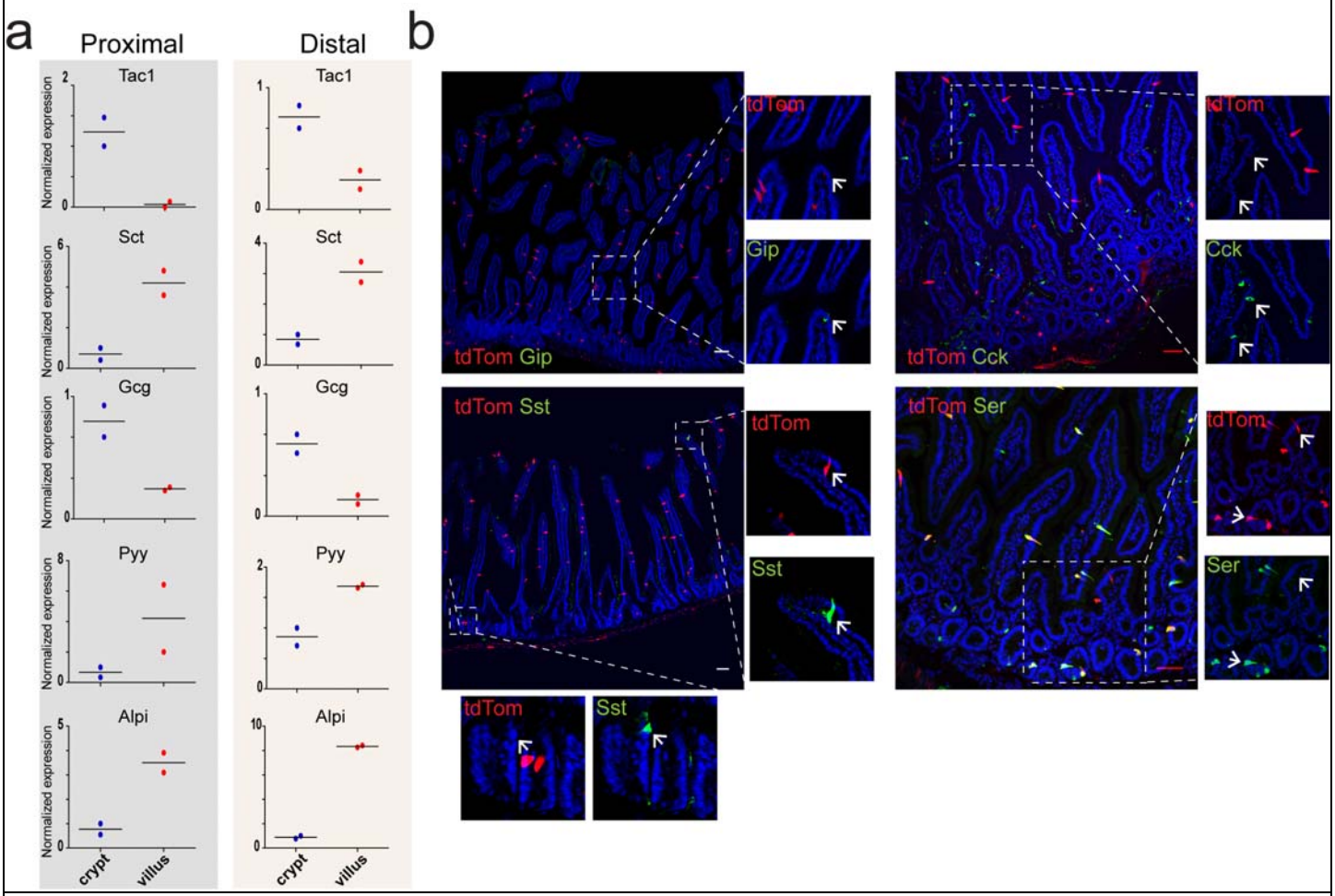
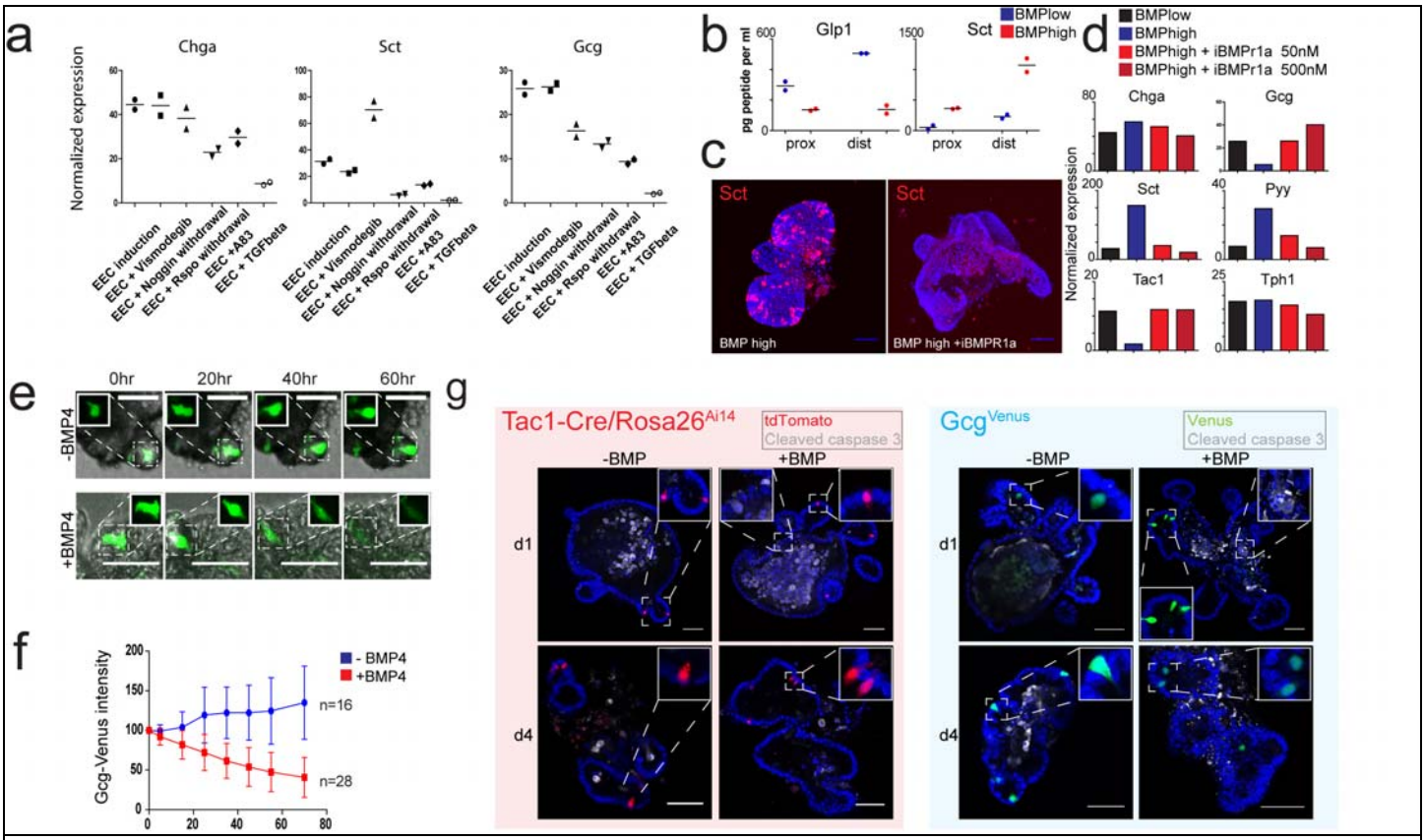


Supplementary Figure 1

Crypt-to-villus heterogeneity and lineage relationship of enteroendocrine cells.

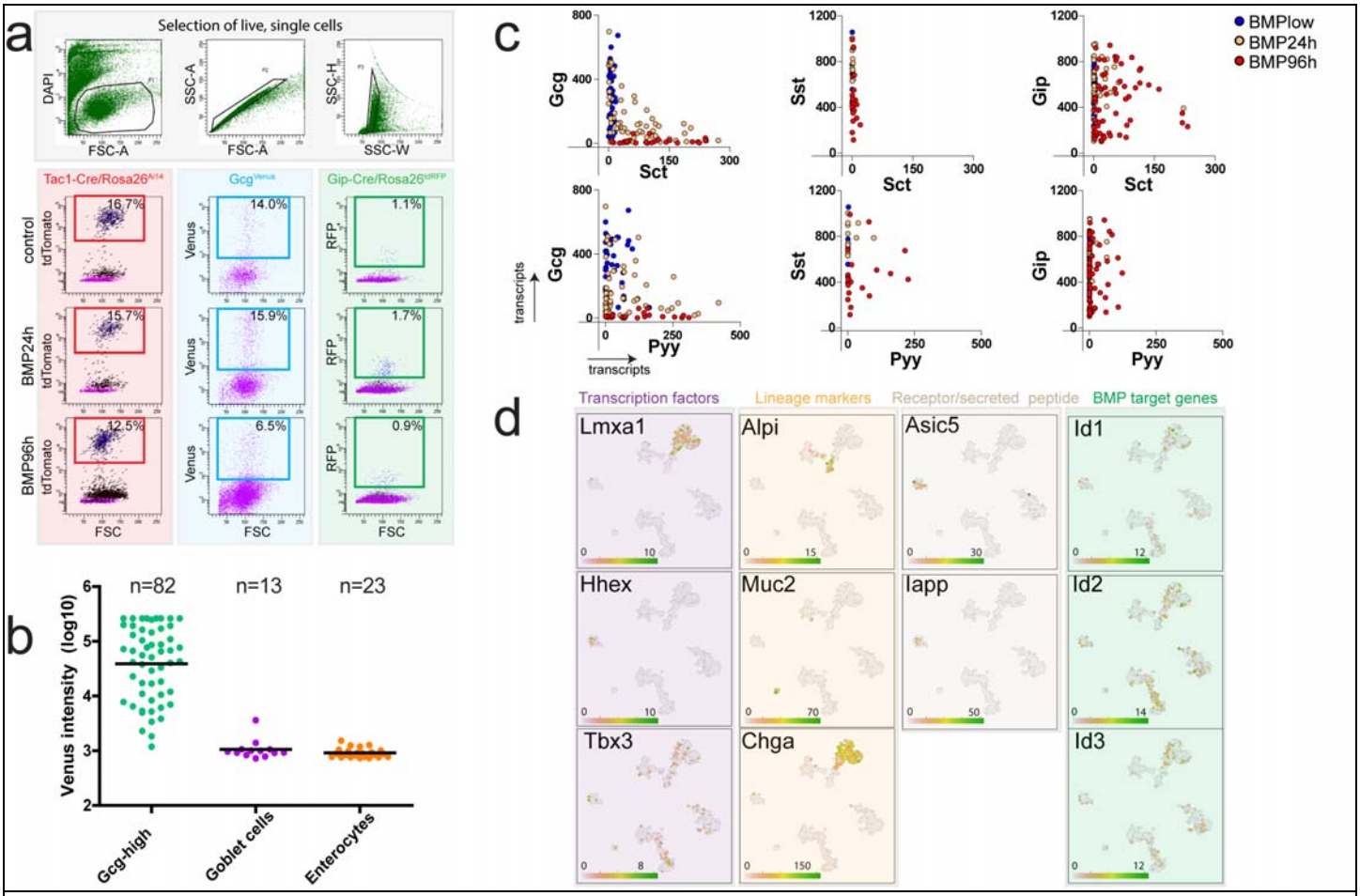
a, RNA was isolated from crypts and villi of the proximal and distal SI. qPCR analysis was performed on selected hormones. Tac1 and Gcg transcripts are enriched in the crypts of mice, while Pyy and Sct are increased in the villus. Alpi, a marker of enterocytes, confirmed enrichment of villus fractions. Sample size represents n=2 mice, and the mean values are shown. **b**, Intestine of $Tac1^{iresCre}$/$Rosa^{Ai14}$ mice reveals that $Tac1^{+}$ expressing cells give rise to Serotonin (Ser) expressing cells and are thus part of the Enterochromaffin lineage. Gip, Sst and Cck expressing EECs are rarely derived from Tac1, while the vast majority of Serotonin producing ECs are derived from Tac1. Quantification in Figure 1g (n=4 mice). Scale bar is 50 μm.



Supplementary Figure 2

Signaling through the BMP4-BMPR1a/BMPR2 axis induces hormone switching in EECs without causing apoptosis

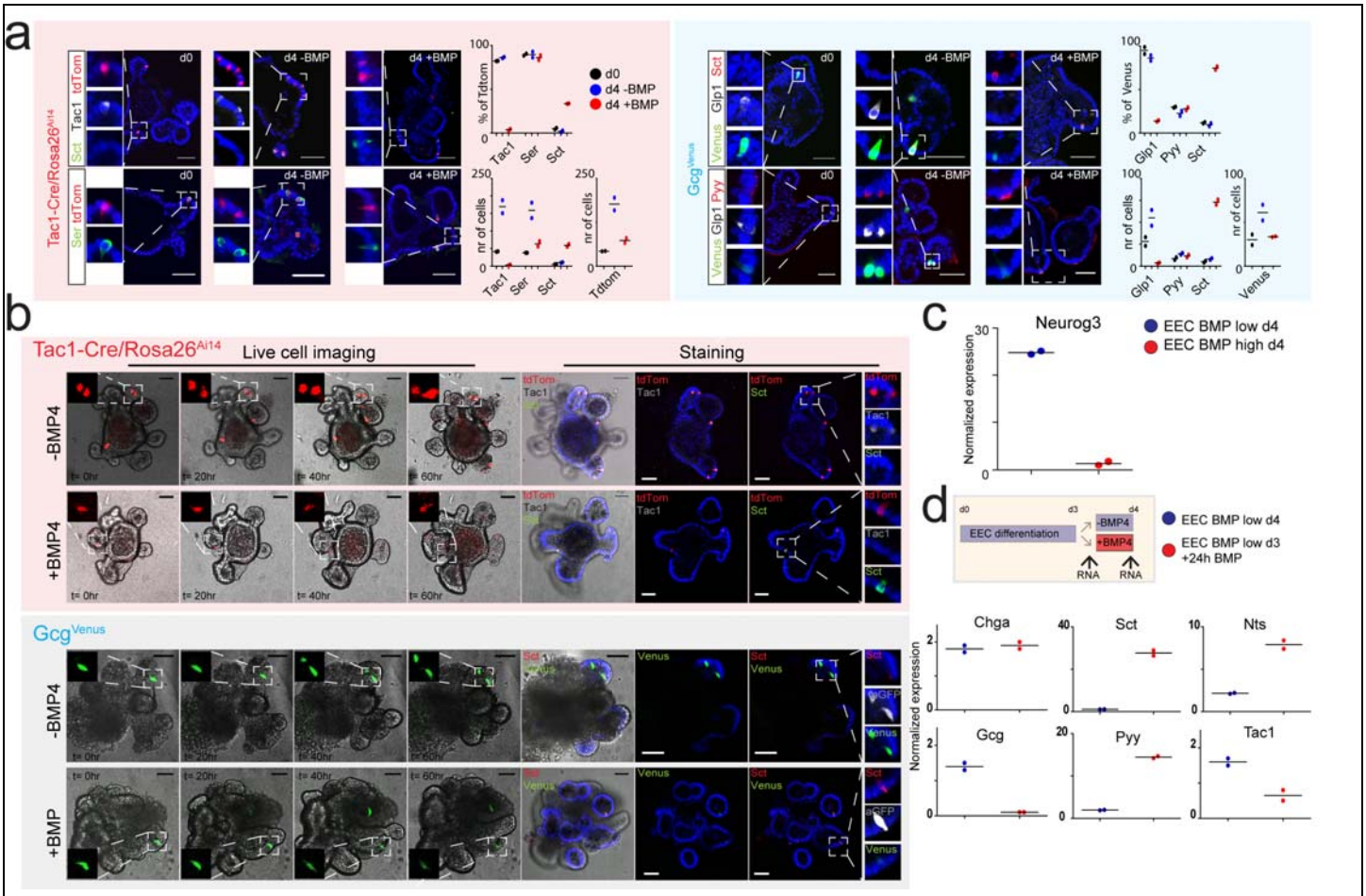
a, Organoids were differentiated into enteroendocrine cells (EECs) for 4 days by the inhibition of Wnt, Notch and MEK signaling. On the background of the EEC differentiation cocktail, we inhibited Hedgehog (Vismodegib), activated BMP (Noggin withdrawal), inhibited Wnt (Rspo withdrawal), inhibited TGFbeta (A83) or activated TGFbeta (TGFβ1) signaling. qPCR analysis of selected hormones shows that activation of BMP signaling selectively induces Sct but represses Gcg expression, while having a neutral effect on Chga. Results are shown relative to control organoids in ENR medium. Sample size represents n=2 biologically independent experiments, and the mean values are shown. **b**, Secreted Glp1 and Sct peptides after stimulation with forskolin were measured by ELISA in EEC differentiation medium with or without BMP4. Secreted Glp1 was diminished in the background of BMP activation, while Sct increased. Experiment was performed in in proximal and distal organoids. Sample size represents n=2 biologically independent experiments, and the mean values are shown. **c**, Addition of BMPR1a inhibitor LDN193189 to a 4-day EEC differentiation protocol containing BMP4 reverses the increase in Sct production, as shown by immunostaining. Image is presented as a maximum projection. Experiment was performed three times independently with similar results. Scale bar is 50 µm. **d**, qPCR analysis of selected hormones of the same experiment as in c. BMPR1a inhibition reverses all changes induced by BMP4 addition to the standard EEC differentiation cocktail (EEC BMPlow). Sample size represents n=1 biologically independent experiment, and the average value of a technical duplicate is shown. **g**, Organoids derived from Gcg^{Venus} mice were imaged for 3 days in the presence and absence of BMP4. Individual L-cells shut down Gcg transcription when stimulated with BMP4, while control cells maintain Gcg positivity throughout the imaging timewindow. Imaging started 6 hours after addition of BMP4. **f**, Quantification of e. The number of $Venus^{+}$ cells imaged and measured is depicted. The mean intensity of all cells at each timepoint is shown, and error bars present SD. **g**, Organoids from Gcg^{Venus} and $Tac1^{iresCre}$ /$Rosa^{Ai14}$ mice were differentiated to EECs in the absence or presence of BMP, and co-stained for cleaved caspase-3 to assess apoptosis. Experiment has been repeated two times with similar results. Scale bar is 50 µm.



Supplementary Figure 3

Single cell transcriptome analyses of EEC subtypes after BMP activation.

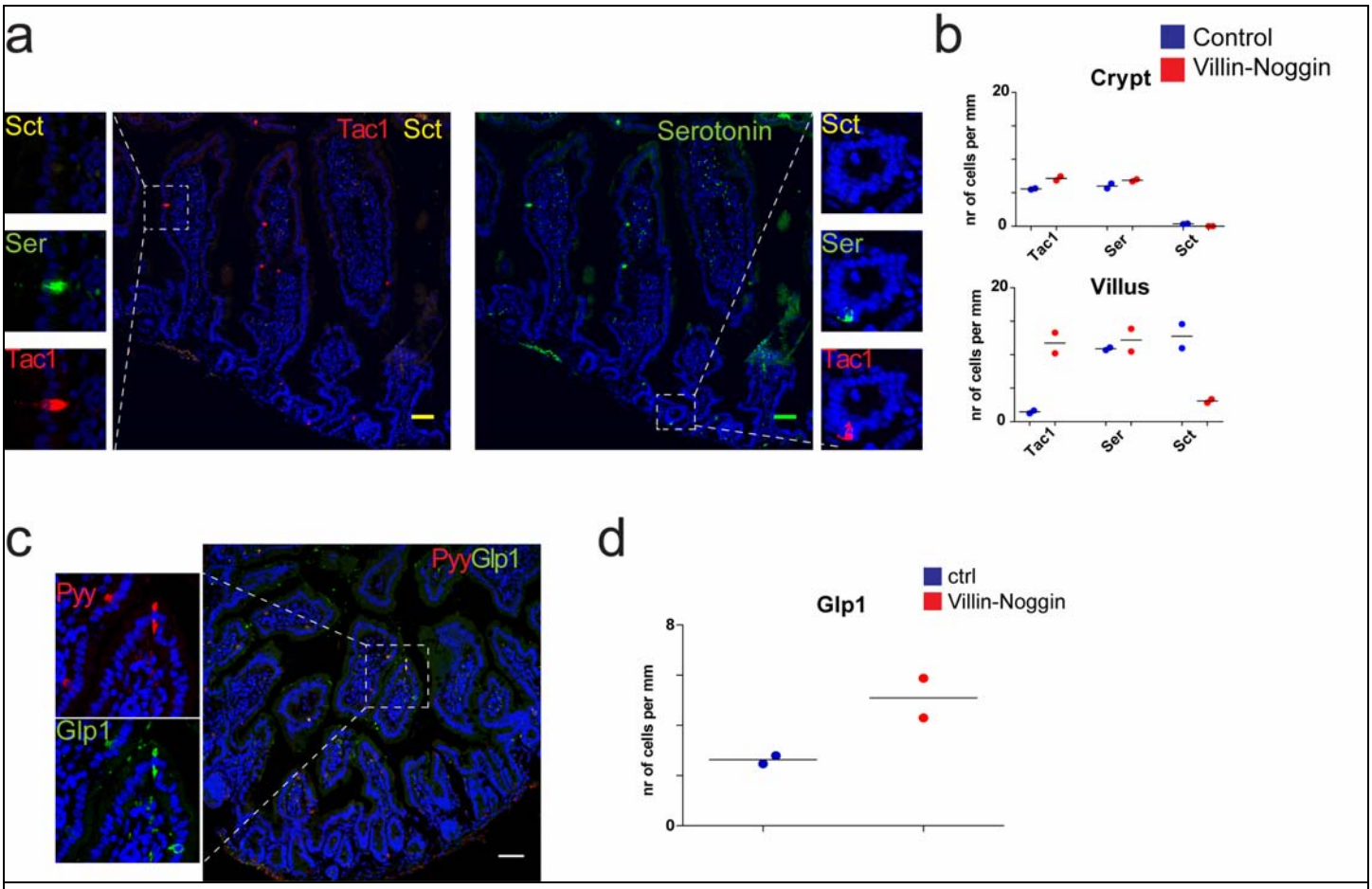
a, FACS plots display the gating strategy used to sort live, single cell (DAPI-negative, right plots) subtypes of EECs from reporter organoids (left plots) in different BMP activation contexts. **b**, Venus intensity levels of Goblet cells (Muc2^{+}), Enterocytes (Alpi^{+}) and Gcg-high cells. Mean intensity is shown for each group, together with the number of cells in each group. **c**, Cells with a Venus intensity of more than 2.5 * 10^{4} , predictive for Gcg expression (Fig. S4a, S4b), were selected from the dataset. Transcripts for Gcg, Sct and Pyy are plotted (upper plots). High Venus positivity is predictive for Gcg expression in BMPlow conditions, but not after BMP treatment. Cells with at least 100 Sst (middle plots) or Gip (lower plots) were selected from the dataset. Gip^{+} and Sst^{+} cells upregulate Pyy expression upon BMP treatment, while only cells producing Gip can co-express Sct. **d**, Expression levels of selected markers in the t-SNE space. Different transcription factors, receptors and secreted peptides were uniquely expressed by subtypes of EECs. BMP target genes associate with BMP4 treated samples.



Supplementary Figure 4

BMP signaling induces switches in peptide profile of individual EECs and blocks initial EEC specification

a, Tac1^{iresCre} /Rosa^{Ai14} and Gcg^{Venus} – reporter organoids were treated with a MEK inhibitor, while receiving Noggin (-BMP) or BMP4 (+BMP). The numbers of each marker positive cells are presented (in 10 organoids), and the co-localization of these hormones with tdTomato or Venus was quantified. Sample size represents n=2 biologically independent experiments, and the mean values are shown. BMP activation dampens the generation of new EECs and induces switches in the peptide profile of individual EECs. **b,** Individual EECs in Tac1^{iresCre} /Rosa^{Ai14} and Gcg^{Venus} – reporter organoids were followed using live cell imaging in the absence and presence of BMP signals. After 60 hours, organoids were fixed inside the BME and stained for different hormones, as indicated. The experiment was performed two times independently with similar results. **c**, Mouse intestinal organoids were EEC differentiated for 4 days in BMP low or high conditions. qPCR analysis reveals Neurog3 is downregulated after BMP treatment (data is shown relative to BMP untreated sample). Sample size represents n=2 biologically independent experiments, and the mean expression values are shown. **d**, Mouse intestinal organoids were EEC differentiated for 4 days in BMP low conditions, or for 3 days in BMP low and 24 hours in BMP high conditions. qPCR analysis of selected hormones (data is shown relative to day 3 EEC differentiation). Initial EEC generation in BMP low conditions and subsequent induction of BMP signaling for 24 hours is most efficient in generating Villus-enriched hormones. Sample size represents n=2 biologically independent experiments, and the mean expression values are shown.



Supplementary Figure 5

A transgenic mouse model with a disrupted BMP gradient displays altered hormone expression.

a, Immunostaining of intestines from Villin^{Noggin} mice. Tac1^{+} cells were scattered along the whole crypt-villus axis, while Sct^{+} cell numbers greatly reduced. **b**, Quantification of a. Sample size represents n=2 mice per genotype. Mean values are depicted. **c**, Villin^{Noggin} mice show an increased number of Glp1^{+} L-cells in the villus. **d**, Quantification of c. Sample size represents n=2 mice per genotype. Mean values are depicted. Scale bar is 50 µm.