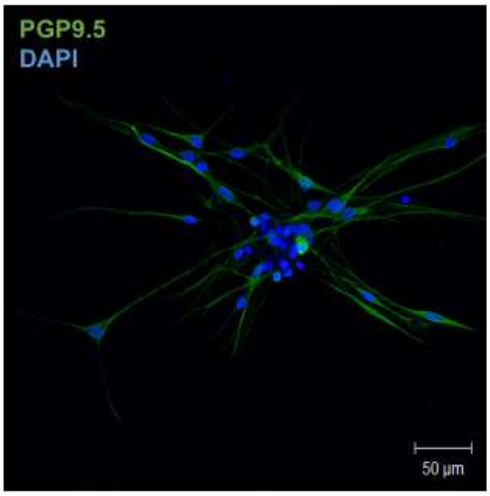
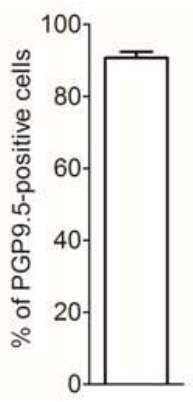
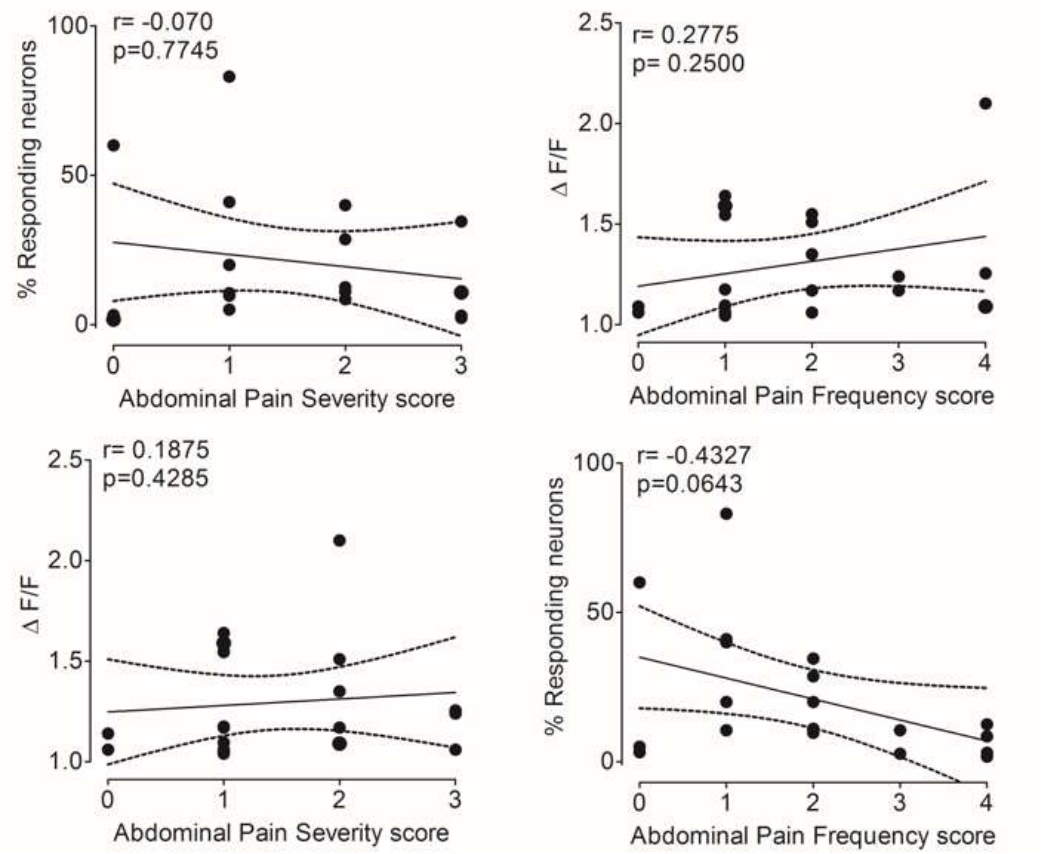
Supplementary Figure 1: DAPI nucleus staining and PGP9.5 neuronal marker staining in cultured human DRG (left panel) and percent of PGP9.5-positive neurons in human DRG cultures (right panel).



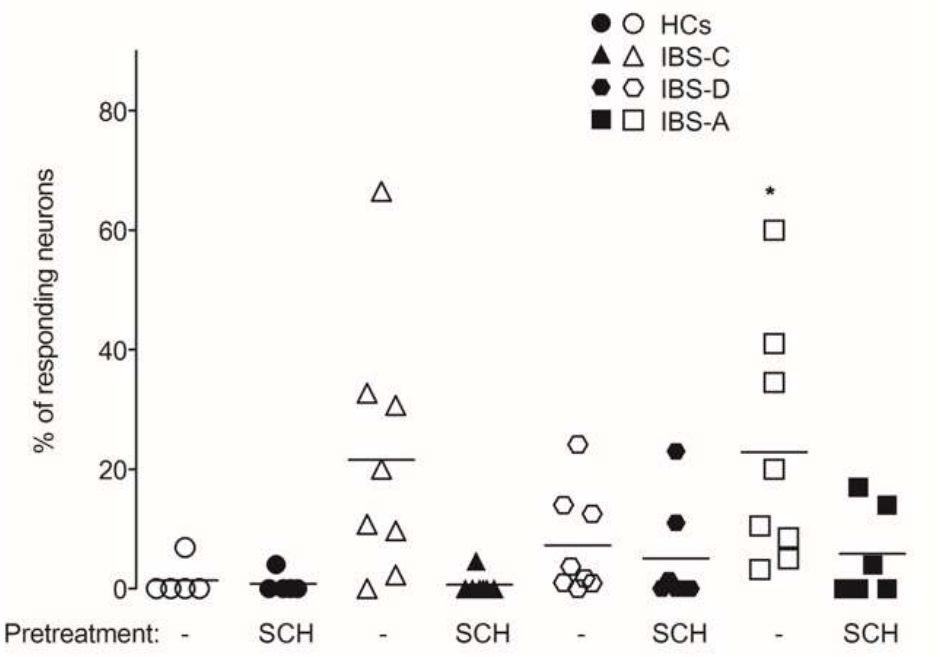


Supplementary Figure 2: Correlation between the amplitude or the percentage of responding neurons to IBS supernatant and abdominal pain. Abdominal pain and frequency scores were determined for each individual (black circle). Coefficient of correlation (r) between the score and the neuronal response were calculated. Data are represented as aligned dot plot with mean and errors linear regression lines. The significance of each correlation is indicated on each graphic by the P value.



Supplementary Figure 3: Effects of supernatants from colonic biopsies of subgroups of IBS patients or healthy controls on the percentage of responding human DRG neurons.

Percentage of responding neurons (measured by calcium mobilization) exposed to supernatants from colonic biopsies of IBS patients: constipation-predominant (-C, triangle), diarrhea-predominant (-D, diamond), alternate (-A, square) or to supernatants from colonic biopsies of healthy control (HC, circle) and pretreated with PAR_1 antagonist (SCH79797, 10 µM, black symbols), or its vehicle (HBSS, white symbols). Data are represented as scattered dot plot with line at mean. Each symbol represented one patient. n=6 independent experiments of 3 wells per condition and 42-53 neurons per well. Statistical analysis was performed using Kruskal-Wallis analysis of variance and subsequent Dunn's post hoc test. * $p<0.05$, significantly different from HC group.



Supplementary Table 1: Characteristics of patients from which biopsies were collected for Human sensory neurons calcium assays.

	Healthy Controls	IBS patients		
		IBS-A	IBS-D	IBS-C
Number (f/m)	(2/3)	(3/5)	(6/2)	(6/2)
Age range (years)	35-70	40-67	22-73	24-50
Pain severity mean score (score range)	0	1.25 (0-3)	1.5 (0-3)	1.9 (1-3)
Pain frequency mean score (score range)	0	1.25 (0-4)	2.25 (0-4)	2.13 (1-4)
Number of patients included in PAR1 antagonist pre-treatment experiments	5	6	7	7