TRPA1-expressing lamina propria mesenchymal cells regulate colonic motility

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The authors have declared that no conflict of interest exists.

Supplemental Table 1

Table 1. Sequence of primers used in ISHH

Gene	Accession No.	Type	Sequence (5'-3')
Trpa1	AY496961	sense antisense	5'-CCCCACTACATTGGGCTGCA-3' 5'-CCGCTGTCCAGGCACATCTT-3'
Cox1	U03388	sense antisense	5'-CTCTGAGGGGCTCTTCTGGA-3' 5'-CTTCTGAGTCACCCGCCAGA-3'
Cox2	AF233596	sense antisense	5'-GGGTGTCCCTTCGCCTCTTT-3' 5'-GTTGCCGGTATCTGCCTTCA-3'
mPges1	AB048730	sense antisense	5'-CCAGGCTGGCTAGCTGAGAT-3' 5'-GGCGAACTGGGCCAGAACAT-3'

Supplemental Table 2

Table 2. Primary antibodies used in immunostaining

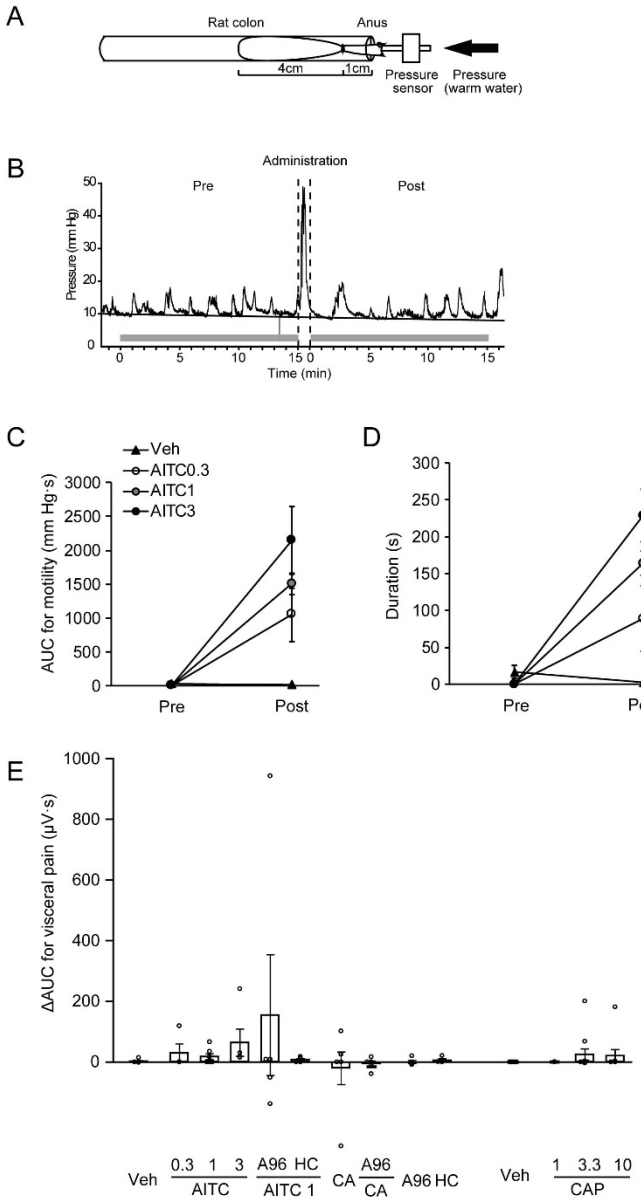
Antigen	Host	Manufacturer	number	Dilution
CGRP	Rabbit	Amersham International plc	RPN 1842	1:2000
COX1	Rabbit	Cayman	160109	1:5000
COX2	Rabbit	Cell Signaling Technology	12282	1:2000
Serotonin	Goat	ImmunoStar	20079	1:5000
SP	Rabbit	Incstar	20064	1:2000
TRPA1	Rabbit	Alomone Labs	ACC-037	1:5000/1:1000/1:500[a]
Vimentin	Mouse	Millipore	MAB3400	1:5000
αSMA	Mouse	Abcam	AB7817	1:200
CD45	Mouse	Bio-Rad	MCA340G	1:1000
CD31	Mouse	Millipore	MAB1393	1:1000
4HNE	Rabbit	Abcam	AB46545	1:200
PGP9.5	Mouse	Abcam	AB8189	1:20

[a,] TRPA1 (1:500) was used for double immunostaining with PGP9.5; (1:5000) was used for other immunostaining for rat tissue; (1:1000) was used for human tissue.

Supplemental Table 3

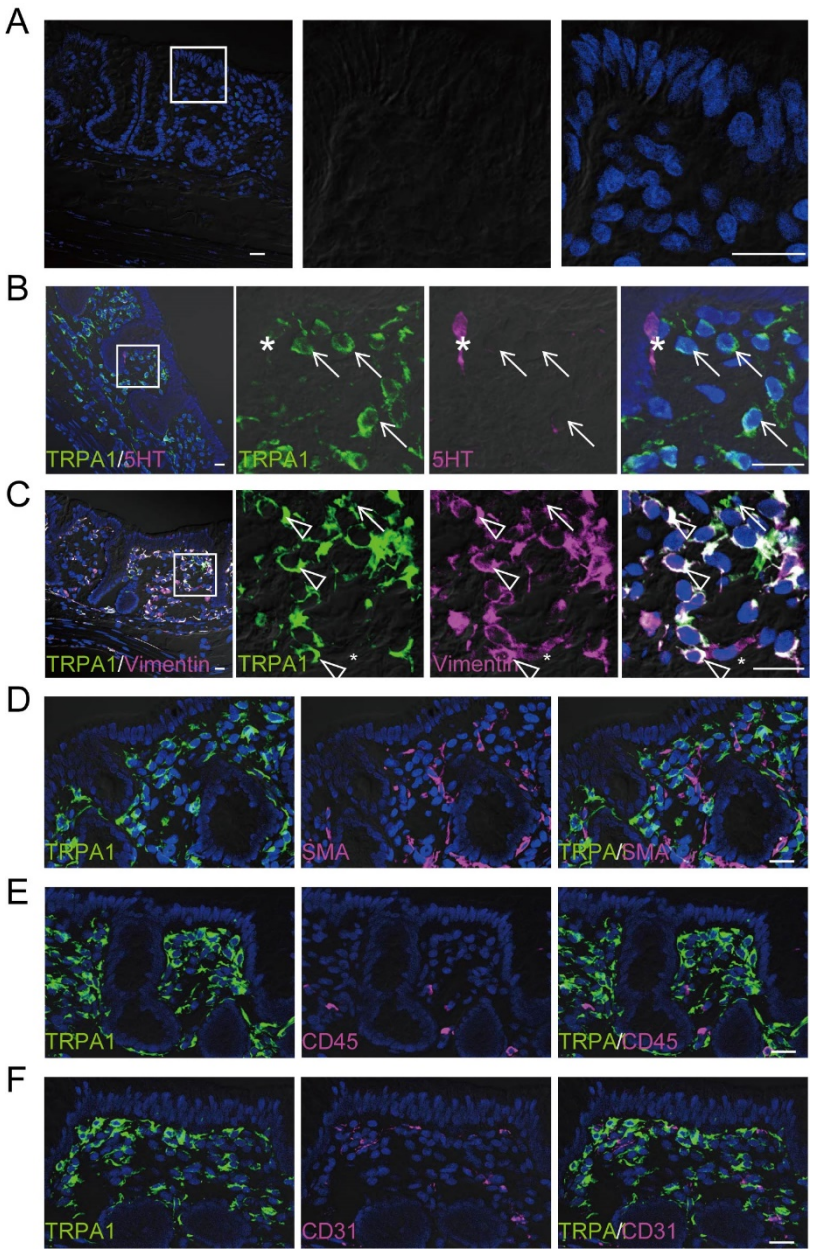
Table 3. Second antibodies used in immunostaining

Antigen	Host	Manufacturer	number	Dilution	Conjugate
Goat IgG	Donkey	Molecular Probe	A11058	1:5000	Alexa 594
Mouse IgG	Goat	Molecular Probe	A11029	1:5000	Alexa 488
Mouse IgG	Goat	Molecular Probe	A11032	1:5000	Alexa 594
Rabbit IgG	Goat	Molecular Probe	A11034	1:5000	Alexa 488
Rabbit IgG	Goat	Molecular Probe	A11037	1:5000	Alexa 594
Rabbit IgG	Donkey	Molecular Probe	A21206	1:5000	Alexa 488



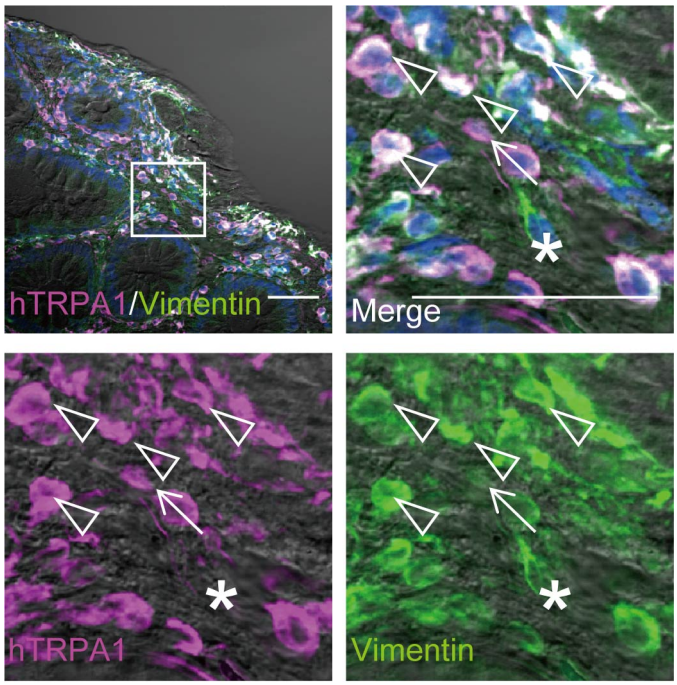
Supplemental Figure 1.

Recording system and the response of intracolonic AITC administration induced colonic motility in naïve rats. (A) Schematic showing the method used to record rat colorectal motility. (B) A representative colorectal motility trace in naïve rats with vehicle treatment. The duration, depicted by a dotted line, shows the time of drug administration. The base pressure was maintained at 10 mmHg. Colorectal motility 15 min pre- and post-administration was used for analyses. (C) Colonic motility (> 20 mmHg) response before (pre) and after (post) drug administration. (D) Duration of colonic contraction (> 20 mmHg) before (pre) and after (post) drug administration. Intracolonic administration of allyl-isothiocyanate (AITC; 0.3, 1, and 3 mM) was performed in C and D. (E) The bar graphs show the agonist- or antagonist-induced visceral pain. The accumulated area under the curve (AUC) value over a 15-min period was calculated for visceromotor response analyses. The ΔAUC value indicates AUC changes after drug administration.



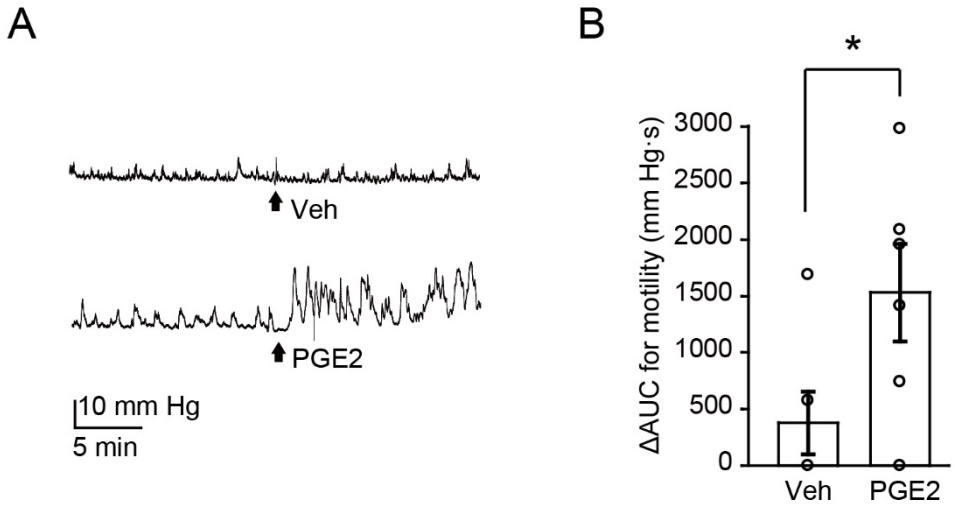
Supplemental Figure 2.

TRPA1 is expressed by fibroblasts but not EC cells, myofibroblasts, leukocytes, or endothelial cells in naïve rat colorectal tissue**.** (A) Immunofluorescence of TRPA1 antibody pretreated with its antigenic peptide in rat colorectal tissues. Note the lack of any positive staining. Tissues were counterstained with DAPI (blue). (B and C) Double immunofluorescence of TRPA1 (green) and serotonin (B) or vimentin (C) (magenta) in rat colorectal tissues. Arrows represent cells only expressing TRPA1. Asterisks represent cells only expressing serotonin or vimentin. (D) Co-expression of α-smooth muscle actin (SMA, magenta) with TRPA1 (green) in colorectal mucosa as assessed by double immunofluorescence. (E) Co-expression of CD45 (magenta) with TRPA1 (green) in colorectal mucosa as assessed by double immunofluorescence. (F) Co-expression of CD31 (magenta) with TRPA1 (green) in colorectal mucosa as assessed by double immunofluorescence. The tissue was counterstained with DAPI. Scale bars are 20 μm.



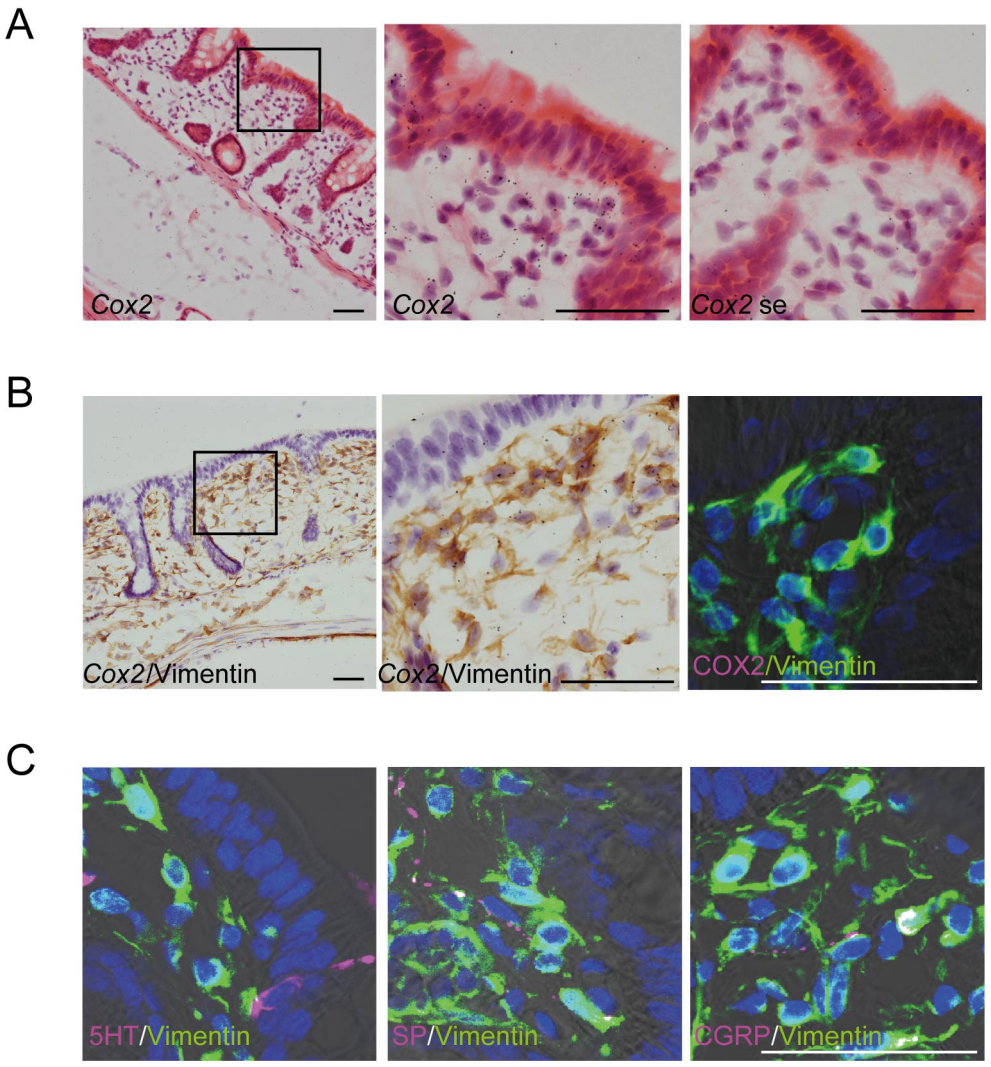
Supplemental Figure 3.

TRPA1 is predominantly expressed by vimentin-positive cells in human colorectal tissue. Double immunofluorescence of TRPA1 (magenta) and vimentin (green) in human tissues. Arrowheads show co-expression. Arrows show cells only expressing TRPA1; asterisks show cells only expressing vimentin. Tissues were counterstained with DAPI (blue). Scale bars are 50 µm.



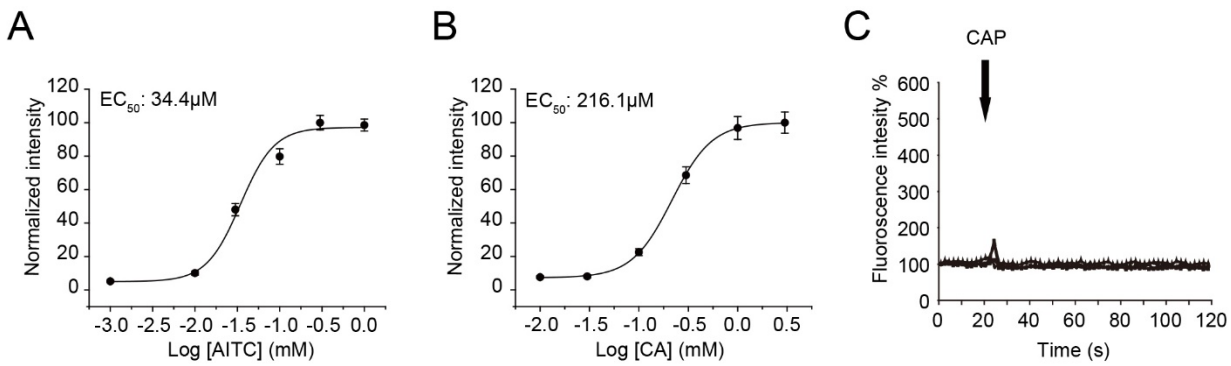
Supplemental Figure 4.

PGE2 increased colorectal motility in naïve rats. (A) Representative traces showing colorectal motility after intraperitoneal administration of PGE2 (1.76 μg·2.5 ml^{-1}·kg^{-1}). Saline with 0.8% DMSO was used as the vehicle. (B) Analyses showing the effect of PGE2 on colorectal contraction; n = 6 rats per group. *P < 0.05 vs. vehicle group (student's t test).



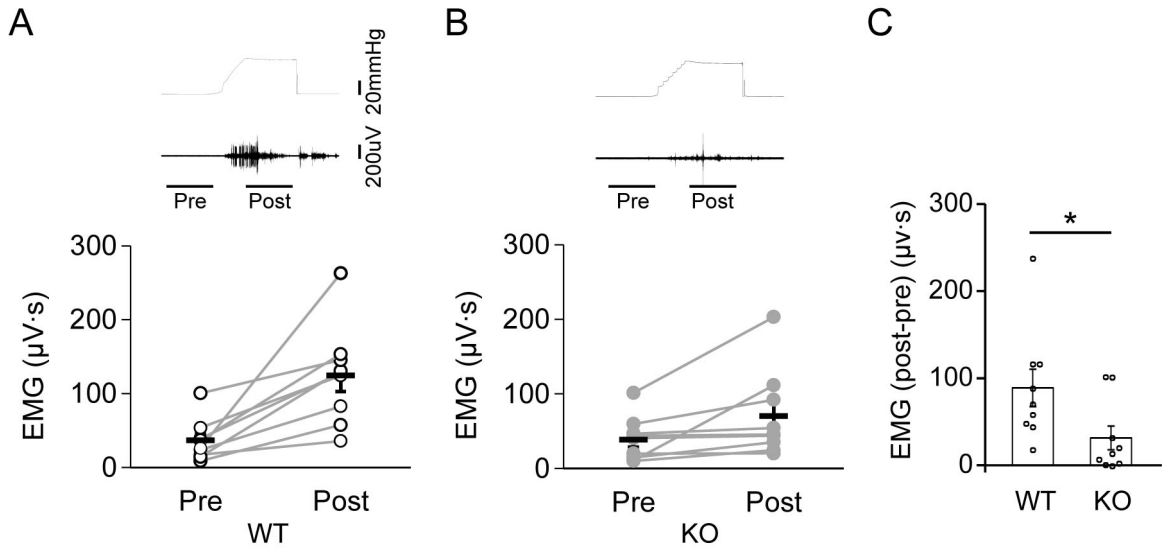
Supplemental Figure 5.

COX2, 5-HT, substance P and CGRP are not expressed in vimentin-positive cells in colorectal tissue of naïve rats. (A) Expression of *Cox2* mRNA in colorectal mucosa as assessed by in situ hybridization. (B) The left and middle panels show double-labeling of *Cox2* mRNA and vimentin (brown) using in situ hybridization. The right panel shows the co-expression of COX2 (magenta) and vimentin (green) using double immunostaining. (C) Co-expression of serotonin (5-HT), substance P (SP), or calcitonin gene-regulated peptide (CGRP) (magenta) with vimentin (green) in colorectal mucosa as assessed by double immunofluorescence. The tissue was counterstained with H&E for single in situ hybridization or hematoxylin for double *in situ* hybridization with IHC experiments. The tissue was counterstained with DAPI for immunostaining experiments. Scale bars are 50 μm.



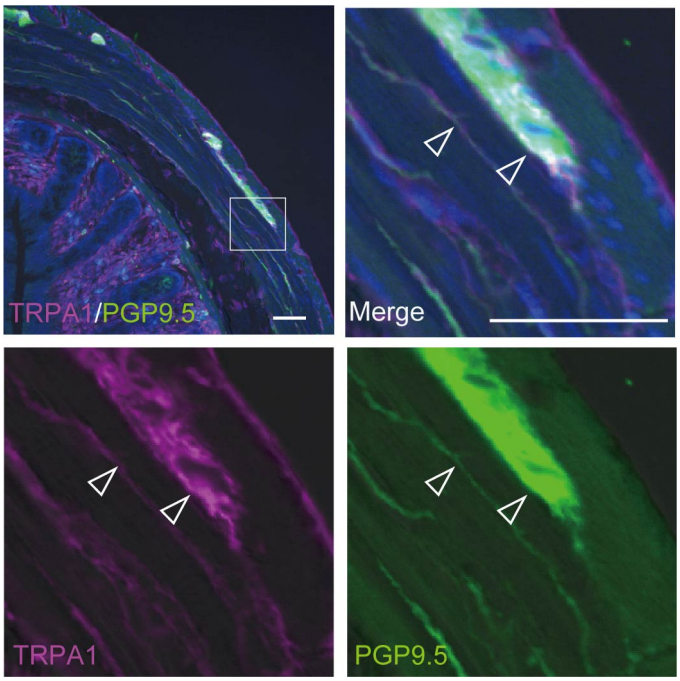
Supplemental Figure 6.

AITC and cinnamaldehyde activate TRPA1 in human colon fibroblastic CCD-18Co cells. (A) The dose-dependent curve of allyl-isothiocyanate (AITC) induced calcium response in CCD-18Co fibroblast cells. The EC_{50} of AITC was found to be 34.4 μM. *n* = 8 for each concentration. (B) The dose-dependent curve of cinnamaldehyde (CA) induced calcium response in CCD-18Co fibroblast cells. The EC_{50} of CA was found to be 216.1 μM. *n* = 6 for each concentration. (C) The effect of capsaicin (CAP, 1 μM) on CCD-18Co fibroblast cells; *n* = 4.



Supplemental Figure 7

Colorectal distention induced pain response in mice using the current balloon apparatus. Colorectal distention was administered at 60 mmHg for 10 sec. The value of AUC (μV·s) for 10 sec showed as pre or post was used for calculation. (A and B) Representative trace of electromyography recordings in WT (A) or *Trpa1*-KO (B) mice, respectively. Upper trace shows distension pressure (up to 60 mmHg); lower trace shows the EMG activity. Line charts show the individual area under the curve (AUC) value of electromyography before (Pre) and after (Post) distension. The circles and blank points represent electromyography of each mouse. The horizontal bars indicate averaged values. (C) Bar graph showing the difference AUC of EMG in WT and TRPA1$^{-/-}$ mice. n = 9 mice per group. *P < 0.05 vs. WT group (student's t test).



Supplemental Figure S8

TRPA1 is expressed in PGP9.5-positive cells in myenteric plexus and muscular layers but not in mucosa. Images showing double immunofluorescence of TRPA1 (magenta) and PGP9.5 (green) in rat colorectal tissues. Arrowheads show co-expression. Rat tissue frozen sections (25 μm) were blocked using 10% normal goat serum for 1 h and then incubated with an anti-TRPA1 antibody (Alomone Labs ACC-037, 1:500) and anti-PGP9.5 antibody (Abcam ab8189, 1:20) for 2 days at 4 °C. After incubation with Alexa 488-conjugated and 594-conjugated secondary antibodies overnight at 4 °C, sections were mounted with DAPI (Vector Laboratories). Images were acquired using a confocal laser-scanning microscope (FV10-ASW Version 03.01.02.02; Olympus Corporation) with a water Plan-Neofluar 20X objective lens. Scale bars are 50 μm.