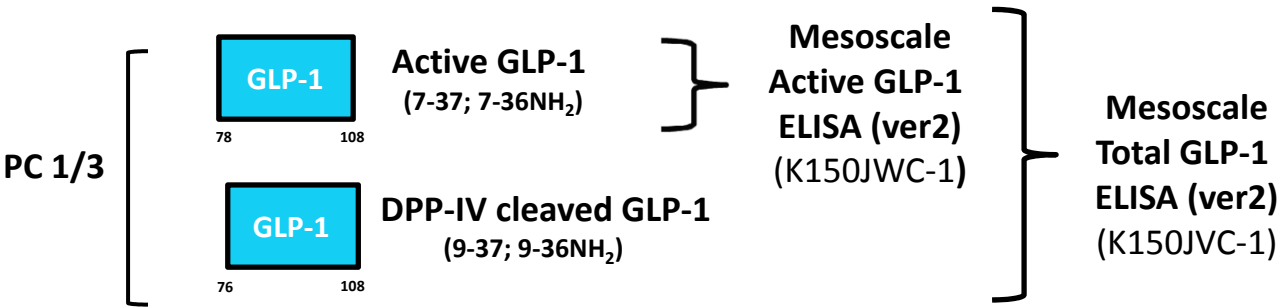
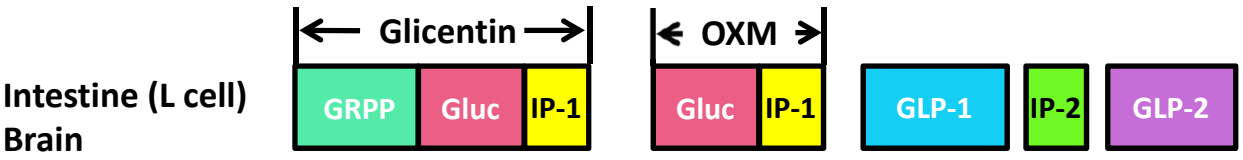
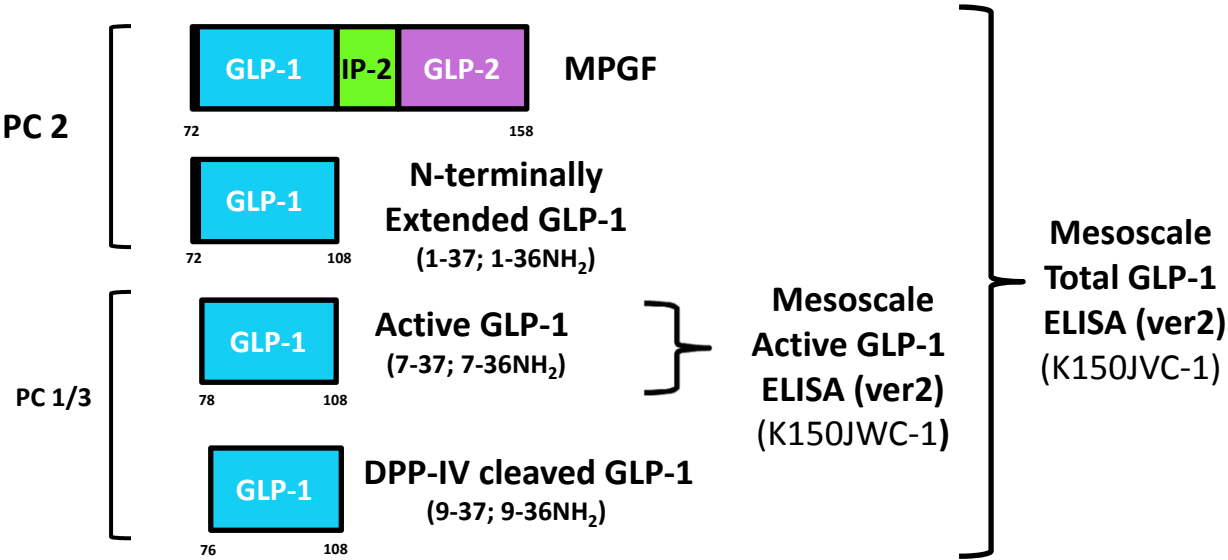
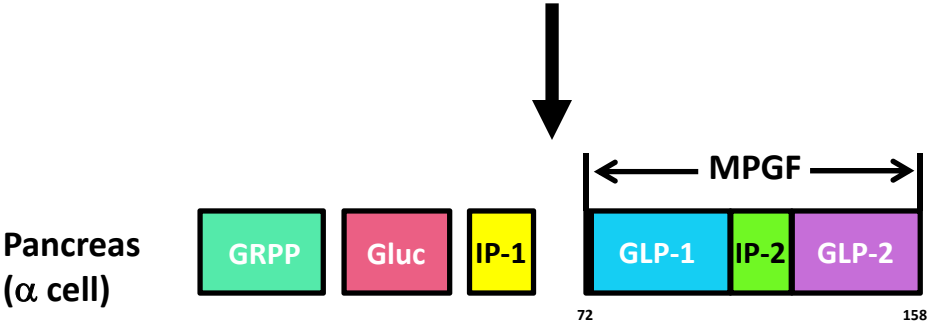


## **Supplemental Information**

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

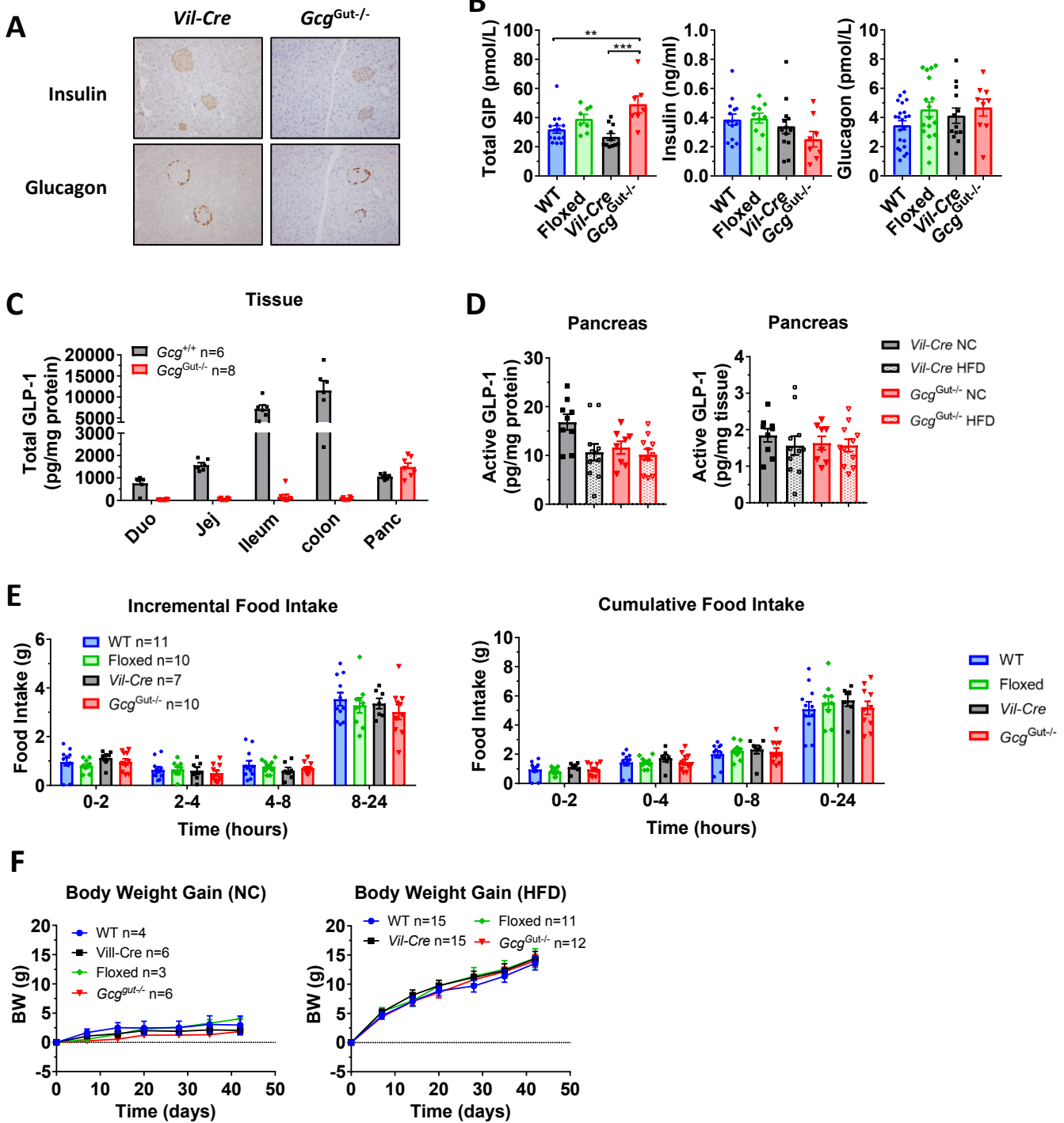
**Youngmi Song, Jacqueline A. Koehler, Laurie L. Baggio, Alvin C. Powers, Darleen A. Sandoval, and Daniel J. Drucker**

Figure S1



**Figure S1 Related to Figures 1-4: Schematic Representation of Tissue-specific Processing of Proglucagon.** In pancreatic  $\alpha$  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<sub>2</sub>/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<sup>Gut-/-</sup>* mice.**

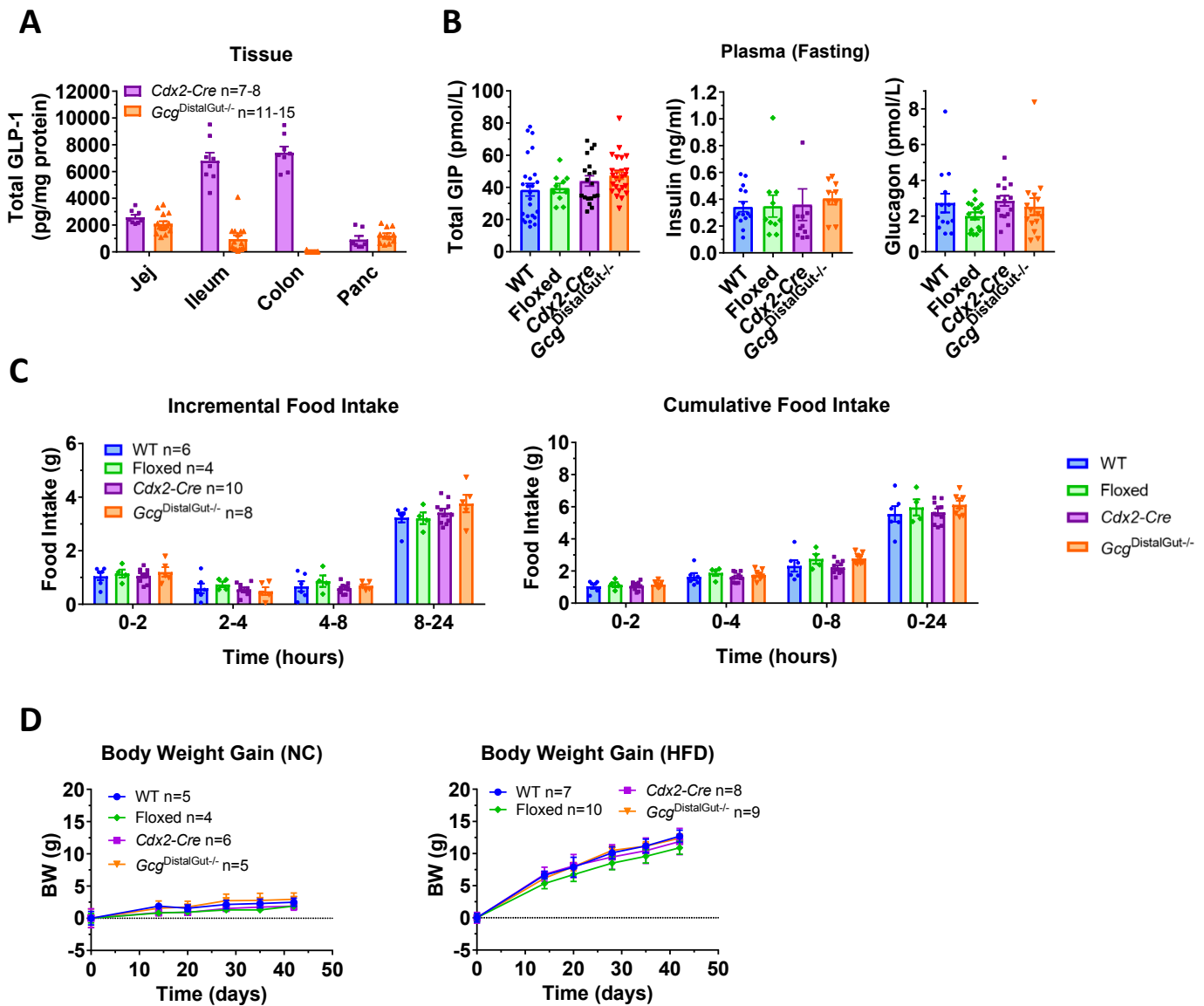
(A) Representative pancreas sections from 22-24-week-old male *Gcg<sup>Gut-/-</sup>* and *Vil-Cre* control mice stained for insulin or glucagon. (B) Fasting plasma levels of total GIP, insulin and glucagon in 14-17-week-old male *Gcg<sup>Gut-/-</sup>* and control mice. (n=7-19/group). (C) Total GLP-1 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<sup>Gut-/-</sup>* 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<sup>Gut-/-</sup>* and control mice.

(F) Weekly body weight gain of male *Gcg<sup>Gut-/-</sup>* 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  $\pm$  SEM. \*\*p<0.01, \*\*\*p<0.001 *Gcg<sup>Gut-/-</sup>* vs. control mice.

**Figure S3**

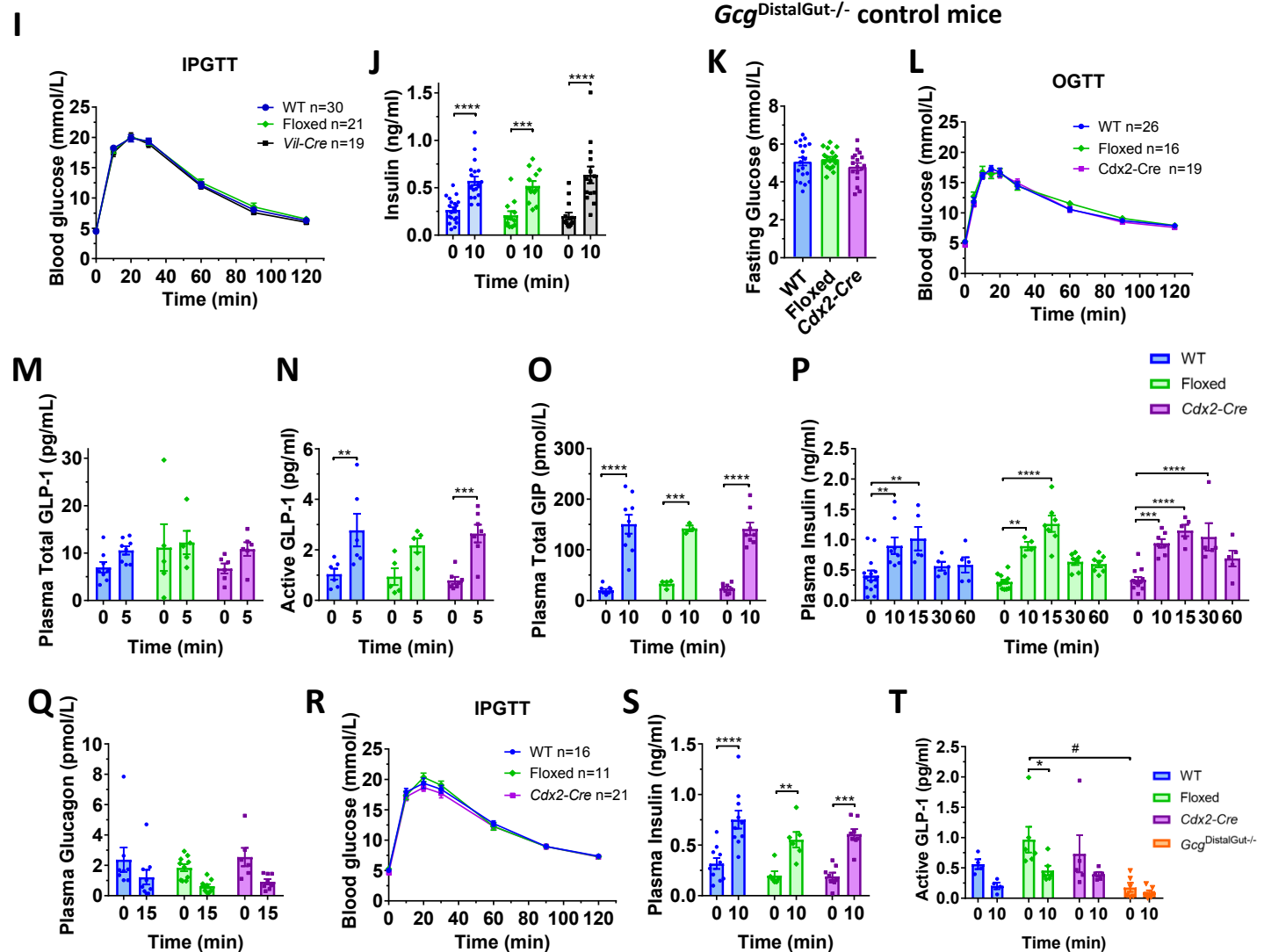
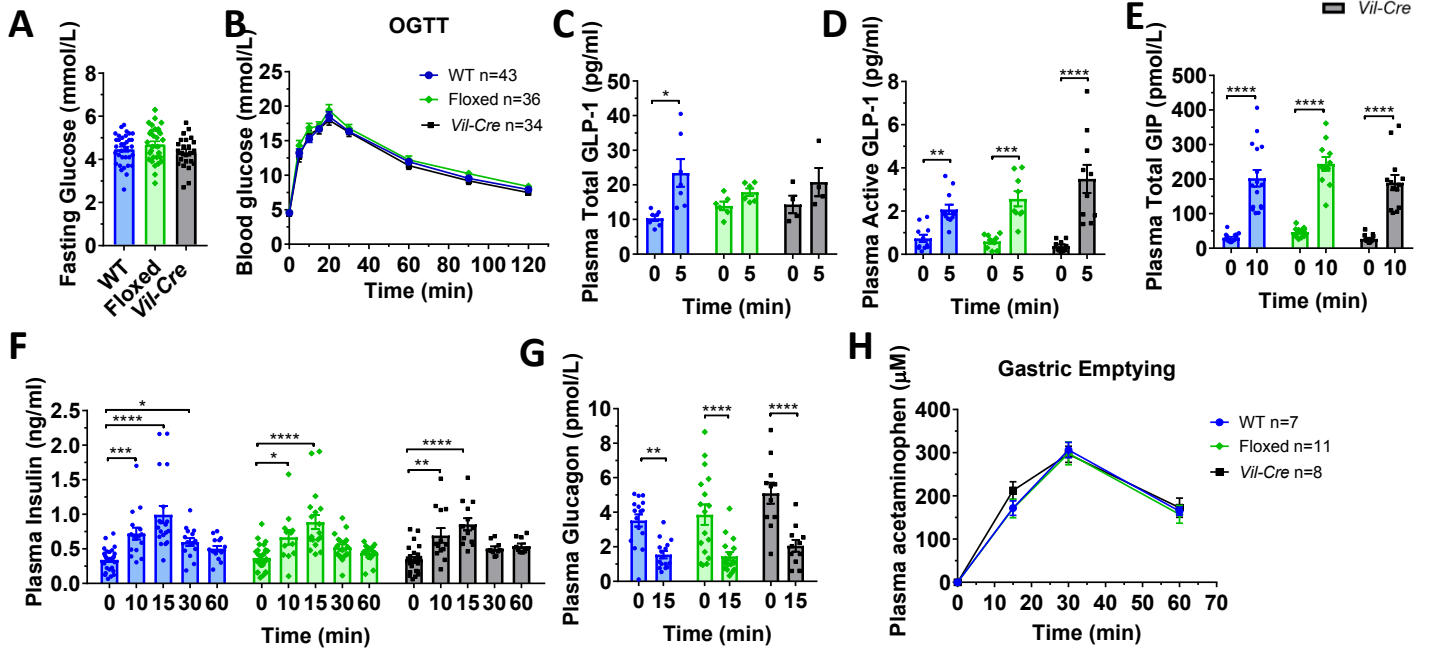


**Figure S3 Related to Figure 2 and 4.**

**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  $\pm$  SEM.

**Figure S4**

*Gcg*<sup>Gut-/-</sup> control mice



**Figure S4 Related to Figures 3 and 4 : Oral and Intraperitoneal Glucose Tolerance in WT, *Gcg<sup>flox/flox</sup>*, *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<sup>Gut-/-</sup>* 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<sup>Gut-/-</sup>* 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<sup>Gut-/-</sup>* 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<sup>DistalGut-/-</sup>* 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<sup>DistalGut-/-</sup>* 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  $\pm$  SEM. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 basal vs. 5-60 min, #p<0.05 *Gcg<sup>DistalGut-/-</sup>* vs. control mice as indicated.

**Table S1**

<b>Donor Information</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
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 <sup>2</sup> )	31.4	25.1	26.9	19.4	24.2	29.8
Donor HbA <sub>1c</sub>	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