Supporting Information

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SI Materials and Methods

Reagents. Chemicals were from Sigma unless specified otherwise. Validated biologically active recombinant mouse SLIT1, SLIT2, and SLIT3 were from R&D Systems. Rabbit polyclonal antibodies to SLIT1 and ROBO2 were from Sigma. Polyclonal antibodies to SLIT2 (rabbit) and ROBO1 (goat) were from Santa Cruz. Purchased antibodies included rabbit polyclonal SLIT3 and guinea pig polyclonal insulin from Millipore, mouse monoclonal glucagon from Sigma, and mouse monoclonal β-actin from Novus. Rabbit polyclonal antibodies to phospho-Akt (Ser473), Akt, Erk1/2, phospho-Ask1 (Thr845), ASK1, caspase-12, cleaved caspase-7, mouse monoclonal antibody phospho-Erk1/2 (Thr202/Tyr204), and rabbit monoclonal antibody cleaved caspase-3 were from Cell Signaling Technology. Mouse monoclonal Chop antibody was from Thermo. ELISA kits used for quantification of SLIT1, SLIT2, and SLIT3 were from Uscn Life Science Inc.

Primary Islet Isolation, Cell Culture, and Perifusion. Pancreatic islets were isolated from 6- to 30-wk-old male C57BL/6J mice (Jax) using collagenase and filtration. Mice were housed and euthanized in accordance with the University of British Columbia Animal Care Committee guidelines. Human islets (>80% purity estimated by dithizone staining) and pancreata were provided by Garth Warnock (University of British Columbia, Vancouver), collected via protocols approved by the University of British Columbia Institutional Advisory Board. Donors, with informed consent, were men or women aged 23–56 y. None of the donors was known to have diabetes. The islets were further handpicked using a bright-field microscope. Islets were cultured overnight (37 °C, 5% CO_2) in RPMI1640 medium (Invitrogen) with 5 mM glucose (Sigma), 100 units/mL penicillin, 100 μg/mL streptomycin (Invitrogen), and 10% vol/vol FBS (Invitrogen) as described in more detail elsewhere (1, 2). MIN6 cells were cultured in DMEM (Invitrogen) containing 22 mM glucose, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% vol/vol FBS as described (2, 3). To measure insulin secretion, mouse islets were perifused (4) and hormone secretion was measured by RIA (Rat Insulin RIA Kit; Millipore).

RT-PCR. Total RNA was isolated from mouse primary islet and MIN6 cells using RNeasy Mini or Micro kits (Qiagen). RT (qScript cDNA SuperMix; Quanta Biosciences) was used to generate cDNA. TaqMan quantitative RT-PCR (qRT-PCR) was conducted using probes from Integrated DNA Technologies and PerfeCTa qPCR SuperMix (Quanta) on a StepOnePlus device (Applied Biosystems). Relative gene expression changes were analyzed by $2^{-\Delta C_t}$ or $2^{-\Delta\Delta C_t}$ methods as indicated in the figure legends. Unless specified otherwise, *Hprt1* or *Ppia* were used as reference genes.

siRNA-Mediated Knockdown of Slits. MIN6 and mouse islet cells dispersed with 0.01% trypsin-EDTA (Invitrogen) were transfected with a combination of Silencer Select siRNA (Ambion) targeting *Slit1*, *Slit2*, and *Slit3*. Cells transfected with scramble siRNA (Ambion) was used as negative control. Neon transfection (Invitrogen) with 100 nM of each siRNA for MIN6 and 200 nM for dispersed mouse islet cells was used. Cells were analyzed by qRT-PCR and immunoblotting at least 48 h following transfection.

Immunofluorescence Imaging. MIN6 and dispersed islet cells were fixed in 4% wt/vol paraformaldehyde (10 min) and permeabilized using 0.1% vol/vol Triton X-100 (10 min). Antigen retrieval was conducted on deparaffinized pancreas sections by boiling for 15 min in sodium citrate buffer (10 mM $Na_3C_6H_5O_7$, 0.05% vol/vol Tween-20, pH 6.0). Normal goat serum (10% vol/vol) was used for blocking. Primary antibodies (1:50–1:200) were incubated overnight at 4 °C. Alexa Fluor 488-, 555-, and 647-conjugated secondary antibodies (1:400; Invitrogen) were incubated for 1 h at 20 °C, before mounting in Vectashield (Vector Laboratories). Cells were imaged using an inverted microscope equipped with 0.75 numerical aperture (NA) 20× and 1.45 NA 100× objectives. For cell death assays, MIN6 and dispersed islet cells were seeded into 96-well plates and stained with 0.05 μg/mL Hoechst 33342 (Invitrogen), 0.5 μg/mL propidium iodide (Sigma), and Alexa Fluor 647-conjugated Annexin V (1:250; Invitrogen) (5). Following treatments, cells were imaged with ImageXpress^{MICRO} (Molecular Devices) every 1 or 2 h at 37 °C and 5% CO_2 (2).

To detect F-actin, mouse islet cells were fixed with Z-FIX (Anatech Ltd.) and stained with Alexa Fluor 488-conjugated phalloidin (Invitrogen). Beta cells were identified by insulin immunostaining (Santa Cruz Biotechnology). Cell images were captured with a Zeiss AxioCam HRm and acquired with AxioVision 4.8 imaging software (Carl Zeiss MicroImaging). Line scans were performed using ImageJ software (version 1.41o; National Institutes of Health) and mean peak-intensity calculated.

Immunoblotting. MIN6 and islet cells were lysed with cell lysis buffer (Cell Signaling) containing protease inhibitors (Calbiochem). Lysates were sonicated for 20 s then centrifuged for 10 min at 10,000 × *g*. Protein concentrations were determined using a bicinchoninic acid assay (Thermo). Proteins were separated on 8 or 12% wt/vol SDS/PAGE gels and transferred to polyvinylidene fluoride membranes. After blocking (0.2% wt/vol I-block, 0.1% vol/vol Tween-20 PBS), membranes were probed with primary antibodies (1:1,000), followed by horseradish peroxidase-conjugated secondary antibodies (1:3,000; Cell Signaling). Immunodetection was performed using enhanced chemiluminescence (Thermo). PathScan intracellular signaling array was used for islet lysates with low protein yield, following the manufacturer's protocol (Cell Signaling).

Cytosolic and Endoplasmic Reticulum Ca^{2+} Imaging. MIN6 and dispersed mouse islet cells seeded onto glass coverslips were transfected with the D1ER FRET probe (5, 6) and imaged 48 h later. Cytosolic Ca^{2+} was imaged using Fura-2-AM (Invitrogen) as described (3). Briefly, dispersed mouse islet cells seeded onto glass coverslips were loaded with 5 μM of Fura-2-AM for 30 min at 37 °C. For cytosolic and endoplasmic reticulum (ER) Ca^{2+} imaging, cells were incubated in Ringer's solution containing (in mM): 5.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 20 Hepes, 141 NaCl, and 3 glucose. Solutions were maintained at 37 °C and cells were imaged using an inverted microscope at 5- or 10-s intervals (Zeiss 200m; Intelligent Imaging Innovations) operated by Slidebook 5.0 software (Intelligent Imaging Innovations). Long-term ER Ca^{2+} changes were imaged with ImageXpress^{MICRO} systems at 1 min intervals under 37 °C and 5% CO_2 conditions.

Statistics. Data are expressed as mean ± SEM unless otherwise indicated. Results were considered statistically significant when $P < 0.05$ using Student *t* test or ANOVA, where appropriate (GraphPad Prism; GraphPad,).

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2. Yang YH, et al. (2011) Paracrine signalling loops in adult human and mouse pancreatic islets: Netrins modulate beta cell apoptosis signalling via dependence receptors. *Diabetologia* 54(4):828–842.
3. Szabat M, Luciani DS, Piret JM, Johnson JD (2009) Maturation of adult beta-cells revealed using a Pdx1/insulin dual-reporter lentivirus. *Endocrinology* 150(4):1627–1635.
4. Dror V, et al. (2007) Notch signalling suppresses apoptosis in adult human and mouse pancreatic islet cells. *Diabetologia* 50(12):2504–2515.
5. Luciani DS, et al. (2009) Roles of IP3R and RyR Ca2+ channels in endoplasmic reticulum stress and beta-cell death. *Diabetes* 58(2):422–432.
6. Gwiazda KS, Yang TL, Lin Y, Johnson JD (2009) Effects of palmitate on ER and cytosolic Ca2+ homeostasis in beta-cells. *Am J Physiol Endocrinol Metab* 296(4):E690–E701.

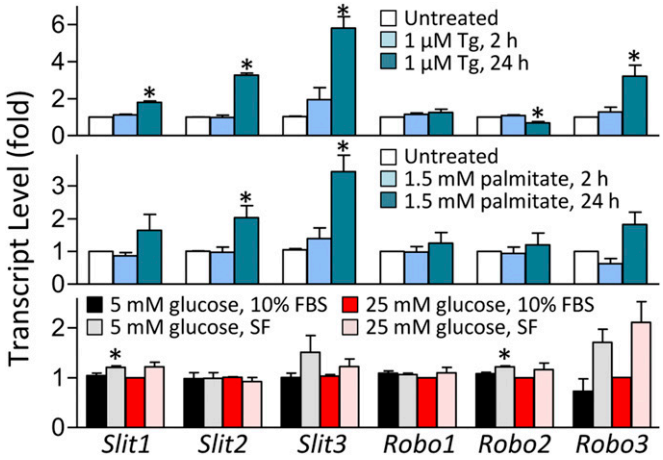

Fig. S1. Expression of Slit and Robo in MIN6 cells under stress conditions. Changes in Slit and Robo expression in MIN6 cells treated with 1 μM thapsigargin (Tg), 1.5 mM palmitate, or 5 mM or 25 mM glucose containing medium supplemented with 10% FBS or under serum-free (SF) conditions. Fold change in transcript level were calculated using $2^{-\Delta\Delta C_t}$ (n = 3–4, mean ± SEM, *P < 0.05 compared with untreated).

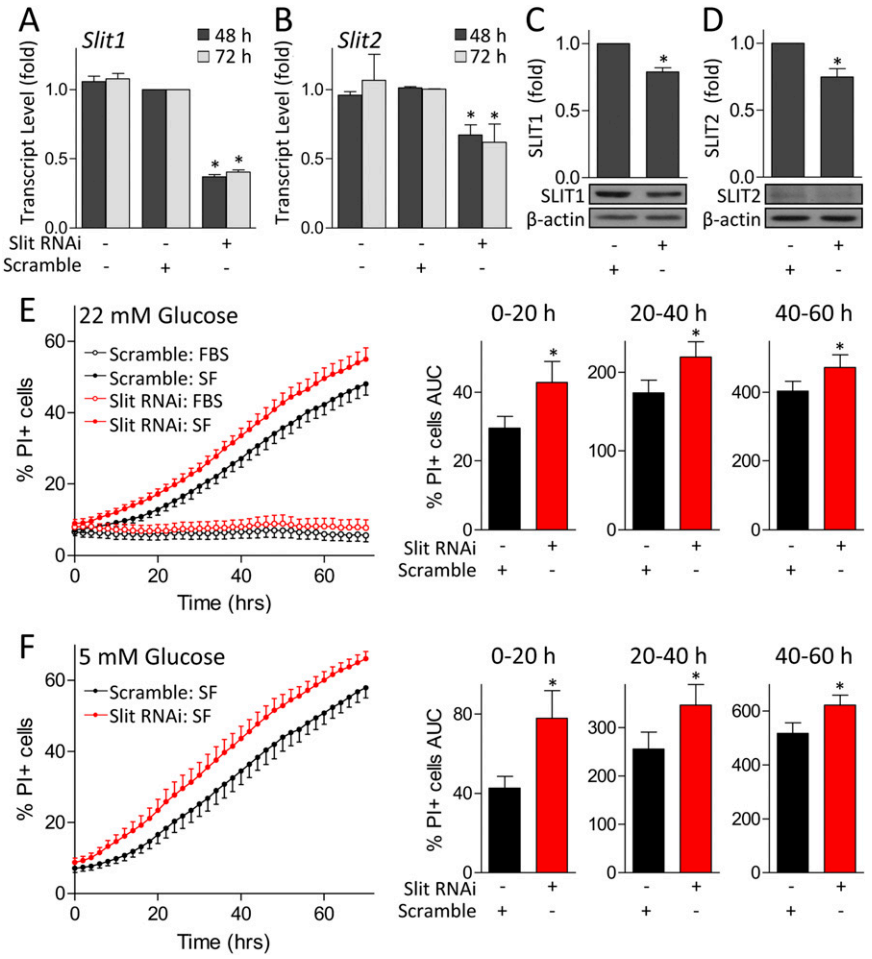

Fig. S2. Knockdown of endogenous Slits increases MIN6 cell death following serum starvation. (*A* and *B*) MIN6 cells were transfected with siRNA for Slit1, Slit2, and Slit3 or scramble siRNA as control. Forty-eight and 72 h following transfection, the fold change in transcript levels of Slit1 and Slit2 were analyzed by qRT-PCR using $2^{-\Delta\Delta C_t}$ ($n = 5$, mean ± SEM, $^*P < 0.05$ compared with control at the same time point). (*C* and *D*) The protein level of SLIT1 and SLIT2 knockdown was analyzed by immunoblotting 48 h following transfection ($n = 4$–5, mean ± SEM, $^*P < 0.05$ compared with control). (*E* and *F*) MIN6 cells transfected with Slit siRNAs were stained with 0.05 μg/mL Hoechst and 0.5 μg/mL propidium iodide (PI) 48 h following transfection. Cells were treated with 22 mM (*E*) and 5 mM (*F*) glucose SF conditions and imaged at 37 °C and 5% CO_2. The percentage of PI-positive cells was determined and area under the curve (AUC) was calculated for the indicated time intervals ($n = 10$, mean ± SEM, $^*P < 0.05$ compared with control).

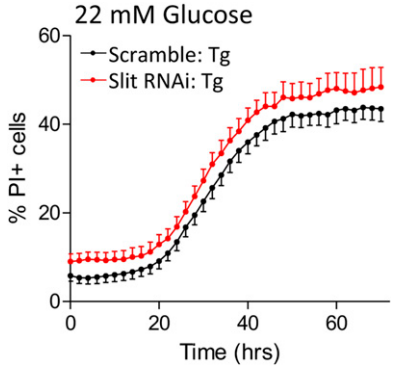

Fig. S3. Knock-down of endogenous Slits has no effect on Tg-induced beta-cell death. MIN6 cells were transfected with siRNA for *Slit1*, *Slit2*, *and Slit3* or scramble siRNA as control, then stained with 0.05 μg/mL Hoechst 33342 and 0.5 μg/mL PI 48 h following transfection. Cells were treated with 1 μM Tg in 22 mM glucose serum-containing conditions and imaged under stable incubation at 37 °C and 5% CO_2. The percentage of PI-positive cells was determined ($n = 10$, mean ± SEM).

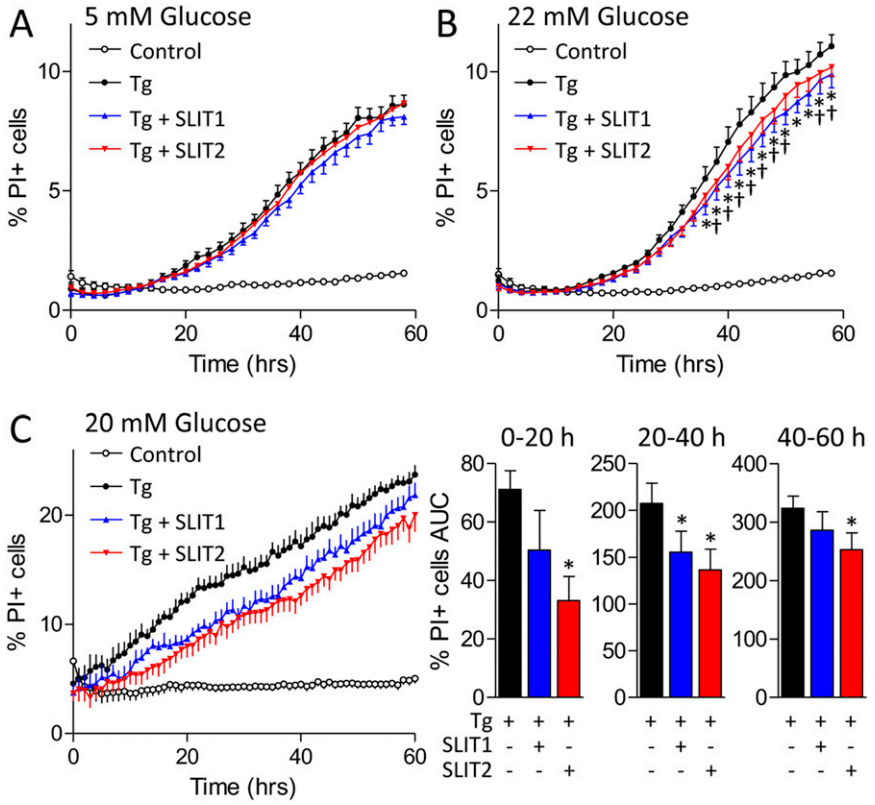

Fig. S4. Slits reduce ER stress–induced beta-cell death under high-glucose conditions. (*A* and *B*) MIN6 cells were stained with 0.05 μg/mL Hoechst and 0.5 μg/mL PI. Cells were imaged under at 37 °C and 5% CO_2. The percentage of PI-positive cells was determined following 0.1 μM Tg treatments with 10 nM SLIT1 or SLIT2 under 5 mM (*A*) and 22 mM (*B*) glucose conditions with serum (n = 14–15, mean ± SEM, $^*P < 0.05$ Tg + SLIT1 compared with Tg treatment; $^{\dagger}P < 0.05$ Tg + SLIT2 compared with Tg treatment at the same time point). (*C*) Dispersed mouse islet cells were stained with 0.05 μg/mL Hoechst and 0.5 μg/mL PI. Cells were imaged at 37 °C and 5% CO_2. The percentage of PI-positive cells was determined following 1 μM Tg treatments with 10 nM SLIT1 or SLIT2 under 20 mM glucose conditions; area under the curve (AUC) was calculated for the indicated time intervals (n = 6–8, mean ± SEM, $^*P < 0.05$ compared with 1 μM Tg or SF treatment).

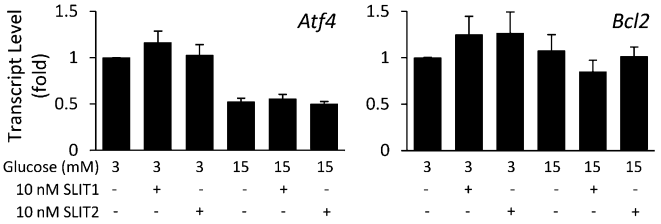

Fig. S5. Slits have no effect on *Atf4* and *Bcl2* expression in mouse islet cells. Mouse islet cells were treated with SLIT1 or SLIT2 for 4 h before RNA isolation. qRT-PCR analysis of *Atf4* and *Bcl2* were expressed as fold change using $2^{-\Delta\Delta C_t}$ calculations (n = 8, mean ± SEM).

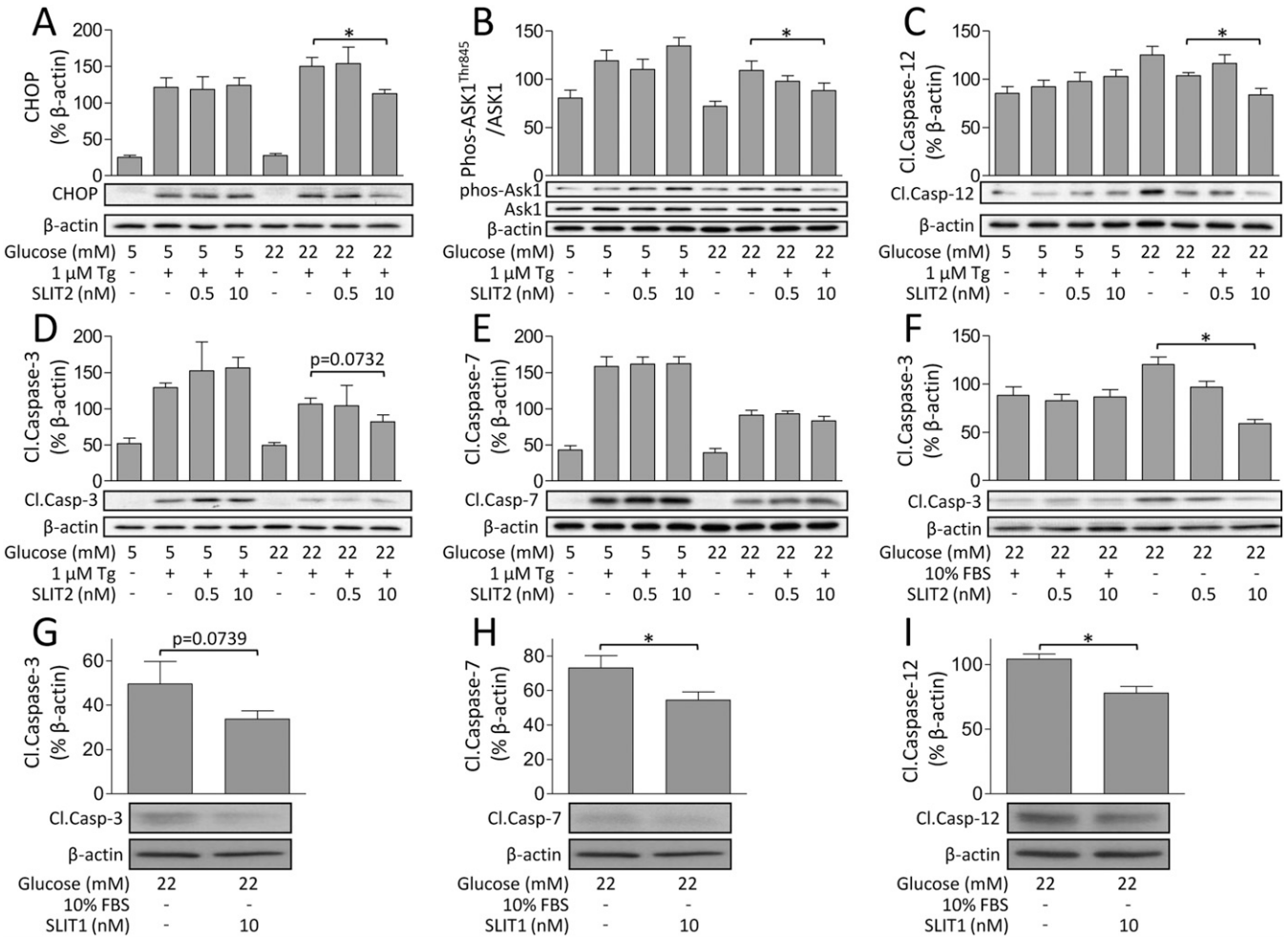

Fig. S6. Slits can down-regulate proapoptotic and ER-stress signaling in MIN6 cells. MIN6 cells were treated with 1 μM Tg in the presence or absence of SLIT2. Immunoblotting for protein levels of CHOP (*A*), ASK1 (*B*) and cleaved (Cl.) Caspase-12 (*C*), Cl. Caspase-3 (*D*) and Cl. Caspase-7 (*E*) ($n = 7$, mean ± SEM, $*P < 0.05$). (*F*) MIN6 cells were cultured in serum containing or serum free conditions in the presence or absence of SLIT2. Immunoblotting for Cl. Caspase-3 ($n = 4$–7, mean ± SEM, $*P < 0.05$). (*G–I*) MIN6 cells were cultured in serum-free conditions in the presence or absence of SLIT1. Immunoblotting for Cl. Caspase-3 (*G*), Cl. Caspase-7 (*H*) and Cl. Caspase-12 (*I*) ($n = 6$–7, mean ± SEM, $*P < 0.05$).

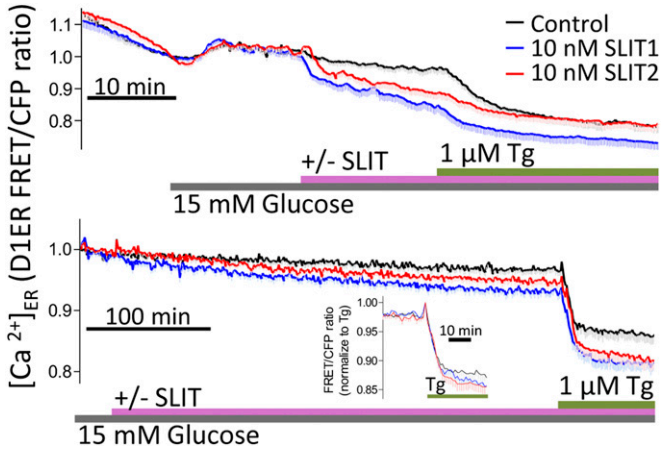

Fig. S7. Slits can modulate ER Ca^{2+} signaling in MIN6 cells. MIN6 cells were transfected with D1ER cameleon and ER Ca^{2+} level was imaged. (*Top*) Cells were exposed to 1 μM Tg 15 min following treatment with 10 nM SLIT1 or SLIT2 under 15 mM glucose ($n = 24$–28, mean ± SEM). (*Bottom*) Cells were exposed to 1 μM Tg 6 h following treatment with 10 nM SLIT1 or SLIT2 under 15 mM glucose (D1ER FRET/CFP ratios were normalized to ratio at first time point; $n = 28$–41, mean ± SEM). (*Inset*) D1ER FRET/CFP ratios were normalized to ratio at time point following Tg addition.

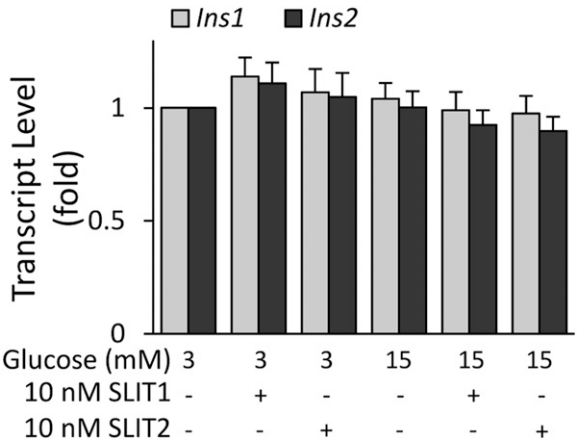

Fig. S8. Slits have no effect on insulin gene expression in mouse islet cells. Mouse islet cells were treated with SLIT1 or SLIT2 for 4 h before RNA isolation. qRT-PCR analysis of *Ins1* and *Ins2* were expressed as fold change using $2^{-\Delta\Delta C_t}$ calculations ($n = 8$, mean ± SEM).