Supplementary Methods

Cell culture

INS-1E cells (generously provided by Claes Wollheim and Pierre Maechler, University of Geneva, Switzerland) and INS-1E-PIG cells (1) were cultured in RPMI 1640 supplemented with 10% FBS, 1% Pen/Strep, 1% sodium pyruvate, and 55 μM β-mercaptoethanol (all from Life Technologies). Cells were maintained in flasks pre-coated with supernatant from rat 804G cell line (804G matrix) as previously described (2). Basal media for INS-1E cells consisted of RPMI-1640 supplemented with 1% FBS, 1% Pen/Strep, 1% fatty acid free BSA, and 50 μM β-mercaptoethanol. GLT media for INS-1E consisted of RPMI 1640 supplemented with 1% FBS, 1% Pen/Strep, 1% fatty acid free BSA, 50 μM β-mercaptoethanol, 25 mM glucose, and 0.5 mM sodium palmitate. Sodium palmitate was dissolved in warmed 4% BSA in PBS before being added to RPMI1640.

Human islets

Islets were obtained from the Integrated Islet Distribution Program (IIDP) and Prodo Laboratories. Dissociation and plating were performed as previously described (3). Basal media for human islets consisted of CMRL 1066 supplemented with 1% fatty acid free BSA and 1% FBS. GLT media for human islets consisted of CMRL 1066 supplemented with 1% fatty acid free BSA, 1% FBS, 30 mM glucose and 1 mM sodium palmitate.

Compounds

All compounds were commercially purchased. CX4945 (Silimitasertib), KD025 (SLx-2119), Y-27632, Fasudil, from Selleckchem; Rho Kinase Inhibitor V, SR3677 from Sigma-Aldrich; and AS-1892802, GSK429286A, (S)-H-1152, RKI-1447 from Cayman Chemicals. Stock solutions were prepared in DMSO and stored as per manufacturer instructions.

Experimental design

INS-1E or INS-1E-PIG or dissociated human islets were seeded in the relevant culture media. After 24 hours, culture media was removed, basal media (+/- compounds) and/or GLT media (+/- compounds) were added and 48 hours later, viability or GSIS experiments were performed.

Insulin Content

INS-1E-PIG cells were subjected to GSIS as previously described (1) Insulin content was inferred by intracellular luciferase, which was measured using Pierce Gaussia luciferase glow assay kit (Thermo Scientific) according to manufacturer's instructions.

Caspase-3/7 activity

INS-1E cells were treated with GLT-inducing media with or without KD025 as previously described (4). Caspase-3/7 activity was measured using Caspase-Glo 3/7 assay kit (Promega) following manufacturer's instructions.

Kinase Profiling

Briefly, streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. Ligand-coated beads were blocked with excess biotin and washed with blocking buffer to remove unbound ligand and reduce non-specific binding. Binding reactions were assembled by combining kinases, ligand affinity beads, and test compounds in binding buffer. Test compounds were prepared as 40x stocks in DMSO and directly diluted into assay reactions. Assay reactions were performed in 384-well plate and incubated at room temperature. Beads were re-suspended in elution buffer and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR and was used to calculate percent kinase activity compared to control (5).

Western Blotting

INS-1E cells transfected with siRNA or *Csnk2a1-Flag* vector were trypsinized, washed twice with cold PBS and pelleted for storage at -80°C. Thawed cell pellets were lysed in RIPA lysis buffer (Thermo Fisher). Protein concentration was quantified using BCA protein assay (Thermo Fisher). Approximately 20-35 μg of protein lysate was run on a Bolt 4-12% Bis-Tris SDS-Page gel (Thermo Fisher) and electrically transferred to PVDF membranes either by wet transfer (ROCK2) using XCell II Blot Module (Thermo Fisher) or semi-dry transfer using iBlot 2 system (Csnk2a1/2) (Thermo Fisher). PVDF membranes were washed with TBST and incubated with SuperBlock Blocking Buffer in TBS (ROCK2) (Thermo Fisher) or Intercept (TBS) Blocking Buffer (Csnk2a1/2) (Li-Cor) for 1 hour at room temperature. Primary antibody (1:1000) incubation was overnight at 4°C: mouse anti-ROCK2 (BD Biosciences, Cat # 610623), rabbit anti-actin-β (Cell Signaling, Cat #4967), rabbit anti-Csnk2a1 (Thermo Fisher, Cat #PA5-28686), rabbit anti-Csnk2a2 (Thermo Fisher, Cat #PA5-109601), rabbit anti-Hprt1 (Thermo Fisher, Cat #PA5-22281). Secondary antibody (1:5000) incubation was for 1 hour at room temperature: anti-Rabbit HRP-linked (Cat #7074), anti-Mouse HRP-linked (Cat #7076) (Cell Signaling), anti-Rabbit IRDye 680 RD (Li-Cor), anti-Rabbit IRDye 800 CW (Li-Cor). Membranes incubated with HRP-linked antibodies were developed using Azure c600 imager (Azure Biosystems). Membranes incubated with Li-Cor antibodies developed using Odyssey DLx imager (Li-Cor). See the Supplementary Fig. 2 for uncropped scans of all western blots.

Immunofluorescence

INS-1E cells were fixed with 4% PFA for 20 minutes, permeabilized with 0.2% Triton X-100 and blocked with 2% BSA in PBS for 2-3 hours at room temperature with gentle shaking. Cells were incubated with mouse anti-Flag antibody (Sigma, Cat# F1804; 1:1000 dilution) overnight at 4°C.

Cells were then incubated with secondary antibody conjugated to Alex Fluor 647 (Invitrogen, #A21235; 1:1000 dilution) for 1 hour at room temperature and after washing twice with PBS, Hoechst 33342 (Invitrogen, #H3570; 1:5000 dilution) was added for 20 mins at room temperature. Cells were washed twice with PBS and then stored at 4°C. Cells were imaged using an Opera Phenix High-Content Imaging Instrument (PerkinElmer).

Human Islet Staining

Human islets were fixed with 3% PFA for 20 minutes, permeabilized with 0.2% Triton X-100, blocked with 2% BSA in PBS for 2-3 hours at room temperature, and then incubated with C-peptide antibody (Developmental Studies Hybridoma Bank, GN-ID4; 1:50 dilution) overnight at 4°C. Cells were incubated with secondary antibody conjugated to AlexaFluor 568 (Invitrogen, #A48262; 1:1000 dilution) and Hoechst 33342, all in 2% BSA for 1 hour at room temperature. Cells were washed five times with PBS and then stored at 4°C. Cells were imaged using an Opera Phenix High-Content Imaging Instrument (PerkinElmer), and percent C-peptide positive cells quantified using Harmony software (PerkinElmer).

References

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