

Supplementary information for:

Impaired glucose tolerance and mild diabetes induce β -cell dysfunction in mice

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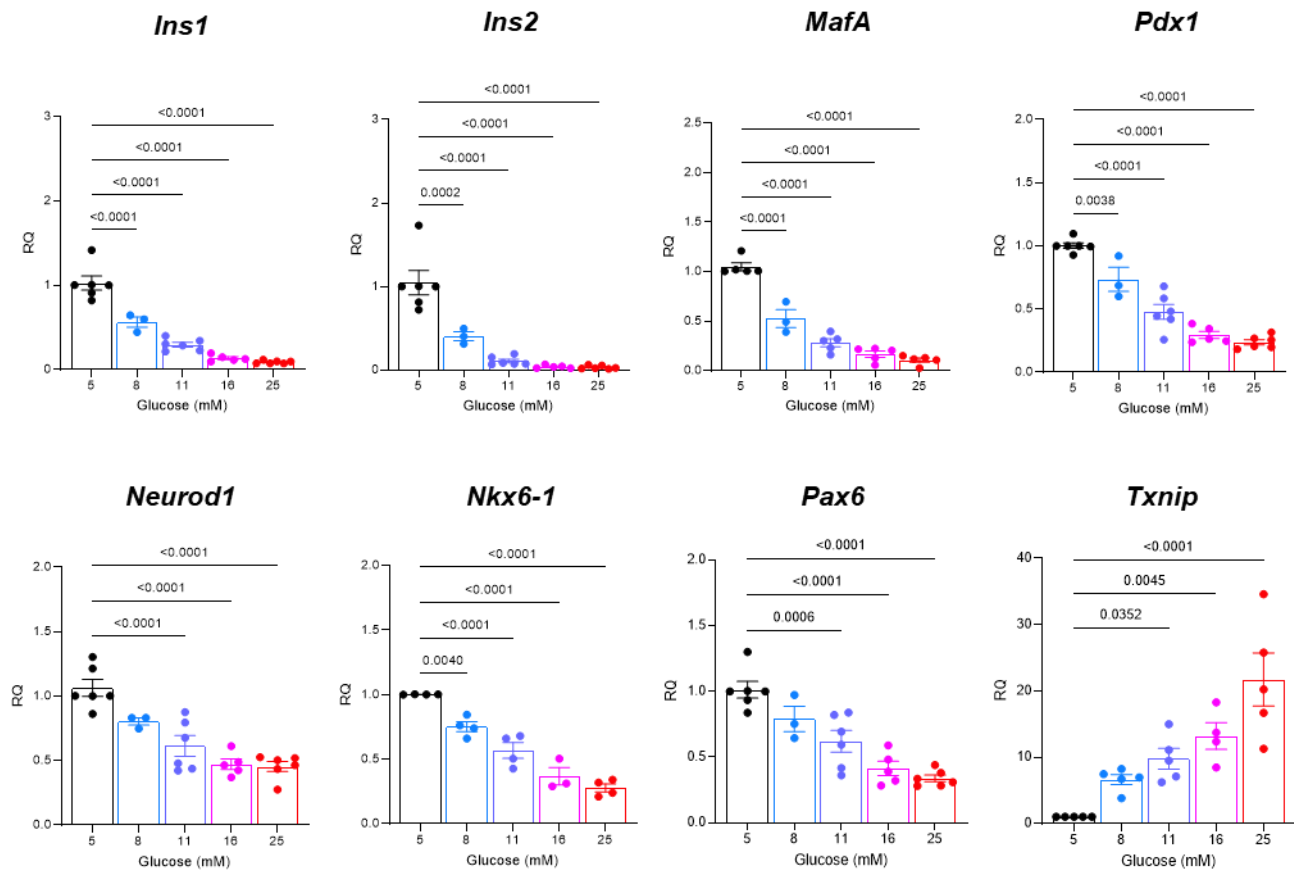
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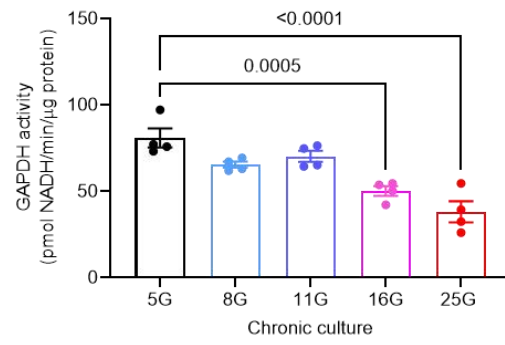
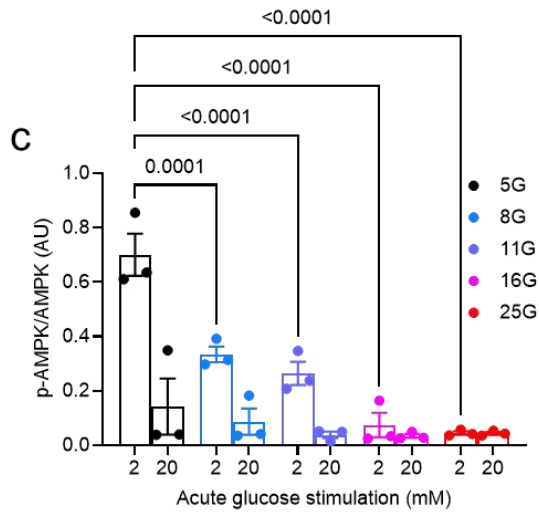
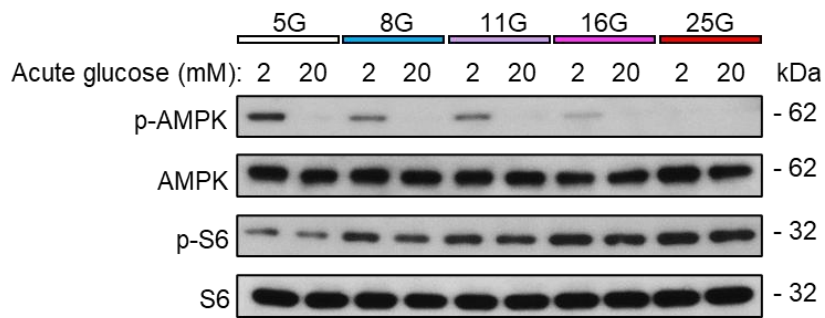
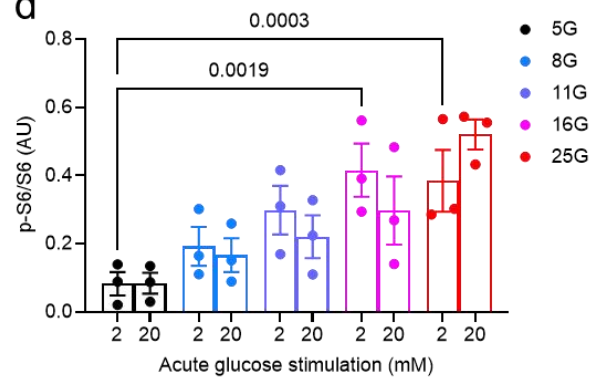
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Supplementary Figure 1 Effects of chronic glucose on expression of selected genes in INS-1 cells

mRNA levels for the indicated genes in INS-1 cells cultured for 48h at the indicated glucose concentrations (n=3-5). All panels show individual data points and mean $\pm$ s.e.m. N values indicate the number of biologically independent experiments. Two-tailed unpaired Student's t test. One-way ANOVA with Bonferroni post hoc test. Source data are provided as a Source Data file.

a**b****d**

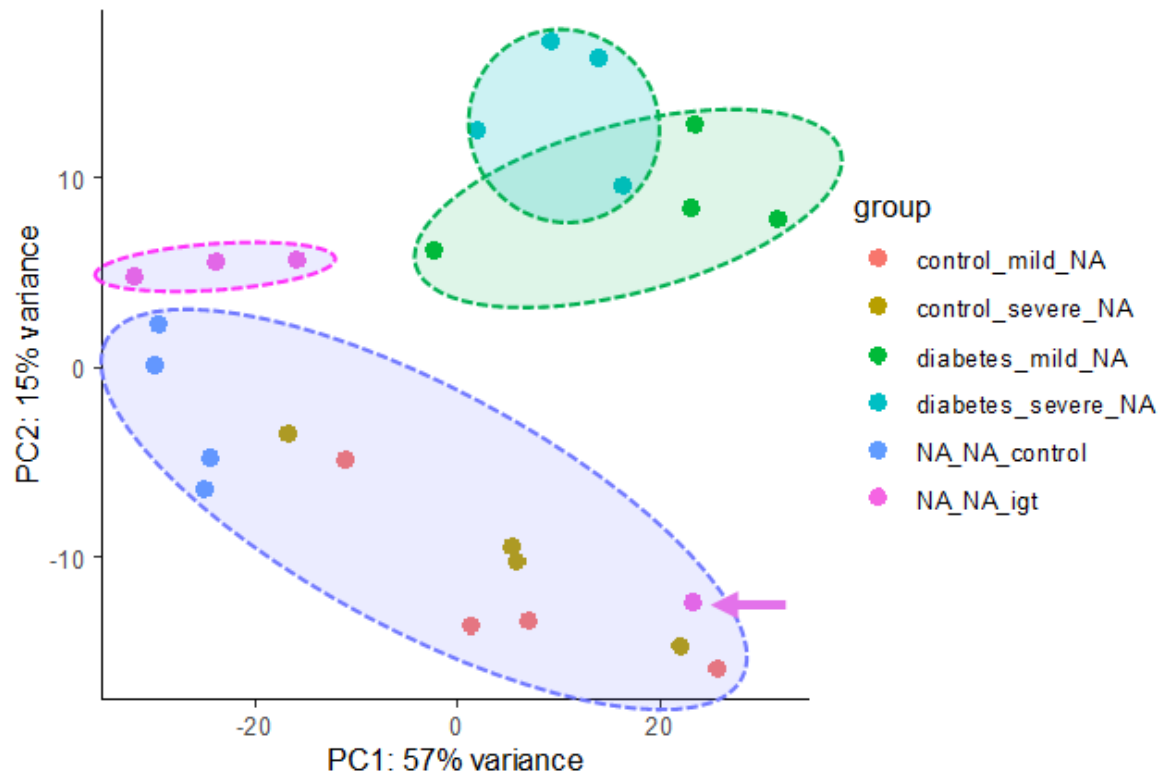
Supplementary Figure 2 *Effects of chronic glucose on GAPDH and AMPK activity*

(a) Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity in INS-1 cells cultured for 48h at the indicated glucose concentrations (n=4 biologically independent experiments).

(b) Representative Western blot of lysates from INS-1 cells cultured at the indicated glucose concentration (G) for 48h and then stimulated with 2mM or 20mM glucose for 30min. Phosphorylated (p) and total AMPK and S6.

(c,d) Quantitative densitometry analysis of p-AMPK/AMPK (c, n=3 experiments) and p-S6/S6 (d, n=3 experiments).

All panels (a,c,d) show individual data points and mean $\pm$ s.e.m. N values indicate the number of biologically independent experiments. Two-way ANOVA with Bonferroni post hoc test (b,c) and one-way ANOVA with Bonferroni post hoc test (d). Source data are provided as a Source Data file.



Supplementary Figure 3 *PCA plot of RNAseq data from IGT, mild-HG and severe-HG mouse islets*

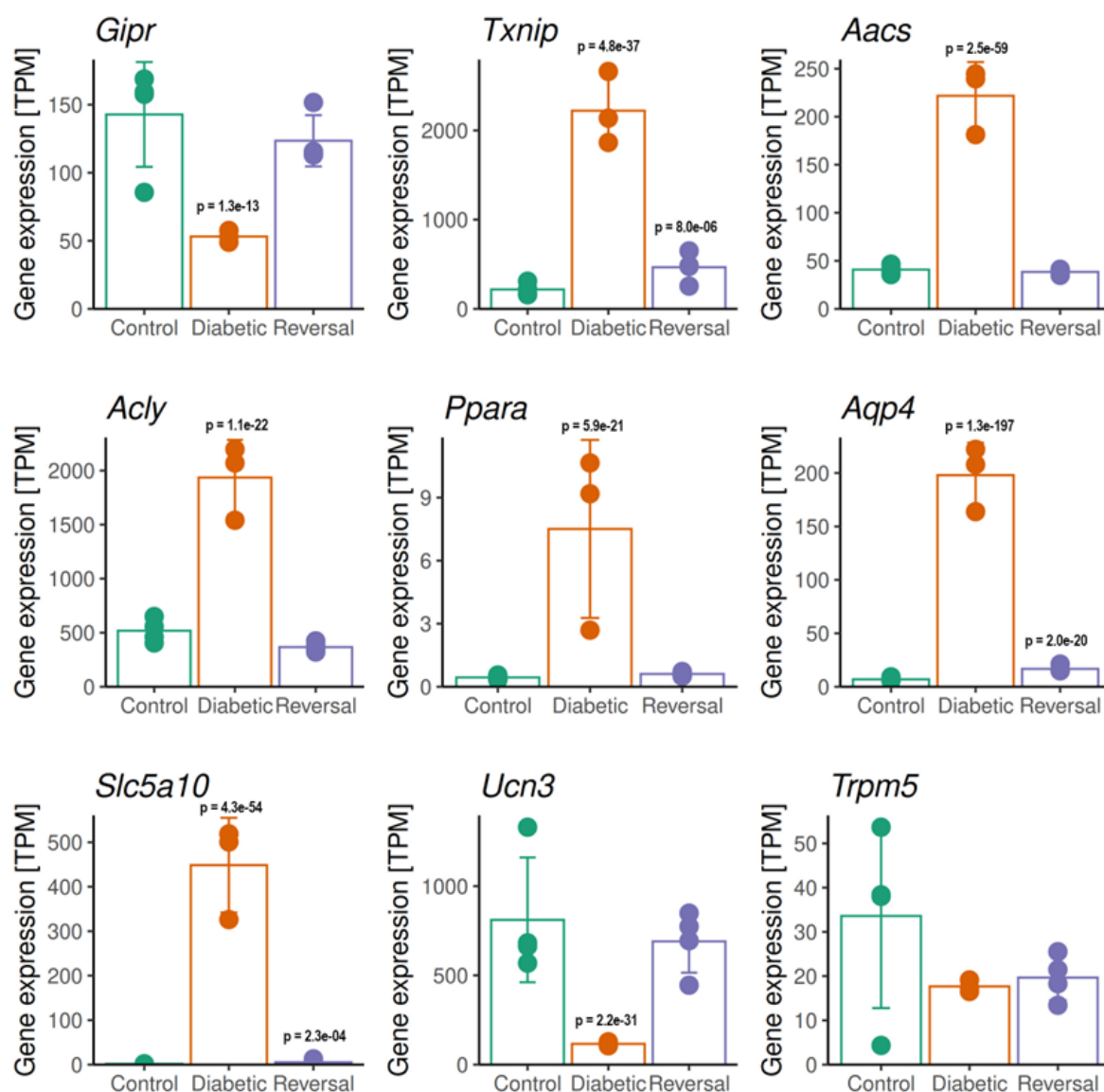
PCA plot of RNAseq data from IGT, mild-HG and severe-HG mouse islets and their respective controls. The plot is based on all genes identified. Control mice were injected with the same concentration of tamoxifen as their respective experimental cohorts. Arrow indicates a mouse that deviated from the others in the IGT set.

KEGG analysis

pathway	severe	mild	IGT
Citrate cycle (TCA cycle)	3.70E-06	3.10E-04	8.19E-02
Collecting duct acid secretion	9.66E-05	3.99E-04	9.59E-02
Lysosome	3.51E-04	1.14E-07	8.66E-03
Glycolysis / Gluconeogenesis	9.19E-04	1.70E-03	2.83E-02
Fatty acid degradation	1.35E-03	5.41E-01	4.15E-01
Central carbon metabolism in cancer	1.35E-03	1.24E-03	2.30E-02
Pentose phosphate pathway	1.42E-03	1.93E-02	8.56E-03
Valine, leucine and isoleucine degradation	1.88E-03	7.97E-03	3.40E-02
Fructose and mannose metabolism	2.03E-03	2.78E-06	1.55E-03
MAPK signaling pathway	2.08E-03	2.20E-08	1.24E-01
Endocytosis	2.27E-03	2.05E-01	7.43E-01
Butanoate metabolism	2.49E-03	6.14E-02	1.84E-01
Oxidative phosphorylation	2.64E-03	1.14E-02	7.38E-01
Alanine, aspartate and glutamate metabolism	3.48E-03	3.06E-01	1.37E-01
HIF-1 signaling pathway	5.86E-03	2.84E-05	1.54E-01
mTOR signaling pathway	6.60E-03	3.34E-02	7.27E-01
Pyruvate metabolism	6.83E-03	3.55E-02	8.56E-02
Colorectal cancer	6.97E-03	5.31E-02	5.26E-01
Gastric cancer	8.01E-03	1.56E-04	1.54E-01
Insulin signaling pathway	8.15E-03	4.94E-03	1.60E-01
total no. of significant pathways	216	240	46

Supplementary Figure 4 *Pathway analysis of RNAseq data*

KEGG analysis of RNAseq data showing the top 20 pathways affected in severe-HG islets (listed in order of significance). The significance of these pathways in mild-HG and IGT islets is included for comparison. Pathways in which $p < 0.05$ are highlighted in yellow. The total number of significant pathways was 216 in severe-HG islets, 240 in mild-HG islets and 46 in IGT islets.

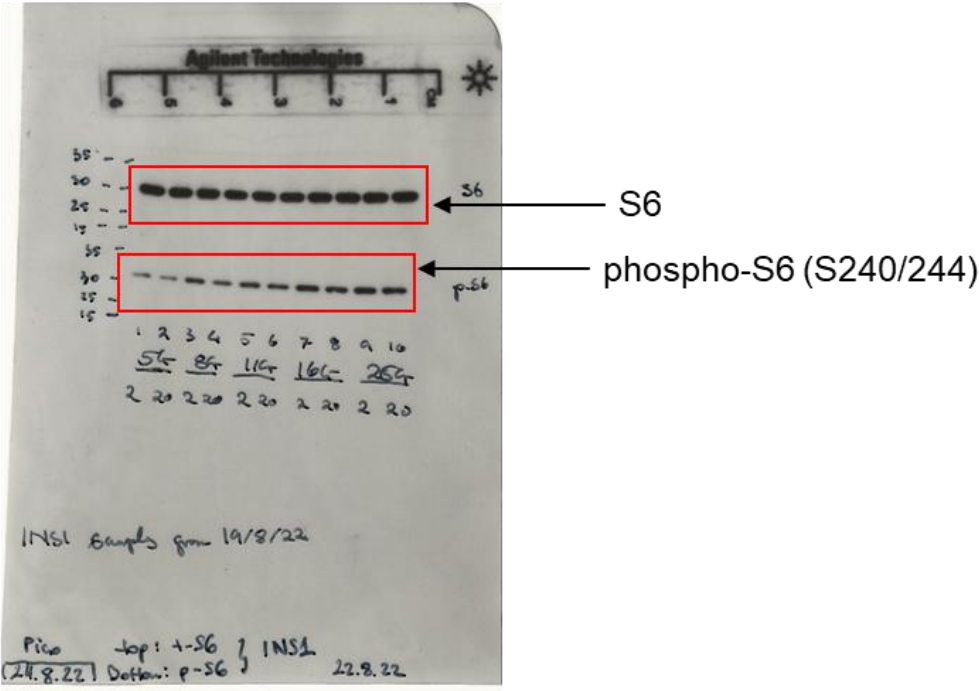


Supplementary Figure 5 *Hyperglycaemia-induced changes in gene expression are reversed following restoration of euglycaemia*

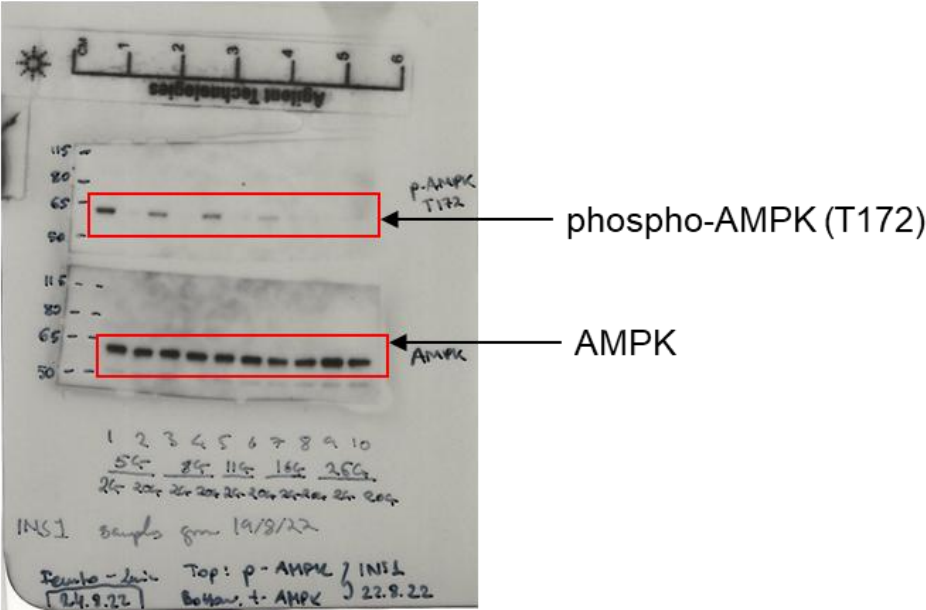
Expression of indicated genes in islets isolated from control mice (green, n=4), 2-wk severe-HG mice (diabetic, red, n=3) and 2-wk severe-HG mice subsequently treated for 2-wk with glibenclamide to restore euglycaemia (reversal, blue, n=4). Genes that show major changes in diabetes have been selected. Expression is shown as transcripts per million (TPM). Mean ($\pm$ SD) and individual data points are shown. Significance against control data is indicated. N values indicate the number of biologically independent experiments. Exact test for a difference in mean between two groups of negative binomial random variables as implemented in the R package *edgeR* with multiple testing correction using the Benjamini–Hochberg procedure. Source data are provided as a Source Data file.

Uncropped blots corresponding to Supplementary Fig.2b

mTORC1 – S6, pS6



AMPK – AMPK, p-AMPK



Supplementary Figure 6 *Original Western blots for the data shown in Supplementary Figure 2b*

(above) Uncropped original Western blot of lysates from INS-1 cells cultured at the indicated glucose concentration (G) for 48h and then stimulated with 2mM or 20mM glucose for 30min. Total S6 and phosphorylated S6 (at S240 and 244) are indicated. The red boxes indicate the parts of the blot shown in Supplementary Figure 2b.

(below) Uncropped original Western blot of lysates from INS-1 cells cultured at the indicated glucose concentration (G) for 48h and then stimulated with 2mM or 20mM glucose for 30min. Total AMPK and phosphorylated AMPK (at T172) are indicated. The red boxes indicate the parts of the blot shown in Supplementary Figure 2b.

Western blot antibodies:

Antibody	Source	Dilution
Rabbit anti-phospho-AMPK α -T172 antibody	Cell Signaling Technology, cat. #2535	1:1000
Rabbit anti-AMPK α antibody	Cell Signaling Technology, cat. #2532	1:1000
Rabbit anti-phospho-S6-S240/244 antibody	Cell Signaling Technology, cat. #5364	1:4000
Rabbit anti-S6 antibody	Cell Signaling Technology, cat. #2217	1:4000

Supplementary Table1

List of antibodies used

Gene	Catalogue number
<i>Actb</i>	Mm02619580_g1
<i>Aldob</i>	Mm00523293_m1 (mouse) and Rn01768292_m1 (rat)
<i>Cox6a2</i>	Mm00438295_g1
<i>Eno1</i>	Mm01619597_g1
<i>Fbp1</i>	Mm00490181_m1
<i>Fbp2</i>	Mm00484280_m1
<i>Fhl</i>	Mm01321349_m1
<i>Gapdh</i>	Rn01775763_g1
<i>G6pc2</i>	Mm00491176_m1
<i>Hspa8</i>	Rn00821191_g1
<i>Hprt1</i>	Rn01527840_m1
<i>Idh2</i>	Rn01478119_m1
<i>Ins1</i>	Rn02121433_g1
<i>Ins2</i>	Mm00731595_gH
<i>Maifa</i>	Rn00845206_s1
<i>Mdh2</i>	Mm00725890_s1
<i>Ndufa4</i>	Mm00809672_s1
<i>Ndufs2</i>	Rn01411711_m1
<i>Ndufs8</i>	Mm00523063_m1
<i>Neurod1</i>	Rn01280117_m1
<i>Nkx6-1</i>	Rn01450076_m1
<i>Ogdh</i>	Mm00803119_m1
<i>Pax6</i>	Rn00689608_m1
<i>Pdk1</i>	Mm00554306_m1 (mouse) and Rn00587598_m1 (rat)
<i>Pdx1</i>	Rn00755591_m1
<i>Pfkfb2</i>	Mm00435575_m1
<i>Pfkfb3</i>	Mm00504650_m1 (mouse) and Rn00678825_m1 (rat)
<i>Pfkf</i>	Mm00435587_m1
<i>Ppargc1a</i>	Mm01208835_m1
<i>Ppp1r3c</i>	Mm01204084_m1
<i>Sdh</i>	Rn00590475_m1
<i>Txnip</i>	Mm00452393_m1

Supplementary Table 2

List of Taqman probes used