Gut

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 P2ArtTA 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/ATPase (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 | | | |
| C57BI/6J | The Jackson Laboratory | 000664 | |
| Gif-rtTA | Applied StemCell | N/A | |

| | The Jackson | |
|--|--------------------|--------------------|
| TetO-H2BGFP | Laboratory | 005104 |
| T 10.0 | The Jackson | 000004 |
| TetO-Cre | Laboratory | 006224 |
| C+(DOCA)26Contm(CAG-tdTomato* -EGEP*)Ees/1022E27 | I ne Jackson | 000507 |
| Gl(ROSA)26501 M(one dramate, 2011) 200/J 023537 | Laboratory | 023537 |
| Oligonucleolides | | |
| GIF-rtTA WT Primers | Sigma-Aldrich | N/A |
| F: CATGAGCACATCACAGCCAAC | Genosys | |
| R: GTTAGTGCAGAAGGTTGCGTC | | |
| TetO-H2BGFP Primers | Sigma Aldrich | N/A |
| F: GCGCTCGAAAATGTCGTTCA | Genosys | |
| R: CGTGTACGGTGGGAGGTCTA | Genosys | |
| TetO-H2BGFP WT Primers | 0. 411.1 | N/A |
| F: CTAGGCCACAGAATTGAAAGATCT | Sigma-Aldrich | |
| R: GTAGGTGGAAATTCTAGCATCATCC | Genosys | |
| TetO-Cre Primers | o: | N/A |
| F: GCGGTCTGGCAGTAAAAACTATC | Sigma-Aldrich | |
| R: GTGAAACAGCATTGCTGTCACTT | Genosys | |
| Rosa nTnG Primers | | N/A |
| WT: GGAGCGGGAGAAATGGATATG | Sigma-Aldrich | |
| Common: AAAGTCGCTCTGAGTTGTTAT | Genosys | |
| Mutant:CCAGGCGGGCCATTTACCGTAAG | | |
| Experimental Models: Organisms/Strains | | |
| Mouse: GIF-rtTA | This paper | N/A |
| Mouse: TetO-H2BGFP | The Jackson | Stock No: |
| | Laboratory | 005104 |
| Mouse: TetO-Cre | The Jackson | Stock No: |
| | Laboratory | 006234 |
| Mouse: Rosa-RnTnG | The Jackson | Stock No: |
| | Laboratory | 023035 |
| Software and Algorithms | | |
| | (Schneider et al., | https://imagej.nih |
| | 2012) | .gov/ij/ |
| Photoshop 2020 | Adobe | Version 21.1.2 |
| GraphPad Prism | GraphPad Software | Version 8.4.2 |
| MATLAB | MathWorks | |