Chemical induction of gut β-like-cells by combined FoxO1/Notch inhibition as a glucose-lowering treatment for diabetes

Takumi Kitamoto[1, 2, 7, †], Yun-Kyoung Lee[3, *], Nishat Sultana[4, *], Hitoshi Watanabe[1, 2],

Wendy M. McKimpson[1, 2], Wen Du[1, 2], Jason Fan[6], Bryan Diaz[4], Hua V. Lin[5],

Rudolph L. Leibel[4], Sandro Belvedere[3], Domenico Accili[1, 2]

[1] Department of Medicine and [2] Naomi Berrie Diabetes Center, [4] Department of Pediatrics

Vagelos College of Physicians and Surgeons, Columbia University, New York, NY 10032;

[3] Forkhead BioTherapeutics, Inc., New York, NY; and

[5] BioFront Therapeutics, Beijing, China

[6] Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami, Miami, FL,

33146,

[7] Chiba University Graduate School of Medicine, Chiba, Japan, 2608670

Running title: FoxO1 inhibitors in murine diabetes

[†] Corresponding Author: Takumi Kitamoto, MD, PhD

Department of Medicine, Vagelos College of Physicians and Surgeons, Columbia

University, New York, NY

Phone: +1-2128515332

Fax: +1-2128515331

E-mail: t.kitamoto@chiba-u.jp

* These authors were equally contributed

Reprint requests: Domenico Accili (da230@cumc.columbia.edu)

INFORMATION

This appendix has been provided by the authors to give extended information about the present study.

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Table S1. List of primers for qPCR

Gene symbol		Primer Sequence
18S	Forward	AAACGGCTACCACATCCAAG
	Reverse	CCTCCAATGGATCCTCGTTA
Foxo1	Forward	TCC AGT TCC TTC ATT CTG CAC T
	Reverse	GCGTGCCCTACTTCAAGGATAA
Ngn3	Forward	TGGCCCATAGATGATGTTCG
	Reverse	AGAAGGCAGATCACCTTCGTG
ChgA	Forward	CAGCTCGTCCACTCTTTCCG
	Reverse	CCTCTCGTCTCCTTGGAGGG
Tph1	Forward	TTCTGACCTGGACTTCTGCG
	Reverse	GGGGTCCCCATGTTTGTAGT
Gcg	Forward	GCTGATTCAAACCAAGATCACTG
	Reverse	ATCCCAAGTGACTGGCACGAG
Ins1	Forward	TTAATGGGCCAAACAGCAAAGTC
	Reverse	TGACCTGCTTGCTGATGGTCTC
Ins2	Forward	CTGGTGGAGGCTCTCTACCTG
	Reverse	CAAGGTCTGAAGGTCACCTGC
Nkx6.1	Forward	CAGGACCAAGTGGAGAAAGAAG
	Reverse	GGGTCCAGAGGTTTGTTGTAAT
Nkx2.2	Forward	CCA GAA CCA TCG CTA CAA GAT
	Reverse	CTTATCCAATCGCTCCACCTT

Table S2. List of antibodies for immunohistochemistry

Name	Vendor	Cat.No	Primary conc	Secondary conc
FoxO1 (C29H4)	Cell signaling	#2880	1:300	1:500
GLP-1	Abcam	ab13329	1:500	1:500
GIP	ABBIOTEC	250668	1:500	1:500
5-HT	Abcam	ab66047	1:1000	1:500
Somatostatin	Dako	A0566	1:500	1:500
GFP	Abcam	Ab13970	1:500	1:500
RFP	MBL	PM005	1:500	1:500
Ki67	Abcam	ab15580	1:500	1:500
Insulin	Mercodia	N/A	1:200	1:500
C-peptide	Thermo	PA5-85595	1:2000	1:500
PC2	Thermo	PA1-058	1:500	1:500
Nkx6.1	R&D	AF5857-SP	1:50	1:500
Donkey anti-goat-Alexa488	Invitrogen	A11055		
Donkey anti-rabbit-Alexa555	Invitrogen	A31572		
Donkey anti-rat-Alexa488	Invitrogen	A21208		
Goat anti-Rabbit-Alexa488	Invitrogen	A11008		
Goat anti-Mouse-Alexa488	Invitrogen	A11029		
Goat anti-Mouse-Alexa555	Invitrogen	A21424		
Goat anti-Chicken-Alexa488	Invitrogen	A11039		
Biotin rat anti mouse igG1	BD	553441		

SUPPLEMENTAL FIGURES

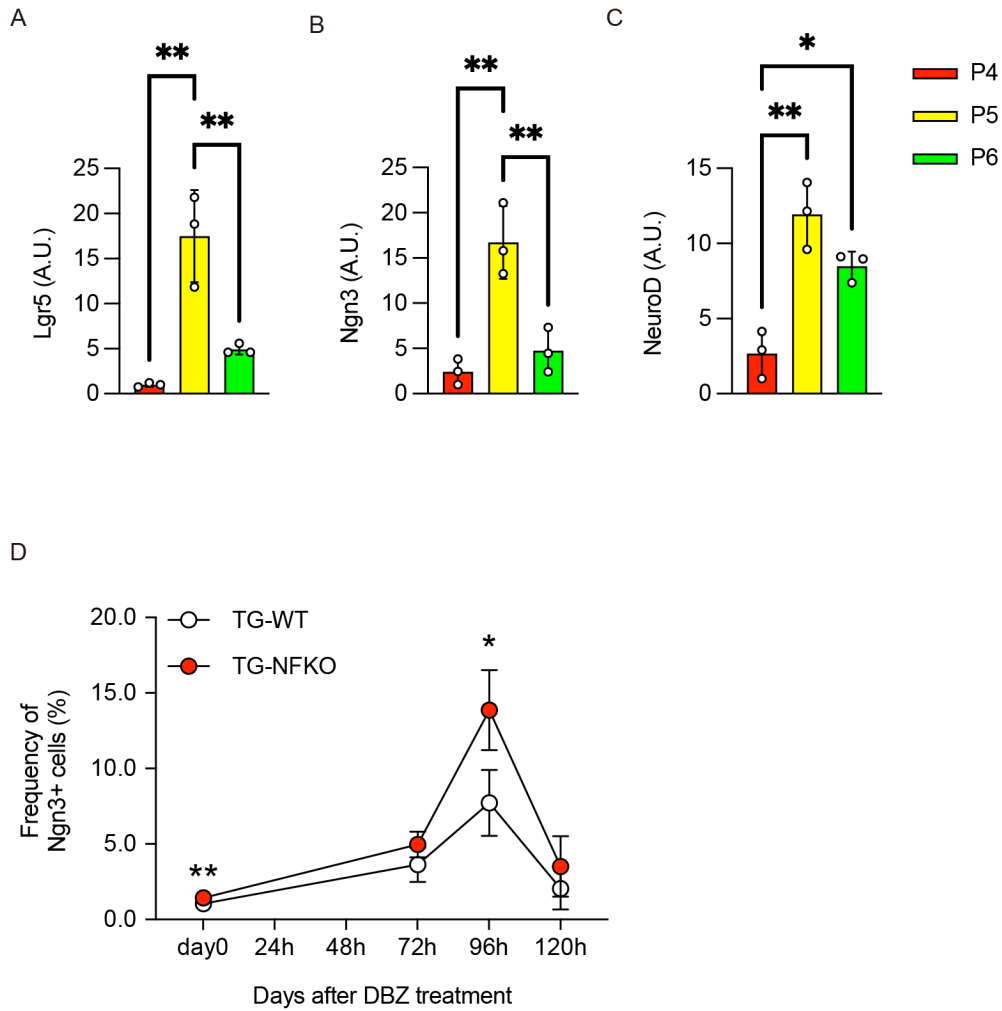Figure S1. Comparisons of progenitor marker expression among subgroups of sorted EECs by RFP and GFP

Fig. S1. (A–C) qPCR of Lgr5 (A), Ngn3 (B), and NeuroD (C) using mRNA from sorted P4, P5, and P6 cells indicated in Fig. 1C. N=3 for each group. (D) Time course of total GFP+ cells, indicating Ngn3+ EECs after DBZ treatment. The data are calculated from the same samples shown in Fig. 1G–J.
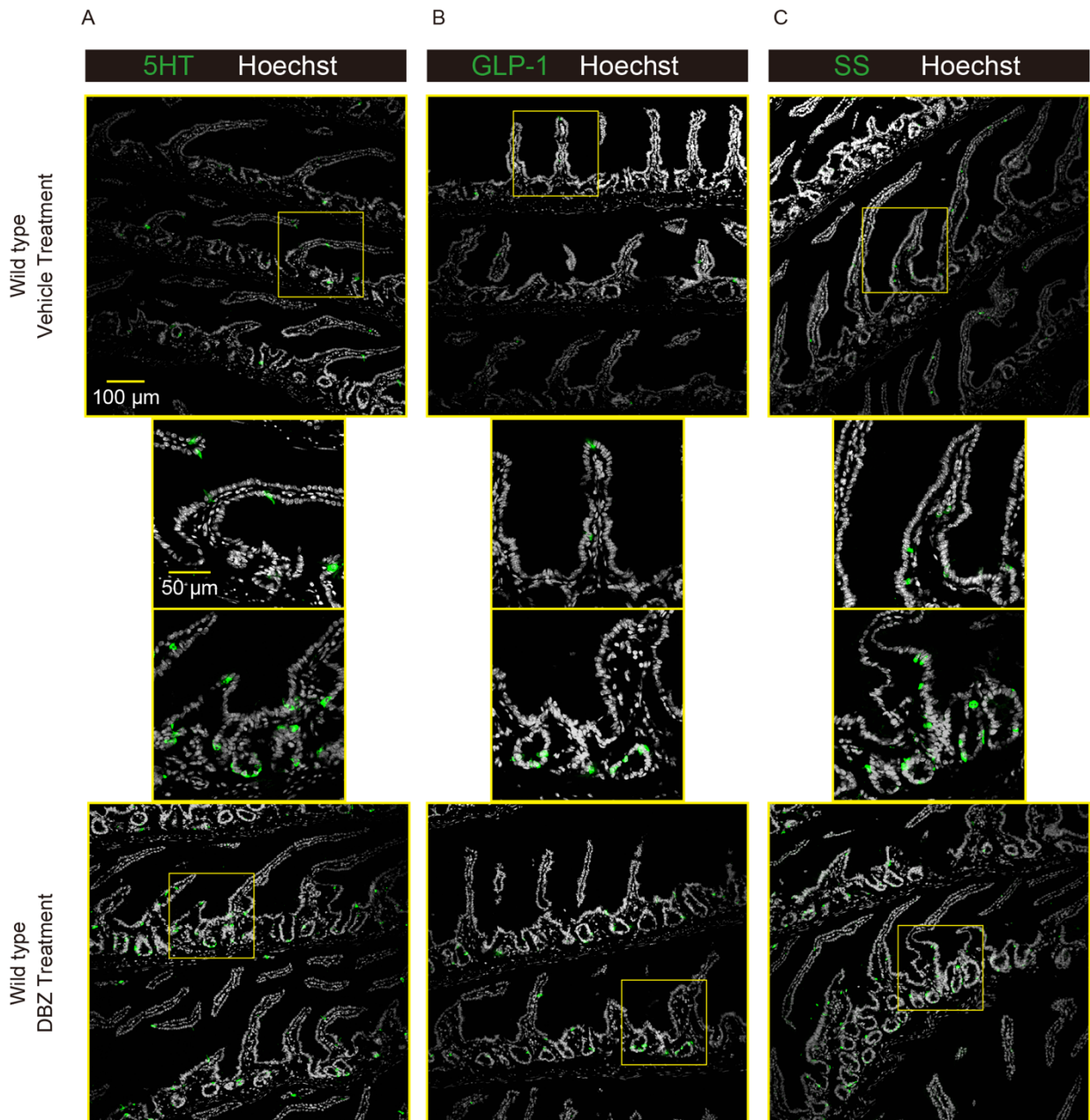
Figure S2. Induction of EECs by DBZ

Fig. S2. 5HT (A), GLP-1 (B), and SS (C) staining (green) of intestinal sections from wild-type

mice 96hr after Vehicle or DBZ treatment. Scale bar = 100 μm in larger pictures, and 50 μm in

magnified pictures. Hoechst counterstains nuclei.

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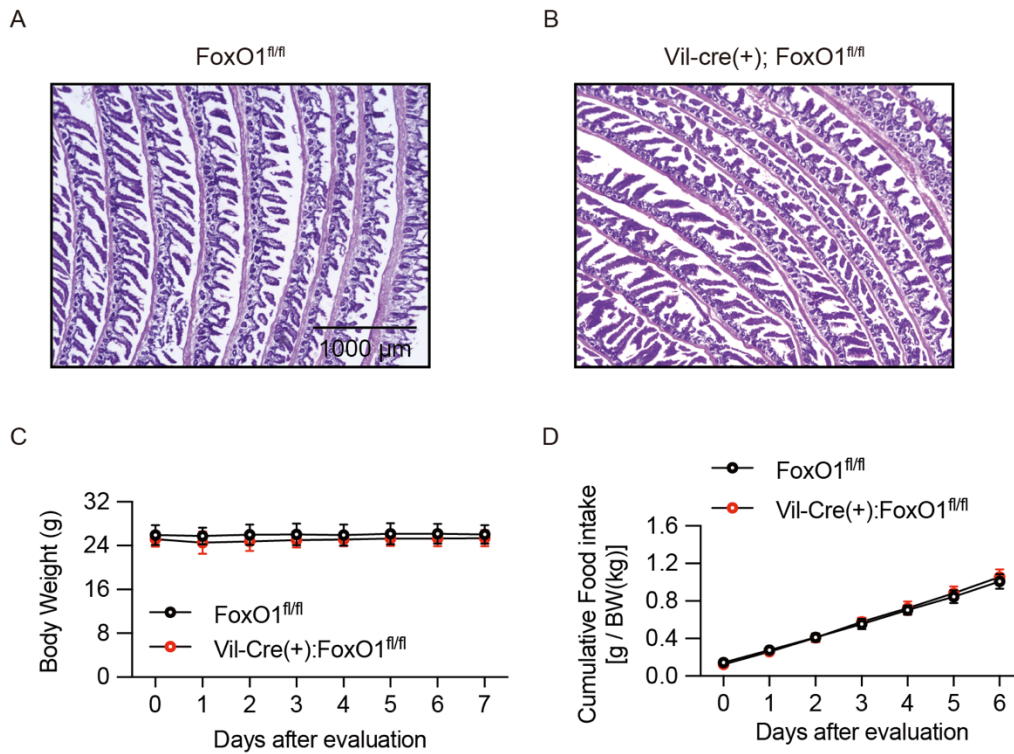Figure S3. Gut structure, weight, and food intake in Vil-FoxO1 knockout and wild-type control mice

Fig. S3. (A, B) H&E of gut from FoxO1$^{fl/fl}$ (A) and Vil-cre (+): FoxO1$^{fl/fl}$ mice (B). (C, D) Body weight and food intake measured daily at 10 AM for a week.

Figure S4. ipGTT and OGTT in Vil-FoxO1 knockout and wild-type control mice
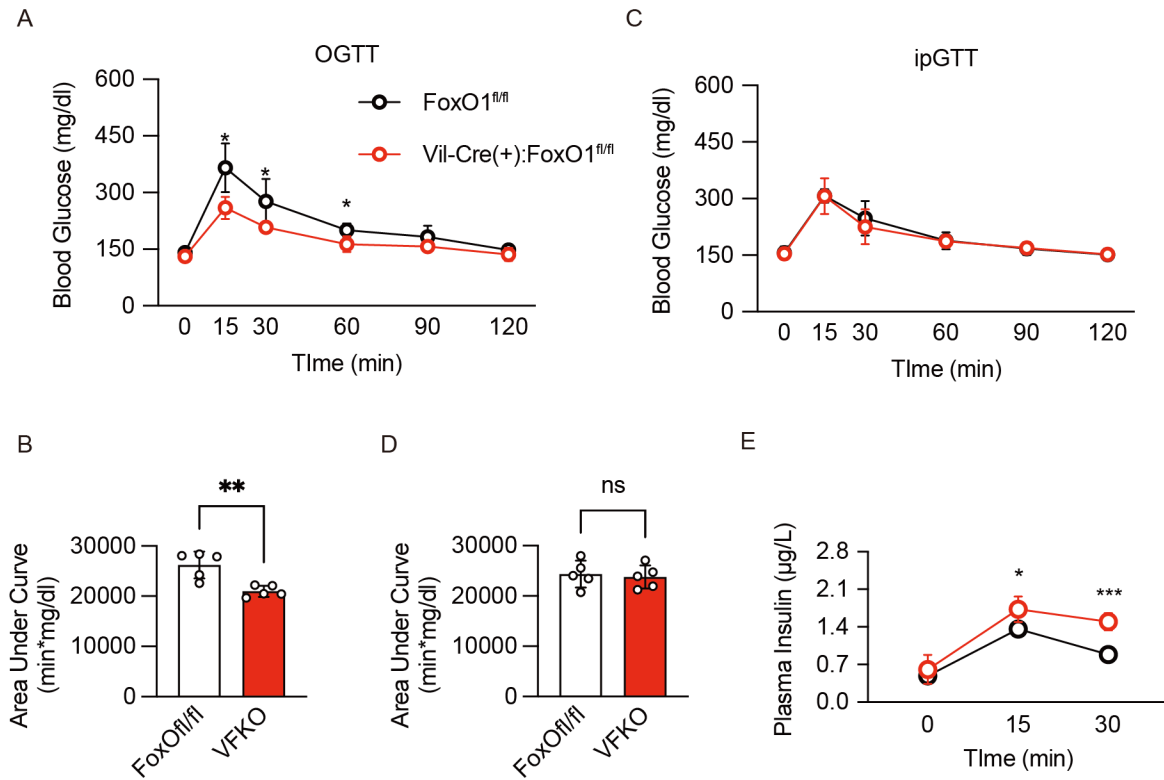

Fig. S4. (A–E) Oral (A) or intra-peritoneal (C) glucose tolerance tests (1g/kg glucose) after a 4-hr fast and area under curve of (B, D). (E) Plasma insulin levels during oral glucose tolerance tests.

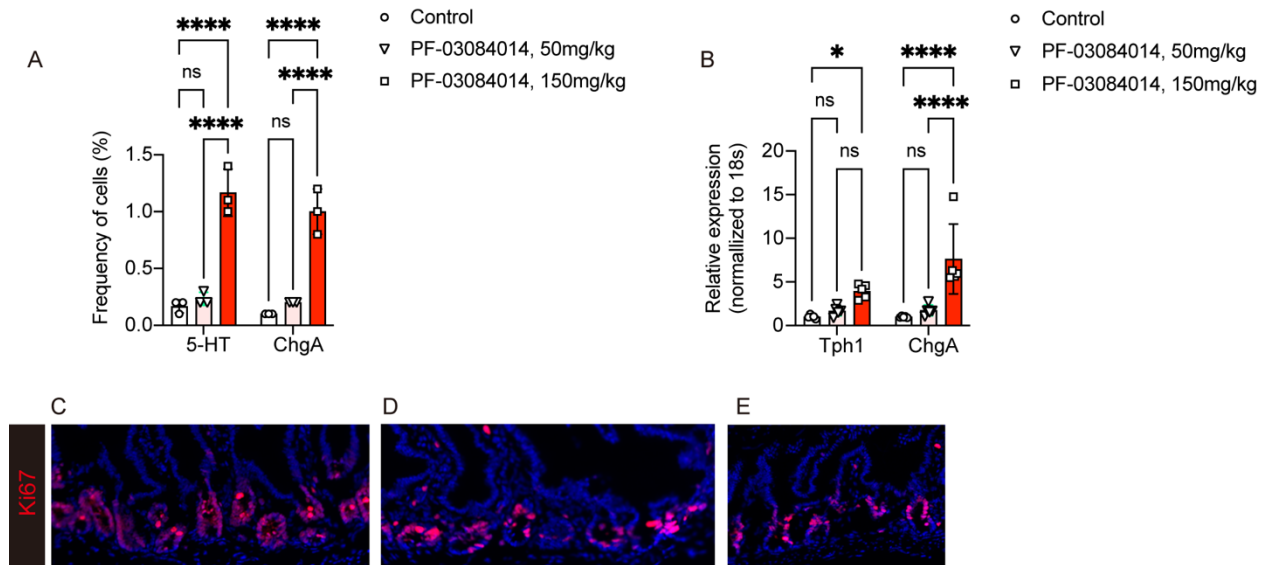Figure S5. Optimization of in vivo PF-03084014 treatment

Fig. S5. (A–E) 7-8 week old C57/BL6 mice were randomized into three groups. Mice were treated with vehicle, PF-03084014 (50mg/kg/dose, PO) or PF-03084014 (150mg/kg/dose, PO) for 5 days, twice daily. (A) 5-HT and Chromogranin A cell analysis by flow cytometry after intra-cellular staining of isolated gut epithelial cells from approximately one third of the proximal end of small intestine cells (n = 3 for each group). (B) Gene expression analysis of enteroendocrine cell marker genes (Tph1, ChgA) (n = 5 for each group). (C-E) Representative images of proliferating cell marker Ki67 immunohistochemistry in tissues collected after 5 days of treatment. (C) Vehicle, (D) PF-03084014 (50mg/kg/dose), (E) PF-03084014 (150mg/kg/dose). Data are presented as means ± SD. Scale bar = 50 μm, DAPI counterstains nuclei. $*= p < 0.05$, $****= p < 0.0001$ by two-factor ANOVA.

Figure S6. Comparison of pancreatic insulin content and quantification of GLP-1^{+}, GIP[+], and C-peptide^{+} cells in INS2[Akita/+] mice with or without pan-intestinal FoxO1 ablation
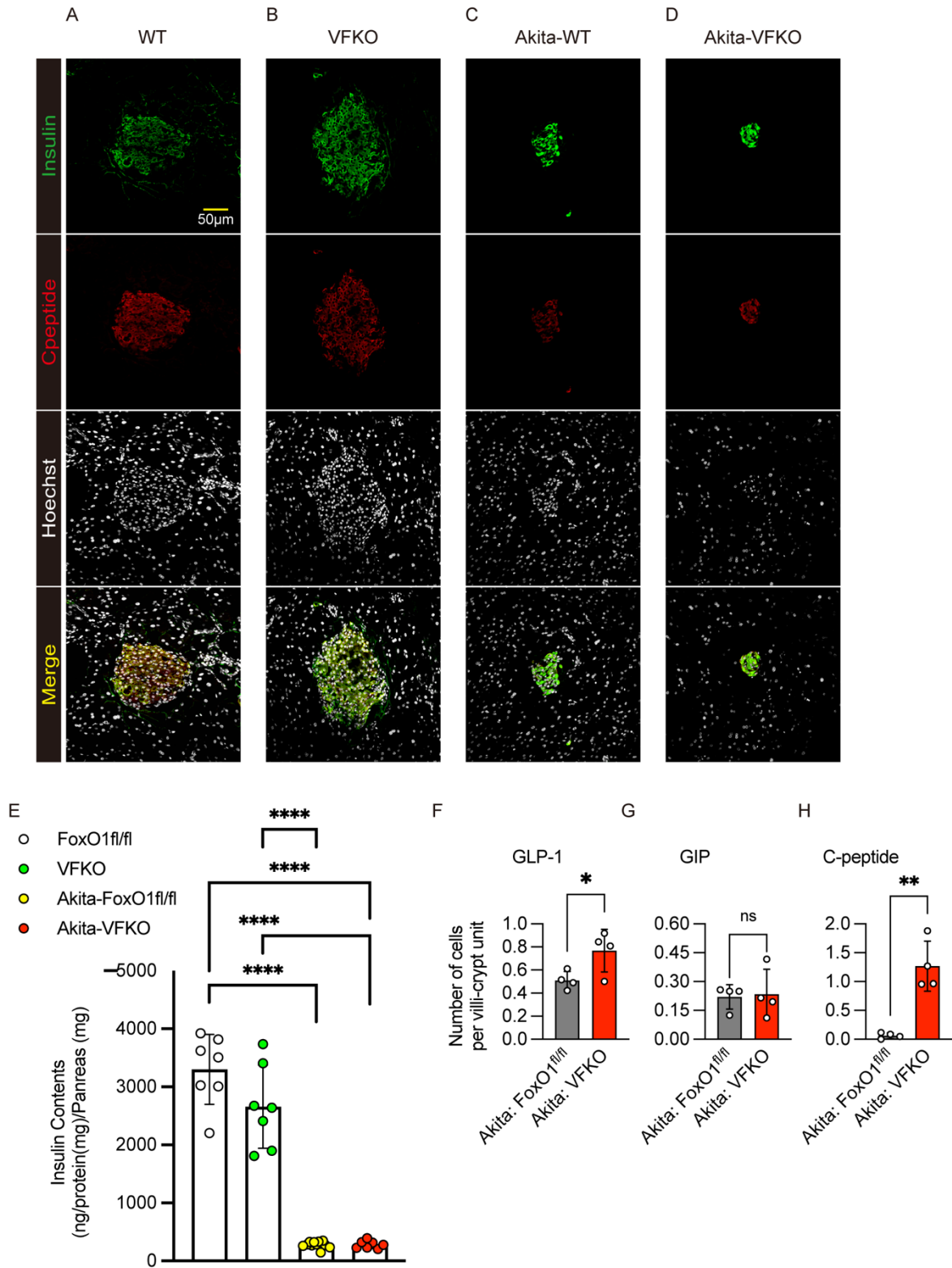
Fig. S6. (A–E) Comparison of whole pancreatic insulin content in $INS2^{Akita/+}$ mice with or without FoxO1$^{fl/fl}$ and VFKO alleles. Representative islet images are stained with insulin and C-peptide (A–D). Quantification of insulin content in whole pancreas (n = 7 for each group) (E).

(F–H) Quantification of GLP-1-, GIP- and C-peptide-IMMUNOREACTIVE gut cells (n = 4 for each group) in Akita-WT, INS2$^{Akita/+}$; FoxO1$^{fl/fl}$: Akita-VFKO, INS2$^{Akita/+}$; Vil-Cre (+): FoxO1$^{fl/fl}$. Data are presented as means ± SD. Scale bar = 50 μm, DAPI counterstains nuclei. *= $p < 0.05$, **= $p < 0.01$, ****= $p < 0.0001$ by two-factor ANOVA.

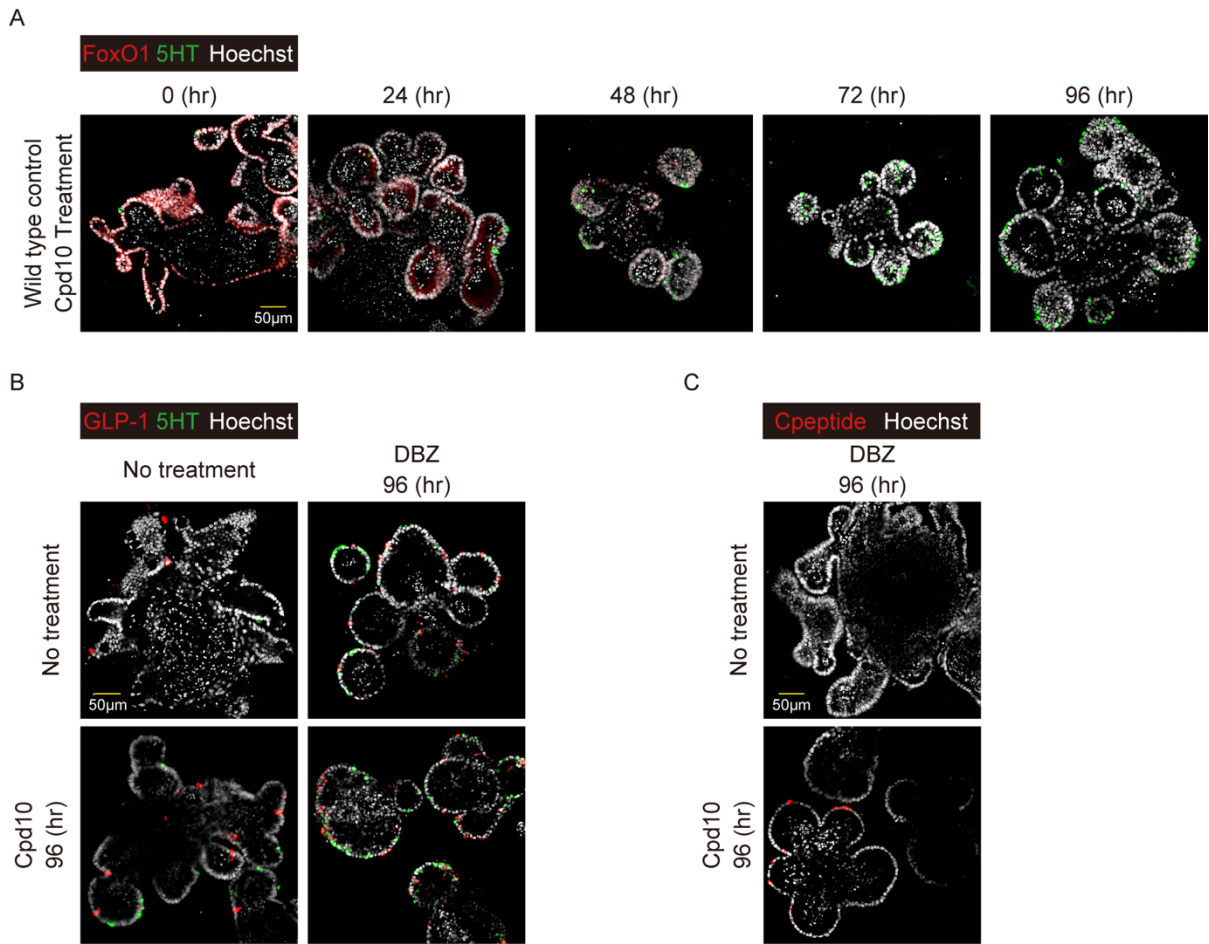Figure S7. Effect of Cpd10 on FoxO1, 5HT, GLP-1, and C-peptide expression

Fig. S7. (A) Time course of FoxO1 and 5HT expression of wild type mGO after Cpd10 and DBZ treatment. (B) GLP-1 immunohistochemistry of wild type mGO with or without Cpd10 treatment before and 96 hr after DBZ treatment. (C) C-peptide staining of wild type mGO 96 hr after Cpd10 and DBZ treatment. Scale bar = 50 μm. Hoechst counterstains nuclei.

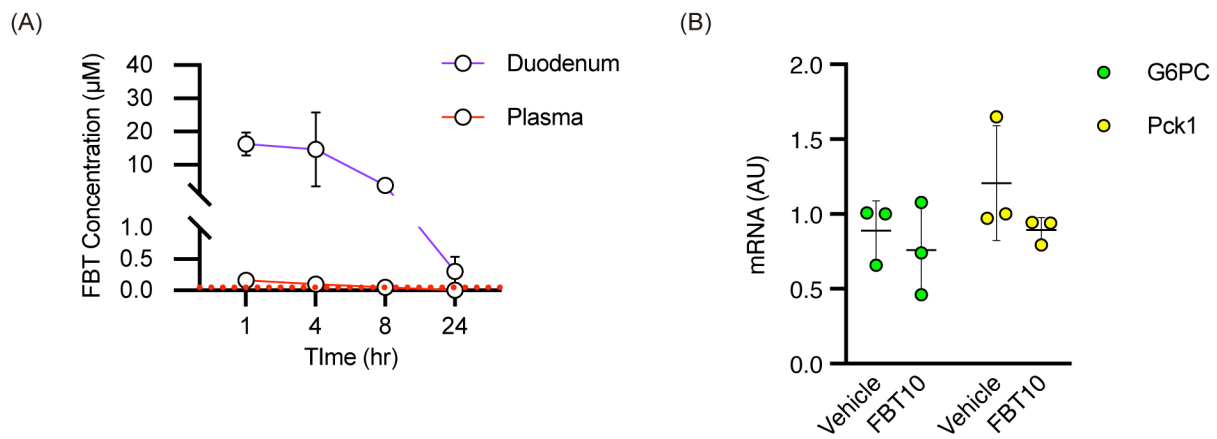Figure S8. Pharmacological effect of Cpd10 *in vivo*

Fig. S8. (A) Gut tissue and plasma concentration of Cpd10 following a single oral dose. (B)

Gluconeogenic gene expression in liver after dosing at 50mg/kg i.p injection of vehicle or Cpd10

twice daily for 4 days. G6PC: glucose-6-phosphatase, Pck1: phosphoenolpyruvate-

carboxykinase. Data are presented as means ± SD. All the experiments were performed in

biological triplicates.

Figure S9. Cpd10 and PF-03084014 dual treatment increases enteroendocrine cells

A

- Control
- Cpd10
- Cpd10+PF-03084014,15 mg/kg
- Cpd10+PF-03084014, 50mg/kg
- Cpd10+PF-03084014, 150mg/kg

B

- Control
- Cpd10
- Cpd10+PF-03084014,15 mg/kg
- Cpd10+PF-03084014, 50mg/kg
- Cpd10+PF-03084014, 150mg/kg

C

- Control
- Cpd10
- Cpd10+PF-03084014,15 mg/kg
- Cpd10+PF-03084014, 50mg/kg
- Cpd10+PF-03084014, 150mg/kg
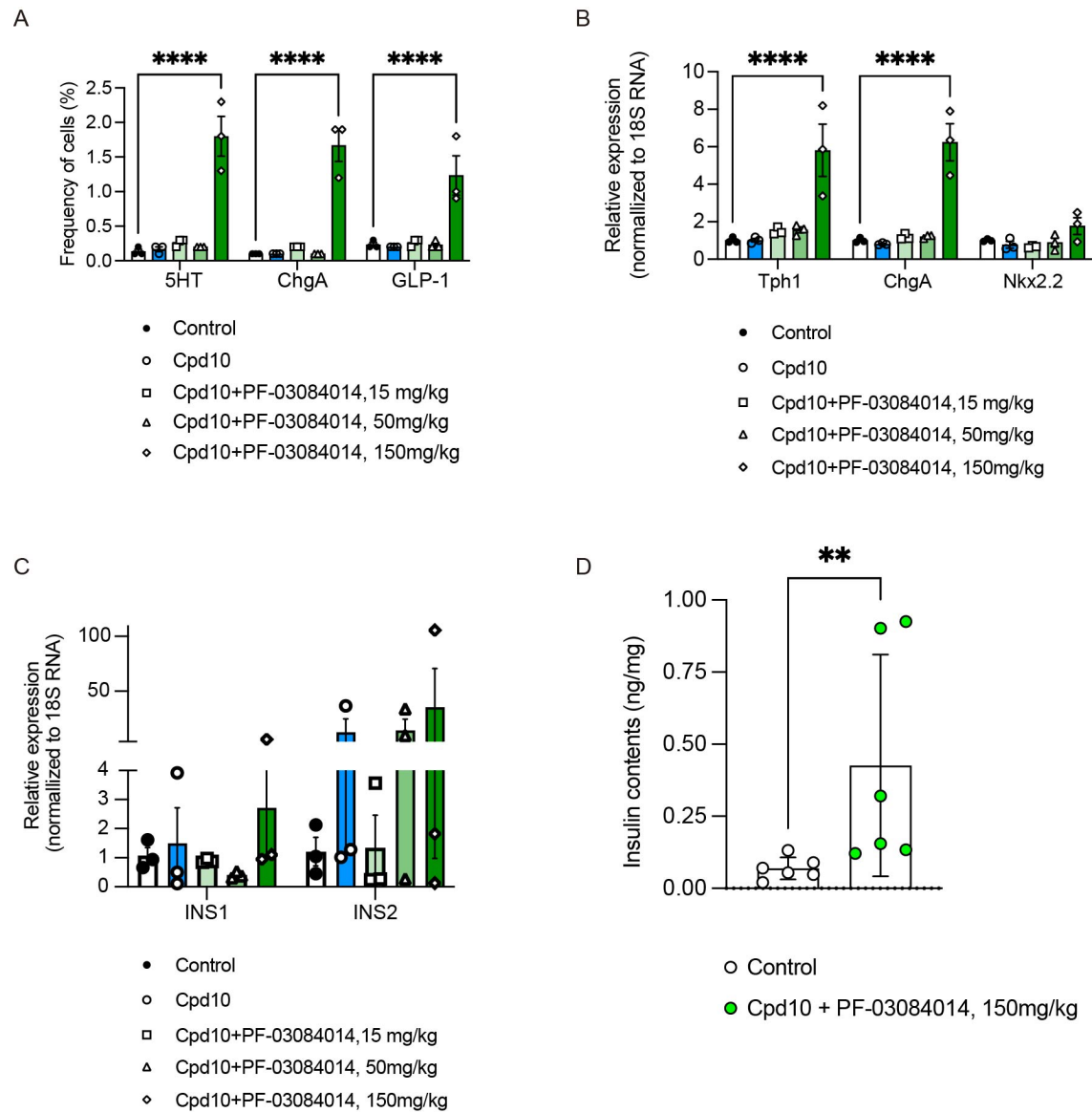
D

- Control
- Cpd10 + PF-03084014, 150mg/kg

Fig. S9. (A–B) 8-9-week old Akita mice were randomized into 5 groups based on normal fed glucose levels and body weight. Mice were treated with vehicle, Cpd10 (50mg/kg/dose, PO), Cpd10 + PF-03084014 (15mg or 50mg or 150mg/kg/dose, PO) twice daily (n = 3 for each group). (A) 5-HT, Chromogranin A, and GLP-1 cell analysis by flow cytometry after intra-

cellular staining of isolated gut epithelial cells from approximately one third of the proximal end of small intestine. (B) Gene expression analysis of enteroendocrine cell (Tph1, ChgA) and β-cell marker of Nkx2.2. (C) Gene expression analysis of INS1 and INS2. (D) Intracellular insulin contents of gut epithelial cells from the mice treated with Vehicle or Cpd10 + PF-03084014 (150mg/kg/dose, PO) twice daily. **= $p < 0.01$, ****= $p < 0.0001$ by Mann-Whitney U test or two-factor ANOVA.

Figure S10. Representative intestinal Immunohistochemistry after 10-day single treatment with Cpd10 or PF-03084014
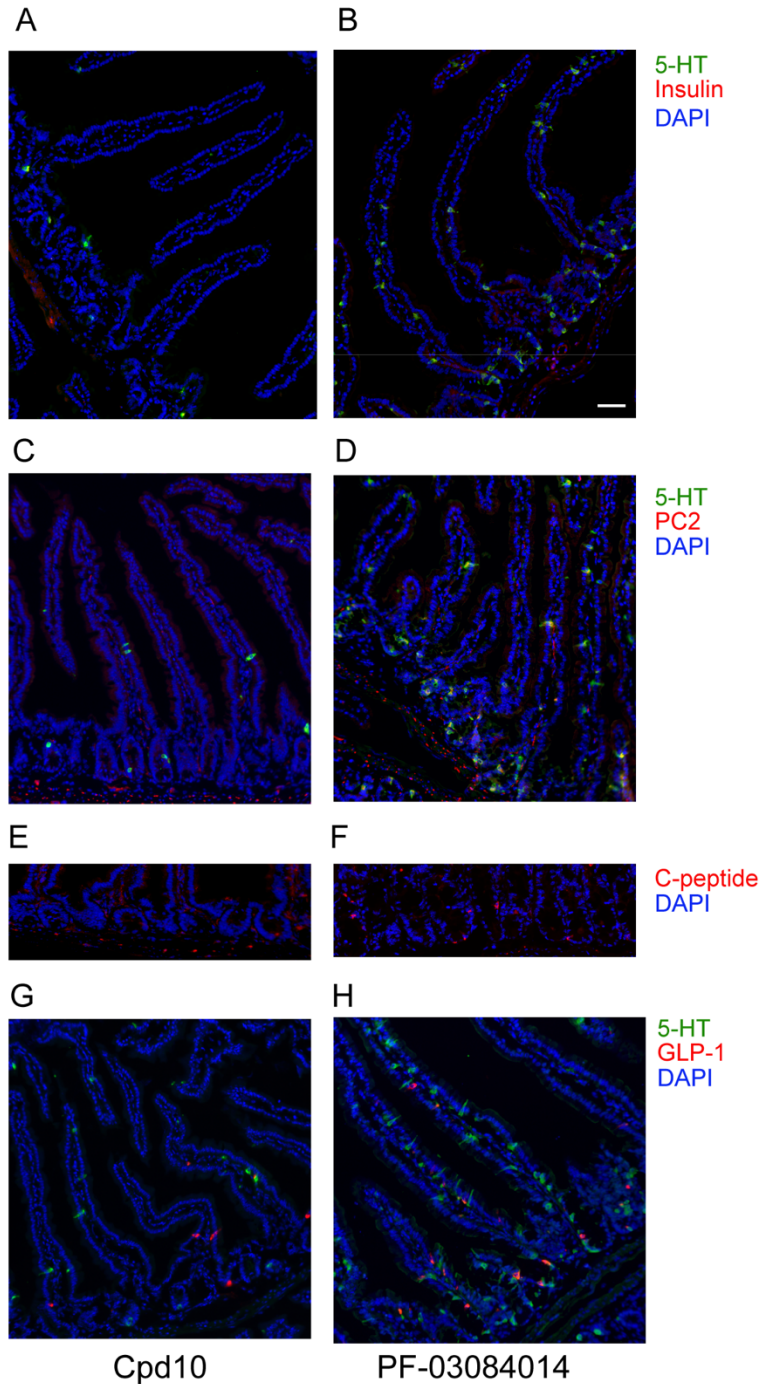

Fig. S10. (A–H) Representative intestinal immunohistochemistry after 10-day treatment. (A, B) Insulin (red) and 5-HT (green), (C, D) PC2 (red) and 5-HT (green), (E, F) C-peptide and (G, H) GLP-1 (red) and 5-HT (green) staining of Cpd10 group (left panel, A, C, E, and G) and PF-03084014-treated group (right panel, B, D, F, H). Scale bar = 50 μm

Figure S11. Images of β-cell specific markers after 10-day treatment
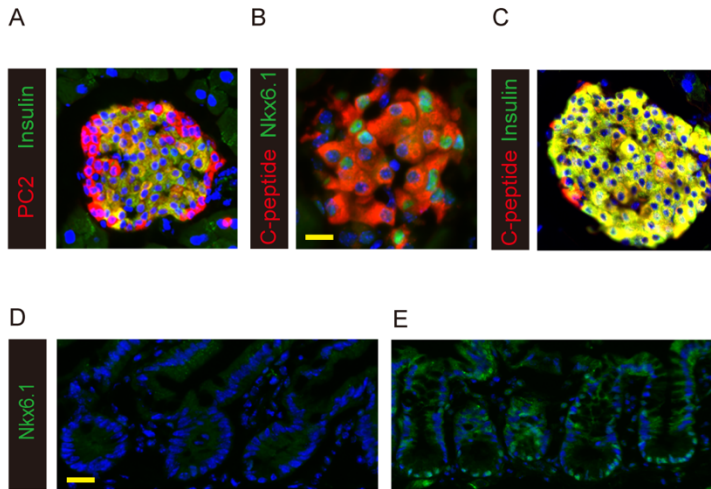

Fig. S11. (A–C) Immunohistochemistry of wild-type pancreas with beta cell markers using the same antibodies employed for gut immunohistochemistry. (A) PC2 and Insulin, (B) C-peptide and Nkx6.1, (C) C-peptide and insulin. (D–E) Representative immunohistochemistry of Nkx6.1 in the gut of 9-week-old $AKITA^{Ins2/+}$ mice after 10 days treatment with Vehicle (D), or Cpd10 (50mg/kg/dose i.p) + PF-03084014 (150mg/kg/dose, PO) (E). Scale bar = 50 μm.