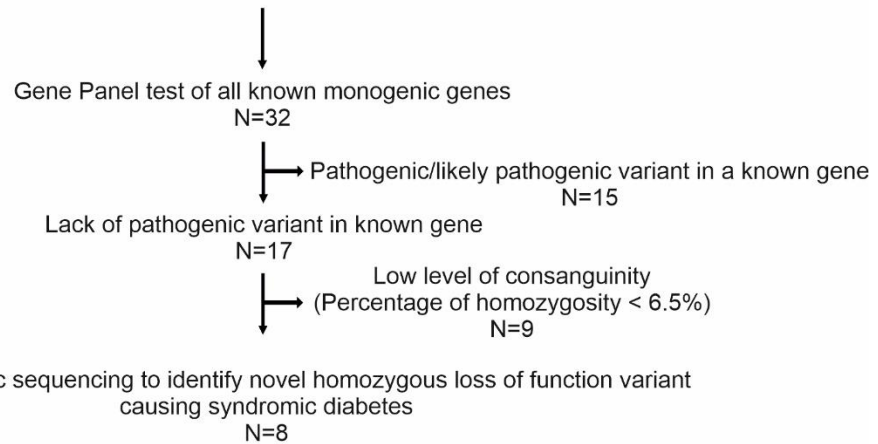


A

Childhood onset syndromic diabetes from Turkey
(Diabetes onset 0.5 - 20 y and any non-autoimmune additional features with less likelihood of type 1 diabetes)

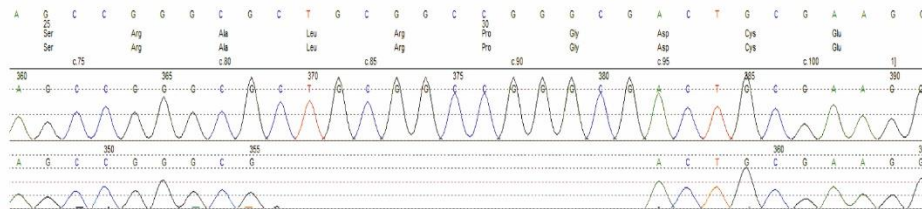


Pathogenic/likely pathogenic variant in a known gene
N=15

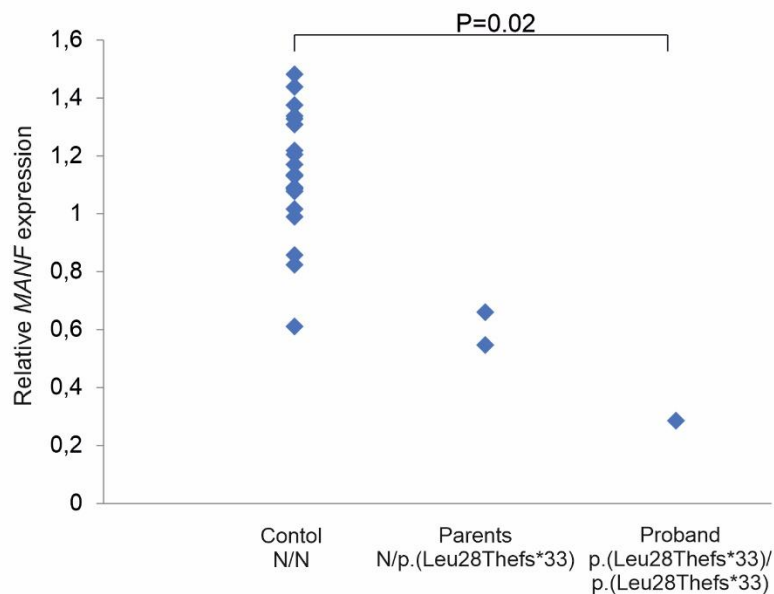
Lack of pathogenic variant in known gene
N=17

Low level of consanguinity
(Percentage of homozygosity < 6.5%)
N=9

Whole genomic sequencing to identify novel homozygous loss of function variant causing syndromic diabetes
N=8

B

C



Relative MANF expression

Contol
N/N

Parents
N/p.(Leu28The

Proband
p.(Leu28Thafs*33)/
p.(Leu28Thafs*33)

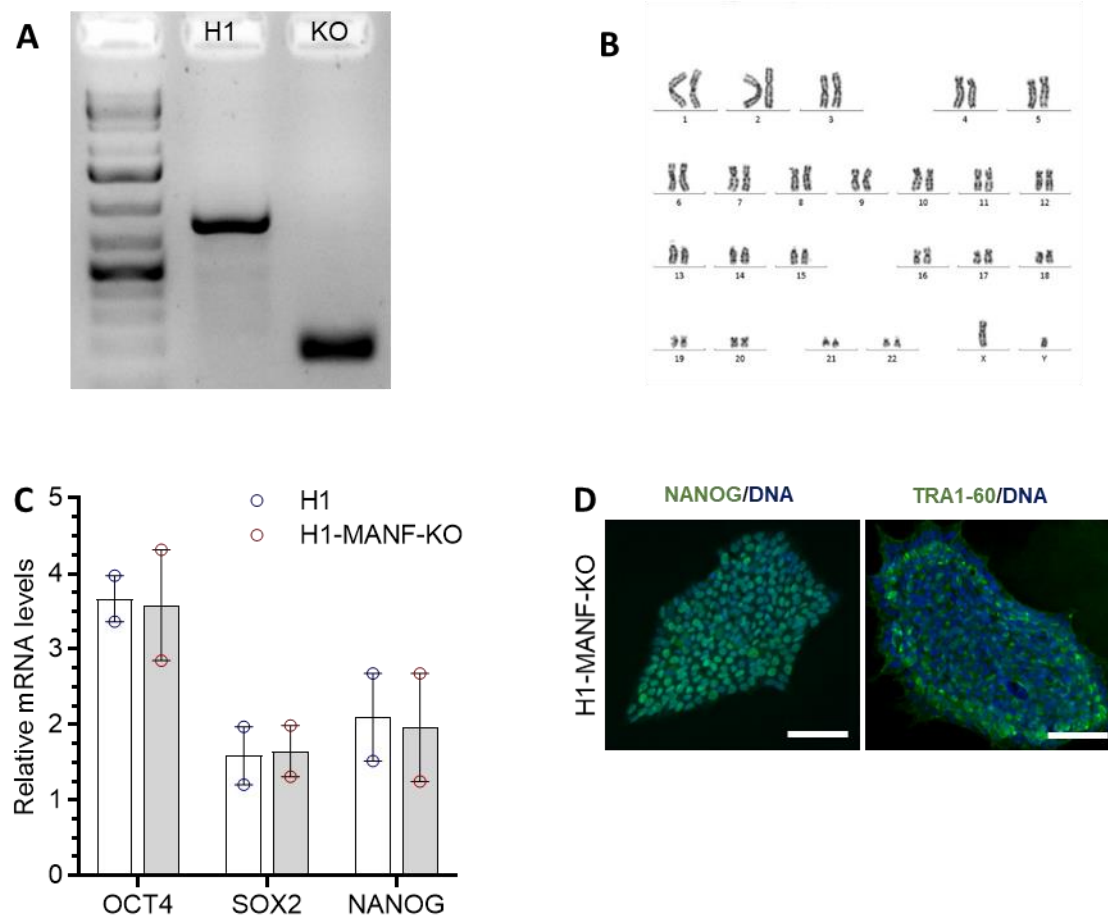
Supplementary Figure 1: Identification of human lacking MANF.

- A. Study plan for molecular genetic analysis
- B. Sequencing Chromatograms confirming the presence of the variant (p.Leu28Thrfs*33, NM_006010.5:c.82_94del) in case 1
- C. Reduced mRNA expression of *MANF* in blood from a proband homozygous for the p.Leu28Thrfs*33 mutation (case 1). Relative expression was determined using TaqMan Gene Expression assays with expression of *MANF* normalised using the geometric mean of five housekeeping genes. *p=0.02 (Grubbs' test).

B. Sequencing Chromatograms confirming the presence of the variant (p.Leu28Thrfs*33, NM_006010.5:c.82_94del) in case 1

C. Reduced mRNA expression of *MANF* in blood from a proband homozygous for the p.Leu28Thrfs*33 mutation (case 1). Relative expression was determined using TaqMan Gene Expression assays with expression of *MANF* normalised using the geometric mean of five housekeeping genes. *p=0.02 (Grubbs' test).

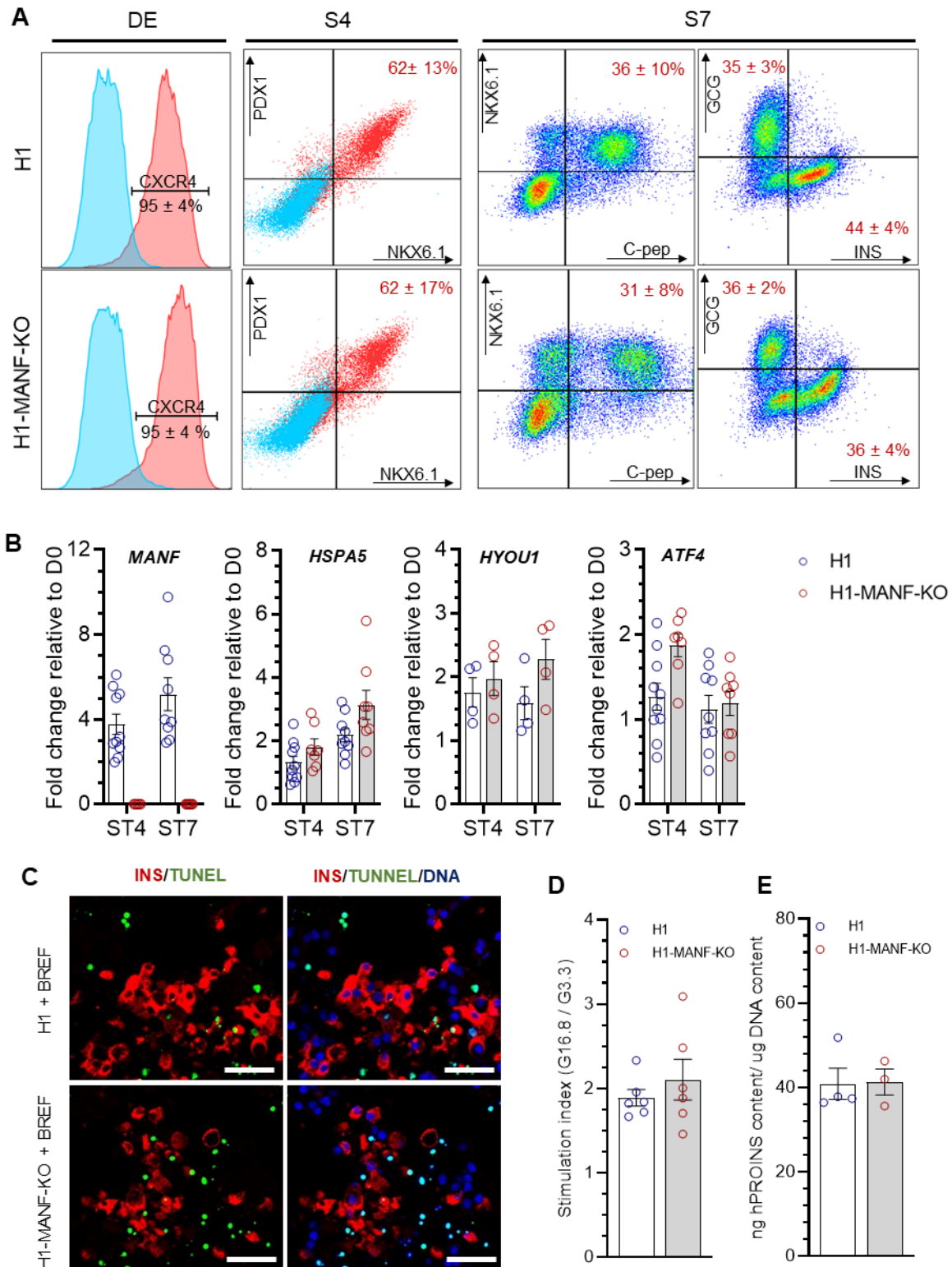
Supplementary Figure 2



Supplementary Figure 2: Characterization of MANF KO human embryonic stem cells.

- A 200 bp amplicon of *MANF* gene showing a 600 bp deletion in the KO clone
- Normal karyotype visualized with G-banding of MANF KO clone
- Relative gene expression levels of pluripotency markers OCT4, SOX2 and NANOG analyzed by RT-qPCR in H1 and H1-MANF-KO (N=2).
- Immunocytochemistry analysis of pluripotency markers NANOG and TRA1-60 in MANF KO clone.

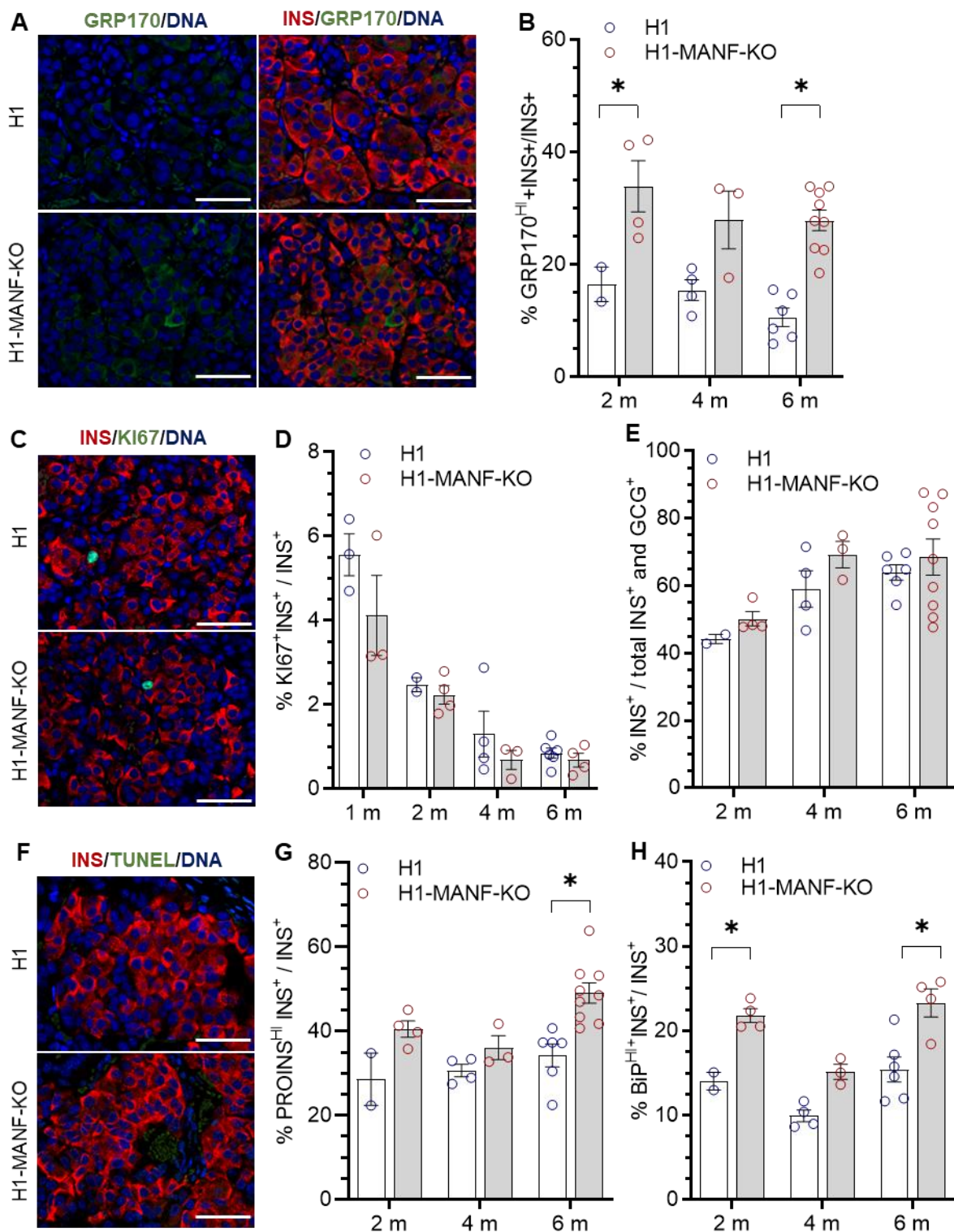
Supplementary Figure 3.



Supplementary Figure 3: Phenotypic characterization of MANF KO cells during *in vitro* differentiation.

- Flow cytometry analysis at different stages during *in vitro* differentiation: CXCR4 at Definitive endoderm (DE); PDX1⁺/NKX6.1⁺ at St4; NKX6.1⁺/C-pep⁺ and INS⁺/GCG⁺ at St7 in H1 and H1-MANF-KO. (N=8 for DE and S7 cells; N=7 For S4 cells)
- qRT-PCR analysis of different ER stress related markers at ST4 and St7.
- TUNEL assay for cell death in beta-like cells after BrefeldinA treatment at St7.
- Stimulation indexes of Insulin secretion in GSIS assay (N=6).
- Human Proinsulin content of S7 SC-islets. Cell mass is normalized by average DNA content of each aggregate. (N=3)

Supplementary Figure 4



Supplementary Figure 4: Further analysis of MANF KO cells implanted under the kidney capsule.

A. Immunohistochemistry analysis for insulin (INS) and GRP170. Scale bar=50 μ M.

B. Quantification of (A) at different time points after implantation.

C. Immunohistochemistry analysis of cell proliferation using KI67 antibody.

D. Quantification (C) at different time points after implantation.

E. Quantification of the percentage of INS⁺ cells at different time points after implantation.

F. TUNEL assay for cell death in beta cells after one month of implantation.

G. Quantification of the proinsulin^{HI} beta cells at different time points of implantation.

H. Quantification of the BiP^{HI} beta cells at different time points of implantation.

Supplementary table 1: PCR Primers used for variant confirmation and family testing for human *MANF*

Exon	Strand	Sequence	Accession number	Chromosome	g. start	g. end
Exon 1	Forward	AGACACCACCAGCCAATGAG	NM_006010.5	3	51422551	51422570
	Reverse	AAGATTTACTAGGGCGGGCG	NM_006010.5	3	51423026	51423045
Exon 2	Forward	AGTGAGTCCCGTCTCAAGGA	NM_006010.5	3	51423332	51423351
	Reverse	TTAACATGAGCCAGGGCCAG	NM_006010.5	3	51423793	51423812
Exon 3	Forward	GGAGGATCACCACCCAACAG	NM_006010.5	3	51424973	51424992
	Reverse	GACCTCCACCCACTTCCATG	NM_006010.5	3	51425450	51425469
Exon 4	Forward	AGTGTGGCCTCTGTCCCTAT	NM_006010.5	3	51426076	51426095
	Reverse	TTTTTCCCCCTCAGGTGCAA	NM_006010.5	3	51426536	51426555

Supplementary table 2: Detail clinical features of cases with MANF pathogenic variants

	Case 1 (c.82_94del/c.82_94del)	Case 2 (c.103+1G>T/c.103+1G>T)
Current age, sex	14y, female	27y, female
Age at diabetes diagnosis	10y	17y
Islet autoantibody	GAD/ICA/IAA – negative	GAD- Negative
T1D-genetic risk score	0.3 centile of T1D population	-
Height and weight at diagnosis of diabetes	Height-120 cm (-2.8SD), weight 23.4 (-2SD), BMI- 16.3 (16 th centile)	Height -143cm (-3.1 SD), Weight-57kg (0.2 SD), BMI 27.9 (93 rd centile)
C-peptide	532 pmol/l at 2y post diagnosis, 244 pmol/L at 4y post diagnosis	330 pmol/l at 6y post diagnosis
Treatment	Initially insulin for 10 days, followed by diet control diabetes for 1.5y. since than on insulin Currently Insulin -0.67u/kg/day	Currently Insulin
Birth weight	weight-2.4 kg at 38 wks of gestation (0.9 centile)	-
Last known height and weight	at 14y Height-134cm (-4.4SD), Weight-37 kg (-2.76SD), BMI 20.66 (52 th centile)	at 17y Height -143cm (-3.1 SD), Weight-57kg (0.2 SD), BMI 27.9 (93 rd centile)
Short stature	Yes	Yes
Microcephaly	Yes	Yes
Developmental delay	Yes, (special education, normal MRI head)	Yes, normal MRI head
Deafness	Bilateral sensorineural	Bilateral sensorineural
Pituitary disease	Not preset at last assessment at 14y	Partial hypopituitarism (deficiency in growth hormone, thyroid stimulating hormone, luteinizing hormone and follicle-stimulating hormone)
Other features	Scoliosis	

Supplementary table 3. Antibodies used for flow cytometry (FC), immunocytochemistry (ICC) and immunohistochemistry (IHC)

Antibody	Supplier	Use
Mouse Anti-CD184 (CXCR4) Monoclonal Antibody, PhycoErythrin Conjugated, Clone 12G5	BD Biosciences Cat# 555974; RRID:AB_396267	FC (1:10)
Mouse IgG2a, kappa Isotype Control, PhycoErythrin Conjugated, Clone G155-178	BD Biosciences Cat# 563023	FC (1:10)
Mouse Anti-PDX1 Phycoerythrin Conjugated	BD Biosciences Cat# 562161; RRID:AB_10893589	FC (1:80)
Mouse Anti-NKX6-1 Alexa Fluor 647 Conjugated	BD Biosciences Cat# 563338	FC (1:80)
Mouse Anti-NKX6.1, Phycoerythrin Conjugated	BD Biosciences Cat# 555574	FC (1:80)
Insulin (C27C9) Rabbit Antibody (Alexa Fluor 647 Conjugate)	Cell Signaling Technology Cat# 9008; RRID:AB_2687822	FC (1:80)
Rabbit IgG Isotype Control (Alexa Fluor 647 Conjugate)	Cell Signaling Technology Cat# 3452S; RRID:AB_10695811	FC (1:80)
Mouse IgG1, kappa Isotype Control, Phycoerythrin Conjugated, Clone MOPC-21 antibody	BD Biosciences Cat# 555749; RRID:AB_396091	FC (1:80)
Mouse anti-TRA1-60	Thermo Fisher Scientific, RRID:AB_2536699	ICC (1:250)
Rabbit anti- NANOG	Santa Cruz Biotechnology Cat# sc-9081; RRID:AB_2167703	ICC (1:250)
Goat anti-PDX1	R and D Systems Cat# AF2419; RRID:AB_355257	ICC (1:250)
Mouse anti-NKX6-1	DSHB Cat# F55A10; RRID:AB_532378	ICC (1:250)
Rabbit anti-CHGA	Dako Cat# A0564	ICC (1:500)
Guinea pig anti-INS	Dako Cat# A0564; RRID:AB_10013624	ICC, IHC (1:500)
Mouse anti-PROINS	DSHB Cat# GS-9A8; RRID:AB_532383	ICC, IHC (1:300)
Mouse anti-GCG	Sigma-Aldrich Cat# G2654; RRID:AB_259852	ICC, IHC (1:500)
Rabbit anti-BiP	Cell Signaling Technology Cat# 3177S; RRID:AB_2119845	ICC, IHC (1:500)
Rabbit anti-GRP170	abcam, Cat#ab134944; RRID:AB_2858190	ICC, IHC (1:250)
Rabbit anti-Ki67	Leica Microsystems Cat# NCL-Ki67p; RRID:AB_442102	IHC (1:250)
Rabbit anti-MANF	Atlas Antibodies Cat#HPA011175	WB (1:500)
Mouse anti- α Tubulin	Sigma; T5168	WB (1:2000)

Supplementary table 4: Primers used for qRT-PCR

<i>Gene</i>	<i>RefSeq</i>	<i>Primers</i>	<i>Amplicon size</i>
<i>PPIG</i>	NM_004792	Fw: TCTTGTCAATGGCCAACAGAG Rv: GCCCATCTAAATGAGGAGTTG	84 bp
<i>PDX1</i>	NM_000209.3	Fw: AAGTCTACCAAAGCTCACGCG Rv: CGTAGGCGCCGCCTGC	52 bp
<i>NKX6.1</i>	NM_006168	Fw: TATTCGTTGGGGATGACAGAG Rv: TGGCCATCTCGGCAGCGTG	91 bp
<i>INS</i>	NM_000207	Fw: CAGAAGCGTGGCATTGTGGA Rv: GCTGCGTCTAGTTGCAGTAG	82 bp
<i>GCG</i>	NM_002054	Fw: GAAGGCGAGATTTCACAGAAG Rv: CCTGGCGGCAAGATTATCAAG	113 bp
<i>BiP (HSPA5)</i>	NM_005347.4	Fw: TGGCTGGAAAGCCACCAAGATGCT Rv: GGGGGAGGGCCTGCACTTCCAT	116 bp
<i>sXBP1</i>	NM_001079539.1	Fw: CTGCTGAGTCCGCAGCAGGTGCA Rv: GGTCCAAGTTGTCCAGAATGC	129 bp
<i>CHOP (DDIT3)</i>	NM_001195053.1	Fw: GCACCTCCCAGAGCCCTCACTC Rv: CCCGGGCTGGGGAATGACCA	120 bp
<i>ATF6</i>	NM_007348.4	Fw: ACCTGCTGTTACCAGCTACCACCCA Rv: GCATCATCACTTCGTAGTCCTGCCC	120 bp
<i>GRP170 (HYOU1)</i>	NM_001130991.2	Fw: GTCCAAGGGCATCAAGGCTC Rv: TTCTGCGCTGTCCTCTACCA	103 bp
<i>MANF</i>	NM_006010.6	Fw: GGCGACTGCGAAGTTTGTAT Rv: TTGCTTCCCGGCAGAACTTT	121
<i>MAFA</i>	NM_201589	Fw: GCCAGGTGGAGCAGCTGAA Rv: CTTCTCGTATTTCTCCTTGAC	77 bp
<i>TXNIP</i>	NM_006472.6	Fw: GGTCTTTAACGACCCTGAAAAGG Rv: ACACGAGTAACTTCACACACCT	87 bp