SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Immunihistochemical analysis of GIF-GFP and GIF-Cre-RnTnG mouse stomachs. A) Scheme for In-frame rtTA fusion of a GIF gene in mice to generate the GIF-rtTA mouse allele using CRISPR technology. CRISPR-Cas9 stimulates homologous recombination between the unmodified chromosome (Chr) and the homologous donor, resulting in an in-frame, 3' terminal 2A-rtTA-tagged gene. CRISPR/Cas9 technology is employed to introduce a double-stranded DNA break (DSB) at a target site before the stop codon. A synthetic guide RNA (sgRNA) was designed in the targeting region. A Donor single strand DNA (ssDNA) containing P2A-rtTA DNA fragment flanked by an upstream (5' arm, ~1kb) and downstream (3' arm,~1kb) homologous fragments designed according to the targeting site was used for site-specific knock-in of the P2A-rtTA through endogenous homology-directed repair. B&C) Immunohistochemistry of GFP in GIF-GFP mice. B) Sections of the liver, pancreas, intestine and lung tissues of GIF-GFP mice were immunostained with antibodies against GFP at 1 week after DOX treatment. No GFP+ cells were observed in the tissues. C) Sections of the stomach tissues of GIF-rtTA or GIF-GFP mice were immunostained with antibodies against GFP at 1 week after DOX treatment. Black and red boxes depict enlarged regions. D) Immunohistochemistry of GFP in GIF-Cre-RnTnG mice. Sections of the stomach tissues of Dox-treated GIF-Cre-RnTnG mice treated with or without L635 were immunostained with antibodies against GFP. Black and red boxes depict regions enlarged. E) Quantitation of glands that contain GFP+ cells in the corpus and antrum in GIF-GFP mouse stomachs with DOX treatment. The graph displays the percentage of GFP+ glands and a total of 100 glands per mouse

were counted in the corpus or antrum. Statistical significance was determined by unpaired Welch's test (P = 0.0012, N = 3 per group). F) Quantitation of glands that contain GFP+ cells in the corpus and antrum in GIF-Cre-RnTnG mouse stomachs with DOX treatment for 1 week. The graph displays the percentage of GFP+ glands and a total of 100 glands per mouse were counted in the corpus or antrum. Statistical significance was determined by unpaired Welch's test (P < 0.0001, N = 3 per group).

Figure S2. Immunofluorescence staining for GFP-labeled cells after long term-lineage tracing. A) Immunostaining for GFP (green), GIF (red) and Ki-67 (blue) at 2 weeks (n=3), 2 (n=3), 6 (n=2), and 12 (n=3) months following Dox treatment in GIF-Cre-RnTnG mouse stomachs. White arrows indicate enlarged area in panel B & C. B) Yellow arrows indicate GIF-negative GFP-labeled cells at 6 months following Dox treatment. C) Yellow arrow indicated co-positive cells for GFP and Ki67 at 12 months following Dox treatment. D) Immunostaining for GFP (green), UEAI (red), GSII (blue) and H/K-ATPase (white) at 12 months following Dox treatment in GIF-Cre-RnTnG mouse stomachs. White arrow indicates enlarged area and yellow arrows indicate co-positive cells for GFP and UEAI, GSII or H/K-ATPase.

Figure S3. Immunofluorescence staining for GFP-labeled cells in the GIF-Cre-RnTnG mouse stomachs. A) GIF-Cre-RnTnG mice were administered without (untreated) or with L635 for 1 or 3 doses (initiation or completion) 2 weeks after the Dox treatment for 1 week. Sections of the stomach tissues were immunostained with antibodies against GFP (green), Ki67 (red), UEAI (white). Nuclei were counterstained with Hoechst (blue). White arrows indicate GFP+Ki67- cells 1 dose after the L635

treatment. Several GFP+ cells were observed at the surface cell zone (yellow arrows) in the glands of stomach tissues treated with L635 for 3 doses, but no GFP+ cells were copositive for Ki67 in this region. Dotted boxes depict enlarged regions. N = 3 mice per group. B) Sections of the stomach tissues treated with L635 for 3 doses (completion) were immunostained with antibodies against GFP (green), Ki67 (red), GSII (blue). Dotted box depicts enlarged region. N = 3 mice per group. C) Quantitation of co-positive cells for GFP, GSII and/or Ki67 per 20 x field. N = 3 mice per group.

Figure S4. Immunofluorescence staining for GFP-labeled cells in the GIF-Cre-RnTnG mouse stomachs. GIF-Cre-RnTnG mice were administered without (untreated) or with L635 for 3 doses (completion) 2 weeks after the Dox treatment for 1 week. A) Sections of the stomach tissues were co-immunostained with antibodies against GFP (green), GIF (red) and GSII (white) or GFP (green), UEAI (red), HK/ATP-ase (HK, gray). Nuclei were counterstained with Hoechst (blue). Dotted boxes depict regions enlarged. White dotted lines depict mucosa regions and yellow dotted lines depict the GFP+ cell zones. White arrows indicate GFP-labeled SPEM cells, co-positive for GIF, and GSII or GFP-labeled surface cells, co-positive for UEAI. B) Sections of the stomach tissues were co-immunostained with antibodies against GFP (green), Muc5ac (red) and UEAI (blue). Dotted boxes depict regions enlarged. White arrows indicate GFP-labeled surface cells, co-positive for both Muc5ac and UEAI. N = 3 mice per group.

SUPPLEMENTAL TABLE 1. KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-GFP (1:5,000)	Novus	NB600-308
Goat anti-GIF (1:1,000)	A gift from Dr. David Alpers	N/A
Mouse anti-H/K-ATPase (1:10,000)	A gift from Dr Adam Smolka	N/A
Rabbit anti-Ki67 (1:1000)	Cell Signaling Technology	9129
Mouse anti-P120 (1:500)	BD Biosciences	610134
UEA1-lectin (1:2000)	Sigma	L9006
GSII-Lectin (1:2,000)	Invitrogen	L32451
Rabbit anti-GPR30 (1:200)	abcam	ab260033
Mouse anti-Muc5ac (1:500)	NeoMarkers	MS-551-P
Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (1:500)	ThermoFisher	A-21209
Donkey anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, DyLight 680 (1:500)	ThermoFisher	SA5-10030
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (1:500)	ThermoFisher	A-21206
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (1:500)	ThermoFisher	A-10040
Donkey anti-Rabbit IgG (H+L) Highly cross-Adsorbed Secondary Antibody, Alexa Fluor 790 (1:500)	ThermoFisher	A-11374
Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluro 488 (1:500)	ThermoFisher	A-11055
Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (1:500)	ThermoFisher	A-11057
Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (1:500)	ThermoFisher	A-21447
Donkey anti-Monkey Highly Cross-Adsorbed Secondary Antibody, Alexa Fluro 790 (1:500)	ThermoFisher	A-11371
Chemicals, Peptides, and Recombinant Proteins		
Viagen Direct PCR (Ear) Lysis Reagent	Fisher Scientific	402-E
Viagen Proteinase K	Fisher Scientific	501-PK
4% paraformaldehyde solution in PBS	Fisher Scientific	AAJ19943K2
L-635	Vanderbilt Chemical Synthesis Core	N/A
Doxycycline Hyclate	Sigma-Aldrich Genosys	D9891
Histoclear	National Diagnostics	HS-200
Critical Commercial Assays		
Platinum II Hot Start PCR Master Mix (2x)	Thermo Fisher	1400014
DreamTaq Green PCR Master Mix (2x)	Thermo Fisher	K1081
Promega GoTaq G2 Green Master Mix (2x)	Thermo Fisher	M7823
Experimental Models: Organisms/Strains		
C57Bl/6J	The Jackson Laboratory	000664
Gif-rtTA	Applied StemCell	N/A

TetO-H2BGFP	The Jackson Laboratory	005104
TetO-Cre	The Jackson Laboratory	006224
Gt(ROSA)26Sor$^{tm(CAG-tdTomato*,-EGFP*)Ees}$/J 023537	The Jackson Laboratory	023537
Oligonucleotides		
GIF-rtTA WT Primers F: CATGAGCACATCACAGCCAAC R: GTTAGTGCAGAAGGTTGCGTC	Sigma-Aldrich Genosys	N/A
TetO-H2BGFP Primers F: GCGCTCGAAAATGTCGTTCA R: CGTGTACGGTGGGAGGTCTA	Sigma-Aldrich Genosys	N/A
TetO-H2BGFP WT Primers F: CTAGGCCACAGAATTGAAAGATCT R: GTAGGTGGAAATTCTAGCATCATCC	Sigma-Aldrich Genosys	N/A
TetO-Cre Primers F: GCGGTCTGGCAGTAAAAACTATC R: GTGAAACAGCATTGCTGTCACTT	Sigma-Aldrich Genosys	N/A
Rosa nTnG Primers WT: GGAGCGGGAGAAATGGATATG Common: AAAGTCGCTCTGAGTTGTTAT Mutant:CCAGGCGGGCCATTTACCGTAAG	Sigma-Aldrich Genosys	N/A
Experimental Models: Organisms/Strains		
Mouse: GIF-rtTA	This paper	N/A
Mouse: TetO-H2BGFP	The Jackson Laboratory	Stock No: 005104
Mouse: TetO-Cre	The Jackson Laboratory	Stock No: 006234
Mouse: Rosa-RnTnG	The Jackson Laboratory	Stock No: 023035
Software and Algorithms		
ImageJ	(Schneider et al., 2012)	https://imagej.nih.gov/ij/
Photoshop 2020	Adobe	Version 21.1.2
GraphPad Prism	GraphPad Software	Version 8.4.2
MATLAB	MathWorks	