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Description and functional validation of human enteroendocrine cell sensors

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Abstract

Enteroendocrine cells (EECs) are gut epithelial cells that respond to intestinal contents by secreting hormones, including incretins GLP-1 and GIP, which regulate multiple physiological processes. Hormone release is controlled through metabolite-sensing proteins. Low expression, interspecies differences, and existence of multiple EEC subtypes have posed challenges to the study of these sensors. We describe differentiation of stomach EECs to complement existing

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Competing interests
HC is head of Roche's Pharma Research and Early Development in Basel, Switzerland since March 2022. JB and HC are inventors on several patents related to organoid technology. HC's full disclosure is given at https://www.uu.nl/staff/JCClevers/.

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intestinal organoid protocols. CD200 emerged as a pan-EEC surface marker, allowing deep transcriptomic profiling from primary human tissue along the stomach-intestinal tract. We generated loss-of-function mutations in 22 receptors and subjected organoids to ligand-induced secretion experiments. We delineate the role of individual human EEC sensors in hormone secretion, including GLP-1. These represent potential pharmacological targets to influence appetite, bowel movement, insulin sensitivity and mucosal immunity.

EECs are GI epithelial cells that constitute part of the gut-brain axis. They regulate physiological responses related to metabolism (such as appetite, insulin release, bowel movement) as well mucosal immunity (1). EECs are relatively rare (~1% of the epithelium) and can be subdivided into 5 major subtypes, each producing a different set of peptide hormones and/or neurotransmitters (2). Each subtype has a distinct distribution along the GI tract. The major EEC subtype, the enterochromaffin cell (EC), produces about 90% of body serotonin (5-HT) and regulates gut motility and inflammation. The other EECs are letter-coded: L-cells produce glucagon-like peptide 1 (GLP-1), neurotensin (NTS), peptide YY (PYY), and cholecystokinin (CCK). MX-cells produce ghrelin (GHRL) and motilin (MLN). D-cells produce somatostatin (SST). K-cells produce gastric inhibitory protein (GIP) while G-cells produce gastrin (GAST) (3). K-, MX-, and G-cells are most abundant in the proximal small intestine, while L-cells are enriched in the distal small intestine and colon. The stomach corpus contains a unique enteroendocrine cell subset, termed enterochromaffin-like cells (ECL-cells). In addition to producing some serotonin, ECL-cells produce histamine to regulate acid secretion by nearby parietal cells (4). Important differences exist between mouse and human EECs. For instance, human MX-cells express motilin only in the small intestine but not in the stomach, while the pertinent gene is a pseudogene in rodents.

EECs are electrically excitable and control hormone secretion through elevation of intracellular calcium (5). Calcium levels are controlled through G protein-coupled receptors (GPCRs) as well as nutrient status, regulating the activity of ATP-sensitive potassium channels. Their products can signal to local neurons, potentially through diffusion or through synaptic interactions (termed neuropods) with nearby neurons (6) and to other EECs.

Drug development based on insights in EEC biology is currently centered around hormone mimetics (7). Such incretin-based drugs - derivatives of GLP-1 and GIP - are turning out to be highly efficacious for the treatment of diabetes and obesity, while additional indications such as Metabolic dysfunction-associated steatohepatitis (MASH), cardiovascular disease, and neurodegenerative diseases are under intense evaluation (8, 9). An alternative therapeutic strategy could involve the stimulation of EEC receptors to control local secretion of multiple hormone products at once (versus systemic individual hormone mimetics). L-cells, for example, produce oxyntomodulin (OXM) – for which the receptor is not established – in addition to GLP-1. OXM administration in mice reduces food intake and increases energy expenditure, and could be beneficial in addition to the incretin effect caused by GLP-1. GLP-2 is another peptide derived from the same cell that exerts beneficial metabolic effects (10). Gastric bypass entails a surgical intervention for obesity that bypasses the SI-proximal EEC types that produce the ‘hunger hormone’ ghrelin.

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Nutrients from the stomach are directly shunted to the more distal SI, inducing hormone secretion by the large L-cell population, improving glucose homeostasis (7). Therapeutic manipulation of other types of EECs or their hormones beyond GLP-1 and GIP remains largely unexplored.

Human EECs have been difficult to study due to their rarity (11); published single cell RNA sequence datasets of primary GI epithelium have provided some insight into the transcriptomes of the various EEC types (12, 13). We have previously built a platform to derive human EECs in large numbers in intestinal organoids using the pulsed overexpression of NEUROG3, a bHLH-transcription factor expressed in the common EEC progenitor. This allowed the generation of a transcriptomic and proteomic description specifically for the main SI EEC subsets (14). In the present study, we pursue the generation of a complete human tissue EEC single cell transcription atlas and -guided by this atlas- define the functions of 22 individual sensors on the various EEC subtypes. To this end, we combine descriptive observations on primary EECs with functional studies on EECs in GI tract region-specific organoids.

Results

Derivation of endocrine cells in stomach organoids

To date, endocrine cells cannot be generated in large quantities in stomach organoids. Murine data suggest that not all stomach endocrine cells derive from a NEUROG3-expressing progenitor cell (15–17). Indeed, X-, EC-, and ECL-cells arise in a NEUROG3-deficient background (15). Lineage tracing additionally suggested that murine 5-HT-producing EC-cells could exclusively be derived from bone marrow cells (18). We generated human stomach organoid lines carrying a doxycycline-inducible NEUROG3 expression construct, as described previously for SI organoids (14). (Fig. 1A) A 2-day overexpression NEUROG3 pulse followed by 3, 5, or 8 days of maturation in EGF-, Noggin-, and Rspondin-containing conditions (ENR) efficiently induced differentiation towards the major stomach endocrine lineages, including EC-cells (Fig. 1B-C, S1A-B). Endocrine cells appeared mature based on transmission electron microscopy, and readily secreted 5-HT and ghrelin when stimulated with cAMP-agonists (Fig. 1D-E). To profile the full spectrum of peptide hormones produced by these organoids, we performed proteomic profiling of the intracellular proteome, as well as the secreted peptides. We noted production of intracellular and secreted endocrine products only in the presence of NEUROG3 overexpression (Fig 1F, S2A-C). Proteins associated with mucus production (for example, Trefoil factors) were enriched in control conditions. For many known stomach hormones, we could confirm the presence of full-length bioactive peptides in the secretome (Table S1).

We previously found that the morphogen BMP profoundly impacts hormone expression along the small intestinal crypt-villus axis (19) and tested its effect on stomach endocrine differentiation using bulk RNA sequencing (Table S2, Fig. 1G). We included organoids derived from the corpus and pylorus region. Corpus organoids expressed much higher levels of ghrelin, confirming that regional identity is maintained in culture (12). Although absolute levels of *MLN* were expectedly very low in stomach organoids, BMPs did increase *MLN* expression at the expense of *GHRL*, mirroring SI patterns (14). Intestinal expression of the

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neuropeptide *NPW* was described before in the small intestine (14) and we now identified this also in the human stomach. Its expression was strongly induced by BMPs. Tachykinin *TAC3* was highly abundant, yet only in the absence of BMPs. Similar to the small intestine, we observed granin proteins to be regulated by BMPs in a similar fashion, including an upregulation of *SCG2* and downregulation of *CHGB* (Fig. 1G). We did not observe any production of ECL markers, such as histamine decarboxylase (HDC), and very low expression of gastrin (Table S2). We conclude that NEUROG3 is sufficient to produce major stomach endocrine lineages except for ECL-cells, and that BMPs modify the repertoire of their secreted products, similar to the small intestine (19).

Description of EEC-subtype GPCR profiles in stomach and intestinal organoids

To track and purify stomach endocrine cell lineages, we introduced fluorescent reporters at the translation termination codon of hormones using CRISPR-Cas9-mediated non-homologous-end-joining (Fig. 1A) (20, 21). Successful tagging of CHGA, SST and GHRL was confirmed by immunofluorescent staining (Fig. 2A). Next, we used these stomach reporter organoids, in combination with a knock-in organoid biobank generated in a previous study, to sort endocrine cells from 14 different cell type and regional identities to build a comprehensive transcriptomic atlas (Fig. S3A) (14). Bulk RNA sequencing (allowing deeper reads per cell type than single cell methods) confirmed the expected hormone expression profiles in the respective reporter populations (Fig. 2B, Table S3). Next, we focused on GPCRs and other receptor genes that could act as sensors regulating hormone release. We found expression of numerous previously described GPCRs in the various EEC populations (Fig. S3B). Additionally, this analysis yielded a number of previously unknown endocrine cell-specific receptor genes, including for example the receptors for the black widow toxin alpha-latrotoxin (*ADGRL1* and *ADGRL2)* (Fig. 2C-D). Dopamine receptors – particularly *DRD2* – were expressed in the majority of endocrine cell subsets, as was the acetylcholine receptor subunit *CHRNB2* (Fig. 2C-D). We observed expression of the galanin receptor *GALR1*, previously described to negatively impact GLP-1 secretion (22), in EC- and L-cells. We additionally found that EC-cells expressed *GALR2*, a receptor that couples to Gq proteins and hence can activate secretion (in contrast to the Gi-coupled *GALR1).* Although both receptors are stimulated by the neuropeptide galanin, *GALR2* can specifically be activated by the 14-amino acid peptide spexin, produced in the hypothalamus (23). We found abundant epithelial production of spexin, particularly in G/K-cells (Table S3), suggestive of signaling from these cells to GALR2-expressing EC-cells.

Finally, we detected expression profiles for multiple receptors that did not agree with previous reports (Fig. S3B). Most notably, previous work has indicated that the melanocortin receptor *MC4R* regulates GLP-1 expression (24), but in agreement with a recent study (25), we found no significant expression in any gut endocrine cell (Table S3). We did observe expression of another melanocortin receptor: *MC1R*. 5-HT secretion from small intestinal EC-cells has been suggested to be predominantly regulated through GLP-1 rather than through nutrient receptors (26). While we did find GLP1R expression in EC-cells, these cells abundantly expressed fatty acid receptors, as well as the bile acid receptor *GPBAR1* in all gut regions (Fig. 2D, S3B), suggestive of direct responsiveness to these dietary components.

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CD200-guided single cell atlas of primary human EECs

We next pursued in vivo validation of the receptor expression profiles as identified in organoids. Endocrine cells are exceedingly sparse and, consequently, single cell signatures for individual EECs appear only rarely in gut atlases (12, 13, 27). From the transcriptomic data from organoids, we identified the surface protein CD200 as a general surface marker of GI endocrine cells (Fig. 3A). We sorted cells enriched for CD200 and EPCAM from human stomach, small intestinal, and colonic biopsies and performed single cell RNA sequencing using the recently developed VASA-seq protocol (28). This method has the key advantage of yielding whole transcript length information and was shown to provide much 'deeper' sequence data than standard protocols, increasing the chances of finding lowly expressed receptor genes (28). We thus generated a GI tract epithelium dataset from stomach, SI, and colon, including 764 endocrine cells and 1314 non-EEC epithelial cells (Fig. 3B-C). The dataset contained an average number of 46897 unique reads and 10057 genes per cell, much deeper than previous datasets containing human endocrine cells (13). EECs represented the largest fraction of CD200-positive cells, confirming successful enrichment (Fig. S4A). Major GI epithelial lineages could readily be identified: enterocytes, goblet cells, stem cells, tuft cells, and chief cells (Fig. S4B-C). The dataset allowed us to validate the expression profiles of most receptors acting as putative sensors (Fig. 3D-E). For example, we did not detect *MC4R*, while *MC1R* was produced (albeit at a low level), and the alpha-latroxin receptors were EEC-enriched (Fig. 3E, Fig. S5A, Table S4). The calcitonin receptor *CALCR* was confirmed in D- and EC-cells, but in tissue was most highly expressed by ECL-cells. As ECL-cells were not present in organoids, we could additionally identify the prostaglandin receptor *PTGER3*, adhesion receptor *ADGRV1*, and the GLP1-receptor *GLP1R* in these cells, while stomach G-cells had high levels of the succinate receptor *SUCNR1* (Fig. S4C). The latter had been previously described to be enriched in murine tuft cells, a finding which we could not confirm in human (Fig. S4C) (29). We confirmed the previously described *GLP1R* expression in D-cells (30). GLP1R is typically difficult to detect in tissue due to paucity of antibodies, complicating the study of its physiological effects. We found that primary tissue ECL-, MX- and G-cells as well as in the immune-regulatory Tuft cells express this hormone receptor (Fig. 3E) and observed this also in organoids (Fig. S3B). We further contrasted EEC subtypes between different parts of the gastrointestinal tract to identify differentially expressed genes reflective of potential regional adaptations to luminal environments. These analyses revealed important region-specific features, such as the enrichment of the fatty acid receptor *FFAR3* in gastric X-cells versus intestinal MX-cells (Table S5). Conversely, MX-cells express much higher levels of the tryptophan receptor *CASR*. We could confirm expression of the majority of GPCRs observed in organoids. Despite a high level of concordance, we did find subtle differences between organoid and tissue data: *GPR12* was strongly enriched in distal gut M/X-cells, while in tissue it was enriched in the proximal counterparts.

We observed a cluster of unknown epithelial cells derived from the duodenum with high expression of peptidases, *GP2* (a known M-cell marker (31)) and *GLP1R* (Fig. 3E, S4C). *GLP1R* was previously identified in murine Brunner glands (32), duodenal submucosal glands that secrete bicarbonate and peptidases. We used multiplexed FISH to assess *GLP1R* expression on human sections and indeed found high expression in Brunner glands, while it

was too low to detect in the endocrine cell populations (Fig. 3F, S5B). While no annotated single cell RNA sequencing dataset for human Brunner glands has been reported, we concluded that this cluster represents Brunner gland cells.

As we were not able to generate ECL cells in our stomach organoid model, we exploited the tissue dataset to identify potential regulators of their differentiation. We noted very high and specific expression of the transcription factors PTF1A and RBPJL, as well as their target gene LHX5 (Fig. S4C). These transcription factors are implicated in development of the acinar cells in the pancreas (33). We thus conditionally overexpressed these factors in the presence and absence of NEUROG3 (Fig. 3G). Surprisingly, organoids displayed massive apoptosis and degradation of the matrigel upon PTF1A overexpression (Fig. 3H). This phenotype was reminiscent of peptidase secretion from mature acinar cells. We could indeed observe high expression of the pancreatic carboxypeptidase *CPA1* (Fig. 3I), suggesting apparent acinar transdifferentiation. We found that LHX5 only in combination with NEUROG3 induced expression of histidine decarboxylase (HDC), the enzyme involved in the synthesis of histamine and a key marker of ECL cells. However, expression remained relatively low in this condition, as well as in other transcription factor combinations.

A multiplexed functional screen for GPCR function in hormone secretion

Having validated conserved gene expression in between organoids and tissue endocrine cells, we applied the organoid models to assess receptor function. We first built a biobank of 22 receptor-deficient organoid lines using cytosine base editors to introduce homozygous nonsense mutations in coding sequences, generating 370 organoid clones (Fig. 4A-C, Fig. S6) (34). We subjected wildtype and receptor-deficient organoids to their respective agonists (Table S6), as well as to a positive control (cAMP agonism, through Forskolin (FSK) and IBMX) to achieve maximal secretion of GLP-1, 5-HT, and somatostatin. A pilot experiment indicated that 24 hours of cAMP activation led to 5-10 fold higher concentrations of GLP-1 in the medium, compared to 2 hours of secretion (Fig. S7A). To increase sensitivity, we therefore opted to perform all secretion assays overnight. As a proof of concept, we further tested bile acids known to regulate GLP-1 secretion through GPBAR1 (35). Indeed, we could induce efficient secretion (~50% of maximum) of GLP-1 that was lost in *GPBAR1* mutant organoids (Fig. 4D). In line with its expression profile, we also observed GPBAR1-mediated release of 5-HT, in contrast to a previous study that did not find such responsiveness in mice (Fig. 4D) (26).

Next, we stimulated the entire biobank of organoids with GPCR agonists, including three genes for which we lacked mutants (FFAR3, GALR2 and TRPV2). We additionally included ghrelin measurements for a selection of MX-cell enriched receptors (Fig. 5A-B, S7B). No significant somatostatin secretion was measurable for the majority of stimulators (Fig. S7C). We found numerous receptors that control secretion of GLP-1, 5-HT, and ghrelin, including the melanocortin receptor *MC1R* (Fig. 5A-B). We uncovered potent release of all gut hormones, including somatostatin (Fig. S6C), with ADGRL1/2-stimulating alpha-latrotoxin. This effect was only partly lost in *ADGRL2*-mutant organoids, suggesting redundancy between the receptors. Of note, black widow envenomation can cause severe GI symptoms including severe vomiting (36). ABCC8 encodes the sulfonylurea receptor, which together

with KCNJ11 forms an ATP-sensitive potassium channel on pancreatic beta cells. Mutations in the gene are associated with Type 2 diabetes. The diabetes drug glibenclamide enhances insulin secretion by inhibiting this complex and thereby inducing membrane depolarization. We found that ABCC8 inhibition similarly controls gut release of GLP-1 and 5-HT, in line with its expression profile. ABCC8 was previously found to induce secretion of GLP-1 in rats and a mouse cell line (37, 38). These and our findings contrast with other observations in intestinal explants or in patients (39). These conflicting observations may arise from differences in assay sensitivity, or indirect effects on L cells that sulfonylureas have in vivo – for example by modulating blood glucose. The tryptophan receptor CasR controlled GLP-1 release, as recently reported in mice (40). GPR19 stimulation repressed secretion of GLP-1 and 5-HT, implying signaling through Gi G-proteins as suggested previously (41). Although we lacked a mutant control, the gut peptide Spexin (identified in this study) induced 5-HT release, presumably through its EC-enriched receptor *GALR2* (Fig. S7B). Besides responsiveness to bile acids (Fig. 4C), intestinal ECs also responded to fatty acids through several receptors, including FFAR2, FFAR4, and GPR119 (Fig. 5A). This contrasts with murine work that showed ECs not to respond to these dietary metabolites (26). GLP-1 release was similarly controlled by these receptors, as shown in earlier work (Fig. 5A) (42). Stomach endocrine cells displayed substantial expression of *TRPV2* (Table S4), which can act as a mechanosensor (43). However, chemical stimulation of this channel did not cause a significant change in stomach 5-HT release compared to receptor-deficient organoids (Fig. S6D).

Finally, we tested the role of the abundantly expressed dopamine receptors in hormone release. We found that dopamine significantly reduced 5-HT release from EC-cells ($p=0.03$), consistent with a dominant role of the Gi-coupled DRD2 (Fig. 5C). Indeed, DRD2 is the highest expressed dopamine receptor in organoids and tissue (Fig. 2 and 3). This effect could be reversed by several approved dopamine receptor inhibitors (Fig. 6C), suggesting that such therapeutics could impact intestinal serotonin release. Not all of these inhibitors reversed the effects of dopamine to a similar degree, potentially a reflection of different inhibitory activities to the numerous dopamine receptors.

Discussion

Enteroendocrine cells represent attractive therapeutic targets for the control of metabolic disease. A potential strategy to exploit these cells involves targeting their naturally occurring metabolite sensors, allowing manipulation of hormone release. Key to such therapeutic intervention is the availability of ‘deep’ mRNA expression datasets. Prior to this work, human EECs were not identifiable without fixation, posing hurdles to their purification from tissue. *Cldn4* has been proposed as a specific surface marker for murine EECs (44), yet this gene is broadly expressed in the human epithelium (13). The enrichment achieved in our study with CD200-based sorting enables deeper analysis of human EECs, in both homeostatic and diseased conditions. A second requirement to predict therapeutic effects of GPCR- or other receptor agonists involves suitable in vitro models for each of the pertinent EEC types. In this study, we have developed a stomach endocrine cell differentiation platform, generated transcriptomic datasets for the complement of EEC populations along the GI tract, and generated a focused receptor-mutant biobank. Thus, we

uncovered functions of EEC receptors as well as unexpected functions for some previously described genes. While previous work has suggested that stomach corpus EC-cells derive from mast cells, we could readily generate these cells from human stomach epithelial progenitors (18).

Incretin-based drugs such as semaglutide and tirzepatide are enjoying immense ever-growing popularity for the treatment of diabetes and obesity, while additional clinical indications such as MASH, cardiovascular disease, and neurodegenerative diseases are under investigation (8, 9). We identified and validated multiple GPCRs and channel proteins controlling human GLP-1 release, including the sulfonylurea receptor ABCC8 and the tryptophan-sensing CasR.

The current work may serve as a basis for exploiting GPCRs for the development of oral small molecules that activate the secretion of desired incretins. Such compounds may complement or broaden the applicability of current incretin-based therapies. CD200-based enrichment may further enable the study of genetic variants – associated with metabolic disease - causing expression changes in EECs. Finally, the presence of this immune checkpoint on intestinal epithelial cells is intriguing, and future work may address whether it plays a role in controlling the activation of tissue-resident or other immune cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data and materials availability

All single cell RNA sequencing data from gastrointestinal tissues are accessible through Dryad (45).

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One-Sentence Summary

Metabolite sensors regulate gut hormone release, suggesting approaches to control metabolism by targeting intestinal EECs.

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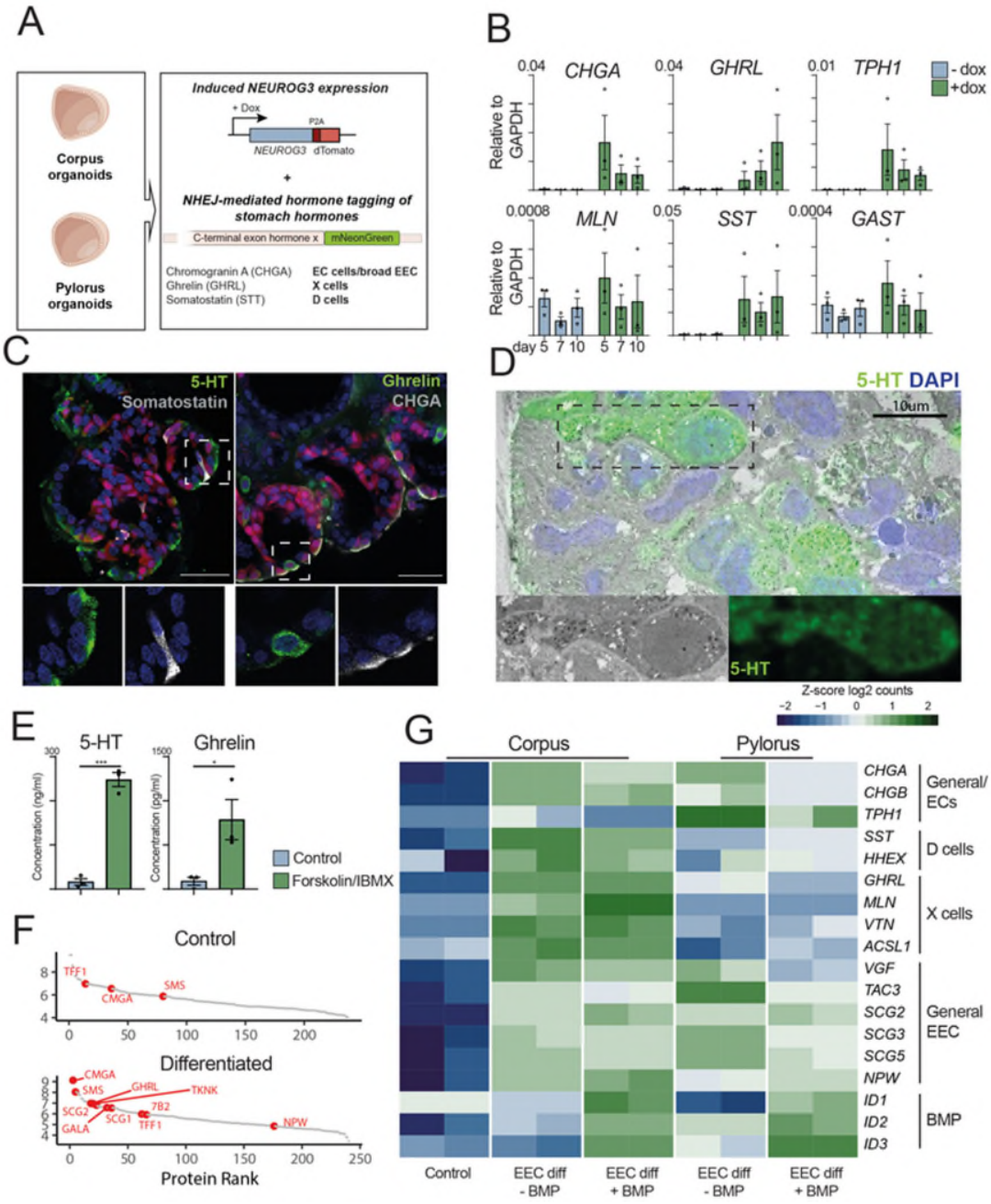

Figure 1. Differentiation of human stomach organoids towards functional endocrine cells

(**A**) Schematic representation of the generation of stomach endocrine cells. Stomach organoids were treated with doxycycline (dox) to induce overexpression of neurogenin-3 (NEUROG3), allowing endocrine cell production. Hormone genes shown were fluorescently tagged.

(**B**) qPCR analysis showing expression of hormones in the presence and absence of dox, at different timepoints. Expression levels are shown relative to *GADPH*. The analysis was performed in n = 3 independent replicates, and the mean expression and SEM are depicted.

(**C**) Immunofluorescent staining of EEC-enriched organoids. Scale bars are 50 μm.
(**D**) Correlative light-electron microscopy image of stomach organoids, showing overlap between TEM image and 5-HT fluorescent staining. Scale bar is 10 μm.
(**E**) Concentration of hormones in supernatant after overnight (16 hours) secretion in stomach organoids with forskolin and IBMX or no stimulus (control). Mean secretion (of n=3 technical replicates) and SEM are depicted.
(**F**) Ranked abundances of proteins detected in the secreted protein fraction, in organoid supernatant. Proteins of interest are highlighted in red. Endocrine products are enriched in differentiated organoids, whereas control organoids secreted trefoil factors.
(**G**) Heatmaps showing expression profiles of hormone genes in stomach organoids. Colored bar represents Z-score of log2 transformed values.

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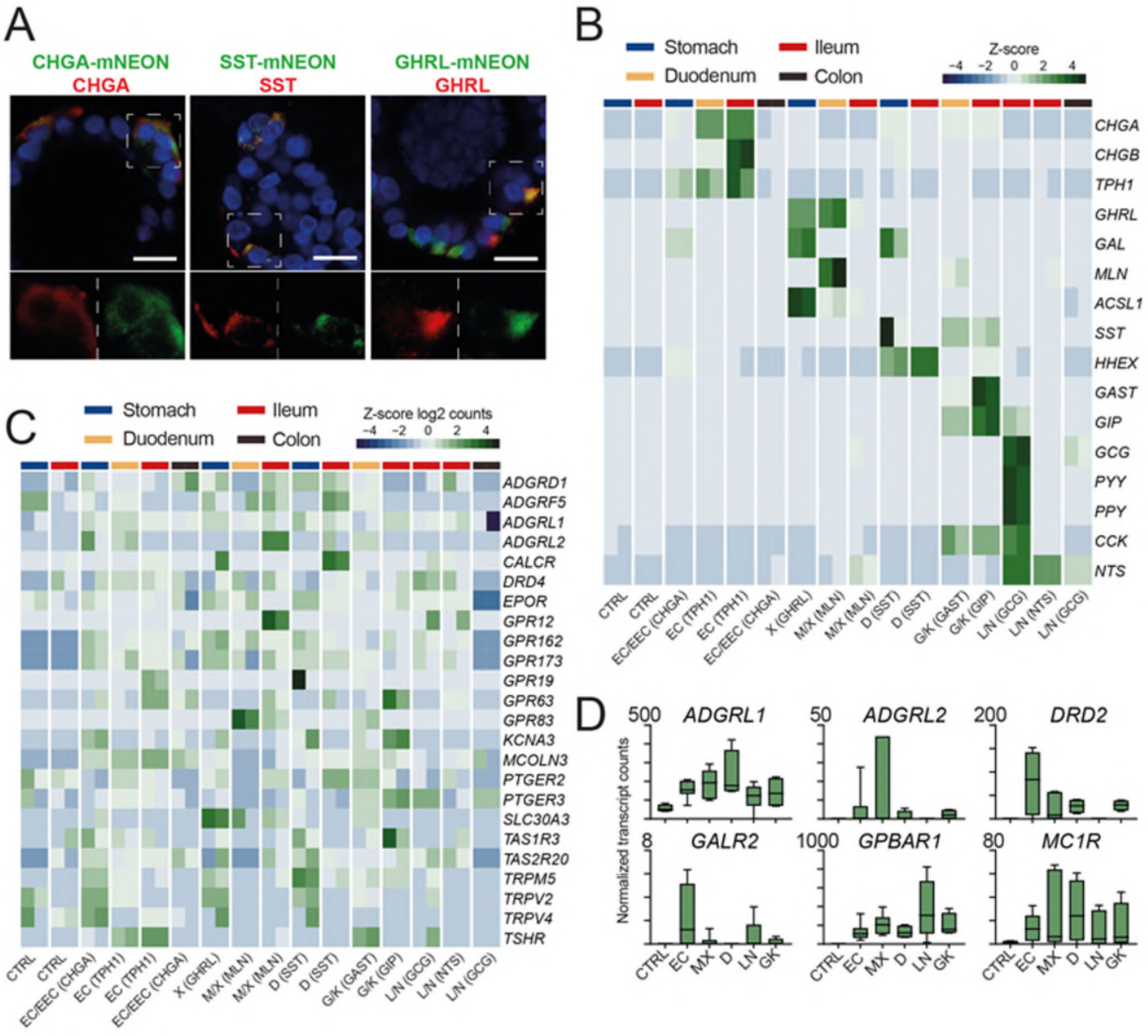

Figure 2. Defining the sensorome of human EEC subtypes in stomach, small intestine, and colon

(**A**) Immunofluorescent staining of hormones in reporter organoids, validating appropriate reporter expression. Zoomed in images of selected cells are shown. Scale bars are 10 μm.

(**B**) Heatmap showing expression profiles of hormones in hormone reporter populations purified from organoids. NTS-positive cells were sequenced from BMP-stimulated organoids. Colored bar represents Z-score.

(**C**) Heatmap showing expression profiles of receptor genes in hormone reporter populations purified from organoids. Colored bar represents Z-score of log2 transformed values.

(**D**) Box and whisker plots depicting expression of individual receptor genes in purified reporter populations. Box plots indicate median, 25th and 75th percentile values, and whiskers the minimum and maximum values.

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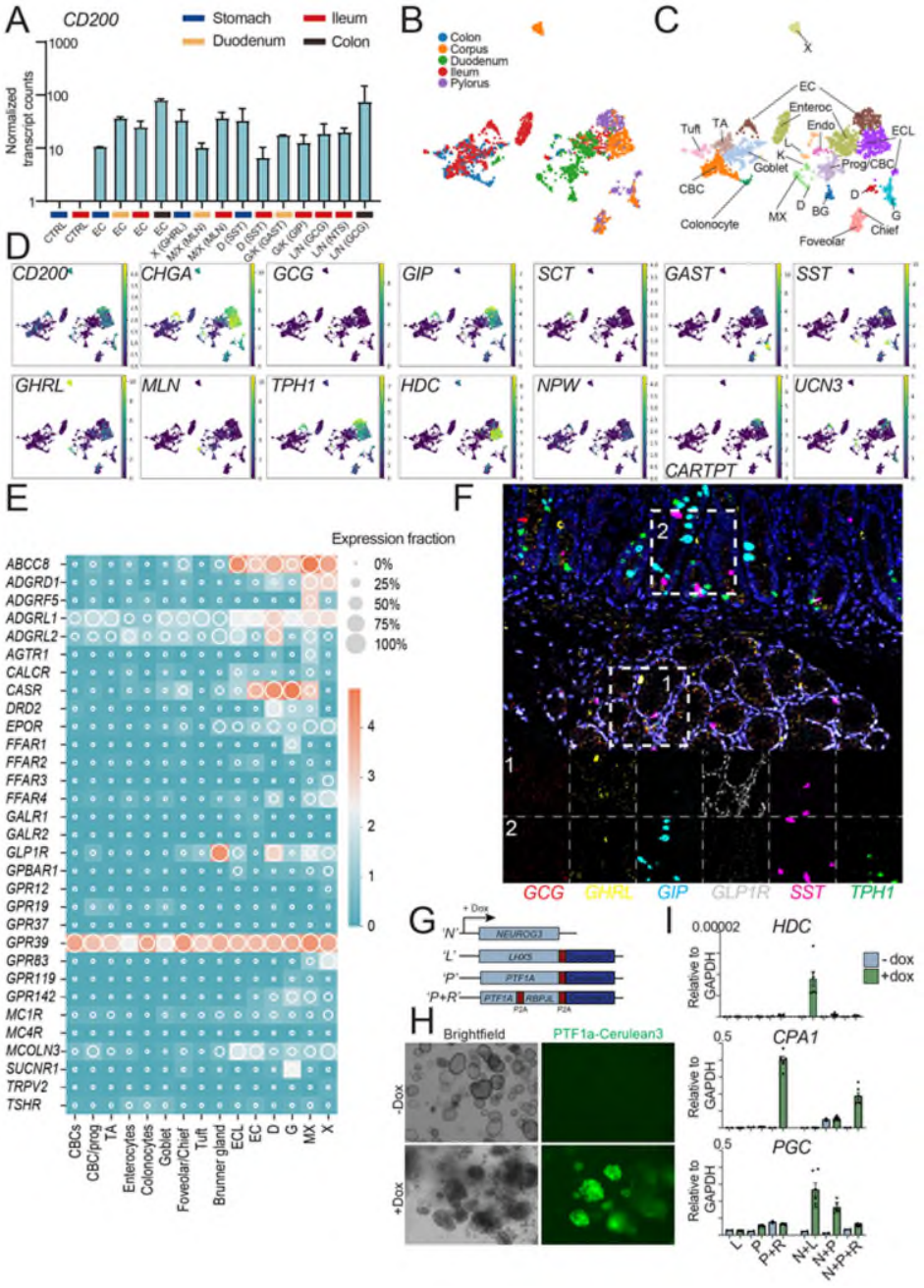

Figure 3. Single cell RNA sequencing and spatial transcriptomics reveal EEC sensors along the human GI tract

(**A**) Bar graphs depicting expression of *CD200* in purified reporter populations.

(**B-C**) UMAP displaying the human VASA-sequencing atlas (2078 cells, including 764 endocrine cells), including tissue identity (**B**) and cell type annotation (**C**).

(**D**) UMAPs displaying expression of different secreted products.

(**E**) Color reflects mean expression (log2) values per population and circles indicate fraction of cells expressing a given markers.

(**F**) Fluorescent in situ hybridization for *GLP1R* and different hormones on a human duodenal section. Zoomed-in images from Brunner gland (1) and duodenal epithelium (2) are shown below.
(**G**) Transcription factor overexpression constructs transduced in stomach organoids. Doxycycline (dox) induces expression of respective transcription factors.
(**H**) Overexpression of PTF1A in stomach organoids disrupted matrigel integrity, and lead to dissociation of organoids.
(**I**) qPCR analysis showing expression of lineage markers in the presence and absence of dox. Expression is shown relative to *GADPH*. The experiment was performed in n = 6 technical replicates, and the mean expression and SEM are depicted.

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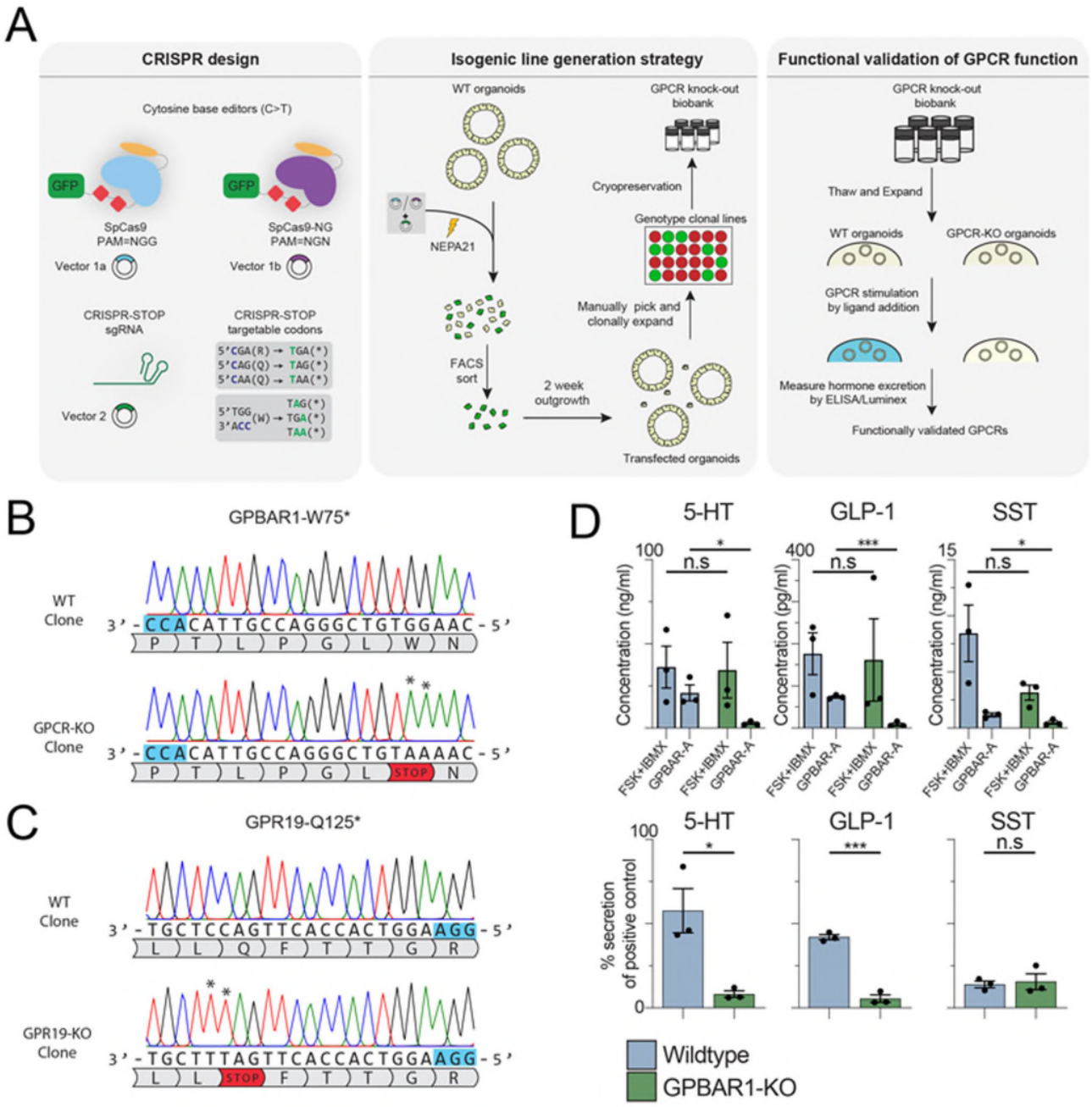

Figure 4. Generation of a human loss-of-function GPCR biobank for functional validation

(**A**) Overview of experimental pipeline.

(**B-C**) Sanger sequencing traces of wildtype and receptor mutant genes. Cytosine base editing induced missense mutations in tryptophan (W, (B)) and glutamine (Q, (C)) amino acids.

(**D**) Concentration of hormones in supernatant after 16 h secretion in wildtype and GPBAR1-mutant intestinal organoids stimulated with forskolin + IBMX or a GPBAR1 agonist. In the lower graphs, values are shown as percentage of maximum secretion

(FSK+IBMX). All data are derived from n = 3 experiments, and the mean concentration and SEM are depicted.

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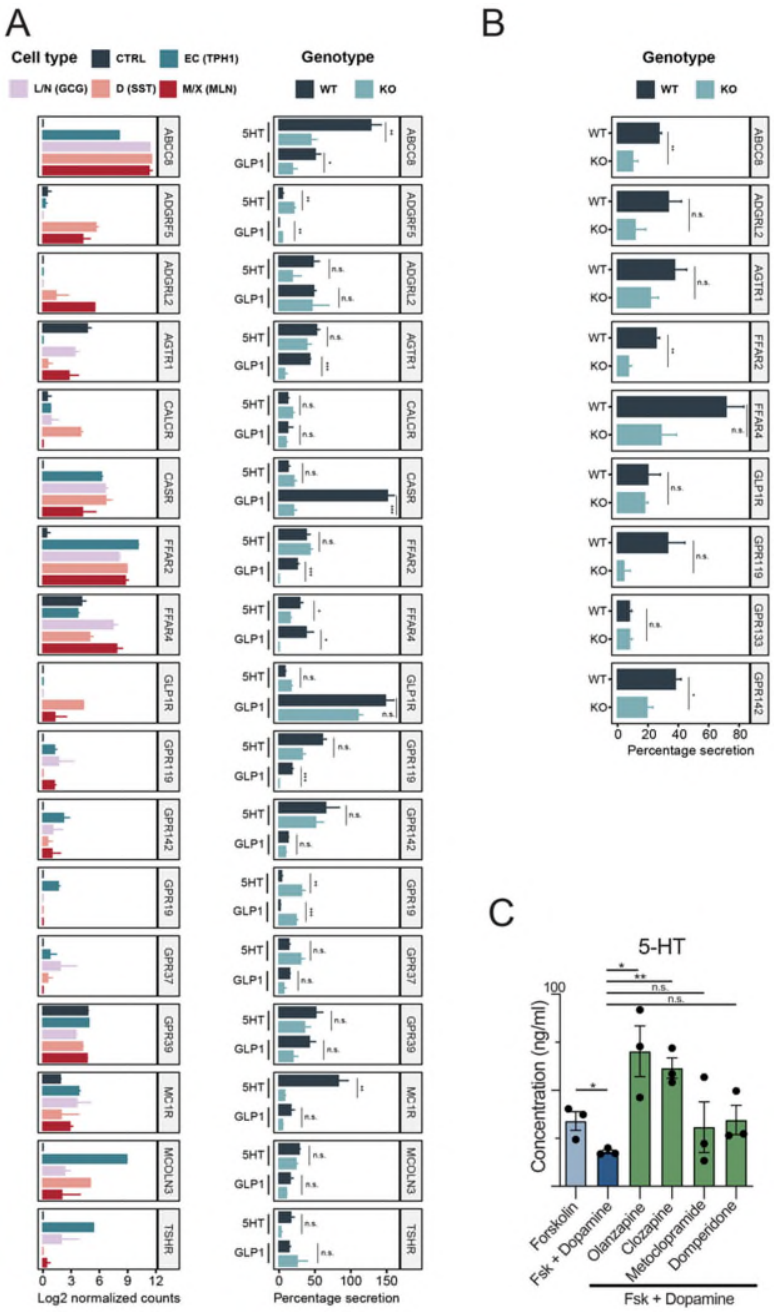

Figure 5. Functional assessment of human EEC sensors

(**A**) Left graphs indicate expression of receptor proteins in respective cell populations in organoids, based on bulk RNA sequencing of purified populations (data from Table S3). Right graphs indicate concentration of hormones in supernatant after 16 h secretion in intestinal organoids, in corresponding receptor wildtype (WT) or mutant (KO). Values depicted indicate the percentage of secretion in the presence of the agonist of the receptor compared to a positive control (FSK+IBMX).

(**B**) Concentration of ghrelin in supernatant after 16 h secretion in intestinal organoids, receptor wildtype (WT) or mutant (KO). Values depicted indicate the percentage of secretion in the presence of the agonist of the receptor compared to a positive control (FSK+IBMX). (**C**) Concentration of 5-HT in supernatant after 16 h secretion in wildtype intestinal organoids stimulated with positive control alone, or with the addition of dopamine with and without dopamine receptor inhibitors. All data are derived from n = 3 experiments, and the mean concentration and SEM are depicted.

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