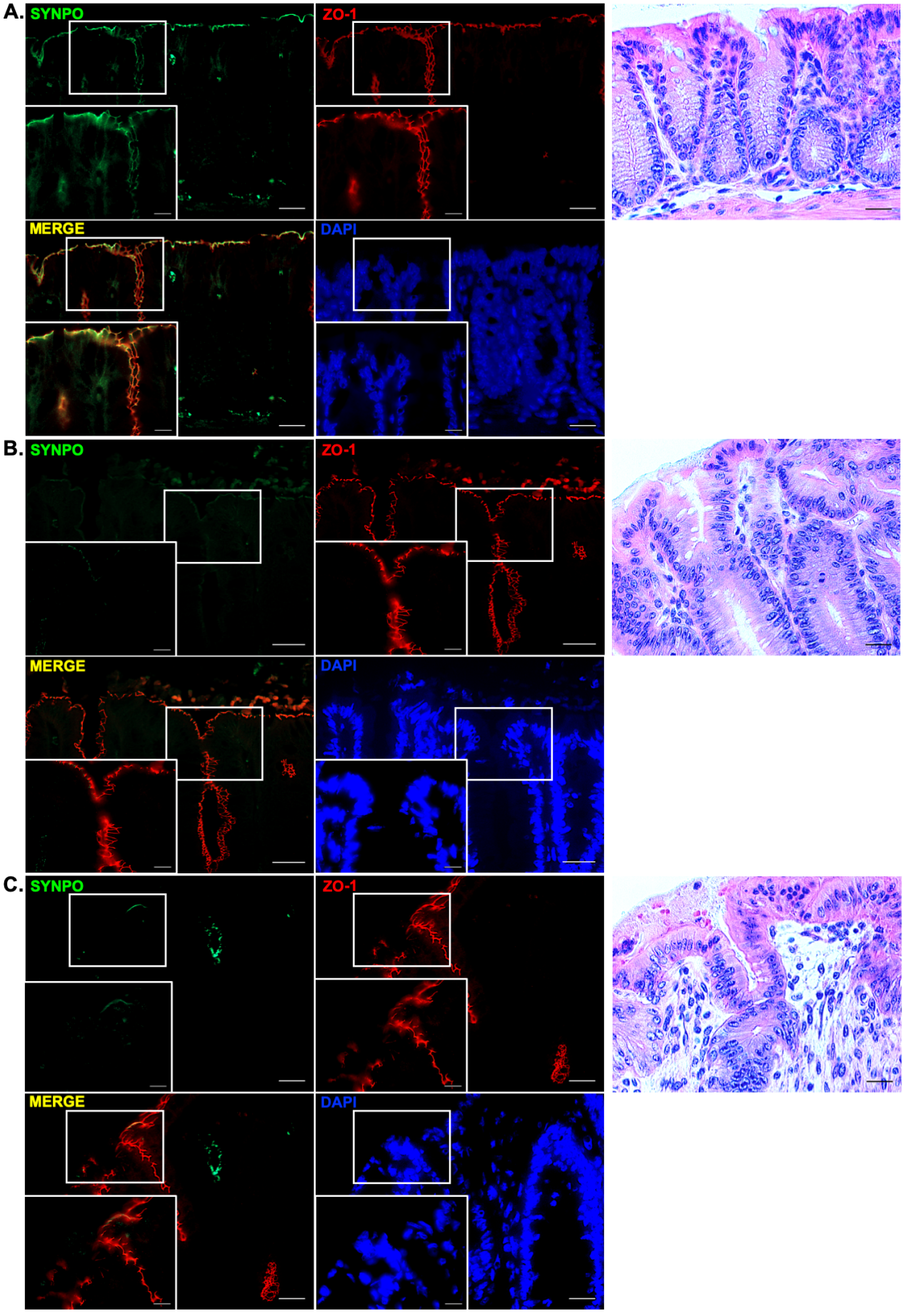
A.
SYNPO
ZO-1
MERGE
DAPI
B.
SYNPO
ZO-1
MERGE
DAPI
C.
SYNPO
ZO-1
MERGE
DAPI

Figure S7: *SYNPO expression in H2O versus DSS mice colon*. (A) Immunofluorescence staining in H2O control mouse colon with corresponding H&E staining. (B) Immunofluorescence staining in DSS mouse colon with corresponding H&E staining in area of low disease (scale bar: 20 μm, 400X, inset: 10 μm, 1000X). (C) Immunofluorescence staining in DSS mouse colon with corresponding H&E staining in area of active disease (scale bar: 20 μm, 400X, inset: 10 μm, 1000X).

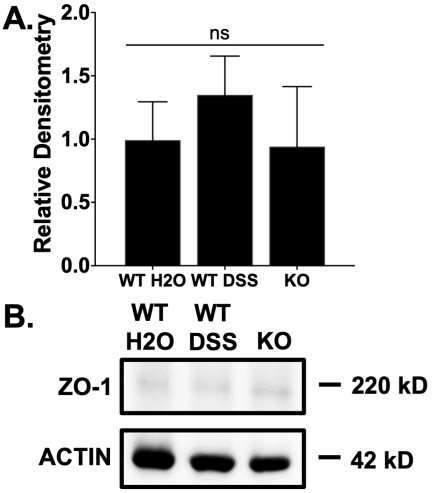

Figure S8: *ZO-1 protein expression in H2O versus DSS mice colon*. (A) ZO-1 protein expression in WT H2O, WT DSS, and KO mice (*n*=5, error bars: SEM, *ns* by 1-way ANOVA, Fisher's multiple comparison). (B) Representative western blot for WT H2O, WT DSS, and KO mice.

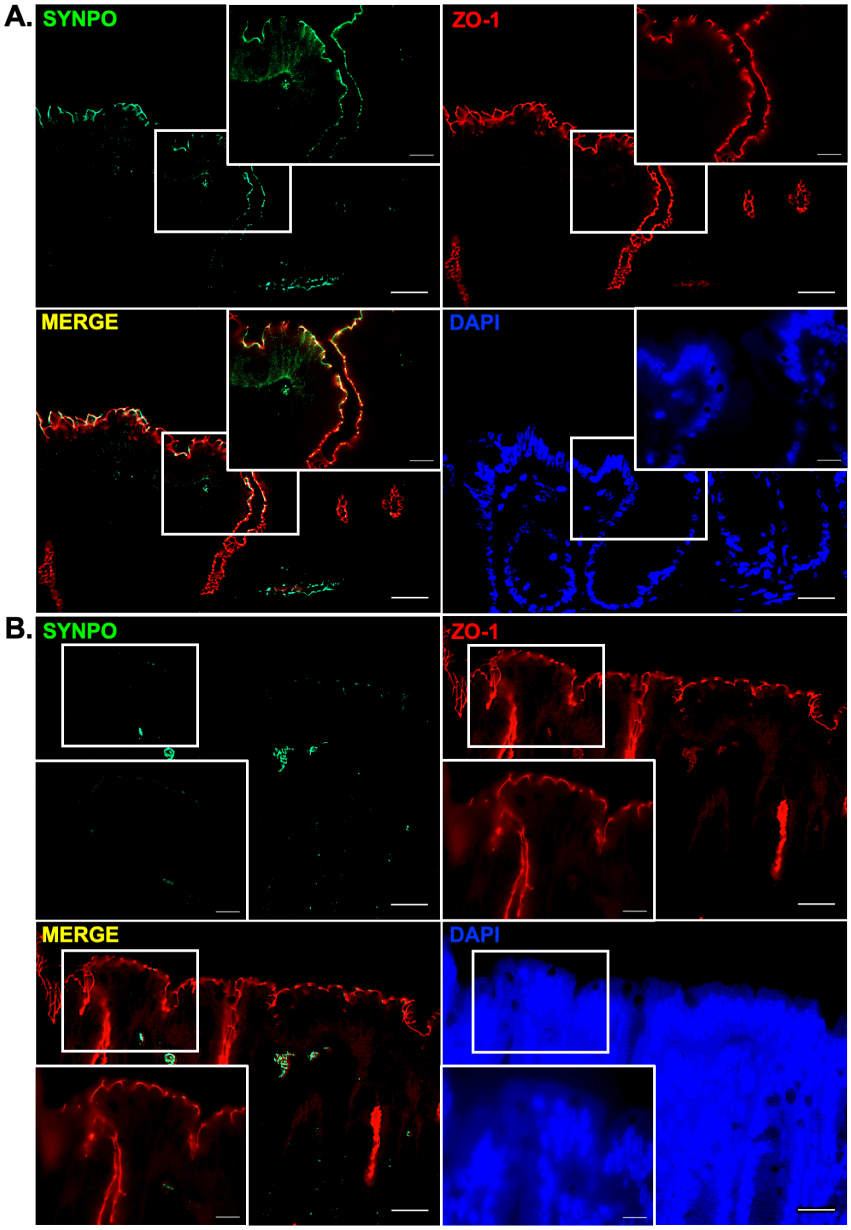

Figure S9: *SYNPO and ZO-1 immunofluorescence in WT and Synpo*$^{-/-}$ *(KO) mice*. (A) SYNPO and ZO-1 staining in WT mouse colonic tissue (scale bar: 20 μm, 400X, inset: 10 μm, 1000X). (B) SYNPO and ZO-1 staining in KO mouse colonic tissue (scale bar: 20 μm, 400X, inset: 10 μm, 1000X).

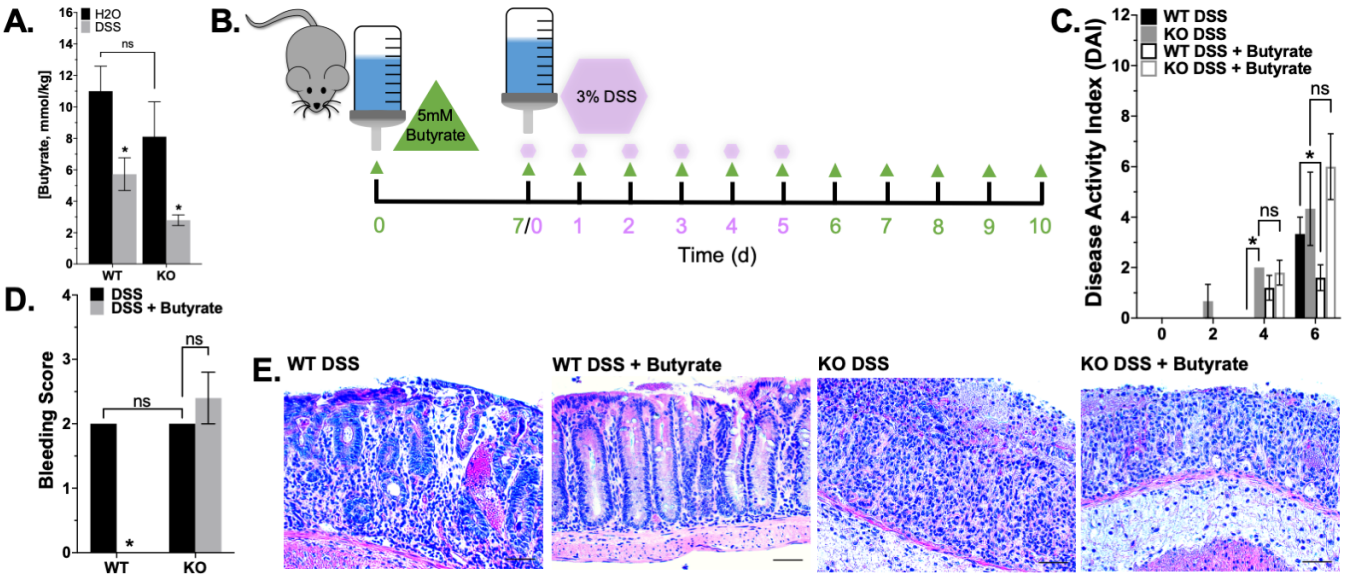

Figure S10: *Butyrate supplementation during DSS colitis in WT and KO mice*. (A) Cecal butyrate content in WT and KO mice subjected to DSS (*n*=5, error bars: SEM, * *p*<0.05 by 2-way ANOVA, Fisher's multiple comparison). (B) Schematic of butyrate supplementation in DSS colitis in WT and KO mice. (C) Disease activity index (DAI) of WT and KO mice subjected to DSS with or without butyrate supplementation up to day 6 when DSS was pulled (*n*=5, error bars: SEM, * *p*<0.05 by 2-way ANOVA, Fisher's multiple comparison). (D) Bleeding scores of WT and KO DSS mice with or without butyrate at peak day of bleeding (*n*=5, error bars: SEM, * *p*<0.05 by 2-way ANOVA, Fisher's multiple comparison). (E) Representative histology images of the extent of damage in colon tissue of WT and KO mice subjected to DSS with or without DSS.

Video S1: *Live cell image video of scratch wound healing in control T84 cells over 38 h*. Intestinal epithelial wound restitution was minimal in control conditions with T84 cells exhibiting limited motility and persistent wound.

Video S2: *Live cell image video of scratch wound healing in T84 cells treated with 5 mM butyrate over 38 h*. Butyrate accelerated intestinal epithelial wound restitution with T84 cells exhibiting markedly increased motility and wound closure.

SI References:

1. T. E. Adolph *et al.*, Paneth cells as a site of origin for intestinal inflammation. *Nature* **503**, 272-276 (2013).
2. L. A. Dieleman *et al.*, Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clin Exp Immunol* **114**, 385-391 (1998).
3. H. Miyoshi, T. S. Stappenbeck, In vitro expansion and genetic modification of gastrointestinal stem cells in spheroid culture. *Nat Protoc* **8**, 2471-2482 (2013).
4. X. Wang, B. Seed, A PCR primer bank for quantitative gene expression analysis. *Nucleic Acids Res* **31**, e154 (2003).
5. J. S. Lee *et al.*, Hypoxanthine is a checkpoint stress metabolite in colonic epithelial energy modulation and barrier function. *J Biol Chem* **293**, 6039-6051 (2018).