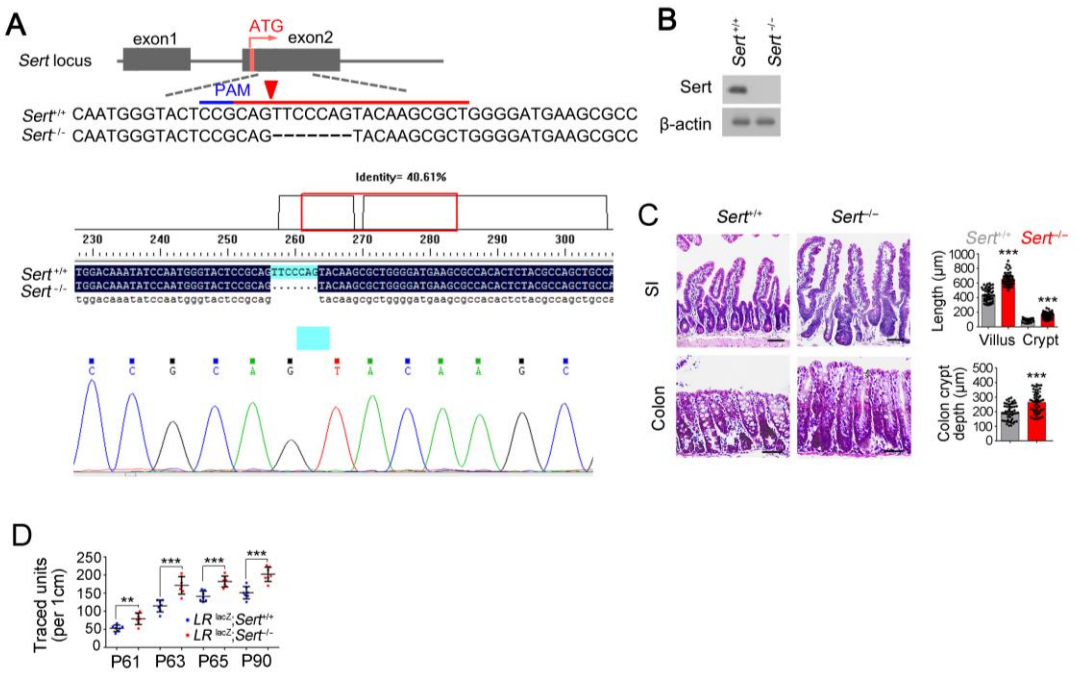
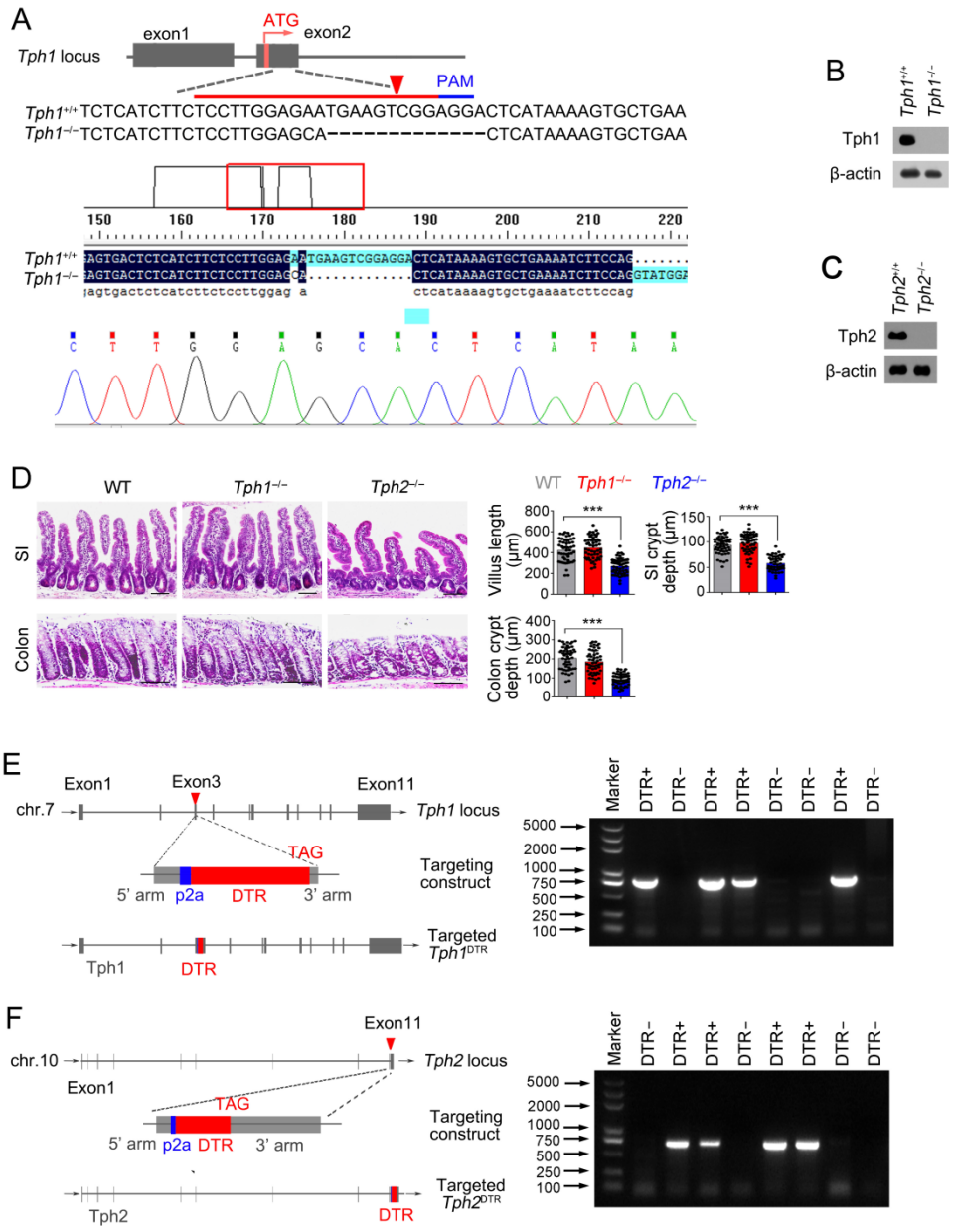
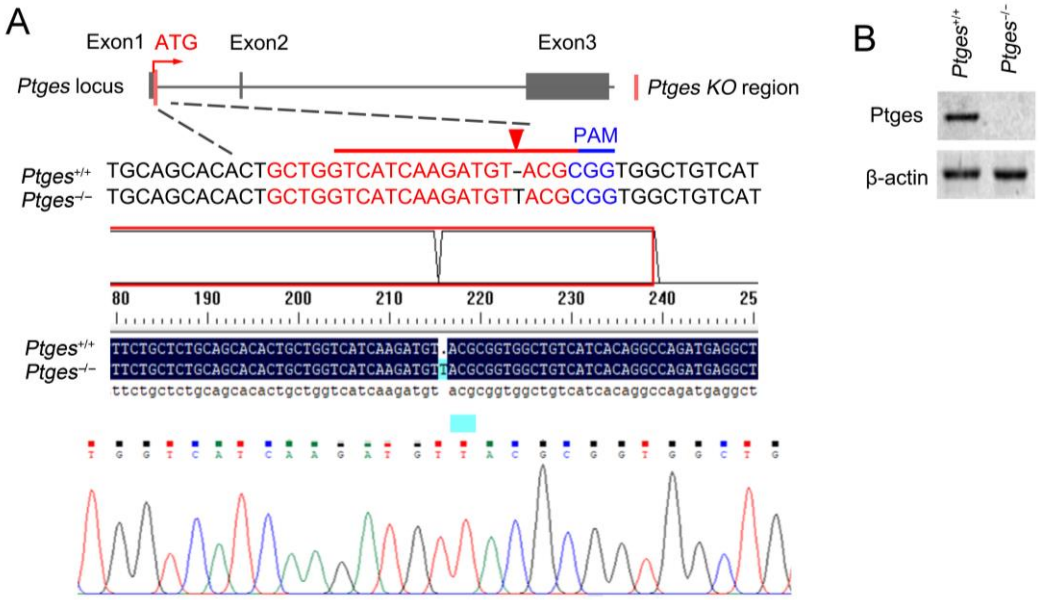
Supplementary Figures & Figure Legends



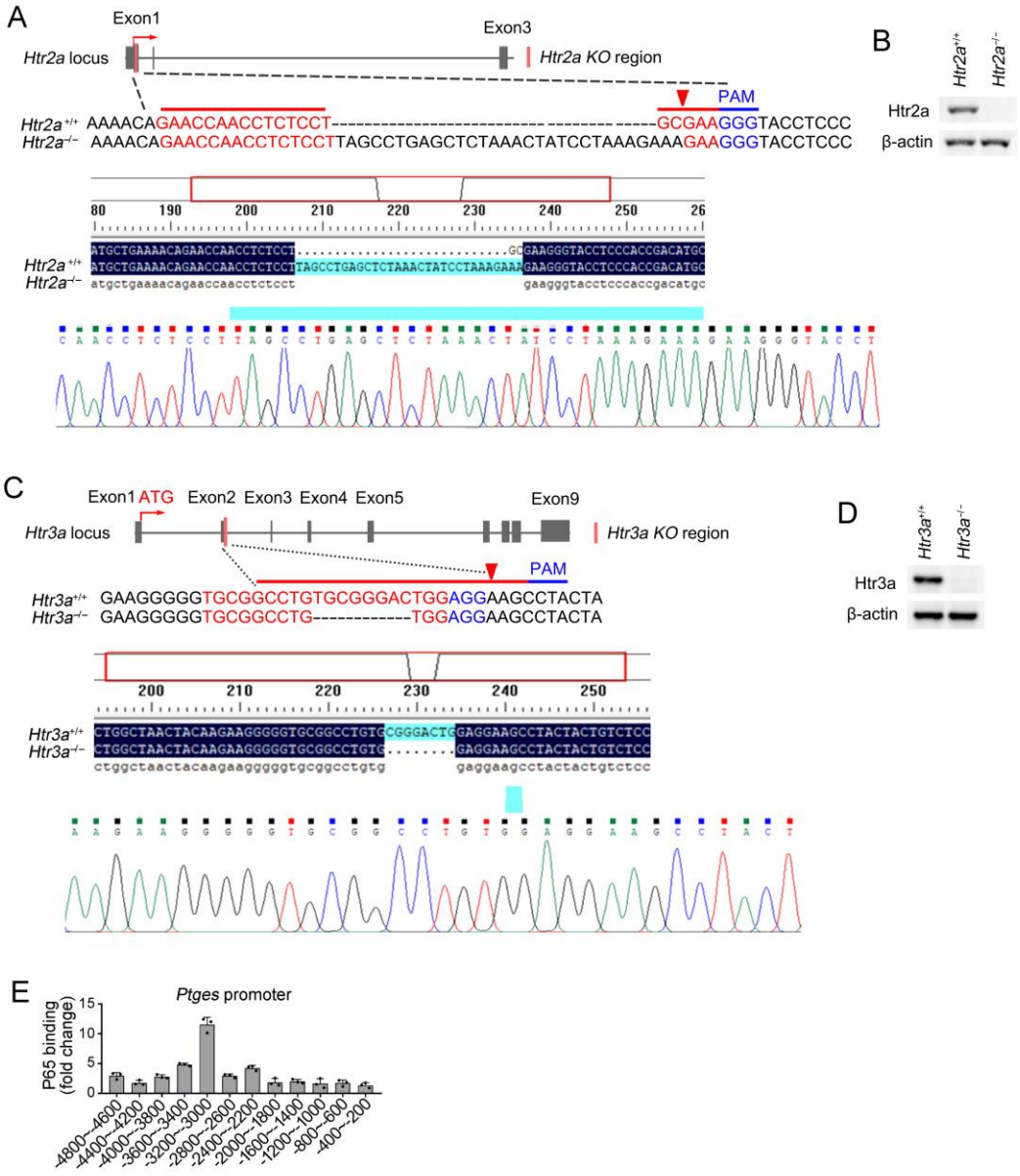
Supplementary Figure 1. Generation of *Sert* KO mice. (A) Schematic representation of *Sert* knockout mice. sgRNA sequence and PAM sequence were marked with red and blue lines, respectively. DNA sequencing results were shown in lower panel. (B) Sert was probed in *Sert* knockout intestines by Western blot. β-actin served as a loading control. (C) H&E staining of small intestine (SI) and colon of *Sert* knockout mice. Typical images were shown in left panel and *n*=50 fields from five mice were observed in right panel. (D) *Lgr5*$^{GFP\text{-}CreERT2}$;*Rosa26*$^{lsl\text{-}lacZ}$ (*LR*^{lacZ}) mice were crossed with *Sert*$^{-/-}$ mice, followed by administration of tamoxifen (TAM) for lineage tracing analysis through intestinal whole-mount staining for β-gal. Numbers of traced crypt-villus units (blue plots) every 1 cm intestine at indicated time points were shown in lower panel (means±s.d).



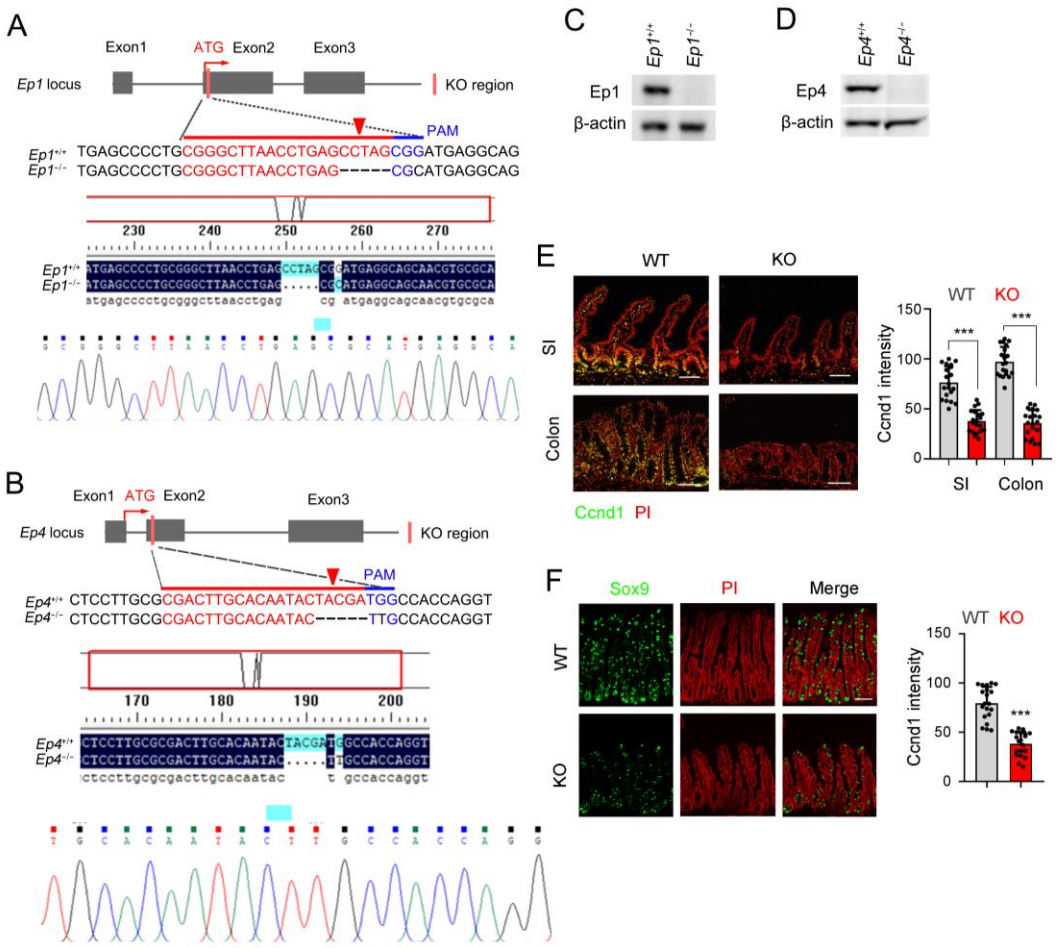
Supplementary Figure 2. Generation of *Tph1* KO, *Tph1*^{DTR} and *Tph2*^{DTR} mice. (A) Schematic representation of *Tph1* knockout mice. sgRNA sequence and PAM sequence were shown. *Tph1* knockout was determined by DNA sequencing (lower panel). (B, C) Tph1 in *Tph1* knockout (B) and Tph2 in *Tph2* knockout (C) intestines were detected by Western blot. β-actin served as loading controls. (D) H&E staining of small intestine (SI) and colon of *Tph1* and *Tph2* knockout mice. Typical images were shown in left panel and *n*=50 fields from five mice were observed and shown in right panel. Scale bars, 100 μm. (E, F) Schematic representation and electrophoresis confirmation of *Tph1*^{DTR} (E) and *Tph2*^{DTR} (F) mice. sgRNA sequence and PAM sequence were shown. DTR insertion was finally confirmed by DNA sequencing.



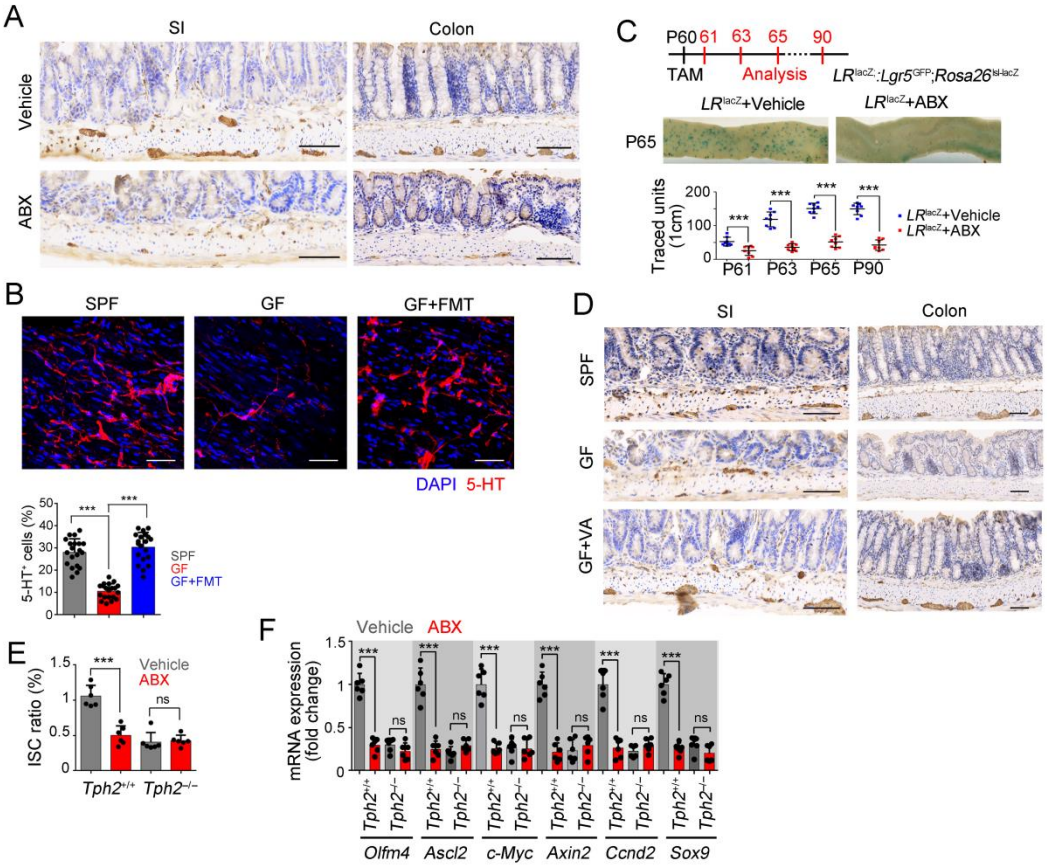
Supplementary Figure 3. Generation of Ptges KO mice. (A, B) *Ptges* knockout mice were generated by CRISPR/Cas9 approach, and knockout efficiency was confirmed by DNA sequencing (A) and Western blot (B).



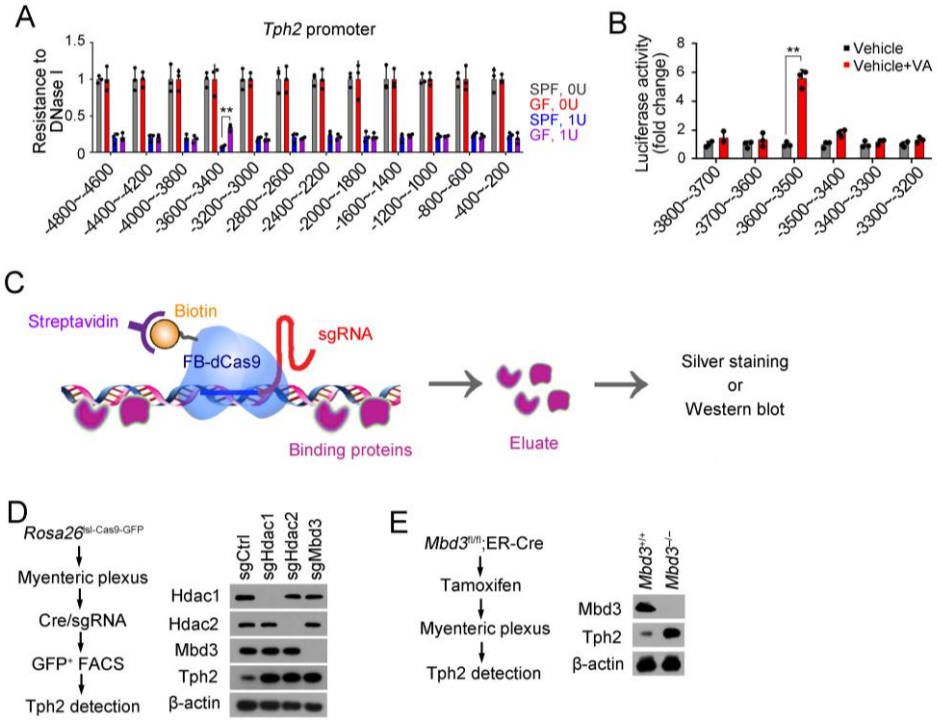
Supplementary Figure 4. Generation of Htr2a and Ktr3a KO mice. (A, B) *Htr2a* KO mice were generated by CRISPR/Cas9 strategy, and examined by DNA sequencing (A) and Western blot as in (B). (C, D) *Htr3a* KO mice were generated as (A, B). (E) Realtime PCR analysis for Ptges promoter enrichment in ChIP eluate, using P65 antibody and indicated treated macrophages. At least three independent experiments were performed with similar results.



Supplementary Figure 5. PGE2 activates Wnt/β-catenin signaling. (A-D) Ep1 and Ep4 knockout mice were generated through CRISPR/Cas9 approach (A, B), and confirmed by Western blot (C, D). (E) Cyclin D1 immunohistochemistry was performed using WT and DKO intestines, and observed by microscope. Scale bars, 100 μm. (F) WT and DKO intestines were used for Sox9 detection, and typical images were shown. Scale bars, 100 μm. At least three independent experiments were performed with similar results, and representative experiments were shown.



Supplementary Figure 6. Gut microbiota promotes Thp2 expression and ISC self-renewal. (A) 2-month-old mice were treated by Ampicillin, Neomycin and Vancomycin (ABX), and expression levels of Tph2 in small intestines (SI) and colons were detected by immunohistochemistry. Scale bars, 100 μm. (B) Myenteric plexus was isolated from indicated mice, followed by 5-HT detection. Typical images were shown in upper panel, and ratios of 5-HT^{+} cells were shown in lower panel. Scale bars, 50 μm. (C) *Lgr5*$^{GFP\text{-}CreERT2}$; *Rosa26*$^{lsl\text{-}lacZ}$ (*LR*^{lacZ}) were treated by ABX or vehicle, followed by administration of tamoxifen (TAM) for lineage tracing analysis through intestinal whole-mount staining for β-gal. *n*=8 mice were sacrificed at indicated time points and typical jejunum sections on P65 (postnatal day 65) were shown. Numbers of traced crypt-villus units (blue plots) at indicated time points were shown in lower panel (means±s.d). (D) Small intestine (SI) and colon tissues from treated mice were detected for Thp2 expression by immunohistochemistry. VA, valeric acid. Scale bars, 100 μm. (E, F) *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ mice were treated with ABX, followed by detection of ISC ratios (E) and mRNA levels of ISCs and Wnt/β-catenin target genes (F). At least three independent experiments were performed with similar results, and representative experiments were shown.



Supplementary Figure 7. Gut microbiota promotes Thp2 expression and ISC self-renewal. (A) Myenteric plexus was isolated from indicated mice for genome extraction. DNase sensibility of different regions of *Tph2* promoter was analyzed by realtime PCR. (B) Indicated regions of *Tph2* promoter were cloned into PGL3 luciferase plasmid, and transfected into myenteric plexus cells and treated with VA. 36 h later, the activation of *Tph2* promoter was detected by luciferase detection. (C) Schematic diagram of CAPTURE assay (CRISPR affinity purification in situ of regulatory elements). (D) *Hdac1*, *Hdac2* and *Mbd3* genes were deleted in myenteric plexus cells from *Rosa26*$^{lsl\text{-}Cas9\text{-}GFP}$ mice through CRISPR/Cas9 approach, followed by Tph2 detection. Procedure diagram was shown in left panel and Western results were in right panel. (E) *Mbd3* knockout cells were established and Tph2 expression was measured by Western blot. At least three independent experiments were performed with similar results, and representative experiments were shown.

Supplementary Table 1. sgRNA sequences used in this study.

sgRNA	Sequences
Sert	5’-AGCGCTTGTACTGGGAACTGCGG-3’
Tph1	5’-TCCTTGGAGAATGAAGTCGGAGG-3’
Htr2a	5’-GAACCAACCTCTCCTGCGAAGGG-3’
Htr3a	5’-TGCGGCCTGTGCGGGACTGGAGG-3’
Ptges	5’-GCTGGTCATCAAGATGTACGCGG-3’
Ep1	5’-CGGGCTTAACCTGAGCCTAGCGG-3’
Ep4	5’-CGACTTGCACAATACTACGATGG-3’

Supplementary Table 2. PCR primers used in this study.

Primers	Sequences
18S (Forward)	5'-AACCCGTTGAACCCCATT-3'
18S (Reverse)	5'-CCATCCAATCGGTAGTAGCG-3'
actin (Forward)	5'-GGCTGTATTCCCCTCCATCG-3'
actin (Reverse)	5'-CCAGTTGGTAACAATGCCATGT-3'
Tph1-CDS1-245 (Forward)	5'-AACTTTCACACTTCAGATTCACC-3'
Tph1-CDS1-245 (Reverse)	5'-ATAGGCCGTCTCTGAGGAAC-3'
Htr1b-250 (Forward)	5'-CCAGGACGAGGAGAGCTATG-3'
Htr1b-250 (Reverse)	5'-GTGTGTAGCTTCCGGGTCC-3'
Htr1d-245 (Forward)	5'-CTTGCTCTGTGGATCGGACT-3'
Htr1d-245 (Reverse)	5'-GACGAAGGCATTGGAGAGGA-3'
Htr1f-234 (Forward)	5'-ACGCATCAGACCAAAACTTGA-3'
Htr1f-234 (Reverse)	5'-ACACAATACTGAAGGGCATCAC-3'
Sert-245 (Forward)	5'-CCCCACACCAGCAGACAA-3'
Sert-245 (Reverse)	5'-CATTCTGGTAGCATATGTAGGGA-3'
Htr1a (Forward)	5'-GACAGGCGGCAACGATACT-3'
Htr1a (Reverse)	5'-CCAAGGAGCCGATGAGATAGTT-3'
Htr1b (Forward)	5'-CGCCGACGGCTACATTTAC-3'
Htr1b (Reverse)	5'-TAGCTTCCGGGTCCGATACA-3'
Htr1d (Forward)	5'-ATCACCGATGCCCTGGAGTA-3'
Htr1d (Reverse)	5'-GCCAGAAGAGTGGAGGGATG-3'
Htr1f (Forward)	5'-ATCAACTCCCTCGTGATCGC-3'
Htr1f (Reverse)	5'-ACACGTACAACAGATGATGTCG-3'
Htr2a (Forward)	5'-TAATGCAATTAGGTGACGACTCG-3'
Htr2a (Reverse)	5'-GCAGGAGAGGTTGGTTCTGTTT-3'
Htr2b (Forward)	5'-GAACAAAGCACAACTTCTGAGC-3'
Htr2b (Reverse)	5'-CCGCGAGTATCAGGAGAGC-3'
Htr2c (Forward)	5'-CTAATTGGCCTATTGGTTTGGCA-3'

Htr2c (Reverse)	5’-CGGGAATTGAAACAAGCGTCC-3’
Htr3a (Forward)	5’-CCTGGCTAACTACAAGAAGGGG-3’
Htr3a (Reverse)	5’-TGCAGAAACTCATCAGTCCAGTA-3’
Htr3b (Forward)	5’-CTGTCTACCTGGACCTTTGCG-3’
Htr3b (Reverse)	5’-AACTCATCGTTCCAAACCTCTC-3’
Htr4 (Forward)	5’-AGTTCCAACGAGGGTTTCAGG-3’
Htr4 (Reverse)	5’-CAGCAGGTTGCCCAAGATG-3’
Htr5a (Forward)	5’-ATGGATCTGCCTGTAAACTTGAC-3’
Htr5a (Reverse)	5’-CACTCGGAAAGCTGAGAGAAAA-3’
Htr5b (Forward)	5’-TTGCTGATCGCTGCCACTTT-3’
Htr5b (Reverse)	5’-GTCGAGGCCACCAAGTTATGT-3’
Htr6 (Forward)	5’-GCTGTGCGTGGTCATCGTA-3’
Htr6 (Reverse)	5’-CATCAGGTCCGACGTGAAGAG-3’
Htr7 (Forward)	5’-CCGACCTCTACGGCCATCT-3’
Htr7 (Reverse)	5’-TCTCGACTCTGCCATAGTTGAT-3’
Tph2 (Forward)	5’-GGTTGTCCTTGGATTCTGCTG-3’
Tph2 (Reverse)	5’-GCCTGGATTCGATATGAAGCAT-3’
Ctnnb1 (Forward)	5’-ATGGAGCCGGACAGAAAAGC-3’
Ctnnb1 (Reverse)	5’-CTTGCCACTCAGGGAAGGA-3’
Tcf3 (Forward)	5’-GGGTGCCAGCGAGATCAAG-3’
Tcf3 (Reverse)	5’-ATGAGCAGTTTGGTCTGCGG-3’
Axin2 (Forward)	5’-TGACTCTCCTTCCAGATCCCA-3’
Axin2 (Reverse)	5’-TGCCCACACTAGGCTGACA-3’
Myc (Forward)	5’-ATGCCCCTCAACGTGAACTTC-3’
Myc (Reverse)	5’-CGCAACATAGGATGGAGAGCA-3’
Lgr5 (Forward)	5’-CCTACTCGAAGACTTACCCAGT-3’
Lgr5 (Reverse)	5’-GCATTGGGGTGAATGATAGCA-3’
Tcf7 (Forward)	5’-AGCTTTCTCCACTCTACGAACA-3’
Tcf7 (Reverse)	5’-AATCCAGAGAGATCGGGGGTC-3’

Ccnd2 (Forward)	5’-GAGTGGGAACTGGTAGTGTTG-3’
Ccnd2 (Reverse)	5’-CGCACAGAGCGATGAAGGT-3’
Sox4 (Forward)	5’-CGGCTGCATCGTTCTCTCC-3’
Sox4 (Reverse)	5’-GGTAGACGTGCTTCACTTTCTTG-3’
Tph2P (-4800 Forward)	5’-TCCTGCATGGCTTCTGTGGA -3’
Tph2P (-4600 Reverse)	5’-CTGGTAACCATCTCTGAAAG -3’
Tph2P (-4400 Forward)	5’-GTGAGAAAGAACTTTGACAA-3’
Tph2P (-4200 Reverse)	5’-AGGATGTATACTTGTTCTGT -3’
Tph2P (-4000 Forward)	5’-TGATATGTAAATAAAAGACT-3’
Tph2P (-3800 Reverse)	5’-CCTATTCGTGTGAAAAGGAC-3’
Tph2P (-3600 Forward)	5’-CAGCAGTCTGGAGTCCTCTG -3’
Tph2P (-3400 Reverse)	5’-ATCCACTGTATTTGTGGTCA -3’
Tph2P (-3200 Forward)	5’-GCTTCTCAGCAACTTTATTG -3’
Tph2P (-3000 Reverse)	5’-TTCAATACTGTAACCACTTT -3’
Tph2P (-2800 Forward)	5’-AGCAAAACAAAAAATAAAGA-3’
Tph2P (-2600 Reverse)	5’-GCCCAGAAGCAGTTGGCCAA-3’
Tph2P (-2400 Forward)	5’-TTGCAGTGCTTGGATAAAAG-3’
Tph2P (-2200 Reverse)	5’-GCTTCTTGGAGTAGATGCTA-3’
Tph2P (-2000 Forward)	5’-ATCTTGAAGTCCTCTTCCAA-3’
Tph2P (-1800 Reverse)	5’-TCTAGATGTAGGGAAAGGAT -3’
Tph2P (-1600 Forward)	5’-TGTGTCTTCTGCAGAGAGGT-3’
Tph2P (-1400 Reverse)	5’-TATACATAAGAACAGATGTA-3’
Tph2P (-1200 Forward)	5’-CACACATATATACACACACA-3’
Tph2P (-1000 Reverse)	5’-CAAGTGTTAATAAATGAACC-3’
Tph2P (-800 Forward)	5’-TTTGGTTAGTTCTTCAGTTA-3’
Tph2P (-600 Reverse)	5’-CATCATTCCTTTGATACTCT-3’
Tph2P (-400 Forward)	5’-CACACACAGGTAAAATTTGC-3’
Tph2P (-200 Reverse)	5’-TCAGGGTTATTATTTTTTTT-3’

Tph2P, Tph2 promoter