Methods

Animal Studies. Animal studies were conducted under an approved Institutional Animal Care and Use Committee (IACUC) protocol at University of California, San Francisco (UCSF). Male and female mice were bred and housed at T_a=22°C or T_a=30°C (as indicated in the text) under a 12-hr light:dark cycle with *ad libitum* access to food and water. Both male and female mice aged 8-12 weeks were used in the experiments. C57BL/6J (Stock No: 000664) and C3H/HeJ (Stock No: 000659) were purchased from Jackson Laboratories and bred in our facility. *Lgr5*$^{GFP\text{-}creERT2/+}$ (B6.129P2-*Lgr5*$^{tm1(cre/ERT2)Cle}$/J), *Rosa26*$^{RFP/+}$ (B6;129S6-*Gt(ROSA)26Sor*$^{tm14(CAG\text{-}tdTomato)Hze}$/J) were kindly provided by Dr. Klein (UCSF), and *Gnas*$^{f/f}$*Adipoq*^{Cre} mice on 129S6/SvEvTac Black Swiss background were kindly provided by Dr. Wang (UCSF).

DSS-induced colitis, *Citrobacter rodentium* infection, and colon tumorigenesis. For acute experimental colitis, mice were administered 3-4% dextran sodium sulfate (DSS; mol. wt. = 36,000–50,000 Da; MP Biomedicals) in sterilized drinking water *ad libitum* for 5-7 days, which was subsequently replaced by regular drinking water. For quantification of bacterial dissemination, organs were harvested by sterile necropsy, mechanically homogenized in sterile PBS with 0.1% TritonX-100 (Sigma-Aldrich), and serial dilutions were plated on LB agar. Colonies were quantified 48 hours after incubation at 37°C. For inflammation-induced colon tumorigenesis, mice were injected with Azoxymethane (10 mg/kg i.p., Sigma-Aldrich) on day 0 and subjected to 3 cycles of DSS (2.5% in drinking water for 5 days) on days 10, 30, and 50. *Citrobacter rodentium* (strain DBS100) was purchased from ATCC (# 51459) and propagated in selective MacConkey media and agar using standard culture methods. On the day of infection, mice were fasted for 6-8 hours before oral gavage with 1x10^6 CFU of *C. rodentium*. For

quantification of *C. rodentium* bacterial burden, organs were harvested by sterile necropsy, mechanically homogenized in sterile PBS, serially plated on MacConkey agar plates, and colonies were enumerated 24 hours after incubation at 37°C. For DSS-induced colitis and *C. rodentium* infection studies, body mass was measured daily, and mice were euthanized if they lost >20% of their initial body weight.

Isolation of large intestine epithelial cells (colonocytes) for RNA-seq. Epithelial cells were isolated from large intestines after flushing with ice-cold sterile PBS. The entire length of the colon was opened longitudinally and washed with Ca^{2+}/Mg^{2+}-free HBSS supplemented with 2% bovine serum albumin and 10 mM HEPES. The colon was then cut into small pieces (5-10 mm) and immersed in 20 ml of Ca^{2+}/Mg^{2+}-free HBSS with 2% fetal calf serum, 1 mM EDTA, 10 mM HEPES, and 1 mM dithiothreitol, and incubated at 37°C at 200 rpm for 20 minutes and the process was repeated twice. The three fractions of epithelial cells were pooled, passed through a 100 μM mesh nylon filter, and centrifuged at 1,000 *g* for 5 minutes at 4°C. Lymphocytes and other $CD45^+$ cells were removed from the epithelial cell fraction by overlaying onto a 40-90% Percoll gradient followed by centrifugation at 2,200 rpm for 20 minutes. The remaining epithelial cells were collected and washed with ice-cold HBSS and centrifuged for 1,000 *g* for 5 minutes at 4°C. The final pellet was then collected as colonocytes, and purity was assessed by flow cytometry using anti-CD45, anti-EpCAM, and anti-cytokeratin-18 antibodies.

Isolation of intestinal crypts for RNA-seq. Large intestine crypts were isolated as described previously (1, 2). Dissected colons were flushed and washed with ice-cold PBS, opened longitudinally, and the lumen was gently scraped with a glass slide to remove fecal matter and mucus. Colon segments were placed in a 50 ml conical tube with 25 ml ice-cold PBS and inverted 10-15 times, and the supernatant was discarded. Colon segments were cut into 5 mm

pieces, placed into 50 ml conical tube containing 10 ml of cold PBS with 5 mM EDTA, and vigorously triturated by pipetting up and down 15 times. Supernatant was aspirated and replaced with 10 ml of cold PBS with 5 mM EDTA and placed at 4°C on a benchtop roller for 15 minutes. Supernatant was removed and pieces were washed twice with ice cold PBS. The washed colon pieces were added to 3 ml of DMEMF/12 basal media (2mM L-glutamine, 100U/ml pencillin, 1000ug/ml streptomycin, 1mM N-acetylcysteine, 10mM HEPES) containing 500 U/ml Collagenase Type IV, vigorous triturated by pipetting up and down 5 times, and incubated in a 37°C water bath for 30 minutes. Subsequently, 10 ml of ice-cold PBS was added and the colon pieces were vigorously triturated by pipetting up and down 10 times to release crypts, which constituted the first fraction. This process was repeated 3 times to obtain a total of 4 crypt fractions. Aliquots of each fraction were assessed using an inverted microscope, and 10 ml of basal media containing 15U/ml DNase I was added to the fraction most enriched with intact crypts. The chosen fraction was then sequentially filtered through 100 μM and 70 μM filters, centrifuged at 300 *g* for 5 minutes, and used for RNA extraction.

Colonoid culture and growth assays. Intestinal crypts were harvested from isolated colon to generate colonoids as previously described (3). Briefly, colon distal to the cecum was removed and luminal contents flushed with cold PBS. The colon was then opened longitudinally and cut into 2mm pieces. Colonic fragments were washed in PBS and transferred to chelation buffer (2mM EDTA in PBS) for 30min at 4°C with shaking. Following mechanical disruption, isolated crypts were collected by centrifugation (800g, 5min, 4°C) and plated in Matrigel. After hardening, Matrigel domes were covered by colonoid culture media consisting of 50% L-WRN conditioned media (4), 50% Advanced DMEM/F12 media with HEPES, GlutaMax, penicillin, and streptomycin. Media was supplemented with EGF (Peprotech), B27 (Life Technologies), N2

(Life Technologies), N-acetyl cysteine (NAC; Sigma). At initial plating, media was further supplemented with CHIR-99021 (Tocris) and Y27623 (Sigma) to support colonoid formation.

Colonoid formation was determined one week after plating an equal number of crypts harvested from mice housed at sub-thermoneutrality or thermoneutrality. To determine colonoid area, random non-overlapping images were taken of colonoids one week after plating. Each colonoid image was analyzed using ImageJ (NIH, Bethesda, MD) following manual determination of colonoid circumference to calculate area (5). Viability was determined by a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay as described (6, 7). Briefly, colonoids were incubated with 500 mg/ml MTT for two hours at 37°C. Media was aspirated and Matrigel dissolved in 2% SDS for 2 h at 37°C with shaking. MTT was then solubilized in DMSO and absorbance measured at OD562. Values were normalized to the average absorbance of untreated control colonoids (taken as 1) and unseeded wells (taken as 0).

Flow cytometric analysis of lamina propria lymphocytes. Large intestines from water or DSS treated mice were isolated, flushed with ice-cold PBS, and cut longitudinally into 5 mm pieces. The cut tissues were shaken in 20 ml of cold PBS with 3 changes, and washed for 20 min at 37°C in 20 ml of Ca^{2+}/Mg^{2+}-free HBSS containing 5 mM DTT, 10 mM HEPES, and 2% FCS with 2 changes followed by wash in 20 ml of Ca^{2+}/Mg^{2+}-replete HBSS containing 10 mM HEPES and 2% FCS. Pieces were then recovered through a 100 μM filter and transferred to GentleMACS C tube with 5 ml of digestion buffer (0.1 W/ml of LiberaseTM, 30 μg/ml Dnase I, 10 mM HEPES in HBSS with 2% FCS) and incubated at 37°C with gentle stirring. The suspension was passed over a 100 μM filter, remaining pieces were mashed through the filter, and washed with 40 ml of ice-cold PBS before centrifugation at 1,500 rpm at 4°C for 5 minutes.

The pellet was resuspended in 5 ml of 40% Percoll, underlayed with a 90% Percoll, and centrifuged at 2,200 rpm for 20 min. The middle-interfaced layer was collected and washed with PBS prior to analysis.

The lamina propria lymphocytes were stained with antibodies against surface antigens at 4°C for 30 minutes in FACS buffer (1xPBS with 2% fetal calf serum, 1mM EDTA, 0.1% sodium azide). Intracellular antigens and cytokines were detected using Fixation/Permeabilization kit (BD Biosciences) as per manufacturers' instructions. Transcription factors and nuclear antigens were detected using Foxp3/Transcription Factor Staining Buffer Set (ThermoFisher Scientific) as per manufacturers' instructions. See Dataset S3 for full list of antibodies used in this study. Stained samples were run on BD FACSVerse flow cytometer and data was analyzed with FlowJo (FlowJo, LLC).

Gates were constructed to identify target populations based on surface marker staining. Immune cells were identified as $CD45^+$. Myeloid cell subsets were identified as alternatively-activated macrophages ($CD11b^+F4/80^+CD206^+CD301^+$), patrolling ($Ly6^{low}$) and inflammatory ($Ly6^{hi}$) monocytes, dendritic cells ($CD11c^+MHCII^{high}$), neutrophils ($Ly6G^+$), and eosinophils ($SiglecF^+$). Lineage negative innate lymphoid cell populations were identified as ILC1s ($ROR\gamma t^-$ $GATA3^-NKp46^{+/-}$), ILC2's ($ROR\gamma t^-GATA3^+CD90.2^+$), and ILC3's ($ROR\gamma t^+$ $GATA3^-$). Adaptive immune cells were identified as natural killer ($NK1.1^+CD3\varepsilon^-$), natural killer T cells ($NK1.1^+CD3\varepsilon^+$), $CD4^+$ and $CD8^+$ T cells, B cells ($B220^+CD3\varepsilon^-$), IFNγ producing cells, type-2 cytokine producing cells ($IL4^+$, $IL5^+$ and $IL13^+$), $CD4^+IL17^+$ T cells ($ROR\gamma t^+IL17A^+$), regulatory T cells ($CD4^+FoxP3^+$), and invariant T cells ($\gamma\delta TCR^+CD4^-$).

Colonization and colitis studies in germ-free mice. Cecal contents from sex matched C57BL/6J mice housed at T_a=22°C or T_a=30°C were isolated under anaerobic conditions and

mixed with 10 ml of BHI media containing 0.05% Cysteine-hydrochloride, 5μg/ml Hemin, 15% glycerol, and 1μg/ml Vitamin K. The resulting solution was passed through a 100 μM filter, transferred into a gnotobiotic isolator, and 100 μl/mouse was used to colonize germ-free mice by oral gavage. Two weeks post colonization, mice were administered 4% DSS (M.W. = 36,000–50,000 Da; MP Biomedicals) in drinking water for 7 days for induction of colitis and body mass was measured daily.

Immunoblotting. Whole cell extracts were prepared in modified RIPA buffer containing 50 mM Tris-HCl pH 7.5, 420 mM NaCl, 0.1% w/v SDS, 0.5% w/v Na-Deoxycholate, 1% v/v Nonidet P-40 substitute (Fluka, #74385), 1mM EDTA, 1mM EGTA and protease inhibitor cocktail (Sigma-Aldrich, #P8340). Protein samples were run into Criterion or mini-PROTEAN TGX Stain-Free precast gels (Biorad), and blotted onto nitrocellulose membrane. Membranes were blocked (5% non-fat milk in 0.1% TBST) for 1h at 22°C and probed for β2- (Abcam, #ab182136, dilution 1:1000) or β3-adrenergic receptor (Abcam, ab94506, dilution 1:1000) in 0.1% TBST containing 5% BSA by overnight incubation at 4°C. After incubation with secondary HRP-linked anti-rabbit antibody (Cell Signaling, #7074) for 1h at 22°C, immunoblotted proteins were detected with ProSignal Pico ECL Reagent (Genesee Scientific, #20-300) and imaged using ChemiDoc MP Imaging System (Bio-Rad).

Treatment with β-adrenoreceptor agonists and antagonist. Mice were administered β2-adrenoceptor agonist bambuterol hydrochloride (1 mg/kg; i.p, daily; Sigma Aldrich), β2-adrenoceptor antagonist butoxamine hydrochloride (1 mg/kg, i.p, daily; Sigma Aldrich), or β3-adrenoceptor agonist CL 316,243 (1 mg/kg, i.p, daily; Sigma Aldrich) for 2 weeks prior and during the one week treatment with DSS.

Measurement of cytokines and tissue damage markers. Plasma and large intestine tissue cytokine levels were measured by cytometric bead array-mouse inflammation kit (BD Biosciences, San Jose, CA), mouse amphiregulin DuoSet ELISA kit (R&D systems), and mouse IL-22 ELISA kit (Thermo Fisher Scientific) as per manufacturers' protocol. Tissue damage during colitis was assessed using lipocalin2/NGAL ELISA kit (R&D Systems), Urea Nitrogen (BUN) colorimetric detection kit (Thermo Fisher Scientific), Alanine Transaminase Colorimetric Activity assay kit (Cayman Chemical), and mouse SAA ELISA kit (Abcam) as per manufacturers' instructions.

Quantification of colonocyte hypoxia. For quantification of colonocyte hypoxia, mice were treated with 100 mg/kg of pimonidazole hydrochloride (i.p., Hypoxyprobe-RedAPC kit, Hypoxyprobe) one hour prior to euthanasia. Large intestine samples were fixed in 10% buffered formalin for 24 hours and stored at 4°C in 30% sucrose in PBS. Paraffin embedded tissues were sectioned at 5 μM, deparaffinized in 2 changes of xylene substitute Histoclear (Sigma-Aldrich) for 5 minutes, and hydrated through a series of ethanol washes (2 changes of 100% ethanol for 3 minutes followed by 1 change each of 95% ethanol for 3 minutes, 90% ethanol for 3 minutes, 80% ethanol for 3 minutes, 70% ethanol for 5 minutes, and distilled water for 3 minutes). Antigens were retrieved by incubation with proteinase K (20 μg/ml in Tris-EDTA buffer) at 37°C for 10 minutes and slides were blocked with 2% BSA in PBS-0.1% Tween20 for 30 minutes at 22°C before overnight incubation with mouse anti-pimonidazole-APC (1:100) overnight at 4°C (MAb 4.3.11.3) in 1% BSA in PBS-0.1% Tween20. Samples were subsequently washed in 4 changes of TBST buffer for 5 minutes with gentle shaking, and counterstained with 4',6-diamidino-2-phenylindole (DAPI). Whole slides were imaged using Zeiss Axioscanner Z1, and tissue area mean fluorescence intensity was quantified using Zen Blue software (Zeiss).

Tissue histology, histologic scoring of inflammation, and immunofluorescence. Large intestines were fixed in 10% formalin for 24 hours and stored at 4°C in 30% sucrose in PBS. Tissues were paraffin embedded and sectioned at 5 μM, deparaffinization as described above. Hematoxylin and eosin (H&E) staining was performed using standard methods. Immunofluorescence staining for RFP, SCA-1, GLI-1, E-Cadherin, and Ki-67 required antigen retrieval, which was performed in citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH6.0) for 20 minutes at 95°C. After cooling at room temperature, slides were washed in PBS-0.1% Tween20 for 5 minutes, permeabilized with PBS-0.1% TritonX-100 for 10 minutes at 22°C, and blocked with 1% BSA, 22.52 mg/ml glycine in PBS-0.1% Tween20 for 1 hour at 22°C. Primary antibodies were incubated in 1% BSA in PBS-0.1% Tween20 overnight at 4°C, followed by 4 changes of TBST buffer for 5 minutes with gentle shaking, and incubation with secondary antibodies in 1% BSA in PBS-0.1% Tween20 at 22°C for 1 hour. Slides were washed in TBST buffer for 5 minutes with gentle shaking (4 times) before counterstaining with 4',6-diamidino-2-phenylindole (DAPI). TUNEL staining was performed using *In Situ* Cell Death Detection Kit, TMR red (Thermo Fisher Scientific) following manufacturer's instructions. For quantification of goblet cells, colon tissue sections were stained with Alcian blue/PAS and Alcian blue^{+} cells were enumerated per villi. Images were acquired with an Olympus BX41 equipped with a Digital Sight DS-Fi1 camera (Nikon) or whole slides were imaged using Zeiss Axioscanner Z1. Data were analyzed using ImageJ software.

The colitis pathology score was generated by assessing total tissue inflammation and epithelial changes in the colon. The total inflammation score (I-score, scale 0-3) was based on the overall severity or extent of inflammation in colon. An epithelial change score (E-score, scale 0–4) was assigned for each of the following categories: goblet cell loss, intraepithelial

neutrophils and/or cryptitis, abscesses, or crypt loss. The I- and E-scores were summed to generate the colitis pathology score (range of 0-19) for each mouse. All histological scoring was performed by a pathologist, who was blinded to the samples.

Tissue oxygen consumption. For measurement of BAT oxygen consumption, freshly isolated tissue was finely minced and the fragments were resuspended in tissue respiration buffer (PBS containing 20 mg/ml BSA, 25 mM glucose, and 1 mM pyruvate). The rate of oxygen consumption was recorded with a Mitocell S200 respirometry system (Strathkelvin Instruments) at 22° C. Data were analyzed with Strathkelvin 782 Oxygen System software (version 4.1).

Energy expenditure and temperature measurements. CLAMS chambers (Columbus Instruments) housed in a temperature controlled environmental chamber were used to quantify energy expenditure, total locomotor activity, food and water consumption, and respiratory exchange ratio. Oxygen consumption rate (VO_2), CO_2 release rate (VCO_2), food intake, and activity were recorded after an acclimation period of 1-2 days in the CLAMS chamber. For measurement of core temperature and movement by telemetry, E-mitters (Starr Life Sciences Corp.) were implanted into the abdominal cavity of mice under anesthesia. After 2 weeks of recovery, individually housed mice were administered DSS, and temperature and movement were recorded using Vital View every 10 mins by placing cages on a receiving platform (ER-4000 Receiver, Starr Life Sciences Corp.) that were housed at T_a=22°C or T_a=30°C.

Next Generation Sequencing Library Preparation and RNA sequencing analysis. RNA sequencing libraries were prepared using TruSeq Stranded mRNA library prep kit (Illumina) according to manufacturer's instructions. The pooled libraries were sequenced on NextSeq 500 Illumina sequencer using NextSeq 500/550 High Output Kit v2.5 (75 cycles) (Illumina #20024906) in a single read mode.

Quality of raw sequencing data was assessed using FastQC, and bases with a per base sequencing quality of less than 28 were trimmed with Trimmomatic (parameters: LEADING:15 TRAILING:15 MINLEN:35) (8). Trimmed reads were pseudoaligned to mouse transcriptome (mm10, Genome Reference Consortium Mouse Build 38) using Kallisto with 100 bootstraps (9). Differential gene expression was defined as a fold change of ≥1.5, with a false discovery rate of q-value <0.05, and was performed using Sleuth in gene-level mode with likelihood-ratio test as the method for statistical testing (10). Gene ontology and pathway enrichment analysis was performed using MetaScape (11). Principal Components Analysis (PCA) plots and heat maps were generated in R (12).

Brown adipose tissue secreted factors were identified as proteins annotated to 'secreted' (UniProt, Keywords), and identified by Villarroya et. al. via comparative proteomic analysis of cAMP and non-cAMP treated murine brown pre-adipocyte cultures using isobaric tag for relative and absolute quantitation (13).

16s rRNA gene sequencing and data analysis. Mouse fecal DNA was purified using ZymoBIOMICS 96 MagBead DNA kit (Zymo Research) according to the manufacturer's instructions with an additional 10 min incubation at 65˚C after mechanical disruption. The sequencing library was prepared using dual-indices and a two-step amplification protocol as previously described (14) with amplicons pooled at equimolar concentrations using SequalPrep Normalization Plates (Life Technologies). The library was sequenced via Illumina MiSeq using V3 reagents and 270x270 reads with ~10% PhiX with demultiplexed performed via Illumina bcl2fastq software using default parameters.

Subsequent sequencing data was processed using QIIME2 (version 2019.4) (15). Briefly, cutadapt was used to strip primers and DADA2 was used to process reads and build a feature

table containing denoised amplicon sequence variants (ASVs). Taxonomy was assigned using the DADA2 classifier against SILVA database version v128. A *de novo* phylogenetic tree was generated using the qiime phylogeny align-to-tree-mafft-fasttree plugin of QIIME2. Vegan and Phyloseq (16) were used to generate diversity analyses and plotting was performed using ggplot2. Differential abundance analysis was carried out using ALDEx2 (17) with 999 Monte Carlo samples and the Wilcoxon Rank Sum test with FDR<0.1 used as the cutoff for statistical significance.

^{1}H NMR-Based Global Metabolomics. Cecal contents, feces, and serum metabolites were extracted as previously described (18, 19). ^{1}H NMR spectra were recorded at 298 K on a Bruker Avance III 600 MHz spectrometer equipped with an inverse cryogenic probe (Bruker Biospin, Germany). NMR spectra of cecal and fecal samples were acquired using the first increment of NOESY pulse sequence with presaturation (Bruker 1D noesygppr1d pulse sequence). The serum spectra were acquired with a Carr-Purcell-Meiboom-Gill pulse sequence [recycle delay-90°-(τ - 180°- τ)$_n$-acquisition]. Quality of all spectra were improved by phase adjustment, baseline correction, and calibration using Topspin 3.0 (Bruker Biospin, Germany). The spectral region δ 0.50-9.50 was integrated into bins with equal width of 0.004 ppm (2.4 HZ) using AMIX package (V3.8, Bruker Biospin) for relative concentration analysis. The metabolites were assigned based on published results (18, 20, 21).

Statistical Analysis. Statistical analysis was performed using Prism 8 (GraphPad Software, San Diego, CA, USA). Data are presented as mean ± s.e.m. Statistical significance was determined using the unpaired two-tailed Student's t test for single variables and two-way ANOVA followed by Bonferroni post-tests for multiple variables. For survival experiments, statistical significance was determined using the Mantel-Cox log-rank test. A p value of <0.05 was considered to be

statistically significant and is listed. Otherwise, p values are presented as *$p < 0.05$, **$p < 0.01$, or ***$p < 0.001$. Biological replicates for each of the studies are indicated in the figure legends as "n=".

Data availability. The RNA-seq data has been deposited at Gene Expression Omnibus (GEO) under GSE158488. 16S rRNA-seq data has been deposited at Sequence Read Archive (SRA) repository under BioProject PRJNA667805.

Supplementary References:

1. T. Sato *et al.*, Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* **141**, 1762-1772 (2011).

2. K. P. O'Rourke, S. Ackerman, L. E. Dow, S. W. Lowe, Isolation, Culture, and Maintenance of Mouse Intestinal Stem Cells. *Bio Protoc* **6** (2016).

3. M. M. Mahe *et al.*, Establishment of Gastrointestinal Epithelial Organoids. *Curr Protoc Mouse Biol* **3**, 217-240 (2013).

4. H. Miyoshi, T. S. Stappenbeck, In vitro expansion and genetic modification of gastrointestinal stem cells in spheroid culture. *Nat Protoc* **8**, 2471-2482 (2013).

5. G. Sorrentino *et al.*, Bile Acids Signal via TGR5 to Activate Intestinal Stem Cells and Epithelial Regeneration. *Gastroenterology* **159**, 956-968 e958 (2020).

6. T. Grabinger *et al.*, Ex vivo culture of intestinal crypt organoids as a model system for assessing cell death induction in intestinal epithelial cells and enteropathy. *Cell Death Dis* **5**, e1228 (2014).

7. J. R. Bayrer *et al.*, LRH-1 mitigates intestinal inflammatory disease by maintaining epithelial homeostasis and cell survival. *Nat Commun* **9**, 4055 (2018).

8. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120 (2014).

9. N. L. Bray, H. Pimentel, P. Melsted, L. Pachter, Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol* **34**, 525-527 (2016).

10. H. Pimentel, N. L. Bray, S. Puente, P. Melsted, L. Pachter, Differential analysis of RNA-seq incorporating quantification uncertainty. *Nat Methods* **14**, 687-690 (2017).

11. S. Tripathi *et al.*, Meta- and Orthogonal Integration of Influenza "OMICs" Data Defines a Role for UBR4 in Virus Budding. *Cell Host Microbe* **18**, 723-735 (2015).

12. R. D. C. Team (2008) R: A language and environment for statistical computing. . (R Foundation for Statistical Computing, Vienna, Austria).

13. J. Villarroya, R. Cereijo, M. Giralt, F. Villarroya, Secretory Proteome of Brown Adipocytes in Response to cAMP-Mediated Thermogenic Activation. *Front Physiol* **10**, 67 (2019).

14. D. M. Gohl *et al.*, Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. *Nat Biotechnol* **34**, 942-949 (2016).

15. E. Bolyen *et al.*, Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**, 852-857 (2019).

16. P. J. McMurdie, S. Holmes, phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**, e61217 (2013).

17. A. D. Fernandes, J. M. Macklaim, T. G. Linn, G. Reid, G. B. Gloor, ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. *PLoS One* **8**, e67019 (2013).

18. J. Cai *et al.*, Antioxidant Drug Tempol Promotes Functional Metabolic Changes in the Gut Microbiota. *J Proteome Res* **15**, 563-571 (2016).

19. X. Shi, C. Xiao, Y. Wang, H. Tang, Gallic acid intake induces alterations to systems metabolism in rats. *J Proteome Res* **12**, 991-1006 (2013).

20. F. Dong, L. Zhang, F. Hao, H. Tang, Y. Wang, Systemic responses of mice to dextran sulfate sodium-induced acute ulcerative colitis using 1H NMR spectroscopy. *J Proteome Res* **12**, 2958-2966 (2013).

21. Y. Tian, L. Zhang, Y. Wang, H. Tang, Age-related topographical metabolic signatures for the rat gastrointestinal contents. *J Proteome Res* **11**, 1397-1411 (2012).

Supplemental Figure Titles and Legends

Fig. S1. Characterization of tissue tolerance during experimental colitis.

(A-M) To induce colitis, mice were administered 4% dextran sodium sulfate (DSS) in drinking water for 7 days. (A-C) Colon length of male (A), colon length of female (B), and colon shortening of female (C) C57BL/6J mice housed at T_a=22°C or T_a=30°C during DSS-induced colitis (n=7-25 per timepoint and temperature, data pooled from 4 independent experiments and analyzed by Student's *t*-test). (D, E) Histological analysis of distal colon on day 7 after administration of DSS to C57BL/6J female mice housed at T_a=22°C or T_a=30°C. (D) Representative pictures of H&E stained sections of colon; scale bar = 100 μM. (E) Colitis pathology score in C57BL/6J female mice administered DSS (n=6-13 per timepoint and temperature, data pooled from 4 independent experiments and analyzed by Student's *t*-test). (F) Plasma levels of lipocalin 2, a tissue damage marker, in C57BL/6J female mice housed at T_a=22°C or T_a=30°C and administered DSS (n=4-9 per timepoint and temperature, data pooled from 3 independent experiments and analyzed by Student's *t*-test). (G) Reaction norm plots of host fitness (colon shortening) and tissue damage (Lipocalin 2) during DSS-induced colitis in C57BL/6J female mice housed at T_a=22°C or T_a=30°C (n=30-31 per temperature, data pooled from 4 independent experiments and analyzed by Student's *t*-test). (H, I) Bacterial colony forming units (CFUs) in spleen, liver, and epididymal white adipose tissue (eWAT) in male (H), and spleen, liver, and gonadal white adipose tissue (gWAT) in female (I) C57BL/6J mice 7 days after administration of DSS (males n=14-25 and females n=9-21 per tissue and temperature, data pooled from 3 independent experiments and analyzed by Student's *t*-test). (J, K) Percent change in fat mass, as compared to water fed controls, on day 7 after administration of DSS. Inguinal WAT (iWAT) and gWAT were quantified for females (J), and eWAT and iWAT were quantified

for males (K) (females n=13-21 and males n=25 per timepoint and temperature, data pooled from 3 independent experiments and analyzed by Student's *t*-test). (L, M) Spleen mass of male (L) and female (M) C57BL/6J mice administered DSS at T_a=22°C or T_a=30°C (males n=7-13 and females n=5-10 per timepoint and temperature, data pooled from 3 independent experiments and analyzed by Student's *t*-test). Data are presented as mean ± SEM.

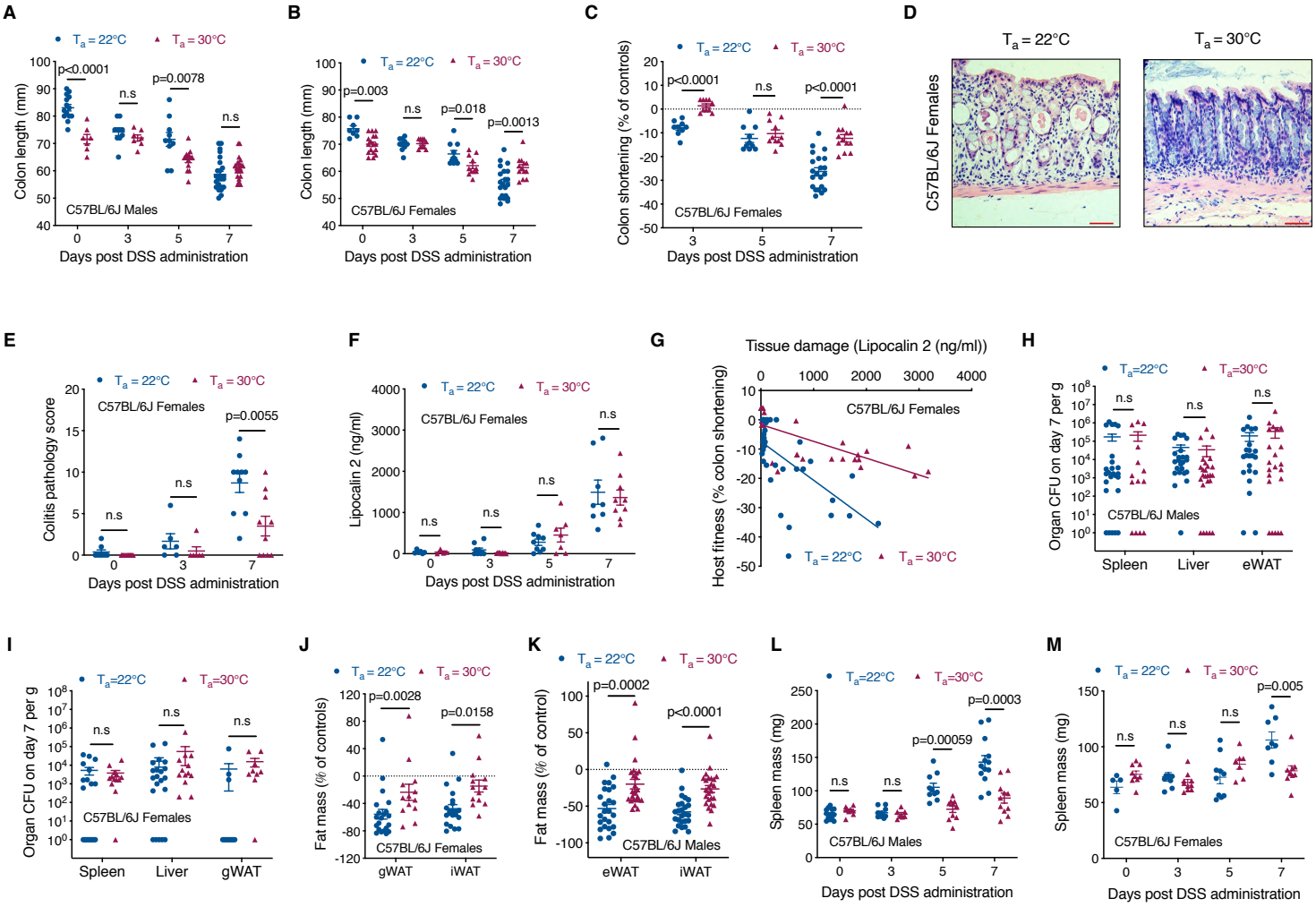


Fig. S2. Quantification of tissue damage and acute inflammatory responses during colitis. (A, B) Plasma alanine transaminase activity (U/ml) in male (A) and (B) female C57BL/6J mice administered DSS at T_a=22°C or T_a=30°C. (C-F) Plasma concentrations of blood urea nitrogen (C, D) and serum amyloid A1 (E, F) in male (C, E) and female (D, F) C57BL/6J mice administered DSS at T_a=22°C or T_a=30°C (males n=5-12 and females n=3-11 per timepoint and temperature, data pooled from 2 independent experiments and analyzed by Student's *t*-test). (G-J) Plasma concentrations of IL-6 (G), IL-10 (H), MCP-1/CCL2 (I), and IL-12p70 (J), IFNγ (K), and TNF (L) in male C57BL/6J mice administered DSS at T_a=22°C or T_a=30°C (n=6-8 per timepoint and temperature, data pooled from 2 independent experiments and analyzed by Student's *t*-test). Data are presented as mean ± SEM.

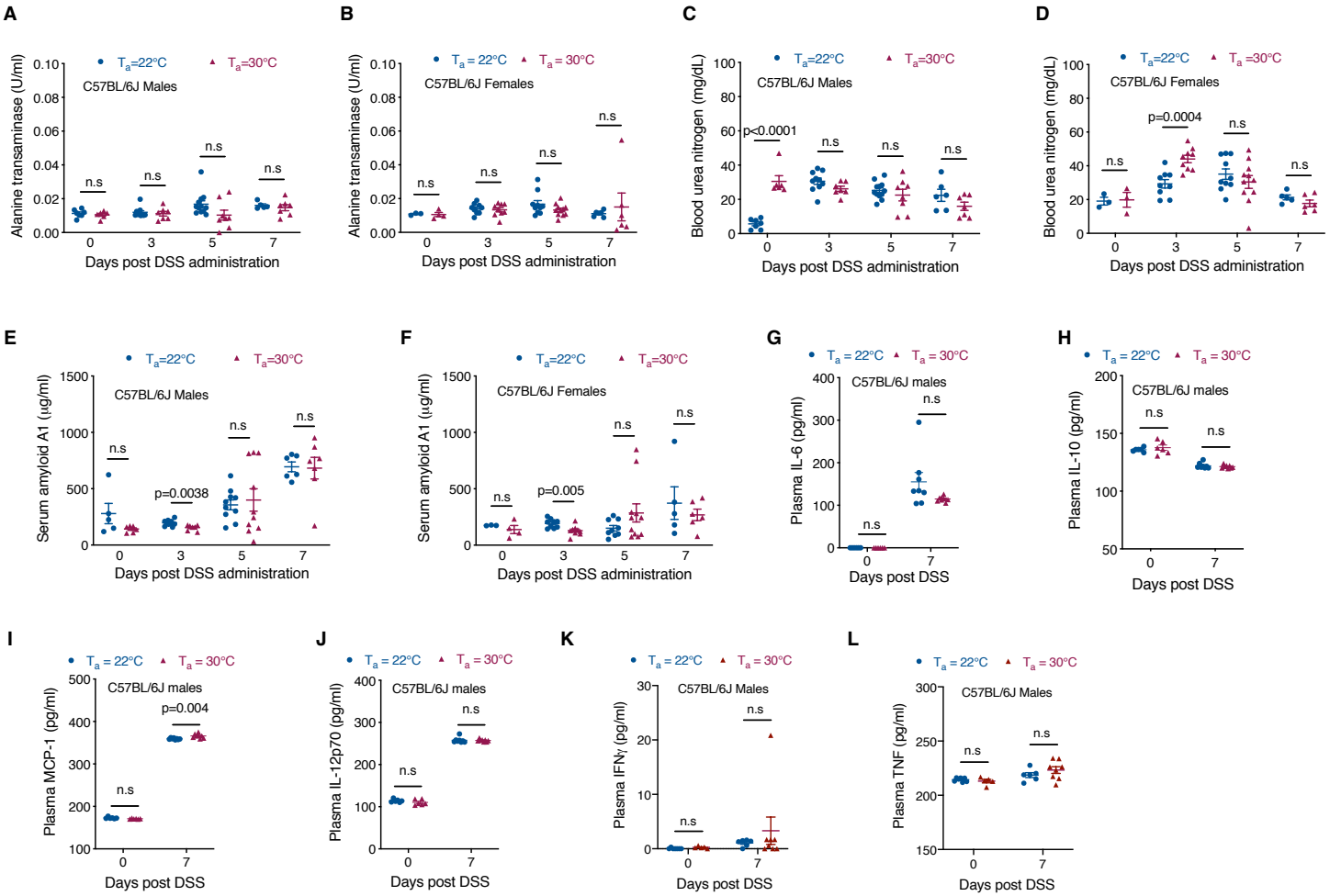


Fig. S3. Metabolic parameters of mice during DSS-induced colitis.

(A, B) Quantification of water intake by CLAMS over a 24-hour period in male (A) and female (B) C57BL/6J mice housed at T_a=22°C or T_a=30°C (A, males n=6-9 and B, females n=12-13 per temperature, data pooled from 2-3 independent experiments and analyzed by Student's *t*-test). (C-F) Radio-telemetry measurement of core temperature (C, D) and total activity counts (E, F) of female C57BL/6J mice administered water or DSS for 7 days at T_a=22°C or T_a=30°C (n=6 per temperature and condition, data representative 2 independent experiments). Data are presented as mean ± SEM.

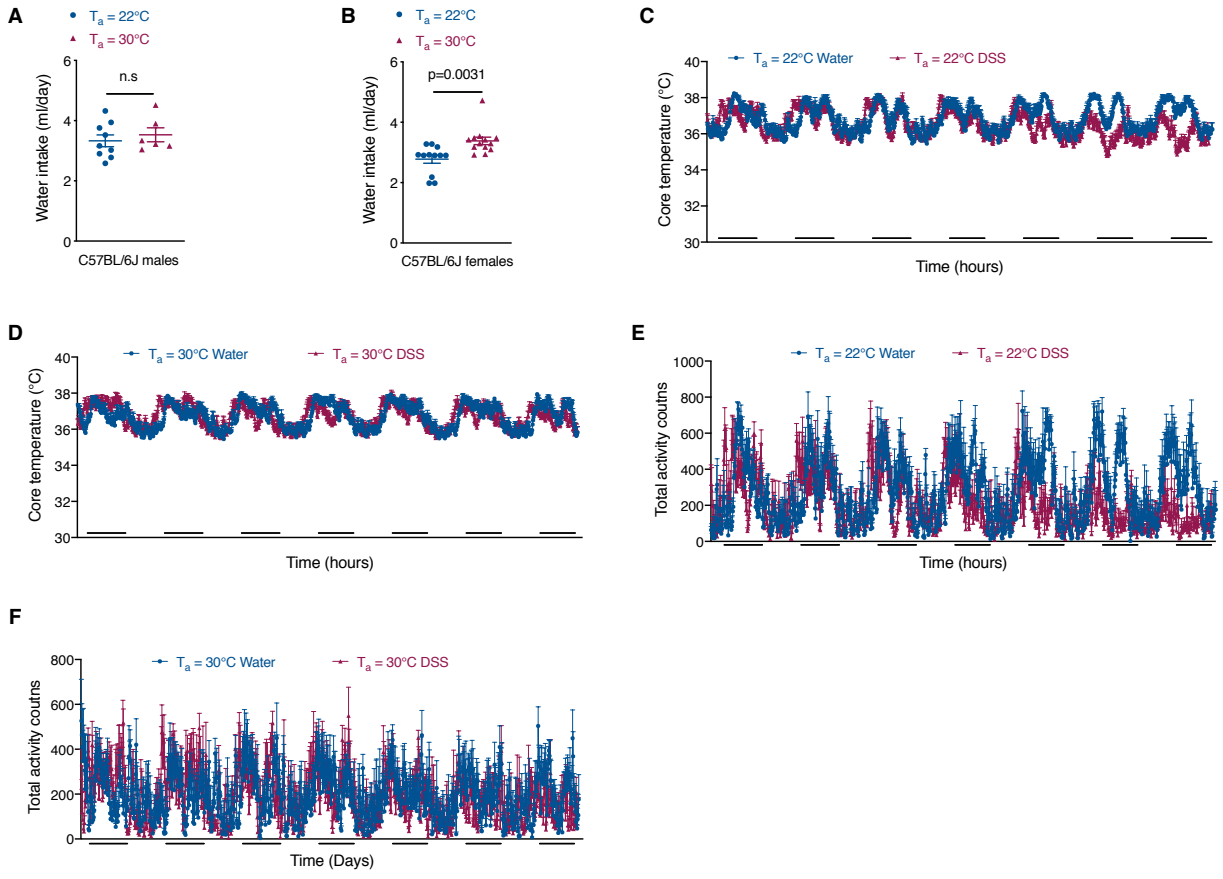


Fig. S4. Characterization of intestinal tissue tolerance in thermoneutral mice.

(A-D) To monitor tissue regeneration, mice were administered 4% DSS in drinking water for 5 days followed by recovery over the next 5 days. (A) Body mass of male C57BL/6J mice housed at T_a=22°C or T_a=30°C during DSS-induced colitis and recovery. (B) Colon shortening in male C57BL/6J mice on day 10. (C) Representative pictures of H&E stained sections of colon on day 10; scale bar = 100 µM. (D) Reaction norm plots of host fitness (weight loss) and tissue damage (Lipocalin 2) during DSS-induced colitis and recovery in male C57BL/6J mice housed at T_a=22°C or T_a=30°C (A-D, n=10-13 per temperature, data pooled from 2 independent experiments and analyzed by Student's *t*-test). (E) Bacterial colony forming units (CFU) in large intestine on day 10 after infection with *C. rodentium* (1x10^6 CFU) in female C3H/HeJ mice housed at T_a=22°C or T_a=30°C (n=5-6 per temperature, data representative of 2 independent experiments and analyzed by Student's *t*-test). Data are presented as mean ± SEM.

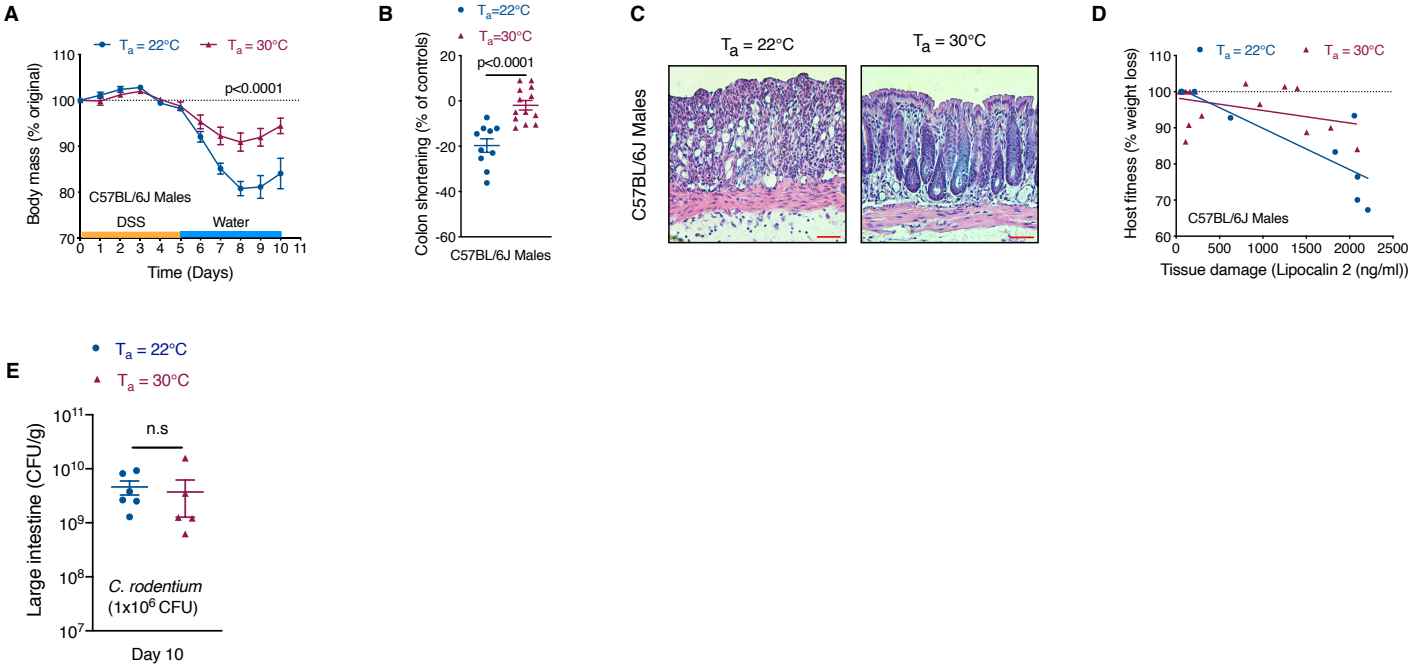


Fig. S5. Flow cytometric analysis of large intestine laminar propria cells during colitis. (A-F) Gating strategies used to identify immune cells in the large intestine lamina propria of female C57BL/6J mice that were untreated or treated with 4% DSS for 5 days. (A) Gating for myeloid cells, including patrolling ($CD115^{+}Ly6^{low}$) and inflammatory ($CD115^{+}Ly6^{hi}$) monocytes, and alternatively activated macrophages ($CD68^{+}CD206^{+}CD301^{+}$). (B) Gating for dendritic cells ($CD11c^{+}MHCII^{high}$), neutrophils ($Ly6G^{+}$), and eosinophils ($SiglecF^{+}$). (C) Gating for lineage negative innate lymphoid cell populations: ILC1 ($ROR\gamma t^{-}GATA3^{-}NKp46^{+}$), ILC2 ($GATA3^{+}CD90.2^{+}$), and ILC3 ($ROR\gamma t^{+}GATA3^{-}$). (D-F) Gating for adaptive immune cells including natural killer ($NK1.1^{+}CD3\varepsilon^{-}$), natural killer T cells ($NK1.1^{+}CD3\varepsilon^{+}$), $CD4^{+}$, and $CD8^{+}$ T cells ($B220^{-}CD3\varepsilon^{+}$), B cells ($B220^{+}CD3\varepsilon^{-}$), and IFNγ producing cells (D); type-2 cytokine producing cells and conventional $CD4^{+}$ T cells ($CD3\varepsilon^{+}$ $CD4^{+}$) (E); and regulatory T cells ($CD4^{+}FoxP3^{+}$) and invariant T cells ($CD4^{-}\gamma\delta TCR^{+}$) (F). Data pooled from 3 independent experiments with n=7-14 per condition and analyzed using Student's *t*-test.

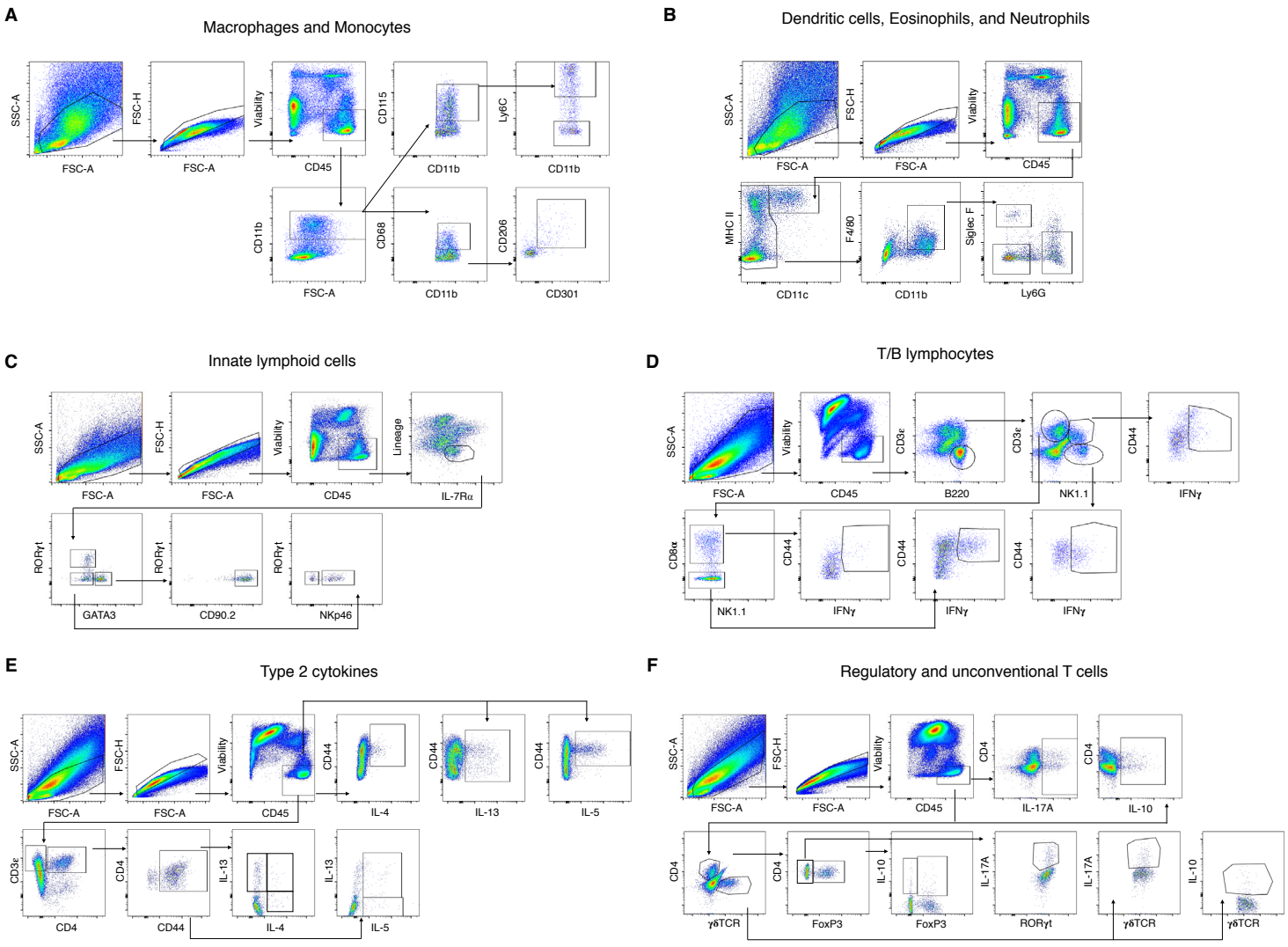

A
Macrophages and Monocytes
SSC-A
FSC-A
FSC-H
FSC-A
Viability
CD45
CD115
CD11b
Ly6C
CD11b
CD11b
FSC-A
CD68
CD11b
CD206
CD301
B
Dendritic cells, Eosinophils, and Neutrophils
SSC-A
FSC-A
FSC-H
FSC-A
Viability
CD45
MHC II
CD11c
F4/80
CD11b
Siglec F
Ly6G
C
Innate lymphoid cells
SSC-A
FSC-A
FSC-H
FSC-A
Viability
CD45
Lineage
IL-7Rα
RORγt
GATA3
RORγt
CD90.2
RORγt
NKp46
D
T/B lymphocytes
SSC-A
FSC-A
Viability
CD45
CD3ε
B220
CD3ε
NK1.1
CD44
IFNγ
CD8α
NK1.1
CD44
IFNγ
CD44
IFNγ
CD44
IFNγ
E
Type 2 cytokines
SSC-A
FSC-A
FSC-H
FSC-A
Viability
CD45
CD44
IL-4
CD44
IL-13
CD44
IL-5
CD3ε
CD4
CD4
CD44
IL-13
IL-4
IL-13
IL-5
F
Regulatory and unconventional T cells
SSC-A
FSC-A
FSC-H
FSC-A
Viability
CD45
CD4
IL-17A
CD4
IL-10
CD4
γδTCR
CD4
FoxP3
IL-10
FoxP3
IL-17A
RORγt
IL-17A
γδTCR
IL-10
γδTCR

Fig. S6. Quantitation of inflammatory and tissue repair cytokines in colon tissues from thermally stressed and thermoneutral mice.

(A-H) Concentration of IL-6 (A), MCP-1/CCL2 (B), IFNγ (C), TNF (D), IL-12p70 (E), IL-10 (F), Amphiregulin (G), and IL-22 (H) in colon tissues of C57BL/6J male mice housed at T_a=22°C or T_a=30°C and treated with DSS (n=3-9 per timepoint and temperature, data pooled from 2 independent experiments and analyzed using Student's *t*-test). (I, J) Representative images of colon sections stained with PAS-Alcian blue for mucus producing goblet cells (I) and quantification of Alcian blue$^+$ cells in the villi (J) at various timepoints after administration of DSS to mice housed at T_a=22°C or T_a=30°C (n=104-189 villi quantified per condition, data pooled from 2 independent experiments and analyzed using Student's *t*-test). Data are presented as mean ± SEM.

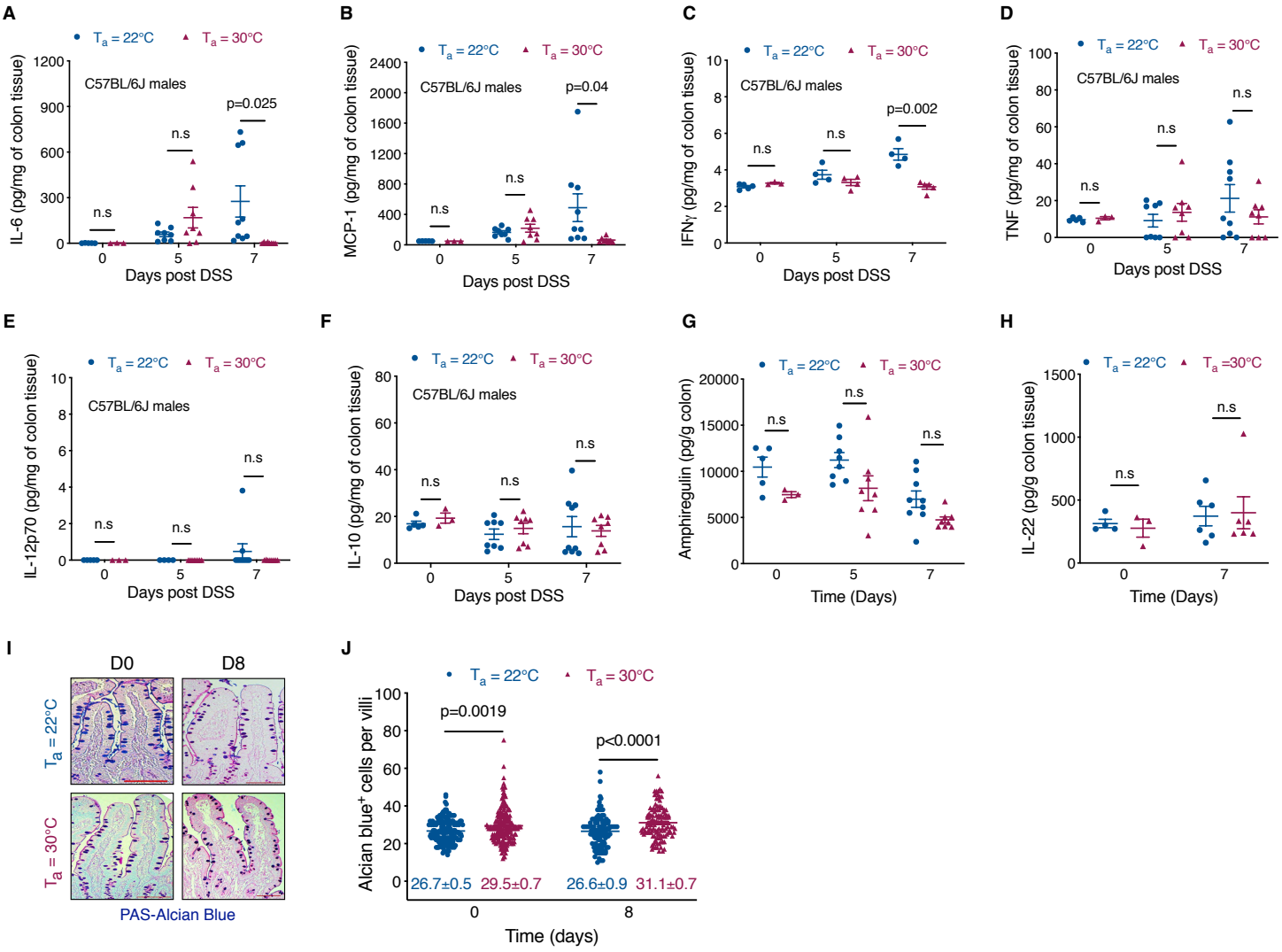

A
B
C
D
E
F
G
H
I
J
T_a = 22°C
T_a = 30°C
C57BL/6J males
IL-6 (pg/mg of colon tissue)
MCP-1 (pg/mg of colon tissue)
IFNγ (pg/mg of colon tissue)
TNF (pg/mg of colon tissue)
IL-12p70 (pg/mg of colon tissue)
IL-10 (pg/mg of colon tissue)
Amphiregulin (pg/g colon)
IL-22 (pg/g colon tissue)
Days post DSS
Time (Days)
n.s
p=0.025
p=0.04
p=0.002
D0
D8
PAS-Alcian Blue
Alcian blue+ cells per villi
p=0.0019
p<0.0001
26.7±0.5
29.5±0.7
26.6±0.9
31.1±0.7
Time (days)

Fig. S7. Fecal composition and colonocyte gene expression in germ-free mice colonized with microbiota of mice housed at T_a=22°C or T_a=30°C.

(A) Phylum-level relative abundance of fecal microbiota in germ-free recipients compared to input at day 14 post colonization. (B) Beta diversity was measured by Bray Curtis dissimilarity metric (analyzed by ADONIS, R^2=0.51677, p-value =0.0002 comparing temperature). (C) Alpha diversity was measured by the Shannon index, expressed as percentage of input (data analyzed by unpaired Student's *t*-test). (D) Colon shortening of germ-free C57BL/6J mice colonized with T_a=22°C or T_a=30°C microbiota during DSS-induced colitis (n=6-8 per condition, data representative of independent experiments and analyzed by Student's *t*-test). (E) Heatmap of differentially expressed colonization and temperature-dependent genes at baseline and after colitis (n=3 per condition, fold change ≥ 1.5, adjusted P value < 0.05). Data are presented as mean ± SEM.

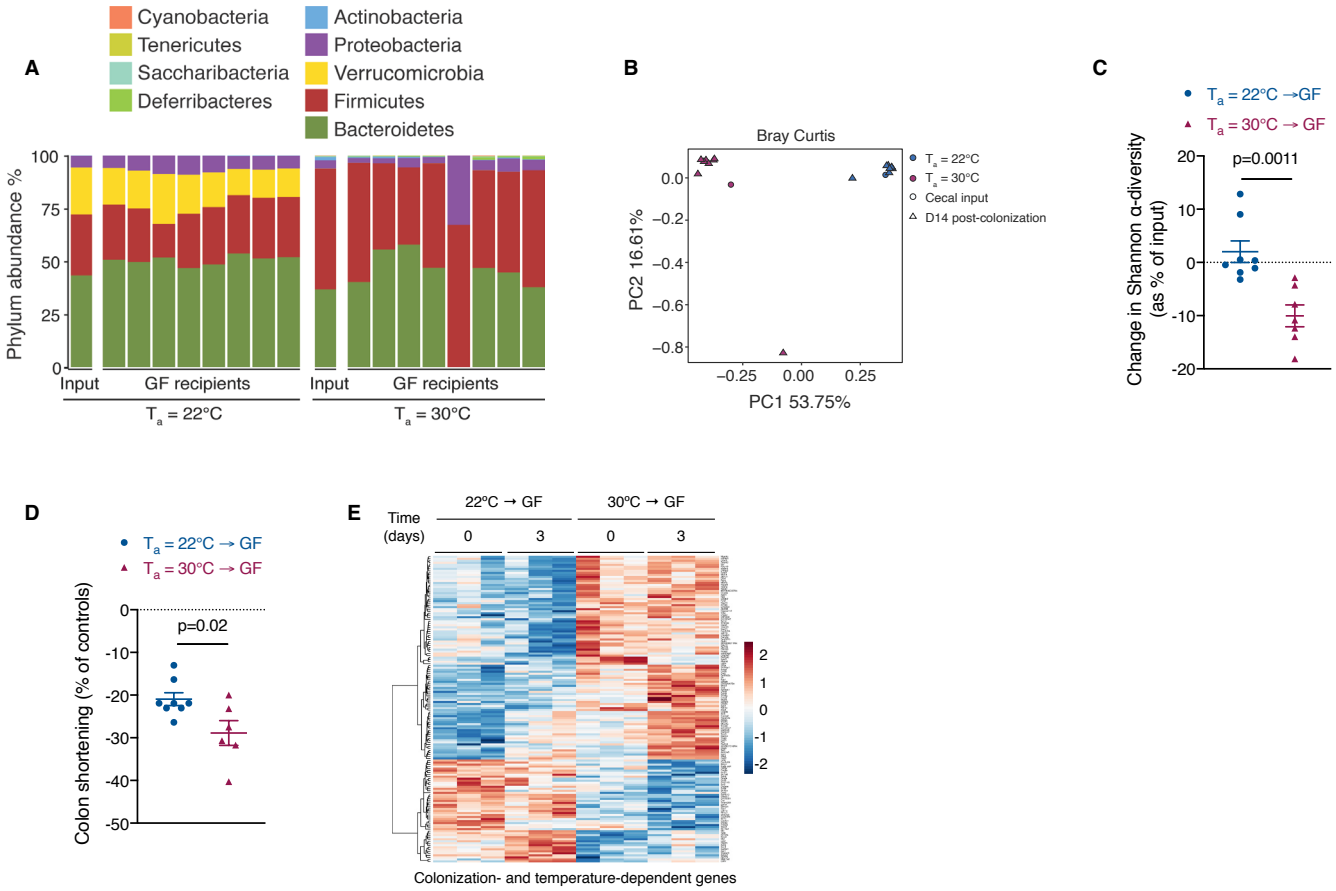

A
Cyanobacteria
Tenericutes
Saccharibacteria
Deferribacteres
Actinobacteria
Proteobacteria
Verrucomicrobia
Firmicutes
Bacteroidetes
Phylum abundance %
100
75
50
25
0
Input
GF recipients
Input
GF recipients
Ta = 22°C
Ta = 30°C
B
Bray Curtis
Ta = 22°C
Ta = 30°C
Cecal input
D14 post-colonization
PC2 16.61%
0.0
−0.2
−0.4
−0.6
−0.8
−0.25
0.00
0.25
PC1 53.75%
C
Ta = 22°C →GF
Ta = 30°C → GF
p=0.0011
Change in Shannon α-diversity
(as % of input)
20
10
0
-10
-20
D
Ta = 22°C → GF
Ta = 30°C → GF
p=0.02
Colon shortening (% of controls)
0
-10
-20
-30
-40
-50
E
Time
(days)
22°C → GF
30°C → GF
0
3
0
3
2
1
0
-1
-2
Colonization- and temperature-dependent genes

Fig. S8. Intestinal stem cell renewal capacity does not contribute to enhanced tissue tolerance of thermoneutral mice.

(A, B) Immunofluorescence for Lgr5-RFP$^+$ cells in the uninjured colon. *Lgr5*$^{\text{GFP-ires-creERT2/+}}$*Rosa26*$^{\text{RFP/+}}$ mice housed at T_a=22°C or T_a=30°C were injected with tamoxifen on D0, and Lgr5-RFP$^+$ cells in crypt and villus were quantified on D1 and D4. Representative images (A) and quantification of LGR5-RFP$^+$ cells per crypt and villus unit (B, n=48-211 crypt and villus units quantified per condition, data pooled from 2 independent experiments and analyzed using Student's *t*-test). (C) Representative immunofluorescence images of Sca-1$^+$ and E-cadherin$^+$ epithelial cells at various timepoints after administration of DSS to mice housed at T_a=22°C or T_a=30°C. (D) Quantification of SCA-1 MFI. (E, F) Representative immunofluorescence images of GLI-1$^+$ cells (E) and quantification of GLI-1$^+$ cells at various timepoints after administration of DSS to mice housed at T_a=22°C or T_a=30°C (F, n=7-14 per timepoint and temperature, data pooled from 2 independent experiments and analyzed using Student's *t*-test). (G, H) Representative immunofluorescence images of Ki-67$^+$ cells (E) and quantification of Ki-67$^+$ cells at various timepoints after administration of DSS to mice housed at T_a=22°C or T_a=30°C (n=7-12 per timepoint and temperature, data pooled from 2 independent experiments and analyzed by Student's *t*-test). (I) Representative immunofluorescence images of TUNEL$^+$ cells at various timepoints after administration of DSS to mice housed at T_a=22°C or T_a=30°C. Data are presented as mean ± SEM.

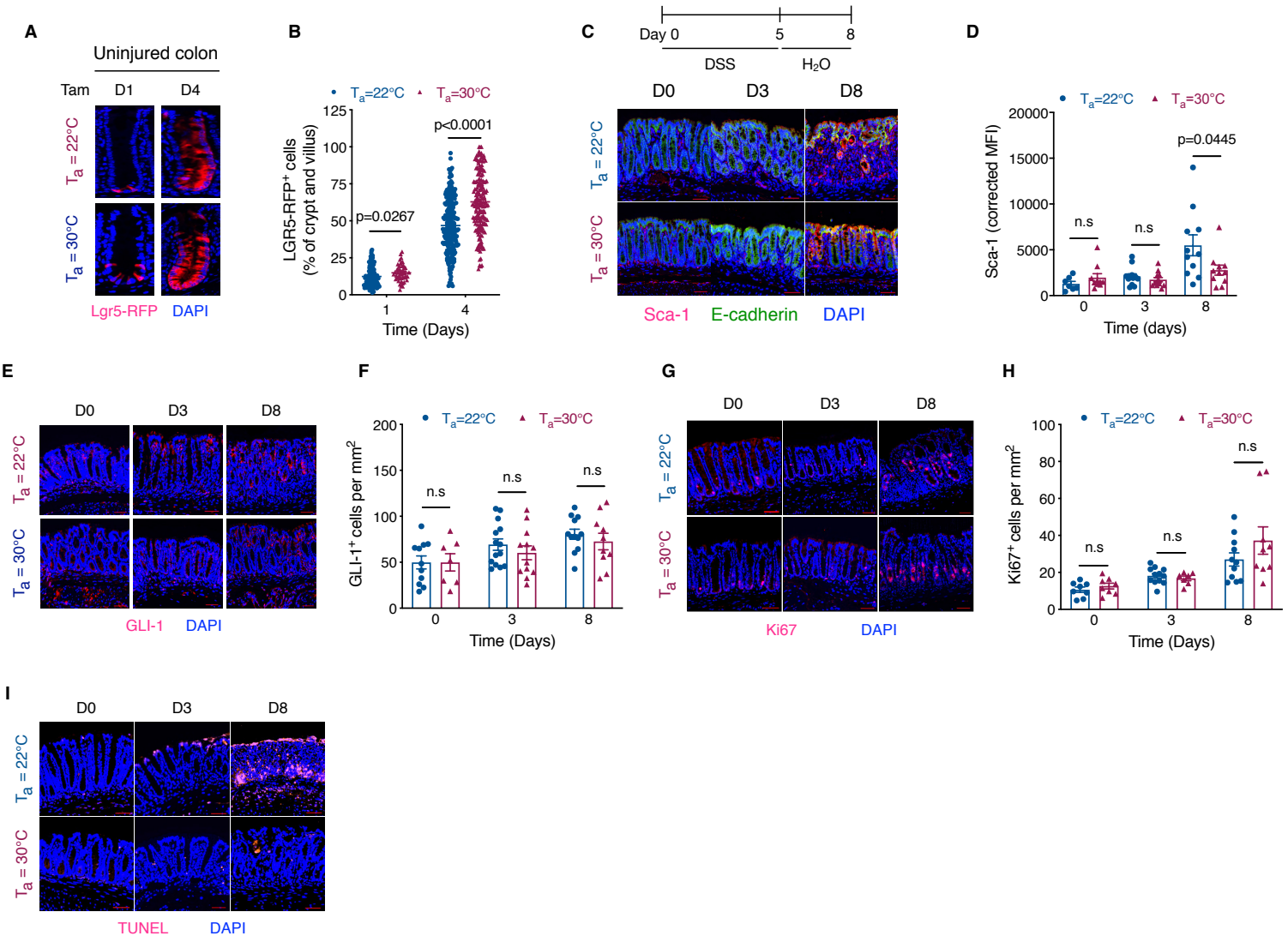

A
Uninjured colon
Tam
D1
D4
Ta = 22°C
Ta = 30°C
Lgr5-RFP
DAPI
B
Ta=22°C
Ta=30°C
LGR5-RFP+ cells (% of crypt and villus)
p=0.0267
p<0.0001
Time (Days)
C
Day 0
5
8
DSS
H2O
D0
D3
D8
Sca-1
E-cadherin
DAPI
D
Sca-1 (corrected MFI)
p=0.0445
n.s
Time (days)
E
GLI-1
DAPI
F
GLI-1+ cells per mm2
Time (Days)
G
Ki67
DAPI
H
Ki67+ cells per mm2
Time (Days)
I
TUNEL
DAPI

Fig. S9. Characterization of colonocytes, crypts, and colon tumorigenesis.

(A) Flow cytometric analysis of colonic epithelial cells (EpCAM1^{+}CD45^{-}Sca1$^{+/-}$), which were isolated for bulk RNA-seq. (B-D) Normalized transcript expression (transcripts per million, TPM) of crypt enriched genes (*Lgr5*) (B), and epithelial enriched genes (*Muc2*, *Krt8*) from RNA-seq data (C-D). (E, F) Heatmap of genes in the "OXPHOS" (E) and "Mitotic cell cycle" (F) GO category in colonocytes of C57BL/6J male mice administered DSS at T_a=22°C and T_a=30°C. (G, H) Tumor burden in C57BL/6J female mice were treated with AOM-DSS. Tumor polyp number (G) and tumor volume (H) at day 90 (n=6 per temperature, data pooled from 2 independent experiments and analyzed using Student's *t*-test). Data are presented as mean ± SEM.

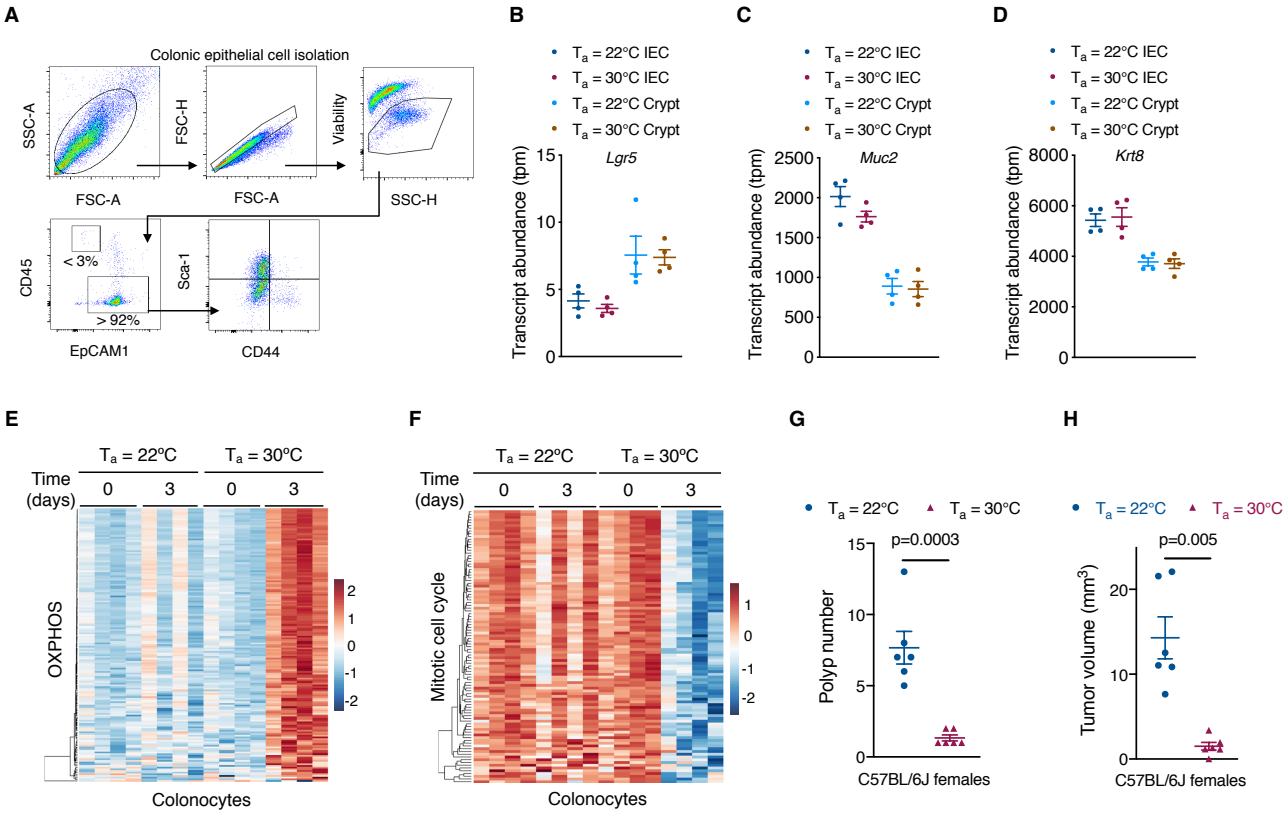


Fig. S10. β2-adrenergic signaling does not regulate intestinal tissue tolerance.

(A) Expression (transcript per million reads, TPM) of adrenergic β receptors in colonic epithelial cells of C57BL/6J male mice administered DSS at T_a=22°C and T_a=30°C by RNA-sequencing. (B-F) Thermoneutral C57BL6/J male mice were treated with β2-adrenoceptor agonist bambuterol prior to oral administration of DSS for 7 days. Colon length (B), BAT mass (C), iWAT mass (D), and eWAT mass (E) of treated mice (n=6-8 per group**,** data pooled from 2 independent experiments and analyzed using Student's *t*-test). (F) Representative pictures of H&E stained sections of BAT and colon of treated mice. (G-K) Thermoneutral C57BL6/J female mice were treated with β2-adrenoceptor agonist bambuterol prior to oral administration of DSS for 7 days. Body mass (G), colon length (H), iWAT mass (I), BAT mass (J), and gWAT mass (K) of treated mice (n=5-9 per group**,** data pooled from 2 independent experiments and analyzed using Student's *t*-test). (L-N) C57BL6/J male mice were treated with β2-adrenoceptor agonist bambuterol prior to oral administration of DSS for 7 days at T_a=22°C. Body mass (L), colon length (M), and BAT mass (N) of treated mice (n=4-6 per condition, representative of 2 independent experiments, data analyzed by Student's *t*-test). Data are presented as mean ± SEM.

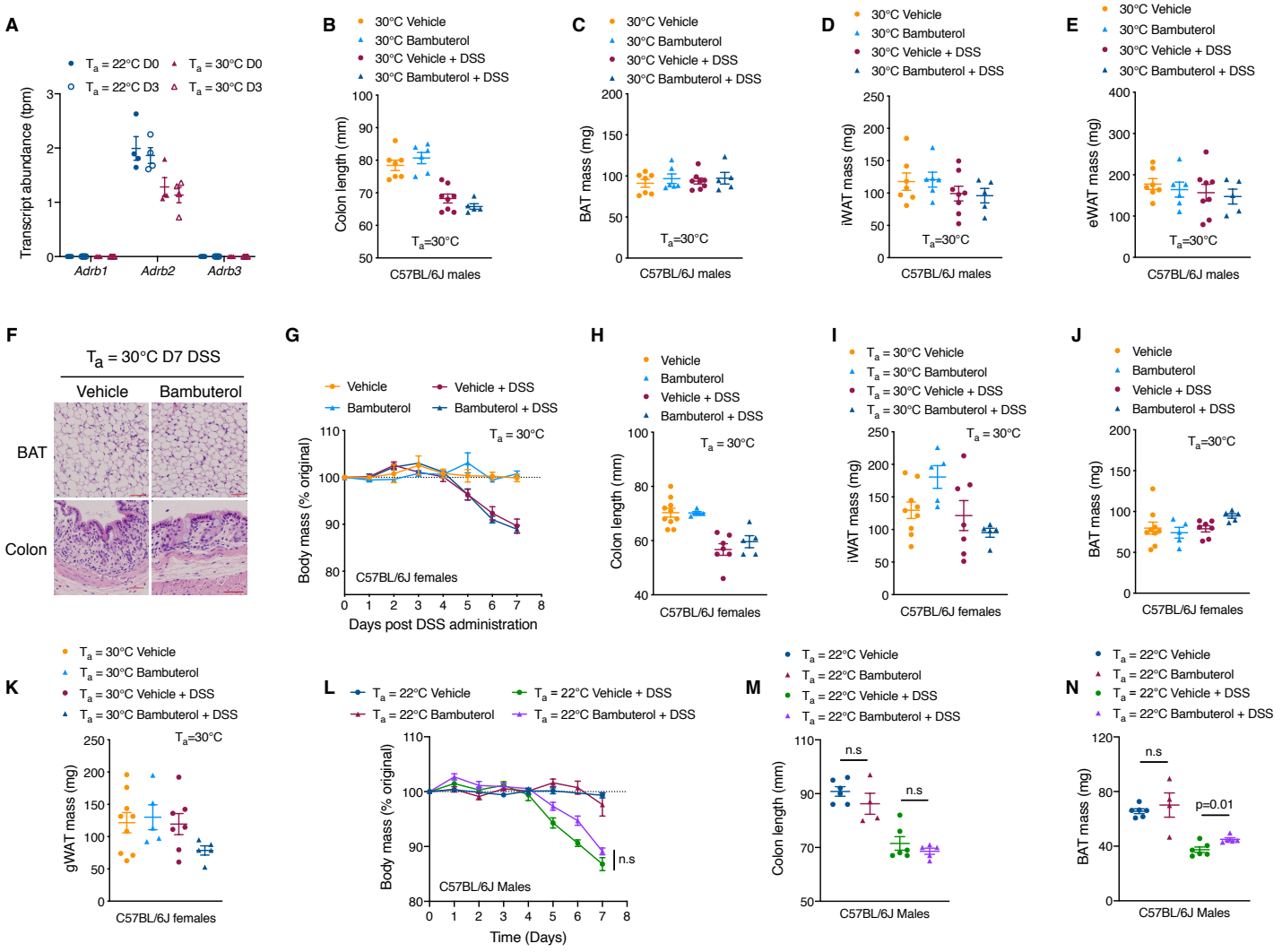

A
Ta = 22°C D0
Ta = 30°C D0
Ta = 22°C D3
Ta = 30°C D3
Transcript abundance (tpm)
Adrb1
Adrb2
Adrb3
B
30°C Vehicle
30°C Bambuterol
30°C Vehicle + DSS
30°C Bambuterol + DSS
Colon length (mm)
Ta=30°C
C57BL/6J males
C
BAT mass (mg)
D
iWAT mass (mg)
E
eWAT mass (mg)
F
Ta = 30°C D7 DSS
Vehicle
Bambuterol
BAT
Colon
G
Vehicle
Bambuterol
Vehicle + DSS
Bambuterol + DSS
Ta = 30°C
Body mass (% original)
C57BL/6J females
Days post DSS administration
H
Ta = 30°C Vehicle
Ta = 30°C Bambuterol
Ta = 30°C Vehicle + DSS
Ta = 30°C Bambuterol + DSS
I
J
K
gWAT mass (mg)
L
Ta = 22°C Vehicle
Ta = 22°C Bambuterol
Ta = 22°C Vehicle + DSS
Ta = 22°C Bambuterol + DSS
C57BL/6J Males
Time (Days)
n.s
M
N
p=0.01

Fig. S11. β2-adrenoceptor antagonists do not affect intestinal tissue tolerance.

(A-C) C57BL6/J mice housed at T_a=22°C were treated with β2-adrenoceptor antagonist butoxamine prior to oral administration of DSS for 7 days. Colon length of treated male mice (A), body mass (B) and colon length (C) of treated female mice (n=4-5 per group**,** data representative of 2 independent experiments and analyzed using Student's *t*-test). (D-F) C57BL6/J mice housed at T_a=30°C were treated with β2-adrenoceptor antagonist butoxamine prior to oral administration of DSS for 7 days. Body mass (D), colon length (E) and BAT mass (F) of treated mice (n=3-5 per condition, representative of 2 independent experiments, data analyzed by Student's *t*-test). Data are presented as mean ± SEM.

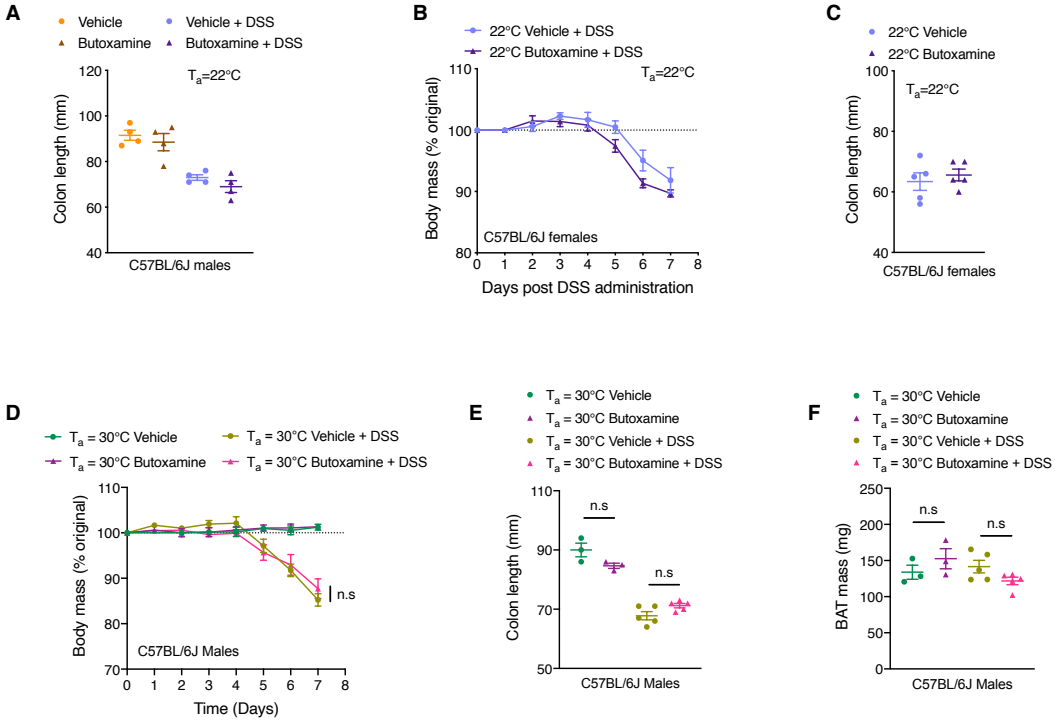


Fig. S12. β3-adrenergic signaling regulates intestinal tissue tolerance.

(A) Thermoneutral C57BL/6J female mice were treated with β3-adrenoceptor agonist CL 316,243 or vehicle and respiratory exchange ratio was measured in CLAMS metabolic chambers (n=4-5 per treatment). (B-G) Thermoneutral C57BL/6J male and female mice were treated with β3-adrenoceptor agonist CL 316,243 or vehicle prior to oral administration of DSS. BAT mass (B) and colon length (C) in DSS treated male mice. Body mass (D), colon length (E), colon shortening (F), and BAT mass (H) in treated female mice (n=6-8 per condition, data pooled from 2 independent experiments and analyzed by Student's *t*-test). (H, I) To monitor tissue regeneration, thermoneutral C57BL6/J female mice were treated with CL 316,243 or vehicle and administered 4% DSS in drinking water for 5 days followed by recovery over the next 5 days. Body mass (H) and colon shortening (I) in treated mice (n=4 per condition, data representative of 2 independent experiments and analyzed by Student's *t*-test). (J-L) C57BL6/J male mice housed at T_a=22°C were treated with β3-adrenoceptor antagonist SR59230A or vehicle prior to oral administration of DSS for 7 days. Body mass (J), colon length (K), and colon shortening (L) of treated mice (n=3-9 per treatment group, data pooled from 2 independent experiments, data analysed by Student's *t*-test). Data are presented as mean ± SEM.

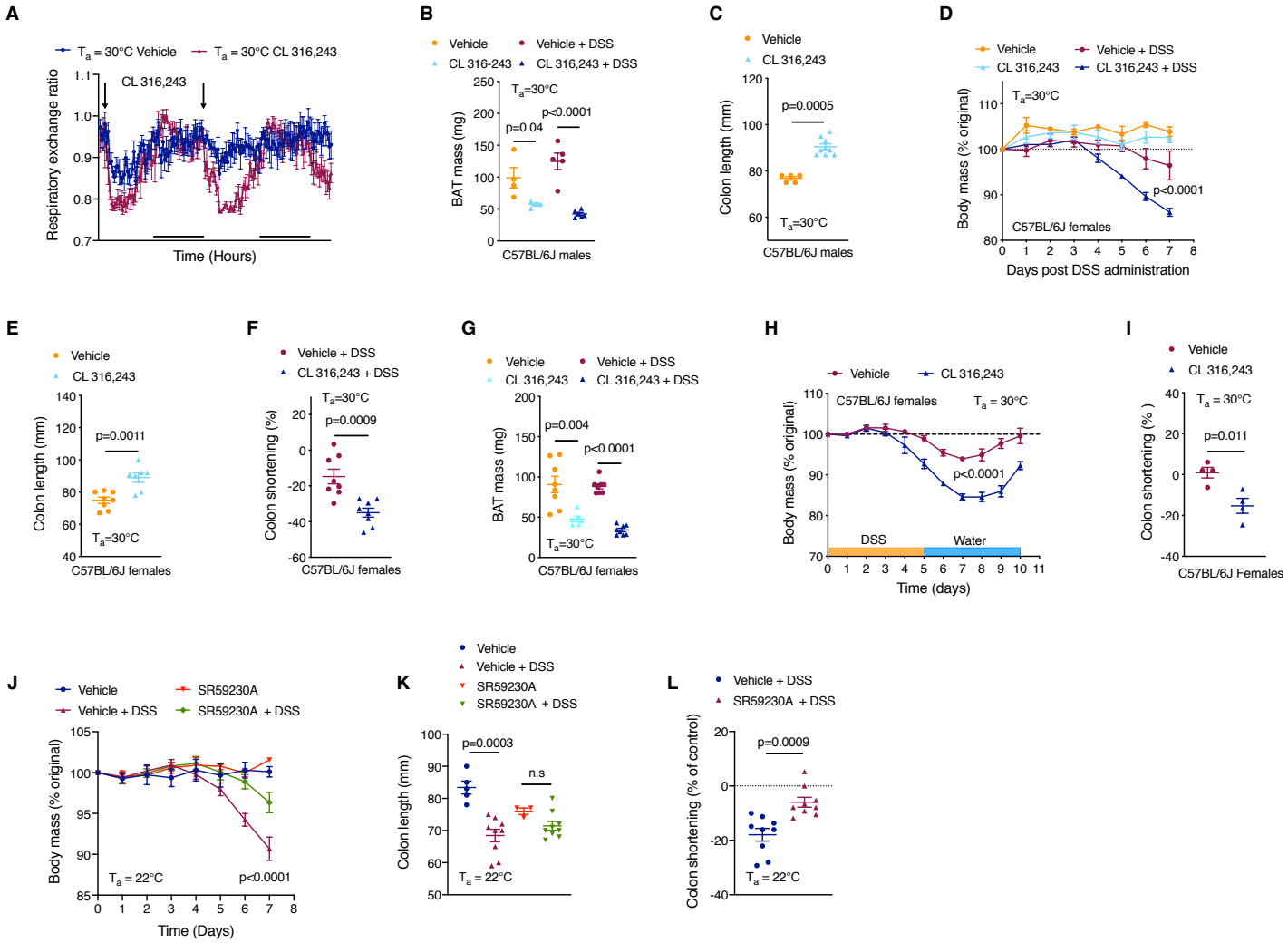

A
Ta = 30°C Vehicle
Ta = 30°C CL 316,243
CL 316,243
Respiratory exchange ratio
Time (Hours)
B
Vehicle
Vehicle + DSS
CL 316-243
CL 316,243 + DSS
Ta=30°C
p=0.04
p<0.0001
BAT mass (mg)
C57BL/6J males
C
Vehicle
CL 316,243
p=0.0005
Colon length (mm)
Ta=30°C
C57BL/6J males
D
Vehicle
Vehicle + DSS
CL 316,243
CL 316,243 + DSS
Ta=30°C
p<0.0001
Body mass (% original)
C57BL/6J females
Days post DSS administration
E
Vehicle
CL 316,243
p=0.0011
Colon length (mm)
Ta=30°C
C57BL/6J females
F
Vehicle + DSS
CL 316,243 + DSS
Ta=30°C
p=0.0009
Colon shortening (%)
C57BL/6J females
G
Vehicle
Vehicle + DSS
CL 316,243
CL 316,243 + DSS
p=0.004
p<0.0001
BAT mass (mg)
Ta=30°C
C57BL/6J females
H
Vehicle
CL 316,243
C57BL/6J females
Ta = 30°C
p<0.0001
Body mass (% original)
DSS
Water
Time (days)
I
Vehicle
CL 316,243
Ta = 30°C
p=0.011
Colon shortening (%)
C57BL/6J Females
J
Vehicle
SR59230A
Vehicle + DSS
SR59230A + DSS
Body mass (% original)
Ta = 22°C
p<0.0001
Time (Days)
K
Vehicle
Vehicle + DSS
SR59230A
SR59230A + DSS
p=0.0003
n.s
Colon length (mm)
Ta = 22°C
L
Vehicle + DSS
SR59230A + DSS
p=0.0009
Colon shortening (% of control)
Ta = 22°C

Fig. S13. β3-adrenergic signaling has minor effects on colonic and systemic inflammatory cytokine expression.

Thermoneutral C57BL/6J male were treated with β3-adrenoceptor agonist CL 316,243 or vehicle for two weeks prior to oral administration of DSS. Concentration of IL-6 (A), MCP-1/CCL2 (B), IFNγ (C), TNF (D), IL-12p70 (E) and IL-10 (F) in colonic tissue. Concentration of IL-6 (G), MCP-1/CCL2 (H), IFNγ (I), TNF (J), IL-12p70 (K) and IL-10 (L) in plasma (n=6-8 per condition, data pooled from 2 independent experiments and analyzed by Student's *t*-test). Data are presented as mean ± SEM.

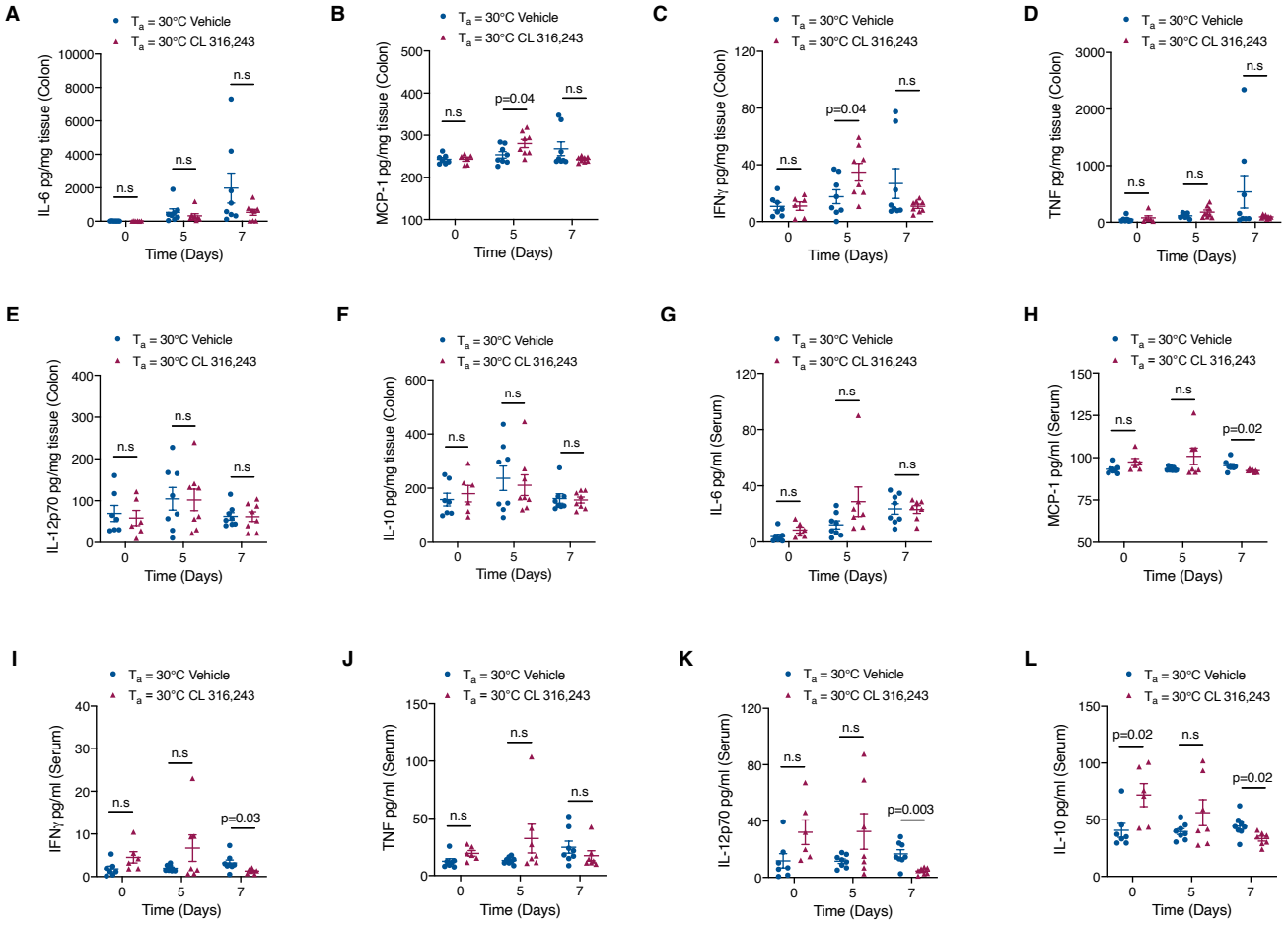


Fig. S14. Treatment with β3-adrenergic agonist alters composition of immune cells in the lamina propria of large intestine.

(A-I) Flow cytometric analysis of large intestine laminar propria immune cells in thermoneutral C57BL/6J mice pre-treated with β3-adrenoceptor agonist CL 316,243 or vehicle prior to oral administration of 4% DSS for 5 days. Gating (A) and quantification of neutrophils ($CD11b^{+}Ly6G^{+}$), total cell number (B) in the large intestine. (C) Total cell number of macrophages ($CD11b^{+}F4/80^{+}$) and dendritic cells ($CD11b^{+}Ly6G^{+}$). (D, E) Gating strategy (D) and quantification (E) of eosinophils ($CD11b^{+}SiglecF^{+}$), patrolling ($CD115^{+}Ly6C^{Low}$) and inflammatory ($CD115^{+}Ly6C^{high}$) monocytes in the large intestine of treated mice. (F, G) Gating strategy (F) and quantification (G) of NK cells ($CD3^{+}NK1.1^{+}$), B cells ($B220^{+}CD3^{-}$), cytotoxic T cells ($CD3^{+}CD8^{+}$), helper T cells ($CD3^{+}CD4^{+}$) and regulatory T cells ($CD4^{+}FoxP3^{+}$). (H, I) Gating strategy (H) and quantification (I) of innate lymphoid cells ($CD45^{+}Lineage^{-}IL7R\alpha^{+}$), ILC1's ($ROR\gamma t^{-}GATA3^{-}NKp46^{+}$), ILC2's ($ROR\gamma t^{-}GATA3^{+}$) and ILC3's ($ROR\gamma t^{+}GATA3^{-}$) in the large intestine of treated mice (n=7 per condition, data pooled from two independent experiments and data analyzed using student's *t* test). Data are presented as mean ± SEM.

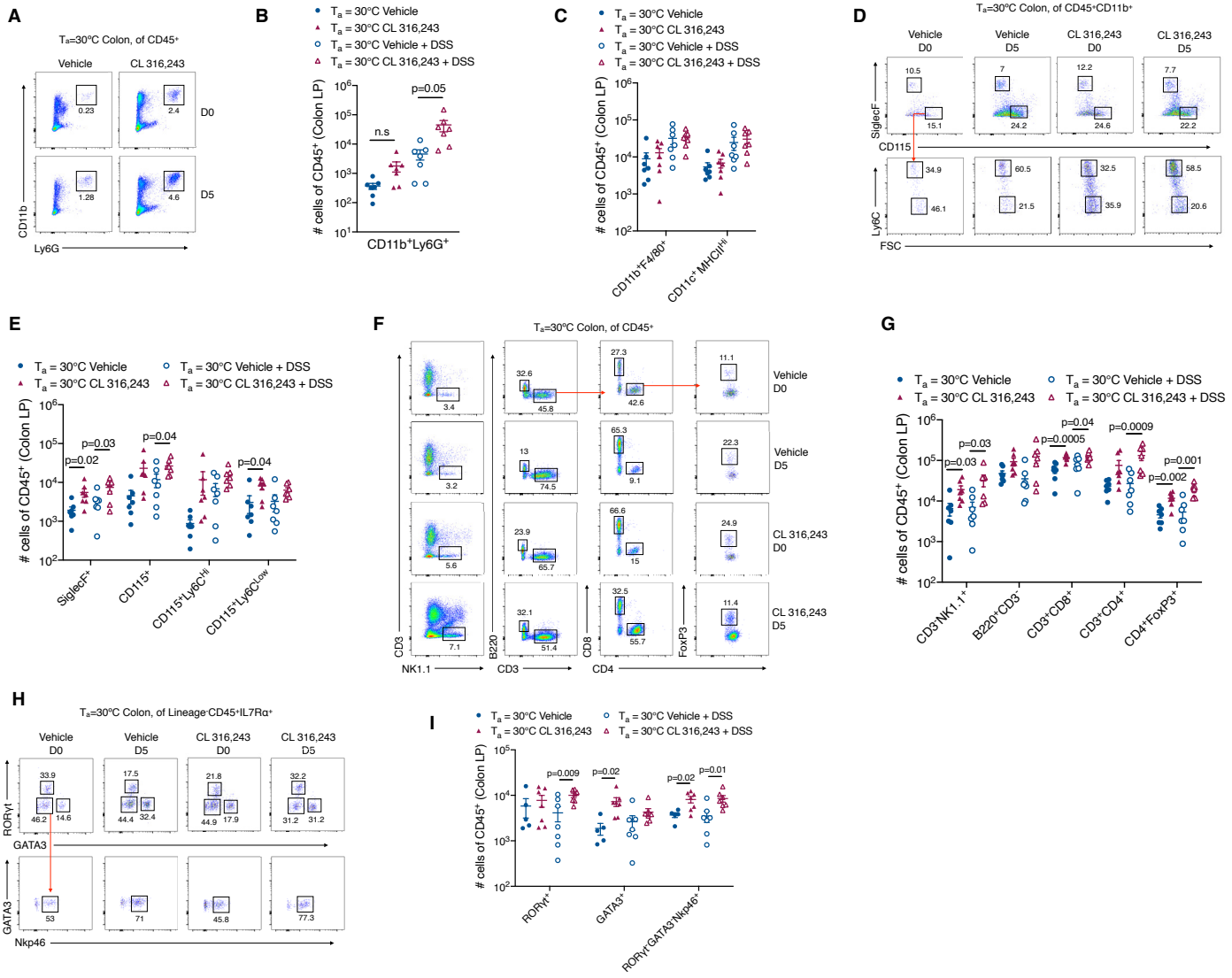

A
Ta=30°C Colon, of CD45+
Vehicle
CL 316,243
D0
D5
CD11b
Ly6G
B
Ta = 30°C Vehicle
Ta = 30°C CL 316,243
Ta = 30°C Vehicle + DSS
Ta = 30°C CL 316,243 + DSS
# cells of CD45+ (Colon LP)
n.s
p=0.05
CD11b+Ly6G+
C
Ta = 30°C Vehicle
Ta = 30°C CL 316,243
Ta = 30°C Vehicle + DSS
Ta = 30°C CL 316,243 + DSS
# cells of CD45+ (Colon LP)
CD11b+F4/80+
CD11c+ MHCIIHi
D
Ta=30°C Colon, of CD45+CD11b+
Vehicle D0
Vehicle D5
CL 316,243 D0
CL 316,243 D5
SiglecF
CD115
Ly6C
FSC
E
Ta = 30°C Vehicle
Ta = 30°C Vehicle + DSS
Ta = 30°C CL 316,243
Ta = 30°C CL 316,243 + DSS
# cells of CD45+ (Colon LP)
p=0.02
p=0.03
p=0.04
p=0.04
SiglecF+
CD115+
CD115+Ly6CHi
CD115+Ly6CLow
F
Ta=30°C Colon, of CD45+
Vehicle D0
Vehicle D5
CL 316,243 D0
CL 316,243 D5
CD3
NK1.1
B220
CD3
CD8
CD4
FoxP3
G
Ta = 30°C Vehicle
Ta = 30°C Vehicle + DSS
Ta = 30°C CL 316,243
Ta = 30°C CL 316,243 + DSS
# cells of CD45+ (Colon LP)
p=0.03
p=0.03
p=0.04
p=0.0005
p=0.0009
p=0.001
p=0.002
CD3-NK1.1+
B220+CD3-
CD3+CD8+
CD3+CD4+
CD4+FoxP3+
H
Ta=30°C Colon, of Lineage-CD45+IL7Rα+
Vehicle D0
Vehicle D5
CL 316,243 D0
CL 316,243 D5
RORγt
GATA3
GATA3
Nkp46
I
Ta = 30°C Vehicle
Ta = 30°C Vehicle + DSS
Ta = 30°C CL 316,243
Ta = 30°C CL 316,243 + DSS
# cells of CD45+ (Colon LP)
p=0.009
p=0.02
p=0.02
p=0.01
RORγt+
GATA3+
RORγt+GATA3+Nkp46+

Fig. S15. β3-adrenergic signalling reprograms BAT function and transcriptome during intestinal injury.

(A) Representative brightfield images of colonoids cultured from mice housed at T_a=22°C or T_a=30°C. Images (4x and 20x (inset) magnification) were taken 7 days after plating of crypts in medium supplemented with vehicle or CL 316,243. (B-D) Quantification of colonoid growth (B), size (C), and crypt budding per colonoid (D) (n > 50 individual colonoids per condition, data analyzed by unpaired Student's *t*-test). (E) Immunoblotting for β2- and β3-adrenergic receptors in isolated colonic crypts from C57BL/6J male housed at T_a=22°C or T_a=30°C. (F-G) Assessment of β3-adrenergic agonist CL 316,243 on colonoid growth. Colonic crypts were isolated from mice housed at T_a=22°C (F) or T_a=30°C (G) and plated in medium containing varying concentrations of CL 316,243 for one week. Colonoid growth was assessed by MTT metabolism and values were normalized to untreated controls (n = 11-14 wells per condition containing ~20 colonoids per well, data analyzed by unpaired Student's *t*-test). (H) Heatmap, gene ontology (GO) enrichment analysis, and corresponding P values of differentially expressed genes in BAT of CL 316,243 and DSS treated C57BL/6J male mice at T_a=22°C or T_a=30°C (n=4 per temperature and condition, fold change ≥1.5, adjusted P value <0.05). (I) Oxygen consumption *ex vivo* in BAT of mice housed at T_a=22°C or T_a=30°C and treated with DSS for 7 days (n=3-7 per temperature and time; data analyzed by Student's *t*-test). (J) Heatmap of differentially expressed secreted factors in BAT of C57BL/6J male mice housed at T_a=22°C or T_a=30°C that were treated with vehicle or CL 316,243 and administered DSS for 7 days (n=4 per timepoint and condition, adjusted P value <0.05). Data are presented as mean ± SEM.

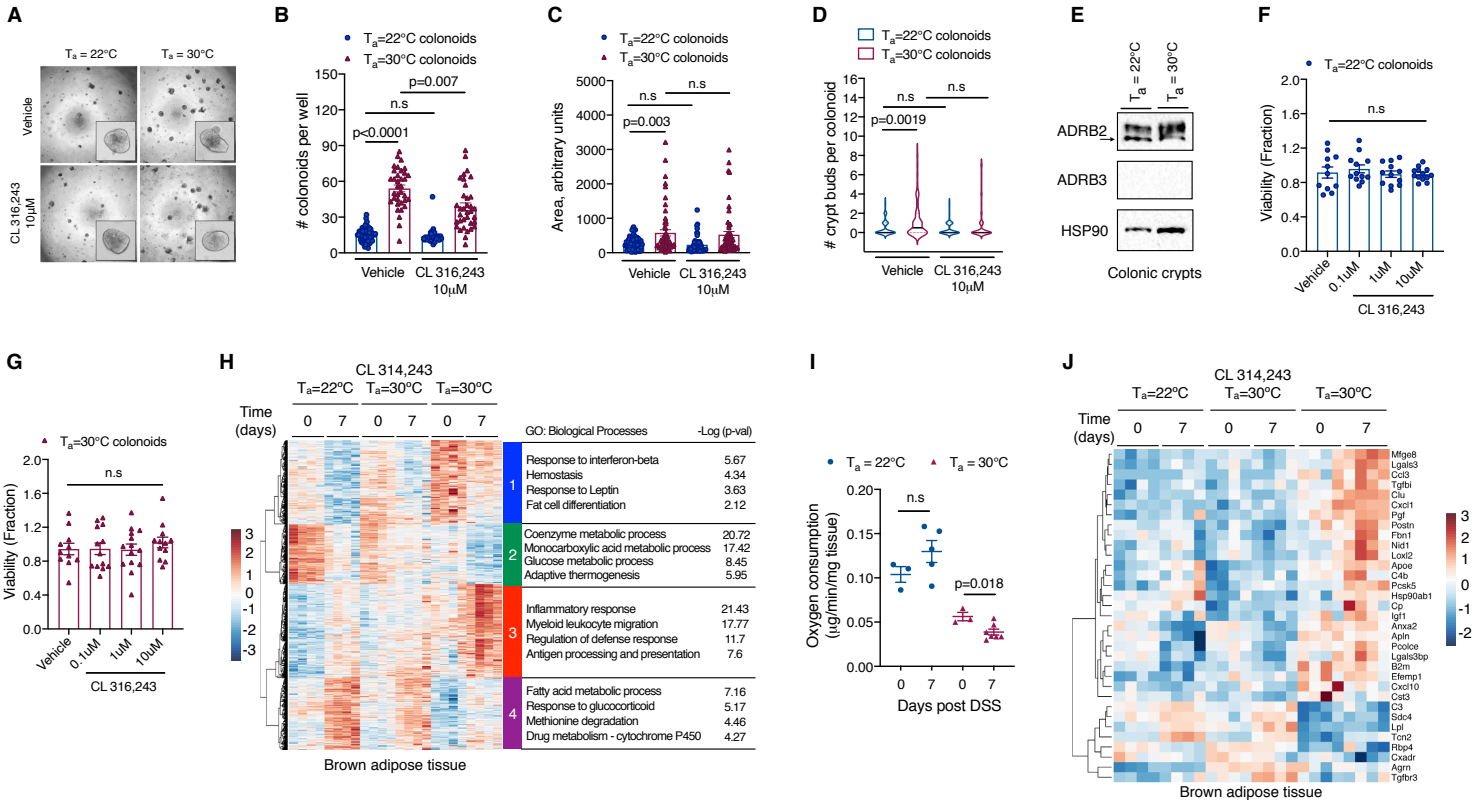

A
Ta = 22°C
Ta = 30°C
Vehicle
CL 316,243 10μM
B
Ta=22°C colonoids
Ta=30°C colonoids
# colonoids per well
p=0.007
n.s
p<0.0001
Vehicle
CL 316,243 10μM
C
Ta=22°C colonoids
Ta=30°C colonoids
Area, arbitrary units
n.s
n.s
p=0.003
Vehicle
CL 316,243 10μM
D
Ta=22°C colonoids
Ta=30°C colonoids
# crypt buds per colonoid
n.s
n.s
p=0.0019
Vehicle
CL 316,243 10μM
E
Ta = 22°C
Ta = 30°C
ADRB2
ADRB3
HSP90
Colonic crypts
F
Ta=22°C colonoids
Viability (Fraction)
n.s
Vehicle
0.1uM
1uM
10uM
CL 316,243
G
Ta=30°C colonoids
Viability (Fraction)
n.s
Vehicle
0.1uM
1uM
10uM
CL 316,243
H
CL 314,243
Ta=22°C
Ta=30°C
Ta=30°C
Time (days)
0
7
0
7
0
7
GO: Biological Processes
-Log (p-val)
1
Response to interferon-beta 5.67
Hemostasis 4.34
Response to Leptin 3.63
Fat cell differentiation 2.12
2
Coenzyme metabolic process 20.72
Monocarboxylic acid metabolic process 17.42
Glucose metabolic process 8.45
Adaptive thermogenesis 5.95
3
Inflammatory response 21.43
Myeloid leukocyte migration 17.77
Regulation of defense response 11.7
Antigen processing and presentation 7.6
4
Fatty acid metabolic process 7.16
Response to glucocorticoid 5.17
Methionine degradation 4.46
Drug metabolism - cytochrome P450 4.27
Brown adipose tissue
I
Ta = 22°C
Ta = 30°C
Oxygen consumption (μg/min/mg tissue)
n.s
p=0.018
0
7
0
7
Days post DSS
J
CL 314,243
Ta=22°C
Ta=30°C
Ta=30°C
Time (days)
0
7
0
7
0
7
Mfge8
Lgals3
Ccl3
Tgfbi
Clu
Cxcl1
Pgf
Postn
Fbn1
Nid1
Loxl2
Apoe
C4b
Pcsk5
Hspb0ab1
Cp
Igf1
Anxa2
Apln
Pcolce
Lgals3bp
B2m
Efemp1
Cxcl10
Cst3
C3
Sdc4
Lpl
Tcn2
Rbp4
Cxadr
Agrn
Tgfbr3
Brown adipose tissue

Fig. S16. β-adrenergic signaling in thermogenic adipose tissue regulates intestinal tissue tolerance.

(A) Male *Gnas*$^{f/f}$ mice on mixed genetic background (129S6/SvEvTac-Black Swiss) were housed at T_a=22°C or T_a=30°C and administered 3% DSS for 7 days. Body mass (A) and colon length (B) were quantified (n=4-7 per condition, data pooled from 2 independent experiments, data analyzed by two-way ANOVA (A) or unpaired Student's *t*-test (B)). (C-E) Female *Gnas*$^{f/f}$ and *Gnas*$^{f/f}$*Adipoq*^{Cre} mice were administered DSS (3%) at T_a=22°C, and colon length (C), gWAT mass (D), and iWAT mass (E) was measured on day 7 (n=7-12 per genotype and treatment, data pooled from 2 independent experiments and analyzed by Student's *t*-test). (F-L) Male and female *Gnas*$^{f/f}$ and *Gnas*$^{f/f}$*Adipoq*^{Cre} mice treated with β3-adrenoceptor agonist CL 316,243 or vehicle and administered DSS (4%) at T_a=30°C. BAT mass (F), iWAT mass (G), and eWAT mass (H) in treated male mice on day 7. Body mass (I), BAT mass (J), iWAT mass (K), and gWAT mass (L) in treated female mice (n=5-12 per genotype, data pooled from 2 independent experiments and analyzed by two-way ANOVA (G) or Student's *t*-test (H to J). Data are presented as mean ± SEM.

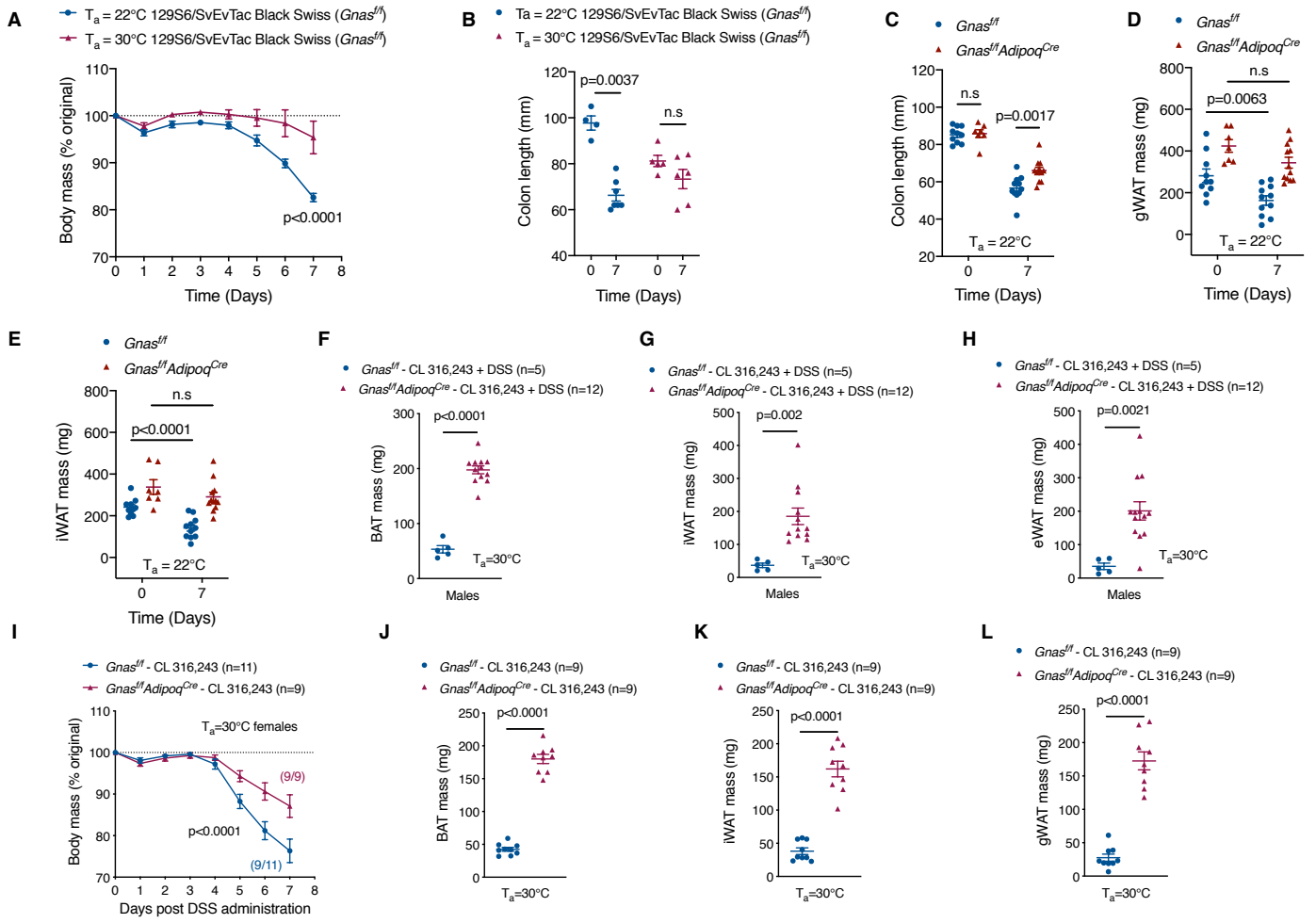
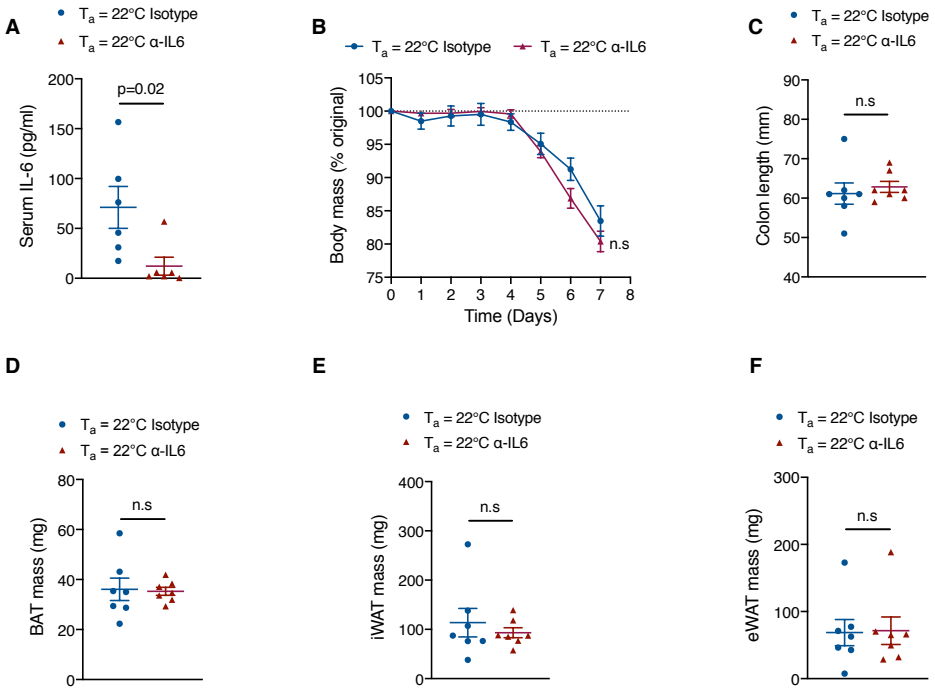

A
Ta = 22°C 129S6/SvEvTac Black Swiss (Gnas f/f)
Ta = 30°C 129S6/SvEvTac Black Swiss (Gnas f/f)
Body mass (% original)
p<0.0001
Time (Days)
B
Ta = 22°C 129S6/SvEvTac Black Swiss (Gnas f/f)
Ta = 30°C 129S6/SvEvTac Black Swiss (Gnas f/f)
Colon length (mm)
p=0.0037
n.s
Time (Days)
C
Gnas f/f
Gnas f/f AdipoqCre
Colon length (mm)
n.s
p=0.0017
Ta = 22°C
Time (Days)
D
Gnas f/f
Gnas f/f AdipoqCre
gWAT mass (mg)
n.s
p=0.0063
Ta = 22°C
Time (Days)
E
Gnas f/f
Gnas f/f AdipoqCre
iWAT mass (mg)
n.s
p<0.0001
Ta = 22°C
Time (Days)
F
Gnas f/f - CL 316,243 + DSS (n=5)
Gnas f/f AdipoqCre - CL 316,243 + DSS (n=12)
BAT mass (mg)
p<0.0001
Ta=30°C
Males
G
Gnas f/f - CL 316,243 + DSS (n=5)
Gnas f/f AdipoqCre - CL 316,243 + DSS (n=12)
iWAT mass (mg)
p=0.002
Ta=30°C
Males
H
Gnas f/f - CL 316,243 + DSS (n=5)
Gnas f/f AdipoqCre - CL 316,243 + DSS (n=12)
eWAT mass (mg)
p=0.0021
Ta=30°C
Males
I
Gnas f/f - CL 316,243 (n=11)
Gnas f/f AdipoqCre - CL 316,243 (n=9)
Body mass (% original)
Ta=30°C females
(9/9)
p<0.0001
(9/11)
Days post DSS administration
J
Gnas f/f - CL 316,243 (n=9)
Gnas f/f AdipoqCre - CL 316,243 (n=9)
BAT mass (mg)
p<0.0001
Ta=30°C
K
Gnas f/f - CL 316,243 (n=9)
Gnas f/f AdipoqCre - CL 316,243 (n=9)
iWAT mass (mg)
p<0.0001
Ta=30°C
L
Gnas f/f - CL 316,243 (n=9)
Gnas f/f AdipoqCre - CL 316,243 (n=9)
gWAT mass (mg)
p<0.0001
Ta=30°C

Fig. S17. Neutralization of IL-6 does not affect intestinal tissue tolerance in mice housed under sub-thermoneutral conditions.

(A-F) C57B/6J male mice housed at T_a=22°C were treated intraperitoneally with neutralizing IL-6 antibody (clone MP5-20F3) or isotype control (200 µg per mouse) every second day starting from the beginning of oral DSS administration (4%). Serum IL-6 (A), body mass (B), colon length (C), BAT mass (D), iWAT mass (E) and eWAT mass (F) were quantified in treated mice. (n=7 per treatment, data pooled from 2 independent experiments and analyzed by two-way ANOVA (B) or Student's *t*-test (A, C-F). Data are presented as mean ± SEM.



Supplementary Datasets

Dataset S1. Quantification of immune cell populations in the large intestine lamina propria in T_a=22℃ and T_a=30℃ housed C57BL/6J mice.

Dataset S2. List of BAT secreted factors used for differential expression analysis in Fig. 5G, 5I, and Fig. S14C.

Dataset S3. List of antibodies, chemicals, assay kits, and software used in these studies.