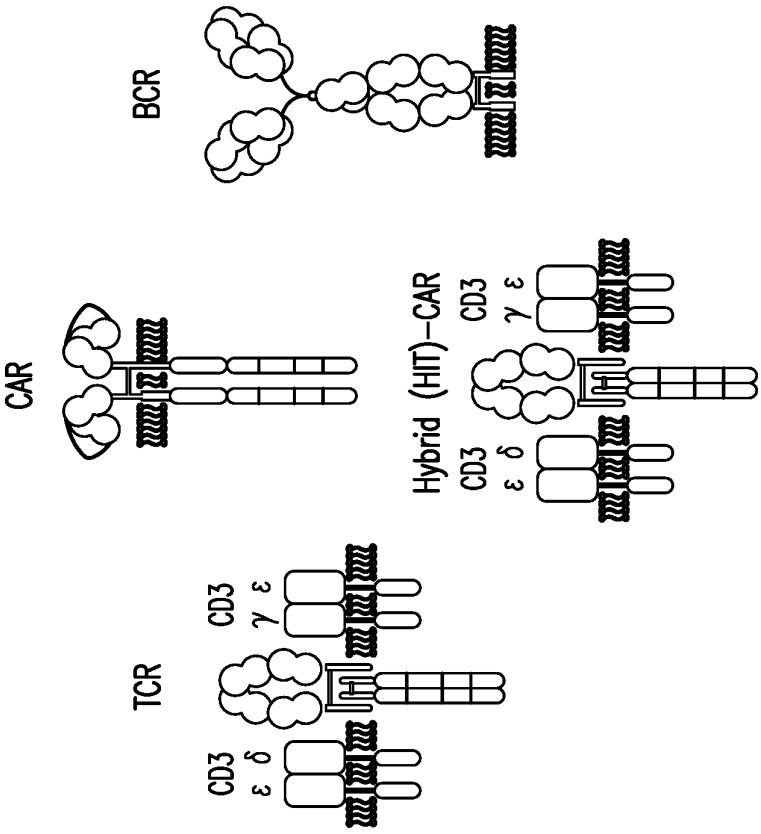
CAR
BCR
TCR
CD3
ε δ
CD3
γ ε
Hybrid (HIT)–CAR
CD3
ε δ
CD3
γ ε

FIG. 1A

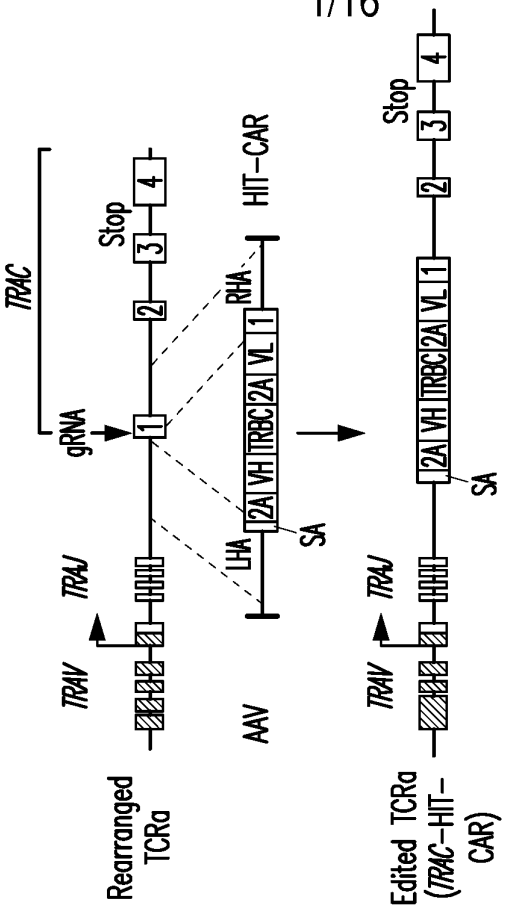
TRAC
gRNA
Stop
HIT–CAR
TRAJ
TRAV
LHA
RHA
2A VH TRBC 2A VL 1
SA
AAV
Rearranged TCRα
Edited TCRα (TRAC–HIT–CAR)

FIG. 1B

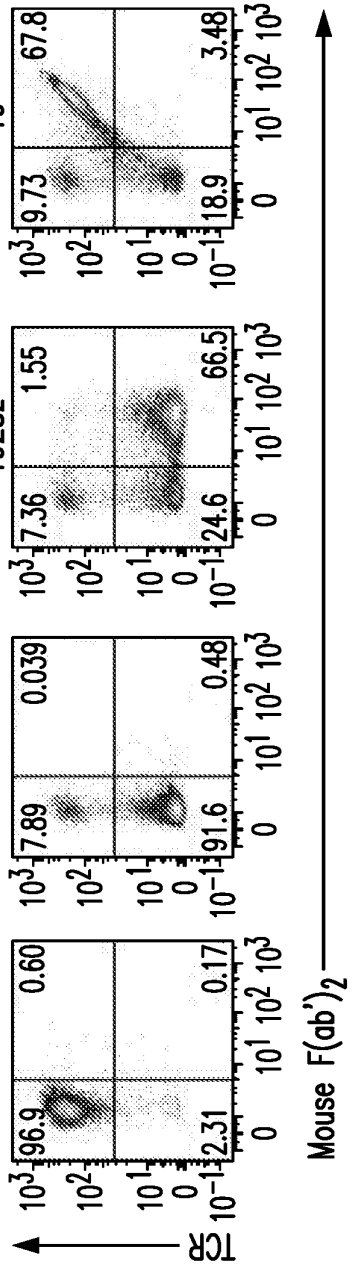
T cells
TRAC KO
TRAC-CAR 1928z
TRAC-HIT-CAR 19
TCR
Mouse F(ab')2
96.9
0.60
2.31
0.17
7.89
0.039
91.6
0.48
7.36
1.55
24.6
66.5
9.73
67.8
18.9
3.48

FIG. 1C

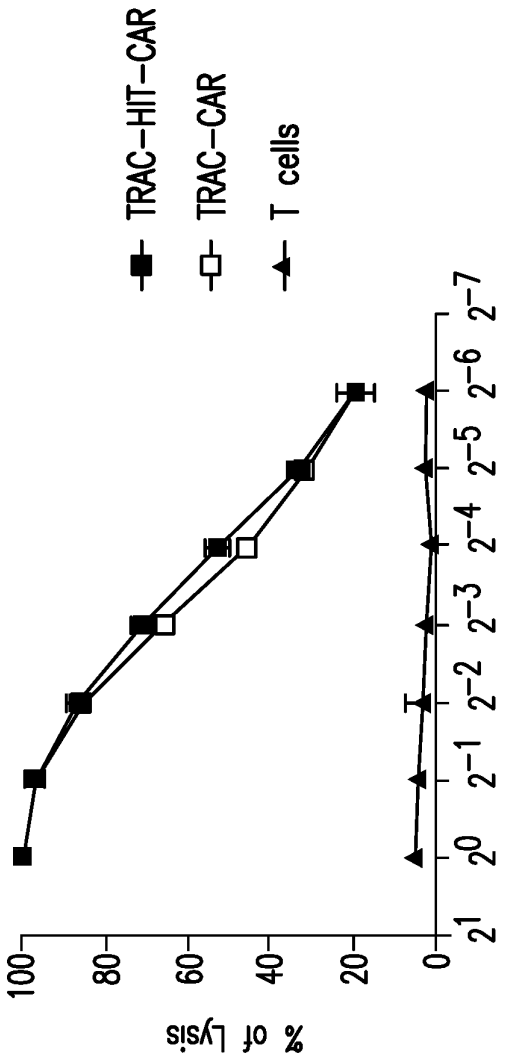
TRAC-HIT-CAR
TRAC-CAR
T cells
% of Lysis
Effector/Target ratio

FIG. 1D

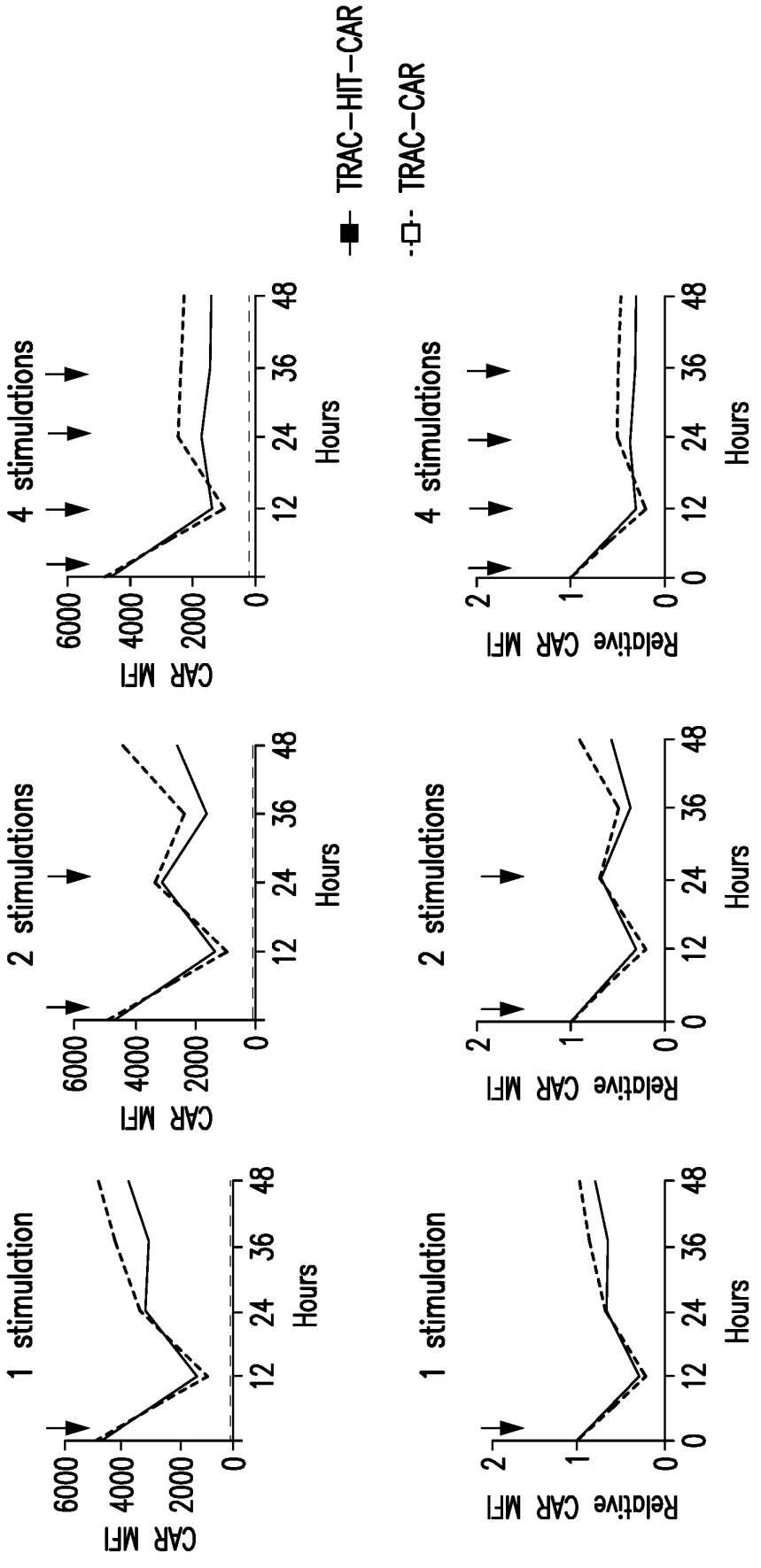
TRAC-HIT-CAR
TRAC-CAR
1 stimulation
2 stimulations
4 stimulations
CAR MFI
Relative CAR MFI
Hours
0
12
24
36
48
2000
4000
6000
1
2

FIG. 1E

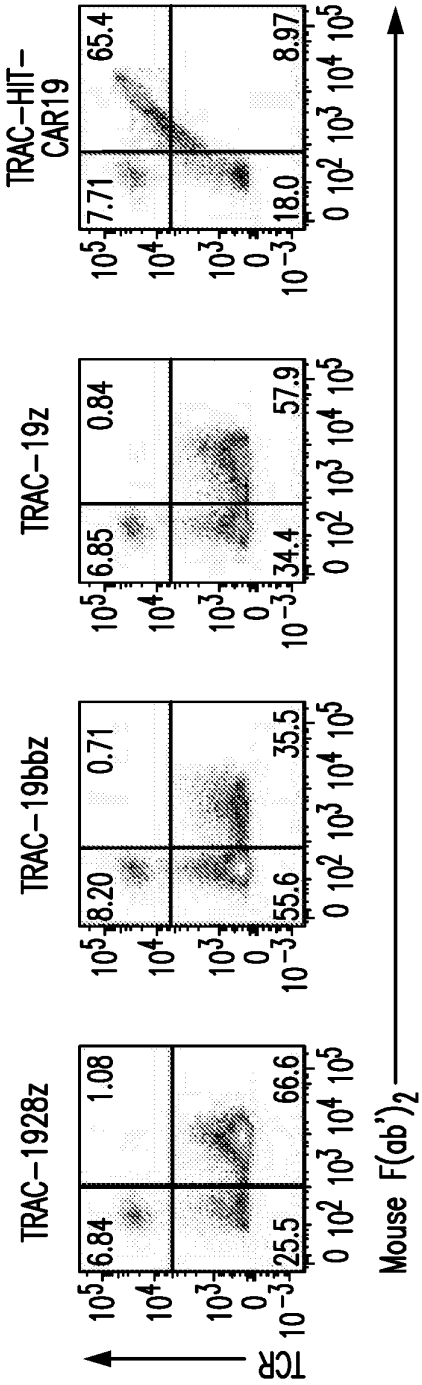
TRAC−1928z
TRAC−19bbz
TRAC−19z
TRAC−HIT−CAR19
TCR
Mouse F(ab')2
6.84
1.08
25.5
66.6
8.20
0.71
55.6
35.5
6.85
0.84
34.4
57.9
7.71
65.4
18.0
8.97

FIG. 2A

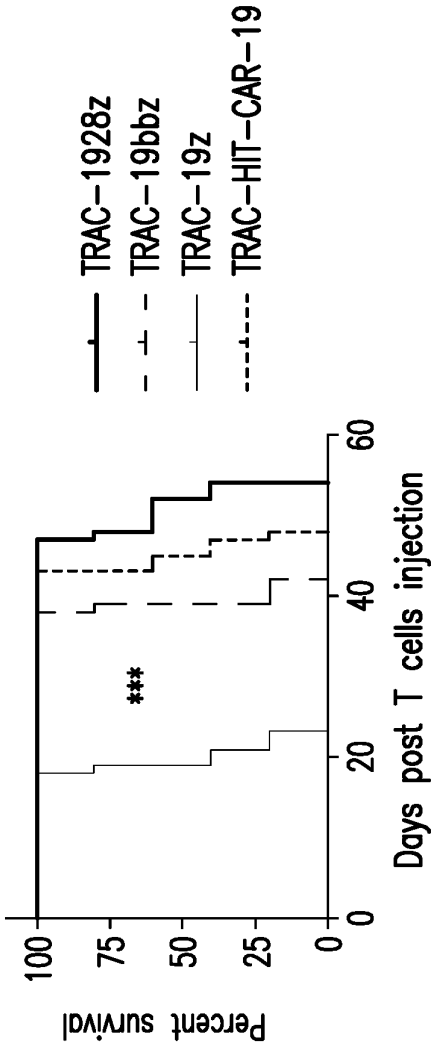
TRAC−1928z
TRAC−19bbz
TRAC−19z
TRAC−HIT−CAR−19
***
Percent survival
Days post T cells injection
0
25
50
75
100
0
20
40
60

FIG. 2B

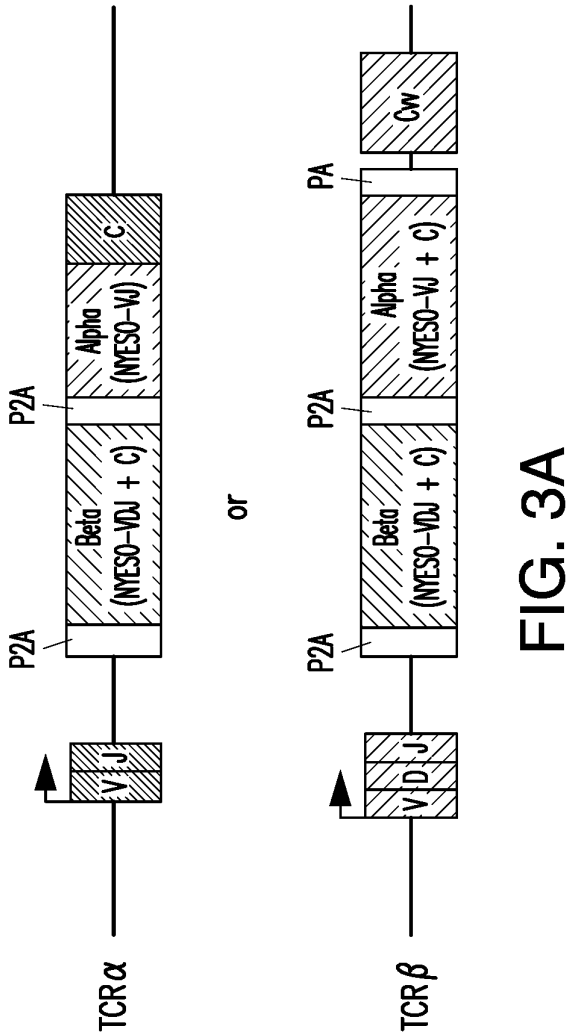

FIG. 3A

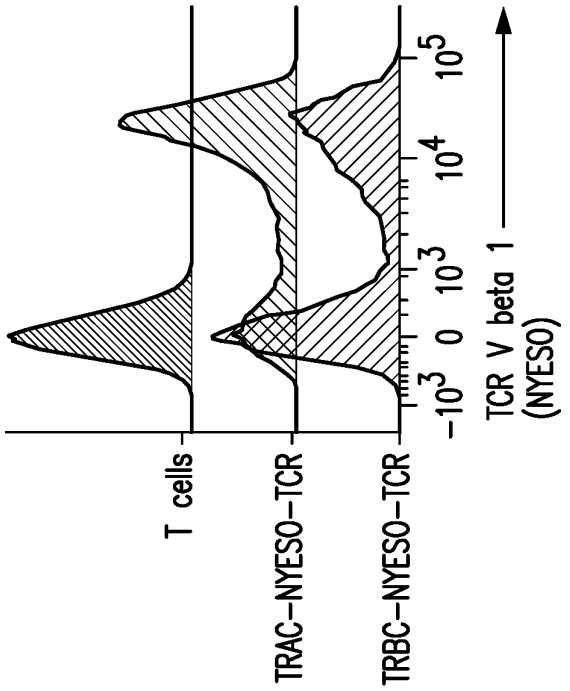

FIG. 3B

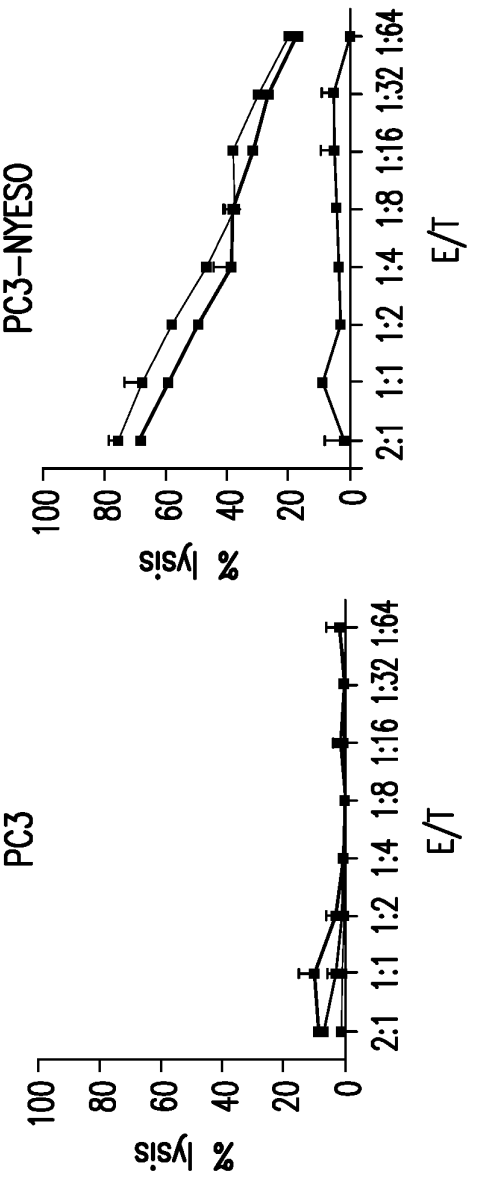

FIG. 3C

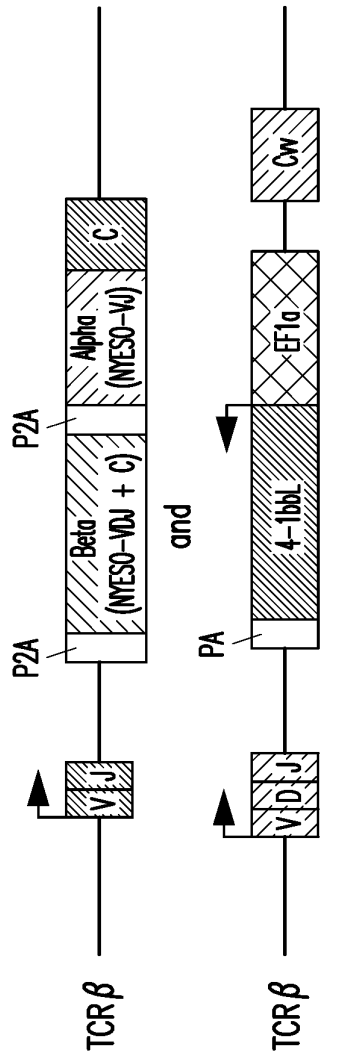
TCR β
V J
P2A
Beta
(NYESO-VDJ + C)
P2A
Alpha
(NYESO-VJ)
C
and
TCR β
V D J
PA
4-1bbL
EF1α
Cw

FIG. 3D

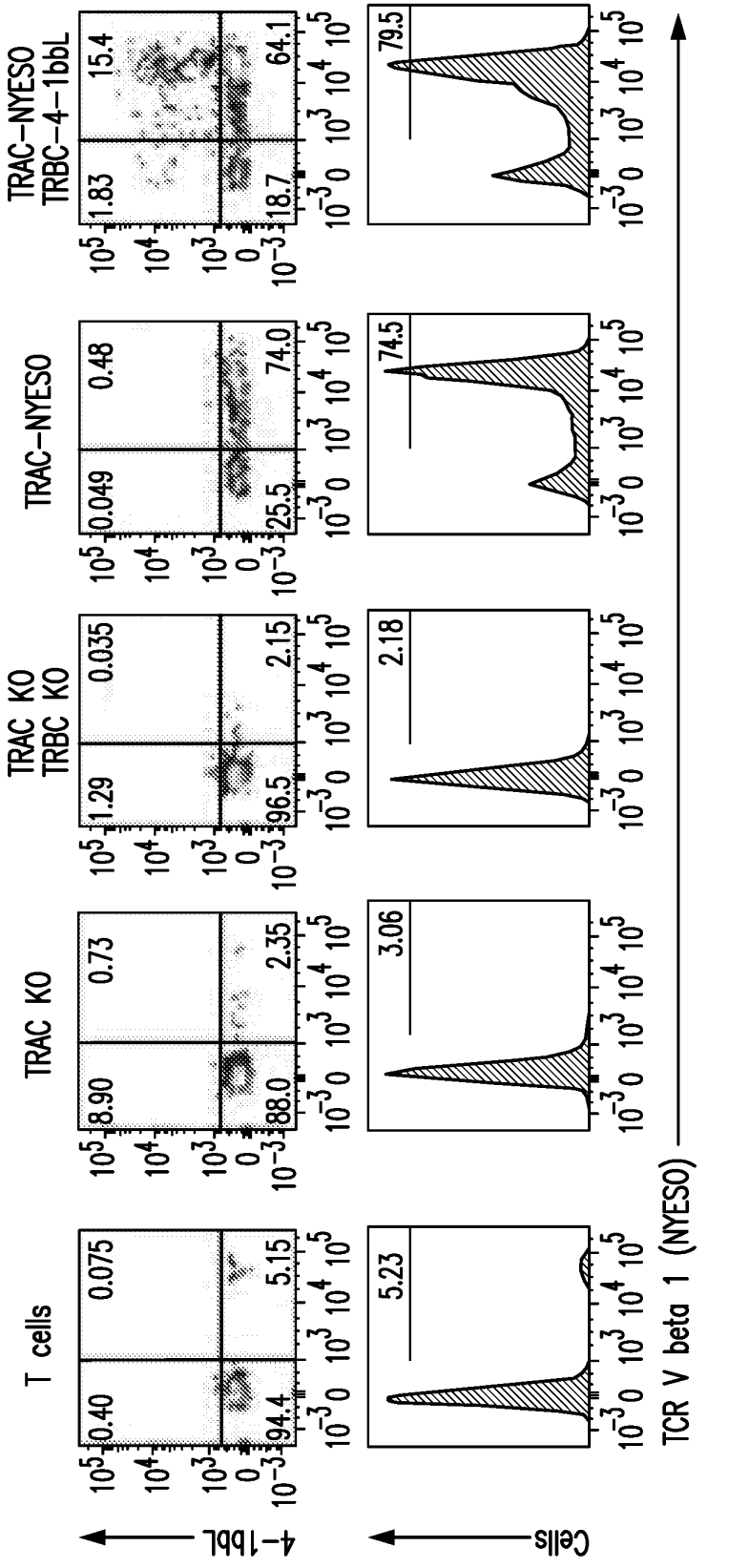
T cells
TRAC KO
TRAC KO TRBC KO
TRAC-NYESO
TRAC-NYESO TRBC-4-1bbL
4-1bbL
Cells
TCR V beta 1 (NYESO)
0.40
0.075
94.4
5.15
8.90
0.73
88.0
2.35
1.29
0.035
96.5
2.15
0.049
0.48
25.5
74.0
1.83
15.4
18.7
64.1
5.23
3.06
2.18
74.5
79.5

FIG. 3E

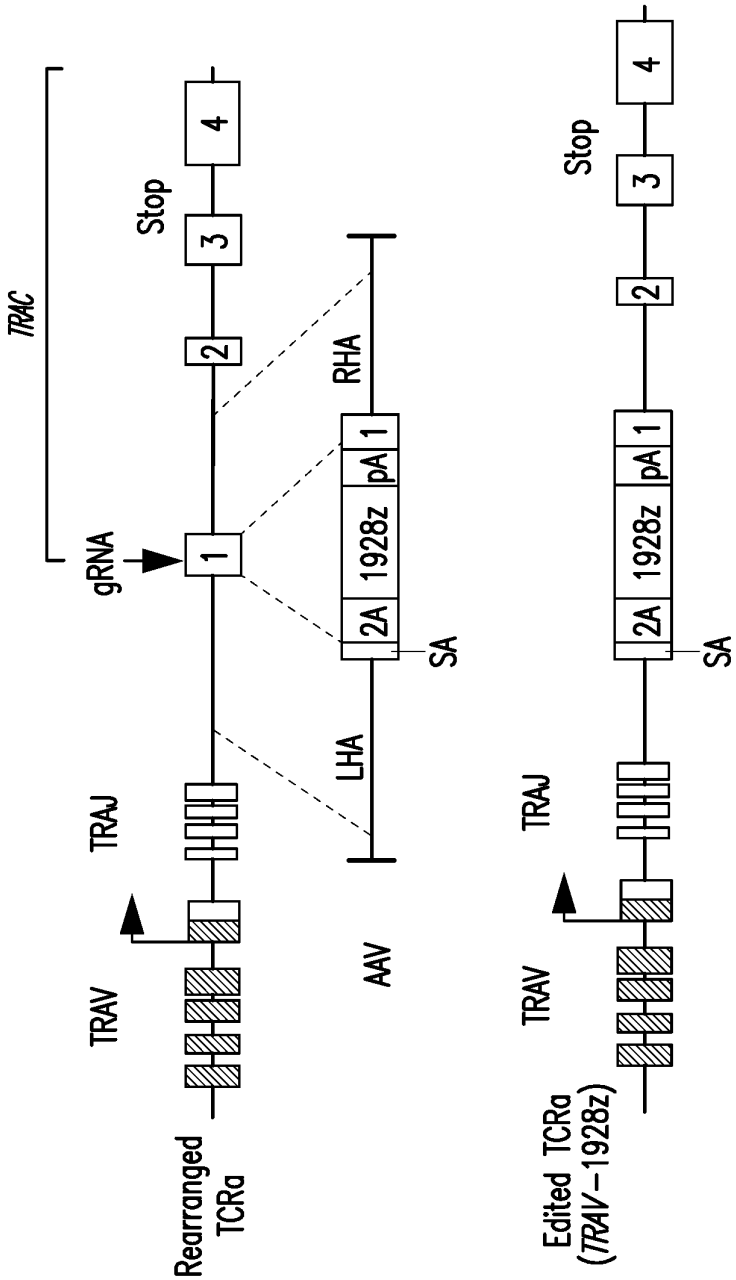
TRAC
gRNA
Stop
1
2
3
4
TRAJ
TRAV
Rearranged TCRα
LHA
RHA
AAV
2A
1928z
pA
SA
Edited TCRα (TRAV–1928z)

FIG. 4A

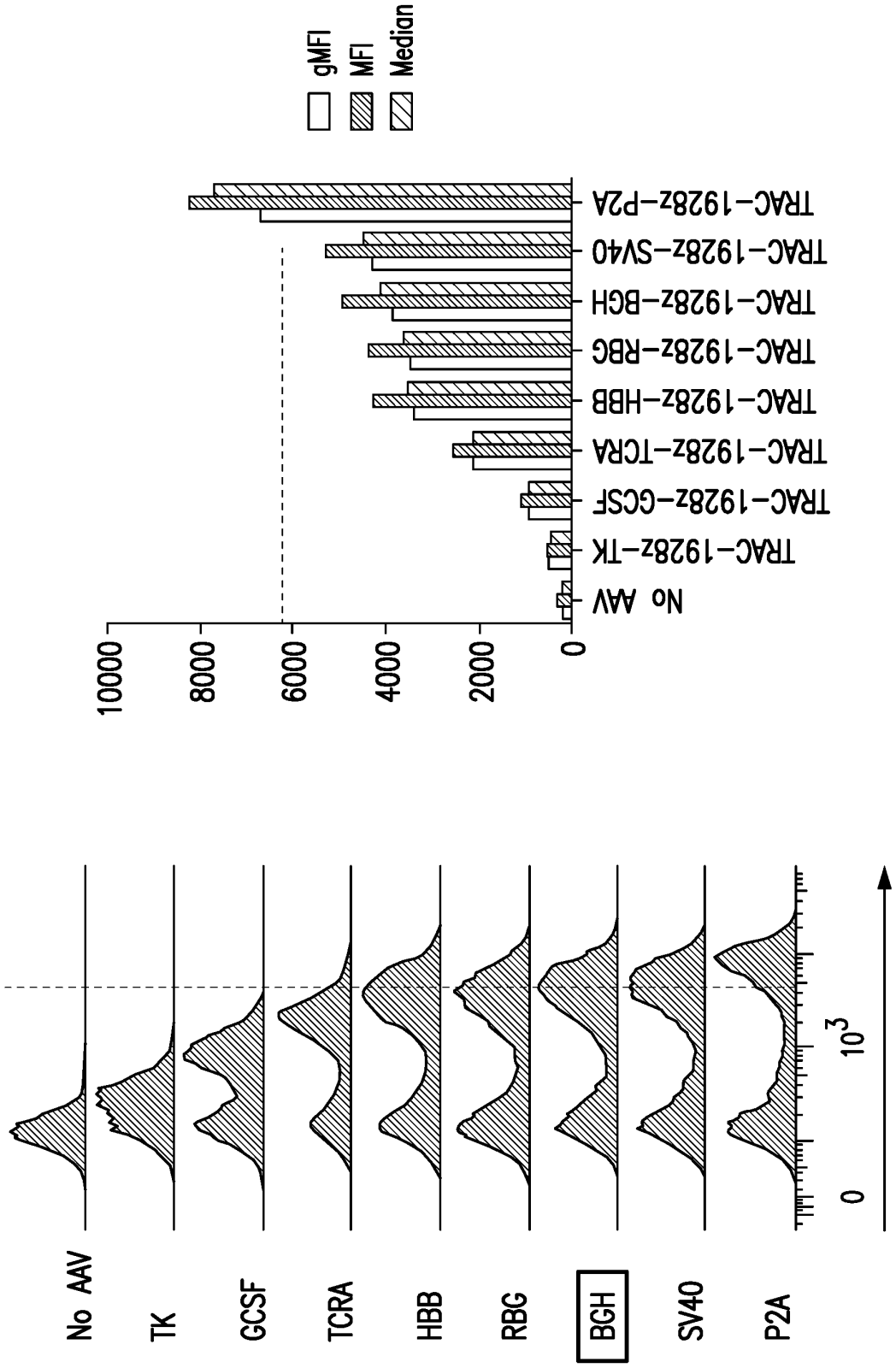
gMFI
MFI
Median
10000
8000
6000
4000
2000
0
No AAV
TRAC-1928z-TK
TRAC-1928z-GCSF
TRAC-1928z-TCRA
TRAC-1928z-HBB
TRAC-1928z-RBG
TRAC-1928z-BGH
TRAC-1928z-SV40
TRAC-1928z-P2A
No AAV
TK
GCSF
TCRA
HBB
RBG
BGH
SV40
P2A
0
10^3
CAR

FIG. 4B

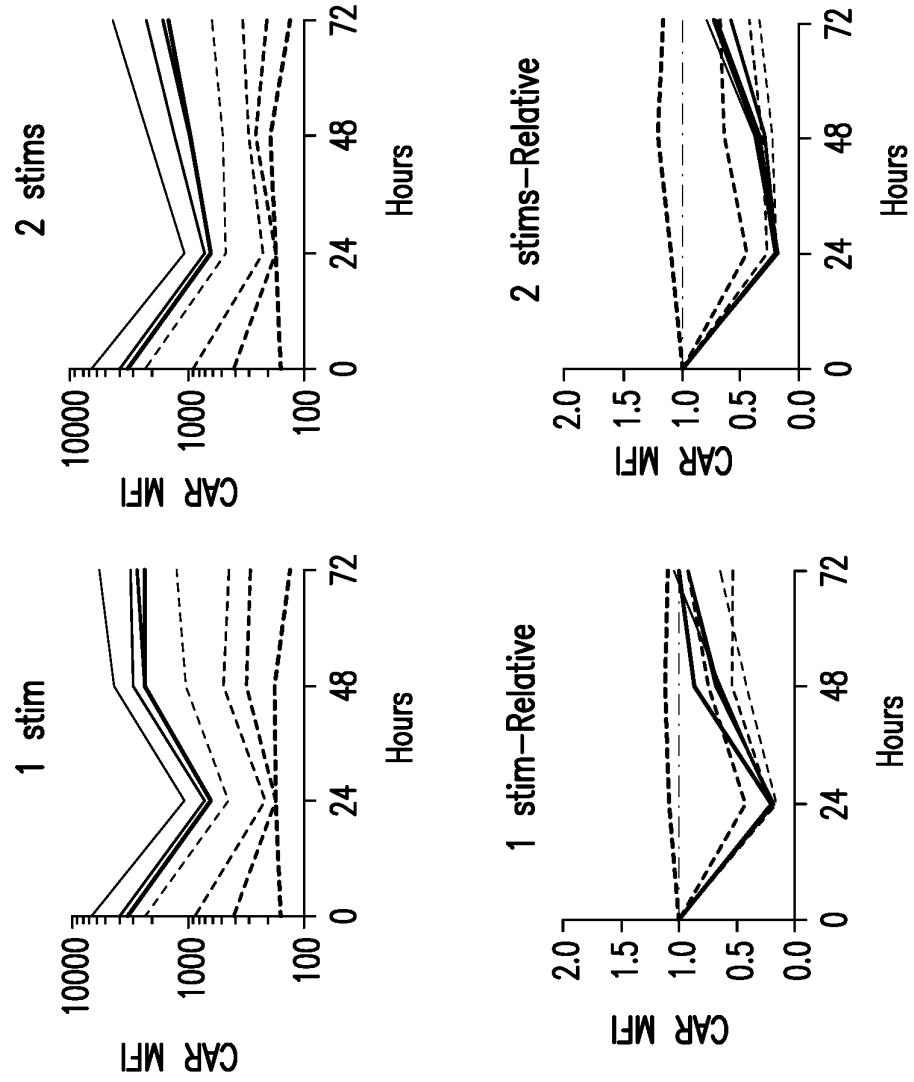
2 stims
1 stim
2 stims–Relative
1 stim–Relative
CAR MFI
Hours
10000
1000
100
2.0
1.5
1.0
0.5
0.0
0
24
48
72

FIG. 4C

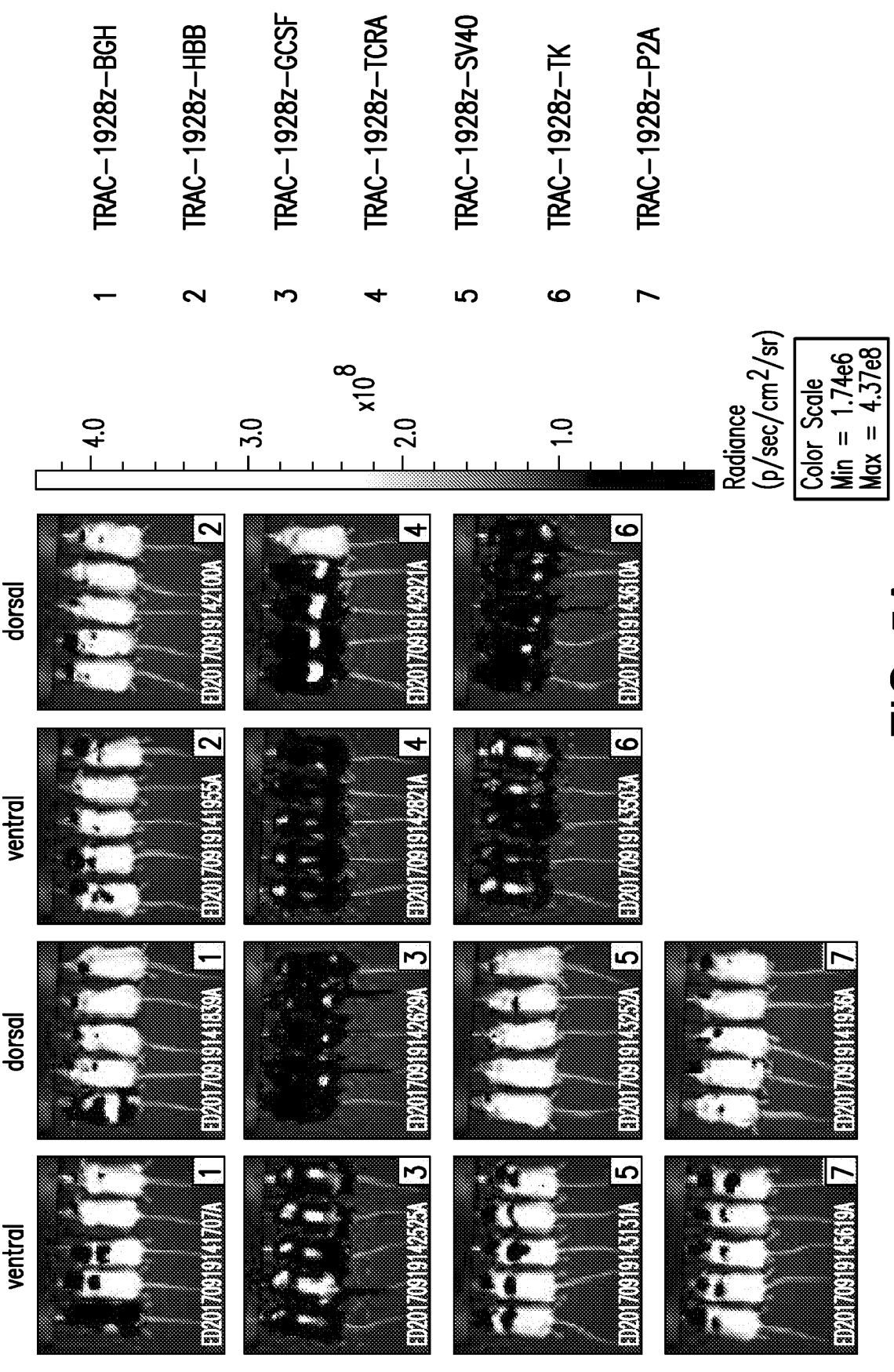
1 TRAC−1928z−BGH
2 TRAC−1928z−HBB
3 TRAC−1928z−GCSF
4 TRAC−1928z−TCRA
5 TRAC−1928z−SV40
6 TRAC−1928z−TK
7 TRAC−1928z−P2A
4.0
3.0
2.0
1.0
x10^8
Radiance (p/sec/cm^2/sr)
Color Scale
Min = 1.74e6
Max = 4.37e8
ventral
dorsal
ventral
dorsal

FIG. 5A

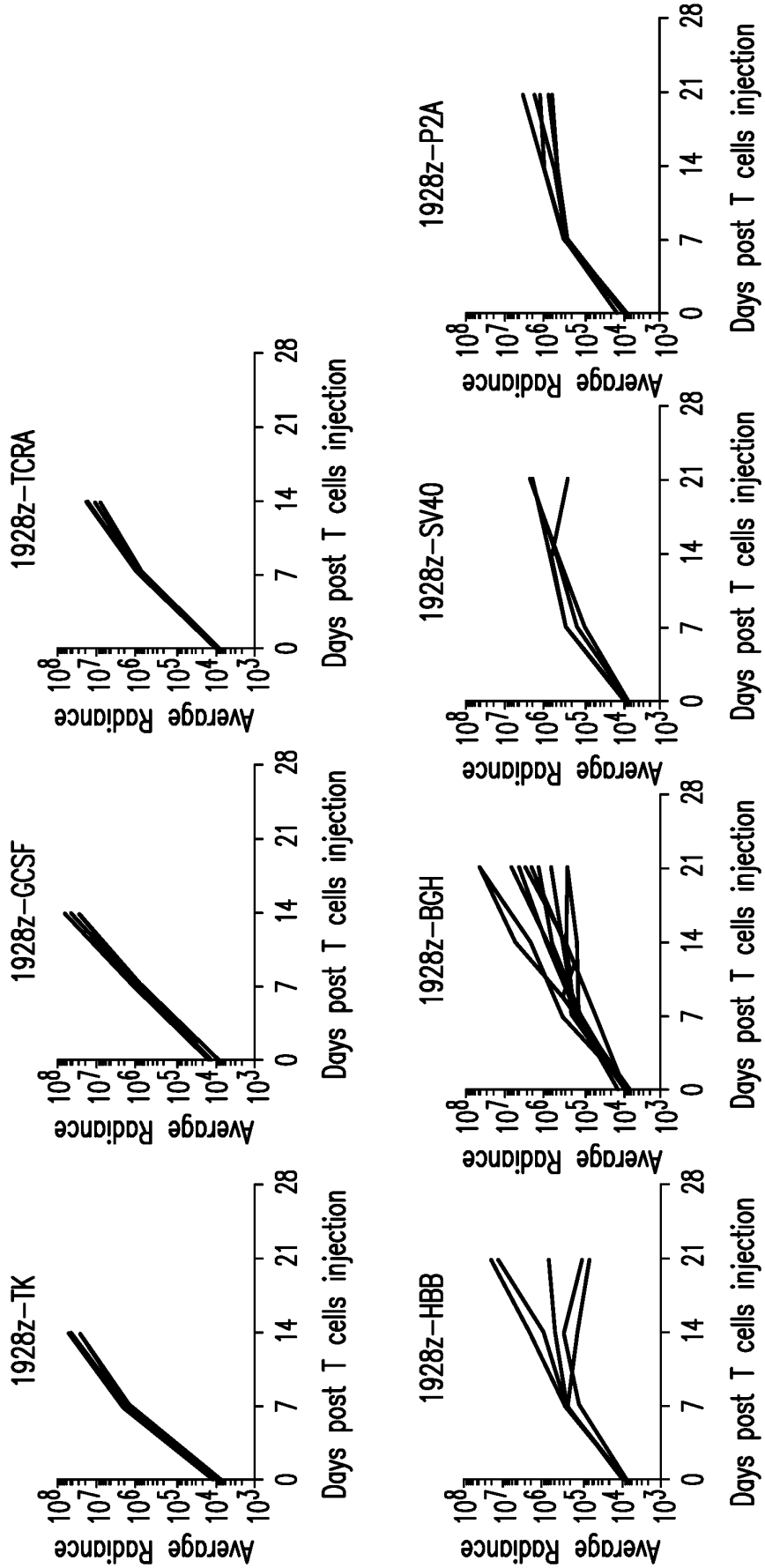
1928z–TK
1928z–GCSF
1928z–TCRA
1928z–HBB
1928z–BGH
1928z–SV40
1928z–P2A
Average Radiance
Days post T cells injection
0
7
14
21
28

FIG. 5B

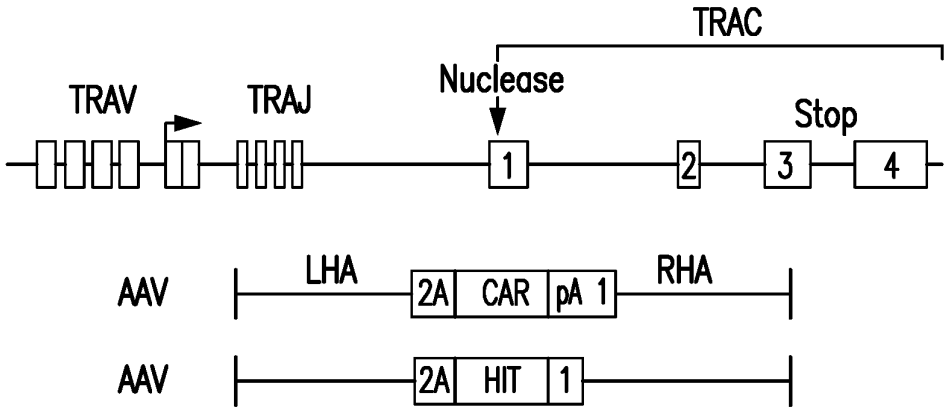

FIG. 6A

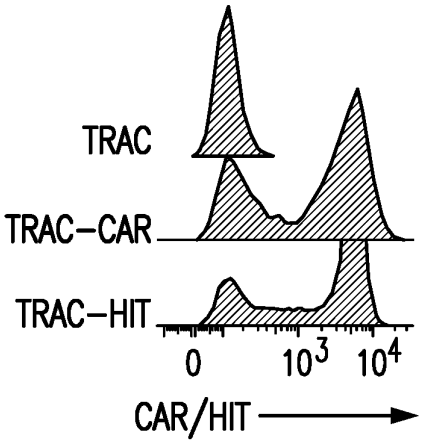

FIG. 6B

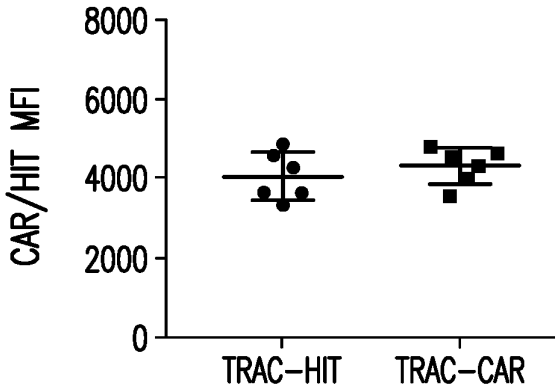

FIG. 6C

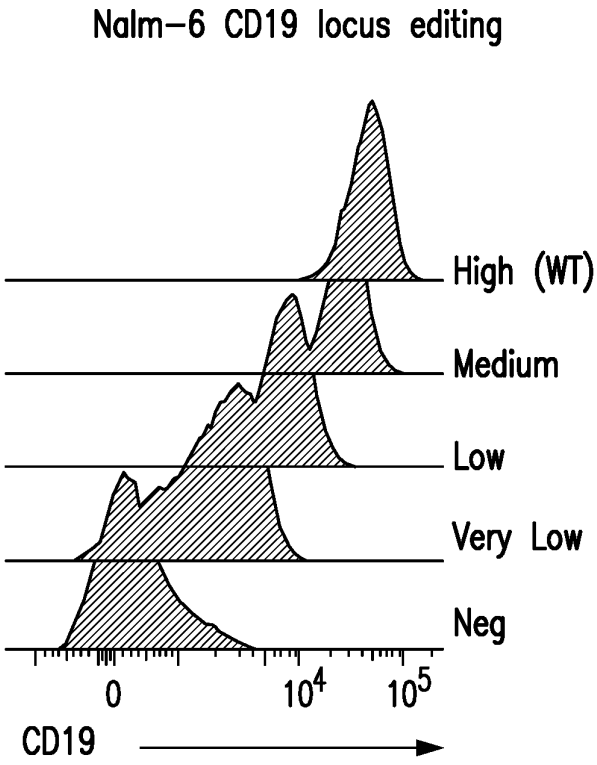
Nalm-6 CD19 locus editing
High (WT)
Medium
Low
Very Low
Neg
0
10^4
10^5
CD19

FIG. 7A

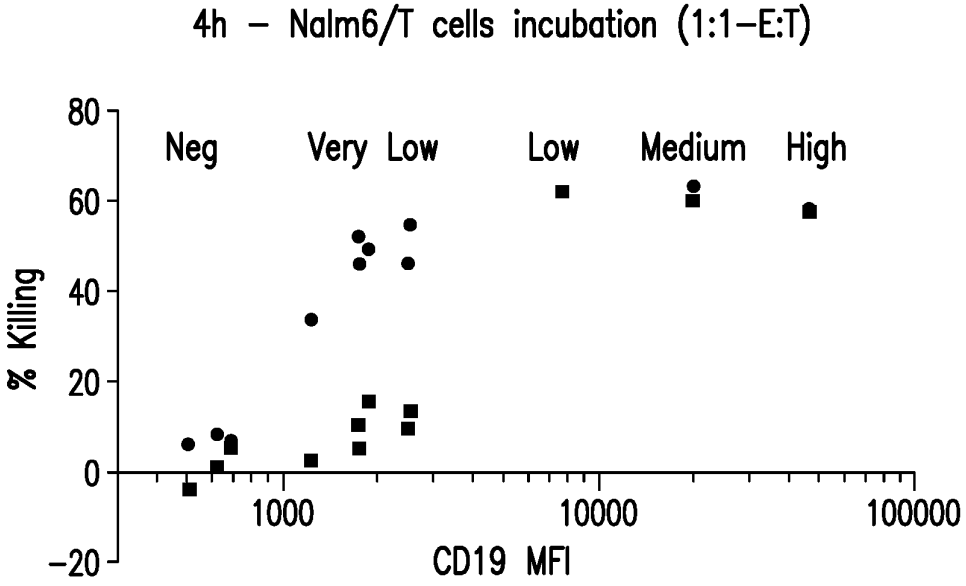
4h – Nalm6/T cells incubation (1:1-E:T)
Neg
Very Low
Low
Medium
High
% Killing
80
60
40
20
0
-20
1000
10000
100000
CD19 MFI

FIG. 7B

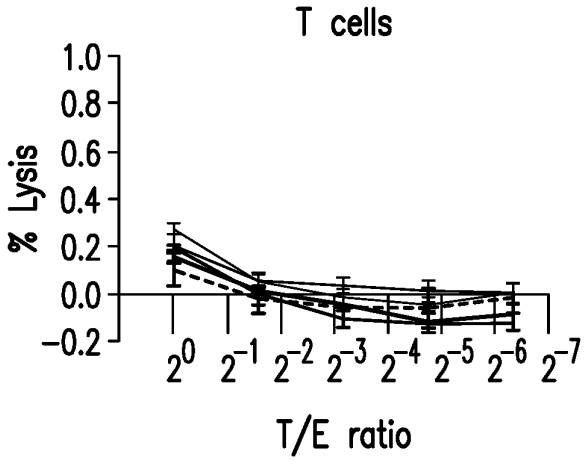

FIG. 8A

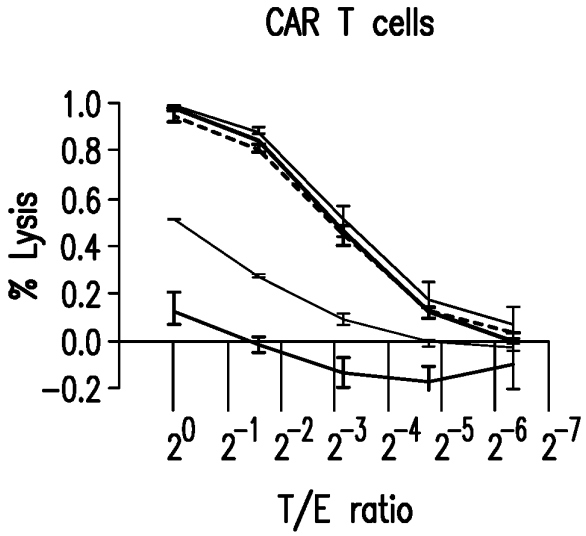

FIG. 8B

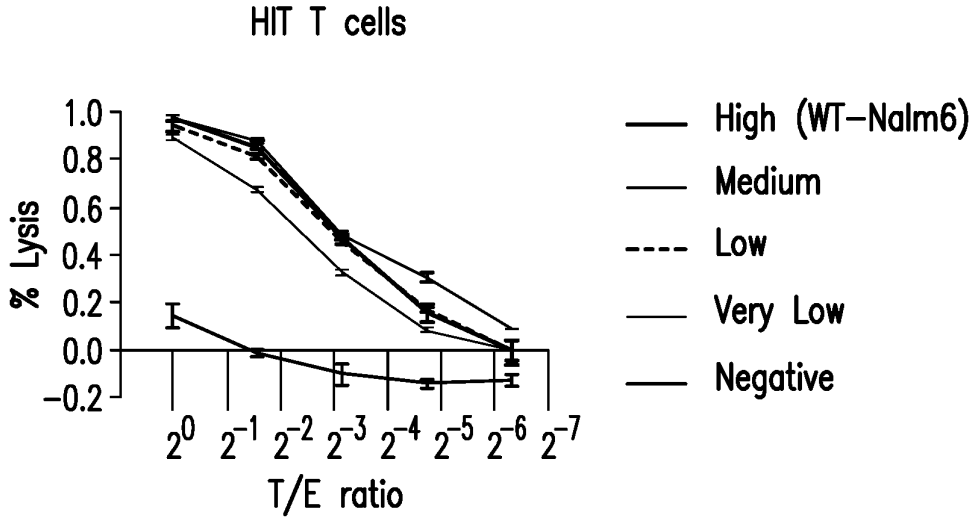

FIG. 8C

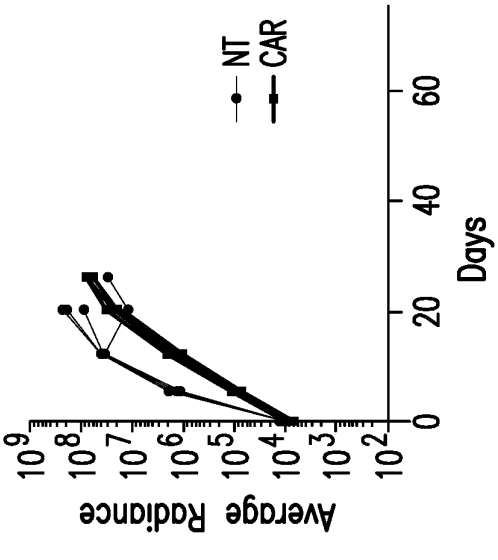

FIG. 9A

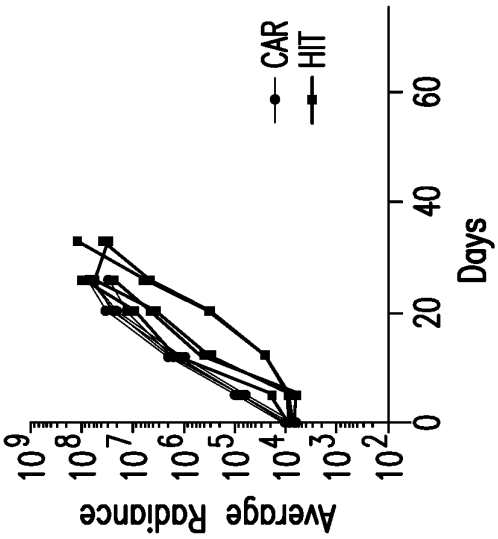

FIG. 9B

FIG. 9C

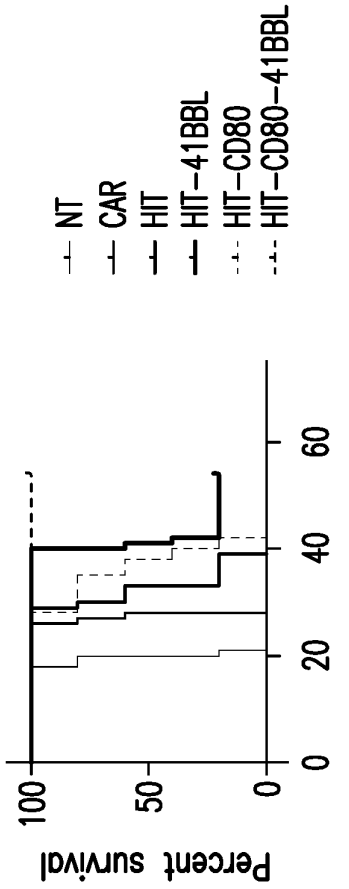

FIG. 9D

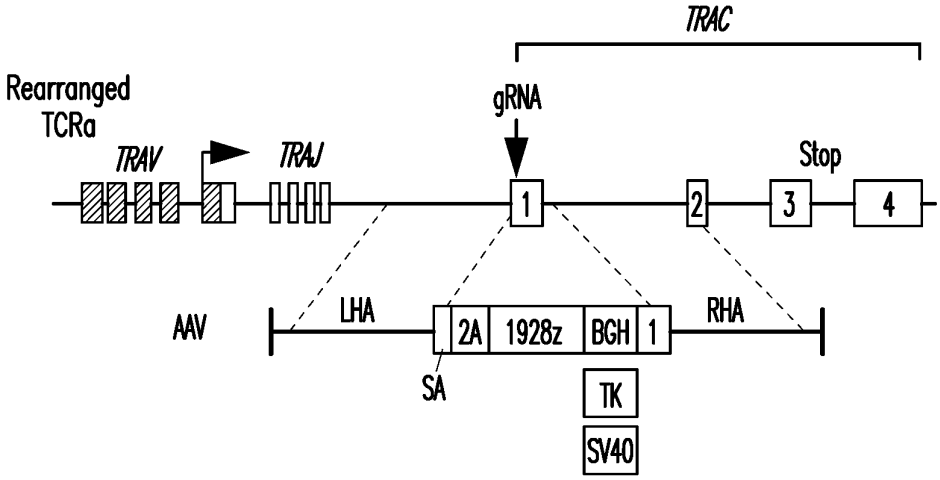

FIG. 10A

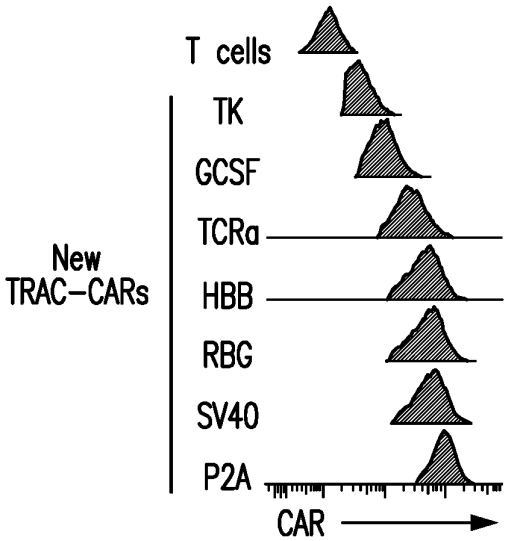

FIG. 10B

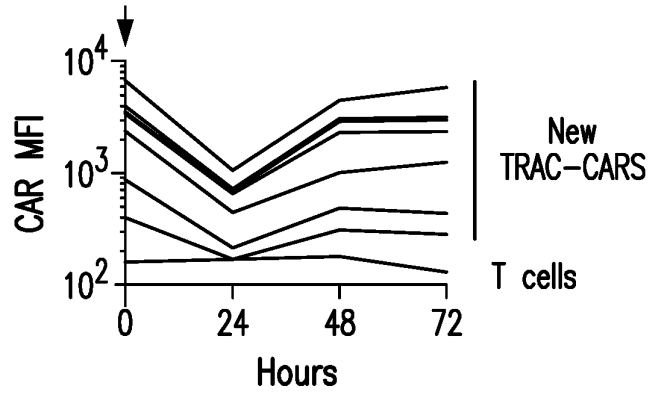

FIG. 10C