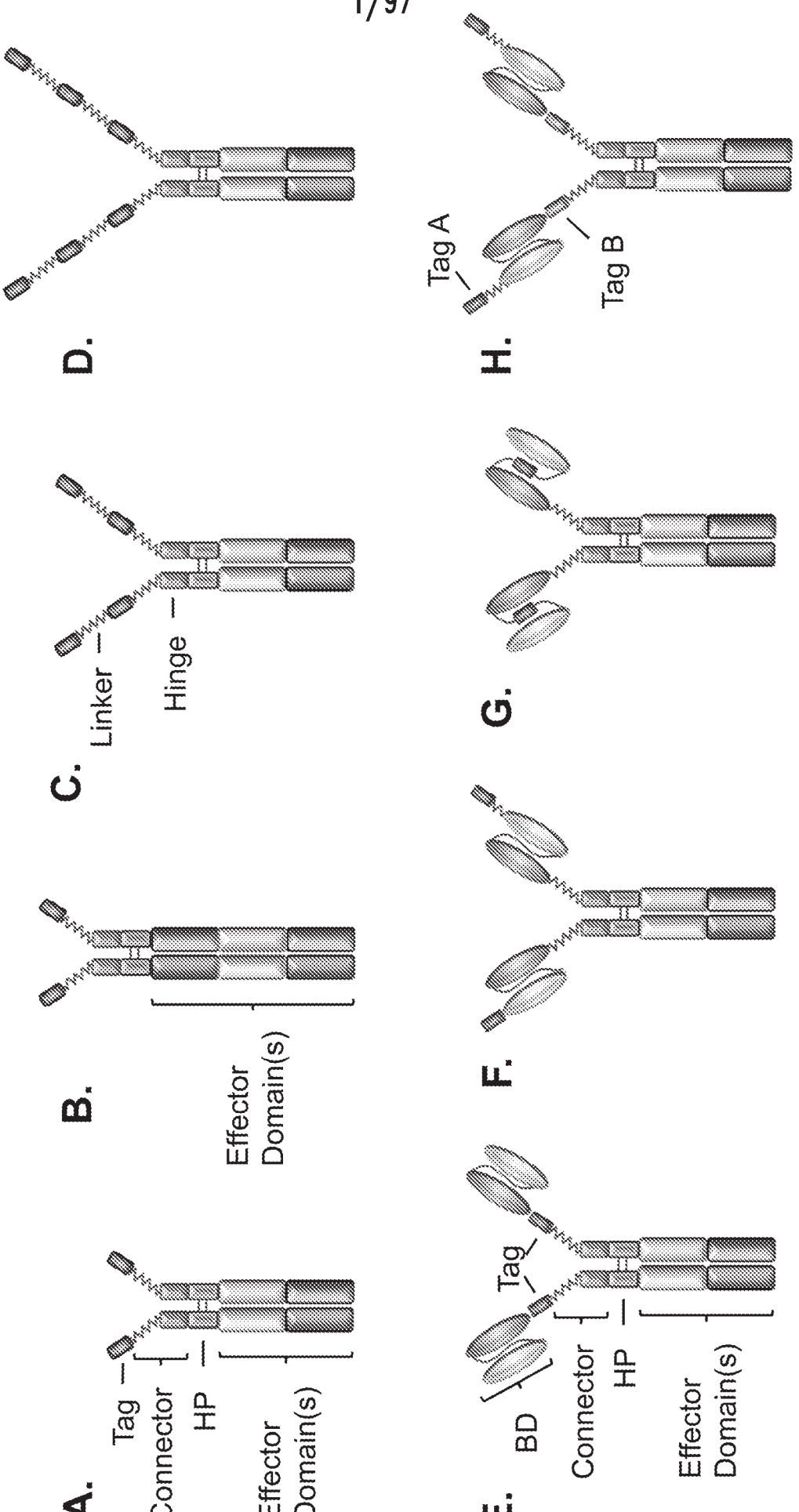

A.
Tag
Connector
HP
Effector
Domain(s)
B.
Effector
Domain(s)
C.
Linker
Hinge
D.
E.
BD
Tag
Connector
HP
Effector
Domain(s)
F.
G.
H.
Tag A
Tag B

Fig. 1

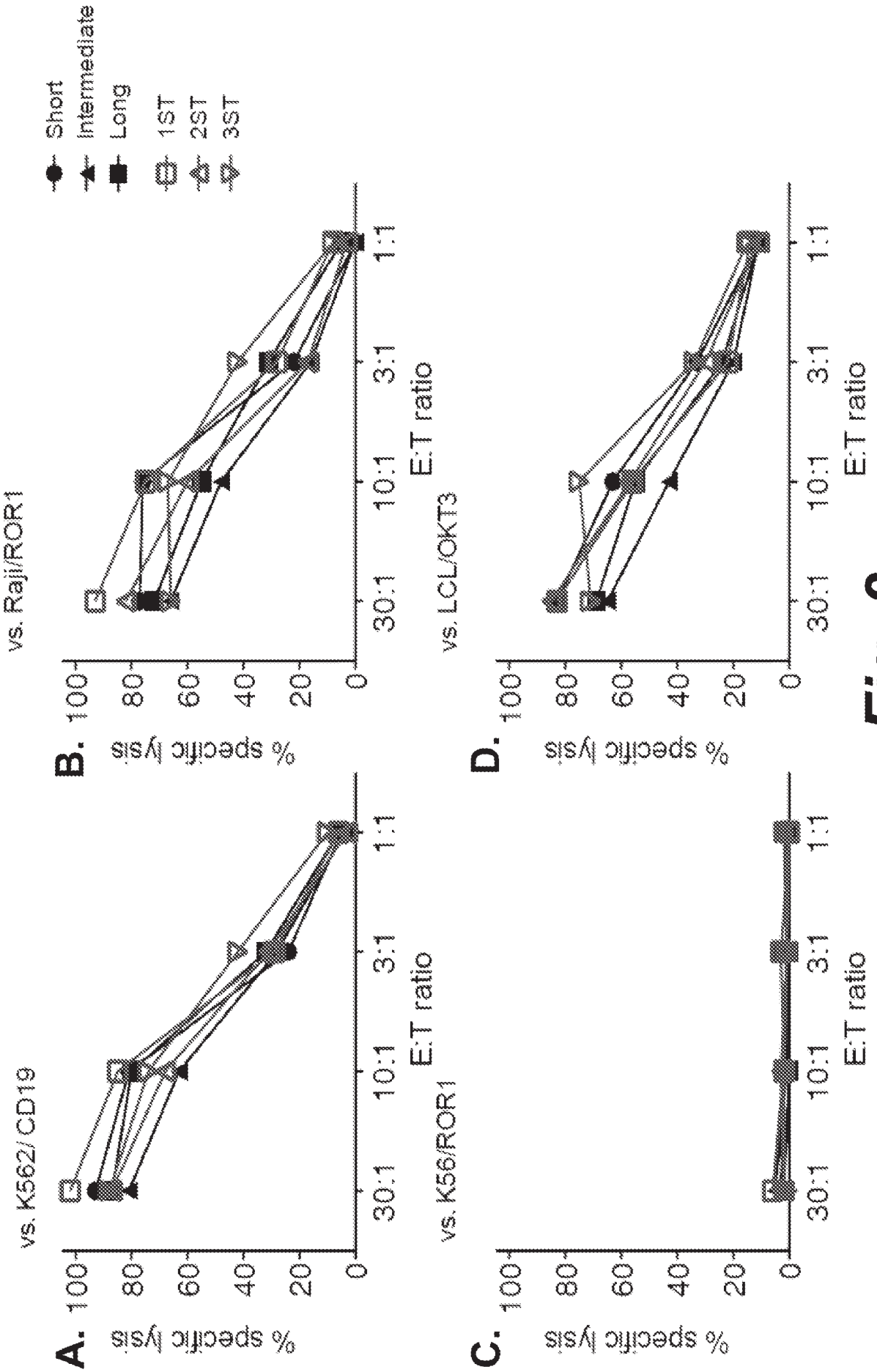
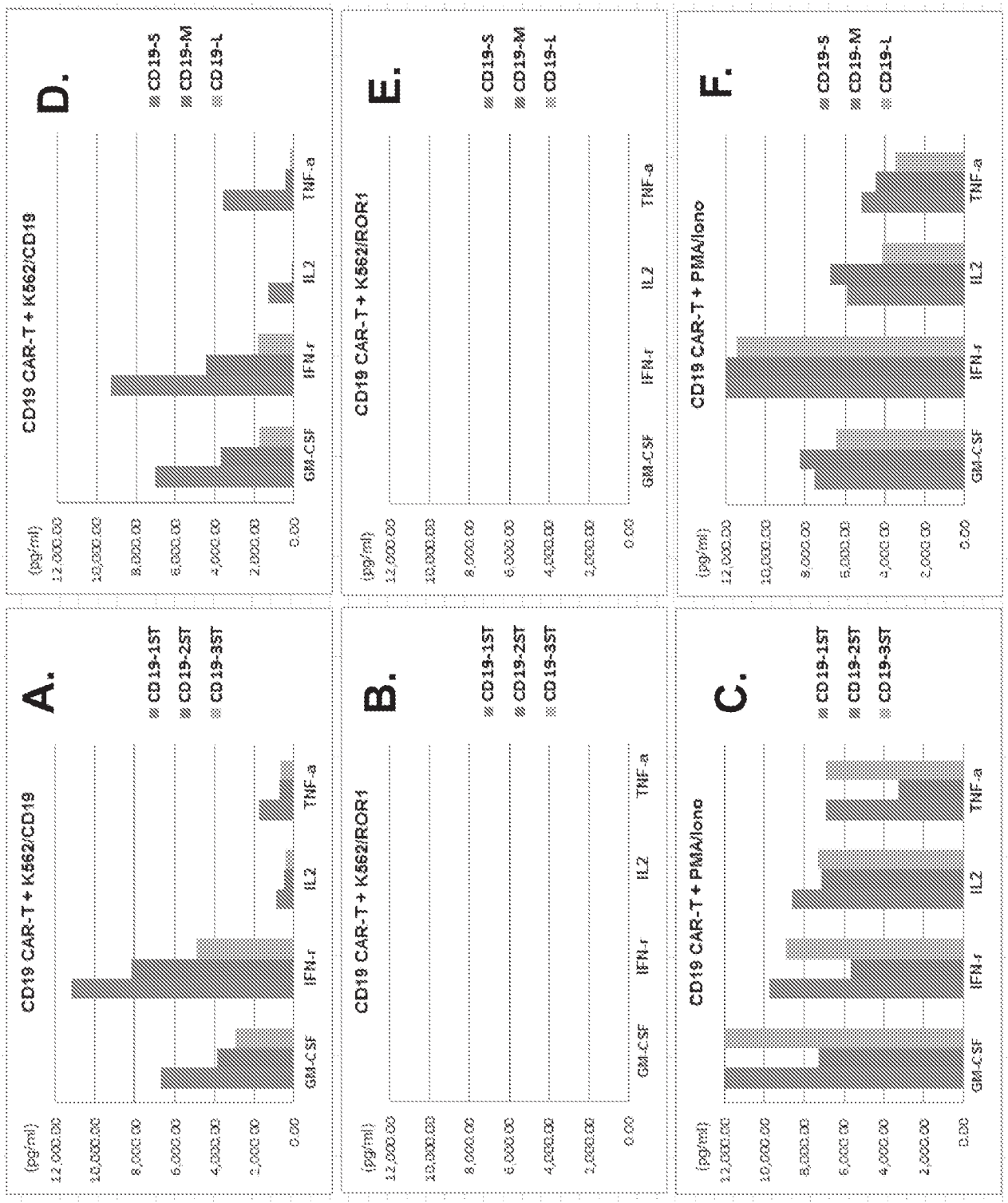

A.
vs. K562/CD19
% specific lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
E:T ratio
B.
vs. Raji/ROR1
Short
Intermediate
Long
1ST
2ST
3ST
C.
vs. K56/ROR1
D.
vs. LCL/OKT3

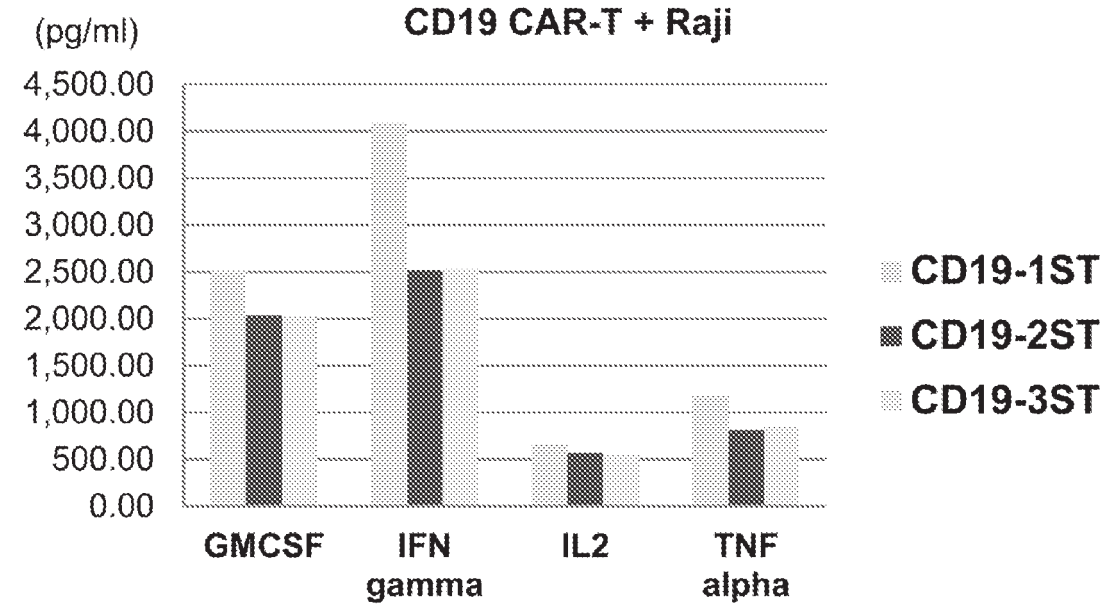
Fig. 2



24h Co-Culture
Cytokine Release

Fig. 3

A.


CD19 CAR-T + Raji
(pg/ml)
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.

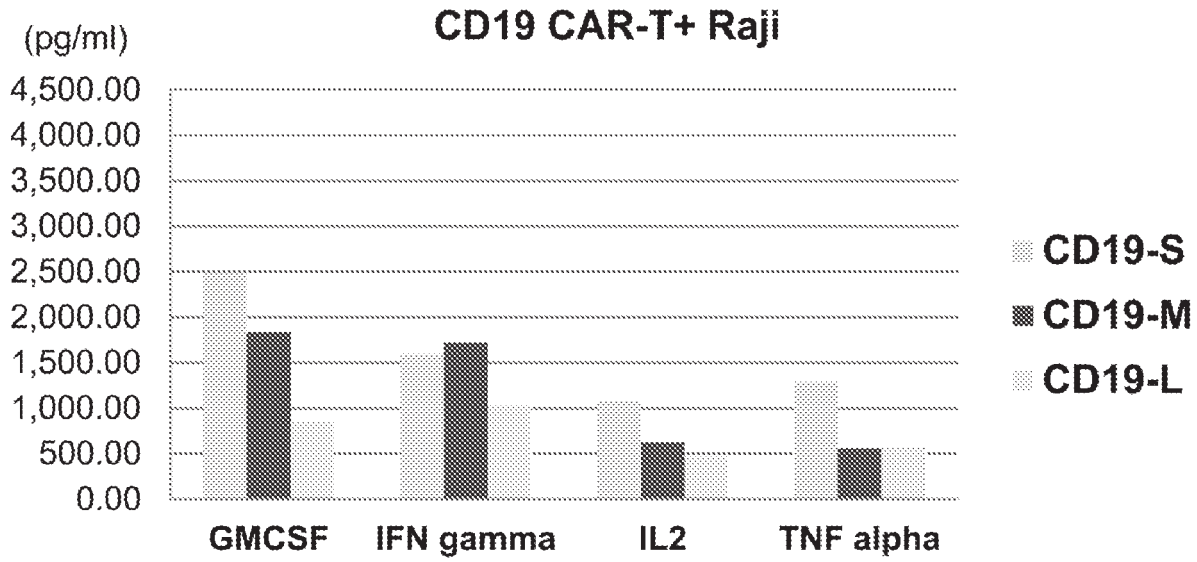

CD19 CAR-T+ Raji
(pg/ml)
4,500.00
4,000.00
3,500.00
3,000.00
2,500.00
2,000.00
1,500.00
1,000.00
500.00
0.00
GMCSF
IFN gamma
IL2
TNF alpha
CD19-S
CD19-M
CD19-L

Fig. 4

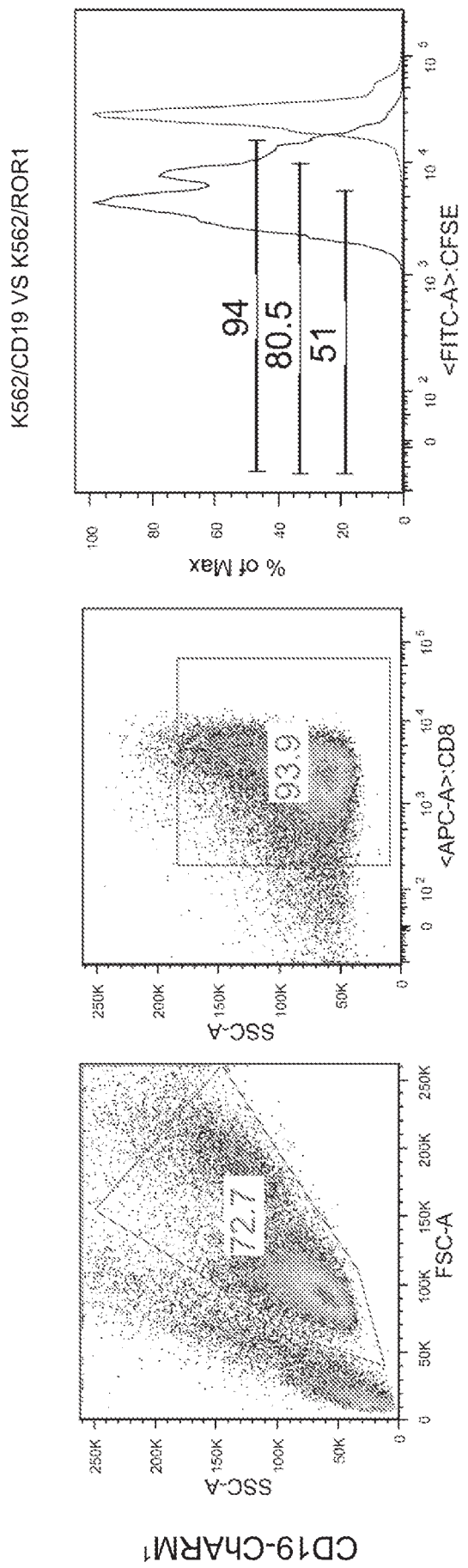

CD19-CHARM1
SSC-A
FSC-A
72.7
SSC-A
<APC-A>:CD8
83.6
K562/CD19 VS K562/ROR1
% of Max
94
80.5
51
<FITC-A>:CFSE

Fig. 5

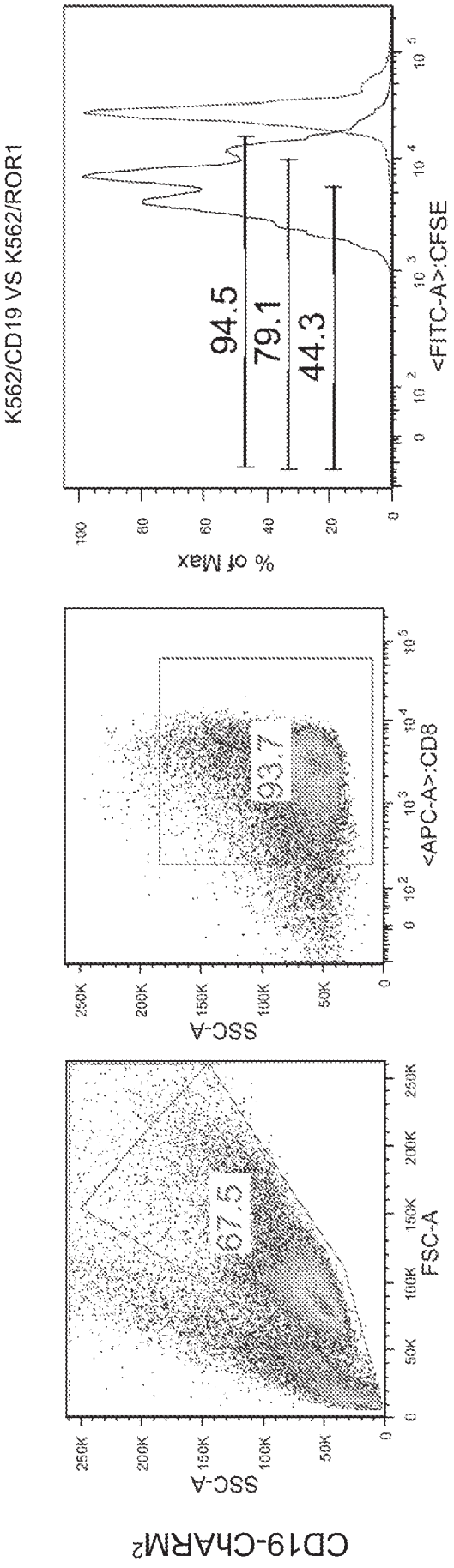


Fig. 5 (Continued)

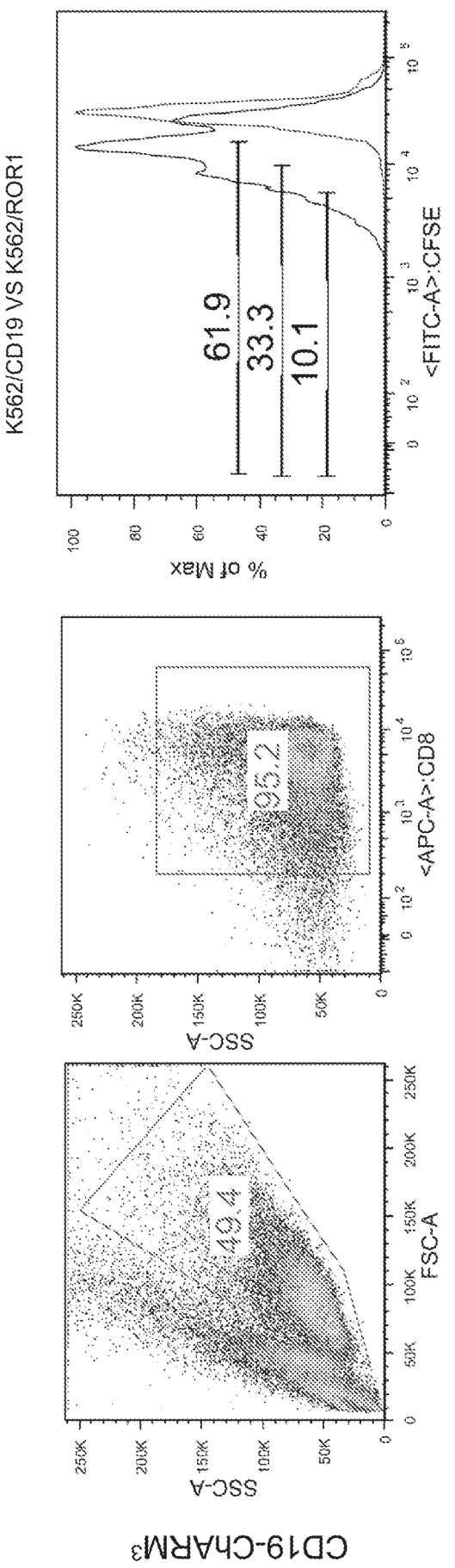


Fig. 5 (Continued)

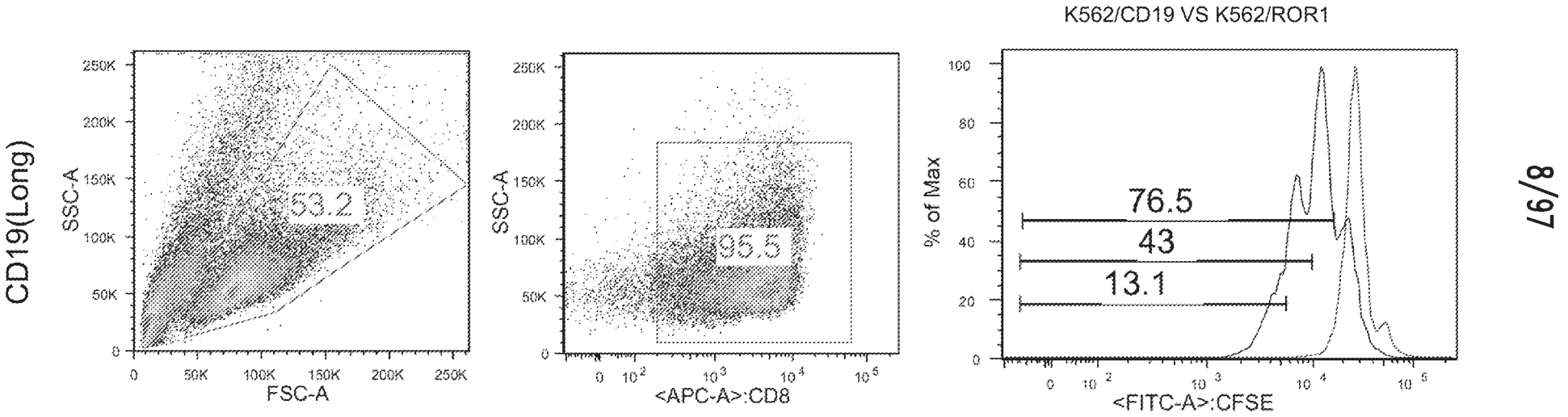
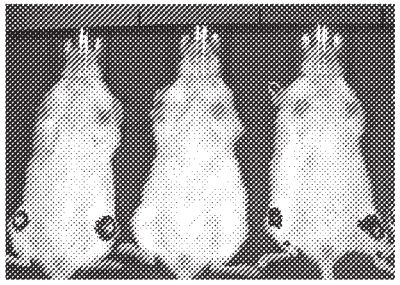


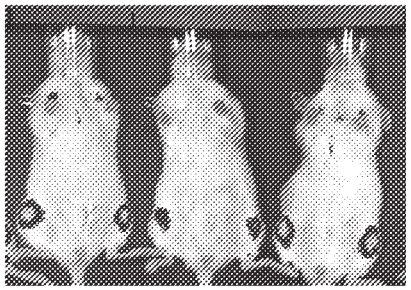
Fig. 5 *(Continued)*

D6 after tumor
D0 after T cells

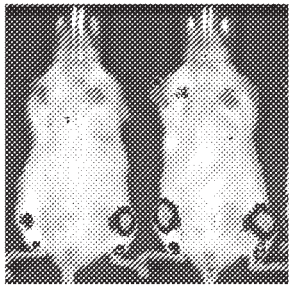
1. CAR(short)



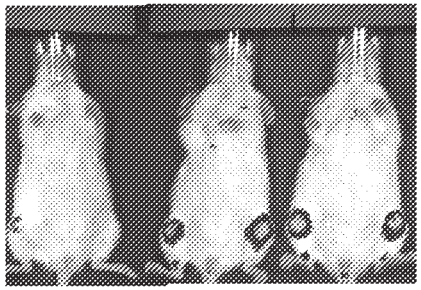
2. CAR(Medium)



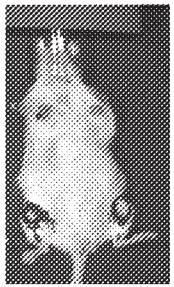
3. CAR(long)



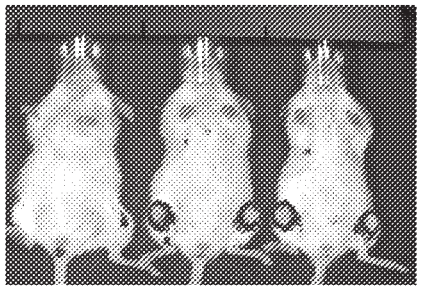
4. ChARM[1] beads+ LCL stim



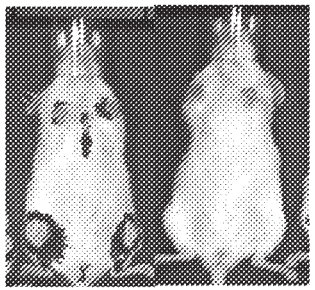
5. ChARM[2] bead stim



6. ChARM[3] LCL stim



7. Untreated



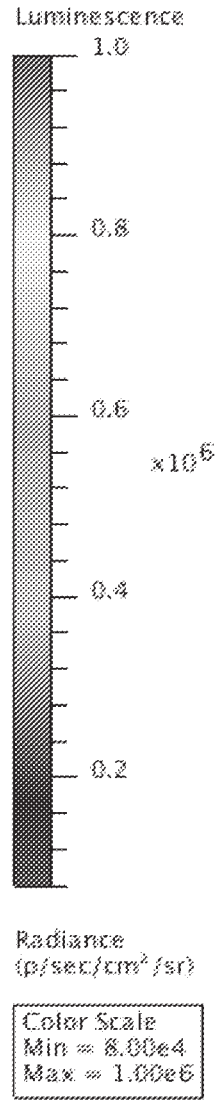
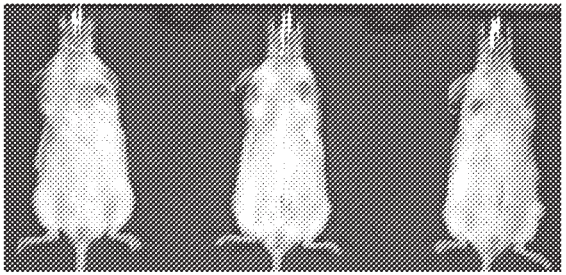


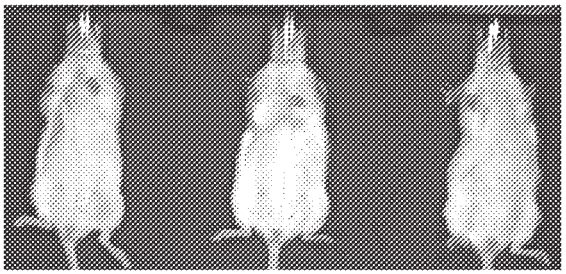
Fig. 6A

D14 after tumor
D7 after T cells

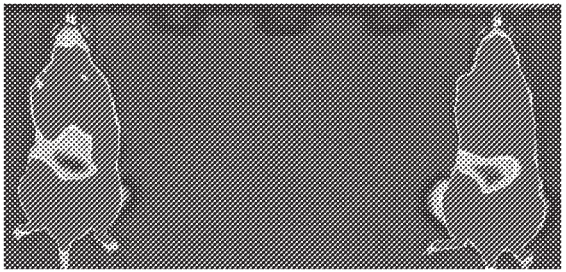
1. CAR(short)



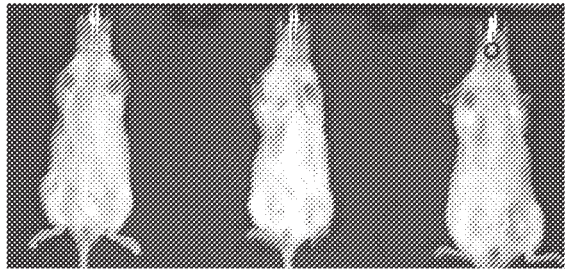
2. CAR(medium)



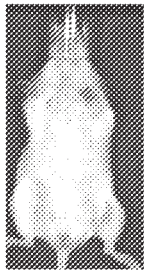
3. CAR(long)



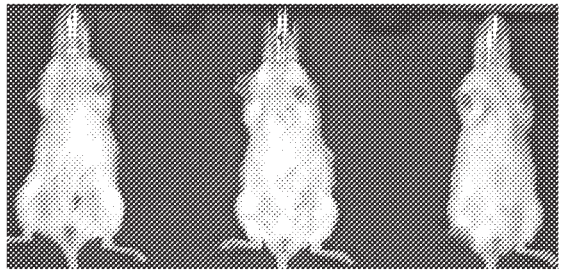
4. ChARM[1] beads+ LCL stim



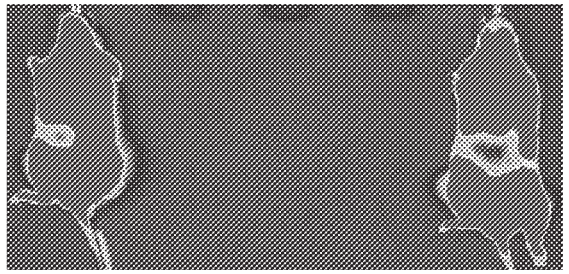
5. ChARM[2] bead stim



6. ChARM[3] LCL stim



7. Untreated



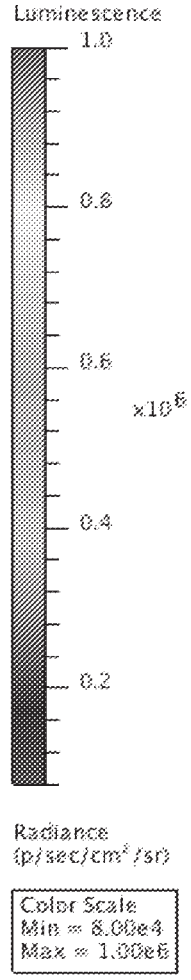
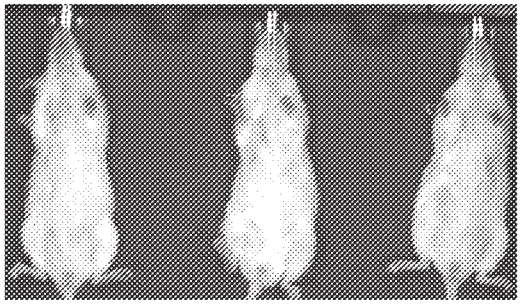


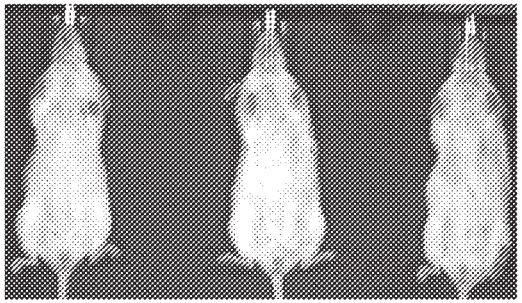
Fig. 6B

D18 after tumor
D11 after T cells

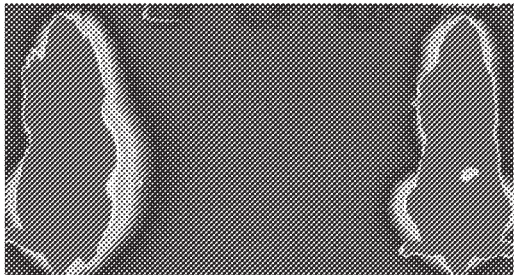
1. CAR(short)



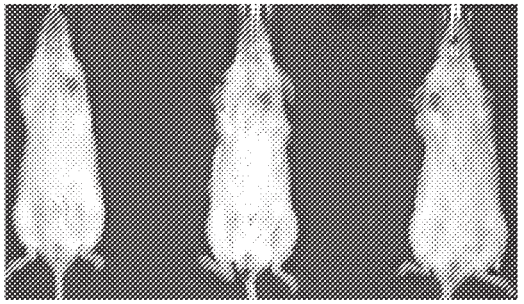
2. CAR(medium)



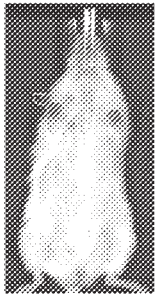
3. CAR(long)



4. ChARM[1] beads+ LCL stim



5. ChARM[2] bead stim



6. ChARM[3] LCL stim



7. Untreated

All dead at Day 17 after tumor

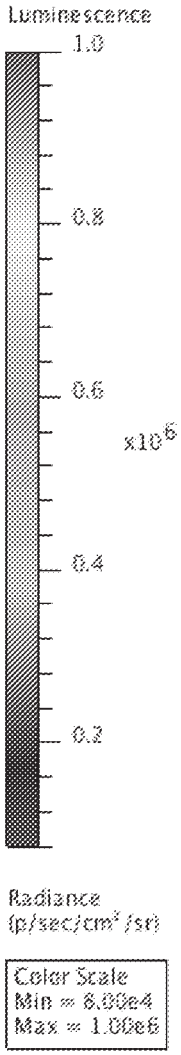


Fig. 6C

Day 25 after tumor
Day 18 after T cells

1. CAR(short)

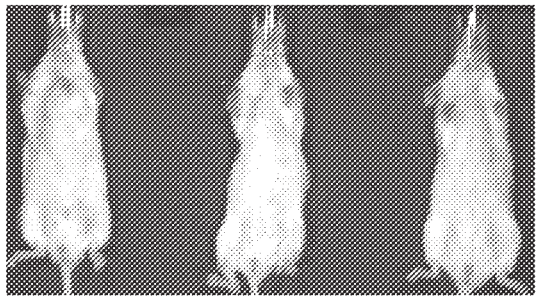


2. CAR(medium)

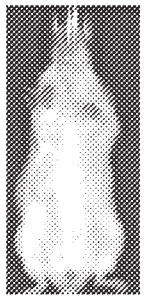


3. CAR(long) **All dead at Day 18 after tumor**

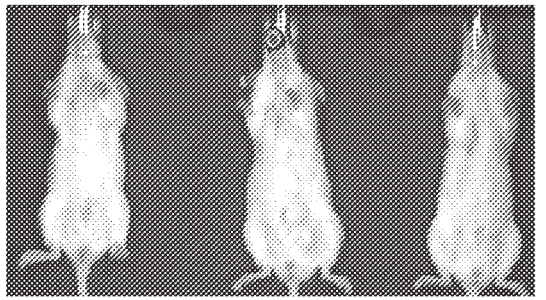
4. ChARM[1] beads+ LCL stim

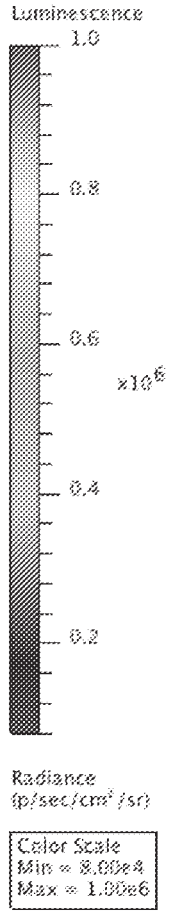


5. ChARM[2] bead stim



6. ChARM[3] LCL stim



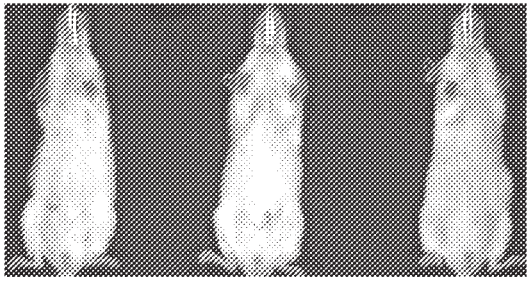


7. Untreated **All dead at Day 17 after tumor**

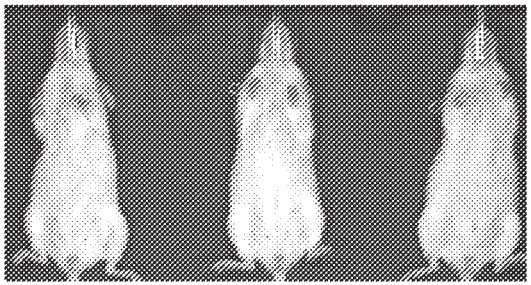
Fig. 6D

Day 33 after tumor
Day 26 after T cells

1. CAR(short)



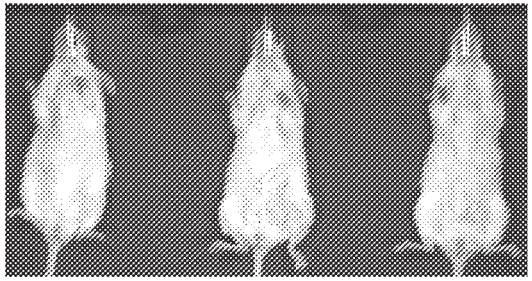
2. CAR(medium



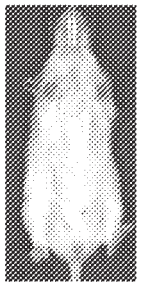
All dead at Day 18 after tumor

3. CAR(long)

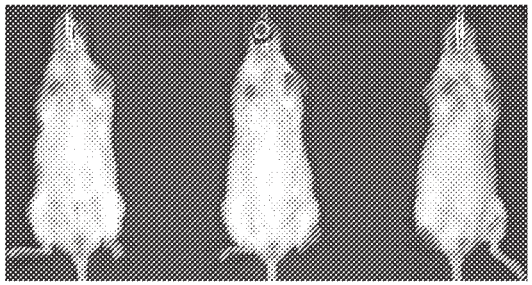
4. ChARM[1] beads+ LCL stim



5. ChARM[2] bead stim



6. ChARM[3] LCL stim





7. Untreated **All dead at Day 17 after tumor**

Fig. 6E

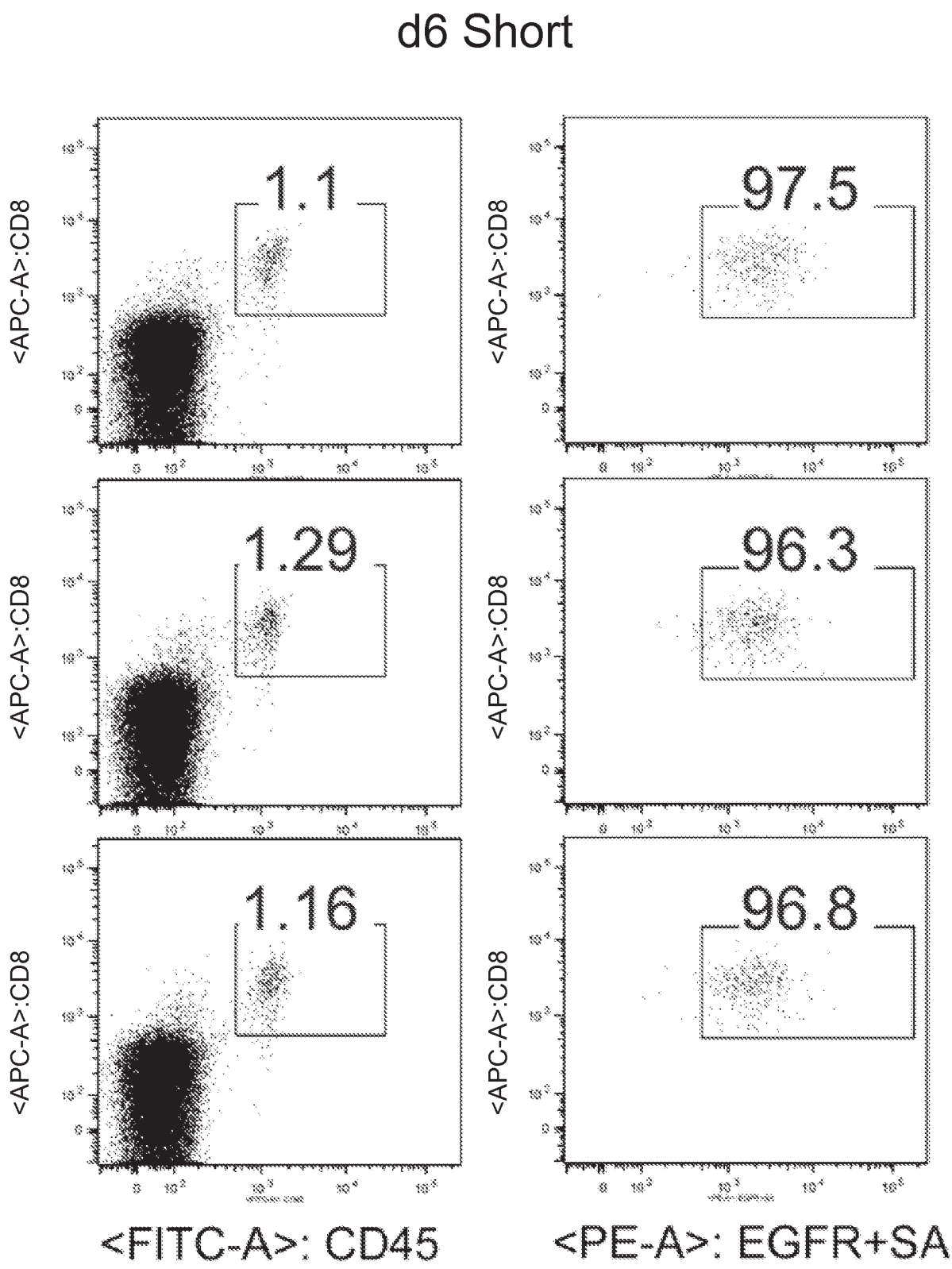

d6 Short
1.1
97.5
1.29
96.3
1.16
96.8
<APC-A>:CD8
<APC-A>:CD8
<APC-A>:CD8
<APC-A>:CD8
<APC-A>:CD8
<APC-A>:CD8
<FITC-A>: CD45
<PE-A>: EGFR+SA

Fig. 7

d6 Intermediate

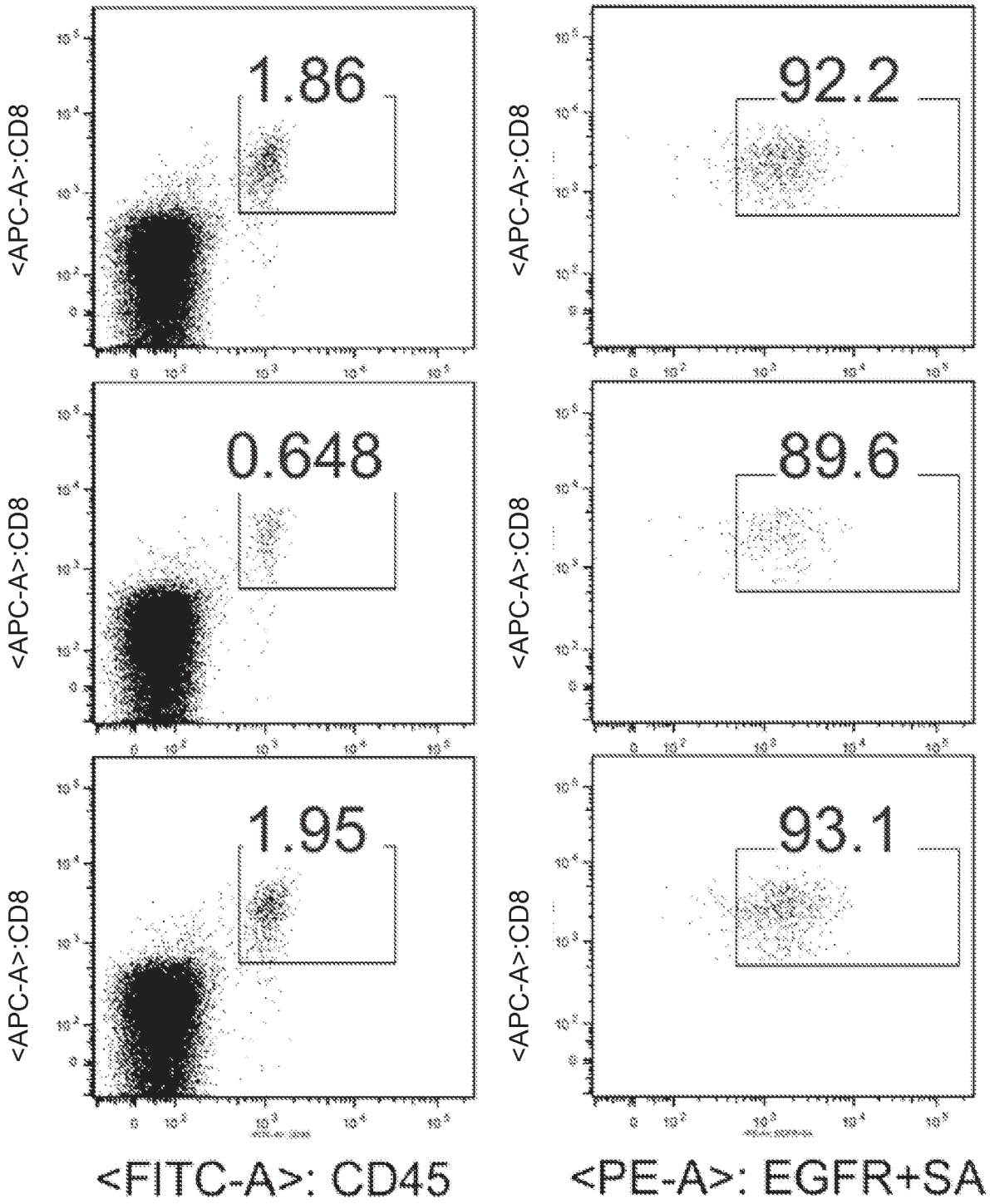


Fig. 7 *(Continued)*

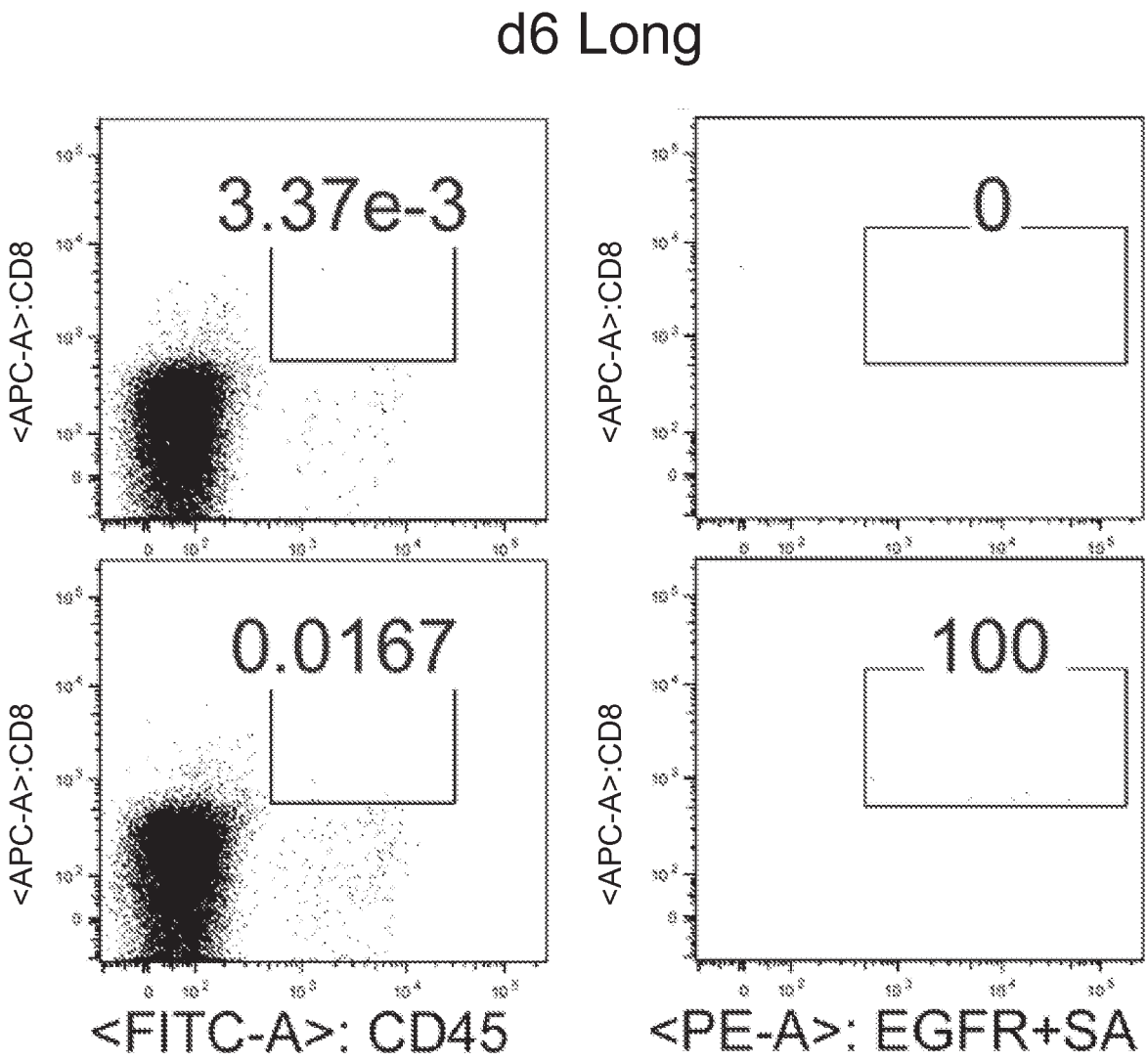


Fig. 7 *(Continued)*

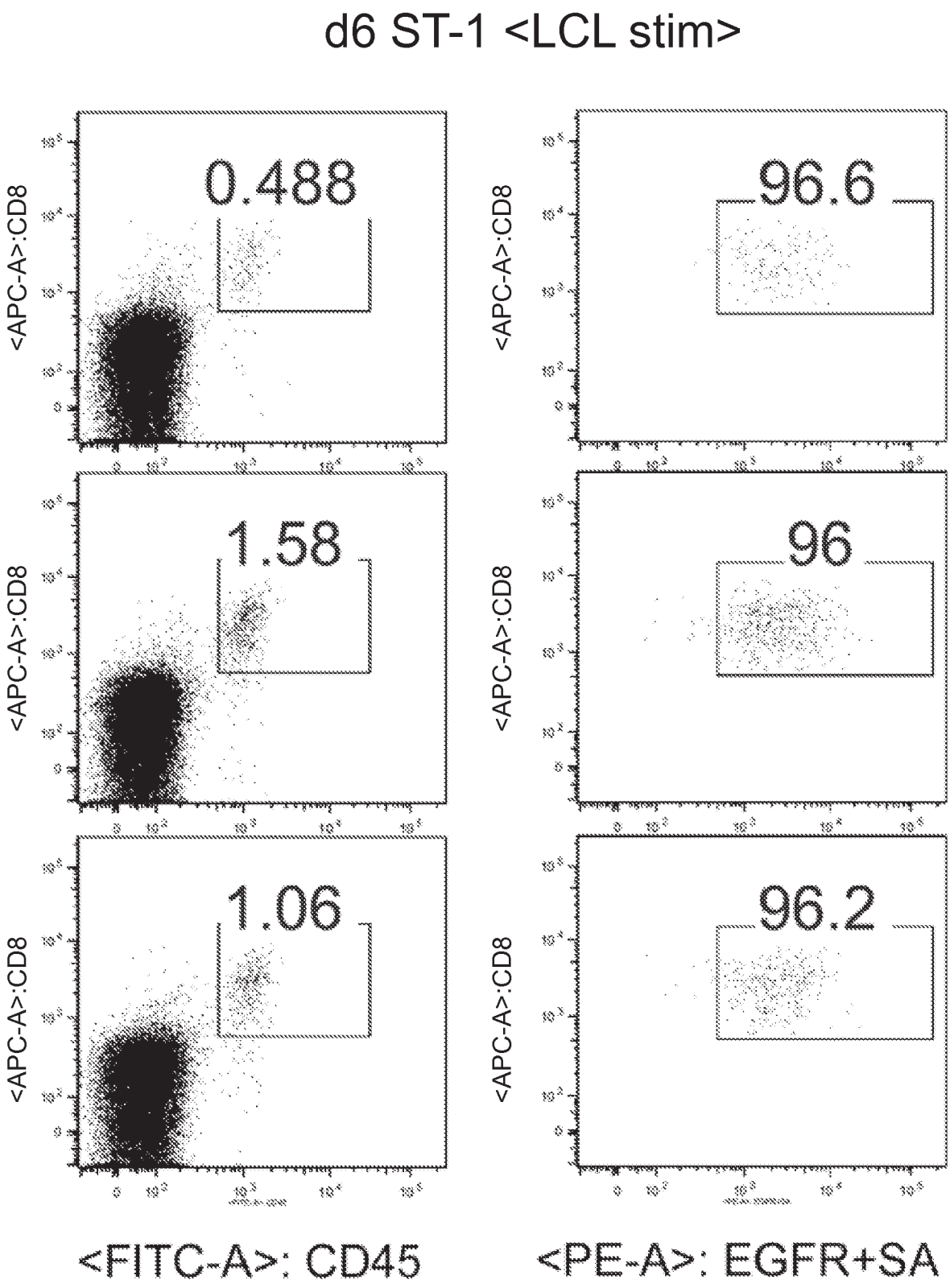


Fig. 7 *(Continued)*

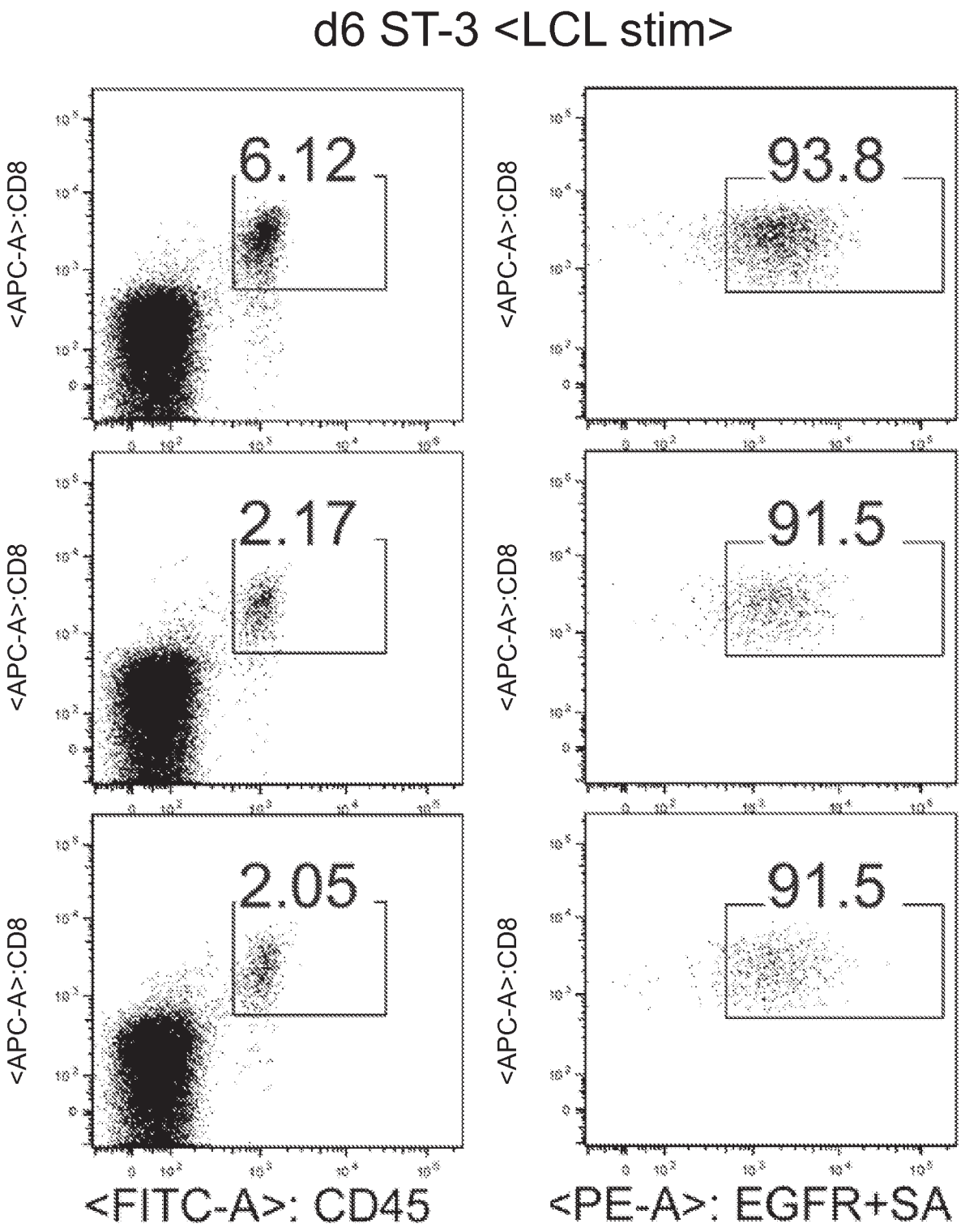


Fig. 7 *(Continued)*

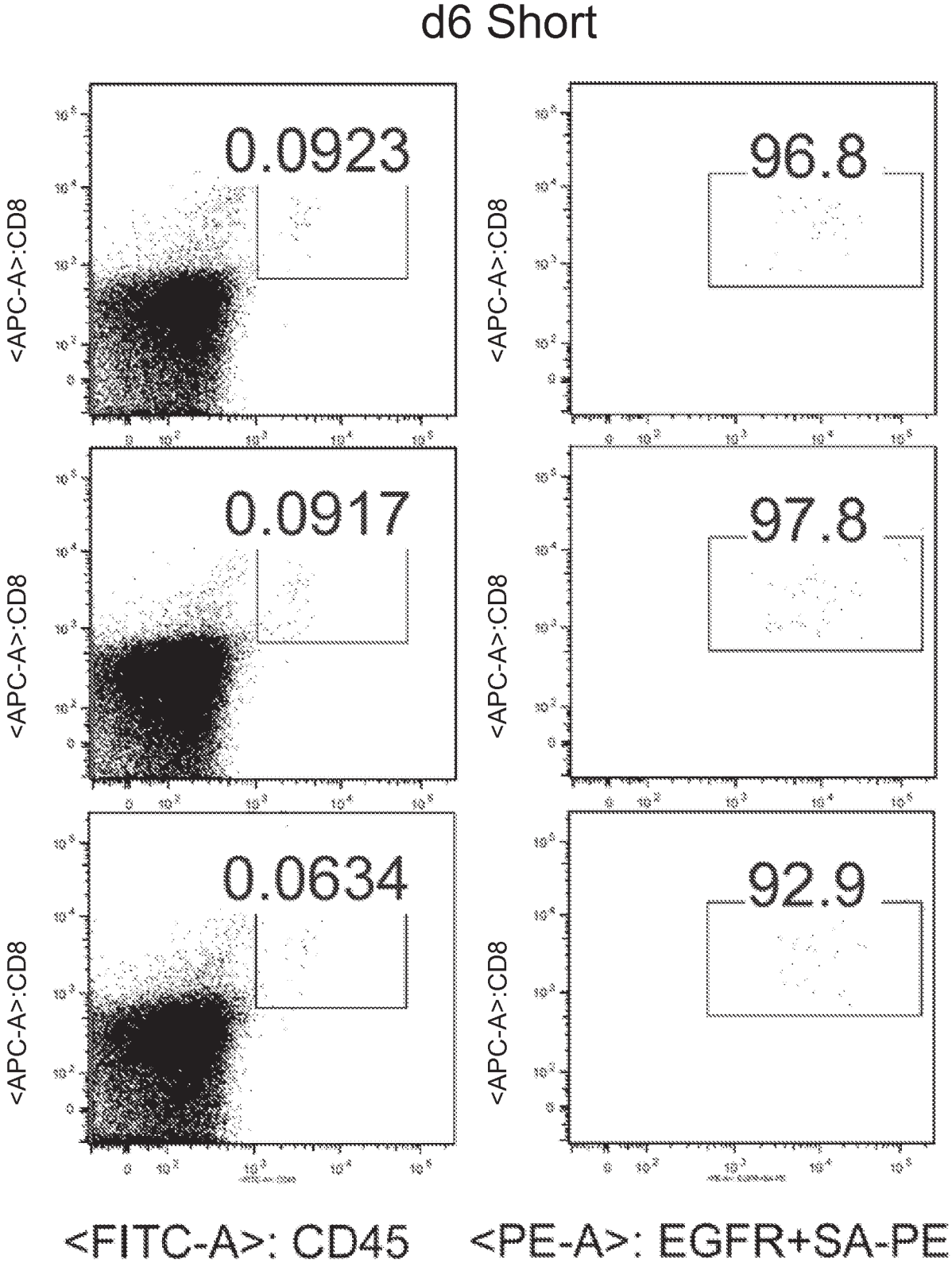


Fig. 7 *(Continued)*

d13 Intermediate

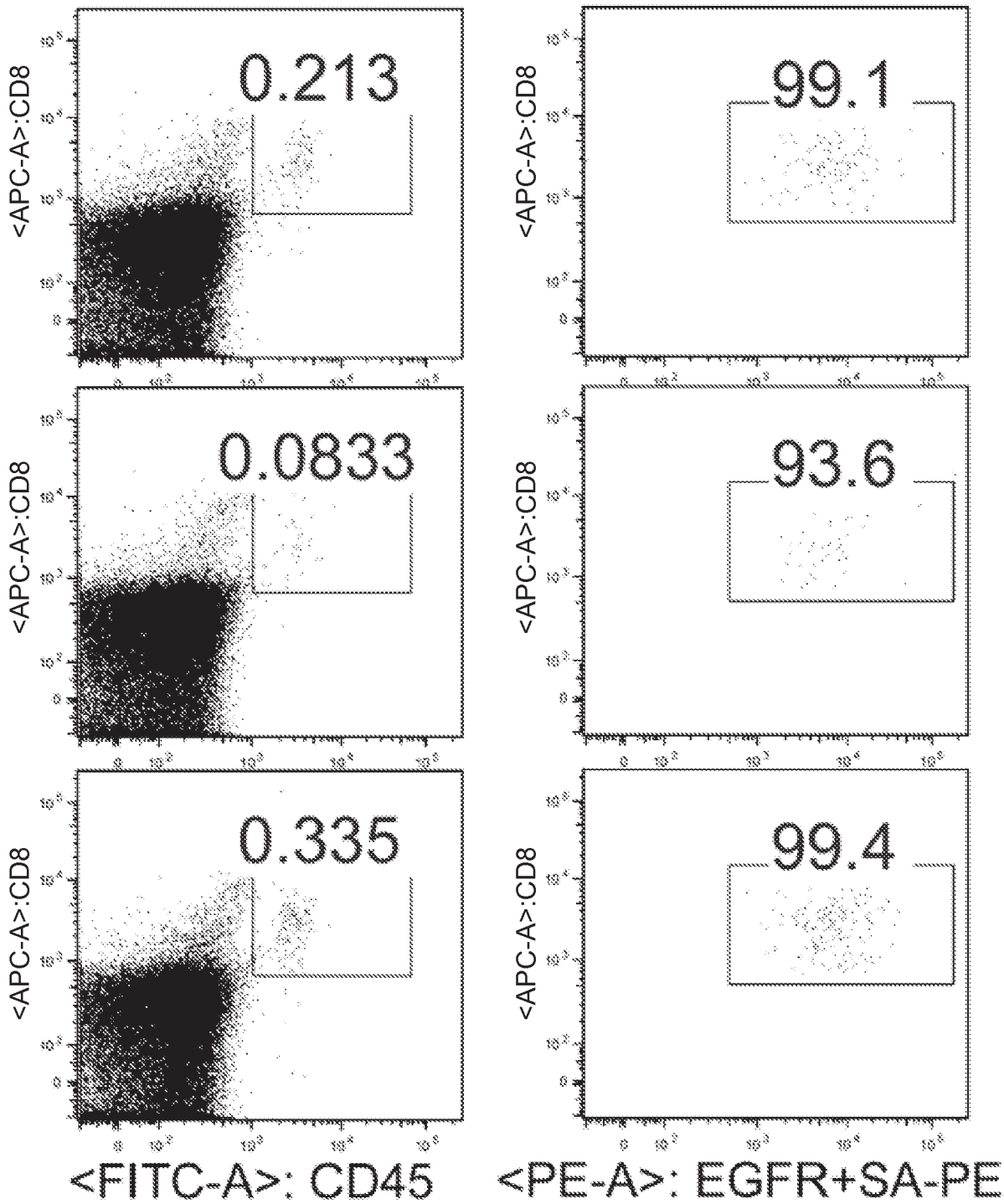


Fig. 7 *(Continued)*

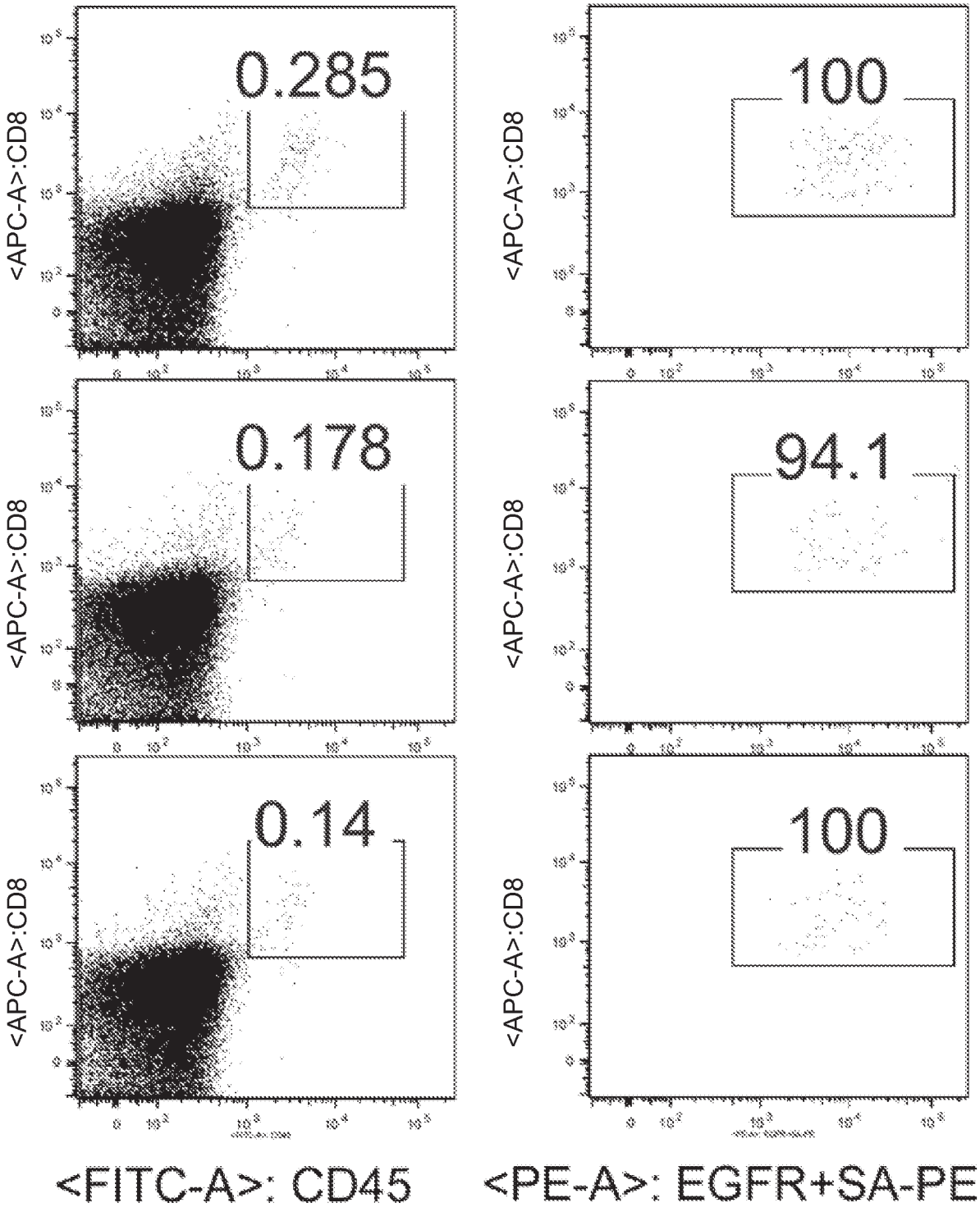


Fig. 7 *(Continued)*

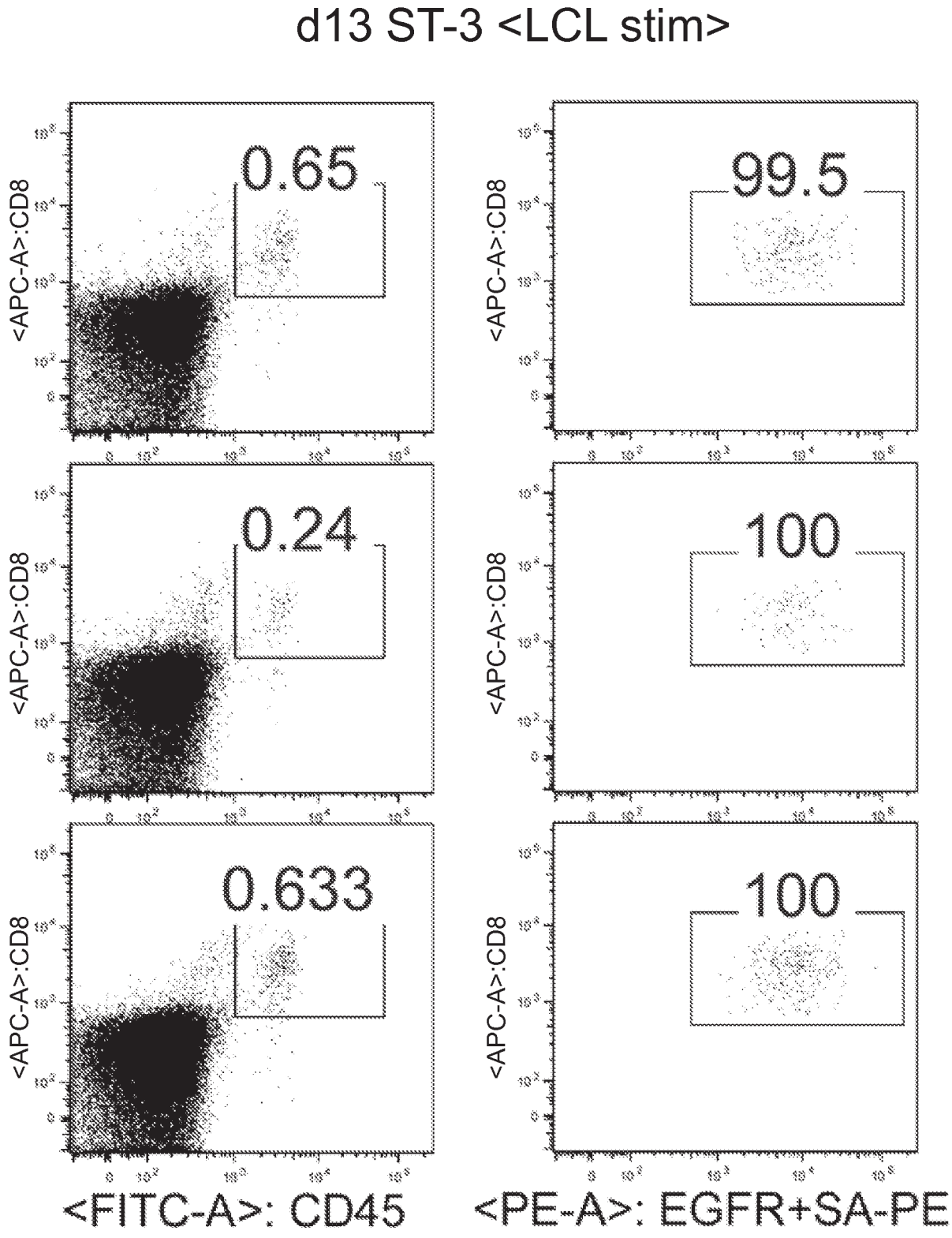


Fig. 7 *(Continued)*

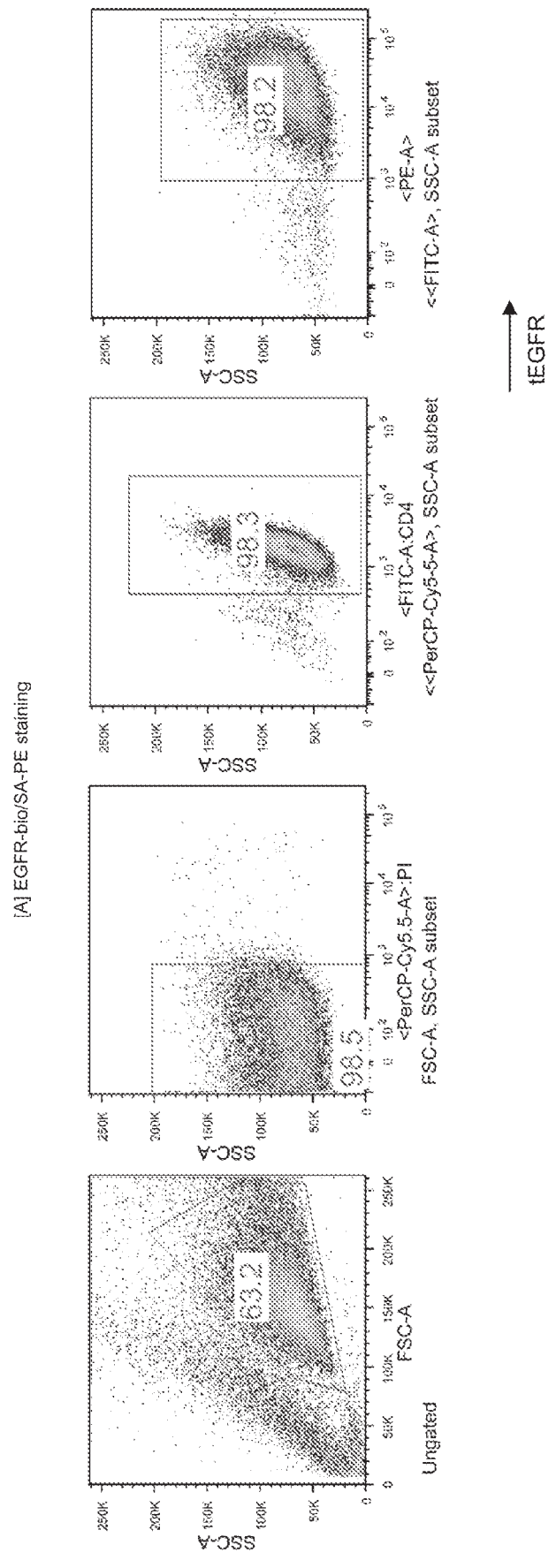

CD19-ChARM1
[A] EGFR-bio/SA-PE staining
Ungated
FSC-A
SSC-A
63.2
FSC-A, SSC-A subset
<PerCP-Cy5.5-A>:PI
98.5
<<PerCP-Cy5-5-A>, SSC-A subset
<FITC-A>:CD4
98.3
<<FITC-A>, SSC-A subset
<PE-A>
98.2
tEGFR

Fig. 8A

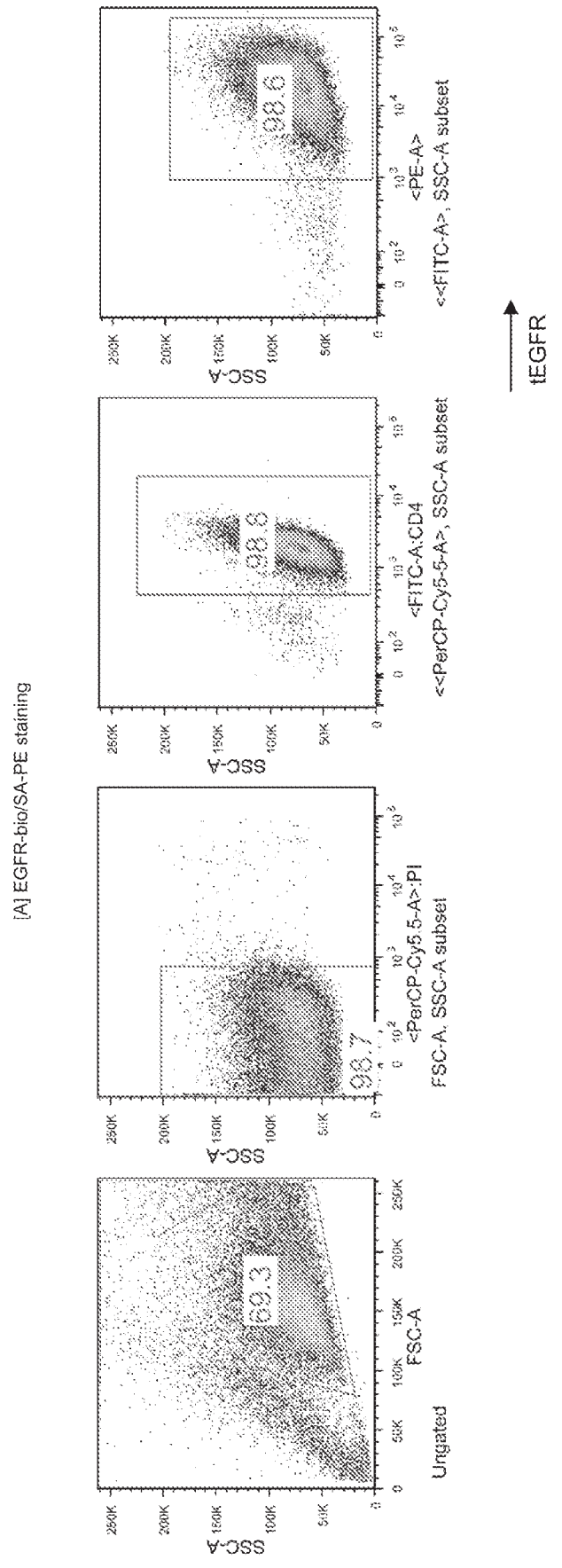


Fig. 8A (Continued)

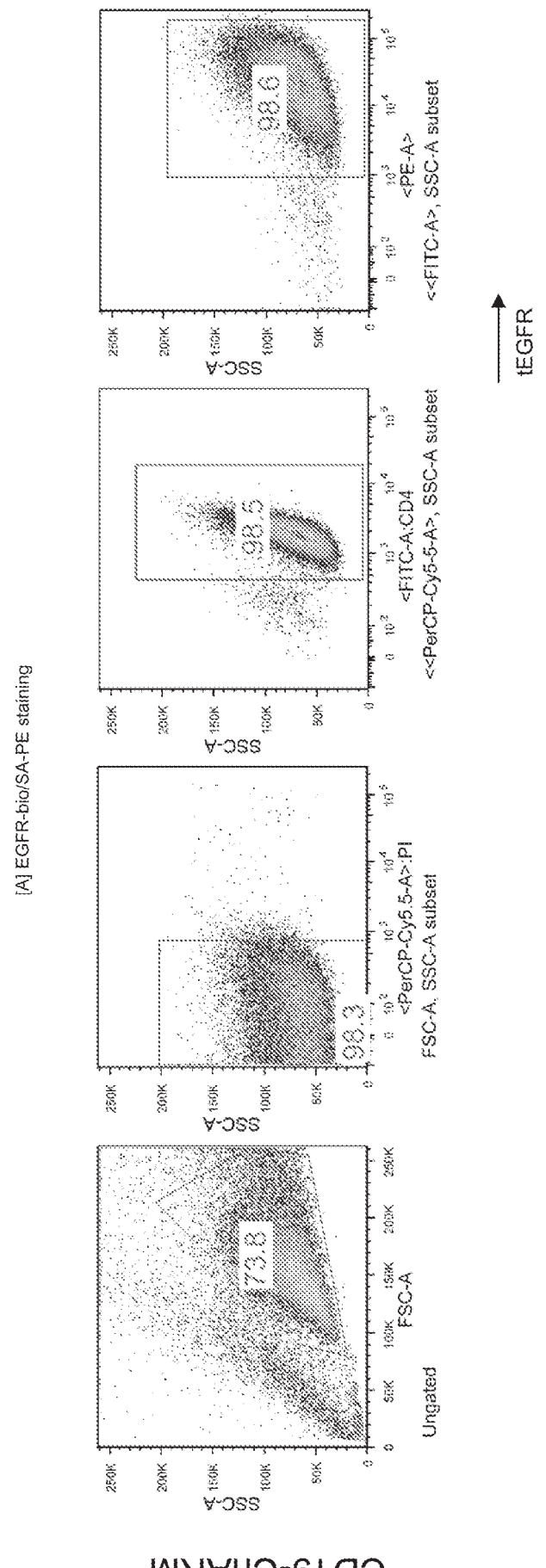


Fig. 8A (Continued)

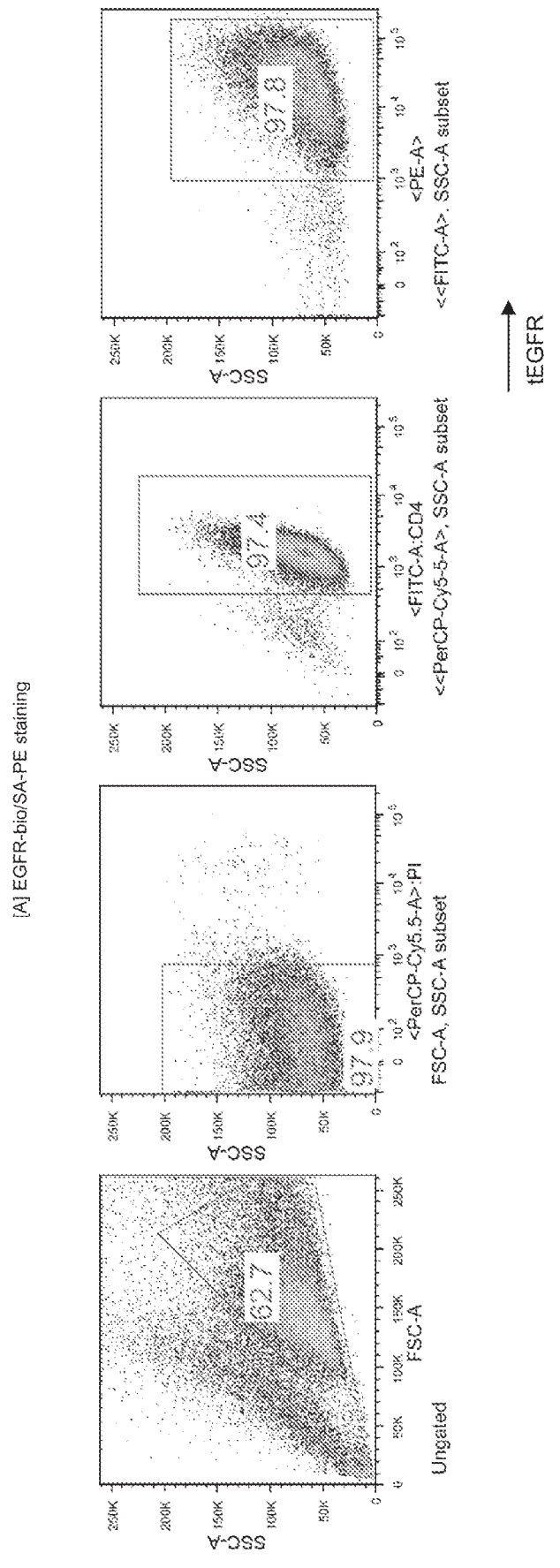


Fig. 8A (Continued)

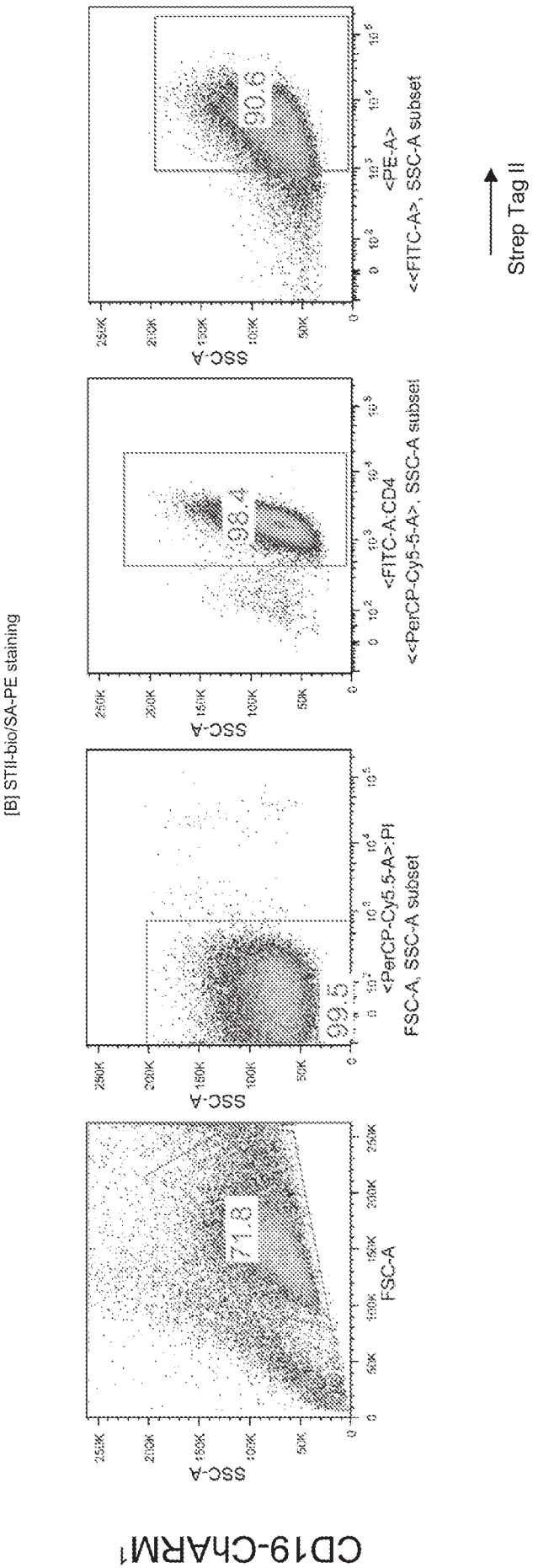

CD19-ChARM1
[B] STII-bio/SA-PE staining
FSC-A
SSC-A
71.8
FSC-A, SSC-A subset
<PerCP-Cy5-5-A>:PI
99.5
<PerCP-Cy5-5-A>, SSC-A subset
<FITC-A:CD4
98.4
<<PerCP-Cy5-5-A>, SSC-A subset
<PE-A>
90.6
<<FITC-A>, SSC-A subset
Strep Tag II

Fig. 8B

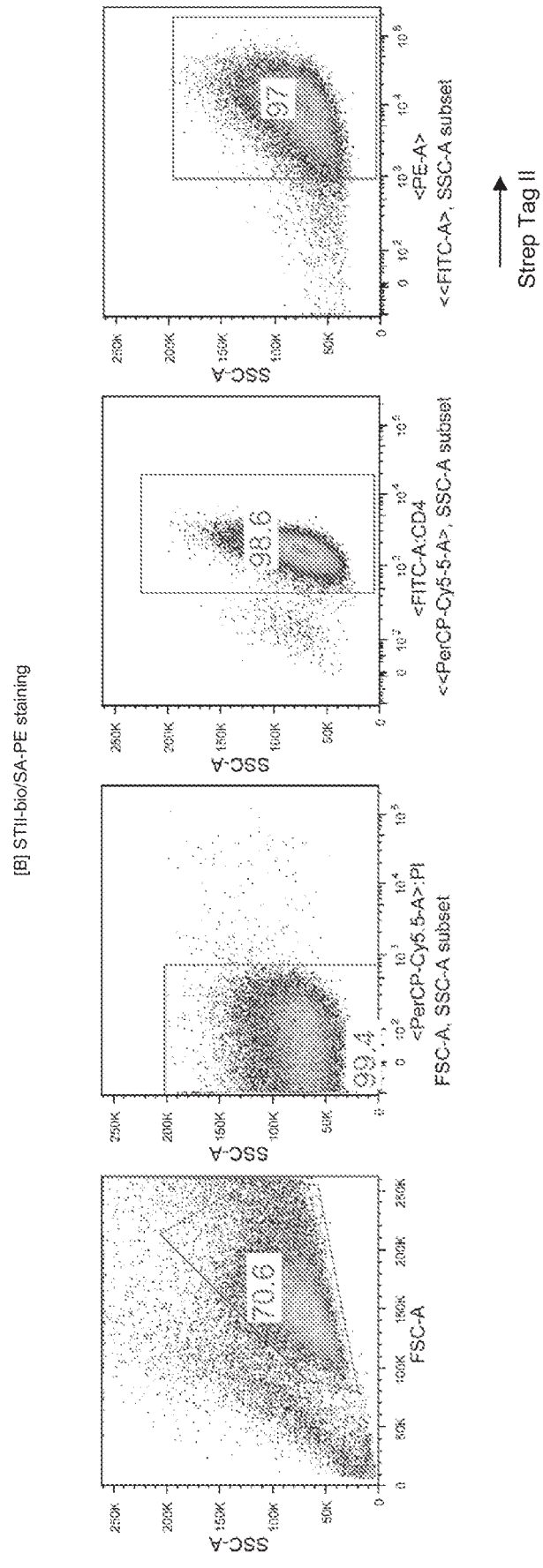


Fig. 8B (Continued)

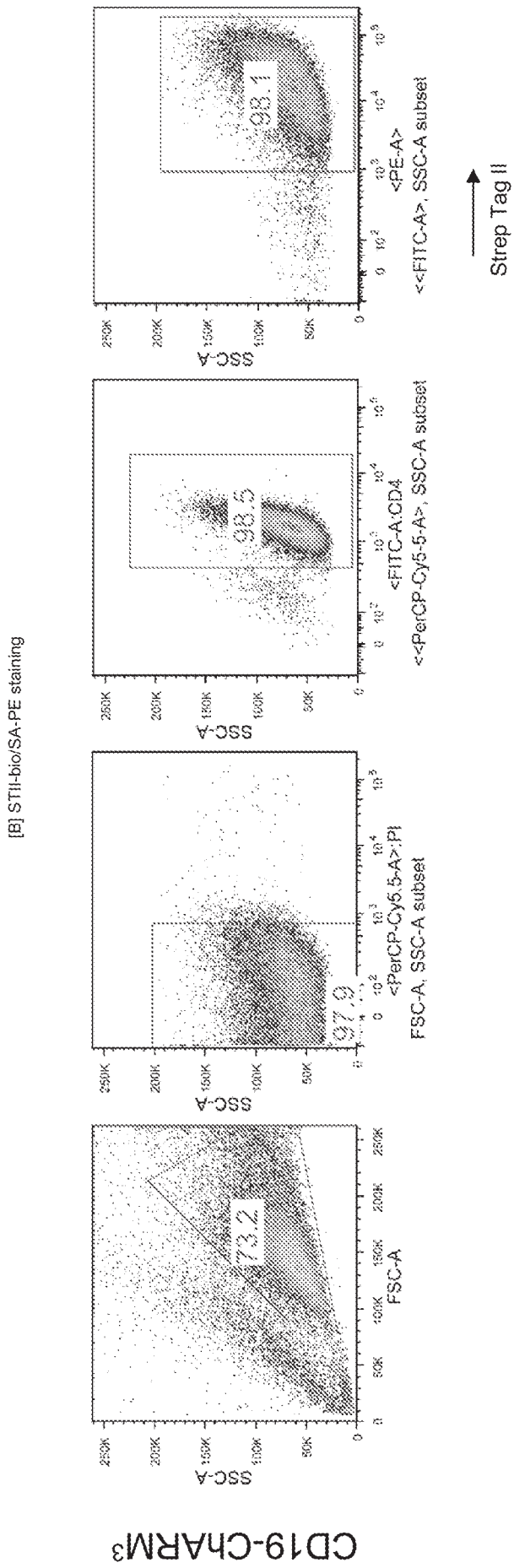


Fig. 8B (Continued)

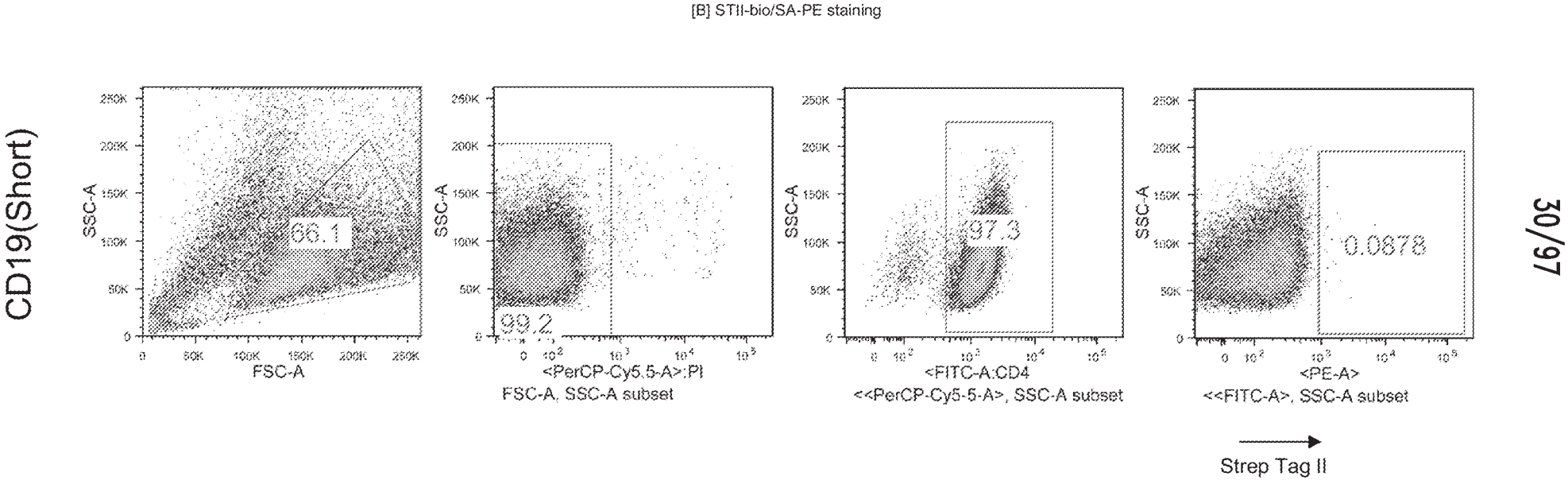


Fig. 8B *(Continued)*

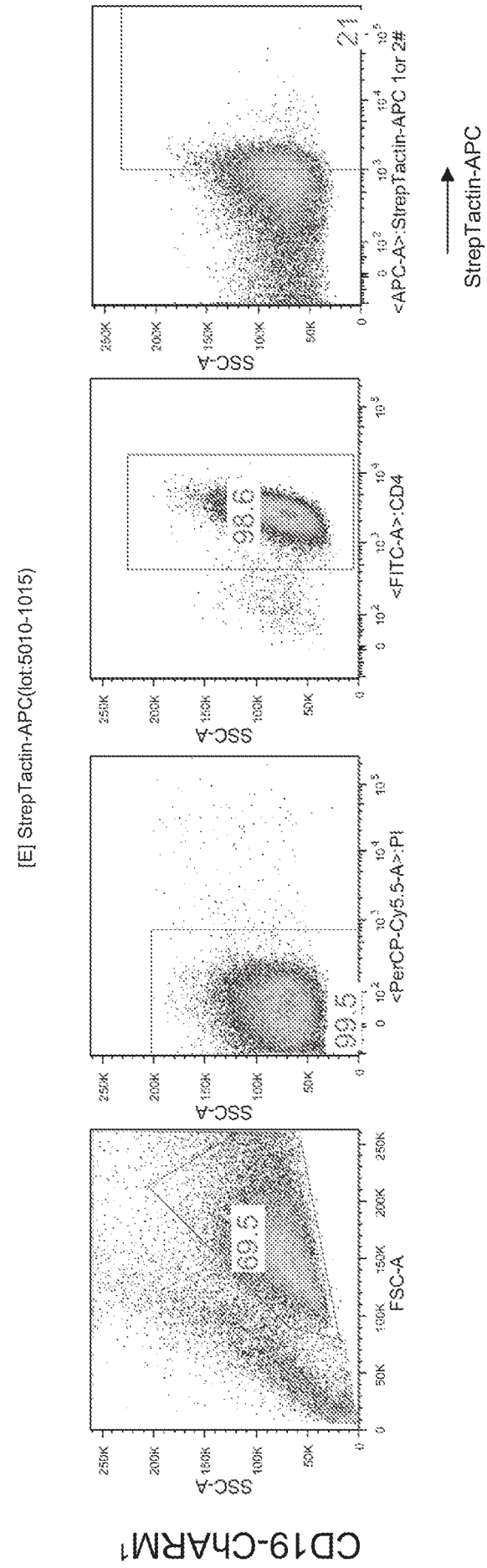

CD19-ChARM1
[E] StrepTactin-APC(lot:5010-1015)
FSC-A
SSC-A
69.5
<PerCP-Cy5.5-A>:PI
99.5
<FITC-A>:CD4
98.6
<APC-A>:StrepTactin-APC 1or 2#
21
StrepTactin-APC

Fig. 8C

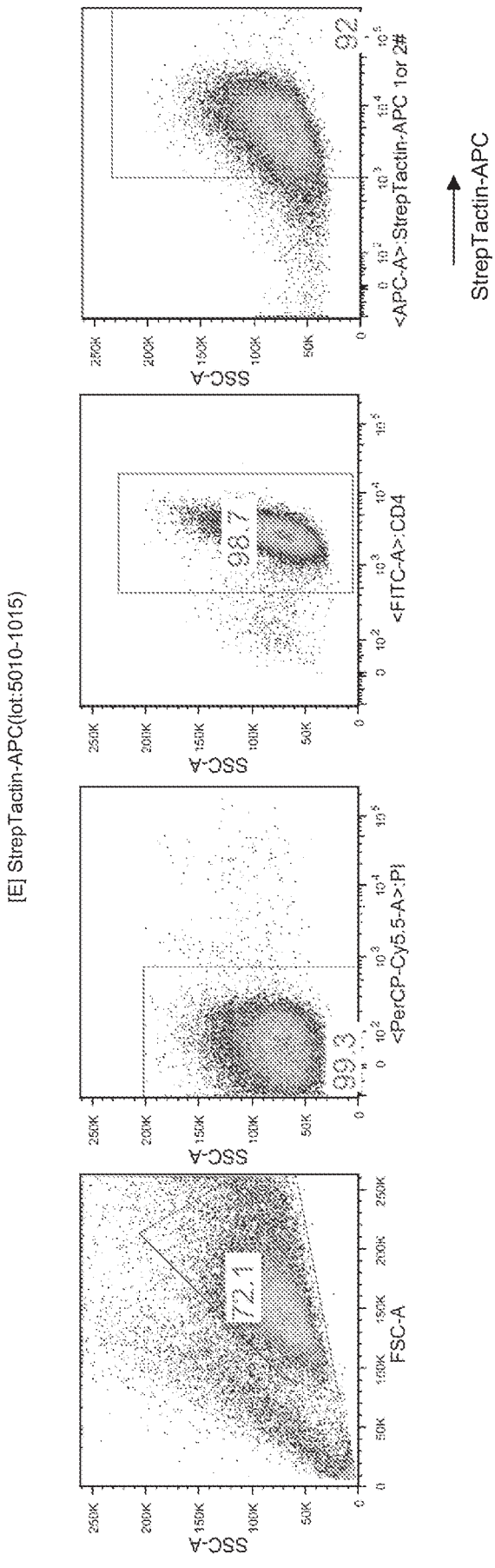


Fig. 8C (Continued)

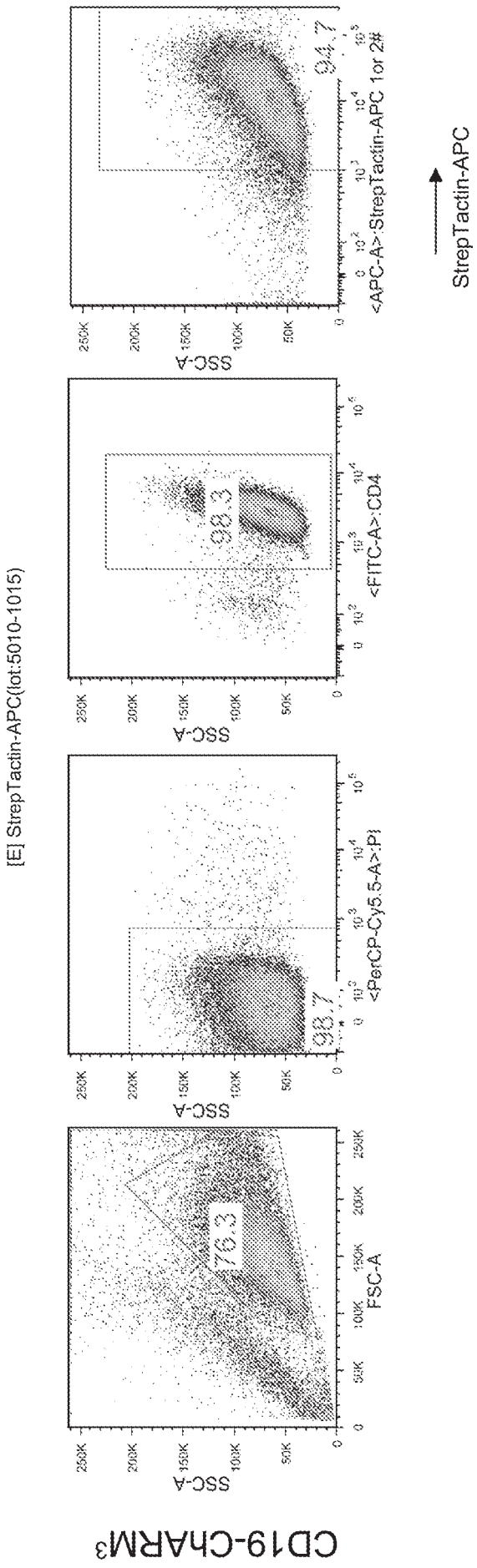


Fig. 8C (Continued)

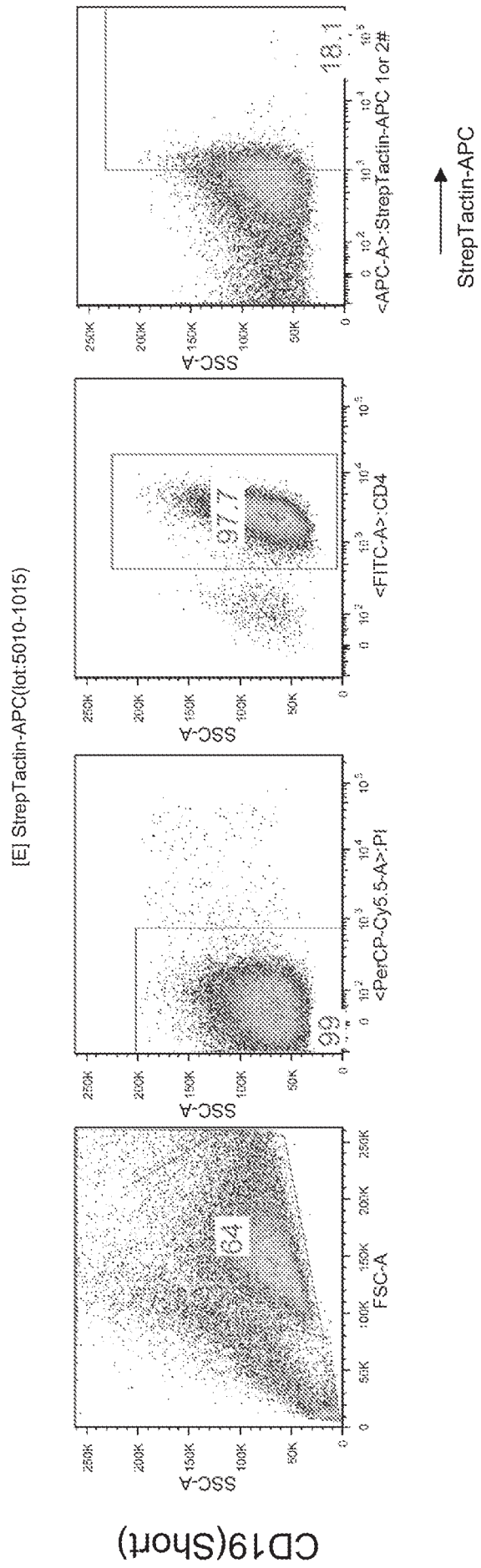


Fig. 8C (Continued)

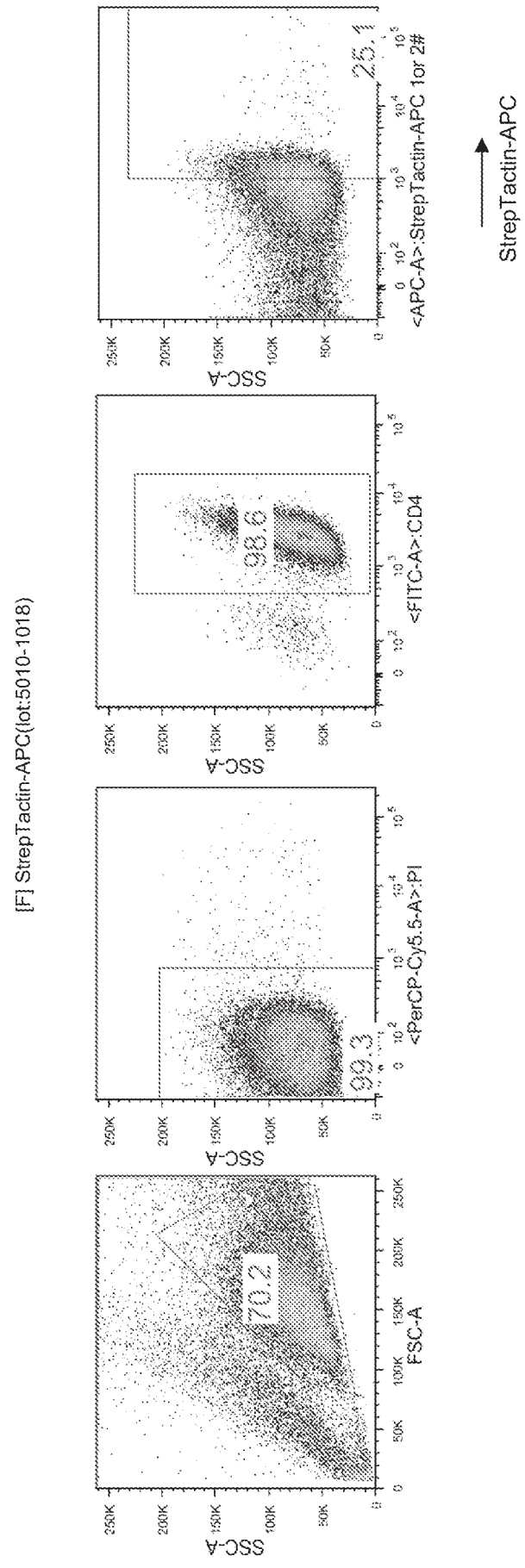

CD19-ChARM1
[F] StrepTactin-APC(lot:5010-1018)
FSC-A
SSC-A
70.2
<PerCP-Cy5.5-A>:PI
89.3
<FITC-A>:CD4
98.6
<APC-A>:StrepTactin-APC 1or 2#
25.1
StrepTactin-APC

Fig. 8D

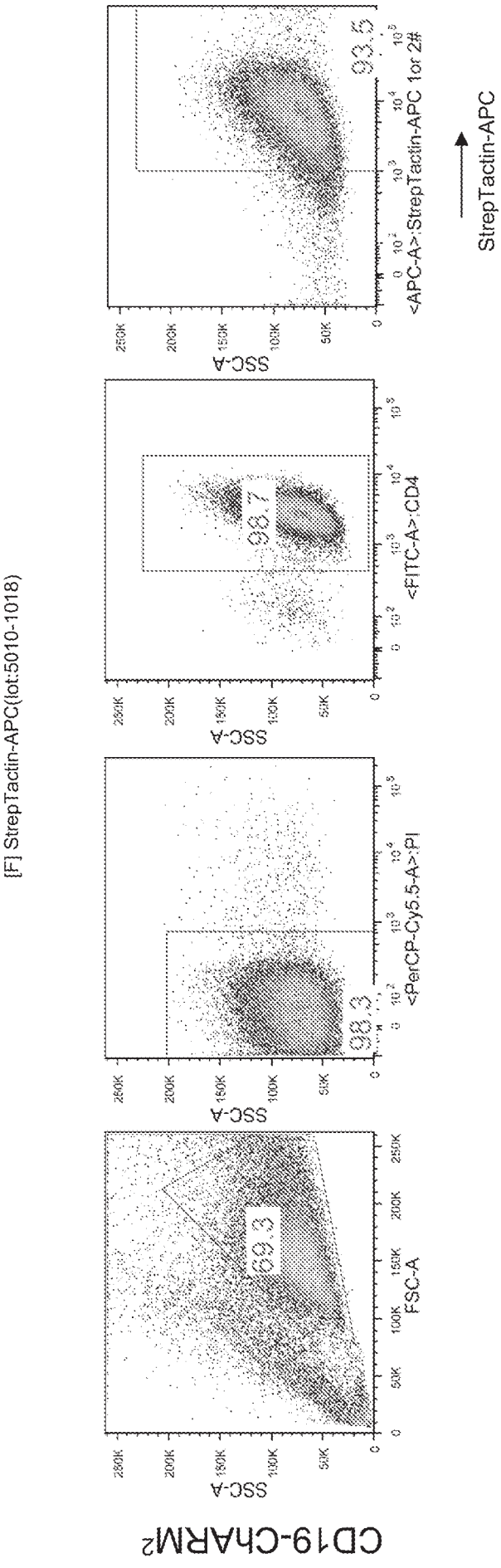


Fig. 8D (Continued)

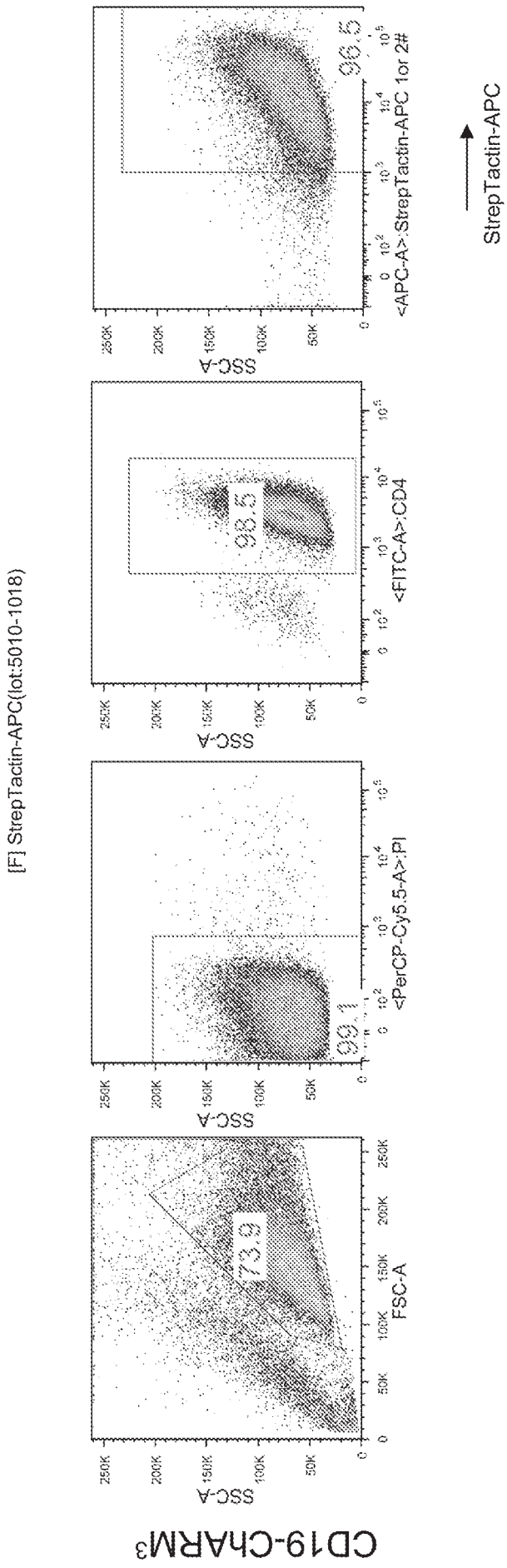


Fig. 8D (Continued)

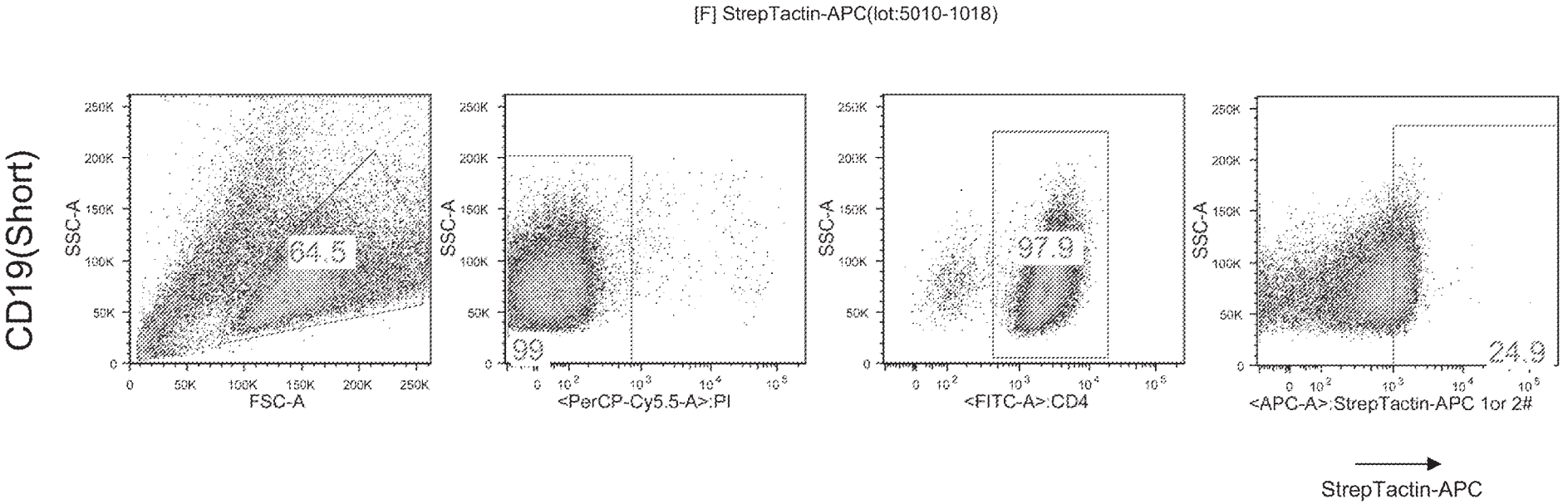


Fig. 8D *(Continued)*

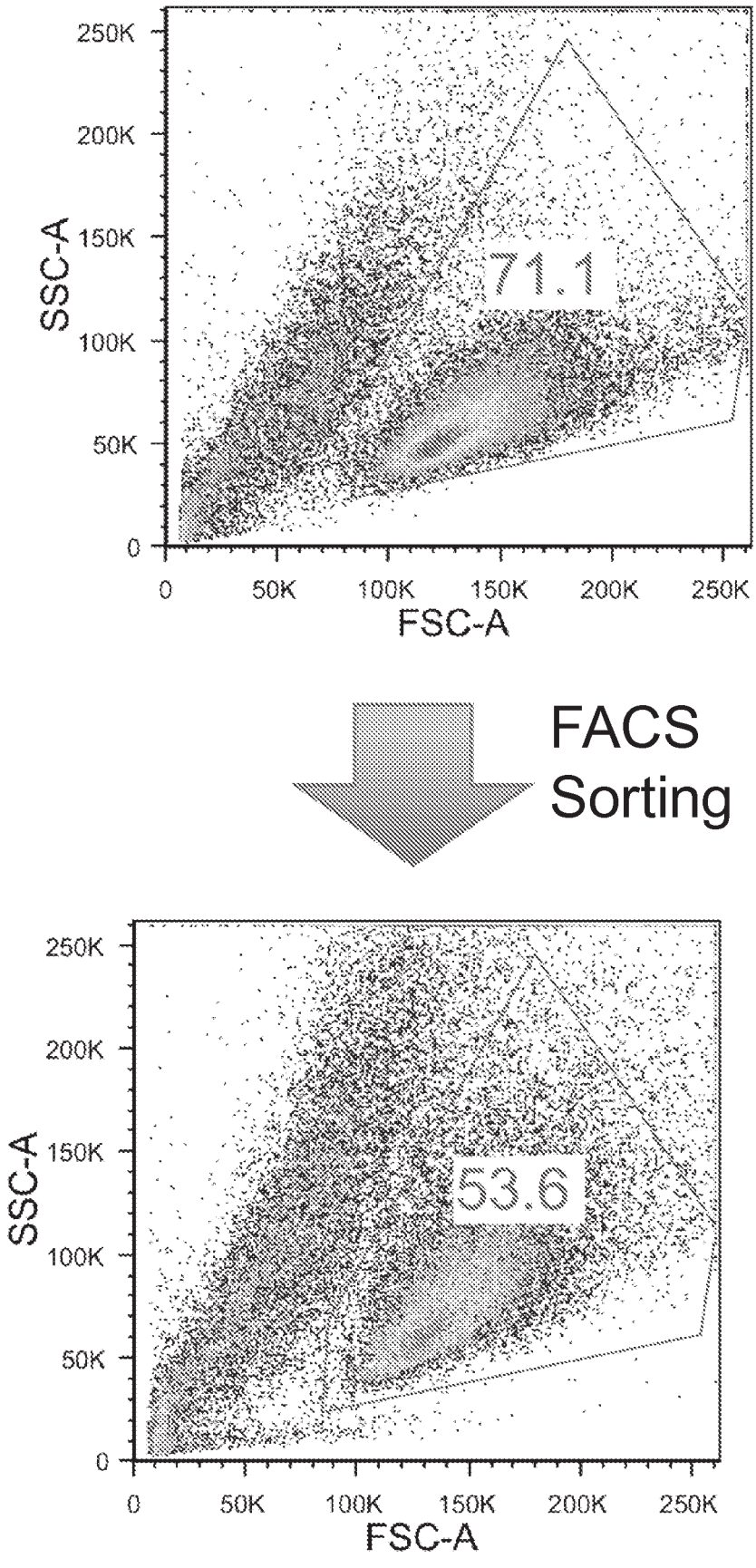

250K
200K
150K
100K
50K
0
SSC-A
71.1
0
50K
100K
150K
200K
250K
FSC-A
FACS
Sorting
250K
200K
150K
100K
50K
0
SSC-A
53.6
0
50K
100K
150K
200K
250K
FSC-A

Fig. 9

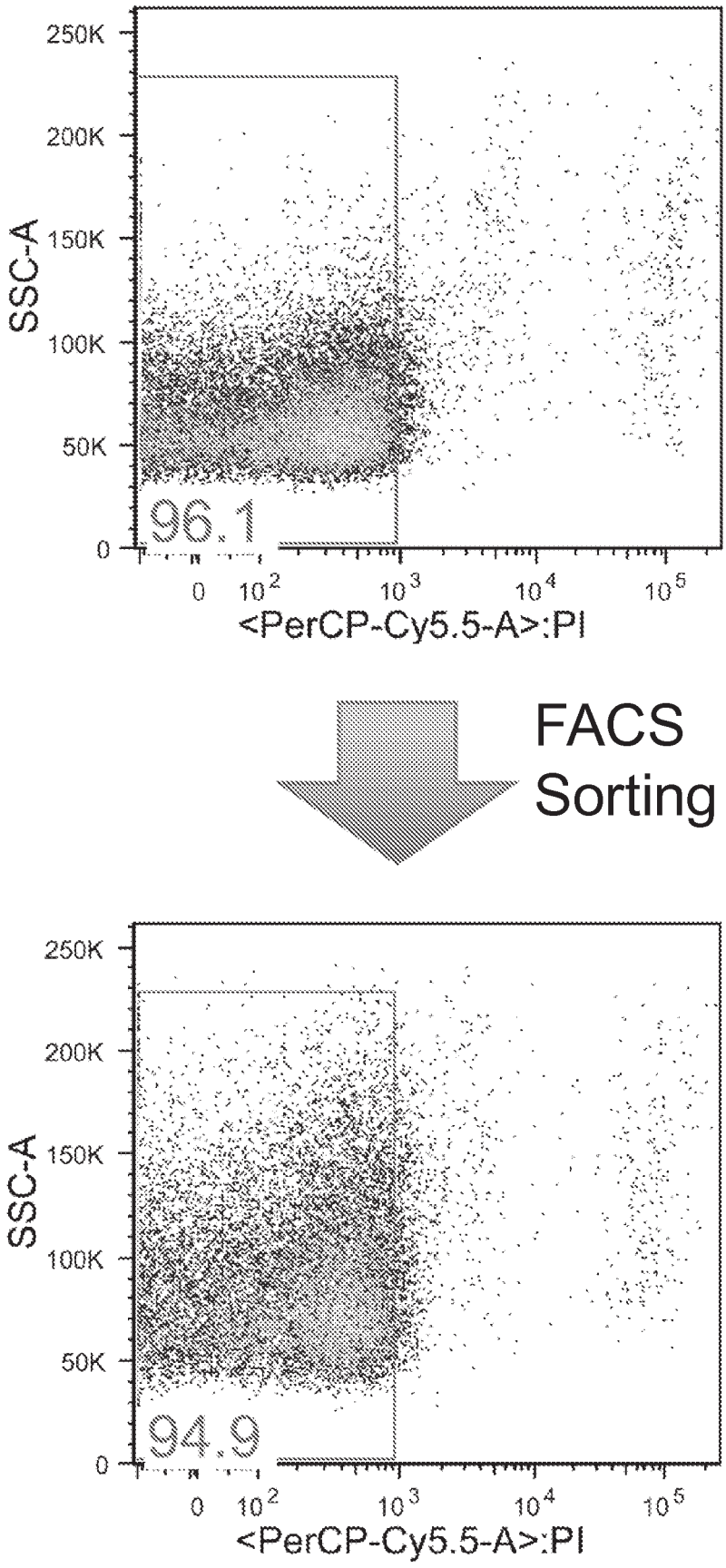


Fig. 9 *(Continued)*

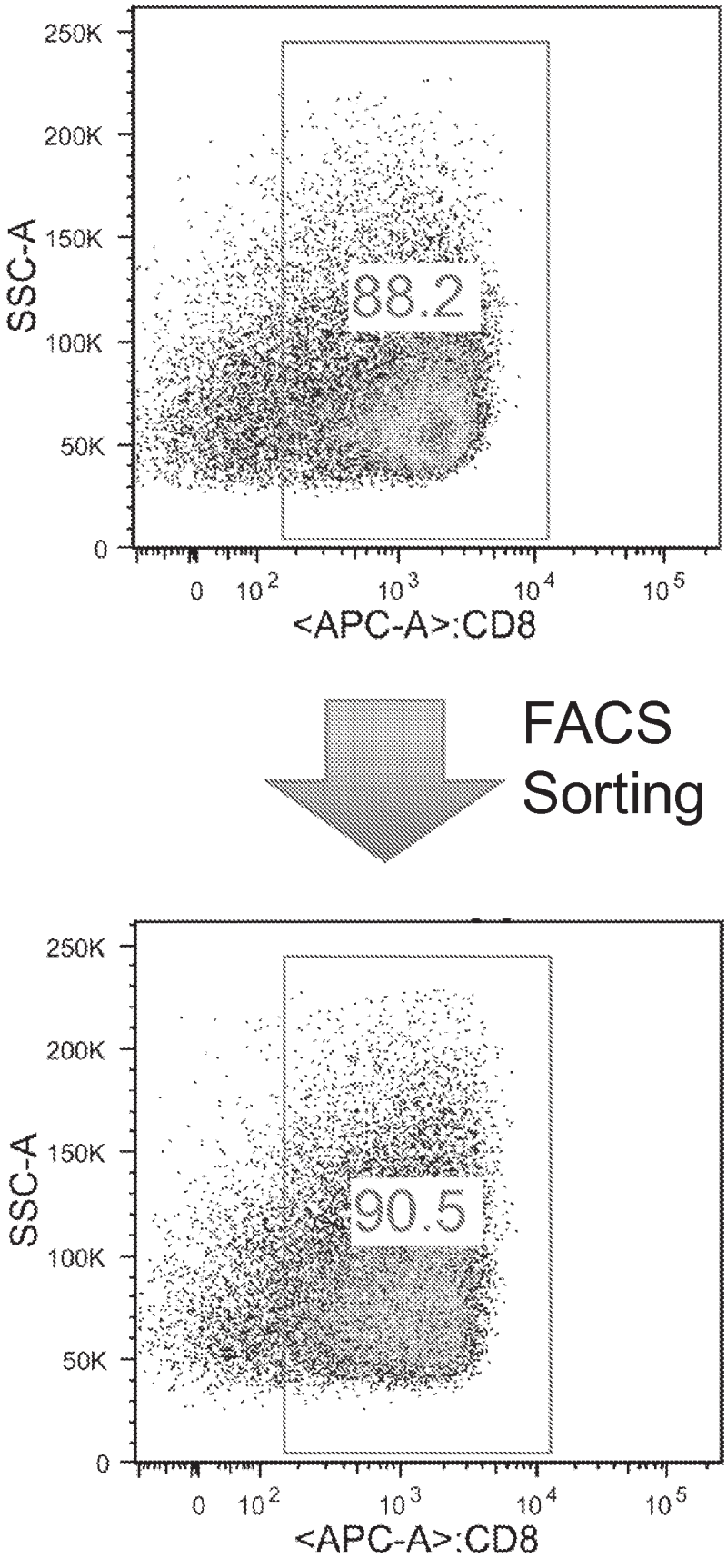
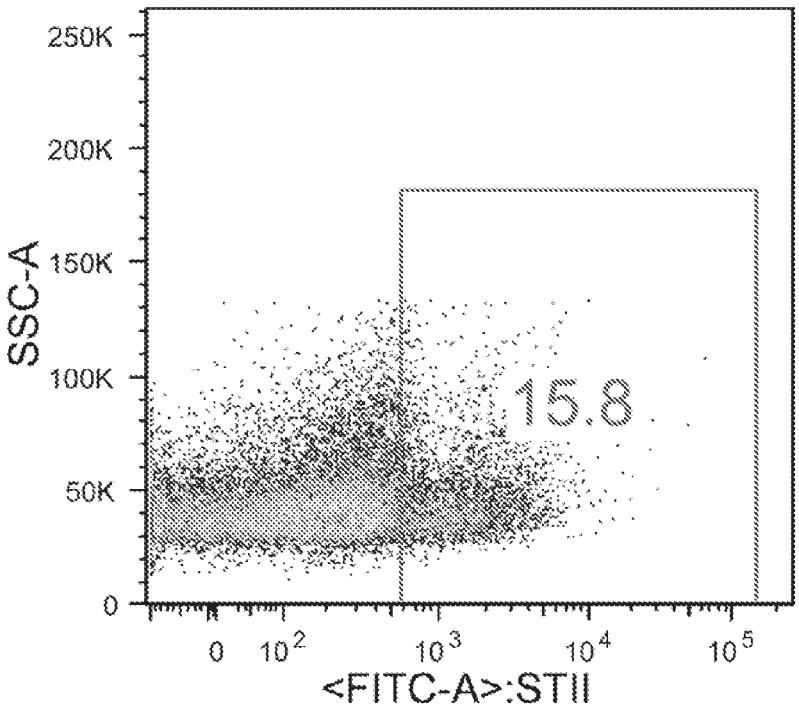


Fig. 9 *(Continued)*



FACS Sorting

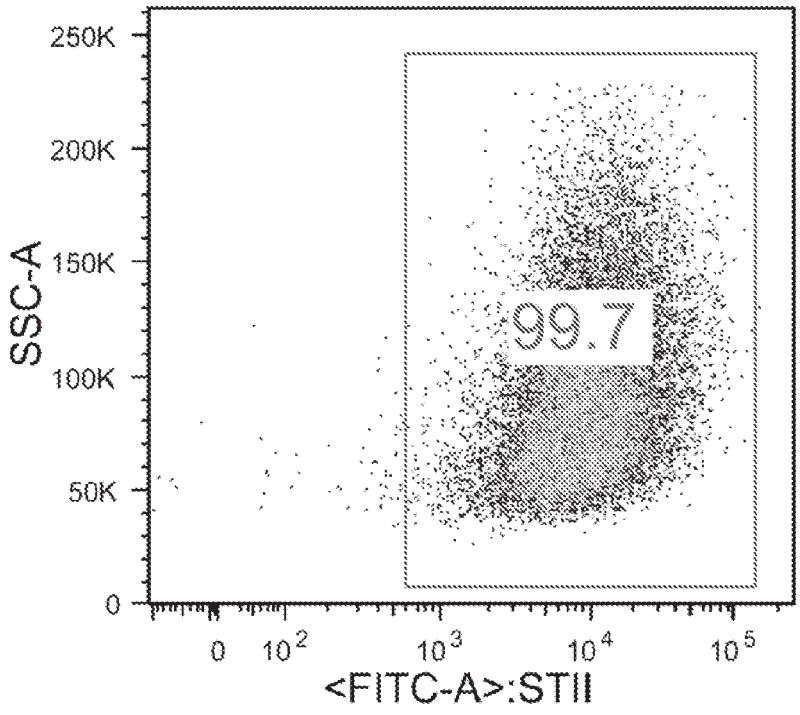


Fig. 9 *(Continued)*

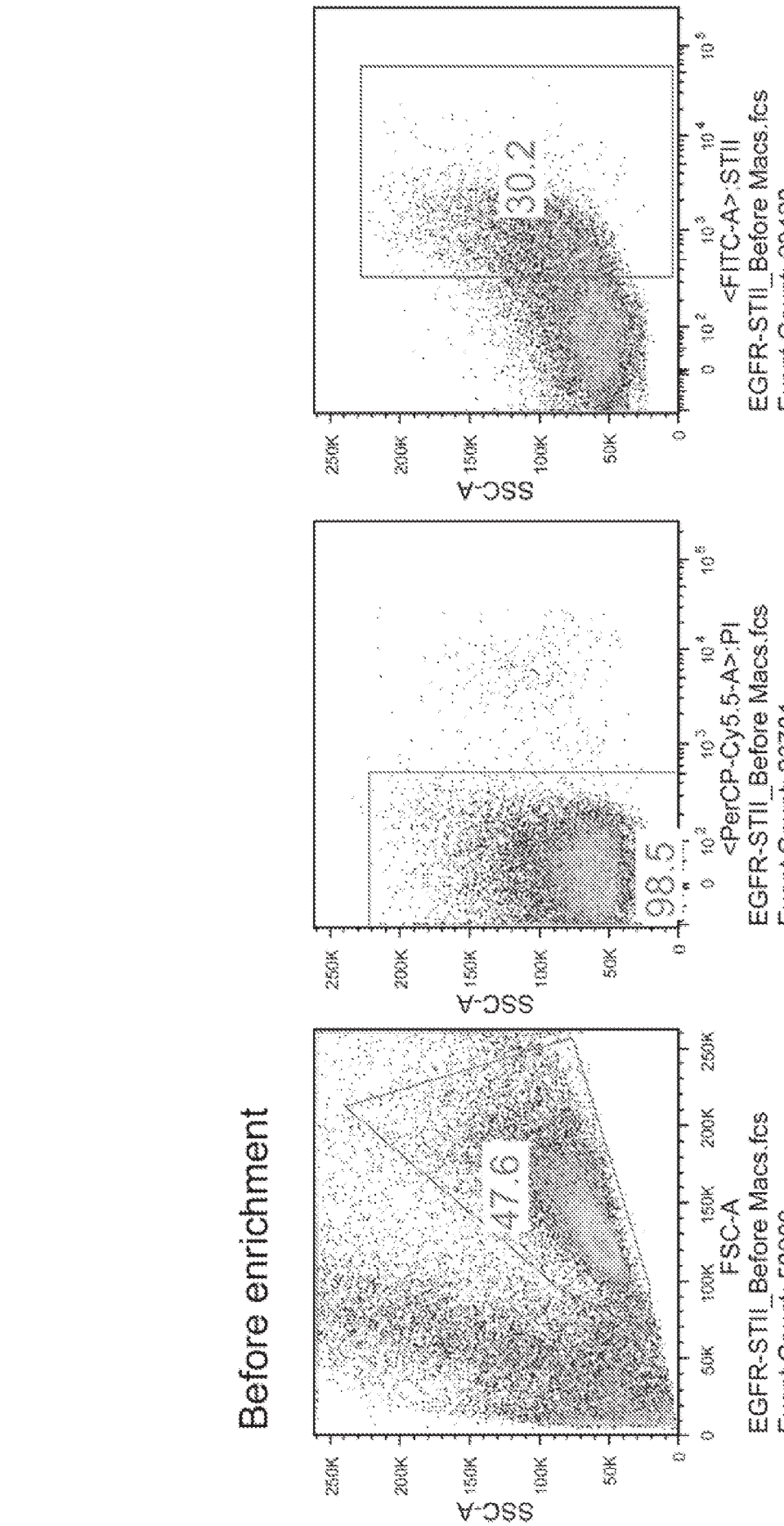

Before enrichment
SSC-A
FSC-A
47.6
EGFR-STII_Before Macs.fcs
Event Count: 50000
98.5
<PerCP-Cy5.5-A>:PI
EGFR-STII_Before Macs.fcs
Event Count: 23781
30.2
<FITC-A>:STII
EGFR-STII_Before Macs.fcs
Event Count: 23428

Fig. 10

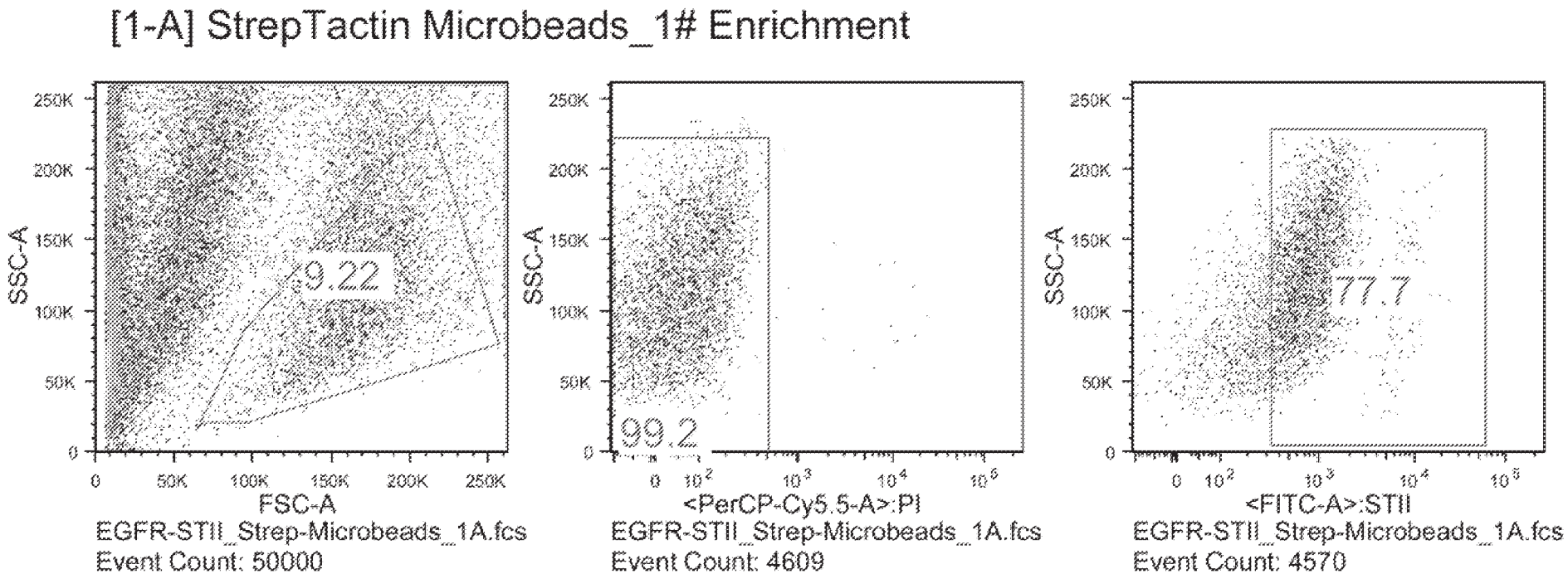


Fig. 10 *(Continued)*

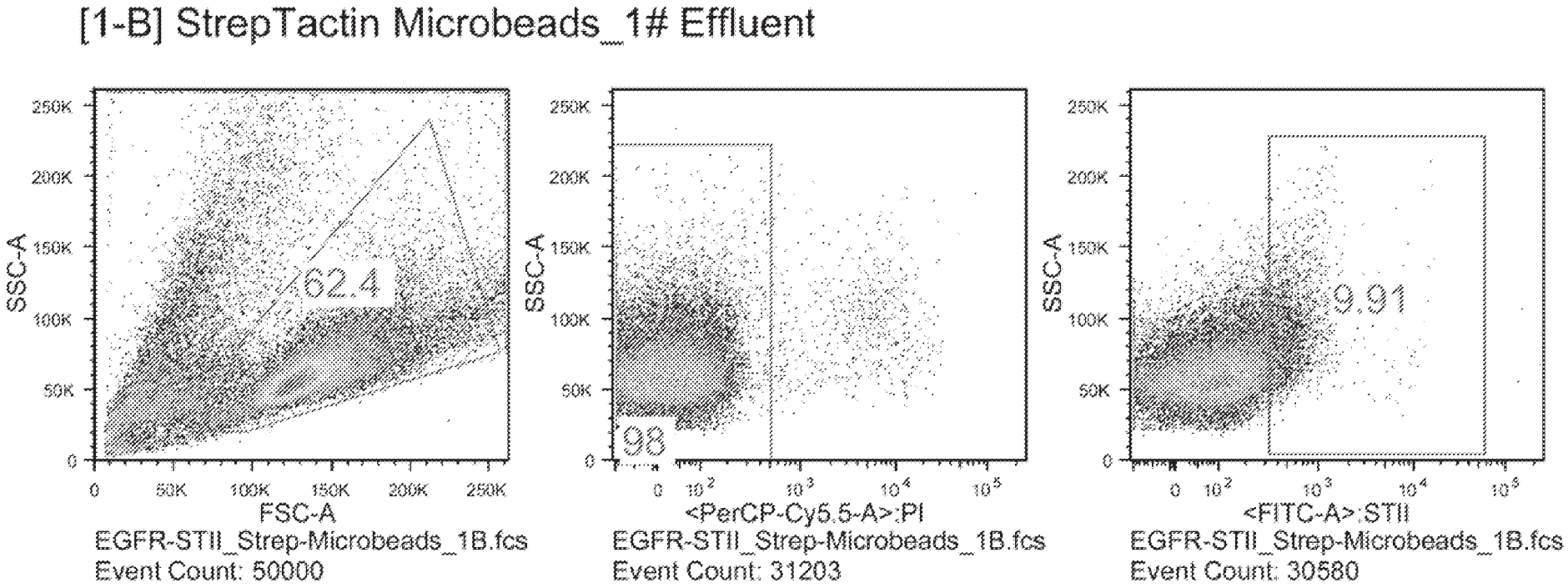


Fig. 10 *(Continued)*

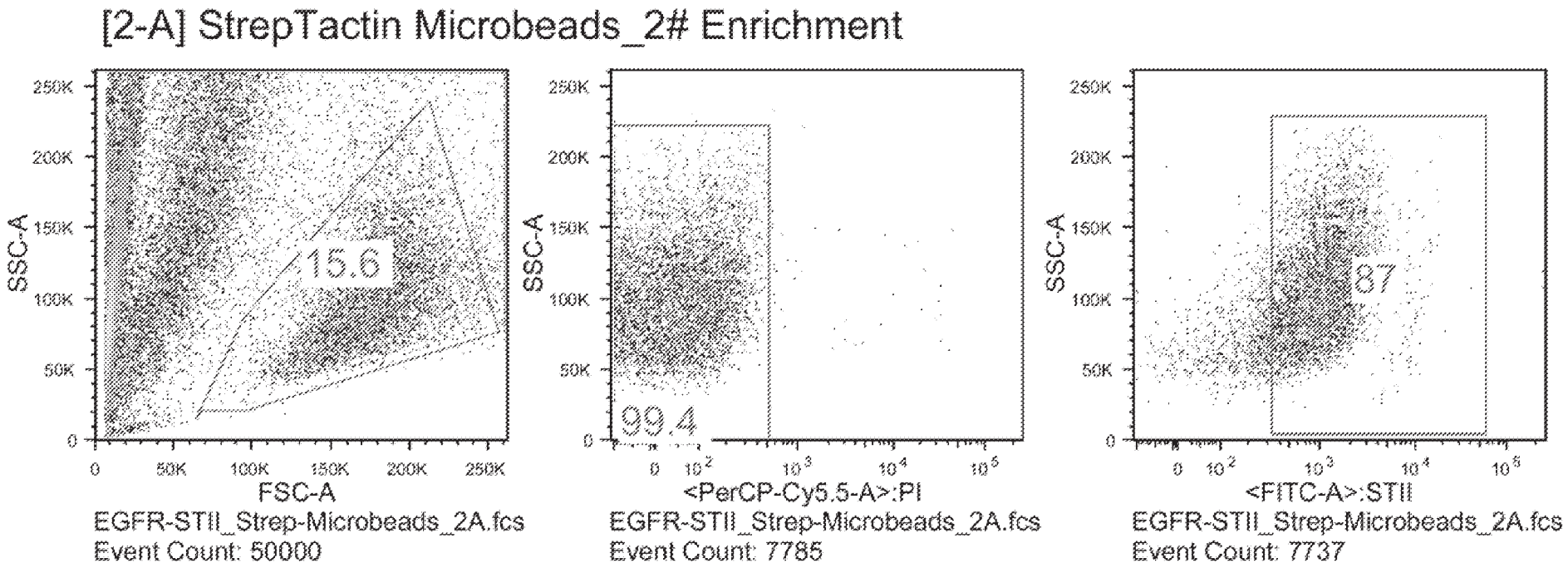


Fig. 10 *(Continued)*

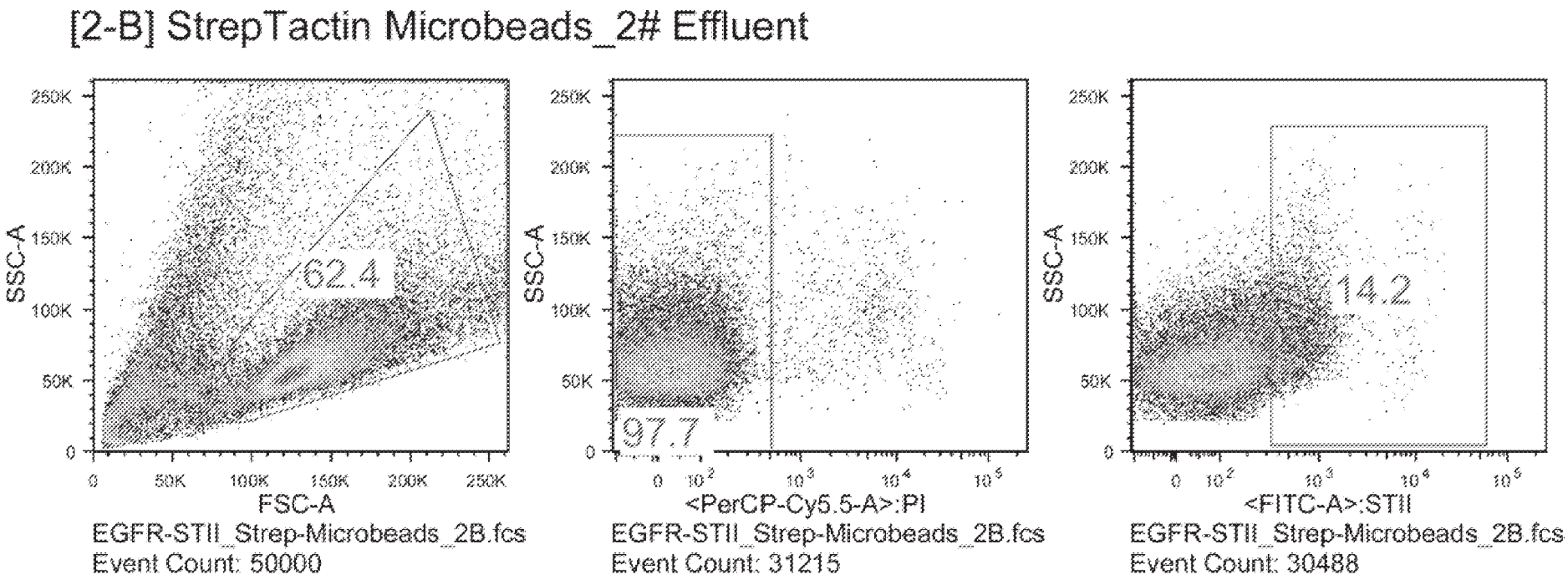


Fig. 10 *(Continued)*

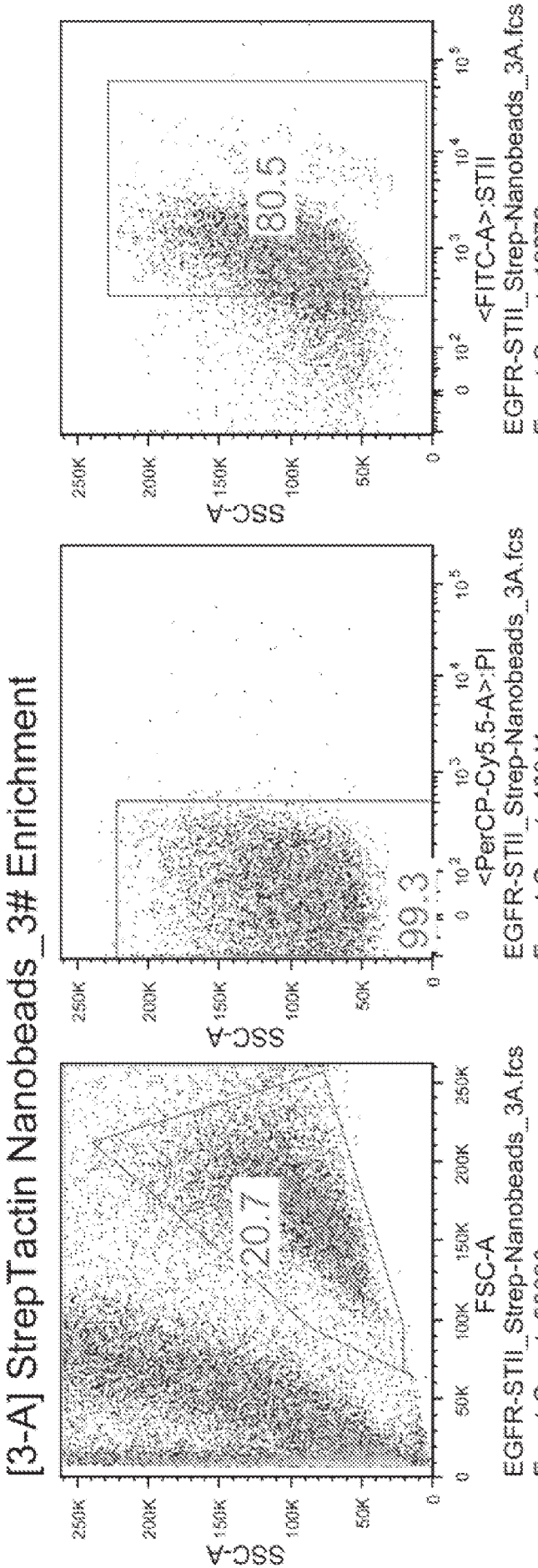


Fig. 10 (Continued)

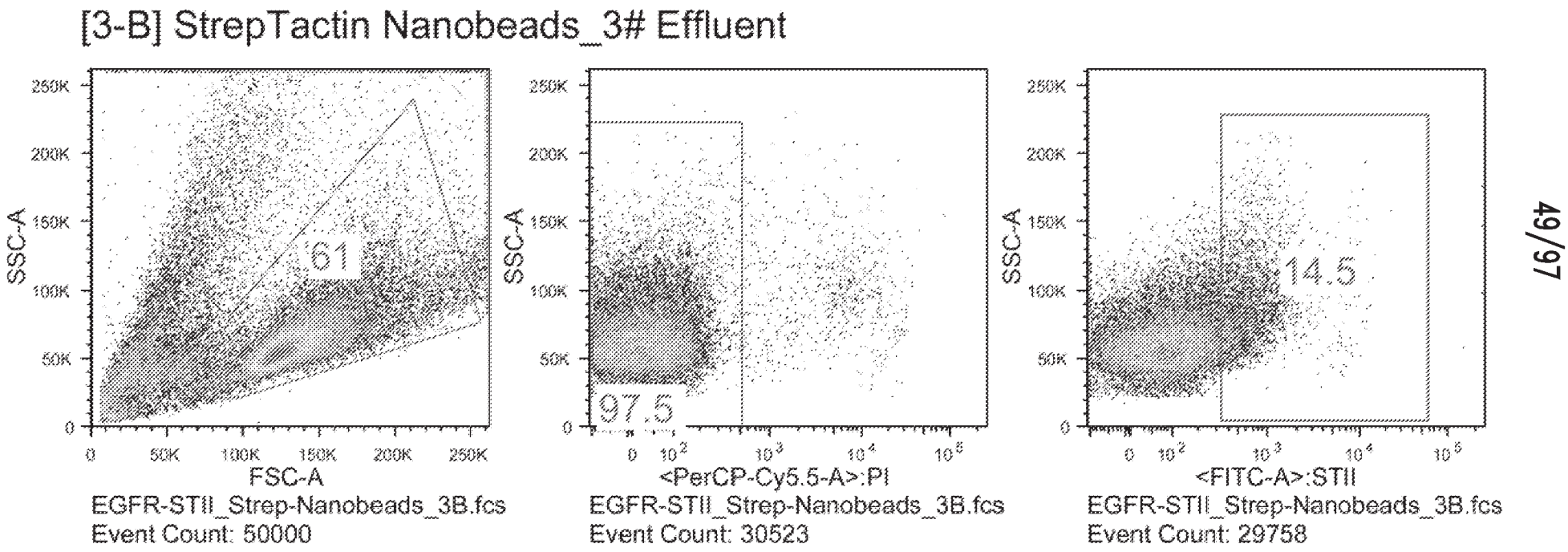


Fig. 10 *(Continued)*

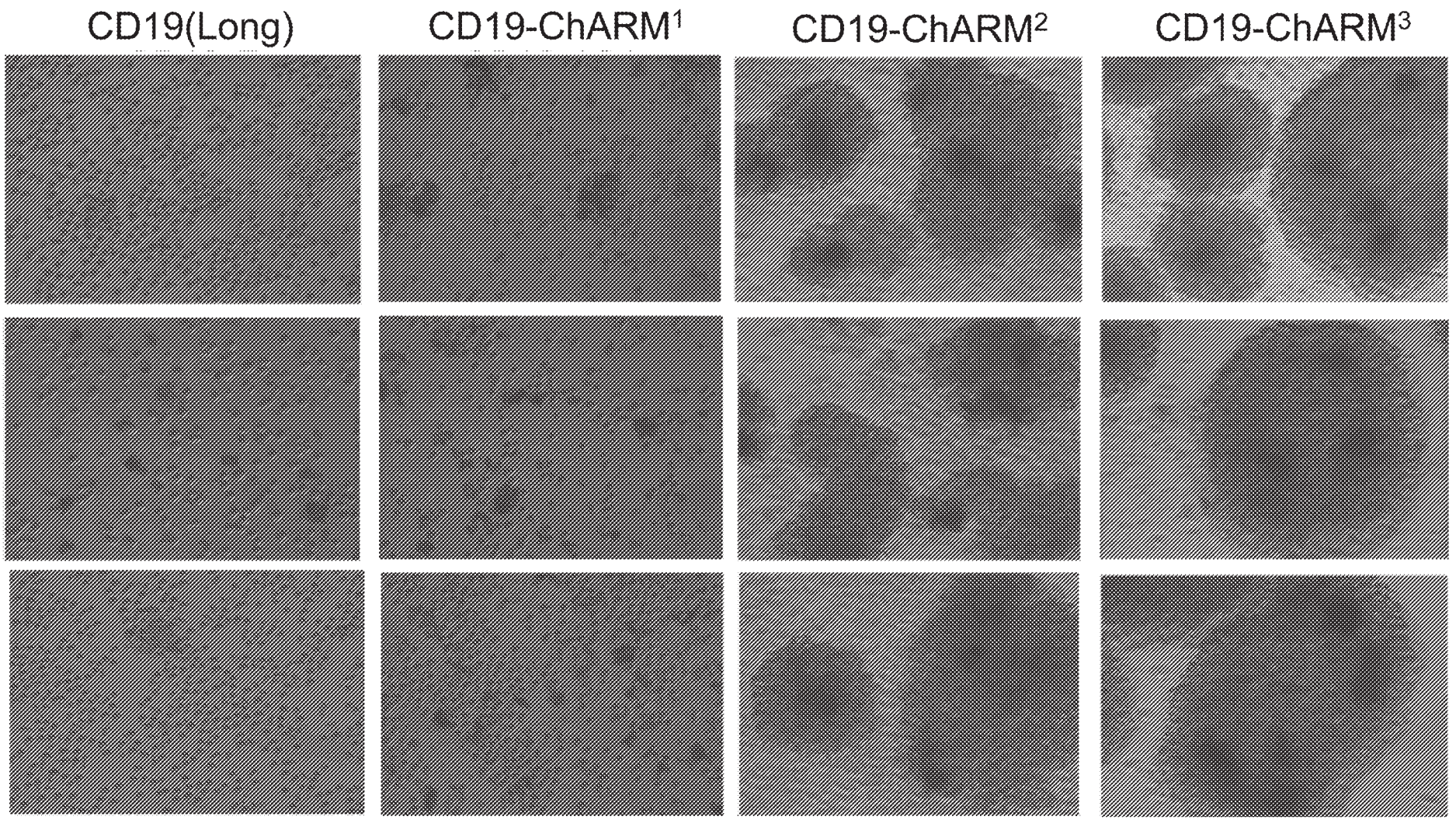


Fig. 11

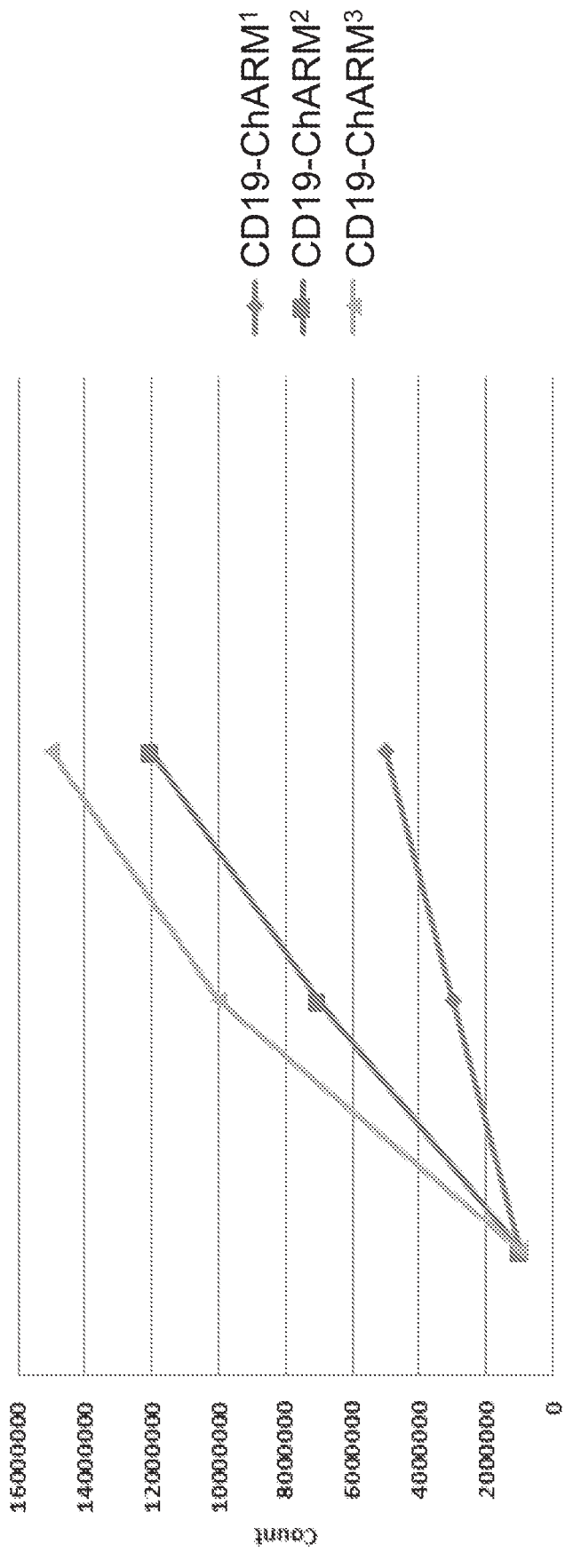

StrepTactin Microbeads (1ul/1X10^6)
Count
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
day1
day5
day10
CD19-ChARM1
CD19-ChARM2
CD19-ChARM3

Fig. 12

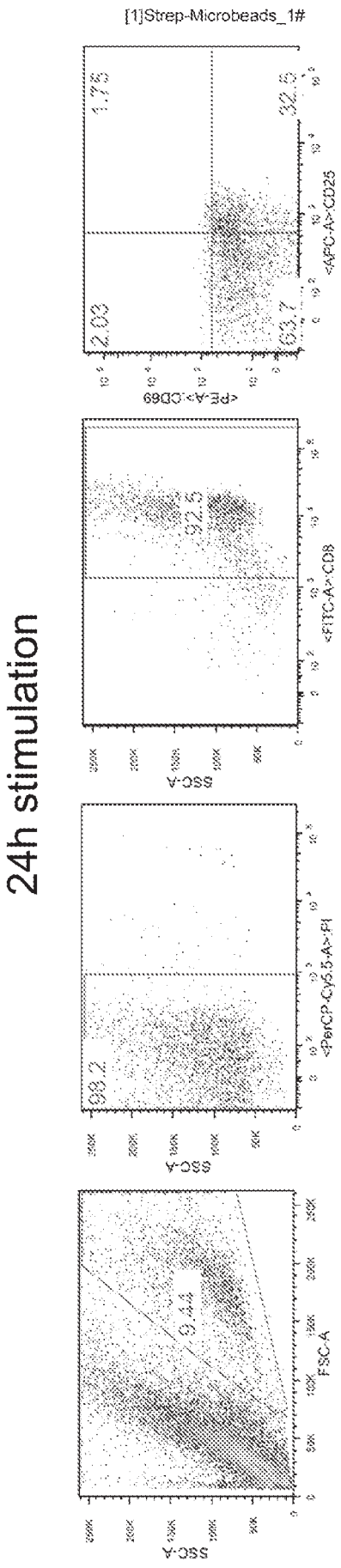

[1]Strep-Microbeads_1#
24h stimulation
FSC-A
SSC-A
9.44
<PerCP-Cy5-5-A>:FI
98.2
<FITC-A>:CD8
92.5
<APC-A>:CD25
1.78
30.6
<PE-A>:CD69
2.03
63.7

Fig. 13A

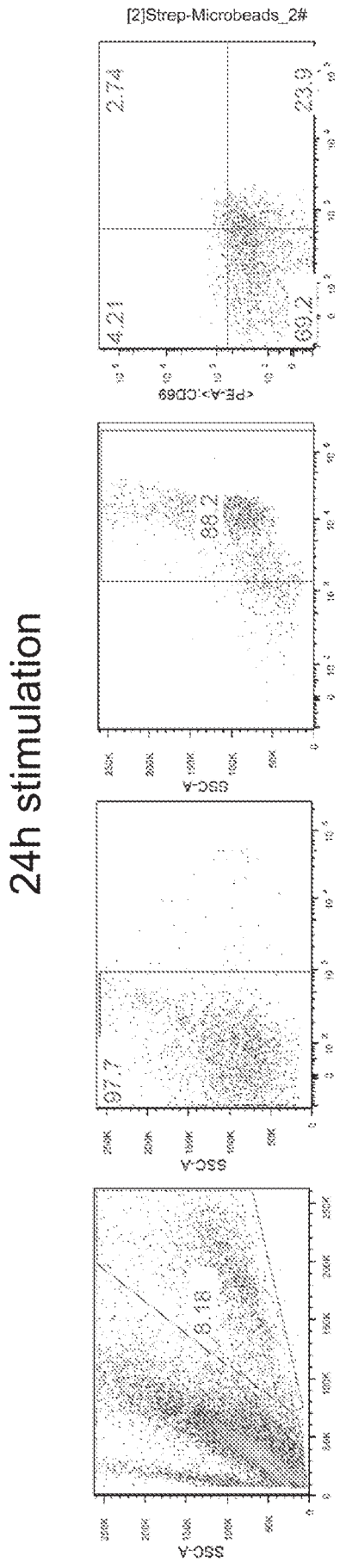


Fig. 13A (Continued)

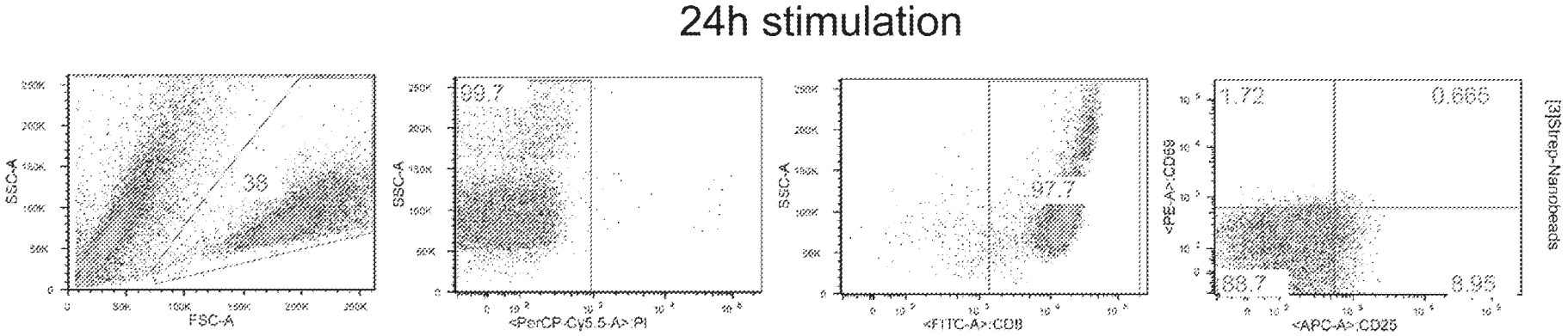


Fig. 13A *(Continued)*

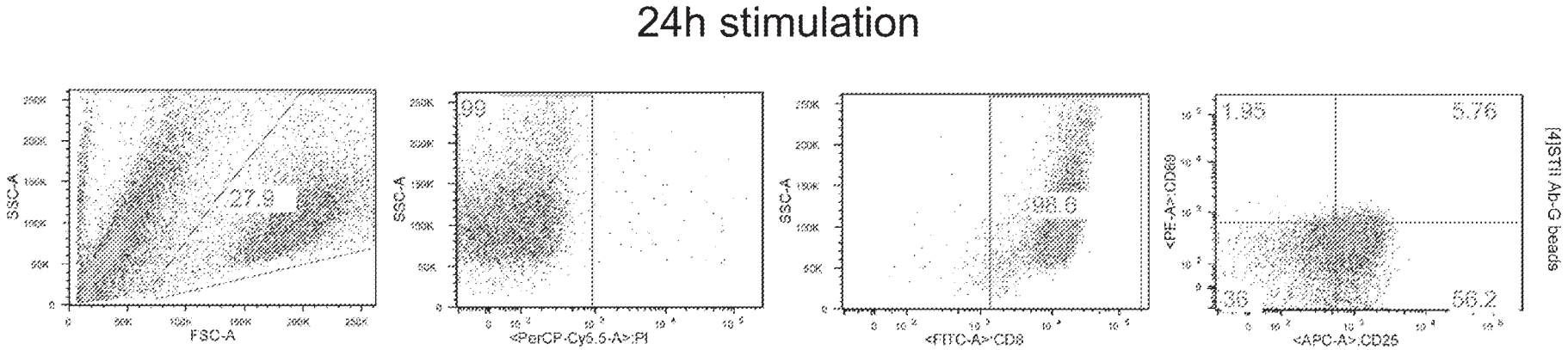


Fig. 13A *(Continued)*

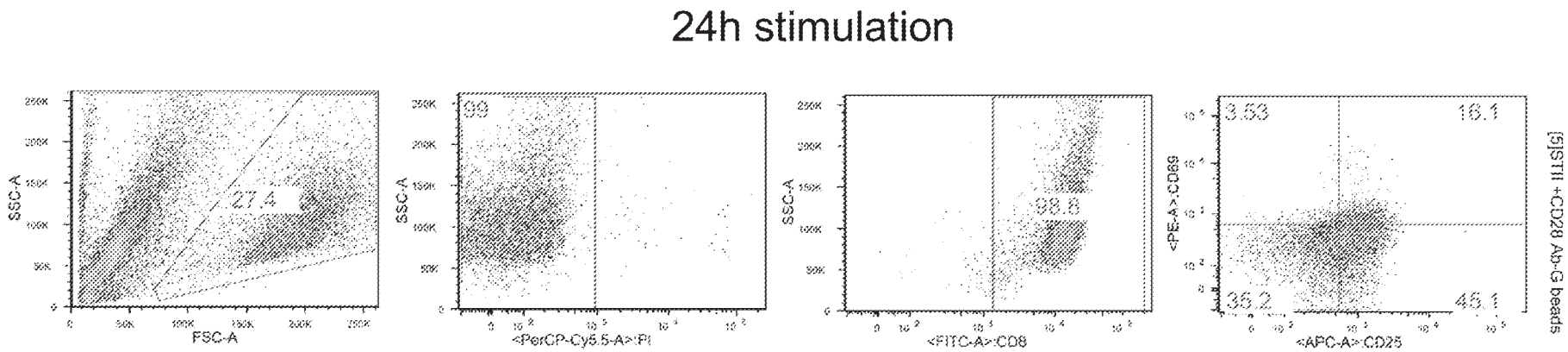


Fig. 13A *(Continued)*

24h stimulation

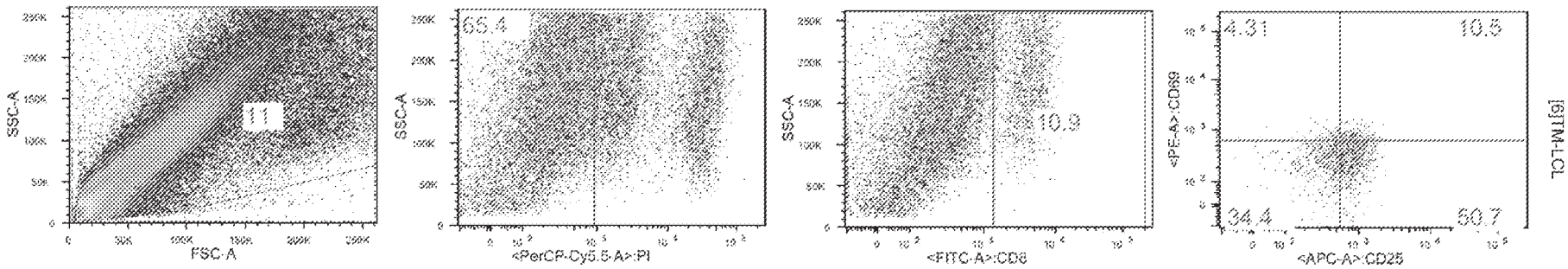


Fig. 13A *(Continued)*

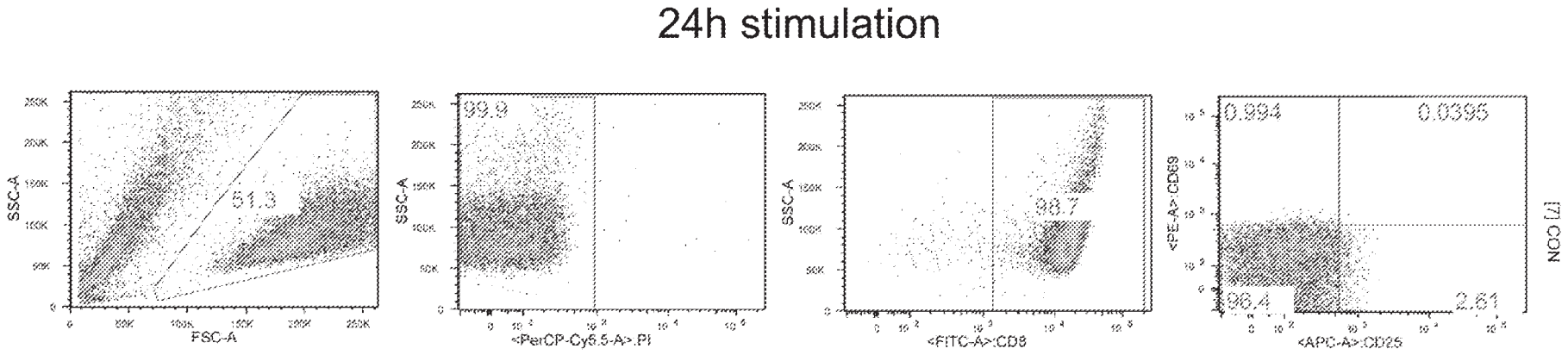


Fig. 13A *(Continued)*

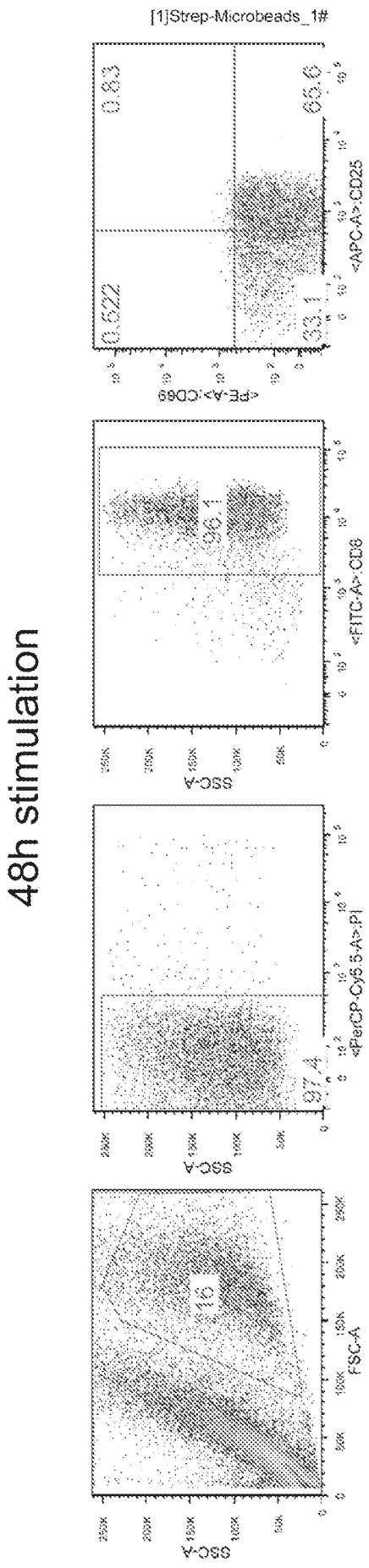

48h stimulation
[1]Strep-Microbeads_1#
SSC-A
FSC-A
10
<PerCP-Cy5-5-A>:PI
97.4
<FITC-A>:CD8
96.1
<PE-A>:CD69
0.622
<APC-A>:CD25
33.1
0.83
65.6

Fig. 13B

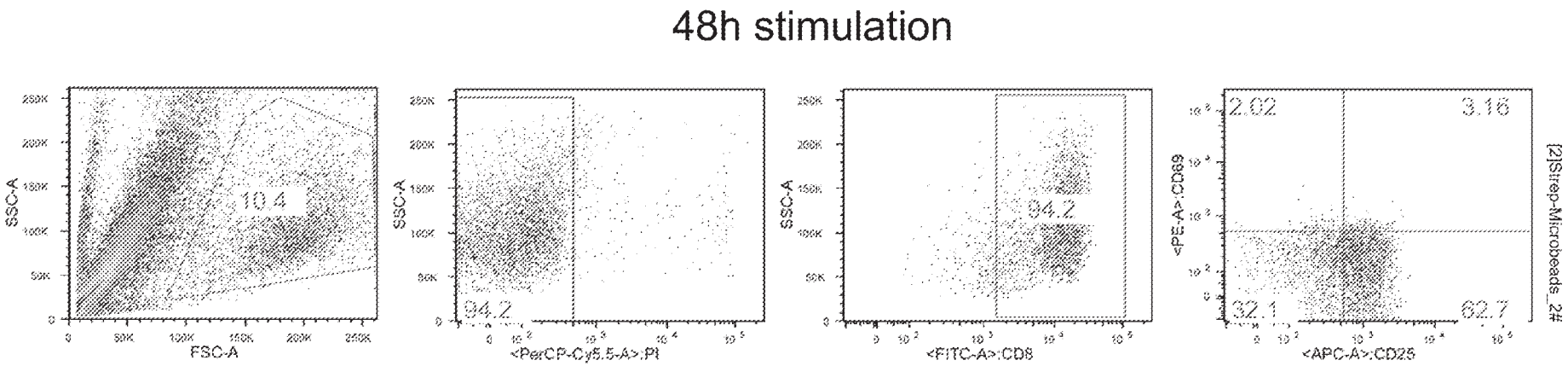


Fig. 13B *(Continued)*

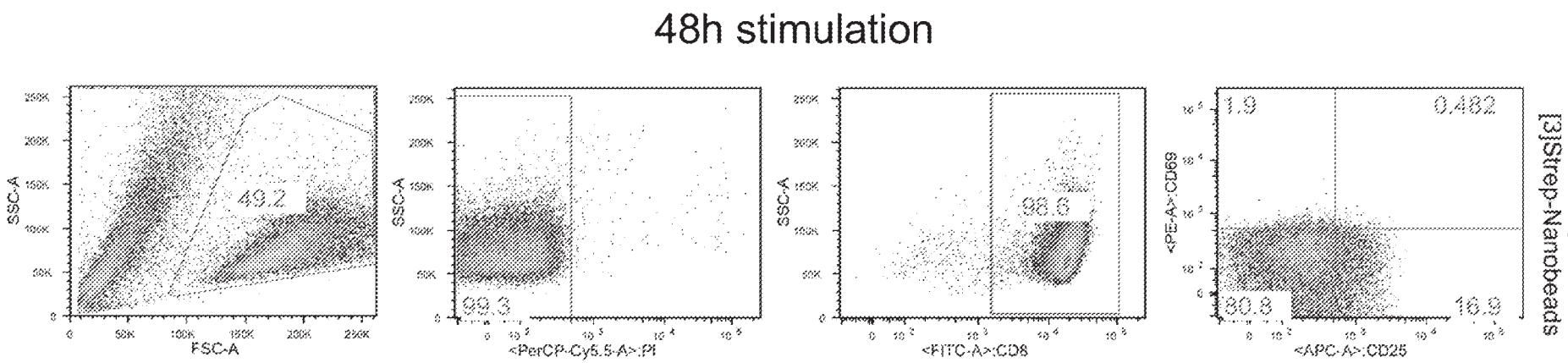


Fig. 13B *(Continued)*

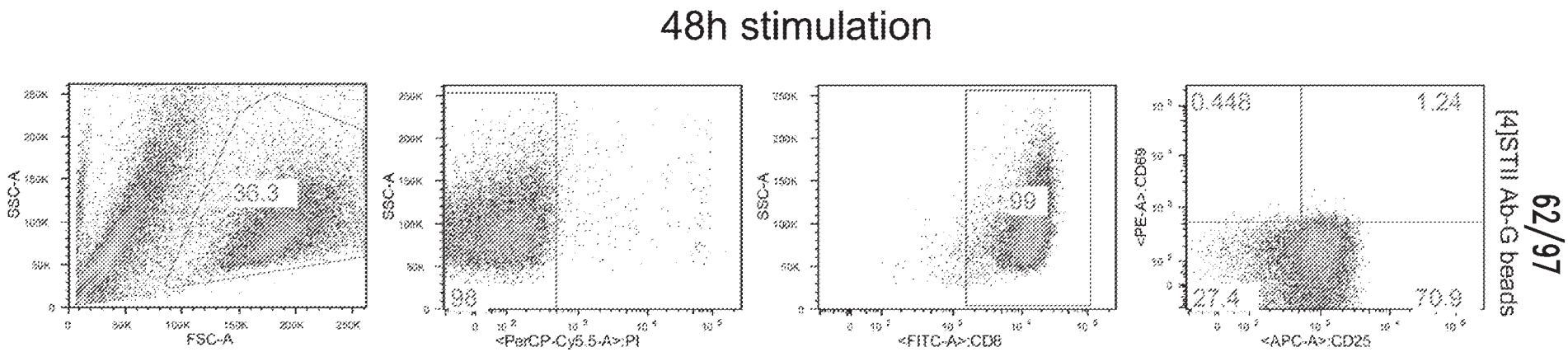


Fig. 13B *(Continued)*

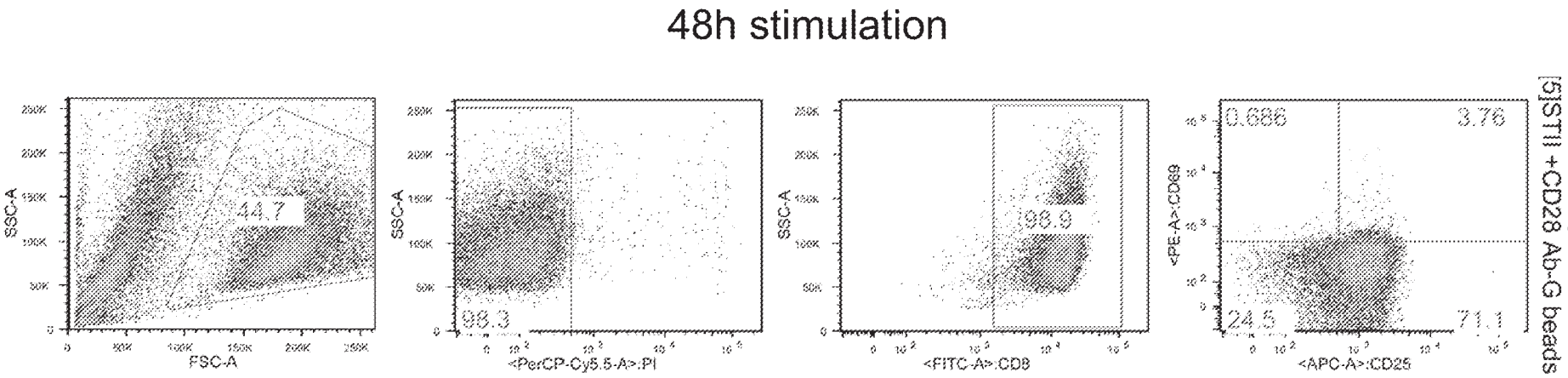


Fig. 13B *(Continued)*

48h stimulation

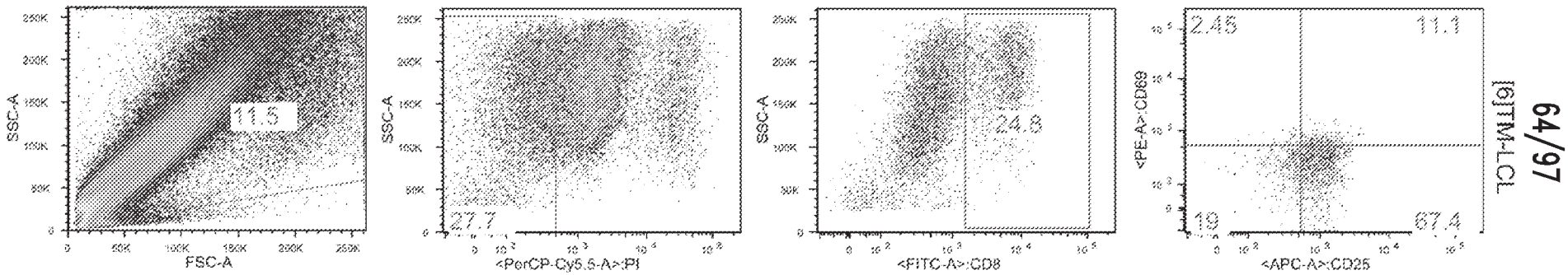


Fig. 13B *(Continued)*

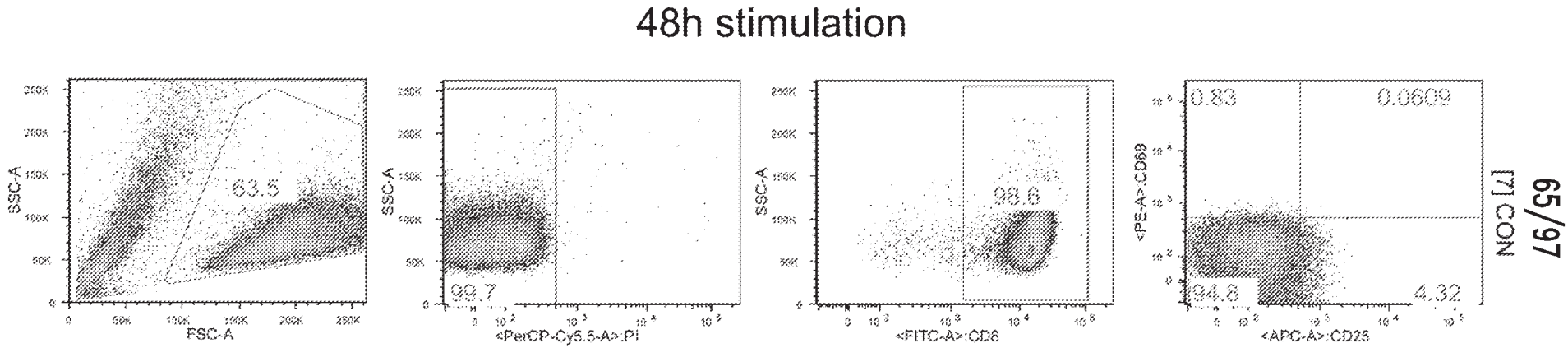


Fig. 13B *(Continued)*

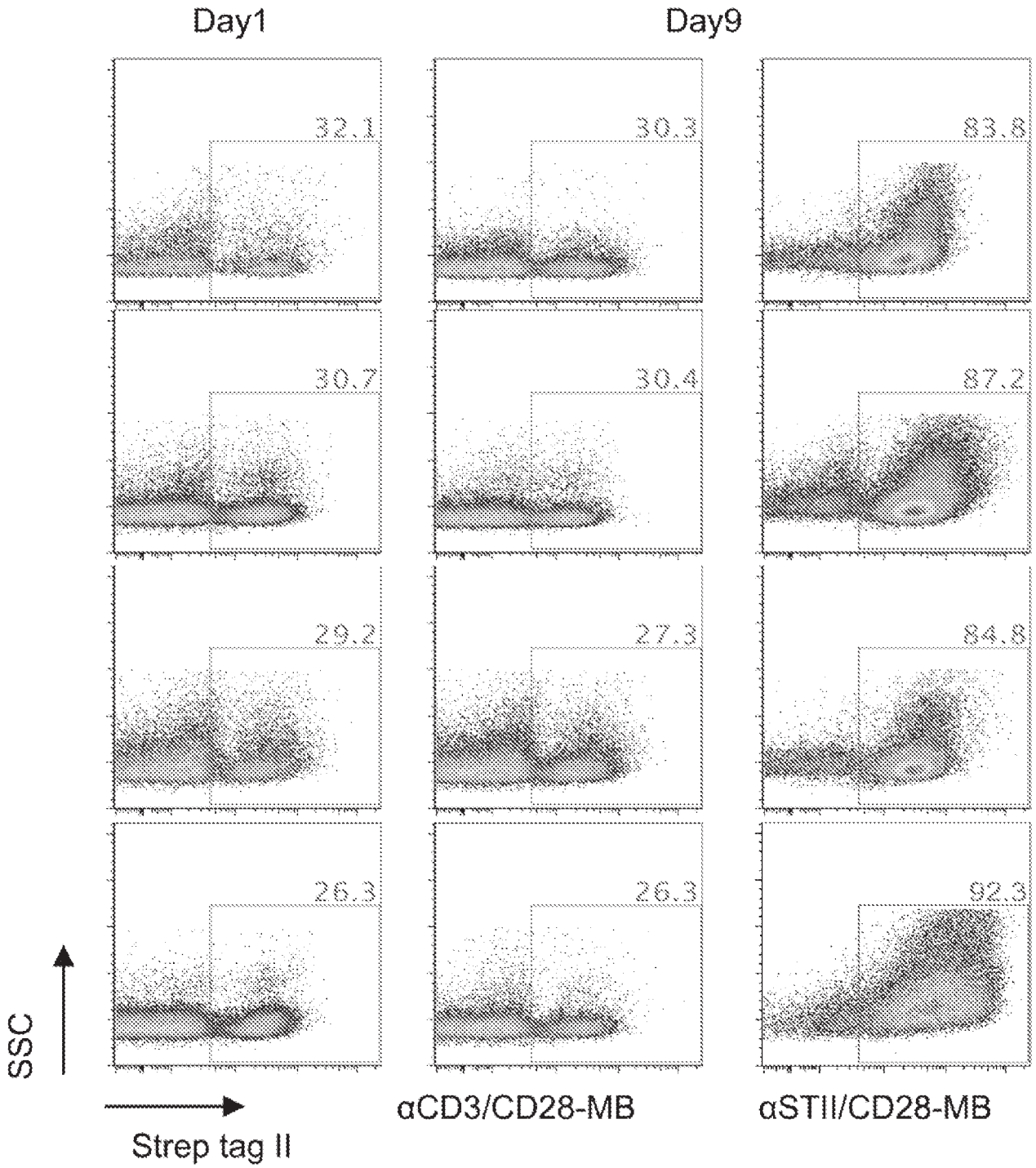

Day1
Day9
32.1
30.3
83.8
30.7
30.4
87.2
29.2
27.3
84.8
26.3
26.3
92.3
SSC
Strep tag II
αCD3/CD28-MB
αSTII/CD28-MB

Fig. 14A

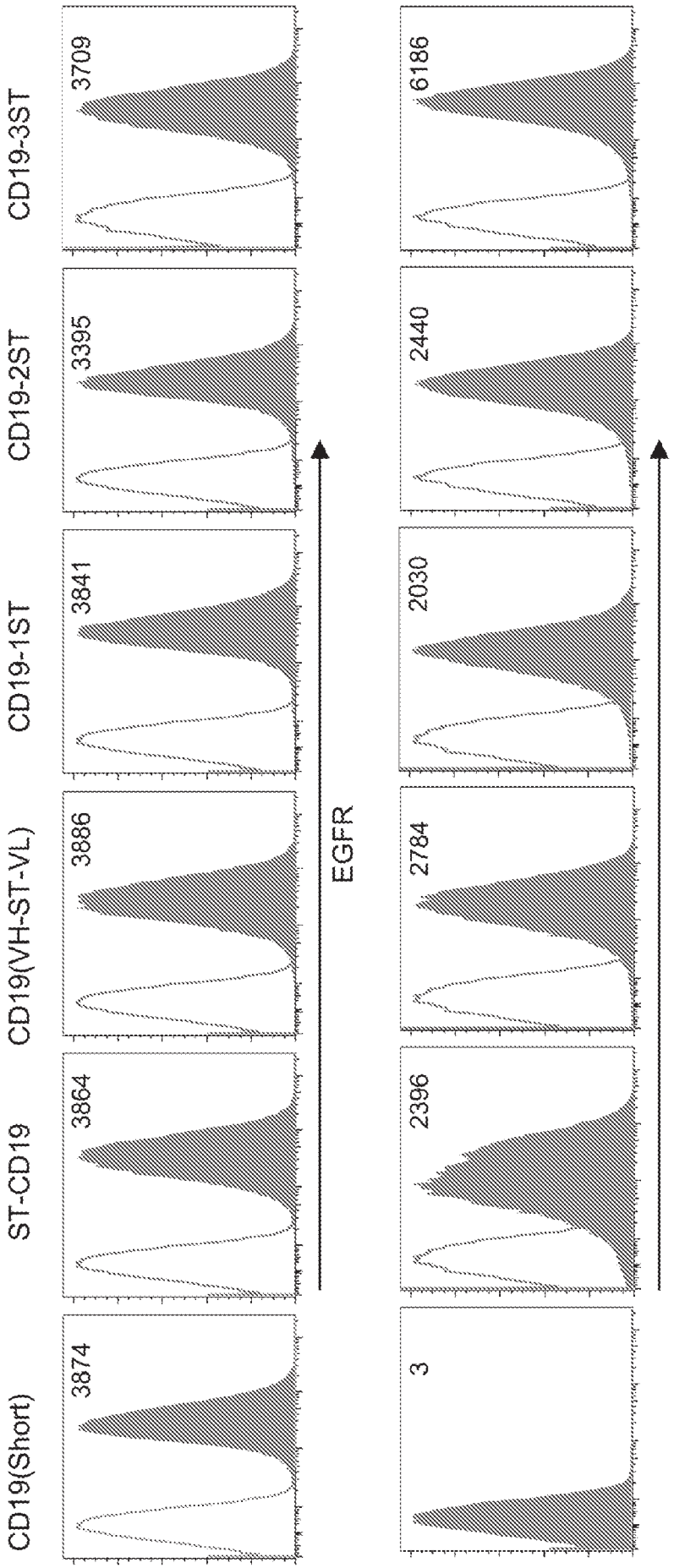

CD19(Short)
ST-CD19
CD19(VH-ST-VL)
CD19-1ST
CD19-2ST
CD19-3ST
3874
3864
3886
3841
3395
3709
EGFR
3
2396
2784
2030
2440
6186
STII

Fig. 14B

CD19-ChARM[1]

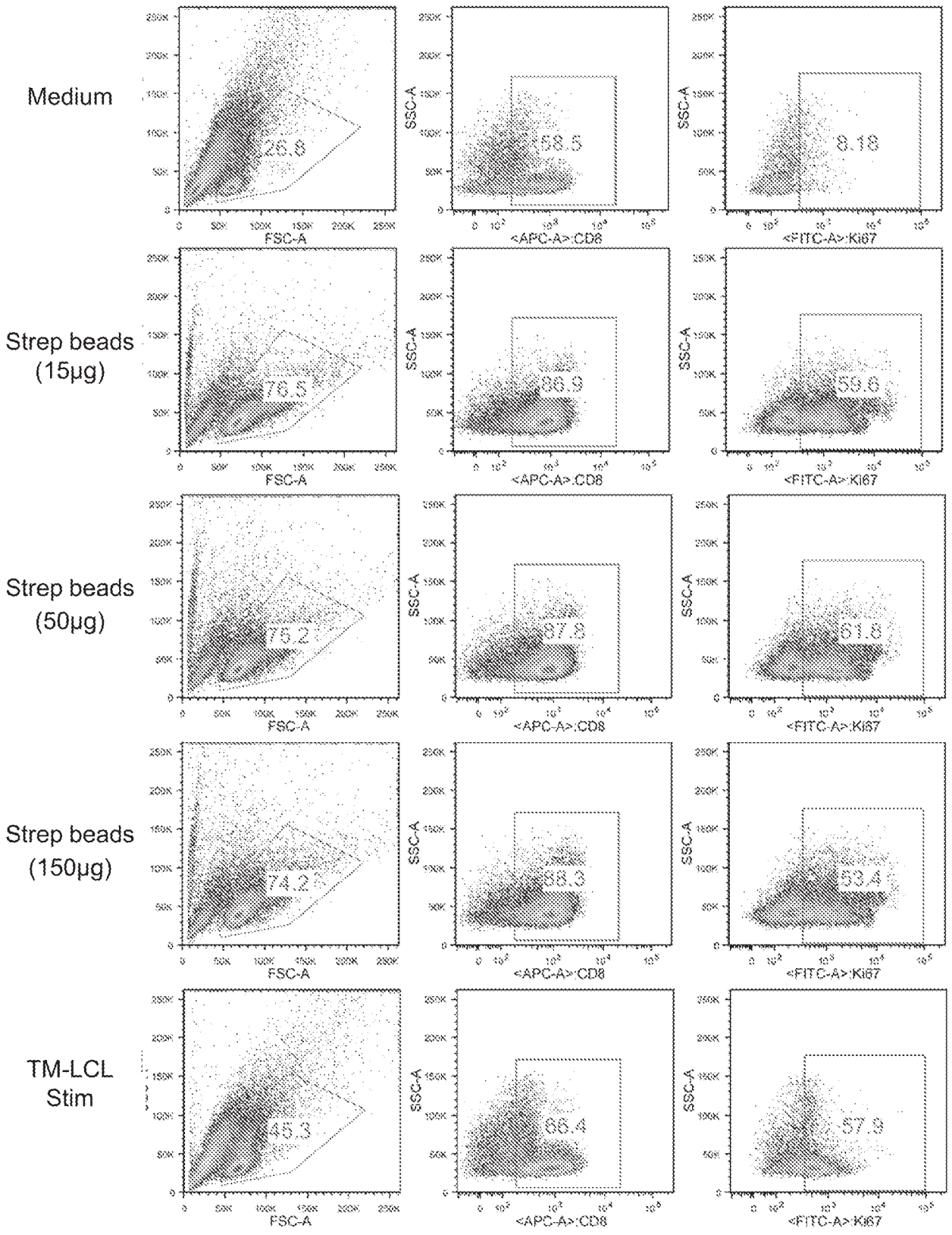


Fig. 15

CD19-ChARM²

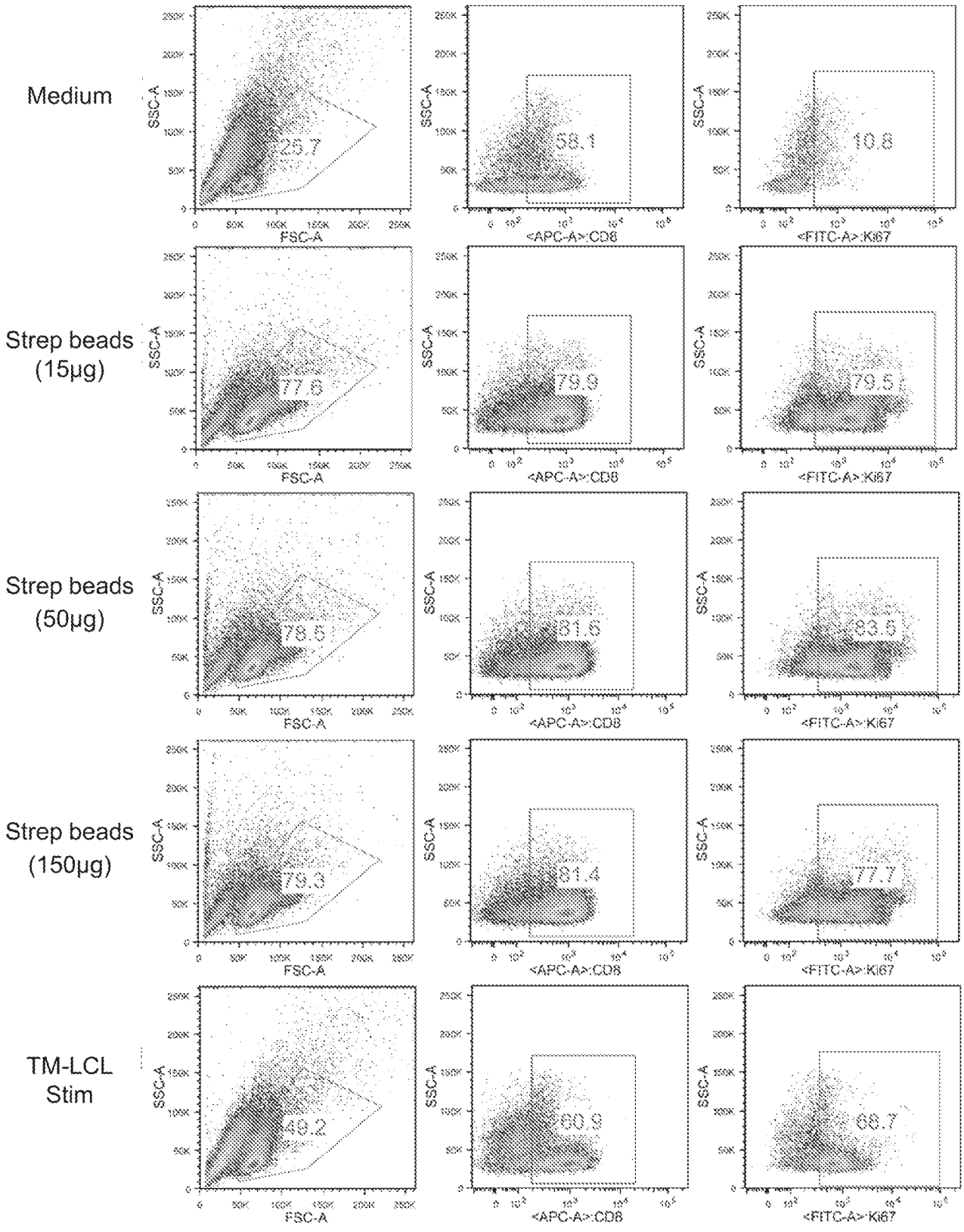


Fig. 15 *(Continued)*

CD19-ChARM^3

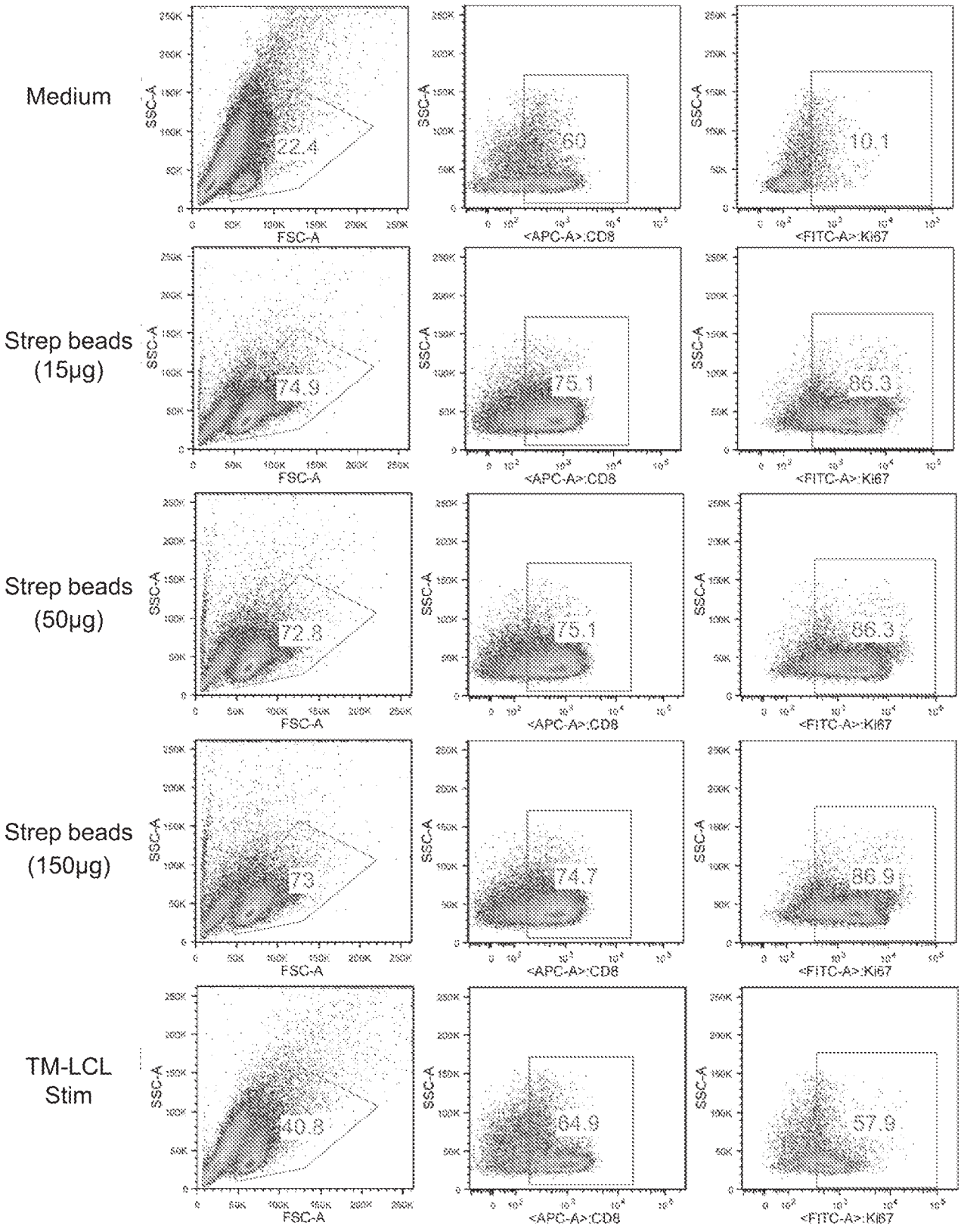


Fig. 15 *(Continued)*

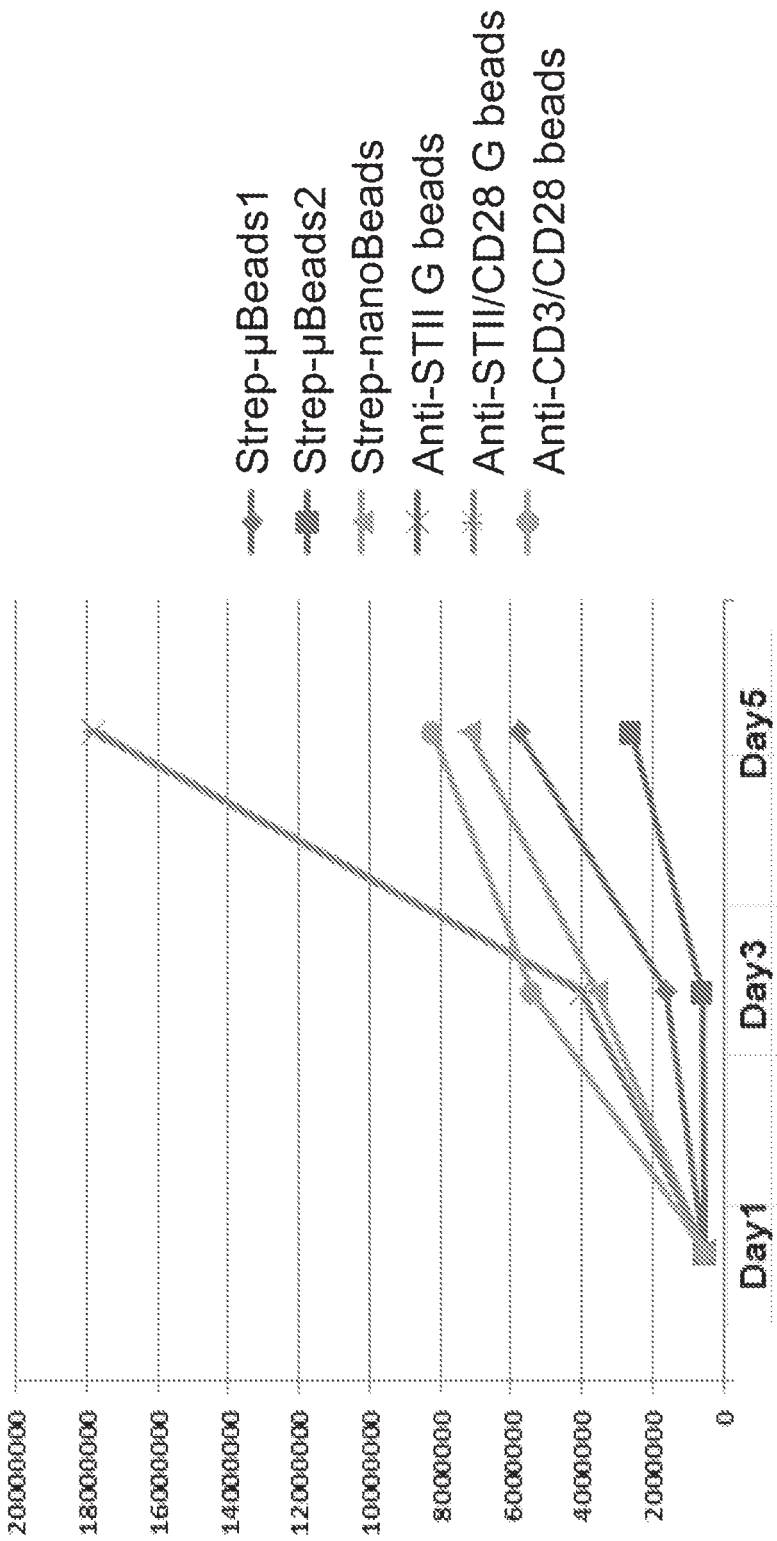

20000000
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Day1
Day3
Day5
Strep-µBeads1
Strep-µBeads2
Strep-nanoBeads
Anti-STII G beads
Anti-STII/CD28 G beads
Anti-CD3/CD28 beads

Fig. 16

A.

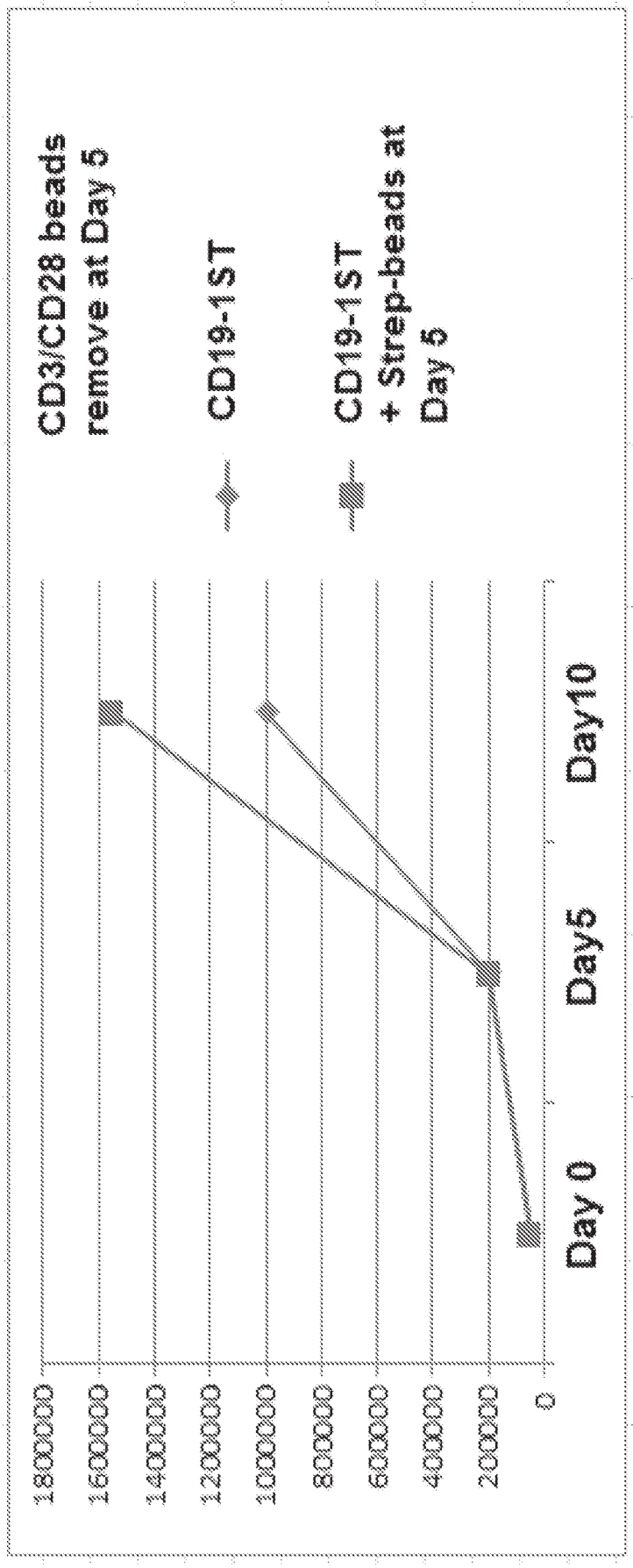

CD3/CD28 beads
remove at Day 5
CD19-1ST
CD19-1ST
+ Strep-beads at
Day 5
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Day 0
Day5
Day10

Fig. 17

B.

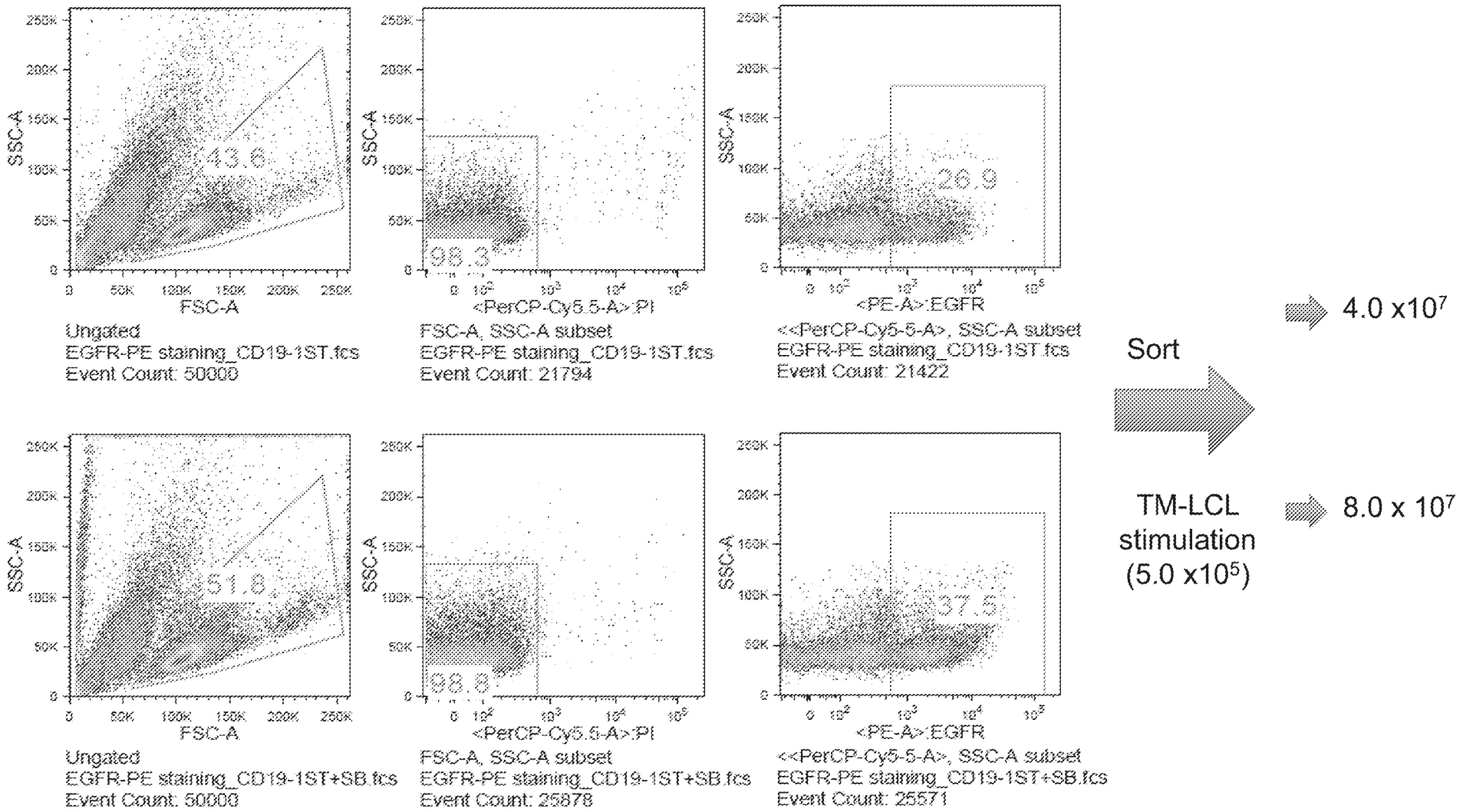

SSC-A
FSC-A
43.6
Ungated
EGFR-PE staining_CD19-1ST.fcs
Event Count: 50000
<PerCP-Cy5.5-A>:PI
98.3
FSC-A, SSC-A subset
EGFR-PE staining_CD19-1ST.fcs
Event Count: 21794
<PE-A>:EGFR
26.9
<<PerCP-Cy5-5-A>, SSC-A subset
EGFR-PE staining_CD19-1ST.fcs
Event Count: 21422
Sort
4.0 x10^7
TM-LCL
stimulation
(5.0 x10^5)
8.0 x 10^7
51.8
Ungated
EGFR-PE staining_CD19-1ST+SB.fcs
Event Count: 50000
98.8
FSC-A, SSC-A subset
EGFR-PE staining_CD19-1ST+SB.fcs
Event Count: 25878
37.5
<<PerCP-Cy5-5-A>, SSC-A subset
EGFR-PE staining_CD19-1ST+SB.fcs
Event Count: 25571

Fig. 17

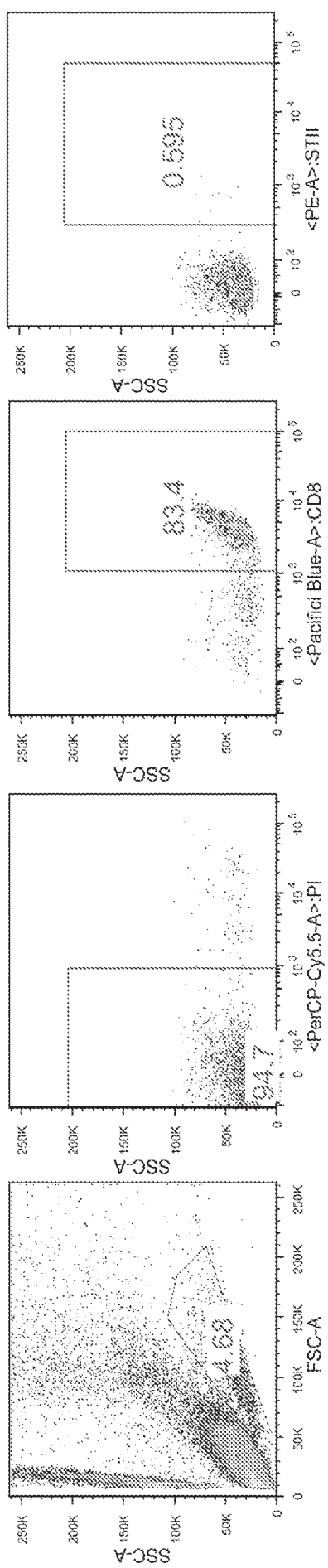

SSC-A
FSC-A
4.68
<PerCP-Cy5.5-A>:PI
94.7
<Pacifici Blue-A>:CD8
83.4
<PE-A>:STII
0.595

Fig. 18A

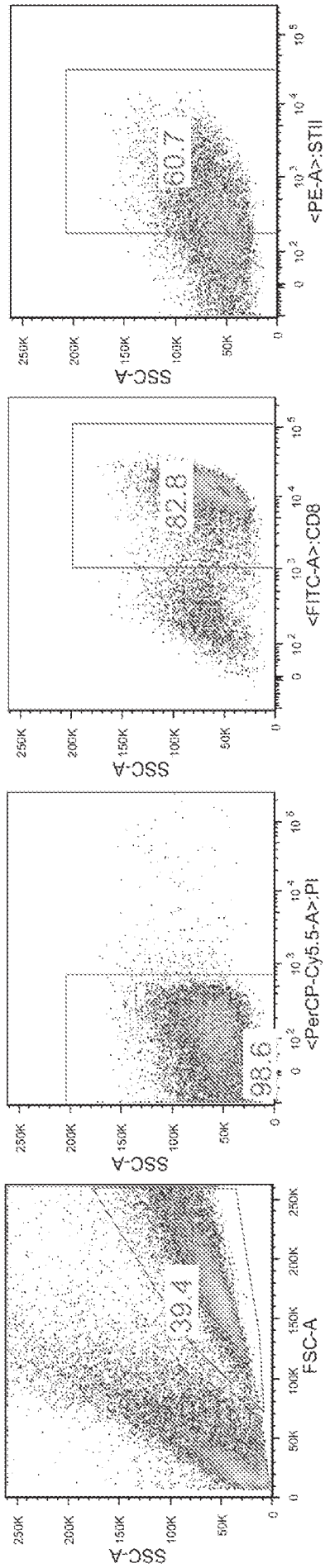


Fig. 18B

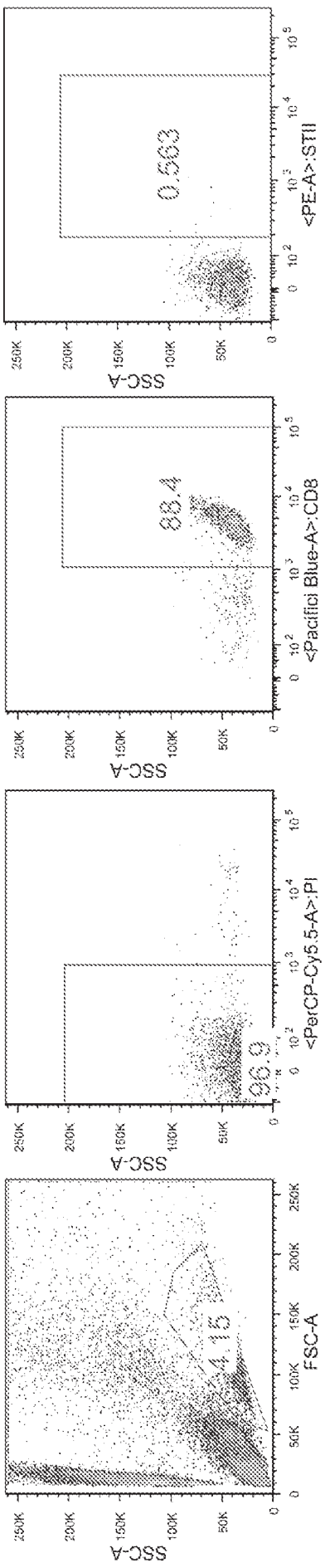

SSC-A
FSC-A
4.15
SSC-A
<PerCP-Cy5.5-A>:PI
96.9
SSC-A
<Pacific Blue-A>:CD8
88.4
SSC-A
<PE-A>:STII
0.563

Fig. 18C

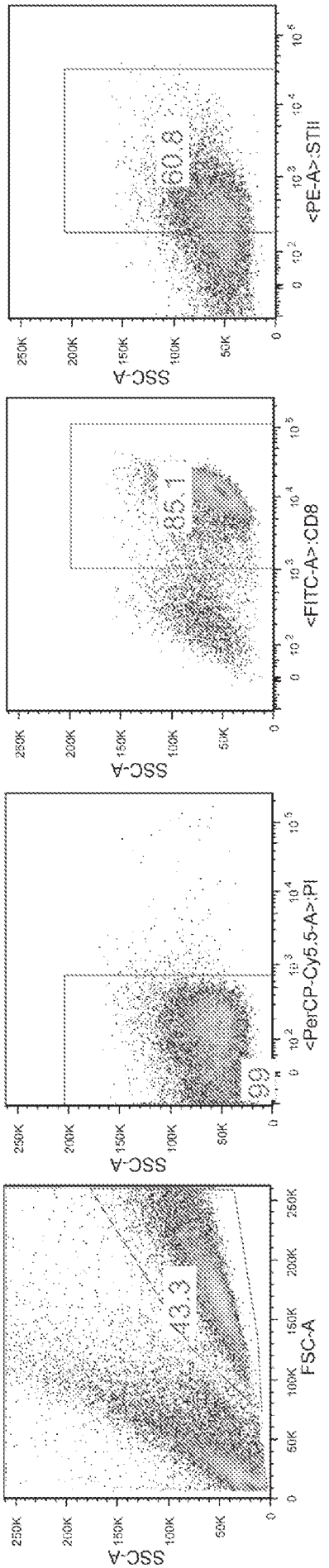

SSC-A
FSC-A
43.3
SSC-A
<PerCP-Cy5.5-A>:PI
99
SSC-A
<FITC-A>:CD8
85.1
SSC-A
<PE-A>:STII
60.8

Fig. 18D

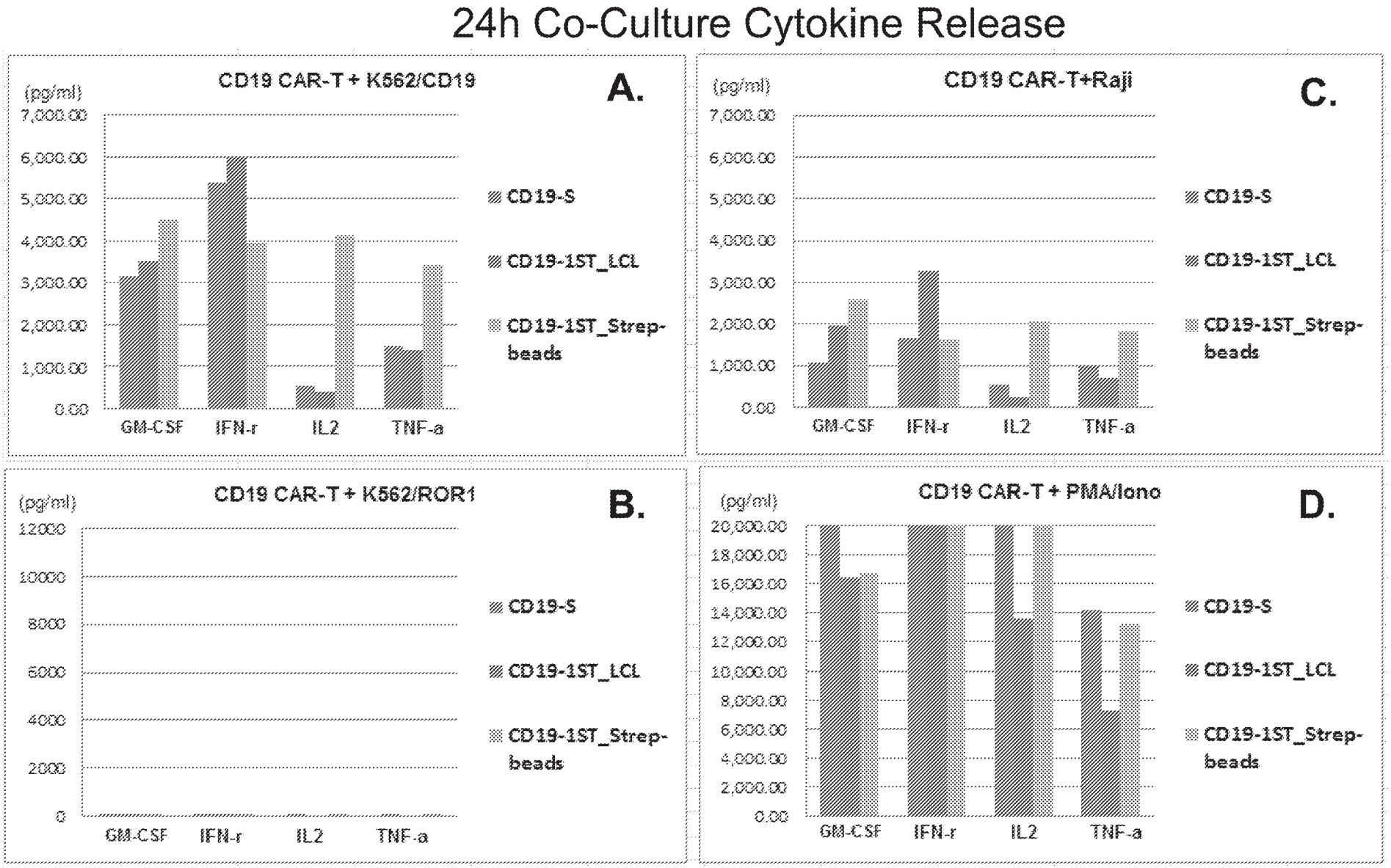
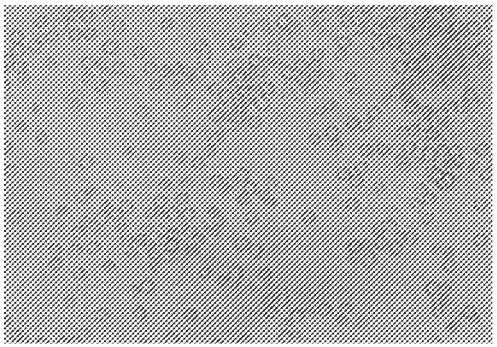

24h Co-Culture Cytokine Release
CD19 CAR-T + K562/CD19
A.
(pg/ml)
7,000.00
6,000.00
5,000.00
4,000.00
3,000.00
2,000.00
1,000.00
0.00
GM-CSF
IFN-r
IL2
TNF-a
CD19-S
CD19-1ST_LCL
CD19-1ST_Strep-beads
CD19 CAR-T+Raji
C.
(pg/ml)
7,000.00
6,000.00
5,000.00
4,000.00
3,000.00
2,000.00
1,000.00
0.00
GM-CSF
IFN-r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

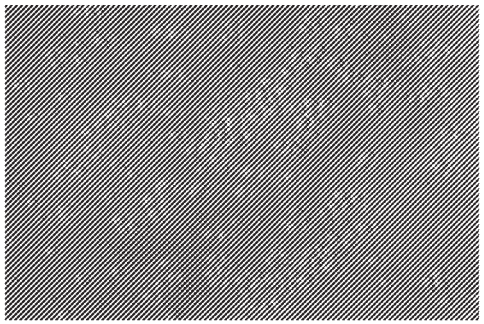
Fig. 19

CD19-ChARM^3 T Cells

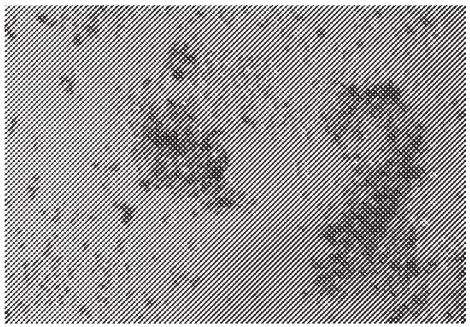
Day 0



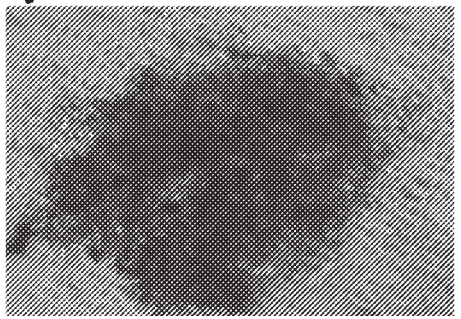
Day 5: Medium only



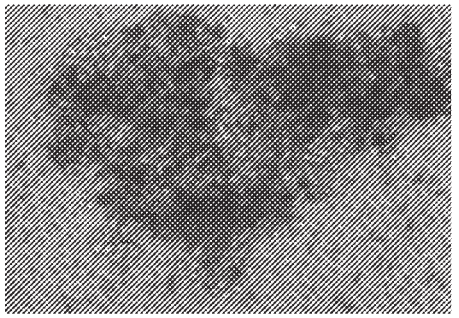
Day 5: Protein G beads only



Day 5: Anti-STII G beads



Day 5: Anti-STII/CD27 G beads



Day 5: Anti-STII/CD28 G beads

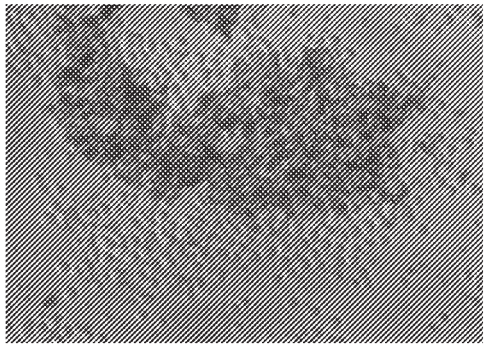


Fig. 20

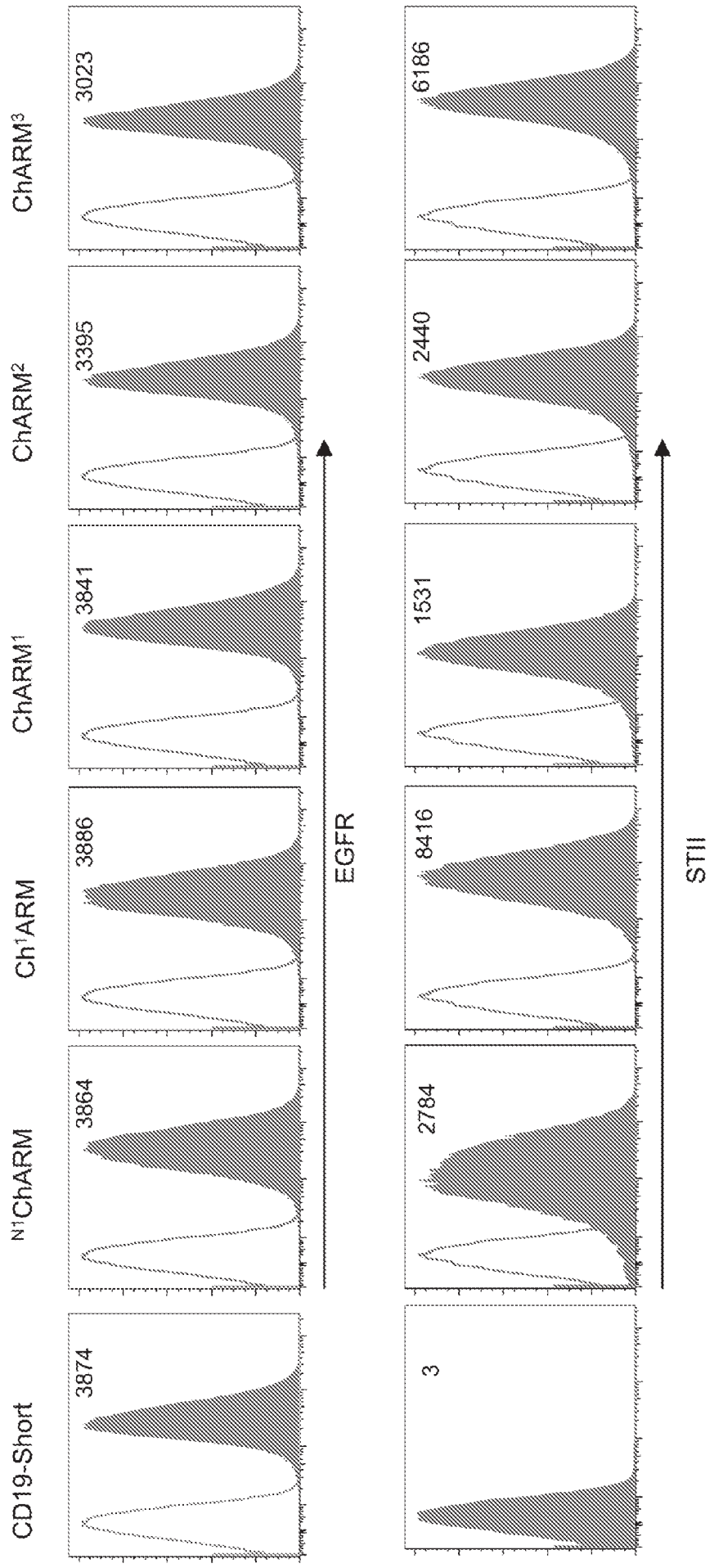

CD19-Short
N1ChARM
Ch1ARM
ChARM1
ChARM2
ChARM3
3874
3864
3886
3841
3395
3023
EGFR
3
2784
8416
1531
2440
6186
STII

Fig. 21

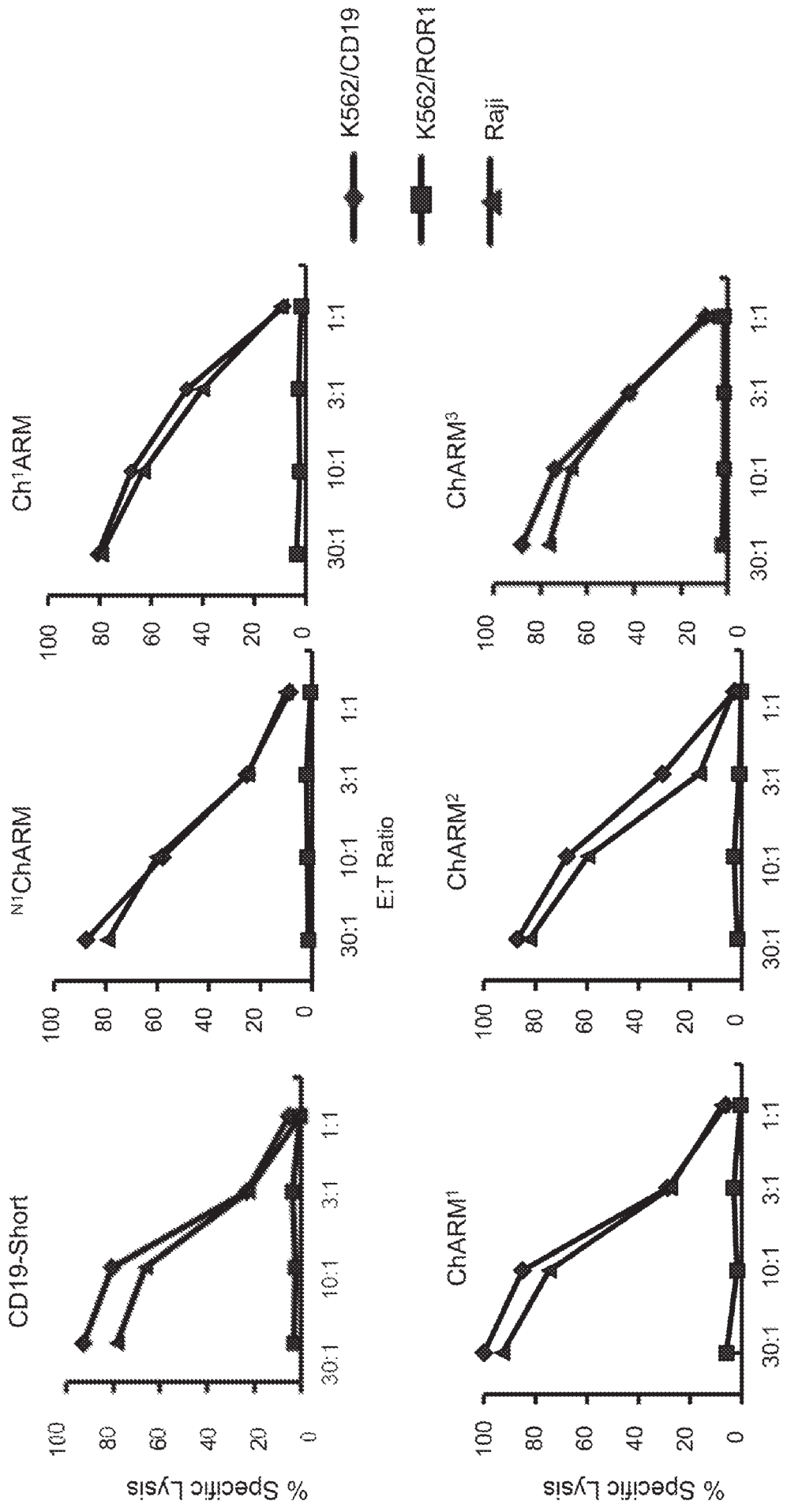

CD19-Short
N1ChARM
Ch1ARM
ChARM1
ChARM2
ChARM3
% Specific Lysis
E:T Ratio
30:1
10:1
3:1
1:1
0
20
40
60
80
100
K562/CD19
K562/ROR1
Raji

Fig. 22

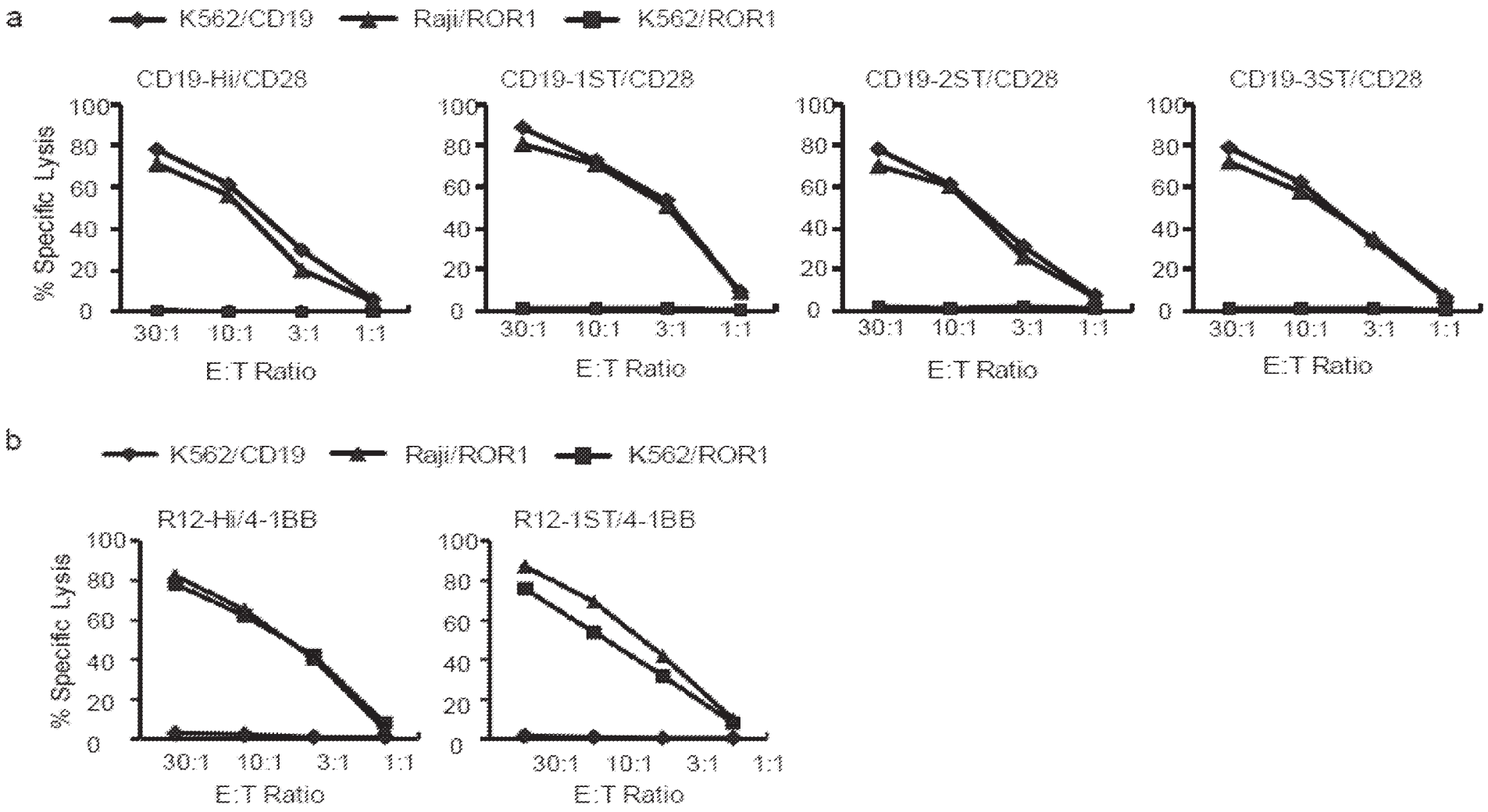

a
K562/CD19
Raji/ROR1
K562/ROR1
CD19-Hi/CD28
CD19-1ST/CD28
CD19-2ST/CD28
CD19-3ST/CD28
% Specific Lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
E:T Ratio
b
K562/CD19
Raji/ROR1
K562/ROR1
R12-Hi/4-1BB
R12-1ST/4-1BB
% Specific Lysis
E:T Ratio

Fig. 23

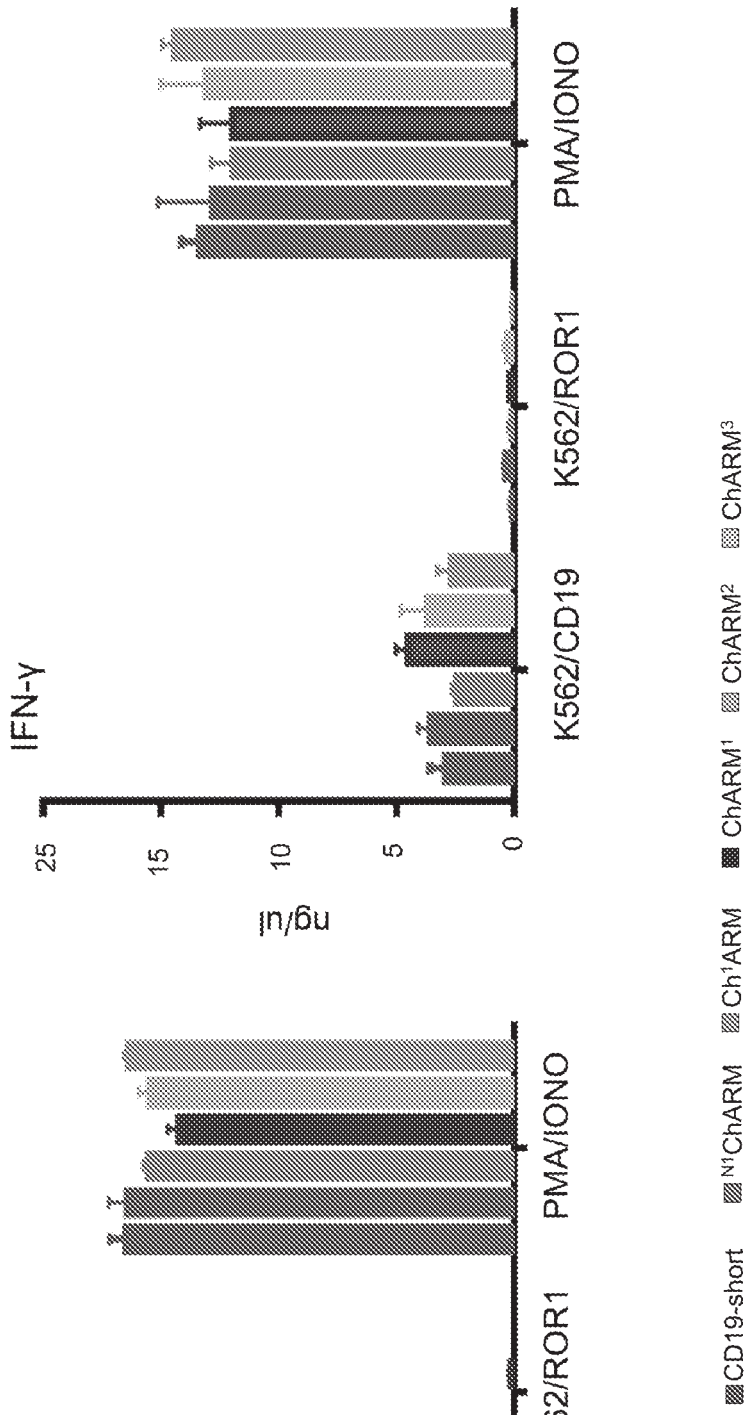

IL2
IFN-γ
pg/ul
0
5
10
15
25
K562/CD19
K562/ROR1
PMA/IONO
CD19-short
N1ChARM
Ch1ARM
ChARM1
ChARM2
ChARM3

Fig. 24

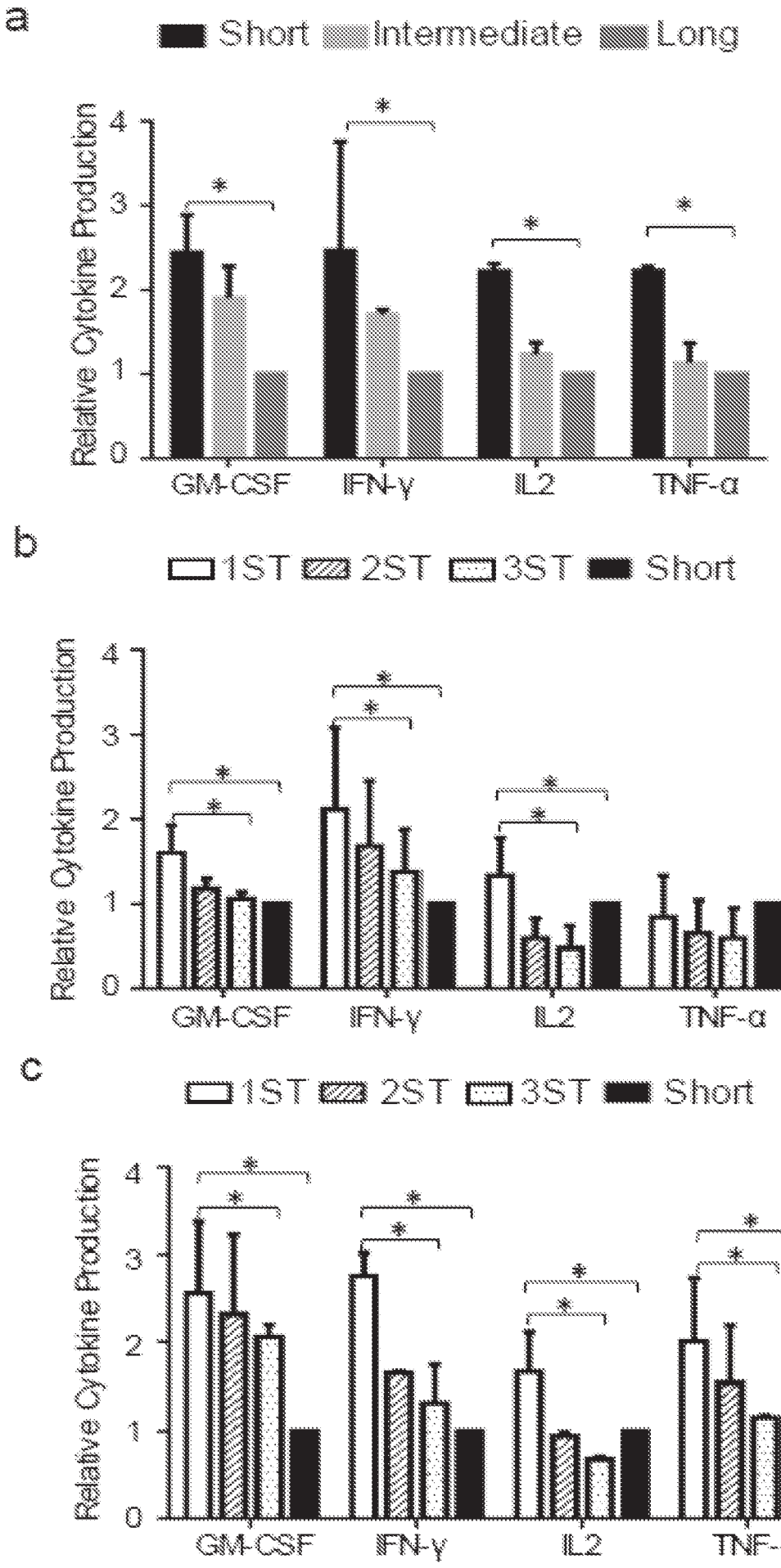

a
Short
Intermediate
Long
Relative Cytokine Production
0
1
2
3
4
GM-CSF
IFN-γ
IL2
TNF-α
b
1ST
2ST
3ST
Short
Relative Cytokine Production
GM-CSF
IFN-γ
IL2
TNF-α
c
1ST
2ST
3ST
Short
Relative Cytokine Production
GM-CSF
IFN-γ
IL2
TNF-α

Fig. 25

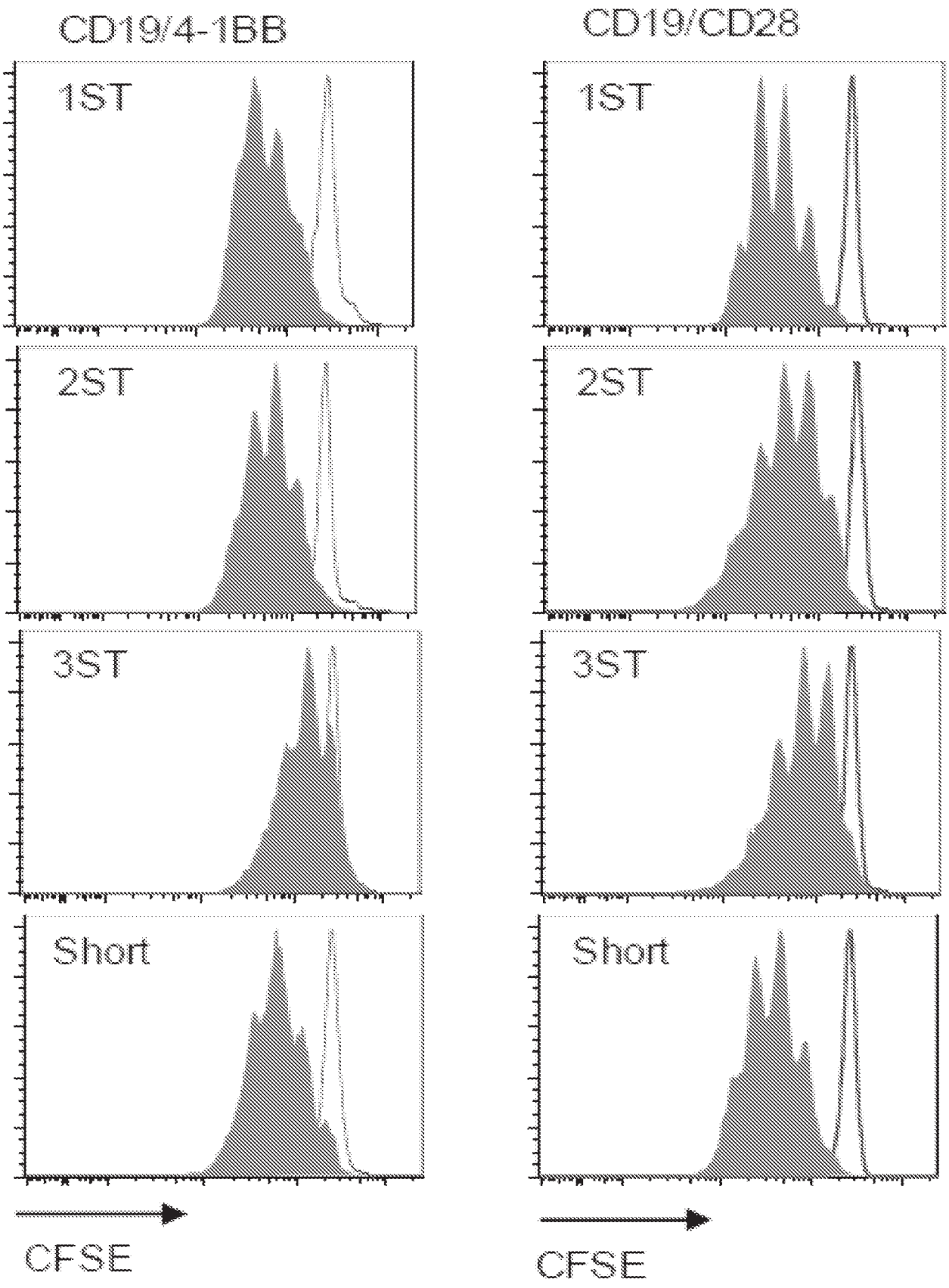

CD19/4-1BB
CD19/CD28
1ST
2ST
3ST
Short
1ST
2ST
3ST
Short
CFSE
CFSE

Fig. 26

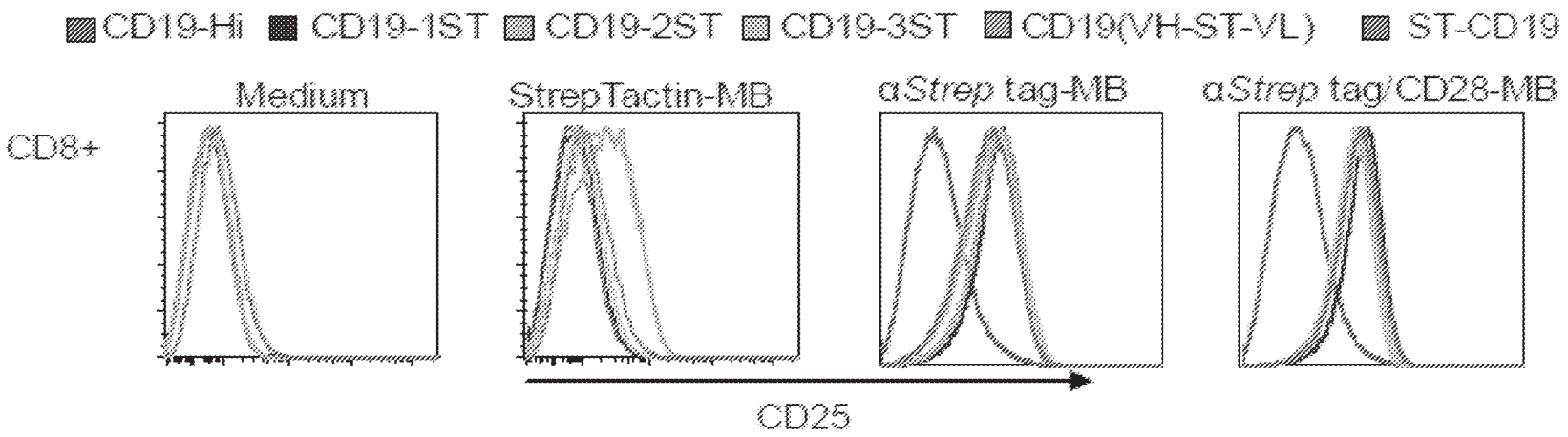


Fig. 27A

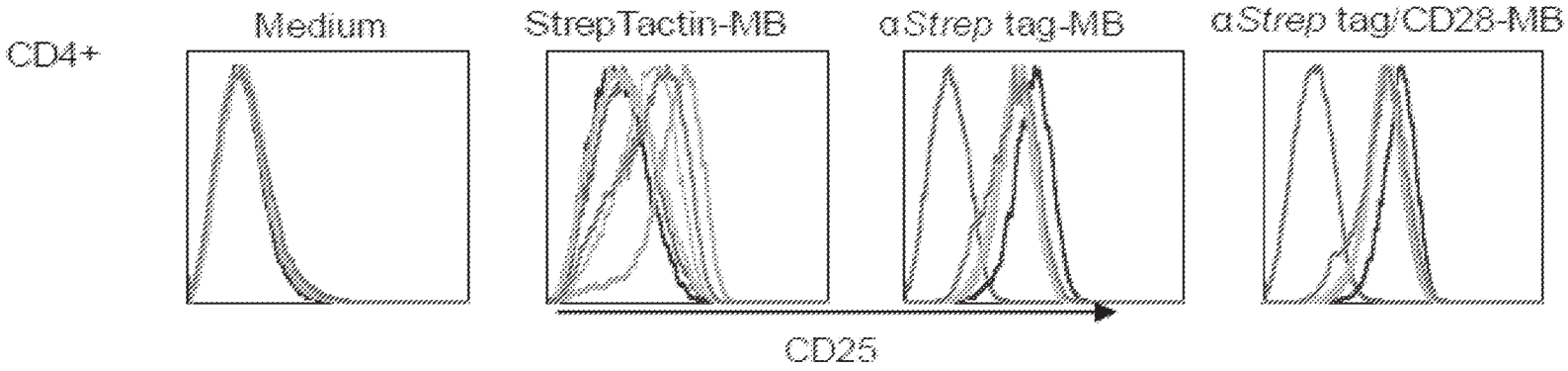


Fig. 27B

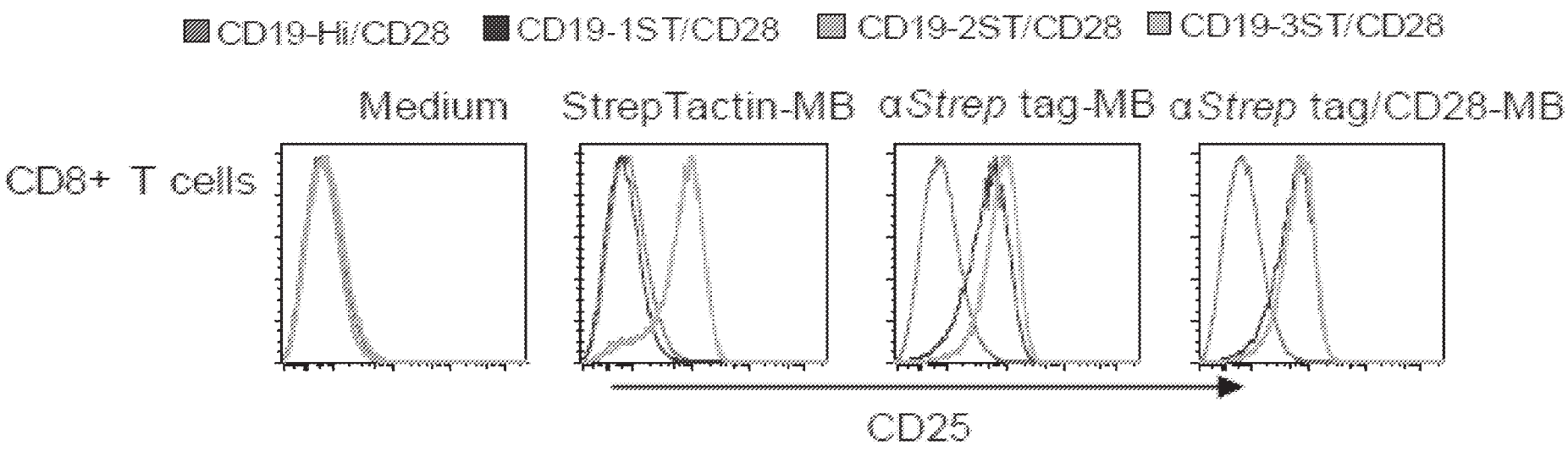


Fig. 27C

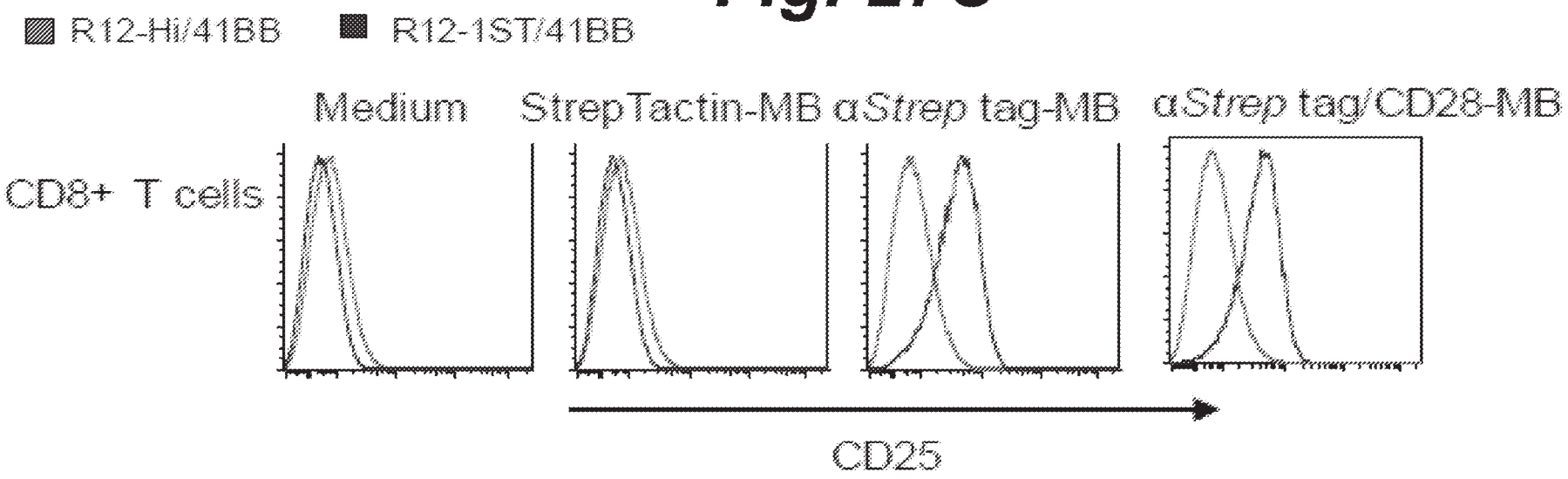


Fig. 27D

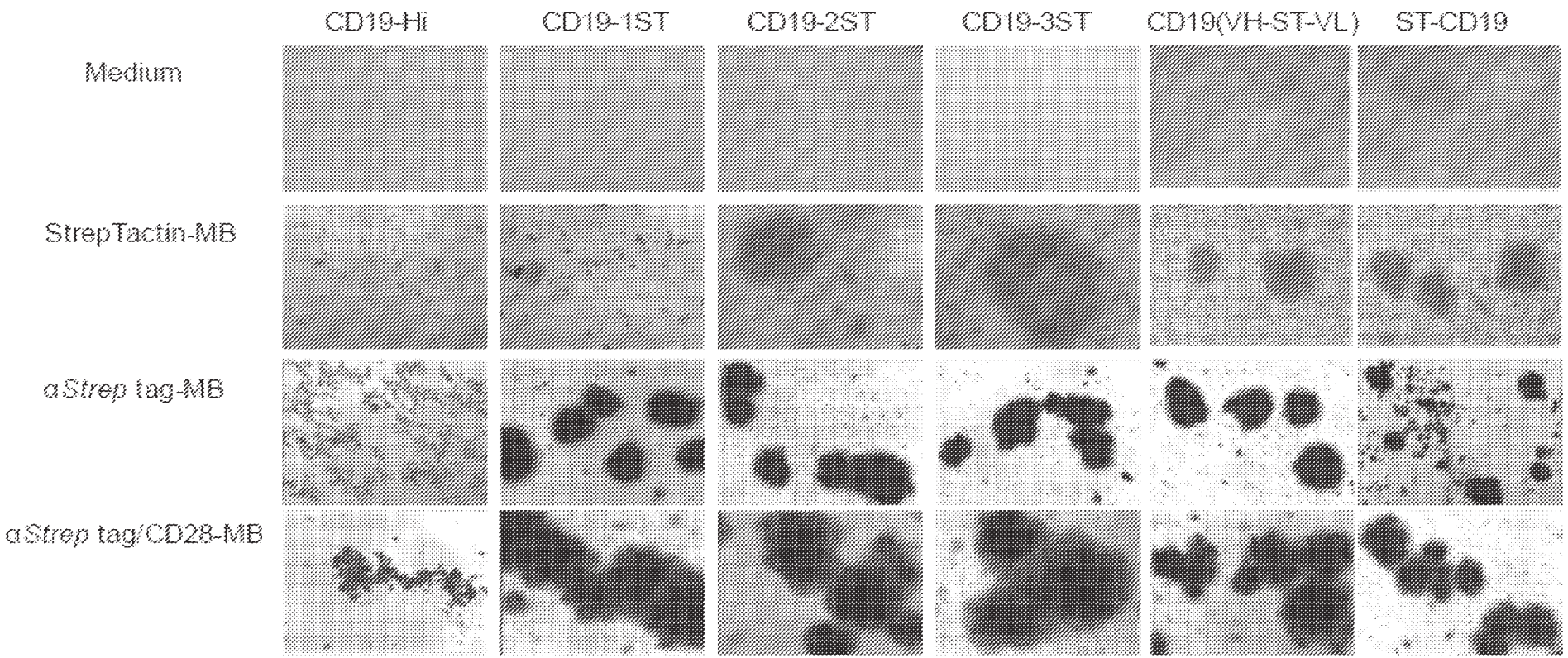

CD19-Hi
CD19-1ST
CD19-2ST
CD19-3ST
CD19(VH-ST-VL)
ST-CD19
Medium
StrepTactin-MB
αStrep tag-MB
αStrep tag/CD28-MB

Fig. 28

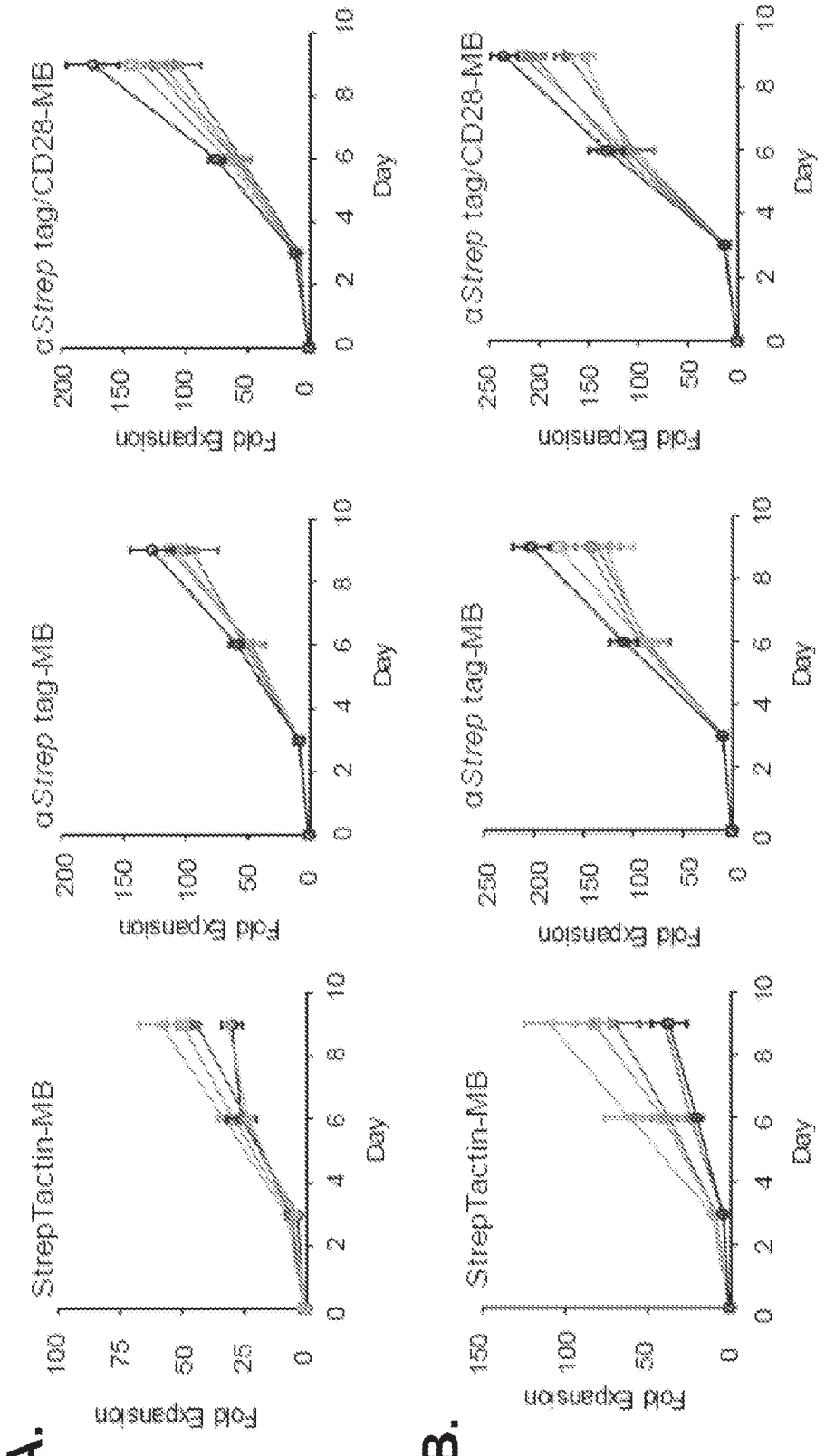

A.
B.
StrepTactin-MB
αStrep tag-MB
αStrep tag/CD28-MB
Fold Expansion
Day
CD19-1ST
CD19-2ST
CD19-3ST
CD19(VH-ST-VL)
ST-CD19

Fig. 29

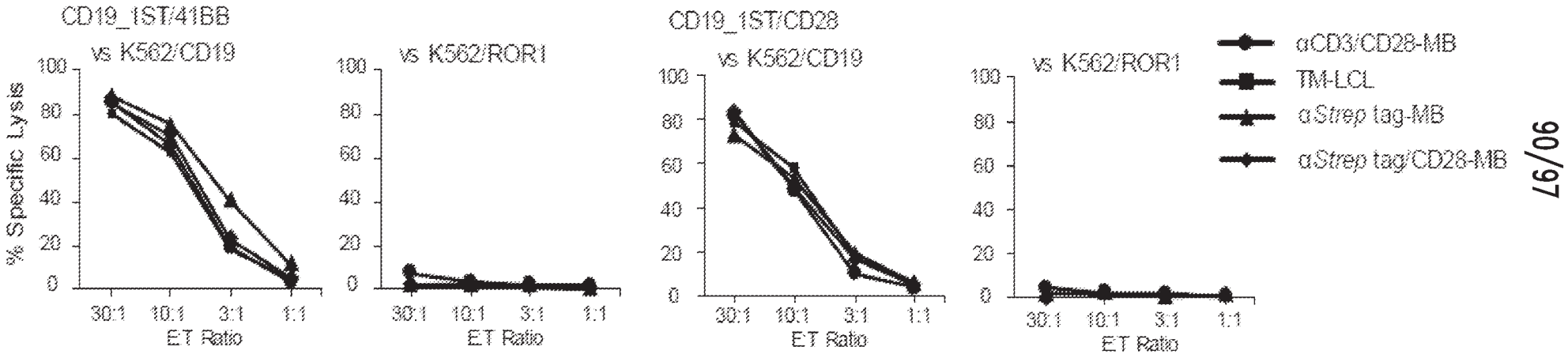

CD19_1ST/41BB
vs K562/CD19
vs K562/ROR1
CD19_1ST/CD28
vs K562/CD19
vs K562/ROR1
% Specific Lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
ET Ratio
αCD3/CD28-MB
TM-LCL
αStrep tag-MB
αStrep tag/CD28-MB

Fig. 30A

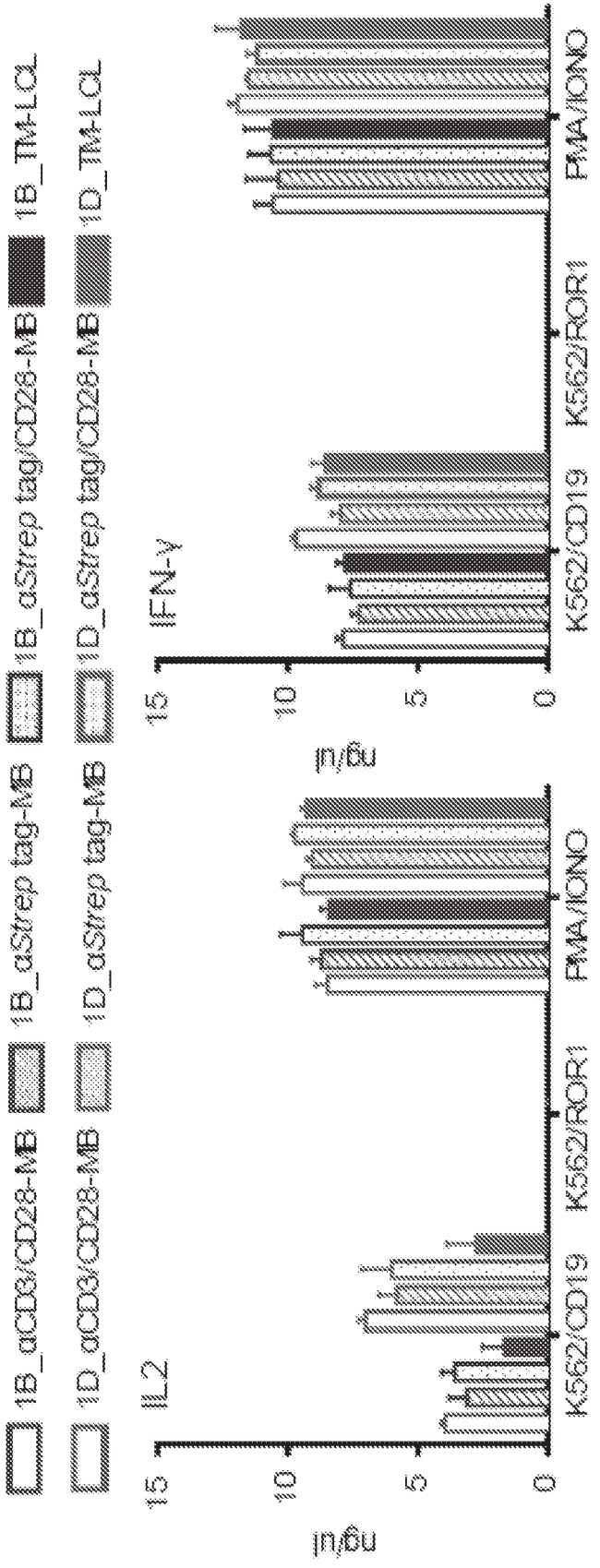

1B_αCD3/CD28-MB
1B_αStrep tag-MB
1B_αStrep tag/CD28-MB
1B_TM-LCL
1D_αCD3/CD28-MB
1D_αStrep tag-MB
1D_αStrep tag/CD28-MB
1D_TM-LCL
IL2
IFN-γ
ng/ul
15
10
5
0
K562/CD19
K562/ROR1
PMA/IONO

Fig. 30B

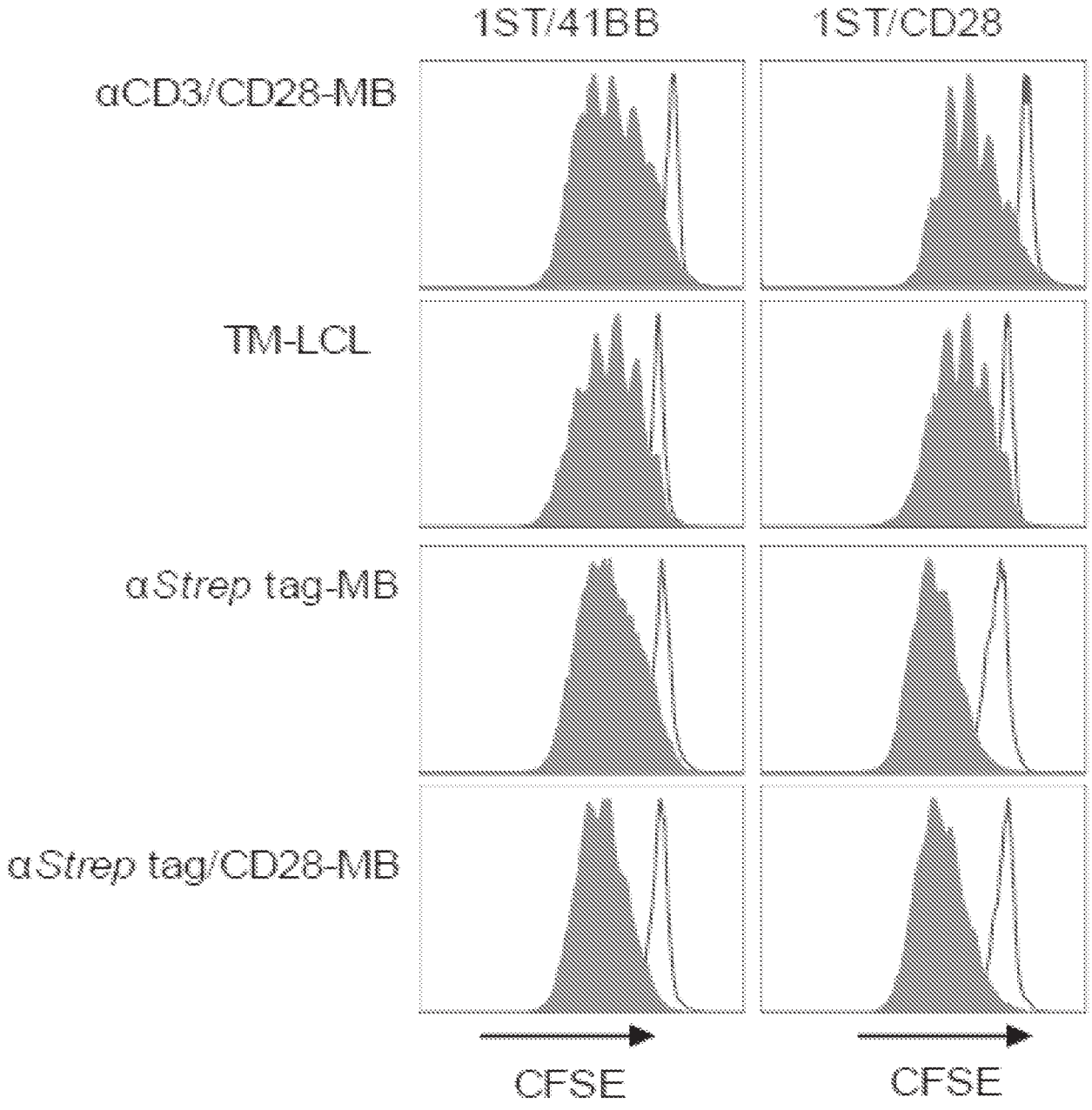

1ST/41BB
1ST/CD28
αCD3/CD28-MB
TM-LCL
αStrep tag-MB
αStrep tag/CD28-MB
CFSE
CFSE

Fig. 30C

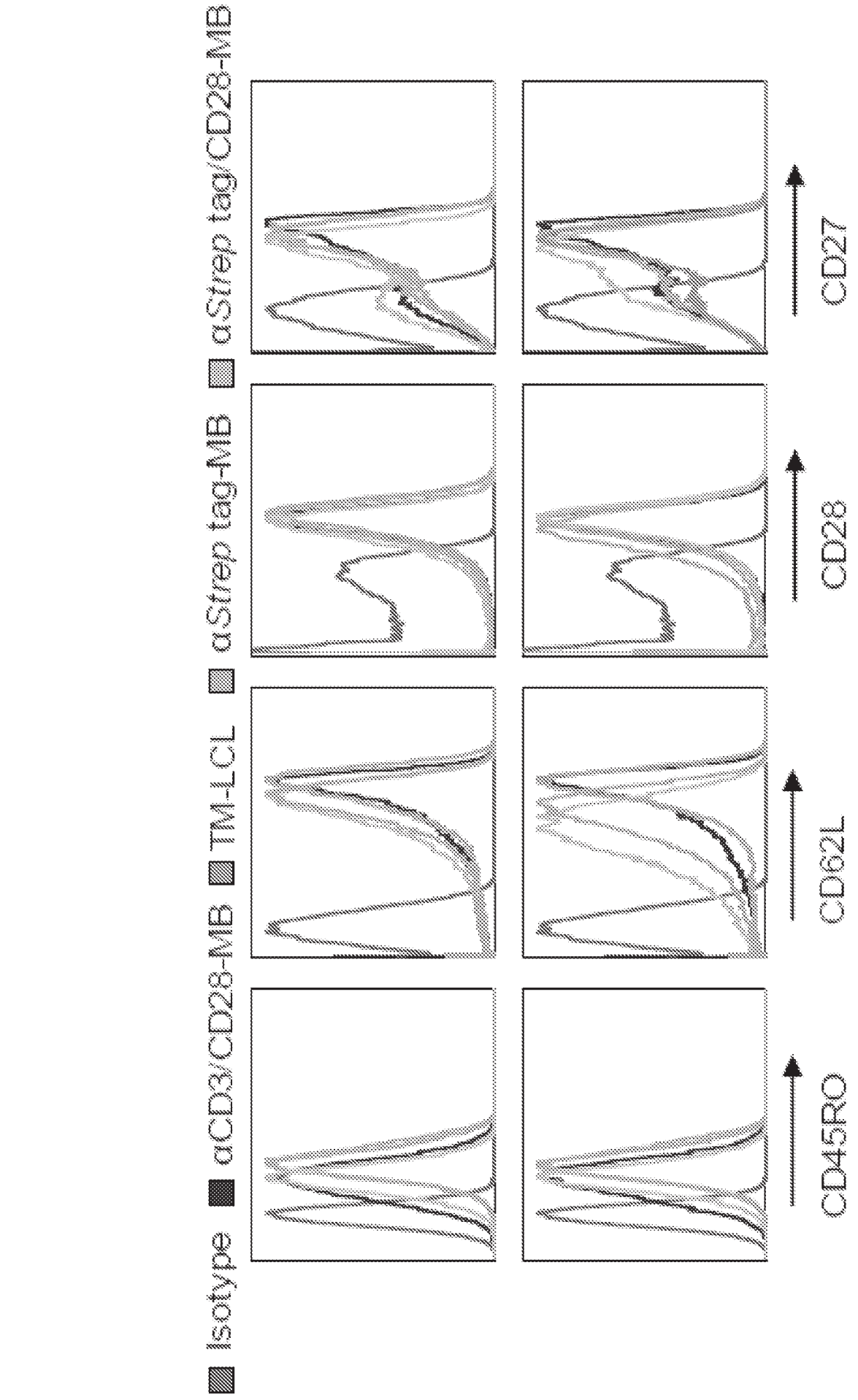

Isotype
αCD3/CD28-MB
TM-LCL
αStrep tag-MB
αStrep tag/CD28-MB
CD45RO
CD62L
CD28
CD27

Fig. 30D

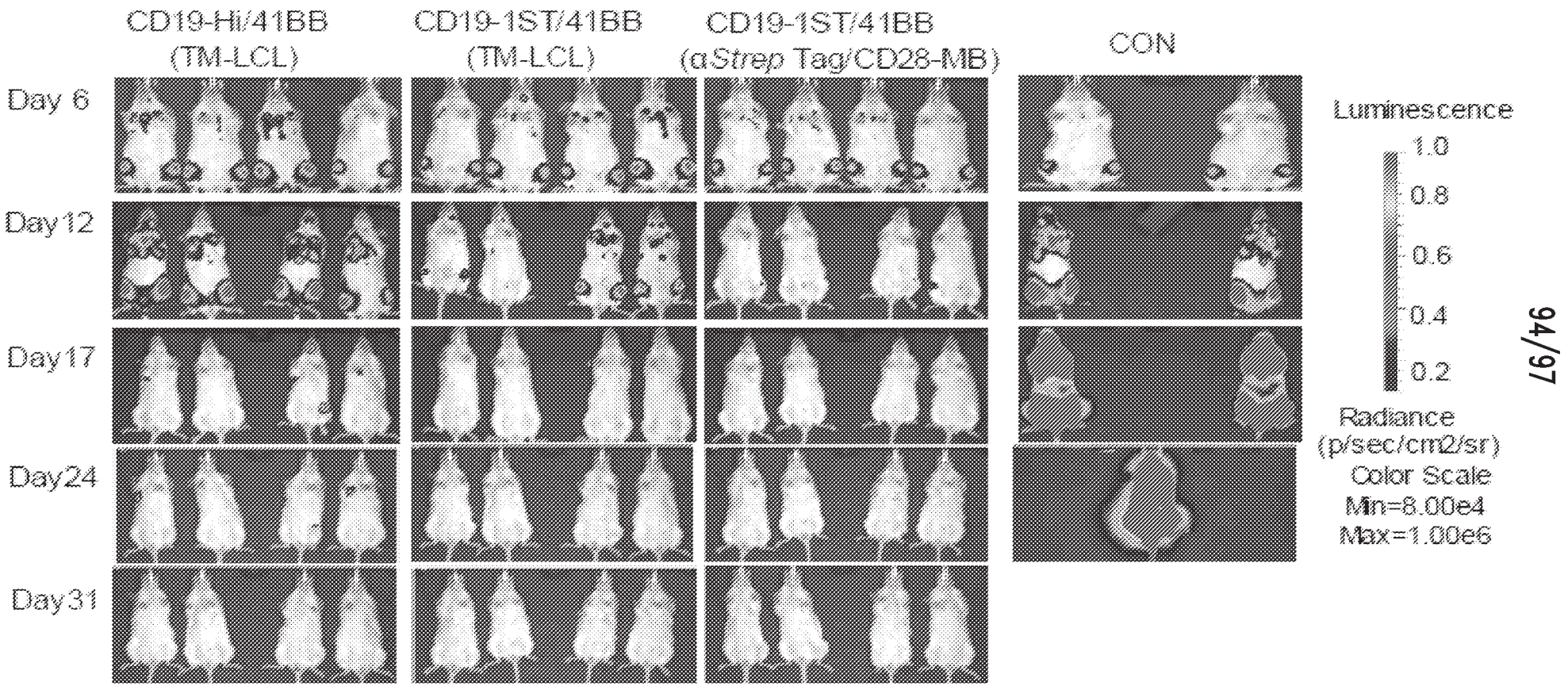

CD19-Hi/41BB
(TM-LCL)
CD19-1ST/41BB
(TM-LCL)
CD19-1ST/41BB
(αStrep Tag/CD28-MB)
CON
Day 6
Day 12
Day 17
Day 24
Day 31
Luminescence
1.0
0.8
0.6
0.4
0.2
Radiance
(p/sec/cm2/sr)
Color Scale
Min=8.00e4
Max=1.00e6

Fig. 30E

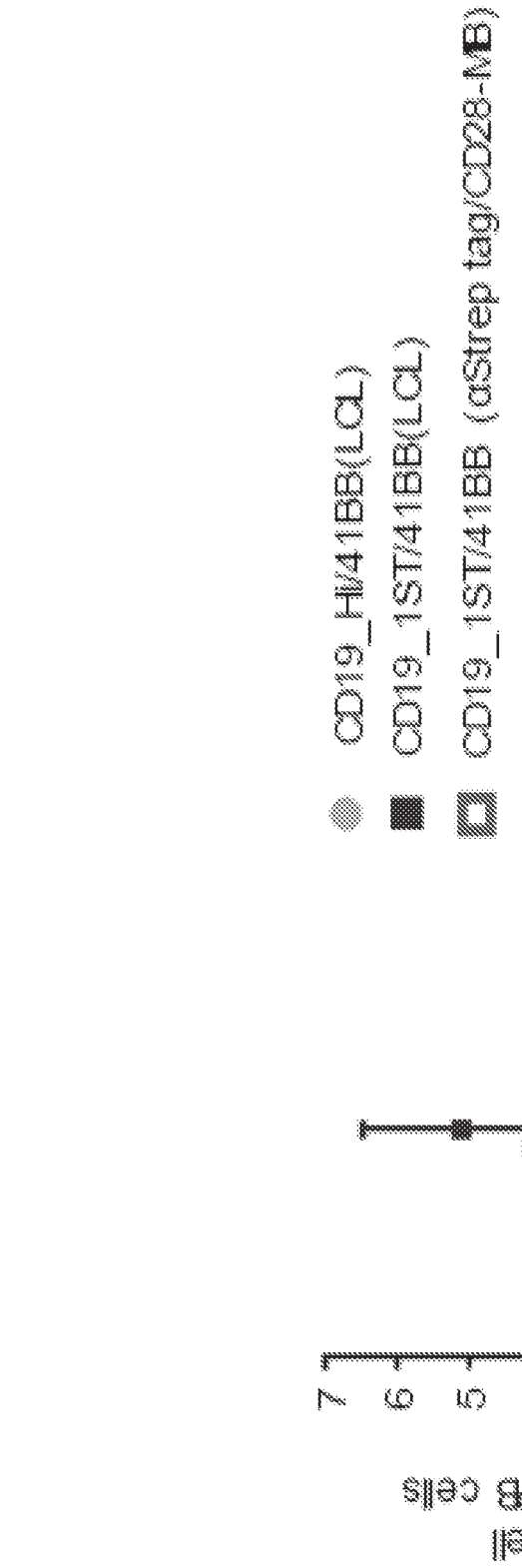

CD19_H/41BB(LCL)
CD19_1ST/41BB(LCL)
CD19_1ST/41BB (αStrep tag/CD28-MB)
CAR+ T cell
% of live FB cells
0
1
2
3
4
5
6
7
Day11
Day18
Day25
Day32

Fig. 30F

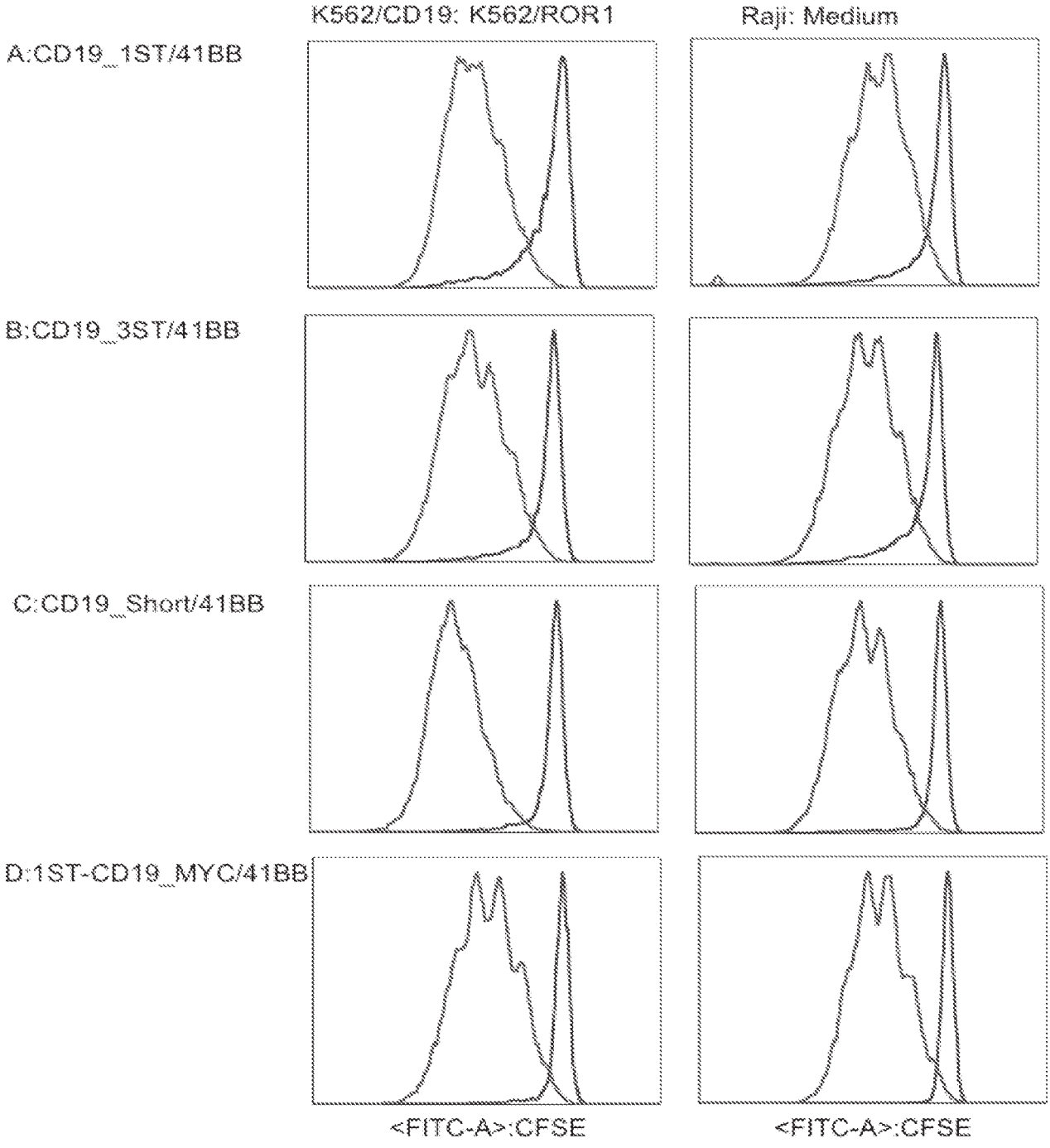

K562/CD19: K562/ROR1
Raji: Medium
A:CD19_1ST/41BB
B:CD19_3ST/41BB
C:CD19_Short/41BB
D:1ST-CD19_MYC/41BB
<FITC-A>:CFSE
<FITC-A>:CFSE

Fig. 31

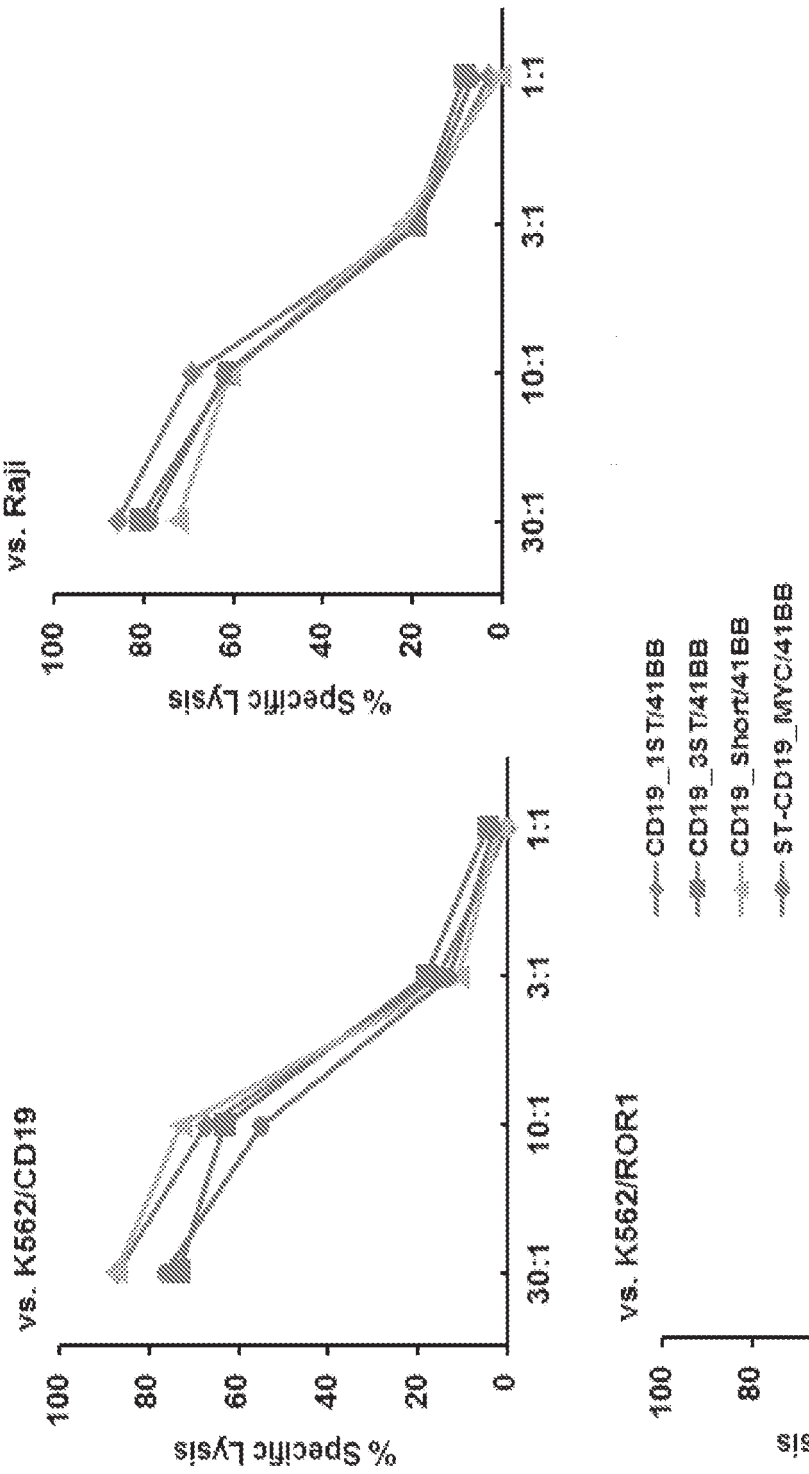

vs. K562/CD19
% Specific Lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
vs. Raji
% Specific Lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
vs. K562/ROR1
% Specific Lysis
100
80
60
40
20
0
30:1
10:1
3:1
1:1
CD19_1ST/41BB
CD19_3ST/41BB
CD19_Short/41BB
ST-CD19_MYC/41BB

Fig. 32