ESM Methods

Animal studies For deletion of the floxed *Sirt6* allele via the MIP1-CreERT system, tamoxifen (TAM) was solubilized in corn oil and 200 mg/kg was delivered into 6-week-old mice by intraperitoneal injection (IP) for 5 consecutive days [1]. For verapamil treatment, mice at 8- to 9-month old (n=4-6) were given verapamil in drinking water (1 mg/ml) per a previous publication [2]. For STZ-induced beta cell death, STZ was dissolved in 0.1 M sodium citrate (pH 4.5) and injected into animals within 30 min [3]. To avoid the reported influence of transgenic CreER construct on beta cell survival [4], we used animals with Cre allele (*Sirt6*$^{f/+}$; MIP1-CreERT or *Sirt6*$^{+/+}$; MIP1-CreERT) as controls. The dose of STZ, 75 mg/kg and 140 mg/kg for *Sirt6* EKO (n=6) and *Sirt6* BKO (n=2) mice, respectively, were determined from pilot experiments.

For H&E staining, pancreas tissues (n>3) were fixed in 4% paraformaldehyde (PFA) (43368; Alfa Aesar) overnight and embedded in paraffin. For immunostaining studies, the PFA fixed pancreases (n>3) were incubated in 30% sucrose overnight at 4 °C and embedded in OCT (4583; SAKURA FINETEK, Torrance, CA, USA) in a manner that the head and tail of the pancreas were present in each slide. The antigen retrieval step was performed with a citrate-based solution (H-3300; Vector lab, Burlingame, CA, USA). Anti-Insulin (ab7842; Abcam), 1:150 and anti-Glucagon (15954-1-AP; Proteintech), 1:200 were used for the quantification of the β-cell areas and α-cell areas. For quantifying islet number and individual endocrine cell type mass, staining of 6-8 slides across the entire pancreas were analysed to obtain a representative value for each animal [5, 6]. The following commercially available antibodies were used for other analysis: anti-Proinsulin (GS-9A8; Developmental Studies Hybridoma Bank, IA, USA), 1:20; anti-cleaved Caspase 3 (9661; CST), 1:100; anti-Somatostatin (sc-7819; Santa Cruz, Dallas, TX, USA), 1:2 000.

Glucose levels were measured by the Bionime Rightest GM550 blood glucose monitoring kit (Bionime, Ontario, CA, USA). Insulin levels were determined by an enzyme-linked immunosorbent assay (ELISA) kit from ALPCO (80-INSMSU-E10; Salem, NH, USA). For total pancreas insulin measurement, pancreases from 2-month-old female *Sirt6* EKO and control littermates (n=3-4) were harvested in 5 ml acid balanced ethanol (2% concentrated HCl in 100% ethanol) and sonicated. The extract was diluted 10 000-fold to determine the insulin level [7].

For GTT, mice of the same gender and similar ages (n>4) were fasted overnight (14-16 hr) and then injected with 1.5 g/kg D-Glucose [8]. For ITT, 8- to 9-month-old male *Sirt6* EKO mice and their control littermates (n=4-6) were fasted for 6 hrs and then injected with 0.75 U/kg Insulin (Novolin, DK-2880; Novo Nordisk, Denmark). Hyperglycaemic clamp assay on conscious, 4- to 5-month-old male *Sirt6* EKO mice and control littermates (n=6-7) were performed as previously described [8].

Pancreatic islet isolation and glucose-stimulated insulin secretion Briefly, the joint point of the common bile duct and intestine was clamped, and 5 ml of 0.7 mg/ml collagenase XI (c7657; Sigma) was injected into the pancreatic duct [9]. After digesting at 37°C for 12 min, islets were enriched by centrifugation with histopaque-1077 (10771; Sigma). Unless otherwise indicated, purified islets were cultured in RPMI 1640 with 10% fetal bovine serum (FBS) for 4 hours before analysis.

For glucose-stimulated insulin secretion in isolated islets (n=4), 15 middle-sized islets were incubated with Krebs-Ringer bicarbonate (KRB) buffer (120 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 25 mmol/l $NaHCO_3$, 1.2 mmol/l KH_2PO_4, 10 mmol/l HEPES, 0.1% BSA) with 2.5 mmol/l D-Glucose for 1hr, and transferred to fresh KRB buffer with 2.5 mmol/l or 16.7 mmol/l D-Glucose for 1 hr. The supernatants were diluted 1:100 to determine the insulin level and the remaining islets were homogenized in acid-ethanol and diluted 1:1000 to determine the total insulin content [5].

Gene expression RNA of isolated islets was extracted with Quick-RNA™ MicroPrep (R1050; ZYMO RESEARCH). Then 50 middle-sized islets were harvested in the lysis buffer provided by the kit and sonicated using a Bioruptor® Pico (Diagenode, Denville, NJ, USA) for 10 cycles (30 seconds ON and 30 seconds OFF). Genomic DNA was removed by in-column DNase I digestion. Equivalent 100 ng of total RNA from different samples were converted to cDNA using Random Primers and the MultiScribe™ Reverse Transcriptase (4311235; Invitrogen, Carlsbad, CA, USA). Primers For quantitative real-time PCR are listed in ESM Table 1. 18s rRNA was used as the endogenous control. The $\Delta\Delta$Ct method was used for relative quantifications.

Immunoblotting Protein was extracted from isolated islets using the Laemmli buffer as previously described [10]. Equivalent 5-10 µg total proteins were electrophoresed on 10-12% SDS-PAGE gel. The following primary antibodies were used: anti-Sirt6 (12486; CST, Danvers, MA, USA), 1:500; anti-H3K9Ac (07-352; EMD Millipore, Billerica, MA, USA), 1:10 000; anti-H3K56Ac (ab76307; Abcam, Cambridge, MA, USA), 1:2 000; anti-H3K27Ac (8173P; CST), 1:500; anti-H3 (ab1791; Abcam), 1:10 000; anti-Tubulin (11224-1-AP; Proteintech, Rosemont, IL, USA), 1:5 000; anti-Txnip (14715; CST), 1:500. In the Sirt6 pancreas tissue distribution study, Ponceau S (P7170; Sigma) staining was used as loading control.

RNA-Seq analysis Total RNA of isolated islets was extracted as described above. For BKO samples, 2 weeks post-tamoxifen control (*Sirt6* $^{f/+}$; MIP1-CreERT) and *Sirt6* BKO (*Sirt6*$^{f/f}$; MIP1-CreERT) mice were used to isolated islets and extracting RNA. For *Sirt6* EKO samples, 2-month-old control (*Sirt6*$^{+/+}$; *Ngn3*-Cre or *Sirt6*$^{f/f}$) and *Sirt6* EKO (*Sirt6*$^{f/f}$; *Ngn3*-Cre) islets were used for RNA extraction. RNA integrity was determined by Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and the samples with RNA Integrity Number > 8 were selected for mRNA isolation. The RNA-seq libraries were prepared using the Truseq kit (Illumina, San Diego, CA, USA) per the manufacturer's instructions. Briefly, the poly-A containing mRNA molecules were purified by poly-T oligo-attached magnetic beads. Following purification, the mRNA is fragmented into small pieces using divalent cations under elevated temperature. The cleaved RNA fragments are copied into first strand cDNA using reverse transcriptase and random primers. This is followed by second strand cDNA synthesis using DNA polymerase I and RNase H. These cDNA fragments then go through an end repair process, the addition of a single 'A' base, and then ligation of the adapters. The products are then purified and enriched with PCR to create the final RNA-Seq library. After RNA-Seq libraries were subjected to quantification process, pooled for cBot amplification and subsequent sequencing run with Illumina HiSeq 3000 (or 2000) platform. Counts generated from raw reads were aligned to the mm9 reference genome using Tophat. Differential expression analysis was performed by the Deseq2 package in R with adjusted p-value < 0.1 and Fold Change > 1.5.

ChIP ChIP was adapted from a published µChIP protocol with minor modification [11]. In brief, control (*Sirt6*$^{+/+}$; *Ngn3*-Cre or *Sirt6* $^{f/f}$) and *Sirt6* EKO (*Sirt6*$^{f/f}$; *Ngn3*-Cre) islets were harvested from 3-month-old mice. Islets were washed with phosphate buffered saline (PBS) twice and

crosslinked in 1% formaldehyde (28908; Fisher Scientific) with 10% FBS for 10 min at room temperature. Then, the Glycine was added to a final concentration of 125 mmol/l for 5 min, followed by rinsing with PBS and snap froze in liquid nitrogen. Frozen cells were thawed on ice and resuspended in SDS lysis buffer (50 mmol/l Tris-HCl, pH 8.0, 10 mmol/l EDTA, 1% SDS) with protease inhibitor cocktail (11836153001; ROCHE, Basel, Switzerland), phosphatase inhibitor cocktail (P3200; GenDEPOT, Katy, TX, USA) and Trichostatin A (T8552; Sigma). Tissue was homogenized by passing through a 30G needle 8 times. After centrifugation at 1000 g for 10 min, pellets were resuspended in RIPA buffer (10 mmol/l Tris-HCl, pH 7.5, 140 mmol/l NaCl, 1 mmol/l EDTA, 0.5 mmol/l EGTA, 1% Triton X-100, 0.1% sodium deoxycholate) and sonicated by Bioruptor® Pico (Diagenode) for 8min (30s ON, 30s OFF, 8 cycle). The resulting chromatin was cleared by centrifuging at 14 000g for 15min and the supernatant was either diluted with RIPA buffer or directly transferred to pre-bound antibody and beads complex in 200 µl tubes. After overnight incubation, the chromatin-antibody-beads complex was washed twice in TSE I (20 mmol/l Tris-HCl, pH 8.0, 150 mmol/l NaCl, 2 mmol/l EDTA, 0.1% SDS, 1% Triton X-100), TSE II (20 mmol/l Tris-HCl, pH 8.0, 500 mmol/l NaCl, 2 mmol/l EDTA, 0.1% SDS, 1% Triton X-100), TSE III (10 mmol/l Tris-HCl, pH 8.0, 250 mmol/l LiCl, 2 mmol/l EDTA, 1% DOC, 1% NP-40), and TE (50 mmol/l Tris-HCl, pH 8.0, 2 mmol/l EDTA). Beads were then captured by a magnetic field (20-400, Millipore), and incubated with Elution buffer (20 mmol/l Tris-HCl, pH 7.5, 5 mmol/l EDTA, 50 mmol/l NaCl, 1% SDS) and Proteinase K (50 µg/ml) at 68°C for at least 4 hours. DNA was extracted using Phenol/Chloroform/Isoamyl Alcohol (BP1753; Fisher Scientific, Hampton, NH, USA) and then eluted with DNase, RNase free-H_2O. The antibodies were obtained from the following suppliers: anti-SIRT6 (D8D12; 12486, CST); anti-H3K9Ac (07-352; EMD Millipore); anti-H3K56Ac (07-677; Millipore); anti-Pol II (05-623B; Millipore); anti-mouse IgG (12-371B; Millipore). Primers for qPCR were listed in ESM Table 1.

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[4] Oropeza D, Jouvet N, Budry L, et al. (2015) Phenotypic Characterization of MIP-CreERT1Lphi Mice With Transgene-Driven Islet Expression of Human Growth Hormone. Diabetes 64: 3798-3807

[5] Chen H, Gu X, Su IH, et al. (2009) Polycomb protein Ezh2 regulates pancreatic beta-cell Ink4a/Arf expression and regeneration in diabetes mellitus. Genes Dev 23: 975-985

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[9] Neuman JC, Truchan NA, Joseph JW, Kimple ME (2014) A method for mouse pancreatic islet isolation and intracellular cAMP determination. J Vis Exp: e50374

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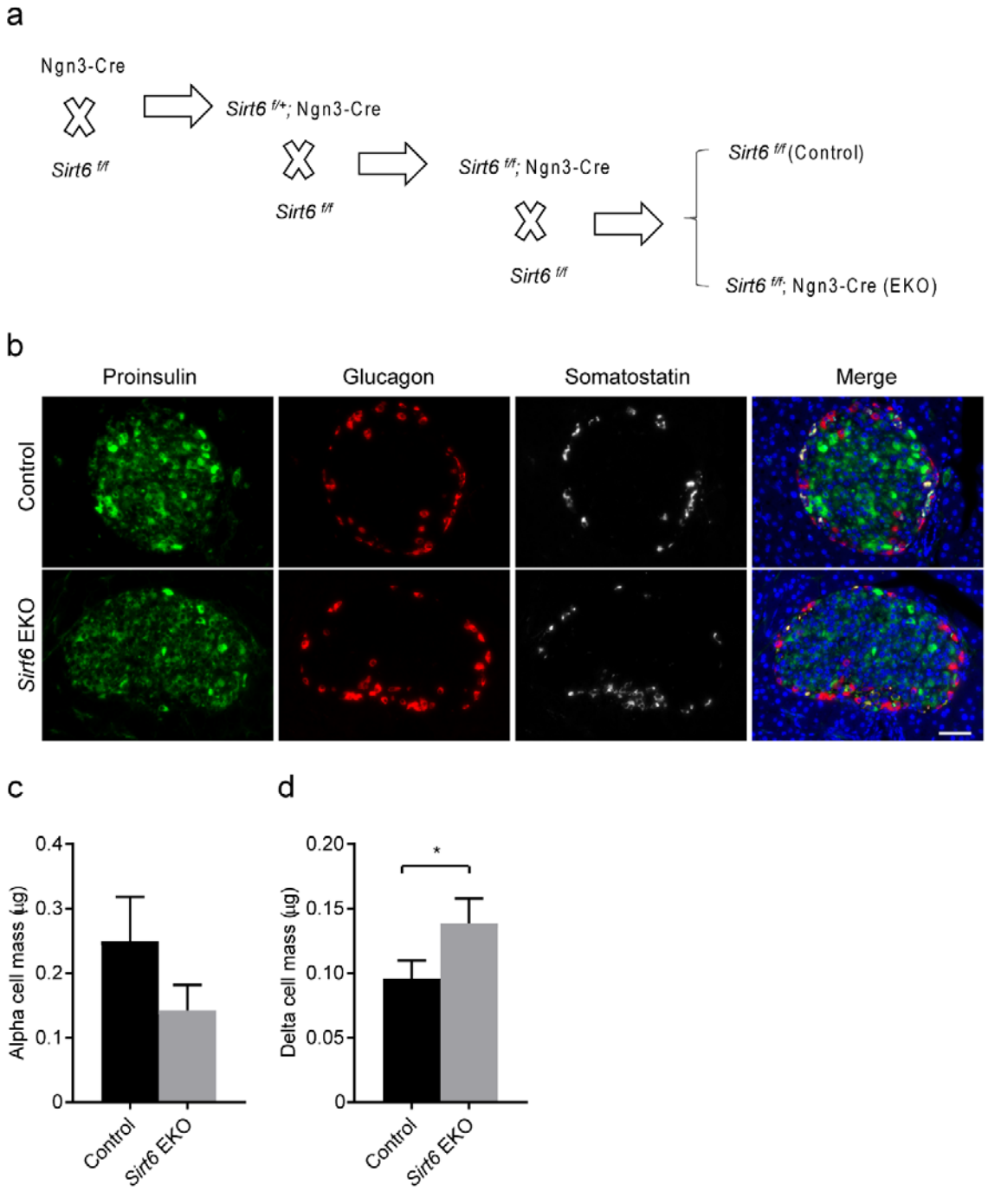
[11] Dahl JA, Collas P (2008) A rapid micro chromatin immunoprecipitation assay (microChIP). Nature protocols 3: 1032-1045

ESM Table 1. Primers

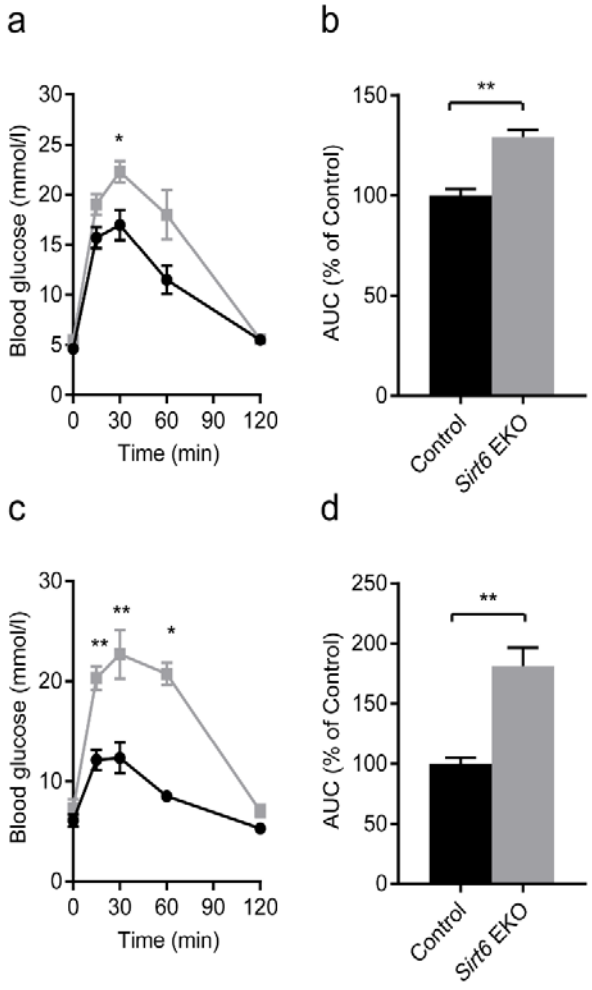
Genotyping primer	
Ngn3-Cre F	GAGGCTCAGCTATCCACTGC
Ngn3-Cre R	AGGCAAATTTTGGTGTACGG
MIP1-CreERT F	CTGGCGATCCCTGAACATGTCCT
MIP1-CreERT R	GGACTATAAAGCTGGTGGGCAT
Sirt6 F	GCTAATGGGAACGAGACCAA
Sirt6 R	ACCCACCTCTCTCCCCTAAA
RT-qPCR primer	
Sirt6 F	GCTGGCCCGGCTAATGTGGC
Sirt6 R	CCCAGTCCAGAATGGTGTCT
Txnip F	GGCCGGACGGGTAATAGTG
Txnip R	AGCGCAAGTAGTCCAAAGTCT
18S rRNA F	AGTCCCTGCCCTTTGTACACA
18s rRNA R	CGATCCGAGGGCCTCACTA
ChIP primer	
Txnip-TSS F	GAACCCACTCGGCTCAATCA
Txnip-TSS R	CGGCTTTGACTCGGGTAACT
Txnip-up2kb F	CCTAGGCCTTGGTGTTGTGTA
Txnip-up2kb R	CCTAACTGGGTTGACACCCTAA

ESM Fig. 1


a
Ngn3-Cre
Sirt6 f/f
Sirt6 f/+; Ngn3-Cre
Sirt6 f/f
Sirt6 f/f; Ngn3-Cre
Sirt6 f/f
Sirt6 f/f (Control)
Sirt6 f/f; Ngn3-Cre (EKO)
b
Proinsulin
Glucagon
Somatostatin
Merge
Control
Sirt6 EKO
c
Alpha cell mass (µg)
0.4
0.3
0.2
0.1
0
Control
Sirt6 EKO
d
Delta cell mass (µg)
0.20
0.15
0.10
0.05
0
*
Control
Sirt6 EKO

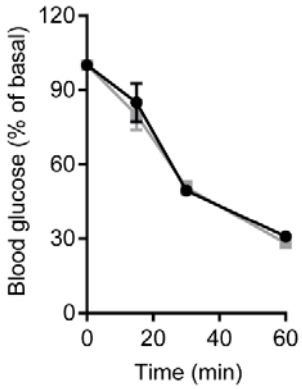
ESM Fig. 1 Deletion of *Sirt6* in pancreatic endocrine progenitor cell does not affect beta cell development (related to Fig. 1). (**a**) The breeding scheme for generating control (Ctrl, *Sirt6*$^{f/f}$) and *Sirt6* EKO mice. (**b**) Immunostaining of pancreatic islets (Green: anti-PROINSULIN, Blue: DAPI, Red: anti-GLUCAGON, White: anti-SOMATOSTATIN) from the control and EKO mice. Scale bar = 50 μm. (**c-d**) Quantification of the alpha cell mass (**c**) and delta cell mass (**d**). Black bars, control mice; grey bars, *Sirt6* EKO mice. Data are expressed as means ± SEM (n=3). *p<0.05 and **p<0.01 for indicated comparisons

ESM Fig. 2



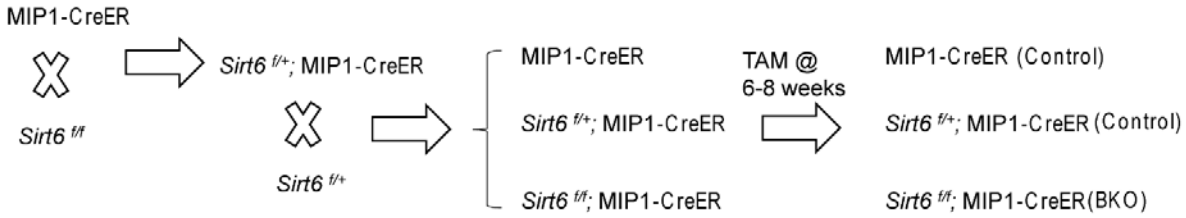
ESM Fig. 2 Deletion of *Sirt6* in pancreatic endocrine progenitor cell leads to progressive glucose intolerance in females (related to Fig. 2). (**a**) Glucose tolerance tests of 2- to 3-month-old control ($Sirt6^{f/f}$) and *Sirt6* EKO female mice (n=4). (**b**) The normalized area of the glucose tolerance test curve of 2- to 3-month-old control and *Sirt6* EKO female mice (n=4). (**c**) Glucose tolerance tests of 5- to 8-month-old control and *Sirt6* EKO female mice (n=3). (**d**) The normalized area of the glucose tolerance test curve of 5- to -8-month-old control and *Sirt6* EKO female mice (n=3). AUC, area under curve. Black bars and circles, control mice; grey bars and squares, *Sirt6* EKO mice. Data are expressed as means ± SEM. *p<0.05 and **p<0.01 vs control mice

ESM Fig. 3



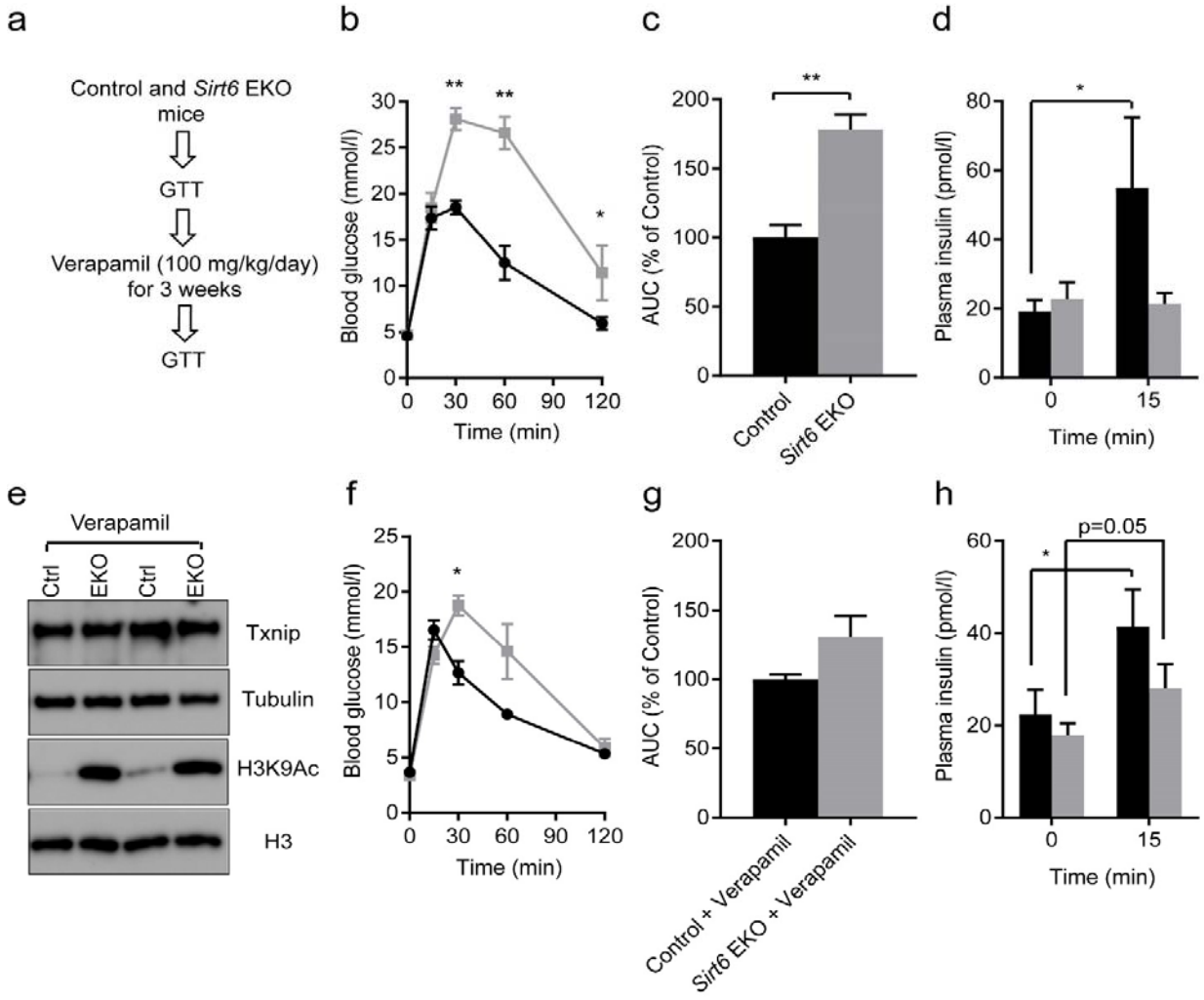
ESM Fig. 3 Insulin tolerance tests of 8- to 10-month-old control (*Sirt6*$^{f/f}$) and *Sirt6* EKO male mice (related to Fig. 3). Black bars and circles, control mice; grey bars and squares, *Sirt6* EKO mice. Data are expressed as means ± SEM (n=4-6). *p<0.05 and **p<0.01 vs control mice

ESM Fig. 4



ESM Fig. 4 The breeding scheme for generating control and *Sirt6* BKO mice (related to Fig. 4). TAM, tamoxifen

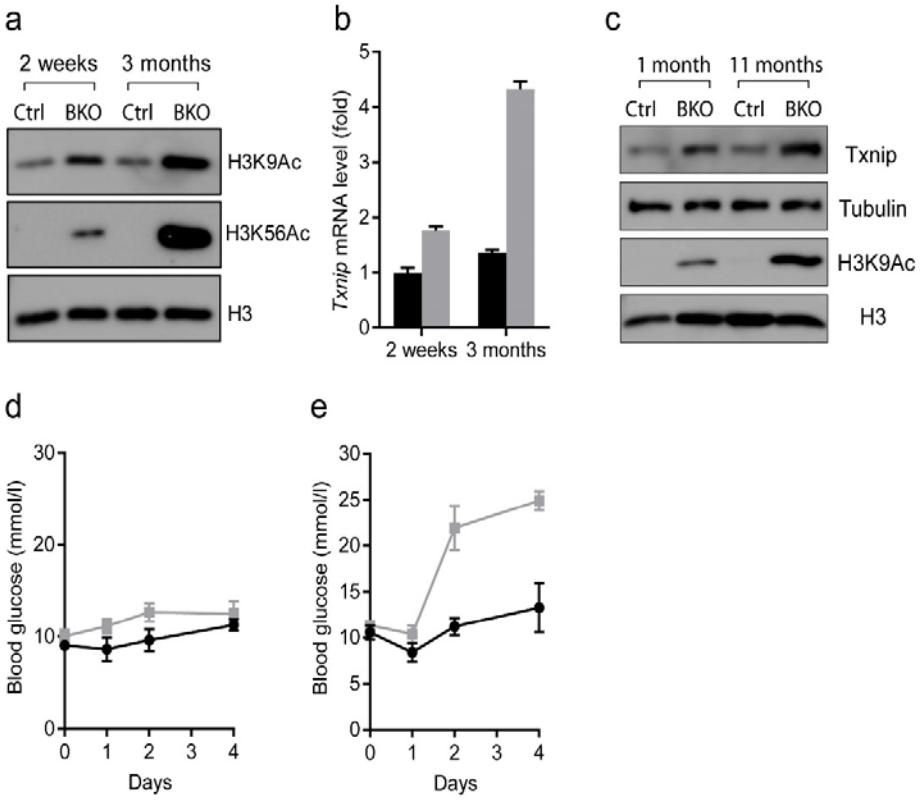
ESM Fig. 5



ESM Fig. 5 Verapamil treatment largely rescues the glucose intolerance in the 8- to 9-month-old *Sirt6* EKO female mice (related to Fig. 5). (**a**) The scheme for evaluating the effect of oral verapamil treatment on *Sirt6* EKO mice. (**b**) Glucose tolerance tests of 8- to 9-month-old control (*Sirt6*$^{f/f}$) and *Sirt6* EKO female mice before verapamil treatment (n=4-6). (c) Normalized area of the glucose tolerance test curve of (**b**). (**d**) Plasma insulin levels of 8- to 9-month-old control and *Sirt6* EKO female mice during the glucose tolerance test (n=3-4). (**e**) Immunoblot of Txnip protein in the islets isolated from the verapamil-treated 8- to 9-month-old control and *Sirt6* EKO mice. (**f**) Glucose tolerance tests of 8- to 9-month-old control and Sirt6 EKO female mice after 3 weeks verapamil treatment (n=4-6). (**g**) Normalized area of the glucose tolerance test curve of (**e**). (**h**) Plasma insulin levels of verapamil treated 8- to 9-month-old control and *Sirt6* EKO

during the glucose tolerance test (n=3-4). Black bars and circles, control mice; grey bars and squares, *Sirt6* EKO mice. Data are expressed as means ± SEM. *p<0.05 and **p<0.01 vs control mice

ESM Fig. 6



ESM Fig. 6 Long-term deletion of Sirt6 in beta cells induces susceptibility to STZ (related to Fig. 6). (**a**) Immunoblots of H3K9Ac and H3K56Ac levels in 2 weeks and 3 months post-tamoxifen control and *Sirt6* BKO islets. Ctrl, a combination of MIP1-CreER or *Sirt6*$^{f/+}$; MIP1-CreER mice after tamoxifen treatment. (**b**) *Txnip* mRNA levels in 2 weeks and 2 months post-tamoxifen control and *Sirt6* BKO islets (n=2). (c) Immunoblots of Txnip and H3K9Ac levels in 1 month and 11 months post-tamoxifen *Sirt6* BKO islets. (d) Blood glucose levels of the 2 weeks post-tamoxifen control and *Sirt6* BKO mice after a single STZ (140 mg/kg) treatment (n=3-4). (e) Blood glucose levels of 6 months post-tamoxifen control and *Sirt6* BKO mice after a single STZ (140 mg/kg) treatment (n=2). Black bars and circles, control mice; grey bars and squares, *Sirt6* BKO mice. Data are expressed as means ± SEM. *p<0.05 and **p<0.01 vs control mice