Supplementary Material

Materials and Methods

Mice

The colony of mice included in this study was housed in a centralized animal facility at the University of Nevada, Reno (UNR) Animal Resources. All procedures that include animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at UNR. UNR is fully accredited by AAALAC International. Animals were air freighted to UNR, where they were housed in the transgenic facility at UNR, School of Medicine. All mice were housed under pathogen-free conditions on a 12 hours light/dark cycle with food and water ad libitum. Mice were euthanized by inhaling CO_2, followed by cervical dislocation. A ventral midline incision was made, and the whole GI tract was carefully excised. These procedures were in accordance with National Institutes of Health guidelines for the care and use of laboratory animals.

Human Specimens

The research participants included in this study were adults 22-69 years of age (Supplementary Table 3). Cases were defined as patients that had been diagnosed with gastroparesis by the Stanford Hospital and Clinics after an upper GI endoscopy. Gastroparesis was confirmed based on well-accepted symptom criteria[1] and delayed gastric emptying, defined as gastric retention of >10% at 4 hours and/or >60% at 2 hours when using the standard low fat, scrambled egg meal as described by Tougas.[2] Additionally, controls were defined as age-matched adults who underwent upper GI endoscopy, did not have a history of functional GI disorders, and did not experience symptoms such as abdominal pain, nausea, or vomiting. Subjects were excluded if they were actively using opiates, were found to have a recent or new diagnosis of *Helicobacter pylori* gastritis, or had active peptic ulcer disease according to endoscopy. Gastric mucosal samples were obtained during upper GI endoscopy by a trained

endoscopist using cold biopsy forceps; the samples were immediately frozen on dry ice and stored at -80°C. No later than 2 hours after phlebotomy, plasma was obtained following a 10-minute centrifugation (800 g) of venous blood in heparin-sulfate collection tubes (BD). Plasma was aliquoted and frozen at -80°C until further use. All experiments with human idiopathic gastroparesis and control biopsy/plasma samples and GCSI-dd were obtained from Stanford University School of Medicine. All human subjects provided informed consent, and all study procedures were approved by the Stanford University Institutional Review Board, and the Institutional Review Board at UNR.

Generation of Tph1$^{CreERT2/+}$, Tph1$^{CreERT2/+}$;Rosa26$^{tdTom/+}$(Tph1-tdTom), and Tph1$^{CreERT2/+}$;Rosa26$^{DTA/+}$(Tph1-DTA) Mice

The BAC clone of *Tph1* Exon 3-11, Chr7: 53,875,573-53,919,578 (UCSC Genome Browser) fosmid clone (*Tph1-pEpi*) WI1-1624F22 (44,006 bp) was purchased from the Children's Hospital Oakland Research Institute. Serial sequences of 5' arm of *Tph1* (1,358 bp) and T2A (234 bp) and 3' arm of *Tph1* (1,115 bp) along with the *kan/neo* cassette was synthesized in *Tph1-pUC57* (4,068 bp) from Bio Basic (Markham). Sequential subclones were performed to obtain *phCreER-Tph1* (7,723 bp). The selected clones were sequenced by Nevada Genomics Center to confirm the sequence. Recombination between *Tph1-pEpi* and linearized *phCreER-Tph1* was performed using Quick & Easy conditional knockout kit (Gene Bridges) to obtain targeting vector *Tph1-pEpi-KI*. The target vector was administrated to the C57BL/6 embryonic stem cells via microinjection at the University of Rochester Department of Ophthalmology. The selected embryonic stem cell clones were confirmed by LR-PCR. Two of the clones were used to generate a heterozygous *Tph1$^{CreERT2\text{-}Kan/Neo/+}$* mouse. The heterozygous mouse was first crossed with *Rosa26^{FLpo}* (The Jackson laboratory, B6.129S4-*Gt(ROSA)26Sor$^{tm2(FLP*)Sor}$*/J, Stock No: 012930) to delete the kan/neo cassette. Next, the mice were crossed separately with *Rosa26^{tdTom}* (The Jackson laboratory, B6;129S6-*Gt(ROSA)26Sor$^{tm9(CAG\text{-}tdTomato)Hze}$*/J, Stock No:

007905), or *Rosa26*^{DTA} (The Jackson laboratory, B6.129P2-*Gt(ROSA)26Sor*$^{tm1(DTA)Lky}$/J, Stock No: 009669) to generate the *Tph1-tdTom* and *Tph1-DTA* mice, respectively. All procedures for animal subjects were approved by the Institutional Animal Care and Use Committee (IACUC) at University of Rochester and UNR.

Tamoxifen Administration

Tph1-tdTom, Tph1-DTA, C57, *Rosa26*$^{DTA/+}$, *Tph1*$^{CreERT2/+}$ mice were given tamoxifen (Sigma-Aldrich; 1.0 mg/20g body weight) solubilized in sunflower oil (Sigma-Aldrich) intraperitoneally for 5 consecutive days at 8-16 weeks of age. The controls were given the same volume of sunflower oil. The analysis was compiled from age-and gender-matched mice from multiple litters.

Immunohistochemistry and Confocal Microscopy Analysis

GI Tissue collection and examination was performed in Krebs buffer (125.35 mmol/L NaCl, 5.9 mmol/L KCl, 1.2 mmol/L $NaHPO_4$, 15.5 mmol/L $NaHCO_3$, 1.2 mmol/L MgCl, 11.5 mmol/L D-glucose, and 2.5 mmol/L $CaCl_2$). Fresh tissue was fixed in 4% paraformaldehyde at 4°C for 20 minutes, followed by overnight incubation in 1 x Tris-buffered saline (TBS) at 4°C. Dehydration was performed in 20% sucrose in TBS at 4°C. Tissue was trimmed and placed in 1:1 OCT/20% sucrose in TBS and super-cooled by liquid nitrogen. 8 µM thickness cryosections were used for immunohistochemistry staining experiments. The section was blocked with 0.5% Triton X-114, 4% slim milk in TBS for 1 hour at room temperature, rinsed with TBS twice for 10 minutes each and then incubated with primary antibodies (Supplementary Table 4) for 48 hours on a rocker at 4°C. The slide was rinsed with TBS twice for 10 minutes each, and then incubated with secondary antibodies conjugated to AlexaFluor-488/594 (1:500) for 2 hours at room temperature. The specimen was rinsed with TBS three times for 10 minutes each, dried, and mounted (Mounting medium with DAPI). An Olympus Fluoview FV1000 confocal laser scanning microscope (Tokyo)

was used for all imaging analysis. Cell counts were quantified using multiple images of the same tissue area under the same magnification via ImageJ software.

ELISA Assay

All mucosal biopsy specimens were collected from the desired region of the GI tract and stored in Pierce RIPA buffer (Thermo-Fisher). The tissues were then homogenized using a homogenizer at 4°C (Bullet Blender). The homogenates were centrifuged at 12,000 rpm at 4°C for 20 minutes, and supernatants were collected. Retro-orbital blood was collected in mice, with ascorbic acid added to the blood samples right after collection. After a 20 minutes incubation at room temperature and subsequent centrifugation, the serum was collected. All biopsy specimens and serum were frozen at -80°C until analysis. For tissue protein extracts, flash-frozen endoscopy biopsy samples were stored at -80°C and thawed in NP-40 lysis buffer (Invitrogen) supplemented with protease and phosphatase inhibitors (cOmplete-Mini, Roche; Halt, Thermo), homogenized on ice (Tissue Master 125, Omni), centrifuged at 1,000 *g*, and the supernatants were aliquoted and frozen until further use. Protein quantification was performed using a BCA assay (Thermo) according to the manufactures protocol. Biopsy specimens and serum were analyzed in duplicate or triplicate with an ELISA specific for 5-HT (BA E-5900, LDN). Protein concentrations of biopsy samples were determined using the BCA assay (Thermo-Fisher). 5-HT content of the biopsy specimens was normalized with the protein concentration of each sample.

Western Blot

Mouse GI tissues (smooth muscle layer and mucosa) were stored in Pierce RIPA Buffer (Thermo-Fisher). The tissues were then homogenized using a homogenizer at 4°C (Bullet Blender). The homogenates were centrifuged at 12,000 rpm at 4°C for 20 minutes, and supernatants were collected. Samples were frozen at -80°C until analysis. Protein concentrations of each sample were determined using the BCA assay (Thermo-Fisher). Transferred polyvinylidene fluoride membranes were pre-blocked in 5% skim milk, then exposed

to a primary antibody at 4 °C in 2% skim milk in 0.05% Tween 20 at varying dilutions. The membranes were then exposed to a secondary antibody at room temperature. Images were taken by the UVP EC3 imaging system. All primary antibodies used in this study are listed in Supplementary Table 4.

Automated Western Blot

Mouse GI tissues (smooth muscle layer and mucosa) were stored in Pierce RIPA Buffer (Thermo-Fisher). The tissues were then homogenized using a homogenizer at 4°C (Bullet Blender). The homogenates were centrifuged at 12,000 rpm at 4°C for 20 minutes, and supernatants were collected. Protein concentrations of each sample were determined using the BCA assay (Thermo-Fisher). Automated Western blots were performed using WES (ProteinSimple). Quantification of banding patterns was performed using Compass software (v4.0.0).

Gastric Emptying Test

The gastric emptying test (GET) test was performed *in vivo* after the mouse received an intragastric gavage with GastroSense 750 (0.25 nmol/100 µL/25g body weight). GastroSense 750 is a near-infrared fluorescent imaging agent used to monitor and quantify gastric emptying rates in real-time using the IVIS Lumina III system (PerkinElmer). GET was performed on mice fed with the control diet (Diet 1, Supplementary Table 2) for three days to rule out the autofluorescence background generated from the chow diet. The mouse was fasted overnight to allow the stomach to empty its food contents. The mouse was anesthetized with a mixture of isoflurane (5%) and oxygen (100-200 mL/minute) until recumbent. IVIS fluorescence gastric imaging of each mouse was taken before applications of GastroSense 750 to ensure that there was no autofluorescence in the stomach area. Each mouse underwent an intragastric gavage with GastroSense 750 mixed with crushed control Diet 1 in 1x Phosphate buffered saline (PBS) or GastroSense 750 in 1 x PBS solution. Fluorescence datasets were acquired by imaging the gastric region at 15, 30, 45, 60, 90, and 120 minutes after the intragastric gavage. Images of the

fluorescence were analyzed using Living Image (PerkinElmer) software (v4.5.5). Percentage gastric emptying after 30 minutes in each mouse was quantified as [1 - (stomach fluorescence/total fluorescence) × 100].[3, 4]

Total GI Transit Time

Overnight fasted mice received an intragastric gavaged of a semiliquid solution containing 5% Evans blue, 0.9% NaCl, and 0.5% methylcellulose in 1 x PBS (100 µL/20g body weight). Mice were monitored for the presence of the first blue fecal pellet at 10 minutes intervals. Total GI transit time was calculated as the time between the intragastric gavage of the dye and the visualization of the first blue fecal pellet.[4, 5, 6]

Colonic Transit Time

Mouse fasted overnight were anesthetized with a mixture of isoflurane (5%) and oxygen (100-200 mL/minutes) until recumbent. Anesthesia was then delivered through a nosecone with 2-3% isoflurane and oxygen throughout the length of the procedure. A 3-mm diameter glass bead was inserted into the anus and pushed gently 3 cm into the colon by using a plastic Pasteur pipette covered with lubricating jelly. The mouse was placed into a recovery cage with thermal support and monitored closely until fully recovered. Colonic transit time was assessed between the time the mouse awoke from anesthesia until the expulsion of the bead.[4, 6]

Fecal Pellet Collection and Analysis

An alpha pad (LBS Biotechnology) was placed in the cage of the mouse instead of the corn cob bedding. A single caged mouse required 24 hours to acclimate to the alpha pad. The fresh fecal pellets were collected and measured within a period of 24 hours. The fecal pellet number was the number of pellets produced by one mouse in 24 hours. The fecal pellet output was calculated as the fecal pellet number multiplied by the weight of the pellets (g) produced by the mouse within 24 hours.

Fecal Pellet Water Content

An alpha pad (LBS Biotechnology) was placed in cage of the mouse instead of the corn cob bedding. A single caged mouse required 24 hours to acclimate to the alpha pad. Pre-weighted Eppendorf tubes were used to collect 3-4 fresh pellets from the mice. The wet weight was measured immediately. The Eppendorf tubes containing the pellets were then opened and placed in an incubator at 60°C overnight (12 hours) to measure the dry weight. Fecal pellet water content to dry pellet mass ratio was measured by subtracting dry weight from wet weight and normalizing it to the dry pellet weight. Pellet water content to dry pellet weight ratio was presented as normalized values.

Body Weight and Blood Glucose Measurements

Body weight was monitored at the same time each day. Fasting blood glucose monitoring was performed in mice fasted for 6 hours by collecting blood from the tail vein via a small needle prick using a blood glucose monitoring system (ReliOnTM Prime). Mice were considered healthy (90-120 mg/dL), prediabetic (>120 to <180 mg/dL) or diabetic (≥ 180 mg/dL) based on 6 hour fasting blood glucose levels.

Preparation of Tissues for CMMCs Recordings

The entire colon was removed and placed in a Sylgard®-lined dissection dish filled with warm (25-30°C) Krebs buffer constantly bubbled with carbogen gas (95% O_2/5% CO_2). The mesentery was carefully trimmed free and the entire colon, containing natural pellets, was placed in an oxygenated organ bath at 35.5 ± 0.5°C. The colon was gently flushed from the proximal end with warm Krebs to expel pellets, then a stainless-steel rod was inserted into the lumen to maintain a horizontal position. The whole colon was then anchored within the organ bath using fine sutures inserted into proximal and distal ends. The time taken for removal of colon from animal to the time at which the colon was anchored in the organ bath was typically <10 minutes.

Mechanical Recordings of Muscle Contractility during CMMCs

We recorded the force generated during each CMMCs contraction, using independent isometric recording transducers connected via fine suture and micro-hooks. To record circular muscle contractility 1 gm of resting tension was applied to preparations of colon at three sites, one site in the proximal colon, one in the mid region and one in the distal colon. Each force transducer was connected to two custom made preamplifiers and then to a PowerLab. LabChart version 6.0 was used for acquisition and analysis of data.

Video Recordings Made from Empty and Stimulated Segments of Mouse Colon

After expulsion of natural pellets had been recorded, the colon was gently flushed with warm Krebs. To maintain consistency for comparison, preparations that did fully empty were also gently flushed with warm Krebs. The empty colon was anchored with a suture at the oral and anal ends. The colon was then inserted with a natural pellet coated with superglue (Gorilla) from the oral ends. Video recordings were made of the colon and the mechanical traces (frequency, amplitude) were measured.

Gut Motility Analysis with 5-HT Administration

GI motility was analyzed after administration of 5-HT (serotonin hydrochloride, Sigma-Aldrich) in *Tph1-DTA* mice that had been injected with oil or Tx. Control groups were treated with the same volume of saline solution. (1) GET: Following overnight fasting the mice were given an intragastric gavage containing GastroSense 750 in 1 x PBS and mixed with 5-HT to the final concentration of 0.5, 1, 2, or 5 mg/mL. Fluorescence datasets were acquired by imaging the gastric region at 15, 30, 45, 60, 90, and 120 minutes after the intragastric gavage. (2) TGITT: Following overnight fasting the mice were given intragastric gavage containing a semiliquid solution containing 5% Evans blue, 0.9% NaCl, and 0.5% methylcellulose in 1 x PBS and mixed with 5-HT to the final concentration of 0.1, 1, or 10 mg/mL. Mice were monitored for the presence of the first blue fecal pellet at 10 minutes intervals. (3) CTT: Following overnight fasting the mice were given an intragastric gavage of 5-HT (1 or 2 mg/mL) 20 minutes prior to bead placement. Colonic transit time was assessed between the time the mouse awoke from

anesthesia until the expulsion of the bead. (4) Fecal pellet water content: An alpha pad (LBS Biotechnology) was placed in the cage of the mouse instead of the corn cob bedding. A single caged mouse required 24 hours to acclimate to the alpha pad. After fasting mice were given an intragastric gavage of 5-HT (1 or 10 mg/mL) and returned to their home cage. Pre-weighted Eppendorf tubes were used to collect 3-4 fresh pellets from the mice.

Dietary Intervention

We have designed and formulated the L-tryptophan (Trp) and 5-Hydroxytryptophan (5-HTP) diets (Supplementary Table 2) from Envigo (Madison) to restore the 5-HT levels in *Tph1-DTA* mice with oil or Tx injection. The *Tph1-DTA* mice were randomly separated into three groups and fed with Trp (Diet 3), 5-HTP (Diet 2) or supplied with a control amino acid defined diet (Diet 1) as a control group. TGITT and fecal pellet output were performed after the administration of the diets.

Statistics

All data are presented as mean ± SEM. The data presented in the figures were collected from multiple independent experiments performed on different days using age-matched mice with both genders unless otherwise mentioned. The inferential statistical significance of differences between sample means was evaluated using two-tailed unpaired *t* tests. The grouped samples were evaluated using one-way or two-way ANOVA tests using GraphPad Prism (v9.0) (GraphPad Software). Based on the sample size power analysis was performed by exploiting Fisher transformation to detect a relevant simple correlation with specified significance level.

References

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Supplementary Figure Legends

Supplementary Figure 1. tdTom$^+$ cells in the GI mucosa are EC cells, not mast cells. (*A*) tdTom$^+$ cells colocalized with 5-HT in duodenum at 3 days post-Tx or oil treatment. (*B*) tdTom$^+$ cells were not colocalized with tryptase in the antrum, corpus, duodenum, and colon at 3 days post-Tx treatment. DAPI staining shows cell nuclei.

Supplementary Figure 2. Mature and self-renewal EC cells in *Tph1-tdTom* mice. (*A*) tdTom$^+$ cells in the 8 regions of the GI tracts at 1 week, 1 month, 2 months, and 6 months post-Tx treatment. (*B-D*) tdTom$^+$ cells stained with Ki67 antibody in the antrum (*B*), duodenum (*C*), and colon (*D*) at 1 month post-Tx treatment. (*E*) tdTom$^+$ cells stained with Lgr5 antibody in colon at 1 month post-Tx treatment. DAPI staining shows cell nuclei. (*F*) Quantification of tdTom$^+$ cells stained with Ki67 antibody at 3 days and 1 month post-Tx treatment. The number of tdTom$^+$ Ki67$^+$/tdTom$^+$ cells was averaged in antrum, duodenum, and colon (n=6). Error bars indicate SEM, unpaired *t*-test. ****p < 0.001*.

Supplementary Figure 3. Depletion of EC cells leads to delayed gastric emptying and STC in *Tph1-DTA* mice. (*A*, *B*) Automated Western blot and quantification of TPH1 expression in the colon of *Tph1-DTA* mice with oil or Tx treatment (n=4). (*C-F*) GET in male and female *Tph1-DTA* mice with Tx or oil treatment. Fluorescent images before and after intragastric gavage of a solid food mixed with GastroSense 750 (*C* and *E*) and percentage of gastric emptying quantified at 30 minutes (*D* and *F*, n=3-5). (*G*) Fecal pellets collected from male and female *Tph1-DTA* mice with oil or Tx treatment. (*H*, *I*) Fecal pellet number in mice with oil or Tx treatment (n=4-5).

(*J*, *K*) Normalized fecal pellet water content in male and female mice with oil or Tx treatment (n=5-6). (*L*, *M*) Body weight of males and females before and after oil or Tx administration (One-way ANOVA, n=4-6). (*N*, *O*) Fasting blood glucose levels of males and females at 7 days post-oil or Tx treatments (n=3-6). Error bars indicate SEM, unpaired *t*-test. ***p < 0.01, ***p < 0.001, ****p < 0.0001*.

Supplementary Figure 4. Tx does not alter *in vivo* and *ex vivo* GI motility. (*A*) Tx injection and analysis plan for *C57*, *Rosa26*$^{DTA/+}$, and *Tph1*$^{CreERT2/+}$ mice. (*B, C*) Fluorescent images before and after an intragastric gavage of a solid food mixed with GastroSense 750 and percentage of gastric emptying quantified at 30 mins (*C*) (n=3-7). (*D*, *E*) TGITT and CTT in no treatment and Tx-treated mice (n=3-5). (*F*) The frequency of the proximal colon contractions per 30 mins in *Tph1-DTA* mice with oil treatment and C57 mice with Tx treatment (n=4-6). (*G*) The propagation of the proximal colon contractions per 30 mins was analyzed in *Tph1-DTA* mice with oil and *Rosa26*$^{DTA/+}$ mice with Tx treatment (n=4-7). Error bars indicate SEM, One-way ANOVA.

Supplementary Figure 5. Delayed GI transit is naturally recovered at 21 days post-Tx treatment in EC cell-depleted mice. (*A*) Tx induction and GI functional analysis plan of *Tph1-DTA* mice. (*B*) TGITT measured at days 3, 7, 14, 21, and 28 post-Tx treatment (n=4-8). (*C*, *D*) Fecal pellet number and output in 24 hours measured at days 3, 7, 14, 21, and 28 post-Tx treatment (n=4). (*E*, *F*) Gastric emptying test at days 3 and 21 post-Tx treatment. Fluorescent images before and after an intragastric gavage of solid food mixed with GastroSense 750 (*E*) and percentage of gastric emptying quantified at 30 minutes (*F*) (n=4-6). (*G*) CTT measured at days 3 and 21 post-Tx treatment (n=4-7). Error bars indicate SEM, One-way ANOVA. ***p < 0.01, ***p < 0.001, ****p < 0.0001*.

Supplementary Figure 6. Exogenous 5-HT reverses impaired gut motility. (*A*) Frequency of initiated CMMCs from the proximal colon with exogenous 5-HT (0.1 µM) in the EC cell-depleted colon (One-way ANOVA, n=4-7). (*B*) CTT measured after an intragastric administration of 5-HT (2 mg/mL) or saline in EC cell-depleted mice (unpaired *t*-test, n=3-7). (*C*) Normalized fecal pellet water content measured after an intragastric administration of 5-HT (1 mg/mL) or saline in EC cell-depleted and control mice (One-way ANOVA, n=4-5). (*D*) TGITT in C57 mice with intragastric administration of 5-HT (1 mg/mL) and saline (unpaired *t*-test, n=4). Error bars indicate SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Supplementary Figure 7. Dietary 5-HTP improves GI motility in EC cell-depleted mice. (*A*) Biogenesis of 5-HT from L-tryptophan (Trp) by TPH1 and aromatic L-amino acid decarboxylase (AADC). (*B*) Schematic diagram of Tx induction and diet administration in *Tph1-DTA* mice. Diet 1 contains neither 5-HTP nor Trp; Diet 2 contains 5-HTP but no Trp; Diet 3 contains no 5-HTP, but Trp. (*C*) Fecal pellet output measured before and after dietary intervention (n=4). (*D*) TGITT measured after dietary intervention (n=3-6). Error bars indicate SEM, One-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Supplementary Figure 8. Correlations among biopsy 5-HT, demographic variables, and the gastroparesis cardinal symptom index-daily diary scores. (*A*) Plasma 5-HT levels measured in patients with IG (n=16) and healthy control samples (n=9) via ELISA assay (error bars indicate SEM, unpaired *t*-test). (*B*) Correlation matrix showing the Spearman's rank correlation among 5-HT in the fundus, body, antrum, duodenum, age, BMI, and gastroparesis cardinal symptoms (abdominal pain, nausea/vomit, fullness/satiety, bloating/abdominal pain, and aggregate) in patients with IG (n=11). *$p < 0.05$, **$p < 0.01$.

Supplementary Table 1. Oligonucleotides used in this study.

Name	Sequence (5' to 3')	Gene	Usage
Tph-1	GCCATGAATGAGTTGCGGTATGAC	Tph1	Genotyping
Tph-1r	GCTCTAGACTGATGCTCAAAGGC	Tph1	Genotyping
5-gt-1	GACCTGATGTACAACAGGGTCGGACTGT	Tph1	LR-PCR
5-gt-1r	GGTTTTGGTGCACAGTCAGCAGGTTGGAGA	Tph1	LR-PCR
3-gt-1	CGCCTTCTTGACGAGTTCTTCTGA	Tph1	LR-PCR
3-gt-1r	GGTATACAAAGTAATGGGTTCCATTGAGGCATGT	Tph1	LR-PCR
Cre-1r	GATATCTCCAACCTGCTGACTGTG	CreERT2	Genotyping
Del-1	GAGGATTGGGAAGACAATAGCAGG	Kan/neo	Genotyping

LR-PCR, Long range Polymerase chain reaction.

Supplementary Table 2. Diet formulation.

Diets	Diet 1	Diet 2	Diet 3
Formula		g/Kg	
L-Alanine	6.89	6.89	6.89
L-Arginine HCl	10.63	10.63	10.63
L-Asparagine	15.9	15.9	15.9
L-Aspartic Acid	4.38	4.38	4.38
L-Cystine	4.0	4.0	4.0
L-Glutamic Acid	48.23	48.23	48.23
Glycine	4.24	4.24	4.24
L-Histidine HCl, monohydrate	8.93	8.93	8.93
L-Isoleucine	13.25	13.25	13.25
L-Leucine	21.2	21.2	21.2
L-Lysine HCl	23.2	23.2	23.2
L-Phenylalanine	11.66	11.66	11.66
L-Proline	23.85	23.85	23.85
L-Serine	13.25	13.25	13.25
L-Threonine	10.07	10.07	10.07
L-Tryptophan	0	0	13.25
L-Tyrosine	12.19	12.19	12.19
L-Valine	15.9	15.9	15.9
Corn Starch	394.852	393.652	381.702
Sucrose	90.0	90.0	90.0
Maltodextrin	100.0	100.0	100.0
Lard	20.0	20.0	20.0
Soybean Oil	20.0	20.0	20.0
Cellulose	65.5	65.5	65.5
Mineral Mix, AIN-93M-MX (94049)	48.0	48.0	48.0
Calcium Phosphate, monobasic, monohydrate	8.0	8.0	8.0
Vitamin A Palmitate (500,000 IU/g)	0.0168	0.0168	0.0168
Vitamin D3, cholecalciferol (500,000 IU/g)	0.0042	0.0042	0.0042
Vitamin E, DL-alpha tocopheryl acetate (500 IU/g)	0.315	0.315	0.315
Vitamin K1, phylloquinone	0.0016	0.0016	0.0016
Thiamin (81%)	0.013	0.013	0.013
Riboflavin	0.013	0.013	0.013
Pyridoxine HCl	0.015	0.015	0.015
Biotin	0.0004	0.0004	0.0004
Niacin	0.063	0.063	0.063
Calcium Pantothenate	0.034	0.034	0.034
Folic Acid	0.002	0.002	0.002
Vitamin B12 (0.1% in mannitol)	0.02	0.02	0.02
Choline Chloride	1.18	1.18	1.18
L-Methionine	3.0	3.0	3.0
Betaine, anhydrous	1.0	1.0	1.0
5-Hydroxytryptophan, customer supplied	0	1.3	0
Food Color	Yellow 0.1	Green 0.1	Orange 0.1

Supplementary Table 3. Clinical information of human samples.

Age	Gender	Race	BMI	GES % 2hr	GES % 4hr	A1C	Scoring Abd Pain	GCSI-dd Aggregate	GCSI-dd Nausea/Vomit	GCSI-dd Fullness/Satiety	GCSI-dd Bloating/Abd Pain	Samples
IG												
34	M	White	21.4	72	40	5	1.5	2.0	1.2	2.5	1.8	Biopsy/Plasma
27	M	White	31.7	29	13	5	2	-	-	-	-	Plasma
56	M	White	26.7	31	11	5.4	0	-	-	-	-	Plasma
34	M	White	-	-	-	-	-	-	-	-	-	Biopsy
35	M	African	-	-	-	-	-	-	-	-	-	Plasma
45	M	White	-	-	-	-	-	-	-	-	-	Plasma
23	F	White	22.3	70	9	5.3	2.9	2.6	1.2	3.4	3.1	Biopsy/Plasma
23	F	White	-	42	20	-	-	-	-	-	-	Plasma
26	F	White	27.7	76	50	4.9	2.1	1.7	1.0	2.3	1.4	Biopsy
26	F	White	18.0	49	18	5	1.0	1.5	0.0	2.0	2.0	Biopsy/Plasma
26	F	White	-	76	-	-	-	-	-	-	-	Plasma
25	F	White	-	64	22	-	-	-	-	-	-	Plasma
29	F	Filipino	24.6	53	26	5.5	4.2	3.9	2.5	4.7	4.4	Biopsy/Plasma
31	F	White	29.3	51	22	5.5	0.6	1.9	1.1	3.0	0.4	Biopsy/Plasma
38	F	White	26.4	65	50	5	2.9	2.0	1.2	2.4	2.4	Biopsy
38	F	White	21.5	80	63	5.3	2.4	2.3	1.1	2.6	3.1	Biopsy/Plasma
50	F	Hispanic	20.8	58	27	5.4	3.0	2.4	0.3	3.5	3.0	Biopsy/Plasma
57	F	White	29.8	46	31	5.5	3.6	3.2	1.3	4.0	4.1	Biopsy/Plasma
58	F	White	27.9	92	61	5.4	0.1	2.4	1.1	3.9	1.2	Biopsy/Plasma
22	F	White	-	-	-	-	-	-	-	-	-	Biopsy
29	F	White	-	-	-	-	-	-	-	-	-	Plasma
37	F	White	-	-	-	-	-	-	-	-	-	Plasma
38	F	White	-	-	-	-	-	-	-	-	-	Biopsy
49	F	Hispanic	-	-	-	-	-	-	-	-	-	Biopsy
59	F	White	-	-	-	-	-	-	-	-	-	Biopsy

Control												
24	M	-	-	-	-	-	-	-	-	-	-	Plasma
26	M	Asian	23.4	-	-	5	0	-	-	-	-	Biopsy
34	M	Hispanic	23.8	-	-	-	0	-	-	-	-	Biopsy
40	M	White	52	-	-	5.9	0	-	-	-	-	Biopsy
41	M	White	-	-	-	-	-	-	-	-	-	Plasma
45	M	African	-	-	-	-	-	-	-	-	-	Plasma
50	M	White	27.8	-	-	5.8	0	-	-	-	-	Biopsy
57	M	White	22.7	-	-	5	0	-	-	-	-	Biopsy
62	M	White	26	-	-	5	0	-	-	-	-	Biopsy
25	F	-	-	-	-	-	-	-	-	-		Plasma
29	F	White	19.4	-	-	-	0	-	-	-	-	Biopsy
36	F	White	-	-	-	-	-	-	-	-	-	Plasma
38	F	White/Hisp	35.7	-	-	5.1	0	-	-	-	-	Biopsy
48	F	Hispanic	32	-	-	5.7	1	-	-	-	-	Biopsy
54	F	Hispanic	-	-	-	-	-	-	-	-	-	Plasma
54	F	Asian	-	-	-	-	-	-	-	-	-	Plasma
57	F	White	-	-	-	-	-	-	-	-	-	Biopsy
63	F	Asian	-	-	-	-	-	-	-	-	-	Plasma
65	F	Asian	28	-	-	-	0	-	-	-	-	Biopsy
66	F	White	28.3	-	-	5	-	-	-	-	-	Biopsy
66	F	White	-	-	-	-	-	-	-	-	-	Biopsy
68	F	White	25.2	-	-	5.3	0	-	-	-	-	Biopsy
69	F	White	-	-	-	-	-	-	-	-	-	Biopsy

IG, idiopathic gastroparesis; BMI, body mass index; GES, gastric emptying scintigraphy; Abd, abdominal; GCSI-dd, gastroparesis cardinal symptom index-daily diary.

Supplementary Table 4. Primary antibodies used in this study.

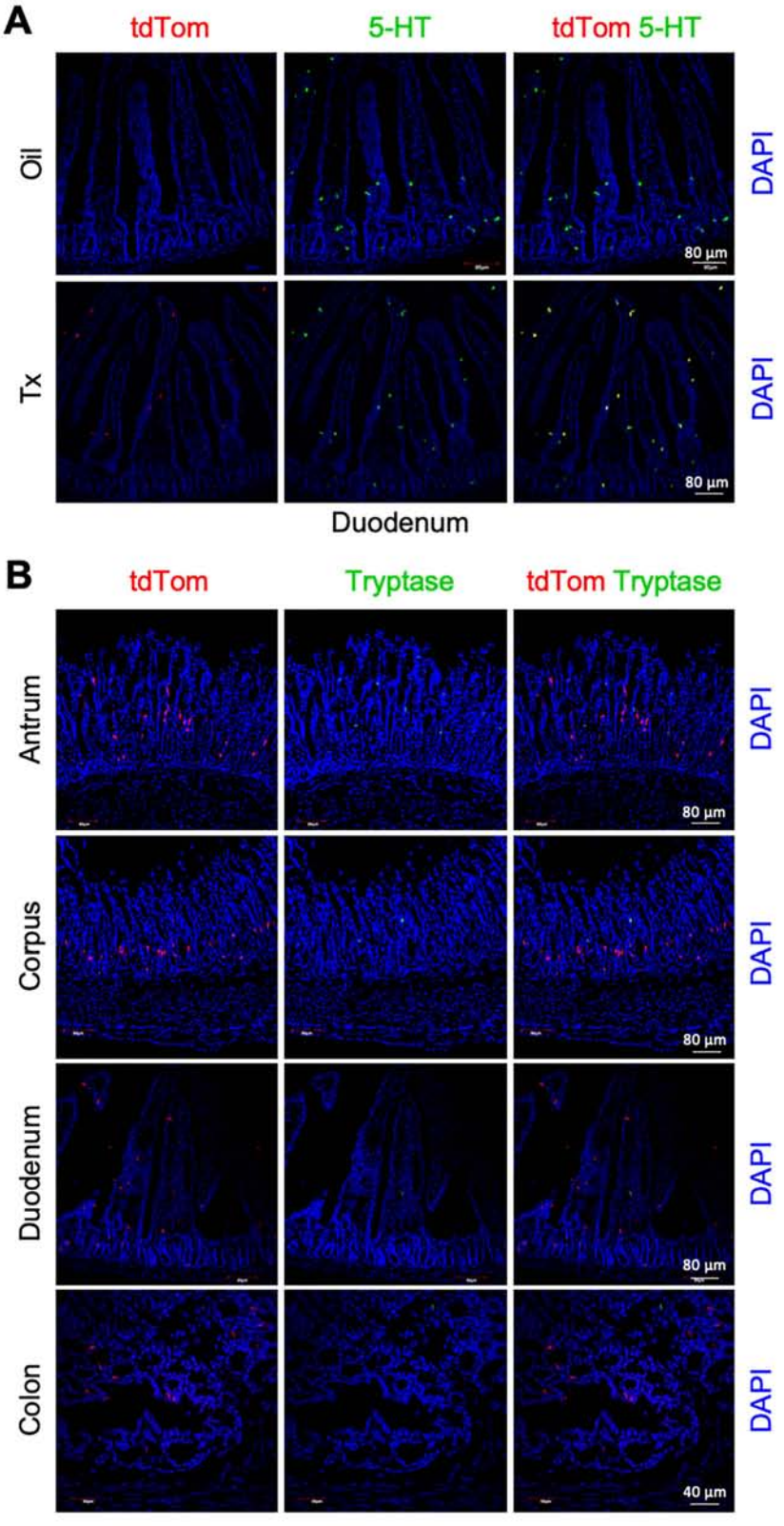
Antibodies	Company	Catalog No.	Specification
TPH1	Abcam	ab52954	IHC 1:200; WB 1:50; Wes,1:20
5-HT	LSBio	LS-C75755	IHC 1:500
Tryptase	Abcam	Ab151757	IHC 1:100
LGR5	Bioss Antibodies	bs-1117R	IHC 1:200
Ki67	Invitrogen	14-5698-82	IHC 1:200
GAPDH	Cell signaling	2118	WB 1:2000; Wes 1:100

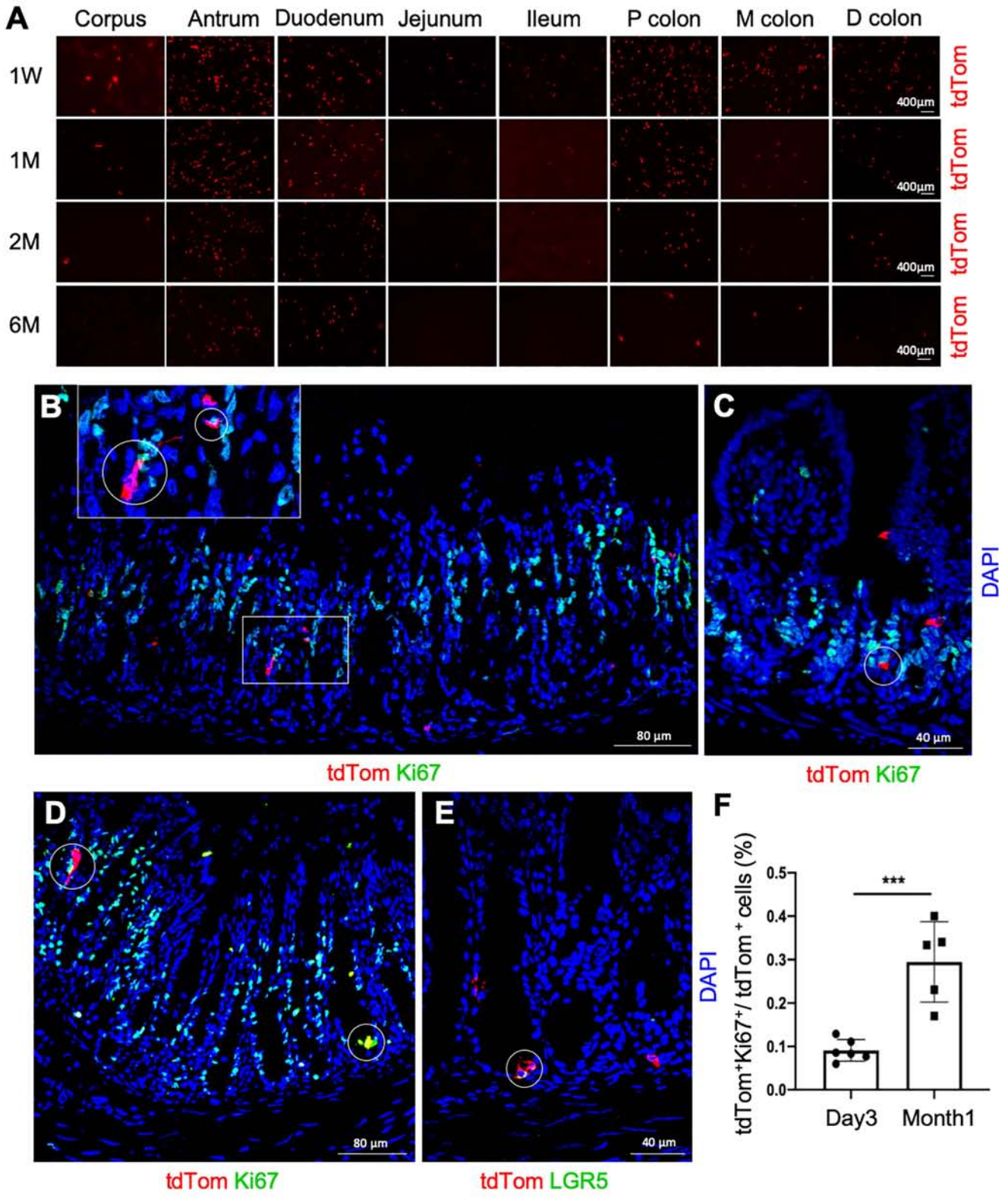
IHC, immunohistochemistry; WB, Western blot; Wes, Simple Western

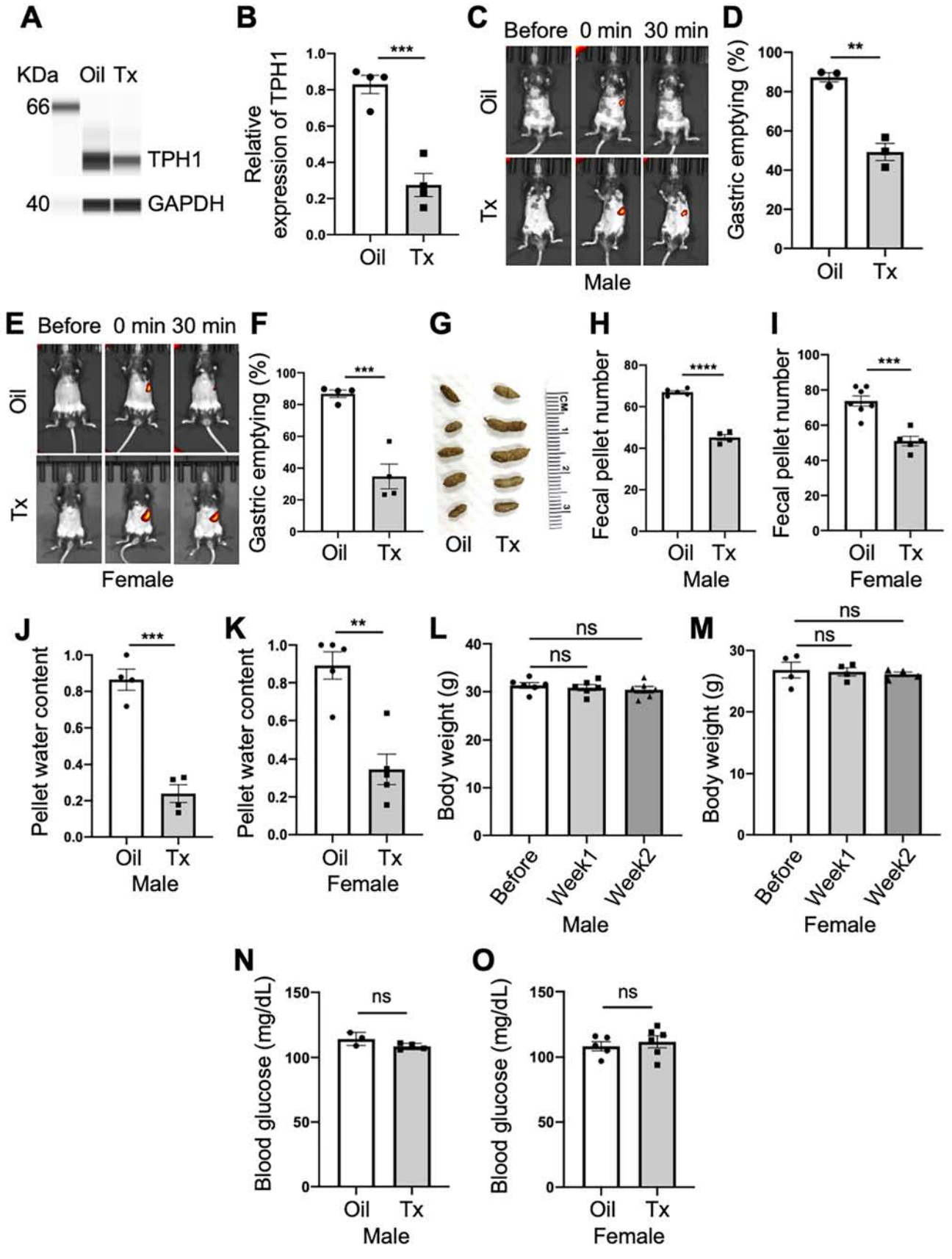
Supplementary Table 5. Correlation power analysis

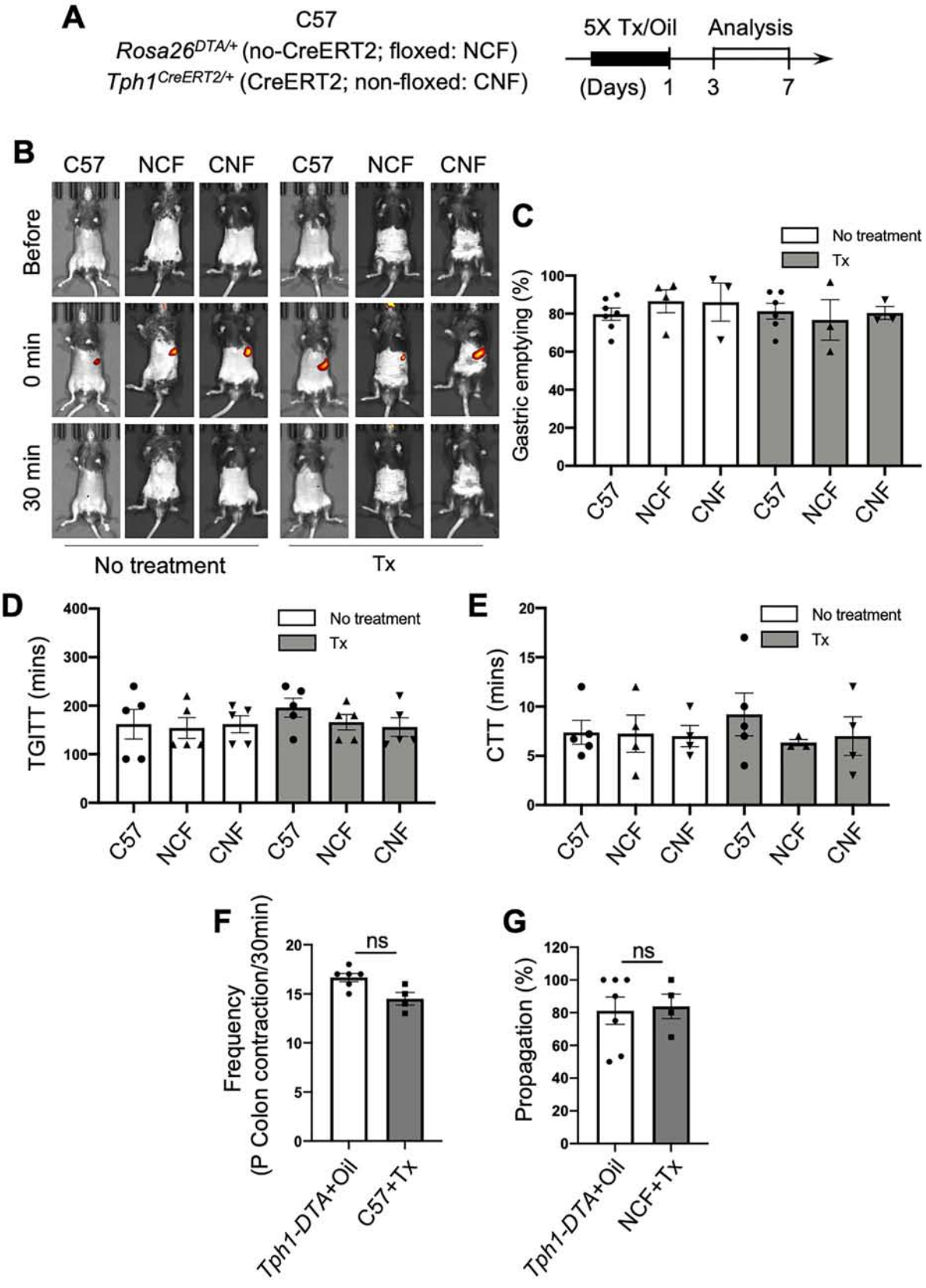
Correlations (N=11)	Coefficient	*p*-value	Power
GES% 4 hr/ 2 hr	0.679	0.025*	0.650
Antral 5-HT/ GES% 2 hr	-0.664	0.031*	0.600
Antral 5-HT/ GES% 4 hr	-0.793	0.005**	0.850
Age/ Duodenum 5-HT	-0.826	0.003**	0.925
BMI/ Body 5-HT	0.718	0.016*	0.750
Abd Pain/Bloating/ Abd Pain	0.893	0.001**	0.978
Aggregate/ Abd Pain	0.680	0.0258*	0.650
Aggregate/ Fullness/Satiety	0.913	0.001**	0.990
Aggregate/ Bloating/Abd Pain	0.723	0.014*	0.750

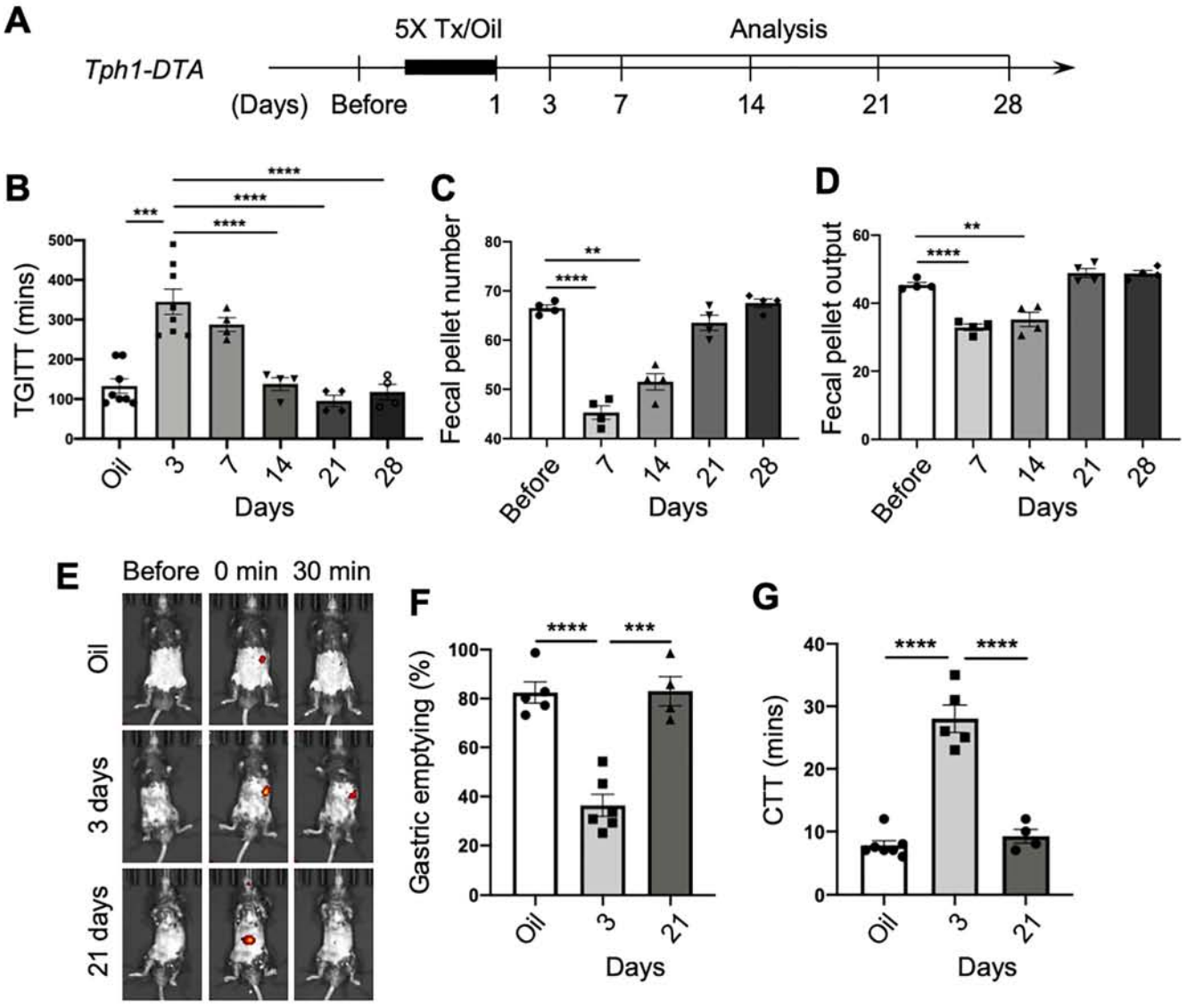
α=0.05, **p<0.05; **p<0.01.*

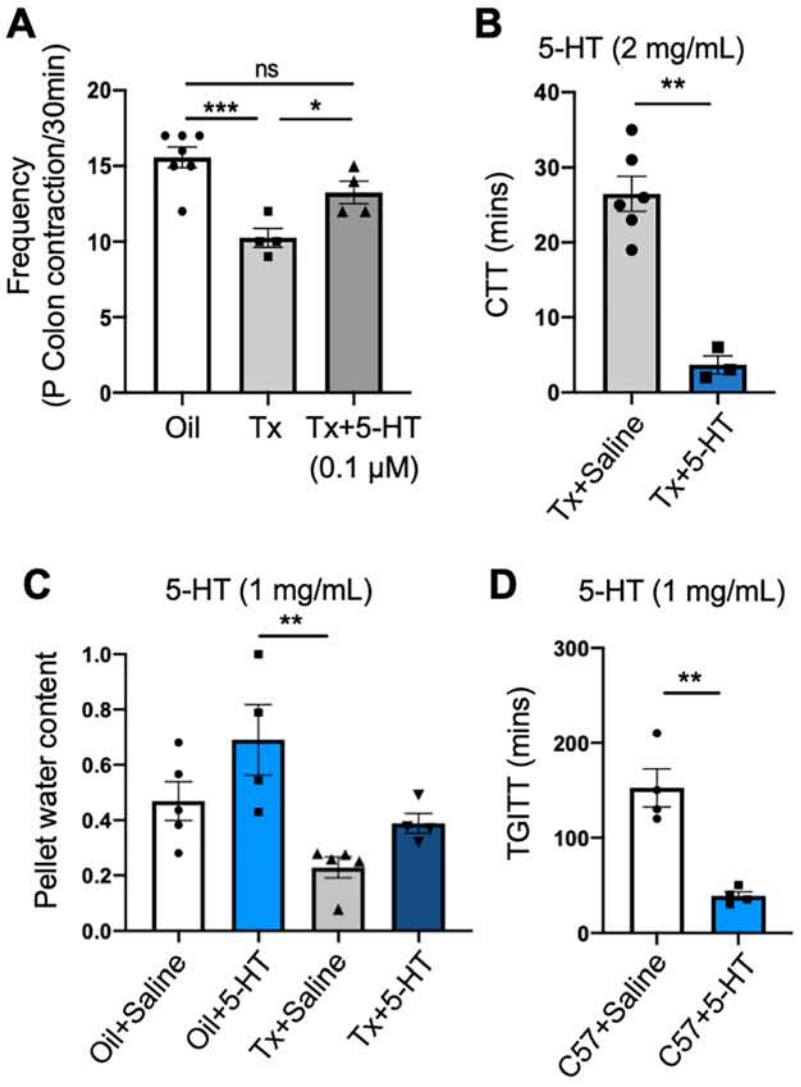

A
tdTom
5-HT
tdTom 5-HT
Oil
Tx
DAPI
DAPI
80 µm
80 µm
Duodenum
B
tdTom
Tryptase
tdTom Tryptase
Antrum
Corpus
Duodenum
Colon
DAPI
DAPI
DAPI
DAPI
80 µm
80 µm
80 µm
40 µm

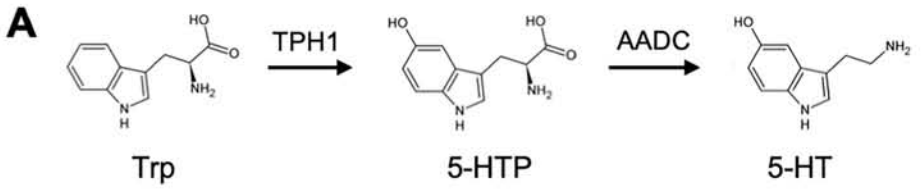

A
Corpus
Antrum
Duodenum
Jejunum
Ileum
P colon
M colon
D colon
1W
1M
2M
6M
tdTom
400μm
B
80 μm
tdTom Ki67
C
40 μm
DAPI
tdTom Ki67
D
80 μm
tdTom Ki67
E
40 μm
tdTom LGR5
F
tdTom+Ki67+/ tdTom+ cells (%)
0.5
0.4
0.3
0.2
0.1
0.0
***
Day3
Month1

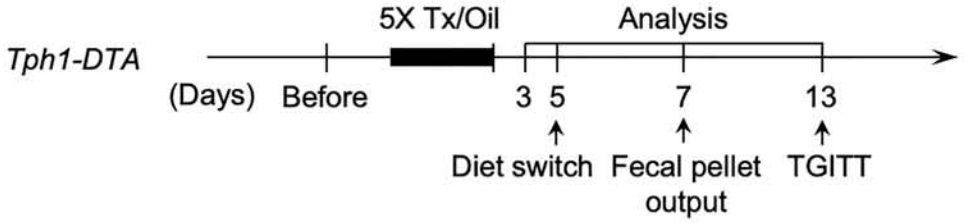

A
KDa
Oil
Tx
66
TPH1
40
GAPDH
B
Relative expression of TPH1
***
Oil
Tx
C
Before
0 min
30 min
Oil
Tx
Male
D
Gastric emptying (%)
**
Oil
Tx
E
Before
0 min
30 min
Oil
Tx
Female
F
Gastric emptying (%)
***
Oil
Tx
G
Oil
Tx
H
Fecal pellet number
****
Oil
Tx
Male
I
Fecal pellet number
***
Oil
Tx
Female
J
Pellet water content
***
Oil
Tx
Male
K
Pellet water content
**
Oil
Tx
Female
L
Body weight (g)
ns
ns
Before
Week1
Week2
Male
M
Body weight (g)
ns
ns
Before
Week1
Week2
Female
N
Blood glucose (mg/dL)
ns
Oil
Tx
Male
O
Blood glucose (mg/dL)
ns
Oil
Tx
Female

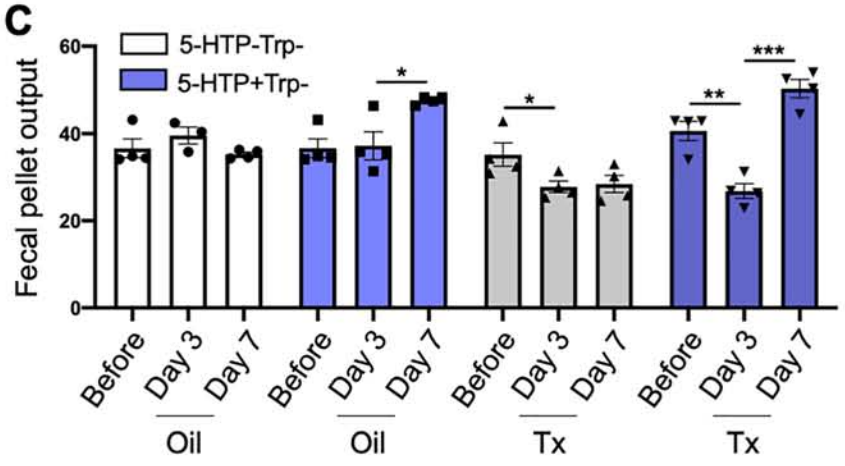

A
C57
Rosa26DTA/+ (no-CreERT2; floxed: NCF)
Tph1CreERT2/+ (CreERT2; non-floxed: CNF)
5X Tx/Oil
Analysis
(Days) 1 3 7
B
C57 NCF CNF C57 NCF CNF
Before
0 min
30 min
No treatment
Tx
C
No treatment
Tx
Gastric emptying (%)
0 20 40 60 80 100
C57 NCF CNF C57 NCF CNF
D
No treatment
Tx
TGITT (mins)
0 100 200 300 400
C57 NCF CNF C57 NCF CNF
E
No treatment
Tx
CTT (mins)
0 5 10 15 20
C57 NCF CNF C57 NCF CNF
F
ns
Frequency
(P Colon contraction/30min)
0 5 10 15 20
Tph1-DTA+Oil
C57+Tx
G
ns
Propagation (%)
0 20 40 60 80 100 120
Tph1-DTA+Oil
NCF+Tx

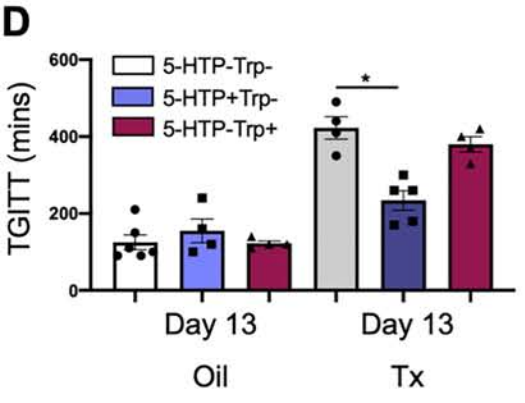

A
5X Tx/Oil
Analysis
Tph1-DTA
(Days) Before 1 3 7 14 21 28
B
TGITT (mins)
Oil 3 7 14 21 28
Days
C
Fecal pellet number
Before 7 14 21 28
Days
D
Fecal pellet output
Before 7 14 21 28
Days
E
Before 0 min 30 min
Oil
3 days
21 days
F
Gastric emptying (%)
Oil 3 21
Days
G
CTT (mins)
Oil 3 21
Days

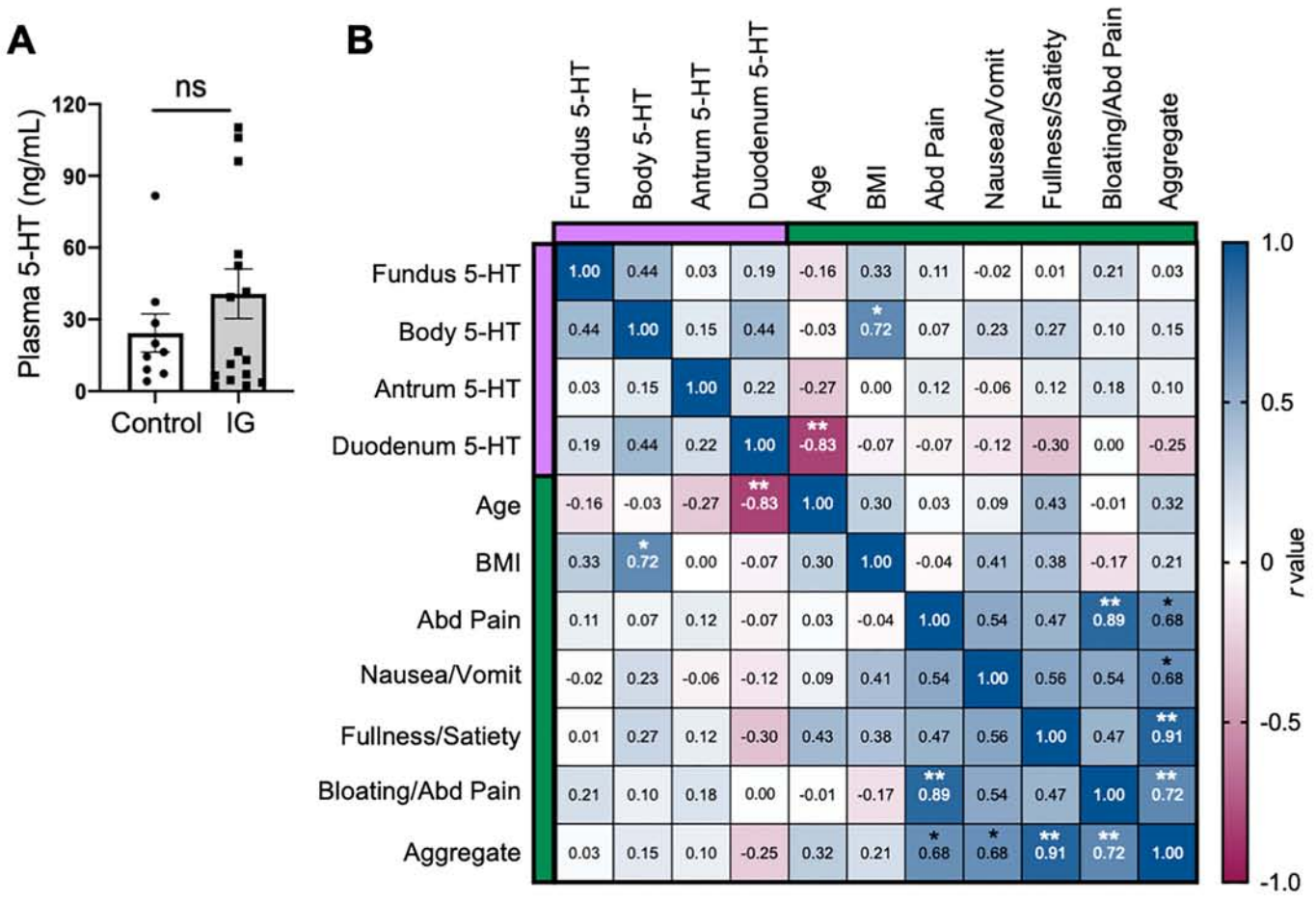

A
Frequency
(P Colon contraction/30min)
ns
***
*
Oil
Tx
Tx+5-HT
(0.1 μM)
B
5-HT (2 mg/mL)
**
CTT (mins)
Tx+Saline
Tx+5-HT
C
5-HT (1 mg/mL)
**
Pellet water content
Oil+Saline
Oil+5-HT
Tx+Saline
Tx+5-HT
D
5-HT (1 mg/mL)
**
TGITT (mins)
C57+Saline
C57+5-HT


A
TPH1
AADC
Trp
5-HTP
5-HT


B
Tph1-DTA
5X Tx/Oil
Analysis
(Days)
Before
3
5
7
13
Diet switch
Fecal pellet output
TGITT


C
Fecal pellet output
5-HTP-Trp-
5-HTP+Trp-
Before
Day 3
Day 7
Oil
Tx


D
TGITT (mins)
5-HTP-Trp-
5-HTP+Trp-
5-HTP-Trp+
Day 13
Oil
Tx


A
ns
Plasma 5-HT (ng/mL)
120
90
60
30
0
Control
IG
B
Fundus 5-HT
Body 5-HT
Antrum 5-HT
Duodenum 5-HT
Age
BMI
Abd Pain
Nausea/Vomit
Fullness/Satiety
Bloating/Abd Pain
Aggregate
1.00 0.44 0.03 0.19 -0.16 0.33 0.11 -0.02 0.01 0.21 0.03
0.44 1.00 0.15 0.44 -0.03 * 0.72 0.07 0.23 0.27 0.10 0.15
0.03 0.15 1.00 0.22 -0.27 0.00 0.12 -0.06 0.12 0.18 0.10
0.19 0.44 0.22 1.00 ** -0.83 -0.07 -0.07 -0.12 -0.30 0.00 -0.25
-0.16 -0.03 -0.27 ** -0.83 1.00 0.30 0.03 0.09 0.43 -0.01 0.32
0.33 * 0.72 0.00 -0.07 0.30 1.00 -0.04 0.41 0.38 -0.17 0.21
0.11 0.07 0.12 -0.07 0.03 -0.04 1.00 0.54 0.47 ** 0.89 * 0.68
-0.02 0.23 -0.06 -0.12 0.09 0.41 0.54 1.00 0.56 0.54 * 0.68
0.01 0.27 0.12 -0.30 0.43 0.38 0.47 0.56 1.00 0.47 ** 0.91
0.21 0.10 0.18 0.00 -0.01 -0.17 ** 0.89 0.54 0.47 1.00 ** 0.72
0.03 0.15 0.10 -0.25 0.32 0.21 * 0.68 * 0.68 ** 0.91 ** 0.72 1.00
1.0
0.5
0
-0.5
-1.0
r value