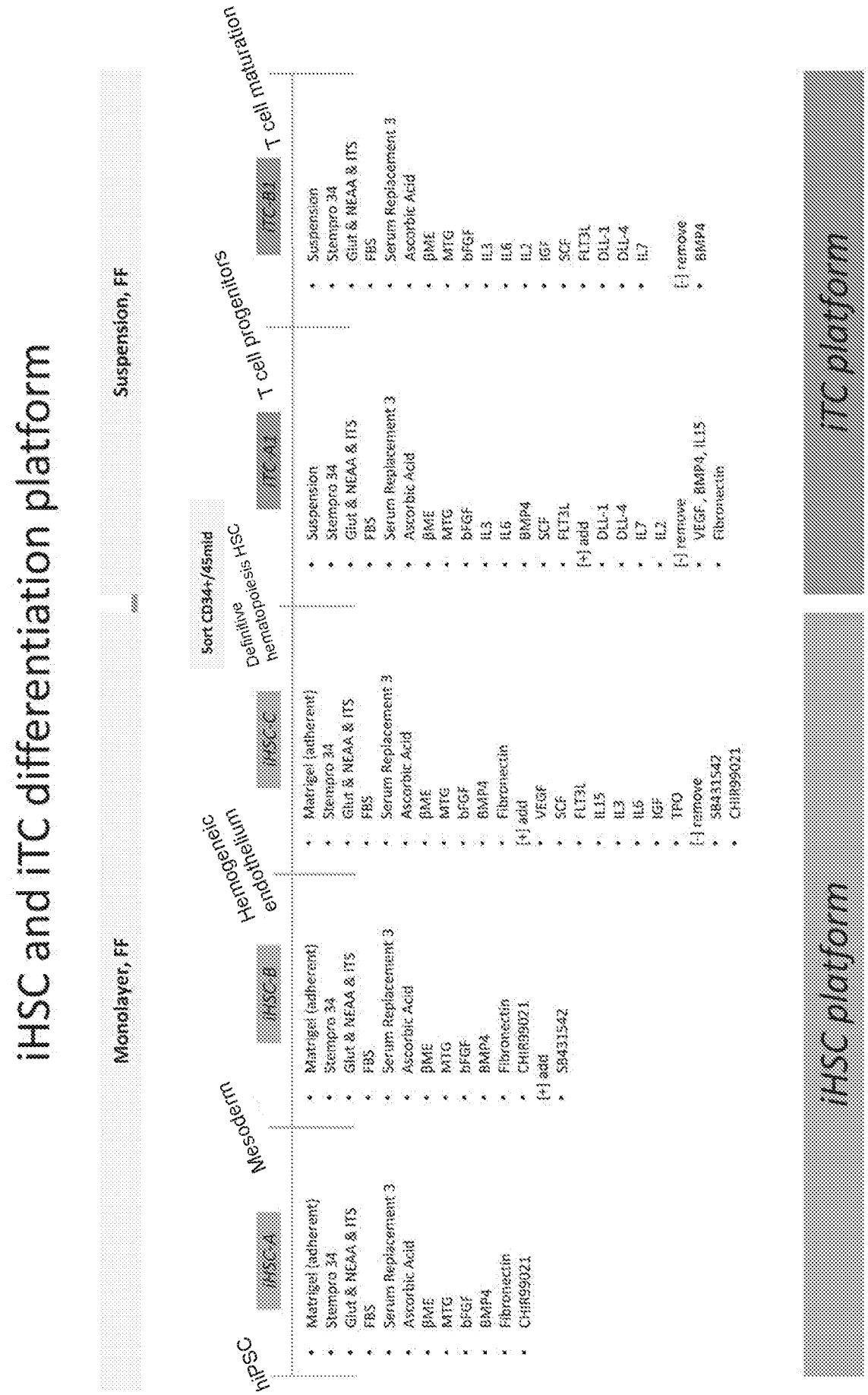

iHSC and iTC differentiation platform
Monolayer, FF
Suspension, FF
hiPSC
iHSC-A
Mesoderm
iHSC-B
Hemogeneic endothelium
iHSC-C
Sort CD34+/45mid
Definitive hematopoiesis HSC
iTC-A1
T cell progenitors
iTC-B1
T cell maturation
Matrigel (adherent)
Stempro 34
Glut & NEAA & ITS
FBS
Serum Replacement 3
Ascorbic Acid
βME
MTG
bFGF
BMP4
Fibronectin
CHIR99021
[+] add
SB431542
VEGF
SCF
FLT3L
IL15
IL3
IL6
IGF
TPO
[-] remove
VEGF, BMP4, IL15
Suspension
IL7
IL2
iHSC platform
iTC platform

FIG. 1

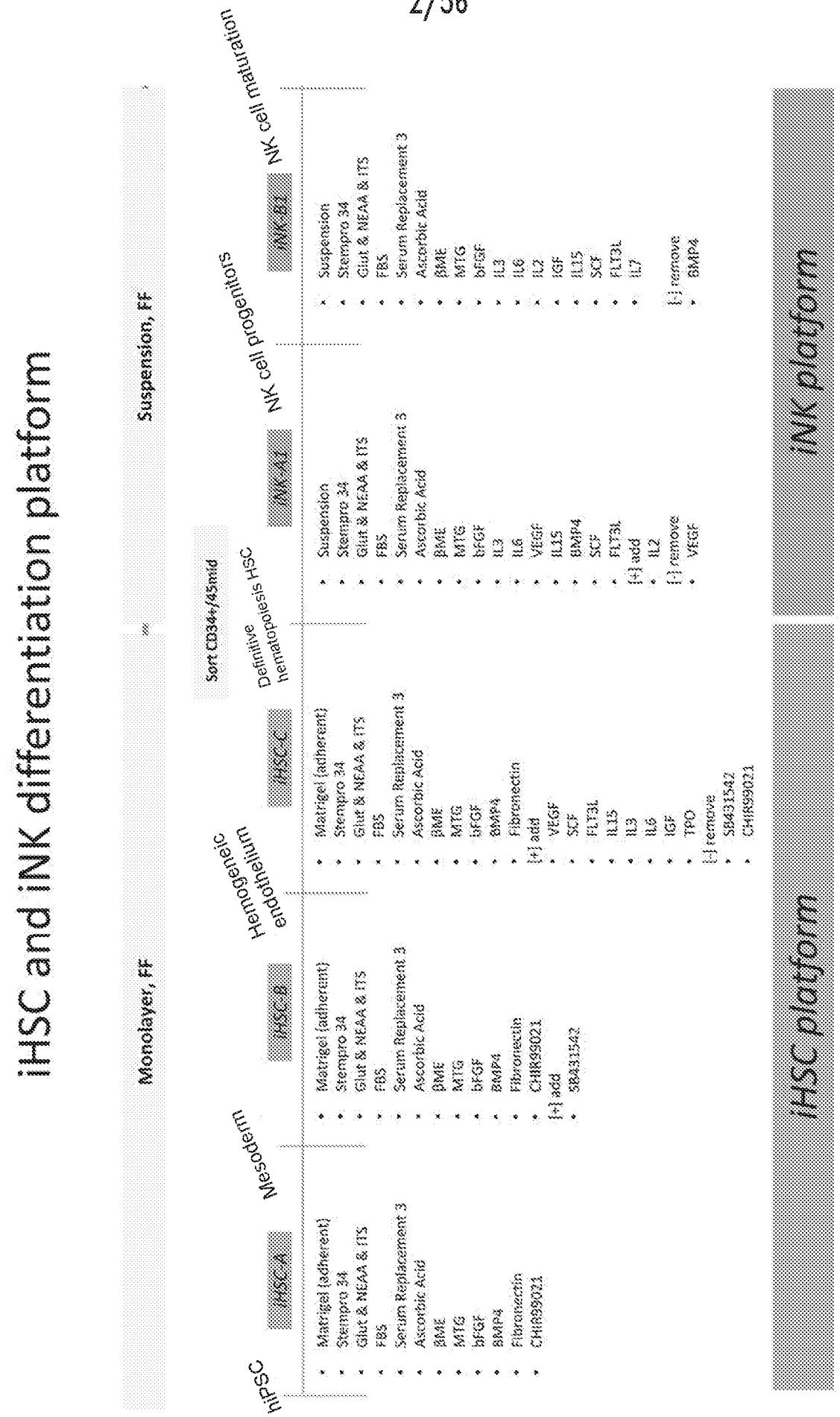

iHSC and iNK differentiation platform
Monolayer, FF
Suspension, FF
Sort CD34+/45mid
hiPSC
iHSC-A
Mesoderm
iHSC-B
Hemogeneic endothelium
iHSC-C
Definitive hematopoiesis HSC
iNK-A1
NK cell progenitors
iNK-B1
NK cell maturation
Matrigel (adherent)
Stempro 34
Glut & NEAA & ITS
FBS
Serum Replacement 3
Ascorbic Acid
βME
MTG
bFGF
BMP4
Fibronectin
CHIR99021
[+] add
SB431542
[+] remove
TPO
VEGF
IL15
IL3
IL6
IGF
IL2
IL7
SCF
FLT3L
Suspension
Stempro 34
Glut & NEAA & ITS
FBS
Serum Replacement 3
Ascorbic Acid
βME
MTG
bFGF
IL3
IL6
IL2
IGF
IL15
SCF
FLT3L
[+] add
IL7
[-] remove
BMP4
iHSC platform
iNK platform

FIG. 2

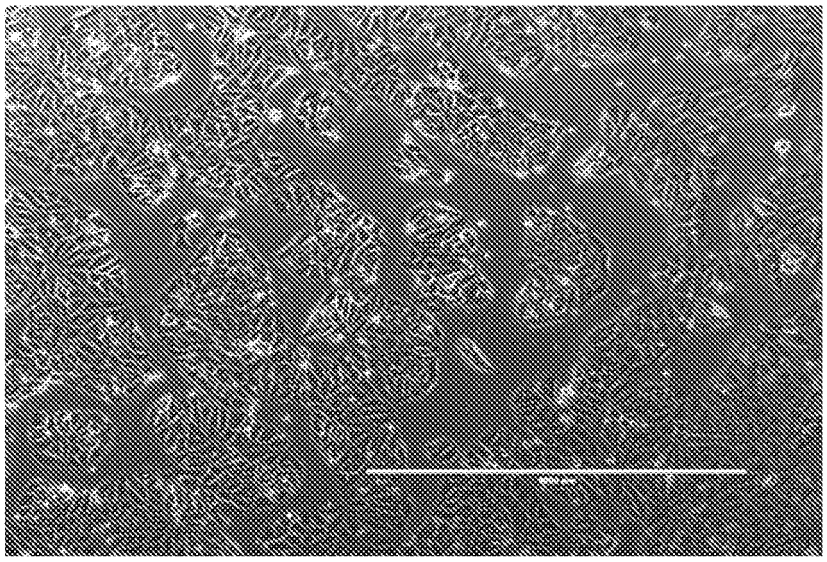


FIG. 3A

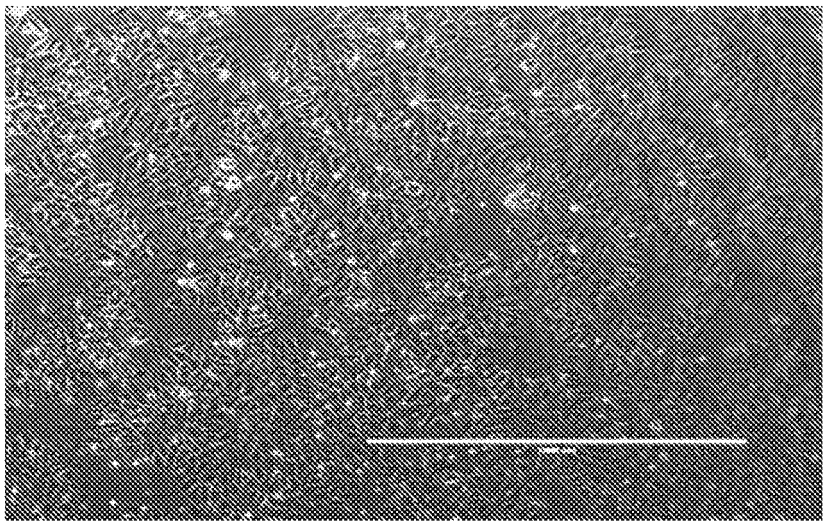


FIG. 3B

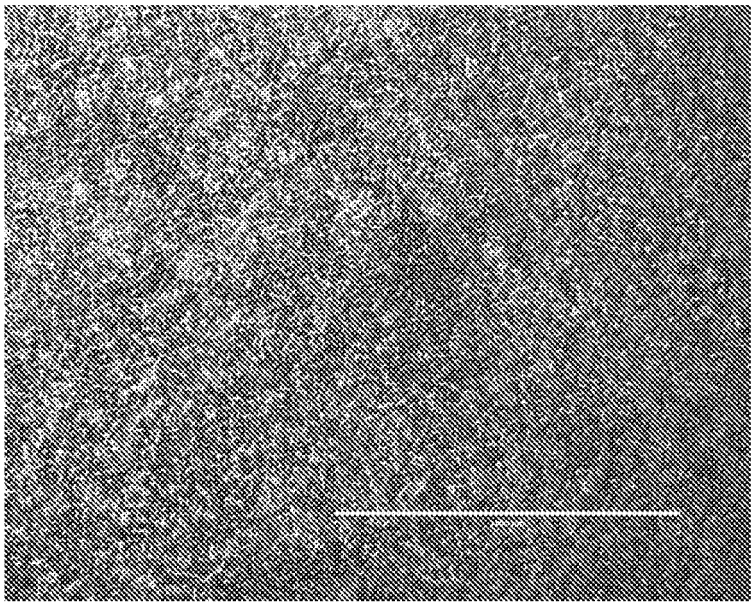


FIG. 3C

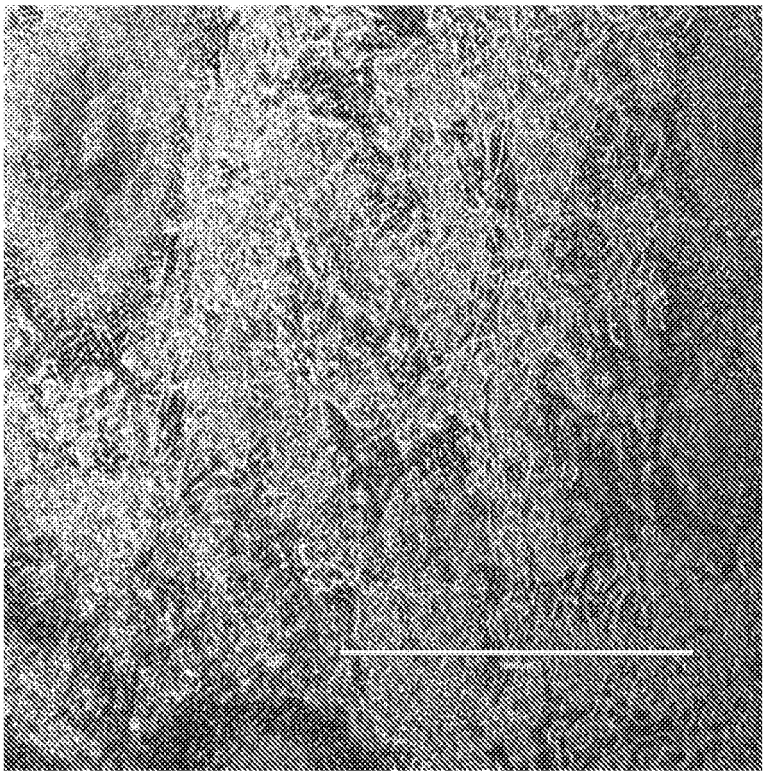


FIG. 3D

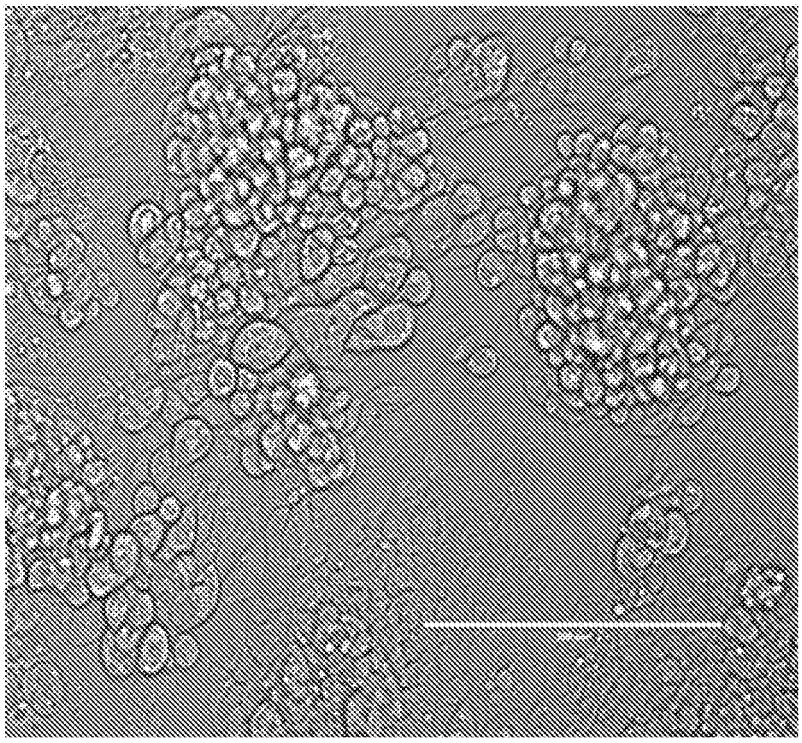


FIG. 3E

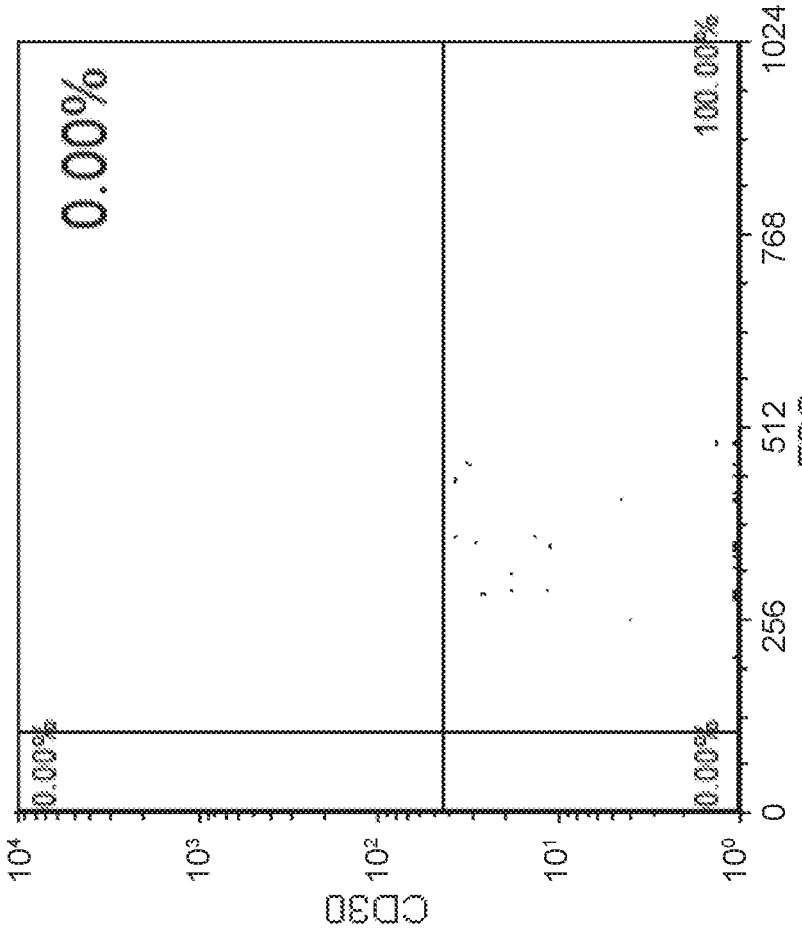

0.00%
0.00%
100.00%
0.00%
CD30
FSC
0
256
512
768
1024

FIG. 4B

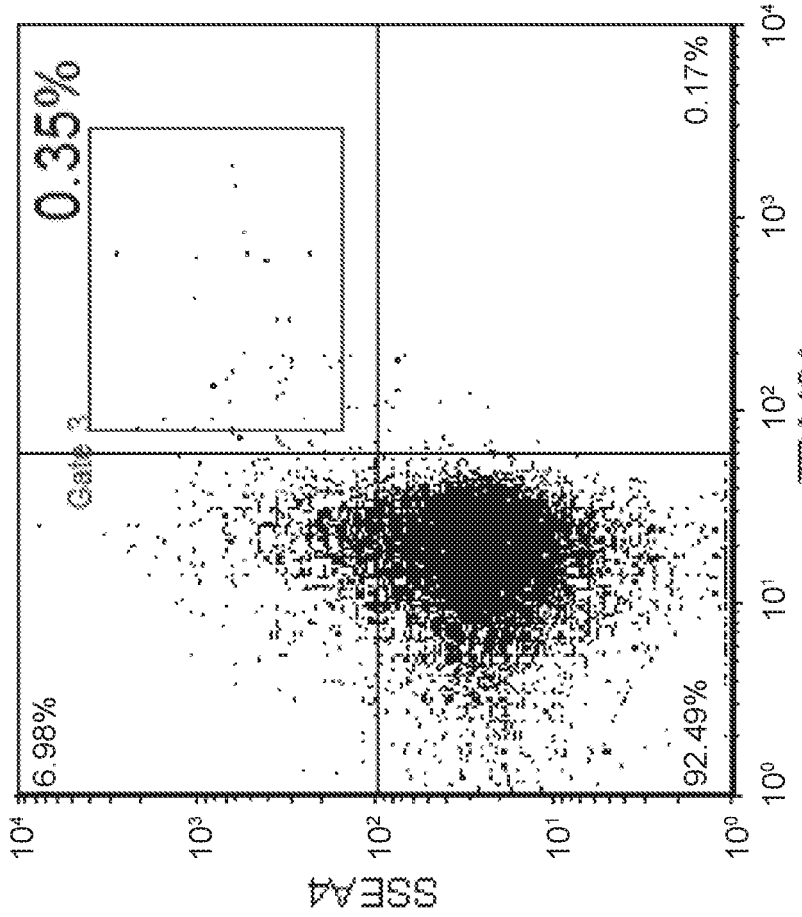

0.35%
Gate 2
6.98%
92.49%
0.17%
SSEA4
TRA181

FIG. 4A

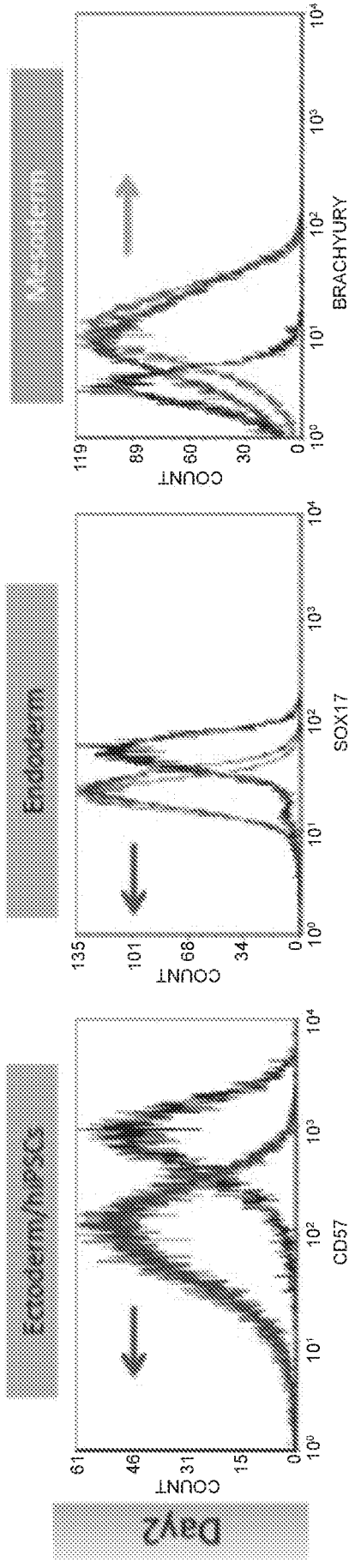

Ectoderm hiPSCs
Endoderm
Mesoderm
day2
COUNT
CD57
SOX17
BRACHYURY

FIG. 4C

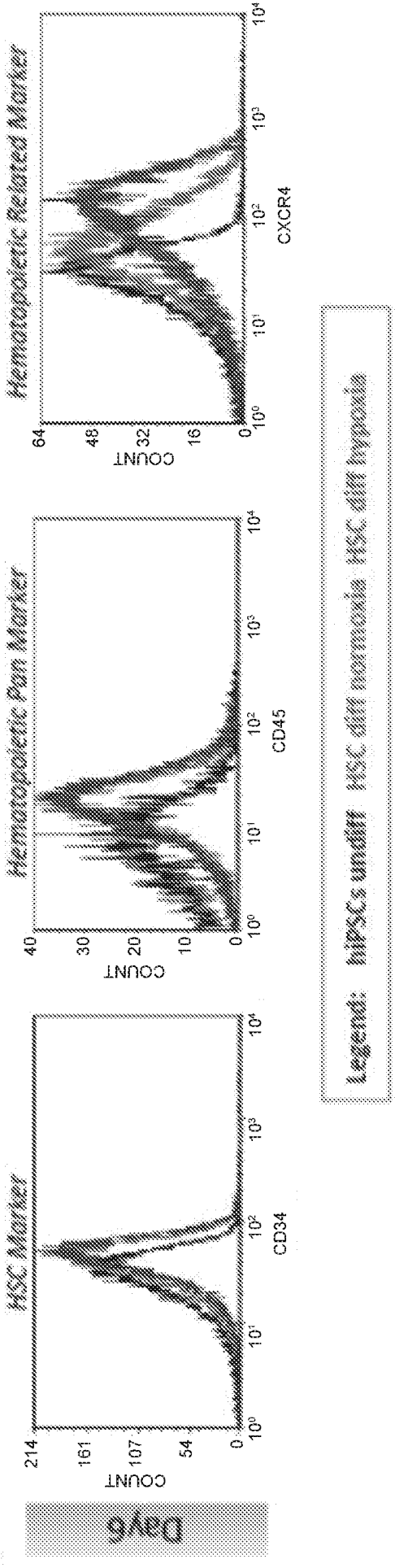
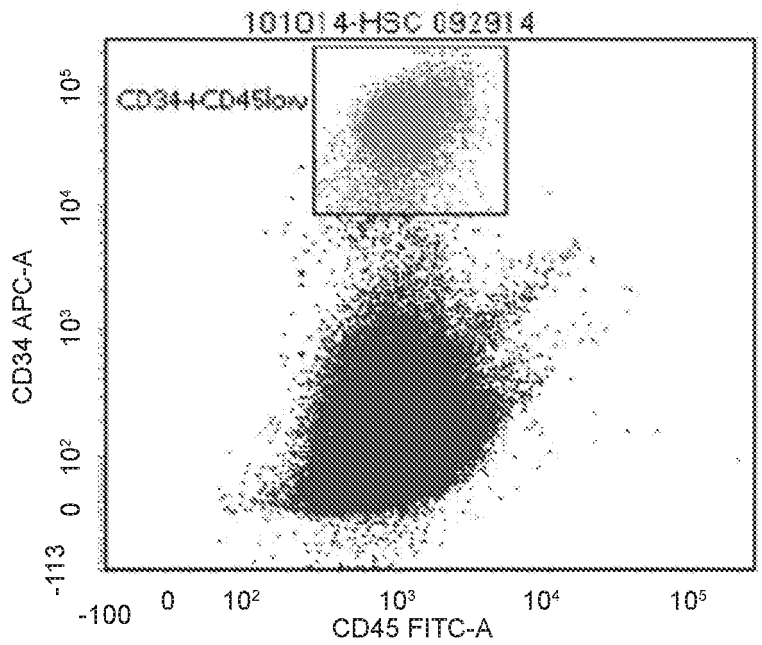
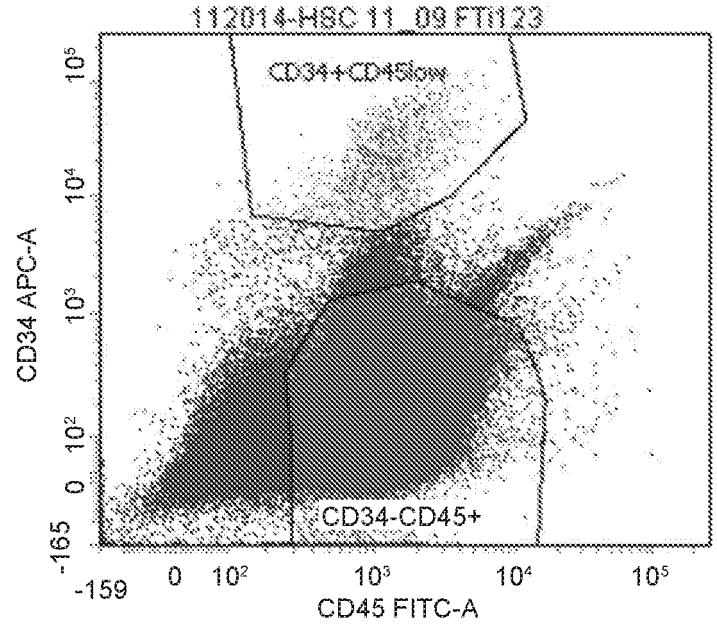

HSC Marker
Hematopoietic Pan Marker
Hematopoietic Related Marker
day6
COUNT
CD34
CD45
CXCR4
Legend: hiPSCs undiff HSC diff normoxia HSC diff hypoxia

FIG. 4D



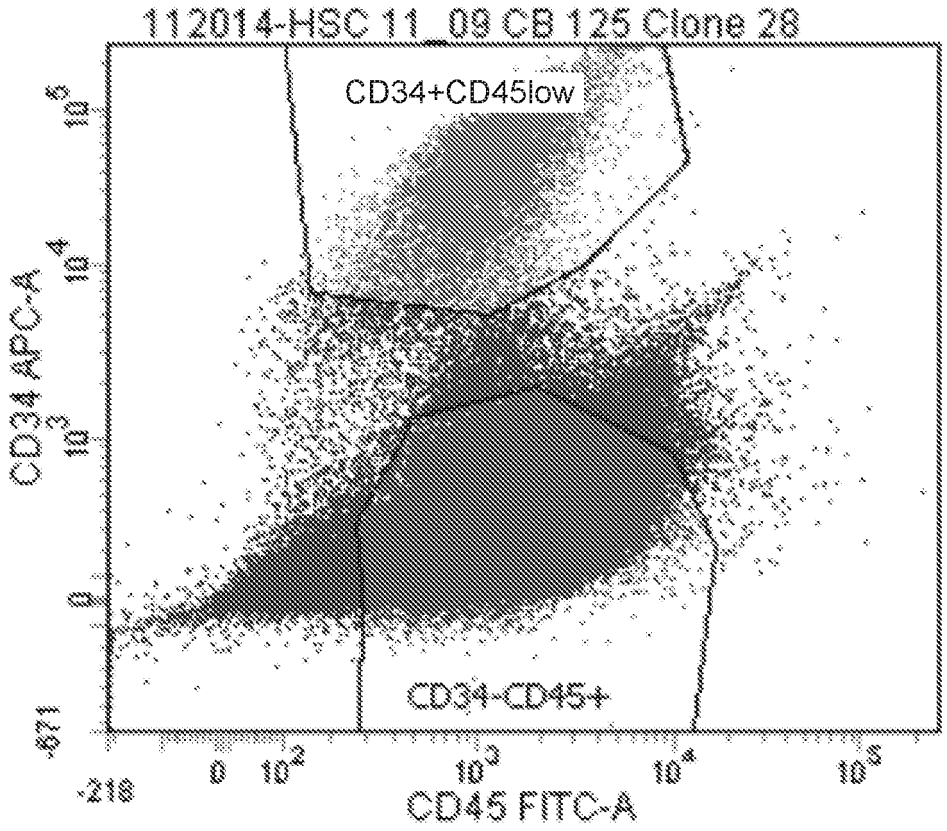
Fibroblast derived
OCT4/SOX2/T

FIG. 5A



Fibroblast derived
OCT4/SOX2/NANOG/T

FIG. 5B



Cord Blood derived
OCT4/SOX2/NANOG/LIN28/MYC/T

FIG. 5C

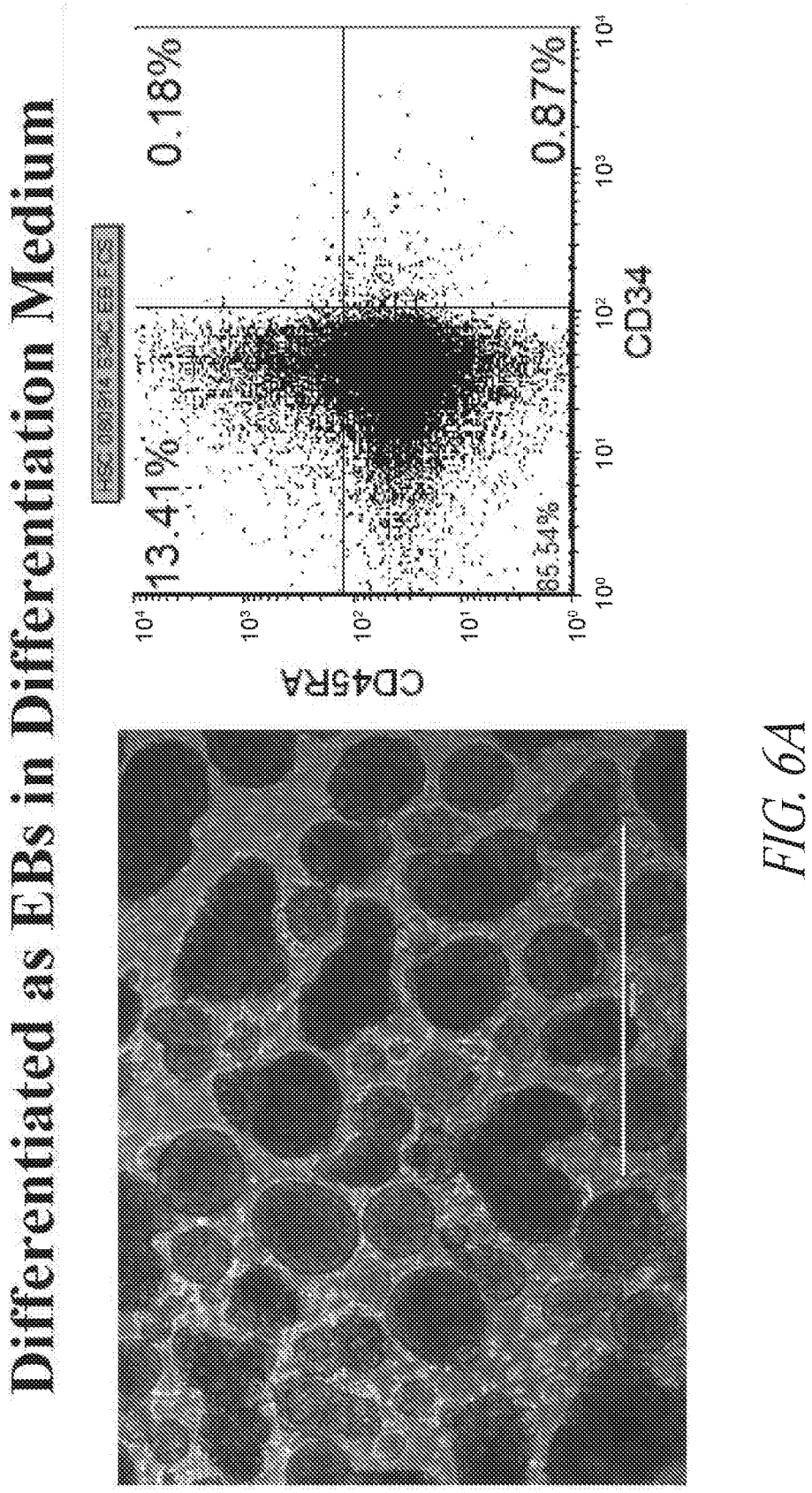

Differentiated as EBs in Differentiation Medium
0.18%
0.87%
13.41%
85.34%
CD45RA
CD34

FIG. 6A

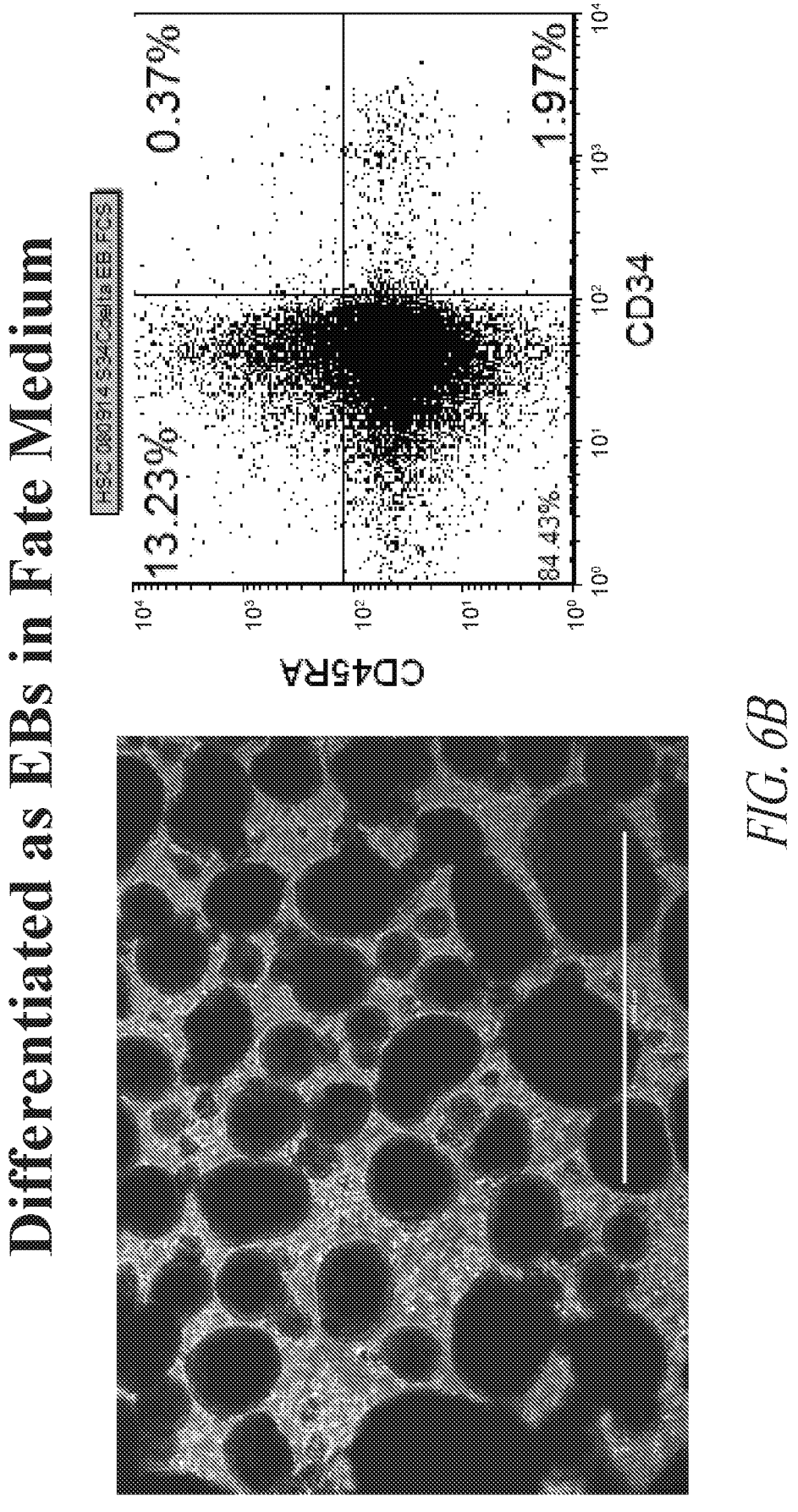

Differentiated as EBs in Fate Medium
0.37%
1.97%
13.23%
84.43%
CD45RA
CD34

FIG. 6B

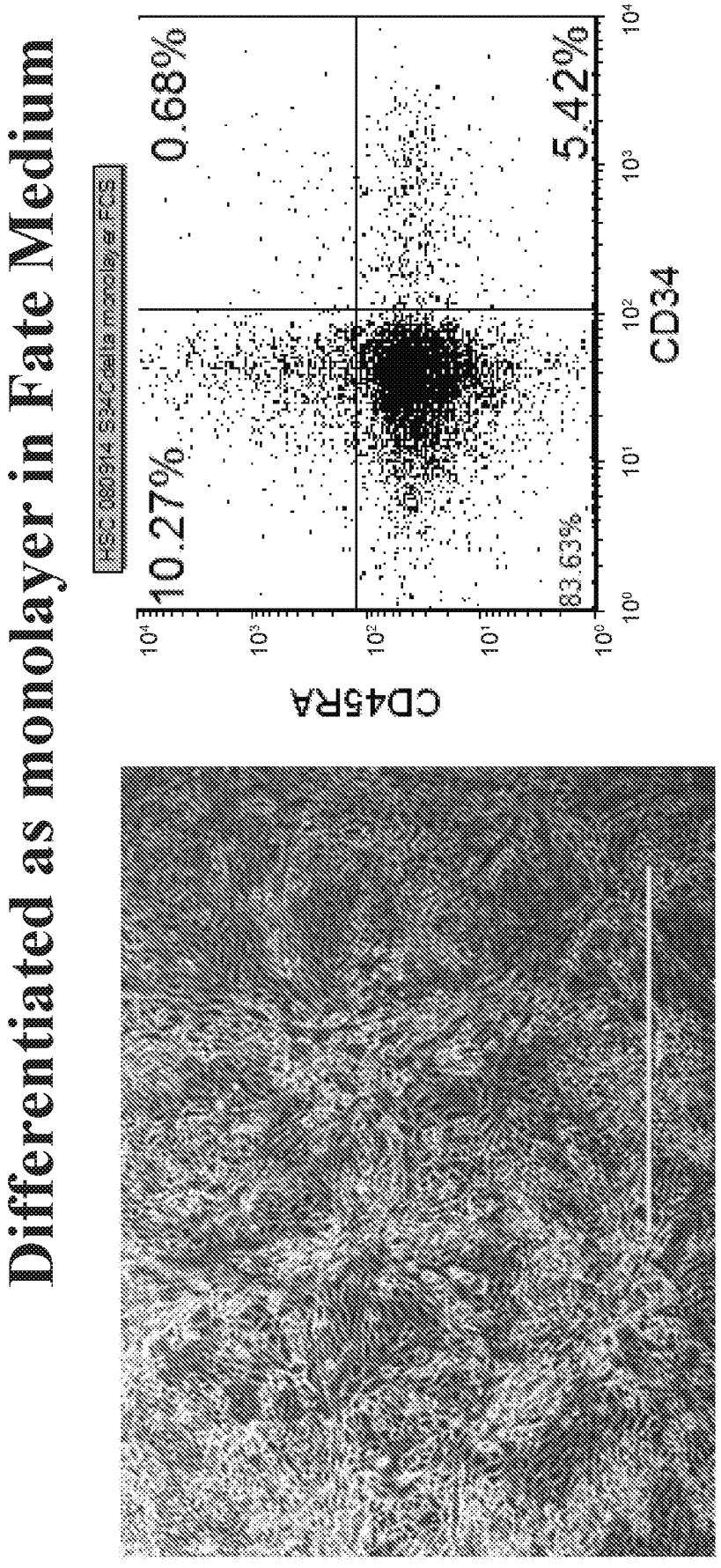

Differentiated as monolayer in Fate Medium
HSC 00514 ESAC cells in media # FCS
0.68%
5.42%
10.27%
83.63%
CD45RA
CD34
10^0
10^1
10^2
10^3
10^4

FIG. 6C

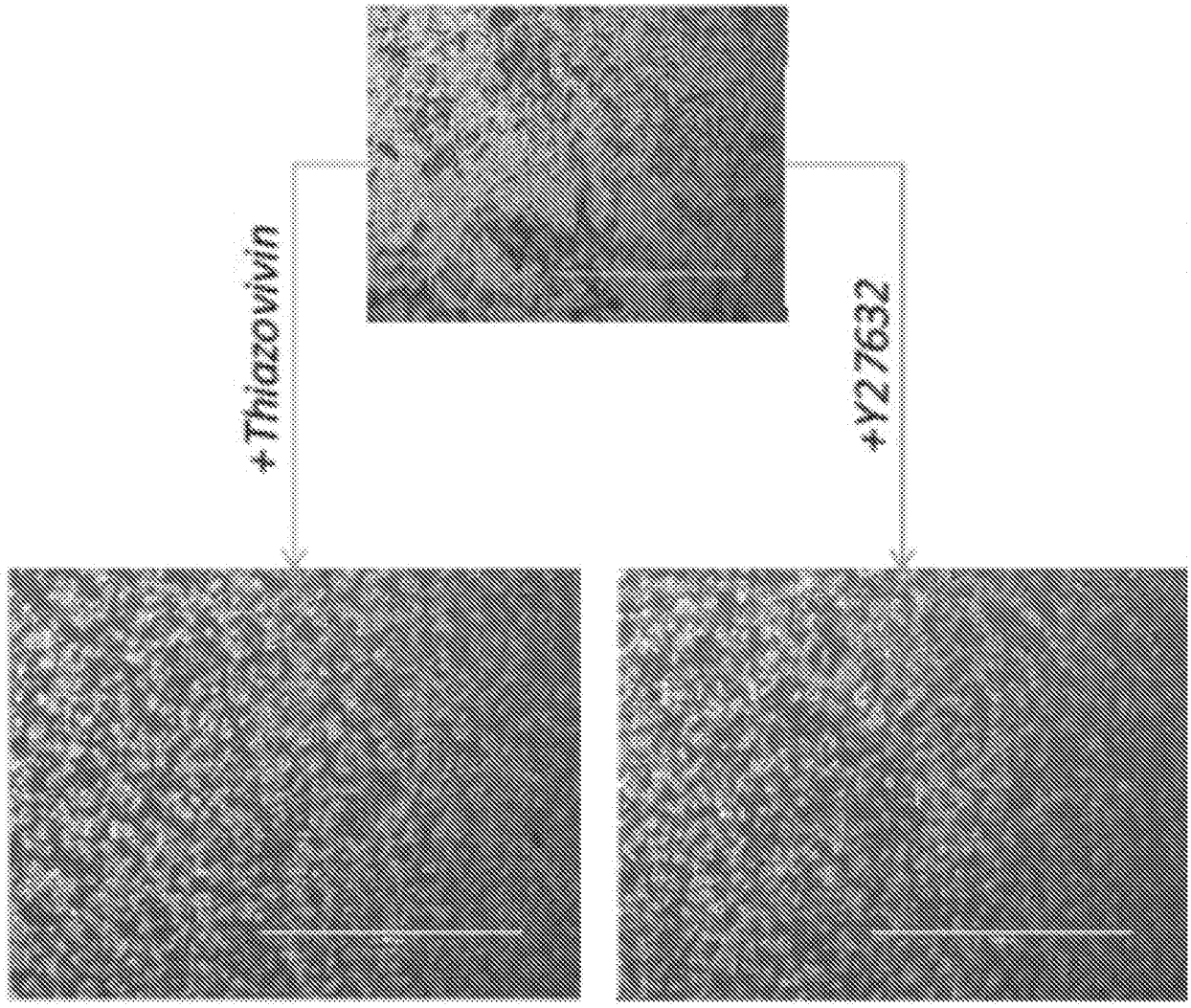


FIG. 7

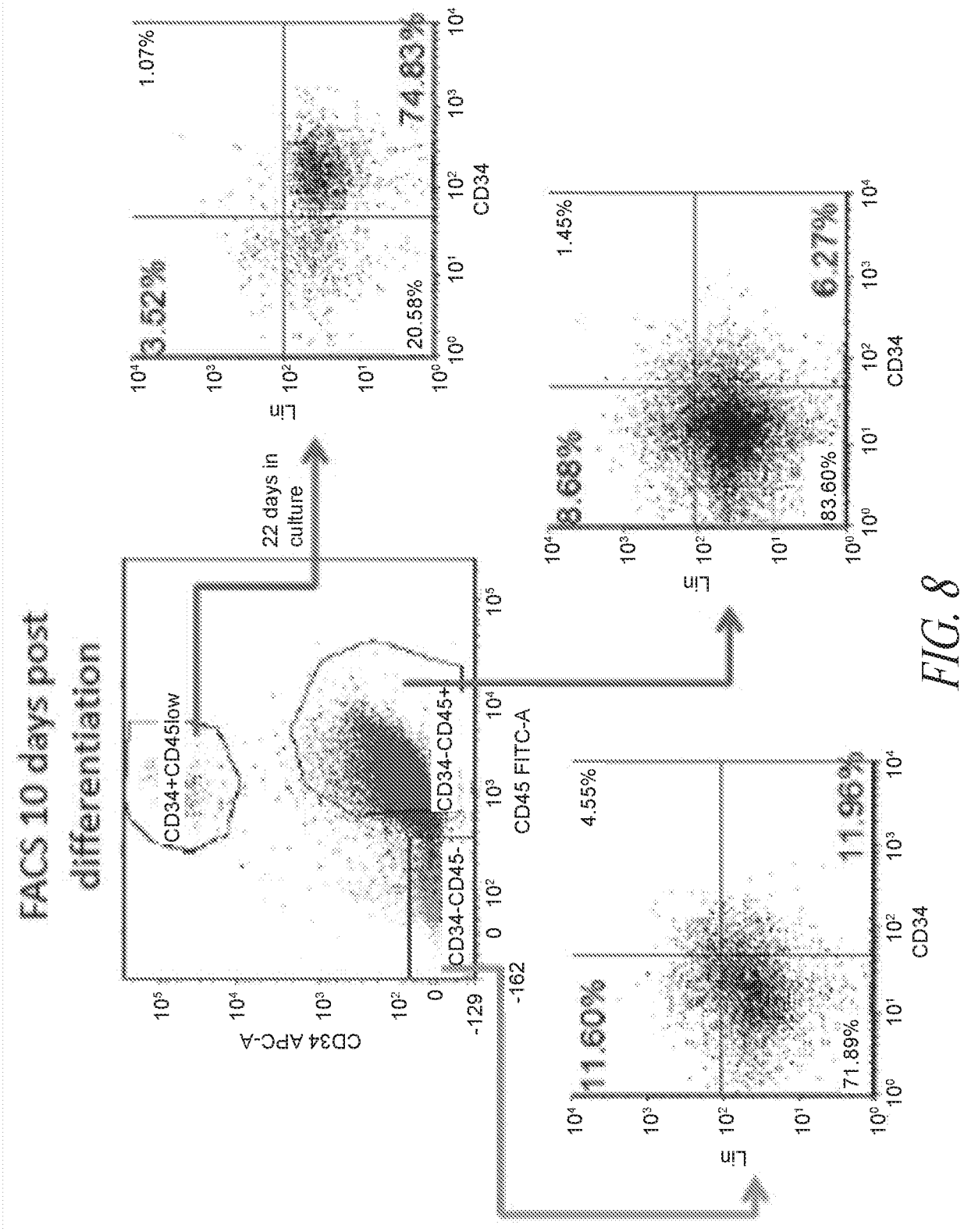

FACS 10 days post differentiation
CD34+CD45low
CD34-CD45+
CD34-CD45-
CD34 APC-A
CD45 FITC-A
22 days in culture
3.52%
20.58%
74.03%
1.07%
3.68%
83.60%
6.27%
1.45%
11.60%
71.89%
4.55%
11.96%
CD34
Lin

FIG. 8

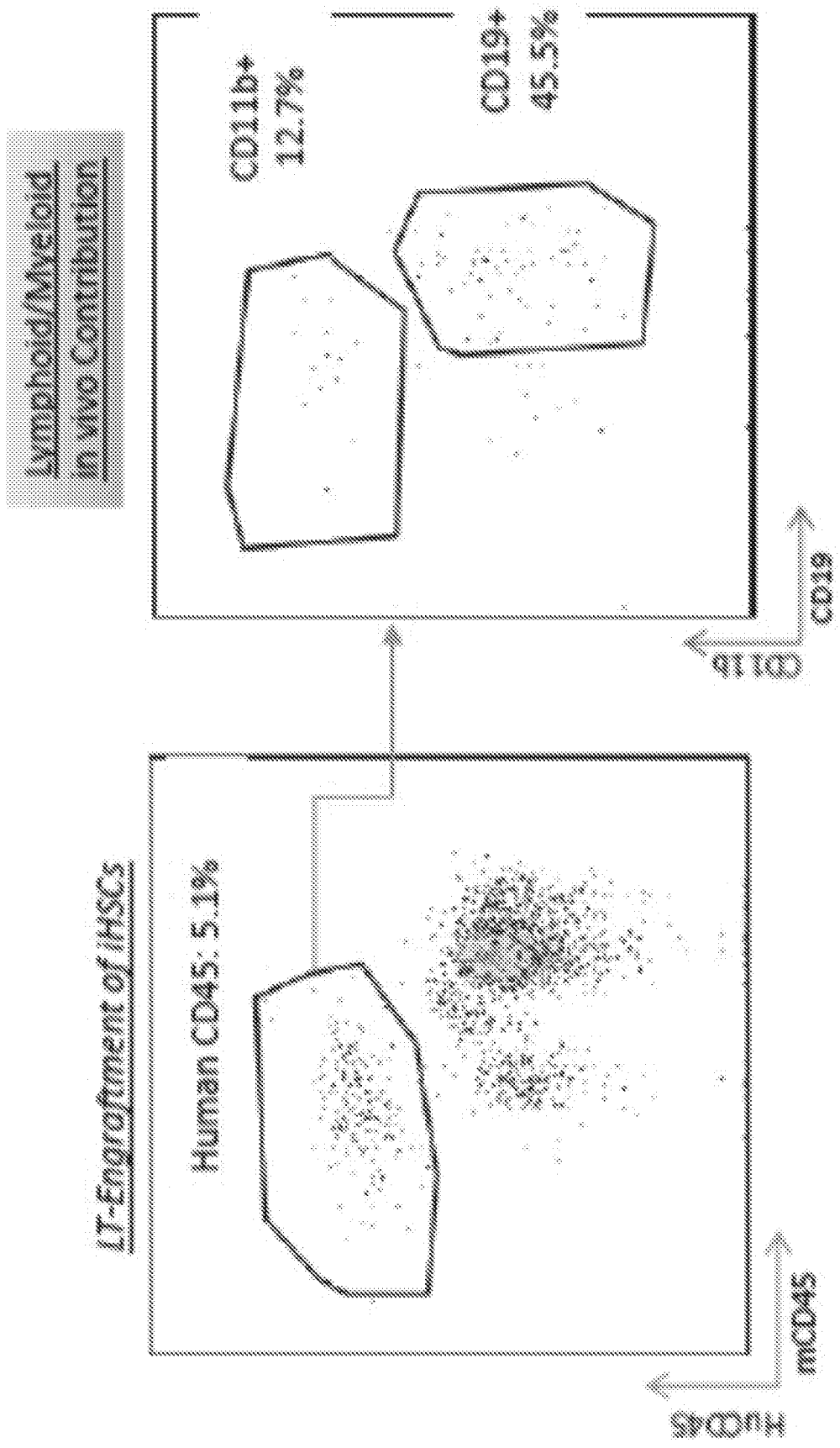

Lymphoid/Myeloid
in vivo Contribution
CD11b+
12.7%
CD19+
45.5%
CD11b
CD19
LT-Engraftment of iHSCs
Human CD45: 5.1%
mCD45
HuCD45

FIG. 9A

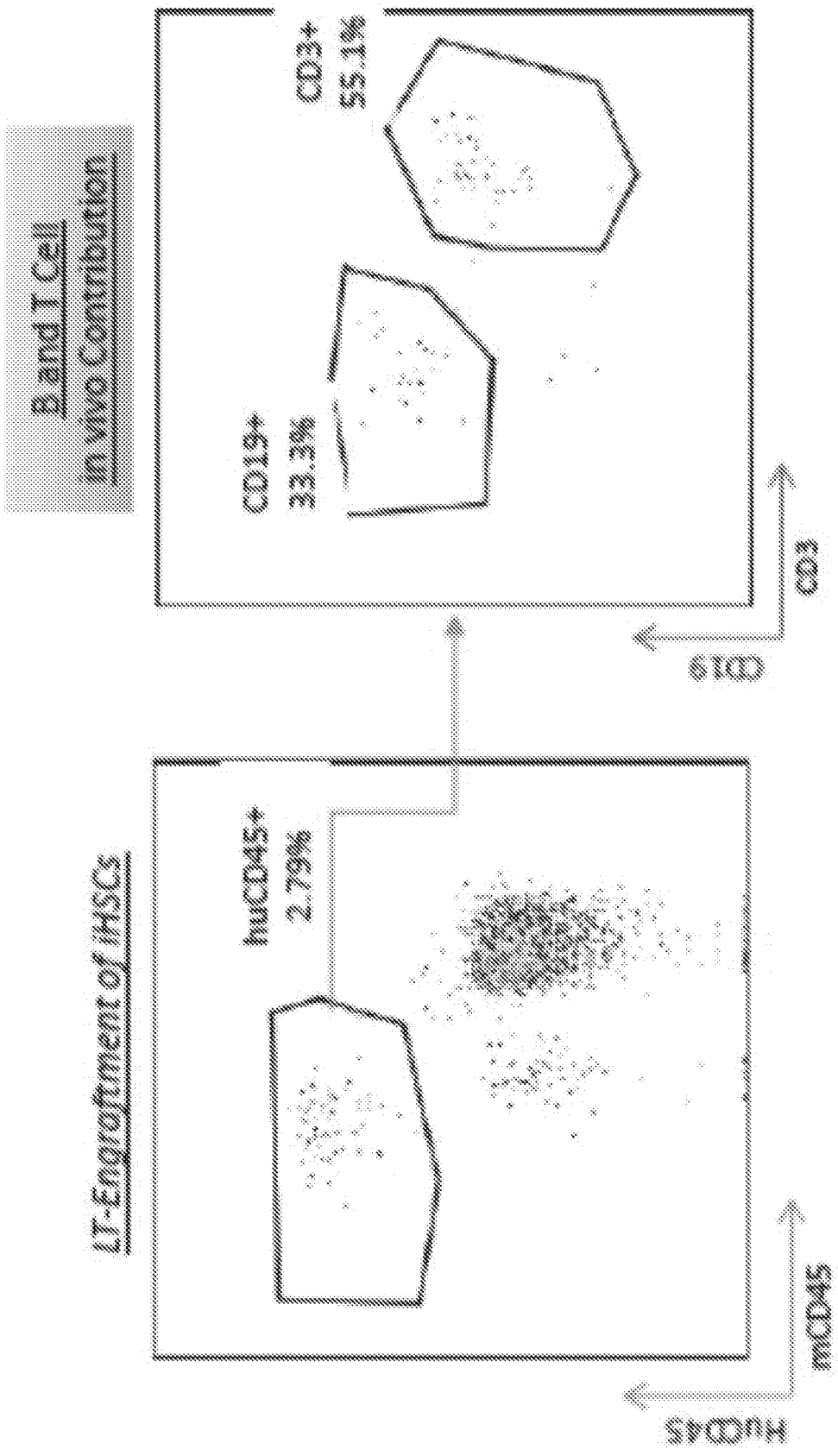

LT-Engraftment of iHSCs
huCD45+
2.79%
huCD45
mCD45
B and T Cell
in vivo Combution
CD19+
33.3%
CD3+
55.1%
CD19
CD3

FIG. 9B

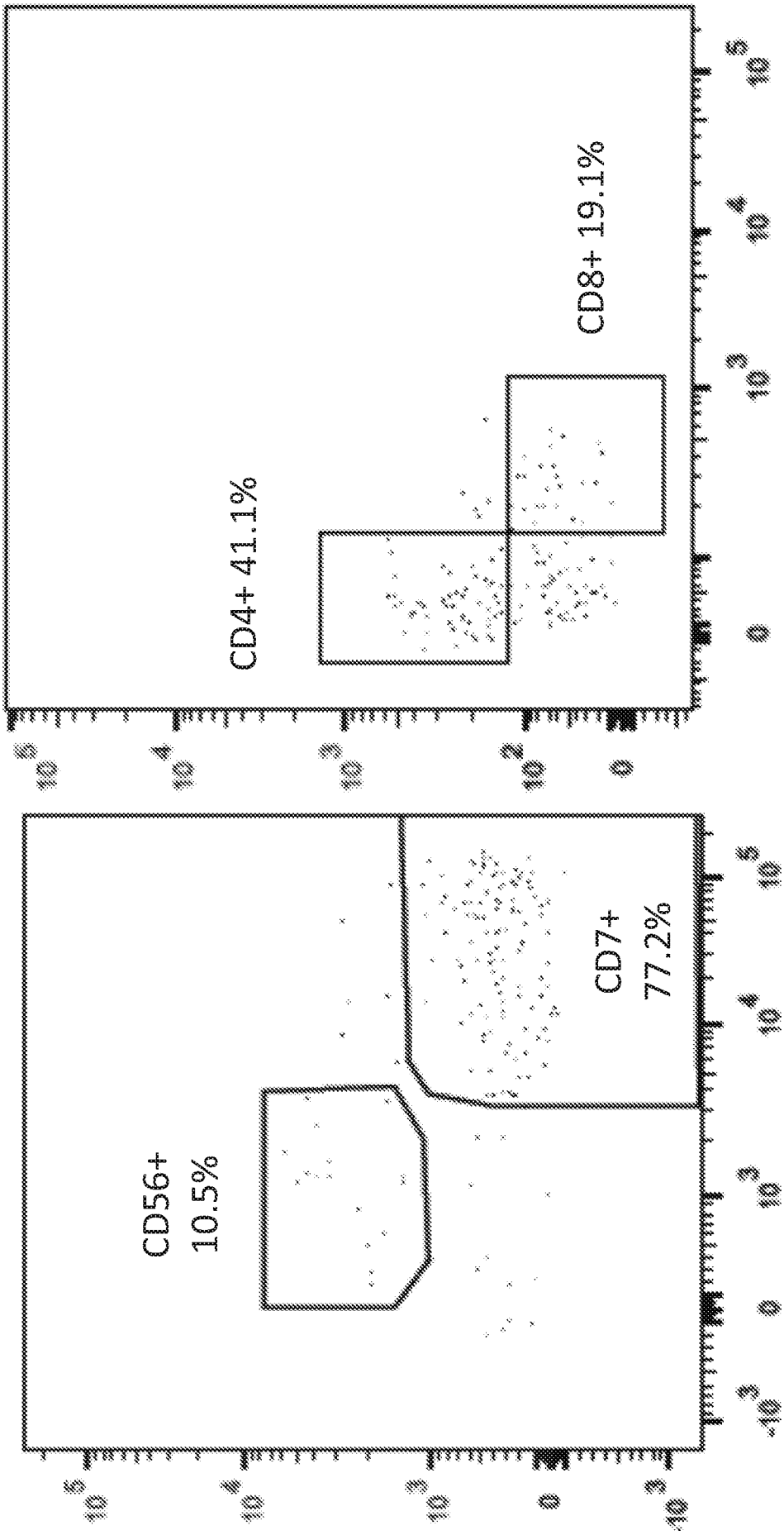


FIG. 10

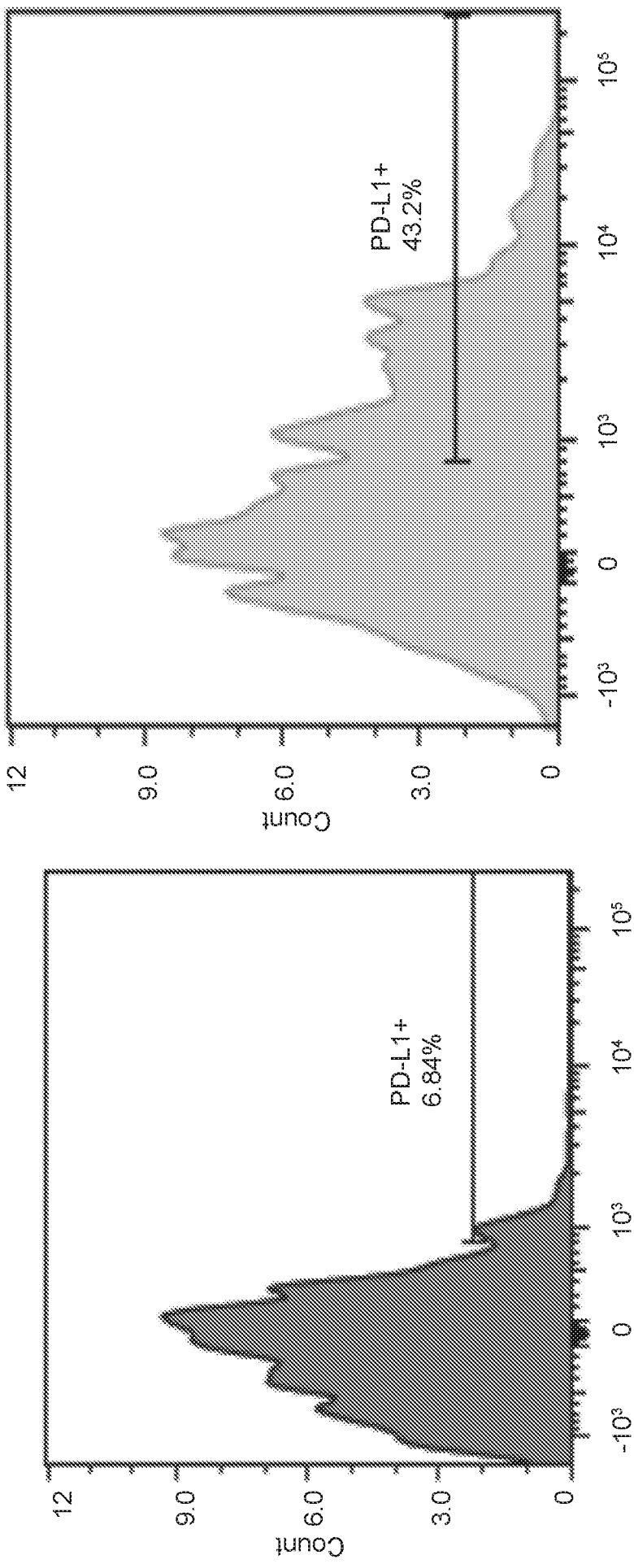


FIG. 11

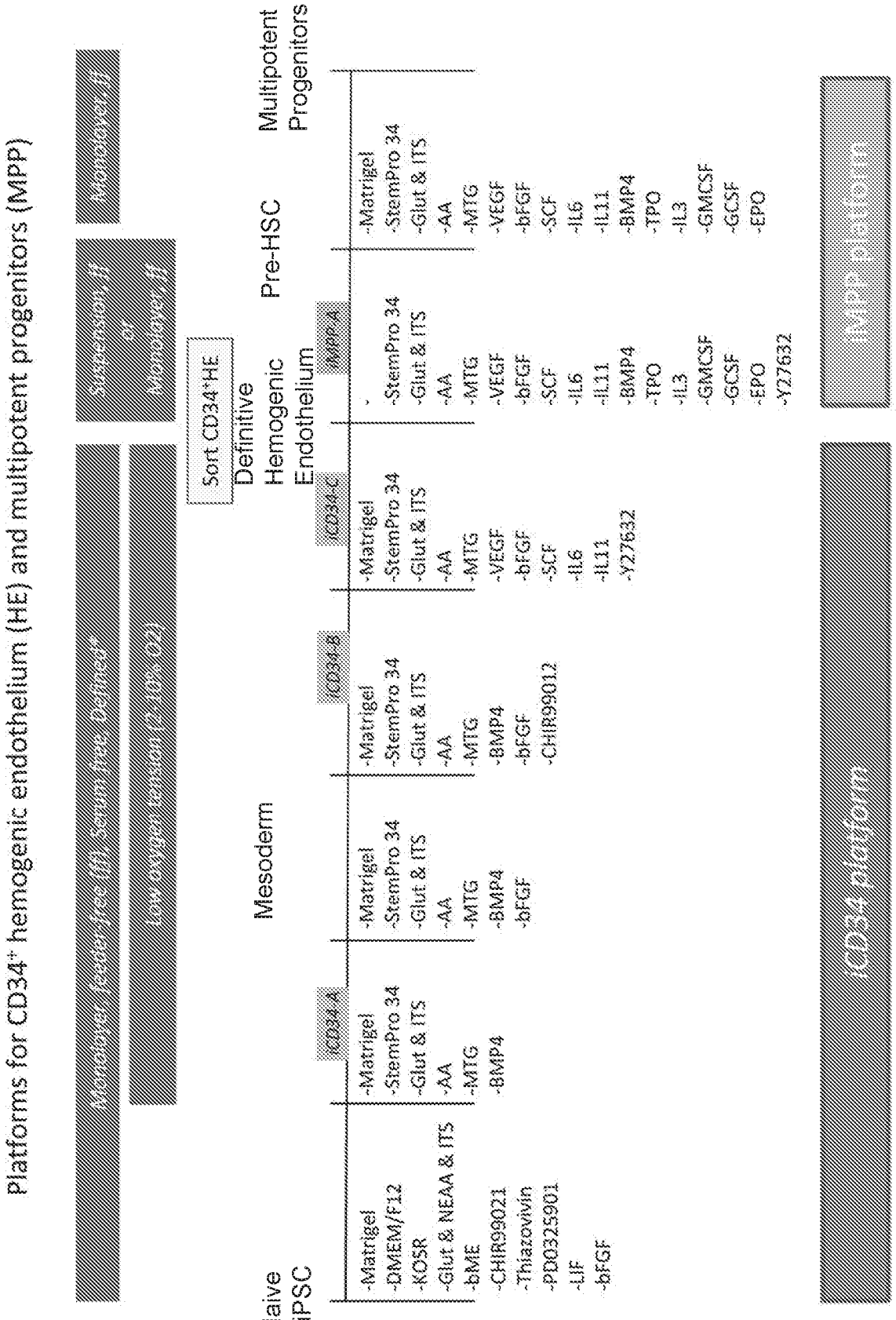

Platforms for CD34+ hemogenic endothelium (HE) and multipotent progenitors (MPP)
Monolayer feeder free (1) seamless Defined
Monolayer feeder free (2) 10% O2
Suspension (1) or Monolayer (2)
Monolayer (1)
Sort CD34+HE
Naive hiPSC
Mesoderm
Definitive Hemogenic Endothelium
Pre-HSC
Multipotent Progenitors
CD34-A
CD34-B
CD34-C
MPP-A
-Matrigel
-DMEM/F12
-KOSR
-Glut & NEAA & ITS
-bME
-CHIR99021
-Thiazovivin
-PD0325901
-LIF
-bFGF
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-BMP4
-bFGF
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-BMP4
-bFGF
-CHIR99012
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-VEGF
-bFGF
-SCF
-IL6
-IL11
-Y27632
-
-StemPro 34
-Glut & ITS
-AA
-MTG
-VEGF
-bFGF
-SCF
-IL6
-IL11
-BMP4
-TPO
-IL3
-GMCSF
-GCSF
-EPO
-Y27632
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-VEGF
-bFGF
-SCF
-IL6
-IL11
-BMP4
-TPO
-IL3
-GMCSF
-GCSF
-EPO
CD34 population
MPP population

FIG. 12

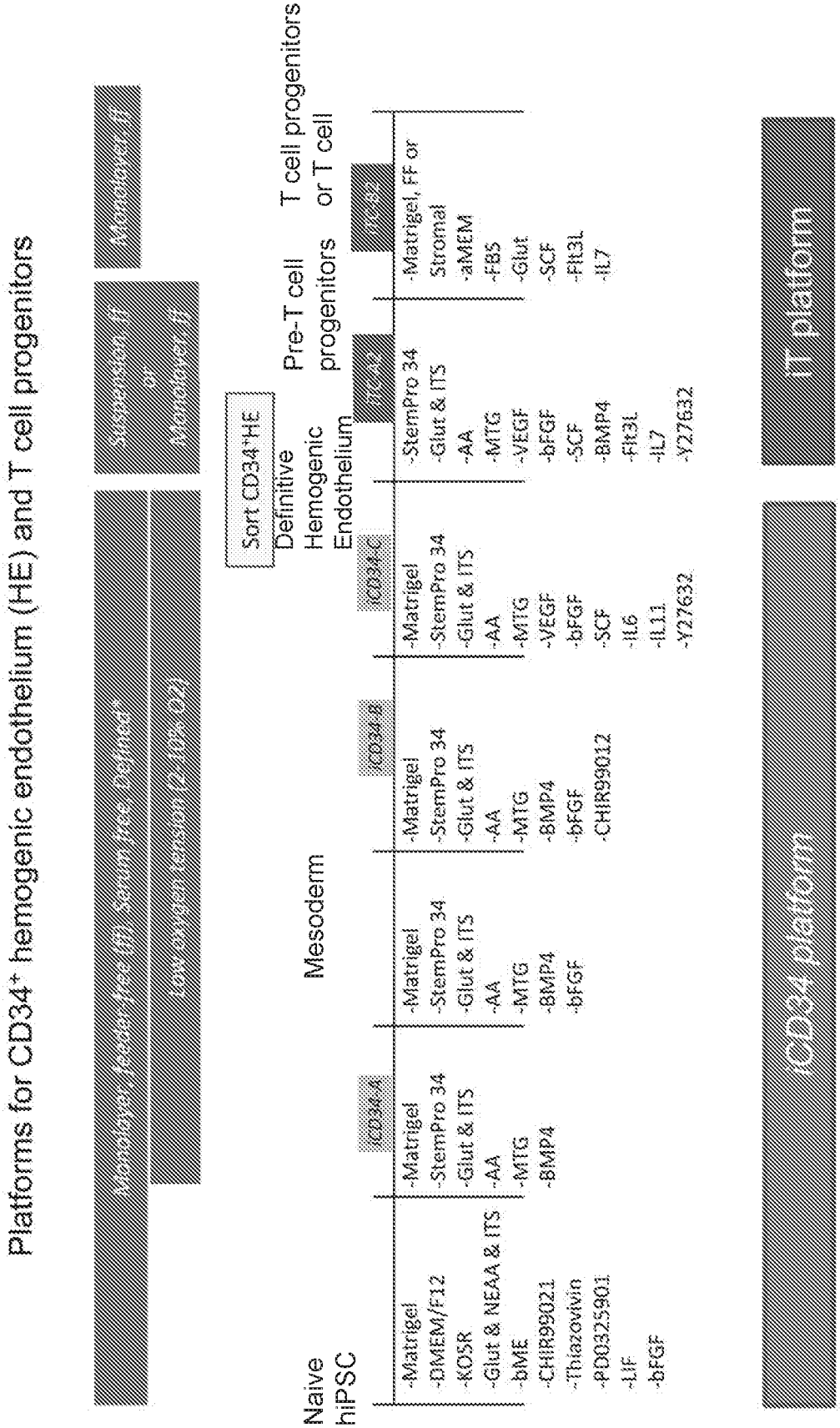

Platforms for CD34+ hemogenic endothelium (HE) and T cell progenitors
Monolayer feeder-free, serum free, defined
Low oxygen tension (2-10% O2)
Mesoderm
Sort CD34+HE
Definitive Hemogenic Endothelium
Pre-T cell progenitors
T cell progenitors or T cell
Suspension
or
Monolayer
Monolayer
Naive hiPSC
CD34-
CD34 a
CD34 c
-Matrigel
-DMEM/F12
-KOSR
-Glut & NEAA & ITS
-bME
-CHIR99021
-Thiazovivin
-PD0325901
-LIF
-bFGF
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-BMP4
-bFGF
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-BMP4
-bFGF
-CHIR99012
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-VEGF
-bFGF
-SCF
-IL6
-IL11
-Y27632
-StemPro 34
-Glut & ITS
-AA
-MTG
-VEGF
-bFGF
-SCF
-BMP4
-Flt3L
-IL7
-Y27632
-Matrigel, FF or Stromal
-aMEM
-FBS
-Glut
-SCF
-Flt3L
-IL7
iCD34 platform
iT platform

FIG. 13

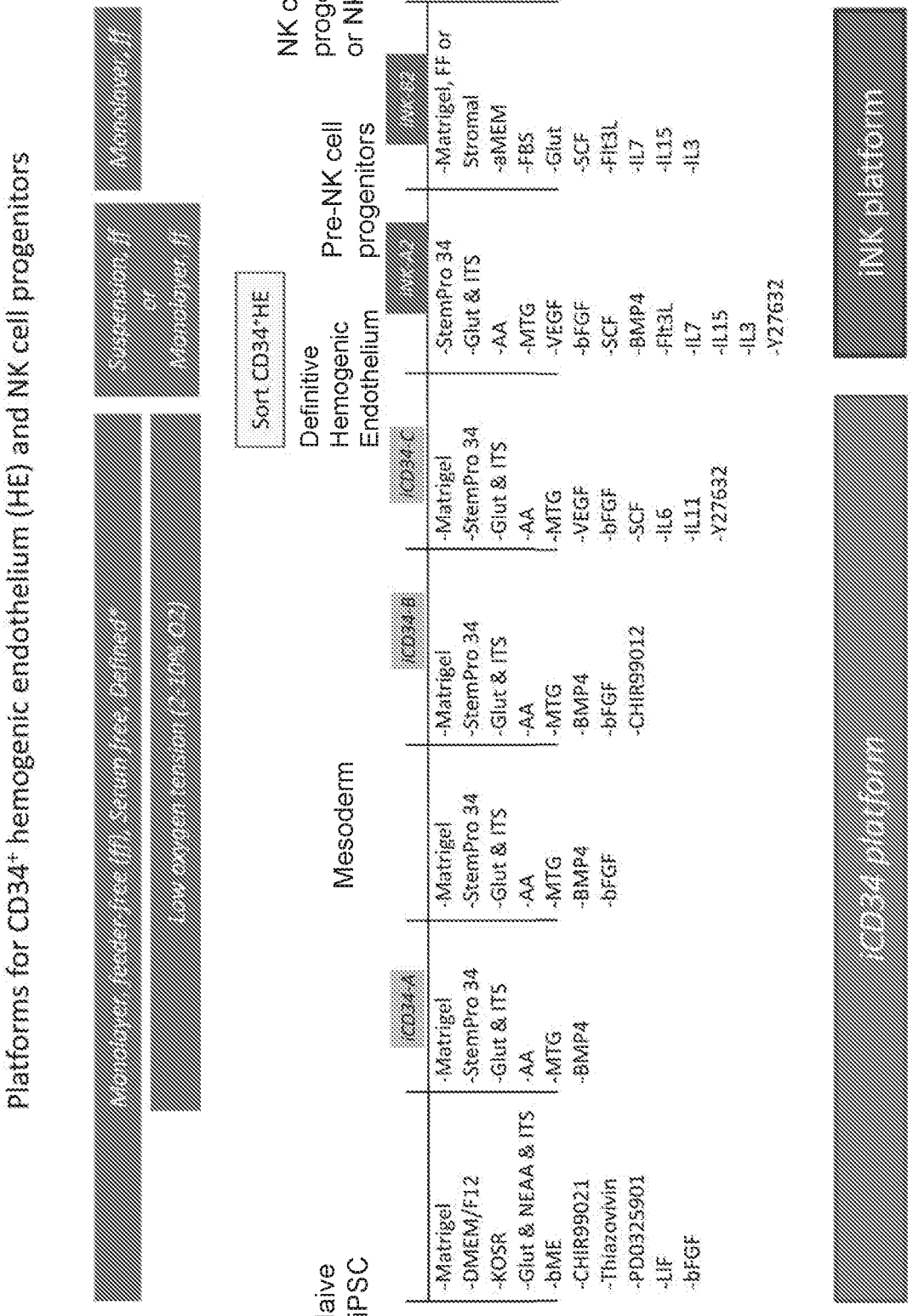

Platforms for CD34+ hemogenic endothelium (HE) and NK cell progenitors
Monolayer (adherent) Serum free Protocol
Low cell density (2-10x10^3)
Suspension or Monolayer
Monolayer
Sort CD34+HE
Naive hiPSC
Mesoderm
Definitive Hemogenic Endothelium
Pre-NK cell progenitors
NK cell progenitors or NK cell
CD34-A
CD34-B
CD34-C
-Matrigel
-DMEM/F12
-KOSR
-Glut & NEAA & ITS
-bME
-CHIR99021
-Thiazovivin
-PD0325901
-LIF
-bFGF
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-BMP4
-bFGF
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-BMP4
-bFGF
-CHIR99012
-Matrigel
-StemPro 34
-Glut & ITS
-AA
-MTG
-VEGF
-bFGF
-SCF
-IL6
-IL11
-Y27632
-StemPro 34
-Glut & ITS
-AA
-MTG
-VEGF
-bFGF
-SCF
-BMP4
-Flt3L
-IL7
-IL15
-IL3
-Y27632
-Matrigel, FF or Stromal
-aMEM
-FBS
-Glut
-SCF
-Flt3L
-IL7
-IL15
-IL3
iCD34 platform
iNK platform

FIG. 14

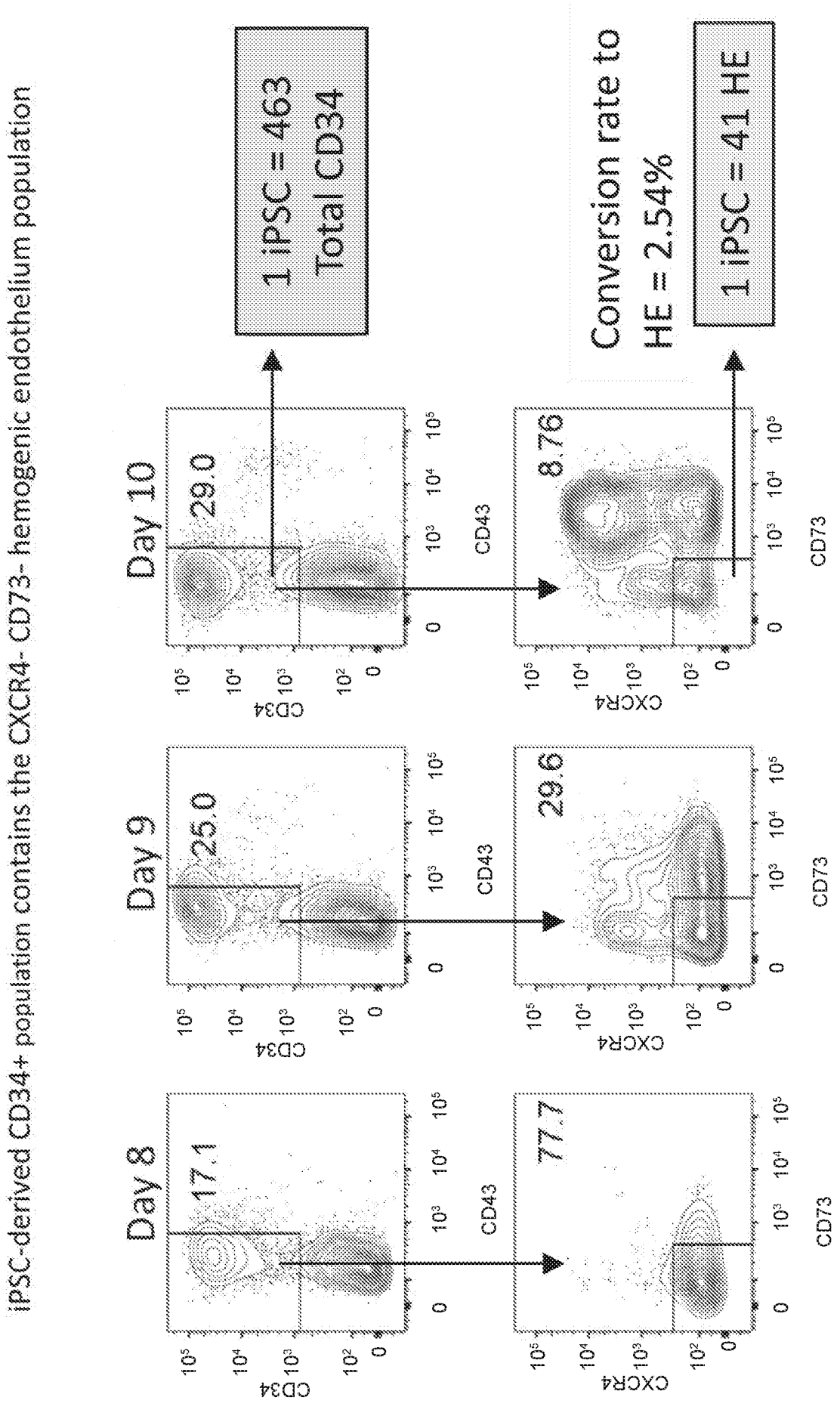

iPSC-derived CD34+ population contains the CXCR4- CD73- hemogenic endothelium population
Day 8
17.1
77.7
Day 9
25.0
29.6
Day 10
29.0
8.76
CD43
CD34
CXCR4
CD73
1 iPSC = 463
Total CD34
Conversion rate to
HE = 2.54%
1 iPSC = 41 HE

FIG. 15A

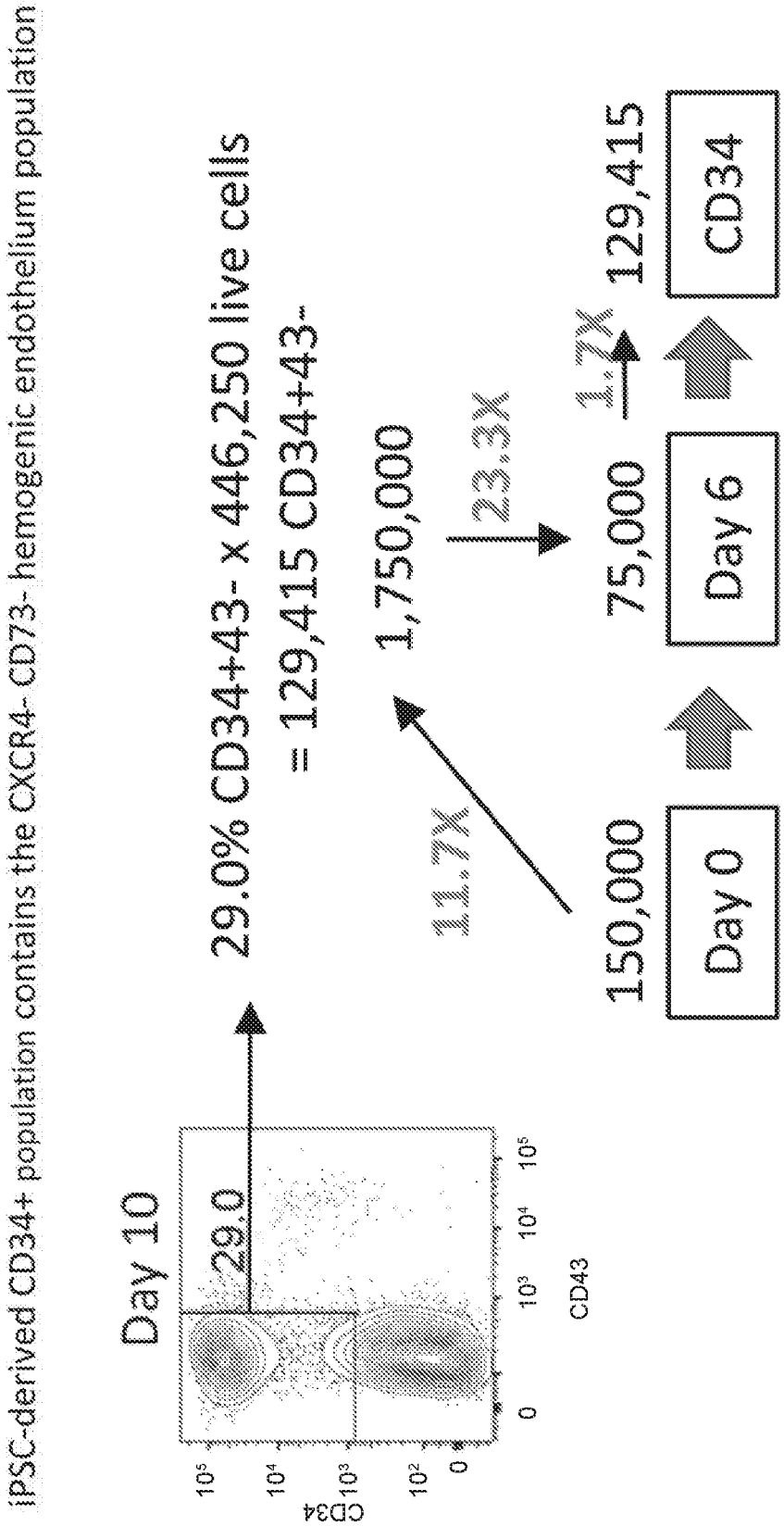

iPSC-derived CD34+ population contains the CXCR4- CD73- hemogenic endothelium population
Day 10
29.0
CD34
CD43
29.0% CD34+43- x 446,250 live cells
= 129,415 CD34+43-
150,000
Day 0
11.7X
1,750,000
23.3X
75,000
Day 6
1.7X
129,415
CD34
11.7 x 23.3 x 1.7 = 463 CD34
1 iPSC = 463 CD34

FIG. 15B

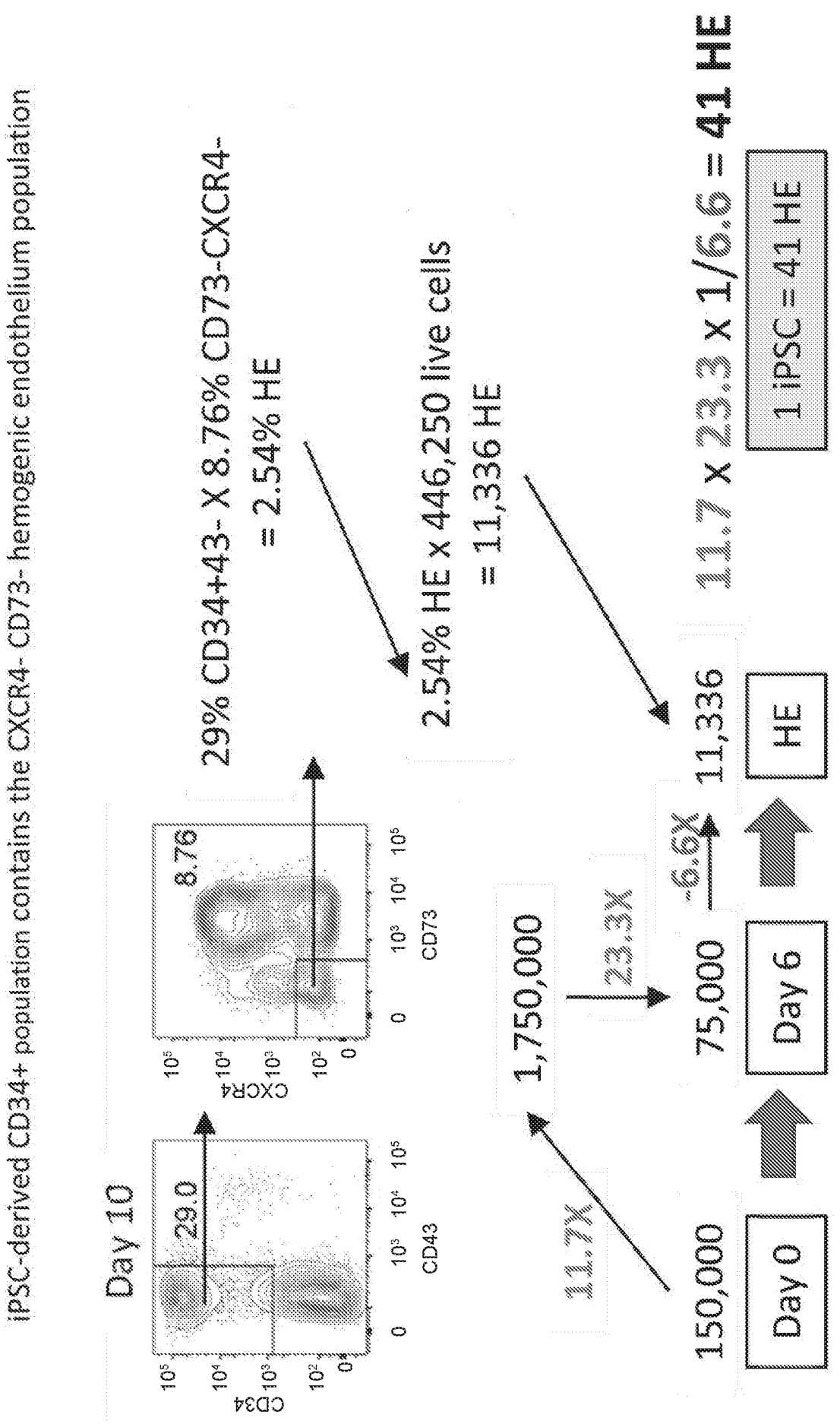

iPSC-derived CD34+ population contains the CXCR4- CD73- hemogenic endothelium population
Day 10
29.0
CD34
CD43
8.76
CXCR4
CD73
29% CD34+43- X 8.76% CD73-CXCR4- = 2.54% HE
2.54% HE x 446,250 live cells = 11,336 HE
11.7 x 23.3 x 1/6.6 = 41 HE
1 iPSC = 41 HE
150,000
Day 0
11.7%
1,750,000
23.3X
75,000
Day 6
-6.6X
11,336
HE

FIG. 15C

Optimization of protocol leads to enhanced differentiation, demonstrating that each component plays a critical role.

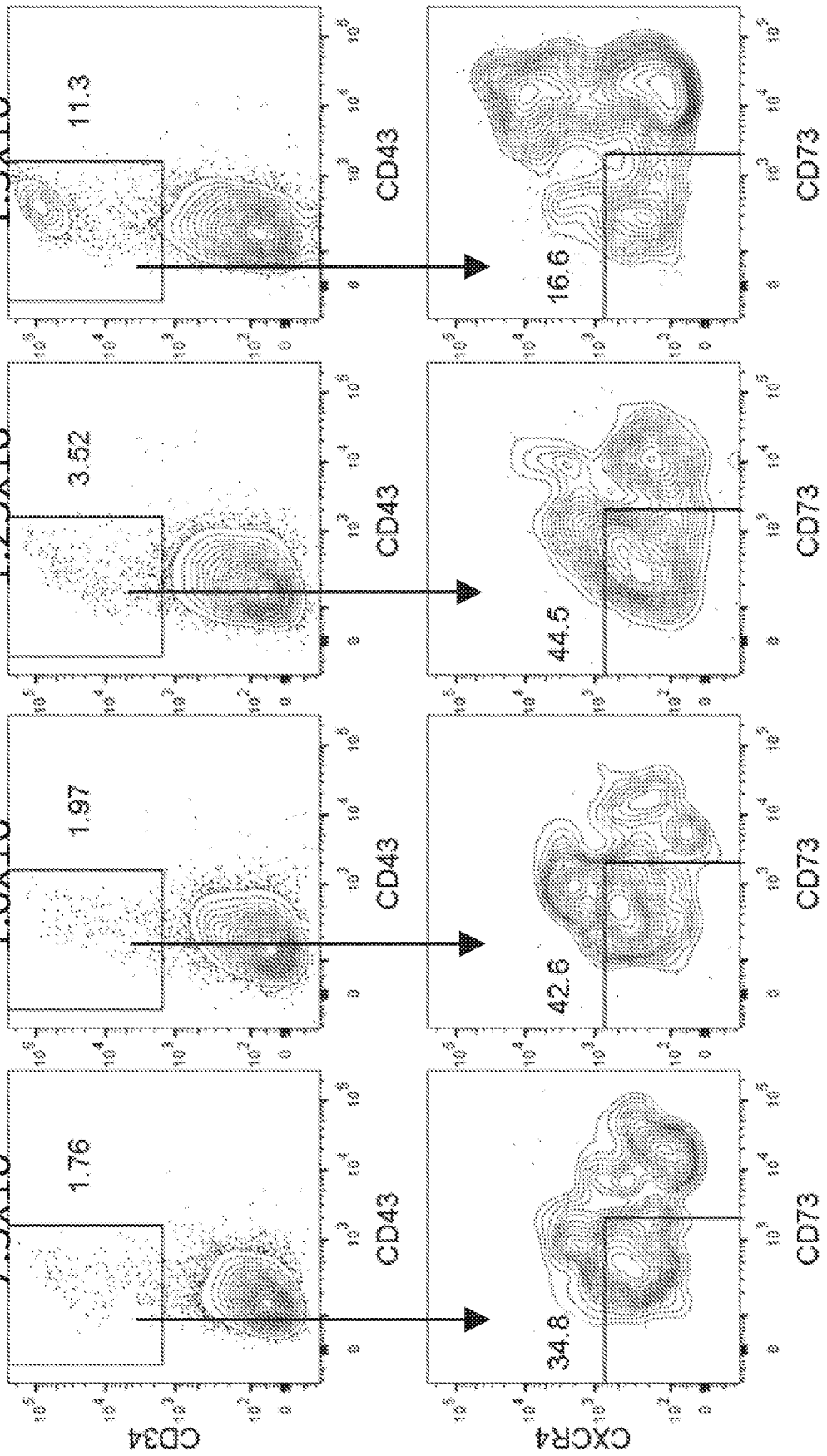


FIG. 16A

Optimization of protocol leads to enhanced differentiation, demonstrating that each component plays a critical role.

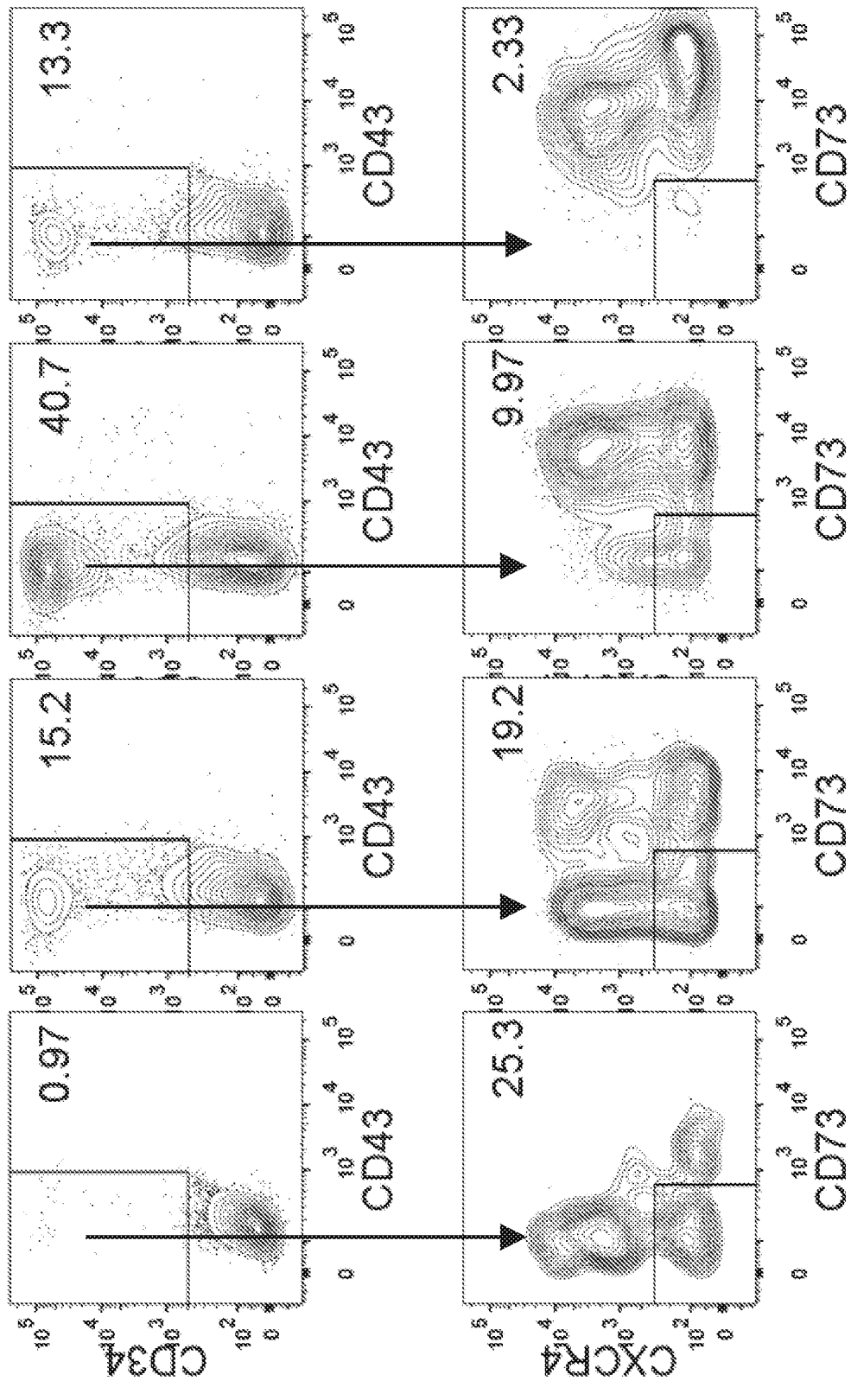


FIG. 16B

Optimization of protocol leads to enhanced differentiation, demonstrating that each component plays a critical role.

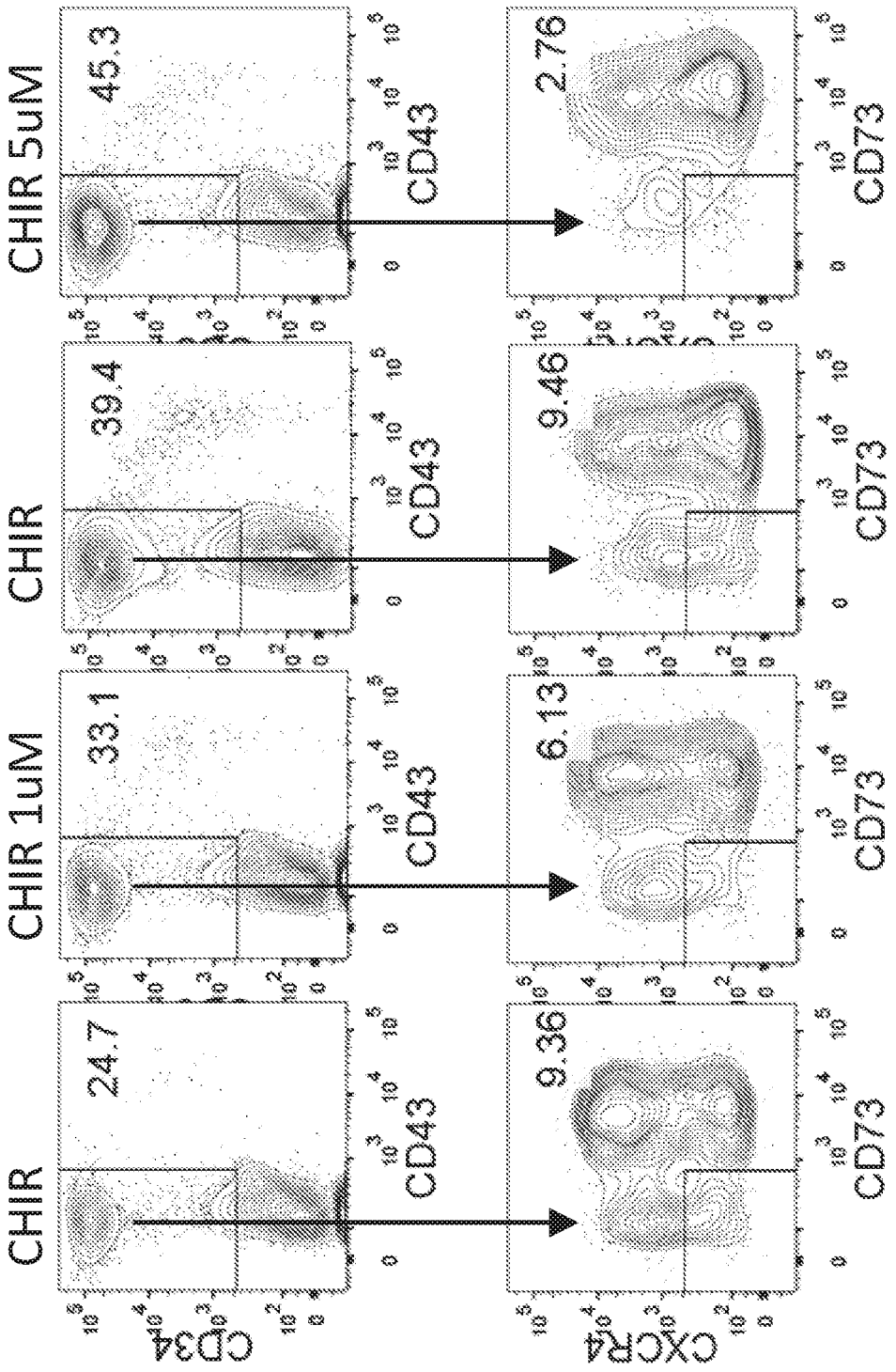


FIG. 16C

Optimization of protocol leads to enhanced differentiation, demonstrating that each component plays a critical role.

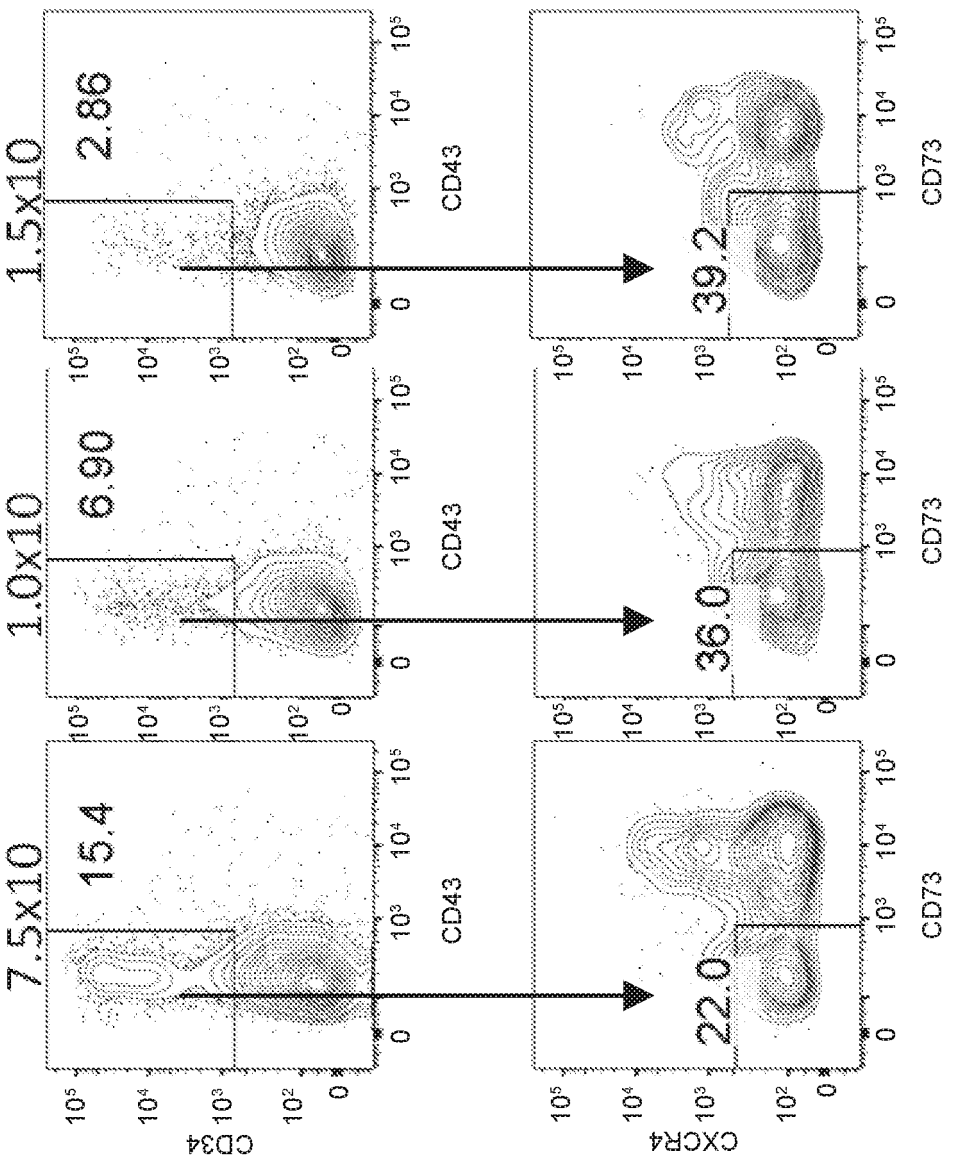


FIG. 16D

Optimization of protocol leads to enhanced differentiation, demonstrating that each component plays a critical role.

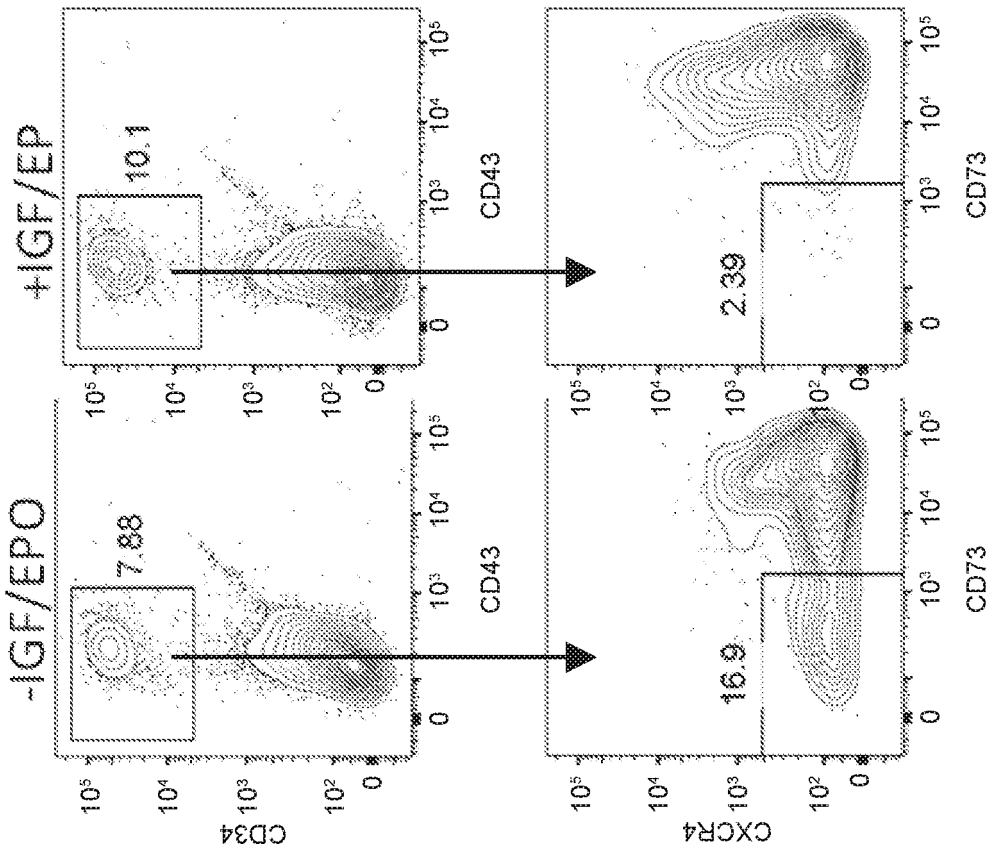


FIG. 16E

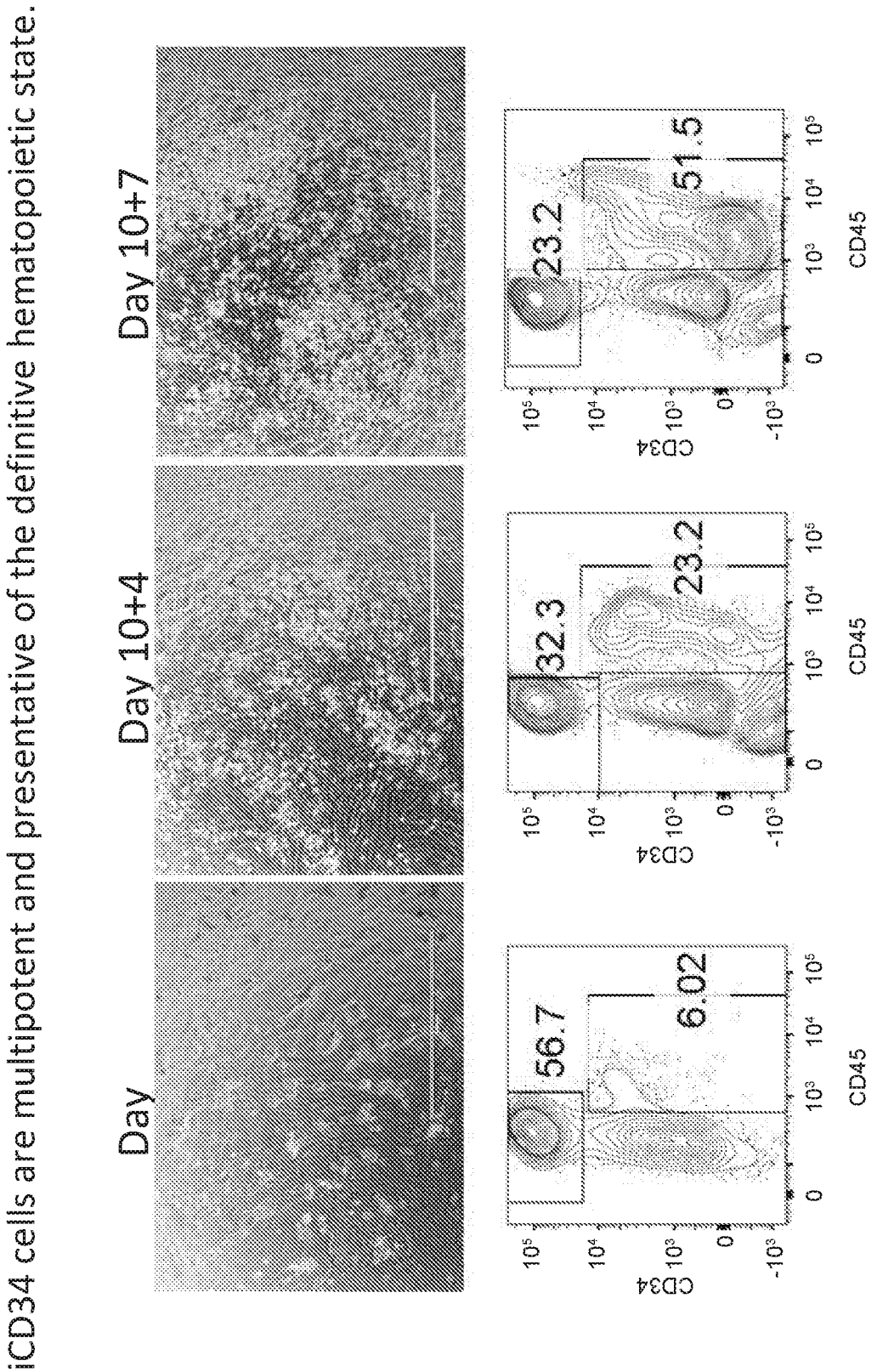

iCD34 cells are multipotent and presentative of the definitive hematopoietic state.
Day
Day 10+4
Day 10+7
56.7
6.02
32.3
23.2
23.2
51.5
CD34
CD45

FIG. 17A

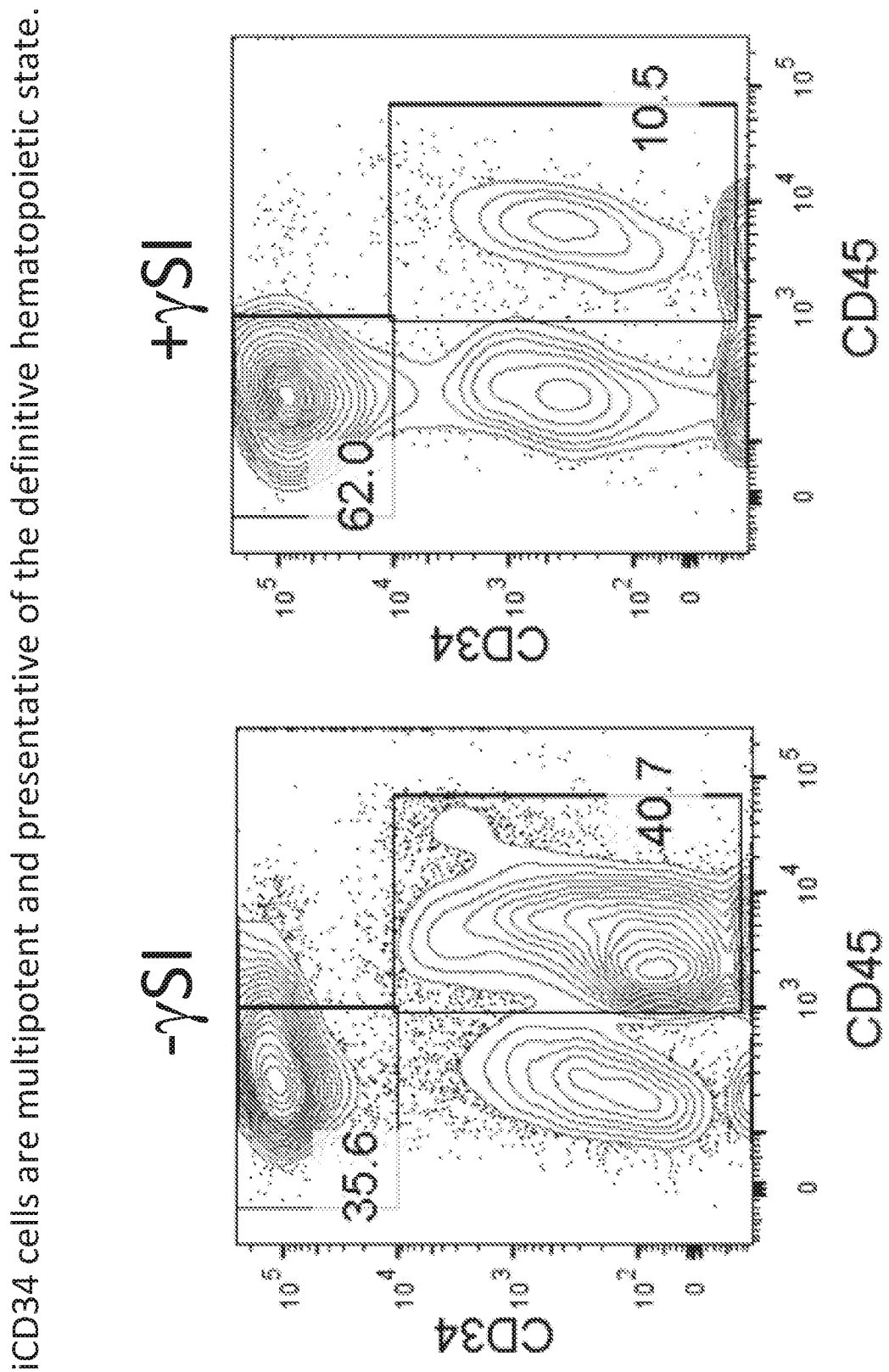

iCD34 cells are multipotent and presentative of the definitive hematopoietic state.
-γSI
+γSI
CD34
CD45
35.6
40.7
62.0
10.5
0
10^2
10^3
10^4
10^5

FIG. 17B

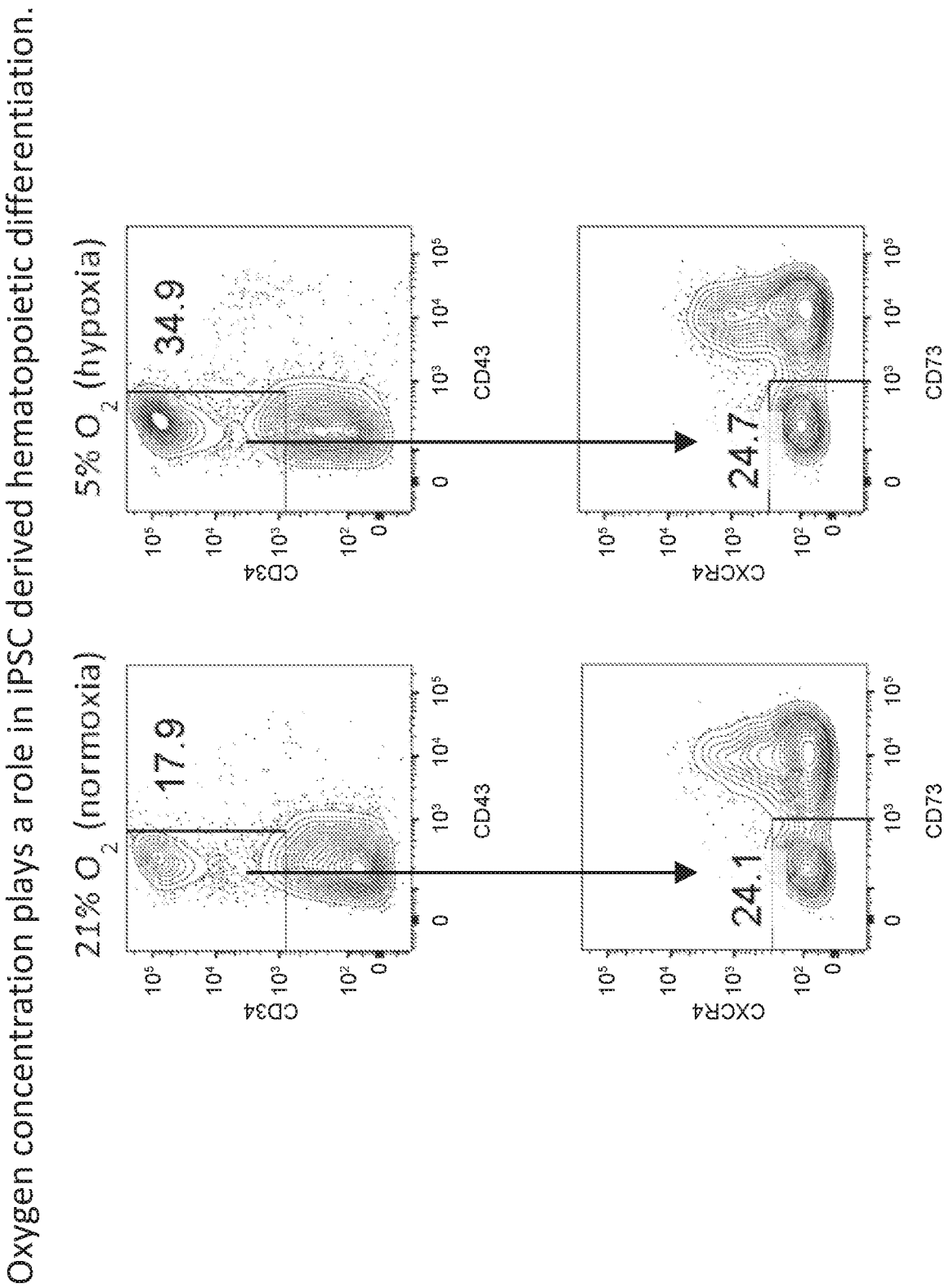

Oxygen concentration plays a role in iPSC derived hematopoietic differentiation.
21% O2 (normoxia)
5% O2 (hypoxia)
17.9
34.9
24.1
24.7
CD34
CD43
CXCR4
CD73
0
10^2
10^3
10^4
10^5

FIG. 18A

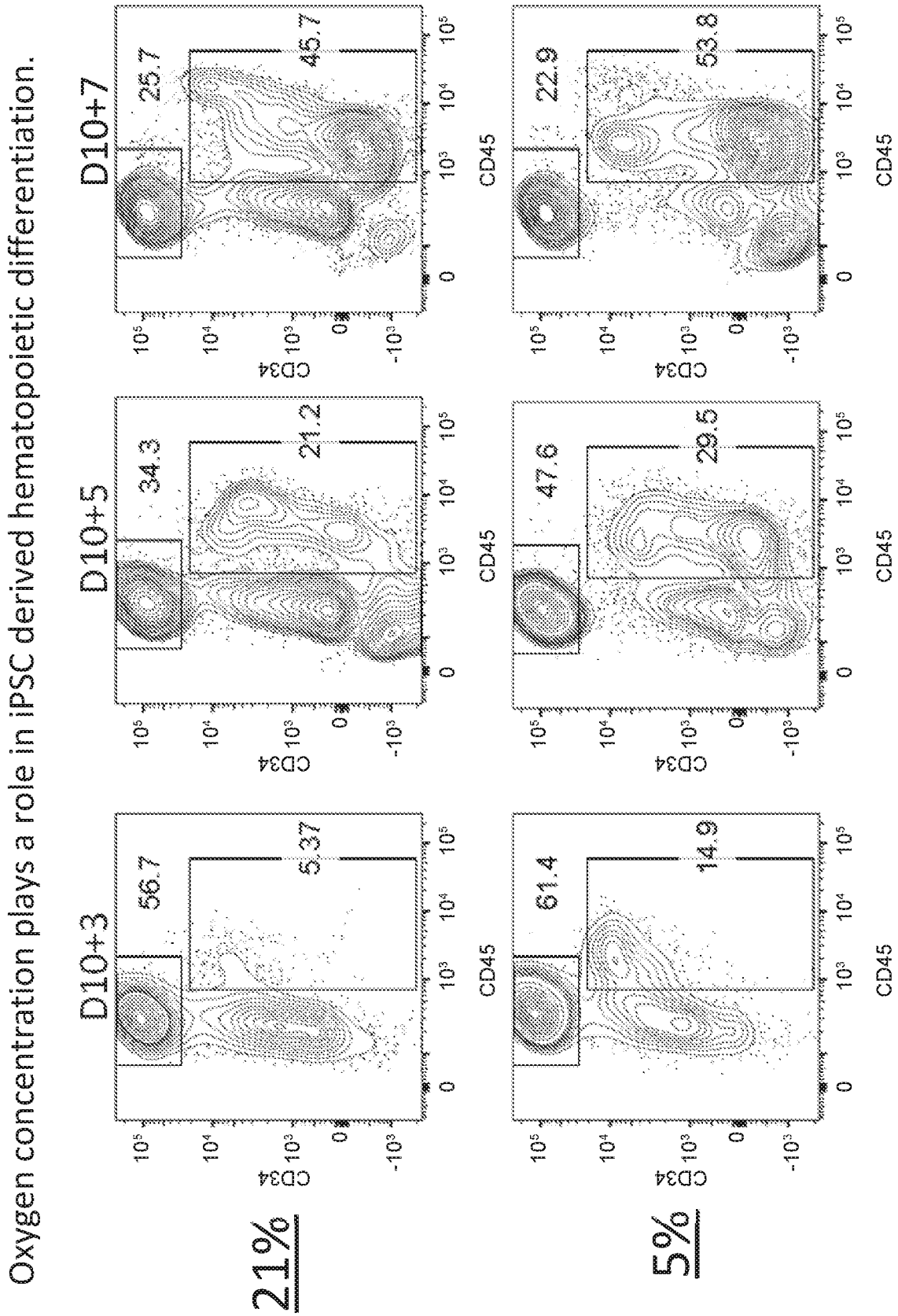

Oxygen concentration plays a role in iPSC derived hematopoietic differentiation.
D10+3
D10+5
D10+7
21%
5%
56.7
5.37
34.3
21.2
25.7
45.7
61.4
14.9
47.6
29.5
22.9
53.8
CD34
CD45

FIG. 18B

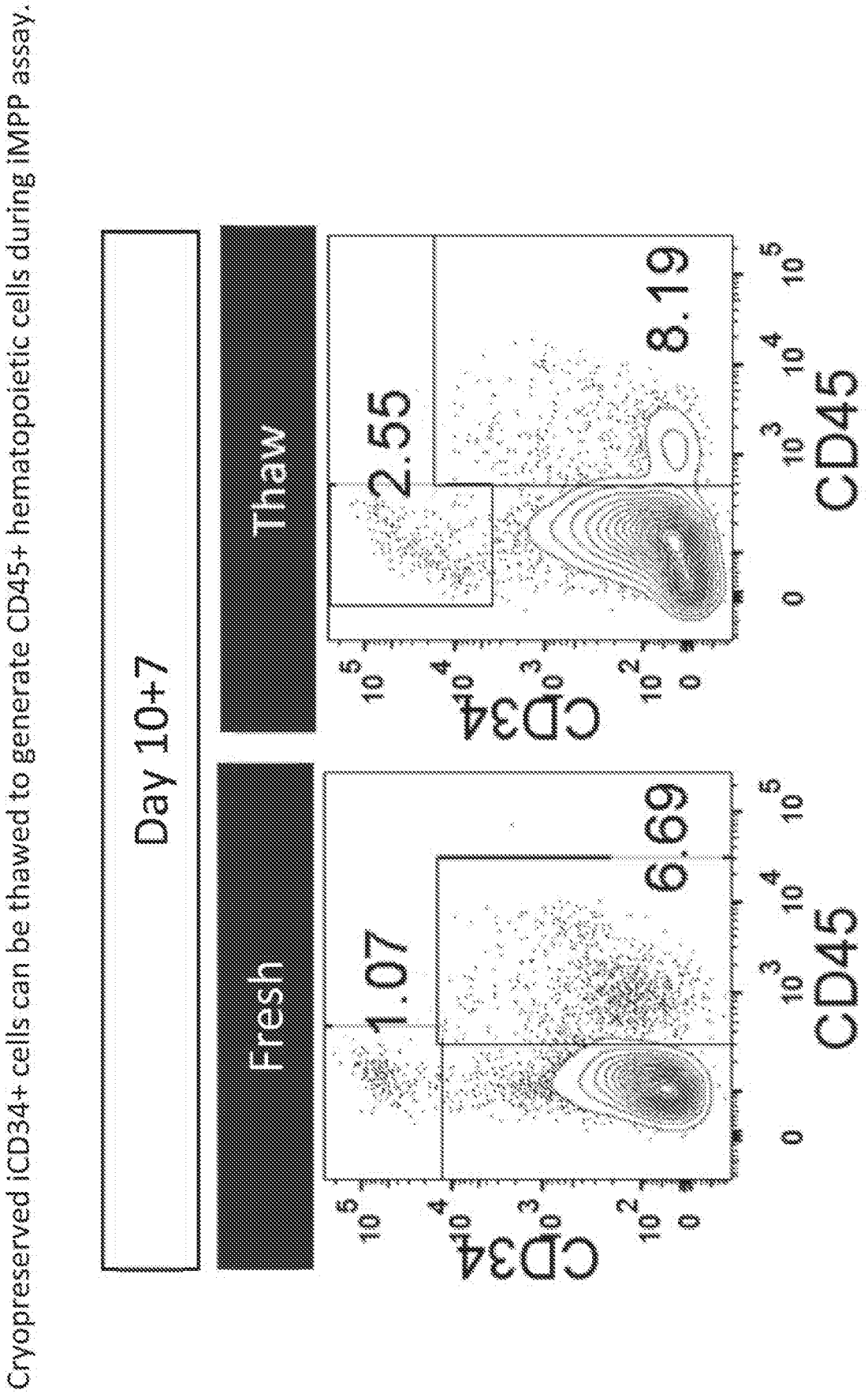

Cryopreserved iCD34+ cells can be thawed to generate CD45+ hematopoietic cells during iMPP assay.
Day 10+7
Fresh
Thaw
1.07
6.69
2.55
8.19
CD34
CD45
0
10^2
10^3
10^4
10^5

FIG. 19A

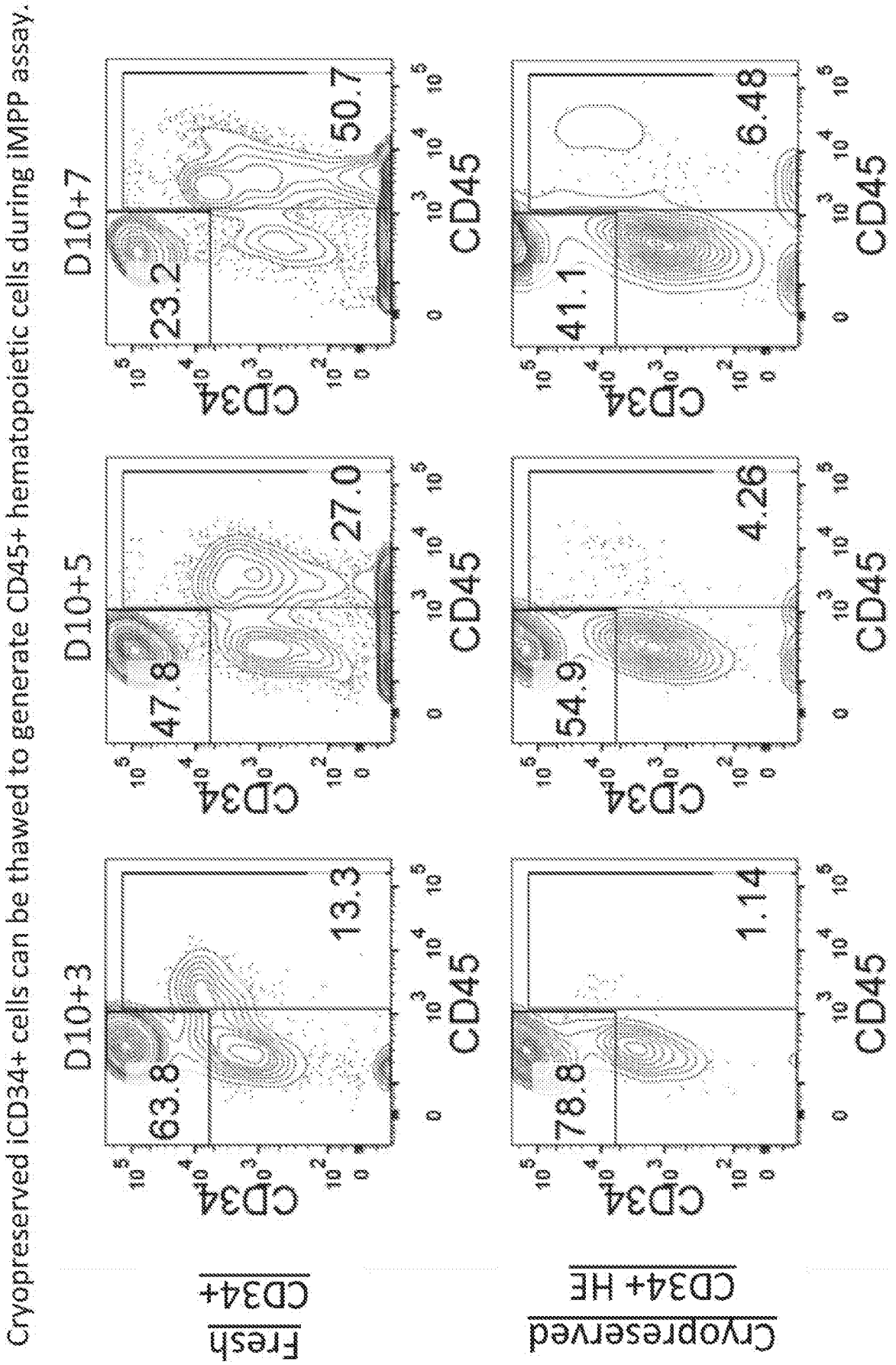

Cryopreserved iCD34+ cells can be thawed to generate CD45+ hematopoietic cells during iMPP assay.
Fresh CD34+
Cryopreserved CD34+ HE
D10+3
D10+5
D10+7
CD34
CD45
63.8
13.3
47.8
27.0
23.2
50.7
78.8
1.14
54.9
4.26
41.1
6.48

FIG. 19B

Cryopreserved iCD34+ cells can be thawed to generate CD45+ hematopoietic cells during iMPP assay.

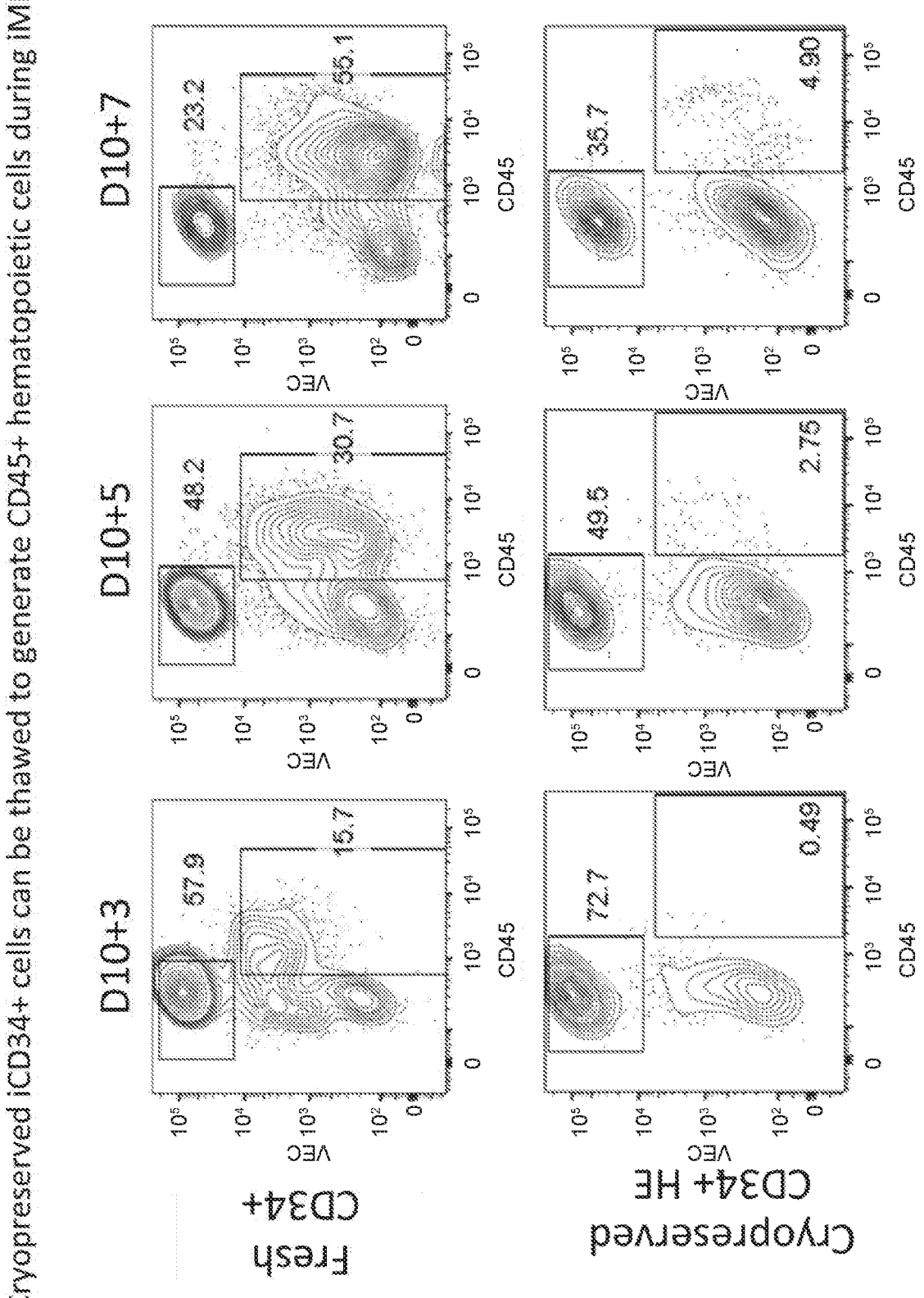


FIG. 19C

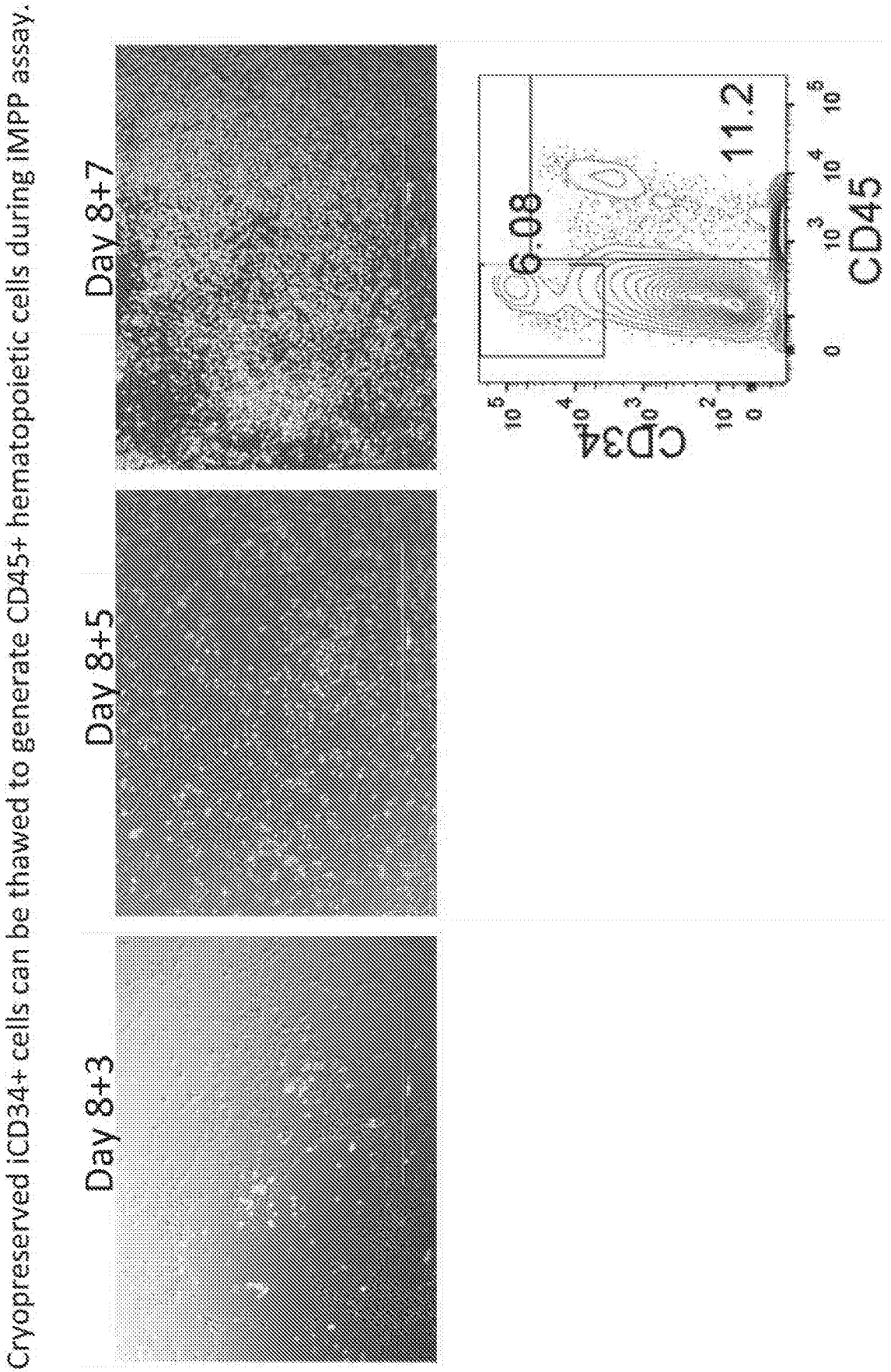

Cryopreserved iCD34+ cells can be thawed to generate CD45+ hematopoietic cells during iMPP assay.
Day 8+3
Day 8+5
Day 8+7
6.08
11.2
CD34
CD45

FIG. 19D

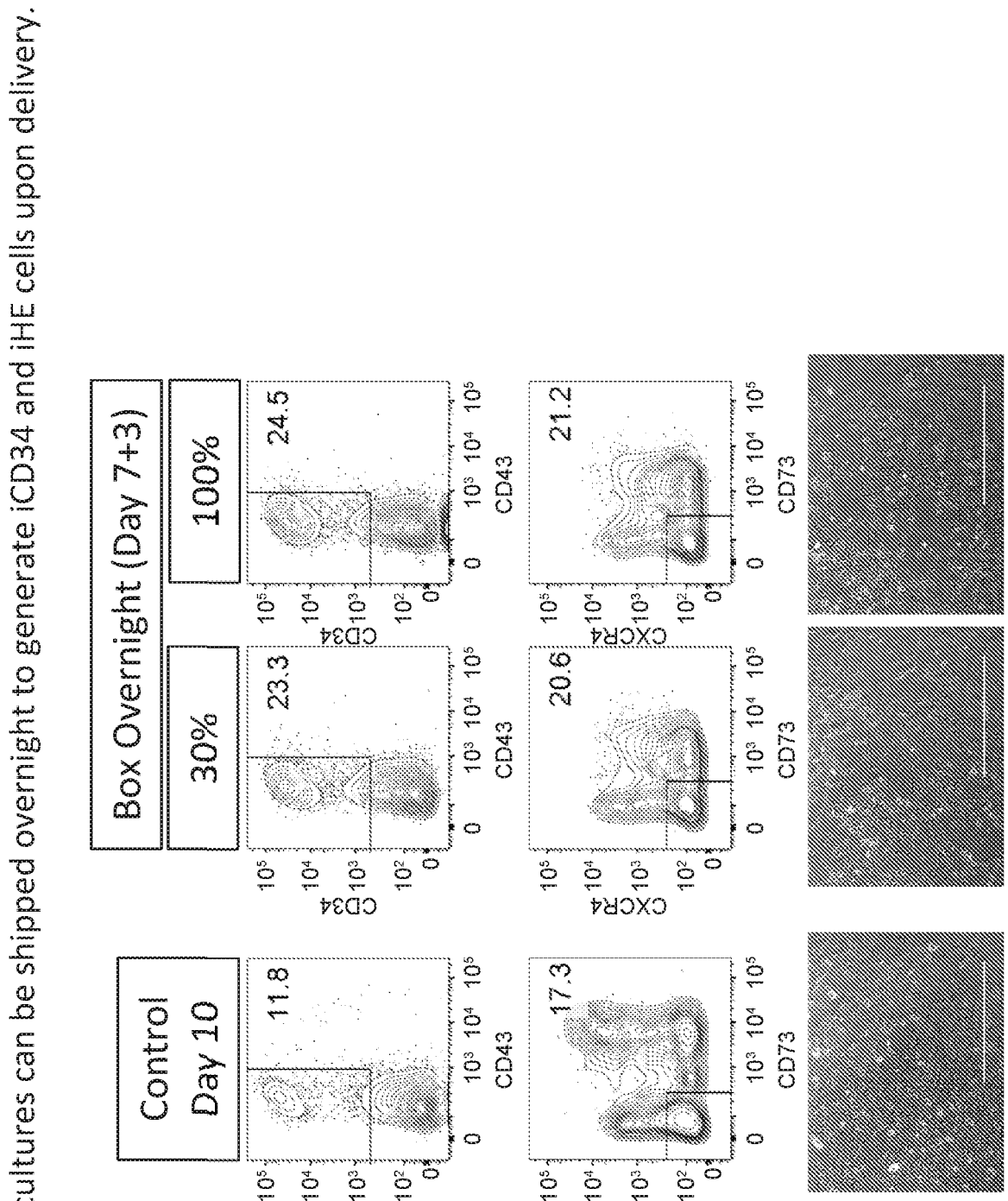

Day 8 differentiation cultures can be shipped overnight to generate iCD34 and iHE cells upon delivery.
Box Overnight (Day 7+3)
Control
Day 10
30%
100%
11.8
23.3
24.5
17.3
20.6
21.2
CD34
CD43
CXCR4
CD73

FIG. 20A

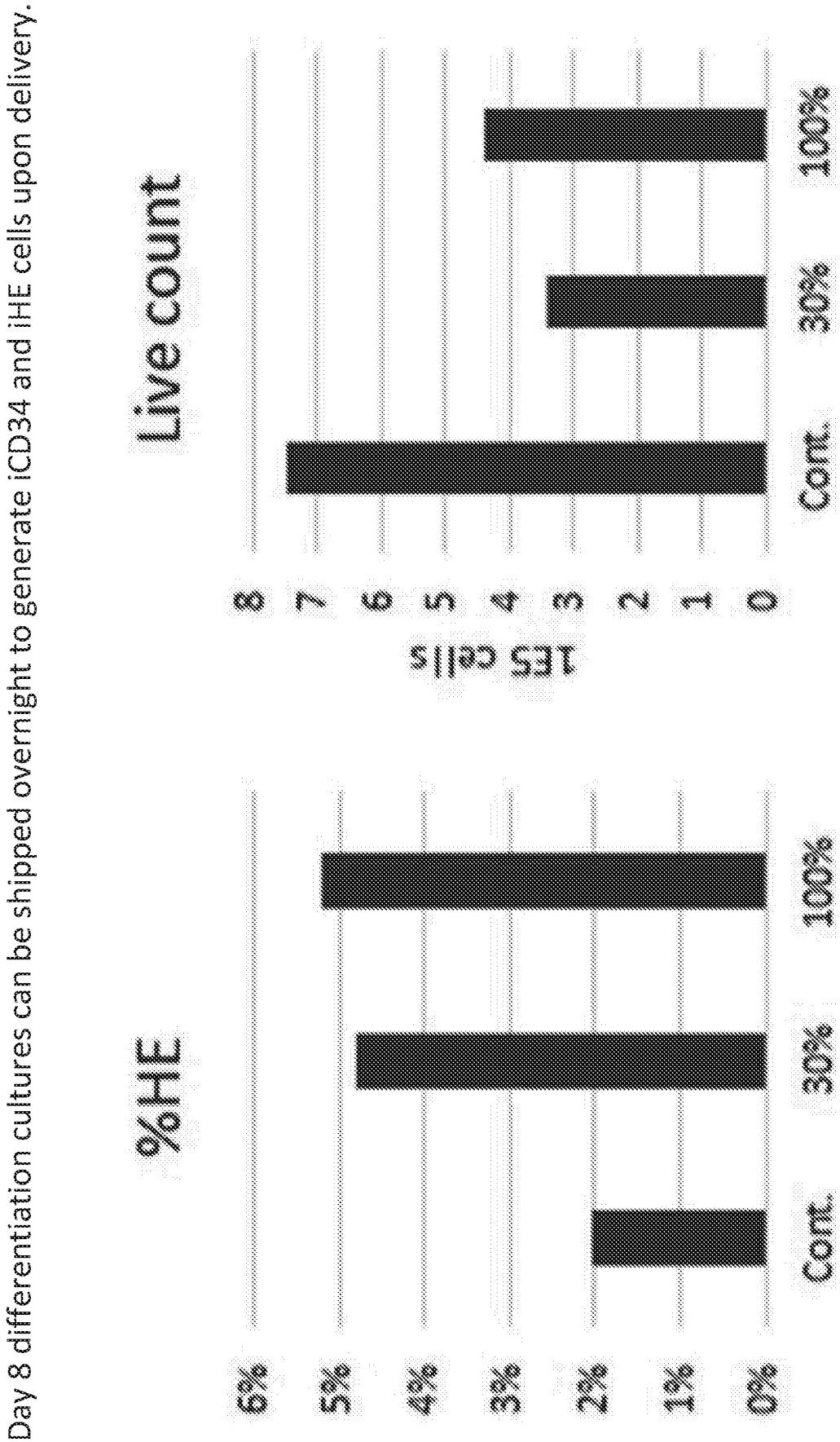

Day 8 differentiation cultures can be shipped overnight to generate iCD34 and iHE cells upon delivery.
%HE
6%
5%
4%
3%
2%
1%
0%
Cont.
30%
100%
Live count
1ES cells
8
7
6
5
4
3
2
1
0
Cont.
30%
100%

FIG. 20B

iPSC-derived, iCD34 differentiated towards T cells.

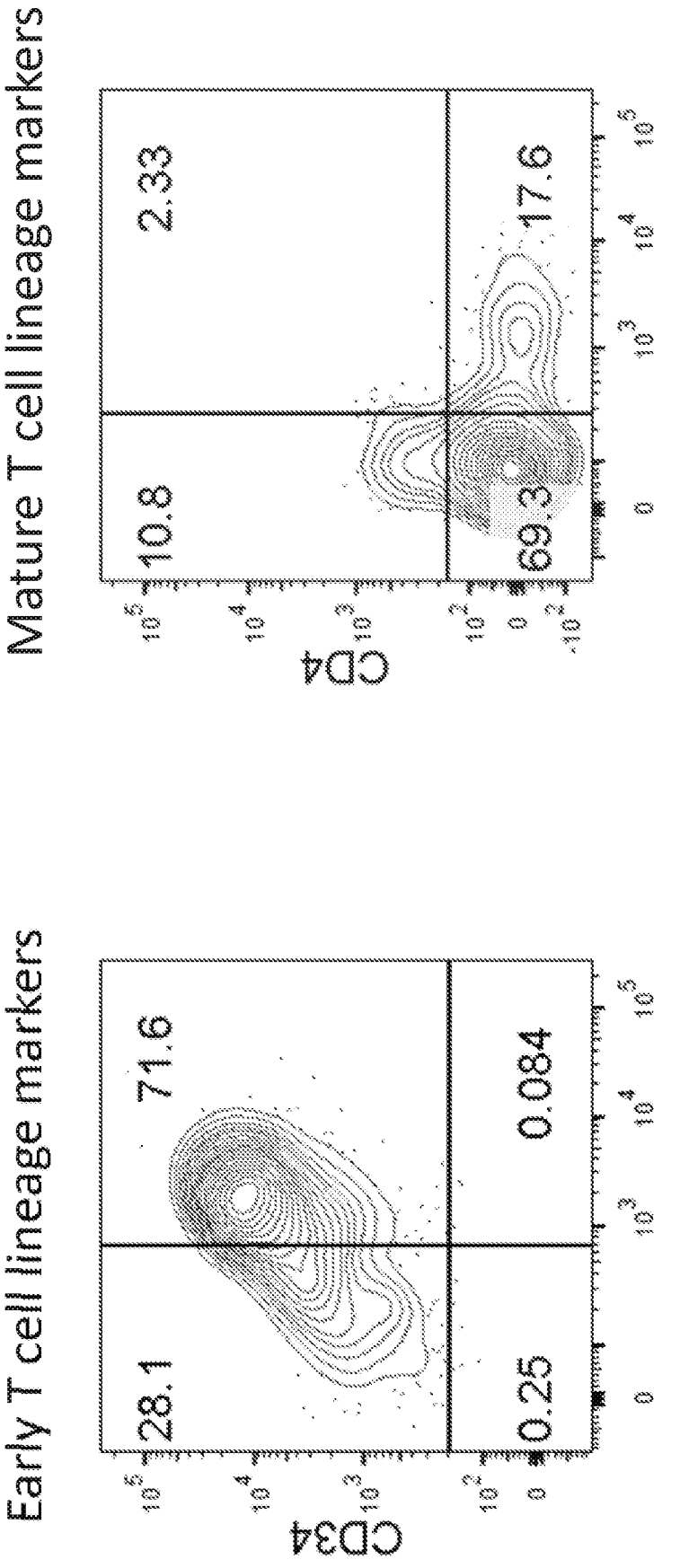


FIG. 21A

FIG. 21B

iPSC-derived, iCD34 differentiated towards T cells.

Day 5: UCB CD34+ cells versus iCD34+ cells

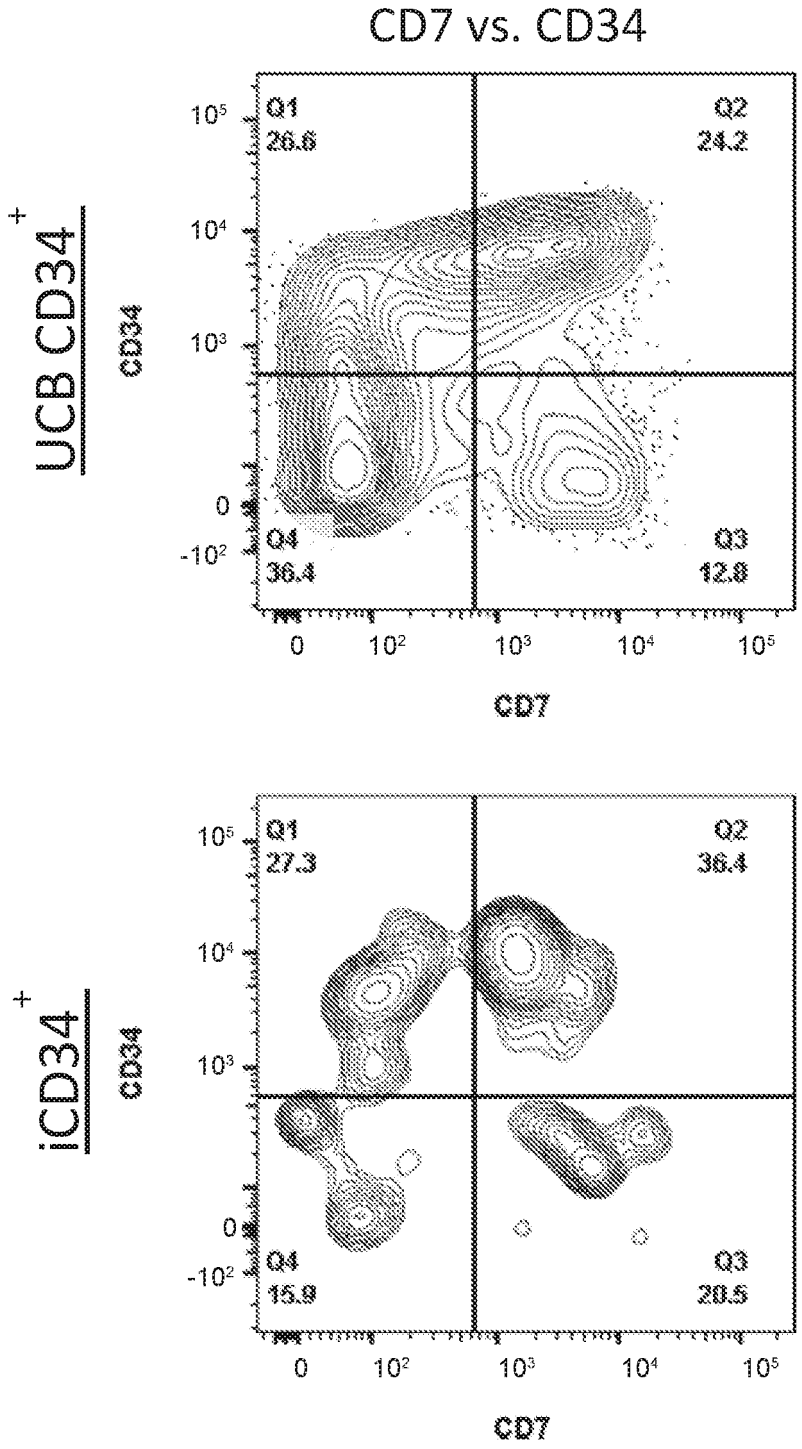


FIG. 21C

iPSC-derived, iCD34 differentiated towards NK cells.

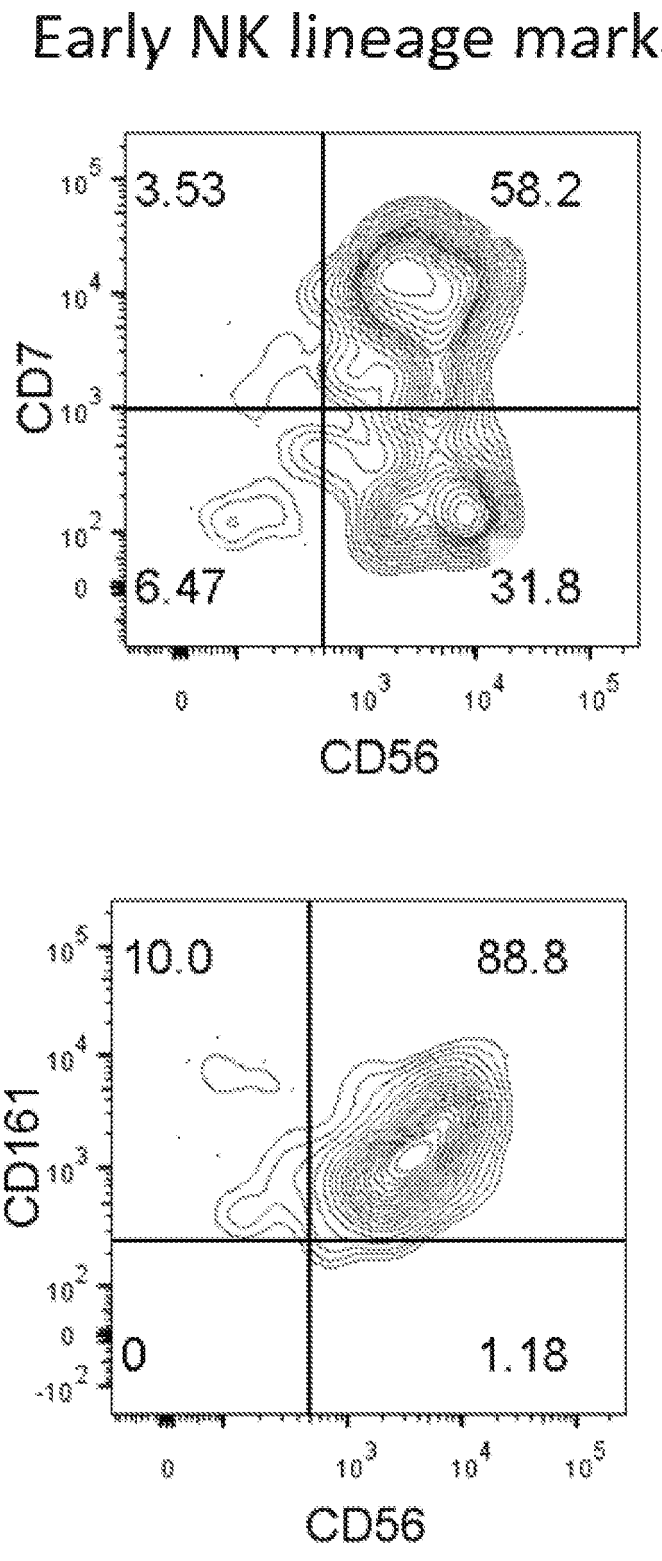


FIG. 22A

iPSC-derived, iCD34 differentiated towards NK cells.

Mature NK lineage markers

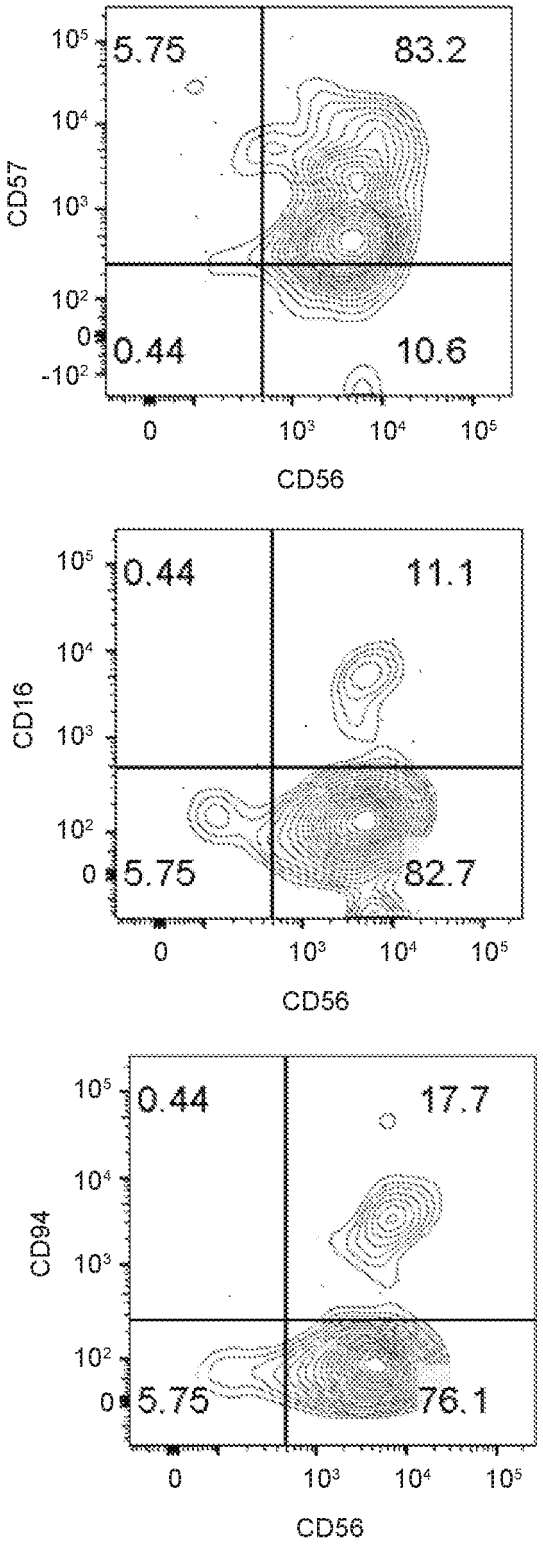


FIG. 22B

iPSC-derived, iCD34 differentiated towards NK cells.

Day 5: UCB CD34+ cells versus iCD34+ cells

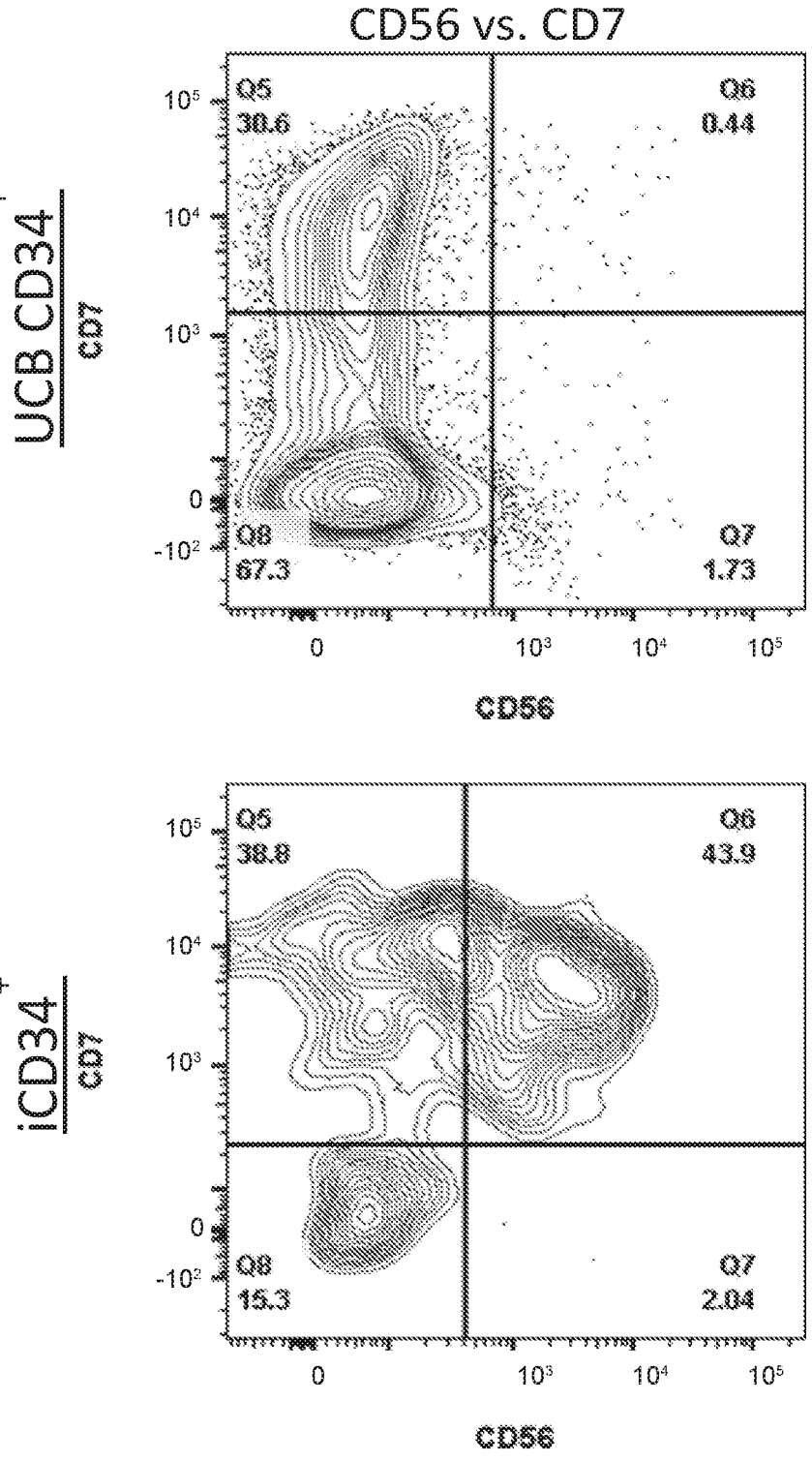
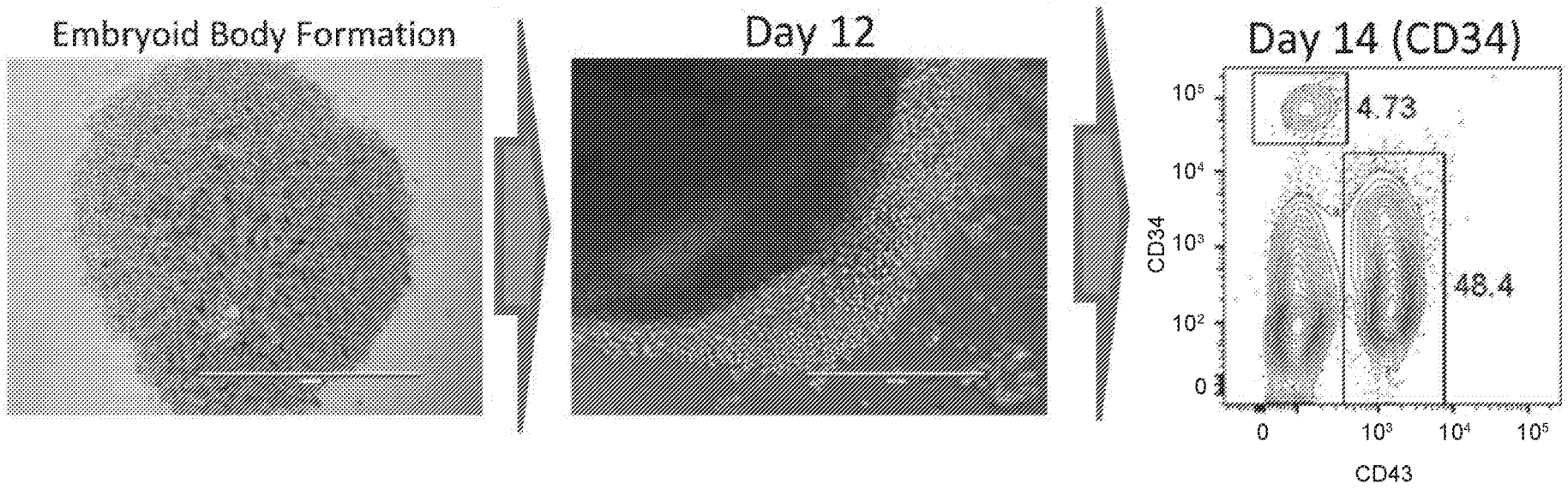


FIG. 22C

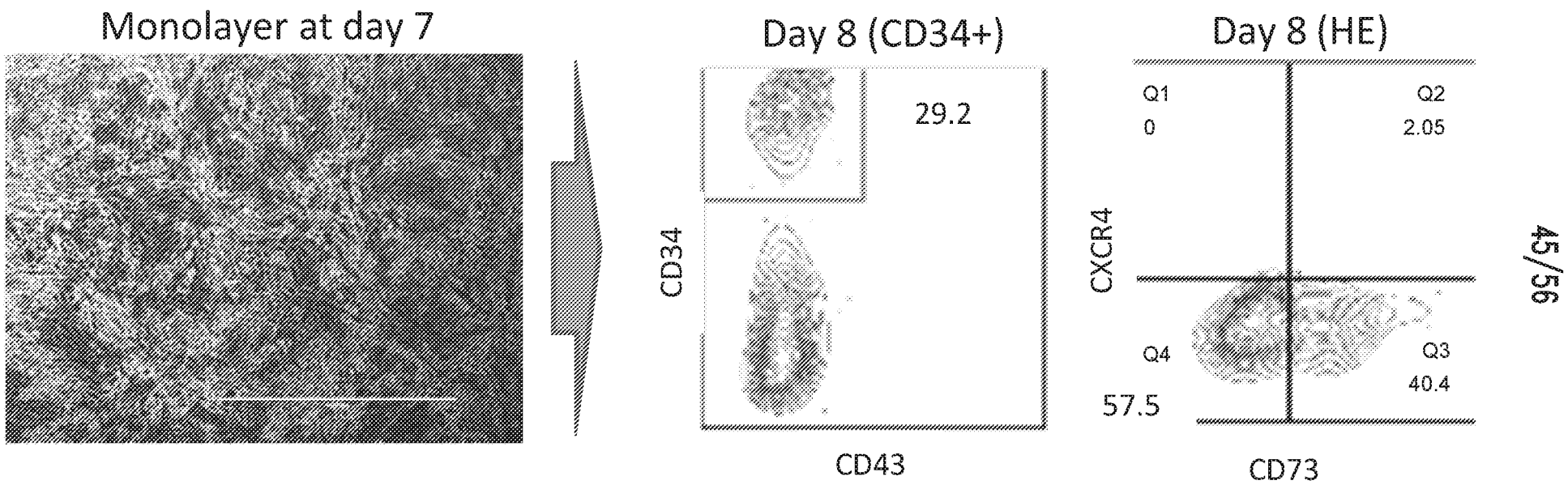
Monolayer hiPSC hematopoietic differentiation platform allows for a scalable expansion strategy that is not seen during EB formation



hiPSCs were aggregated to form Embryoid bodies and differentiated for 14 days prior to analysis for CD34 and expression.

FIG. 23A

Monolayer hiPSC hematopoietic differentiation platform allows for a scalable expansion strategy that is not seen during EB formation



hiPSCs were seeded as monolayer and differentiated for 8 days prior to analysis for CD34, 43, CXCR4 and CD73.

FIG. 23B

CD34 positive cells were counted and plotted over time for both monolayer and EB mediated hematopoietic differentiation.

FIG. 23C

Pluripotent Cell Clonal Expansion: Off-the-Shelf Scalability and Product Consistency
1 Pluripotent Clone ---> ~1M vials of 1x10^6 iCD34+ cells
Single Engineered Human Pluripotent Cell Clone
30 Days
6 passages
Master Cell Bank
~50 vials
Each of 1 x 10^6 Cells
~1:100
14 Days
3 passages
~5,000 vials
Each of 1 x 10^6 Cells
Working Cell Bank
~1:200
10 Days
1 passage
~1,000,000 vials
Each of 1 x 10^6 Cells
iNK
iT
iBanking
iProducts
Extensive homogeneity, scalability, quality control and quality assurance
100,000s of therapeutic doses

FIG. 24

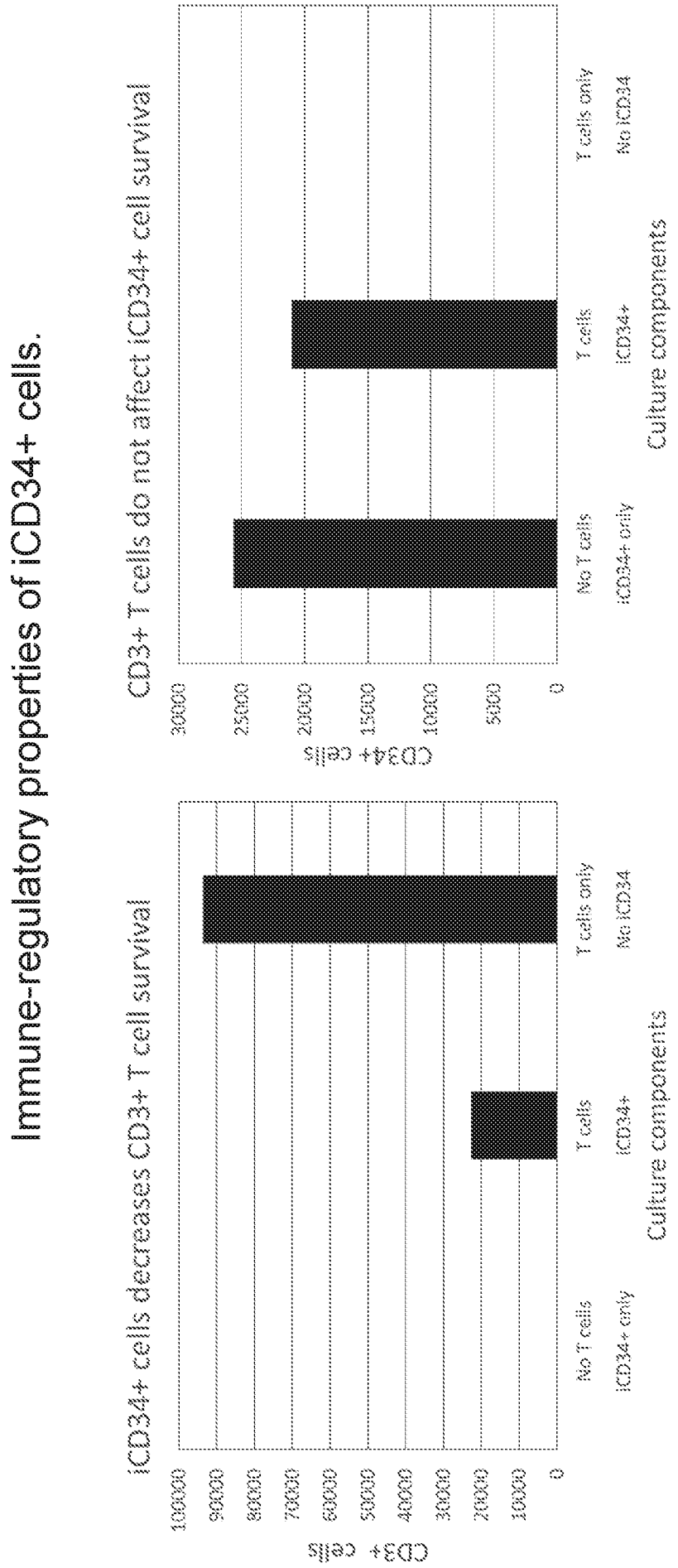

Immune-regulatory properties of iCD34+ cells.
iCD34+ cells decreases CD3+ T cell survival
CD3+ cells
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
No T cells
iCD34+ only
T cells
iCD34+
T cells only
No iCD34
Culture components
CD3+ T cells do not affect iCD34+ cell survival
CD34+ cells
30000
25000
20000
15000
10000
5000
0
No T cells
iCD34+ only
T cells
iCD34+
T cells only
No iCD34
Culture components

FIG. 25

iPSC-derived, iCD34 cells differentiated towards T cells.

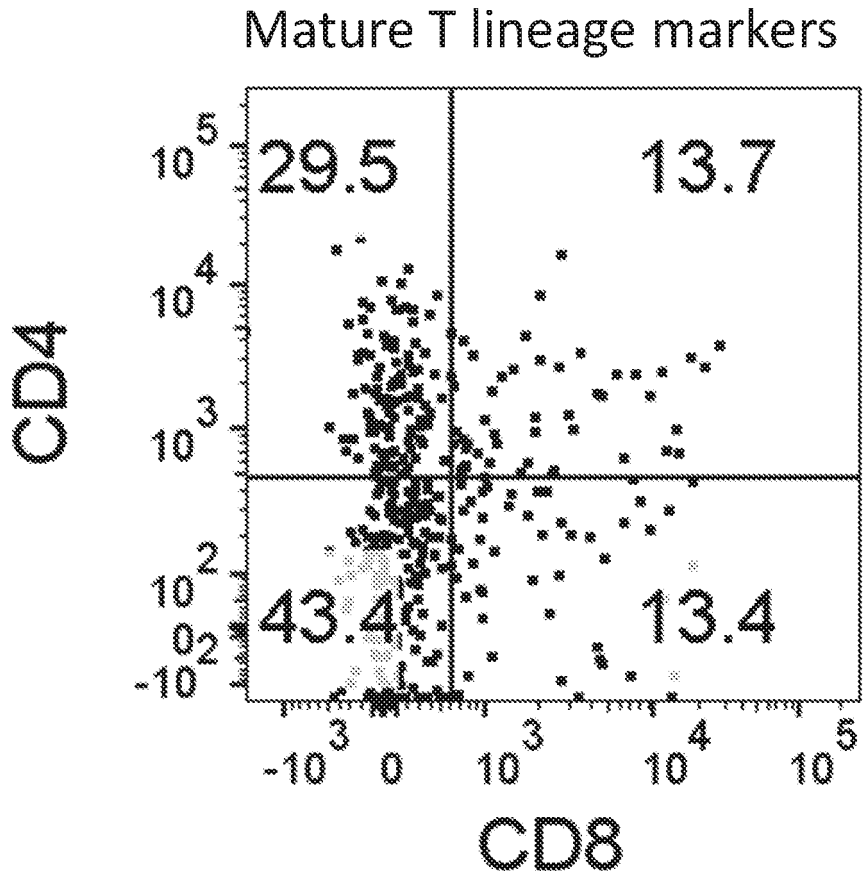


FIG. 26

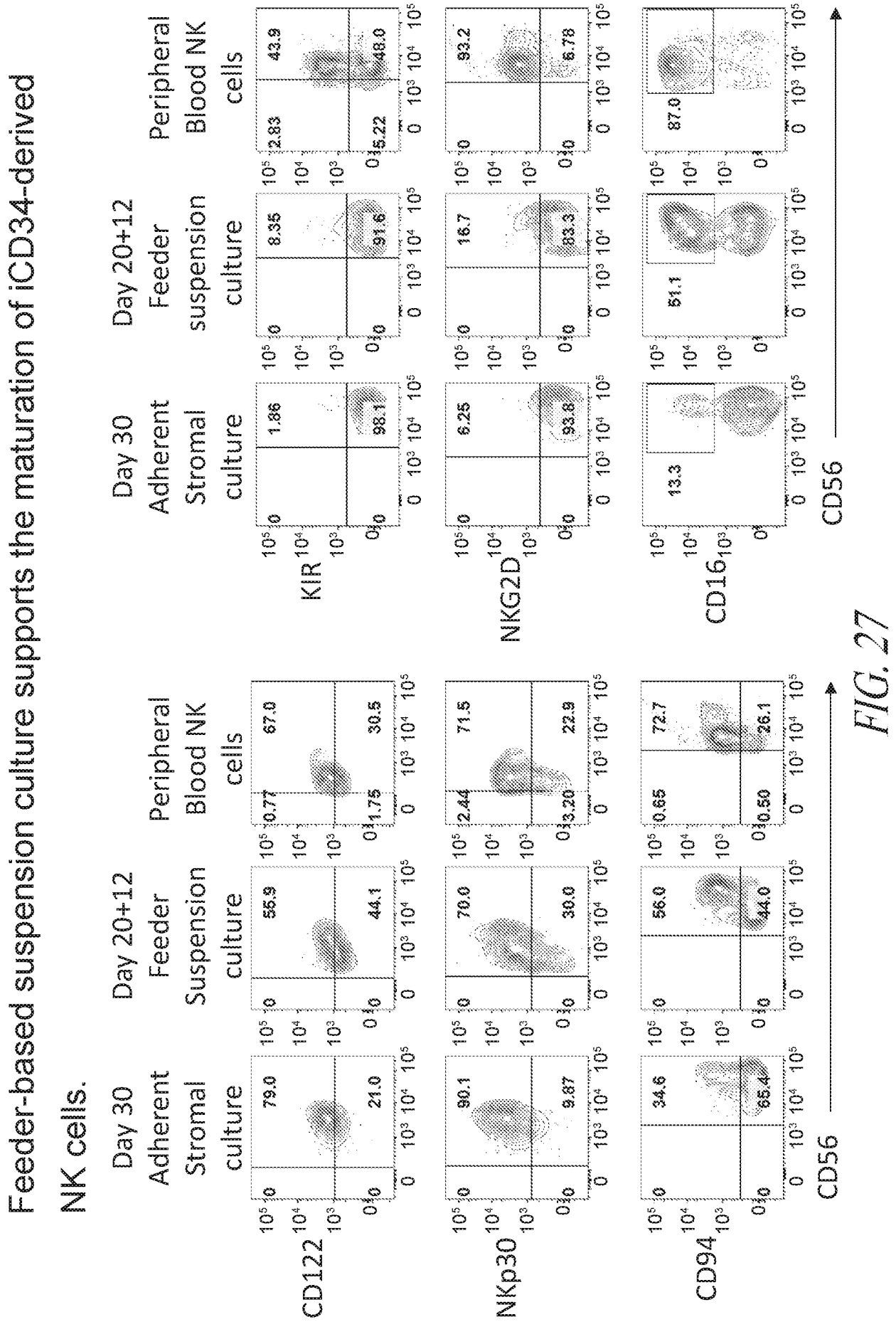

Feeder-based suspension culture supports the maturation of iCD34-derived NK cells.
Day 30 Adherent Stromal culture
Day 20+12 Feeder Suspension culture
Peripheral Blood NK cells
Day 30 Adherent Stromal culture
Day 20+12 Feeder suspension culture
Peripheral Blood NK cells
CD122
KIR
NKp30
NKG2D
CD94
CD16
CD56
CD56

FIG. 27

iCD34-derived iNK respond to cytokine stimulation to secrete pro-inflammatory cytokines in a similar manner to peripheral blood NK cells.

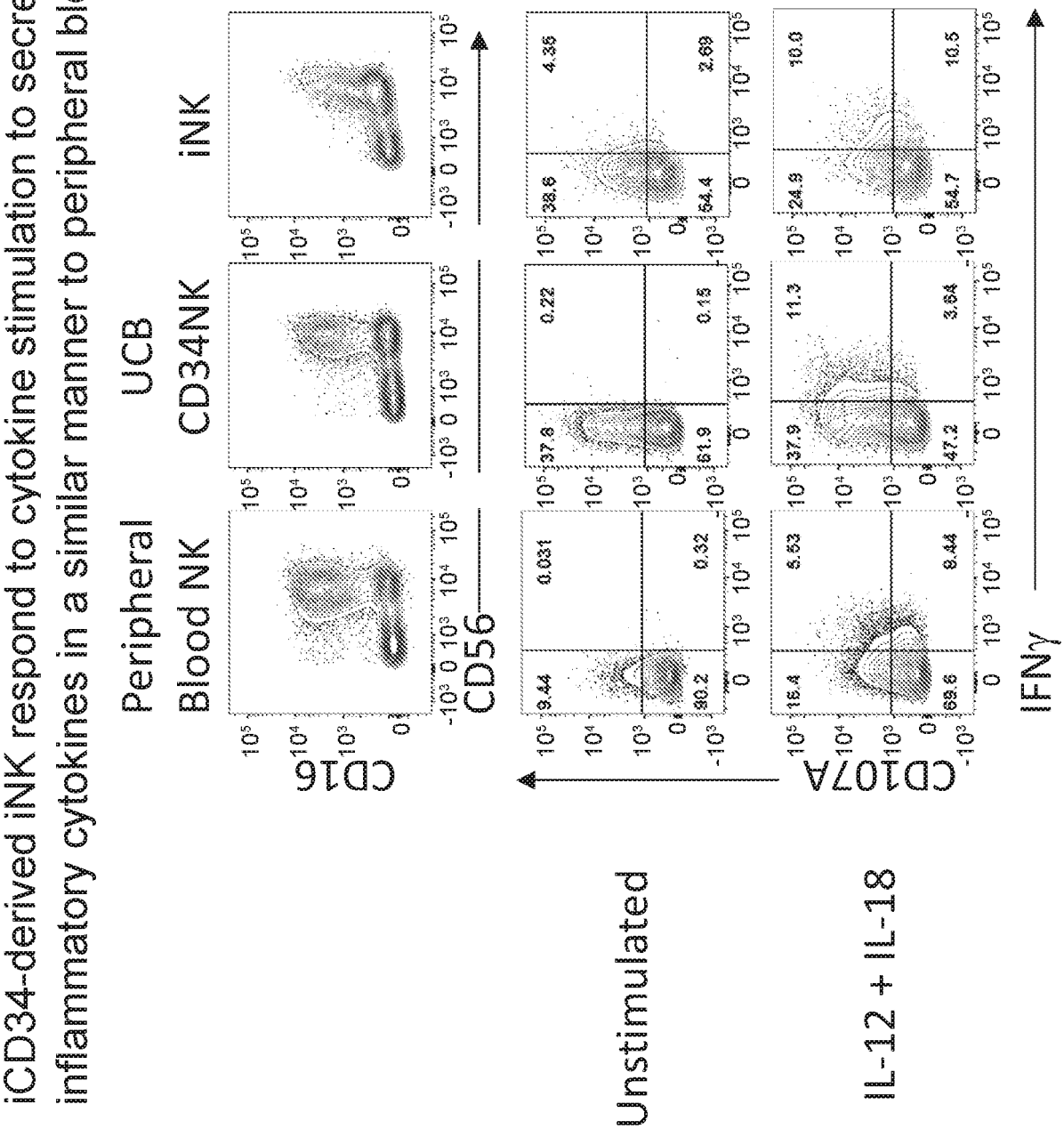


FIG. 28

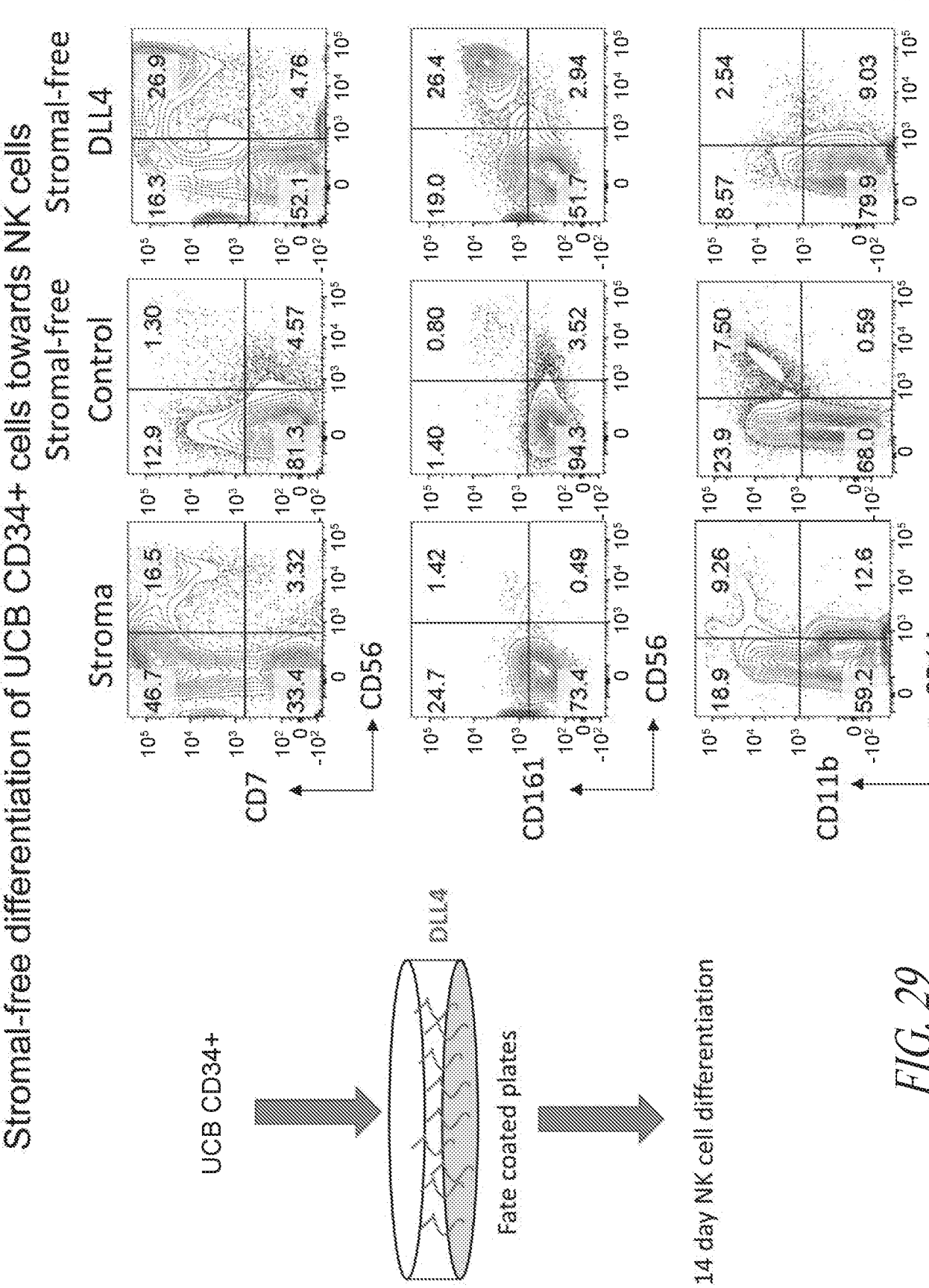

Stromal-free differentiation of UCB CD34+ cells towards NK cells
Stroma
Stromal-free Control
Stromal-free DLL4
CD7
CD56
CD161
CD56
CD11b
CD14
46.7
16.5
33.4
3.32
12.9
1.30
81.3
4.57
16.3
26.9
52.1
4.76
24.7
1.42
73.4
0.49
1.40
0.80
94.3
3.52
19.0
26.4
51.7
2.94
18.9
9.26
59.2
12.6
23.9
7.50
68.0
0.59
8.57
2.54
79.9
9.03
UCB CD34+
DLL4
Fate coated plates
14 day NK cell differentiation

FIG. 29

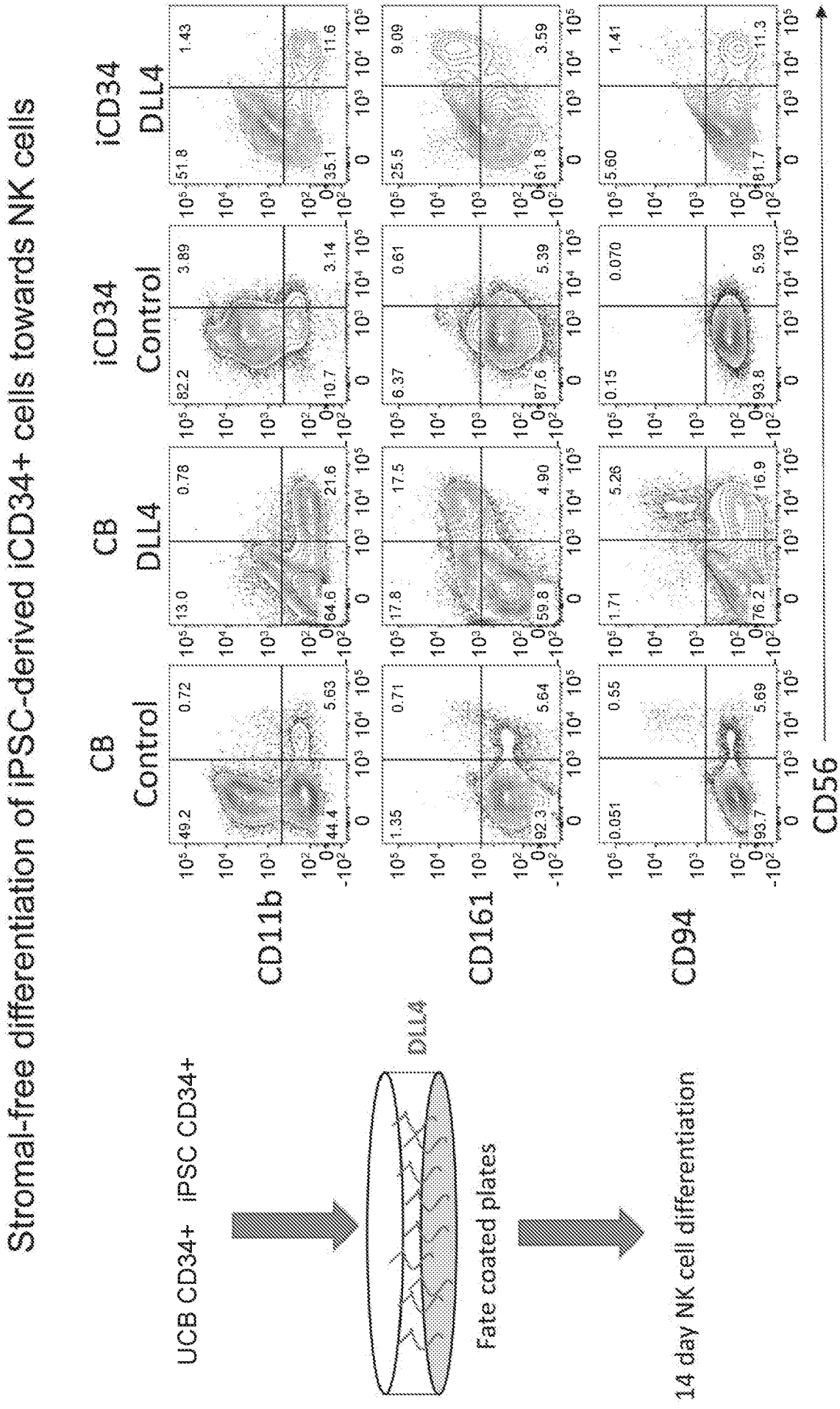

Stromal-free differentiation of iPSC-derived iCD34+ cells towards NK cells
UCB CD34+ iPSC CD34+
DLL4
Fate coated plates
14 day NK cell differentiation
CB Control
CB DLL4
iCD34 Control
iCD34 DLL4
CD11b
CD161
CD94
CD56

FIG. 30

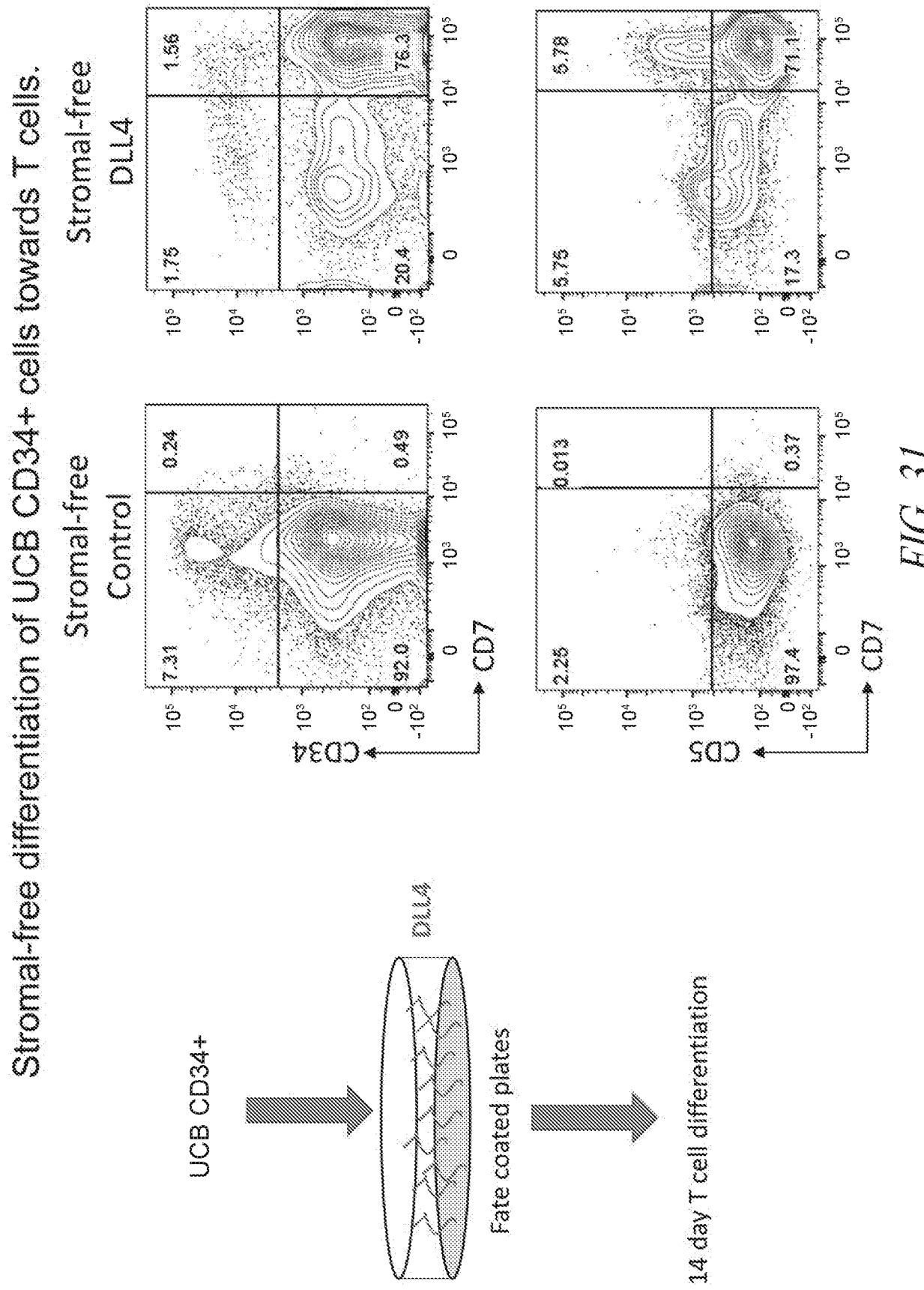

Stromal-free differentiation of UCB CD34+ cells towards T cells.
Stromal-free
Control
Stromal-free
DLL4
7.31
0.24
92.0
0.49
1.75
1.56
20.4
76.3
2.25
0.013
97.4
0.37
5.75
6.78
17.3
71.1
CD34
CD5
CD7
UCB CD34+
DLL4
Fate coated plates
14 day T cell differentiation

FIG. 31

SUBSTITUTE SHEET (RULE 26)

Stromal-free differentiation of iPSC-derived iCD34+ cells towards T cells.

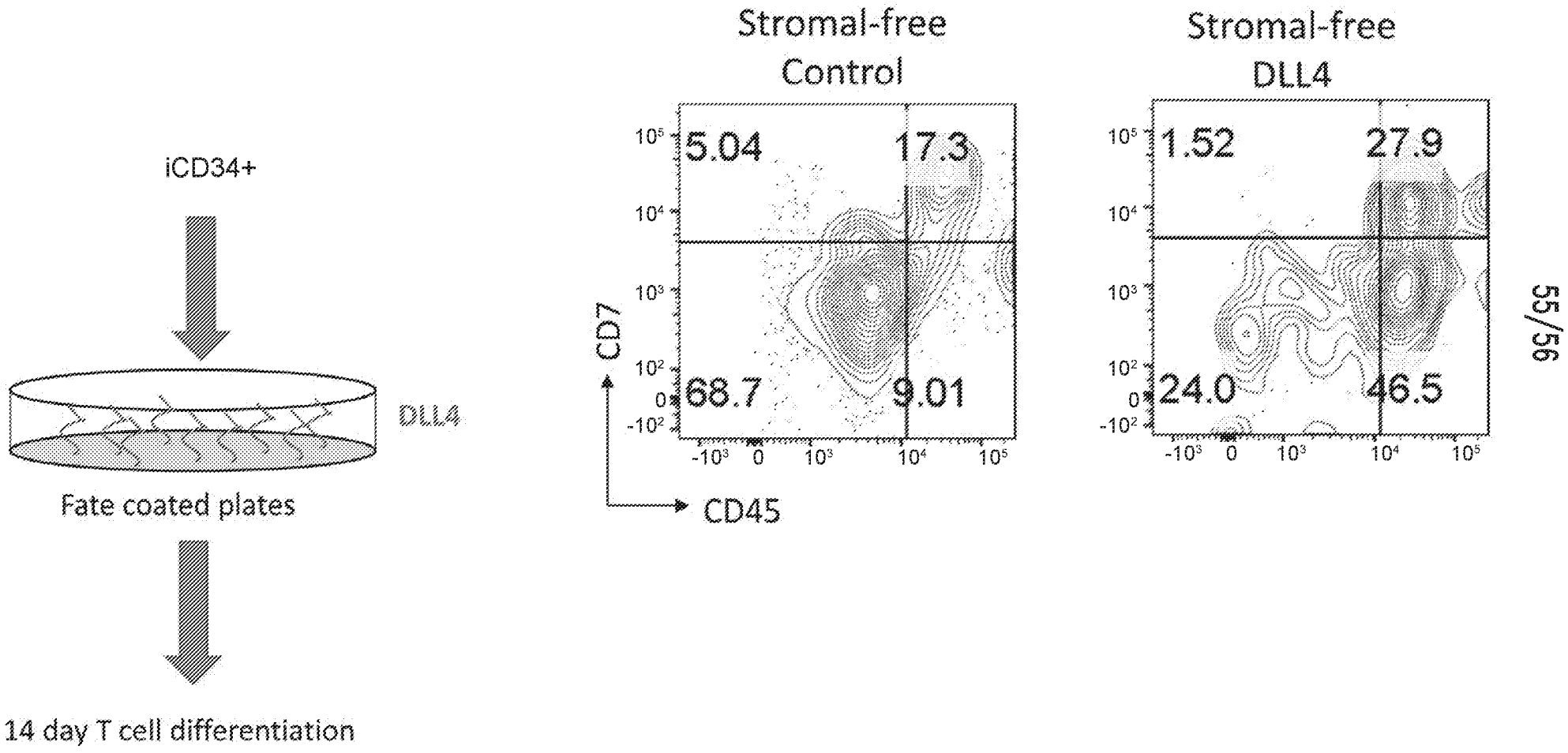


FIG. 32

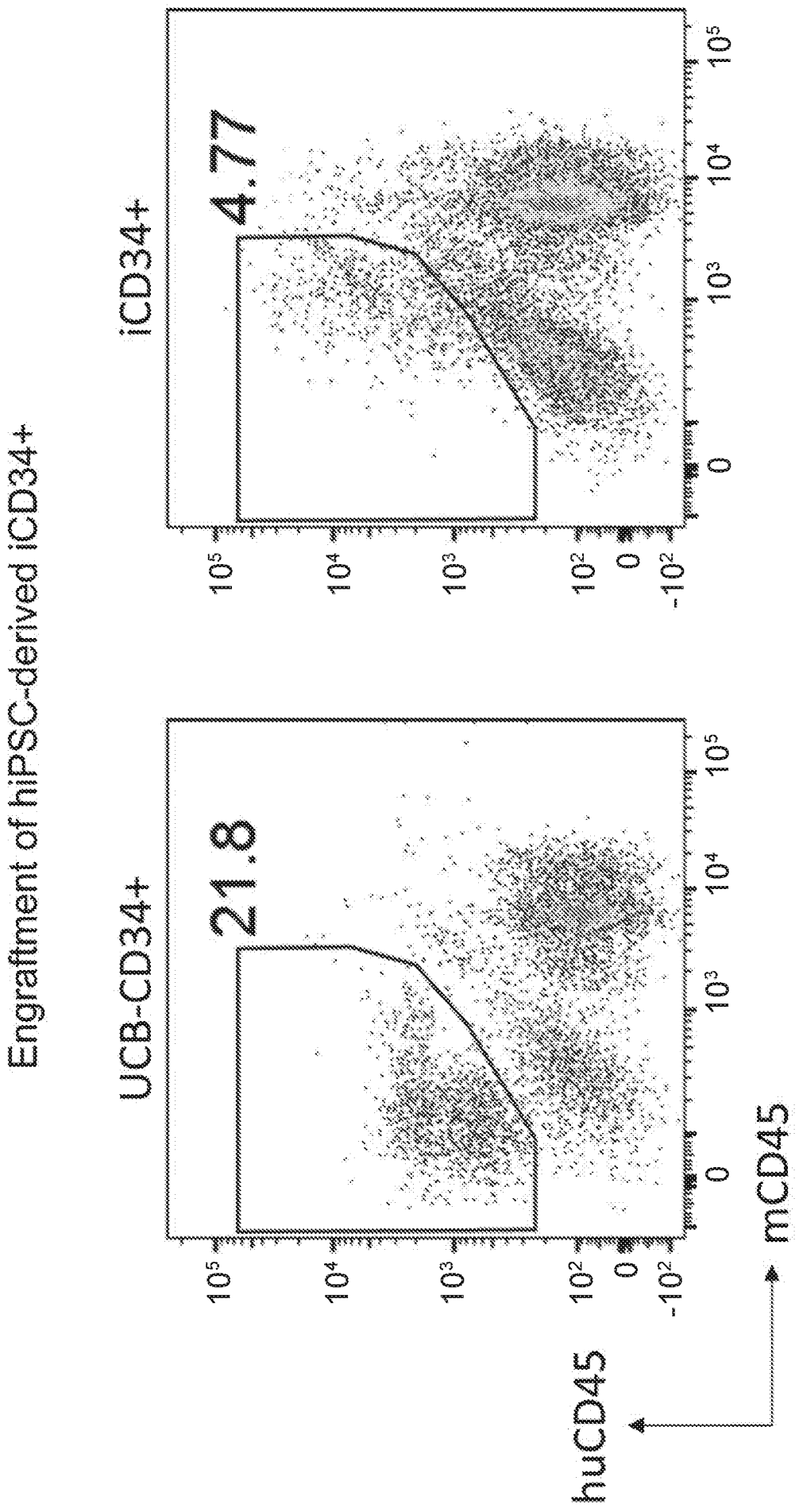

Engraftment of hiPSC-derived iCD34+
UCB-CD34+
21.8
iCD34+
4.77
huCD45
mCD45
10^5
10^4
10^3
10^2
0
-10^2

FIG. 33