Supporting Information

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SI Materials and Methods

Physical Studies. All blood glucose measurements were determined on whole venous blood by using an automated glucose monitor (One Touch Basic, Lifescan) (1). Glucose tolerance tests were performed on mice after 16 h of fasting. Mice were injected intraperitoneally (i.p.) with D-glucose (2 g/kg of body weight) and blood was obtained at indicated time points. For insulin release, glucose (3 g/kg of body weight) was injected i.p., and blood was collected at indicated time points (2). Serum insulin levels were measured by ELISA with a rat insulin standard (Crystal Chem) (3). Statistical analysis was performed using a 2-tailed unpaired *t* test.

Islet Isolation and Insulin Content Measurement. Mice were killed, the common bile duct was cannulated with a 27-mm needle in the anterograde direction, and its duodenal end was occluded by clamping (2). We subsequently injected collagenase P (2–3 mg/mL) into the duct to distend the pancreas. The pancreas was removed and incubated at 37 °C for 15–24 min, then put in 10 mL of Hanks balanced salt solution to dilute the enzyme and shaken vigorously to break the tissue completely. Cellular components were obtained by centrifugation (220 × *g* for 1 min) and resuspended in 10 mL of RPMI medium 1640. We handpicked islets under a light microscope and washed the islets once in RPMI medium 1640. Islets were spun in a centrifuge at 15,600 × *g* for 5 min and cultured in RPMI medium 1640 supplemented with 10% FBS overnight. After incubation, islets can be used for experiments immediately or stored at −80 °C for DNA or protein extraction. We measured insulin content in islets isolated from knockout mice and littermate controls in acid/ethanol extracts by using an ELISA kit (Crystal Chem).

Immunohistochemistry. Mice were killed and pancreata were immediately isolated. After rinsing in PBS, tissues were fixed in 4% paraformaldehyde for 18 h and embedded in paraffin (3). Sections were cut and stained with antibodies to Glut2 (Chemicon) 1:200; insulin (Santa Cruz Biotechnology) 1:500; Shp2 (Santa Cruz Biotechnology) 1:300; Pdx1 (gift of C. V. Wright, Vanderbilt University, Nashville, TN) 1:500. Secondary antibodies included Alexa Fluor 594 anti-rabbit IgG and Alexa Fluor 488 anti-mouse IgG (1:200 dilution, Molecular Probes). Appropriate positive and negative controls for each antibody were included. We used normal mouse pancreas sections/INS1 832–13 cells as positive control for PDX1, Glut2, and insulin. Meanwhile, the primary antibody was replaced by PBS as the negative control.

Cell Culture, Transfection, and Immunocytochemistry. INS-1 832/13 cells were cultured in regular RPMI medium 1640 containing 10% FBS, at 37 °C and 5% CO_2 as reported (4). Shp2 expression was knocked down using 200 pmol of Qiagen siRNA (sense: 5′-GGA CUA UGA CCU CUA Utt-3′, antisense: 5′-AUA GAG GUC AUA GUA GUC Ctt-3′) in an Amaxa nucleoporator with solution T using program T-20 (4). All assays were performed ≈72 h after siRNA treatment. For immunocytochemistry, cells were washed with PBS and fixed in 4% paraformaldehyde for 15 min. After 3 PBS washes, cells were blocked in 5% normal goat serum in PBS/Triton X-100 for 60 min and incubated overnight with antibodies to insulin, 1:500; Pdx1, 1:500; Shp2, 1:300; FoxO1, 1:500. Secondary antibodies were as described previously.

In Vitro Insulin Secretion Assay. INS-1 832/13 cells were seeded in 12-well culture plates after 72 h of siRNA treatment. After washing twice with PBS, cells were starved for 2 h in standard assay buffer (SAB) (4). Subsequently, the medium was replaced by SAB containing 3 mM glucose (for basal secretion) or high glucose (15 mM) to measure insulin secretion during a 2-h incubation. Supernatants were removed to measure secreted insulin, and insulin secretion results were normalized to cell number. The amount of insulin secretion was assayed by using an RIA kit (Linco Research).

ATP Determination. After 72 h of siRNA treatment, cells were starved and treated with low or high glucose and then lysed in lysis buffer (10 mM Tris, pH 7.5, 0.1 M NaCl, 1 mM EDTA, and 0.01% Triton X-100). After centrifugation, supernatants were obtained for quantitative ATP determination using an ATP Determination Kit (Molecular Probes). ATP levels were standardized to protein concentrations.

Quantitative Real-Time RT-PCR (qRT-RCR). Total RNA was isolated from INS-1 832/13 cells by using TRIzol (Invitrogen) and reverse-transcribed by using SuperScript II reverse transcriptase (Invitrogen) (5). qRT-PCR was performed using SYBR Green dye (Applied Biosystems) in a MX3000P thermal cycler (Stratagene). Primers for qRT-PCR analysis of samples from mouse islets and INS-1 832/13 cells are listed in Table S1 and Table S2, respectively. Each reaction was carried out in triplicate and gene expression was normalized to Cph. Results are expressed as the mean ± SEM.

Chromatin Immunoprecipitation (ChIP) Assay. The ChIP assay was performed following standard protocol. Quantitative PCR was performed on a Mx3000P machine from Stratagene. PCR conditions were: 1 cycle at 95 °C for 10 min, followed by 40 cycles at 95 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s, and finally 1 cycle at 95 °C for 1 min, 55 °C for 30 s, 95 °C for 30 s. The primers used for Pdx1 binding to Ins1 and Ins2 promoter ChIP assay were: *Ins1*, 5′-actgcttcatcaggccatct-3′ and 5′-aggaggggtaggtaggcaga-3′; *Ins2*, 5′- acccaggagcccctattaag-3′ and 5′-ccctggactttgctgtttgt-3′; and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), 5′-tgagagaggcccagctactc-3′, and 5′-gaacagggaggagcagagag-3′. The primers used for FoxO1 binding to Pdx1 promoter assay were: *Pdx1*, 5′-ggatcaggcgactgagagag-3′ and 5′-cccagatcgctttgacagtt-3′, and negative control, 5′-tgatggagtcccttggaaac-3′ and 5′-tcctactccagggcctacct-3′.

Plasmid Construction and Transfection. Mouse Pdx-1 cDNA was PCR amplified from the plasmid pZL1-Pdx-1 (a kind gift of C. V. Wright) by using primers 5′-ACGCGGATCCATGAACAGTGAGGAGCAGTA-3′ (BamHI) and 5′-ACCGCTCGAGTCACCGGGGTTCCTGC-3′ (XhoI), and subcloned into pCMV-Tag2B (Flag), thus creating the plasmid pCMV-Tag2B-mPDX1. Correct clones were confirmed by sequencing. pCMV-Tag2B-mPDX1 plasmid was transfected into INS-1 832/13 cells by using an Amaxa nucleoporator with solution T using program T-20. After 72 h, harvested cells were extracted with acid/ethanol to determine insulin content. Insulin content was assayed by ELISA as described above.

Immunoblotting and Immunoprecipitation. For protein extraction of tissue samples, we used ice-cold tissue lysis buffer containing 50 mM Tris·HCl, 0.15 M NaCl, 1% Triton X-100, 0.25% sodium

deoxycholate, 1 μg/mL leupeptin, 2 μg/mL pepstatin A, 10 μg/mL aprotinin, 1 mM PMSF, 1 mM sodium orthovanadate, 1 mM benzamidine, and 0.1 mM benzethonium chloride, pH 8.0; 20 μg of protein was resolved by SDS-polyacrylamide gel electrophoresis (SDS/PAGE).

Mouse islets or INS-1 832/13 cells were lysed in RIPA buffer (50 mM Tris·HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate, and 0.02% sodium azide) supplemented with protease and phosphatase inhibitors (1 mM Na_3VO_4, 1 mM PMSF, 10 μg/mL aprotinin and leupeptin), and protein was quantified by standard techniques. Immunoblots of INS-1 832/13 cell lysates were treated with antibodies against p-FoxO1 (Ser-256) (Cell Signaling Technology), FoxO1 (Cell Signaling Technology), p-Akt (Ser-473) (Cell Signaling Technology), Akt (Cell Signaling Technology), p-Erk1/2 (Cell Signaling Technology), Erk1/2, Pdx1 (gift of C. V. Wright), and Shp2. For immunoprecipitation, INS-1 832/13 cell lysates containing 1 mg of total protein was treated with rabbit Shp2 antibody and protein A/G plus agarose (Santa Cruz Biotechnology), and the agarose was washed 3 times with cold HNTG buffer (20 mM Hepes, pH 7.5; 150 mM NaCl, 0.1% Triton X-100, 10% glycerol; 1 mM Na3VO4, 0.1% ZnC12; 1 mM PMSF; 1 μg/ml leupeptin; and 10 μg/ml aprotinin). Samples of immunoprecipitates were immunoblotted with antibodies against IRS2 (Upstate), P85 (Cell Signaling Technology), or Shp2 (Santa Cruz Biotechnology). Immunoblots were developed by using an ECL system (Amersham).

Cell Proliferation Assay. INS-1 832/13 cells were seeded in 96-well culture plates after siRNA treatment. At days 2, 3, and 4, cell culture medium was removed and the cells were frozen at −80 °C until the cell-counting assay. Cell growth was analyzed by using the CyQuant cell proliferation assay kit (Molecular Probes), as described previously. Data were expressed as percentages of controls.

1. Zhang EE, Chapeau E, Hagihara K, Feng GS (2004) Neuronal Shp2 tyrosine phosphatase controls energy balance and metabolism. *Proc Natl Acad Sci USA* 101:16064–16069.
2. Kulkarni RN, et al. (2002) Beta-cell-specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter beta-cell mass. *Nat Genet* 31:111–115.
3. Ueki K, et al. (2006) Total insulin and IGF-I resistance in pancreatic beta cells causes overt diabetes. *Nat Genet* 38:583–588.
4. Pagliarini DJ, et al. (2005) Involvement of a mitochondrial phosphatase in the regulation of ATP production and insulin secretion in pancreatic beta cells. *Mol Cell* 19:197–207.
5. Heit JJ, et al. (2006) Calcineurin/NFAT signalling regulates pancreatic beta-cell growth and function. *Nature* 443:345–349.

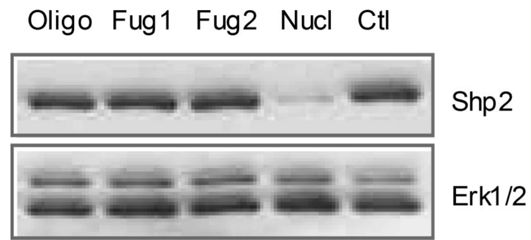

Fig. S1. Nucleoporation of siRNA is the most efficient method for Shp2 knockdown. Shown is an immunoblot analysis of Shp2 and Erk1/2 proteins in lysates of INS-1 832/13 cells treated with Shp2-specific or a scrambled (Ctl) siRNA using Oligofectamine Reagent (Oligo), FuGENE 6 Transfection Reagent at two different dosages, or nucleoporation (Nucl).

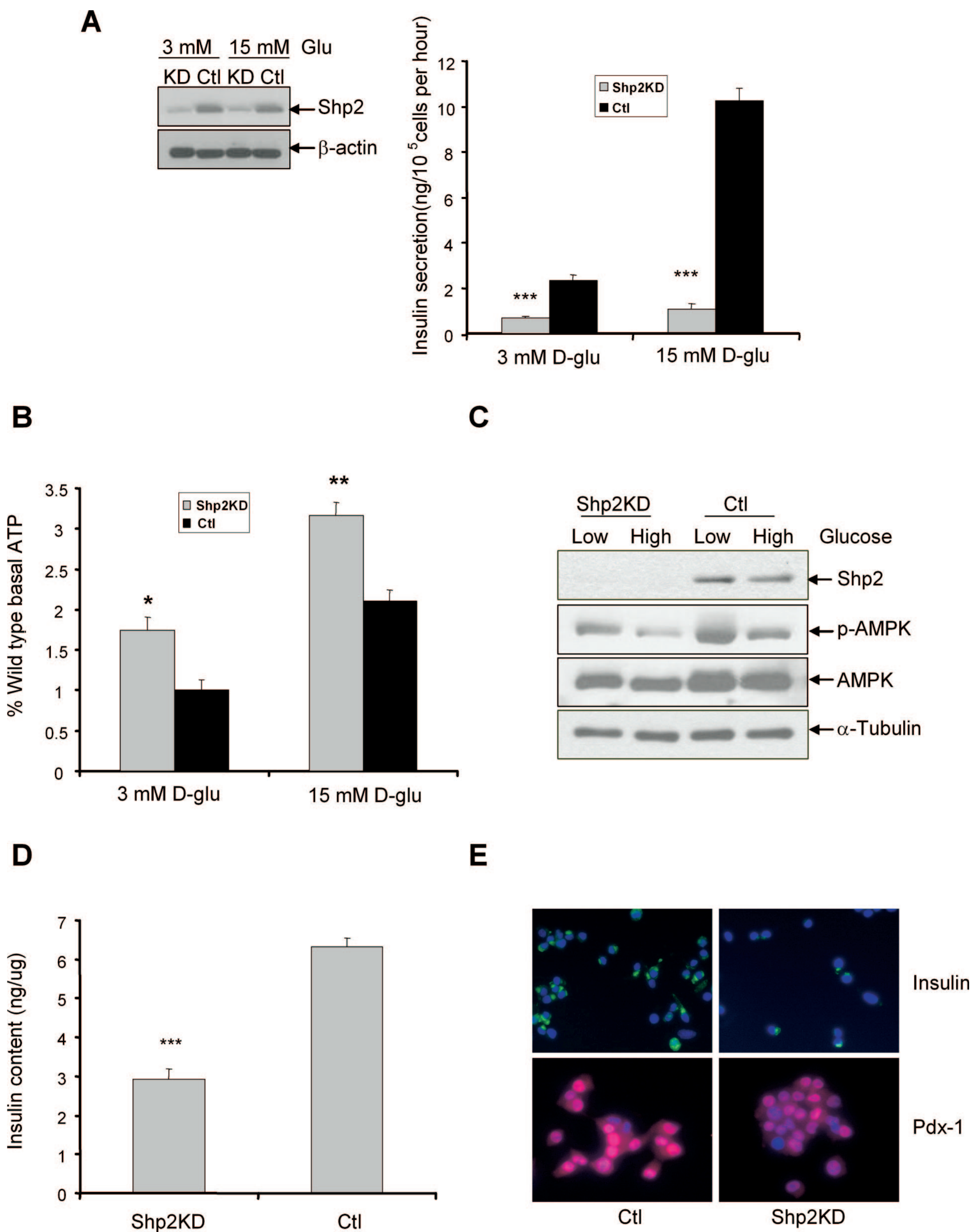

Fig. S2. Shp2 knockdown leads to markedly reduced insulin secretion, altered ATP generation, and suppressed insulin production in INS-1 832/13 cells. (*A*) (*Left*) Immunoblot analysis of Shp2 or β-actin (loading control) from whole-cell lysates from INS-1 832/13 cells treated with siRNA against either Shp2 (KD) or a scrambled siRNA (Ctl). (*Right*) Insulin secretion in INS-1 832/13 cells after stimulation with medium containing 3 or 15 mM glucose for 2 h after a 2-h preincubation with 3 mM glucose. ***, $P < 0.001$ versus controls. All data are presented as means $\pm$ SEM. (*B*) ATP content of INS-1 832/13 cells treated with siRNA against either Shp2 (KD) or a scrambled siRNA (Ctl) incubated in medium containing 3 or 15 mM glucose for 2 h after a 2-h preincubation with 3 mM glucose. *, $P < 0.05$; **, $P < 0.01$. (*C*) Immunoblots of AMPK, phosphothreonine 172 AMPK, Shp2, and α-tubulin from Shp2KD and control cells incubated with 3 or 15 mM glucose for 2 h after a 2-h preincubation with 3 mM glucose. (*D*) Insulin content (ng/μg of total protein) in Shp2 knockdown and control INS-1 832/13 cells. ***, $P < 0.001$ versus controls. (*E*) Immunofluorescence indicating insulin and Pdx1 in Shp2KD and control INS-1 832/13 cells.

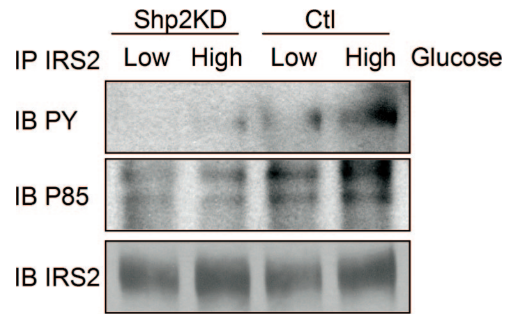

Fig. S3. Detection of IRS2 tyrosine phosphorylation and interaction with P85 in Shp2-knockdown and control cells. IRS2 immunoprecipitates from control and Shp2-knockdown cells under low or high glucose status were subjected to immunoblotting with anti-phosphotyrosine (PY), p85, and IRS2 antibodies.

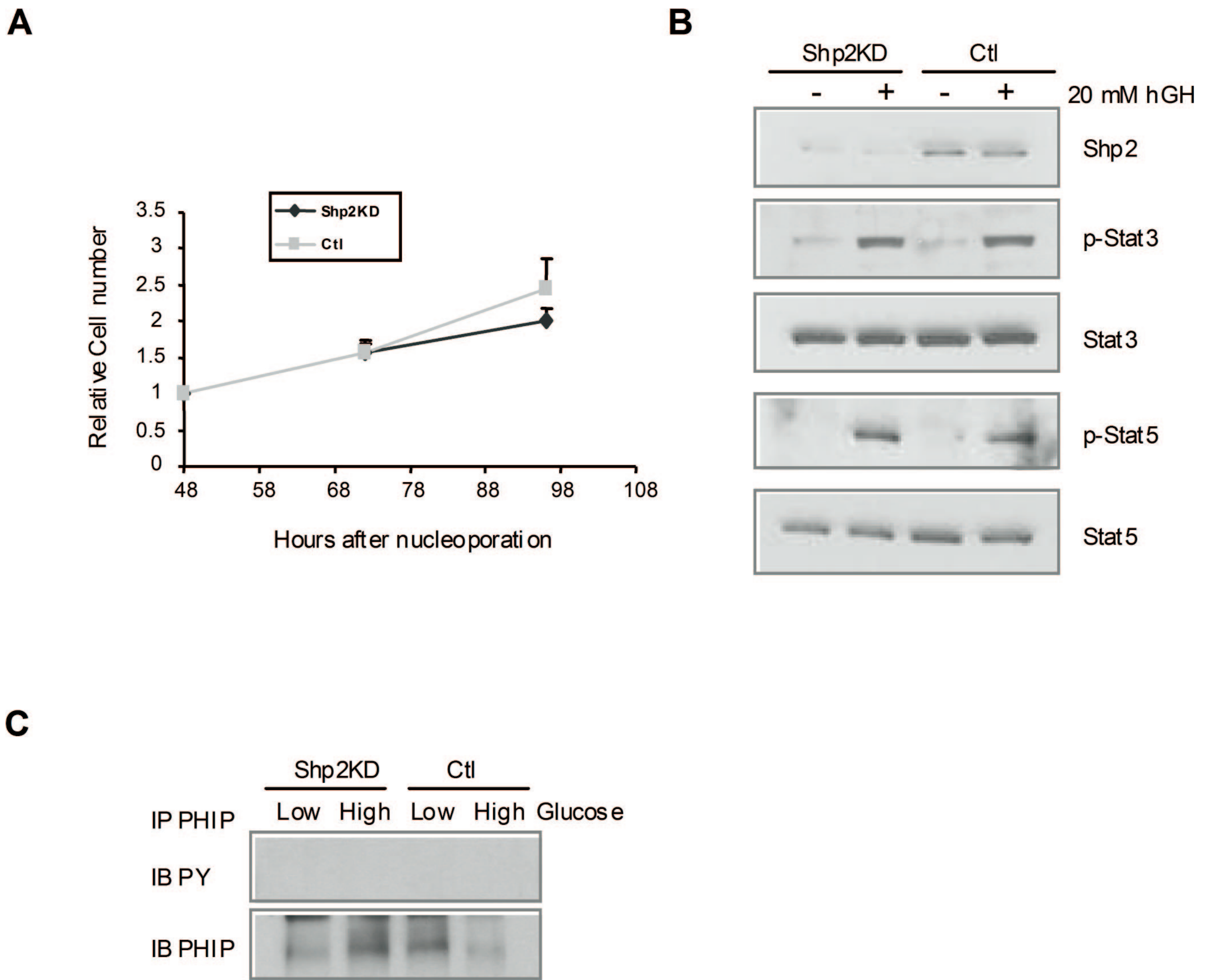

Fig. S4. Effects of Shp2 knockdown on cell proliferation and cell signaling. (*A*) CyQuant cell proliferation analysis of INS-1 832/13 cells treated with siRNA against either Shp2 or scrambled (Ctl) at indicated time points. (*B*) Immunoblots of Shp2, p-Stat3, Stat3, p-Stat5, and Stat5 in Shp2-knockdown and control cells treated with (+) or without (−) 20 mM human growth hormone. (*C*) Immunoprecipitation and immunoblot analysis of tyrosine phosphorylation of PHIP in Shp2-knockdown and control cells in low (3 mM) or high (15 mM) glucose states.

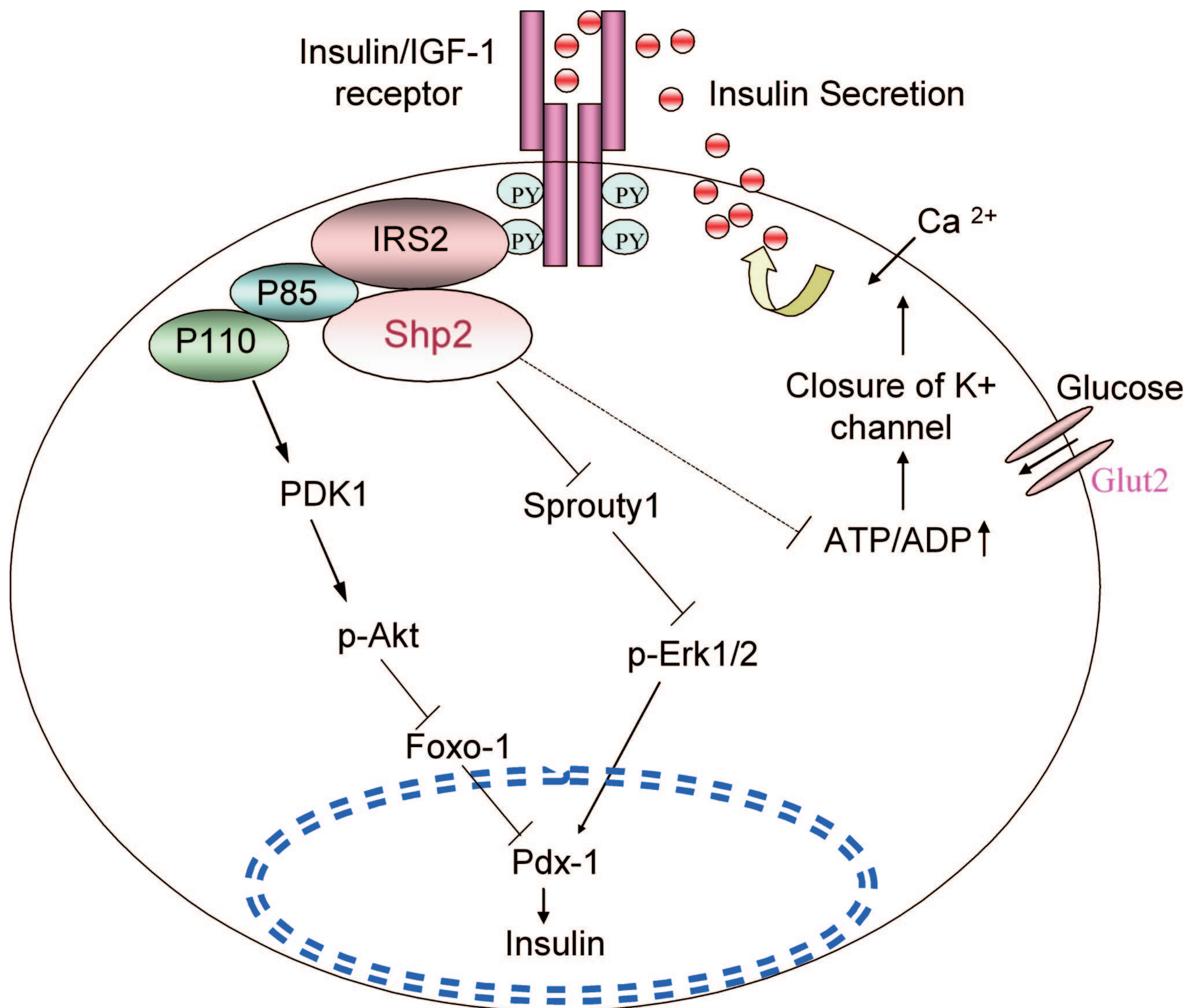

Fig. S5. A model for Shp2 orchestration of signaling events controlling insulin biosynthesis in pancreatic β-cells. Shp2 acts downstream of IRS2 in coordinated regulation of glucose and insulin signals in pancreatic β-cells. Shp2 promotes signaling through both the PI3K-Akt-FoxO1 and Erk pathways in control of Pdx1 expression and activity. Pdx1 is a major transcription factor controlling expression of insulin biosynthesis.

Table S1. List of qRT-PCR primers used for mouse islets

Primer	Sequence
Glut2-F	CTGTCGGTAATTGGCATCCG
Glut2-R	GGCATCGACTGAGCAGAAGGT
Pdx1-F	ACCCGTACTGCCTACACCCG
Pdx1-R	GGGCCGGGAGATGTATTTGT
INS1-F	GCTGGTAGAGGGAGCAGATG
INS1-R	CAGAGACCATCAGCAAGCAG
INS2-F	GAAGTGGAGGACCCACAAGT
INS2-R	AGTGCCAAGGTCTGAAGGTC
GcK-F	AGTTCCTCCTGGAGTACGACCG
GcK-R	CCCATGTACTTTCCGCCAATG
Beta2-F	CGTCAGTTTCACTATTCCCG
Beta2-R	GCCTTCTGTAAACAGGACAGTCAC
Ccnd1-F	CTCTCCTGCTACCGCACAACG
Ccnd1-R	GCAGTCCGGGTCACACTTGAT
Ccnd2-F	CAAGCTGAAAGAGACCATCCCG
Ccnd2-R	CAGTTCCCACTCCAGCAGCTC
Cdk4-F	GCACAGACATCCATCAGCCG
Cdk4-R	CGTGAGGTGGCCTTGTTAAGGA
Shp2-F	CATGGCTGTCCAGCACTACA
Shp2-R	TGTCCTTTTCTTTTGCTTTTCTG
c-Myc-F	TTTGTCTATTTGGGGACAGTGTT
c-Myc-R	CATCGTCGTGGCTGTCTG

Table S2. List of qRT-PCR primers used for INS-1 832/13 cells

Primer	Sequence
Glut2-F	TTTCTGTGCCGTCTTCATGT
Glut2-R	CCGTCATGCTCACATAACTCA
Pdx1-F	CTCTCGTGCCATGTGAACC
Pdx1-R	TTCTCTAAATTGGTCCCAGGAA
INS1-F	GACCTTGGCACTGGAGGTT
INS1-R	CCAGTTGGTAGAGGGAGCAG
INS2-F	CGAAGTGGAGGACCCACA
INS2-R	TGCTGGTGCAGCACTGAT
GcK-F	GCCCAGTTGTTGACTCTGGT
GcK-R	CATCACCTTCTTCAGGTCTTCC
Beta2-F	ACGCAGAAGGCAAGGTGT
Beta2-R	TTTGGTCATGTTTCCACTTCC
Hnf1α-F	CTCAGCACCAGTCCCACAG
Hnf1α-R	CGTTGGAGTCAGAACTCTGGT
Hnf4α-F	CAAGAGGTCCATGGTGTTCA
Hnf4α-R	CCGAGGGACGATGTAGTCAT
NFATc1-F	AGTTATGGCCAGACAGCACCATCT
NFATc1-R	TGTGCAGCTACACGGTTACTTGGA
MafA-F	CGAGTACGTCAACGACTTCG
MafA-R	AAGAGGGCACCGAGGAGCAG
Ccnd1-F	GCACAACGCACTTTCTTTCC
Ccnd1-R	TCCAGAAGGGCTTCAATCTG
Ccnd2-F	CACCGACAACTCTGTGAAGC
Ccnd2-R	CCACTTCAGCTTACCCAACAC
Cdk4-F	GTCAGTGGTGCCGGAGAT
Cdk4-R	GGATTAAAGGTCAGCATTTCCA
c-Myc	GCTCCTCGCGTTATTTGAAG
c-Myc	GCATCGTCGTGACTGTCG