ESM Methods

Glucose Stimulated gene expression assay in fresh mouse islets- Islets from 3–4 months old sex matched mice were isolated as previously described in [21] with slight modifications according to Collagenase P (Roche, Indianapolis, IN, USA) manufacturer's protocol. Briefly, mice were anaesthetised by intraperitoneal injection with ketamine (50 mg/kg). Collagenase P solution (1mg/ml) was injected into the bile duct to inflate the pancreas followed by removal and incubation of inflated pancreata at 37℃ for digestion until the pancreas form a milky solution with only a few clumps. After digestion, Islets were purified in density gradient Histopaque1077, 1083, and 1119 (Sigma, Saint Louis, MO, USA). Finally, islets were manually selected and washed in Krebs–Ringer HEPES buffer, and cultured overnight in RPMI-1640 full medium (supplemented with 15% Heat Inactivated Fetal Bovine Serum, 100 U/ml penicillin, 0.1mg/ml streptomycin) containing 5mmol/l glucose. Islets were washed twice in buffer A (5 mmol/l KCl, 120 mmol/l NaCl, 24 mmol/l $NaHCO_3$, 1 mmol/l $MgCl_2$, 2 mmol/l $CaCl_2$, 1 mg/ml Ovalbumin, 15 mmol/l HEPES at pH 7.4) and then transferred to RPMI-1640 full medium containing 3.3 mmol/l glucose overnight to stabilize basal insulin secretion. Islets were washed twice with buffer A without glucose and glucose stimulation was performed in RPMI-1640 full medium with either 3.3mmol/l glucose or 16.7mmol/l glucose for 72 hours. Following glucose stimulation, islets were washed twice with PBSx1 and total RNA and miRNA were extracted using miRNeasy Mini kit (Qiagen) following manufacturer's protocols. RT-PCR for both mRNA and miRNA was performed using miScript II RT kit (Qiagen) and quantitative PCR was performed using miScript SYBR Green PCR kit (Qiagen) for miRNA, and SYBR Green PCR Master Mix (Applied Biosystems) for mRNA on a 7500 Real-Time PCR system (Applied Biosystems).

Glucose Stimulated gene expression assay in MINB6 cells – MIN6B cells were transferred to RPMI 1640 (with L-glutamine and without glucose) medium supplemented with 15% Heat Inactivated Fetal Bovine Serum, 100 U/ml penicillin, 0.1mg/ml streptomycin and with 5mmol/lglucose for 72-90 hours prior to glucose stimulation in order to acclimate these cells (usually grown in media containing 25mmol/l glucose) to relatively normal levels of glucose. Then cells were washed once and incubated for 1 hour in buffer A (5 mmol/l KCl, 120 mmol/l NaCl, 24 mmol/l $NaHCO_3$, 1 mmol/l $MgCl_2$, 2 mmol/l $CaCl_2$, 1 mg/ml Ovalbumin, 15 mmol/l HEPES at pH 7.4) supplemented with 3.3mmol/l glucose. Cells were washed twice with buffer A without glucose and glucose stimulation was performed in buffer A supplemented with either 5mmol/l glucose (control) or 25mmol/l glucose (high glucose) for 4 hours in 37°C. Following glucose stimulation, cells were washed twice with PBSx1 and total RNA and miRNA were extracted using miRNeasy Mini kit (Qiagen) following manufacturer's instructions. RT-PCR for both mRNA and miRNA was performed using miScript II RT kit (Qiagen) and quantitative PCR was performed using miScript SYBR Green PCR kit (Qiagen) for miRNA, and SYBR Green PCR Master Mix (Applied Biosystems) or Taqman gene expression assay (Applied Biosystems) for mRNA on a 7500 Real-Time PCR system (Applied Biosystems).

Protein extraction and Western blot analysis- Cultured cells were rinsed with cold PBS and lysed in the cell disruption buffer from the mirVANA PARIS kit (Applied Biosystems). Note that this extraction procedure allows co-isolation of proteins and high quality RNA (including the small RNA species) from the same sample allowing direct comparative measurements (of miRNAs, mRNA and protein) within a given tissue or cells. This protocol was used to extract RNA and proteins from SH-SY5Y cells, and and from adult wild type, *Ia-2* single knockout, *Ia-2beta* single knockout and *Ia-2/Ia-2beta* double knockout

mouse brains. Protein concentrations were quantified, samples were mixed with reducing loading buffer, heated at 70°C for 10 min, and equal amounts of protein was loaded in each lane on Novex Bis-Tris PAGE gels (Invitrogen). Proteins were transferred by wet transfer using the XCell II™ Blot Module CE Mark according to manufacturer's instructions. All blots were blocked in blocking buffer (TBS/ 0.1% Tween20 (TBS-T) / 5% nonfat-milk). After blocking for 1 hour, blots were incubated over night at 4°C in blocking buffer. After washing, the blots were incubated for 1 hour at room temperature with HRP conjugated secondary antibodies directed against the species of origin from the primary antibody. After the secondary antibody incubation, the blot was washed in TBS-T for 6x 10minutes. The blots were subsequently processed for enhanced chemiluminescence (ECL) using the Western lightning plus ECL kit (Perkin Elmer) and the blots were imaged using the Fuji LAS300 imaging systems (Fuji). Densitometry was performed using the Advanced Image Data Analyzer (AIDA) software (version 4.22.034).

Transfections, DNA cloning and luciferase assay- For analysis of regulation endogenous alpha-synuclein by microRNAs, 400,000 SH-SY5Y cells per well were plated in 6 well plates. The next day, cells were transfected with 50 nmol/l of pre-mir-mir153 (miR-153) (Applied Biosystems) or a scrambled sequence (SCR) (NEG2, Applied Biosystems) using lipofectamine 2000 (Invitrogen) following manufacturer's instructions. 48 hours post-transfection, cells were processed for immunoblot analysis. MIN6 cells were plated in 12 well plates, 70% confluency and transfected with 50nmol/l of either mir-153 mimic (Syn-mmu-miR153, Qiagen) or AllStars Negative Control (Qiagen) using HiPerFect Transfection Reagent (Qiagen) following manufacturer's protocol. Cells were harvested for quantitative RT-PCR analyses 72 hours after transfection. For analysis of regulation of Firefly Luciferase reporter construct containing alpha-synuclein 3'UTR by microRNA, 200,000 SH-SY5Y were plated in 12-well plates. The 3'UTR of either *Alpha-synuclein (SNCA), Bassoon* (*BSN*) or *Piccolo* (*PCLO*) were amplified from human cDNA and the *TK* promoter from human genomic DNA and cloned into the pGL3-luciferase basic vector (Promega). Sequences of primers and cloning strategy are available on request. Inserts were confirmed by sequencing. The luciferase miR-153 seed mutant constructs (seed mutant) were generated using the QuickChange II XL Site-Directed Mutagenesis kit (Stratagene) according to manufacturer's instructions. For luciferase assays, 50 nmol/l of pre-mirs (miR-153) (Applied Biosystems) were co-transfected with the Firefly Luciferase wildtype 3'UTR or seed mutant and the Renilla control vector (Promega). 26-28 hours post-transfection, the measurements were performed using the Dual luciferase reporter assay kit following manufacturer's instructions (Promega).