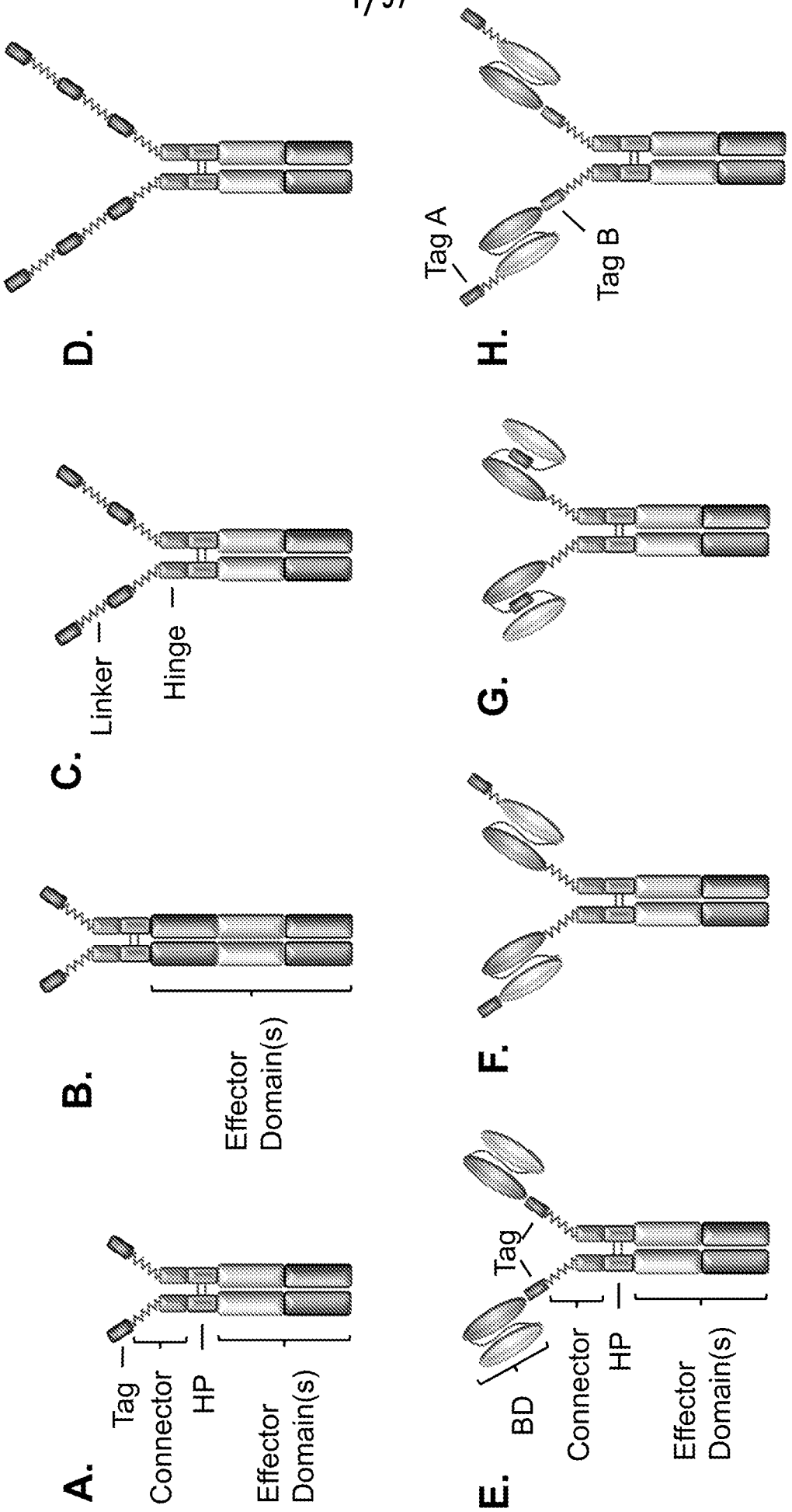

A.
Tag
Connector
HP
Effector Domain(s)
B.
Effector Domain(s)
C.
Linker
Hinge
D.
E.
BD
Tag
Connector
HP
Effector Domain(s)
F.
G.
H.
Tag A
Tag B

Fig. 1

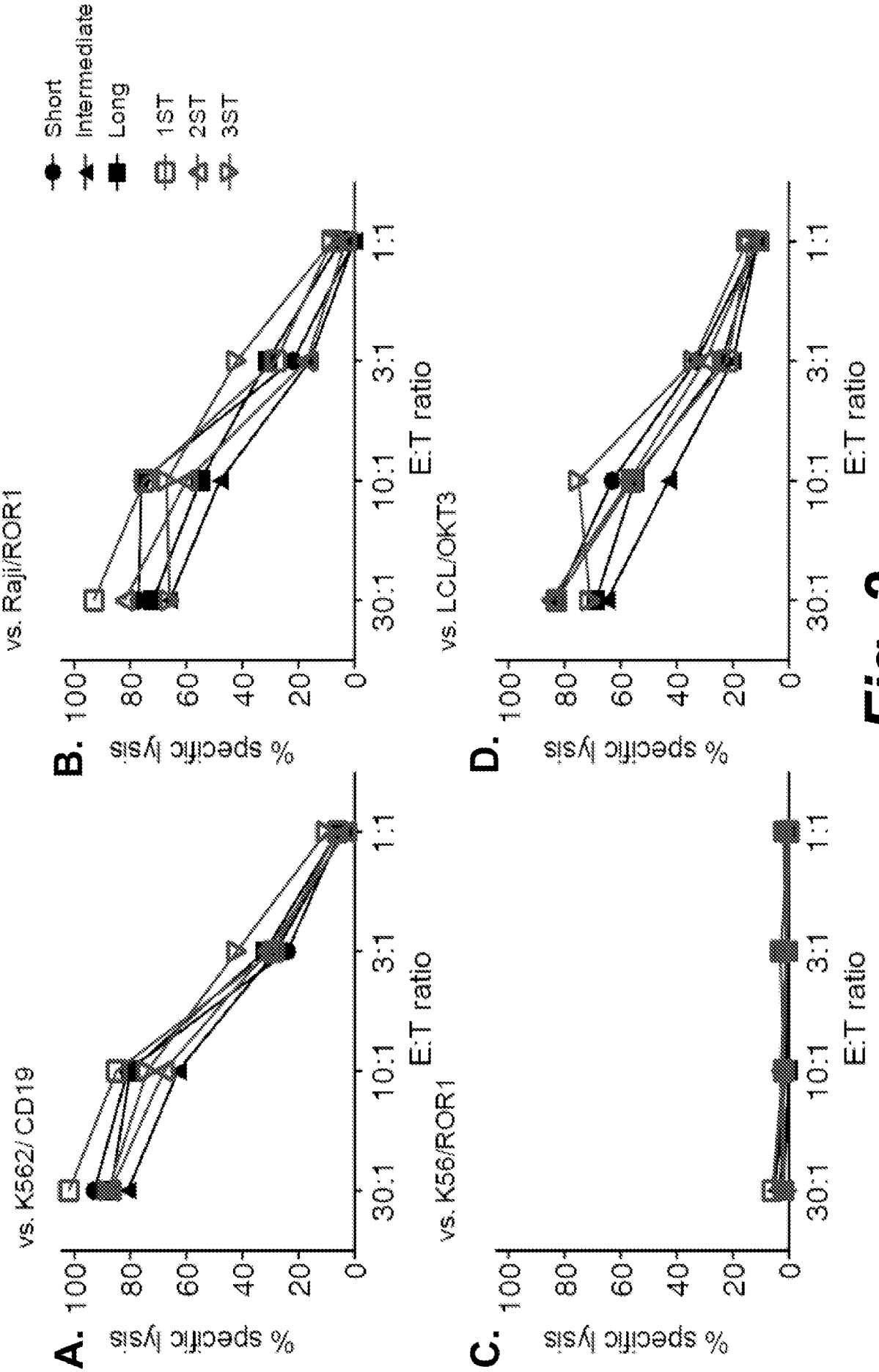
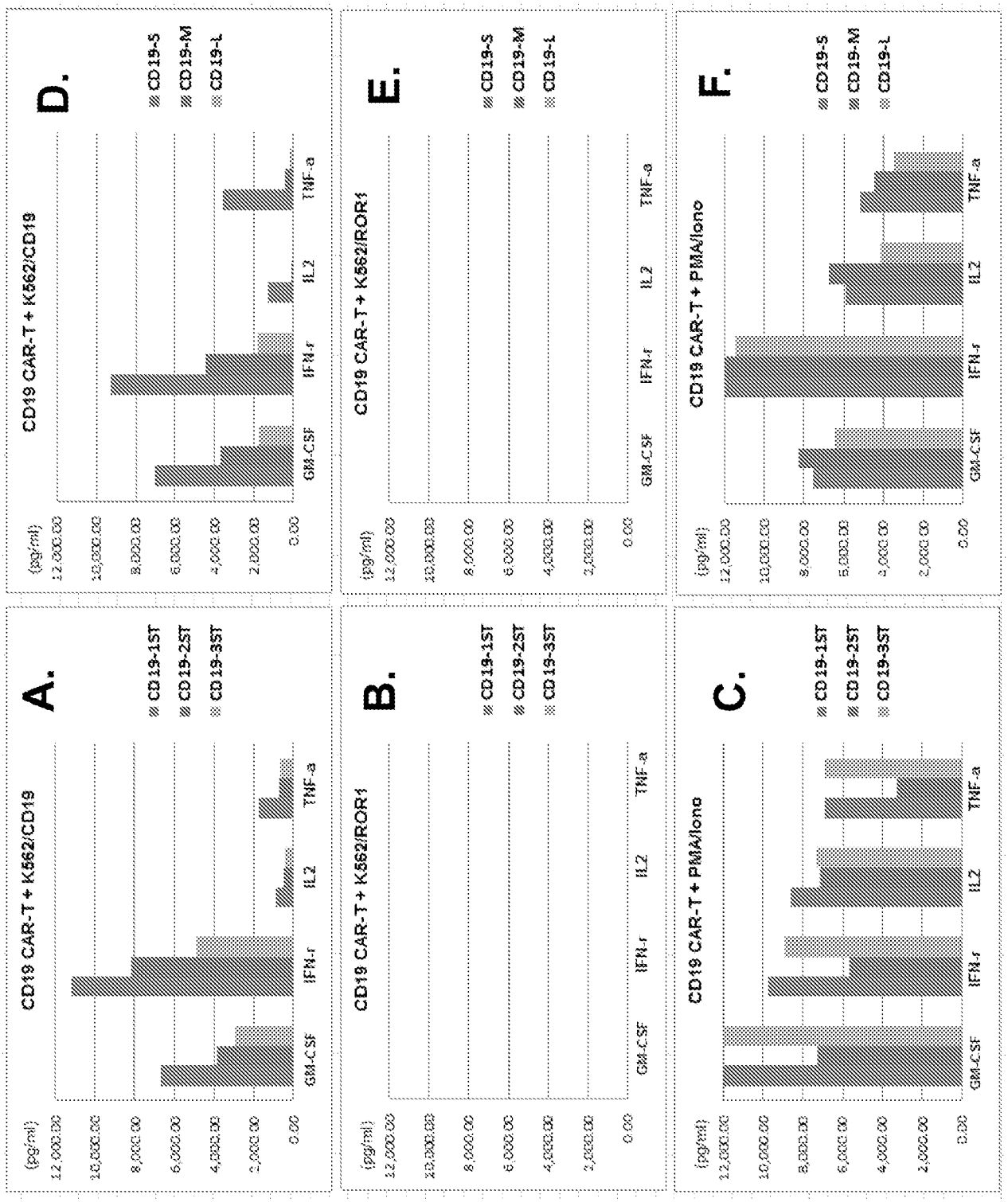

A.
vs. K562/CD19
% specific lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
E:T ratio
B.
vs. Raji/ROR1
Short
Intermediate
Long
1ST
2ST
3ST
C.
vs. K56/ROR1
D.
vs. LCL/OKT3

Fig. 2



24h Co-Culture
Cytokine Release

Fig. 3

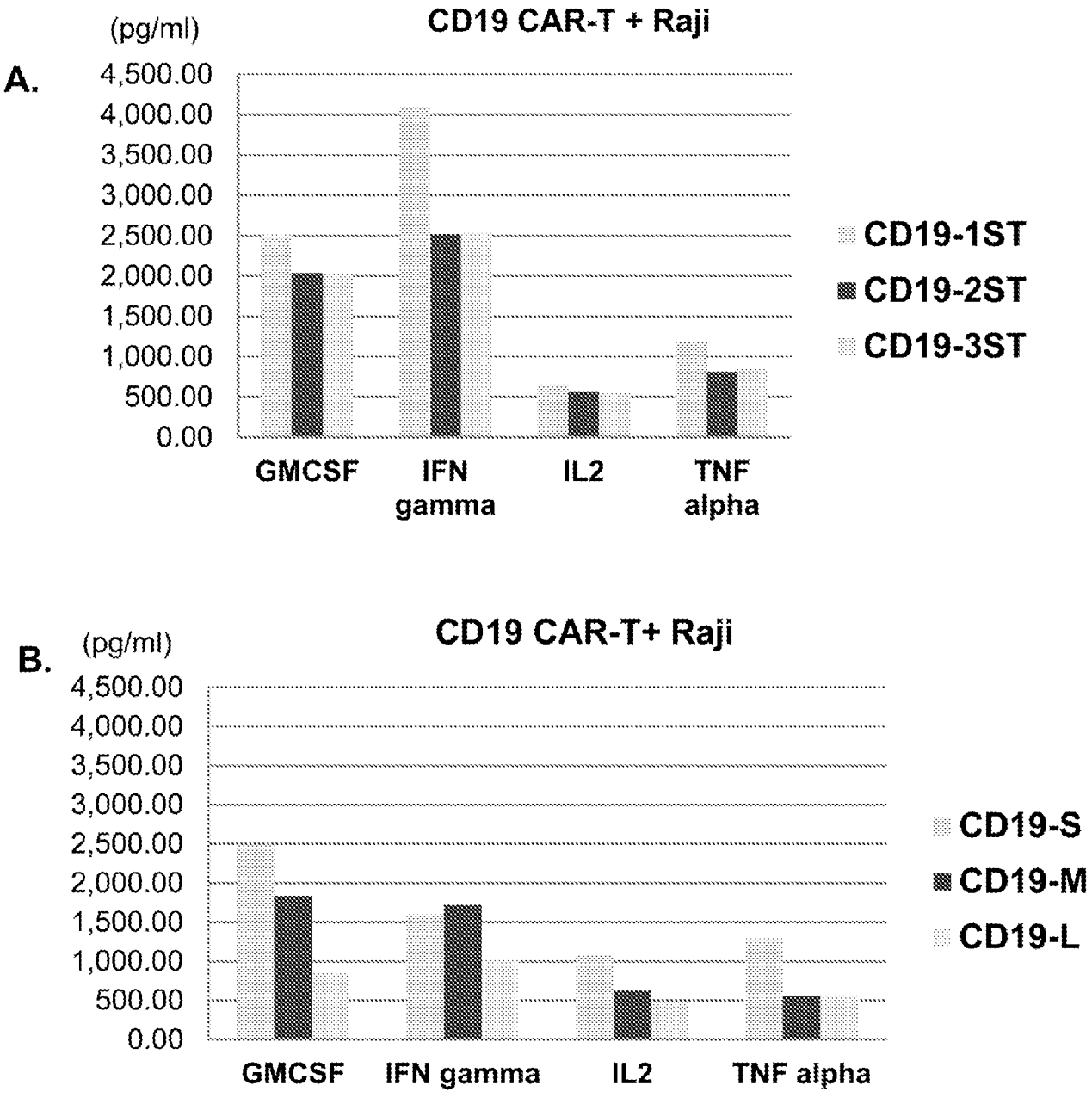

A.
(pg/ml)
CD19 CAR-T + Raji
4,500.00
4,000.00
3,500.00
3,000.00
2,500.00
2,000.00
1,500.00
1,000.00
500.00
0.00
GMCSF
IFN gamma
IL2
TNF alpha
CD19-1ST
CD19-2ST
CD19-3ST
B.
(pg/ml)
CD19 CAR-T+ Raji
4,500.00
4,000.00
3,500.00
3,000.00
2,500.00
2,000.00
1,500.00
1,000.00
500.00
0.00
GMCSF
IFN gamma
IL2
TNF alpha
CD19-S
CD19-M
CD19-L

Fig. 4

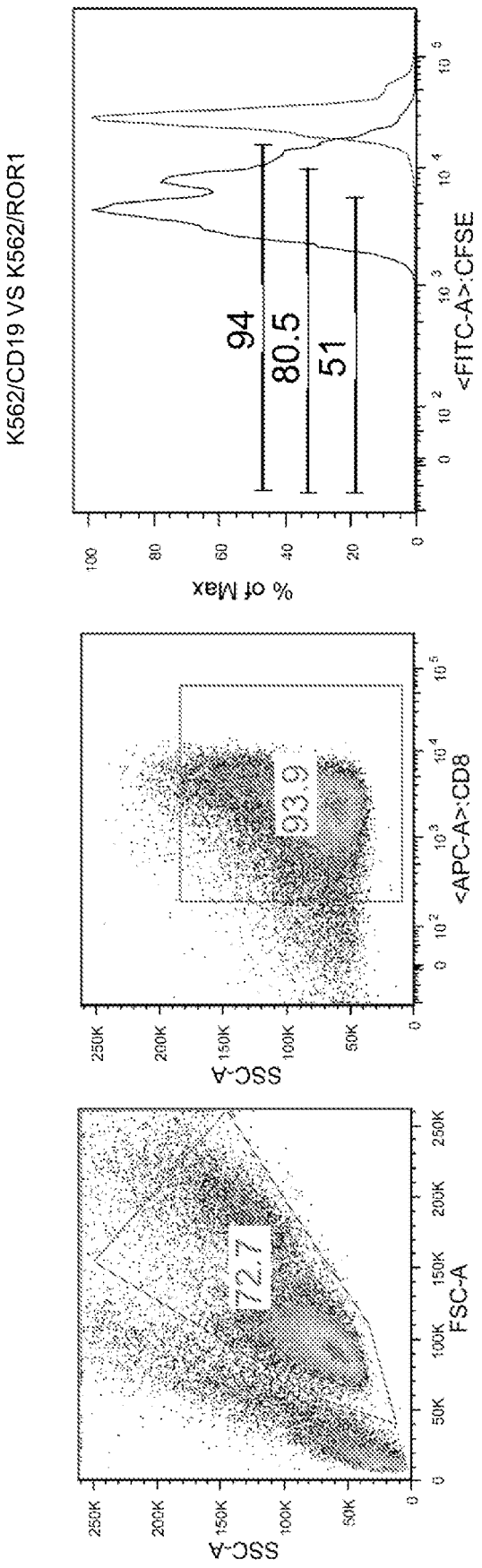

CD19-ChARM1
SSC-A
FSC-A
72.7
SSC-A
<APC-A>:CD8
63.0
K562/CD19 VS K562/ROR1
% of Max
<FITC-A>:CFSE
94
80.5
51

Fig. 5

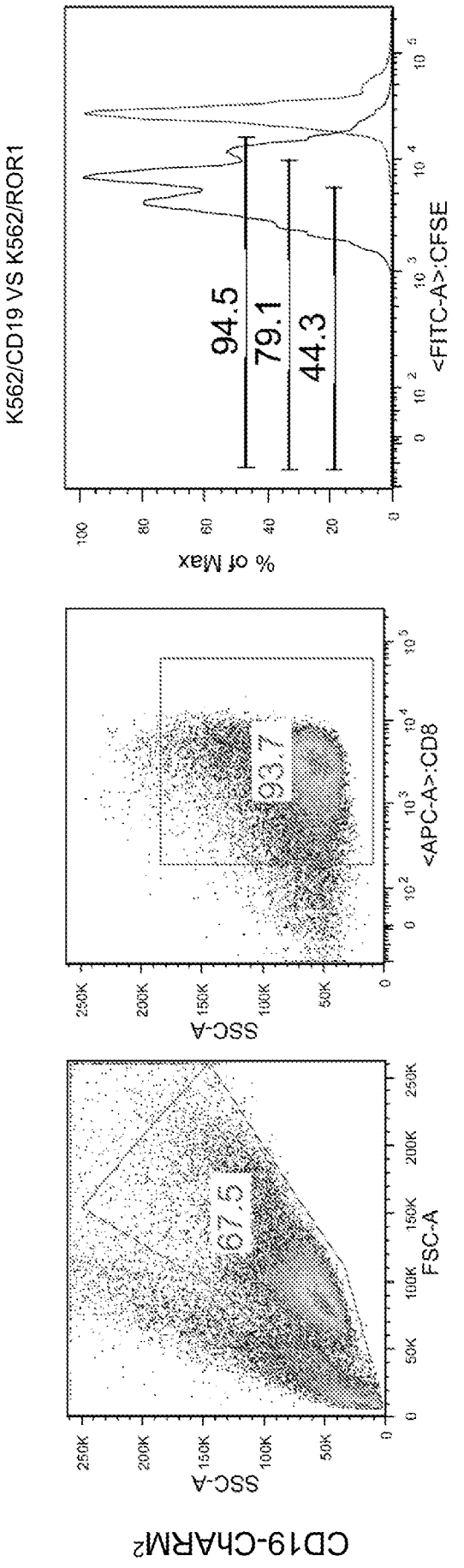


Fig. 5 (Continued)

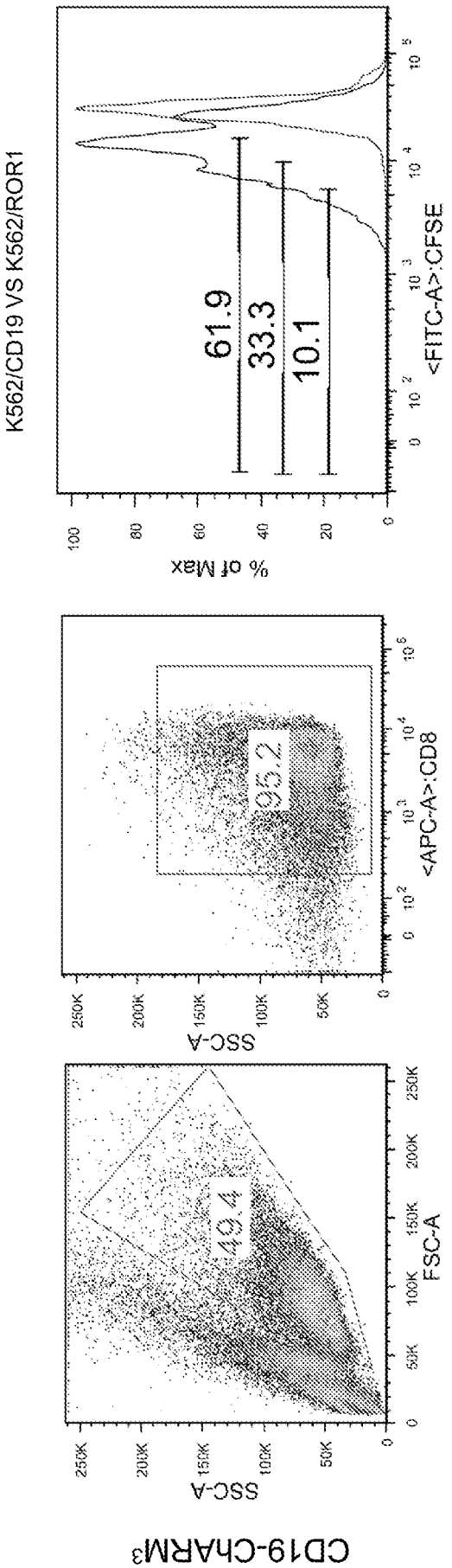


Fig. 5 (Continued)

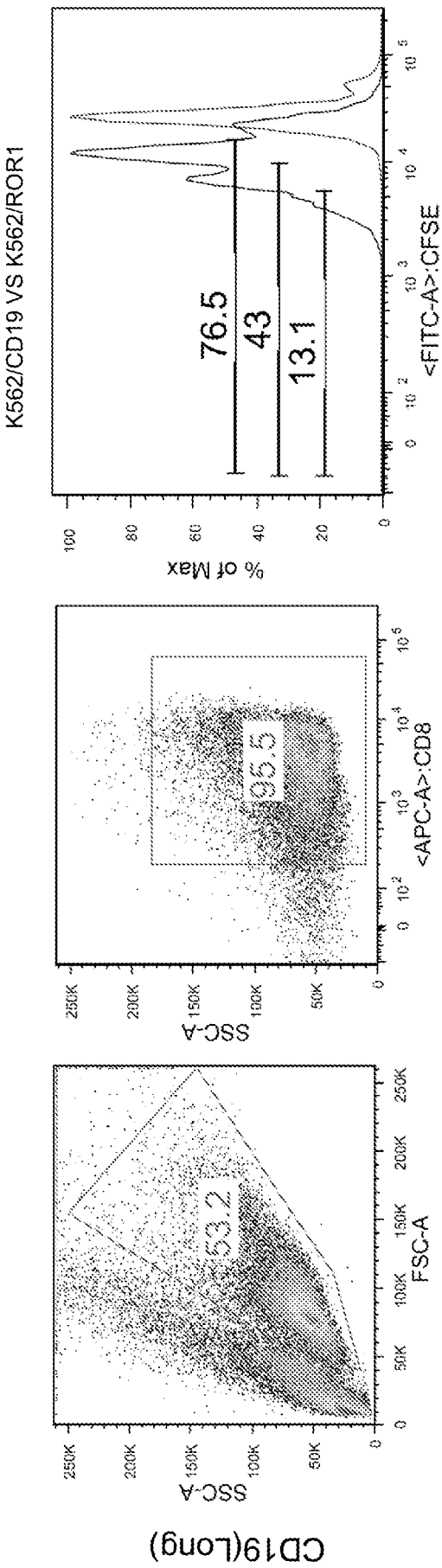


Fig. 5 (Continued)

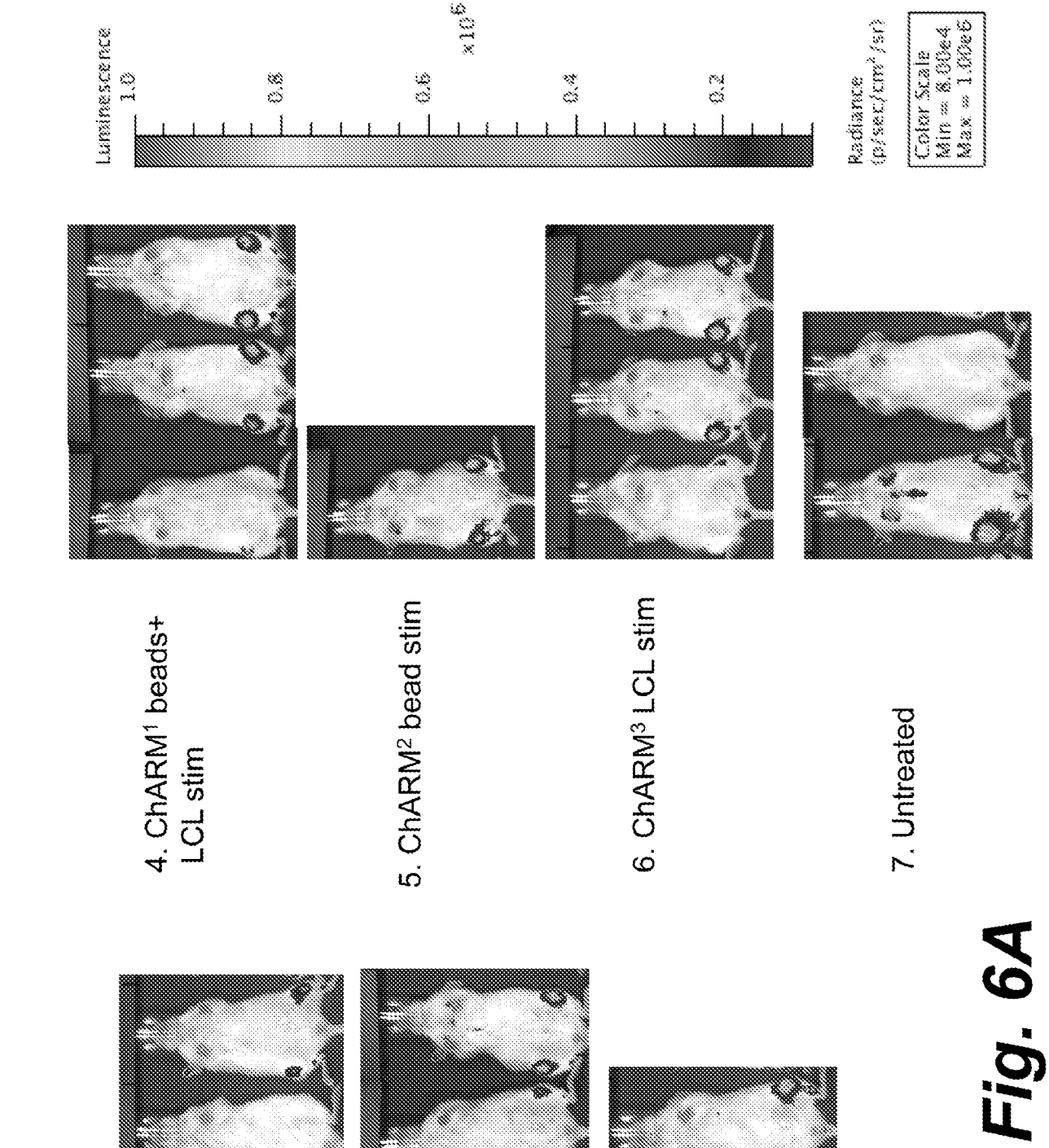

D6 after tumor
D0 after T cells
1. CAR(short)
2. CAR(Medium)
3. CAR(long)
4. ChARM[1] beads+ LCL stim
5. ChARM[2] bead stim
6. ChARM[3] LCL stim
7. Untreated
Luminescence
1.0
0.8
0.6
0.4
0.2
x10^6
Radiance (p/sec/cm²/sr)
Color Scale
Min = 8.00e4
Max = 1.00e6

Fig. 6A

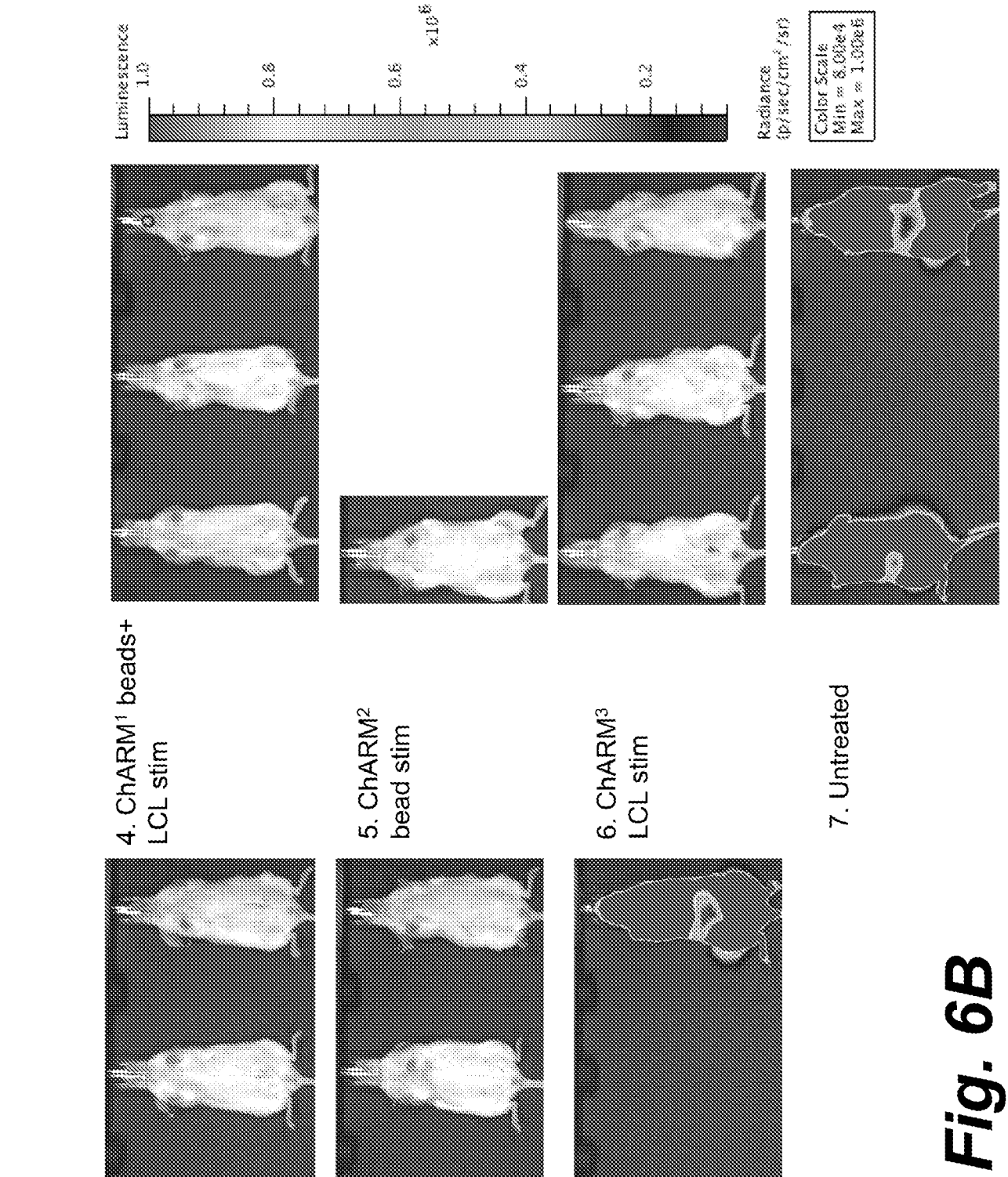

D14 after tumor
D7 after T cells
1. CAR(short)
2. CAR(medium)
3. CAR(long)
4. ChARM1 beads+ LCL stim
5. ChARM2 bead stim
6. ChARM3 LCL stim
7. Untreated
Luminescence
1.0
0.8
0.6
0.4
0.2
x10^6
Radiance (p/sec/cm²/sr)
Color Scale
Min = 8.00e4
Max = 1.00e6

Fig. 6B

SUBSTITUTE SHEET (RULE 26)

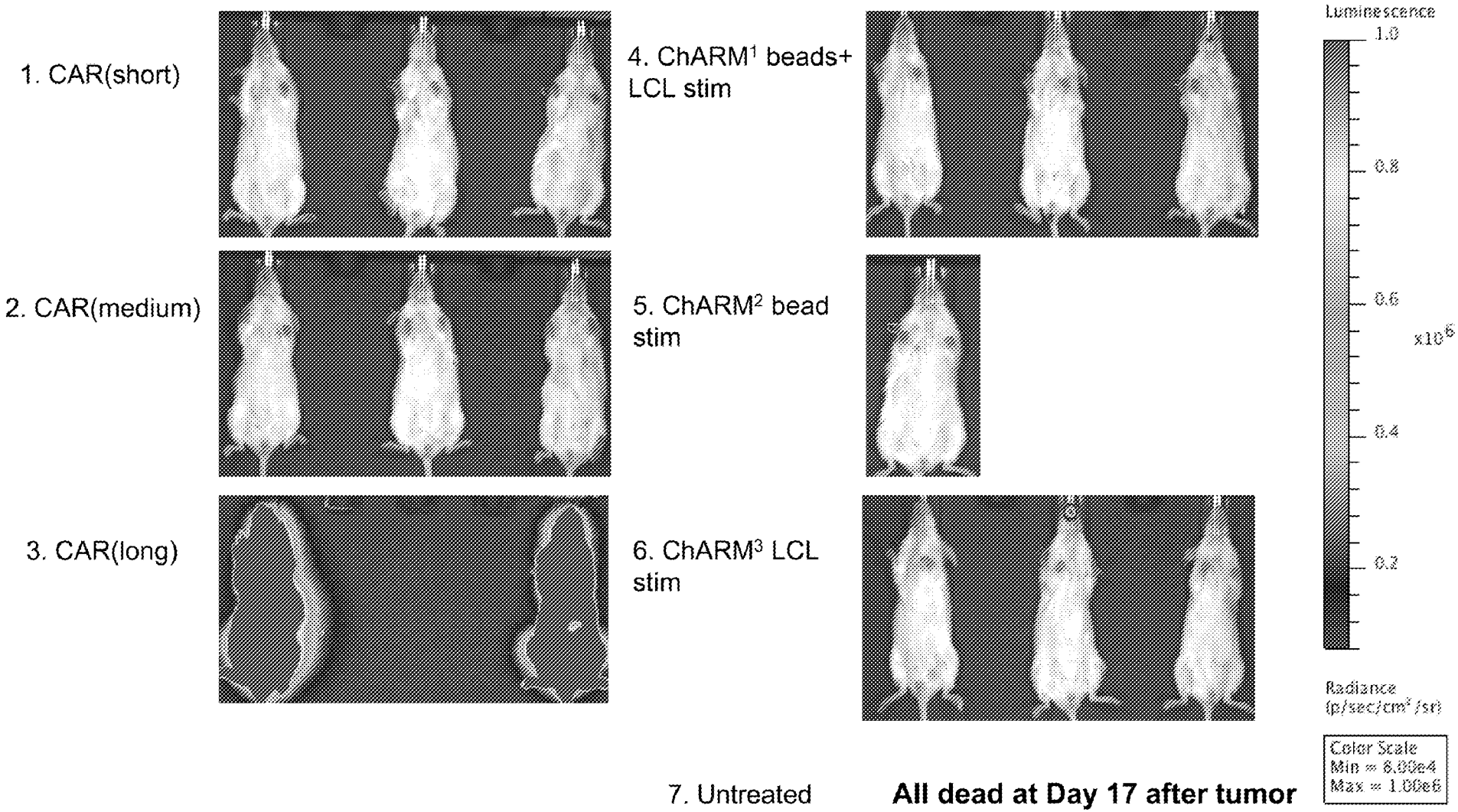


Fig. 6C

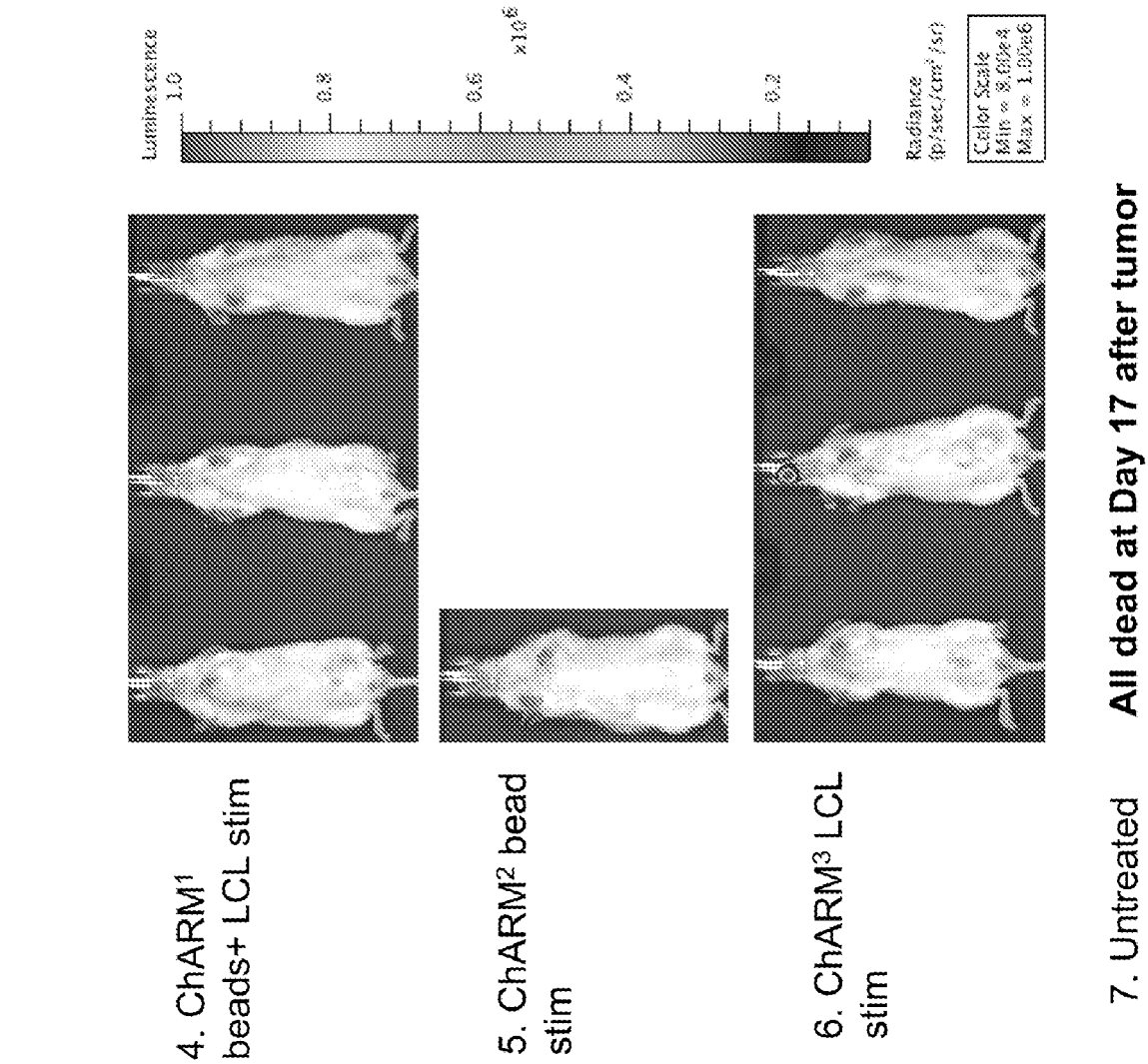

Day 25 after tumor
Day 18 after T cells
1. CAR(short)
2. CAR(medium)
3. CAR(long) All dead at Day 18 after tumor
4. ChARM1 beads+ LCL stim
5. ChARM2 bead stim
6. ChARM3 LCL stim
7. Untreated All dead at Day 17 after tumor
Luminescence
1.0
0.8
0.6
0.4
0.2
×10^6
Radiance (p/sec/cm²/sr)
Color Scale
Min = 8.08e4
Max = 1.00e6

Fig. 6D

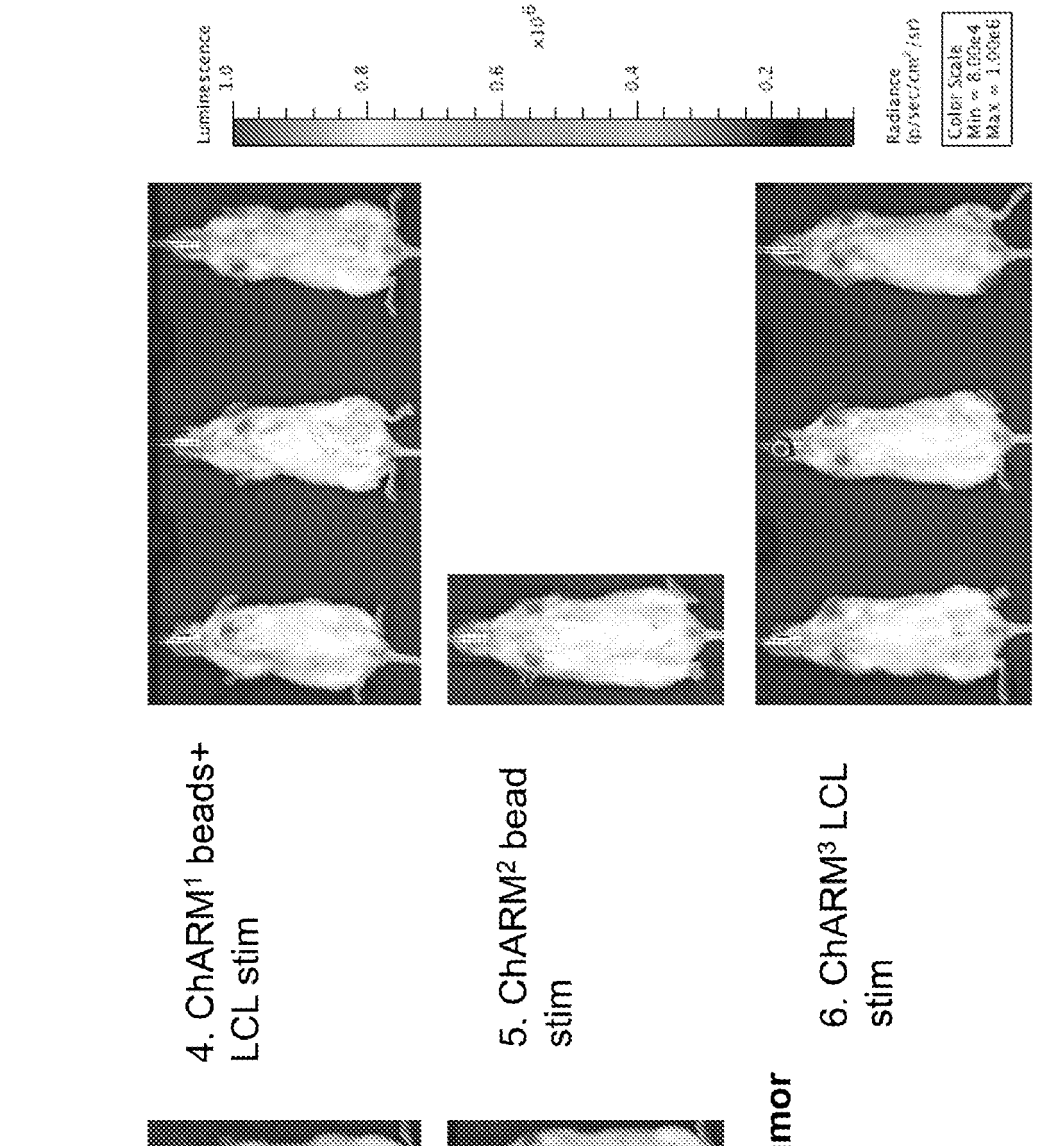

Day 33 after tumor
Day 26 after T cells
1. CAR(short)
2. CAR(medium
3. CAR(long)
All dead at Day 18 after tumor
4. ChARM1 beads+ LCL stim
5. ChARM2 bead stim
6. ChARM3 LCL stim
7. Untreated
All dead at Day 17 after tumor
Luminescence
Radiance (p/sec/cm²/sr)
Color Scale
Min = 8.89e+4
Max = 1.06e6

Fig. 6E

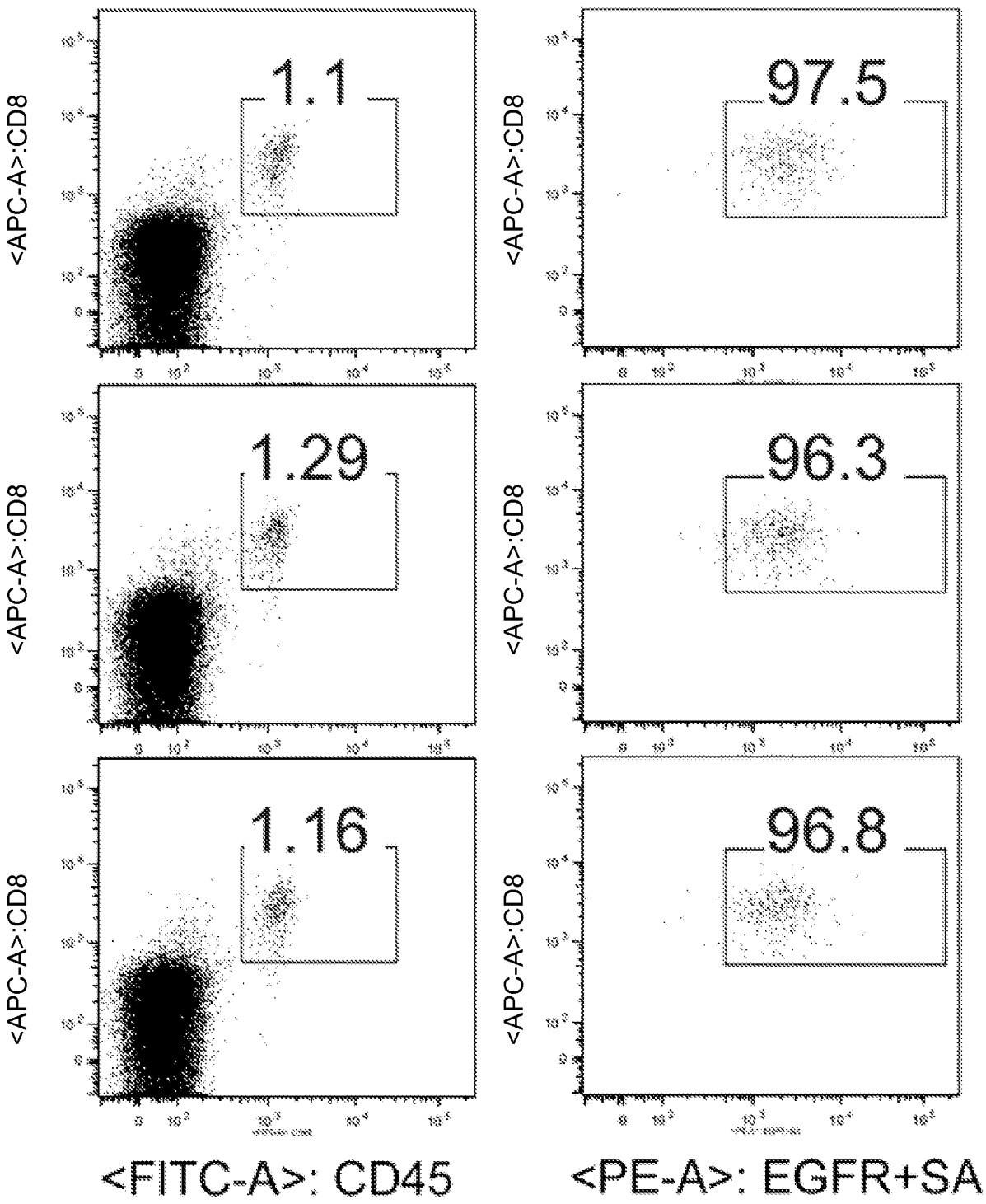

d6 Short
1.1
97.5
1.29
96.3
1.16
96.8
<APC-A>:CD8
<APC-A>:CD8
<APC-A>:CD8
<APC-A>:CD8
<APC-A>:CD8
<APC-A>:CD8
<FITC-A>: CD45
<PE-A>: EGFR+SA

Fig. 7

d6 Intermediate

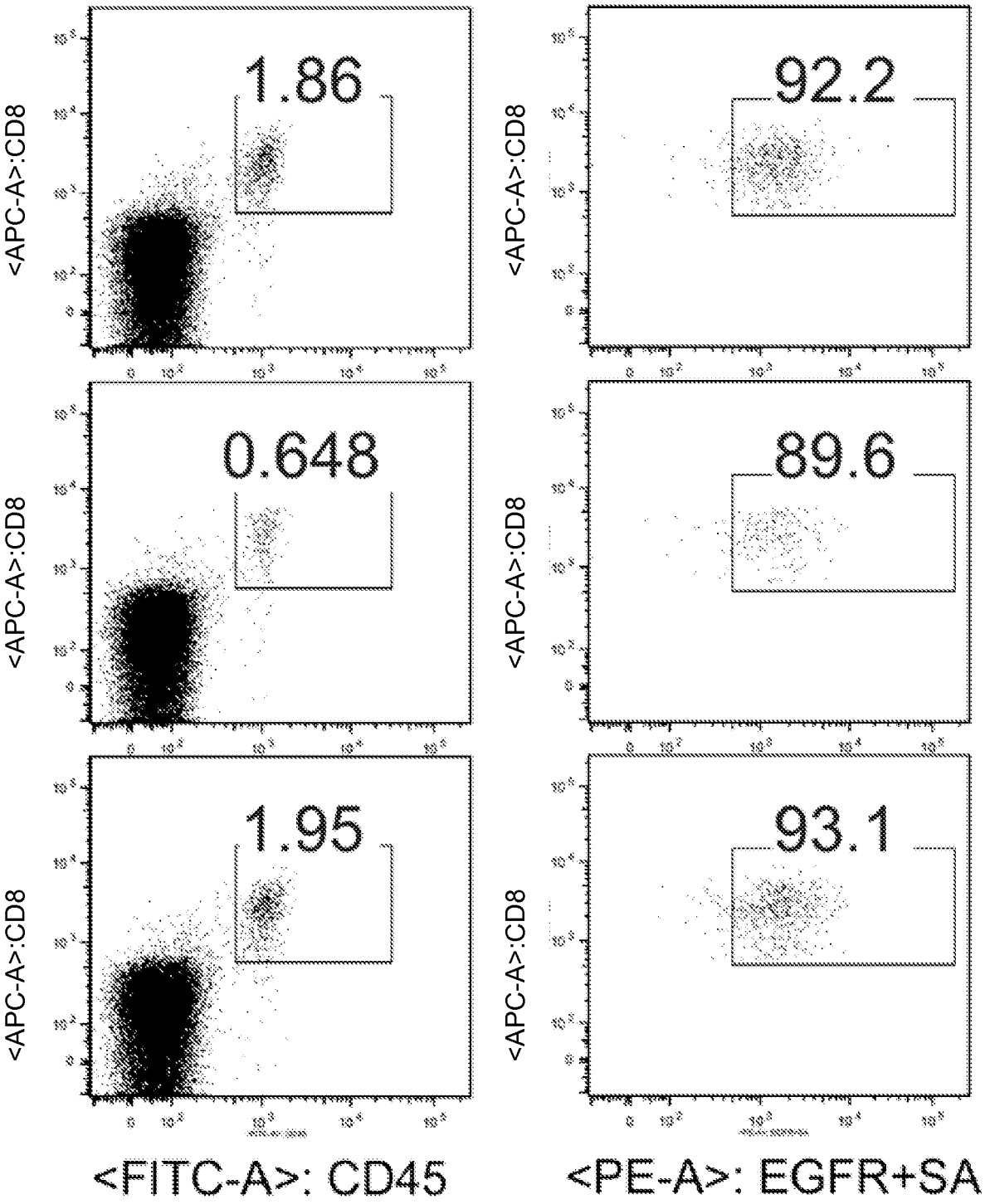


Fig. 7 *(Continued)*

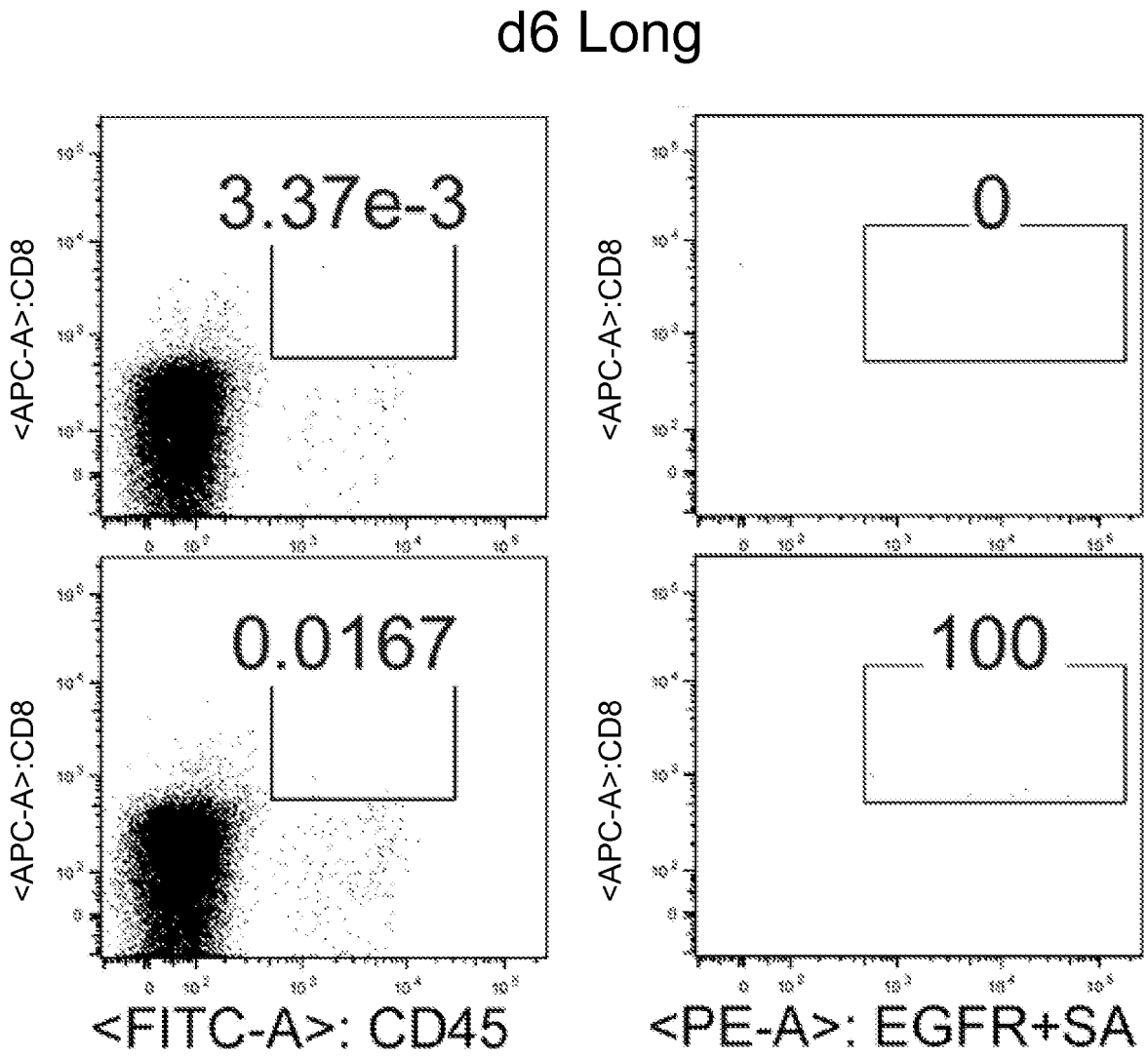


Fig. 7 *(Continued)*

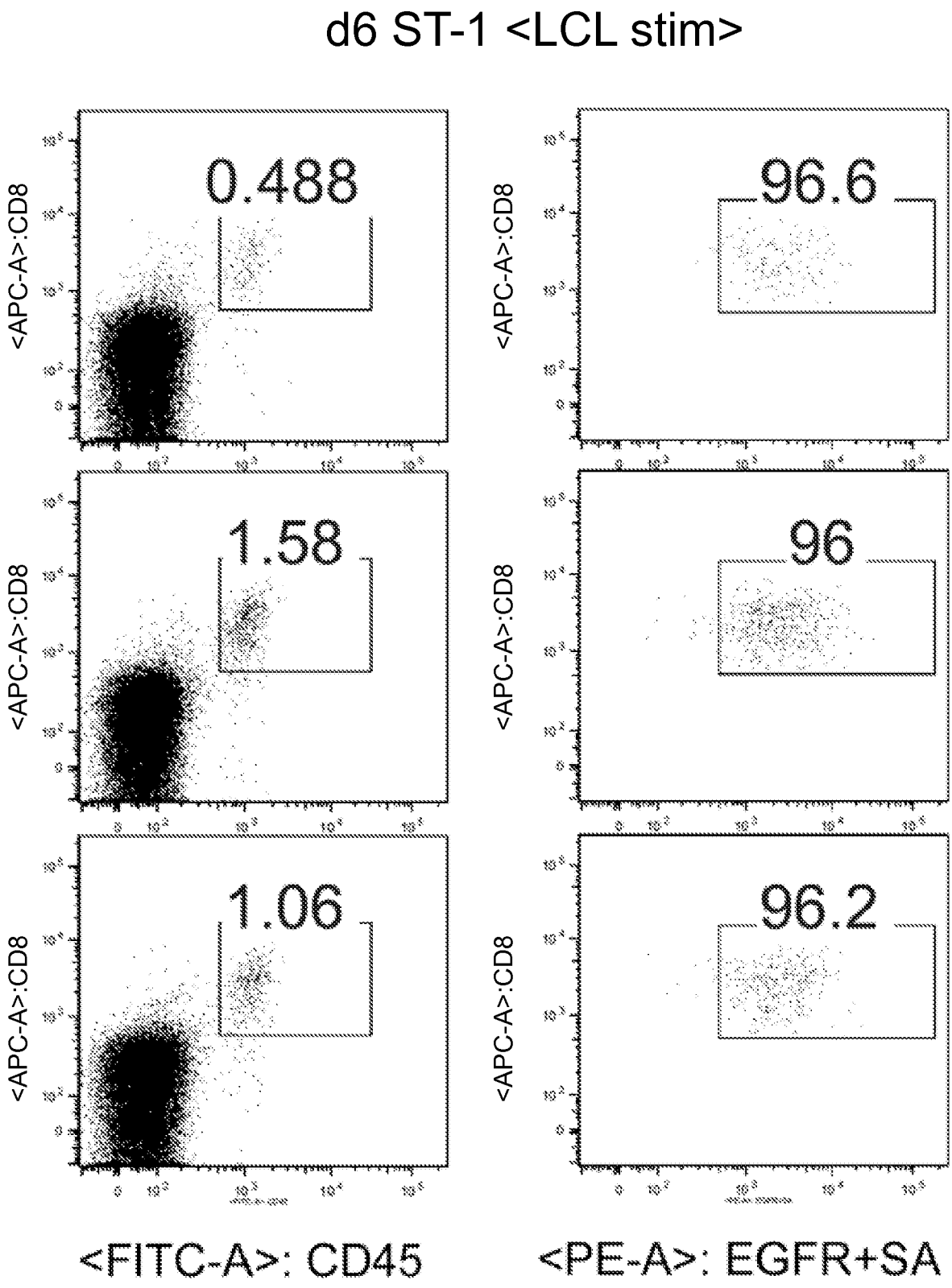


Fig. 7 *(Continued)*

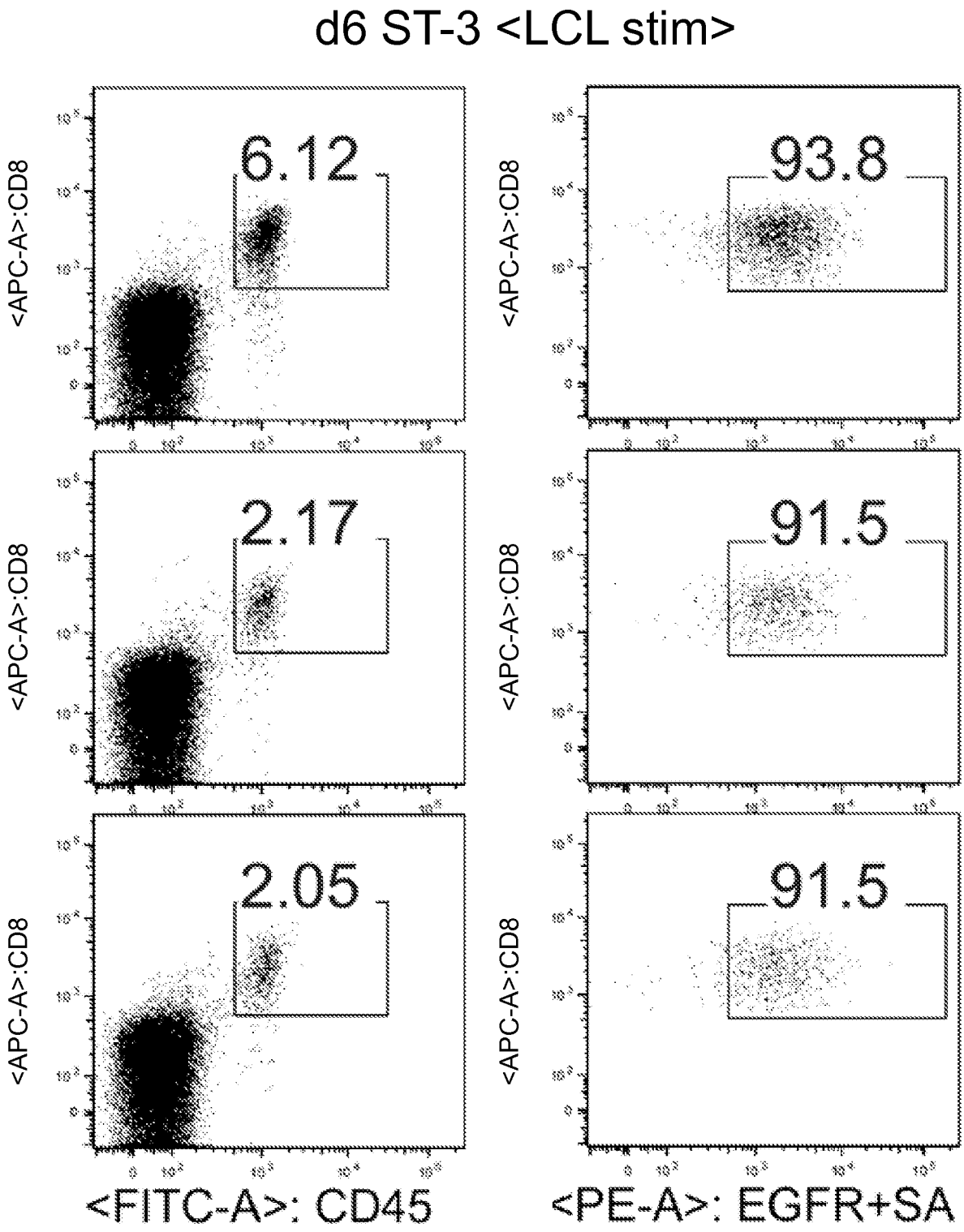


Fig. 7 *(Continued)*

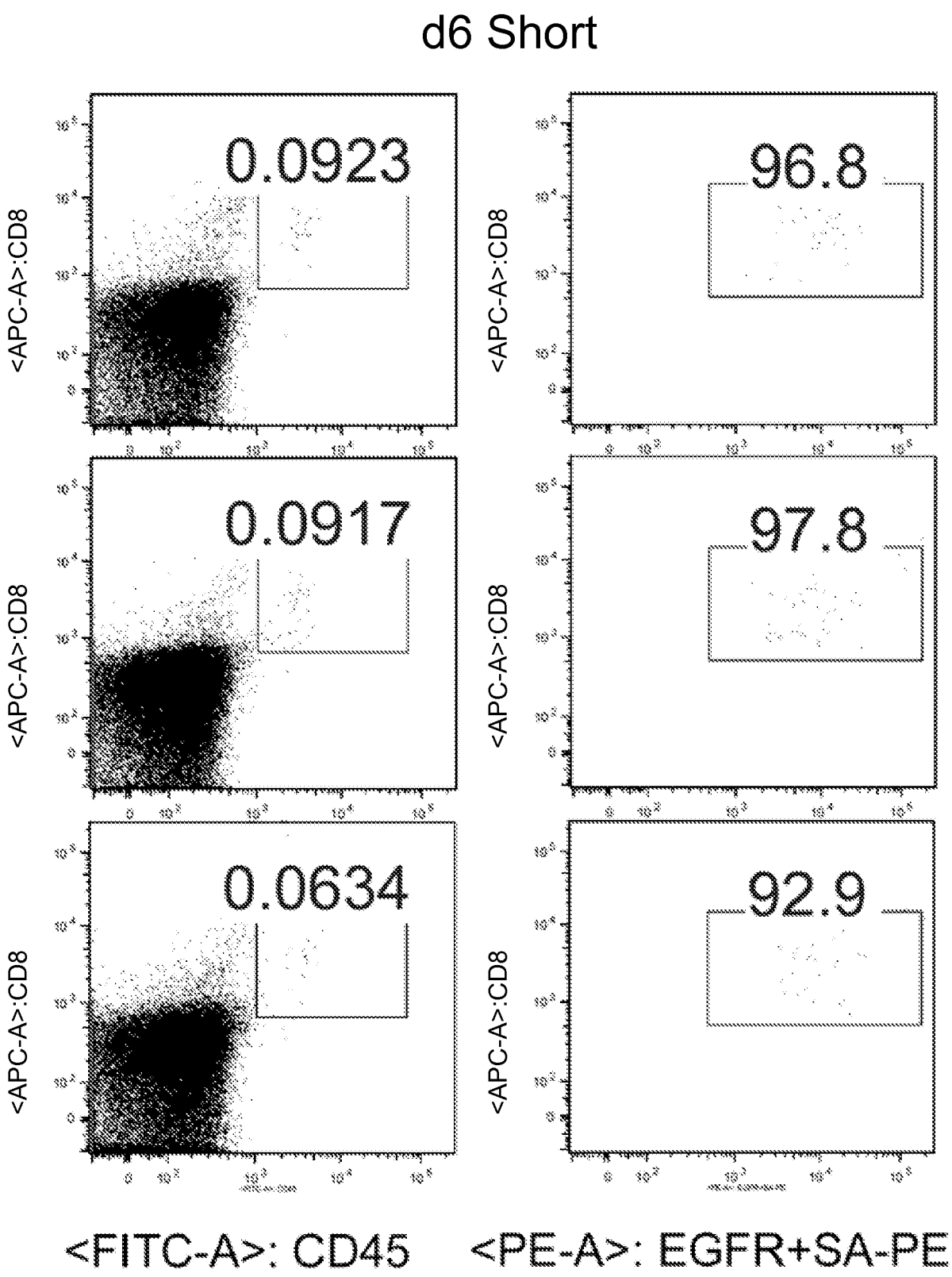


Fig. 7 *(Continued)*

d13 Intermediate

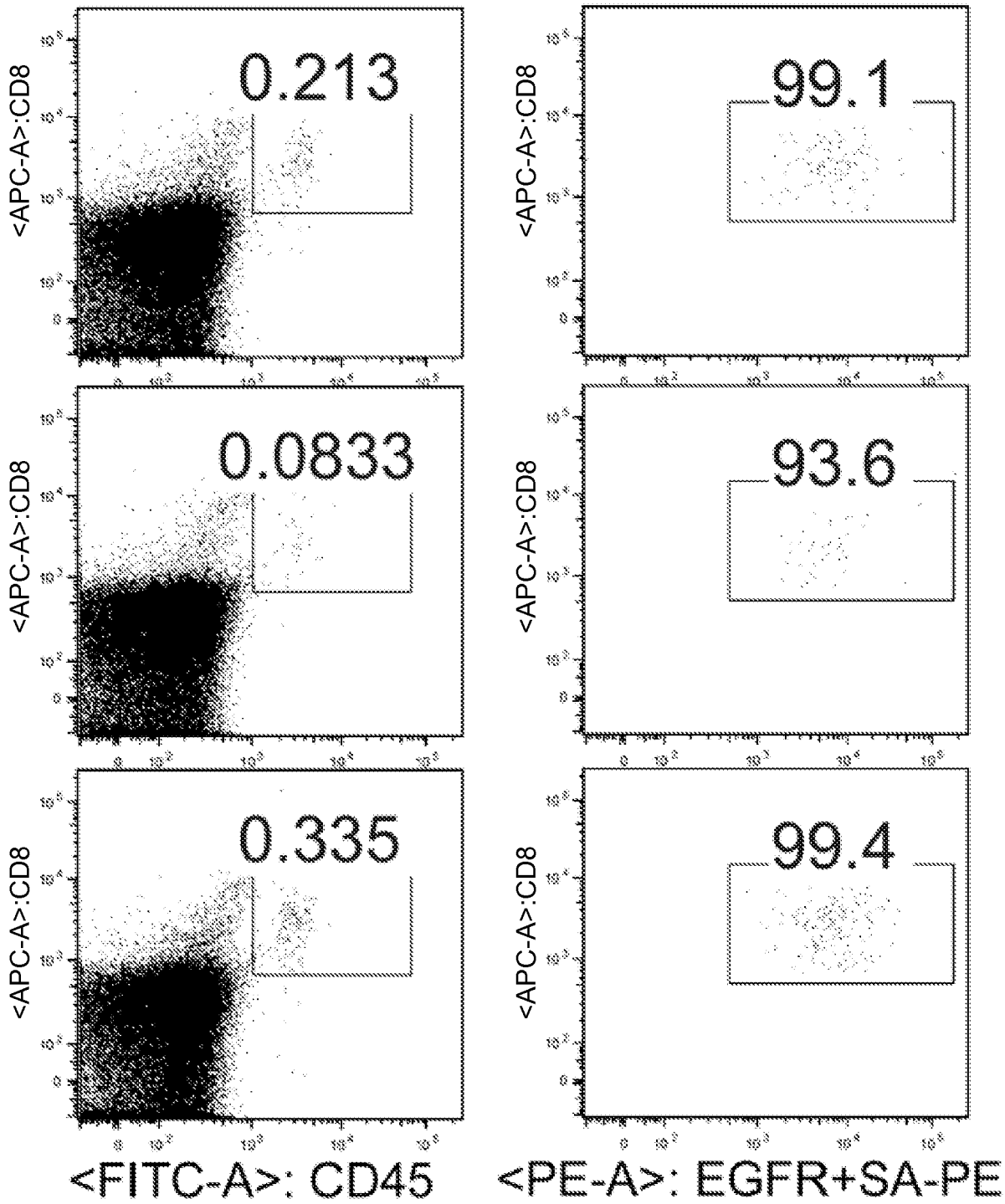


Fig. 7 *(Continued)*

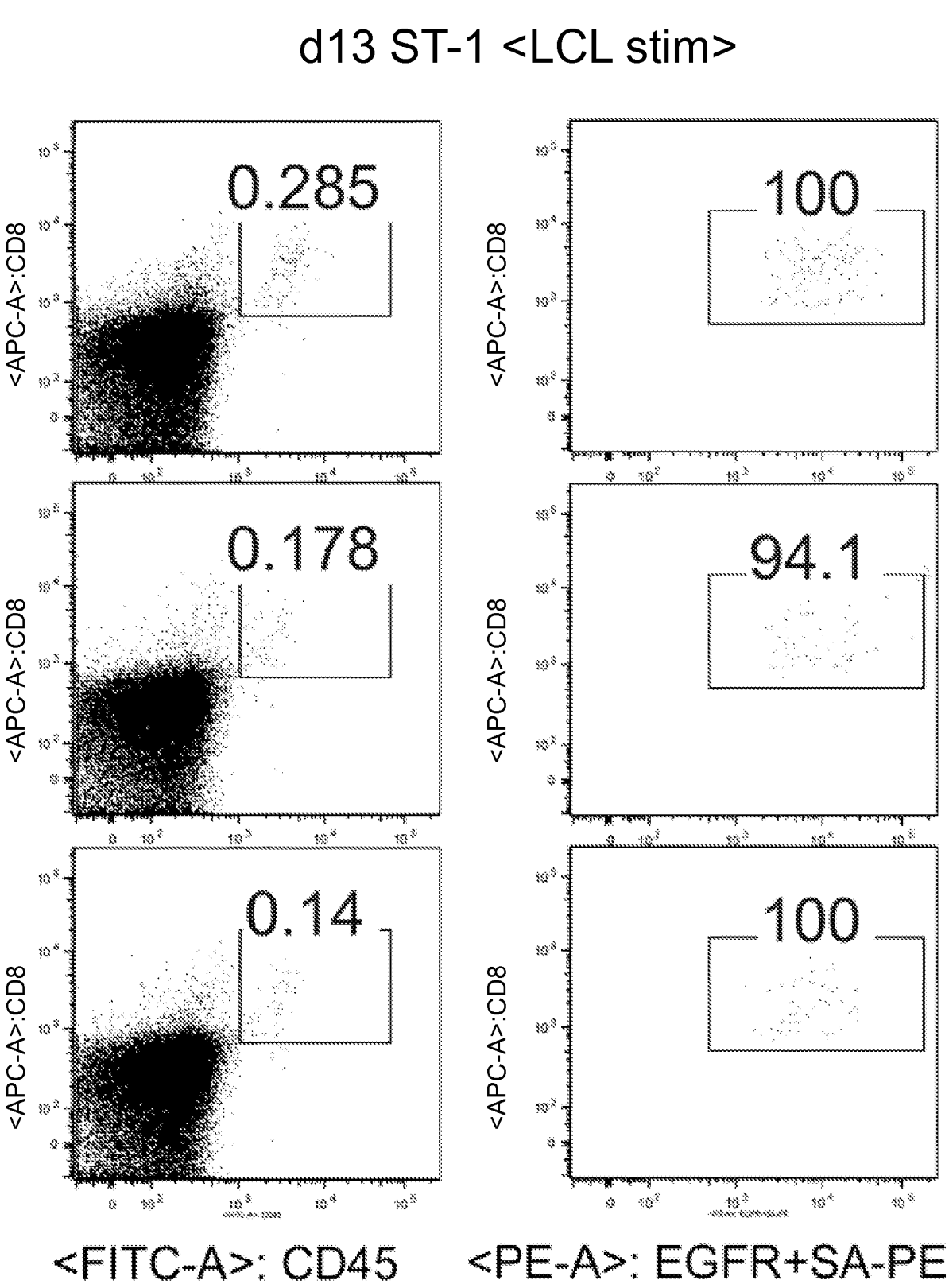


Fig. 7 *(Continued)*

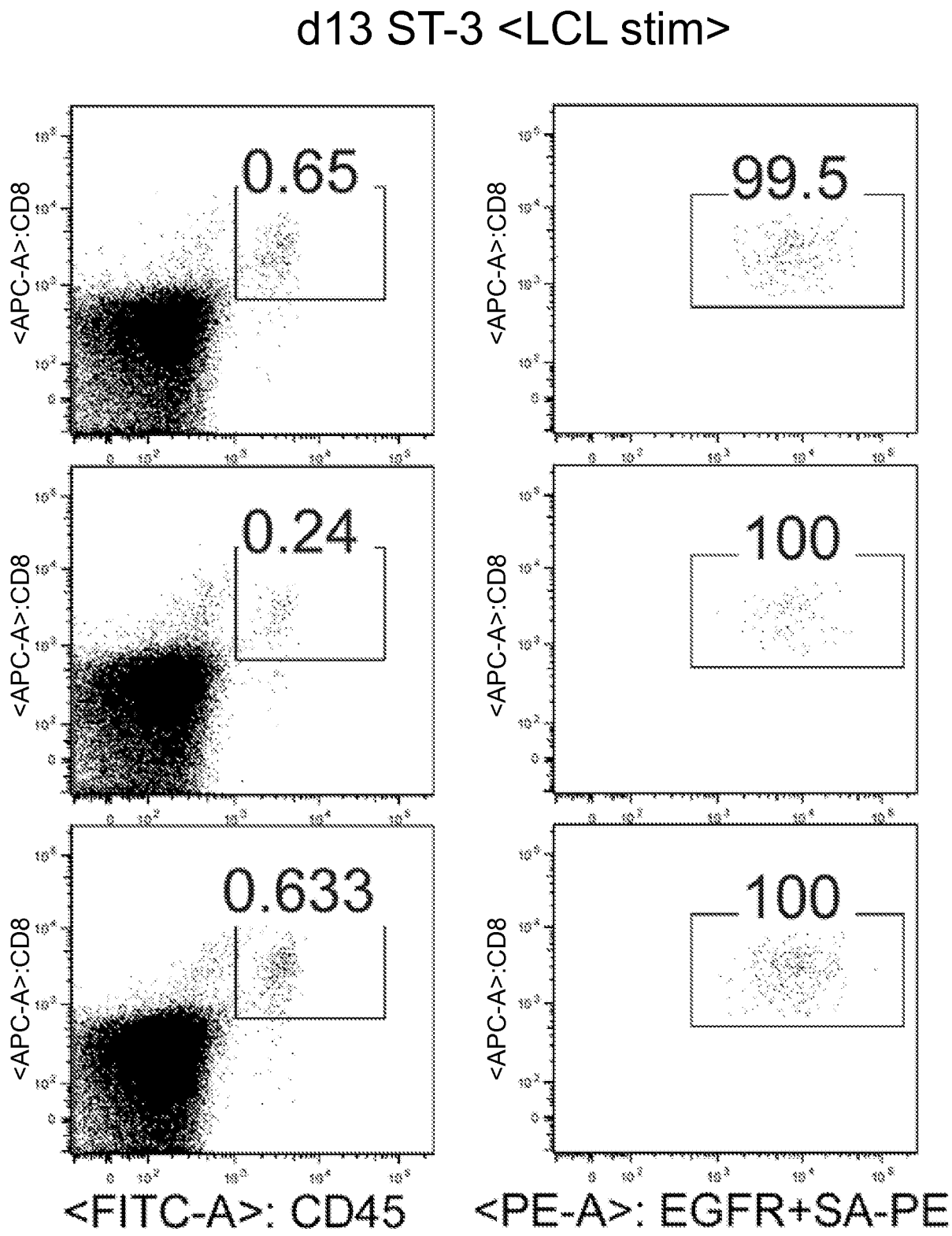


Fig. 7 *(Continued)*

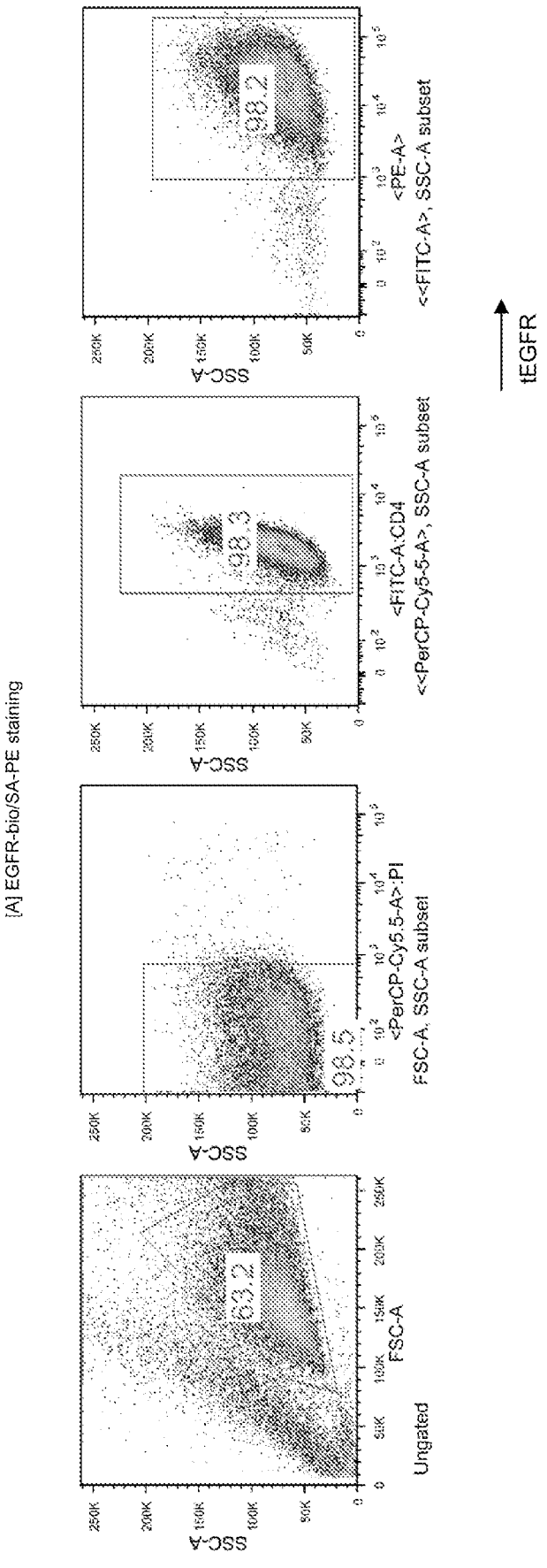

CD19-ChARM1
[A] EGFR-bio/SA-PE staining
Ungated
FSC-A
SSC-A
63.2
FSC-A, SSC-A subset
<PerCP-Cy5.5-A>:PI
98.5
<<PerCP-Cy5-5-A>, SSC-A subset
<FITC-A:CD4
98.3
<<FITC-A>, SSC-A subset
<PE-A>
98.2
tEGFR

Fig. 8A

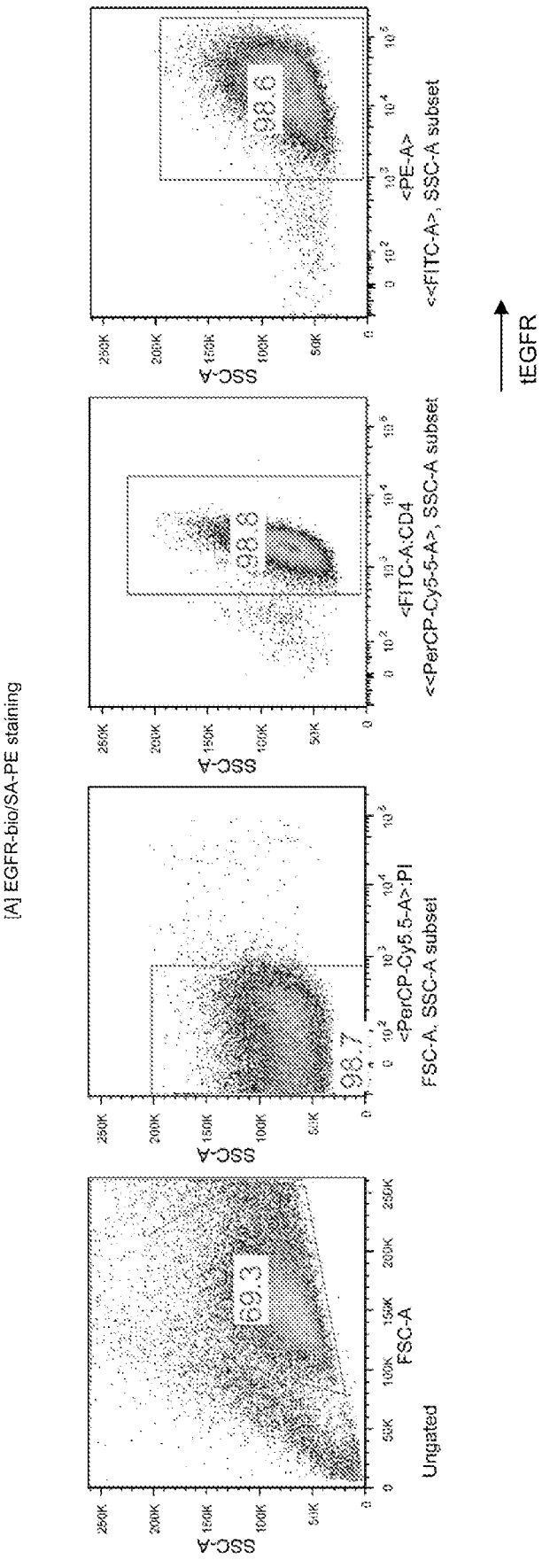


Fig. 8A (Continued)

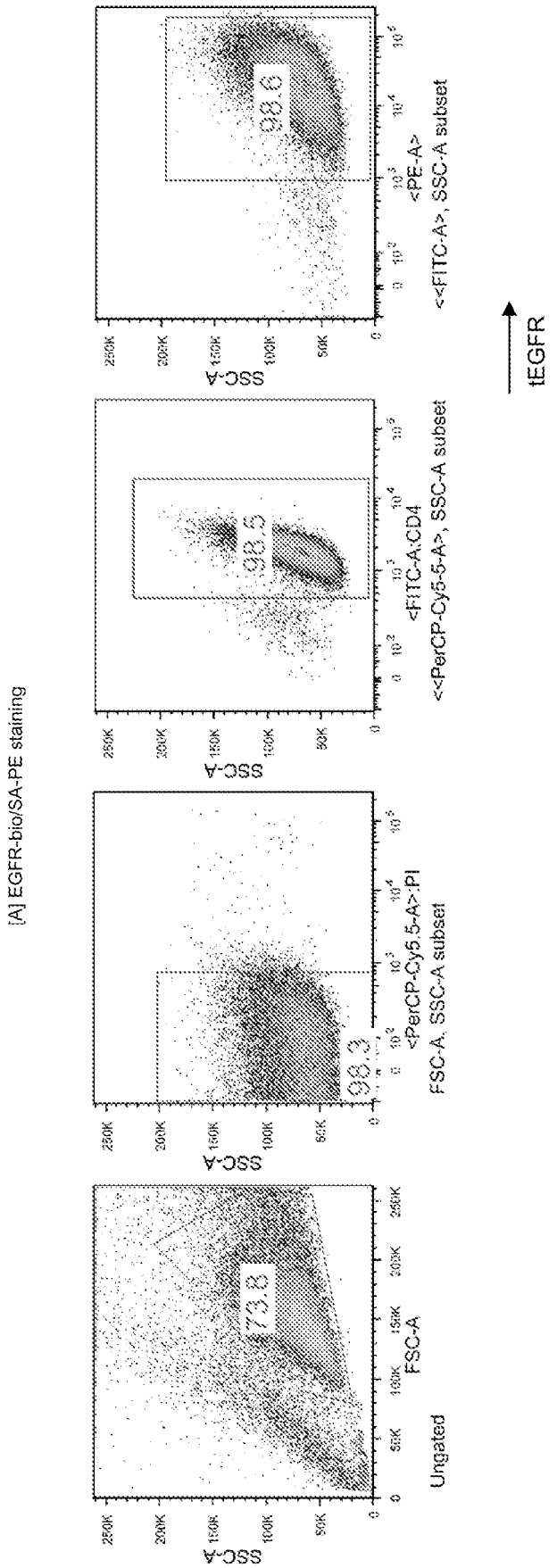


Fig. 8A (Continued)

CD19-ChARM3

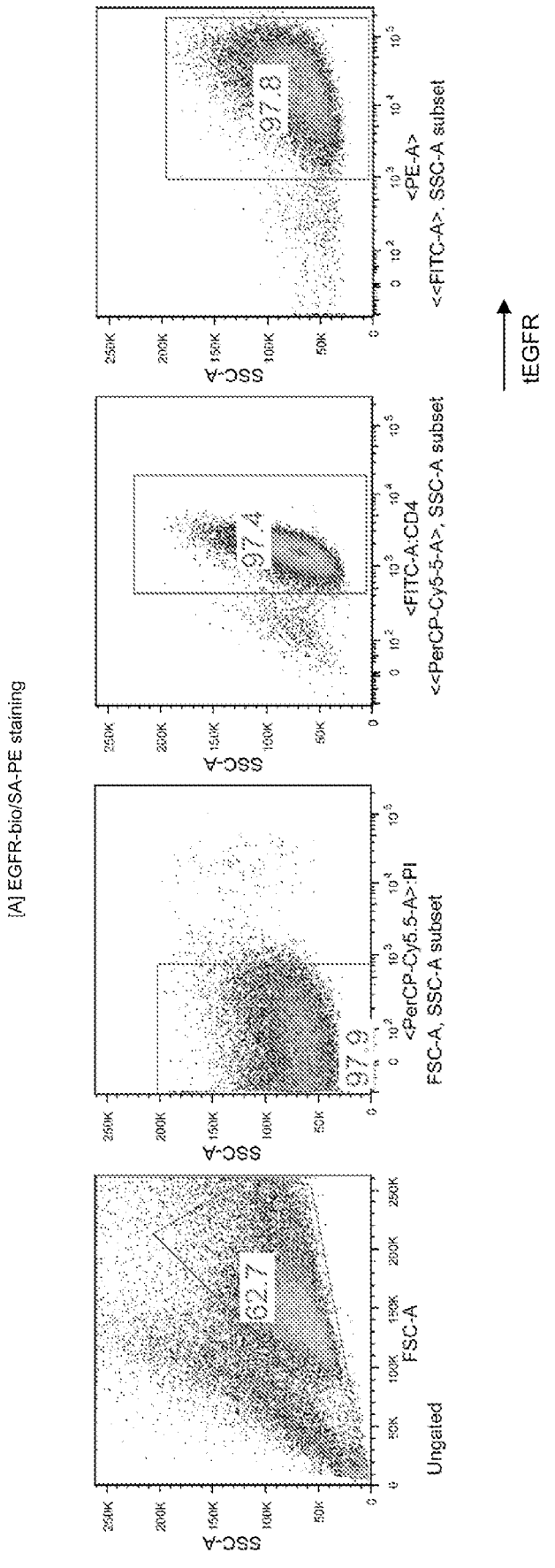


Fig. 8A (Continued)

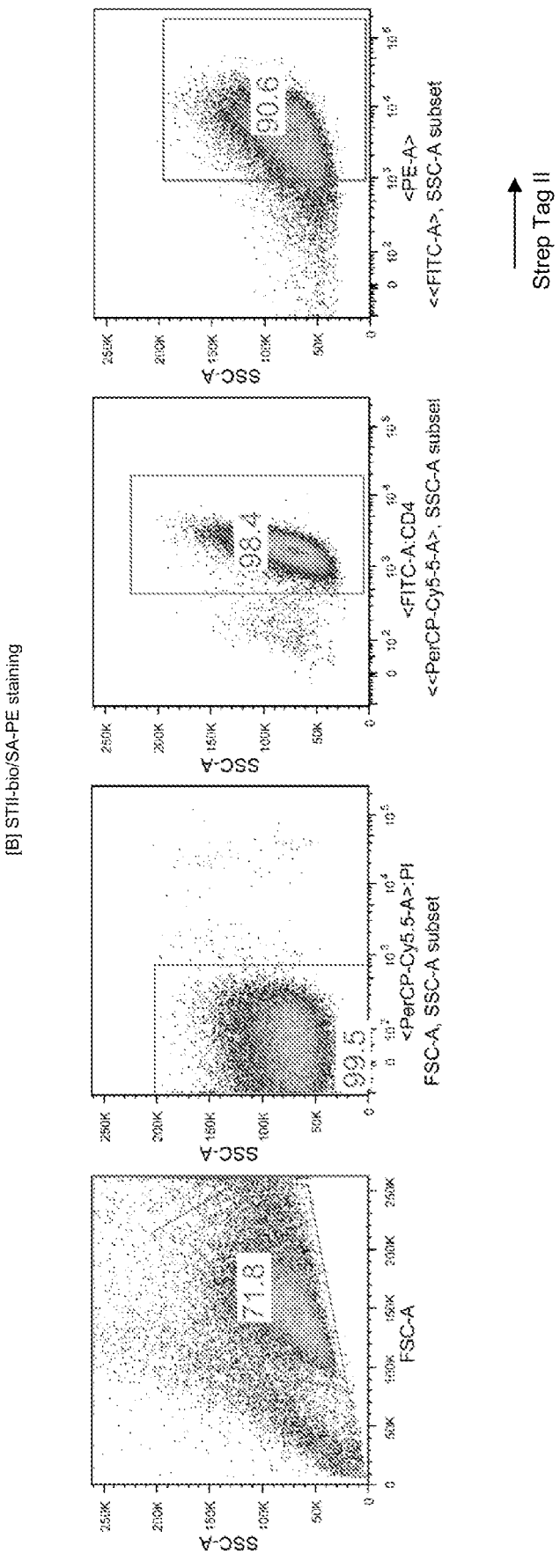

[B] STII-bio/SA-PE staining
CD19-ChARM1
SSC-A
FSC-A
71.8
FSC-A, SSC-A subset
<PerCP-Cy5-5-A>:PI
99.5
<FITC-A:CD4
<<PerCP-Cy5-5-A>, SSC-A subset
98.4
<PE-A>
<<FITC-A>, SSC-A subset
90.6
Strep Tag II

Fig. 8B

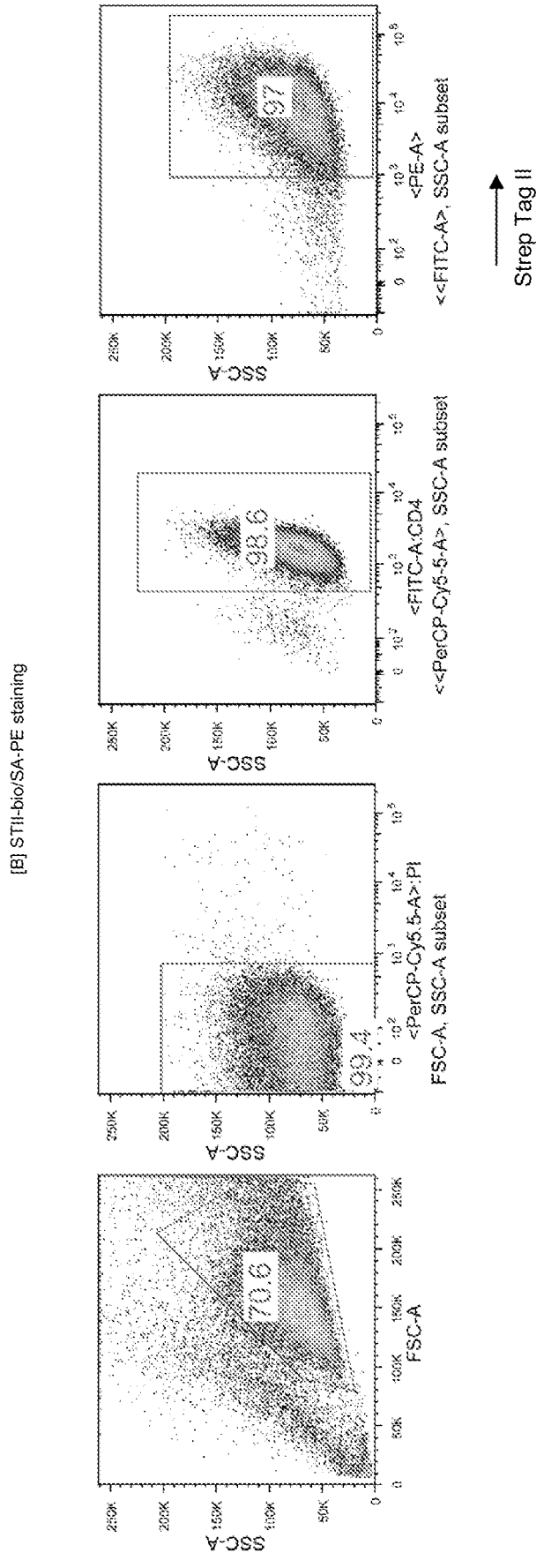


Fig. 8B (Continued)

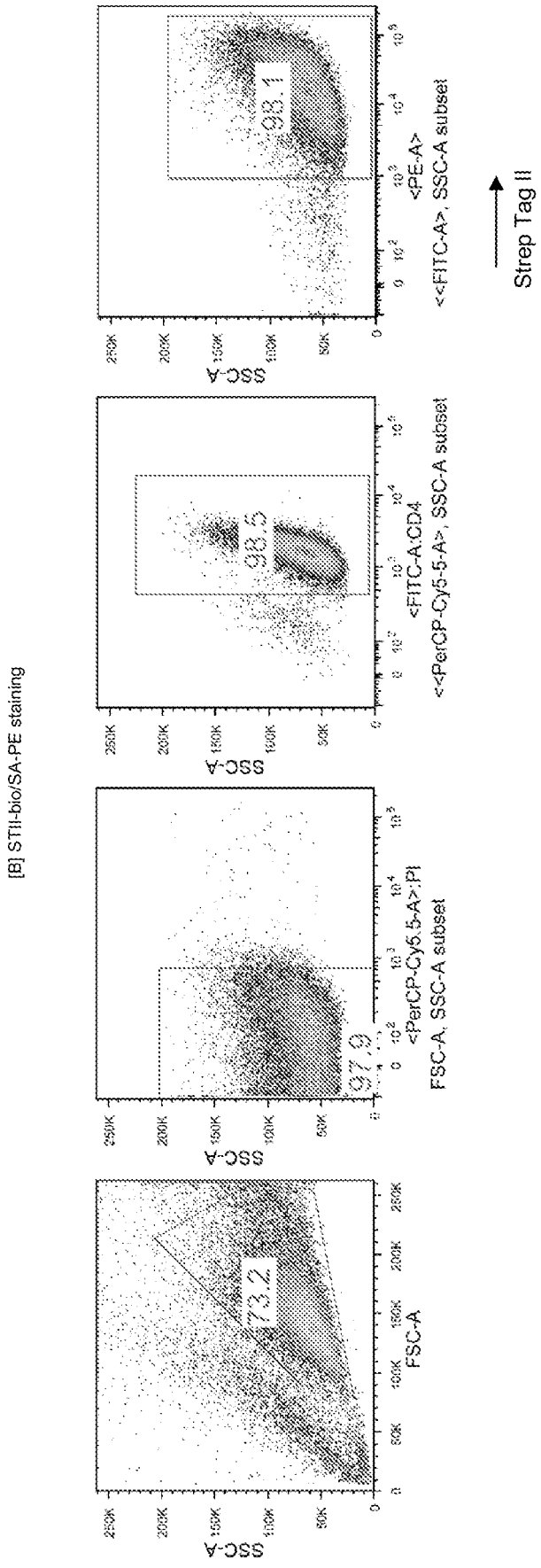


Fig. 8B (Continued)

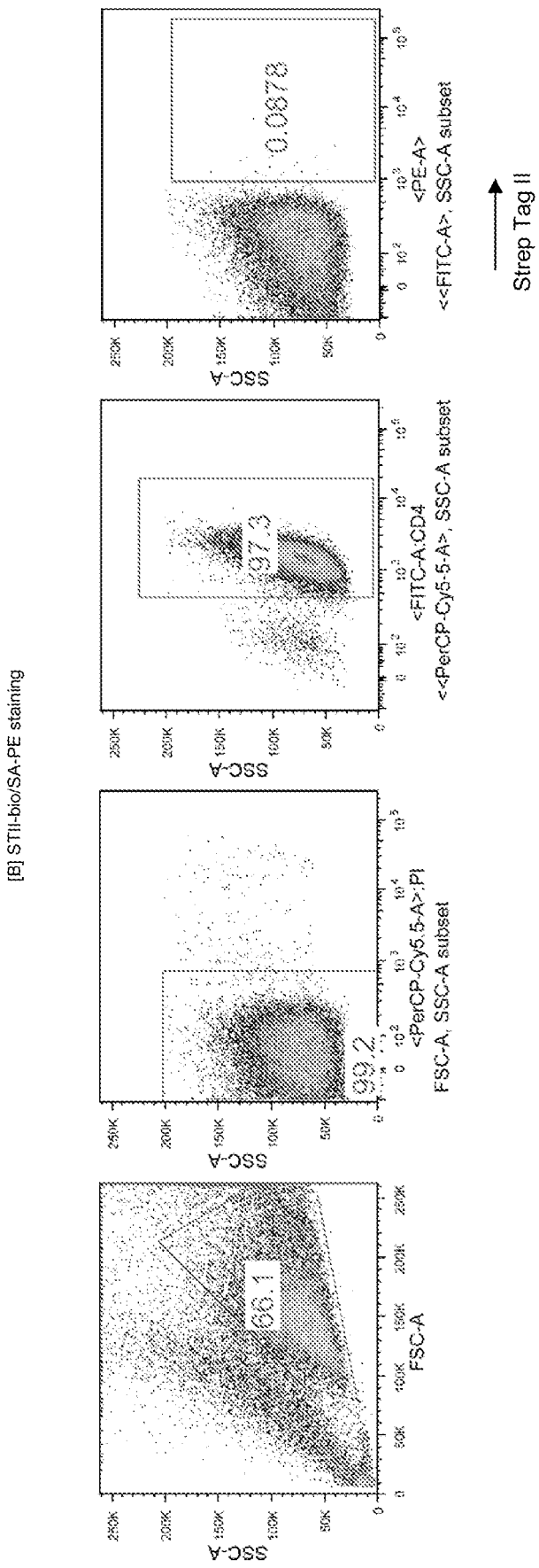


Fig. 8B (Continued)

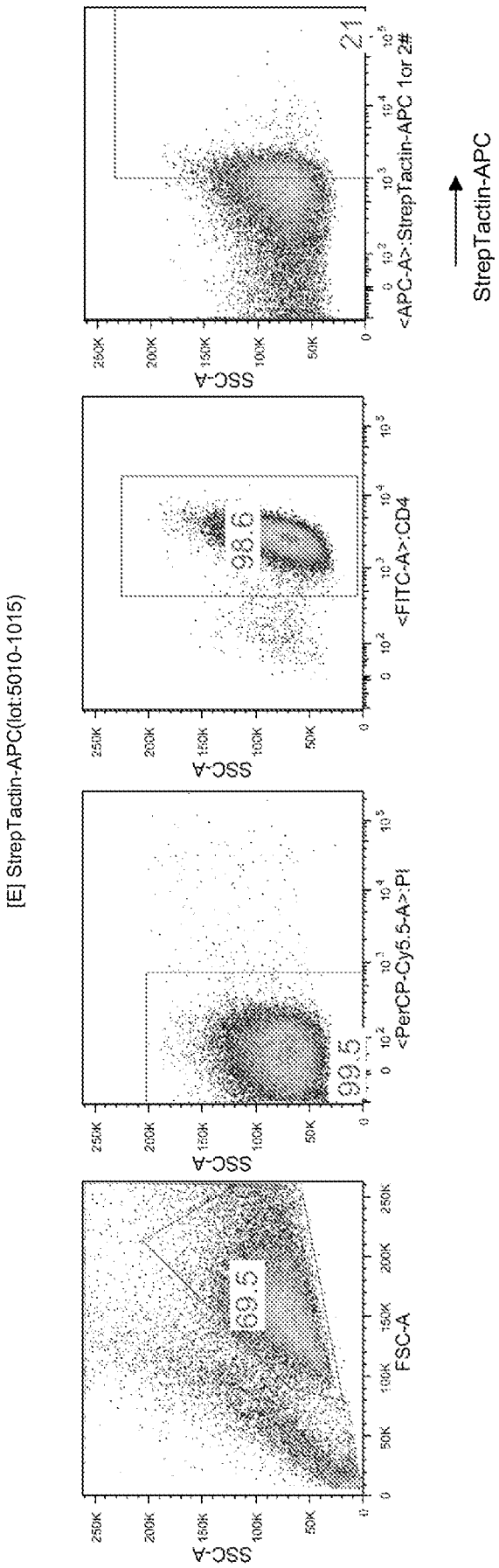

CD19-ChARM1
[E] StrepTactin-APC(lot:5010-1015)
FSC-A
SSC-A
69.5
<PerCP-Cy5.5-A>:PI
89.5
<FITC-A>:CD4
98.6
<APC-A>:StrepTactin-APC 1or 2#
21
StrepTactin-APC

Fig. 8C

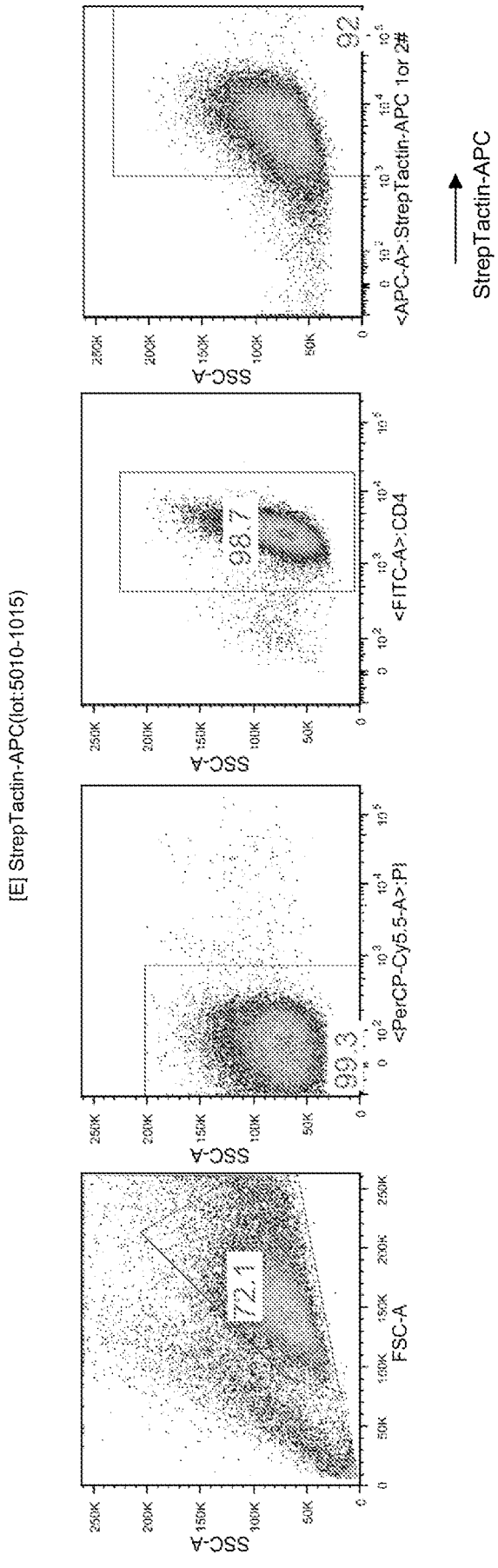


Fig. 8C (Continued)

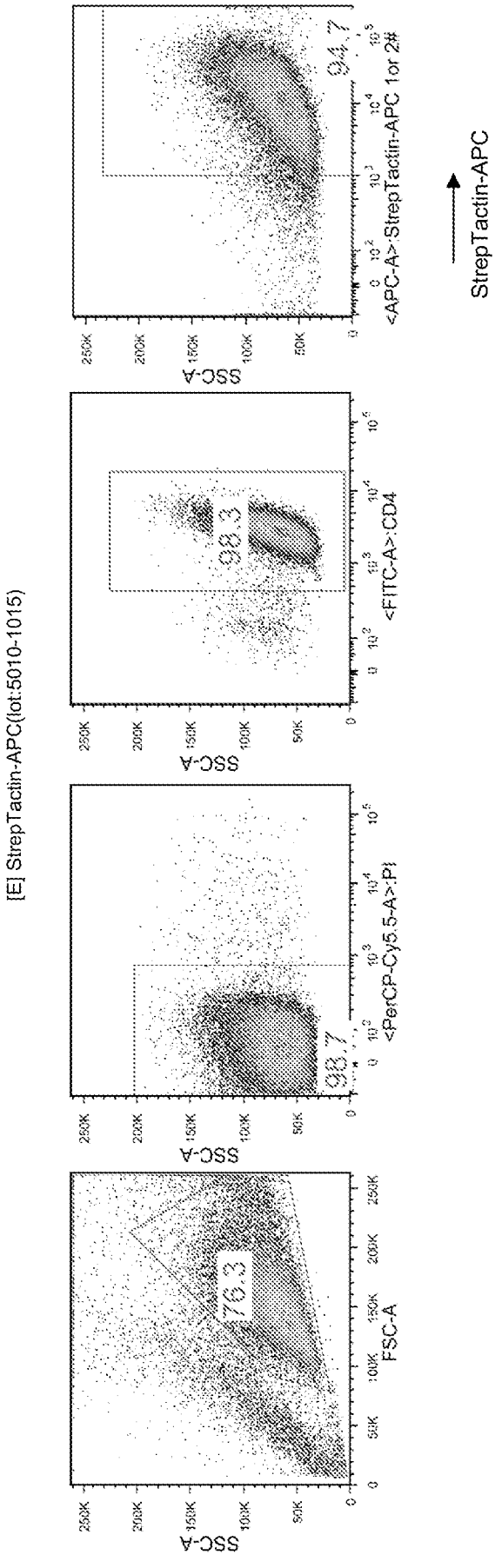


Fig. 8C (Continued)

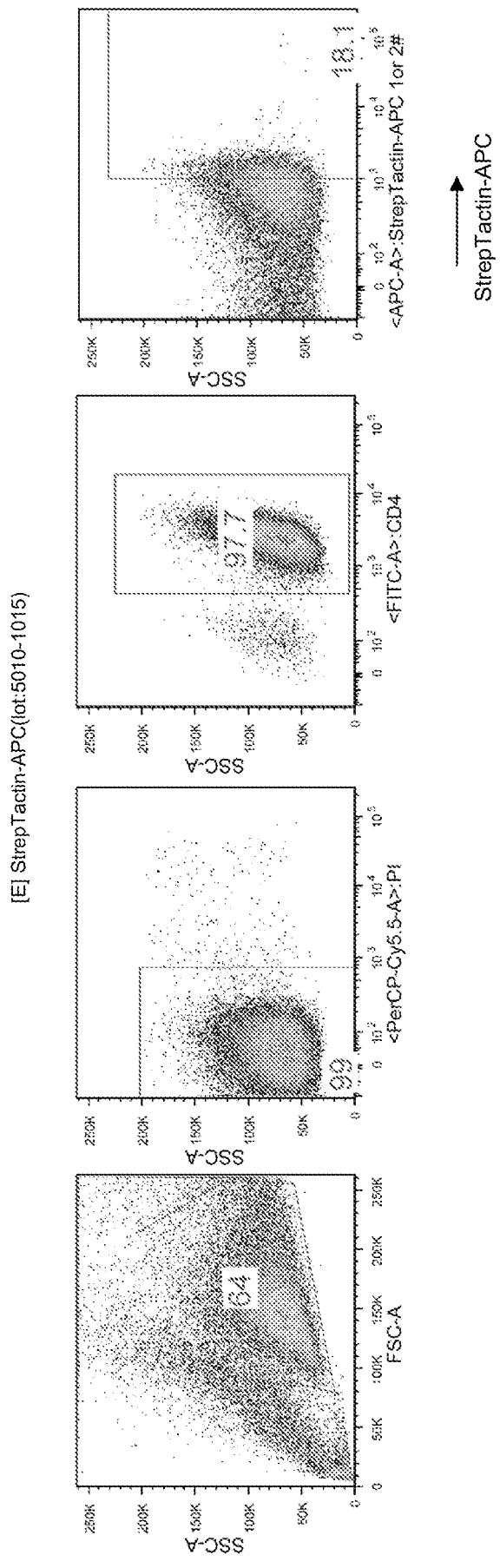


Fig. 8C (Continued)

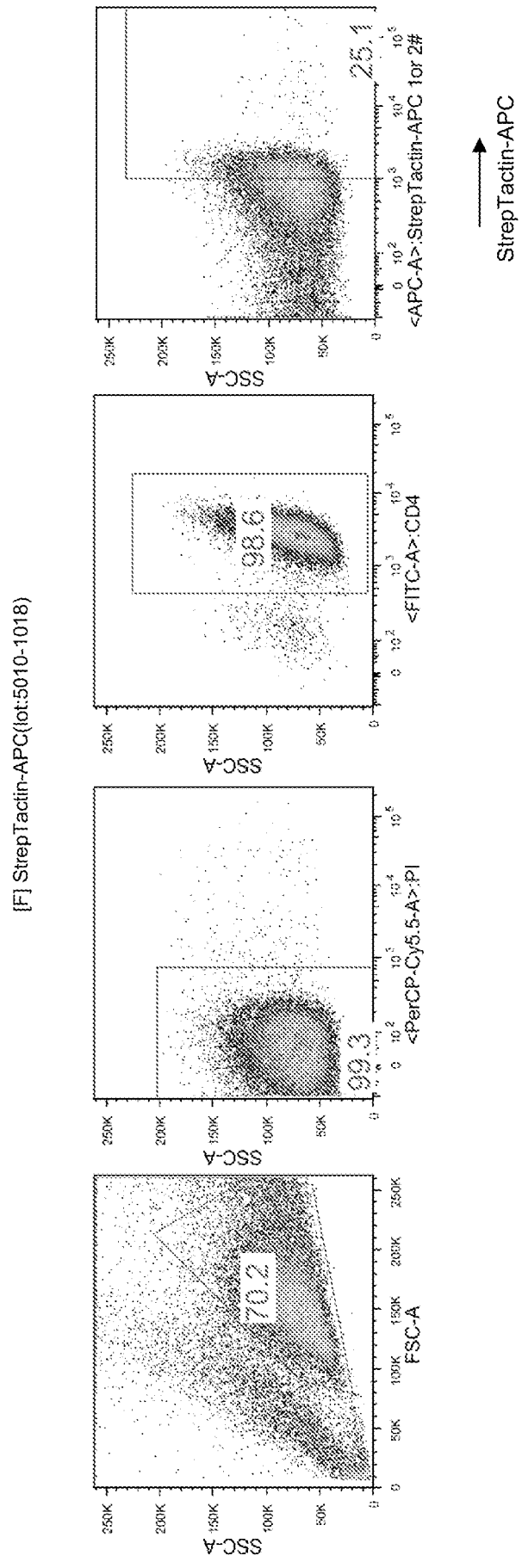

CD19-ChARM1
[F] StrepTactin-APC(lot:5010-1018)
SSC-A
FSC-A
70.2
<PerCP-Cy5.5-A>:PI
99.3
<FITC-A>:CD4
98.6
<APC-A>:StrepTactin-APC 1or 2#
25.1
StrepTactin-APC

Fig. 8D

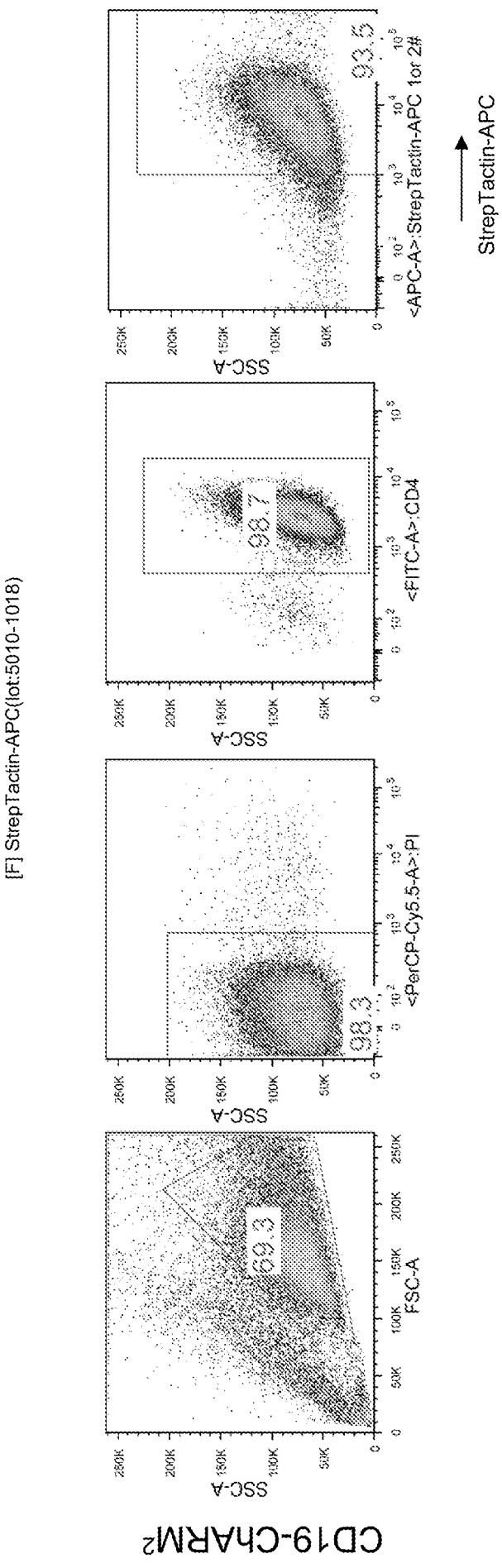


Fig. 8D (Continued)

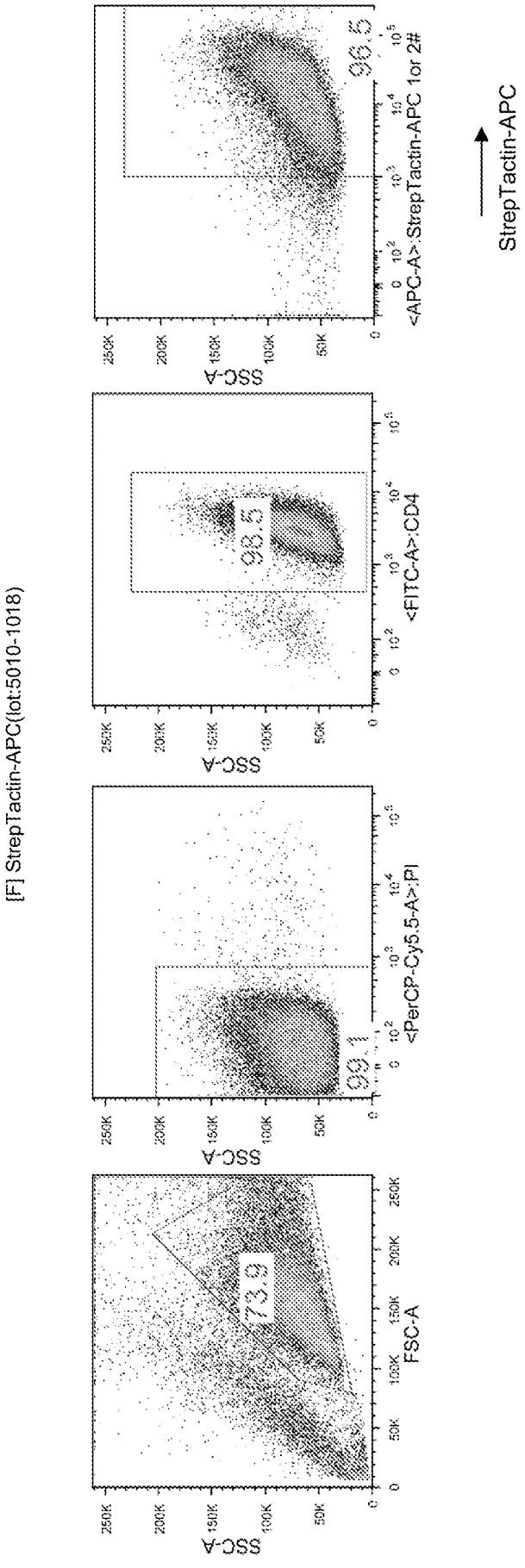


Fig. 8D (Continued)

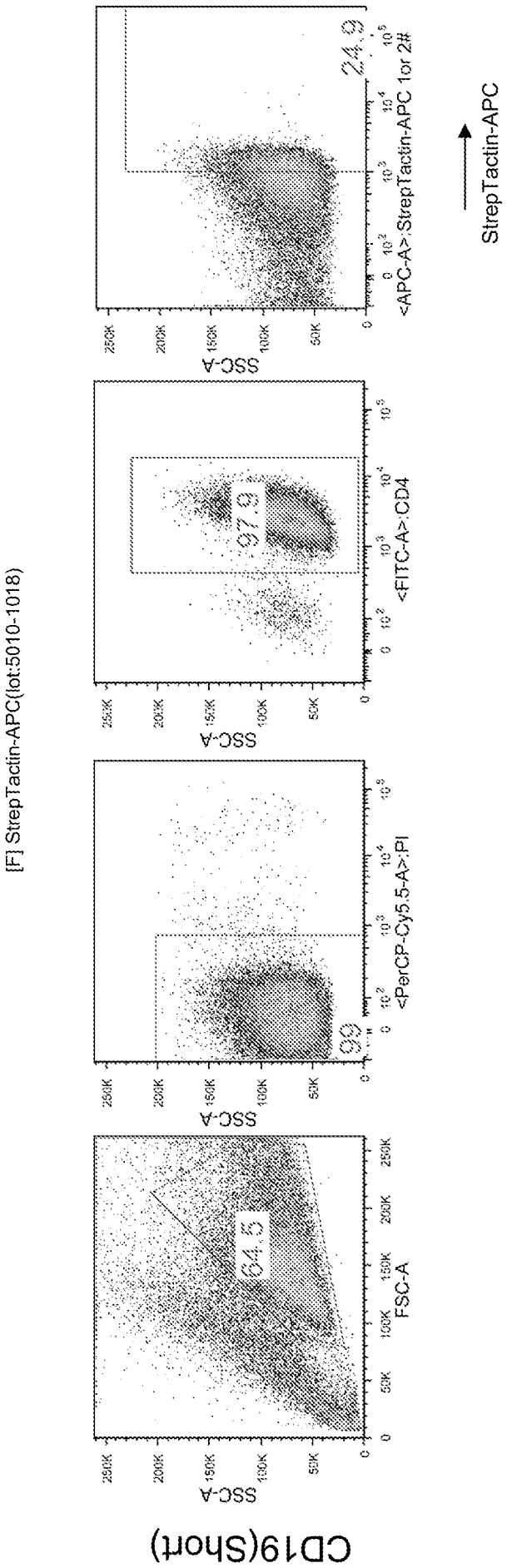


Fig. 8D (Continued)

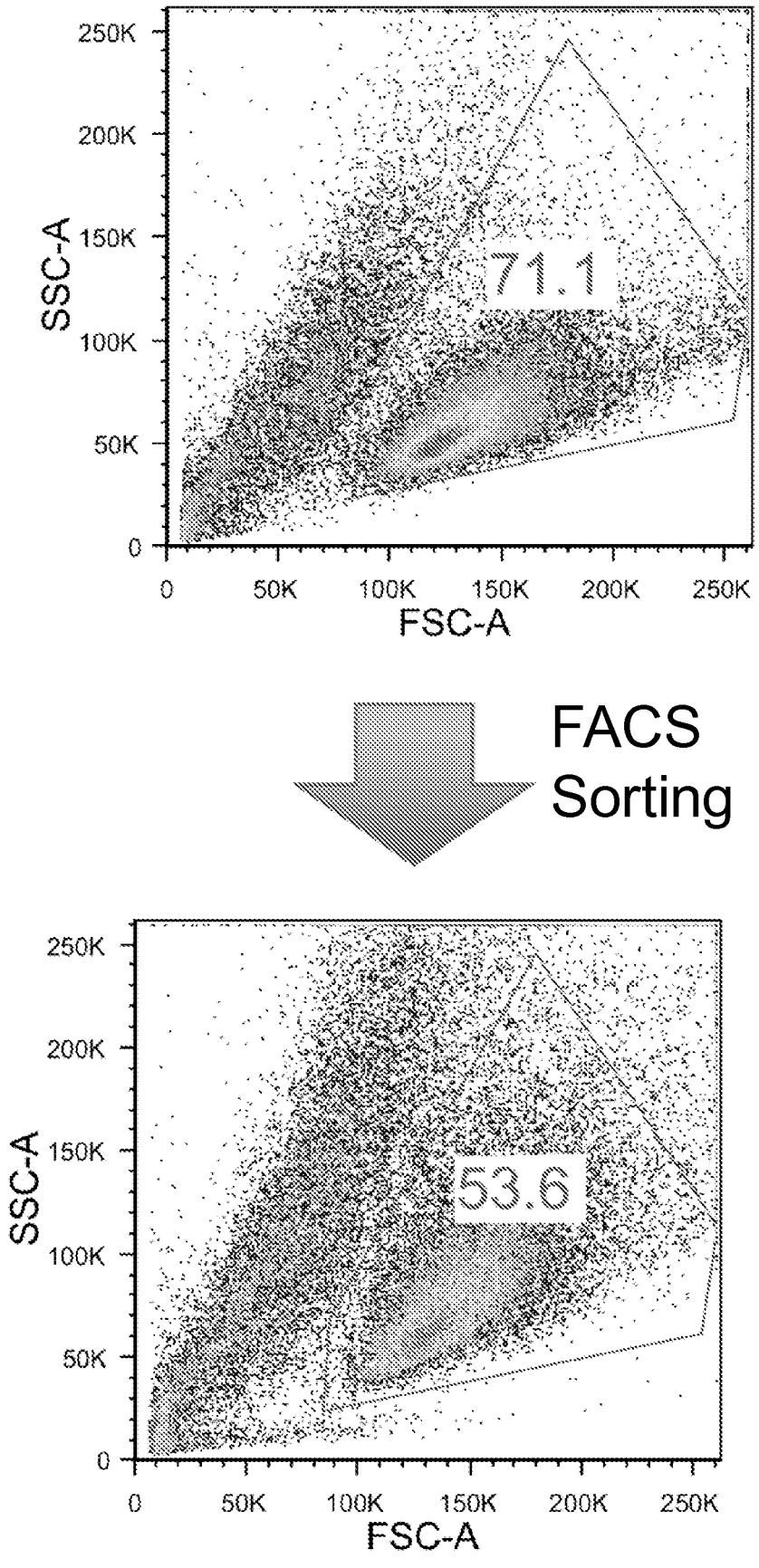

250K
200K
150K
100K
50K
0
SSC-A
71.1
0
50K
100K
150K
200K
250K
FSC-A
FACS
Sorting
250K
200K
150K
100K
50K
0
SSC-A
53.6
0
50K
100K
150K
200K
250K
FSC-A

Fig. 9

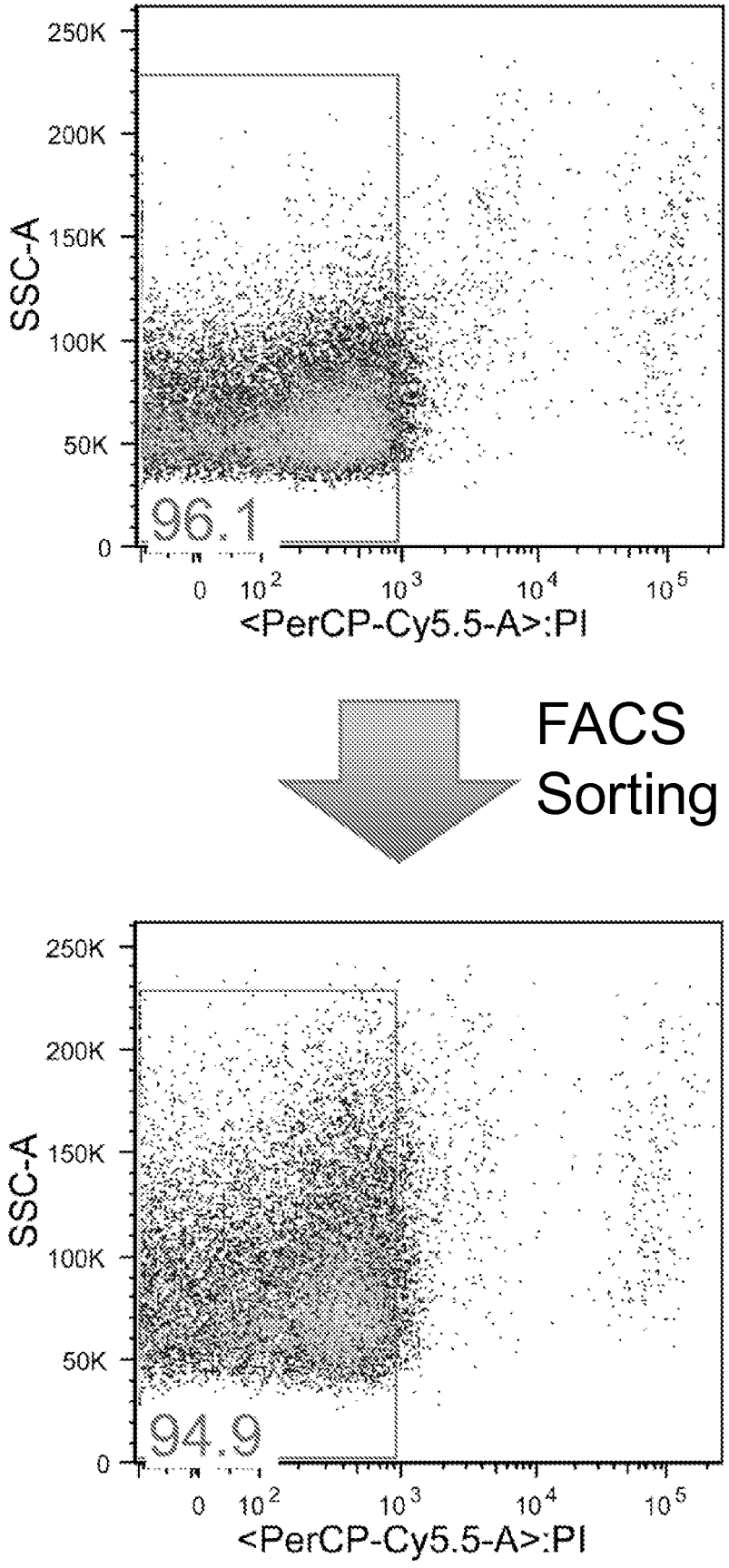


Fig. 9 *(Continued)*

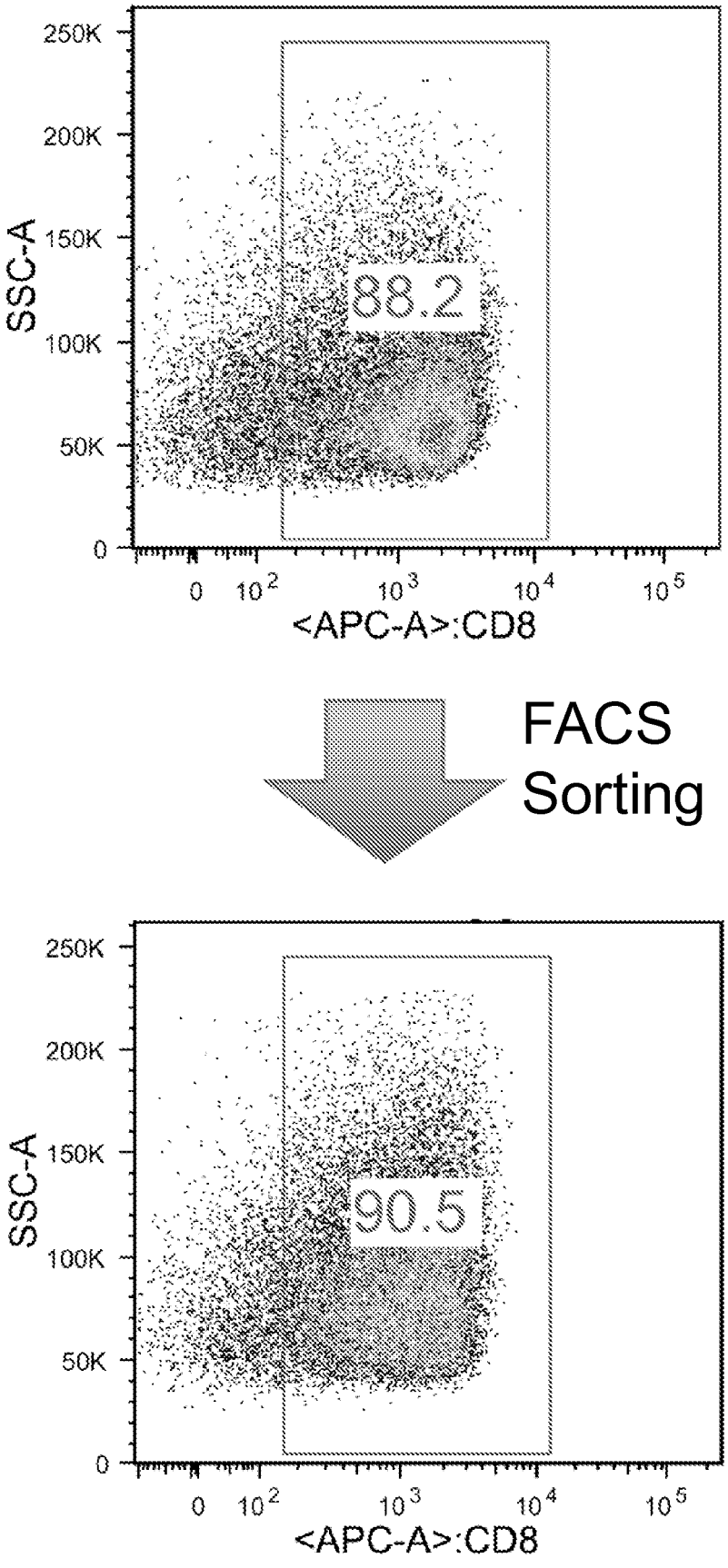


Fig. 9 *(Continued)*

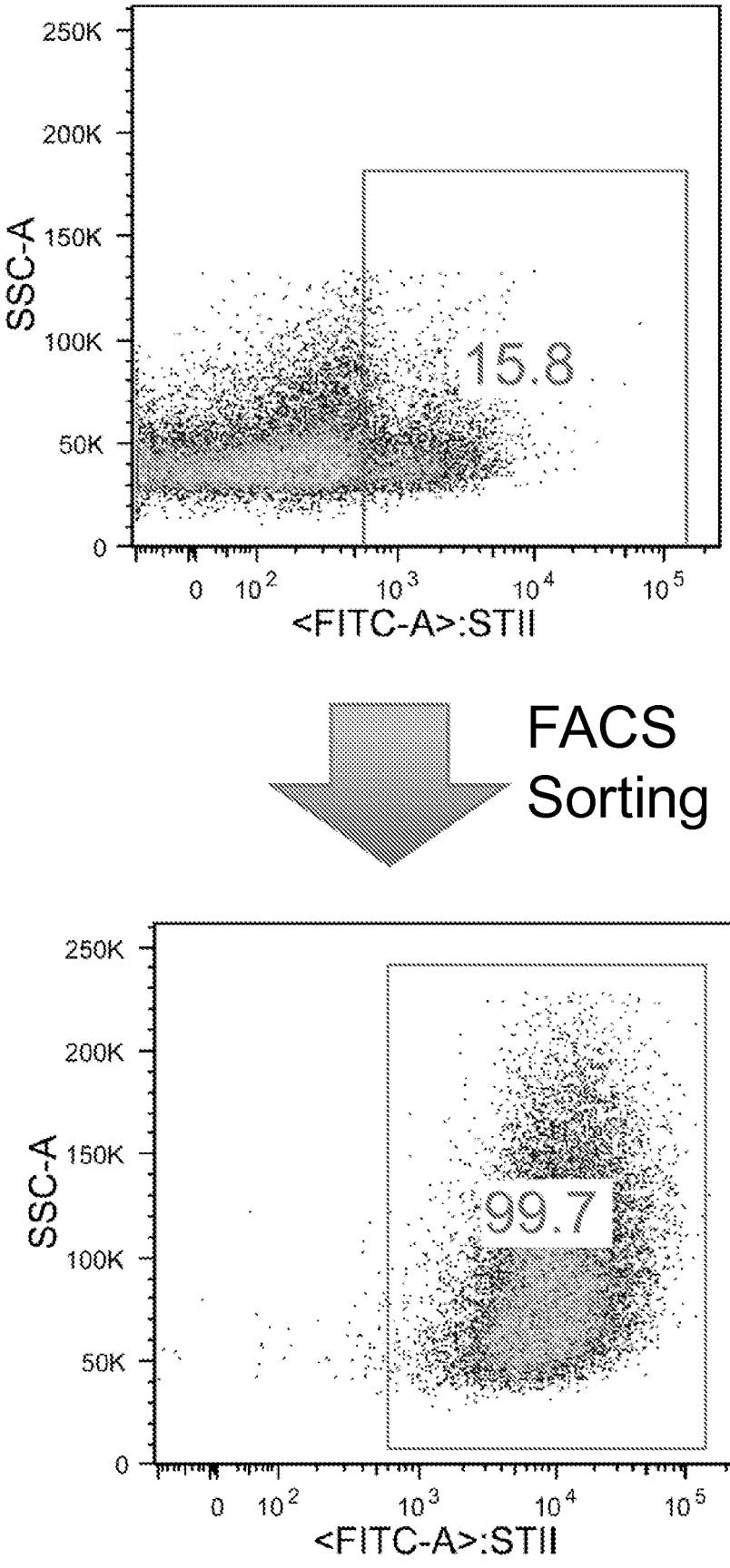


Fig. 9 *(Continued)*

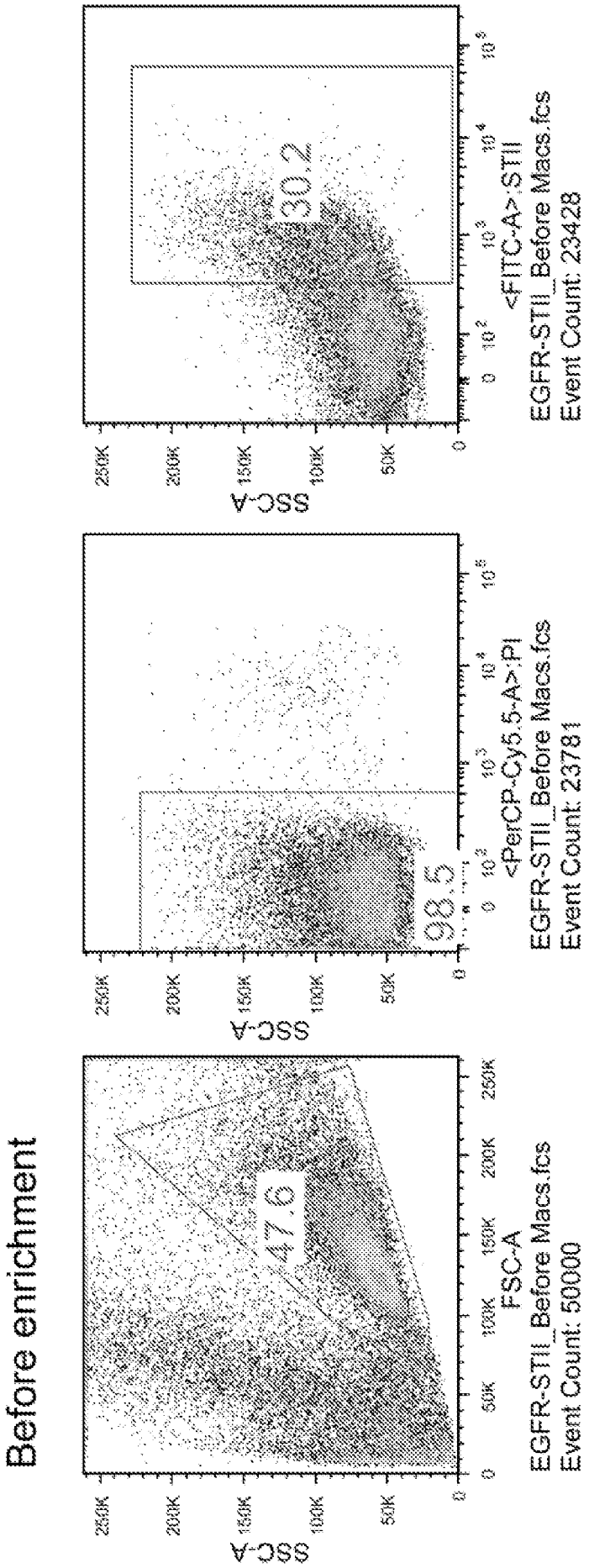

Before enrichment
SSC-A
FSC-A
47.6
EGFR-STII_Before Macs.fcs
Event Count: 50000
98.5
<PerCP-Cy5.5-A>:PI
EGFR-STII_Before Macs.fcs
Event Count: 23781
30.2
<FITC-A>:STII
EGFR-STII_Before Macs.fcs
Event Count: 23428

Fig. 10

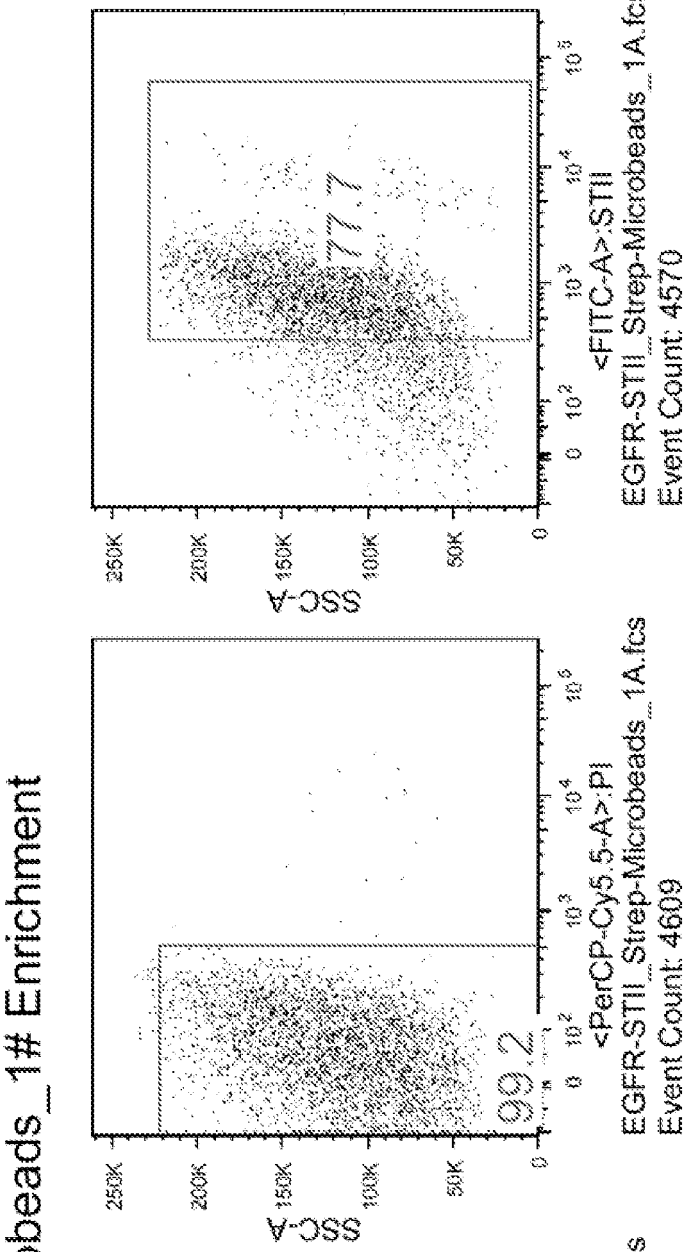


Fig. 10 (Continued)

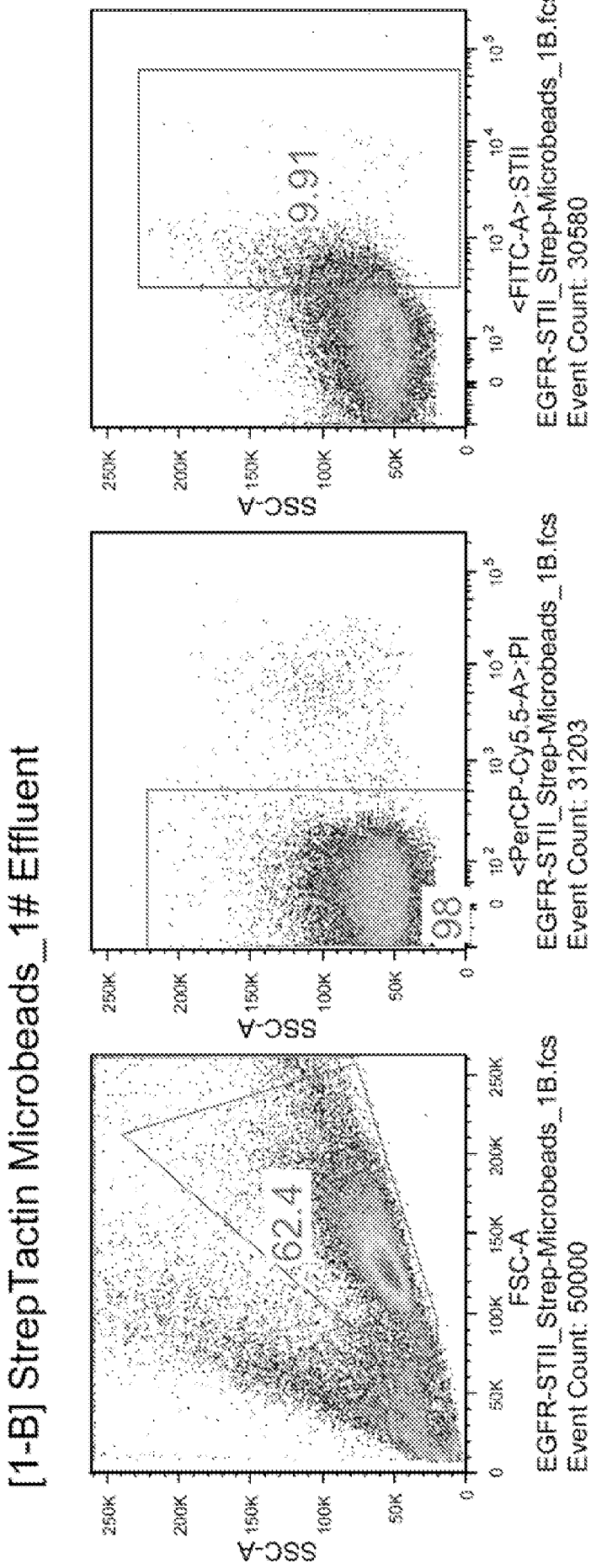


Fig. 10 (Continued)

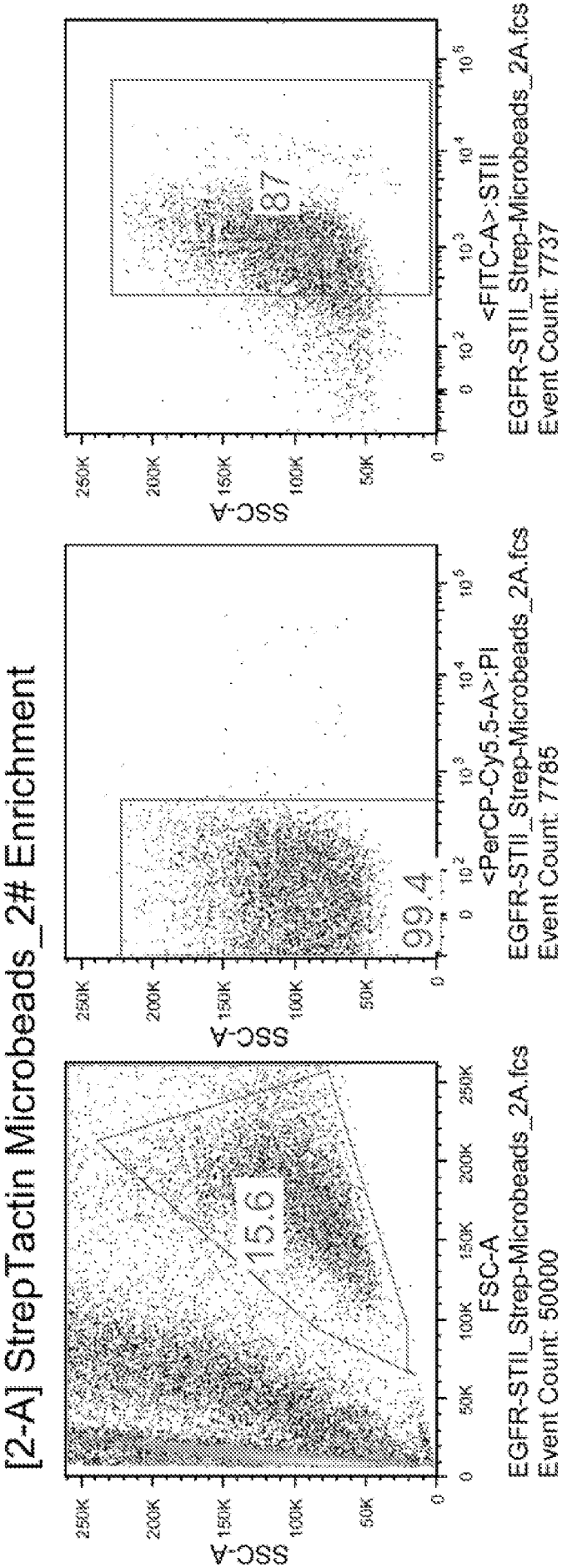


Fig. 10 (Continued)

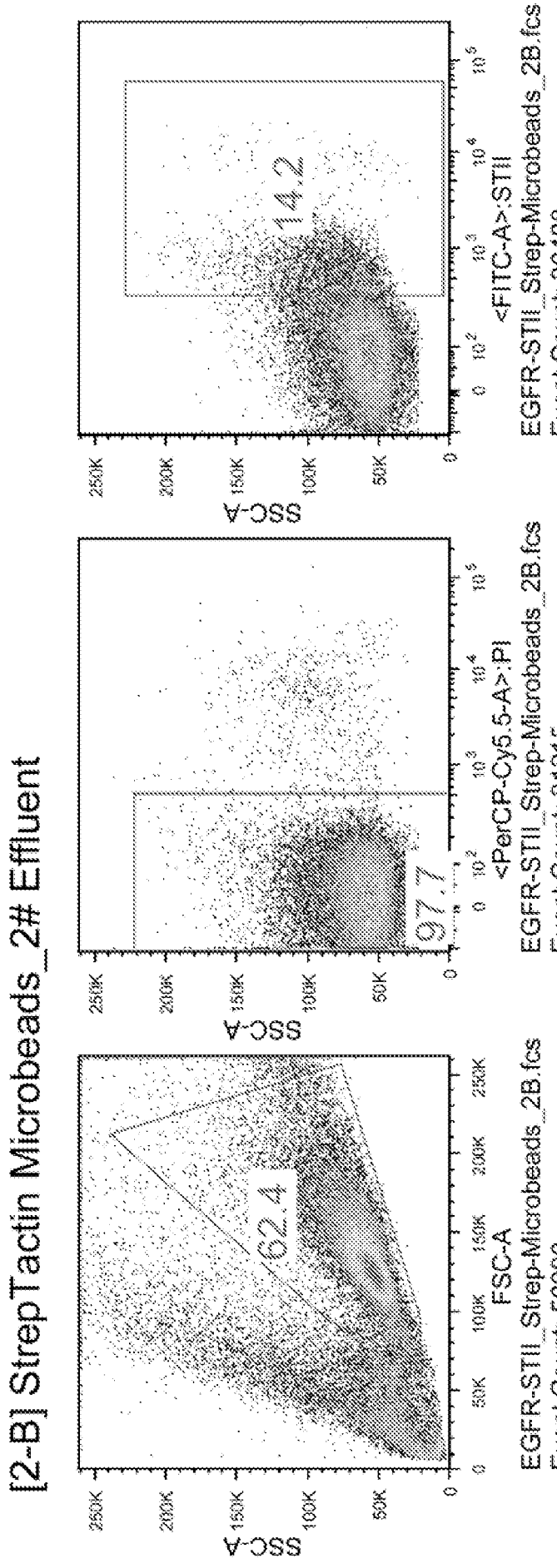


Fig. 10 (Continued)

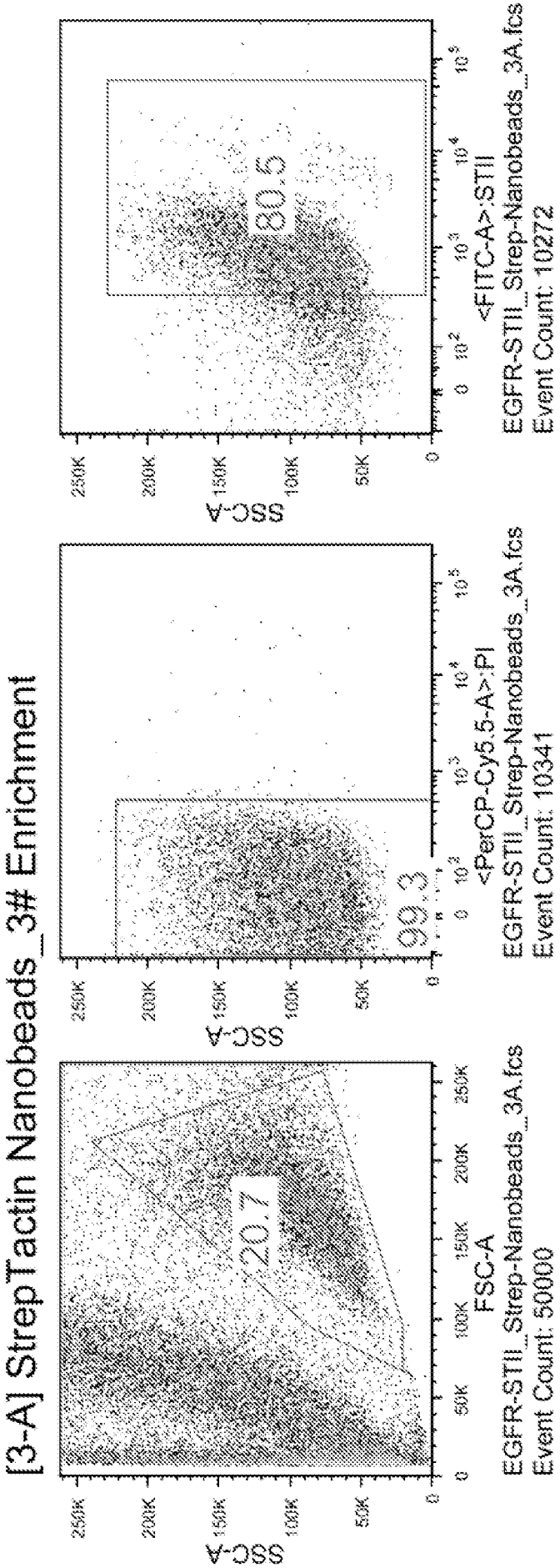


Fig. 10 (Continued)

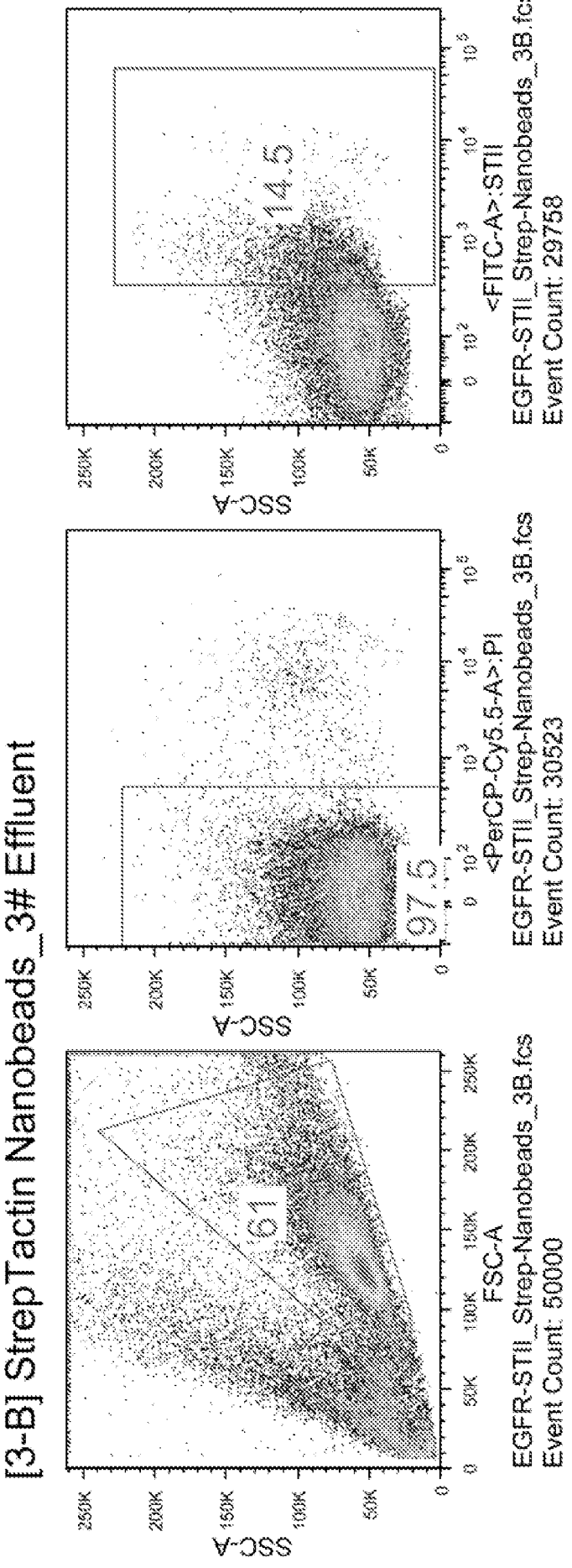


Fig. 10 (Continued)

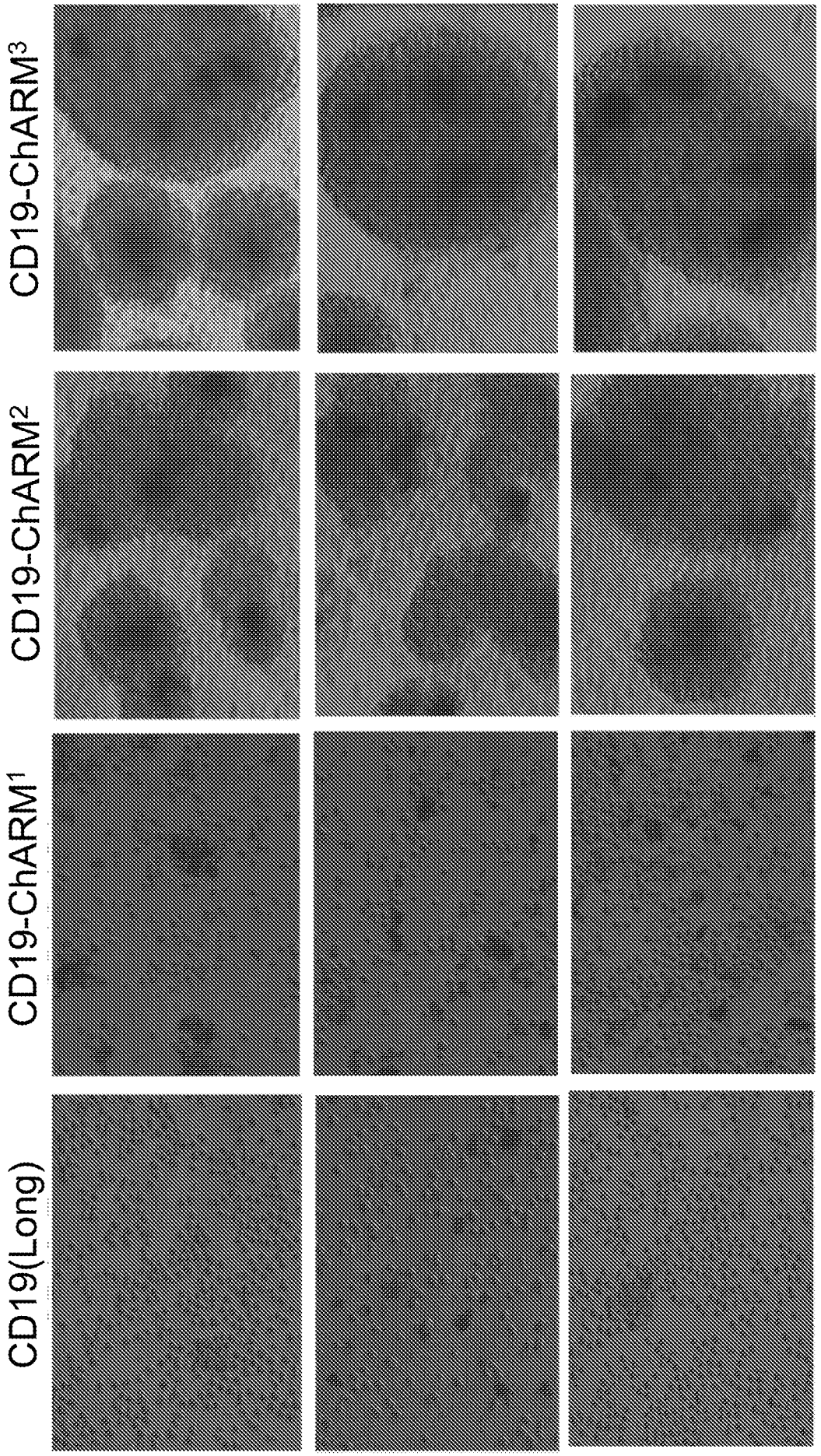


Fig. 11

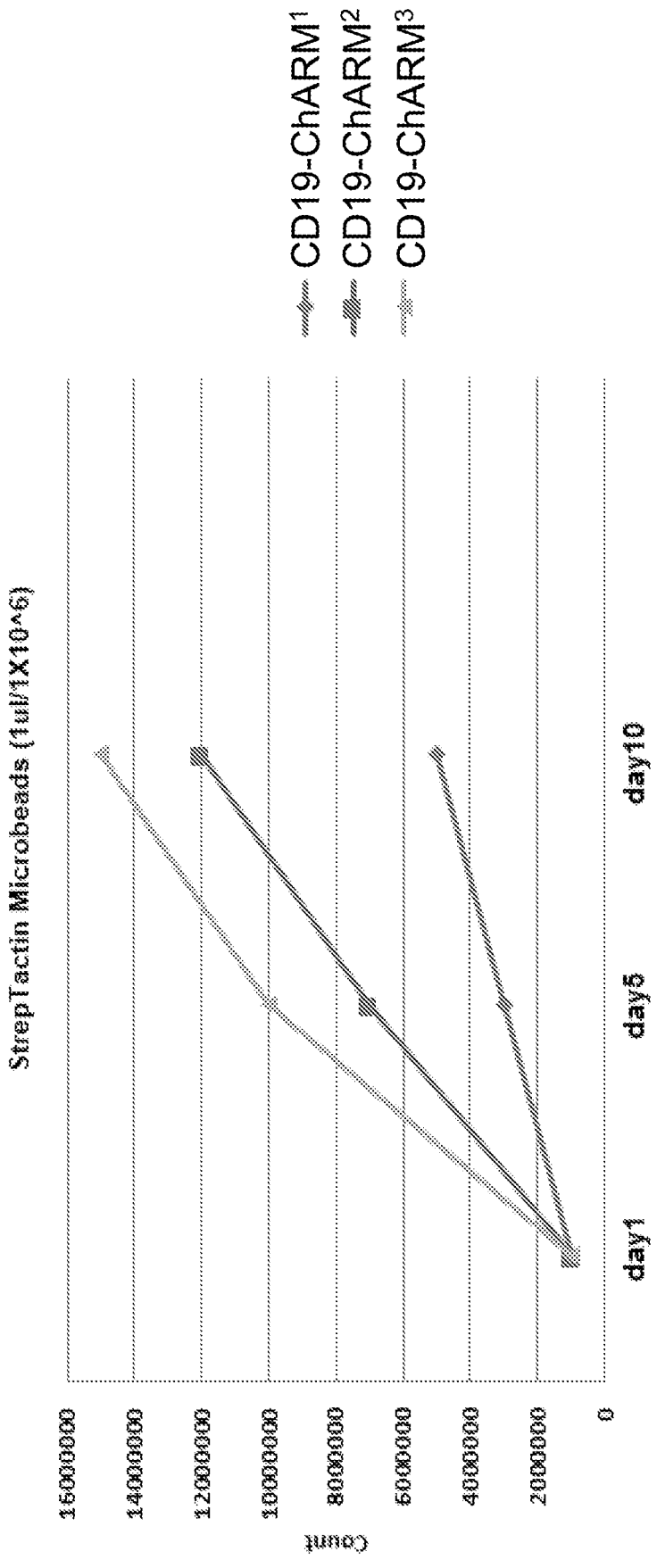

CD19-ChARM1
CD19-ChARM2
CD19-ChARM3
StrepTactin Microbeads (1ul/1X10^6)
Count
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
day1
day5
day10

Fig. 12

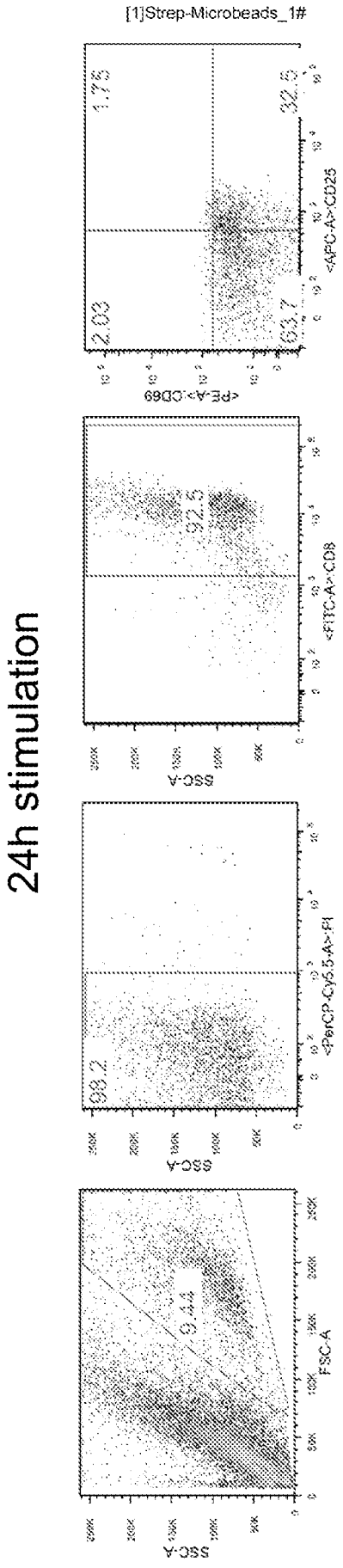

[1]Strep-Microbeads_1#
24h stimulation
1.76
32.6
2.03
63.7
<PE-A>:CD69
<APC-A>:CD25
92.3
<FITC-A>:CD8
98.2
<PerCP-Cy5.5-A>:PI
9.44
SSC-A
FSC-A

Fig. 13A

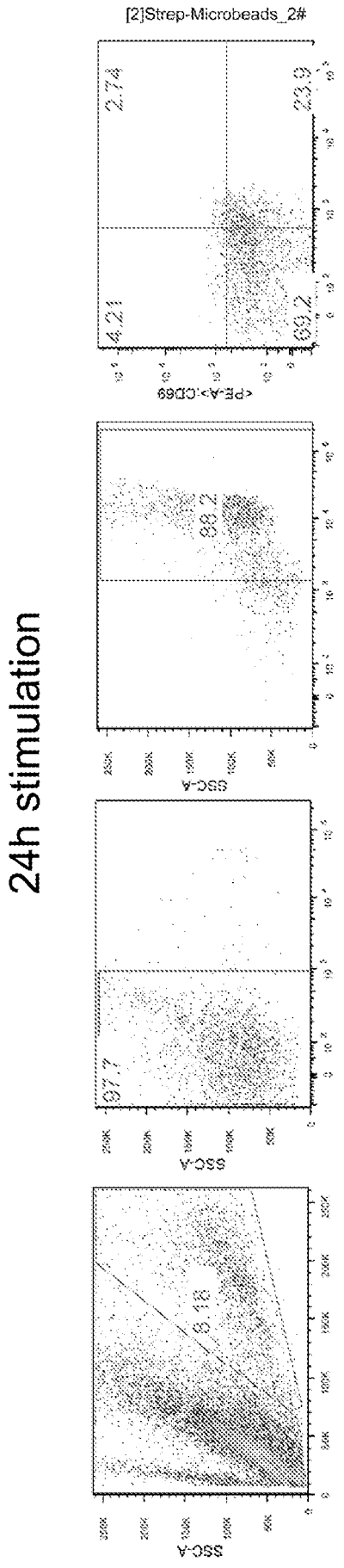


Fig. 13A (Continued)

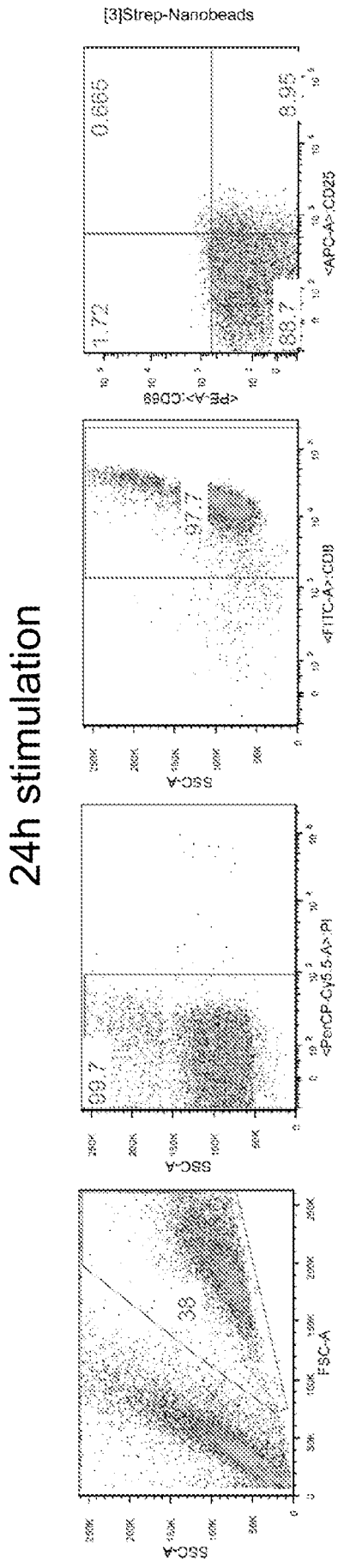


Fig. 13A (Continued)

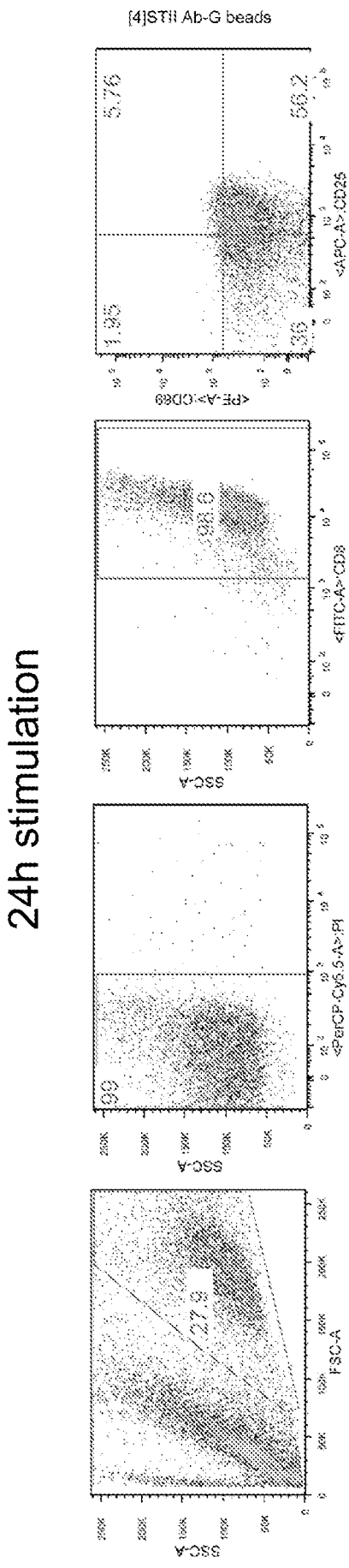


Fig. 13A (Continued)

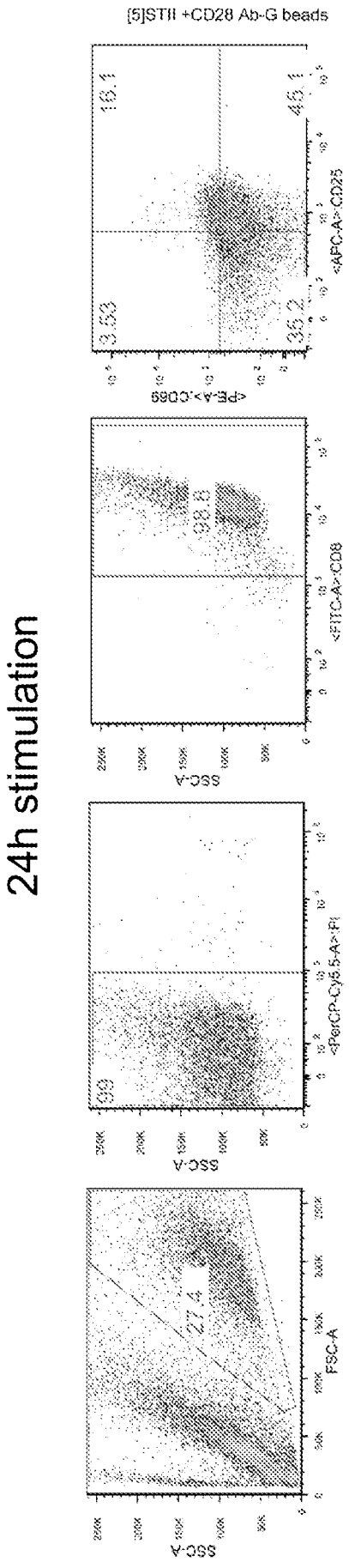


Fig. 13A (Continued)

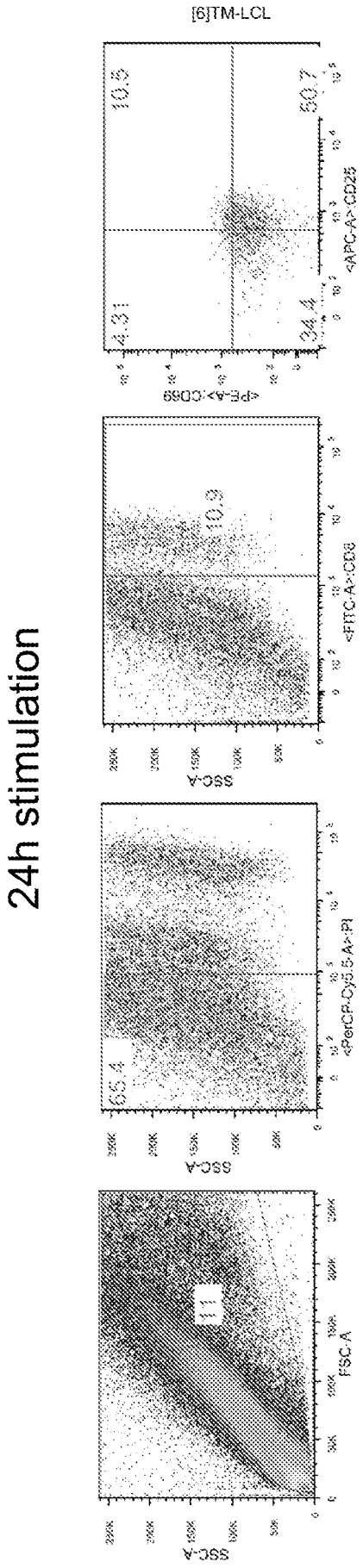


Fig. 13A (Continued)

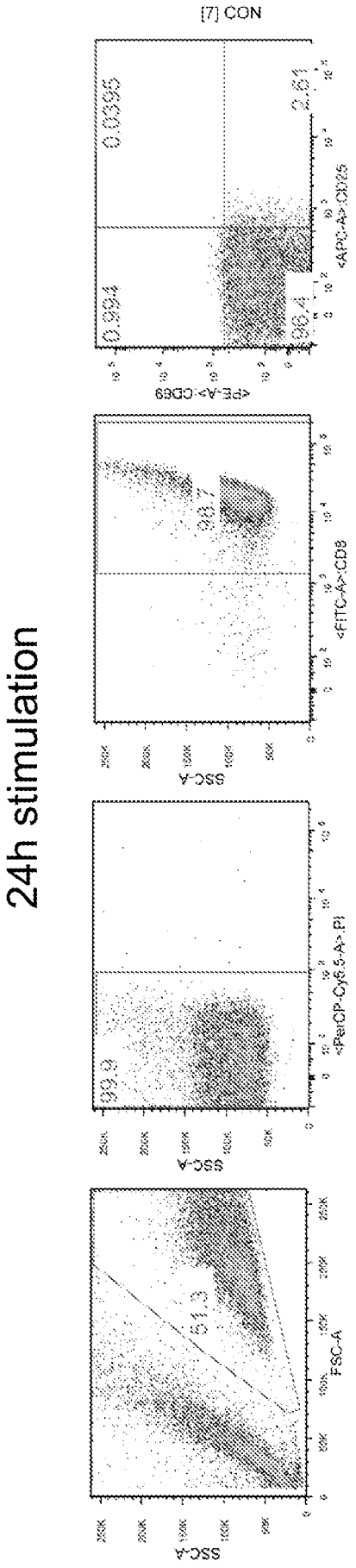


Fig. 13A (Continued)

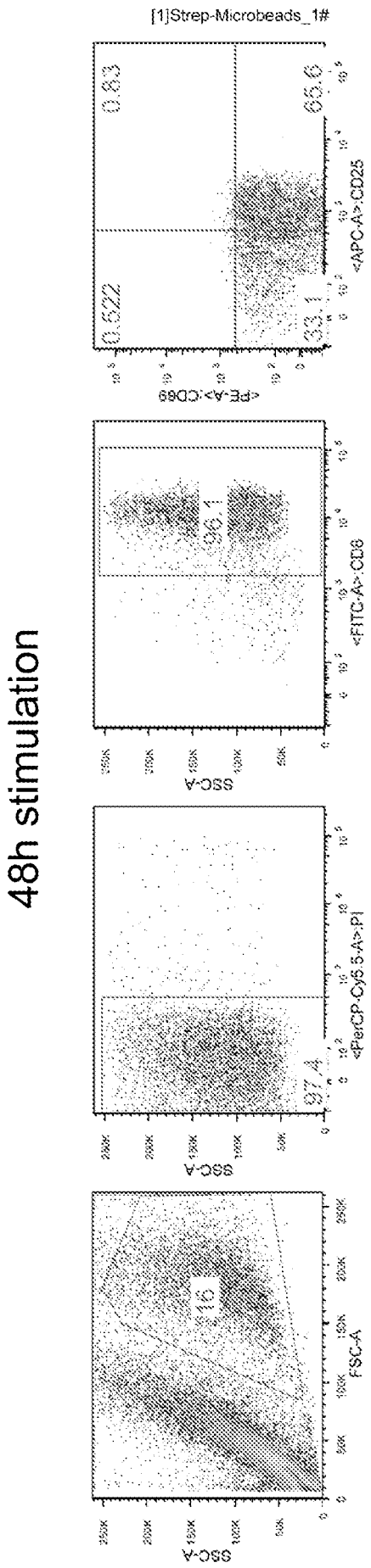

[1]Strep-Microbeads_1#
48h stimulation
FSC-A
SSC-A
<PerCP-Cy5-5-A>:PI
97.4
<FITC-A>:CD8
96.1
<APC-A>:CD25
<PE-A>:CD69
0.522
33.1
0.83
65.6

Fig. 13B

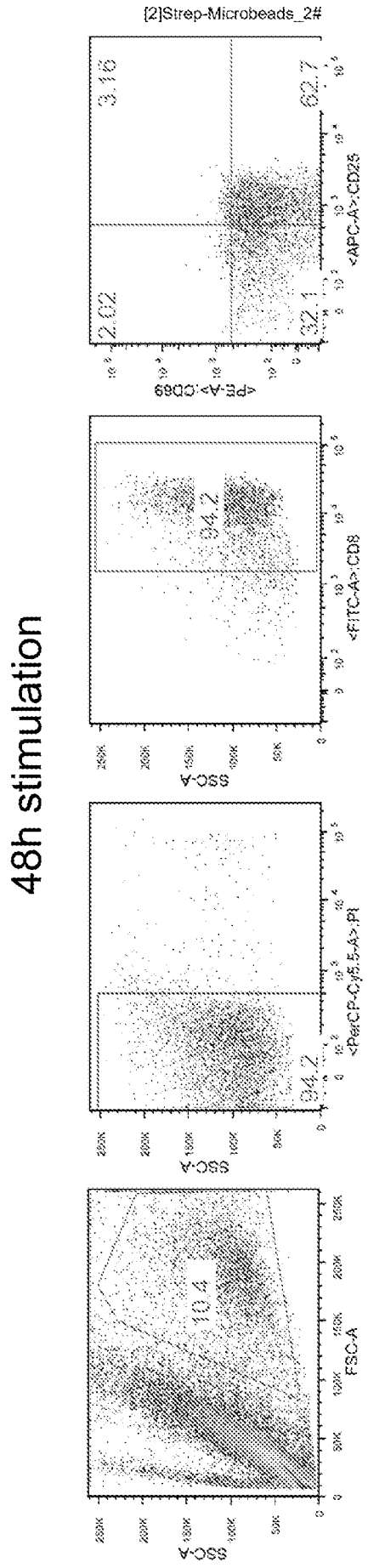


Fig. 13B (Continued)

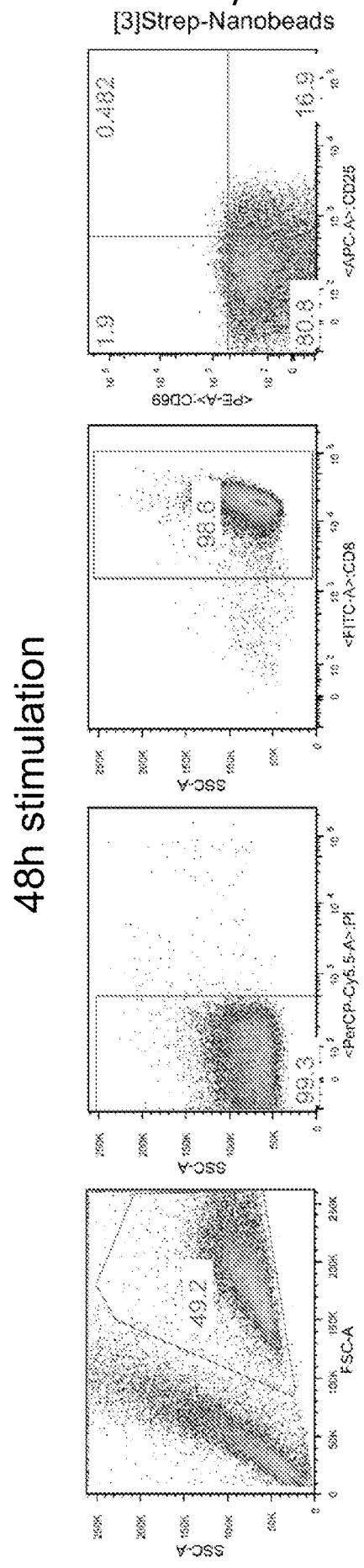


Fig. 13B (Continued)

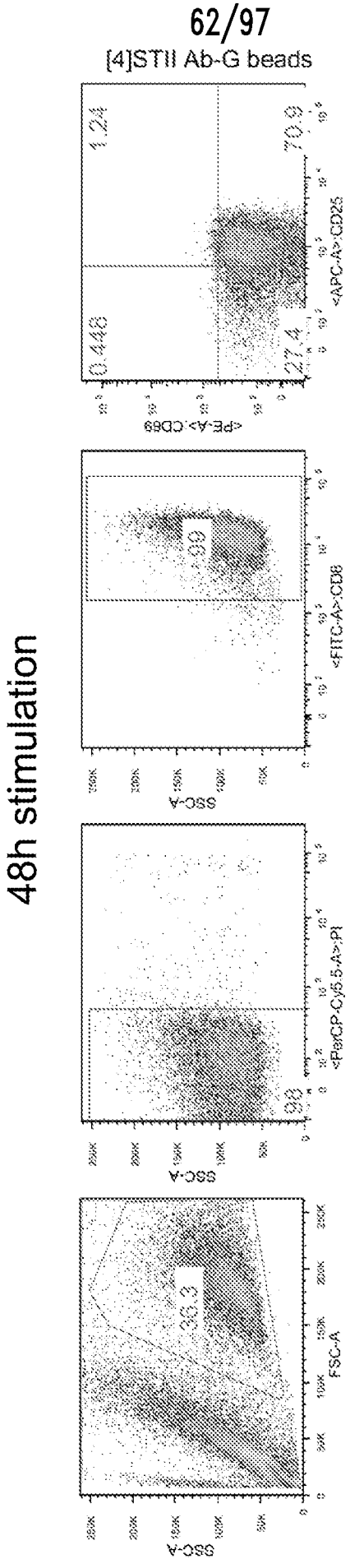


Fig. 13B (Continued)

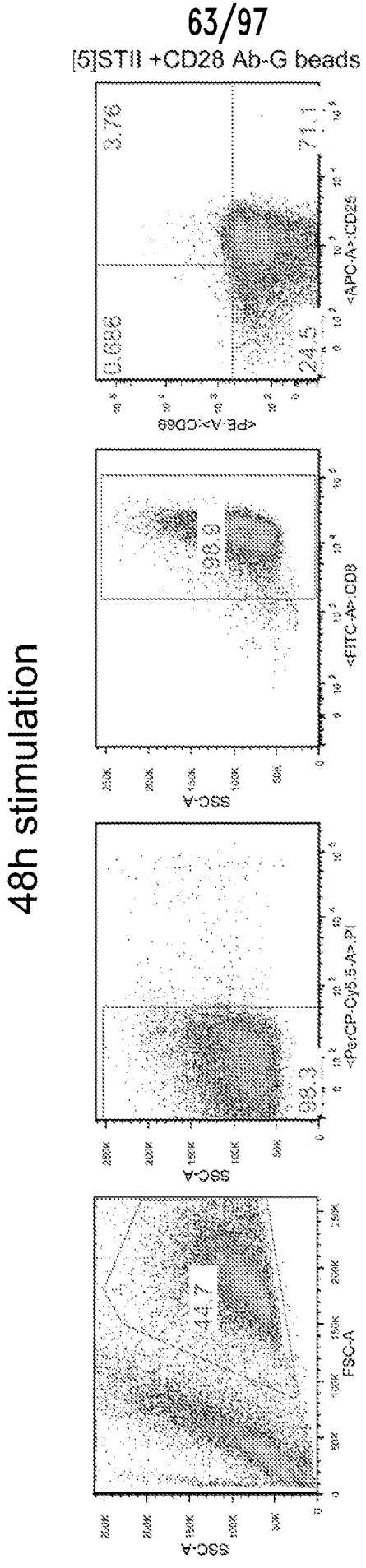


Fig. 13B (Continued)

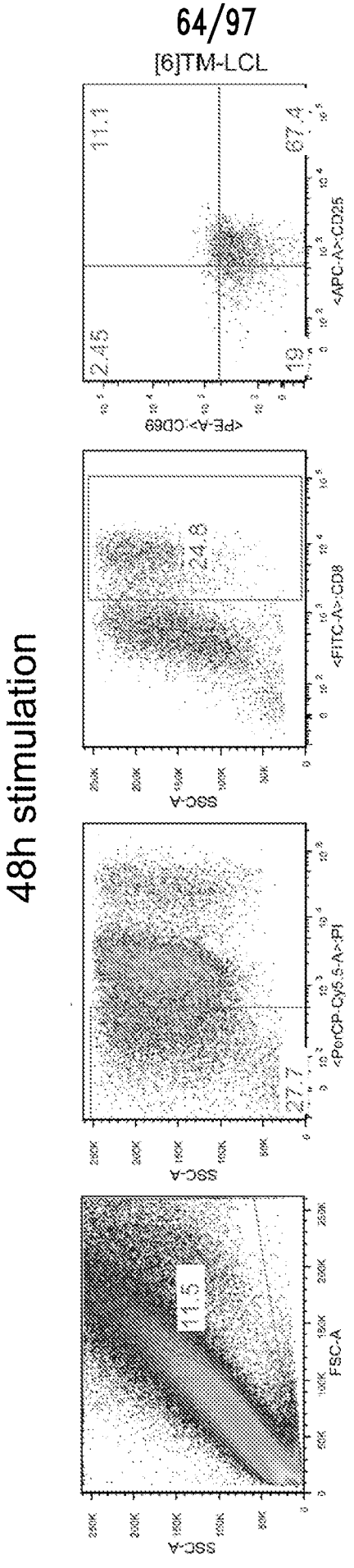


Fig. 13B (Continued)

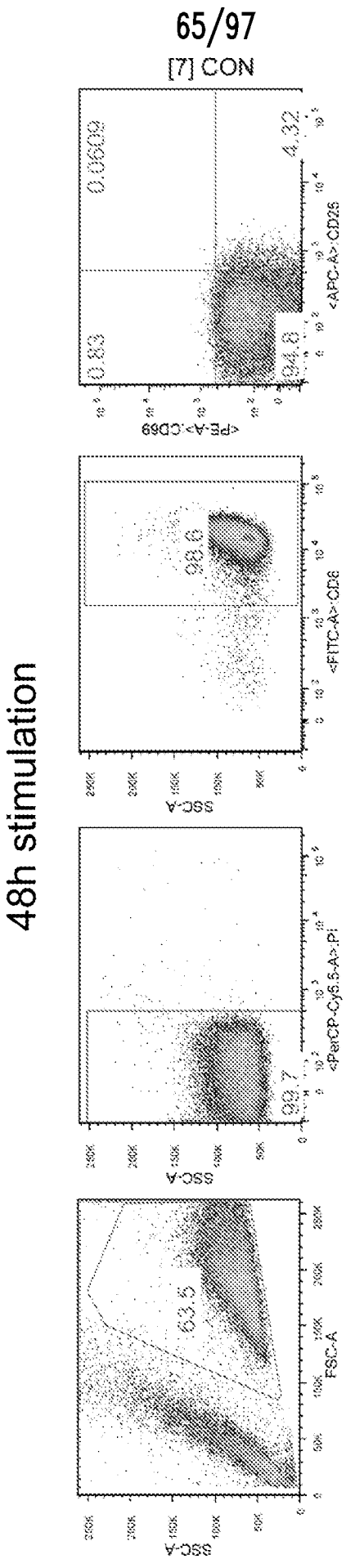


Fig. 13B (Continued)

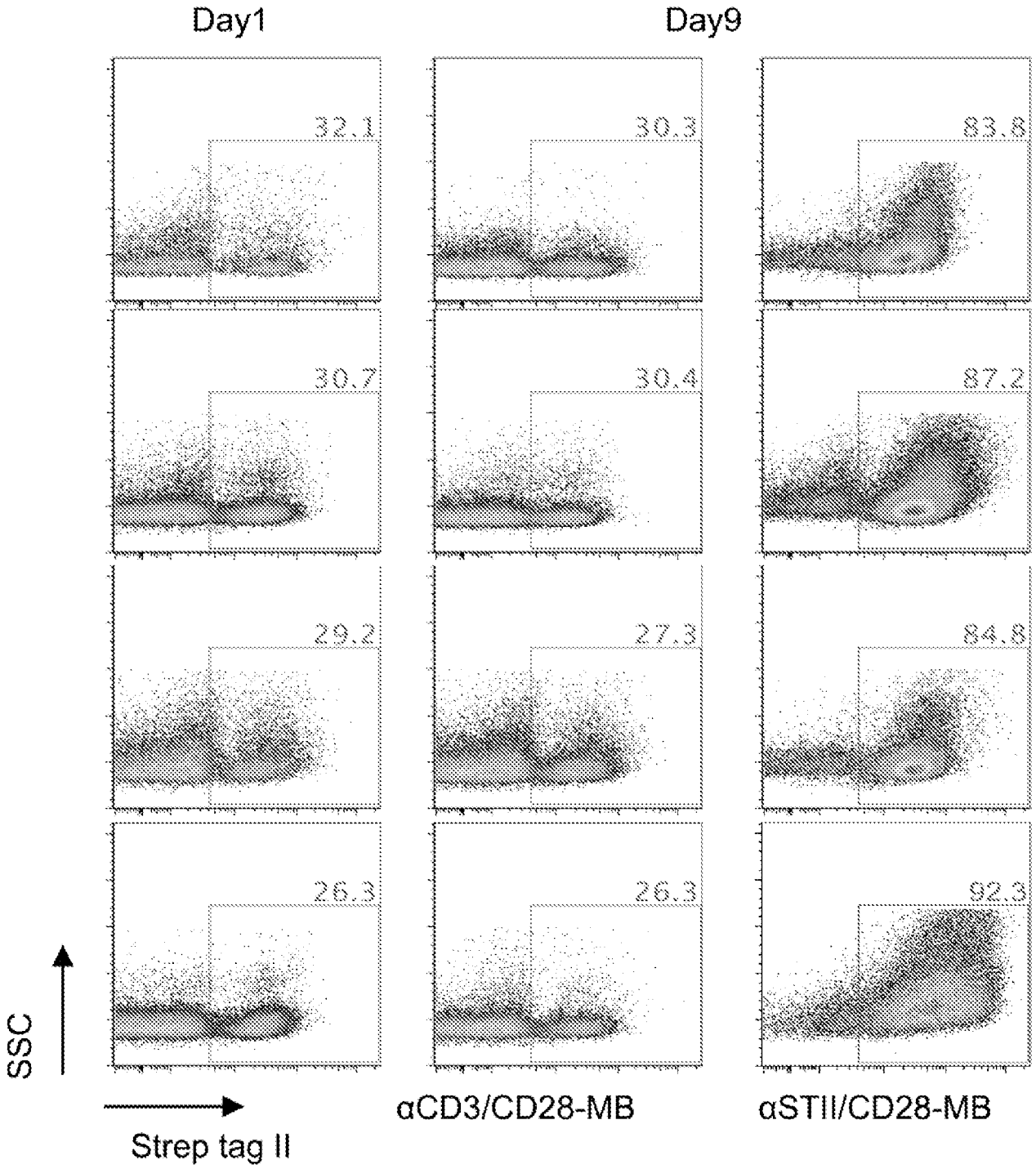

Day1
Day9
32.1
30.3
83.8
30.7
30.4
87.2
29.2
27.3
84.8
26.3
26.3
92.3
SSC
Strep tag II
αCD3/CD28-MB
αSTII/CD28-MB

Fig. 14A

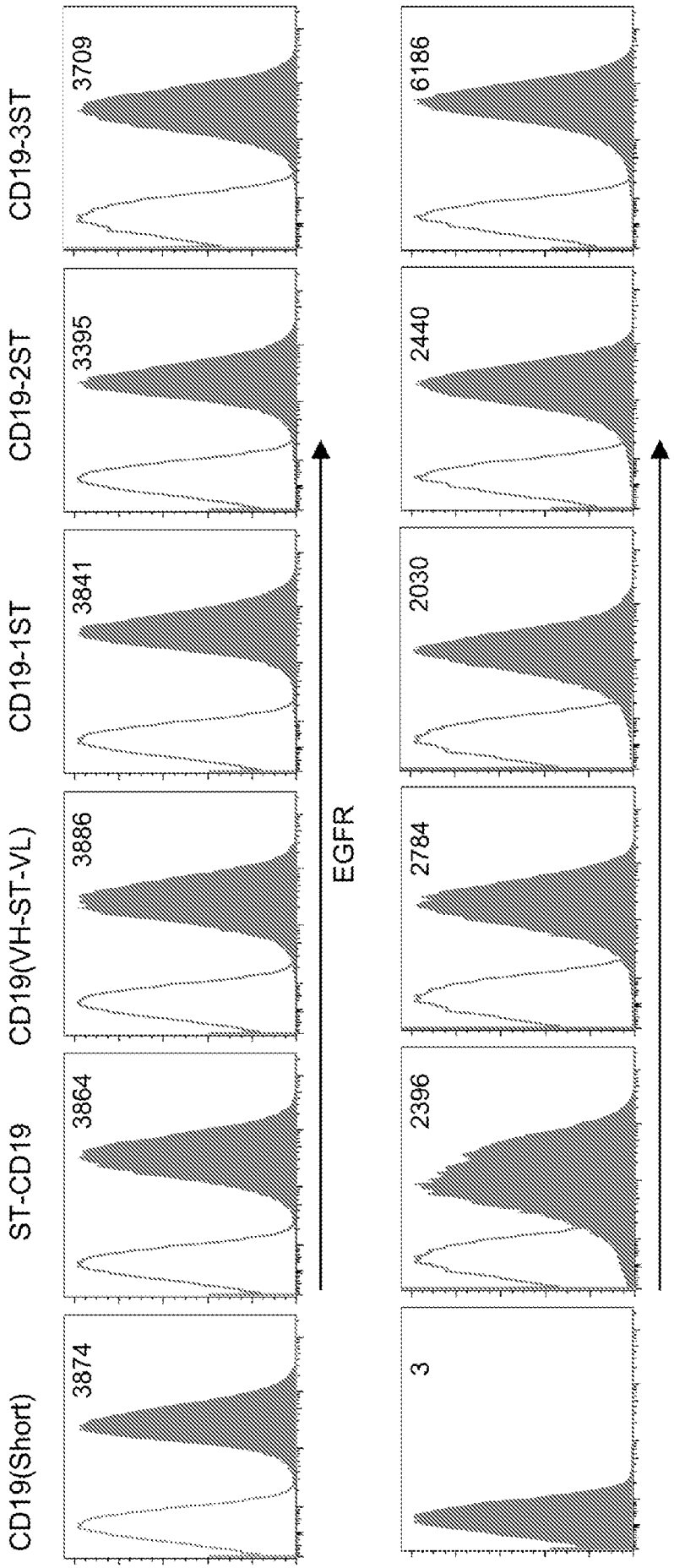

CD19(Short)
ST-CD19
CD19(VH-ST-VL)
CD19-1ST
CD19-2ST
CD19-3ST
3874
3864
3886
3841
3395
3709
EGFR
3
2396
2784
2030
2440
6186
STII

Fig. 14B

CD19-ChARM[1]

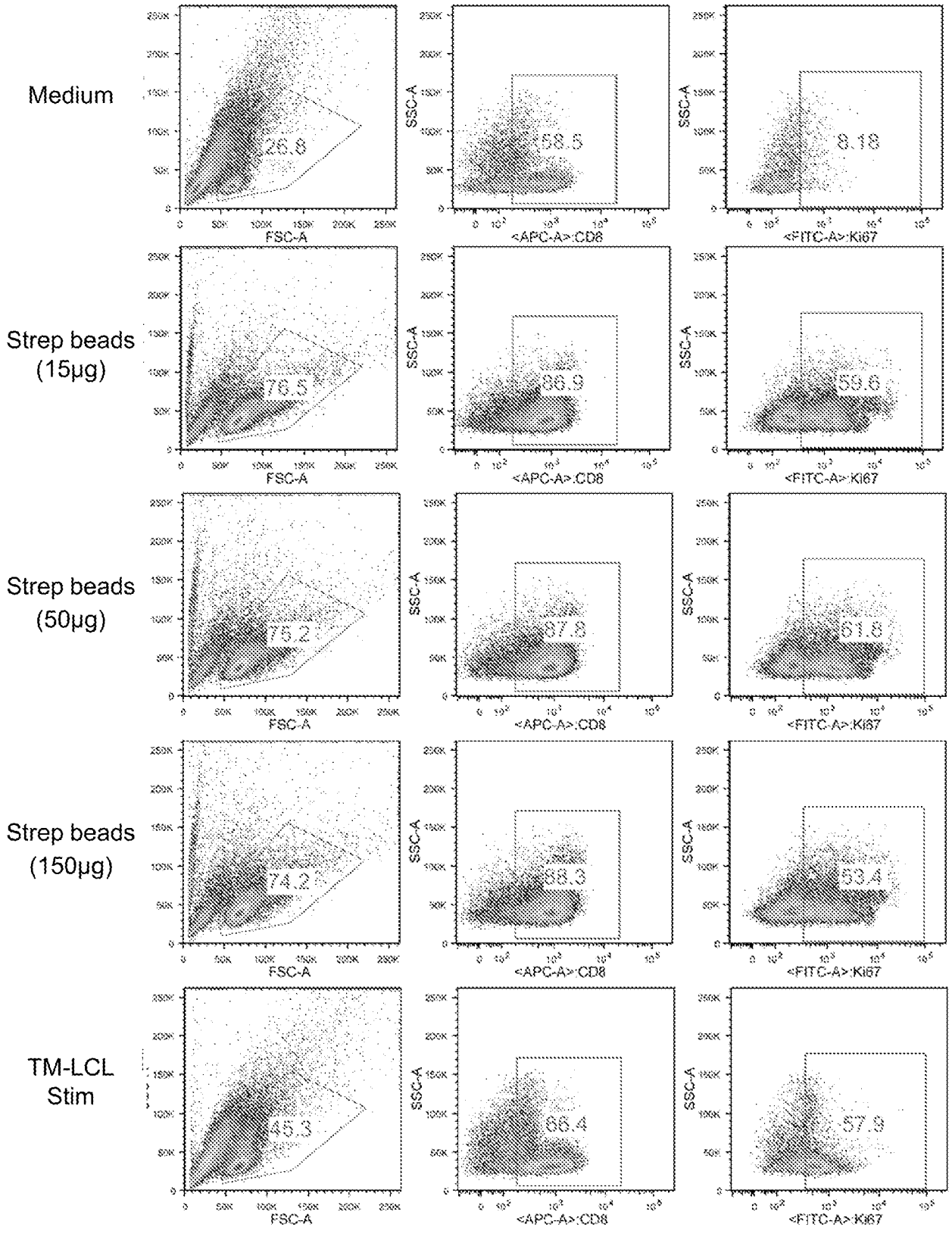


Fig. 15

CD19-ChARM²

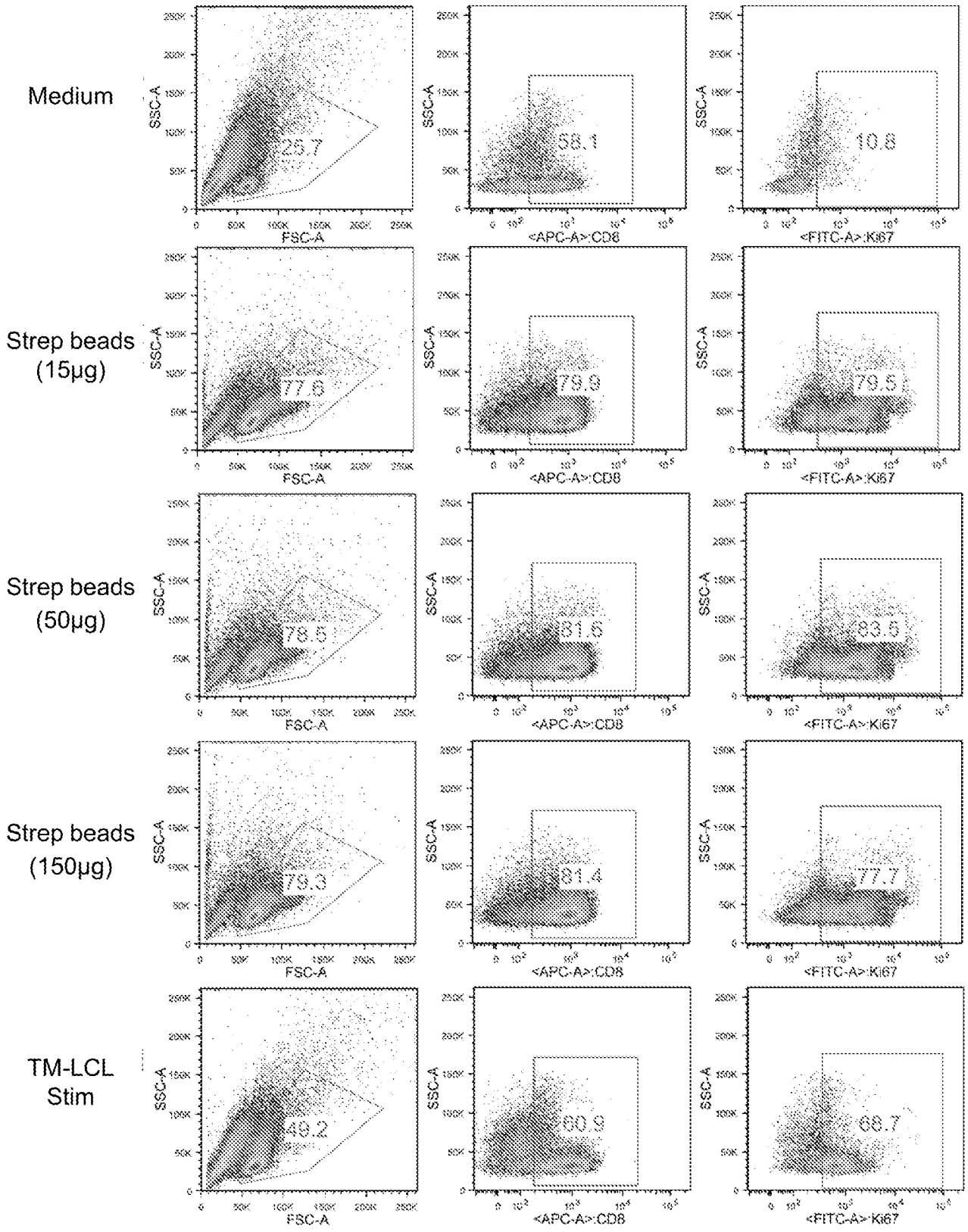


Fig. 15 *(Continued)*

CD19-ChARM^3

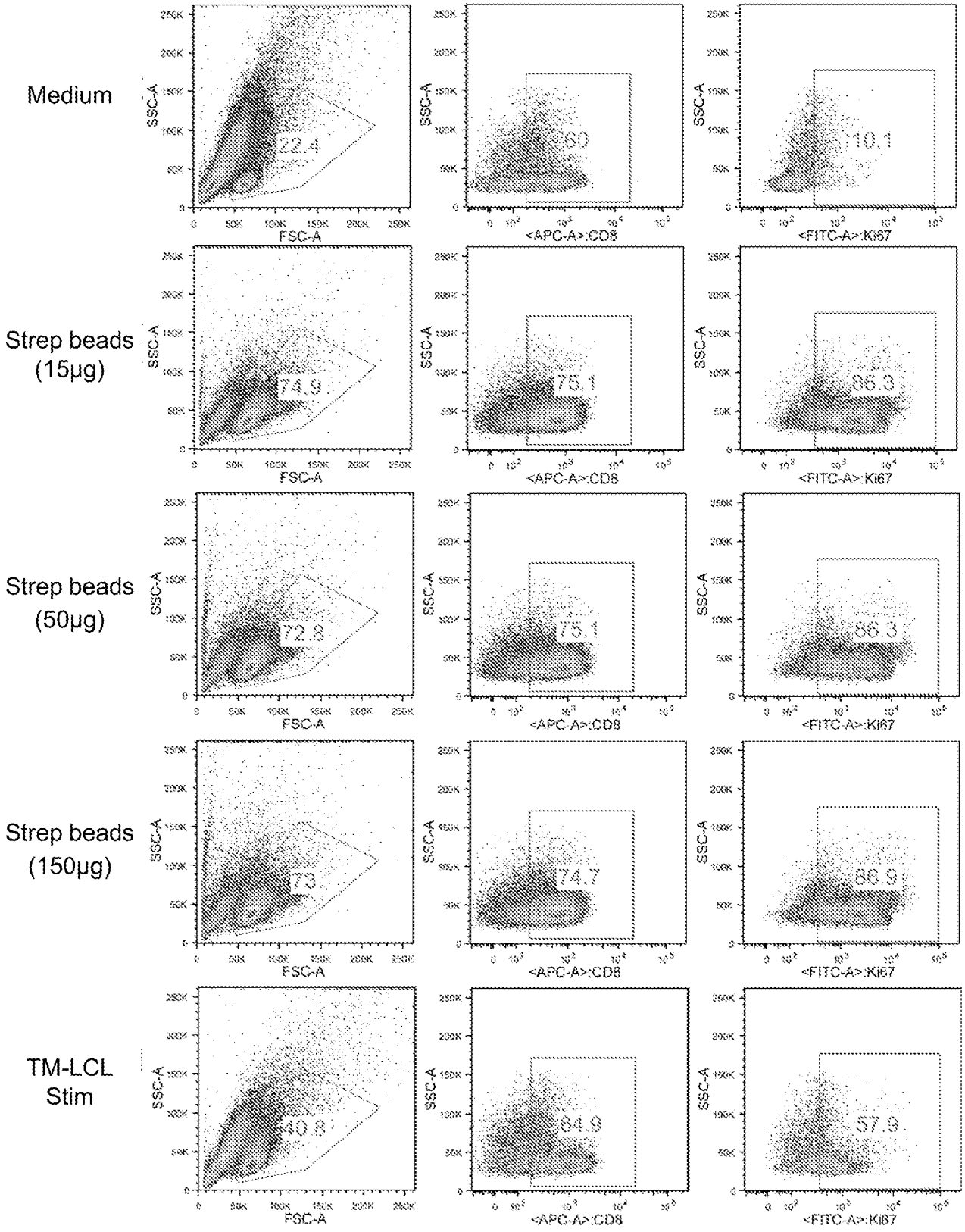


Fig. 15 *(Continued)*

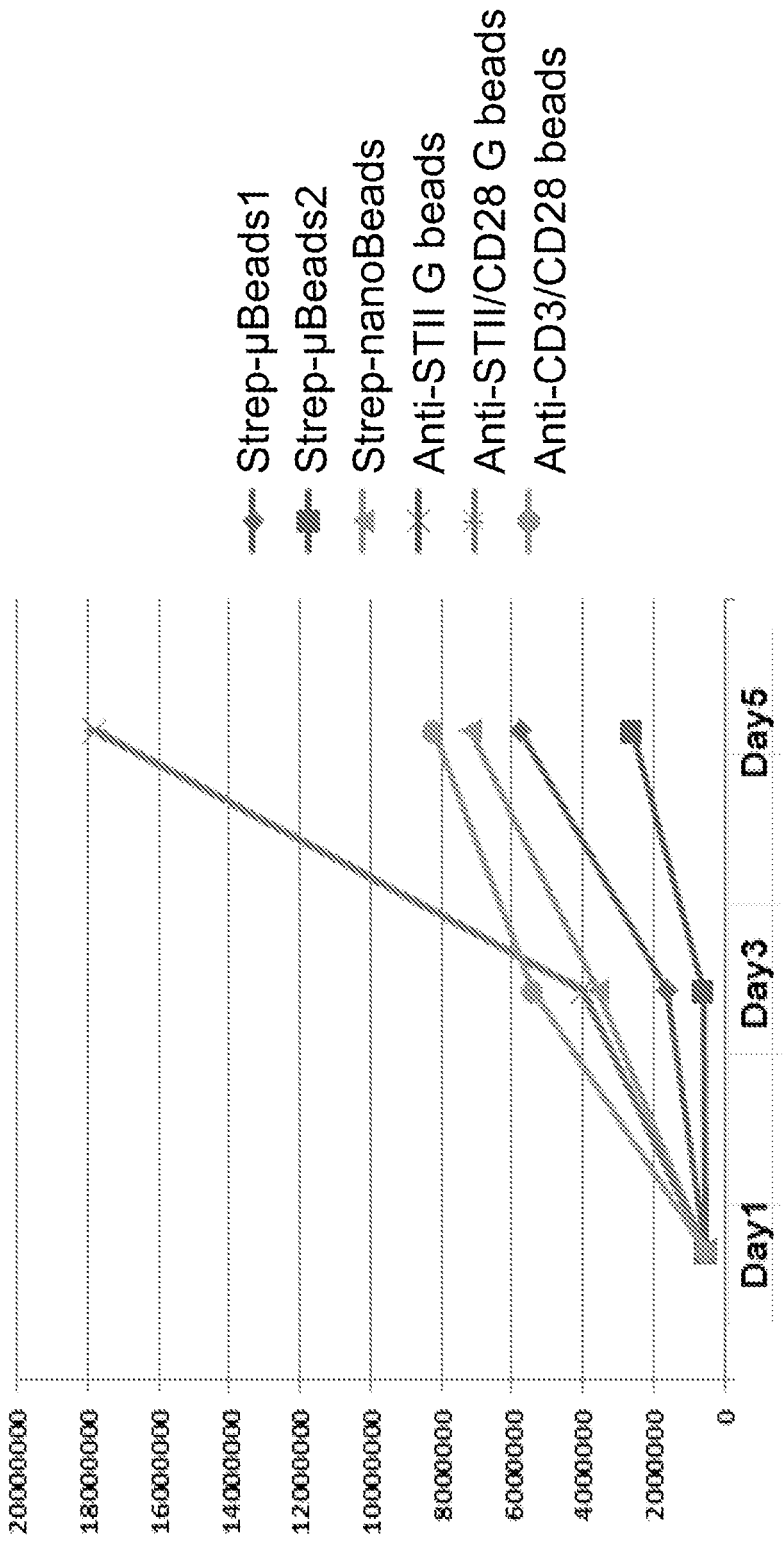

Strep-µBeads1
Strep-µBeads2
Strep-nanoBeads
Anti-STII G beads
Anti-STII/CD28 G beads
Anti-CD3/CD28 beads
20000000
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Day1
Day3
Day5

Fig. 16

A.

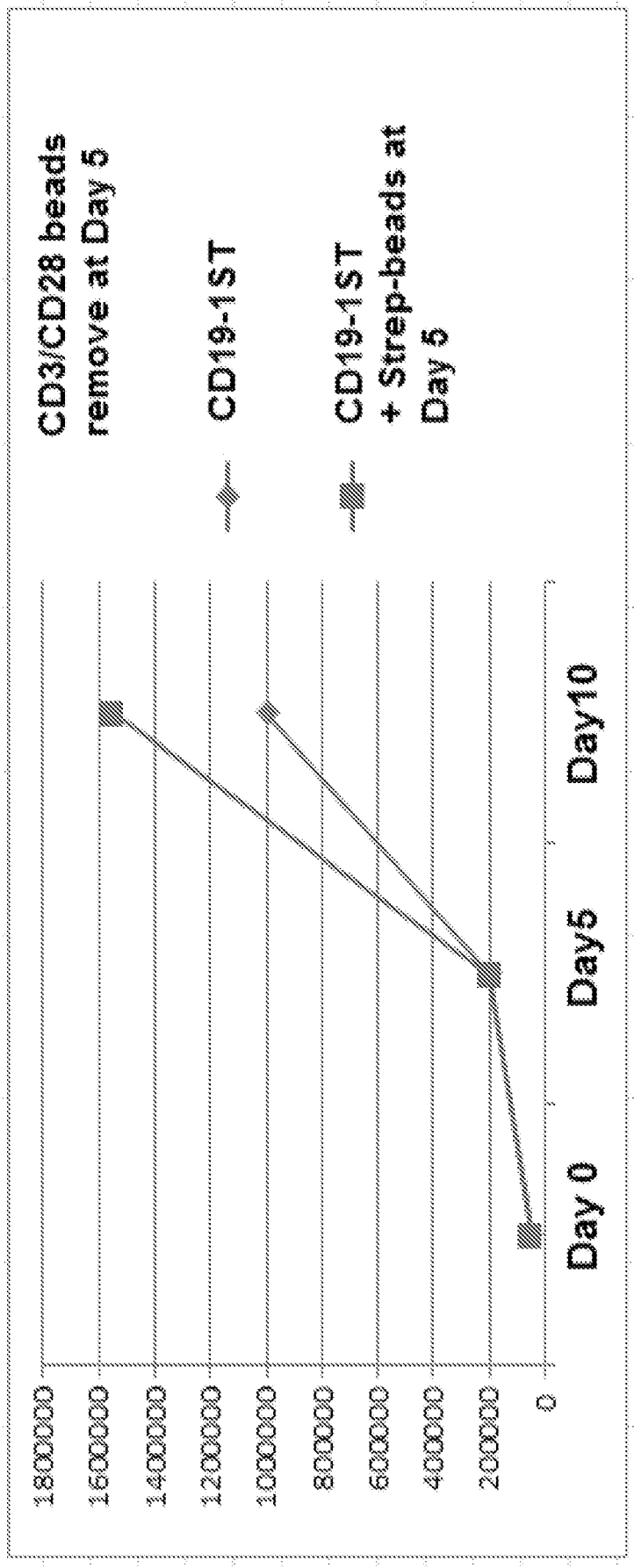

CD3/CD28 beads
remove at Day 5
CD19-1ST
CD19-1ST
+ Strep-beads at
Day 5
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Day 0
Day5
Day10

Fig. 17

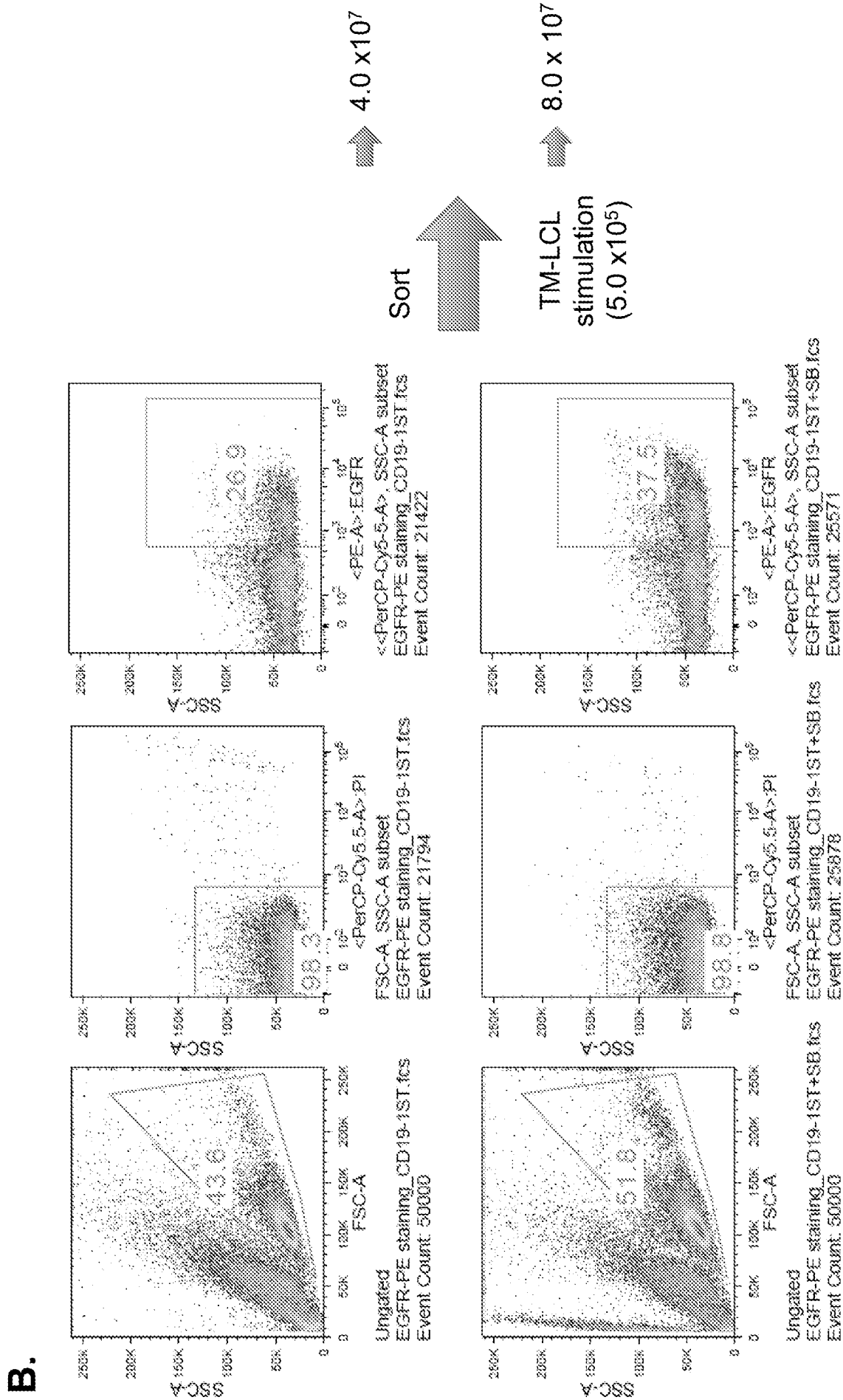

B.
Ungated
EGFR-PE staining_CD19-1ST.fcs
Event Count: 50000
FSC-A
SSC-A
43.6
FSC-A, SSC-A subset
EGFR-PE staining_CD19-1ST.fcs
Event Count: 21794
<PerCP-Cy5-5-A>:PI
98.3
<<PerCP-Cy5-5-A>, SSC-A subset
EGFR-PE staining_CD19-1ST.fcs
Event Count: 21422
<PE-A>:EGFR
20.9
Sort
4.0 x10^7
TM-LCL stimulation (5.0 x10^5)
8.0 x 10^7
Ungated
EGFR-PE staining_CD19-1ST+SB.fcs
Event Count: 50000
FSC-A
SSC-A
51.8
FSC-A, SSC-A subset
EGFR-PE staining_CD19-1ST+SB.fcs
Event Count: 25878
<PerCP-Cy5-5-A>:PI
98.8
<<PerCP-Cy5-5-A>, SSC-A subset
EGFR-PE staining_CD19-1ST+SB.fcs
Event Count: 25571
<PE-A>:EGFR
37.5

Fig. 17

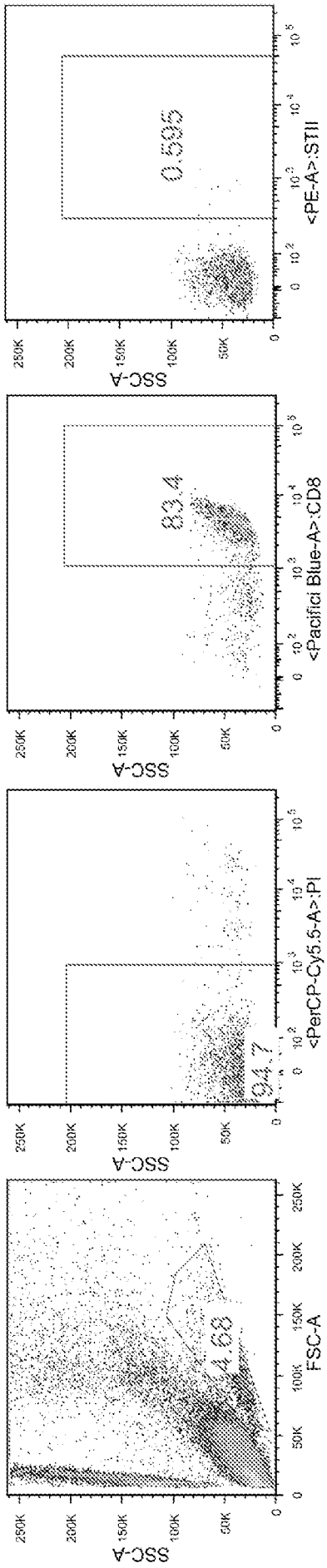

FSC-A
SSC-A
4.68
94.7
<PerCP-Cy5.5-A>:PI
83.4
<Pacific Blue-A>:CD8
0.595
<PE-A>:STII

Fig. 18A

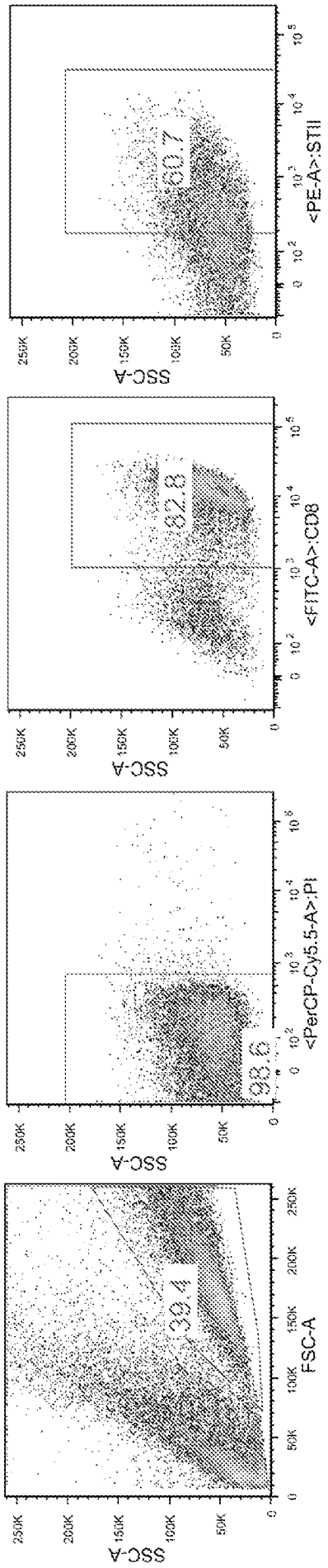

SSC-A
FSC-A
39.4
SSC-A
<PerCP-Cy5.5-A>:PI
98.6
SSC-A
<FITC-A>:CD8
82.8
SSC-A
<PE-A>:STII
60.7

Fig. 18B

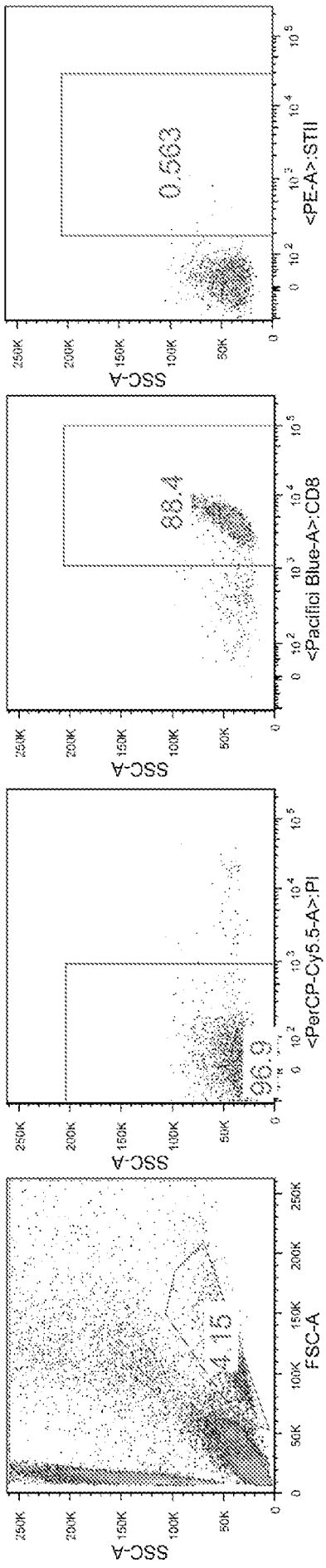

FSC-A
SSC-A
4.15
<PerCP-Cy5.5-A>:PI
96.9
<Pacific Blue-A>:CD8
88.4
<PE-A>:STII
0.563

Fig. 18C

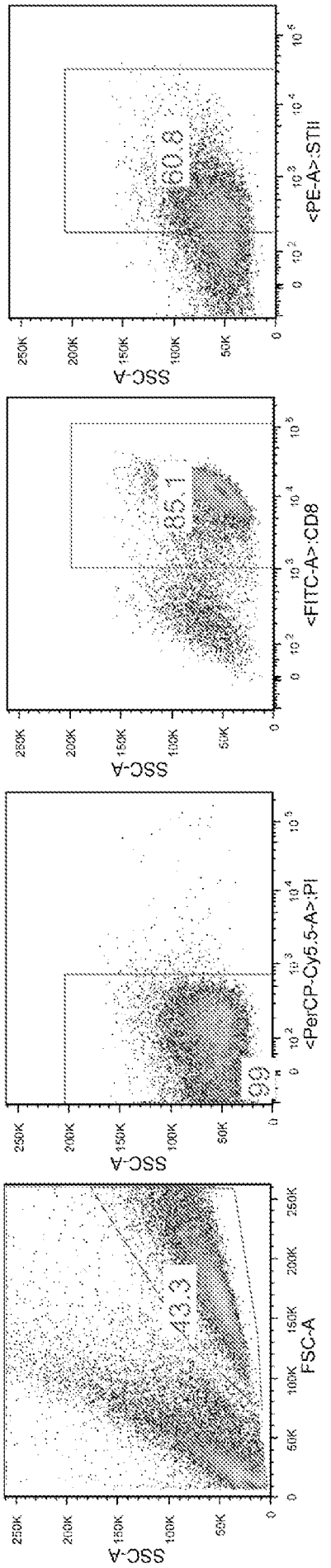


Fig. 18D

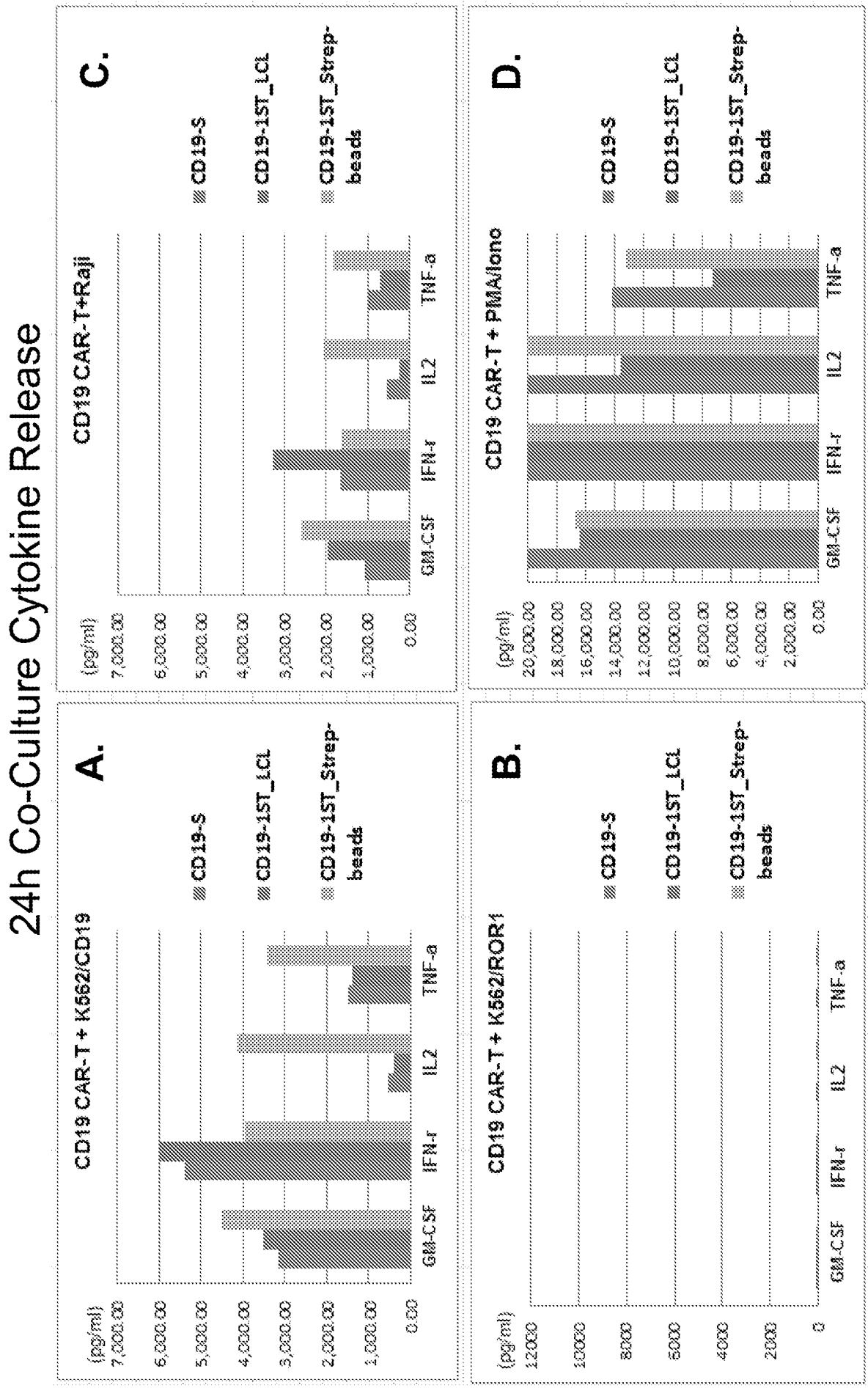

24h Co-Culture Cytokine Release
A.
CD19 CAR-T + K562/CD19
(pg/ml)
7,000.00
6,000.00
5,000.00
4,000.00
3,000.00
2,000.00
1,000.00
0.00
GM-CSF
IFN-r
IL2
TNF-a
CD19-S
CD19-1ST_LCL
CD19-1ST_Strep-beads
B.
CD19 CAR-T + K562/ROR1
(pg/ml)
12000
10000
8000
6000
4000
2000
0
C.
CD19 CAR-T+Raji
D.
CD19 CAR-T + PMA/Iono
(pg/ml)
20,000.00
18,000.00
16,000.00
14,000.00
12,000.00
10,000.00
8,000.00
6,000.00
4,000.00
2,000.00
0.00

Fig. 19

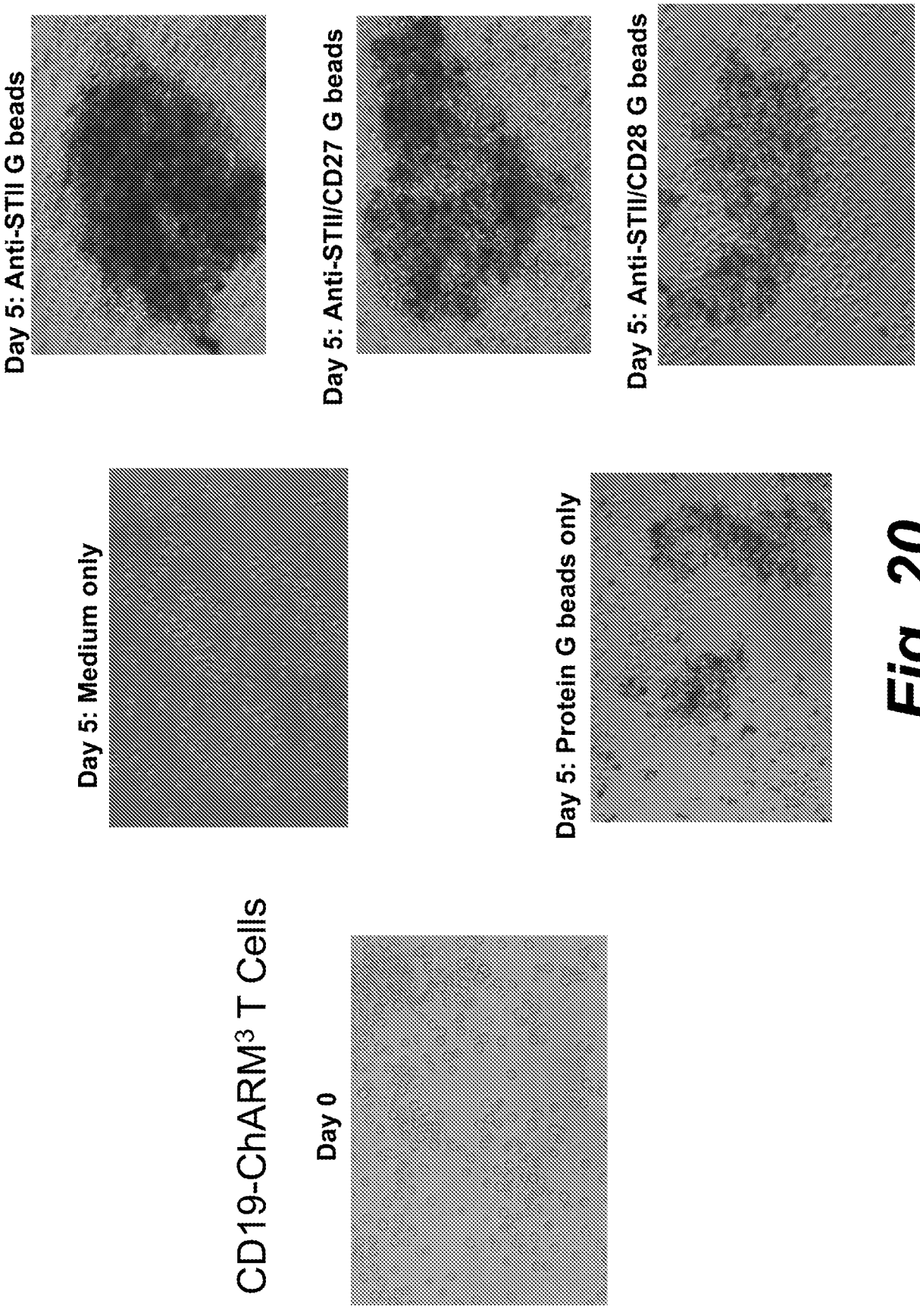

Day 5: Anti-STII G beads
Day 5: Anti-STII/CD27 G beads
Day 5: Anti-STII/CD28 G beads
Day 5: Medium only
Day 5: Protein G beads only
CD19-ChARM3 T Cells
Day 0

Fig. 20

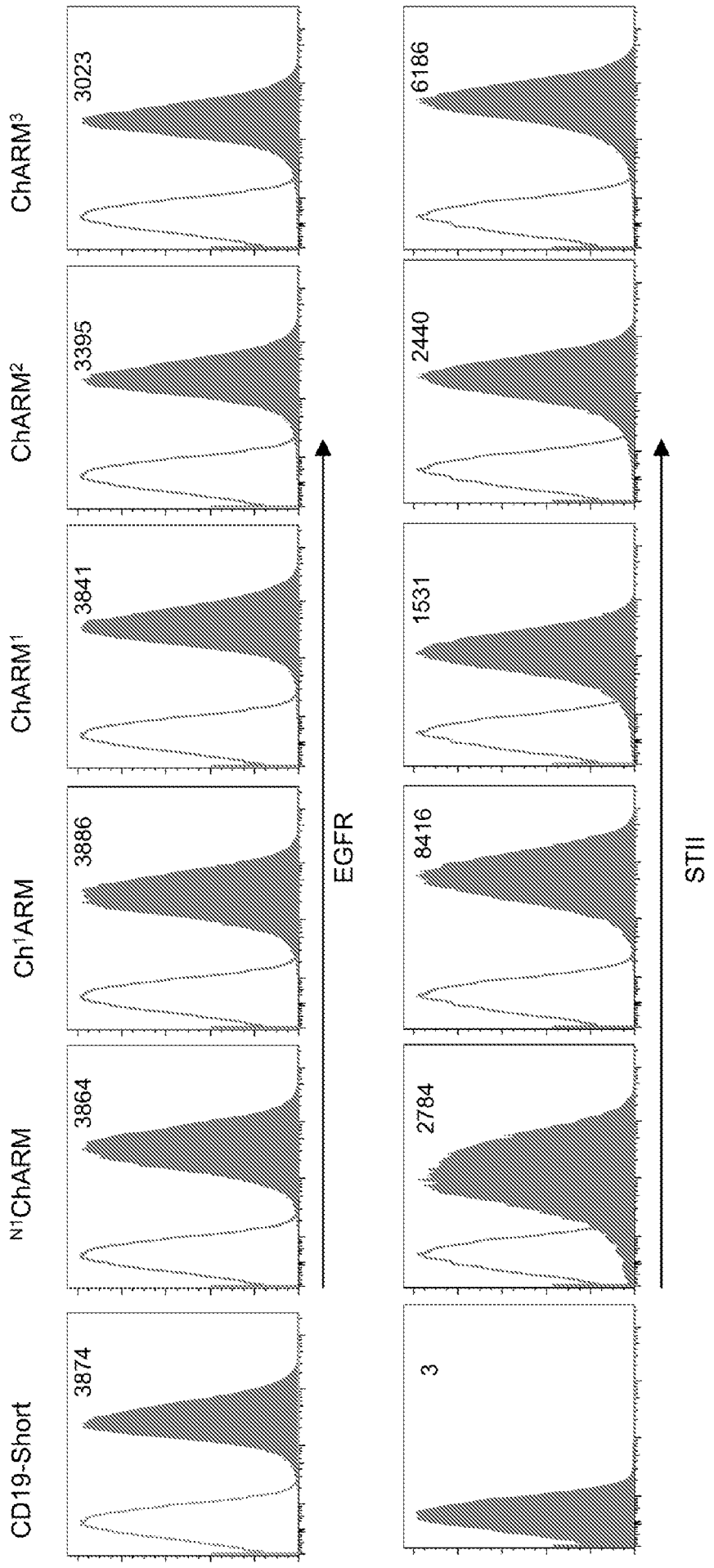

CD19-Short
N1ChARM
Ch1ARM
ChARM1
ChARM2
ChARM3
3874
3864
3886
3841
3395
3023
EGFR
3
2784
8416
1531
2440
6186
STII

Fig. 21

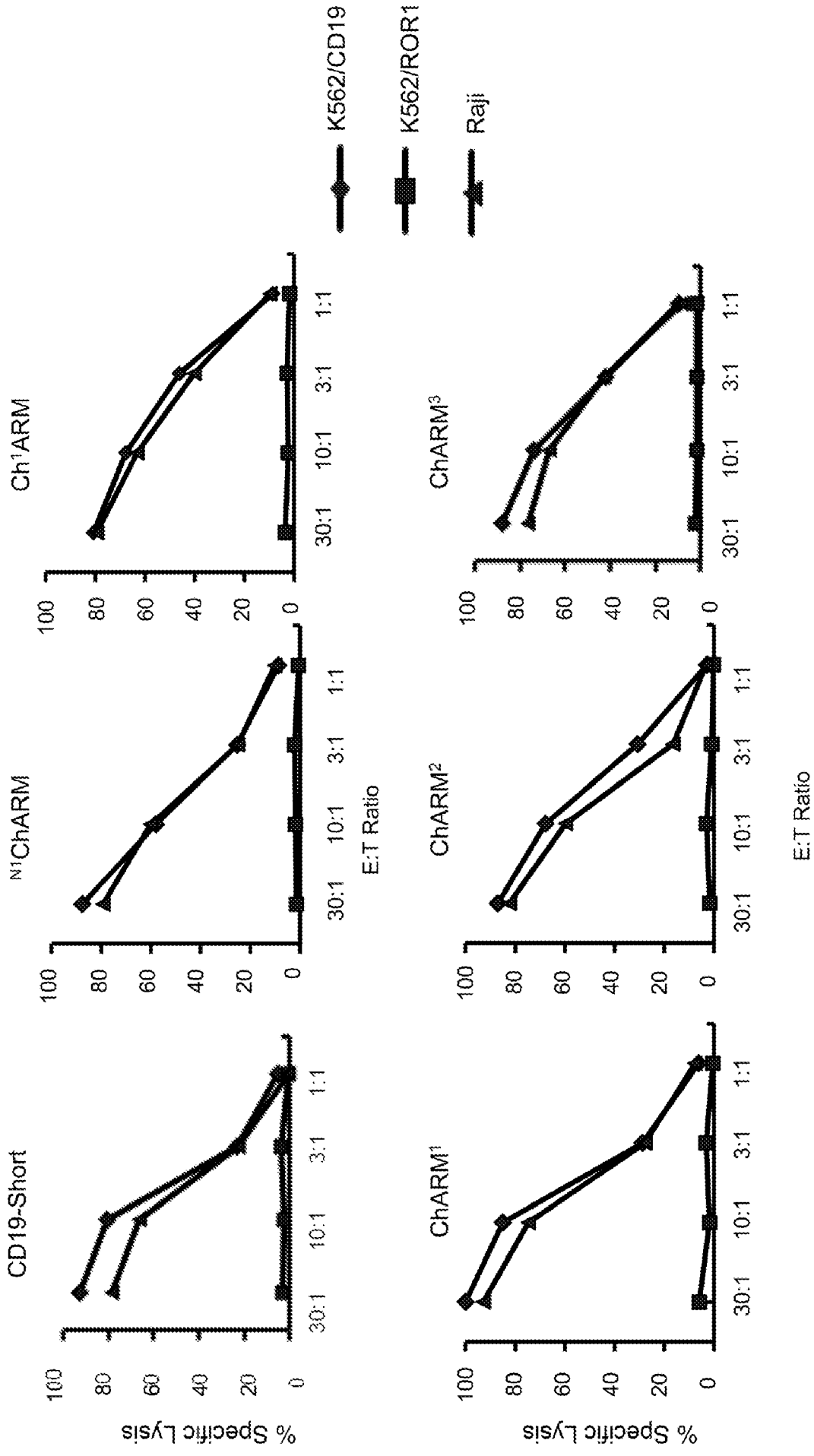

K562/CD19
K562/ROR1
Raji
CD19-Short
N1ChARM
Ch1ARM
ChARM1
ChARM2
ChARM3
% Specific Lysis
E:T Ratio
100
80
60
40
20
0
30:1
10:1
3:1
1:1

Fig. 22

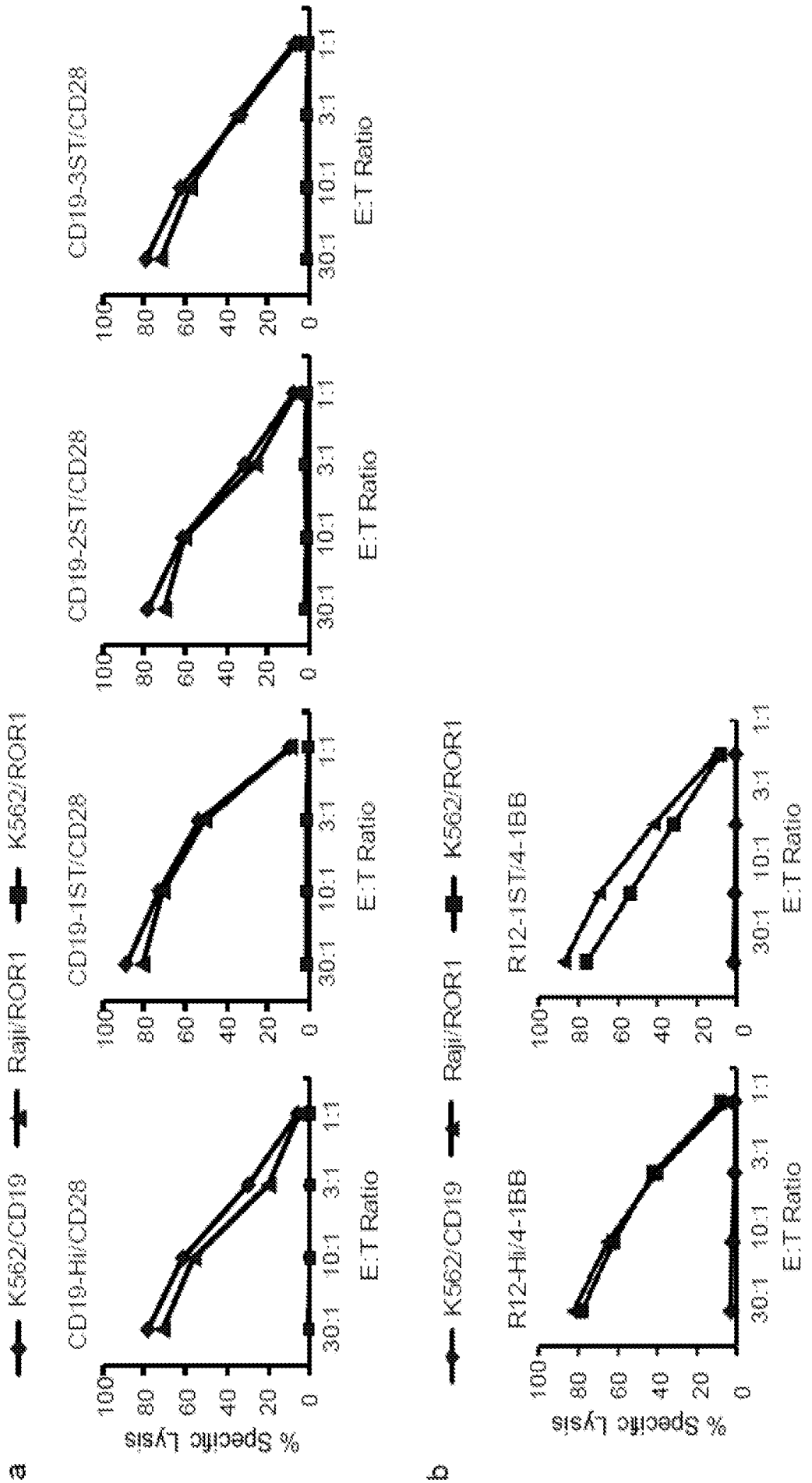

a
K562/CD19
Raji/ROR1
K562/ROR1
CD19-Hi/CD28
CD19-1ST/CD28
CD19-2ST/CD28
CD19-3ST/CD28
% Specific Lysis
E:T Ratio
30:1
10:1
3:1
1:1
0
20
40
60
80
100
b
K562/CD19
Raji/ROR1
K562/ROR1
R12-Hi/4-1BB
R12-1ST/4-1BB
% Specific Lysis
E:T Ratio

Fig. 23

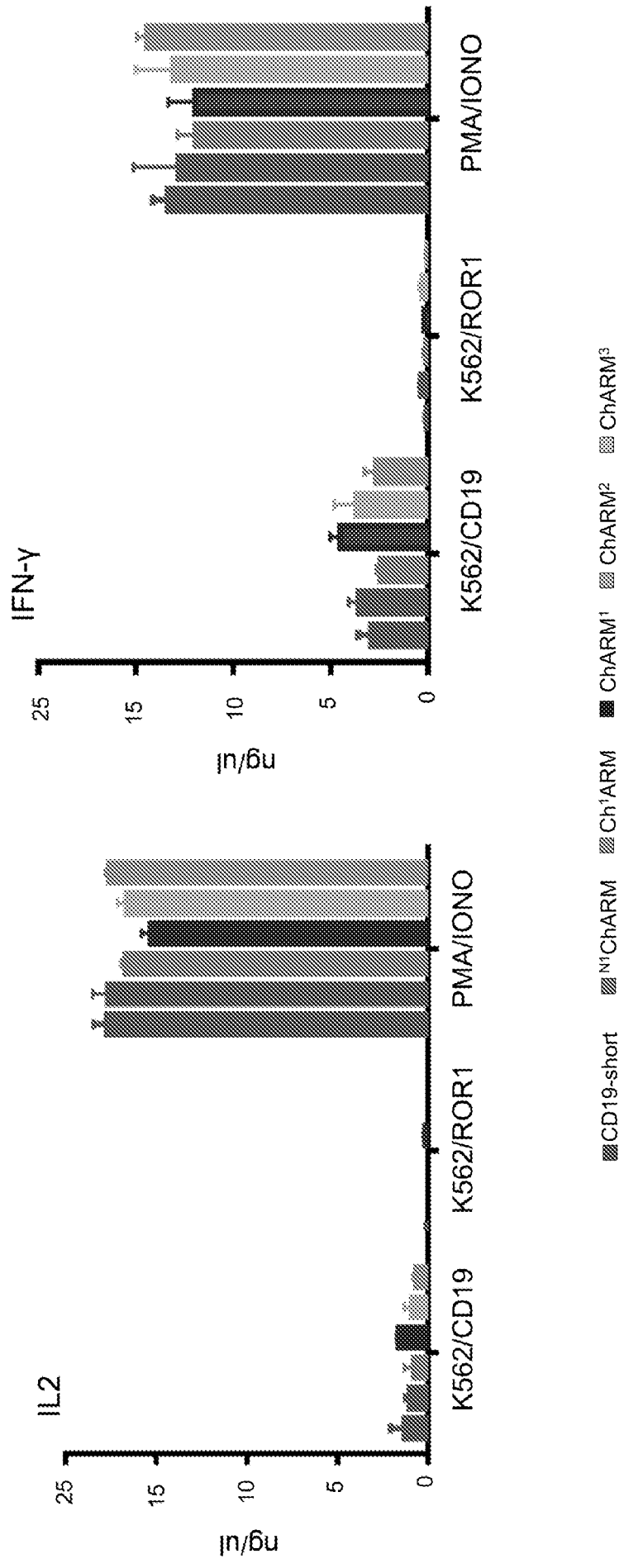

IFN-γ
IL2
pg/ul
25
15
10
5
0
K562/CD19
K562/ROR1
PMA/IONO
CD19-short
N1ChARM
Ch1ARM
ChARM1
ChARM2
ChARM3

Fig. 24

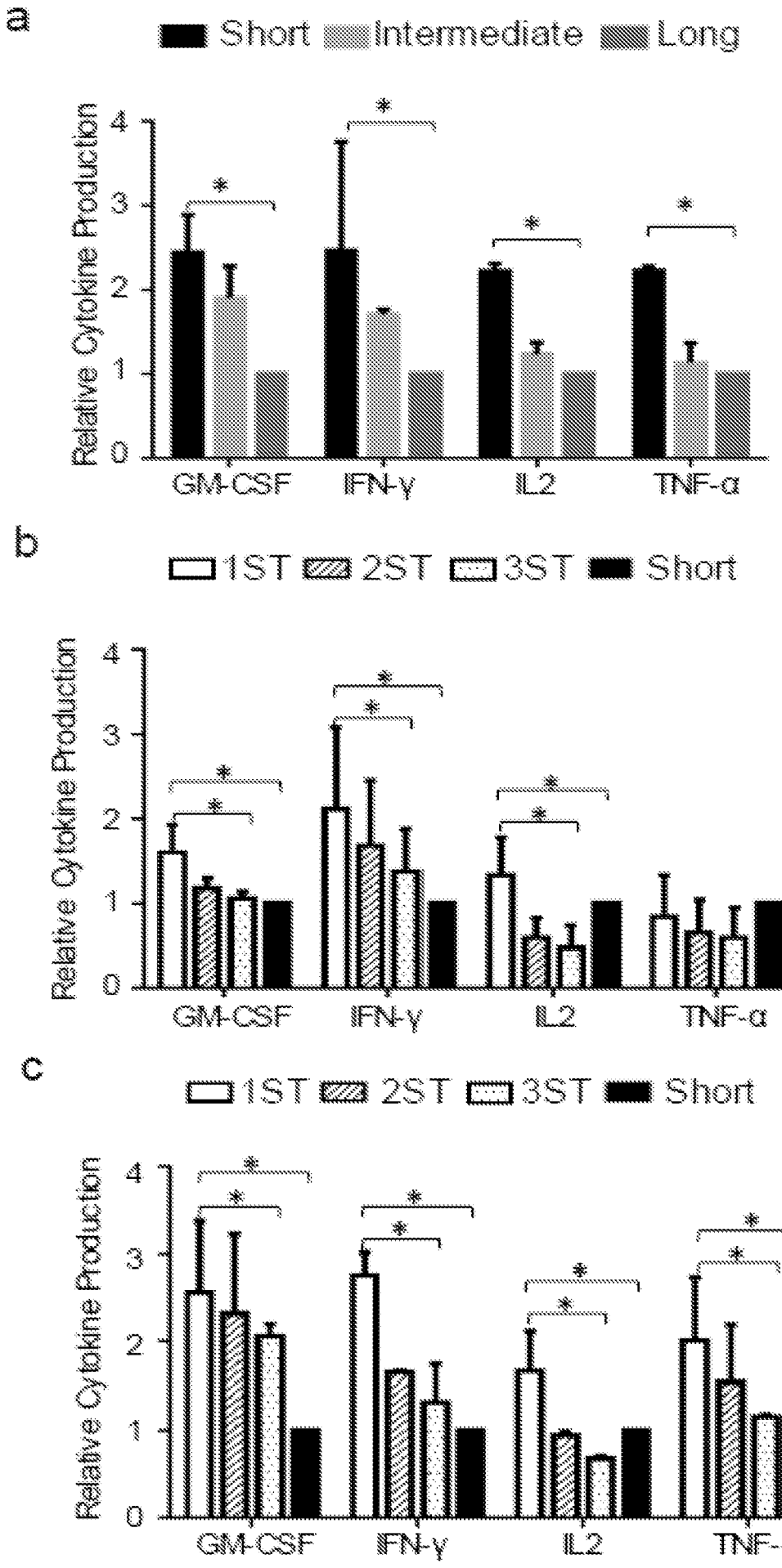

a
Short
Intermediate
Long
Relative Cytokine Production
0
1
2
3
4
GM-CSF
IFN-γ
IL2
TNF-α
b
1ST
2ST
3ST
Short
Relative Cytokine Production
GM-CSF
IFN-γ
IL2
TNF-α
c
1ST
2ST
3ST
Short
Relative Cytokine Production
GM-CSF
IFN-γ
IL2
TNF-α

Fig. 25

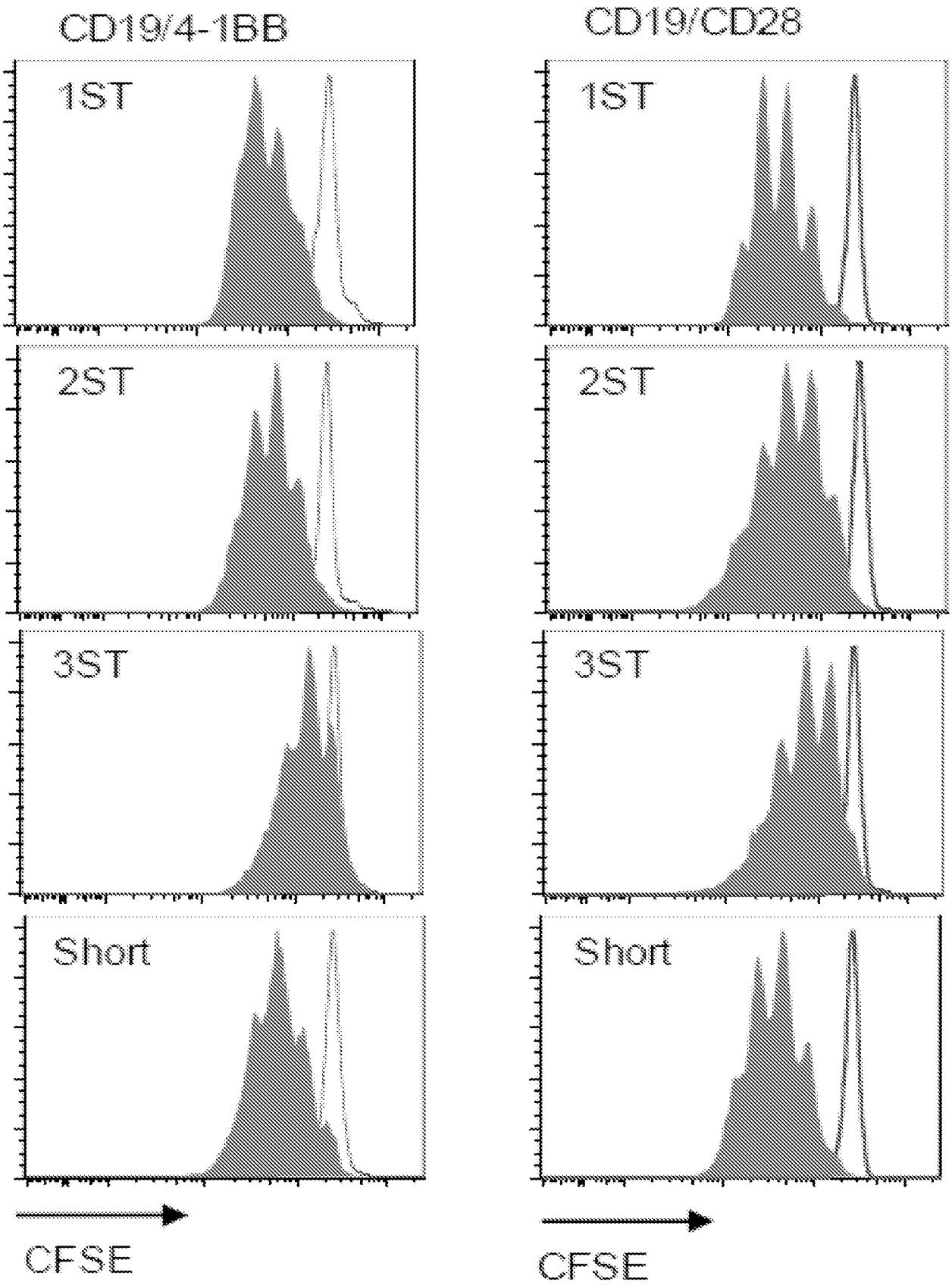

CD19/4-1BB
CD19/CD28
1ST
2ST
3ST
Short
1ST
2ST
3ST
Short
CFSE
CFSE

Fig. 26

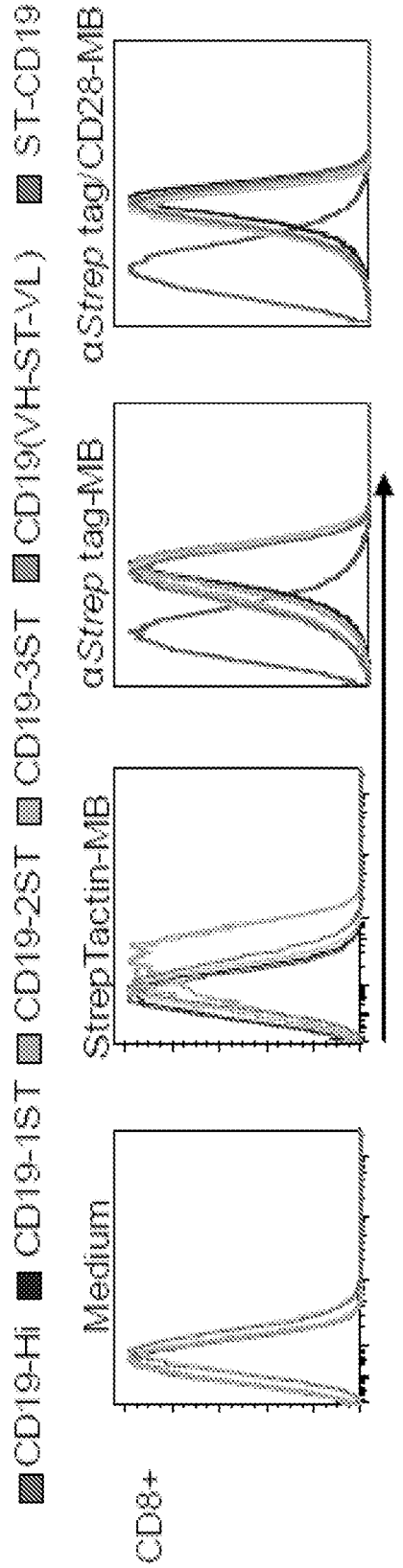


Fig. 27A

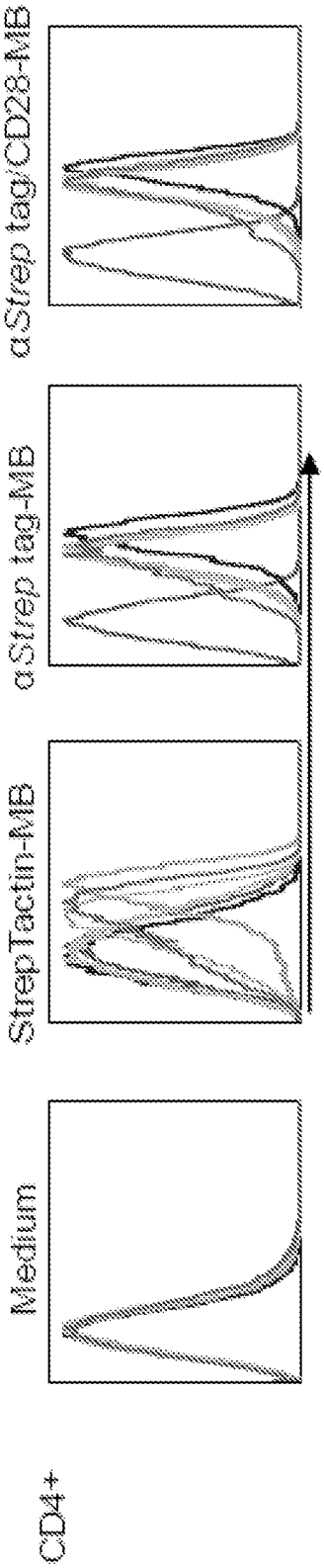


Fig. 27B

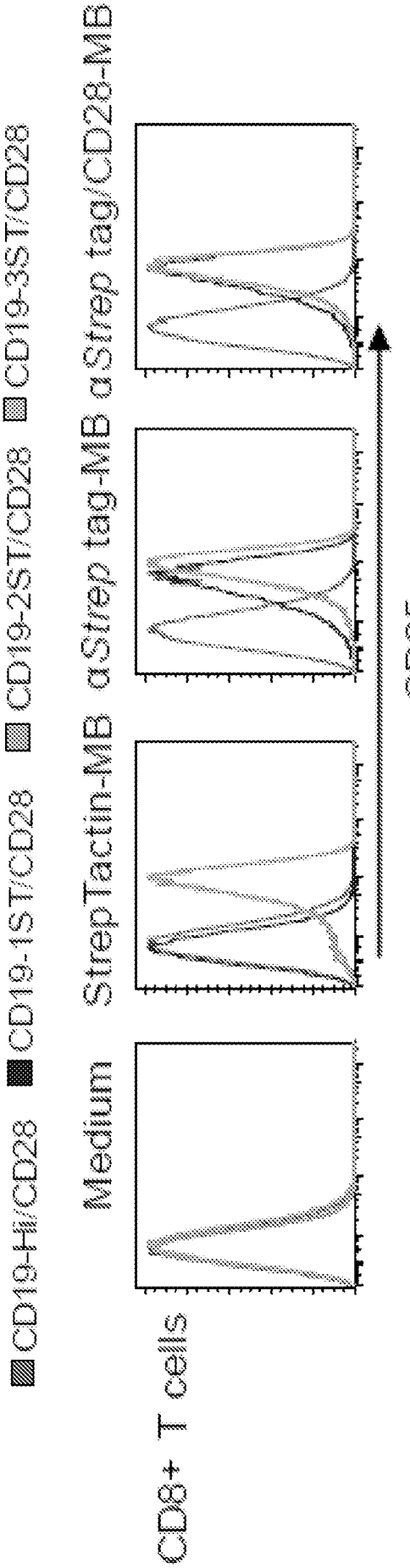


Fig. 27C

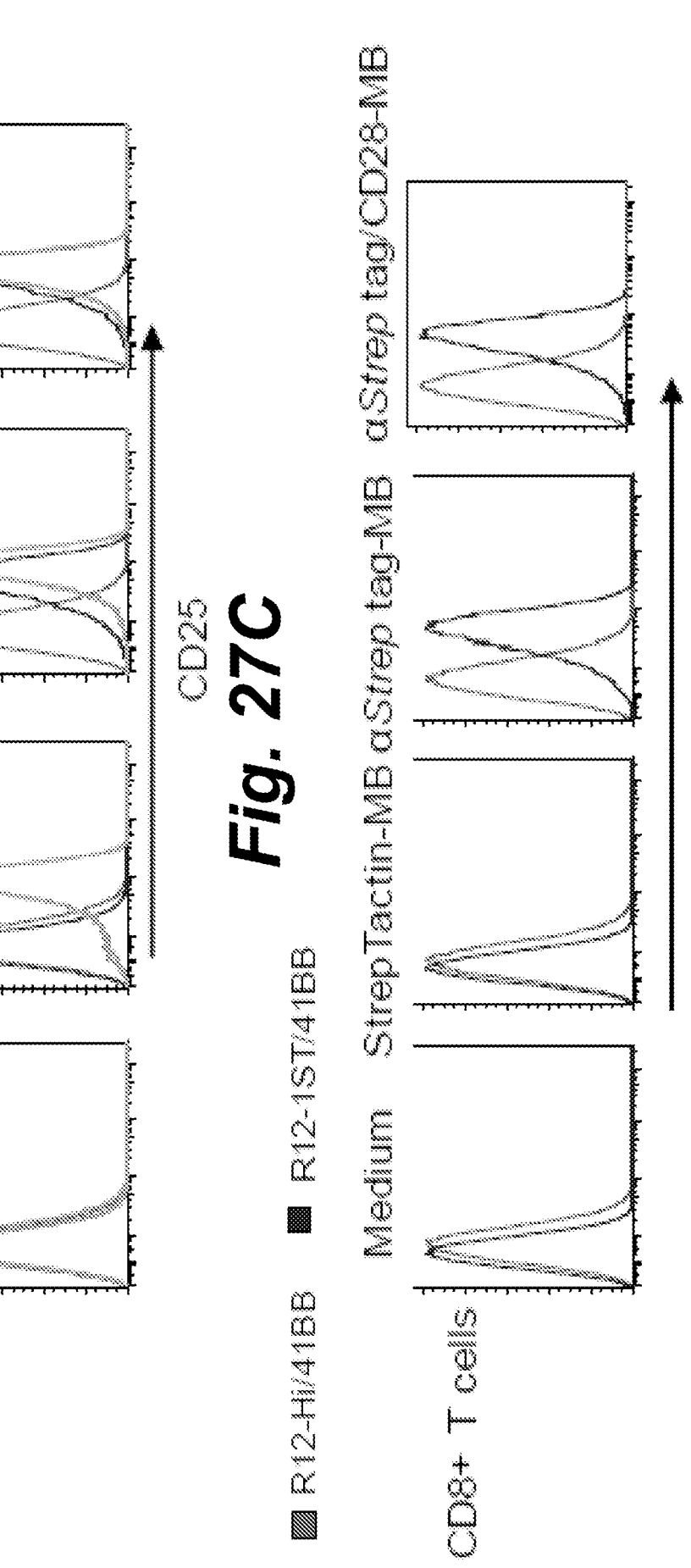


Fig. 27D

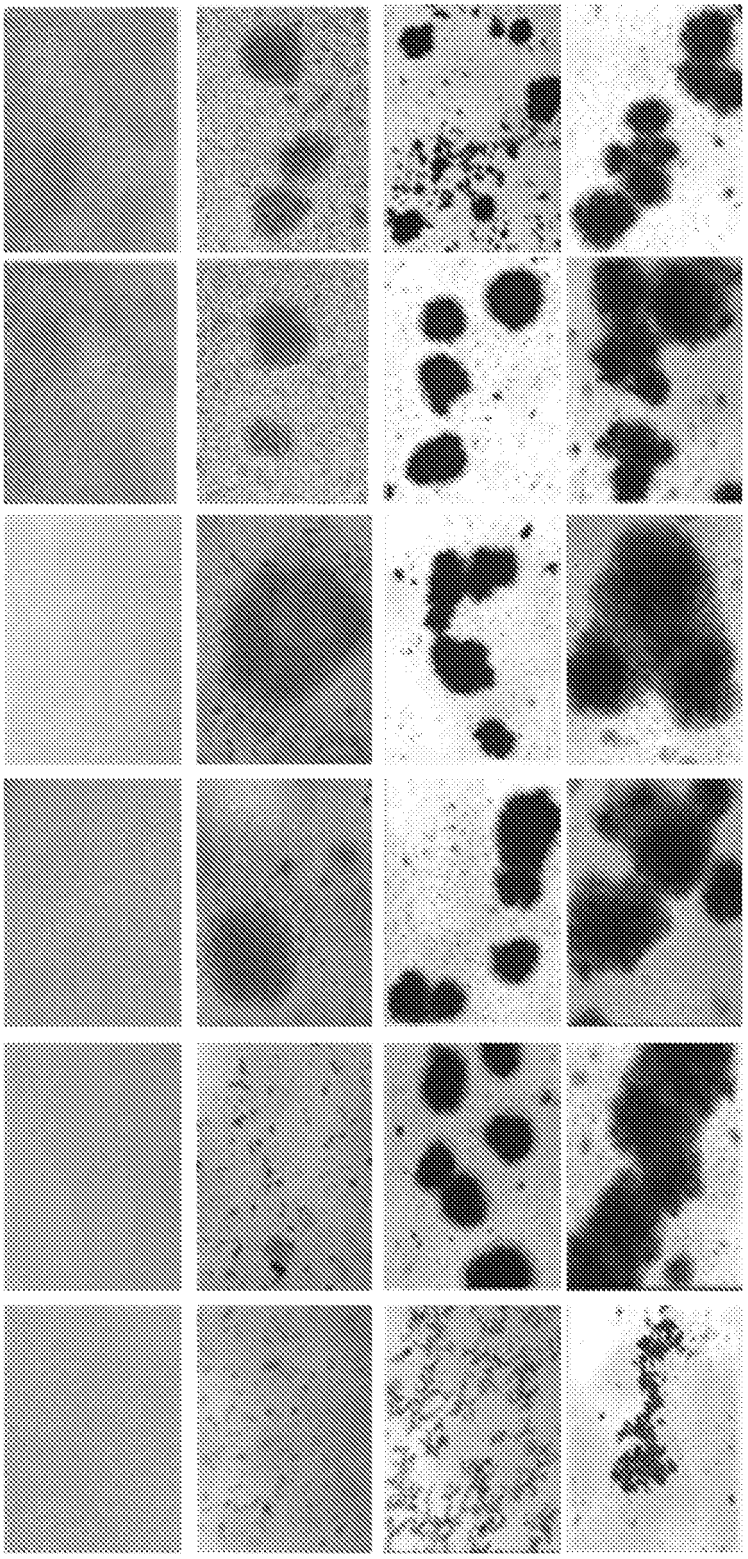

CD19-Hi
CD19-1ST
CD19-2ST
CD19-3ST
CD19(VH-ST-VL)
ST-CD19
Medium
StrepTactin-MB
αStrep tag-MB
αStrep tag/CD28-MB

Fig. 28

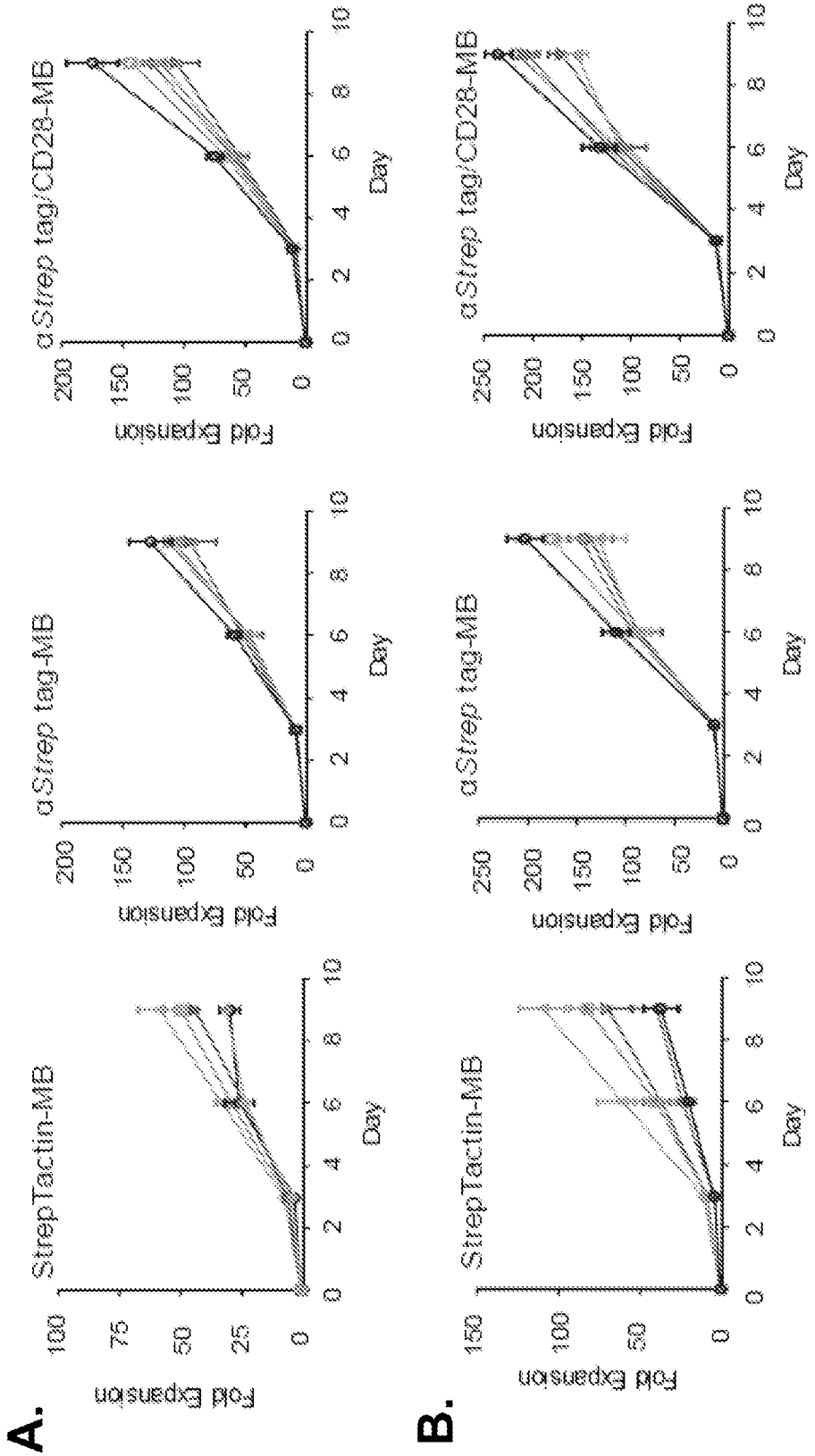

A.
B.
StrepTactin-MB
αStrep tag-MB
αStrep tag/CD28-MB
Fold Expansion
Day
CD19-1ST
CD19-2ST
CD19-3ST
CD19(VH-ST-VL)
ST-CD19

Fig. 29

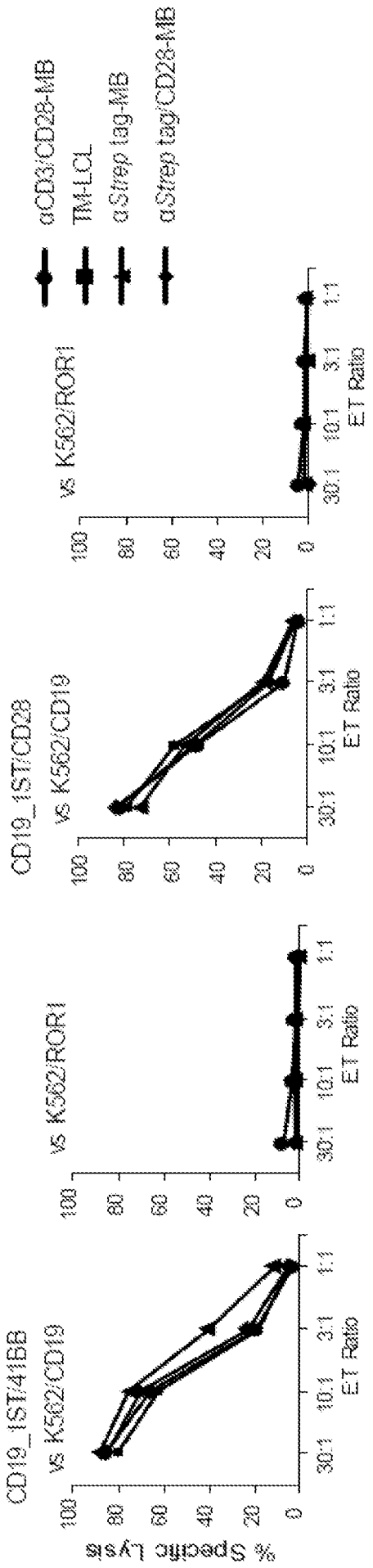

% Specific Lysis
CD19_1ST/41BB
vs K562/CD19
CD19_1ST/CD28
vs K562/CD19
vs K562/ROR1
vs K562/ROR1
αCD3/CD28-MB
TM-LCL
αStrep tag-MB
αStrep tag/CD28-MB
E:T Ratio
30:1
10:1
3:1
1:1
0
20
40
60
80
100

Fig. 30A

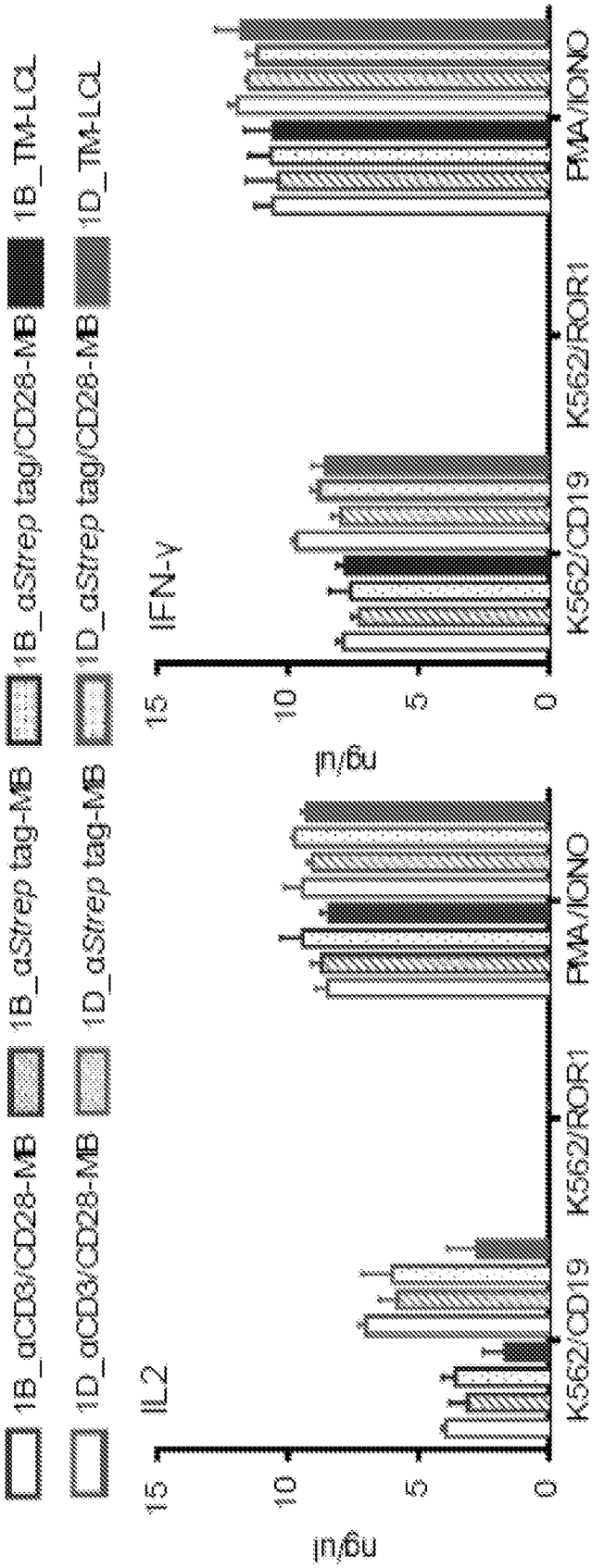

1B_αCD3/CD28-MB
1B_αStrep tag-MB
1B_αStrep tag/CD28-MB
1B_TM-LCL
1D_αCD3/CD28-MB
1D_αStrep tag-MB
1D_αStrep tag/CD28-MB
1D_TM-LCL
IL2
IFN-γ
ng/ul
15
10
5
0
K562/CD19
K562/ROR1
PMA/IONO

Fig. 30B

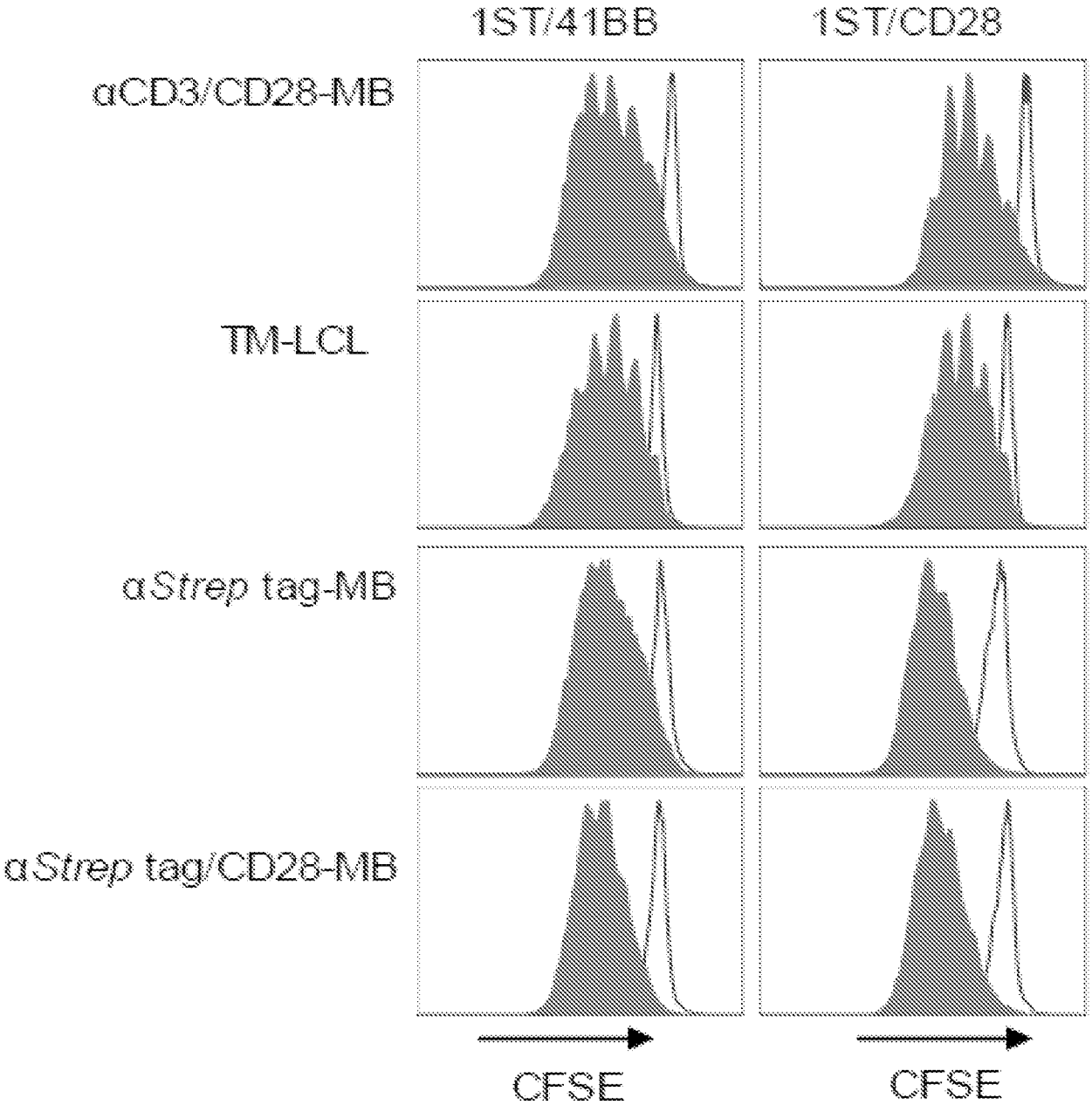

1ST/41BB
1ST/CD28
αCD3/CD28-MB
TM-LCL
αStrep tag-MB
αStrep tag/CD28-MB
CFSE
CFSE

Fig. 30C

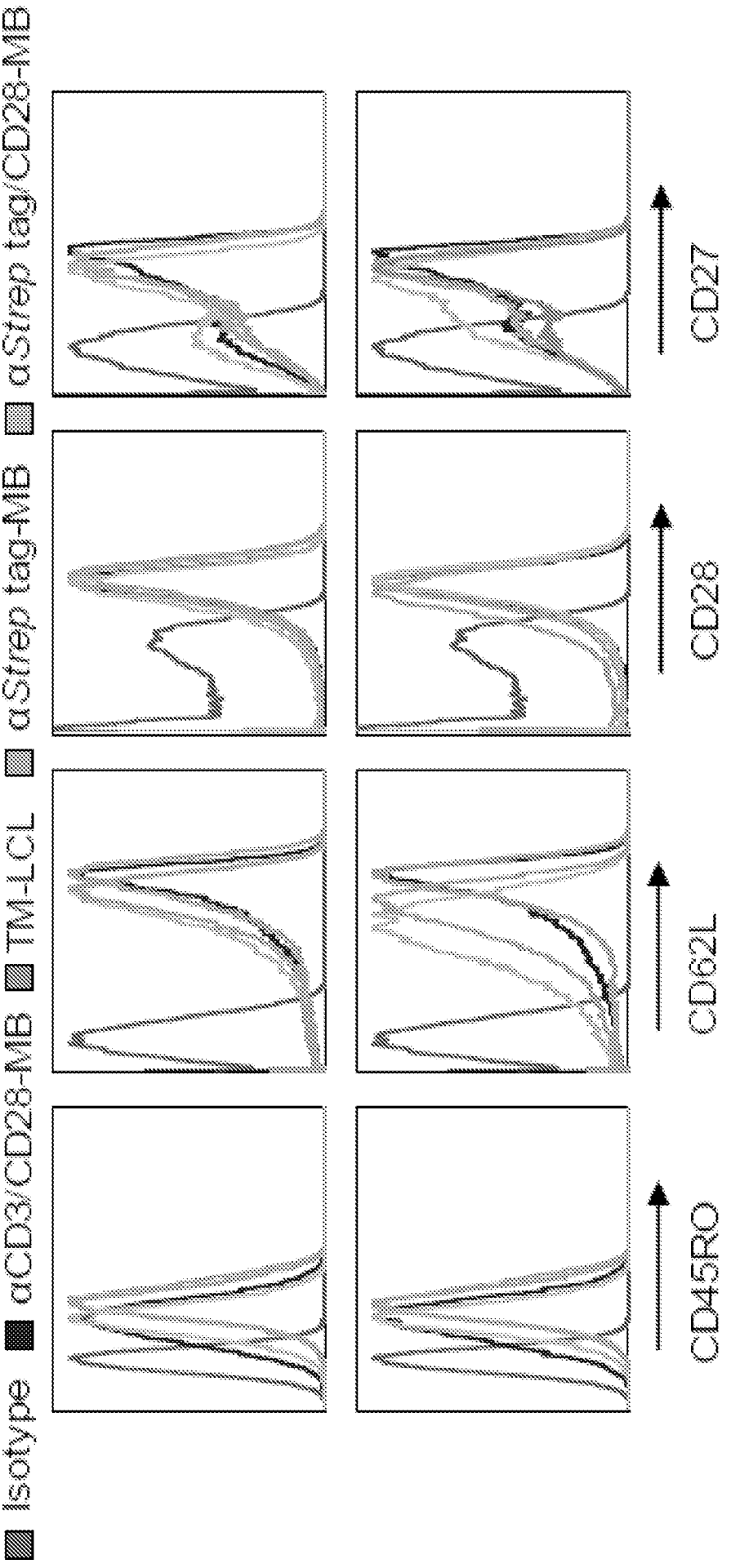

Isotype
αCD3/CD28-MB
TM-LCL
αStrep tag-MB
αStrep tag/CD28-MB
CD45RO
CD62L
CD28
CD27

Fig. 30D

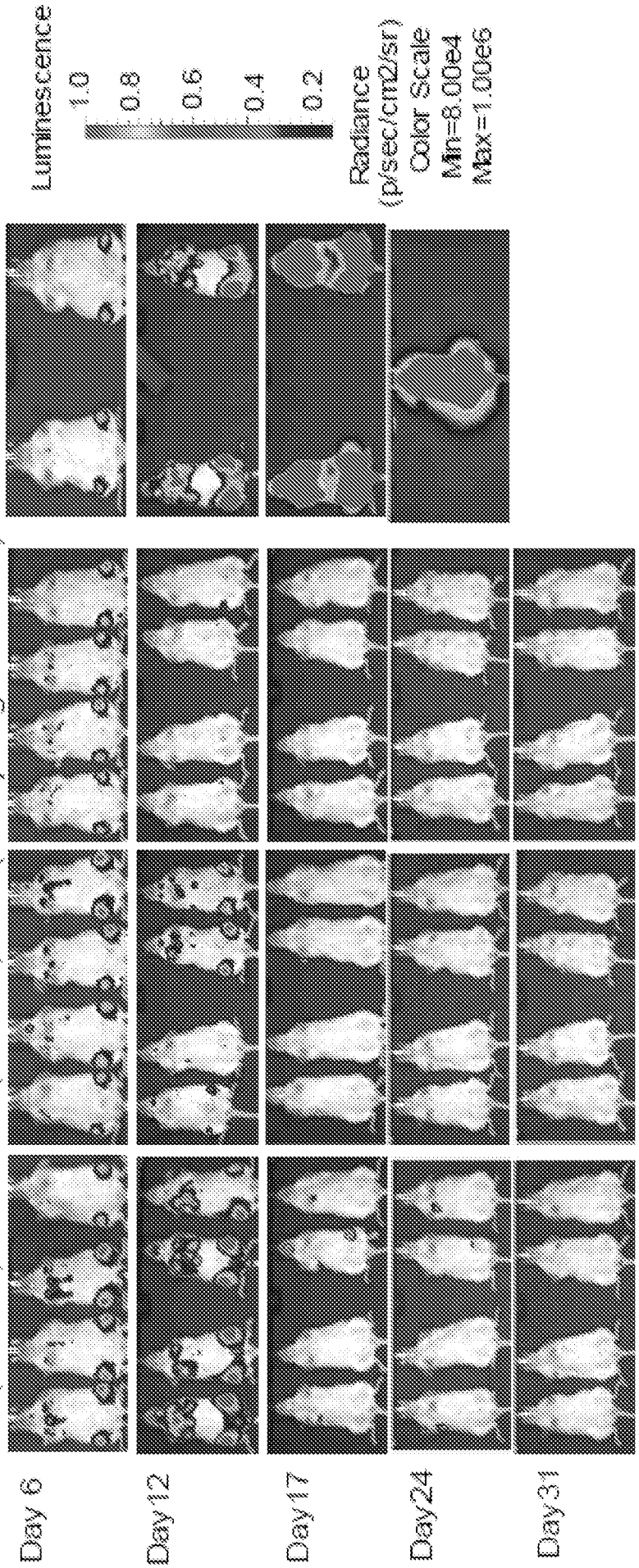

CD19-Hi/41BB (TM-LCL)
CD19-1ST/41BB (TM-LCL)
CD19-1ST/41BB (aStrep Tag/CD28-MB)
CON
Luminescence
1.0
0.8
0.6
0.4
0.2
Radiance (p/sec/cm2/sr)
Color Scale
Min=8.00e4
Max=1.00e6
Day 6
Day12
Day17
Day24
Day31

Fig. 30E

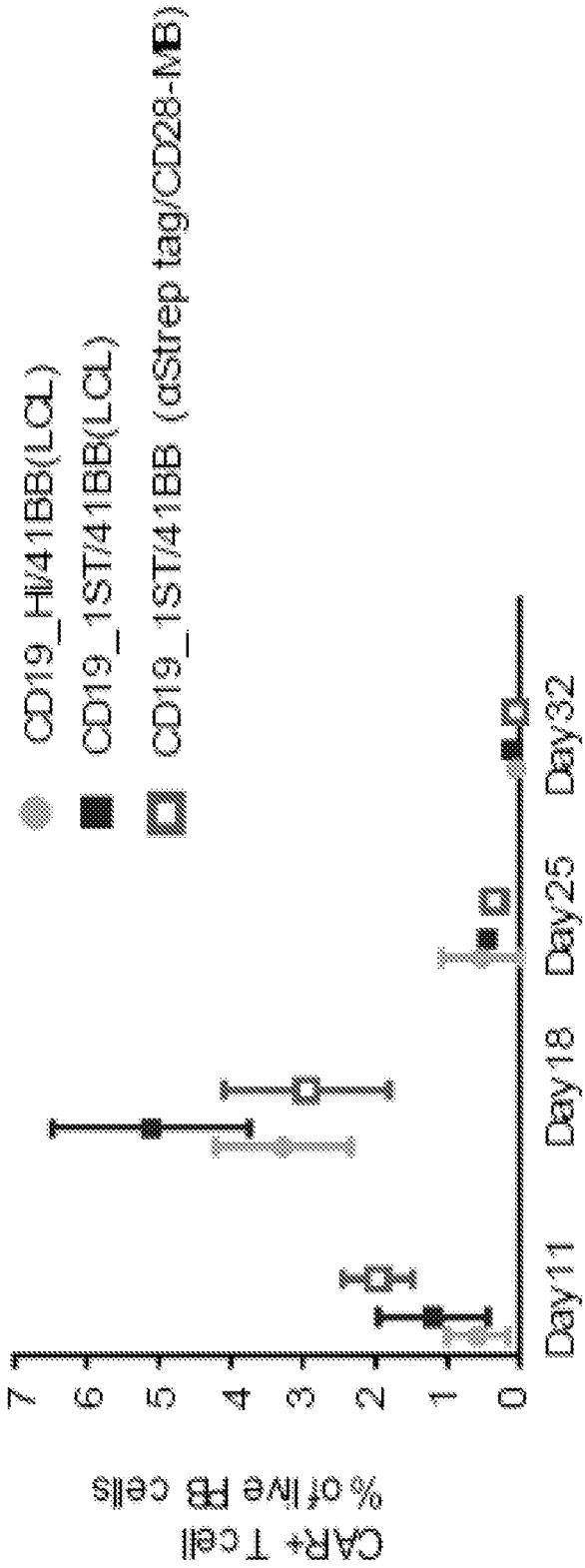

CD19_H/41BB(LCL)
CD19_1ST/41BB(LCL)
CD19_1ST/41BB (αStrep tag/CD28-MB)
CAR+ T cell
% of live PB cells
0
1
2
3
4
5
6
7
Day11
Day18
Day25
Day32

Fig. 30F

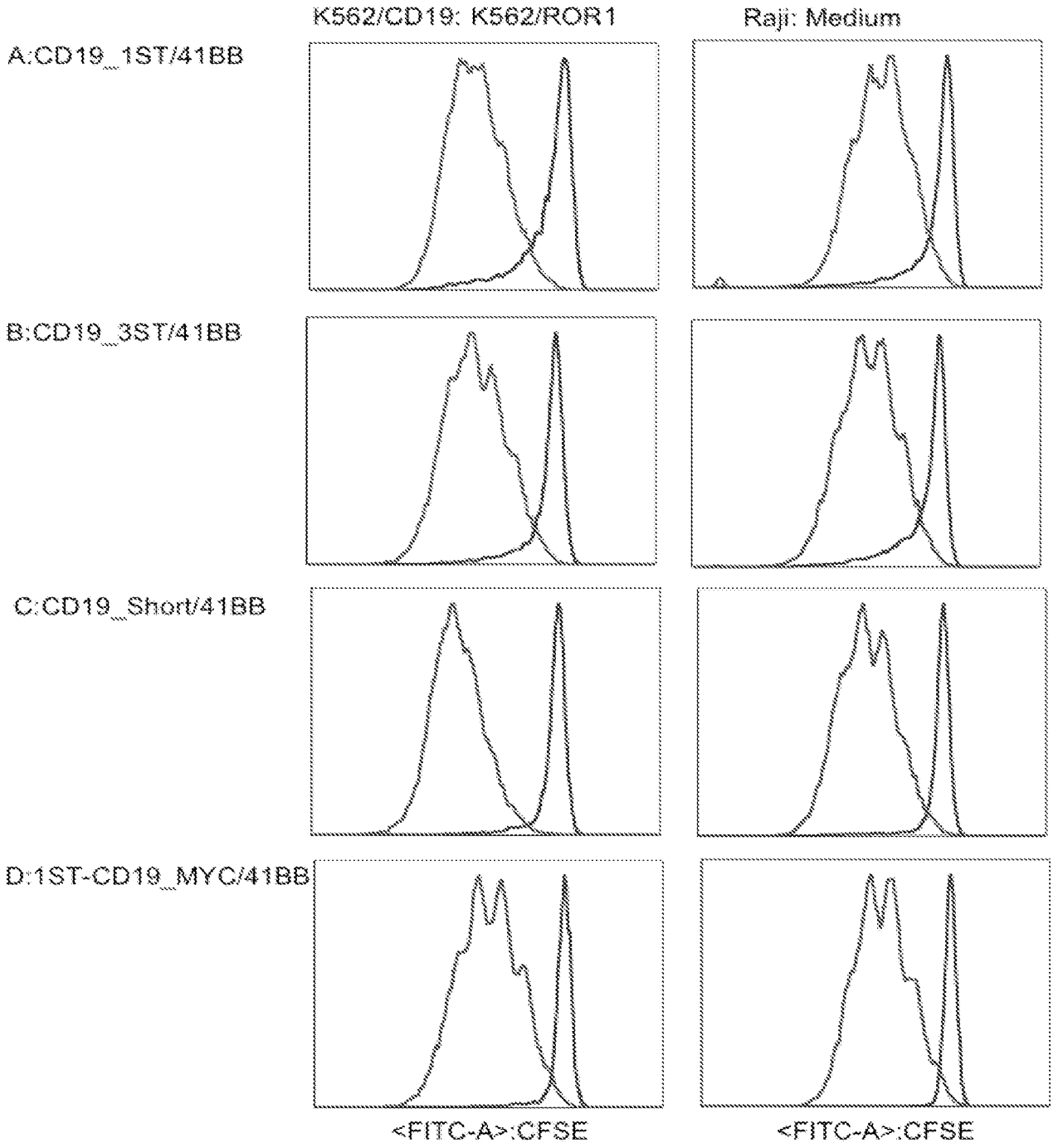
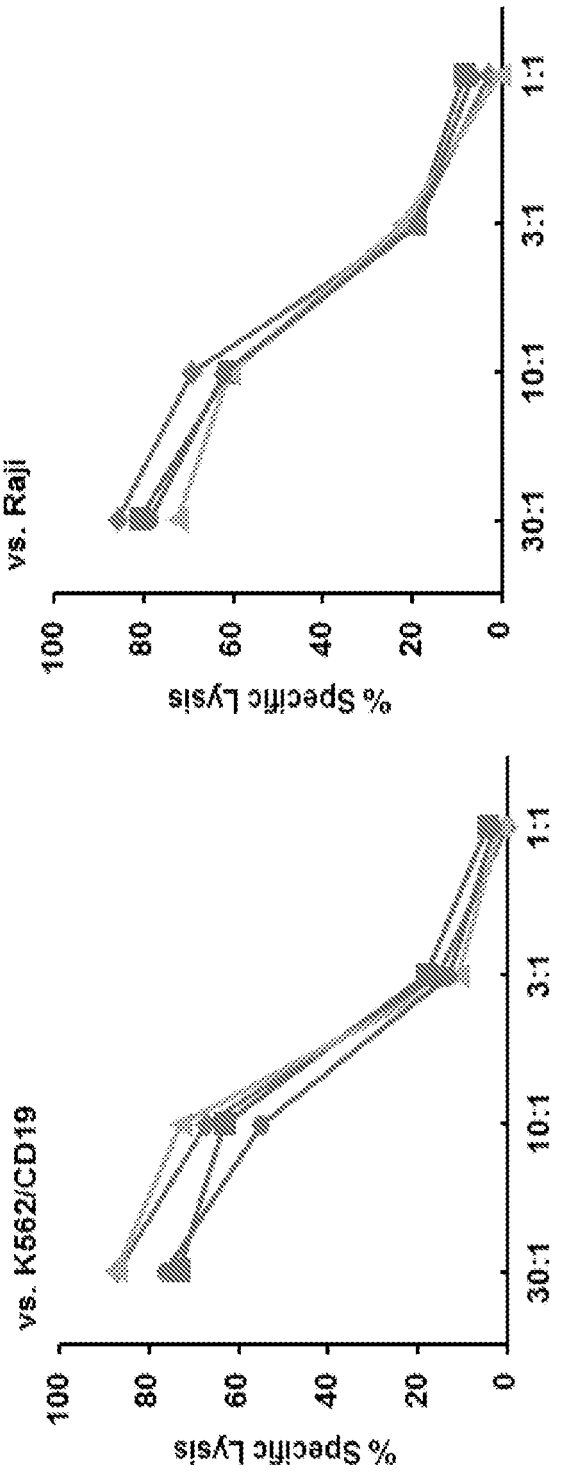

K562/CD19: K562/ROR1
Raji: Medium
A:CD19_1ST/41BB
B:CD19_3ST/41BB
C:CD19_Short/41BB
D:1ST-CD19_MYC/41BB
<FITC-A>:CFSE
<FITC-A>:CFSE

Fig. 31


vs. Raji
% Specific Lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
vs. K562/CD19
% Specific Lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1

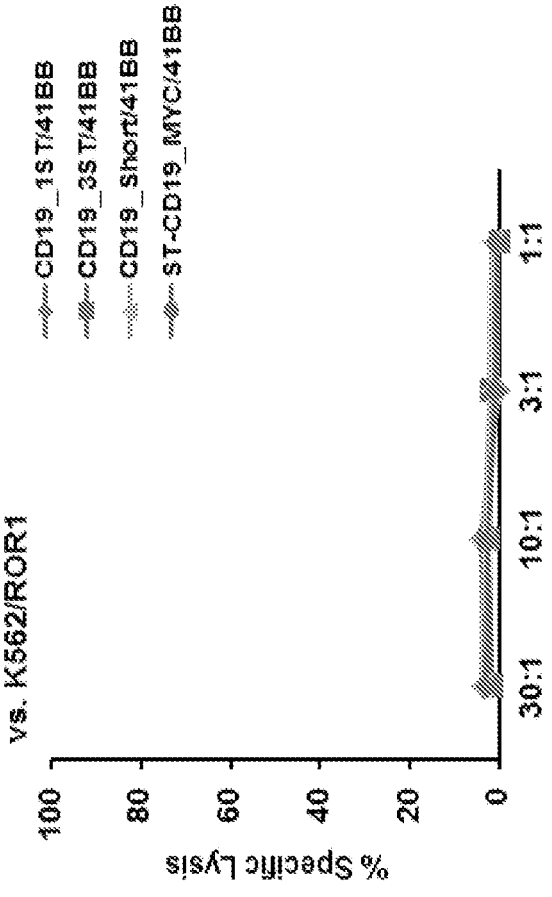

vs. K562/ROR1
% Specific Lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
CD19_1ST/41BB
CD19_3ST/41BB
CD19_Short/41BB
ST-CD19_MYC/41BB

Fig. 32