

## **Supplemental Information**

### **An insulin hypersecretion phenotype precedes pancreatic $\beta$ cell failure in MODY3 patient-specific cells**

**Florian M. Hermann, Maya Friis Kjærgaard, Chenglei Tian, Ulf Tiemann, Abigail Jackson, Lars Rønn Olsen, Maria Kraft, Per-Ola Carlsson, Iina M. Elfving, Jarno L.T. Kettunen, Tiinamaija Tuomi, Ivana Novak, and Henrik Semb**

## **Supplemental Information**

### **Insulin hypersecretion is the primary defect in MODY3 pancreatic $\beta$ cells**

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#### **Supplemental Figures**

Figure S1, Related to Figure 1.

Figure S2. Related to Figure 1.

Figure S3. Related to Figures 1 and 2.

Figure S4. Related to Figure 4.

Figure S5. Related to Figures 4 and 6.

Figure S6. Related to Figure 5.

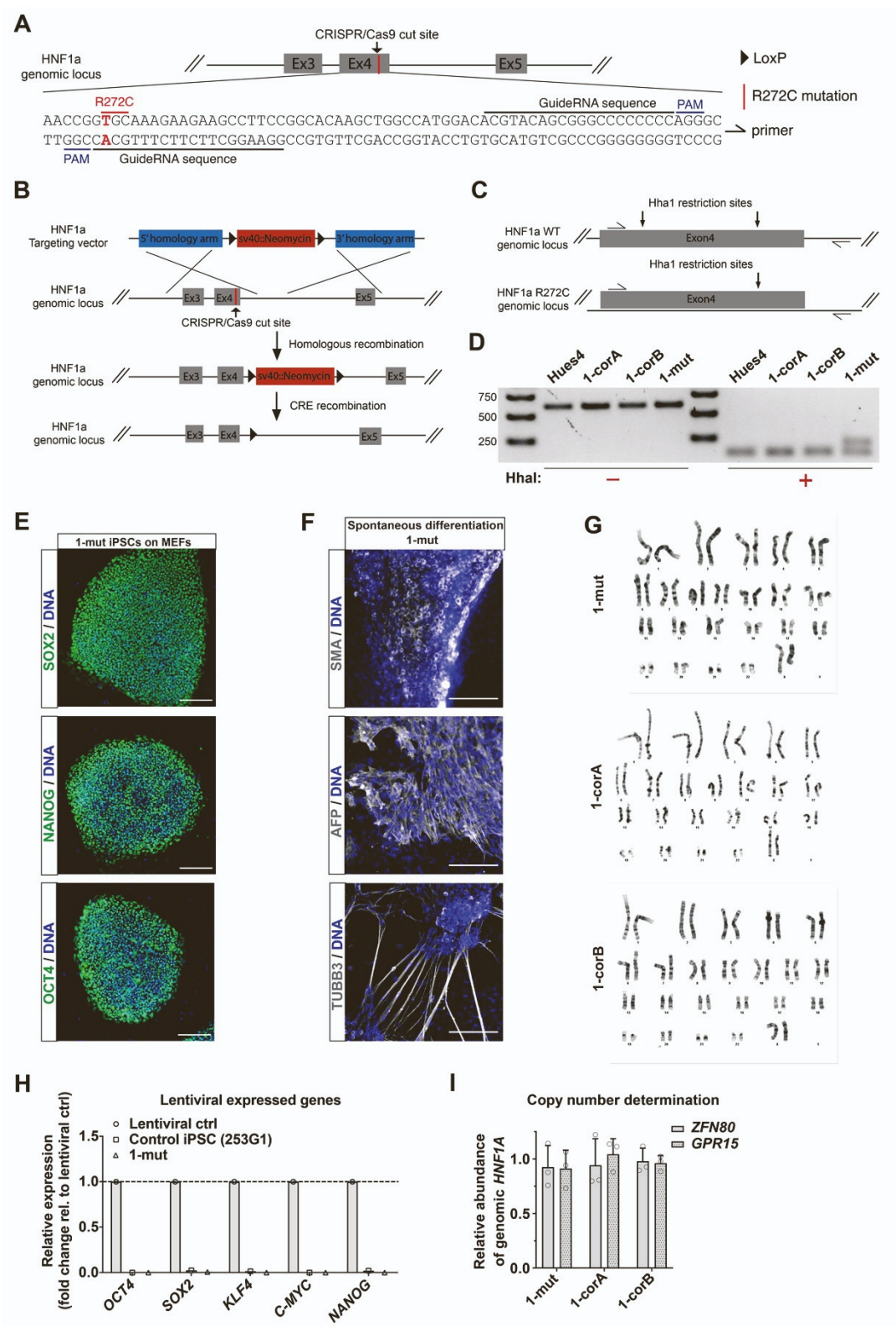
#### **Supplemental Table**

Table S1, Related to Figure 3

Table S2, Related to Figures S1 and S2, Key Resources Table

Table S3, Related to Figures 4, S3, S4 and S5, Key Resources Table

Figure S1



**Supplementary Figure 1. CRISPR-Cas9-facilitated genetic correction and characterisation of a MODY3 hiPSC line.**

(A and B) Schematics illustrating the design of the guide RNAs for double nicking in exon4 of *HNFI1A* (A), and the homologous recombination strategy for correction of the T nucleotide (B).

(C) Schematic explaining the strategy for restriction fragment length experiment verifying correction of the heterozygous R272C mutation (loss of one HhaI restriction site in R272C MODY3 allele).

(D) PCR products of genomic DNA from Hues4 hESCs (WT control), corrected isogenic control hiPSCs (1-corA and 1-corB) and patient-specific 1-mut hiPSCs before (left) and after (right) digestion with the restriction enzyme HhaI.

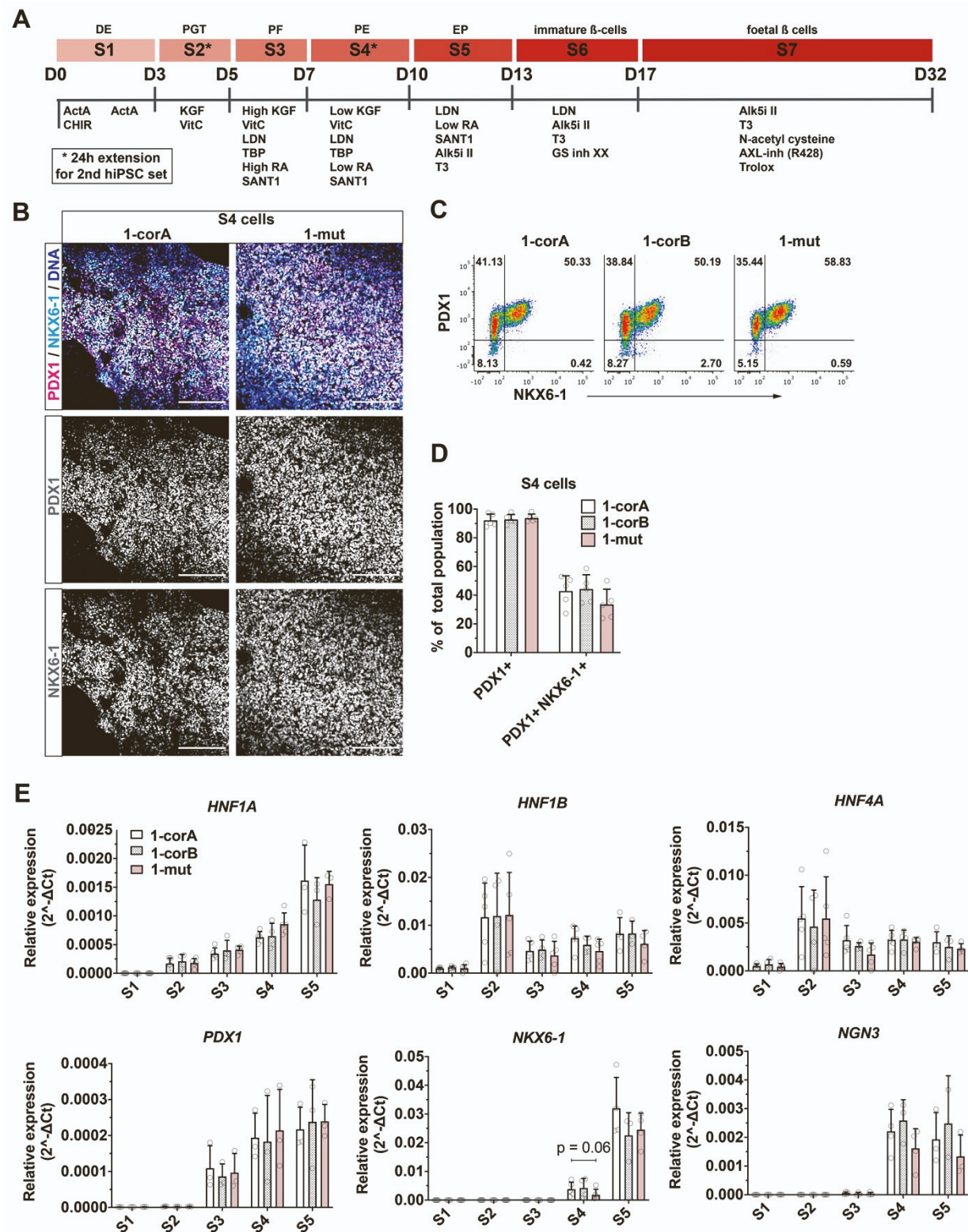
(E and F) Representative immunofluorescence images of MODY3 hiPSCs (1-mut) stained for pluripotency markers (SOX2, NANOG and OCT4) (E), or for endodermal (AFP), mesodermal (SMA) and ectodermal (TUBB3) germ layer markers following spontaneous embryoid body differentiation. Scale bars: 100  $\mu$ m (F).

(G) Karyotype of 1-corA, 1-corB and 1-mut hiPSC lines (normal 46XX).

(H) RT-qPCR of the lentiviral reprogramming factors (*OCT4*, *SOX2*, *KLF4*, *CMYC* and *NANOG*) in a positive lentiviral control, control hiPSCs and 1-mut. n = 1 independent experiment.

(I) The copy number of the integrated targeting vector in the two corrected clones compared to the mutant cell line was determined by quantifying exon four of *HNFI1A* and two reference genes (*ZFN80* and *GPR15*) by qPCR. Data were collected from 3 technical replicates. Data are represented as mean + SD.

Figure S2



**Supplementary Figure 2. Directed differentiation of pancreatic progenitors from hiPSCs.**

(A) Schematic of the differentiation protocol used throughout the study, with optimisations required for the second hiPSC set noted by asterisks. S, stage; D, day(s); DE, definitive endoderm; PGT, primitive gut tube; PF, posterior foregut; PE, pancreatic endoderm; EP, endocrine precursors.

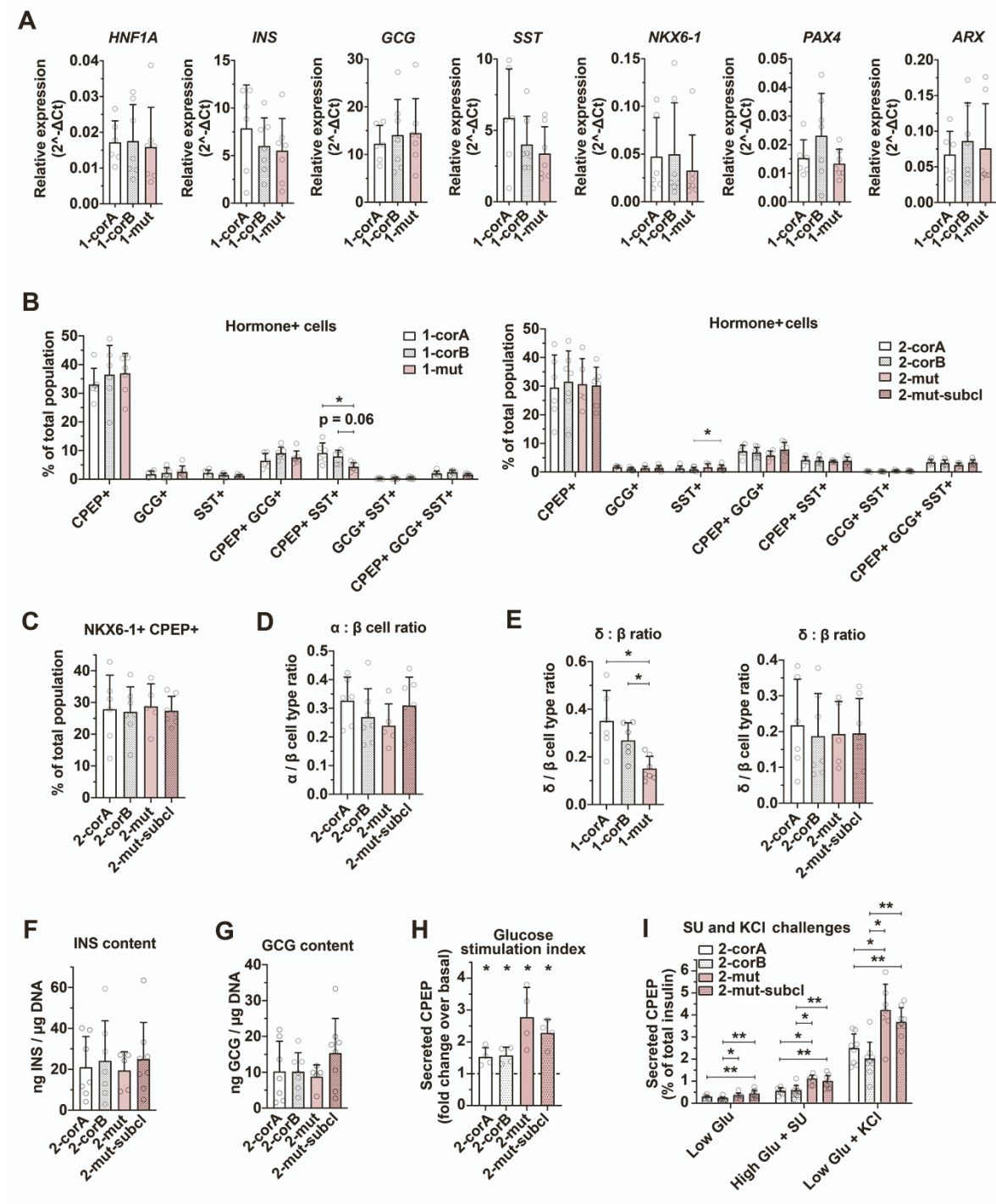
(B) Representative immunofluorescence images of S4 cells stained for PE markers (PDX1 and NKX6-1). Scale bar: 100  $\mu$ m.

(C and D) Representative flow cytometry density plots (C) and quantifications (D) of S4 cells stained for PDX1 and NKX6-1. A Mann-Whitney test was used to assess significance. n = 5 independent experiments. Data are represented as mean + SD.

(E) RT-qPCR time course analyses (S1-S5) of *HNF1A* and closely related genes (*HNF1B* and *HNF4A*), as well as PE and EP markers (*PDX1*, *NKX6-1* and *NGN3*). A Mann-Whitney test was used to assess significance. n = 3-5 independent experiments. Data are represented as mean + SD.



Figure S3



**Supplementary Figure 3. Directed differentiation of pancreatic endocrine cells from hiPSCs and the characterisation of hiPSC-derived pancreatic endocrine cells.**

(A) RT-qPCR analysis of *HNF1A*, pancreatic hormones (*INS*, *GCG* and *SST*) and endocrine transcription factors (*NKX6-1*, *PAX4* and *ARX*) in S7. n = 6 (1-corA) or 7 (1-corB and 1-mut) independent experiments.

(B and C) Flow quantifications of the mono- and polyhormonal CPEP<sup>+</sup>, GCG<sup>+</sup> and / or SST<sup>+</sup> populations (B) and the CPEP<sup>+</sup> / NKX6-1<sup>+</sup>  $\beta$  cell populations (C). n = 5 (2-mut), 6 (1-corA, 1-corB, and 2-corA) or 7 (1-mut, 2-corB, 2-mut-subcl) independent experiments. Wilcoxin matched-pairs signed-rank test analysis.

(D and E) The ratio of  $\alpha$  cell (single GCG<sup>+</sup> and double GCG<sup>+</sup> / CPEP<sup>+</sup>) to  $\beta$  cell populations (single CPEP<sup>+</sup>; D) and  $\delta$  cell (single SST<sup>+</sup> and double SST<sup>+</sup> / CPEP<sup>+</sup>) to  $\beta$  cell population (E). n = 5 (2-mut), 6 (1-corA, 1-corB, and 2-corA) or 7 (1-mut, 2-corB, 2-mut-subcl) independent experiments. Wilcoxin matched-pairs signed-rank test analysis.

(F and G) ELISA measurements of total INS (F) and GCG content (G) normalized to DNA content in S7. n = 5 (2-mut), 6 (1-corA, 1-corB, and 2-corA) or 7 (1-mut, 2-corB, 2-mut-subcl) independent experiments.

(H) The fold change of CPEP secretion in 30 minutes 16.67 mM glucose relative to 1.67 mM glucose in S7. Asterisks indicate significance between low and high glucose, calculated with a Mann-Whitney test. n = 4 independent experiments.

(I) ELISA measurements of secreted CPEP after 30 minutes in 1.67 mM glucose (Low Glu), 16.67 mM glucose with 100  $\mu$ M Tolbutamide (High Glu + SU) and 1.67 mM glucose with 30

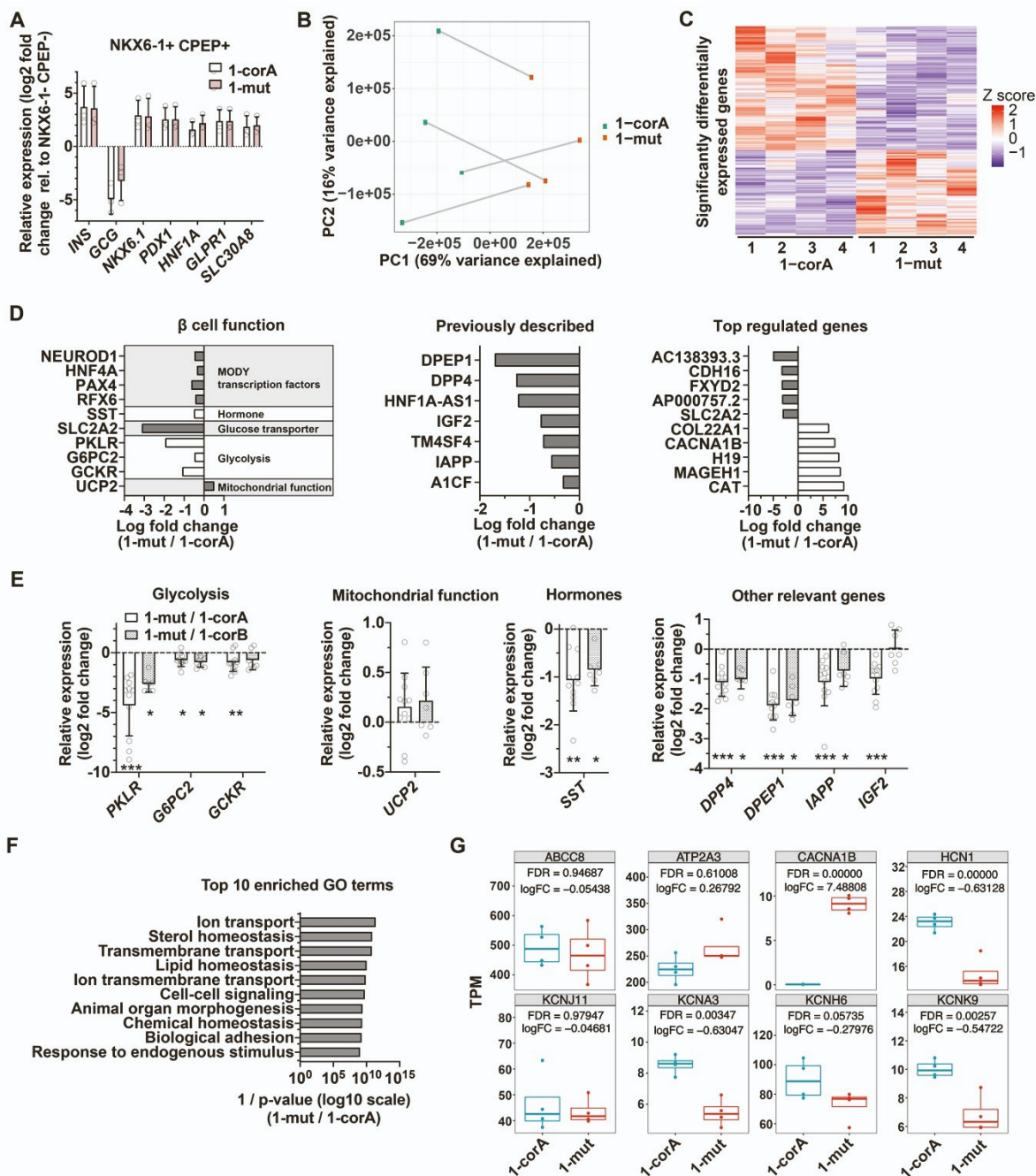


mM KCl normalized to the total INS content in S7 cells. n = 7 (2-mut), 8 (2-corA) or 9 (2-corB and 2-mut-subcl) independent experiments. Wilcoxin matched-pairs signed-rank test analysis.

Data are represented as mean + SD.

\*, and \*\* represent statistical significance at  $p \leq 0.05$ ,  $p \leq 0.01$ , respectively.

Figure S4



**Supplementary Figure 4. Validation of the RNA sequencing data set.**

(A) RT-qPCR analysis of  $\alpha$  cell (*GCG*) and  $\beta$  cell (*INS*, *NKX6-1*, *PDX1*, *HNF1A*, *GLP1R*, *SLC30A8*) markers in CPEP<sup>+</sup> / NKX6-1<sup>+</sup>  $\beta$  cells relative to the double negative population. n = 3 independent experiments. Data are represented as mean + SD.

(B) Principal component analysis of the RNA sequencing data set showing consistent separation of HNF1A<sup>+R272C</sup> cells (1-mut) and corrected control cells (1-corA) in each experiment. n = 4 independent experiments.

(C) Heatmap of 691 differentially expressed genes (281 upregulated and 410 downregulated in 1-mut). n = 4 independent experiments.

(D) Barplots from the RNA sequencing data of differentially expressed MODY genes, hormones, glucose transporters, glycolytic genes, mitochondrial function-related genes, selected genes previously described in human *HNF1A* deficient  $\beta$  cells (Cardenas-Diaz et al., 2019; González et al., 2022; Haliyur et al., 2019; Low et al., 2021) and the top 5 down- and upregulated genes.

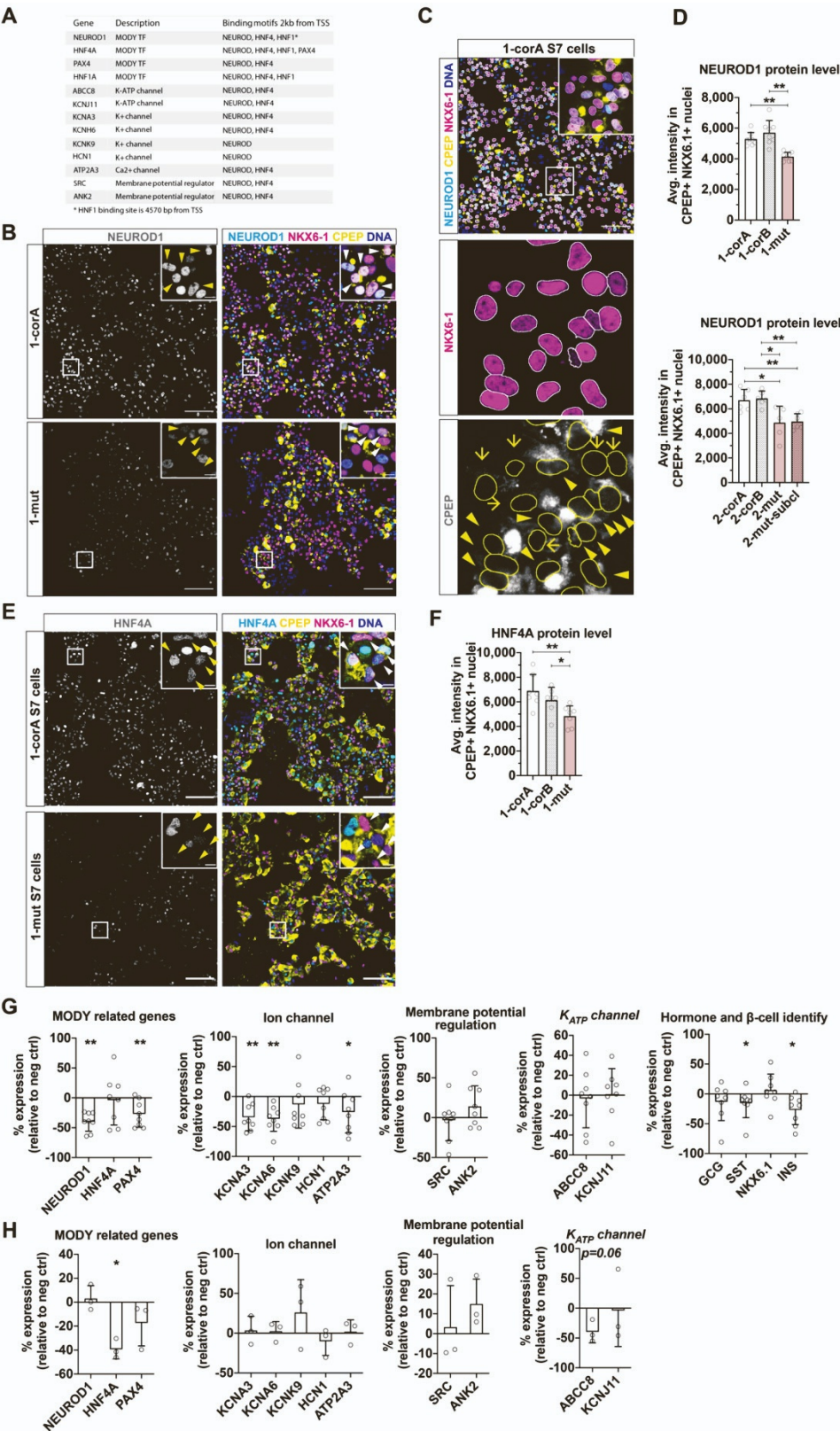
(E) Validation of selected RNAseq hits by RT-qPCR of CPEP<sup>+</sup> / NKX6-1<sup>+</sup>  $\beta$  cells for glycolytic genes (*PKLR*, *G6PC2* and *GCKR*), the mitochondrial function-related gene *UCP2*, the pancreatic hormone *SST* and other relevant genes (*DPP4*, *DPEP1*, *IAPP* and *IGF2*). n = 7 (1-corB) or 12 (1-mut and 1-corA) independent experiments. Data are represented as mean + SD. Wilcoxin matched-pairs signed-rank test analysis.

(F) Barplot of the top 10 GO terms in the GO enrichment analysis.

(G) RNA sequencing expression levels (TPM) of ion channels. The RNA sequencing data shows no significant difference in *ABCC8*, *KCNJ11*, *ATP2A3* and *KCNH6* expression between 1-mut and 1-corA cells. See Figure 4D for validation of the ion channels by RT-qPCR.

\*, \*\*, and \*\*\* represent statistical significance at  $p \leq 0.05$ ,  $p \leq 0.01$ , and  $p \leq 0.001$ , respectively.

Figure S5



### **Supplementary Figure 5. Validation and knockdown of NEUROD1 and HNF4A.**

(A) Table showing the results of an *in silico* transcription factor (TF) binding motif analysis 2,000 base pairs upstream and downstream of the transcriptional start site (TSS) of selected genes using the Gene Search function of the online tool at <http://motifmap.ics.uci.edu><sup>58,59</sup>.

(B) Representative immunofluorescence confocal images of S7 cells stained for NEUROD1 (cyan), CPEP (yellow), NKX6-1 (magenta) and DNA (blue). Scale bars represent 100  $\mu\text{m}$  and 10  $\mu\text{m}$  (insets) and arrowheads mark CPEP<sup>+</sup> / NKX6-1<sup>+</sup>  $\beta$  cells.

(C) Representative immunofluorescence confocal images of S7 hiPSCs stained for NKX6-1 (magenta), CPEP (yellow), NEUROD1 (cyan) and DNA (blue), showcasing the segmentation of NKX6-1 (middle panel) and which nuclei are considered part of CPEP<sup>+</sup> cells (arrowhead) or CPEP<sup>-</sup> cells (arrows) based on the average CPEP nuclear intensity. The pinhole was opened to 3.5 airy units to obtain scattering of the cytoplasmic CPEP signal in the nuclei.

(D) Image-based quantifications of the average intensity of NEUROD1 in the nuclei of CPEP<sup>+</sup> / NKX6-1<sup>+</sup>  $\beta$  cells.  $n = 8$  independent images. Data are represented as mean + SD. Wilcoxin matched-pairs signed-rank test analysis.

(E) Representative immunofluorescence confocal images of S7 cells stained for HNF4A (cyan), CPEP (yellow), NKX6-1 (magenta) and DNA (blue). Scale bars represent 100  $\mu\text{m}$  and 10  $\mu\text{m}$  (insets) and arrowheads mark CPEP<sup>+</sup> / NKX6-1<sup>+</sup>  $\beta$  cells.

(F) Image-based quantification of the average intensity of HNF4A in the nuclei of CPEP<sup>+</sup> / NKX6-1<sup>+</sup>  $\beta$  cells.  $n = 6$  independent images. Data are represented as mean + SD. Wilcoxin matched-pairs signed-rank test analysis.

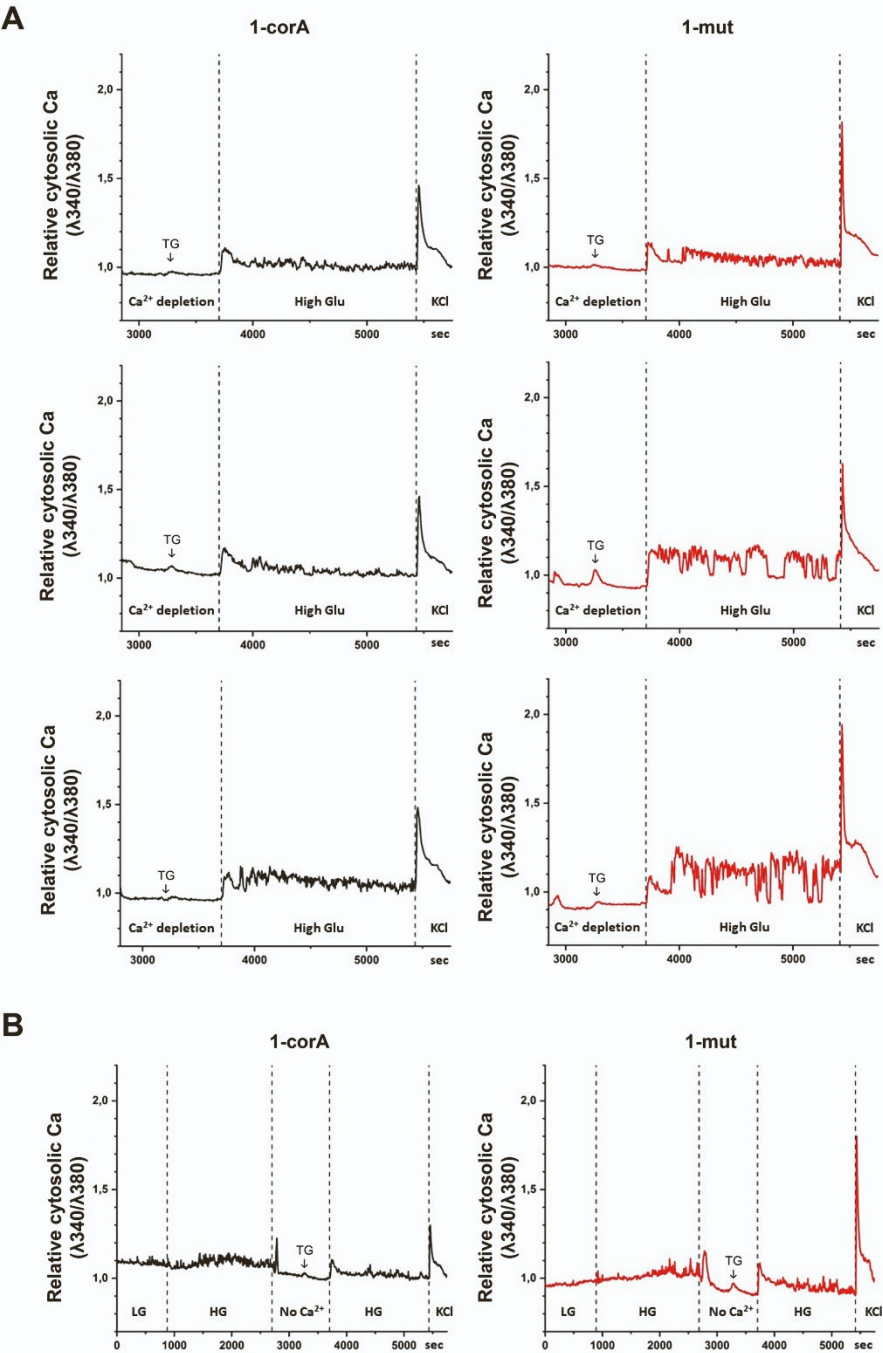


(G) RT-qPCR analysis of the HNF1A mutant-related gene expression in Ctrl and *NEUROD1* KD cells in S7. n = 8 independent experiments. Data are represented as mean + SD. Wilcoxin matched-pairs signed-rank test analysis.

(H) RT-qPCR analysis of the HNF1A mutant-related gene expression in Ctrl and *HNF4A* KD cells in S7. n = 3 independent experiments. Data are represented as mean + SD.

\*, and \*\* represent statistical significance at p % 0.05, and p % 0.01, respectively.

Figure S6



**Supplementary Figure 6. Increased calcium signaling in MODY3  $\beta$  cells.**

(A) Three representative tracks of single, live-imaged S7 cells (1-mut and 1-corA) after staining with an intracellular calcium dye (Fura2-AM) showing relative cytosolic intracellular  $\text{Ca}^{2+}$  levels during  $\text{Ca}^{2+}$  depletion (0 mM  $\text{Ca}^{2+}$ ; 1.67 mM glucose; 5  $\mu\text{M}$  thapsigargin to empty intracellular  $\text{Ca}^{2+}$  stores), high glucose (2.2 mM  $\text{Ca}^{2+}$ ; 16.7 mM glucose) and KCl (2.2 mM  $\text{Ca}^{2+}$ ; 16.7 mM glucose; 30 mM KCl) challenges. Related to Figure 4A.

(B) Representative tracks of single, live-imaged S7 cells (1-corA in black and 1-mut in red) loaded with an intracellular calcium dye showing relative cytosolic intracellular  $\text{Ca}^{2+}$  levels during low glucose (LG; 2.2 mM  $\text{Ca}^{2+}$ ; 1.67 mM glucose), high glucose (HG; 2.2 mM  $\text{Ca}^{2+}$ ; 16.7 mM glucose),  $\text{Ca}^{2+}$  depletion (No  $\text{Ca}^{2+}$ ; 0 mM  $\text{Ca}^{2+}$ ; 1.67 mM glucose; 5  $\mu\text{M}$  Thapsigargin to empty intracellular  $\text{Ca}^{2+}$  stores), high glucose (HG; 2.2 mM  $\text{Ca}^{2+}$ ; 16.7 mM glucose) and KCl (2.2 mM  $\text{Ca}^{2+}$ ; 16.7 mM glucose; 30 mM KCl) challenges.

**Supplementary Table 1. Summary of the total estimated volume per graft.**

<b>Cohort</b>	<b>Graft Sample /Section replicate</b>	<b>INS volume <math>\mu\text{m}^3</math> (Imaris quantified)</b>	<b>GCG volume <math>\mu\text{m}^3</math> (Imaris quantified)</b>
MODY3	ID 6 replicate 1	47369149	49105535
MODY3	ID 6 replicate 2	197441720	137598108
MODY3	ID 13 replicate 1	46304432	40853026
MODY3	ID 13 replicate 2	110530754	102550395
MODY3	ID 9 replicate 1	159030669	167458378
MODY3	ID 9 replicate 2	132615754	135042997
Corrected	ID 15 replicate 1	77538710	30994040
Corrected	ID 15 replicate 2	54380645	74885977
Corrected	ID 11 replicate 1	168365581	63494190
Corrected	ID 11 replicate 2	108130253	145544579

**Supplementary Table 2. Summary of oligonucleotides for RT-qPCR (refer to KEY RESOURCES TABLE).**

Name	Forward	Reverse
<i>LentiOct4</i>	CCC TGT CTC TGT CAC CAC T	CCA CAT AGC GTA AAA GGA GCA
<i>LentiSox2</i>	ACA CTG CCC CTC TCA CAC AT	CAT AGC GTA AAA GGA GCA ACA
<i>LenticMyc</i>	AAG AGG ACT TGT TGC GGA AA	TTG TAA TCC AGA GGT TGA TTA TCG
<i>LentiKlf4</i>	GAC CAC CTC GCC TTA CAC AT	CAT AGC GTA AAA GGA GCA ACA
<i>LentiNanog</i>	ACA TGC AAC CTG AAG ACG TG	CAC ATA GCG TAA AAG GAG CAA
<i>HNF4A</i>	CGA AGG TCA AGC TAT GAG GAC A	ATC TGC GAT GCT GGC AAT CT
<i>HNF1B</i>	ACC AAG CCG GTC TTC CAT ACT	GGT GTG TCA TAG TCG TCG CC
<i>PDX1</i>	CAA AGC TCA CGC GTG GAA	GCG TCC GCT TGT TCT CC
<i>NKX6-1</i>	ATT CGT TGG GGA TGA CAG AG	CGA GTC CTG CTT CTT CTT GG
<i>PAX4</i>	GGG TCT GGT TTT CCA ACA GAA G	TCA GCC CCT GGG AAG CA
<i>HNF1A</i>	GTG GTG GAG ACC CTT CTG C	CTG GTT GAG GCC AGT GGT AT

**Supplementary Table 3. Summary of TaqMan probes (refer to KEY RESOURCES TABLE).**

<b>TaqMan probe</b>	<b>SOURCE</b>	<b>IDENTIFIER</b>
<i>GAPDH</i>	Thermo Fisher	Hs02758991
<i>HNF1A</i>	Thermo Fisher	Hs00167041
<i>INS</i>	Thermo Fisher	Hs02741908
<i>GCG</i>	Thermo Fisher	Hs01031536
<i>SST</i>	Thermo Fisher	Hs00356144_m1
<i>NKX6-1</i>	Thermo Fisher	Hs00232355
<i>PAX4</i>	Thermo Fisher	Hs00173014
<i>ARX</i>	Thermo Fisher	Hs00992303_m1
<i>NEUROD1</i>	Thermo Fisher	Hs00159598
<i>HNF4A</i>	Thermo Fisher	Hs002308853
<i>RFX6</i>	Thermo Fisher	Hs00942164_m1
<i>KCNA3</i>	Thermo Fisher	Hs04403047_m1
<i>KCNH6</i>	Thermo Fisher	Hs002292215
<i>KCNK9</i>	Thermo Fisher	Hs04397236
<i>HCN1</i>	Thermo Fisher	Hs01085412_m1
<i>ATP2A3</i>	Thermo Fisher	Hs00193090_m1
<i>CACNA1B</i>	Thermo Fisher	Hs04996252
<i>SRC</i>	Thermo Fisher	Hs01082246_m1
<i>ANK2</i>	Thermo Fisher	Hs00153998_m1
<i>G6PC2</i>	Thermo Fisher	Hs01549773_m1
<i>PKLR</i>	Thermo Fisher	Hs00176075_m1
<i>GCKR</i>	Thermo Fisher	Hs00609806_m1
<i>UCP2</i>	Thermo Fisher	Hs01549773_m1
<i>DPP4</i>	Thermo Fisher	Hs00897391_m1



<i>DPEP1</i>	Thermo Fisher	Hs01116757_g1
<i>IAPP</i>	Thermo Fisher	Hs00169095_m1
<i>IGF2</i>	Thermo Fisher	Hs01005963_m1
<i>ABCC8</i>	Thermo Fisher	Hs01093752_m1
<i>KCNJ11</i>	Thermo Fisher	Hs00265026_s1

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