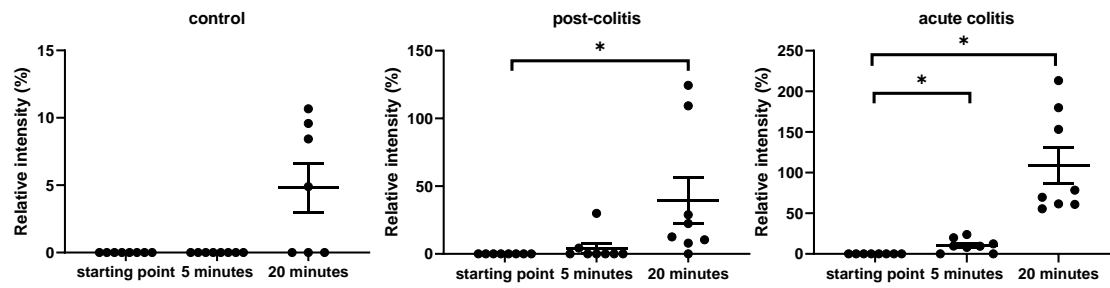


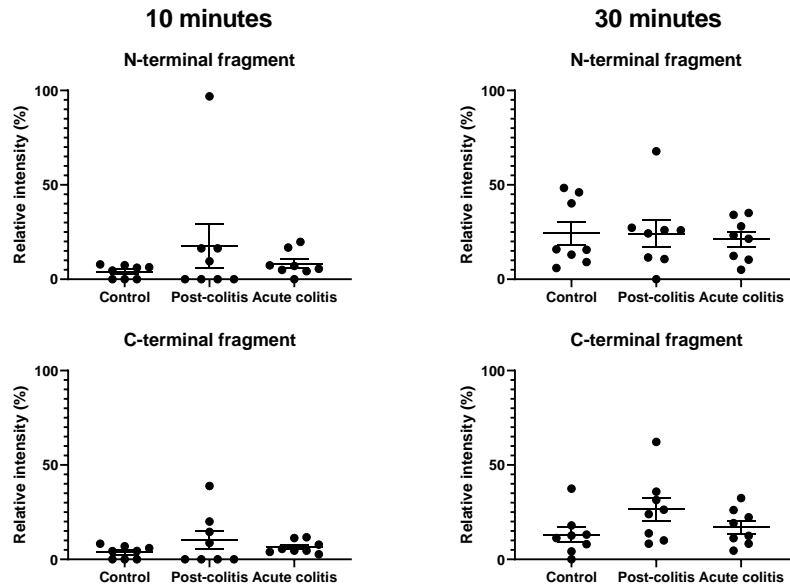
## Supplementary materials



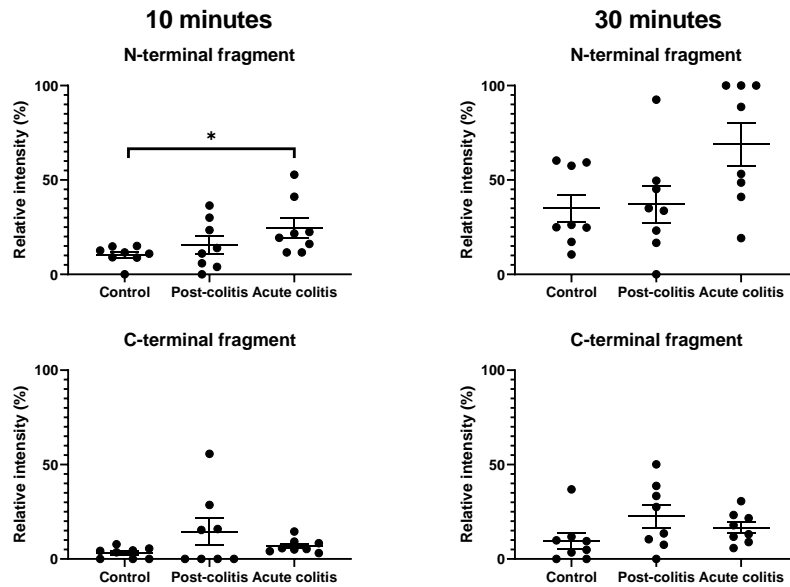
**Figure S 1:** Bradykinin was processed to BK1-7 by colonic samples from acute and post-colitis rats. The relative intensities of bradykinin(BK)1-7 at the starting point and after 5 and 20 min of incubation with colon samples from control, post-colitis and acute colitis animals are shown here. The increase of BK1-7 is significant after 20 min of incubation in post-colitis samples ( $p = 0.02$ ) and after 5 min of incubation in acute colitis samples ( $p = 0.03$ ). Statistics: Friedman test followed by Wilcoxon signed-rank test with Bonferroni correction. The mean and SEM are shown.  $n = 8$  animals per group \* $p < 0.05$



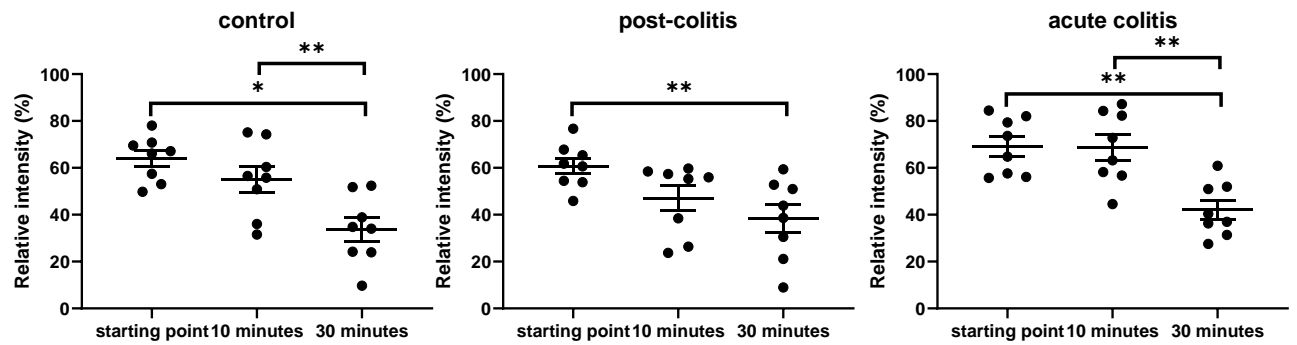
(b) Cleavage after L13



(c) Cleavage after R14



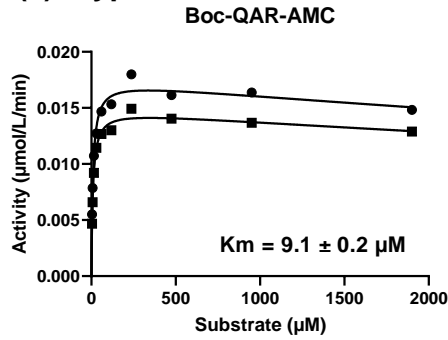
**Figure S 2:** (a) Cleavage sites in VIP after incubation with colonic samples from control, acute colitis and post-colitis rats. (b) Relative intensities of the N and C-terminal fragment after cleavage at leucine in position 13 after 10 and 30 min of incubation with the colonic samples. (c) Relative intensities of the N and C-terminal fragment after cleavage at arginine in position 14 after 10 and 30 min of incubation with the colonic samples. Statistics: Kruskal-Wallis test with post-hoc Dunnett-Bonferroni. The mean and SEM are shown. n = 8 animals per group \*p<0.05



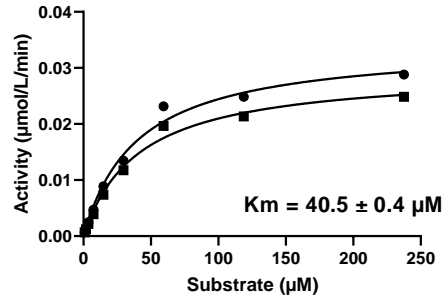
**Figure S 3:** Relative intensities of the full-length substance P at the starting point and after 10 and 30 min of incubation with colonic samples from control, acute colitis or post-colitis rats. The decrease of the full-length peptide is significant in control, acute and post-colitis samples after 30 min of incubation. Statistics: Friedman test followed by Wilcoxon signed-rank test with Bonferroni correction. The mean and SEM are shown. n = 8 animals per group \* $p < 0.05$  \*\* $p < 0.01$



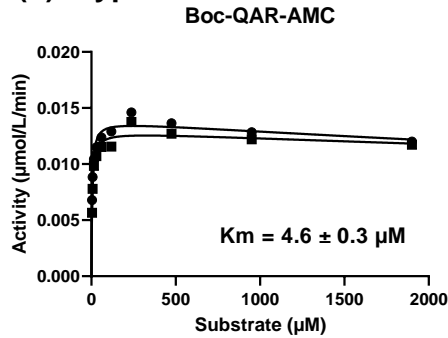
**(a) Trypsin-1**



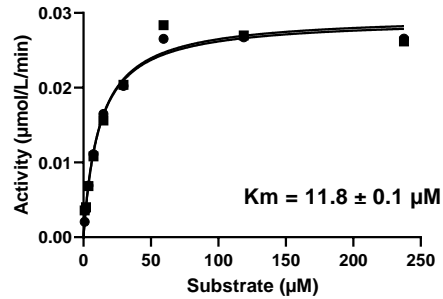
**Tos-GPR-AMC**



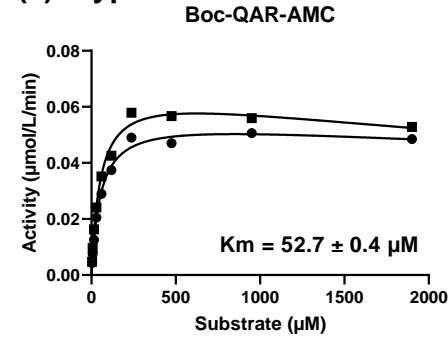
**(b) Trypsin-2**



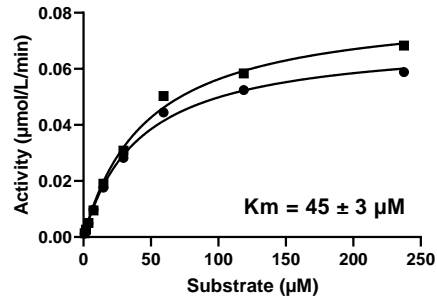
**Tos-GPR-AMC**



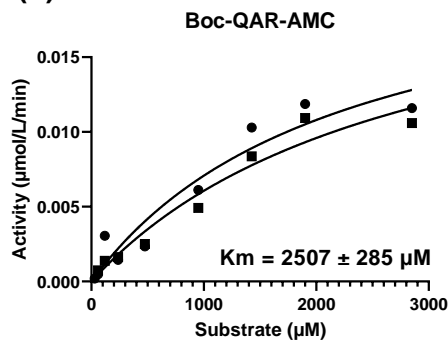
**(c) Trypsin-3**



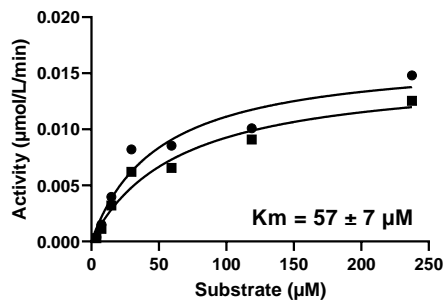
**Tos-GPR-AMC**



**(d) Thrombin**



**Tos-GPR-AMC**



**Figure S 5:** Michaelis-Menten constant ( $K_m$ ) for (a) trypsin-1, (b) trypsin-2, (c) trypsin-3 and (d) thrombin with Boc-QAR-AMC and Tos-GPR-AMC. The  $K_m$  was determined with Boc-QAR-AMC and Tos-GPR-AMC by adding trypsin-1 (0.015 nM), trypsin-2 (0.015 nM), trypsin-3 (0.015 nM) or thrombin (0.19 nM) to a range of substrate concentrations at 37°C. The fluorescence was measured kinetically for 20 min at 37°C on a Tecan F200 multi-plate reader. The results in RFU/min were recalculated by dividing them by the slope of a calibration curve with AMC (0.78 – 10  $\mu\text{M}$ ) to obtain results in  $\mu\text{mol/L/min}$ . These activities were plotted against the substrate concentrations and GraphPad Prism 9.2.0. was used to fit a Michaelis-Menten curve to the data and calculate  $K_m$ . The experiment was repeated twice.