

Supplementary table 1: cell lines information

Name	UCSF001-A	WTSI028-A	HMGU001-A hINS-T2A-H2B-Cherry (+/-)
Aberration in this study	WTC11	Babk2	InsCherry*
Vender	hPSCreg	hPSCreg	Helmholtz Zentrum München
Cell line type	Human induced pluripotent stem cell (hiPSC)		
Sex	Male	Female	Female
Age of donor (at collection)	30-34	50-54	NA
Ethnicity	Asian	White - White British	Caucasian
Source cell tyoe, origin	Blood, Derived Peripheral Blood Mononuclear Cell	Zone Of Skin, Fibroblast	Upper Arm, Fibroblast
Reprogramming Vector type	Episomal, Non-integrating	Sendai virus, Non-integrating	Episomal, Non-integrating
Reprogramming Genes	POU5F1, SOX2, KLF4, MYC, LIN28	POU5F1, SOX2, KLF4, MYC	POU5F1, SOX2, KLF4, MYC, LIN28, NANOG

*Using CRISPR/Cas9, a T2A-H2B-Cherry construct was inserted in one allele of INS exon 3. Information was shown from its parent cell line: HMGU001-A (hPSCreg Name).

Supplementary table 2: Islets donor characteristics

Islet preparation	1	2	3	4	5	6
Unique identifier	O79	O80	O88	O99	O100	O104
Donor age (years)	49	35	57	57	36	43
Donor sex (M/F) ^a	F	M	M	F	F	M
Donor BMI (kg/m2)	37.6	25.2	24.5	24.2	20.8	32.7
Donor HbA1c (mmol/mol)	34	NA	NA	NA	NA	NA
Origin/source of islets ^b	ECIT ^b	ECIT	ECIT	ECIT	ECIT	ECIT
Islet isolation centre	Oslo	Oslo	Oslo	Oslo	Oslo	Oslo
Donor history of diabetes?	No	No	No	No	No	No
For RNA Isolation and analysis	√	√	NA	√	√	NA
For DNA Isolation and analysis	NA	NA	√	√	√	NA
Dynamic GSIS	NA	NA	NA	√	√	√

a. M, Male; F, Female.

b. European Consortium for Islet Transplantation

Supplementary Table 3: Formulation of the differentiation medium

Stage 1 medium: 3days		
Basal medium	Pen/Strep	1%
	NaHCO ₃	1.174g/L
	Glutamax (100x)	1x
	Glucose	+4.5mM to 10mM
Supplementary	BSA	0.50%
	Activin A	100ng/ml
	Chir99021	3μM*
* only added in the first 24 hours		
Stage 2 medium: 2 days		
Basal medium	Pen/Strep	1.00%
	NaHCO ₃	1.174g/L
	Glutamax (100x)	1x
	Glucose	+4.5mM to 10mM
	BSA	0.50%
	vitamin C	0.25mM
Supplementary	FGF7	50ng/ml
Stage 3 medium: 2 days		
Basal medium	Pen/Strep	1%
	NaHCO ₃	1.754g/L
	Glutamax (100x)	1x
	Glucose	+4.5mM to 10mM
	BSA	2%
	ITS-X	1:200
	vitamin C	0.25mM
	Retinoid Acid	1μM
Supplementary	SANT1	0.25μM
	FGF7	50ng/ml
	LDN193189	0.1μM
	TPB	0.2μM
Stage 4 medium: 3 days		
Basal medium	Pen/Strep	1%
	NaHCO ₃	1.754g/L
	Glutamax (100x)	1x
	Glucose	+4.5mM to 10mM
	BSA	2%
	ITS-X	1:200
	vitamin C	0.25mM
Supplementary	Retinoid Acid	0.1μM
	SANT1	0.25μM
	FGF7	2ng/ml
	LDN 193189	0.2μM
	TPB	0.1nM

Stage 5 medium: 3 days		
Basal medium	Pen/Strep	1%
	NaHCO ₃	1.754g/L
	Glutamax (100x)	1x
	Glucose	Final conc. at 5.5mM or 20mM
	BSA	2%
	ITS-X	1:200
	Heparin	10ug/ml
Supplementary	vitamin C	0.25mM
	ZnSO ₄	10μM
	Retinoid Acid	0.05μM
	SANT1	0.25μM
	ALK5 inh II	10μM
	T3	1μM
	LDN 193189	0.1μM
Stage 6 medium: 7 days		
Basal medium	Pen/Strep	1%
	NaHCO ₃	1.754g/L
	Glutamax (100x)	1x
	Glucose	Final conc. at 5.5mM or 20mM
	BSA	2%
	ITS-X	1:200
	Heparin	10ug/ml
	vitamin C	0.25mM
Supplementary	ZnSO ₄	10μM
	ALK5 inh II	10μM
	T3	1 μM
	LDN	100nM
	Xxi	0.1μM
Stage 7 medium: 7days		
Basal medium	Pen/Strep	1%
	NaHCO ₃	1.754g/L
	Glutamax (100x)	1x
	Glucose	Final conc. at 5.5mM or 20mM
	BSA	2%
	ITS-X	1:200
	Heparin	10ug/ml
	vitamin C	0.25mM
Supplementary	ZnSO ₄	10μM
	ALK5 inh II	10μM
	T3	1 uM
	N-acetyl cysteine	1mM
	Trolox	10μM
	R428	2μM
Stage 7+: 7-10 days		
Same medium as in Stage 7, cells were dissociated and resuspended in Ultra Low Attachment plates at ~1*10 ⁶ cells/ml and maintained in orbital shaker (90RPM) in 5% CO ₂ , 37°C incubator.		

Supplementary Table 4: List of antibodies

Antibody	Catalog No.	Vendor	Dilution factor	Application ¹
Guinea Pig anti-insulin	A0564	Dako	1:500	IF,FCS
Goat anti Human PDX-1	AF2419	R&D Systems	1:200	IF,FCS
Mouse anti Nkx-6.1	F55A12-S	University of Iowa,DHB	1:100	IF,FCS
Mouse anti YAP1 Antibody (63.7)	SC-101199	Santa Cruz	1:1000	WB
Rabbit anti Phospho-YAP (Ser127)	4911	Celll Signaling Technology	1:1000	WB
Mouse anti Angiotonin (AMOT, B-4)	SC-166924	Santa Cruz	1:500	WB
Mouse anti AMPKalpha	2793S	Celll Signaling Technology	1:1000	WB
Rabbit p-AMPKalpha (40H9)(T172)	2535S	Celll Signaling Technology	1:1000	WB
Rabbit anti actin	A2066	Sigma-Aldrich	1:5000	WB

¹IF: Immunofluorescence; WB: Western blot; FCS: Flow cytometry

Supplementary Table 5: List of oligonucleotides for qRT-PCR

Gene name	Forward primer sequence	Reverse primer sequence
<i>TBP</i>	TGTATCCACAGTGAATCTTGGTTG	GGTTCGTGGCTCTCTTATCC
<i>SOX9</i>	GATTAGCACACTGATCACACGA	TTAACCCTCTTCAGAGCAAGC
<i>NGN3</i>	CTAAGAGCGAGTTGGCACTG	TGCCGAGTTGAGGTTGTGCA
<i>PAX4</i>	GGCAGTATCCTGATTCAGTGCC	CTTCTCTTGCCGACGCCATTT
<i>ARX</i>	CTGCTGAAACGCAAACAGAGG	CTCGGTCAAGTCCAGCCTCAT
<i>NEUROD1</i>	ATCAGCCCACTCTCGCTGTA	GCCCCAGGGTTATGAGACTAT
<i>NKX2-2</i>	GGAGCTTGAGTCCTGAGGG	TCTACGACAGCAGCGACAAC
<i>PDX1</i>	CGTCCGCTTGTTCTCCTC	CCTTTCCCATGGATGAAGTC
<i>NKX6.1</i>	CTATTGCTTGGGGATGACAGAG	TGTCTCCGAGTCCTGCTTCT
<i>INS</i>	ACGAGGCTTCTTCTACACACC	TCCACAATGCCACGCTTCTG
<i>GCG</i>	CGTTCCCTTCAAGACACAGAG	GCCTGGAGTCCAGATACTTG
<i>SST</i>	TGGGTTCAGACAGCAGCTC	CCCAGACTCCGTCAGTTTCT
<i>MAFA</i>	GAGAGCGAGAAGTGCCAACT	TTCTCCTTGTAAGGTCCCG
<i>MAFB</i>	ACGCCTACAAGGTCAAGTGC	CGACTCACAGAAAGAACTCGG
<i>ISL1</i>	TCACGAAGTCGTTCTTGCTG	CATGCTTTGTTAGGGATGGG
<i>UCN3</i>	GGAGGGAAGTCCACTCTCG	TGTAGAACTGTGGGGGAGG
<i>LDHA</i>	GGCCTGTGCCATCAGTATCT	GGAGATCCATCATCTCTCCC
<i>ABCC8</i>	GAGAAGTCGGCCTCTTTGAA	GGGCCTTTGCCATCTATACC
<i>YAP</i>	TGTCCCAGATGAACGTCACAG	TGGTGGCTGTTTCACTGGAG
<i>VDAC1</i>	GCAAAAATCCCGAGTGACCCA	TCCAGGCAAGATTGACAGCG
<i>VDAC2</i>	CTTTGCAGTGGGCTACAGGA	GAGTGCAGTTGGTACCTGATG
<i>GLUT1</i>	GCAGGCTTCTCCAAGTGGAC	GAACCAGGAGCACAGTGAAG
<i>Mdh1</i>	GGTGTCTAATGGAACTGCAAG	TCCAGGTCTTTGAAGGCAACG
<i>PSCK1</i>	CCAGATGTGCAGGAGAAATTGC	CGTCACAATGCCATCCAGCAT
<i>β2M</i>	TGCTGTCTCCATGTTTGATGTATCT	TCTCTGCTCCCCACCTCTAAGT
<i>mtDNA 16S rRNA</i>	GCCTTCCCCCGTAAATGATA	TTATGCGATTACCGGGCTCT