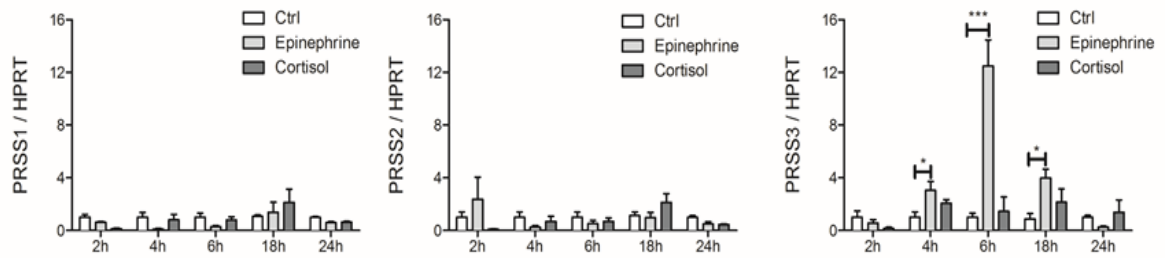
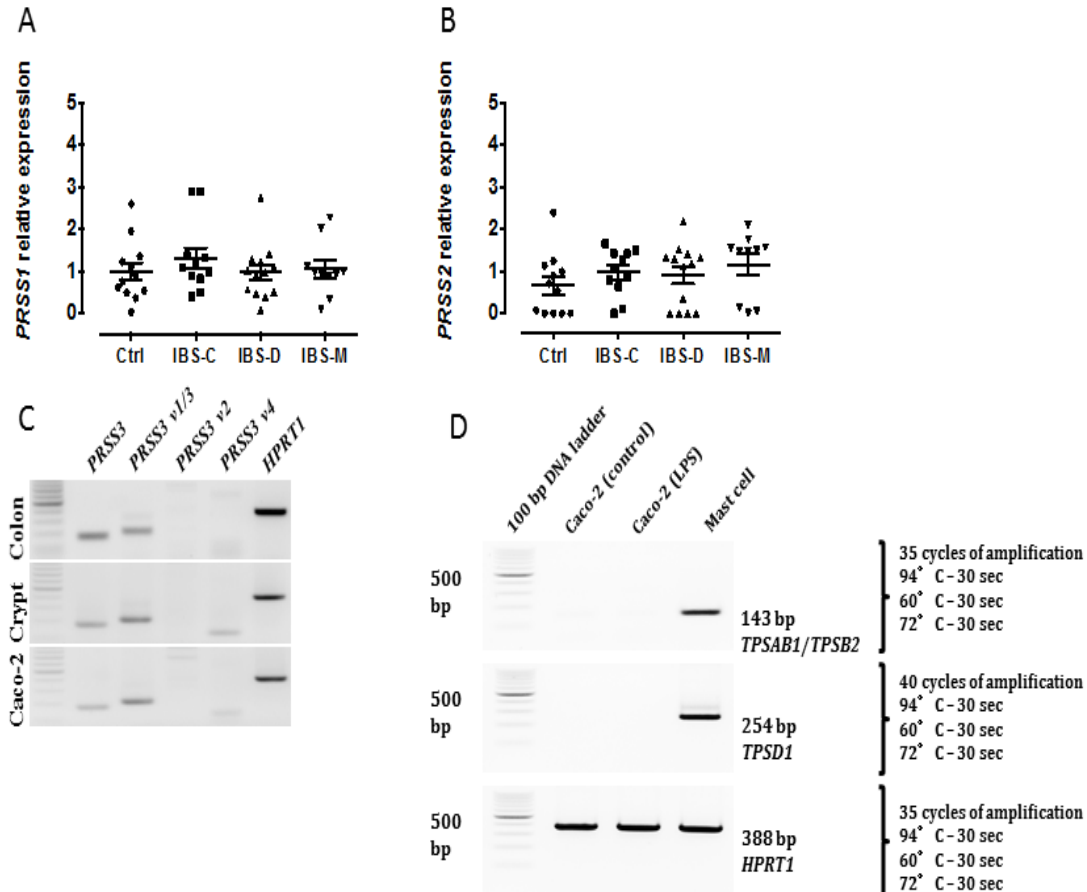


Supplementary figures:

Supplementary Figure 1: Kinetic expression of the Trypsin genes PRSS1, PRSS2 and PRSS3 alternative splice variants in intestinal epithelial cells (Caco-2) at different times after their exposure to Epinephrine or to Cortisol. Data are expressed as mean \pm SEM.



Supplementary Figure 2: Expression of the Trypsin genes PRSS1, PRSS2 and PRSS3 alternative splice variants, and the Trypsin genes TPSAB1, TPSB2, TPSD1. Relative gene expression of **A. PRSS1** (cationic trypsin: Trypsin-1 precursor) and **B. PRSS2** (anionic trypsin: Trypsin-2 precursor) in colonic biopsies from control and IBS patients. Data are expressed as mean \pm SEM and were compared using Student's *t*-test. **C.** Analytical agarose-gel electrophoresis of RT-PCR products performed with RNA extracted from human colonic biopsies (control patient), isolated colonic crypts or Caco2 cells. Amplicons were amplified with oligonucleotides for total PRSS3 (170 bp), or the alternative splice variants 1 (coding for Trypsinogen IV; 202 bp), 2 (coding for Mesotrypsinogen; 667 bp), 3 (330 bp) and 4 (122 bp) and *HPRT1* (388 bp). **D.** Representative agarose-gel electrophoresis of RT-PCR products performed on RNA extracted from Caco-2 cells stimulated or not with LPS, or primary mast cells derived from human CD34⁺ precursor cells. The amplicon in the top panel was amplified with oligonucleotides common for the genes coding for trypsinase- β 1 and trypsinase- β 2 (*TPSAB1* and *TPSB2*, respectively). The amplicon in the middle panel was amplified with oligonucleotides specific for trypsinase- δ 1 (*TPSD1*). Amplicons in the bottom panel were amplified with oligonucleotides specific for the house-keeping gene *HPRT1*.



Supplementary Figure 3. Trypsin-3-evoked calcium signals in mouse nociceptive DRG neurons and human submucosal neurons are PAR1 and PAR4 independent. **A.** Average amplitude of $[Ca^{2+}]_i$ rises and percentage of responding neurons to Trypsin-3 (10 nM) exposure in mouse nociceptive DRG neurons in the presence or absence of the PAR1 (SCH79797) or the PAR4 (ML-354) specific antagonists. Data are expressed as mean \pm SEM and were analyzed by One-Way ANOVA followed by Kruskal Wallis's post-test, (n=6 mice per group), *** and * different from basal for $p < 0.001$, $P < 0.05$ respectively. **B.** Average amplitude of $[Ca^{2+}]_i$ rises and percentage of responding neurons to Trypsin-3 (10 nM) exposure in human submucosal neurons in the presence or absence of the PAR1 (SCH79797) or the PAR4 (ML-354) specific antagonists. Data are expressed as mean \pm SEM and were analyzed by One-Way ANOVA followed by Kruskal Wallis's post-test, (n=3 subjects per group), *** $p < 0.001$.

