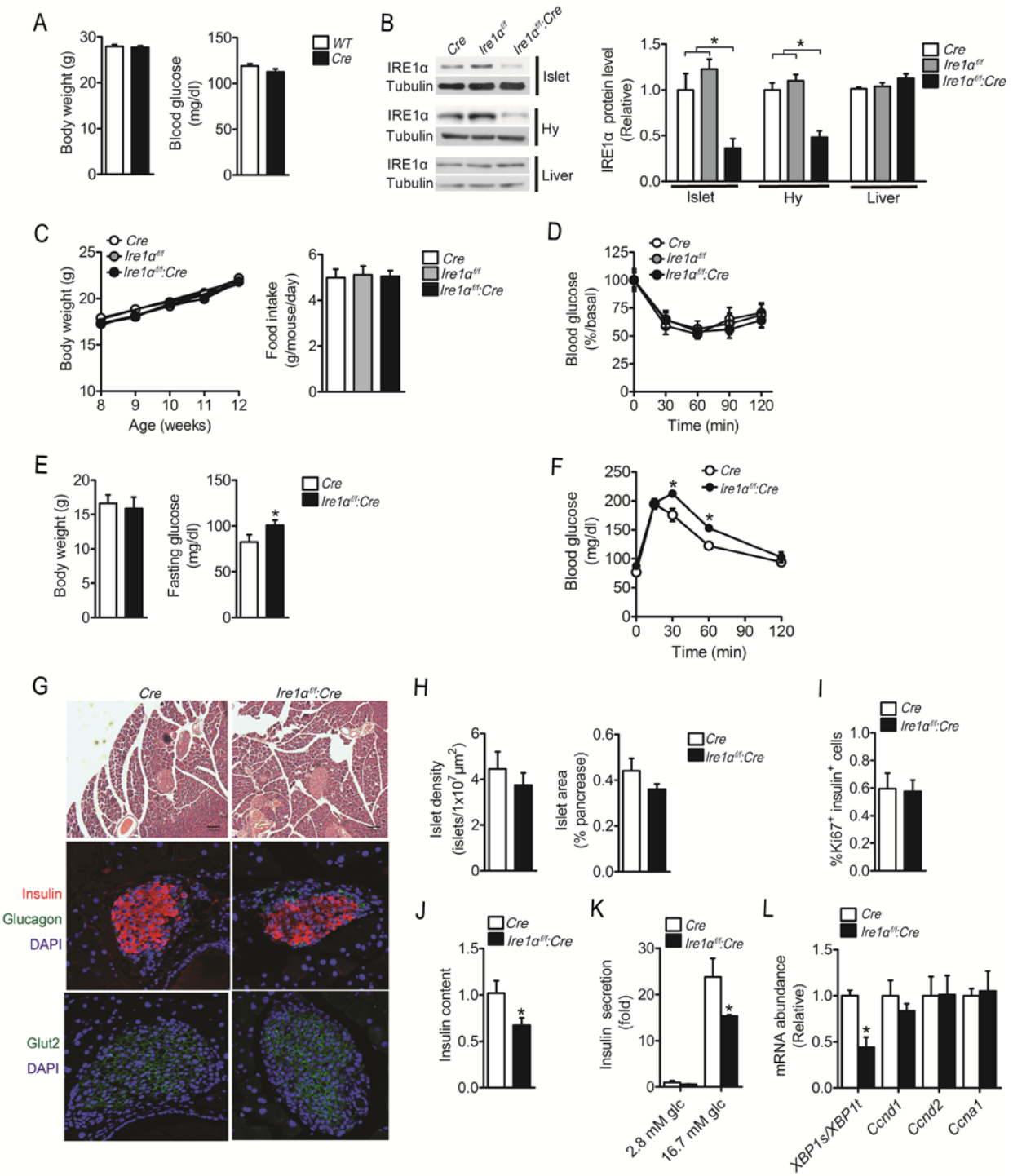
A
Body weight (g)
Blood glucose (mg/dl)
WT
Cre
B
Cre
Ire1a^f/f
Ire1a^f/f:Cre
IRE1α
Tubulin
Islet
Hy
Liver
IRE1α protein level (Relative)
C
Body weight (g)
Age (weeks)
Food intake (g/mouse/day)
D
Blood glucose (%/basal)
Time (min)
E
Body weight (g)
Fasting glucose (mg/dl)
F
Blood glucose (mg/dl)
Time (min)
G
Insulin
Glucagon
DAPI
Glut2
DAPI
H
Islet density (islets/1x10^7 μm^2)
Islet area (% pancrease)
I
%Ki67+ insulin+ cells
J
Insulin content
K
Insulin secretion (fold)
2.8 mM glc
16.7 mM glc
L
mRNA abundance (Relative)
Xbp1s/Xbp1t
Ccnd1
Ccnd2
Ccna1

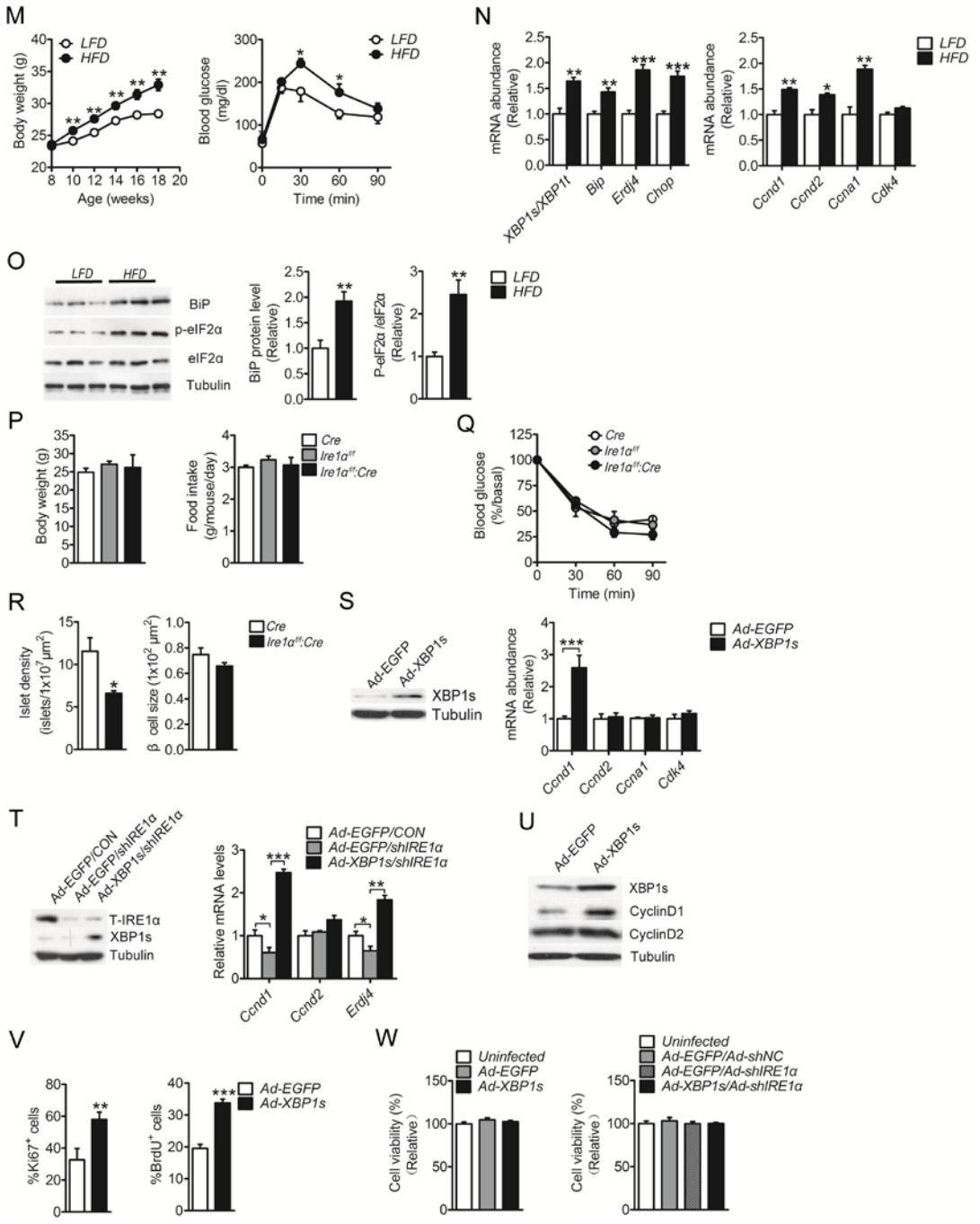

Figure S1 (A) Comparison of body weight and blood glucose levels in male wild-type (WT) and *Cre* littermates. Mice were maintained on a normal chow diet (*n* = 7 per genotype). Body weight and fed blood glucose levels were measured at 16 weeks of age. Results are presented as means ± SEM. **(B-D)** Abrogation of *Ire1α* in β-cells does not affect body weight, food intake or insulin's blood glucose-lowering ability in mice fed a normal chow diet. Male *Ire1α*$^{f/f}$, *Cre* and *Ire1α*$^{f/f}$*:Cre* mice were maintained on a normal chow diet (*n* = 5-6 per genotype). (B) RIP-*Cre*-mediated abrogation of *Ire1α* in pancreatic β-cells. Immunoblotting analysis of IRE1α protein

in pancreatic islets, hypothalamus (Hy) and liver of mice of the indicated genotype at 8 weeks of age. Shown are representative results, and tubulin was used as the loading control. Densitometric quantifications are presented as means ± SEM after normalization to the IRE1α protein level in *Cre* mice (n = 3 for each genotype). **(C)** Body weight of mice was monitored at the indicated ages, and daily food intake was measured at 10 weeks of age. **(D)** Insulin tolerance tests. Blood glucose was determined for mice at 11 weeks of age at the indicated time points after i.p. injection of 0.75 U/kg insulin. Data were normalized to the initial values before insulin injection. Results are presented as means ± SEM. **(E-F)** Ablation of *Ire1α* in β-cells disrupts glucose homeostasis in female mice. Female mice of the indicated genotype are maintained on a normal chow diet (n = 5-6 per genotype). **(E)** Body weight was determined at 7 weeks of age, and blood glucose levels were measured after a 6-hour fast at 9 weeks of age. Results are presented as means ± SEM. *$P < 0.05$ by unpaired two-tailed Student's t-test. (F) Glucose tolerance tests. Blood glucose was determined for mice at 10 weeks of age at the indicated time points after i.p. injection of 1 g/kg glucose. Results are presented as means ± SEM. *$P < 0.05$ by one-way ANOVA. **(G-L)** Deletion of *Ire1α* in β-cells decreases islet insulin content in NC-fed mice. *Cre* and *Ire1α*$^{f/f}$*:Cre* mice were maintained on a normal chow (NC) diet. (G) Morphological analyses of islets of male mice at 10 weeks of age (n = 3-5 per genotype). Shown are representative images of hematoxylin and eosin staining of the pancreatic sections (scale bar: 100 μm), immunohistochemical staining of pancreatic sections using antibodies against insulin (red), glucagon (green) and Glut2 (green), with nuclei visualized by DAPI staining. (H) Islet density was determined and shown as the average number of islets per 1×10^7 μm^2 of pancreas area (10 sections/mouse, n = 5 per genotype), and islet areas were measured as the percentage of total pancreas areas examined (10 sections/mouse, n = 5 per genotype). (I) β-cell proliferation was estimated by quantification of Ki67-positive β-cells in pancreas. Shown are percentage ratios of insulin and Ki67 double-positive cells to insulin-positive cells. Data are presented as means ± SEM (10 sections/mouse, n = 3 per genotype). (J-L) Primary islets were isolated from female mice of the indicated genotype at 10 weeks

of age (n = 3 per genotype). (J) Islet insulin content was determined and normalized to islet protein concentration. Data are presented as relative to the value of *Cre* islets. $^{*}P < 0.05$ by unpaired two-tailed Student's *t*-test. (K) Fold stimulation of insulin secretion was measured in response to 16.7 mM glucose relative to the basal level at 2.8 mM glucose. After normalization to islet protein concentration, results are presented as relative to the value of *Cre* islets at 2.8 mM glucose. $^{*}P < 0.05$ by two-way ANOVA. Data are shown as means ± SEM. (L) Primary islets were isolated from 3 individual mice at 11 weeks of age. Quantitative RT-PCR analyses of islet *Xbp1* mRNA splicing, shown as spliced (s) relative to total (t), and the mRNA abundance of genes encoding cyclin D1, cyclin D2 and cyclin A1. 18S ribosomal RNA was used as the internal control. $*P < 0.05$ by unpaired two-tailed Student's t-test. Data in (H-L) are shown as means ± SEM. **(M-O)** HFD-fed obese mice exhibit increased ER stress and expression of cell cycle regulators in islets. Male C57BL/6J mice at 8 or 10 weeks of age were fed a low-fat diet (LFD, 10% fat) or high-fat diet (HFD, 60% fat) for 10 weeks (n = 5 per group). (M) Body weight was monitored weekly. $**P < 0.01$ by one-way ANOVA. Glucose tolerance tests were also performed. Blood glucose was determined at the indicated time points after i.p. injection of 1 g/kg glucose. $*P < 0.05$ by one-way ANOVA. (N) Primary islets were isolated and pooled from each individual mouse (n = 3 per group). Quantitative RT-PCR analyses of *Xbp1* mRNA splicing, shown as spliced (s) relative to total (t), and the mRNA abundance of the indicated ER stress-related genes, as well as the mRNA abundance of genes encoding the indicated cyclins and cyclin-dependent kinase (CDK) 4. 18S ribosomal RNA was used as the internal control. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by unpaired two-tailed Student's *t*-test. (O) Immunoblotting analysis of islet BiP protein and eIF2α phosphorylation with densitometric quantifications (n = 3 per group). Tubulin was used as the loading control. $**P < 0.01$ by unpaired two-tailed Student's *t*-test. Data are shown as means ± SEM. **(P-R)** Effects of *Ire1α* deletion in β-cells upon body weight, food intake, insulin sensitivity, total islet density and β-cell size of HFD-fed mice. (P and Q) Male mice of the indicated genotype at 12 weeks of age (n = 5-6 per genotype) were challenged with a high-fat diet (HFD). (P) Body weight was

determined after 8 weeks of HFD feeding, and daily food intake was determined after 4 weeks of HFD feeding. (Q) Insulin tolerance tests were performed through i.p. injection of 0.75 U/kg insulin in mice fed HFD for 9 weeks. Data were normalized to the initial values before insulin injection. Results are shown as means ± SEM. (R) Male *Cre* and *Ire1α$^{f/f}$:Cre* mice at 12 weeks of age were fed HFD for 10 weeks (n = 4 per genotype). Total islet density was determined, presented as the average number of islets per 1×10^7 μm^2 of pancreas area. *P < 0.05 by unpaired two-tailed Student's t-test. β-cell sizes were also determined from sections immunostained for Glut2. Data are shown as means ± SEM (10 sections/mouse, n = 3 mice). **(S-V)** XBP1s upregulates the expression of cyclin D1 and promotes β-cell proliferation. (S) INS-1 cells were infected for 48 hours with adenoviruses Ad-XBP1s or Ad-EGFP. The mRNA abundance of the indicated genes was determined by quantitative RT-PCR using *cyclophilin A* as the internal control. (T) INS-1 cells were co-infected for 48 or 72 hours with adenoviruses expressing a scramble control shRNA (Ad-shCON) or shRNA directed against IRE1α (Ad-shIRE1α) with Ad-EGFP or Ad-XBP1s. IRE1α and XBP1s proteins were analyzed by immunoblotting, and the mRNA abundance of the indicated genes was determined by quantitative RT-PCR with *Cyclophilin A* as the internal control. (U and V) INS-1 cells were infected for 48 hours with adenoviruses Ad-XBP1s or Ad-EGFP. (U) Protein expression levels of XBP1s, cyclin D1 and D2 were analyzed by immunoblotting. Tubulin was used as the loading control. (V) Quantification of Ki67-positive and BrdU-positive cells. Data are shown as means ± SEM (n = 3 independent experiments). *P < 0.05, **P < 0.01, ***P < 0.001 by unpaired two-tailed Student's t-test. **(W)** Effects of adenoviral infections on the viability of INS-1 β-cells. INS-1 cells were infected with Ad-EGFP or Ad-XBP1s at an MOI of 20 for 48 hours or left uninfected. INS-1 cells were left uninfected, or infected with Ad-EGFP plus Ad-shNC, Ad-EGFP plus Ad-shIRE1α, or Ad-XBP1s plus Ad-shIRE1α at a total MOI of 40 for 72 hours. Each adenovirus was used at an MOI of 20. Cell viability was determined by the Cell Counting Kit-8, presented as the averaged percentage of viable cells after normalization to the uninfected control. Data are shown as means ± SEM (n = 3 independent experiments).

Table S1 Sequences of the oligonucleotide primers used for quantitative RT-PCR analysis.

Rat *Cyclophilin A* (+)	5’-ATGGCAAATGCTGGACCAAA-3’
Rat *Cyclophilin A* (-)	5’-CATGCCTTCTTTCACCTTCCC-3’
Rat *Erdj4* (+)	5’-ATAAAAGCCCTGATGCTGAAGC-3’
Rat *Erdj4* (-)	5’-GCCATTGGTAAAAGCACTGTGT-3’
Rat *Ccnd1* (+)	5’-GCACAACGCACTTTCTTTCC-3’
Rat *Ccnd1* (-)	5’-TCCAGAAGGGCTTCAATCTG-3’
Rat *Ccnd2* (+)	5’-CACCGACAACTCTGTGAAGC-3’
Rat *Ccnd2* (-)	5’-CCACTTCAGCTTACCCAACAC-3’
Rat *Ccna1* (+)	5’-GACAAACTCATCGACATCGGGCGGG-3’
Rat *Ccna1* (-)	5’-GACCCTGTACTTGGCTGTCAACTTC-3’
Rat *Cdk4* (+)	5’-GTCAGTGGTGCCGGAGAT-3’
Rat *Cdk4* (-)	5’-GGATTAAAGGTCAGCATTTCCA-3’
Mus *Ribosomal 18s* (+)	5’- AGGGAGAGCGGGTAAGAGA-3’
Mus *Ribosomal 18s* (-)	5’- GGACAGGACTAGGCGGAACA-3’
Mus *XBP1s* (+)	5’-CTGAGTCCGAATCAGGTGCAG-3’
Mus *XBP1s* (-)	5’-CCATGGGAAGATGTTCTGG-3’
Mus *XBP1t* (+)	5’-TGGCCGGGTCTGCTGAGTCCG-3’
Mus *XBP1t* (-)	5’-TCCATGGGAAGATGTTCTGG-3’
Mus *Erdj4* (+)	5’-TAAAAGCCCTGATGCTGAAGC-3’
Mus *Erdj4* (-)	5’-TCCGACTATTGGCATCCGA-3’
Mus *Bip* (+)	5’-TCATCGGACGCACTTGGAA-3’
Mus *Bip* (-)	5’-CAACCACCTTGAATGGCAAGA-3’
Mus *Chop* (+)	5’-CTGGAAGCCTGGTATGAGGAT-3’
Mus *Chop* (-)	5’-CAGGGTCAAGAGTAGTGAAGGT-3’
Mus *Ccnd1* (+)	5’-GCGTACCCTGACACCAATCTC-3’
Mus *Ccnd1* (-)	5’-CTCCTCTTCGCACTTCTGCTC-3’
Mus *Ccnd* 2 (+)	5’-ATCCGGCGTTATGCTGCTCT-3’
Mus *Ccnd* 2 (-)	5’-ATCCGGCGTTATGCTGCTCT-3’
Mus *Ccna1* (+)	5’-ACCTAAGGCGTCAAGGAGTGT-3’
Mus *Ccna1* (-)	5’-CAGCAACCAAGGAAGGAAGATA-3’
Mus *Cdk4* (+)	5’-GCACAGACATCCATCAGCCG-3’
Mus *Cdk4* (-)	5’-CGTGAGGTGGCCTTGTTAAGGA-3’

Materials and Methods

Creation of mice with deletion of IRE1α in β-cells

Mice in which exon 2 of the *Ire1α* allele was flanked with *loxP* sites (denoted *Ire1α*$^{f/f}$ mice) were generated at Shanghai Research Center for Model Organisms as described [1]. *Ire1α*$^{f/f}$ mice were subsequently backcrossed for 6 generations into the genetic background of C57BL/6J mice (purchased from Shanghai Laboratory Animal Co. Ltd). To generate β-cell-specific IRE1α knockout mice (denoted *Ire1α*$^{f/f}$*:Cre* mice), *RIP-Cre* transgenic mice (*B6.Cg-Tg(Ins2-cre)25Mgn/J*, denoted *Cre* mice; The Jackson Laboratory, Bar Harbor, ME), which express Cre recombinase under the control of the rat insulin promoter, was intercrossed with *Ire1α*$^{f/f}$ mice to produce *Ire1α*$^{flox/+}$*:Cre* mice. *Ire1α*$^{flox/+}$*:Cre* mice were then bred with *Ire1α*$^{flox/+}$ mice to generate *Ire1α*$^{f/f}$*:Cre* along with *Ire1α*$^{f/f}$ and *Cre* control mice. Genotyping was done as described [1].

Mice were maintained on a 12-hour light/dark cycle with free access to a regular chow diet (Shanghai Laboratory Animal Co. Ltd, China) and water at an accredited animal facility at Shanghai Institutes for Biological Sciences. For diet-induced obesity, male mice were fed a low-fat diet (LFD) or a high-fat diet (HFD) containing 10% or 60% kcal fat (Research Diets Inc., New Brunswick, NJ, USA). All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committees at the Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences.

Physiologic measurements

The body weight of animals was monitored weekly. Food consumption was measured for individually caged mice at 7-8 weeks of age by weighing food daily before the dark cycle for one week. Fasting glucose was determined from tail vein blood for male mice after a 6-hour fast (9:00–15:00) using a glucometer (FreeStyle, Alameda, CA, USA). Serum insulin was measured using a rat/mouse insulin ELISA kit (Millipore, St. Charles, MO, USA) according to the manufacturer’s instructions.

Glucose tolerance test (GTT) and insulin tolerance test (ITT)

For GTT, male mice were fasted for overnight (16 hours) and then injected i.p. with D-glucose at 1g/kg of body weight. Blood glucose was measured from tail vein blood at the indicated time interval after the injection using a glucometer (FreeStyle, Alameda, CA, USA). For ITT, male mice were fasted for 6 hours and injected i.p. with 0.75 U/kg body weight of human insulin (Roche, Rotkreuz, Switzerland) before blood glucose measurement.

Pancreatic islet isolation and insulin secretion/content measurement

Mouse pancreatic islets were isolated using the Liberase (Roche, Rotkreuz, Switzerland) digestion method, and glucose-stimulated insulin secretion was determined as previously described in detail [2]. To determine the insulin content, primary islets were lysed in acid-ethanol buffer (0.18 M HCl in 70% ethanol) by mild sonication on ice. Insulin content was determined using the rat/mouse ELISA kit (Millipore, St. Charles, MO, USA).

Histological analysis and immunofluorescence staining of pancreatic sections

Pancreas was fixed in 4% paraformaldehyde, embedded in paraffin, and cut into consecutive 5-μm sections. Pancreatic sections were stained with hematoxylin and eosin at 200-μm intervals to avoid repeated measurements of the same islet. Images of the islets and the entire pancreas were acquired and analyzed using the Image J Software (http://rsb.info.nih.gov/ij). Islet mass was expressed as the percentage of the total pancreatic area that was surveyed.

For immunostaining, fixed pancreatic sections were heated for 15 min in boiling 10 mM citrate buffer (PH=6.0) for antigen retrieval. Sections were subsequently probed with rabbit anti-glucagon (Cell Signaling Technology, Danvers, MA,USA), guinea pig anti-insulin (Dako, Produktionsvej, Denmark), rabbit anti-Glut2 (Millipore, St. Charles, MO,USA) or mouse anti-Ki67 (BD, Franklin Lakes, New Jersey, USA) antibody, followed by incubation with specific secondary antibodies conjugated to Alexa 488 or 594 **(**Invitrogen, Carlsbad, CA, USA). DAPI (Sigma, St. Louis, MO,

USA) was used to visualize the nucleus. Sections were analyzed by laser confocal microscopy (Olympus, Tokyo, Japan).

Cell culture and adenovirus infections

The rat insulinoma INS-1 cell line was kindly provided by Dr. Christopher B. Newgard (Duke University Medical Center) and maintained in RPMI 1640 as described [3]. INS-1 cells between passages 20-30 were used, which possessed robust glucose-stimulated insulin secretion properties. Recombinant adenoviruses Ad-EGFP, Ad-shCON and Ad-shIRE1 that were used for infection of INS-1 cells were generated as previously described in detail [4, 5]. Ad-XBP1s was a generous gift from Dr. Ling Qi at Cornell University. INS-1 cells were infected with adenoviruses at an MOI of 20-40 for the indicated periods of time, and adenoviral infections under these conditions did not show significant effects on cellular viability as analyzed using the Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) (Fig. S1W). Isolated primary islets from *Ire1α*$^{f/f}$*:Cre* mice were infected with the indicated adenoviruses at an MOI of 200 for 2 days.

Cell proliferation analysis

For Ki67 staining analysis, infected INS-1 cells were washed with PBS and then fixed with 4% paraformaldehyde for 30 minutes at room temperature. Following incubation in 0.2% Triton X-100 for 5 minutes, cells were blocked with 1xPBST containing 5% donkey serum for 1 hour. Cells were then incubated with Ki67 antibody for 1 hour, followed by staining with DAPI (Sigma, St.Louis, MO, USA) and Alexa 488-conjugated secondary antibody for 30 minutes. Pancreatic sections were incubated with Ki67 and guinea pig anti-insulin antibody, followed by incubation with secondary antibodies conjugated to Alexa 488 or Alexa 594. Cells or sections were analyzed by laser confocal microscopy (Olympus, Tokyo, Japan).

For BrdU incorporation analysis, infected INS-1 cells were treated with BrdU (Sigma, St. Louis, MO, USA; final concentration 3 μg/ml) for 3 hours. After washing with PBS, cells were fixed with 4% paraformaldehyde for 30 minutes at room temperature.

Cells were then incubated in 2N HCl for 30 minutes and blocked with 1xPBST containing 5% donkey serum for 1 hour. After incubation with BrdU antibody (Dako, Produktionsvej, Denmark) for 1 hour, cells were stained with DAPI and Alexa-conjugated secondary antibody for 30 minutes before analysis by laser confocal microscopy (Olympus, Tokyo, Japan).

Luciferase reporter assay

The pGL3 plasmid containing the promoter of human *Ccnd1* gene, which corresponds to the region of -1748 to +134 with respect to the putative transcription start site (denoted nucleotide +1), was purchased from Addgene (Cambridge, MA, USA). Deletion of the ACGT core from the *Ccnd1* promoter was done by a PCR-based strategy. HEK293T cells were co-transfected with the desired plasmids. Luciferase activity was measured using Dual-luciferase Assay Kit (Promega, Madison, WI) according to the manufacturer's instructions. Renilla activity was used as an internal control for normalization.

Antibodies and immunoblotting

IRE1α, p-eIF2α, eIF2α, Cyclin D1 and D2 antibodies were purchased from Cell Signaling Technologies, BiP antibody from Enzo Life Sciences (New York, USA), XBP1s antibody from Biolegend (San Diego, CA, USA). Tubulin antibody was from Sigma (St. Louis, MO, USA). Protein extracts were prepared from isolated primary islets or livers of mice and INS-1 cells. Western immunoblotting was performed as described previously [4].

Quantitative real-time RT-PCR analysis

Total RNA from mouse liver or islets was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized with Moloney murine leukemia virus (M-MLV) reverse transcriptase and random hexamer primers (Invitrogen, Carlsbad, CA, USA). Real-time quantitative PCR was conducted using the SYBR Green PCR system, following the manufacturer's recommendations

(Applied Biosystems). 18S rRNA or Cyclophilin A was used as an internal control for normalization. Primers used were as shown in Table S1.

Statistical analysis

Results are presented as means ± SEM. Statistical analysis of differences was conducted using the Student's *t*-test, one-way analysis of variance (ANOVA). $P < 0.05$ was considered significant.

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