Supplemental Material

Gut Microbiota-Derived Short-Chain Fatty Acids Promote Post-Stroke Recovery in Aged Mice

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Running title: Post-stroke gut microbiome transplant and recovery

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Methods

Animals

Aged male mice (C57BL/6, 16-18 months) were obtained from the National Institute on Aging (NIA). Mice were acclimated in our animal facilities for at least two months before use. Mice were housed 4-5 per cage with a 12-h light/dark schedule in a temperature- and humidity-controlled vivarium, with ad libitum access to food and water. Mice received irradiated, nutritionally balanced and pelleted rodent diet (PicoLab® rodent diet 20 5053, LabDiet) and filtered tap water. All experiments were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at Houston. This study was performed in accordance with the guidelines provided by the National Institute of Health (NIH) and followed RIGOR guidelines.[48] Only male animals were used in this study to allow comparability to earlier studies and to exclude potential sex and estrogen-related effects. All animals were randomly assigned into groups and behavioral analyses were performed blinded to treatment groups. Sample size was determined by power analysis and based on our previous experiments.

Health Surveillance Program and Facility Practices

Microenvironment: Mice are housed in individually ventilated cages on irradiated 1/8" corncob bedding and receive irradiated, nutritionally balanced, pelleted rodent diet and filtered tap water. Cages are serviced under HEPA-filtered animal-transfer stations. Required protective wear for entry into room includes a disposable gown, shoe covers, hair cover, mask and gloves.

Health surveillance: One cage of two sentinel mice is placed on each rack housing up to 71 cages; sentinel cages receive soiled bedding from cages of colony mice on the corresponding rack. Sentinels in each housing room are processed and necropsied quarterly in house; blood, feces and fur swabs submitted to an outside diagnostic laboratory for serology and parasitology for verification of health status.

Excluded pathogen list:

Species	
Virus	Ectromelia, EDIM, LCMV, MAV1, MAV2, MHV, MPV, MVM, Polyoma, PVM, REO3, Sendai, TMEV
Prokaryote	CAR bacillus, *Clostrodium piliforme*, *Mycoplasma pulmonis*
Eukaryote	*Aspiculuris tetraptera*, *Encephalitozoon cuniculi*, *Myocoptes* spp., *Radfordia* spp./*Myobia* spp., *Syphacia muris, Syphacia obvelata*

For this study, all aged C57BL/6 mice were on standard lab chow for 2 months before FTG studies.

Experimental Stroke

Middle cerebral artery occlusion (MCAO) was used to induce reversible cerebral ischemia in aged male mice as previously described.[49] Mice were subjected to a 60-minute MCAO followed by reperfusion. We used 0.23 mm silicone-coated sutures for the occlusion (Doccol Corporation) and isoflurane for anesthesia. Animal temperature was maintained at 37°C during the surgery and for 24 hours after surgery. Post-operative care was performed by injecting saline (0.5 ml, subcutaneously) for 1 week and providing animals with soft food. Data were excluded from mice that died during the MCAO surgery. Mice that died during recovery were excluded from behavioral analysis but were used for survival plots to show mortality rates in each cohort.

Fecal Transplant Gavage (FTG)

Fresh fecal samples were collected from naïve young or aged mice (n=5-10 per group) between 9-10 am and homogenized in ice-cold PBS (120 mg feces/1 ml buffer) followed by 800g centrifugation for 3 min at 4°C. The supernatant was transferred into new tubes and used for transplantation. Mice were orally gavaged with streptomycin HCl (500 mg, Sigma-Aldrich) in 50 µl sterile for the first two days after the MCAO. To determine the reduction of bacterial load after streptomycin treatment, fecal samples were collected at post-stroke day 3 prior to FTG. Bacterial DNA was extracted from fecal materials using the ZR fecal DNA Miniprep Kit (Zymo Research, USA) and used for the quantification of all bacterial strains present in the

gut fecal contents. Bacteria were quantified using quantitative real-time PCR targeting the 16S ribosomal RNA (rRNA) gene. All primers were purchased from Integrated DNA Technologies (IDT), USA. Standard curves were obtained by spiking feces from germ-free mice with known cell numbers of *Lactobacillus sps*. bacteria. The Quant Studio 3 Real-Time PCR system (Applied Biosystems, USA) was used for amplification and fluorescent data collection. The supplied software was used to calculate absolute cell numbers according to the calibration curves. The master mix consisted of 10 µl of Power SYBR Green 2000 (ABI systems, USA), 0.5 µl of each primer (10 µM), 1 µl of sample and adjusted with water to a final volume of 20 µl per well. After PCR amplification, the specificity of the primers was checked by inspecting the melting curve and determining the size of the amplicon by agarose gel electrophoresis (1%). On days 3 and 4 after stroke, 100 µl of the fecal supernatant was gavaged to randomly assigned mice.[4]

16S rRNA Sequencing

Fecal samples were collected from mice between 9-10 am at baseline and post-stroke day 14 and immediately frozen at -80°C until use. Additionally, 16S rRNA sequencing was performed on collected samples as previously described.[4]

DADA2 (Divisive Amplicon Denoising Algorithm 2, https://github.com/benjjneb/dada2) and phyloseq were used to process Illumina MiSeq paired-end amplicon sequences of the V4 region of the 16S rRNA gene. Demultiplexed FASTQ files were uploaded into R. After visual inspection of FASTQ quality plots, Read1 and Read2 FASTQ files were filtered and trimmed using these parameters (truncLen=c(220,150), maxEE=2, truncQ=11, maxN=0). Sequences were inferred, de-replicated and merged. Sequence table was constructed, chimeras were removed, and taxonomy was assigned using pre-trained Naïve Bayes classifier based on the Silva 132 99% OTUs database (https://www.arbsilva.d-e/documentation/release-132/). Phylogenetic tree of the data was made from sequencing table using the package "phangorn" (Phylogenetic analysis in R, http://cran.rproject.org/web/packages/-phangorn). Sequence table, taxonomy table, and phylogenetic tree were imported into phyloseq (R-package for microbiome data, https://github.com/joey711/-phyloseq) for downstream analyses. Samples were rarefied to 26,696 (lowest sample reads in dataset) reads to perform beta diversity and multivariate analyses. Beta diversity matrices were used (unweighted and weighted Unifrac) to calculate dissimilarity distances among samples. Principal coordinates analysis (PCoA) distance calculation and ordination were performed in phyloseq. The PCoA plots were visualized using the package ggplot2 (R package for data visualization, https://ggplot2.tidyverse.org). Adonis function was used in the vegan package (vegan: Community Ecology Package, https://CRAN.R-project.org/package=vegan) to perform PERMANOVA test on pairwise distances based of the four distance matrices used to make PCoA plots.

Behavioral Assessment

Based on our knowledge and previous experience in aged stroke mice, we chose a battery of behavioral tests to provide a comprehensive comparison between the groups.[38] Mice were acclimated in the testing room for 1 hour prior to all tests. Each test was performed by blinded investigators at the same time each day to avoid bias and circadian variations. All apparatus used for behavioral tests was cleaned with 70% ethanol between animals to remove olfactory cues.

Open field test (OFT): To measure spontaneous locomotor activity, the OFT was evaluated on day 3, 7 and 14 post-stroke. Mice were placed in separate arenas (16" x 16" in dimension) in a dark room. Mice activity was tracked for 20 min and analyzed using EthoVision behavior tracking software (Noldus).

Novel objective recognition test (NORT): To measure changes in cognitive function, the NORT was performed on post-stroke day 14. Mice were placed in the center of the arena with two identical objects and allowed to explore for 10 min. After an hour, mice were placed back into the same arena with a novel and a familiar object (the same object used in the training phase). Exploratory activity was measured by monitoring the time spent with the novel objects. If memory function is normal, mice will spend more time exploring the novel object. The percent of the time with the novel vs. the familiar object was calculated.

Tail suspension test (TST): To assess depressive-like behaviors, the TST was performed by suspending the mice by their tail from a suspension apparatus. The experiment was recorded for 6 min using a video camera and the duration of immobility was analyzed, as a measure of depressive-like behavior.

Neurological deficit score (NDS): NDS was assessed at post-stroke day 14. The Bederson-score system [0 (normal) to 4 (the most severe)] was employed with minor modifications.

Hangwire test: To measure post-stroke limb strength and balance, the hangwire test was performed using a wire cage top (18" x 9" in size) which was held 36 inches above a cage with soft bedding. Mice were monitored for up to 5 min and the latency to fall from the inverted cage top was recorded.

Cresyl Violet Quantification

Mice were perfused with PBS containing 1% heparin followed by 4% paraformaldehyde (PFA). Brains were post-fixed in 4% PFA for 6 hours, and then placed in sucrose and processed as in. Sections were thin cut at 30 micron thickness and collected into 96 well plates for immunohistochemistry and atrophy analysis.[38]

Intestinal Lamina Propria (LP) Cell Isolation and Flow Cytometry

Small (SI) and large intestinal (LI) tissues was isolated, opened longitudinally, and tissues were rinsed with ice-cold RPMI-1640 (Corning) to remove luminal contents including feces. For SI, Peyer's patches were removed. To remove epithelial cells (ECs) and intraepithelial lymphocytes, tissue pieces were incubated with RPMI-1640 containing 2 mM EDTA (Invitrogen) and 10% FBS (Gibco) for 30 min at 37°C. The resulting suspension was used for the isolation of ECs. The remaining tissues including LP cells were digested with collagenase/dispase (1.0 mg/ml, Roche) and DNase I (20 ug/ml, Sigma-Aldrich) for 60 min at 37°C.[50]

For the analysis of LP cells, cells were stained with the following antibodies: anti-CD45 (clone: 30-F11, Invitrogen 48-0451-82), anti-CD4 (clone: RM4-5, BioLegend 100516), anti-TCRβ (clone: H57-597, BioLegend 109228), anti-TCRγδ (clone: GL3, BioLegend 118124), anti-CD3ε (clone: 145-2C11, BioLegend 100362), anti-CD8α (clone: 53-6.7, BioLegend 100734), anti-CD19 (clone: 6D5, BioLegend 115520), anti-NK-1.1 (clone: PK136, BioLegend 108753), anti-CD11b (clone: M1/70, Invitrogen 53-0112-82) and an amine reactive Live/Dead Aqua viability stain (Invitrogen L34966). For intracellular staining, cells were fixed and permeabilized using Foxp3/Transcription Factor Staining Buffer Set (eBioscience) and stained with anti-Foxp3 (clone: 150D/E4, eBioscience 12-4774-42). Flow cytometry was performed using CytoFLEX (BECKMAN COULTER) and data was analyzed using FlowJo software (Tree Star, Inc.).

Gut Histology

Intestinal tissues were fixed in 4% PFA or Carnoy's solution overnight and embedded in paraffin. PFA-fixed tissues were sectioned at 5 μm and stained with hematoxylin and eosin. Carnoy's solution-fixed tissues were sectioned at 5 μm and stained with 1) alcian blue and periodic acid-Schiff (AB-PAS) for mucins or 2) a primary antibody targeting mouse Muc2 (Novus Biologicals, NBP1-31231) and HRP anti-rabbit IgG (Abcam, ab205718) for Muc2. Immunoperoxidase staining was performed using diaminobenzidine (DAB) system (Nichirei Biosciences). To validate the specificity of the primary antibody, only secondary antibody with no treatment of primary antibody was used as control. Histologic changes were evaluated using light microscopy by an investigator blinded to treatment group (Leica DM2000 LED).

In vivo Intestinal Permeability Assay

To assess the intestinal permeability of aged stroke mice after FTG, mice were orally gavaged with 0.5 ml of FITC-dextran in saline at post-stroke day 14. After two hours, plasma was collected and analyzed for fluorescence intensity at an excitation of 470 nm and emission of 520 nm.

Intestinal EC Isolation and Real-Time Quantitative PCR

Intestinal ECs were isolated as previously described.[51] As mentioned earlier, LI tissues were incubated with RPMI-1640 (Corning) including 2 mM EDTA (Invitrogen) and 10% FBS (Gibco). The resulting suspension

was intermixed with Percoll (GE Healthcare) density gradients: 25%, 40% and 75%. The cells were centrifuged at 500g for 20 min and the interface between the gradients (25% and 40%) was collected as ECs.

Total RNA was isolated using Trizol (Invitrogen) according to manufacturer's provided protocol. cDNA was synthesized using 500 ng of RNA using iScript Reverse Transcription Supermix for RT-qPCR kit (Bio-Rad). Beta-actin was used to normalize the expression level of mRNAs. qPCR was performed using CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) with SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad). The thermal cycling protocol used for qPCR was as follows: 1) polymerase activation and DNA denaturation for 30 sec at 95°C, 2) denaturation for amplification for 10 sec at 95°C, 3) annealing, extension and plate read for 30 sec at 60°C (step 2 and 3: 40 cycles) and 4) melt-curve analysis at 65-95°C with 0.5°C increment. Primer sequences used in this study are listed in Online Table 1.

Brain Cell Isolation and Flow Cytometry

Blood was removed using transcardial perfusion with ice-cold PBS. The brain tissue was isolated and the ipsilateral stroke hemisphere was used for the flow cytometry. Tissues were minced and incubated with RPMI-1640 (Corning) including collagenase/dispase (1 mg/ml, Roche) and DNase I (10 mg/ml, Roche) for 45 min at 37°C. The resulting cell suspensions was filtrated through a 70 μm cell strainer. The filtrate was applied to a Percoll (GE Healthcare) gradients: 30% and 70%. After centrifugation at 500g for 20 min, the interphase was collected to obtain brain cells.

For the analysis of brain cells, isolated cells were stained with the following antibodies: anti-CD45 (clone: 30-F11, Invitrogen 48-0451-82), anti-CD11b (clone: M1/70, Invitrogen 53-0112-82), anti-TCRβ (clone: H57-597, BioLegend 109228), anti-TCRγδ (clone: GL3, BioLegend 118124), anti-CD4 (clone: RM4-5, BioLegend 100516) and an amine reactive Live/Dead Aqua viability stain (Invitrogen L34966). For intracellular staining, cells were fixed and permeabilized using Foxp3/Transcription Factor Staining Buffer Set (eBioscience) and stained with anti-Foxp3 (clone: 150D/E4, eBioscience 12-4774-42). Flow cytometry was performed using CytoFLEX (BECKMAN COULTER) and data was analyzed using FlowJo software (Tree Star, Inc.).

IL-17 Analysis Using Flow Cytometry

For IL-17 analysis, isolated brain or intestinal LP cells were incubated with Cell Activation Cocktail (BioLegend) including optimized concentration of PMA (phorbol 12-myristate-13-acetate), ionomycin and Brefeldin A, for 4 h at 37°C in a CO_2 incubator. After stimulation, cells were stained with cell surface markers as indicated above. For intracellular staining, cells were fixed and permeabilized using Intracellular Fixation & Permeabilization Buffer Set (eBioscience) and stained with anti-IL-17A (clone: TC11-18H10.1, BioLegend 506904). Throughout, fluorescence-minus-one (FMO) controls were used to identify positive cell populations for each antibodies. Flow cytometry was performed using CytoFLEX (BECKMAN COULTER) and data was analyzed using FlowJo software (Tree Star, Inc.).

Brain Immunohistochemistry Staining

Brain sections were thin cut at 30 micron thickness and incubated with pre-conjugated antibodies [Alexa Fluor® 488-anti-Iba1 antibody (clone: EPR16588, abcam ab225260) and PE-anti-P2RY12 antibody (clone: S16007D, BioLegend 848004), 1:200 for both] for two hours in TBS + 0.25% Triton X-100 at room temperature. No treatment of primary antibody was used as a control. The sections were washed with PBS for 10 min three times. After staining with DAPI (Vector Laboratories H-1500-10), images were collected using a Leica confocal microscope (Leica, Heerbrugg, Switzerland). All slides were imaged and analyzed using ImageJ by an investigator blinded to treatment condition.

Bacteria Culture

The bacterial strains used in this study were *Bifidobacterium longum, Clostridium symbiosum, Faecalibacterium prausnitzii and Lactobacillus fermentum*. All strains were routinely cultured at 37°C in

brain heart infusion (BHI) medium. All strains were cultured under strictly anoxic conditions using N_2:CO_2 (80:20; v:v) as the gas phase. Briefly, the culture medium is autoclaved in a Hungat tube to maintain anaerobic bacteria. Under sterile conditions, bacteria of interest were inoculated individually in a 5 ml culture medium (initial bacterial inoculation is 100 ul of frozen stock) and left overnight at 37°C. The next day, bacteria are in the elongation phase. 100 ul of the overnight culture was inoculated into a fresh 5 ml culture medium and left overnight. From the fresh overnight culture, 1 ml was used to record the optical density at 600 nm before the bacteria were used to prepare the gavage cocktail. Optical density at 600 nm of the inoculum is close to "zero" at initiation of inoculation, and an overnight culture yielded an optical density (OD) close to 0.8 to 1 before use for transplant. An OD of one determines that bacteria are in the elongation phase. *F. prausnitzii* takes 2 days to get to the desired OD. In addition, the absolute numbers from the inoculum were tested using qPCR in addition to OD measurements with appropriate standard curves as explained.[52]

Short-Chain Fatty Acid (SCFA)-Producing Bacteria Gavage

For the SCFA study, we elected to gavage four bacterial strains that produce key SCFAs including acetate, propionate and butyrate. To this end, *B. longum*, *C. symbiosum*, *F. prausnitzii* and *L. fermentum* were purchased from the American Type Culture Collection (ATCC) and cultured in an anaerobic chamber at 37°C for 24 to 48 hours. For the oral gavage, the number of bacterial cells were counted and mixed to prepare a cocktail: *B. longum* ($1x10^7$), *C. symbiosum* ($5x10^6$), *F. prausnitzii* ($1x10^6$) and *L. fermemtum* ($1x10^9$). Mice were treated with 50 µl of antibiotics consisting of 500 mg of streptomycin HCl (Sigma-Aldrich) in sterile water for the first two days after the MCAO and then on days 3 and 4 the oral gavage was performed using the cocktail including inulin (1 ml/day of 10 mg/ml, Sigma-Aldrich). Aforementioned bacterial cocktail, concentrations in 200 µl medium, was administered to randomly assigned mice by oral gastric gavage. Control mice received 200 µl of sterile medium. Successful bacterial colonization after inoculation was validated in the feces by qPCR as described.[53]

Metabolomics for SCFAs

SCFAs of mouse feces, plasma and brain samples were analyzed as previously described.[4, 54] Briefly, samples were diluted (1:10, w/v) and homogenized with 50% aqueous acetonitrile. The supernatant was used to detect SCFAs using liquid chromatography-mass spectrometry and Acquity UPLC HSS T3 column (100Å, 1.8 µm, 2.1 mm x 100 mm).

Statistics

For group comparisons, we provided bar graphs with individual dots for each group of each experiment. Normality was checked by Shapiro-Wilk test and outliers were identified by Tukey's fences method based on the interquartile range. Two-sample *t* test was used for two-group comparison. When the normality does not hold or outliers present, we use the Mann-Whitney *U* test instead. For analysis with more than two groups, ordinary one-way ANOVA or Kruskal-Wallis test was used depending on the normality of the data, followed by Dunnett's multiple comparison test. For repeatedly measured outcomes such as body weight and OFT, we provided mean plots along time. Linear mixed model was used to account for within-subject correlation. Group comparisons were further performed at each time adjusted for multiple testing. Significance was defined by *P* value less than 0.05. All analyses were performed in GraphPad Prism 7.03 or SAS 9.4 software (Cary, NC).

Please see the Major Resources Table in the Supplemental Materials.

ONLINE FIGURE LEGENDS

Online Figure I. Temporal changes in the gut microbiome of recipient aged stroke mice.
A, Reduced bacterial load in the fecal samples after treatment of antibiotics (Abx) for two consecutive days after MCAO. The bacterial load was assessed using fecal samples which were collected at baseline and at day 3 after MCAO (i.e., day 1 after Abx treatment). n=8 per group. **B**, Temporal changes in the family-level fecal microbiome of donors and recipients at baseline, post-stroke day 7 (PS-D7) and post-stroke day 14 (PS-D14). n=4 per group. To determine the effect of Abx on the colonization of newly introduced microbiota in recipient mice, the fecal samples from recipients (young FTG without Abx, n=2; aged FTG without Abx, n=3) which were not treated with Abx (i.e., only FTG after MCAO) were collected at post-MCAO day 14 and analyzed. **C**, Change of *Bacteroides*, *Parabacteroides*, *Prevotella* and *Odoribacter* at day 14 after MCAO in aged mice with FTG (n=4 per group). Throughout, error bars represent mean±SEM. Mann-Whitney *U* test (**A**) and Student's *t* test (**C**) were used based on the normality of data assessed by the Shapiro-Wilk normality test.

Online Figure II. Behavioral outcomes and brain atrophy in aged stroke mice with FTG.
A, Neurological deficit score (NDS) 2 weeks after MCAO in aged stroke mice after Abx treatment and FTG (n=11 per group). **B**, Hanging time using hangwire test at post-MCAO day 14 (aged FTG, n=7; young FTG, n=5). **C**, Brain atrophy was measured using cresyl violet staining at post-MCAO day 14 (aged FTG, n=8; young FTG, n=9). Data was obtained from independent experimental cohorts. Throughout, error bars represent mean±SEM. Mann-Whitney *U* test (**A**) and Student's *t* test (**B** and **C**) were used based on the normality of data assessed by the Shapiro-Wilk normality test.

Online Figure III. Changes in intestinal immune cells after FTG in aged stroke mice.
Levels of $CD3^+$ T cells (T cell lineage), $CD8^+$ cytotoxic T cells, $CD19^+$ B cells, $NK1.1^+$ natural killer cells, Th17 cells and γδ T cells did not differ in the small (SI LP) and large intestinal lamina propria (LI LP) between young and aged FTG groups. Throughout, error bars represent mean±SEM. n=5 per group. Student's *t* test was used after the normality of data was confirmed by the Shapiro-Wilk normality test.

Online Figure IV. Young FTG reduced microglia activation in aged stroke brain.
Brain microglia ($CD45^{int}CD11b^+$) (**A**) and myeloid cells ($CD45^+CD11b^{high}$) (**B**) were analyzed using flow cytometry at day 14 after MCAO in the aged stroke mice with young (n=5) and aged (n=7) FTG. Throughout, error bars represent mean±SEM. **C**, Immunohistochemistry on brain sections from aged stroke mice with young and aged FTG demonstrated that young FTG reduced microglial activation (3 sections/brain; n=5 per group). Inset scale bars, 100 μm. Student's *t* test (**A**) and Mann-Whitney *U* test (**B**) were used based on the normality of data assessed by the Shapiro-Wilk normality test.

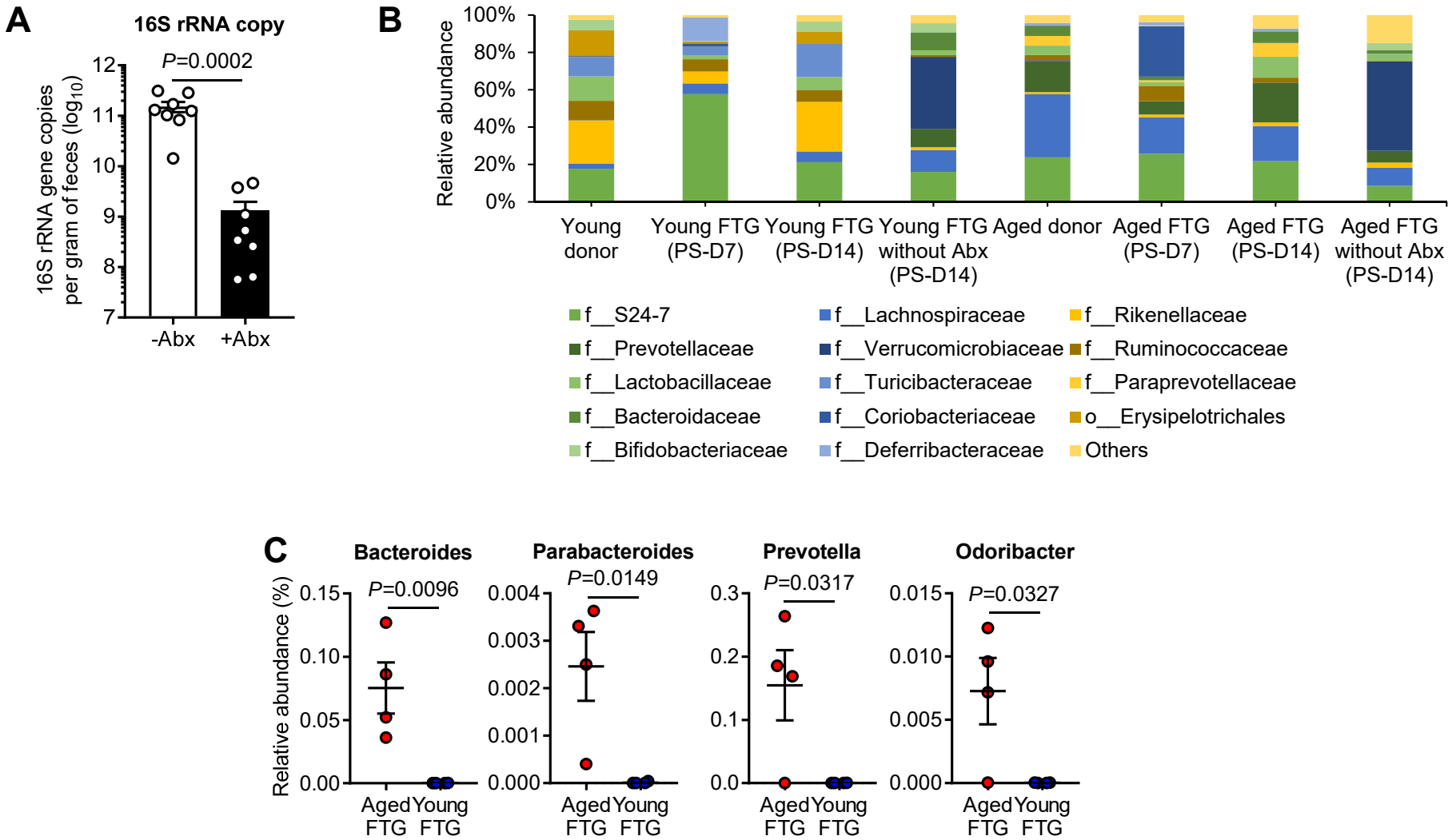
Online Figure V. Enhanced gut SCFAs improves intestinal histology and improves mucin production in aged stroke mice.
At post-MCAO day 14 after Abx (days 1 and 2) and bacterial (days 3 and 4) gavage, large intestinal (LI) tissues were collected from mice receiving vehicle and SCFA-producers (vehicle, n=3; inulin + bacteria, n=5). Intestinal morphology was assessed using hematoxylin and eosin staining (**A**) and Muc2 immunohistochemistry (**B**). Data are representative of three independent experiments. Scale bars, 250 μm. **C**, Muc2 signal was measured for 10 crypts per mouse and analyzed using Image-J. Error bars represent mean±SEM. Student's *t* test (**C**) was used after the normality of data was confirmed by the Shapiro-Wilk normality test.

Online Figure VI. Changes in fecal, plasma and brain metabolites of aged stroke mice after oral administration of SCFA-producers and inulin.
Mass spectrometry analysis was performed to measure fecal (vehicle, n=7; inulin+bacteria, n=8), plasma (vehicle, n=7; inulin+bacteria, n=8) and brain (vehicle, n=5; inulin+bacteria, n=7) metabolites including valerate, isovalerate, 3-methylvalerate, caproate and isocaproate. Throughout, error bars represent mean±SEM. Student's *t* test (fecal isovalerate and caproate, plasma valerate and brain valerate, caproate and isocaproate) and Mann-Whitney *U* test (other metabolites) were used based on the normality of data assessed by the Shapiro-Wilk normality test.

Online Table I. Primers sequences of the genes used in qPCR.

	Forward (5’ → 3’)	Reverse (5’ → 3’)
β-actin	GATCTGGCACCACACCTTCT	GGGGTGTTGAAGGTCTCAAA
Muc2	CAAGTGATTGTGTTTCAGGCTC	TGGAGATGTTCTTGGTGCAG
Muc4	GACAAAGCACCAATTCCATCC	CCTTAGAGTTGCTGGTGATCTC
Reg3β	CTCTCCTGCCTGATGCTCTT	GTAGGAGCCATAAGCCTGGG
Reg3γ	CCTGATGCTCCTTTCTCAGG	ATGTCCTGAGGGCCTCTTTT

Online Figure I



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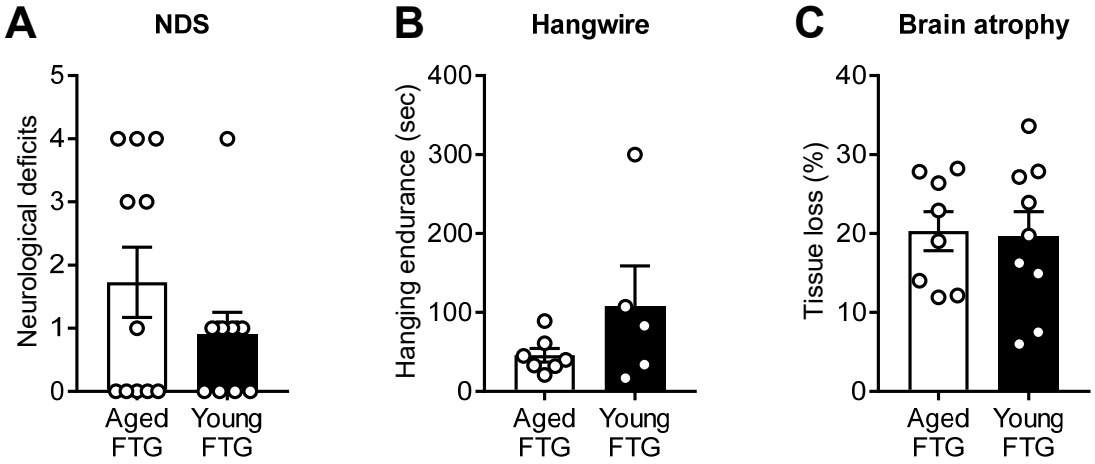

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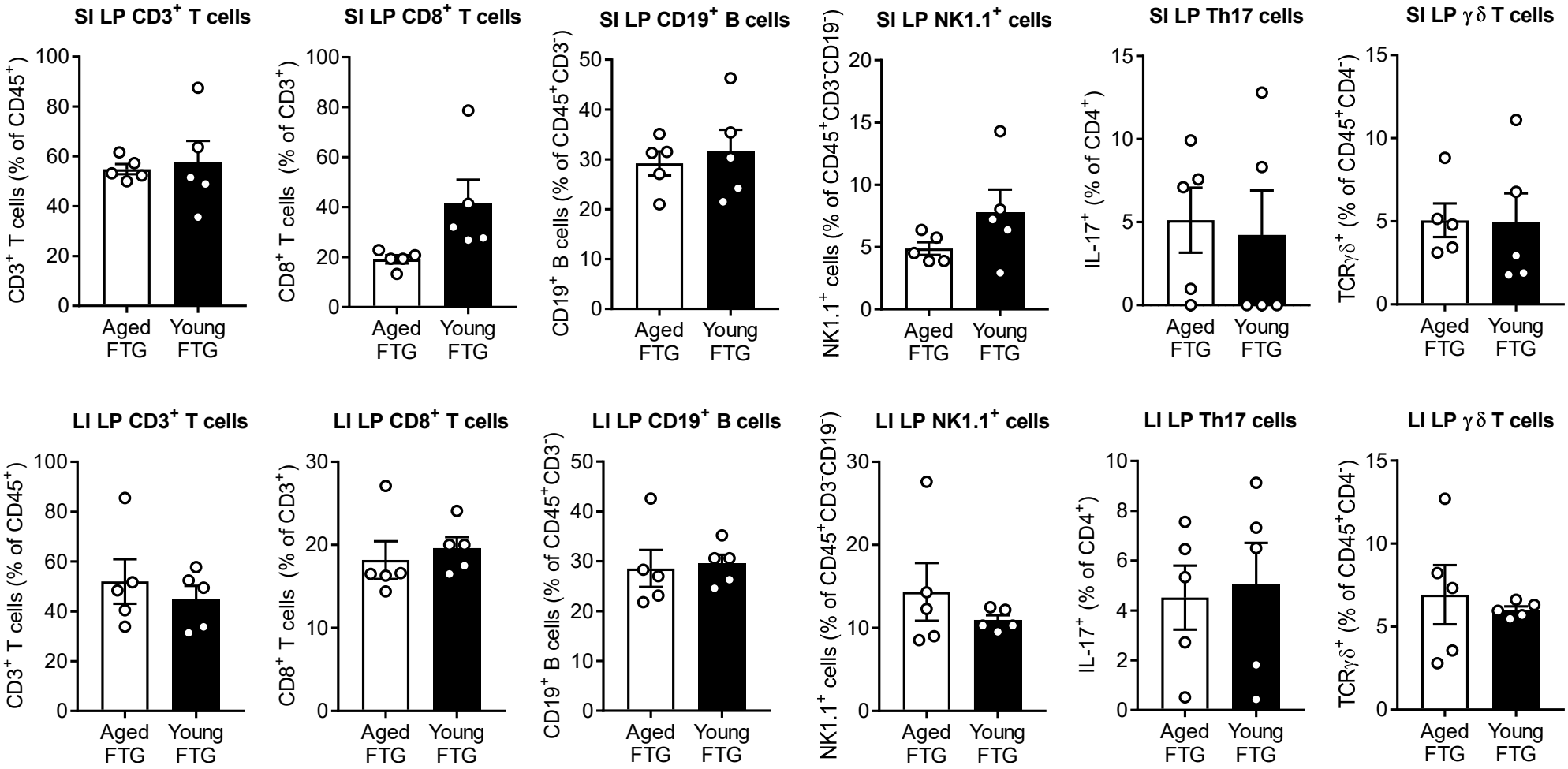

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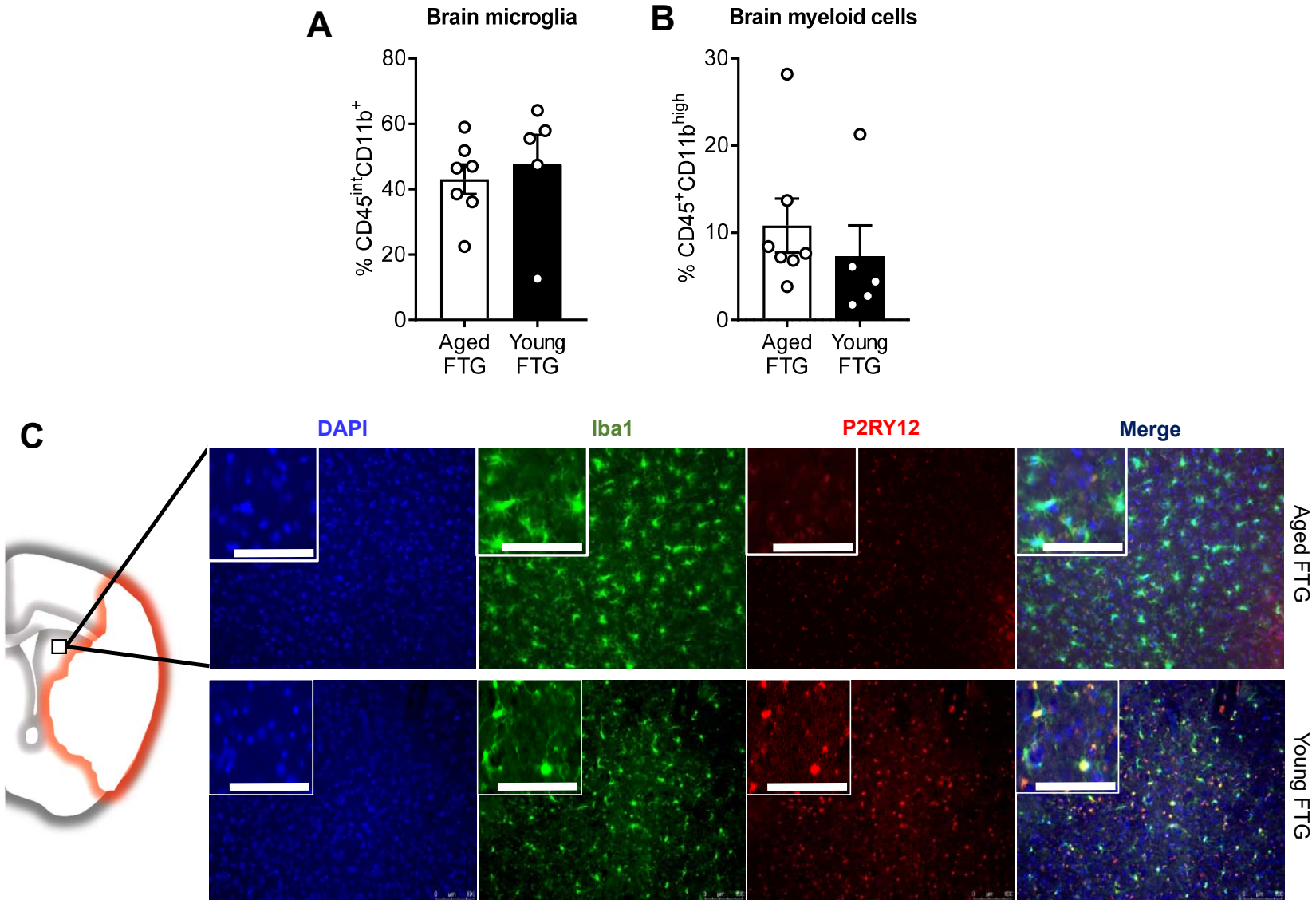
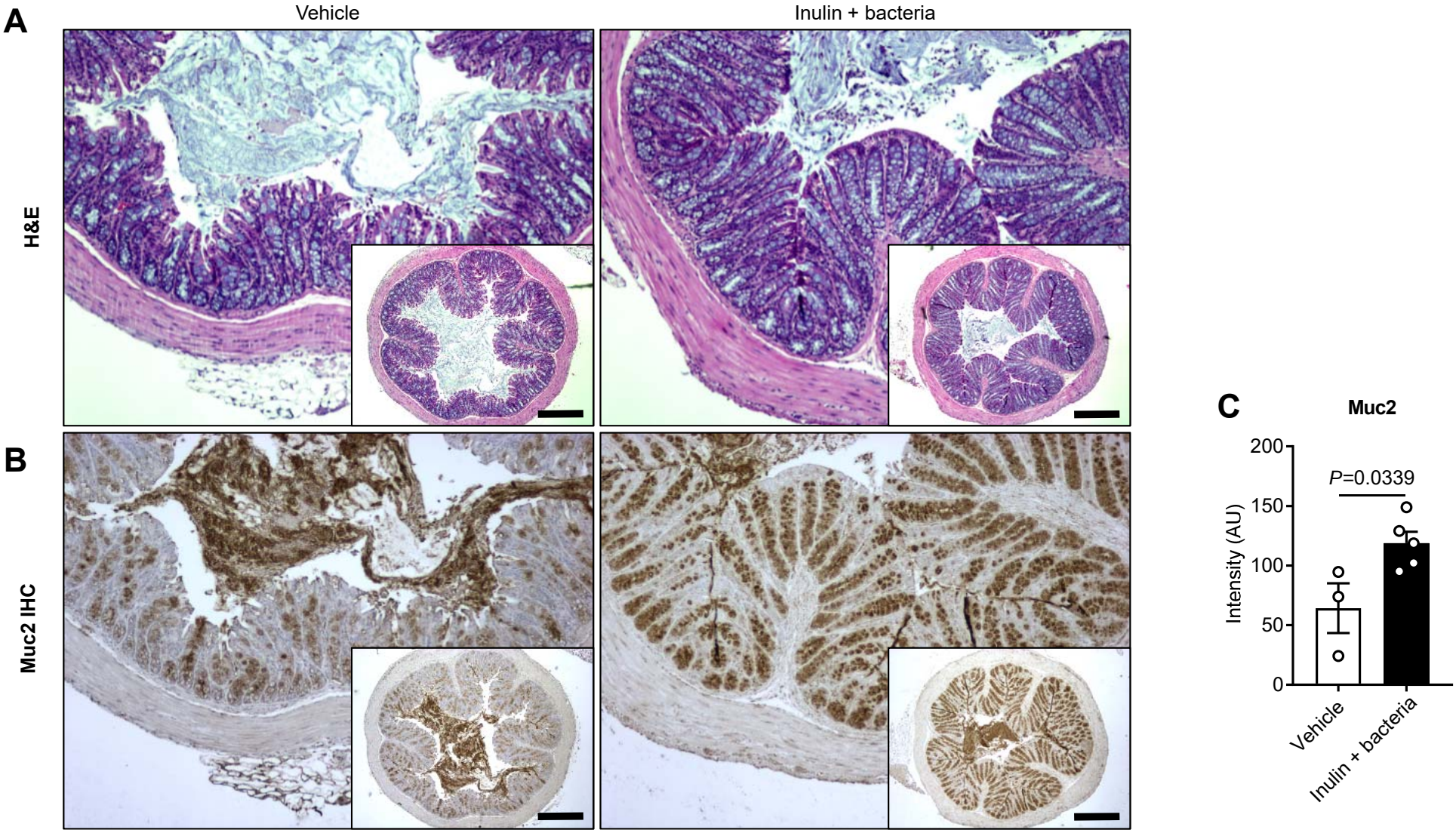


Online Figure V



Online Figure VI