Supplemental Data

Intra-islet Glucagon Signaling is Critical for Maintaining Glucose Homeostasis

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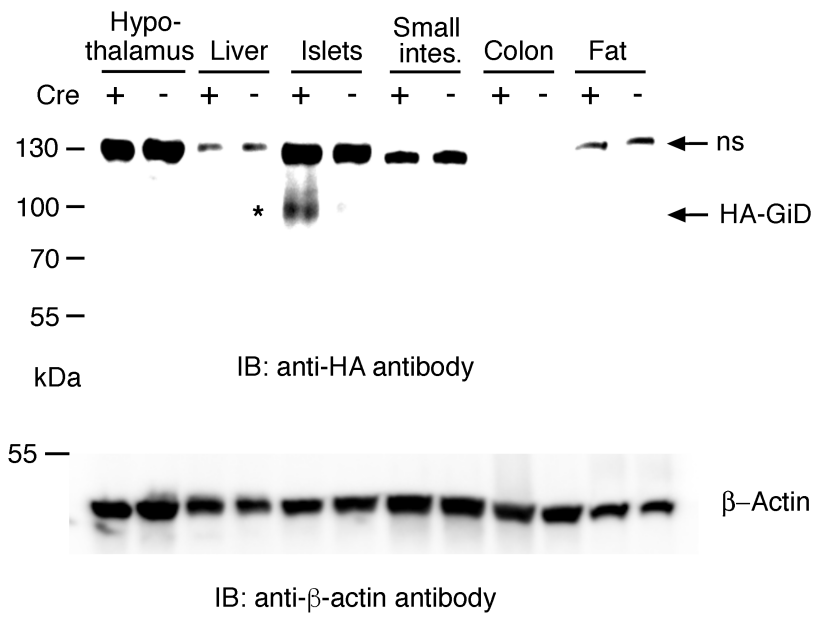
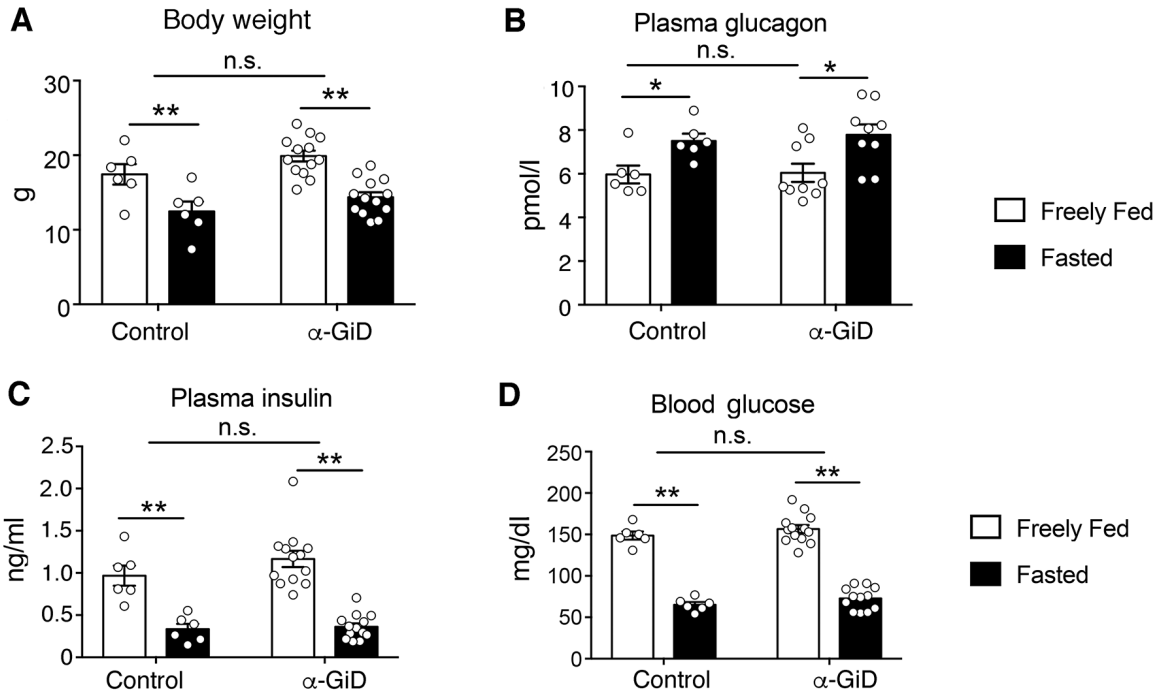
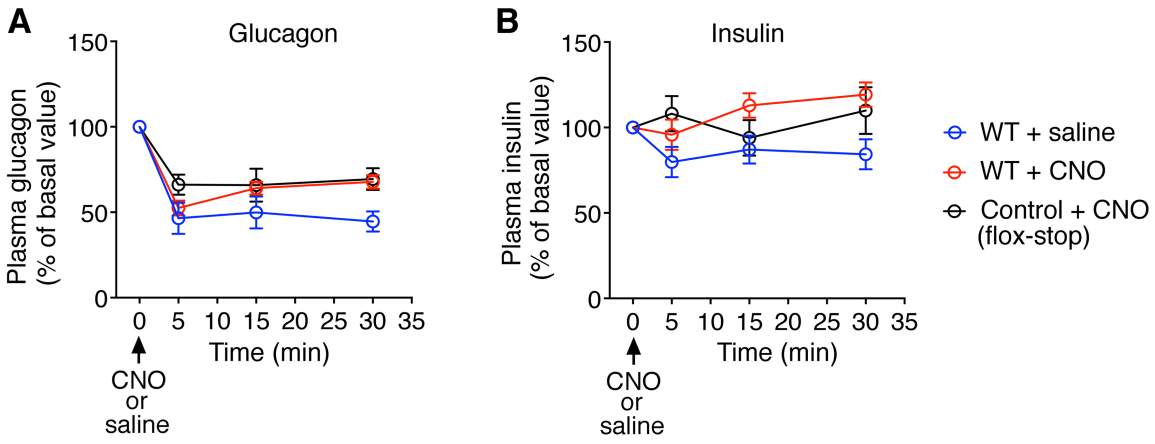


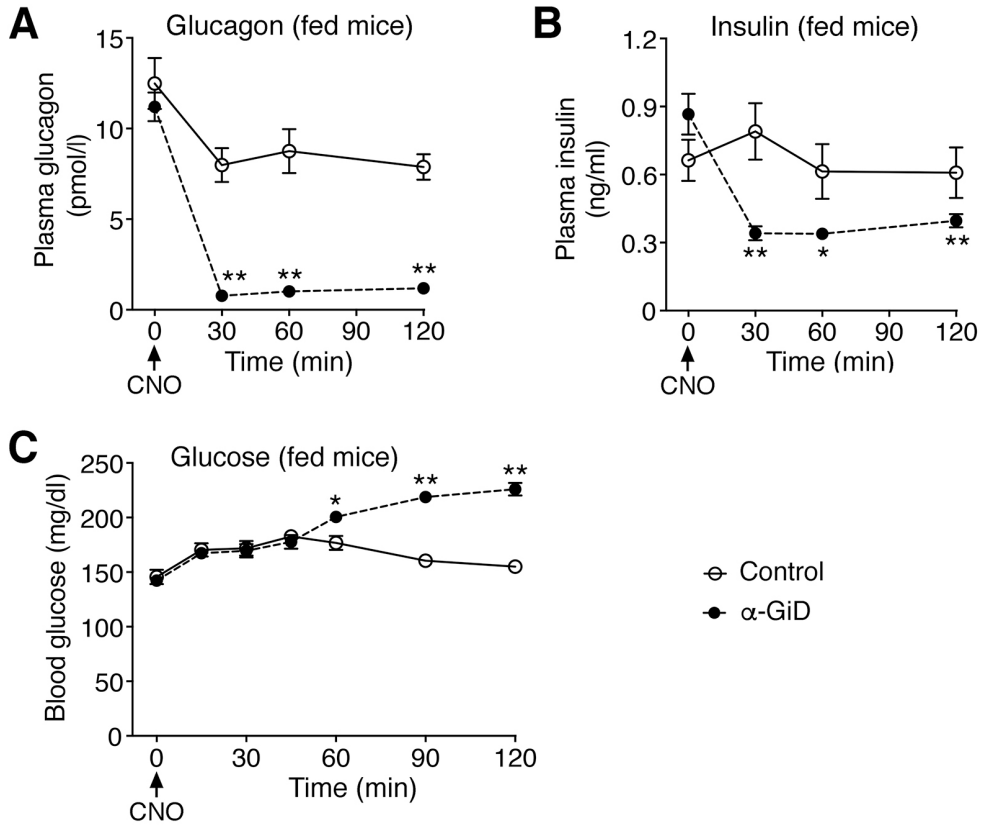
Supplemental Fig. 1. Selective expression of GiD in pancreatic islets of α–GiD mice, as detected by western blotting. Western blotting studies were carried out using lysates from the indicated tissues of α-GiD transgenic mice and control littermates lacking the Cre transgene (see Supplemental Methods for details). Blots were probed with an anti-HA antibody directed against the HA-epitope tag fused to the N-terminus of GiD. Note that the GiD receptor could be detected as a ~90 kDa band (*) only in pancreatic islets from α-GiD mice (Cre+). A ~130 kDa non-specific (ns) band was observed in most lanes. A representative blot is shown. Two additional blots gave similar results. Tissues were harvested from adult male mice four weeks after the last tamoxifen injection. Fat, white adipose epididymal fat.



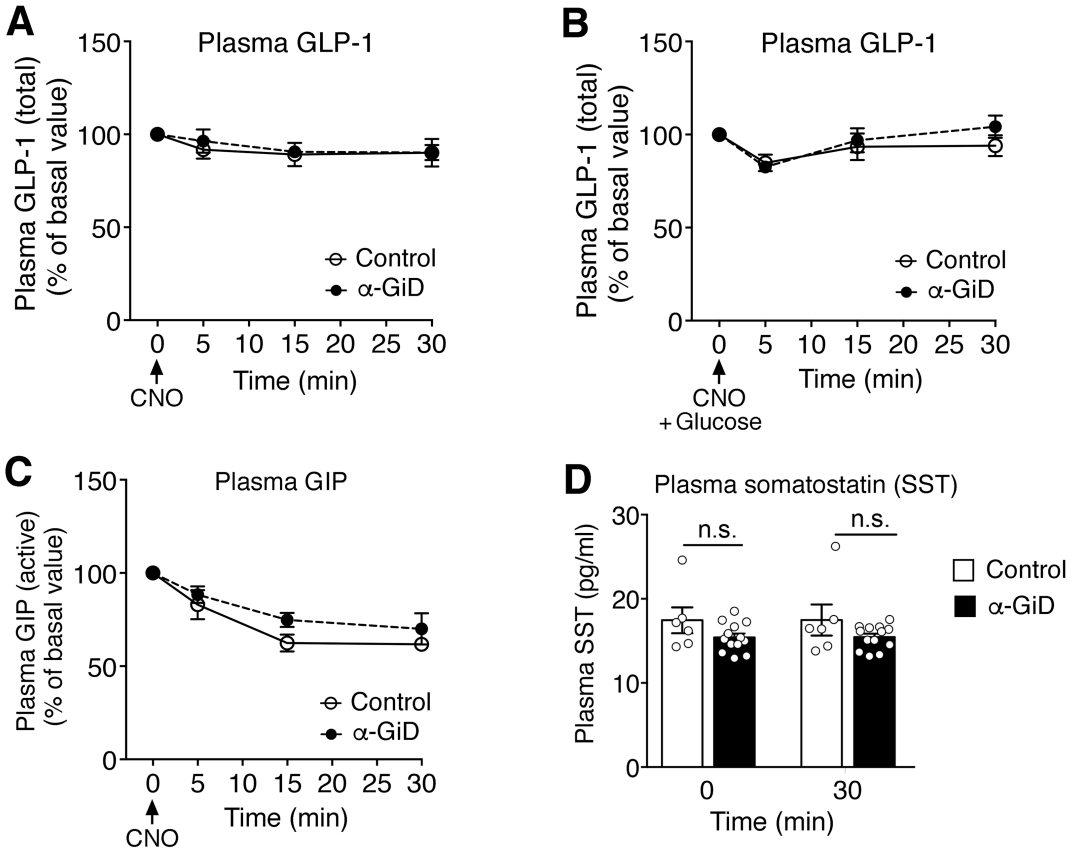
Supplemental Fig. 2. Body weight, plasma glucagon, plasma insulin, and blood glucose levels in freely fed and fasted α-GiD and control mice. Male α-GiD mice and control littermates (age: 14 weeks) had free access to food (fed mice) or were fasted overnight for 12 hr. (**A**) Body weight. (**B**) Plasma glucagon levels. (**C**) Plasma insulin levels. (**D**) Blood glucose levels. Data are given as means ± SEM (control: n=6; α-GiD: n=13;). **P*<0.05; ***P*<0.01 (two-way ANOVA followed by Tukey's post-hoc test). n.s., no statistically significant difference between control and mutant mice.



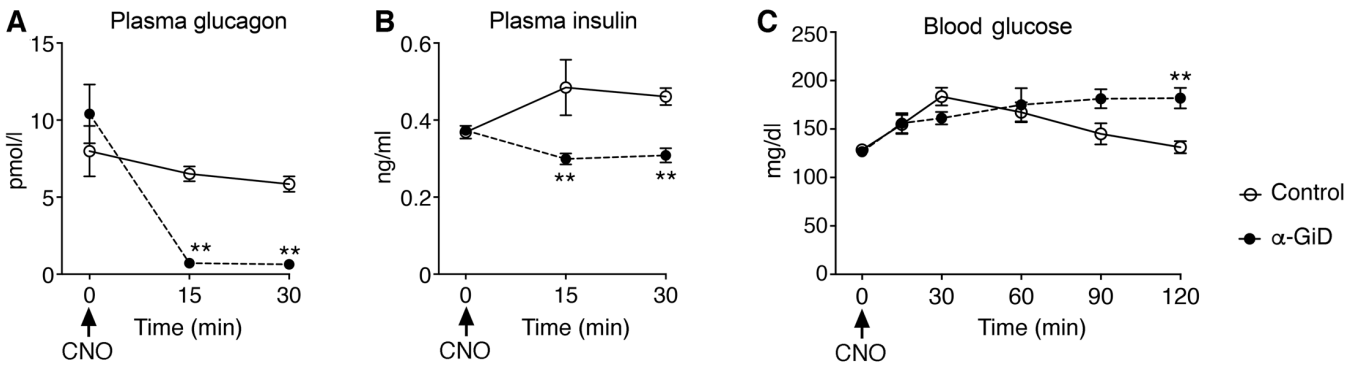
Supplemental Fig. 3. Saline or CNO injections (i.p.) of fasted wild-type (WT) mice cause reduced plasma glucagon levels. WT mice (14-week-old males) were injected i.p. with CNO (1 mg/kg) or normal saline, followed by the measurement of plasma glucagon and insulin levels at the indicated time points. For comparison, the data obtained with CNO-treated flox-stop control mice (14-week-old males; see Fig. 1) were also included in the two panels. (**A**) Plasma glucagon levels (% of pre-injection levels). (**B**) Plasma insulin levels (% of pre-injection levels). Data are given as means ± SEM (n=6-8 per group).



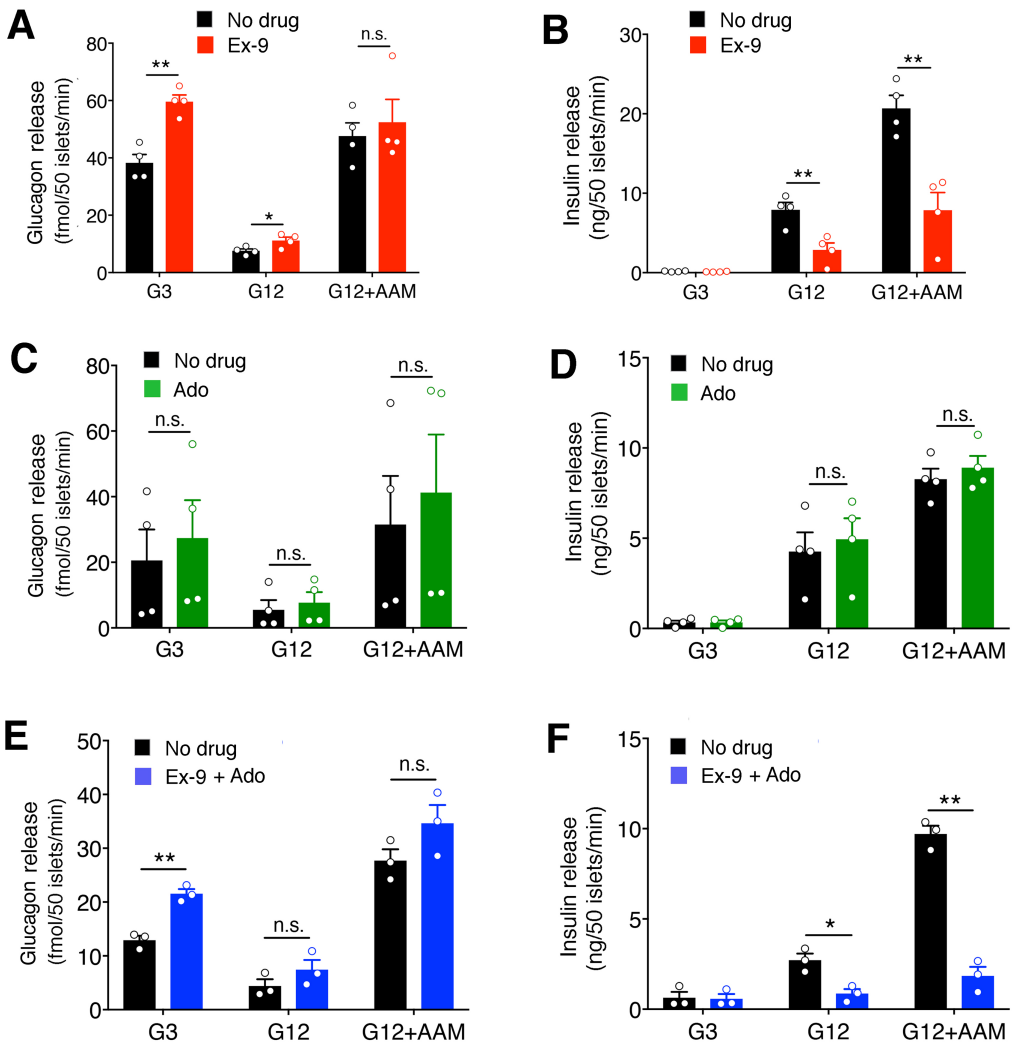
Supplemental Fig. 4. CNO treatment of α-GiD mice causes long-lasting reductions in plasma glucagon and insulin levels. Male α-GiD mice and control littermates (age: 12-20 weeks) were injected with CNO (1 mg/kg i.p.), followed by the measurement of plasma glucagon, plasma insulin, and blood glucose levels at the indicated time points. Mice had free access to food. (**A**) Plasma glucagon levels after CNO injection. (**B**) Plasma insulin levels after CNO injection. (**C**) Blood glucose levels after CNO injection. Data are given as means ± SEM α-GiD: n=13; control: n=6). **P*<0.05; ***P*<0.01 (mixed-effects repeated measures ANOVA for post-injection differences).



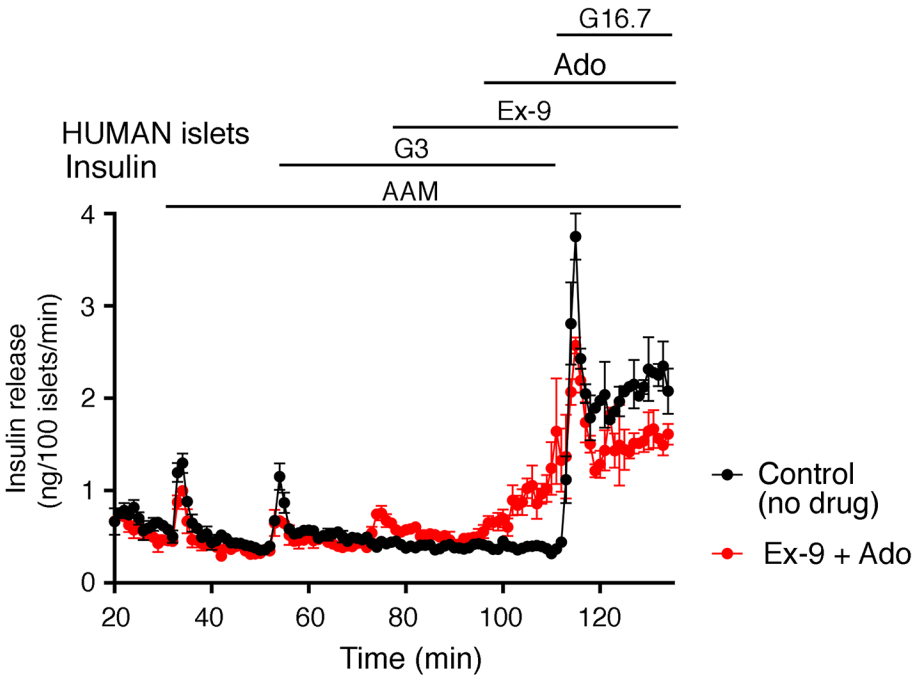
Supplemental Fig. 5. Plasma GLP-1, GIP and somatostatin (SST) levels after CNO Treatment of α-GiD mice. Male α-GiD mice and control littermates (age: 20-30 weeks) were injected with CNO (1 mg/kg i.p.), followed by the measurement of plasma hormone levels at the indicated time points. Mice had free access to food, except for panel (B) where fasted mice were used. (**A**) Plasma GLP-1 levels after CNO injection. (**B**) Plasma GLP-1 levels after coinjection of CNO with glucose (2 g/kg i.p.). (**C**) Plasma GIP levels after CNO injection. (**D**) Plasma SST levels determined 30 min after CNO injection. Data in (A-C) were normalized to basal (pre-injection) levels. All experiments were carried out with male littermates that were 20-30 weeks old. Data are given as means ± SEM (α-GiD: n=13; control: n=6). n.s., no statistically significant difference.



Supplemental Fig. 6. CNO treatment of female α-GiD mice causes similar reductions in plasma glucagon and insulin levels as observed with male α-GiD mice.
Female α-GiD mice and control littermates (age: 12 weeks) were injected with CNO (1 mg/kg i.p.), followed by the measurement of plasma glucagon, plasma insulin, and blood glucose levels at the indicated time points. Mice had free access to food. (**A**) Plasma glucagon levels. (**B**) Plasma insulin levels. (**C**) Blood glucose levels. Data are given as means ± SEM (α-GiD: n=7; control: n=5). **P*<0.05; ***P*<0.01 (mixed-effects repeated measures ANOVA for post-injection differences).



Supplemental Fig. 7. Quantitative analysis of the glucagon and insulin release data shown in Fig. 4. Wild-type (WT) mouse pancreatic islets were perifused with 3 or 12 mM of glucose (G3 and G12, respectively) and an amino acid mixture (AAM). Hormone (glucagon and insulin) secretion during a specific time interval was expressed as the average of all values measured during this particular perfusion period: G3, 0-20 min; G12, 20-40 min; G12+AAM: 40-60 min. (**A, B**) Glucagon (A) and insulin (B) secretion in the presence or absence of Ex9 (1 μM), a selective GLP-1 receptor antagonist. (**C, D**) Glucagon (C) and insulin (D) secretion in the presence or absence of adomeglivant (Ado; 1 μM), a selective glucagon receptor antagonist. (**E, F**) Glucagon (E) and insulin (F) secretion in the presence or absence of a mixture of Ex9 (1 μM) and Ado (1 μM). The amounts of secreted glucagon and insulin were normalized to islet DNA content. All islets were prepared from 12-20-week-old male mice. Data are presented as means ± SEM (three or four perifusions with 50 islets per perfusion chamber; islets from two mice were pooled per perfusion experiment). *P<0.05; **P<0.01 (two-tailed Student's t-test). n.s., no statistically significant difference.



Supplemental Fig. 8. Insulin release studies performed with perifused human islets in the presence of Ex-9 and adomeglivant. Human islets were perifused with 3 and 16.7 mM of glucose (G3 and G16.7, respectively), in the presence of a physiological amino acid mixture (AAM). Insulin secretion was monitored continuously throughout experiments. A mixture of 1 μM Ex-9 (selective GLP-1 receptor antagonist) and 1 μM adomeglivant (Ado; selective glucagon receptor antagonist) was added prior to stimulation of islets with G16.7. In the control group, both drugs were omitted from the perfusate. Data represent means ± SEM from two independent perfusions.

Supplemental Table S1. Source of Reagents and Animals

Reagent/resource	Source	
Antibodies		
Rabbit anti-HA antibody	Cell Signaling	Cat# 3724
Guinea pig anti-insulin antibody	Abcam	Cat# 7842
Mouse anti-glucagon	Sigma-Aldrich	Cat# G2654
Mouse anti-β-actin antibody	Cell Signaling	Cat# 3700
Anti-rabbit IgG, HRP-linked secondary antibody	Cell Signaling	Cat# 7074
Anti-mouse IgG, HRP-linked secondary antibody	Cell Signaling	Cat# 7076
Alexa Fluor 555 goat anti-guinea secondary antibody	Thermo Fisher Scientific	Cat# A21435
Alexa Fluor 555 goat anti-mouse secondary antibody	Thermo Fisher Scientific	Cat# A28180
Alexa Fluor 488 goat anti-rabbit secondary antibody	Thermo Fisher Scientific	Cat# A11034
Drugs, etc.		
Clozapine-N-oxide (CNO)	Rapid Access to Investigative Drug Program (NINDS)	N/A
Exendin (9-39) amide	Abcam	Cat# ab141101
Adomeglivant	ChemScene	Cat# CS-5729
Tamoxifen	Sigma-Aldrich	Cat# T5648
Aprotinin	Sigma-Aldrich	Cat# A3428
DPP-4 inhibitor (KR-62436 hydrate)	Sigma-Aldrich	Cat# K4264
Corn oil	Sigma-Aldrich	Cat# C8267
Human insulin (Humulin R U-100)	Eli Lilly	Cat# NDC 0002-8215-17
ProLong™ Gold antifade reagent with DAPI	Thermo Fisher Scientific	Cat# P36931
BCA protein assay kit	Pierce	Cat# 23225
ECL Western blotting substrate	Pierce	Cat# 32106

Bovine serum albumin (fatty acid-free)	Sigma-Aldrich	Cat# A7030
Triton X-100	Fisher Scientific	Cat# BP151
Assay kits		
Glucagon ELISA kit	Mercodia	Cat# 10-1281-01
Ultra-Sensitive Mouse Insulin ELISA kit	Crystal Chem	Cat# 90080
Somatostatin ELISA kit	LifeSpan Biosciences	Cat# LS-F4026
GLP-1 ELISA kit	Crystal Chem	Cat# 81508
GIP ELISA kit	Crystal Chem	Cat# 81511
Mouse strains		
Gcg-CreER^{T2} mice	Dr. Klaus H. Kaestner (University of Pennsylvania)(1)	N/A
Rosa26-LSL- hM4Di mice	Dr. Ute Hochgeschwender (Central Michigan University)(2)	N/A

References

1. Ackermann AM, Zhang J, Heller A, Briker A, and Kaestner KH. High-fidelity Glucagon-CreER mouse line generated by CRISPR-Cas9 assisted gene targeting. *Mol Metab.* 2017;6(3):236-44.
2. Zhu H, Aryal DK, Olsen RH, Urban DJ, Swearingen A, Forbes S, et al. Cre-dependent DREADD (Designer Receptors Exclusively Activated by Designer Drugs) mice. *Genesis (New York, NY : 2000).* 2016;54(8):439-46.