FIGURE 1A

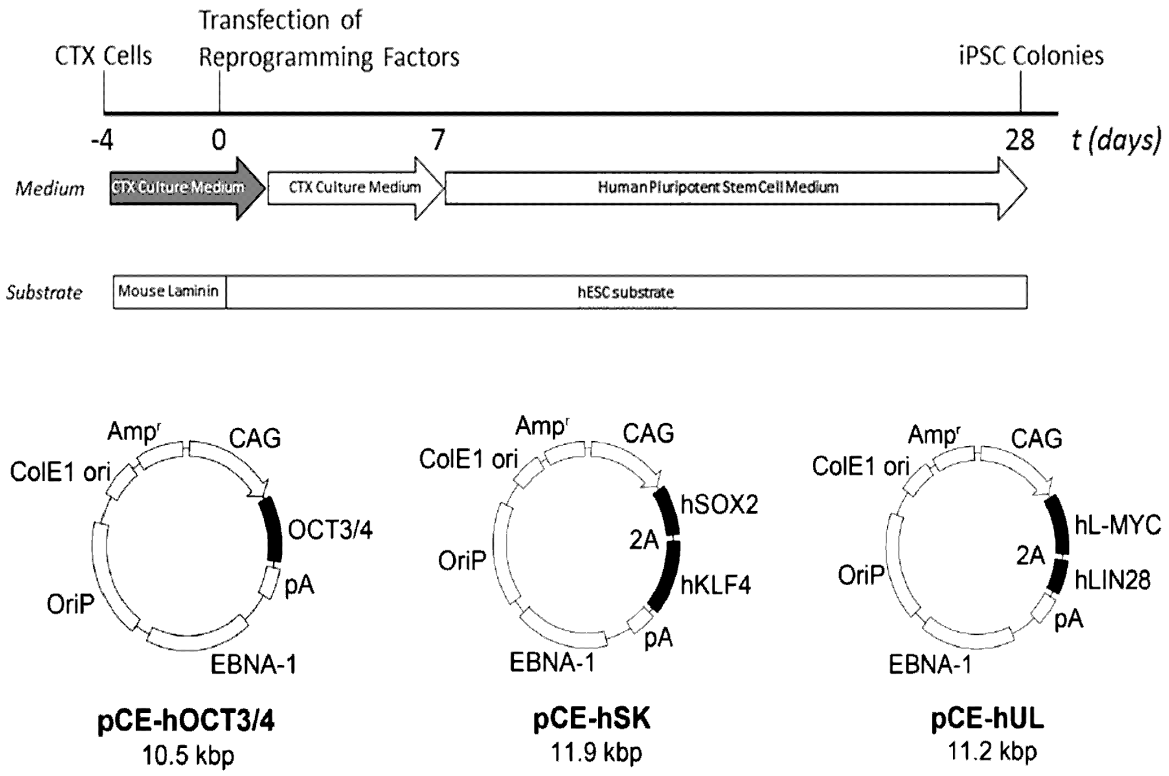


FIGURE 1B

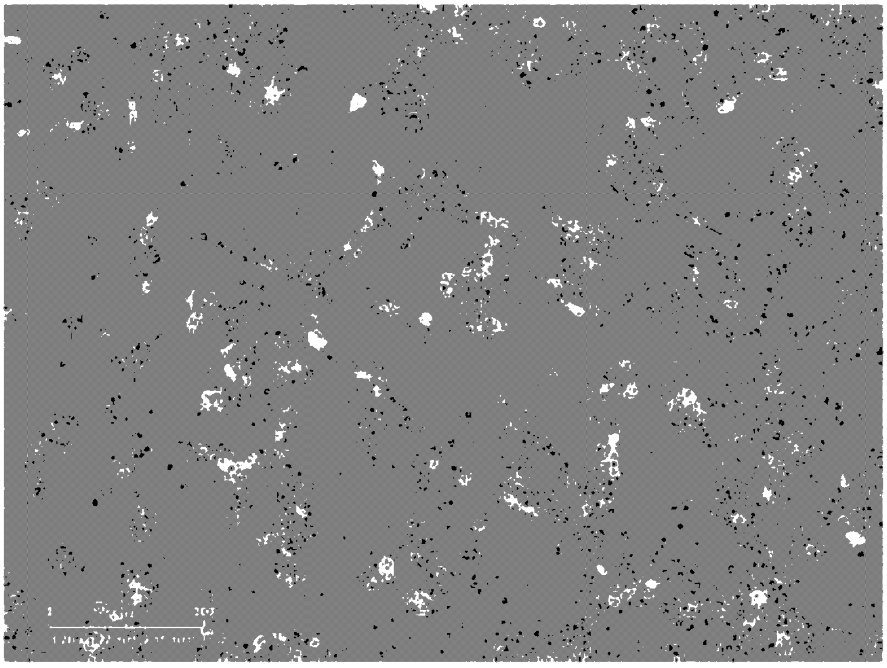


Figure 1C

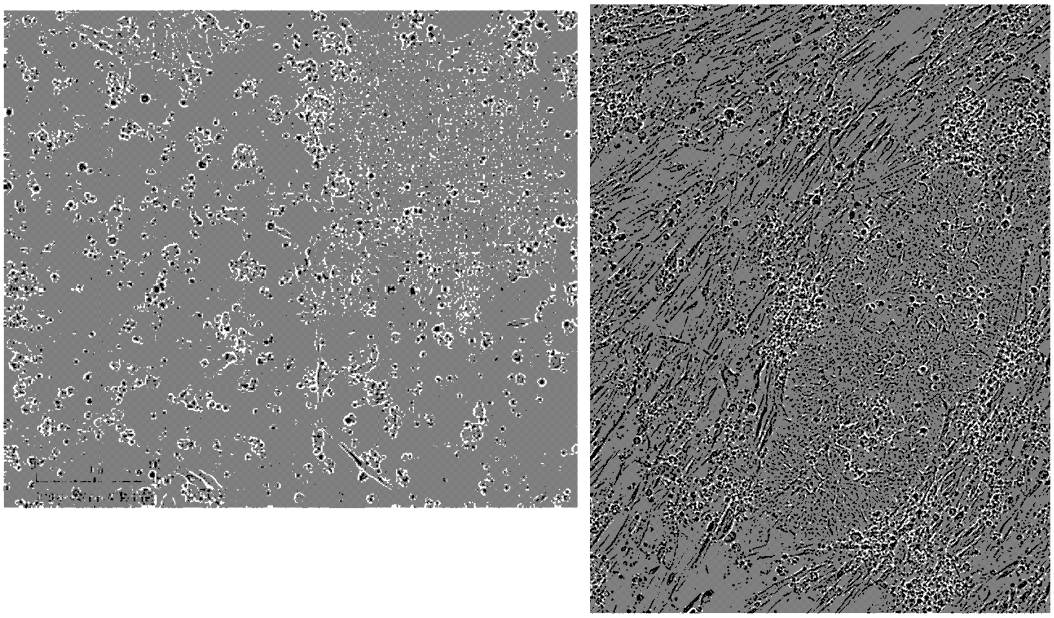


Figure 1D

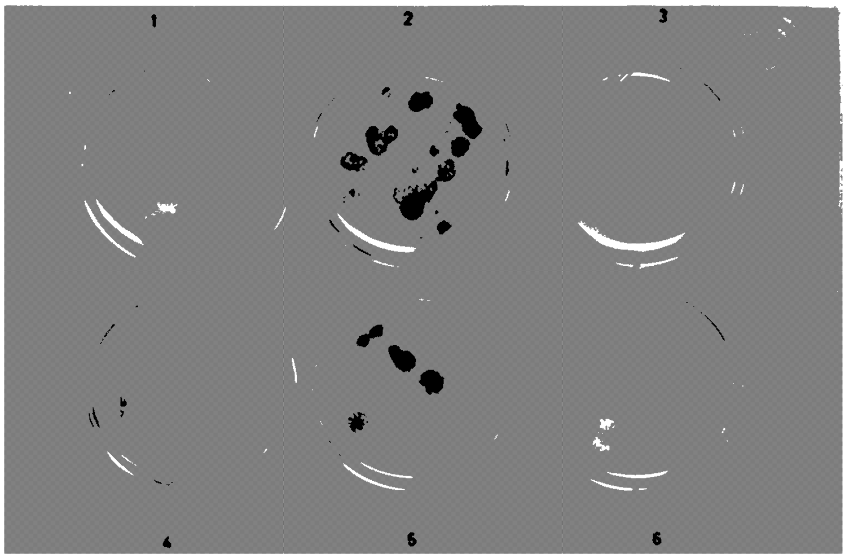
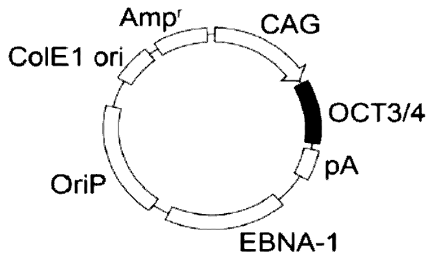
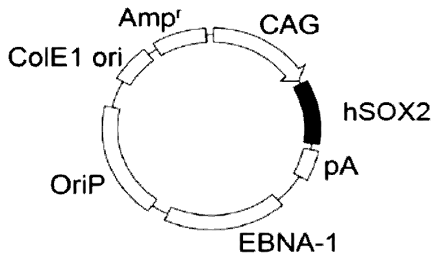


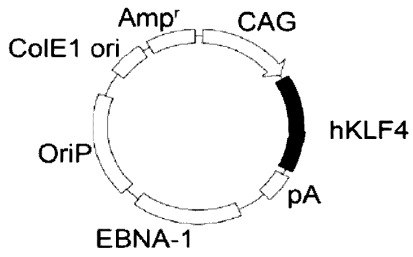
Figure 2A



pCE-hOCT3/4
10.5 kbp



pCE-hSOX2
10.3 kbp



pCE-hKLF4
10.8 kbp

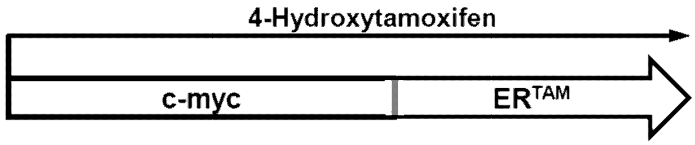


Figure 2B

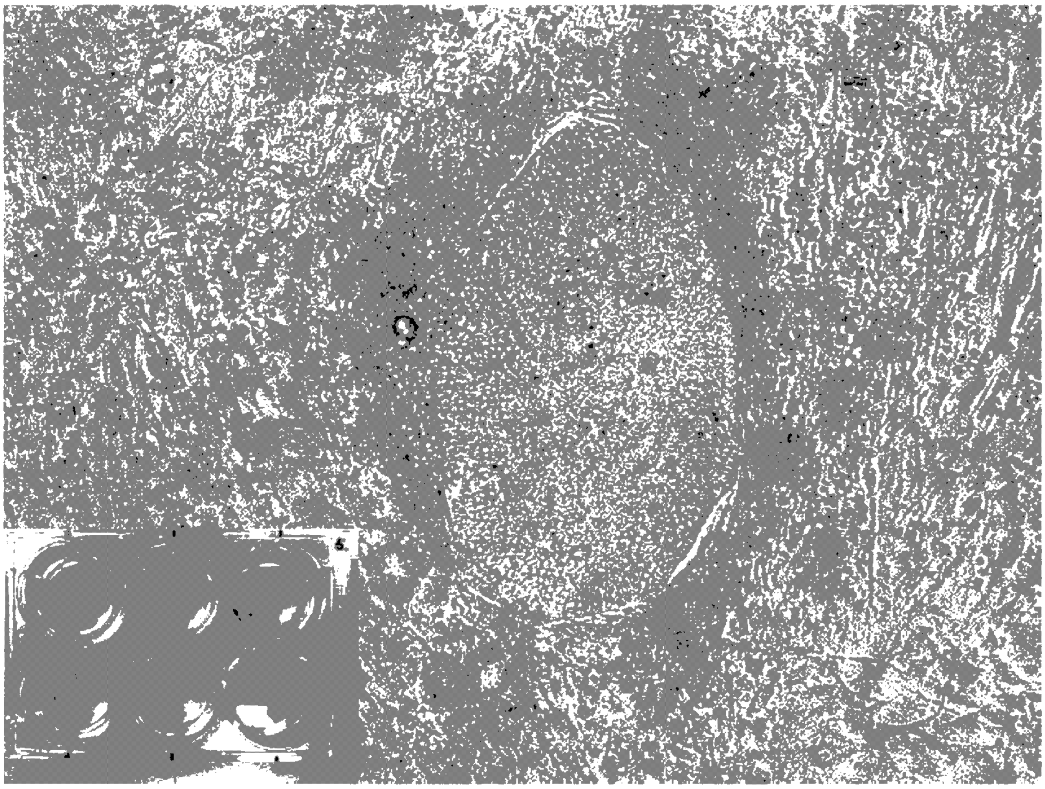


FIGURE 2C

Factors	N_{wells}	$N_{colonies}$	$Mean_{colonies}$ $well^{-1}$
(Epi5): O, S-K, M-L	1	38	38
O, S-K, M	5	43	8.6
O, S-K	5	22	4.4
O, M	5	0	0
O	4	1	0.25
S-K, M	5	0	0
S-K	5	0	0
O, S, K, M	5	0	0
O, S, K	4	3	0.75
O, S, M	5	0	0
O, S	5	5	1
O, K, M	5	0	0
O, K	5	0	0

FIGURE 2D

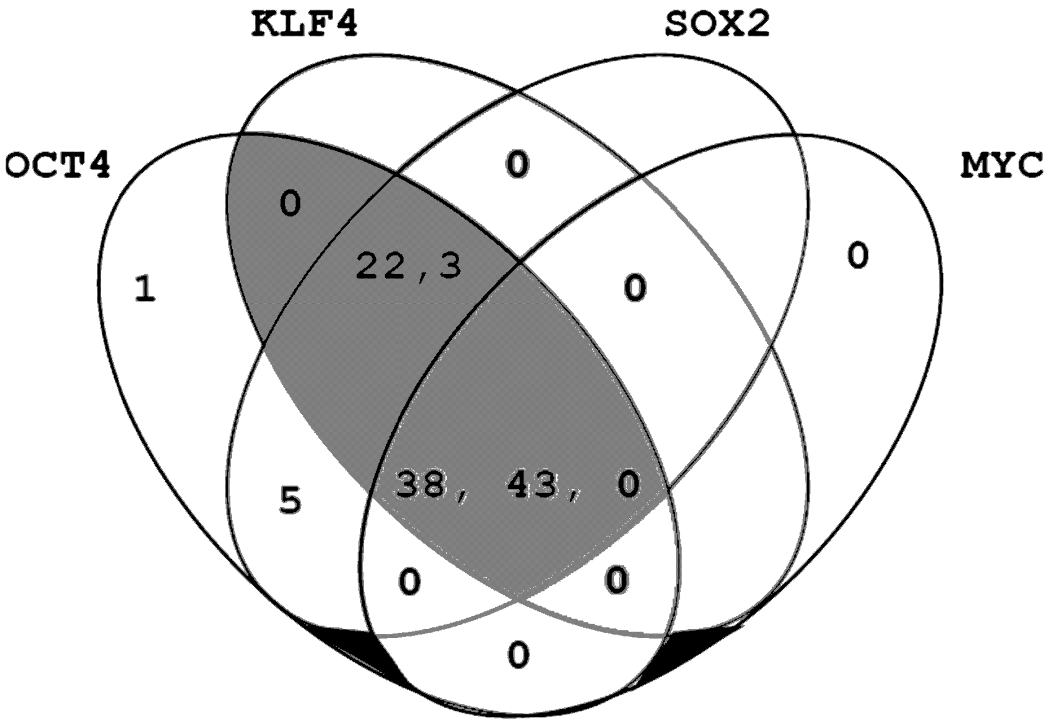


FIGURE 3A

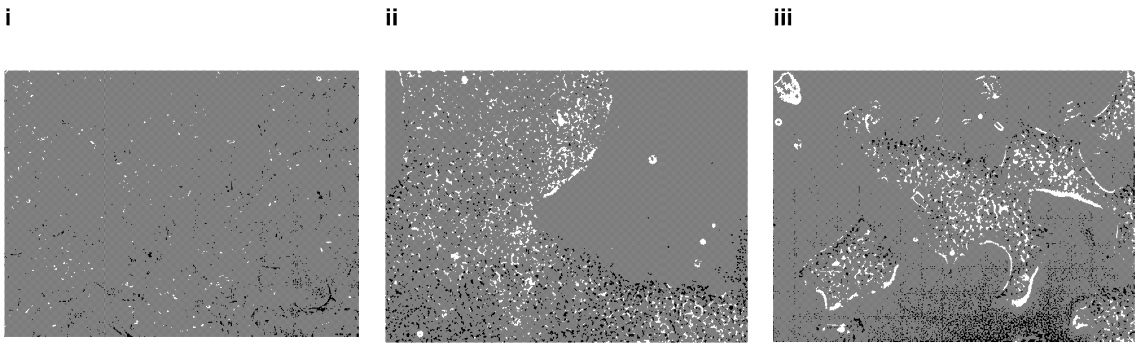


FIGURE 3B

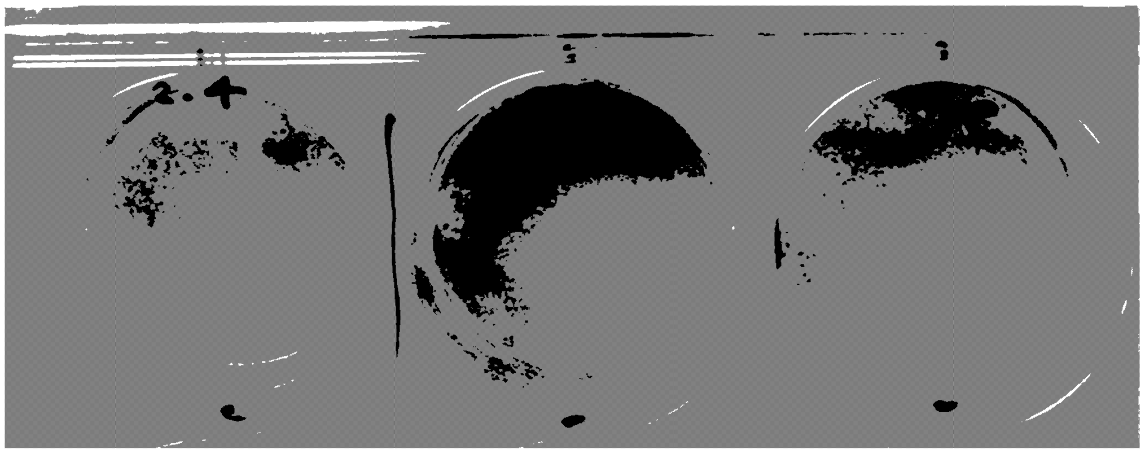


Figure 3C

(i)

Line	Marker	%+ve
CTX-iPSC-1.1	SSEA-1	2.31
	OCT4	87.02
	SSEA-4	98.2
	TRA-1-60	90.9
CTX-iPSC-1.3	SSEA-1	11.24
	OCT4	68.86
	SSEA-4	96.92
	TRA-1-60	61.87
CTX-iPSC-1.4	SSEA-1	1.11
	OCT4	77.24
	SSEA-4	99.6
	TRA-1-60	92.4
CTX-iPSC-1.5	SSEA-1	26.16
	OCT4	65.47
	SSEA-4	98.19
	TRA-1-60	41.63
CTX-iPSC-1.6	SSEA-1	1.7
	OCT4	88.79
	SSEA-4	99.46
	TRA-1-60	55.13
CTX-iPSC-vx1	SSEA-1	47.37
	OCT4	16.44
	SSEA-4	65.24
	TRA-1-60	12.26

(ii)

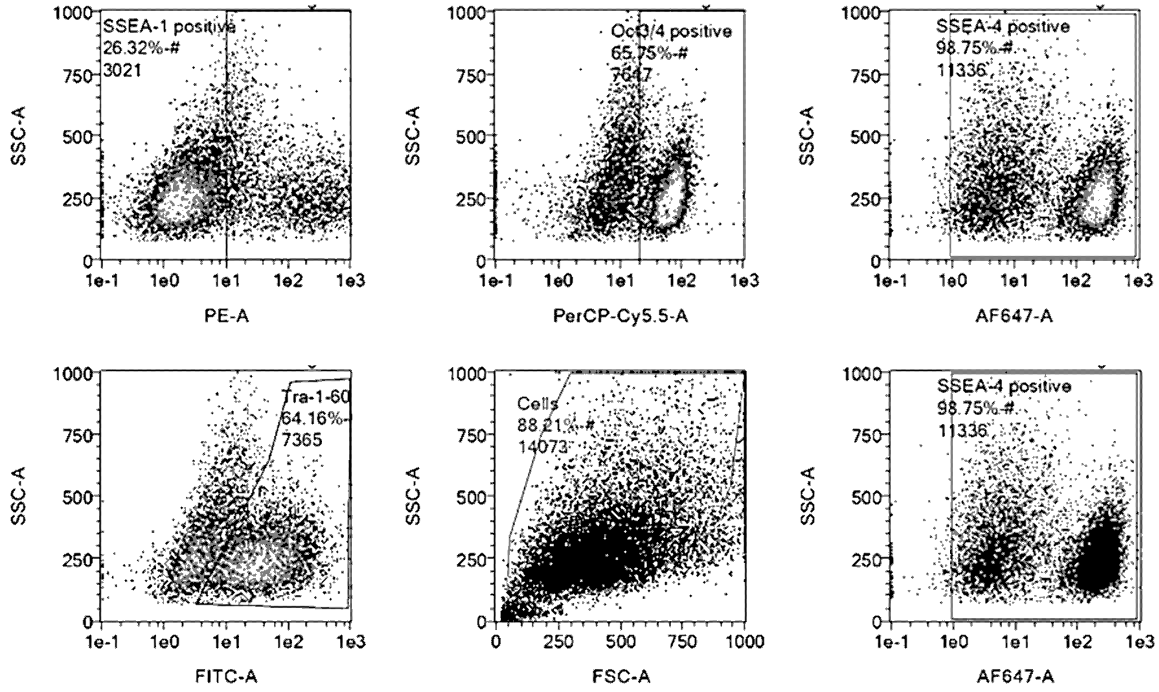
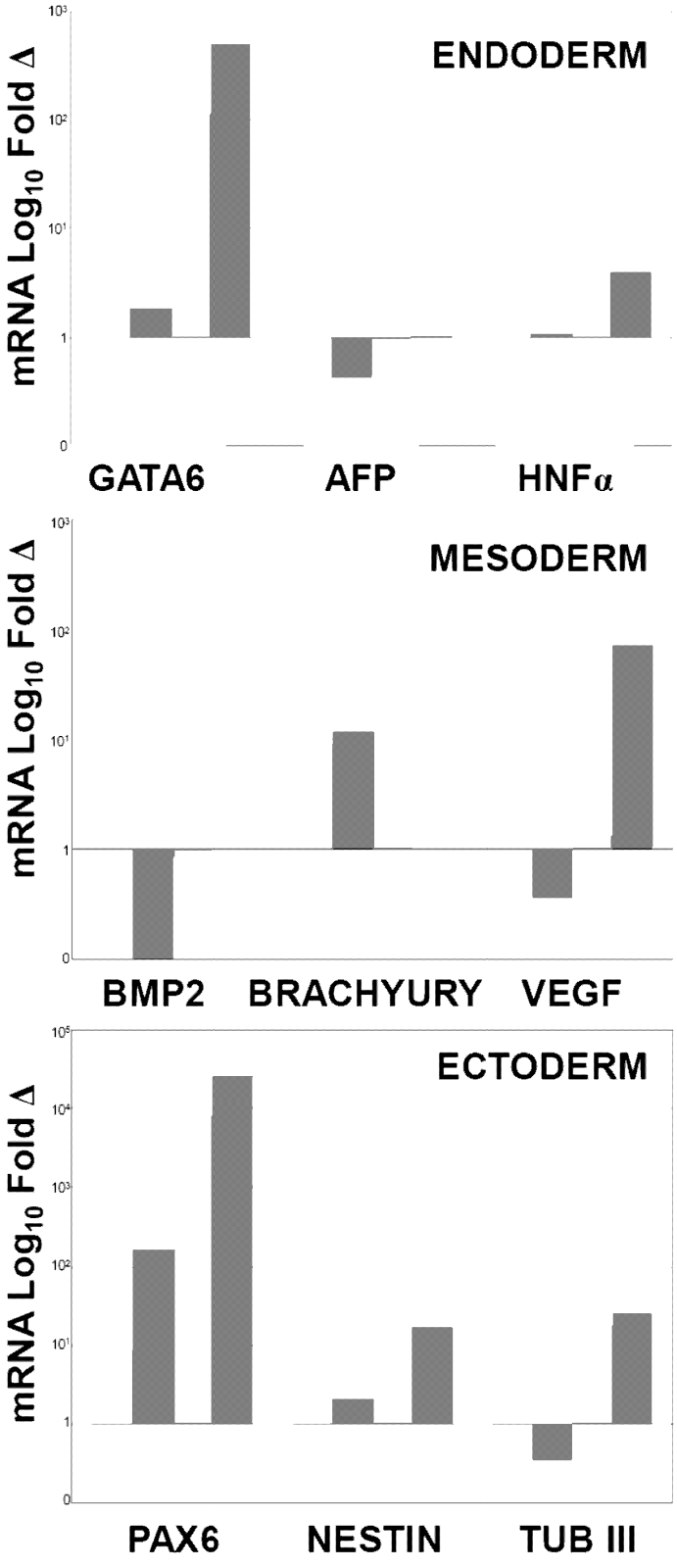


Figure 3D


ENDODERM
mRNA Log10 Fold Δ
GATA6
AFP
HNFα
MESODERM
mRNA Log10 Fold Δ
BMP2
BRACHYURY
VEGF
ECTODERM
mRNA Log10 Fold Δ
PAX6
NESTIN
TUB III

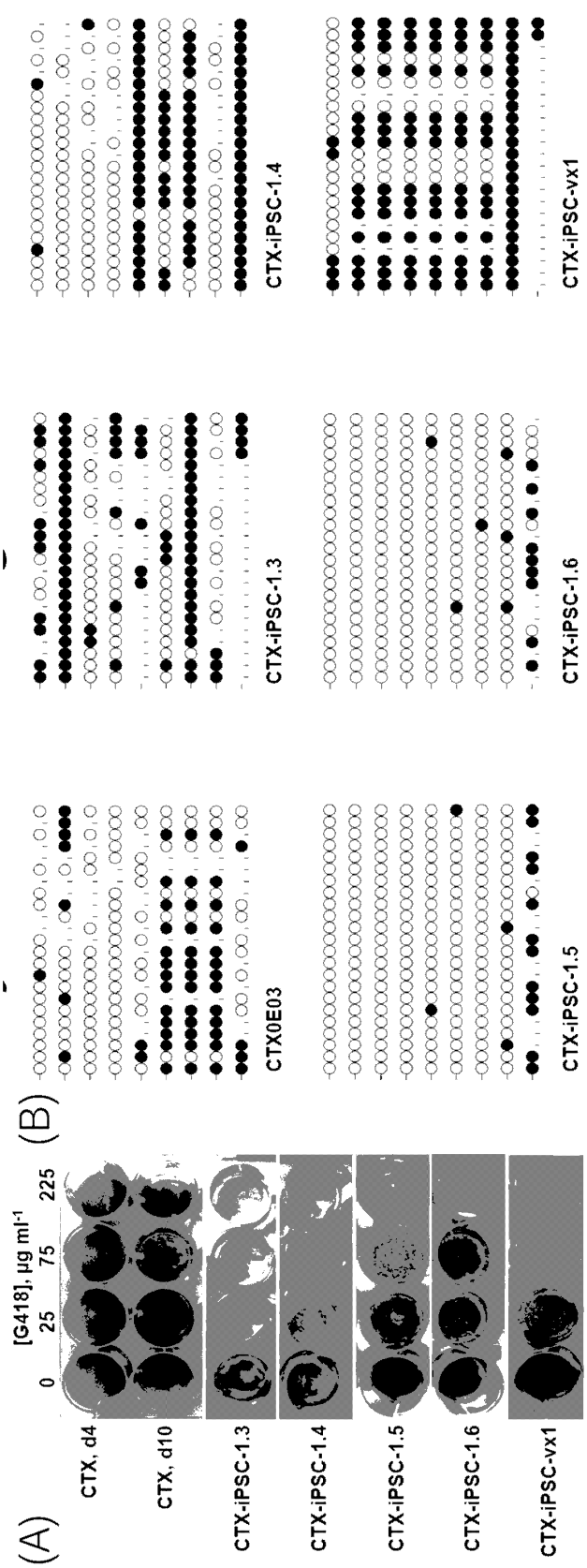

(A)
[G418], µg ml-1
0
25
75
225
CTX, d4
CTX, d10
CTX-iPSC-1.3
CTX-iPSC-1.4
CTX-iPSC-1.5
CTX-iPSC-1.6
CTX-iPSC-vx1
(B)
CTX0E03
CTX-iPSC-1.5
CTX-iPSC-1.3
CTX-iPSC-1.6
CTX-iPSC-1.4
CTX-iPSC-vx1

Figure 4

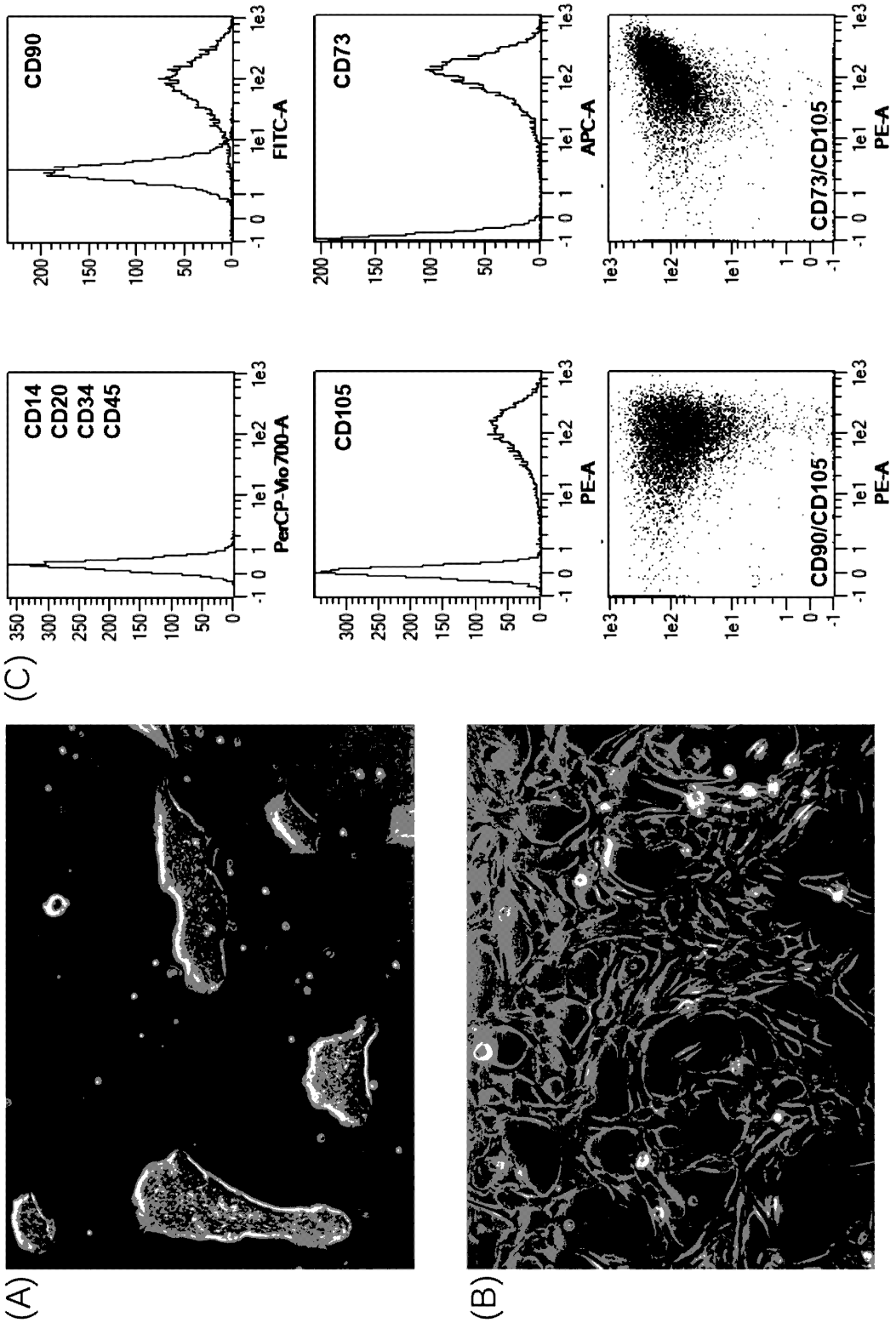

(A)
(B)
(C)
CD14
CD20
CD34
CD45
PerCP-Vio700-A
CD105
PE-A
CD90/CD105
PE-A
CD90
FITC-A
CD73
APC-A
CD73/CD105
PE-A

Figure 5

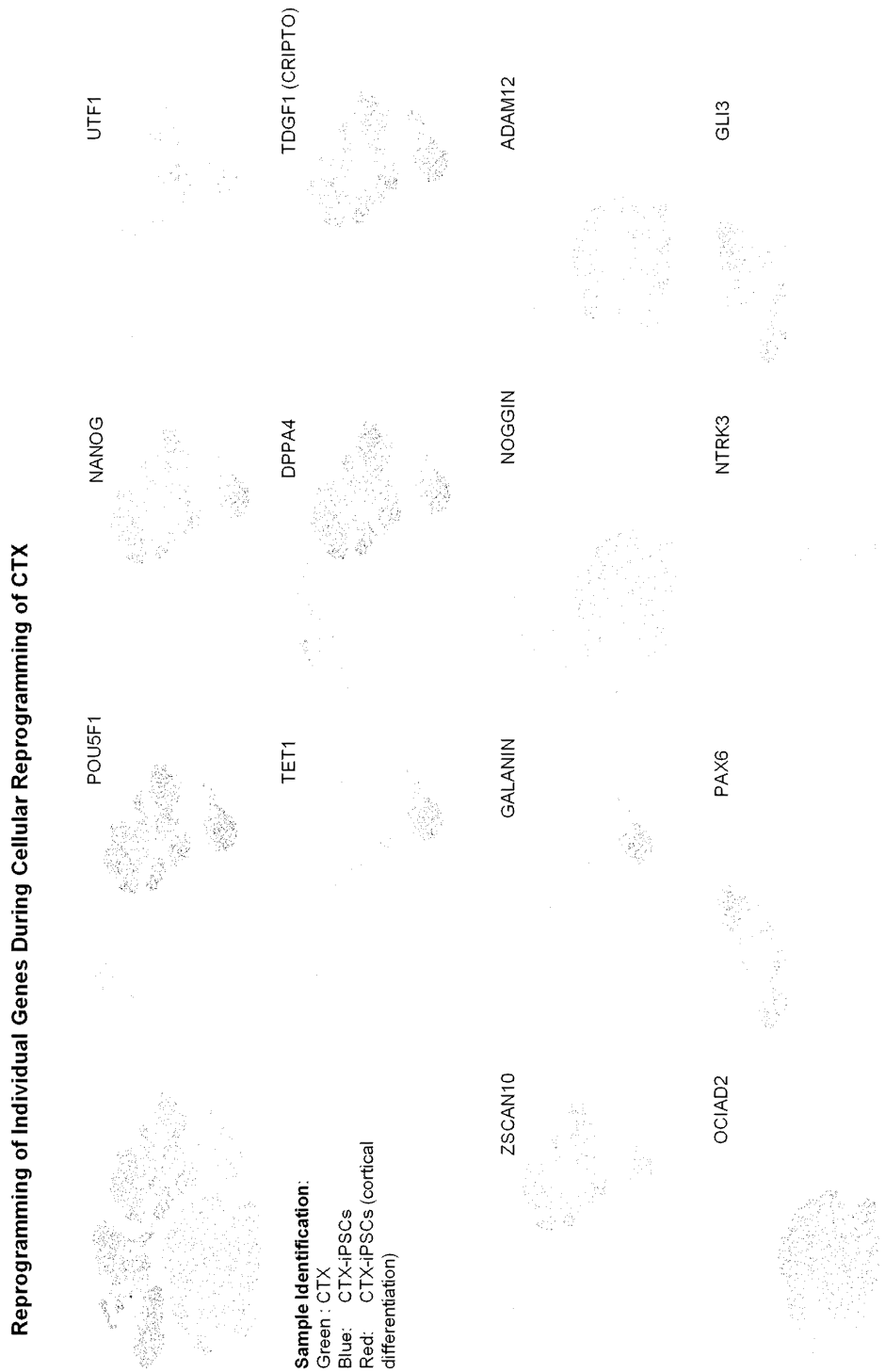

Reprogramming of Individual Genes During Cellular Reprogramming of CTX
POU5F1
NANOG
UTF1
TDGF1 (CRIPTO)
TET1
DPPA4
GALANIN
NOGGIN
ADAM12
ZSCAN10
PAX6
NTRK3
GLI3
OCIAD2
Sample Identification:
Green : CTX
Blue: CTX-iPSCs
Red: CTX-iPSCs (cortical differentiation)

FIGURE 6

CTX-iPSCs: Lineage Potency

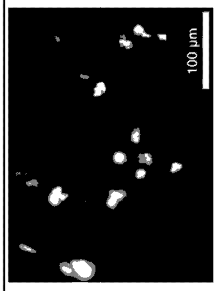
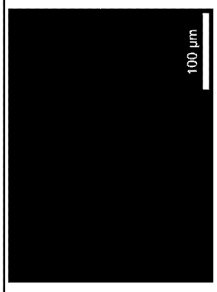
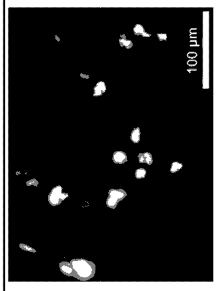
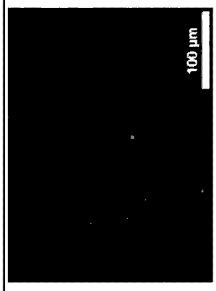
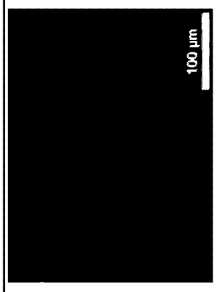
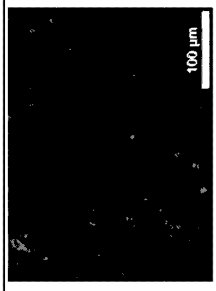
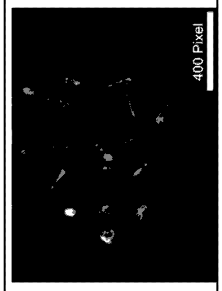
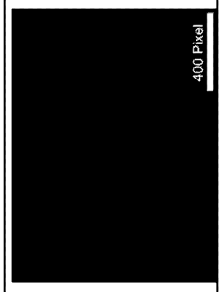
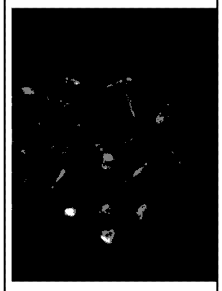
	Green	Red	Merge
Endoderm (*Green: SOX17 Red: FOXA2*)			
Mesoderm (*Green: Brachyury Red: CXCR4*)			
Ectoderm (*Green: Nestin Red: PAX6*)			

FIGURE 7

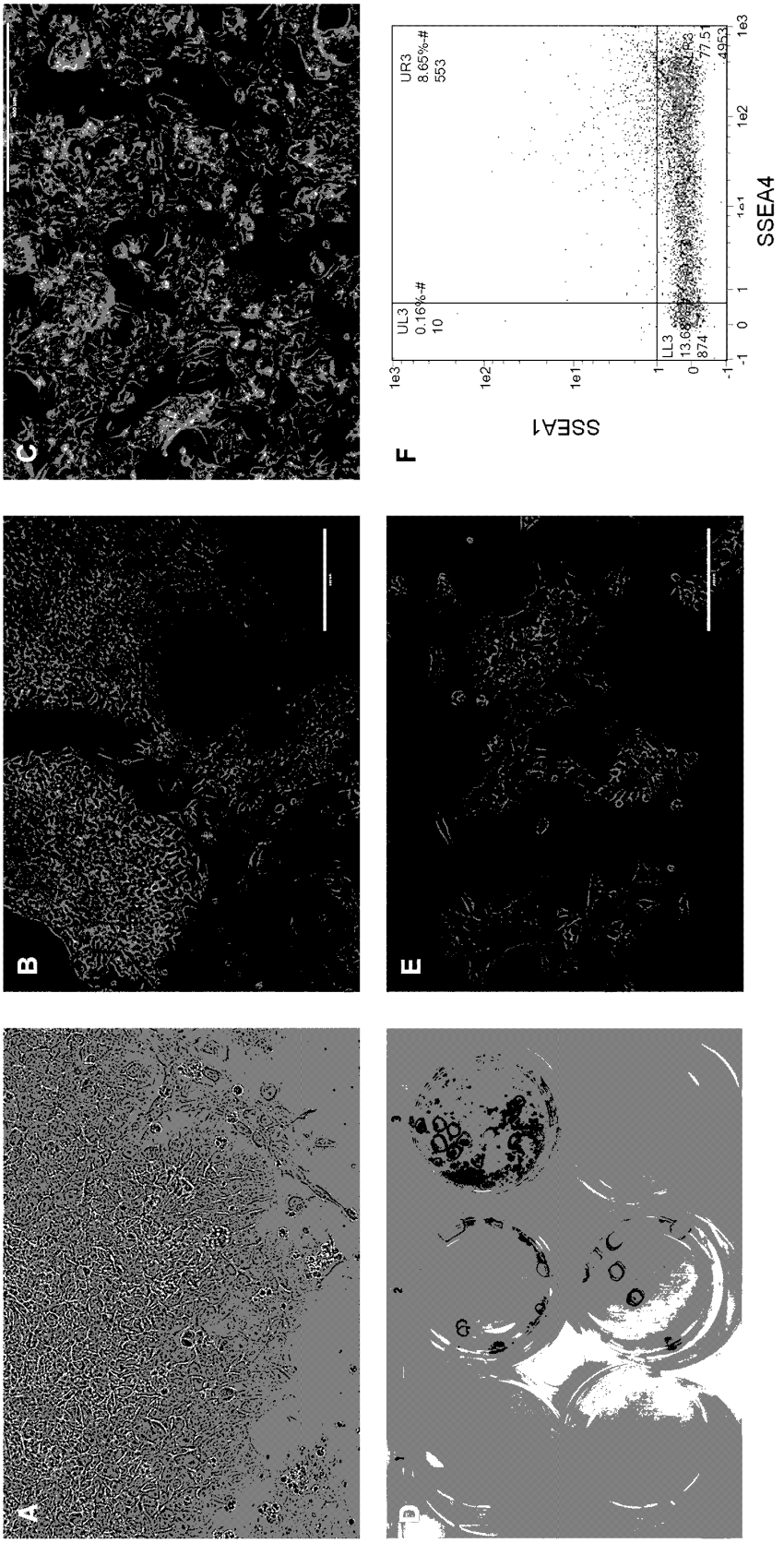

A
B
C
D
E
F
SSEA1
SSEA4
UL3
0.16%-#
10
UR3
8.65%-#
553
LL3
13.68
874
77.51
4953

FIGURE 8

STR0C05-iPSCs: Lineage Potency

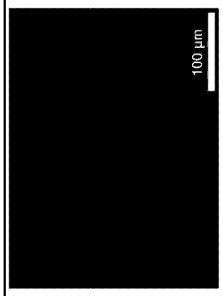
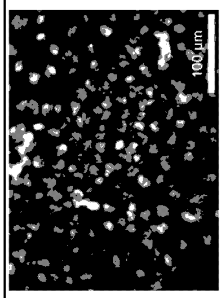
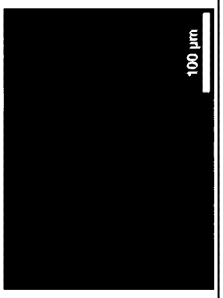
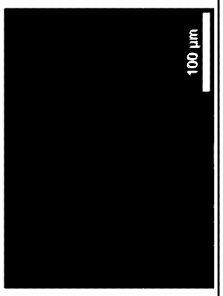
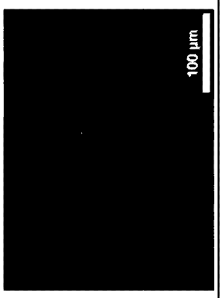
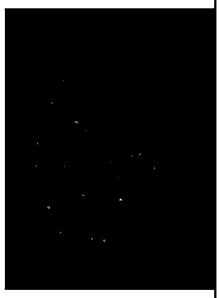
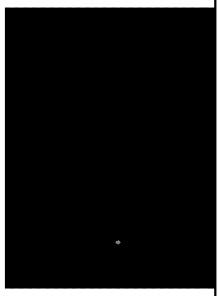
	Green	Red	Merge
Endoderm (*Green: SOX17 Red: FOXA2*)			
Mesoderm (*Green: Brachyury Red: CXCR4*)			
Ectoderm (*Green: Nestin Red: PAX6*)			

FIGURE 9

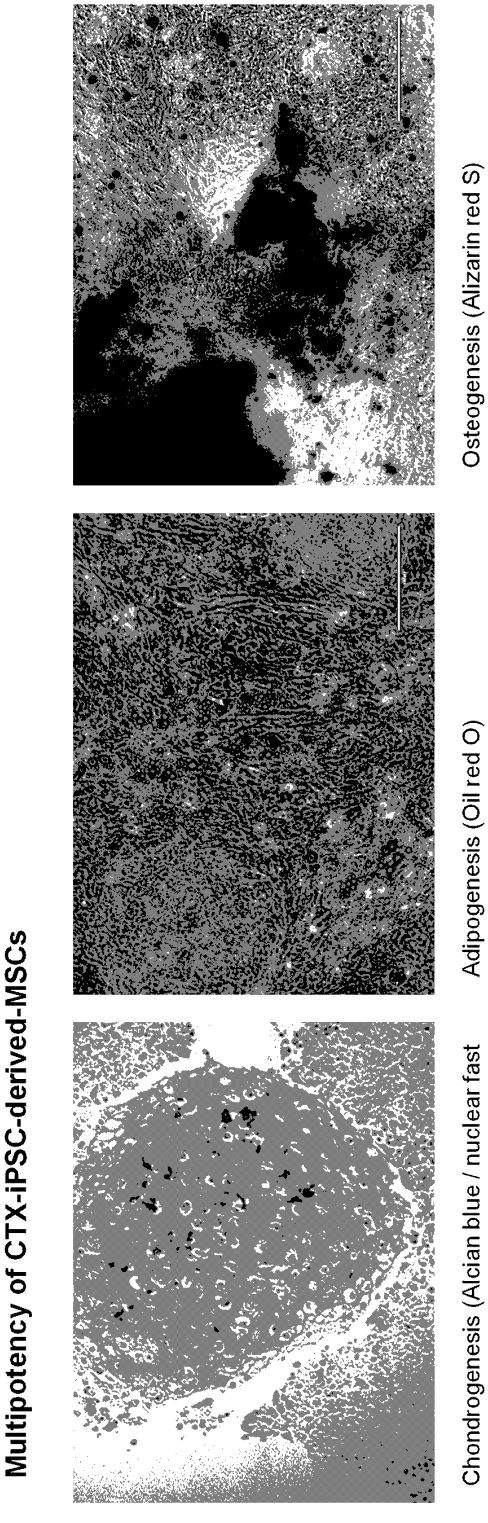

Multipotency of CTX-iPSC-derived-MSCs
Chondrogenesis (Alcian blue / nuclear fast red)
Adipogenesis (Oil red O)
Osteogenesis (Alizarin red S)

FIGURE 10

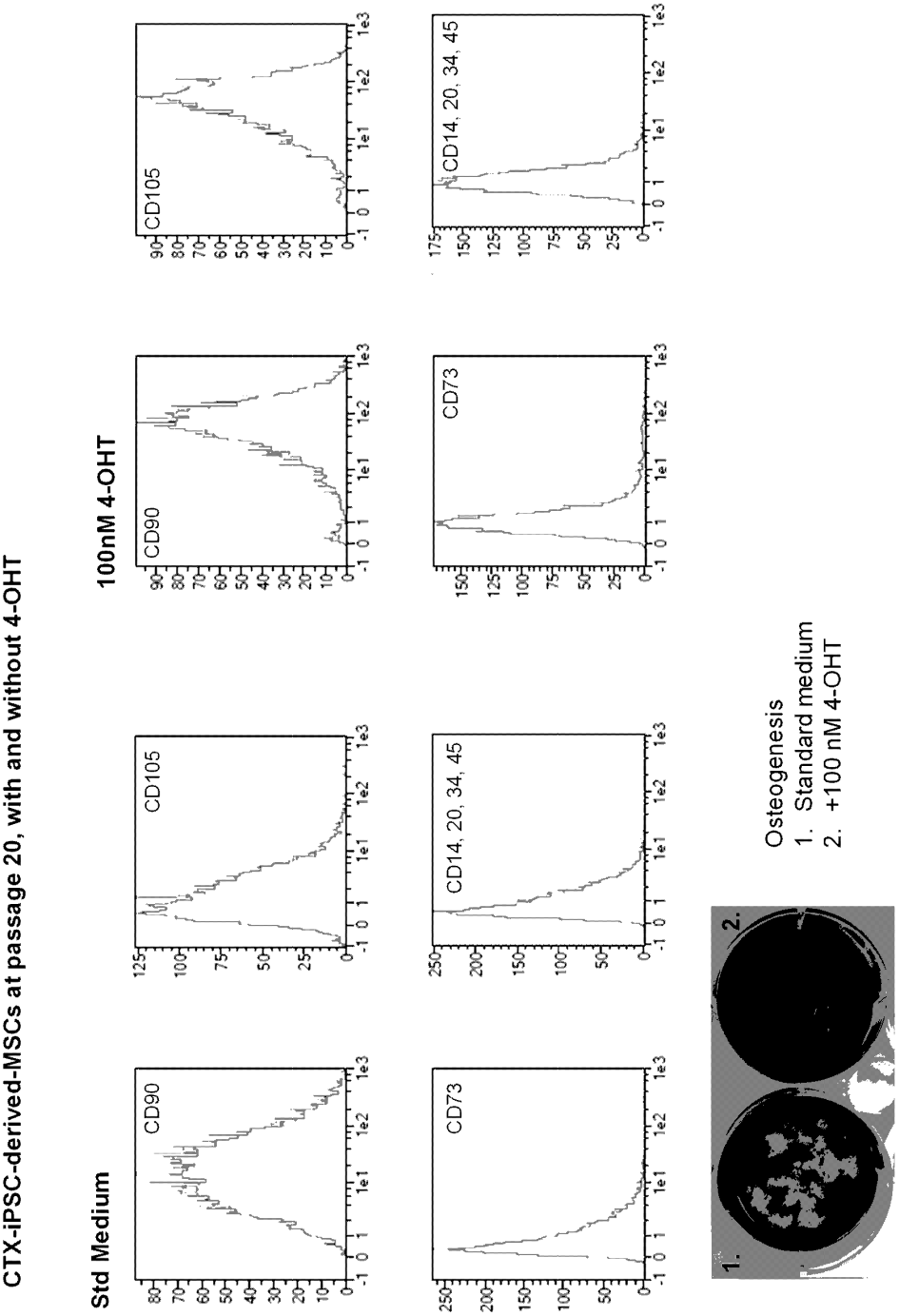

CTX-iPSC-derived-MSCs at passage 20, with and without 4-OHT
Std Medium
100nM 4-OHT
CD90
CD105
CD73
CD14, 20, 34, 45
Osteogenesis
1. Standard medium
2. +100 nM 4-OHT
1.
2.

FIGURE 11

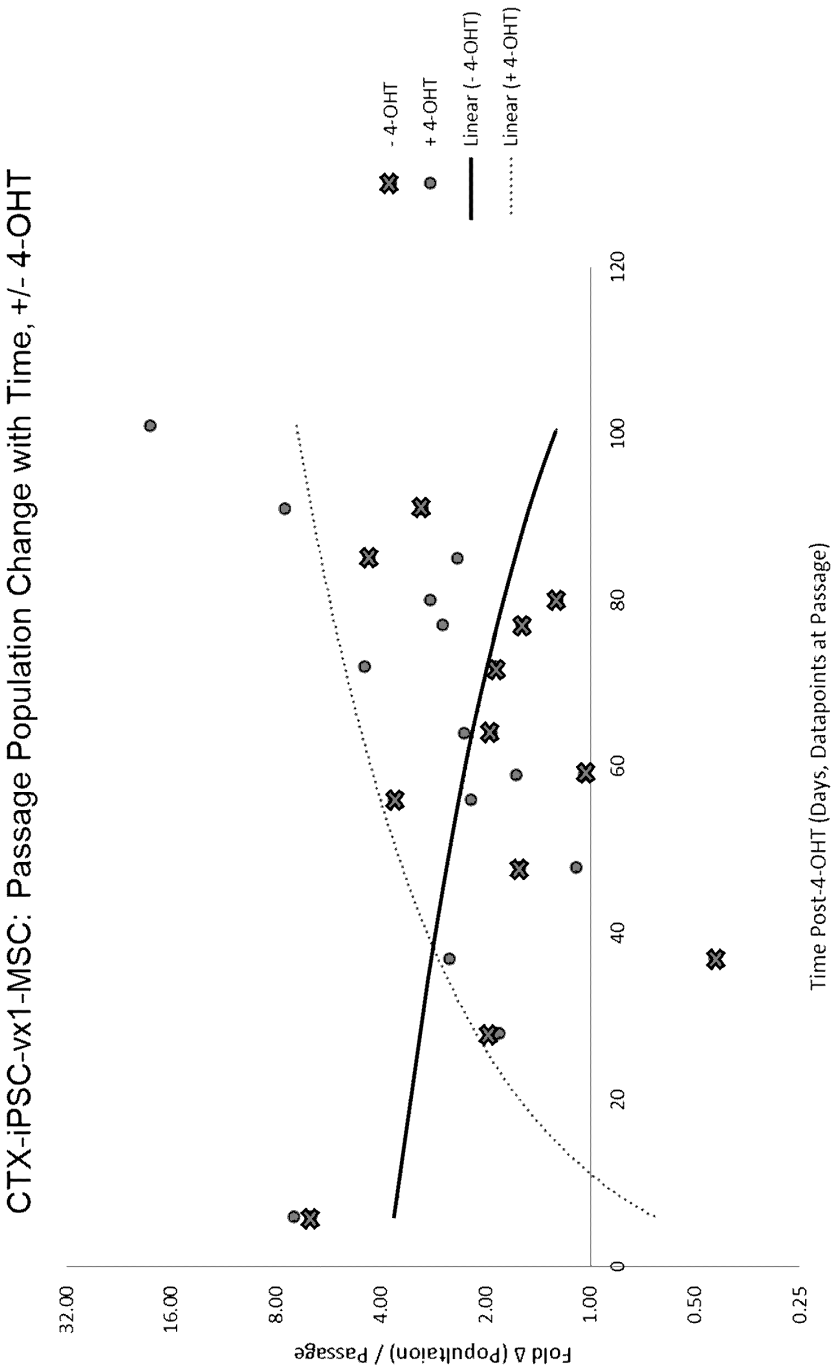

CTX-iPSC-vx1-MSC: Passage Population Change with Time, +/- 4-OHT
- 4-OHT
+ 4-OHT
Linear (- 4-OHT)
Linear (+ 4-OHT)
Fold Δ (Population) / Passage
32.00
16.00
8.00
4.00
2.00
1.00
0.50
0.25
0
20
40
60
80
100
120
Time Post-4-OHT (Days, Datapoints at Passage)

FIGURE 12

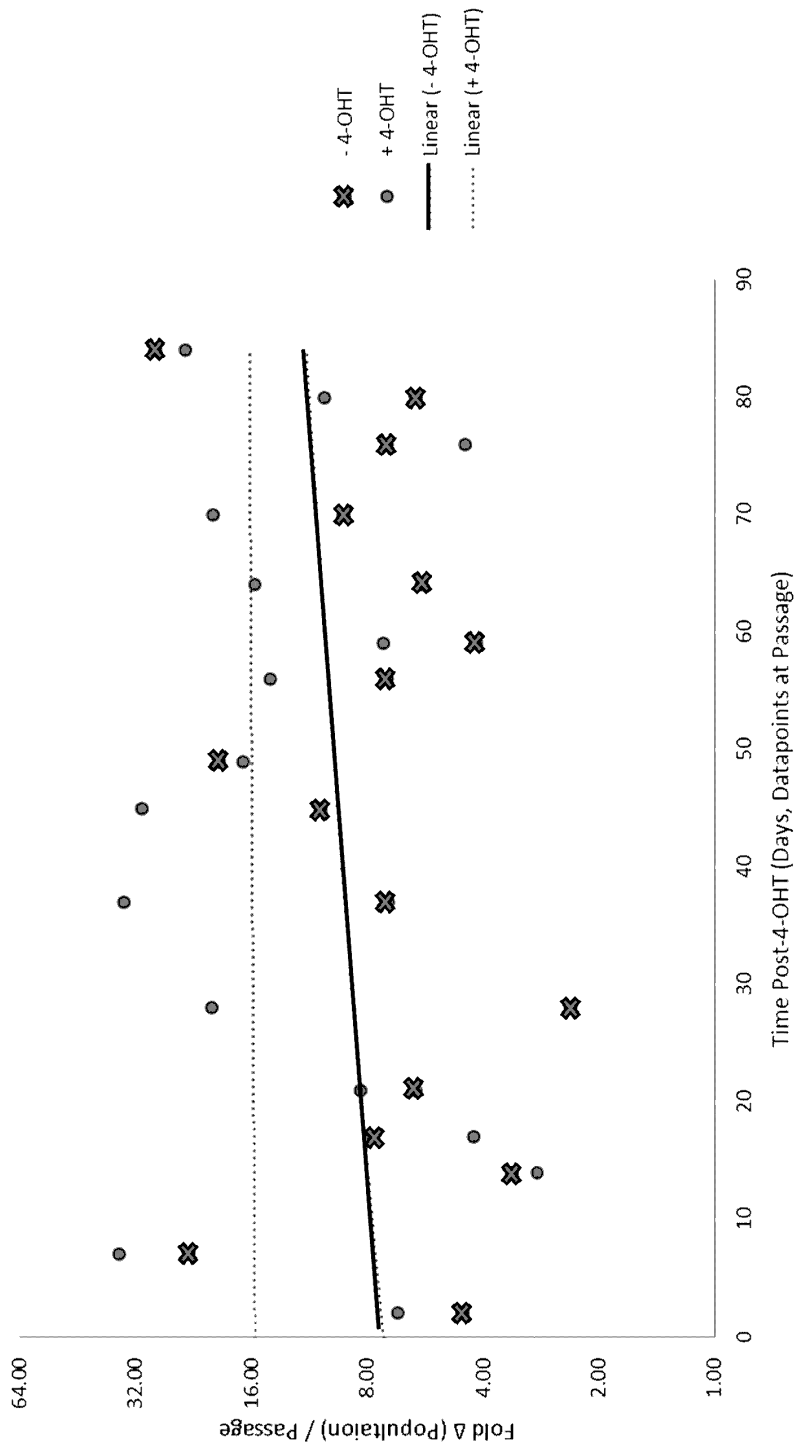

CTX-iPSC-1.6
-MSC: Passage Population Change with Time, +/- 4-OHT
- 4-OHT
+ 4-OHT
Linear (- 4-OHT)
Linear (+ 4-OHT)
Fold Δ (Population) / Passage
64.00
32.00
16.00
8.00
4.00
2.00
1.00
0
10
20
30
40
50
60
70
80
90
Time Post-4-OHT (Days, Datapoints at Passage)

FIGURE 13

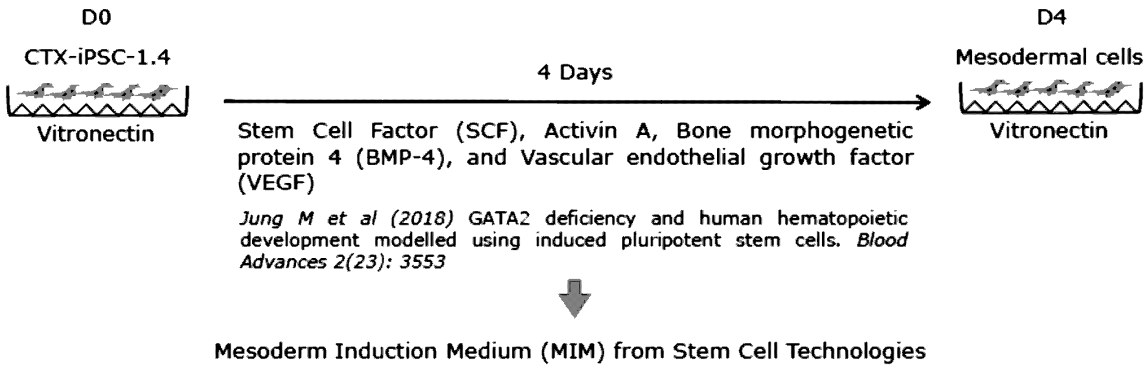


Figure 14A

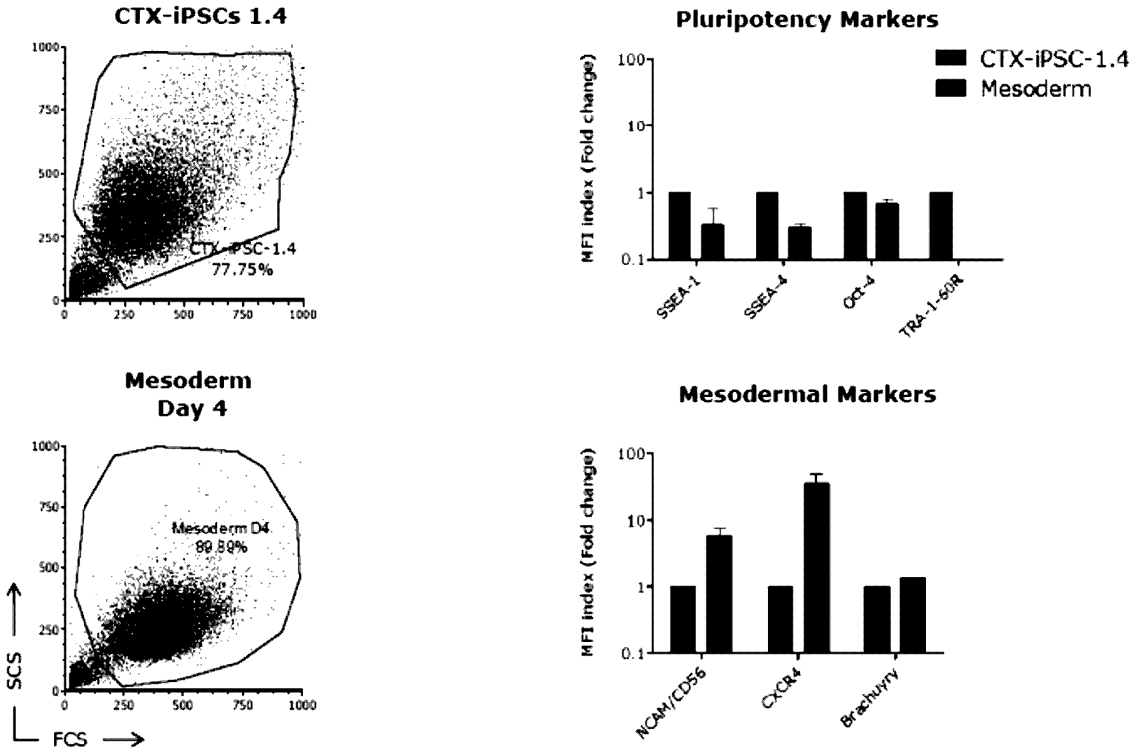
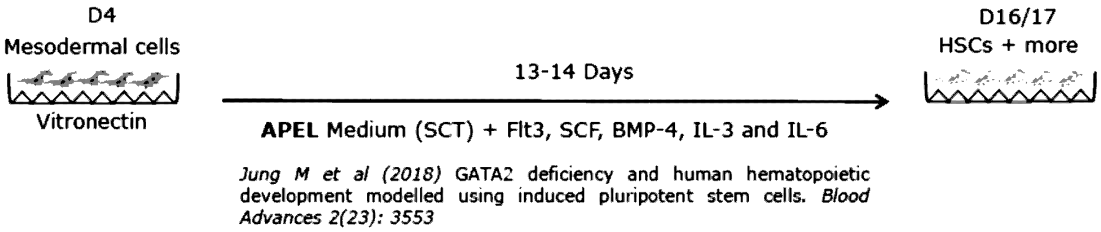


Figure 14B



Jung M et al (2018) GATA2 deficiency and human hematopoietic development modelled using induced pluripotent stem cells. *Blood Advances 2(23): 3553*

Figure 15A

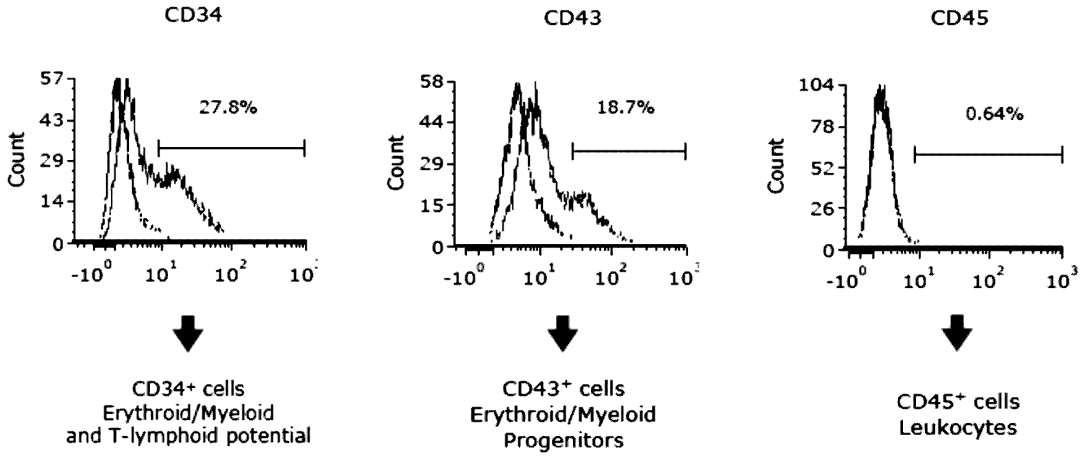


Figure 15B

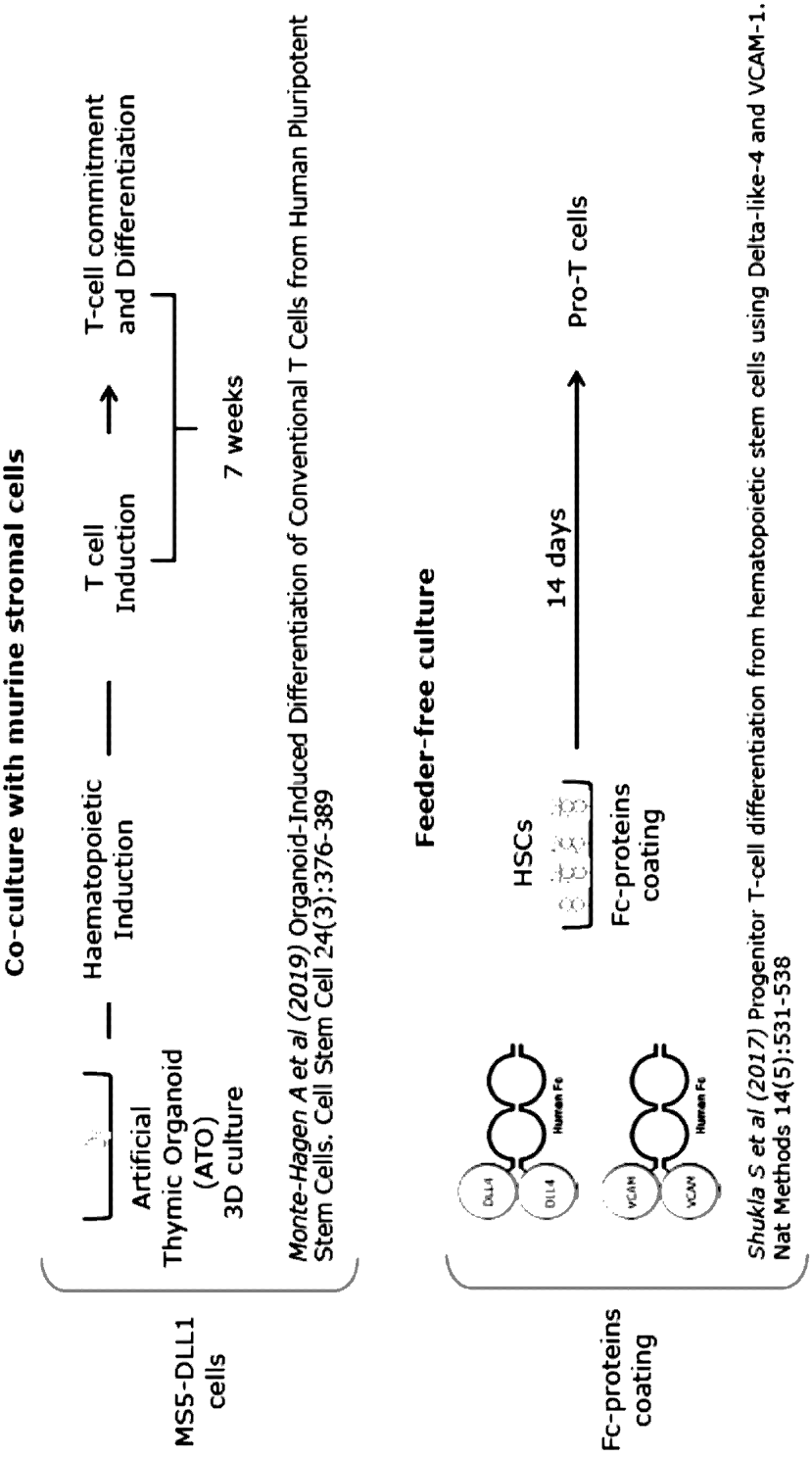

Co-culture with murine stromal cells
MS5-DLL1 cells
Artificial Thymic Organoid (ATO) 3D culture
Haematopoietic Induction
T cell Induction
T-cell commitment and Differentiation
7 weeks
Monte-Hagen A et al (2019) Organoid-Induced Differentiation of Conventional T Cells from Human Pluripotent Stem Cells. Cell Stem Cell 24(3):376-389
Feeder-free culture
Fc-proteins coating
HSCs
Fc-proteins coating
14 days
Pro-T cells
DLL4
Human Fc
VCAM
Shukla S et al (2017) Progenitor T-cell differentiation from hematopoietic stem cells using Delta-like-4 and VCAM-1. Nat Methods 14(5):531-538

Figure 16

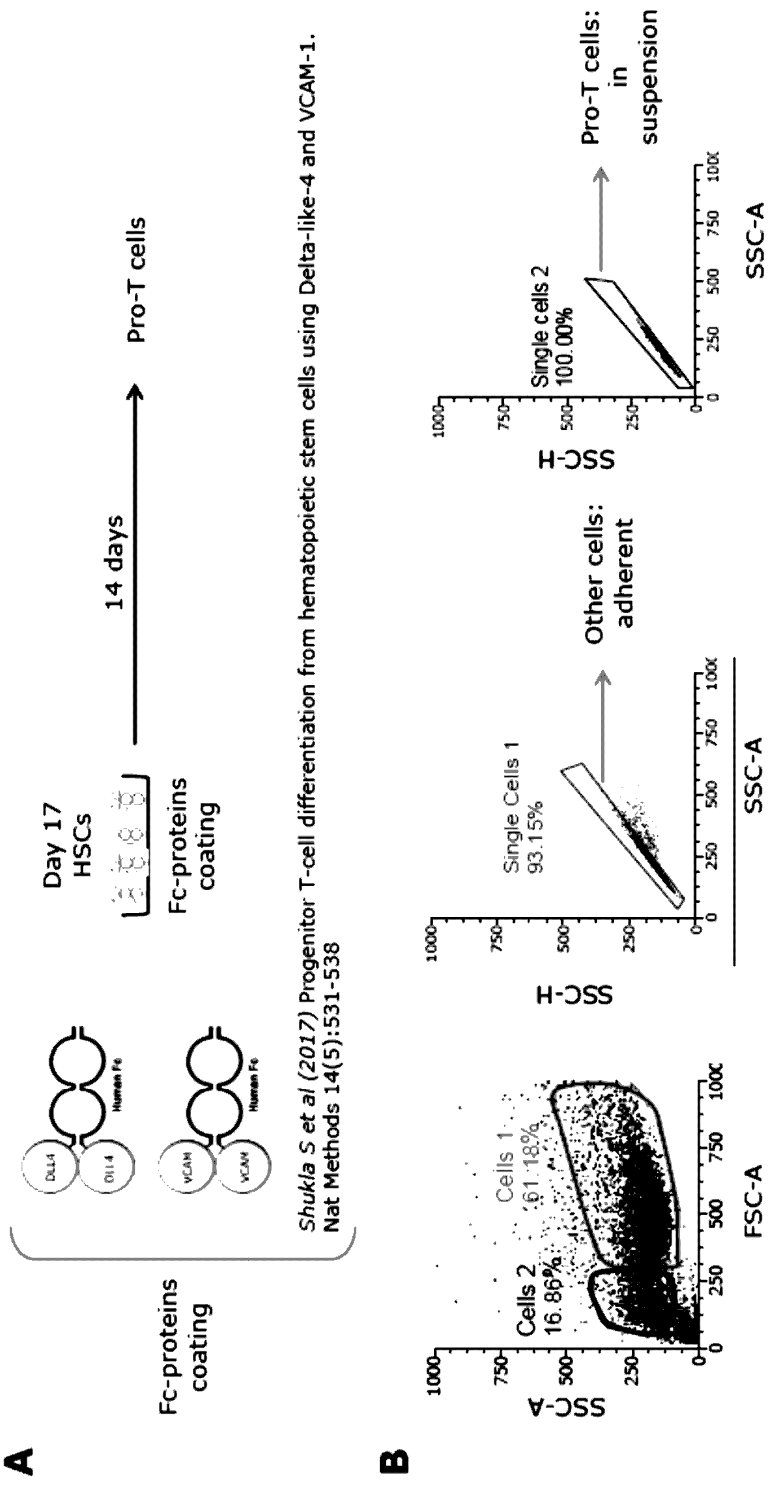

A
Fc-proteins coating
DLL4
DLL4
Human Fc
VCAM
VCAM
Human Fc
Day 17 HSCs
Fc-proteins coating
14 days
Pro-T cells
Shukla S et al (2017) Progenitor T-cell differentiation from hematopoietic stem cells using Delta-like-4 and VCAM-1. Nat Methods 14(5):531-538
B
SSC-A
FSC-A
Cells 1
61.18%
Cells 2
16.86%
SSC-H
SSC-A
Single Cells 1
93.15%
Other cells: adherent
SSC-H
SSC-A
Single cells 2
100.00%
Pro-T cells: in suspension

Figure 17

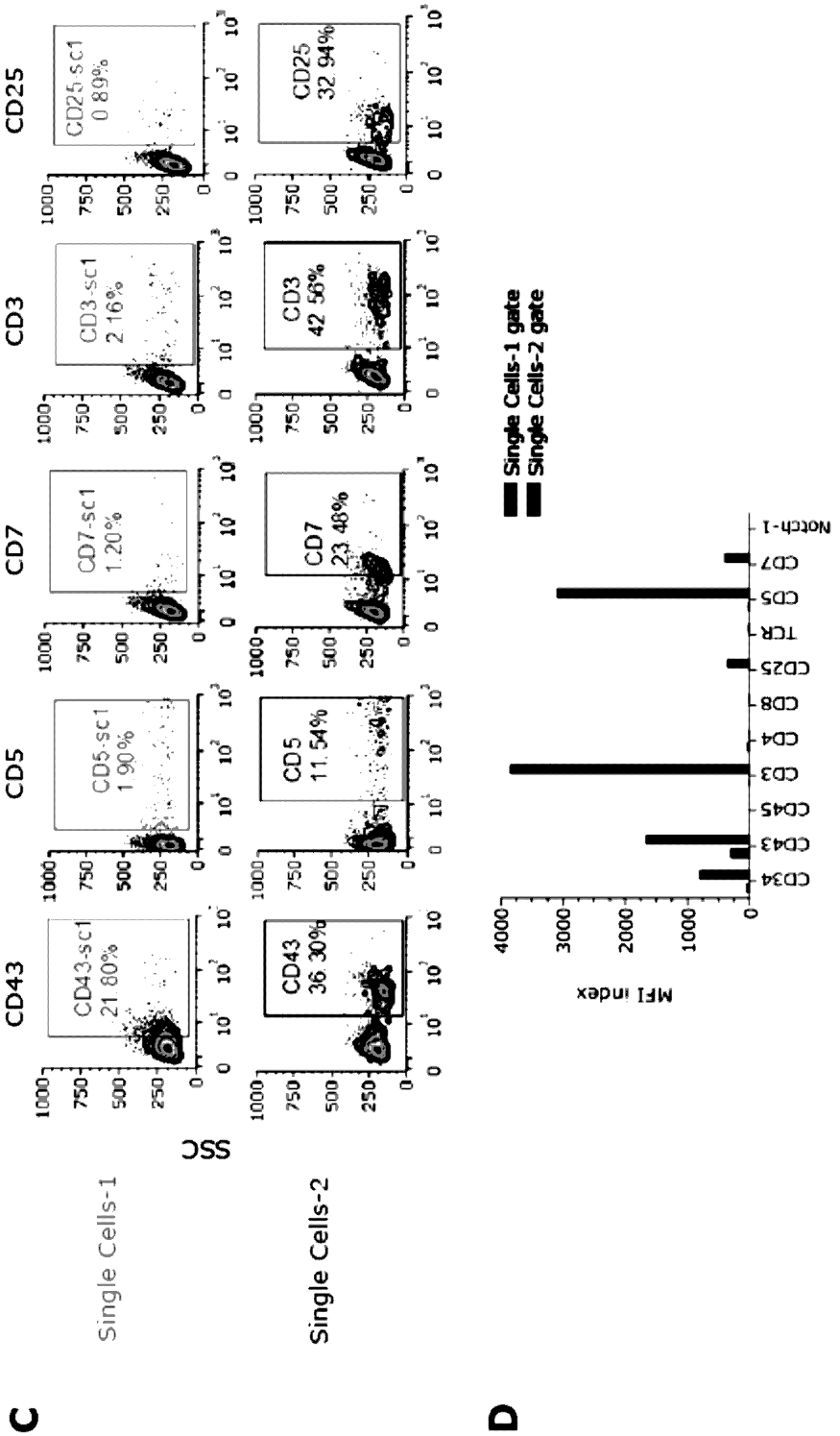

C
D
CD43
CD5
CD7
CD3
CD25
SSC
Single Cells-1
Single Cells-2
CD43-sc1 21.80%
CD5-sc1 1.90%
CD7-sc1 1.20%
CD3-sc1 2.16%
CD25-sc1 0.89%
CD43 36.30%
CD5 11.54%
CD7 23.48%
CD3 42.56%
CD25 32.94%
Single Cells-1 gate
Single Cells-2 gate
MFI index
CD34
CD43
CD45
CD3
CD4
CD8
CD25
TCR
CD5
CD7
Notch-1

Figure 17

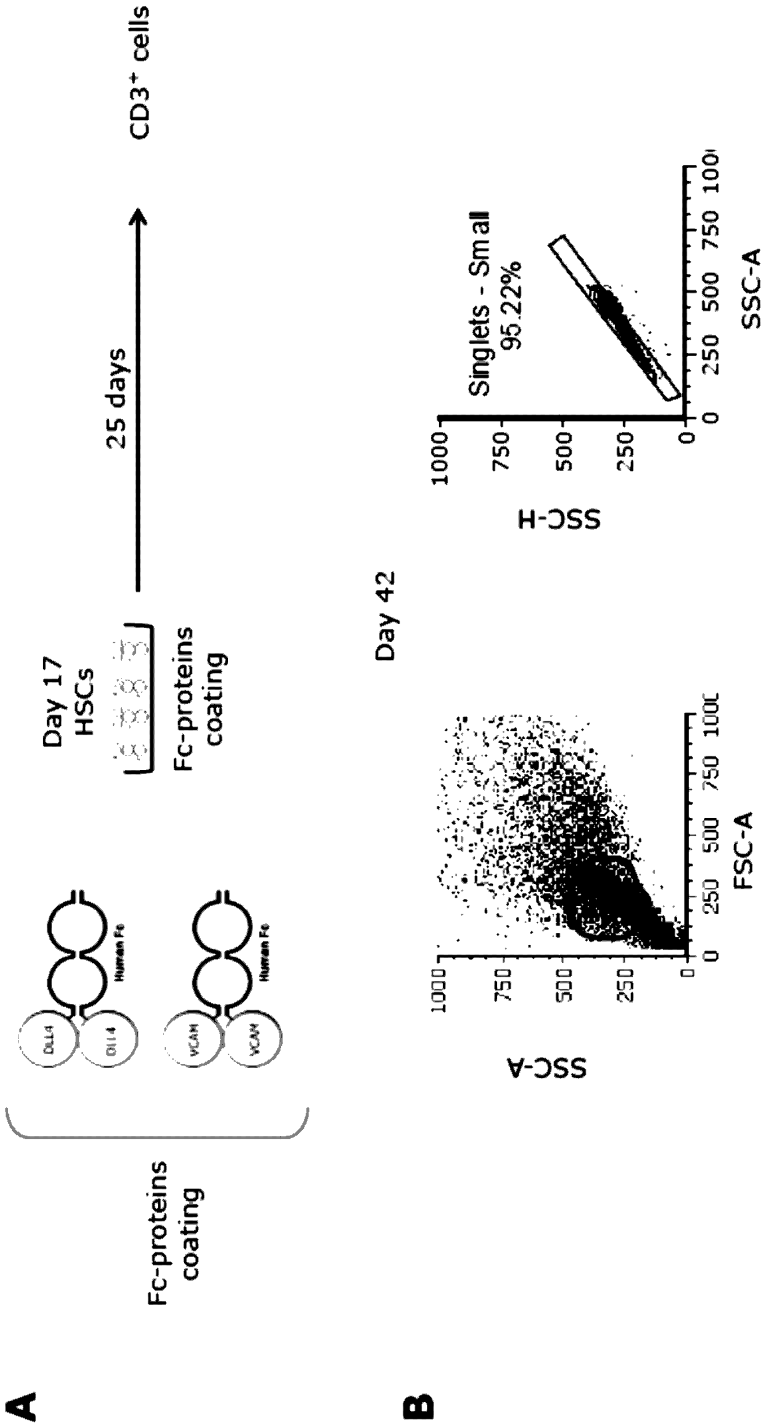

A
Fc-proteins coating
Human Fc
DLL4
DLL4
VCAM
VCAM
Day 17 HSCs
Fc-proteins coating
25 days
CD3+ cells
B
Day 42
SSC-A
FSC-A
SSC-H
SSC-A
Singlets - Small
95.22%

Figure 18

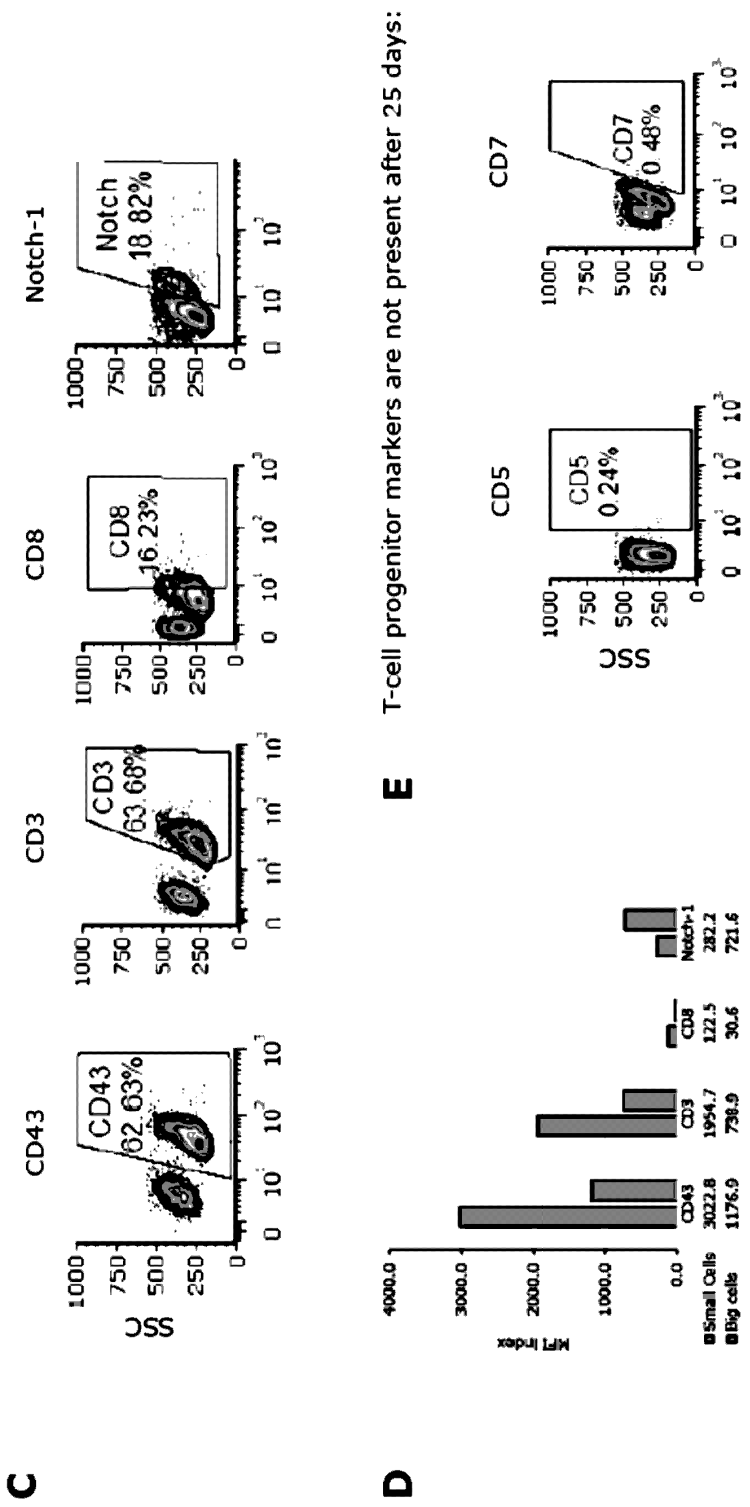

C
CD43
CD43
62.63%
CD3
CD3
63.68%
CD8
CD8
16.23%
Notch-1
Notch
18.82%
SSC
D
E
T-cell progenitor markers are not present after 25 days:
CD5
CD5
0.24%
CD7
CD7
0.48%
SSC
MFI Index
4000.0
3000.0
2000.0
1000.0
0.0
CD43
CD3
CD8
Notch-1
Small Cells
3022.8
1954.7
122.5
282.2
Big cells
1176.9
738.9
30.6
721.6

Figure 18

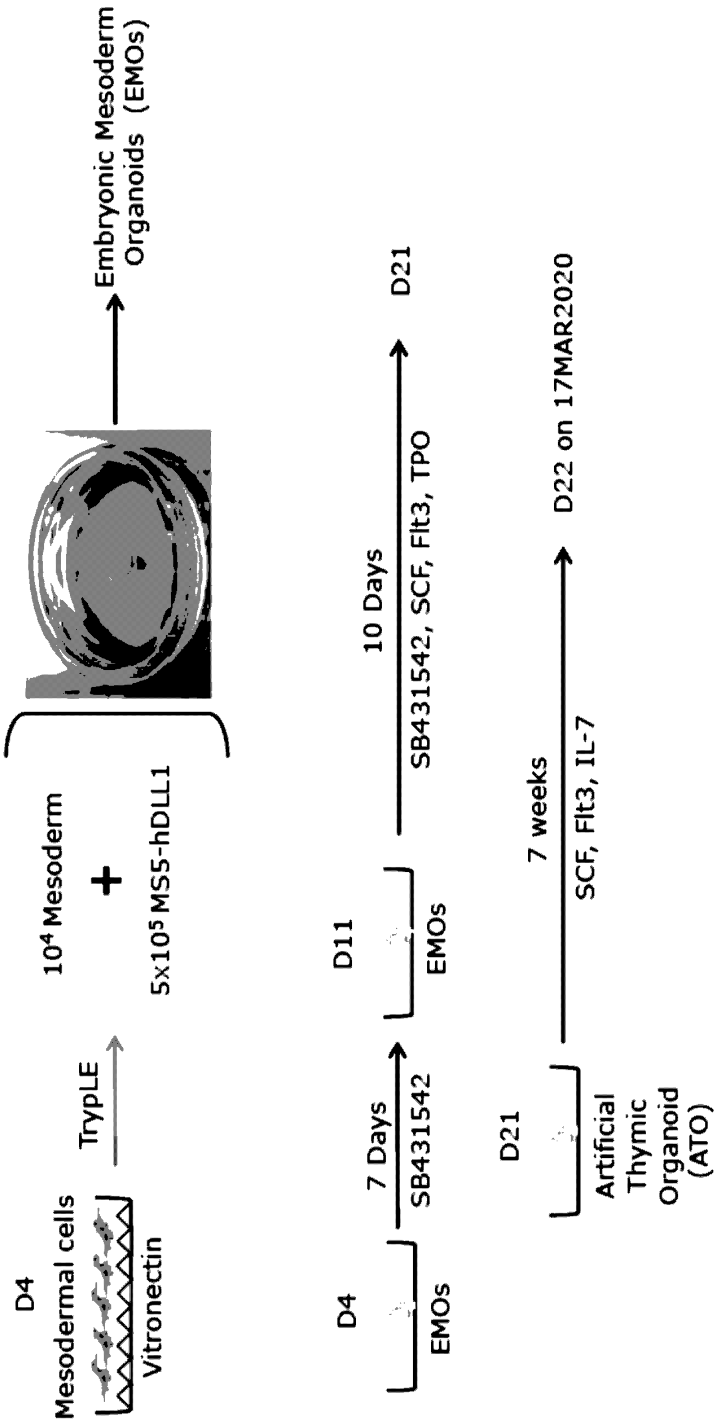

D4
Mesodermal cells
Vitronectin
TrypLE
10⁴ Mesoderm
+
5x10⁵ MS5-hDLL1
Embryonic Mesoderm Organoids (EMOs)
D4
EMOs
7 Days
SB431542
D11
EMOs
10 Days
SB431542, SCF, Flt3, TPO
D21
D21
Artificial Thymic Organoid (ATO)
7 weeks
SCF, Flt3, IL-7
D22 on 17MAR2020

Figure 19

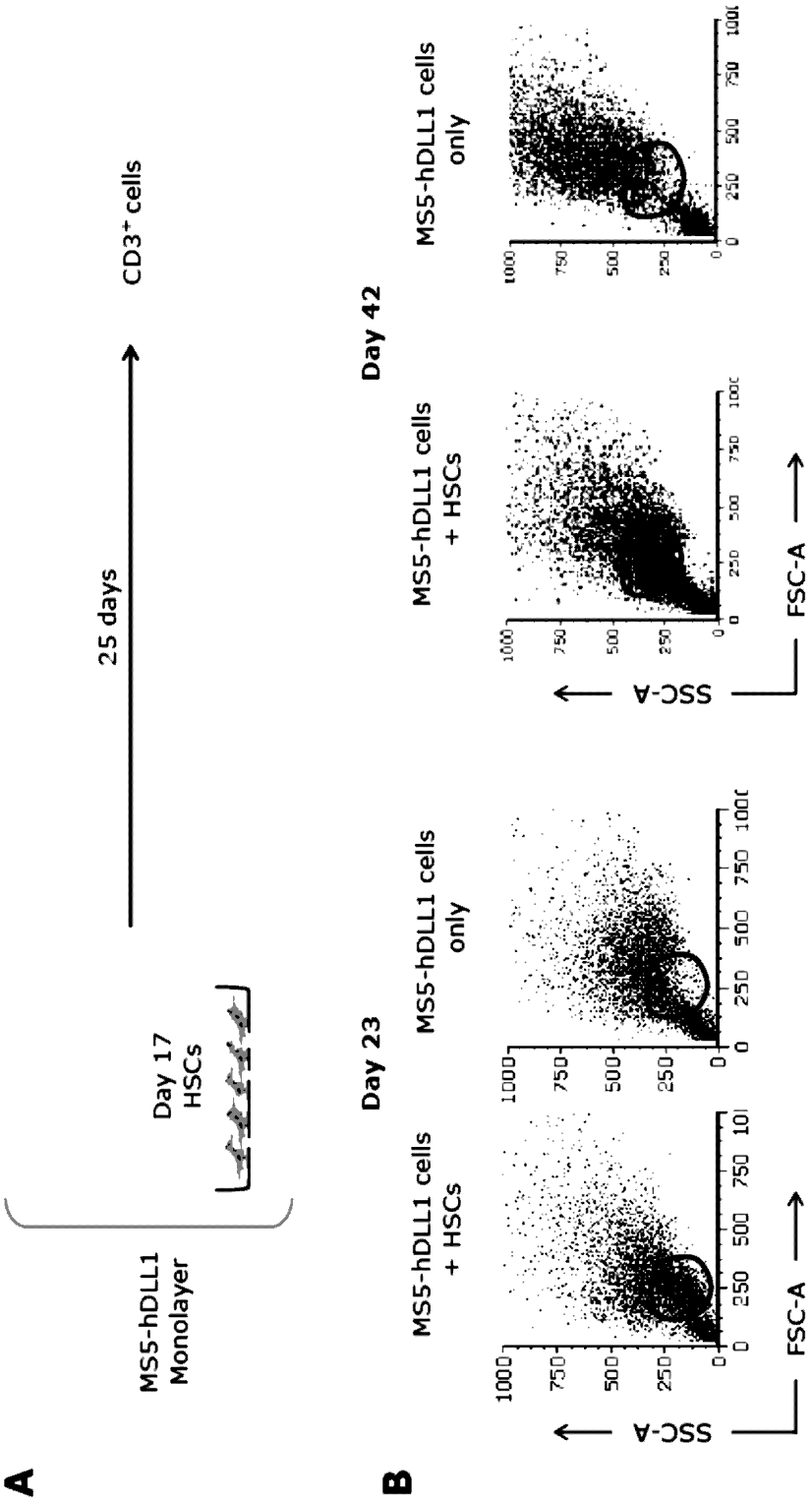

A
MS5-hDLL1
Monolayer
Day 17
HSCs
25 days
CD3⁺ cells
B
Day 23
MS5-hDLL1 cells
+ HSCs
MS5-hDLL1 cells
only
Day 42
MS5-hDLL1 cells
+ HSCs
MS5-hDLL1 cells
only
SSC-A
FSC-A
0
250
500
750
1000

Figure 20

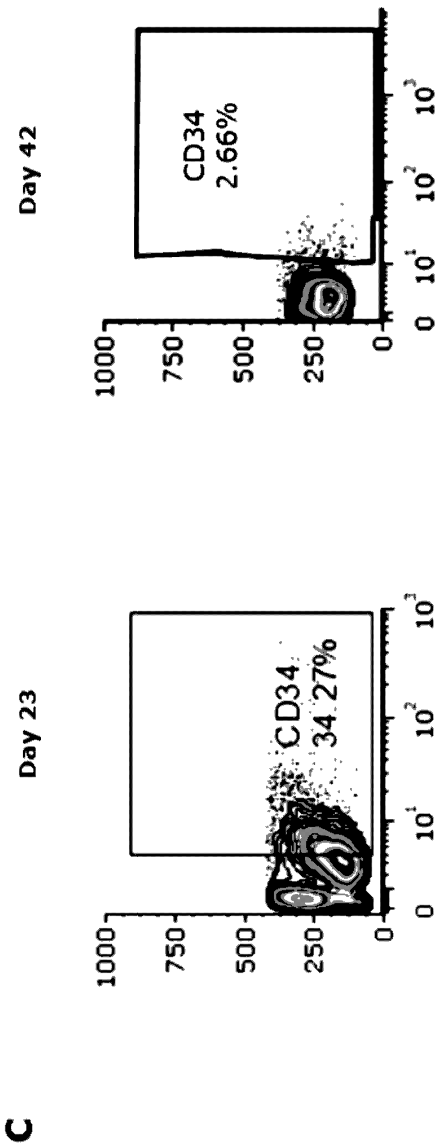

C
Day 23
CD34
34.27%
Day 42
CD34
2.66%
1000
750
500
250
0
0
10^1
10^2
10^3

Figure 20

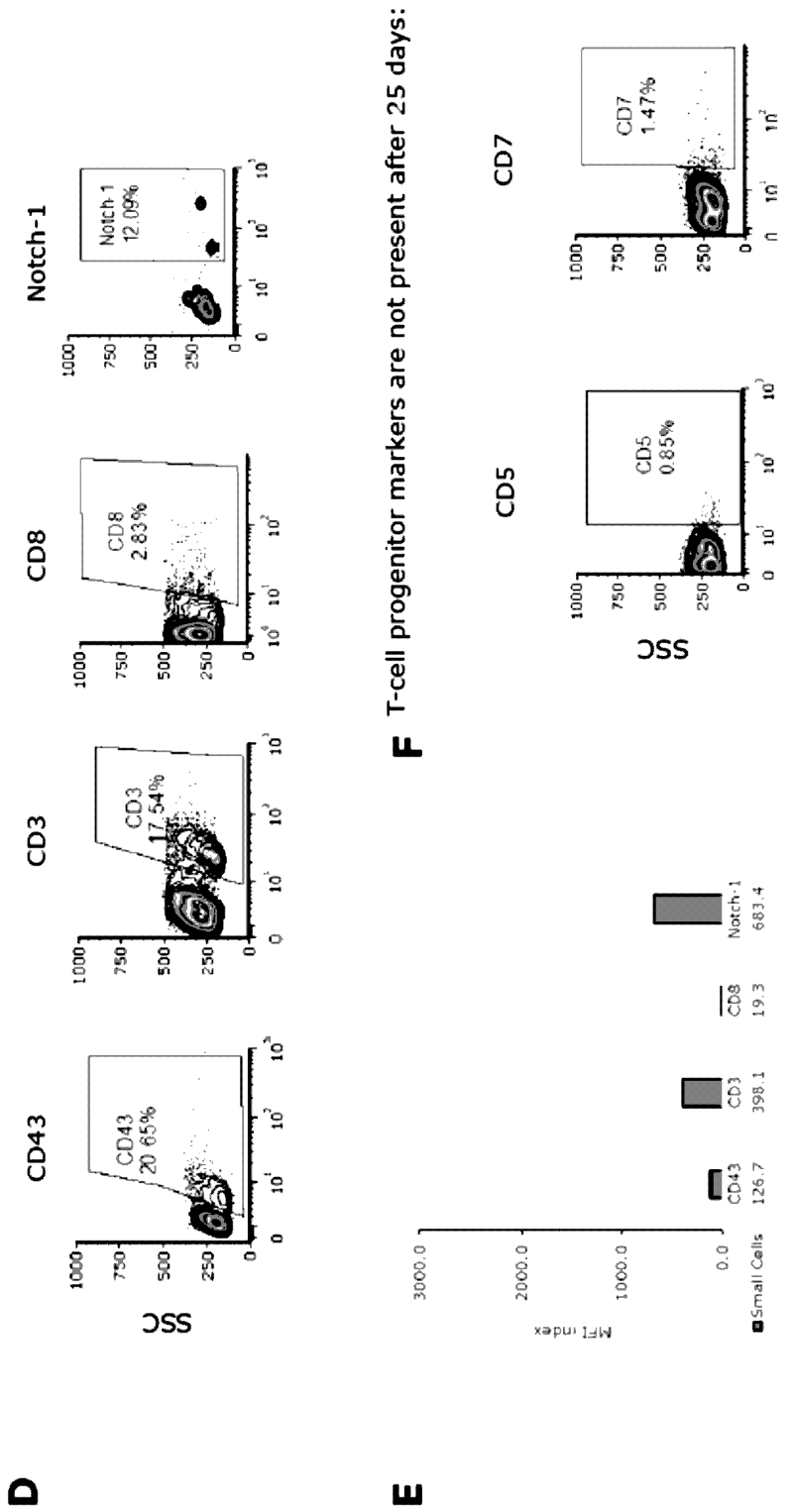

D
CD43
CD43
20.65%
CD3
CD3
17.54%
CD8
CD8
2.83%
Notch-1
Notch 1
12.09%
SSC
E
F
T-cell progenitor markers are not present after 25 days:
CD5
CD5
0.85%
CD7
CD7
1.47%
SSC
MFI index
3000.0
2000.0
1000.0
0.0
CD43 126.7
CD3 398.1
CD8 19.3
Notch-1 683.4
Small Cells

Figure 20

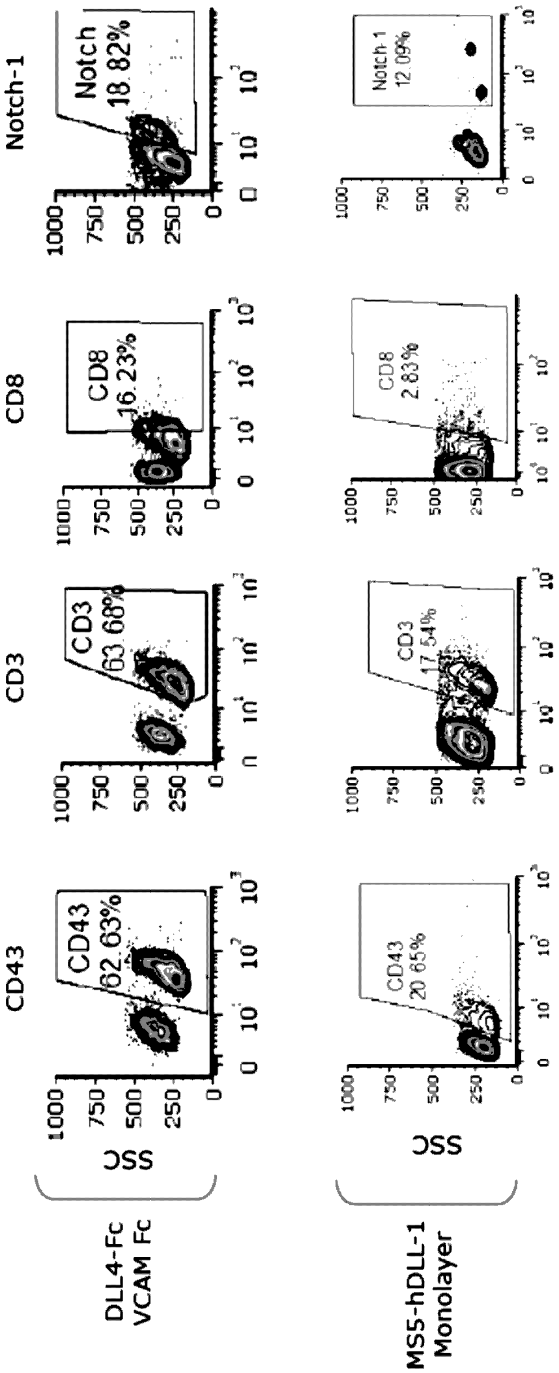

CD43
CD3
CD8
Notch-1
CD43 62.63%
CD3 63.68%
CD8 16.23%
Notch 18.82%
CD43 20.65%
CD3 17.54%
CD8 2.83%
Notch-1 12.09%
SSC
DLL4-Fc VCAM Fc
MS5-hDLL-1 Monolayer

Figure 21

Increased Expression of CD56/NCAM: NK cell potential

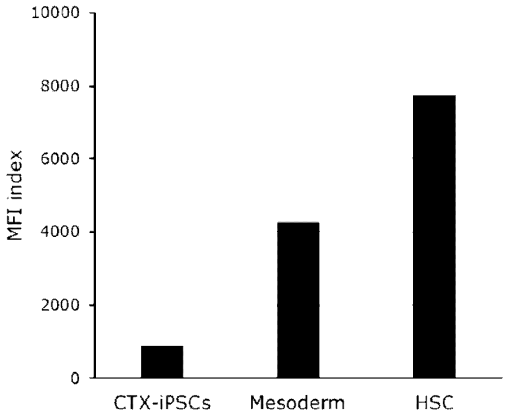


Figure 22

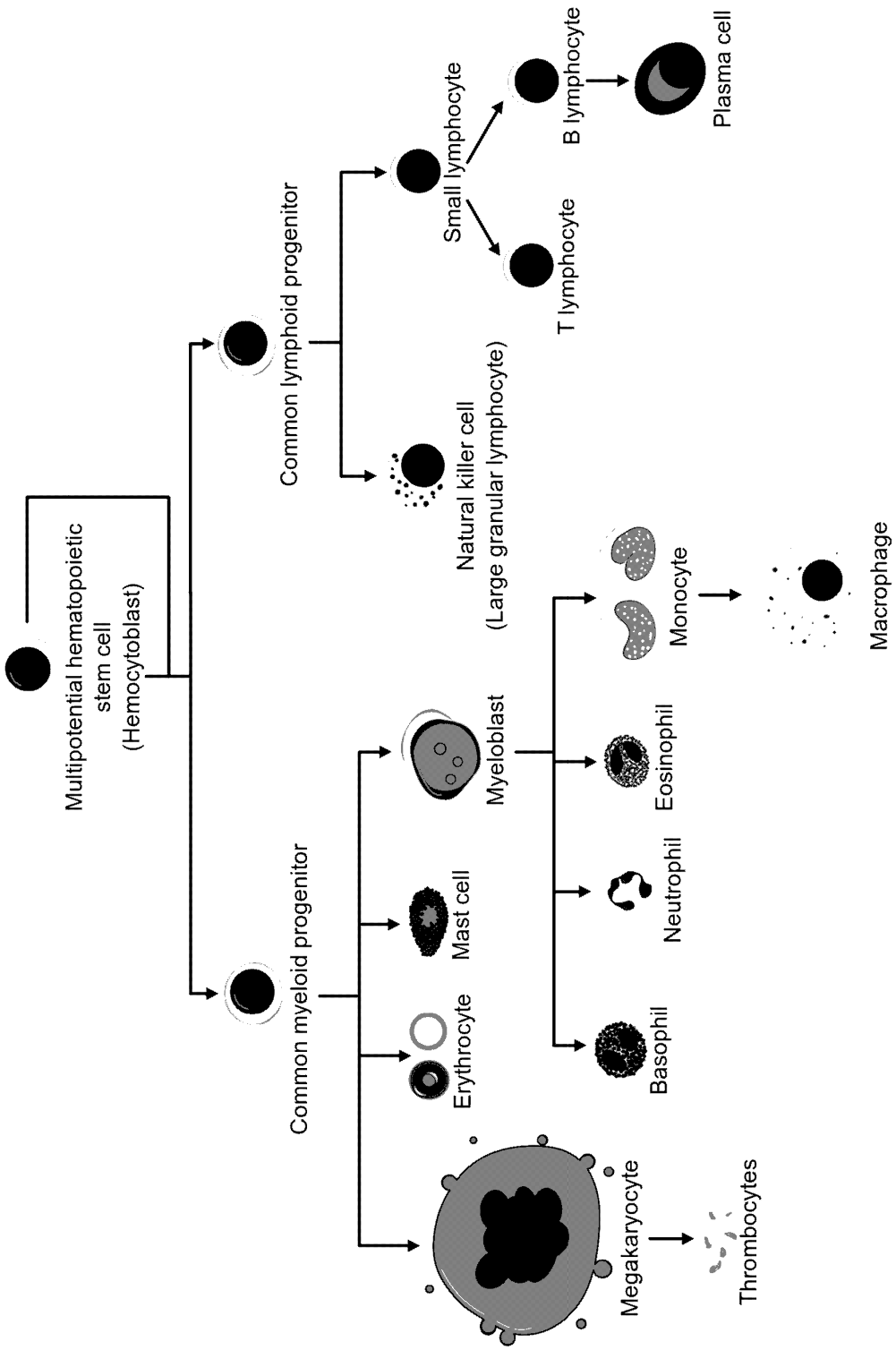

Multipotential hematopoietic stem cell (Hemocytoblast)
Common lymphoid progenitor
Small lymphocyte
B lymphocyte
Plasma cell
T lymphocyte
Natural killer cell (Large granular lymphocyte)
Common myeloid progenitor
Myeloblast
Monocyte
Macrophage
Eosinophil
Neutrophil
Basophil
Mast cell
Erythrocyte
Megakaryocyte
Thrombocytes

Figure 23

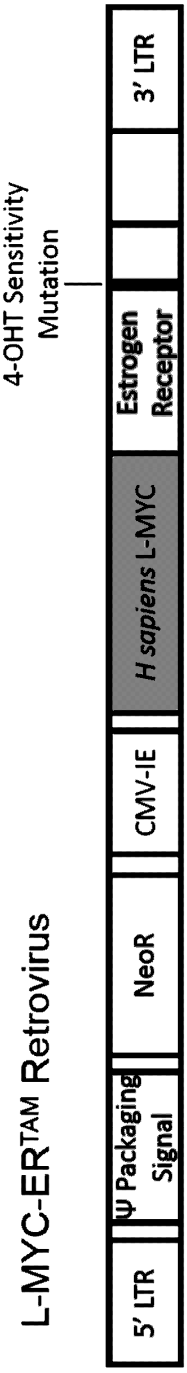

L-MYC-ER^{TAM} Retrovirus
5' LTR
Ψ Packaging Signal
NeoR
CMV-IE
H sapiens L-MYC
Estrogen Receptor
3' LTR
4-OHT Sensitivity Mutation

Figure 24

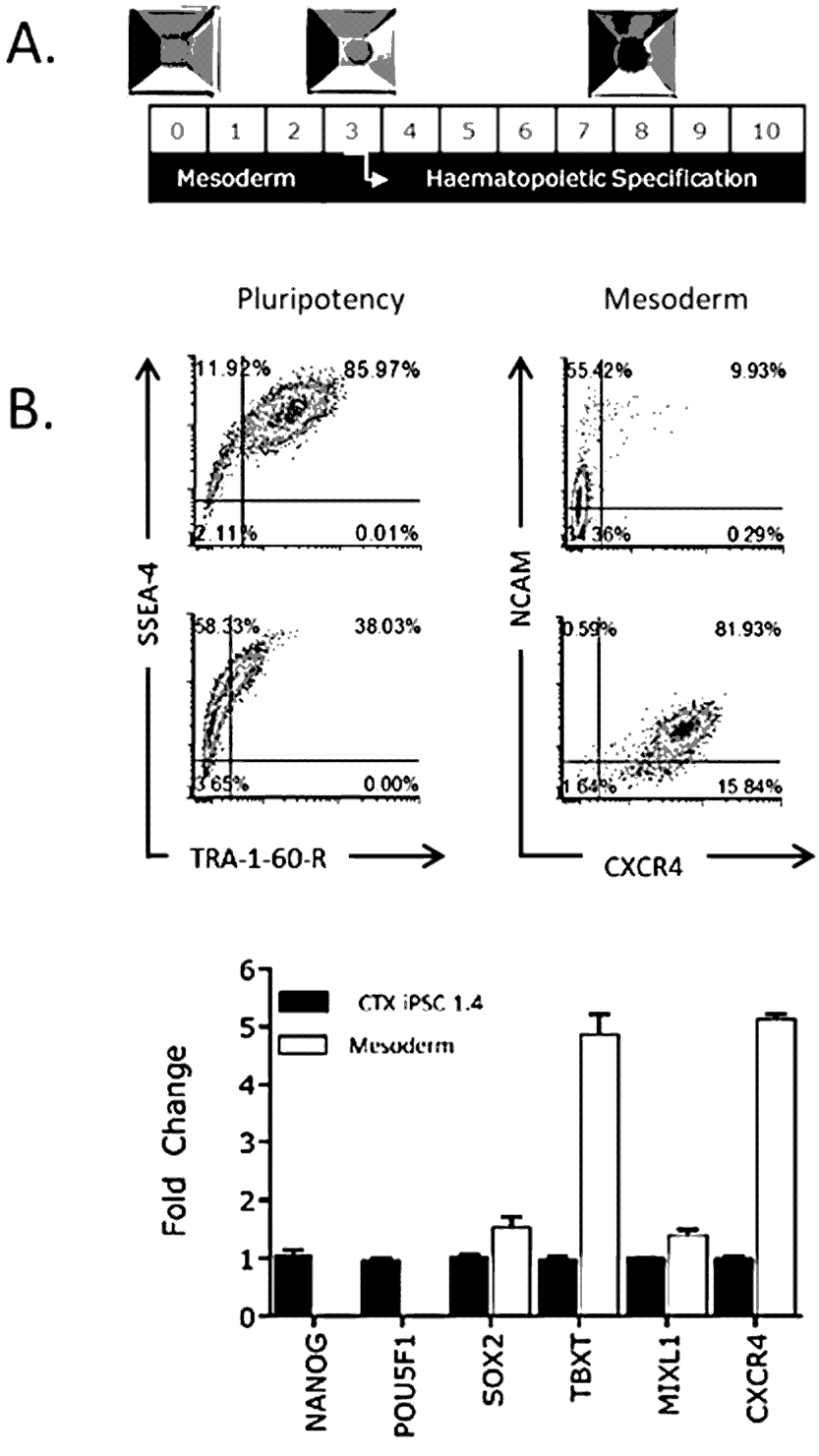

A.
0
1
2
3
4
5
6
7
8
9
10
Mesoderm
Haematopoietic Specification
B.
Pluripotency
Mesoderm
11.92%
85.97%
2.11%
0.01%
55.42%
9.93%
14.36%
0.29%
58.33%
38.03%
3.65%
0.00%
0.59%
81.93%
1.64%
15.84%
SSEA-4
TRA-1-60-R
NCAM
CXCR4
CTX iPSC 1.4
Mesoderm
Fold Change
NANOG
POU5F1
SOX2
TBXT
MIXL1
CXCR4

Figure 25

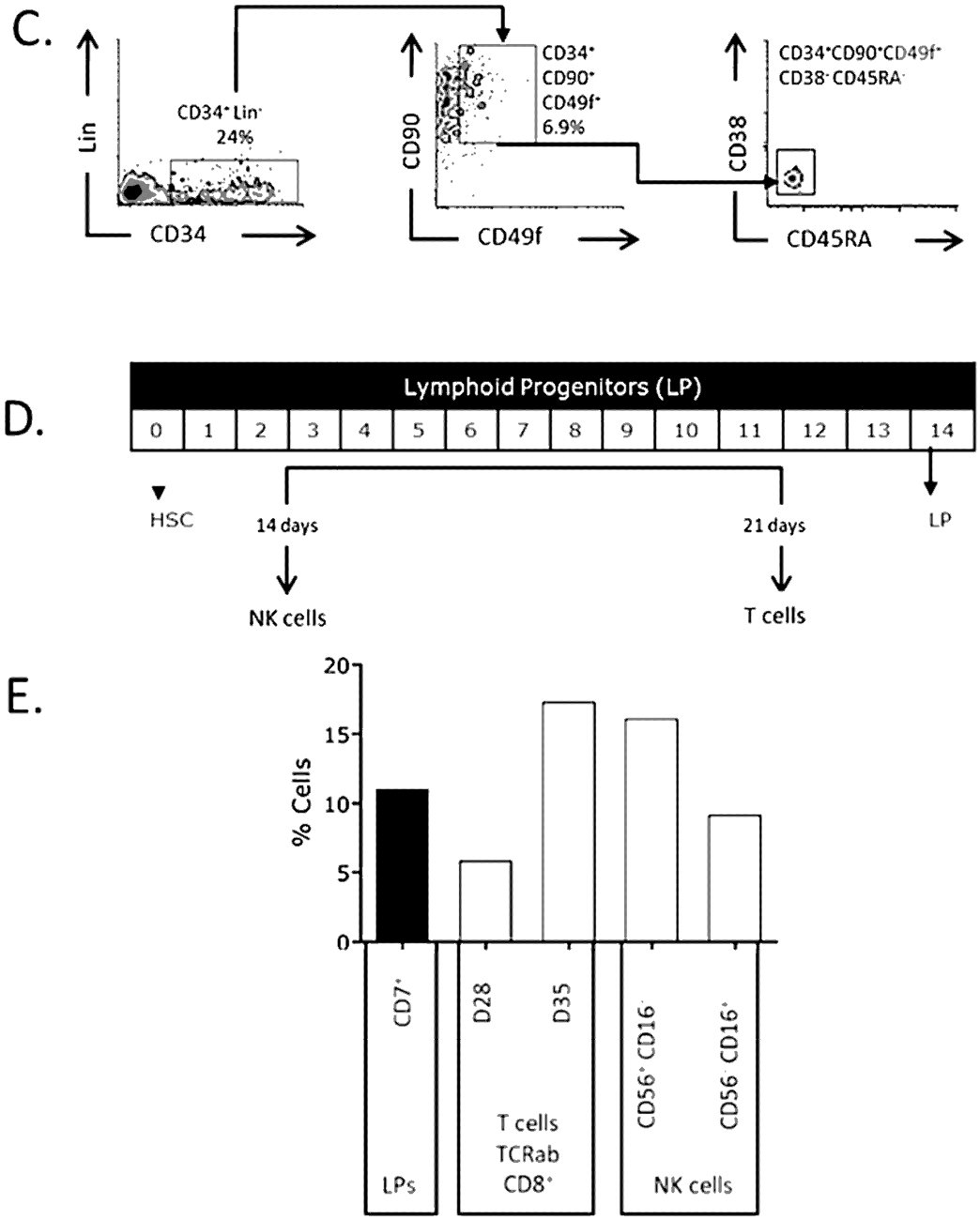


Figure 25 (contd.)