

FIG. 1A

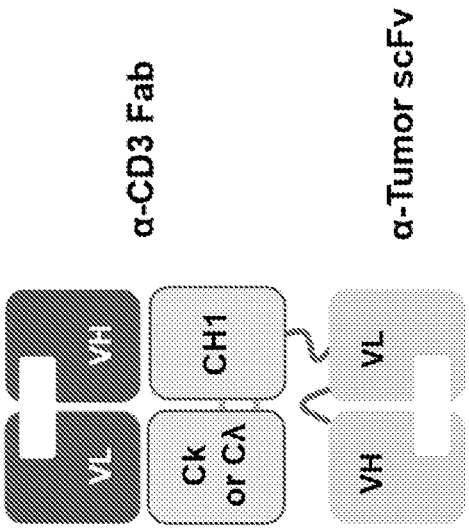


FIG. 1B

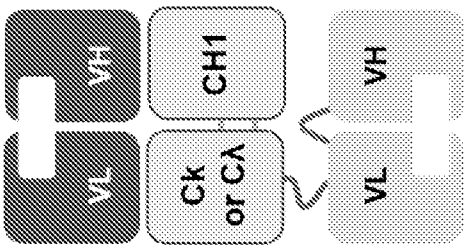


FIG. 1C

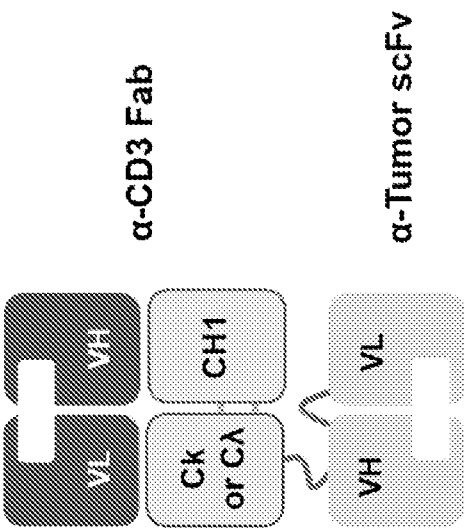


FIG. 1D

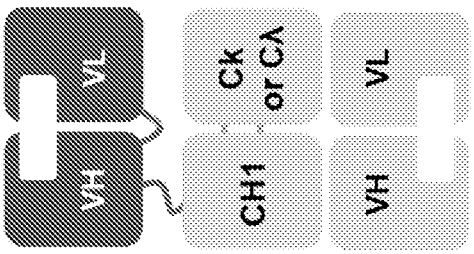


FIG. 1E

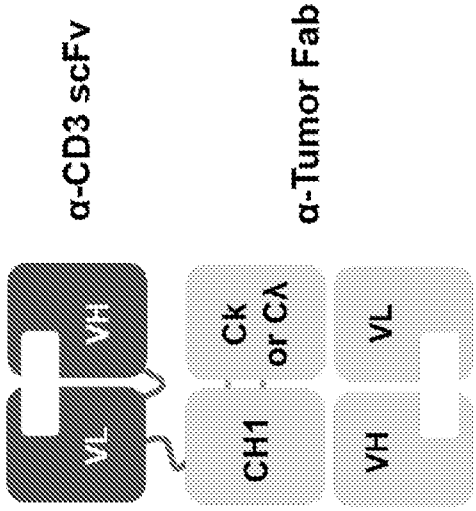


FIG. 1F

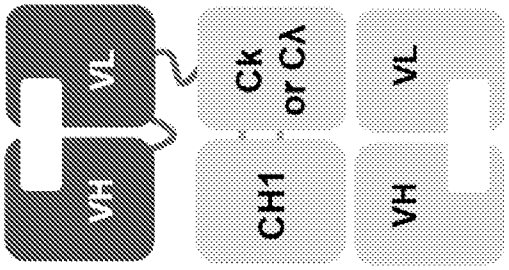


FIG. 1G

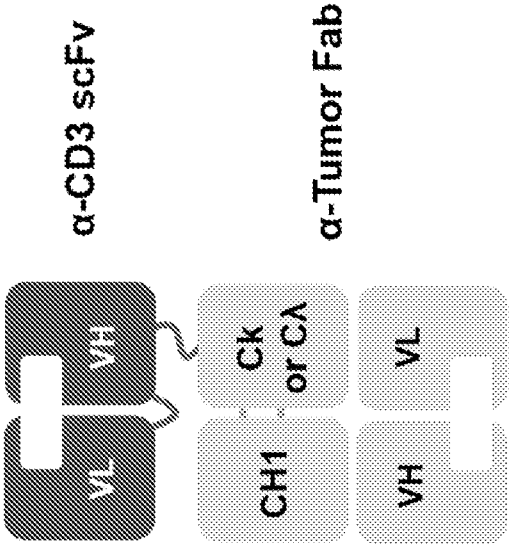


FIG. 1H

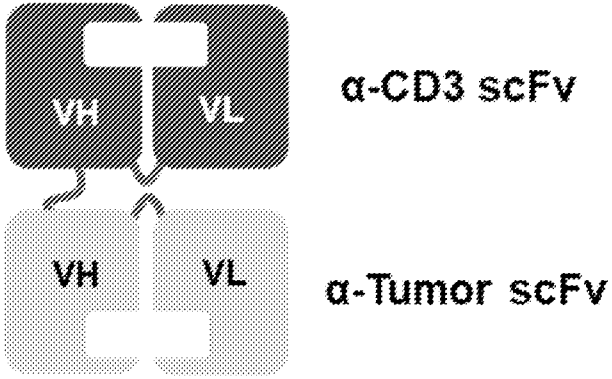


FIG. 1I

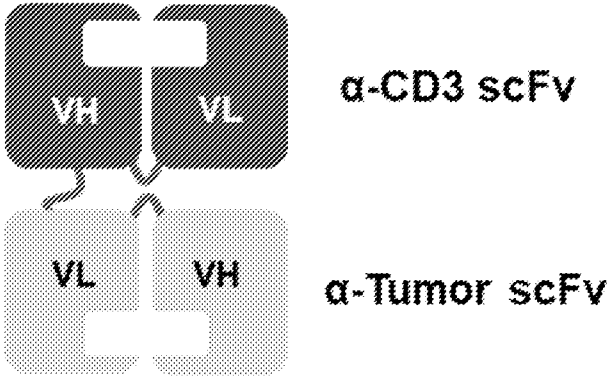


FIG. 1J

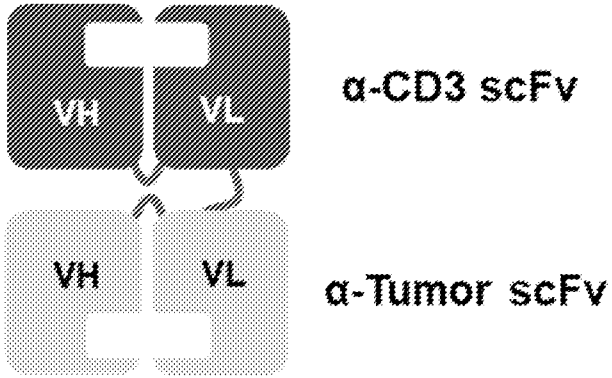


FIG. 1K

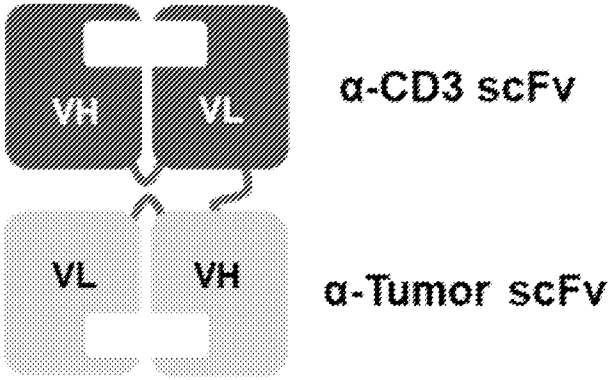


FIG. 1L

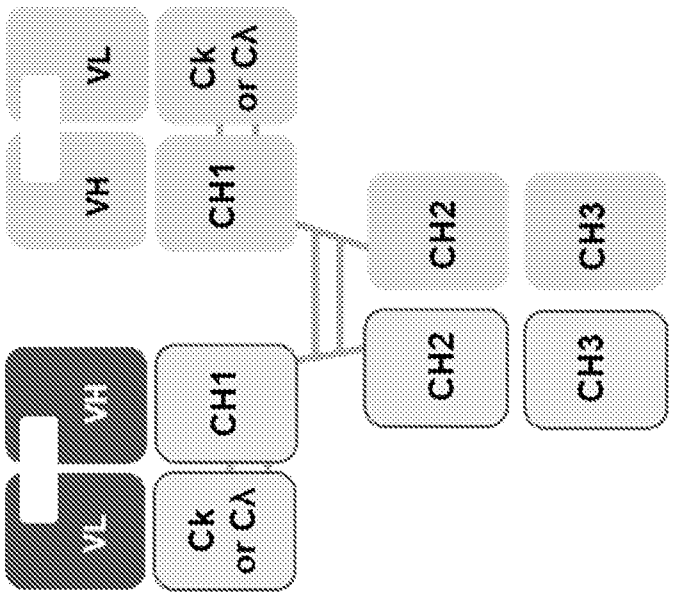

VL
VH
Ck
or Cλ
CH1
CH2
CH2
CH3
CH3

FIG. 1M

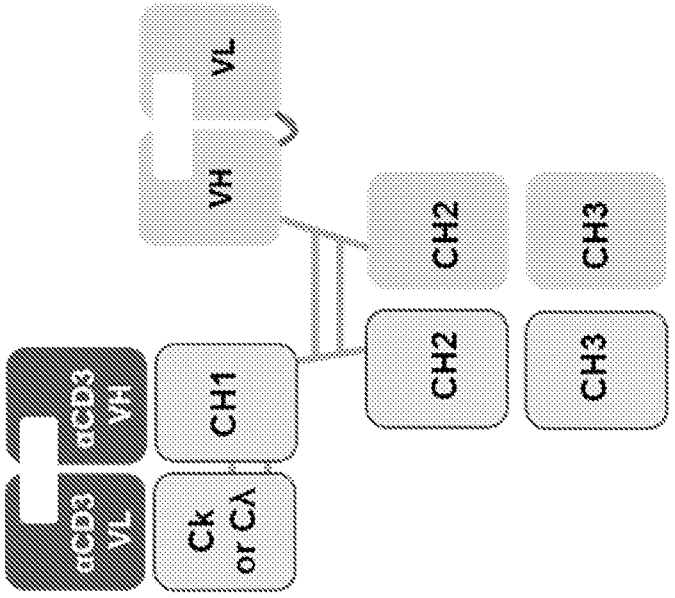

αCD3
VL
αCD3
VH
Ck
or Cλ
CH1
VH
VL
CH2
CH2
CH3
CH3

FIG. 1N

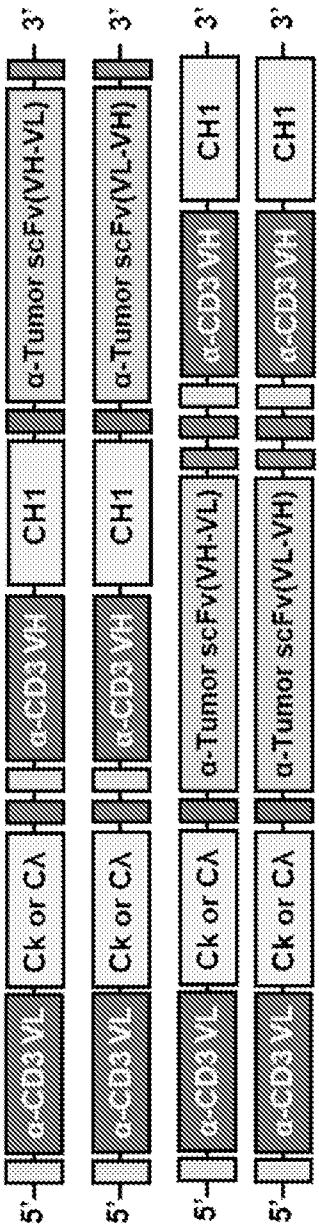


FIG. 2A

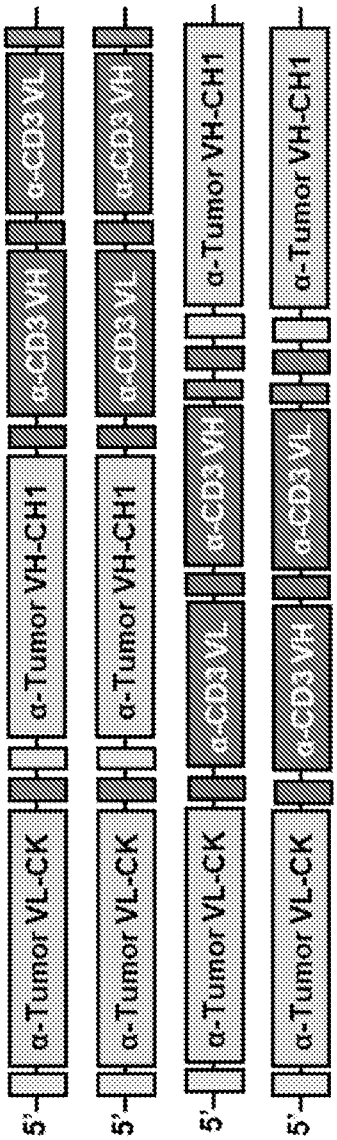


FIG. 2B

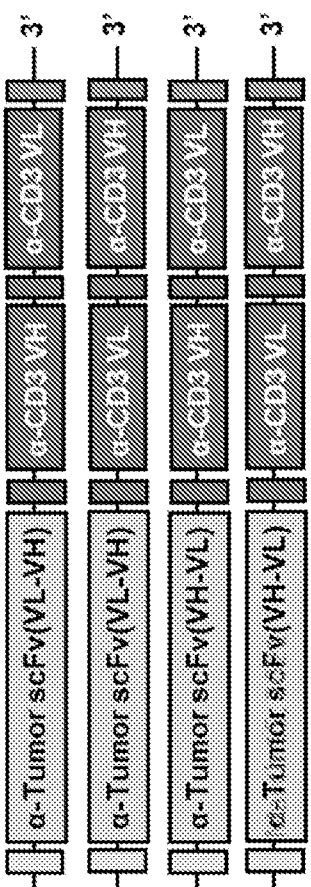


FIG. 2C

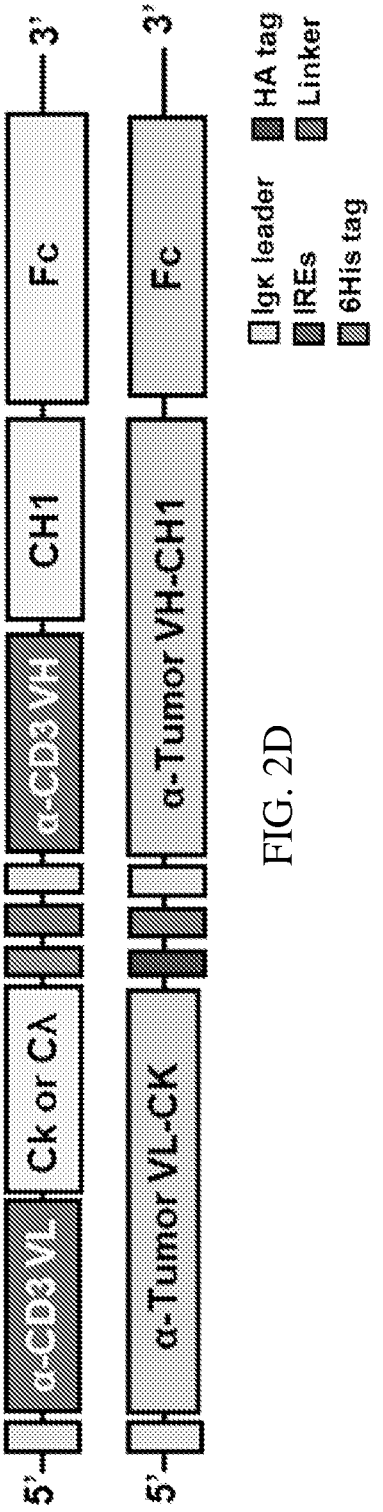

5'
α-CD3 VL
Ck or Cλ
α-CD3 VH
CH1
Fc
3'
5'
α-Tumor VL-CK
α-Tumor VH-CH1
Fc
3'
Igκ leader
IREs
6His tag
HA tag
Linker

FIG. 2D

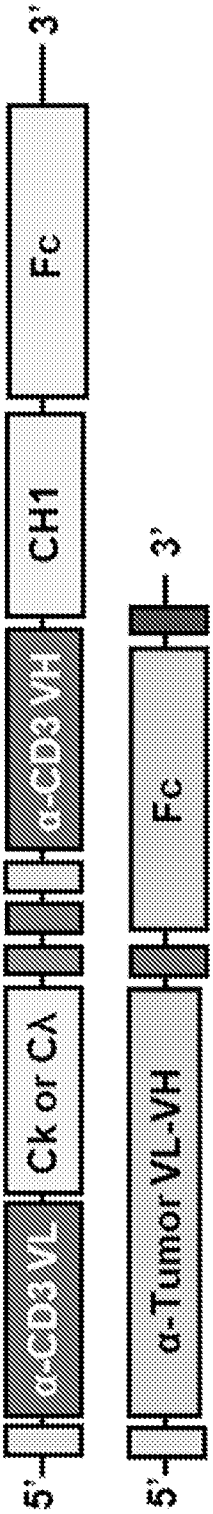

5'
α-CD3 VL
Ck or Cλ
α-CD3 VH
CH1
Fc
3'
5'
α-Tumor VL-VH
Fc
3'

FIG. 2E

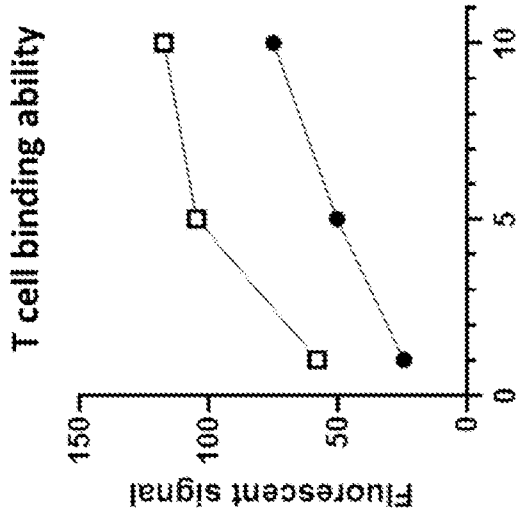

T cell binding ability
Fluorescent signal
150
100
50
0
0
5
10
BsAb (ug/mL)
CTA02scFv/CTAT03Fab
CTA01scFv/CTAT03Fab

FIG. 3

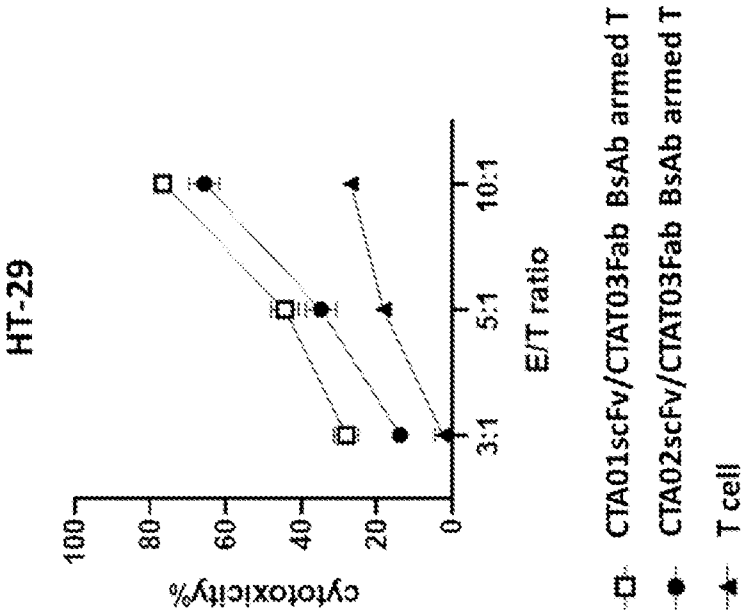

HT-29
cytotoxicity%
100
80
60
40
20
0
3:1
5:1
10:1
E/T ratio
CTA01scFv/CTAT03Fab BsAb armed T
CTA02scFv/CTAT03Fab BsAb armed T
T cell

FIG. 4

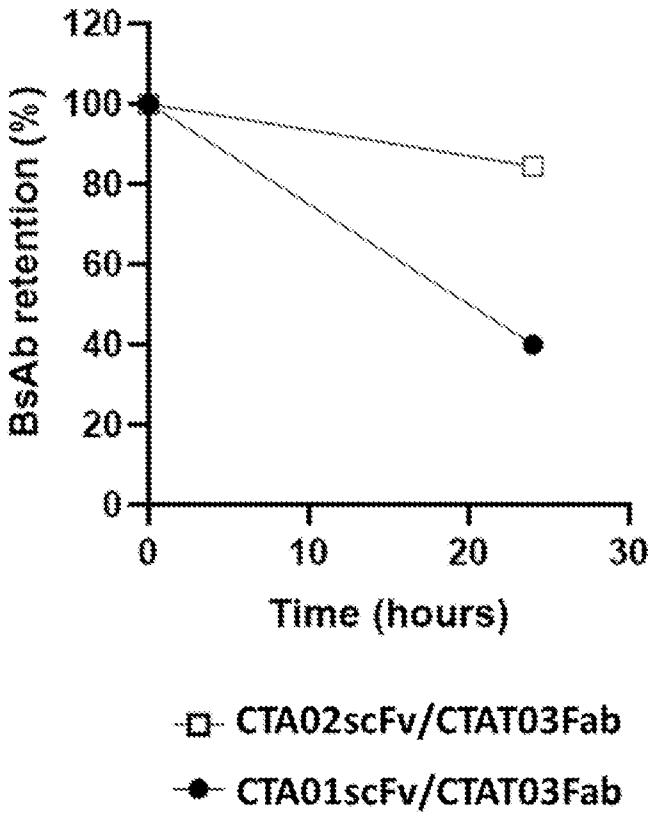

120
100
80
60
40
20
0
BsAb retention (%)
0
10
20
30
Time (hours)
CTA02scFv/CTAT03Fab
CTA01scFv/CTAT03Fab

FIG. 5

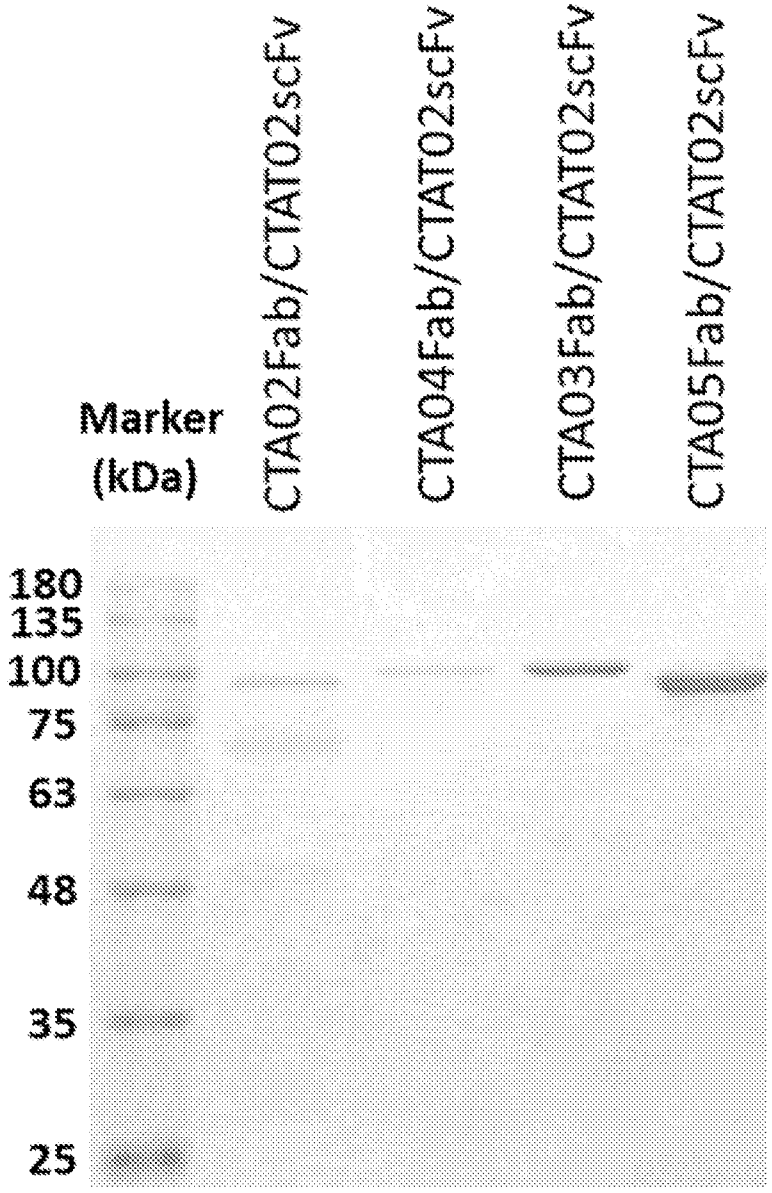

Marker
(kDa)
CTA02Fab/CTAT02scFv
CTA04Fab/CTAT02scFv
CTA03Fab/CTAT02scFv
CTA05Fab/CTAT02scFv
180
135
100
75
63
48
35
25

FIG. 6A

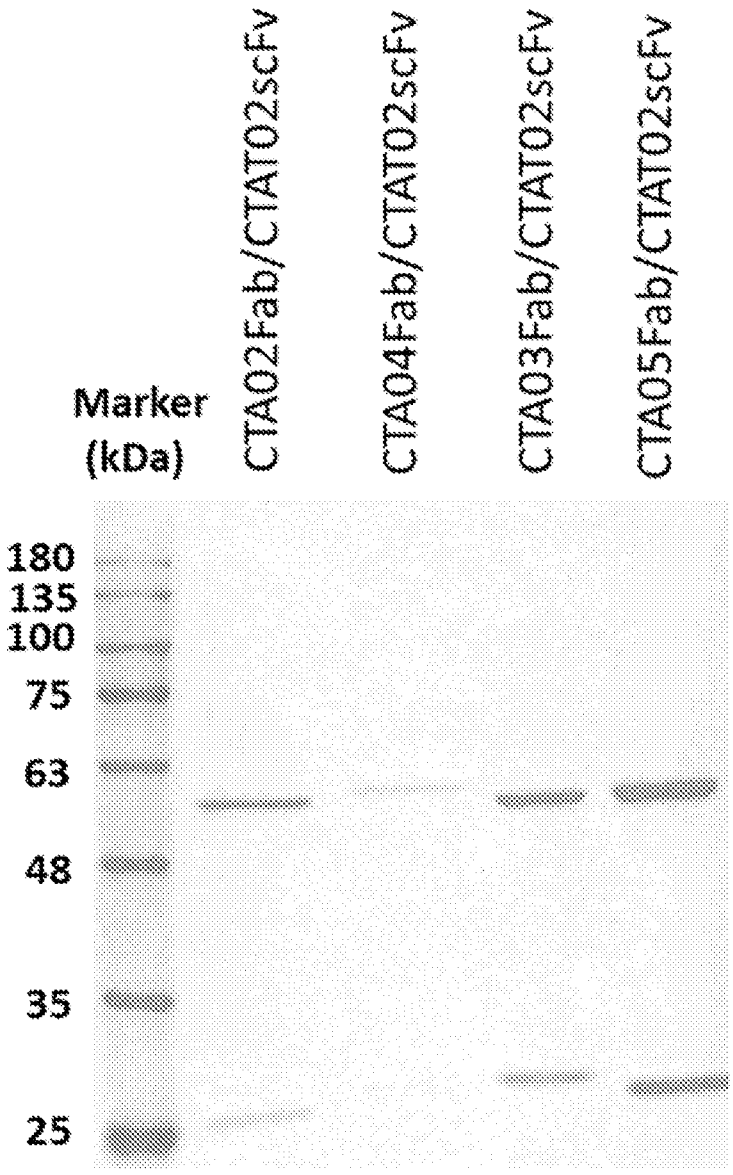

Marker
(kDa)
CTA02Fab/CTAT02scFv
CTA04Fab/CTAT02scFv
CTA03Fab/CTAT02scFv
CTA05Fab/CTAT02scFv
180
135
100
75
63
48
35
25

FIG. 6B

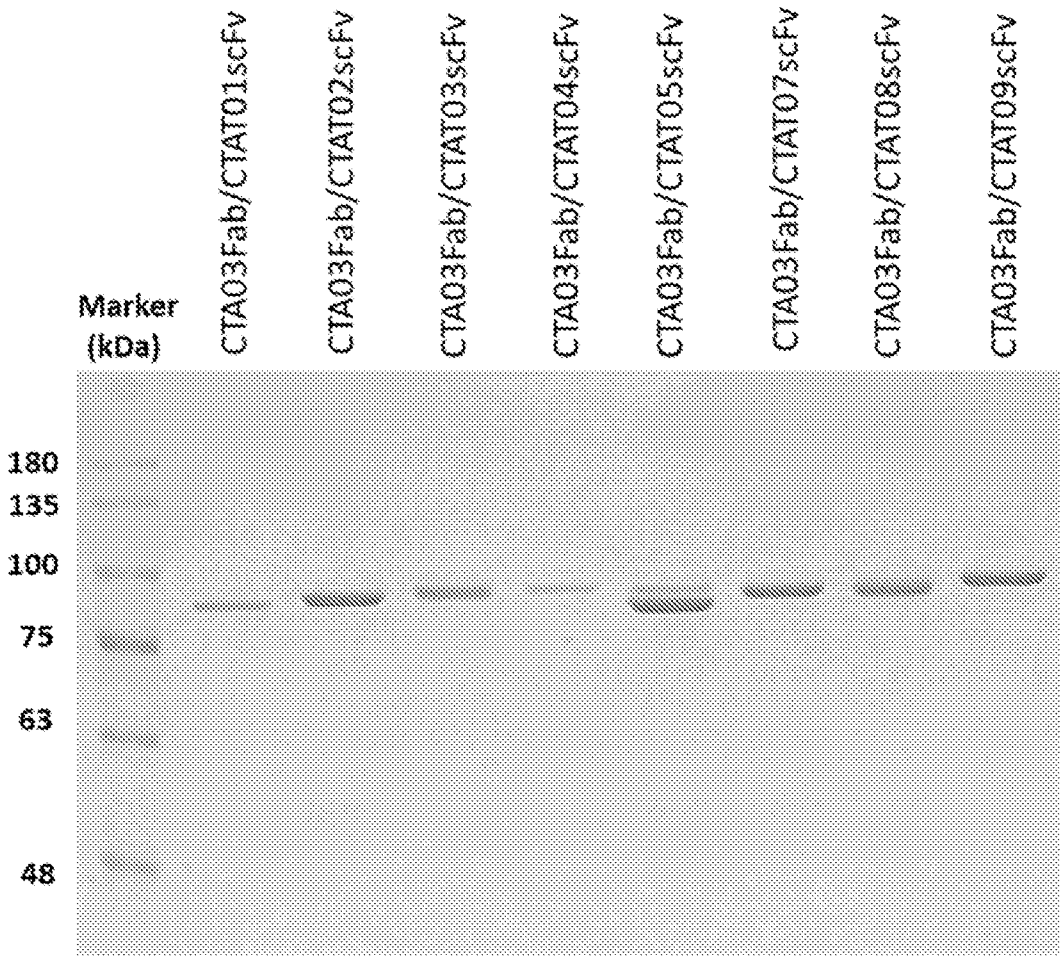

Marker
(kDa)
CTA03Fab/CTAT01scFv
CTA03Fab/CTAT02scFv
CTA03Fab/CTAT03scFv
CTA03Fab/CTAT04scFv
CTA03Fab/CTAT05scFv
CTA03Fab/CTAT07scFv
CTA03Fab/CTAT08scFv
CTA03Fab/CTAT09scFv
180
135
100
75
63
48

FIG. 7A

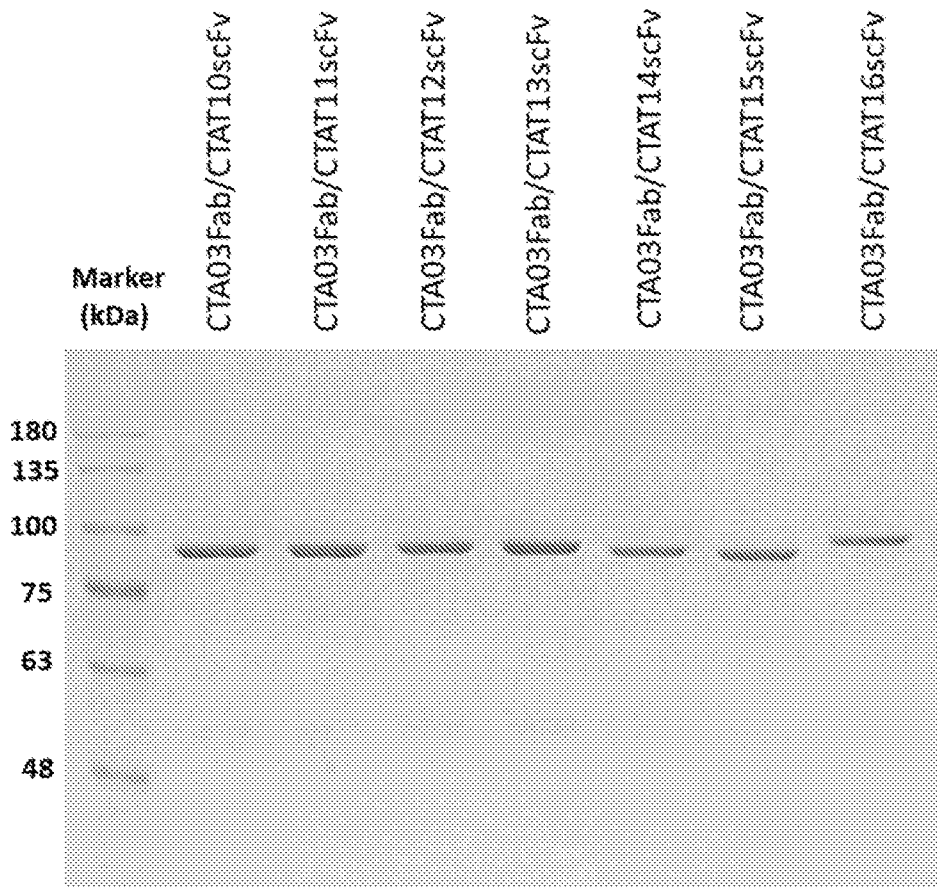

Marker
(kDa)
CTA03Fab/CTAT10scFv
CTA03Fab/CTAT11scFv
CTA03Fab/CTAT12scFv
CTA03Fab/CTAT13scFv
CTA03Fab/CTAT14scFv
CTA03Fab/CTAT15scFv
CTA03Fab/CTAT16scFv
180
135
100
75
63
48

FIG. 7B

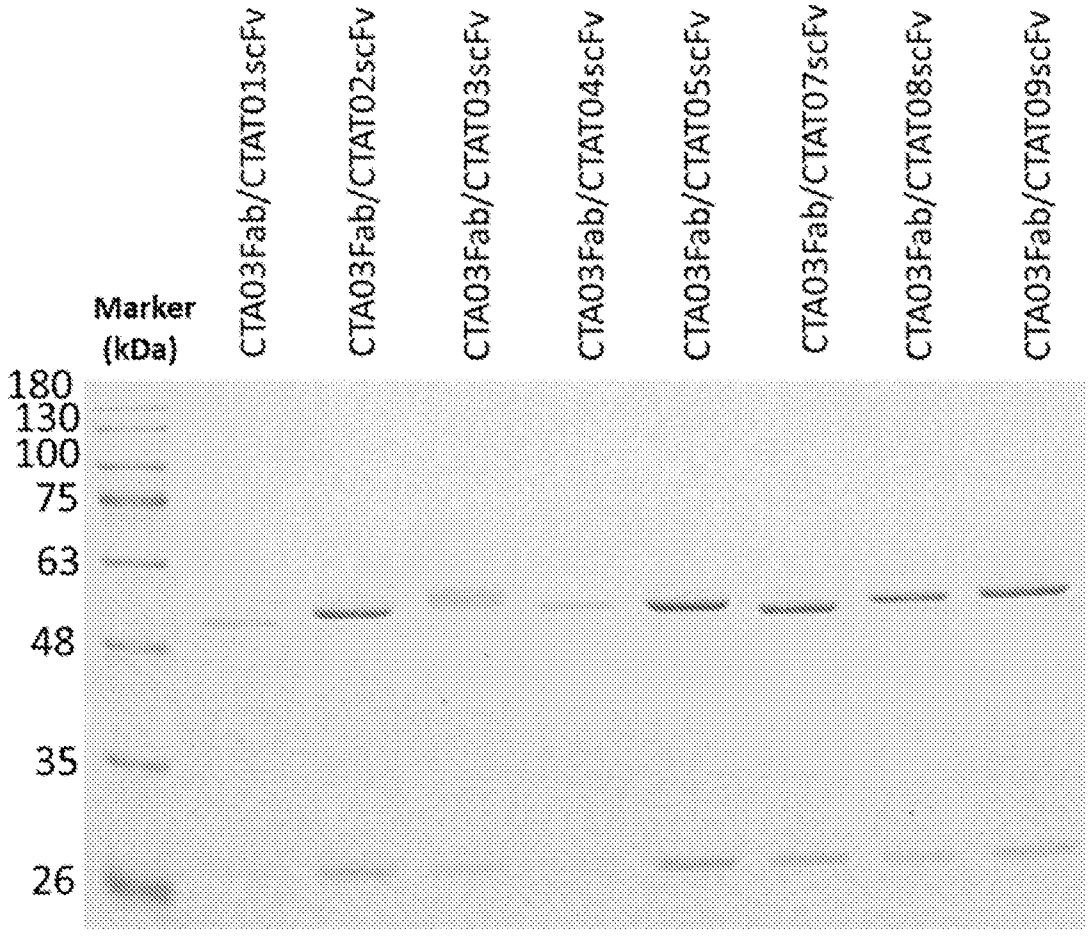

Marker
(kDa)
180
130
100
75
63
48
35
26
CTA03Fab/CTAT01scFv
CTA03Fab/CTAT02scFv
CTA03Fab/CTAT03scFv
CTA03Fab/CTAT04scFv
CTA03Fab/CTAT05scFv
CTA03Fab/CTAT07scFv
CTA03Fab/CTAT08scFv
CTA03Fab/CTAT09scFv

FIG. 7C

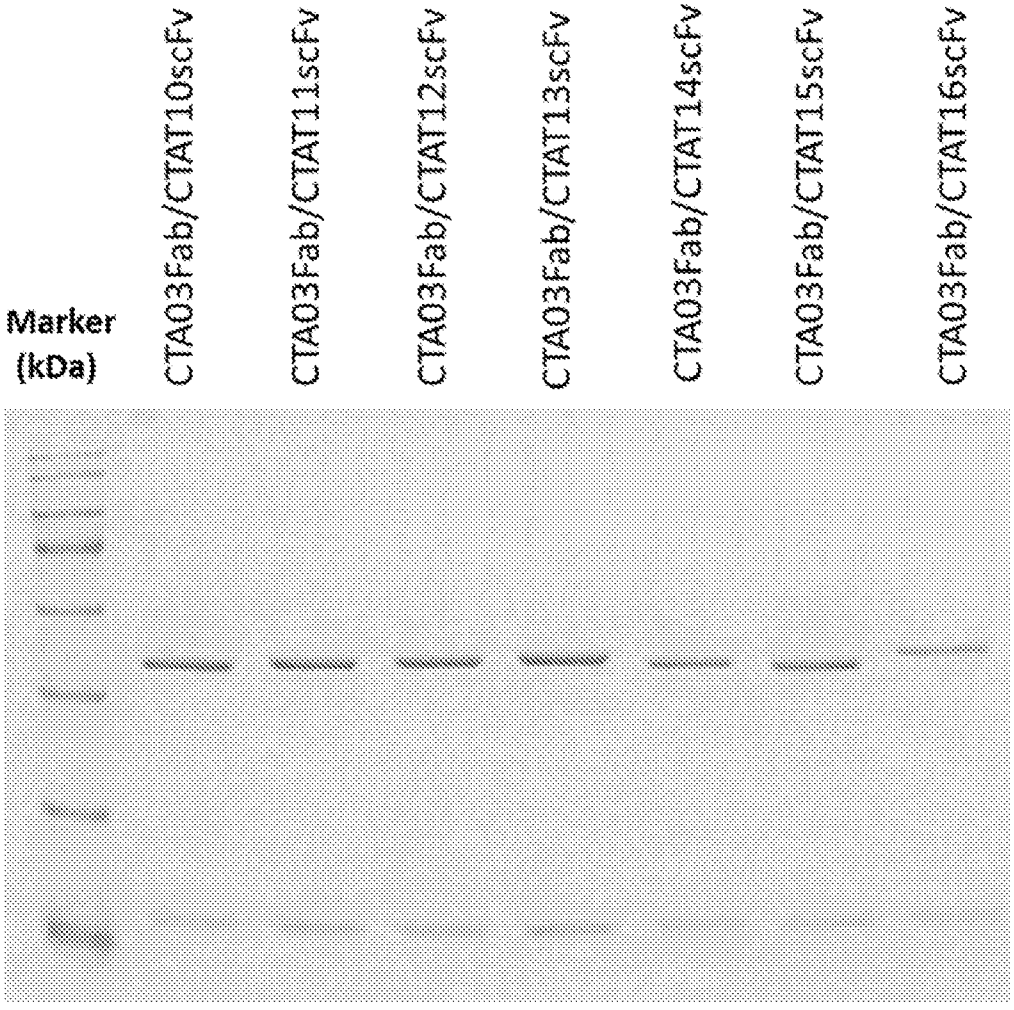

Marker
(kDa)
CTA03Fab/CTAT10scFv
CTA03Fab/CTAT11scFv
CTA03Fab/CTAT12scFv
CTA03Fab/CTAT13scFv
CTA03Fab/CTAT14scFv
CTA03Fab/CTAT15scFv
CTA03Fab/CTAT16scFv

FIG. 7D

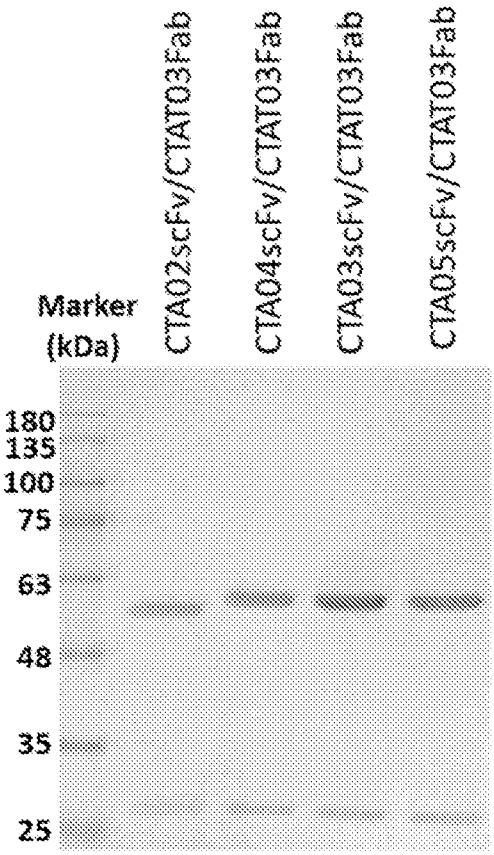

Marker
(kDa)
CTA02scFv/CTAT03Fab
CTA04scFv/CTAT03Fab
CTA03scFv/CTAT03Fab
CTA05scFv/CTAT03Fab
180
135
100
75
63
48
35
25

FIG. 7F

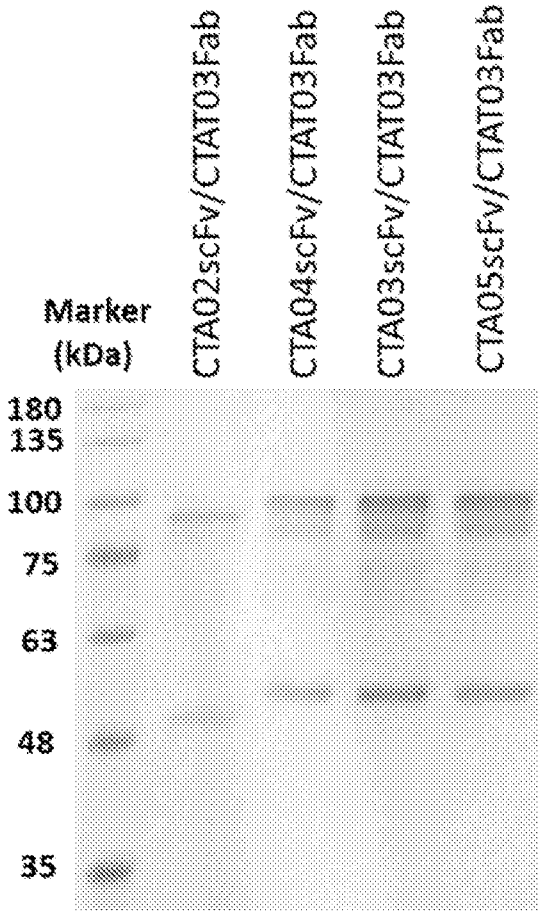

Marker
(kDa)
CTA02scFv/CTAT03Fab
CTA04scFv/CTAT03Fab
CTA03scFv/CTAT03Fab
CTA05scFv/CTAT03Fab
180
135
100
75
63
48
35

FIG. 7E

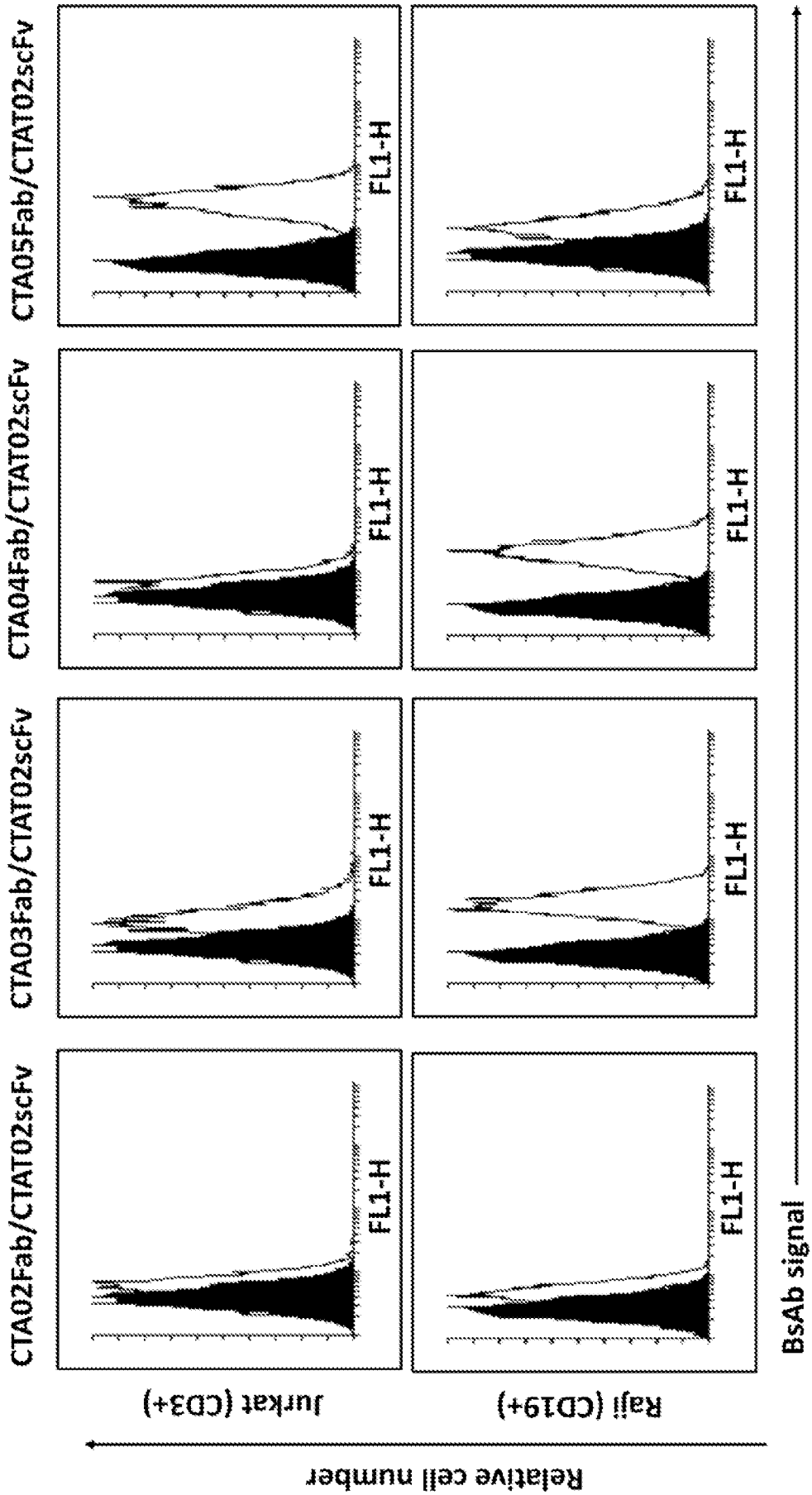

CTA02Fab/CTAT02scFv
CTA03Fab/CTAT02scFv
CTA04Fab/CTAT02scFv
CTA05Fab/CTAT02scFv
FL1-H
Jurkat (CD3+)
Raji (CD19+)
Relative cell number
BsAb signal

FIG. 8

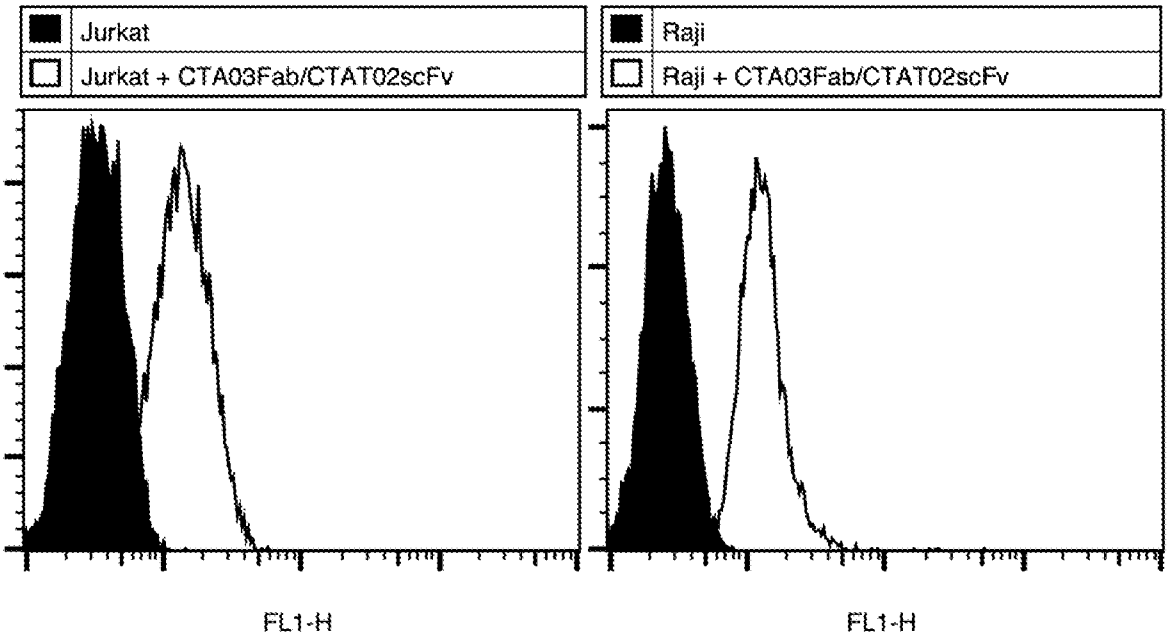

Jurkat
Jurkat + CTA03Fab/CTAT02scFv
Raji
Raji + CTA03Fab/CTAT02scFv
FL1-H
FL1-H

FIG. 9A

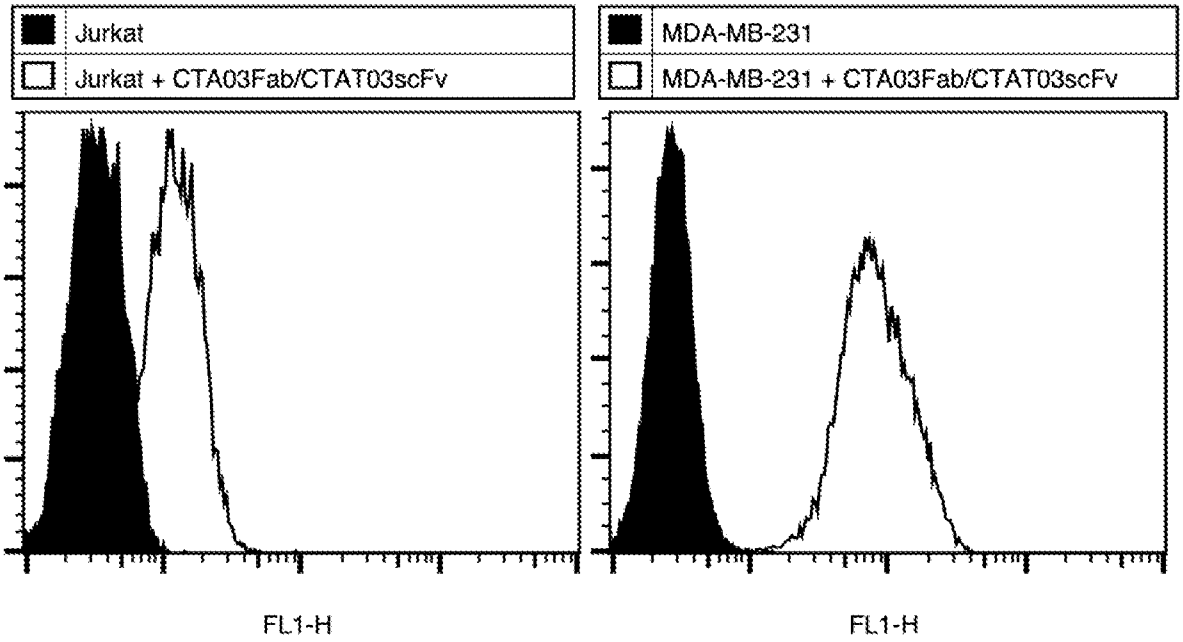

Jurkat
Jurkat + CTA03Fab/CTAT03scFv
MDA-MB-231
MDA-MB-231 + CTA03Fab/CTAT03scFv
FL1-H
FL1-H

FIG. 9B

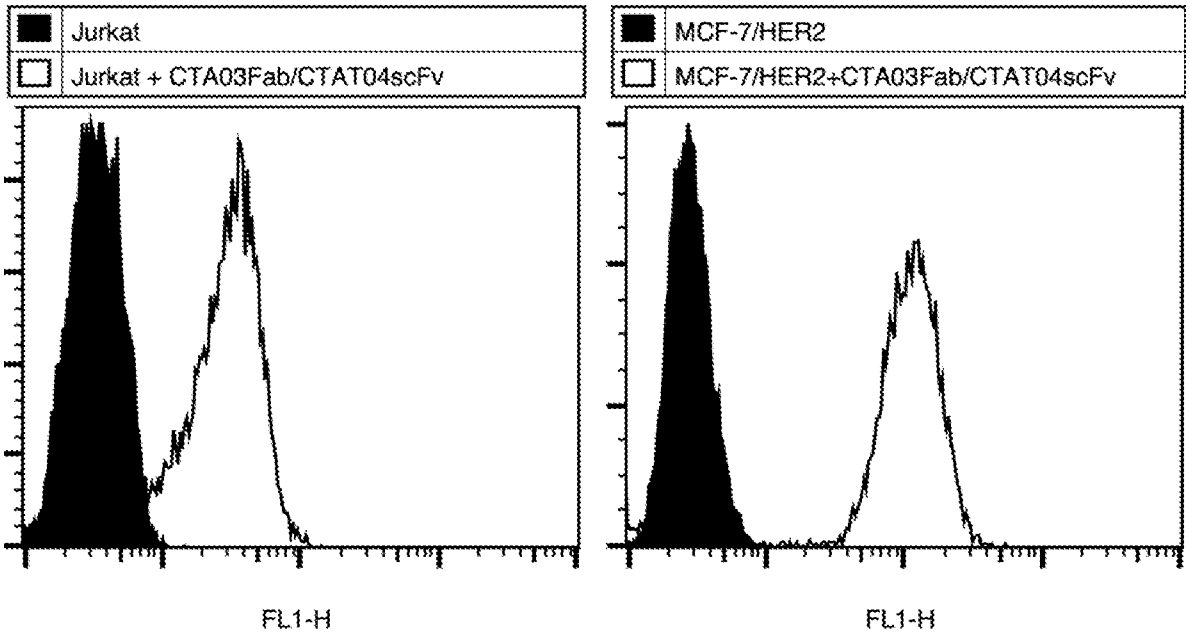

Jurkat
Jurkat + CTA03Fab/CTAT04scFv
MCF-7/HER2
MCF-7/HER2+CTA03Fab/CTAT04scFv
FL1-H
FL1-H

FIG. 9C

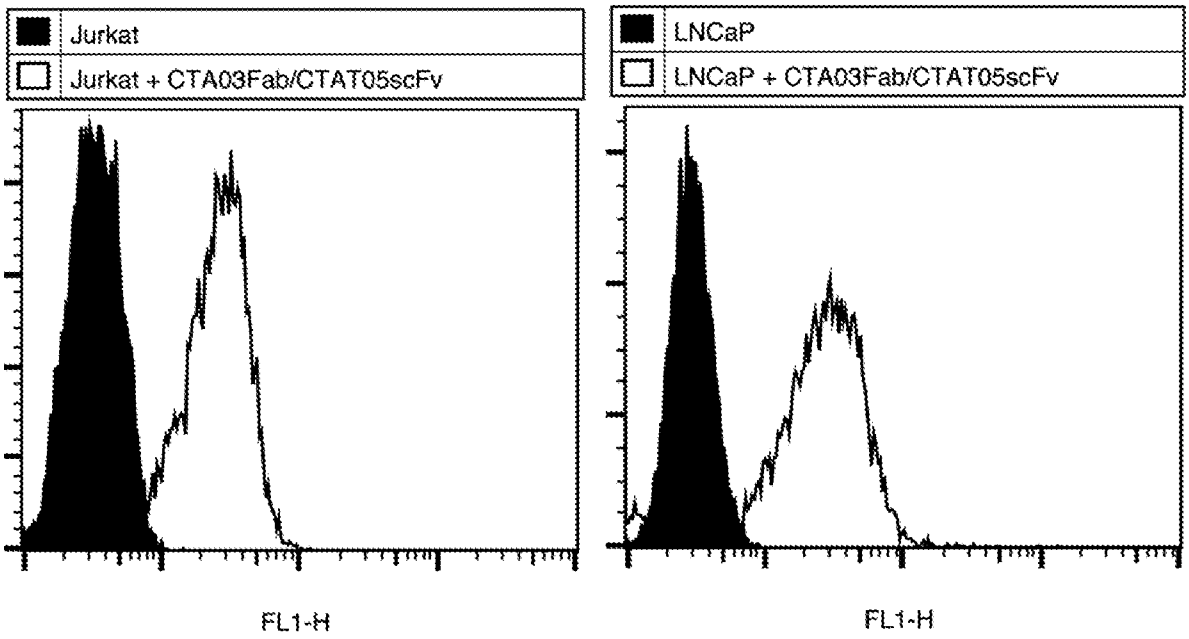

Jurkat
Jurkat + CTA03Fab/CTAT05scFv
LNCaP
LNCaP + CTA03Fab/CTAT05scFv
FL1-H
FL1-H

FIG. 9D

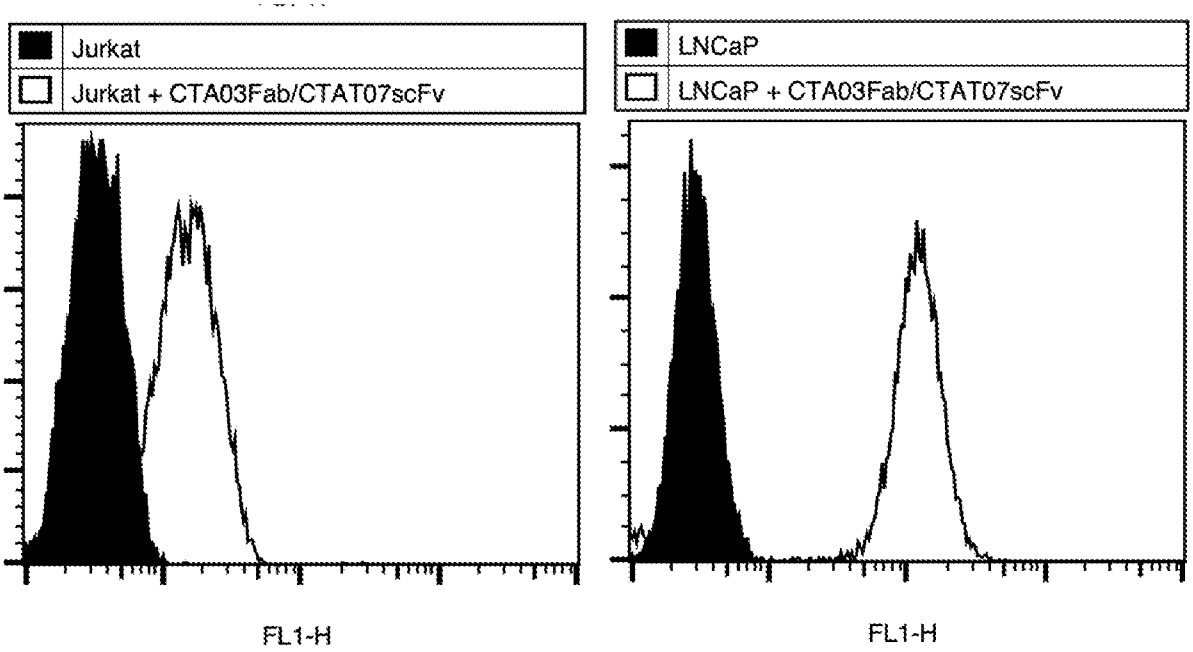

Jurkat
Jurkat + CTA03Fab/CTAT07scFv
LNCaP
LNCaP + CTA03Fab/CTAT07scFv
FL1-H
FL1-H

FIG. 9E

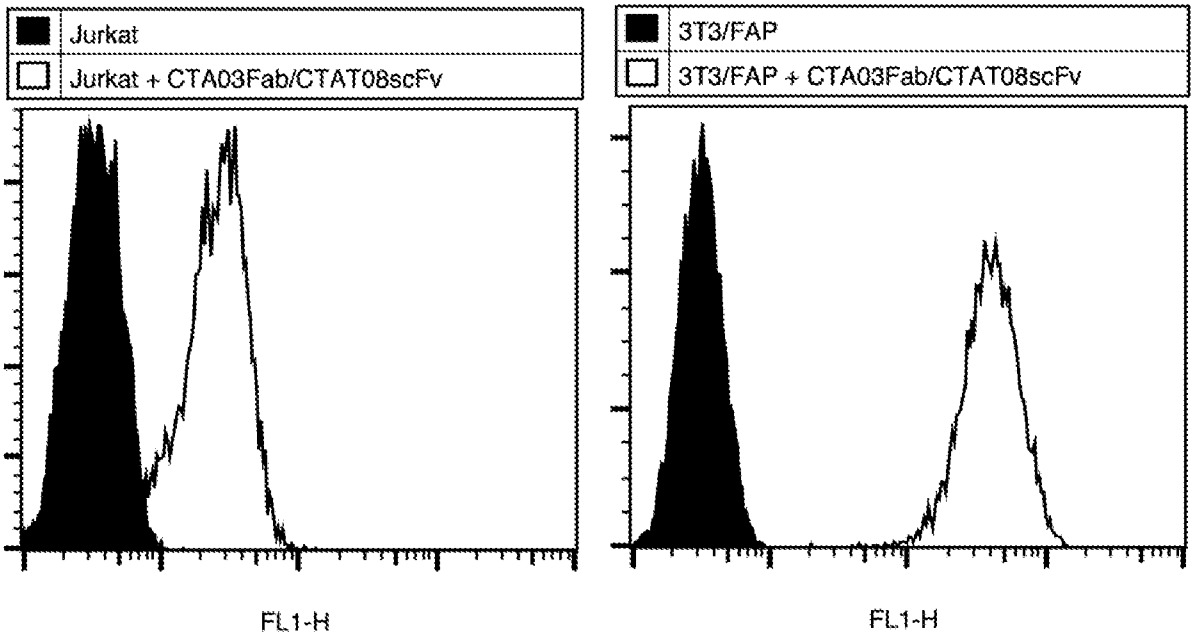

Jurkat
Jurkat + CTA03Fab/CTAT08scFv
3T3/FAP
3T3/FAP + CTA03Fab/CTAT08scFv
FL1-H
FL1-H

FIG. 9F

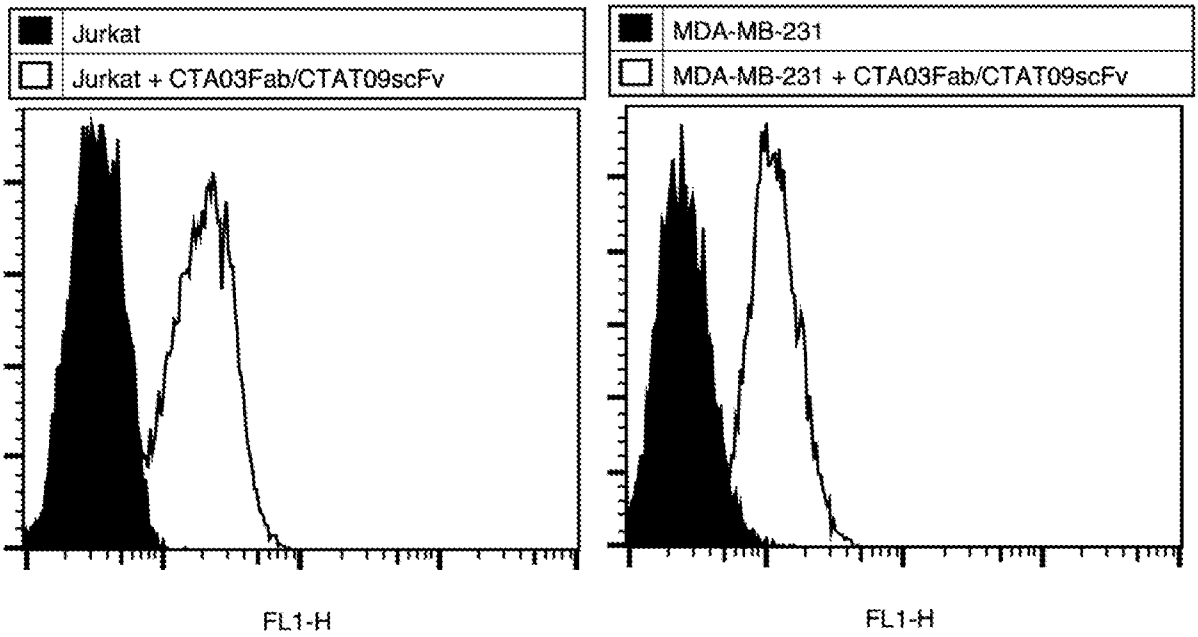

Jurkat
Jurkat + CTA03Fab/CTAT09scFv
MDA-MB-231
MDA-MB-231 + CTA03Fab/CTAT09scFv
FL1-H
FL1-H

FIG. 9G

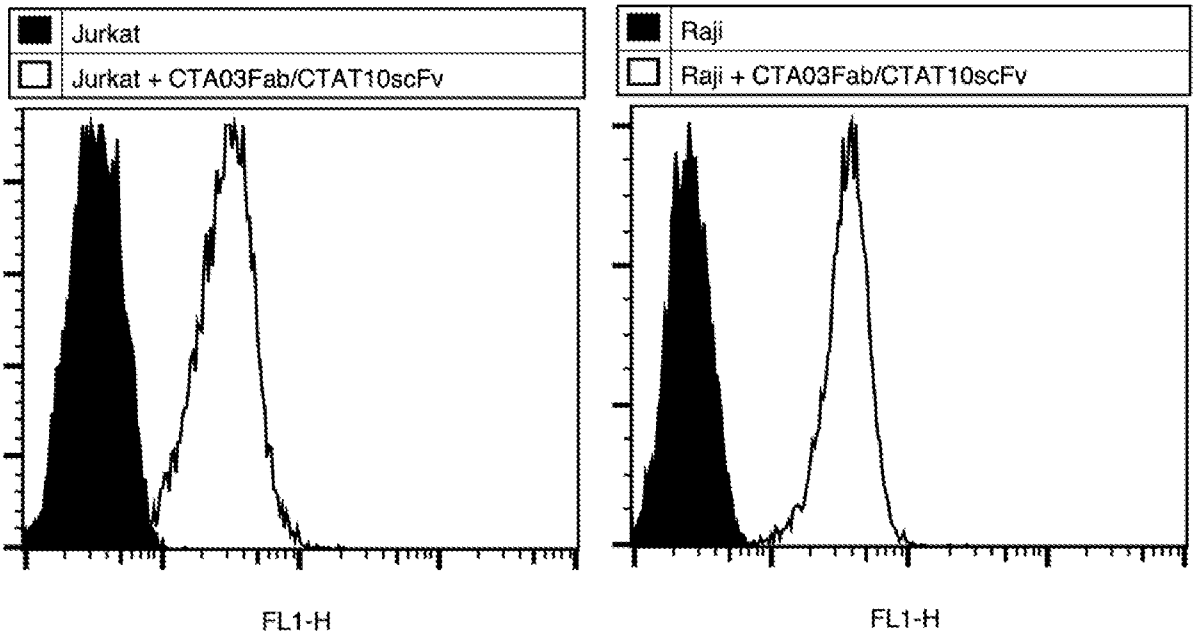

Jurkat
Jurkat + CTA03Fab/CTAT10scFv
Raji
Raji + CTA03Fab/CTAT10scFv
FL1-H
FL1-H

FIG. 9H

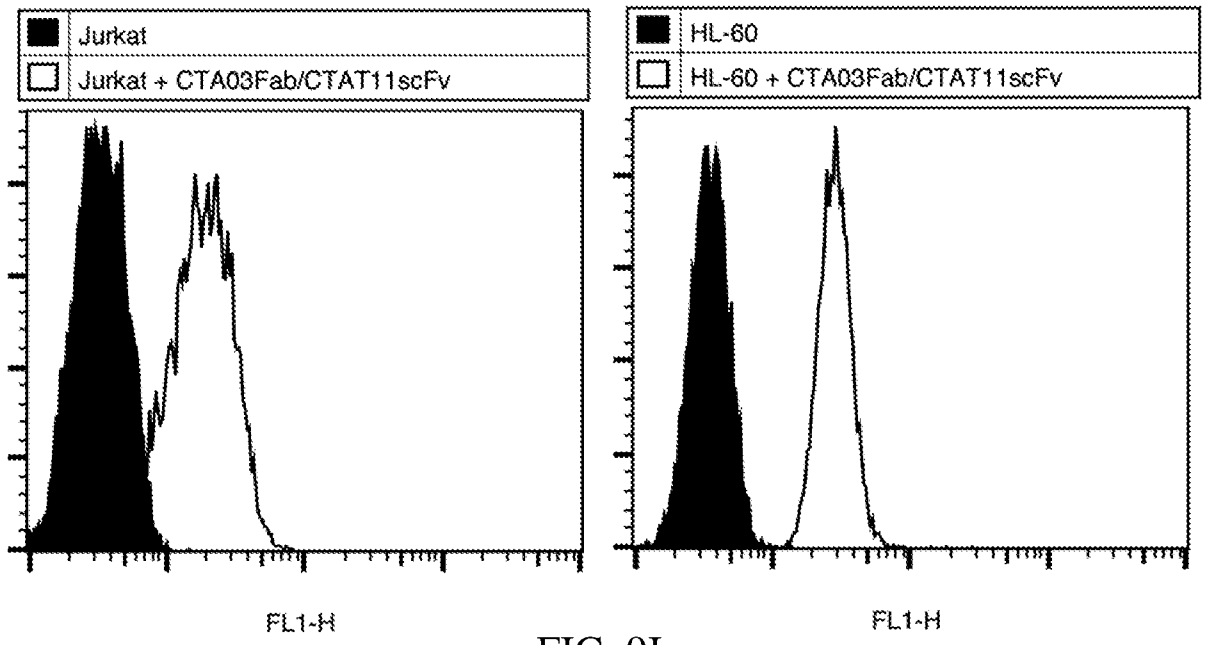

Jurkat
Jurkat + CTA03Fab/CTAT11scFv
HL-60
HL-60 + CTA03Fab/CTAT11scFv
FL1-H
FL1-H

FIG. 9I

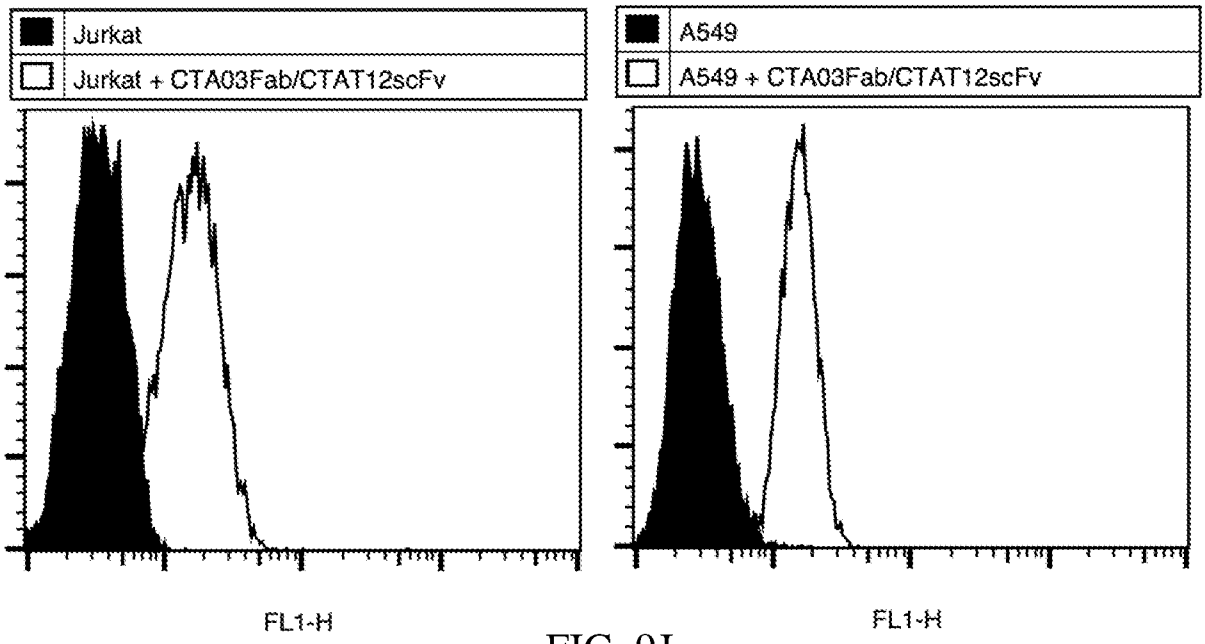

Jurkat
Jurkat + CTA03Fab/CTAT12scFv
A549
A549 + CTA03Fab/CTAT12scFv
FL1-H
FL1-H

FIG. 9J

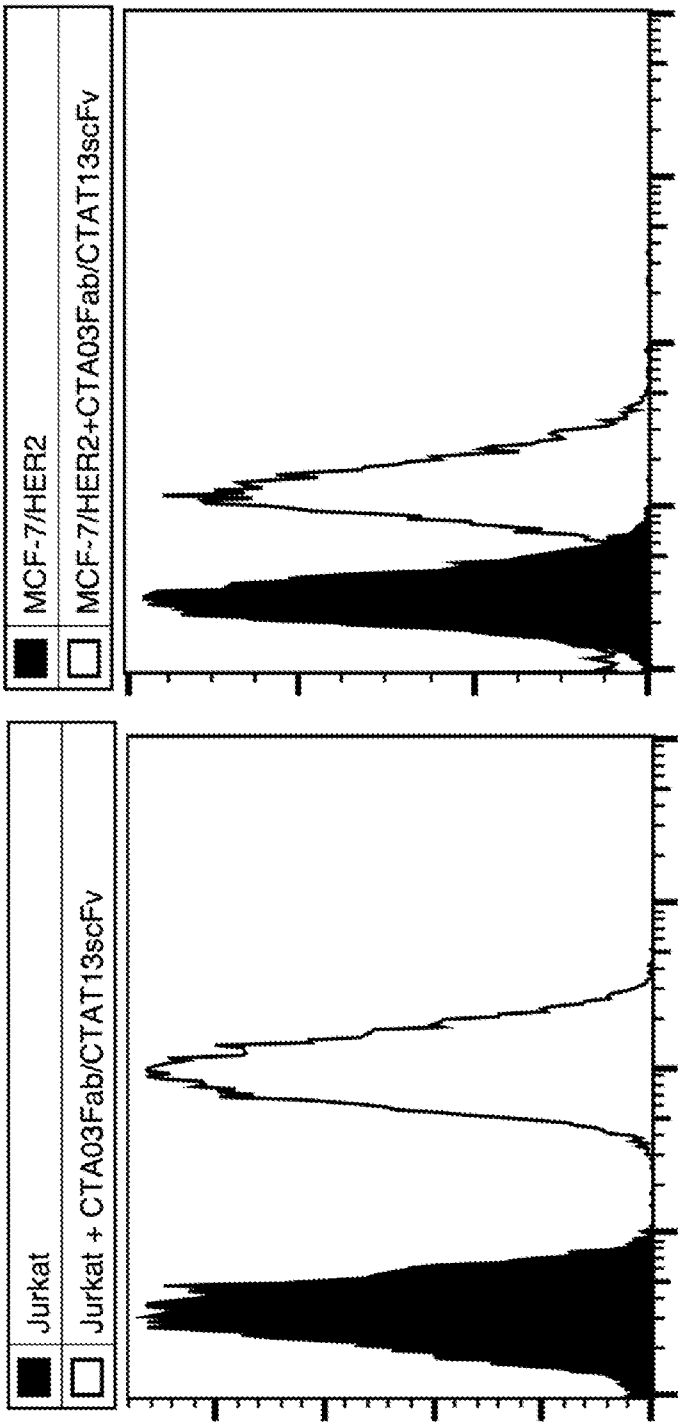

Jurkat
Jurkat + CTA03Fab/CTAT13scFv
FL1-H
MCF-7/HER2
MCF-7/HER2+CTA03Fab/CTAT13scFv
FL1-H

FIG. 9K

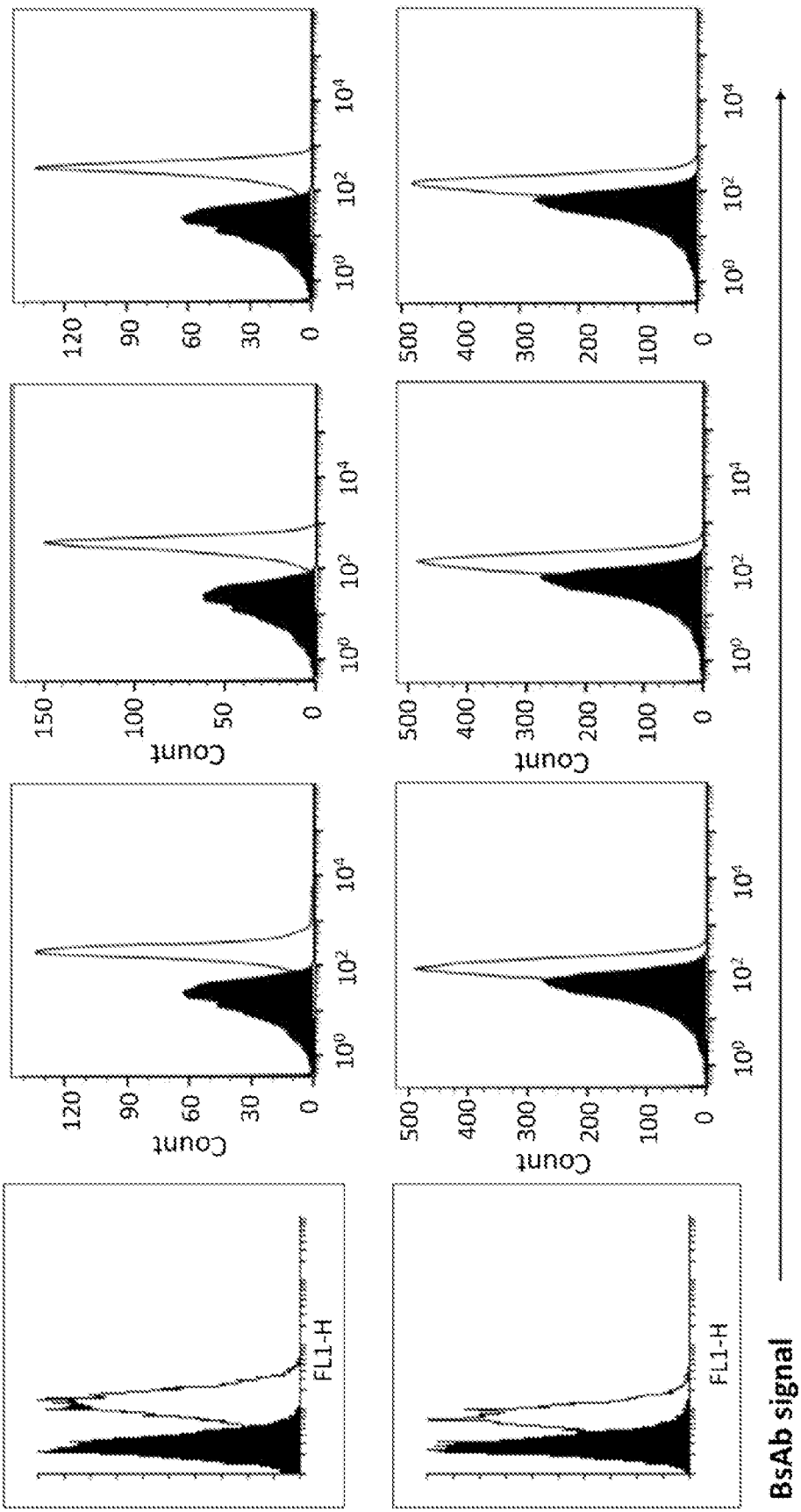

CTA02scFv/CTAT03Fab
CTA03scFv/CTAT03Fab
CTA04scFv/CTAT03Fab
CTA05scFv/CTAT03Fab
Jurkat (CD3+)
HT-29 (EGFR+)
Relative cell number
BsAb signal
FL1-H
Count

FIG. 9L

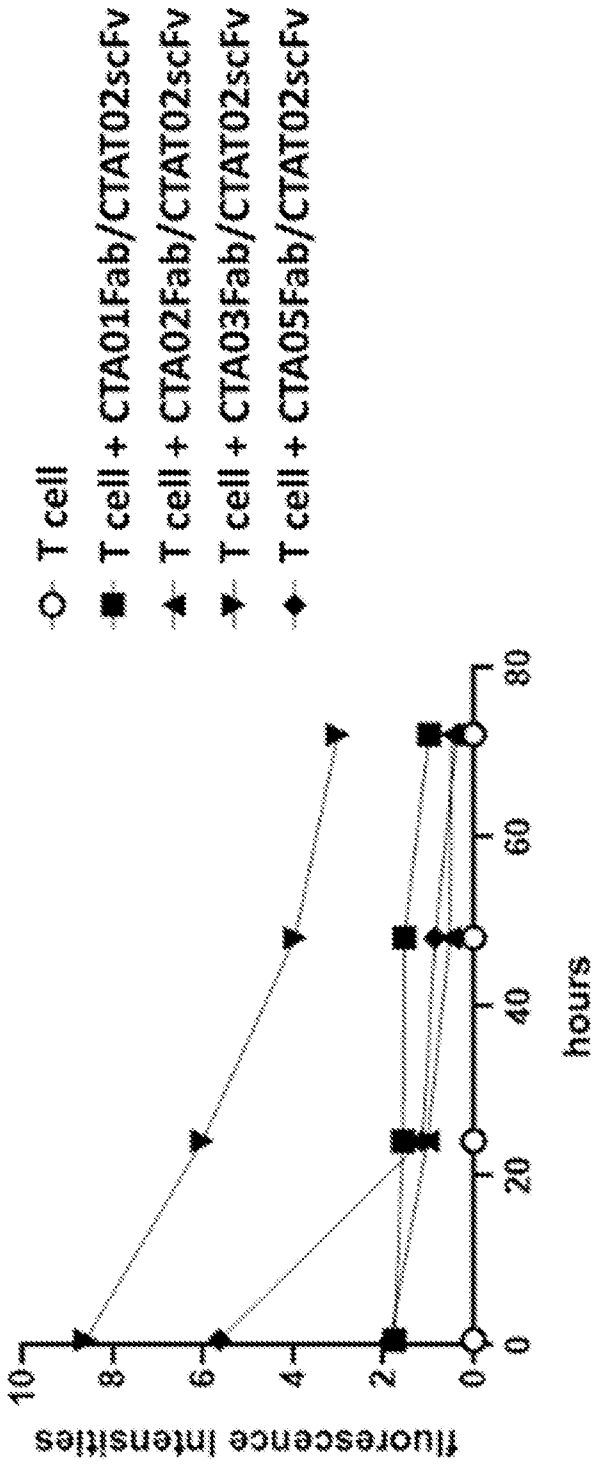

T cell
T cell + CTA01Fab/CTAT02scFv
T cell + CTA02Fab/CTAT02scFv
T cell + CTA03Fab/CTAT02scFv
T cell + CTA05Fab/CTAT02scFv
fluorescence intensities
0
2
4
6
8
10
0
20
40
60
80
hours

FIG. 10

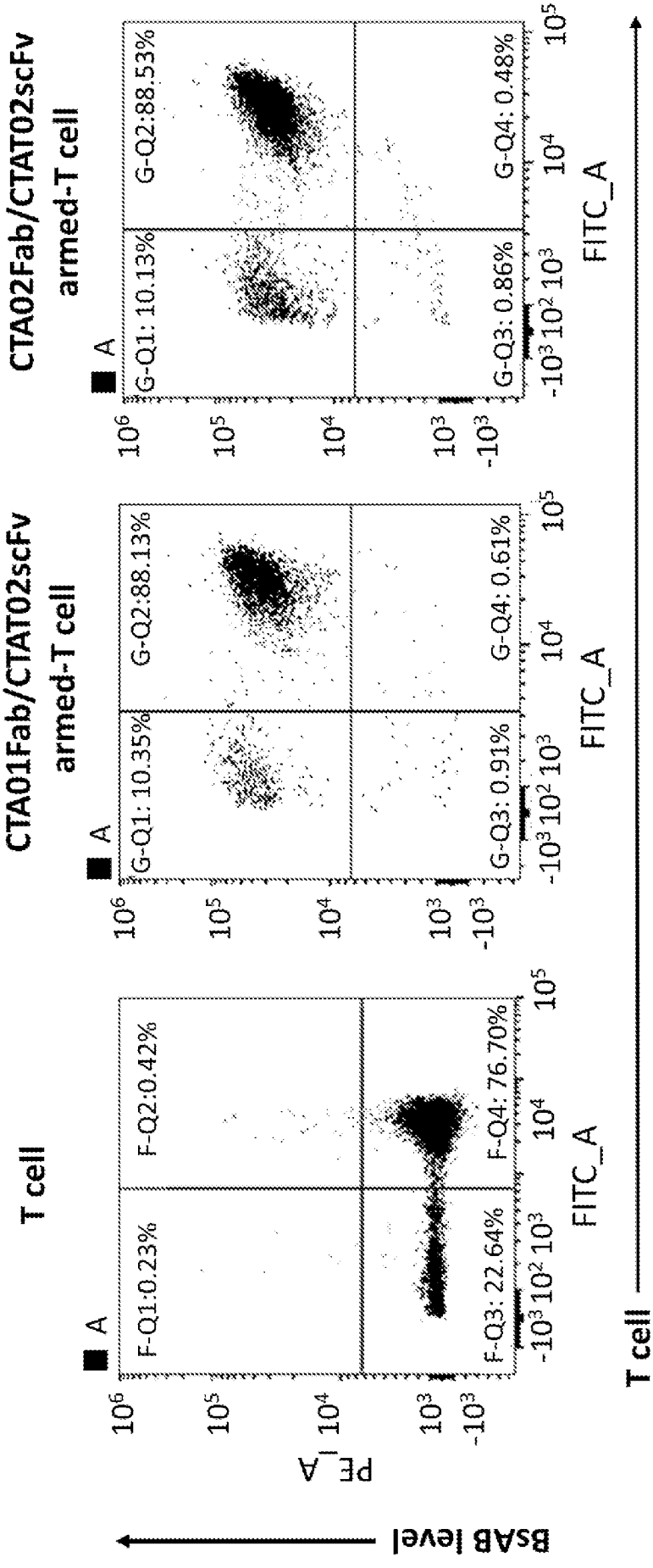

T cell
CTA01Fab/CTAT02scFv armed-T cell
CTA02Fab/CTAT02scFv armed-T cell
A
F-Q1:0.23%
F-Q2:0.42%
F-Q3: 22.64%
F-Q4: 76.70%
G-Q1: 10.35%
G-Q2:88.13%
G-Q3: 0.91%
G-Q4: 0.61%
G-Q1: 10.13%
G-Q2:88.53%
G-Q3: 0.86%
G-Q4: 0.48%
PE_A
FITC_A
BsAB level
T cell

FIG. 11A

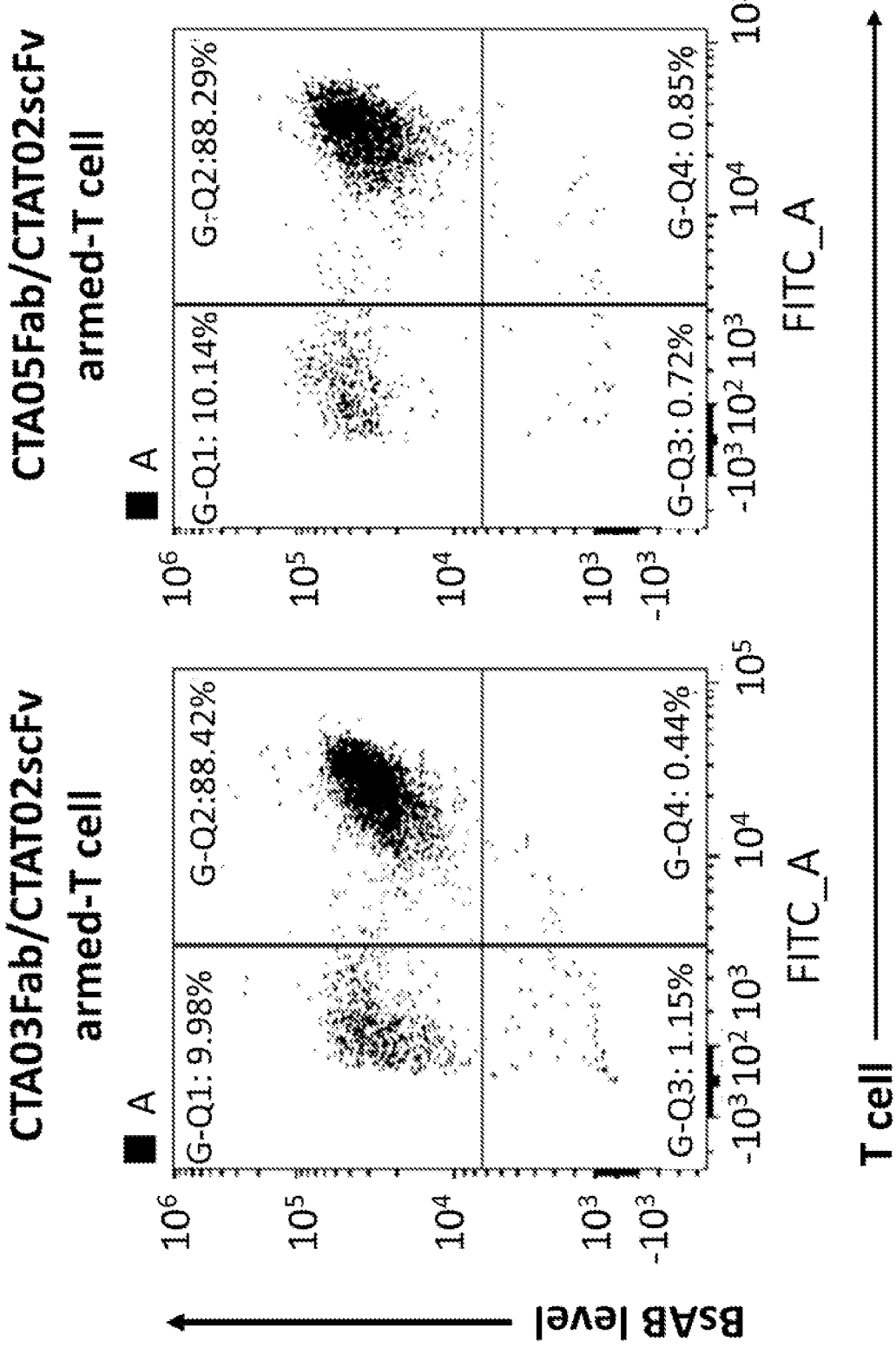

CTA03Fab/CTAT02scFv
armed-T cell
CTA05Fab/CTAT02scFv
armed-T cell
A
G-Q1: 9.98%
G-Q2:88.42%
G-Q3: 1.15%
G-Q4: 0.44%
G-Q1: 10.14%
G-Q2:88.29%
G-Q3: 0.72%
G-Q4: 0.85%
BsAB level
T cell
FITC_A
10^6
10^5
10^4
10^3
-10^3
-10^3 10^2 10^3 10^4 10^5

FIG. 11B

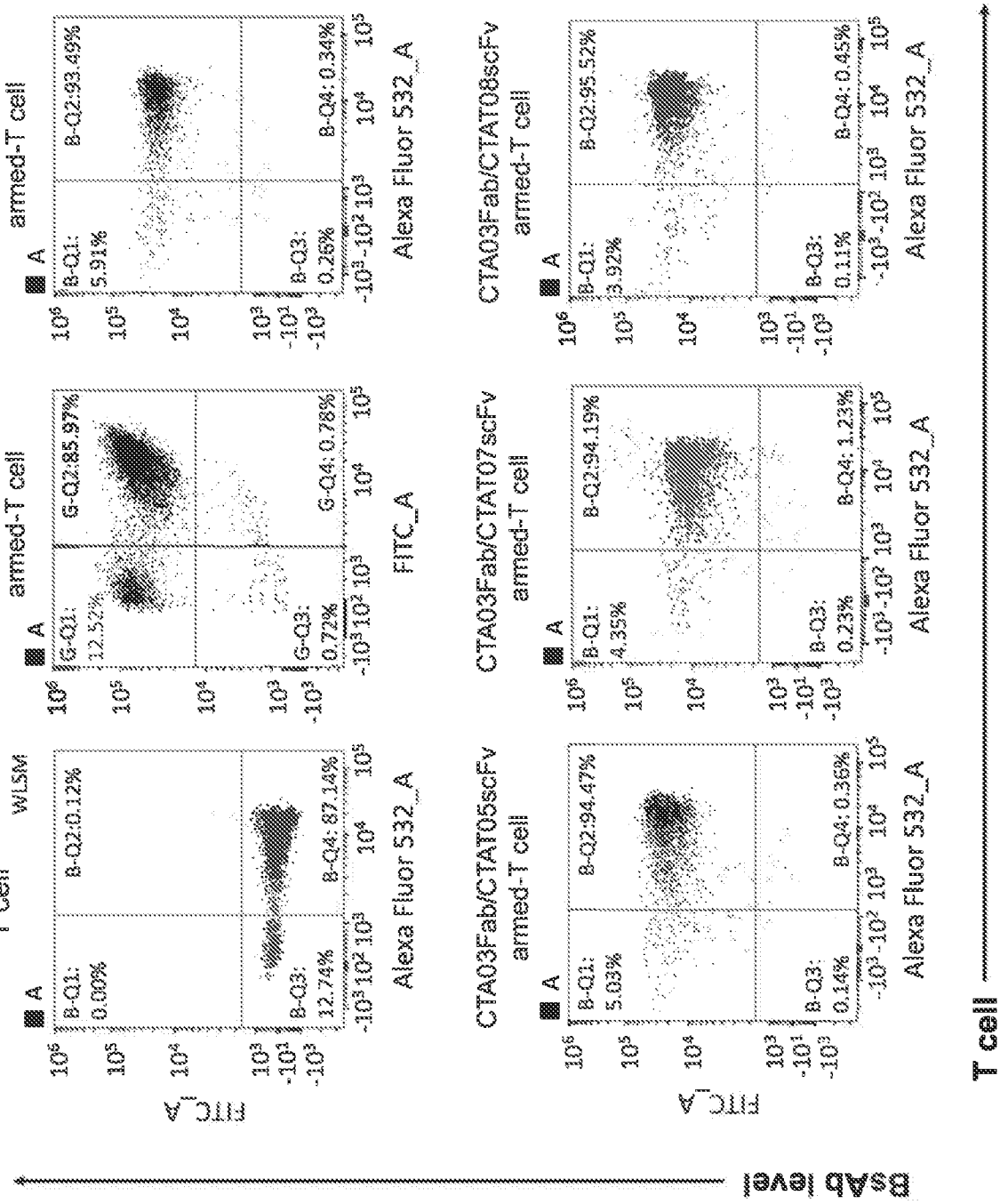

T cell
WLSM
CTA03Fab/CTAT03scFv armed-T cell
CTA03Fab/CTAT04scFv armed-T cell
CTA03Fab/CTAT05scFv armed-T cell
CTA03Fab/CTAT07scFv armed-T cell
CTA03Fab/CTAT08scFv armed-T cell
B-Q1: 0.00%
B-Q2:0.12%
B-Q3: 12.74%
B-Q4: 87.14%
G-Q1: 12.52%
G-Q2:85.97%
G-Q3: 0.72%
G-Q4: 0.78%
B-Q1: 5.91%
B-Q2:93.49%
B-Q3: 0.26%
B-Q4: 0.34%
B-Q1: 5.03%
B-Q2:94.47%
B-Q3: 0.14%
B-Q4: 0.36%
B-Q1: 4.35%
B-Q2:94.19%
B-Q3: 0.23%
B-Q4: 1.23%
B-Q1: 3.92%
B-Q2:95.52%
B-Q3: 0.11%
B-Q4: 0.45%
FITC_A
Alexa Fluor 532_A
BsAb level
T cell

FIG. 12A

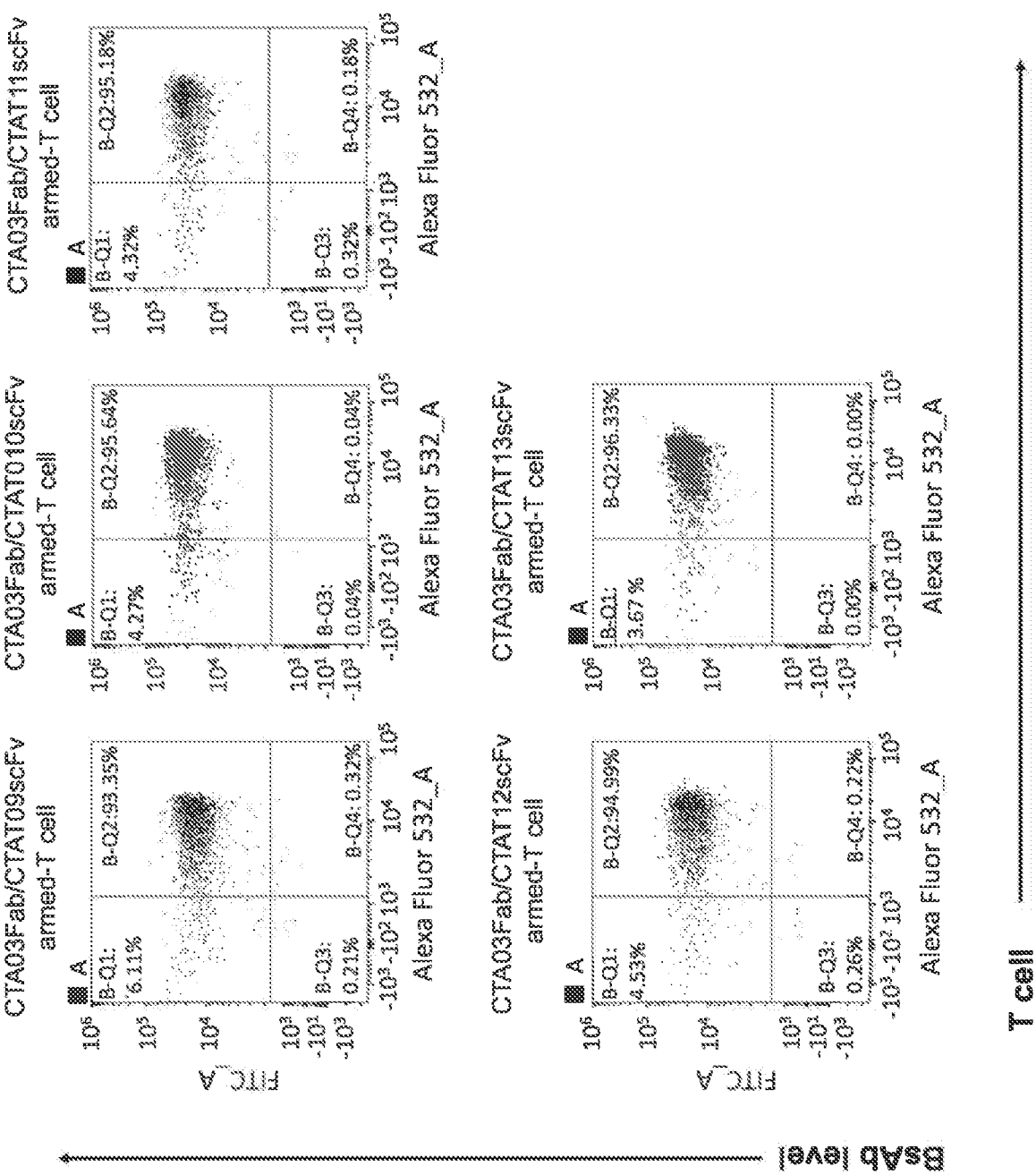

CTA03Fab/CTAT09scFv
armed-T cell
CTA03Fab/CTAT010scFv
armed-T cell
CTA03Fab/CTAT11scFv
armed-T cell
CTA03Fab/CTAT12scFv
armed-T cell
CTA03Fab/CTAT13scFv
armed-T cell
A
B-Q1: 6.11%
B-Q2:93.35%
B-Q3: 0.21%
B-Q4: 0.32%
B-Q1: 4.27%
B-Q2:95.64%
B-Q3: 0.04%
B-Q4: 0.04%
B-Q1: 4.32%
B-Q2:95.18%
B-Q3: 0.32%
B-Q4: 0.18%
B-Q1: 4.53%
B-Q2:94.99%
B-Q3: 0.26%
B-Q4: 0.22%
B-Q1: 3.67 %
B-Q2:96.33%
B-Q3: 0.00%
B-Q4: 0.00%
FITC_A
Alexa Fluor 532_A
BsAb level
T cell

FIG. 12B

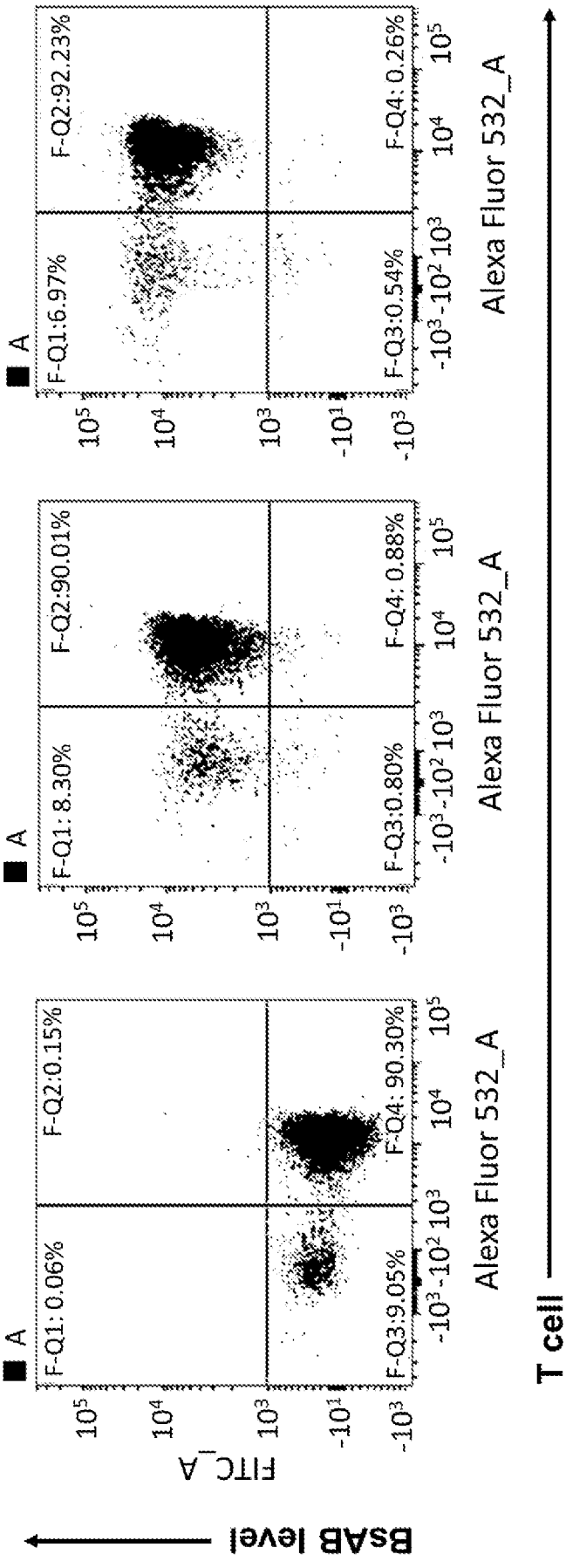

T cell
CTA01scFv/CTAT03Fab armed-T cell
CTA02scFv/CTAT03Fab armed-T cell
A
F-Q1: 0.06%
F-Q2:0.15%
F-Q3:9.05%
F-Q4: 90.30%
F-Q1: 8.30%
F-Q2:90.01%
F-Q3:0.80%
F-Q4: 0.88%
F-Q1:6.97%
F-Q2:92.23%
F-Q3:0.54%
F-Q4: 0.26%
FITC_A
Alexa Fluor 532_A
BsAB level
T cell

FIG. 12C

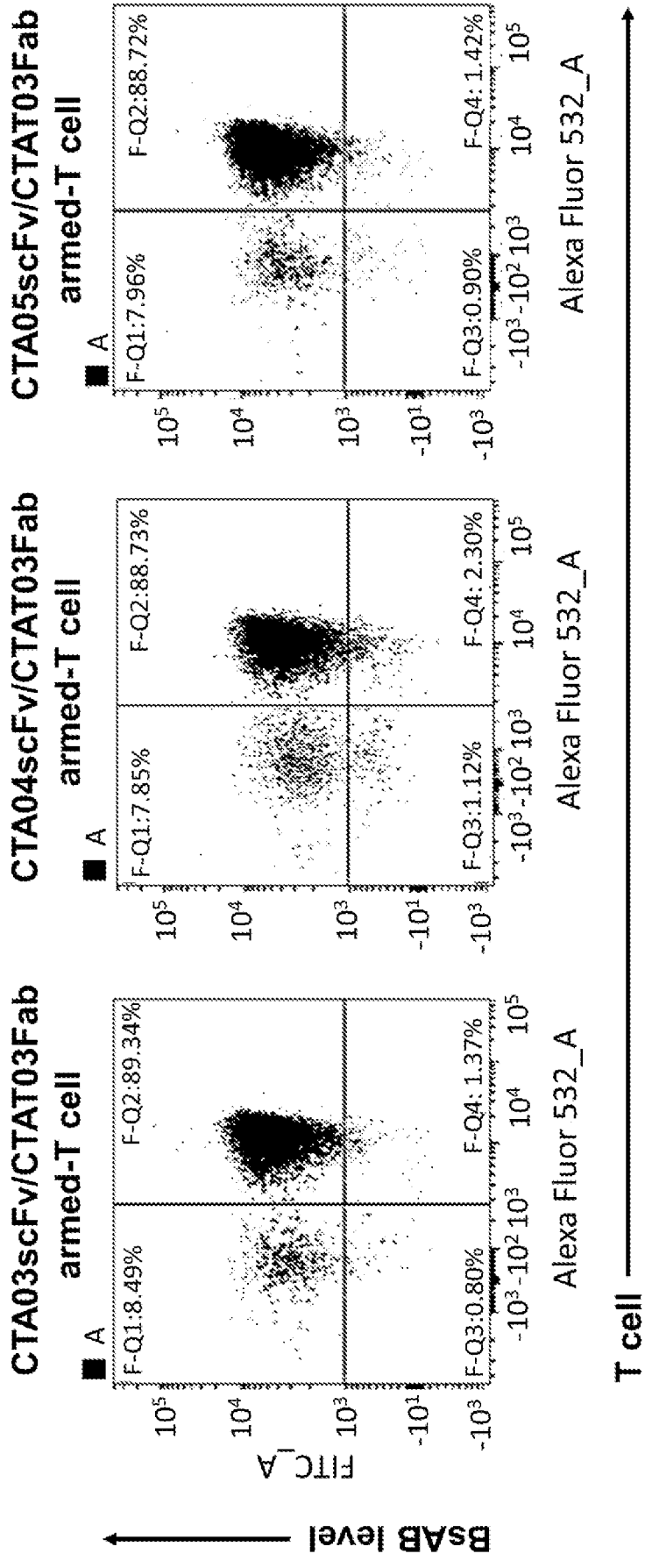

CTA03scFv/CTAT03Fab armed-T cell
CTA04scFv/CTAT03Fab armed-T cell
CTA05scFv/CTAT03Fab armed-T cell
A
F-Q1:8.49%
F-Q2:89.34%
F-Q3:0.80%
F-Q4: 1.37%
F-Q1:7.85%
F-Q2:88.73%
F-Q3:1.12%
F-Q4: 2.30%
F-Q1:7.96%
F-Q2:88.72%
F-Q3:0.90%
F-Q4: 1.42%
FITC_A
Alexa Fluor 532_A
BsAB level
T cell

FIG. 12D

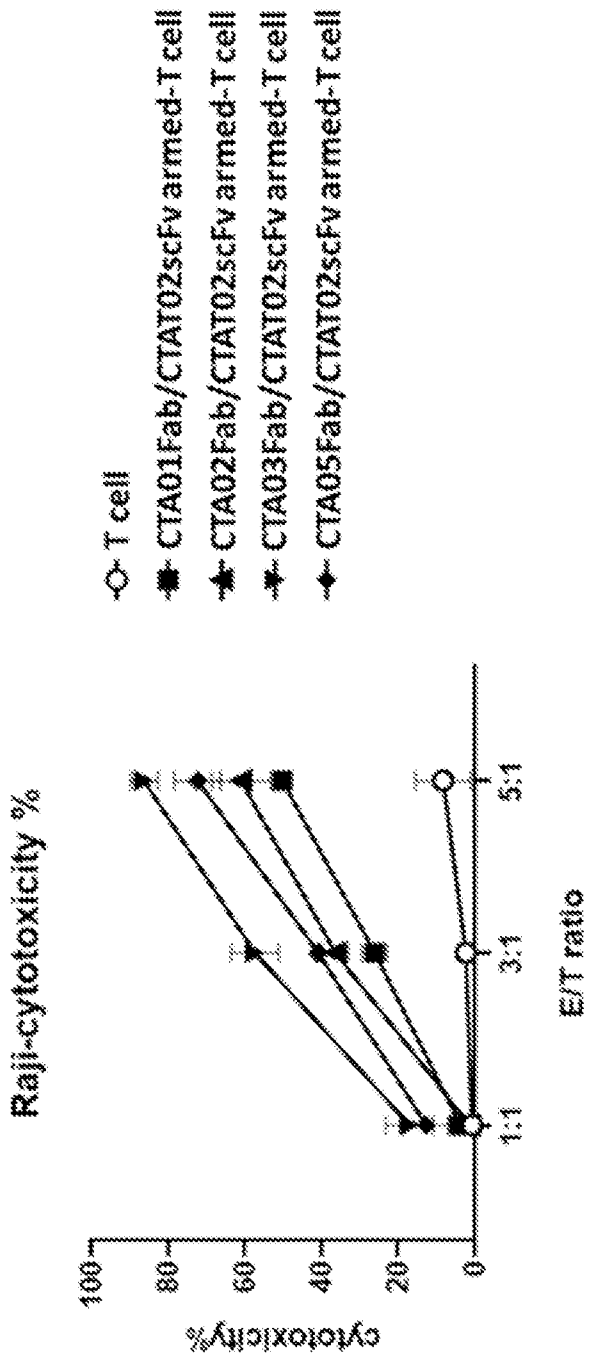

Raji-cytotoxicity %
T cell
CTA01Fab/CTAT02scFv armed-T cell
CTA02Fab/CTAT02scFv armed-T cell
CTA03Fab/CTAT02scFv armed-T cell
CTA05Fab/CTAT02scFv armed-T cell
cytotoxicity%
100
80
60
40
20
0
1:1
3:1
5:1
E/T ratio

FIG. 13A

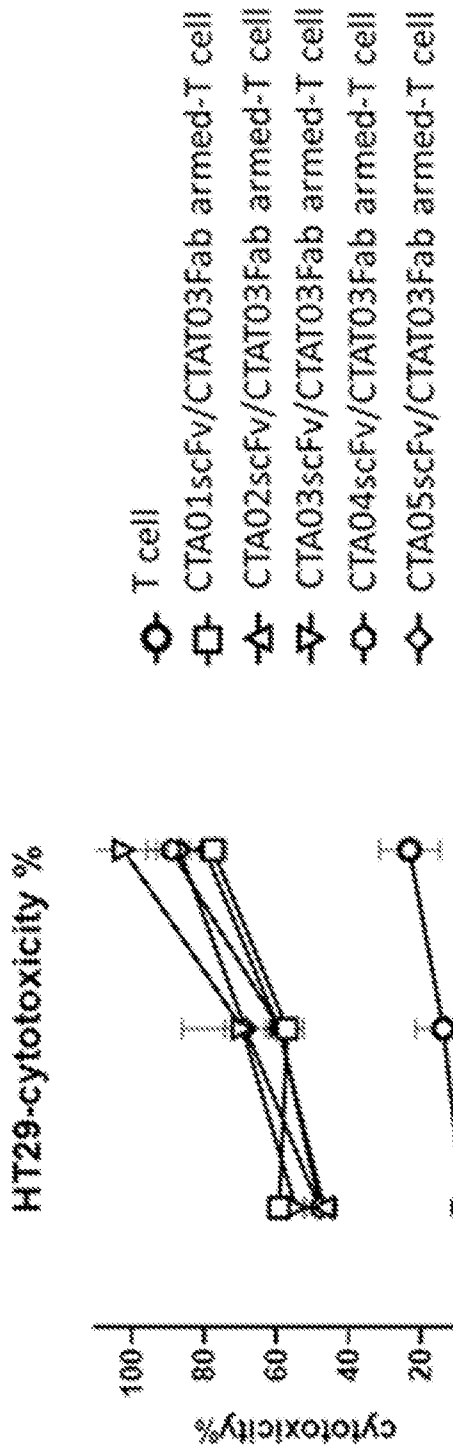

HT29-cytotoxicity %
cytotoxicity%
100
80
60
40
20
0
3:1
5:1
10:1
E/T ratio
T cell
CTA01scFv/CTAT03Fab armed-T cell
CTA02scFv/CTAT03Fab armed-T cell
CTA03scFv/CTAT03Fab armed-T cell
CTA04scFv/CTAT03Fab armed-T cell
CTA05scFv/CTAT03Fab armed-T cell

FIG. 13B

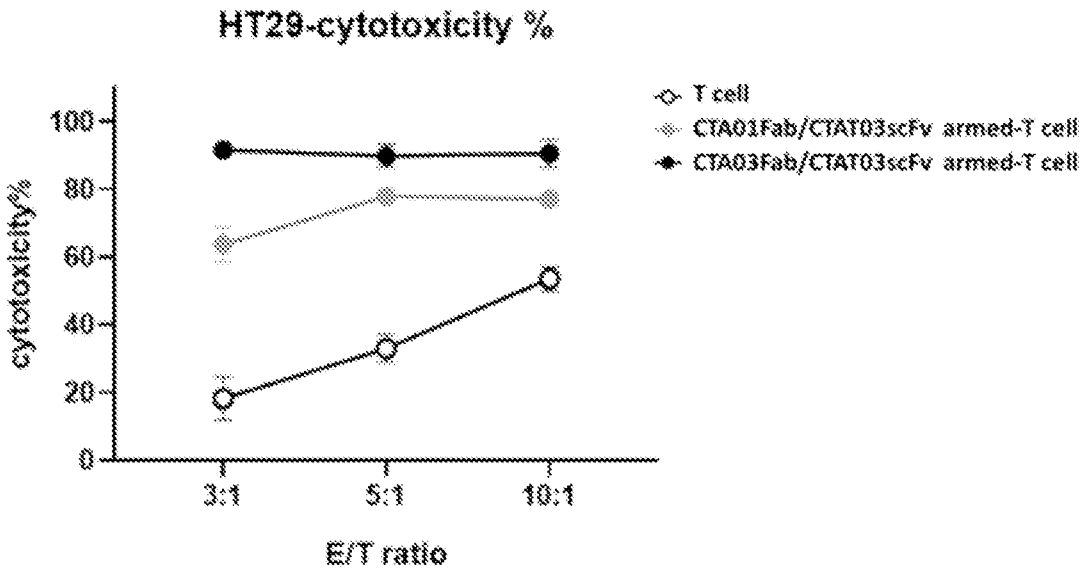


FIG. 14A

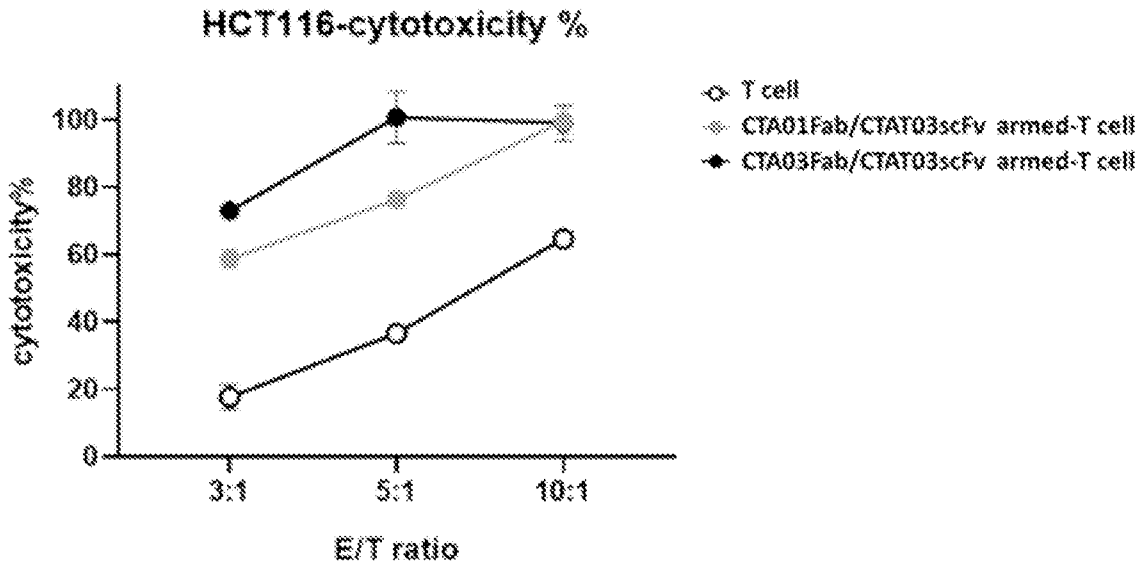


FIG. 14B

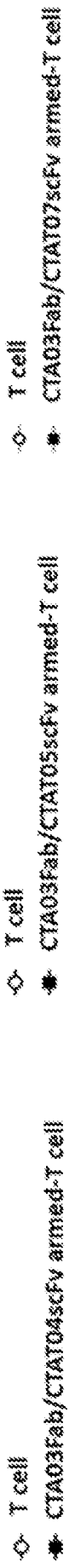


FIG. 14C

FIG. 14D

FIG. 14E

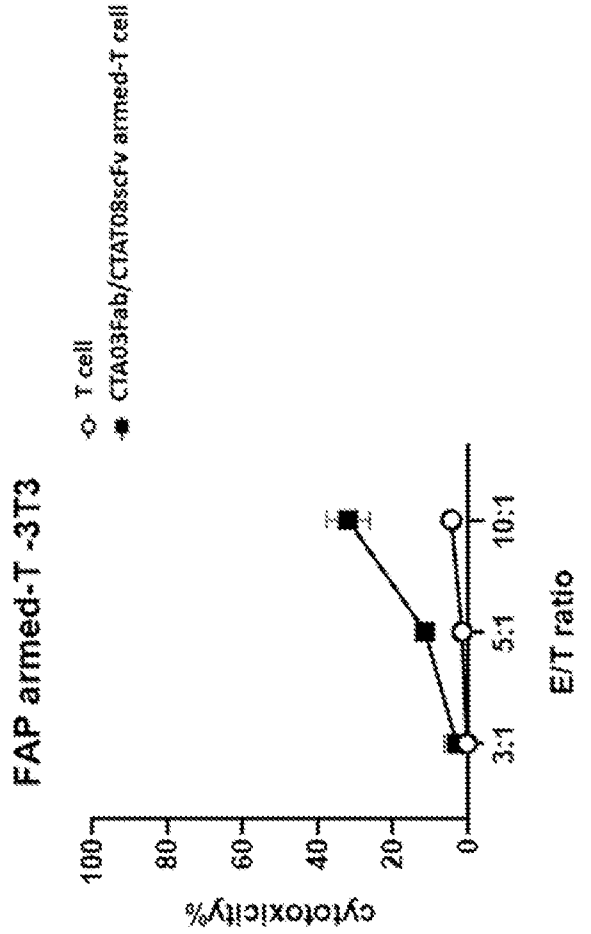


FIG. 14F

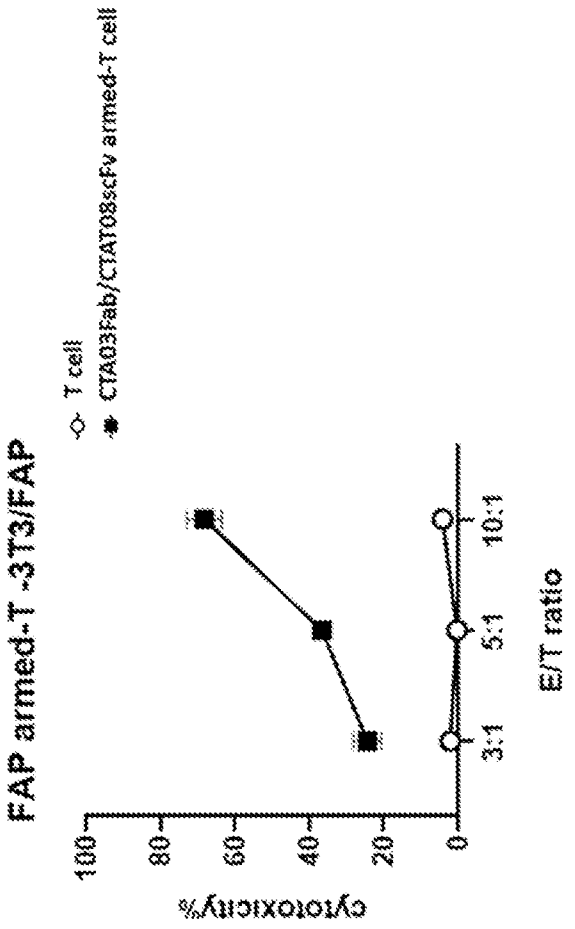


FIG. 14G

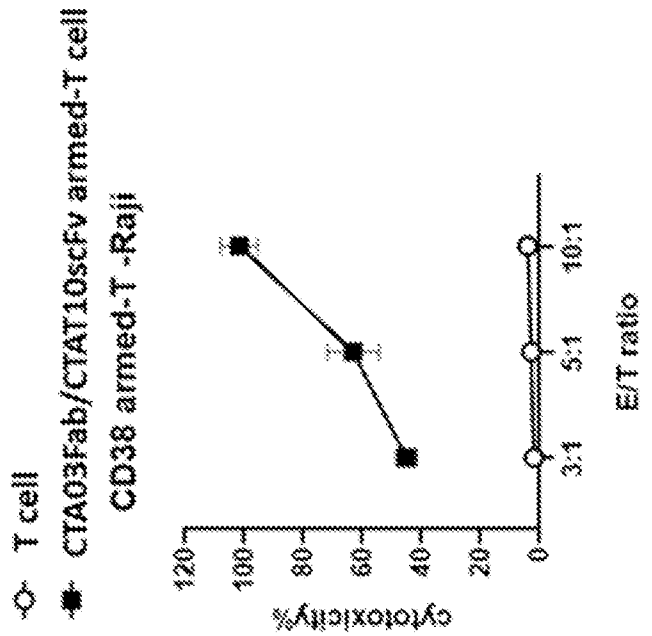


FIG. 15B

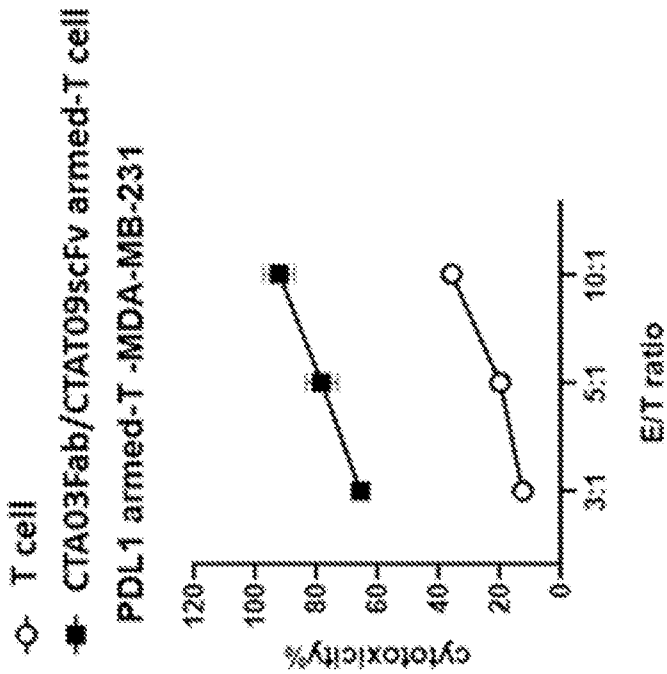


FIG. 15A

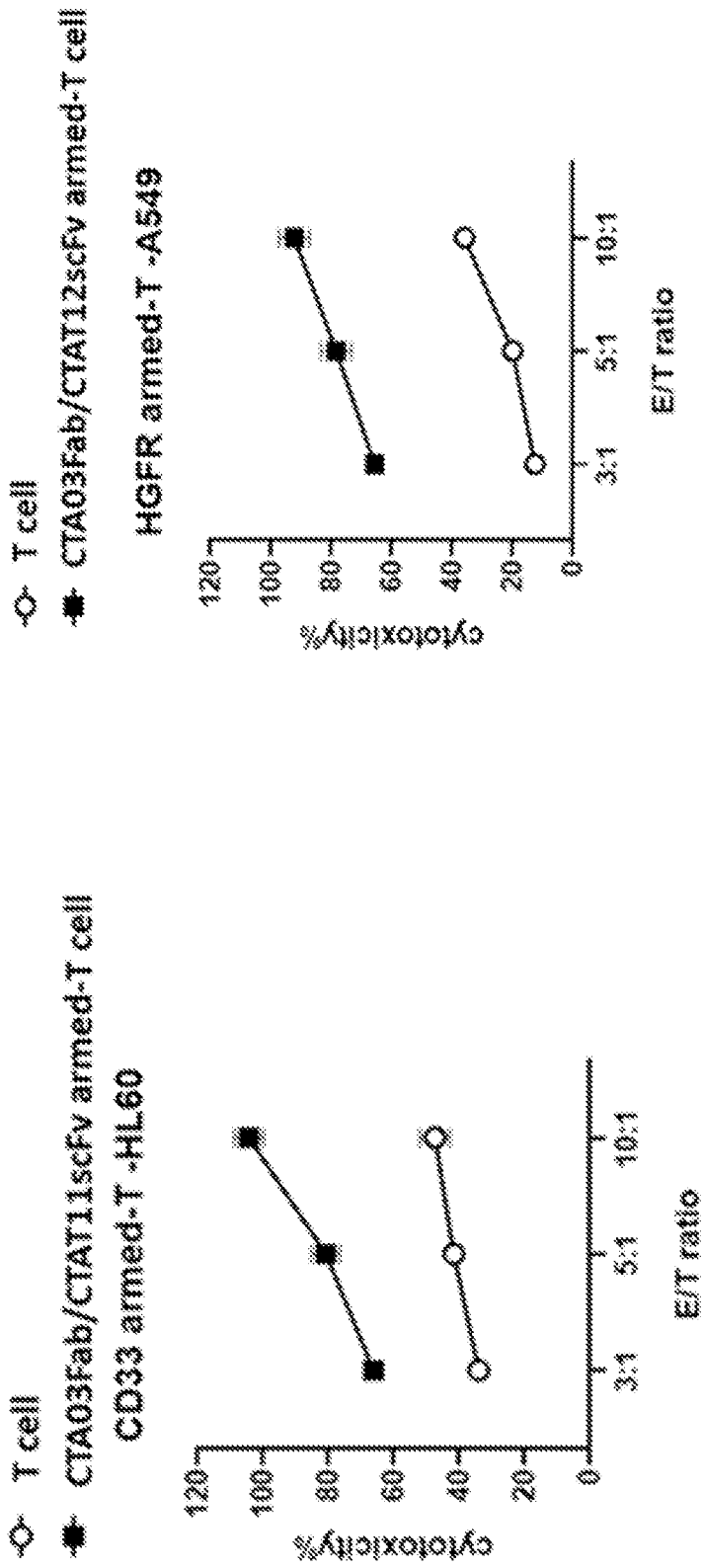


FIG. 15C

FIG. 15D

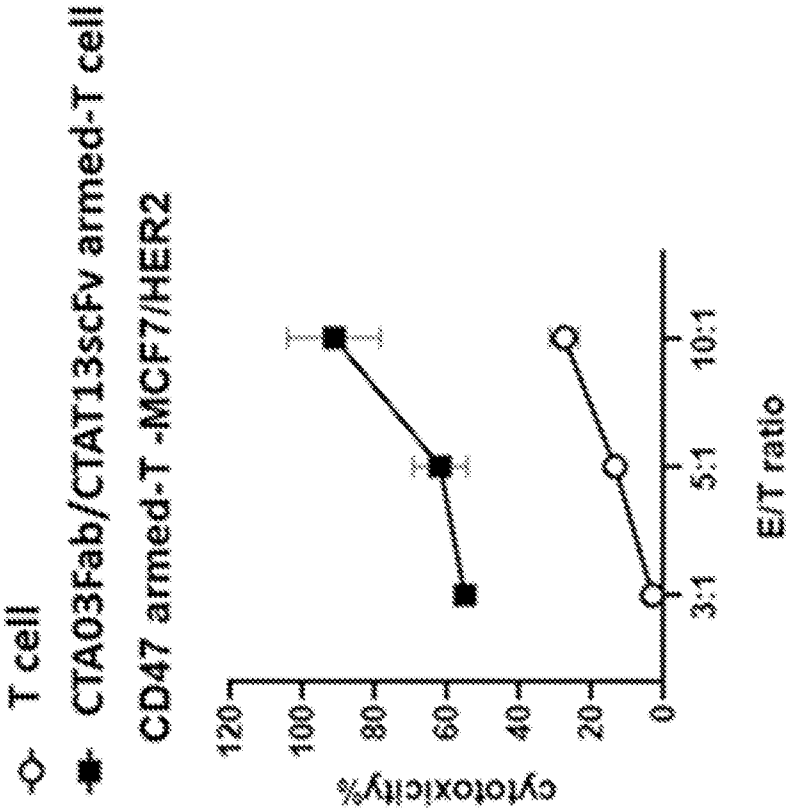

T cell
CTA03Fab/CTAT13scFv armed-T cell
CD47 armed-T -MCF7/HER2
cytotoxicity%
120
100
80
60
40
20
0
3:1
5:1
10:1
E/T ratio

FIG. 15E

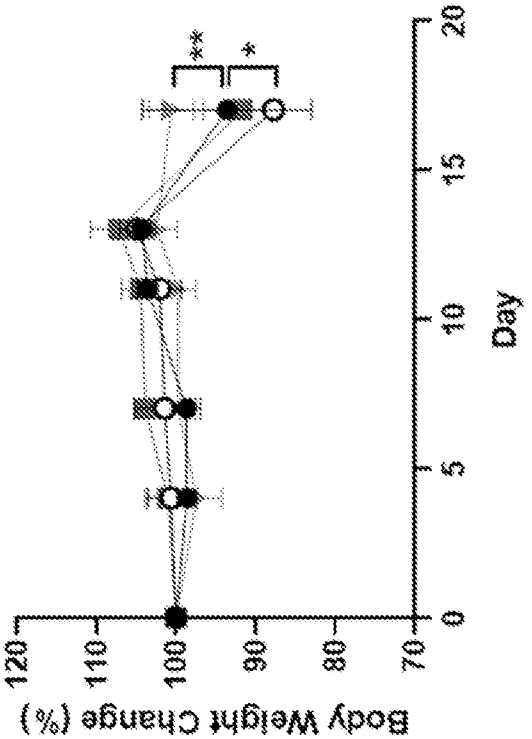


FIG. 16A

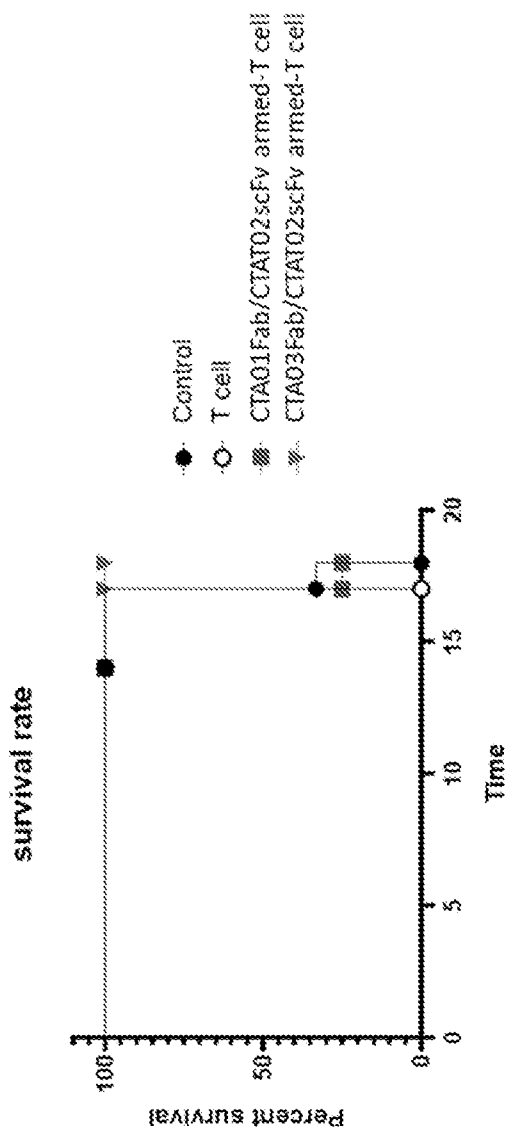


FIG. 16B

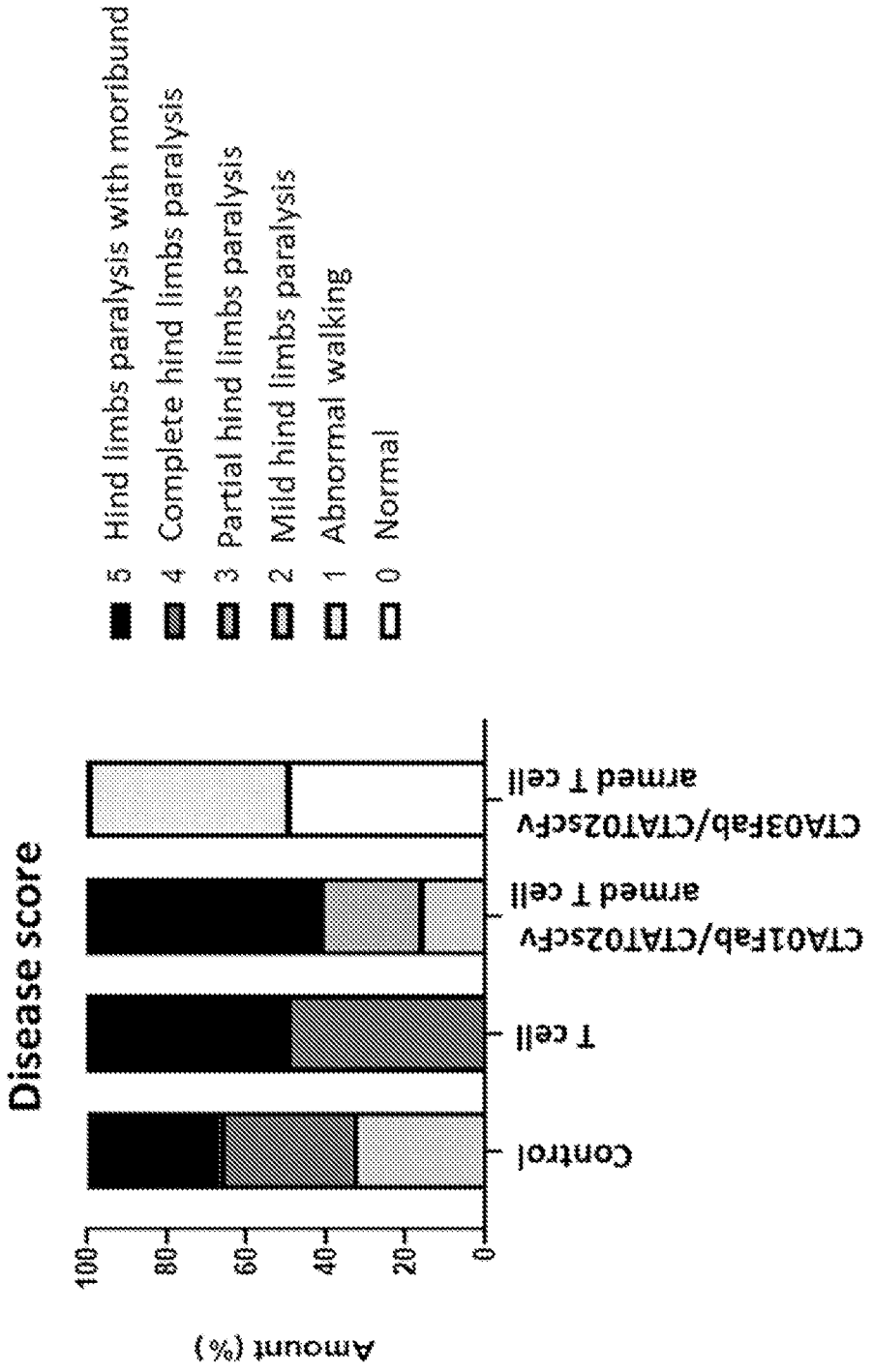

Disease score
5 Hind limbs paralysis with moribund
4 Complete hind limbs paralysis
3 Partial hind limbs paralysis
2 Mild hind limbs paralysis
1 Abnormal walking
0 Normal
Amount (%)
100
80
60
40
20
0
Control
T cell
CTA01Fab/CTAT02scFv armed T cell
CTA03Fab/CTAT02scFv armed T cell

FIG. 16C

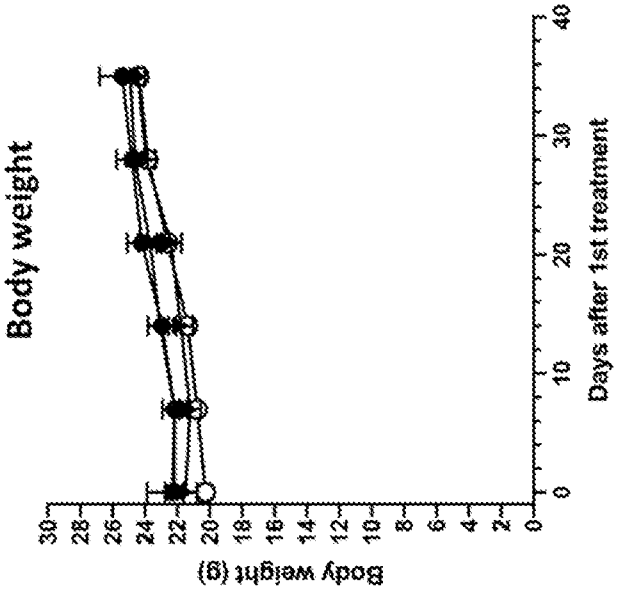


FIG. 17A

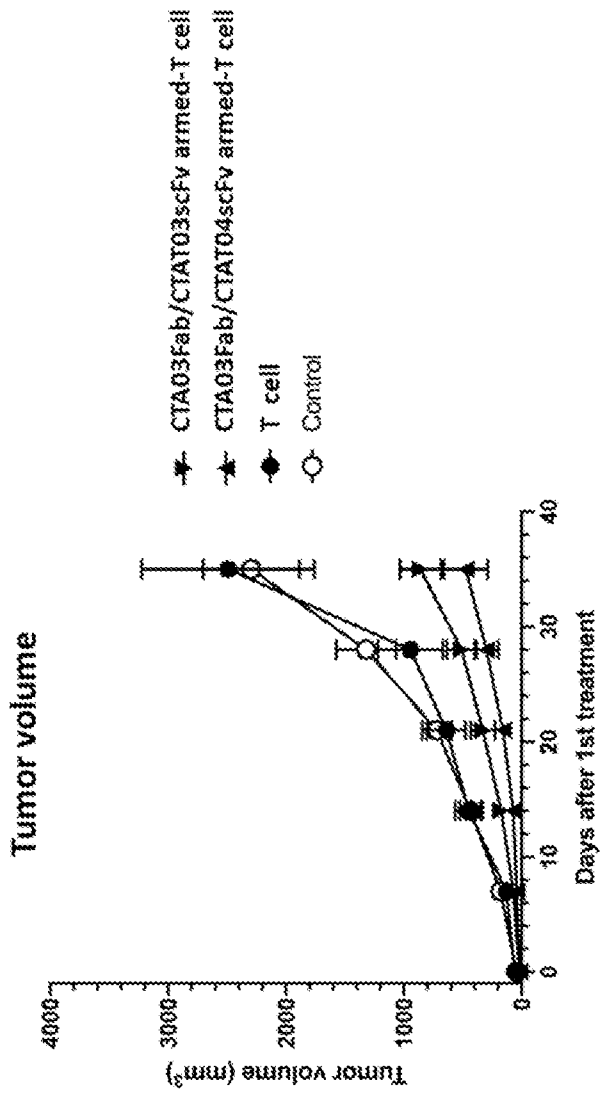


FIG. 17B

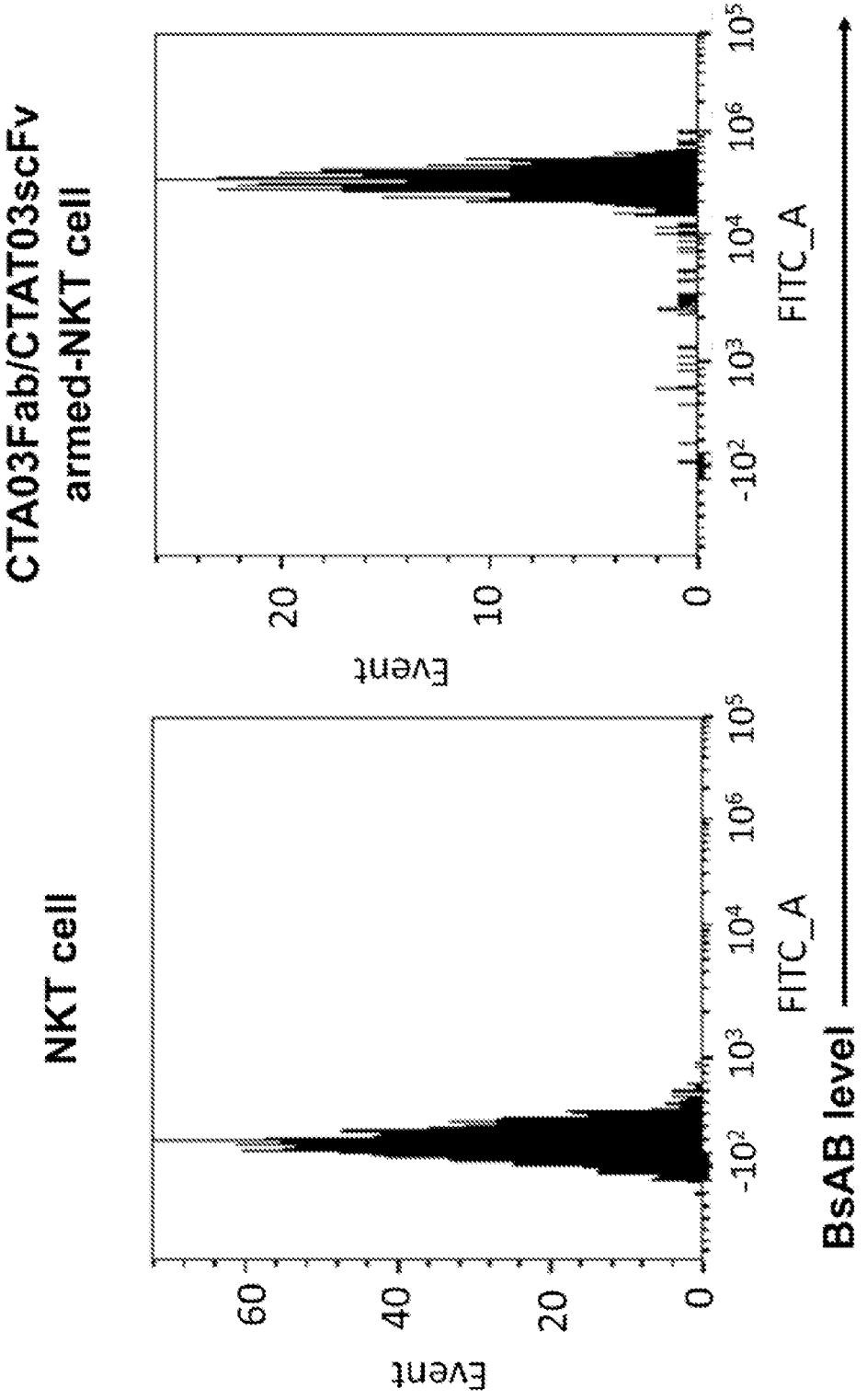

NKT cell
CTA03Fab/CTAT03scFv
armed-NKT cell
Event
FITC_A
BsAB level
0
20
40
60
10
-10²
10³
10⁴
10⁵
10⁶

FIG. 18A

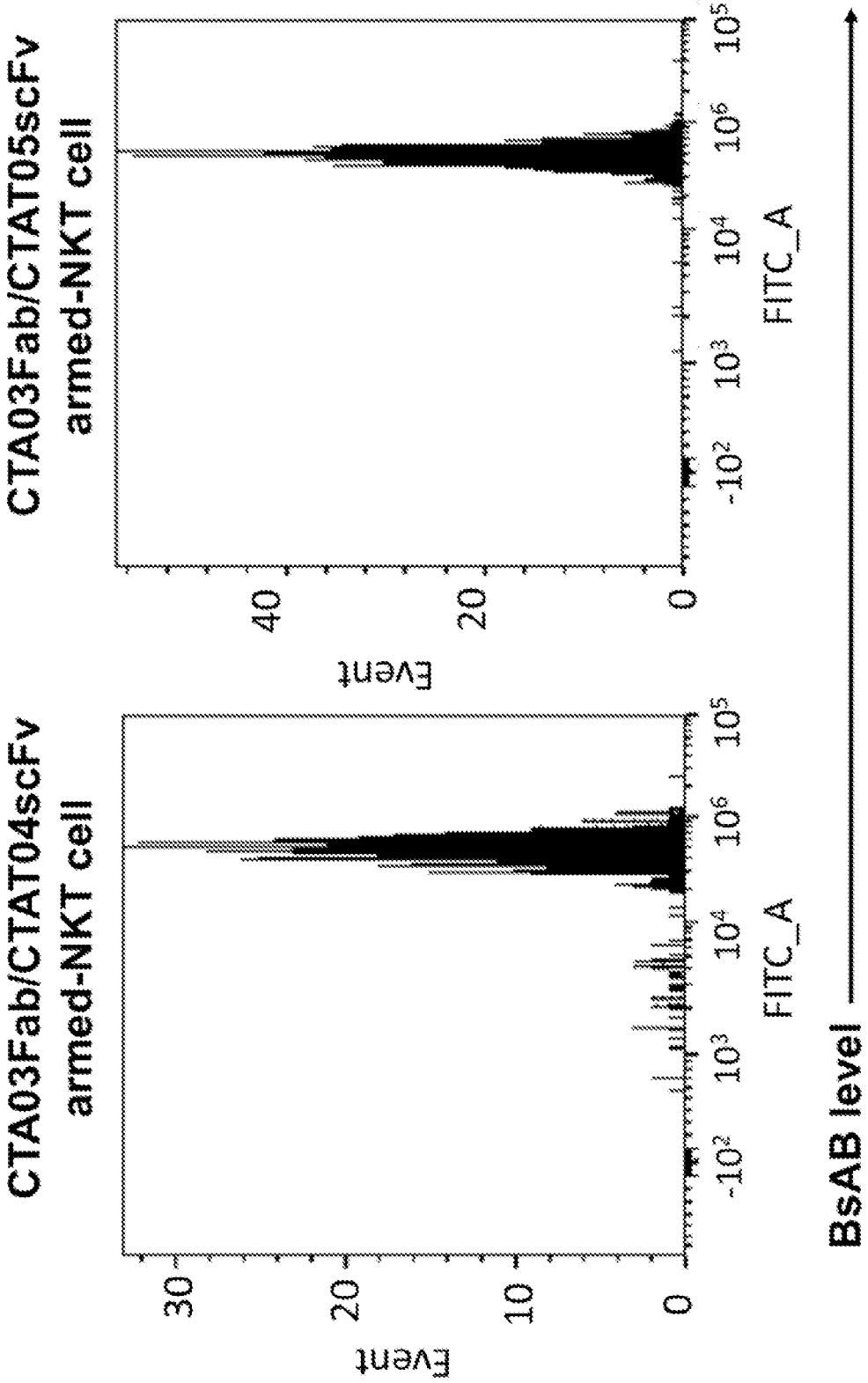

CTA03Fab/CTAT04scFv
armed-NKT cell
CTA03Fab/CTAT05scFv
armed-NKT cell
Event
FITC_A
-10²
10³
10⁴
10⁵
10⁶
0
10
20
30
40
BsAB level

FIG. 18B

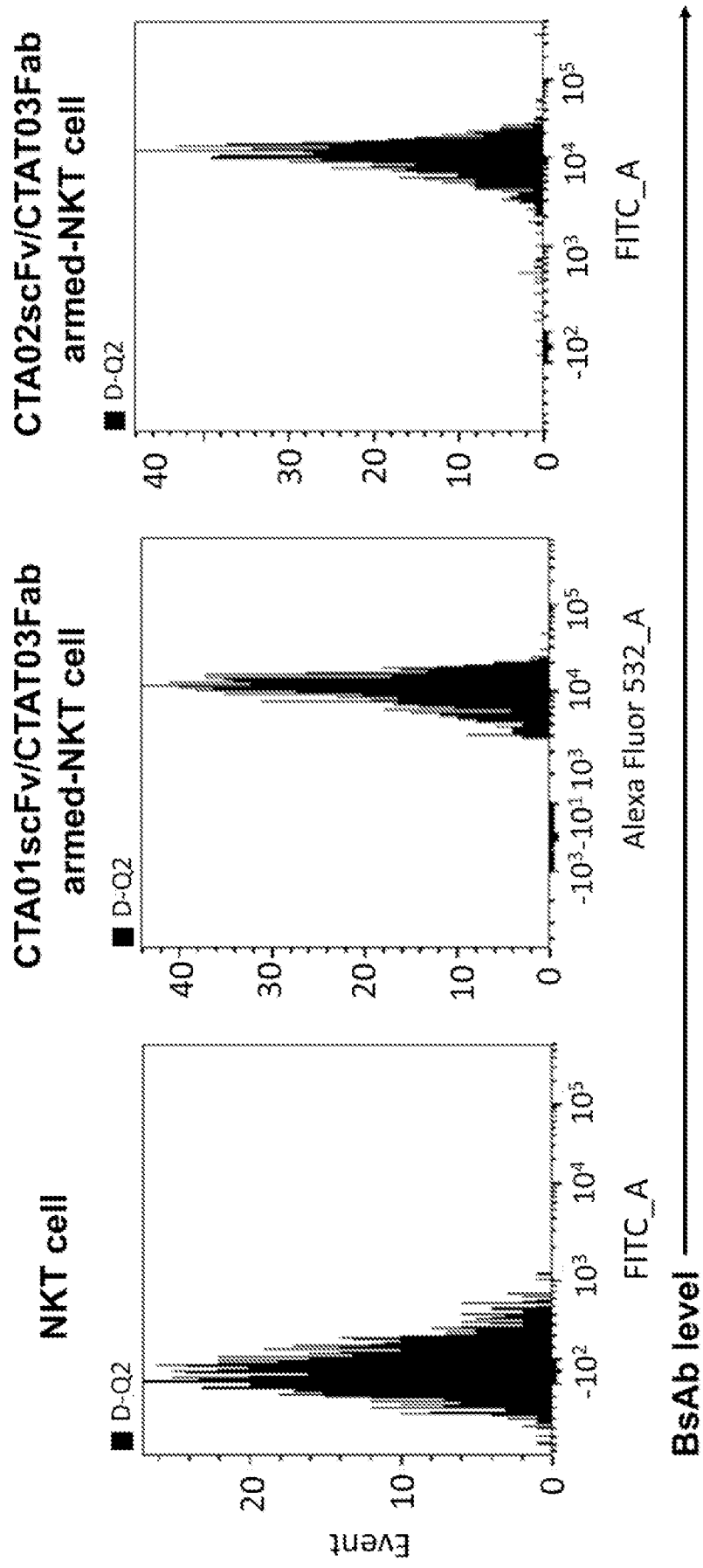

NKT cell
CTA01scFv/CTAT03Fab armed-NKT cell
CTA02scFv/CTAT03Fab armed-NKT cell
D-Q2
Event
FITC_A
Alexa Fluor 532_A
BsAb level

FIG. 18C

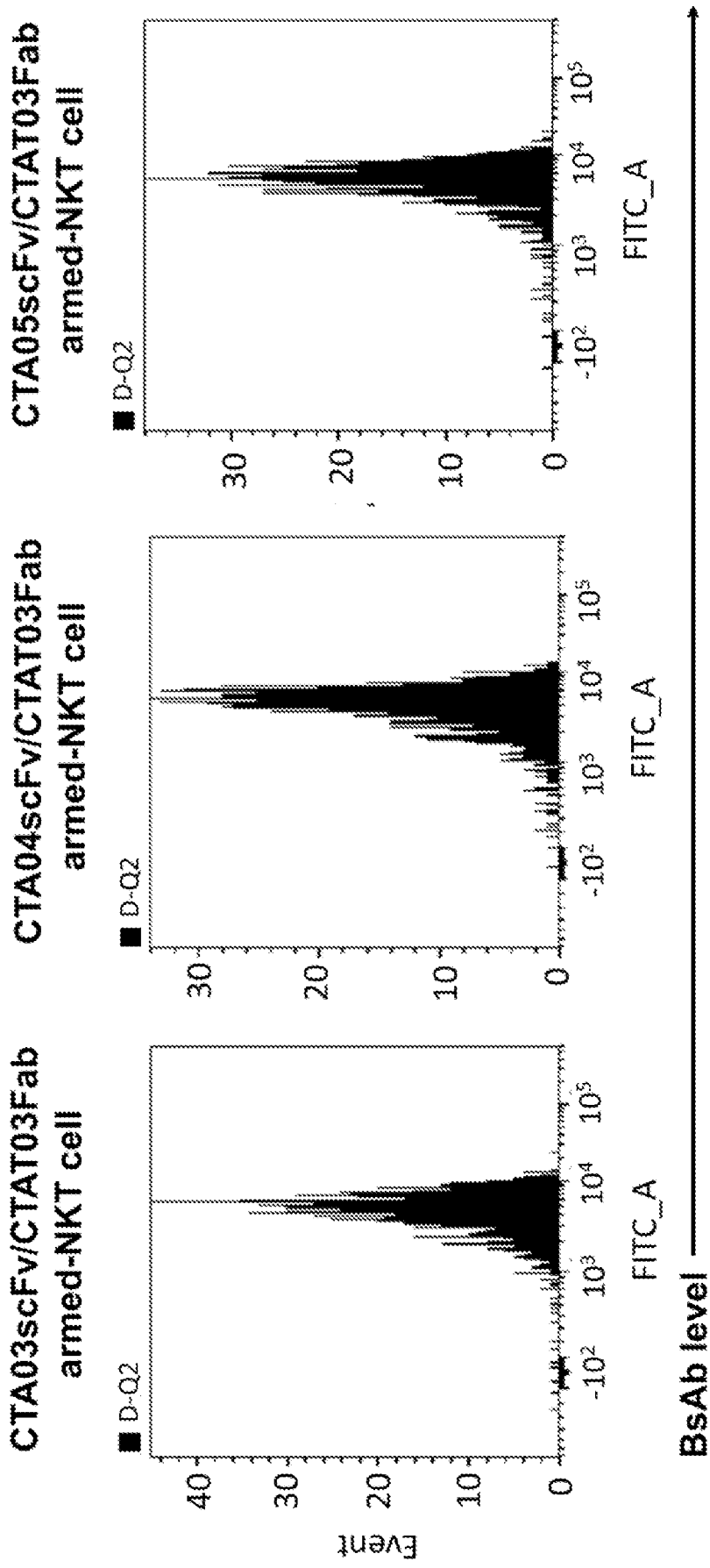

CTA03scFv/CTAT03Fab armed-NKT cell
CTA04scFv/CTAT03Fab armed-NKT cell
CTA05scFv/CTAT03Fab armed-NKT cell
D-Q2
Event
FITC_A
BsAb level

FIG. 18D

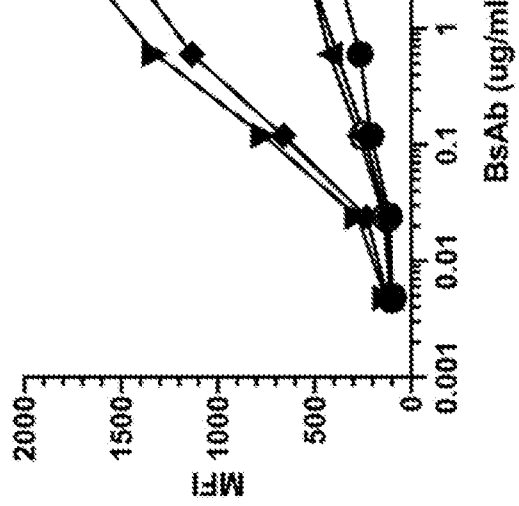


FIG. 19A

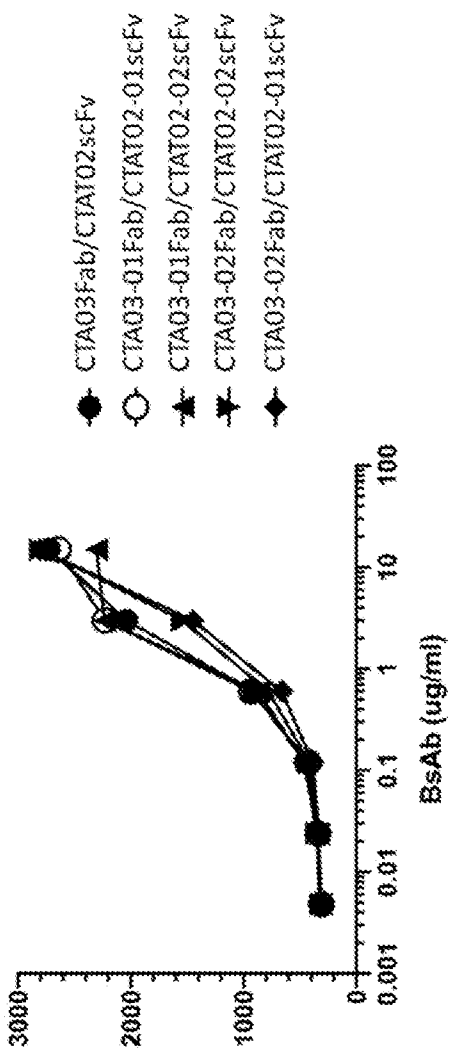


FIG. 19B

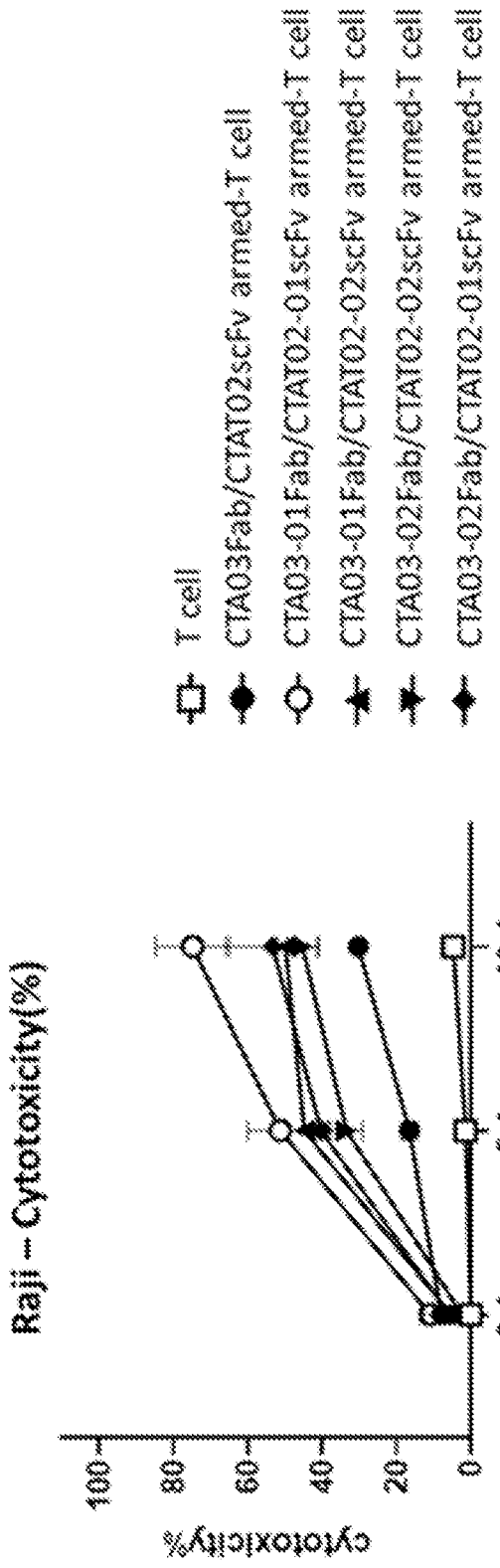

Raji – Cytotoxicity(%)
cytotoxicity%
100
80
60
40
20
0
3:1
5:1
10:1
E/T ratio
T cell
CTA03Fab/CTAT02scFv armed-T cell
CTA03-01Fab/CTAT02-01scFv armed-T cell
CTA03-01Fab/CTAT02-02scFv armed-T cell
CTA03-02Fab/CTAT02-02scFv armed-T cell
CTA03-02Fab/CTAT02-01scFv armed-T cell

FIG. 19C