

Supplemental Information for: Cryopreservation of stem cell-derived beta-like cells enriches for insulin-producing cells with improved function

Supplemental Figure Legends:

Supplemental Figure 1: hPSC efficiently differentiate into stem cell-derived beta-like cells.

A) Schematic of cluster formation and differentiation of hPSCs into sBC. **B)** Representative live brightfield (BF) images of clusters on day 0, day 3, and day 9. **C-E)** Representative flow cytometry plot and flow quantification of SOX2 and TRA-1-60 expression on day 0 (**C**), SOX17 and FOXA2 expression on day 3 (**D**), and PDX-1 and NKX-6.1 expression on day 9 (**E**) of the differentiation protocol. **F&G)** Representative live brightfield (BF) and GFP insulin reporter (pINS.GFP) images of clusters on day 16 and day 23 of differentiation. **H-K)** Flow cytometry analysis of PDX1⁺ NKX6.1⁺ (**H**), C-peptide⁺ (**I**), C-peptide⁺ NKX6.1⁺ (**J**), and C-peptide⁺ Glucagon⁺ frequencies on day 23 of differentiation. **L)** Quantification of total cluster count on day 23 (prior to freezing) and on day 30 in F/T sBC. **M)** Representative flow cytometry plot and cumulative quantification of the FSC-H (size) of day 30 control or F/T sBC. Data is representative of 3-7 independent experiments. White circles indicate ESC, triangles indicate iPSC.

Supplemental Figure 2: Single cell RNA sequencing reveals distinct cell populations.

A) Heatmap of 200 differentially expressed genes between samples. **B)** Heatmap of 10 most differentially regulated genes in different clusters from single cell RNA sequencing experiment. **C-D)** t-SNE plot of positive reads and quantification of frequency of events/sample ID for expression of *GCG* and *SST*. **E)** Normalized gene expression analysis of *MKI67* by qPCR in control or F/T sBC on day 30. Purple triangles indicate iPSC-derived sBC, white circles indicate ESC-derived sBC. Data is from 6 independent experiments. Data analyzed by paired t-test. ** $p < 0.01$. **F-I)** t-SNE plot of positive reads and quantification of frequency of events/sample ID for expression of *MKI67*, *TOP2A*, *ISL1*, and *IAPP*.

Supplemental Figure 3: Single cell RNA sequencing reveals distinct cell populations.

A-F) t-SNE plot of positive reads and quantification of frequency of events/sample ID for expression of *TPH1*, *CXCL14*, *SCL18A1*, *ADRA2A*, *TAC1*, and *MUC1*.

Supplemental Tables:

Supplemental Table 1: List of antibodies and dyes used for flow cytometry

Antigen	Clone	Fluorophore	Dilution	Vendor	Catalog #
TRA-1-60	TRA-1-60-R	AF647	1:100	BioLegend	330606
SOX2	14A6A34	AF594	1:100	BioLegend	656106
SOX17	P7-969	AF488	1:200	BD Biosciences	562205
FOXA2	N17-280	PE	1:200	BD Biosciences	561589
C-peptide	C-PEP-01	AF488*	1:300	Origene	BM270
NKX-6.1	R11-560	AF647	1:50	BD Biosciences	563338
PDX-1	658A5	PE	1:50	BD Biosciences	562161
SOX9	polyclonal	AF350*	1:50	Millipore Sigma	AB5535
Glucagon	U16-850	BV421	1:500	BD Biosciences	565891

* antibodies were conjugated in house

Supplemental Table 2: List of antibodies and dyes used for immunofluorescence

Antigen	Clone	Fluorophore	Dilution	Vendor	Catalog #
Insulin	Polyclonal	N/A	1:10	DAKO	A0564
PDX-1	Polyclonal	N/A	1:200	R&D Systems	AF2419
NKX-6.1	F55A10	N/A	1:200	DSHB	F55A10
Glucagon	K79bb10	N/A	1:2000	Millipore Sigma	G2654
Human Nuclear Antigen	235-1	PE	1:200	Abcam	ab191181
NKX-2.2	74.5A5	N/A	1:20	DSHB	74.5A5
Synaptophysin	Snp88	N/A	1:50	BioGenex	MU363-UC

SOX9	Polyclonal	N/A	1:1000	Millipore Sigma	AB5535
Mouse IgG	polyclonal	AF647	1:500	ThermoFisher	A31571
Goat IgG	Polyclonal	AF555	1:500	ThermoFisher	A21432
Guinea pig IgG	Polyclonal	AF488	1:500	ThermoFisher	A11073

Supplemental Table 3: List of primers used for quantitative PCR.

Probe	Vendor	Catalog #
<i>INS</i>	BioRad	qHsaCPE5192383
<i>GCG</i>	BioRad	qHsaCEP0050851
<i>SST</i>	BioRad	qHsaCPE5046148
<i>MKi67</i>	BioRad	qHsaCPE5050322
<i>TBP</i>	BioRad	qHsaCIP0036255

Supplemental Table 4: List of UMI thresholds for single cell RNA sequencing

Gene	LogUMINorm Range
INS	3-8
GCG	3-9
SST	3-9
FEV	3-5
ENTPD3	0.1-4
IAPP	0.1-6
SOX9	0.5-4

CHGA	3-9
NKX6.1	0.5-4
PDX1	0.5-4
TPH1	3-5
ISL1	0.5-4
NUEROD1	0.5-5
NKX2.2	0.5-4
MKi67	1-4
TOP2A	1-4
SLC18A1	1-4
ADRA2A	1-3
TAC1	1-4
CXCL14	1-4
MUC1	1-3

Supplemental Table 5: Top 10 differentially expressed genes per cluster within t-sne plot

Gene	Cluster 1 Log2 Fold Change	Cluster 2 Log2 Fold Change	Cluster 3 Log2 Fold Change	Cluster 4 Log2 Fold Change	Cluster 5 Log2 Fold Change	Cluster 6 Log2 Fold Change	Cluster 7 Log2 Fold Change
MFAP2	4.98483809	-3.2829503	0.47968975	-3.655798	-3.6429391	-3.3988531	-2.661469
HSPG2	5.55684927	-3.7504126	-2.0553095	-2.7713501	-2.9996921	-2.9673279	-5.7768301
CYR61	8.35522632	-6.4822229	-0.5336771	-5.3461616	-6.9800042	-7.2534819	-7.3027632
VTCN1	7.25453394	-4.5886956	0.09012092	-5.13931	-5.3181727	-5.2355135	-6.6794939
NOTCH2	5.86586927	-4.7515848	-0.9490407	-2.947646	-3.0767999	-4.4904334	-4.7858522
NES	5.85609164	-3.8031262	1.59421706	-3.5818849	-5.7992798	-5.7529767	-3.2056844
KIRREL1	6.72817824	-4.6738008	-0.4556203	-2.4269171	-4.5515845	-5.1268746	-5.8981813
NR5A2	6.91533876	-4.316411	-0.8800689	-3.2092589	-5.4772794	-5.0092234	-6.4533317
PTPN14	6.22803312	-3.8958054	-0.6051841	-2.8263707	-3.8993476	-4.8864407	-5.466109
LINC01829	5.84877758	-3.9461198	-0.6549624	-1.486176	-4.3723682	-5.106966	-3.9826264

Extended Detailed Methods:

Single Cell RNA sequencing:

scRNA-seq libraries were sequenced via paired-end sequencing through the Illumina® Novaseq platform by Novogene Corporation Inc. Sequencing reads were uploaded to

10X Genomic's cloud analysis platform and processed with the Cell Ranger Count v7.1.0 pipeline using the Human GRCh38 v3.0.0 reference genome to create unique molecular identifier (UMI) gene count matrices per sample. These individual sample matrices were then aggregated together and processed using the 10X Genomics Loupe Browser v7.0.1 platform to perform filtering, normalization, clustering and to create t-SNE projections. Cells that contained a UMI count lower than 260 or higher than 200,000, had fewer than 20 or greater than 12,000 genes expressed, or a proportion of mitochondrial genes greater than 20% were removed from the analysis.

Kidney capsule transplantation of sBC:

Mice were first anesthetized with isoflurane, followed by lubricant application on the eyes and subcutaneous injections of analgesics (buprenorphine and meloxicam). After shaving and applying Nair, the surgical area was sterilized with povidone-iodine and alcohol swabs. The surgery was then performed aseptically, first by making an incision in the skin and then in the peritoneal layer. The kidney was then exposed and 1000 sBC transplanted into the kidney capsule. The kidney was returned to its original position, the peritoneal incision closed with surgical stitches, and the skin incision closed with surgical clips. Experimental animals were monitored and received additional subcutaneous injections of analgesics in the following days as per approved UF IACUC protocol.