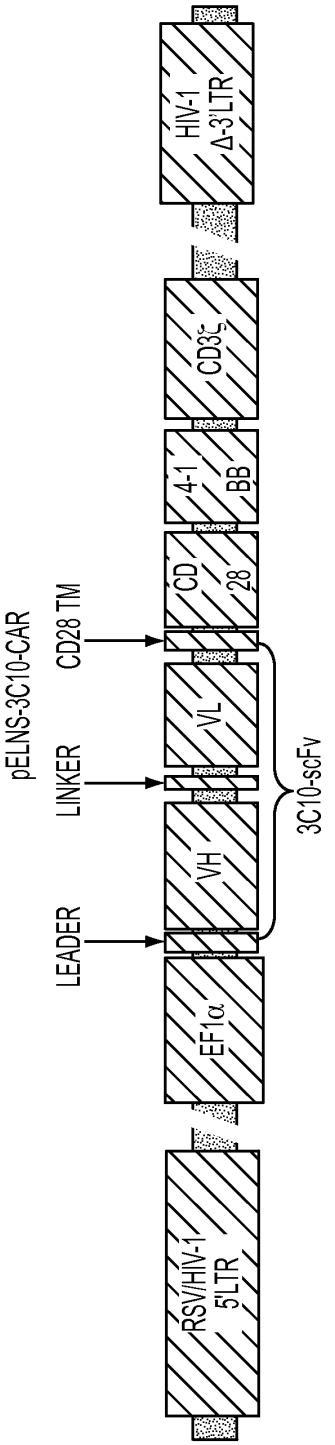

pELNS-3C10-CAR
CD28 TM
LINKER
LEADER
RSV/HIV-1 5'LTR
EF1α
V/H
V/L
3C10-scFv
CD 28
4-1 BB
CD3ζ
HIV-1 Δ-3'LTR

FIG. 1A

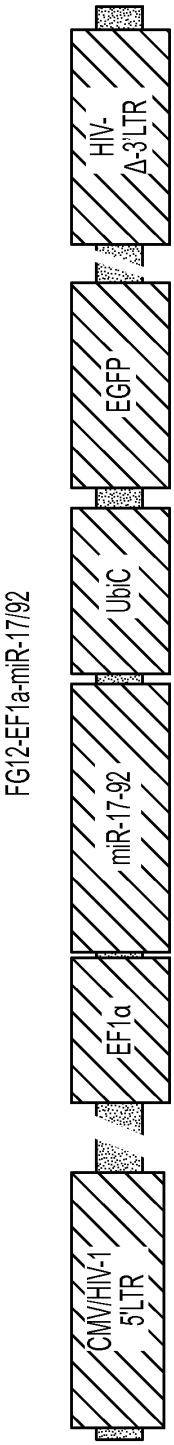

FG12-EF1a-miR-17/92
CMV/HIV-1 5'LTR
EF1α
miR-17-92
UbiC
EGFP
HIV- Δ-3'LTR

FIG. 1B

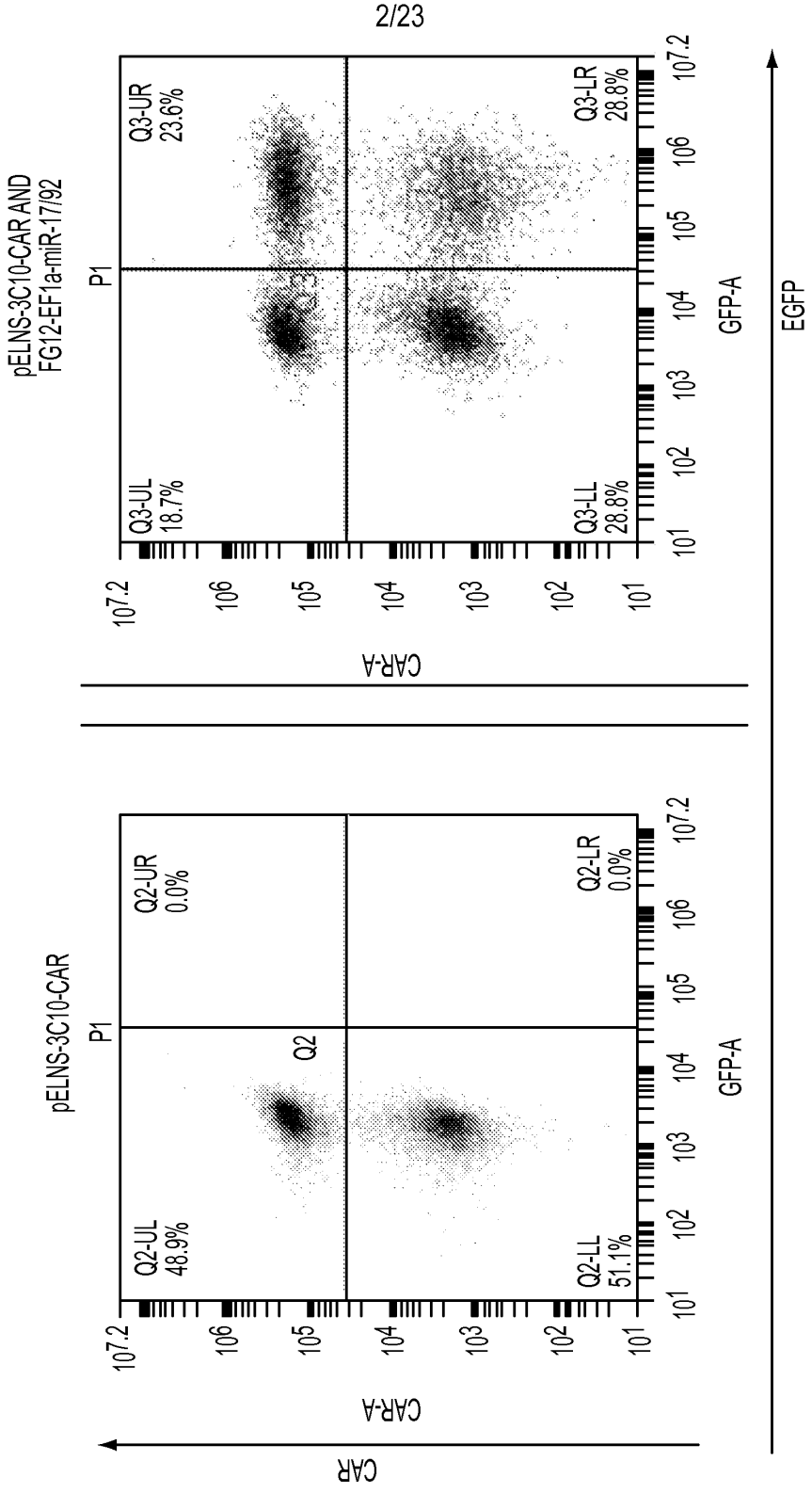

pELNS-3C10-CAR AND
FG12-EF1a-miR-17/92
P1
Q3-UR
23.6%
Q3-LR
28.8%
Q3-UL
18.7%
Q3-LL
28.8%
CAR-A
GFP-A
EGFP
pELNS-3C10-CAR
P1
Q2-UR
0.0%
Q2-LR
0.0%
Q2
Q2-UL
48.9%
Q2-LL
51.1%
CAR-A
GFP-A
CAR

FIG. 2A

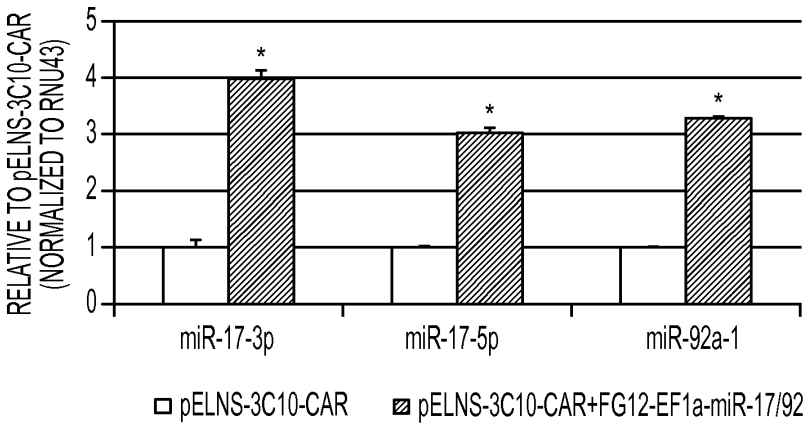

RELATIVE TO pELNS-3C10-CAR (NORMALIZED TO RNU43)
5
4
3
2
1
0
*
*
*
miR-17-3p
miR-17-5p
miR-92a-1
pELNS-3C10-CAR
pELNS-3C10-CAR+FG12-EF1a-miR-17/92

FIG. 2B

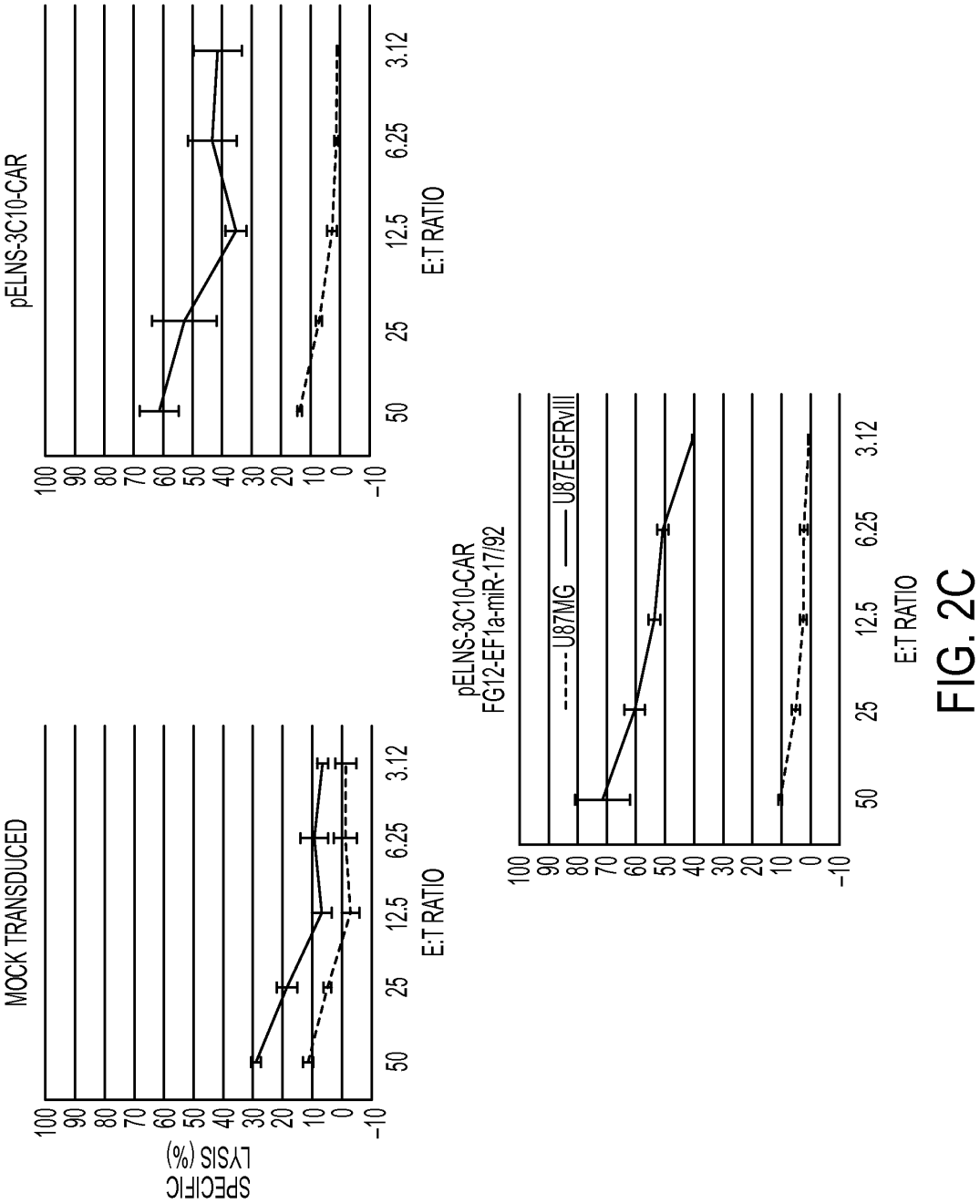

MOCK TRANSDUCED
pELNS-3C10-CAR
pELNS-3C10-CAR
FG12-EF1a-miR-17/92
U87MG
U87EGFRvIII
SPECIFIC LYSIS (%)
E:T RATIO
100
90
80
70
60
50
40
30
20
10
0
-10
50
25
12.5
6.25
3.12

FIG. 2C

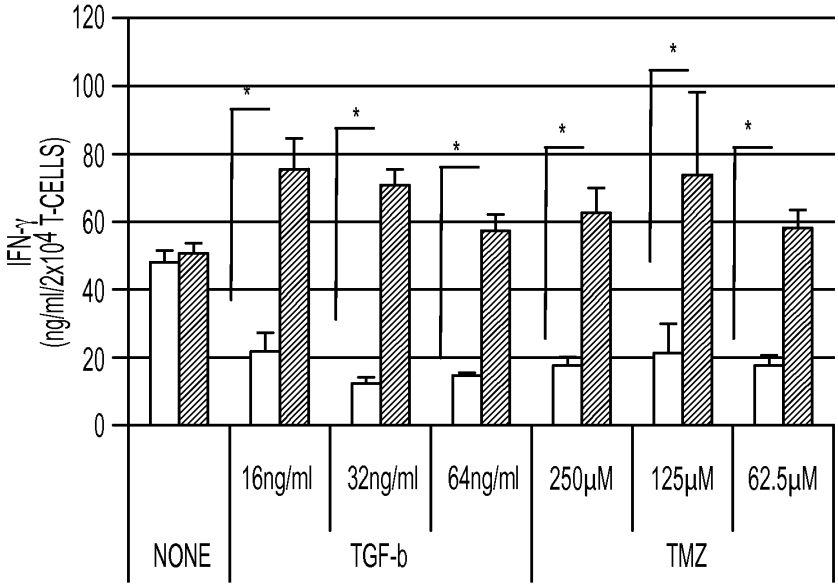

IFN-γ
(ng/ml/2x10^4 T-CELLS)
120
100
80
60
40
20
0
*
NONE
16ng/ml
32ng/ml
64ng/ml
TGF-b
250μM
125μM
62.5μM
TMZ

FIG. 3A

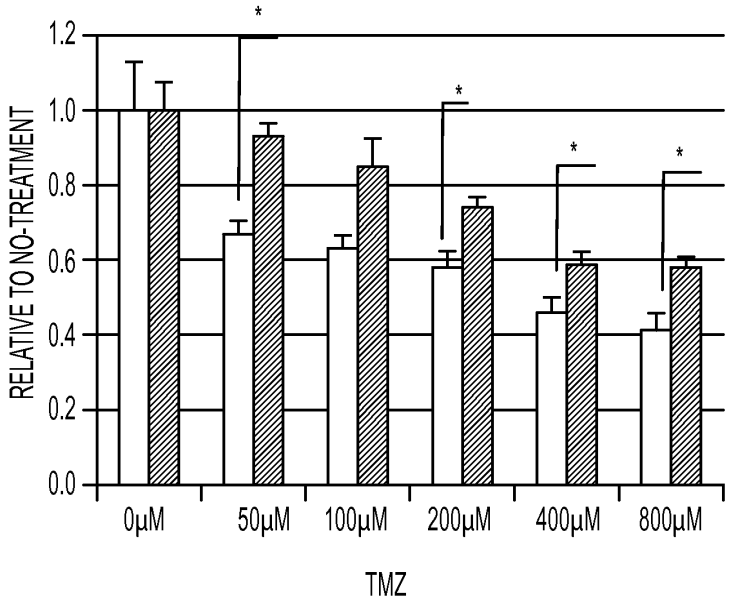

RELATIVE TO NO-TREATMENT
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
0μM
50μM
100μM
200μM
400μM
800μM
TMZ

FIG. 3B

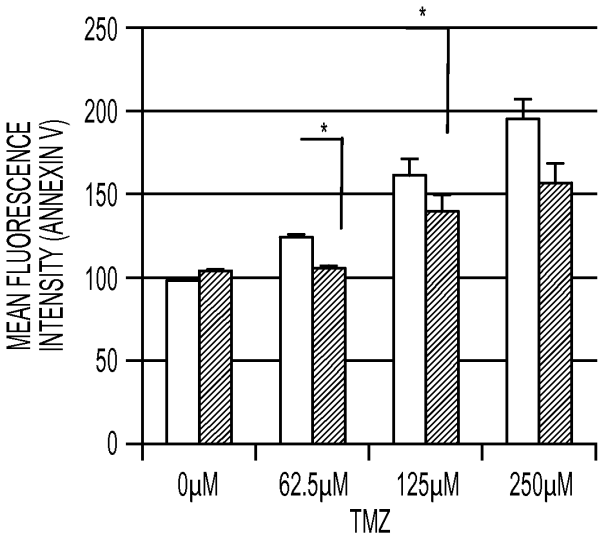

MEAN FLUORESCENCE INTENSITY (ANNEXIN V)
250
200
150
100
50
0
*
*
0μM
62.5μM
125μM
250μM
TMZ

FIG. 3C

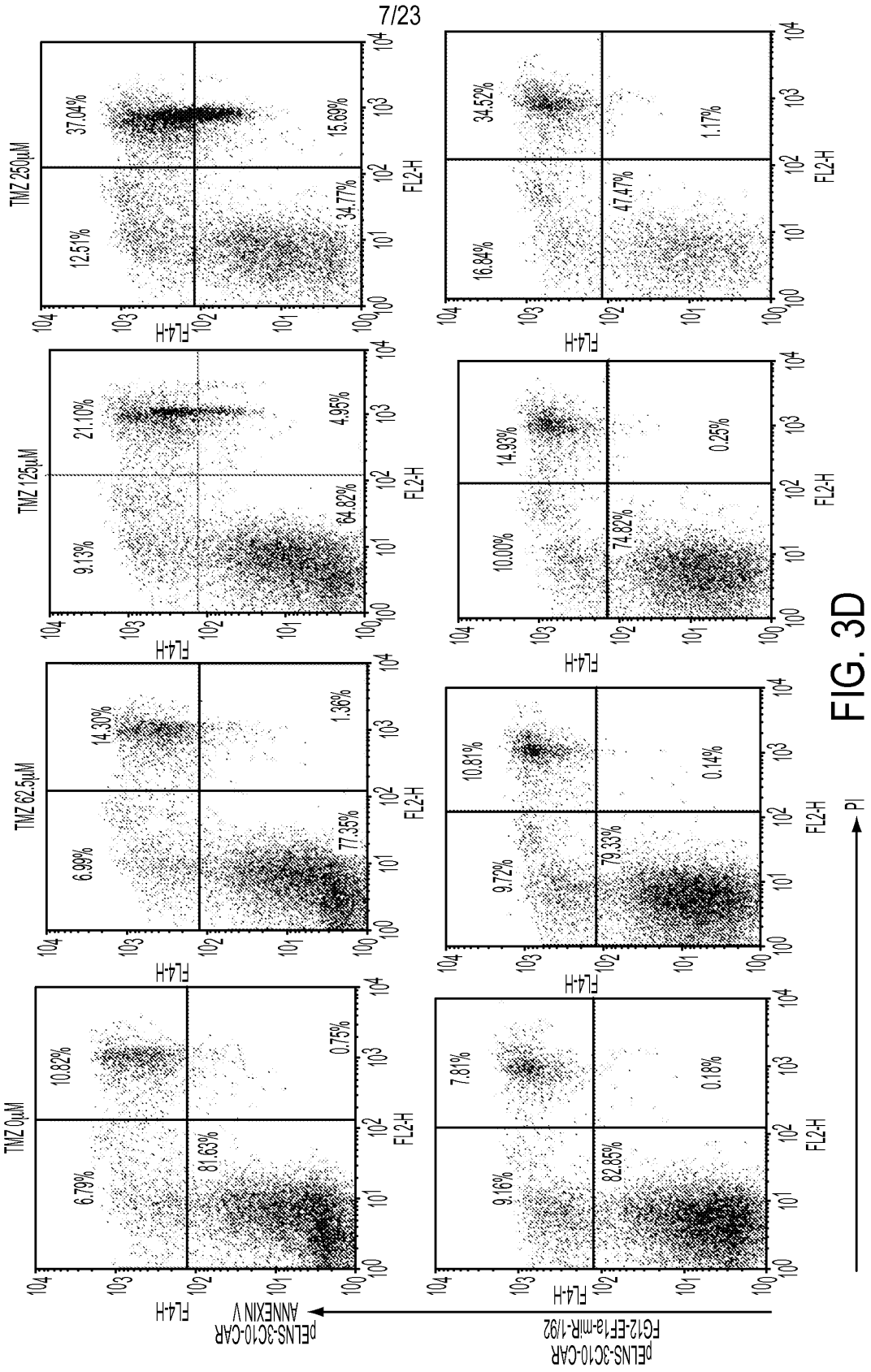

TMZ 0μM
TMZ 62.5μM
TMZ 125μM
TMZ 250μM
6.79%
10.82%
81.63%
0.75%
6.99%
14.30%
77.35%
1.36%
9.13%
21.10%
64.82%
4.95%
12.51%
37.04%
34.77%
15.69%
9.16%
7.81%
82.85%
0.18%
9.72%
10.81%
79.33%
0.14%
10.00%
14.93%
74.82%
0.25%
16.84%
34.52%
47.47%
1.17%
FL4-H
FL2-H
pELNS-3C10-CAR
ANNEXIN V
pELNS-3C10-CAR
FG12-EF1a-miR-1/92
PI

FIG. 3D

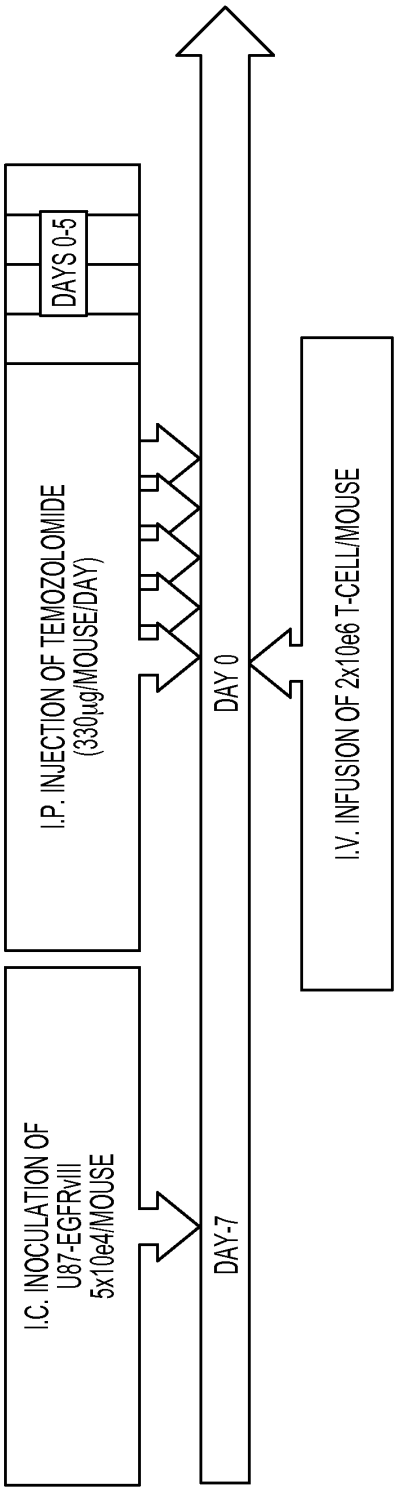

I.C. INOCULATION OF
U87-EGFRvIII
5x10e4/MOUSE
I.P. INJECTION OF TEMOZOLOMIDE
(330µg/MOUSE/DAY)
DAYS 0-5
I.V. INFUSION OF 2x10e6 T-CELL/MOUSE
DAY-7
DAY 0

FIG. 4A

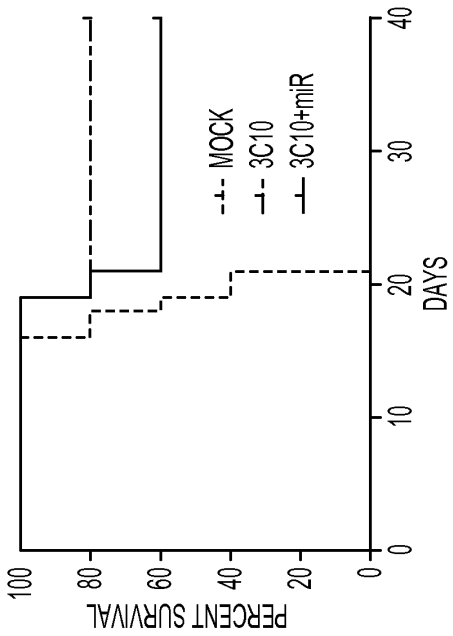

PERCENT SURVIVAL
DAYS
MOCK
3C10
3C10+miR
0
20
40
60
80
100
0
10
20
30
40

FIG. 4B

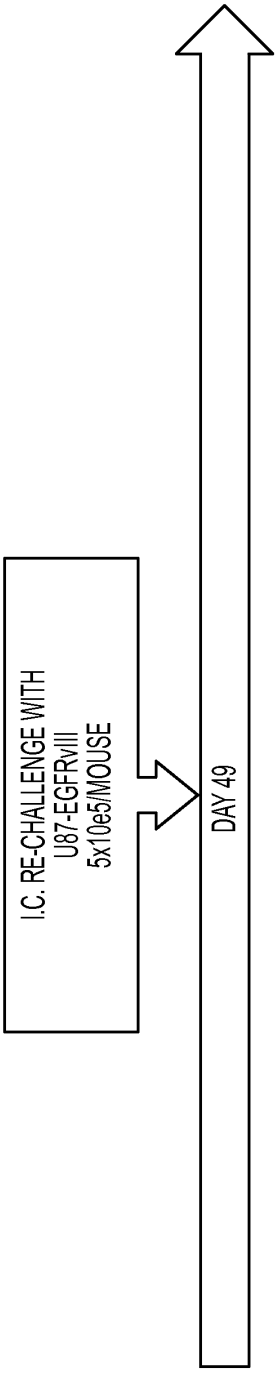


FIG. 5A

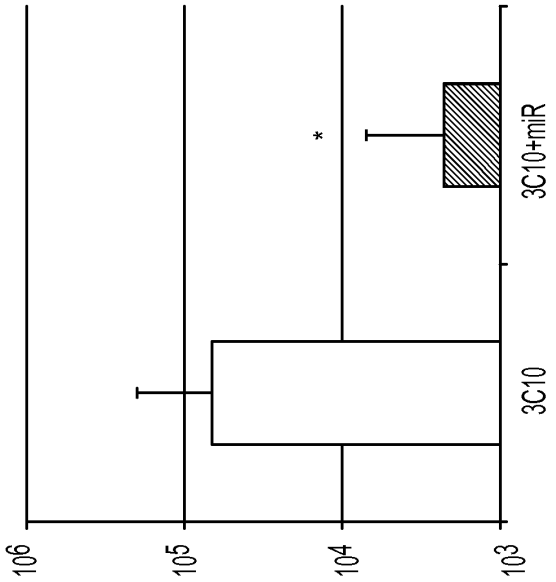


FIG. 5C

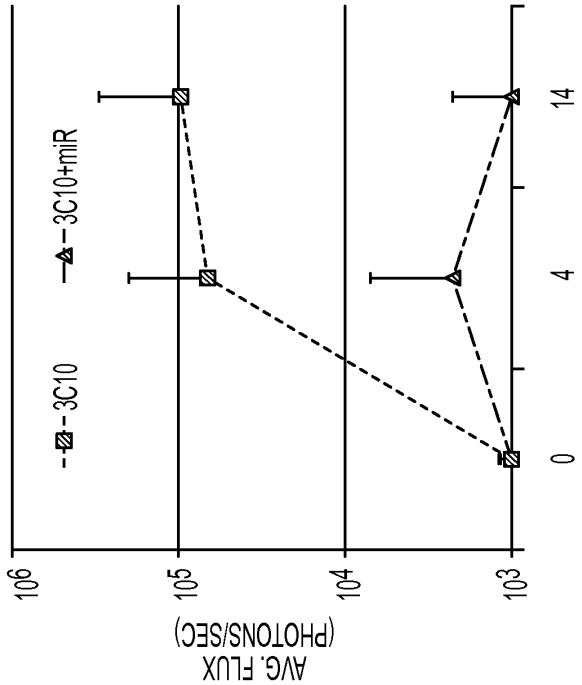


FIG. 5B

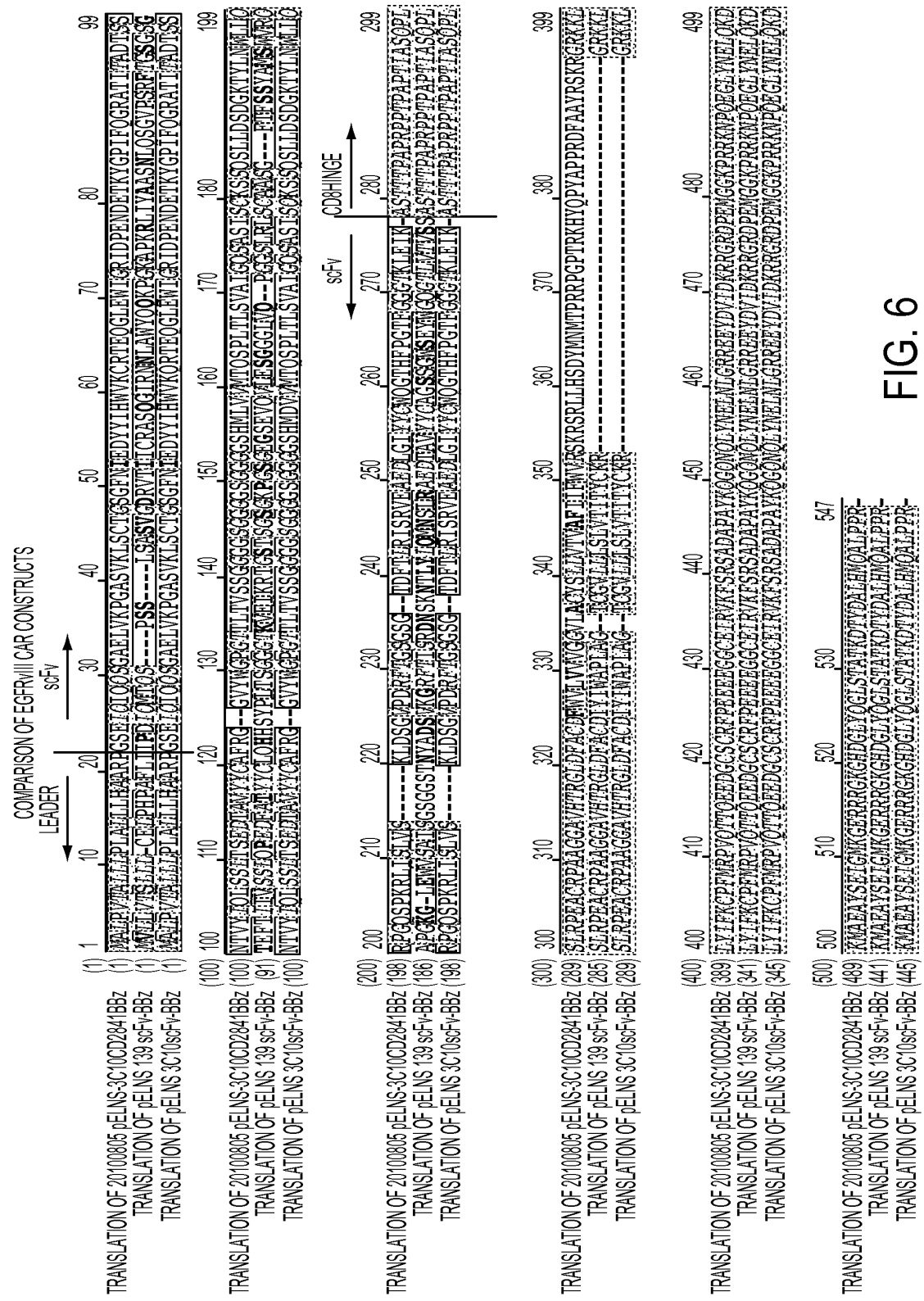

COMPARISON OF EGFRvIII CAR CONSTRUCTS
LEADER
scFv
scFv
CD8HINGE
TRANSLATION OF 20100805 pELNS-3C10CD2841BBz
TRANSLATION OF pELNS 139 scFv-BBz
TRANSLATION OF pELNS 3C10scFv-BBz

FIG. 6

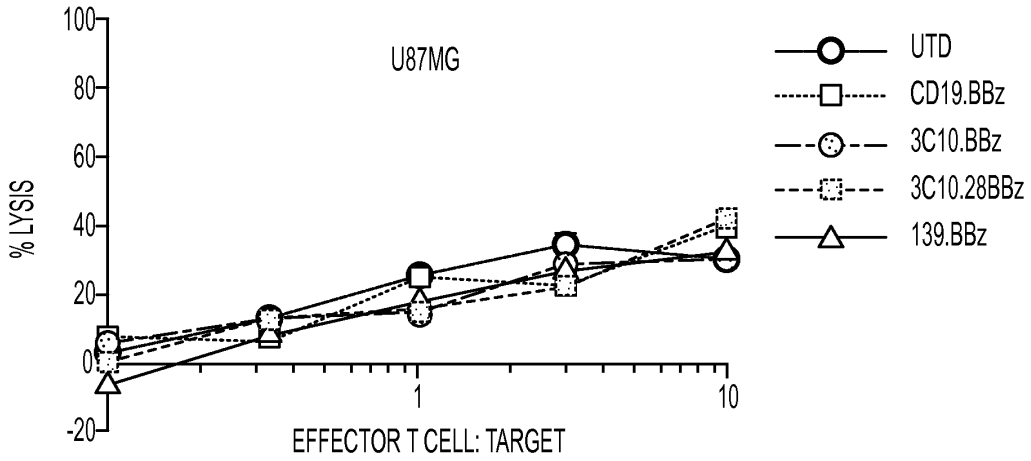

U87MG
% LYSIS
100
80
60
40
20
0
-20
1
10
EFFECTOR T CELL: TARGET
UTD
CD19.BBz
3C10.BBz
3C10.28BBz
139.BBz

FIG. 7A

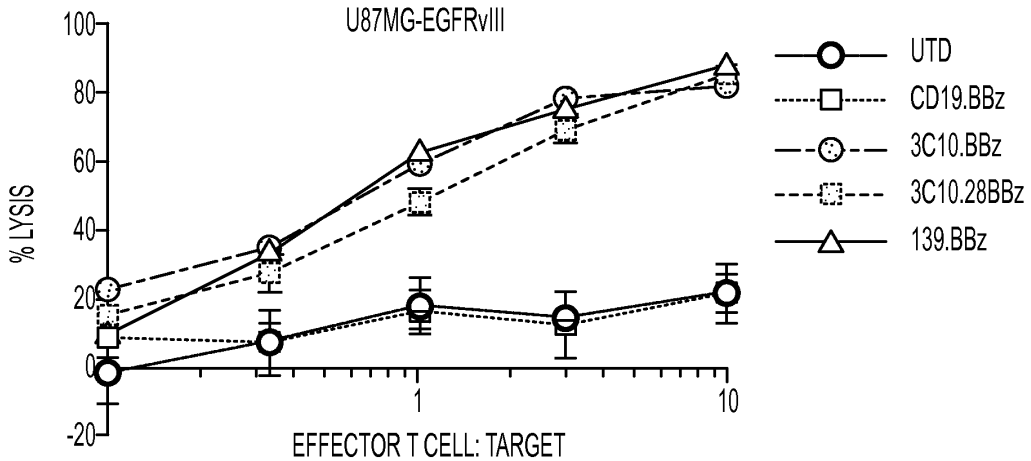

U87MG-EGFRvIII
% LYSIS
100
80
60
40
20
0
-20
1
10
EFFECTOR T CELL: TARGET
UTD
CD19.BBz
3C10.BBz
3C10.28BBz
139.BBz

FIG. 7B

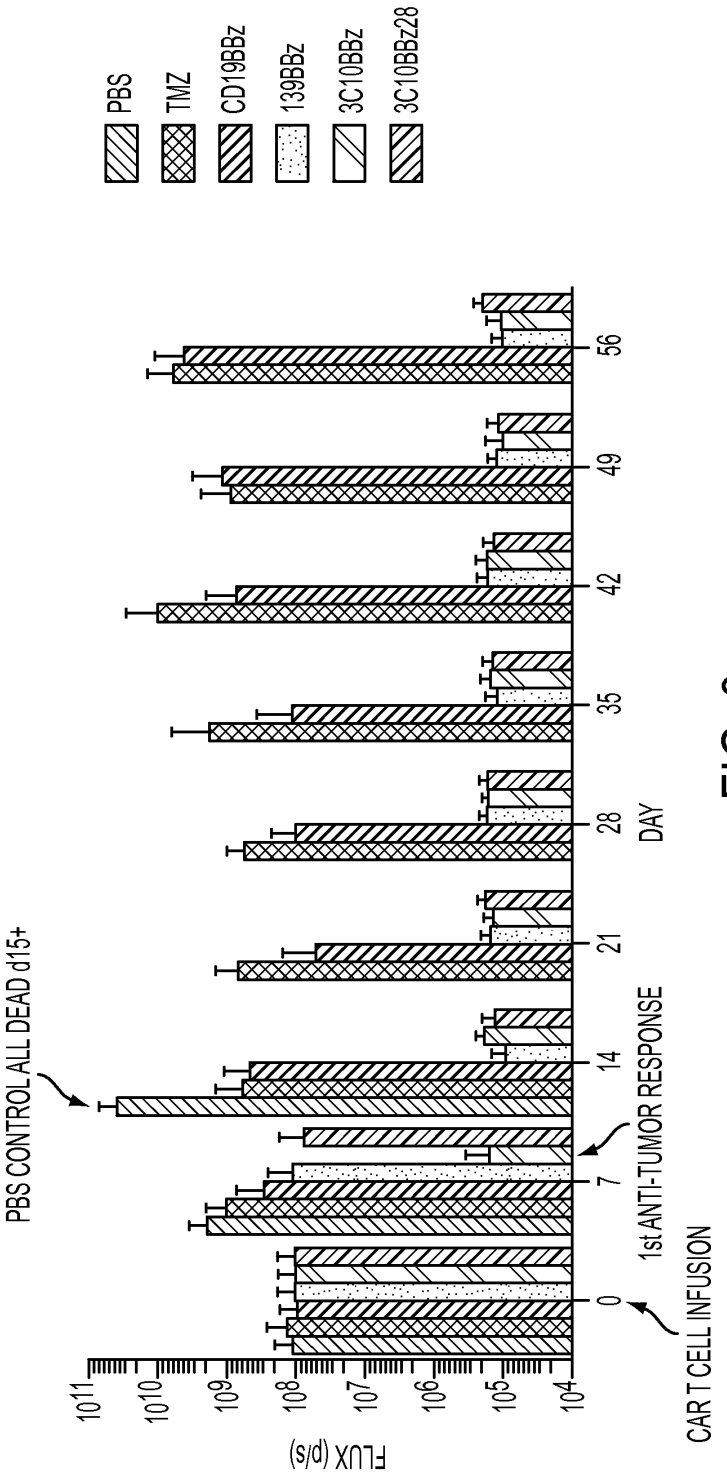

PBS
TMZ
CD19BBz
139BBz
3C10BBz
3C10BBz28
FLUX (p/s)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
0
7
14
21
28
35
42
49
56
DAY
PBS CONTROL ALL DEAD d15+
1st ANTI-TUMOR RESPONSE
CAR T CELL INFUSION

FIG. 8

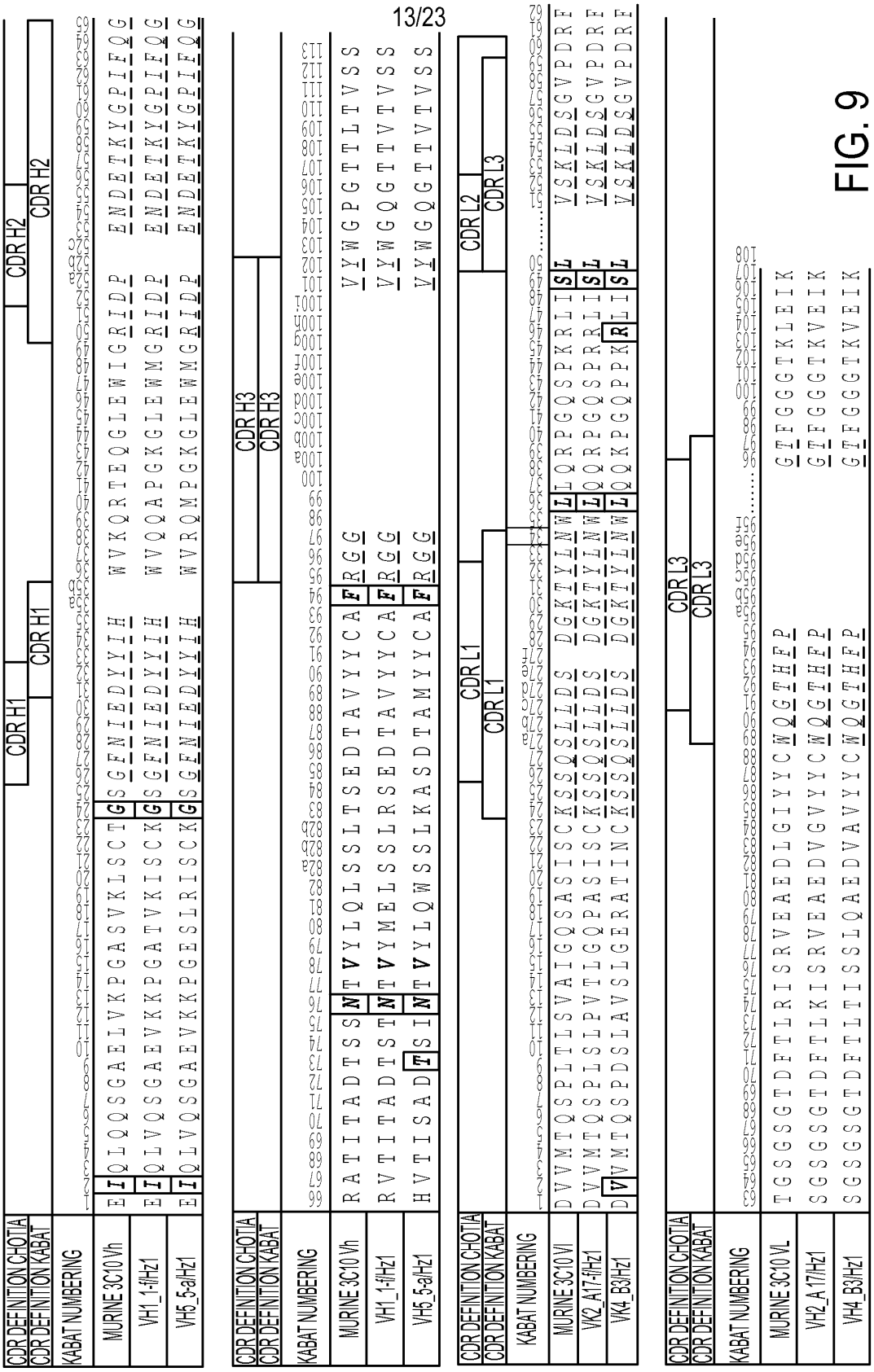

CDR DEFINITION CHOTIA
CDR DEFINITION KABAT
KABAT NUMBERING
MURINE 3C10 Vh
VH1_1-f/Hz1
VH5_5-a/Hz1
CDR H1
CDR H2
MURINE 3C10 Vl
VK2_A17-f/Hz1
VK4_B3/Hz1
CDR L1
CDR L2
CDR L3
MURINE 3C10 VL
VH2_A17/Hz1
VH4_B3/Hz1

FIG. 9

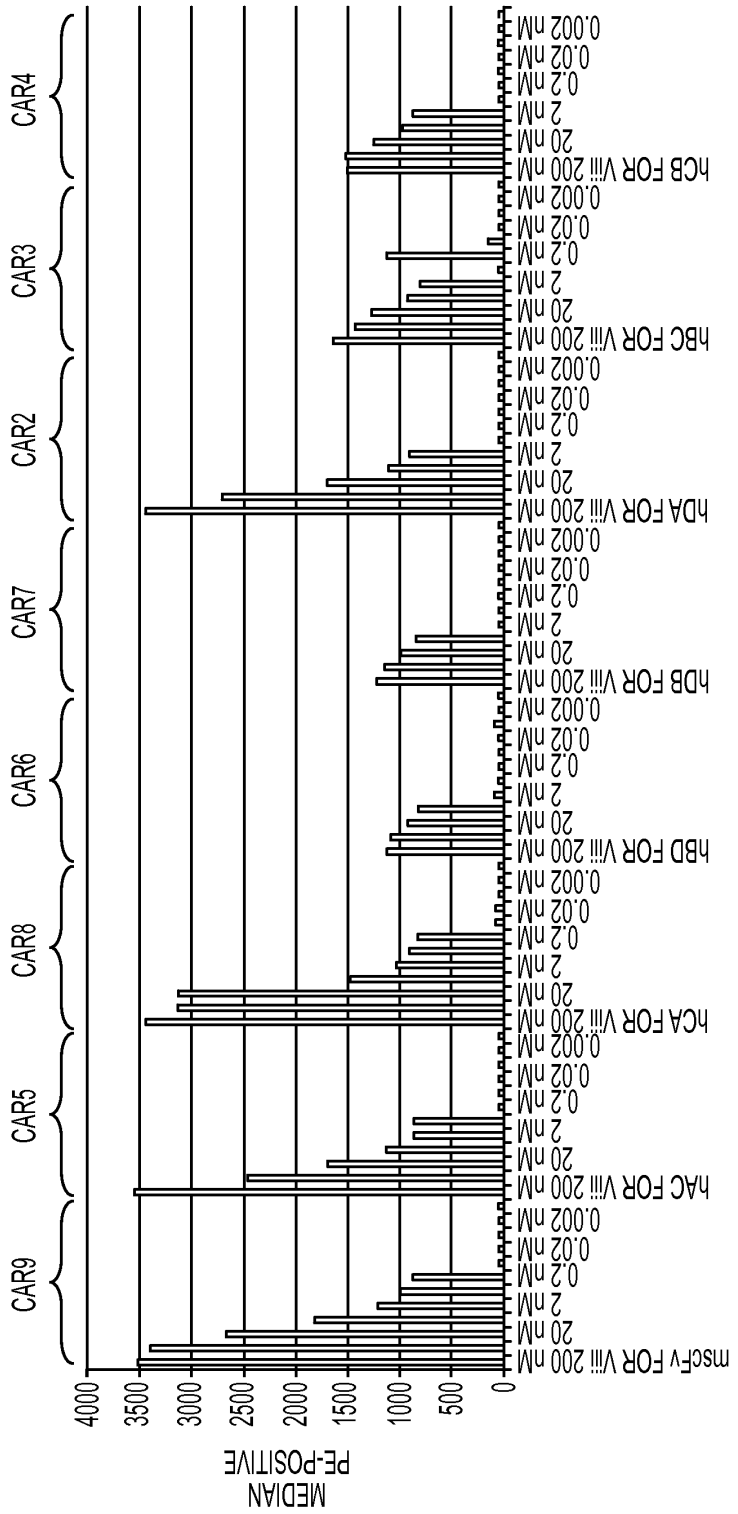

MEDIAN
PE-POSITIVE
4000
3500
3000
2500
2000
1500
1000
500
0
CAR9
CAR5
CAR8
CAR6
CAR7
CAR2
CAR3
CAR4
mscFv FOR V/iii 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM
hAC FOR V/iii 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM
hCA FOR V/iii 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM
hBD FOR V/iii 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM
hDB FOR V/iii 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM
hDA FOR V/iii 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM
hBC FOR V/iii 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM
hCB FOR V/iii 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM

FIG. 10

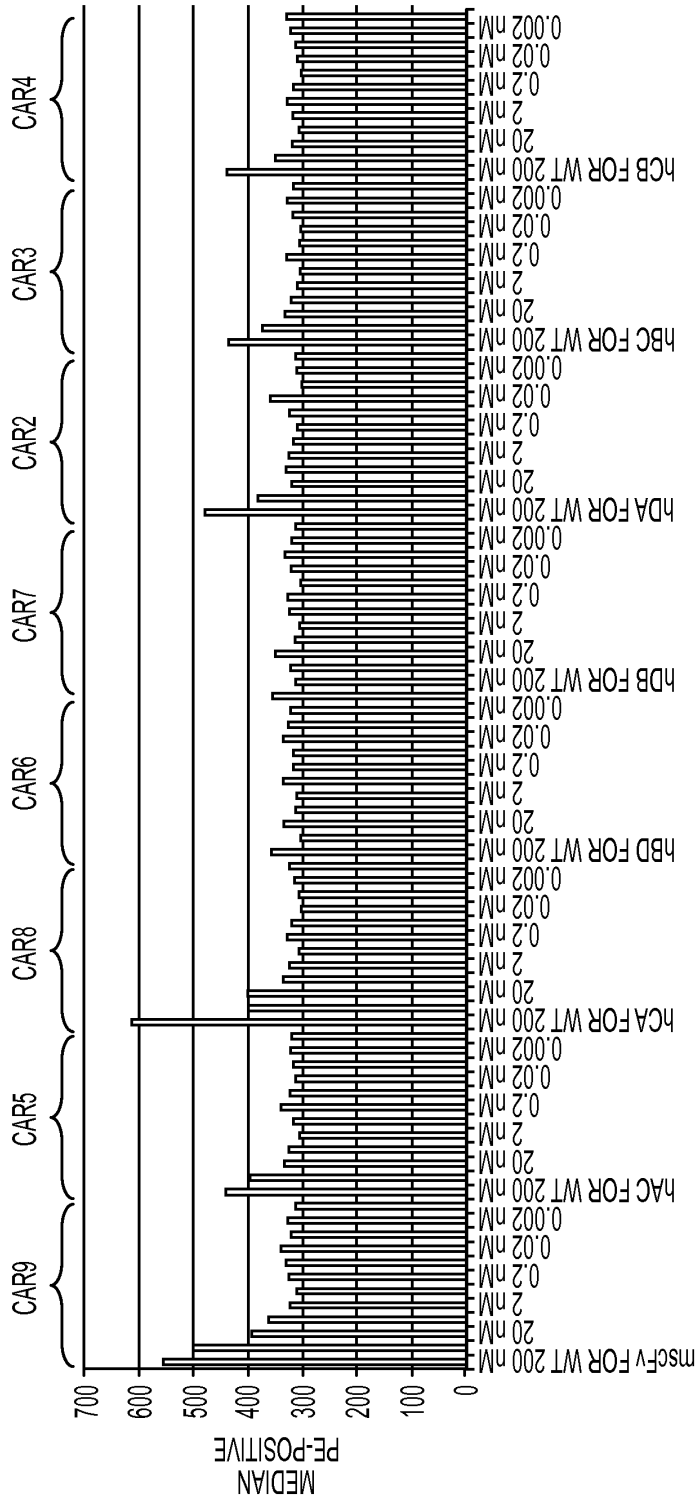

MEDIAN
PE-POSITIVE
700
600
500
400
300
200
100
0
CAR9
CAR5
CAR8
CAR6
CAR7
CAR2
CAR3
CAR4
mscFv FOR WT 200 nM
20 nM
2 nM
0.2 nM
0.02 nM
0.002 nM
hAC FOR WT 200 nM
hCA FOR WT 200 nM
hBD FOR WT 200 nM
hDB FOR WT 200 nM
hDA FOR WT 200 nM
hBC FOR WT 200 nM
hCB FOR WT 200 nM

FIG. 11

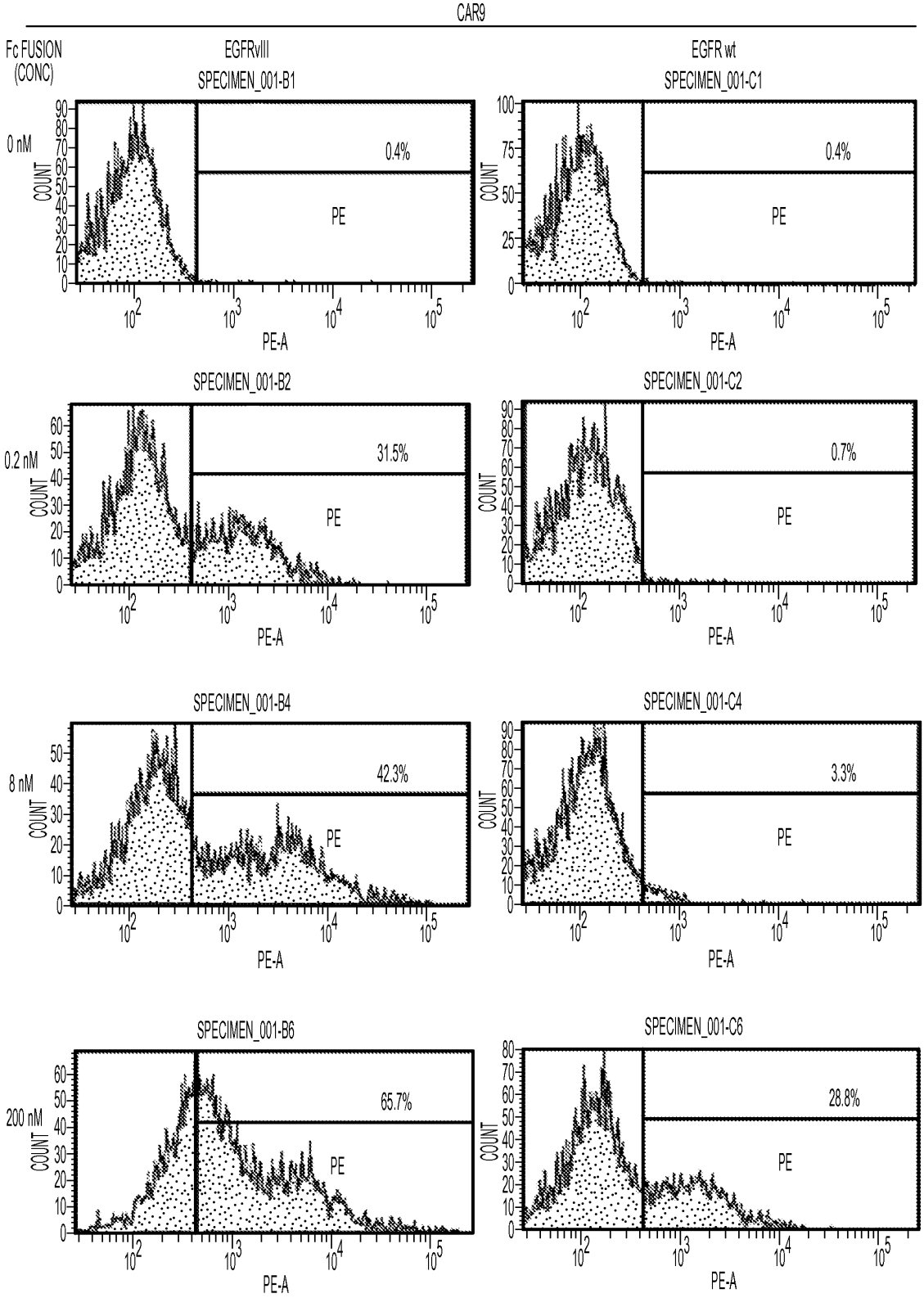

CAR9
Fc FUSION (CONC)
EGFRvIII
EGFR wt
SPECIMEN_001-B1
SPECIMEN_001-C1
SPECIMEN_001-B2
SPECIMEN_001-C2
SPECIMEN_001-B4
SPECIMEN_001-C4
SPECIMEN_001-B6
SPECIMEN_001-C6
0 nM
0.2 nM
8 nM
200 nM
COUNT
PE-A
PE
0.4%
0.4%
31.5%
0.7%
42.3%
3.3%
65.7%
28.8%

FIG. 12A

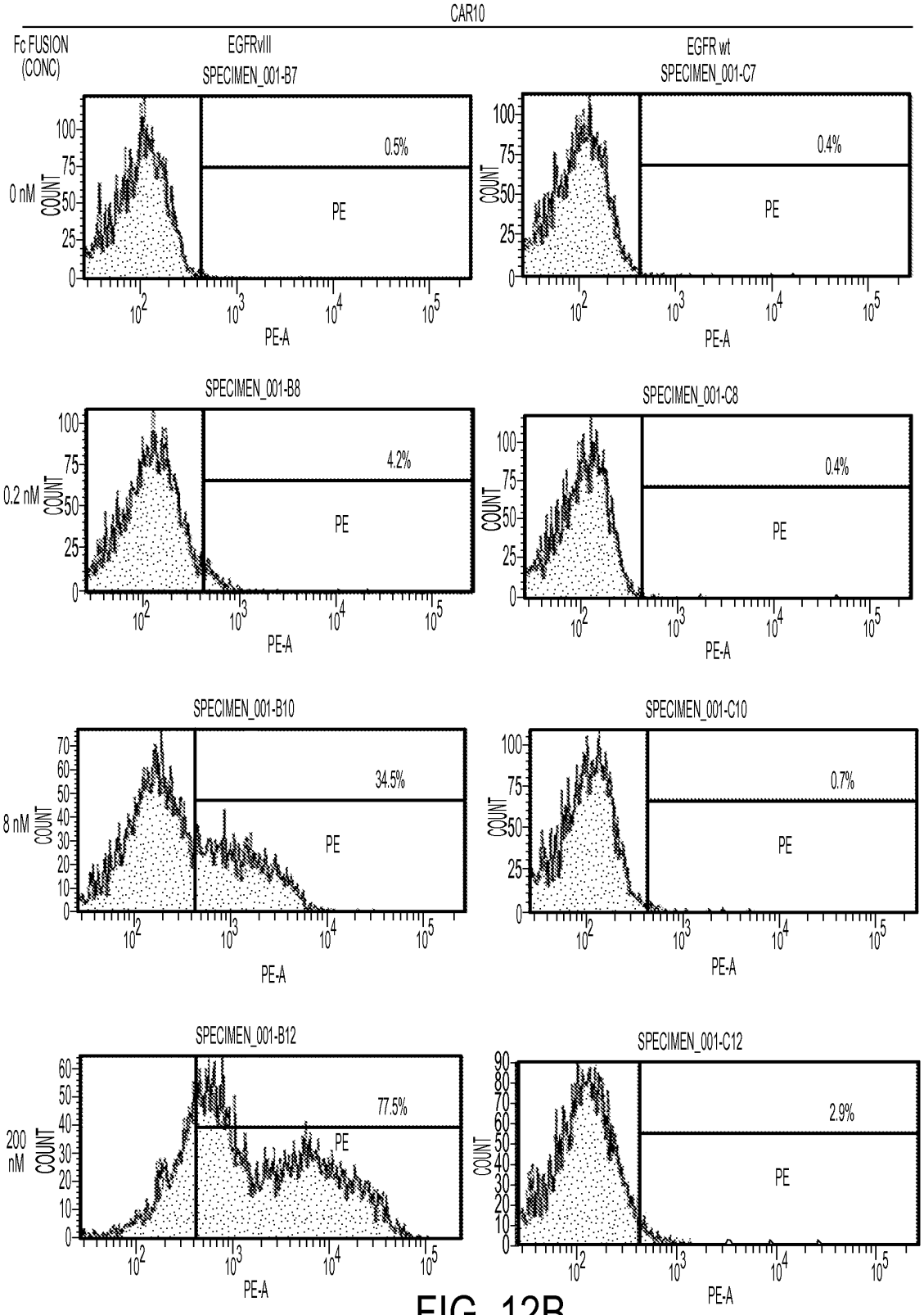

CAR10
Fc FUSION (CONC)
EGFRvIII
EGFR wt
SPECIMEN_001-B7
SPECIMEN_001-C7
SPECIMEN_001-B8
SPECIMEN_001-C8
SPECIMEN_001-B10
SPECIMEN_001-C10
SPECIMEN_001-B12
SPECIMEN_001-C12
0 nM
0.2 nM
8 nM
200 nM
COUNT
PE-A
PE
0.5%
0.4%
4.2%
0.4%
34.5%
0.7%
77.5%
2.9%

FIG. 12B

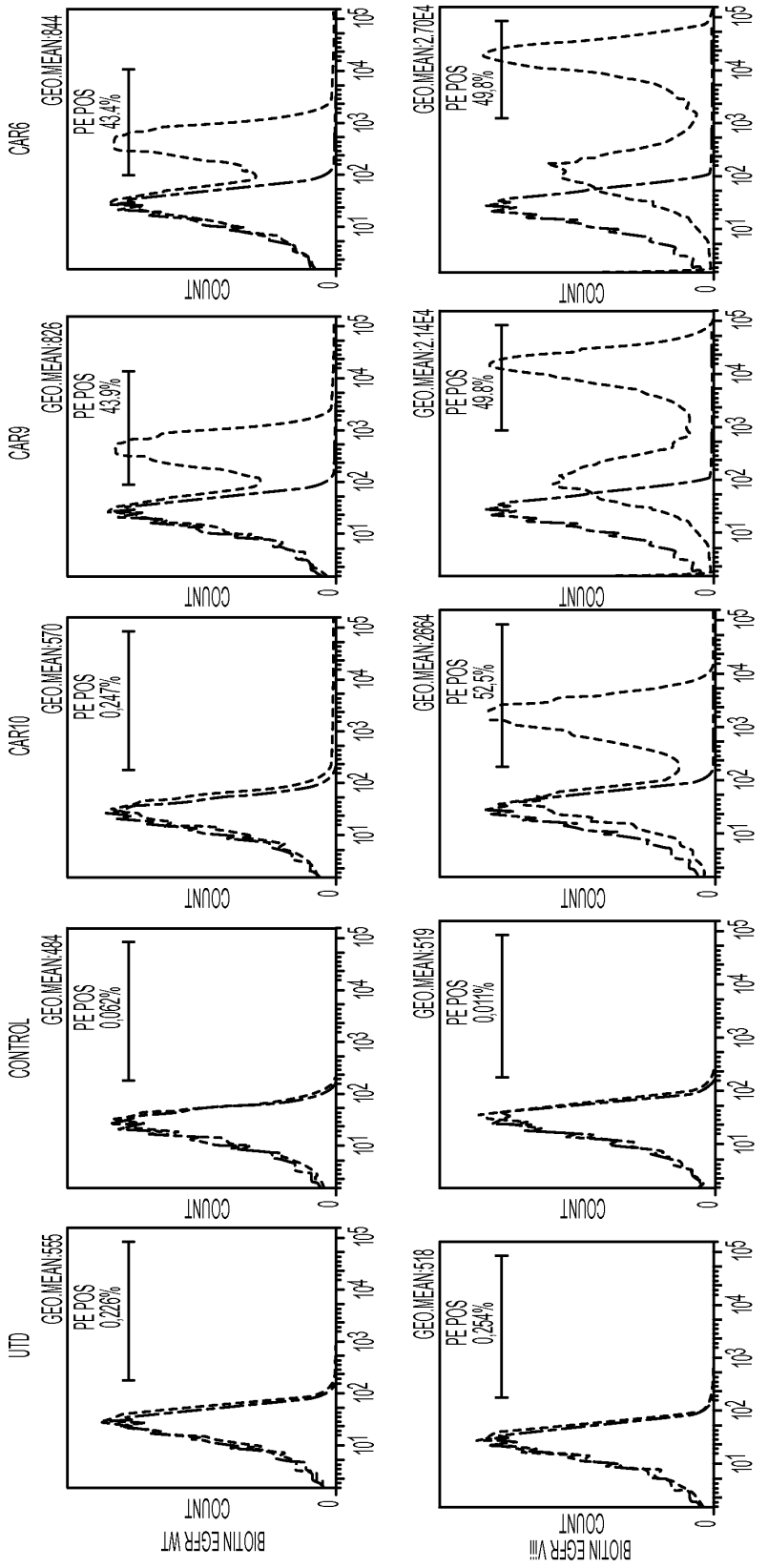

UTD
CONTROL
CAR10
CAR9
CAR6
GEO.MEAN:555
PE POS
0,226%
GEO.MEAN:484
PE POS
0,062%
GEO.MEAN:570
PE POS
0,247%
GEO.MEAN:826
PE POS
43,9%
GEO.MEAN:844
PE POS
43,4%
GEO.MEAN:518
PE POS
0,254%
GEO.MEAN:519
PE POS
0,011%
GEO.MEAN:2684
PE POS
52,5%
GEO.MEAN:2.14E4
PE POS
49,8%
GEO.MEAN:2.70E4
PE POS
49,8%
COUNT
0
10^1
10^2
10^3
10^4
10^5
BIOTIN EGFR WT
BIOTIN EGFR VIII

FIG. 13

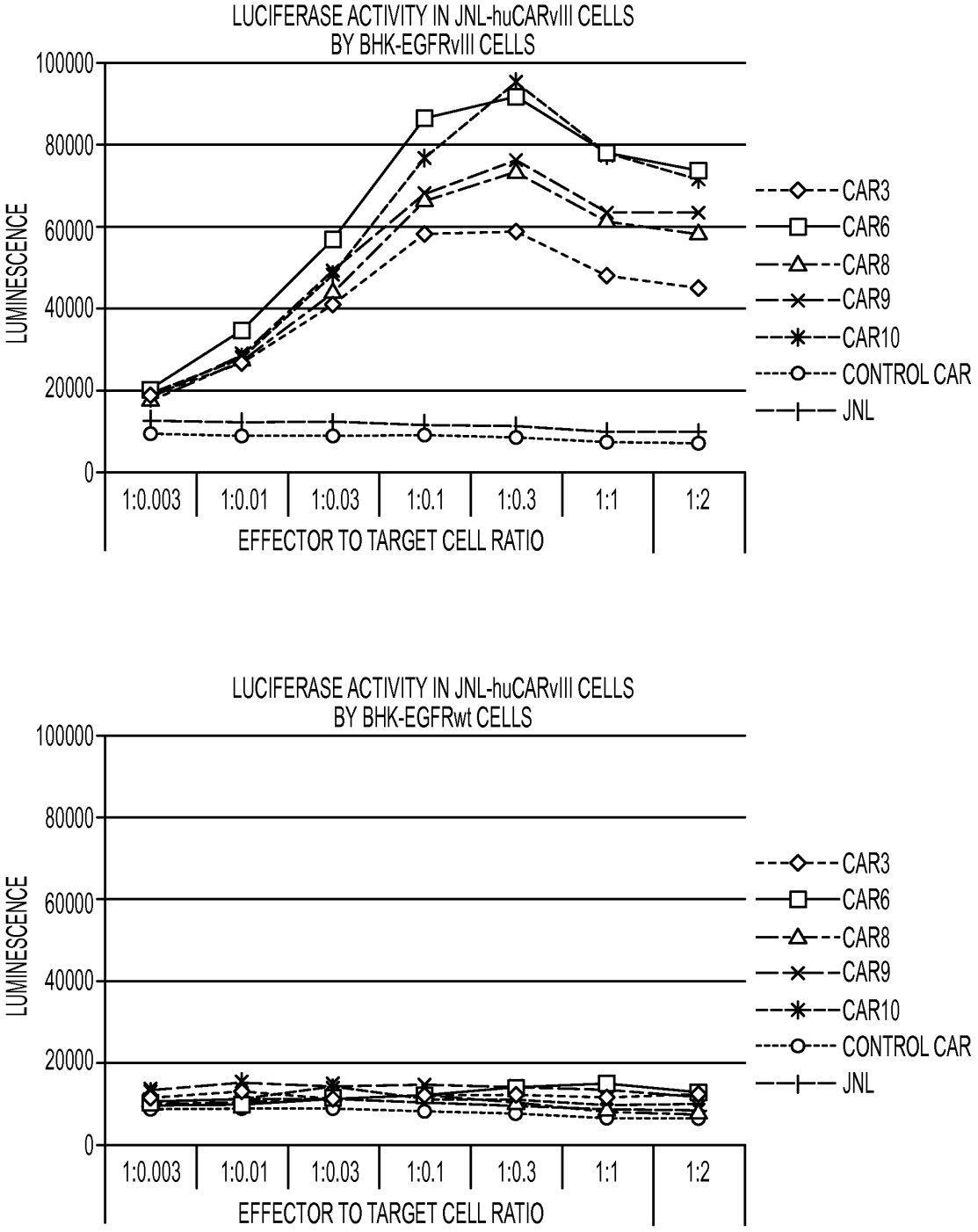

LUCIFERASE ACTIVITY IN JNL-huCARvIII CELLS
BY BHK-EGFRvIII CELLS
LUMINESCENCE
100000
80000
60000
40000
20000
0
1:0.003
1:0.01
1:0.03
1:0.1
1:0.3
1:1
1:2
EFFECTOR TO TARGET CELL RATIO
CAR3
CAR6
CAR8
CAR9
CAR10
CONTROL CAR
JNL
LUCIFERASE ACTIVITY IN JNL-huCARvIII CELLS
BY BHK-EGFRwt CELLS
LUMINESCENCE
100000
80000
60000
40000
20000
0
1:0.003
1:0.01
1:0.03
1:0.1
1:0.3
1:1
1:2
EFFECTOR TO TARGET CELL RATIO
CAR3
CAR6
CAR8
CAR9
CAR10
CONTROL CAR
JNL

FIG. 14

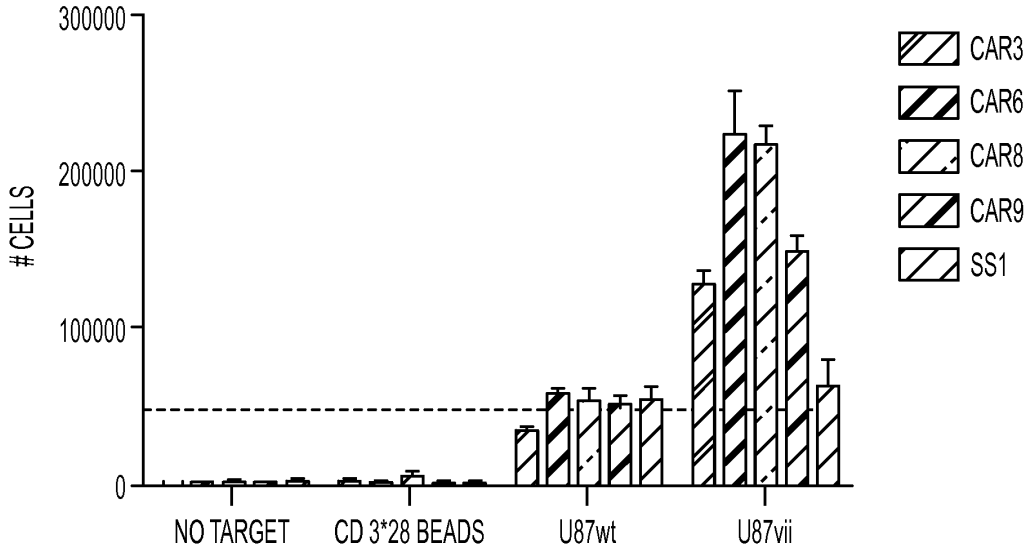

300000
200000
100000
0
CELLS
NO TARGET
CD 3*28 BEADS
U87wt
U87vii
CAR3
CAR6
CAR8
CAR9
SS1

FIG. 15

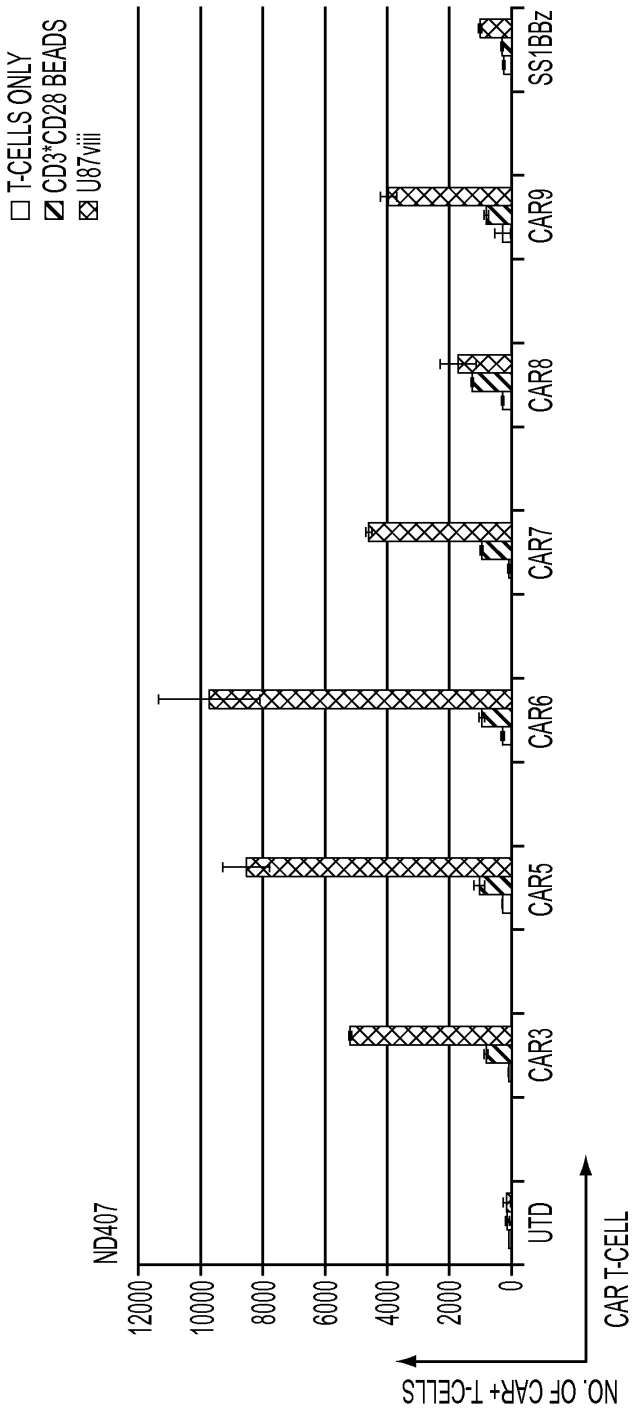

T-CELLS ONLY
CD3*CD28 BEADS
U87viii
ND407
12000
10000
8000
6000
4000
2000
0
NO. OF CAR+ T-CELLS
CAR T-CELL
UTD
CAR3
CAR5
CAR6
CAR7
CAR8
CAR9
SS1BBz

FIG. 16

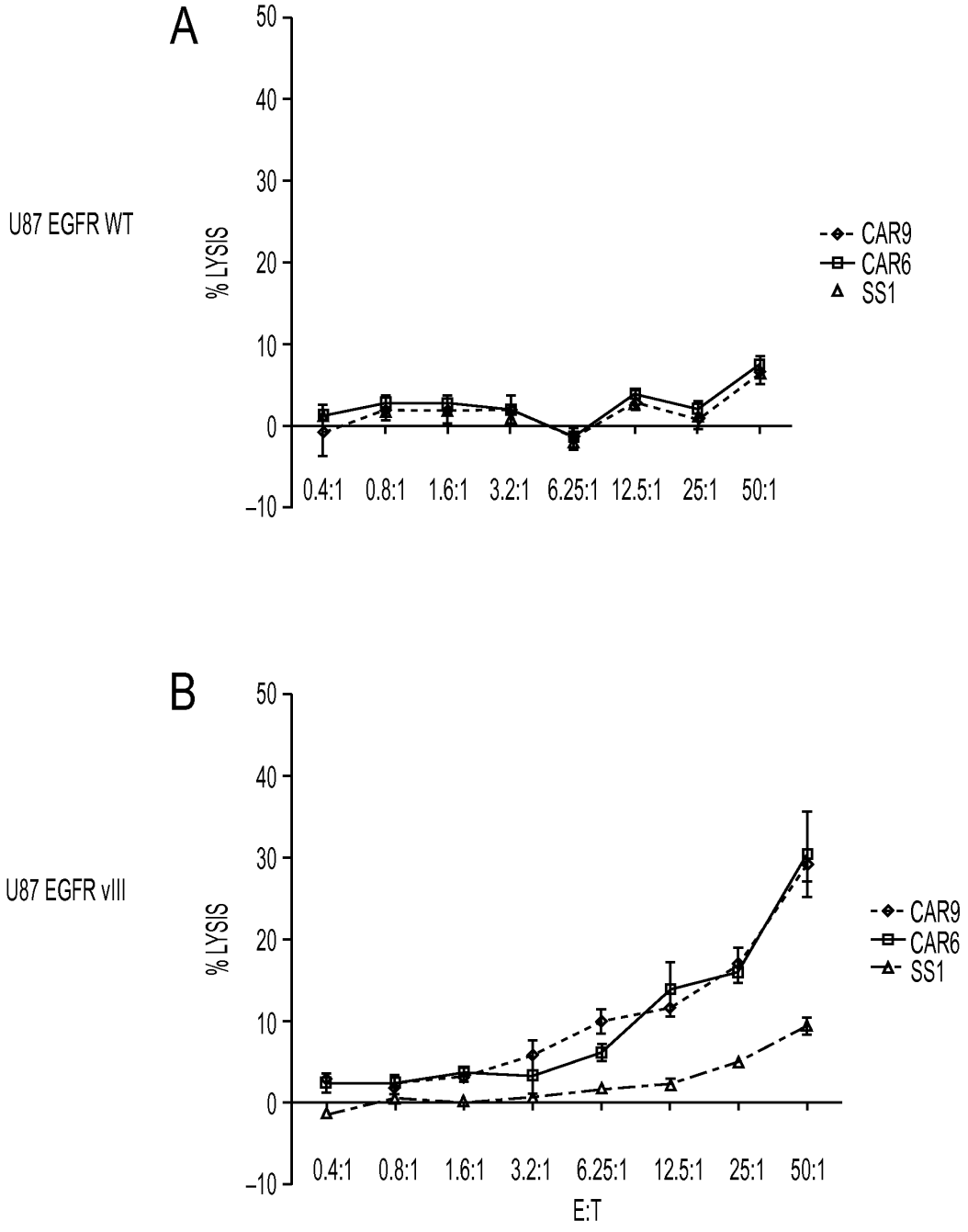

A
U87 EGFR WT
% LYSIS
50
40
30
20
10
0
−10
0.4:1
0.8:1
1.6:1
3.2:1
6.25:1
12.5:1
25:1
50:1
CAR9
CAR6
SS1
B
U87 EGFR vIII
% LYSIS
50
40
30
20
10
0
−10
0.4:1
0.8:1
1.6:1
3.2:1
6.25:1
12.5:1
25:1
50:1
E:T
CAR9
CAR6
SS1

FIG. 17

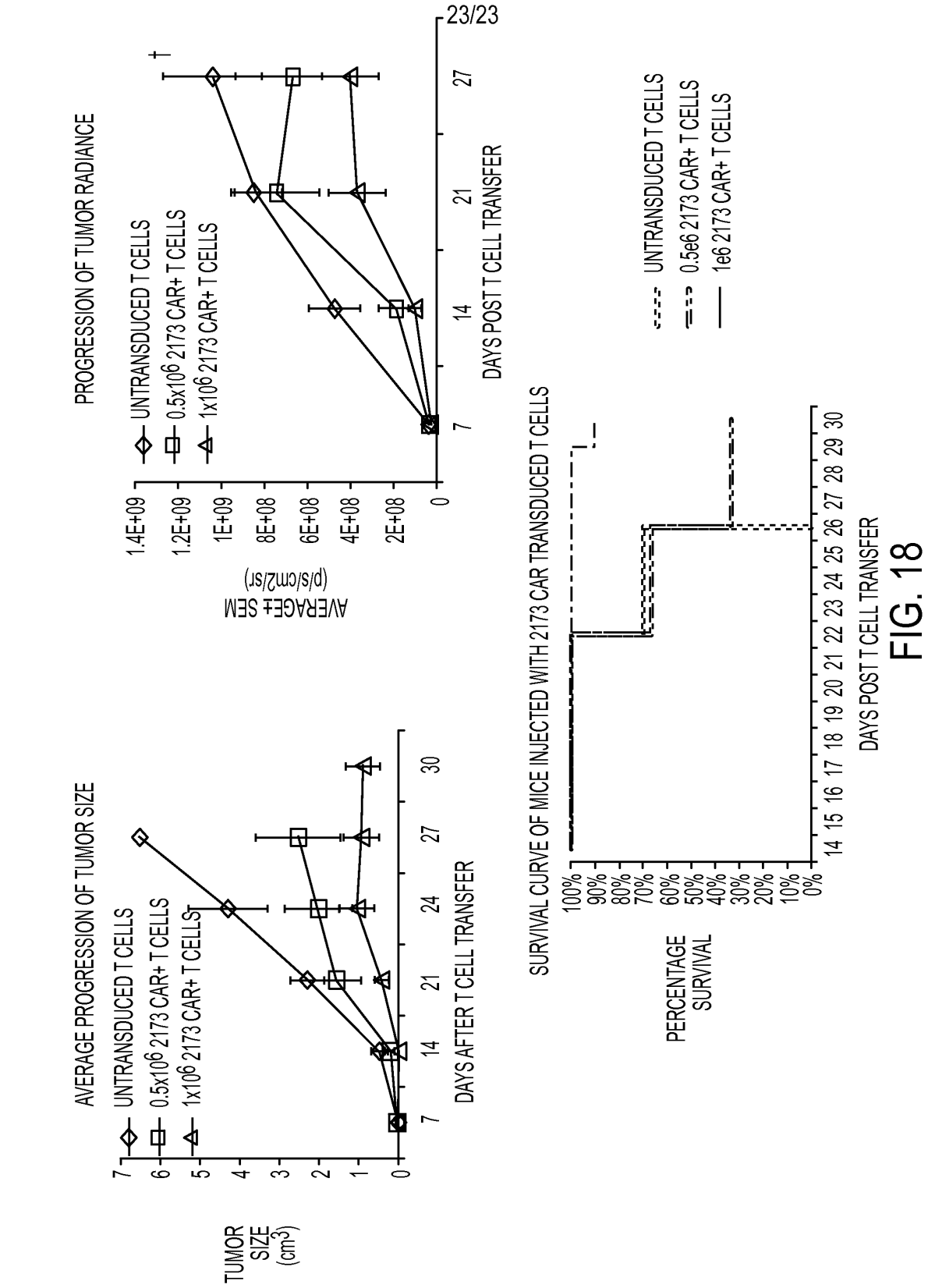

AVERAGE PROGRESSION OF TUMOR SIZE
UNTRANSDUCED T CELLS
0.5x10^6 2173 CAR+ T CELLS
1x10^6 2173 CAR+ T CELLS
TUMOR SIZE (cm3)
DAYS AFTER T CELL TRANSFER
PROGRESSION OF TUMOR RADIANCE
AVERAGE± SEM (p/s/cm2/sr)
DAYS POST T CELL TRANSFER
SURVIVAL CURVE OF MICE INJECTED WITH 2173 CAR TRANSDUCED T CELLS
PERCENTAGE SURVIVAL
UNTRANSDUCED T CELLS
0.5e6 2173 CAR+ T CELLS
1e6 2173 CAR+ T CELLS
DAYS POST T CELL TRANSFER

FIG. 18