SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1: Quantification of proteins in the islets of *db/db* mice, *misty/misty* mice, DIO mice, and lean mice, and immunofluorescence staining of human samples

(A) Quantification of the immunoblots of MEK1, MEK2, pMEK1/2, AKT and pAKT in the islets of 6, 10 and 14-week-old *db/db* and *misty/misty* mice. **P* < 0.05, ***P* < 0.01, *n* = 3. All data are represented as mean ± SEM.

(B) Quantification of the immunoblots of MEK1, MEK2, pMEK1/2, AKT and pAKT in the islets of DIO mice fed on HFD for 2, 10 and 20 weeks (HFD) and age-matched control mice on NCD (NCD). **P* < 0.05, ***P* < 0.01, *n* = 3-4. All data are represented as mean ± SEM.

(C) Immunofluorescence staining with a pERK1/2-specific antibody (red), an insulin-specific antibody (green) and DAPI (blue) of pancreatic sections obtained from the subjects with or without type 2 diabetes (T2DM and non-DM)

Scale bar, 100 μm.

Figure S2, related to Figure 2: Evaluation of MEK1 and MEK2 deletion in islets and other organs obtained from *MIP-Cre/ERT*$^{+}$*/Mek1*$^{f/f}$ mice (Tmx+), *Mek2*$^{ko/ko}$ mice, β*Mek1/2*DKO mice and their controls, and ITTs on DIO β*Mek1/2*DKO mice

(A) Immunoblots of proteins of interest in the islets of the 11- to 12-week-old *MIP-Cre/ERT*$^{+}$*/Mek1*$^{f/f}$ and *MIP-Cre/ERT*$^{-}$*/Mek1*$^{f/f}$ mice with Tmx injection

(B) Immunoblots of proteins of interest in the islets of the 12-week-old *Mek2*$^{ko/ko}$ and *Mek2*$^{wt/wt}$ mice

(C) Quantification of the immunoblots of ERK1/2 and pERK1/2 in the islets of 14-week-old β*Mek1/2*DKO and Tmx^{-} control mice. **P* < 0.05, *n* = 3. All data are represented as mean ± SEM.

(D) Immunoblots of MEK1 in hypothalami, livers, epididymal white adipose tissues (eWATs) and muscles from β*Mek1/2*DKO and Tmx^{-} control mice

(E-G) Blood glucose levels after intraperitoneal injection of insulin in β*Mek1/2*DKO and Tmx^{-} control mice fed on HFD for 6 weeks (1.0 U/kg-BW) (E), 12 weeks (1.5 U/kg-BW) (F), and 18 weeks (1.5 U/kg-BW) (G). *n* = 7-10. All data are represented as mean ± SEM.

Figure S3, related to Figure 2: The metabolic features of *Mek2*$^{ko/ko}$ mice fed on HFD

(A - C) Profiles of *Mek2*$^{ko/ko}$, *Mek2*$^{wt/ko}$ and *Mek2*$^{wt/wt}$ mice fed on HFD: body weight (A), blood glucose levels (B), and plasma insulin concentrations (C). *n* = 12-17. All data are represented as mean ± SEM.

(D and E) Blood glucose levels (D) and area under the blood concentration time curves (AUC) (E) after intraperitoneal injection of glucose (1.0 g/kg-BW) in *Mek2*$^{ko/ko}$, *Mek2*$^{wt/ko}$ and *Mek2*$^{wt/wt}$ mice fed on HFD for 16 weeks. *n* = 6-7. All data are represented as mean ± SEM.

(F and G) Plasma insulin concentrations (F) and area under the plasma concentration

time curves (AUC) (G) after intraperitoneal injection of glucose (1.0 g/kg-BW) in *Mek2*$^{ko/ko}$, *Mek2*$^{wt/ko}$ and *Mek2*$^{wt/wt}$ mice fed on HFD for 16 weeks. *n* = 6-7. All data are represented as mean ± SEM.

(H and I) Blood glucose levels (H) and area under the blood concentration time curves (AUC) (I) after intraperitoneal injection of insulin (1.5 U/kg-BW) in *Mek2*$^{ko/ko}$, *Mek2*$^{wt/ko}$ and *Mek2*$^{wt/wt}$ mice fed on HFD for 16-18 weeks. *n* = 12-17. All data are represented as mean ± SEM.

Figure S4, related to Figure 2: Assessment of the effects of Tmx injection on DIO mice

(A and B) Profiles of *Mek1*$^{f/f}$*Mek2*$^{ko/ko}$ mice with or without Tmx injection fed on HFD: body weight (A) and blood glucose levels (B). *n* = 9-11. All data are represented as mean ± SEM.

(C-E) Blood glucose levels after intraperitoneal injection of glucose (1.0 g/kg-BW) in *Mek1*$^{f/f}$*Mek2*$^{ko/ko}$ mice fed on HFD for 6 weeks (C), 12 weeks (D) and 19 weeks (E) with or without Tmx injection. *n* = 9-11. All data are represented as mean ± SEM.

(F-H) Blood glucose levels after intraperitoneal injection of insulin in *Mek1*$^{f/f}$*Mek2*$^{ko/ko}$ mice with or without Tmx injection fed on HFD for 6 weeks (1.0 U/kg-BW) (F), 12 weeks (1.5 U/kg-BW) (G) and 18 weeks (1.5 U/kg-BW) (H). *n* = 9-11. All data are represented as mean ± SEM.

(I) Plasma insulin concentrations after intraperitoneal injection of glucose (3.0 g/kg-BW) in *Mek1*$^{f/f}$*Mek2*$^{ko/ko}$ mice fed on HFD for 22 weeks with or without Tmx

injection. *n* = 9-11. All data are represented as mean ± SEM.

Figure S5, related to Figure 3: Immunohistochemical staining of E14.5 mouse pancreatic sections, ratio of Gcg$^+$ area to insulin$^+$ area in β*Mek1/2*DKO islets, and gene-expression analyses of *Gcg* and *Arx* in the islets from β*Mek1/2*DKO mice

(A) Immunohistochemical staining of E14.5 mouse pancreatic sections with a Sox9-specific antibody (red), an Ngn3-specific antibody (green) and DAPI (blue). Scale bar, 10 μm

(B) Immunohistochemical staining with an insulin-specific antibody (green), Gcg-specific antibody (red) and DAPI (blue) (Left), quantified ratios of Gcg$^+$ area to insulin$^+$ area (Middle) and quantified proportions of Gcg$^+$ area in whole pancreas (Right) assessed in the pancreatic sections from β*Mek1/2*DKO and Tmx$^-$ control mice fed on HFD for 18 weeks. The proportion of Gcg$^+$ area in whole pancreas was calculated from the ratio of Gcg$^+$ to insulin$^+$ area and the proportion of insulin$^+$ area in whole pancreas. **P* < 0.05, *n* = 7; Scale bar, 50 μm. All data are represented as mean ± SEM.

(C) Levels of expression of *Gcg* and *Arx* in the islets from β*Mek1/2*DKO and Tmx$^-$ control mice fed on NCD (NCD) or HFD for indicated periods. **P* < 0.05, *n* = 4-9. All data are represented as mean ± SEM.

Figure S6, related to Figure 4 and 5: Insulin contents of isolated islets from β*Mek1/2*DKO mice and Tmx$^-$ control mice fed on HFD or NCD

(A) Insulin contents per islet isolated from β*Mek1/2*DKO and Tmx$^-$ control mice fed on HFD for 24-25 weeks. After incubating in recovery buffer over night, we pooled 6 isolated islets and maintained them in KRB buffer with 0.2% BSA supplemented with 2.8 mM glucose for 1.5 hours at 37°C. Subsequently, insulin was extracted from the islets by sonication and subsequent incubation in acid ethanol at 4℃. Insulin contents were measured using ELISA kits (Morinaga).

n = 5 (the quadruple of 6 pooled islets was counted as *n* = 1). All data are represented as mean ± SEM.

(B) DNA contents per islet isolated from β*Mek1/2*DKO and Tmx$^-$ control mice fed on HFD for 24-25 weeks. After incubating in recovery buffer over night, we pooled 6 isolated islets, washed them, and DNAs were extracted from the pooled islets using Tissue Genomic DNA Extraction Mini Kit (Chiyoda Science).

n = 5 (the quadruple of 6 pooled islets was counted as *n* = 1). All data are represented as mean ± SEM.

(C) Insulin contents normalized by DNA contents of islet isolated from β*Mek1/2*DKO and Tmx$^-$ control mice fed on HFD for 24-25 weeks.

n = 5. All data are represented as mean ± SEM.

(D) Insulin contents per islet isolated from 14- to 15-week-old β*Mek1/2*DKO and Tmx-control mice fed on NCD

n = 6-7 (the triplicate of 6 pooled islets was counted as *n* = 1). All data are represented as mean ± SEM.

(E) DNA contents per islet isolated from 14- to 15-week-old β*Mek1/2*DKO and Tmx-

control mice fed on NCD

n = 6-7 (the triplicate of 6 pooled islets was counted as *n* = 1). All data are represented as mean ± SEM.

(F) Insulin contents normalized by DNA contents of islet isolated from 14- to 15-week-old β*Mek1/2*DKO and Tmx- control mice fed on NCD

n = 6-7. All data are represented as mean ± SEM.

Figure S7, related to Figure 5: Metabolic and histological analyses on β*Mek1/2*DKO mice and *Mek2*$^{ko/ko}$ mice fed on NCD, and assessment of the effects of Tmx injection on NCD mice

(A) Blood glucose levels after intraperitoneal injection of insulin (1.0 U/kg-BW) in 11-week-old β*Mek1/2*DKO and Tmx$^-$ control mice fed on NCD. *n* = 6-7. All data are represented as mean ± SEM.

(B) Plasma human insulin levels after intraperitoneal injection of insulin (1.0 U/kg-BW) in 11-week-old β*Mek1/2*DKO and Tmx$^-$ control mice fed on NCD. *n* = 8-9. All data are represented as mean ± SEM.

(C) Proportions of islets in whole pancreas assessed in the pancreatic sections from β*Mek1/2*DKO and Tmx$^-$ control mice fed on NCD. Four whole pancreatic sections per animal (400 μm apart) stained with HE or aldehyde fuchsin were used for the analyses. **P* < 0.05, *n* = 4-9. All data are represented as mean ± SEM.

(D) Number of islets with a cross-sectional area of interest in the pancreas sections from 23-week-old β*Mek1/2*DKO and Tmx$^-$ control mice fed on NCD. **P* < 0.05, *n* = 4. All

data are represented as mean ± SEM.

(E - G) Profiles of 11-week-old *Mek2*$^{ko/ko}$ and *Mek2*$^{wt/wt}$ mice fed on NCD: body weight (E), blood glucose levels (F) and plasma insulin levels (G). *n* = 8. All data are represented as mean ± SEM.

(H and I) Blood glucose levels (H) and area under the blood concentration time curves (AUC) (I) after intraperitoneal injection of insulin (1.0 U/kg-BW) in the 11-week-old *Mek2*$^{ko/ko}$ and *Mek2*$^{wt/wt}$ mice fed on NCD. *n* = 6-8. All data are represented as mean ± SEM.

(J) Plasma insulin concentrations after intraperitoneal injection of glucose (3.0 g/kg-BW) in the 12-week-old *Mek2*$^{ko/ko}$ and *Mek2*$^{wt/wt}$ mice on NCD. *n* = 8. All data are represented as mean ± SEM.

(K and L) Blood glucose levels (K) and area under the blood concentration time curves (AUC) (L) after intraperitoneal injection of glucose (2.0 g/kg-BW) in the 18-week-old *Mek2*$^{ko/ko}$ and *Mek2*$^{wt/wt}$ mice fed on NCD. *n* = 7-8. All data are represented as mean ± SEM.

(M and N) Plasma insulin concentrations (M) and area under the plasma concentration time curves (AUC) (N) after intraperitoneal injection of glucose (2.0 g/kg-BW) in the 18-week-old *Mek2*$^{ko/ko}$ and *Mek2*$^{wt/wt}$ mice on NCD. *n* = 7-8. All data are represented as mean ± SEM.

(O) Plasma insulin concentrations after intraperitoneal injection of glucose (3.0 g/kg-BW) in the 11- to 12-week-old *Mek1*$^{f/f}$*Mek2*$^{ko/ko}$ mice fed on NCD with or without Tmx injection. *n* = 9. All data are represented as mean ± SEM.

Figure S8 related to Figure 6: Gene-expression analyses on exocytotic molecules in β*Mek1/2*DKO islets and GO analyses of the molecules with significantly less-phosphorylated site(s) in β*Mek1/2*DKO islets

(A) Levels of expression of *Snap25, Pclo, Itpr3, Rab37*, *Sytl4, Rims2, Stx1a*, *Vamp2* and *Rab27a* in the islets from β*Mek1/2*DKO and Tmx^- control mice fed on NCD. *n* =7-9. All data are represented as mean ± SEM.

(B) The results of GO analyses of the 190 molecules harboring the 241 sites which were phosphorylated at significantly lower levels in β*Mek1/2*DKO mouse islets than in the Tmx^- control mouse islets (*P* value < 0.05, fold change < -1.5). Top 10 GO terms in the GOTERM_CC_DIRECT and UP_KEYWORD categories were shown with raw *P* values. CC stands for cellular component.

Figure S9 related to Figure 7: The GO analyses based on phosphoproteomic data of isolated islets and Mincl4 cells

(A) The results of GO analyses of the 282 molecules harboring the 373 sites which were phosphorylated at significantly higher levels in HG condition than in LG condition (*P* value < 0.05, fold change > 1.5). Top 10 GO terms in the GOTERM_CC_DIRECT and UP_KEYWORD categories were shown with raw *P* values.

(B) The results of GO analyses of the 112 molecules harboring the 155 phosphosites with significantly higher phosphorylation levels in HG condition compared to both in LG and HGi conditions (*P* value < 0.05). Top 10 GO terms in the

GOTERM_CC_DIRECT and UP_KEYWORD categories were shown with raw *P* values.