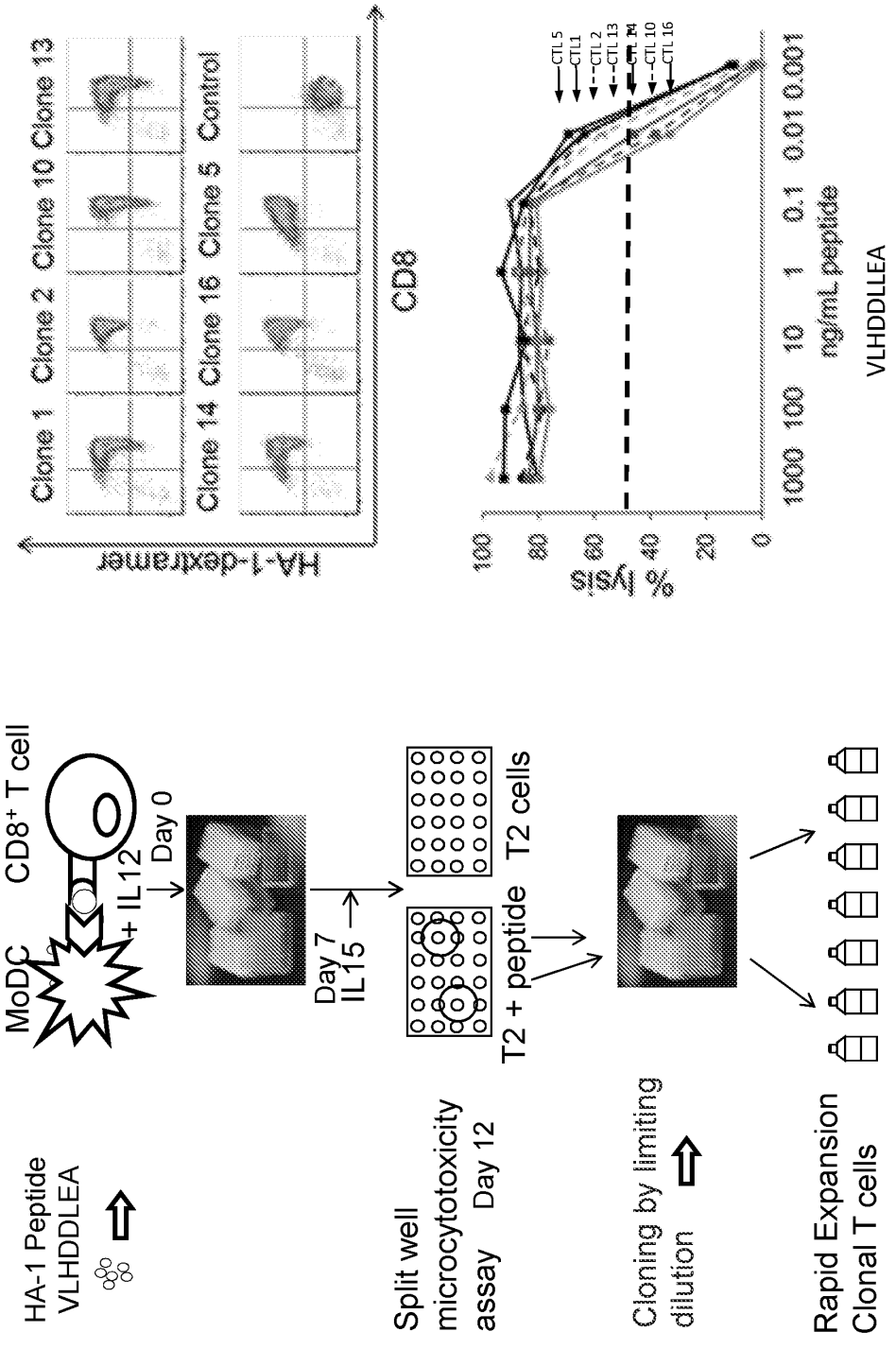

HA-1 Peptide
VLHDDLEA
MoDC
CD8+ T cell
+ IL12
Day 0
Day 7
IL15
Split well
microcytotoxicity
assay Day 12
T2 + peptide
T2 cells
Cloning by limiting
dilution
Rapid Expansion
Clonal T cells
HA-1-dextramer
Clone 1
Clone 2
Clone 10
Clone 13
Clone 14
Clone 16
Clone 5
Control
CD8
% lysis
100
80
60
40
20
0
CTL 5
CTL1
CTL 2
CTL 13
CTL 10
CTL 16
1000 100 10 1 0.1 0.01 0.001
ng/mL peptide
VLHDDLLEA

FIG. 1

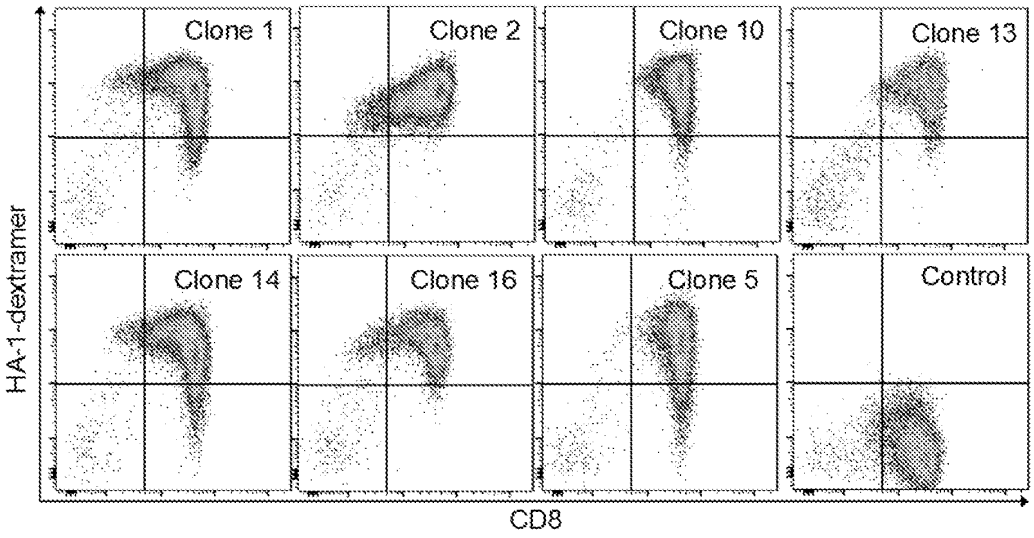


FIG. 2A

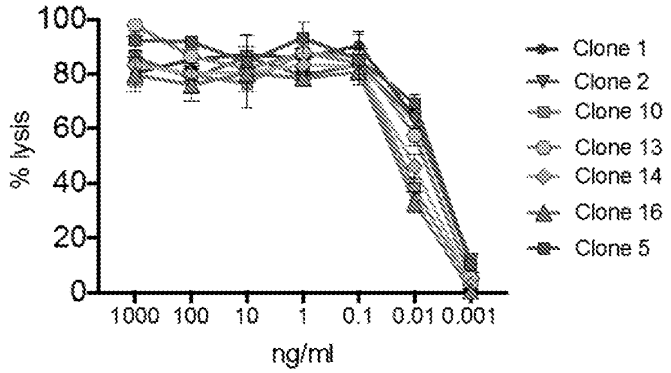


FIG. 2B

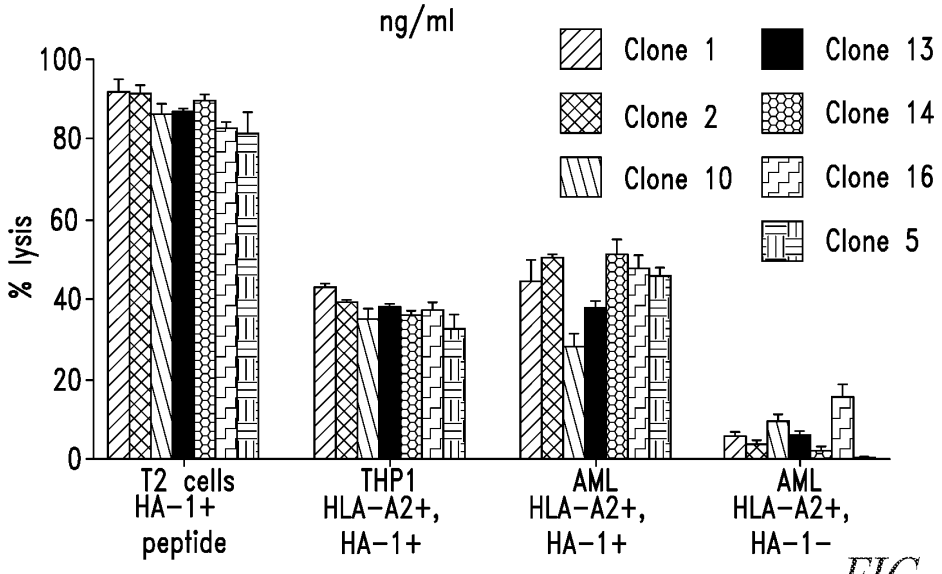


FIG. 2C

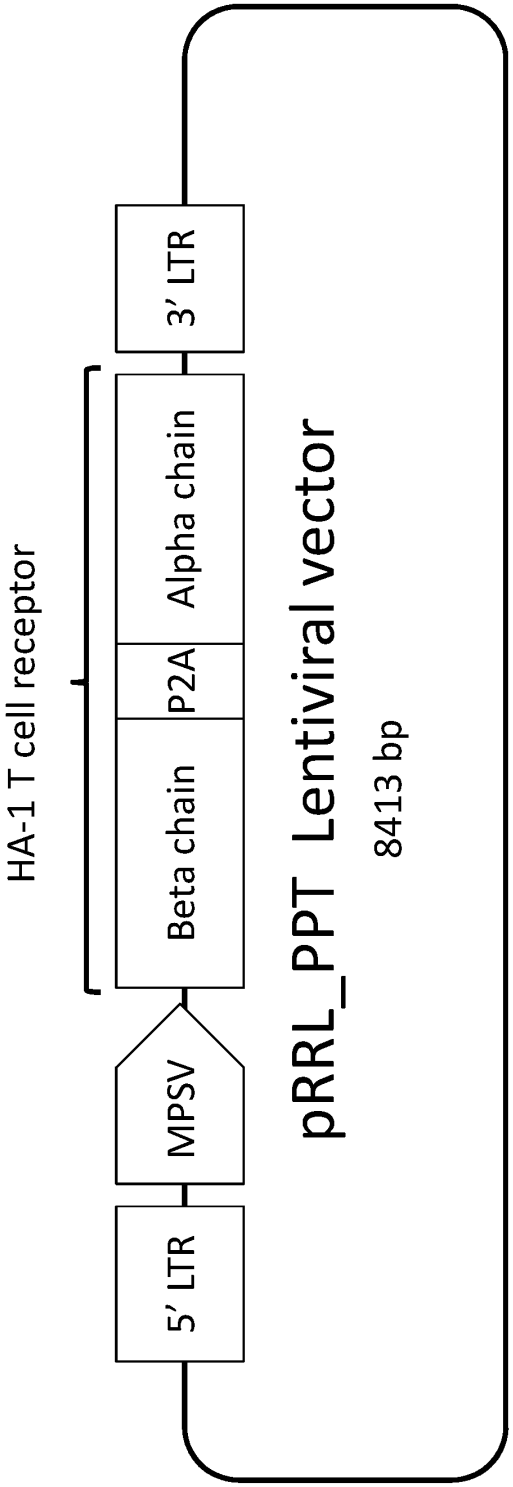

pRRL_PPT Lentiviral vector
8413 bp
5' LTR
MPSV
Beta chain
P2A
Alpha chain
3' LTR
HA-1 T cell receptor

FIG. 3

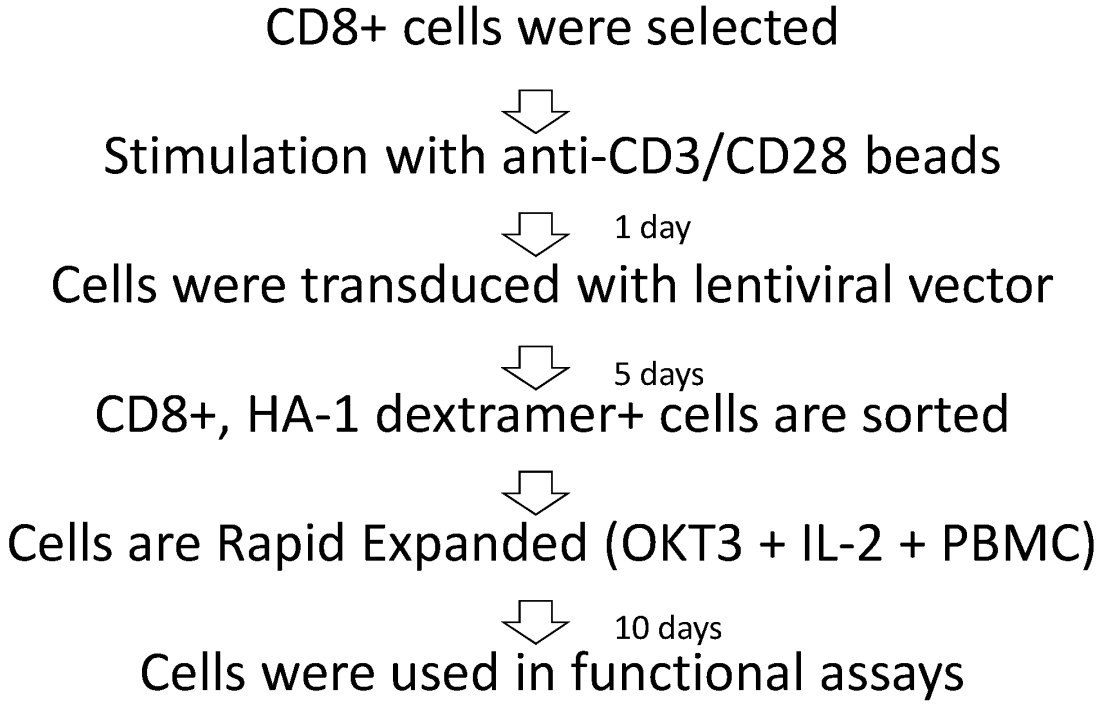

CD8+ cells were selected
Stimulation with anti-CD3/CD28 beads
1 day
Cells were transduced with lentiviral vector
5 days
CD8+, HA-1 dextramer+ cells are sorted
Cells are Rapid Expanded (OKT3 + IL-2 + PBMC)
10 days
Cells were used in functional assays

FIG. 4

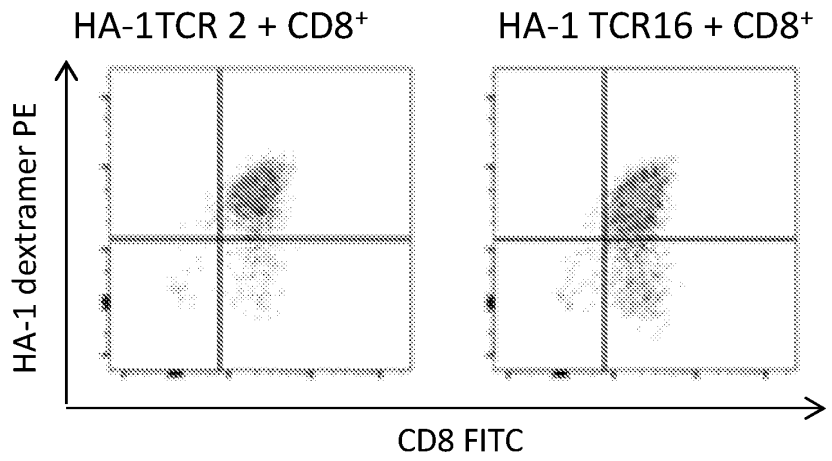

HA-1TCR 2 + CD8+
HA-1 TCR16 + CD8+
HA-1 dextramer PE
CD8 FITC

FIG. 5A

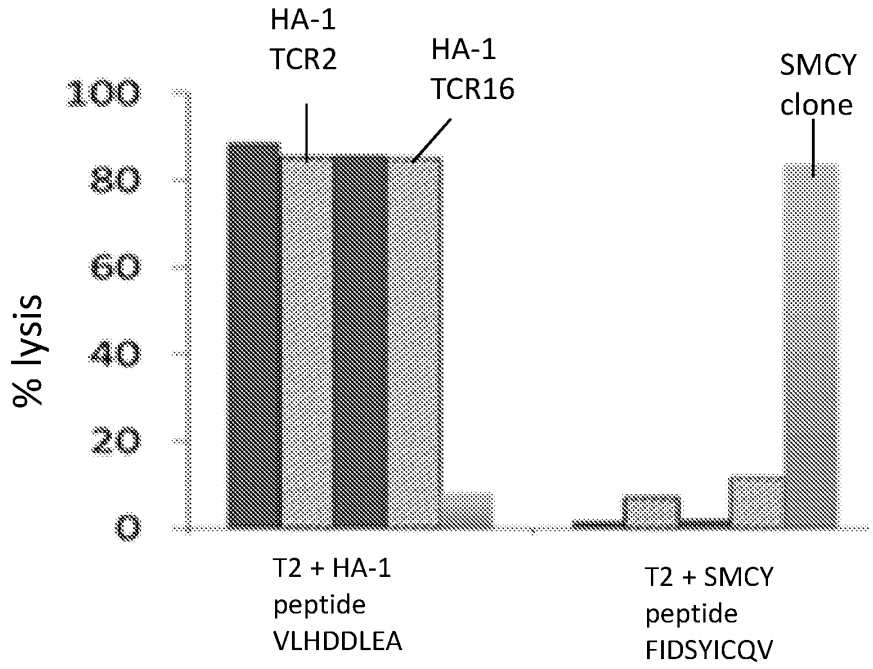

HA-1
TCR2
HA-1
TCR16
SMCY
clone
100
80
60
40
20
0
% lysis
T2 + HA-1
peptide
VLHDDLEA
T2 + SMCY
peptide
FIDSYICQV

FIG. 5B

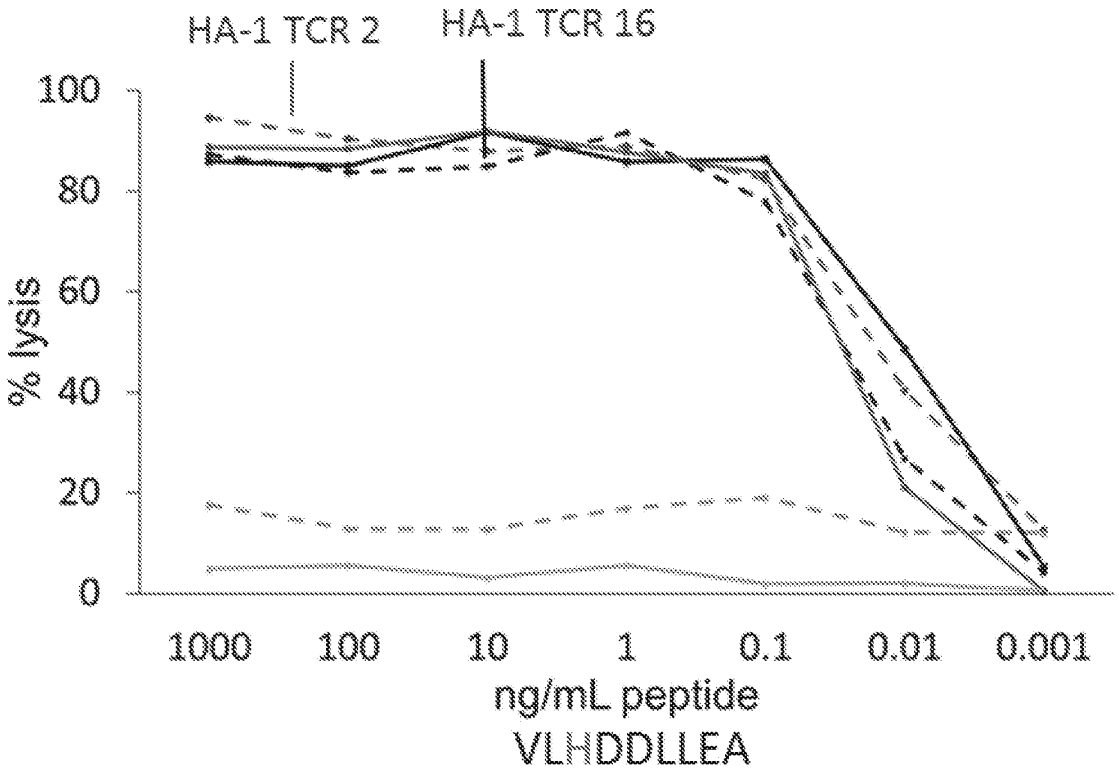

HA-1 TCR 2
HA-1 TCR 16
100
80
60
40
20
0
% lysis
1000
100
10
1
0.1
0.01
0.001
ng/mL peptide
VLHDDLLEA

FIG. 5C

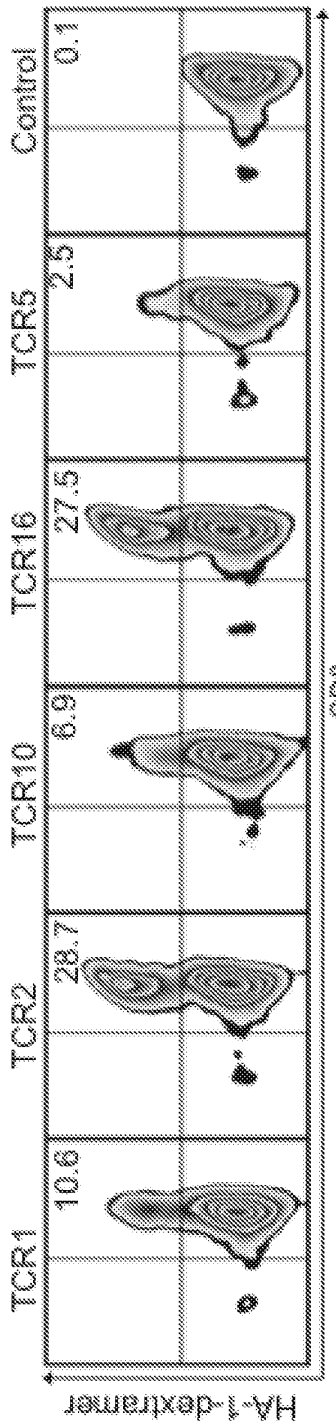


FIG. 6A

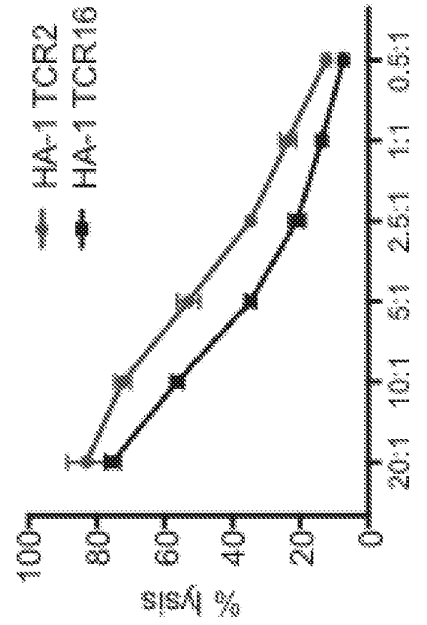


FIG. 6C

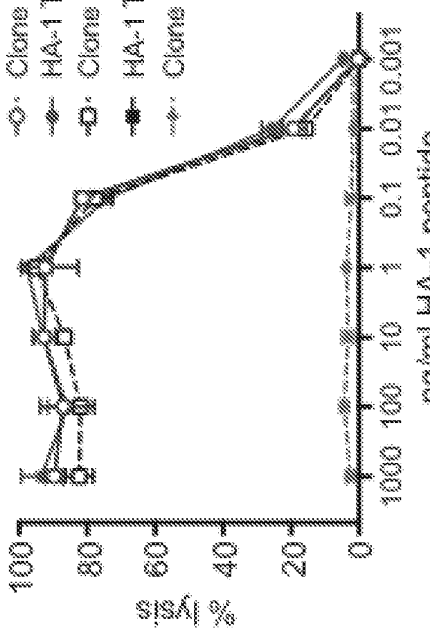


FIG. 6B

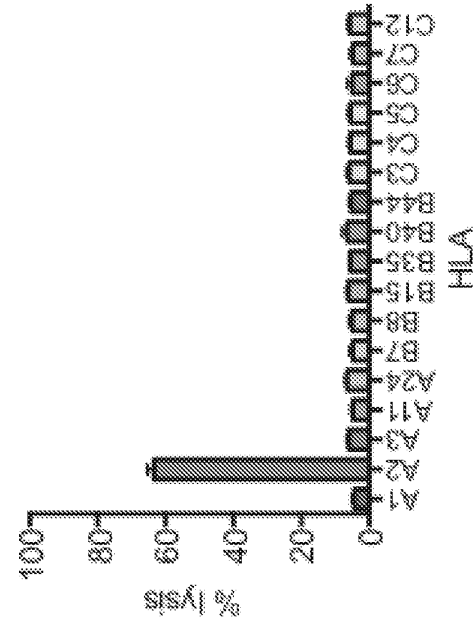

% lysis
100
80
60
40
20
0
A1
A2
A3
A11
A24
B7
B8
B15
B35
B40
B44
Cw3
Cw4
Cw5
Cw7
Cw12
HLA

FIG. 6E

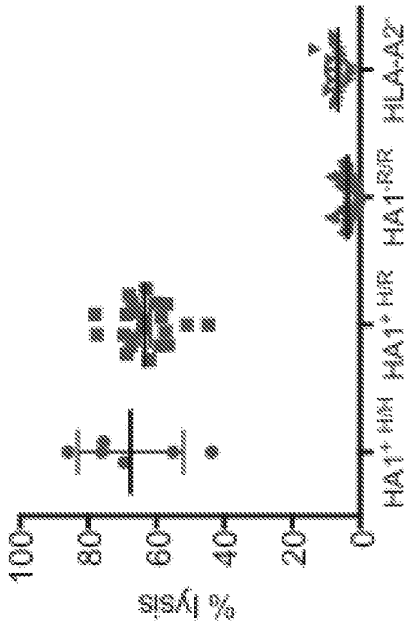

% lysis
100
80
60
40
20
0
HA1+ HIM
HA1+ HIR
HA1+R/R
HLA-A2+

FIG. 6D

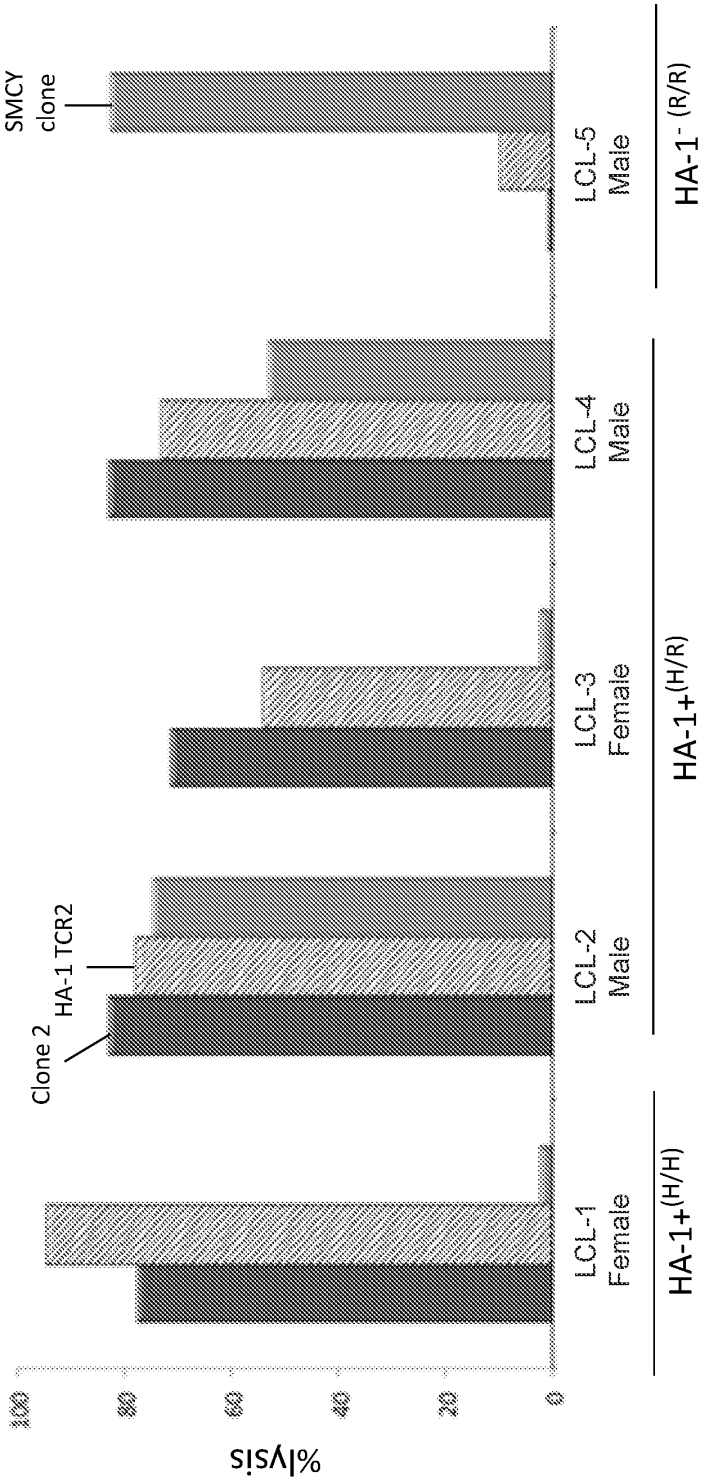

%lysis
100
80
60
40
20
0
LCL-1 Female
LCL-2 Male
LCL-3 Female
LCL-4 Male
LCL-5 Male
HA-1+(H/H)
HA-1+(H/R)
HA-1- (R/R)
Clone 2
HA-1 TCR2
SMCY clone

FIG. 7

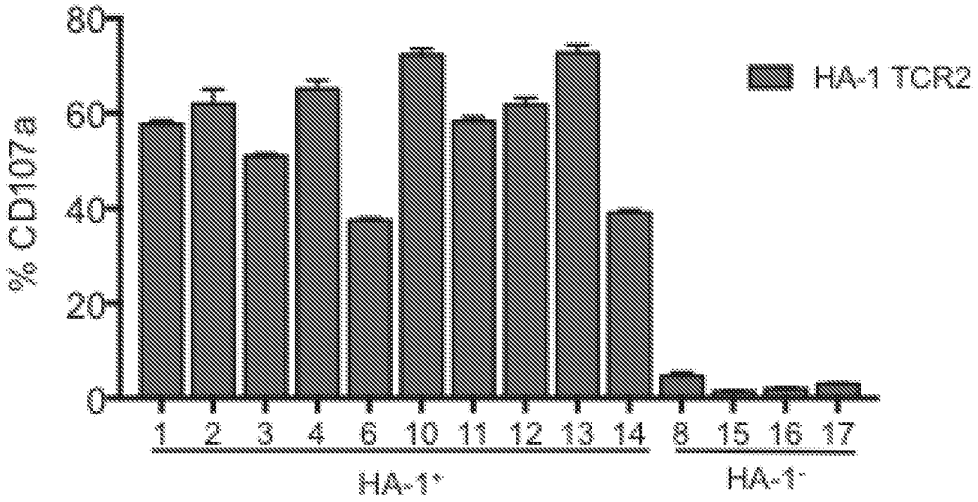


FIG. 8A

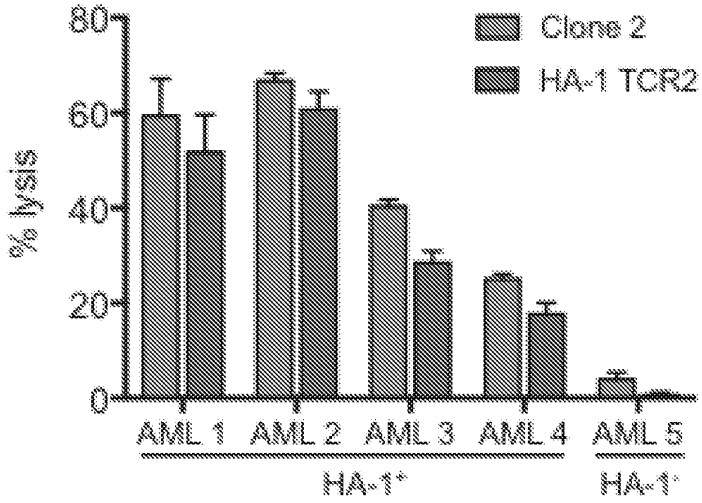


FIG. 8B

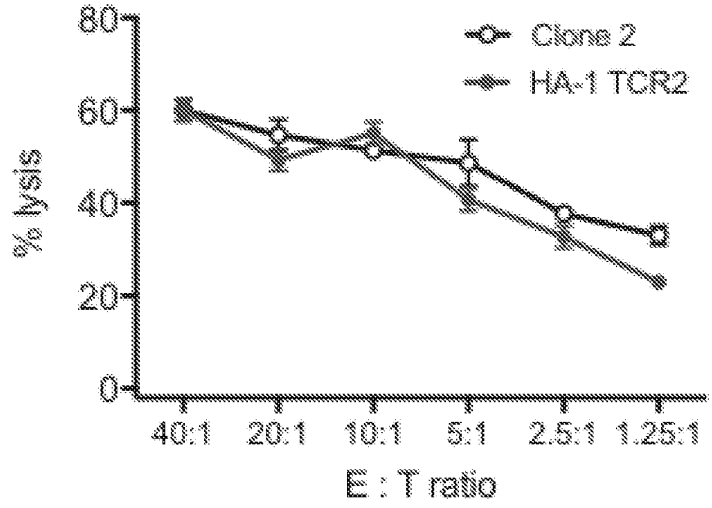


FIG. 8C

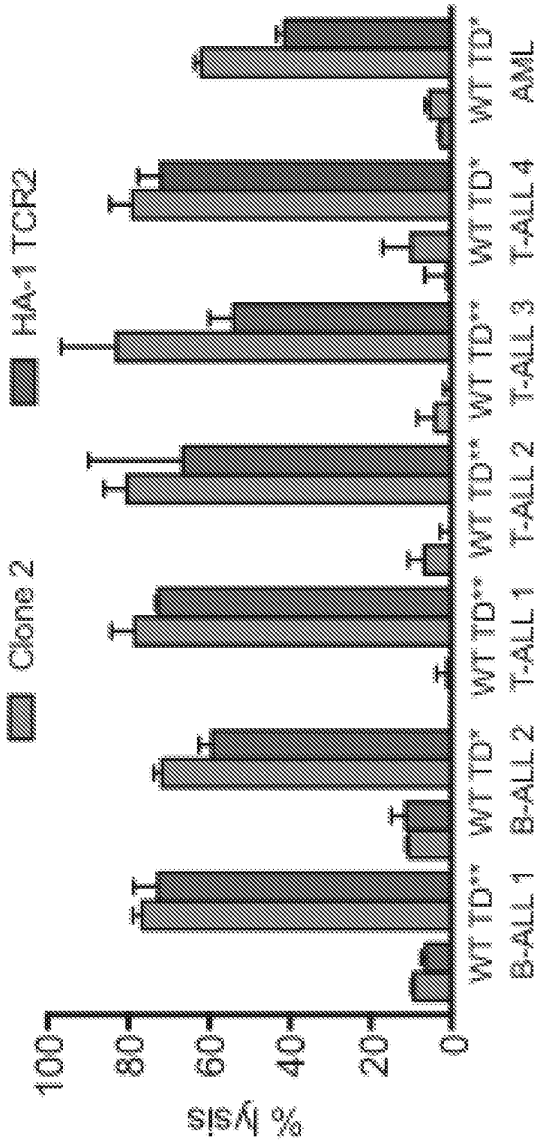

% lysis
100
80
60
40
20
0
Clone 2
HA-1 TCR2
B-ALL 1
WT TD''
B-ALL 2
WT TD'
T-ALL 1
WT TD''
T-ALL 2
WT TD''
T-ALL 3
WT TD''
T-ALL 4
WT TD'
AML
WT TD'

FIG. 8D

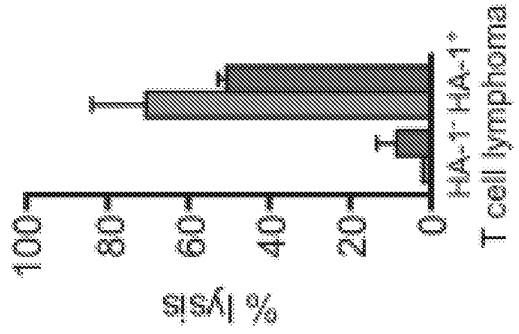
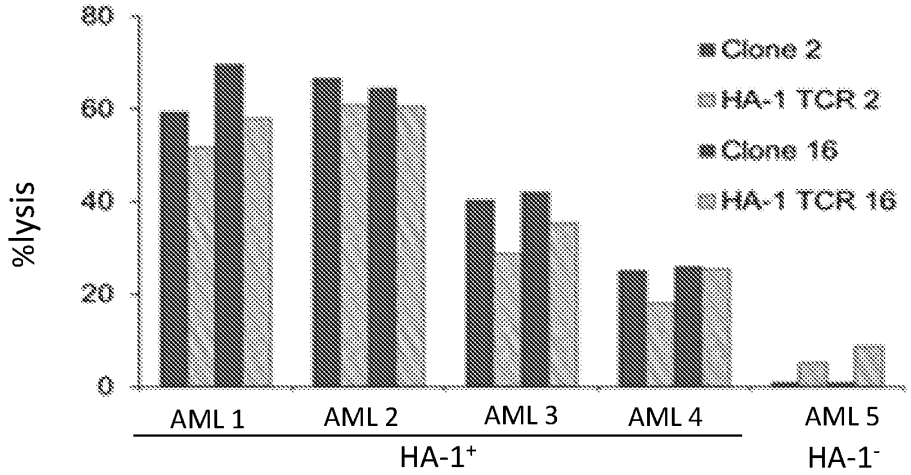

% lysis
100
80
60
40
20
0
HA-1+ HA-1+
T cell lymphoma

FIG. 8E



4hr CRA

FIG. 9A

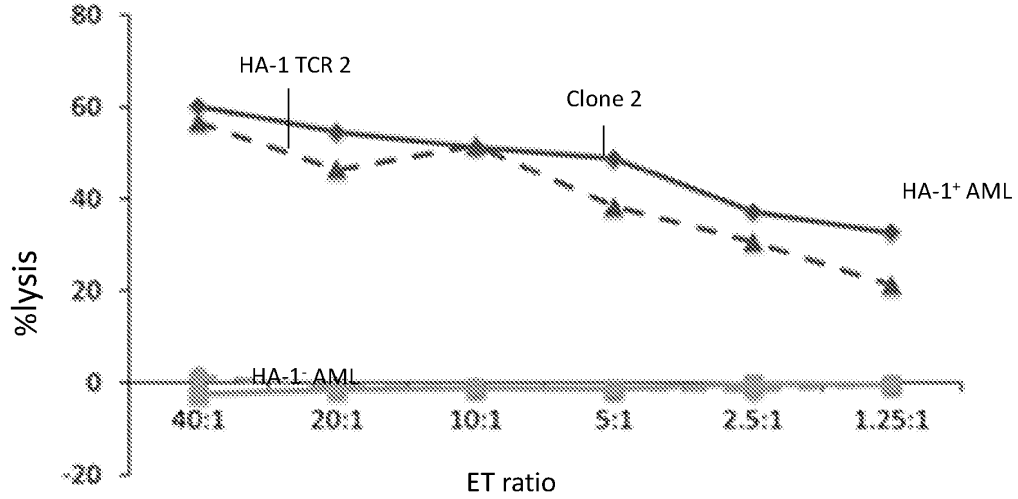


FIG. 9B

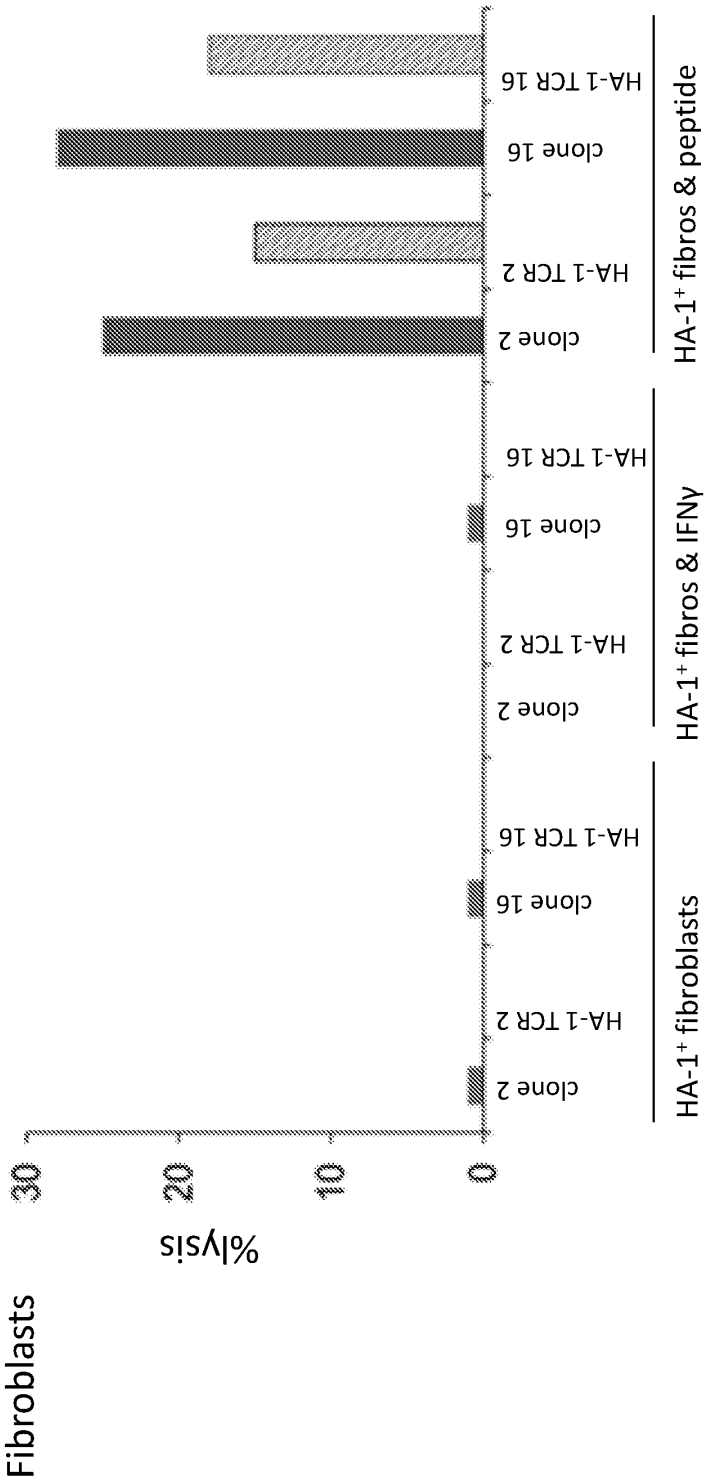

Fibroblasts
%lysis
0
10
20
30
HA-1+ fibroblasts
clone 2
HA-1 TCR 2
clone 16
HA-1 TCR 16
HA-1+ fibros & IFNγ
clone 2
HA-1 TCR 2
clone 16
HA-1 TCR 16
HA-1+ fibros & peptide
clone 2
HA-1 TCR 2
clone 16
HA-1 TCR 16

FIG. 10

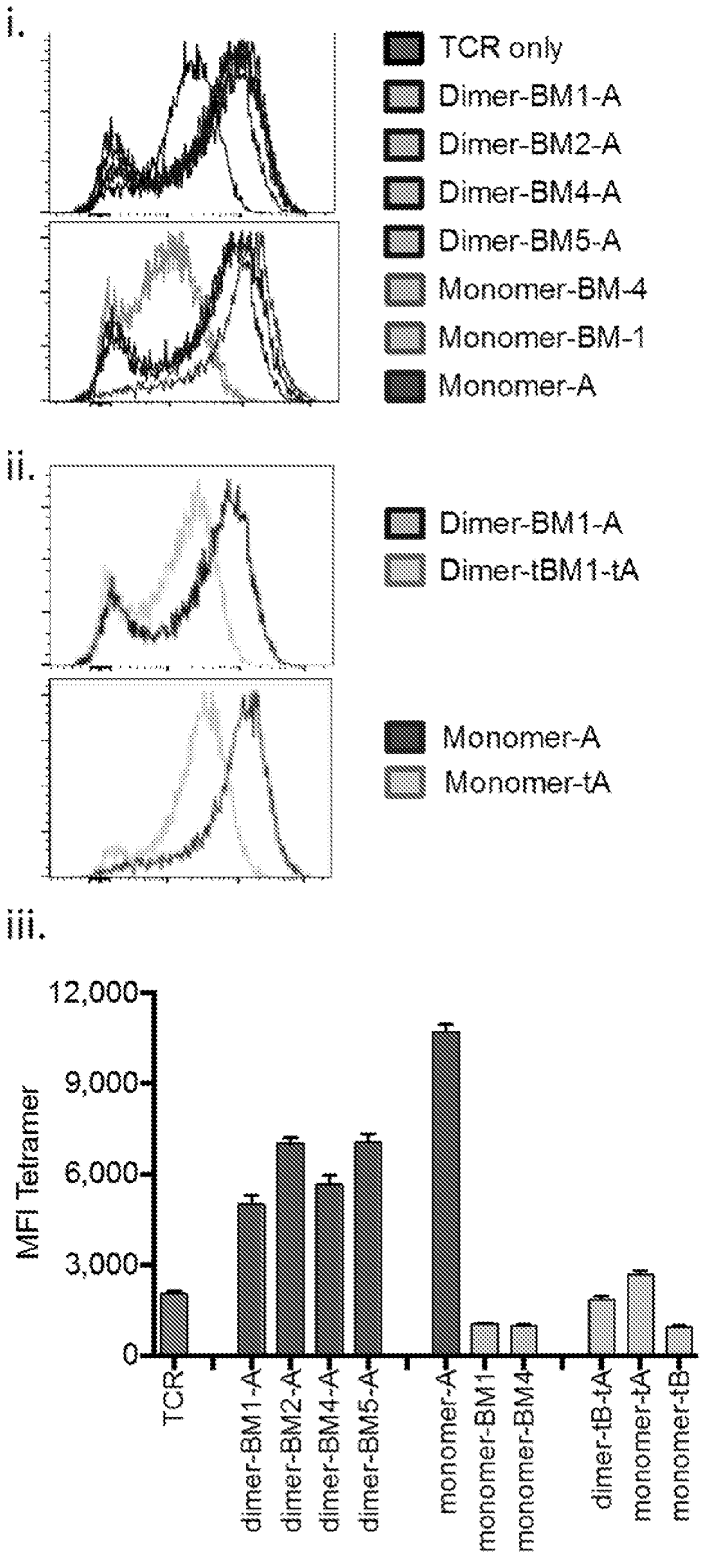

i.
TCR only
Dimer-BM1-A
Dimer-BM2-A
Dimer-BM4-A
Dimer-BM5-A
Monomer-BM-4
Monomer-BM-1
Monomer-A
ii.
Dimer-BM1-A
Dimer-tBM1-tA
Monomer-A
Monomer-tA
iii.
MFI Tetramer
12,000
9,000
6,000
3,000
0
TCR
dimer-BM1-A
dimer-BM2-A
dimer-BM4-A
dimer-BM5-A
monomer-A
monomer-BM1
monomer-BM4
dimer-tB-tA
monomer-tA
monomer-tB

FIG. 11A

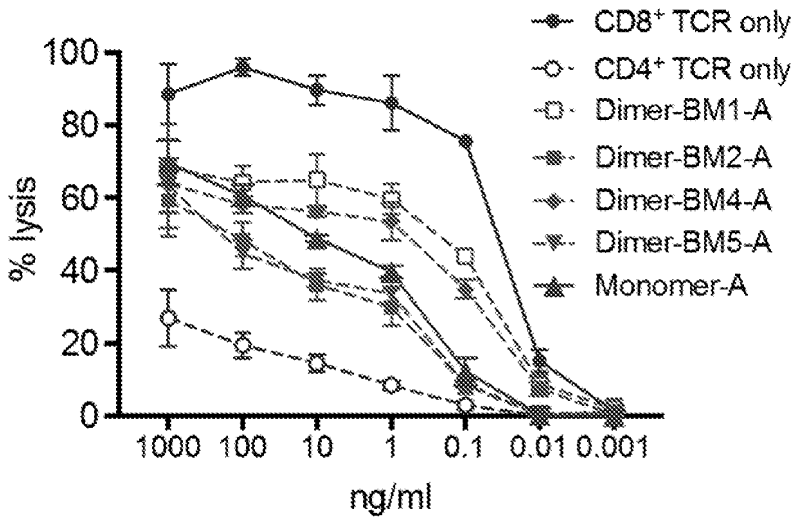


FIG. 11B

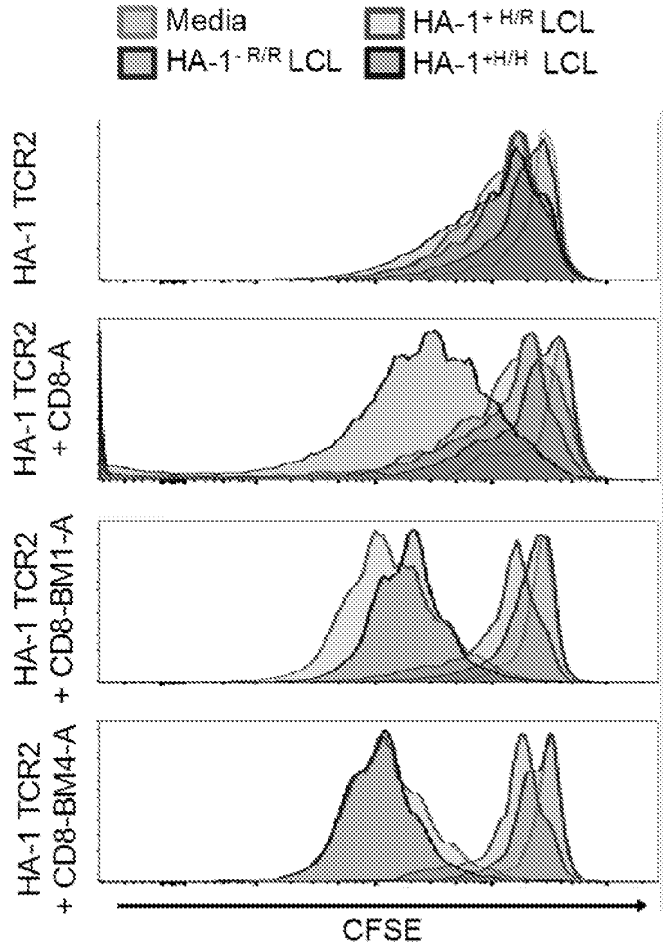


FIG. 11C

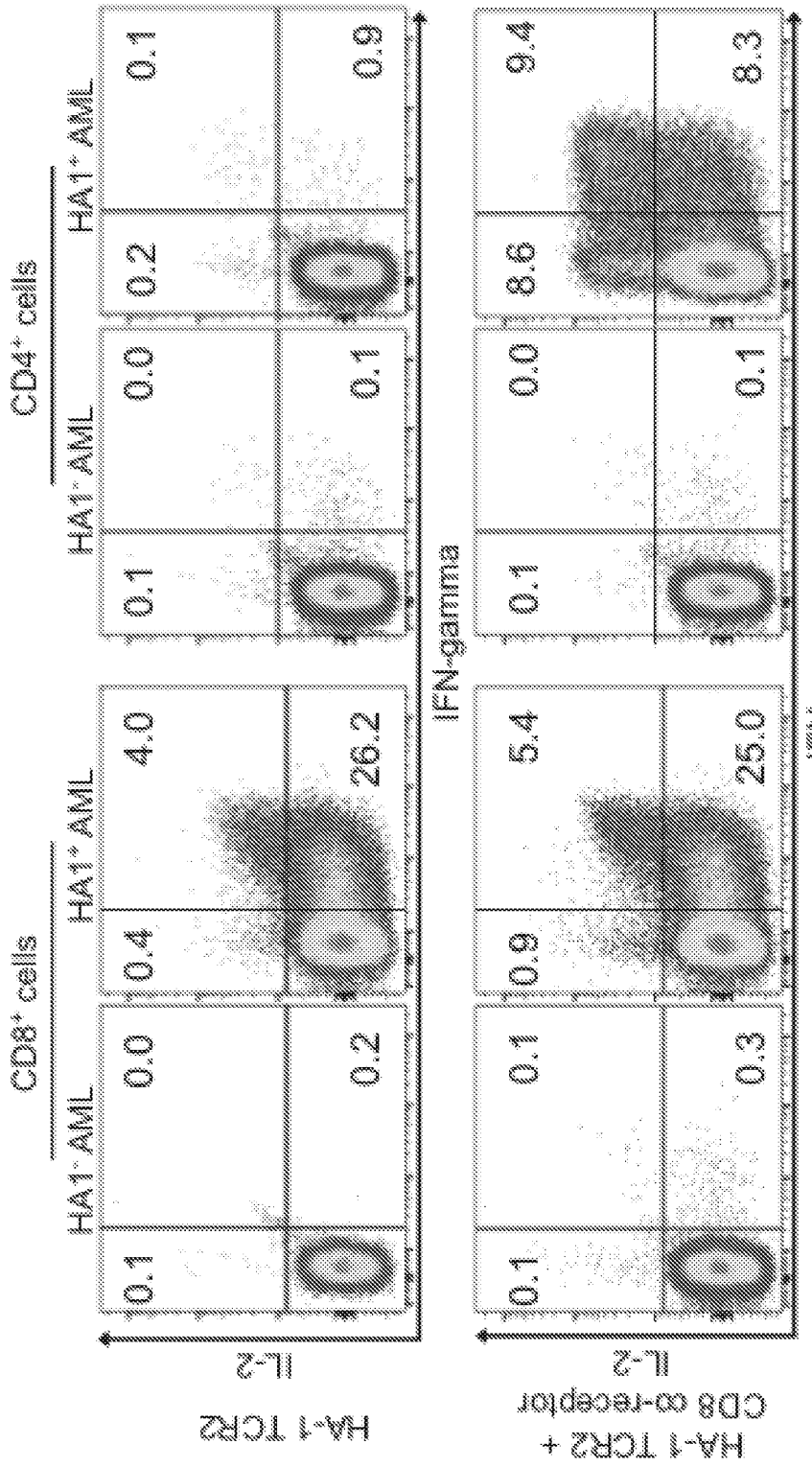

CD8+ cells
HA1- AML
HA1+ AML
0.1
0.0
0.4
4.0
0.2
26.2
CD4+ cells
HA1- AML
HA1+ AML
0.1
0.0
0.2
0.1
0.1
0.9
IL-2
IFN-gamma
HA-1 TCR2
HA-1 TCR2 + CD8 co-receptor
0.1
0.1
0.9
5.4
0.3
25.0
0.1
0.0
8.6
9.4
0.1
0.1
8.3

FIG. 12A

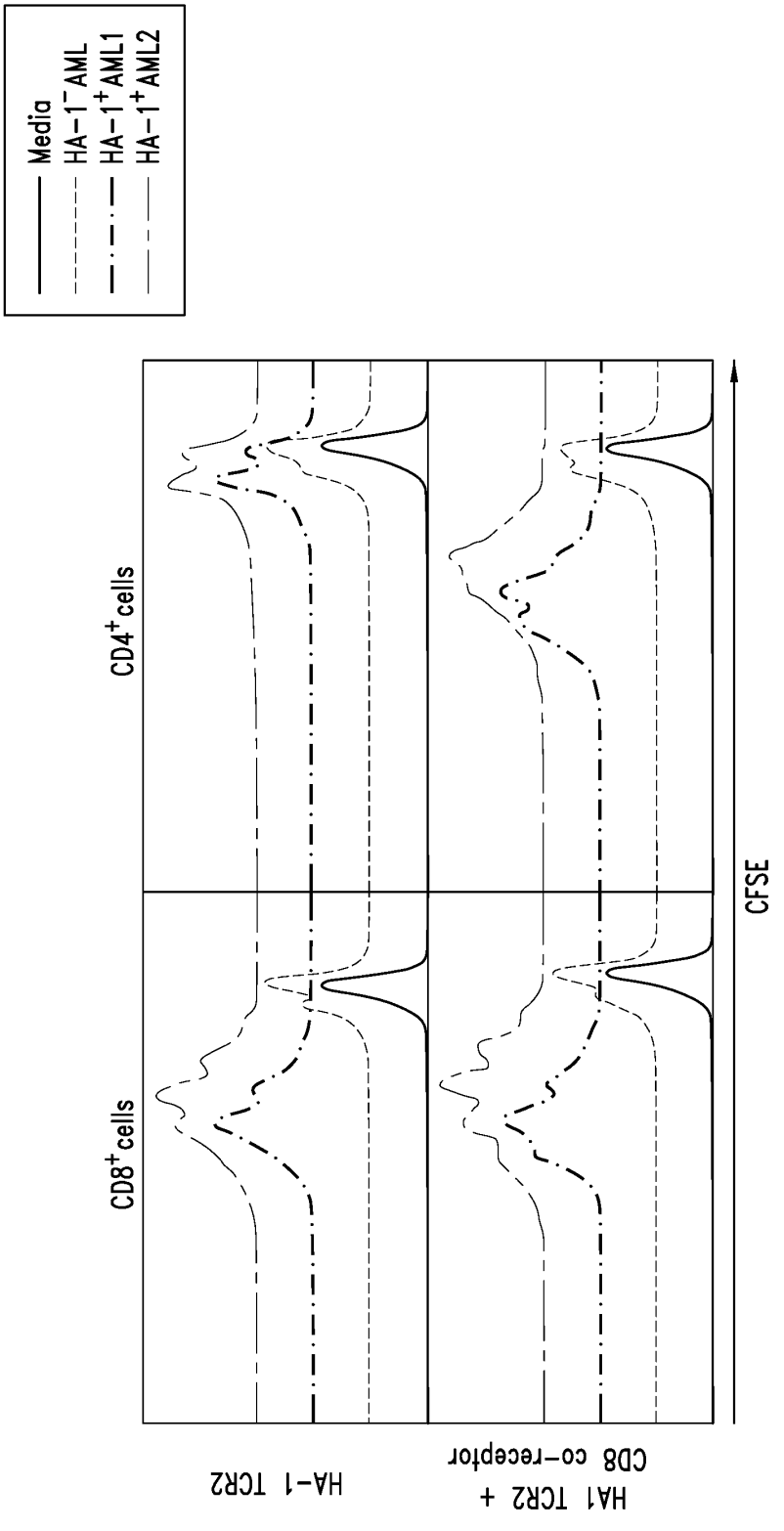

Media
HA-1⁻AML
HA-1⁺AML1
HA-1⁺AML2
CD4⁺cells
CD8⁺cells
HA-1 TCR2
HA1 TCR2 +
CD8 co-receptor
CFSE

FIG. 12B

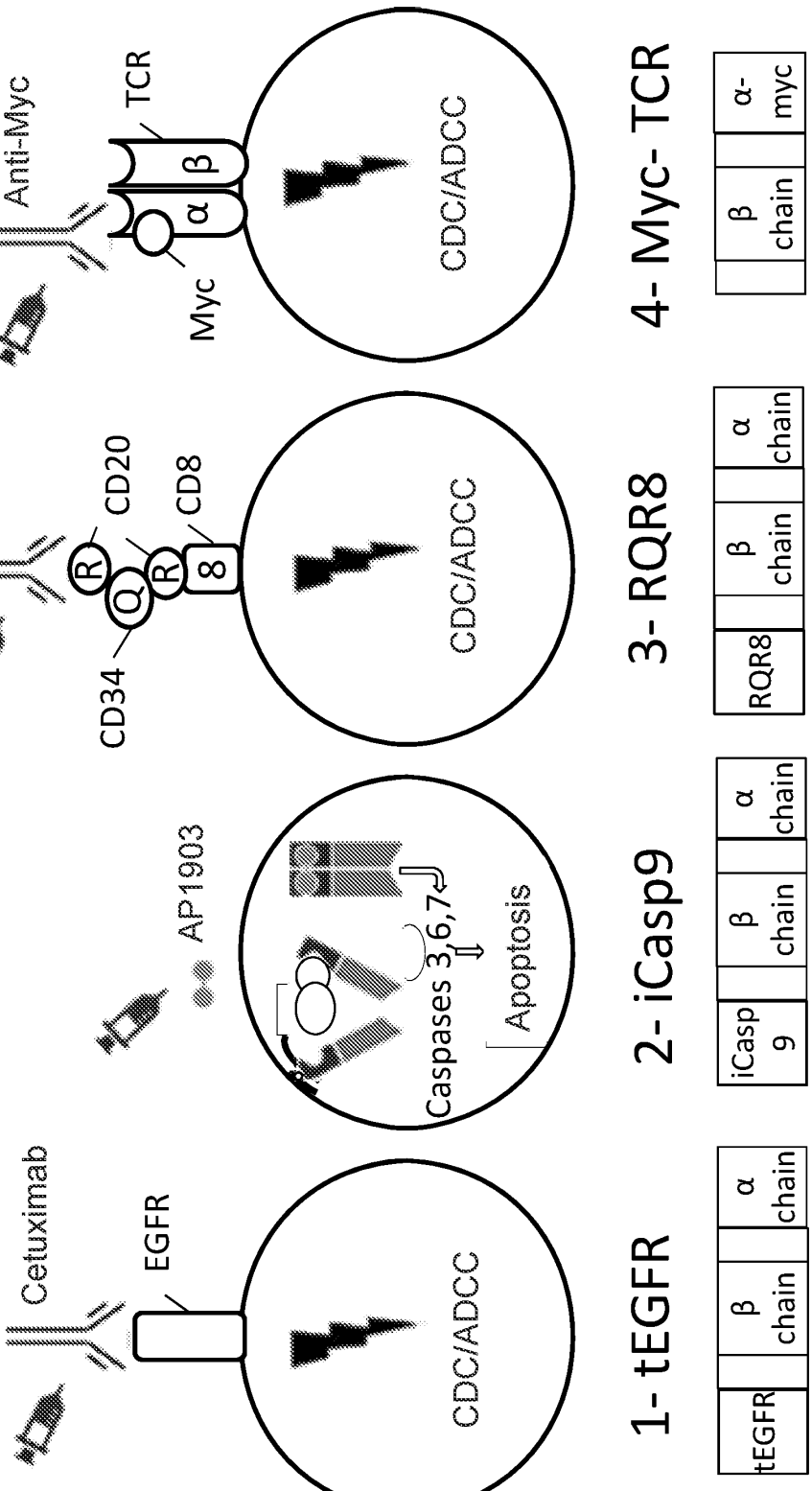

Cetuximab
EGFR
CDC/ADCC
AP1903
Caspases 3,6,7
Apoptosis
Rituximab
CD34
CD20
CD8
CDC/ADCC
Anti-Myc
Myc
TCR
α
β
CDC/ADCC
1- tEGFR
tEGFR
β chain
α chain
2- iCasp9
iCasp 9
β chain
α chain
3- RQR8
RQR8
β chain
α chain
4- Myc- TCR
β chain
α-myc

FIG. 13

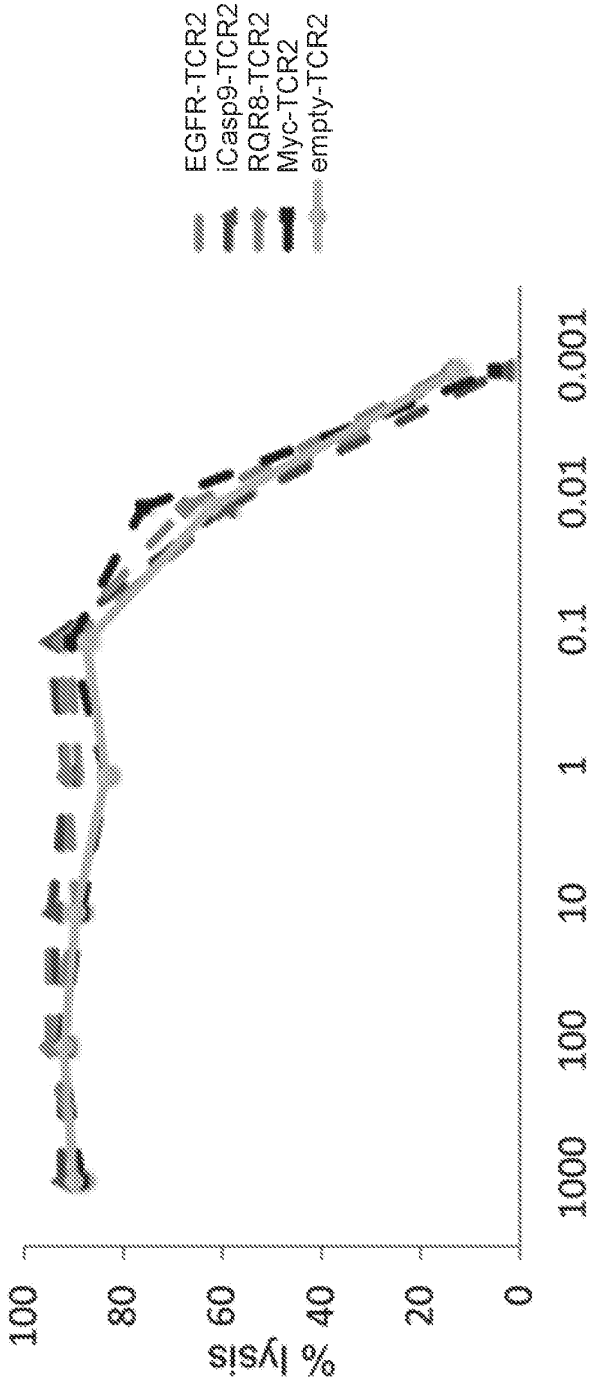

EGFR-TCR2
iCasp9-TCR2
RQR8-TCR2
Myc-TCR2
empty-TCR2
% lysis
100
80
60
40
20
0
1000
100
10
1
0.1
0.01
0.001
ng/mL peptide

FIG. 14

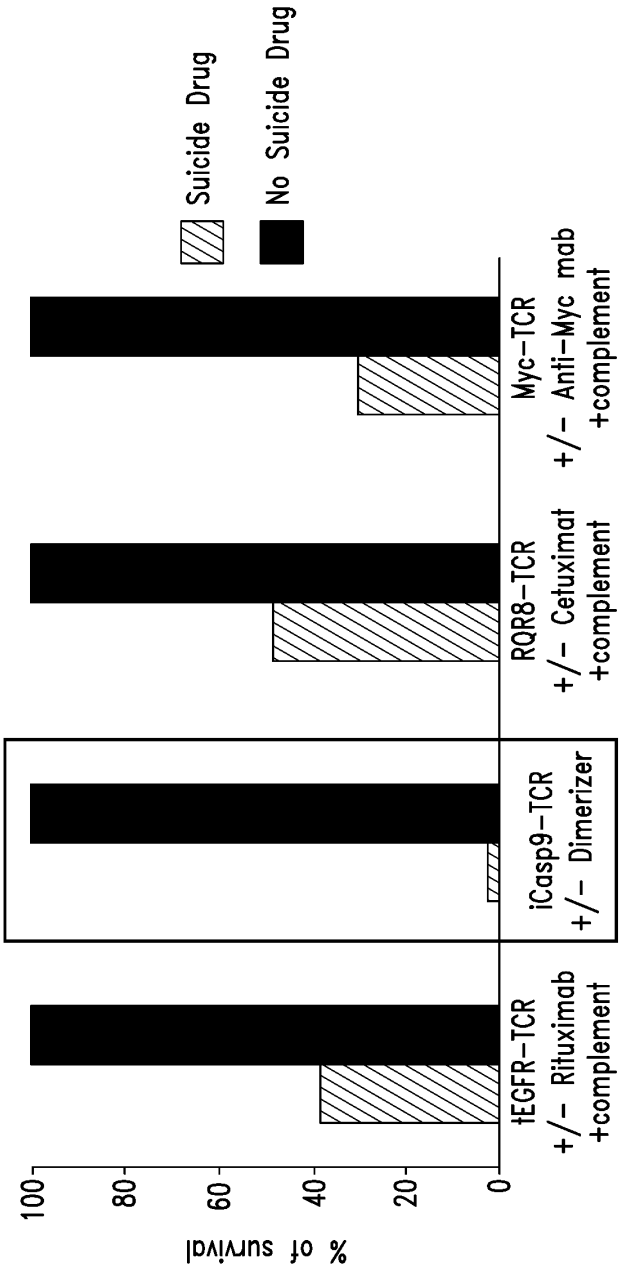

Suicide Drug
No Suicide Drug
% of survival
100
80
60
40
20
0
tEGFR-TCR
+/- Rituximab
+complement
iCasp9-TCR
+/- Dimerizer
RQR8-TCR
+/- Cetuximat
+complement
Myc-TCR
+/- Anti-Myc mab
+complement

FIG. 15

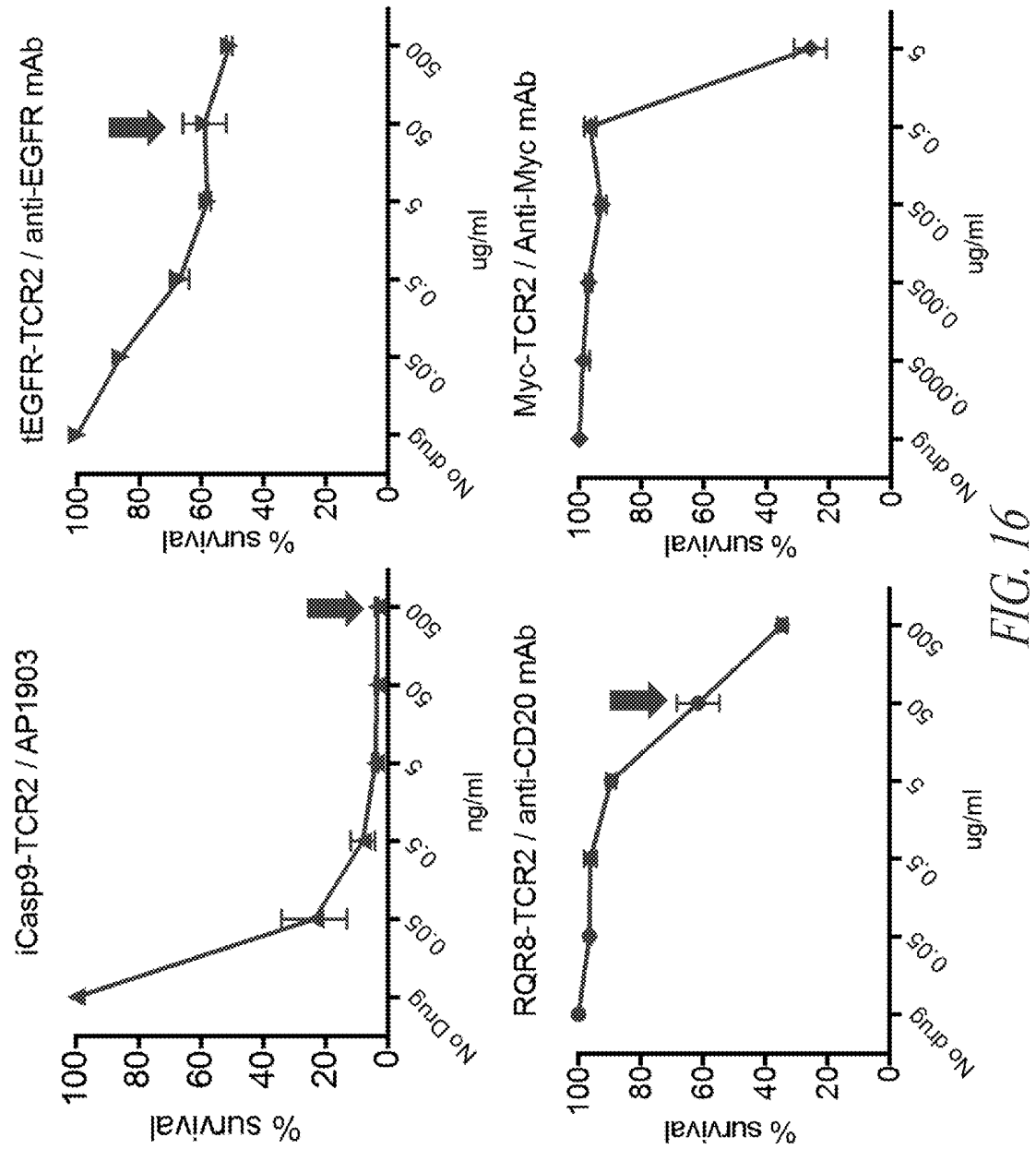

iCasp9-TCR2 / AP1903
% survival
100
80
60
40
20
0
No Drug
0.05
0.5
5
50
500
ng/ml
tEGFR-TCR2 / anti-EGFR mAb
% survival
100
80
60
40
20
0
No drug
0.05
0.5
5
50
500
ug/ml
RQR8-TCR2 / anti-CD20 mAb
% survival
100
80
60
40
20
0
No drug
0.05
0.5
5
50
500
ug/ml
Myc-TCR2 / Anti-Myc mAb
% survival
100
80
60
40
20
0
No drug
0.0005
0.005
0.05
0.5
5
ug/ml

FIG. 16

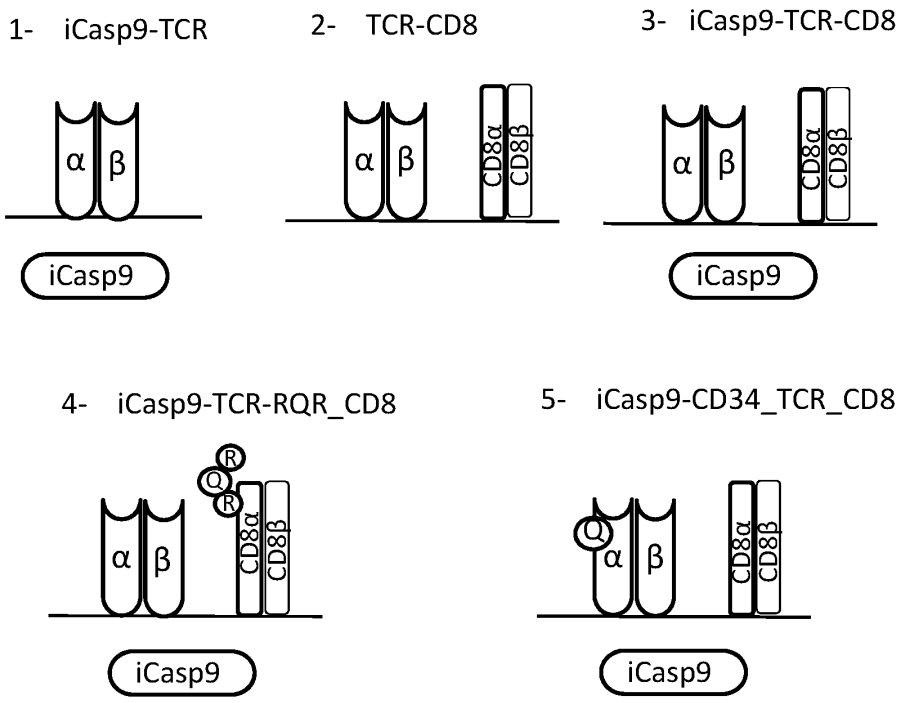

1- iCasp9-TCR
2- TCR-CD8
3- iCasp9-TCR-CD8
α
β
CD8α
CD8β
iCasp9
4- iCasp9-TCR-RQR_CD8
5- iCasp9-CD34_TCR_CD8
R
Q
R
Q

FIG. 17

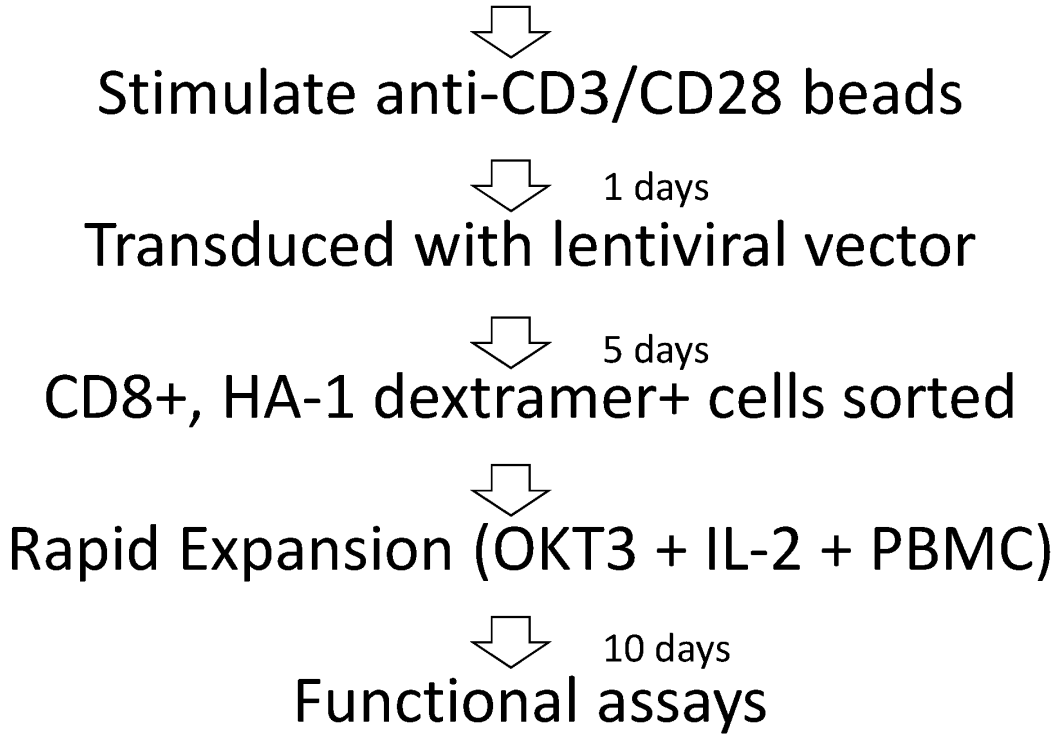

CD8+ cells selected
Stimulate anti-CD3/CD28 beads
1 days
Transduced with lentiviral vector
5 days
CD8+, HA-1 dextramer+ cells sorted
Rapid Expansion (OKT3 + IL-2 + PBMC)
10 days
Functional assays

FIG. 18

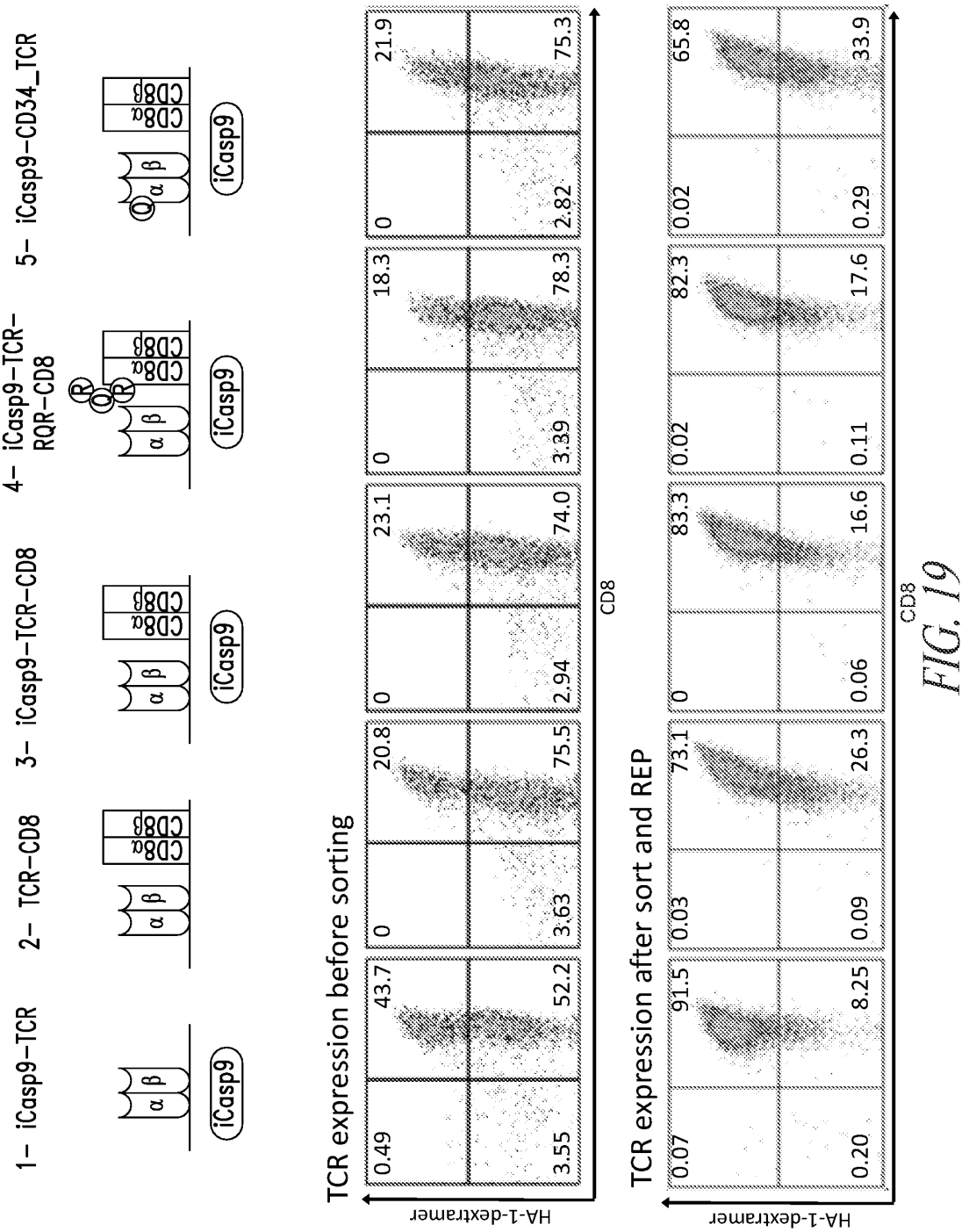

1- iCasp9-TCR
2- TCR-CD8
3- iCasp9-TCR-CD8
4- iCasp9-TCR-RQR-CD8
5- iCasp9-CD34_TCR
iCasp9
α β
CD8α CD8β
TCR expression before sorting
TCR expression after sort and REP
HA-1-dextramer
CD8
0.49 43.7 0 20.8 0 23.1 0 18.3 0 21.9
3.55 52.2 3.63 75.5 2.94 74.0 3.39 78.3 2.82 75.3
0.07 91.5 0.03 73.1 0 83.3 0.02 82.3 0.02 65.8
0.20 8.25 0.09 26.3 0.06 16.6 0.11 17.6 0.29 33.9

FIG. 19

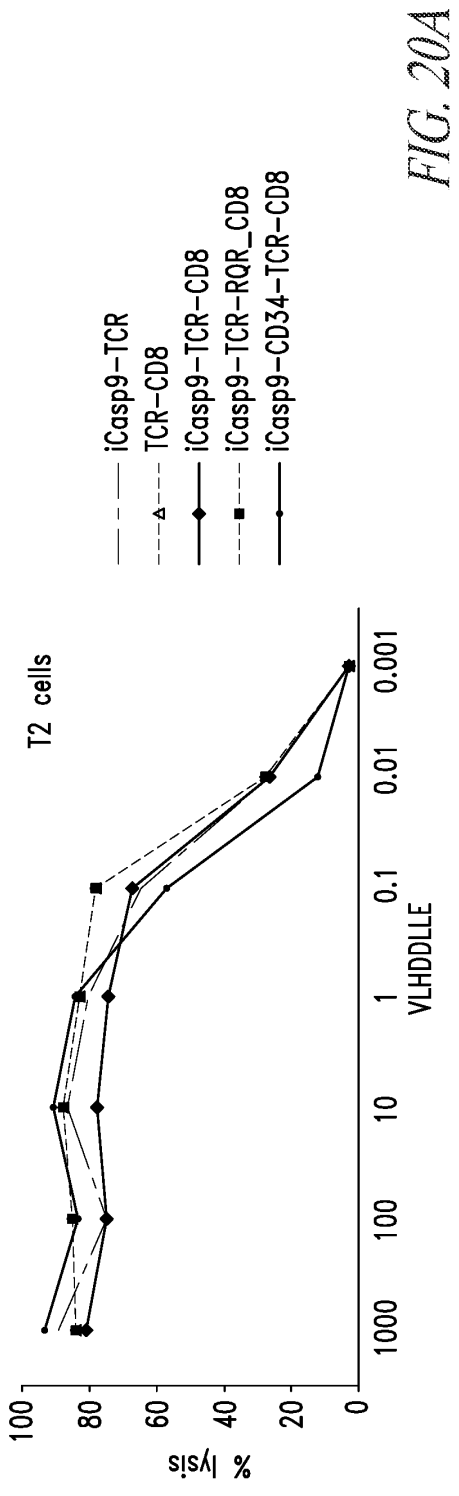


FIG. 20A

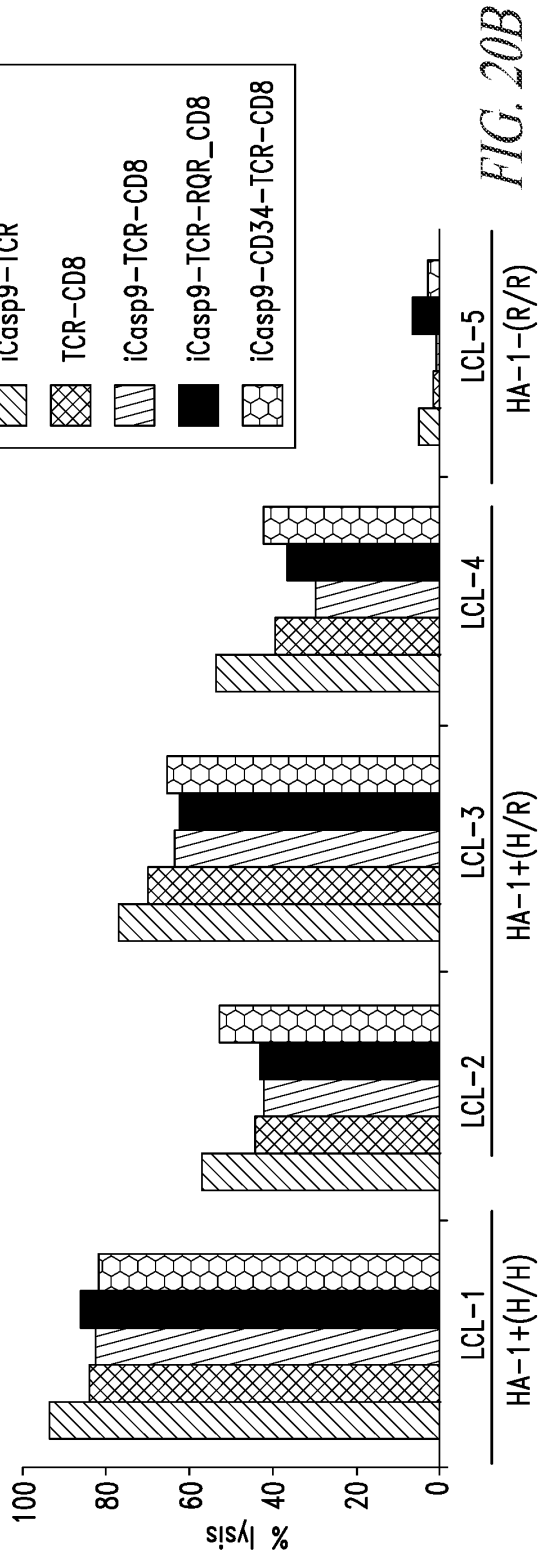


FIG. 20B

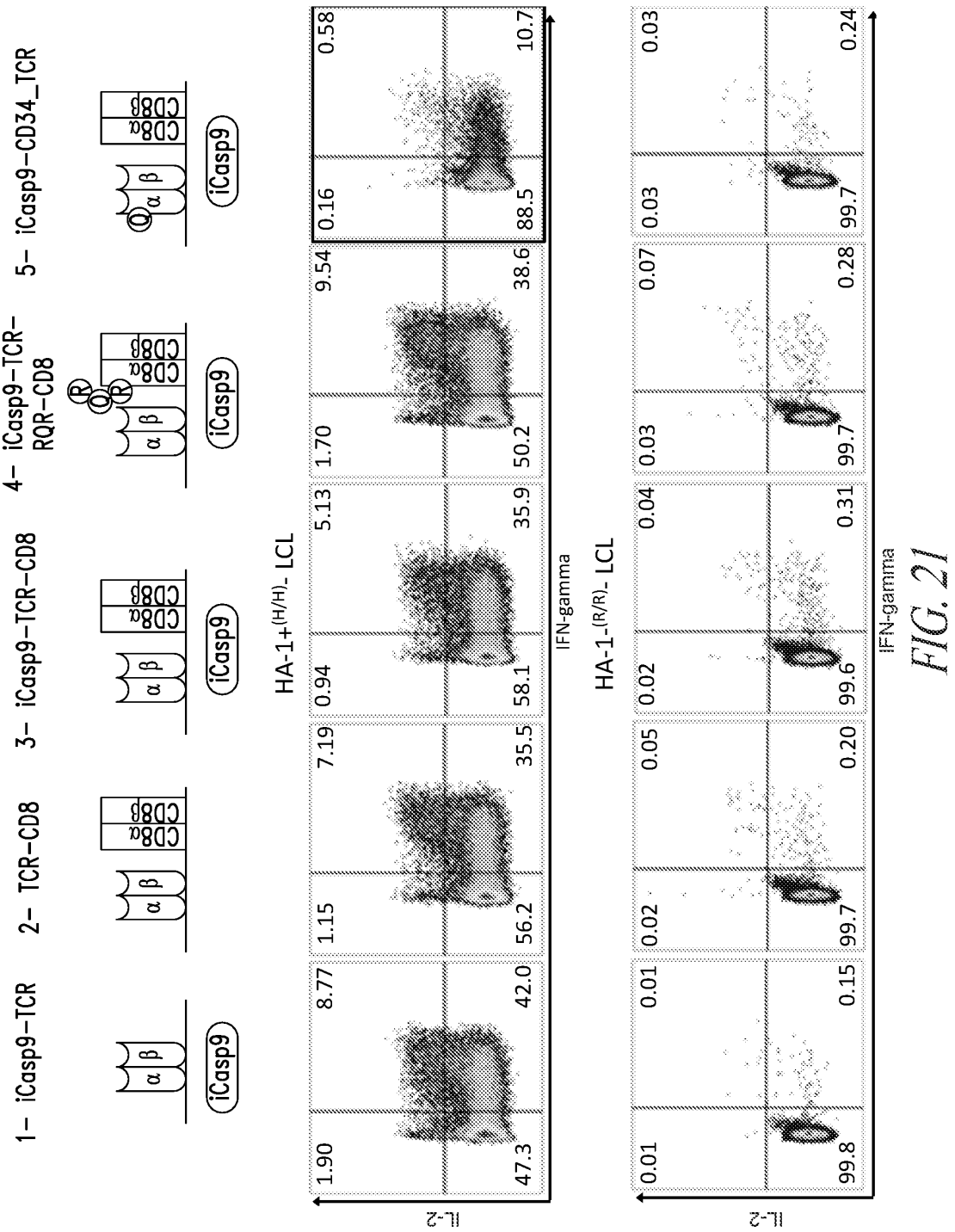

1- iCasp9-TCR
2- TCR-CD8
3- iCasp9-TCR-CD8
4- iCasp9-TCR-RQR-CD8
5- iCasp9-CD34_TCR
iCasp9
α β
CD8α
CD8β
HA-1+(H/H)- LCL
HA-1-(R/R)- LCL
IL-2
IFN-gamma
1.90
8.77
1.15
7.19
0.94
5.13
1.70
9.54
0.16
0.58
47.3
42.0
56.2
35.5
58.1
35.9
50.2
38.6
88.5
10.7
0.01
0.01
0.02
0.05
0.02
0.04
0.03
0.07
0.03
0.03
99.8
0.15
99.7
0.20
99.6
0.31
99.7
0.28
99.7
0.24

FIG. 21

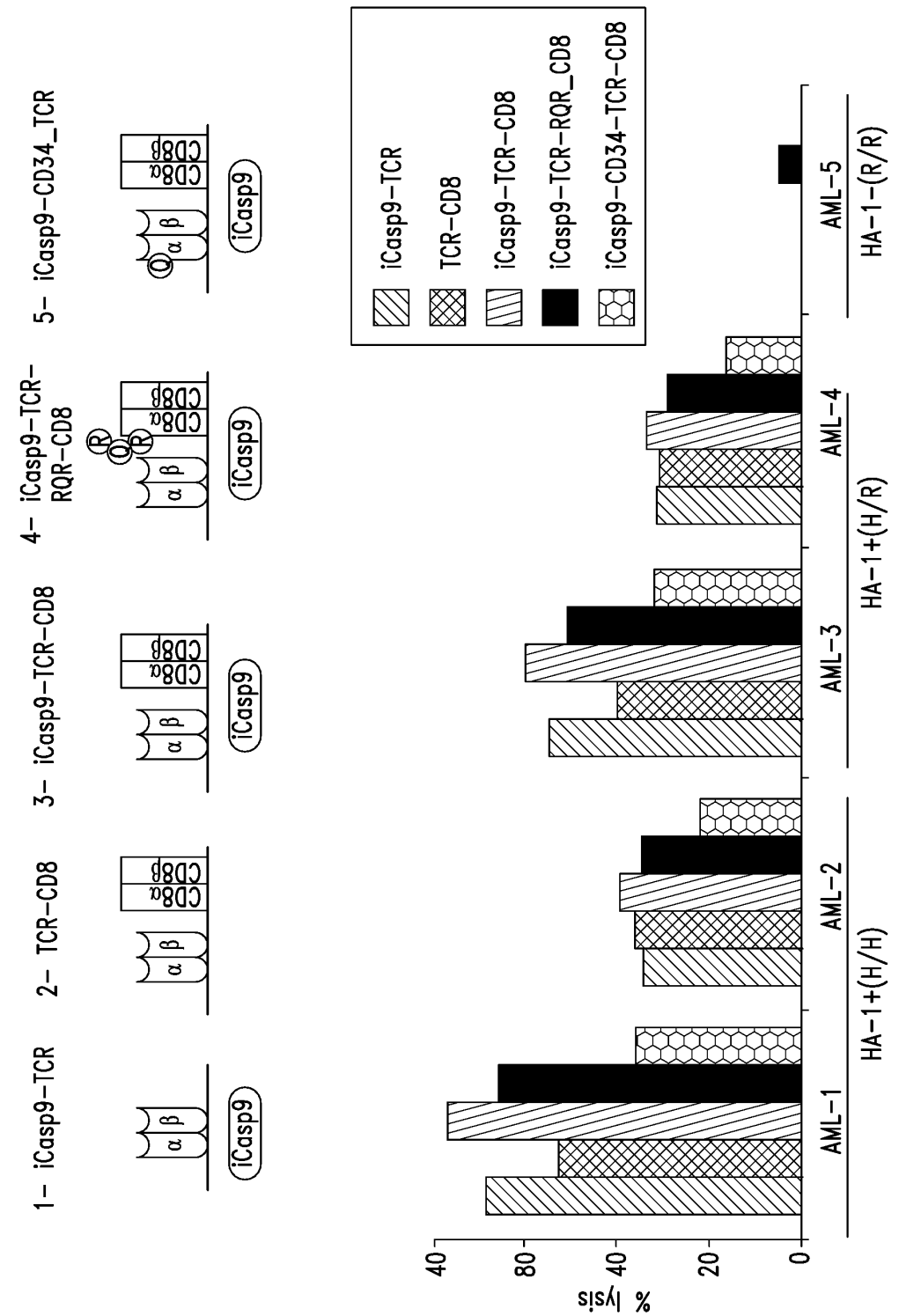

1- iCasp9-TCR
2- TCR-CD8
3- iCasp9-TCR-CD8
4- iCasp9-TCR-RQR-CD8
5- iCasp9-CD34_TCR
iCasp9
α β
CD8α CD8β
R
Q
iCasp9-TCR
TCR-CD8
iCasp9-TCR-CD8
iCasp9-TCR-RQR_CD8
iCasp9-CD34-TCR-CD8
% lysis
0
20
40
80
40
AML-1
AML-2
AML-3
AML-4
AML-5
HA-1+(H/H)
HA-1+(H/R)
HA-1-(R/R)

FIG. 22

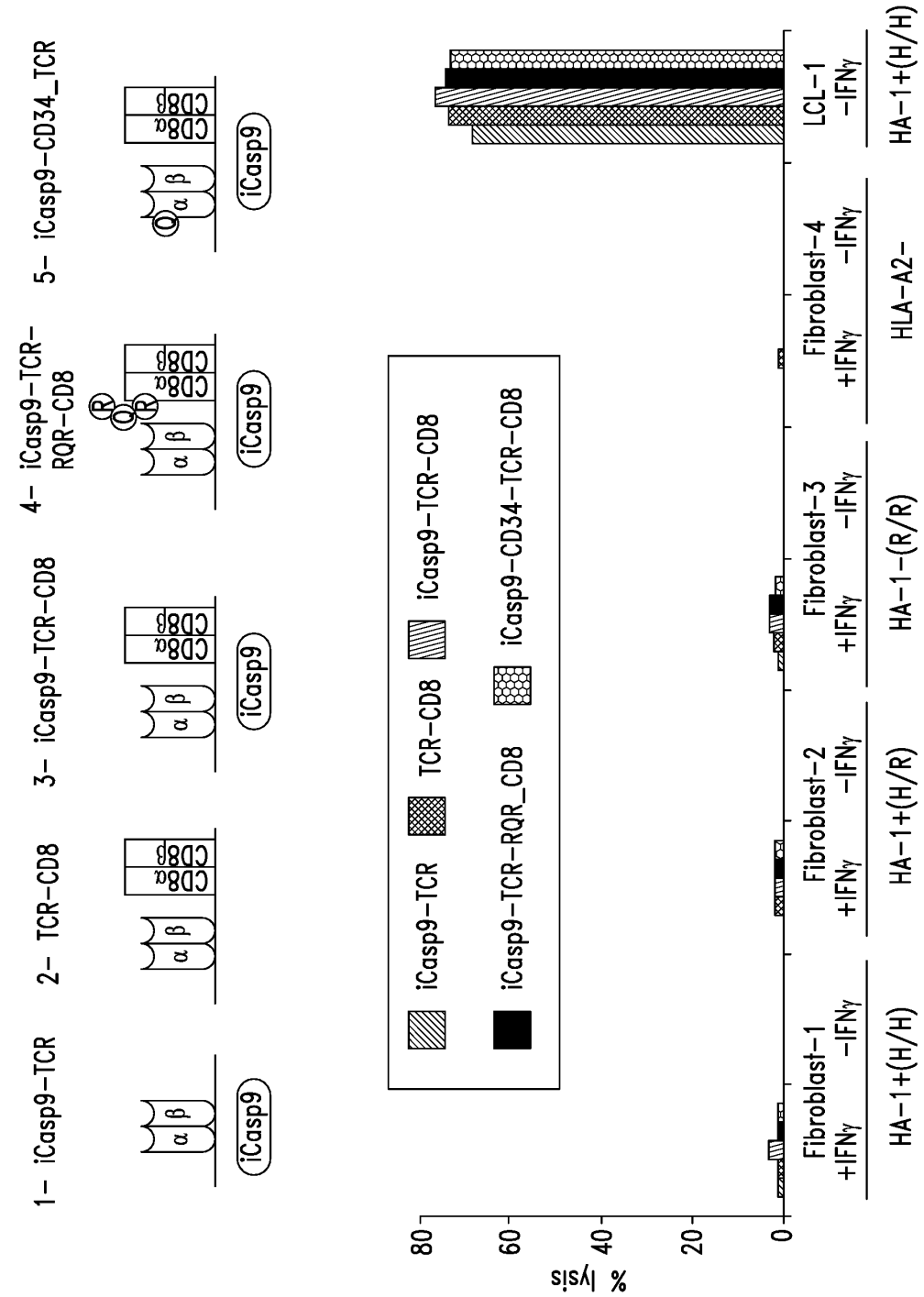

1- iCasp9-TCR
2- TCR-CD8
3- iCasp9-TCR-CD8
4- iCasp9-TCR-RQR-CD8
5- iCasp9-CD34_TCR
iCasp9
α β
CD8α
CD8β
R
Q
iCasp9-TCR
TCR-CD8
iCasp9-TCR-CD8
iCasp9-TCR-RQR_CD8
iCasp9-CD34-TCR-CD8
% lysis
0
20
40
60
80
Fibroblast-1 +IFNγ -IFNγ
Fibroblast-2 +IFNγ -IFNγ
Fibroblast-3 +IFNγ -IFNγ
Fibroblast-4 +IFNγ -IFNγ
LCL-1 -IFNγ
HA-1+(H/H)
HA-1+(H/R)
HA-1-(R/R)
HLA-A2-
HA-1+(H/H)

FIG. 23

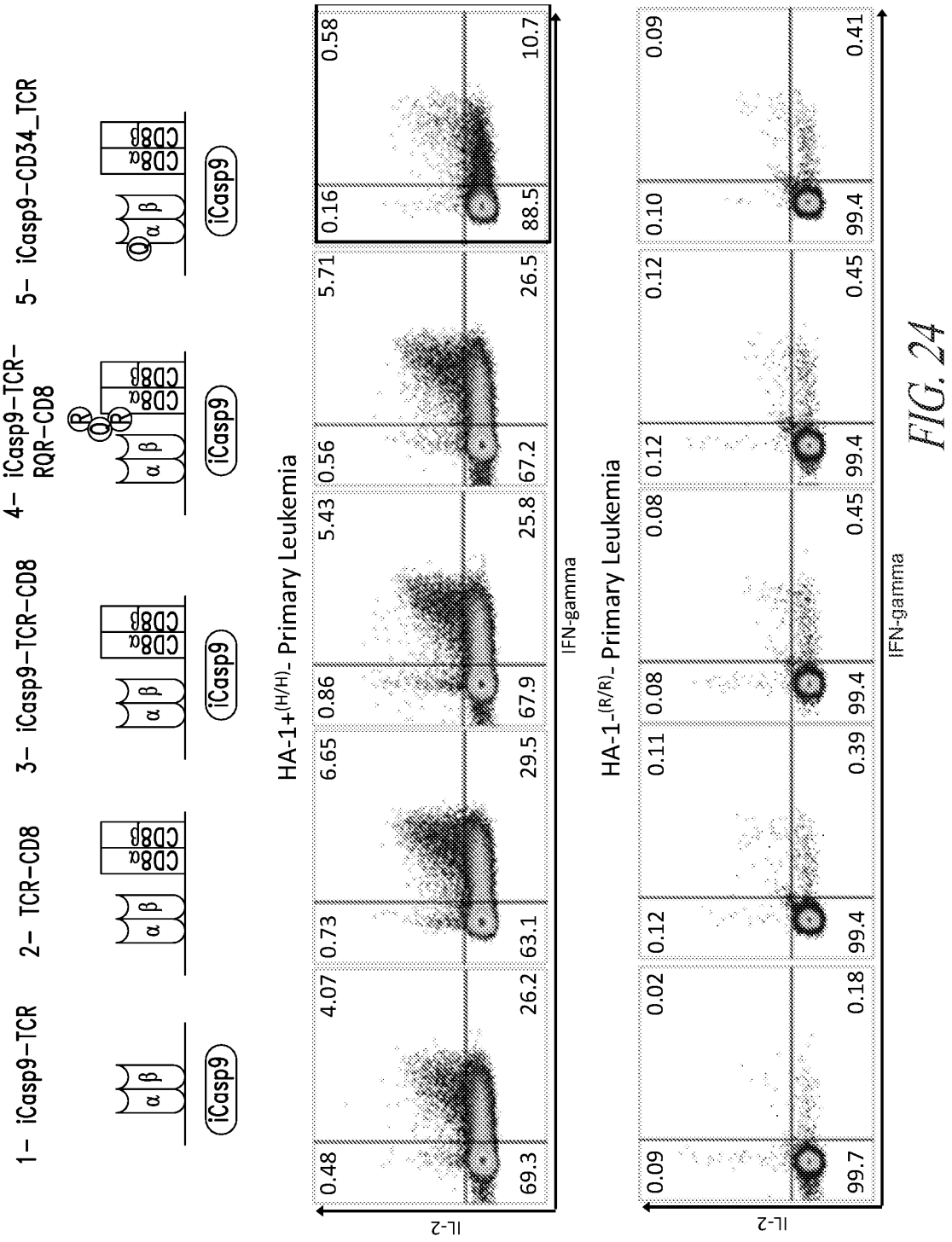

1- iCasp9-TCR
2- TCR-CD8
3- iCasp9-TCR-CD8
4- iCasp9-TCR-RQR-CD8
5- iCasp9-CD34_TCR
iCasp9
HA-1+(H/H)- Primary Leukemia
HA-1-(R/R)- Primary Leukemia
IL-2
IFN-gamma
0.48
4.07
0.73
6.65
0.86
5.43
0.56
5.71
0.16
0.58
69.3
26.2
63.1
29.5
67.9
25.8
67.2
26.5
88.5
10.7
0.09
0.02
0.12
0.11
0.08
0.08
0.12
0.12
0.10
0.09
99.7
0.18
99.4
0.39
99.4
0.45
99.4
0.45
99.4
0.41

FIG. 24

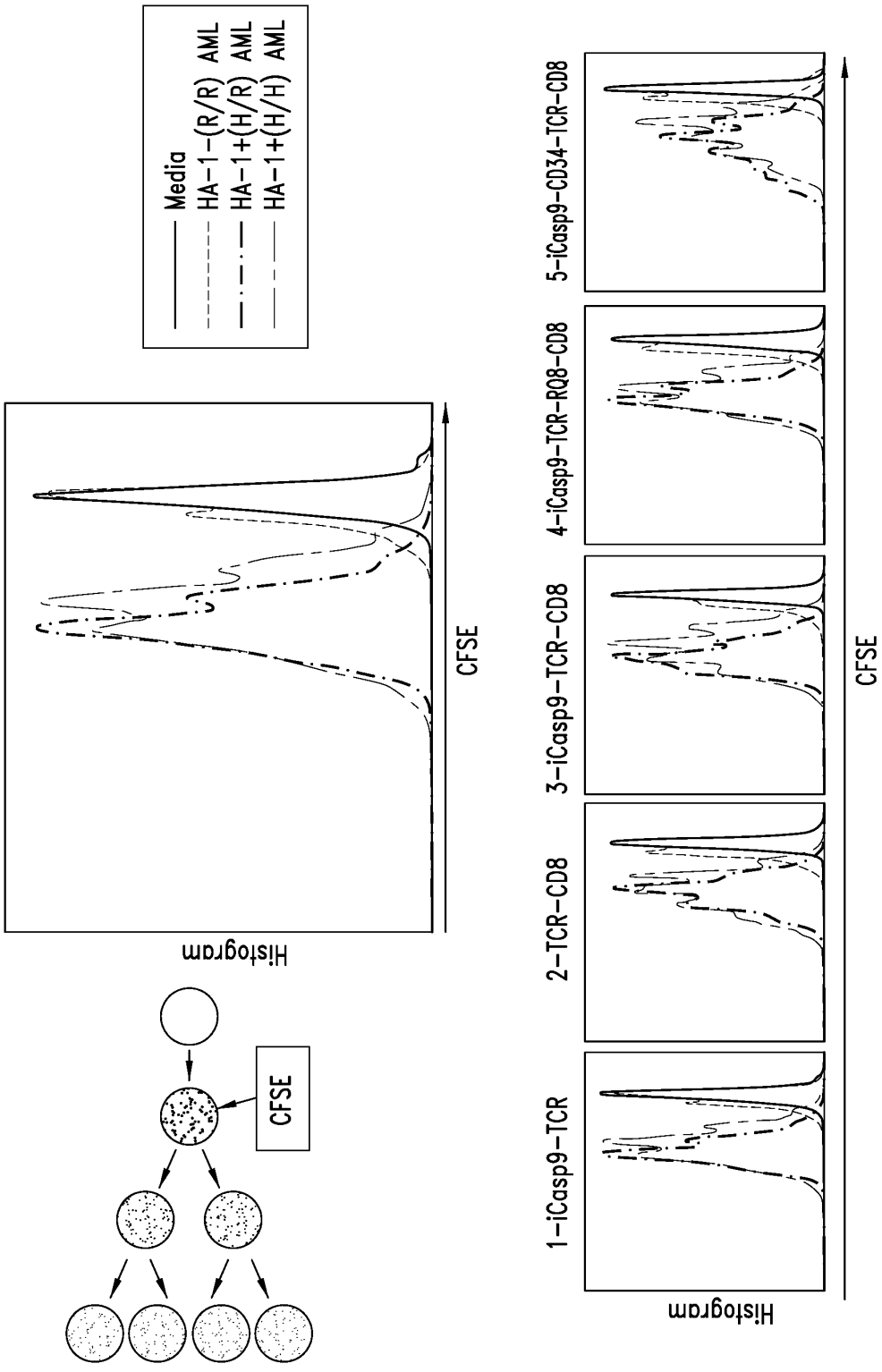

Media
HA-1-(R/R) AML
HA-1+(H/R) AML
HA-1+(H/H) AML
Histogram
CFSE
CFSE
1-iCasp9-TCR
2-TCR-CD8
3-iCasp9-TCR-CD8
4-iCasp9-TCR-RQ8-CD8
5-iCasp9-CD34-TCR-CD8
Histogram
CFSE

FIG. 25

1- iCasp9-TCR
2- TCR-CD8
3- iCasp9-TCR-CD8
4- iCasp9-TCR-RQR-CD8
5- iCasp9-CD34_TCR
iCasp9
α β
CD8α
CD8β
Q
R
5ug/ml
0.05ug/ml
0.0005ug/ml
No dimerizer
% of survival
0
20
40
60
80
100
iCasp9-TCR
TCR-CD8
iCasp9-TCR-CD8
iCasp9-TCR-RQR_CD8
iCasp9-CD34-TCR-CD8

FIG. 26

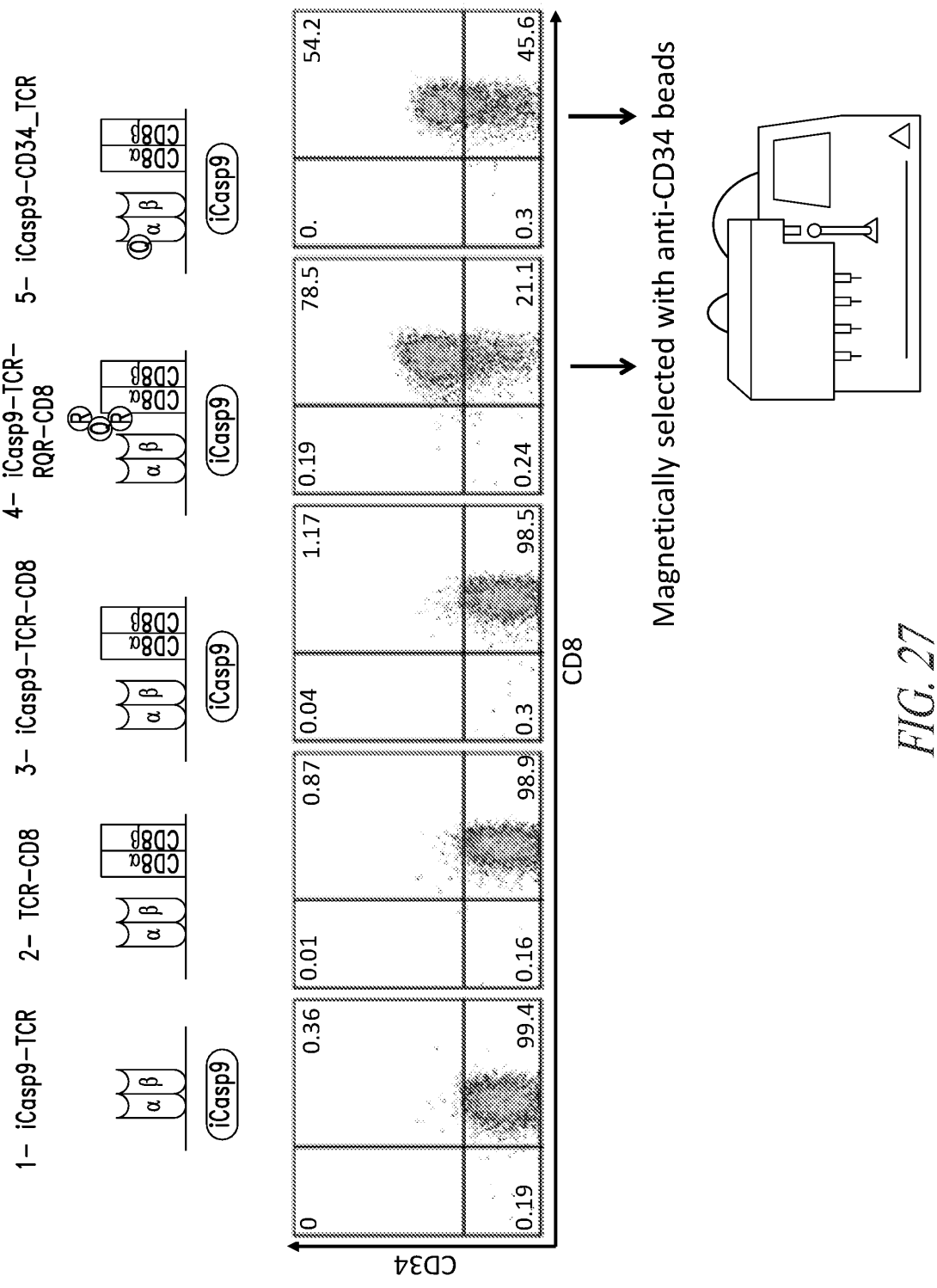

1- iCasp9-TCR
2- TCR-CD8
3- iCasp9-TCR-CD8
4- iCasp9-TCR-RQR-CD8
5- iCasp9-CD34_TCR
iCasp9
α β
CD8α
CD8β
CD34
CD8
0
0.36
0.01
0.87
0.04
1.17
0.19
78.5
0.
54.2
0.19
99.4
0.16
98.9
0.3
98.5
0.24
21.1
0.3
45.6
Magnetically selected with anti-CD34 beads

FIG. 27

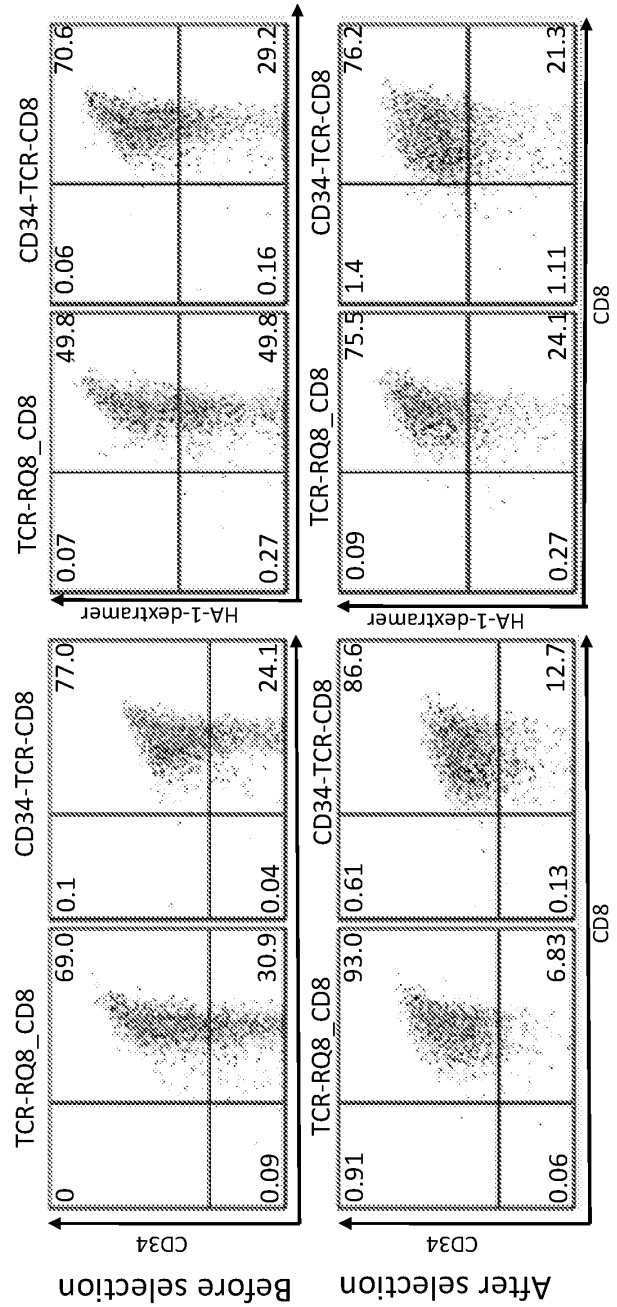


FIG. 28A

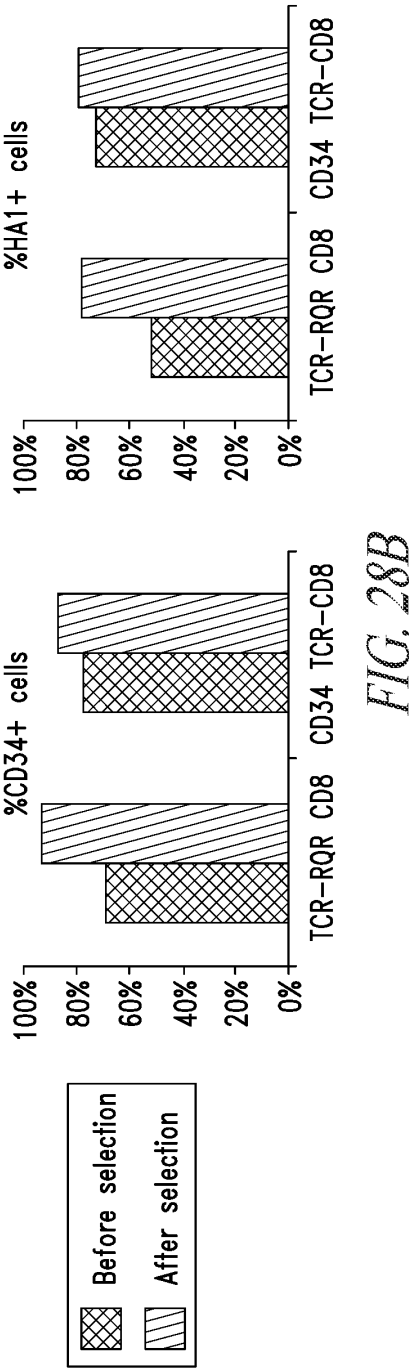


FIG. 28B

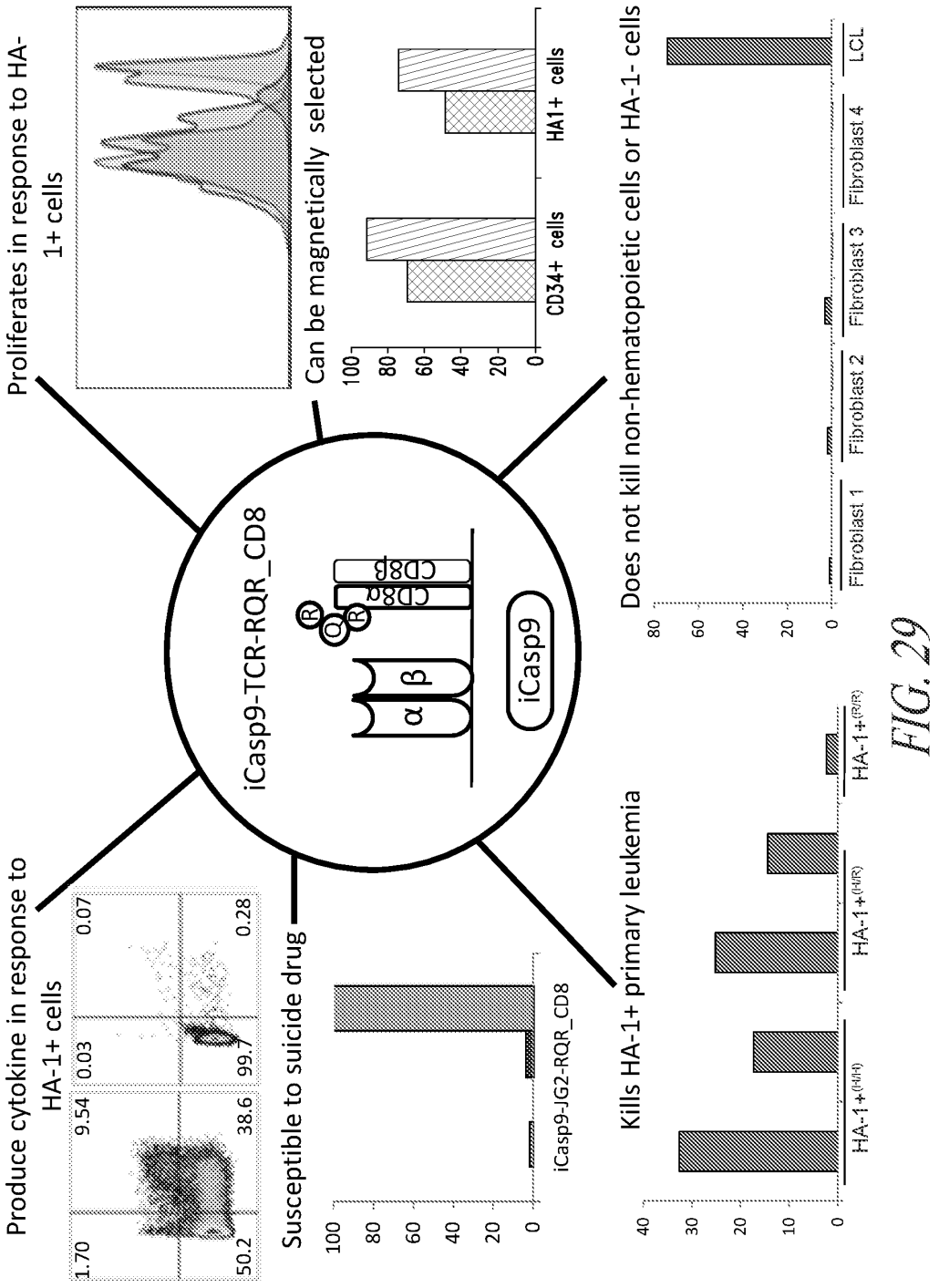

Produce cytokine in response to HA-1+ cells
1.70
9.54
0.03
0.07
50.2
38.6
99.7
0.28
Susceptible to suicide drug
100
80
60
40
20
0
iCasp9-JG2-RQR_CD8
iCasp9-TCR-RQR_CD8
α
β
CD8α
CD8β
iCasp9
Proliferates in response to HA-1+ cells
Can be magnetically selected
100
80
60
40
20
0
CD34+ cells
HA1+ cells
Kills HA-1+ primary leukemia
40
30
20
10
0
HA-1+(MM)
HA-1+(MR)
HA-1+(RR)
Does not kill non-hematopoietic cells or HA-1- cells
80
60
40
20
0
Fibroblast 1
Fibroblast 2
Fibroblast 3
Fibroblast 4
LCL

FIG. 29

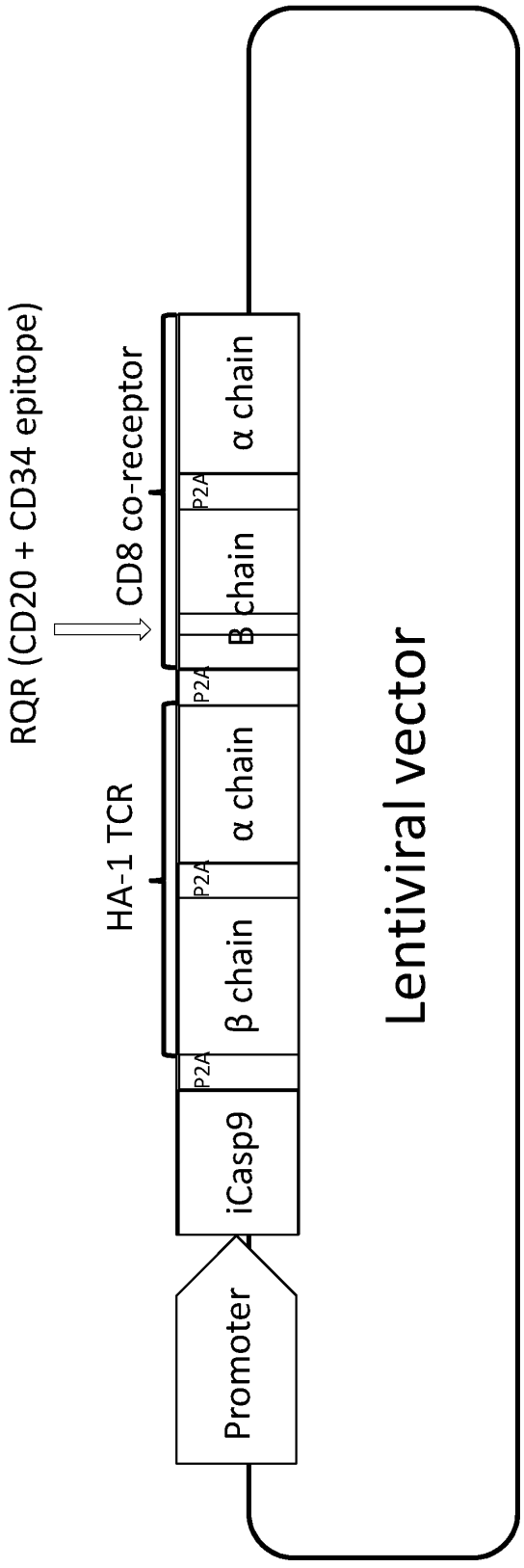

Lentiviral vector
Promoter
iCasp9
P2A
β chain
P2A
α chain
HA-1 TCR
P2A
β chain
P2A
α chain
CD8 co-receptor
RQR (CD20 + CD34 epitope)

FIG. 30

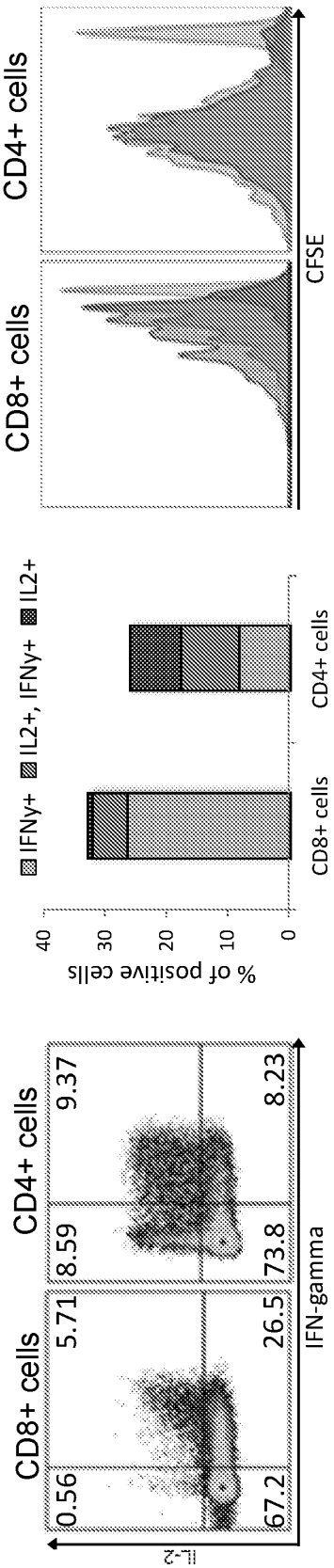

CD8+ cells
CD4+ cells
CFSE
IFNy+
IL2+, IFNy+
IL2+
% of positive cells
40
30
20
10
0
CD8+ cells
CD4+ cells
CD8+ cells
CD4+ cells
0.56
5.71
8.59
9.37
67.2
26.5
73.8
8.23
IL-2
IFN-gamma

FIG. 31

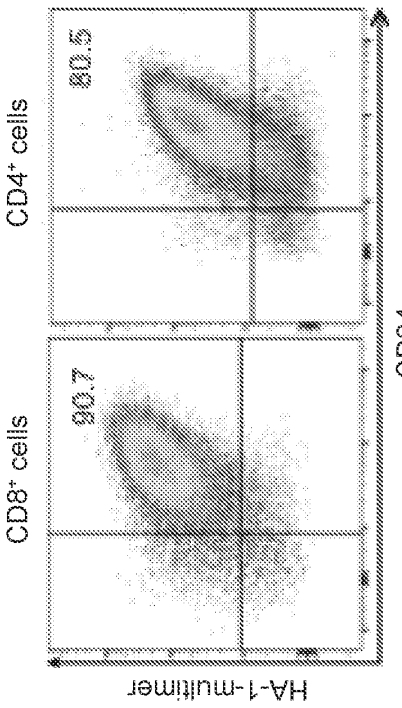


FIG. 32B

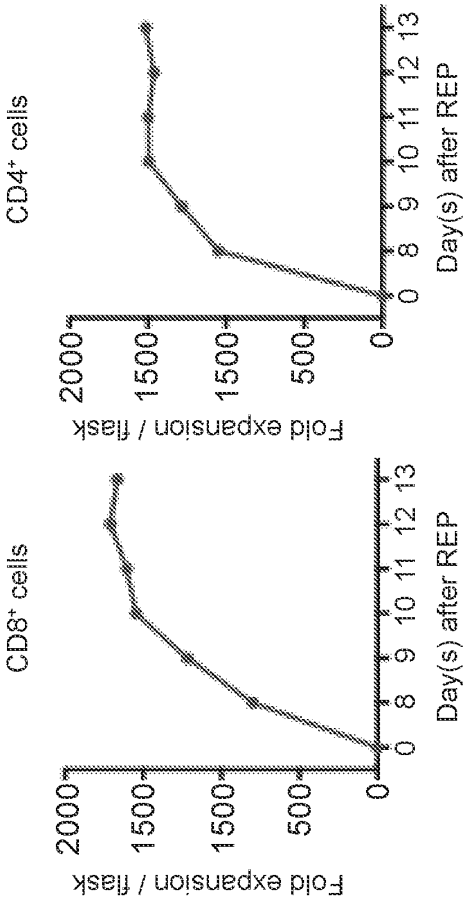


FIG. 32A

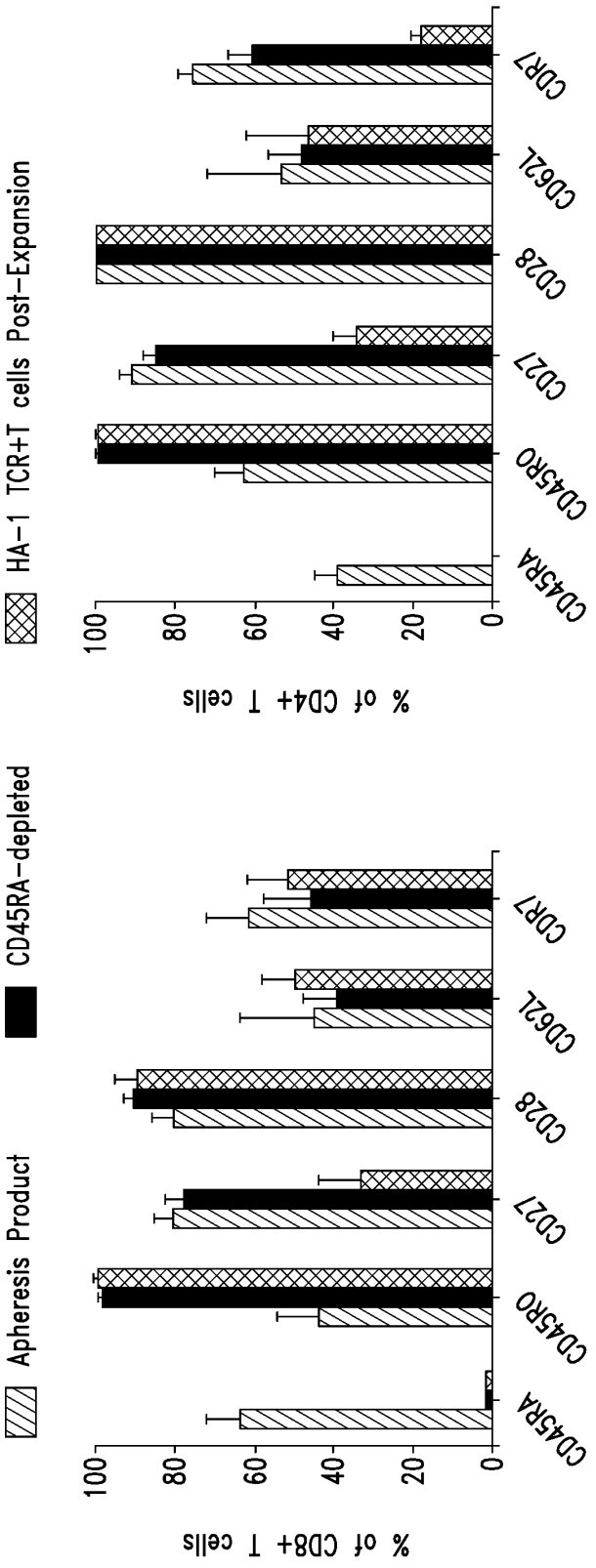

% of CD8+ T cells
100
80
60
40
20
0
CD45RA
CD45RO
CD27
CD28
CD62L
CCR7
Apheresis Product
CD45RA-depleted
% of CD4+ T cells
100
80
60
40
20
0
CD45RA
CD45RO
CD27
CD28
CD62L
CCR7
HA-1 TCR+T cells Post-Expansion

FIG. 32C

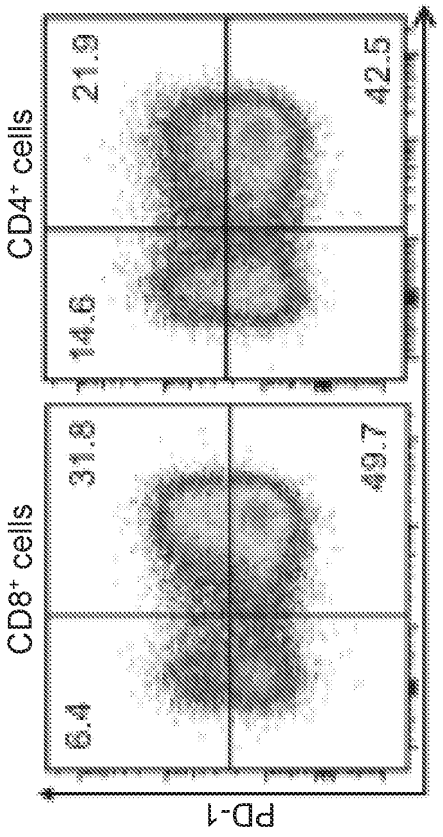


FIG. 32E

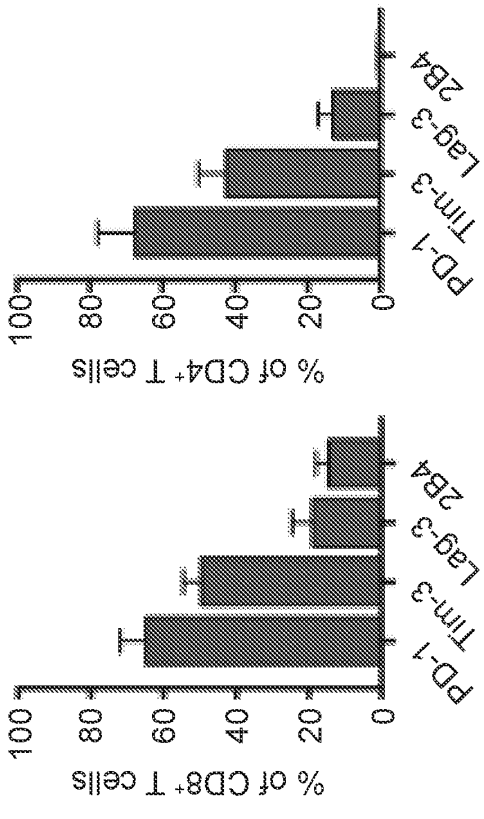


FIG. 32D

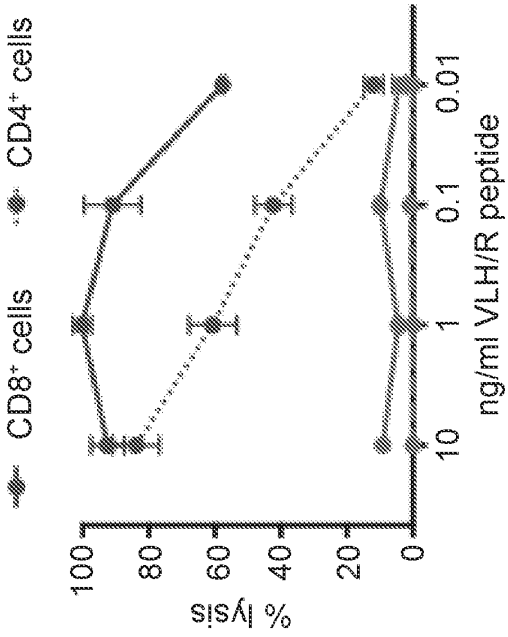


FIG. 33A

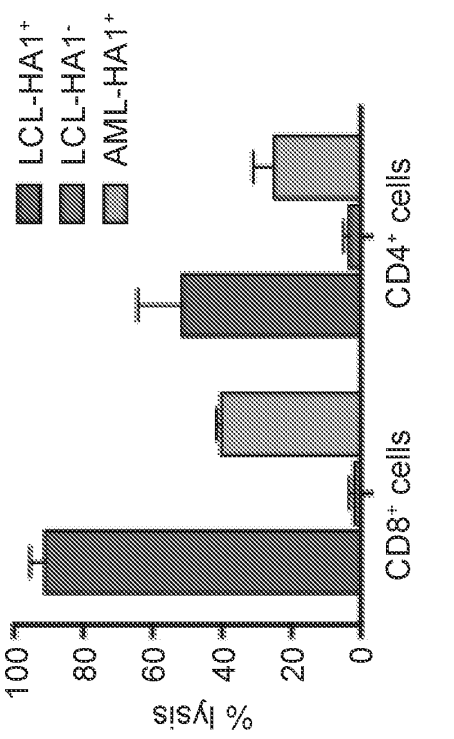


FIG. 33B

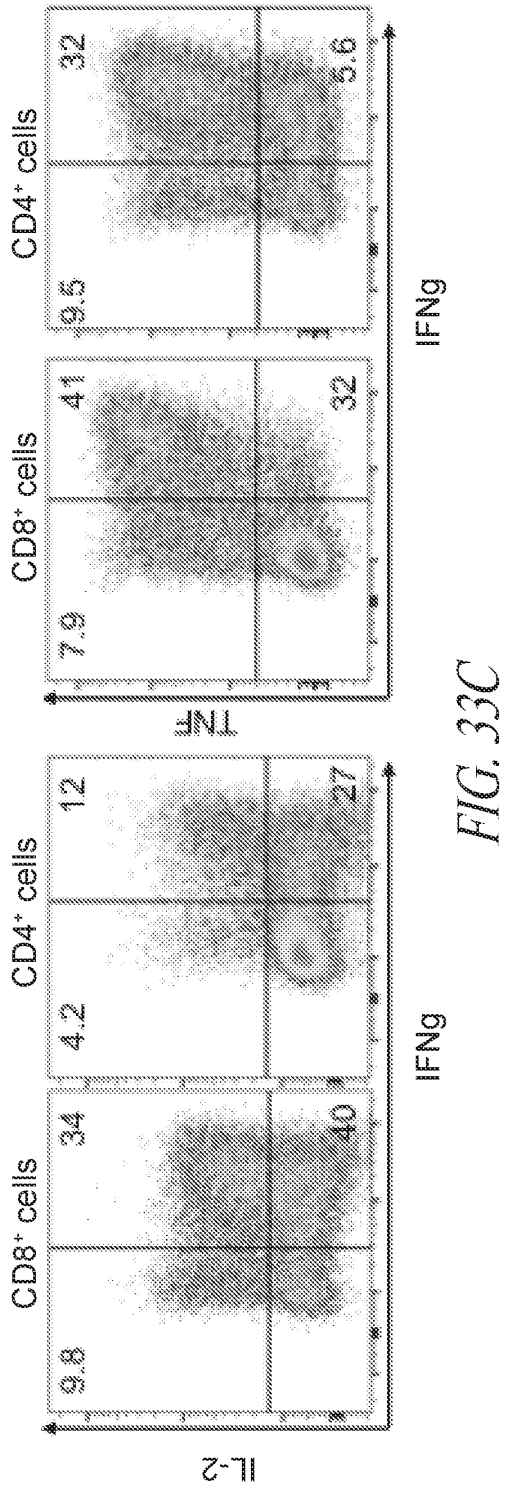


FIG. 33C

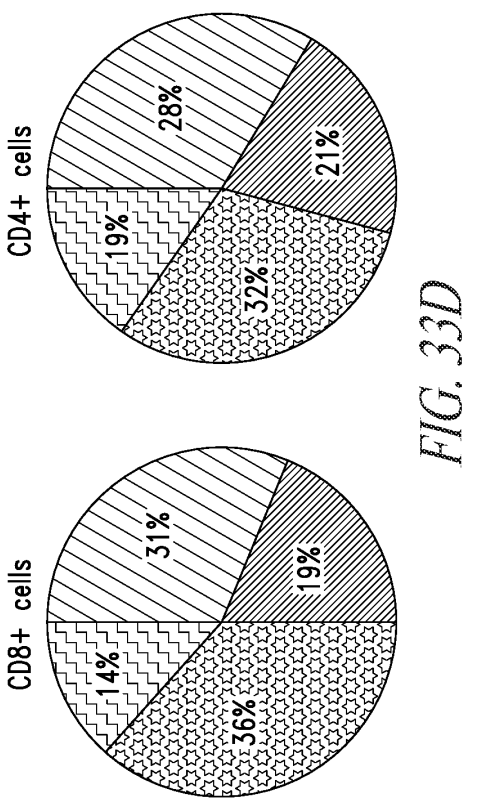


FIG. 33D

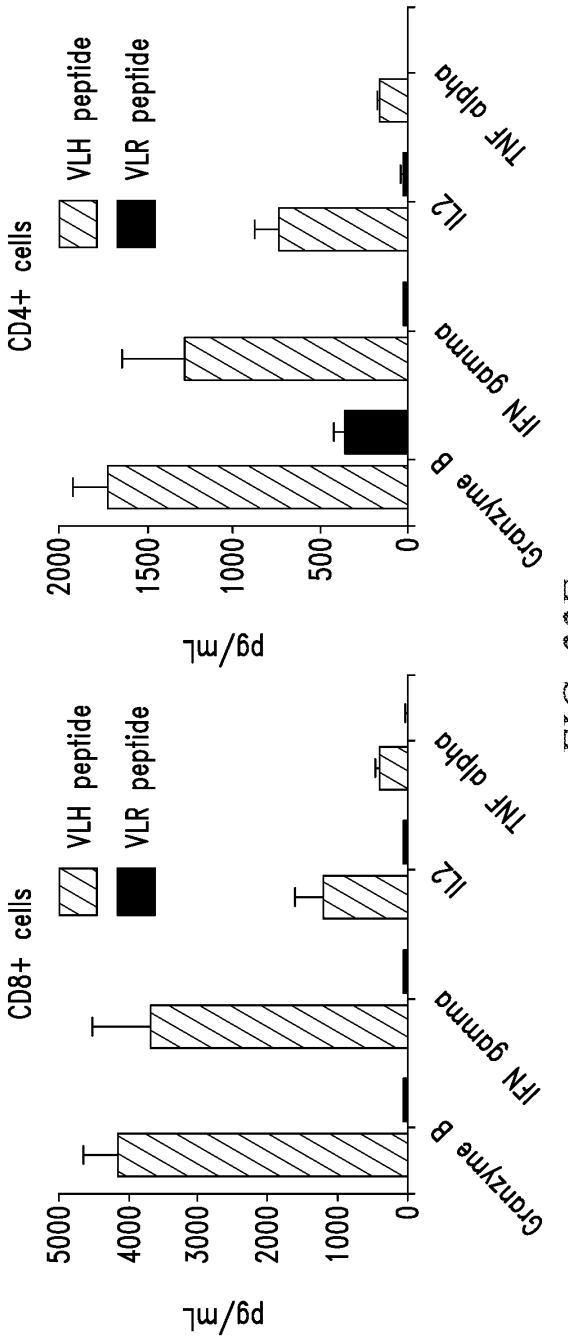


FIG. 33E

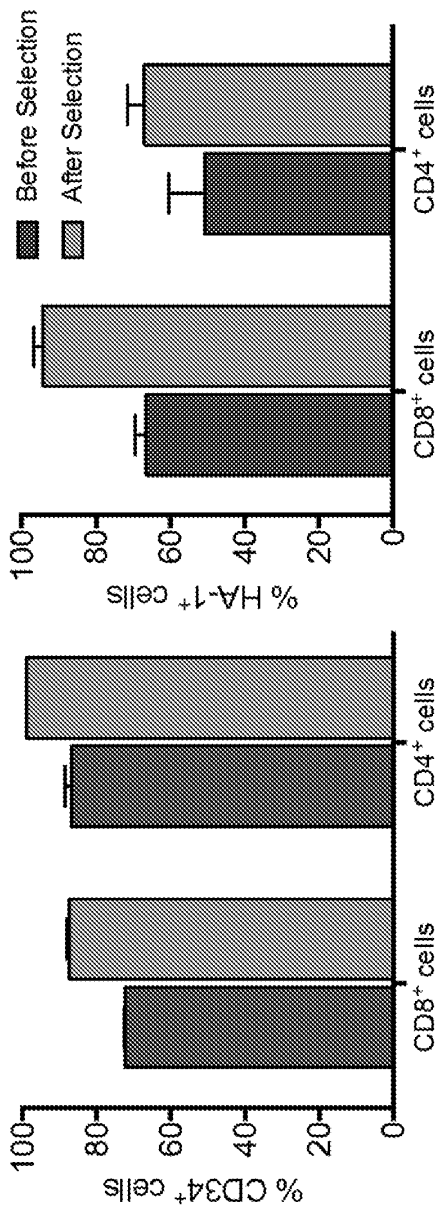


FIG. 34A

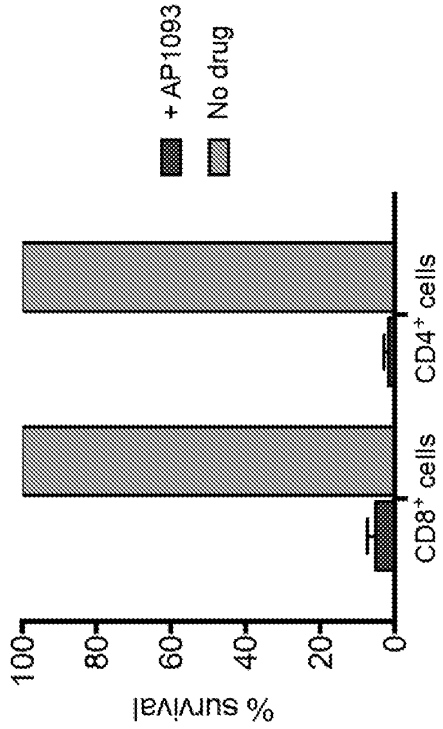


FIG. 34B

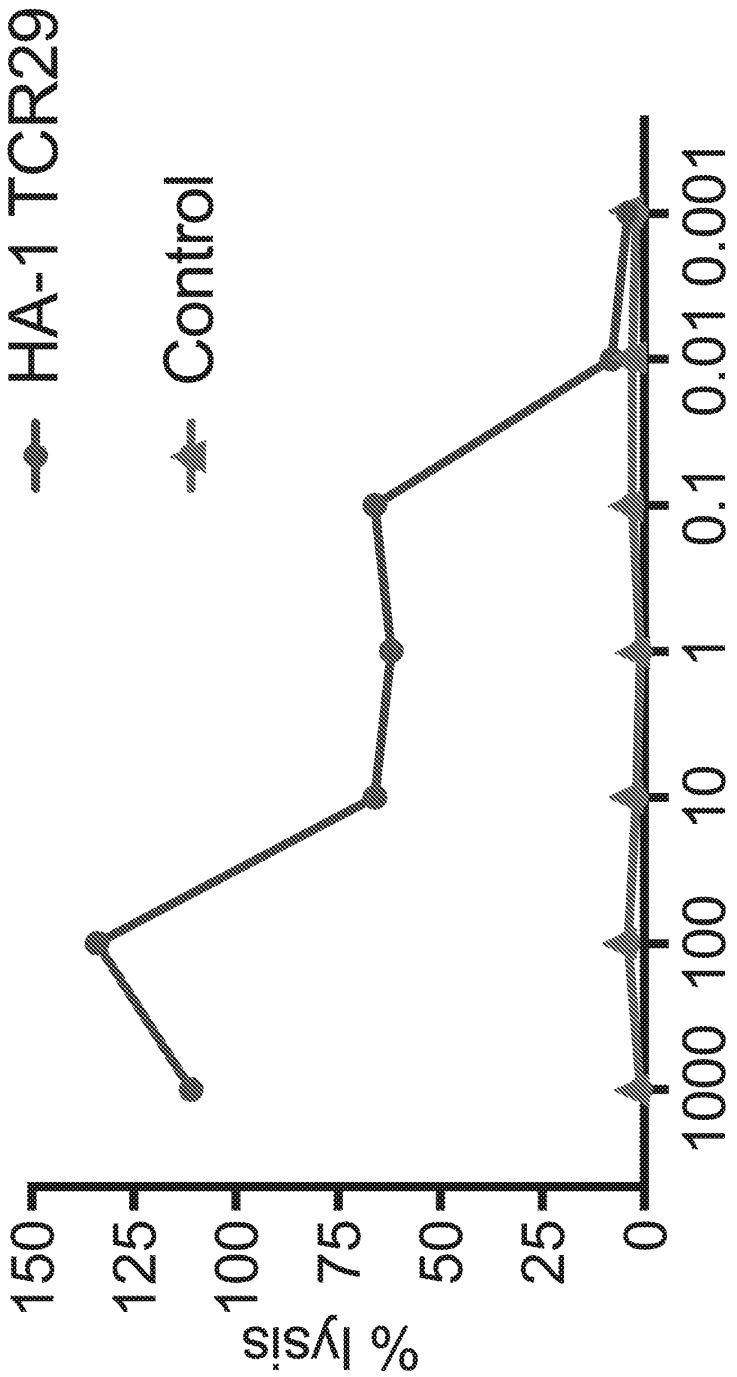

HA-1 TCR29
Control
% lysis
150
125
100
75
50
25
0
1000
100
10
1
0.1
0.01
0.001
ng/ml HA-1+ VLH peptide

FIG. 35