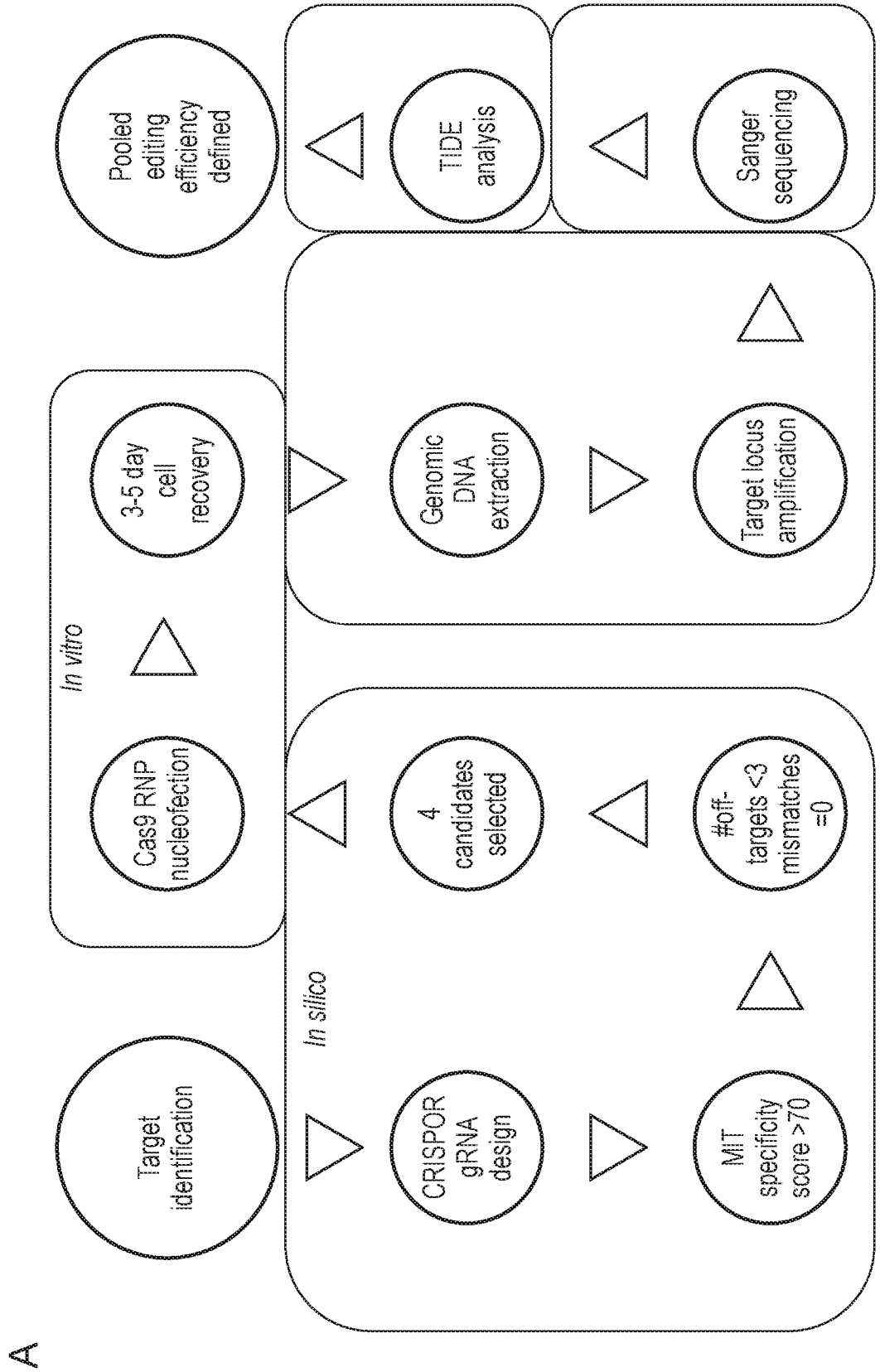

A
Target identification
In silico
CRISPOR gRNA design
MIT specificity score >70
4 candidates selected
#off-targets <3 mismatches =0
In vitro
Cas9 RNP nucleofection
3-5 day cell recovery
Genomic DNA extraction
Target locus amplification
TIDE analysis
Sanger sequencing
Pooled editing efficiency defined

FIG. 1

B

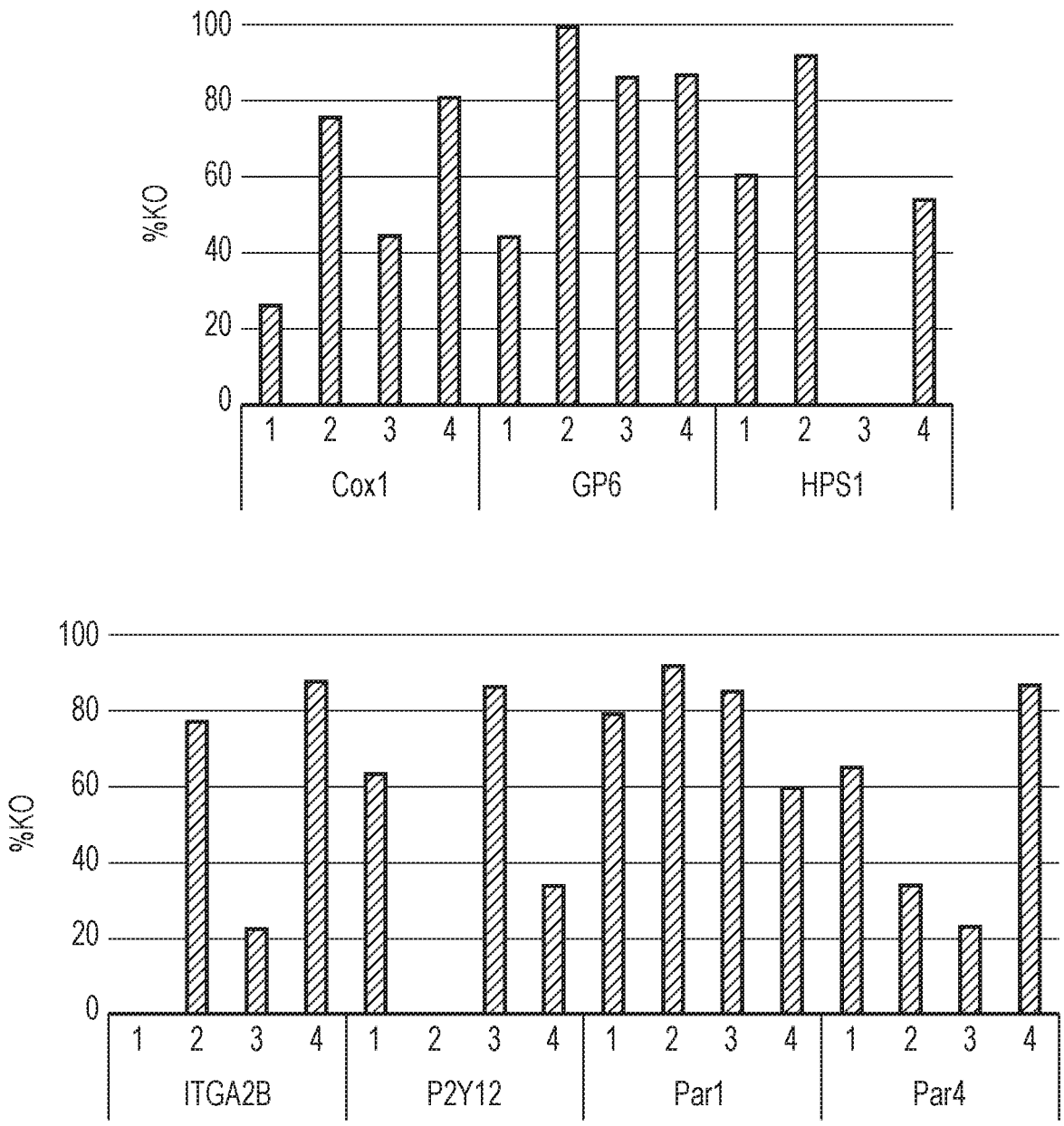


FIG. 1 (Continued)

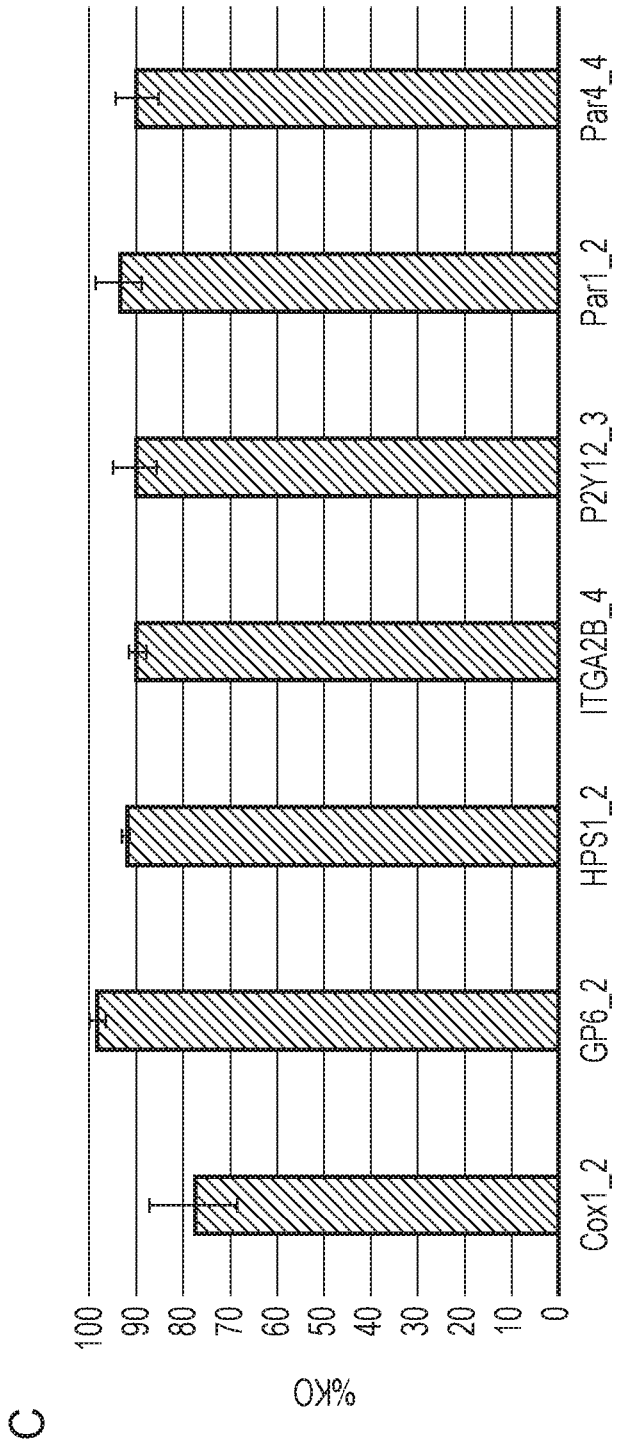


FIG. 1 (Continued)

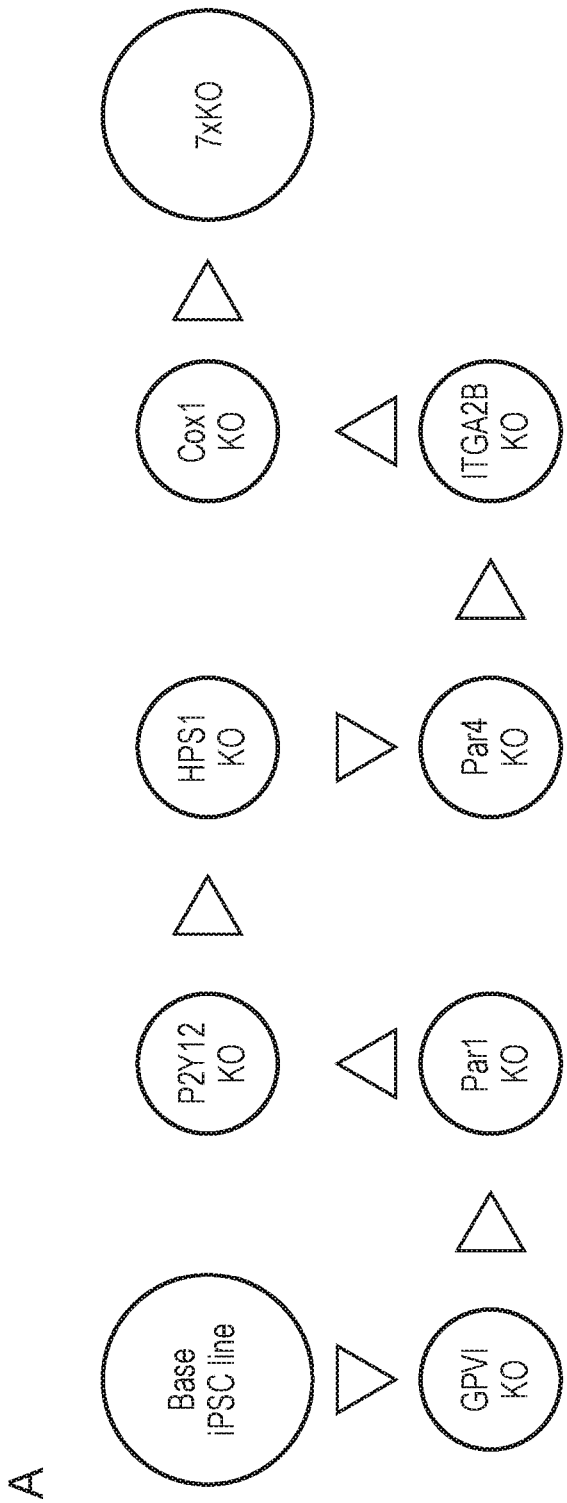
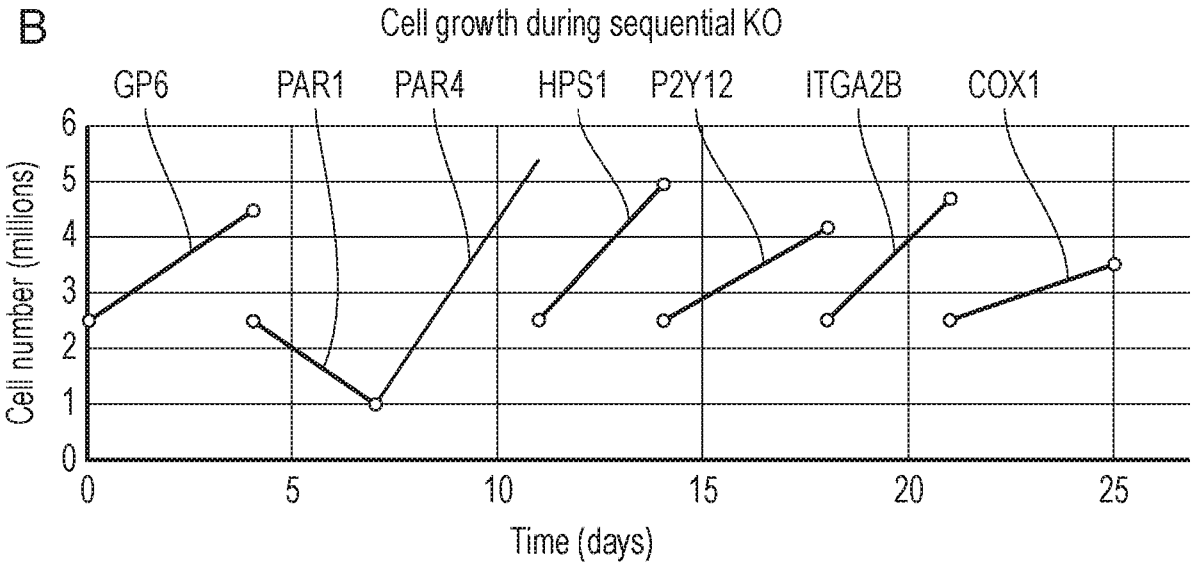

A
Base iPSC line
GPVI KO
Par1 KO
P2Y12 KO
Par4 KO
HPS1 KO
ITGA2B KO
Cox1 KO
7xKO

FIG. 2

B



C

	%KO GP6	%KO PAR1	%KO PAR4	%KO HPS1	%KO P2Y12	%KO ITGA2B	%KO COX1
GP6	100%						
PAR1	100%	98%					
PAR4	100%	97%	96%				
HPS1	100%	99%	97%	95%			
P2Y12	100%	99%	96%	96%	95%		
ITGA2B	100%	99%	95%	96%	95%	96%	
COX1	100%	99%	97%	96%	94%	97%	59%

Single-cell sort & clonal expansion

FIG. 2 (Continued)

A

Sequencing round 1:

	GP6	Par1	Par4	HPS1	P2Y12	ITGA2B	Cox1
1				99		99	
2	100			99	100	97	97
3	100	1	49	97	98	99	96
4	100	96	11	88	49	91	
5	86	94		100	95	62	43
6	100	89	99	99		99	
7	100	95	99	99		99	
8			95	4		100	
9	52	99	99		100	99	
10	100	0	95	97	98	39	0
11	95	96	99	47	30	99	100
12	100	96	47	99	98	99	3
13	100	94	89	96	49	93	
14	100	38	99	99	99	94	
15	100	96	96	90	98	100	100
16	100	99	96	90	49	91	53
17	100	38	95	53	97	99	
18	51	93	96	42	15	63	
19	100	99	99	96	94	94	
20	100	99	17	99	96	94	95
21	96	46	99	96	96	50	99
22	100	52	68	97	38	96	0

	GP6	Par1	Par4	HPS1	P2Y12	ITGA2B	Cox1
23	97	97	99	99	99	92	6
24	100	97		97	98	96	0
25	96	99	99	97	72	100	84
26	100	97		99	98	92	100
27	100	51	99	97	16	28	
28	100	96	99	98	48	99	0
29	100	28	95	95	89	41	47
30	100	99	100	99	97	99	0
31	100	0	73	99	97	99	47
32	96	86	95	47	98	90	100
33	56	96	32	97	98	99	0
34	100	99	96	99	99	99	
35	97	99	67	99	94	99	
36	100	93	22	89	54		95
37	10	95	95	99	52	49	43
38	100	1	99	98	89	48	
39	49	99	100	47	98	50	0
40	100	99	26	97	98	99	54
41	1	99	100		98		
42	42	96		85	48	99	
43	49	98		99	51	43	81
44	100	99		96	98	91	

B

Sequencing round 2:

clone ID	GP6	Par1	Par4	HPS1	P2Y12	ITGA2B	Cox1
1		0	98	99	0	99	100
2	100		95	99	100	97	97
5	86	92		100	95	99	43
6	100	89	99	99	100	99	90
7	100	95	99	99		99	
8	99	2	95	100	100	100	
9	100	99	99	99	100	99	
12	100	96	48	99	98	99	3
14	100	39	99	99	99	94	100
17	100	26	95	99	97	99	
19	100	99	99	96	97	94	

clone ID	GP6	Par1	Par4	HPS1	P2Y12	ITGA2B	Cox1
20	100	99	20	99	96	94	95
23	97	97	99	99	99	92	6
26	100	97	79	99	98	92	100
29	100	12	95	99	90	100	47
34	100	99	96	99	99	99	100
35	97	99	67	99	100	99	100
38	100	1	99	98	89	99	
40	100	99	26	97	98	99	50
41	20	99	100	97	98	48	100
44	100	99	100	96	98	99	50

FIG. 3

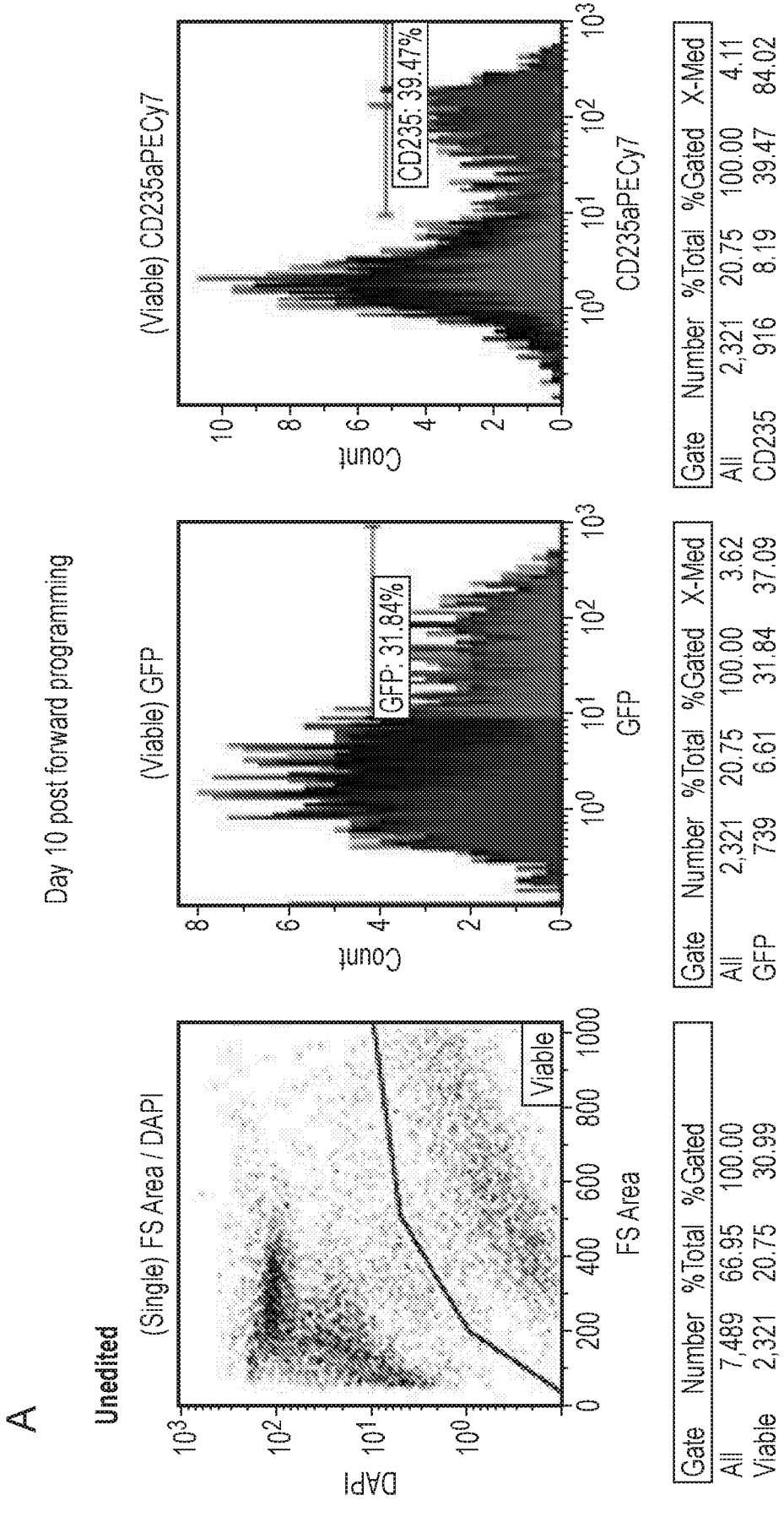
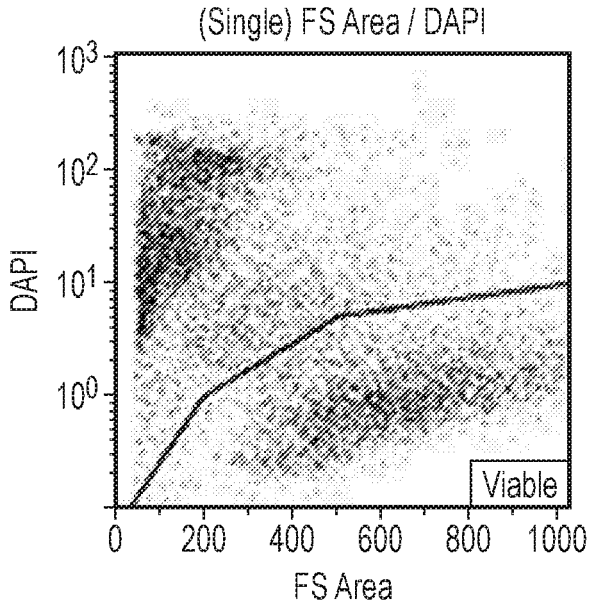

A
Unedited
Day 10 post forward programming
(Single) FS Area / DAPI
DAPI
FS Area
Viable
(Viable) GFP
Count
GFP
GFP: 31.84%
(Viable) CD235aPECy7
CD235aPECy7
CD235: 39.47%
Gate Number %Total %Gated
All 7,489 66.95 100.00
Viable 2,321 20.75 30.99
Gate Number %Total %Gated X-Med
All 2,321 20.75 100.00 3.62
GFP 739 6.61 31.84 37.09
Gate Number %Total %Gated X-Med
All 2,321 20.75 100.00 4.11
CD235 916 8.19 39.47 84.02

FIG. 4

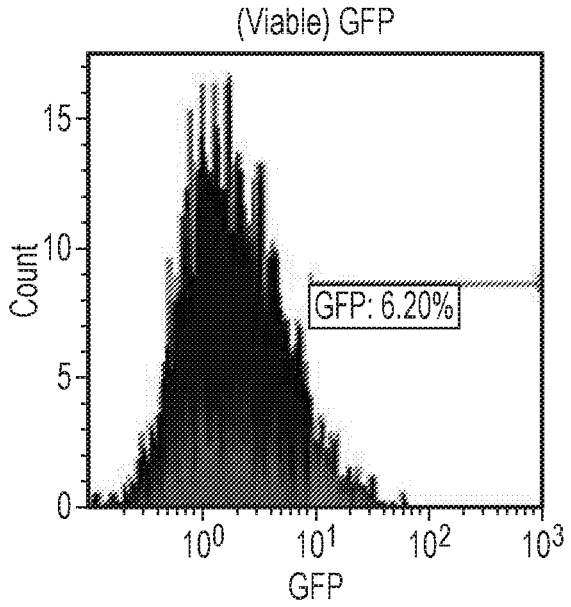
A

Day 10 post forward programming

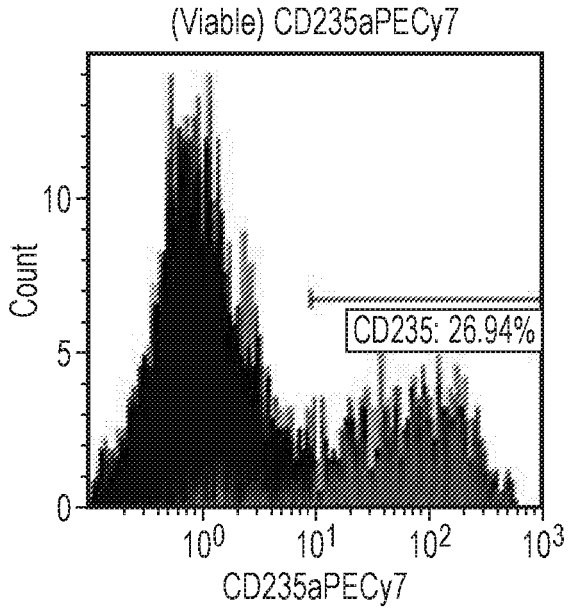
7xKO Pool



Gate	Number	%Total	%Gated
All	8,215	64.50	100.00
Viable	3,356	26.35	40.85



Gate	Number	%Total	%Gated	X-Med
All	3,356	26.35	100.00	1.75
GFP	208	1.63	6.20	13.17

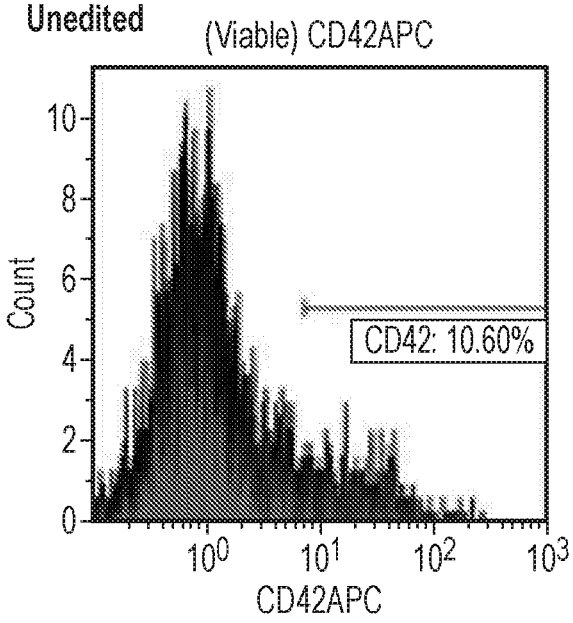


Gate	Number	%Total	%Gated	X-Med
All	3,356	26.35	100.00	1.46
CD235	904	7.10	26.94	74.21

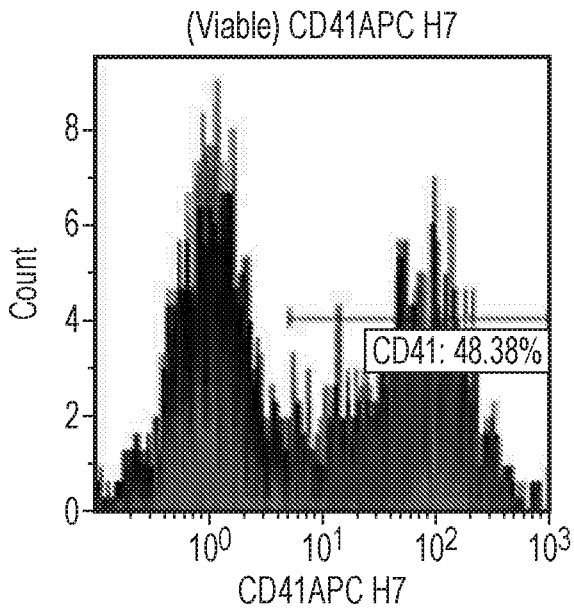
FIG. 4 (Continued)

A

Unedited

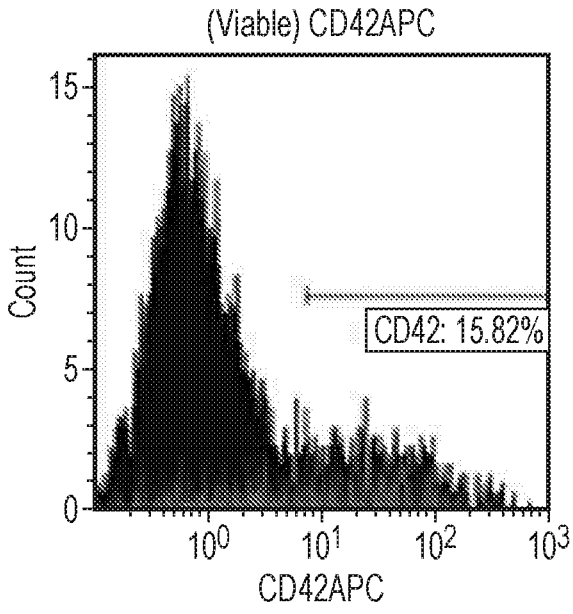


Gate	Number	%Total	%Gated	X-Med
All	2,321	20.75	100.00	0.79
CD42	246	2.20	10.60	22.46

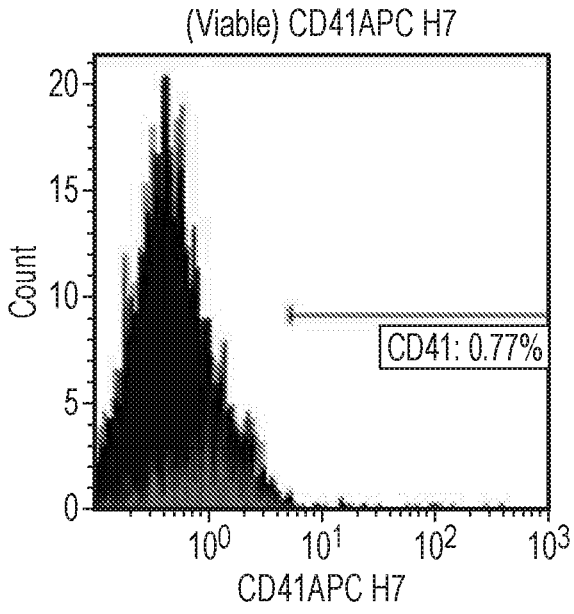


Gate	Number	%Total	%Gated	X-Med
All	2,321	20.75	100.00	4.31
CD41	1,123	10.04	48.38	65.68

7xKO Pool



Gate	Number	%Total	%Gated	X-Med
All	3,356	26.35	100.00	0.83
CD42	531	4.17	15.82	34.37

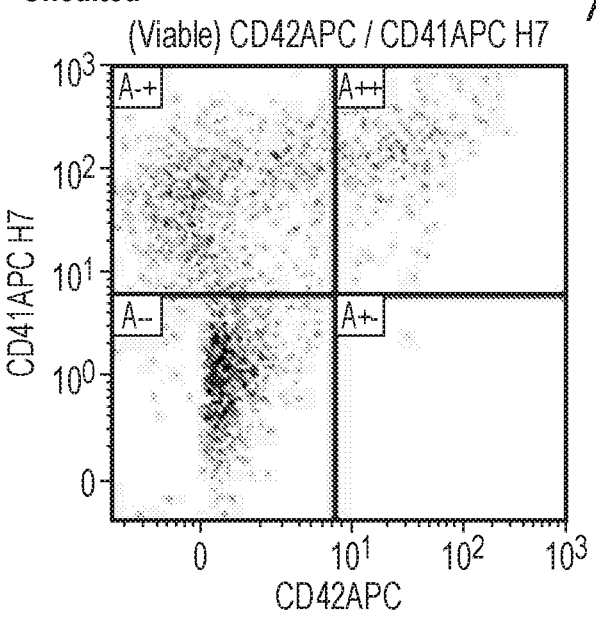


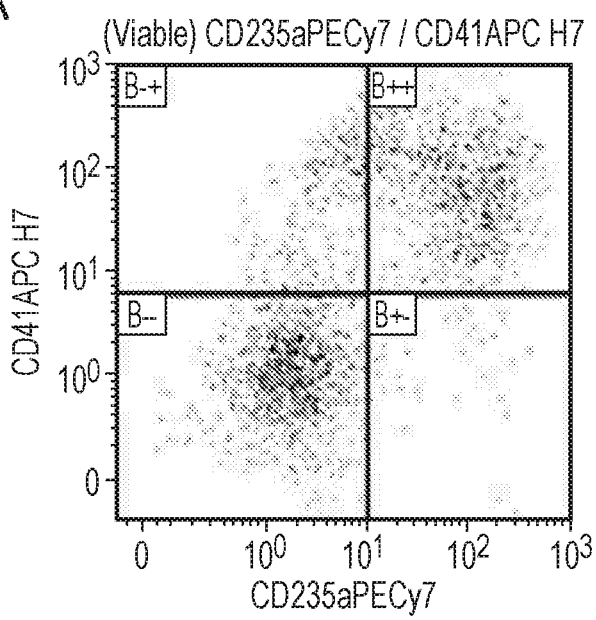
Gate	Number	%Total	%Gated	X-Med
All	3,356	26.35	100.00	0.43
CD41	26	0.20	0.77	18.54

FIG. 4 (Continued)

A

Unedited

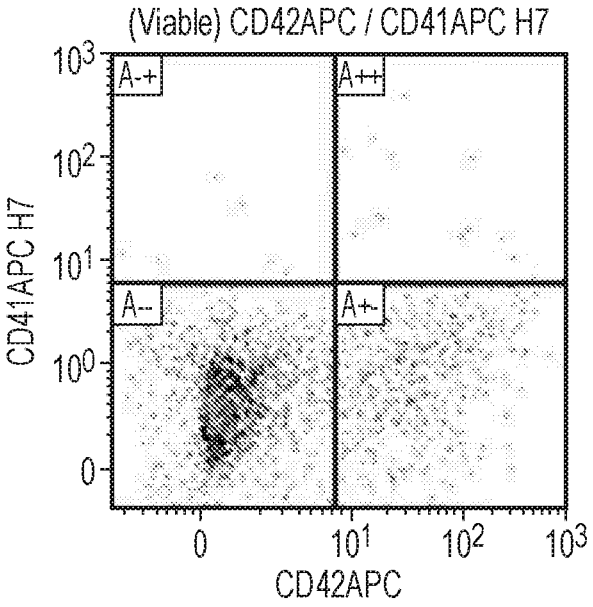


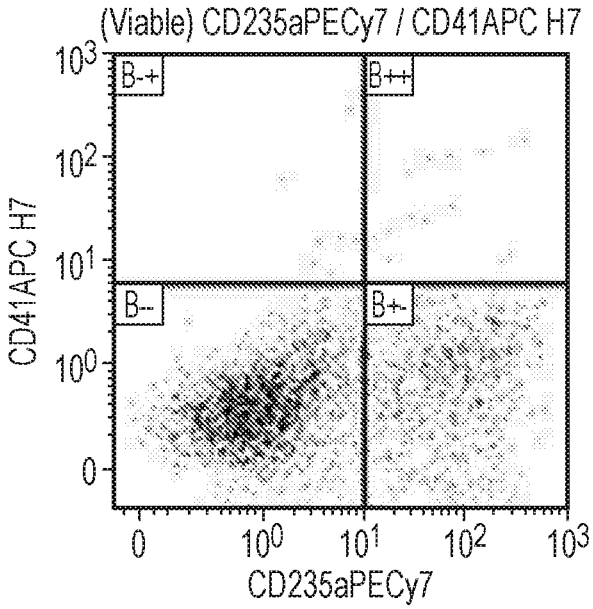


Gate	Number	%Total	%Gated
All	2,321	20.75	100.00
A--	1,195	10.68	51.49
A-+	880	7.87	37.91
A+-	3	0.03	0.13
A++	243	2.17	10.47

Gate	Number	%Total	%Gated
All	2,321	20.75	100.00
B--	1,156	10.33	49.81
B-+	249	2.23	10.73
B+-	42	0.38	1.81
B++	874	7.81	37.66

7xKO Pool

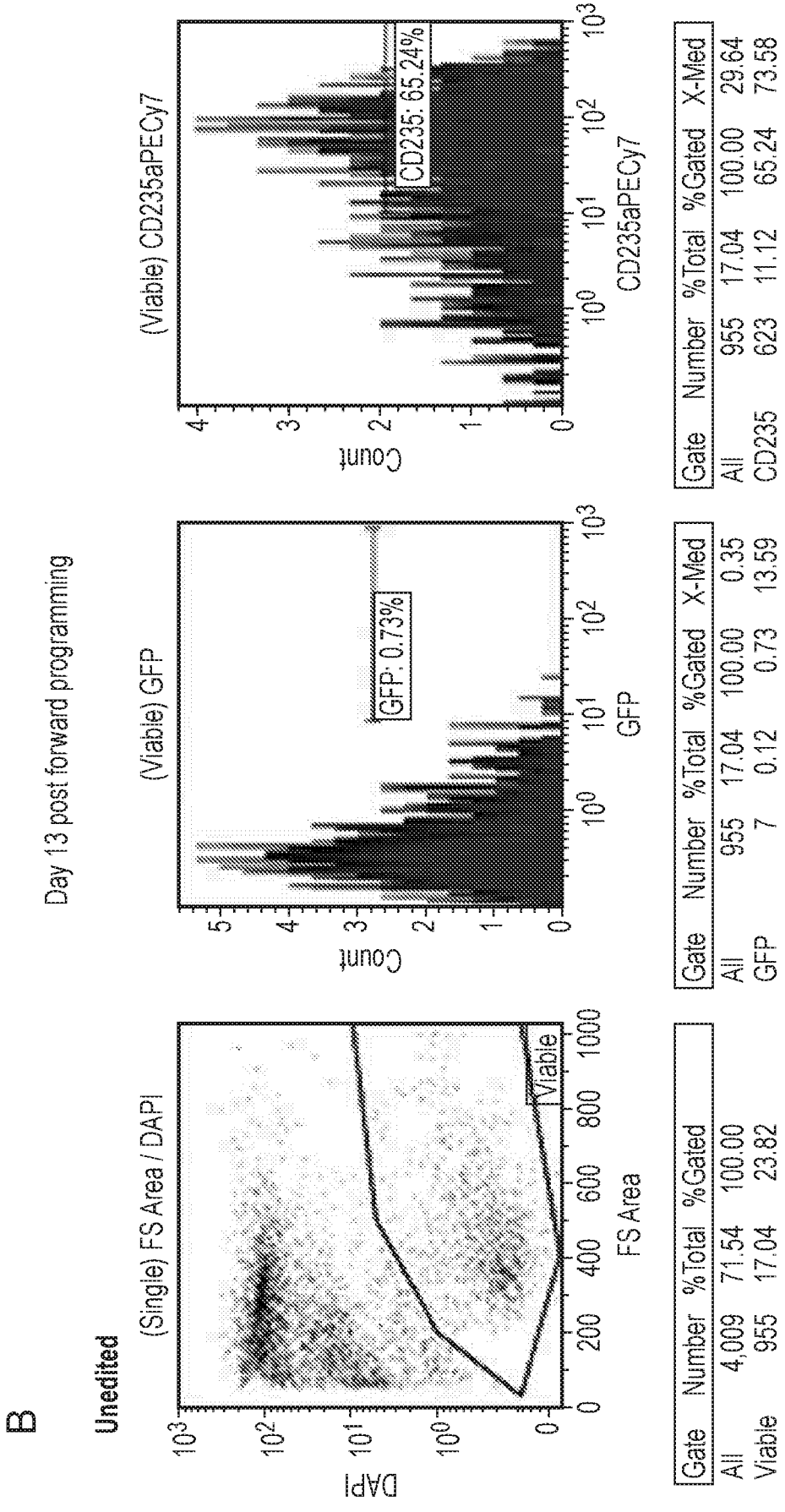




Gate	Number	%Total	%Gated
All	3,356	26.35	100.00
A--	2,816	22.11	83.91
A-+	9	0.07	0.27
A+-	514	4.04	15.32
A++	17	0.13	0.51

Gate	Number	%Total	%Gated
All	3,356	26.35	100.00
B--	2,442	19.17	72.77
B-+	10	0.08	0.30
B+-	888	6.97	26.46
B++	16	0.13	0.48

FIG. 4 (Continued)

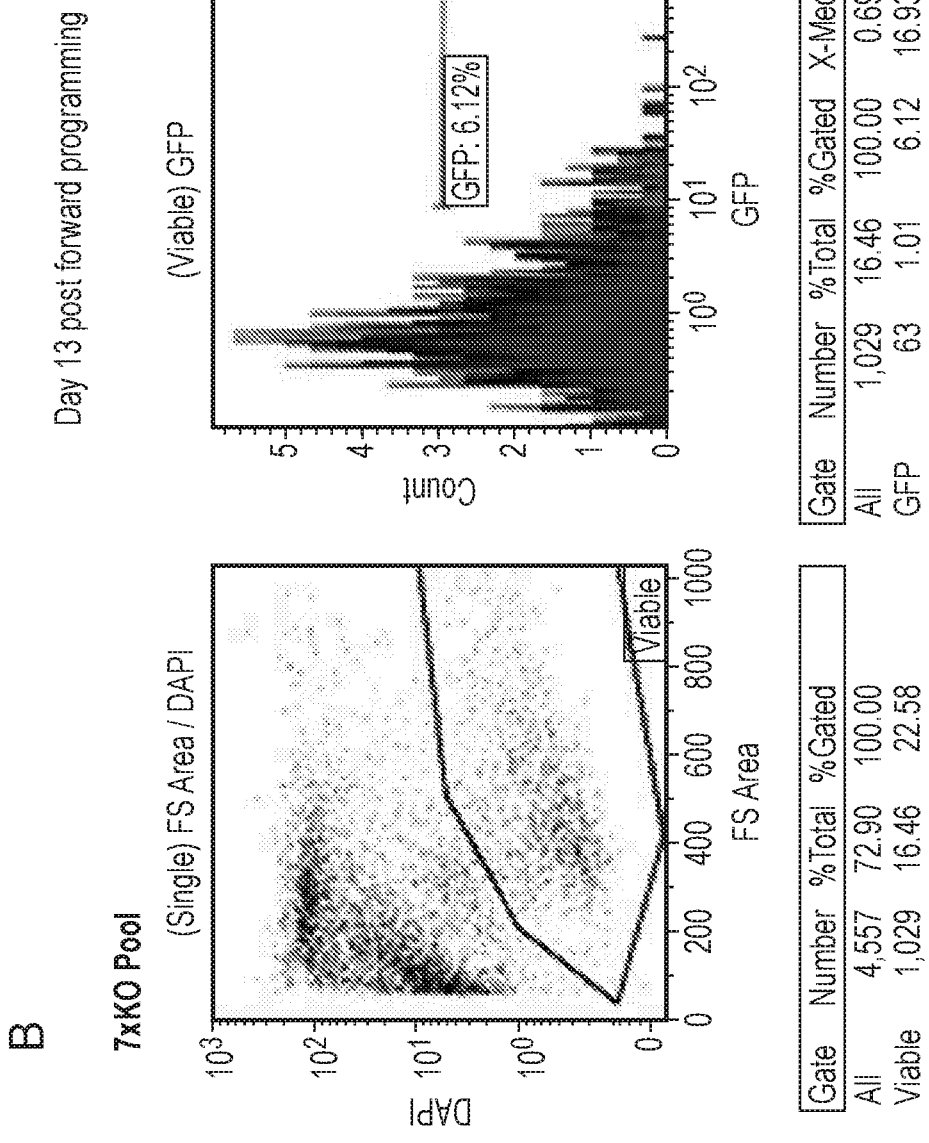


Gate	Number	%Total	%Gated
All	4,009	71.54	100.00
Viable	955	17.04	23.82

Gate	Number	%Total	%Gated	X-Med
All	955	17.04	100.00	0.35
GFP	7	0.12	0.73	13.59

Gate	Number	%Total	%Gated	X-Med
All	955	17.04	100.00	29.64
CD235	623	11.12	65.24	73.58

FIG. 4 (Continued)



Gate	Number	%Total	%Gated
All	4,557	72.90	100.00
Viable	1,029	16.46	22.58

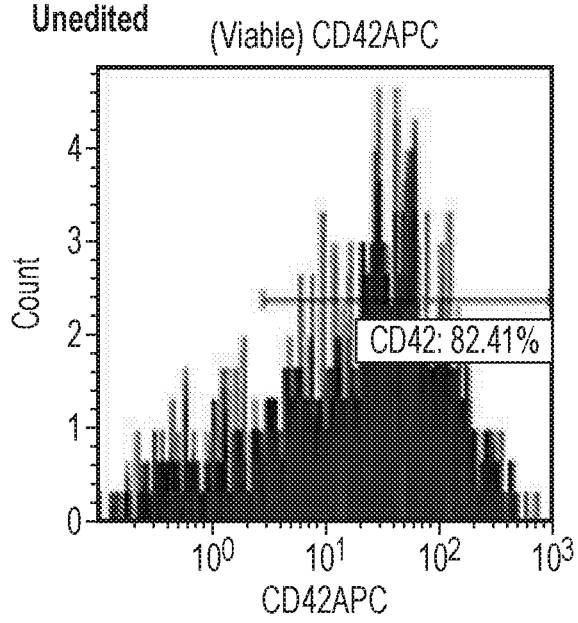
Gate	Number	%Total	%Gated	X-Med
All	1,029	16.46	100.00	0.69
GFP	63	1.01	6.12	16.93

Gate	Number	%Total	%Gated	X-Med
All	1,029	16.46	100.00	32.60
CD235	661	10.57	64.24	75.08

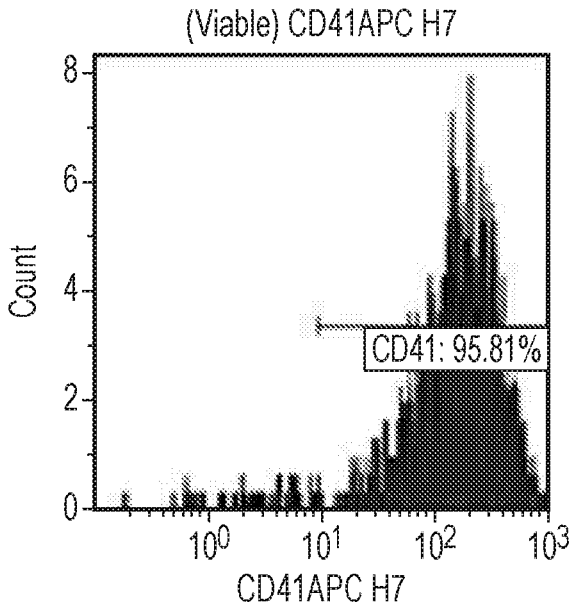
FIG. 4 (Continued)

B

Unedited

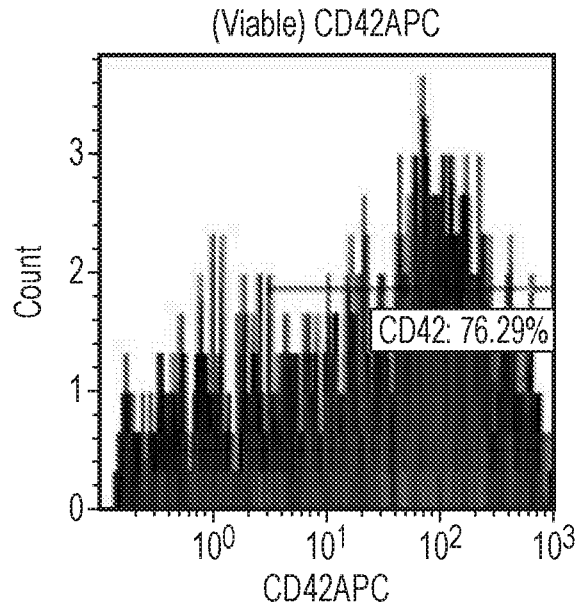


Gate	Number	%Total	%Gated	X-Med
All	955	17.04	100.00	28.47
CD42	787	14.04	82.41	39.03

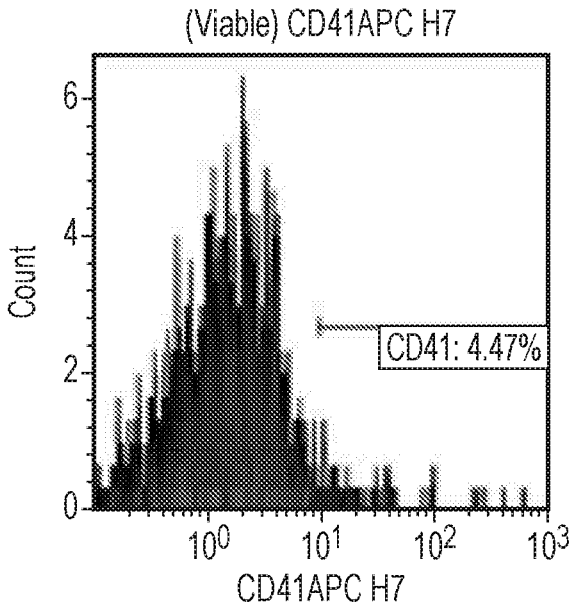


Gate	Number	%Total	%Gated	X-Med
All	955	17.04	100.00	157.83
CD41	915	16.33	95.81	167.18

7xKO Pool



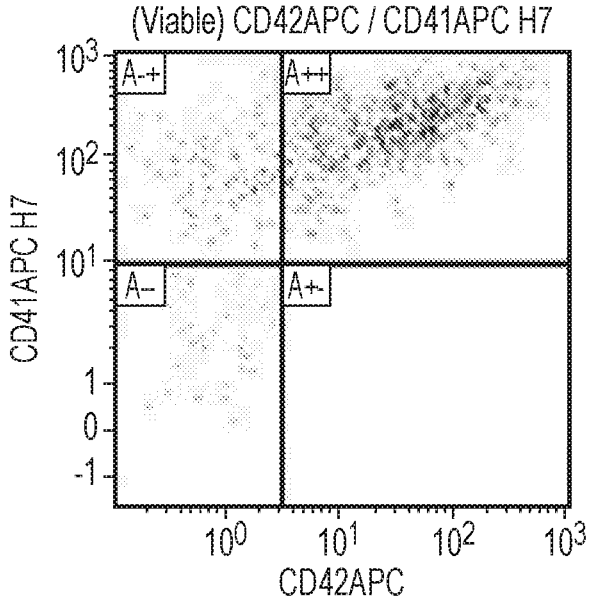
Gate	Number	%Total	%Gated	X-Med
All	1,029	16.46	100.00	36.32
CD42	785	12.56	76.29	70.92



Gate	Number	%Total	%Gated	X-Med
All	1,029	16.46	100.00	1.40
CD41	46	0.74	4.47	20.10

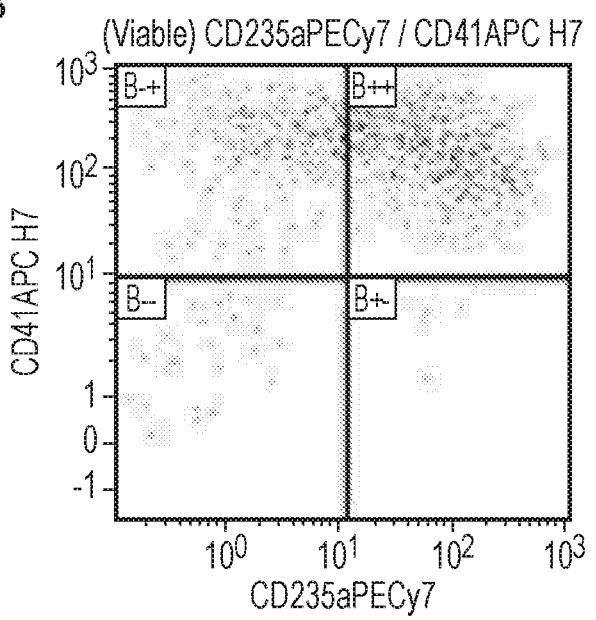
FIG. 4 (Continued)

Unedited



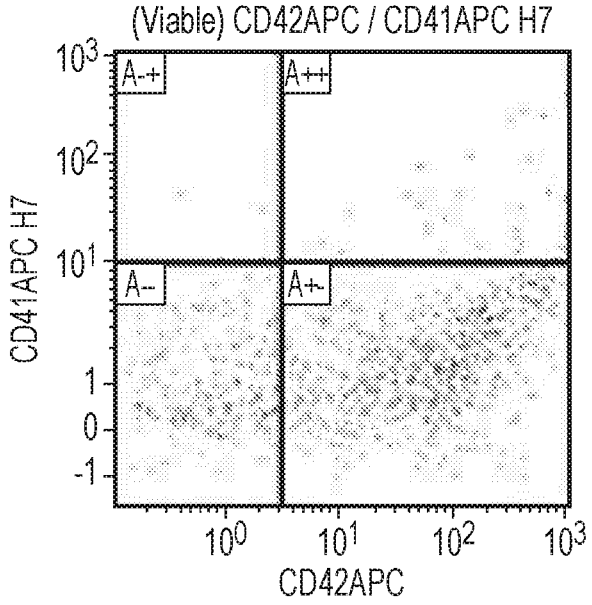
Gate	Number	%Total	%Gated
All	955	17.04	100.00
A--	36	0.64	3.77
A-+	132	2.36	13.82
A+-	4	0.07	0.42
A++	783	13.97	81.99

B

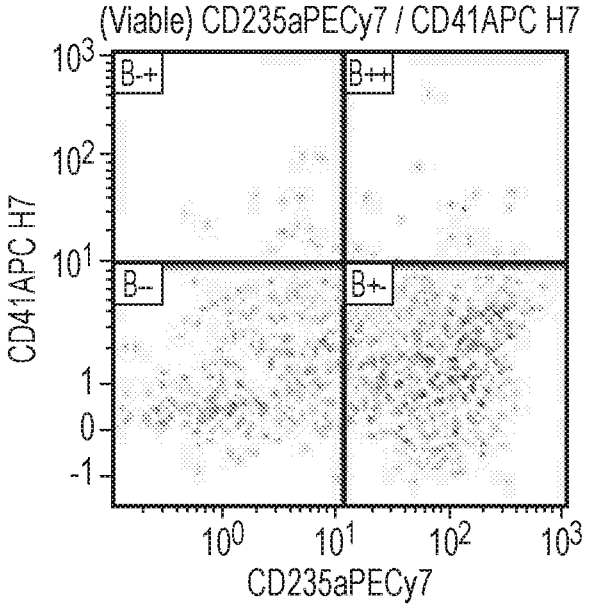


Gate	Number	%Total	%Gated
All	955	17.04	100.00
B--	34	0.61	3.56
B-+	298	5.32	31.20
B+-	6	0.11	0.63
B++	617	11.01	64.61

7xKO pool

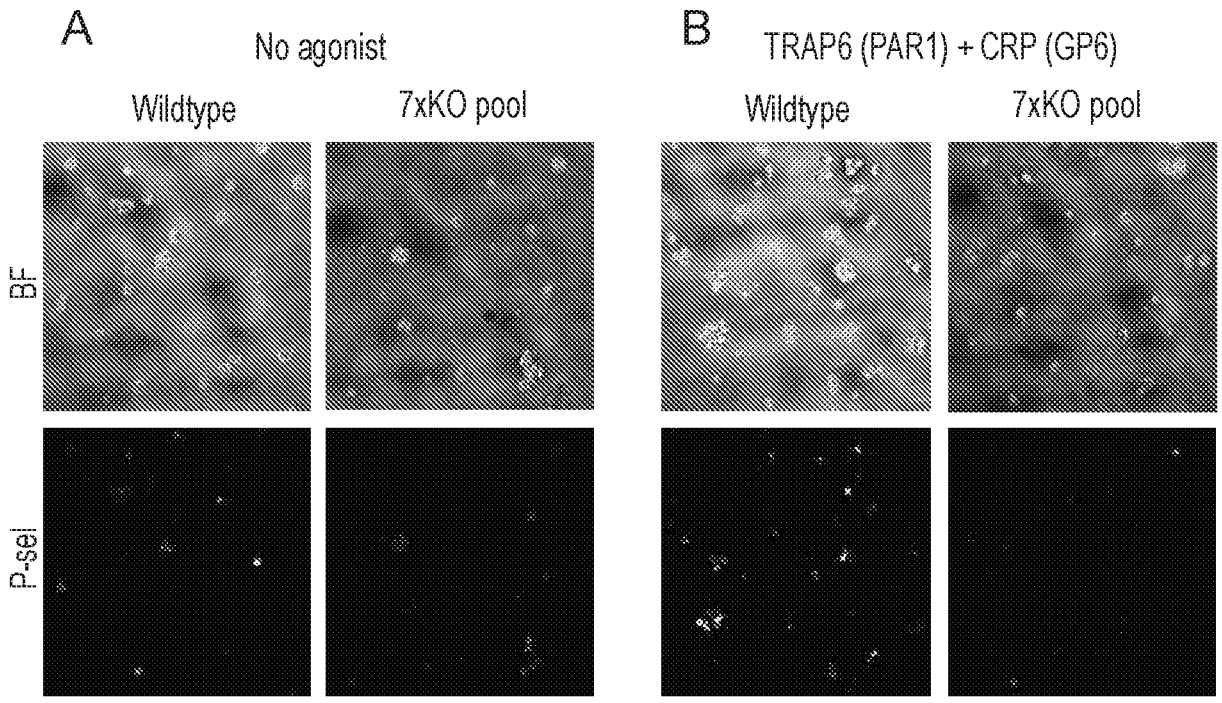


Gate	Number	%Total	%Gated
All	1,029	16.46	100.00
A--	238	3.81	23.13
A-+	6	0.10	0.58
A+-	745	11.92	72.40
A++	40	0.64	3.89



Gate	Number	%Total	%Gated
All	1,029	16.46	100.00
B--	347	5.55	33.72
B-+	21	0.34	2.04
B+-	636	10.17	61.81
B++	25	0.40	2.43

FIG. 4 (Continued)


A
No agonist
Wildtype
7xKO pool
B
TRAP6 (PAR1) + CRP (GP6)
Wildtype
7xKO pool
BF
P-sel

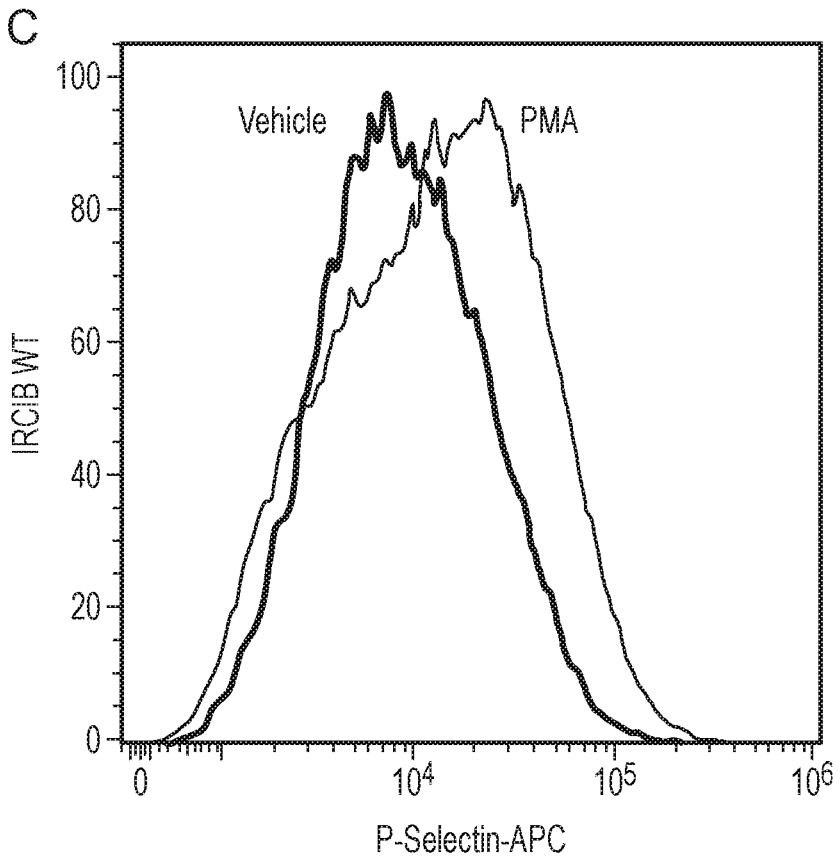

C
Vehicle
PMA
IRCIB WT
100
80
60
40
20
0
0
10^4
10^5
10^6
P-Selectin-APC

FIG. 5

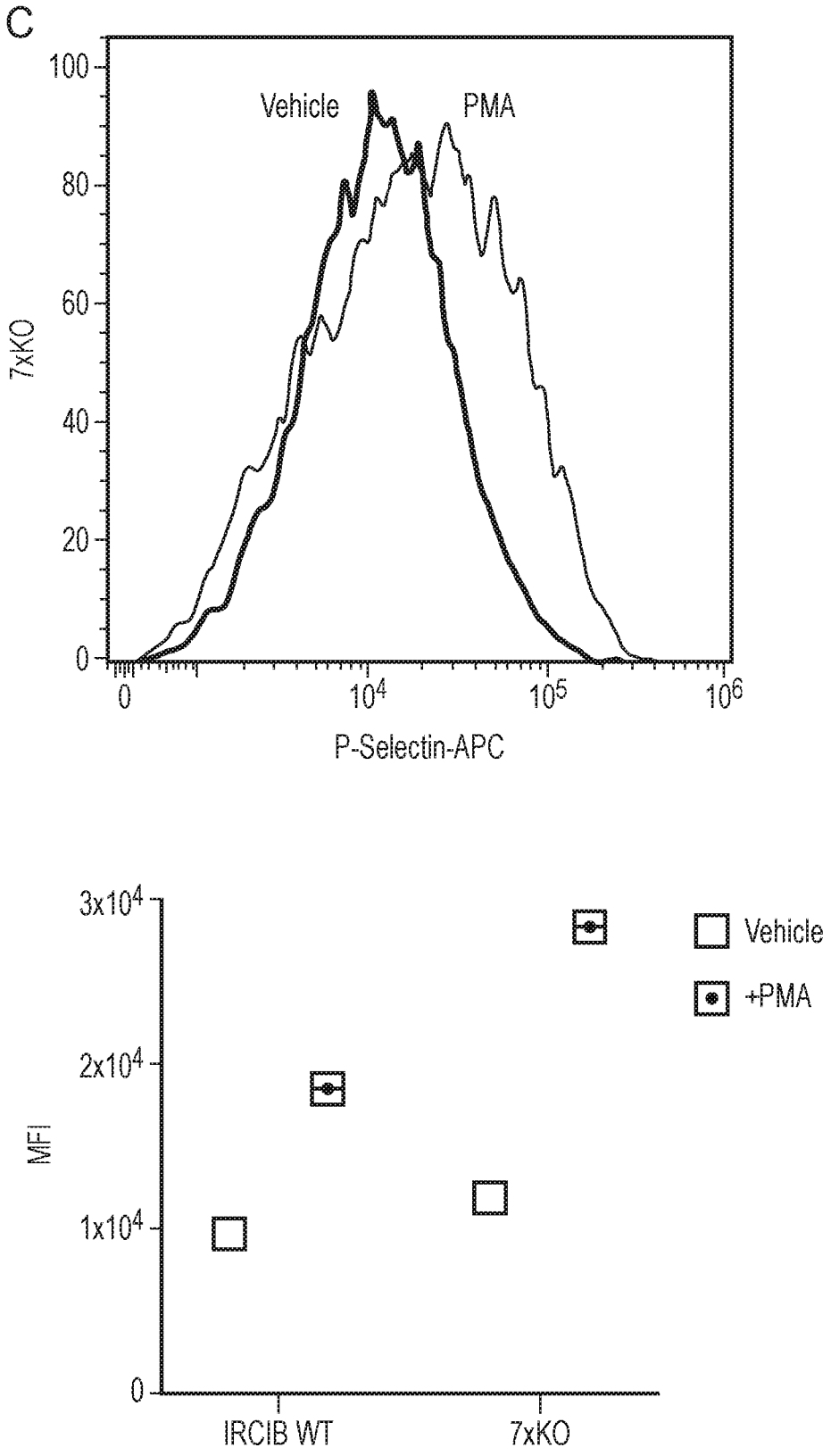
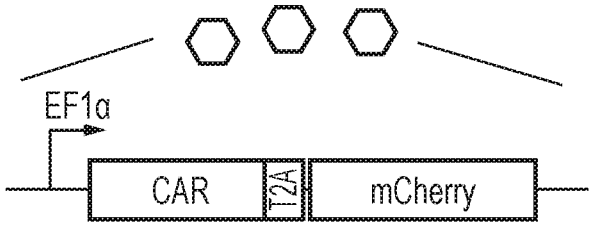


FIG. 5 (Continued)

A

1	Anti-CD19_IgG-Hinge_FCERG
2	Anti-CD19_CD28-Hinge_FCERG
3	Anti-CD19_IgG-Hinge_FCGR2A
4	Anti-CD19_CD28-Hinge_FCGR2A

B



C

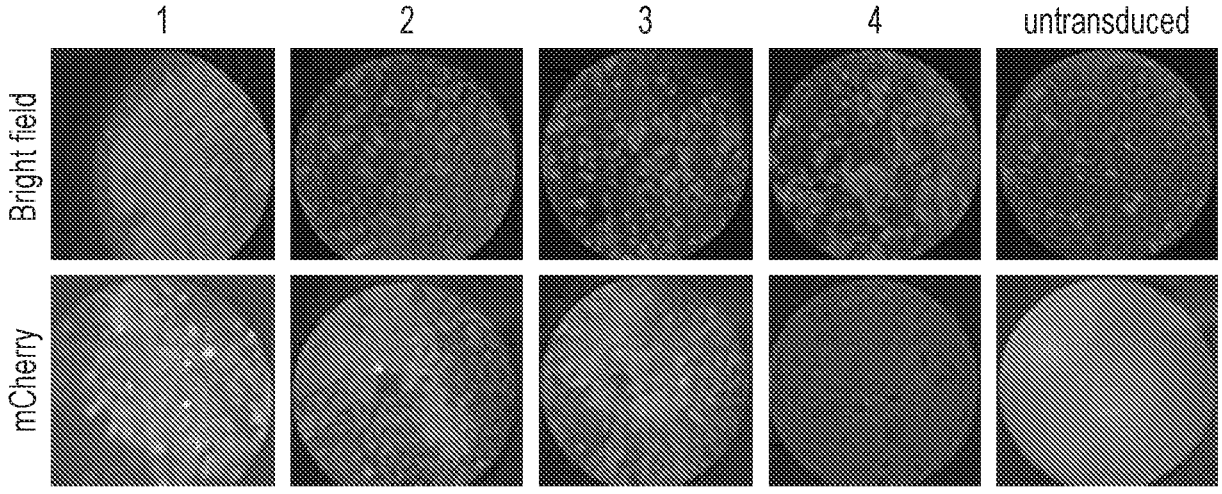


FIG. 6

A

1	Anti-CD19_IgG-Hinge_FCERG
2	Anti-CD19_CD28-Hinge_FCERG
3	Anti-CD19_IgG-Hinge_FCGR2A
4	Anti-CD19_CD28-Hinge_FCGR2A

B

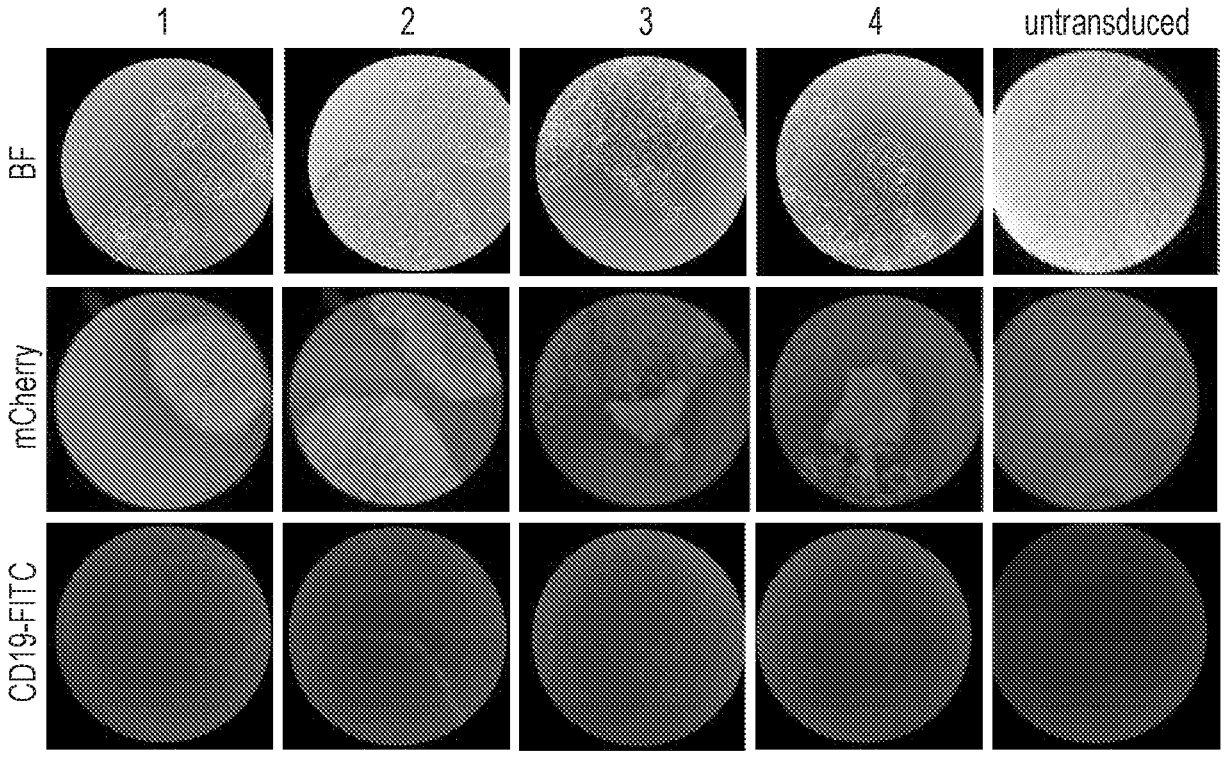


FIG. 7

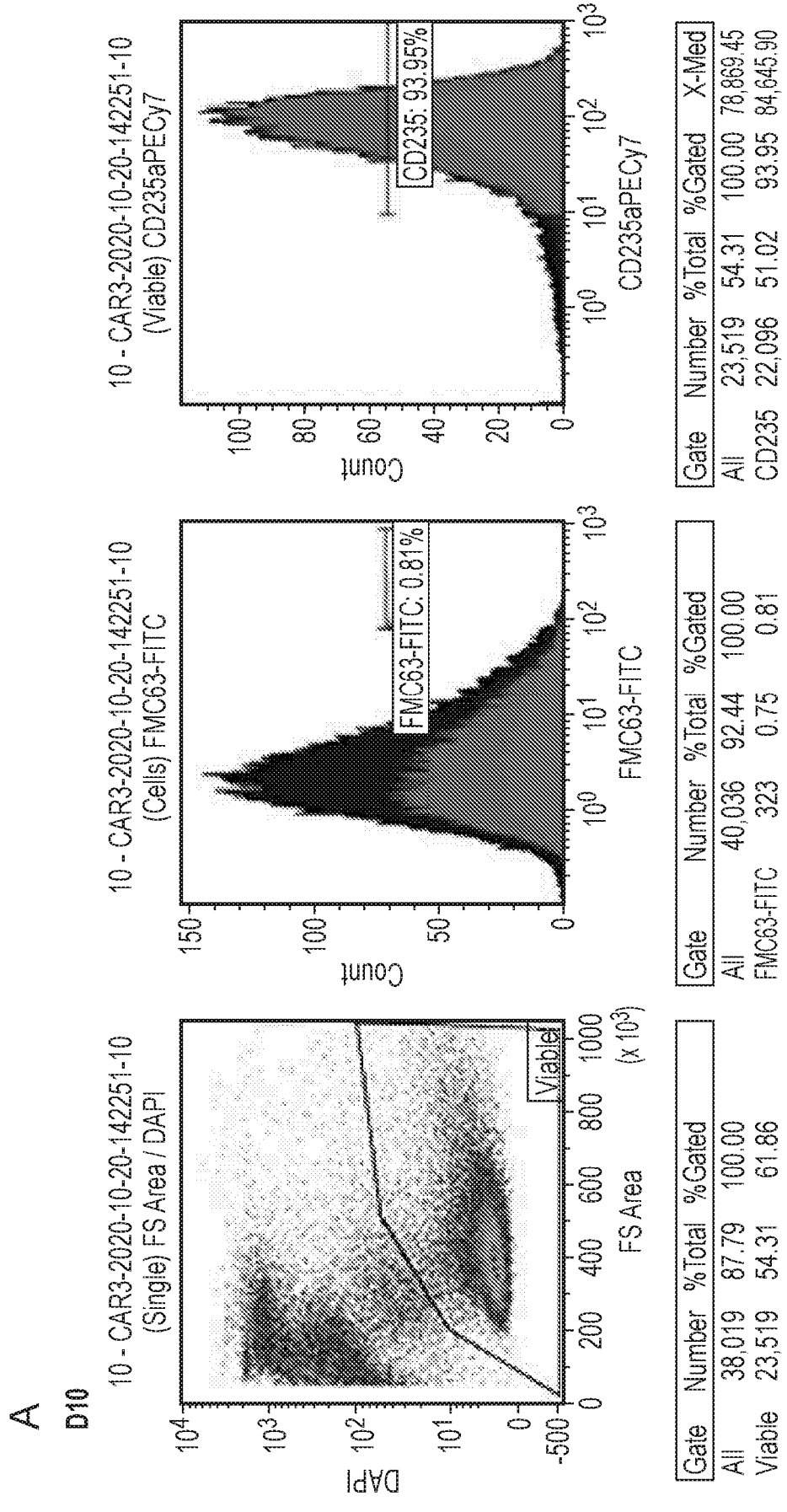
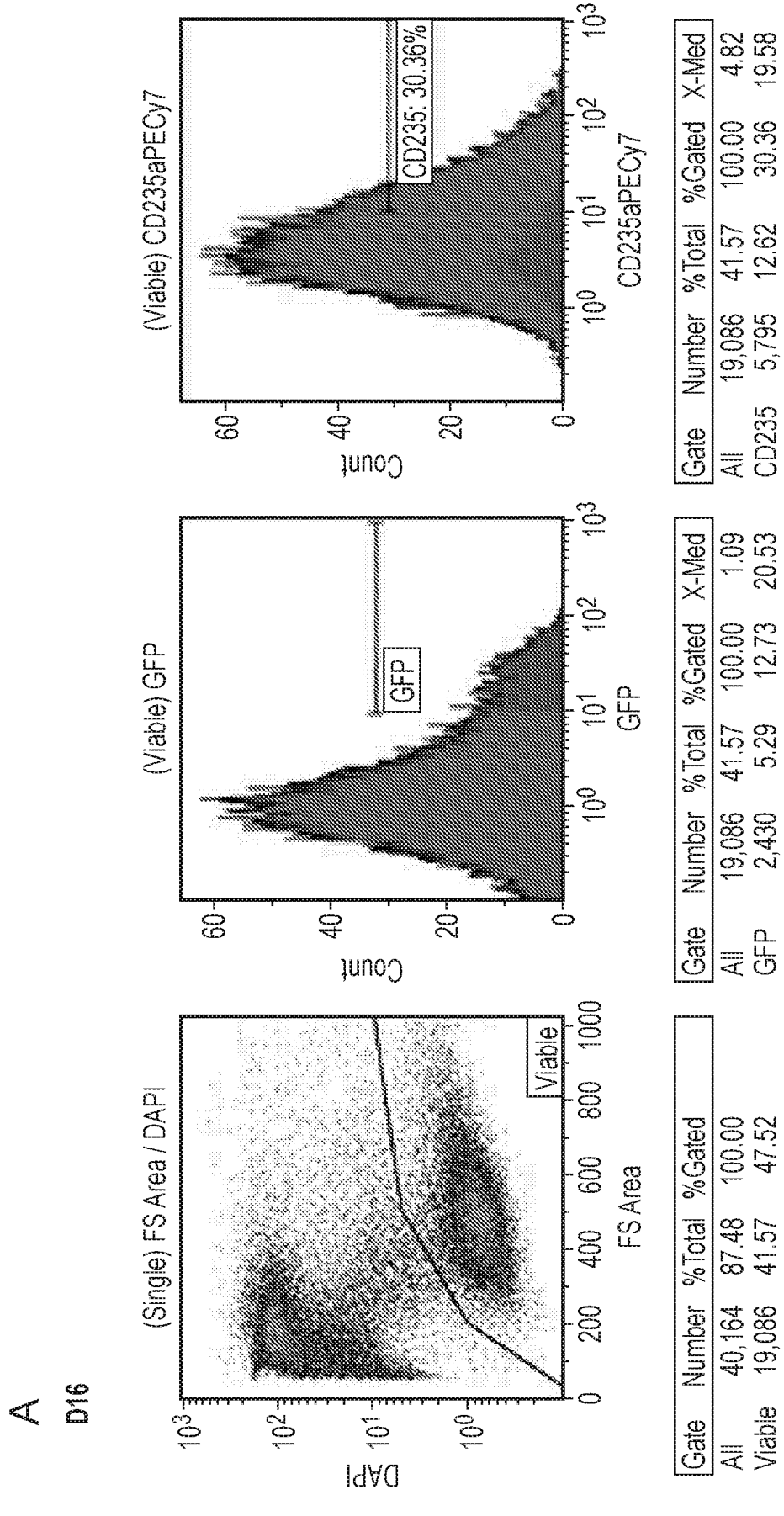

A
D10
10 - CAR3-2020-10-20-142251-10
(Single) FS Area / DAPI
DAPI
FS Area
(x 10³)
Viable
Gate Number %Total %Gated
All 38,019 87.79 100.00
Viable 23,519 54.31 61.86
10 - CAR3-2020-10-20-142251-10
(Cells) FMC63-FITC
Count
FMC63-FITC
FMC63-FITC: 0.81%
Gate Number %Total %Gated
All 40,036 92.44 100.00
FMC63-FITC 323 0.75 0.81
10 - CAR3-2020-10-20-142251-10
(Viable) CD235aPECy7
Count
CD235aPECy7
CD235: 93.95%
Gate Number %Total %Gated X-Med
All 23,519 54.31 100.00 78,869.45
CD235 22,096 51.02 93.95 84,645.90

FIG. 8



Gate	Number	%Total	%Gated
All	40,164	87.48	100.00
Viable	19,086	41.57	47.52

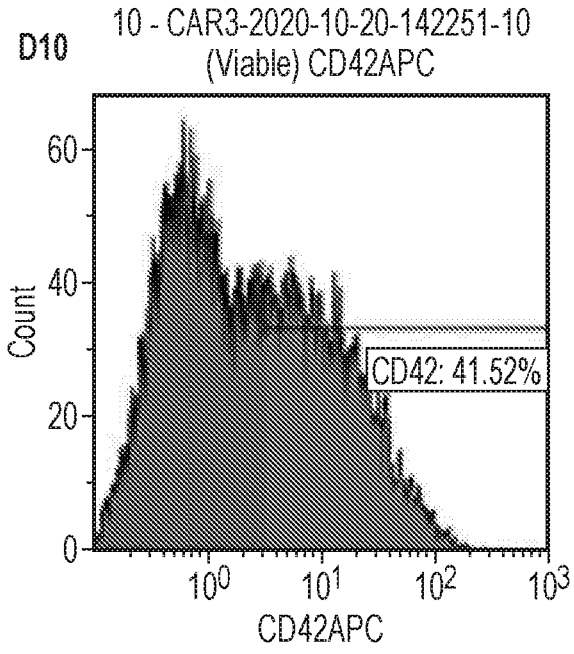
Gate	Number	%Total	%Gated	X-Med
All	19,086	41.57	100.00	1.09
GFP	2,430	5.29	12.73	20.53

Gate	Number	%Total	%Gated	X-Med
All	19,086	41.57	100.00	4.82
CD235	5,795	12.62	30.36	19.58

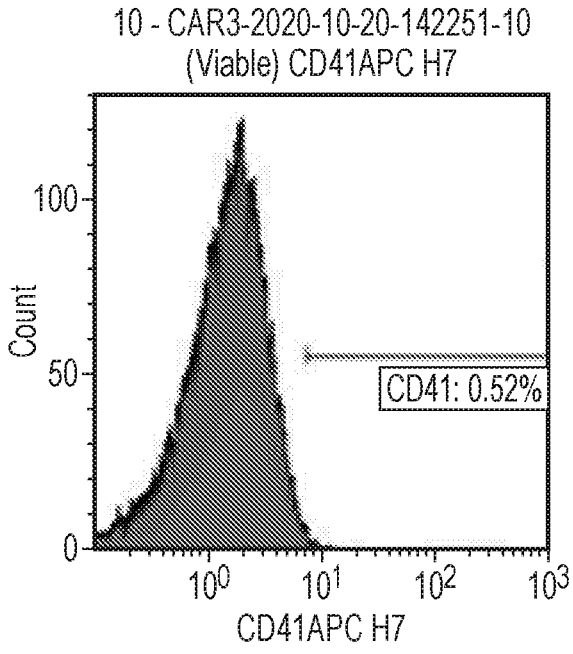
FIG. 8 (Continued)

A

D10

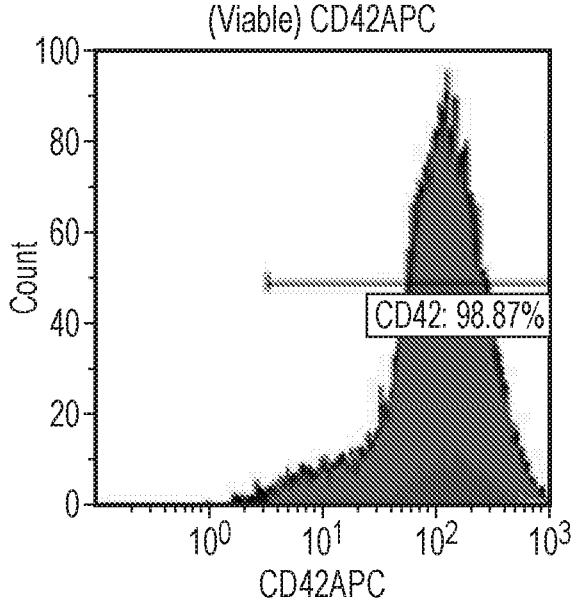


Gate	Number	%Total	%Gated	X-Med
All	23,519	54.31	100.00	2,153.10
CD42	9,764	22.55	41.52	11,588.77

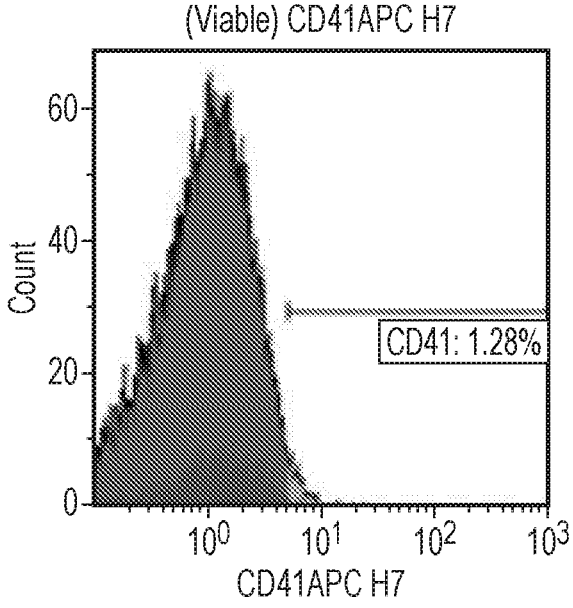


Gate	Number	%Total	%Gated	X-Med
All	23,519	54.31	100.00	1,519.84
CD41	123	0.28	0.52	8,893.38

D16



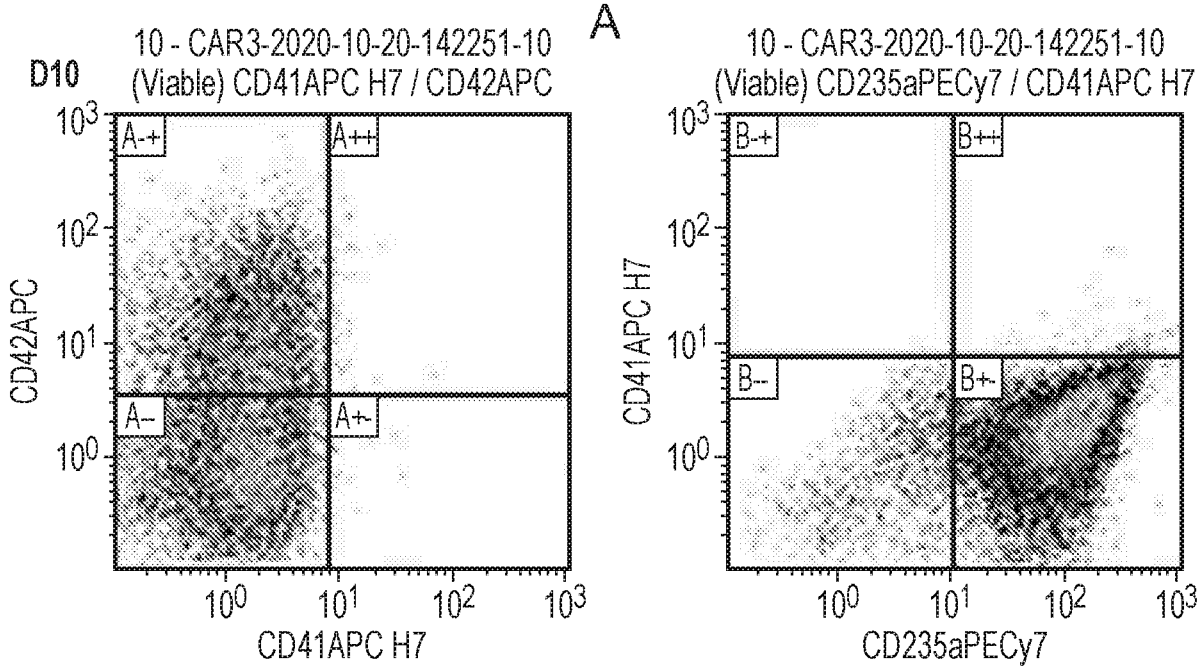
Gate	Number	%Total	%Gated	X-Med
All	19,086	41.57	100.00	111.91
CD42	18,871	41.10	98.87	113.37



Gate	Number	%Total	%Gated	X-Med
All	19,086	41.57	100.00	0.77
CD41	244	0.53	1.28	6.68

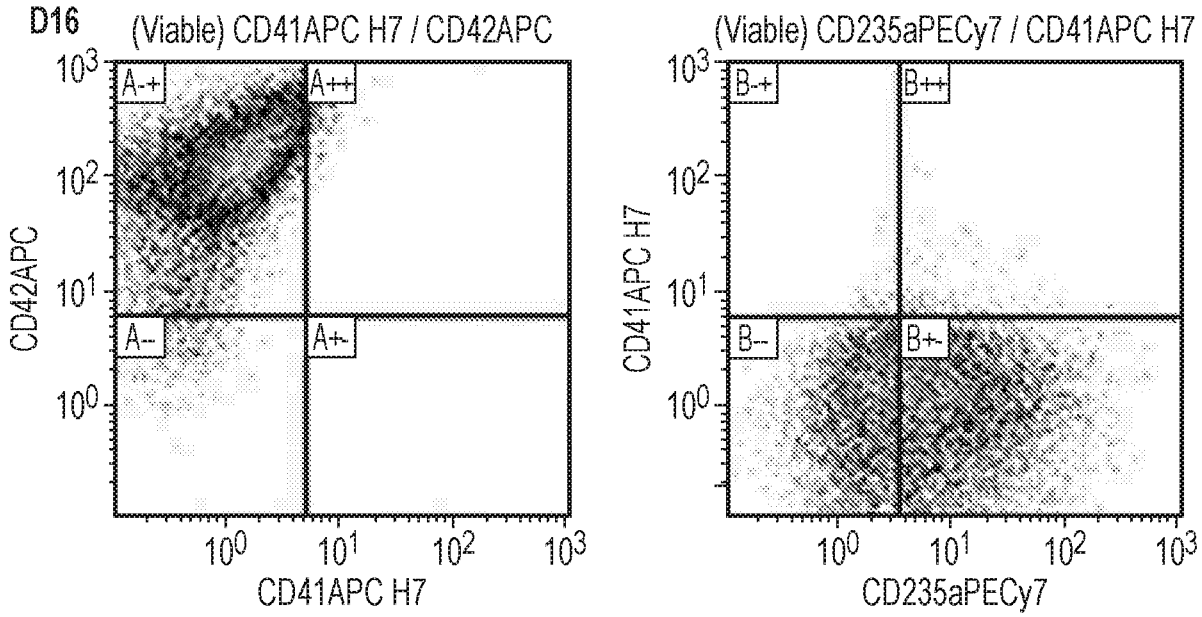
FIG. 8 (Continued)

A



Gate	Number	%Total	%Gated
All	23,519	54.31	100.00
A--	13,709	31.65	58.29
A-+	9,687	22.37	41.19
A+-	45	0.11	0.20
A++	77	0.18	0.33

Gate	Number	%Total	%Gated
All	23,519	54.31	100.00
B--	1,420	3.28	6.04
B-+	3	0.01	0.01
B+-	21,976	50.74	93.44
B++	120	0.28	0.51



Gate	Number	%Total	%Gated
All	19,086	41.57	100.00
A--	480	1.05	2.51
A-+	18,212	39.67	95.42
A+-	0	0.00	0.00
A++	394	0.86	2.06

Gate	Number	%Total	%Gated
All	19,086	41.57	100.00
B--	7,347	16.00	38.49
B-+	59	0.13	0.31
B+-	11,525	25.10	60.38
B++	155	0.34	0.81

FIG. 8 (Continued)

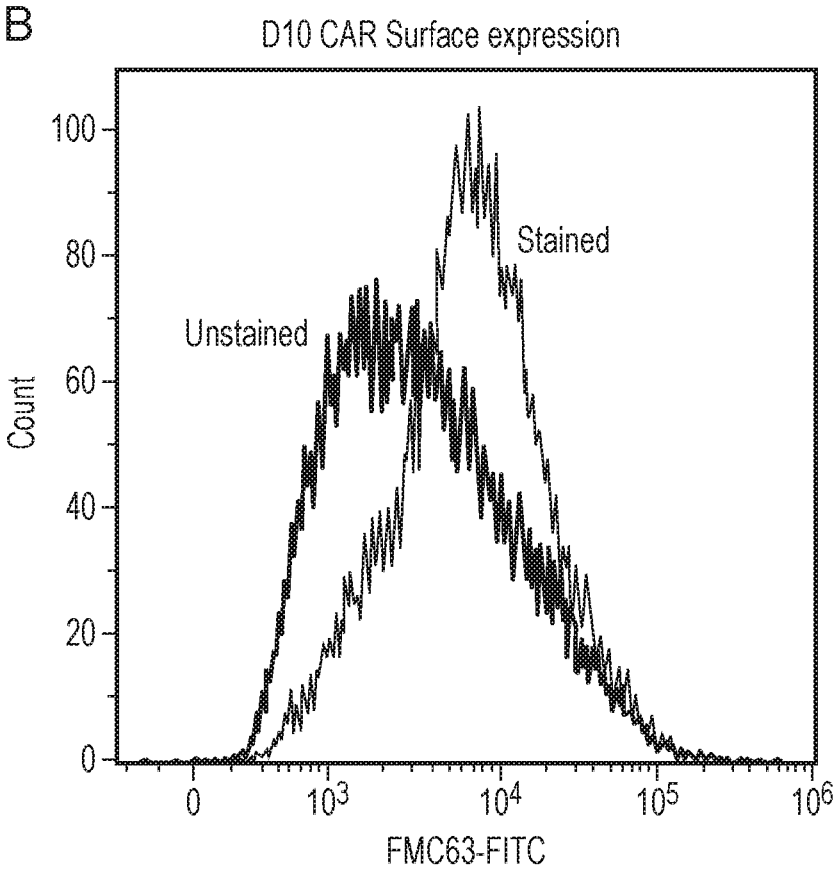


FIG. 8 (Continued)

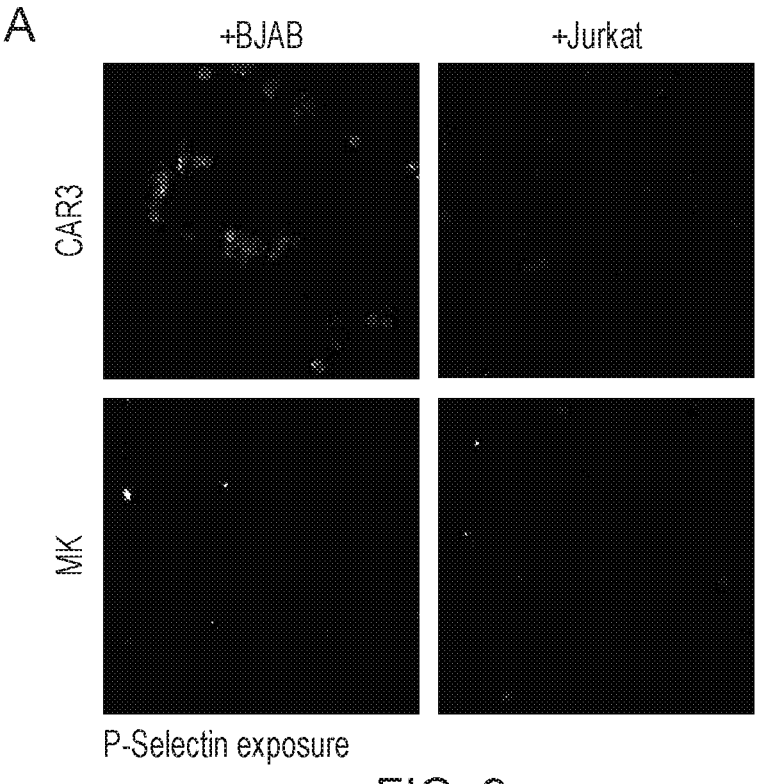


FIG. 9

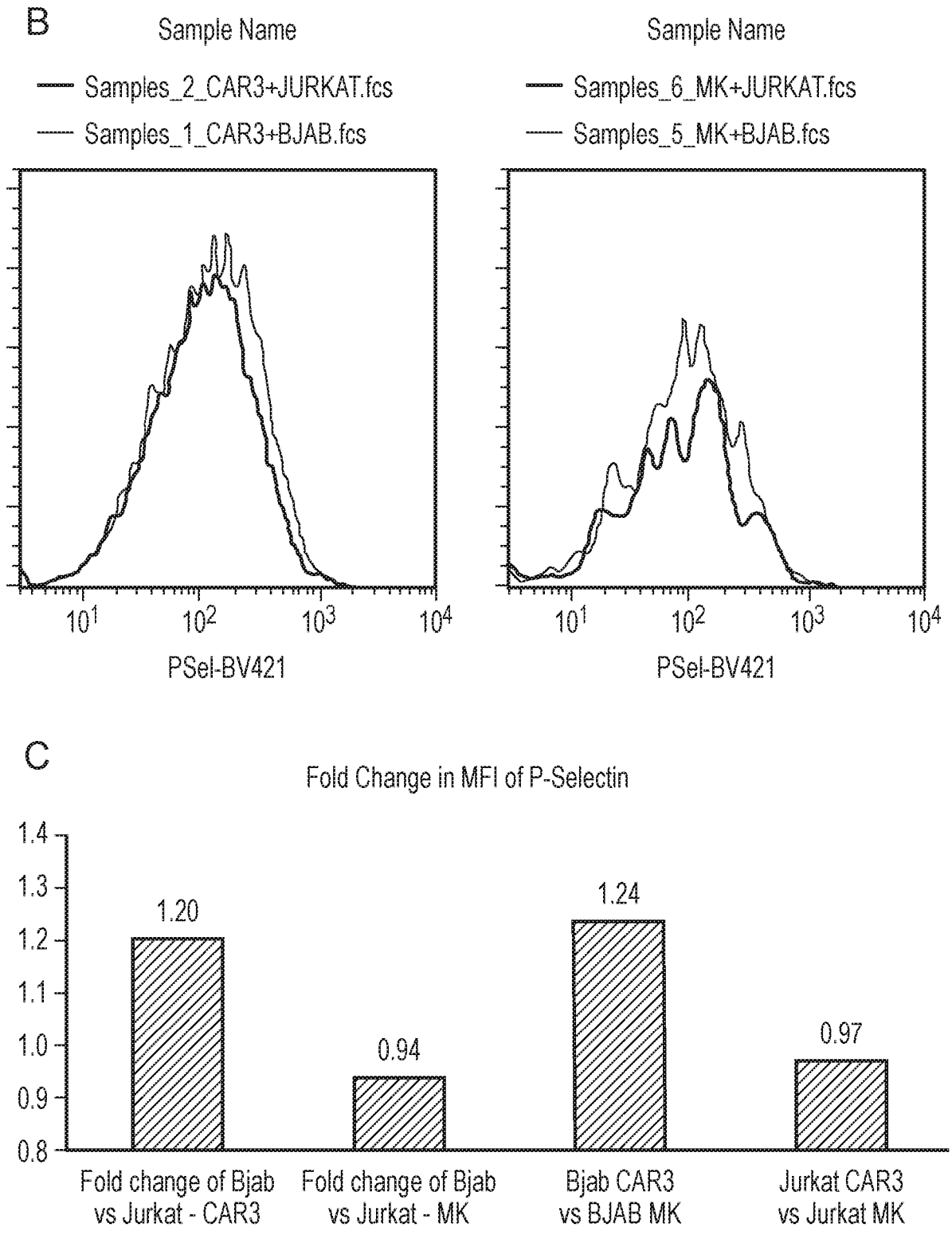


FIG. 9 (Continued)

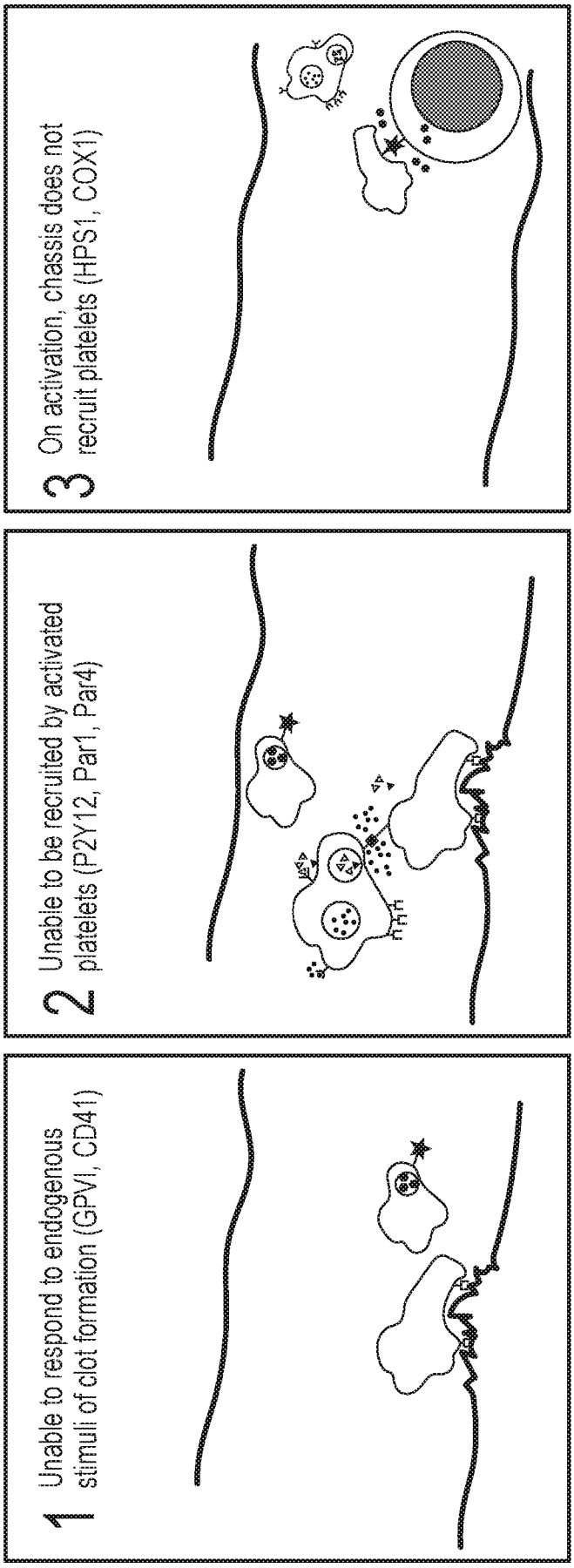

1 Unable to respond to endogenous stimuli of clot formation (GPVI, CD41)
2 Unable to be recruited by activated platelets (P2Y12, Par1, Par4)
3 On activation, chassis does not recruit platelets (HPS1, COX1)

FIG. 10

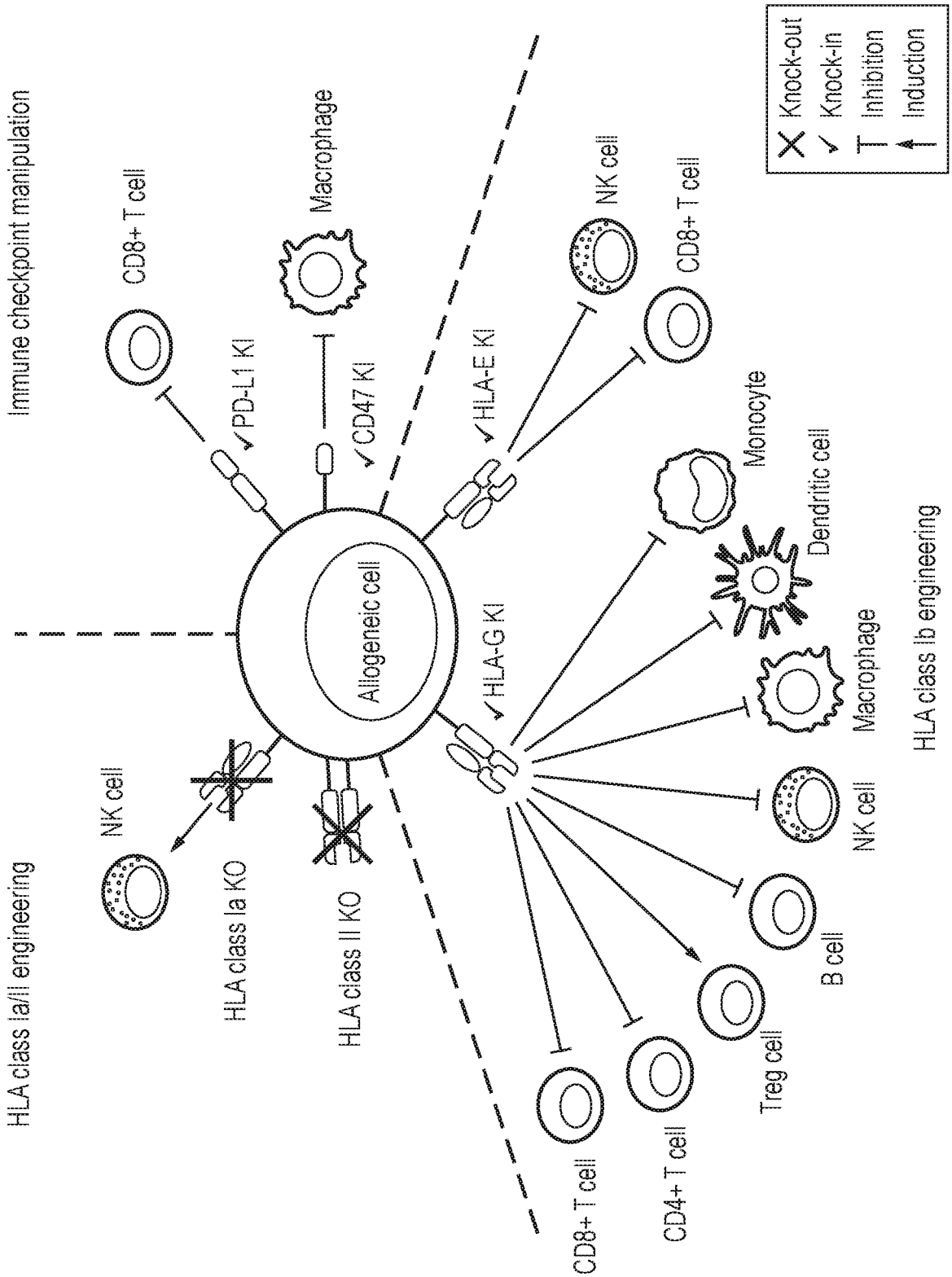

HLA class Ia/II engineering
Immune checkpoint manipulation
HLA class Ib engineering
NK cell
HLA class Ia KO
HLA class II KO
CD8+ T cell
PD-L1 KI
Macrophage
CD47 KI
HLA-E KI
HLA-G KI
Allogeneic cell
NK cell
CD8+ T cell
Monocyte
Dendritic cell
Macrophage
NK cell
B cell
Treg cell
CD4+ T cell
CD8+ T cell
Knock-out
Knock-in
Inhibition
Induction

FIG. 11

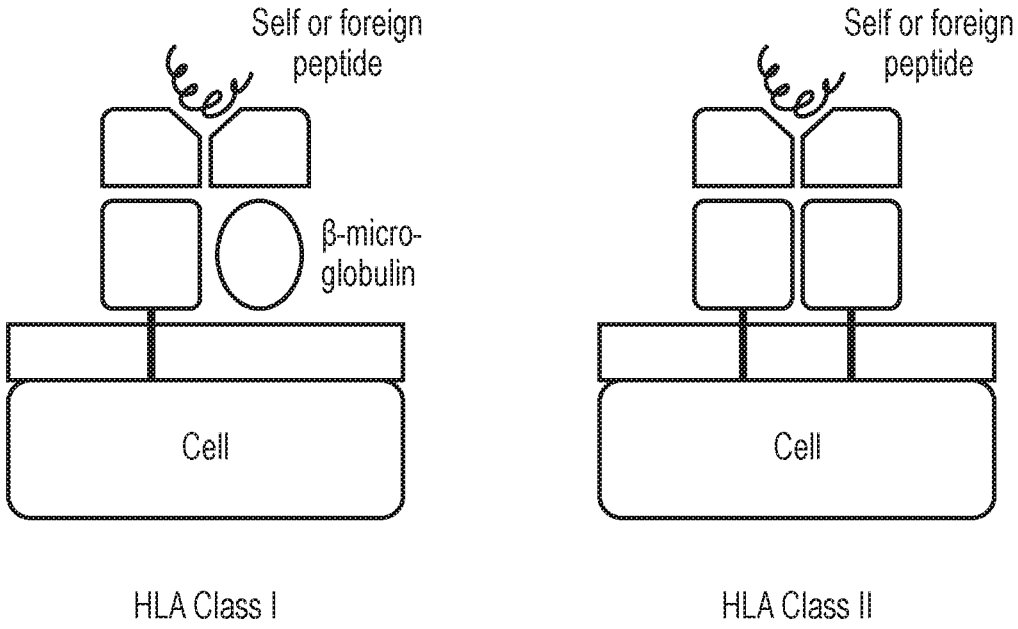

Self or foreign peptide
β-micro-globulin
Cell
HLA Class I
Self or foreign peptide
Cell
HLA Class II

FIG. 12(A)

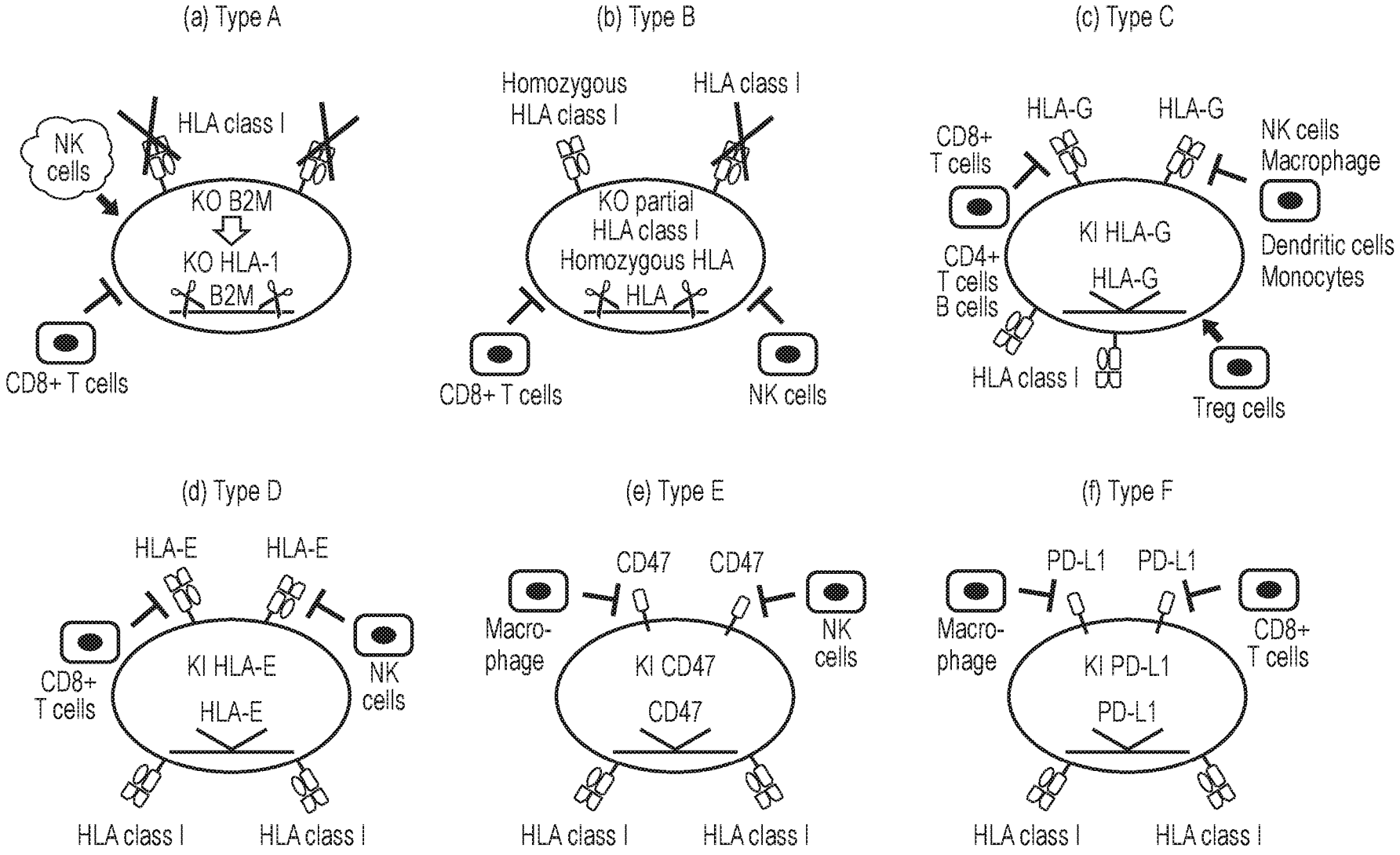

(a) Type A
NK cells
HLA class I
KO B2M
KO HLA-1
B2M
CD8+ T cells
(b) Type B
Homozygous HLA class I
HLA class I
KO partial HLA class I
Homozygous HLA
HLA
CD8+ T cells
NK cells
(c) Type C
HLA-G
HLA-G
CD8+ T cells
NK cells
Macrophage
KI HLA-G
HLA-G
CD4+ T cells
B cells
Dendritic cells
Monocytes
HLA class I
Treg cells
(d) Type D
HLA-E
HLA-E
CD8+ T cells
KI HLA-E
HLA-E
NK cells
HLA class I
HLA class I
(e) Type E
CD47
CD47
Macro-phage
KI CD47
CD47
NK cells
HLA class I
HLA class I
(f) Type F
PD-L1
PD-L1
Macro-phage
KI PD-L1
PD-L1
CD8+ T cells
HLA class I
HLA class I

FIG. 12(B)

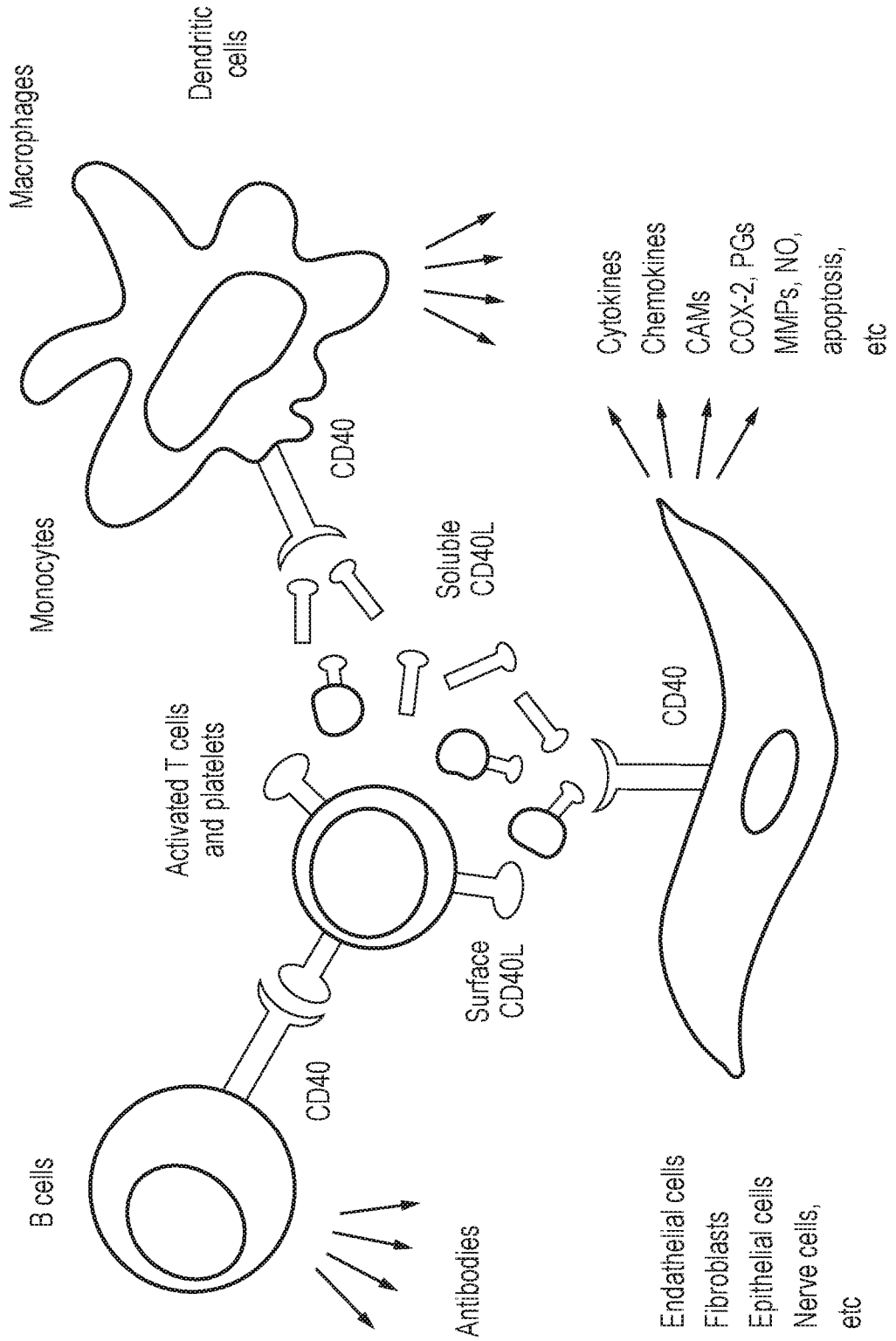

Macrophages
Dendritic cells
Monocytes
CD40
Soluble CD40L
Activated T cells and platelets
CD40
Surface CD40L
B cells
Antibodies
CD40
Cytokines
Chemokines
CAMs
COX-2, PGs
MMPs, NO,
apoptosis,
etc
Endathelial cells
Fibroblasts
Epithelial cells
Nerve cells,
etc

FIG. 13

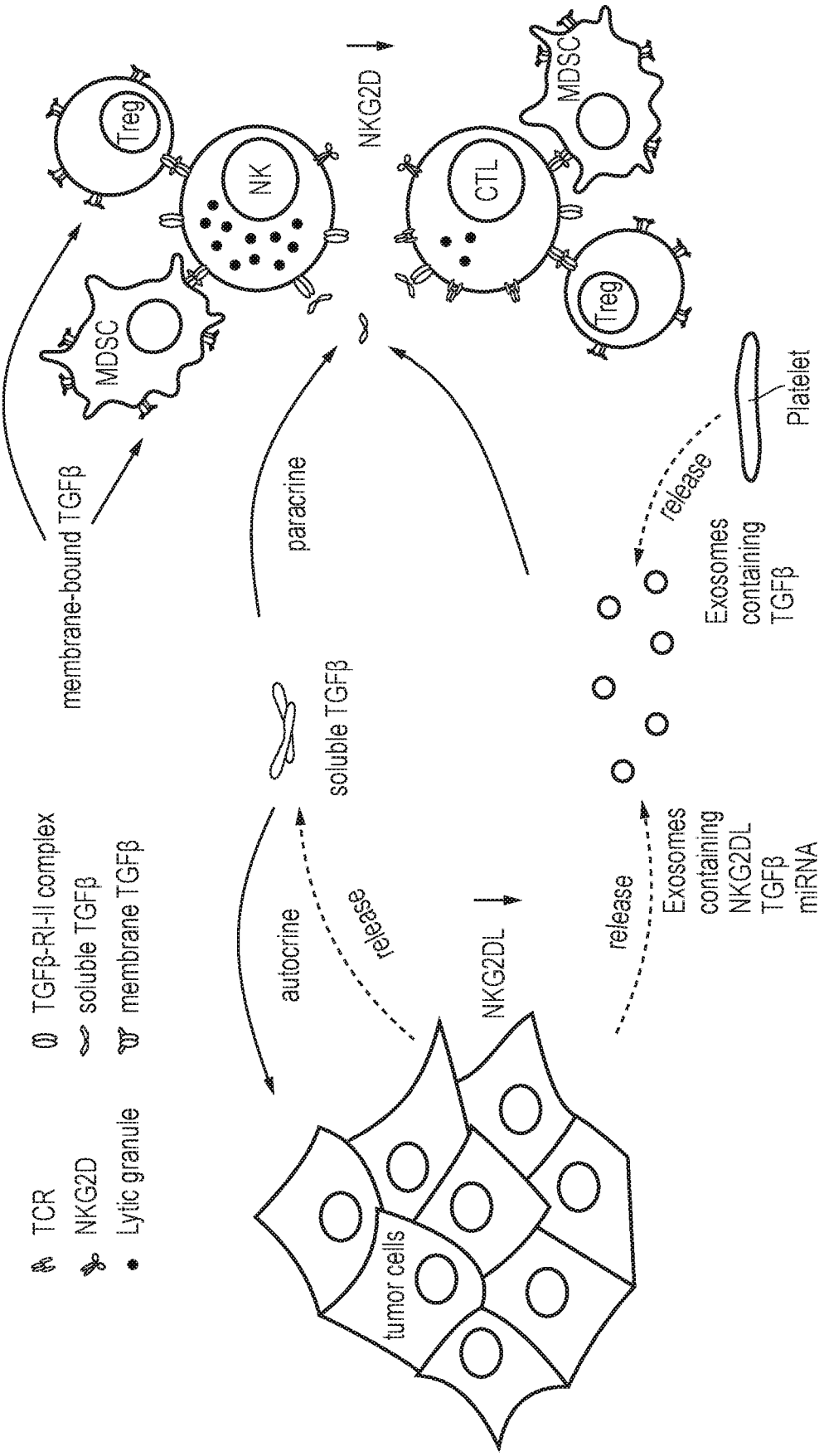

TCR
NKG2D
Lytic granule
TGFβ-RI-II complex
soluble TGFβ
membrane TGFβ
Treg
MDSC
NK
CTL
NKG2D
membrane-bound TGFβ
paracrine
soluble TGFβ
autocrine
release
NKG2DL
tumor cells
release
Exosomes containing NKG2DL TGFβ miRNA
release
Exosomes containing TGFβ
Platelet

FIG. 14

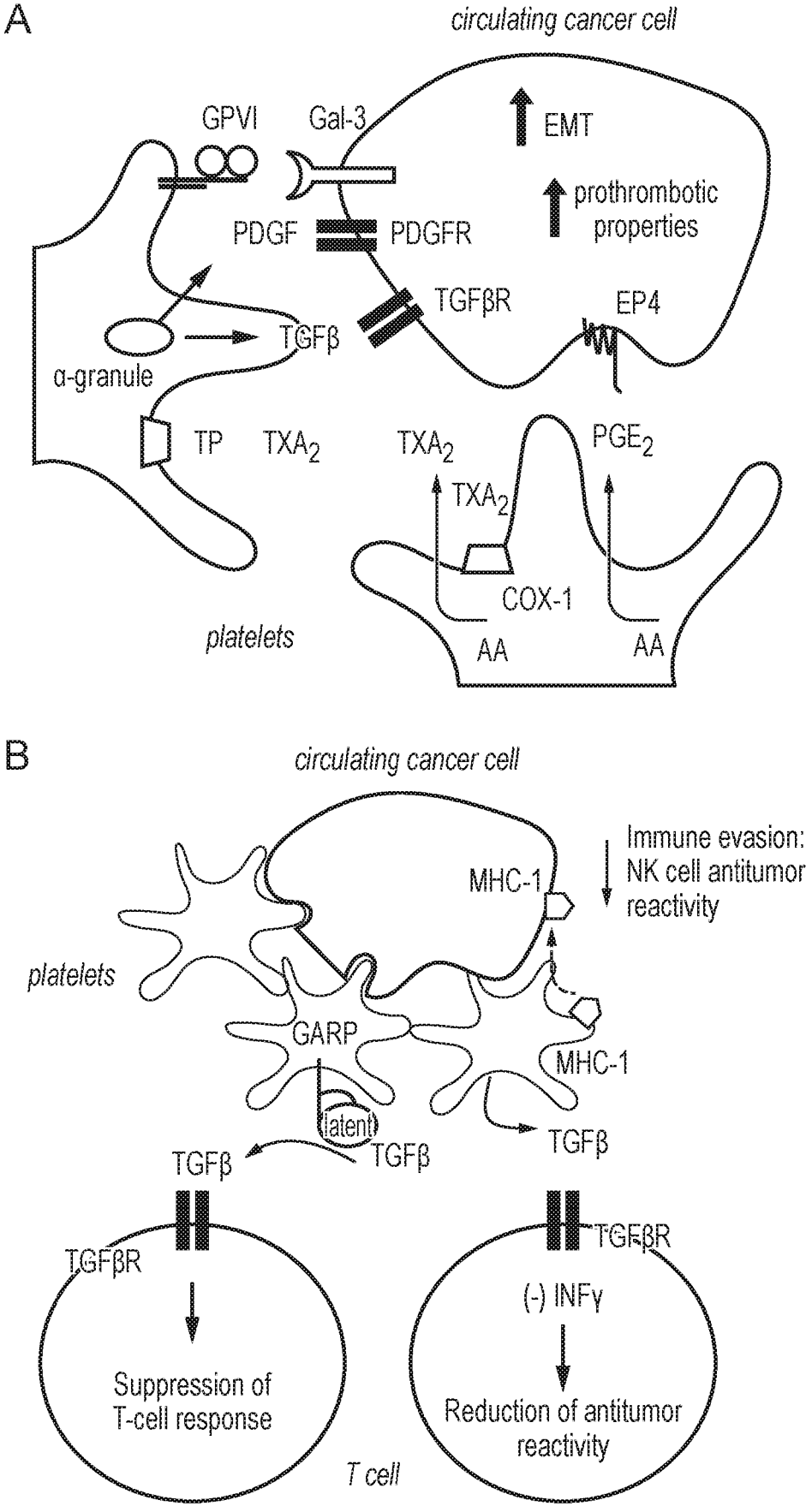

A
circulating cancer cell
GPVI
Gal-3
EMT
prothrombotic properties
PDGF
PDGFR
TGFβR
EP4
TGFβ
α-granule
TP
TXA2
TXA2
PGE2
TXA2
COX-1
AA
AA
platelets
B
circulating cancer cell
Immune evasion: NK cell antitumor reactivity
MHC-1
platelets
GARP
MHC-1
latent
TGFβ
TGFβ
TGFβ
TGFβR
TGFβR
(-) INFγ
Suppression of T-cell response
Reduction of antitumor reactivity
T cell

FIG. 15

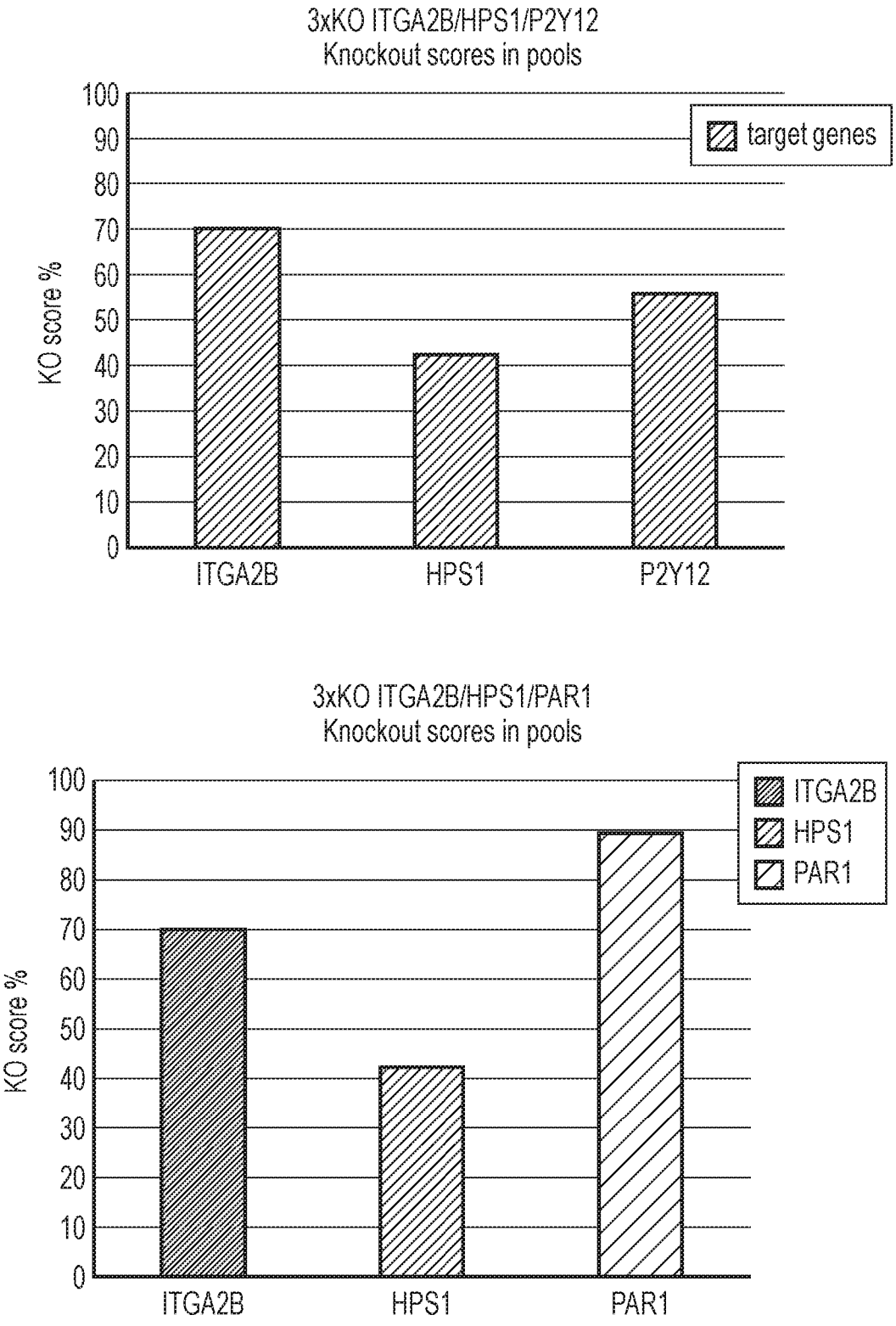

3xKO ITGA2B/HPS1/P2Y12
Knockout scores in pools
KO score %
100
90
80
70
60
50
40
30
20
10
0
target genes
ITGA2B
HPS1
P2Y12
3xKO ITGA2B/HPS1/PAR1
Knockout scores in pools
KO score %
ITGA2B
HPS1
PAR1
ITGA2B
HPS1
PAR1

FIG. 16

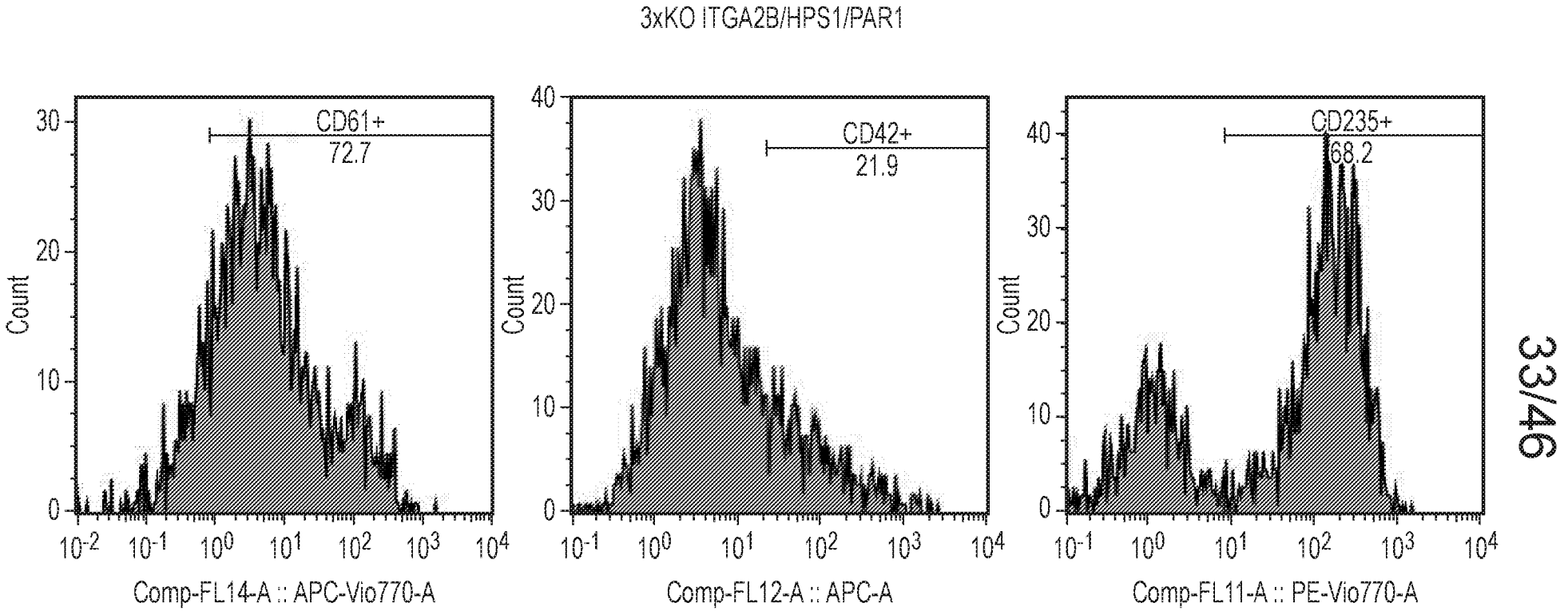

3xKO ITGA2B/HPS1/PAR1
CD61+
72.7
CD42+
21.9
CD235+
68.2
Count
Comp-FL14-A :: APC-Vio770-A
Comp-FL12-A :: APC-A
Comp-FL11-A :: PE-Vio770-A
XT_2022-05-27_D3_Plate 2 PAR1.0001.mqd
DAPI-
2201

FIG. 17A

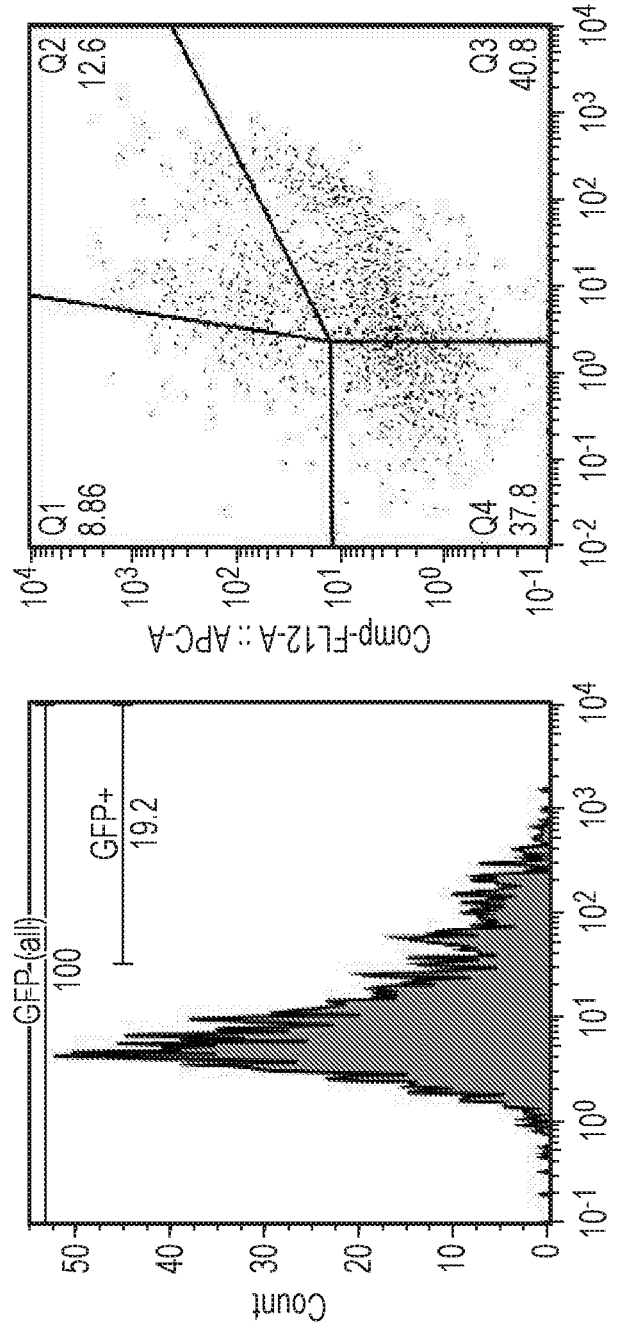


FIG. 17A (Continued)

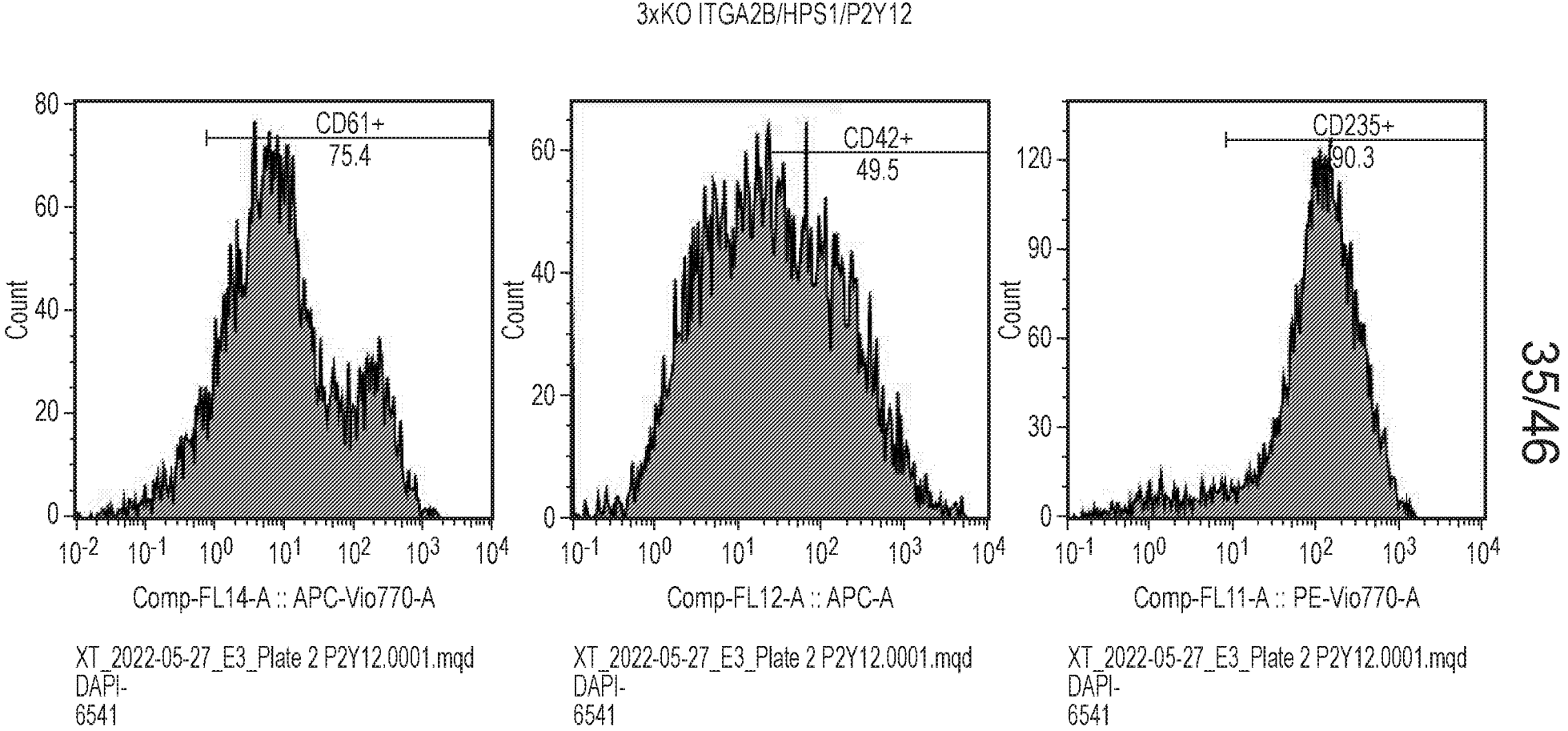

3xKO ITGA2B/HPS1/P2Y12
CD61+
75.4
Count
Comp-FL14-A :: APC-Vio770-A
XT_2022-05-27_E3_Plate 2 P2Y12.0001.mqd
DAPI-
6541
CD42+
49.5
Count
Comp-FL12-A :: APC-A
XT_2022-05-27_E3_Plate 2 P2Y12.0001.mqd
DAPI-
6541
CD235+
90.3
Count
Comp-FL11-A :: PE-Vio770-A
XT_2022-05-27_E3_Plate 2 P2Y12.0001.mqd
DAPI-
6541

FIG. 17B

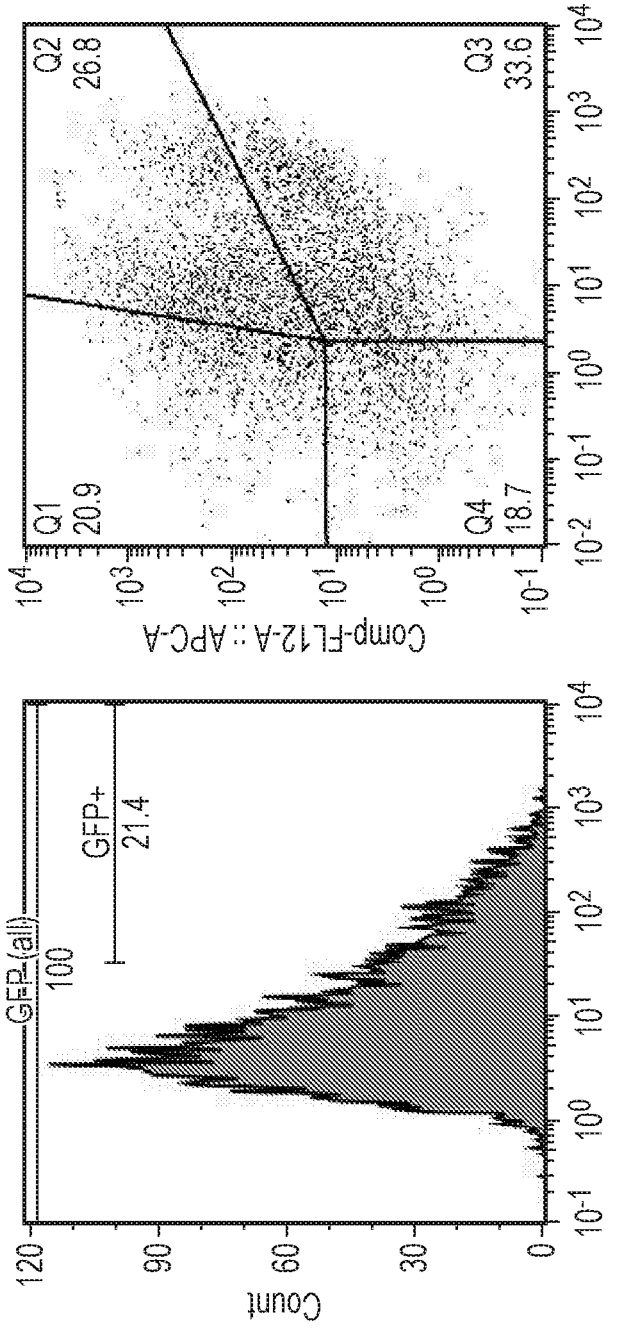


FIG. 17B (Continued)

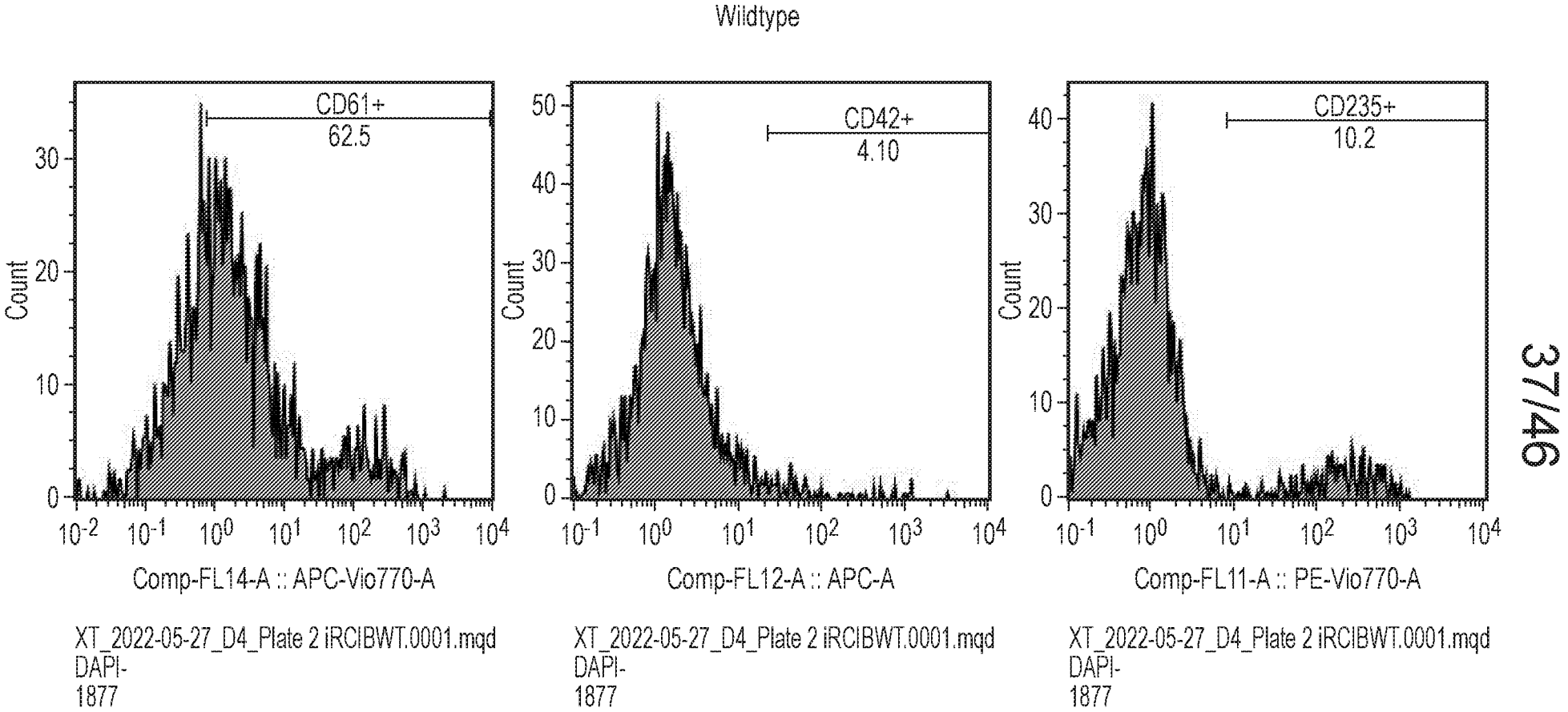

Wildtype
CD61+
62.5
Count
Comp-FL14-A :: APC-Vio770-A
XT_2022-05-27_D4_Plate 2 iRCIBWT.0001.mqd
DAPI-
1877
CD42+
4.10
Count
Comp-FL12-A :: APC-A
XT_2022-05-27_D4_Plate 2 iRCIBWT.0001.mqd
DAPI-
1877
CD235+
10.2
Count
Comp-FL11-A :: PE-Vio770-A
XT_2022-05-27_D4_Plate 2 iRCIBWT.0001.mqd
DAPI-
1877

FIG. 17C

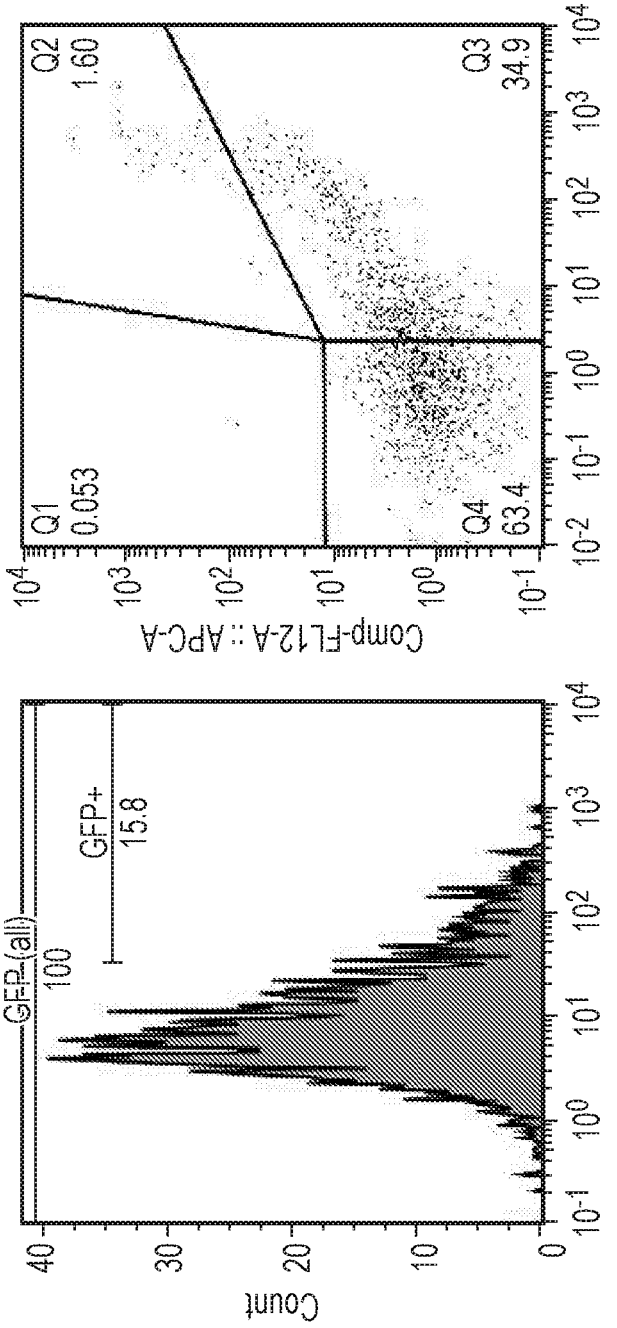


FIG. 17C (Continued)

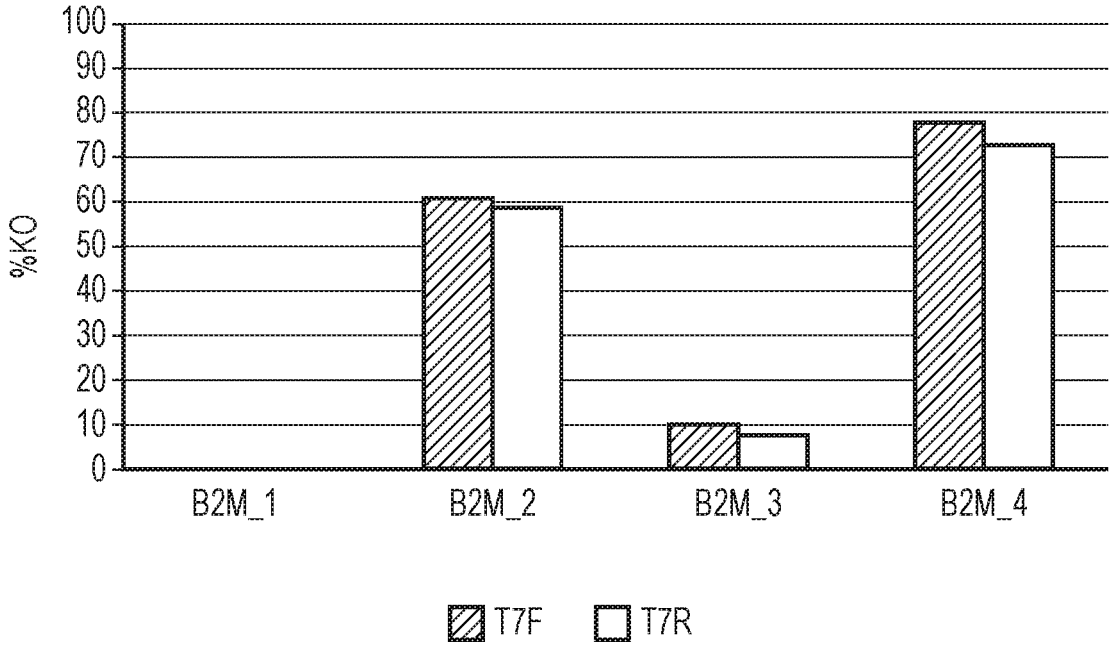

100
90
80
70
60
50
40
30
20
10
0
%KO
B2M_1
B2M_2
B2M_3
B2M_4
T7F
T7R

FIG. 18

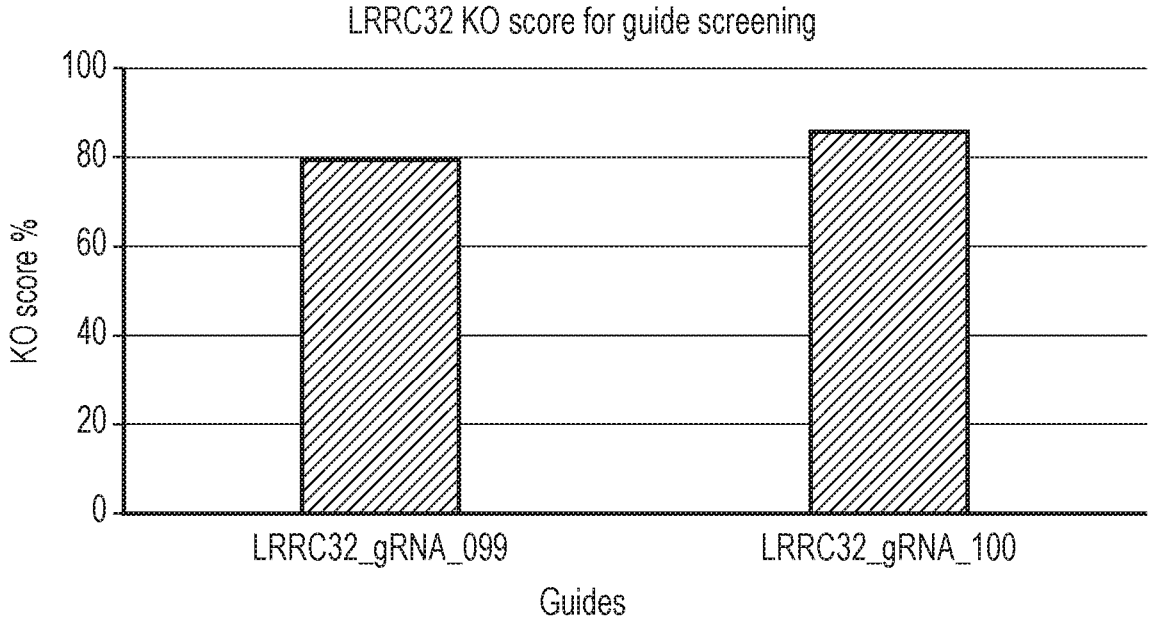
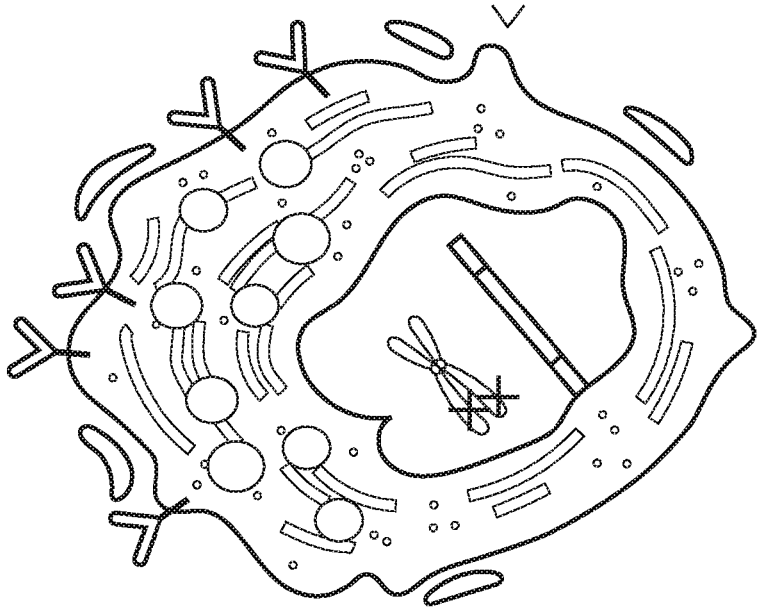

LRRC32 KO score for guide screening
100
80
60
40
20
0
KO score %
LRRC32_gRNA_099
LRRC32_gRNA_100
Guides

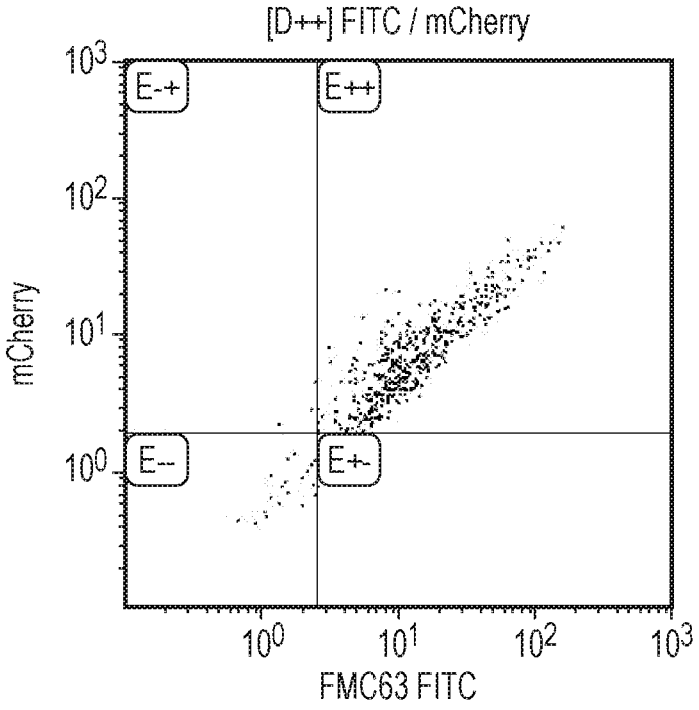
FIG. 19



MK expressing CAR

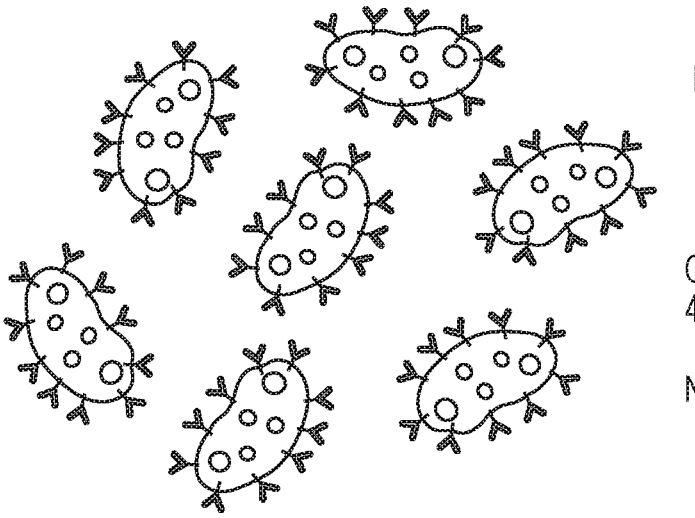
CD41+/CD42+MK,
2 days post-transduction

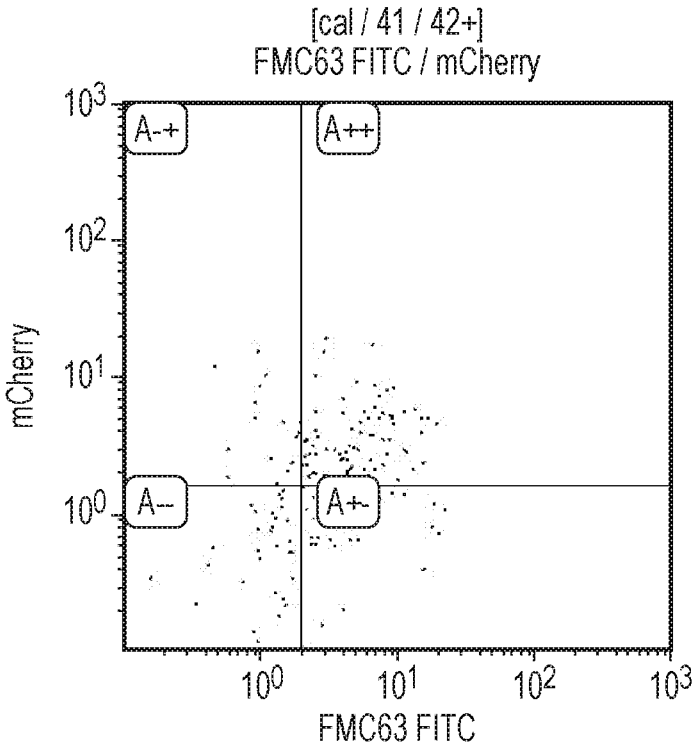
N=3



Gate	Number	%Total	%Gated
All	494	24.37	100.00
E--	19	0.94	3.85
E-+	3	0.15	0.61
E+-	25	1.23	5.06
E++	447	22.05	90.49

FIG. 20





Gate	Number	%Total	%Gated
All	427	3.34	100.00
A--	154	1.20	36.07
A-+	18	0.14	4.22
A+-	174	1.36	40.75
A++	81	0.63	18.97

FIG. 20 (Continued)

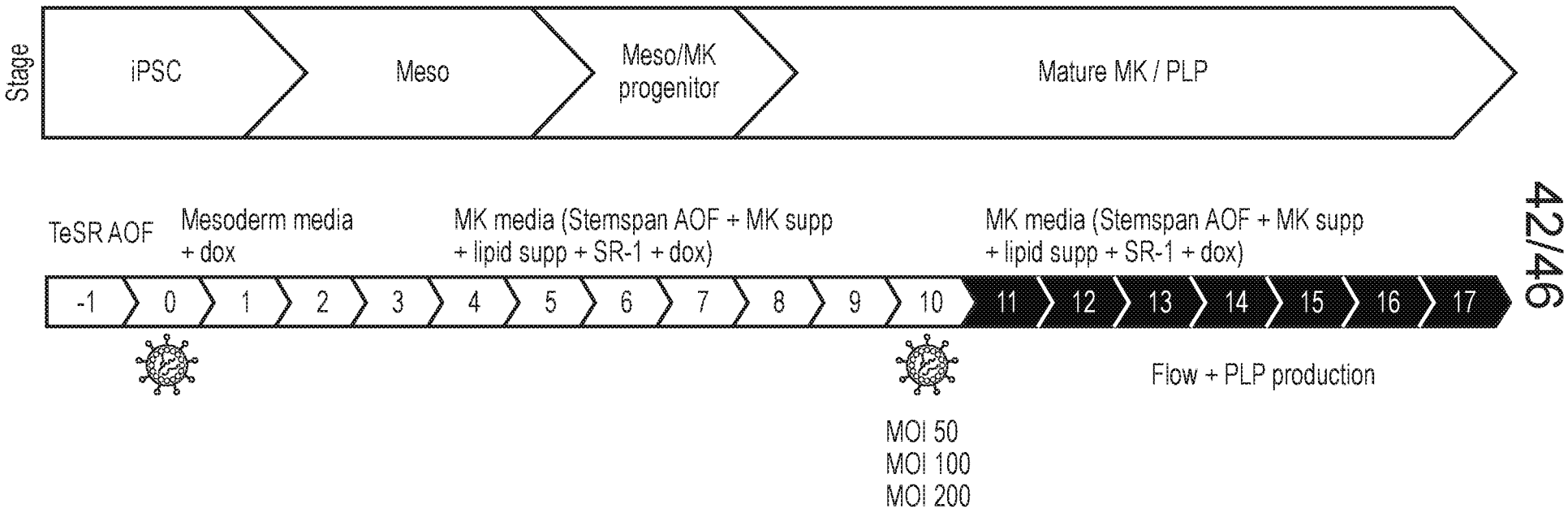

Stage
iPSC
Meso
Meso/MK progenitor
Mature MK / PLP
TeSR AOF
Mesoderm media + dox
MK media (Stemspan AOF + MK supp + lipid supp + SR-1 + dox)
MK media (Stemspan AOF + MK supp + lipid supp + SR-1 + dox)
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Flow + PLP production
MOI 50
MOI 100
MOI 200

FIG. 21

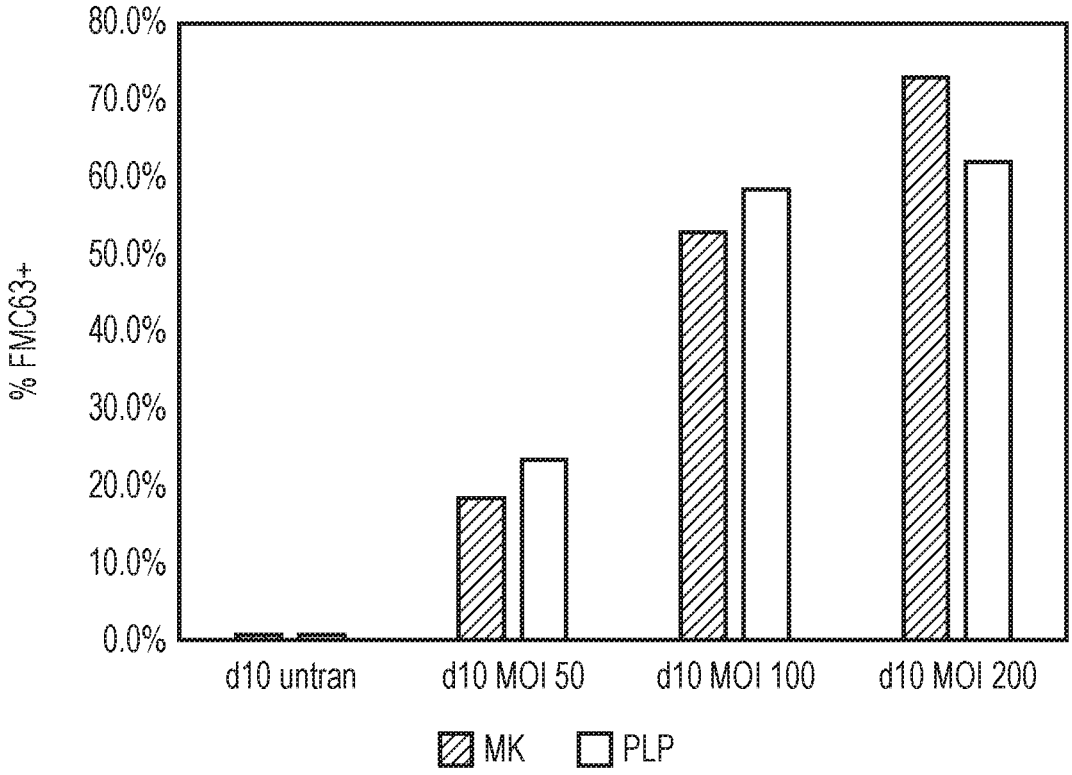


FIG. 22

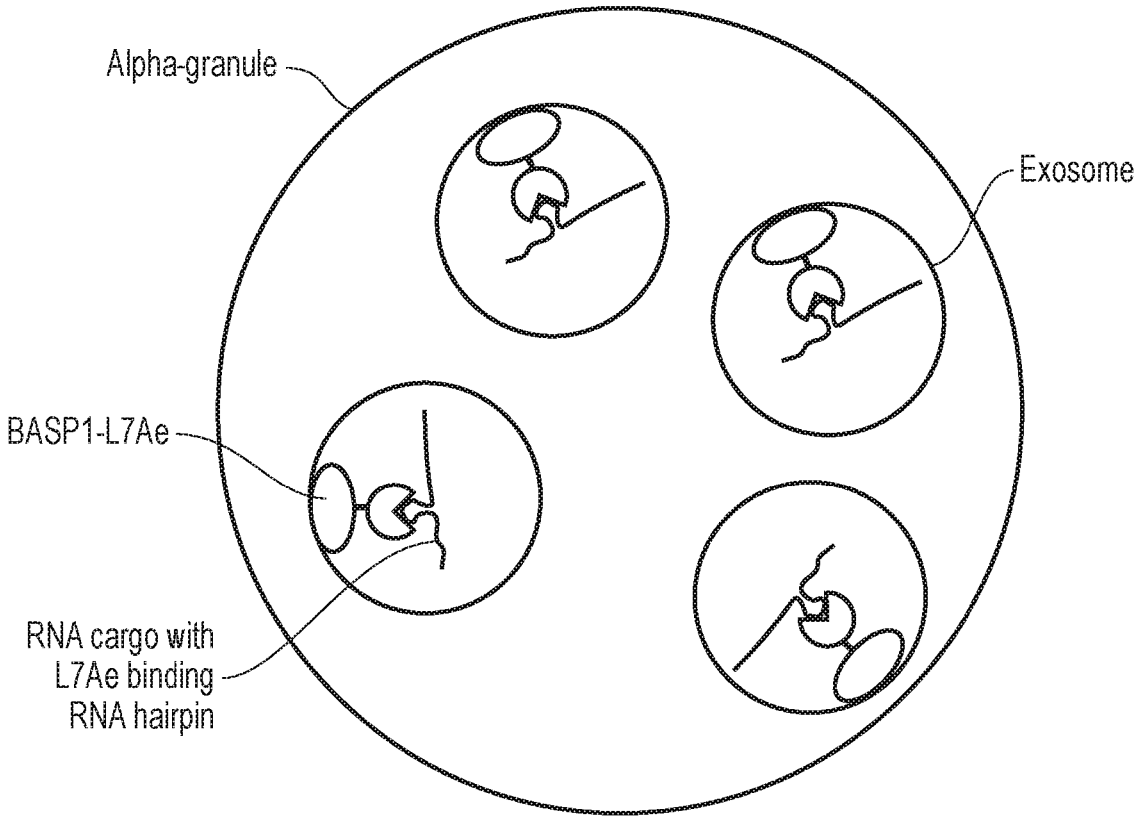


FIG. 23A

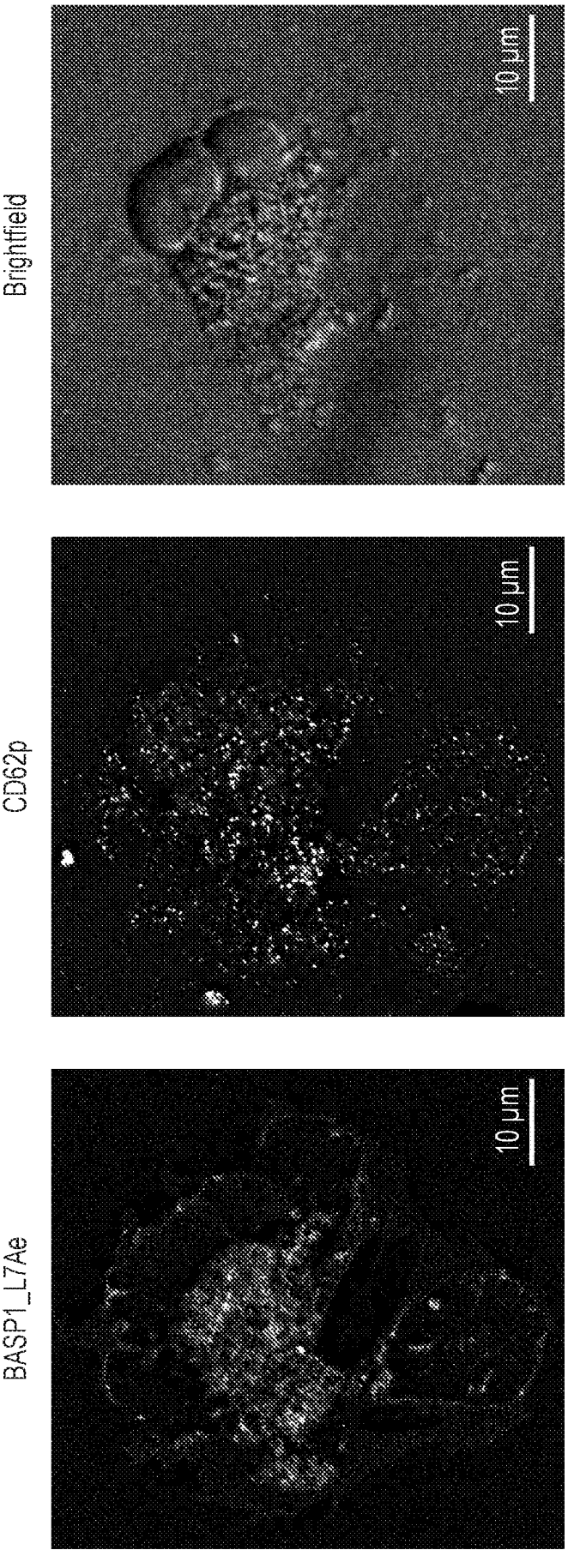


FIG. 23B

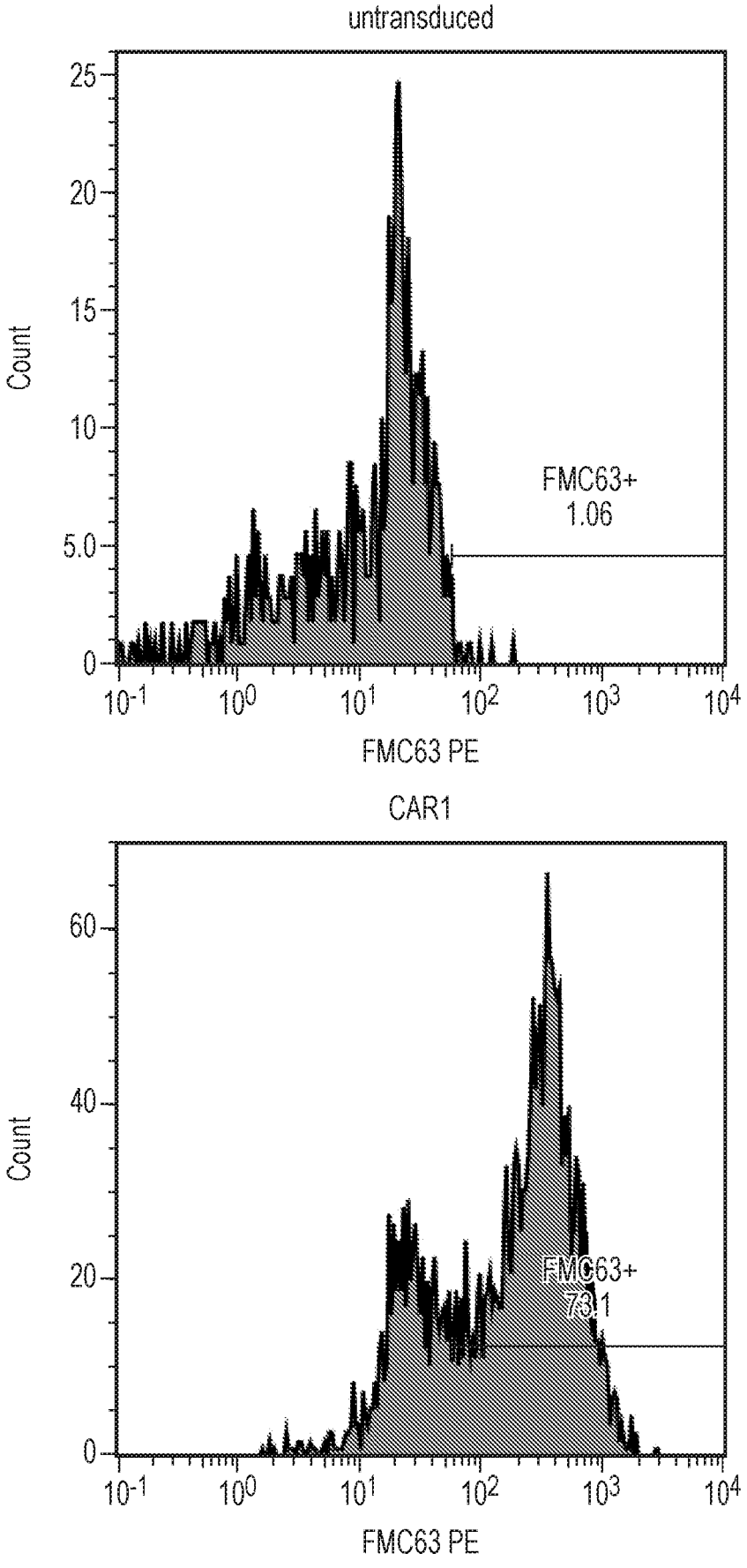

untransduced
Count
25
20
15
10
5.0
0
FMC63+
1.06
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
FMC63 PE
CAR1
Count
60
40
20
0
FMC63+
73.1
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
FMC63 PE

FIG. 24

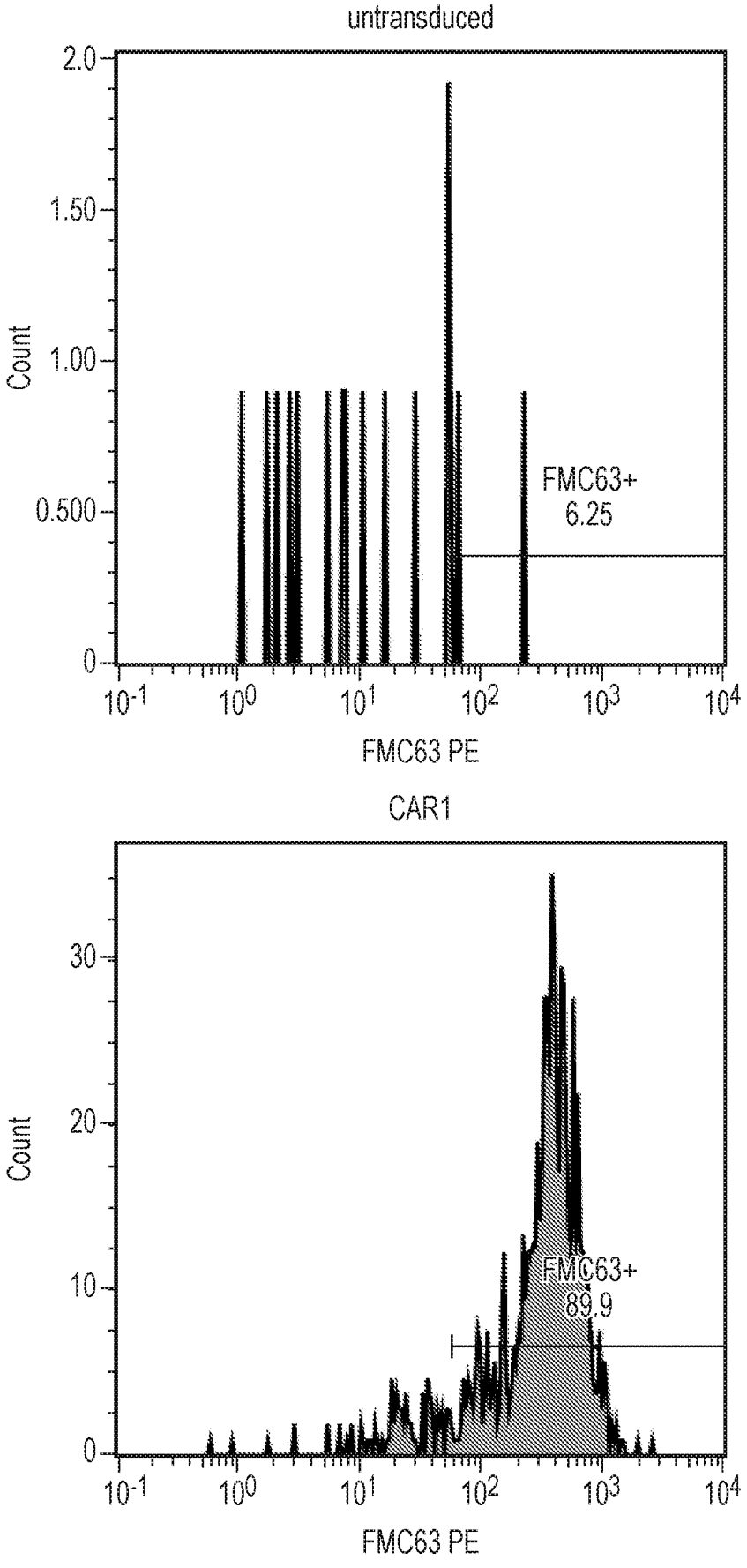

untransduced
2.0
1.50
1.00
0.500
0
Count
FMC63+
6.25
10-1
100
101
102
103
104
FMC63 PE
CAR1
30
20
10
0
Count
FMC63+
89.9
10-1
100
101
102
103
104
FMC63 PE

FIG. 25