Supplementary material

***Mouse studies*.** Generation of TCPTP-floxed ($TCPTP^{fl/fl}$) mice on C57Bl/6J background has been reported previously [1]. Pdx1-*Cre* mice on C57Bl/6J background were provided by Dr. D. Melton (Harvard University) [2]. Mice were maintained on a 12 h light-dark cycle in a temperature-controlled facility, with ad lib access to water and food. Mice were fed regular laboratory chow (Purina laboratory chow, # 5001) at weaning, and in a separate cohort, switched to a high fat diet (HFD) (60% kcal from fat, # D12492, Research Diets, New Brunswick, NJ, USA) at four weeks of age. Genotyping for the TCPTP floxed allele and *Cre* was performed as described previously [1]. Glucose was measured in blood collected from the tail vein using a glucometer (Home Aide Diagnostics, Deerfield Beach, FL, USA). Insulin concentration in serum and cell culture media was determined using the ultra-sensitive mouse insulin ELISA kit (Crystal Chem, Downers Grove, IL, USA). Plasma glucagon was measured using RIA (Linco Research, St. Charles, MO, USA). Fed glucose measurements were taken between 7-9 AM and, where indicated, from mice fasted for 12 h. For insulin tolerance tests (ITTs), mice were fasted for 4 h and injected intraperitoneally (IP) with 0.75-1 mU/g human insulin (HumulinR; Eli Lilly, Indianapolis, IN, USA). Blood glucose values were measured before and at 15, 30, 60 and 90 minutes post-injection. For glucose tolerance tests (GTTs), overnight-fasted mice were injected with 20% D-glucose (2 mg/g body weight), and blood glucose was measured before and at 15, 30, 60, and 120 minutes following injection. *In vivo* glucose-stimulated insulin secretion (GSIS) was performed on overnight-fasted mice injected with glucose (3 mg/g body weight) and blood was collected before and at 2, 20 and 60 minutes following injection for determination of insulin concentrations. For *ex vivo* GSIS, primary islets from panc-TCPTP KO and control mice were isolated using the intra-ductal collagenase method [3]. Following overnight culture, 20 size-

matched islets were hand-picked and starved for 1 h in Krebs Ringer Buffer (KRB) containing 2 mmol/l glucose. After starvation, medium was exchanged with 3.3 mM or 16.7 mM glucose in the absence or presence of 10 nmol/l glucagon-like peptide-1 (GLP-1) (Sigma, St. Charles, MO, USA). Media were collected for insulin measurements and islets lysed to determine total protein content, and insulin levels were normalized to islet protein content.

***Cell culture*.** MIN6 β-cell lines were maintained in DMEM containing 25 mmol/l glucose, 15% fetal bovine serum, 50 U/ml penicillin and 50 μg/ml streptomycin. TCPTP silencing was achieved by testing five different hairpins in individual lentiviral vectors (Open Biosystems, Huntsville, AL, USA) to generate cell lines by puromycin-induced selection. Packaging (psPAX2) and envelope (pMD2.G) vectors were obtained from Addgene. Lentiviruses were generated by co-transfection of vectors into HEK293FT cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following manufacturer's guidelines. MIN6 beta-cells were infected with the generated viruses, selected using puromycin (2 μg/ml) and drug-resistant pools of cells propagated. Reconstitution was performed by transfecting TCPTP knockdown cells with shRNA-resistant human TCPTP wild type (WT) and neomycin-resistant pools of cells were generated. STAT1 and STAT3 silencing was achieved using respective siRNAs (Santa Cruz Biotechnology, Santa Cruz, CA, USA) according to manufacturer instructions. Briefly, cells were seeded in 6-well culture plates at a density of 2.0×10^4 cells/cm^2 and cultured in antibiotic-free DMEM supplemented with 10% FBS. Cells were then transfected using a solution of 0.1μmol/l siRNA and 0.5% liposome transfection reagent (Santa Cruz Biotechnology) for 6 h. Media were changed 24 h after transfection and cells were used following an additional 24 h incubation period.

Pancreas immunostaining. For immunofluorescence pancreata were fixed in Z-fix (Fisher Scientific, Pittsburgh, PA, USA) and imbedded in paraffin. Sections of the pancreas were stained with guinea pig insulin (Zymed Laboratories, San Francisco, CA, USA) or TCPTP (Medimabs, Montreal, Quebec, Canada) antibodies overnight at 4°C. Detection was performed with appropriate fluorescein-conjugated secondary antibodies (Jackson Immunoresearch, West Grove, PA, USA) and visualized using Olympus BX51 microscope. Islet size and number were determined by using ImageJ software (NIH; http://rsb.info.nih.gov/ij/). Briefly, three consecutive sections (200 μm apart) of each pancreas were stained with guinea pig insulin antibodies. Pictures of every islet (insulin-stained area) were acquired to determine islet size and number. Each islet was manually outlined using ImageJ and islet surface area was calculated and recorded. The surface area of each of the three pancreas sections (per animal) was also determined using this method. Percentage of islet surface area to pancreas surface area was calculated by dividing the average surface area of islets in a pancreas section by the surface area of that section. Final islet surface area to pancreas surface area was represented by the average of the percent values. Islet number was represented by the average number of islets per pancreas section in three different sections. Islet size was presented as number of islets per range of sizes.

Biochemical analyses. MIN6 beta-cells and pancreatic islets were lysed using radio-immunoprecipitation assay (RIPA) buffer (10 mmol/l Tris-HCl, pH: 7.4, 150 mmol/l NaCl, 0.1% sodium dodecyl sulfate [SDS], 1% Triton X-100, 1% sodium deoxycholate, 5 mmol/l EDTA, 1 mmol/l NaF, 1 mmol/l sodium orthovanadate and protease inhibitors). Lysates were clarified by centrifugation and protein concentrations were determined using a bicinchoninic acid protein assay kit (Pierce Biotechnology, Rockford, IL, USA). Proteins were resolved by SDS-PAGE and transferred to PVDF membranes. For substrate-trapping experiments, cells were lysed in 1%

NP40 buffer with a protease inhibitor cocktail (without sodium orthovanadate). Immune complexes were collected on PureProteome beads (Millipore, Temecula, CA, USA) and washed three times with lysis buffer and once with PBS. Immunoblotting of lysates and immunoprecipitates was performed with antibodies for PTP1B, PDX1 (Abcam, Cambridge, MA, USA), GLUT2, TCPTP, SHP2, phosphotyrosine (PY99), pSTAT3 Tyr705, STAT3, pSTAT1 Tyr701, STAT1, CaCna1d, pSTAT3 Tyr705, STAT3, GK, Kir6.2 and Tubulin (all from Santa Cruz Biotechnology), and ERK (Cell signaling Technology, Danvers, MA, USA). After incubation with appropriate secondary antibodies, proteins were visualized using enhanced chemiluminescence (ECL, Amersham Biosciences, Piscataway, NJ, USA). Pixel intensities of immunoreactive bands were quantified using ImageQuant 5.0 software (Alpha Innotech, San Jose, CA, USA).

For reverse transcription-quantitative real-time PCR total RNA was extracted from cells using TRIzol reagent (Invitrogen). cDNA was generated using high-capacity cDNA synthesis kit from Applied Biosystems (High Capacity cDNA Reverse Transcription Kit). *CaCna1d, Gk, Glut2*, *Ins1*, *Ins2*, *Kir6.2*, *Pdx1* and *Tcptp* mRNA were assessed by SYBR Green quantitative real time PCR (iCycler, BioRad, Hercules, CA, USA) using the ΔCT method with appropriate primers (ESM Table 1) and normalized to *Tata-box binding protein* (*Tbp*).

TCPTP enzymatic activity. MIN6 beta-cells were pre-treated with TCPTP inhibitor (compound 8; 50 nmol/l) for 2 h then lyzed in NP40 buffer (10 mmol/l Tris-HCl pH: 7.4, 150 mmol/l NaCl, 1 mmol/l EDTA, 1% NP-40, 5% glycerol and proteases inhibitors). One mg of total protein lysates was used to immunoprecipitate TCPTP using PureProteome beads (Millipore). The precipitate was washed twice with ice-cold lysis buffer then resuspended in 50 μl reaction buffer (50 mmol/l HEPES, pH: 5.0, 1 mmol/l EDTA, 100 mmol/l NaCl and 5 mM DTT) for 10

minutes, and the reaction was initiated by the addition of p-Nitrophenyl Phosphate (pNNP, 5 mmol/l). TCPTP activity was monitored by measuring the absorbance of pNNP at 405 nm using Wallac Victor 2 plate reader.

References

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