

Online Supplemental Materials

Supplemental Research Design and Methods

Electrophysiology

Pancreatic islets were dissociated into single β -cells and plated onto glass coverslips, as previously described [1]. Electrophysiological recordings were performed in perforated patch-clamp configuration using an EPC9 patch-clamp amplifier controlled by Pulse acquisition software (Heka Elektronik, Pfalz, Germany). β -cells were identified morphologically and by depolarisation of the membrane potential in response to 17 mM glucose. β -cells were constantly perfused at 32°C with normal saline solution (mM): 135 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 HEPES and 16.7 glucose (pH 7.4). Recording electrodes had resistances of 8-10 M Ω and were filled with a solution comprised of (mM): 140 KCl, 5 MgCl₂, 3.8 CaCl₂, 10 HEPES, 10 EGTA (pH 7.2) and 20–25 μ g/ml amphotericin B (Sigma-Aldrich).

Ca²⁺ imaging and connectivity analysis

Ca²⁺ (with Fluo-2-AM, Invitrogen) imaging were performed as previously described [2,3]. Briefly, Calcium imaging was performed in Krebs-HEPES-Bicarbonate (KHB) buffer (40 mM NaCl, 3.6 mM KCl, 0.5 mM NaH₂PO₄, 0.2 mM MgSO₄, 1.5 mM CaCl₂, 10 mM HEPES, 25 mM NaHCO₃), saturated with 95% O₂/5% CO₂ and adjusted to pH 7.4. Islets were incubated at 37 °C 95% O₂/5% CO₂ for 45 min. in 10 μ M Fluo-2-AM (Invitrogen). Islets were then transferred in a perfusion chamber, mounted on a Zeiss Axiovert confocal microscope and perfused continuously at 34-36°C. Images were acquired every 2 s with a Hamamatsu ImagEM camera and Volocity software (Perkin-Elmer) was used for data capture. Significantly correlated cell pairs were measured as described previously [4]. Correlation coefficient (R) and heatmaps were generated using an in house Matlab script (available upon request).

Generation of GFP-STARD10 and STARD10-GFP constructs

STARD10 open reading frame (NM_006645) was amplified by PCR from a MCF7 (ATCC HTB-22) cDNA library using the following primers: P1: 5'- CCC CAT GGA GAA GCT GGC GGC CTC T-3' and P2: 5'- TCA GGT GAG CGA GGT GTC GTC GTC G-3', and cloned into the pSTBlue-1 Blunt vector. STARD10 cDNA was then amplified by PCR with the primers: P1 and 5'- CGG TGA GCG AGG TGT CGT CGT C -3', or 5'- GAG AAG CTG GCG GCC TCT ACA -3' and P2. The PCR fragments were phosphorylated and cloned into the DraI and EcoRV sites of the pENTR1A gateway entry vector, to generate entry plasmids compatible with C-terminal and N-terminal fusions, respectively. STARD10 was further cloned by gateway recombination into pEGFP-N1 and pEGFP-C2 destination vectors to generate STARD10-EGFP and EGFP-STARD10 expression vectors, respectively.

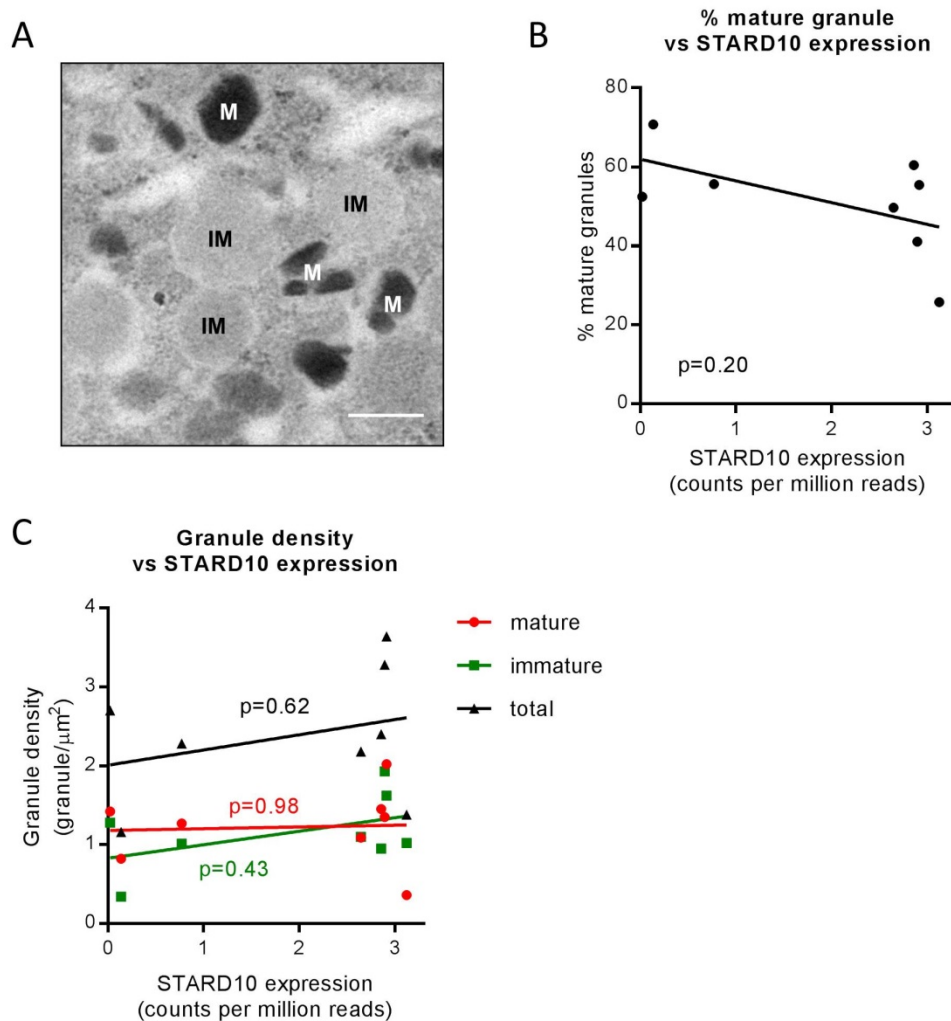
STARD10 subcellular localisation.

Cells from the human β -cell line EndoC- β H1 were seeded on 24 mm diameter coverslips in a 6 well-plate and transfected with the GFP-STARD10 or STARD10-GFP constructs using Lipofectamine2000. 48 h after transfection, the cells were imaged live on a spinning disk confocal microscope (Nikon Eclipse Ti, Crest spinning disk, 60x oil objective; Cairn instruments).

Supplemental references:

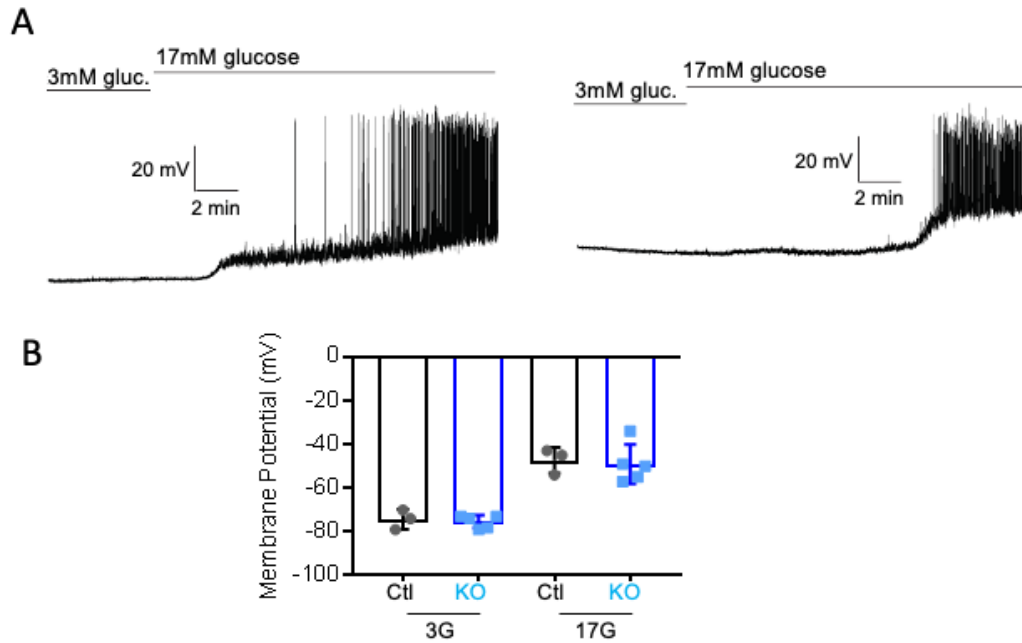
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- [2] Sun, G., Tarasov, A.I., McGinty, J.A., French, P.M., McDonald, A., Leclerc, I., et al., 2010. LKB1 deletion with the RIP2.Cre transgene modifies pancreatic beta-cell morphology and enhances insulin secretion in vivo. *Am J Physiol Endocrinol Metab* 298(6): E1261-73, Doi: 10.1152/ajpendo.00100.2010.
- [3] Hodson, D.J., Tarasov, A.I., Gimeno Brias, S., Mitchell, R.K., Johnston, N.R., Haghollahi, S., et al., 2014. Incretin-Modulated Beta Cell Energetics in Intact Islets of Langerhans. *Molecular Endocrinology* 28(6): 860–71, Doi: 10.1210/me.2014-1038.
- [4] Hodson, D.J., Mitchell, R.K., Bellomo, E.A., Sun, G., Vinet, L., Meda, P., et al., 2013. Lipotoxicity disrupts incretin-regulated human β cell connectivity. *The Journal of Clinical Investigation* 123(10): 4182–94, Doi: 10.1172/JCI68459.

Supplemental figures



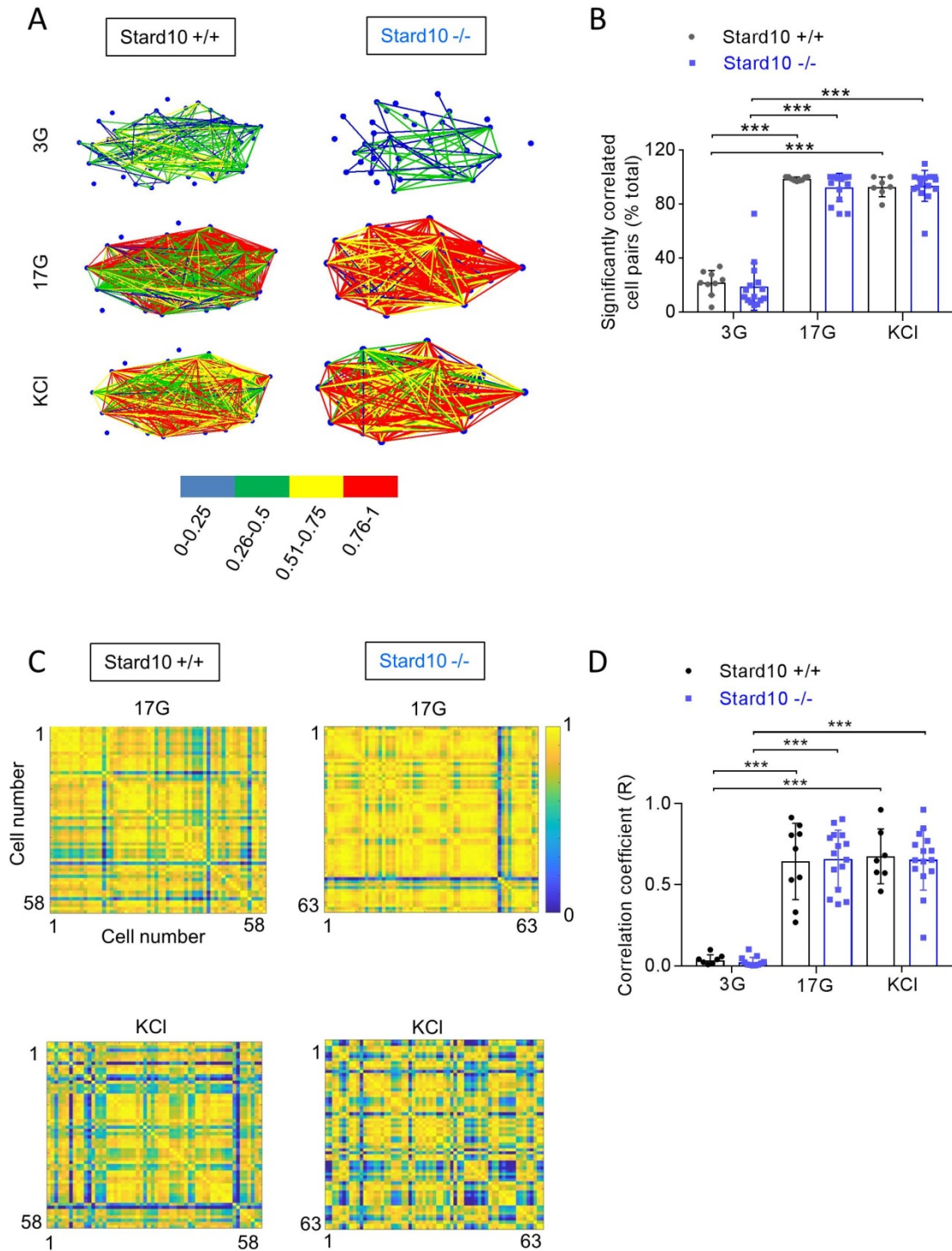
Supplemental Figure 1. Association of *STARD10* expression and granule morphology in human islets

A, Representative Transmission Electron Microscopy image of a human β -cell taken from a partial pancreatectomy sample showing examples of mature (M) and immature (IM) insulin secretory granules. Scale bar = 200 nm. B, No significant correlation could be observed between the percentage of mature insulin granules and the level of *STARD10* expression measured by RNASeq ($n = 8$ human donors; ns by non-parametric Spearman correlation, p value shown on graph). C, Identical to B but with density of mature, immature and total insulin granules (granule/ μm^2) ($n = 8$ human donors; ns by non-parametric Spearman correlation, p -value shown on graph).



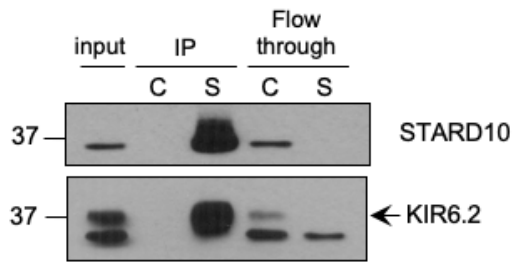
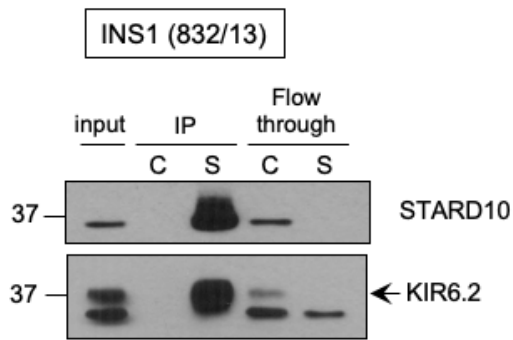
Supplemental Figure 2: Deletion of *Stard10* does not affect membrane potential in mouse islet.

A, Representative current clamp recordings of isolated β -cells from Ctl and β *Stard10*KO mice in response to a 3 and 17 mM glucose application. B, Mean membrane potential at 3 and 17 mM glucose ($n = 4$ to 5 cells from 2 animals per genotype, ns by Student's t-test).



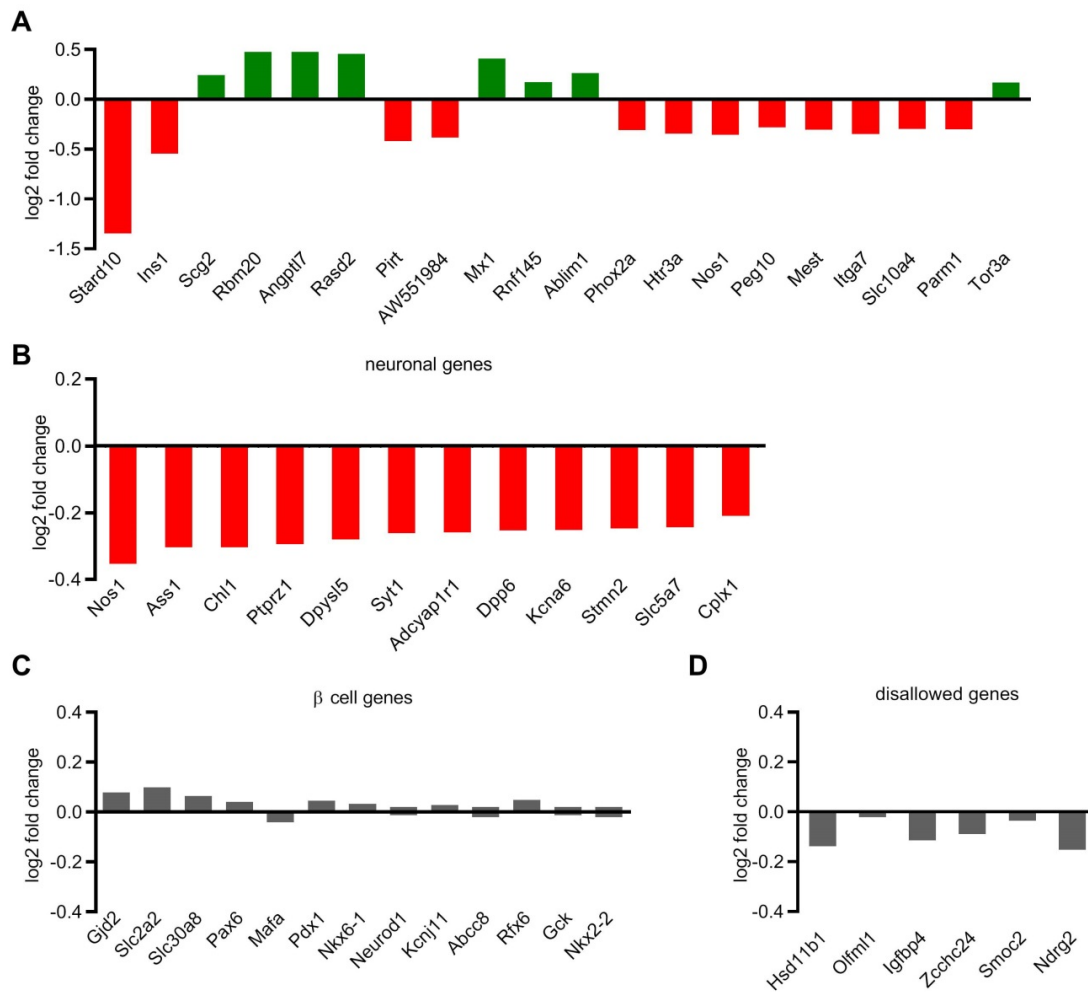
Supplemental Figure 3: Impact of *Stard10* deletion on intercellular connectivity

A, Representative maps of the recorded cells with coded connectivity strength. The maps show a Cartesian representation of the cells. A colour coded line connects two cells according to the strength of the Pearson R statistic. B, Percentage of correlated cell pairs at 3, 17 mM glucose or 20 mM KCl ($n = 7$ to 15 islets per genotype; ns by 2-way ANOVA). C, Representative heatmaps depicting connectivity strength (Pearson R statistic) of all cell pairs (R values color coded from -1 to 0, blue to yellow respectively). D, The average correlation coefficient (R) between β -cells rises significantly in WT islets in response to the addition of glucose or KCl. This response was not attenuated in *Stard10KO* islets.



Supplemental Figure 4: The ATP-sensitive K^+ (K_{ATP}) channel KIR6.2 is a STARD10 binding partner in INS1(832/13) β -cell line

Anti-STARD10 antibody (S: STARD10 condition) or Rabbit IgG isotype control (C: Control condition) were used for the immunoprecipitation from 1mg of INS1 (832/13) cell lysate. Western-(immune)-blotting of the eluates show the specific immunoprecipitation of STARD10 (IP, S vs. C condition) and co-elution of KIR6.2 (IP, S condition). In parallel, we can observe a strong reduction of STARD10 and KIR6.2 presence in the Flow through after immunoprecipitation of STARD10, but not in the control condition.



Supplemental Figure 5: Identification of differentially regulated genes in β Stard10KO islets by RNA seq

A, 20 first differentially regulated genes in β Stard10KO, ranked by increasing adjusted p-value with their relative expression *versus* Ctl (log2 fold change, n = 6 animals per genotype)

B, Genes identified by the GO consortium as component of “neuron projection” and “neuron part”, found enriched among the downregulated genes identified in the β Stard10KO islets, shown as relative expression *versus* Ctl (log2 fold change). Neither β -cell signature genes (C) nor “disallowed” β -cell gene family (D) were differentially regulated in β Stard10KO vs. Ctl islets.

Supplemental Table 1. Characteristics of the human donors used for the electron microscopy analysis of insulin granules

ND: normoglycemic donor; IGT: donor with impaired glucose tolerance; T2D: donor with type 2 diabetes; T3D: donor with type 3 diabetes

Gender	Diabetes status	STARD10 expression (Counts per million reads)
F	T2D	3.1216
F	ND	2.9139
F	T2D	2.8928
F	T2D	2.8561
F	T2D	2.6440
M	T2D	0.7689
M	T3D	0.1353
F	IGT	0.0206

Supplemental Table 2. Statistics from STARD10 crystallographic analysis

Complex	StarD10
PDB ID	6SER
Data collection	
Source	I03
Wavelength (Å)	0.9762
Resolution (Å)	60.06-2.30 (2.34-2.30)
Space group	I 2 2 2
Cell dimensions : a, b, c	68.3, 101.2, 125.8
Observations	256184 (11210)
Unique reflections	19758 (925)
R _{merge} (%)	8.5 (44.0)
I/σI	19.2 (5.6)
Completeness (%)	99.9 (97.7)
Redundancy	13.0
Refinement statistics	
Resolution (Å)	60.06-2.30 (2.34-2.30)
R _{factor} (%) / R _{free} (%)	20.98/24.77
rmsd bonds (Å)/angles (°)	0.007/1.0
Ramachandran plot : Favored (%)	96.0

The values in parentheses refer to the last shell.

$R_{\text{factor}} = \Sigma ||F(\text{obs}) - F(\text{calc})| / \Sigma |F(\text{obs})|$.

R_{free} = R factor calculated using 5.0% of the reflection data randomly chosen and omitted from the start of refinement.

Supplemental Table 3: lipidomic analysis of Ctl and β Stard10KO islets

The raw data of the analysis of 24 classes and 280 species of lipids is presented in a separate excel spreadsheet. The data was normalised as nmol/g (protein) and % total lipids.

Supplemental Table 4. Gene ontology analysis of STARD10 binding partners in INS1 (832/13) cells: biological process

Top 10 most significant gene ontology terms for biological processes

GO biological process	Number of proteins	Fold Enrichment	P-value
RNA processing (GO:0006396)	85	11.19	1.99E-62
gene expression (GO:0010467)	114	6.4	2.66E-62
RNA splicing (GO:0008380)	63	22.47	5.73E-62
mRNA processing (GO:0006397)	67	18.66	2.16E-61
mRNA metabolic process (GO:0016071)	71	14.76	6.70E-59
RNA metabolic process (GO:0016070)	93	7.16	5.13E-53
cellular nitrogen compound metabolic process (GO:0034641)	123	4	1.83E-46
nucleic acid metabolic process (GO:0090304)	96	5.17	3.55E-43
mRNA splicing, via spliceosome (GO:0000398)	43	21.42	3.54E-41
RNA splicing, via transesterification reactions with bulged adenosine as nucleophile (GO:0000377)	43	21.42	3.54E-41

Supplemental Table 5. Gene ontology analysis of STARD10 binding partners in INS1

(832/13) cells: cellular components

Top 10 most significant gene ontology terms for cellular components

GO cellular component	Number of proteins	Fold Enrichment	P-value
ribonucleoprotein complex (GO:1990904)	100	8.18	1.04E-62
spliceosomal complex (GO:0005681)	52	28.97	9.68E-56
catalytic step 2 spliceosome (GO:0071013)	40	41.38	7.06E-48
protein-containing complex (GO:0032991)	159	2.72	1.45E-43
intracellular organelle part (GO:0044446)	179	2.19	4.59E-39
nuclear speck (GO:0016607)	48	13.14	3.93E-37
organelle part (GO:0044422)	180	2.11	4.48E-37
nuclear part (GO:0044428)	128	3.04	2.57E-36
U2-type spliceosomal complex (GO:0005684)	31	36.48	8.32E-36
nucleus (GO:0005634)	159	2.36	1.90E-35

Supplemental Table 6. Gene ontology analysis of STARD10 binding partners in INS1

(832/13) cells: molecular functions

Top 10 most significant gene ontology terms for molecular functions

GO molecular function	Number of proteins	Fold Enrichment	P-value
RNA binding (GO:0003723)	93	7.99	6.96E-57
nucleic acid binding (GO:0003676)	112	3.33	6.80E-34
mRNA binding (GO:0003729)	39	13.91	5.38E-31
heterocyclic compound binding (GO:1901363)	132	2.45	5.21E-28
organic cyclic compound binding (GO:0097159)	132	2.4	3.06E-27
binding (GO:0005488)	184	1.43	1.51E-14
mRNA 3'-UTR binding (GO:0003730)	14	16.9	8.21E-13
ATP-dependent RNA helicase activity (GO:0004004)	11	28.77	1.70E-12
RNA helicase activity (GO:0003724)	11	27.99	2.18E-12
RNA-dependent ATPase activity (GO:0008186)	11	27.99	2.18E-12

Supplemental Table 7. Enrichment of neuronal genes among the downregulated genes in β Stard10KO islets

Genes identified by the GO consortium as components of “neuron projection” and “neuron part”, found enriched among the downregulated genes identified in β Stard10KO islets, listed as relative expression versus Ctl (log2 fold change).

	symbol	log2FoldChange	pvalue	padj	entrezID
ENSMUSG00000029361	Nos1	-0.3533	4.34E-07	0.000468	18125
ENSMUSG00000076441	Ass1	-0.3040	4.67E-05	0.014264	11898
ENSMUSG00000030077	Chl1	-0.3032	1.91E-05	0.009003	12661
ENSMUSG00000068748	Ptprz1	-0.2947	6.26E-06	0.00394	19283
ENSMUSG00000029168	Dpysl5	-0.2805	0.00017	0.034308	65254
ENSMUSG00000035864	Syt1	-0.2618	0.0002	0.038729	20979
ENSMUSG00000029778	Adcyap1r1	-0.2588	8.67E-05	0.022575	11517
ENSMUSG00000061576	Dpp6	-0.2531	2.89E-05	0.011209	13483
ENSMUSG00000038077	Kcna6	-0.2514	0.000115	0.028134	16494
ENSMUSG00000027500	Stmn2	-0.2474	3.69E-05	0.012594	20257
ENSMUSG00000023945	Slc5a7	-0.2435	2.18E-05	0.009472	63993
ENSMUSG00000033615	Cplx1	-0.2093	0.000111	0.027526	12889