Supporting Information

IER3IP1 is critical for maintaining glucose homeostasis through regulating the endoplasmic reticulum function and survival of β cells

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Supplemental Materials and Methods

Oral or Intraperitoneal Glucose Tolerance Test. Oral or intraperitoneal glucose tolerance tests (OGTTs or IPGTT) were performed by orally/intraperitoneally giving 2g glucose/kg body weight to mice after fasting for 5 h. Glucose measure results higher than 33.3 mmol/L were recorded as 33.3 mmol/L. Serum insulin levels were determined with insulin ELISA kits (EZassay, China) according to the manufacturer's protocol. All OGTT/IPGTT experiments were performed on age- and sex-matched cohorts.

Islet Isolation and Insulin Secretion Assay. Islet isolation was performed as previous described (1). For glucose-stimulated Insulin secretion (GSIS) experiments, after recovered overnight, islets were incubated for 1 h in HEPES - balanced Krebs-Ringer bicarbonate buffer (KRBH) (135 mmol/L NaCl, 3.6 mmol/L KCl, 0.5 mmol/L $MgSO_4 \cdot 7\ H_2O$, 0.5 mmol/L NaH_2PO_4, 2 mmol/L $NaHCO_3$, 10 mmol/L HEPES, 1.5 mmol/L $CaCl_2$, 0.1% BSA) without glucose. Thereafter, the islets were incubated in KRBH containing low glucose (2.8 mmol/L) for 2 h followed by a stimulatory glucose (16.7 mmol/L) for additional 2 h. At the end of the stimulation, supernatants were collected and the islets were homogenized in acid alcohol. Secreted insulin and insulin content in the islets were measured using ELISA kits (EZassay, China). The secretion efficiency was calculated with secreted insulin in the media normalized to islet insulin content.

Immunofluorescence and Immunohistochemical Staining. Paraffin-embedded human (The information of donors was shown in ***SI Appendix,*** **Table S2**) and mouse pancreases were cut into 5 μm sections. Immunohistochemical/immunofluorescent staining were performed using appropriate antibodies as previously described (1-2). The antibodies used were as follows: anti-IER3IP1 (homemade), anti-IER3IP1 (Cat No. ab181247, Abcam, USA), anti-proinsulin/CCI-17 (Cat No. NB100-73013, Novus,

USA), anti-insulin (homemade), anti-glucagon (Cat No. G2654, Sigma, USA), anti-somatostatin (Cat No. ab30788, Abcam, USA), anti-Ki67 (Cat No. 9129, CST, USA), anti-Nkx6.1 (Cat No. NBP1-82553, Novus, USA) and anti-Pdx-1 (Cat No. 5679T, CST, USA). Fluorescent images were visualized using Axio Imager M2 (Carl Zeiss, Oberkochen, Germany). Quantification of percentages of insulin positive cells in pancreas: the areas of insulin positive cells and the corresponding fields of pancreases were quantified, and the percentages of insulin positive cell areas in pancreases were calculated (mouse numbers per group, section numbers per pancreas, and islet numbers per section were indicated in the corresponding figure legends).

RNA Extraction, cDNA Synthesis, and Real-Time Quantitative PCR. Total RNA of islets was extracted using TRIzol reagent (Invitrogen, USA) and cDNA was synthesized using a PrimeScript^{TM}RT reagent Kit with gDNA Eraser (Takara, Japan, Cat No. RR047A). Real-time PCR was conducted with the TB Green Premix Ex Taq^{TM} (Tli RNaseH Plus) (Takara, Japan, Cat No. RR420A). GAPDH was used as internal controls. Primers were designed and chemically synthesized by TSINGKE Biological Technology (Beijing, China) Co. Ltd. The sequence information of the primers used in this study were shown in ***SI Appendix,* Table S3**.

Protein Extraction and Western Blot Analysis. Isolated islets were lysed in RIPA buffer. Equal amounts of protein samples were loaded on 4-12% NuPage gradient gels (Thermo Fisher Scientific), transferred to nitrocellulose membrane (BioTrace NT nitrocellulose membrane, 66485, PALL, Mexico), and blotted with appropriated first antibodies followed by secondary antibodies conjugated with horseradish peroxidases (HRP). The antibodies used were as follows: anti-proinsulin/CCI-17 (Cat No. NB100-73013, Novus, USA), anti-insulin (homemade), anti-β-Tubulin (Cat No. KM9003, Sungene, China), anti-PDI (Cat No. ab2792, Abcam, USA), anti-ERO1α (Cat No. 31231332, Saierbio, China), anti-P58IPK (Cat No. ab70840, Abcam, USA), anti-BiP (Cat No. sc-376768, Santa Cruz, USA), anti-peIF2α (Cat No. 9721S, CST,

USA), anti-eIF2α (Cat No. 9722S, CST, USA), anti-pIRE1α (Cat No. AP0878, ABclonal, China), anti-IRE1α (Cat No. 3294S, CST, USA), anti-CHOP (Cat No. A0221, ABclonal, China), and anti-flag (Cat No. F1804, Sigma, USA). Polyclonal antibody against IER3IP1 was raised using a peptide consists of WGTDQGIGGFGEEPG corresponding to the residues 33-47 of mouse IER3IP1 (conserved in human IER3IP1). The antibody was validated by western blot using 293T cells transfected with the plasmids encoding IER3IP1 and by immunofluorescent staining of IER3IP1-βKO mouse pancreatic sections (***SI Appendix,* Fig. S1**).

Procedure of TEM (Transmission Electron Microscopy). Mouse pancreas were perfused in fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.15 M sodium cacodylate buffer, pH 7.4), cut into small size of 1 mm^3, and fixed at 4 ℃ for 4 h. Tissues were dehydrated, embedded in epoxy resin and polymerized. The resin blocks were cut into 60-80 nm thin on the ultra-microtome, and the tissues were fished out onto the 150 meshes cuprum grids with formvar film. 2% uranium acetate saturated alcohol solution avoid light staining for 8 min, rinsed in 70% ethanol for 3 times and then rinsed in ultra-pure water for 3 times. 2.6% lead citrate avoid CO_2 staining for 8 min. After dried by the filer paper, the cuprum grids were put into the grids board and dried overnight at room temperature. The cuprum grids are observed and images were captured under HITACHI TEM (HT7800).

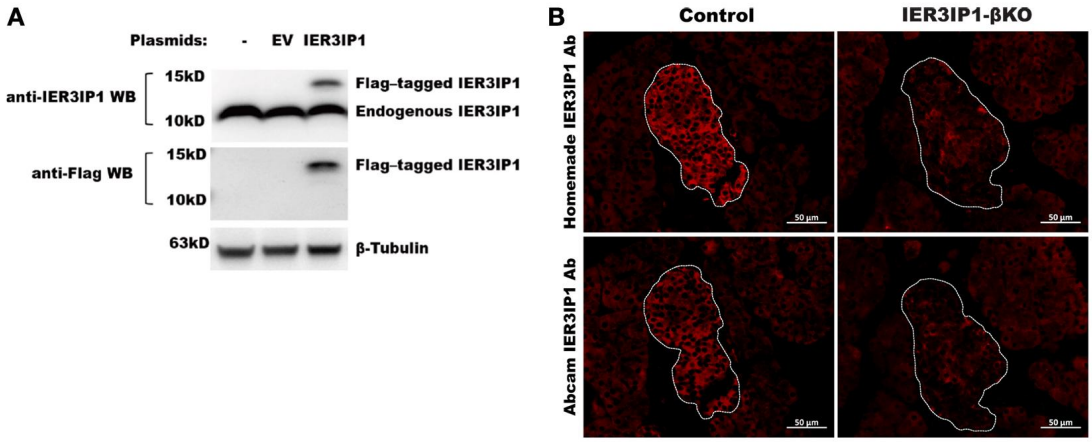
Statistics. All experiments described above were performed independently at least three times. All data are presented as the means ± SEM. Graph-Pad Prism 8 software was used for all statistical analyses. We analyzed data using unpaired *Student's t test* and used the traditional threshold $P < 0.05$ to declare statistical significance.

References

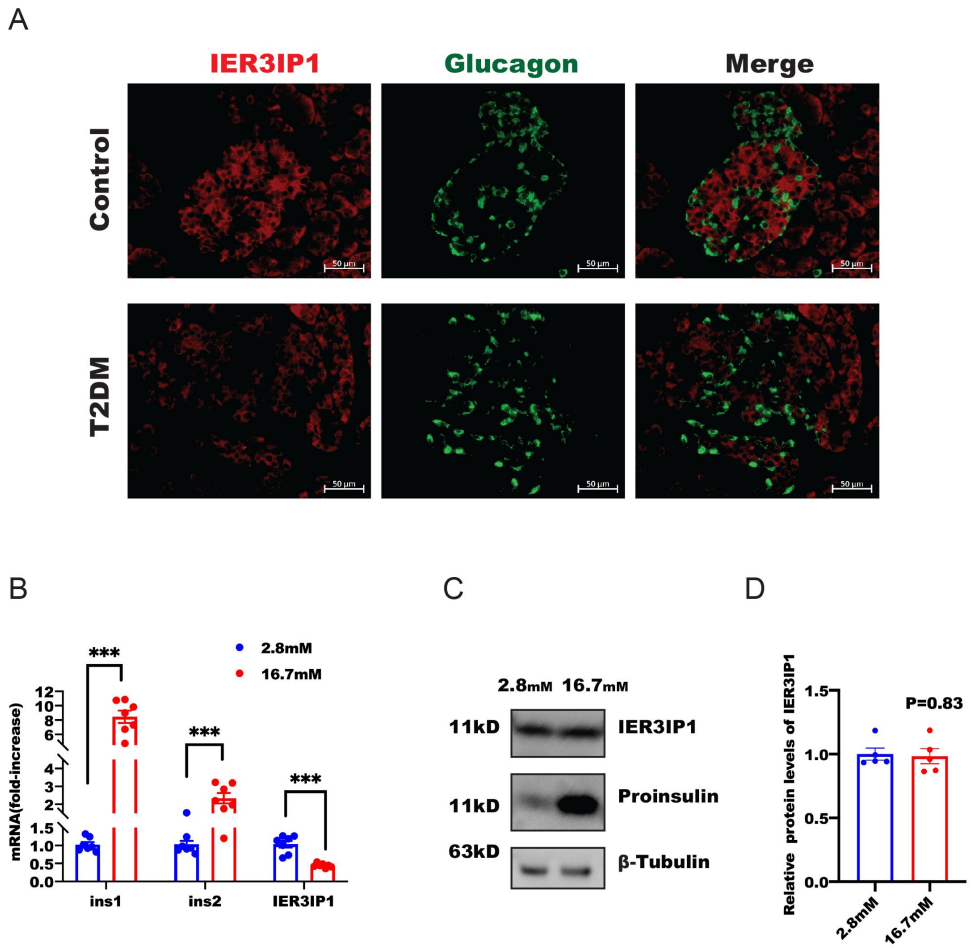
1. Qiao J *et al.*, A distinct role of STING in regulating glucose homeostasis through insulin sensitivity and insulin secretion. *Proc Natl Acad Sci U S A*. 2022;119(7):e2101848119.

2. Huang Y *et al.*, Defective insulin maturation in patients with type 2 diabetes. *Eur J Endocrinol*. 2021;185(4):565-76.

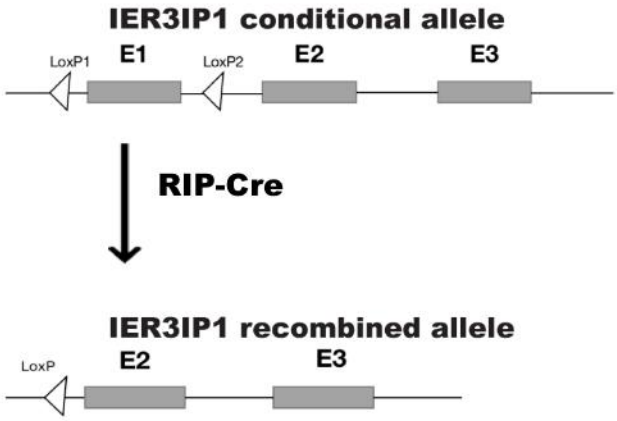
Supplemental Figures



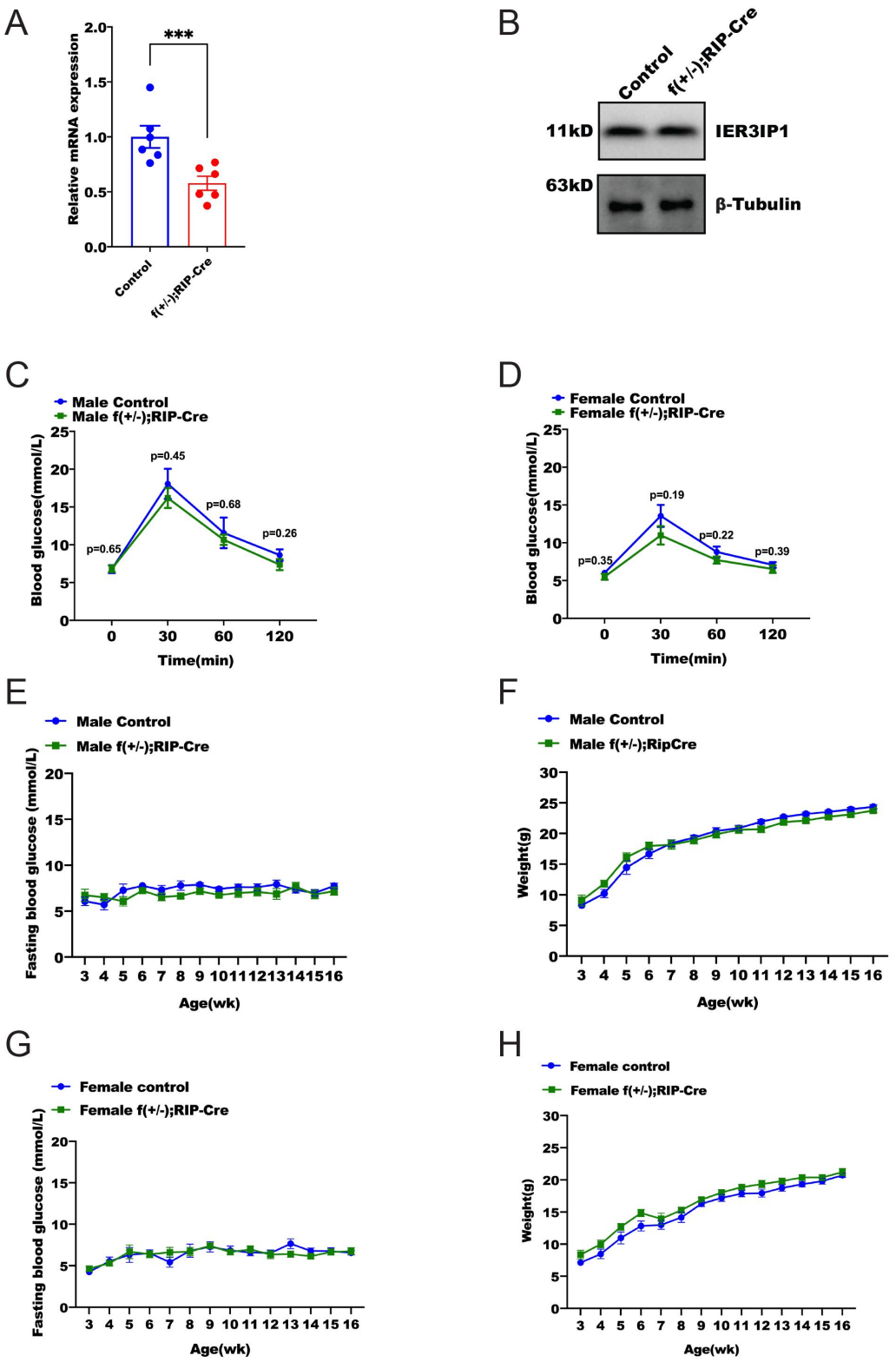
Supplemental Figure S1. Validation of homemade rabbit polyclonal antibody against mouse IER3IP1. (**A)** The 293T cells were transiently transfected with empty vector (EV) or plasmids encoding Flag-tagged wild-type IER3IP1. After 48 h post-transfection, the cells were lysed and subjected to western blot using homemade rabbit polyclonal anti-IER3IP1 antibody (*upper panel*) and anti-flag antibody (*bottom panel*). (**B)** Two serial pancreatic sections of control and IER3IP1-βKO mice were immunostaining with homemade anti-IER3IP1 (*upper panel*) and anti-IER3IP1 from Abcam (*bottom panel*). The areas of islets were circled.



Supplemental Figure S2. IER3IP1 expression in donors with T2D and its response to different glucose treatment in mouse islets. (A) The expression of IER3IP1 in T2DM pancreases were detected via the immunostaining with anti-IER3IP1 (red) with anti-glucagon (green). **(B)** The islets of 4-8 wk old mice were incubated in the media with either 2.8 mM or 16.7 mM glucose for 24 h. The expression of mRNA of *Ins1*, *Ins2* and *IER3IP1* in the treated islets was determined by qRT-PCR. The mRNA levels in islets incubated with 2.8 mM glucose were set as 1 (n = 7 in each group). (**C**) The protein expression of IER3IP1 and proinsulin from wild-type islets treated with 2.8 mM and 16.7 mM for 12 h was examined by western blots. (n = 5 in each group). (**D)** Quantification of IER3IP1 protein levels in (**C**). Values were shown as mean ± SEM. ***$P < 0.001$.

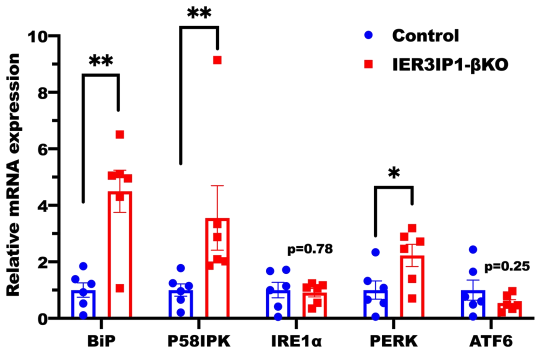


Supplemental Figure S3. Schematic diagram showing IER3IP1 gene deletion by RIP-Cre.

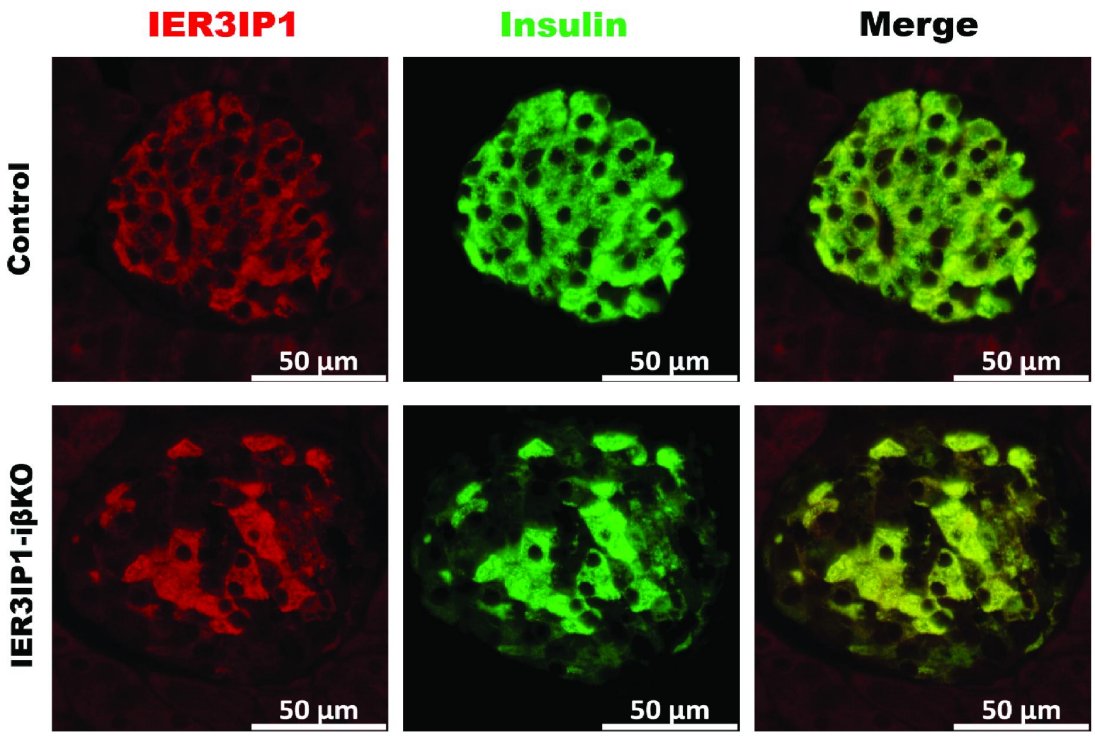


Supplemental Figure S4. Heterozygous deletion of IER3IP1 does not affect blood glucose and body weight. (A) mRNA levels of IER3IP1 in heterozygous mice flox$^{+/-}$;

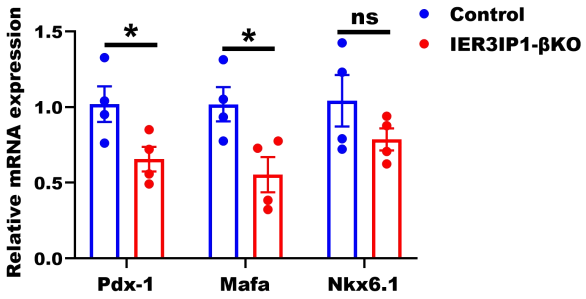
RIP-Cre and control mice ($flox^{+/+}$). (**B)** Western blot detected protein levels of IER3IP1 in heterozygous and control mouse islets. (**C-D)** OGTTs of 3-wk-old (**C**) male (n = 7) or (**D**) female (n = 10) mice. (**E)** Fasting blood glucose changes in male mice (control n = 13, heterozygous n = 10). (**F)** Body weight of male mice (control n = 13, heterozygous n = 10). (**G)** Fasting blood glucose in female mice (control n = 10, heterozygous n = 7). (**H)** Body weight in female (control n = 10, heterozygous n = 7). Values were shown as mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



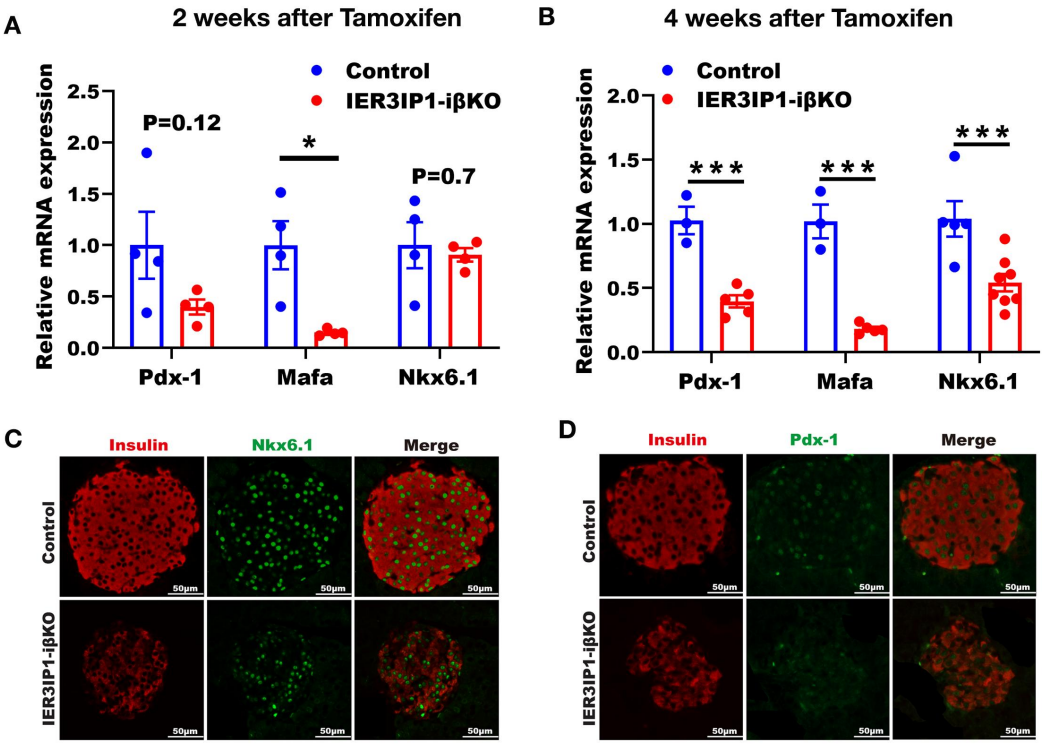
Supplemental Figure S5. IER3IP1-βKO induces ER stress. The expression of ER stress markers (BiP and P58IPK) and genes involved in UPR were examined by real-time PCR in isolated islets from 14-16 wk old diabetic IER3IP1-βKO mice and control mice. n = 6, Values were shown as mean ± SEM. *$P < 0.05$, **$P < 0.01$.



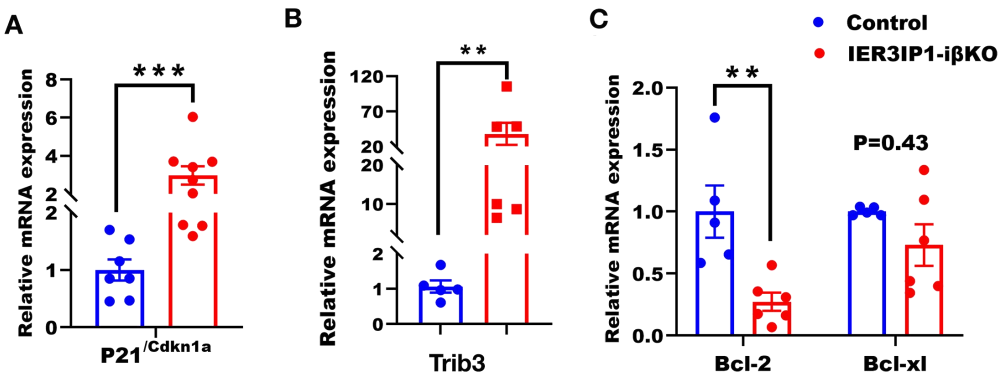
Supplemental Figure S6. Inducible deletion of IER3IP1 decreases insulin content in IER3IP1-iβKO mice islets. Representative immunofluorescence images of anti-IER3IP1 (red) and anti-insulin (green) in pancreatic sections from IER3IP1-iβKO (IER3IP1 flox$^{+/+}$;MIP-CreERT) and control (IER3IP1 flox$^{+/+}$) mice after 4 wk of tamoxifen administration at 8 wk of age.



Supplemental Figure S7. IER3IP1-βKO results in decreased expression of β-cell transcription factors. Islets from 2-wk-old control and IER3IP1-βKO mice were used to examine the expression of β-cell key transcription factors, including Pdx-1, Mafa, and Nkx6.1. The mRNA expression of these genes in control mice were set as "1". *$P < 0.05$.



Supplemental Figure S8. Inducible IER3IP1 knockdown decreases expression of β-cell transcription factors. (A-B) Isolated islets from 2-wk-old (**A**) and 4-wk-old (**B**) Male control and IER3IP1-βKO mice were used to examined mRNA expression of key transcription factors of β cells. (**C-D)** The expression and localization of Nkx6.1 and Pdx-1 were examined by immunostaining after 4 wk of tamoxifen injection in male control and IER3IP1-iβKO mice. n = 4-6, **P* < 0.05, and ****P* < 0.001.



Supplemental Figure S9. Inducible IER3IP1 knockdown increases the expression of P21 and Trib3, but decreases the expression of Bcl-2. (**A-C**) Islets from male control and IER3IP1-iβKO mice were isolated after 4 wk of tamoxifen injection. mRNA levels of indicated transcription factors of β cells were examined by real-time PCR. n = 5-9, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Supplemental Table S1. Primers for genotyping (5'- 3')

IER3IP1^{flox} forward	CGC TTC CTC AAG AAC AGT
IER3IP1^{flox} reverse	CTG TAA ATC GGC AAC AAG AA
RIP-Cre forward	TAA GGC TAA GTA GAG GTGT
RIP-Cre reverse	TCC ATG GTG ATA CAA GGG AC
Ins1-cre/ERT 15832	TGC GAA CCT CAT CAC TCG T
Ins1-cre/ERT 19101	TGG ACT ATA AAG CTG GTG GGC AT
Ins1-cre/ERT oIMR7338	CTA GGC CAC AGA ATT GAA AGA TCT
Ins1-cre/ERT oIMR7339	GTA GGT GGA AAT TCT AGC ATC ATC C

Supplemental Table S2. The information of donors.

	Control	T2D
N	3	4
Age (years)	49.5±8.5	51.5±2.5
Sex	male	male
BMI	23.5±2.65	26.8±2.60
HbA1c (%)	5.25±0.15	7.05±0.55

Supplemental Table S3. Primer sequences for qRT-PCR (5'- 3')

Gene	Forward	Reverse
GAPDH	AAGAGGGATGCTGCCCTTAC	TACGGCCAAATCCGTTCACA
IER3IP1	GAGGAGCGCTTCCTCAAGAACA	TCTCATCACGGTTCTTACAGACC
Mafa	AAGCGGCGACGCTCAAGAA	GGTCCCGCTCCTTGGCCAGA
Pdx-1	AGAGAGGAGCAGGGCAAAAC	TGAAATCAGCCAGGTTGCCT
Nkx6.1	GGCTGTGGGATGTTAGCTGT	TCATCTCGGCCATACTGTGC
Plk-1	TGGAGCAACTTCGGCATCAT	GCCTGCGAACACCTCTTTTG
Ckd1	GGAAGGCCTGCTGAAGAGTT	TCCCTTAGCGCTTGTTCCTG
Cdk-2	AGCTCTCCTTGCGTTCCATC	TCGGATGGCAGTACTGGGTA
p21	ACTACCAGCTGTGGGGTGAG	TCGGACATCACCAGGATTGG
p16	CGAACTCGAGGAGAGCCATC	TACGTGAACGTTGCCCATCA
Bcl-2	CTGCAAATGCTGGACTGAAA	TCAGGAGGGTTTCCAGATTG
Bcl-xl	GCCACCTATCTGAATGACCACCTA	AGAACCACACCAGCCACAGT
Mcl-1	AACTGGGGCAGGATTGTGAC	CGCCTTCTAGGTCCTGTACG
Trib3	ACCTTCAGAGCGACTTGTGG	GCTTGGCCCAAAAAGTCAGG
BiP	TGTGTGTGAGACCAGAACCG	TAGGTGGTCCCCAAGTCGAT
IRE1α	CCCAGCACAGACCTCAAGTT	CTCGATGTTTGGGCAGGTTG
PERK	AGCAGTGGGATTTGGACGTG	GAGGCACAGCTGTAGGTTGG
ATF6	GGGACCCTGTCTTACCATGT	AAGCCACAGGTCCTCTTTAGG