

Supplementary Material

Table S1. Primers used for qRT-PCR assay.

Primer	Primer sequences (5' → 3')
<i>Scn7a</i> -F	TGAAGCTGTTTGGTCGAAG
<i>Scn7a</i> -R	TCGGAACACGGTCATGTA
<i>Kcna1</i> -F	AAGGGCTCCCGTAGTGTT
<i>Kcna1</i> -R	ACGCTGTCCTCTCTCTGG
<i>Gfap</i> -F	AGCGTGCAGAGATGATGG
<i>Gfap</i> -R	AGTTTGGTGGGCTCCTTG
<i>Pdgfrα</i> -F	ATGACAGCAGGCAGGGCTTCAACG
<i>Pdgfrα</i> -R	CGGCACAGGTCACCACGATCGTTT
<i>c-Kit</i> -F	CGCCTGCCGAAATGTATGACG
<i>c-Kit</i> -R	GGTTCTCTGGGTTGGGGTTGC
<i>Nos1</i> -F	ATCTGTCTCGCCAGCCATCAGCCA
<i>Nos1</i> -R	GGAGCTTTGTGCAGTTTGCCGTCG
<i>P2ry1</i> -F	ACCGAGGTGCCTTGGTCGGT
<i>P2ry1</i> -R	CCGGTCTTGGTCAGGGCACA
<i>Slc18a3</i> -F	TCATGTTTCGCCTCCACAGTC
<i>Slc18a3</i> -R	GCTCCTCGGGATACTTGTCG
<i>Scn5a</i> -F	GTTCGGCCACCTCACAG
<i>Scn5a</i> -R	AAAGGGTCCAGGTCTTCCA
<i>Snap25</i> -F	TCATCCGCAGGGTAACA
<i>Snap25</i> -R	GCGATTCTGGGTGTCAAT
<i>Syn2</i> -F	ATTCCCTGCGTGTGATAGA
<i>Syn2</i> -R	GGGGTGTGACTTGTTGAG
<i>Syt1</i> -F	CTTCTCCCTCCGCTACG
<i>Syt1</i> -R	TTCAGTCTCTTGCCGTTCT
<i>Gapdh</i> -F	GCCGATGCCCCCATGTTTGTGA
<i>Gapdh</i> -R	GGGTGGCAGTGATGGCATGGAC
<i>Sox10</i> -F	AACACATCGCTGCCCCTTTA
<i>Sox10</i> -R	GCAGGCAACCAGAAGCATTG
<i>Ret</i> -F	GCATGTCAGACCCGAAGTGG
<i>Ret</i> -R	CGCTGAGGGTGAAACCATCC
<i>Phox2b</i> -F	GCGAGAGTCCAGGTGTGGTT
<i>Phox2b</i> -R	CTCTTTGCTCTCGTCGTCCC
<i>Chat</i> -F	AAAATGGCGTCCAACGAGGA
<i>Chat</i> -R	CCCGGTTGGTGGAGTCTTTTA
<i>S100b</i> -F	CGAGAGGGTGACAAGCACAA
<i>S100b</i> -R	TCCTGCTCCTTGATTTCCTCCA

Table S2. All antibodies used for immunoblotting and immunohistochemistry.

Primary/secondary antibody	Catalog	Supplier	Application (Work concentration)
Anti-GFAP	ab7260	ABCAM	WB (1:5000)
Anti-PDGFR α	D1E1E XP	Cell Signaling Technology	Wes (1:100)
Anti-c-Kit	AF1356	R&D systems	Wes (1:100)
Anti-ANO1	ab53212	ABCAM	Wes (1:100)
Anti-NOS1	SC-5302	Santa Cruz Biotechnology	Wes (1:100)
Anti-P2RY1	ER1914-59	HuaBio Technology	WB (1:1000)
Anti-VACHT / SLC18A3	ab235201	ABCAM	WB (1:1000)
Anti-SK3	ab28631	ABCAM	WB (1:5000)
Anti- γ -ACTIN	GTX101794	Genetex	Wes (1:200)
Anti- β -ACTIN	ab8226	ABCAM	WB (1:10000)
HRP-conjugated Anti-rabbit IgG	AF008	R&D systems	Wes (1:1000), WB (1:5000)
HRP-conjugated Anti-mouse IgG	AF007	R&D systems	WB (1:5000)

Table S3. All drugs used in isometric force recording and CMCs assay.

Drug	Catalog	Supplier	Application (Work concentration)
LNNA	No.0665	Tocris	IFR & CMCs (100.0 μ M)
MRS2500	No.2159	Tocris	IFR & CMCs (1.0 μ M)
Atropine	A-046	Sigma-Aldrich	IFR & CMCs (1.0 μ M)

Supplementary Figure Legends

Figure S1. The differentially expressed genes in the whole colon of young mice. (A) The volcano plot of differentially expressed genes between PW1 and PW3 sample. (B) The volcano plot of differentially expressed genes between PW1 and PW5 sample. FC, fold change. The red and blue dots represent significantly up-regulated and down-regulated genes ($\text{Log}_2(\text{FC}) \geq 1.0$ or ≤ -1.0 , $P < 0.05$).

Figure S2. The gene ontology and pathway enrichment of differentially expressed genes. (A), (B) and (C) The top ten biological processes, cellular components, and molecular function of differentially expressed genes in the colon of PW3 mice, respectively. (D) The top five enriched KEGG pathway of differentially expressed genes in the colon of PW3 mice. (E), (F) and (G) The top ten biological processes, cellular components, and molecular function of differentially expressed genes in the colon of PW3 mice, respectively. (H) The top five enriched KEGG pathway of differentially expressed genes in the colon of PW5 mice.

Figure S3. The quantitative real time PCR results of representative genes in enteric neuron system. Relative quantitative mRNA level of *Scn5a* (A), *Snap25* (B), *Syn2* (C), *Syt1* (D), *Sox10* (E), *Phox2b* (F), *Ret* (G), *Chat* (H) and *S100b* (I) in the whole colon of PW1, PW3, and PW5 mice. The box graphs shown in panels A-I represent mean \pm SD. Five samples were used for testing each transcript.

Figure S4. The inhibitory effects of the P2Y1 receptor antagonist MRS2500 on CMC of the young murine colon. A-C: Contractile responses of isolated proximal (P), middle (M), and distal colons (D) treated with MRS2500 (1.0 μM) from PW1 (A), PW3 (B), and PW5 (C) mice. D: The CMC frequency of each colon segment treated with MRS2500 from PW1, PW3 and PW5 mice. ND represents not determined. E: The CMC amplitude of each colon segment treated with MRS2500 from PW1, PW3, and PW5 mice. The bar graphs shown in panel D and E represent mean \pm SE ($n = 6$). “n” means the number of animals. * $P < 0.05$.

Fig. S1

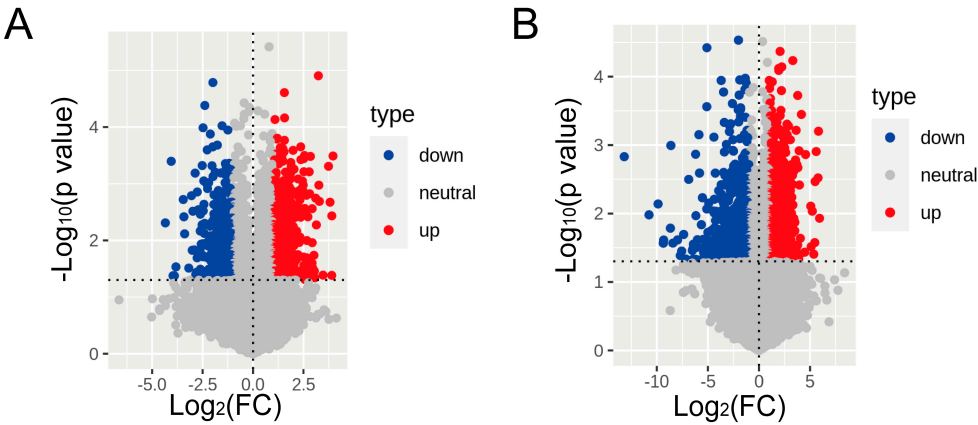


Fig. S2

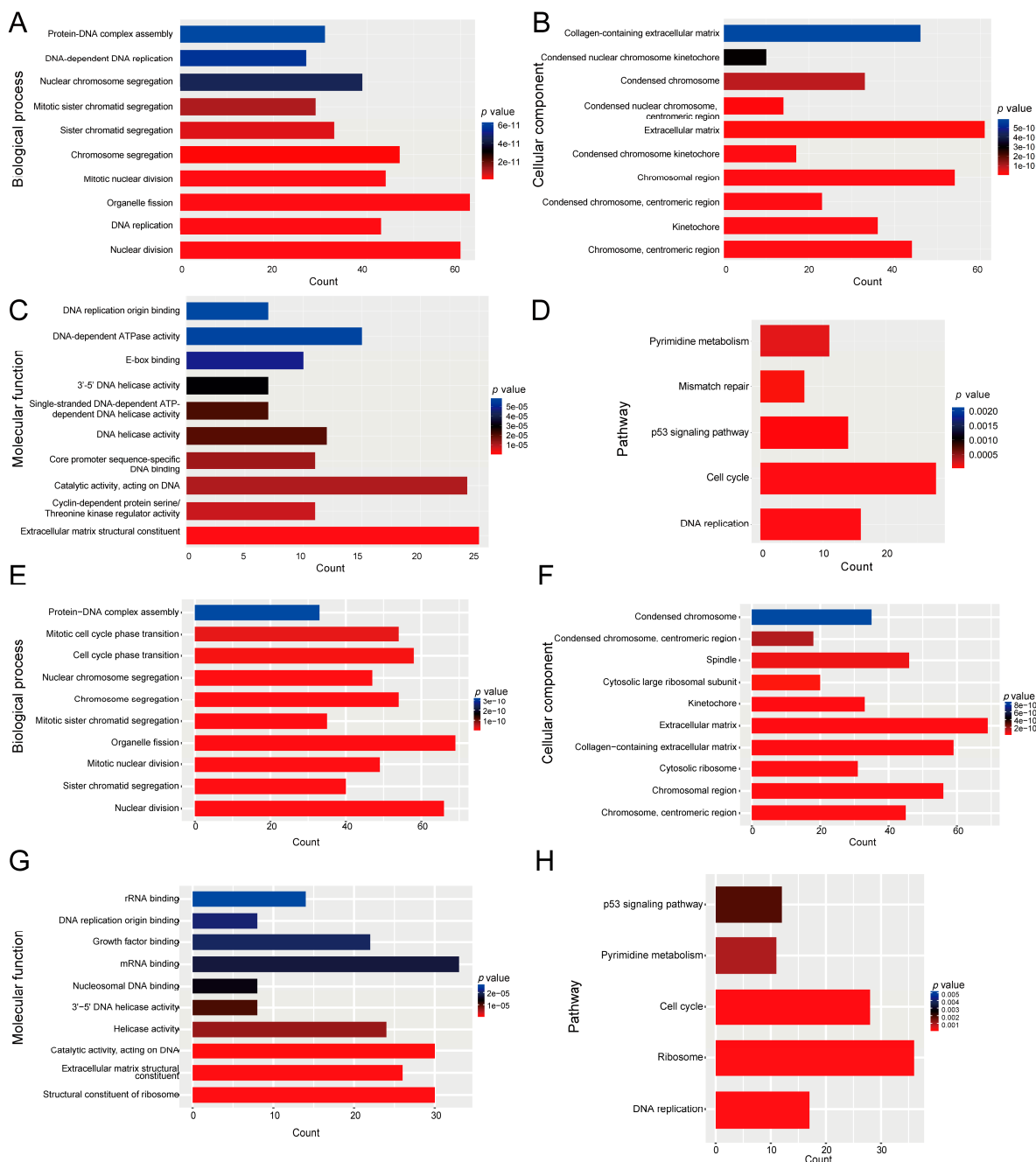


Fig. S3

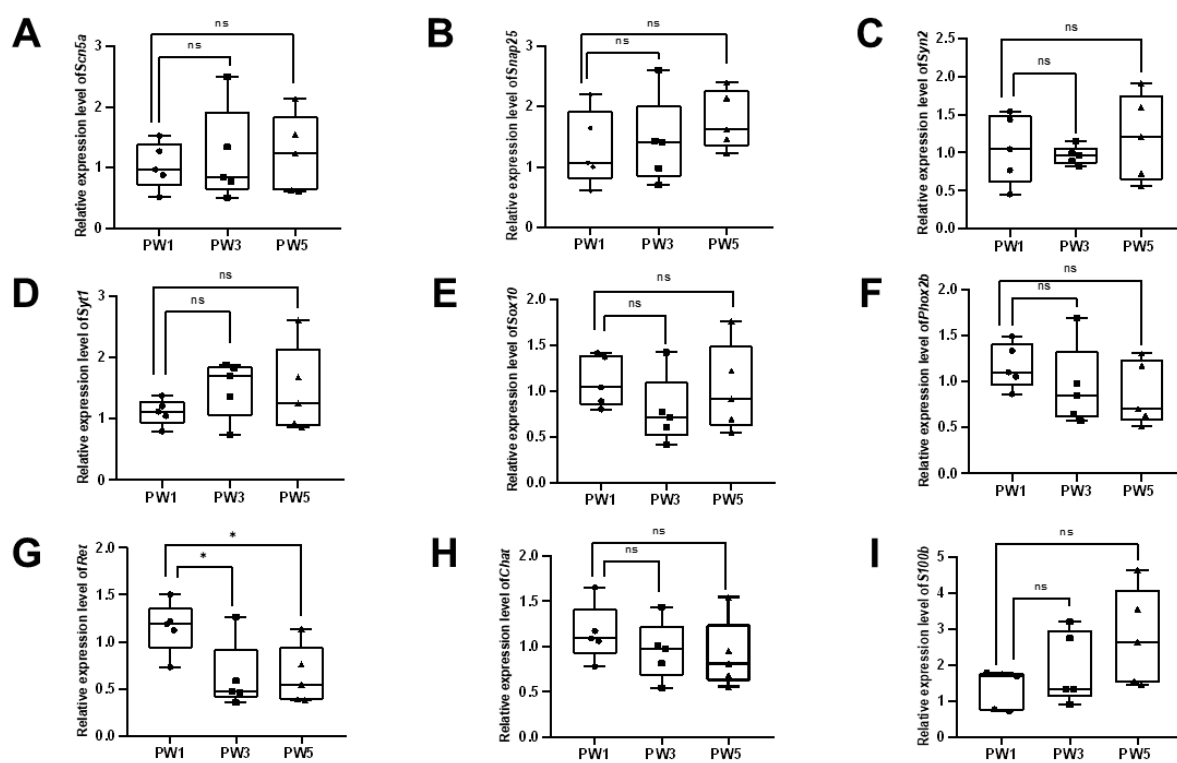


Fig.S4

