

Platforms for CD34⁺ hemogenic endothelium (HE) and multipotent progenitors (MPP)

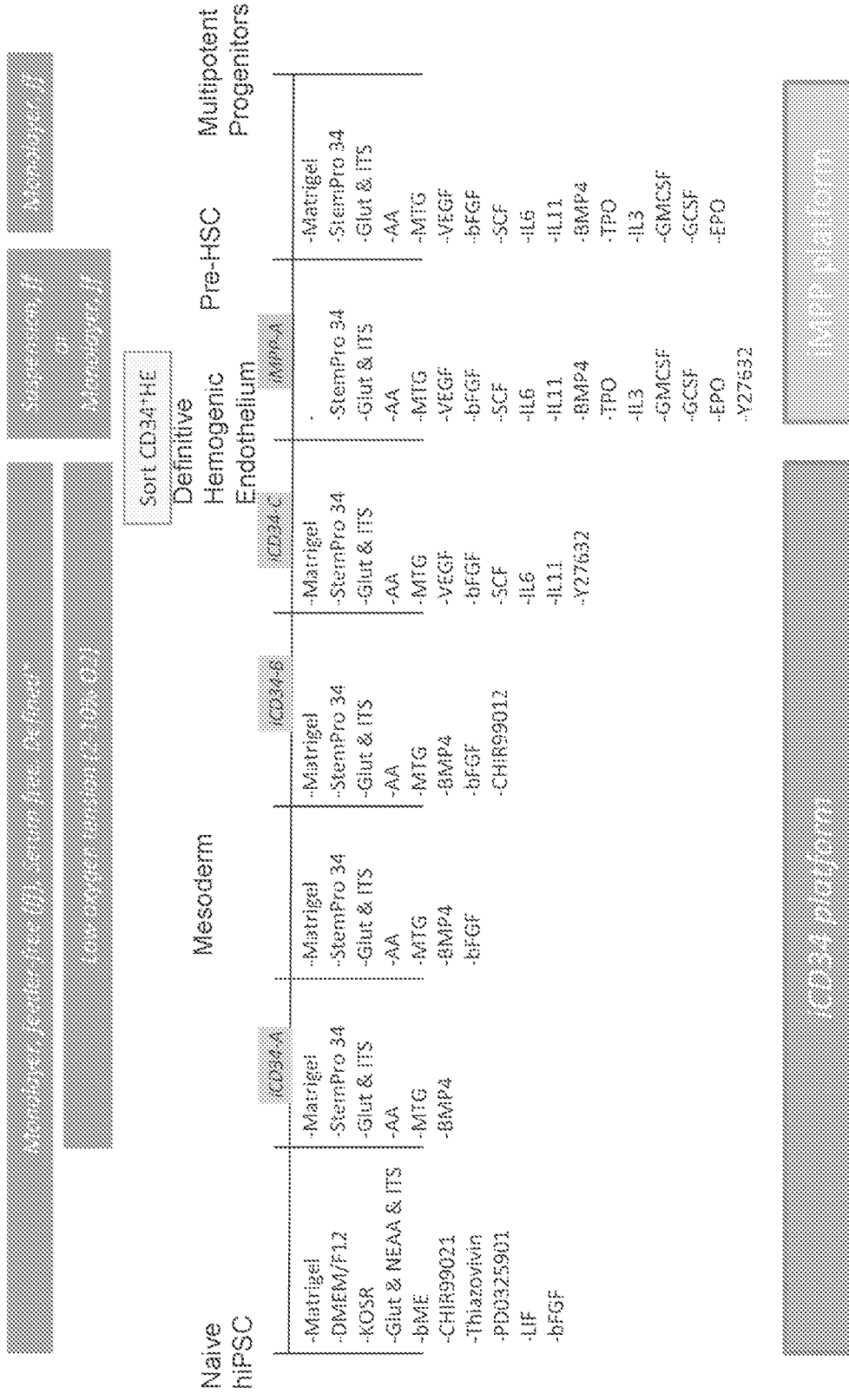


FIG. 1



FIG. 2

Platforms for CD34⁺ hemogenic endothelium (HE) and NK cell progenitors

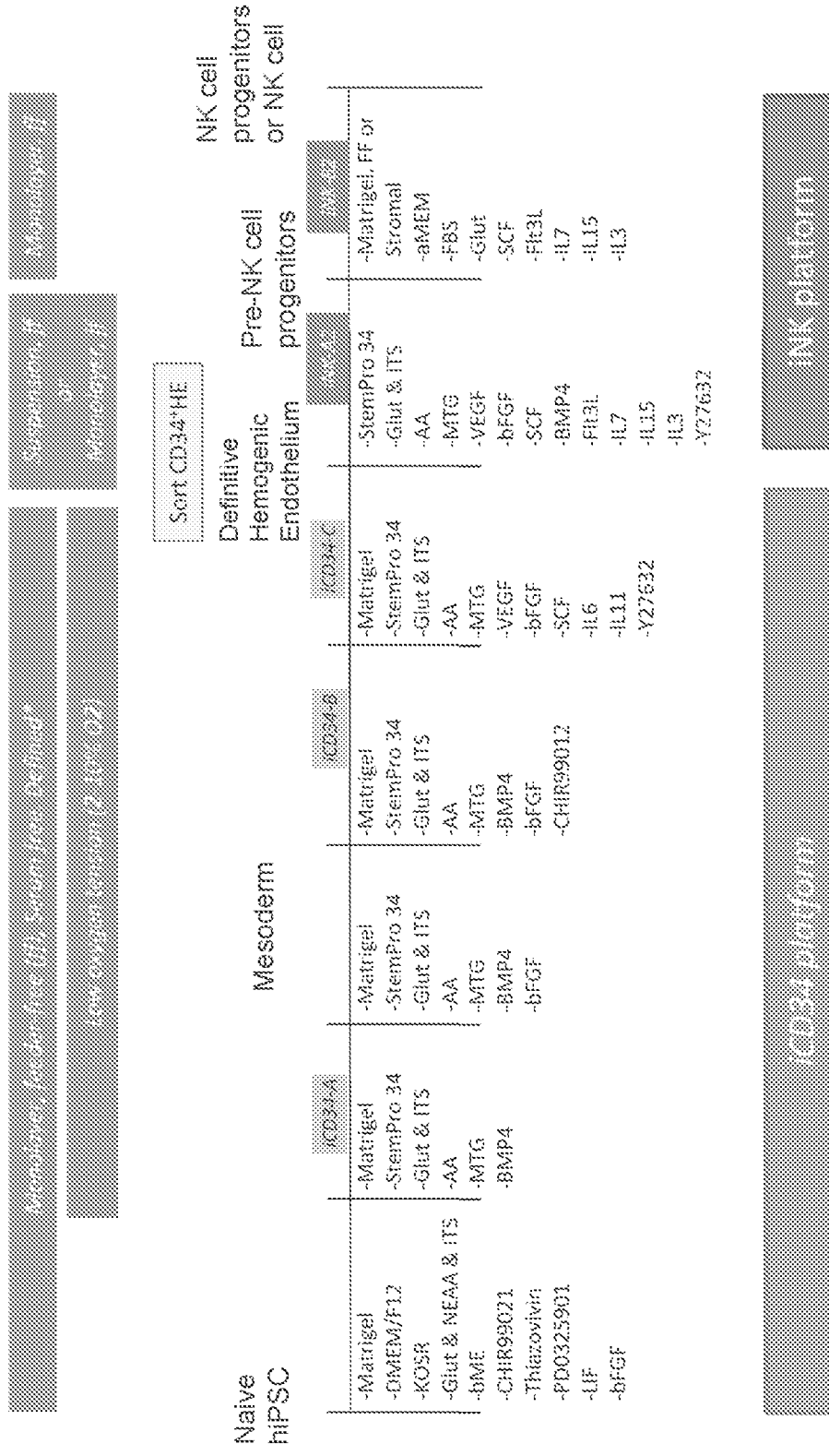


FIG. 3

ipSC-derived CD34⁺ population contains the CXCR4- CD73- hemogenic endothelium population

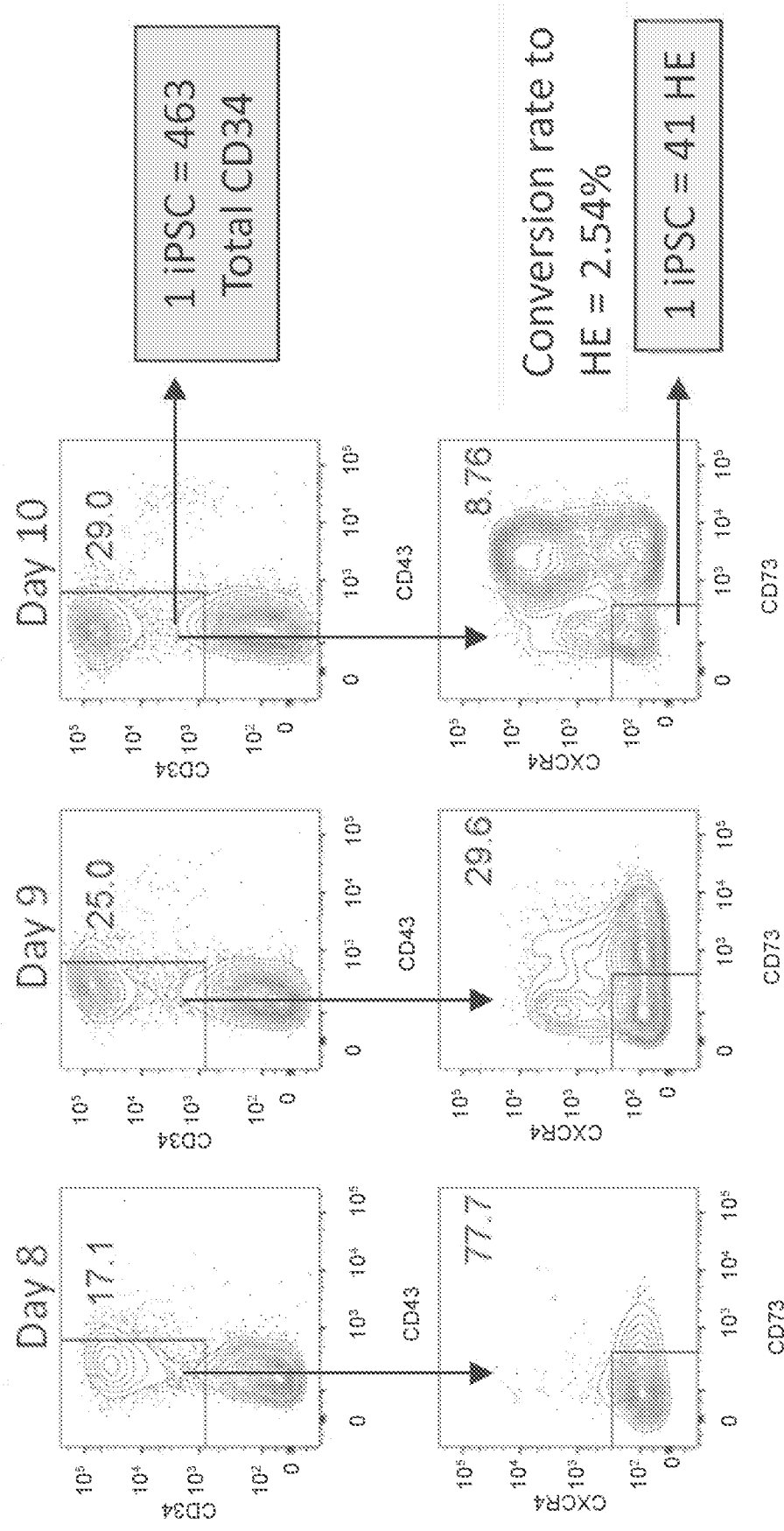


FIG. 4A

iPSC-derived CD34⁺ population contains the CXCR4⁺ CD73⁺ hemogenic endothelium population

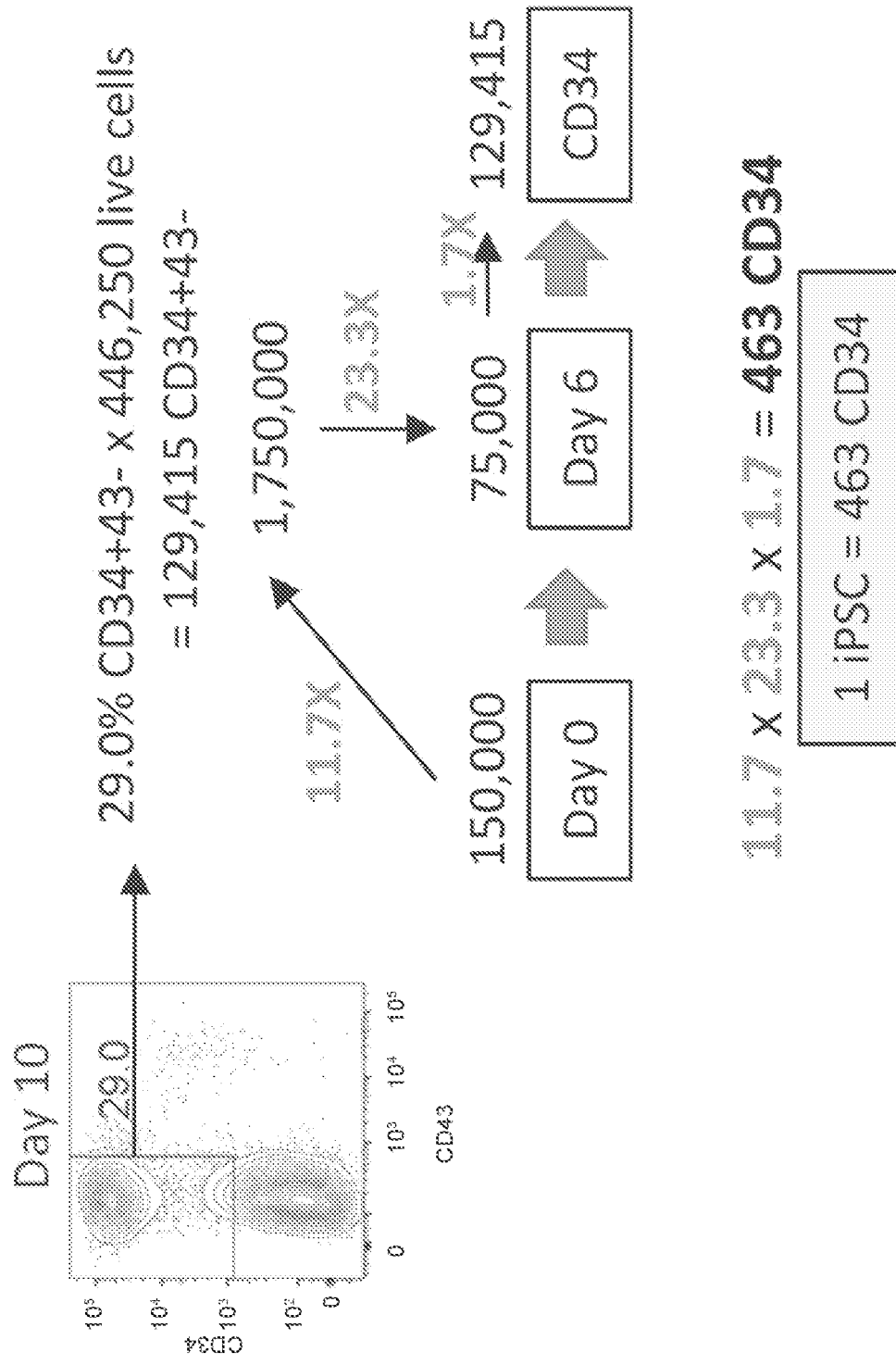


FIG. 4B

iPSC-derived CD34+ population contains the CXCR4- CD73- hemogenic endothelium population

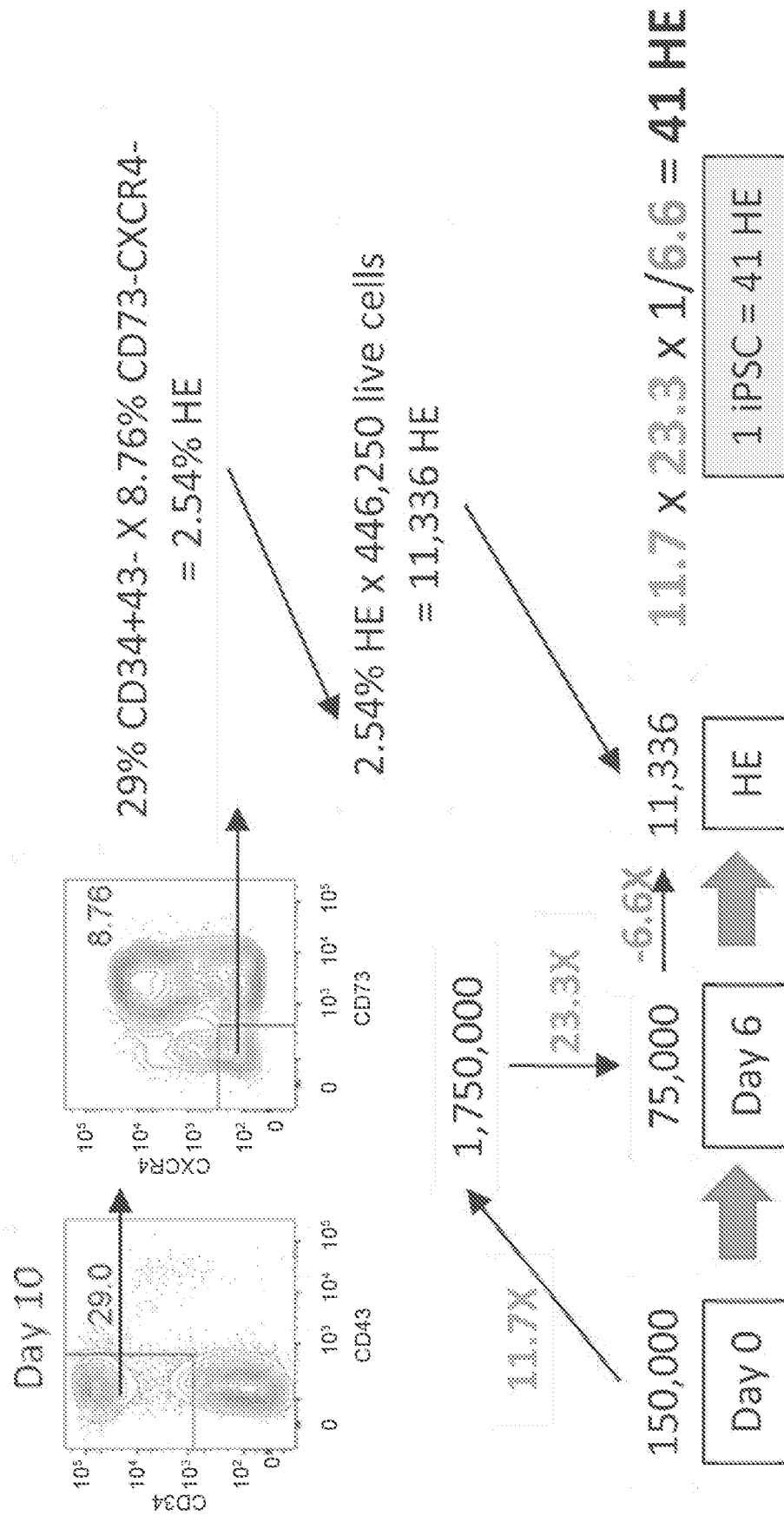


FIG. 4C

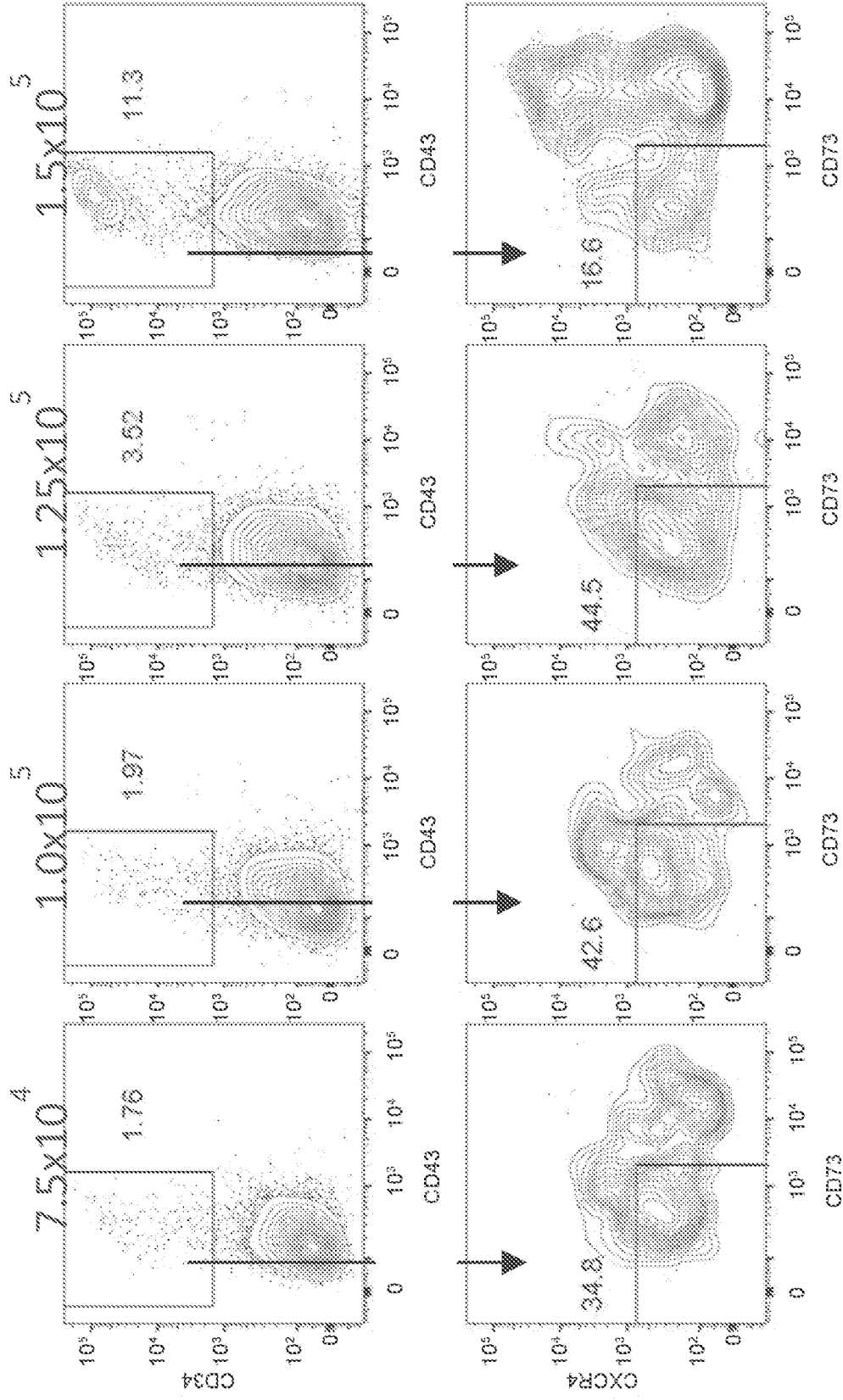


FIG. 5A

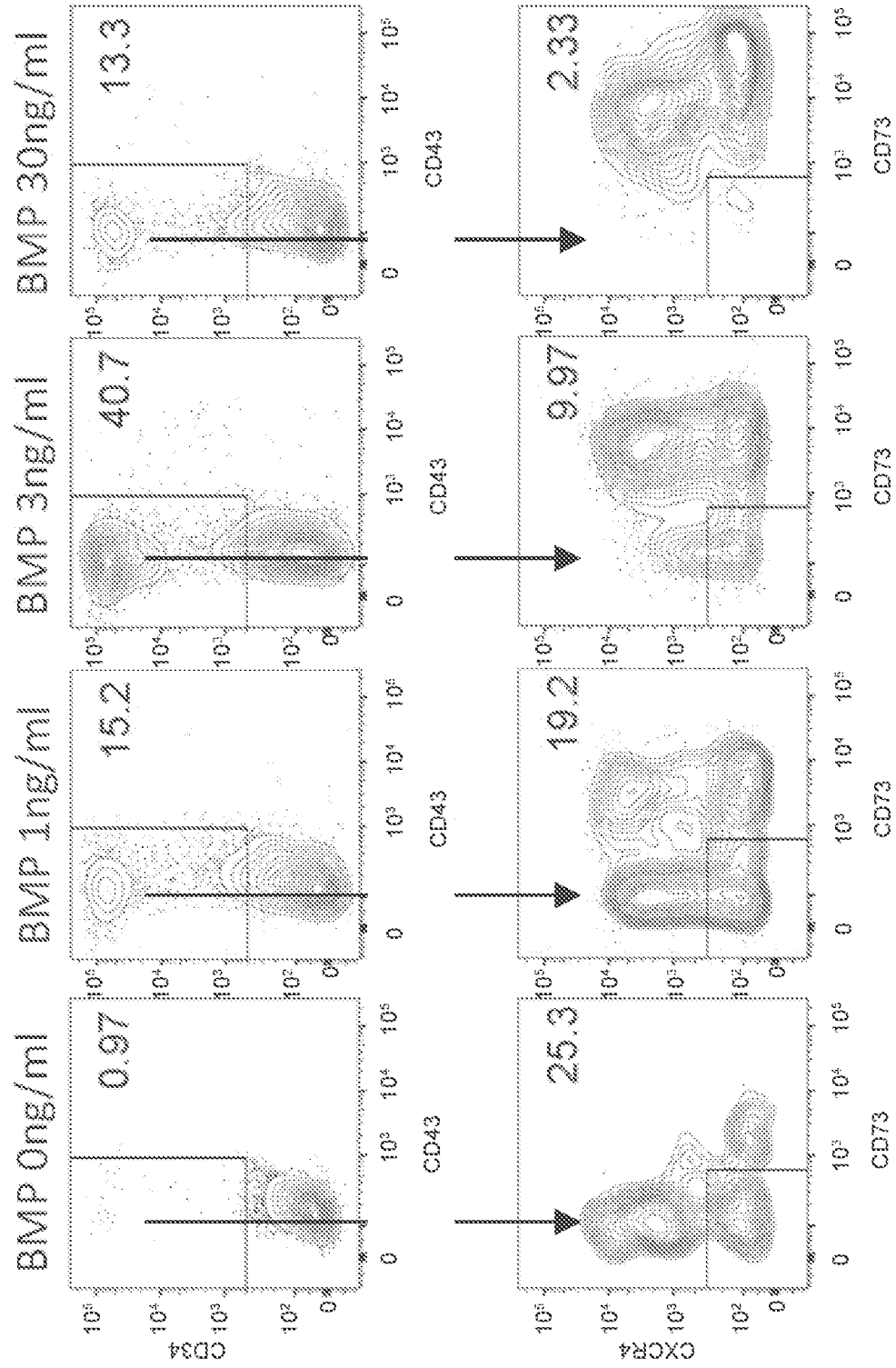


FIG. 5B

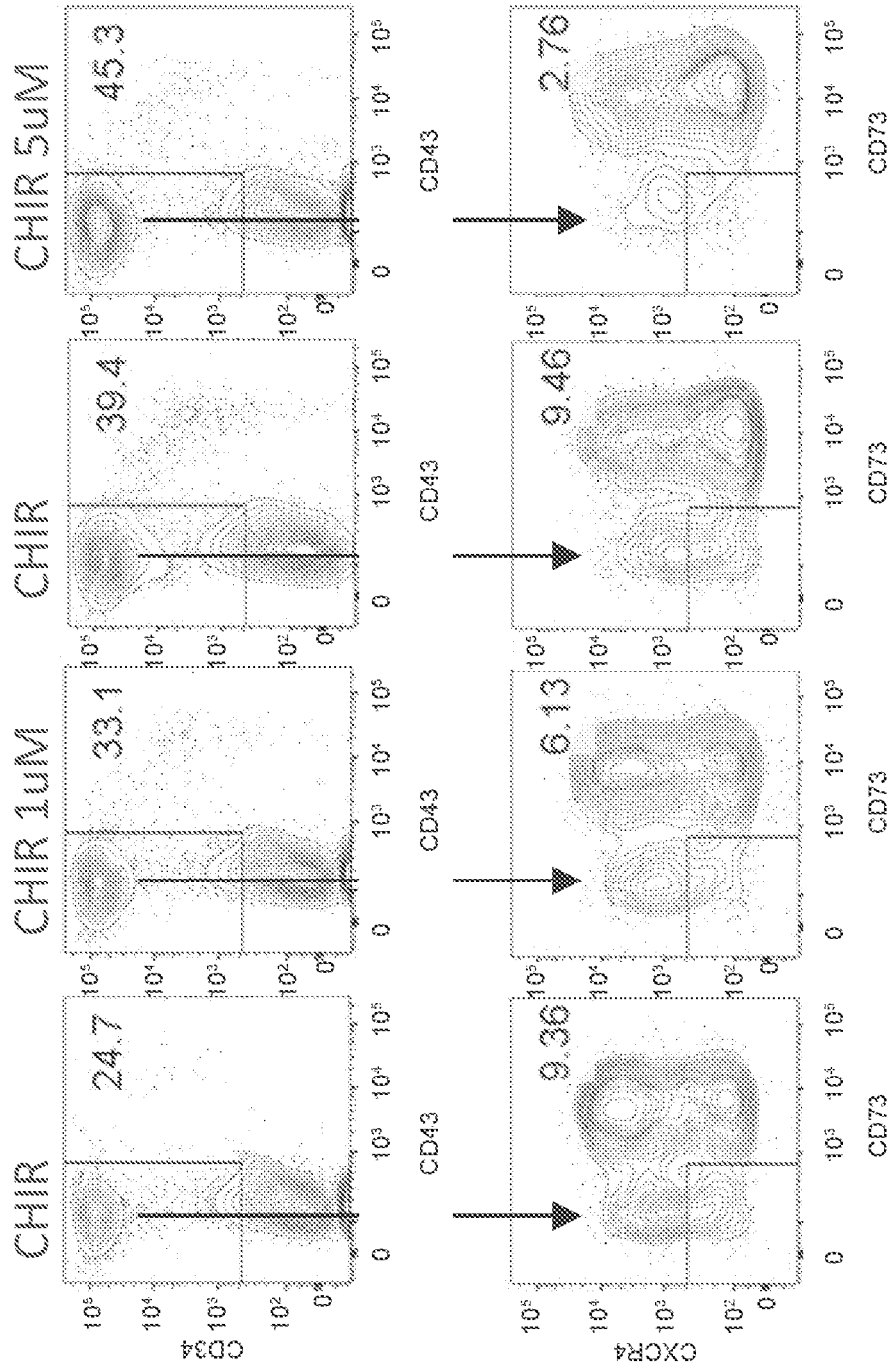


FIG. 5C

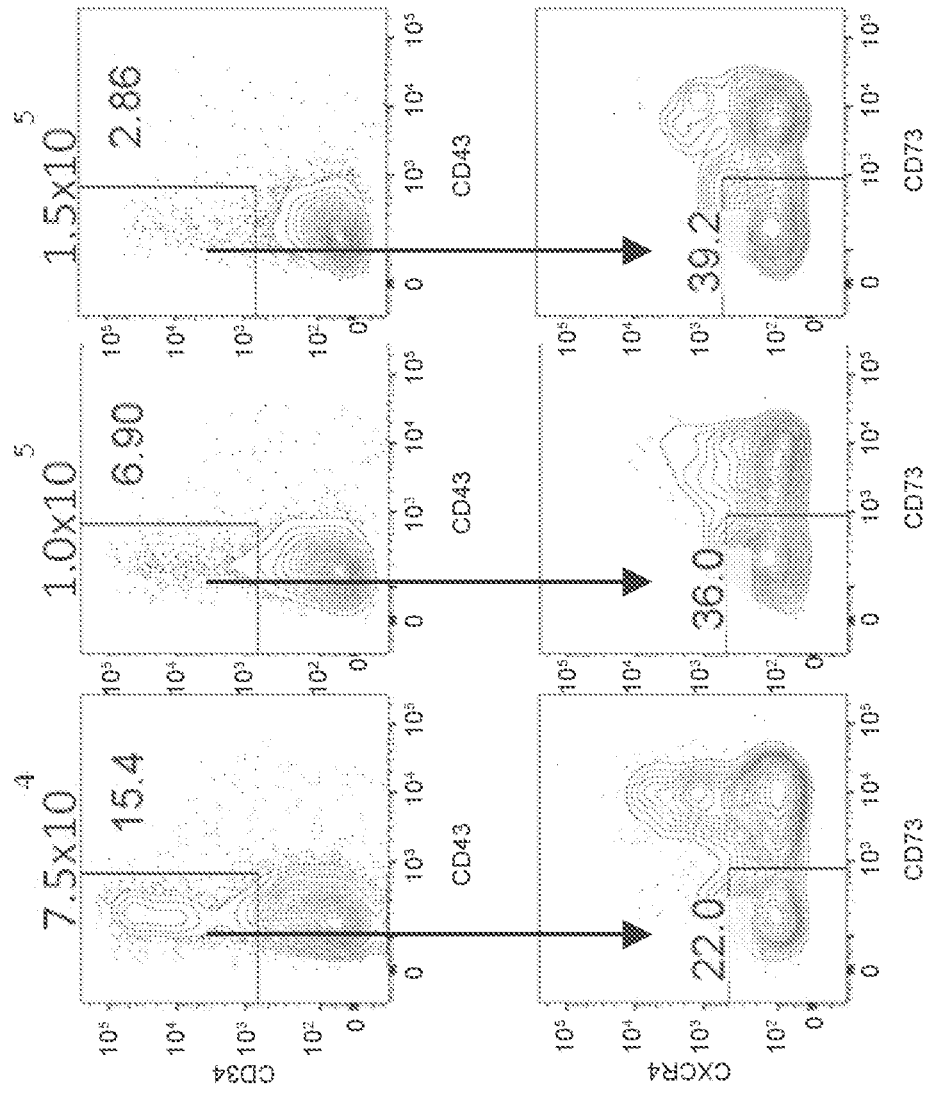


FIG. 5D

iCD34 cells are multipotent and presentative of the definitive hematopoietic state.

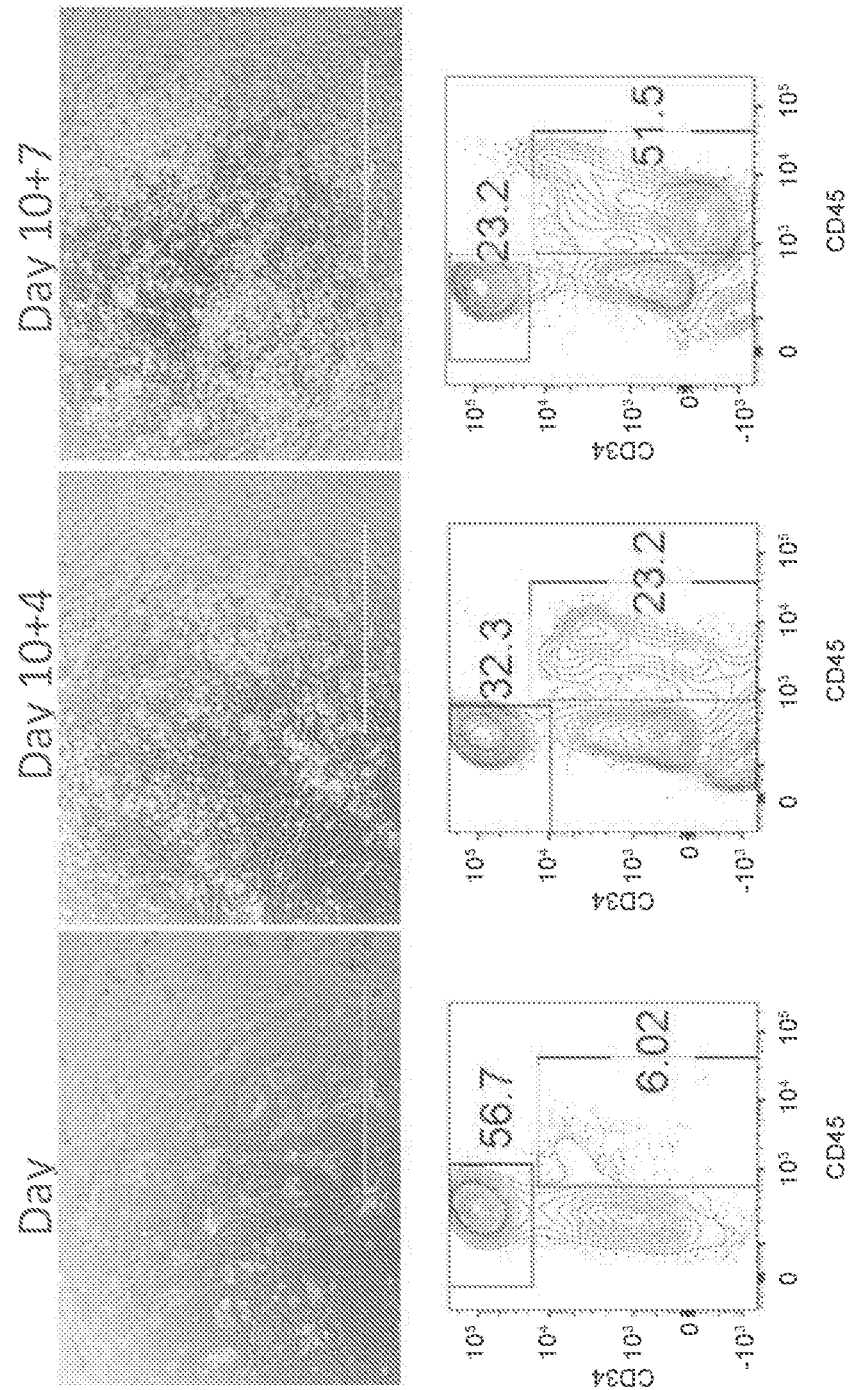


FIG. 6A

iCD34 cells are multipotent and presentative of the definitive hematopoietic state.

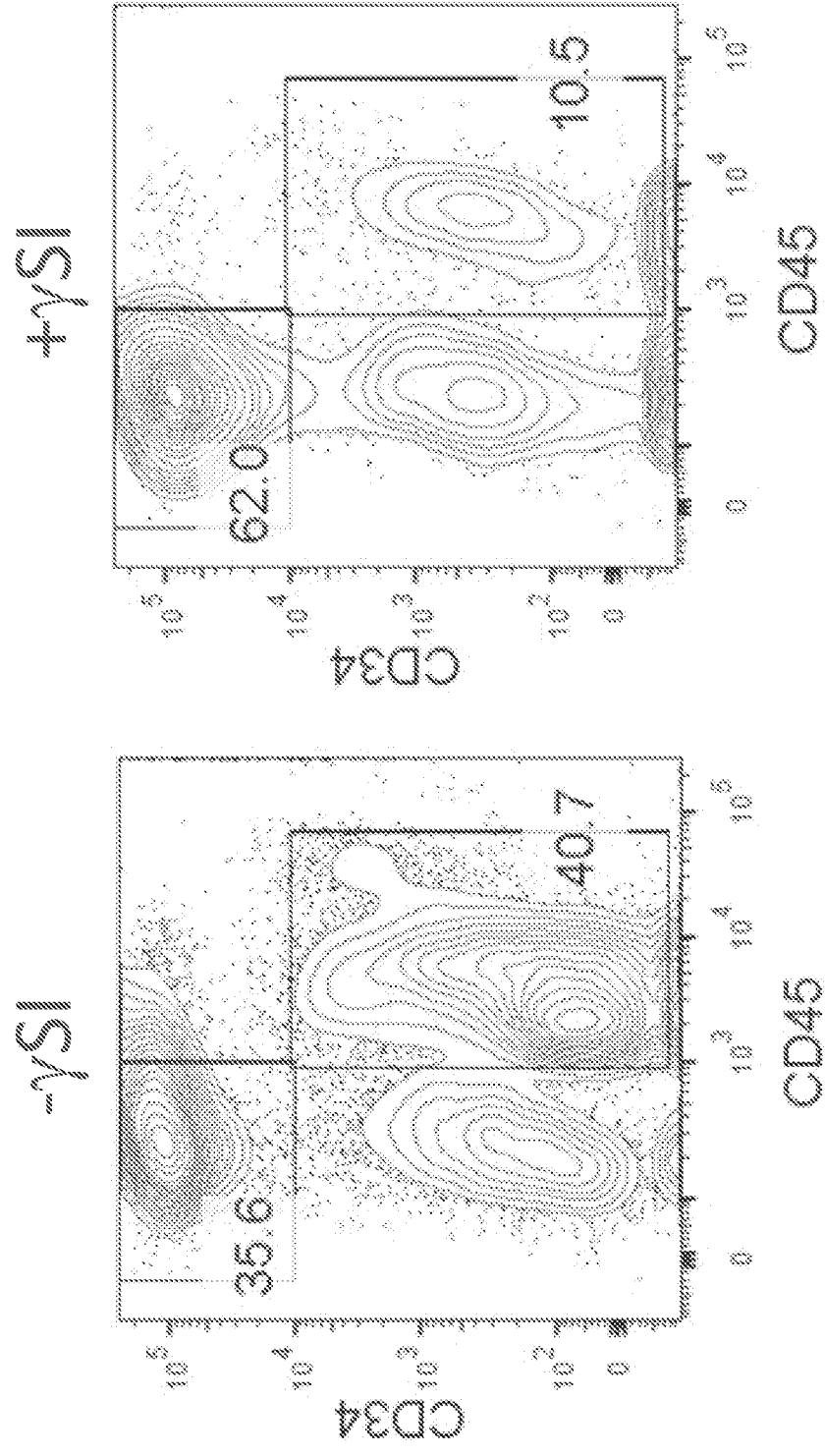


FIG. 6B

Oxygen concentration plays a role in iPSC derived hematopoietic differentiation.

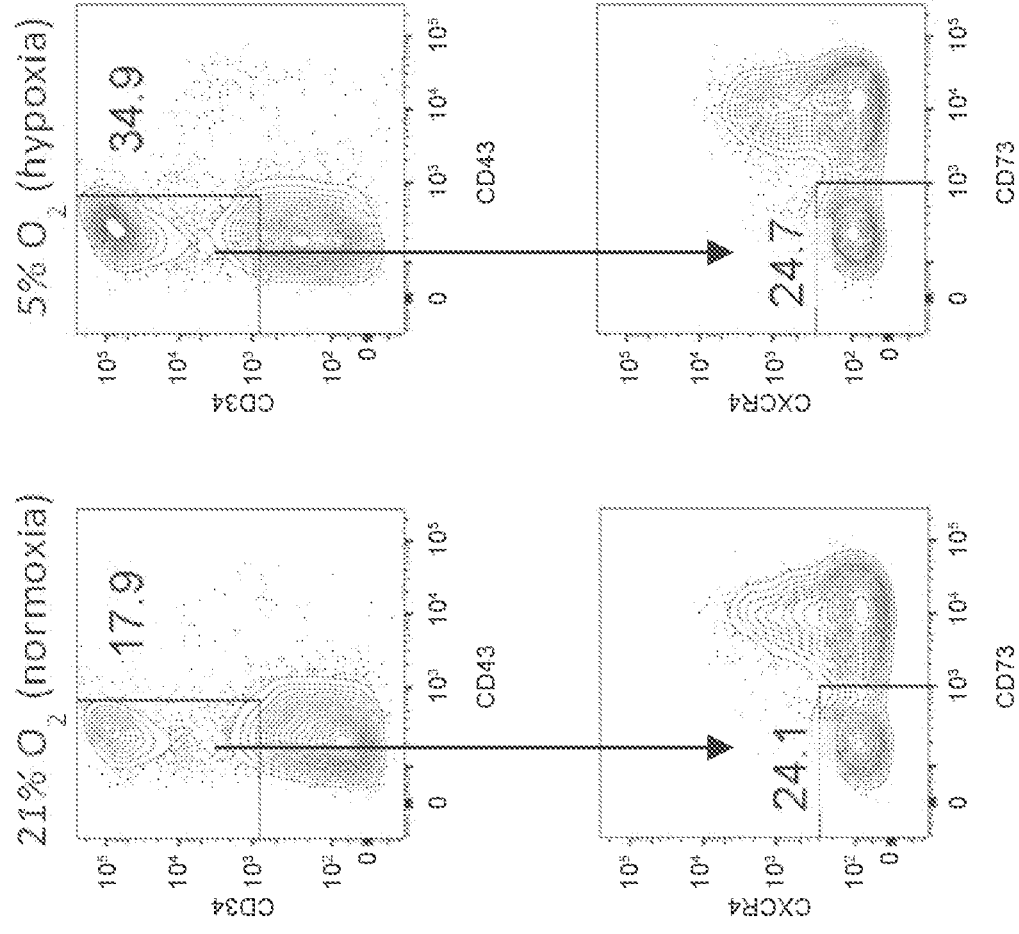


FIG. 7A

Oxygen concentration plays a role in iPSC derived hematopoietic differentiation.

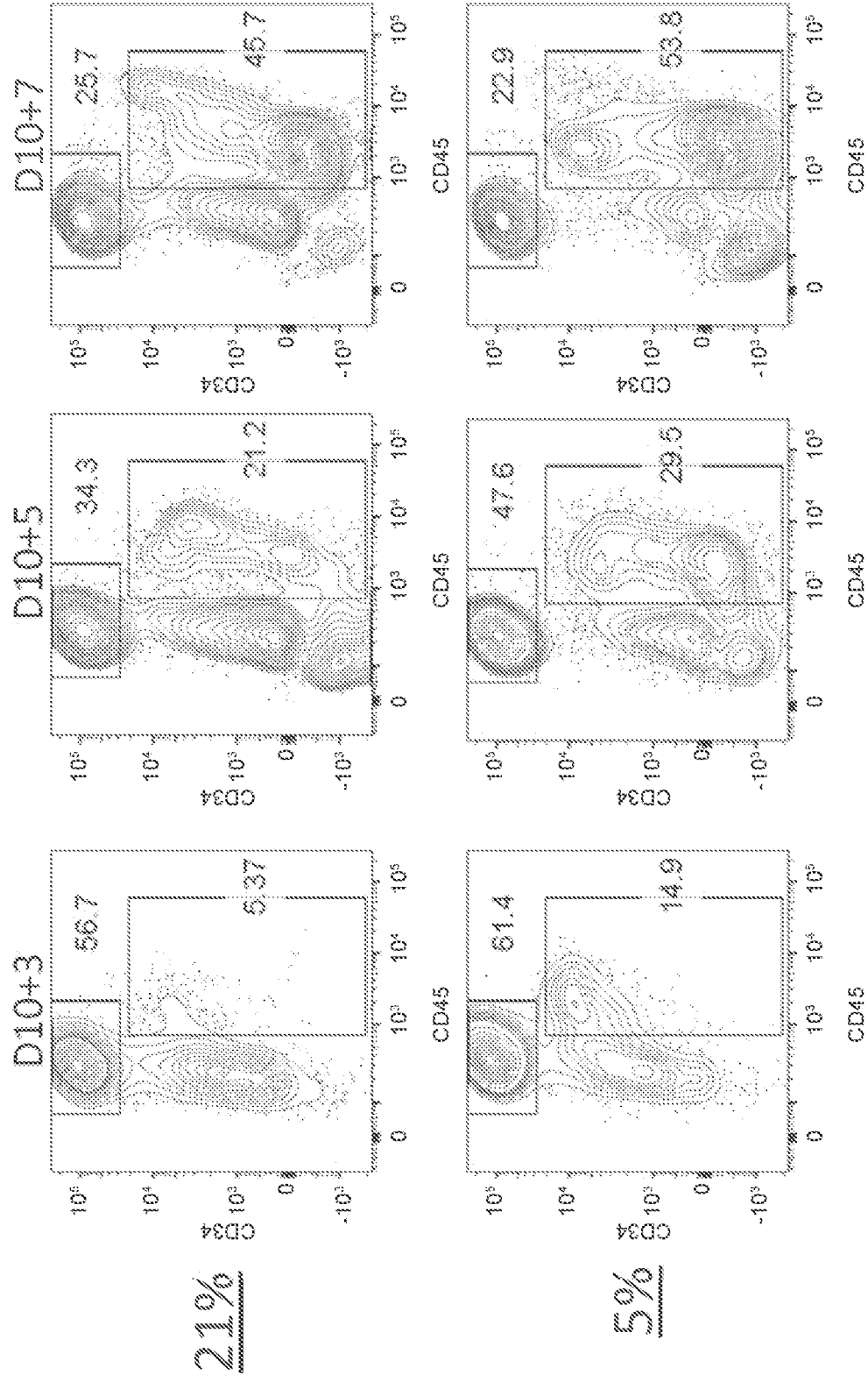
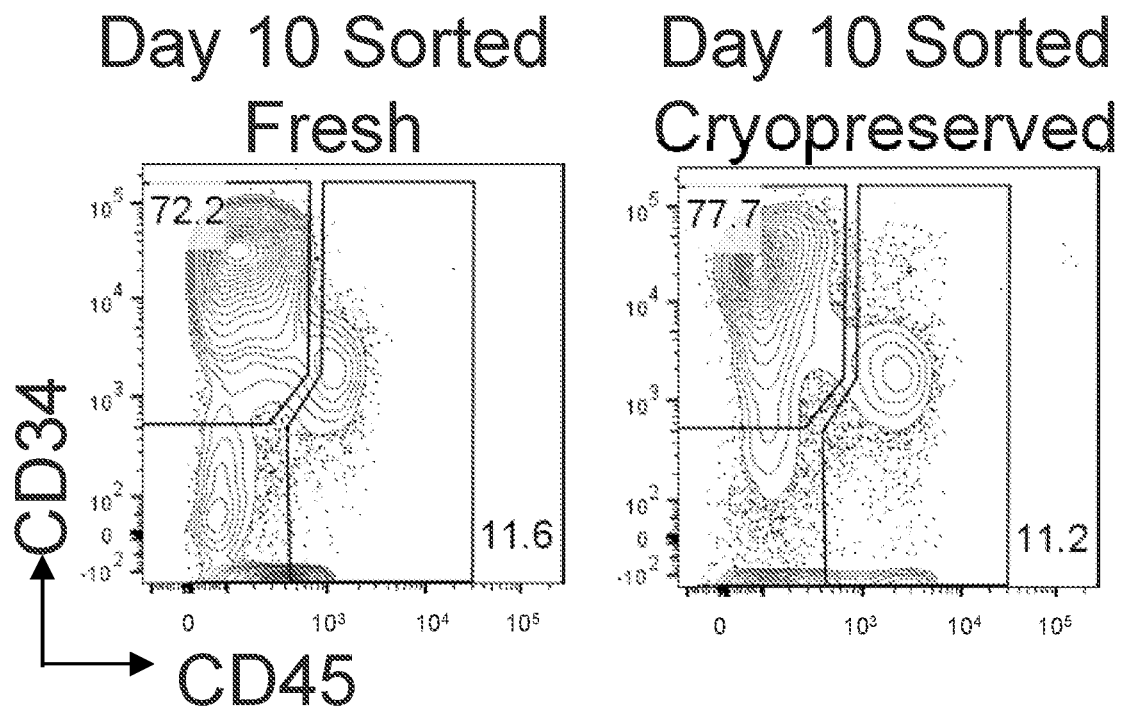
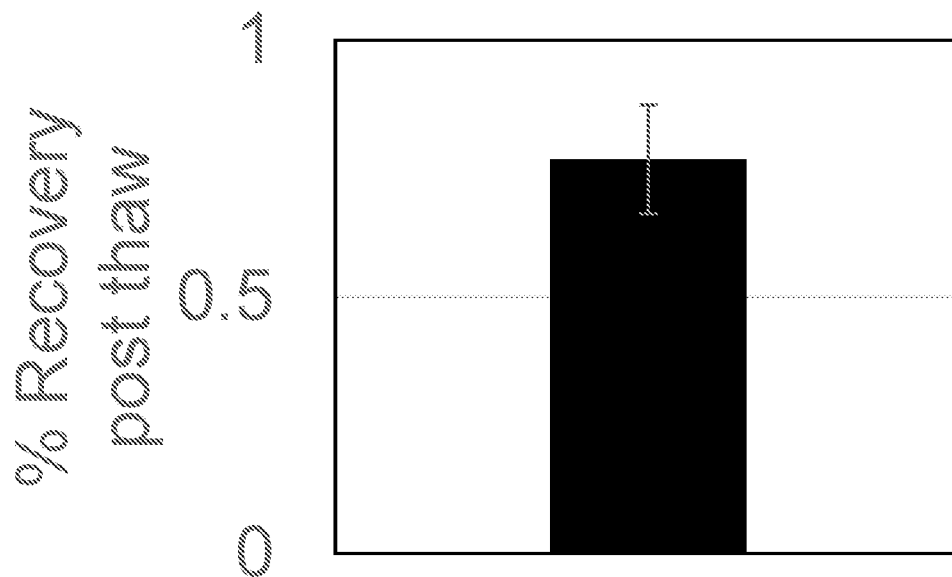


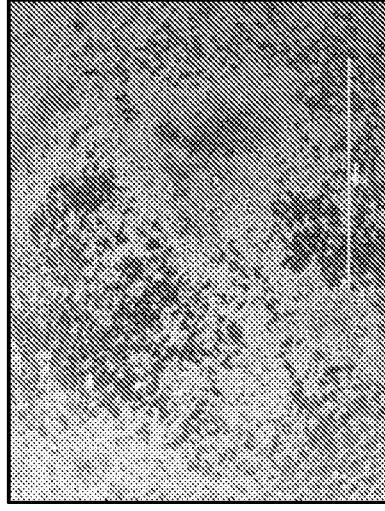
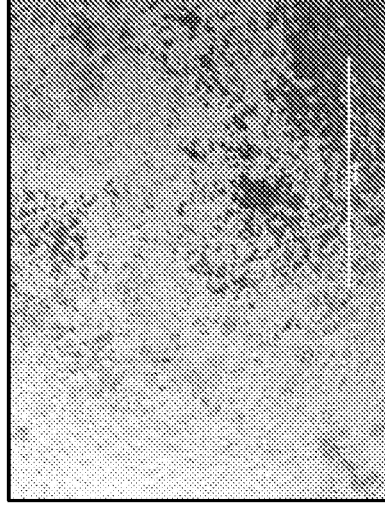
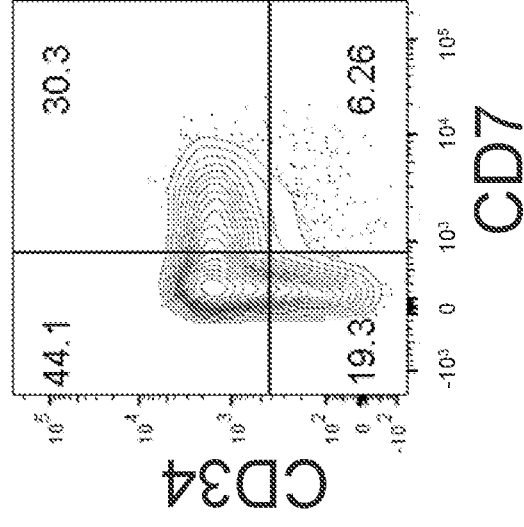
FIG. 7B

*FIG. 8A**FIG. 8B*

MPP

MPP Fresh

Cryopreserved



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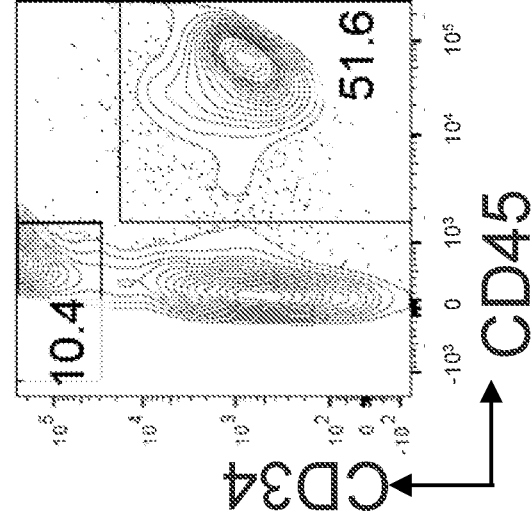


FIG. 8C

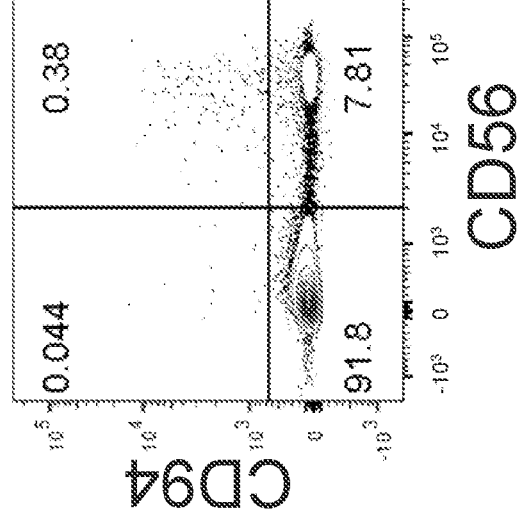


FIG. 8D

Day 8 differentiation cultures can be shipped overnight to generate iCD34 and iHE cells upon delivery.

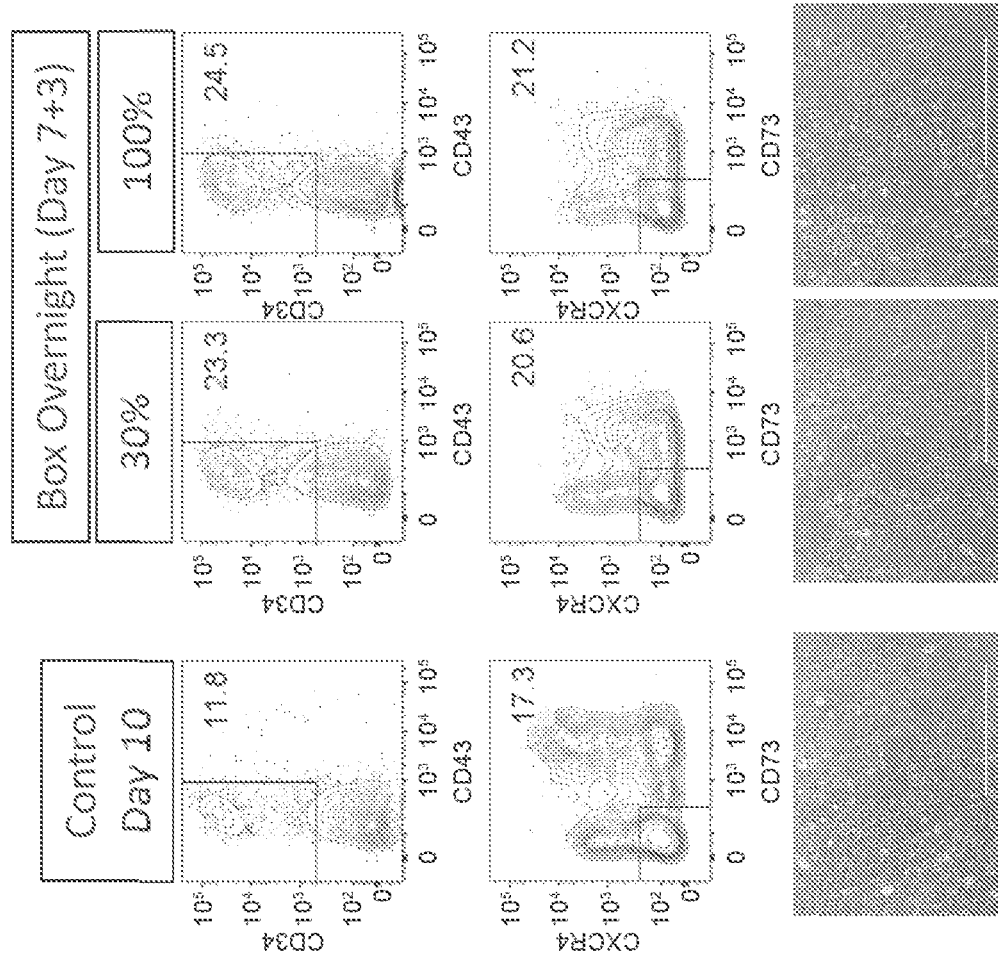


FIG. 9A

Day 8 differentiation cultures can be shipped overnight to generate iCD34 and iHE cells upon delivery.

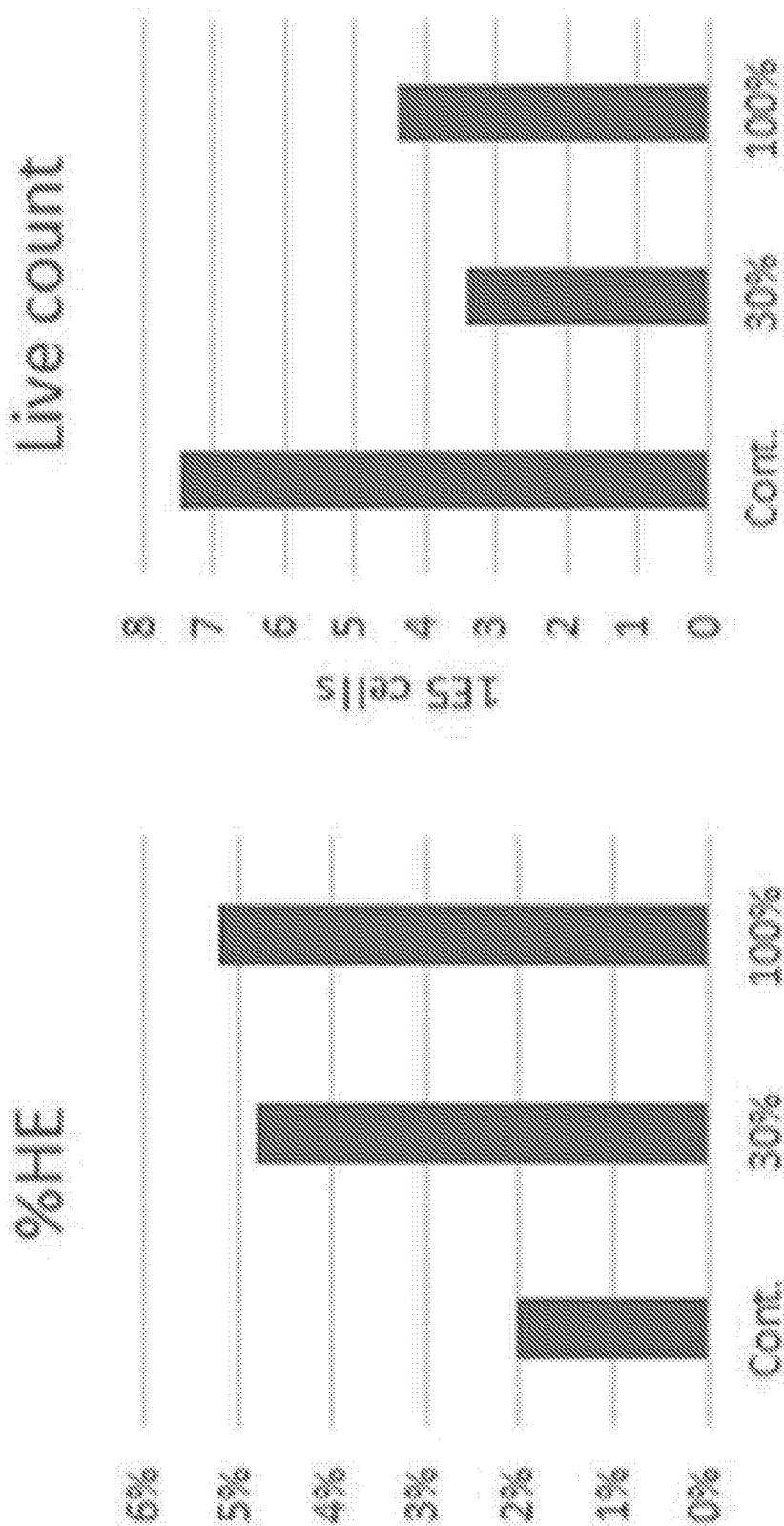


FIG. 9B

ipSC-derived, iCD34 differentiated towards T cells.

Early T cell lineage markers Mature T cell lineage markers

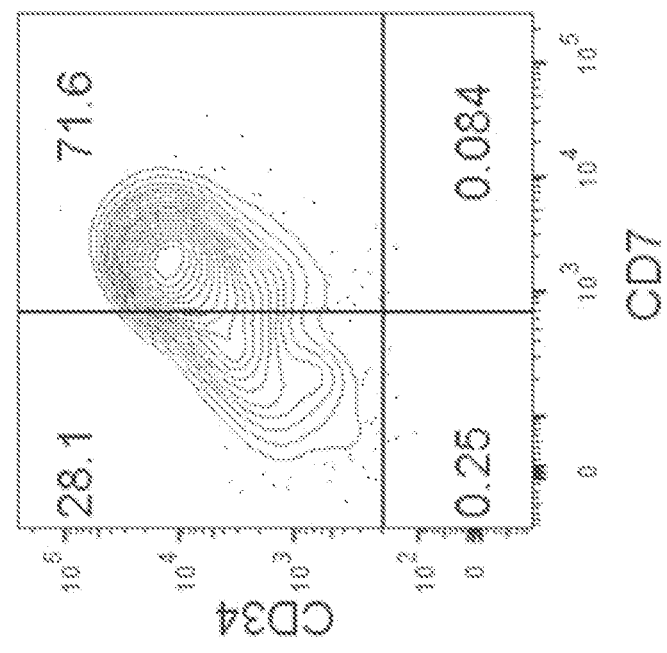


FIG. 10A

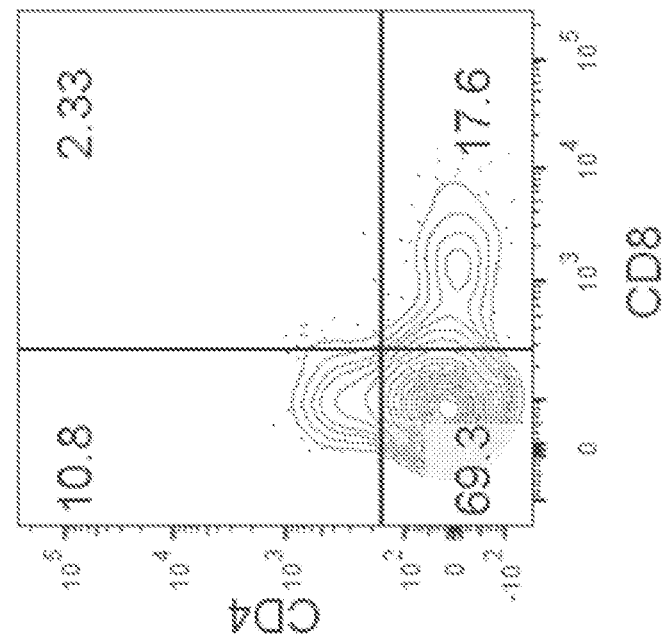


FIG. 10B

iPSC-derived, iCD34 differentiated towards T cells.

Day 5: UCB CD34⁺ cells versus
iCD34⁺ cells

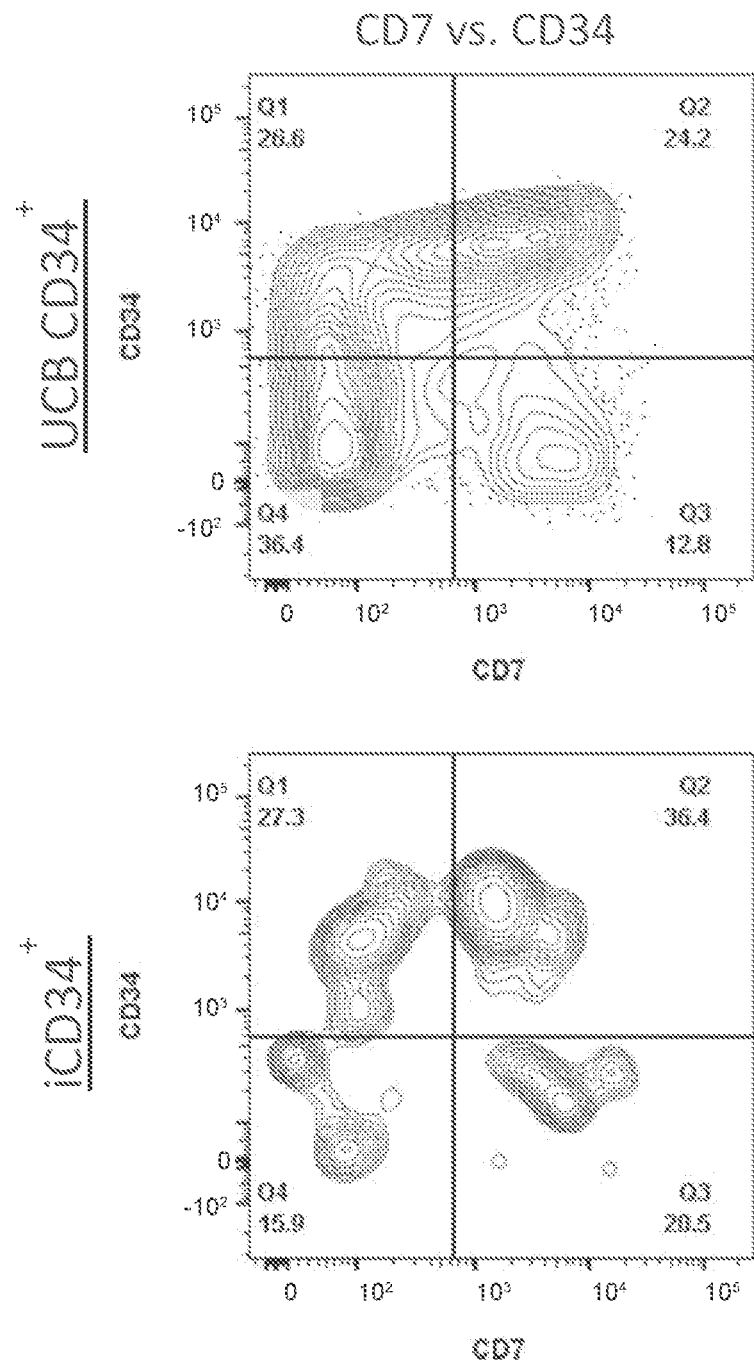


FIG. 10C

iPSC-derived, iCD34 differentiated towards NK cells.

Early NK lineage markers

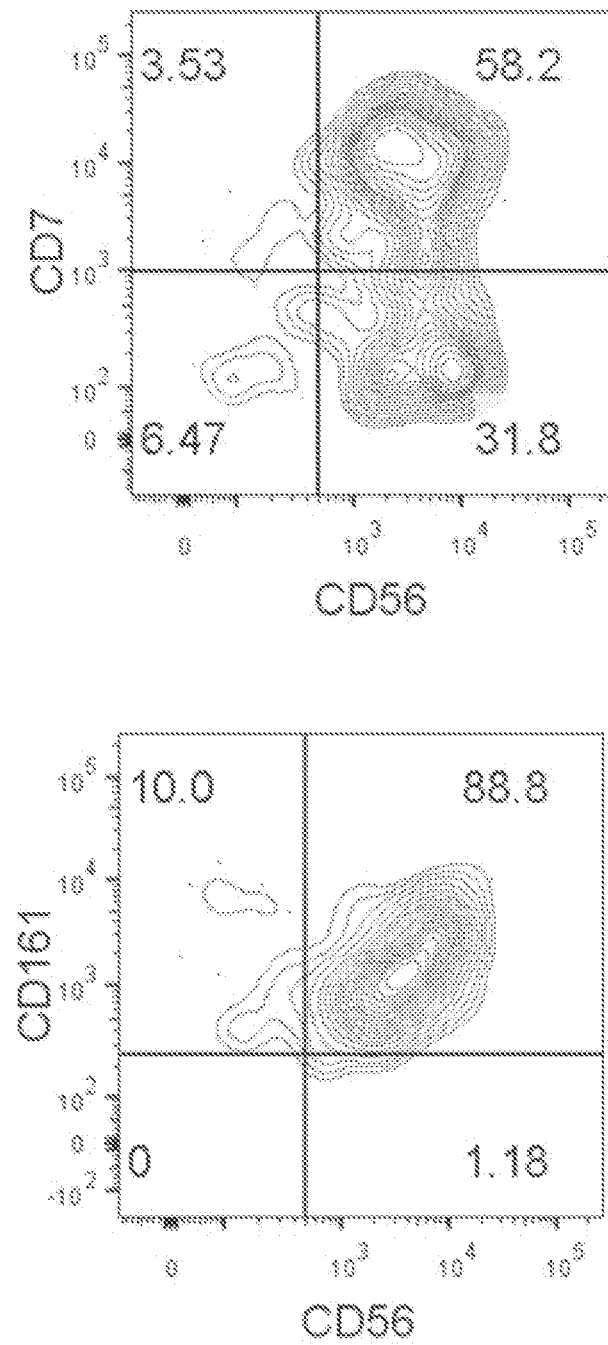


FIG. 11A

iPSC-derived, iCD34 differentiated towards NK cells.

Mature NK lineage markers

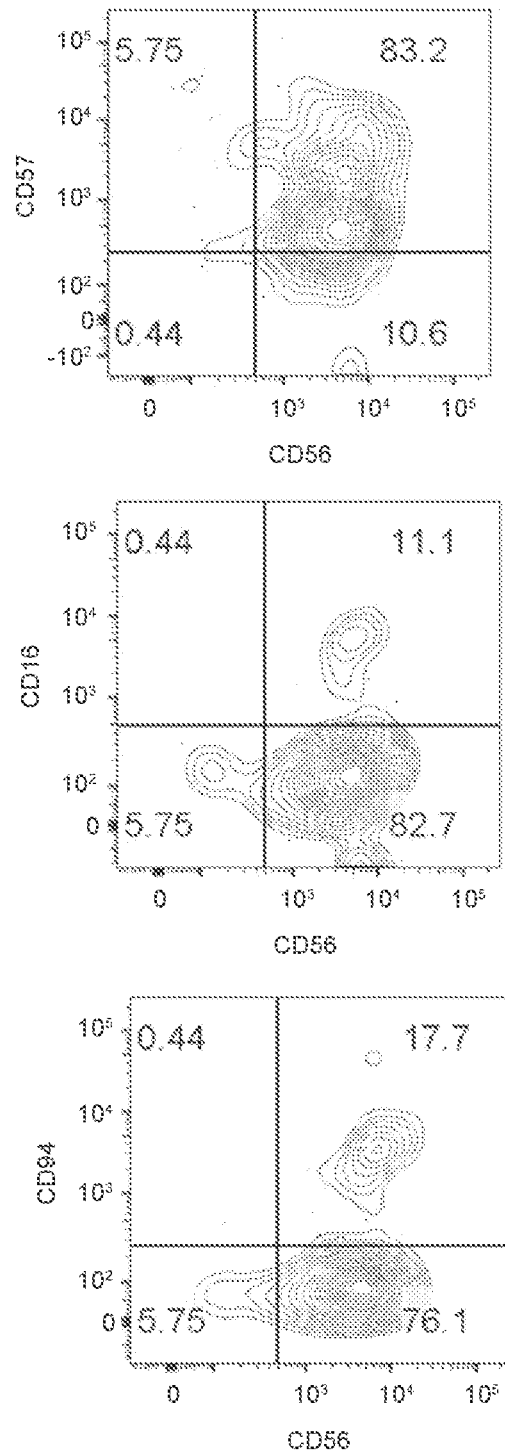


FIG. 11B

iPSC-derived, iCD34 differentiated towards NK cells.

Day 5: UCB CD34⁺ cells versus iCD34⁺ cells

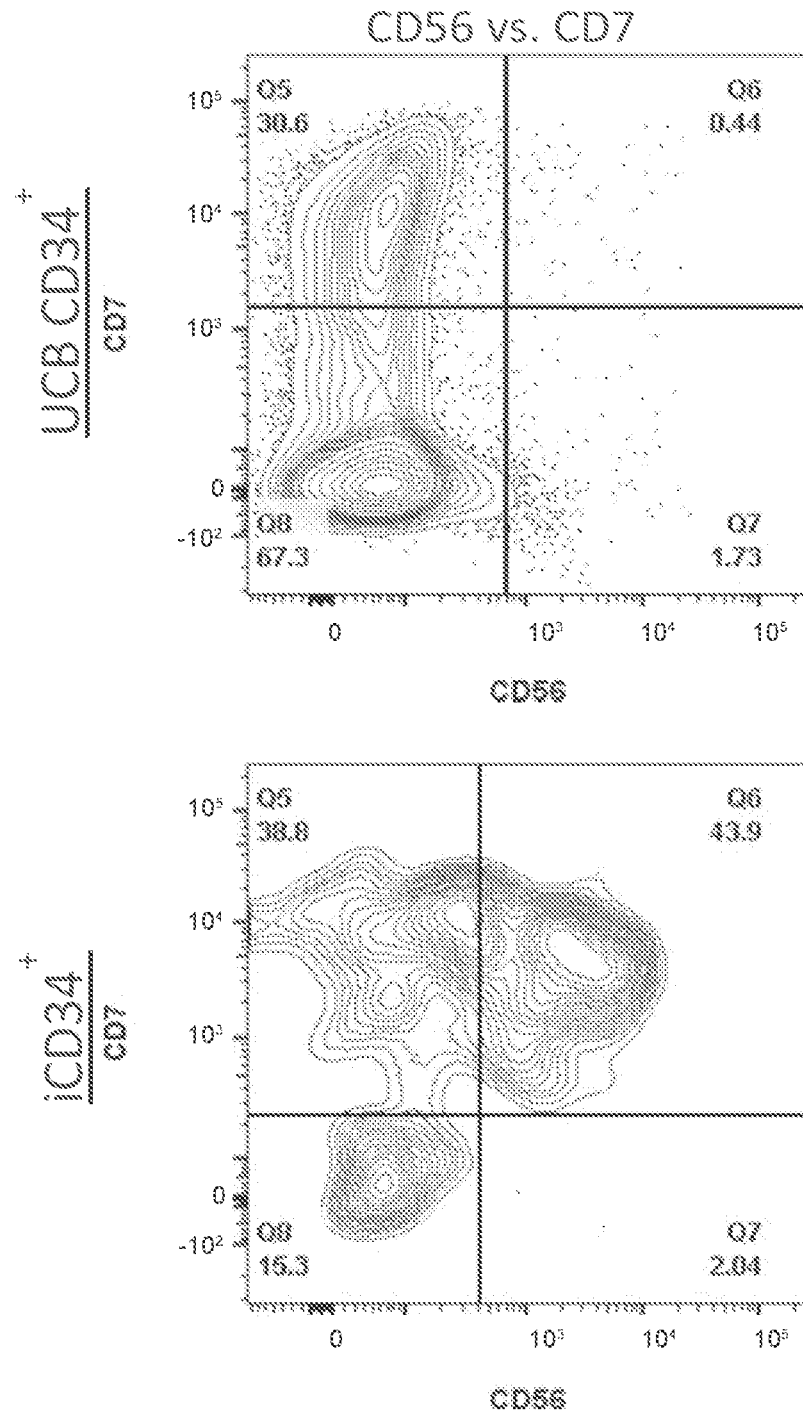
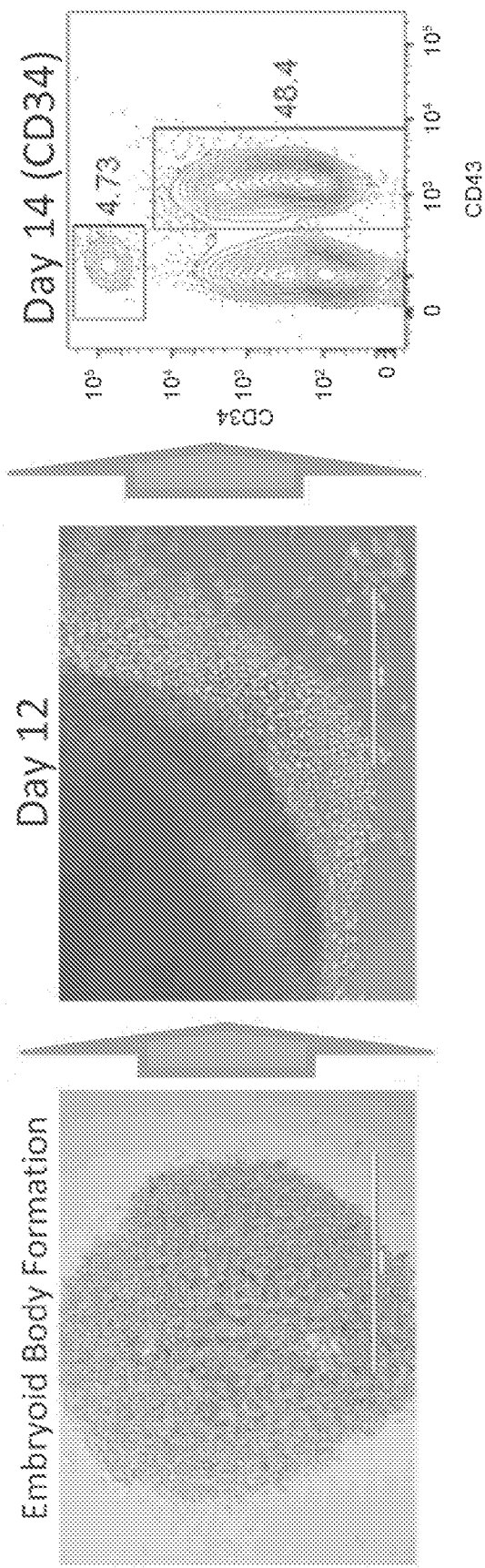


FIG. 11C

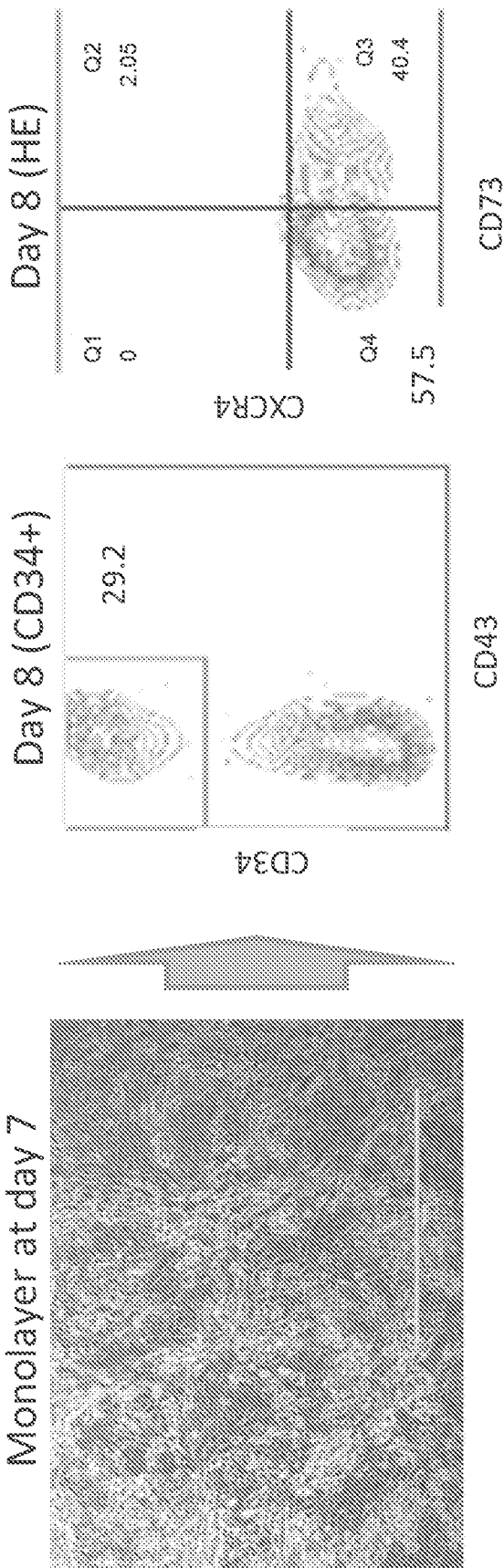
Monolayer hiPSC hematopoietic differentiation platform allows for a scalable expansion strategy that is not seen during EB formation



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

FIG. 12A

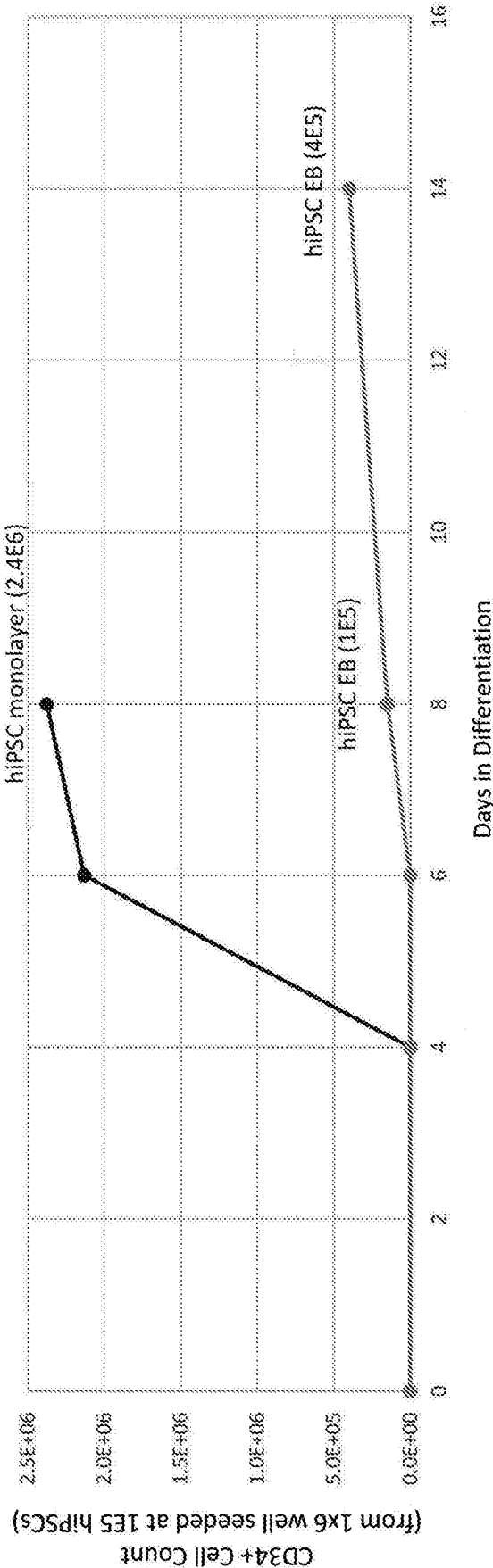
Monolayer hiPSC hematopoietic differentiation platform allows for a scalable expansion strategy that is not seen during EB formation



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

FIG. 12B

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



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

FIG. 12C

Pluripotent Cell Clonal Expansion: Off-the-Shelf Scalability and Product Consistency

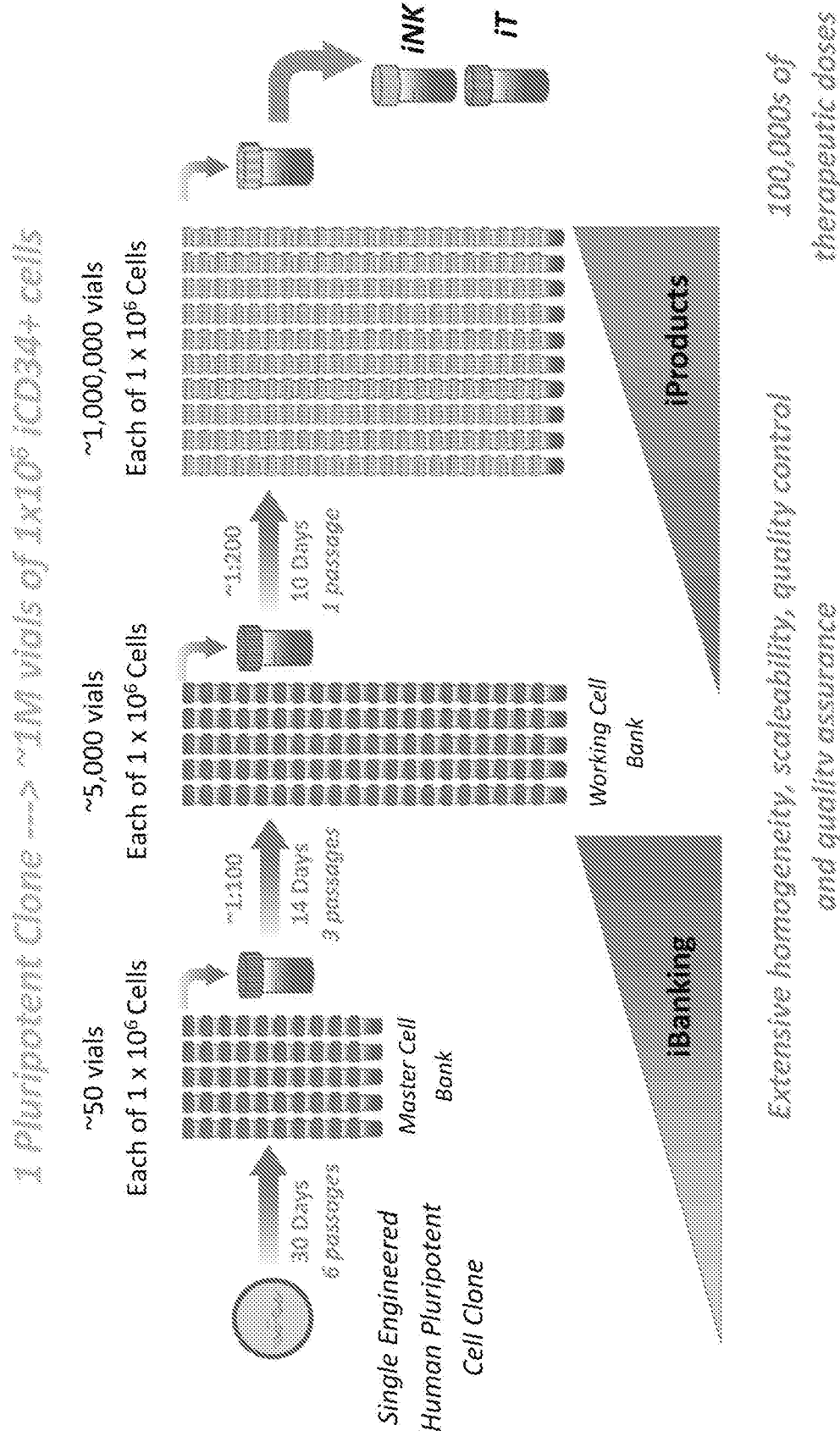


FIG. 13

Immune-regulatory properties of iCD34+ cells.

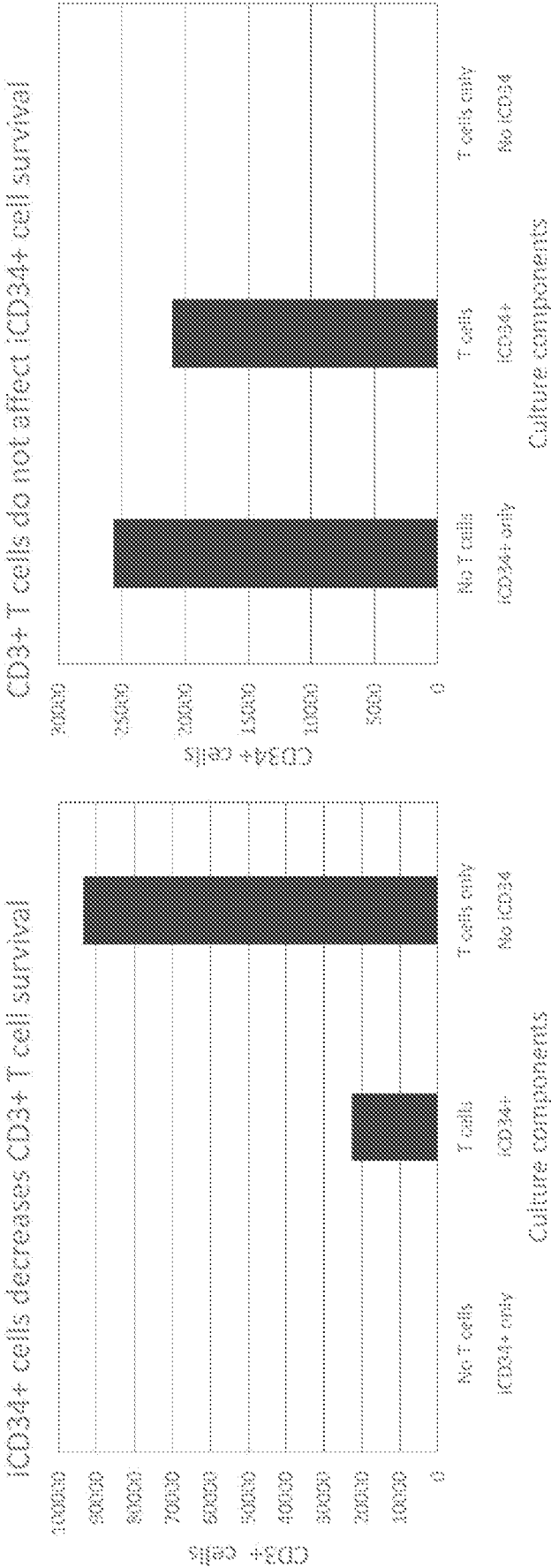


FIG. 14

iPSC-derived, iCD34 cells differentiated towards T cells.

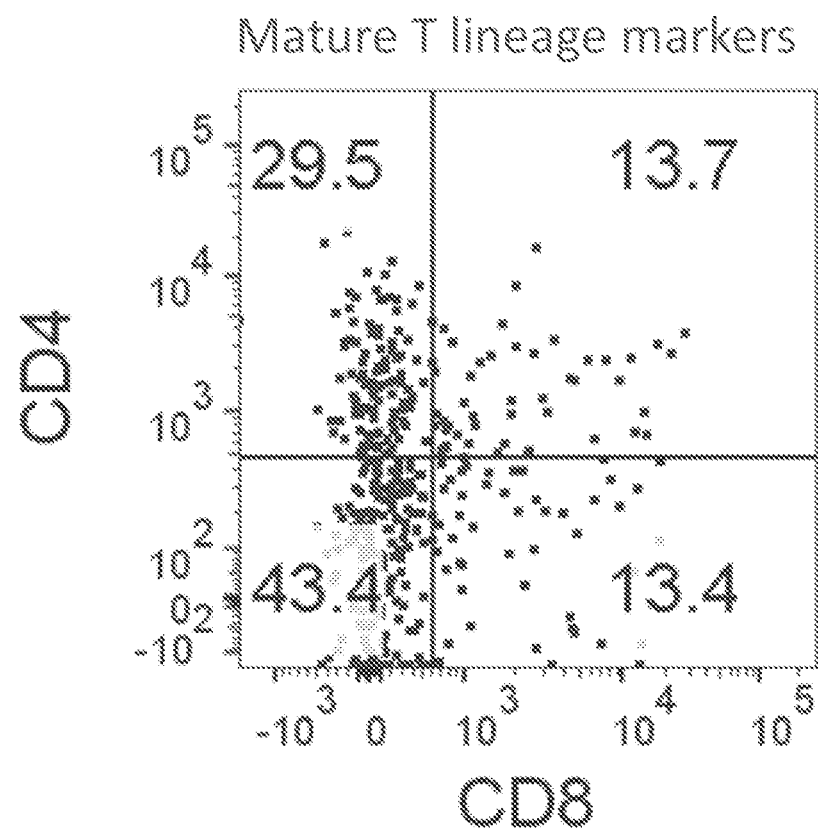


FIG. 15

Feeder-based suspension culture supports the maturation of iCD34-derived NK cells.

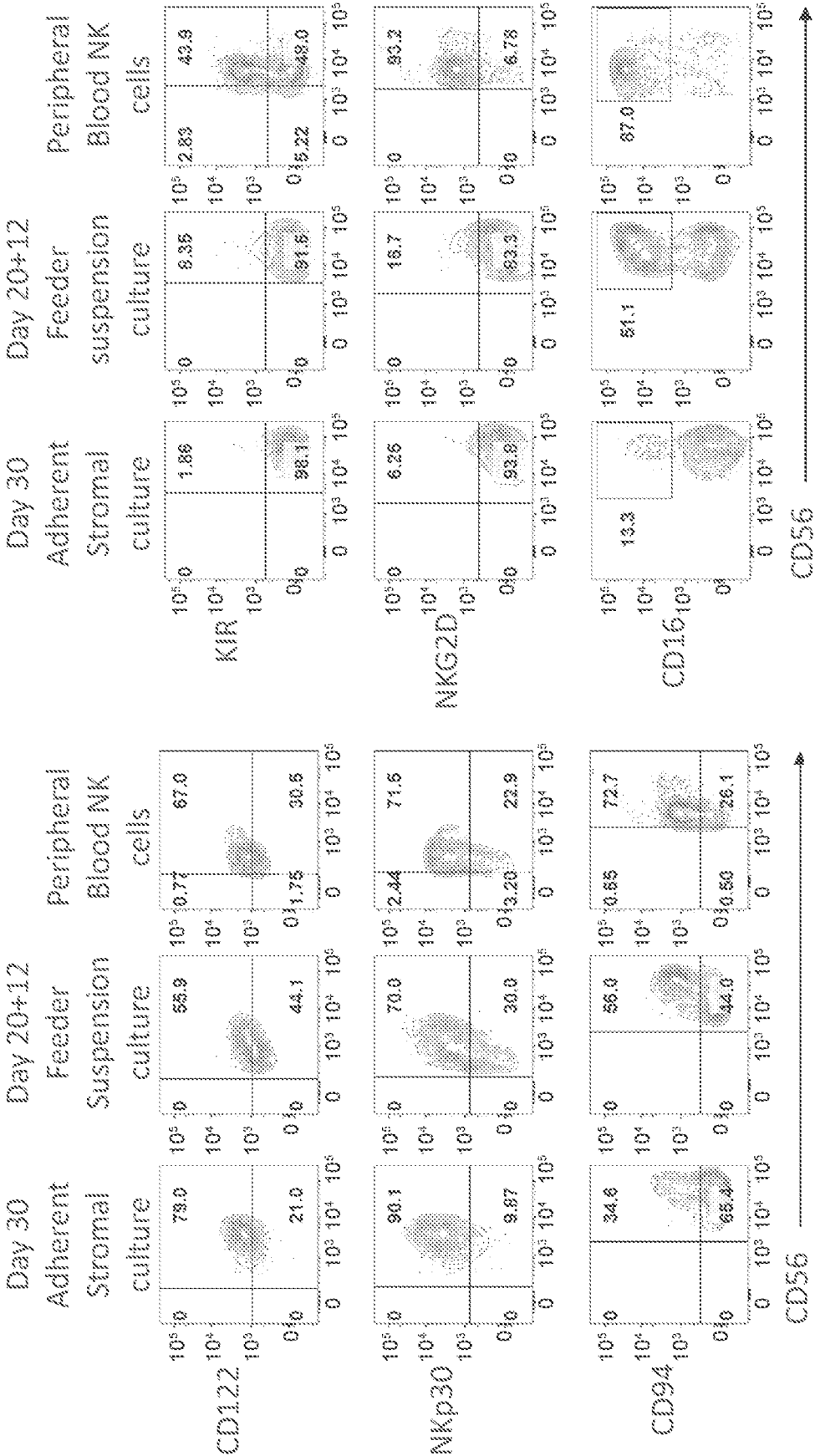


FIG. 16

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

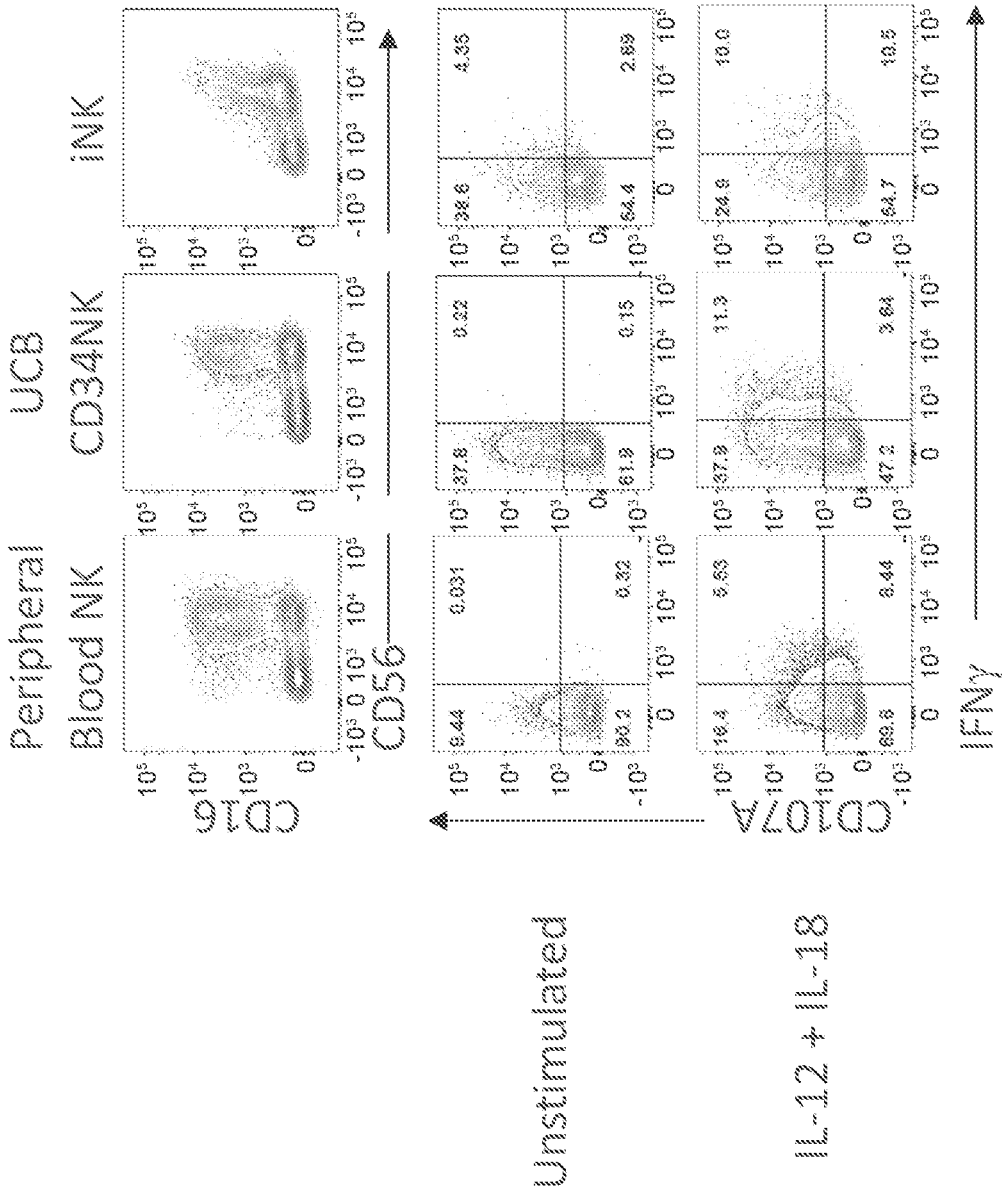


FIG. 17

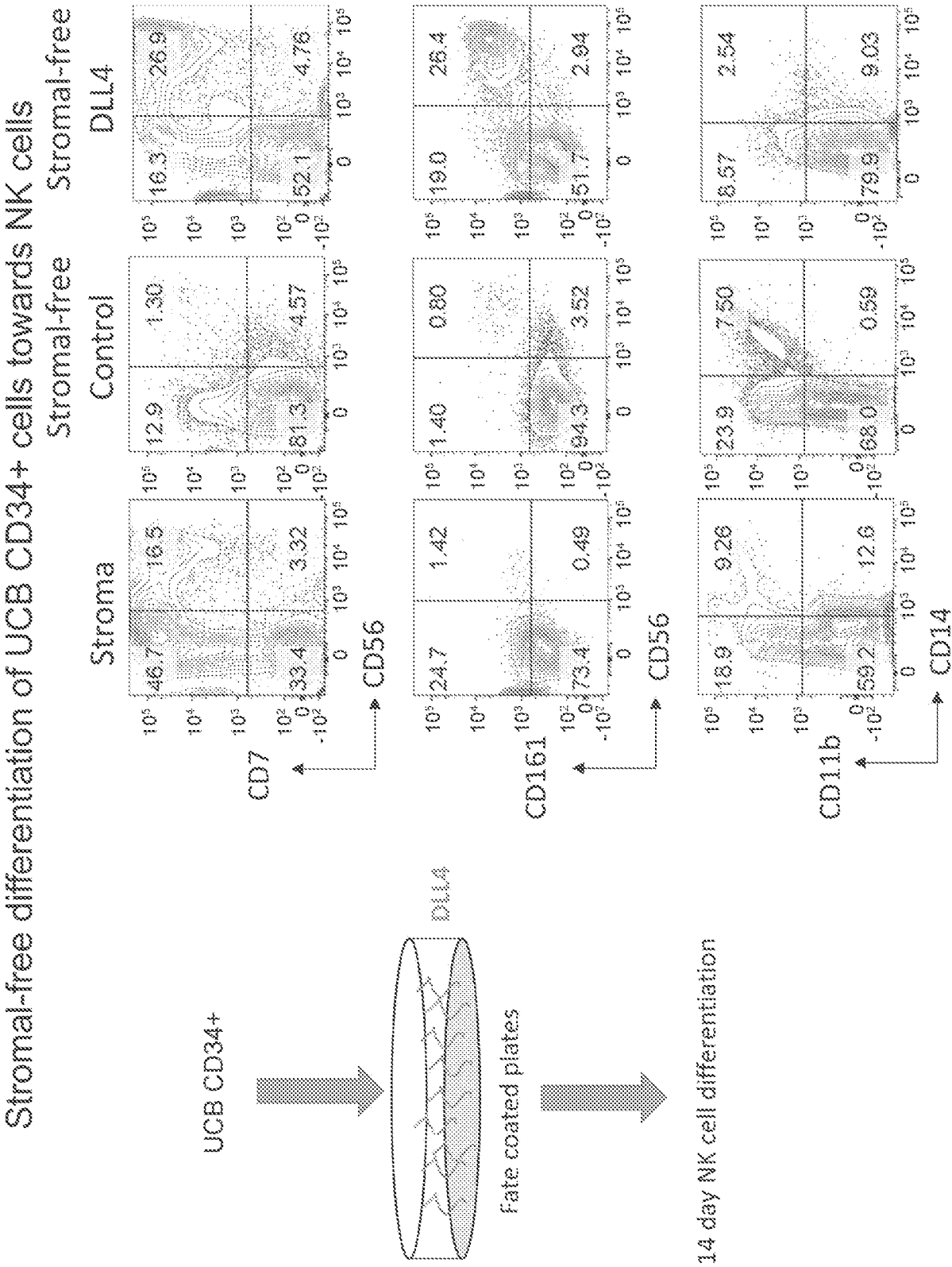


FIG. 18

Stromal-free differentiation of iPSC-derived iCD34+ cells towards NK cells

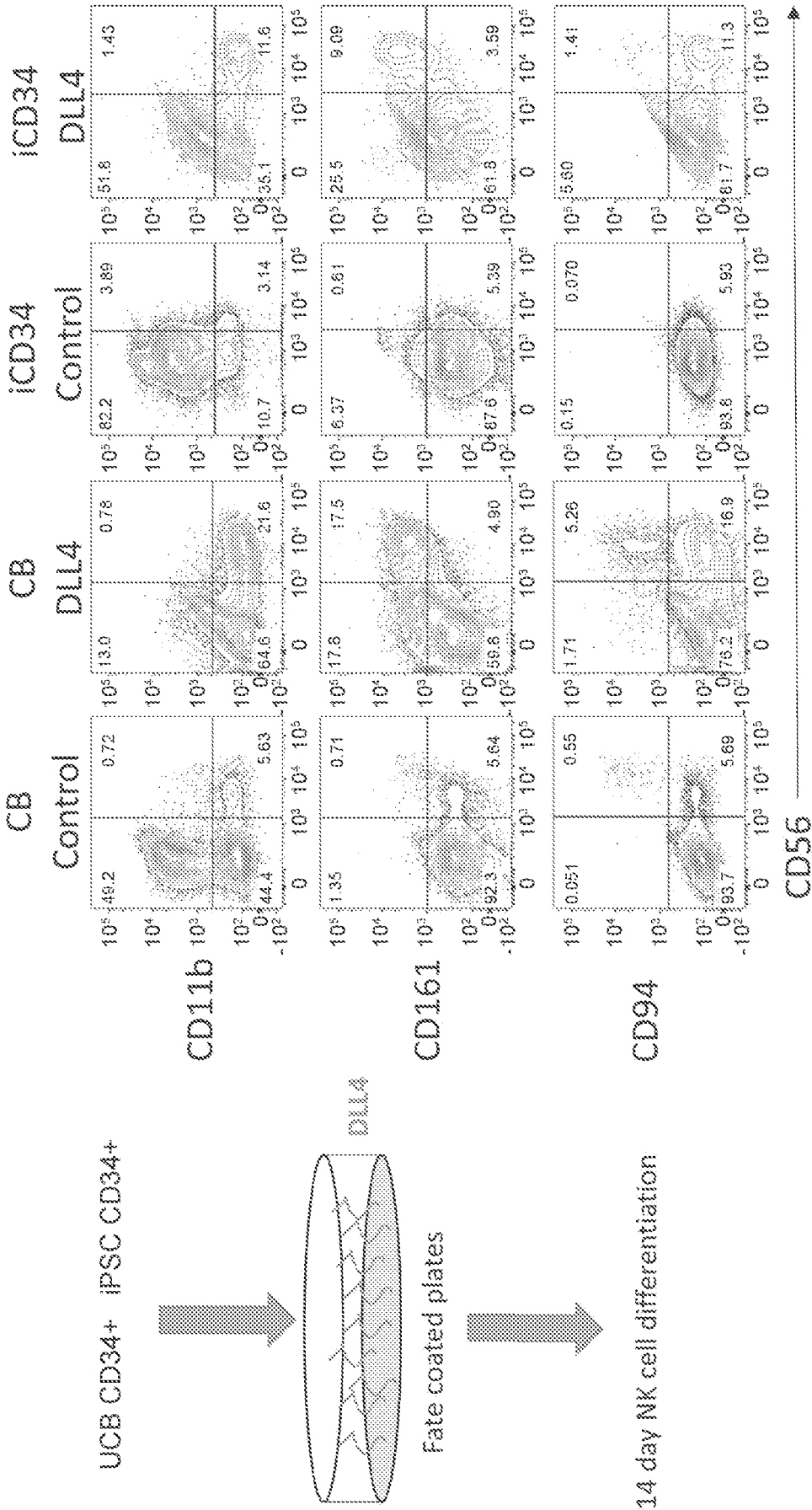


FIG. 19

Stromal-free differentiation of UCB CD34+ cells towards T cells.

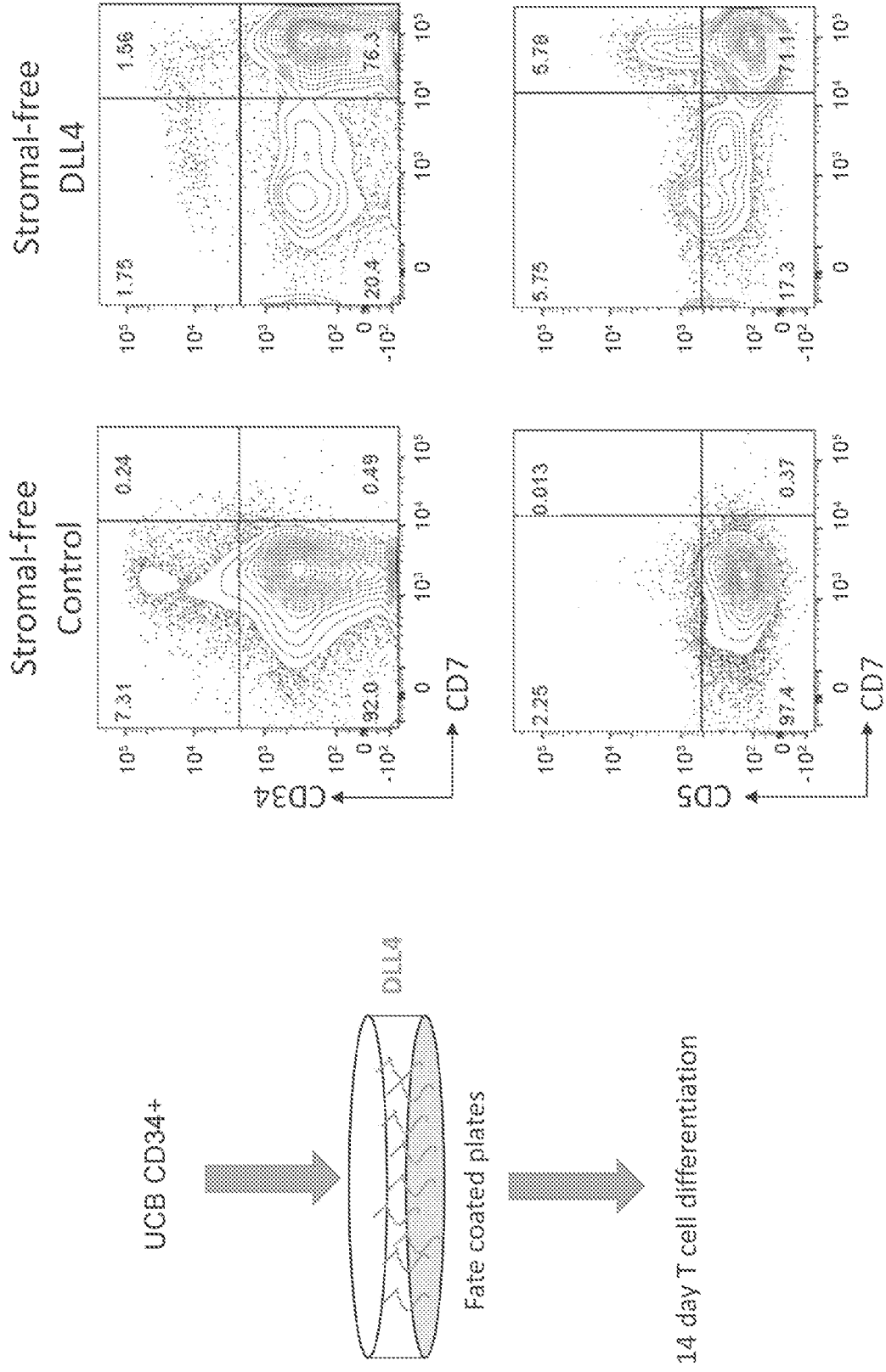
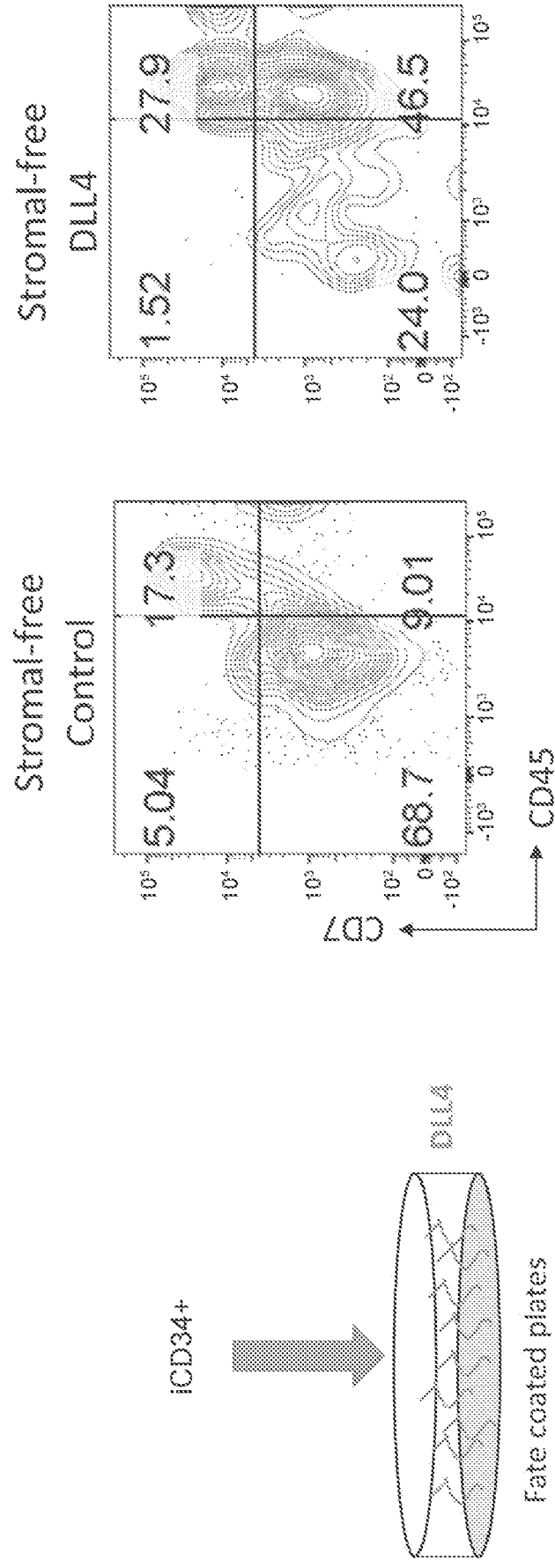


FIG. 20

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



14 day T cell differentiation

FIG. 21

Engraftment of hiPSC-derived iCD34+

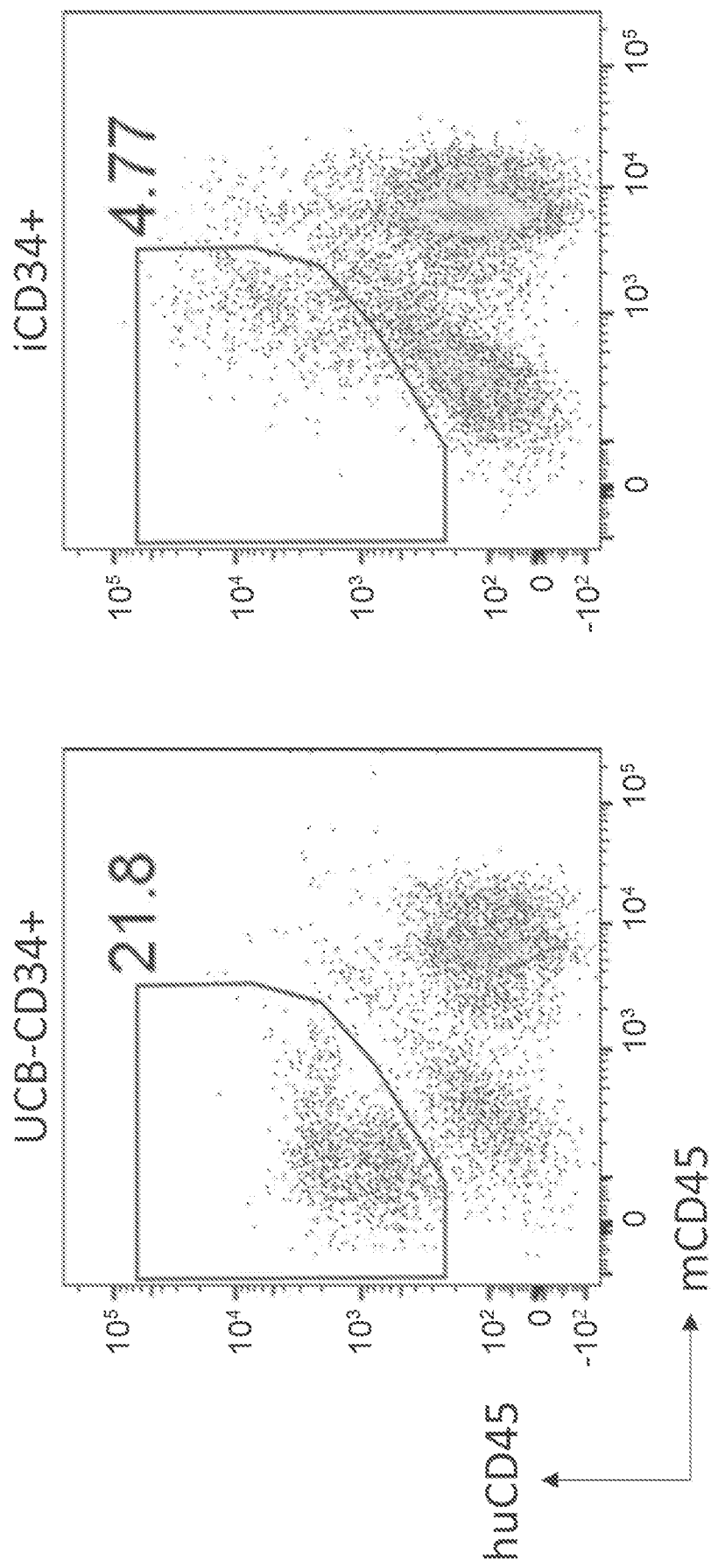


FIG. 22

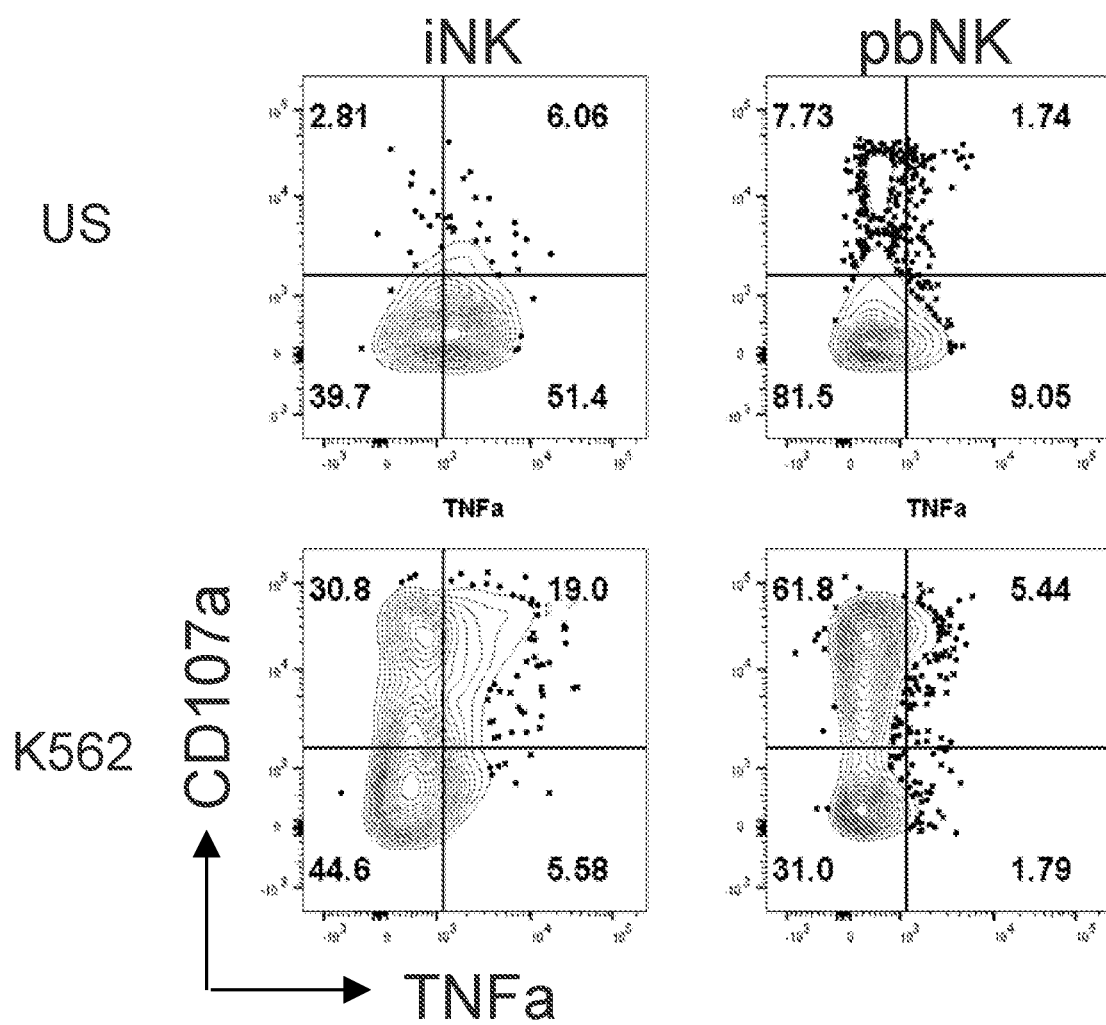


FIG. 23A

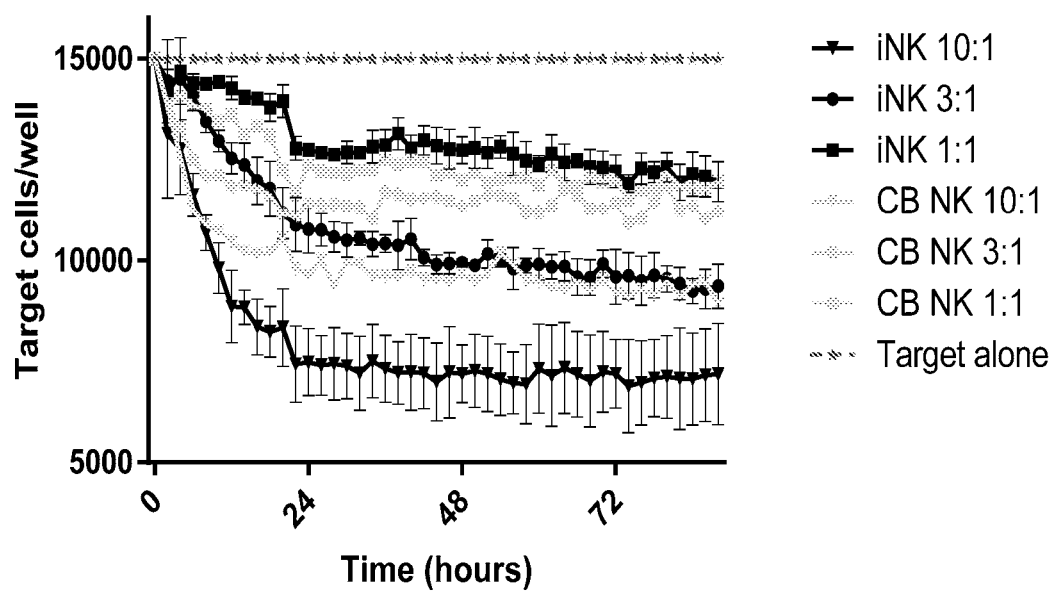


FIG. 23B

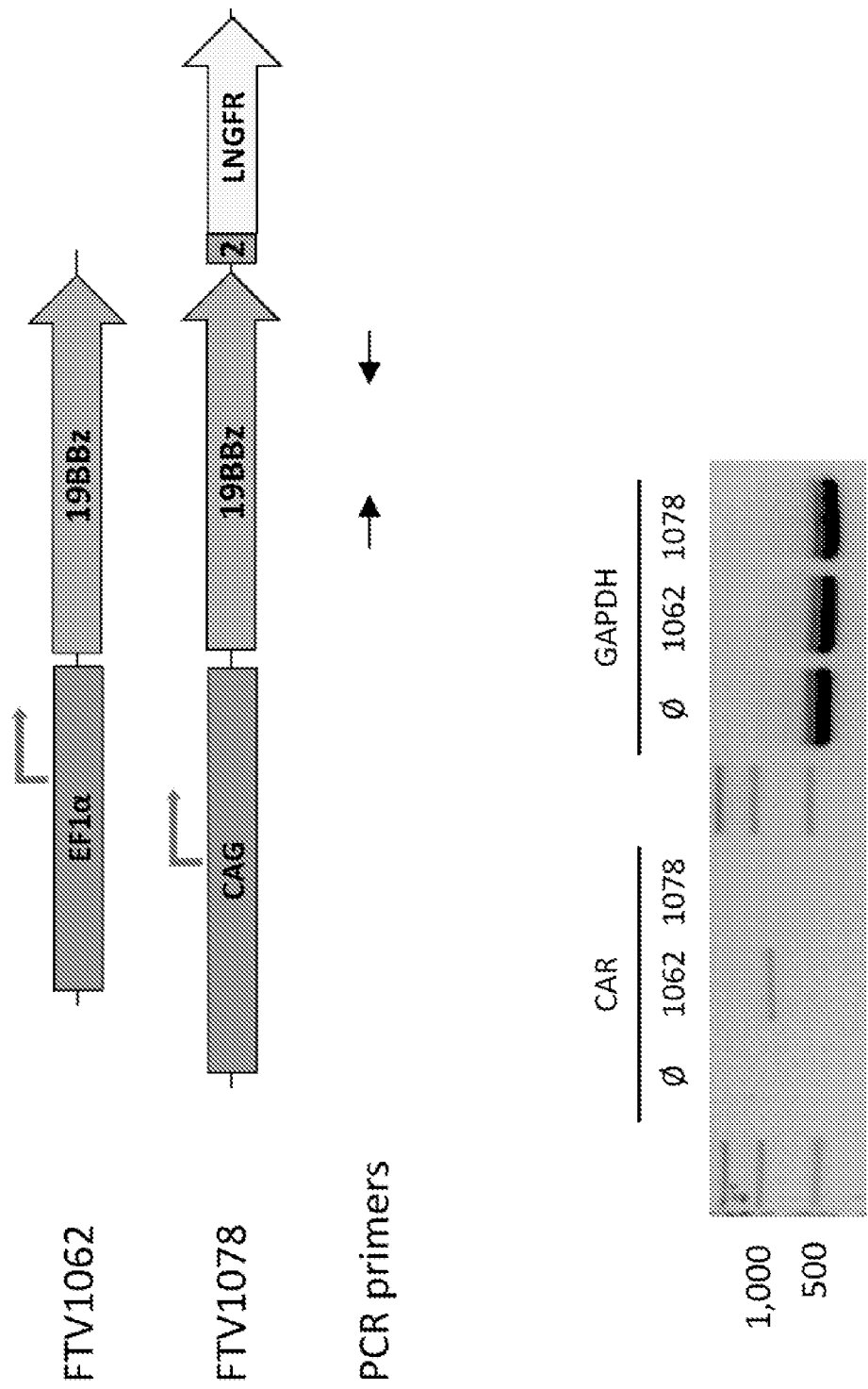


FIG. 24

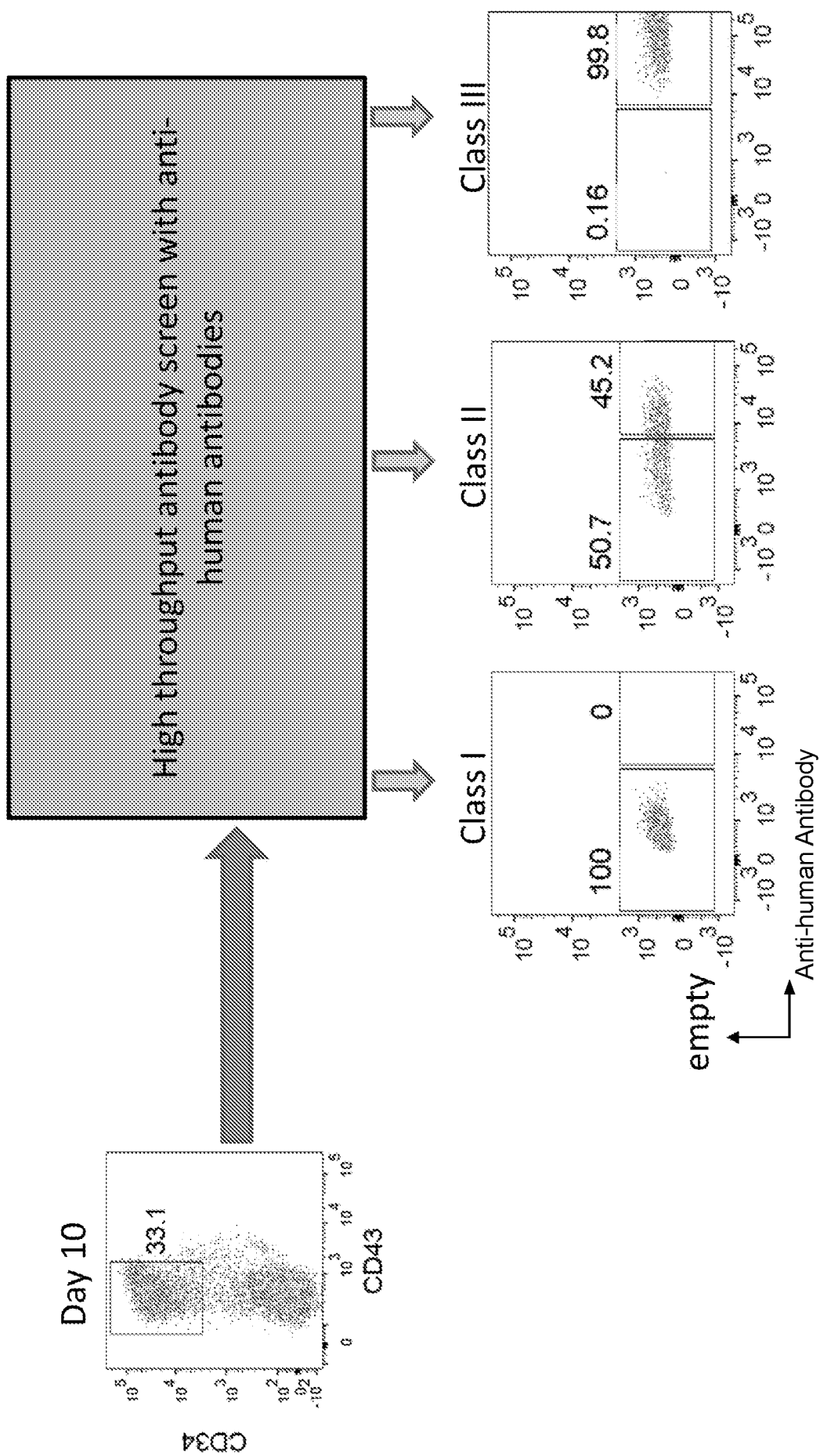


FIG. 25

Name	Clone	CD34+ CD43- Marker+	CD34+ CD43- Marker+	CD34+ CD43- Marker+	Name	Clone	CD34+ CD43- Marker+	CD34+ CD43- Marker+	CD34+ CD43- Marker+	Name	Clone	CD34+ CD43- Marker+	CD34+ CD43- Marker+	CD34+ CD43- Marker+	Name	Clone	CD34+ CD43- Marker+	CD34+ CD43- Marker+	CD34+ CD43- Marker+	
CD29	TS2/16	100	0	51.7	44.7	CD44	BJ18	51.7	44.7	CD97	VM3b	4.26	93.9	CXCR7	CXCR7	8F11-M16	0.53	99.3	CD34+	CD43- Marker++
CD49e	NK1-SAM-1	100	0	45.7	47.2	CD82	ASL-24	45.7	47.2	CD319	162.1	3.95	95.7	CD74	CD74	LN2	0.51	99.3	CD34+	CD43- Marker++
CD59	p282 (H19)	100	0.027	42	51.2	CD54	HA58	42	51.2	CD69	FN50	3.34	95.7	CD274	CD274	29E.2A3	0.48	99.1	CD34+	CD43- Marker++
CD34	581	99.9	0.1	41.2	51.2	SSEA-5	8E11	41.2	51.2	CD338	5D3	3.29	95.7	4-1BB Ligand	4-1BB Ligand	5F4	0.46	99.3	CD34+	CD43- Marker++
CD24	M15	99.9	0.12	39.3	51.4	CD56	HCD56	39.3	51.4	Podoplanin	NC-08	3.16	95.5	CD215	CD215	IM7A4	0.46	99.4	CD34+	CD43- Marker++
CD90	5E10	99.7	0.2	39.7	52	CD206	15-2	39.7	52	CD111	R1.302	2.78	93.3	Tim-1	Tim-1	1D12	0.45	99.6	CD34+	CD43- Marker++
CD276	MIH42	99.7	0.23	42.3	52.8	CD73	AD2	42.3	52.8	CD304	12C2	2.7	94.6	CD140b	CD140b	18A2	0.44	99.4	CD34+	CD43- Marker++
CD298	LNH-94	99.7	0.28	35	55.9	CCR10	6588-5	35	55.9	CD326	9C4	2.49	95.2	CD277	CD277	BT3.1	0.41	98.8	CD34+	CD43- Marker++
CD81	5A6	99.5	0.31	32.1	59.7	CD164	67D2	32.1	59.7	CD257	T7-241	2.14	97.4	CD231	CD231	SN1a (M3-3D9)	0.4	99.4	CD34+	CD43- Marker++
CD112	TX31	99.6	0.34	30.5	61.2	CD95	DX2	30.5	61.2	CD100	A8	2.08	96.4	CD179b	CD179b	HSL11	0.35	99	CD34+	CD43- Marker++
CD156c	SHM14	99.4	0.43	25.1	66.1	CD144	BV9	25.1	66.1	CD32	FUN-2	2.02	97.5	CD344	CD344	CH3A4A7	0.35	99.5	CD34+	CD43- Marker++
CD63	H5C6	99	0.76	21.4	74.5	CD166	3A6	21.4	74.5	CD253	RIK-2	1.73	97.8	TNAP	TNAP	W8B2	0.32	99.7	CD34+	CD43- Marker++
CD99	HCD99	99.1	0.78	21.2	67.5	Lymphotoxin β Receptor	31G4D8	21.2	67.5	CD79b	CB3-1	1.59	98.3	CD278	CD278	C398.4A	0.31	99.5	CD34+	CD43- Marker++
CD31	WM59	98.8	1	19.7	74	CD252	11C3.1	19.7	74	CD33	WM53	1.5	97.8	gamma/delta TCR	gamma/delta TCR	B1	0.31	99.6	CD34+	CD43- Marker++
CD165	SN2 (N6-D11)	98.4	1.37	19	73.8	CD55	JS11	19	73.8	CD83	H815e	1.37	98.5	CD220	CD220	B6.220	0.3	99.5	CD34+	CD43- Marker++
CD102	C8B-IC2/2	98.3	1.54	17.8	74.9	HLA-A,B,C	W6/32	17.8	74.9	GARP	7B11	1.34	96.6	CD158b	CD158b	DX27	0.28	99.2	CD34+	CD43- Marker++
CD146	SHM-57	97.5	1.82	17.1	78.5	CD40	H814	17.1	78.5	CD183	G025H7	1.33	98.5	CD197	CD197	G043H7	0.28	99.6	CD34+	CD43- Marker++
CD49c	ASC-1	96.5	2.62	17	75.3	CD46	TRA2-10	17	75.3	CD357	621	1.03	98.9	MICA/MCIB	MICA/MCIB	6D4	0.28	99	CD34+	CD43- Marker++
CD13	WM15	94.6	4.16	17	74.9	CD340	24D2	17	74.9	CD261	DIR1	0.98	98.8	CD84	CD84	CD84.1.21	0.27	99.6	CD34+	CD43- Marker++
CD58	TS2/9	92.7	4.24	15.7	76.3	CD119	GIR-208	15.7	76.3	CD196	G034E3	0.97	98.9	Jagged 2	Jagged 2	MH12-523	0.27	99.6	CD34+	CD43- Marker++
β2-microglobulin	2M2	90.9	5.66	14.8	80	CD106	STA	14.8	80	MSC-NPC	W4A5	0.97	99	CD124	CD124	G077F6	0.26	99.6	CD34+	CD43- Marker++
Integrin α9β1	V9A2	91.5	5.94	14	82.5	CD66a/c/e	ASL-32	14	82.5	CD218a	H44	0.96	98.9	CD1d	CD1d	5.1.1	0.25	99.7	CD34+	CD43- Marker++
CD51	NK1-M9	90.7	6.46	13.9	79.6	CD49d	9F10	13.9	79.6	CD115	9-4D2-1E4	0.94	99	CD48	CD48	BJ40	0.25	99.6	CD34+	CD43- Marker++
CD10	H110s	91.4	6.53	13.7	81	CD45RB	MEM-55	13.7	81	CD266	ITEM-1	0.9	98.6	CD158f	CD158f	UP-R1	0.24	99.7	CD34+	CD43- Marker++
CD202b	33.1 (Ab33)	91.1	7.59	13.5	81	DL4	MHD4-46	13.5	81	EGFR	AV13	0.84	98.9	CD62L	CD62L	DREG-56	0.23	99.5	CD34+	CD43- Marker++
CD141	M80	88.6	9.28	12.2	82	CD107a	H4A3	12.2	82	HVEM	122	0.8	99	CD80	CD80	2D10	0.23	99.7	CD34+	CD43- Marker++
CD49a	TS2/7	87	9.66	9.98	84.7	CD116	4H1	9.98	84.7	CD271	ME20.4	0.79	98.8	CD85d	CD85d	42D1	0.23	99.6	CD34+	CD43- Marker++
CD9	H19a	85.6	11.9	5.41	87.4	CD324	67A4	5.41	87.4	CX3CR1	2A9-1	0.79	99	CD210	CD210	3F9	0.23	99.7	CD34+	CD43- Marker++
CD201	RCR-401	77.9	17.2	9.18	87.5	CD123	6H6	9.18	87.5	C5L2	1D9-M12	0.78	98.9	IFN-γ R b chain	IFN-γ R b chain	2HUB-159	0.23	99.6	CD34+	CD43- Marker++
CD47	CC2C6	74.5	18.1	9.17	86.8	CD49f	GoH3	9.17	86.8	NPC	57D2	0.76	99.2	SUSD2	SUSD2	W5C5	0.23	99.3	CD34+	CD43- Marker++
CD262	DJR2.4 (7-8)	73.5	18.2	8.17	87.8	CD200	OX-104	8.17	87.8	CD244	C1.7	0.75	99.1	TCR α/β	TCR α/β	IP26	0.23	99.8	CD34+	CD43- Marker++
CD109	W7C5	74.2	20.2	8	87.6	CD71	CY16A	8	87.6	CD301	H037G3	0.75	99.1	CD1a	CD1a	H149	0.22	99.6	CD34+	CD43- Marker++
CD39	A1	74.2	23	7.44	87.8	CD172a	5E5A5	7.44	87.8	CD226	11A8	0.69	98.9	CD28.2	CD28.2	CD28.2	0.22	99.6	CD34+	CD43- Marker++
CD317	RS38E	69.1	26.4	7.39	89.2	CD21	Bu32	7.39	89.2	CD337	P30-15	0.68	99.3	CD85	CD85	GHI/75	0.22	99.5	CD34+	CD43- Marker++
CD143	5-369	67.9	26.5	7.16	91.9	CD184	12G5	7.16	91.9	SUSD2	W3D5	0.62	99.1	CD108	CD108	MEM-150	0.22	99.7	CD34+	CD43- Marker++
Integrin β5	AST-3T	65.4	27.1	6.18	91.1	CD263	DIR3	6.18	91.1	CD138	DL-101	0.6	99.3	HLA-E	HLA-E	3D12	0.22	99.7	CD34+	CD43- Marker++
CD105	43A3	67.4	27.2	5.98	89.6	HLA-A2	BB7.2	5.98	89.6	CD181	8F1/CXCR1	0.6	99.2	CD64	CD64	10.1	0.21	99.7	CD34+	CD43- Marker++
CD155	SK11.4	61.2	28.3	5.74	89.9	CD221	1H7/CD221	5.74	89.9	CD167a	51D6	0.58	99.2	DR3	DR3	JD3	0.21	99.7	CD34+	CD43- Marker++
CD93	VIMD2	62.4	32.6	5.44	91	Notch 4	MHN4-2	5.44	91	CD158d	mAB 33 (33)	0.56	99.2	Siglec-9	Siglec-9	K8	0.21	99.8	CD34+	CD43- Marker++
SSEA-4	MC-813-70	59.2	34.2	4.51	92.3	MSC	W7C6	4.51	92.3	CD352	NT-7	0.56	99.3	F38-2E2	F38-2E2	Tim-3	0.21	99.8	CD34+	CD43- Marker++

FIG. 26

Name	Clone	CD34+ CD43- Marker+	CD34+ CD43- Marker+	Name	Clone	CD34+ CD43- Marker+	CD34+ CD43- Marker+	Name	Clone	CD34+ CD43- Marker+	CD34+ CD43- Marker+
CD61	VI-PL2	0.2	99.8	CD170	1A5	0.1	99.8	CD38	H12	0.048	99.9
CD158a/h	HP-MA4	0.2	99.3	CD200R	OX-108	0.1	99.8	CD148	A3	0.048	99.8
CD161	HP-3G10	0.2	99.8	CD104	58XB4	0.098	99.9	CLEC9A	8F9	0.048	99.9
CD205	HD30	0.2	99.8	Integrin $\beta 7$	FIB504	0.098	99.9	CD85	17G10.2	0.046	99.8
CD255	CARL-1	0.2	99.7	Notch 1	MHN1-519	0.092	99.9	CD3	HIT3a	0.045	99.9
CD300F	UP-D2	0.2	99.4	IgG2a, Ctrl	RTK2758	0.091	99.8	CD131	1C1	0.045	99.9
TCR VB23	gHUT7	0.2	99.8	CD43	CD43-10G7	0.089	99.9	IgLC- κ	MHK-49	0.045	99.9
CD62E	HAe-1f	0.19	99.7	CD180	MHR73-11	0.087	99.9	CD85	MKT5.1	0.044	99.8
CD62P	AK4	0.19	99.6	CD229	Hly-9.1.25	0.044	100	CD229	Hly-9.1.25	0.044	100
CD213a2	SHM38	0.19	99.7	NKp80	5D12	0.084	99.9	CD35	E11	0.042	99.9
CD258	T5-39	0.19	99.7	CD284	HTA125	0.083	99.8	CD235ab	H1R2	0.042	99.9
CD45RO	UCHL1	0.18	99.7	CD314	1D11	0.082	99.9	CD360	2G1-K12	0.042	99.9
CD132	TUGH4	0.18	99.7	CD272	MH26	0.082	99.8	CD27	O323	0.038	100
CD85h	24	0.17	99.7	CD179a	HSL96	0.081	99.9	Pre-BCR	HSL2	0.035	99.9
N/A	DY12	0.17	99.7	IgM, Ctrl	MM-30	0.081	99.9	CD127	A01905	0.034	99.9
SSEA-1	MC-480	0.17	99.8	CD303	201A	0.08	99.8	CD282	TL2.1	0.033	99.9
CD20	2H7	0.16	99.8	CD7	CD7-6B7	0.079	99.8	CD158e1	DX9	0.032	100
CD26	BA5b	0.16	99.6	CD135	BV10A4H2	0.078	99.8	CD300e	UP-H2	0.032	99.7
CD117	104D2	0.16	99.5	CD52	H186	0.077	99.8	HLA-G	87G	0.032	99.9
CD273	24F.10C12	0.16	99.8	CD318	CUB1	0.077	99.9	CD334	4FRGD3	0.031	100
CD66b	G10F5	0.15	99.7	CD351	TXD1	0.077	99.9	CD126	UV4	0.03	99.9
CD70	113-16	0.15	99.8	CD172g	LSB2.20	0.073	99.9	CD140a	16A1	0.03	100
CD85k	ZM4.1	0.14	99.8	CD203c	NP4D6	0.073	99.8	CD336	P44-8	0.029	99.9
CD88	55/1	0.14	99.7	IgM, Ctrl	RTK2118	0.072	99.9	erbB3	184C3	0.029	99.9
Siglec-8	7C9	0.14	99.9	CD11a	H111	0.071	99.8	Siglec-10	566	0.029	99.9
IgG2a, Ctrl	MOPC-173	0.14	99.7	CD15	W6D3	0.071	99.9	CD14	M5E2	0.028	99.9
CD129	AH9H7	0.13	99.7	TCR VB2	B6	0.071	99.9	CD193	5E8	0.028	99.9
CD163	GH1/61	0.13	99.8	CD41	HIP8	0.069	99.9	DL11	MHD1-314	0.028	100
CD209	9E9A8	0.13	99.8	CD254	MH24	0.069	99.8	CD11c	3.9	0.027	99.9
LAP	Tw4-6H10	0.13	99.7	IgG1, Ctrl	RTK2071	0.068	99.9	CD137	4B4-1	0.027	99.9
CD114	LMM741	0.12	99.8	CD18	TS1/18	0.067	99.9	CD286	TLR6.127	0.027	99.9
FcRL4	413D12	0.12	99.9	TCR Va7.2	3C10	0.067	99.9	CD325	8C11	0.027	99.8
C3aR	hC3aR28	0.12	99.7	CD328	6-434	0.061	99.9	CLEC12A	50C1	0.027	99.9
IgD	1Ae-2	0.12	99.9	IgG3, Ctrl	MG3-35	0.06	99.9	DOR	DOR7D2A4	0.026	100
Mac-2	Gal397	0.12	99.8	IgG5, Ctrl	HTK888	0.059	99.9	CD8a	HIT8a	0.025	100
IgG2b, Ctrl	MPC-11	0.12	99.9	CD182	5E8/CXCR2	0.058	99.8	CD86	IT2.2	0.024	100
CD1b	SN13 (K5-188)	0.11	99.8	Blank		0.057	99.9	CD11b	C6RM1/5	0.023	99.9
CD57	HCD57	0.11	99.8	CD4	RPA-74	0.056	99.9	CD290	3C10C5	0.023	99.9
CD178	NOK-1	0.11	99.7	CD25	BC96	0.056	99.9	CD268	11C1	0.022	100
CD355	Cr24.1	0.11	99.9	CD30	BY88	0.053	99.9	CD45	H130	0.021	100
IgG1, Ctrl	MOPC-21	0.11	99.8	CD150	A12 (7D4)	0.052	99.9	CD45RA	H100	0.02	99.8
CD36	5-271	0.1	99.8	CD152	L3D10	0.051	99.9	CD172b	B4B6	0.019	100
CD96	NK92.39	0.1	99.9	IL-28RA	MHLICR2a	0.051	99.9	CD1c	IL161	0	100
CD122	TU27	0.1	99.9	TRA-1-60-R	TRA-1-60-R	0.049	100	CD2	RPA-2.10	0	100

FIG. 26 (Continued)

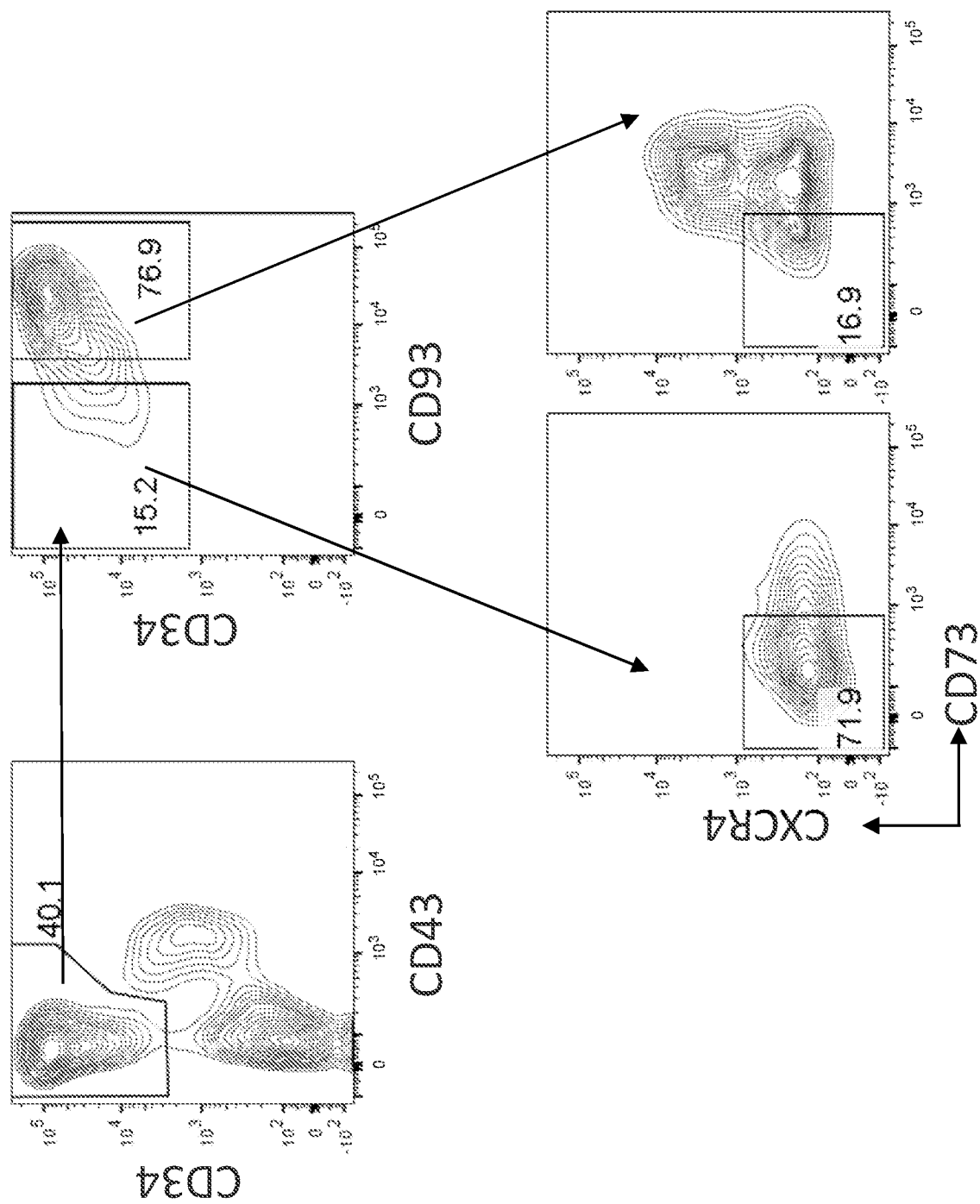
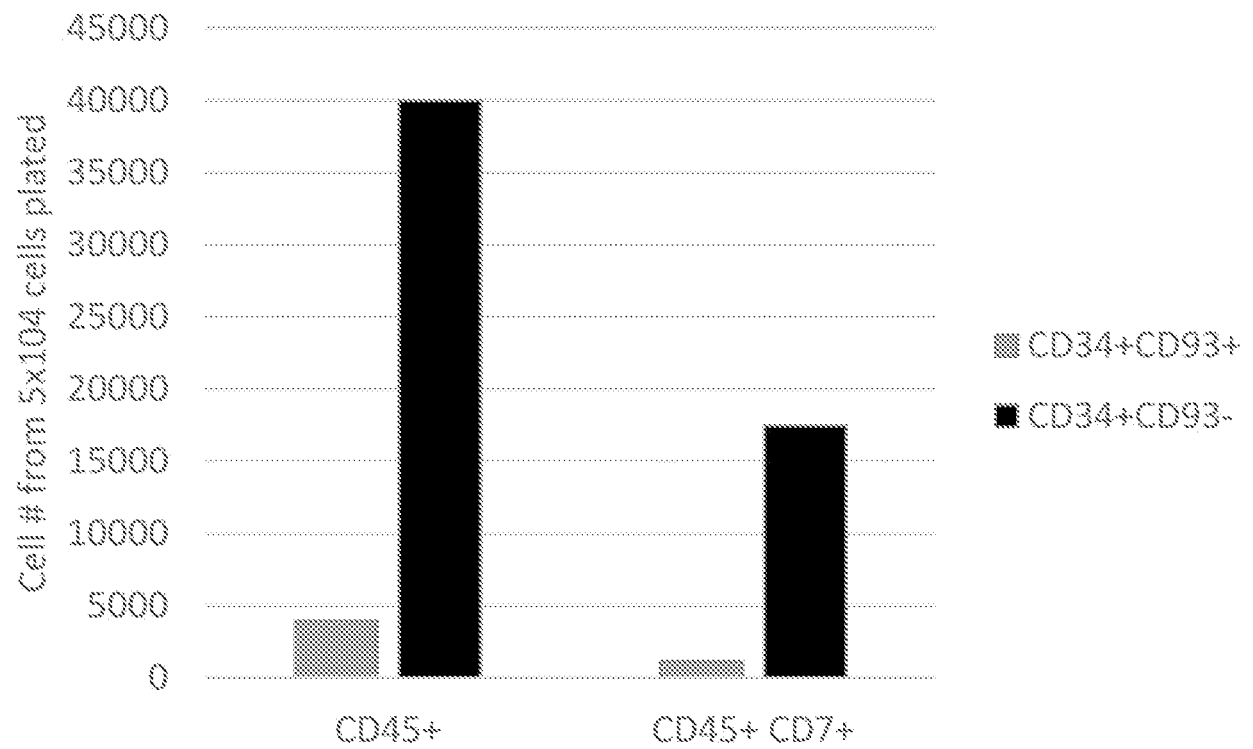
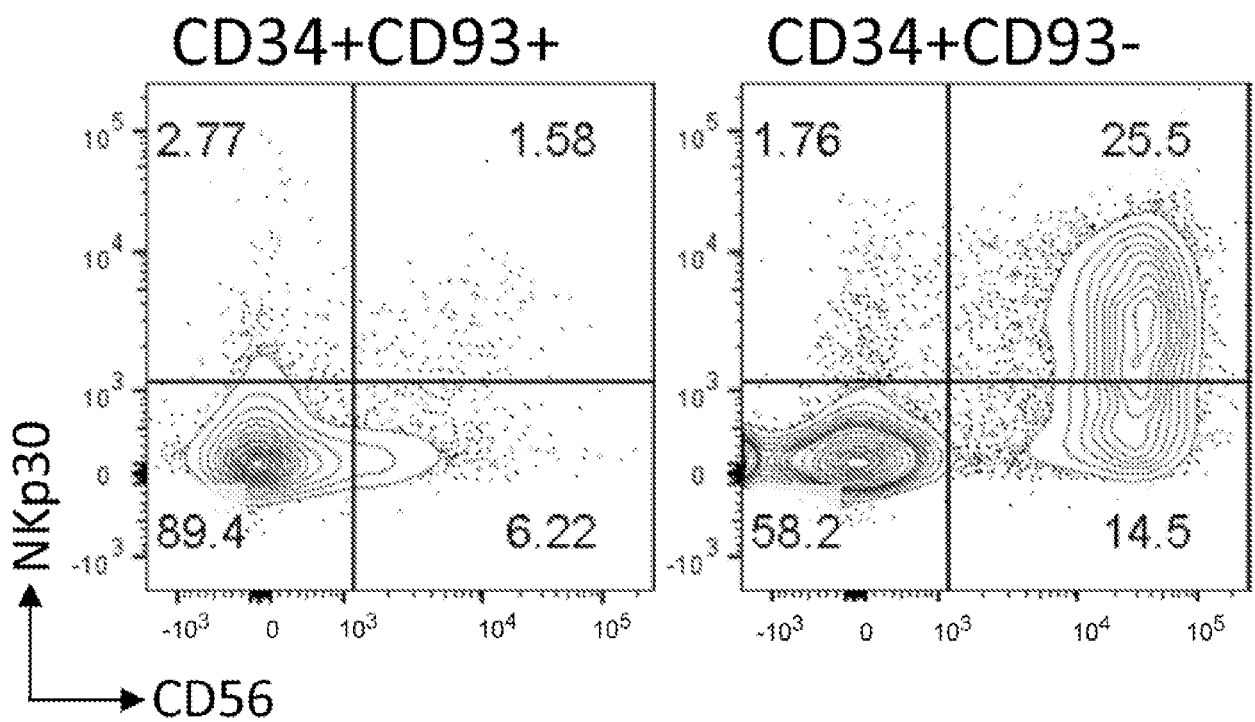


FIG. 27

*FIG. 28A**FIG. 28B*

Modulation of WNT signaling improves the output of iCD34 cells

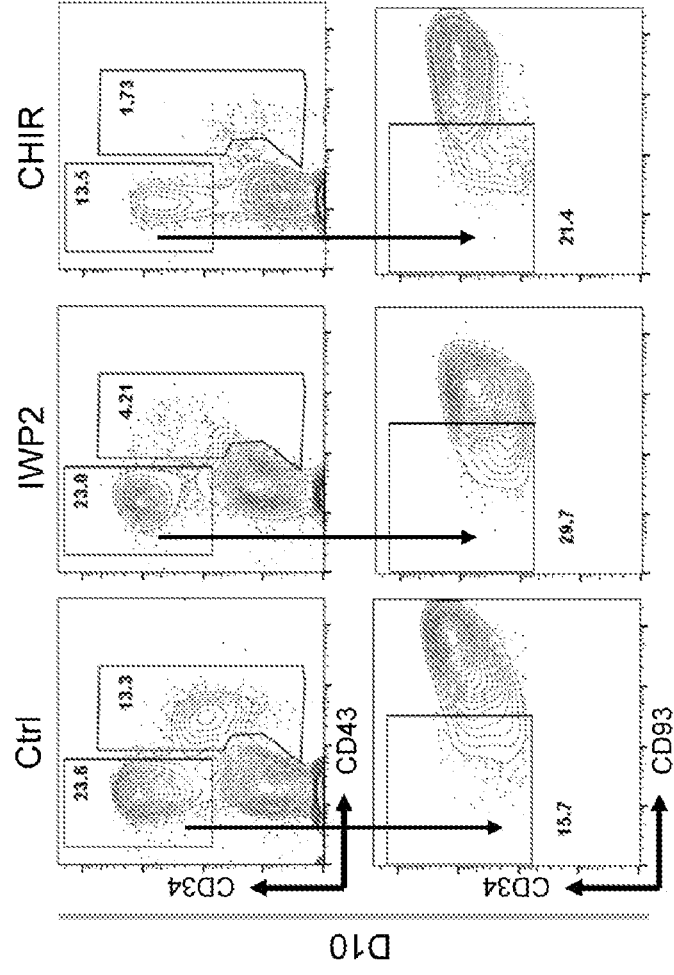


FIG. 29A

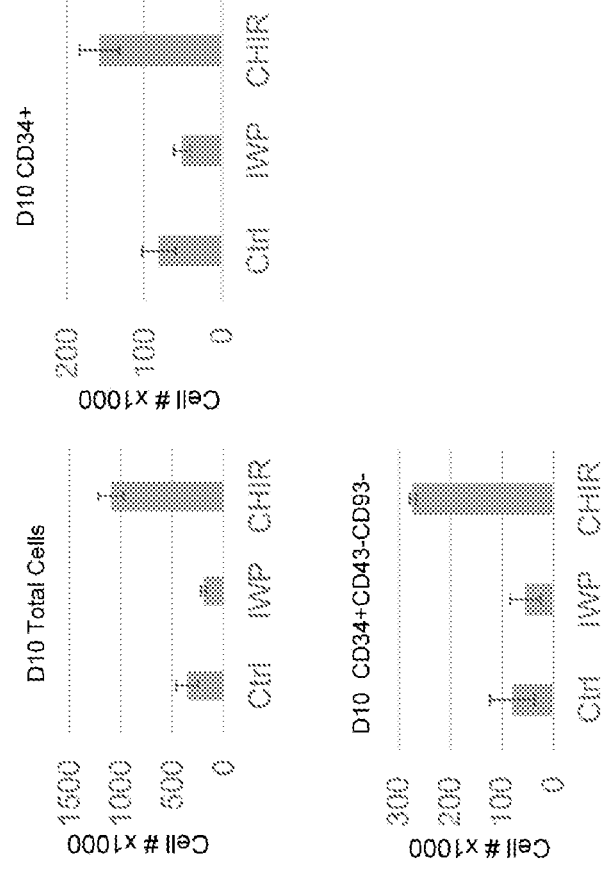


FIG. 29B

Modulation of WNT signaling improves pan hematopoietic and lymphoid output from iCD34 cells

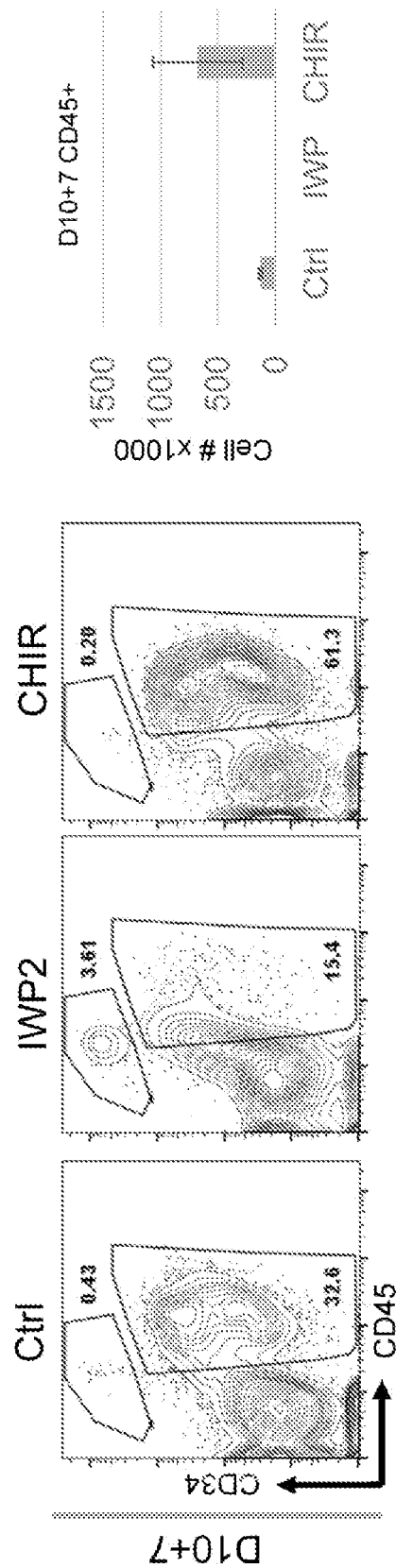


FIG. 30A

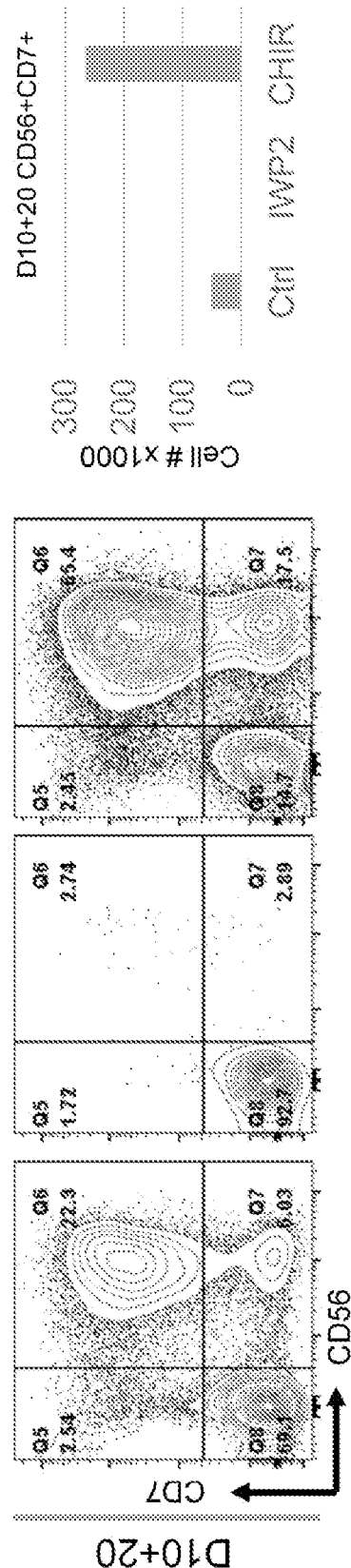


FIG. 30B

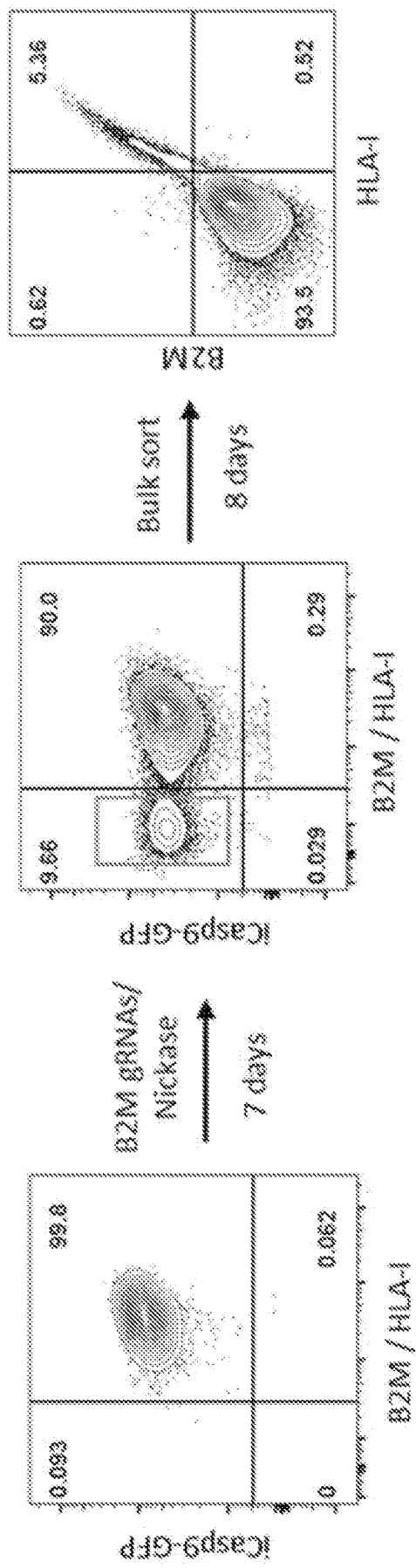


FIG. 31

HLA class I null iPSCs differentiate into iCD34 HE and can be further differentiated into pan-hematopoietic and lymphoid progenitors

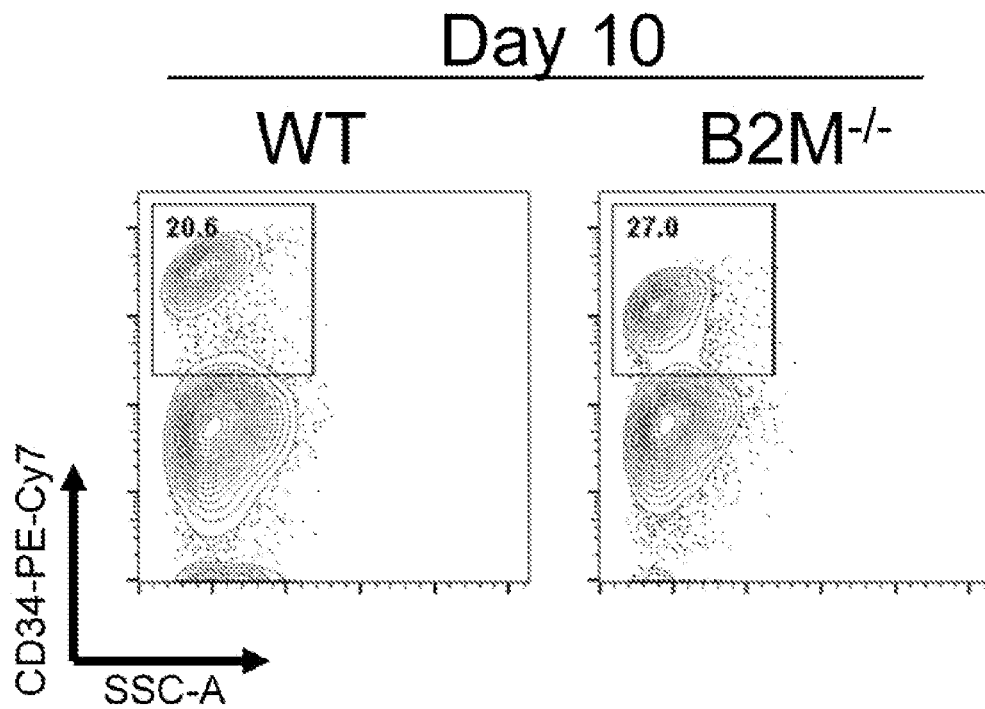


FIG. 32A

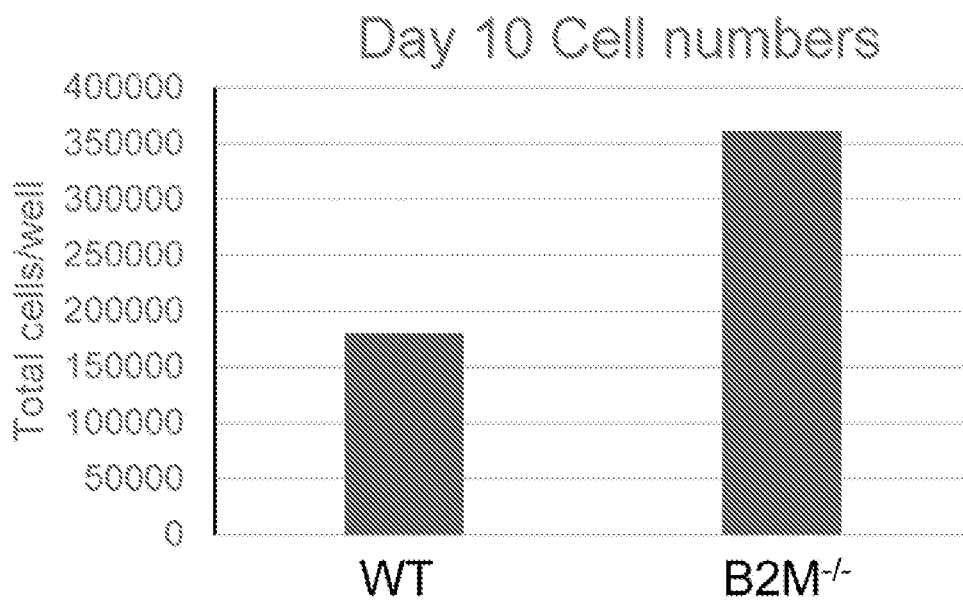


FIG. 32B

HLA class I null iPSCs differentiate into iCD34⁺ HE and can be further differentiated into pan-hematopoietic and lymphoid progenitors

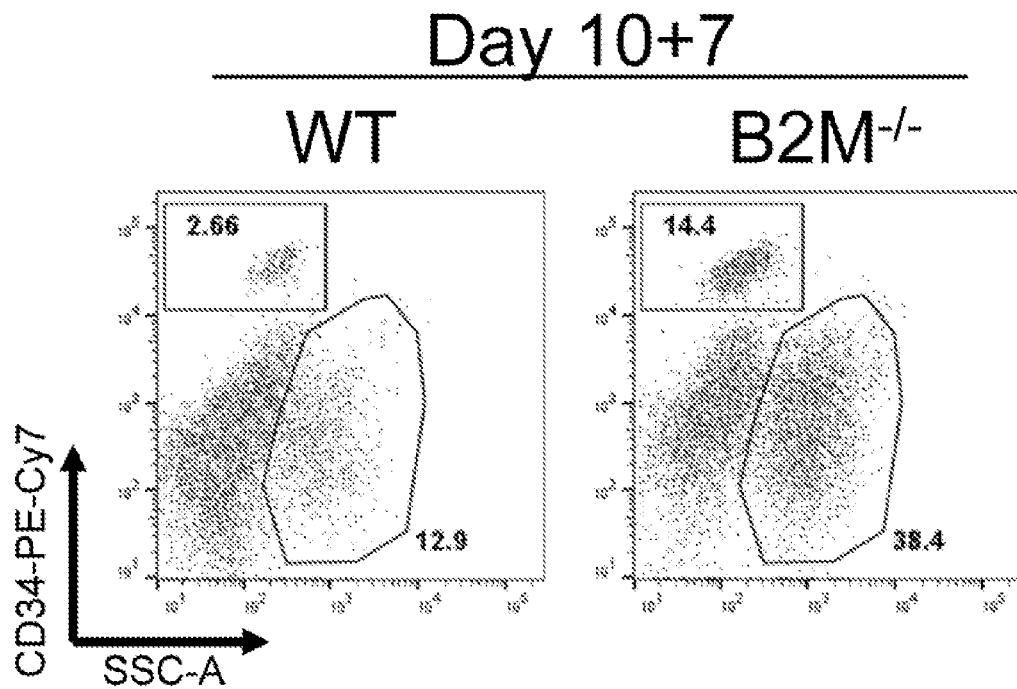


FIG. 32C

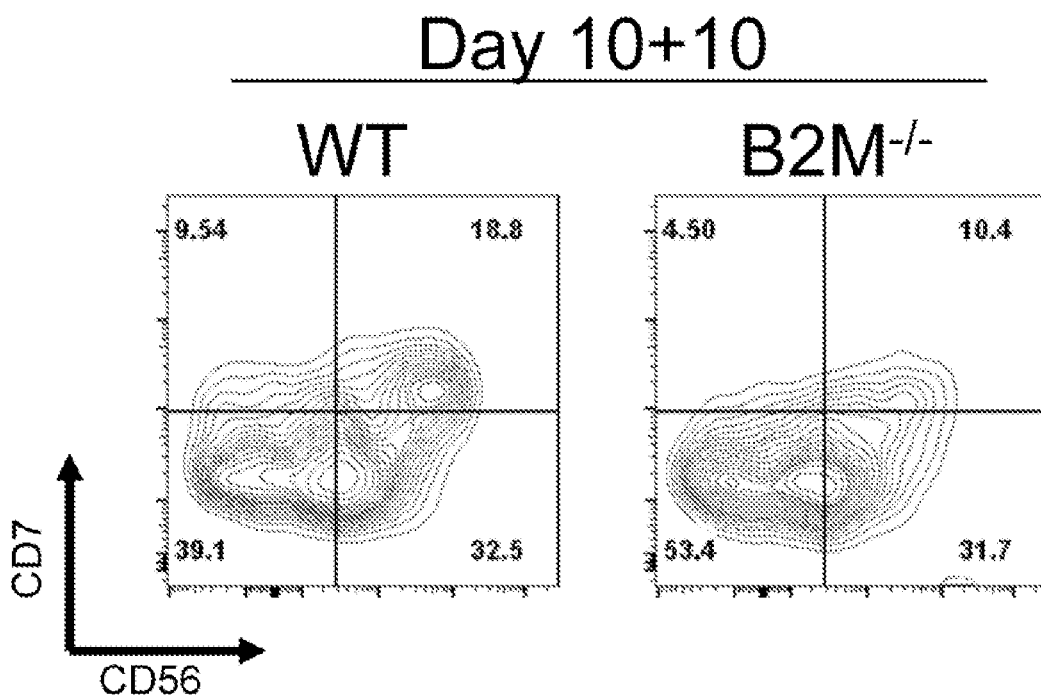


FIG. 32D

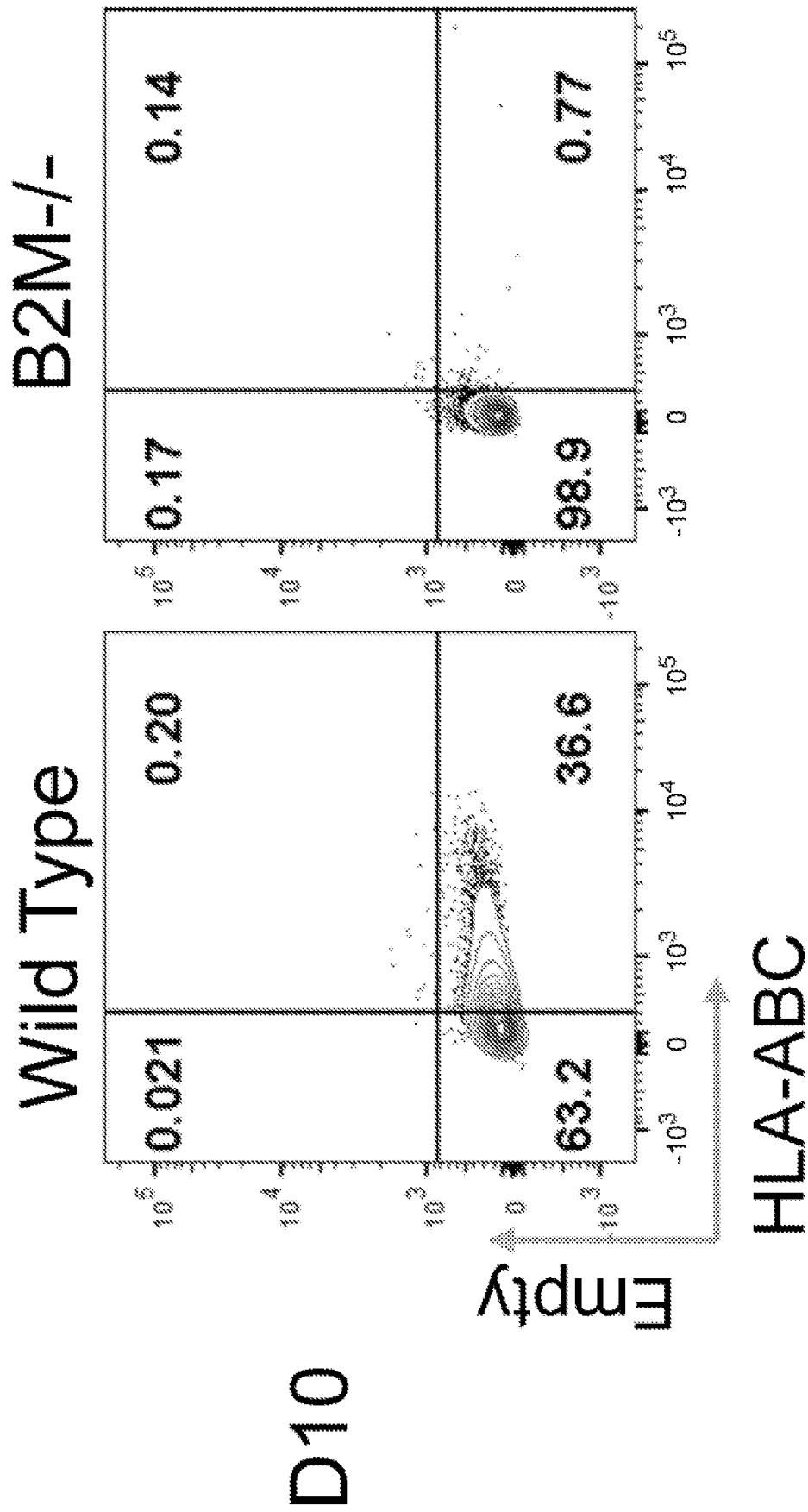


FIG. 33

Modulation of HLA class I on iPSC increases persistence of iPSC in immune-competent recipients

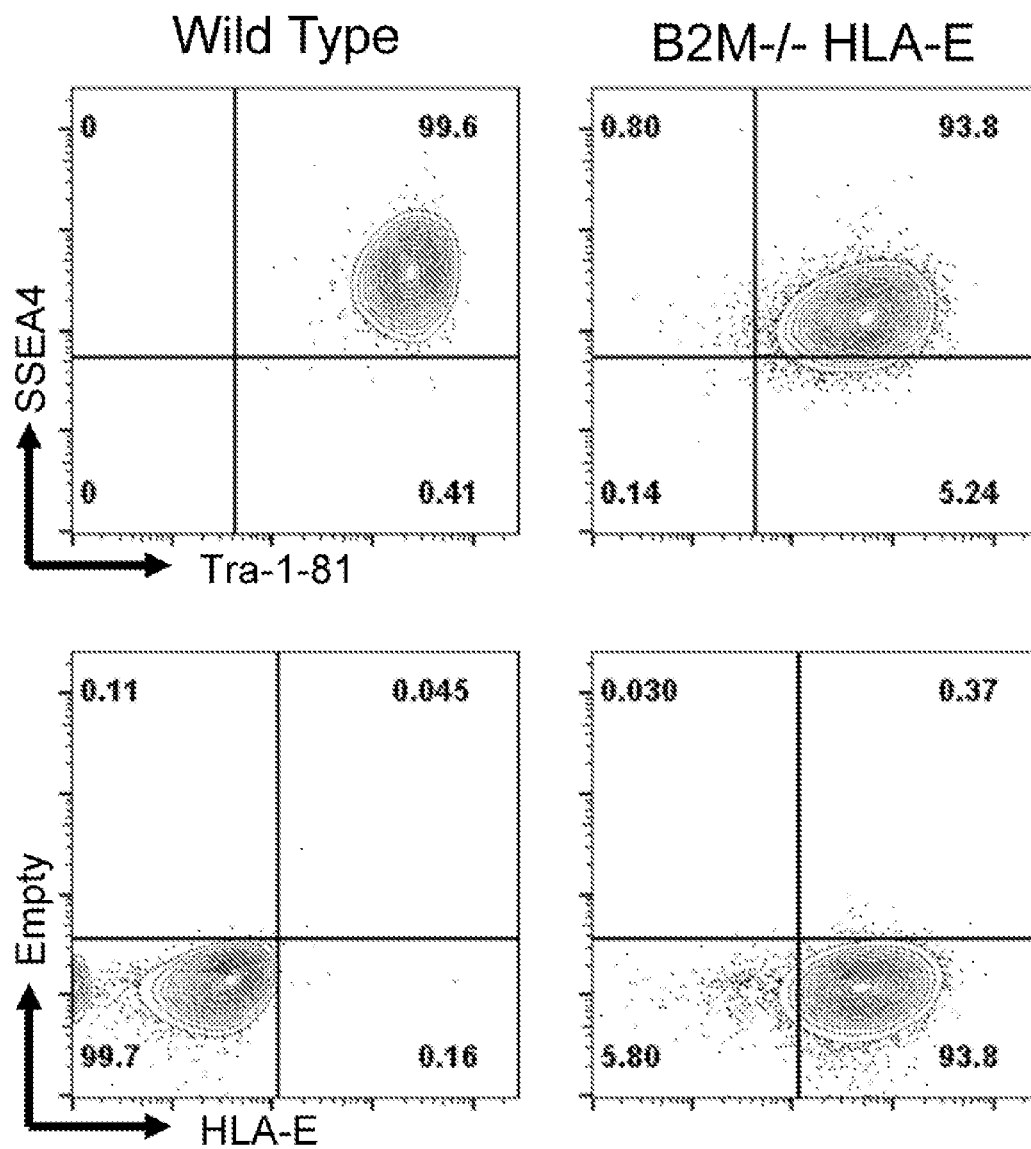
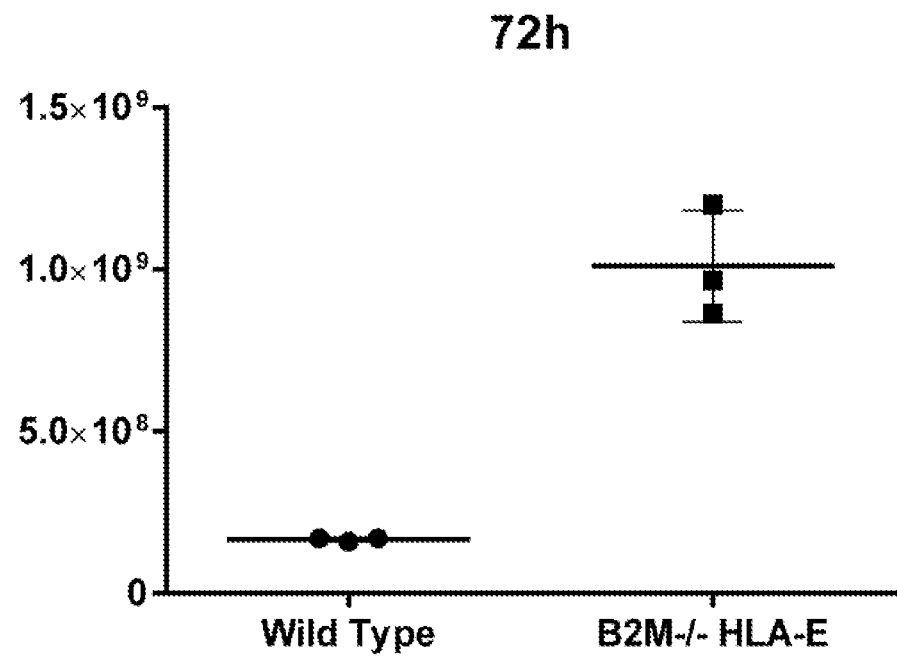


FIG. 34A

Modulation of HLA class I on iPSC increases persistence of iPSC in immune-competent recipients



Left: B2M-/- HLA-E
Right: Wild Type

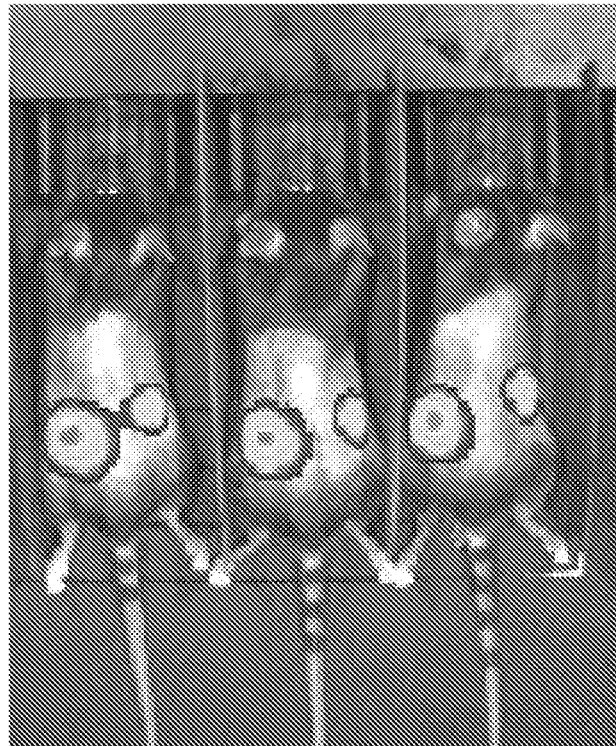


FIG. 34B

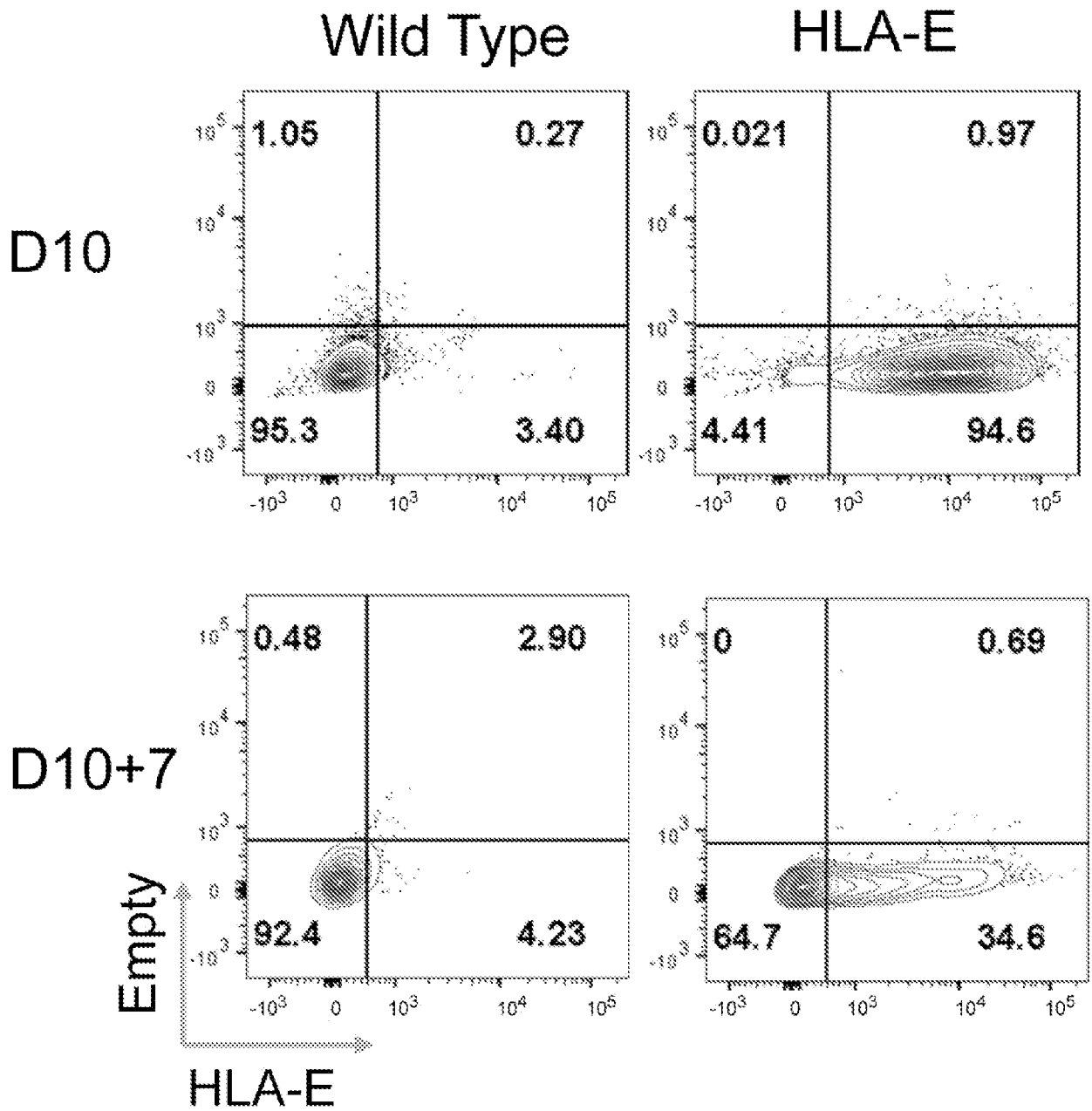


FIG. 35

HLA class I modulated iPSC can generate
functional CD34⁺HE

Day 10

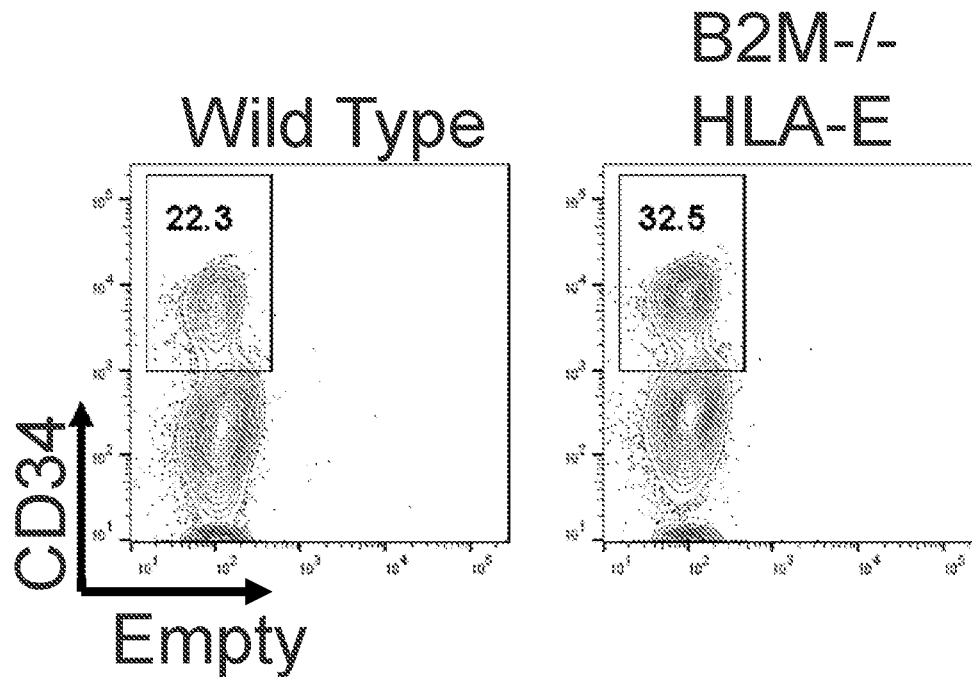


FIG. 36A

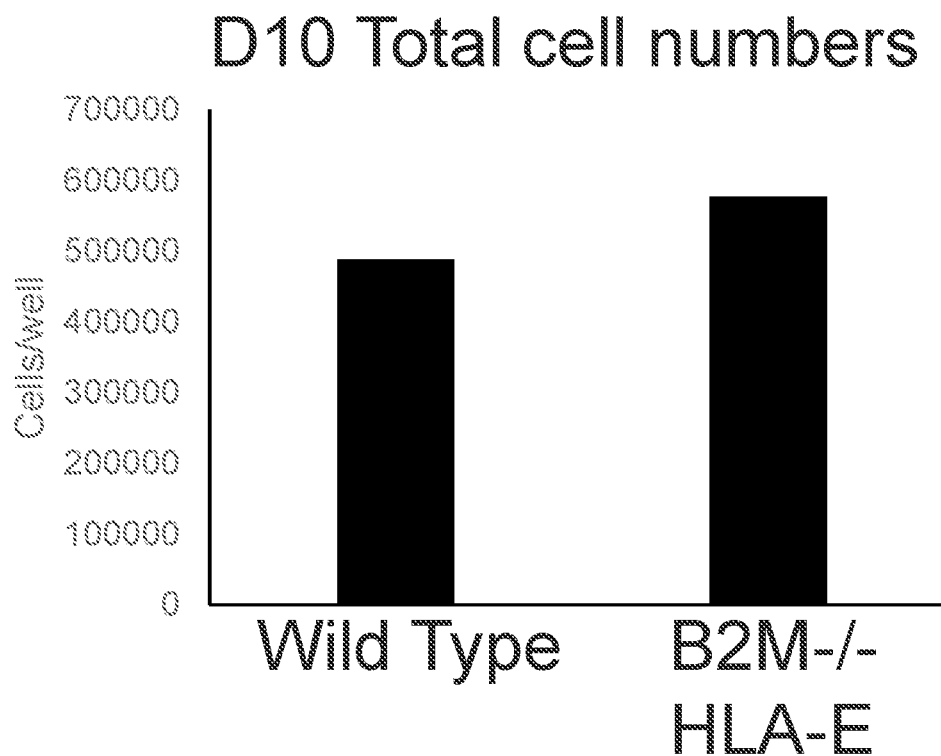


FIG. 36B

HLA class I modulated iPSC can generate
functional CD34+HE

Day 10+7

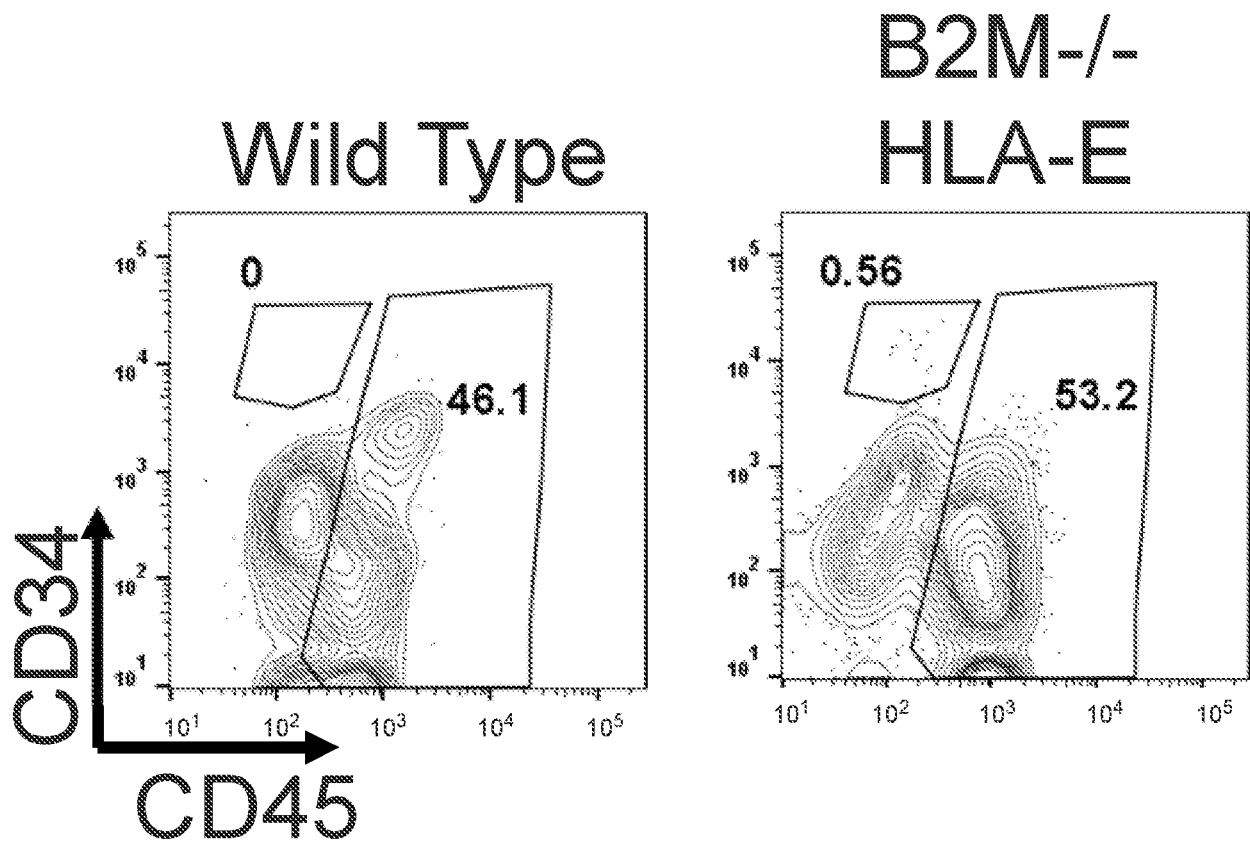


FIG. 36C

iPSC genetically engineered to express the high-affinity CD16 receptor and 41BBL costimulatory molecule retain expression throughout differentiation to iCD34 cells

Day 0

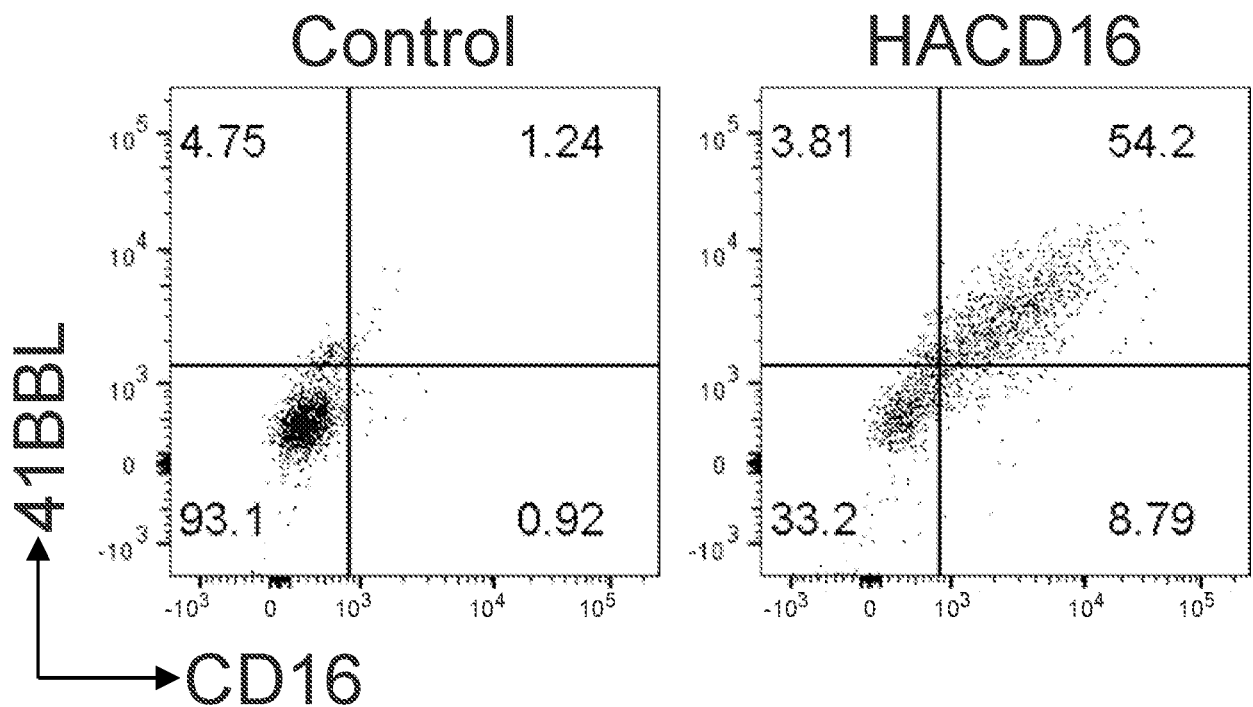


FIG. 37A

iPSC genetically engineered to express the high-affinity CD16 receptor and 41BBL costimulatory molecule retain expression throughout differentiation to iCD34 cells

Day 10

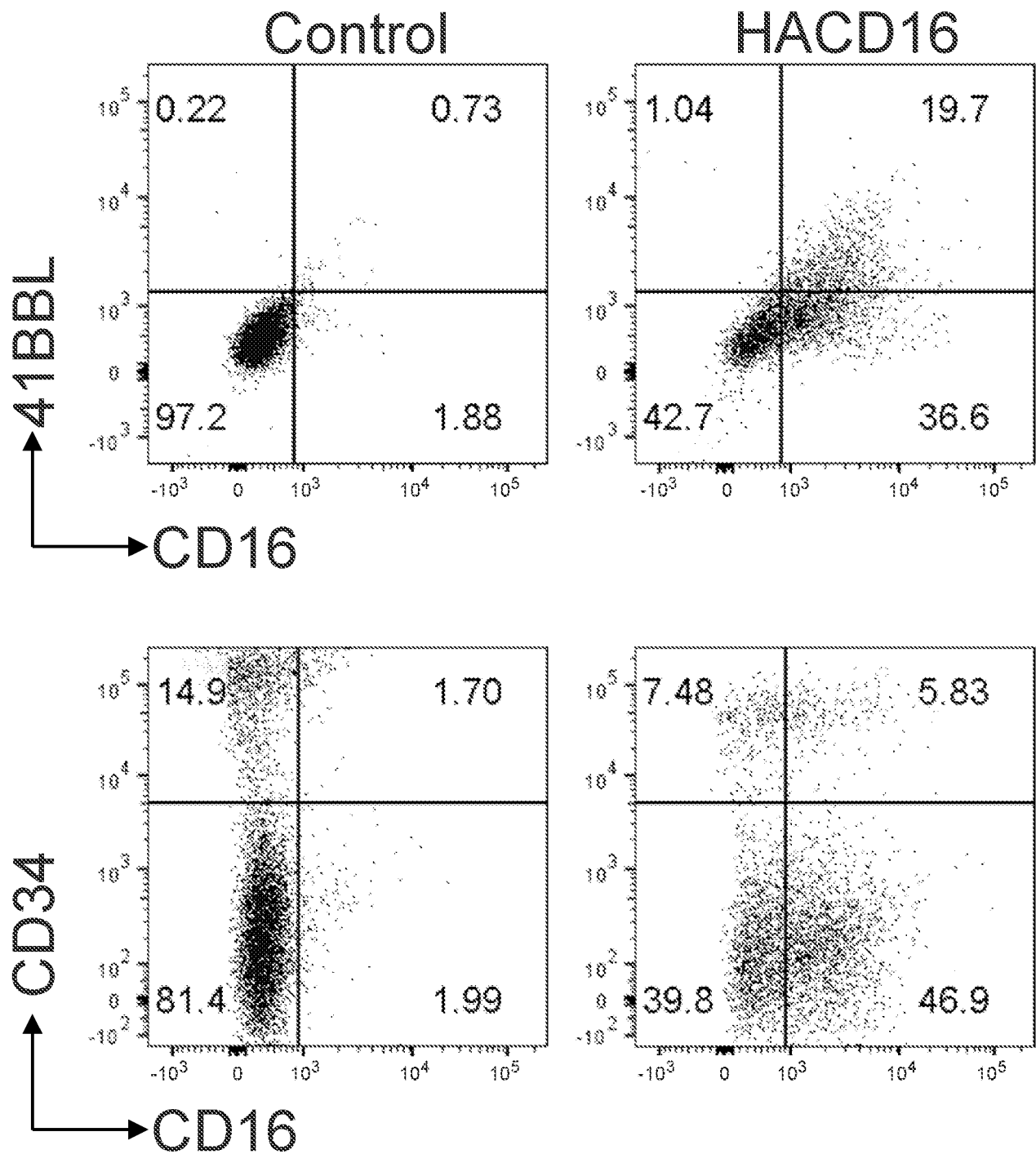


FIG. 37B

CAR-T derived iPSC retains the CAR

Day 0

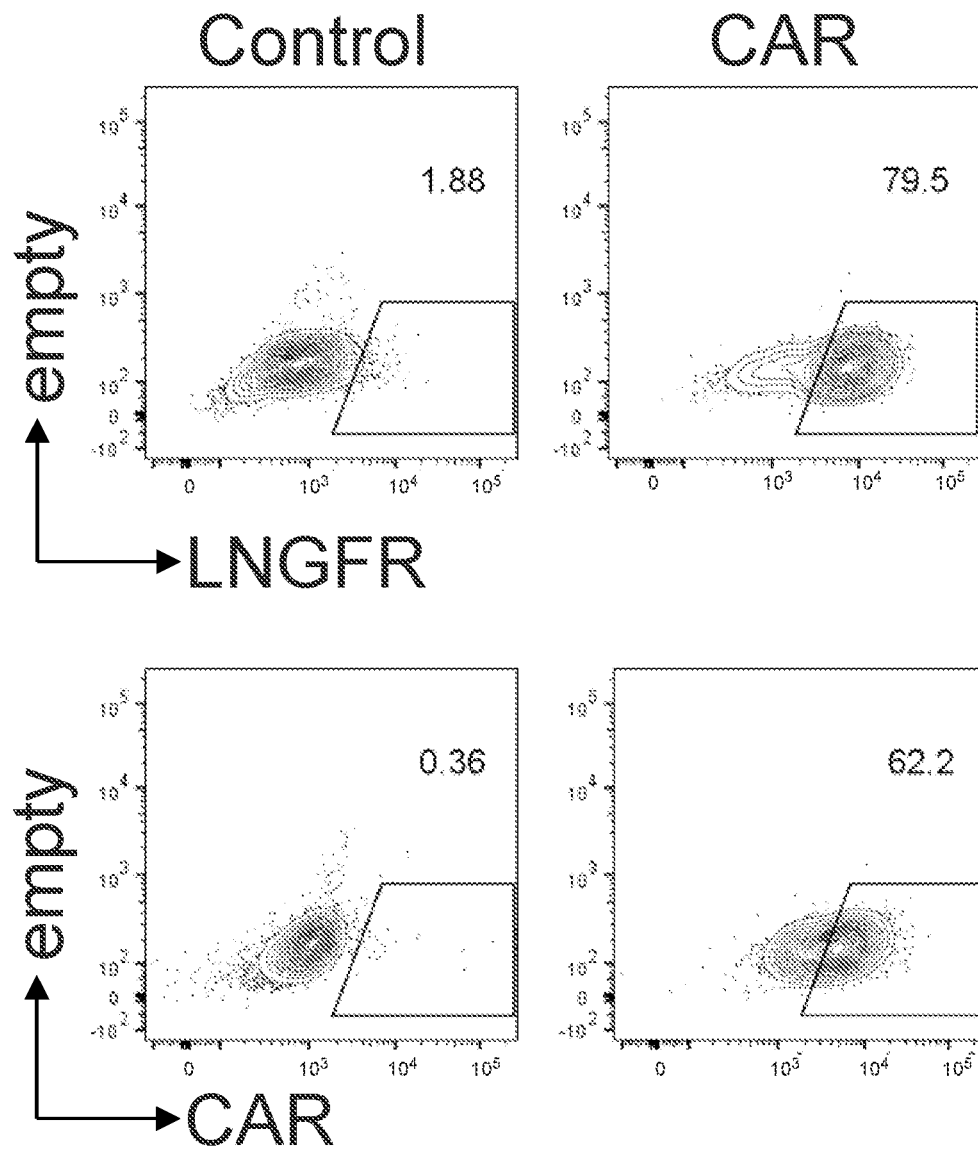
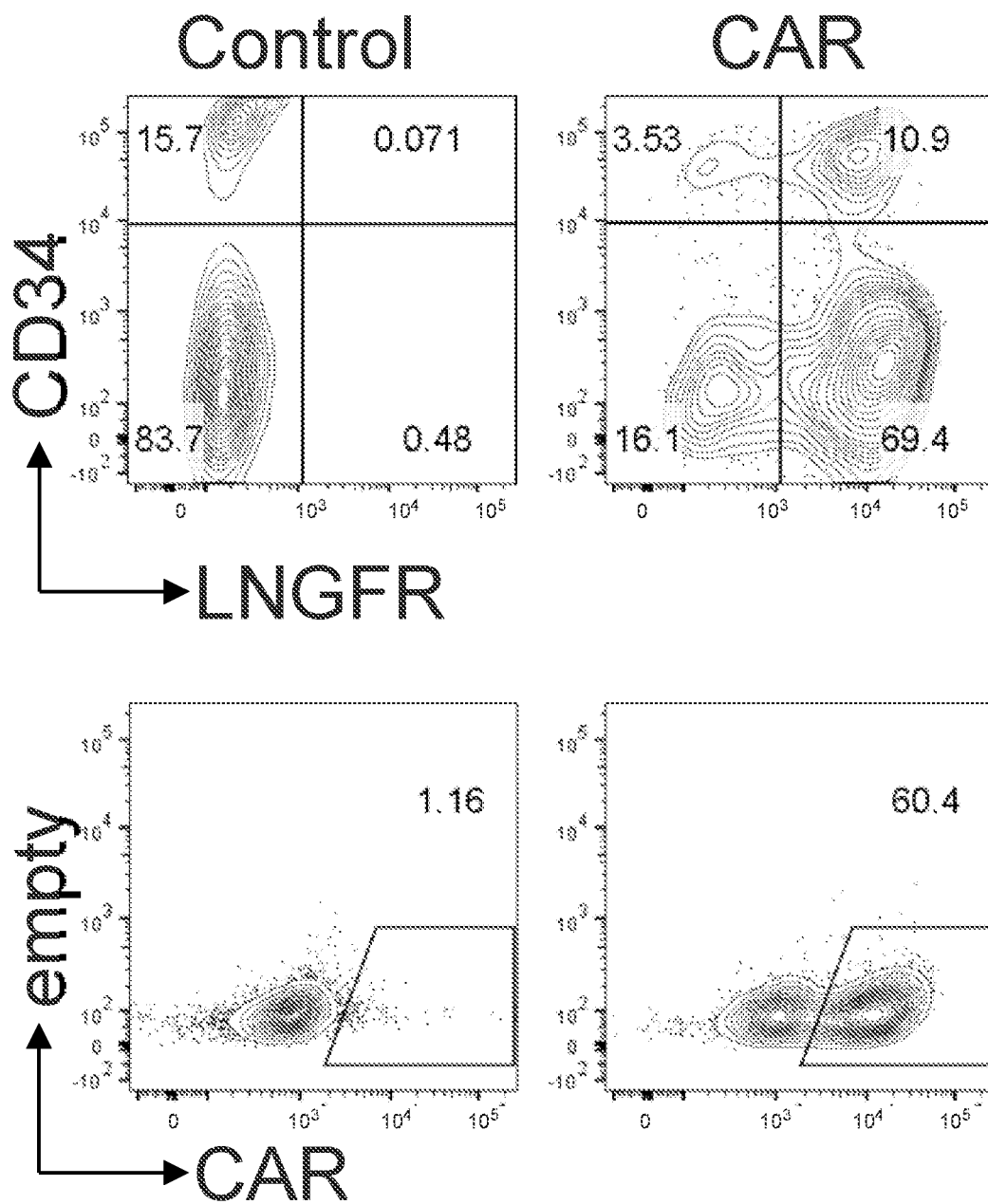


FIG. 38A

CAR-T derived iPSC retains the CAR**Day 10**

Temporal expression of CAR at endogenous TCR α locus

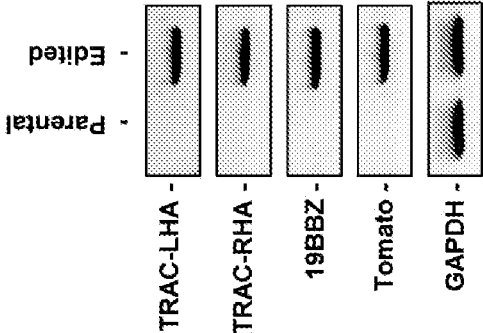
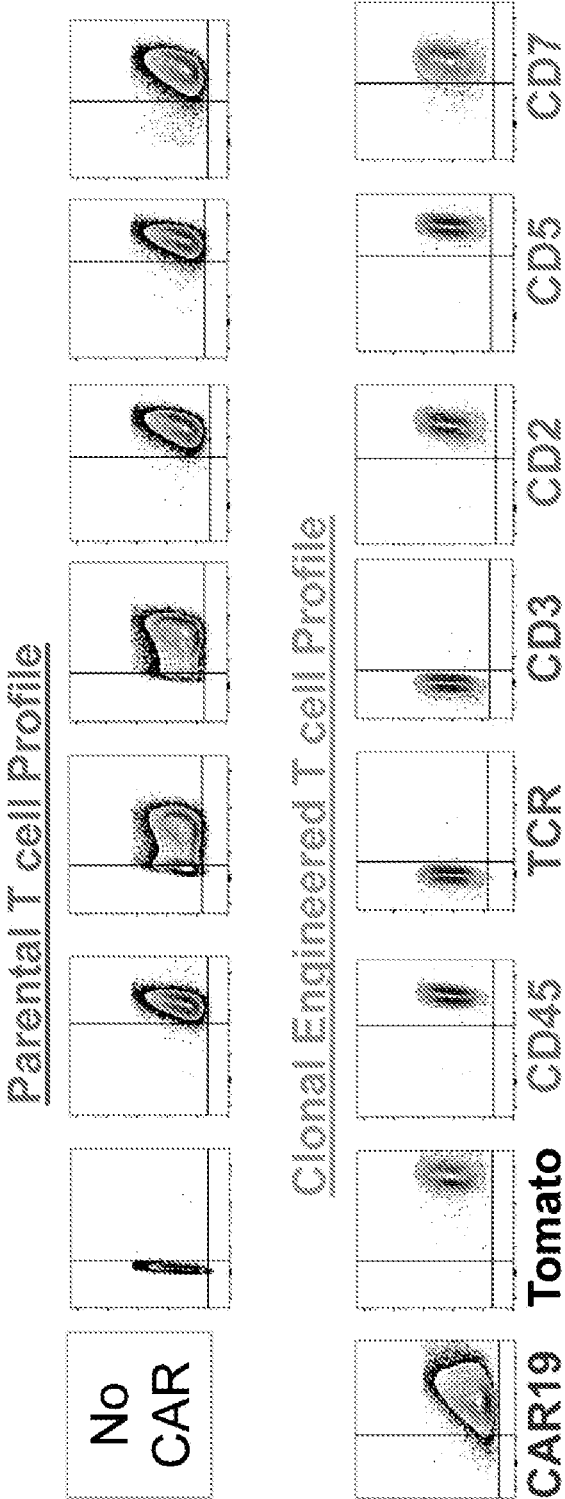


FIG. 39

Off-The-Shelf Targeting Strategy for Engaging Cancer and Other Diseases with the Entire Effector Repertoire

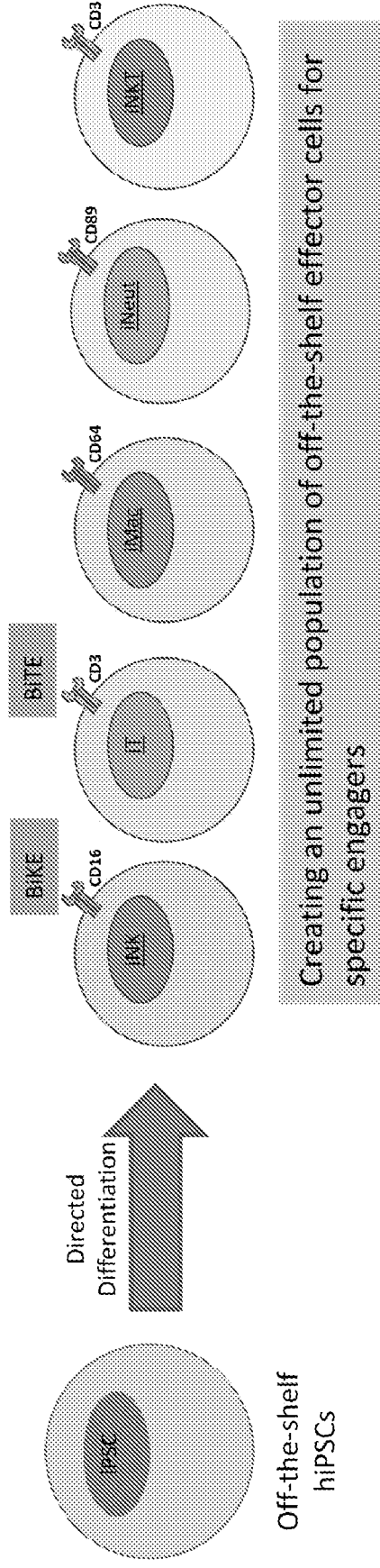


FIG. 40A

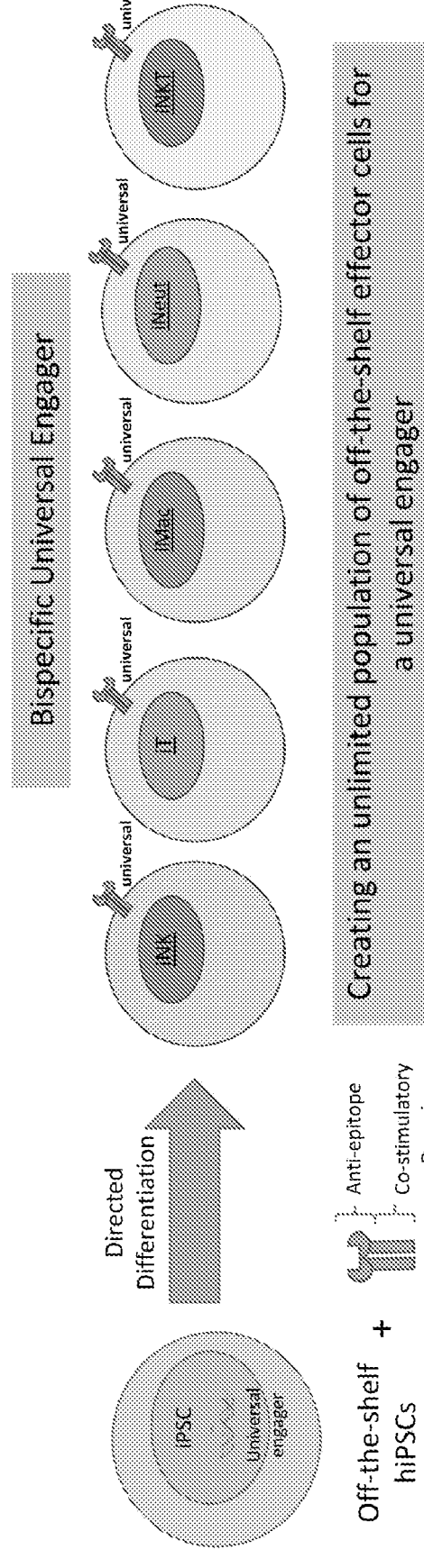


FIG. 40B

HACD16 iPSC maintain CD16 expression during iNK differentiation

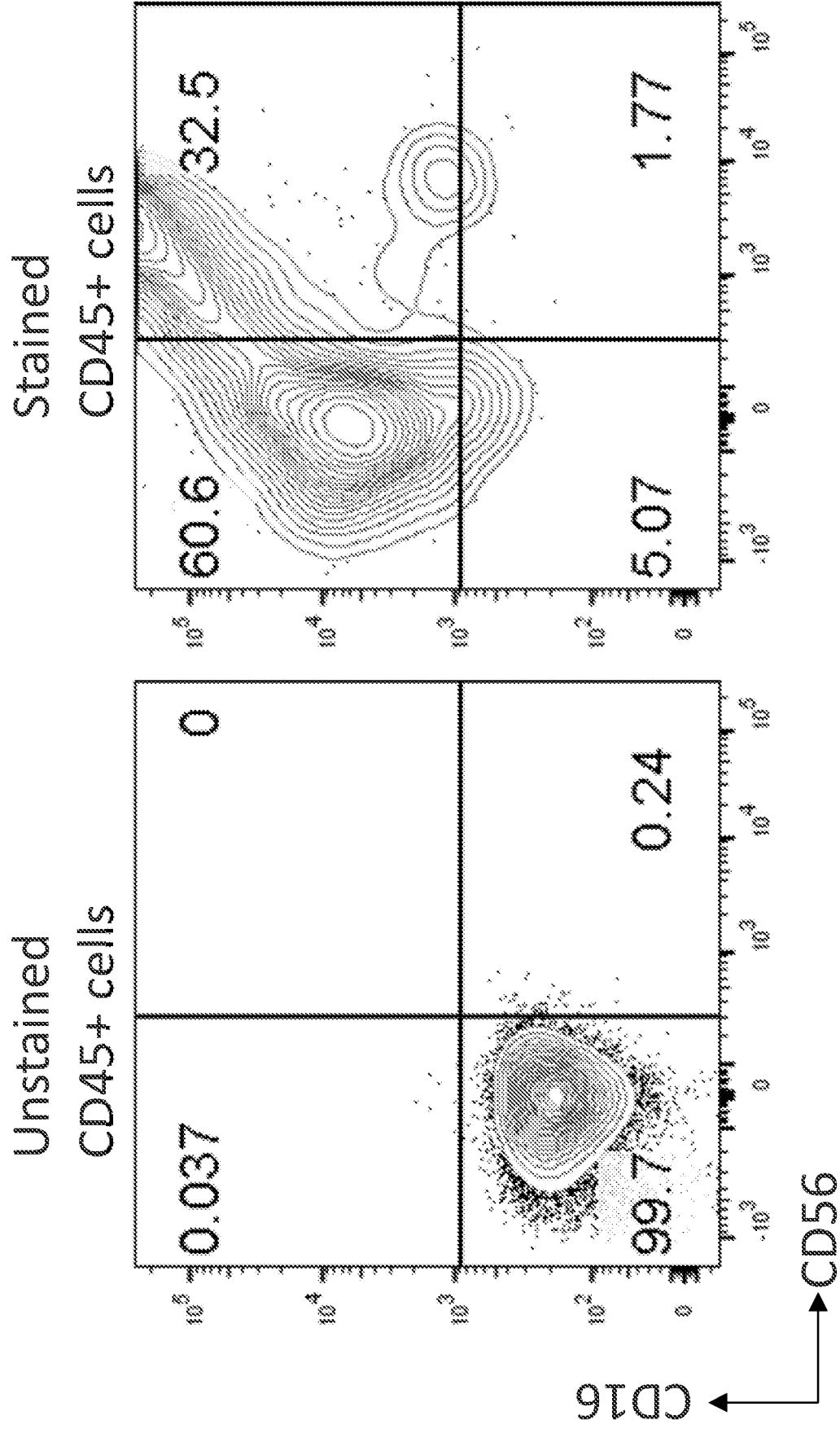


FIG. 41