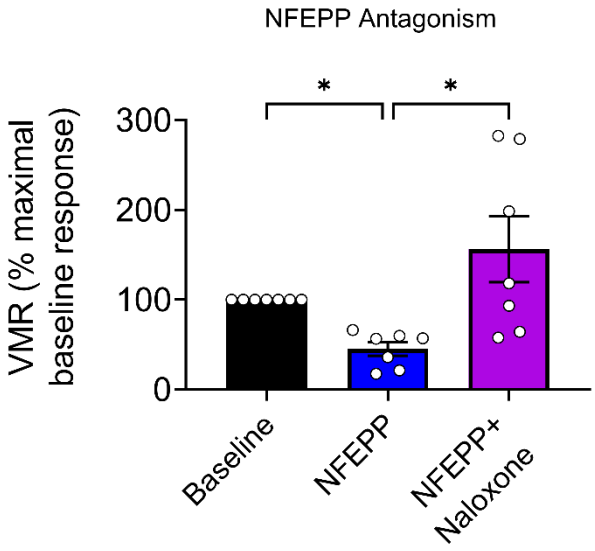
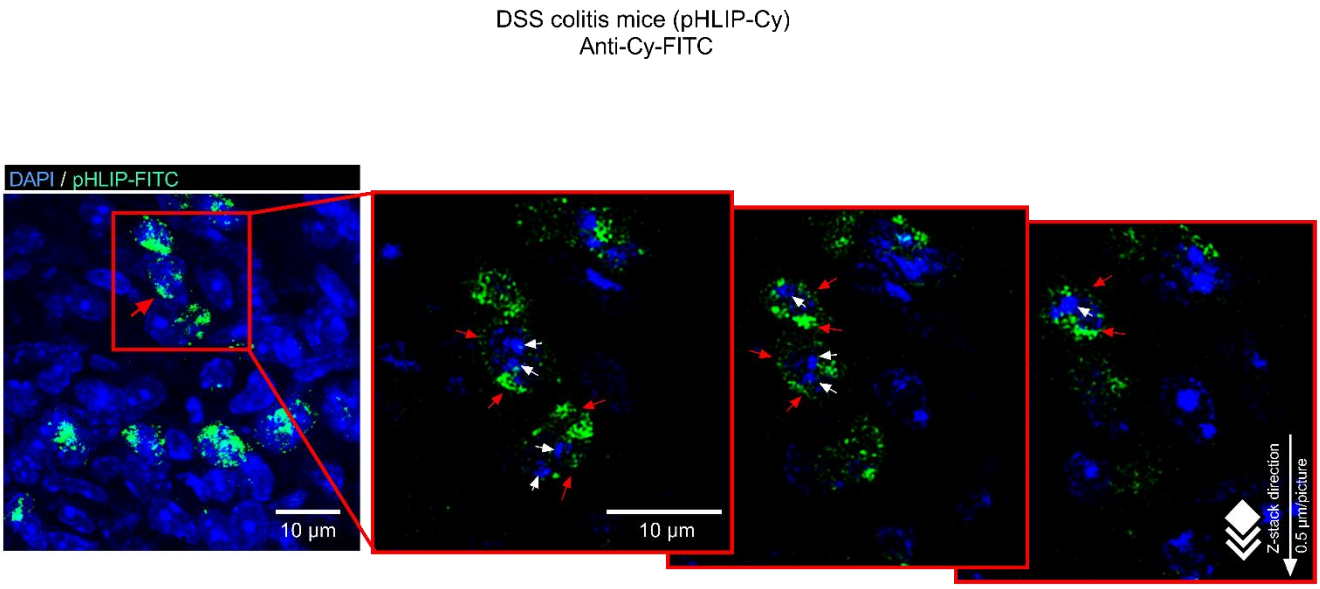
Supplementary digital content: Degro et al., Evolving acidic microenvironments during acute colitis provide selective analgesic targets for a pH-sensitive opioid

Supplementary digital content Figure 1:



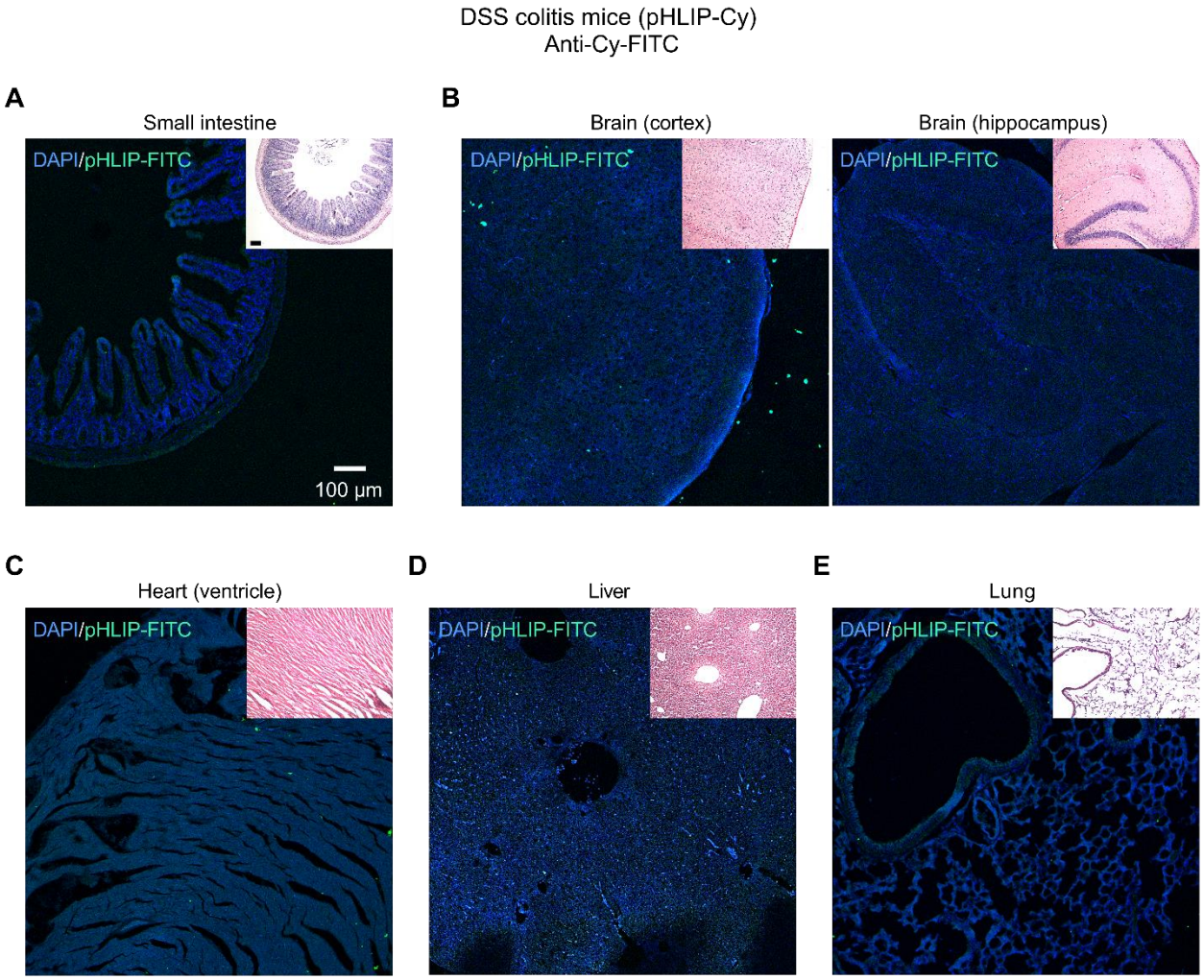
Supplementary digital content Fig. 1: Reversal of NFEPP induced inhibition of VMRs (80 µl colorectal distension) by the opioid antagonist naloxone in DSS colitis mice. Mice received NFEPP 0.4 mg/kg s.c. and after 4 hours a re-administration of NFEPP (0.4 mg/kg s.c.) together with the opioid antagonist naloxone hydrochloride (1 mg/kg s.c., 15 min prior to VMR recording). *Abbreviations: DSS, dextran sulphate sodium. VMR, visceromotor response. * p<0.05, N=7, Friedman test, Dunn´s test.*

Supplementary digital content Figure 2:



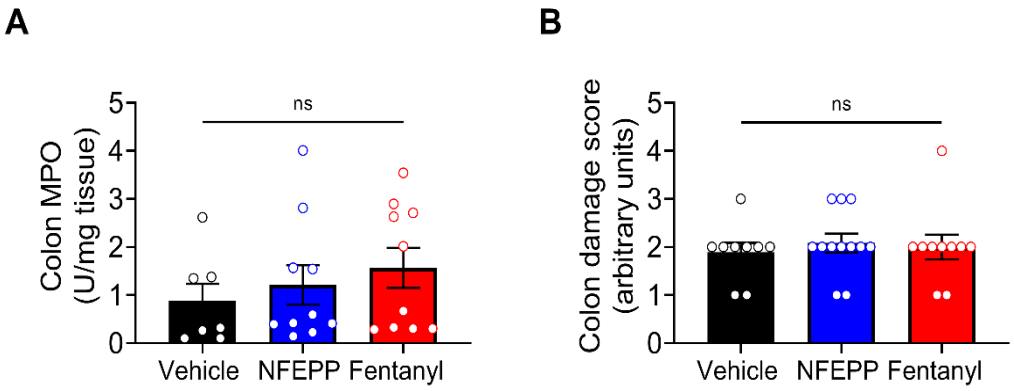
Supplementary digital content Fig. 2: Subcellular localization of pHLIP. Z-axis sections (0.5 µm) of the inflamed colon from a DSS colitis mouse, obtained by confocal microscopy. White arrows denote the nucleus labelled with DAPI. Red arrows indicate the pHLIP-FITC signal in the plasma membrane surrounding the nucleus. *Abbreviations: Cy, cyanin. DSS, dextran sulphate sodium. DAPI, 4',6-diamidino-2-phenylindole. FITC, fluorescein isothiocyanate. pHLIP, pH low insertion peptide.*

Supplementary digital content Figure 3:



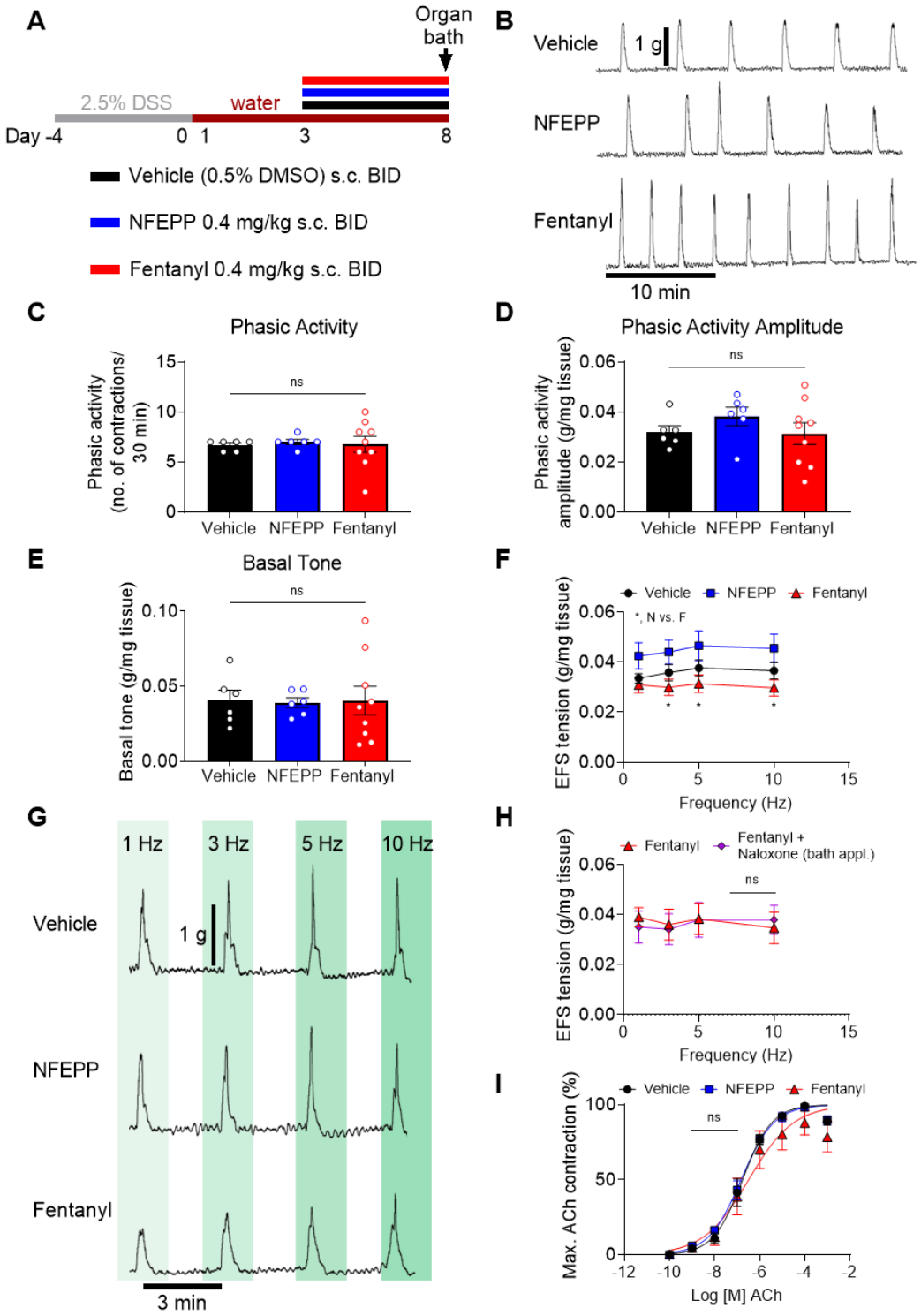
Supplementary digital content Fig. 3: pHLIP detection in extraintestinal organs from DSS colitis mice. DAPI/ anti-Cy-FITC labelling of the small intestine (**A**), the brain (**B**, cortex and hippocampus), the heart (**C**, ventricle), the liver (**D**) and the lung (**E**) from DSS colitis mice treated with pHLIP. Insets illustrate H&E stained sections of the corresponding organ (black scale bar: 100 µm). *Abbreviations: Cy, cyanin. DSS, dextran sulphate sodium. DAPI, 4',6-diamidino-2-phenylindole. FITC, fluorescein isothiocyanate. H&E, haematoxylin and eosin. pHLIP, pH low insertion peptide.*

Supplementary digital content Figure 4:



Supplementary digital content Fig. 4: MPO activity and colon damage score. Combined MPO activity (**A**) and histological damage score (**B**) of the inflamed colon from the CT- and VMR- group (cohort 1). *Abbreviations: MPO, myeloperoxidase. VMR, visceromotor response. A: N=7-10. B: N=9-12. Kruskal-Wallis test, Dunn´s test.*

Supplementary digital content Figure 5:



Supplementary digital content Fig. 5: Effects of repeated NFEPP and fentanyl application on colonic contractility during acute DSS colitis assessed by in-vitro isometric tension recordings and electrical field stimulation (EFS). A. Study design with injection regimen (color coded). Isometric tension recordings were performed at day 8 after chronic treatment with vehicle (0.5% DMSO s.c., N=6), NFEPP (0.4 mg/kg s.c., N=6) or fentanyl (0.4 mg/kg s.c., N=9) BID. **B.** Representative traces of phasic contractions of colon segments from vehicle, NFEPP and fentanyl treated DSS colitis mice. **C-E.** Phasic activity frequency (**C**), phasic activity amplitude (**D**) and basal tone (**E**) of colon segments from vehicle, NFEPP and fentanyl treated DSS colitis mice. **F.** EFS-induced contractility of colon segments from vehicle, NFEPP and fentanyl treated DSS colitis mice. **G.** Representative traces of colon segment contractions induced by EFS with different stimuli (1, 3, 5 and 10 Hertz, Hz) **H.** EFS-induced contractility of colon segments before and after bath application of naloxone hydrochloride (10 µM) in a subset of fentanyl treated DSS colitis mice (N=4). **I.** Smooth muscle contractility in response to bath application of cumulative increasing concentrations of ACh chloride of colon segments from vehicle (N=6), NFEPP (N=6) and fentanyl (N=5) treated DSS colitis mice. *Abbreviations: BID, bis in die (twice daily). DMSO, dimethyl sulfoxide. DSS, dextran sulphate sodium. ACh, acetylcholine. * p<0.05. C: Kruskal-Wallis test. D, E: Welch ANOVA. F, H, I: Two-way ANOVA, Bonferroni test.*