Supporting Information

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SI Materials and Methods

Generation of β-Cell-Specific XBP1 KO Mice. $Xbp1^{flox}$ mice that contain two loxP sites flanking exon 2 of the Xbp1 gene were described previously (1). $Xbp1^{flox}$ mice which were originally generated using 129/SvJ-originated embryonic stem cells were backcrossed to the C57BL/6 strain of mice for more than five generations and then crossed with B6.Cg-Tg(Ins2-cre)25Mgn/J (RIP-cre) (Jackson Laboratory) mice that expressed cre recombinase under the control of the rat insulin gene promoter (2). Two consecutive breedings generated $Xbp1^{f/f}$ and $Xbp1^{f/f}$;RIP-cre mice, which were intercrossed to amplify the control and β-cell-specific XBP1 KO mice. For some experiments, Xbp1 heterozygous $Xbp1^{f/+}$;RIP-cre mice were generated by crossing $Xbp1^{f/f}$ and RIP-cre mice. $Xbp1^{f/f}$;RIP-cre mice were also mated to $Ins2^{+/Akita}$ mice (Jackson Laboratory) to generate compound mutant mice. Animal studies and experiments were approved and carried out according to Harvard University's Standing Committee on Animals and National Institutes of Health guidelines for animal use and care. Mice were housed in a specific pathogen-free animal facility at the Harvard School of Public Health and fed regular rodent chow containing 4.5% fat (PicoLab Rodent Diet 20; LabDiet).

Histological Analysis and Electron Microscopy. For histological analysis, pancreata were fixed in 10% formalin, paraffin-embedded, and sectioned in 5 μm. Pancreas sections were stained with H&E for routine histological study. To determine islet area, H&E-stained sections were photographed at low magnification. Images were imported to Photoshop (Adobe), and total pancreas and islet areas were identified by the Magic Wand tool. Pixel numbers in selected areas were counted and used to calculate relative islet area to total pancreas.

For immunohistochemistry, slides were heated for 20 min in boiling 10 mM citrate buffer (pH 6.0) for antigen retrieval. Sections were probed with guinea pig anti-insulin (4011-01; Linco), rabbit anti-glucagon (AB932; Chemicon), mouse anti-CPE (610758; BD Biosciences), or rabbit anti-Glut2 (07-1402; Millipore), followed by specific secondary antibodies conjugated to Texas Red, FITC, or Alexa Fluor 488. For β-cell proliferation assay, BrdU (Sigma) was administered to 4-wk-old male mice by i.p. injection at 50 mg/kg, 24 h before sacrifice. Pancreas sections fixed in 4% formaldehyde were double stained with BrdU (Biolegend) and insulin antibody.

For transmission electron microscopy (TEM), pancreata were cut into small pieces and then fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Islets were separated from exocrine tissue under a dissecting microscope and embedded in epoxy resin. Ultrathin sectioning and TEM imaging were performed by the Advanced Microscopy Core facility (Diabetes and Endocrinology Research Center) at the Joslin Diabetes Center (Boston, MA).

Cell Culture and Transfection. Min6 cells were cultured in DMEM/high glucose supplemented with 15% FBS, nonessential amino acids, 50 μM β-mercaptoethanol, and penicillin/streptomycin. pLKO.1 puro lentiviral vectors containing shRNA for mouse XBP1 (target sequence, 5′-CCAGGAGTTAAGAACACGCTT-3′) or luciferase were obtained from the Broad Institute. Min6 cells were infected with recombinant lentiviruses produced from 293T cells and selected with 2 μg/mL puromycin. For some experiments, Min6 cells were transfected with luciferase or XBP1 and IRE1α siRNAs provided by Alnylam Pharmaceuticals using Lipofectamine RNAiMAX reagent (Invitrogen). Min6 cells expressing a dominant-negative XBP1 (dnXBP) were generated as described previously (3). Transfection into 293T cells was performed using Lipofectamine 2000 (Invitrogen). EGFP, Ins1 (BC098468), PC1 (BC108983), PC2 (BC057348), and CPE (BC053612) cDNA clones in CMV-SPORT6 vector were obtained from Open Biosystems or generated by standard subcloning techniques.

RNA Cleavage Assay. The cytoplasmic domain of human IRE1α produced in insect cells was kindly provided by Mannkind. IRE1α was added to a reaction mixture containing the total RNA purified from Min6 cells in a buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 5 mM β-mercaptoethanol, and 2 mM ATP, as described previously (4). After incubation with IRE1α at 37 °C for 30 min, the substrate RNAs were separated on denaturing agarose gels and transferred onto GeneScreen nylon membranes (PerkinElmer). Hybridization of the RNA to ^{32}P-labeled probes was performed using UltraHyb buffer (Ambion), as described previously (5). Radiolabeled probes were prepared using the Rediprime II random prime labeling system (GE Healthcare). Templates for random priming were generated by PCR amplification of the fragments of XBP1 (+614 to +1235), Ins (+209 to +324), PC1 (+88 to +540), PC2 (+290 to +722), CPE (+652 to +797), and β-actin (+987 to +1083) cDNAs.

Chromatin Immunoprecipitation Assays. Chromatin immunoprecipitation (ChIP) assays were performed as described previously (1). Primer sequences are shown in Table S1.

Western Blot. Cell lysates were separated by SDS/PAGE and transferred to polyvinylidene difluoride membranes. Phos-tag reagent (NARD Institute) was added to the gel at 7.5 μM to separate the native and phosphorylated IRE1α (6). Western blot was performed using anti-XBP1s (BioLegend) and anti-IRE1α (Cell Signaling) antibodies.

1. Lee AH, Scapa EF, Cohen DE, Glimcher LH (2008) Regulation of hepatic lipogenesis by the transcription factor XBP1. *Science* 320:1492–1496.
2. Postic C, et al. (1999) Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic β cell-specific gene knock-outs using Cre recombinase. *J Biol Chem* 274:305–315.
3. Iwakoshi NN, et al. (2003) Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. *Nat Immunol* 4:321–329.
4. Iwawaki T, Akai R (2006) Analysis of the XBP1 splicing mechanism using endoplasmic reticulum stress-indicators. *Biochem Biophys Res Commun* 350:709–715.
5. Lee AH, Iwakoshi NN, Glimcher LH (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* 23:7448–7459.
6. Sha H, et al. (2009) The IRE1α-XBP1 pathway of the unfolded protein response is required for adipogenesis. *Cell Metab* 9:556–564.

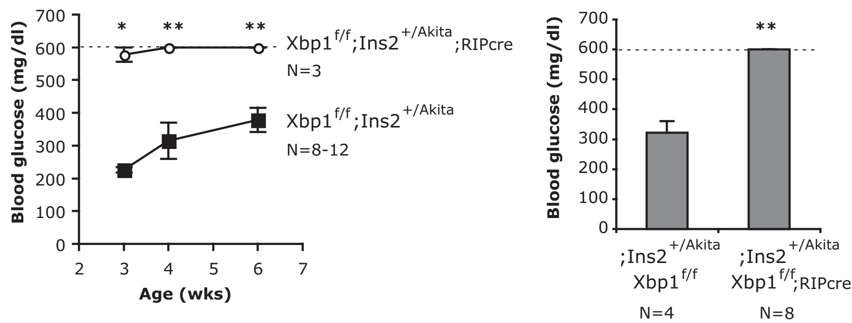

Fig. S1. Ablation of XBP1 in Akita mice producing C96Y mutant insulin aggravates hyperglycemia. Blood glucose levels of mice with the indicated genotypes were measured at fed state. Error bars represent SEM. (*Left*) Male. (*Right*) Five-week-old female mice. Two (*) and all (**) mice had glucose levels greater than the detection limit of the glucometer (600 mg/dL), and were represented as 600 mg/dL.

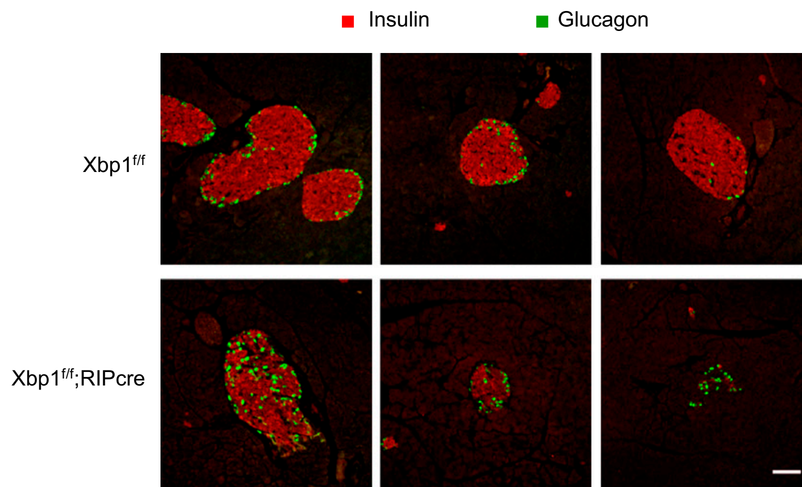

Fig. S2. Representative images of WT and XBP1 KO islets exhibiting decreased abundance of β-cells relative to α-cells to varying extents. Pancreas sections of WT and Xbp1$^{f/f}$;RIP-cre mice were double stained with insulin and glucagon antibodies. (Scale bar, 50 μm.)

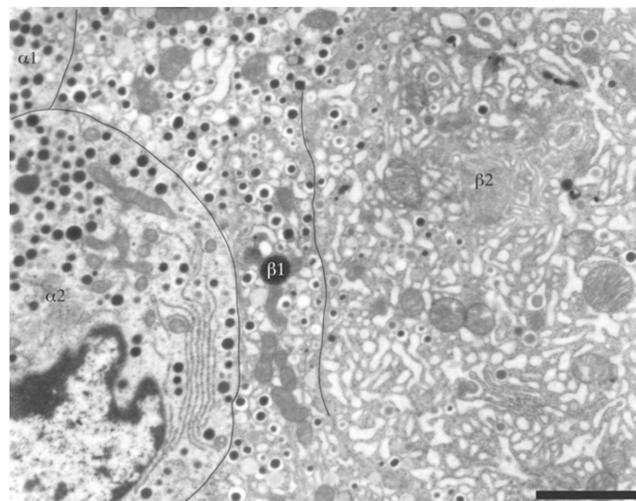

Fig. S3. A representative TEM image of an XBP1 KO islet exhibiting α- and β-cells. Cell boundaries are highlighted by thin lines. Two α-cells contain abundant glucagon granules, which are characterized by uniformly stained granule contents surrounded by membranes with little vacant space. One α-cell (α2) exhibits normal morphology of mitochondria and the endoplasmic reticulum (ER). Insulin secretory granules and mitochondria appear to be normal in one β-cell (β1). The other β-cell, labeled β2, contains severely swollen mitochondria, dilated ER, and scarce secretory granules. (Scale bar, 2 μm.)

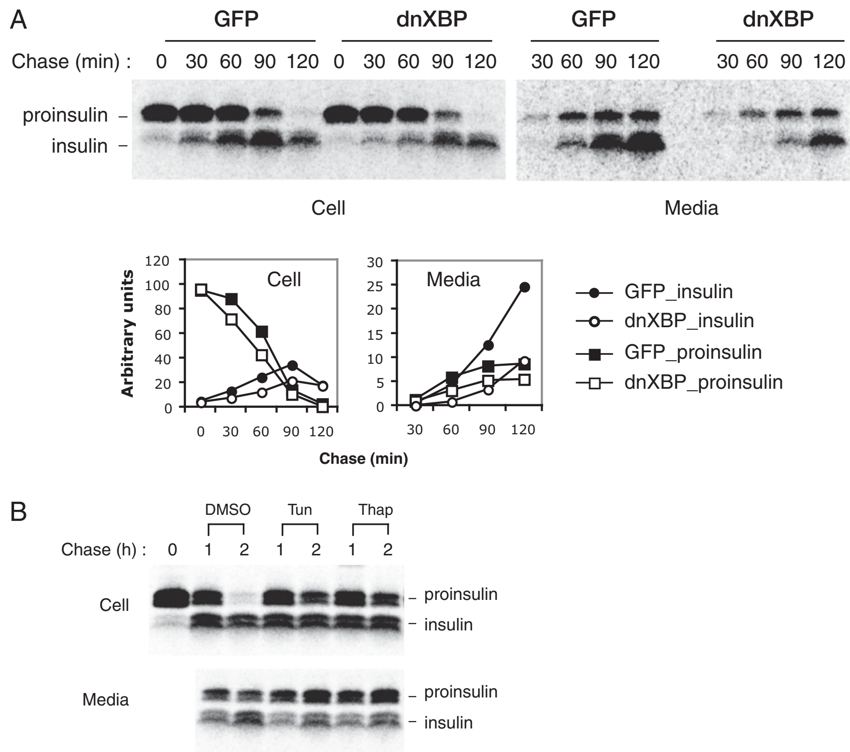

Fig. S4. Impaired proinsulin maturation by XBP1 inhibition or ER stress. (*A*) Stable cell lines were generated by transducing Min6 cells with retroviral vectors expressing GFP or dominant-negative XBP1 (dnXBP). Pulse-chase experiments were performed as described in Fig. 4. ^{35}S-labeled intracellular and secreted insulin species were quantified for plotting. (*B*) Min6 cells were pulse-labeled for 30 min with [^{35}S]Met/Cys. During chase, DMSO, tunicamycin (10 μg/mL; Tun), or thapsigargin (1 μM; Thap) were added to the media. Cells and media were harvested to measure ^{35}S-labeled proinsulin and insulin contents.

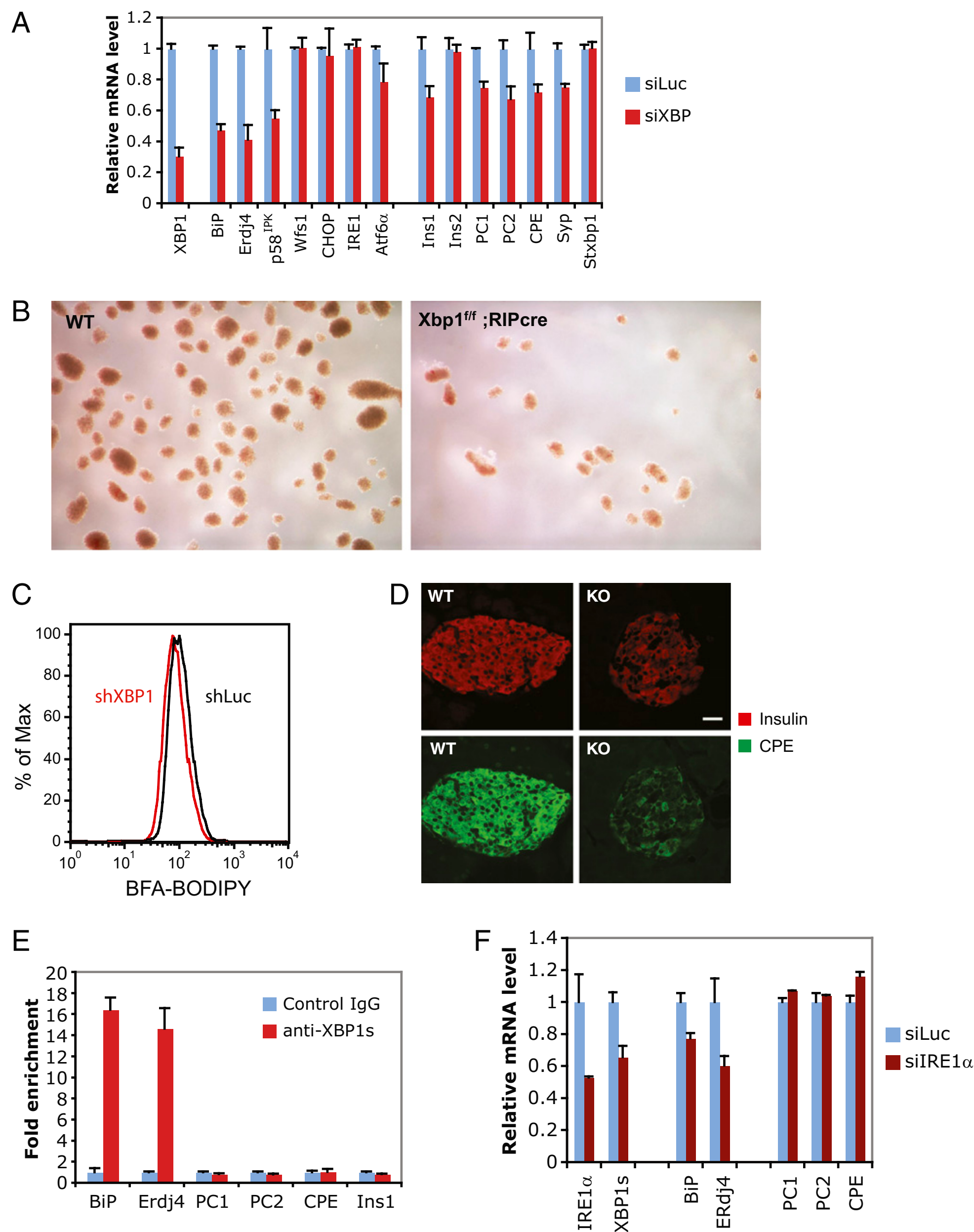

Fig. S5. Regulation of proinsulin-processing enzymes by IRE1α. (*A*) Min6 cells were transfected with siRNAs targeting luciferase or XBP1. Cells were harvested 72 h after transfection, and mRNA levels of the indicated genes were determined by quantitative RT-PCR. (*B*) Primary pancreatic islets isolated from WT and Xbp1$^{f/f}$;RIP-cre mice using librase. (*C*) BODIPY-brefeldin A (BFA) staining analyzed by fluorescent cell sorting. (*D*) CPE expression in WT and Xbp1$^{f/f}$;RIP-cre mice was examined by immunofluorescence staining of islets. (Scale bar, 50 μm.) (*E*) Chromatin immunoprecipitation followed by PCR quantification to demonstrate the binding of XBP1 to proximal promoter regions of indicated genes. (*F*) mRNA levels were measured by quantitative RT-PCR 72 h after siRNA transfection into Min6 cells.

Table S1. Sequences of PCR primers

Gene	Forward (5′-3′)	Reverse (5′-3′)	Position*
Quantitative RT-PCR primers			
ATF6	TGCCTTGGGAGTCAGACCTAT	GCTGAGTTGAAGAACACGAGTC	+240/+380
BiP	TCATCGGACGCACTTGGAA	CAACCACCTTGAATGGCAAGA	+528/+596
CHOP	GTCCCTAGCTTGGCTGACAGA	TGGAGAGCGAGGGCTTTG	+341/+413
CPE	GAACCGTAACTTCCCAGACCT	TCCAGTGAATGACAGCCTTGG	+652/+797
EDEM	AAGCCCTCTGGAACTTGCG	AACCCAATGGCCTGTCTGG	+1085/+1161
ERdj4	TAAAAGCCCTGATGCTGAAGC	TCCGACTATTGGCATCCGA	+313/+389
Ins1	GCAAGCAGGTCATTGTTTCAAC	AAGCCTGGGTGGGTTTGG	+162/+256
Ins2	CGTGGCTTCTTCTACACACCC	AGCTCCAGTTGTGCCACTTGT	+209/+276
IRE1	CCGAGCCATGAGAAACAAGAA	GGGAAGCGGGAAGTGAAGTAG	+2823/+2933
PC1	CTTTCGCCTTCTTTTGCGTTT	TCCGCCGCCCATTCATTAAC	+88/+166
PC2	AGAGAGACCCCAGGATAAAGATG	CTTGCCCAGTGTTGAACAGGT	+579/+722
Stxbp1	GTGGACCAGTTAAGCATGAGG	GCGCAGCCCGATATTTAGC	+238/+448
Syp	CAGTTCCGGGTGGTCAAGG	ACTCTCCGTCTTGTTGGCAC	+34/+152
Wfs1	CGGGAAGAAACGGACAGAGC	CGTAGGTAGTGTTTGCCCAC	+214/+319
XBP1	GACAGAGAGTCAAACTAACGTGG	GTCCAGCAGGCAAGAAGGT	+852/+1041
XBP1s	AAGAACACGCTTGGGAATGG	CTGCACCTGCTGCGGAC	+406/+544
ChIP primers			
Cpe (a)	AGGATGATCTTGGGTCGTGGTTC	CAGGGATGTTGGGTTTATTTCTTG	−270/−130
Cpe (b)	AGACCTTGCTGTAGATGAAAT	AACCCGCTGCTTGCCTGGAC	−429/−357
Dnajb9	AGTGACGCAAGGACCAAACG	CTACACGAAACGCTTCCCCA	−142/−42
Hspa5	TGACGTGAGTTGCGGAGGA	TCCGATTGGTGAAGTCGCTAC	−159/−59
Ins1	TCTCACCTTCTGGGACAAT	TGGACATCAACACATCTACTTA	−283/−176
Ins2	CCTGGGGAATGATGTGGAAAAA	CCCTGATGGCCTGATGAACC	−331/−229
Pcsk1 (a)	AGGCGGGGGTGGGAGGTAGG	TGGTGGGATCGGTCAGTTC	−298/−139
Pcsk1 (b)	TGACCGATCCCACCAGTT	TCAAATCGACGAAAGCCATCT	−153/−16
Pcsk2	GGGAAGACAGGGGAAGGGAAGAGA	CCCGGTGATGTCATTGTTTGTGGA	−466/−276

*Position from the transcription start site.