SUPPLEMENTAL MATERIALS

Supplemental Methods

Bone Marrow Transplantation

8-10 week old Balb/C were irradiated with (850 cGy). Four hours later they received 10 million bone marrow (BM) cells from MHC-EGFP donor mice via tail vein injection. 12 weeks post BM transplant, the stomach tissues were collected for analysis.

Tissue preparation and Immunofluorescence*:*

Mouse stomach tissues were processed for immunohistochemistry and incubated with primary and secondary antibodies as previously described[1]. Guinea pig anti-HDC at 1:300 (EuroProxima) was used to stain HDC expressing cells.

Gastric epithelial single cell isolation and cell sortin*g*

Gastric corpus epithelial cells were isolated using mixed methods of mechanical and enzymatic dissociation [2]. The mouse stomach corpus mucosa cell suspension was obtained by protease buffer containing 50 µg pronase (Sigma-Aldrich) per 100 ml Basal Medium Eagle (Gibco). A MoFlo Beckman Coulter Cytomation sorter was gated to select CFP-positive cells using side and forward scatter to select single cells excluding nonviable cells with 7-AAD. Analysis of sorted HDC^+ cells by subsequent analytical flow cytometry for CFP showed that approximately 82% of sorted cells expressed the fluorescent protein. Analysis of HDC enrichment in CFP^+ cells versus CFP^- cells by RNAseq approached 388-fold with reduction in expression H+/K+ATPase ß subunit, TFF2, and pepsinogen by 84%, 95%, and 78% respectively. The ECL enrichment achieved in the present work is considerably higher than described previously, indicating lower contamination by parietal, mucous pit, and chief cells[3] and was sufficiently

enriched to identify genes that were significantly upregulated in ECL cells. For corpus Tph1-CFP^{+} cells isolated by FACS, Tph1 and Mcpt1 expression, measured by qPCR, were enriched by >270-fold and 256-fold respectively versus unsorted stomach corpus cells with greater than 60% reduction in H+/K+ATPase and pepsinogen expression measured by real time RT-PCR.

For analytical flow cytometry for cell surface proteins, cell samples were centrifuged at 800 rpm, decanted, and incubated for 10 minutes with selected fluorescently-conjugated antibodies (all from eBiosciences): rat anti-CD45-APC, rat anti-c-Kit-PE, rat anti-Gr-1-FITC, and rat anti-CD11b-PE-Cy7. Analytical flow cytometry was performed on a BD LSR II flow cytometer.

RNA extraction and RT-PCR

Total RNA from FACS sorted cells was isolated using Qiagen RNeasy mini purification kit (Valencia, CA, USA) and reverse transcribed using iScript II cDNA synthesis (Biorad). To analyze the expression of transcripts, primers (sequences provided upon request) were designed against several targets and quantitative or semiquantitative PCR were performed accordingly.

Legends to Supplemental Figures

Supplemental Figure 1. **Contribution of Neurog3-expressing cells to gastric serotonin, ghrelin, and somatostatin cells in Neurog3Cre;ROSA^{tdTom} mice.** (Top row) Coexpression of tdTomato with serotonin (5HT) in the antrum (B) but not in the corpus (A). (Bottom row) tdTom coexpression in the majority of ChgA^{+} cells (C) and Ghrelin-cells (D) in the corpus.

Supplemental Figure 2. Correlation between RNAseq variance normalized read counts and relative gene expression measured by qPCR. Expression measured by qPCR for 6 genes with normalized read counts between 1000 and 60000. Logarithmic scale.

Suppl Figure 3. Coexpression of somatostatin (Sst) and ghrelin in some HDC+ cells from HDC-CFP mice. Green arrows, CFP^+ cells, yellow arrows, double positive cells, red arrows, $ghrelin^+$ or Sst^+ cells, red arrowhead, CFP^- cell with strong ghrelin staining.

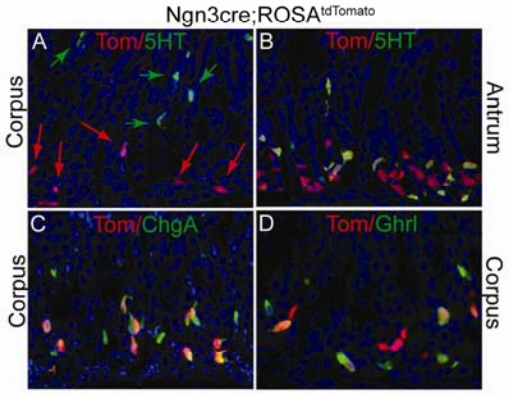
References for Supplemental Materials

1. **Li HJ, Kapoor** A, Giel-Moloney M, et al. Notch signaling differentially regulates the cell fate of early endocrine precursor cells and their maturing descendants in the mouse pancreas and intestine. Dev Biol 2012;371:156-69.
2. Jain RN, Brunkan CS, Chew CS, et al. Gene expression profiling of gastrin target genes in parietal cells. Physiological Genomics 2006;24:124-32.
3. Walker AK, Park WM, Chuang JC, et al. Characterization of gastric and neuronal histaminergic populations using a transgenic mouse model. PLoS One 2013;8:e60276.

Suppl. Table 2 GO enrichment analysis of top 1200 upregulated genes
Top 60 GO terms ranked by pvalue out of 234

GO ID	Pvalue	Odds Ratio	Exp. no. of genes	Observed	Size	Term (Biological Process)
GO:0019226	5.54E-17	7.30	5.8	34	584	transmission of nerve impulse
GO:0044765	1.72E-16	3.87	25.2	70	2549	single-organism transport
GO:0035637	1.96E-16	6.98	6.0	34	609	multicellular organismal signaling
GO:0007268	3.11E-16	7.98	4.6	30	466	synaptic transmission
GO:0007267	5.31E-16	6.14	7.5	37	756	cell-cell signaling
GO:0051179	1.36E-13	3.07	40.5	86	4098	localization
GO:0006810	1.50E-13	3.22	32.3	75	3268	transport
GO:0051234	3.94E-13	3.14	32.9	75	3329	establishment of localization
GO:0051049	9.96E-13	4.41	11.1	40	1121	regulation of transport
GO:0046903	4.98E-11	4.97	6.9	29	693	secretion
GO:0006836	2.48E-10	12.73	1.2	13	122	neurotransmitter transport
GO:0034220	3.33E-10	5.22	5.5	25	561	ion transmembrane transport
GO:0055085	4.52E-10	4.37	8.0	30	812	transmembrane transport
GO:0032879	6.98E-10	3.38	15.0	42	1512	regulation of localization
GO:0006811	1.18E-09	3.73	11.1	35	1120	ion transport
GO:0032940	1.58E-09	4.81	6.0	25	605	secretion by cell
GO:0043269	2.45E-09	5.56	4.3	21	436	regulation of ion transport
GO:0003001	3.60E-09	6.44	3.2	18	322	generation of a signal involved in cell-cel
GO:0023061	3.60E-09	6.44	3.2	18	322	signal release
GO:0051046	5.62E-09	5.29	4.5	21	457	regulation of secretion
GO:0006812	1.72E-08	3.98	7.8	27	786	cation transport
GO:0001505	3.30E-08	10.56	1.2	11	121	regulation of neurotransmitter levels
GO:0030001	4.73E-08	4.28	6.1	23	615	metal ion transport
GO:0007269	8.52E-08	13.45	0.8	9	79	neurotransmitter secretion
GO:0048489	3.14E-07	14.12	0.7	8	67	synaptic vesicle transport
GO:0007399	3.36E-07	2.86	14.5	36	1469	nervous system development
GO:0034765	8.26E-07	6.08	2.4	13	240	regulation of ion transmembrane transpo
GO:0007270	9.98E-07	9.80	1.0	9	105	neuron-neuron synaptic transmission
GO:0065008	1.03E-06	2.44	22.2	46	2245	regulation of biological quality
GO:0003008	1.11E-06	2.37	24.5	49	2474	system process

GO:0034762	1.19E-06	5.87	2.5	13	248	regulation of transmembrane transport
GO:0060341	1.25E-06	3.62	6.8	22	685	regulation of cellular localization
GO:0051649	2.34E-06	2.69	14.4	34	1459	establishment of localization in cell
GO:0010817	2.48E-06	4.73	3.5	15	353	regulation of hormone levels
GO:0044057	3.27E-06	3.95	5.0	18	509	regulation of system process
GO:0015672	3.85E-06	4.55	3.6	15	366	monovalent inorganic cation transport
GO:0022008	4.64E-06	2.93	10.3	27	1045	neurogenesis
GO:0006887	4.86E-06	6.15	2.0	11	199	exocytosis
GO:0006813	5.58E-06	6.80	1.6	10	164	potassium ion transport
GO:0050877	7.08E-06	2.32	20.9	42	2109	neurological system process
GO:0030182	7.42E-06	3.05	8.8	24	885	neuron differentiation
GO:0016486	8.60E-06	40.86	0.1	4	14	peptide hormone processing
GO:0045956	8.60E-06	40.86	0.1	4	14	positive regulation of calcium ion-depend
GO:0071705	9.84E-06	3.78	4.9	17	498	nitrogen compound transport
GO:0051641	1.08E-05	2.42	16.9	36	1707	cellular localization
GO:0042391	1.14E-05	4.71	3.0	13	305	regulation of membrane potential
GO:0050804	1.26E-05	5.53	2.2	11	220	regulation of synaptic transmission
GO:0048699	1.35E-05	2.86	9.7	25	980	generation of neurons
GO:0048666	1.62E-05	3.24	6.8	20	686	neuron development
GO:0031644	1.78E-05	4.86	2.7	12	272	regulation of neurological system proces
GO:0015837	2.31E-05	9.17	0.9	7	86	amine transport
GO:0044708	2.53E-05	4.07	3.7	14	378	single-organism behavior
GO:0035249	3.06E-05	11.24	0.6	6	61	synaptic transmission, glutamatergic
GO:0051050	3.33E-05	3.42	5.4	17	548	positive regulation of transport
GO:0023052	3.45E-05	1.85	50.4	76	5093	signaling
GO:0044700	3.45E-05	1.85	50.4	76	5093	single organism signaling
GO:0032501	3.70E-05	1.82	58.6	85	5921	multicellular organismal process
GO:0051969	3.95E-05	4.85	2.5	11	249	regulation of transmission of nerve impul
GO:0017157	4.12E-05	8.32	0.9	7	94	regulation of exocytosis


Ngn3cre;ROSA^tdTomato
A
Tom/5HT
B
Tom/5HT
Corpus
Antrum
C
Tom/ChgA
D
Tom/Ghrl
Corpus
Corpus

Supplemental Table 1.

Selected GO term analysis from1,200 top enriched biological processes for HDC+ and HDC-

GO	Biological Process	*p*-value
A. Endocrine & Neuronal Function enriched in HDC+		
GO:0016486	peptide hormone processing	8.60E-06
GO:0046879	hormone secretion	0.0003
GO:0006836	Neurotransmitter transport	2.50E-10
GO:0022008	Neurogenesis	4.60E-06
GO:0030182	Neuron differentiation	7.40E-06
B. Mast Cell Function enriched in HDC- cells		
GO:0045576	Mast Cell Activation	2.90E-06
GO:00043304	Regulation of Mast Cell Degranulation	2.95E-05
GO:0002448	Mast Cell Mediated Immunity	0.0002
GO:0032762	Mast Cell Cytokine production	0.0050