Supplementary Table 1. Primary Antibodies Used in Immunohistochemical Staining

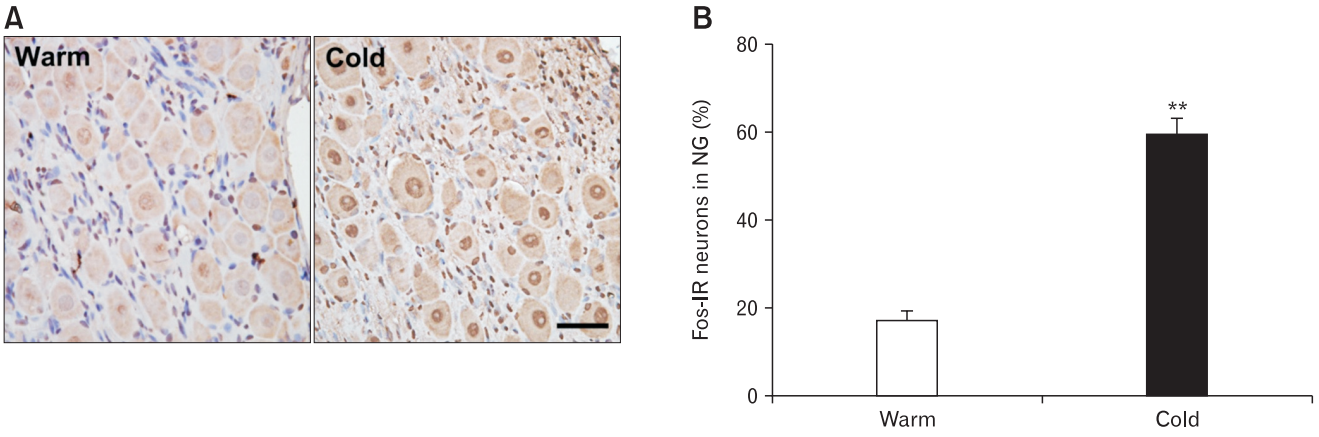
Primary antibody	Catalog No.	Dilution	Company	Reference
Rabbit anti-p-ERK1/2	9101	1:200	Cell Signaling Technology	PMID: 27920126
Mouse anti-p-ERK1/2	5726	1:300	Cell Signaling Technology	PMID: 27809309
Rabbit anti-c-Fos	ab102699	1:200	Abcam	PMID: 25756354
Rabbit anti-TRPA1	ab58844	1:400	Abcam	PMID: 25023795
Rabbit anti-TRPA1	GTX54765	1:200	GeneTex	-
Rabbit anti-TRPM8	ab74845	1:400	Abcam	PMID: 21489690

p-ERK1/2, phospho-extracellular signal-regulated protein kinase 1/2; c-Fos, FBJ osteosarcoma oncogene; TRPA1, transient receptor potential ankyrin 1; TRPM8, transient receptor potential melastatin 8.

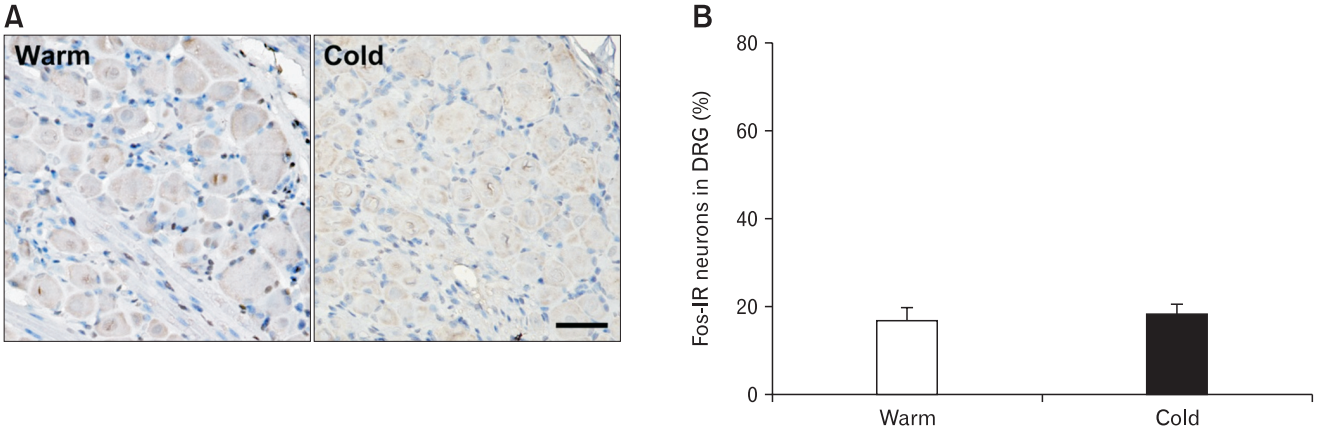
Supplementary Table 2. Chronic Stress-induced Inflammatory Cell Infiltration in Antral Mucosa

	Neutrophils (cells/HP)	Mononuclear cells (cells/HP)
Sham stress	4.1 ± 0.6	9.6 ± 2.0
Stress	10.2 ± 3.3	16.5 ± 2.1
P-value	0.047	0.033

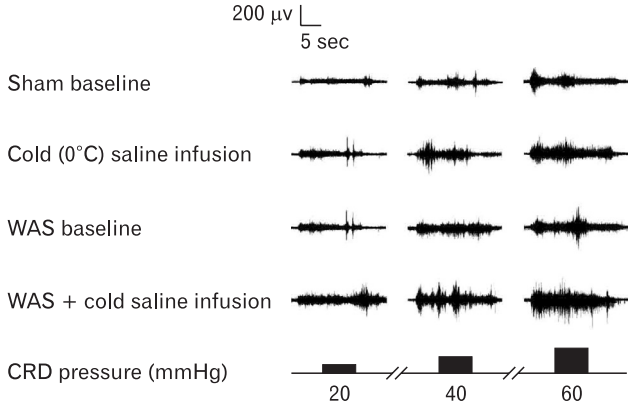
HP, high power field.
Rats were subjected to water avoidance stress 1 hour each day or sham stress for 10 consecutive days. Cell populations were counted at ×400 magnification in 8 different areas of mucosa and submucosa in each section.
Values are mean ± SEM; n = 5 per group.



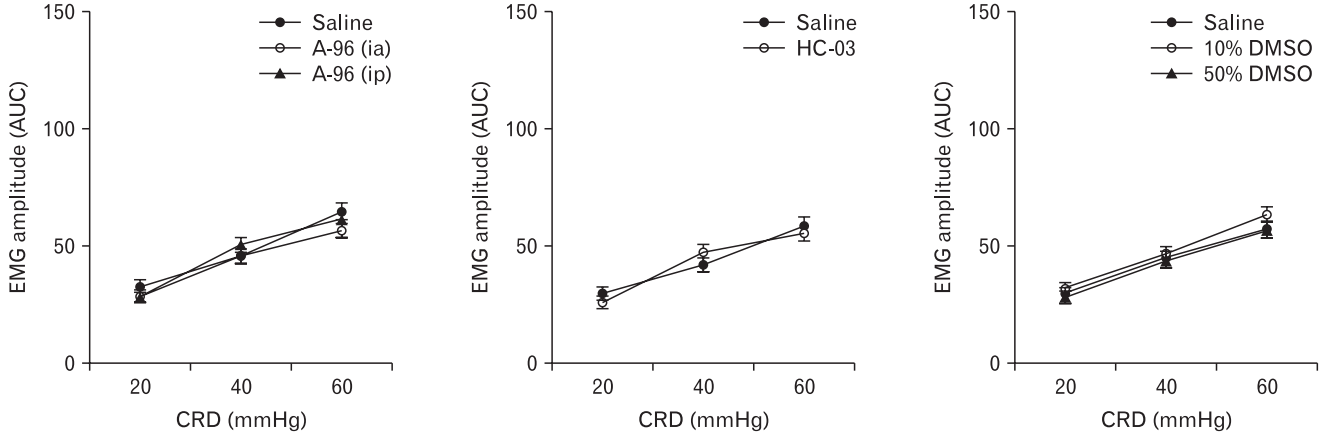
Supplementary Figure 1. Representative photomicrographs showing FBJ osteosarcoma cellular oncogene (c-Fos) expression in nodose neurons. (A) Fos immunoreactivity (IR) was not evident in nodose ganglia from rats receiving intra-antral infusion of warm saline (at 37°C). In contrast, intra-antral infusion of cold saline (at 4°C) markedly increased the number of Fos-IR neurons in nodose ganglia. (B) The percentage of c-Fos-IR neurons was calculated in 3 rats (6 ganglia) per group. $^{**}P < 0.01$ (Student's *t* test). Scale bar = 100 μm. NG, nodose ganglia.



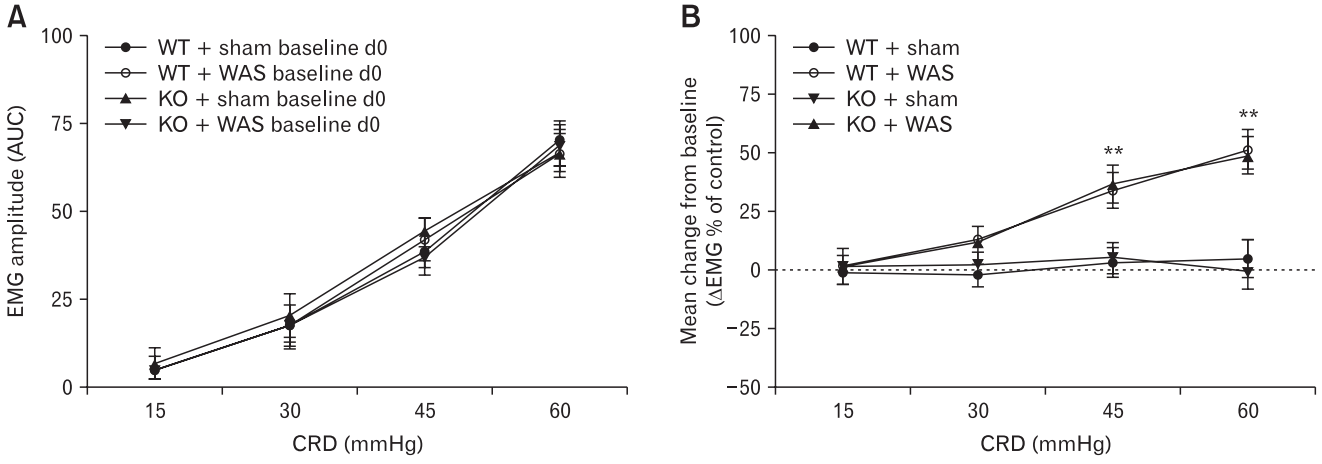
Supplementary Figure 2. Representative photomicrographs showing FBJ osteosarcoma cellular oncogene (c-Fos) expression in thoracic (T6-10) dorsal root neurons. (A) Fos immunoreactivity (IR) was similar in dorsal root ganglia (DRG) from rats receiving intra-antral infusion of warm and cold saline. (B) The percentage of c-Fos-IR neurons was calculated in 3 rats (6 ganglia) per group. Scale bar = 100 μm.



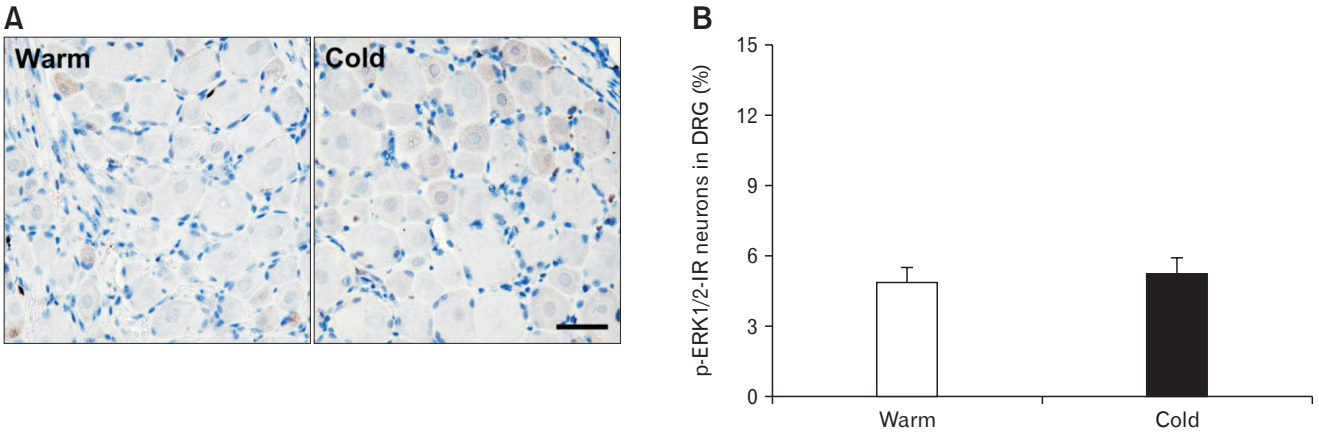
Supplementary Figure 3. Representative electromyogram (EMG) recordings depicting the visceromotor response (VMR) to colorectal distention (CRD) at 20, 40, and 60 mmHg before and after antral infusion of cold saline in rats previously subjected to a 10-day sham stress or water avoidance stress (WAS).



Supplementary Figure 4. The inhibitors of transient receptor potential ankyrin 1 had no effect on the visceromotor response (VMR) to colorectal distention (CRD). A-967079 (A-96) (A), HC-030031 (HC-03) (B), and the vehicles (50% and 10% dimethyl sulfoxide [DMSO], respectively; C) alone did not affect the VMR to CRD (n = 5 rats per group). EMG. Electromyogram; AUC, area under the curve.



Supplementary Figure 5. Effect of chronic stress on the visceromotor response (VMR) to colorectal distention (CRD) in wild type (WT) and transient receptor potential ankyrin 1 (TRPA1)-knockout (KO) mice. (A) The baseline VMR to CRD before sham stress or stress (on day 0) in WT and TRPA1- KO mice. (B) Effect of a 9-day water avoidance stress (WAS) on VMR to CRD in WT and TRPA1-KO mice. n = 8 per group. Data are expressed as mean ± SEM of VMR expressed as mean change of EMG amplitude from the baseline response on day 0. $^{**}P < 0.01$ vs sham stress groups (2-way ANOVA followed by Bonferroni post-test comparisons). EMG. Electromyogram; AUC, area under the curve.



Supplementary Figure 6. Intra-antral cold stimulation had no effect on phosphorylation of extracellular signal-regulated protein kinase 1/2 (p-ERK1/2) in rat dorsal root ganglia (DRG). (A) Phospho-ERK labelling in dorsal root neurons 10 minutes after intra-antral infusion of warm or cold saline. (B) The percentage of p-ERK1/2-immunoreactive (IR) neurons was calculated in 3 rats (6 ganglia) per group. Scale bar = 100 μm.