
5' TRAC
HA (660bp)
JeT
CAR INSERT
3' TRAC
HA (650bp)
pAAV VECTOR

FIG. 1A

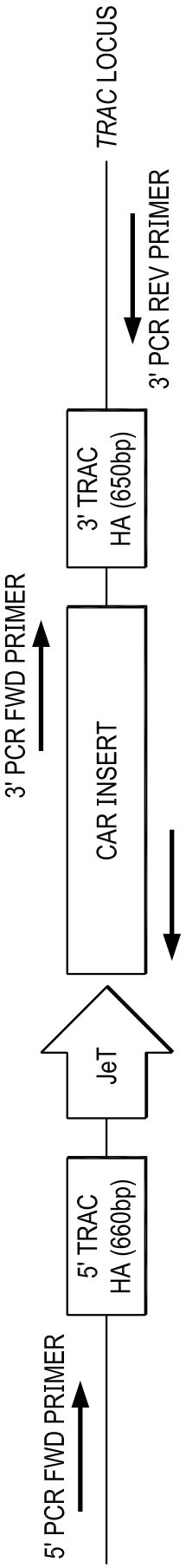

5' PCR FWD PRIMER
5' TRAC
HA (660bp)
JeT
CAR INSERT
5' PCR REV PRIMER
3' PCR FWD PRIMER
3' TRAC
HA (650bp)
3' PCR REV PRIMER
TRAC LOCUS

FIG. 1B

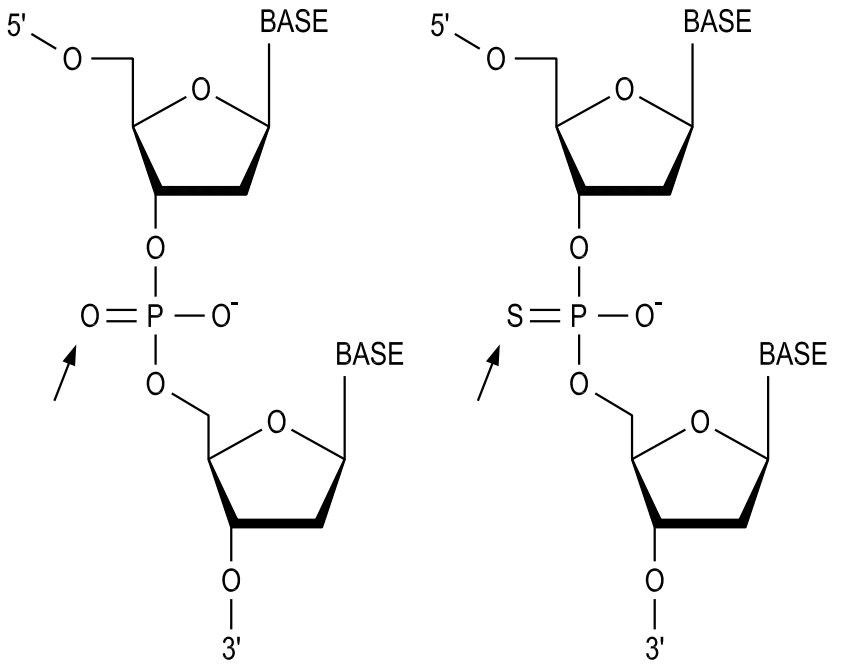

5'
BASE
O
O
O
O=P—O⁻
O
BASE
O
O
3'
PHOSPHODIESTER BOND
5'
BASE
O
O
O
S=P—O⁻
O
BASE
O
O
3'
PHOSPHOROTHIOATE BOND

FIG. 2A

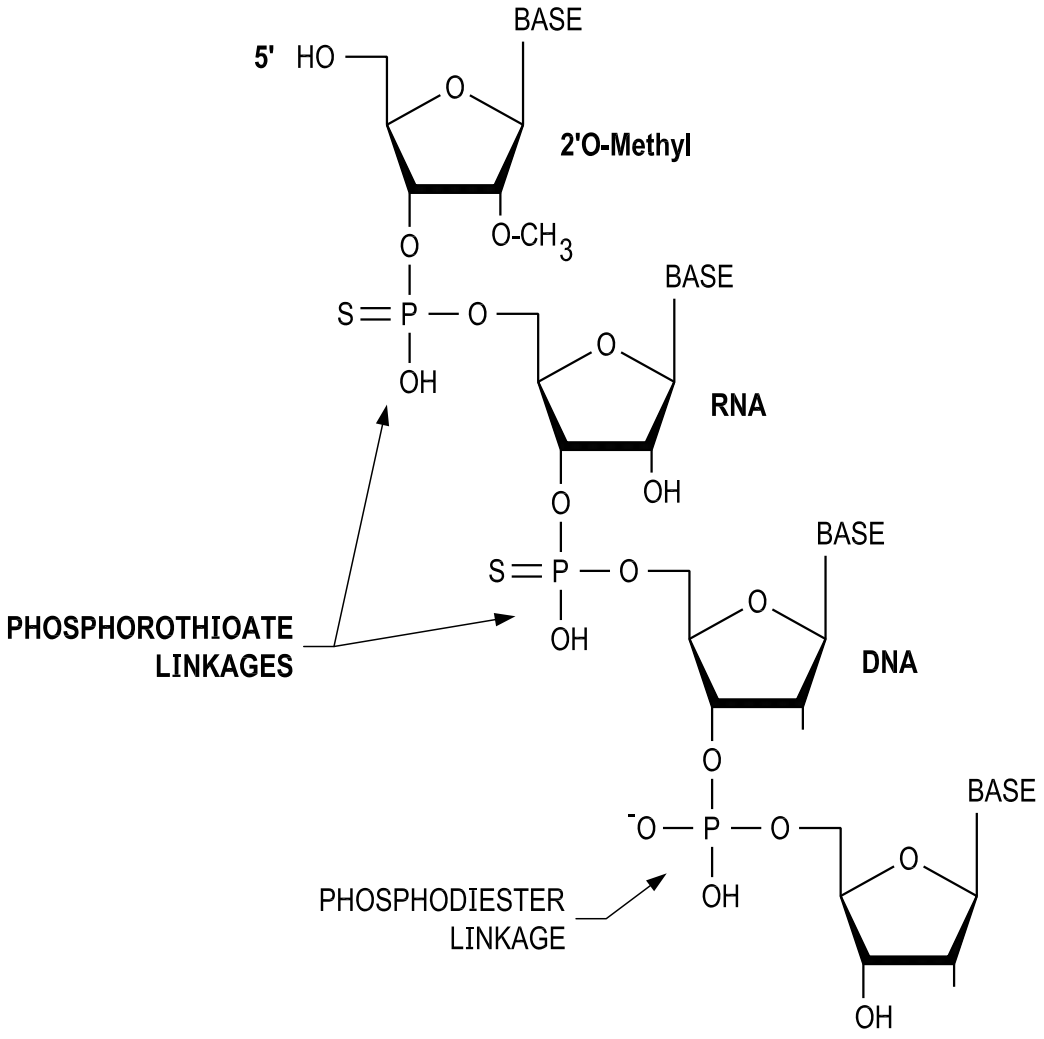

5' HO
BASE
O
2'O-Methyl
O-CH3
O
S=P—O
OH
BASE
O
RNA
OH
O
S=P—O
OH
BASE
O
DNA
PHOSPHOROTHIOATE LINKAGES
O
-O—P—O
OH
BASE
O
OH
PHOSPHODIESTER LINKAGE

FIG. 2B

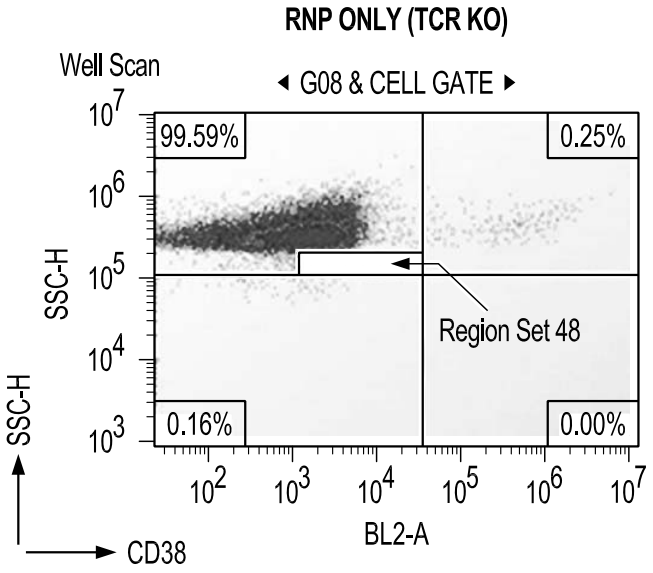


FIG. 3A

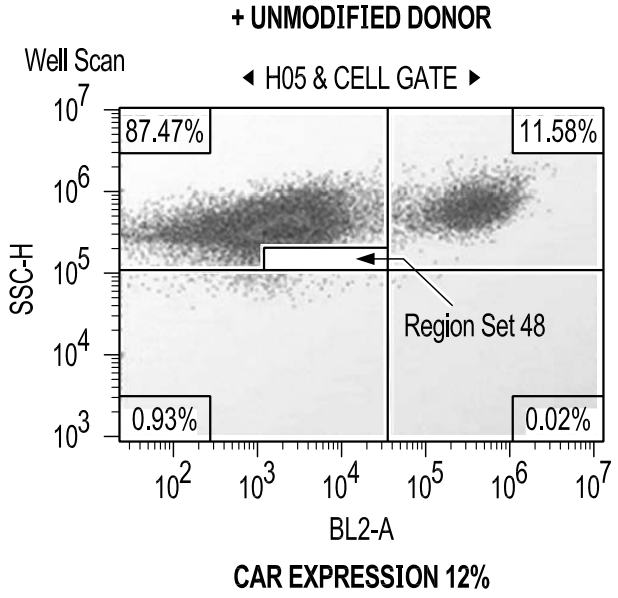


FIG. 3B

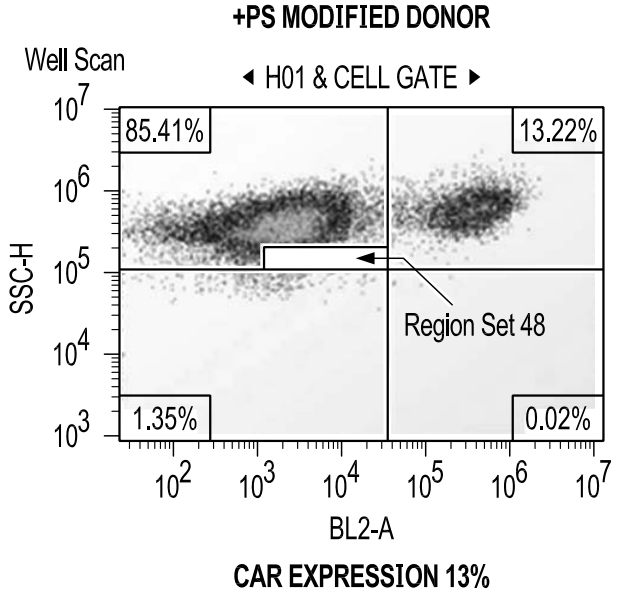


FIG. 3C

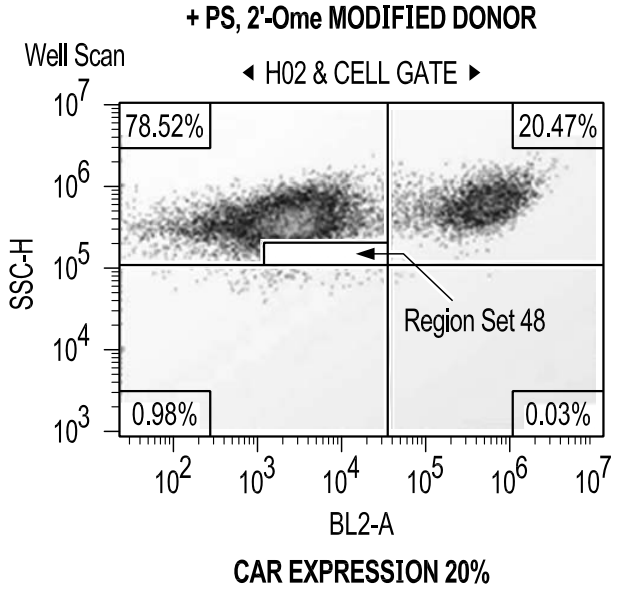

+ PS, 2'-Ome MODIFIED DONOR
Well Scan
◄ H02 & CELL GATE ►
78.52%
20.47%
Region Set 48
0.98%
0.03%
SSC-H
BL2-A
CAR EXPRESSION 20%

FIG. 3D

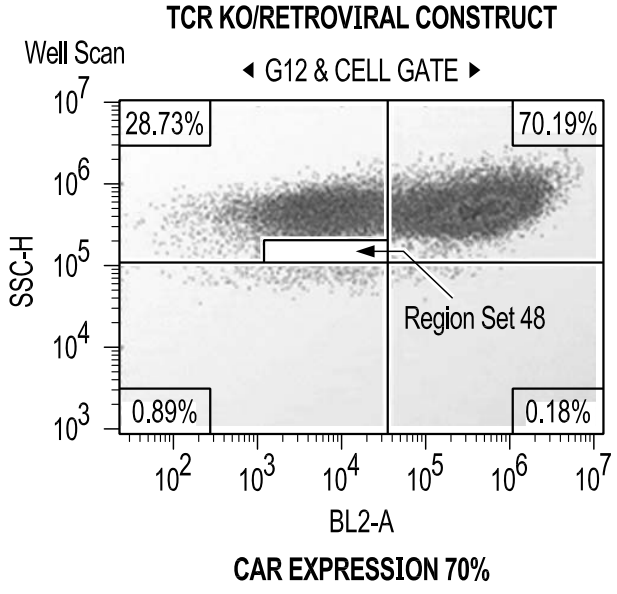

TCR KO/RETROVIRAL CONSTRUCT
Well Scan
◄ G12 & CELL GATE ►
28.73%
70.19%
Region Set 48
0.89%
0.18%
SSC-H
BL2-A
CAR EXPRESSION 70%

FIG. 3E

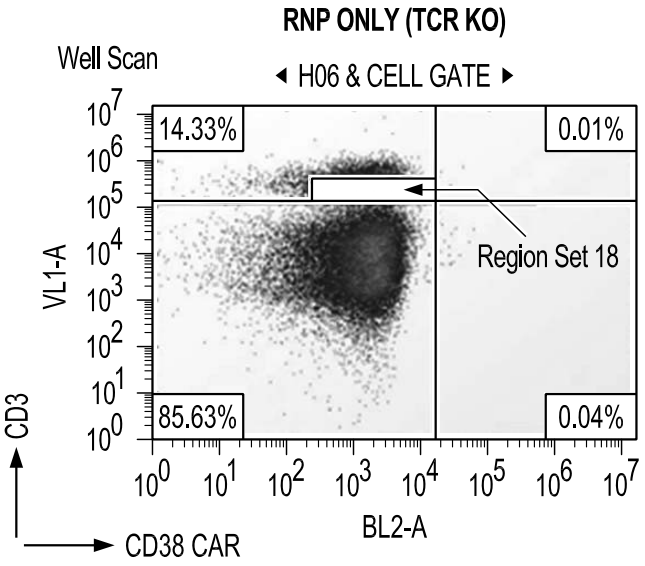


FIG. 3F

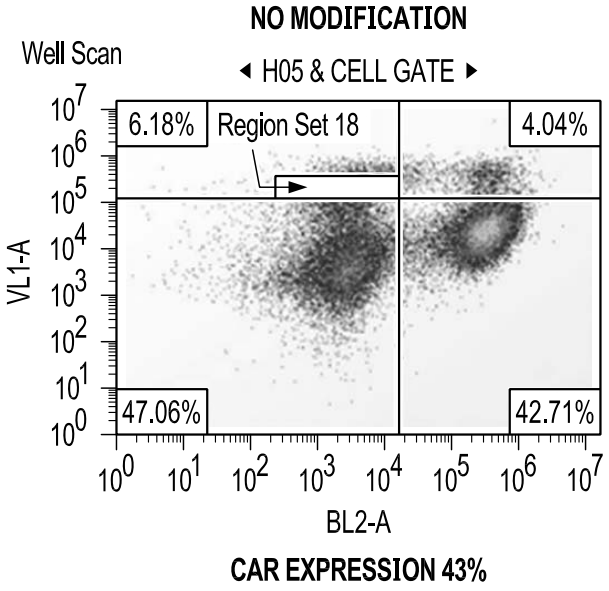


FIG. 3G

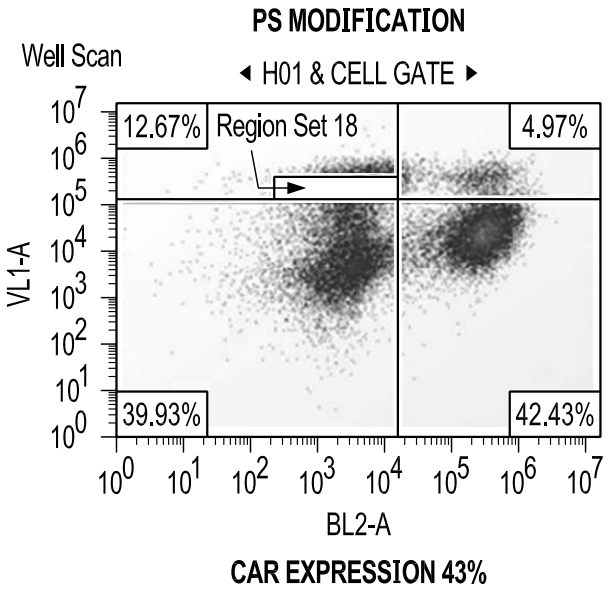


FIG. 3H

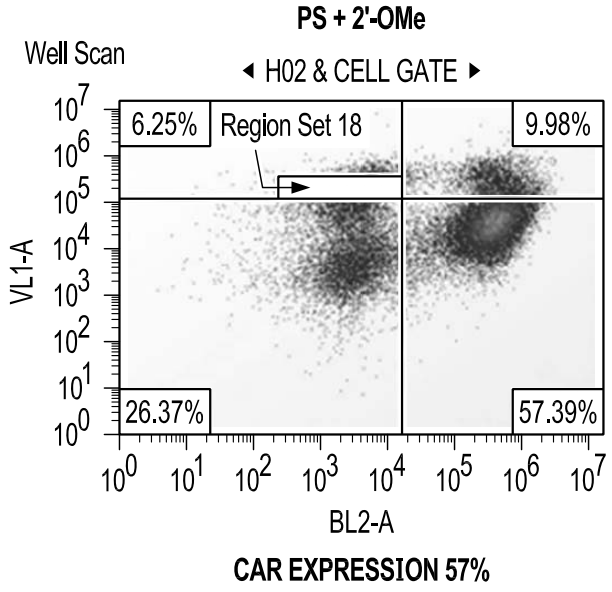

PS + 2'-OMe
Well Scan
H02 & CELL GATE
6.25%
Region Set 18
9.98%
26.37%
57.39%
VL1-A
BL2-A
CAR EXPRESSION 57%

FIG. 3I

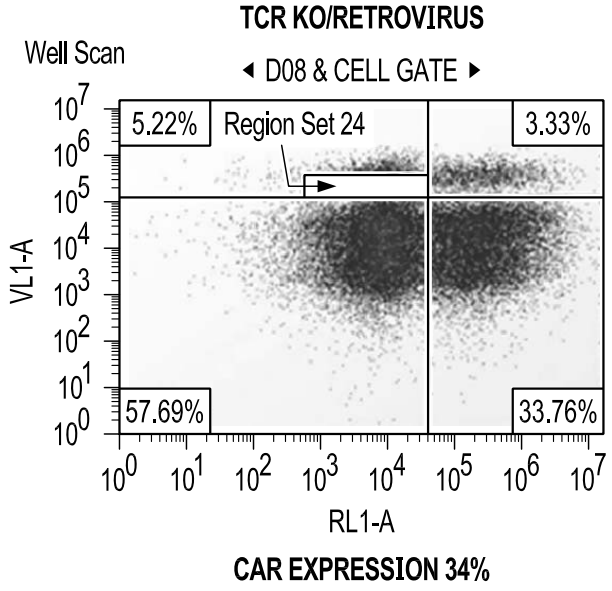

TCR KO/RETROVIRUS
Well Scan
D08 & CELL GATE
5.22%
Region Set 24
3.33%
57.69%
33.76%
VL1-A
RL1-A
CAR EXPRESSION 34%

FIG. 3J

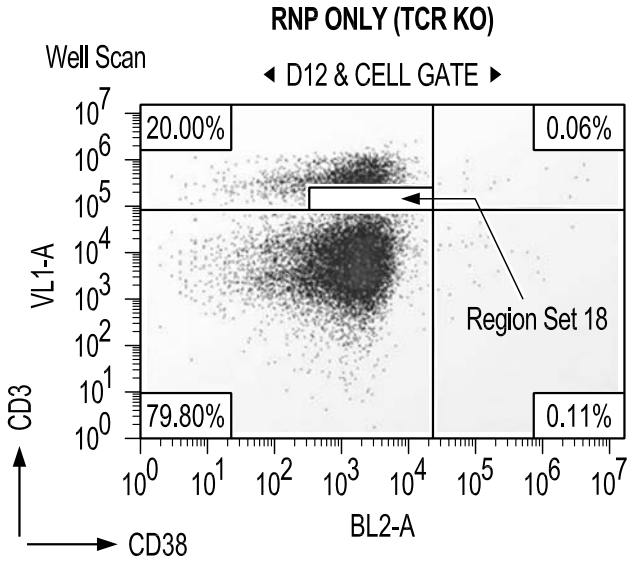


FIG. 3K

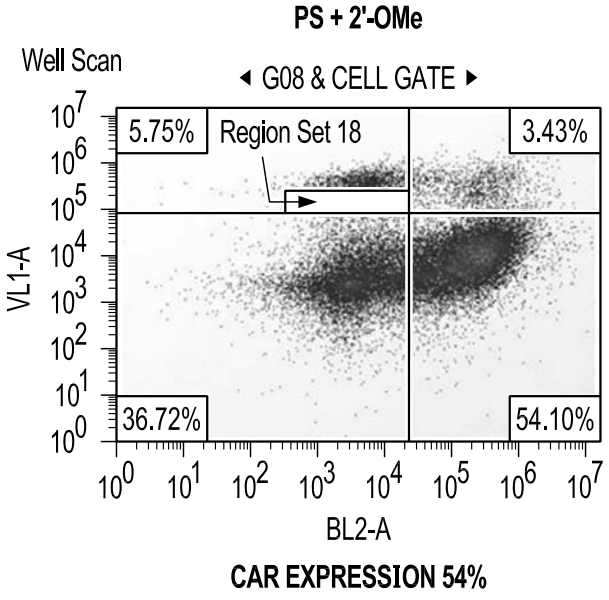


FIG. 3L

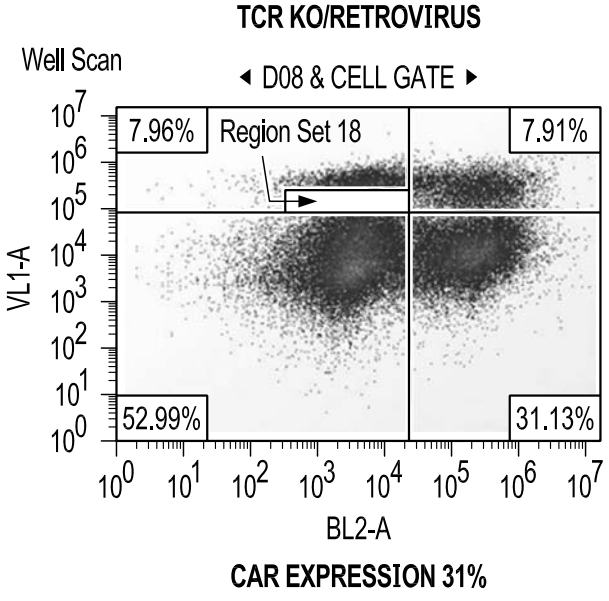


FIG. 3M

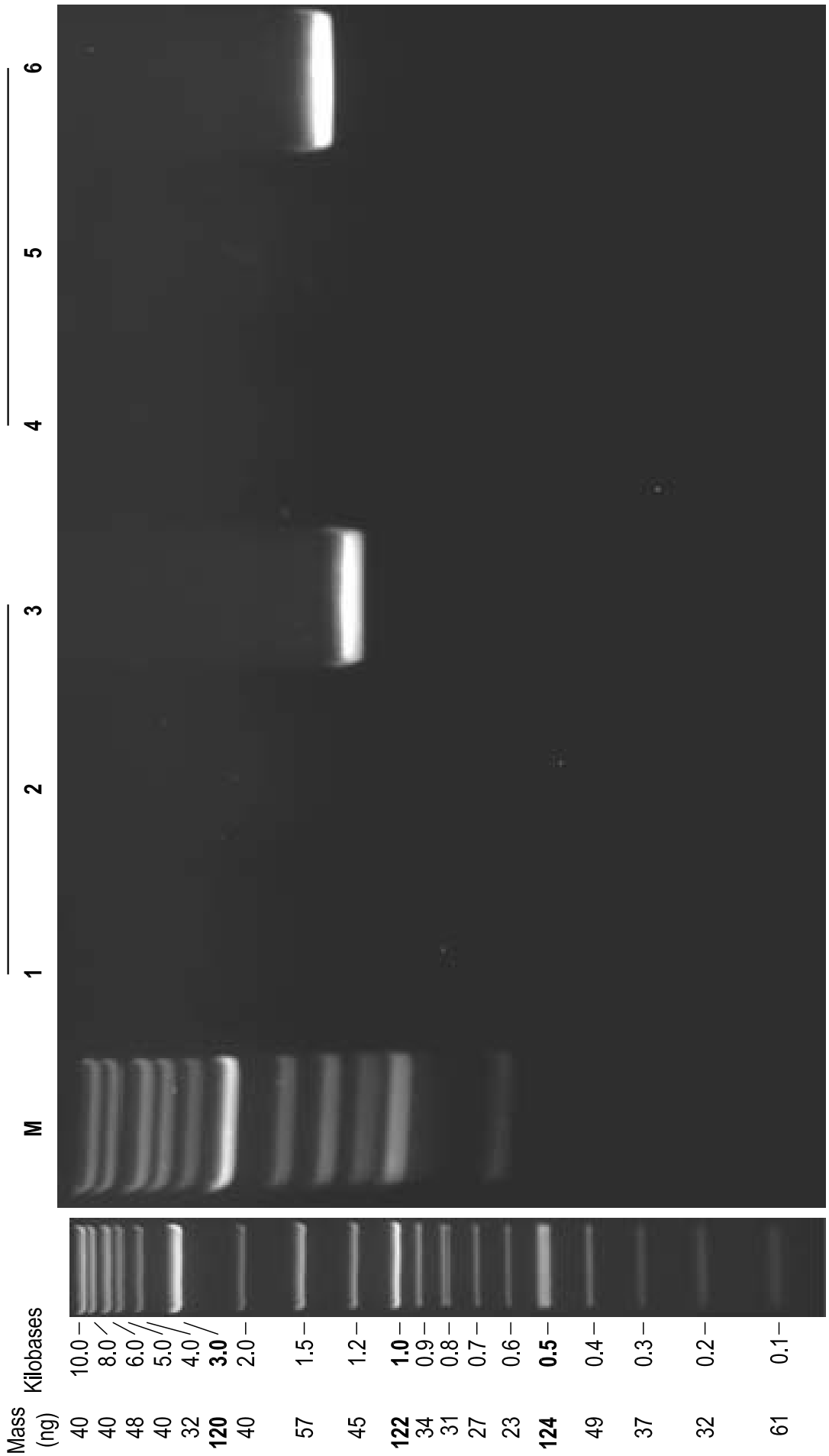

5' HA
3' HA
M
1
2
3
4
5
6
Mass (ng)
Kilobases
40 10.0
40 8.0
48 6.0
40 5.0
32 4.0
120 3.0
40 2.0
57 1.5
45 1.2
122 1.0
34 0.9
31 0.8
27 0.7
23 0.6
124 0.5
49 0.4
37 0.3
32 0.2
61 0.1

FIG. 4

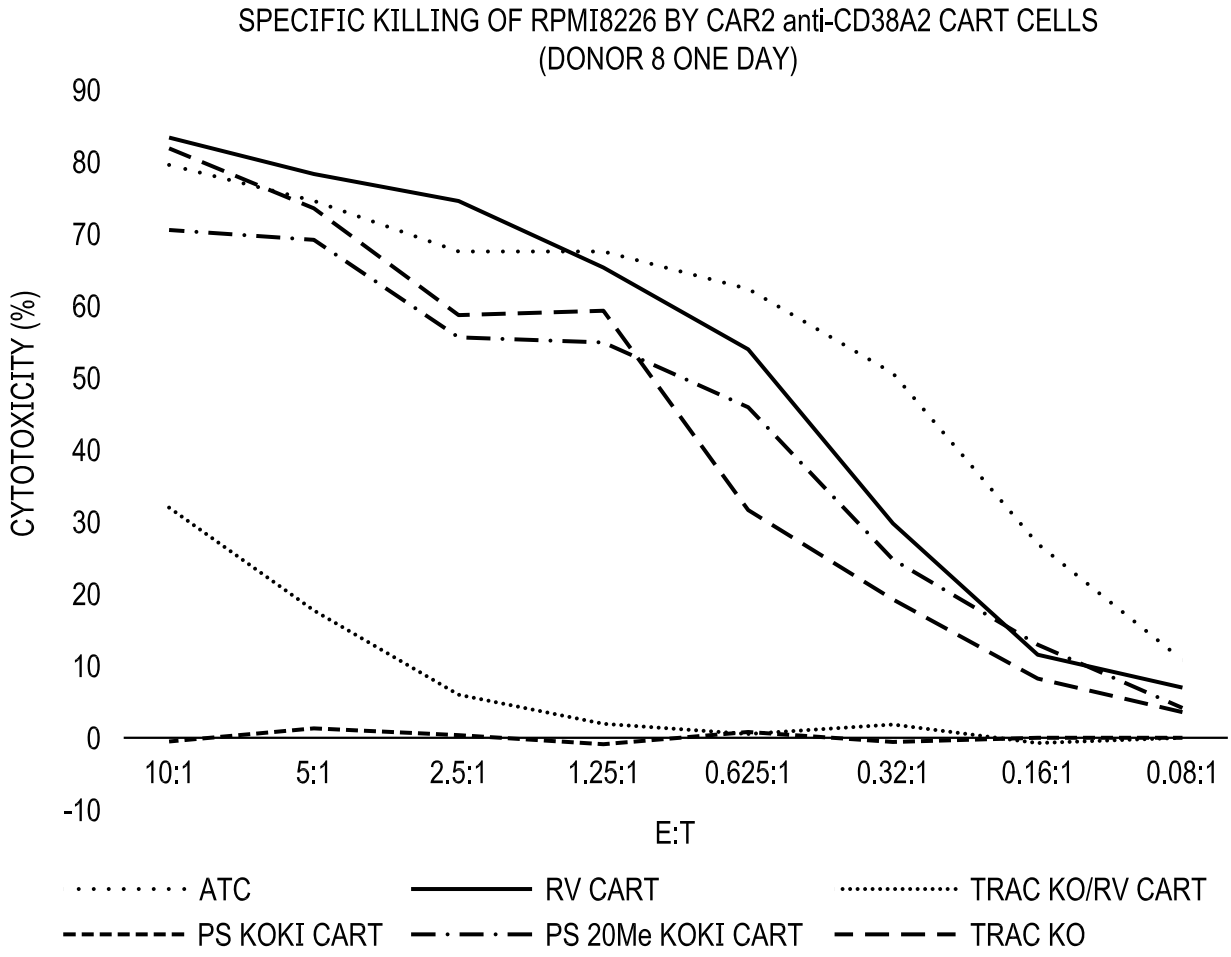

SPECIFIC KILLING OF RPMI8226 BY CAR2 anti-CD38A2 CART CELLS
(DONOR 8 ONE DAY)
CYTOTOXICITY (%)
90
80
70
60
50
40
30
20
10
0
-10
10:1
5:1
2.5:1
1.25:1
0.625:1
0.32:1
0.16:1
0.08:1
E:T
ATC
RV CART
TRAC KO/RV CART
PS KOKI CART
PS 20Me KOKI CART
TRAC KO

FIG. 5

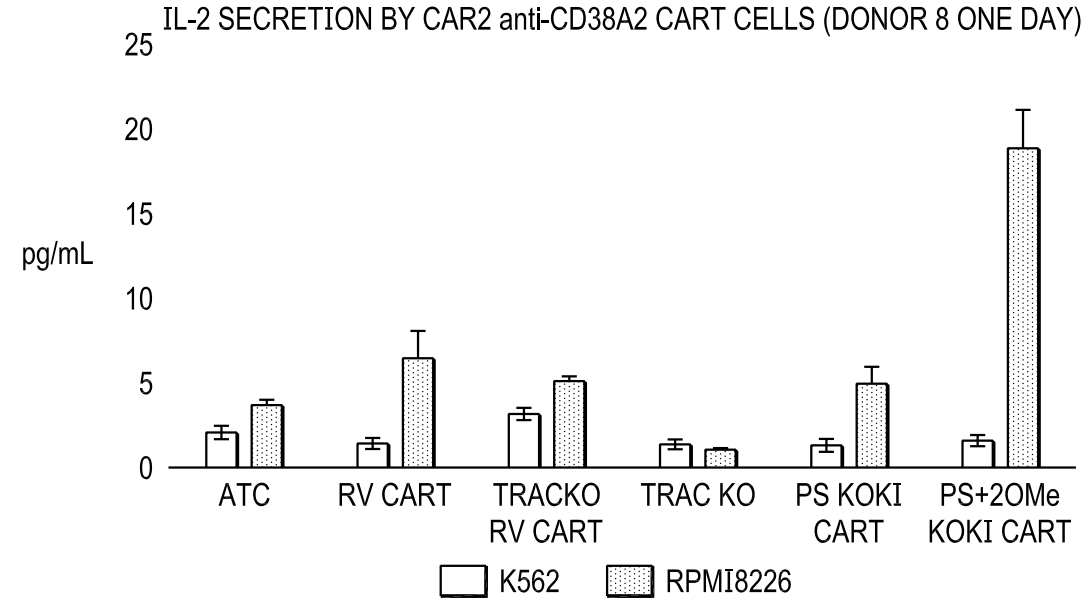


FIG. 6A

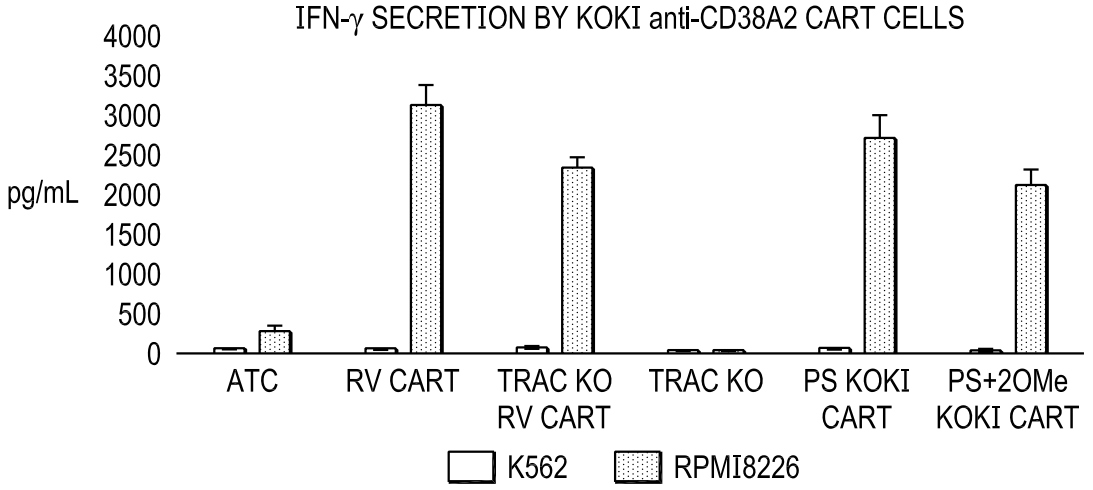


FIG. 6B

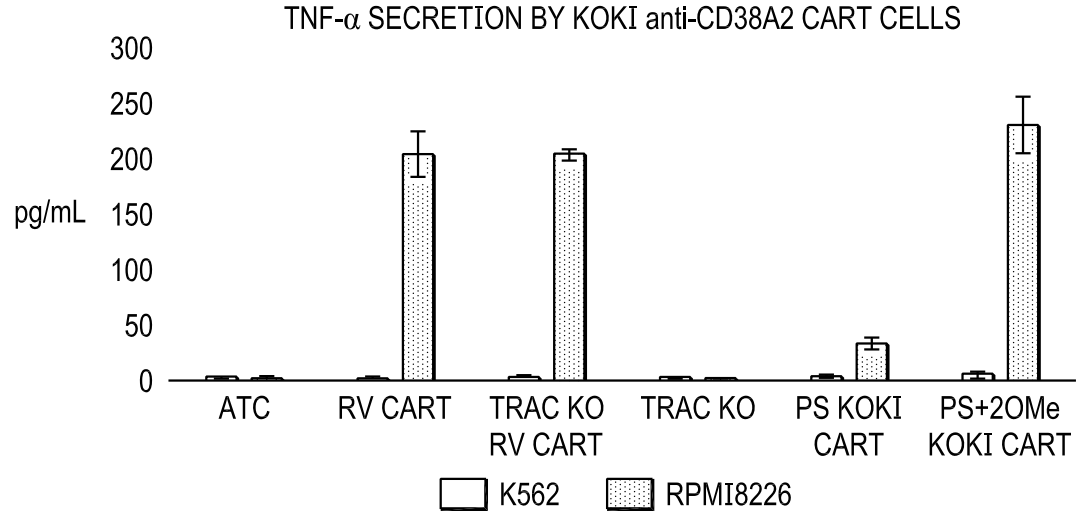


FIG. 6C

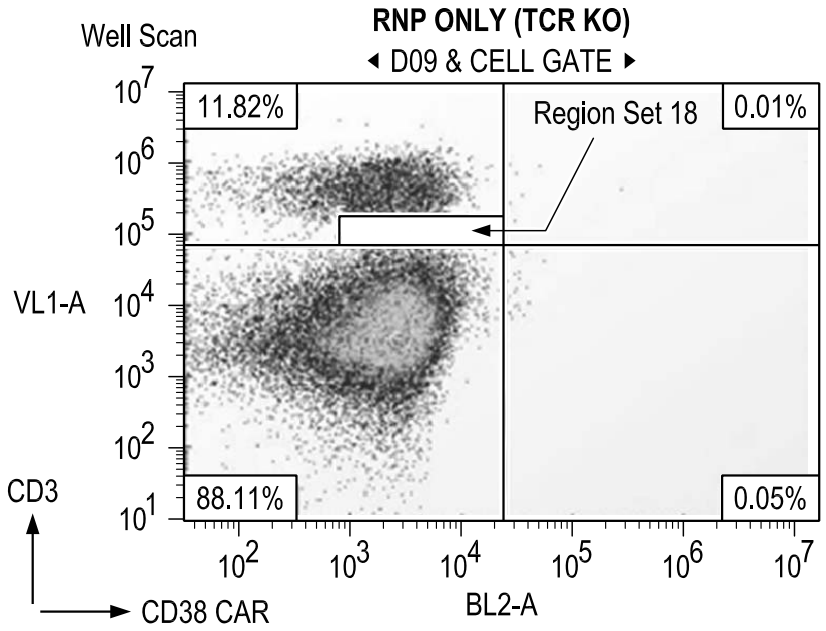

RNP ONLY (TCR KO)
Well Scan
◄ D09 & CELL GATE ►
11.82%
Region Set 18
0.01%
88.11%
0.05%
VL1-A
CD3
CD38 CAR
BL2-A

FIG. 7A

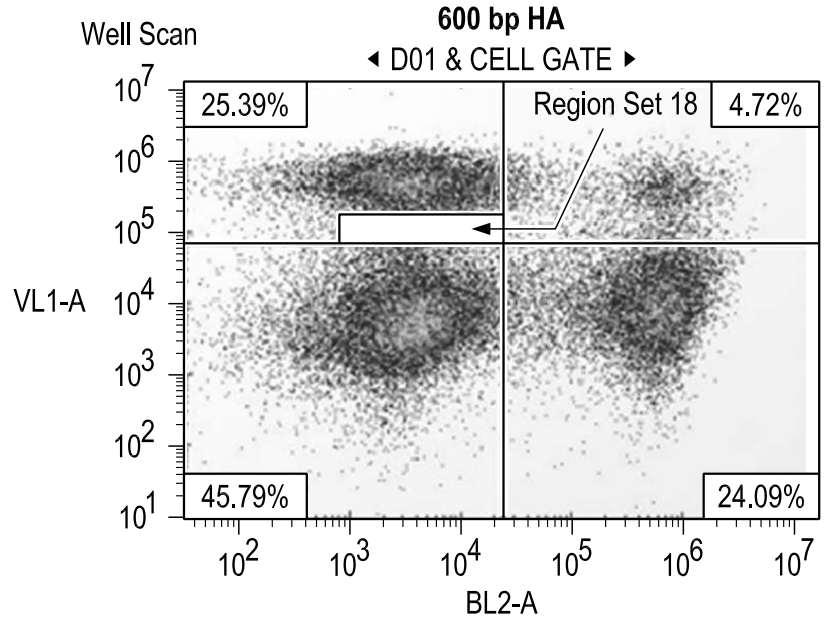

600 bp HA
Well Scan
◄ D01 & CELL GATE ►
25.39%
Region Set 18
4.72%
45.79%
24.09%
VL1-A
BL2-A

FIG. 7B

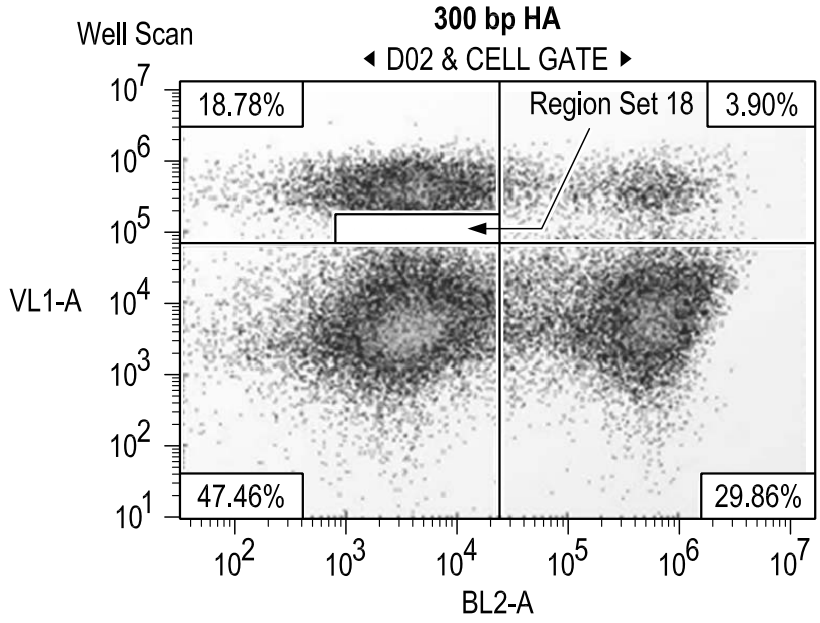

Well Scan
300 bp HA
D02 & CELL GATE
18.78%
Region Set 18
3.90%
47.46%
29.86%
VL1-A
BL2-A
10^1
10^2
10^3
10^4
10^5
10^6
10^7

FIG. 7C

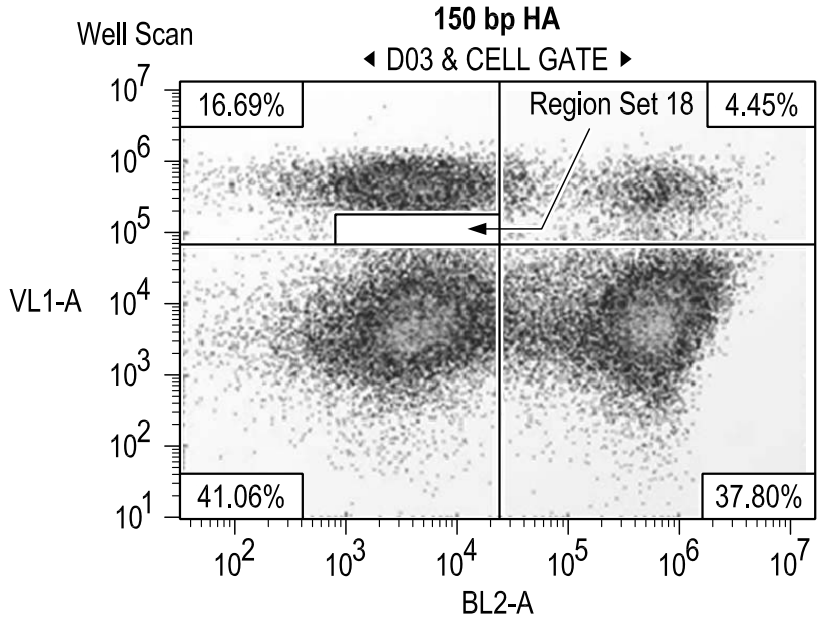

Well Scan
150 bp HA
D03 & CELL GATE
16.69%
Region Set 18
4.45%
41.06%
37.80%
VL1-A
BL2-A
10^1
10^2
10^3
10^4
10^5
10^6
10^7

FIG. 7D

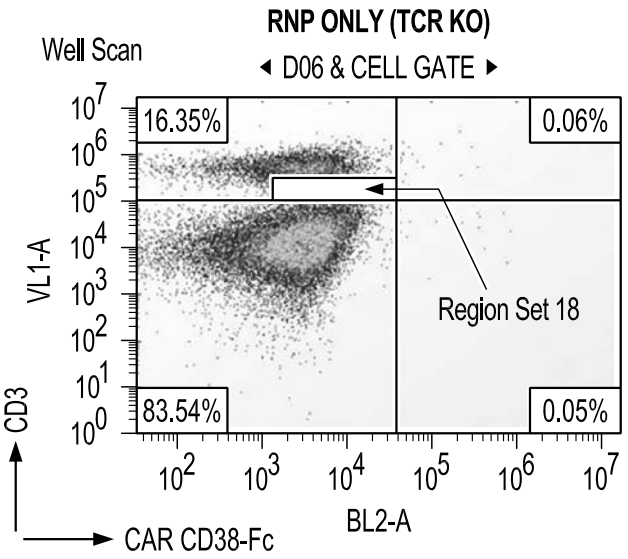


FIG. 8A

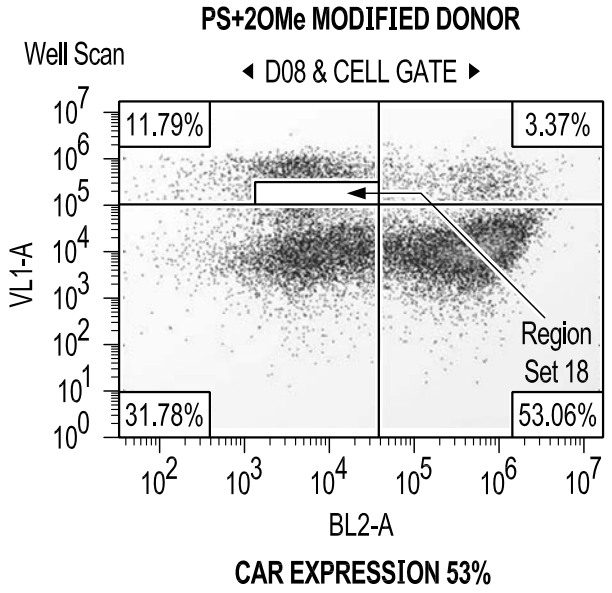


FIG. 8B

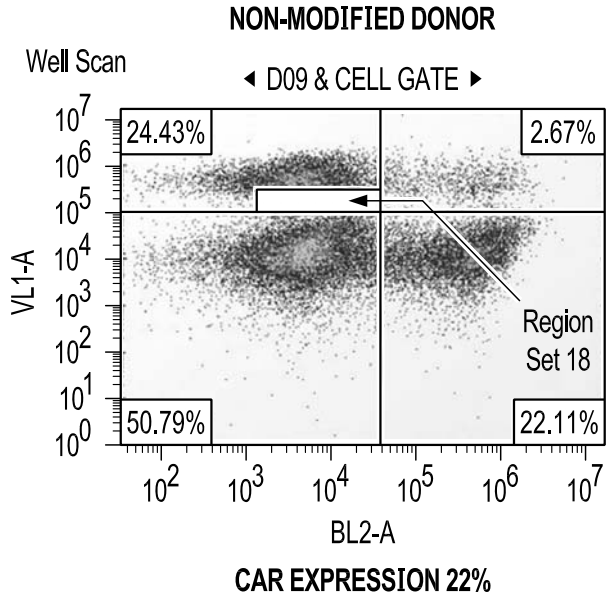


FIG. 8C

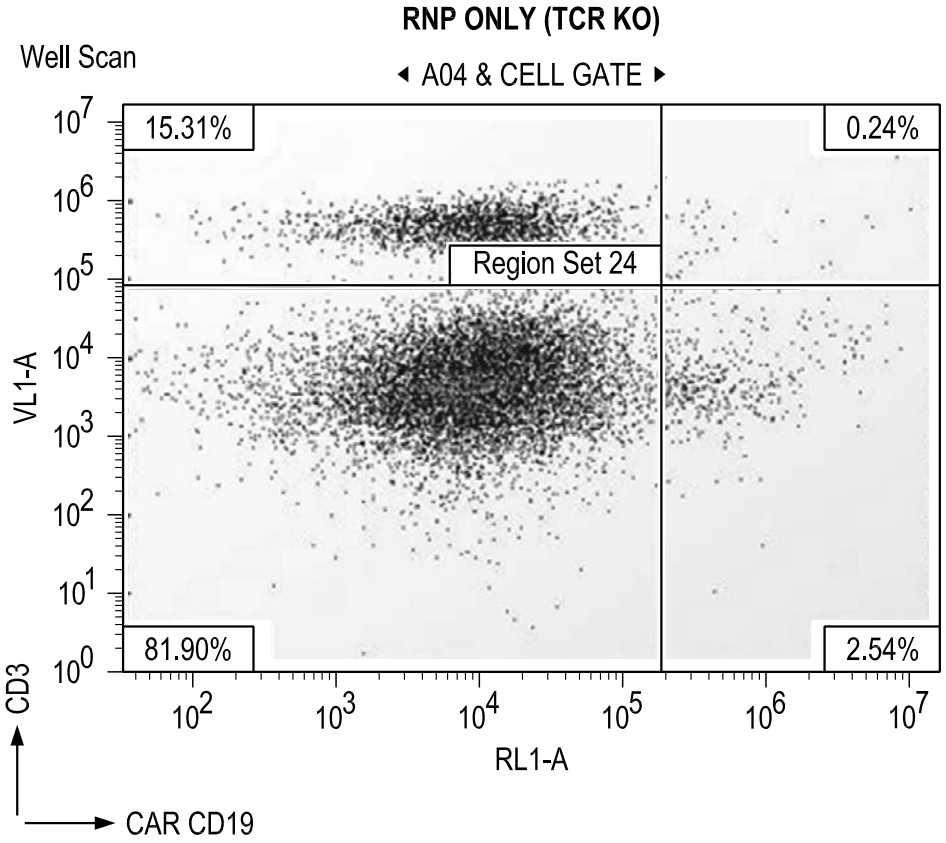

RNP ONLY (TCR KO)
Well Scan
A04 & CELL GATE
15.31%
0.24%
Region Set 24
81.90%
2.54%
VL1-A
RL1-A
CD3
CAR CD19

FIG. 9A

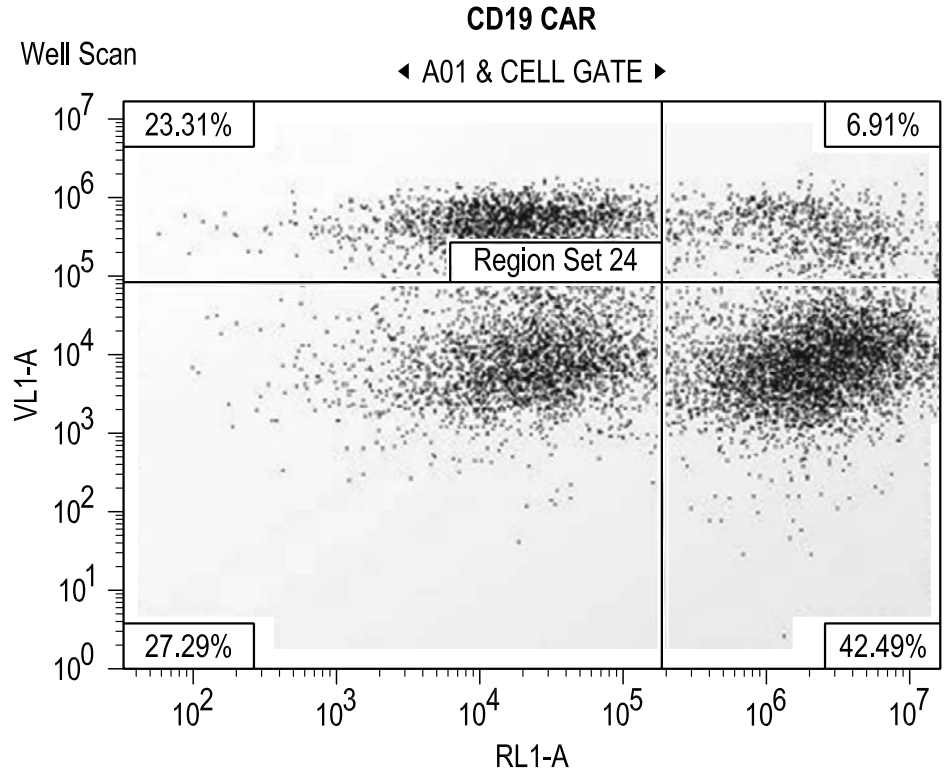

CD19 CAR
Well Scan
A01 & CELL GATE
23.31%
6.91%
Region Set 24
27.29%
42.49%
VL1-A
RL1-A

FIG. 9B

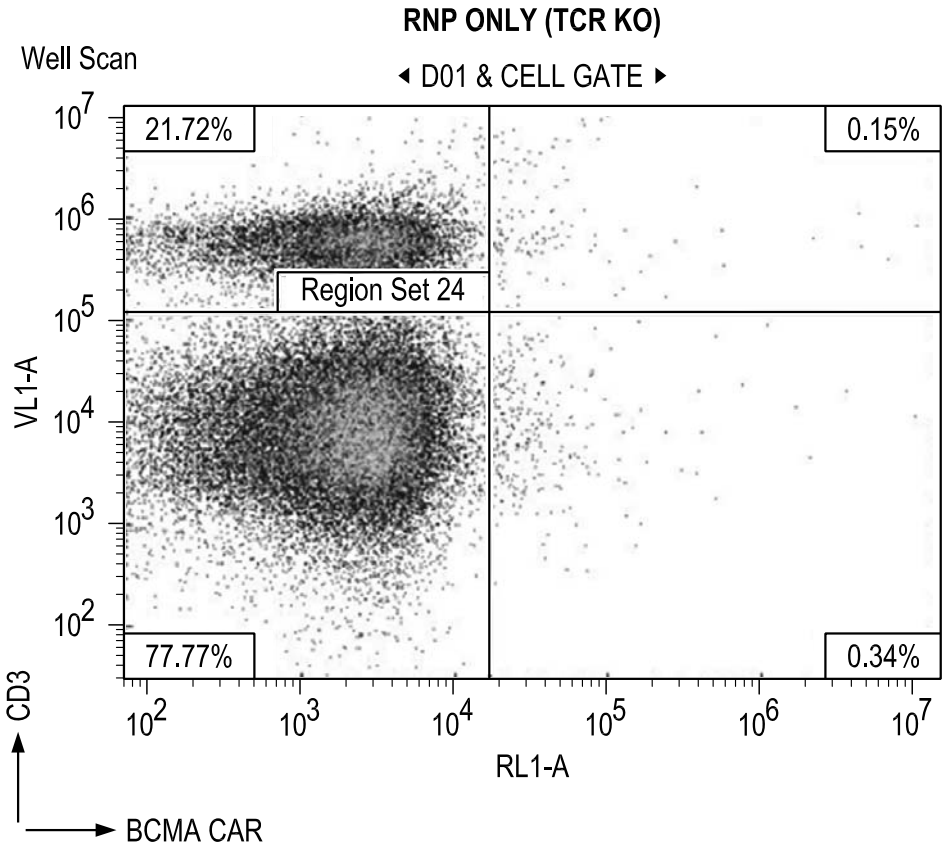


FIG. 10A

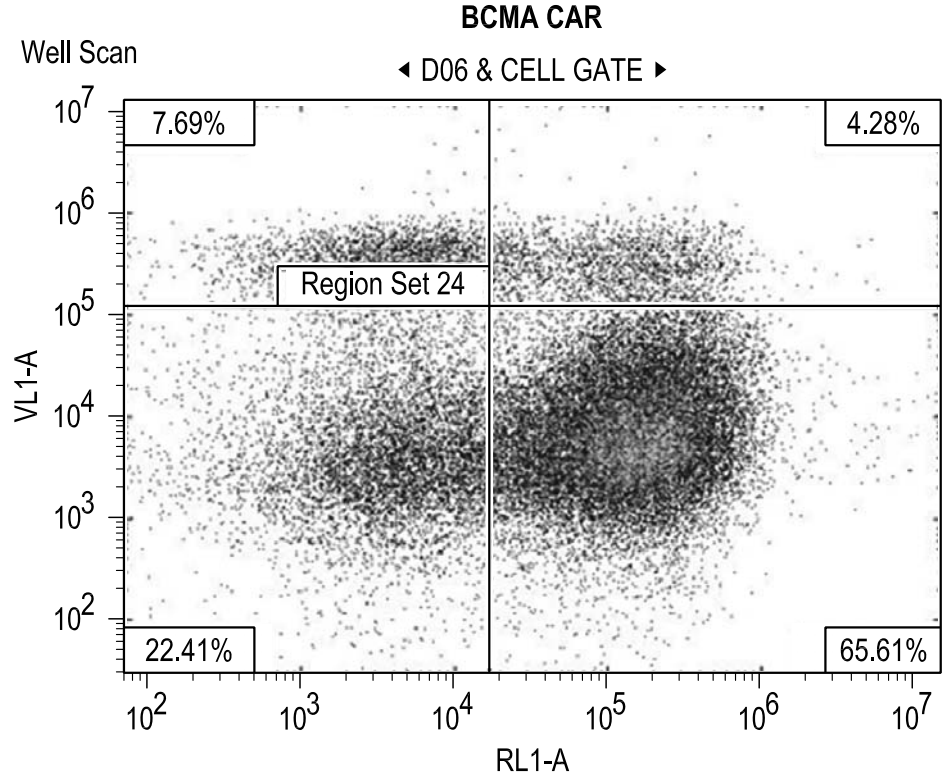


FIG. 10B

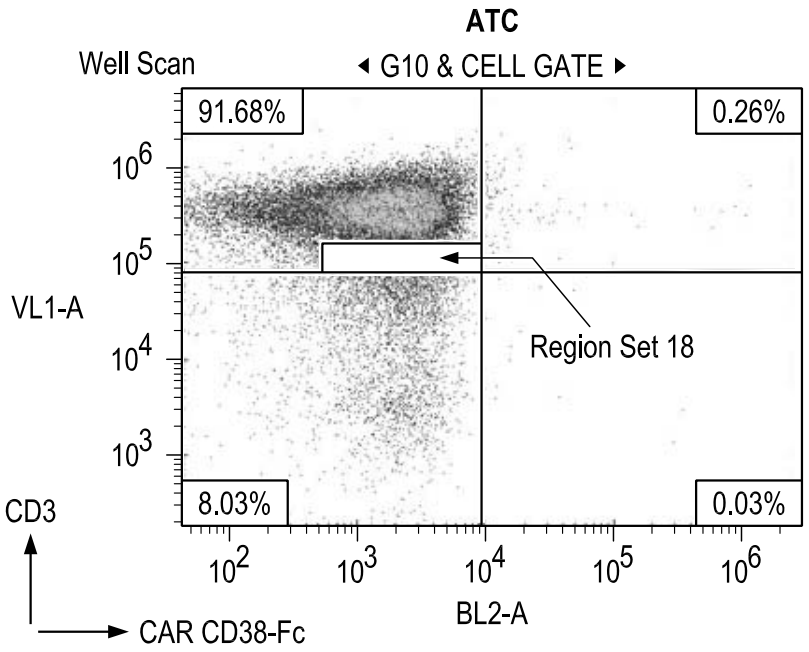


FIG. 11A

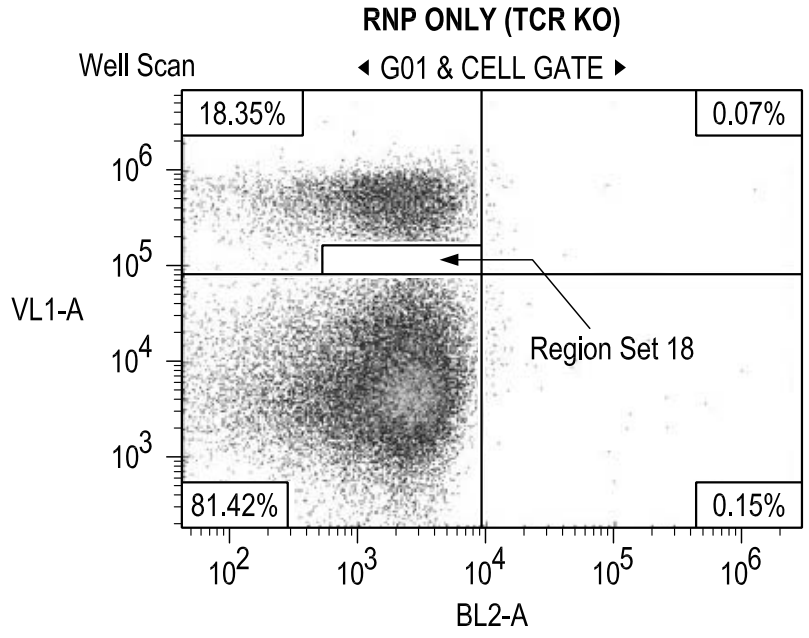


FIG. 11B

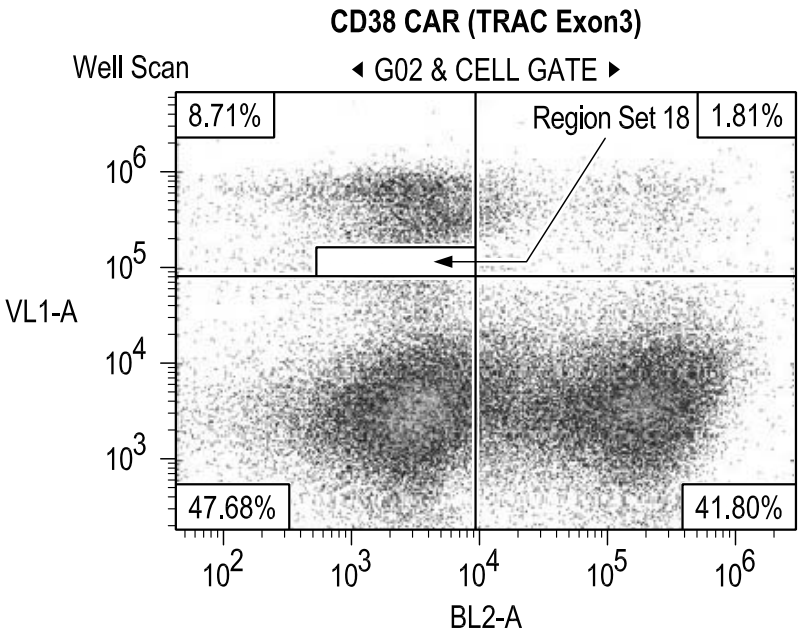

CD38 CAR (TRAC Exon3)
Well Scan
◂ G02 & CELL GATE ▸
8.71%
Region Set 18
1.81%
47.68%
41.80%
VL1-A
BL2-A
10^2
10^3
10^4
10^5
10^6

FIG. 11C

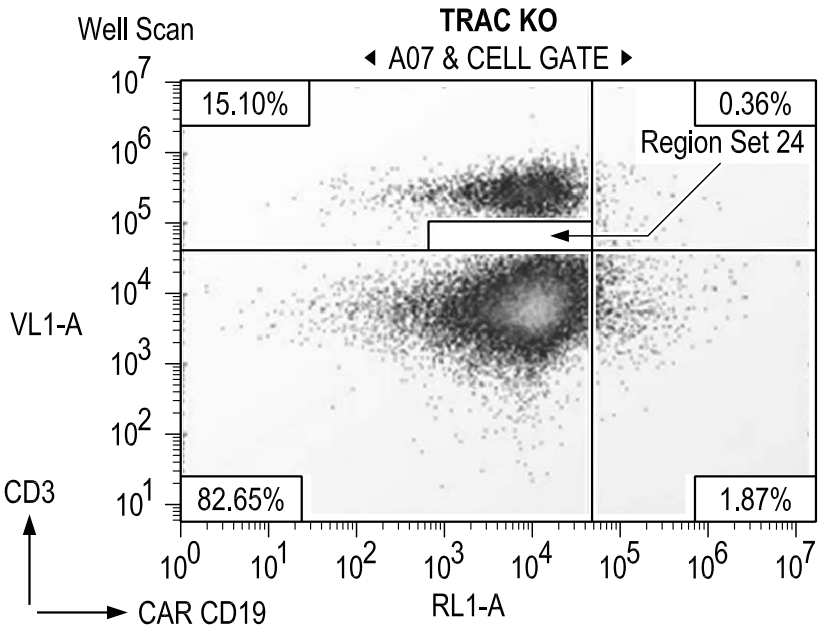


FIG. 12A

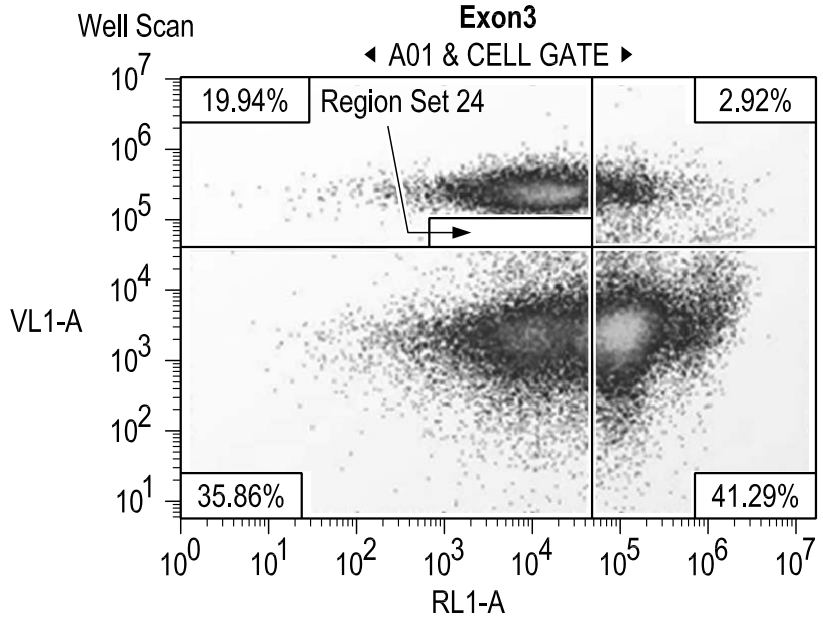


FIG. 12B

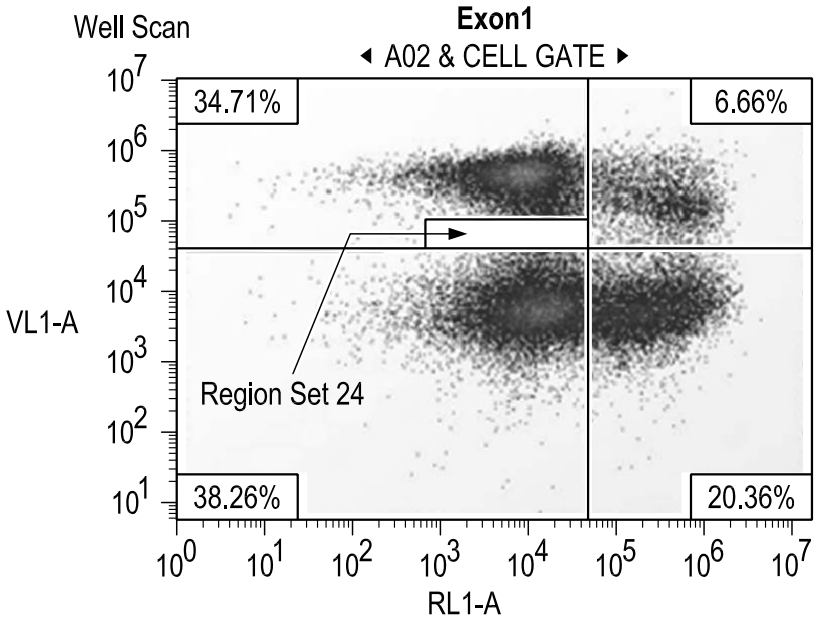


FIG. 12C

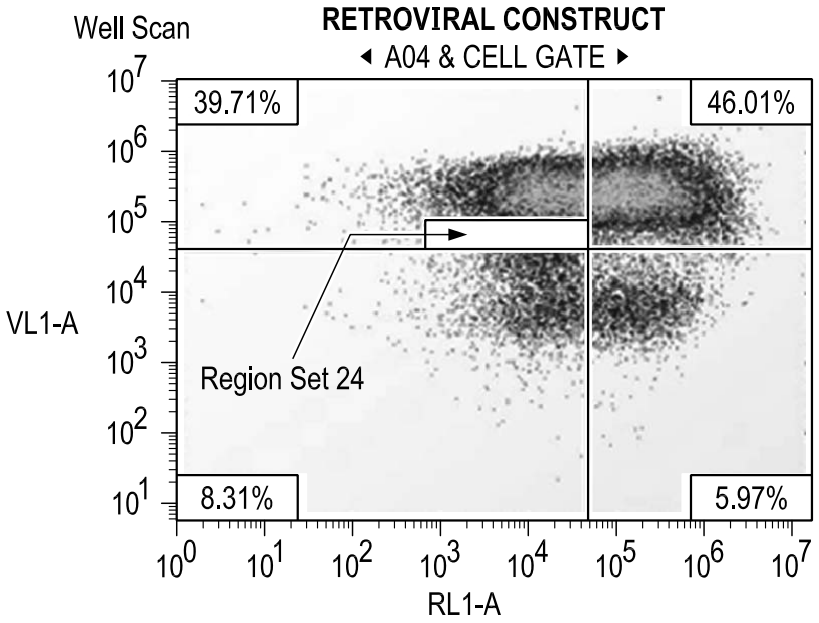


FIG. 12D

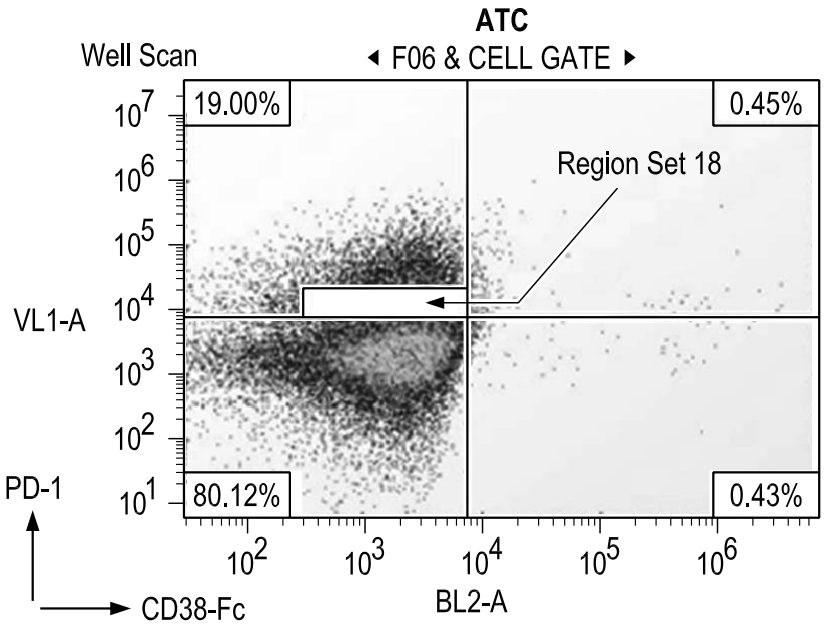


FIG. 13A

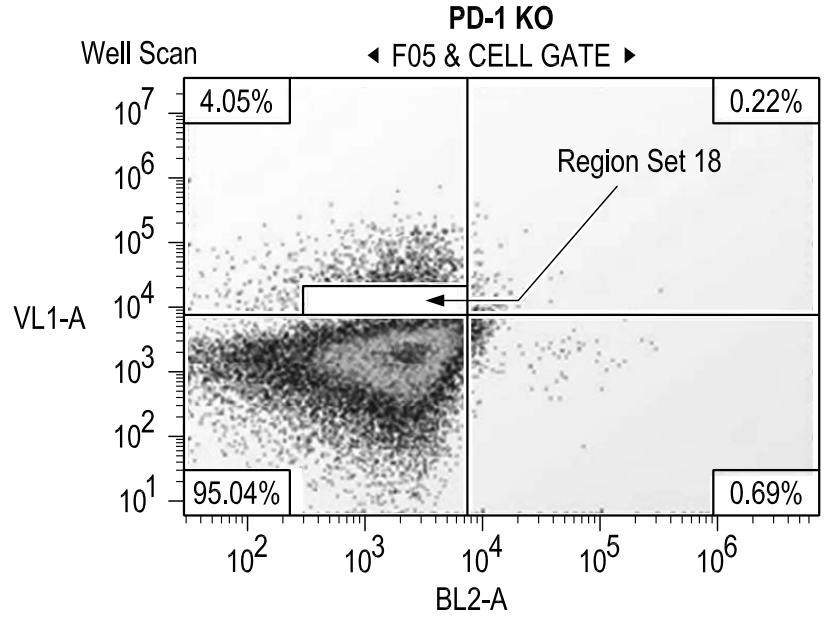


FIG. 13B

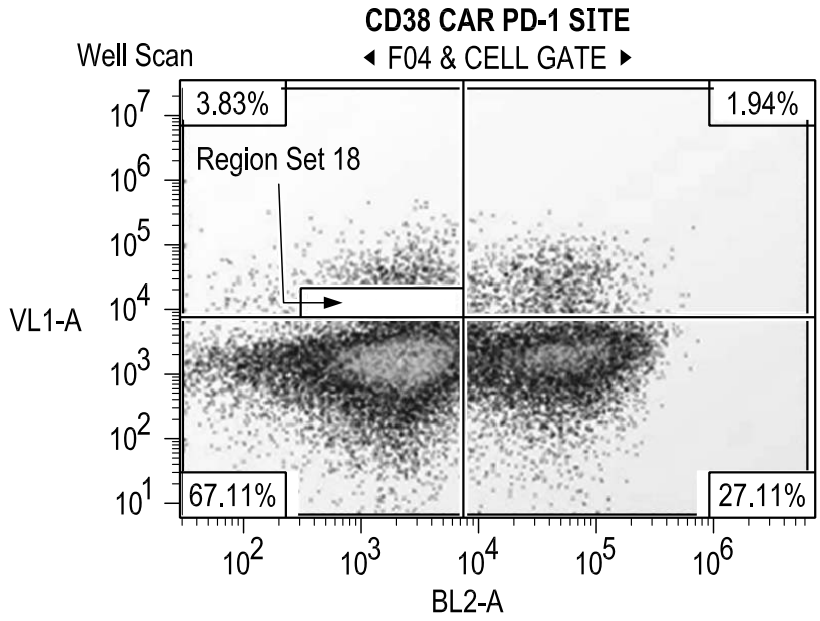


FIG. 13C

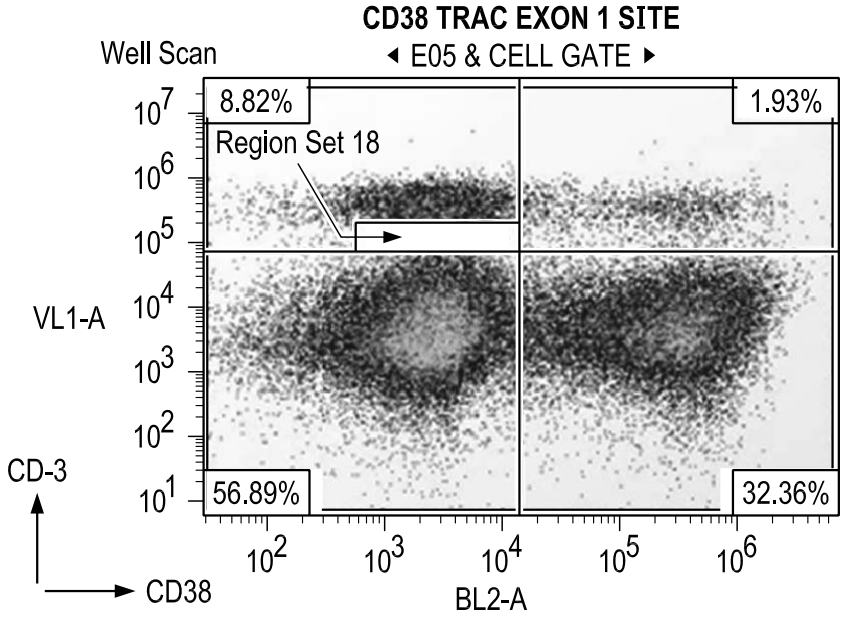


FIG. 13D

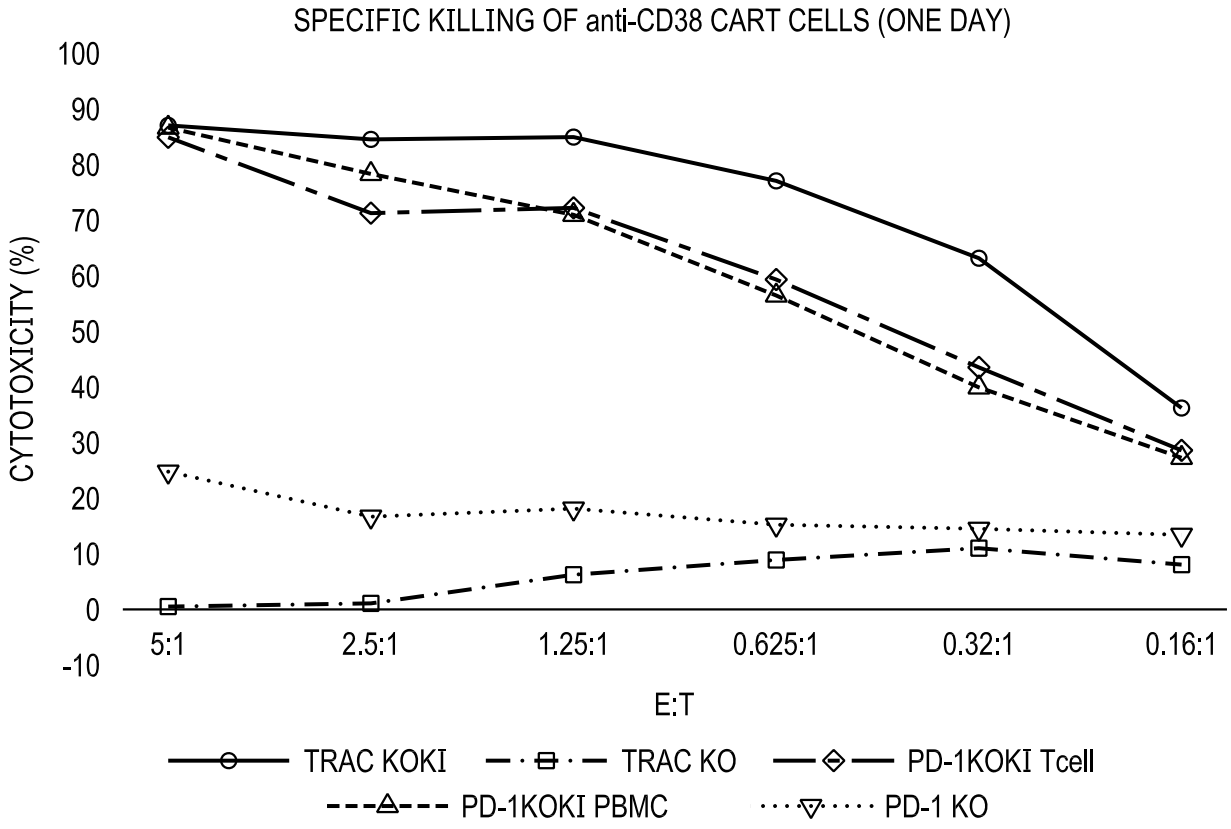

SPECIFIC KILLING OF anti-CD38 CART CELLS (ONE DAY)
CYTOTOXICITY (%)
100
90
80
70
60
50
40
30
20
10
0
-10
5:1
2.5:1
1.25:1
0.625:1
0.32:1
0.16:1
E:T
TRAC KOKI
TRAC KO
PD-1KOKI Tcell
PD-1KOKI PBMC
PD-1 KO

FIG. 14

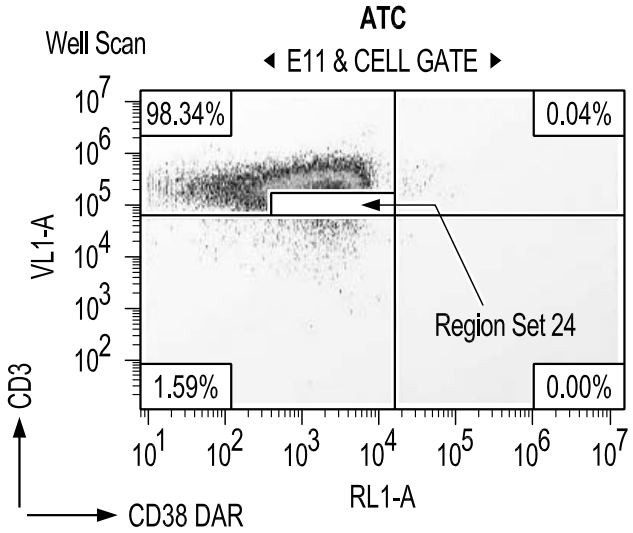


FIG. 15A

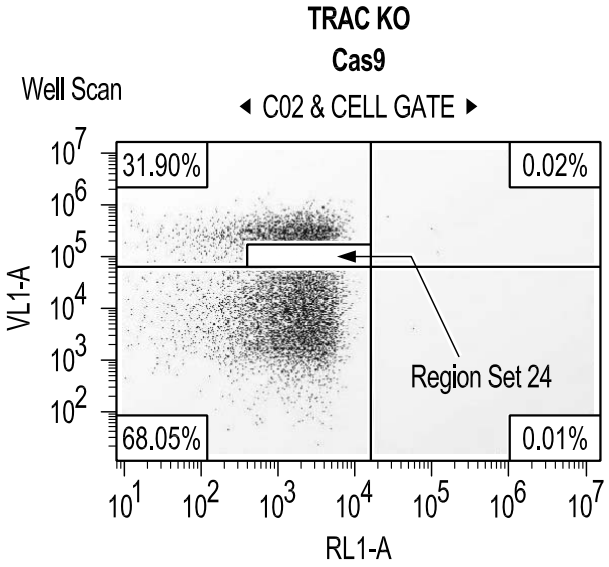


FIG. 15B

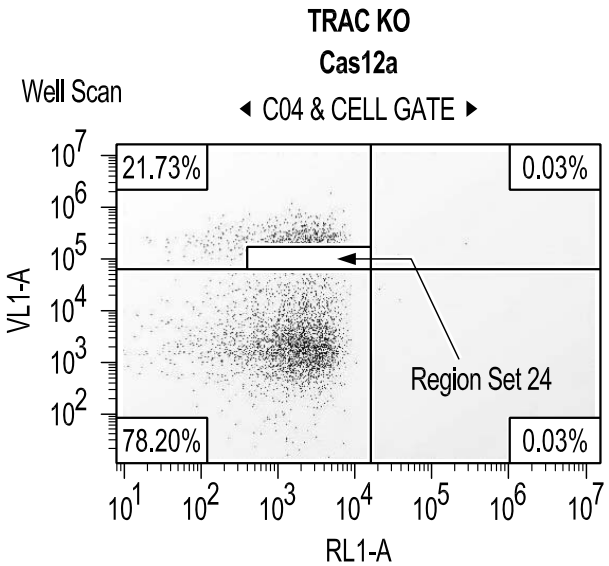


FIG. 15C

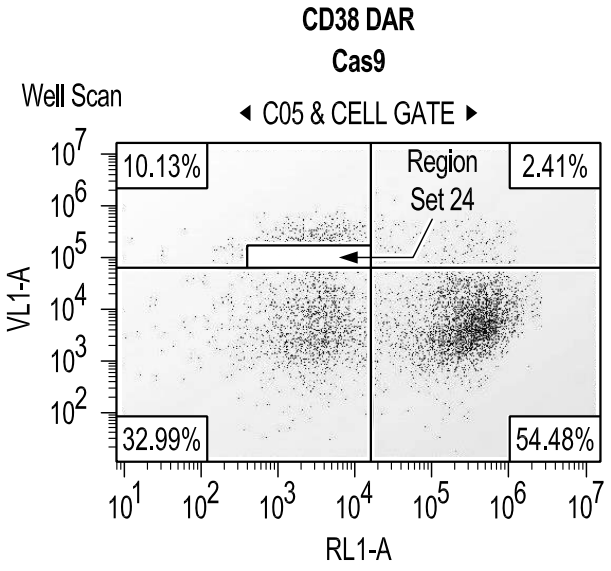


FIG. 15D

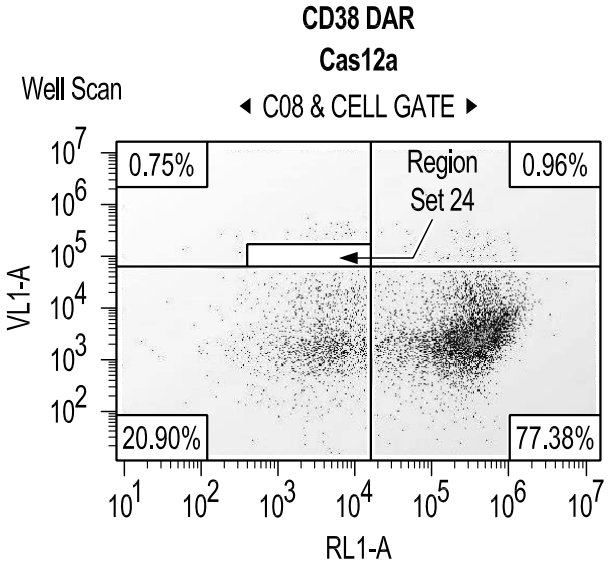


FIG. 15E

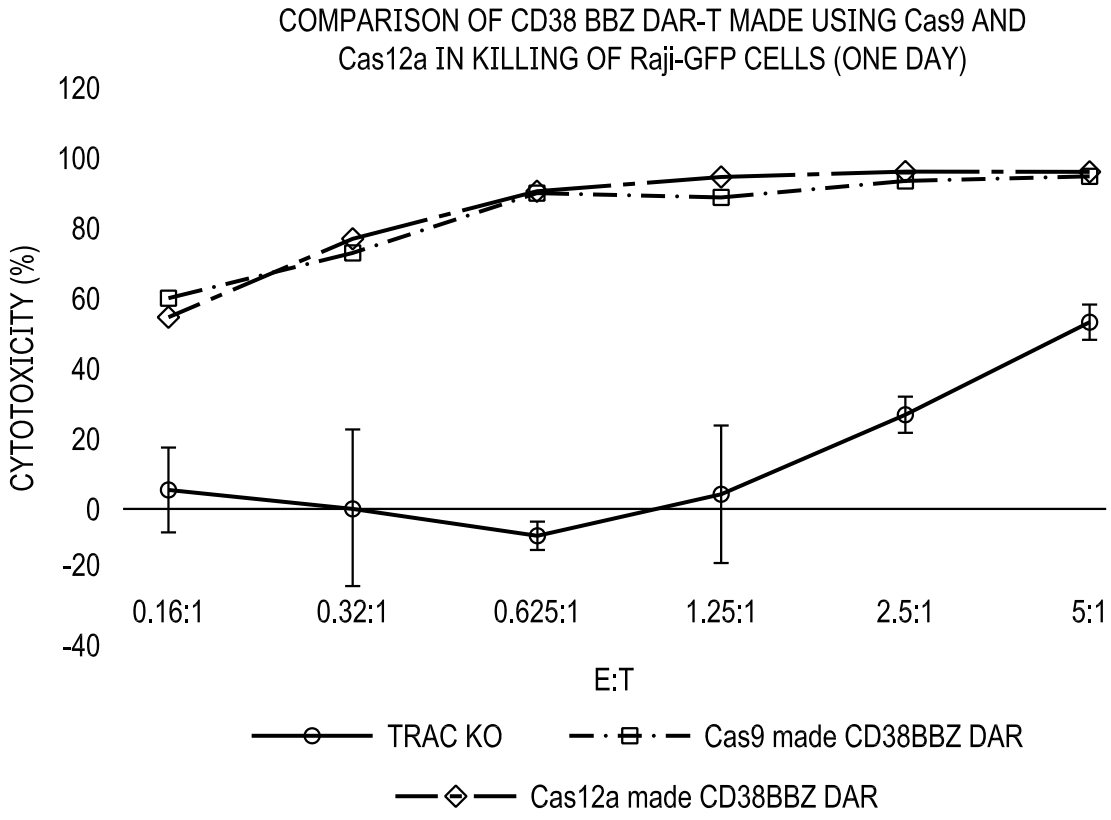

COMPARISON OF CD38 BBZ DAR-T MADE USING Cas9 AND Cas12a IN KILLING OF Raji-GFP CELLS (ONE DAY)
CYTOTOXICITY (%)
120
100
80
60
40
20
0
-20
-40
0.16:1
0.32:1
0.625:1
1.25:1
2.5:1
5:1
E:T
TRAC KO
Cas9 made CD38BBZ DAR
Cas12a made CD38BBZ DAR

FIG. 16

SOMATIC InDel DETECTION RESULTS FROM WGS DAT OF Cas9 / anti-CD38-DART

CHROM	POS	ID	REF	ALT	Vaf	FILTER	GeneNam	Func	Gene
10	51484776	rs1462741	TTA	T	0.325	PASS	AGAP7P	ncRNA_in	NR_12658
11	10063345	rs1454722	TGATGATA	T	0.5	PASS	SBF2	intronic	NM_03096
GL000229.	3418	·	G	GAGGGAA	0.0506	PASS	·	intergenic	NONE,NO
GL000225.	189989	·	TGGAATG	T	0.0678	PASS	·	intergenic	NONE,NO

FIG. 17

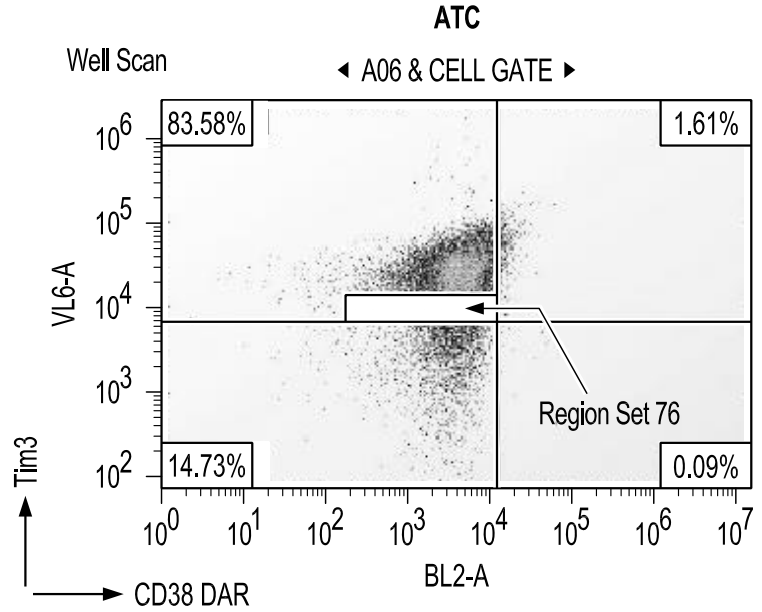


FIG. 18A

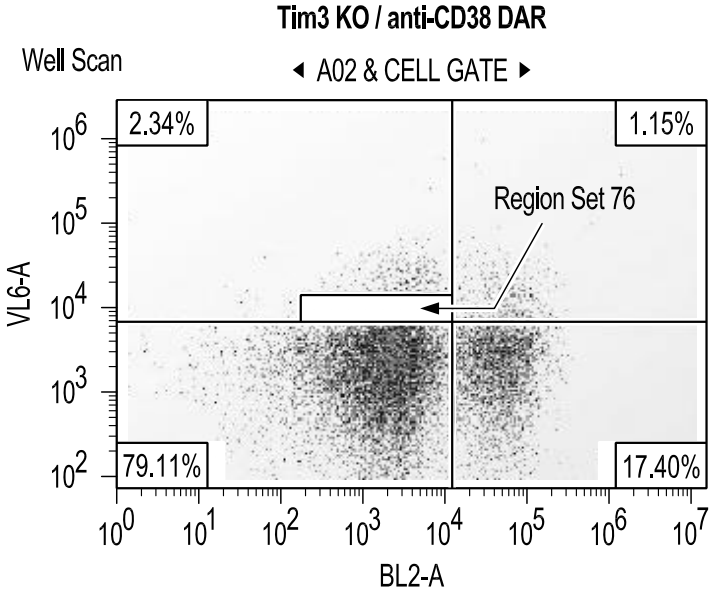


FIG. 18B

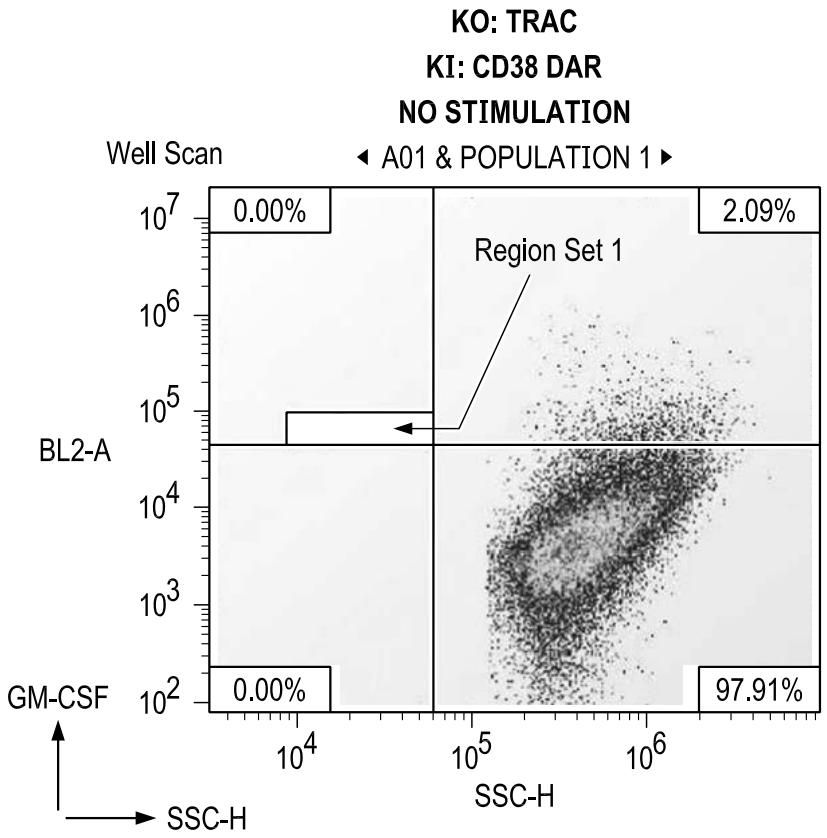


FIG. 19A

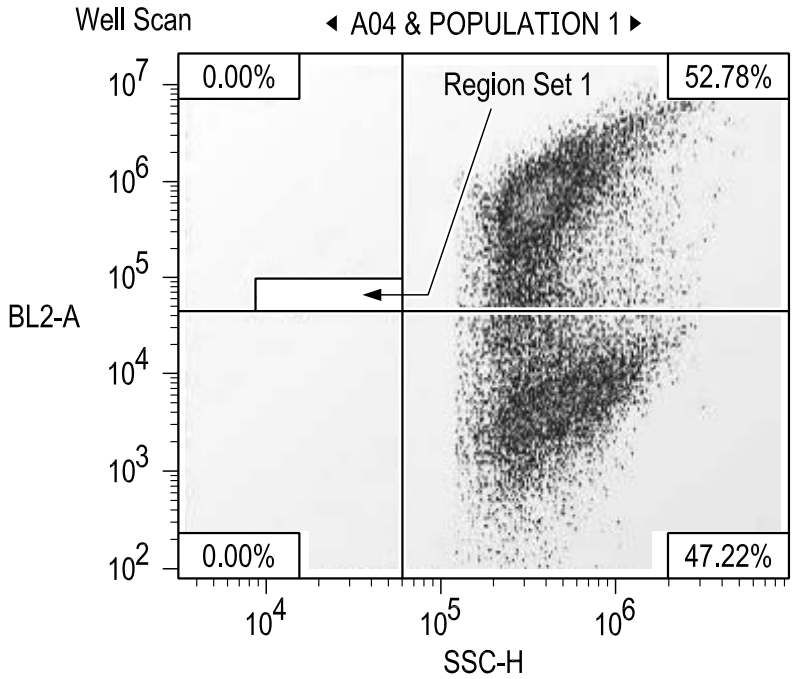


FIG. 19B

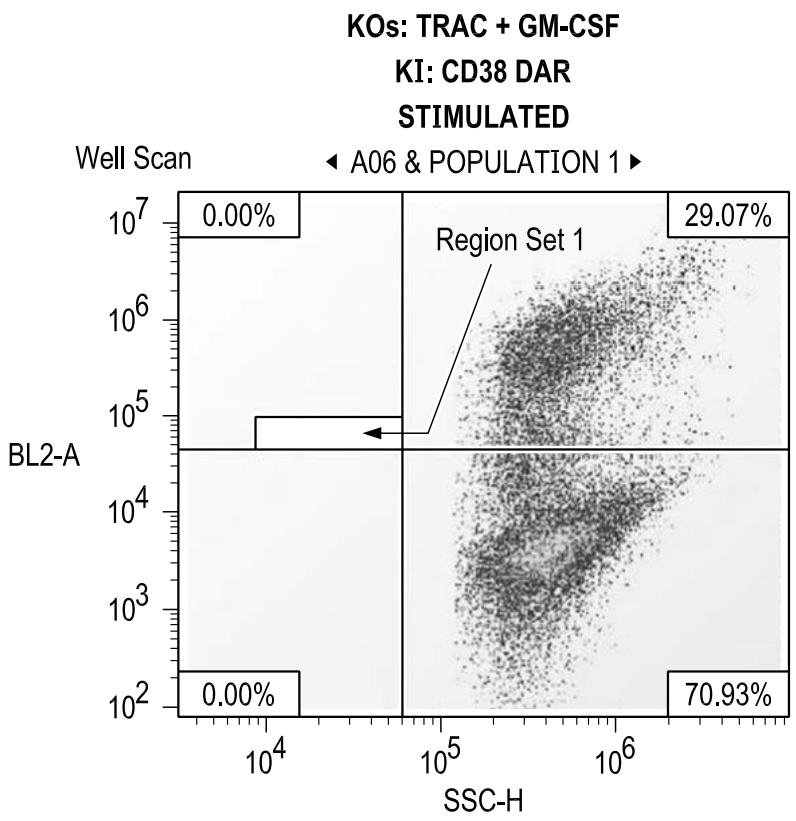


FIG. 19C

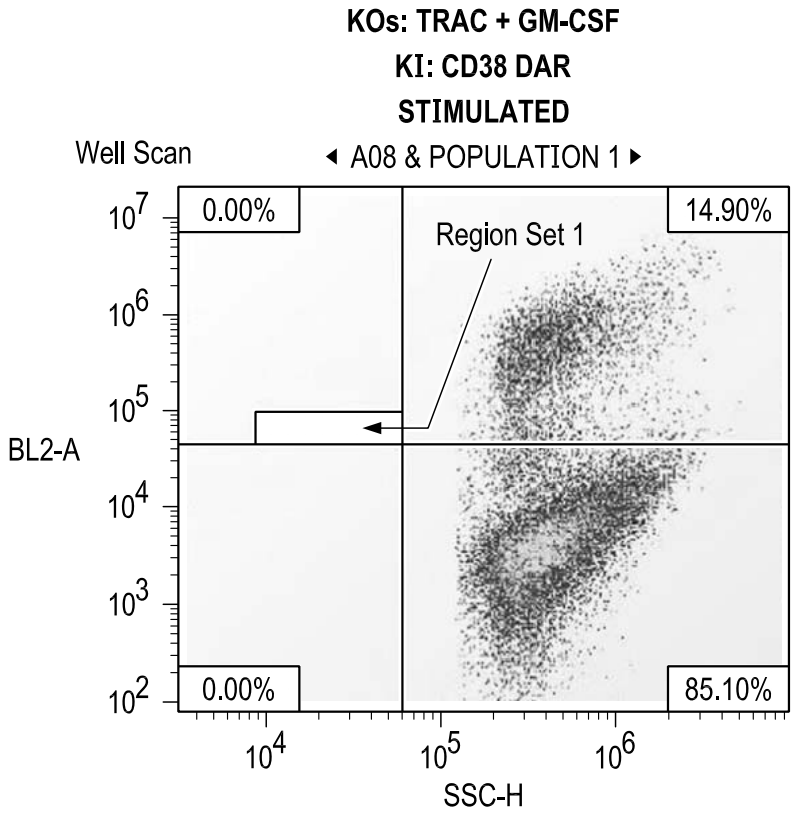


FIG. 19D

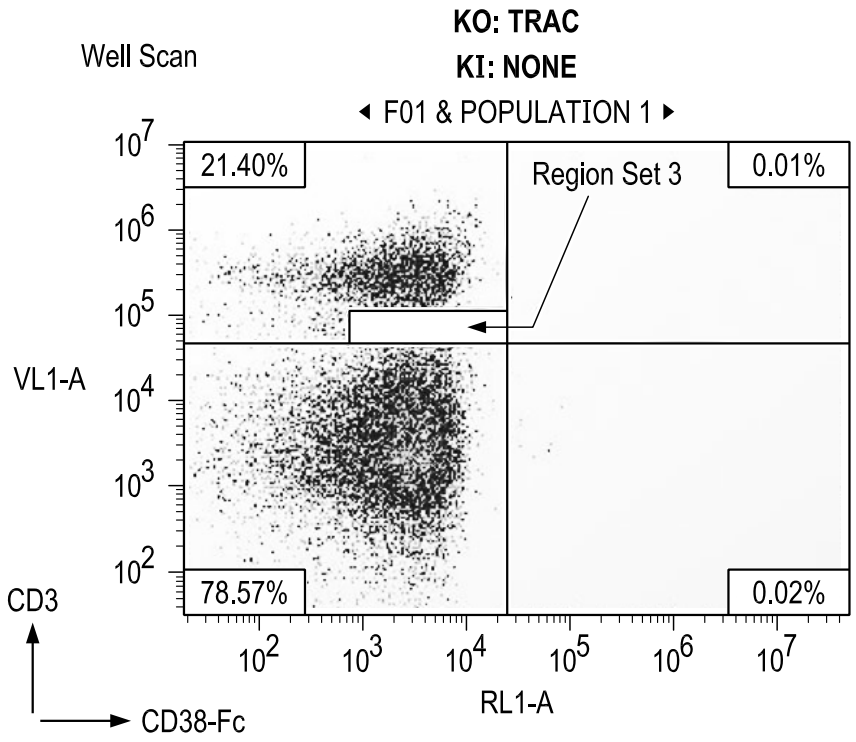


FIG. 19E

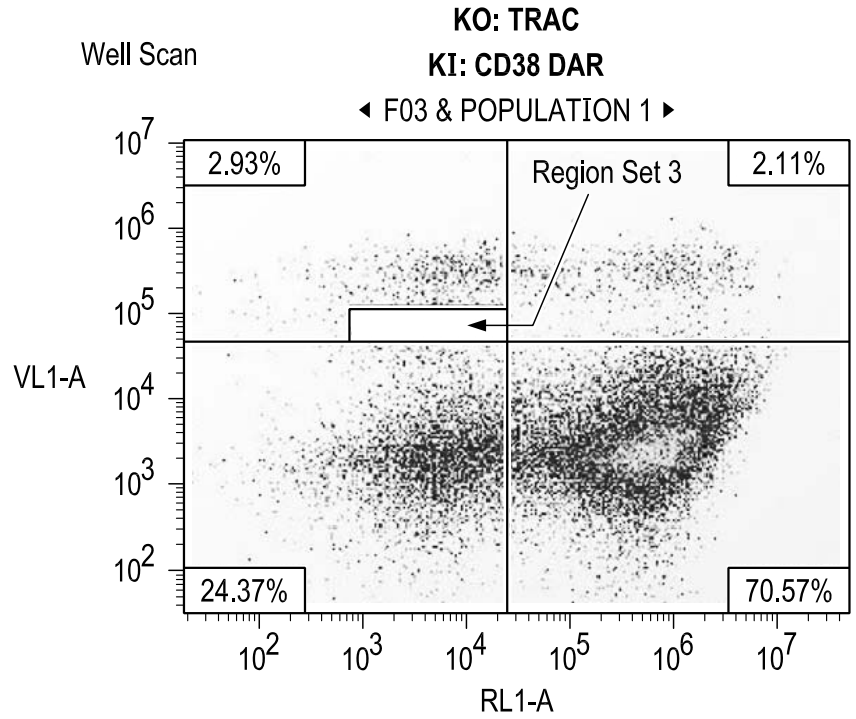


FIG. 19F

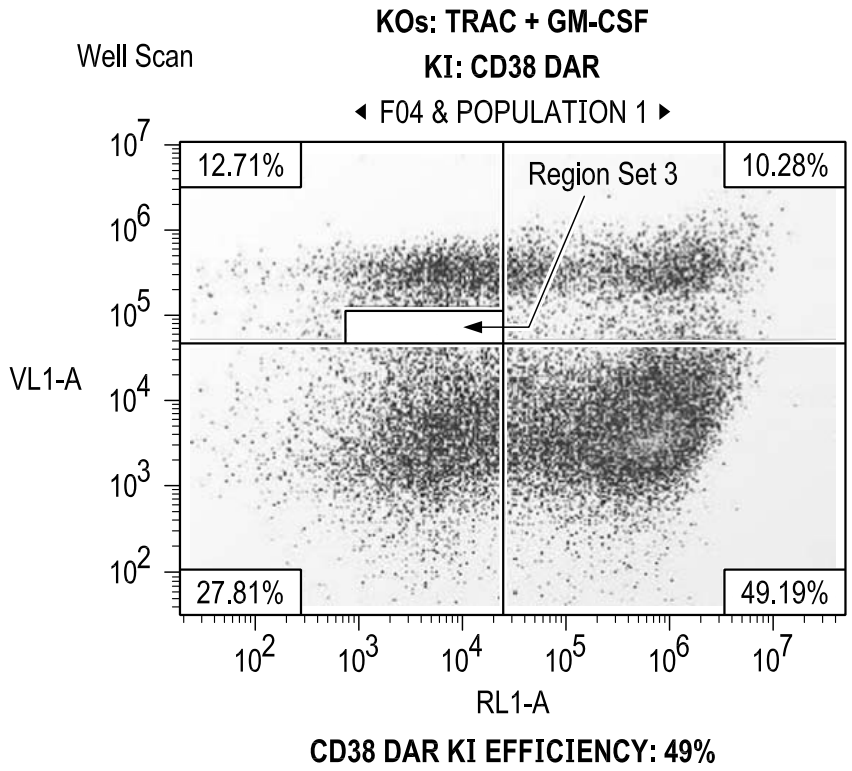


FIG. 19G

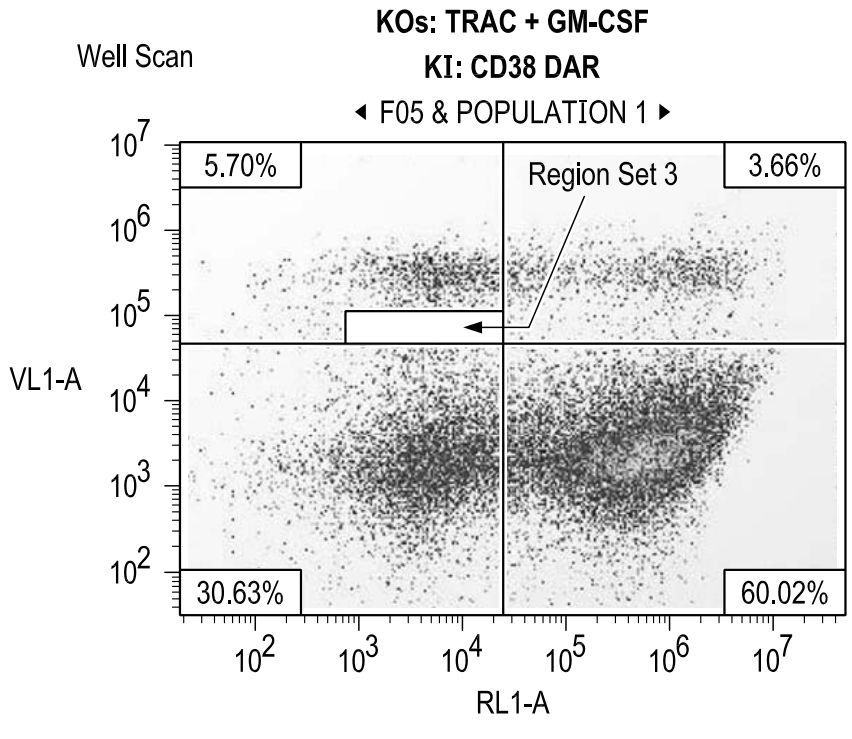


FIG. 19H

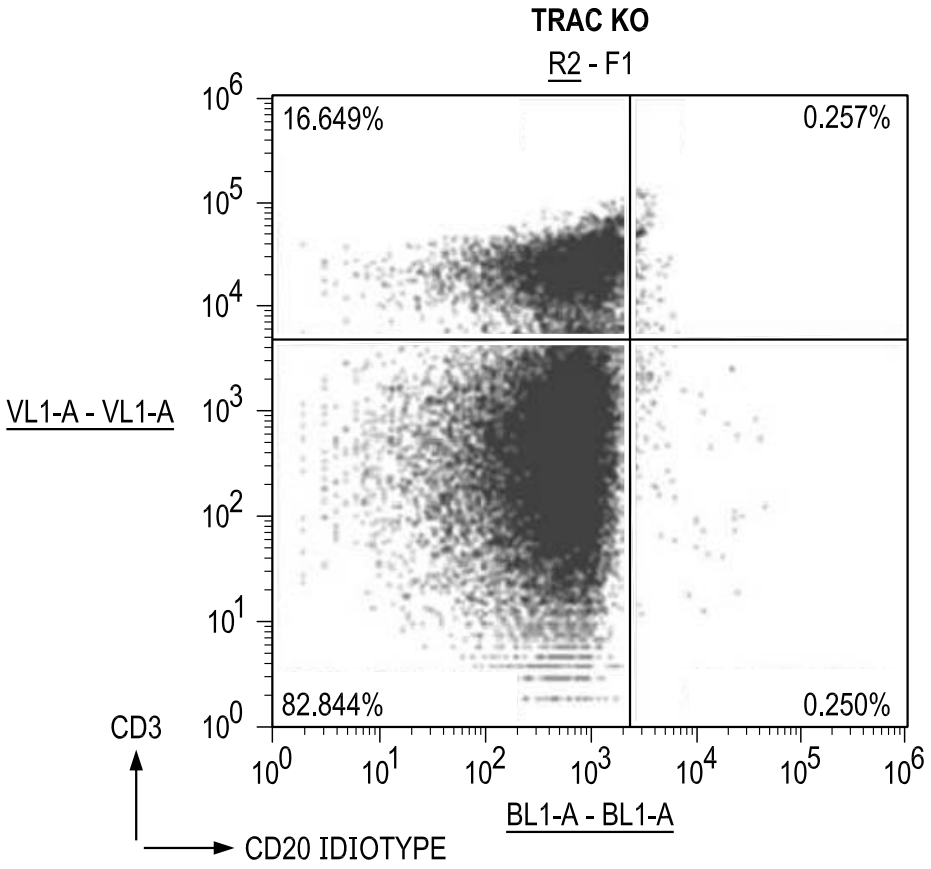


FIG. 20A

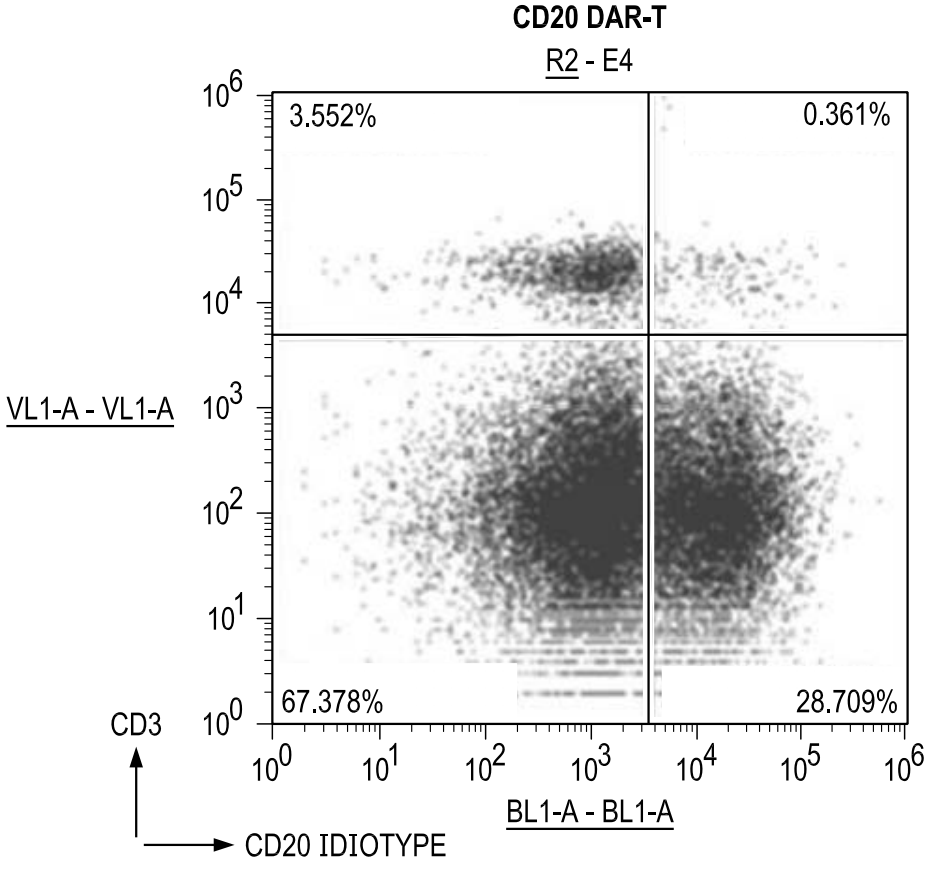


FIG. 20B

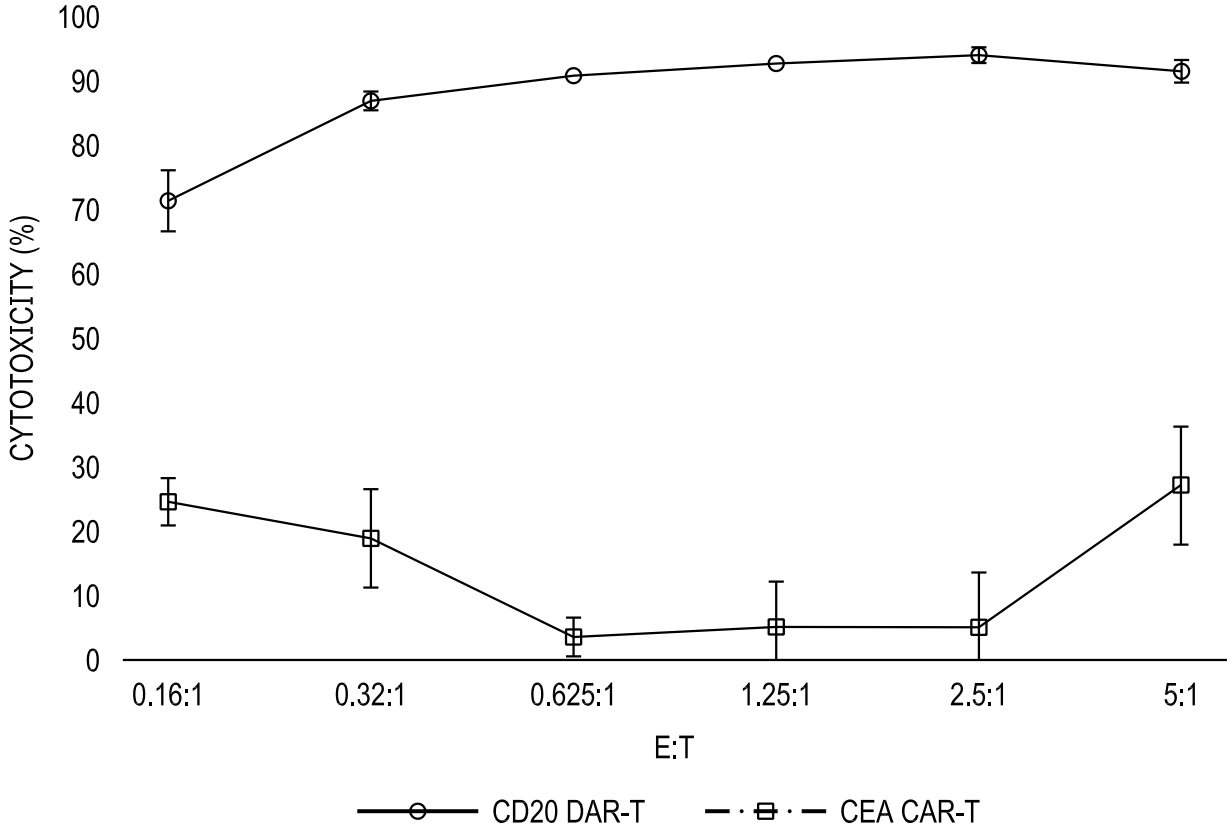


FIG. 21

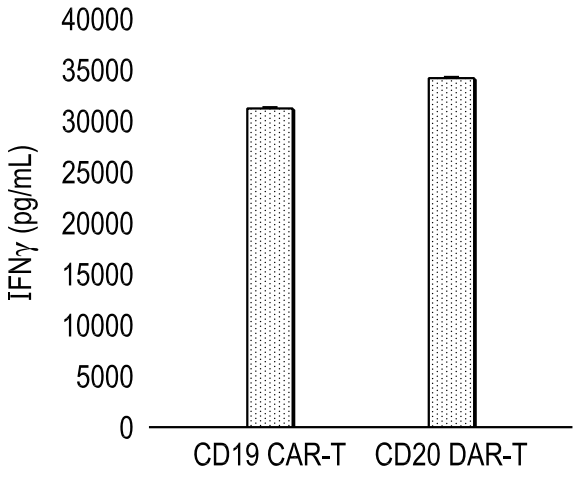


FIG. 22A

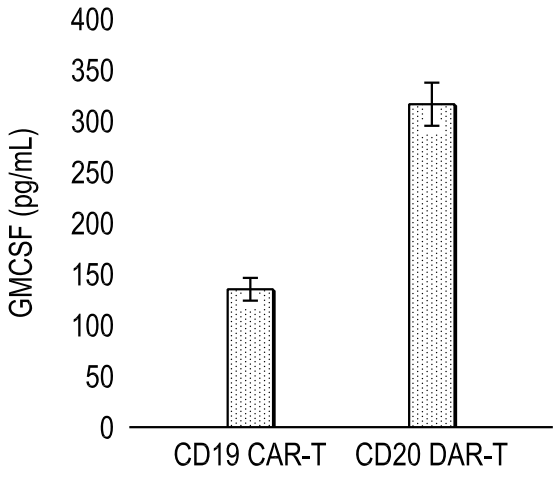


FIG. 22B

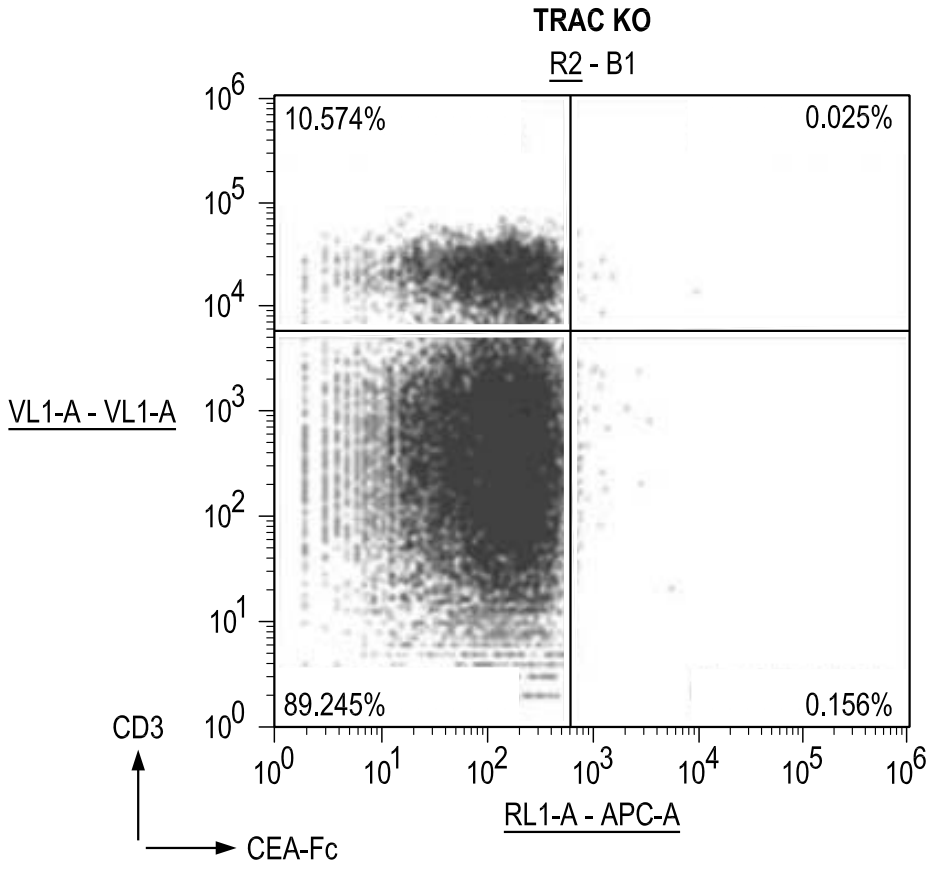


FIG. 23A

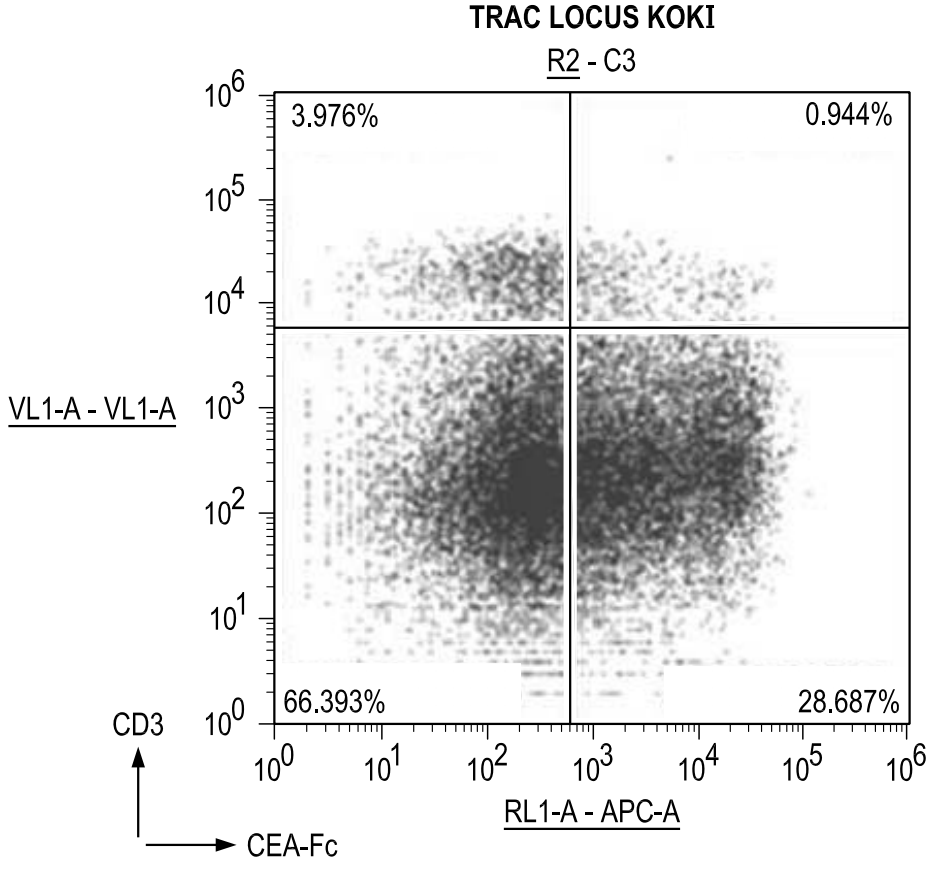


FIG. 23B

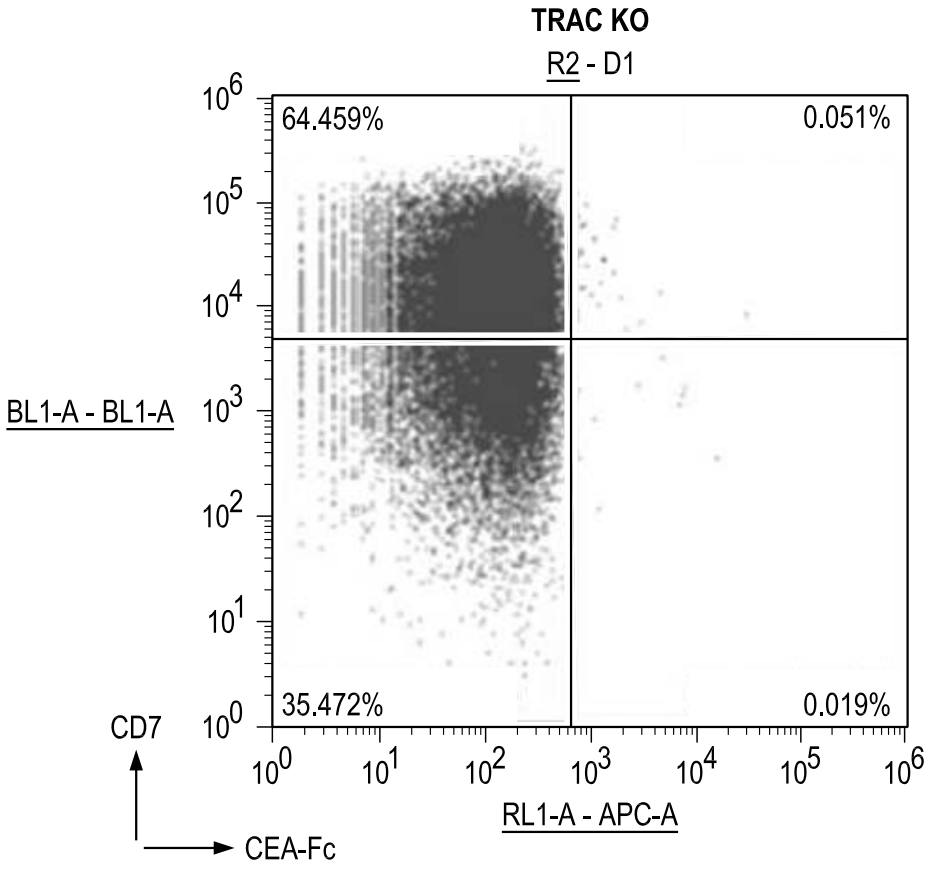


FIG. 23C

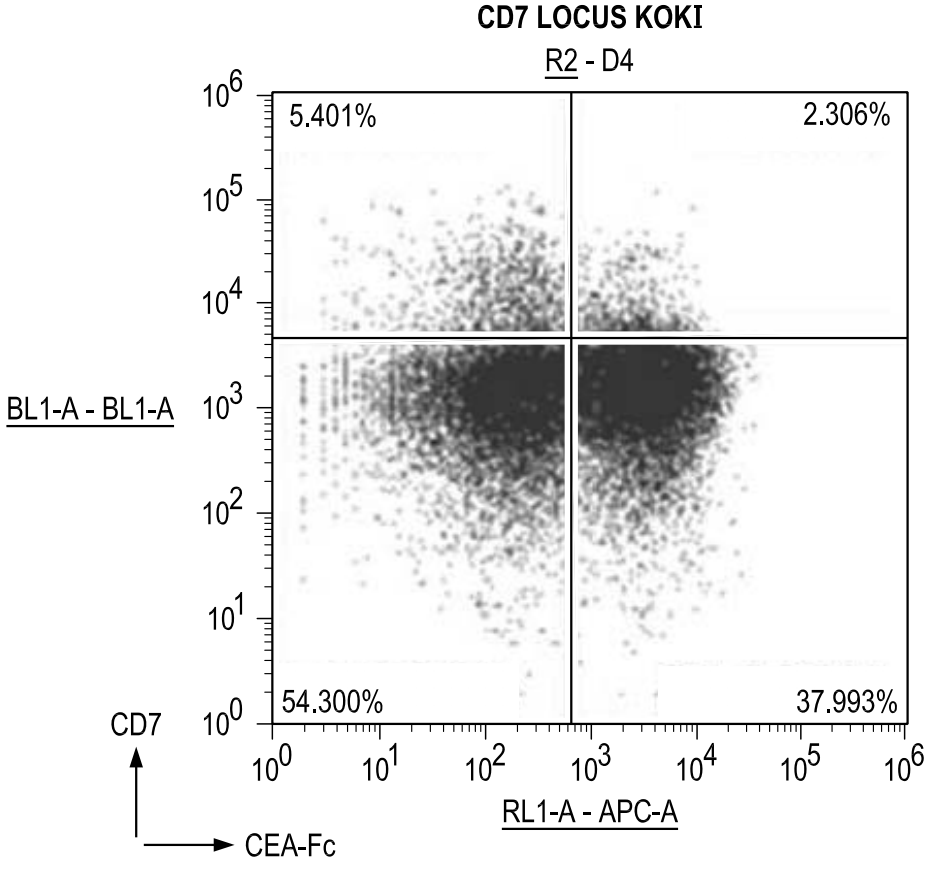


FIG. 23D

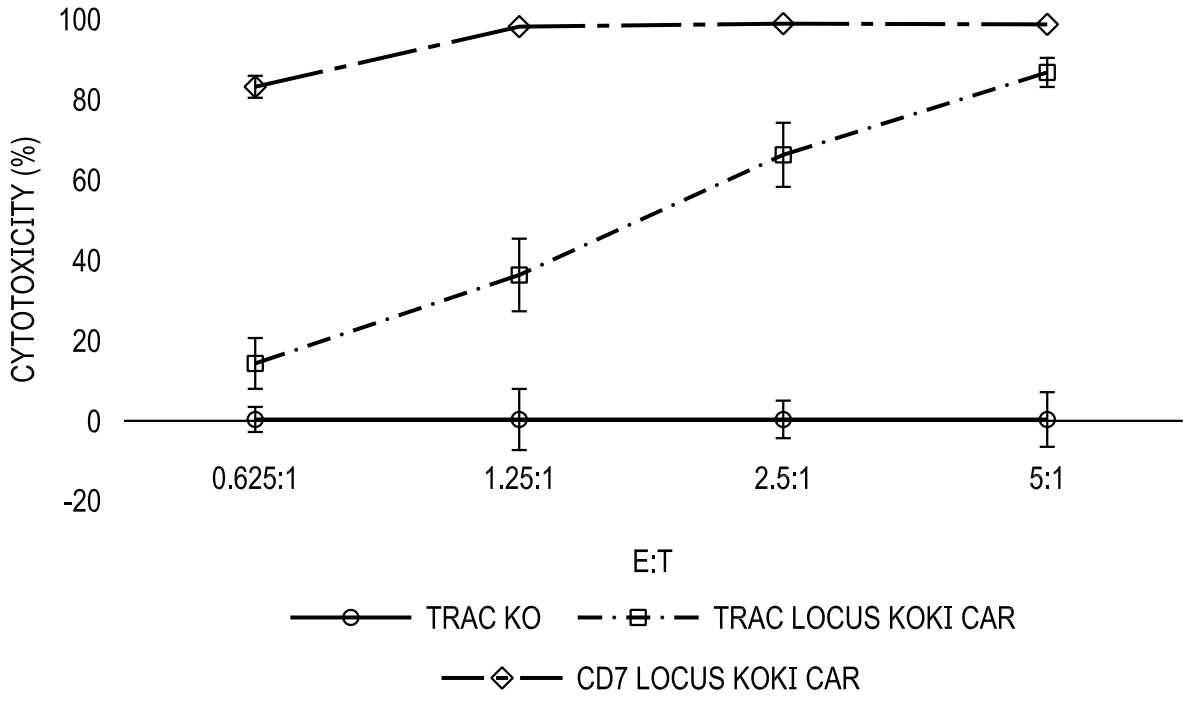

100
80
60
40
20
0
-20
CYTOTOXICITY (%)
0.625:1
1.25:1
2.5:1
5:1
E:T
TRAC KO
TRAC LOCUS KOKI CAR
CD7 LOCUS KOKI CAR

FIG. 24

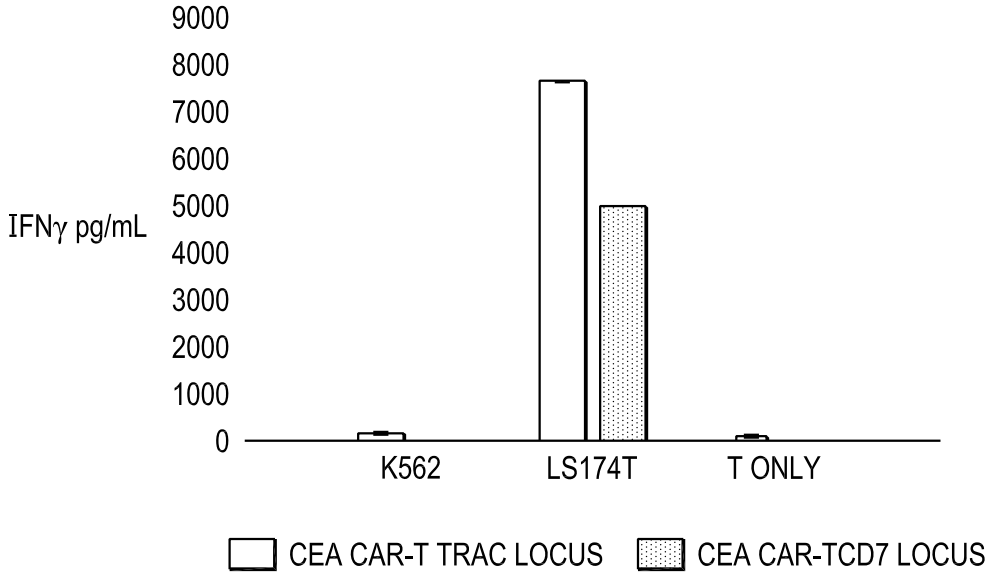

9000
8000
7000
6000
5000
4000
3000
2000
1000
0
IFNγ pg/mL
K562
LS174T
T ONLY
CEA CAR-T TRAC LOCUS
CEA CAR-TCD7 LOCUS

FIG. 25