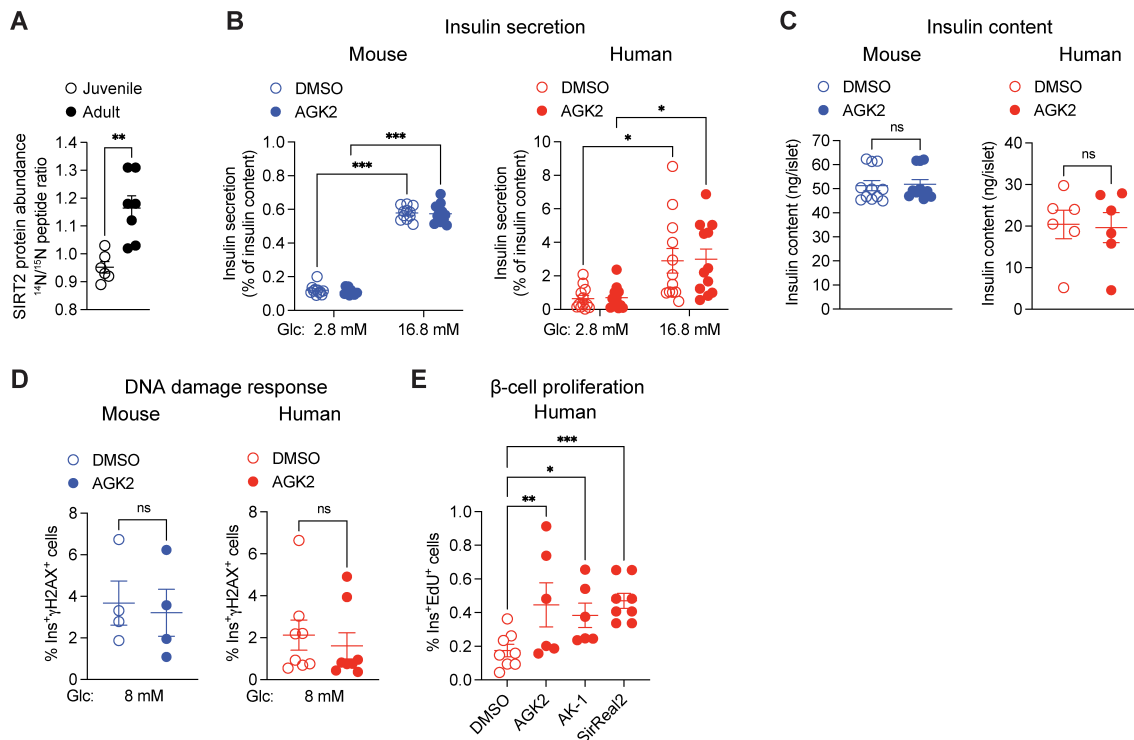


Supplemental Information

Supplemental Figures



Supplemental Figure 1. SIRT2 inhibition does not affect β -cell function.

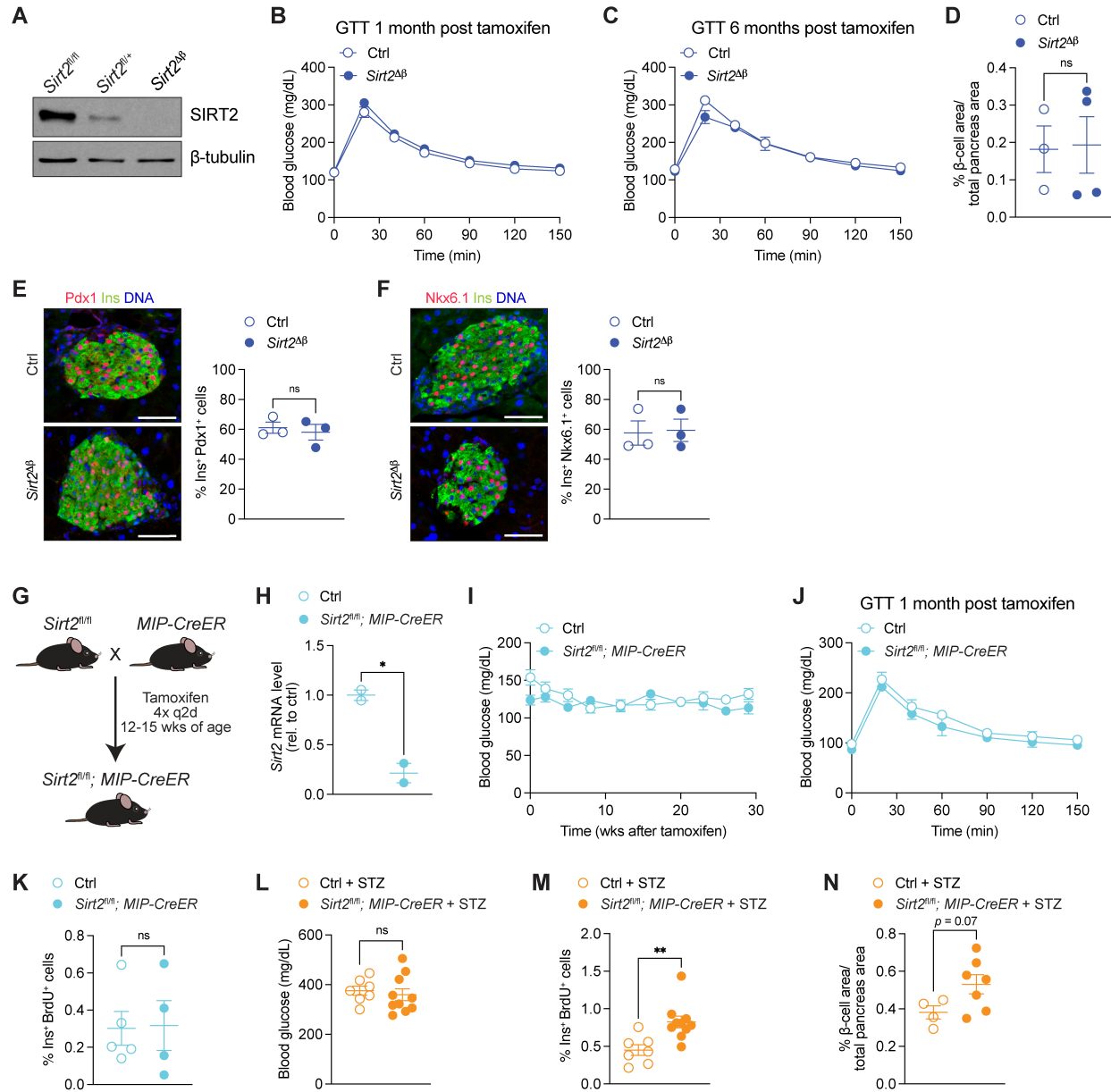
(A) Relative protein abundance for SIRT2 in islets from juvenile (one-month-old) and adult (one-year-old) mice ($n = 4$ pools of islets from distinct biological replicates/group with each peptide of SIRT2 shown as an individual data point).

(B and C) Glucose stimulated insulin secretion (B) and insulin content (C) were measured in isolated mouse (blue, $n = 11$ islet preparations/group) and human (red, B: $n = 12$, C: $n = 6$ islet preparations/group) islets after DMSO or AGK2 treatment in the indicated glucose concentrations.

(D) Quantification of γ H2AX-positive β -cells in isolated mouse (blue, $n = 4$ islet preparations/group) and human (red, $n = 8$ islet preparations/group) islets after DMSO or AGK2 treatment in 8 mM glucose.

(E) Quantification of β -cell proliferation as a percentage of insulin-positive and EdU-positive cells relative to total β -cell numbers in human islets (red, $n = 6-8$ islet preparations/group) after treatment with DMSO, AGK2, AK-1, and SirReal2 in 8 mM glucose.

Data are shown as mean \pm SEM. Statistical differences were calculated using unpaired t-test (A), two-way ANOVA with Tukey post hoc analysis (B), paired t-test (C, D), or one-way ANOVA with Tukey post hoc analysis (E). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant.



Supplemental Figure 2. Effect of *Sirt2* deletion on glucose homeostasis and β -cell proliferation.

(A) Immunoblot analysis of SIRT2 and β -tubulin in mouse islets of control or $Sirt2^{\Delta\beta}$ mice. Islets were pooled from 3 mice/genotype.

(B and C) Blood glucose levels at indicated time points after an intraperitoneal glucose injection 1 month (B) and 6 months (C) after tamoxifen treatment ($n = 5-9$ mice/group). Tamoxifen-treated $Sirt2^{+/+}; Pdx1CreER$ and $Sirt2^{+/+}; Pdx1CreER$ mice were used as controls.

(D) Quantification of β -cell area relative to whole pancreas area ($n = 3-4$ mice/group).

(E and F) Left, representative immunofluorescence staining for the indicated proteins. Right, quantification of the percentage of β -cells expressing Pdx1 (E) or Nkx6.1 (F) for the indicated genotypes 4-6 weeks following tamoxifen treatment. DAPI was used for DNA counterstain. Scalebar is $50 \mu m$. $n = 3$ mice per genotype.

(G) Schematic for generating β -cell-specific *Sirt2* deficient mice. Tamoxifen-treated *Sirt2*^{+/+}; *MIPCreER* and *Sirt2*^{fl/+}; *MIPCreER* mice were used as controls.

(H) qPCR analysis of *Sirt2* mRNA level in islets from β -cell-specific *Sirt2* deficient mice relative to control mice ($n = 2$ mice/group).

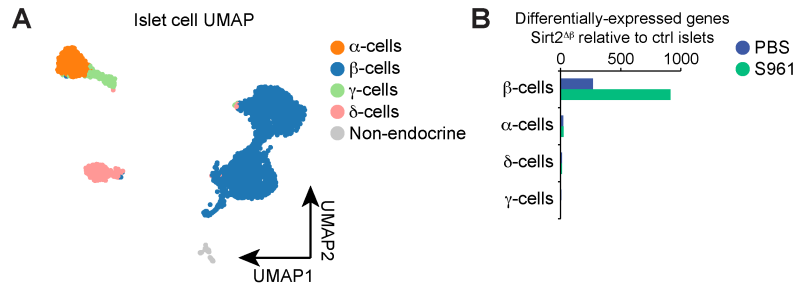
(I) Blood glucose levels measured for 30 weeks following tamoxifen treatment ($n = 11$ mice/group).

(J) Blood glucose levels at indicated time points after an intraperitoneal glucose injection ($n = 4-8$ mice/group).

(K) Quantification of β -cell proliferation as percentage of insulin-positive and BrdU-positive cells relative to total β -cell numbers 4-6 weeks post tamoxifen treatment ($n = 4-5$ mice/group).

(L-N) Hyperglycemia was induced in control and β -cell-specific *Sirt2* deficient mice by intraperitoneal injection on STZ (200 mg/kg body weight). After 3 weeks, blood glucose levels (L; $n = 7-10$ mice/group), β -cell proliferation (M; $n = 7-10$ mice/group) and β -cell area (N; $n = 4-7$ mice/group) were measured.

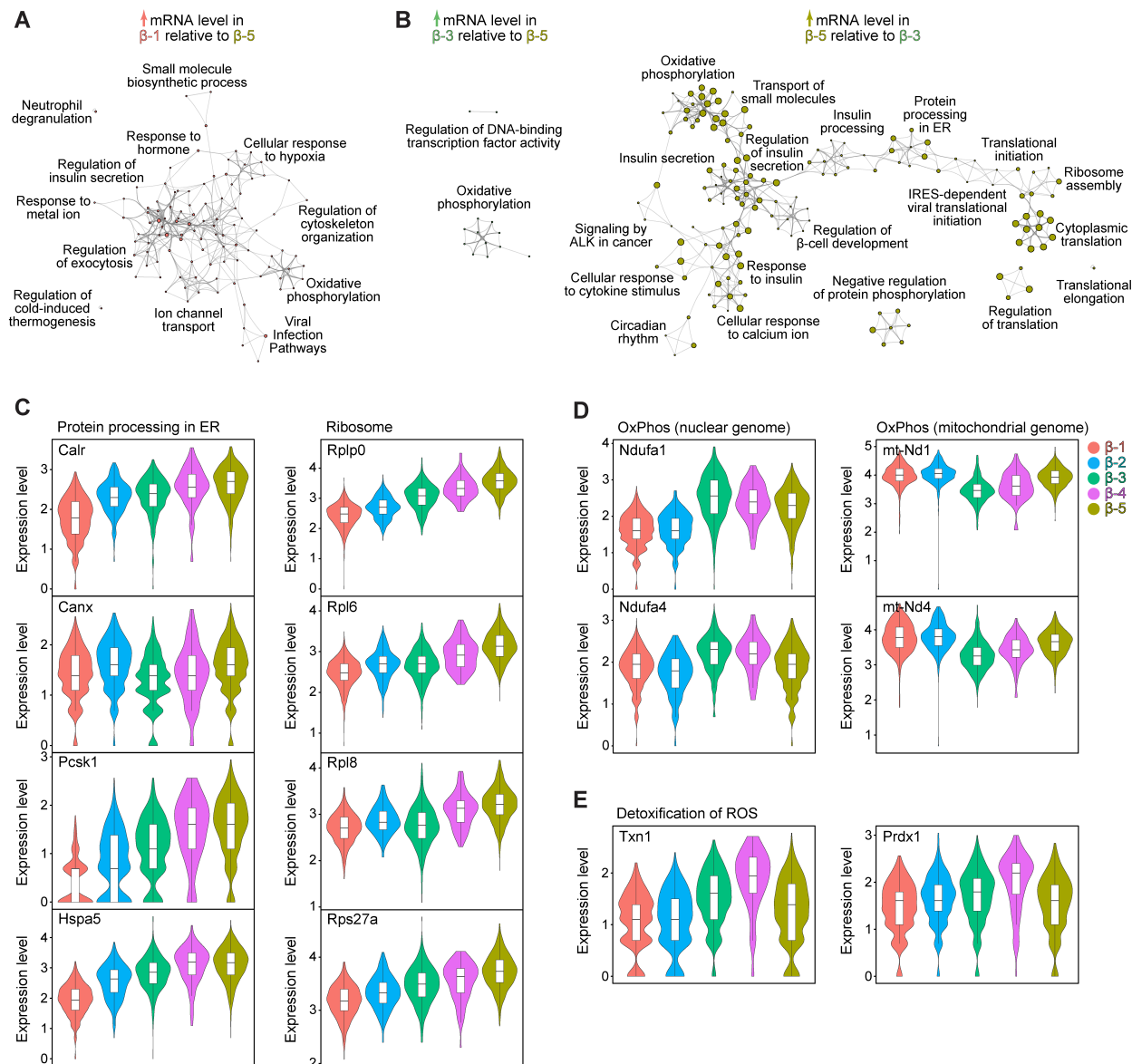
Data are shown as mean \pm SEM. Statistical differences were calculated using a two-way ANOVA with Tukey post hoc analysis (B, C, I, J) or an unpaired t-test (D-F, H, K-N). * $p < 0.05$, ** $p < 0.01$; ns, not significant. q2d, every 2 days; wks, weeks.



Supplemental Figure 3. Effect of β-cell *Sirt2* deletion upon transcriptomes of other islet cell types.

(A) UMAP (uniform manifold approximation and projection) plot of all islet cells colored by cell type.

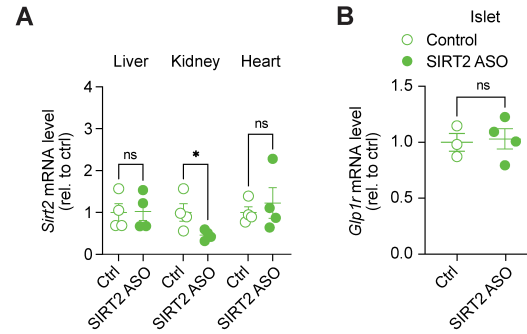
(B) Bar plot indicating numbers of differentially expressed genes (FDR < 0.05) between control and *Sirt2*^{Δβ} islet cells for each cell type in the indicated conditions. FDR was determined by Wilcoxon rank-sum test. Ctrl, control.



Supplemental Figure 4. Transcriptional differences between β -cell states.

(A and B) Networks of gene ontologies and pathways for mRNAs more highly expressed in β -1 compared to β -5 cells (A) and for mRNAs more highly expressed for the indicated comparisons (B). FDR < 0.05.

(C-E) Violin plots of the indicated mRNAs for each β -cell subset.



Supplemental Figure 5. Selectivity of *Sirt2* knockdown by GLP1-*Sirt2*-ASO followed by S961 treatment.

(**A** and **B**) qPCR analysis of *Sirt2* (**A**) and *Glp1r* (**B**) mRNA levels for the indicated tissues from S961-treated mice.

Data are shown as mean \pm SEM. Statistical differences were calculated using two-way ANOVA followed by Fisher's LSD test (**A**) or unpaired t-test (**B**). * $p < 0.05$, ns, not significant.

Supplemental Tables

Supplemental Table 1. Acetylome analysis in human islets.

(A) All detected peptides and corresponding fold changes in intensity between AGK2- and DMSO-treated human islet lysates enriched for acetyl-Lys.

(B) Peptides identified as being acetylated from (A).

(C) GO term and pathway enrichment analysis of proteins whose corresponding peptide fragments were identified as being acetylated that also exhibited ≥ 1.5 -fold differences in abundance between AGK2- and DMSO-treated human islet acetyl-Lys fractions.

(Supplied as Excel file: Supplementary_Table_1.xlsx)

Supplemental Table 2. Single cell RNA-seq analysis of control and *Sirt2*^{Δβ} mice treated with PBS or S961.

(A) mRNAs enriched in the indicated β -cell subsets relative to all other β -cells.

(B-F) GO term and pathway enrichment analysis of mRNAs highly expressed in each subset relative to all other β -cells (from A) for mRNAs enriched in β -1 (B), β -2 (C), β -3 (D), β -4 (E), and β -5 cells (F).

(G and H) Pairwise differential expression analysis of β -cell subsets comparing β -1 normal-OxPhos cells with β -5 translation-stress cells (G) or comparing β -3 OxPhos-ROS cells with β -5 translation-stress cells (H).

(Supplied as Excel file: Supplementary_Table_2.xlsx)

Supplemental Table 3. Human islet donor information.

Donor information, isolation center, and data generated for each human islet prep. All tissue was obtained through the Integrated Islet Distribution Program. All donors were nondiabetic.

Human islet donor information (proliferation and GSIS experiments)

RRID:	SAMN087 83909	SAMN087 83902	SAMN087 76506	SAMN087 76503	SAMN087 75096	SAMN087 75090	SAMN087 75023	SAMN087 75047	SAMN087 74967	SAMN087 74953	SAMN087 74820
Age	53	50	40	56	47	32	61	52	46	29	23
Sex	M	F	M	M	F	M	F	M	F	M	F
BMI	27.3	27.8	35.4	23.8	22.5	23.1	30.8	36.7	20.3	32.6	24.7
HbA1c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cause of death	CV/stroke	CV/stroke	Head trauma	Head trauma	CV/stroke	Anoxia	CV/stroke	CV/stroke	CV/stroke	Head trauma	Head trauma
Source	Prodo	UPenn	UPenn	COH	UPenn	Prodo	Prodo	UPenn	Miami	Miami	Prodo
Use in study	AGK2 prolif	AGK2 prolif	AGK2 prolif	AGK2 prolif	AGK2 prolif	AGK2 prolif	AGK2 prolif	AGK2 prolif	AGK2 prolif	AGK2 prolif	AGK2, AK1/ SR2, prolif, γ H2AX

Human islet donor information, continued (proliferation and GSIS experiments)

RRID:	SAMN087 74480	SAMN087 74472	SAMN087 74194	SAMN087 84507	SAMN087 73854	SAMN089 30712	SAMN087 73781	SAMN087 69836	SAMN087 69806	SAMN087 69393	SAMN087 69390
Age	24	35	68	30	49	26	42	56	36	53	33
Sex	F	M	M	F	M	M	F	F	M	F	F
BMI	35.4	32.9	26.7	18.4	40.1	44.8	23.2	33.5	28.5	32.0	34.2
HbA1c	ND	5.6	5.3	ND	5.4	4.7	5.4	5.2	5.6	5.7	5.5
Cause of death	CV/stroke	Head trauma	Head trauma	Anoxia	Anoxia	Head trauma	CV/stroke	Head trauma	Head trauma	CV/stroke	CV/stroke
Source	Miami	Miami	Prodo	UPenn	COH	Illinois	UPenn	Prodo	Miami	Prodo	Wisconsin
Use in study	AGK2, AK1/SR2 prolif, γ H2AX	AGK2, AK1/SR2 prolif, γ H2AX	AGK2, AK1/SR2 prolif, γ H2AX	AGK2, AK1/SR2, NMN, non β -cell prolif, γ H2AX	AGK2 prolif	AGK2, NMN, non β -cell prolif, γ H2AX, GSIS	AGK2, AK1/SR2, NMN, non β -cell prolif, γ H2AX, GSIS	GSIS	AGK2 prolif, GSIS	GSIS	GSIS

**Human islet donor information, continued
(proliferation and GSIS experiments)**

RRID:	SAMN087 69206	SAMN087 69132	SAMN087 68969	SAMN087 69031	SAMN087 69028	UNOS AEK1071	SAMN087 43022	SAMN090 91256	SAMN093 93858	SAMN445 71547	SAMN447 79828
Age	52	23	33	62	63	59	33	45	43	49	58
Sex	F	M	M	M	F	M	F	M	F	M	F
BMI	25.8	24.8	30.9	28.9	20.0	27.2	32.3	29.3	34.3	24.7	26.3
HbA1c	5.6	5.3	5.7	5.5	5.0	5.1	4.9	5.0	4.6	5.0	5.5
Cause of death	CV/stroke	Anoxia	Head trauma	CV/stroke	CV/stroke	CV/stroke	Anoxia	CV/stroke	CV/stroke	CV/stroke	CV/stroke
Source	Miami	Prodo	Prodo	COH	Miami	COH	Prodo	Prodo	Prodo	Prodo	Wisconsin
Use in study	GSIS	AK1/ SR2, prolif, GSIS	AGK2, AGK2+/- NMN prolif	AGK2, AGK2+/- NMN prolif	AGK2, AGK2+/- NMN prolif	AGK2, AGK2+/- NMN prolif	AGK2, AGK2+/- NMN prolif	AGK2, prolif, non β -cell prolif	AGK2, AGK2+/- NMN prolif, non β -cell prolif	AGK2 prolif	AGK2 prolif

**Human islet donor information, continued
(proliferation and GSIS experiments)**

RRID:	SAMN451 49991	SAMN462 23636
Age	66	57
Sex	M	M
BMI	32	30.5
HbA1c	5.6	5.3
Cause of death	Anoxia	CV/stroke
Source	COH	Prodo
Use in study	AGK2 prolif	AGK2 prolif

ND, not determined; CV, cardiovascular; Prodo, Prodo Labs Human Islet Isolation Center; Illinois, University of Illinois; Miami, University of Miami; UPenn, University of Pennsylvania; COH, Southern California Islet Cell Resource Center at City of Hope; Wisconsin, University of Wisconsin Human Islet Core; SR2, SirReal2.

**Human islet donor information, continued
(acetyl-Lys study)**

RRID:	SAMN130 50553	SAMN130 49263	SAMN135 70019	SAMN137 39565	SAMN138 36615	SAMN139 72304
Age	25	38	37	42	58	49
Sex	M	M	F	M	M	M
BMI	29.3	24.5	24.0	37.4	23.3	34.8
HbA1c	5.3	5.3	5.2	5.6	5.7	5.5
Cause of death	Head trauma	Anoxia	Anoxia	Anoxia	CV/stroke	CV/stroke
Source	UPenn	UPenn	UPenn	Prodo	Prodo	COH
Use in study	Acetyl-Lys	Acetyl-Lys	Acetyl-Lys	Acetyl-Lys	Acetyl-Lys	Acetyl-Lys

ND, not determined; CV, cardiovascular; Prodo, Prodo Labs Human Islet Isolation Center; UPenn, University of Pennsylvania; COH, Southern California Islet Cell Resource Center at City of Hope.

Supplemental Table 4. RT-qPCR primer sequences.

mRNA	F Primer	R Primer
Sirt2	GCACCTTCTACACATCACACT	ACACGATATCAGGCTTTACCAC
Glp1r	ACGGTGTCCCTCTCAGAGAC	ATCAAAGGTCCGGTTGCAGAA
Tbp	GAAGCTGCGGTACAATTCCAG	CCCCTTGTACCCTTCACCAAT