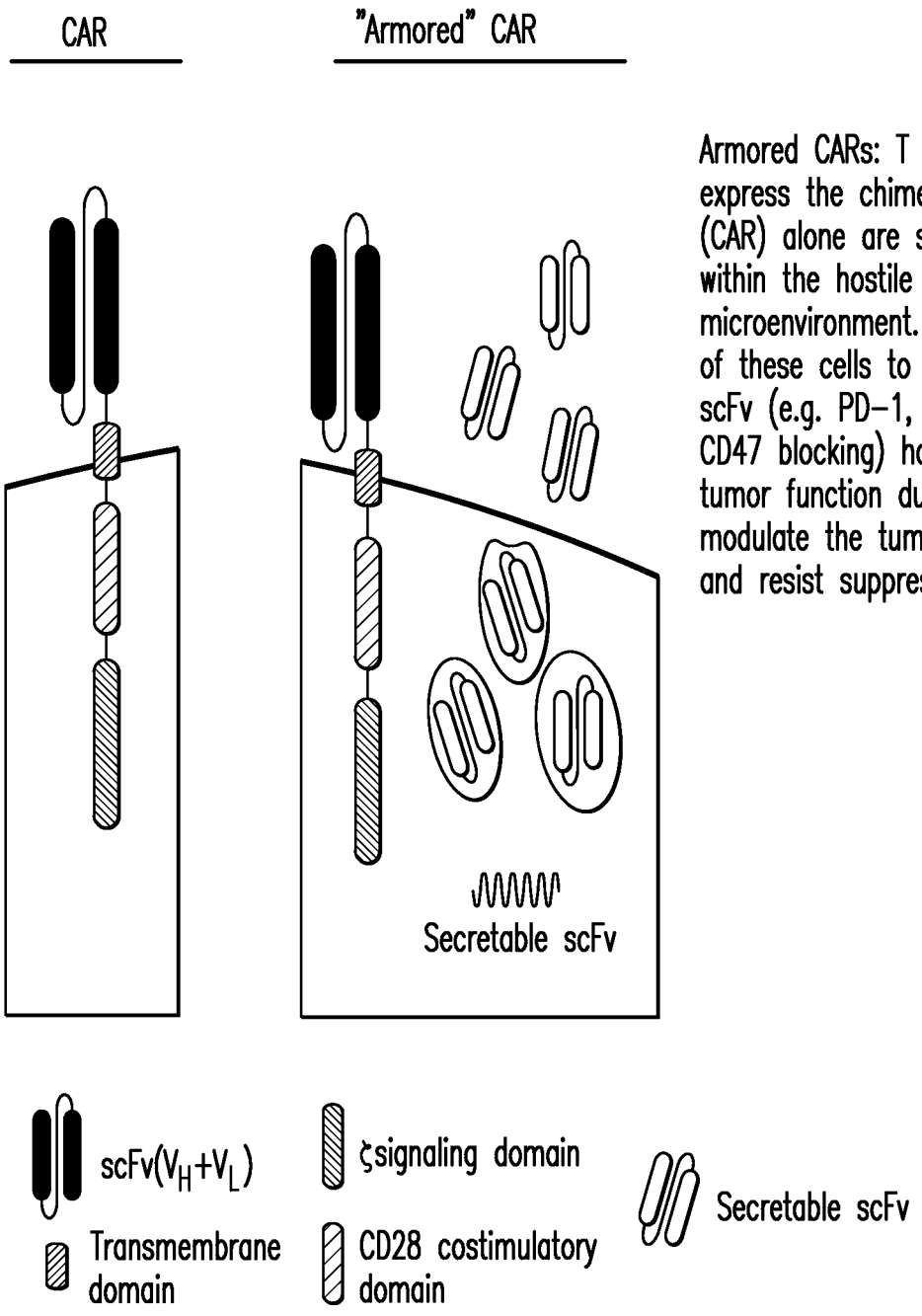


Armored CARs: T Cells modified to express the chimeric antigen receptor (CAR) alone are subject to suppression within the hostile tumor microenvironment. Further modification of these cells to express secretable scFv (e.g. PD-1, PD-L1, CTLA-4 or CD47 blocking) have improved anti-tumor function due to their ability to modulate the tumor microenvironment and resist suppressive factors

FIG. 1

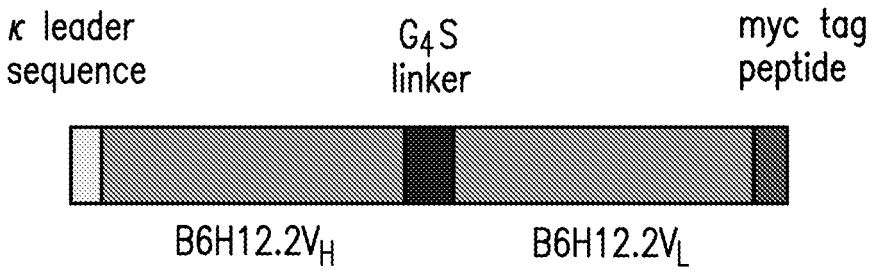


FIG. 2A

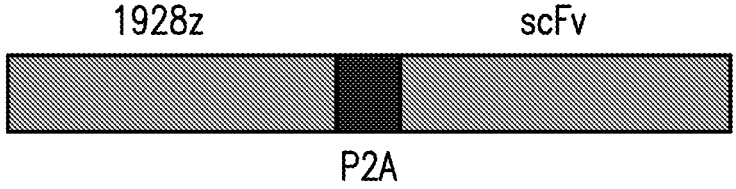


FIG. 2B

Secretable anti-CD47 scFv structure

(a) Secretable anti-CD47 scFv was designed to include a kappa(κ) leader sequence to allow exportation of this protein. The variable heavy(V_H) and light(V_L) were linked with a serine glycine linker(G_4S) and a myc-tag peptide was included to allow detection of the scFv. (b) The secretable scFv was linked to the 1928z CAR construct using a P2A element as shown.

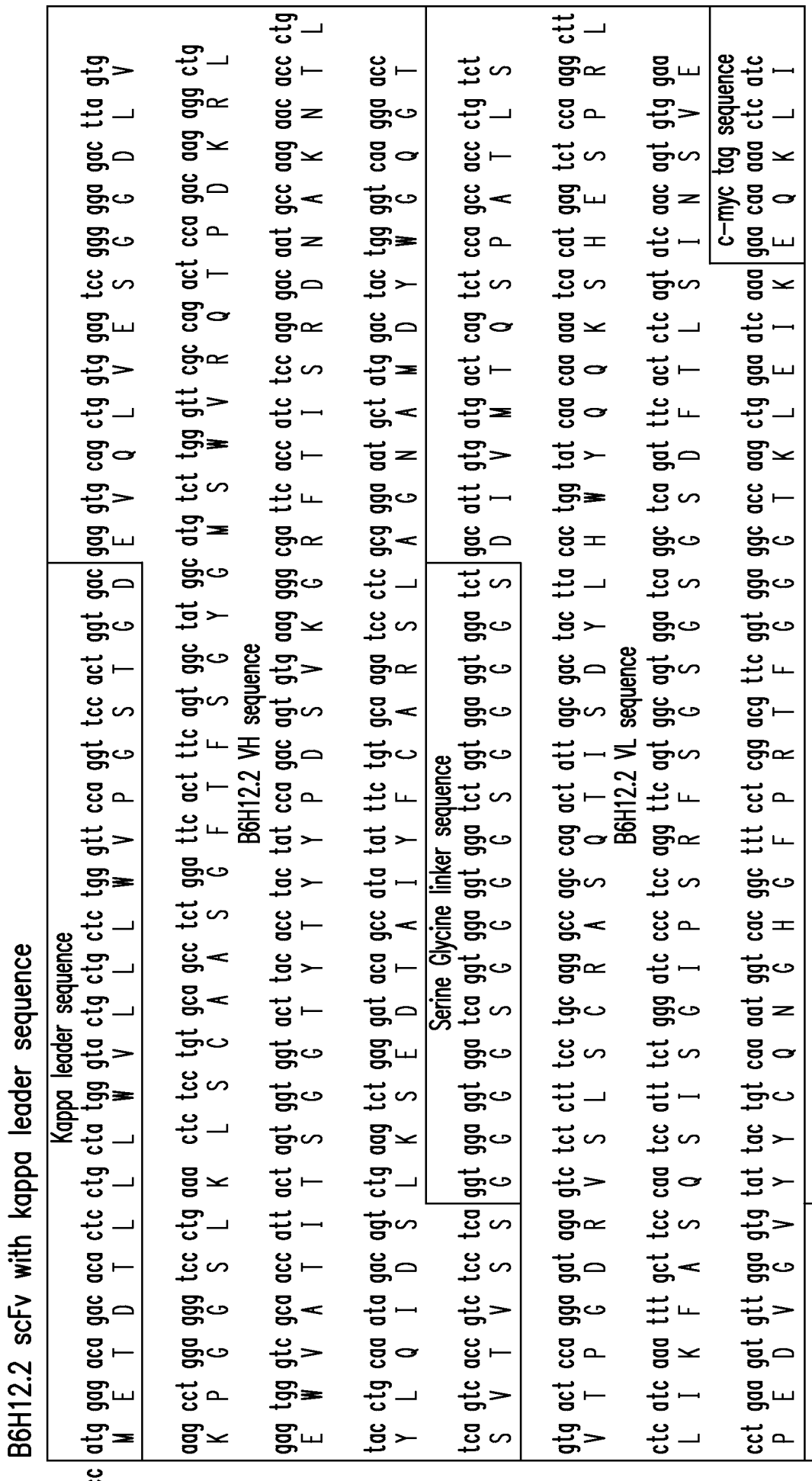

B6H12.2 scFv with kappa leader sequence
Kappa leader sequence
B6H12.2 VH sequence
Serine Glycine linker sequence
B6H12.2 VL sequence
c-myc tag sequence

FIG. 3

B6H12.2scFv with CD8 leader sequence

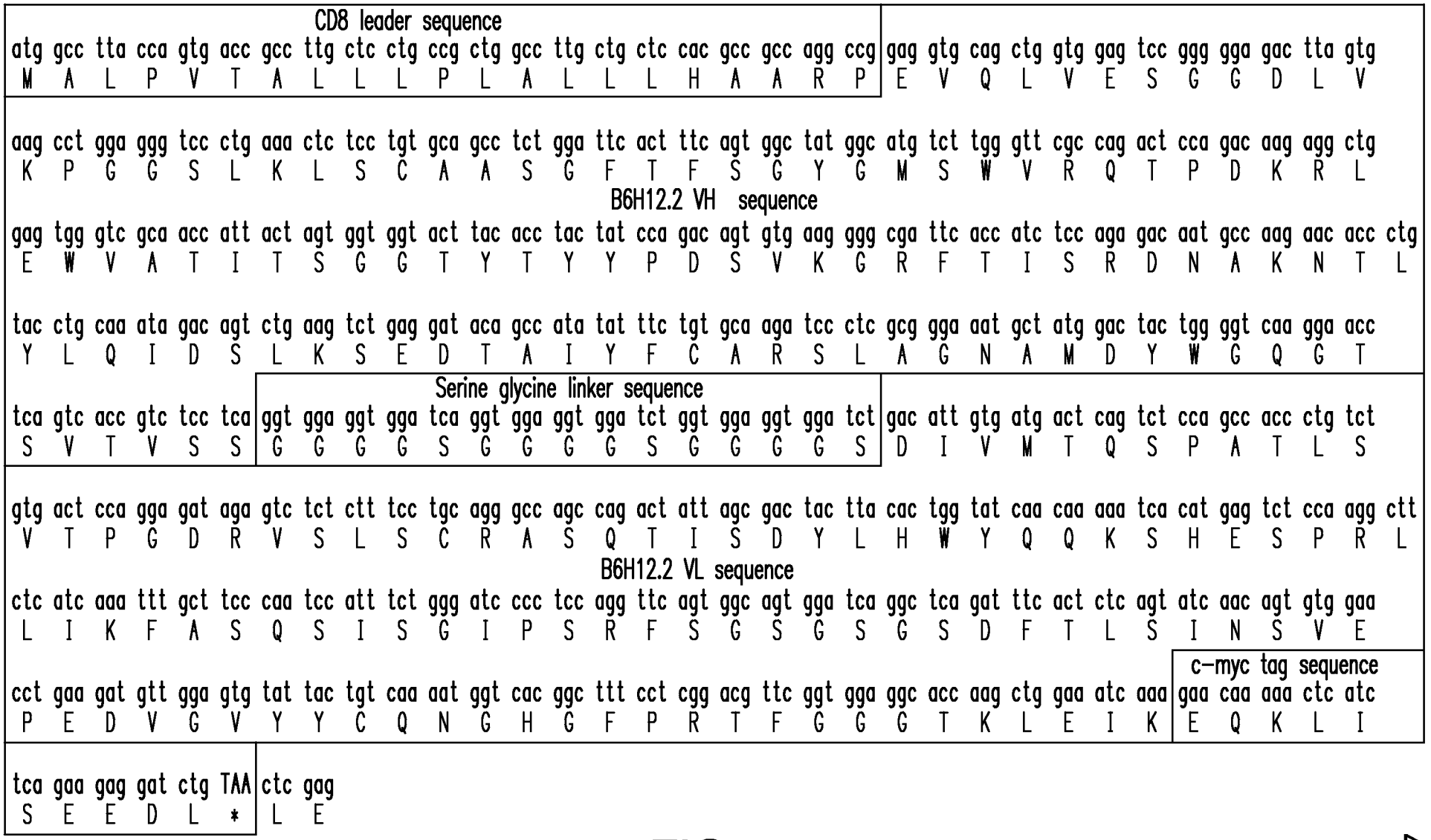


FIG. 4

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1928z–2A–B6H12.2 (kappa leader) sequence

atggctctcccagtgactgccctactgcttcccctagcgcttctcctgcatgcagaggtgaagctgcagcagtctggg
gctgagctggtgaggcctgggtcctcagtgaagatttcctgcaaggcttctggctatgcattcagtagctactggatgaa
ctgggtgaagcagaggcctggacagggtcttgagtggattggacagatttatcctggagatggtgatactaactacaat
ggaaagttcaagggtcaagccacactgactgcagacaaatcctccagcacagcctacatgcagctcagcggcctaacat
ctgaggactctgcggtctatttctgtgcaagaaagaccattagttcggtagtagatttctactttgactactggggccaagg
gaccacggtcaccgtctcctcaggtggaggtggatcaggtggaggtggatctggtggaggtggatctgacattgagc
tcacccagtctccaaaattcatgtccacatcagtaggagacagggtcagcgtcacctgcaaggccagtcagaatgtgggt
actaatgtagcctggtatcaacagaaaccaggacaatctcctaaaccactgatttactcggcaacctaccggaacagtgg
agtccctgatcgcttcacaggcagtggatctgggacagatttcactctcaccatcactaacgtgcagtctaaagacttggc
agactatttctgtcaacaatataacaggtatccgtacacgtccggaggggggaccaagctggagatcaaacgggcggc
cgcaattgaagttatgtatcctcctccttacctagacaatgagaagagcaatggaaccattatccatgtgaaagggaaaca
cctttgtccaagtcccctatttcccggaccttctaagccctttgggtgctggtggtggttggtggagtcctggcttgctata
gcttgtagtaacagtggcctttattattttctgggtgaggagtaagaggagcaggctcctgcacagtgactacatgaac
atgactccccgccgccccgggcccacccgcaagcattaccagccctatgccccaccacgcgacttcgcagcctatcgctc
cagagtgaagttcagcaggagcgcagagcccccgcgtaccagcagggccagaaccagctctataacgagctcaatct
aggacgaagagaggagtacgatgttttggacaagagacgtggccgggaccctgagatgggggaaagccgagaag
gaagaaccctcaggaaggcctgtacaatgaactgcagaaagataagatggcggaggcctacagtgagattgggatga
aaggcgagcgccggaggggcaaggggcacgatggcctttaccagggtctcagtacagccaccaaggacacctacga
cgcccttcacatgcaggccctgcccctcgcggatctggagcaacaaacttctcactactcaaacaagcaggtgacgtgg -
aggagaatcccggaccc**atggagacagacacactcctgctatgggtactgctgctctgggttccaggttccactggtgac**
gaggtgcagctggtggagtccgggggagacttagtgaagcctggagggtccctgaaactctcctgtgcagcctctgga
ttcactttcagtggctatggcatgtcttgggttcgccagactccagacaagaggctggagtgggtcgcaaccattactagt
ggtggtacttacacctactatccagacagtgtgaaggggcgattcaccatctccagagacaatgccaagaacaccctgta
cctgcaaatagacagtctgaagtctgaggatacagccatatatttctgtgcaagatccctcgcgggaaatgctatggacta
ctggggtcaaggaacctcagtcaccgtctcctcaggtggaggtggatcaggtggaggtggatctggtggaggtggatc
tgacattgtgatgactcagtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggccagccag
actattagcgactacttacactggtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatttgcttcccaatccattt
ctgggatcccctccaggttcagtggcagtggatcaggctcagatttcactctcagtatcaacagtgtggaacctgaagatg
ttggagtgtattactgtcaaaatggtcacggctttcctcggacgttcggtggaggcaccaagctggaaatcaaagaacaa
aaactcatctcagaagaggatctgtaa

1928z CAR

P2A

B6H12.2 scFv

FIG. 5

4H1128z–2A–B6H12.2 (kappa leader) sequence

CATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAG
CTGCAGGAGTCAGGGGGAGGCTTCGTGAAGCCTGGAGGGTCCCTCAAAGTCTCCTGTGCAGC
CTCTGGATTCACTTTCAGTAGCTATGCCATGTCCTGGGTTCGCCTGAGTCCGGAGATGAGGCTG
GAGTGGGTCGCAACCATTAGCAGTGCTGGTGGTTACATCTTCTATTCTGACAGTGTGCAGGGA
CGATTCACCATTTCCAGAGACAATGCCAAGAACACCCTGCACCTGCAAATGGGCAGTCTGAGG
TCTGGGGACACGGCCATGTATTACTGTGCAAGGCAGGGATTTGGTAACTACGGTGATTACTATG
CTATGGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTGGAGGTGGATCAGGT
GGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCTCACCCAGTCTCCATCCTCCCTGGCT
GTGTCAGCAGGAGAGAAGGTCACTATGAGCTGCAAATCCAGTCAGAGTCTGCTCAACAGTAG
AACCCGAAAGAACCAGTTGGCTTGGTACCAGCAAAAACCAGGACAGTCTCCTGAACTGCTG
ATCTACTGGGCATCCACTAGGCAATCTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGG
ACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGCCAG
CAATCTTATAATCTACTgcaacaaacttctcactactcaaacaagcaggtgacgtggaggagaatcccggaccc**at**
ggagacagacacactcctgctatgggtactgctgctctgggttccaggttccactggtgacgaggtgcagctggtggag
tccgggggagacttagtgaagcctggagggtccctgaaactctcctgtgcagcctctggattcactttcagtggctatgg
catgtcttgggttcgccagactccagacaagaggctggagtgggtcgcaaccattactagtggtggtacttacacctact
atccagacagtgtgaaggggcgattcaccatctccagagacaatgccaagaacaccctgtacctgcaaatagacagtct
gaagtctgaggatacagccatatatttctgtgcaagatccctcgcgggaaatgctatggactactggggtcaaggaacc
tcagtcaccgtctcctcaggtggaggtggatcaggtggaggtggatctggtggaggtggatctgacattgtgatgact
cagtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggccagccagactattagcgacta
cttacactggtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatttgcttcccaatccatttctgggatcccct
ccaggttcagtggcagtggatcaggctcagatttcactctcagtatcaacagtgtggaacctgaagatgttggagtgtat
tactgtcaaaatggtcacggctttcctcggacgttcggtggaggcaccaagctggaaatcaaagaacaaaaactcatct
cagaagaggatctgtaa

4H1128z CAR

P2A

B6H12.2 scFv

FIG. 6

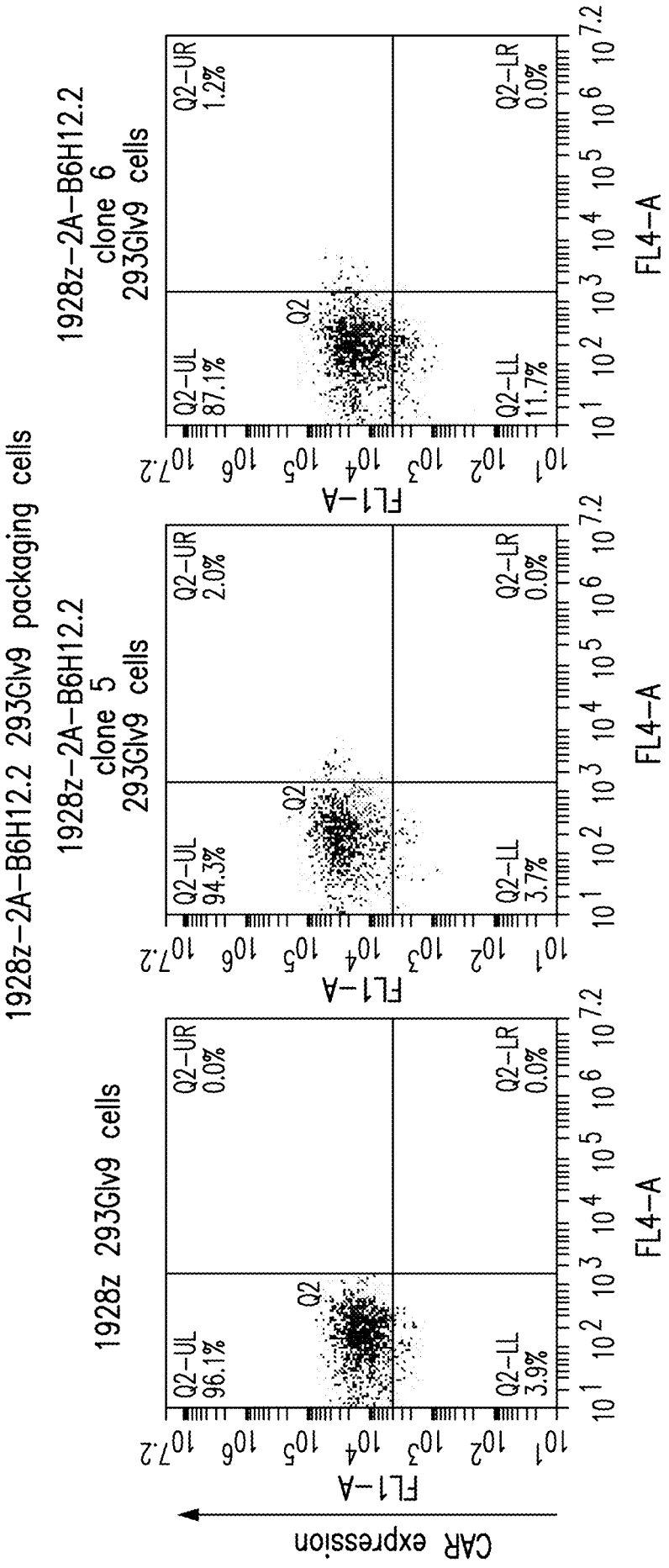

1928z-2A-B6H12.2 293Glv9 packaging cells
1928z 293Glv9 cells
Q2-UL 96.1%
Q2
Q2-UR 0.0%
Q2-LL 3.9%
Q2-LR 0.0%
1928z-2A-B6H12.2 clone 5 293Glv9 cells
Q2-UL 94.3%
Q2
Q2-UR 2.0%
Q2-LL 3.7%
Q2-LR 0.0%
1928z-2A-B6H12.2 clone 6 293Glv9 cells
Q2-UL 87.1%
Q2
Q2-UR 1.2%
Q2-LL 11.7%
Q2-LR 0.0%
FL1-A
FL4-A
CAR expression

FIG. 7A

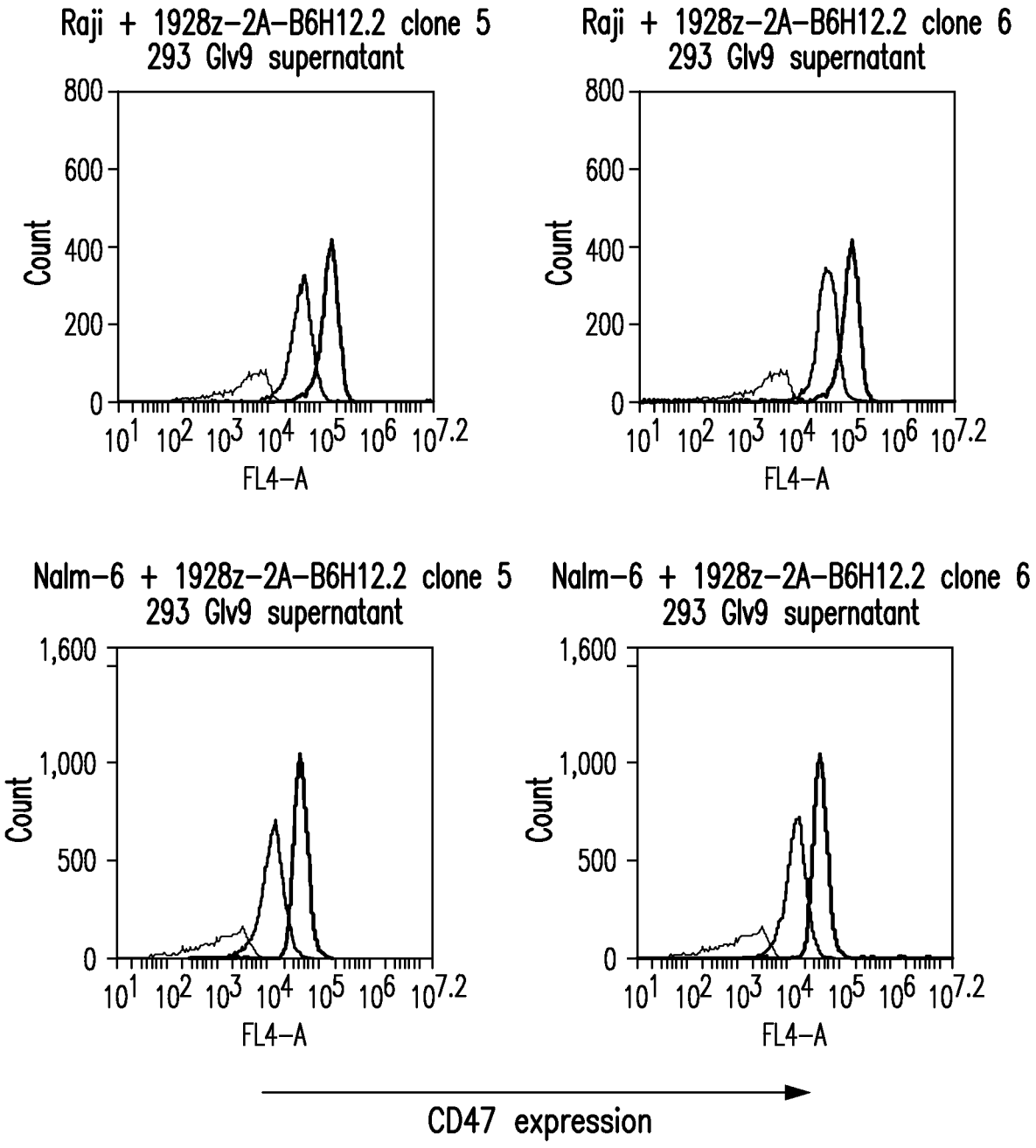

Raji + 1928z-2A-B6H12.2 clone 5
293 Glv9 supernatant
Raji + 1928z-2A-B6H12.2 clone 6
293 Glv9 supernatant
Nalm-6 + 1928z-2A-B6H12.2 clone 5
293 Glv9 supernatant
Nalm-6 + 1928z-2A-B6H12.2 clone 6
293 Glv9 supernatant
Count
FL4-A
CD47 expression

FIG. 7B

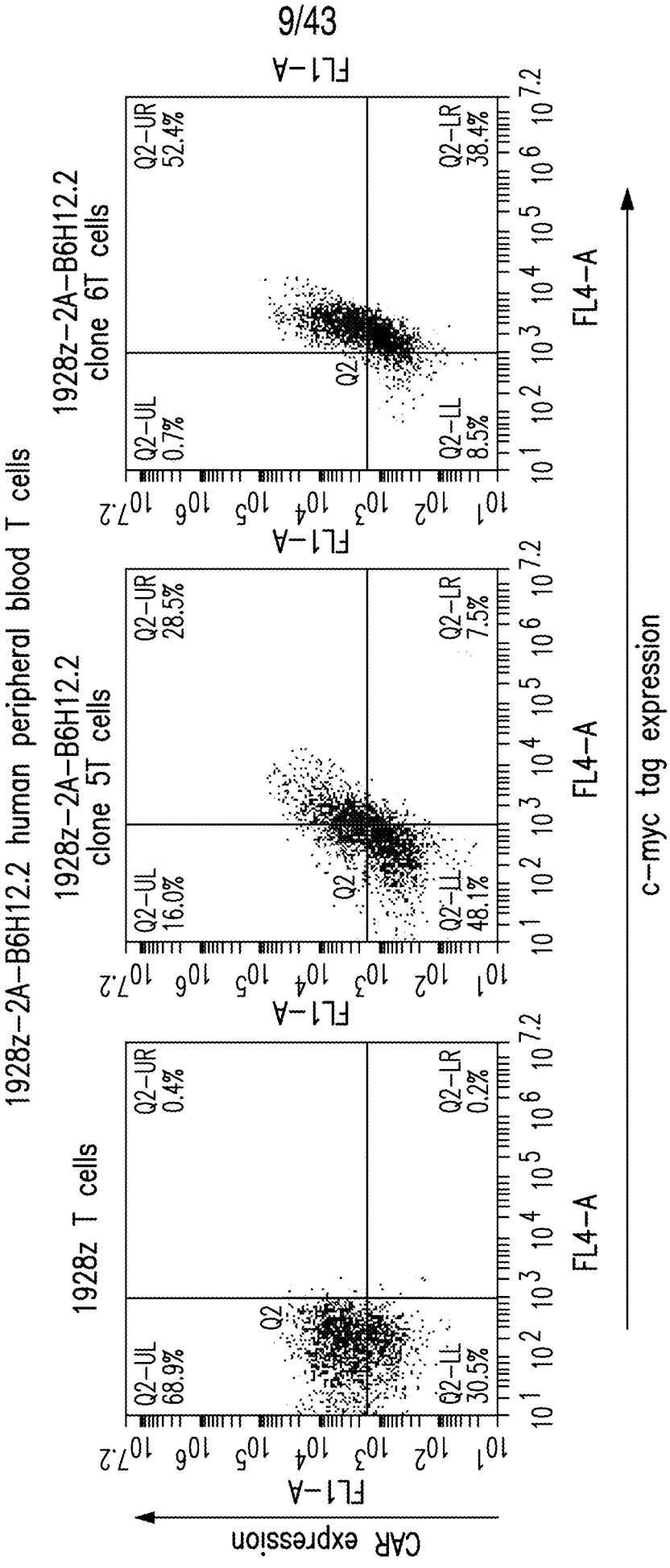

1928z−2A−B6H12.2 human peripheral blood T cells
1928z T cells
Q2−UL 68.9%
Q2−UR 0.4%
Q2−LL 30.5%
Q2−LR 0.2%
1928z−2A−B6H12.2 clone 5T cells
Q2−UL 16.0%
Q2−UR 28.5%
Q2−LL 48.1%
Q2−LR 7.5%
1928z−2A−B6H12.2 clone 6T cells
Q2−UL 0.7%
Q2−UR 52.4%
Q2−LL 8.5%
Q2−LR 38.4%
Q2
FL1−A
FL4−A
CAR expression
c−myc tag expression

FIG. 8A

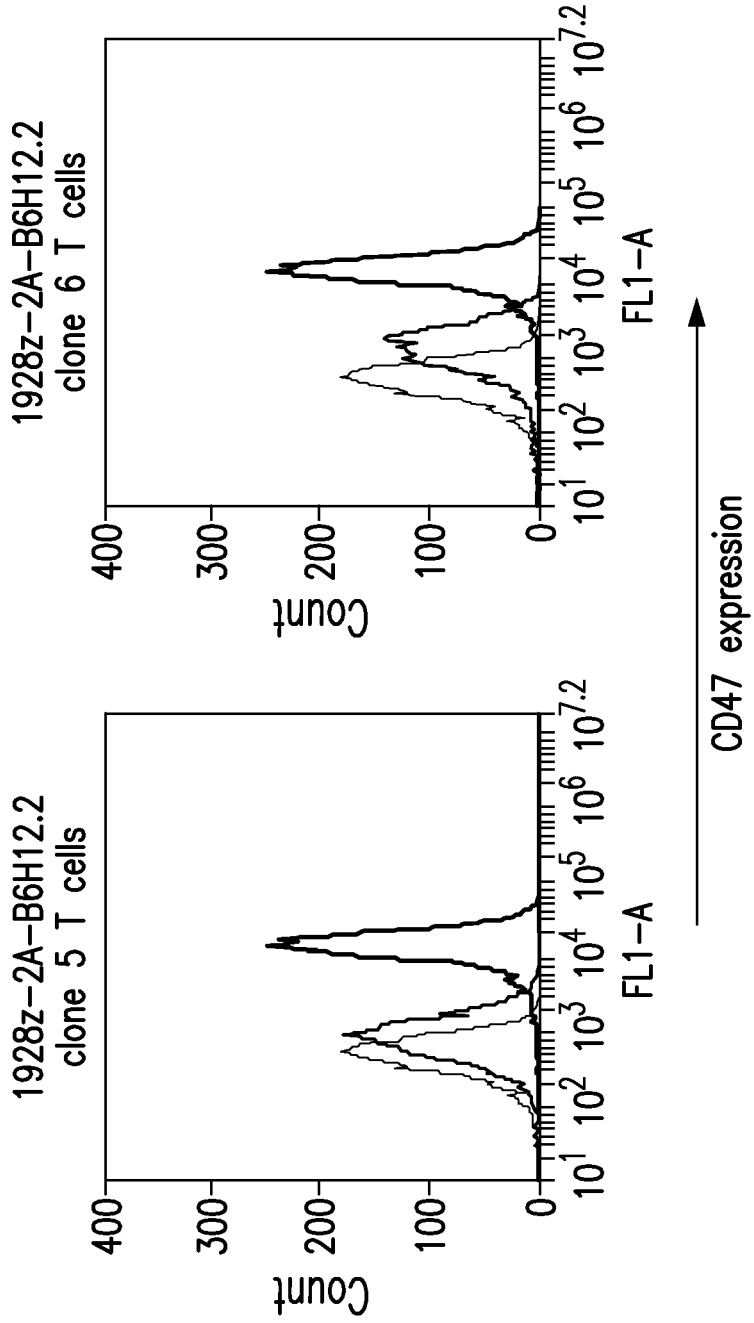

1928z-2A-B6H12.2
clone 6 T cells
1928z-2A-B6H12.2
clone 5 T cells
Count
FL1-A
CD47 expression

FIG. 8B

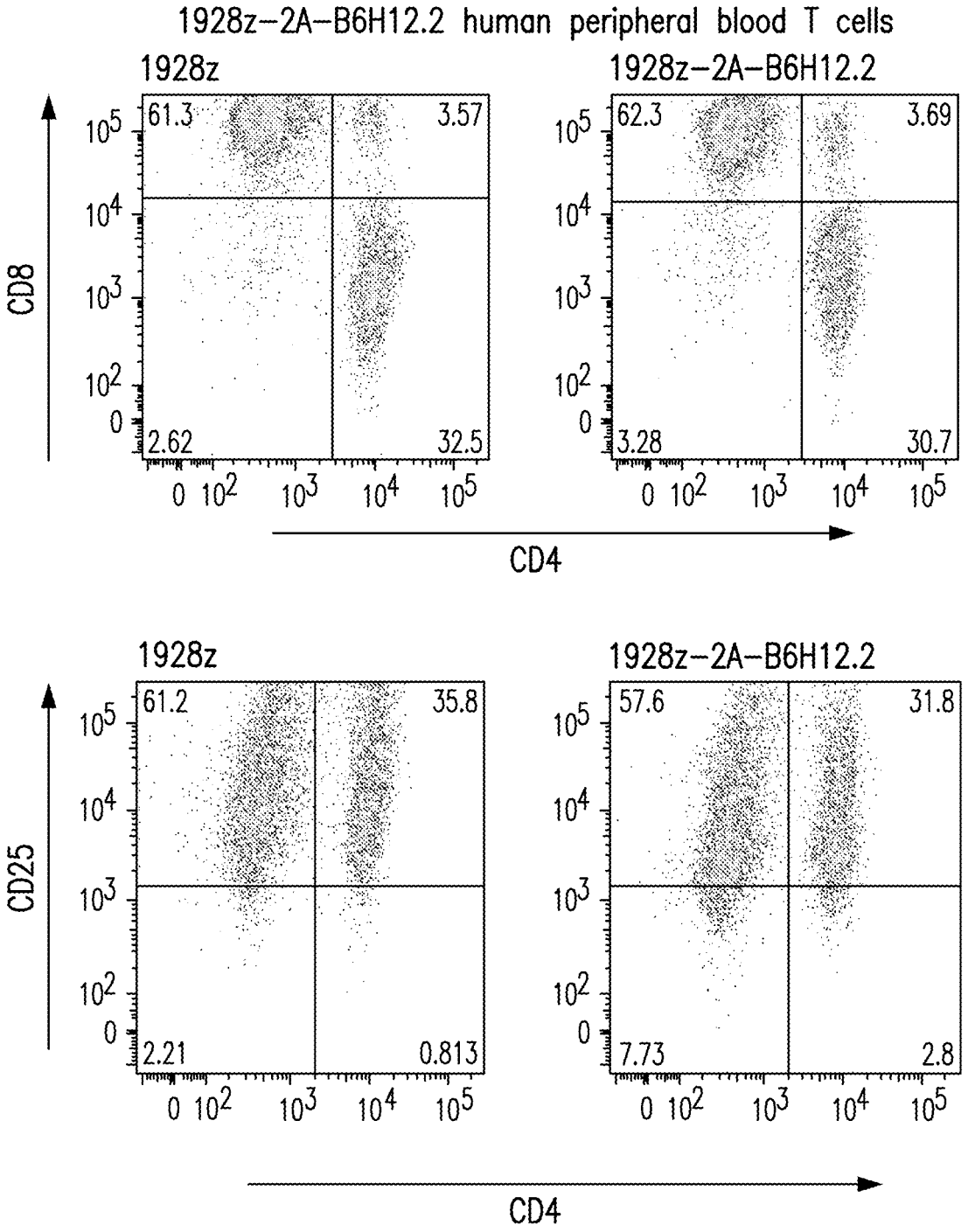

1928z–2A–B6H12.2 human peripheral blood T cells
1928z
1928z–2A–B6H12.2
CD8
CD4
61.3
3.57
2.62
32.5
62.3
3.69
3.28
30.7
CD25
CD4
61.2
35.8
2.21
0.813
57.6
31.8
7.73
2.8
0
10^2
10^3
10^4
10^5

FIG. 9A-1

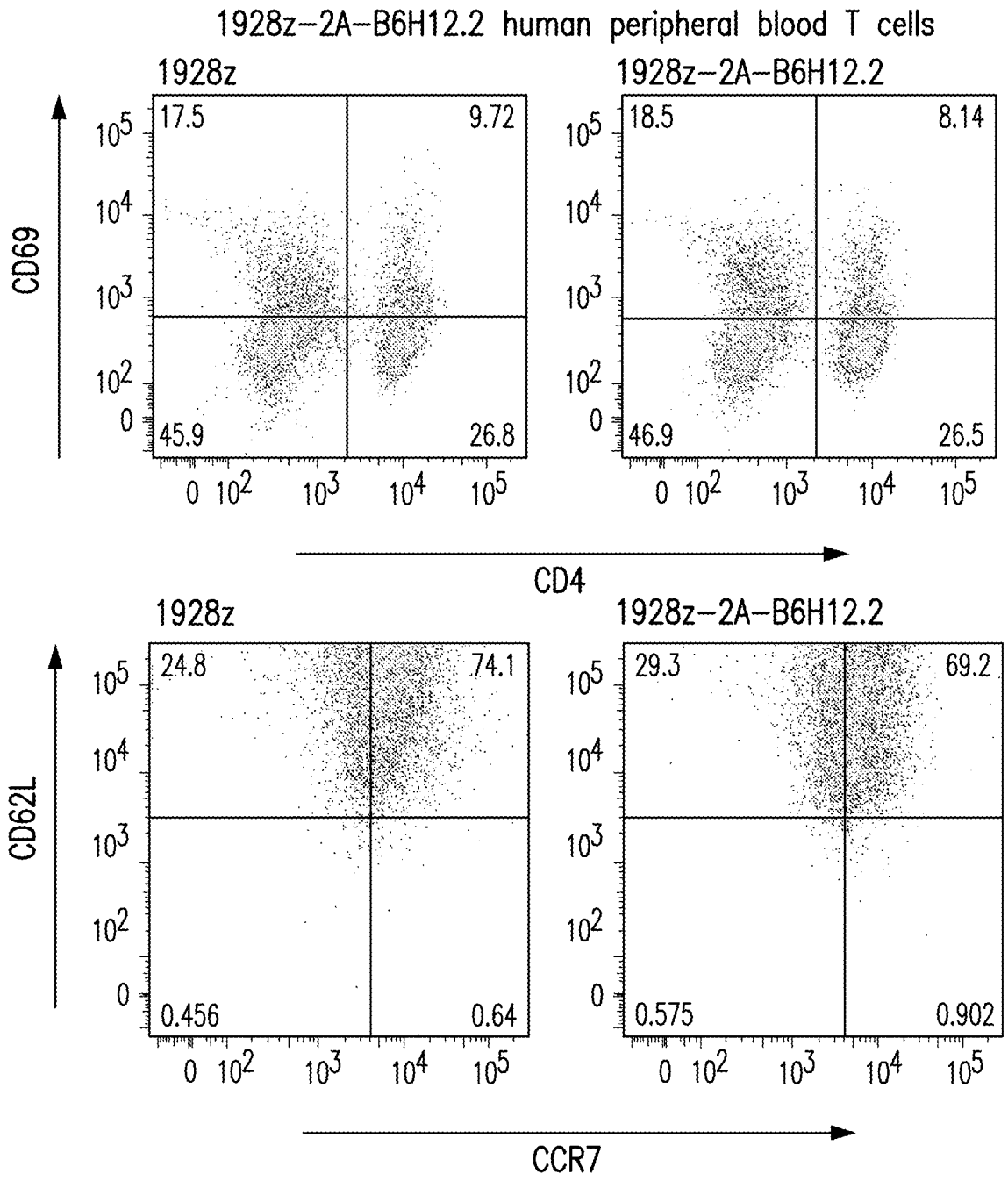

1928z–2A–B6H12.2 human peripheral blood T cells
1928z
1928z–2A–B6H12.2
CD69
17.5
9.72
45.9
26.8
18.5
8.14
46.9
26.5
CD4
1928z
1928z–2A–B6H12.2
CD62L
24.8
74.1
0.456
0.64
29.3
69.2
0.575
0.902
CCR7

FIG. 9A-2

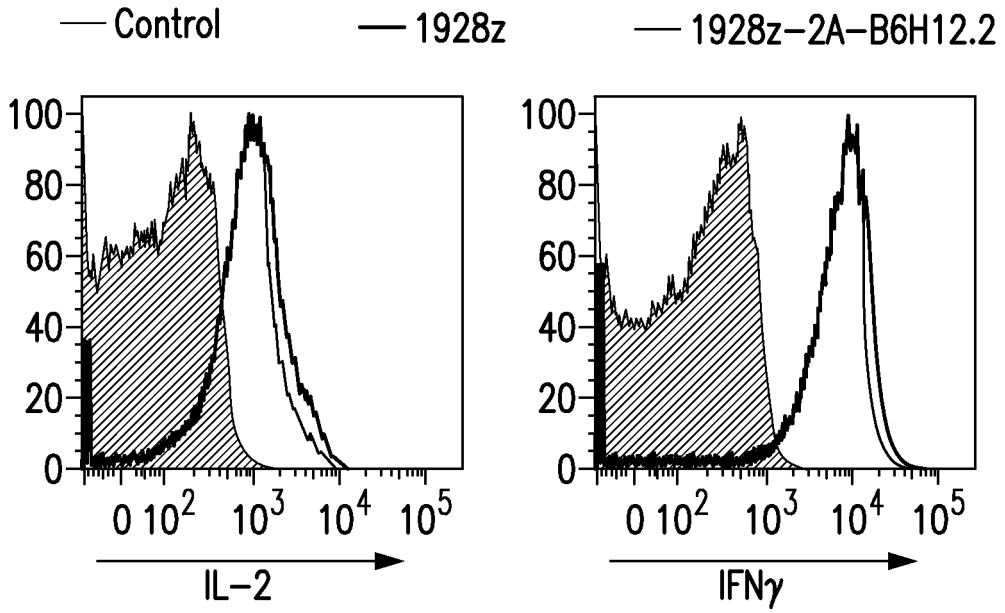

Control
1928z
1928z-2A-B6H12.2
100
80
60
40
20
0
0 10^2 10^3 10^4 10^5
IL-2
IFNγ

FIG. 9B

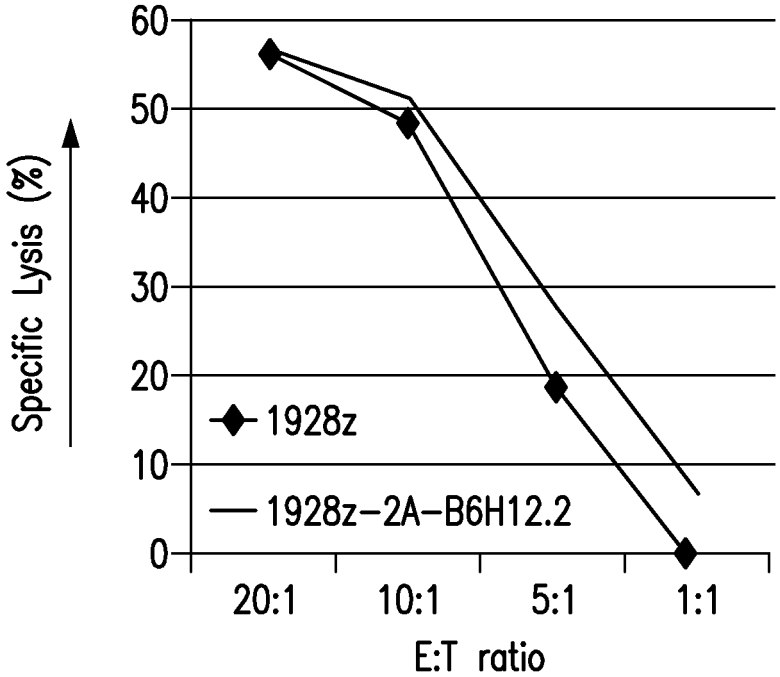

60
50
40
30
20
10
0
Specific Lysis (%)
1928z
1928z-2A-B6H12.2
20:1
10:1
5:1
1:1
E:T ratio

FIG. 9C

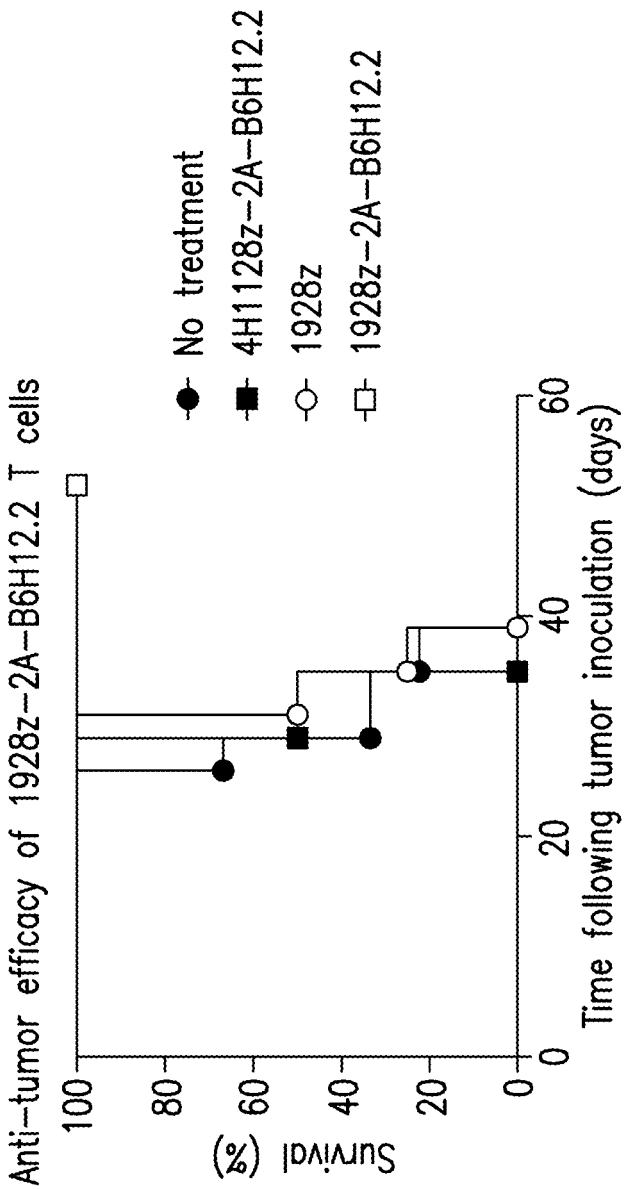


FIG. 10A

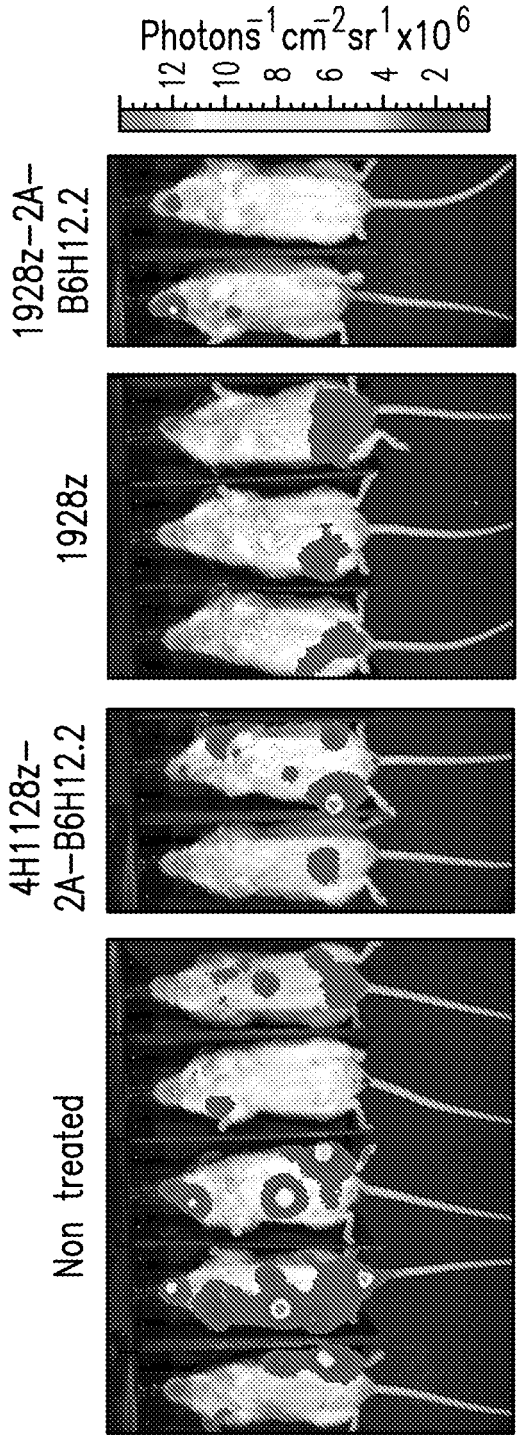


FIG. 10B

5C4scFv with Kappa leader sequence

Kappa leader sequence

ATG GAG ACA GAC ACA CTC CTG CTA CTG GTA CTG CTC TGG GTT CCA GGT TCC ACT GGT GAC CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC
M E T D T L L L L W V L L L W V P G S T G D Q V Q L V E S G G G V V

CAG CCT GGG AGG TCC CTG AGA CTC GAC TGT AAA GCG TCT GGA ATC ACC TTC AGT AAC TCT GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG
Q P G R S L R L D C K A S G I T F S N S G M H W V R Q A P G K G L

5C4 VH sequence

GAG TGG GTG GCA GTT ATT TGG TAT GAT GGA AGT AAA AGA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG
E W V A V I W Y D G S K R Y Y A D S V K G R F T I S R D N S K N T

CTG TTT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCT GTG TAT TAC TGT GCG ACA AAC GAC GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC
L F L Q M N S L R A E D T A V Y Y C A T N D D Y W G Q G T L V T V

Serine glycine linker sequence

TCC TCA GGT GGA GGT GGA TCA GGT GGA GGT GGA TCT GGT GGA GGT GGA TCT GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG
S S G G G G S G G G G S G G G G S E I V L T Q S P A T L S L S P G

GAA AGA GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGT AGT TAC TTA GCC TGG TAC CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT
E R A T L S C R A S Q S V S S Y L A W Y Q Q K P G Q A P R L L I Y D

5C4 VL sequence

GCA TCC AAC AGG GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA
A S N R A T G I P A R F S G S G S G T D F T L T I S S L E P E D F A

c-myc tag sequence

GTT TAT TAC TGT CAG CAG AGT AGC AAC TGG CCT CGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG TAA
V Y Y C Q Q S S N W P R T F G Q G T K V E I K E Q K L I S E E D L *

FIG. 11

1928z-2A-5C4 (kappa leader) sequence



FIG. 12

4H1128Z-2A-5C4 (kappa leader) sequence

CATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAG
CTGCAGGAGTCAGGGGGAGGCTTCGTGAAGCCTGGAGGGTCCCTCAAAGTCTCCTGTGCAGC
CTCTGGATTCACTTTCAGTAGCTATGCCATGTCCTGGGTTCGCCTGAGTCCGGAGATGAGGCTG
GAGTGGGTCGCAACCATTAGCAGTGCTGGTGGTTACATCTTCTATTCTGACAGTGTGCAGGGA
CGATTCACCATTTCCAGAGACAATGCCAAGAACACCCTGCACCTGCAAATGGGCAGTCTGAGG
TCTGGGGACACGGCCATGTATTACTGTGCAAGGCAGGGATTTGGTAACTACGGTGATTACTATG
CTATGGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTGGAGGTGGATCAGGT
GGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCTCACCCAGTCTCCATCCTCCCTGGCT
GTGTCAGCAGGAGAGAAGGTCACTATGAGCTGCAAATCCAGTCAGAGTCTGCTCAACAGTAG
AACCCGAAAGAACCAGTTGGCTTGGTACCAGCAAAAACCAGGACAGTCTCCTGAACTGCTG
ATCTACTGGGCATCCACTAGGCAATCTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGG
ACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGCCAG
CAATCTTATAATCTACTgcaacaaacttctcactactcaaacaagcaggtgacgtggaggagaatcccggaccc
ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGA
CCAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CGACTGTAAAGCGTCTGGAATCACCTTCAGTAACTCTGGCATGCACTGGGTCCGCCAGGCTC
CAGGCAAGGGGCTGGAGTGGGTGGCAGTTATTTGGTATGATGGAAGTAAAAGATACTATGC
AGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGACAAACGACGACTA
CTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGA
TCTGGTGGAGGTGGATCTGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCA
GGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGTAGTTACTTAGCCTGGTA
CCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCACTG
GCATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAG
CCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGAGTAGCAACTGGCCTCGGACGT
TCGGCCAAGGGACCAAGGTGGAAATCAAAGAACAAAAACTCATCTCAGAAGAGGATCTGTA
ACTCGAGGATCC

4H1128z CAR

P2A

SC4 scFv

FIG. 13

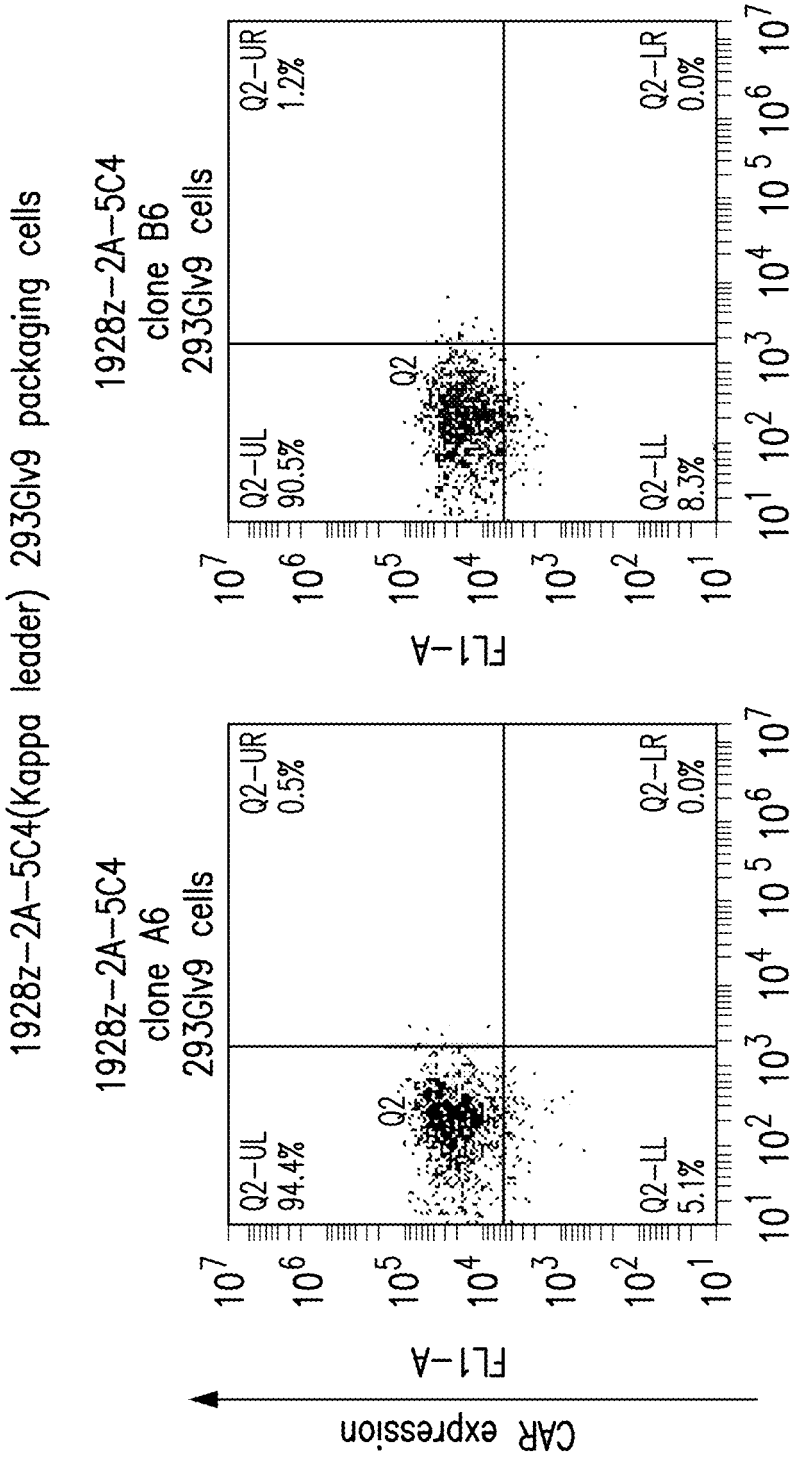

1928z-2A-5C4(Kappa leader) 293Glv9 packaging cells
1928z-2A-5C4
clone A6
293Glv9 cells
Q2-UL
94.4%
Q2-UR
0.5%
Q2
Q2-LL
5.1%
Q2-LR
0.0%
1928z-2A-5C4
clone B6
293Glv9 cells
Q2-UL
90.5%
Q2-UR
1.2%
Q2
Q2-LL
8.3%
Q2-LR
0.0%
CAR expression
FL1-A
FL4-A

FIG. 14

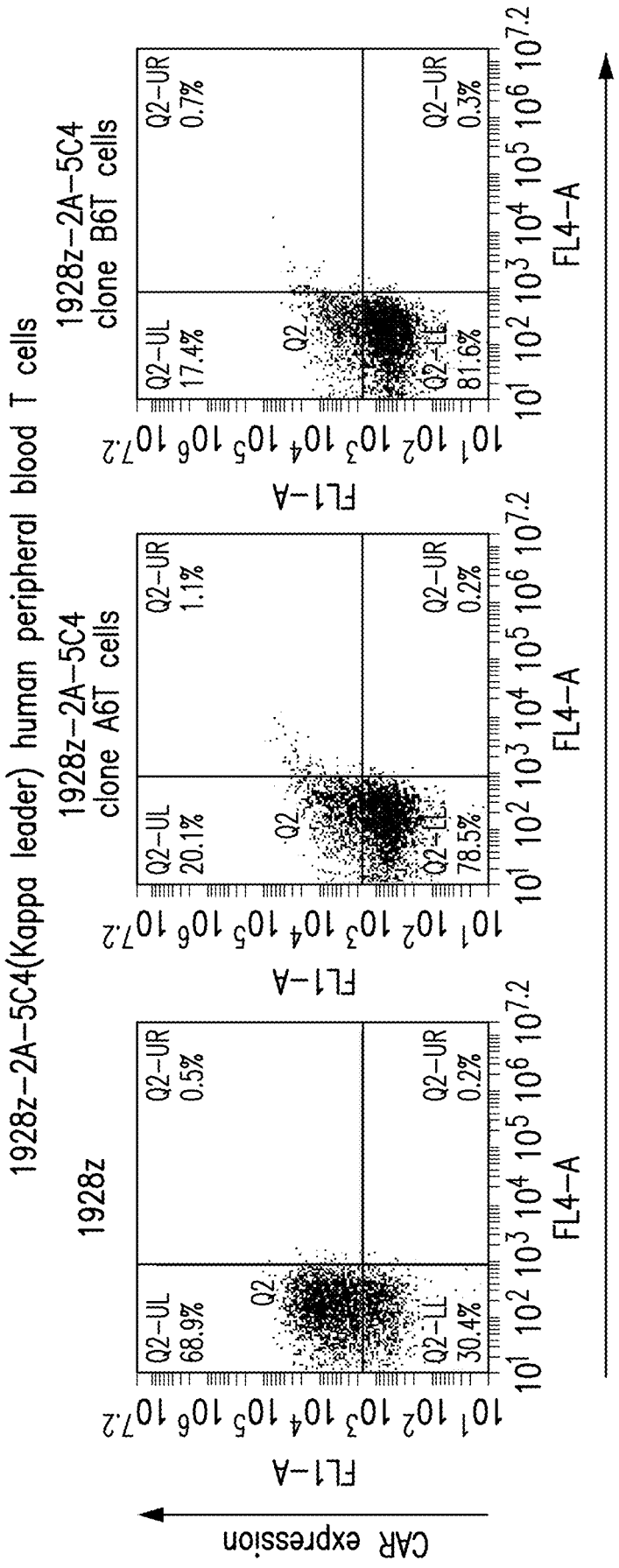

1928z-2A-5C4(Kappa leader) human peripheral blood T cells
1928z
1928z-2A-5C4 clone A6T cells
1928z-2A-5C4 clone B6T cells
Q2-UL 68.9%
Q2-UR 0.5%
Q2-LL 30.4%
Q2-UR 0.2%
Q2-UL 20.1%
Q2-UR 1.1%
Q2-LL 78.5%
Q2-UR 0.2%
Q2-UL 17.4%
Q2-UR 0.7%
Q2-LL 81.6%
Q2-UR 0.3%
FL1-A
FL4-A
CAR expression
c-myc tag expression

FIG. 15

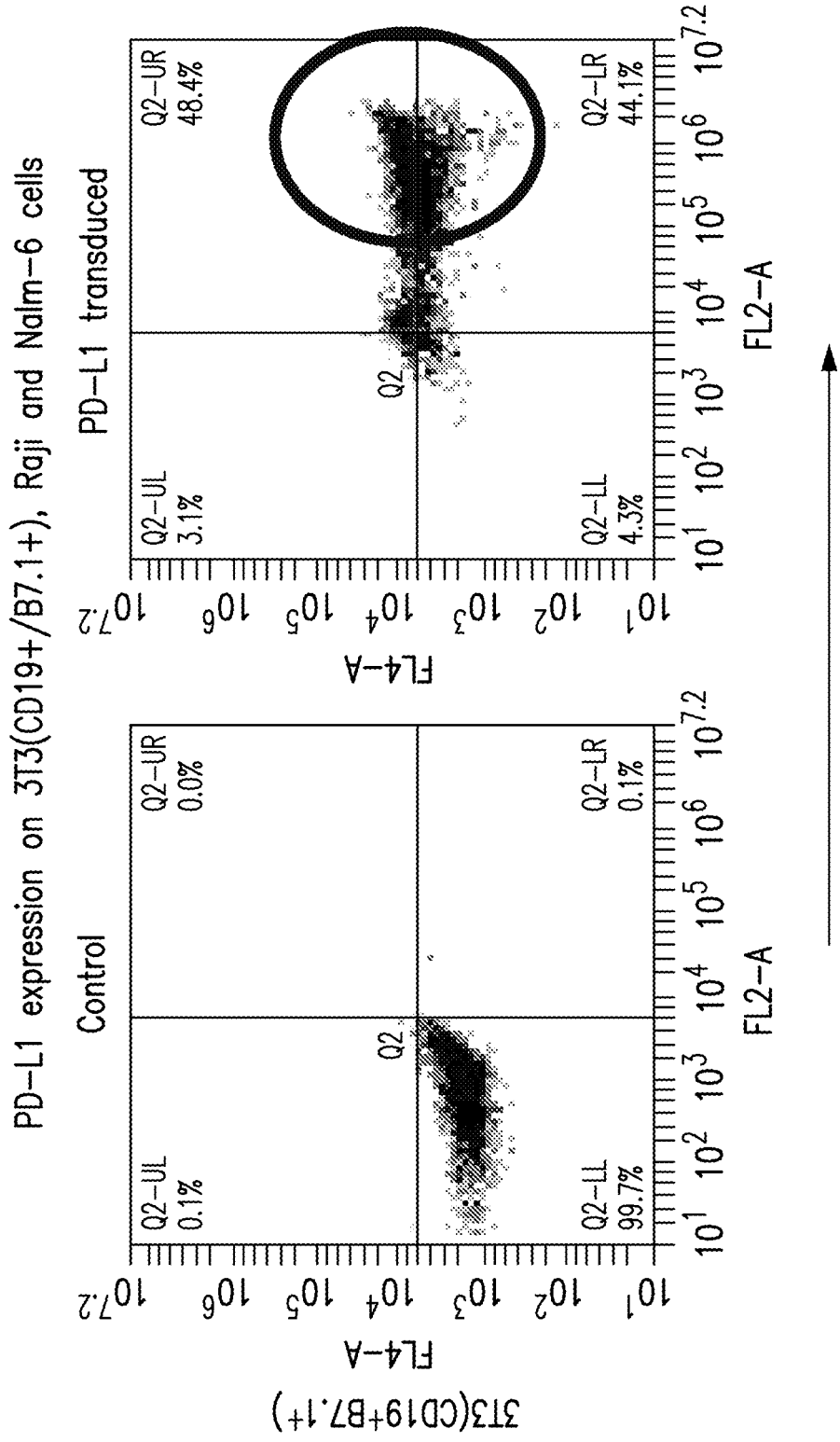

PD-L1 expression on 3T3(CD19+/B7.1+), Raji and Nalm-6 cells
Control
PD-L1 transduced
Q2-UL 0.1%
Q2-UR 0.0%
Q2-LL 99.7%
Q2-LR 0.1%
Q2-UL 3.1%
Q2-UR 48.4%
Q2-LL 4.3%
Q2-LR 44.1%
Q2
FL4-A
FL2-A
3T3(CD19+B7.1+)
PD-L1

FIG. 16A

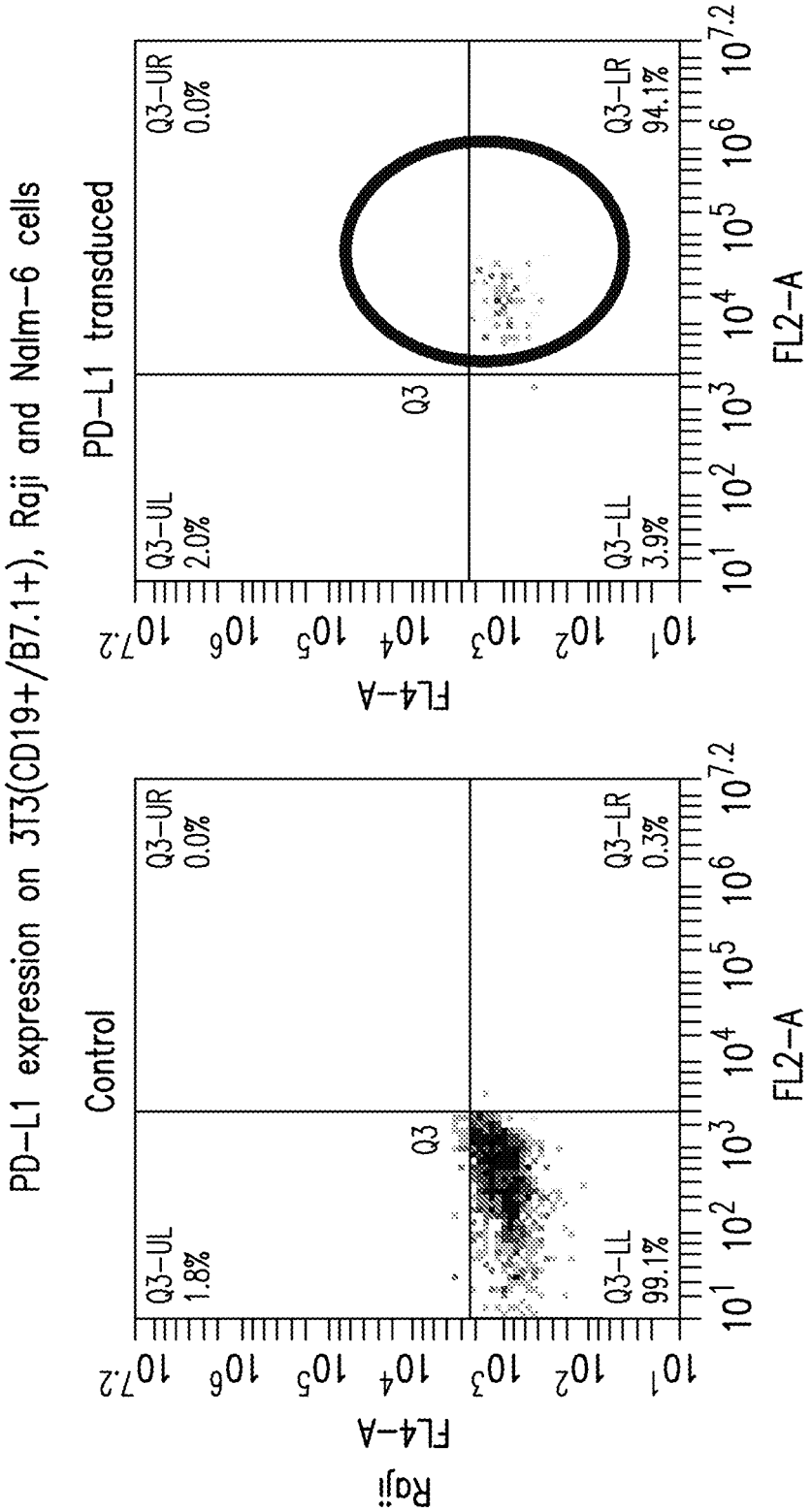

PD-L1 expression on 3T3(CD19+/B7.1+), Raji and Nalm-6 cells
Control
PD-L1 transduced
Raji
Q3-UL
1.8%
Q3-UR
0.0%
Q3-LL
99.1%
Q3-LR
0.3%
Q3-UL
2.0%
Q3-UR
0.0%
Q3-LL
3.9%
Q3-LR
94.1%
Q3
FL4-A
FL2-A
PD-L1

FIG. 16B

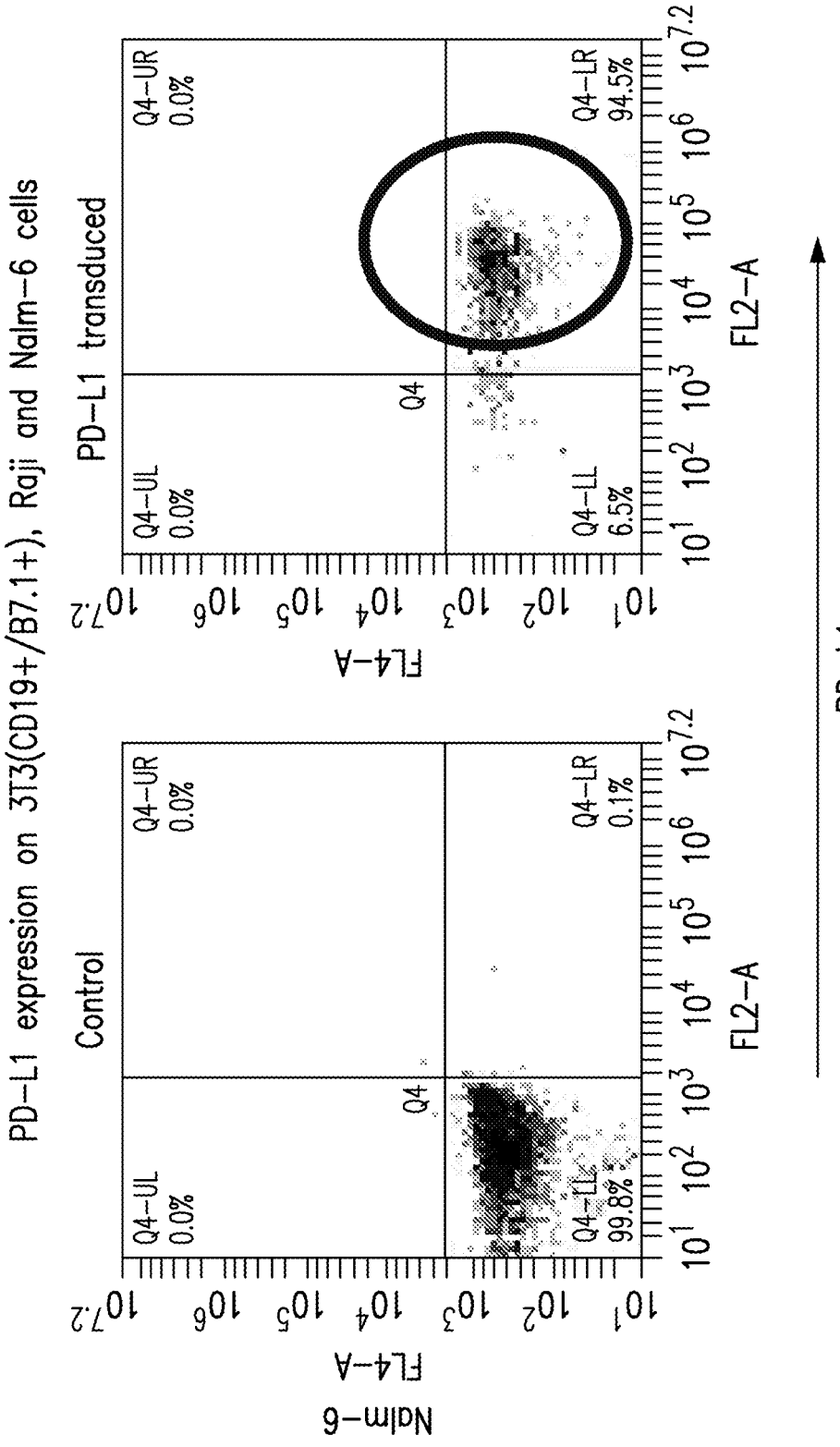

PD-L1 expression on 3T3(CD19+/B7.1+), Raji and Nalm-6 cells
Control
PD-L1 transduced
Nalm-6
FL4-A
FL2-A
Q4-UL 0.0%
Q4-UR 0.0%
Q4-LL 99.8%
Q4-LR 0.1%
Q4-UL 0.0%
Q4-UR 0.0%
Q4-LL 6.5%
Q4-LR 94.5%
Q4
PD-L1

FIG. 16C

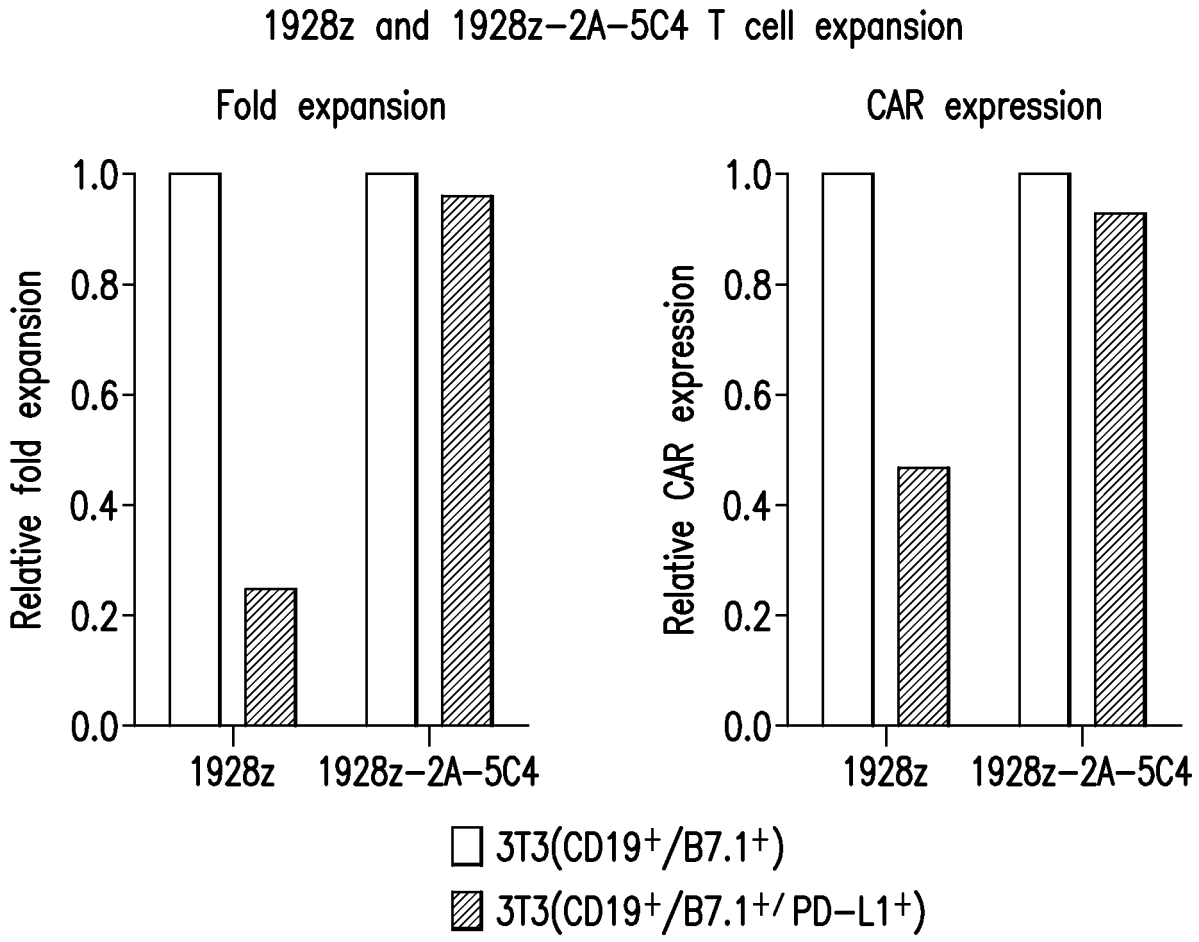

1928z and 1928z-2A-5C4 T cell expansion
Fold expansion
CAR expression
Relative fold expansion
Relative CAR expression
1.0
0.8
0.6
0.4
0.2
0.0
1928z
1928z-2A-5C4
3T3(CD19+/B7.1+)
3T3(CD19+/B7.1+/ PD-L1+)

FIG. 17

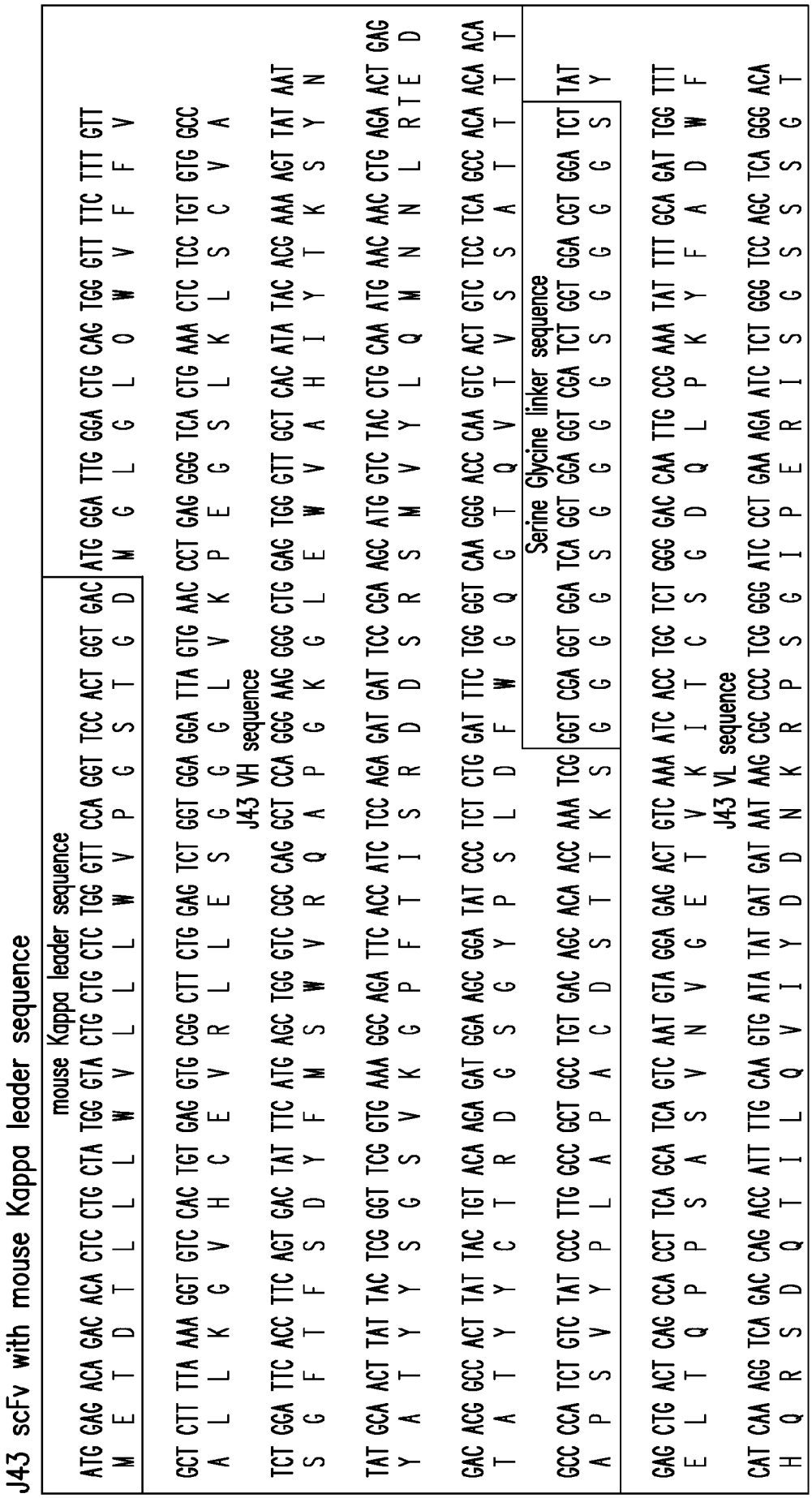

J43 scFv with mouse Kappa leader sequence
mouse Kappa leader sequence
ATG GAG ACA GAC ACA CTC CTG CTA TGG GTA CTG CTC CTC TGG GTT CCA GGT TCC ACT GGT GAC
M E T D T L L L W V L L L W V P G S T G D
ATG GGA TTG GGA CTG CAG TGG GTT TTC TTT GTT
M G L G L Q W V F F V
GCT CTT TTA AAA GGT GTC CAC TGT GAG GTG CGG CTT CTG GAG TCT GGT GGA GGA TTA GTG AAC CCT GAG GGG TCA CTG AAA CTC TCC TGT GTG GCC
A L L K G V H C E V R L L E S G G G L V K P E G S L K L S C V A
J43 VH sequence
TCT GGA TTC ACC TTC AGT GAC TAT TTC ATG AGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG GAG TGG GTT GCT CAC ATA TAC ACG AAA AGT TAT AAT
S G F T F S D Y F M S W V R Q A P G K G L E W V A H I Y T K S Y N
TAT GCA ACT TAT TAC TCG GGT TCG GTG AAA GGC AGA TTC ACC ATC TCC AGA GAT GAT TCC CGA AGC ATG GTC TAC CTG CAA ATG AAC AAC CTG AGA ACT GAG
Y A T Y Y S G S V K G P F T I S R D D S R S M V Y L Q M N N L R T E D
GAC ACG GCC ACT TAT TAC TGT ACA AGA GAT GGA AGC GGA TAT CCC TCT CTG GAT TTC TGG GGT CAA GGG ACC CAA GTC ACT GTC TCC TCA GCC ACA ACA ACA
T A T Y Y C T R D G S G Y P S L D F W G Q G T Q V T V S S A T T T
Serine Glycine linker sequence
GCC CCA TCT GTC TAT CCC TTG GCC GCT GCC TGT GAC AGC ACA ACC AAA TCG GGT CGA GGT GGA TCA GGT GGA GGT CGA TCT GGA CGT GGA TCT TAT
A P S V Y P L A P A C D S T T K S G G G G S G G G G S G G G G S Y
GAG CTG ACT CAG CCA CCT TCA GCA TCA GTC AAT GTA GGA GAG ACT GTC AAA ATC ACC TGC TCT GGG GAC CAA TTG CCG AAA TAT TTT GCA GAT TGG TTT
E L T Q P P S A S V N V G E T V K I T C S G D Q L P K Y F A D W F
J43 VL sequence
CAT CAA AGG TCA GAC CAG ACC ATT TTG CAA GTG ATA TAT GAT GAT AAT AAG CGC CCC TCG GGG ATC CCT GAA AGA ATC TCT GGG TCC AGC TCA GGG ACA
H Q R S D Q T I L Q V I Y D D N K R P S G I P E R I S G S S S G T

FIG. 18

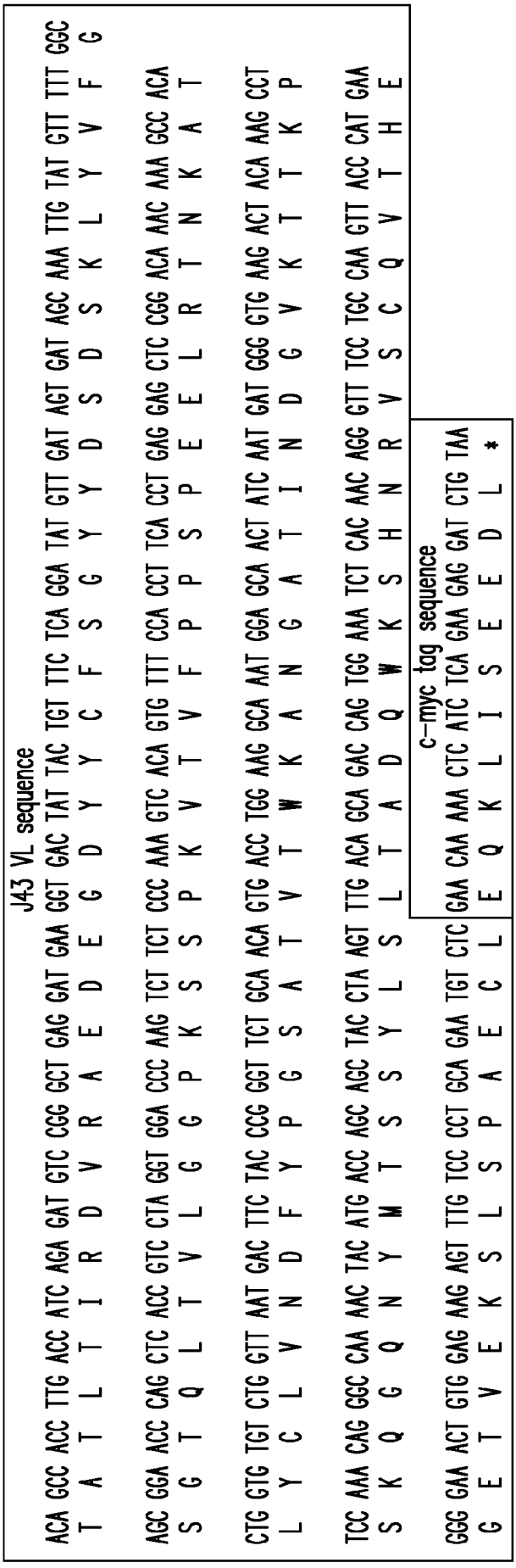


FIG. 18 continued

19m28mzIRESJ43(mouse Kappa leader)sequence

CATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAGCTGCAGCAGTCTGGGG
CTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATGCATTCAGTAGCTACTGGATGAAC
TGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAGTGGATTGGACAGATTTATCCTGGAGATGGTGATACTAACTACAATGG
AAAGTTCAAGGGTCAAGCCACACTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCGGCCTAACATCTG
AGGACTCTGCGGTCTATTTCTGTGCAAGAAAGACCATTAGTTCGGTAGTAGATTTCTACTTTGACTACTGGGGCCAAGGG
ACCACGGTCACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCTCAC
CCAGTCTCCAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTCAGAATGTGGGTACTA
ATGTAGCCTGGTATCAACAGAAACCAGGACAATCTCCTAAACCACTGATTTACTCGGCAACCTACCGGAACAGTGGAGTC
CCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCACTAACGTGCAGTCTAAAGACTTGGCAGA
CTATTTCTGTCAACAATATAACAGGTATCCGTACACGTCCGGAGGGGGGACCAAGCTGGAGATCAAACGGGCGGCCCCAA
TTGAGTTCATGTACCCTCCGCCTTACCTAGACAACGAGAGGAGCAATGGAACTATTATTCACATAAAAGAGAAACATCTT
TGTCATACTCAGTCATCTCCTAAGCTGTTTTGGGCACTGGTCGTGGTTGCTGGAGTCCTGTTTTGTTATGGCTTGCTAGT
GACAGTGGCTCTTTGTGTTATCTGGACAAATAGTAGAAGGAACAGACTCCTTCAAAGTGACTACATGAACATGACTCCCC
GGAGGCCTGGGCTCACTCGAAAGCCTTACCAGCCCTACGCCCCTGCCAGAGACTTTGCAGCGTACCGCCCCAGAGCAAAA
TTCAGCAGGAGTGCAGAGACTGCTGCCAACCTGCAGGACCCCAACCAGCTCTACAATGAGCTCAATCTAGGGCGAAGAGA
GGAATATGACGTCTTGGAGAAGAAGCGGGCTCGGGATCCAGAGATGGGAGGCAAACAGCAGAGGAGGAGGAACCCCCAGG
AAGGCGTATACAATGCACTGCAGAAAGACAAGATGGCAGAAGCCTACAGTGAGATCGGCACAAAAGGCGAGAGACGGAGA
GGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGCACTGCCACCAAGGACACCTATGATGCCCTGCATATGCAGACCCT

19m28mzCAR

GGCCCCTCGCTAACAGCCACTCGAGGATCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAA
TAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGG
CCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAGGGTCTGTTGAATGTCGTGAAGG
AAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGC
GACAGGTGCCTCTGCGACCAAAGGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAG
TTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCC
ATTGTATGGGATCTGATCTGGGGCCTCGGTCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCC

IRES

CGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATATGGCCACAAACTGCCATGGAGACAGACACACTCC
TGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACATGGGATTGGGACTGCAGTGGGTTTTCTTTGTTGCT
CTTTTAAAAGGTGTCCACTGTGAGGTGCGGCTTCTGGAGTCTGGTGGAGGATTAGTGAAGCCTGAGGGGTCACTGAAACT
CTCCTGTGTGGCCTCTGGATTCACCTTCAGTGACTATTTCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGT
GGGTTGCTCACATATACACGAAAAGTTATAATTATGCAACTTATTACTCGGGTTCGGTGAAAGGCAGATTCACCATCTCC
AGAGATGATTCCCGAAGCATGGTCTACCTGCAAATGAACAACCTGAGAACTGAGGACACGGCCACTTATTACTGTACAAG
AGATGGAAGCGGATATCCCTCTCTGGATTTCTGGGGTCAAGGGACCCAAGTCACTGTCTCCTCAGCCACAACAACAGCCC
CATCTGTCTATCCCTTGGCCCCTGCCTGTGACAGCACAACCAAATCGGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGT
GGAGGTGGATCTTATGAGCTGACTCAGCCACCTTCAGCATCAGTCAATGTAGGAGAGACTGTCAAAATCACCTGCTCTGG
GGACCAATTGCCGAAATATTTTGCAGATTGGTTTCATCAAAGGTCAGACCAGACCATTTTGCAAGTGATATATGATGATA
ATAAGCGCCCCTCGGGGATCCCTGAAAGAATCTCTGGGTCCAGCTCAGGGACAACAGCCACCTTGACCATCAGAGATGTC
CGGGCTGAGGATGAAGGTGACTATTACTGTTTCTCAGGATATGTTGATAGTGATAGCAAATTGTATGTTTTTGGCAGCGG
AACCCAGCTCACCGTCCTAGGTGGACCCAAGTCTTCTCCCAAAGTCACAGTGTTTCCACCTTCACCTGAGGAGCTCCGGA
CAAACAAAGCCACACTGGTGTGTCTGGTTAATGACTTCTACCCGGGTTCTGCAACAGTGACCTGGAAGGCAAATGGAGCA
ACTATCAATGATGGGGTGAAGACTACAAAGCCTTCCAAACAGGGCCAAAACTACATGACCAGCAGCTACCTAAGTTTGAC
AGCAGACCAGTGGAAATCTCACAACAGGGTTTCCTGCCAAGTTACCCATGAAGGGGAAACTGTGGAGAAGAGTTTGTCCC
CTGCAGAATGTCTCGAACAAAAACTCATCTCAGAAGAGGATCTGTAACTCGAG

J43 scFv

FIG. 19

4H11m28mzIRESJ43(mouse Kappa leader)sequence

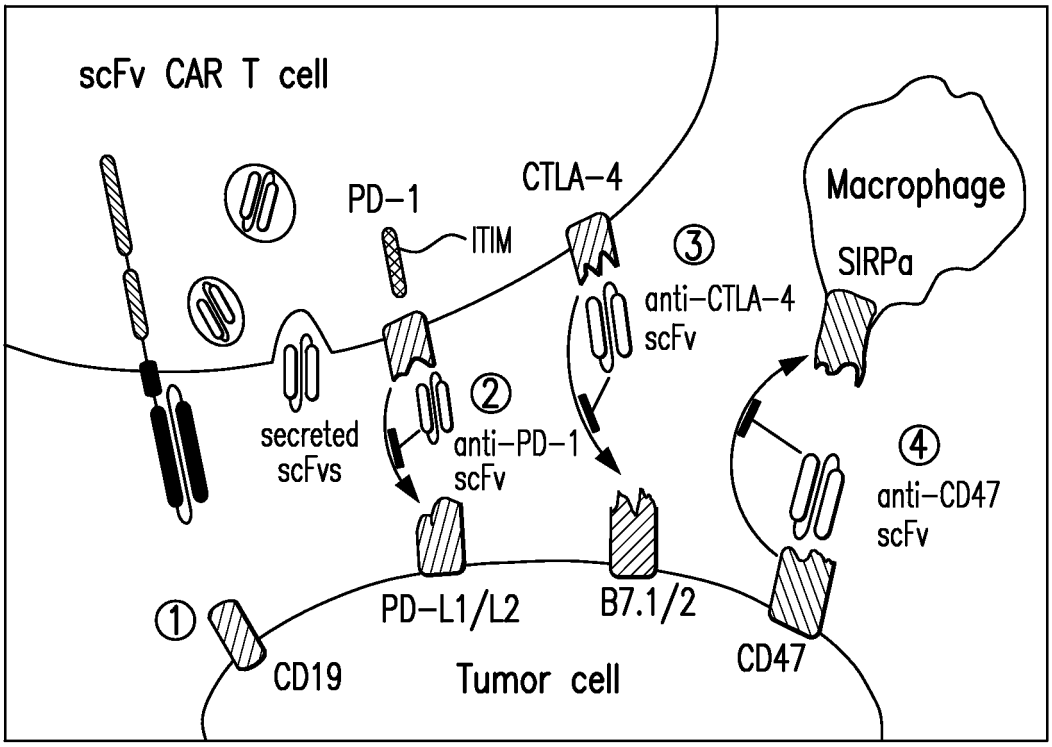
CTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGGTGAAGCTGCAGGAGTCAGGGGGAGGC
TTCGTGAAGCCTGGAGGGTCCCTCAAAGTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAGCTATGCCATGTCCTGGGT
TCGCCTGAGTCCGGAGATGAGGCTGGAGTGGGTCGCAACCATTAGCAGTGCTGGTGGTTACATCTTCTATTCTGACAGTG
TGCAGGGACGATTCACCATTTCCAGAGACAATGCCAAGAACACCCTGCACCTGCAAATGGGCAGTCTGAGGTCTGGGGAC
ACGGCCATGTATTACTGTGCAACGCAGGGATTTGGTAACTACGGTGATTACTATGCTATGGACTACTGGGGCCAAGGGAC
CACGGTCACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATCTGACATTGAGCTCACCC
AGTCTCCATCCTCCCTGGCTGTGTCAGCAGGAGAGAAGGTCACTATGAGCTGCAAATCCAGTCAGAGTCTGCTCAACAGT
AGAACCCGAAAGAACCAGTTGGCTTGGTACCAGCAAAAACCAGGACAGTCTCCTGAACTGCTGATCTACTGGGCATCCAC
TAGGCAATCTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGCAGG
CTGAAGACCTGGCAGTTTATTACTGCCAGCAATCTTATAATCTACTGGGACCAAGCTGGAGATCAAACGGGCGGCCGCAA
TTGAGTTCATGTACCCTCCGCCTTACCTAGACAACGAGAGGAGCAATGGAACTATTATTCACATAAAAGAGAAACATCTT
TGTCATACTCAGTCATCTCCTAAGCTGTTTTGGGCACTGGTCGTGGTTGCTGGAGTCCTGTTTTGTTATGGCTTGCTAGT
GACAGTGGCTCTTTGTGTTATCTGGACAAATAGTAGAAGGAACAGACTCCTTCAAAGTGACTACATGAACATGACTCCCC
GGAGGCCTGGGCTCACTCGAAAGCCTTACCAGCCCTACGCCCCTGCCAGAGACTTTGCAGCGTACCGCCCCAGAGCAAAA
TTCAGCAGGAGTGCAGAGACTGCTGCCAACCTGCAGGACCCCAACCAGCTCTACAATGAGCTCAATCTAGGGCGAAGAGA
GGAATATGACGTCTTGGAGAAGAAGCGGGCTCGGGATCCAGAGATGGGAGGCAAACAGCAGAGGAGGAGGAACCCCCAGG
AAGGCGTATACAATGCACTGCAGAAAGACAAGATGGCAGAAGCCTACAGTGAGATCGGCACAAAAGGCGAGAGGCGGAGA
GGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGCACTGCCACCAAGGACACCTATGATGCCCTGTATATGCAGACCCT
GGCCCCTCGCTAACAGCCACTCGAGGATCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAA
TAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGG
CCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAGGGTCTGTTGAATGTCGTGAAGG
AAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGC
GACAGGTGCCTCTGCGACCAAAGGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAG
TTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCC
ATTGTATGGGATCTGATCTGGGGCCTCGGTCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCC
CGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATATGGCCACAAACTGCCATGGAGACAGACACACTCC
TGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACATGGGATTGGGACTGCAGTGGGTTTTCTTTGTTGCT
CTTTTAAAAGGTGTCCACTGTGAGGTGCGGCTTCTGGAGTCTGGTGGAGGATTAGTGAAGCCTGAGGGGTCACTGAAACT
CTCCTGTGTGGCCTCTGGATTCACCTTCAGTGACTATTTCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTCGAGT
GGGTTGCTCACATATACACGAAAAGTTATAATTATGCAACTTATTACTCGGGTTCGGTGAAAGGCAGATTCACCATCTCC
AGAGATGATTCCCGAAGCATGGTCTACCTGCAAATGAACAACCTGAGAACTGAGGACACGGCCACTTATTACTGTACAAG
AGATGGAAGCGGATATCCCTCTCTGGATTTCTGGGGTCAAGGGACCCAAGTCACTGTCTCCTCAGCCACAACAACAGCCC
CATCTGTCTATCCCTTGGCCCCTGCCTGTGACAGCACAACCAAATCGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGT
GGAGGTGGATCTTATGAGCTGACTCAGCCACCTTCAGCATCAGTCAATGTAGGAGAGACTGTCAAAATCACCTGCTCTGG
GGACCAATTGCCGAAATATTTTGCAGATTGGTTTCATCAAAGGTCAGACCAGACCATTTTGCAAGTGATATATGATGATA
ATAAGCGCCCCTCGGGGATCCCTGAAAGAATCTCTGGGTCCAGCTCAGGGACAACAGCCACCTTGACCATCAGAGATGTC
CGGGCTGAGGATGAAGGTGACTATTACTGTTTCTCAGGATATGTTGATAGTGATAGCAAATTGTATGTTTTTGGCAGCGG
AACCCAGCTCACCGTCCTAGGTGGACCCAAGTCTTCTCCCAAAGTCACAGTGTTTCCACCTTCACCTGAGGAGCTCCGGA
CAAACAAAGCCACACTGGTGTGTCTGGTTAATGACTTCTACCCGGGTTCTGCAACAGTGACCTGGAAGGCAAATGGAGCA
ACTATCAATGATGGGGTGAAGACTACAAAGCCTTCCAAACAGGGCCAAAACTACATGACCAGCAGCTACCTAAGTTTGAC
AGCAGACCAGTGGAAATCTCACAACAGGGTTTCCTGCCAAGTTACCCATGAAGGGGAAACTGTGGAGAAGAGTTTGTCCC
CTGCAGAATGTCTCGAACAAAAACTCATCTCAGAAGAGGATCTGTAACTCGAG

4H11m28mz CAR

IRES

J43 scFv

FIG. 20



Genetically engineering "armored" CAR T cells designed to overcome the "hostile tumor microenvironment"

CAR-T cells may alternatively be additionally modified to secrete antagonistic scFvs with immune regulatory functions. Upon activation of the CAR to cognate antigen(1), armored CAR modified T cells may be induced to secrete scFvs antagonistic to the inhibitory PD-1 T cell receptor on both infused CAR modified T cells and endogenous anti-tumor T cells enhancing anti-tumor effector function(2), induced to secrete scFvs antagonistic to the inhibitory CTLA-4 T cell receptor on both infused CAR modified T cells and endogenous anti-tumor T cells enhancing anti-tumor effector function(3), or induced to secrete an scFv antagonistic to the CD47 receptor expressed on the tumor cell reversing the cloaking the tumor cell from recognition by the host innate anti-tumor imune response leading to recognition and eradication of tumor by host macrophages.

FIG. 21

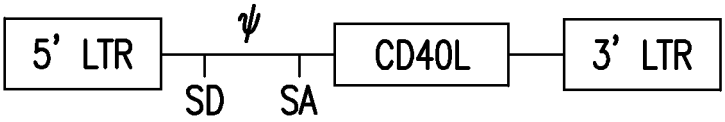


FIG. 22A

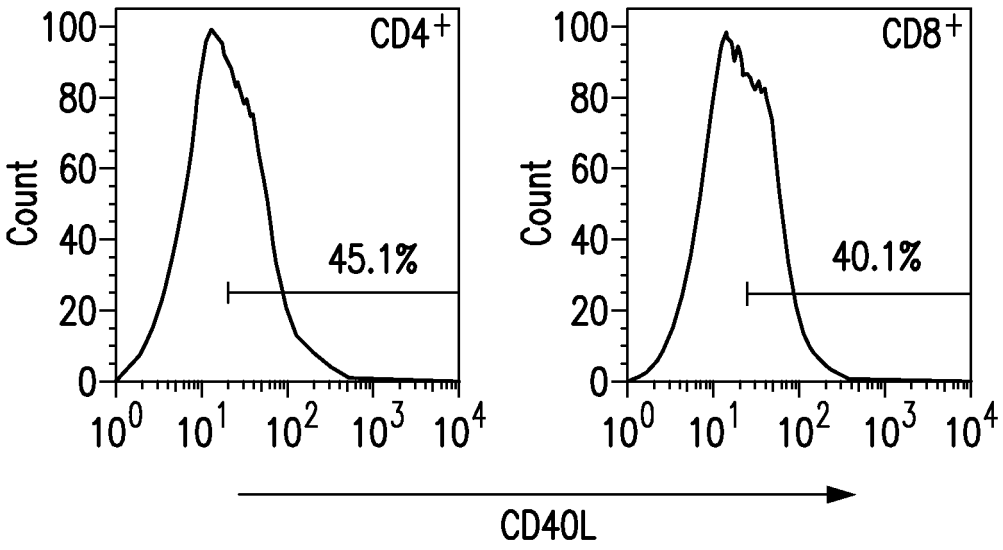


FIG. 22B

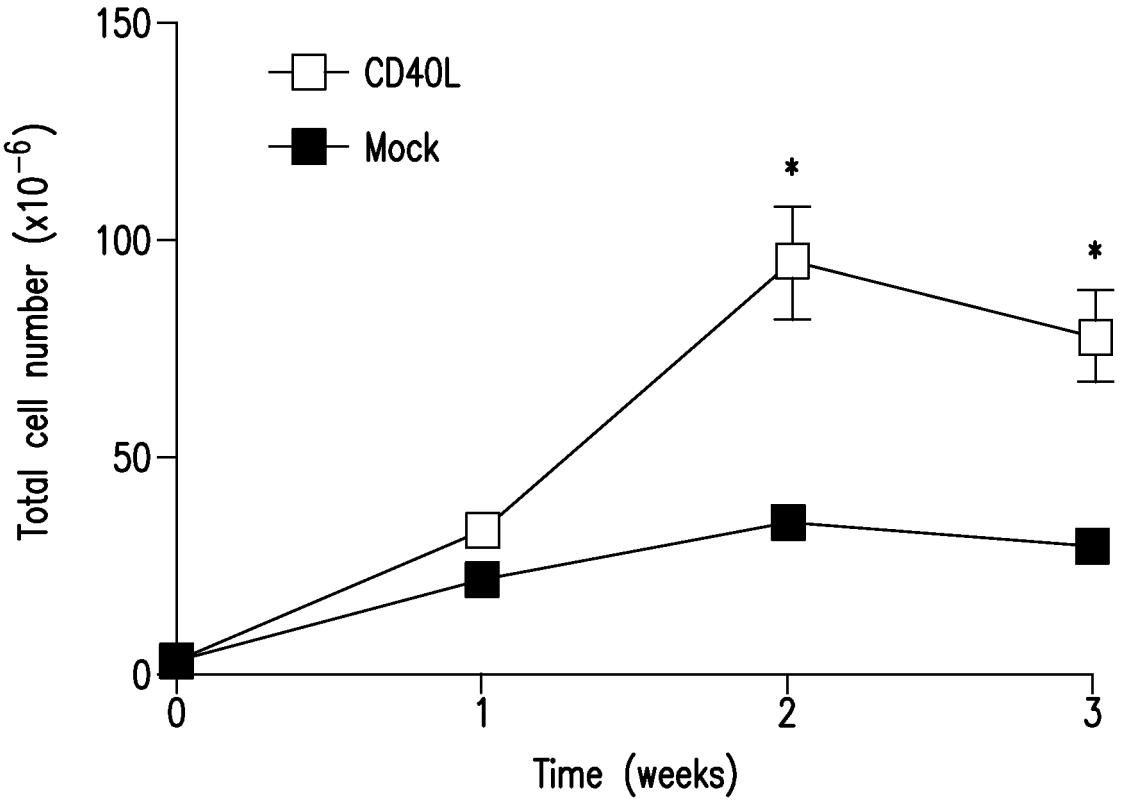

150
100
50
0
Total cell number (x10^{-6})
CD40L
Mock
*
*
0
1
2
3
Time (weeks)

FIG. 22C

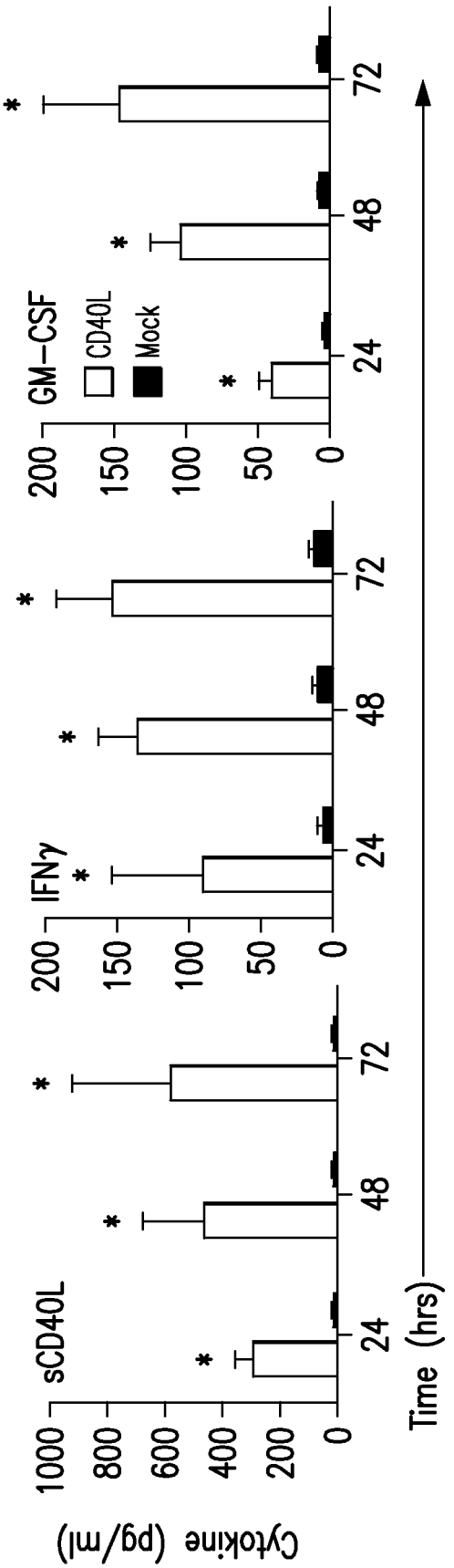

Cytokine (pg/ml)
sCD40L
IFNγ
GM-CSF
CD40L
Mock
Time (hrs)
0
200
400
600
800
1000
50
100
150
200
24
48
72
*

FIG. 22D

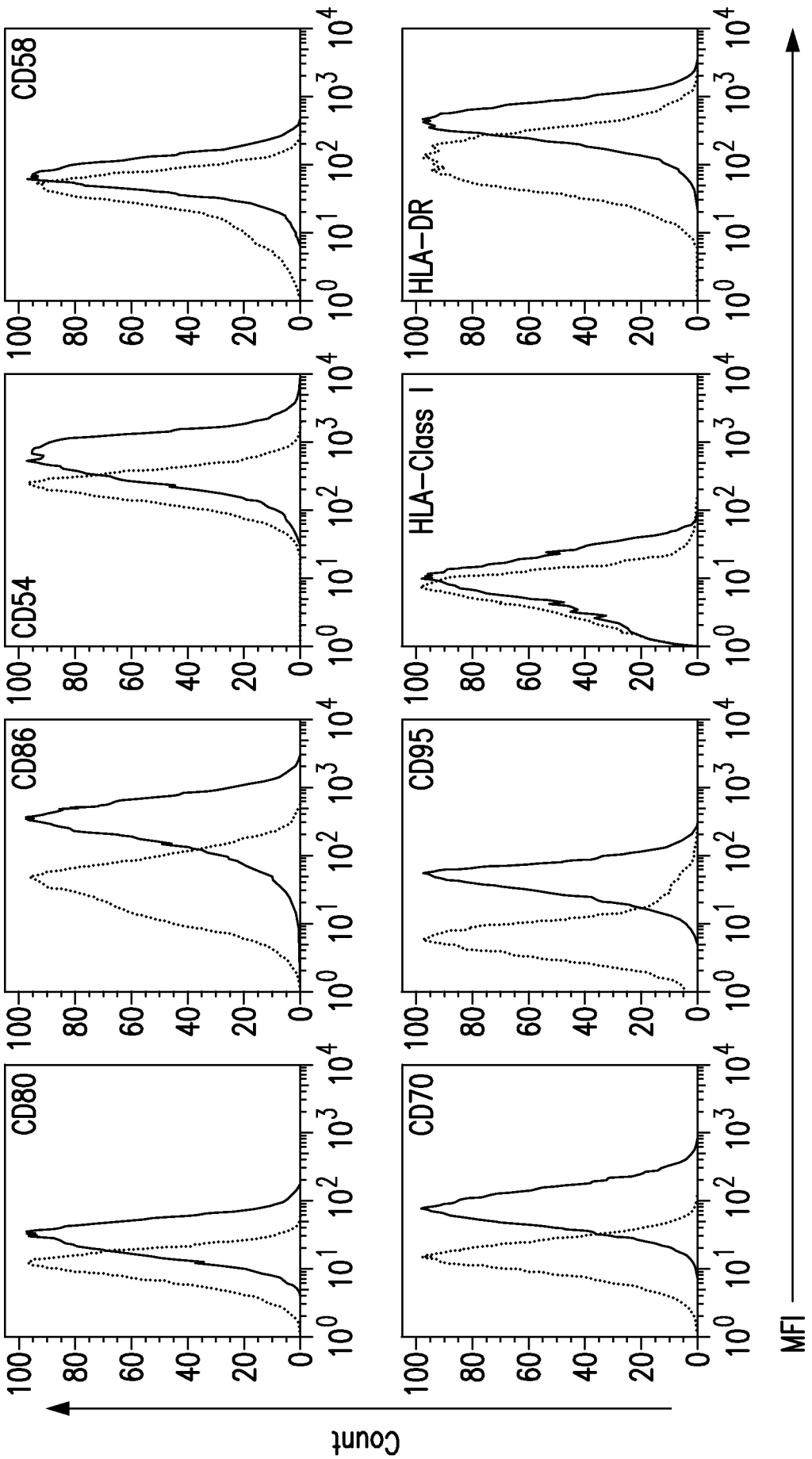


FIG. 23A

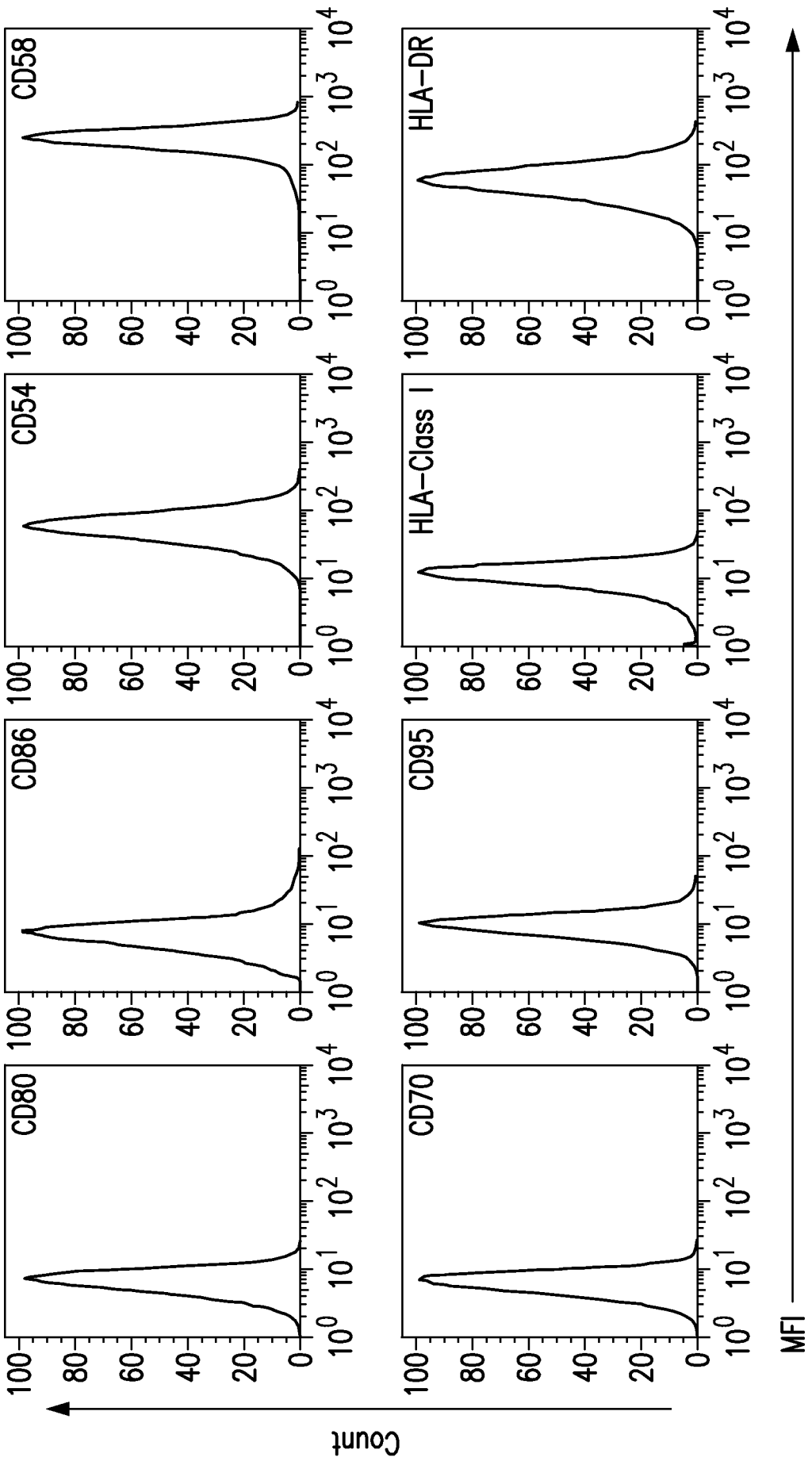


FIG. 23B

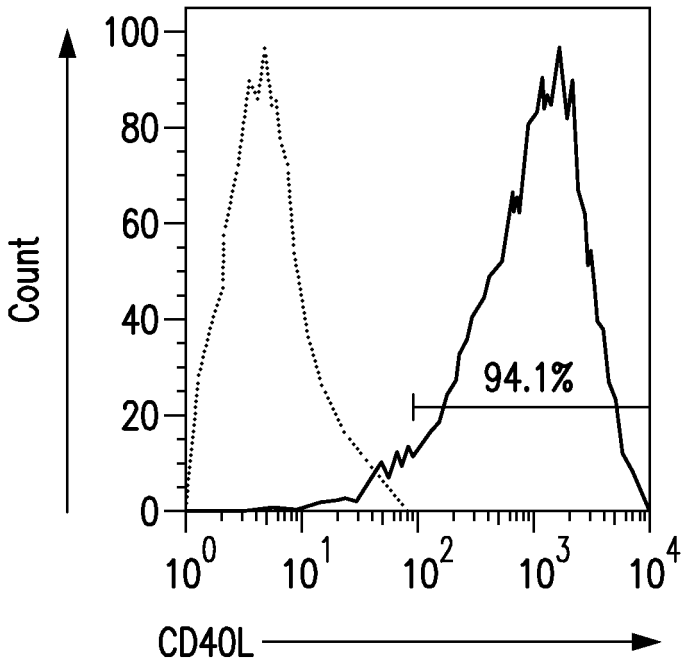


FIG. 24A

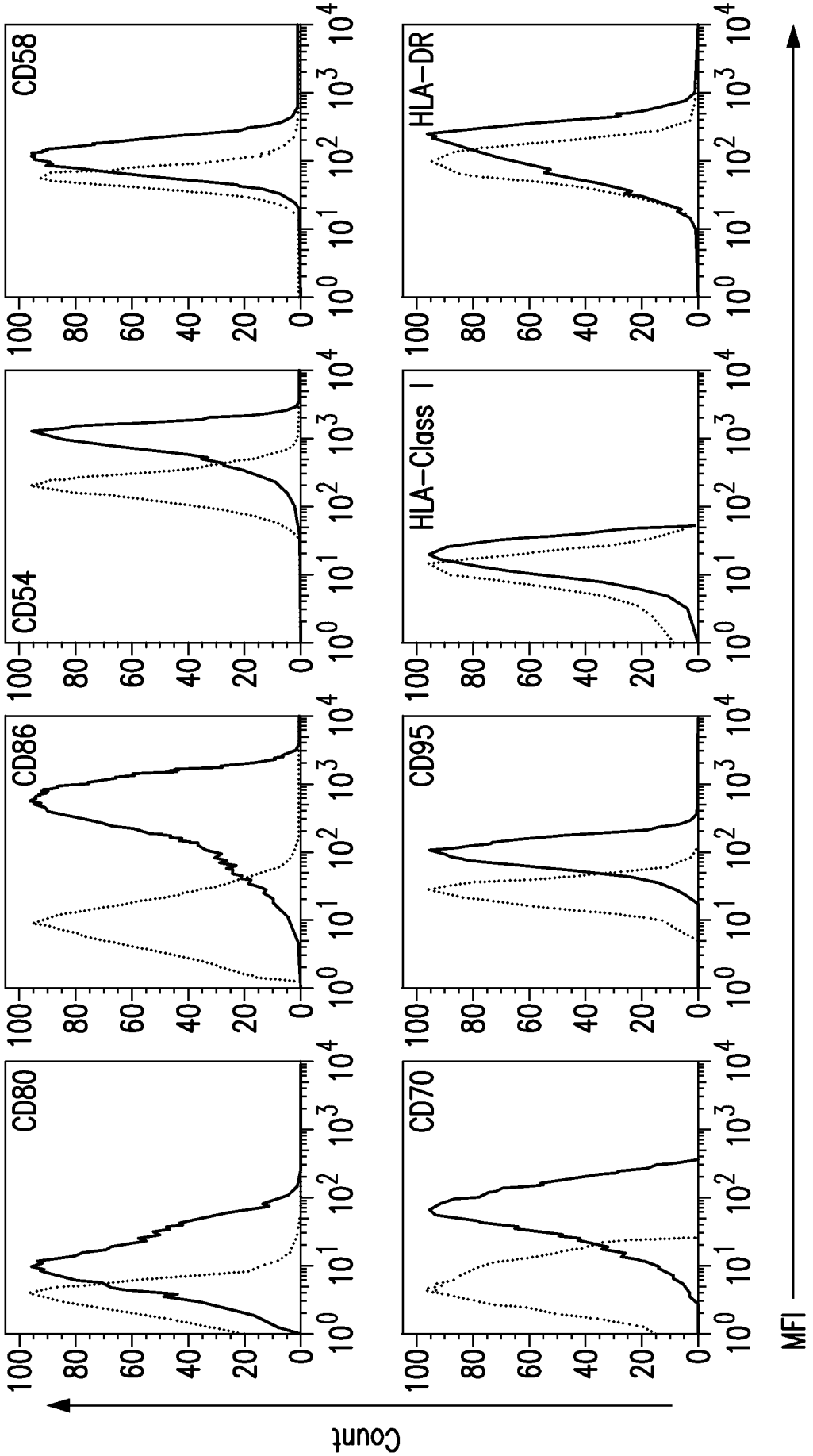


FIG. 24B

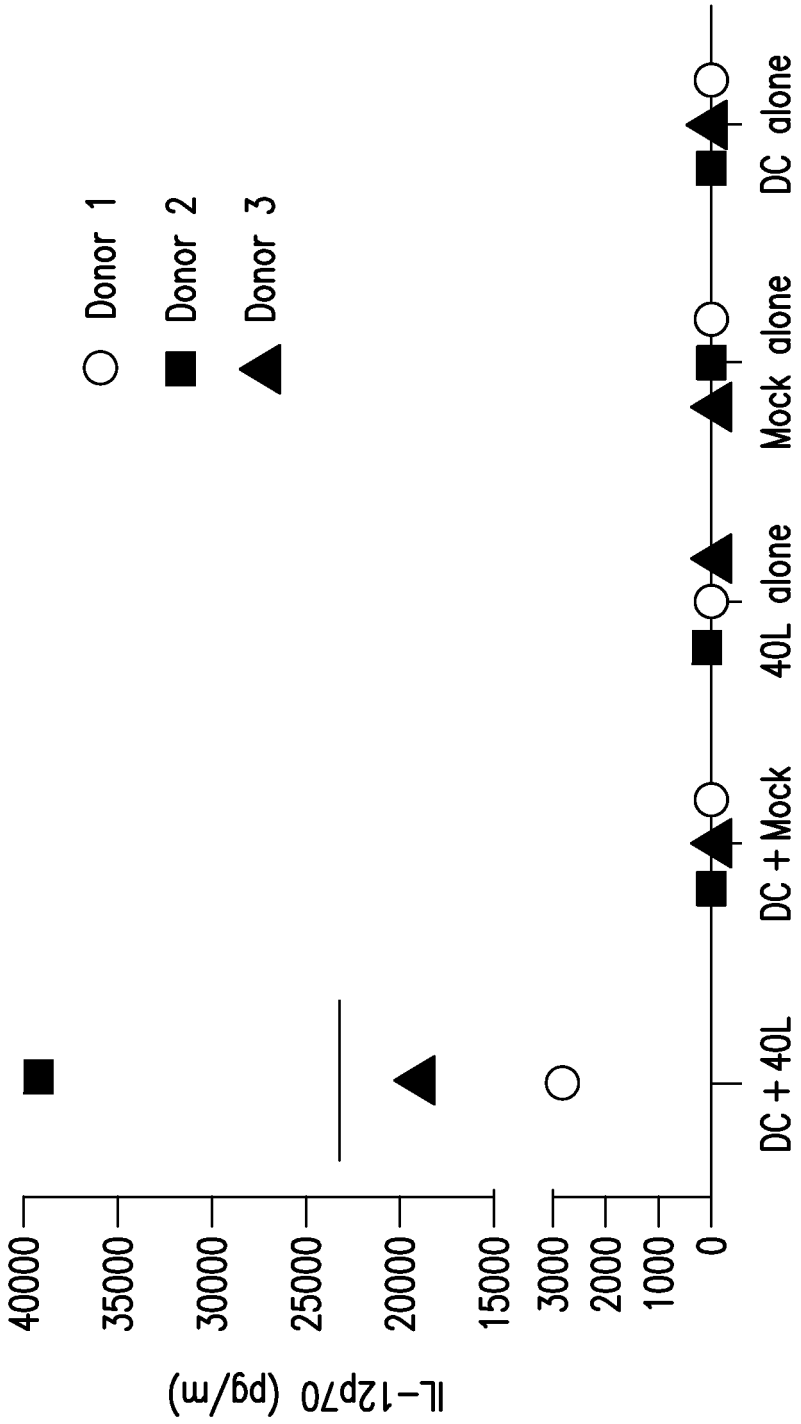

IL-12p70 (pg/m)
40000
35000
30000
25000
20000
15000
3000
2000
1000
0
DC + 40L
DC + Mock
40L alone
Mock alone
DC alone
Donor 1
Donor 2
Donor 3

FIG. 25A

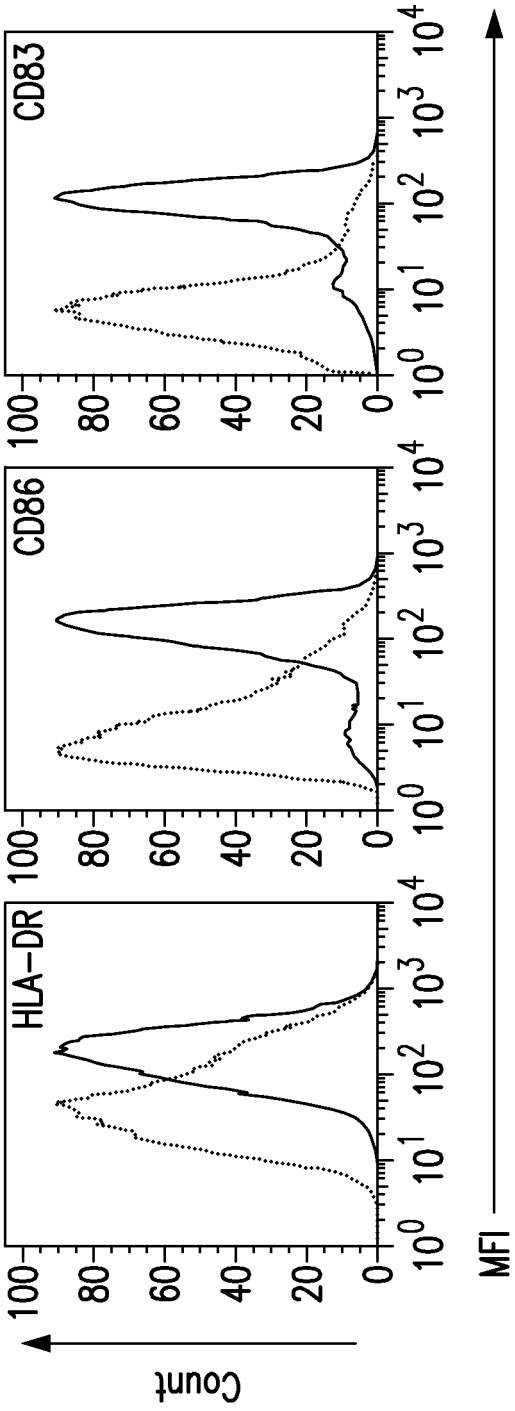


FIG. 25B

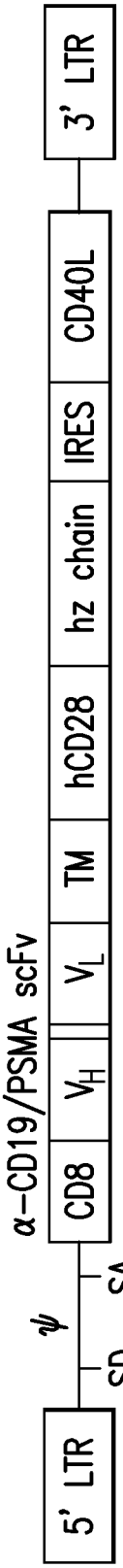

5' LTR
SD
ψ
SA
α-CD19/PSMA scFv
CD8
V_H
V_L
TM
hCD28
hz chain
IRES
CD40L
3' LTR

FIG. 26A

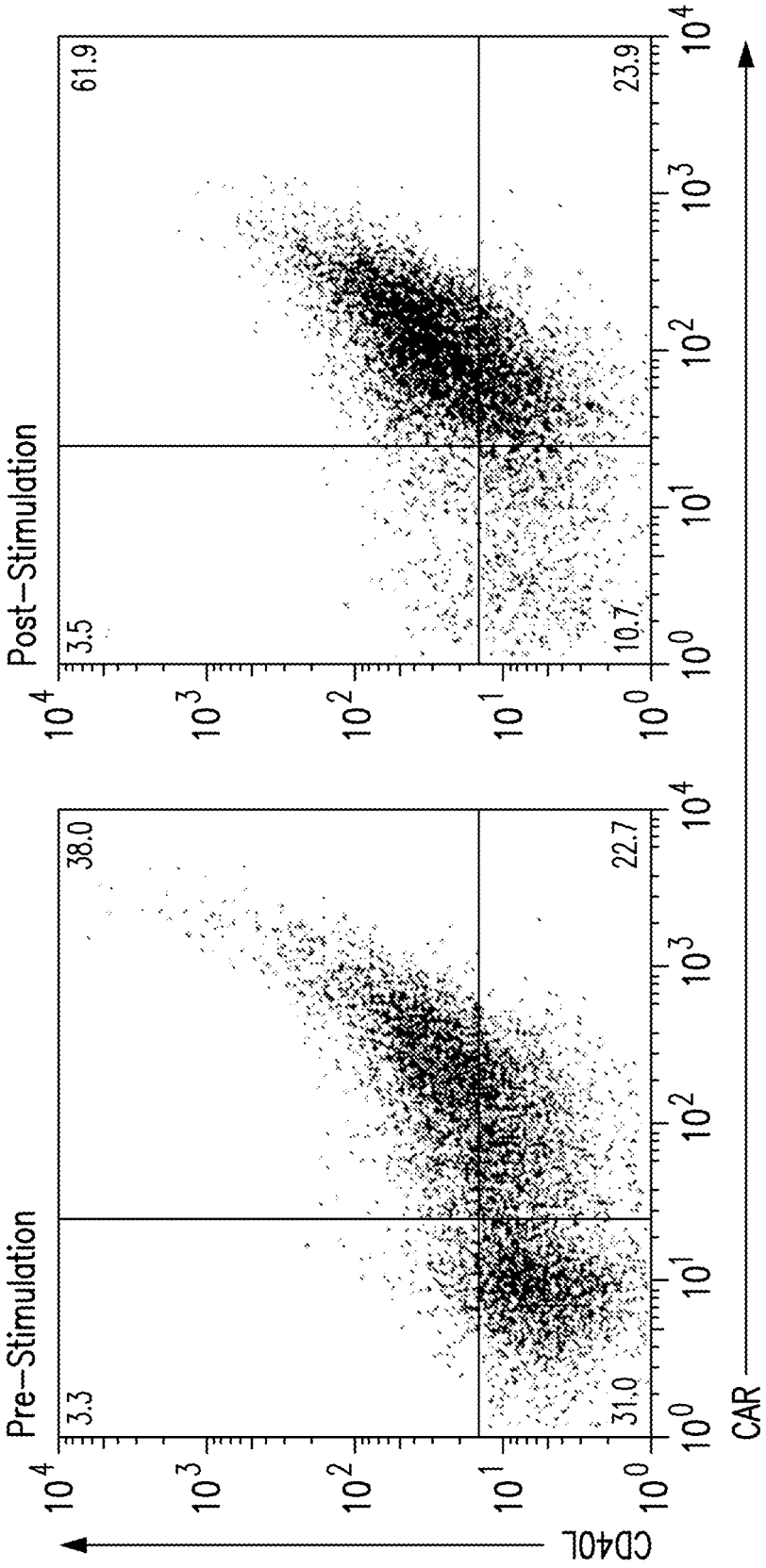

Pre−Stimulation
Post−Stimulation
CD40L
CAR
3.3
38.0
31.0
22.7
3.5
61.9
10.7
23.9
10^0
10^1
10^2
10^3
10^4

FIG. 26B

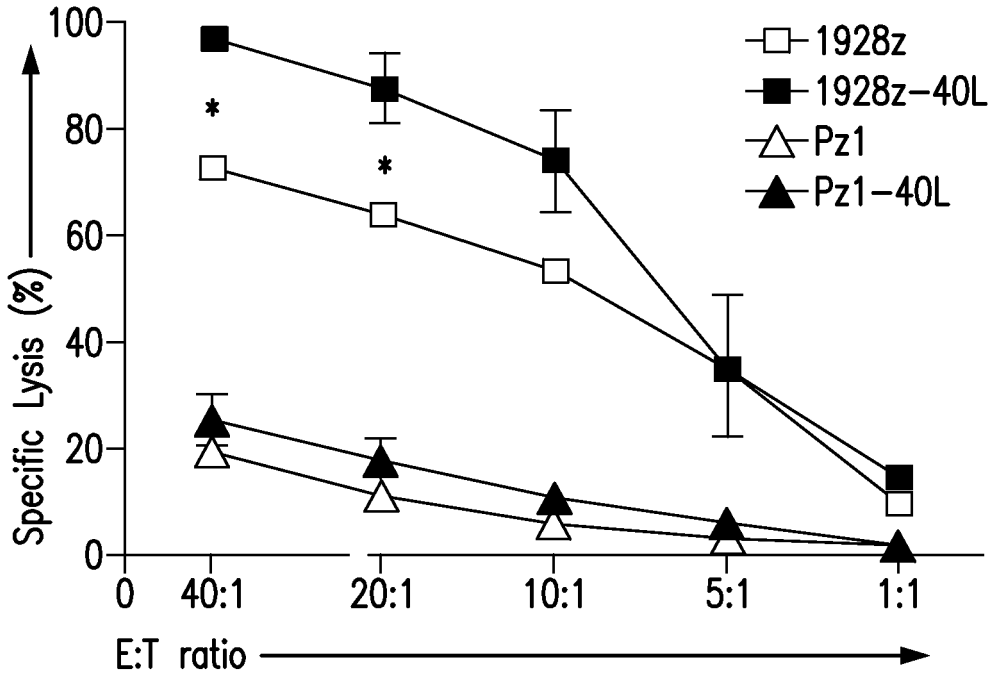

100
80
60
40
20
0
Specific Lysis (%)
*
*
1928z
1928z-40L
Pz1
Pz1-40L
0
40:1
20:1
10:1
5:1
1:1
E:T ratio

FIG. 26C

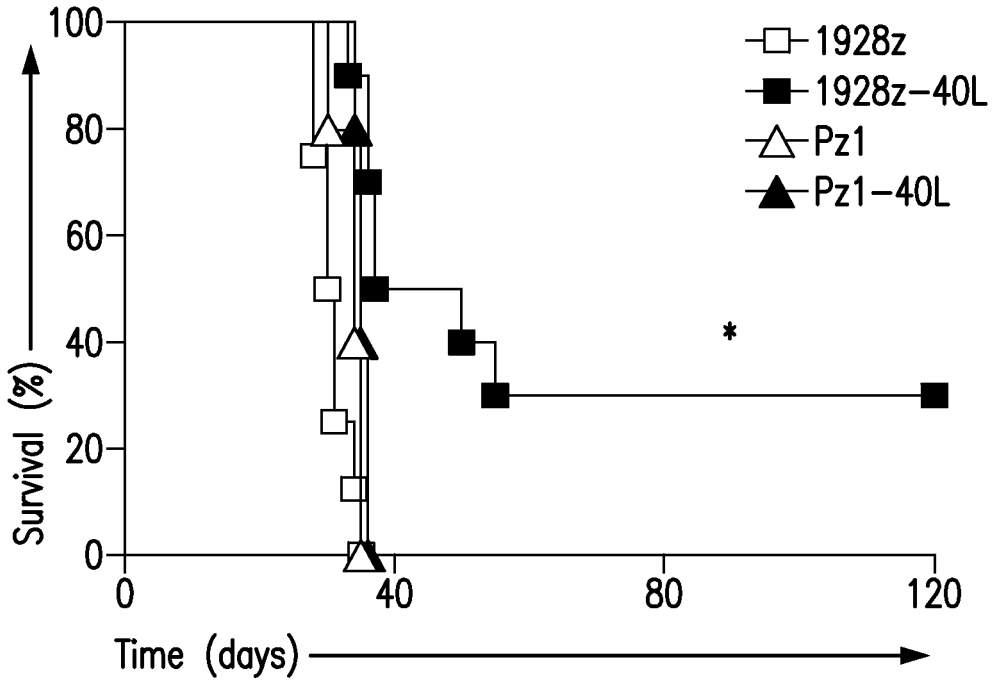

100
80
60
40
20
0
Survival (%)
0
40
80
120
Time (days)
1928z
1928z-40L
Pz1
Pz1-40L
*

FIG. 27

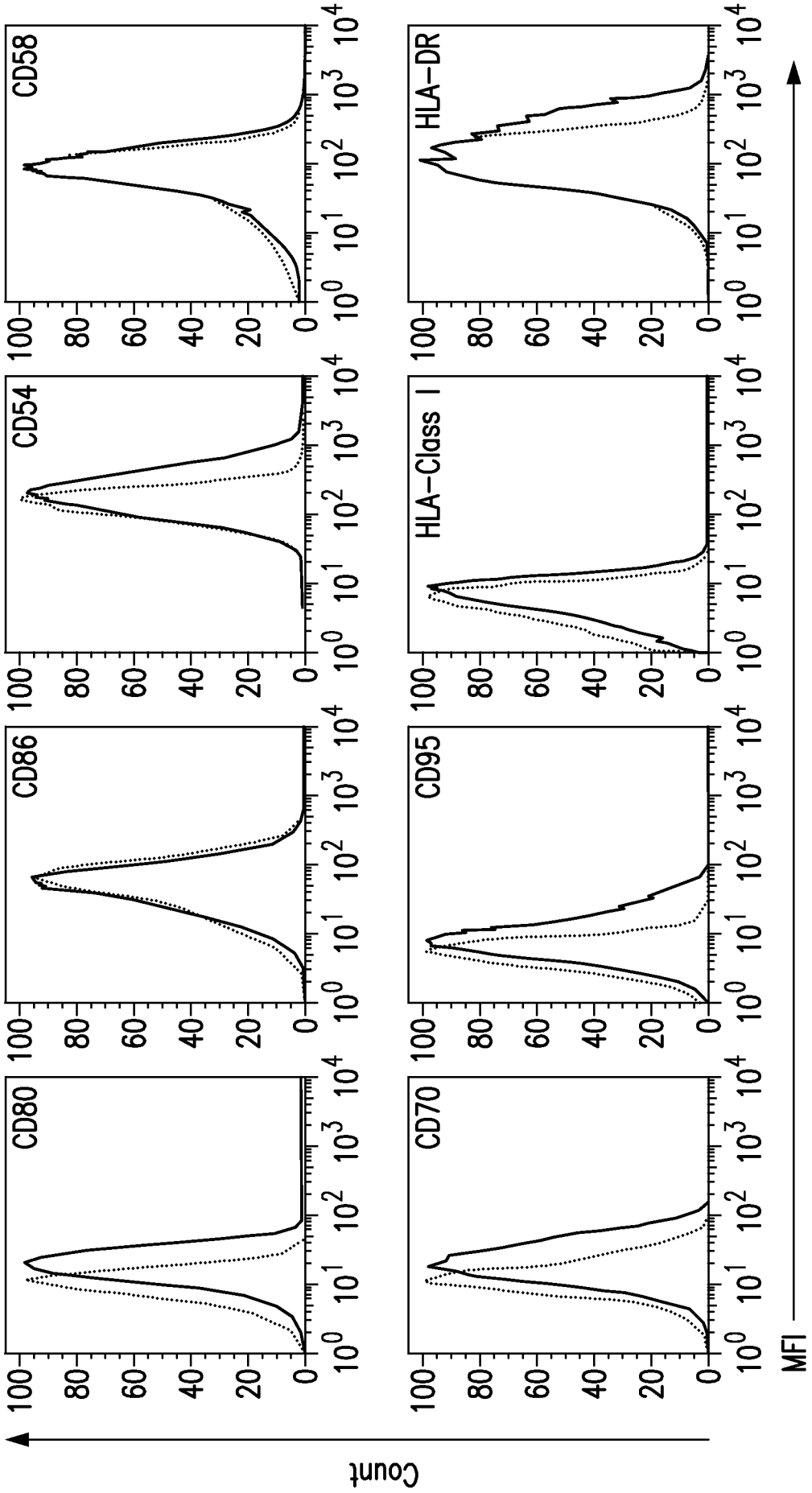

CD58
CD54
CD86
CD80
HLA-DR
HLA-Class I
CD95
CD70
Count
MFI
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4

FIG. 28

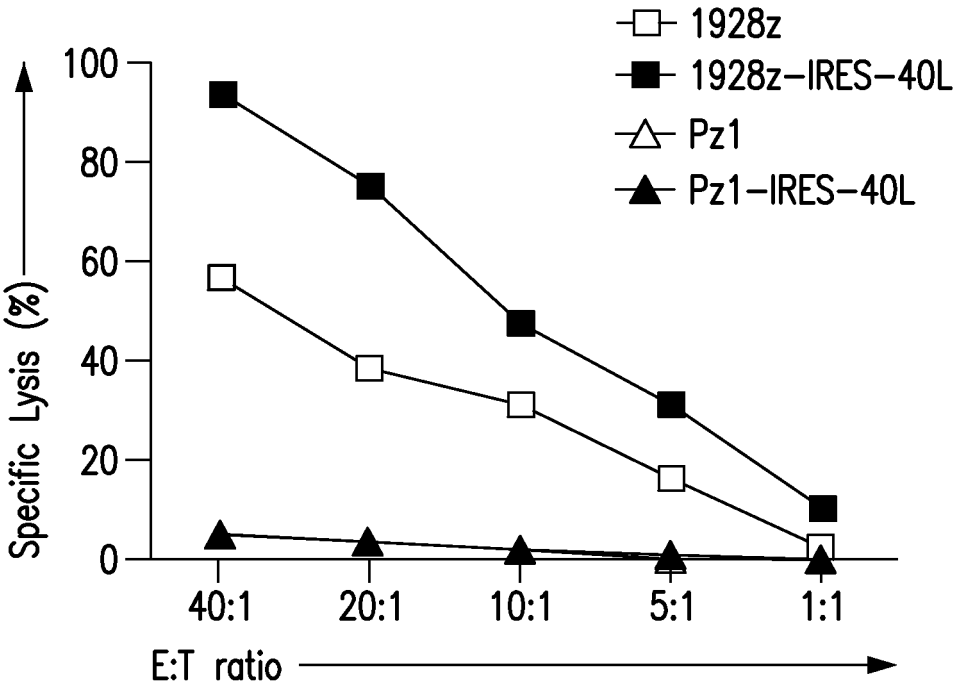

1928z
1928z-IRES-40L
Pz1
Pz1-IRES-40L
Specific Lysis (%)
100
80
60
40
20
0
40:1
20:1
10:1
5:1
1:1
E:T ratio

FIG. 29