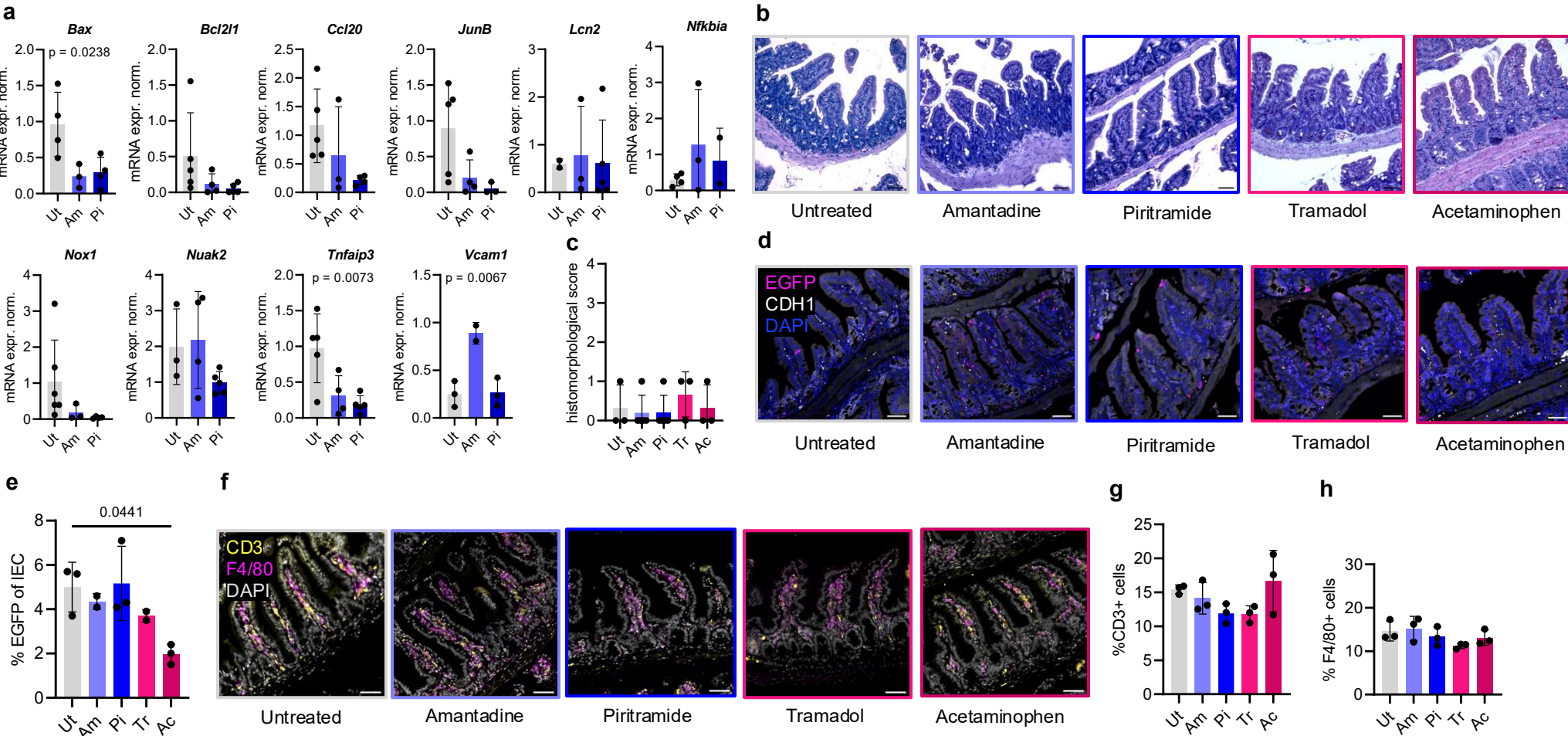
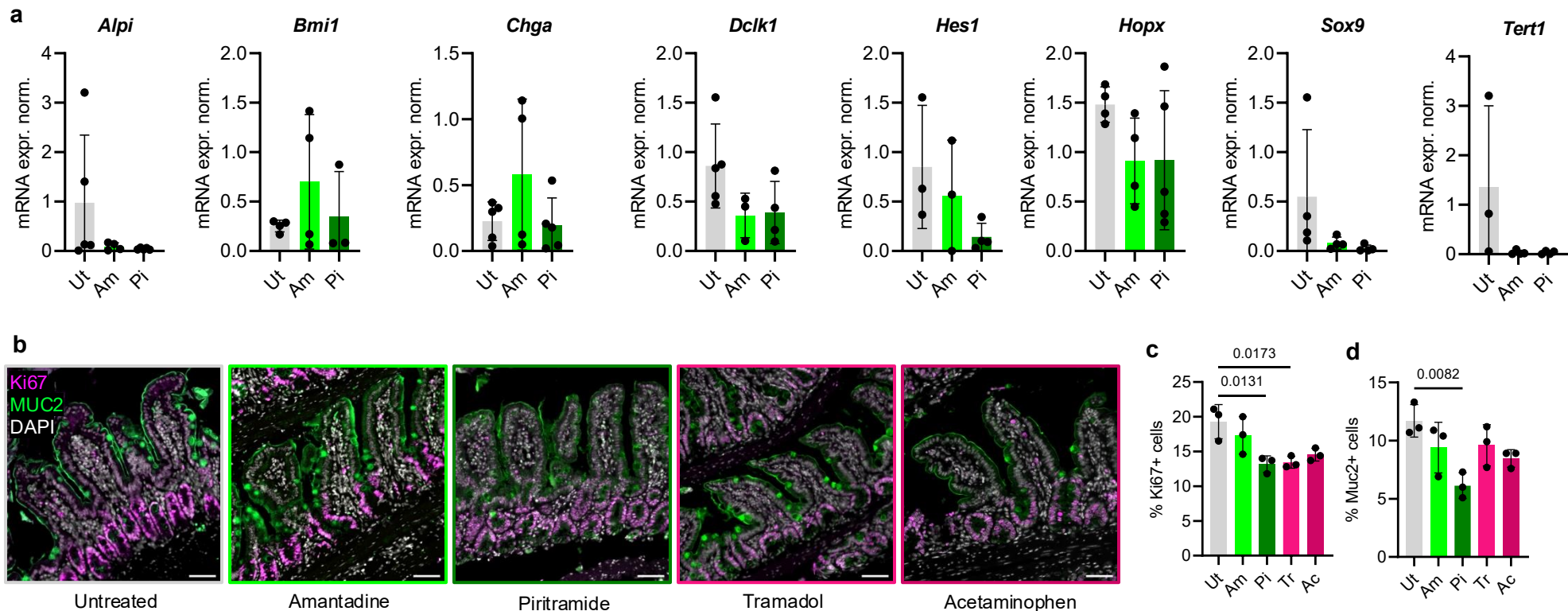


**Figure S2: PEDL<sup>+</sup>-selected analgesics show no interference with NF-κB signaling or immune cell composition in the small intestine**



**Figure S2: a.** qPCR analysis of bulk colonic tissue of mice treated as in 2a. with targets of NF-κB. Normalized expression values ( $\Delta\Delta C_q$ ) shown. Ut (Untreated), Am (Amantadine), Pi (Piritramide). Analysis: One-way ANOVA with Tukey's multiple comparison test, only significant p-values shown horizontally. **b.** H&E of ileum from mice treated with analgesics for 5 days or left untreated. Representative sections shown from at least  $n = 3$  mice per group. Analysis as in a. Scale: 50μm. **c.** Histomorphological score from b. Analysis as in a.  $F(4, 14) = 0.4912$ ;  $p = 0.7423$ . Tr (Tramadol), Ac (Acetaminophen). **d.** Ileal immunofluorescence staining (IF) for EGFP and CDH1 from mice treated as in b. Representative sections shown from at least  $n = 2$  mice per group. Scale: 50μm. **e.** Quantitation of c. shown as percentage of EGFP+ cells in ileal CDH1+ intestinal epithelial cells. Analysis as in a.  $F(4, 8) = 4.532$ ;  $p = 0.0332$ . **f.** Ileal IF for CD3 and F4/80 from mice treated as in a.  $n = 3$ . Scale: 50μm. **g.** Quantitation of f. shown as percentage of CD3+ cells in colonic lamina propria (LP). Analysis as in a.  $F(4, 10) = 2.288$ ;  $p = 0.1315$ . **h.** Quantitation of f. shown as percentage of F4/80+ cells in colonic LP. Analysis as in a.  $F(4, 10) = 1.707$ ;  $p = 0.2243$ .

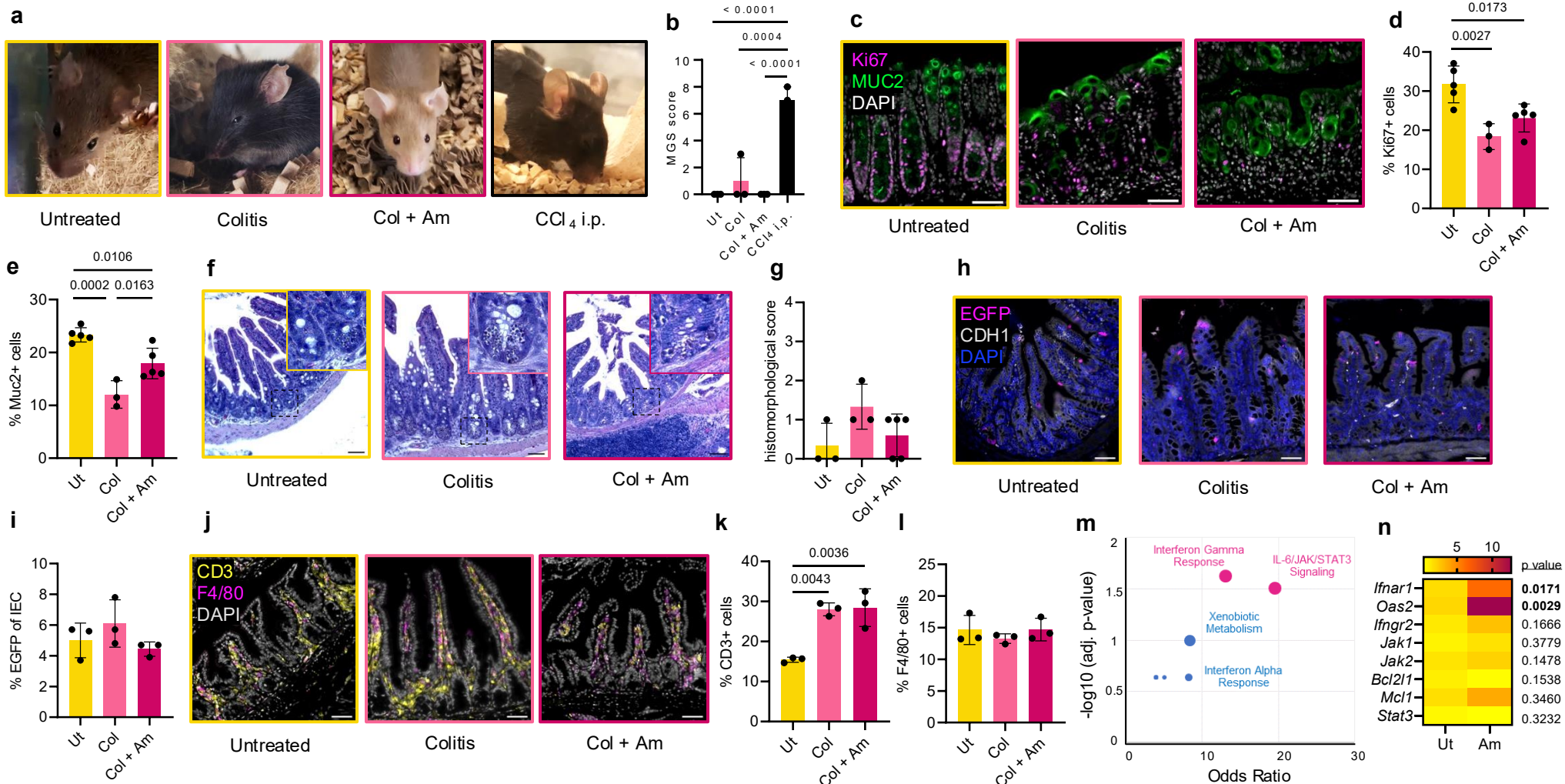
## Figure S3: Ileal differentiation of secretory cells is affected in piritramide and tramadol treated mice



**Figure S3:** **a.** qPCR analysis of bulk colonic tissue of mice treated as in 3a. with markers of epithelial cell type and differentiation. Ut (Untreated), Am (Amantadine), Pi (Piritramide). Normalized expression values ( $\Delta\Delta Cq$ ) shown. Analysis: One-way ANOVA with Tukey's multiple comparison test, only significant p-values shown horizontally. **b.** Ileal immunofluorescence staining (IF) for Ki67 and MUC2 from mice treated with analgesics for 5 days or left untreated. Representative sections shown from at least  $n = 3$  mice per group. Scale: 50µm. **c.** Quantitation of **b.** shown as percentage of Ki67+ cells of intestinal epithelial cells (IECs). Tr (Tramadol), Ac (Acetaminophen). Analysis as in **a.**  $F(4, 10) = 6.541$ ;  $p = 0.0075$ . **d.** Quantitation of **b.** shown as percentage of MUC2+ cells of IECs. Analysis as in **a.**  $F(4, 10) = 5.255$ ;  $p = 0.0153$ .



**Figure S4: Mouse grimace scale in validation of amantadine as analgesic**



**Figure S4: a.** Images of mice treated as in Figure 4a. Control mice received an i.p. injection of carbon tetrachloride (CCl<sub>4</sub>). **b.** Quantitation of mouse grimace scale (MGS) score from a. Analysis: One-way ANOVA with Tukey's multiple comparison test, only significant p-values shown horizontally. F (3, 8) = 34.00; p < 0.0001. **c.** Colon immunofluorescence staining (IF) for Ki67 and MUC2 from mice treated as in a. Representative sections shown from at least n = 3 mice per group. Scale: 50µm. **d.** Quantitation of c. shown as percentage of Ki67+ cells of intestinal epithelial cells (IECs). Analysis as in b. F (2, 10) = 11.61; p = 0.0025. **e.** Quantitation of c. shown as percentage of MUC2+ cells of IECs. Analysis as in b. F (2, 10) = 22.34; p = 0.0002. **f.** H&E of ileum from mice treated as in a. Representative sections shown from at least n = 3 mice per group. Scale: 50µm. Insets show magnified views of dotted squares. **g.** Histomorphological score from f. Analysis as in b. F (2, 8) = 2.603; p = 0.1347. **h.** Ileal IF for EGFP and CDH1 from mice treated as in a. n = 3 mice per group. Scale: 50µm. **i.** Quantitation of h shown as percentage of EGFP+ cells from CDH1+ epithelial cells. Analysis as in b. F (2, 6) = 1.677; p = 0.2639. **j.** Ileal IF for CD3 and F4/80 from mice treated as in a. n = 3. Scale: 50µm. **k.** Quantitation of j. shown as percentage of CD3+ cells in colonic lamina propria (LP). Analysis as in b. F (2, 6) = 19.61; p = 0.0023. **l.** Quantitation of j. shown as percentage of F4/80+ cells in colonic LP. Analysis as in b. F (2, 6) = 0.6489; p = 0.5557. **m.** Scatter plot of terms from Molecular Signatures Database Hallmark 2020 using Enrichr, based on chemical-target interactions for amantadine (PubChem). Each point represents a single term, plotted by odds ratio and -log<sub>10</sub>(adjusted p-value). Significantly enriched terms with adjusted p-value < 0.05 in magenta. **n.** qPCR analysis of bulk colonic tissue of mice treated as in 2a. Normalized expression values (ΔΔCq) shown in heat map. Unpaired t-test. Significant p-values < 0.05 in bold.