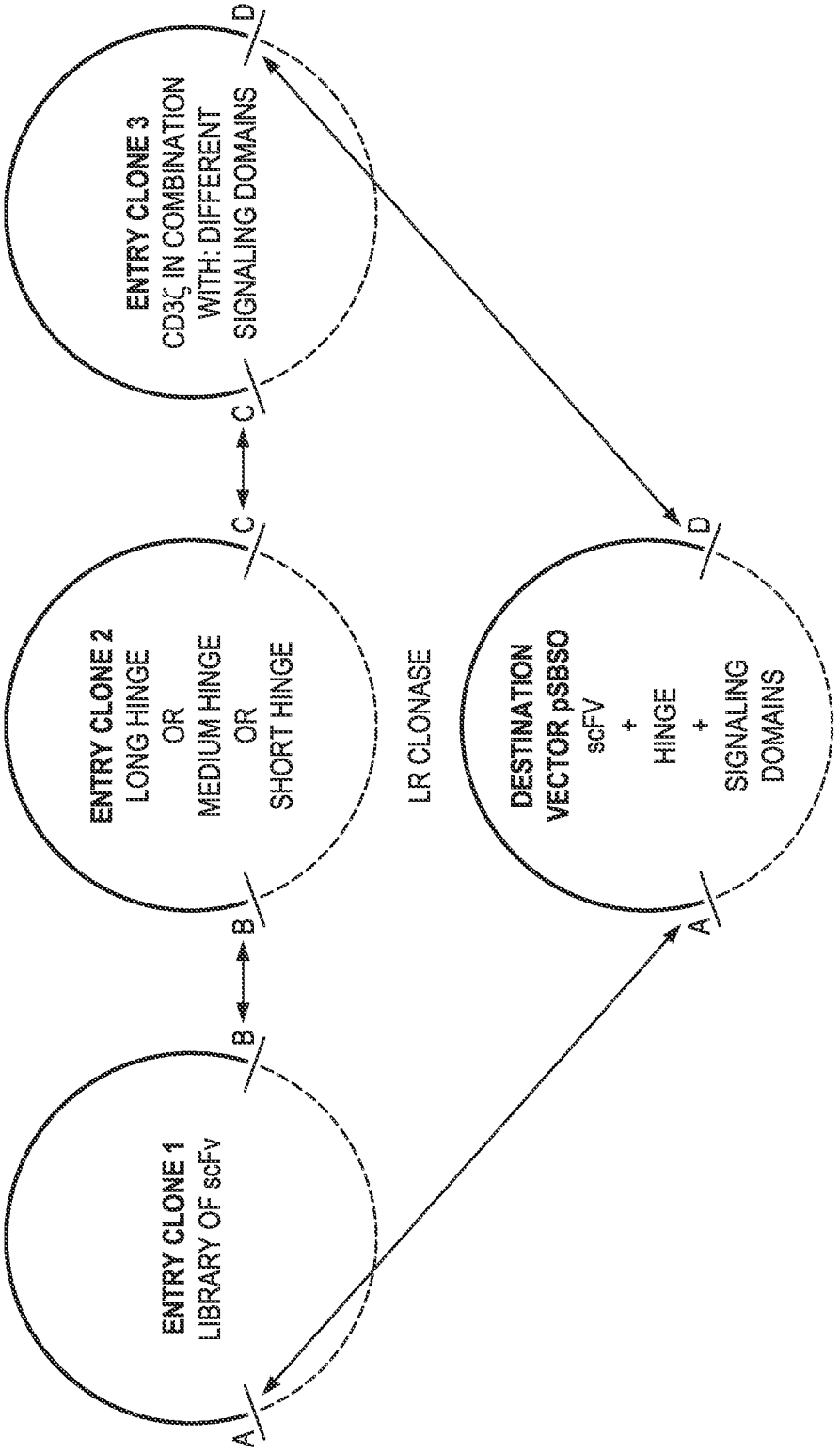

ENTRY CLONE 1
LIBRARY OF scFv
ENTRY CLONE 2
LONG HINGE
OR
MEDIUM HINGE
OR
SHORT HINGE
ENTRY CLONE 3
CD3ζ IN COMBINATION
WITH: DIFFERENT
SIGNALING DOMAINS
LR CLONASE
DESTINATION
VECTOR pSBSO
scFV
+
HINGE
+
SIGNALING
DOMAINS
A
B
B
C
C
D
D
A

FIG. 1

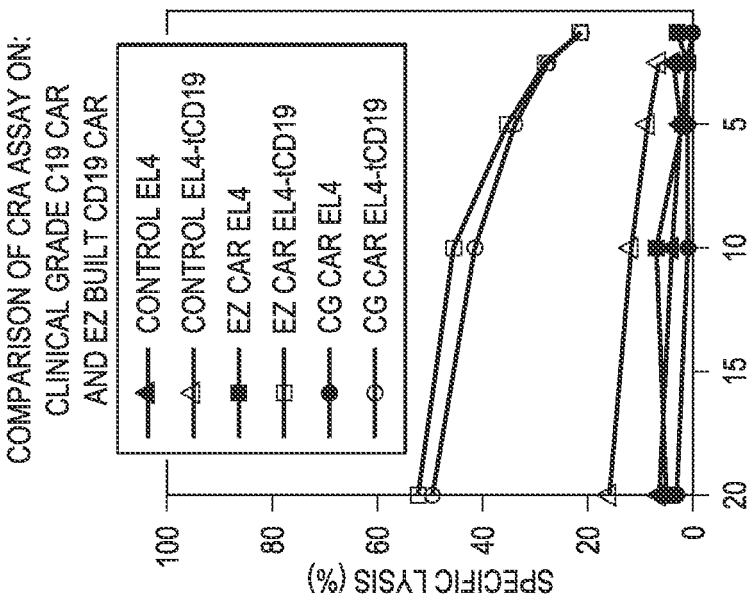

COMPARISON OF CRA ASSAY ON:
CLINICAL GRADE C19 CAR
AND EZ BUILT CD19 CAR
CONTROL EL4
CONTROL EL4-iCD19
EZ CAR EL4
EZ CAR EL4-iCD19
CG CAR EL4
CG CAR EL4-iCD19
SPECIFIC LYSIS (%)
E:T (X:1)

FIG. 2B

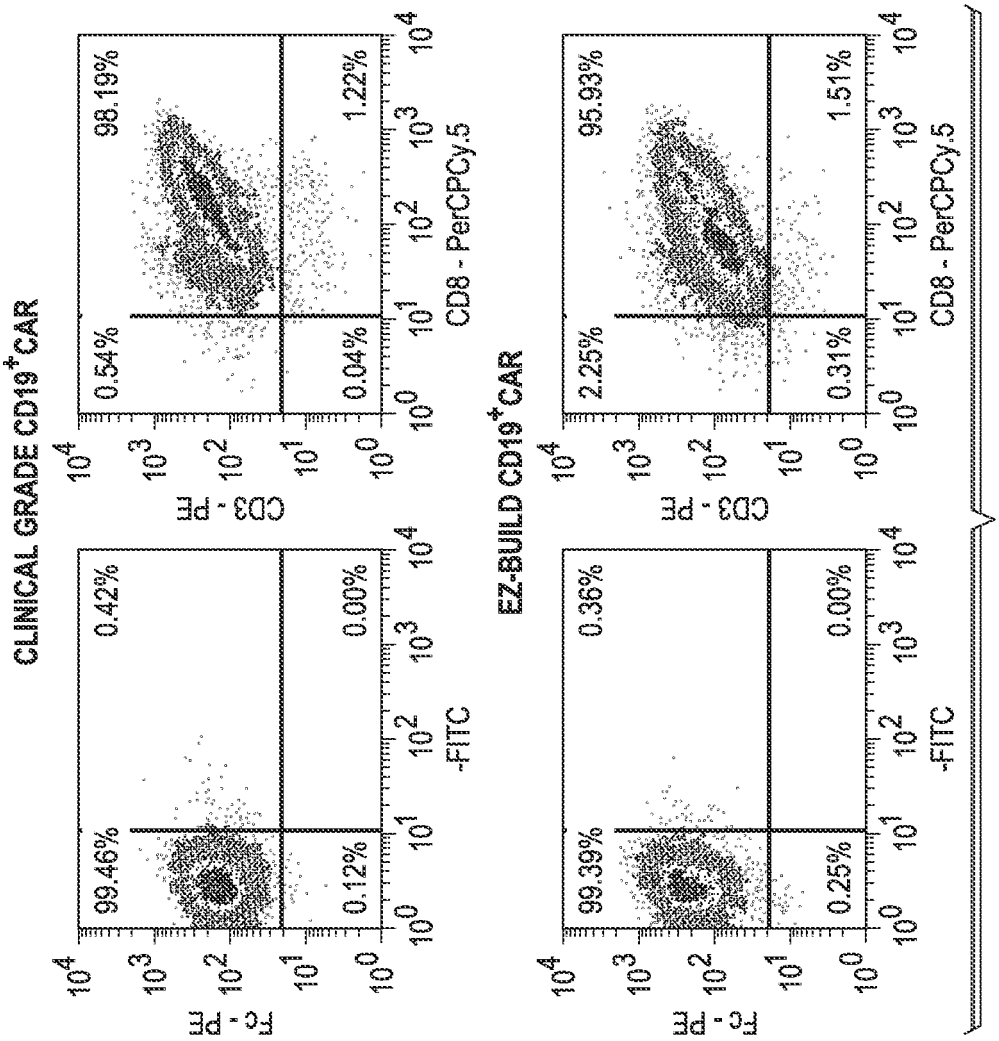

CLINICAL GRADE CD19+CAR
EZ-BUILD CD19+CAR
CD3 - PE
CD8 - PerCPCy.5
Fc - PE
-FITC
99.46%
0.42%
0.12%
0.00%
0.54%
98.19%
0.04%
1.22%
99.39%
0.36%
0.25%
0.00%
2.25%
95.93%
0.31%
1.51%

FIG. 2A

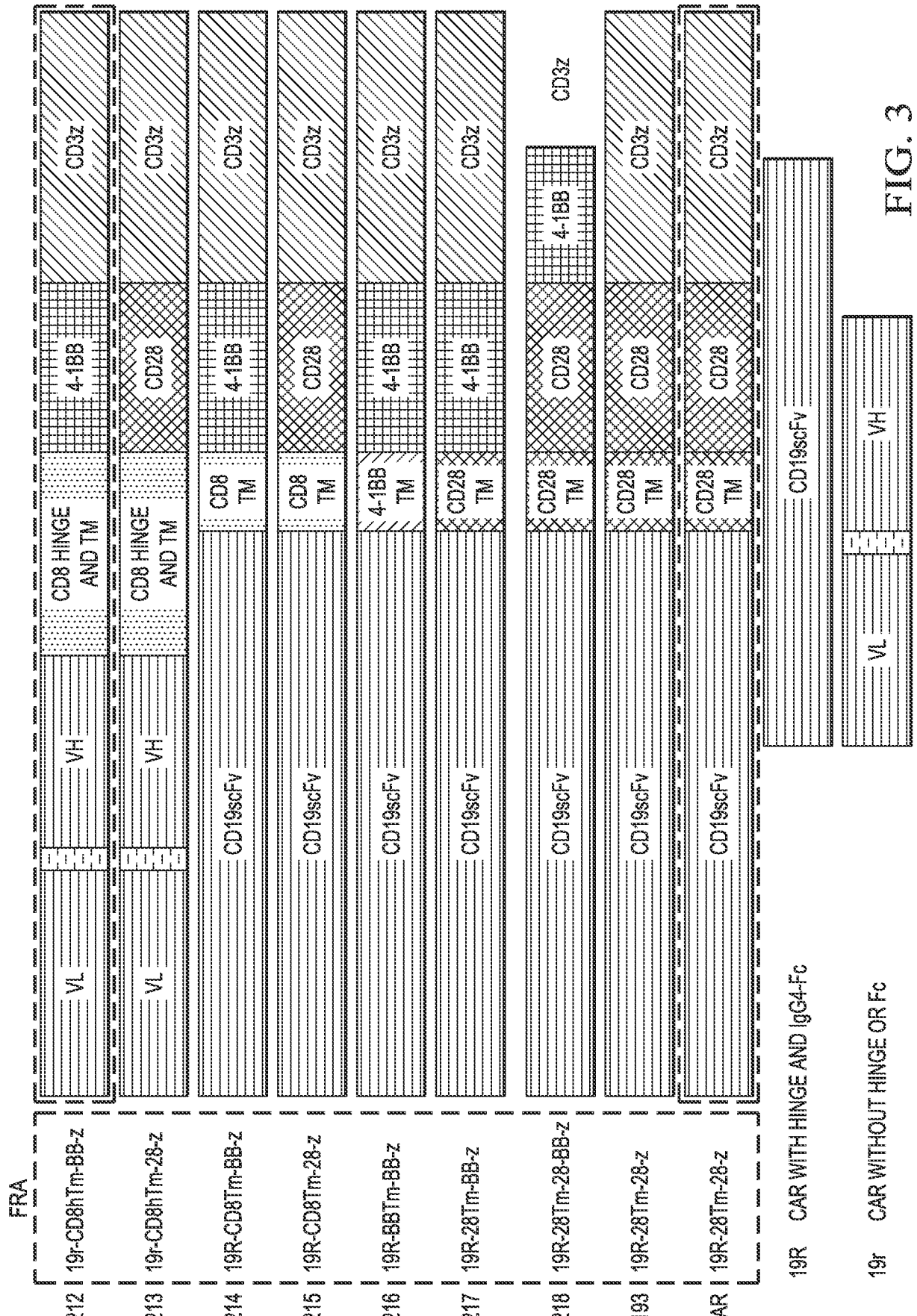

FRA
212 19r-CD8hTm-BB-z
VL
VH
CD8 HINGE AND TM
4-1BB
CD3z
213 19r-CD8hTm-28-z
VL
VH
CD8 HINGE AND TM
CD28
CD3z
214 19R-CD8Tm-BB-z
CD19scFv
CD8 TM
4-1BB
CD3z
215 19R-CD8Tm-28-z
CD19scFv
CD8 TM
CD28
CD3z
216 19R-BBTm-BB-z
CD19scFv
4-1BB TM
4-1BB
CD3z
217 19R-28Tm-BB-z
CD19scFv
CD28 TM
4-1BB
CD3z
218 19R-28Tm-28-BB-z
CD19scFv
CD28 TM
CD28
4-1BB
CD3z
193 19R-28Tm-28-z
CD19scFv
CD28 TM
CD28
CD3z
CAR 19R-28Tm-28-z
CD19scFv
CD28 TM
CD28
CD3z
19R CAR WITH HINGE AND IgG4-Fc
CD19scFv
19r CAR WITHOUT HINGE OR Fc
VL
VH

FIG. 3

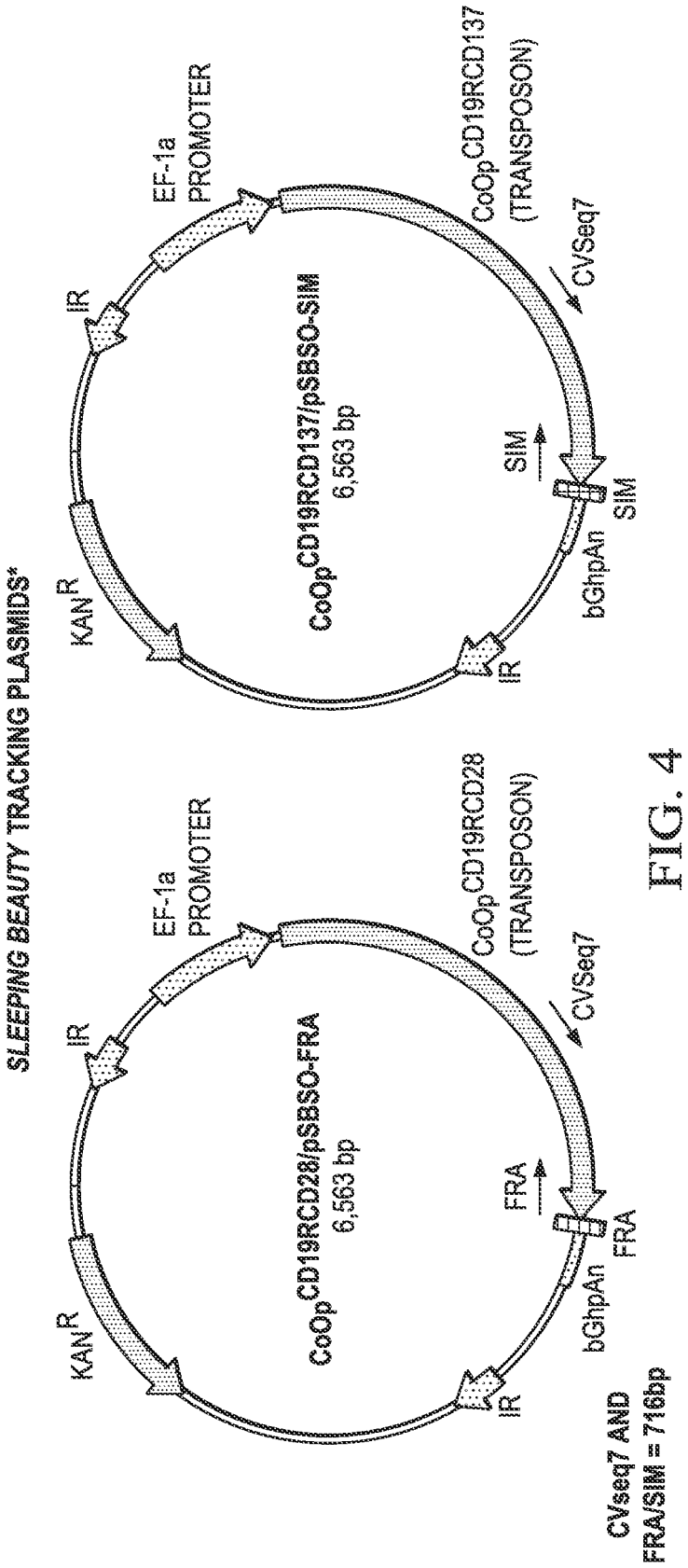

SLEEPING BEAUTY TRACKING PLASMIDS*
EF-1a PROMOTER
IR
KAN^R
CoOpCD19RCD137/pSBSO-SIM
6,563 bp
CoOpCD19RCD137 (TRANSPOSON)
CVSeq7
SIM
SIM
bGhpAn
IR
EF-1a PROMOTER
IR
KAN^R
CoOpCD19RCD28/pSBSO-FRA
6,563 bp
CoOpCD19RCD28 (TRANSPOSON)
CVSeq7
FRA
FRA
bGhpAn
IR
CVseq7 AND FRA/SIM = 716bp

FIG. 4

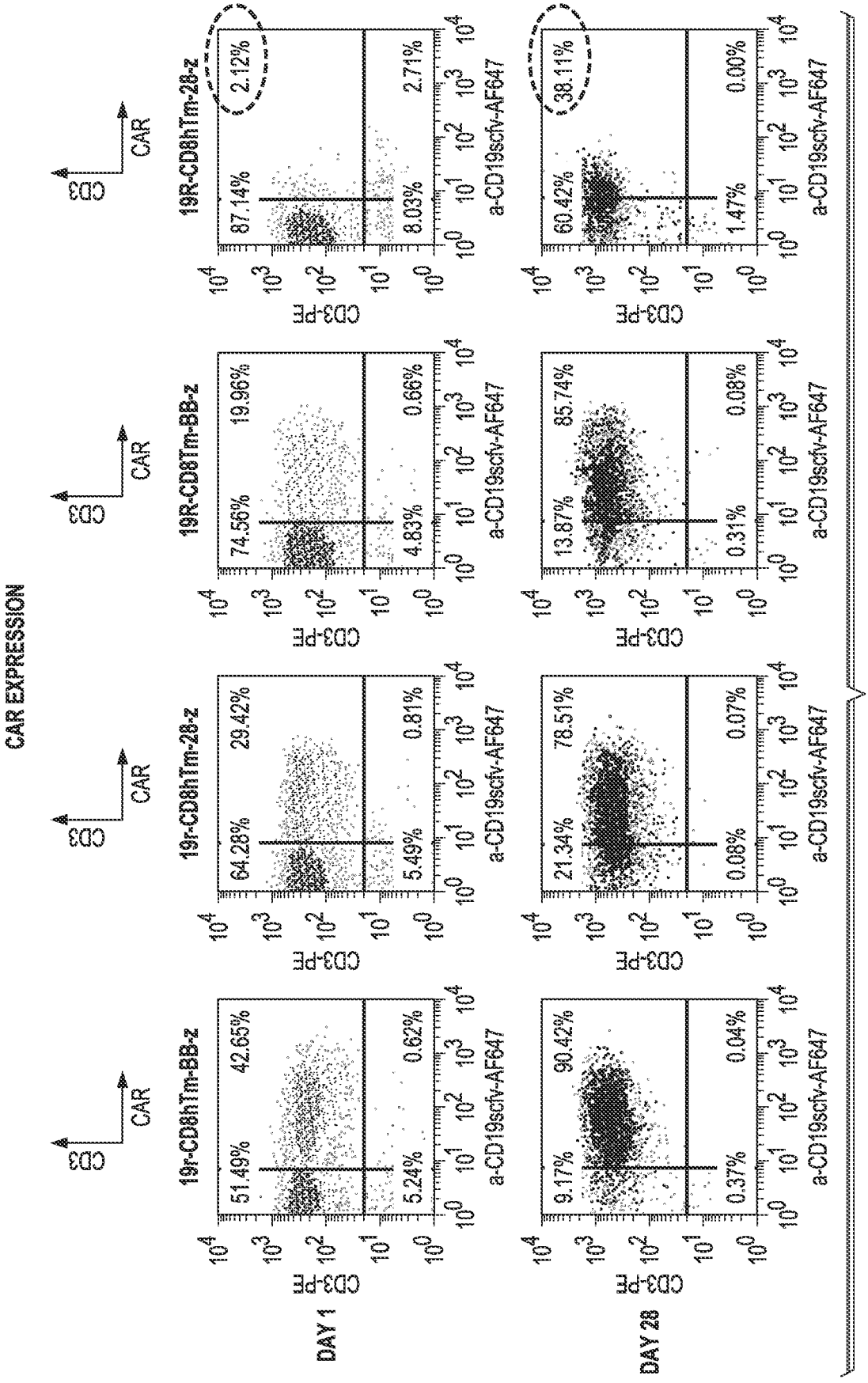

CAR EXPRESSION
DAY 1
DAY 28
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8hTm-28-z
CD3
CAR
CD3-PE
a-CD19scfv-AF647
51.49%
42.65%
5.24%
0.62%
64.28%
29.42%
5.49%
0.81%
74.56%
19.96%
4.83%
0.66%
87.14%
2.12%
8.03%
2.71%
9.17%
90.42%
0.37%
0.04%
21.34%
78.51%
0.08%
0.07%
13.87%
85.74%
0.31%
0.08%
60.42%
38.11%
1.47%
0.00%

FIG. 5-1

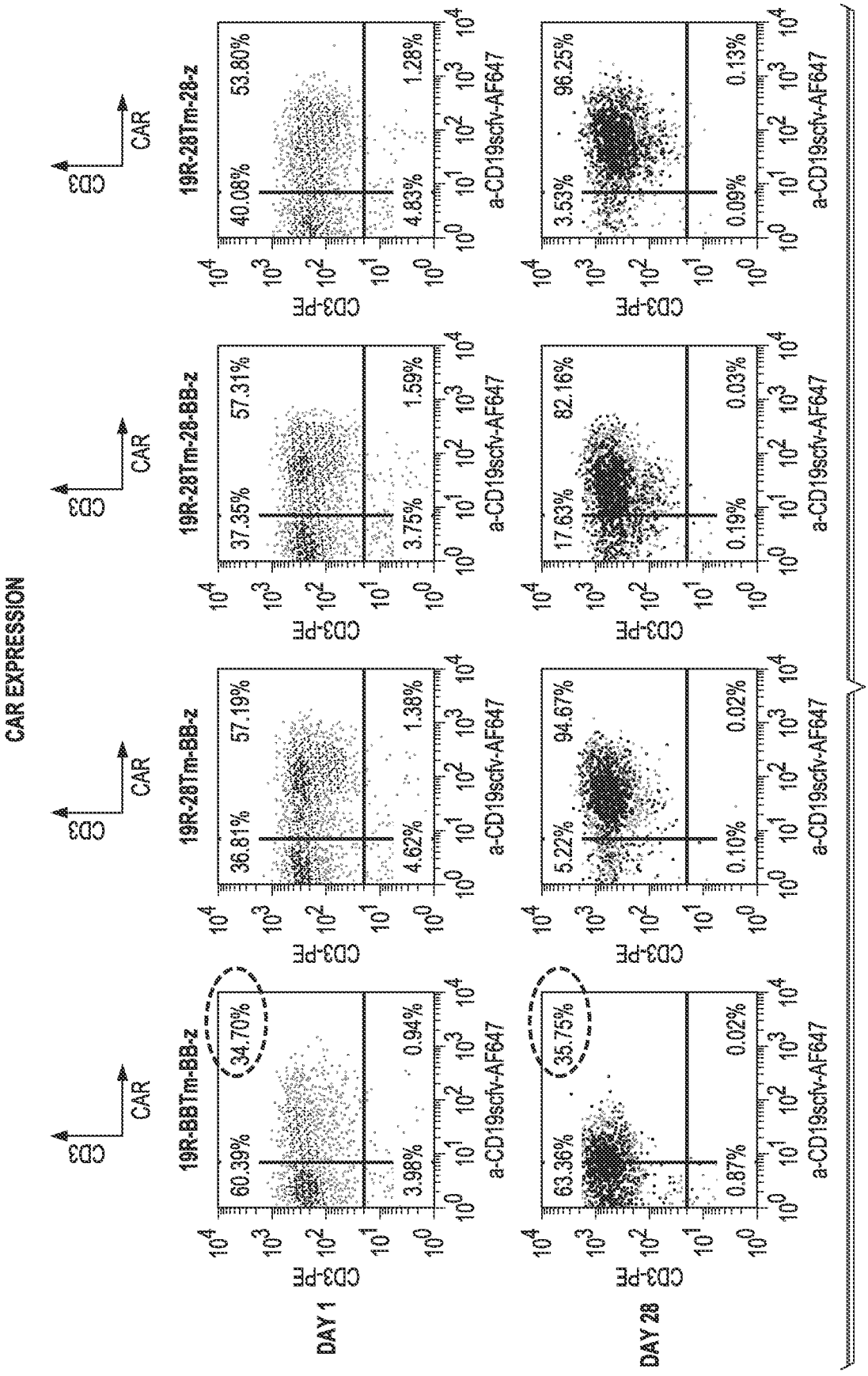

CAR EXPRESSION
DAY 1
DAY 28
CD3
CAR
CD3-PE
a-CD19scfv-AF647
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z
60.39%
34.70%
3.98%
0.94%
0.87%
0.02%
36.81%
57.19%
4.62%
1.38%
0.10%
0.02%
37.35%
57.31%
3.75%
1.59%
0.19%
0.03%
40.08%
53.80%
4.83%
1.28%
0.09%
0.13%
63.36%
35.75%
5.22%
94.67%
17.63%
82.16%
3.53%
96.25%

FIG. 5-2

FIG. 6

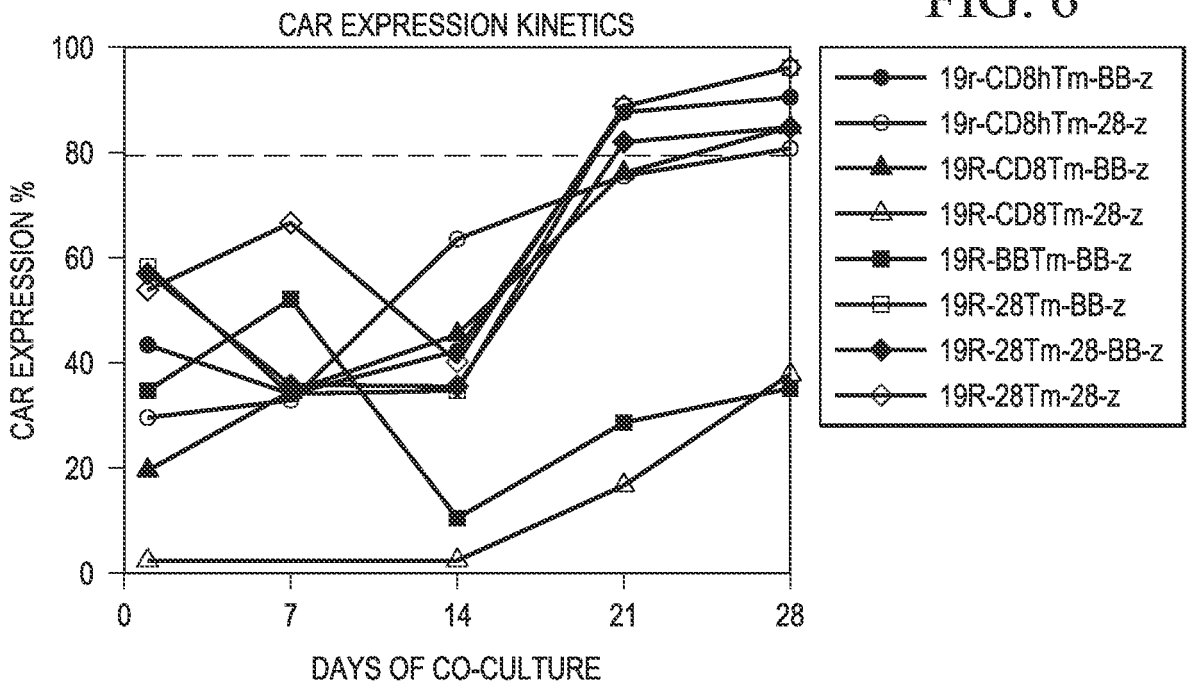
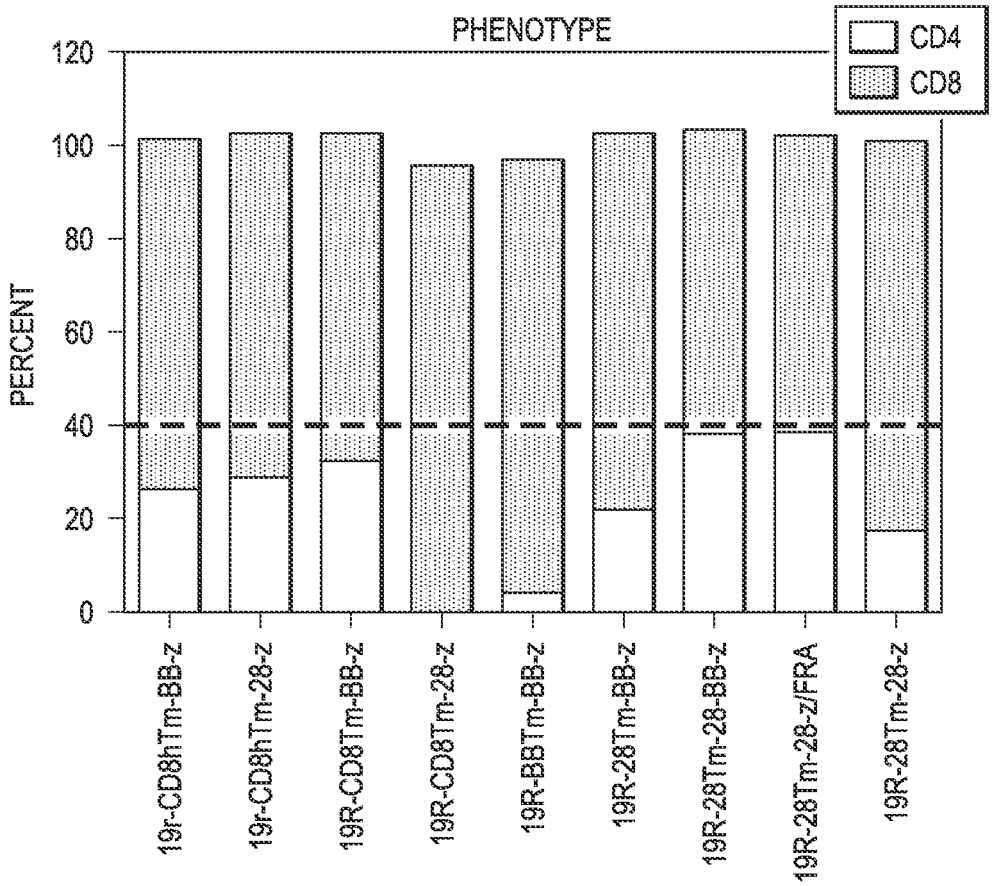

CAR EXPRESSION KINETICS
CAR EXPRESSION %
100
80
60
40
20
0
0
7
14
21
28
DAYS OF CO-CULTURE
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z

FIG. 7


PHENOTYPE
CD4
CD8
PERCENT
120
100
80
60
40
20
0
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z/FRA
19R-28Tm-28-z

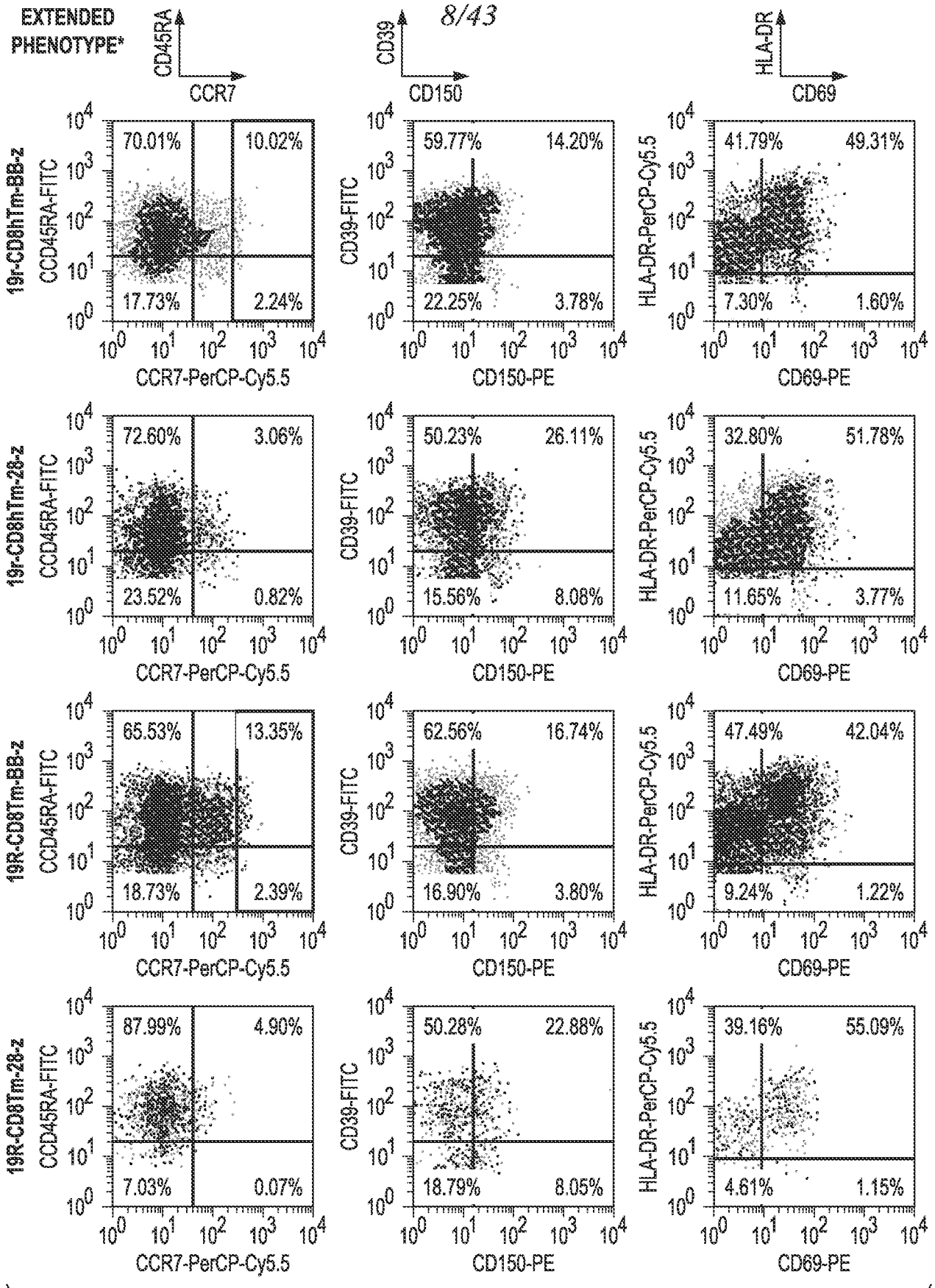

EXTENDED PHENOTYPE*
CD45RA
CCR7
CD39
CD150
HLA-DR
CD69
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
CCD45RA-FITC
CD39-FITC
HLA-DR-PerCP-Cy5.5
CCR7-PerCP-Cy5.5
CD150-PE
CD69-PE
70.01%
10.02%
17.73%
2.24%
59.77%
14.20%
22.25%
3.78%
41.79%
49.31%
7.30%
1.60%
72.60%
3.06%
23.52%
0.82%
50.23%
26.11%
15.56%
8.08%
32.80%
51.78%
11.65%
3.77%
65.53%
13.35%
18.73%
2.39%
62.56%
16.74%
16.90%
3.80%
47.49%
42.04%
9.24%
1.22%
87.99%
4.90%
7.03%
0.07%
50.28%
22.88%
18.79%
8.05%
39.16%
55.09%
4.61%
1.15%

FIG. 8A-1

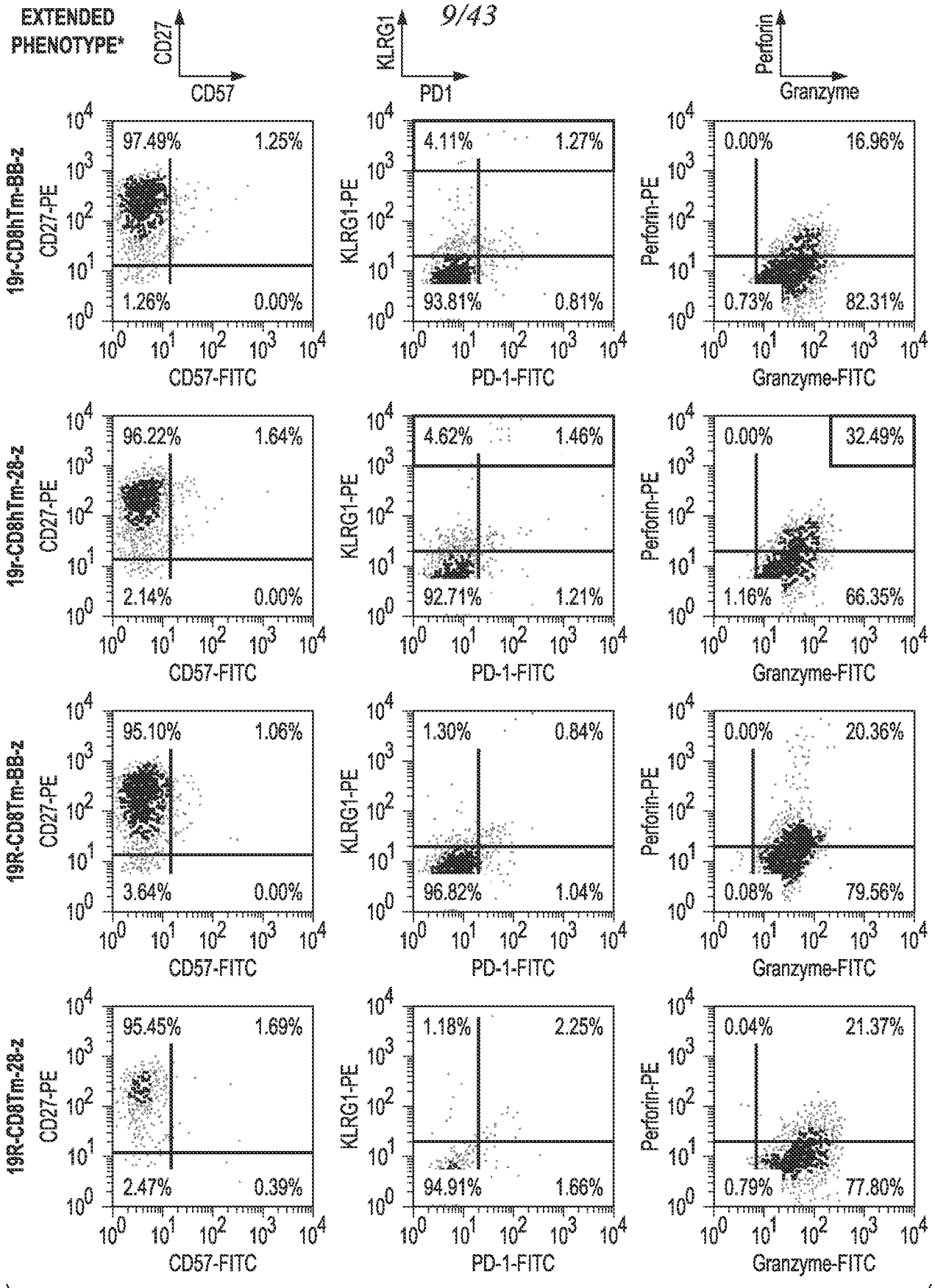

EXTENDED PHENOTYPE*
CD27
CD57
KLRG1
PD1
Perforin
Granzyme
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
CD27-PE
KLRG1-PE
Perforin-PE
CD57-FITC
PD-1-FITC
Granzyme-FITC
97.49%
1.25%
1.26%
0.00%
4.11%
1.27%
93.81%
0.81%
0.00%
16.96%
0.73%
82.31%
96.22%
1.64%
2.14%
0.00%
4.62%
1.46%
92.71%
1.21%
0.00%
32.49%
1.16%
66.35%
95.10%
1.06%
3.64%
0.00%
1.30%
0.84%
96.82%
1.04%
0.00%
20.36%
0.08%
79.56%
95.45%
1.69%
2.47%
0.39%
1.18%
2.25%
94.91%
1.66%
0.04%
21.37%
0.79%
77.80%

FIG. 8A-2

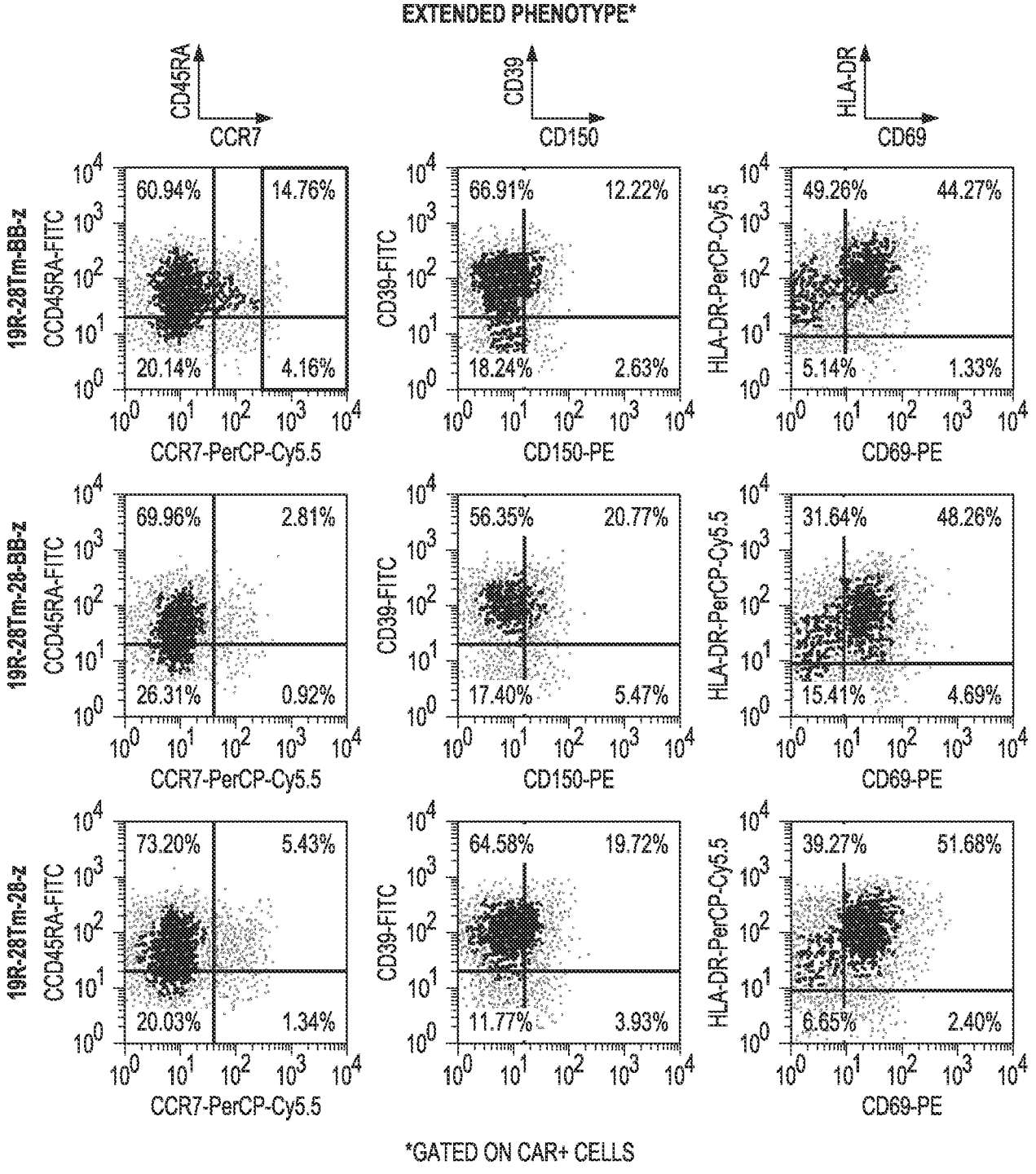

EXTENDED PHENOTYPE*
CD45RA
CCR7
CD39
CD150
HLA-DR
CD69
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z
CCD45RA-FITC
CD39-FITC
HLA-DR-PerCP-Cy5.5
CCR7-PerCP-Cy5.5
CD150-PE
CD69-PE
60.94%
14.76%
20.14%
4.16%
66.91%
12.22%
18.24%
2.63%
49.26%
44.27%
5.14%
1.33%
69.96%
2.81%
26.31%
0.92%
56.35%
20.77%
17.40%
5.47%
31.64%
48.26%
15.41%
4.69%
73.20%
5.43%
20.03%
1.34%
64.58%
19.72%
11.77%
3.93%
39.27%
51.68%
6.65%
2.40%
*GATED ON CAR+ CELLS

FIG. 8B-1

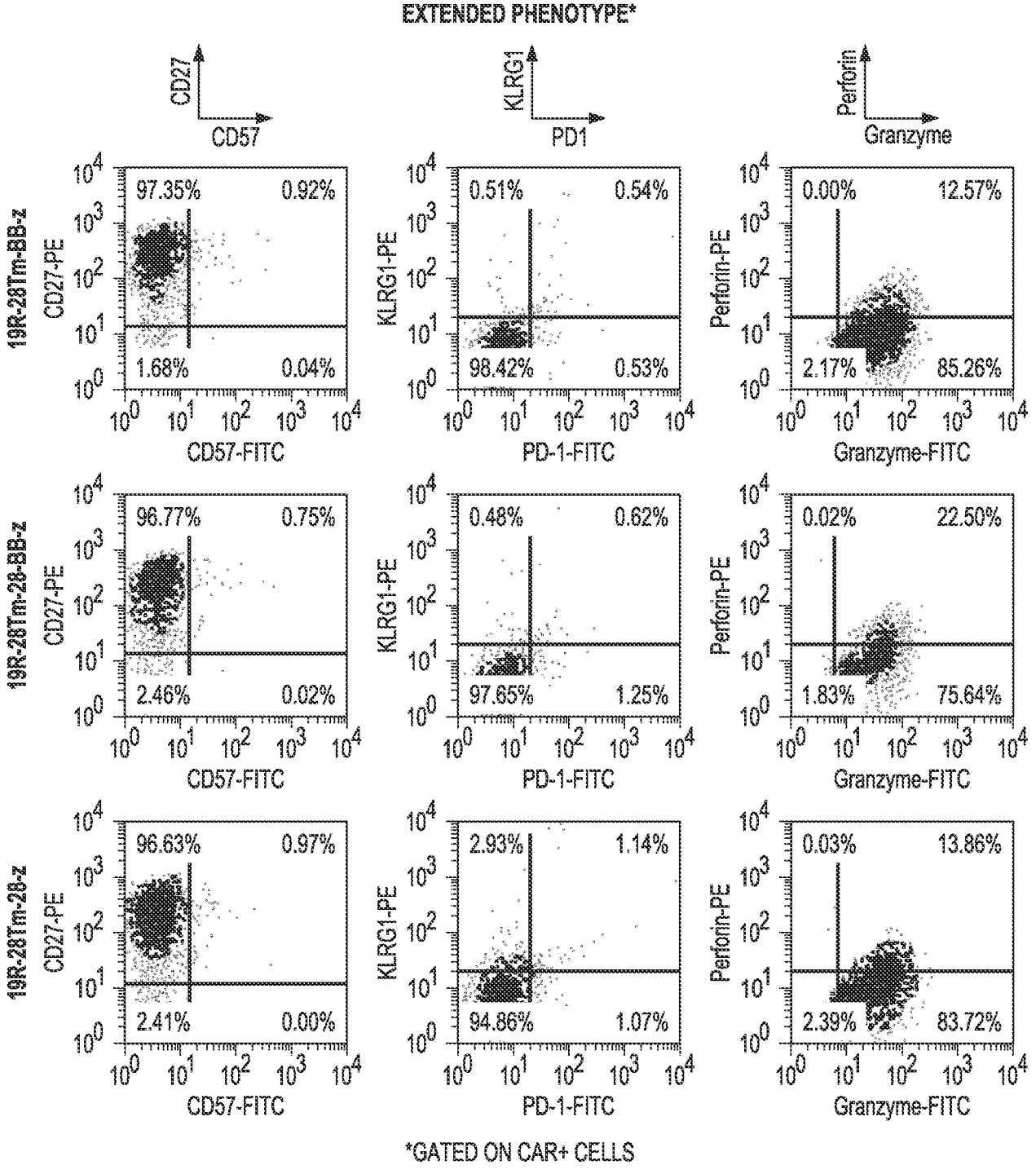

EXTENDED PHENOTYPE*
CD27
CD57
KLRG1
PD1
Perforin
Granzyme
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z
CD27-PE
KLRG1-PE
Perforin-PE
CD57-FITC
PD-1-FITC
Granzyme-FITC
97.35%
0.92%
1.68%
0.04%
0.51%
0.54%
98.42%
0.53%
0.00%
12.57%
2.17%
85.26%
96.77%
0.75%
2.46%
0.02%
0.48%
0.62%
97.65%
1.25%
0.02%
22.50%
1.83%
75.64%
96.63%
0.97%
2.41%
0.00%
2.93%
1.14%
94.86%
1.07%
0.03%
13.86%
2.39%
83.72%
*GATED ON CAR+ CELLS

FIG. 8B-2

CONSTRUCT	MOLECULAR WT (kDS)	
	EXPTD	OBSD
19R-28Tm-28-z		71.4
19R-28Tm-28-z/FRA	73.1	70.1
19r-CD8hTm-BB-z	52.2	51.4
19r-CD8hTm-28-z	52.1	51.9
19R-CD8Tm-BB-z	73.3	73.5
19R-CD8Tm-28-z	73.2	70.7
19R-BBTm-BB-z	73.3	72.8
19R-28Tm-BB-z	73.2	72.8
19R-28Tm-28-BB-z	78.0	77.9

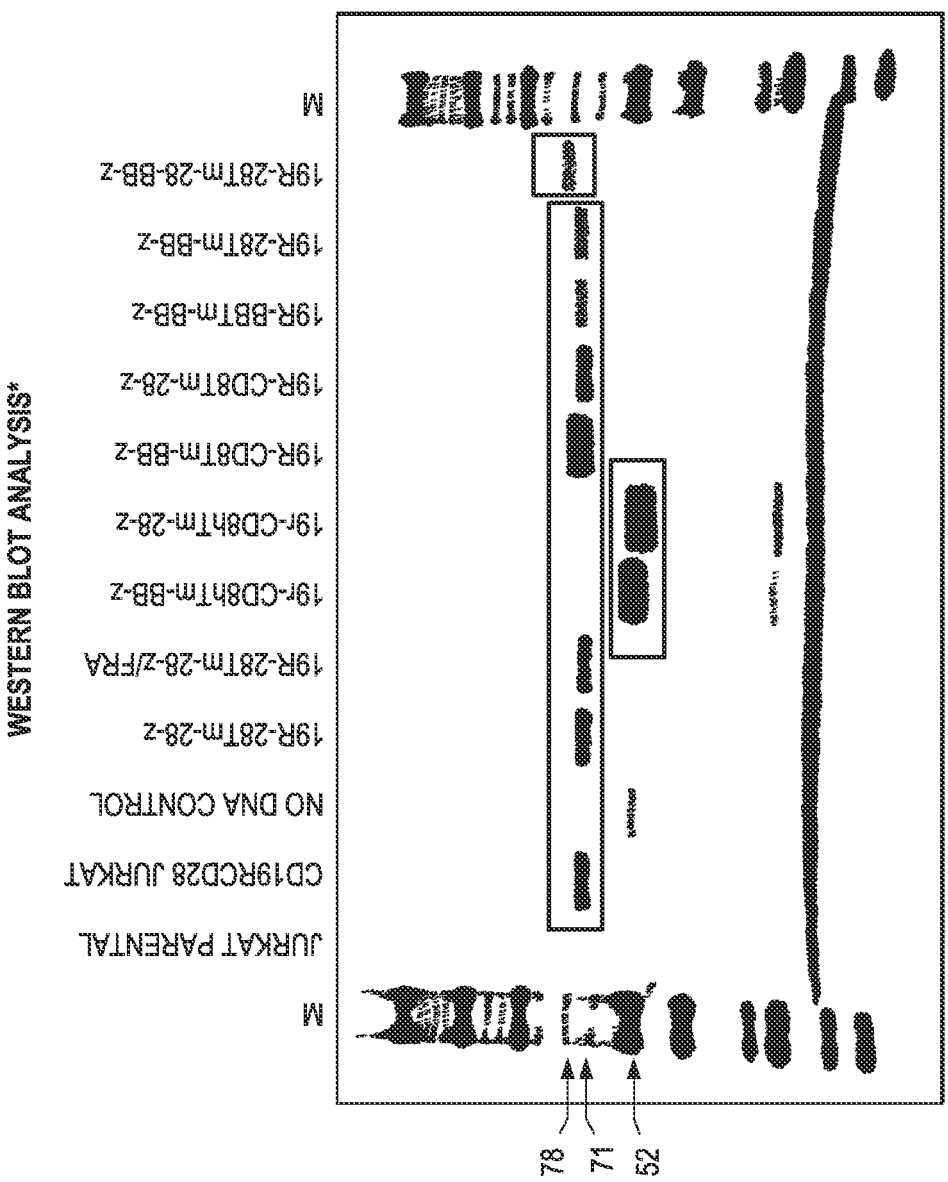
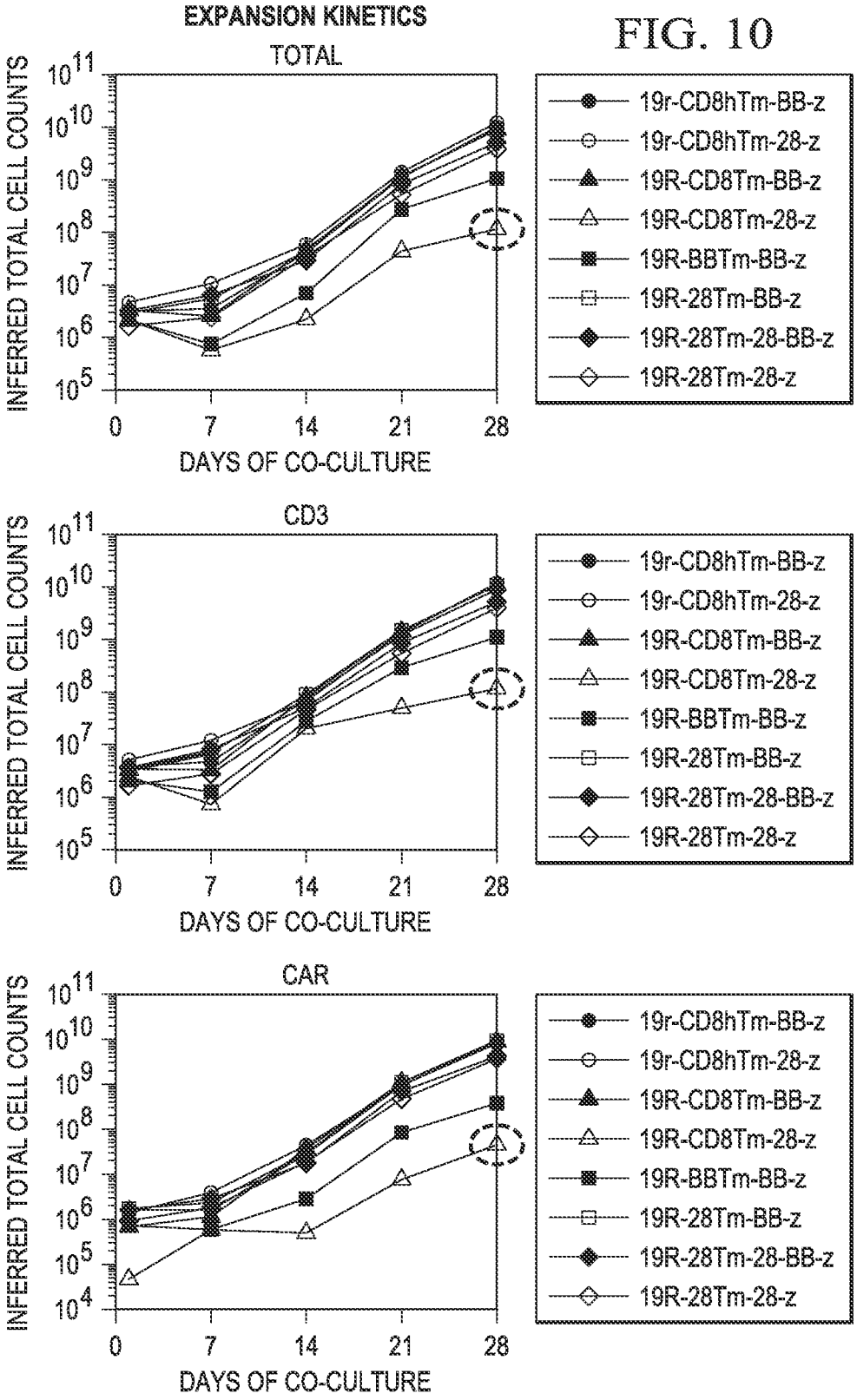


FIG. 9

FIG. 10


EXPANSION KINETICS
TOTAL
INFERRED TOTAL CELL COUNTS
DAYS OF CO-CULTURE
CD3
CAR
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z

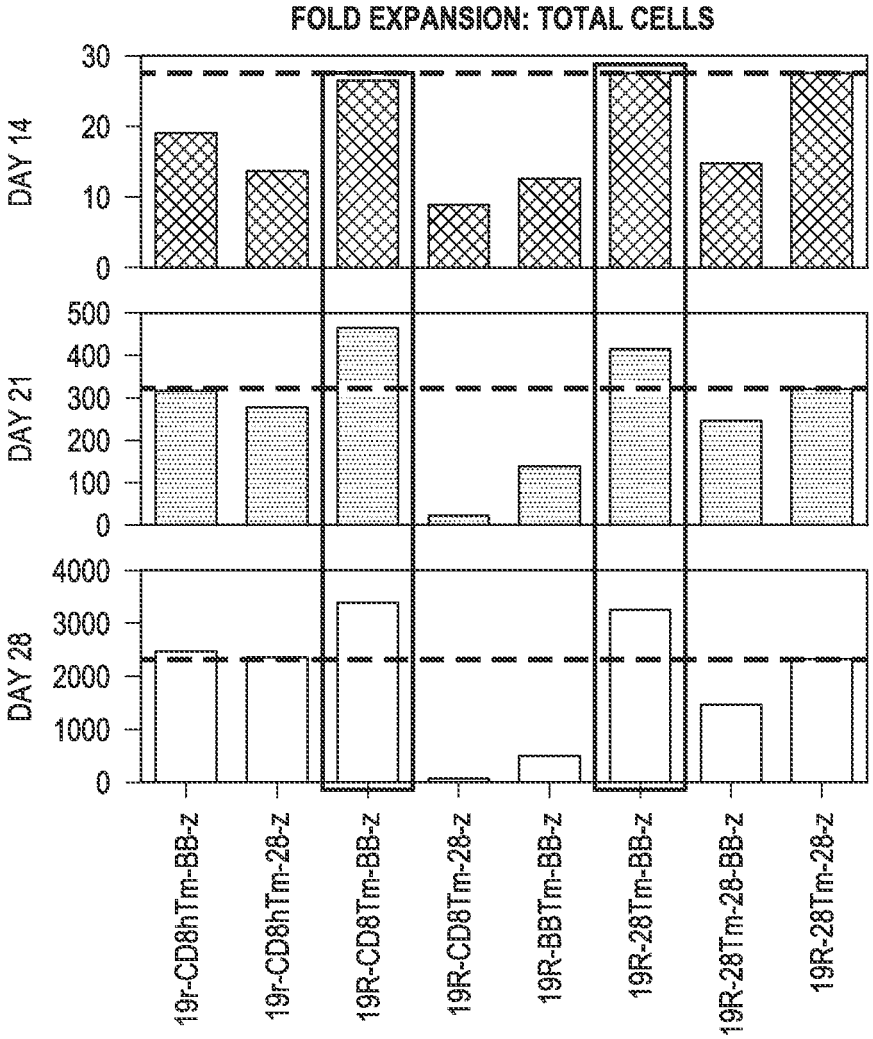

FOLD EXPANSION: TOTAL CELLS
DAY 14
DAY 21
DAY 28
30
20
10
0
500
400
300
200
100
0
4000
3000
2000
1000
0
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z

FIG. 11

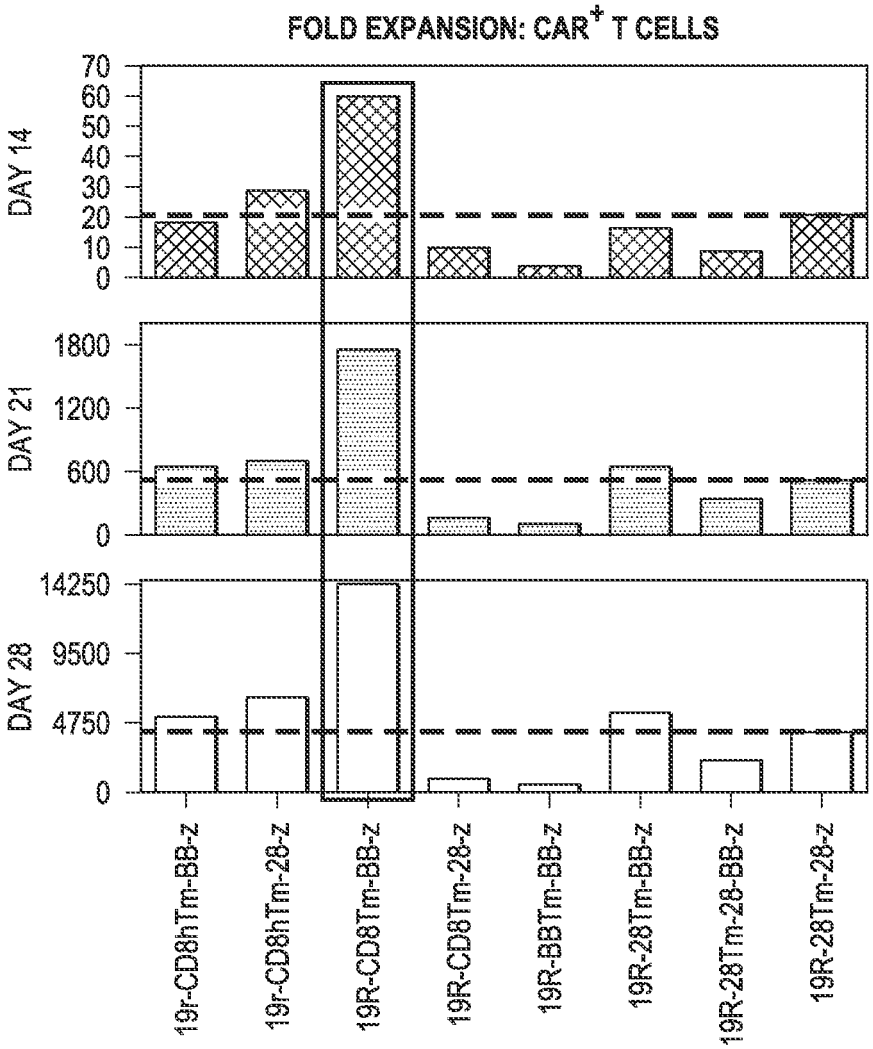
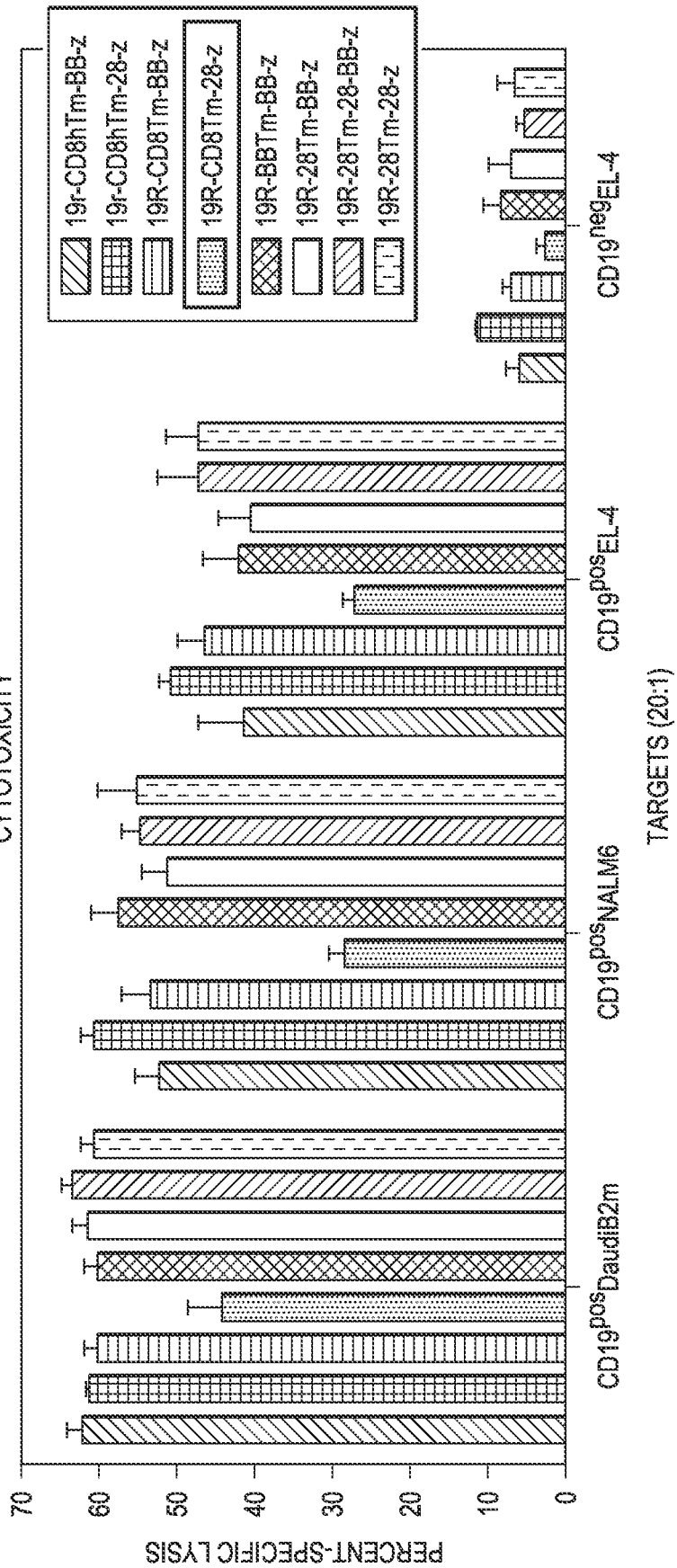

FOLD EXPANSION: CAR+ T CELLS
DAY 14
70
60
50
40
30
20
10
0
DAY 21
1800
1200
600
0
DAY 28
14250
9500
4750
0
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z

FIG. 12

FIG. 13


19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z
CYTOTOXICITY
PERCENT-SPECIFIC LYSIS
70
60
50
40
30
20
10
0
CD19pos DaudiB2m
CD19pos NALM6
CD19pos EL-4
CD19neg EL-4
TARGETS (20:1)

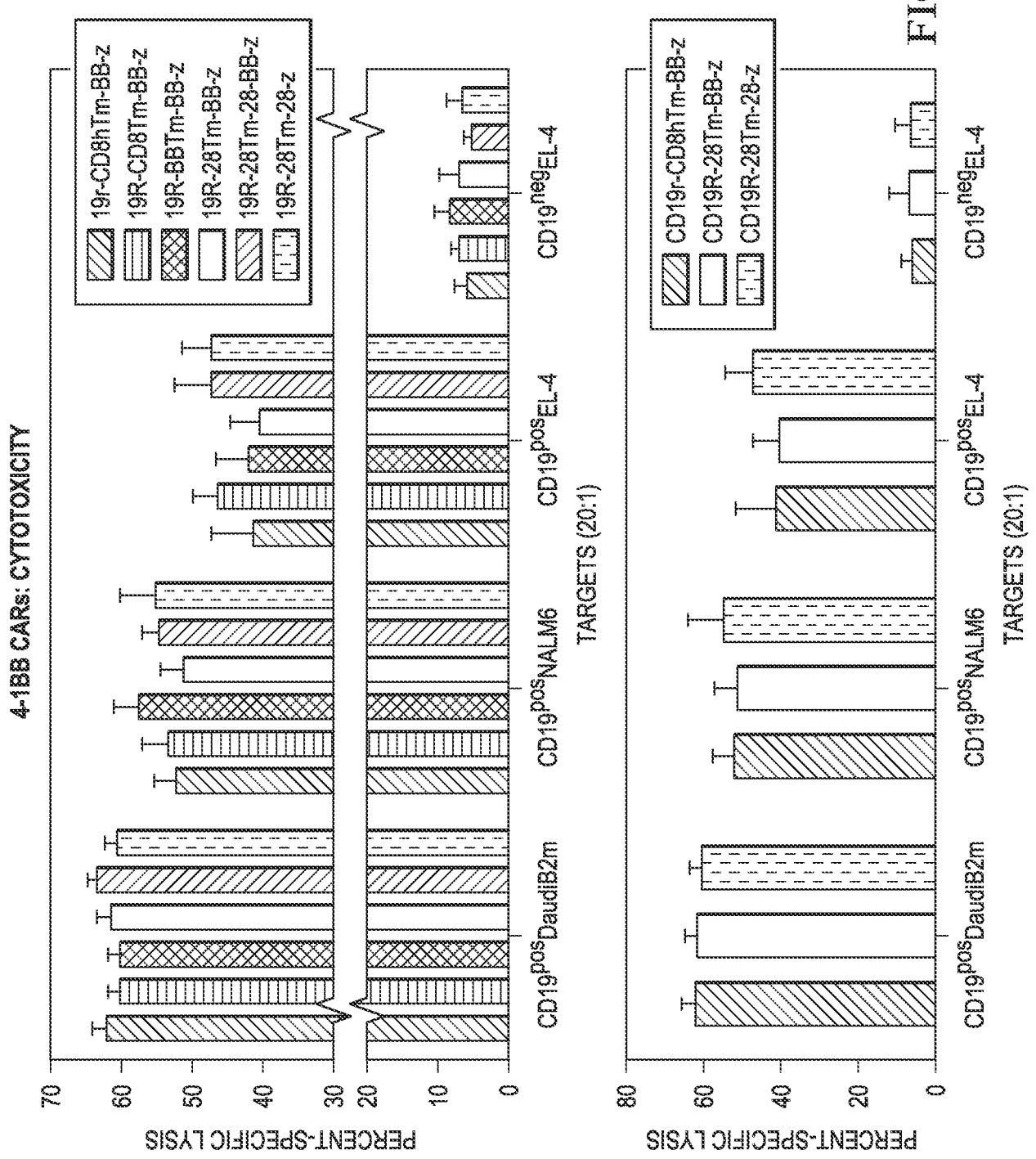

4-1BB CARs: CYTOTOXICITY
19r-CD8hTm-BB-z
19R-CD8Tm-BB-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z
PERCENT-SPECIFIC LYSIS
70
60
50
40
30
20
10
0
CD19posDaudiB2m
CD19posNALM6
CD19posEL-4
CD19negEL-4
TARGETS (20:1)
CD19r-CD8hTm-BB-z
CD19R-28Tm-BB-z
CD19R-28Tm-28-z
PERCENT-SPECIFIC LYSIS
80
60
40
20
0
CD19posDaudiB2m
CD19posNALM6
CD19posEL-4
CD19negEL-4
TARGETS (20:1)

FIG. 14

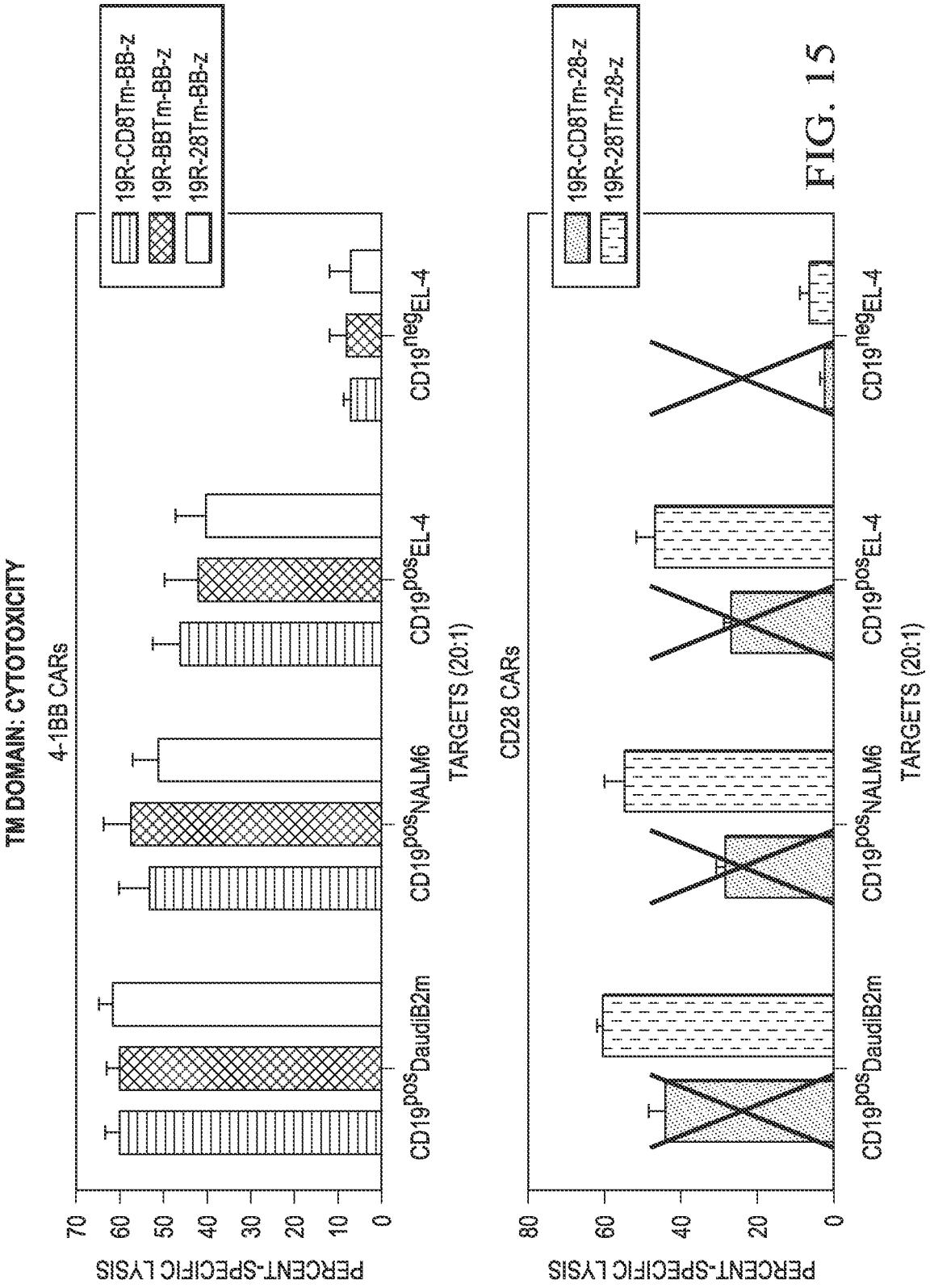

TM DOMAIN: CYTOTOXICITY
4-1BB CARs
19R-CD8Tm-BB-z
19R-BBTm-BB-z
19R-28Tm-BB-z
PERCENT-SPECIFIC LYSIS
70
60
50
40
30
20
10
0
CD19posDaudiB2m
CD19posNALM6
CD19posEL-4
CD19negEL-4
TARGETS (20:1)
CD28 CARs
19R-CD8Tm-28-z
19R-28Tm-28-z
PERCENT-SPECIFIC LYSIS
80
60
40
20
0
CD19posDaudiB2m
CD19posNALM6
CD19posEL-4
CD19negEL-4
TARGETS (20:1)

FIG. 15

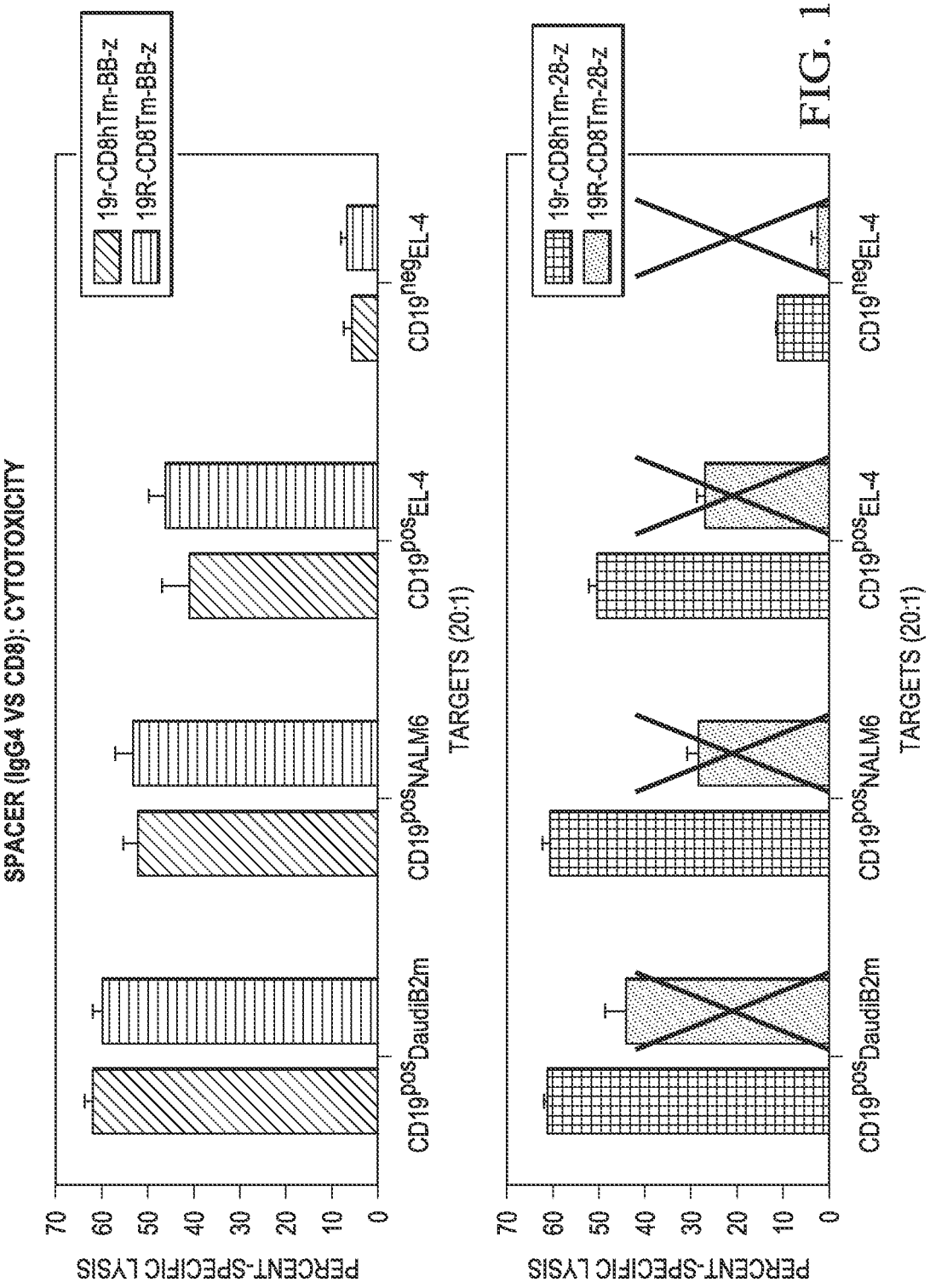

SPACER (IgG4 VS CD8): CYTOTOXICITY
19r-CD8hTm-BB-z
19R-CD8Tm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-28-z
PERCENT-SPECIFIC LYSIS
70
60
50
40
30
20
10
0
CD19pos DaudiB2m
CD19pos NALM6
CD19pos EL-4
CD19neg EL-4
TARGETS (20:1)

FIG. 16

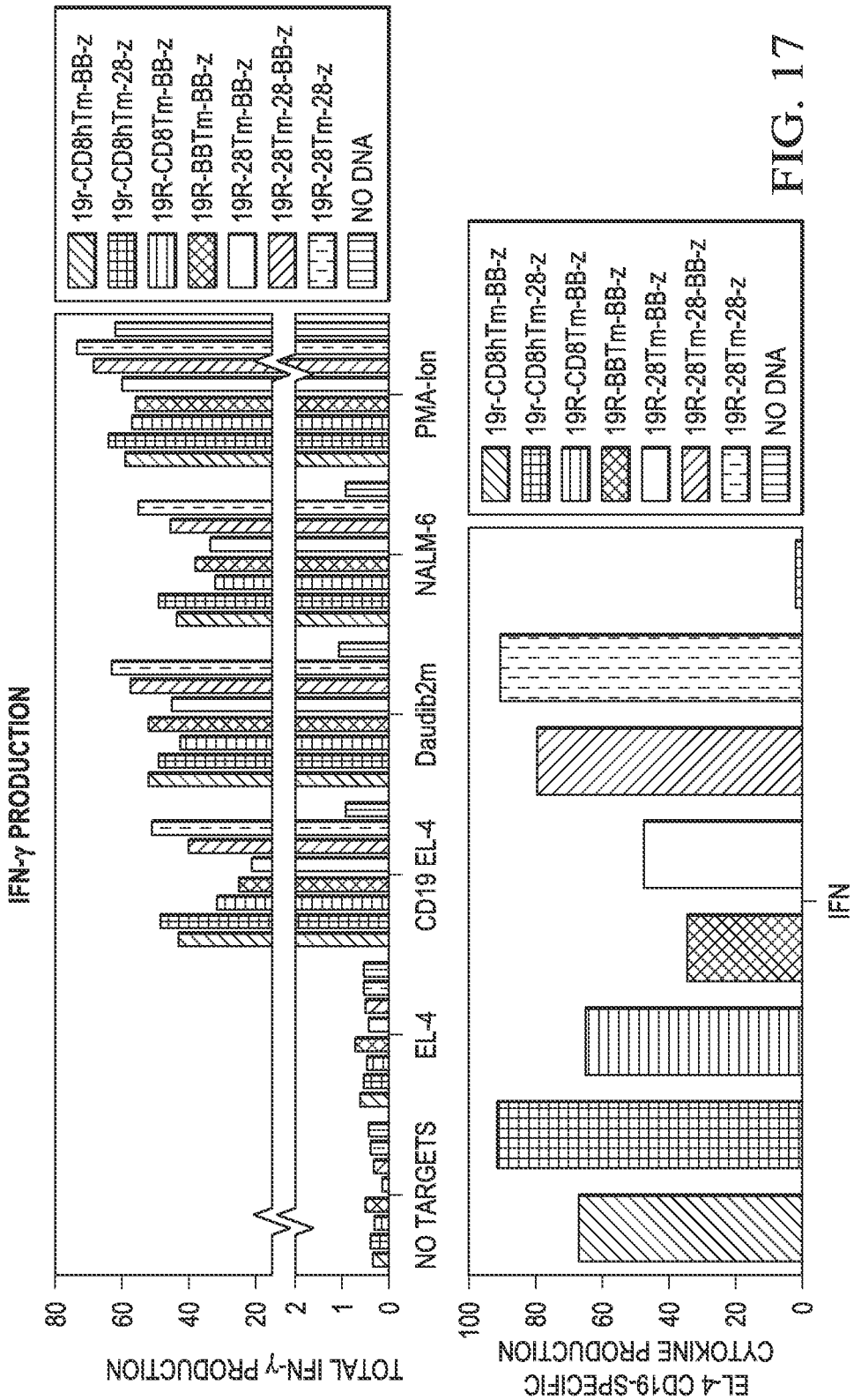

IFN-γ PRODUCTION
TOTAL IFN-γ PRODUCTION
80
60
40
20
2
1
0
NO TARGETS
EL-4
CD19 EL-4
Daudib2m
NALM-6
PMA-Ion
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z
NO DNA
EL-4 CD19-SPECIFIC CYTOKINE PRODUCTION
100
80
60
40
20
0
IFN

FIG. 17

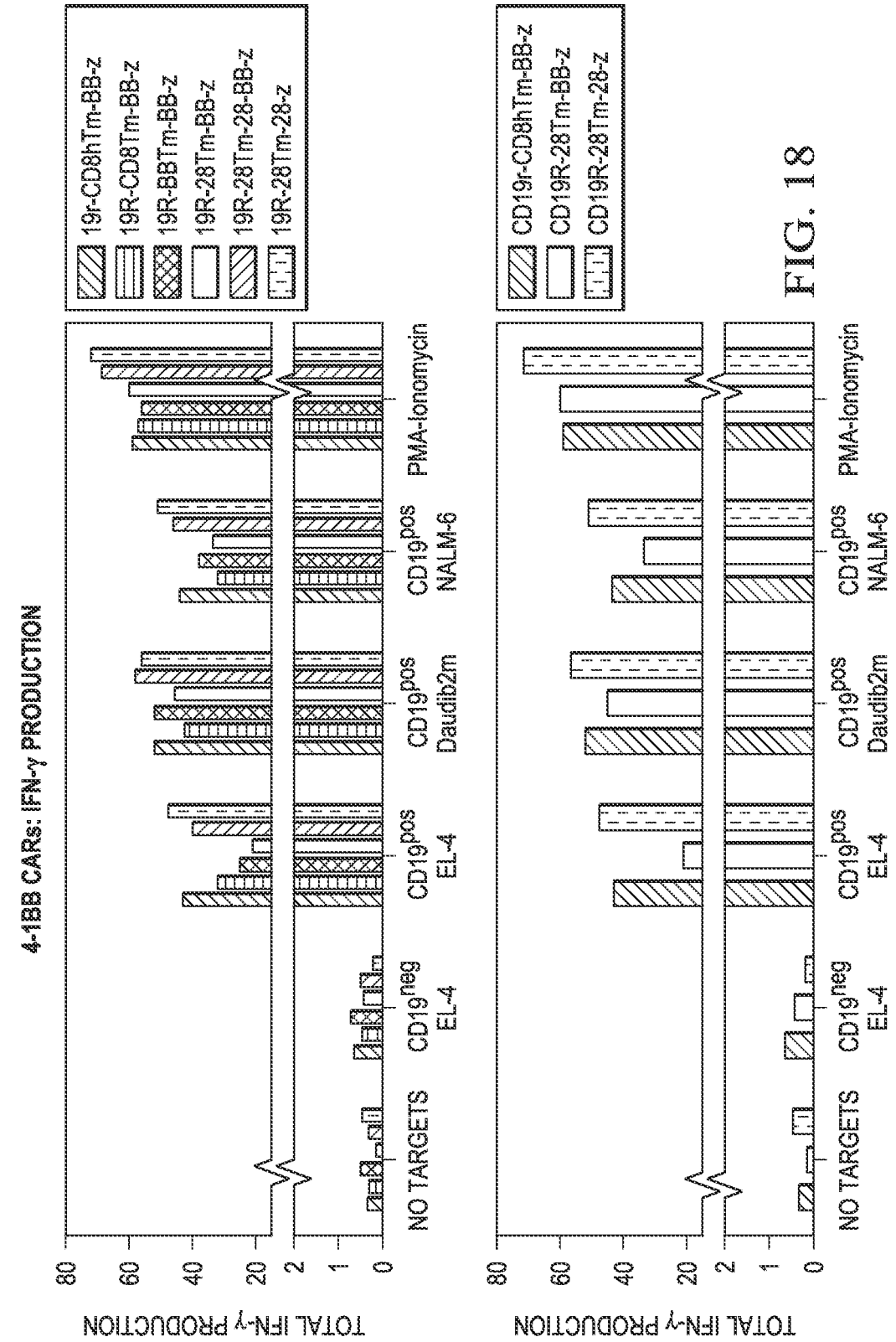

4-1BB CARs: IFN-γ PRODUCTION
TOTAL IFN-γ PRODUCTION
80
60
40
20
2
1
0
19r-CD8hTm-BB-z
19R-CD8Tm-BB-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z
NO TARGETS
CD19neg EL-4
CD19pos EL-4
CD19pos Daudib2m
CD19pos NALM-6
PMA-Ionomycin
CD19r-CD8hTm-BB-z
CD19R-28Tm-BB-z
CD19R-28Tm-28-z

FIG. 18

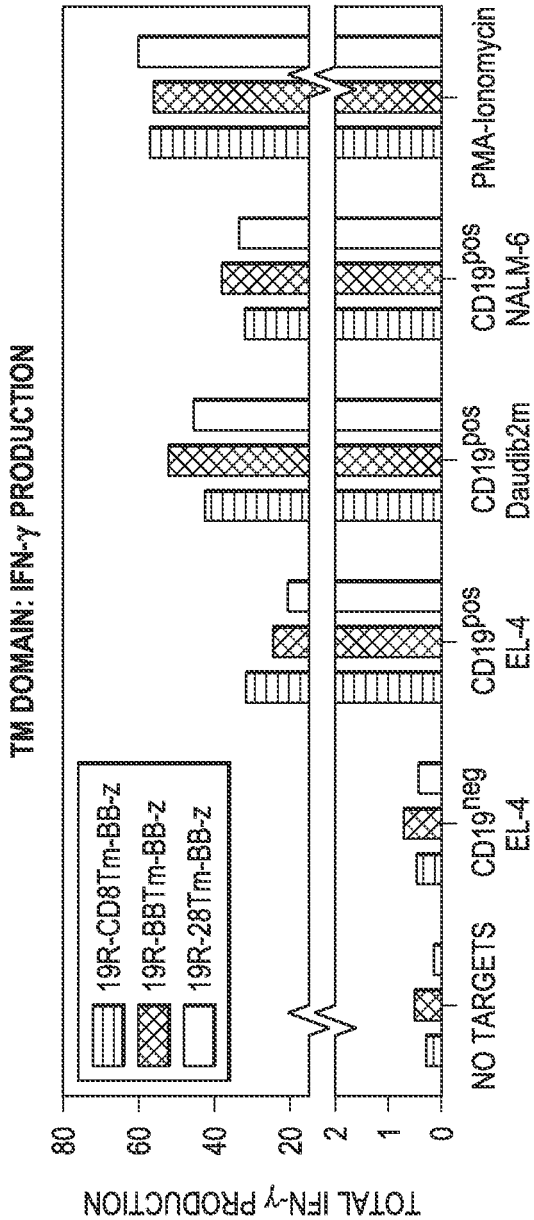

TM DOMAIN: IFN-γ PRODUCTION
19R-CD8Tm-BB-z
19R-BBTm-BB-z
19R-28Tm-BB-z
TOTAL IFN-γ PRODUCTION
80
60
40
20
2
1
0
NO TARGETS
CD19neg EL-4
CD19pos EL-4
CD19pos Daudib2m
CD19pos NALM-6
PMA-Ionomycin

FIG. 19

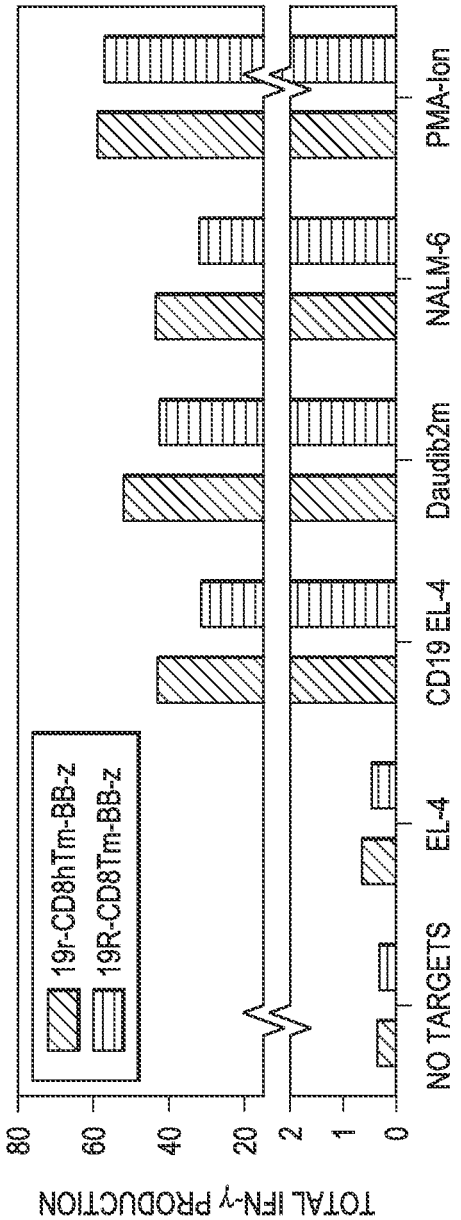

SPACER (IgG4 VS CD8): IFN-γ PRODUCTION
19r-CD8hTm-BB-z
19R-CD8Tm-BB-z
TOTAL IFN-γ PRODUCTION
80
60
40
20
2
1
0
NO TARGETS
EL-4
CD19 EL-4
Daudib2m
NALM-6
PMA-Ion

FIG. 20

FIG. 21

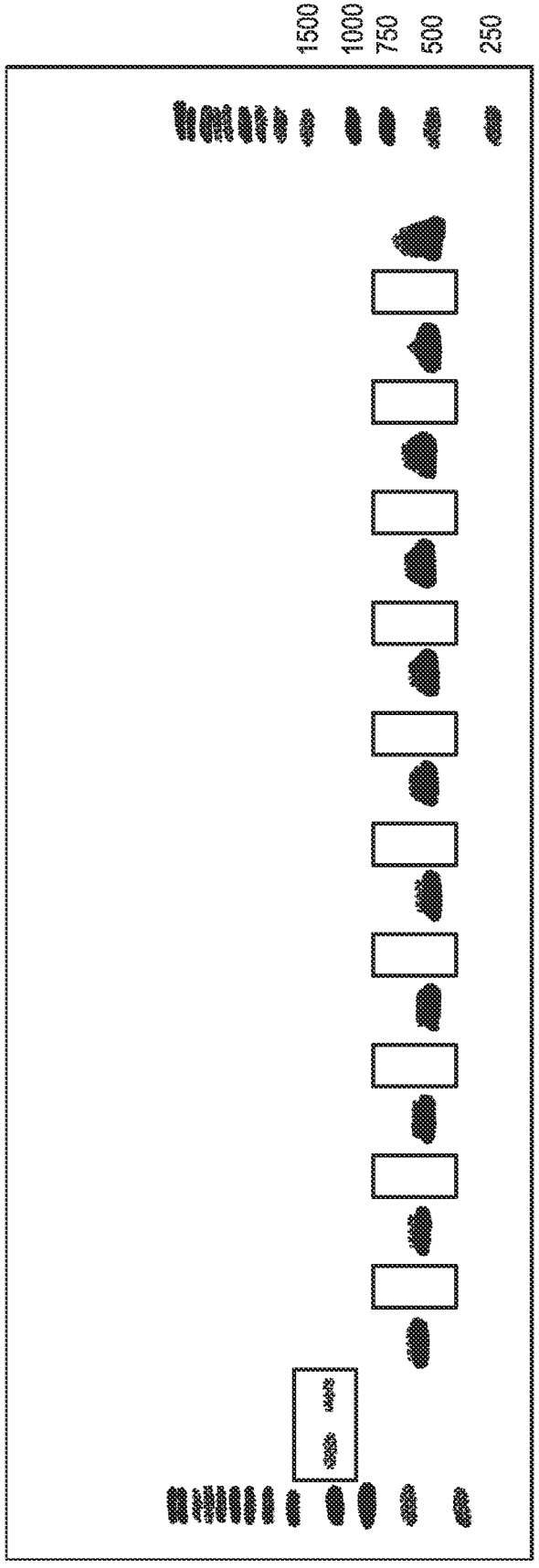
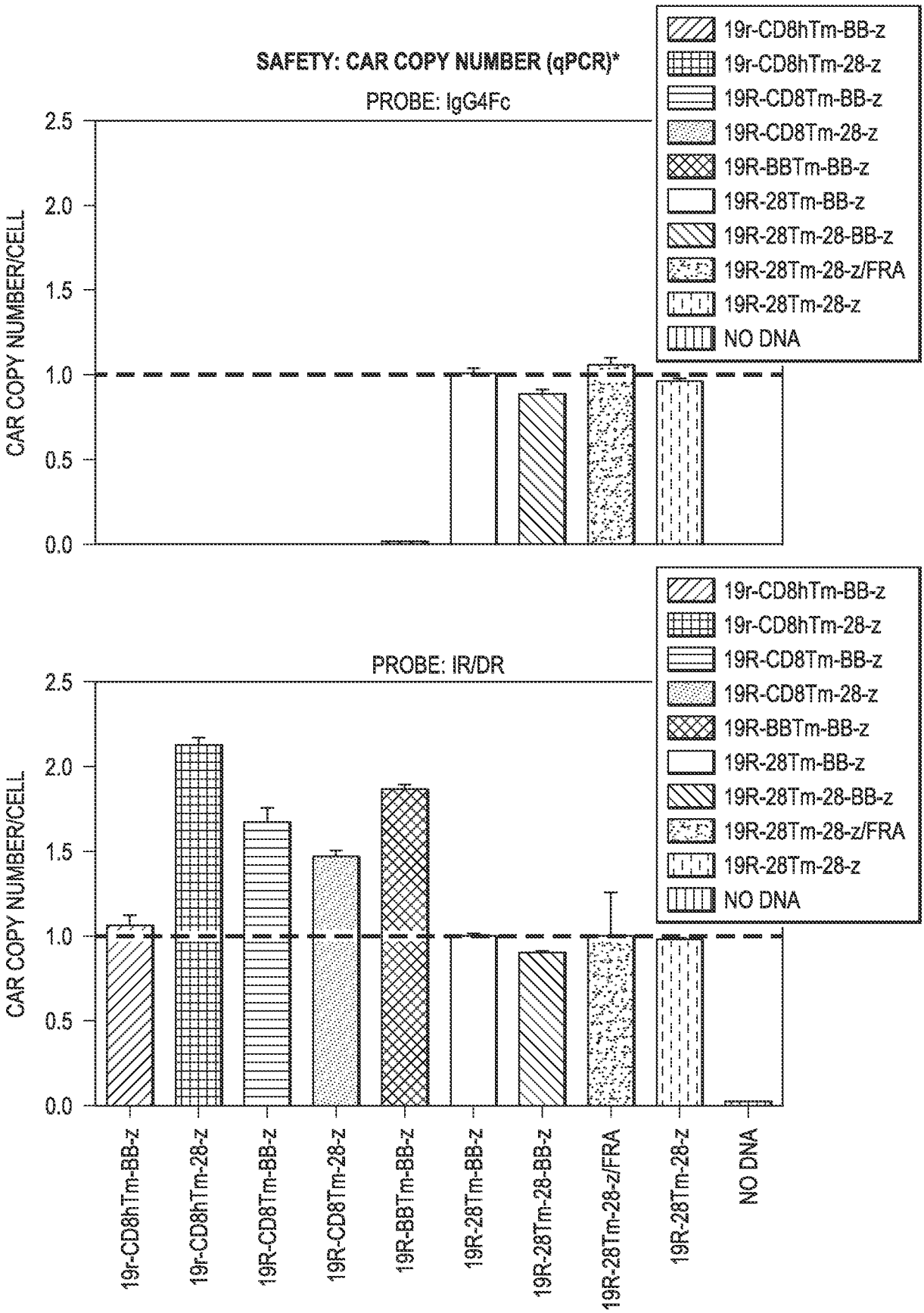

SAFETY: SB11 PCR*
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
1500
1000
750
500
250
SB11 PRIMERS
GAPDH PRIMERS
1,26 = 1kb LADDER
25 = BLANK
2 = SB11-IRES2-EGFP PLASMID
3 = JURKAT/SB11-IRES2-EGFP
5 = NO DNA
7 = 217
9 = 215
11 = 213
13 = 218
15 = 214
17 = CAR
19 = 193
21 = 216
23 = 212
4 = JURKAT/SB11-IRES2-EGFP
6 = NO DNA
8 = 217
10 = 215
12 = 213
14 = 218
16 = 214
18 = CAR
20 = 193
22 = 216
24 = 212

FIG. 22


SAFETY: CAR COPY NUMBER (qPCR)*
PROBE: IgG4Fc
CAR COPY NUMBER/CELL
2.5
2.0
1.5
1.0
0.5
0.0
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z/FRA
19R-28Tm-28-z
NO DNA
PROBE: IR/DR
CAR COPY NUMBER/CELL
2.5
2.0
1.5
1.0
0.5
0.0
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z/FRA
19R-28Tm-28-z
NO DNA

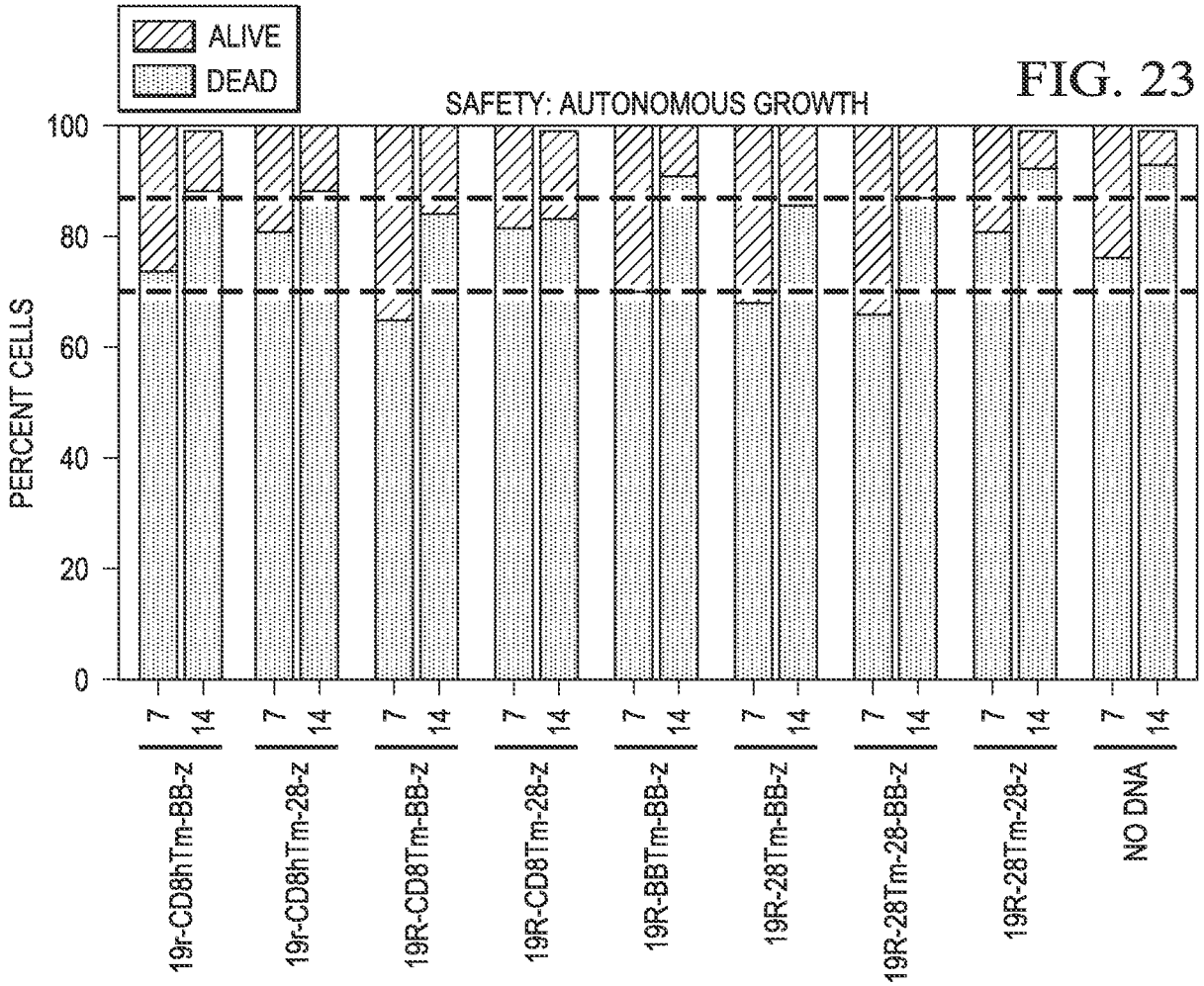

ALIVE
DEAD
SAFETY: AUTONOMOUS GROWTH
PERCENT CELLS
100
80
60
40
20
0
7
14
19r-CD8hTm-BB-z
19r-CD8hTm-28-z
19R-CD8Tm-BB-z
19R-CD8Tm-28-z
19R-BBTm-BB-z
19R-28Tm-BB-z
19R-28Tm-28-BB-z
19R-28Tm-28-z
NO DNA

FIG. 23

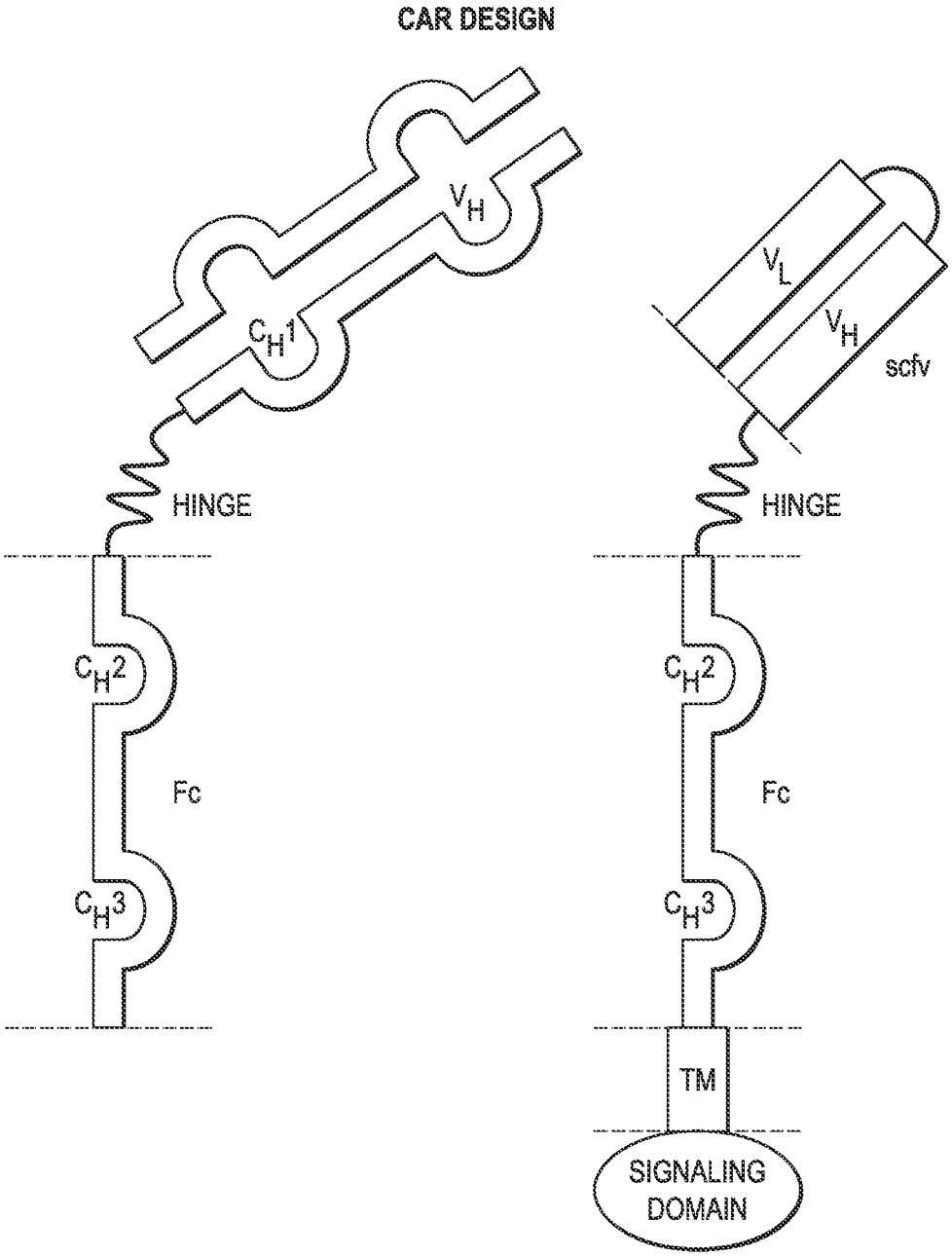

CAR DESIGN
V_H
C_H1
HINGE
C_H2
Fc
C_H3
V_L
V_H
scfv
HINGE
C_H2
Fc
C_H3
TM
SIGNALING DOMAIN

FIG. 24

CD3ζ

HOMO SAPIENS CD247 MOLECULE (CD247), TRANSCRIPT VARIANT 1, mRNA
NCBI REFERENCE SEQUENCE: NM_198053.2

```
Query    1   RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP-RRKNPQEGLY  59
Sbjct        RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLY

Query   60   NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR  112
Sbjct        NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
```

T-CELL SURFACE GLYCOPROTEIN CD3 ZETA CHAIN ISOFORM 1 PRECURSOR [HOMO SAPIENS]
NCBI REFERENCE SEQUENCE: NP_932170.1

```
Query    1   RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP-RRKNPQEGLY  59
Sbjct   52   RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLY  111

Query   60   NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR  112
Sbjct  112   NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR  164
```

T-CELL SURFACE GLYCOPROTEIN CD3 ZETA CHAIN ISOFORM 2 PRECURSOR [HOMO SAPIENS]
NCBI REFERENCE SEQUENCE: NP_000725.1

ITAM1 ITAM2

```
Query    1   RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN  60
Sbjct   52   RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN  111
```

ITAM3

```
Query   61   ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR  112
Sbjct  112   ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR  163
```

FIG. 25

FIG. 26

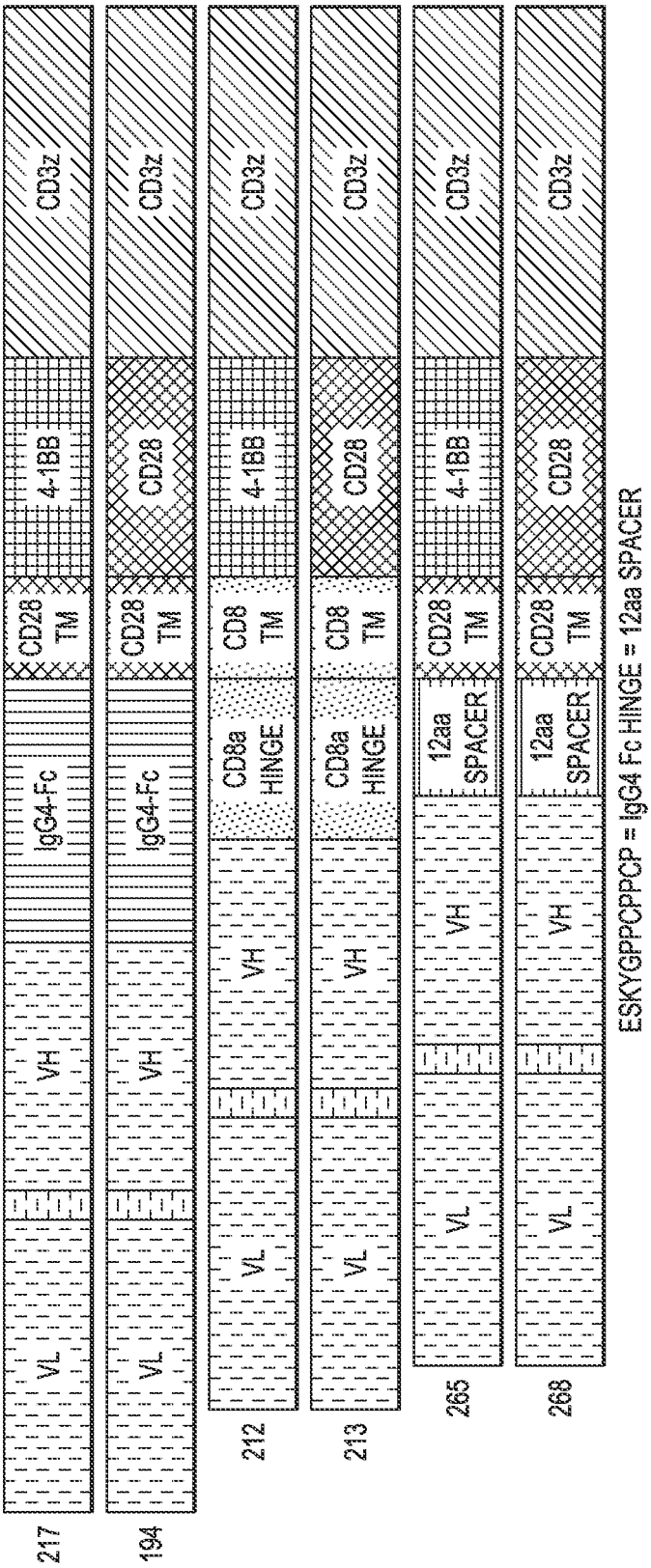


FIG. 27

CARs

CAR →	COOPER	
SPACER	CD28	CD137
Fc (230aa)	194	217
CD8 (47aa)	213	212
SHORT (12aa)	265	268
CD3ζ	WT	WT

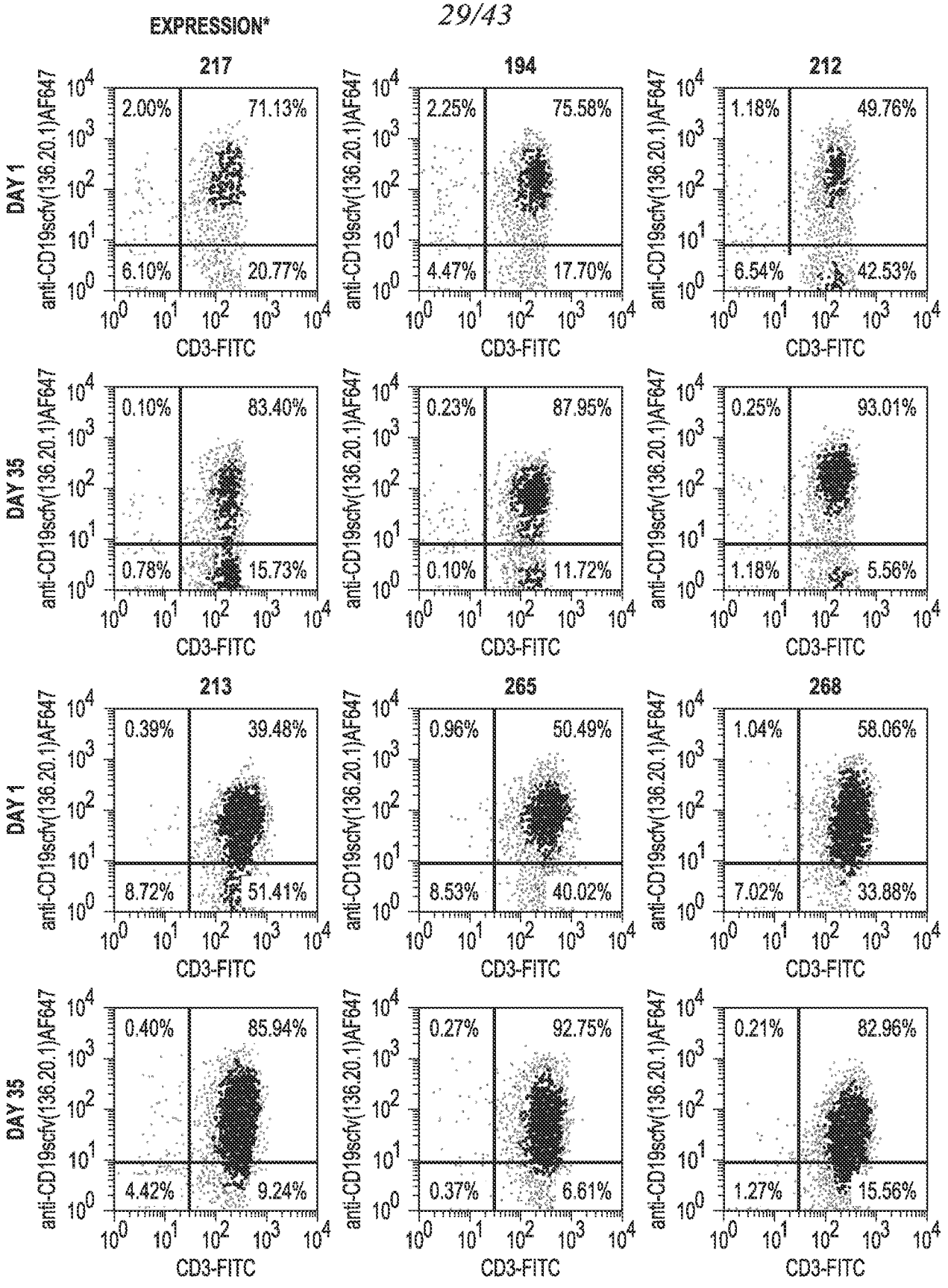

EXPRESSION*
217
194
212
213
265
268
DAY 1
DAY 35
anti-CD19scfv(136.20.1)AF647
CD3-FITC
2.00%
71.13%
6.10%
20.77%
2.25%
75.58%
4.47%
17.70%
1.18%
49.76%
6.54%
42.53%
0.10%
83.40%
0.78%
15.73%
0.23%
87.95%
0.10%
11.72%
0.25%
93.01%
1.18%
5.56%
0.39%
39.48%
8.72%
51.41%
0.96%
50.49%
8.53%
40.02%
1.04%
58.06%
7.02%
33.88%
0.40%
85.94%
4.42%
9.24%
0.27%
92.75%
0.37%
6.61%
0.21%
82.96%
1.27%
15.56%

FIG. 28

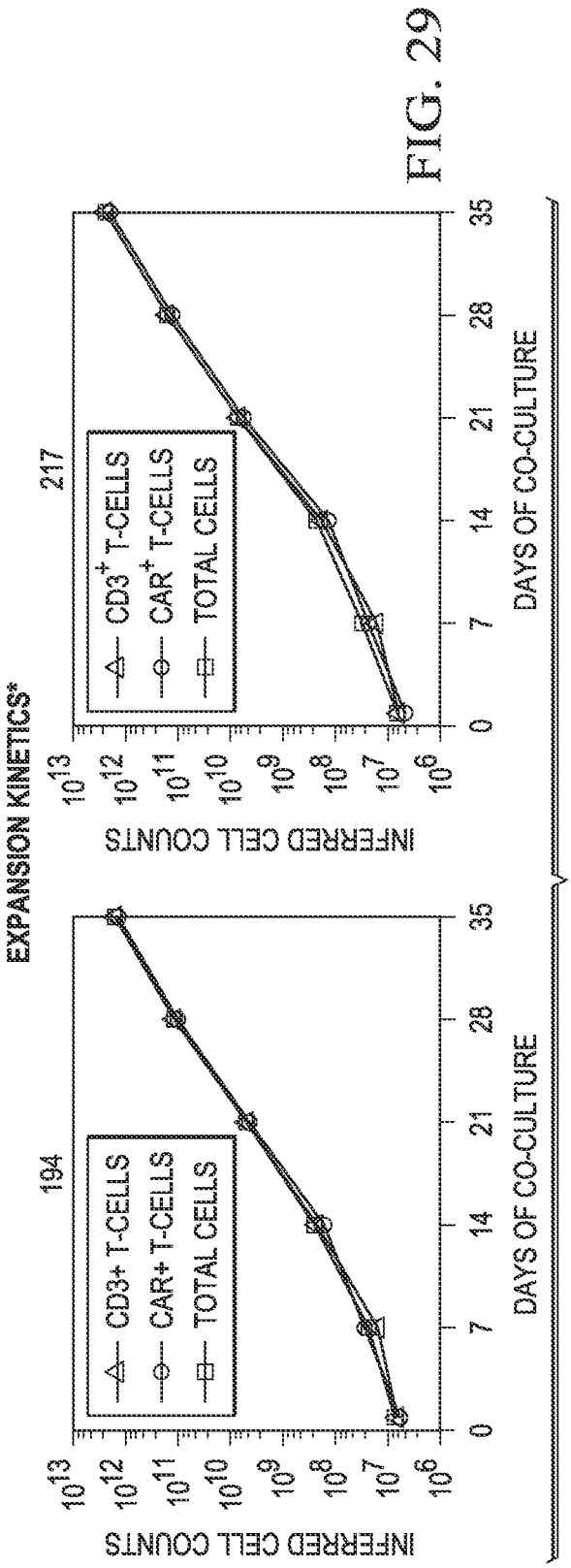

EXPANSION KINETICS*
194
217
CD3+ T-CELLS
CAR+ T-CELLS
TOTAL CELLS
INFERRED CELL COUNTS
DAYS OF CO-CULTURE

FIG. 29

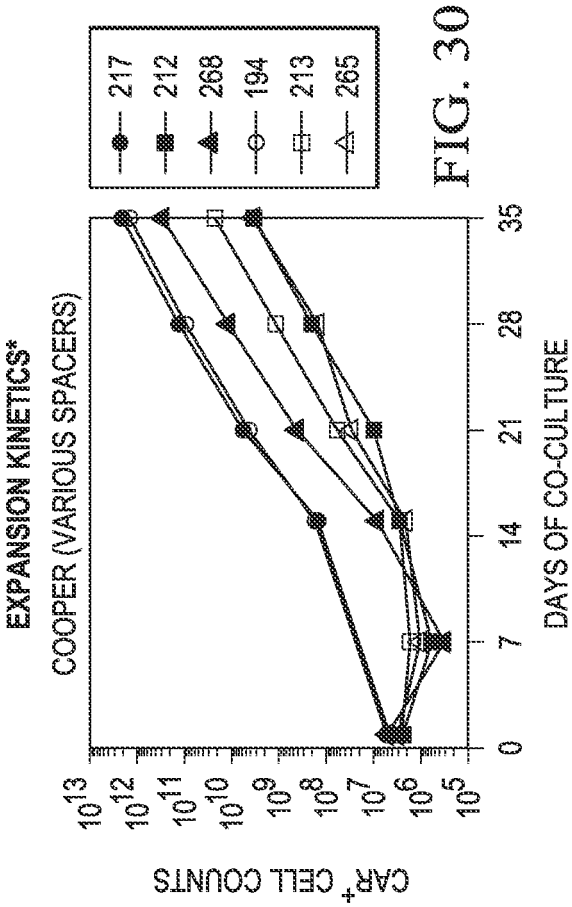

EXPANSION KINETICS*
COOPER (VARIOUS SPACERS)
217
212
268
194
213
265
CAR+ CELL COUNTS
DAYS OF CO-CULTURE

FIG. 30

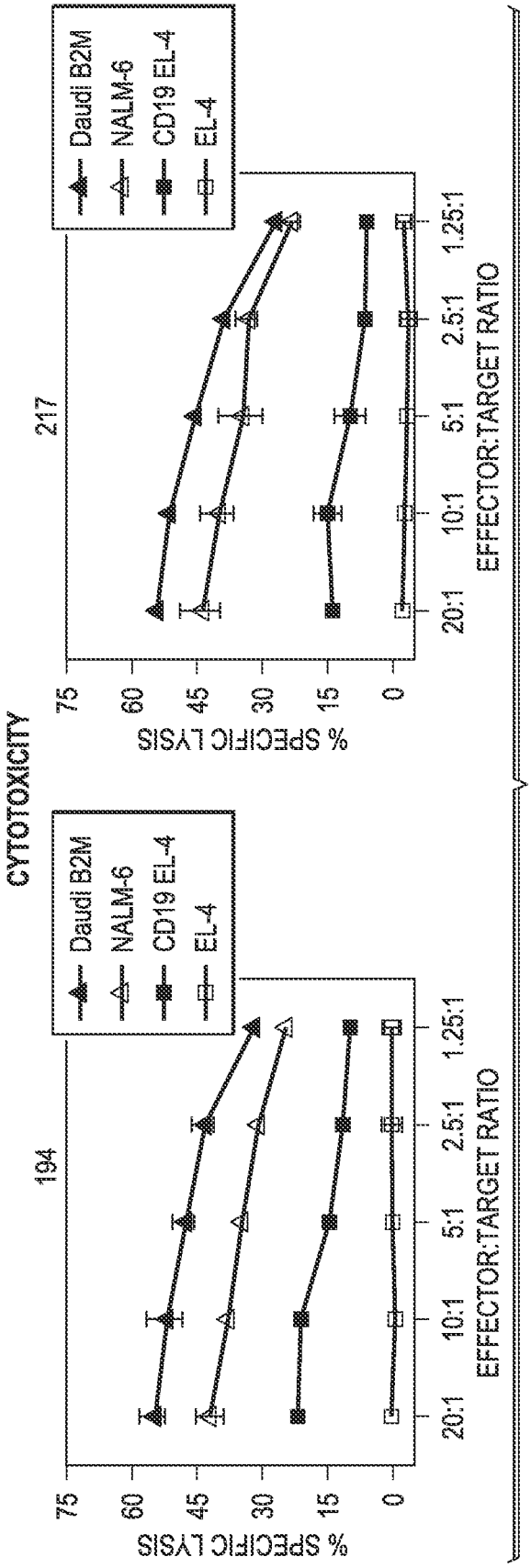


FIG. 31

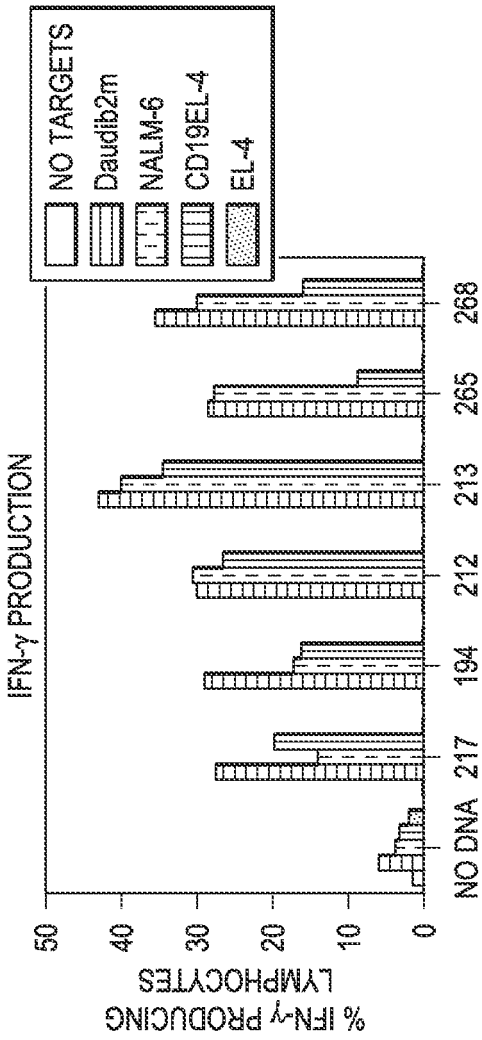


FIG. 34

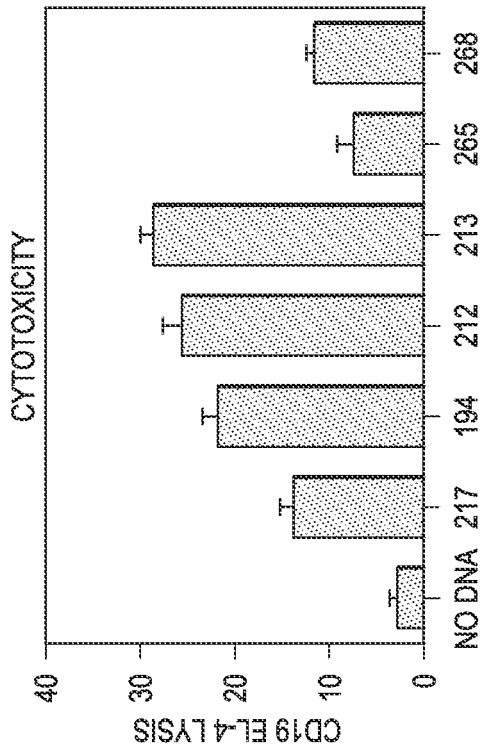


FIG. 32

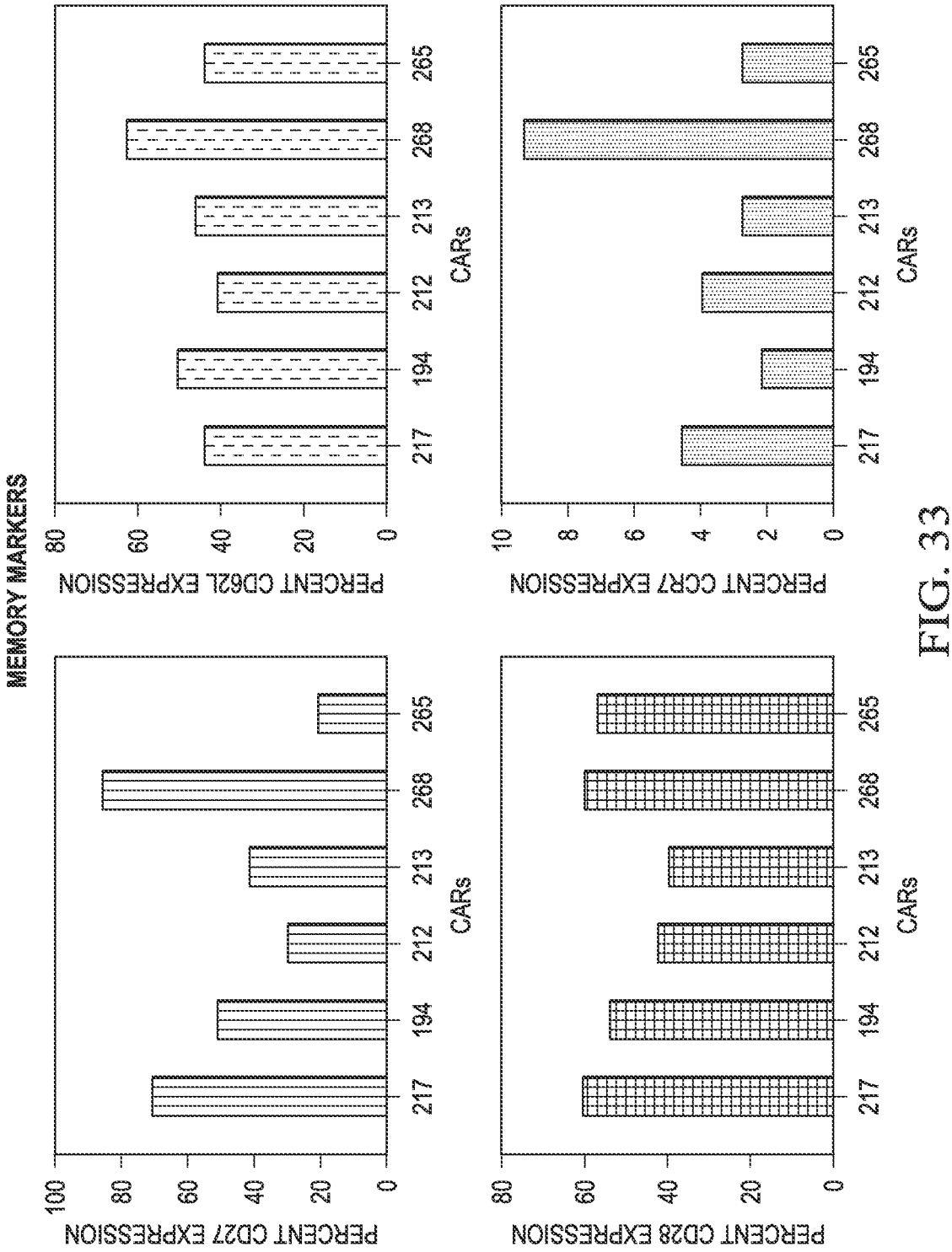

MEMORY MARKERS
PERCENT CD62L EXPRESSION
PERCENT CCR7 EXPRESSION
PERCENT CD27 EXPRESSION
PERCENT CD28 EXPRESSION
CARs
217
194
212
213
268
265
0
20
40
60
80
100
2
4
6
8
10

FIG. 33

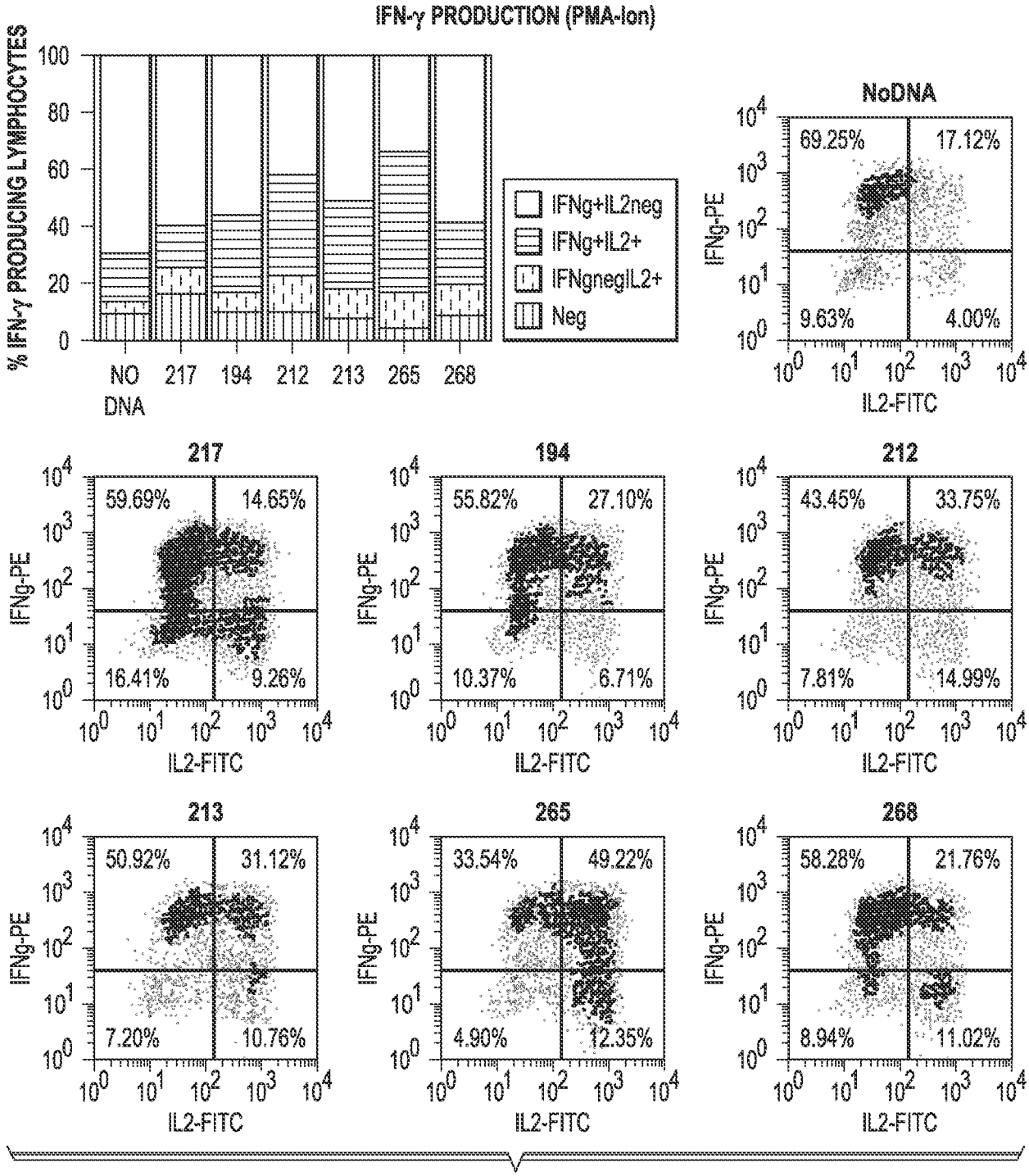

IFN-γ PRODUCTION (PMA-Ion)
% IFN-γ PRODUCING LYMPHOCYTES
100
80
60
40
20
0
NO DNA
217
194
212
213
265
268
IFNg+IL2neg
IFNg+IL2+
IFNgnegIL2+
Neg
NoDNA
69.25%
17.12%
9.63%
4.00%
217
59.69%
14.65%
16.41%
9.26%
194
55.82%
27.10%
10.37%
6.71%
212
43.45%
33.75%
7.81%
14.99%
213
50.92%
31.12%
7.20%
10.76%
265
33.54%
49.22%
4.90%
12.35%
268
58.28%
21.76%
8.94%
11.02%
IFNg-PE
IL2-FITC

FIG. 35

FIG. 36

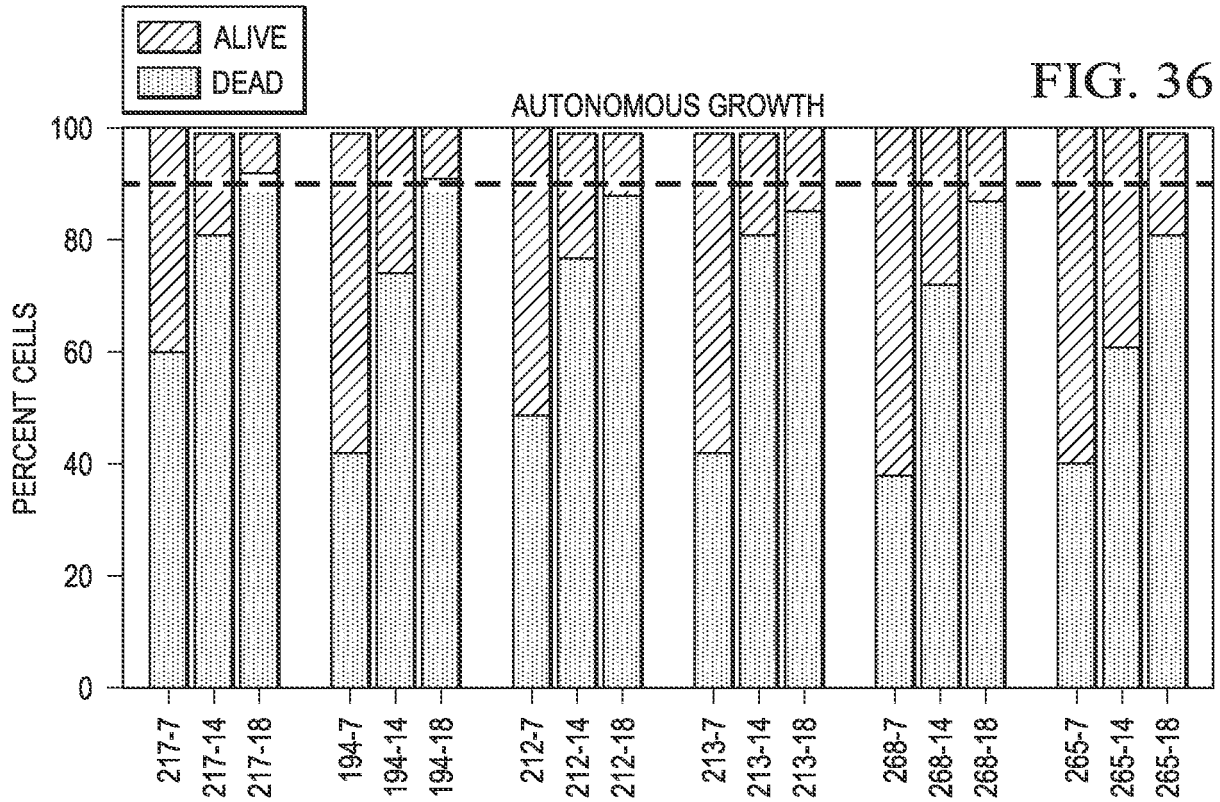
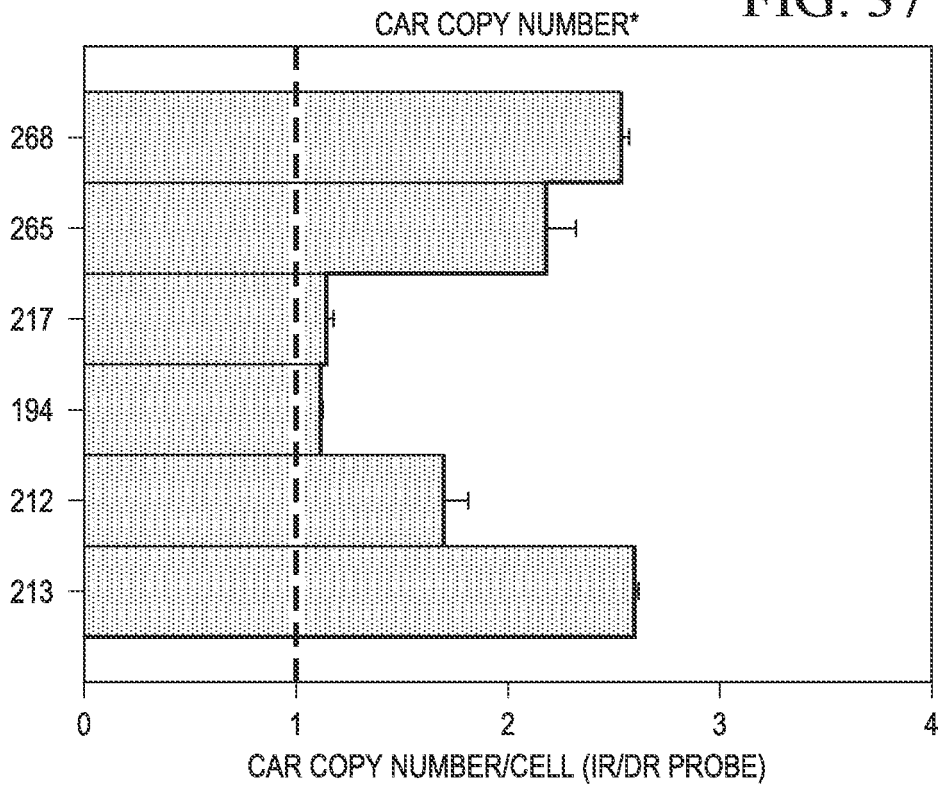

ALIVE
DEAD
AUTONOMOUS GROWTH
PERCENT CELLS
100
80
60
40
20
0
217-7
217-14
217-18
194-7
194-14
194-18
212-7
212-14
212-18
213-7
213-14
213-18
268-7
268-14
268-18
265-7
265-14
265-18

FIG. 37


CAR COPY NUMBER*
268
265
217
194
212
213
0
1
2
3
4
CAR COPY NUMBER/CELL (IR/DR PROBE)

DONOR P491C714
DONOR GCR357861

FIG. 38

CAR COPY NUMBER

DOMAINS	COOPER						
INTRACELLULAR SIGNALING	CD28			CD137			CD28+ CD137
TM/SPACER	CD8	CD28	CD137	CD8	CD28	CD137	CD28
Fc (230aa)	215 (1.48)	194 (1.12) 193 (1.01) 118 (0.98)		214 (1.67)	217 (1.01, 1.14)	216 (1.87)	218 (0.90)
CD8 (47aa)	213 (2.13, 2.6)			212 (1.06, 1.7)			
SHORT (12aa)		265 (2.18)			268 (2.53)		
CD3ζ	WT	WT	WT	WT	WT	WT	WT

FIG. 39

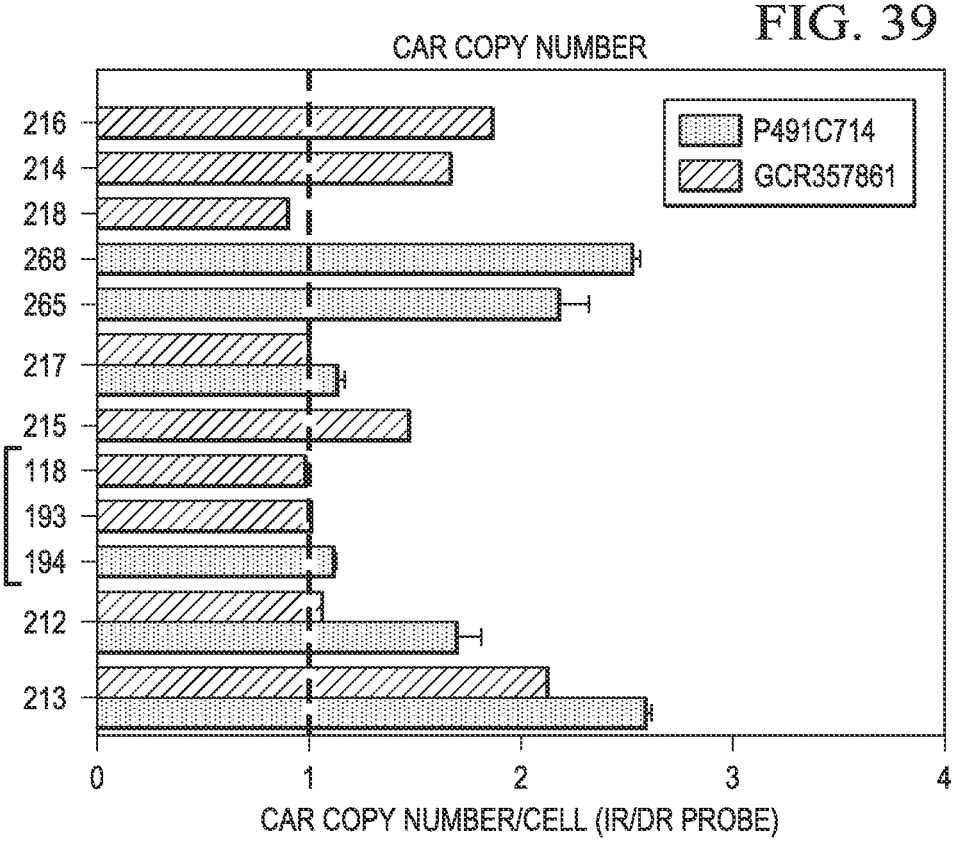
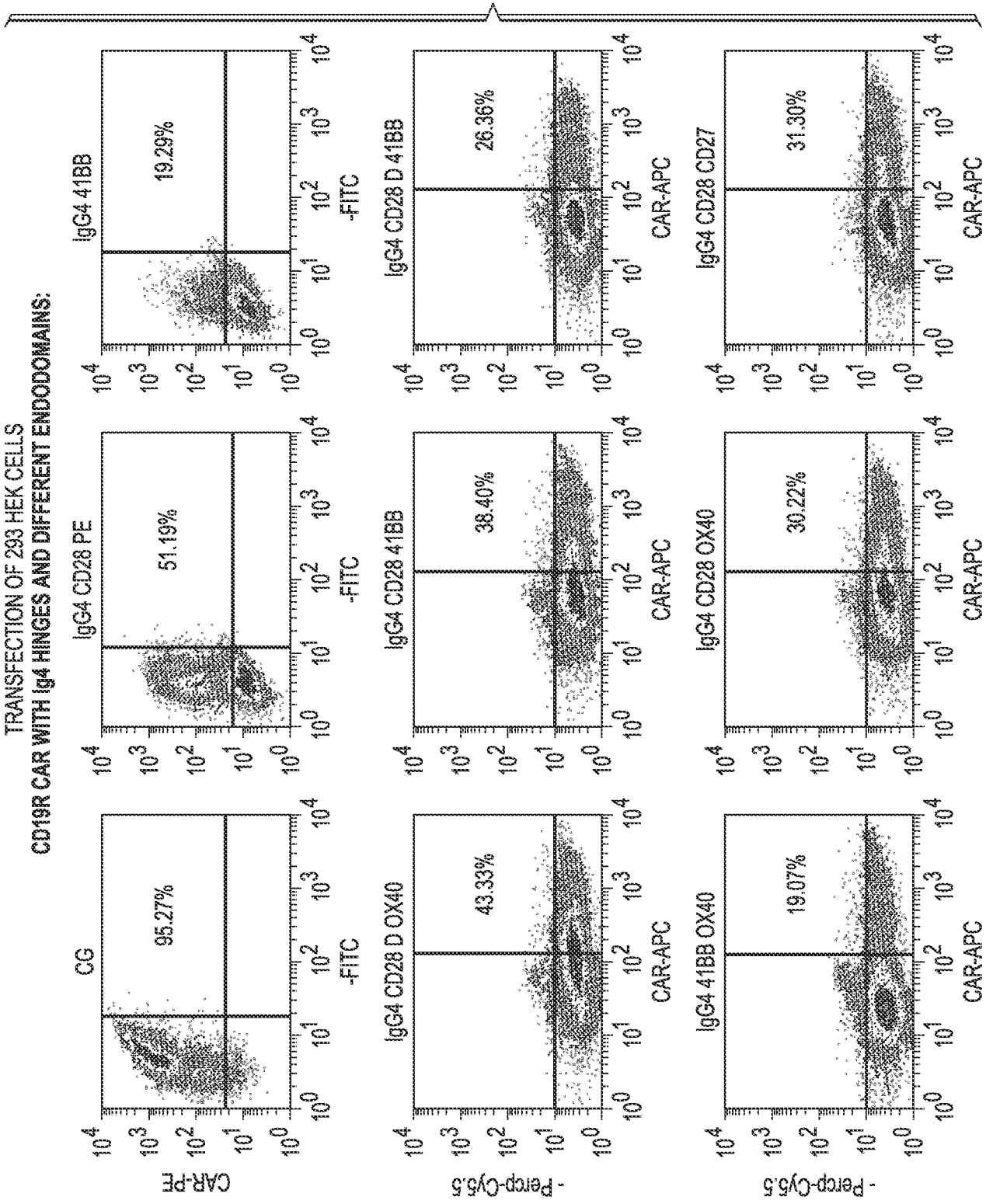


FIG. 40A


TRANSFECTION OF 293 HEK CELLS
CD19R CAR WITH Ig4 HINGES AND DIFFERENT ENDODOMAINS:
CG
95.27%
IgG4 CD28 PE
51.19%
IgG4 41BB
19.29%
IgG4 CD28 D OX40
43.33%
IgG4 CD28 41BB
38.40%
IgG4 CD28 D 41BB
26.36%
IgG4 41BB OX40
19.07%
IgG4 CD28 OX40
30.22%
IgG4 CD28 CD27
31.30%
CAR-PE
-FITC
- Percp-Cy5.5
CAR-APC

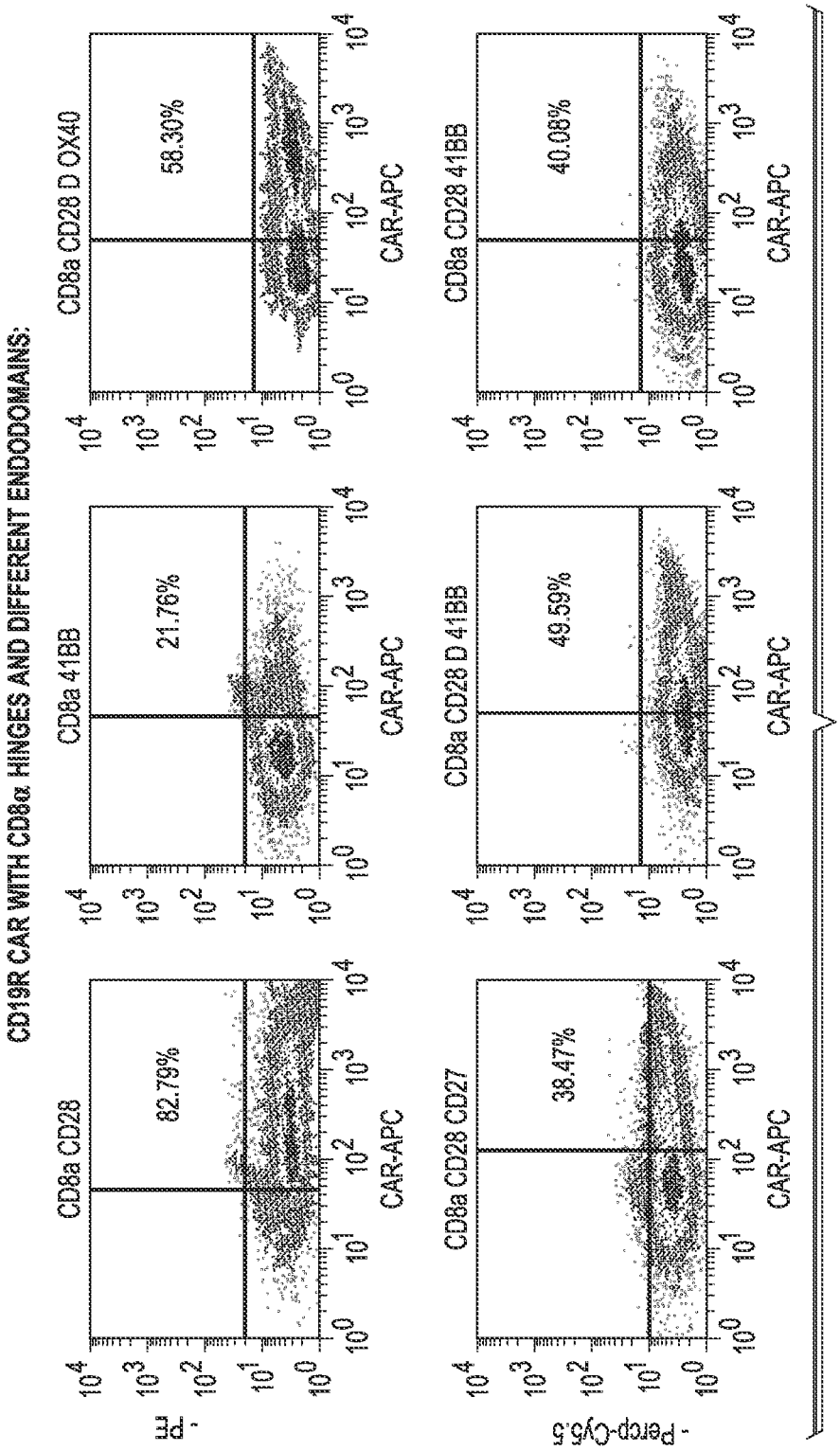

CD19R CAR WITH CD8α HINGES AND DIFFERENT ENDODOMAINS:
CD8a CD28
82.79%
CD8a 41BB
21.76%
CD8a CD28 D OX40
58.30%
CD8a CD28 41BB
40.08%
CD8a CD28 D 41BB
49.59%
CD8a CD28 CD27
38.47%
CAR-APC
PE
Percp-Cy5.5

FIG. 40B

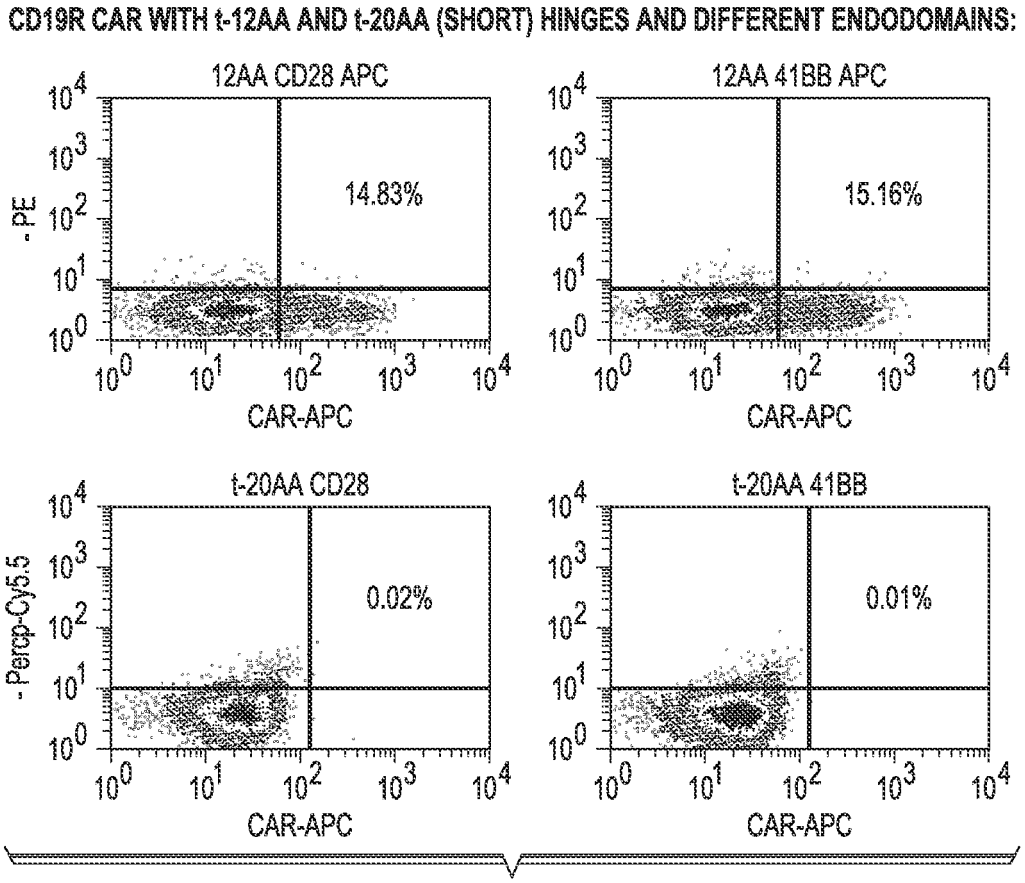

CD19R CAR WITH t-12AA AND t-20AA (SHORT) HINGES AND DIFFERENT ENDODOMAINS:
12AA CD28 APC
14.83%
12AA 41BB APC
15.16%
t-20AA CD28
0.02%
t-20AA 41BB
0.01%
-PE
-Percp-Cy5.5
CAR-APC

FIG. 40C

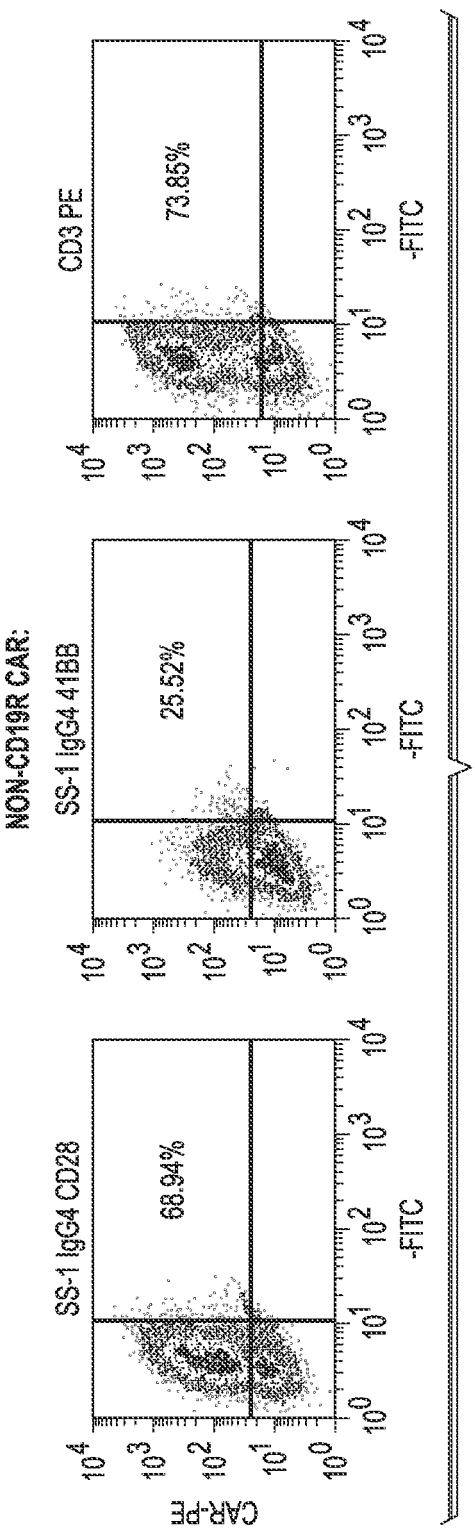


FIG. 40D

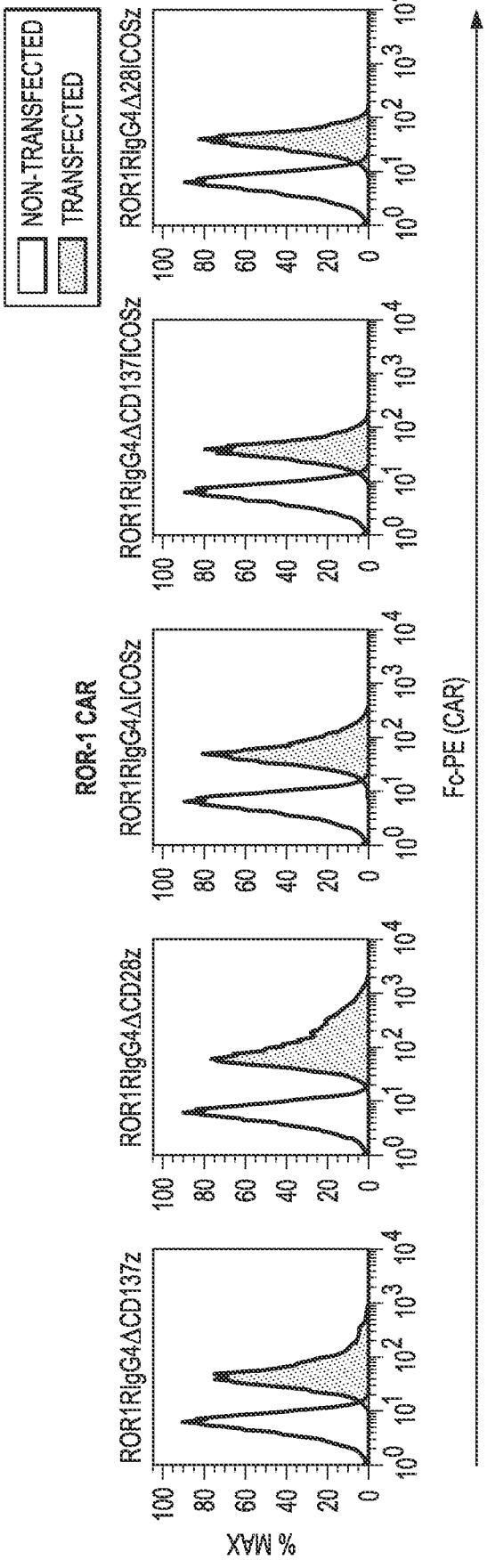


FIG. 40E

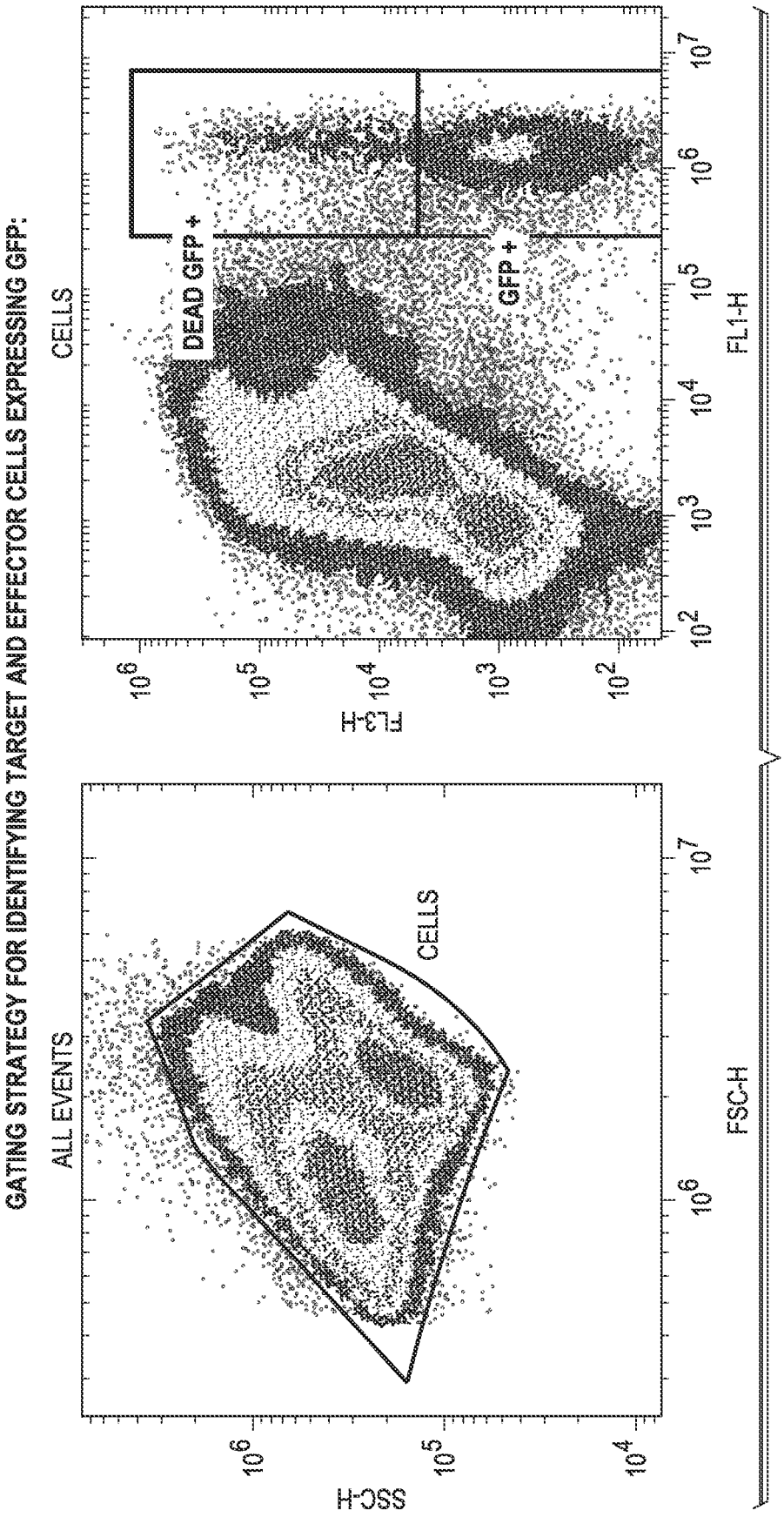

GATING STRATEGY FOR IDENTIFYING TARGET AND EFFECTOR CELLS EXPRESSING GFP:
ALL EVENTS
CELLS
SSC-H
FSC-H
CELLS
DEAD GFP +
GFP +
FL3-H
FL1-H

FIG. 41A-1

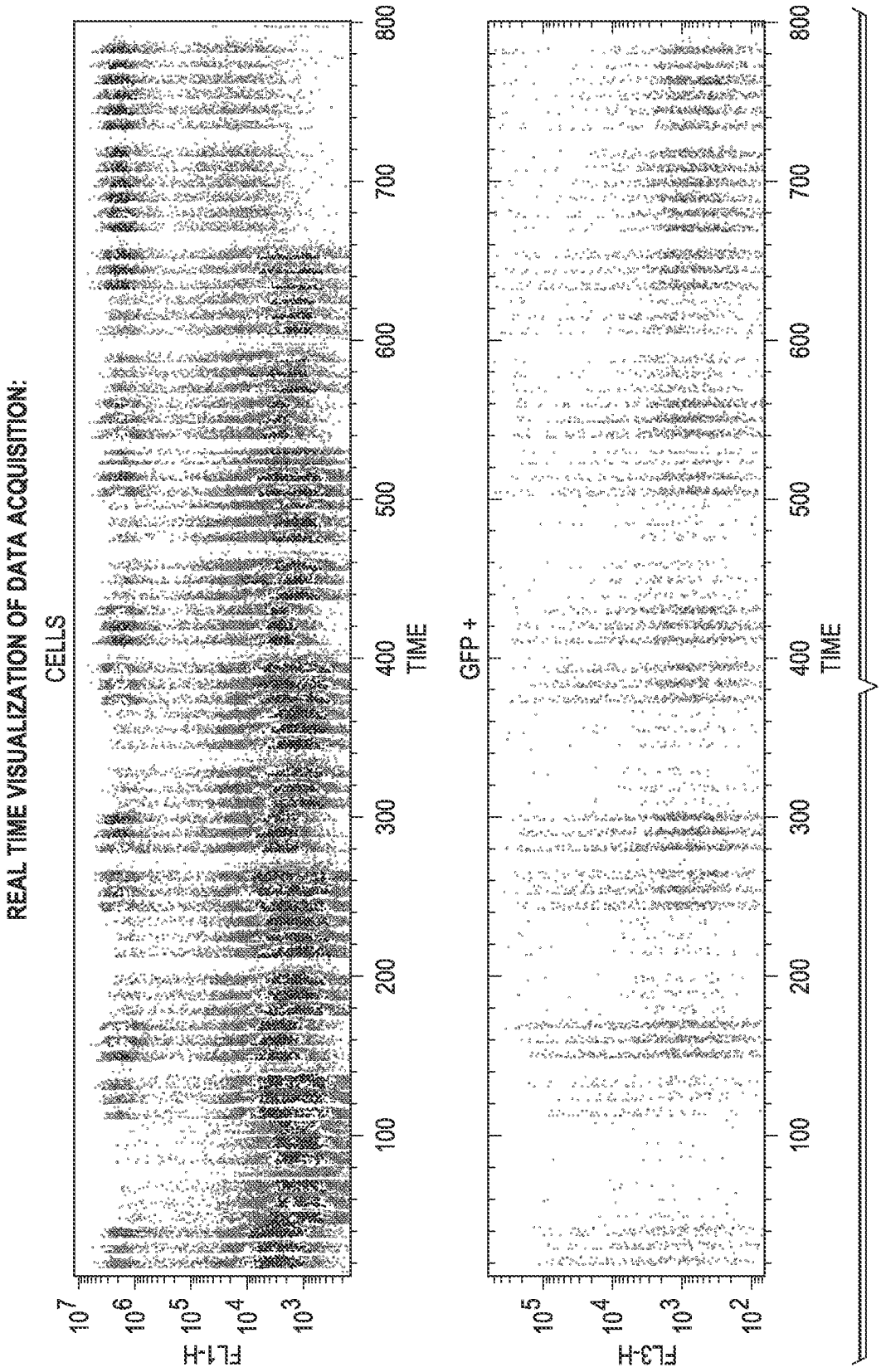

REAL TIME VISUALIZATION OF DATA ACQUISITION:
CELLS
TIME
FL1-H
GFP +
TIME
FL3-H

FIG. 41A-2

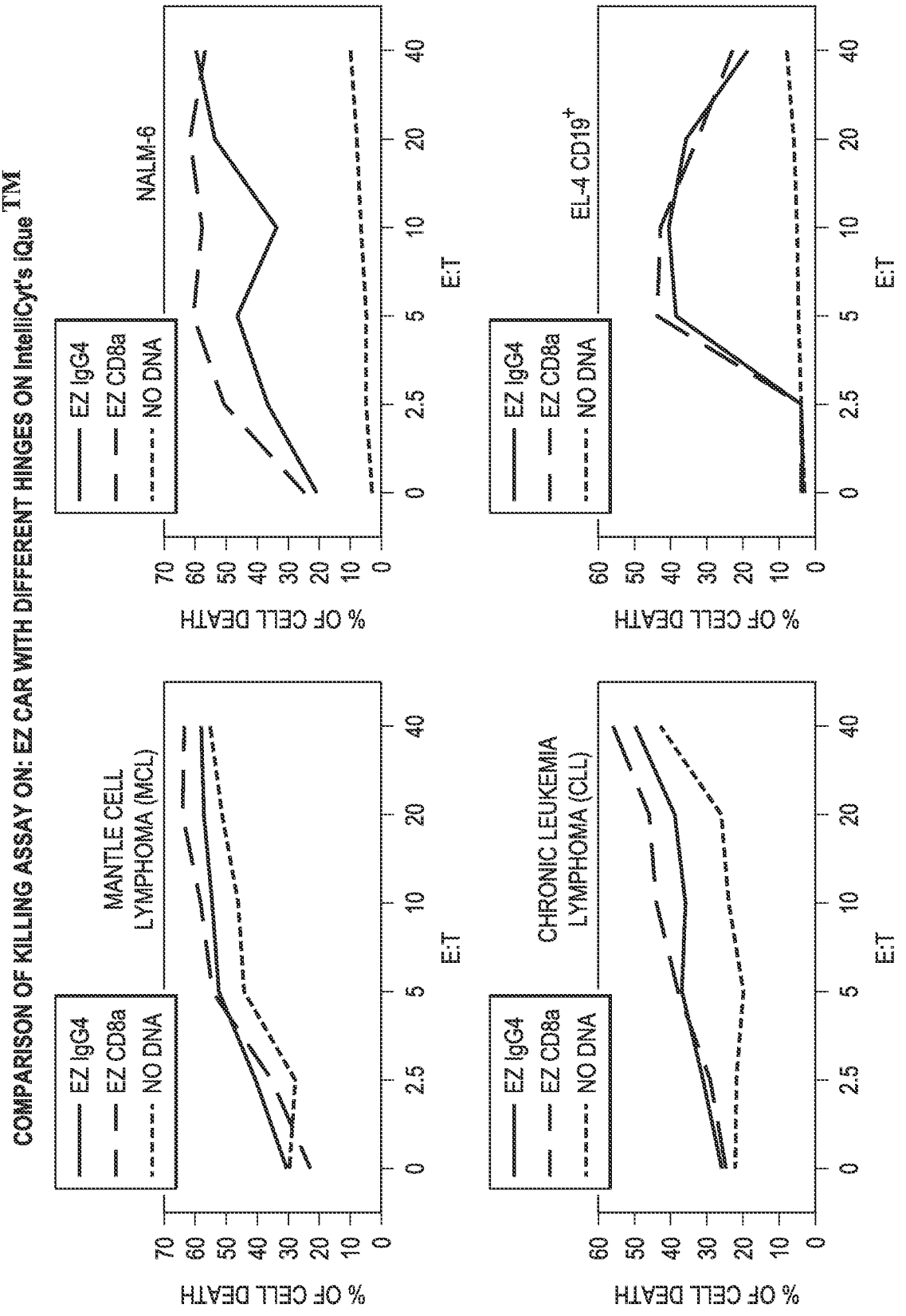
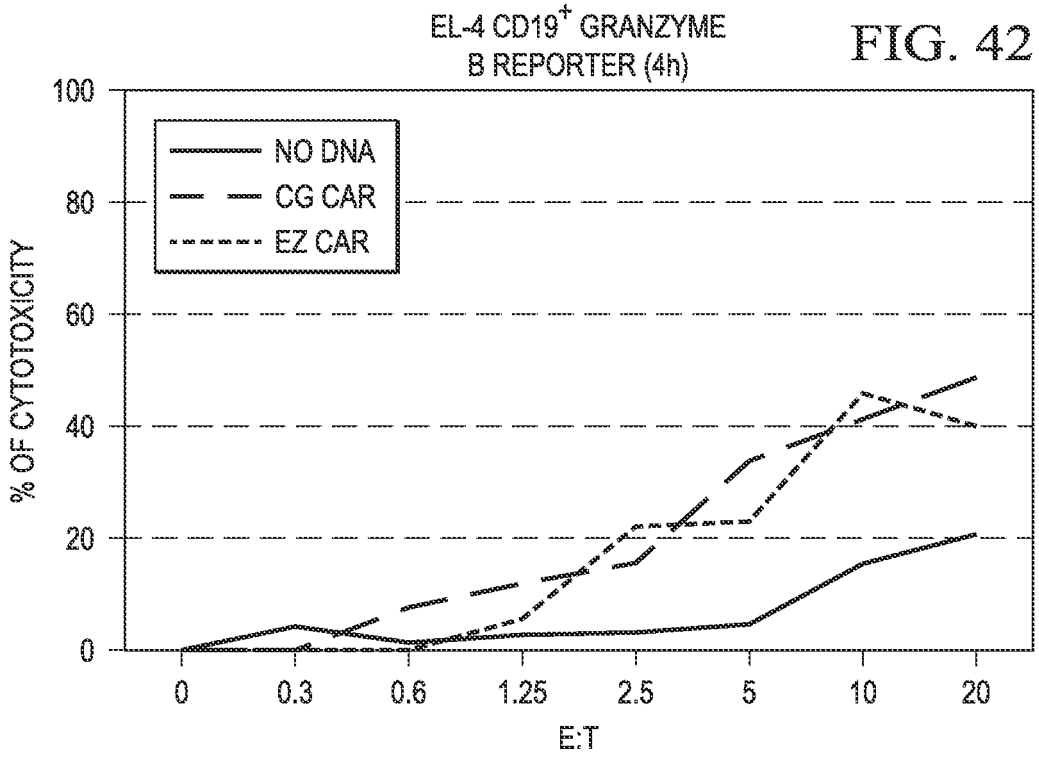
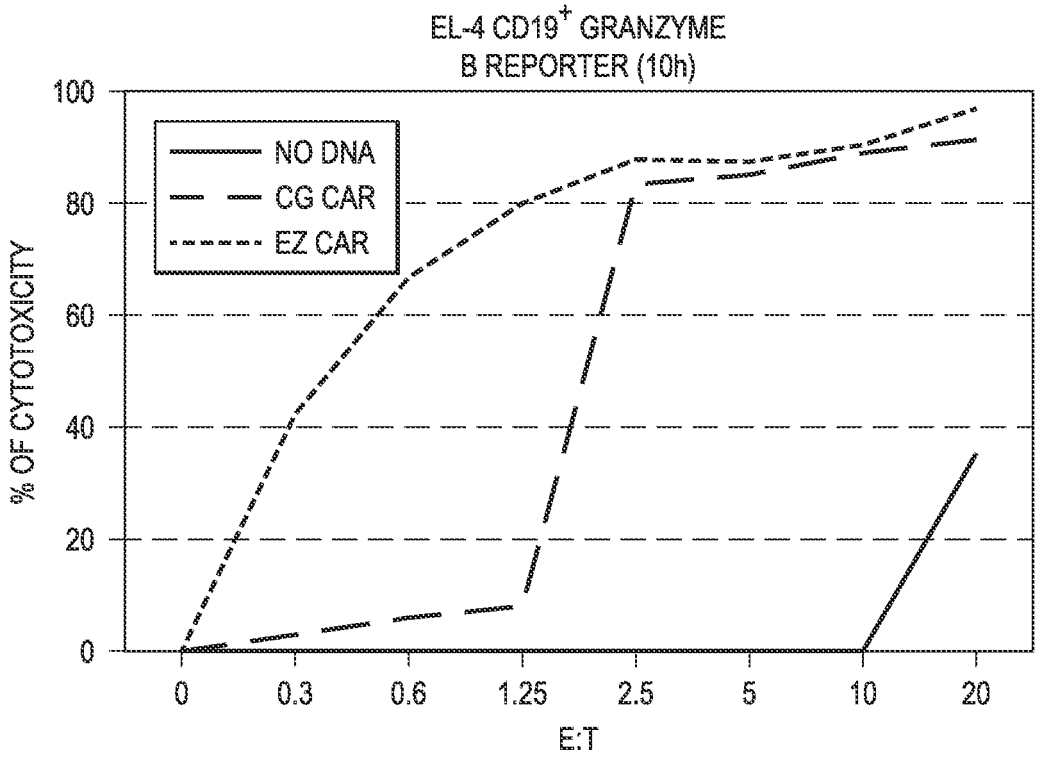

COMPARISON OF KILLING ASSAY ON: EZ CAR WITH DIFFERENT HINGES ON IntelliCyt's iQue™
EZ IgG4
EZ CD8a
NO DNA
NALM-6
EL-4 CD19+
MANTLE CELL LYMPHOMA (MCL)
CHRONIC LEUKEMIA LYMPHOMA (CLL)
% OF CELL DEATH
E:T
0
2.5
5
10
20
40
10
20
30
40
50
60
70

FIG. 41B

FIG. 42


EL-4 CD19+ GRANZYME B REPORTER (4h)
% OF CYTOTOXICITY
100
80
60
40
20
0
NO DNA
CG CAR
EZ CAR
0
0.3
0.6
1.25
2.5
5
10
20
E:T


EL-4 CD19+ GRANZYME B REPORTER (10h)
% OF CYTOTOXICITY
100
80
60
40
20
0
NO DNA
CG CAR
EZ CAR
0
0.3
0.6
1.25
2.5
5
10
20
E:T