SUPPLEMENTARY MATERIAL:

Human dermal fibroblasts reprogramming to iPSCs

Fibroblasts were reprogrammed into iPSCs using a non-integration method based on modified mRNA[1] using a StemRNA reprogramming kit (StemGent; Cambridge, MA). Skin fibroblasts were plated at three different densities on irradiated feeder human dermal fibroblasts and transfected with modified mRNA encoding human OCT4, KLF4, SOX2, LIN28, c-MYC, and control GFP. The transfection was repeated daily over 10–14-day periods as iPSC colonies appeared. Newly derived iPSCs were passaged at least three times before banking. qPCR: RNA was extracted using the RNeasy Mini Kit (Qiagen; Hilden, Germany). cDNA was synthesized using KAPA cDNA Mix, and qPCR was performed using KAPA SYBR FAST (Kapa Biosystems; Indianapolis, Indiana) on a Connect CFX light cycler (Bio-Rad; Hercules, CA) with ≤40 PCR cycles. Using qPrimerDepot, primers were designed so the PCR products spanned exon junctions. Primer specificity was checked using CFX Manager Software v3.1 (Applied Biosystems; Foster City, CA) and PCR product agarose gel electrophoresis. Threshold data were analyzed with the CFX Manager Software v3.1 using the Comparative Ct relative quantitation method with TATA-Box binding protein as an internal control.

Human induced pluripotent stem cells (iPSCs) maintenance

The iPSCs were maintained on Matrigel (BD Biosciences; San Jose, CA) in Essential 8 media (E8, StemCell Technologies; Vancouver, Canada). Cells were passaged every 3–5 days at 80% confluency with TrypLE Express (Invitrogen, Carlsbad, CA). After passage, cells were kept in 10 μM Y-27632 (StemGent) for 24 h.

Flow cytometry

Cells were dissociated, washed with phosphate-buffered saline (PBS), filtered through a 40-μm cell strainer, and fixed with 4% PFA at 4°C for 30 min on a rotating platform. One percent BSA, 0.1% saponin in PBS was used to dilute antibodies and permeabilize cells. Primary antibodies (**Table S3)** were added and the sample incubated overnight at 4°C. After primary antibody incubation, samples were washed once with 1% BSA, 0.1% saponin in PBS and pelleted at 1,200 ×g for 5 min. Secondary fluorophore-conjugated antibodies were added, and the sample was incubated overnight at 4°C. Cells were centrifuged at 1,200 ×g for 5 min and washed with a 10× volume of 1% BSA, 0.1% saponin in PBS. Stained cells were filtered through a 40-μm cell strainer before flow cytometry. FACS analysis was performed using an LSRII and the Diva software package (BD Biosciences). For all samples, at least 5,000 events were captured, and FlowJo (BD Biosciences) was used for gating and analysis.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde/formaldehyde (PFA) in PBS, washed three times with PBS, and incubated with 5% donkey serum (Jackson ImmunoResearch Laboratories; West Grove, PA) in PBS + 0.1% Triton X (PBST) for 30 min to permeabilize the cells and avoid nonspecific binding of antibody. Primary antibodies, diluted in 5% donkey serum in PBST, were added and incubated at 4°C overnight with shaking. After primary antibody incubation, cells were washed three times with PBST, and secondary antibodies were conjugated with Alexa-Fluor Dyes (Jackson ImmunoResearch Laboratories) diluted in 5% donkey serum in PBST were added to cells for 30 min at room temperature. Then, cells were washed three times with PBST, and nuclei were stained with DAPI (Roche Diagnostics; Indianapolis, IN). Antibody sources, catalog numbers, and dilutions are listed in **Table S3**. For imaging, we used a Leica DMI6000 or confocal Leica TCS SPE. Images were processed using LAS X software (Leica Biosystems; Wetzlar Germany).

The pancreatic spheroids at the beta-cell stage were imaged with confocal microscopy and compressed Z-stack images are shown. Quantification was performed using 8 individual Z-stacks from each spheroid for three biological repeats (independent differentiation). Quantification was also confirmed using flow cytometry analysis of dispersed spheroid cells stained with anti-INS or C-peptide antibodies.

Immunoblotting

One million endodermal cells were pelleted, washed with PBS and resuspended in 250 µl of lysis buffer composed of 10 mM HEPES pH 7.5, 10 mM $MgCl_2$, 5 mM KCl, 0.1 mM EDTA pH 8, 0.1% Triton X-100, 0.2 mM PMSF, 1 mM DTT, and one tab of Complete Protease Inhibitor Cocktail (Roche Diagnostics). Cell lysates were centrifuged at 12,000 ×g at 4°C for 15 min, and the supernatants were collected. Protein concentration was determined using a Pierce BCA protein assay kit (Thermo Fisher Scientific; Waltham MA). For western blot, 30 µg of protein were denatured with 4x Laemmli buffer (40% glycerol, 8% SDS, 240 mM Tris-HCl pH 6.8, 5% β-mercaptoethanol, 12.5 mM EDTA, 0.04% bromophenol blue) at 95°C for 3 min and resolved on 8% SDS-PAGE. Proteins were transferred to PVDF membranes (Bio-Rad), and the membranes were blocked with 5% BSA in Tris-buffered saline with 1% Tween 20 (TBST) for 1 h before applying primary antibody. Membranes were washed three times for 10 min each, anti-rabbit IgG-HRP (GE Life Science; Marlborough, MA) was added for 3 h at room temperature, then membranes were washed three times. HyGLO™ Quick Spray Chemiluminescent HRP Antibody Detection Reagent (Denville Scientific Inc.; Metuchen, NJ) was used to detect antigen, and the membrane was developed using CL-XPosure Film (Thermo Fisher Scientific). Membrane stripping was performed using a mild stripping buffer (200 mM glycine, 0.1% SDS, 1% Tween 20, pH 2.2) according to Abcam's instructions.

Introduction of PDX1-variant into iCas9 human embryonic stem cells (hESCs).

Single guide RNA (sgRNAs) were delivered as in vitro-transcribed RNAs using the method of Verma et al.[2] and hESC Hues8-iCas9 cells were previously described [3].

Metabolomics Gas Chromatography-Mass Spectrometry (GC/MS) Array and analysis

All extraction protocols were described previously [4,5]. To ensure quality data, quality controls (liver tissue extract) were used to monitor the extraction efficiency, instrument variation, and reproducibility as described earlier [6].

RNA-Sequencing analysis

Raw sequencing data was prepared by confirming the initial quality control was satisfactory. Adapter sequences were trimmed using TrimGalore (Babraham Bioinformatics; Cambridge, UK) [7]. rRNA sequences were depleted in silico using the SortMeRNA package (Bonsai Bioinformatics; Lille, France) [8]. Filtered sequences were then aligned to the human Gencode release 34 genome (GRCH38.p13) using STAR aligner [9]. Aligned bam files were summarized for gene counts using featureCount [10]. Count data was analyzed using the R package DESeq2 [11]. Differential expression across all three cell types was estimated using the Likelihood ratio test controlling for batch. The resulting genes were analyzed for enrichment in KEGG and WIkiPathways using WebGestalt [12]. Heatmaps were created in R using the package pheatmap.

EMSA

DNA oligomers of wildtype and mutant 5'-UTR sequences of the PDX-1 promoter region were ordered from Sigma (Woodlands, TX) and generated by annealing the two complementary oligonucleotides. Probe set 1 (wild-type 5'-UTR sequence) - forward 5'-CGG CTC CCG GCT CCC GGT GCC CAA TCC CGG GCC GCA GCC-3', reverse 5'-CGG GGC TGC GGC CCG

GGA TTG GGC ACC GGG AGC CGG GAG-3'. Probe set 2 (mutant 5'-UTR sequence) – forward 5'-CGG CTC CCG GCT CCC GGT GCC TAA TCC CGG GCC GCA GCC-3', reverse 5'-CGG GGC TGC GGC CCG GGA TTA GGC ACC GGG AGC CGG GAG-3'. Probe sets were labeled with Klenow DNA polymerase and ^{32}P-dCTP. Then 5 µg of nuclear extract from cultured cells was incubated with the probe in binding buffer containing 25 mM Tris-HCl, 100 mM KCl, 5 mM dithiothreitol, 10% glycerol, 2 mM $MgCl_2$, 0.2 µg of dIdC non-specific competitor, and 50,000 cpm of the probe. To determine the identity of binding proteins, specific antibodies were added before probe addition. Antibodies to NF-YA (H-209), USF-1 (H-86) and PDX-1 (A-17) were used (Santa Cruz Biotechnology, Inc, Santa Cruz, CA). DNA-protein complexes were separated by non-denaturing polyacrylamide gel electrophoresis (6% polyacrylamide) in 0.5X Tris-borate-EDTA (TBE) buffer at 4°C. After electrophoresis, the gel was dried and exposed to X-ray film.

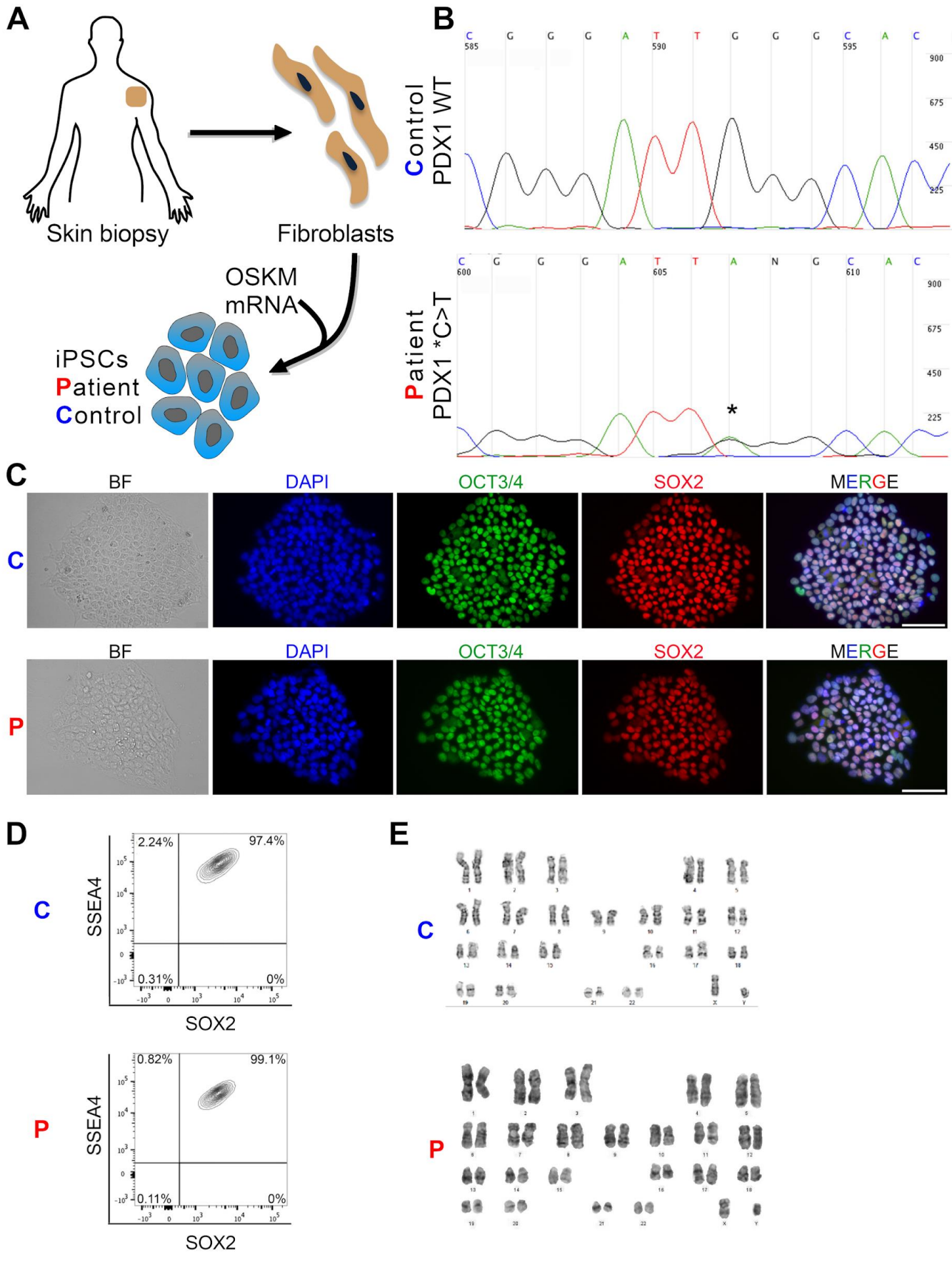
qPCR

RNA was isolated from cells using RNAzol RT (MRC, Cincinnati, OH) and 500 ng was subjected to reverse transcription using RevertAid Reverse Transcriptase (Thermo Fisher Scientific). qPCR was performed using Power SYBR™ Green PCR Master Mix (Thermo Fisher Scientific), run and analysed using QuantStudio™ 7 Flex Real-Time PCR System (Thermo Fisher). ACTB was used as an internal reference. Primers used for qPCR were: PDX1_mut CGGCTCCCGGTGCCT, PDX1_wt CGGCTCCCGGTGCCCAATCC, PDX1_rev CGCATGGGTCCTTGTAAAGC, PDX1_fwd CCAGTGGGCAGGCGG, PDX1_rev2 GCCGTGAGATGTACTTGTTGA, NFYB-fwd CAACCAAACAGCCGATTGGAG, NFYB-rev ACTGTCACCATCCATTGTCATG; ACTB-fwd ACAGAGCCTCGCCTTTGCCGAT, ACTB-rev ATCATCCATGGTGAGCTGGCGG.

Supplemental Figures

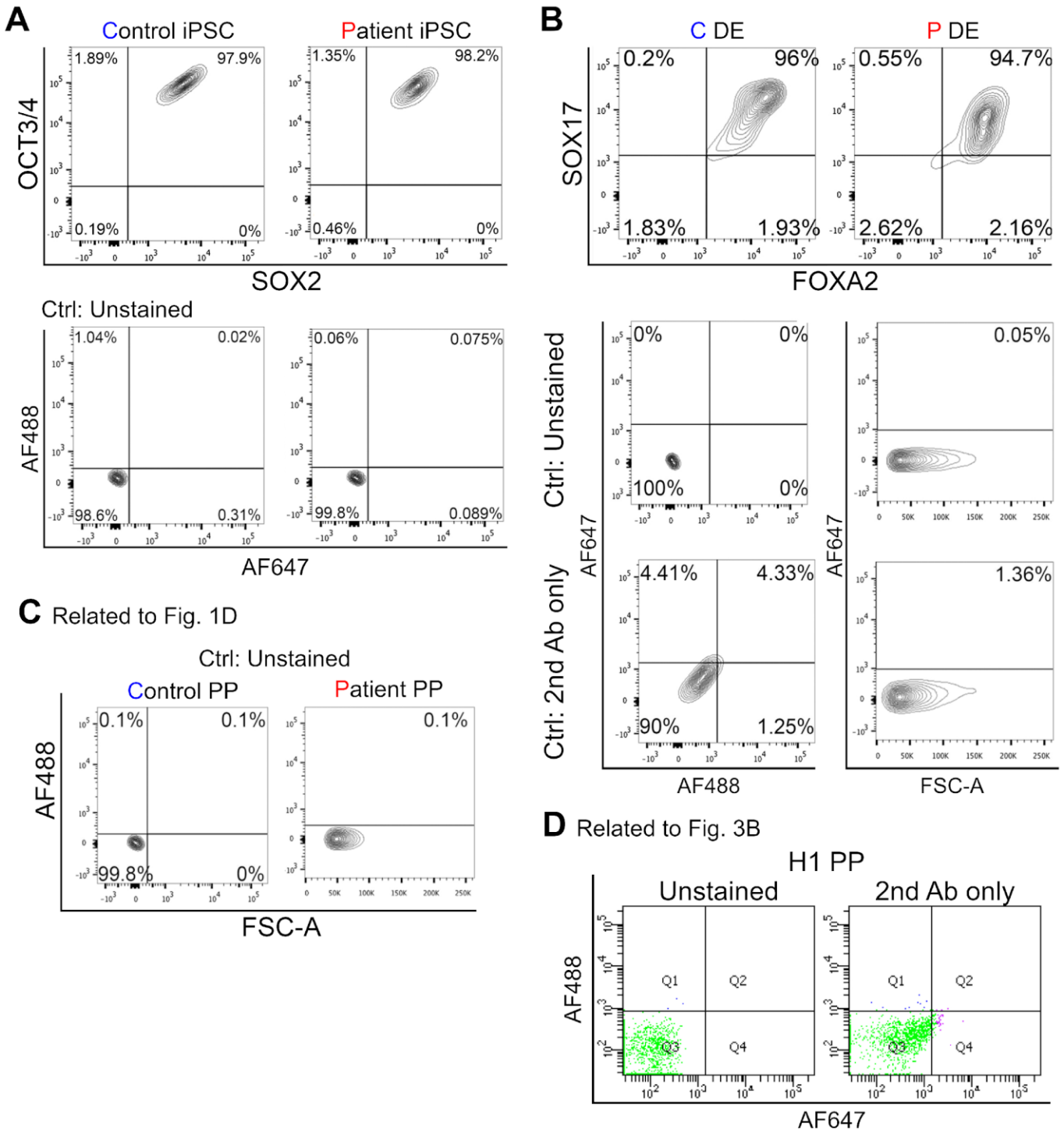
Suppl Fig. 1: Generation of induced pluripotent stem cells (iPSCs) from control and patient using non-integrating modified mRNA-based reprograming.

A. Scheme of dermal fibroblast reprogramming with modified mRNA encoding OCT4, SOX2, KLF4, c-MYC and control GFP.
B. Sanger sequencing of PDX1 upstream region showing a CCAAT motif in control and CTAAT motif in patient iPSCs.
C. Pluripotency marker expression in control and patient iPSCs: BF, bright field; DAPI, blue; OCT3/4, green; SOX2, red; Scale bar = 100 μm
D. Flow cytometry plot showing % live cells expressing SSEA4 pluripotency marker in control and patient iPSCs.
E. Karyotype analysis showing normal karyotypes in control and patient iPSCs


A
Skin biopsy
Fibroblasts
OSKM
mRNA
iPSCs
Patient
Control
B
Control
PDX1 WT
Patient
PDX1 *C>T
C
BF
DAPI
OCT3/4
SOX2
MERGE
C
P
D
C
P
SSEA4
SOX2
2.24%
97.4%
0.31%
0%
0.82%
99.1%
0.11%
0%
E
C
P

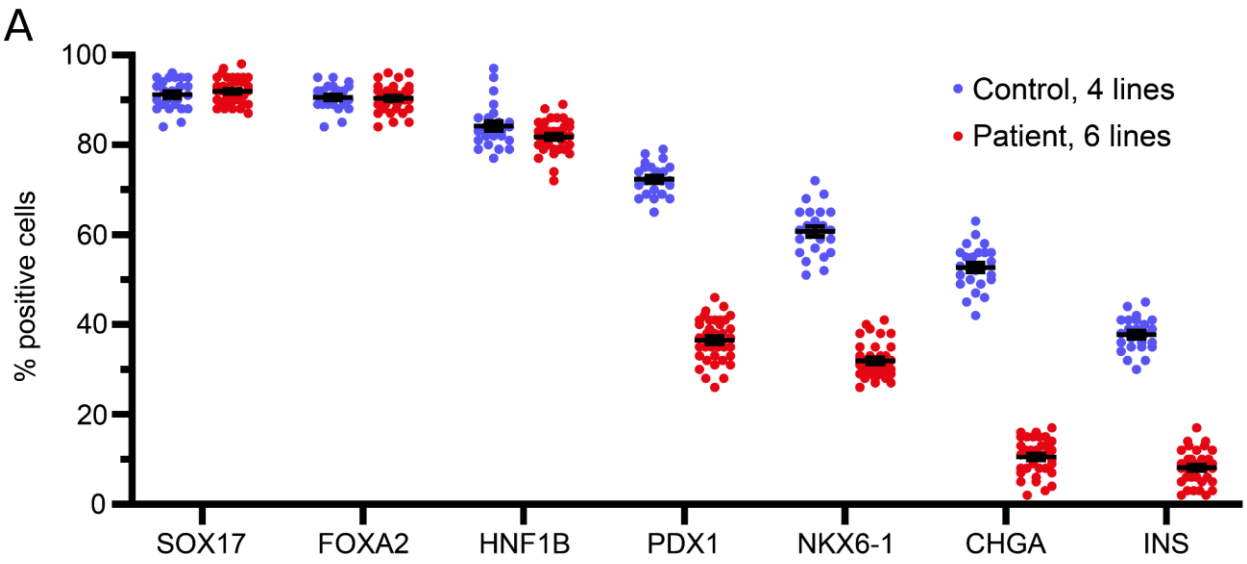
Suppl Fig. 2. **Flow cytometry analysis of control and patient iPSCs and pancreatic progenitors.**

A. Flow cytometry plot showing % live cells expressing OCT3/4 pluripotency markers in control and patient iPSCs at the start of pancreatic differentiation. Below are flow plots of unstained control cells used for gate-setting during immunofluorescence for OCT3/4 detection in control and patient iPSCs.
B. Flow cytometry plot showing % live cells expressing SOX17 and FOXA2 definitive endoderm (DE) markers in control and patient cells at DE stage. Below are flow plots of unstained and secondary antibody-stained control cells used for gate-setting during immunofluorescence for SOX17 and FOXA2 in control and patient DE cells.
C. Flow plots of unstained controls used for gate-setting during immunofluorescence for PDX1 in control and patient PP cells (data shown in Figure 1D).
D. Flow cytometry plots of unstained controls used for gate-setting during immunofluorescence for PDX1 in H1-derived PP cells (data shown in Figure 3B).


A
Control iPSC
Patient iPSC
OCT3/4
1.89%
97.9%
0.19%
0%
1.35%
98.2%
0.46%
0%
SOX2
Ctrl: Unstained
AF488
1.04%
0.02%
98.6%
0.31%
0.06%
0.075%
99.8%
0.089%
AF647
B
C DE
P DE
SOX17
0.2%
96%
1.83%
1.93%
0.55%
94.7%
2.62%
2.16%
FOXA2
Ctrl: Unstained
Ctrl: 2nd Ab only
AF647
0%
0%
100%
0%
0.05%
4.41%
4.33%
90%
1.25%
1.36%
AF488
FSC-A
C Related to Fig. 1D
Ctrl: Unstained
Control PP
Patient PP
AF488
0.1%
0.1%
99.8%
0%
0.1%
FSC-A
D Related to Fig. 3B
H1 PP
Unstained
2nd Ab only
AF488
Q1
Q2
Q3
Q4
AF647

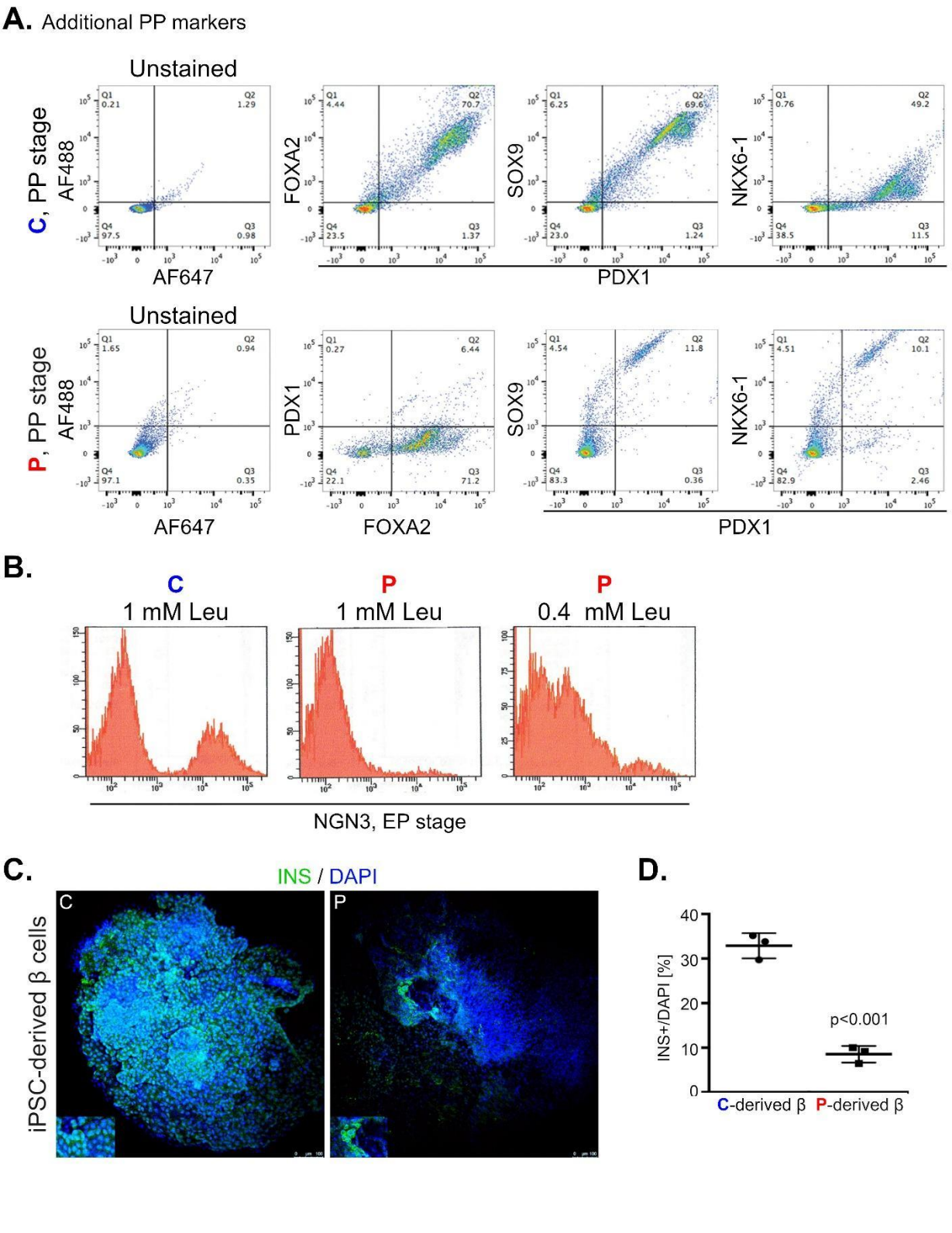
Suppl Fig. 3. The efficiency of generating different pancreatic progenitors from multiple control and patient-specific iPSC lines.

A. Pancreatic differentiation and stage-efficiency assessment for 6 patient-specific clonal lines and 4 control clonal lines based on antibody staining against stage-specific markers and flow cytometry analysis. n = 6 for each cell line.



Suppl Fig. 4. Pancreatic differentiation of control and patient iPSCs.

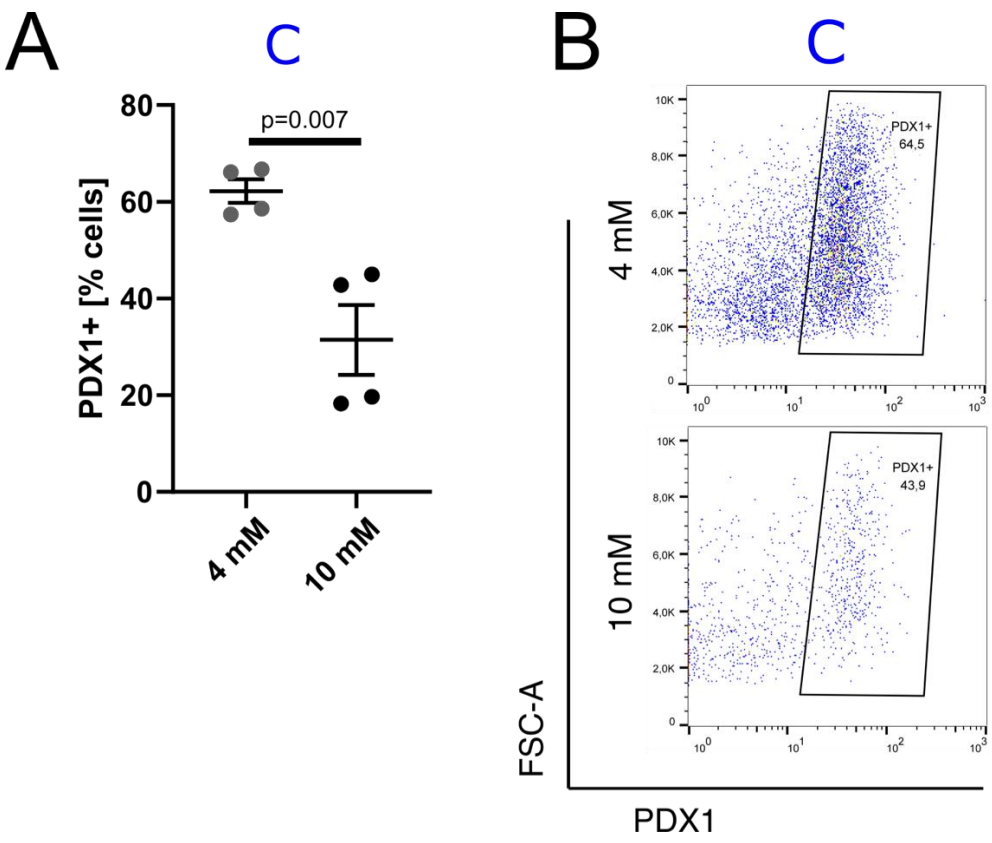
A. Flow cytometry to detect the FOXA2, SOX9, NKX6-1 and PDX1 co-expression in control and patient PP cells.
B. Flow cytometry to detect NGN3 expression in control and patient cells at the endocrine progenitor (EP) stage.
C. Induction of INS+ beta-like cells from control and patient iPSCs. Scale bar = 100 μm
D. Quantification of % INS+ cells at the end of three-dimensional pancreatic differentiation of control and patient iPSCs. n=3.


A. Additional PP markers
Unstained
C, PP stage
AF488
AF647
FOXA2
SOX9
NKX6-1
PDX1
Unstained
P, PP stage
AF488
AF647
PDX1
FOXA2
SOX9
NKX6-1
PDX1
B.
C
1 mM Leu
P
1 mM Leu
P
0.4 mM Leu
NGN3, EP stage
C.
INS / DAPI
iPSC-derived β cells
C
P
D.
INS+/DAPI [%]
40
30
20
10
0
p<0.001
C-derived β
P-derived β

Suppl. Fig. 5. High leucine concentration decreases the efficiency of PP differentiation of control iPSCs.

A. Flow cytometry analysis of PDX1+ cells of control PP cells differentiated in 4 mM and 10 mM Leu. n = 4

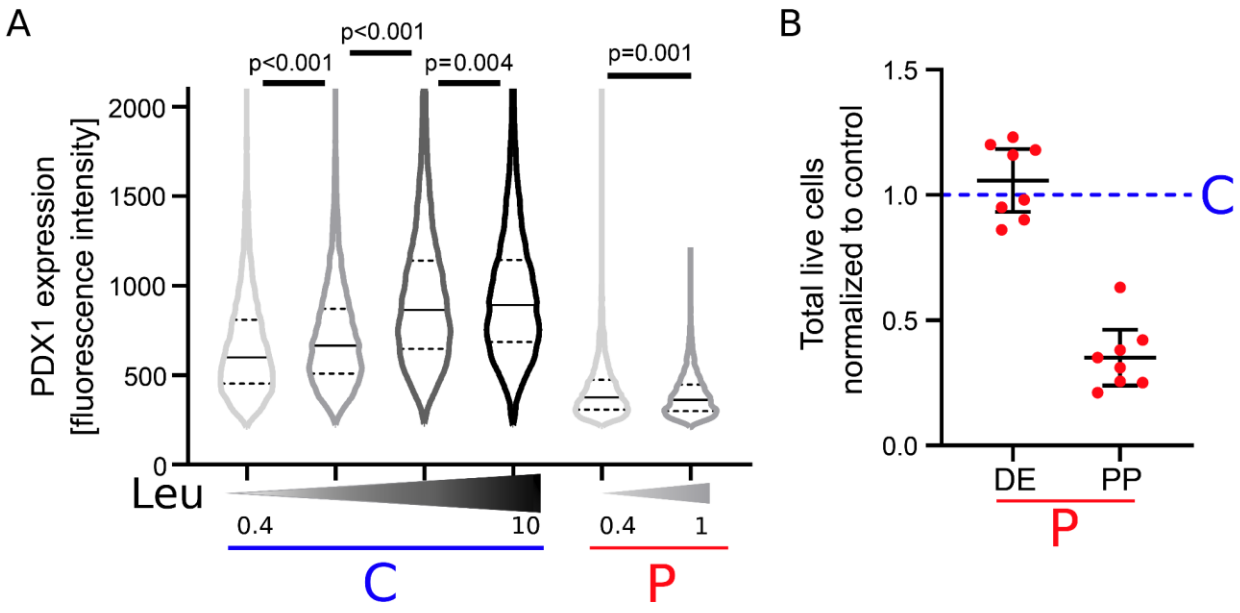
B. Example of flow cytometry plots for control PP cells differentiated in 4 mM and 10 mM Leu.



Suppl. Fig 6. Leu concentration influences the PDX1 expression levels and total cell number in patient PPs.

A. Violin plots of PDX1 signal intensities in individual PDX1+ PP cells analysed by flow cytometry. PDX1 expression significantly increased in control cells with increasing Leu concentration (0.4 to 10 mM), while the opposite effect is observed in patient cells (0.4 to 1 mM). Solid line – median, dotted lines – 1st and 3rd quartiles. n = 14,664, 16,062, 17,453 and 18,150 single cells for control (C) 0.4, 1, 4, and 10 mM Leu, respectively, n =9,563 and 3,302 single cells for patient (P) 0.4 and 1 mM Leu, respectively; cells were analyzed from 3 independent pancreatic differentiations.

B. Loss of patient cells differentiated in 1 mM Leu concentration between the DE stage and the end of the PP stage. n=8.

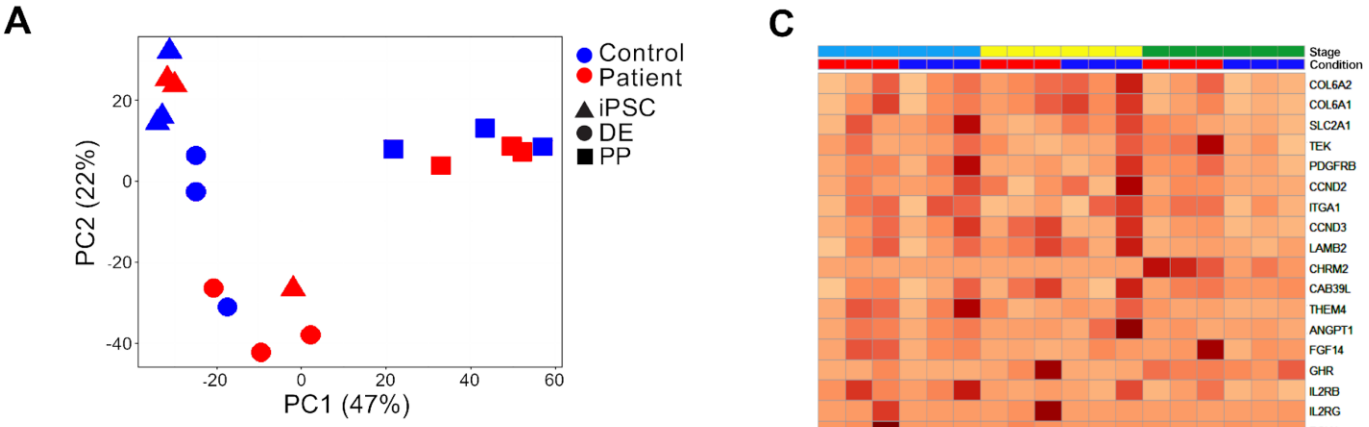


Suppl Fig. 7. Supporting data for RNA-Sequencing analysis.

A. Principal Components Analysis after batch corrections of the raw RNA-Seq data demonstrates tight clustering of samples by differentiation stage.

B. Pathway analysis using the WikiPathways database of the ~700 differentially expressed genes shows enrichment in PI3 kinase-AKT-mTOR pathways in the patient's cells.

C. Row-normalized heatmap of genes within the PI3K-AKT-mTOR Wiki Pathways database identified in the pathway enrichment analysis.



B

WikiPathways enrichment

Gene Set	Description	Size	Expect	Ratio	p Value	FDR
WP4172	PI-3K-Akt Signaling Pathway	345	9,7568	2,1523	0,0007	0,16959
WP49	IL-2 Signaling Pathway	42	1,1878	5,0514	0,001	0,16959
WP3932	Focal Adhesion-PI3K-Akt-mTOR-signaling pathway	309	8,7387	2,1742	0,0011	0,16959
WP558	Complement and Coagulation Cascades	59	1,6686	4,1953	0,0013	0,16959
WP4541	Hippo-Merlin Signaling Dysregulation	123	3,4785	2,8748	0,0024	0,2486
WP3995	Prion disease pathway	37	1,0464	4,7784	0,0036	0,2486
WP272	Blood Clotting Cascade	23	0,65045	6,1496	0,0036	0,2486
WP4341	Non-genomic actions of Vitamin D3	71	2,0079	3,4862	0,0037	0,2486
WP3941	Oxidative Damage	41	1,1595	4,3122	0,0056	0,28258
WP4223	Ras Signaling	185	5,2319	2,2936	0,006	0,28258

Suppl Fig 8. Effect of mTOR inhibition on PDX1 expression in patient-specific PP cells.

Violin plots of PDX1 flow cytometry signal intensities in individual PDX1+ **(A)** control or **(B)** patient PP cells treated with broad (PI-103) and specific (Torin2) mTOR inhibitors during days 4-9 of PP differentiation. PDX1 expression significantly increases in patient PP cells with modest (0.01x EC50) mTOR inhibition, while further increasing inhibition (up to 1x EC50) decreases PDX1 content per cell. Solid line – median, dotted lines – 1st and 3rd quartiles. n was between 8,751 and 11,366 single cells from 3 biological replicates each.

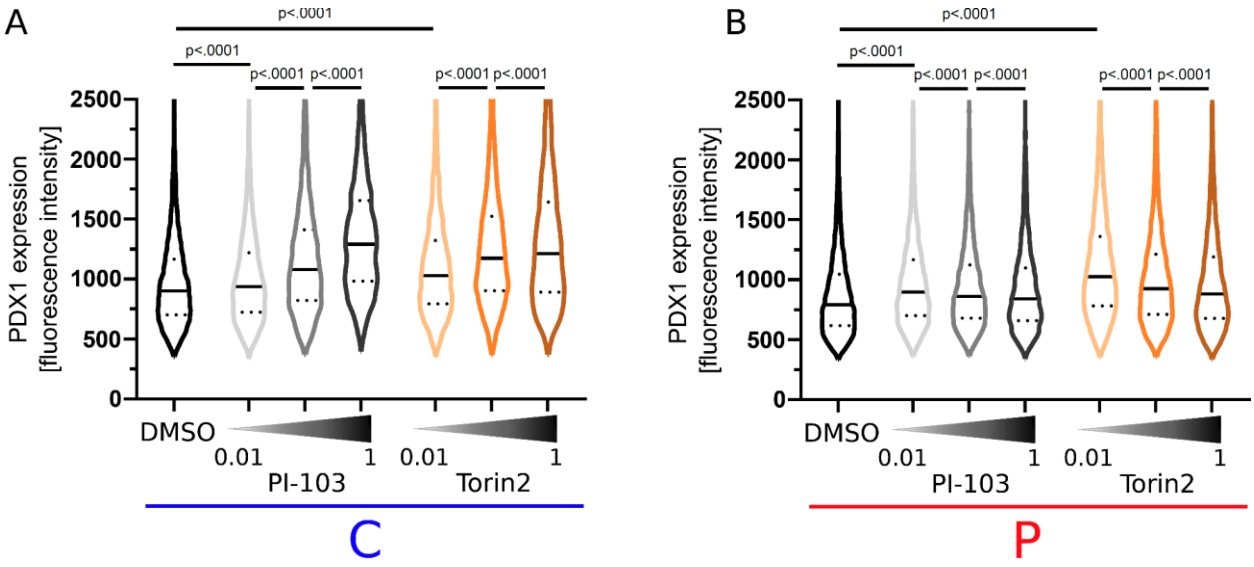


Table S1. All internal standard-normalized metabolomics data.

Data is provided in a separate Excel file.

Table S2. Significantly different RNA-Seq results with gene annotation.

Data is provided in a separate Excel file.

Table S3. List of antibodies

Antibody	Manufacturer	Catalog number	Dilution
AKT	Cell Signaling Technology	9272	1:1000
Beta-actin	MilliporeSigma	A5441	1:5000
Chromogranin A (CHGA)	Abcam	Ab15160	1:100
C-peptide	Developmental Studies Hybridoma Bank (DSHB)	GN104-s	1:100
FOXA2	MilliporeSigma	07-633	1:250
HNF1B	Santa Cruz Biotechnology	SC-8987	1:100
INS	Dako	A056401	1:100
mTOR	Cell Signaling Technology	2972	1:100
NGN3	R&D Systems	AF3444	1:200
NKX6.1	DSHB	F64A6B4	1:100
OCT3/4	Santa Cruz Biotechnology	sc-5279	1:100
p-AKT (Ser473)	Cell Signaling Technology	4060	1:100
PDK1	Cell Signaling Technology	3062	1:1000
PDX1	R&D Systems	AF2419	1:100
p-mTOR (Ser2448)	Cell Signaling Technology	2971	1:150
p-PDK1 (Ser241)	Cell Signaling Technology	3438	1:1000
SOX17	R&D Systems	BAF1924	1:100
SOX2	Abcam	Ab79351	1:1000
SOX9	MilliporeSigma	AB5535	1:100
SSEA4	MilliporeSigma	MAB4304	1:100

Table S4. Sequences of sgRNAs, donors and primers.

PDX1 variant correction	
sgRNA 1	5’-CATGGCTGCGGCCCGGGATTGGG-3’
sgRNA 2	5’-CGGCGAGGAGCAGTACTACGCGG-3’
Primer_Fwd	5’-CCTGGGCCTAGCCTCTTAGT-3’
Primer_Rev	5’-GGTGGGAAAGATGCTTCAAA-3’
PDX1 variant introduction	
sgRNA	5’ - T7 RNA polymerase promoter – **sgRNA** – scaffold – 3’
sgRNA 1	5’-TAATACGACTCACTATAGGG**TGCGGCCCGGGATTGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT-3’
sgRNA 2	5’-TAATACGACTCACTATAGGG**CGGCTCCCGGTGCCCAATCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT-3’
sgRNA 3	5’TAATACGACTCACTATAGGG**CGGCGAGGAGCAGTACTACG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT-3’
Donors	5’ – left homology – **C>T mutation –** right homology – 3’
donor 1	5’CTCCCGGCTCCCGGCTCCCGGCTCCCGGTGCC**T**AATCCCGGGCCGCAGCCATGAACGGCGAGGAG-3’
donor 2	5’CCCGACTCCCGGCTCCCGGCTCCCGGCTCCCGGTGCC**T**AATCCCGGGCCGCAGCCATGAACGGCGAGGAGCAGTACTACGCGGCCACGCAGCTTTACAAGGACCCATGCGCG-3’
Primers	

T7_F	5’-TAATACGACTCACTATAGGG-3’
Tracr_R	5’-AAAAGCACCGACTCGGTGCC-3’
PDX1_F	5’-CCTGGGCCTAGCCTCTTAGT-3’
PDX1_F3	5’-CGGCTCCCGGTGCCT-3’
PDX1_R	5’-GGGTGGGAAAGATGCTTCAAAC-3’

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