Supplemental Materials

Supplementary Table. 1: Human pancreas donors obtained from Network for Pancreatic Organ Donors with Diabetes (nPOD).

Supplementary Fig. 1: Characterization of SDHB$^{\beta KO}$ mice.

Supplementary Fig. 2: Immunofluorescent staining in ND Human Pancreas.

Supplementary Fig. 3: Islet Insulin Content in Control and SDHB$^{\beta KO}$ islets.

Supplementary Fig. 4: Metabolomic analysis in Control and SDHB$^{\beta KO}$ islets.

Supplementary Fig. 5: RNA Sequencing in Control and SDHB$^{\beta KO}$ islets.

Supplementary Fig. 6: Pathway Changes of SDHB$^{\beta KO}$ Islets based on Metabolomics and Transcriptomics Analyses.

Supplementary Fig. 7: Rapamycin reduces succinate levels, $\Delta\Psi m$ and lipid content in 3-NPA- and DMS-treated R7T1 β-cells.

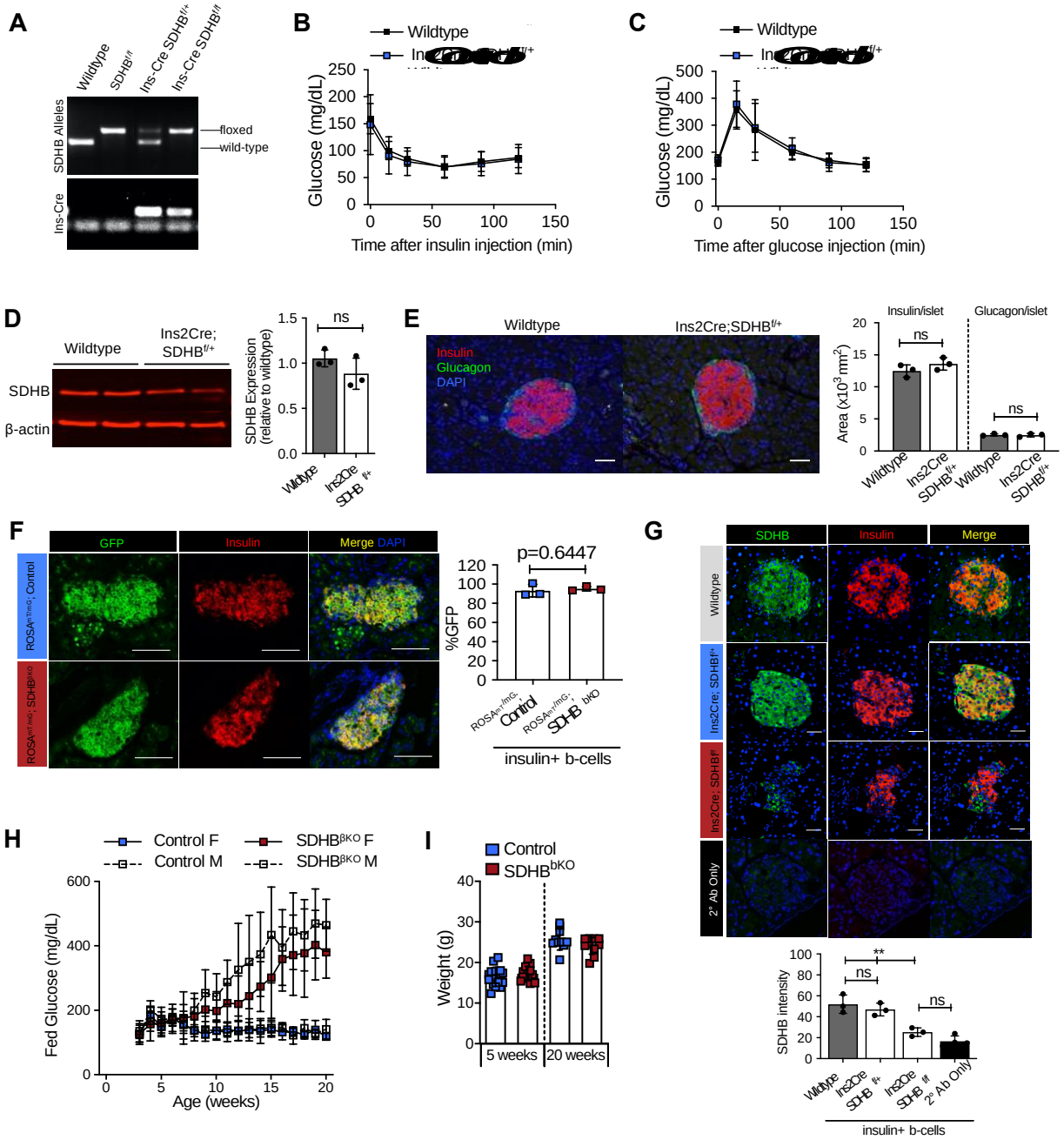
Supplementary Fig. 8: Rapamycin effect on GSIS *in vivo.*

Supplementary Table 1

CaseID	Age (yrs)	Sex	BMI	Race	Donor Type
6020	60	M	29.8	Caucasian	ND
6102	45	F	35.1	Caucasian	ND
6015	39	F	32.2	Caucasian	ND
6288	55	M	37.7	Caucasian	ND
6009	45	M	30.6	Caucasian	ND
6251	33	F	29.5	Caucasian	ND
6254	38	M	30.5	Caucasian	ND
6234	20	F	25.6	Caucasian	ND
6108	58	M	30.4	Asian	T2D
6127	45	F	30.4	Caucasian	T2D
6139	37	F	45.4	Hispanic/Latino	T2D
6259	57	M	32.3	Caucasian	T2D
6275	48	M	41.0	Hispanic/Latino	T2D
6249	45	F	32.3	Asian	T2D
6252	20	M	37.8	Caucasian	T2D

Supplementary Table. 1: Human pancreas donors obtained from Network for Pancreatic Organ Donors with Diabetes (nPOD). Organ donor information: Age (years), Sex, Body Mass Index (BMI), No Diabetes (ND) and Type 2 Diabetes (T2D).

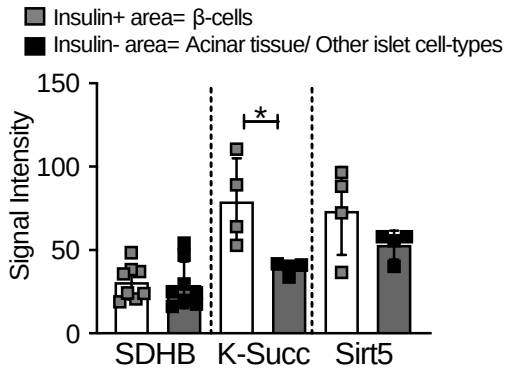
Supplementary Figure 1



Supplementary Fig. 1: Characterization of SDHB$^{\beta KO}$ mice. A: Agarose gel of *sdhb* DNA isolated from tail snips for verification of mouse genotype. **B,C:** Glucose levels following (B) intraperitoneal insulin (0.75 IU/kg) and (C) intraperitoneal glucose (2 mg/kg) administration in 5-week-old wild-type and Ins2Cre; SDHB$^{f/+}$ (heterozygous Control) mice (n=9/group). **D:** Representative immunoblot of SDHB protein in islet lysates from 5-week-old wild-type and

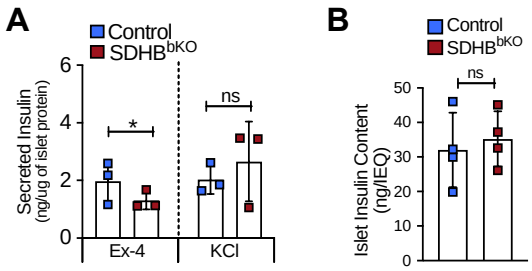
Ins2Cre; SDHB$^{f/+}$ (n=3 mice/group). Quantification of SDHB expression normalized to β-actin loading control. **E:** Representative immunofluorescent images pancreatic sections from 5-week-old wild-type and Ins2Cre; SDHB$^{f/+}$ mice stained with insulin (red) and glucagon (green). Nuclei were counterstained with DAPI. Scale Bar, 50 μM. Quantification of insulin and glucagon positive areas per islet in pancreatic sections are shown in the adjacent graph (n=3 mice/group). **F:** Percentage of Cre-expressing β-cells. Representative immunofluorescent images of GFP (green) and insulin (red) staining in pancreatic sections from 5-week-old fluorescent Cre-reporter mice-ROSA^{mTmG} Control and ROSA^{mTmG} SDHB$^{\beta KO}$. Quantification of mean %GFP-positive cells within insulin+ β-cells ± SD are shown in adjacent graphs (n= five images/mice from 3 mice/group). **G:** Representative immunofluorescent images of SDHB in pancreatic sections from 5-week-old wild-type, Ins2Cre; SDHB$^{f/+}$ (heterozygous Control) and Ins2Cre; SDHB$^{f/f}$ (SDHB$^{\beta KO}$) mice. Quantification of mean staining intensity ± SD within insulin+ β-cells are shown in adjacent graph (n= five images/mice from 3 mice/group). Secondary antibody only (2° Ab) serves as no primary antibody background control. **H:** Sex-specific representation of mean random (free-fed) blood glucose levels ± SD in female verse male Control and SDHB$^{\beta KO}$ mice (3-20 weeks), n=20/group. **I:** Mixed gender animal weights (g) of control and SDHB$^{\beta KO}$ mice at 5 and 20 weeks, n=10-16 mice/group.

Supplementary Figure 2



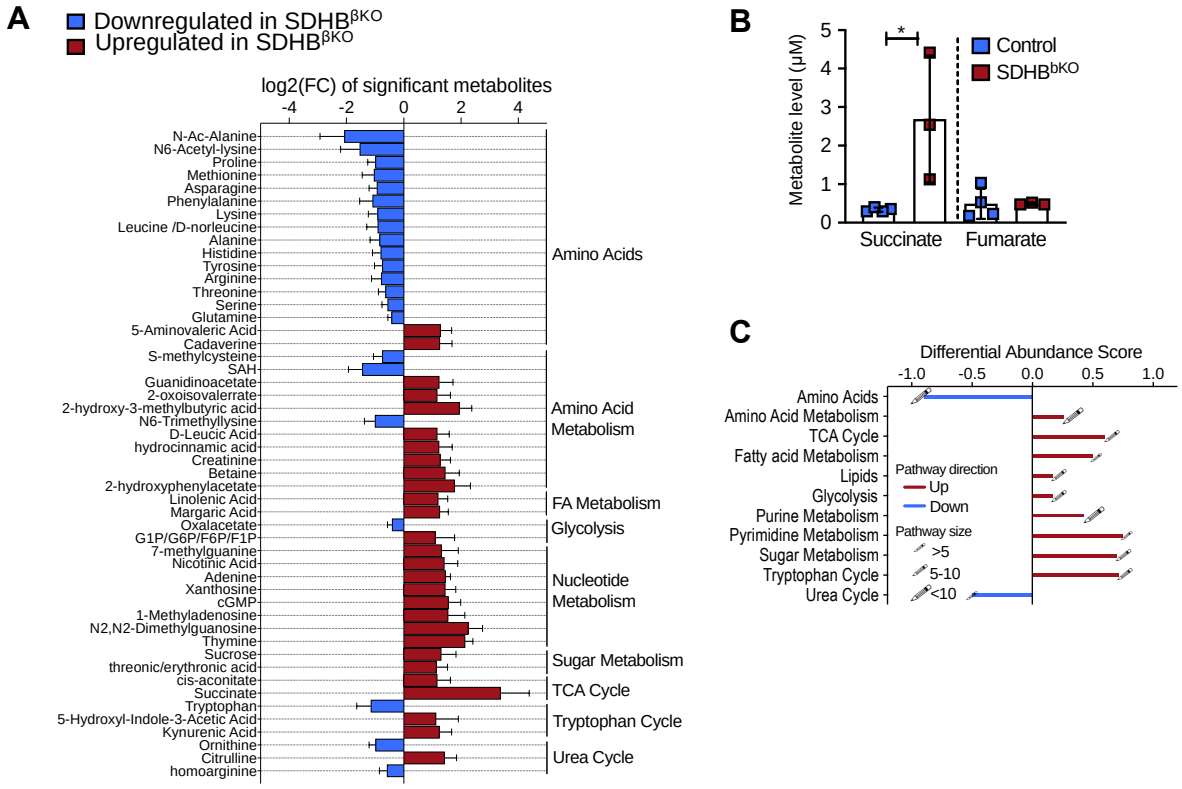
Supplementary Fig. 2: Immunofluorescent staining in ND Human Pancreas. Quantification of mean SDHB, K-Succ and Sirt5 staining intensity in either the insulin-positive β-cells or insulin-negative areas (acinar tissue and other islet-cell types) of pancreas sections from human donor (n=4-6 islets/donor from n=4-8 donors/group). Data represented as mean ± SD and were analyzed by unpaired t-test. *, $p<0.05$.

Supplementary Figure 3



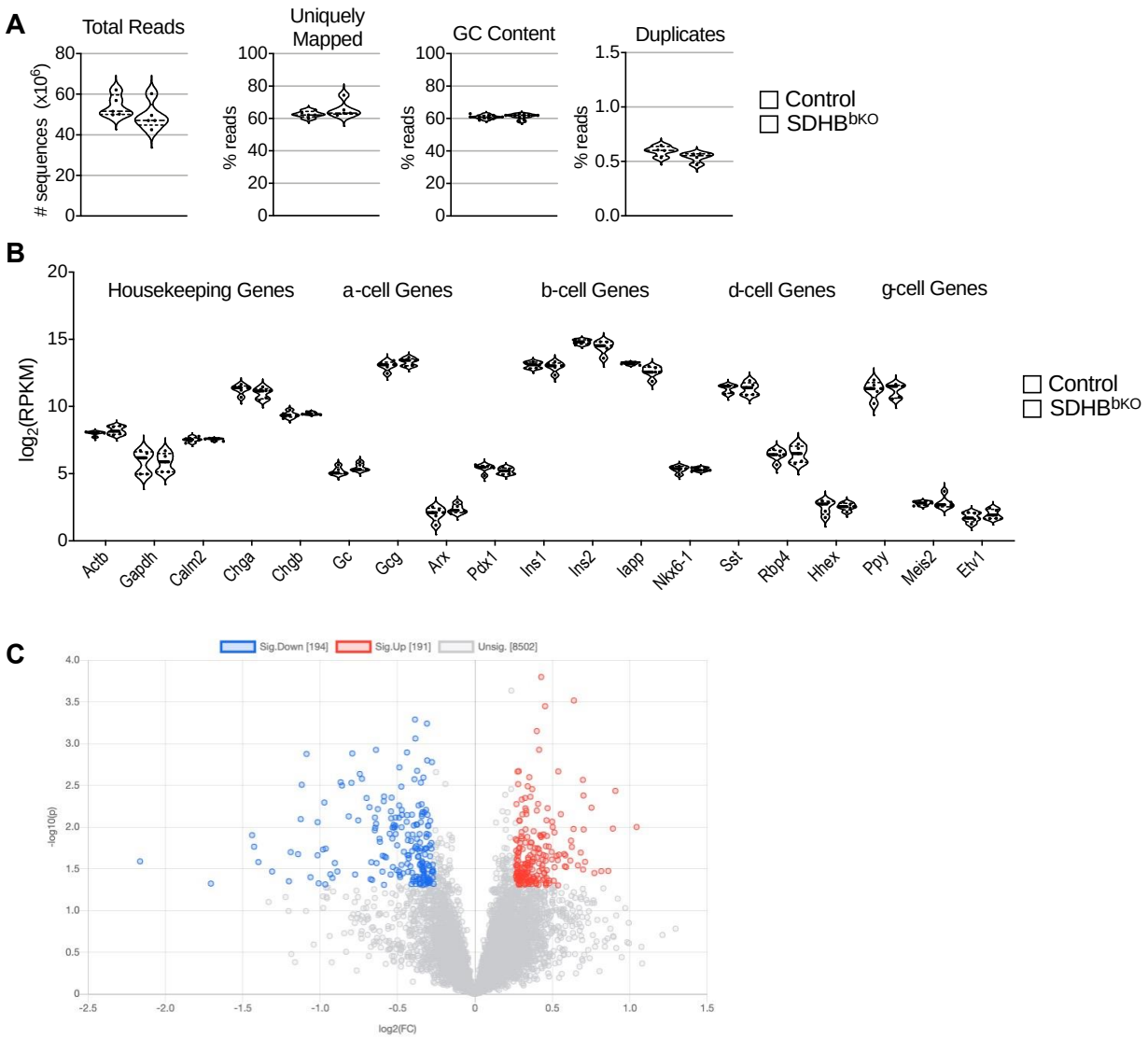
Supplementary Fig. 3: Islet Insulin Content. A: Static insulin secretion response to high glucose [16.7 mM] + 20 nM exendin-4 (Ex-4) and basal glucose [5.6 mM] + 20 mM potassium chloride (KCl) in isolated islets from 5-week-old Control and SDHB$^{\beta KO}$, n=3 mice/group. **B:** Insulin content of 5-week-old Control and SDHB$^{\beta KO}$ islets used for islet perifusion assay, n=4/group.

Supplementary Figure 4



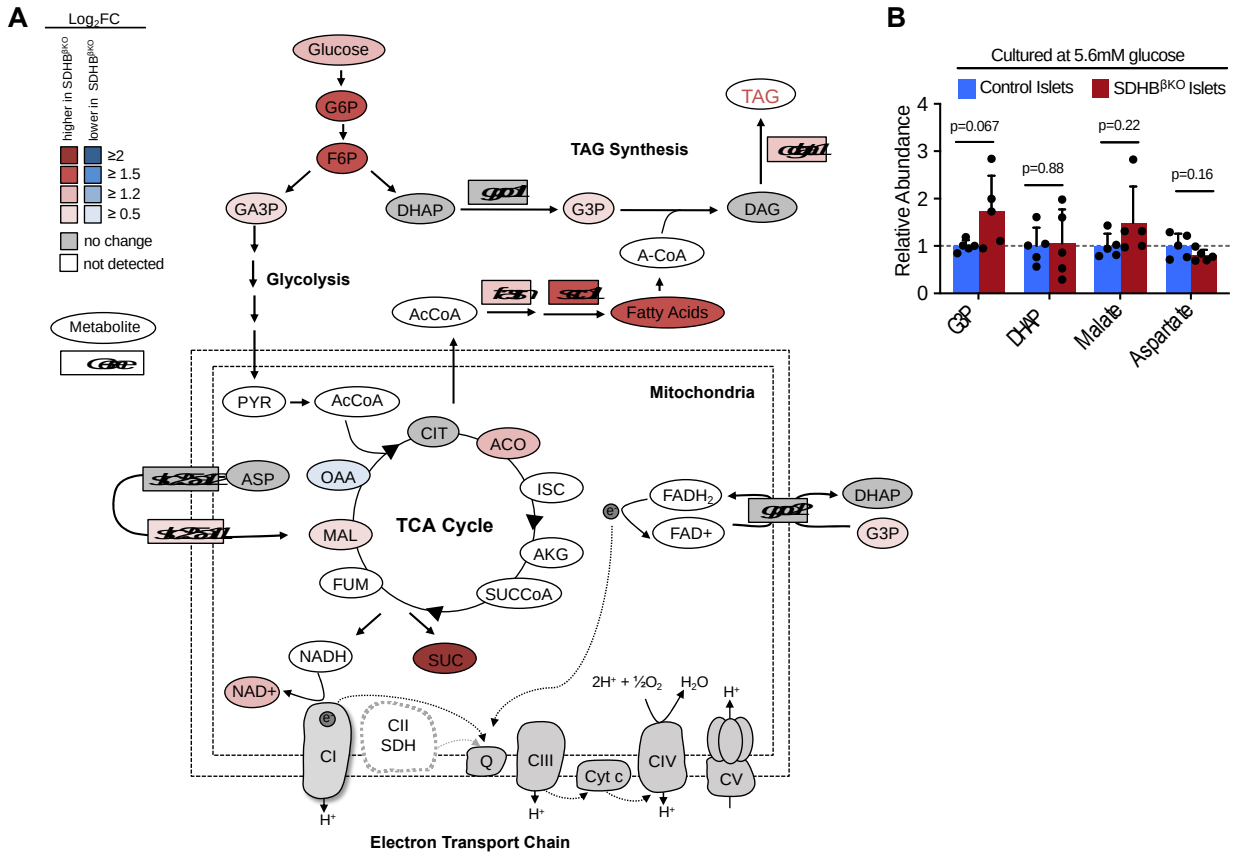
Supplementary Fig. 4: Metabolomic analysis. From 148 identified metabolites, we observed significant changes in 50 metabolites ($p < 0.05$), where 28 were increased (Log2FC ≥ 1) and 22 were decreased (Log2FC ≥ -1) in SDHB$^{\beta KO}$ islets. **A:** Fold-change ± SEM of significantly (p-value<0.05) downregulated and upregulated metabolites identified in SDHB$^{\beta KO}$ islets, compared to Ins2-Cre SDHB$^{fl/wt}$ (Control) islets, n=5/group. Metabolites are categorized into specific metabolic pathways. **B:** Succinate and fumarate levels (uM) measured by LC-MS/MS in isolated islets from 5-week-old Ins2-Cre SDHB$^{fl/wt}$ (Control; n=4) and SDHB$^{\beta KO}$ mice (n=3). **C:** Data represented as mean ± SD and were analyzed by unpaired t-test. *, p<0.05. Metabolic pathway-based analysis with differential abundance scores. The differential abundance score captures the average, gross changes for all metabolites in a pathway. A score of 1 indicates all measured metabolites in the pathway increase, and -1 indicates all measured metabolites in a pathway decrease.

Supplementary Figure 5



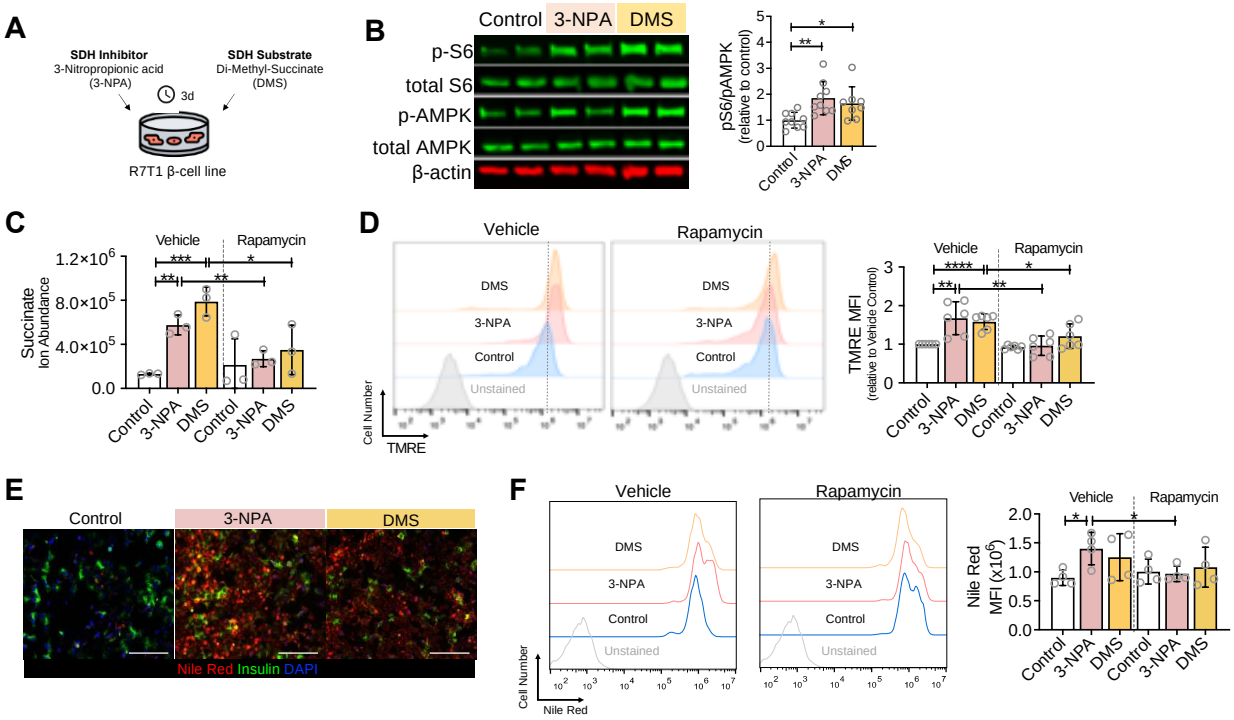
Supplementary Fig. 5: RNA Sequencing in Control and SDHB$^{\beta KO}$ islets. From 30,447 identified genes, 385 were differentially expressed in SDHB$^{\beta KO}$ islets compared to Ins2Cre; SDHB$^{f/+}$ (Control) islets ($p < 0.05$). Of these, 194 genes were up-regulated and 191 genes were down-regulated**. A:** RNA-seq data quality control metrics: total reads (# of sequences reads) and uniquely mapped, duplicates and GC content (as % of total reads). **B:** Key expression genes (*Camunas-Soler et al, Cell Metab, 2020*) for each islet cell-type identified in transcriptomic analysis: α–, β-, δ-, and γ-cells. **C:** Volcano plot of differentially expressed genes in SDHB$^{\beta KO}$ compared to Control. Red: up-regulated genes (LogFC ≥ 1.2); Blue: down-regulated genes (LogFC ≥- 1.2).

Supplementary Figure 6



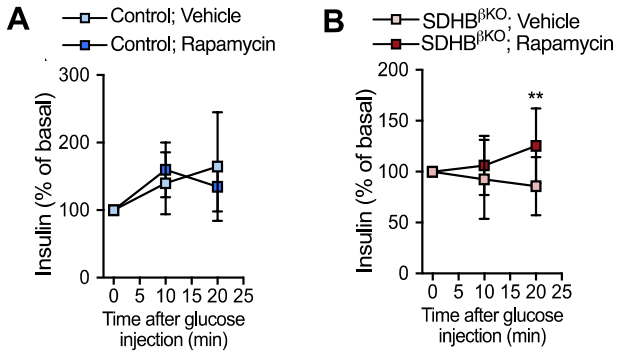
Supplementary Fig. 6: Pathway Changes of SDHB$^{\beta KO}$ Islets based on Metabolomics and Transcriptomics Analyses. A: Schematic representation of pathway changes. Color corresponds to the Log2 fold changes between in SDHB$^{\beta KO}$ and Ins2Cre; SDHB$^{f/+}$ (Control) islets. Red, increase; blue, decrease; gray, no change; white, not detected/measured. Metabolites are labelled as color-coded ovals: A-Coa, acyl-CoA; AcCoA, acetyl-CoA; ACO, cis-aconitate; AKG, alpha-ketoglutarate; ASP, aspartate; CIT, citrate; DAG, diacylglyceride; DHAP, dihydrxyacetone phosphate; F6P, fructose 6-phosphate; FUM, fumarate; G6P, glucose 6-phosphate; G3P, glyceraldehyde 3-phosphate; GA3P, glyceraldehyde 3-phosphate; ISC, isocitrate; MAL, malate; OAA, oxaloacetate; PYR, pyruvate; SUCCoA, succinyl-CoA; SUC, succinate. Transcript genes are labelled as color-coded rectangles: *dgat1*, diacylglycerol o-acyltransferase 1; *fasn*, fatty acid synthase; *gpd1/2*, glycerol-3-phosphate dehydrogenase 1/2; *slc25a11*, 2-oxoglutarate/malate carrier; *slc25a12*, aspartate/glutamate carrier. **B:** Basal metabolite levels associated with G3P and malate-aspartate shuttle in isolated islets from 5-week-old Ins2-Cre SDHB$^{fl/wt}$ (Control) and SDHB$^{\beta KO}$ mice (n=5/group). Data represented as mean ± SD and were analyzed by unpaired t-test.

Supplementary Figure 7



Supplementary Fig. 7: Rapamycin reduces succinate levels, ΔΨm and lipid content in 3-NPA- and DMS-treated R7T1 β-cells. A: Graphical representation of treatment experiments in R7T1 β-cell culture. R7T1 β-cells are treated with 3-Nitropropionic acid (3-NPA) and cell-permeable dimethyl-succinate (DMS) for 3 days. **B:** Representative immunoblot of cell lysates from control, 3-NPA and DMS-treated R7T1 β-cells. β-actin serves as the loading control. Quantification of immunoblot as a ratio of p-S6 over phospho-AMPKα shown in adjacent graphs, n=8-9/group from three independent experiments. **C:** Cellular succinate levels in 3-NPA and DMS-treated R7T1 β-cells with a 24h vehicle or rapamycin [50 nM] treatment, n=3/group. **D:** Representative FACS analyses of mitochondrial membrane potential (TMRE) in 3-NPA and DMS-treated R7T1 β-cells following a vehicle or rapamycin treatment. Median Fluorescence Intensity (MFI) of TMRE relative to vehicle control shown in adjacent graph, n=6/group from three independent experiments. **E:** Representative immunofluorescent images of control, 3-NPA and DMS-treated R7T1 β-cells stained with Nile Red (red) and insulin (green). Nuclei were counterstained with DAPI. **F:** Representative FACS analyses of Nile Red in control, 3-NPA and DMS-treated R7T1 β-cells after a 24h treatment with vehicle or rapamycin. Median Fluorescence Intensity (MFI) of Nile Red is shown in adjacent graph, n=4/group from two independent experiments.

Supplementary Figure 8



Supplementary Fig. 8: Rapamycin effect on GSIS *in vivo*. Serum insulin levels following intraperitoneal glucose (10 mg/kg) injection in (**A)** control and (**B**) SDHB$^{\beta KO}$ mice, n=12-14/group.