

Supplemental Information

Single-cell transcriptome profiling of pancreatic islets from early diabetic mice identifies *Anxa10* for Ca^{2+} homeostasis into β -cell failure

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Supplemental Methods

Bulk RNA sequencing

Total RNA was extracted from five to ten pancreatic islets isolated from *db*^{+/+} and *db/db* mice. ImmunoGeneTecs, Inc (Noda, Japan) was entrusted with the RNA amplification and bulk RNA-seq. The mapped reads per gene (raw tag counts) were quantified as gene expression. Between-sample normalization of gene expression was conducted against raw count data by iDEP 1.1 (<http://bioinformatics.sdstate.edu/idep/>) with DESeq2. Genes with adjusted p-value <0.05 and fold-change of ≥ 2 between at least three samples were determined as statistically significant DEGs. Raw data from the experiment have been recorded in the DDBJ database.

Cell culture

The mouse insulinoma MIN6 cells were regulated in Dulbecco's Modified Eagle Medium (DMEM) comprising 25-mM glucose supplemented with 15% fetal bovine serum (FBS), 100-units/ml penicillin, 100- μ g/ml streptomycin, 100- μ g/ml L-glutamine, and 71- μ M β -mercapto-ethanol. INS-1 832/13 cells were cultured in RPMI-1640 medium containing 11-mM glucose supplemented with 10% FBS, 50- μ M β -mercapto-ethanol, 1-mM sodium pyruvate, 10-mM HEPES, 100- μ g/mL streptomycin, and 100-IU/ml penicillin.

Treatment of MIN6 cells and isolated islets with palmitate and chemicals

As previously described, MIN6 cells and isolated islets were treated with palmitate and chemicals (1). Insulin content and insulin secretion of MIN6 cells and isolated islets were measured as detailed elsewhere (1).

RNA extraction and quantitative real-time PCR

Total RNA extraction from isolated islets and MIN6 cells, cDNA synthesis, and quantitative real-time PCR (qRT-PCR) were carried out as previously described (1). The primer sequences that we employed were shown in Supplemental Table 11. mRNA expression levels were normalized to that of cyclophilin B mRNA.

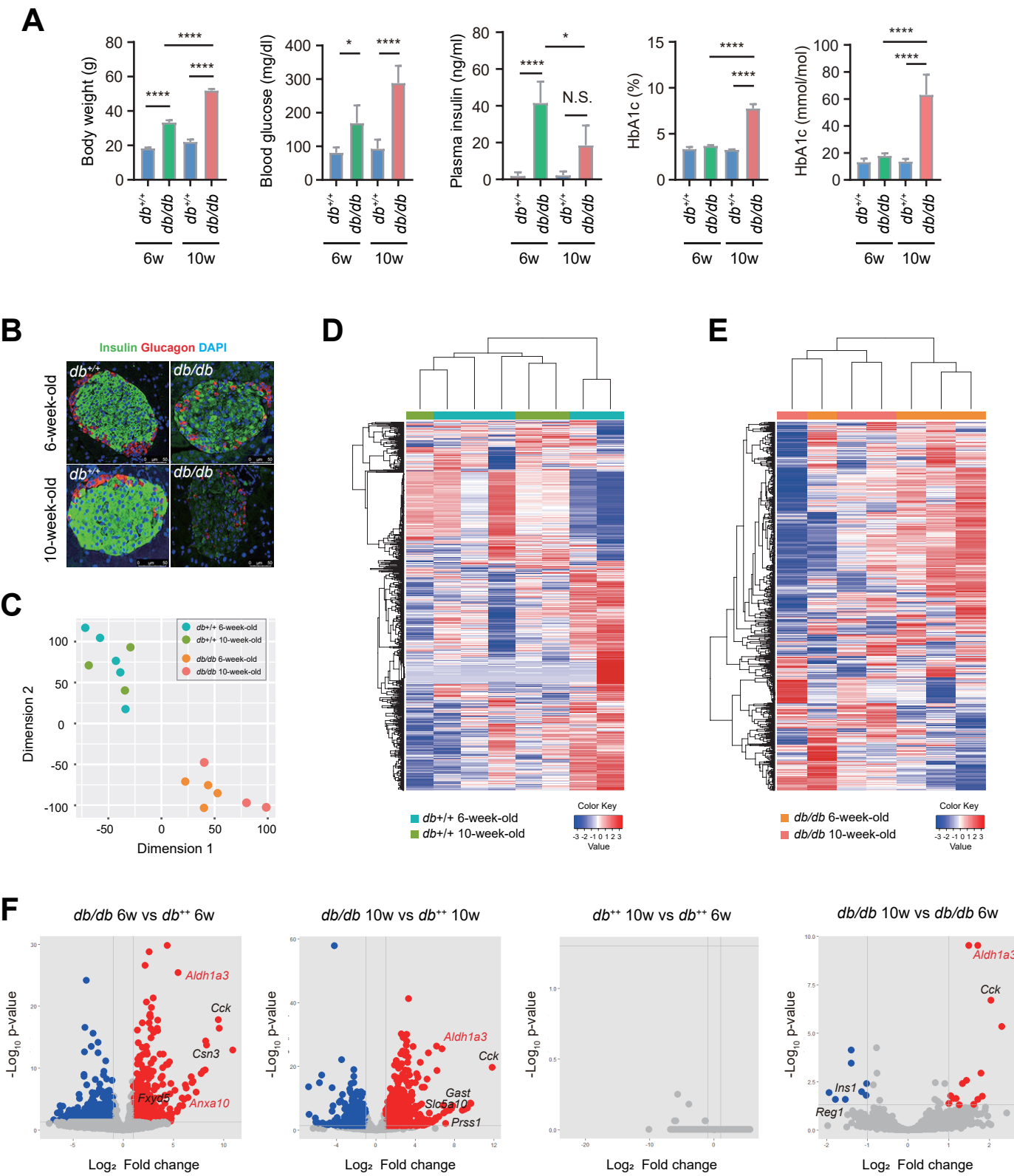
Immunoblot analysis

Protein extraction from the cells and immunoblot analysis was carried out as previously described (2).

Supplemental References

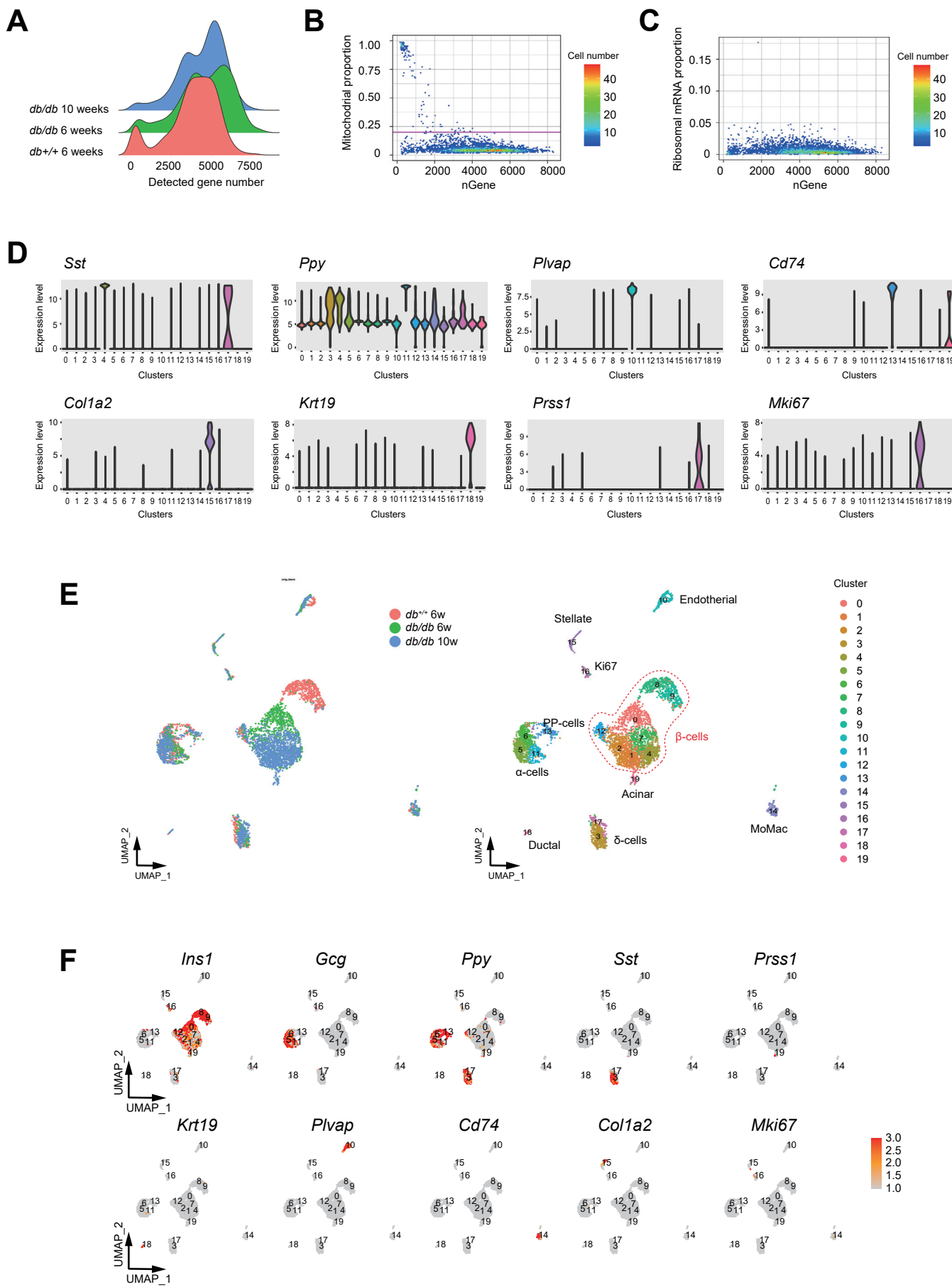
1. Zhao H, Matsuzaka T, Nakano Y, Motomura K, Tang N, Yokoo T, Okajima Y, Han SI, Takeuchi Y, Aita Y, Iwasaki H, Yatoh S, Suzuki H, Sekiya M, Yahagi N, Nakagawa Y, Sone H, Yamada N, Shimano H: Elovl6 Deficiency Improves Glycemic Control in Diabetic db/db Mice by Expanding beta-Cell Mass and Increasing Insulin Secretory Capacity. *Diabetes* 2017;66:1833-1846
2. Takeuchi Y, Yahagi N, Aita Y, Murayama Y, Sawada Y, Piao X, Toya N, Oya Y, Shikama A, Takarada A, Masuda Y, Nishi M, Kubota M, Izumida Y, Yamamoto T, Sekiya M, Matsuzaka T, Nakagawa Y, Urayama O, Kawakami Y, Iizuka Y, Gotoda T, Itaka K, Kataoka K, Nagai R, Kadowaki T, Yamada N, Lu Y, Jain MK, Shimano H: KLF15 Enables Rapid Switching between Lipogenesis and Gluconeogenesis during Fasting. *Cell Rep* 2016;16:2373-2386

Supplemental Figure 1



Supplemental Figure 1. Phenotypic characteristics of *db*^{+/+} and *db/db* mice and bulk RNA-seq analysis of pancreatic islet cells isolated from *db*^{+/+} and *db/db* mice. (A) Body weights, fasted blood glucose levels, fed plasma insulin levels, and hemoglobin A1c (HbA1c) levels of 6- and 10-week-old *db*^{+/+} and *db/db* mice used in this study. N = 3–5 per group., **P* < 0.05, *****P* < 0.0001. (B) Representative confocal images of pancreatic sections from *db*^{+/+} and *db/db* mice at indicated ages stained with antibodies against insulin (green, β-cell marker), glucagon (red, α-cell marker), and DAPI (blue, nuclei). Scale bar = 50 μm. (C) t-SNE plot of pancreatic islet cells isolated from 6- and 10-week-old *db*^{+/+} and *db/db* mice as determined by bulk RNA-seq. (D, E) Unsupervised hierarchical clustering of bulk RNA-seq data from pancreatic islet cells isolated from 6- and 10-week-old *db*^{+/+} (D) and *db/db* (E) mice. (F) Volcano plots depicting differentially expressed genes (FDR < 0.05) in 6-week-old *db*^{+/+} versus 6-week-old *db/db* islets (left), 10-week-old *db*^{+/+} versus 10-week-old *db/db* islets (left), and 6-week-old *db/db* versus 10-week-old *db/db* islets (right), as determined by bulk RNA-seq.

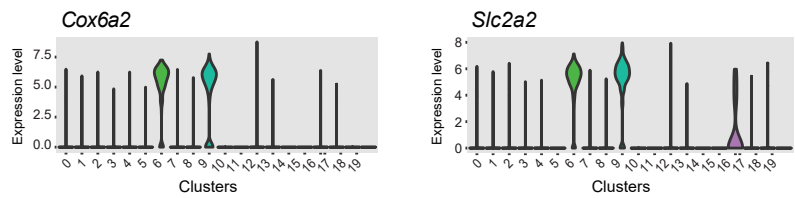
Supplemental Figure 2



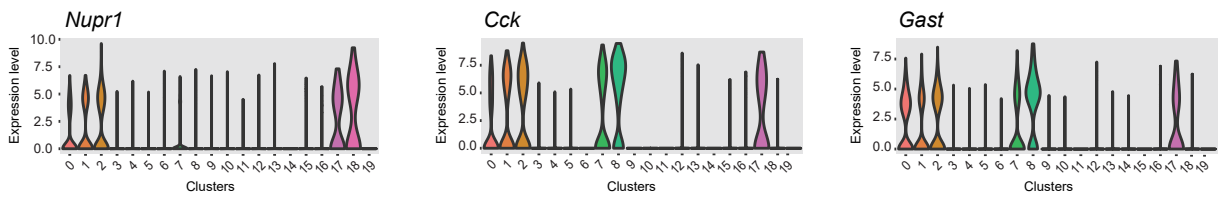
Supplemental Figure 2. scRNA-seq analysis of pancreatic islet cells isolated from *db*^{+/+} and *db/db* mice. (A) Ridgeline plot representation of the distribution of detected gene number of islet cells from 6-week-old *db*^{+/+}, 6-week-old *db/db*, and 10-week-old *db/db* mice. (B, C) Hexagonal pseudocolor plots of library quality metrics of mitochondrial gene (B) and ribosomal RNA (C) proportions. (D) Violin plots showing the expression levels of *Sst* (delta cell), *Ppy* (PP cell), *Plvap* (endothelial cell), *Cd74* (monocyte-derived macrophage), *Colla2* (pancreatic stellate cell), *Prss1* (acinar cell), *Krt19* (ductal cell), and *Mki67* (Ki67 positive cell). (E) UMAP plot of 4,956 islet cells from 6-week-old *db*^{+/+}, 6-week-old prediabetic *db/db*, and 10-week-old diabetic *db/db* mice. All cells are colored by genotype and age (left). Unsupervised clustering identified 20 clusters (right). (F) Expression levels of known markers of β -cell (*Ins1*), α -cell (*Gcg*), PP-cell (*Ppy*), δ -cell (*Sst*), acinar cell (*Prss-1*), ductal cell (*Krt19*), endothelial cell (*Plvap*), monocyte-derived macrophage (*Cd74*), pancreatic stellate cell (*Colla2*), and Ki67-positive cell (*Mki67*) in UMAP space.

Supplemental Figure 3

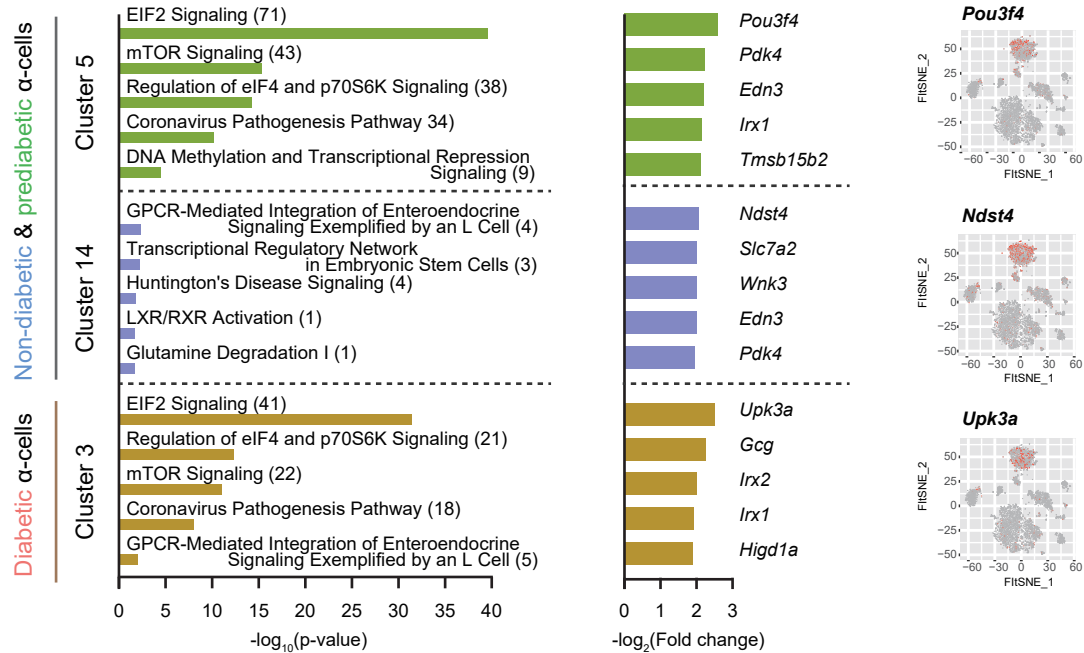
A DEGs in nondiabetic β -cell clusters



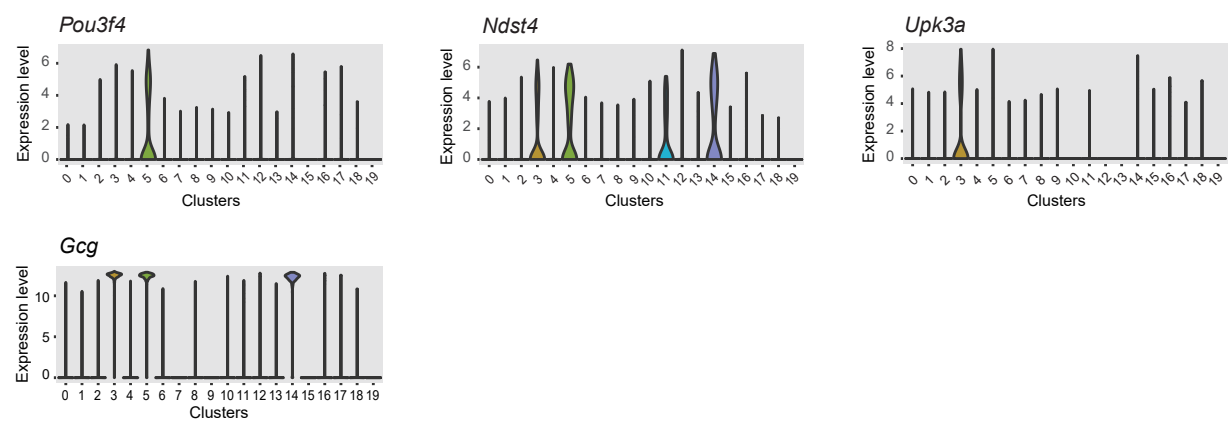
B DEGs in diabetic β -cell clusters



C

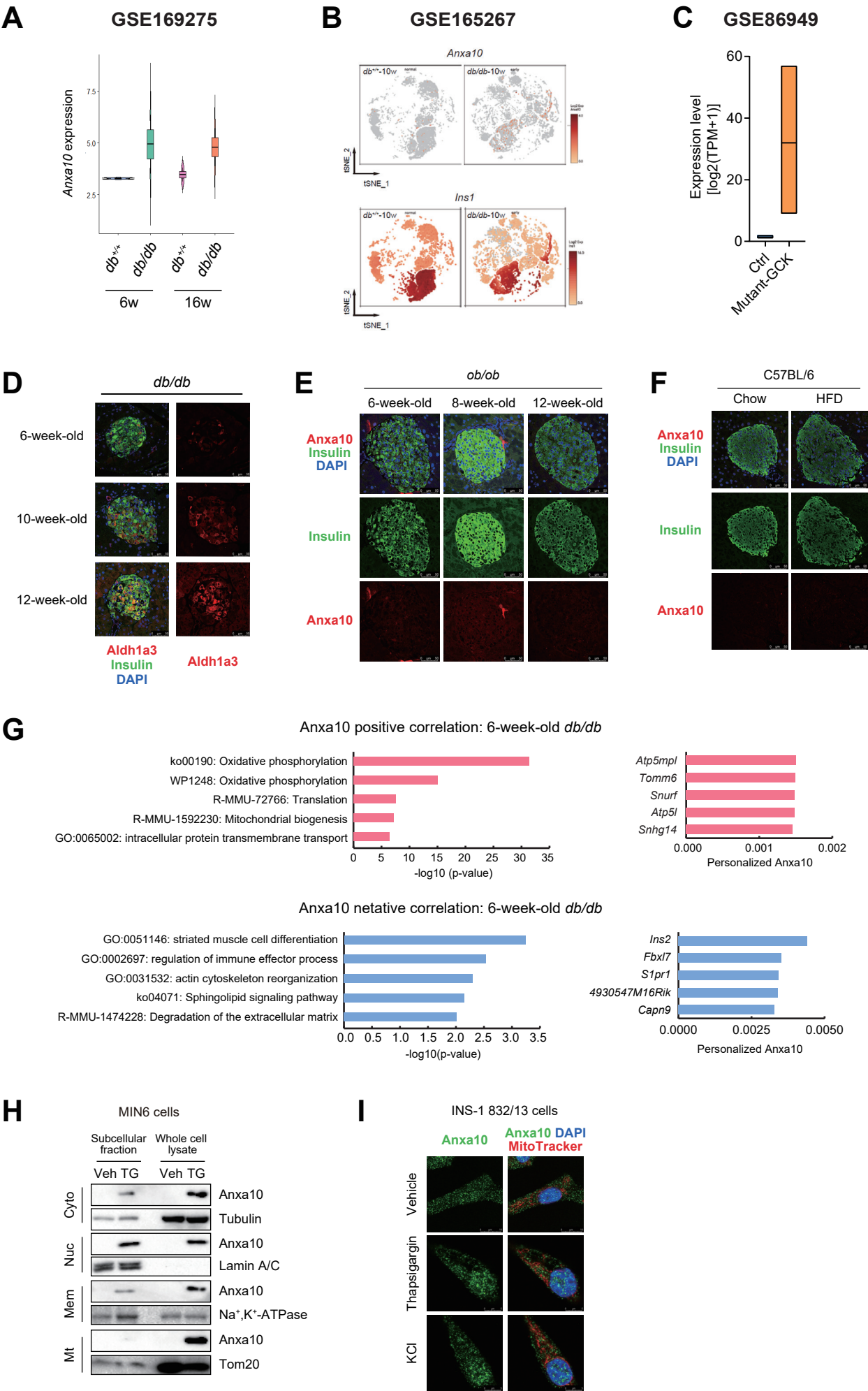


D DEGs in α -cell clusters



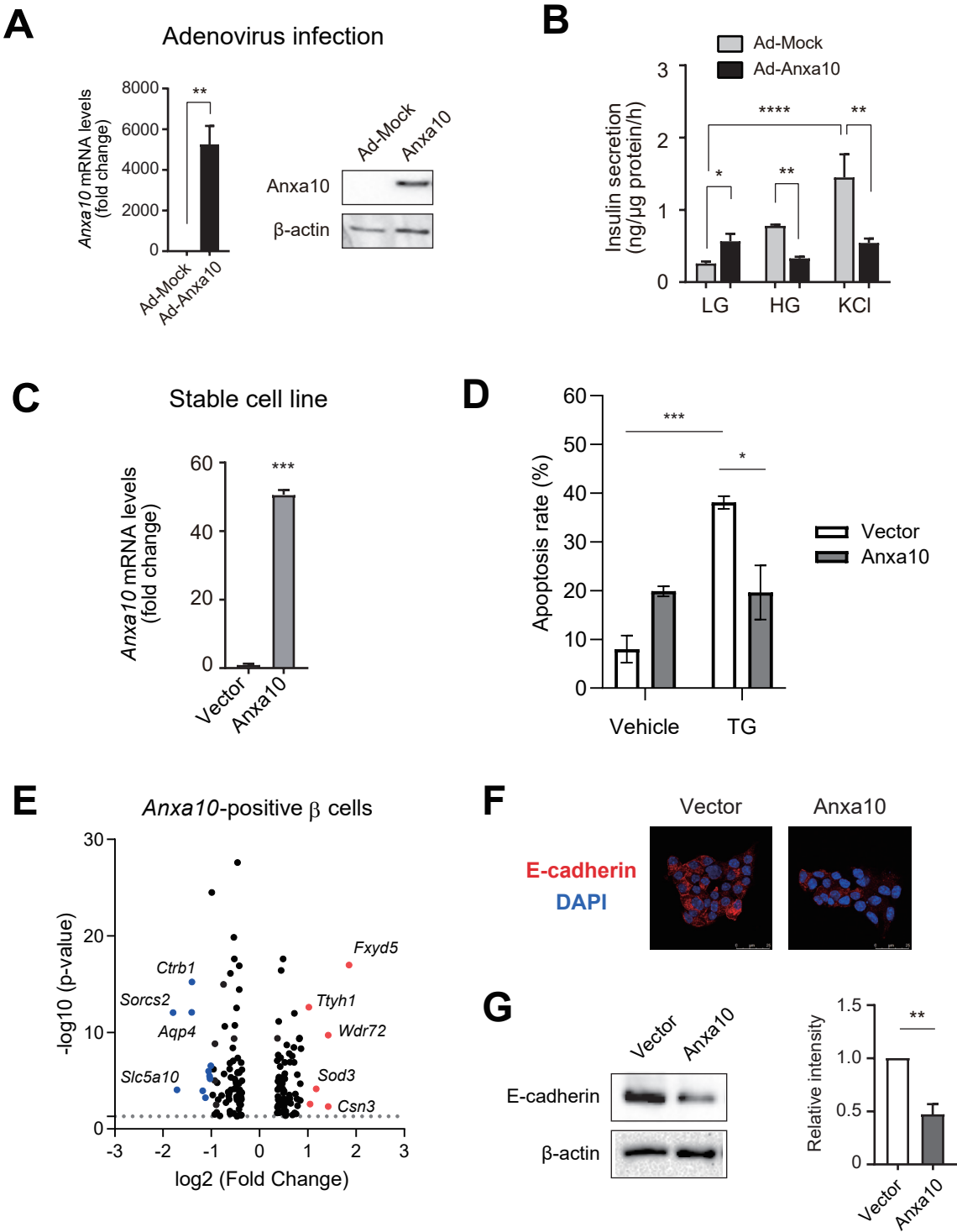
Supplemental Figure 3. Expression patterns of DEGs in pancreatic islet cells isolated from *db*^{+/+} and *db/db* mice. (A, B) Violin plots of the most upregulated genes in nondiabetic (A) and diabetic (B) β -cell clusters. (C) Ingenuity pathway analysis (IPA) of DEGs (left panel), DEGs (middle panel), and feature plots of the most upregulated genes (right panel) in α -cell clusters. The number of genes which exhibited a significantly altered expression in each IPA pathway is shown within the parenthesis. (D) Violin plots of the most upregulated genes in α -cell clusters.

Supplemental Figure 4



Supplemental Figure 4. Analyses of *Anxa10* in pancreatic islets and β cells. (A) A violin and box plot of the *Anxa10* expression (log2 TPM+1) in islets from 6- and 16-week-old *db*^{+/+} and *db/db* mice using the NCBI dataset GSE169275. (B) Feature plots of *Anxa10* and *Ins1* in islet cells from 10-week-old *db*^{+/+} and *db/db* mice using the NCBI dataset GSE165267. (C) A box plot of the *Anxa10* expression (log2 TPM+1) in islets isolated from transgenic mice expressing mutant glucokinase (Y214C) (Mutant-GCK) and control (Ctrl) mice using the NCBI dataset GSE86949. (D) Representative confocal images of pancreatic sections from *db*^{+/+} and *db/db* mice at indicated ages stained with antibodies against Aldh1a3, insulin, and DAPI. (E) Representative confocal images of pancreatic sections from *ob/ob* mice at indicated ages stained with antibodies against *Anxa10*, insulin, and DAPI. (F) Representative confocal images of pancreatic sections from C57BL/6 mice fed a chow or high-fat diet (HFD) for 12 weeks that were stained with antibodies to *Anxa10* and insulin, and DAPI. (G) Functional enrichment pathways and top 5 genes for *Anxa10*-positive correlation and *Anxa10*-negative correlation in β cells from 6-week-old *db/db* mice revealed by Gene correlation network analysis. (H) Immunoblot analysis of *Anxa10* in subcellular fractions isolated from MIN6 cells stably expressing GFP or GFP-*Anxa10*. Cells were treated with vehicle or 1- μ M thapsigargin (TG) for 24 hours. Known cytoplasmic (Cyto, α -tubulin), nuclear (Nuc, Lamin A/C), membrane (Mem, Na⁺, K⁺-ATPase), and mitochondria (Mt, Tom20) proteins were used to validate the fractions. (I) Representative confocal images of INS-1 832/13 cells stained with *Anxa10*, MitoTracker, and DAPI. Cells were treated with vehicle, 1- μ M thapsigargin, or 30-mM KCl for 24 hours. Scale bar = 10 and 5 μ m for vehicle- and thapsigargin- or KCl-treated cells, respectively.

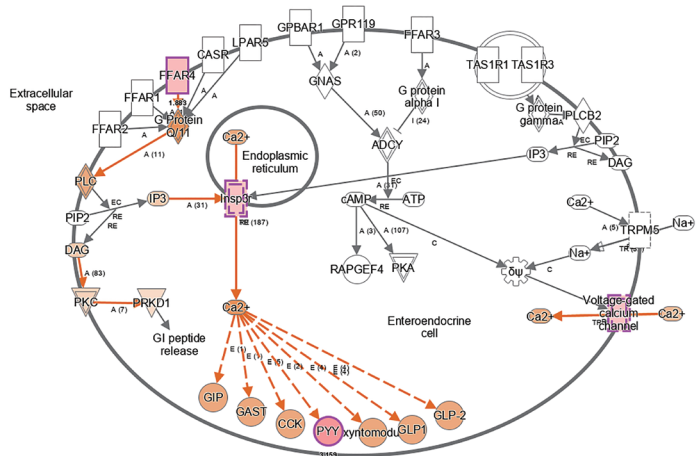
Supplemental Figure 5



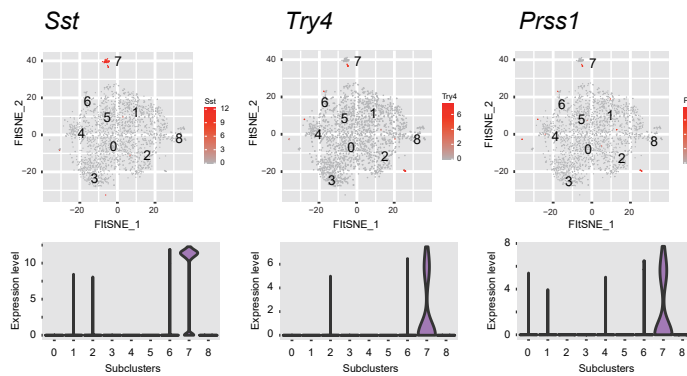
Supplemental Figure 5. Effects of Anxa10 overexpression in MIN6 cells. (A) Messenger RNA and protein levels of Anxa10 in MIN6 cells infected with full-length Anxa10 expressing adenovirus (Ad-Anxa10) or control adenovirus (Ad-Mock) for 48 hours. (B) GSIS and KSIS in MIN6 cells infected with Ad-mock or Ad-Anxa10 for 48 hours (n = 3–6). (C) Messenger RNA levels of Anxa10 in MIN6 cells stably expressing GFP or GFP-Anxa10 (n = 3). (D) The proportion of apoptosis cells in MIN6 cells stably expressing GFP or GFP-Anxa10 treated with or without 1 μ M TG for 24 hours (n = 3). (E) The volcano plot of scRNA-seq for differentially expressed transcripts in Anxa10-positive β cells. (F) Representative confocal images of MIN6 cells stably expressing GFP or GFP-Anxa10 that were stained with E-cadherin antibody and DAPI. Scale bar, 25 μ m. (G) Immunoblot analysis of E-cadherin in MIN6 cells stably expressing GFP or GFP-Anxa10. The results were quantified by densitometry (n = 3). * P < 0.05, *** P < 0.001.

Supplemental Figure 6

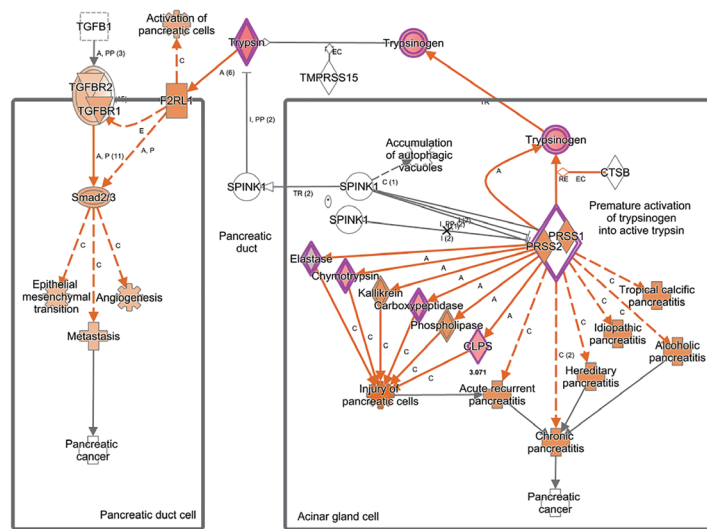
A GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells



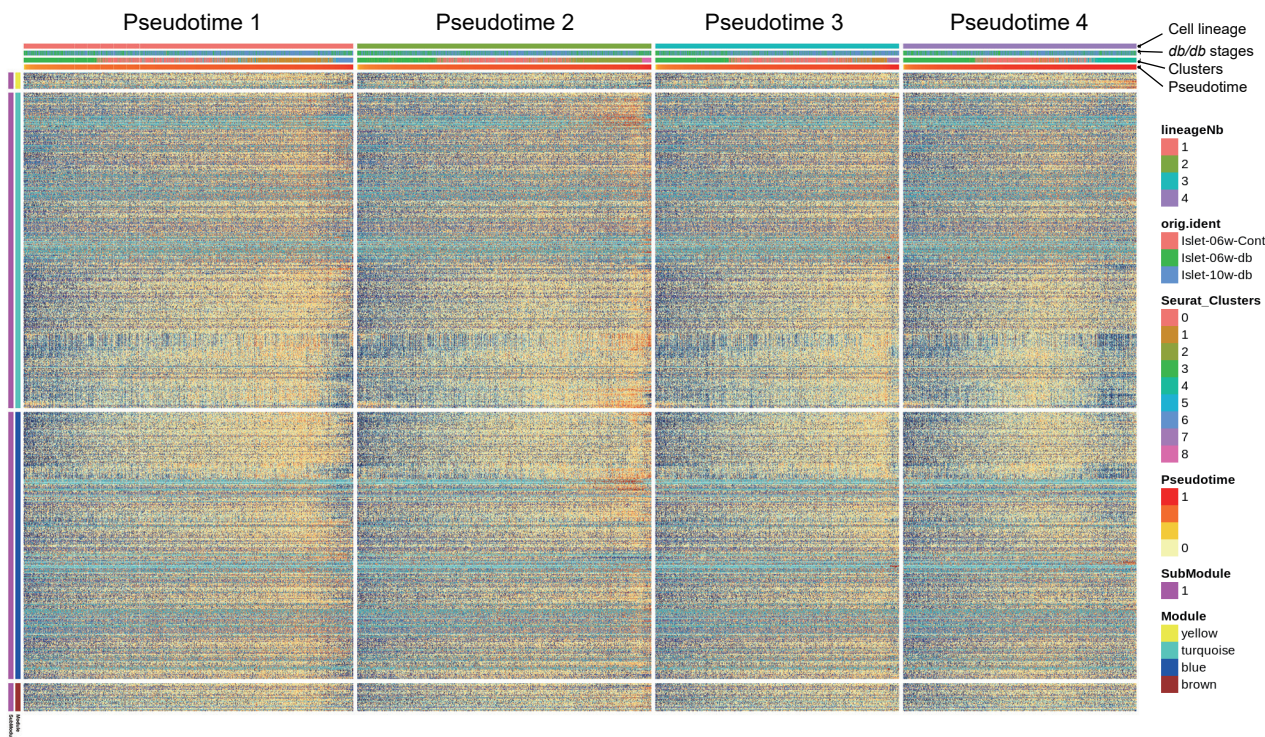
B β -cell subcluster



C SPINK1 Pancreatic Cancer Pathway of β -cell subcluster 7

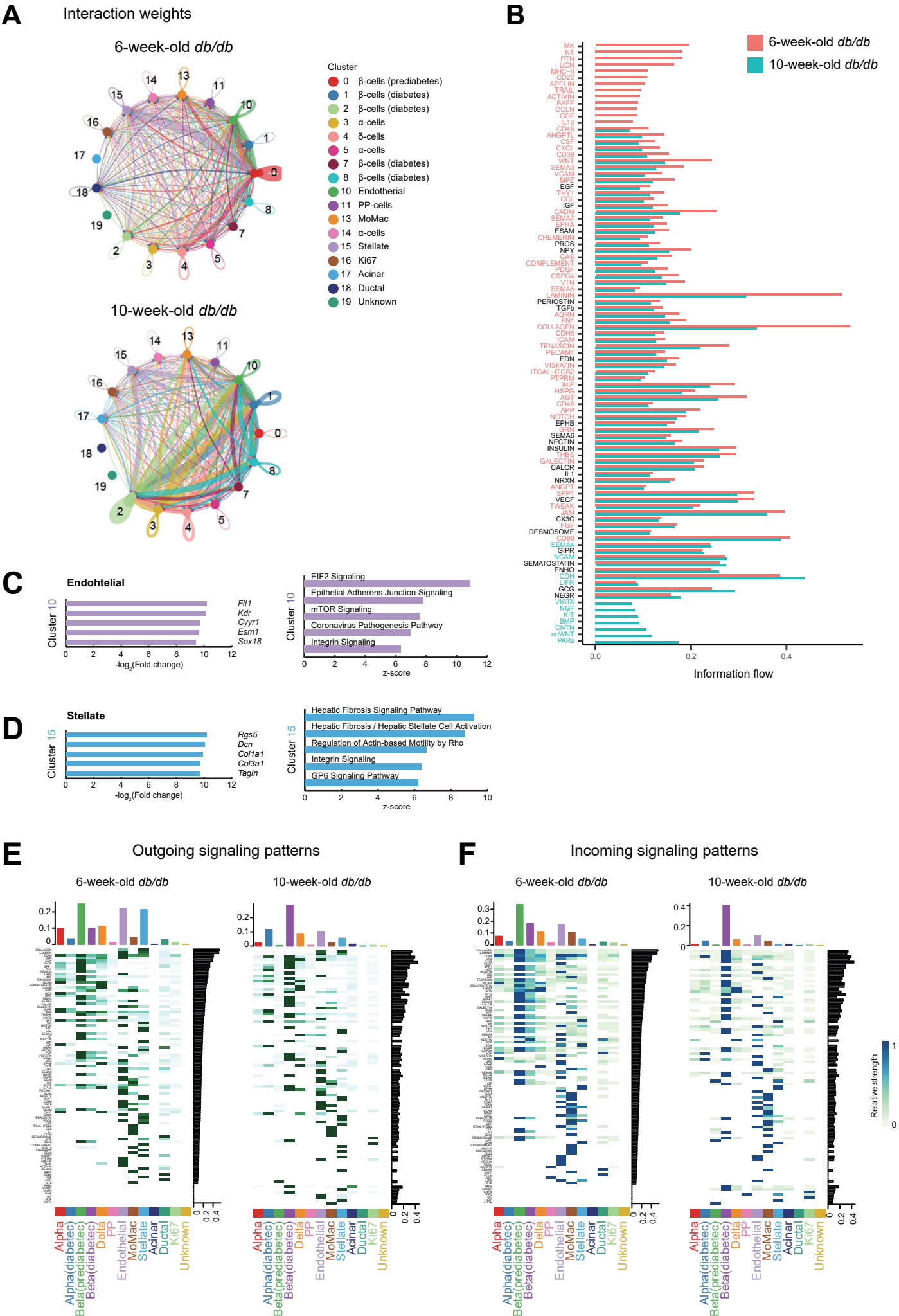


D



Supplemental Figure 6. Gene expression of acinar- and δ -cell markers during β -cell transdifferentiation. (A) Pathway diagram for GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells provided by Ingenuity Pathway Analysis. DEGs in β -cell subcluster-2 are shown in magenta. Inferred nodes and edges upregulated in β -cell subcluster-2 are depicted in orange. (B) Feature and violin plots of δ -cell (*Sst*) and acinar-cell (*Trp4*, *Prss1*) marker genes in β -cell subclusters. (C) Pathway diagram for SPINK1 Pancreatic Cancer Pathway provided by Ingenuity Pathway Analysis. DEGs in Beta-7 cluster are shown in magenta. Inferred nodes and edges upregulated in β -cell subcluster-7 are depicted in orange. (D) Heatmap showing pseudotime ordering of DEGs in *db/db* β -cell subclusters.

Supplemental Figure 7



Supplemental Figure 7. Cell–cell communications in *db/db* islets predicted by the CellChat. (A) Circle plot visualizations of all cell–cell interaction strength among individual cell types in 6- and 10-week-old *db/db* islets. Circle size of each cell type is normalized to the cell number of each subset. Arrows and edge color represent direction. The thickness of the lines connecting cells represents the interaction strength. Clusters are consistent with Figure 1. (B) Signaling pathway networks that are active in 6- and 10-week-old *db/db* islets based on the differences in the overall information flow. (C, D) Top 5 upregulated DEGs and ingenuity pathways in endothelial (C) and stellate (D) cell clusters. (E, F) The outgoing (E) and incoming (F) signaling patterns within 6- and 10-week-old *db/db* islet cells. glucagon (Gcg), endothelin (EDN), fibronectin 1 (FN1), vascular endothelial growth factor (VEGF), protease-activated receptors (PARs), chemokine ligand (CXCL), monocyte-derived macrophage (MoMac).

Supplemental Table 1. Cell number of each islet cell cluster.

Supplemental Table 2. Marker genes used for cell type annotation.

Supplemental Table 3. Differentially expressed genes of each islet cell cluster.

Supplemental Table 4. Gene list for Ingenuity Pathway Analysis of each islet cell cluster.

Supplemental Table 5. Clinical characteristics of study subjects.

Supplemental Table 6. Gene network analysis of the correlation of *Anxa10* with genes in pancreatic β cells from 6-week-old *db/db* mice.

Supplemental Table 7. Cell number of each β -cell subcluster.

Supplemental Table 8. Differentially expressed genes of each β -cell subcluster.

Supplemental Table 9. Modules and differentially expressed genes of pseudotime analysis of *db/db* β -cell subclusters.

Supplemental Table 10. Gene list for Ingenuity Pathway Analysis of each β -cell subcluster.

Supplemental Table 11. Primer sequences used for the quantitative real-time PCR analysis.