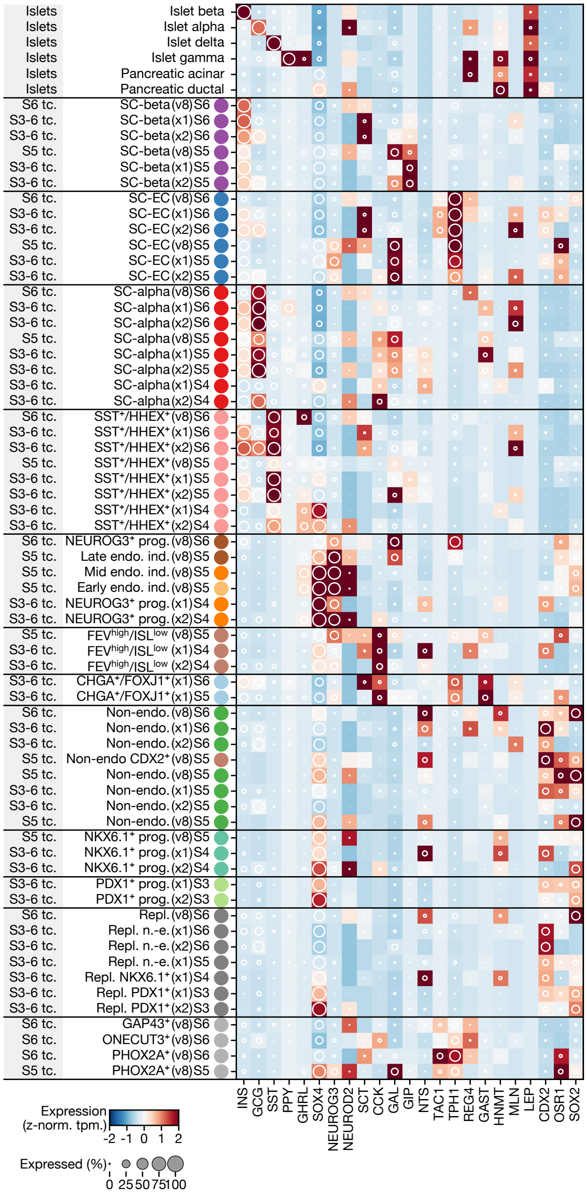
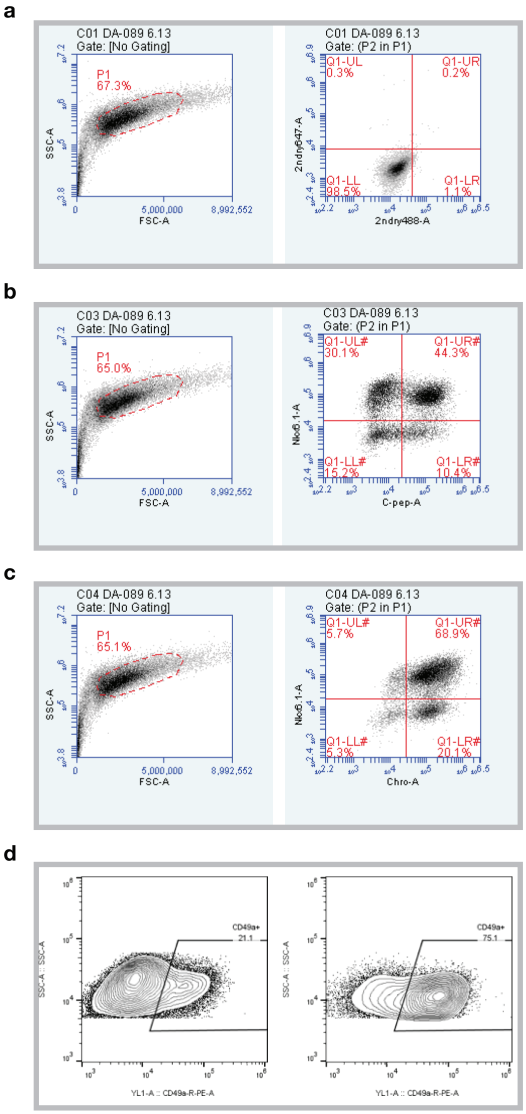
Supplementary Figure 1


Islets Islet beta
Islets Islet alpha
Islets Islet delta
Islets Islet gamma
Islets Pancreatic acinar
Islets Pancreatic ductal
S6 tc. SC-beta (v8) S6
S3-6 tc. SC-beta (x1) S6
S3-6 tc. SC-beta (x2) S6
S5 tc. SC-beta (v8) S5
S3-6 tc. SC-beta (x1) S5
S3-6 tc. SC-beta (x2) S5
S6 tc. SC-EC (v8) S6
S3-6 tc. SC-EC (x1) S6
S3-6 tc. SC-EC (x2) S6
S5 tc. SC-EC (v8) S5
S3-6 tc. SC-EC (x1) S5
S3-6 tc. SC-EC (x2) S5
S6 tc. SC-alpha (v8) S6
S3-6 tc. SC-alpha (x1) S6
S3-6 tc. SC-alpha (x2) S6
S5 tc. SC-alpha (v8) S5
S3-6 tc. SC-alpha (x1) S5
S3-6 tc. SC-alpha (x2) S5
S3-6 tc. SC-alpha (x1) S4
S3-6 tc. SC-alpha (x2) S4
S6 tc. SST$^+$/HHEX$^+$ (v8) S6
S3-6 tc. SST$^+$/HHEX$^+$ (x1) S6
S3-6 tc. SST$^+$/HHEX$^+$ (x2) S6
S5 tc. SST$^+$/HHEX$^+$ (v8) S5
S3-6 tc. SST$^+$/HHEX$^+$ (x1) S5
S3-6 tc. SST$^+$/HHEX$^+$ (x2) S5
S3-6 tc. SST$^+$/HHEX$^+$ (x1) S4
S3-6 tc. SST$^+$/HHEX$^+$ (x2) S4
S6 tc. NEUROG3$^+$ prog. (v8) S6
S5 tc. Late endo. ind. (v8) S5
S5 tc. Mid endo. ind. (v8) S5
S5 tc. Early endo. ind. (v8) S5
S3-6 tc. NEUROG3$^+$ prog. (x1) S4
S3-6 tc. NEUROG3$^+$ prog. (x2) S4
S5 tc. FEV^{high}/ISL^{low} (v8) S5
S3-6 tc. FEV^{high}/ISL^{low} (x1) S4
S3-6 tc. FEV^{high}/ISL^{low} (x2) S4
S3-6 tc. CHGA$^+$/FOXJ1$^+$ (x1) S6
S3-6 tc. CHGA$^+$/FOXJ1$^+$ (x1) S5
S6 tc. Non-endo. (v8) S6
S3-6 tc. Non-endo. (x1) S6
S3-6 tc. Non-endo. (x2) S6
S5 tc. Non-endo CDX2$^+$ (v8) S5
S5 tc. Non-endo. (v8) S5
S3-6 tc. Non-endo. (x1) S5
S3-6 tc. Non-endo. (x2) S5
S5 tc. Non-endo. (v8) S5
S5 tc. NKX6.1$^+$ prog. (v8) S5
S3-6 tc. NKX6.1$^+$ prog. (x1) S4
S3-6 tc. NKX6.1$^+$ prog. (x2) S4
S3-6 tc. PDX1$^+$ prog. (x1) S3
S3-6 tc. PDX1$^+$ prog. (x2) S3
S6 tc. Repl. (v8) S6
S3-6 tc. Repl. n.-e. (x1) S6
S3-6 tc. Repl. n.-e. (x2) S6
S3-6 tc. Repl. n.-e. (x1) S5
S3-6 tc. Repl. NKX6.1$^+$ (x1) S4
S3-6 tc. Repl. PDX1$^+$ (x1) S3
S3-6 tc. Repl. PDX1$^+$ (x2) S3
S6 tc. GAP43$^+$ (v8) S6
S6 tc. ONECUT3$^+$ (v8) S6
S6 tc. PHOX2A$^+$ (v8) S6
S5 tc. PHOX2A$^+$ (v8) S5
INS
NKX6-1
NPTX2
ITGA1
PCSK1
ISL1
TPH1
SLC18A1
LMX1A
ADRA2A
MME
GCG
DPP4
ARX
IRX2
ETV1
HHEX
SST
NEUROG3
LMX1B
RFX3
FOXJ1
ENKUR
CHGA
FEV
MNX1
SYP
CPE
CDKN1C
SOX9
PTF1A
PDX1
CDX2
TOP2A
AURKB
GAP43
DPYSL3
ONECUT3
GC
PHOX2A
KLK1
ENC1
Expression (z-norm. tpm.)
-2 -1 0 1 2
Expressed (%)
0 25 50 75 100

Supplementary Figure 2


Islets
Islet beta
Islets
Islet alpha
Islets
Islet delta
Islets
Islet gamma
Islets
Pancreatic acinar
Islets
Pancreatic ductal
S6 tc.
SC-beta (v8) S6
S3-6 tc.
SC-beta (x1) S6
S3-6 tc.
SC-beta (x2) S6
S5 tc.
SC-beta (v8) S5
S3-6 tc.
SC-beta (x1) S5
S3-6 tc.
SC-beta (x2) S5
S6 tc.
SC-EC (v8) S6
S3-6 tc.
SC-EC (x1) S6
S3-6 tc.
SC-EC (x2) S6
S5 tc.
SC-EC (v8) S5
S3-6 tc.
SC-EC (x1) S5
S3-6 tc.
SC-EC (x2) S5
S6 tc.
SC-alpha (v8) S6
S3-6 tc.
SC-alpha (x1) S6
S3-6 tc.
SC-alpha (x2) S6
S5 tc.
SC-alpha (v8) S5
S3-6 tc.
SC-alpha (x1) S5
S3-6 tc.
SC-alpha (x2) S5
S3-6 tc.
SC-alpha (x1) S4
S3-6 tc.
SC-alpha (x2) S4
S6 tc.
SST$^+$/HHEX$^+$ (v8) S6
S3-6 tc.
SST$^+$/HHEX$^+$ (x1) S6
S3-6 tc.
SST$^+$/HHEX$^+$ (x2) S6
S5 tc.
SST$^+$/HHEX$^+$ (v8) S5
S3-6 tc.
SST$^+$/HHEX$^+$ (x1) S5
S3-6 tc.
SST$^+$/HHEX$^+$ (x2) S5
S3-6 tc.
SST$^+$/HHEX$^+$ (x1) S4
S3-6 tc.
SST$^+$/HHEX$^+$ (x2) S4
S6 tc.
NEUROG3$^+$ prog. (v8) S6
S5 tc.
Late endo. ind. (v8) S5
S5 tc.
Mid endo. ind. (v8) S5
S5 tc.
Early endo. ind. (v8) S5
S3-6 tc.
NEUROG3$^+$ prog. (x1) S4
S3-6 tc.
NEUROG3$^+$ prog. (x2) S4
S5 tc.
FEV^{high}/ISL^{low} (v8) S5
S3-6 tc.
FEV^{high}/ISL^{low} (x1) S4
S3-6 tc.
FEV^{high}/ISL^{low} (x2) S4
S3-6 tc.
CHGA$^+$/FOXJ1$^+$ (x1) S6
S3-6 tc.
CHGA$^+$/FOXJ1$^+$ (x1) S5
S6 tc.
Non-endo. (v8) S6
S3-6 tc.
Non-endo. (x1) S6
S3-6 tc.
Non-endo. (x2) S6
S5 tc.
Non-endo CDX2$^+$ (v8) S5
S5 tc.
Non-endo. (v8) S5
S3-6 tc.
Non-endo. (x1) S5
S3-6 tc.
Non-endo. (x2) S5
S5 tc.
Non-endo. (v8) S5
S5 tc.
NKX6.1$^+$ prog. (v8) S5
S3-6 tc.
NKX6.1$^+$ prog. (x1) S4
S3-6 tc.
NKX6.1$^+$ prog. (x2) S4
S3-6 tc.
PDX1$^+$ prog. (x1) S3
S3-6 tc.
PDX1$^+$ prog. (x2) S3
S6 tc.
Repl. (v8) S6
S3-6 tc.
Repl. n.-e. (x1) S6
S3-6 tc.
Repl. n.-e. (x2) S6
S3-6 tc.
Repl. n.-e. (x1) S5
S3-6 tc.
Repl. NKX6.1$^+$ (x1) S4
S3-6 tc.
Repl. PDX1$^+$ (x1) S3
S3-6 tc.
Repl. PDX1$^+$ (x2) S3
S6 tc.
GAP43$^+$ (v8) S6
S6 tc.
ONECUT3$^+$ (v8) S6
S6 tc.
PHOX2A$^+$ (v8) S6
S5 tc.
PHOX2A$^+$ (v8) S5
INS
GCG
SST
PPY
GHRL
SOX4
NEUROG3
NEUROD2
SCT
CCK
GAL
GIP
NTS
TAC1
TPH1
REG4
GAST
HNMT
MLN
LEP
CDX2
OSR1
SOX2
Expression (z-norm. tpm.)
-2
-1
0
1
2
Expressed (%)
0
25
50
75
100

Supplementary Figure 3


a
C01 DA-089 6.13
Gate: [No Gating]
P1
67.3%
SSC-A
FSC-A
0
5,000,000
8,992,552
C01 DA-089 6.13
Gate: (P2 in P1)
Q1-UL
0.3%
Q1-UR
0.2%
Q1-LL
98.5%
Q1-LR
1.1%
2ndry647-A
2ndry488-A
b
C03 DA-089 6.13
Gate: [No Gating]
P1
65.0%
SSC-A
FSC-A
0
5,000,000
8,992,552
C03 DA-089 6.13
Gate: (P2 in P1)
Q1-UL#
30.1%
Q1-UR#
44.3%
Q1-LL#
15.2%
Q1-LR#
10.4%
Nkx6.1-A
C-pep-A
c
C04 DA-089 6.13
Gate: [No Gating]
P1
65.1%
SSC-A
FSC-A
0
5,000,000
8,992,552
C04 DA-089 6.13
Gate: (P2 in P1)
Q1-UL#
5.7%
Q1-UR#
68.9%
Q1-LL#
5.3%
Q1-LR#
20.1%
Nkx6.1-A
Chro-A
d
CD49a+
21.1
SSC-A :: SSC-A
YL1-A :: CD49a-R-PE-A
CD49a+
75.1
SSC-A :: SSC-A
YL1-A :: CD49a-R-PE-A

Supplementary Table 2

	Purpose	Cells used	Cells sequenced	Data presented in
Stages 3-6 time course	• Identify populations in each stage • Compare two protocol variants	2 differentiations HUES8, protocol x1 HUES8, protocol x2	42,097 cells from 16 libraries inDrops v2 (Harvard Single Cell core)	Figure 1; Extended Data Figure 1
Stage 6 weekly time course	• Track long-term gene expression changes after differentiation	3 differentiations HUES8, protocol v8	38,494 cells from 36 libraries inDrops v2 (1Cell Bio)	Figures 2, 3, 4; Extended Data Figures 3,4,6;
Stage 5 daily time course	• Establish lineage map of endocrine induction during Stage 5	2 differentiations HUES8, protocol v8	51,274 cells from 32 libraries inDrops v2 (1Cell Bio)	Figure 5; Extended Data Figure 8
hESC vs hiPSC comparison	• Compare cell populations produced by different pluripotent stem cell lines	3 differentiations HUES8, protocol v4 HUES8, protocol x3 iPS1016/31, protocol v4	4,312 cells from 6 libraries inDrops v3 (Harvard Single Cell core)	Extended Data Figure 1
Re-aggregation analysis	• Identify which populations are retained by re-aggregation	1 differentiation HUES8, protocol v4 processed into two conditions at the end of Stage 5: - native, in MCDB131 - RA, in MCBD131	5,636 cells from 4 libraries inDrops v3 (Harvard Single Cell core)	Extended Data Figure 7