Supplemental Information

Gut-Proglucagon-Derived Peptides Are Essential for Regulating Glucose Homeostasis in Mice

Youngmi Song, Jacqueline A. Koehler, Laurie L. Baggio, Alvin C. Powers, Darleen A. Sandoval, and Daniel J. Drucker

Figure S1

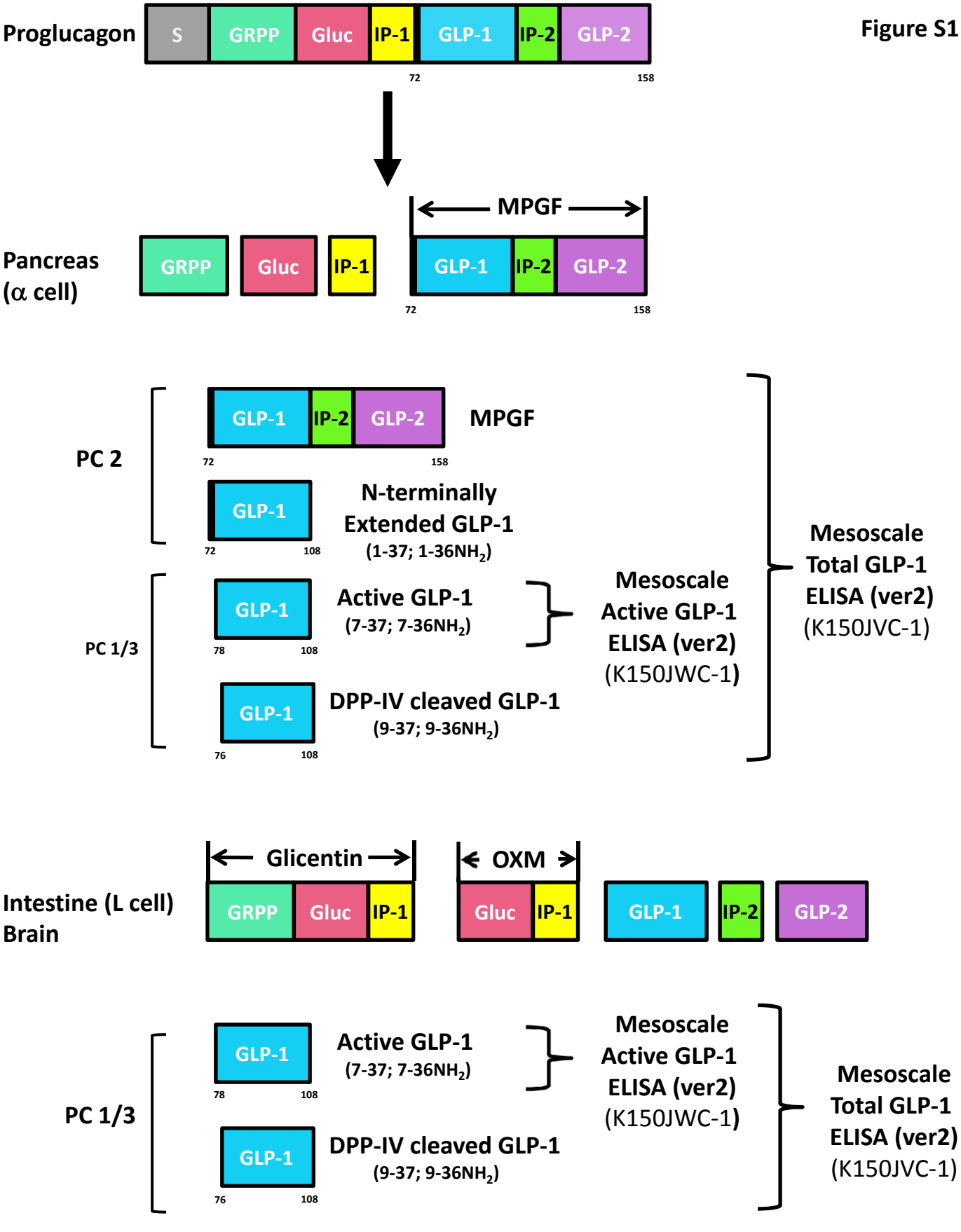
Proglucagon
S
GRPP
Gluc
IP-1
GLP-1
IP-2
GLP-2
72
158
Pancreas
(α cell)
GRPP
Gluc
IP-1
MPGF
GLP-1
IP-2
GLP-2
72
158
PC 2
GLP-1
IP-2
GLP-2
MPGF
72
158
GLP-1
N-terminally
Extended GLP-1
(1-37; 1-36NH2)
72
108
PC 1/3
GLP-1
Active GLP-1
(7-37; 7-36NH2)
78
108
GLP-1
DPP-IV cleaved GLP-1
(9-37; 9-36NH2)
76
108
Mesoscale
Active GLP-1
ELISA (ver2)
(K150JWC-1)
Mesoscale
Total GLP-1
ELISA (ver2)
(K150JVC-1)
Intestine (L cell)
Brain
Glicentin
GRPP
Gluc
IP-1
OXM
Gluc
IP-1
GLP-1
IP-2
GLP-2
PC 1/3
GLP-1
Active GLP-1
(7-37; 7-36NH2)
78
108
GLP-1
DPP-IV cleaved GLP-1
(9-37; 9-36NH2)
76
108
Mesoscale
Active GLP-1
ELISA (ver2)
(K150JWC-1)
Mesoscale
Total GLP-1
ELISA (ver2)
(K150JVC-1)

Figure S1 Related to Figures 1-4: Schematic Representation of Tissue-specific Processing of Proglucagon. In pancreatic α cells, proglucagon is processed by PC 2 into glicentin-related pancreatic polypeptide (GRPP), glucagon (Gluc), the major proglucagon fragment (MPGF), and to some degree to an N-terminally extended form of GLP-1 (1-36NH_2/1-37). In intestinal L cells, proglucagon is cleaved by prohormone convertase 1/3 (PC 1/3) to produce glicentin, oxyntomodulin (OXM), glucagon-like peptide-1 (GLP-1) and GLP-2. Also depicted are the potential products recognized by the two GLP-1 ELISAs utilized in this study. DPP-IV: Dipeptidyl peptidase-4. IP: Intervening peptide.

Figure S2

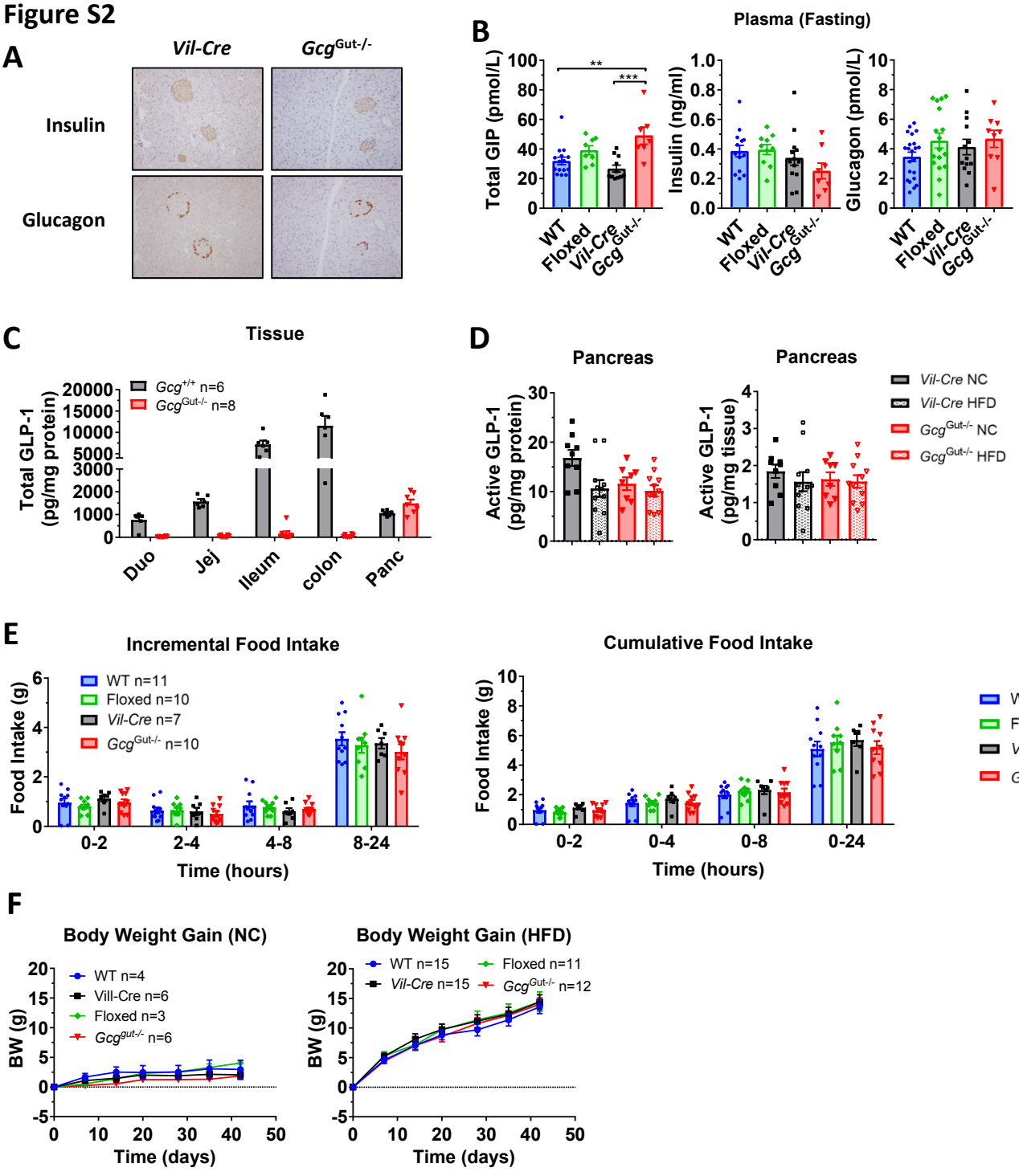

Figure S2 Related to Figures 2 and 3: Glucoregulatory Hormones, Food Intake and Body Weight Gain in *Gcg*$^{Gut-/-}$ mice.
(A) Representative pancreas sections from 22-24-week-old male *Gcg*$^{Gut-/-}$ and *Vil-Cre* control mice stained for insulin or glucagon. (B) Fasting plasma levels of total GIP, insulin and glucagon in 14-17-week-old male *Gcg*$^{Gut-/-}$ and control mice. (n=7-19/group). (C) Total GLP-1 levels, normalized to protein content, in tissue extracts from different regions of the intestine and pancreas of 16-18-week-old female mice. (n=6-8/group).
(D) Active GLP-1 levels, normalized to protein content (*left panel*), or tissue weight (*right panel*), in extracts from pancreas of male *Gcg*$^{Gut-/-}$ and *Vil-Cre* mice fed normal chow (NC), or a 60% high-fat-diet (HFD) for 7 weeks. (n=8-11/group). (E) Incremental (*left panel*) and cumulative (*right panel*) food intake over 24 hours in overnight fasted 11-14-week-old male *Gcg*$^{Gut-/-}$ and control mice.
(F) Weekly body weight gain of male *Gcg*$^{Gut-/-}$ and control mice fed normal chow (NC, *left panel*), or a 60% high-fat-diet (HFD, *right panel*). (n=3-15/group). Data are presented as the mean ± SEM. **p<0.01, ***p=<0.001 *Gcg*$^{Gut-/-}$ *vs.* control mice.

Figure S3

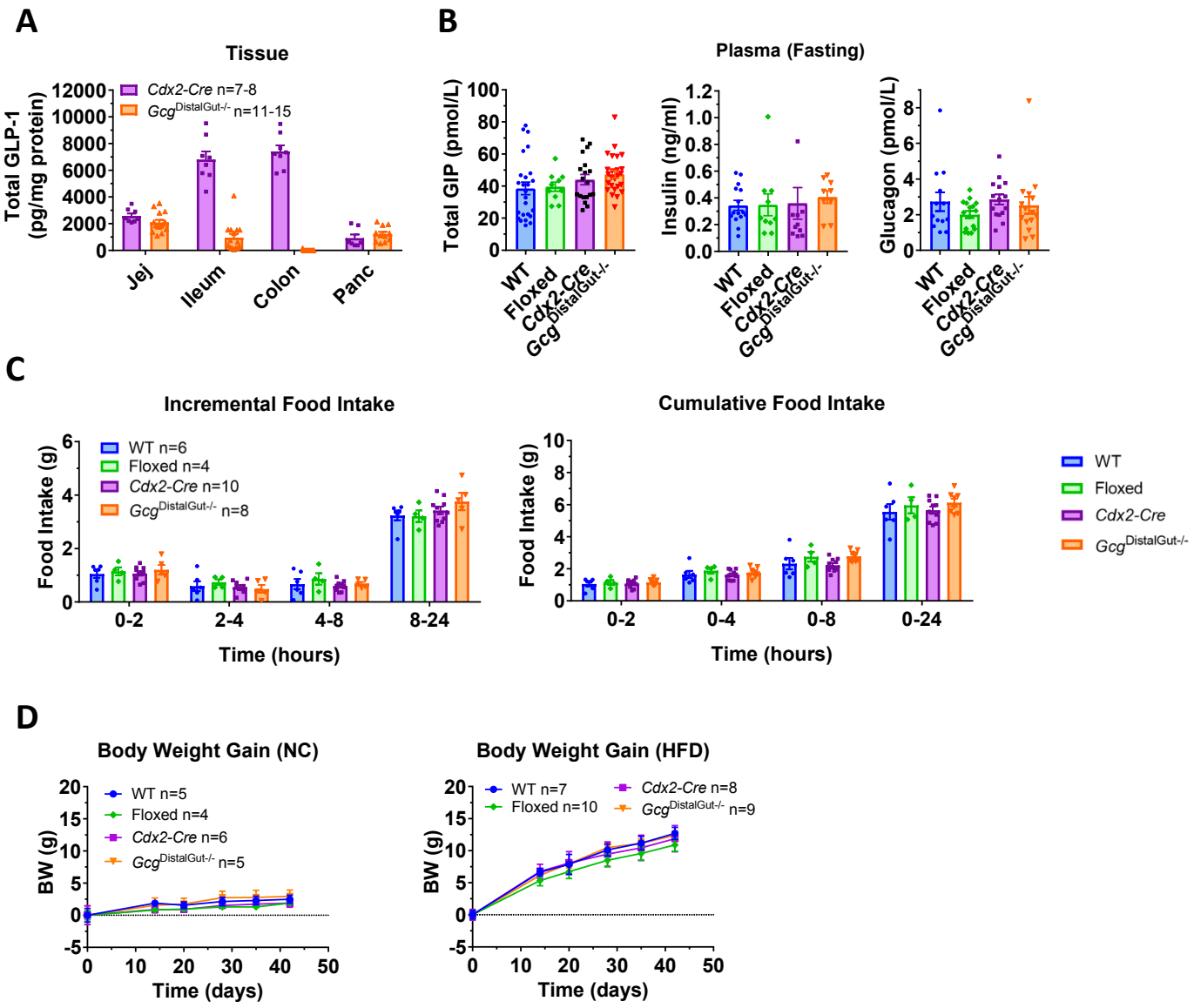

Figure S3 Related to Figure 2 and 4.

Glucoregulatory Hormones, Food Intake and Body Weight Gain in *Gcg*$^{DistalGut-/-}$ mice. (A) Total GLP-1 levels, normalized to protein content, in whole tissue extracts from different regions of the intestine and pancreas of 20-23-week-old male *Gcg*$^{DistalGut-/-}$ and control mice. (n=7-15/group). (B) Fasting plasma levels of total GIP, insulin and glucagon in 10-14-week-old male *Gcg*$^{DistalGut-/-}$ and control mice. (n=5-25/group). (C) Incremental (*left panel*) and cumulative (*right panel*) food intake in overnight fasted 8-week-old male *Gcg*$^{DistalGut-/-}$ and control mice over 24 hours. (n=4-10/group). (D) Weekly body weight gain of *Gcg*$^{DistalGut-/-}$ and control mice fed normal chow (NC, *left panel*), or a 60% high-fat-diet (HFD, *right panel*). (n=3-15/group). Data are presented as the mean ± SEM.

Figure S4

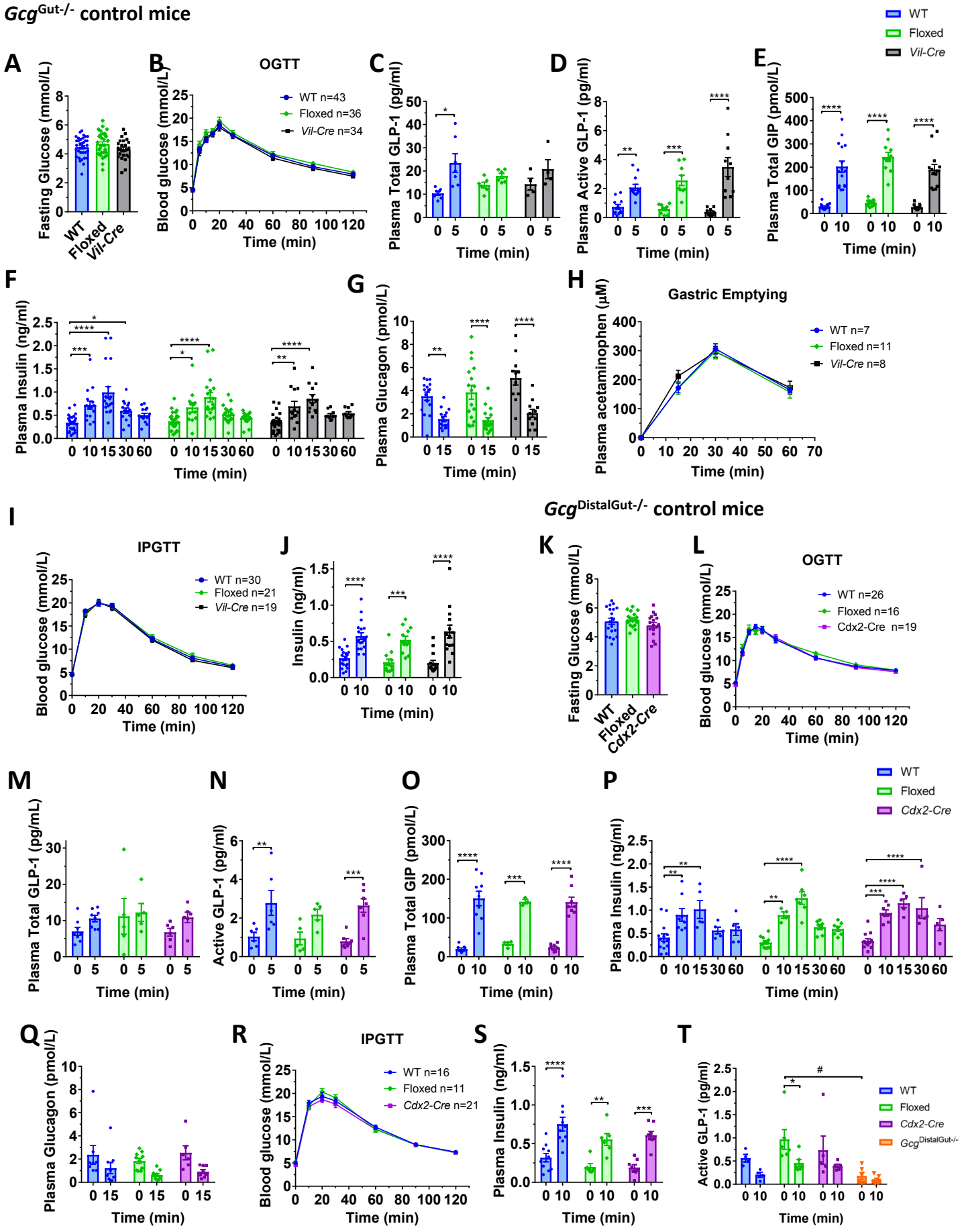
Gcg Gut-/- control mice
WT
Floxed
Vil-Cre
A
Fasting Glucose (mmol/L)
B
OGTT
Blood glucose (mmol/L)
WT n=43
Floxed n=36
Vil-Cre n=34
Time (min)
C
Plasma Total GLP-1 (pg/ml)
D
Plasma Active GLP-1 (pg/ml)
E
Plasma Total GIP (pmol/L)
F
Plasma Insulin (ng/ml)
G
Plasma Glucagon (pmol/L)
H
Gastric Emptying
Plasma acetaminophen (μM)
WT n=7
Floxed n=11
Vil-Cre n=8
Gcg DistalGut-/- control mice
I
IPGTT
WT n=30
Floxed n=21
Vil-Cre n=19
J
Insulin (ng/ml)
K
Fasting Glucose (mmol/L)
WT
Floxed
Cdx2-Cre
L
OGTT
WT n=26
Floxed n=16
Cdx2-Cre n=19
M
Plasma Total GLP-1 (pg/mL)
N
Active GLP-1 (pg/ml)
O
Plasma Total GIP (pmol/L)
P
Plasma Insulin (ng/ml)
Cdx2-Cre
Q
Plasma Glucagon (pmol/L)
R
IPGTT
WT n=16
Floxed n=11
Cdx2-Cre n=21
S
Plasma Insulin (ng/ml)
T
Active GLP-1 (pg/ml)
GcgDistalGut-/-
Time (min)

Figure S4 Related to Figures 3 and 4 : Oral and Intraperitoneal Glucose Tolerance in WT, *Gcg*$^{flox/flox}$, *Vil-Cre* and *Cdx2-Cre* Control Mice. (A-B) Fasting glucose and blood glucose levels after oral glucose (1.5g/kg) in overnight fasted 12-17-week-old male *Gcg*$^{Gut-/-}$ control mice. (n=34-43/group). (C) Plasma total GLP-1, (D) active GLP-1, (E) total GIP, (F) insulin, and (G) glucagon measured before and 5-60 min after oral glucose challenge as indicated. (n=4-20/group). (H) Plasma acetaminophen levels, as a measurement of gastric emptying, 0-60 min after co-administration of oral acetaminophen and glucose in overnight fasted 14-17-week-old male *Gcg*$^{Gut-/-}$ control mice. (n=7-11/group). (I) Blood glucose levels taken after intraperitoneal (*i.p.*) administration of glucose (1.5g/kg), and (J) plasma insulin 10 min after *i.p.* glucose challenge in overnight fasted 14-19-week-old male *Gcg*$^{Gut-/-}$ control mice. For (I) n=19-30/group, and (J) n=12-19/group. K-L) Fasting glucose and blood glucose levels after oral glucose (1.5g/kg) in overnight fasted 12-15-week-old male *Gcg*$^{DistalGut-/-}$ control mice. (n=16-26/group). (M) Plasma total GLP-1, (N) active GLP-1, (O) total GIP, (P) insulin, and (Q) glucagon measured before and 5-60 min after oral glucose challenge as indicated. (R) Blood glucose levels taken after intraperitoneal (*i.p.*) administration of glucose (1.5g/kg). (S) Plasma insulin, and (T) active GLP-1 10 min after *i.p.* glucose challenge in overnight fasted 14-17-week-old male control mice (S and T), and *Gcg*$^{DistalGut-/-}$ mice (T). For (R) n=11-21/group and (S) n=6-10/group, (T) n=4-6/group. Data are presented as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 basal *vs.* 5-60 min, #p<0.05 *Gcg*$^{DistalGut-/-}$ *vs.* control mice as indicated.

Table S1

Donor Information	1	2	3	4	5	6
Unique identifier	ABGH375	ABHB279	ABI2259	ACEZ011	AFEF080	AFG1440
Donor age (years)	18	18	35	20	55	45
Donor sex (M/F)	M	M	M	M	F	F
Donor BMI (kg/m^2)	31.4	25.1	26.9	19.4	24.2	29.8
Donor HbA_{1c}	N/A	N/A	5.1	5.6	N/A	5.6
Processing center	Pittsburgh	Pittsburgh	VUMC	Vanderbilt	Pittsburgh	Pittsburgh
Donor history of diabetes?	No	No	No	No	No	No
Donor cause of death	Head Trauma / Blunt Injury	Head Trauma / Blunt Injury	Head Trauma / Gunshot wound / Suicide	Head Trauma/Blunt Injury/MVA	Cerebrovascular / Stroke / Intracranial Hemorrhage	Anoxia / Cardiovascular
Warm ischaemia time (h)	N/A	N/A	N/A	N/A	N/A	N/A
Cold ischaemia time (h)	N/A	N/A	N/A	N/A	3.8	9

Table S1 Related to Figure 1

Information related to the human pancreatic samples analyzed in Figure 1. N/A – not available