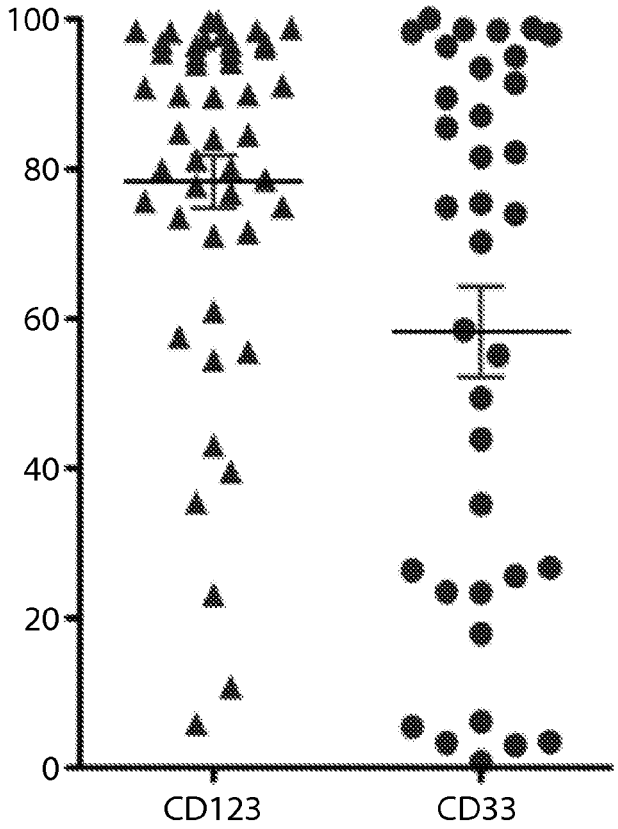


FIG. 1

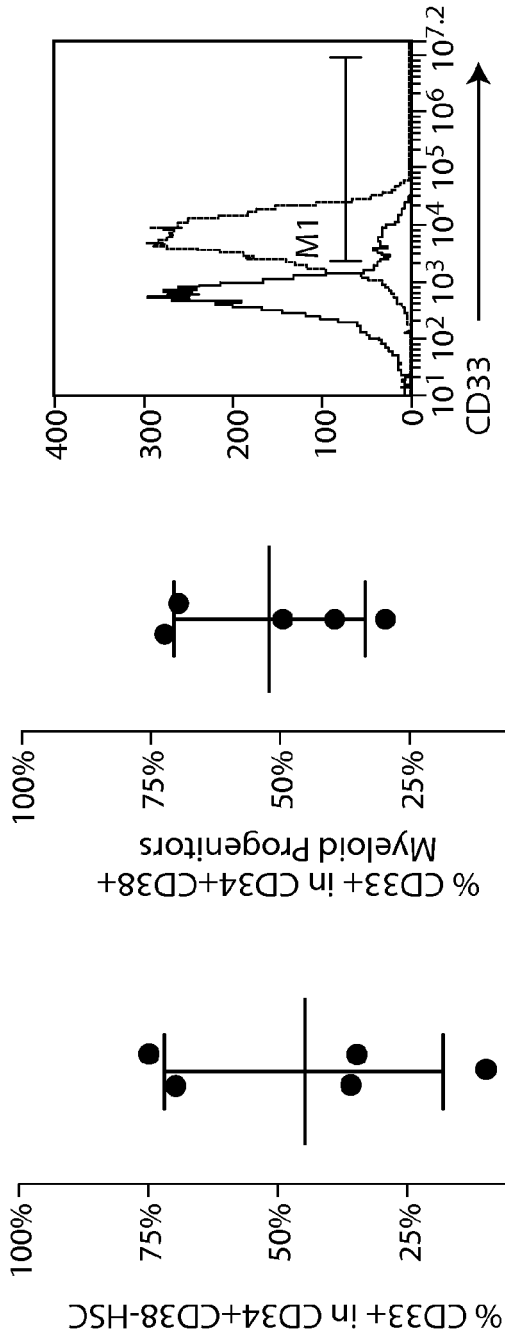


FIG. 2A

FIG. 2B

FIG. 2C

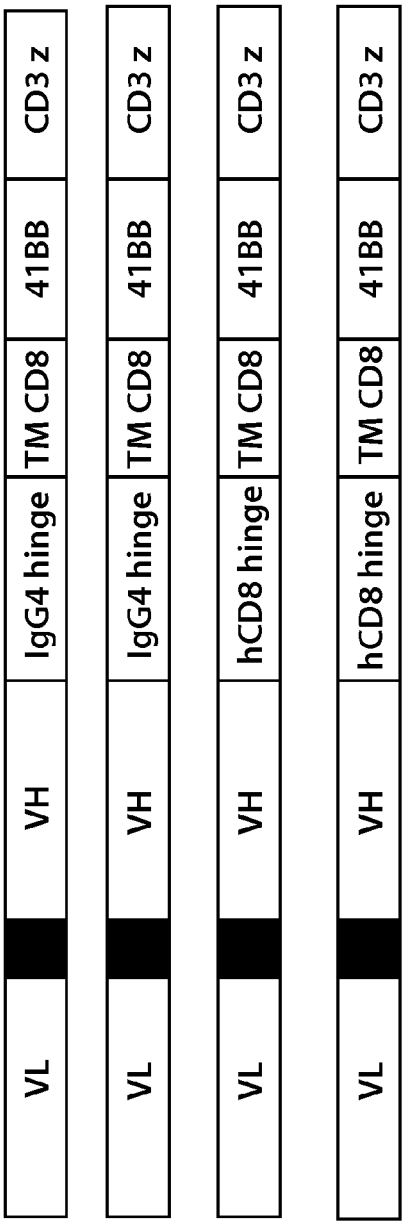

Murine CD33 – IgG4 hinge
VL
VH
IgG4 hinge
TM CD8
41BB
CD3 z
Humanized CD33 – IgG4 hinge
VL
VH
IgG4 hinge
TM CD8
41BB
CD3 z
Humanized CD33 – CD8 hinge
VL
VH
hCD8 hinge
TM CD8
41BB
CD3 z
CD123 – CD8 hinge
VL
VH
hCD8 hinge
TM CD8
41BB
CD3 z

FIG. 3

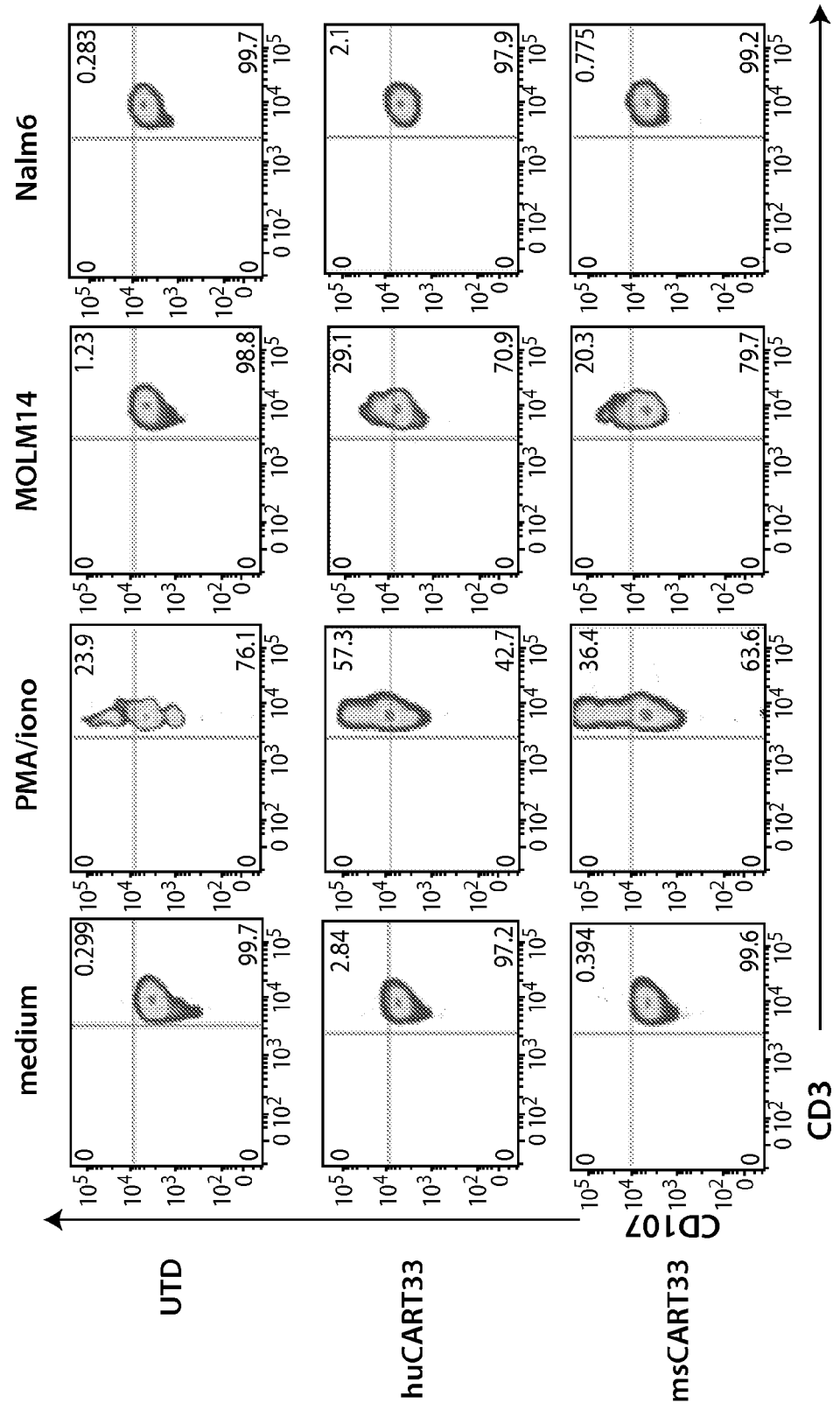

medium
PMA/iono
MOLM14
Nalm6
UTD
huCART33
msCART33
CD107
CD3
0.299
99.7
23.9
76.1
1.23
98.8
0.283
99.7
2.84
97.2
57.3
42.7
29.1
70.9
2.1
97.9
0.394
99.6
36.4
63.6
20.3
79.7
0.775
99.2

FIG. 4-1

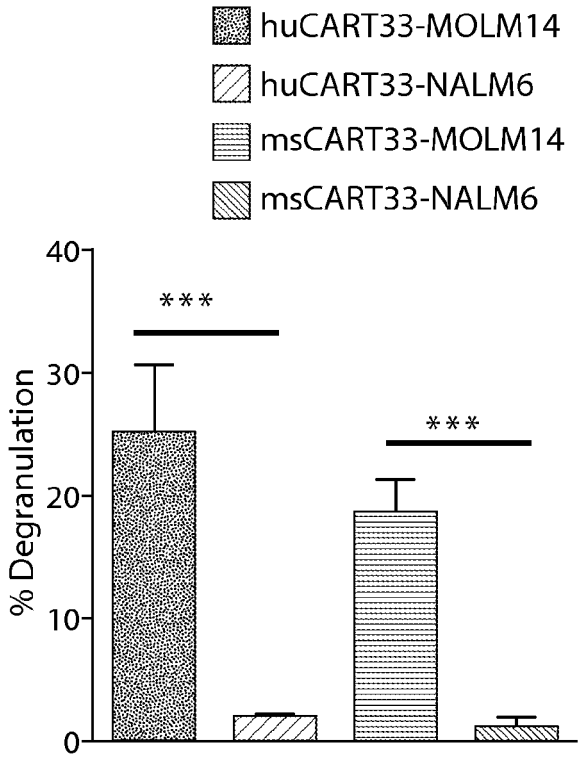

huCART33-MOLM14
huCART33-NALM6
msCART33-MOLM14
msCART33-NALM6
% Degranulation
40
30
20
10
0
***
***

FIG. 4-2

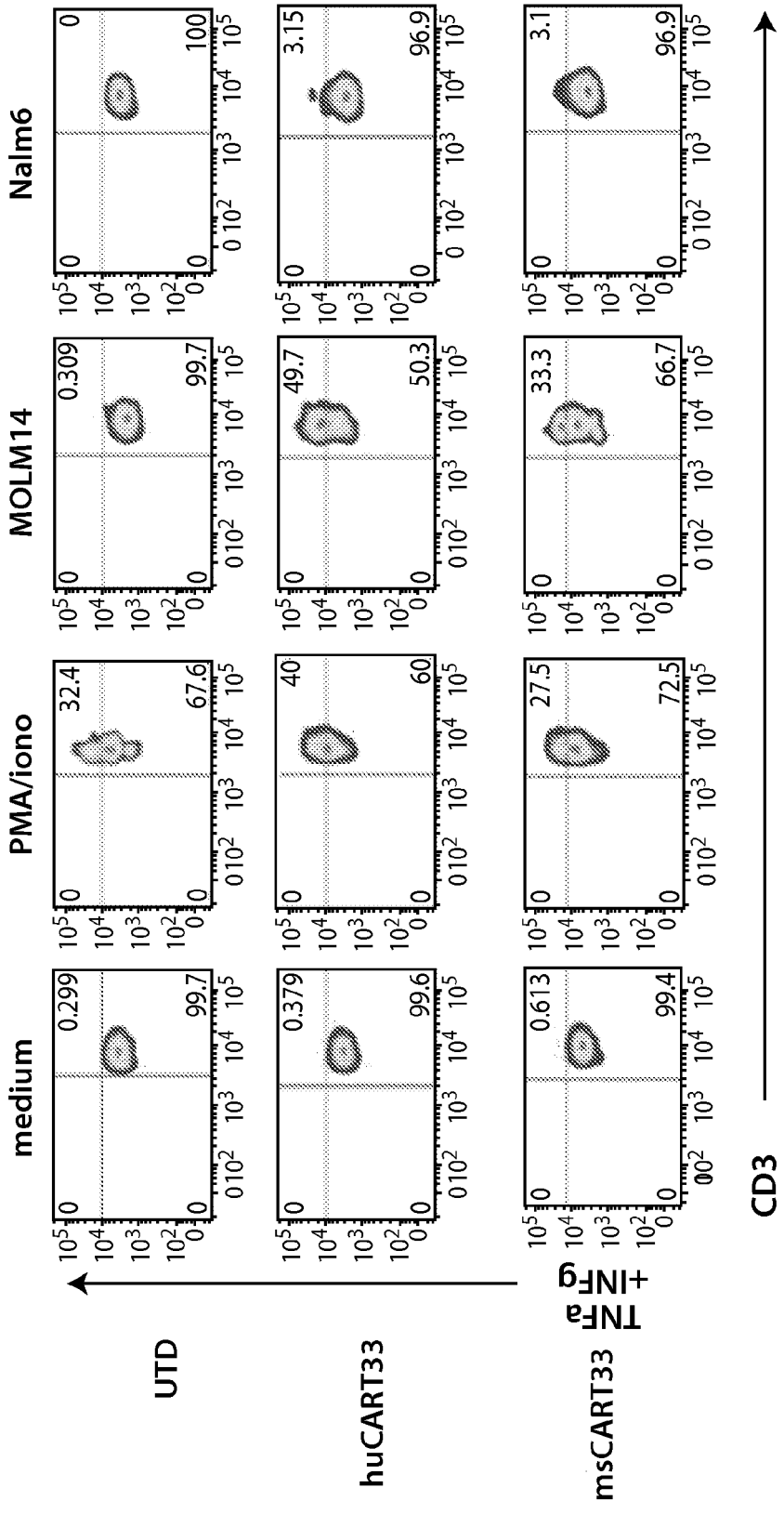

medium
PMA/iono
MOLM14
Nalm6
UTD
huCART33
msCART33
TNFa
+INFg
CD3
0.299
99.7
32.4
67.6
0.309
99.7
0
100
0.379
99.6
40
60
49.7
50.3
3.15
96.9
0.613
99.4
27.5
72.5
33.3
66.7
3.1
96.9

FIG. 5-1

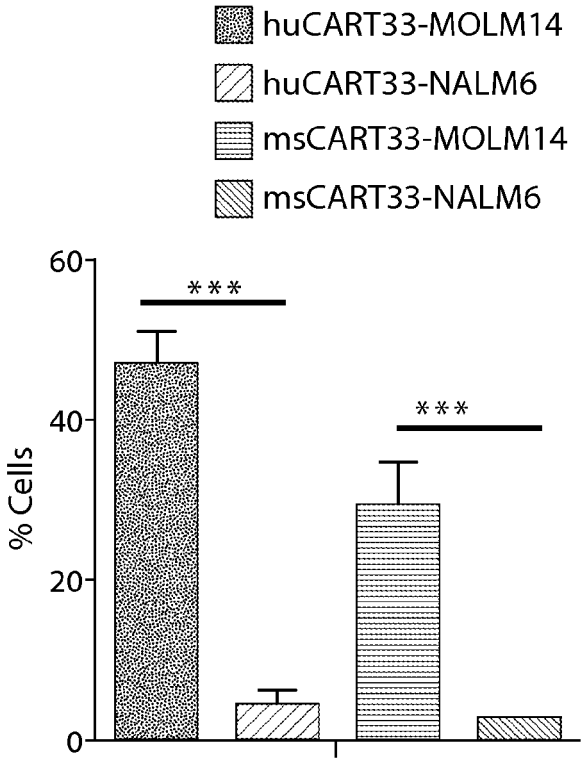

huCART33-MOLM14
huCART33-NALM6
msCART33-MOLM14
msCART33-NALM6
60
40
20
0
% Cells
***
***

FIG. 5-2

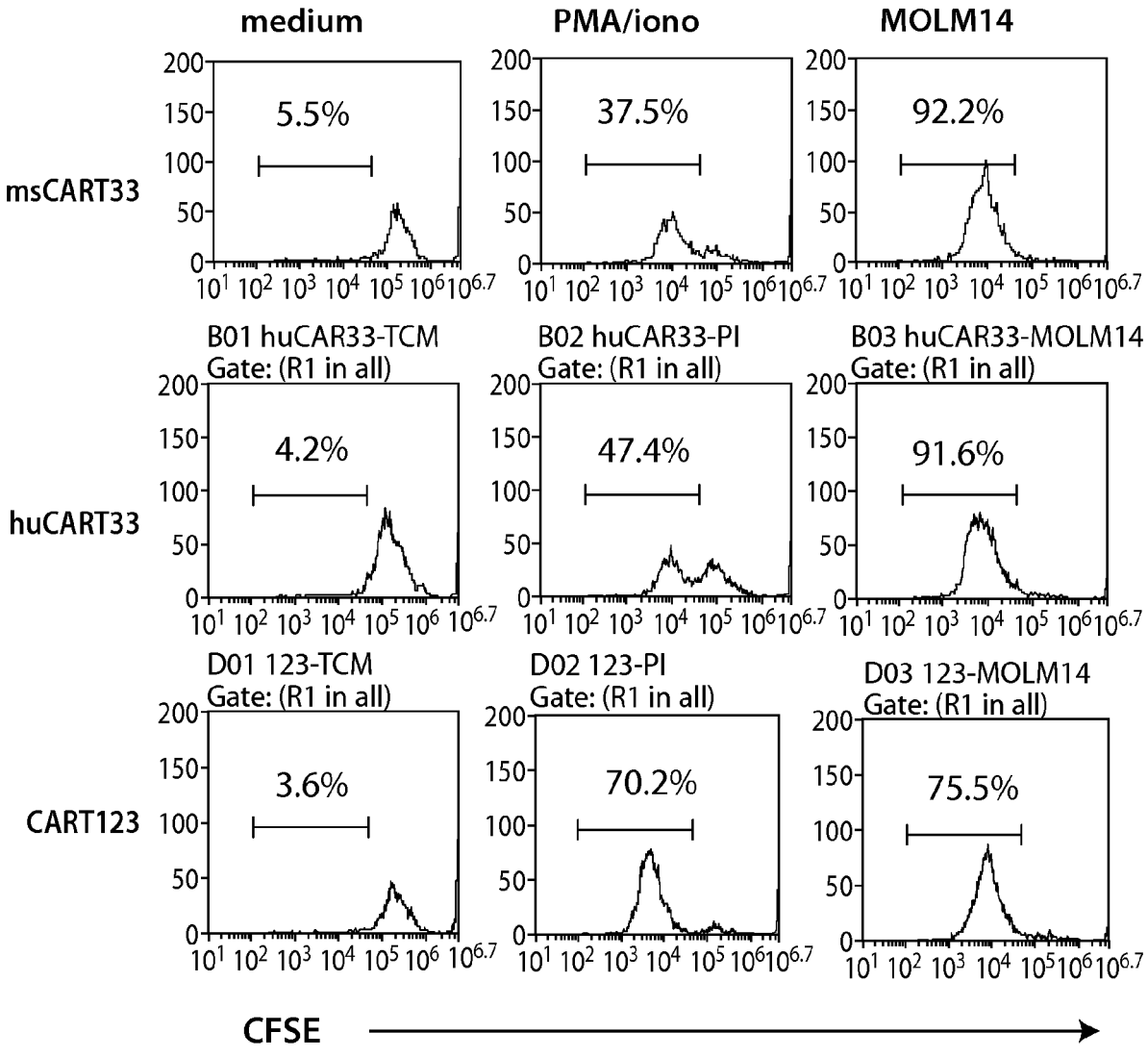

medium
PMA/iono
MOLM14
msCART33
5.5%
37.5%
92.2%
B01 huCAR33-TCM
Gate: (R1 in all)
B02 huCAR33-PI
Gate: (R1 in all)
B03 huCAR33-MOLM14
Gate: (R1 in all)
huCART33
4.2%
47.4%
91.6%
D01 123-TCM
Gate: (R1 in all)
D02 123-PI
Gate: (R1 in all)
D03 123-MOLM14
Gate: (R1 in all)
CART123
3.6%
70.2%
75.5%
CFSE

FIG. 6

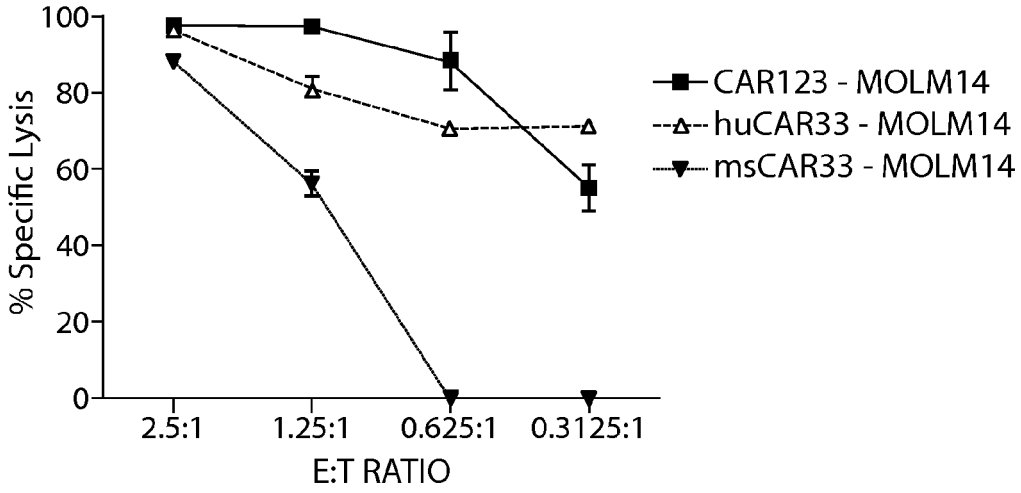

100
80
60
40
20
0
% Specific Lysis
2.5:1
1.25:1
0.625:1
0.3125:1
E:T RATIO
CAR123 - MOLM14
huCAR33 - MOLM14
msCAR33 - MOLM14

FIG. 7A

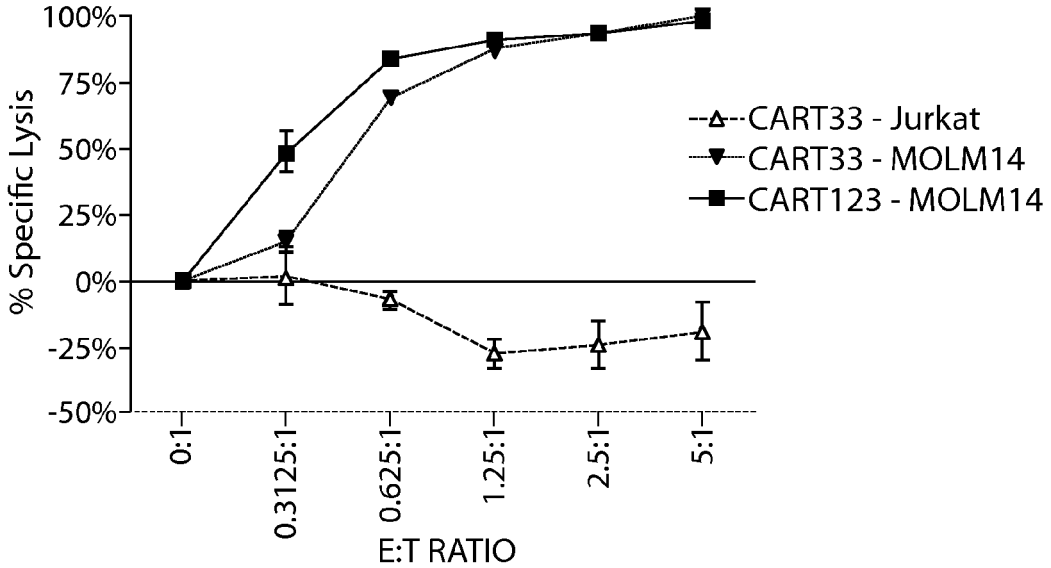

100%
75%
50%
25%
0%
-25%
-50%
% Specific Lysis
0:1
0.3125:1
0.625:1
1.25:1
2.5:1
5:1
E:T RATIO
CART33 - Jurkat
CART33 - MOLM14
CART123 - MOLM14

FIG. 7B

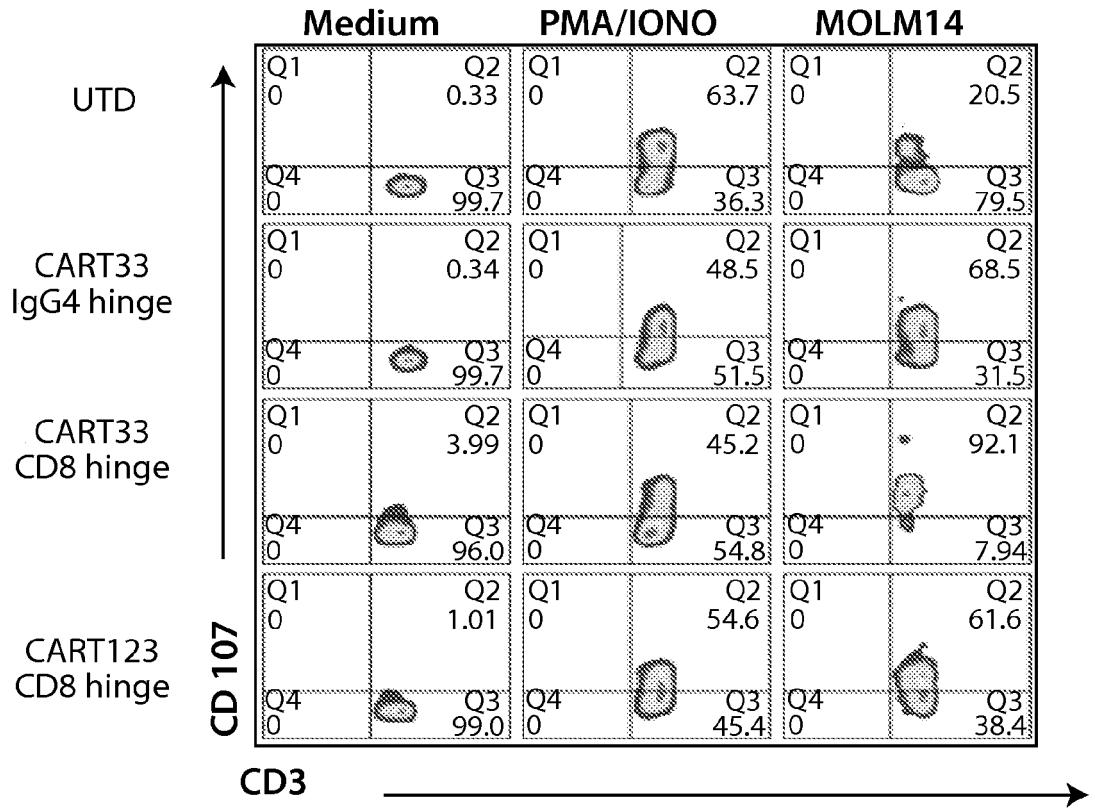

Medium
PMA/IONO
MOLM14
UTD
CART33 IgG4 hinge
CART33 CD8 hinge
CART123 CD8 hinge
CD 107
CD3
Q1 0
Q2 0.33
Q4 0
Q3 99.7
Q1 0
Q2 63.7
Q4 0
Q3 36.3
Q1 0
Q2 20.5
Q4 0
Q3 79.5
Q1 0
Q2 0.34
Q4 0
Q3 99.7
Q1 0
Q2 48.5
Q4 0
Q3 51.5
Q1 0
Q2 68.5
Q4 0
Q3 31.5
Q1 0
Q2 3.99
Q4 0
Q3 96.0
Q1 0
Q2 45.2
Q4 0
Q3 54.8
Q1 0
Q2 92.1
Q4 0
Q3 7.94
Q1 0
Q2 1.01
Q4 0
Q3 99.0
Q1 0
Q2 54.6
Q4 0
Q3 45.4
Q1 0
Q2 61.6
Q4 0
Q3 38.4

FIG. 8

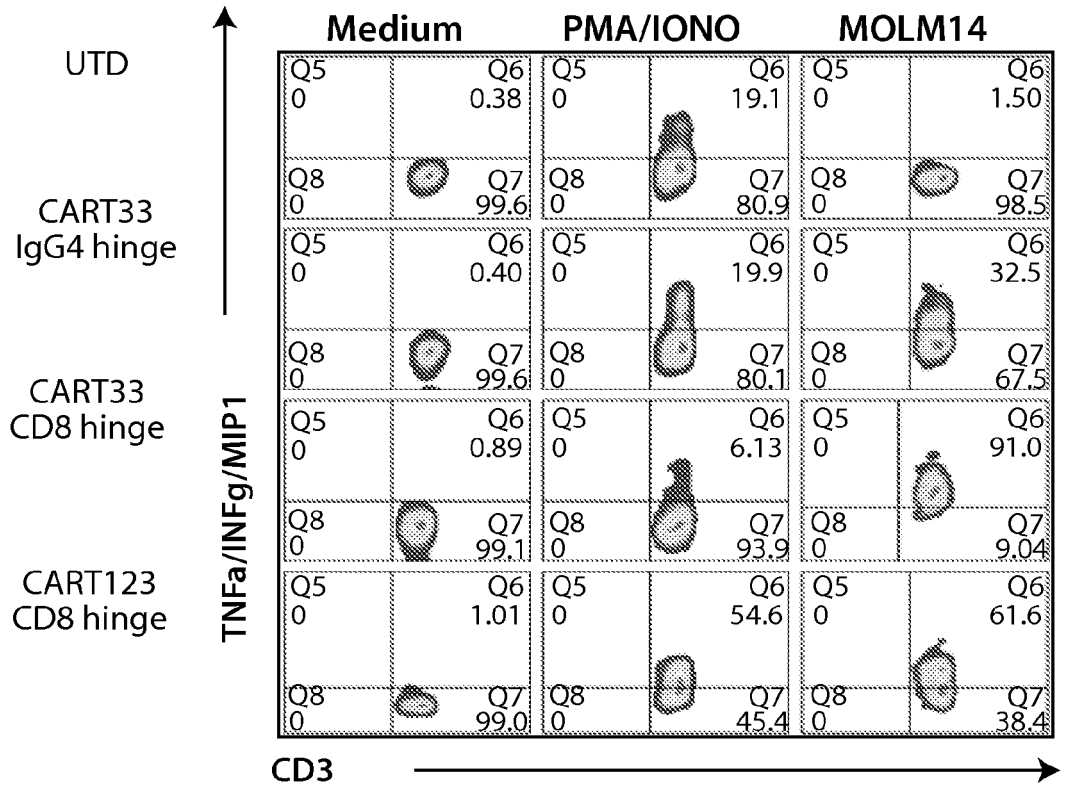
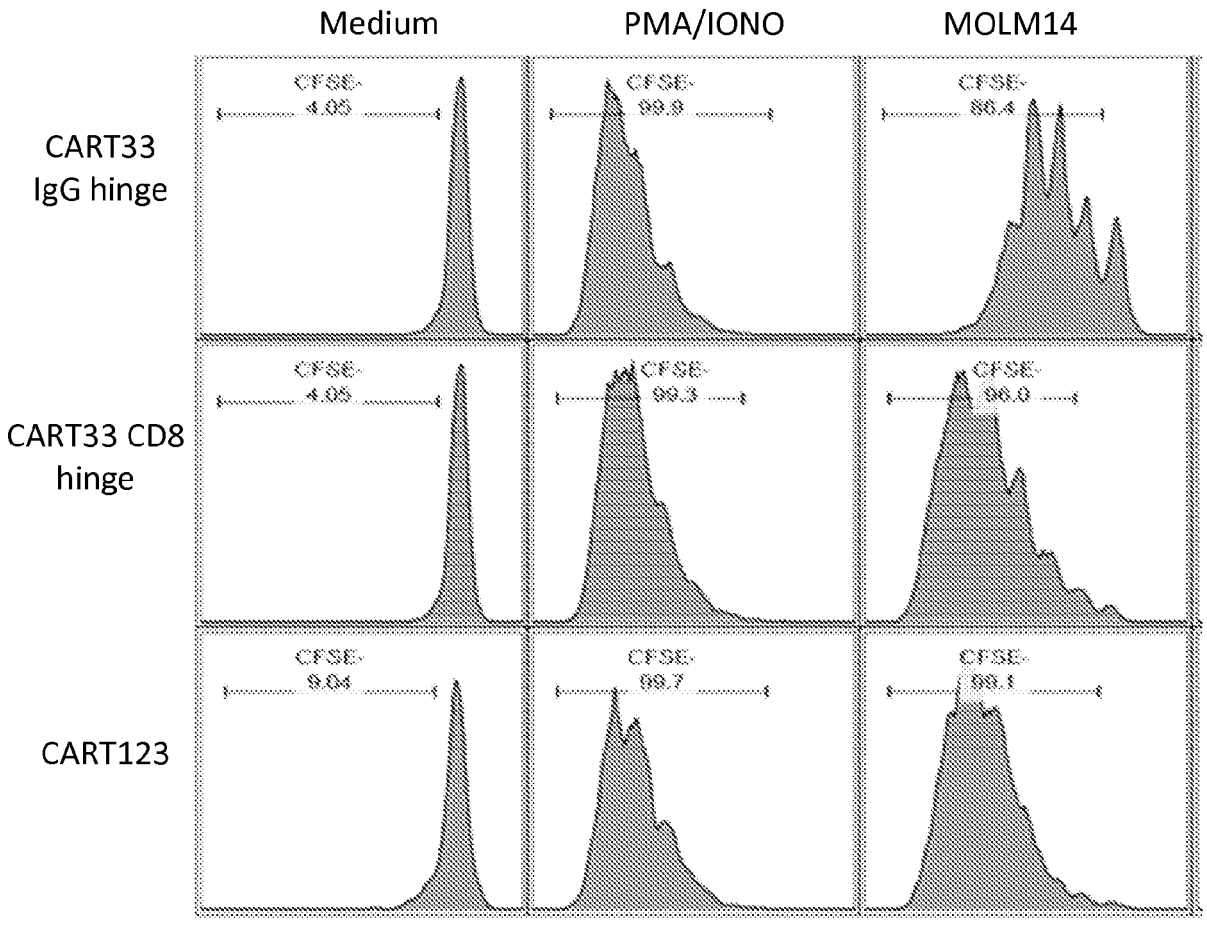

Medium
PMA/IONO
MOLM14
UTD
CART33 IgG4 hinge
CART33 CD8 hinge
CART123 CD8 hinge
TNFa/INFg/MIP1
CD3
Q5 0
Q6 0.38
Q8 0
Q7 99.6
Q5 0
Q6 19.1
Q8 0
Q7 80.9
Q5 0
Q6 1.50
Q8 0
Q7 98.5
Q5 0
Q6 0.40
Q8 0
Q7 99.6
Q5 0
Q6 19.9
Q8 0
Q7 80.1
Q5 0
Q6 32.5
Q8 0
Q7 67.5
Q5 0
Q6 0.89
Q8 0
Q7 99.1
Q5 0
Q6 6.13
Q8 0
Q7 93.9
Q5 0
Q6 91.0
Q8 0
Q7 9.04
Q5 0
Q6 1.01
Q8 0
Q7 99.0
Q5 0
Q6 54.6
Q8 0
Q7 45.4
Q5 0
Q6 61.6
Q8 0
Q7 38.4

FIG. 9

FIG. 10


Medium
PMA/IONO
MOLM14
CART33 IgG hinge
CART33 CD8 hinge
CART123
CFSE-
4.05
CFSE-
99.9
CFSE-
86.4
CFSE-
4.05
CFSE-
99.3
CFSE-
96.0
CFSE-
9.04
CFSE-
99.7
CFSE-
99.1

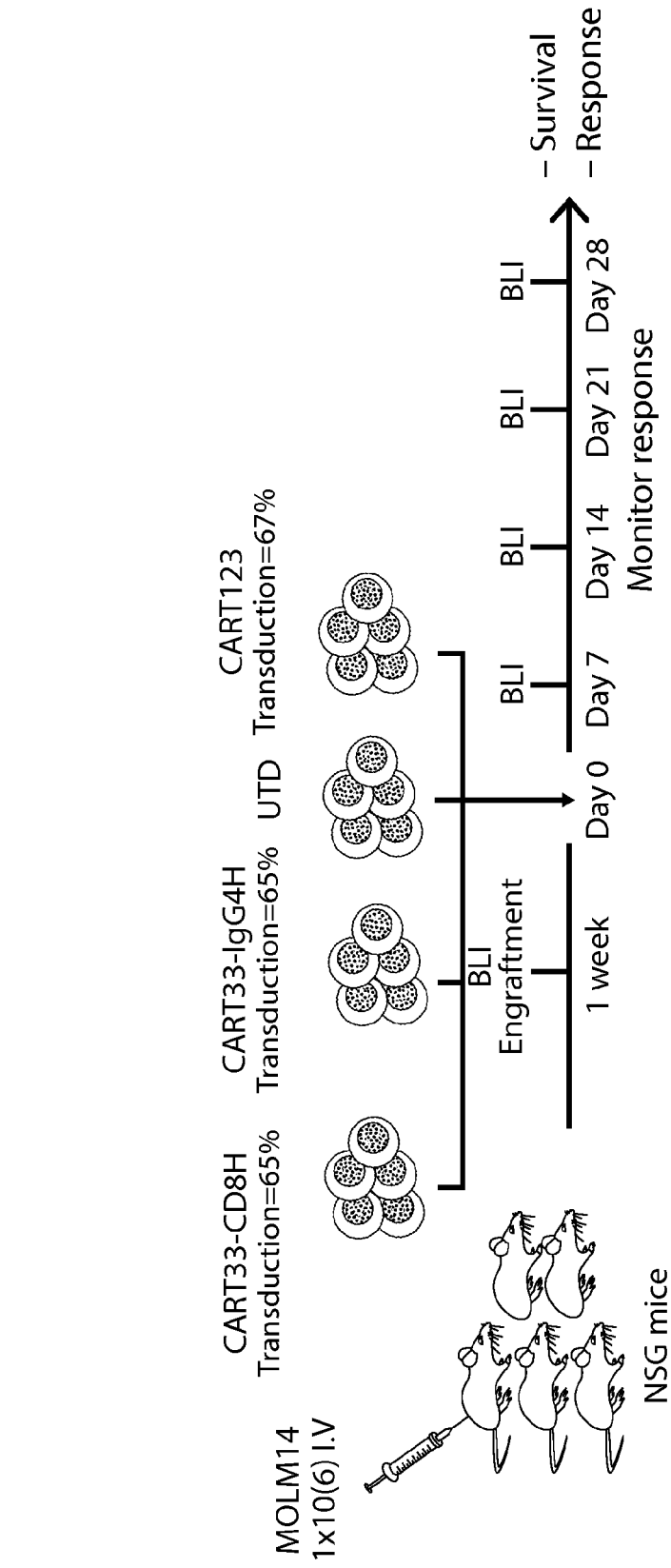

MOLM14
1x10(6) I.V
NSG mice
CART33-CD8H
Transduction=65%
CART33-IgG4H
Transduction=65%
UTD
CART123
Transduction=67%
BLI
Engraftment
1 week
Day 0
Day 7
Day 14
Day 21
Day 28
BLI
BLI
BLI
BLI
Monitor response
– Survival
– Response

FIG. 11

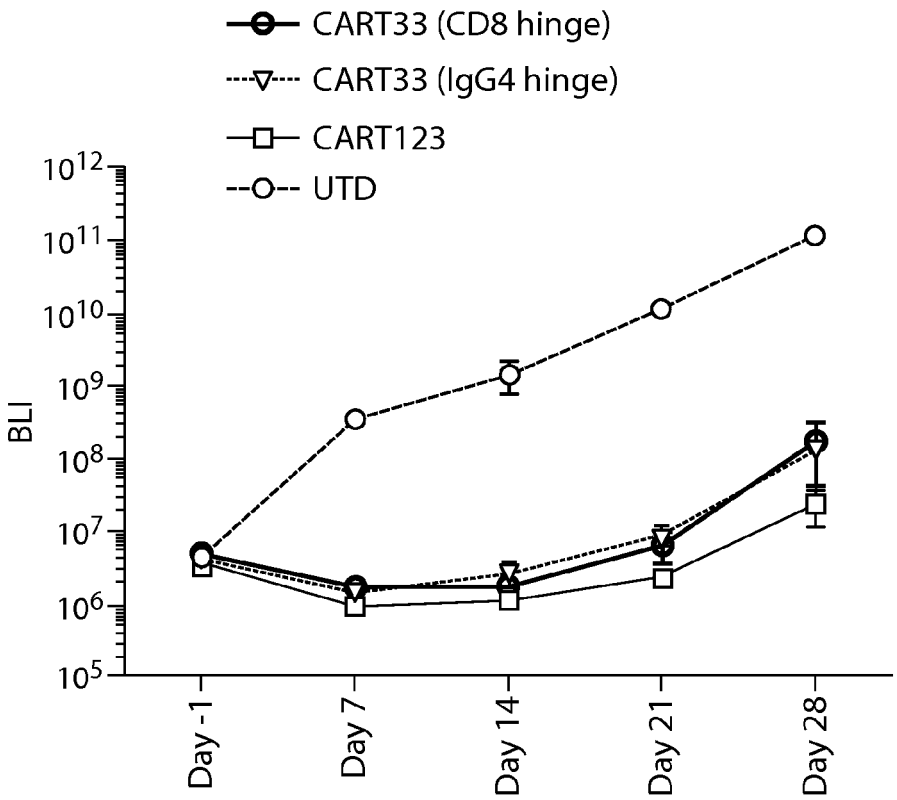

CART33 (CD8 hinge)
CART33 (IgG4 hinge)
CART123
UTD
BLI
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
Day -1
Day 7
Day 14
Day 21
Day 28

FIG. 12

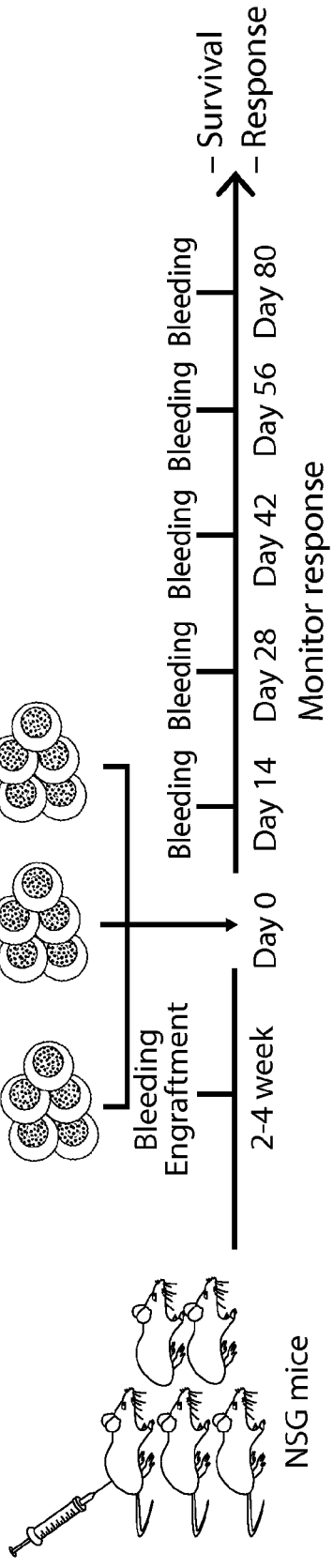

CART33
Transduction=65%
UTD
CART123
Transduction=67%
Bleeding
Engraftment
2-4 week
Day 0
Day 14
Day 28
Day 42
Day 56
Day 80
Bleeding
Bleeding
Bleeding
Bleeding
Bleeding
Monitor response
– Survival
– Response
NSG mice

FIG. 13

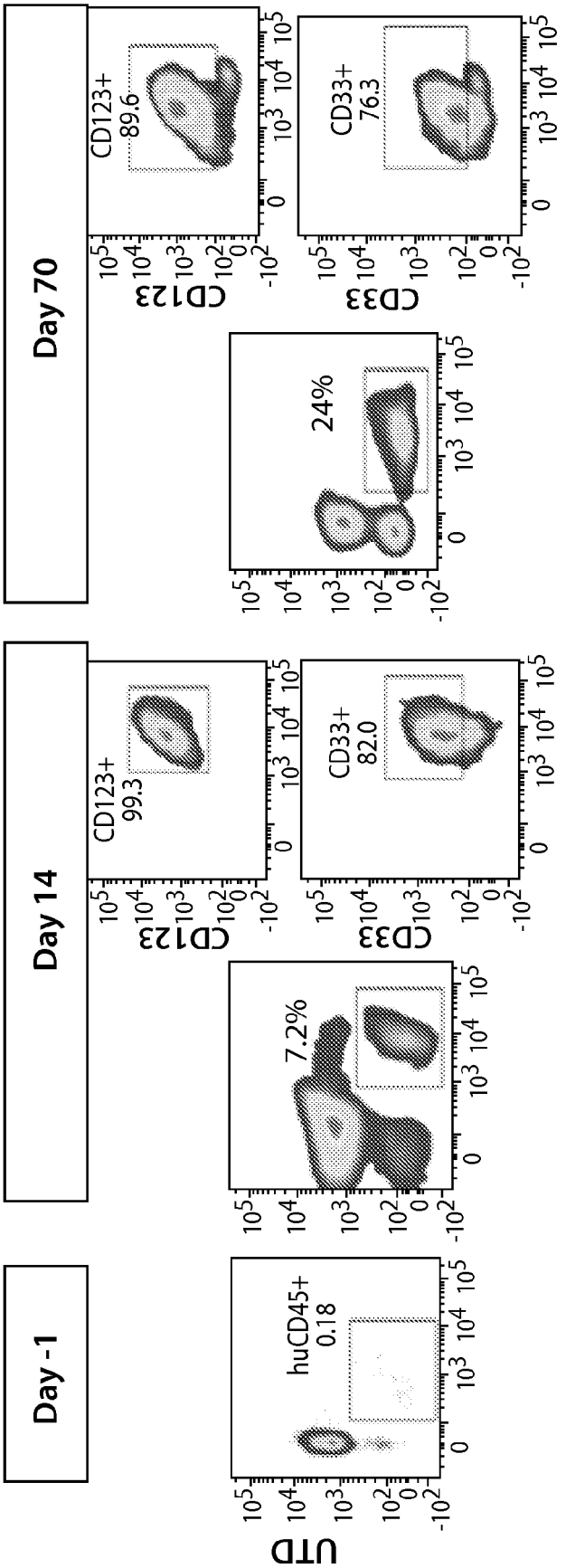

Day -1
Day 14
Day 70
UTD
huCD45+
0.18
7.2%
CD123+
99.3
CD33+
82.0
24%
CD123+
89.6
CD33+
76.3
CD123
CD33

FIG. 14-1

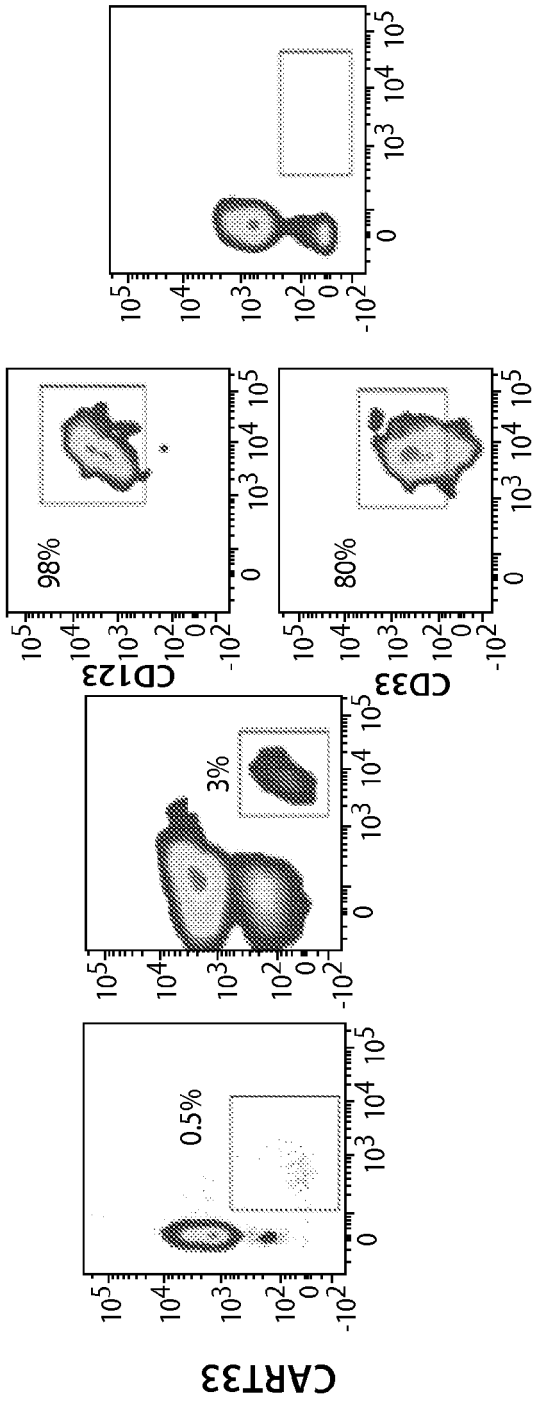


FIG. 14-2

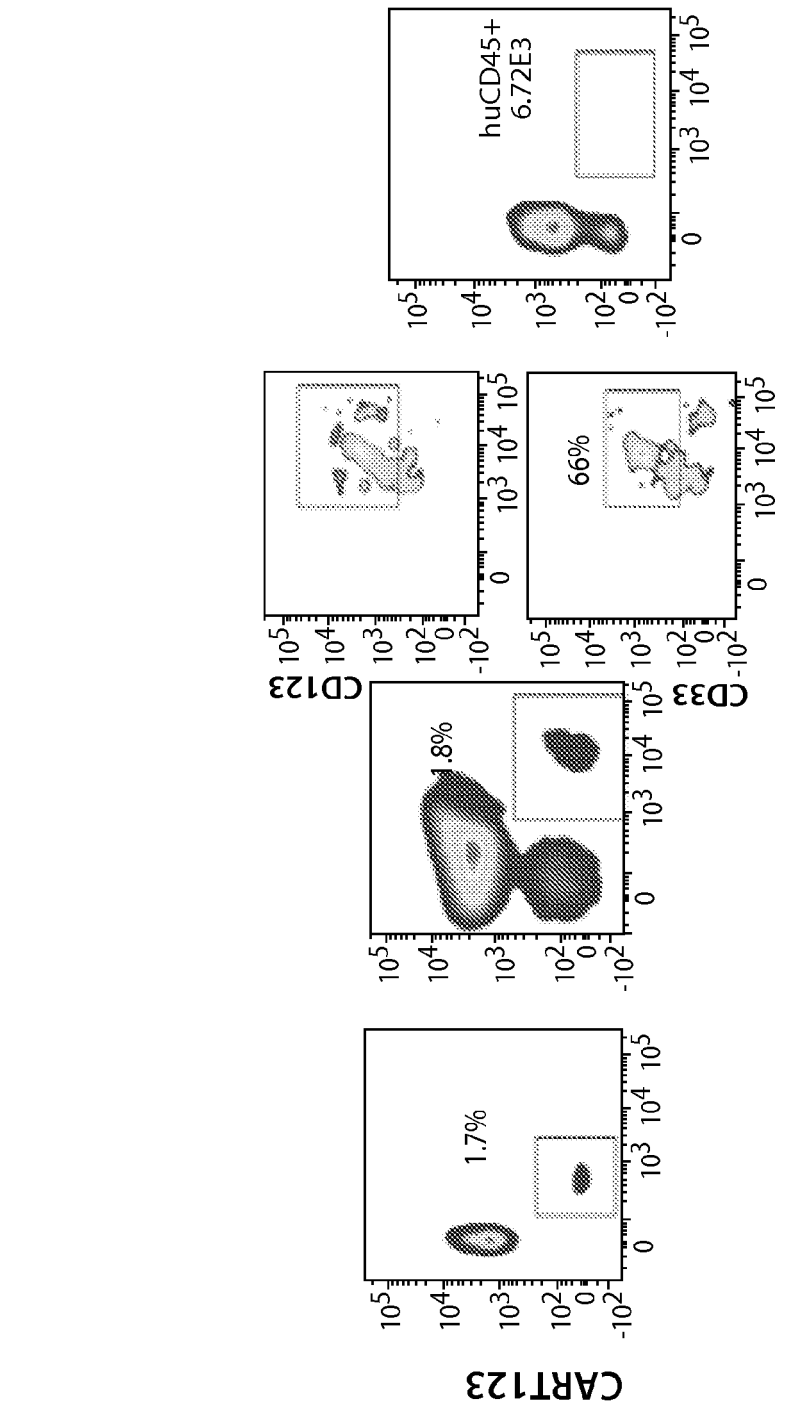
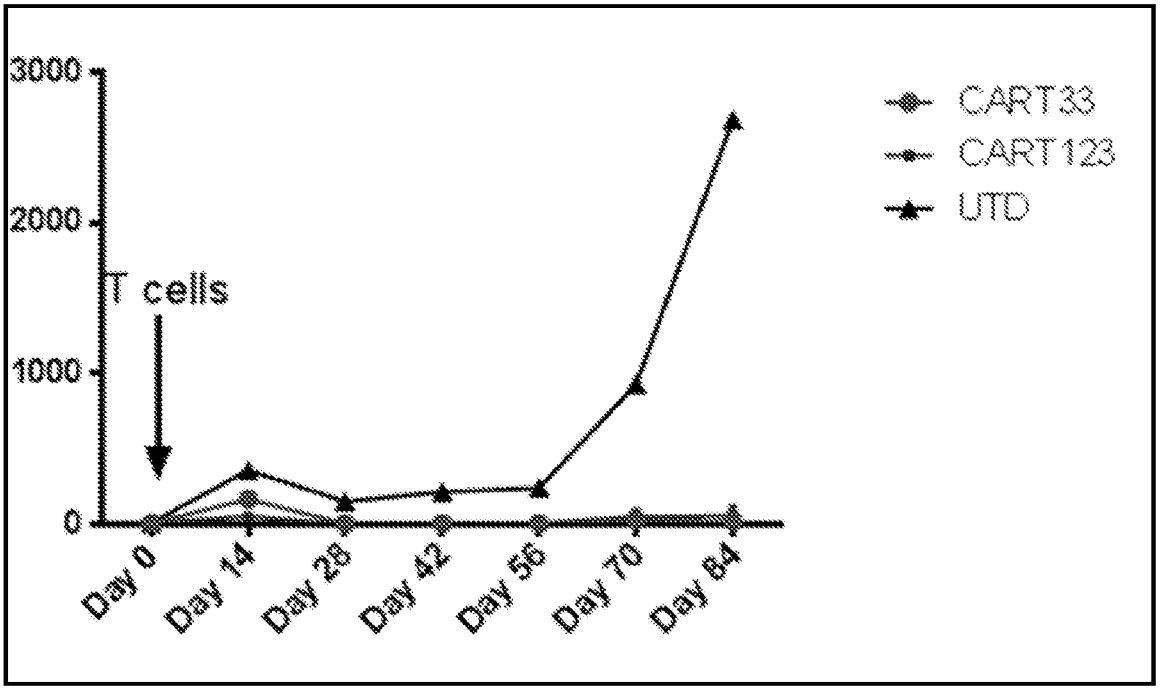


FIG. 14-3

FIG. 15


3000
2000
1000
0
T cells
Day 0
Day 14
Day 28
Day 42
Day 56
Day 70
Day 84
CART33
CART123
UTD

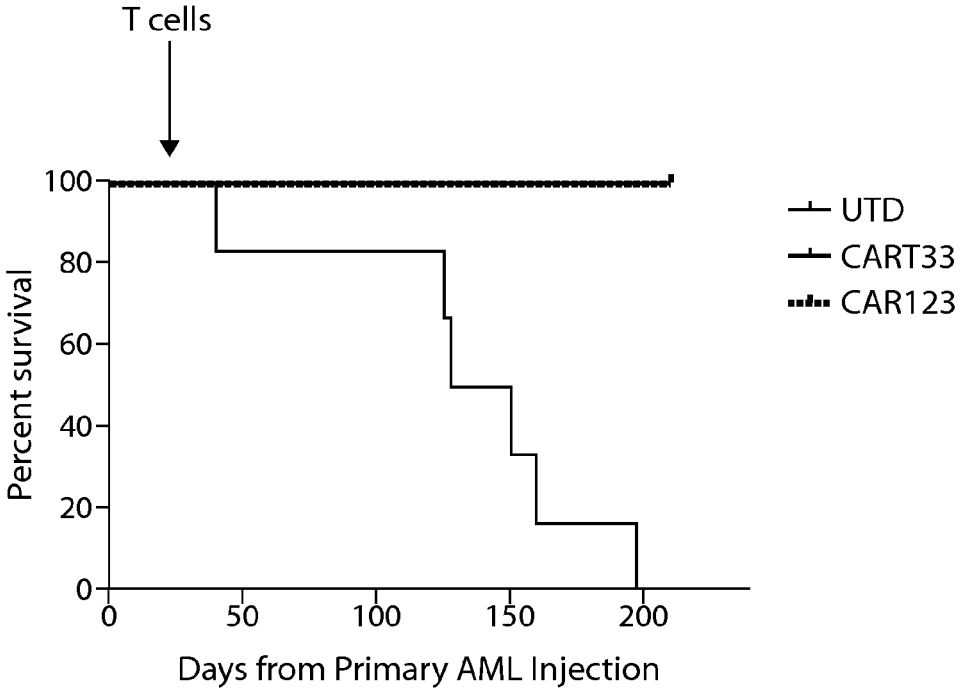

T cells
100
80
60
40
20
0
Percent survival
0
50
100
150
200
Days from Primary AML Injection
UTD
CART33
CAR123

FIG. 16

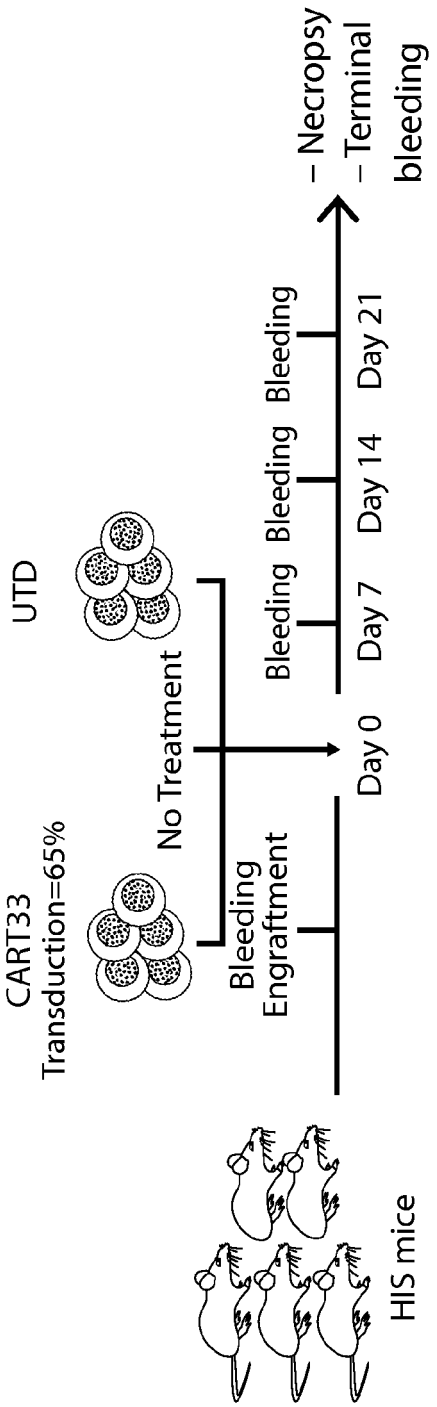

HIS mice
CART33
Transduction=65%
UTD
No Treatment
Bleeding
Engraftment
Day 0
Day 7
Day 14
Day 21
Bleeding
Bleeding
Bleeding
– Necropsy
– Terminal
bleeding

FIG. 17

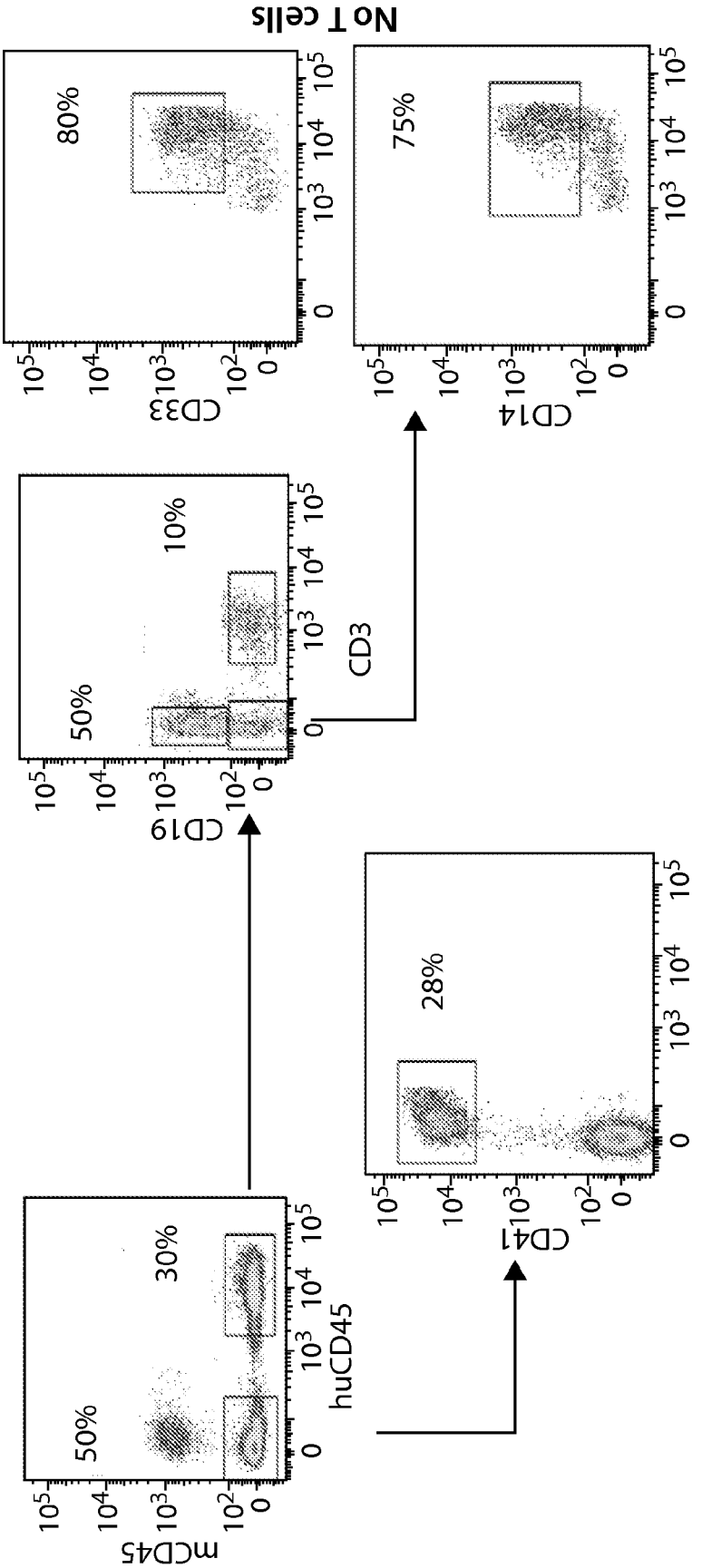

No T cells
mCD45
huCD45
50%
30%
CD19
50%
10%
CD3
CD33
80%
CD14
75%
CD41
28%

FIG. 18-1

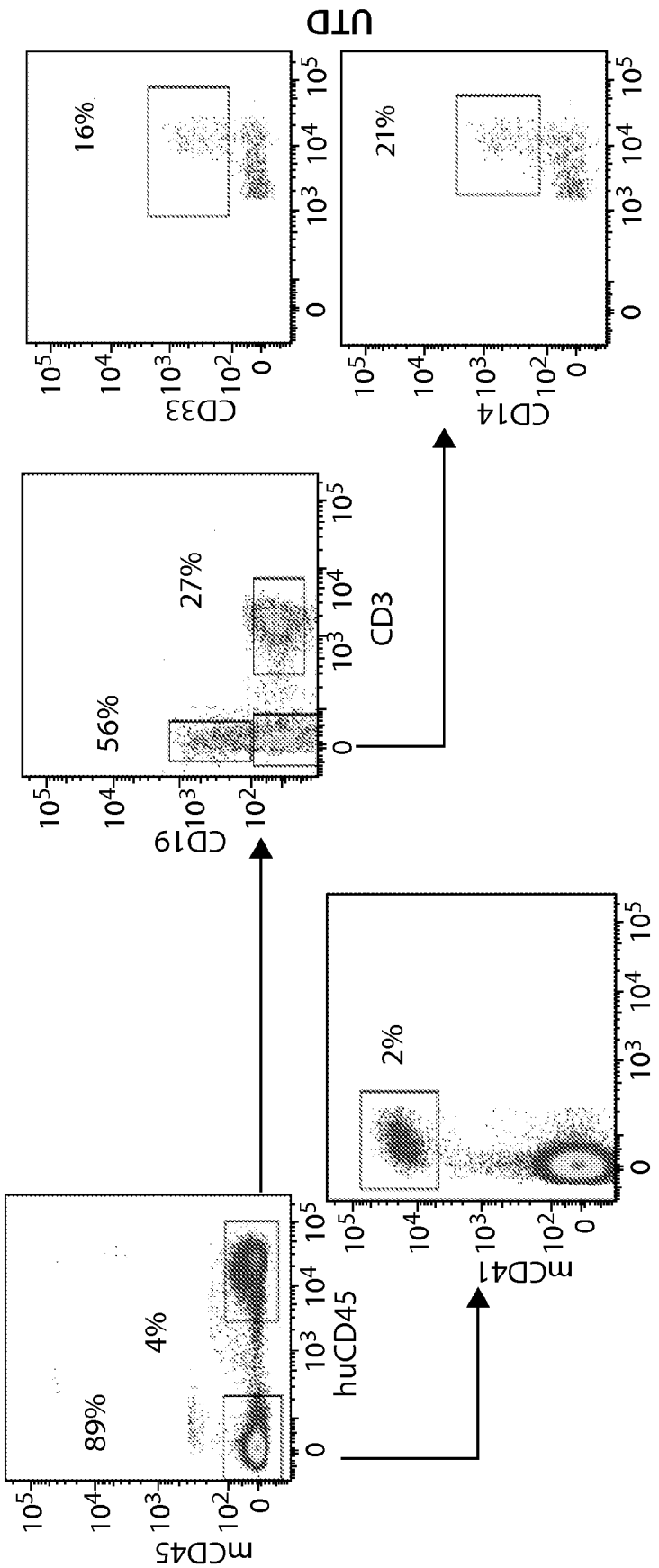


FIG. 18-2

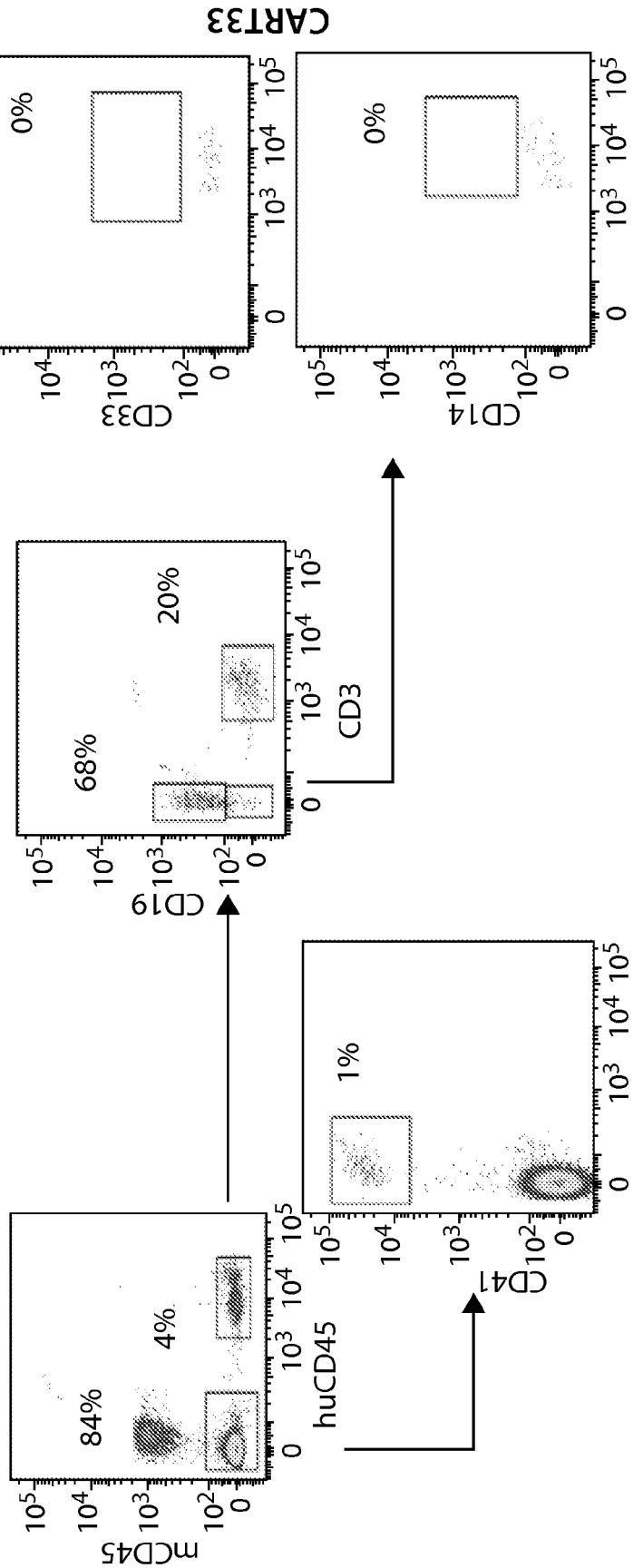


FIG. 18-3

FIG. 19

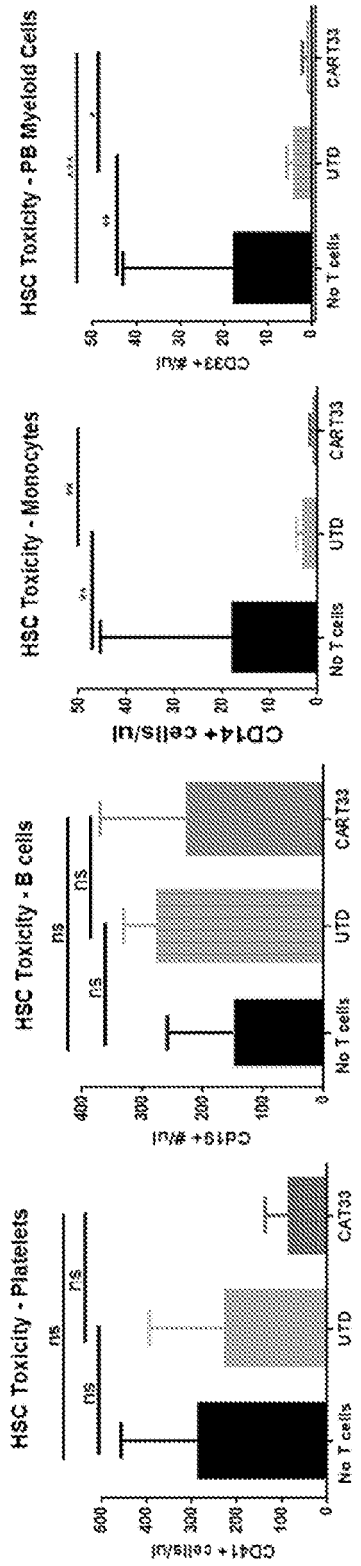
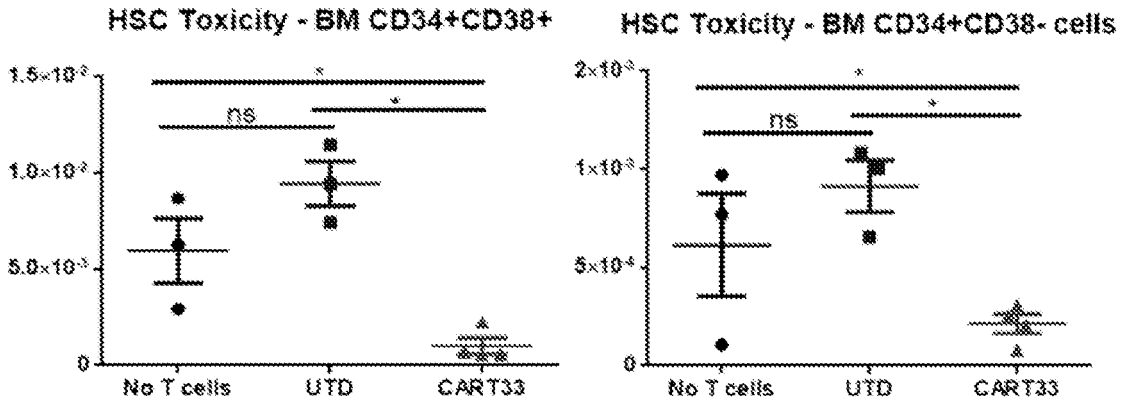

HSC Toxicity - Platelets
CD41+ cells/ul
HSC Toxicity - B cells
CD19+ #/ul
HSC Toxicity - Monocytes
CD14+ cells/ul
HSC Toxicity - PB Myeloid Cells
CD33+ #/ul
No T cells
UTD
CAR33
ns
**
***

FIG. 20


HSC Toxicity - BM CD34+CD38+
1.5×10⁴
1.0×10⁴
5.0×10³
0
ns
*
*
No T cells
UTD
CART33
HSC Toxicity - BM CD34+CD38- cells
2×10⁴
1×10⁴
5×10³
0
ns
*
*
No T cells
UTD
CART33

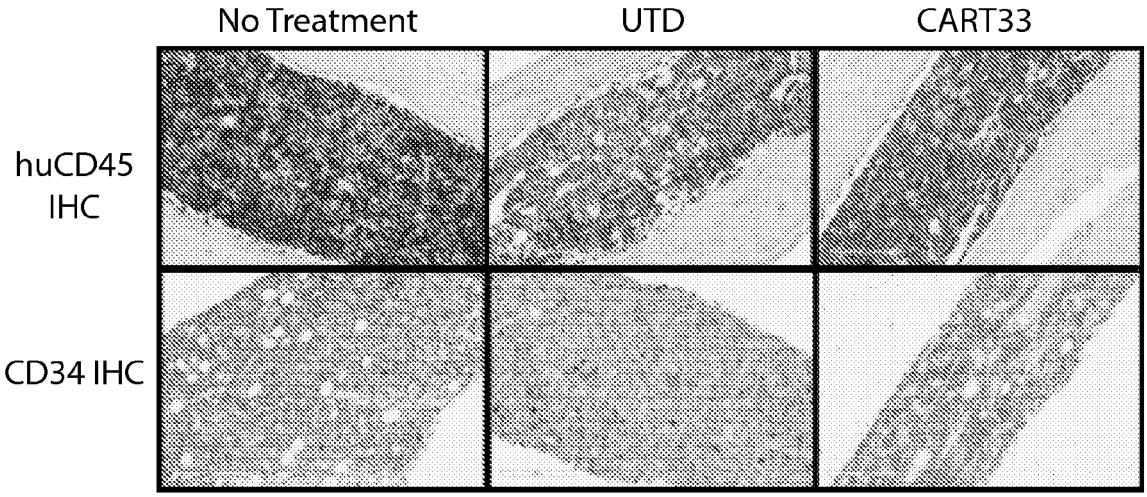


FIG. 21

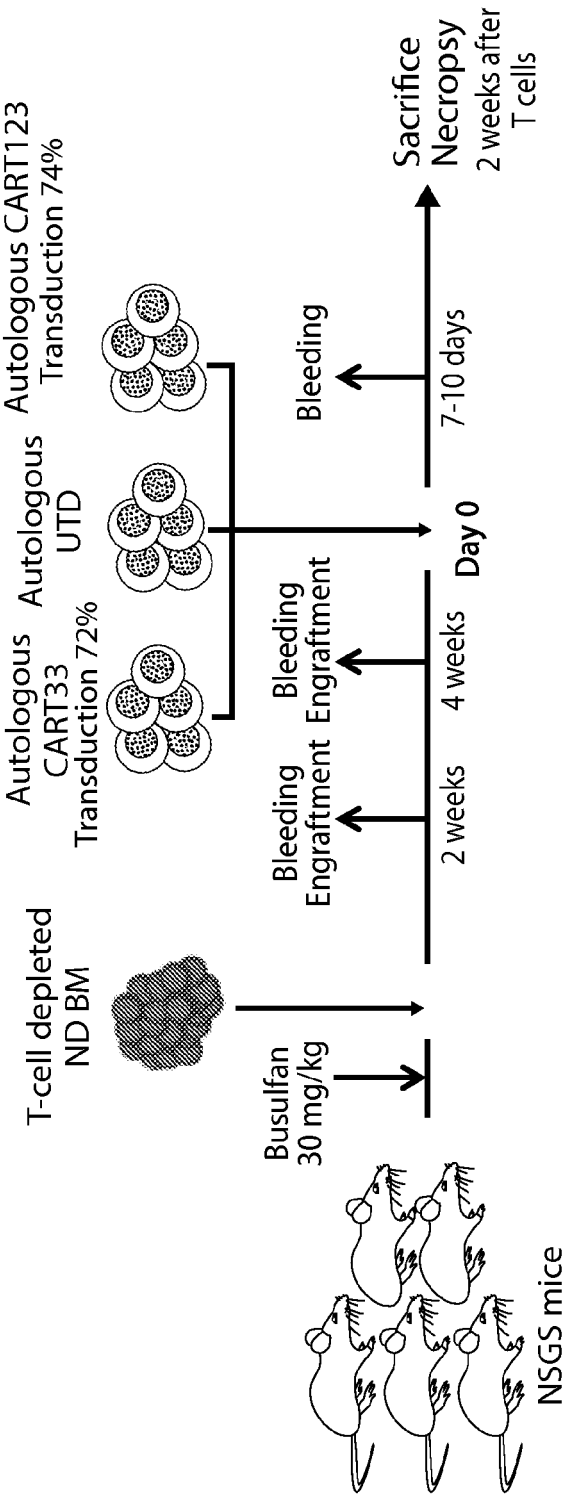

T-cell depleted
ND BM
Busulfan
30 mg/kg
NSGS mice
Autologous
CART33
Transduction 72%
Autologous
UTD
Autologous CART123
Transduction 74%
Bleeding
Engraftment
2 weeks
Bleeding
Engraftment
4 weeks
Day 0
Bleeding
7-10 days
Sacrifice
Necropsy
2 weeks after
T cells

FIG. 22

FIG. 23

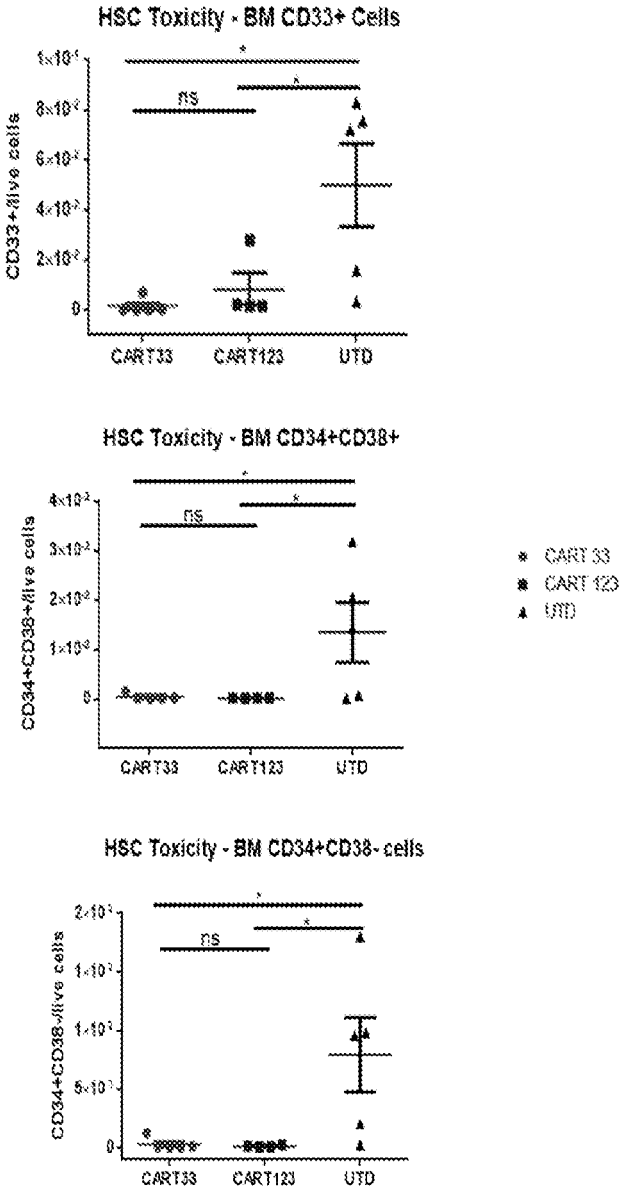

HSC Toxicity - BM CD33+ Cells
CD33+/live cells
ns
CART33
CART123
UTD
HSC Toxicity - BM CD34+CD38+
CD34+CD38+/live cells
ns
CART33
CART123
UTD
CART 33
CART 123
UTD
HSC Toxicity - BM CD34+CD38- cells
CD34+CD38-/live cells
ns
CART33
CART123
UTD

FIG. 24

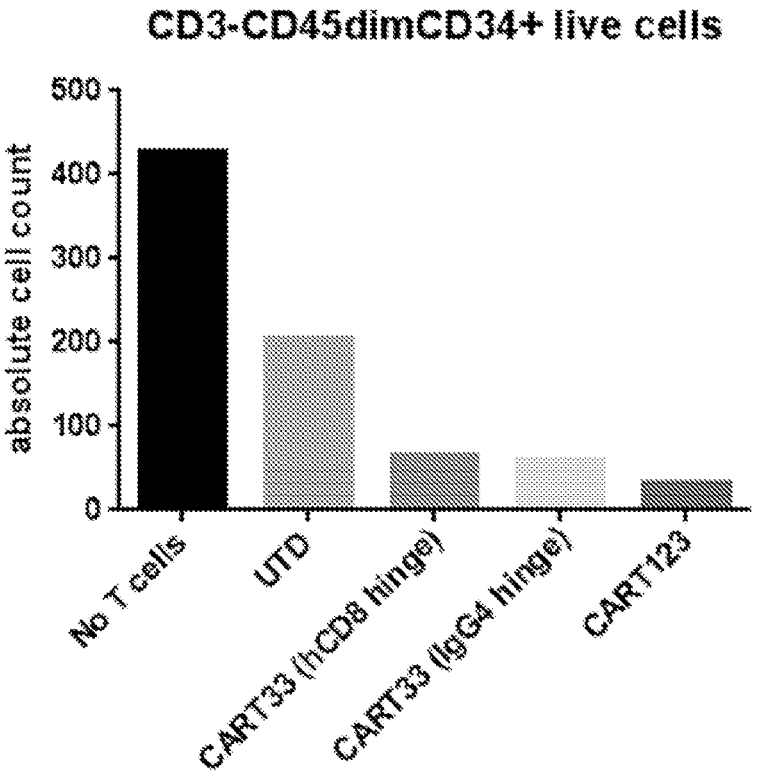

CD3-CD45dimCD34+ live cells
absolute cell count
500
400
300
200
100
0
No T cells
UTD
CART33 (hCD8 hinge)
CART33 (IgG4 hinge)
CART123

FIG. 25

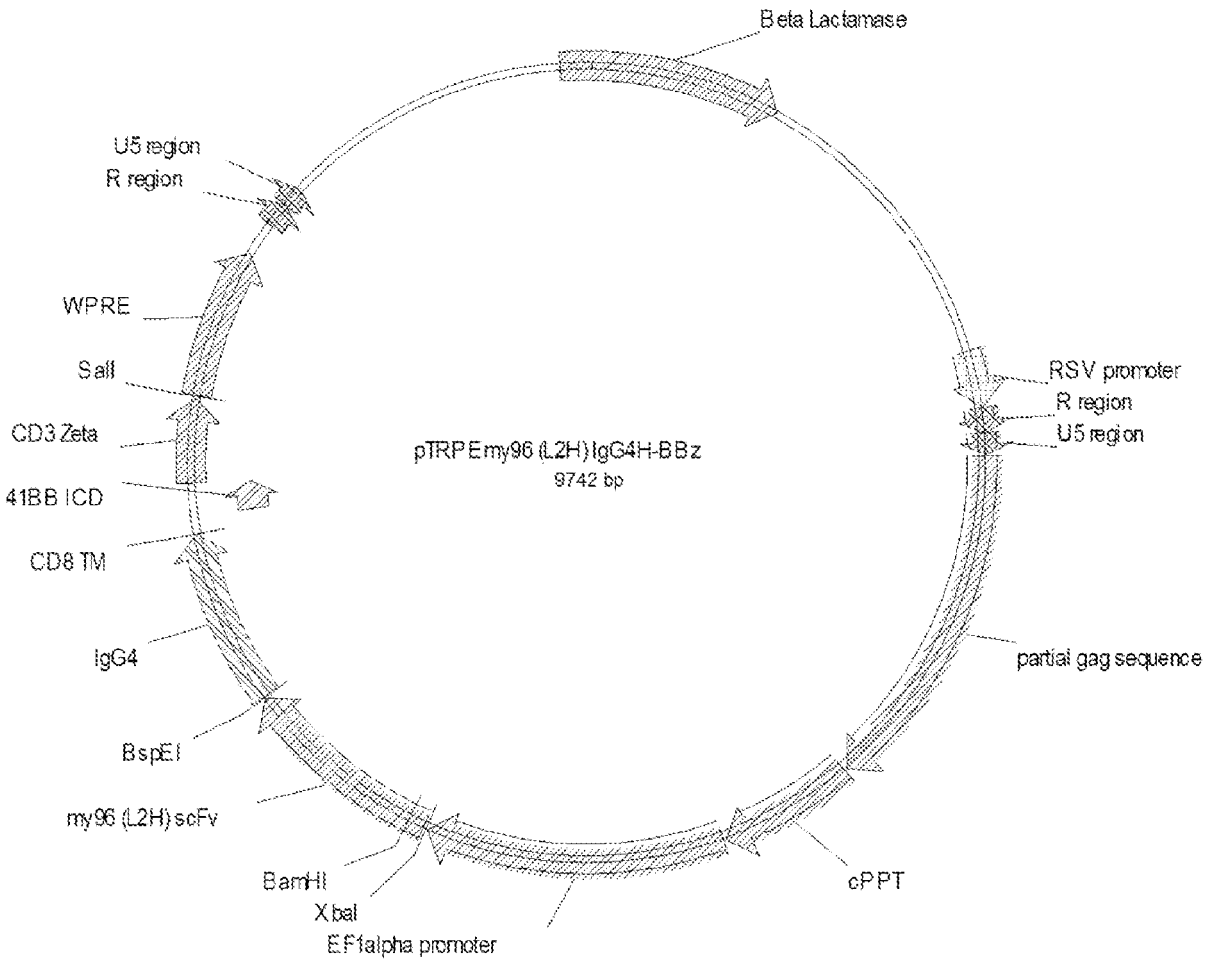
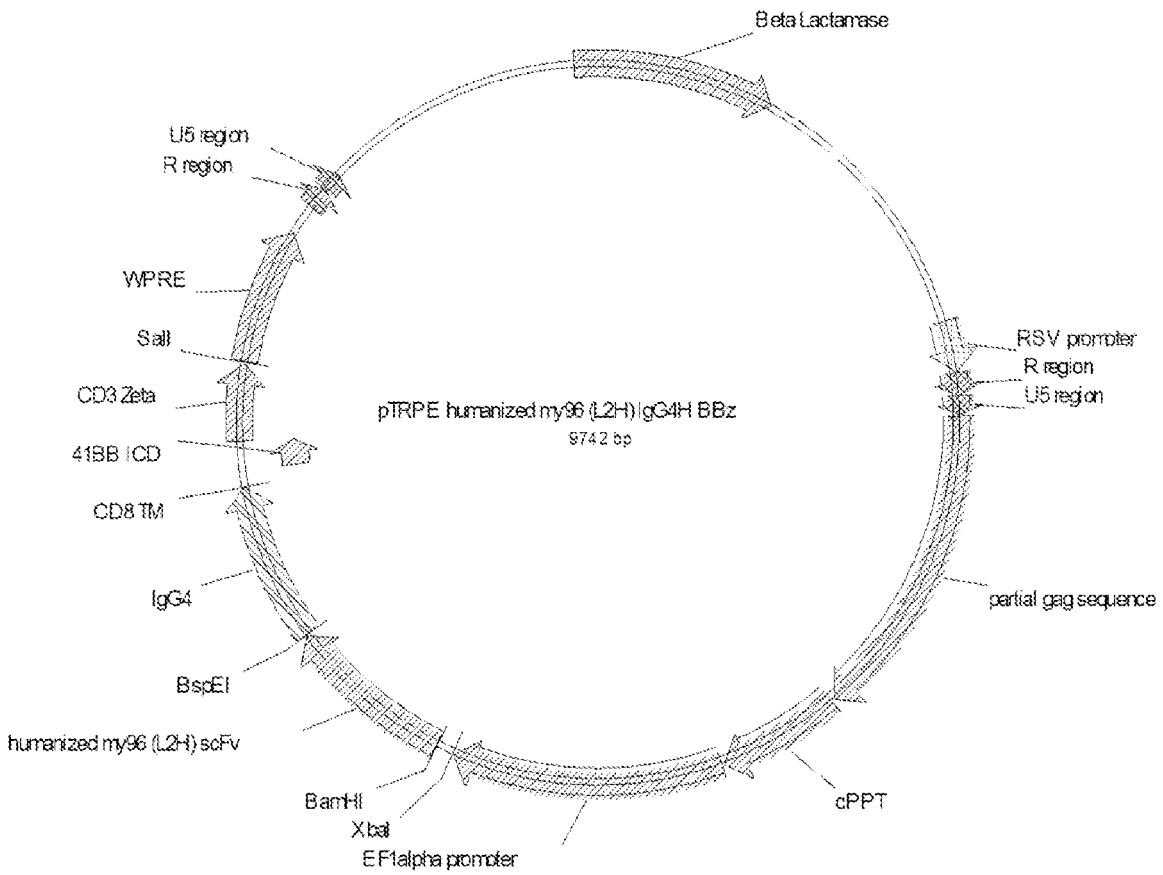

Beta Lactamase
U5 region
R region
WPRE
SalI
CD3 Zeta
41BB ICD
CD8 TM
IgG4
BspEI
my96 (L2H) scFv
BamHI
XbaI
EF1alpha promoter
cPPT
partial gag sequence
RSV promoter
R region
U5 region
pTRPEmy96 (L2H) IgG4H-BBz
9742 bp

FIG. 26


Beta Lactamase
U5 region
R region
WPRE
SalI
CD3 Zeta
41BB ICD
CD8 TM
IgG4
BspEI
humanized my96 (L2H) scFv
BamHI
XbaI
EF1alpha promoter
cPPT
partial gag sequence
RSV promoter
R region
U5 region
pTRPE humanized my96 (L2H) IgG4H BBz
9742 bp

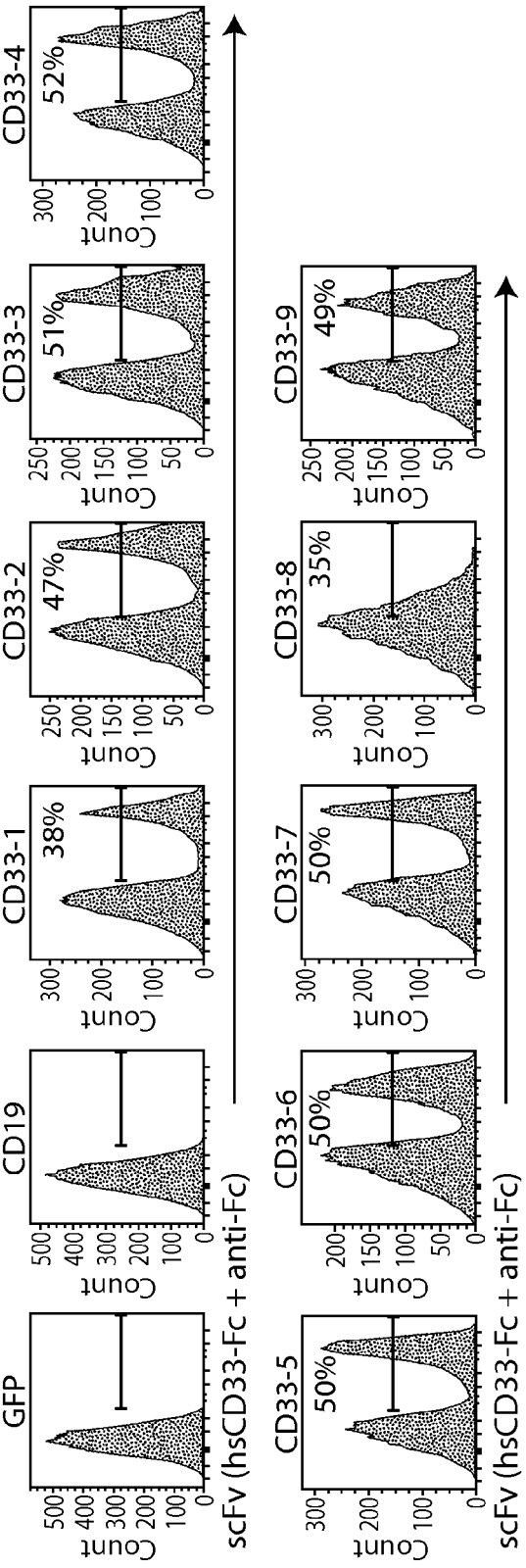

GFP
CD19
CD33-1
38%
CD33-2
47%
CD33-3
51%
CD33-4
52%
CD33-5
50%
CD33-6
50%
CD33-7
50%
CD33-8
35%
CD33-9
49%
Count
scFv (hsCD33-Fc + anti-Fc)

FIG. 27

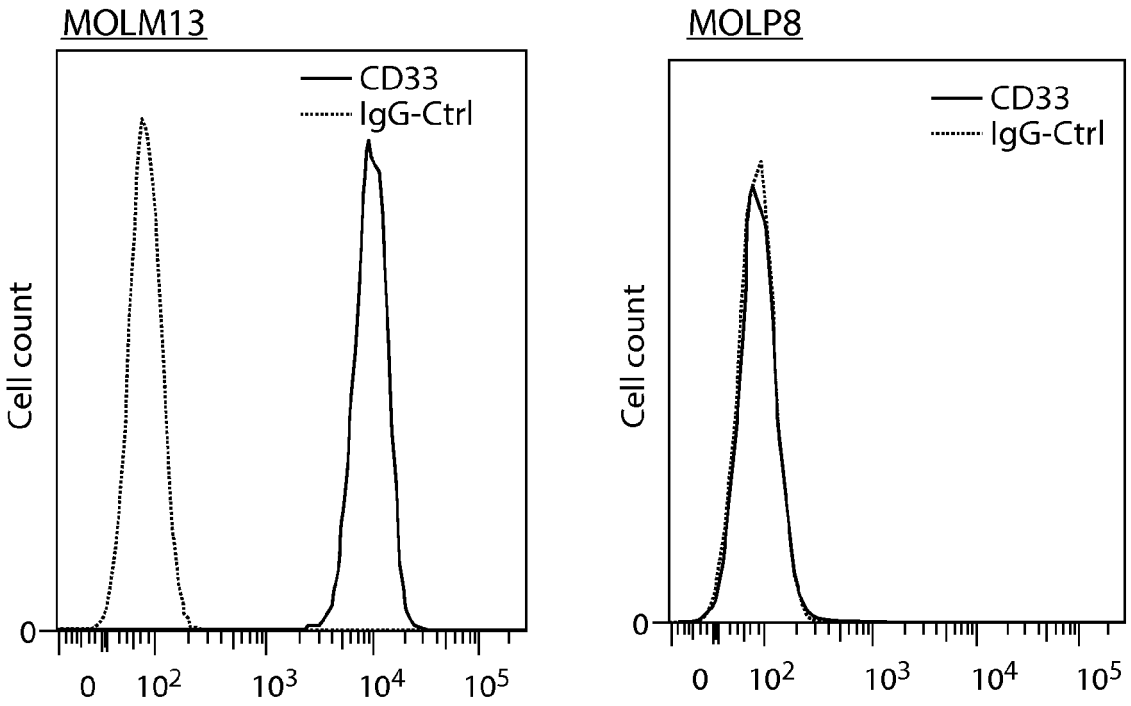

MOLM13
CD33
IgG-Ctrl
Cell count
0
0
10^2
10^3
10^4
10^5
MOLP8
CD33
IgG-Ctrl
Cell count
0
0
10^2
10^3
10^4
10^5

FIG. 28A

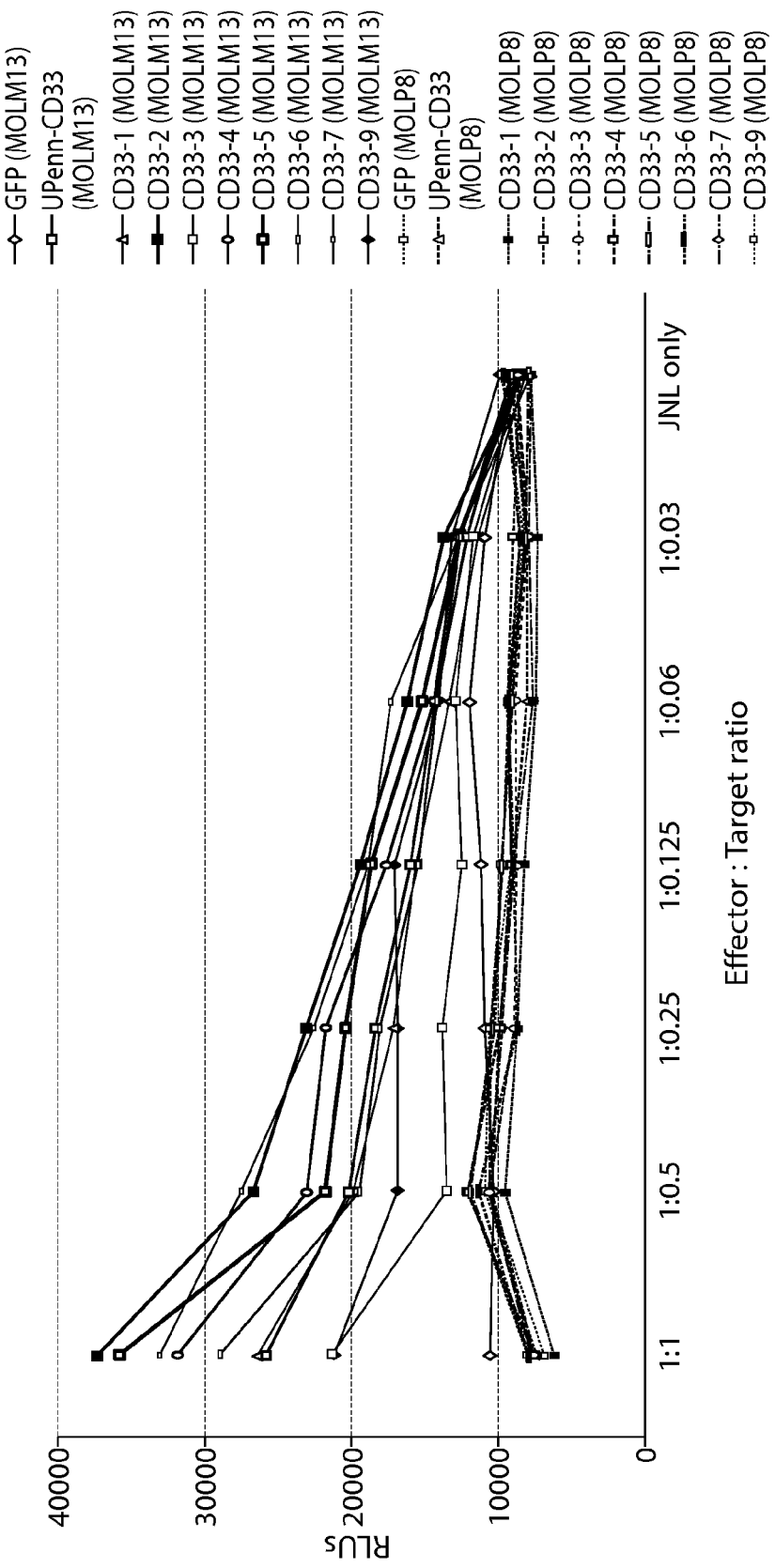

GFP (MOLM13)
UPenn-CD33 (MOLM13)
CD33-1 (MOLM13)
CD33-2 (MOLM13)
CD33-3 (MOLM13)
CD33-4 (MOLM13)
CD33-5 (MOLM13)
CD33-6 (MOLM13)
CD33-7 (MOLM13)
CD33-9 (MOLM13)
GFP (MOLP8)
UPenn-CD33 (MOLP8)
CD33-1 (MOLP8)
CD33-2 (MOLP8)
CD33-3 (MOLP8)
CD33-4 (MOLP8)
CD33-5 (MOLP8)
CD33-6 (MOLP8)
CD33-7 (MOLP8)
CD33-9 (MOLP8)
RLUs
40000
30000
20000
10000
0
1:1
1:0.5
1:0.25
1:0.125
1:0.06
1:0.03
JNL only
Effector : Target ratio

FIG. 28B

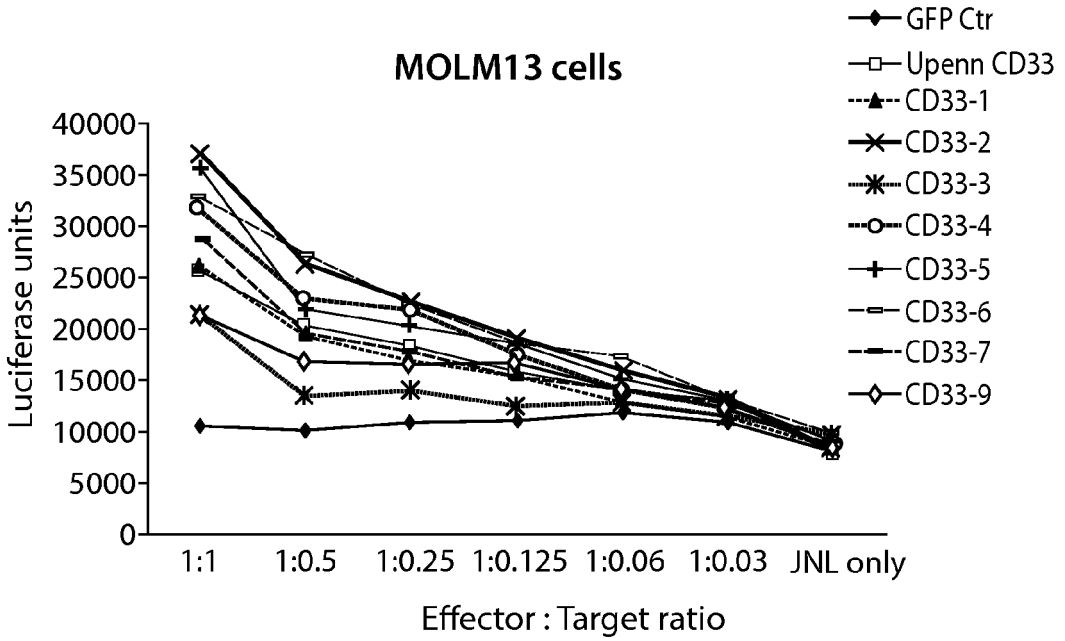


FIG. 28C

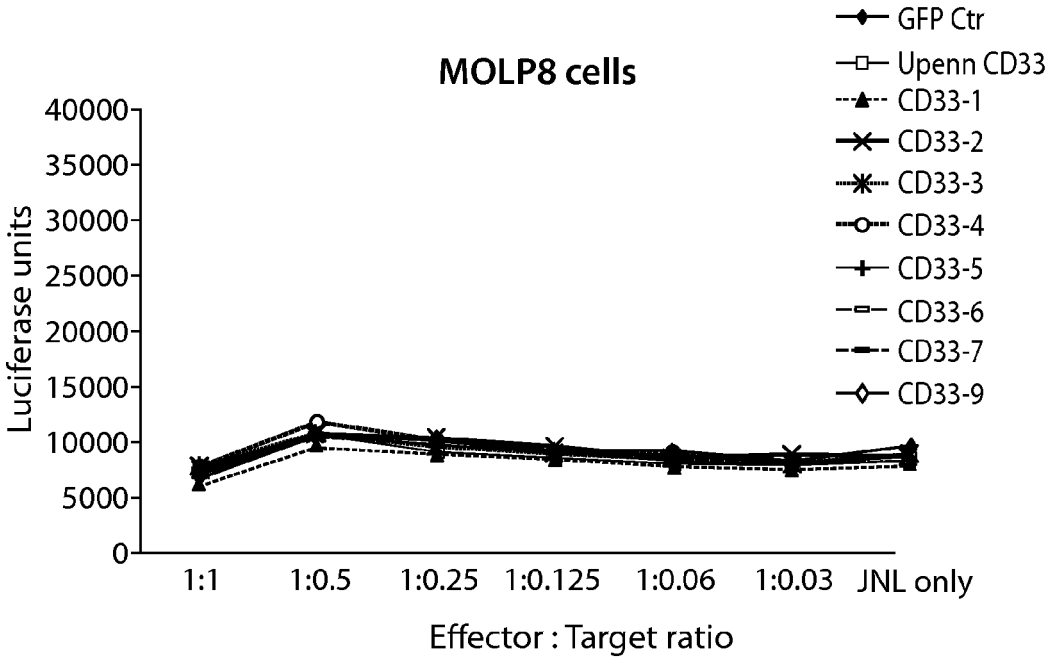


FIG. 28D

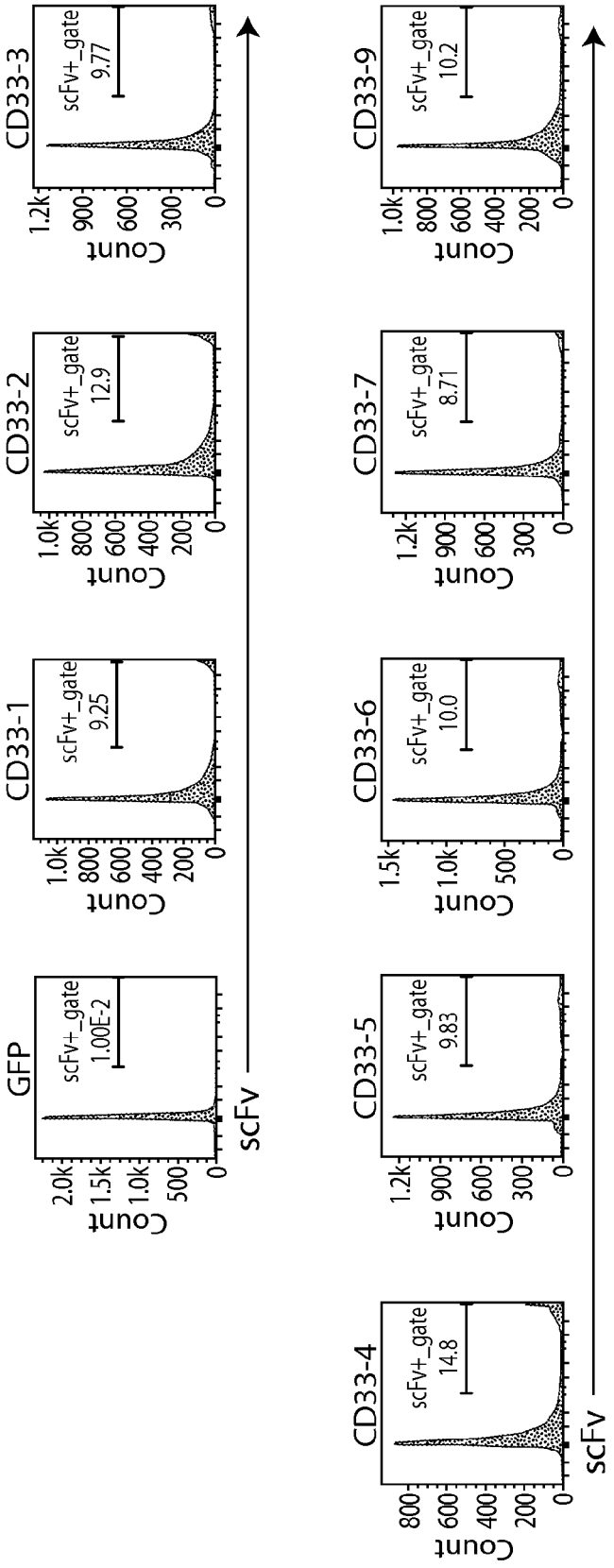

GFP
scFv+_gate
1.00E-2
CD33-1
scFv+_gate
9.25
CD33-2
scFv+_gate
12.9
CD33-3
scFv+_gate
9.77
CD33-4
scFv+_gate
14.8
CD33-5
scFv+_gate
9.83
CD33-6
scFv+_gate
10.0
CD33-7
scFv+_gate
8.71
CD33-9
scFv+_gate
10.2
Count
scFv

FIG. 29A

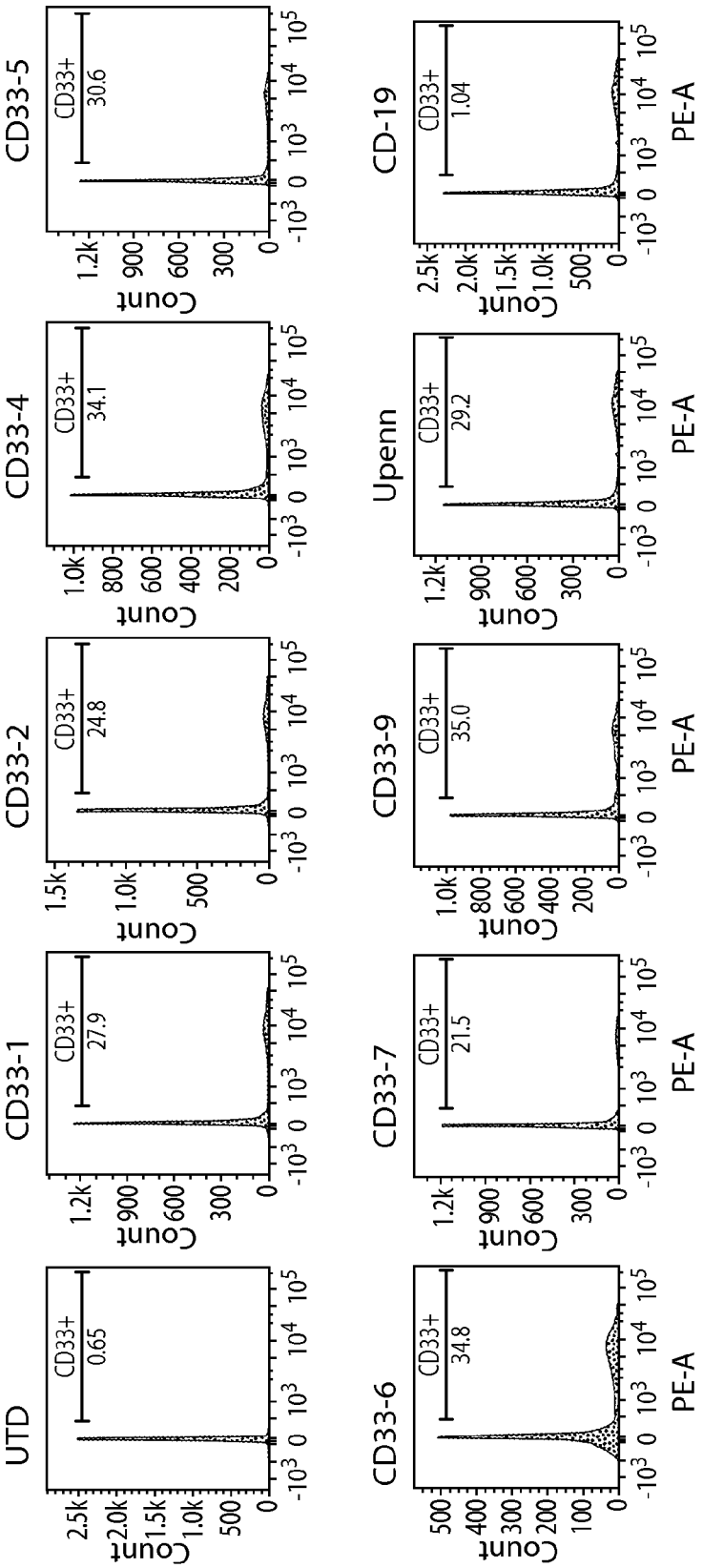
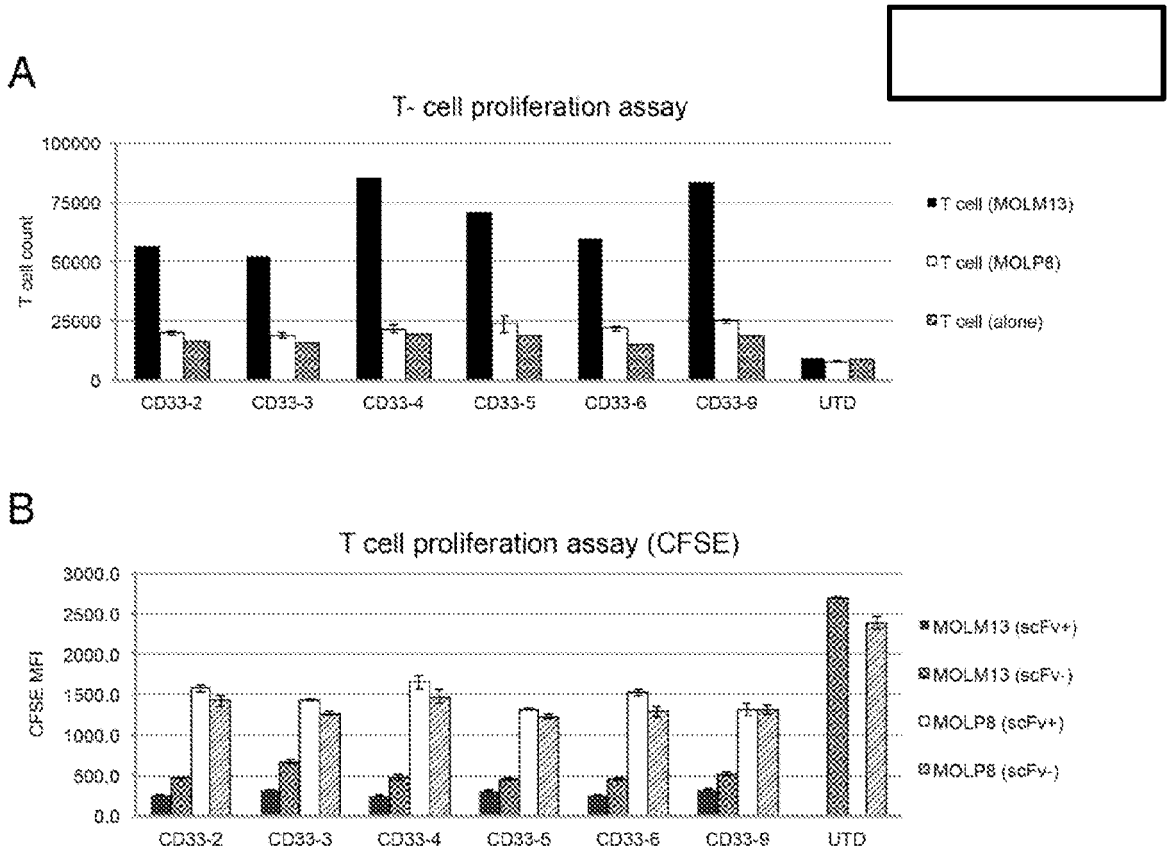

UTD
CD33+
0.65
CD33-1
CD33+
27.9
CD33-2
CD33+
24.8
CD33-4
CD33+
34.1
CD33-5
CD33+
30.6
CD33-6
CD33+
34.8
CD33-7
CD33+
21.5
CD33-9
CD33+
35.0
Upenn
CD33+
29.2
CD-19
CD33+
1.04
Count
PE-A

FIG. 29B

FIG. 30


A
T- cell proliferation assay
T cell count
100000
75000
50000
25000
0
CD33-2
CD33-3
CD33-4
CD33-5
CD33-6
CD33-9
UTD
T cell (MOLM13)
T cell (MOLP8)
T cell (alone)
B
T cell proliferation assay (CFSE)
CFSE MFI
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
CD33-2
CD33-3
CD33-4
CD33-5
CD33-6
CD33-9
UTD
MOLM13 (scFv+)
MOLM13 (scFv-)
MOLP8 (scFv+)
MOLP8 (scFv-)

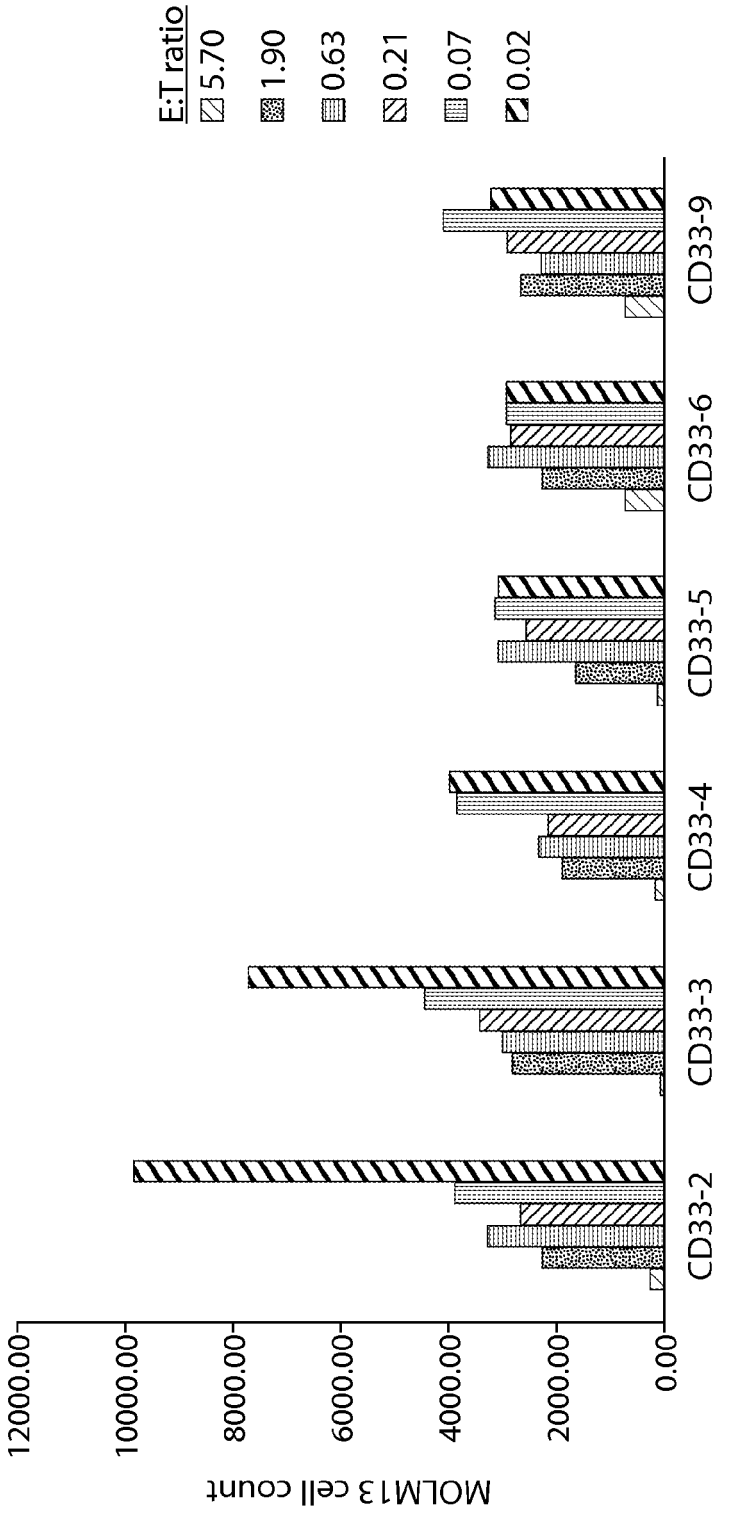

E:T ratio
5.70
1.90
0.63
0.21
0.07
0.02
MOLM13 cell count
12000.00
10000.00
8000.00
6000.00
4000.00
2000.00
0.00
CD33-2
CD33-3
CD33-4
CD33-5
CD33-6
CD33-9

FIG. 31

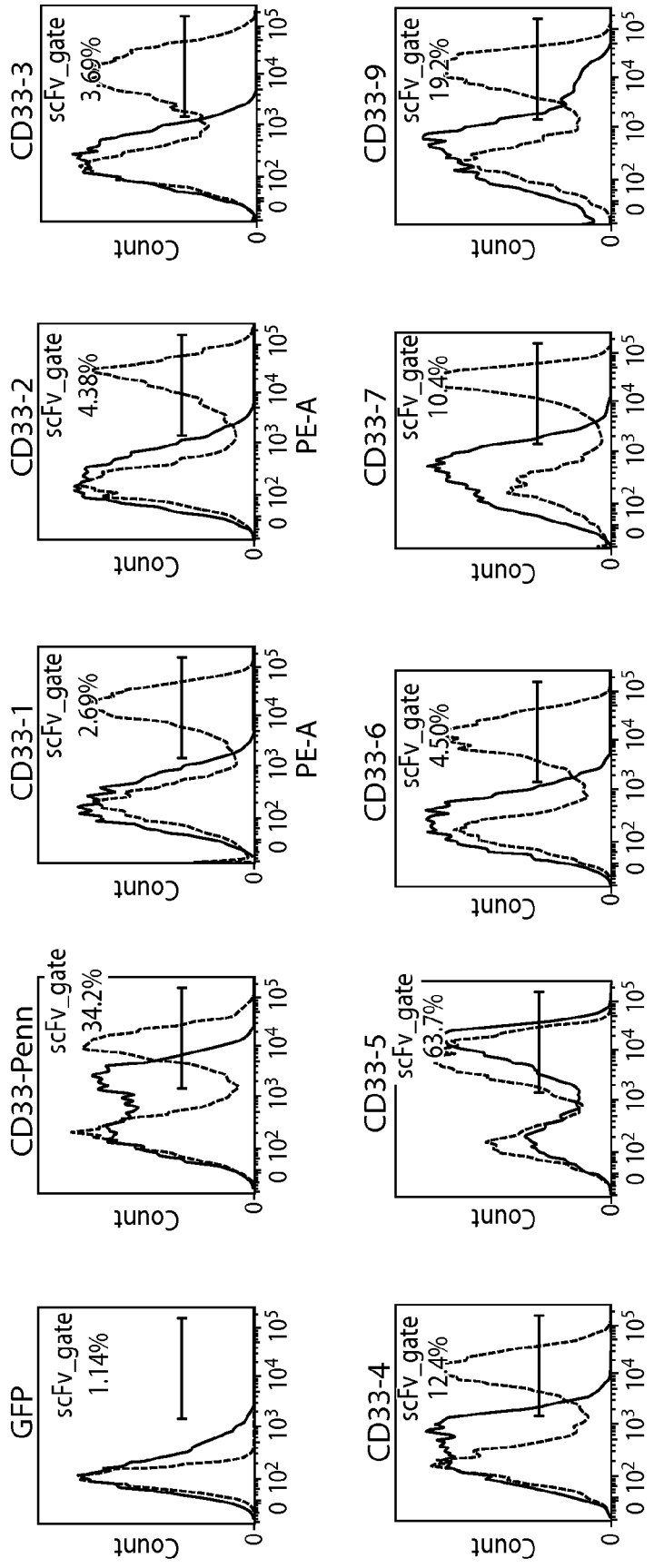

GFP
scFv_gate
1.14%
CD33-Penn
scFv_gate
34.2%
CD33-1
scFv_gate
2.69%
CD33-2
scFv_gate
4.38%
CD33-3
scFv_gate
3.69%
CD33-4
scFv_gate
12.4%
CD33-5
scFv_gate
63.7%
CD33-6
scFv_gate
4.50%
CD33-7
scFv_gate
10.4%
CD33-9
scFv_gate
19.2%
Count
PE-A
0
0 10^2 10^3 10^4 10^5

FIG. 32

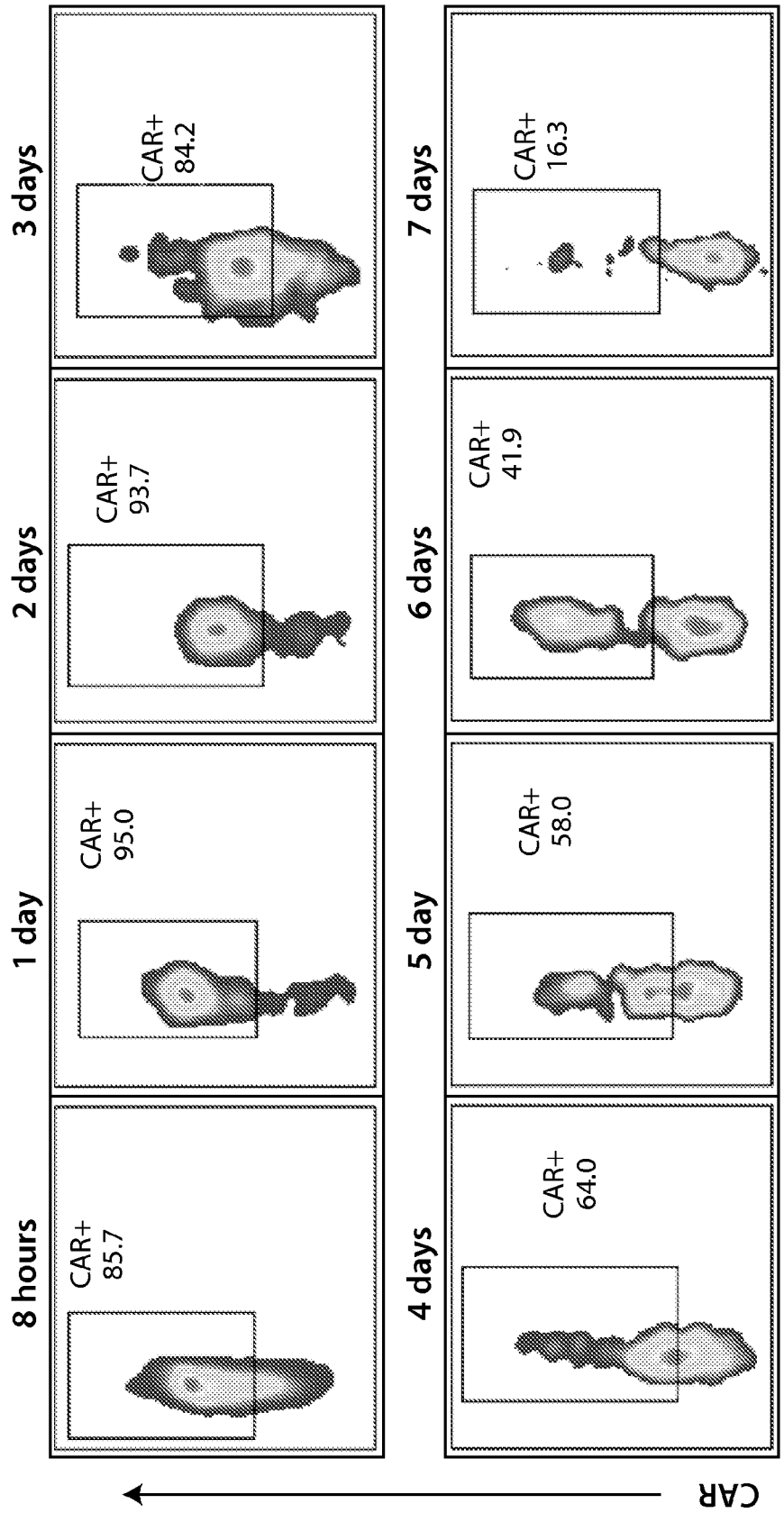

8 hours
CAR+
85.7
1 day
CAR+
95.0
2 days
CAR+
93.7
3 days
CAR+
84.2
4 days
CAR+
64.0
5 day
CAR+
58.0
6 days
CAR+
41.9
7 days
CAR+
16.3
CAR

FIG. 33A

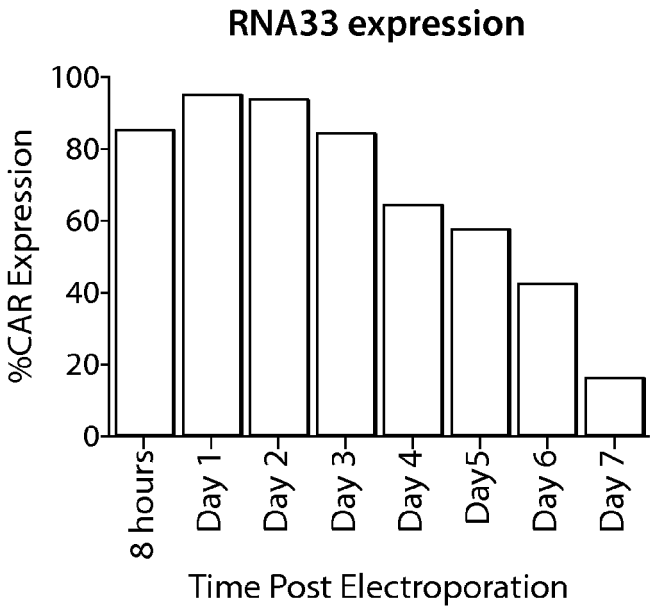

RNA33 expression
%CAR Expression
100
80
60
40
20
0
8 hours
Day 1
Day 2
Day 3
Day 4
Day5
Day 6
Day 7
Time Post Electroporation

FIG. 33B

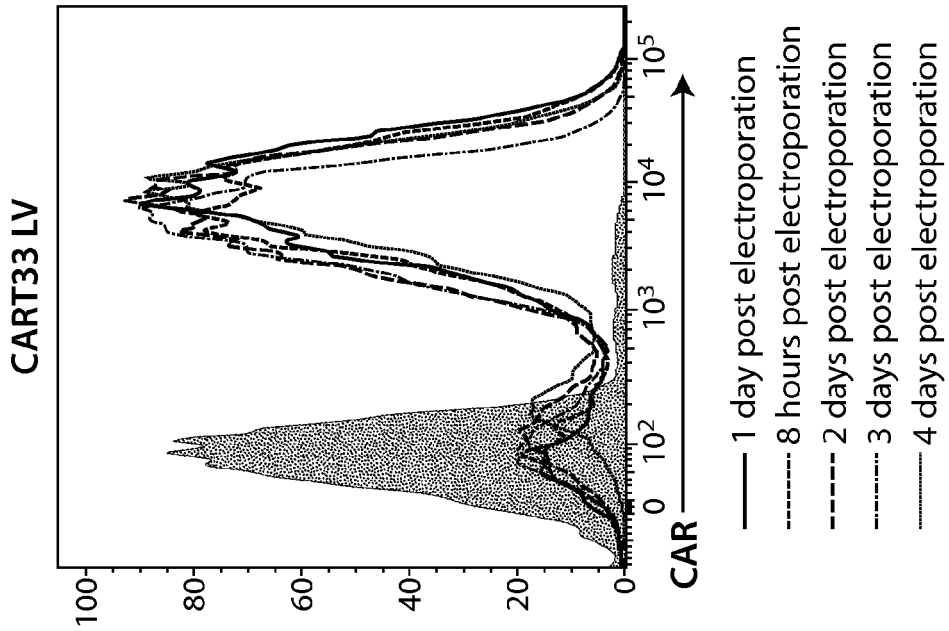


FIG. 34A

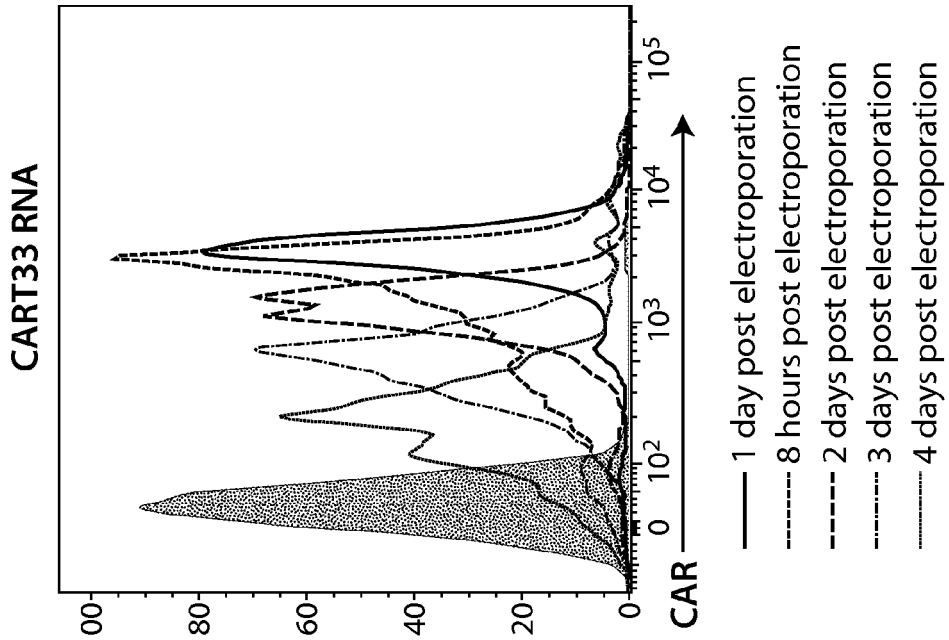


FIG. 34B

FIG. 35

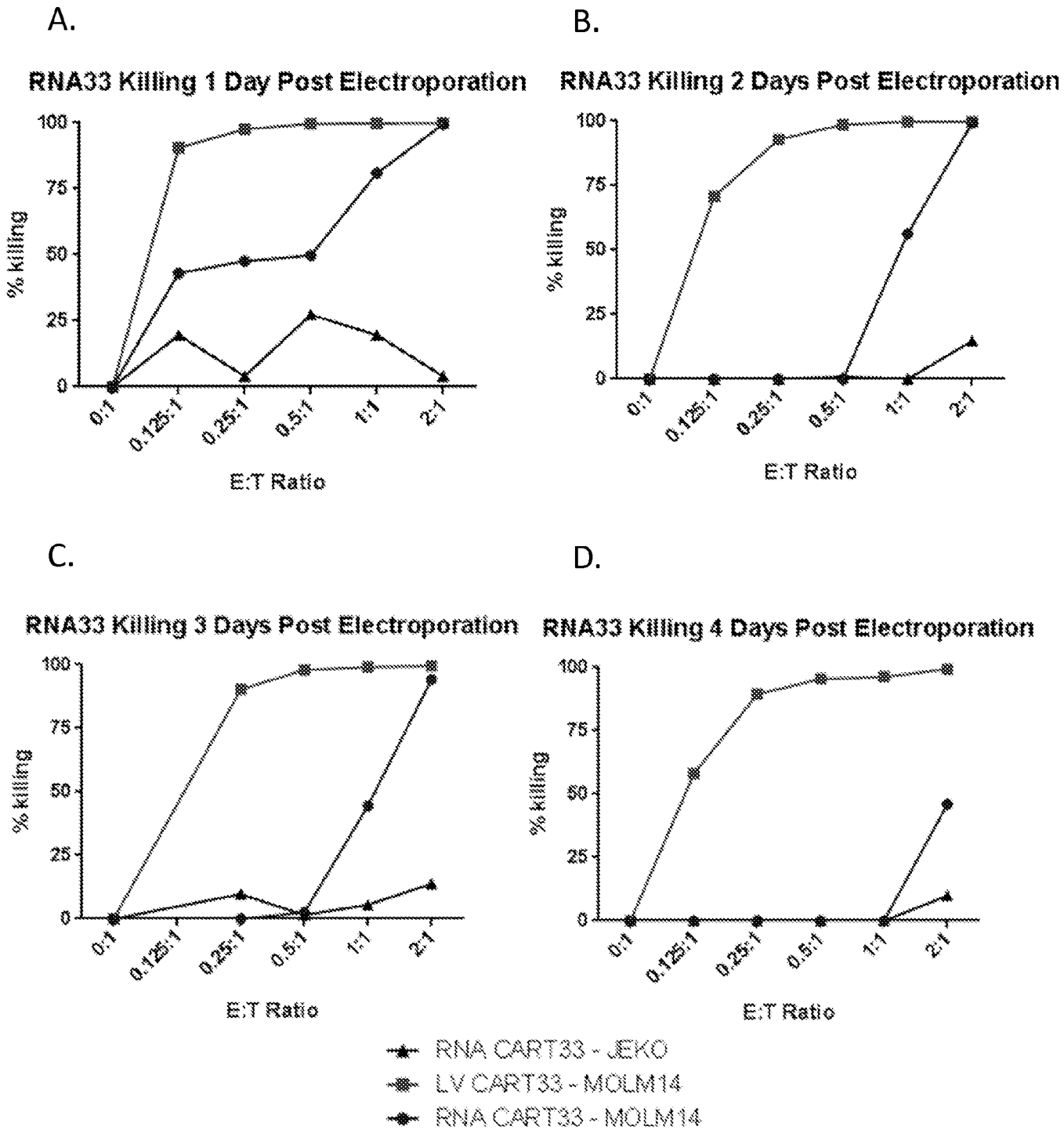
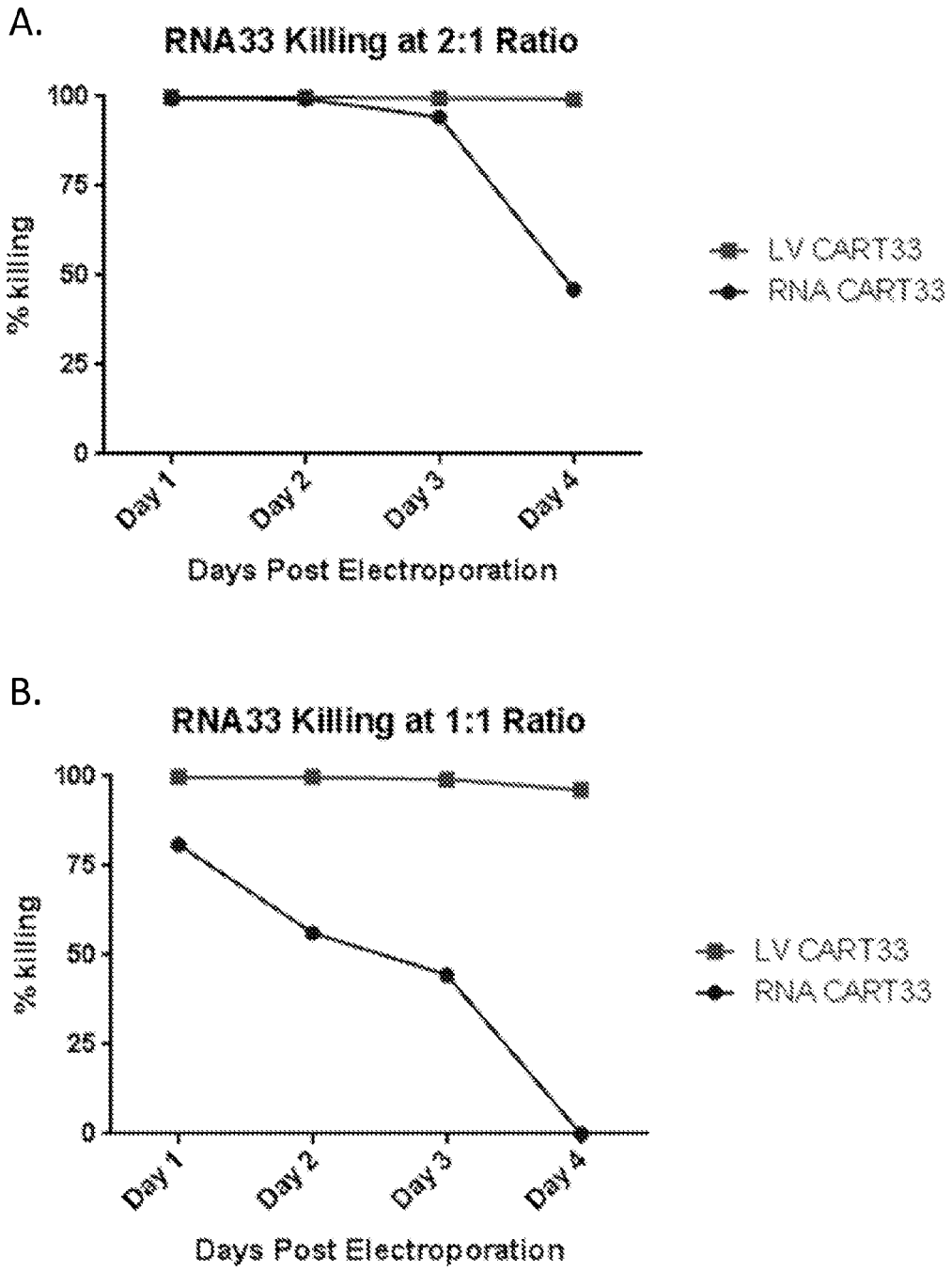

A.
RNA33 Killing 1 Day Post Electroporation
% killing
100
75
50
25
0
0:1
0.125:1
0.25:1
0.5:1
1:1
2:1
E:T Ratio
B.
RNA33 Killing 2 Days Post Electroporation
% killing
E:T Ratio
C.
RNA33 Killing 3 Days Post Electroporation
% killing
E:T Ratio
D.
RNA33 Killing 4 Days Post Electroporation
% killing
E:T Ratio
RNA CART33 - JEKO
LV CART33 - MOLM14
RNA CART33 - MOLM14

FIG. 36


A.
RNA33 Killing at 2:1 Ratio
% killing
100
75
50
25
0
Day 1
Day 2
Day 3
Day 4
Days Post Electroporation
LV CART33
RNA CART33
B.
RNA33 Killing at 1:1 Ratio
% killing
100
75
50
25
0
Day 1
Day 2
Day 3
Day 4
Days Post Electroporation
LV CART33
RNA CART33

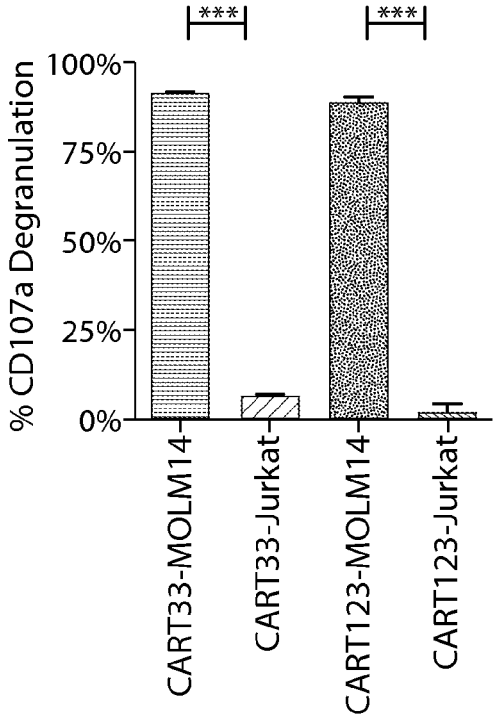


FIG. 37A

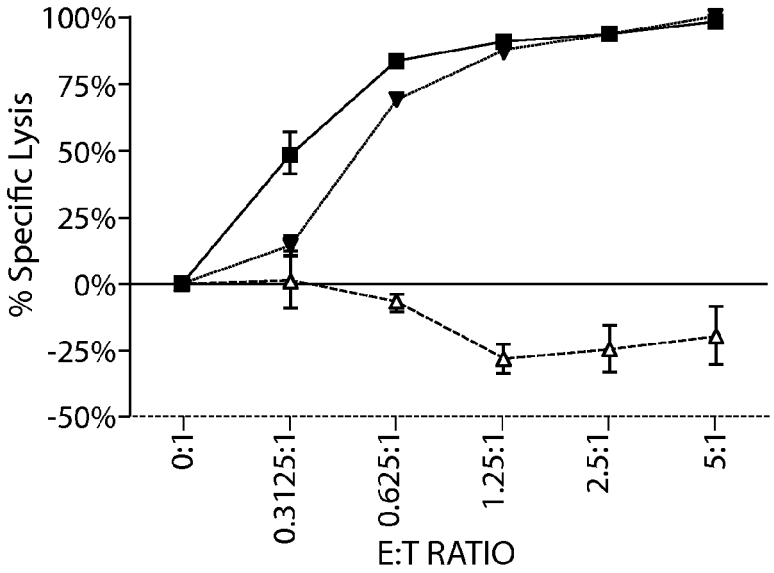


FIG. 37B

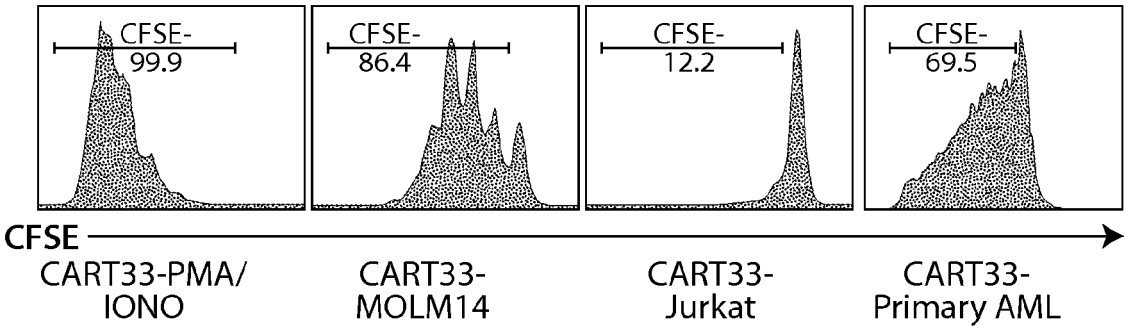


FIG. 37C

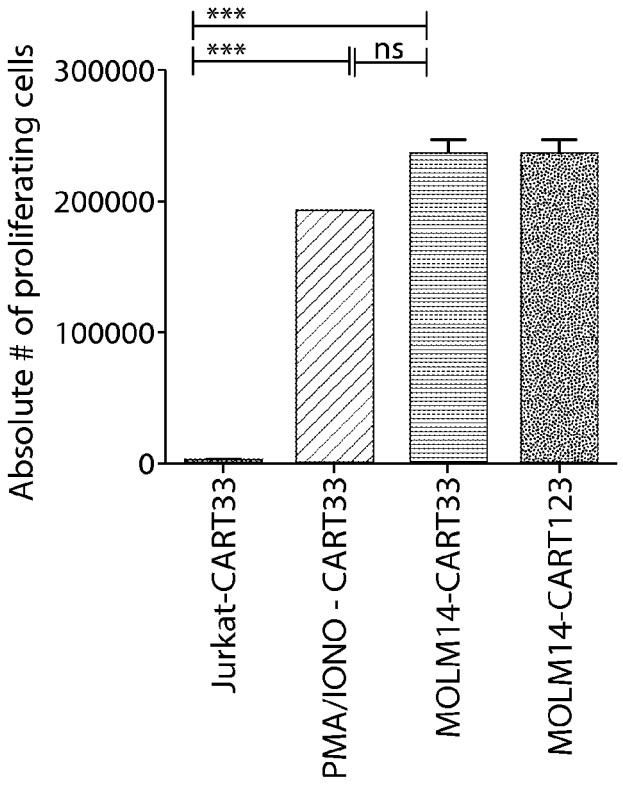
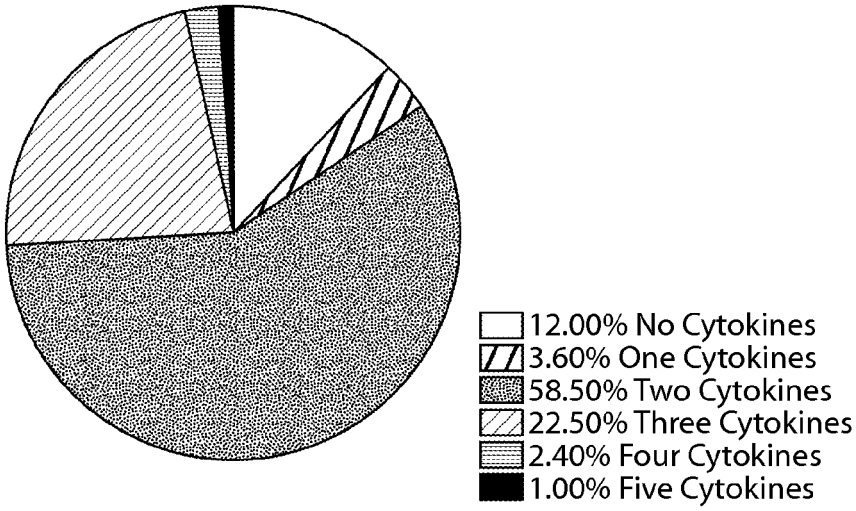
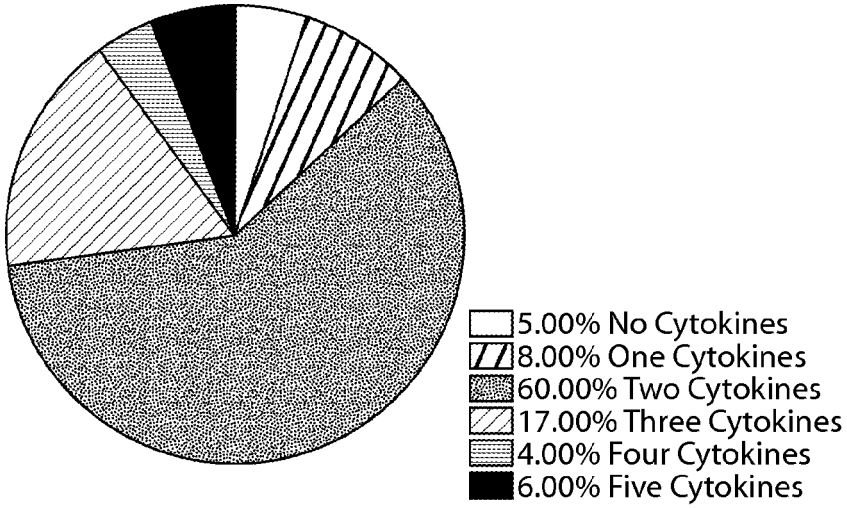


FIG. 37D



CART33 in response to MOLM14

FIG. 38A



CART33 in response to PMA/IONA

FIG. 38B

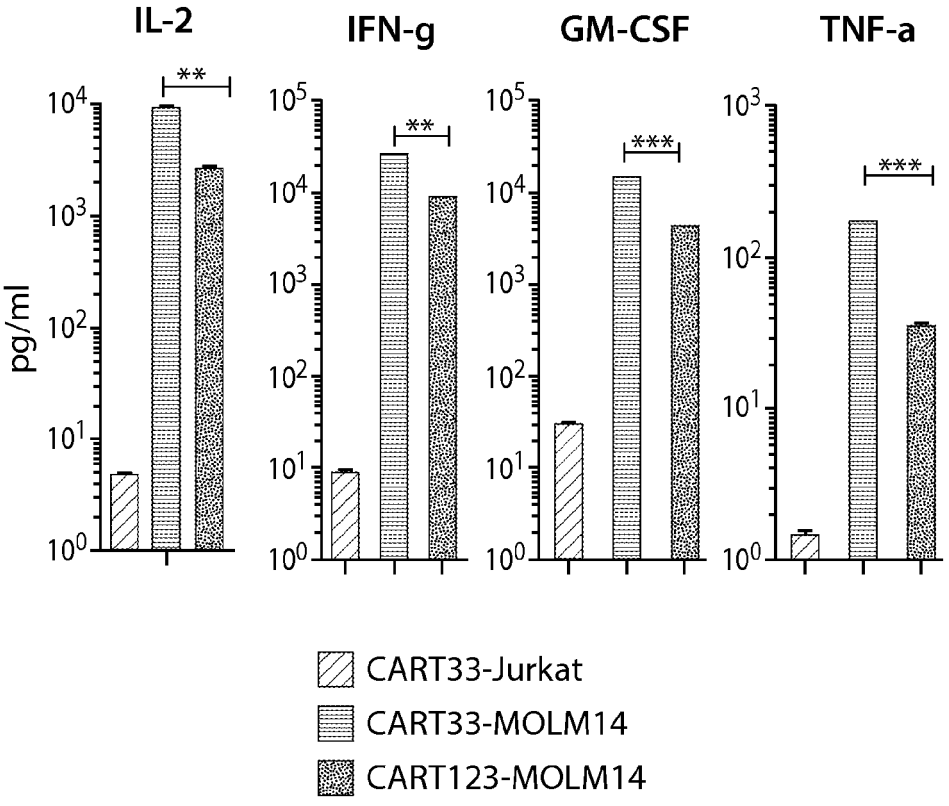

IL-2
IFN-g
GM-CSF
TNF-a
pg/ml
**
**
***
***
CART33-Jurkat
CART33-MOLM14
CART123-MOLM14

FIG. 38C

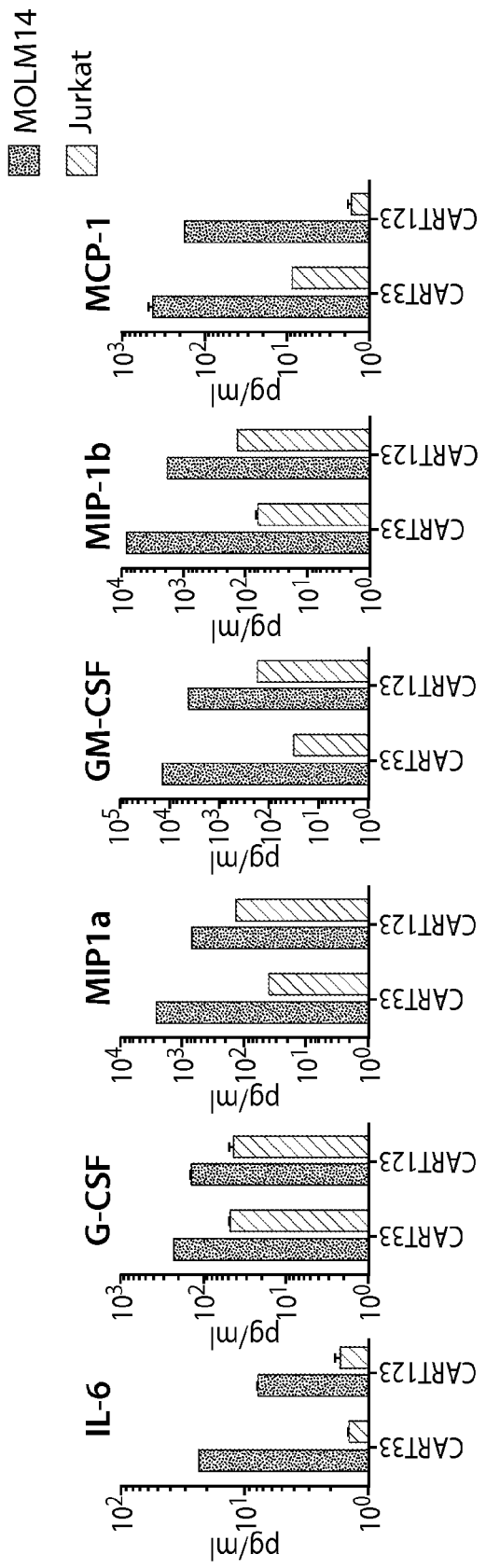

MOLM14
Jurkat
IL-6
G-CSF
MIP1a
GM-CSF
MIP-1b
MCP-1
pg/ml
CART33
CART123

FIG. 39-1

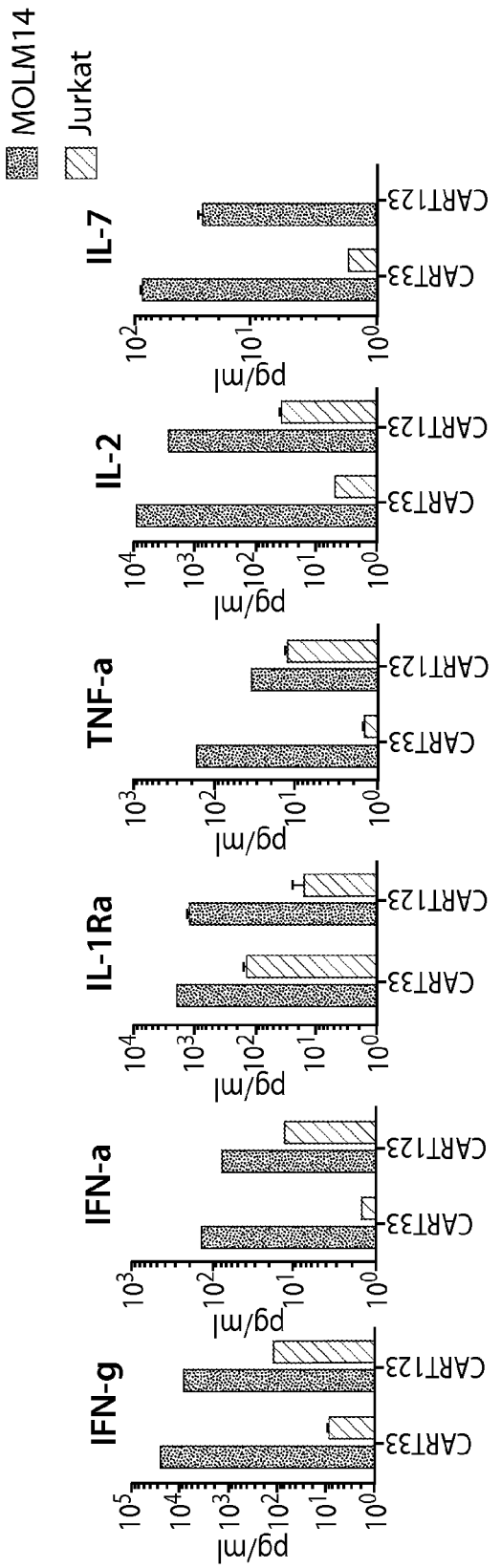

MOLM14
Jurkat
IFN-g
IFN-a
IL-1Ra
TNF-a
IL-2
IL-7
pg/ml
CART33
CART123

FIG. 39-2

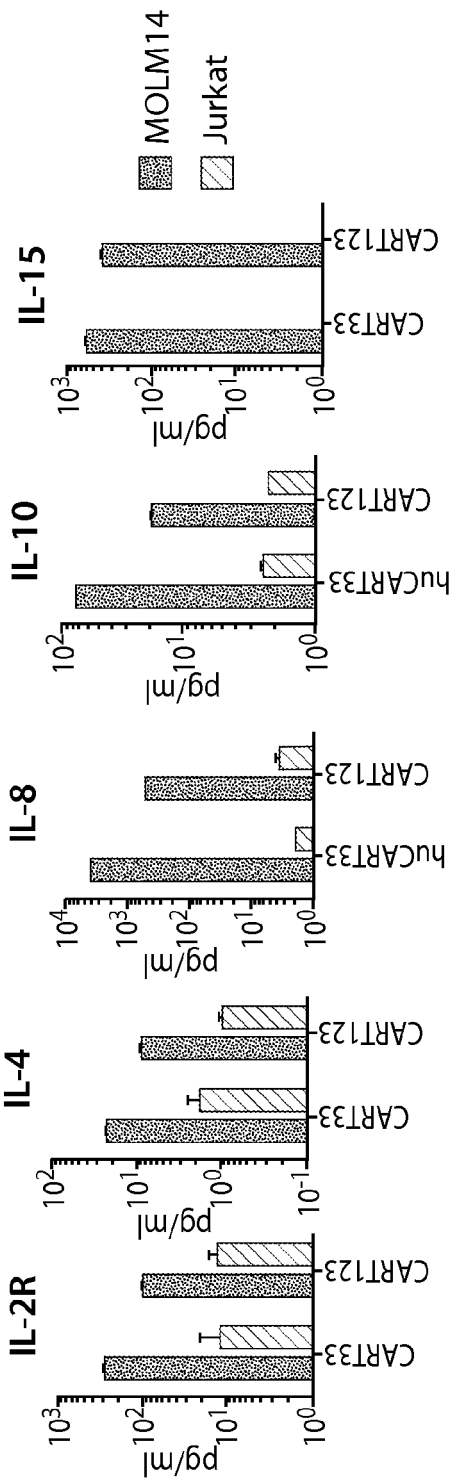

MOLM14
Jurkat
IL-15
IL-10
IL-8
IL-4
IL-2R
pg/ml
CART33
CART123
huCART33

FIG. 39-3

FIG. 40

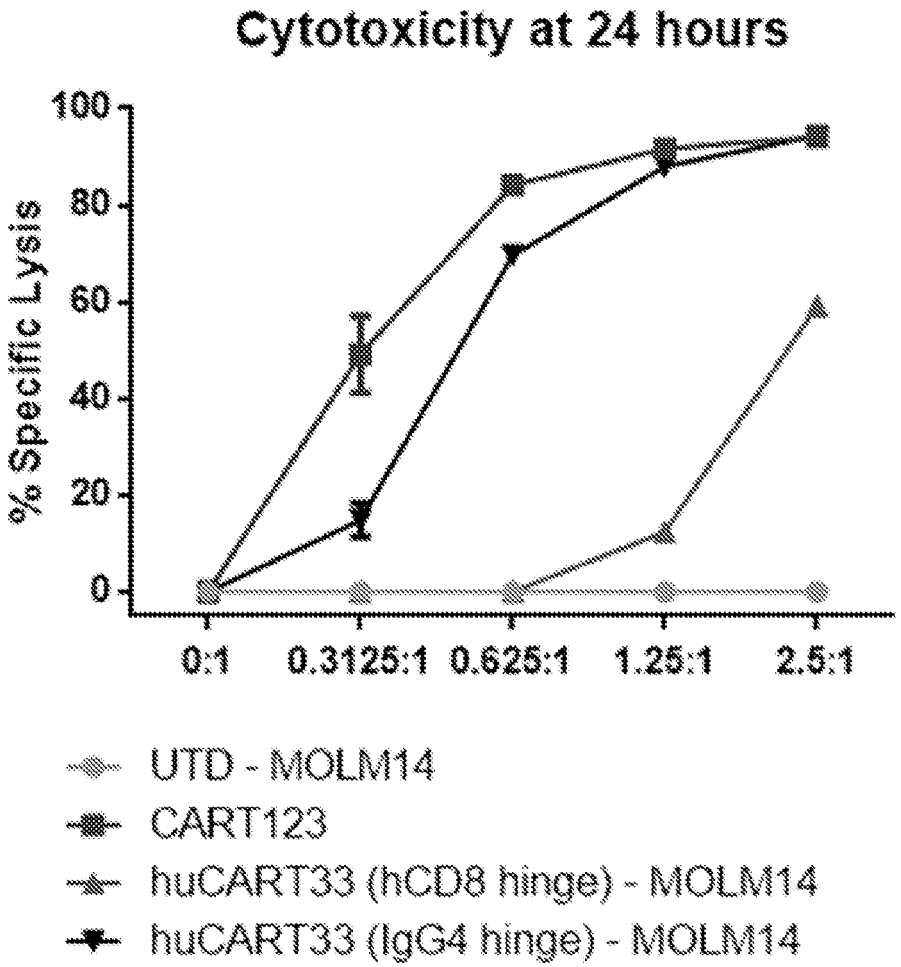
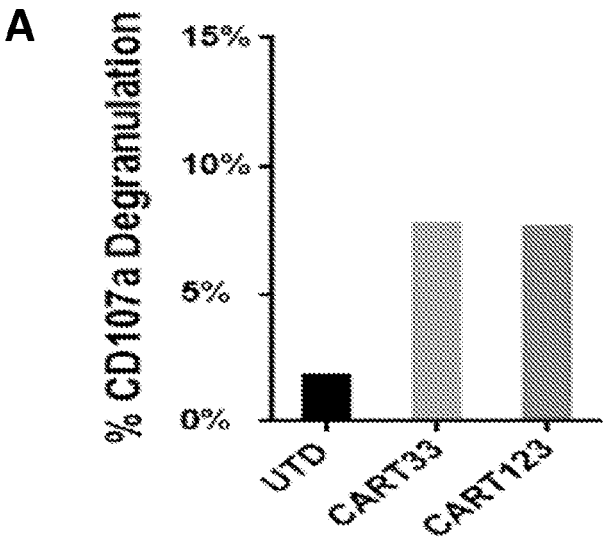
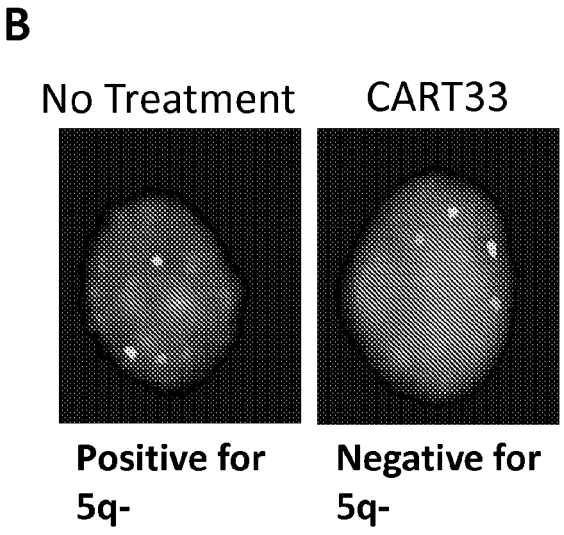

Cytotoxicity at 24 hours
% Specific Lysis
100
80
60
40
20
0
0:1
0.3125:1
0.625:1
1.25:1
2.5:1
UTD - MOLM14
CART123
huCART33 (hCD8 hinge) - MOLM14
huCART33 (IgG4 hinge) - MOLM14

FIG. 41


A
% CD107a Degranulation
15%
10%
5%
0%
UTD
CART33
CART123


B
No Treatment
CART33
Positive for 5q-
Negative for 5q-

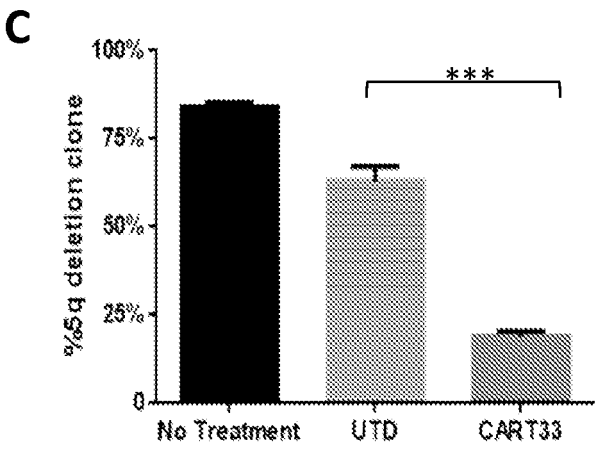
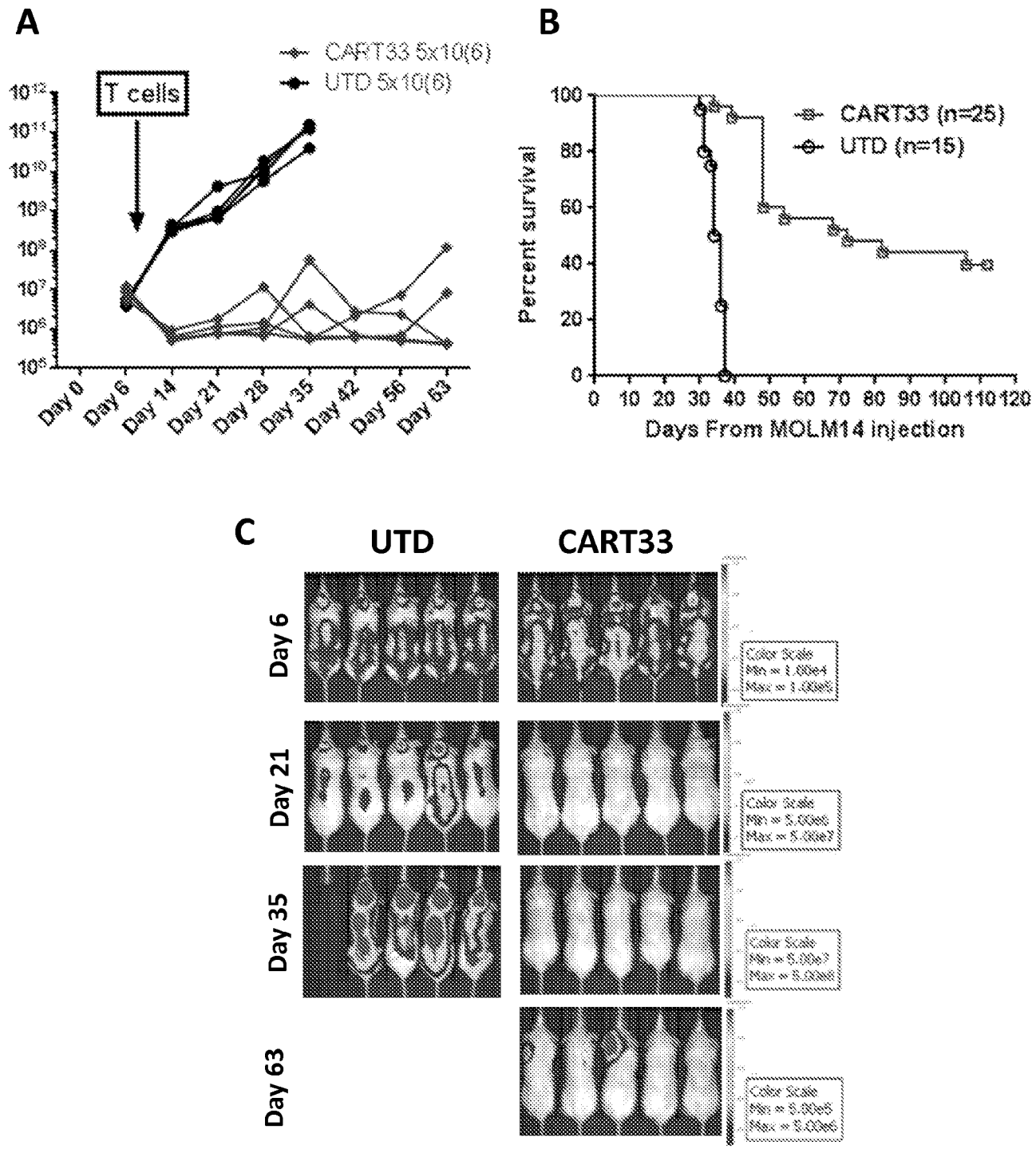

C
%5q deletion clone
100%
75%
50%
25%
0
***
No Treatment
UTD
CART33

FIG. 42


A
CART33 5x10(6)
UTD 5x10(6)
T cells
Day 0
Day 6
Day 14
Day 21
Day 28
Day 35
Day 42
Day 56
Day 63
B
CART33 (n=25)
UTD (n=15)
Percent survival
Days From MOLM14 injection
C
UTD
CART33
Day 6
Day 21
Day 35
Day 63

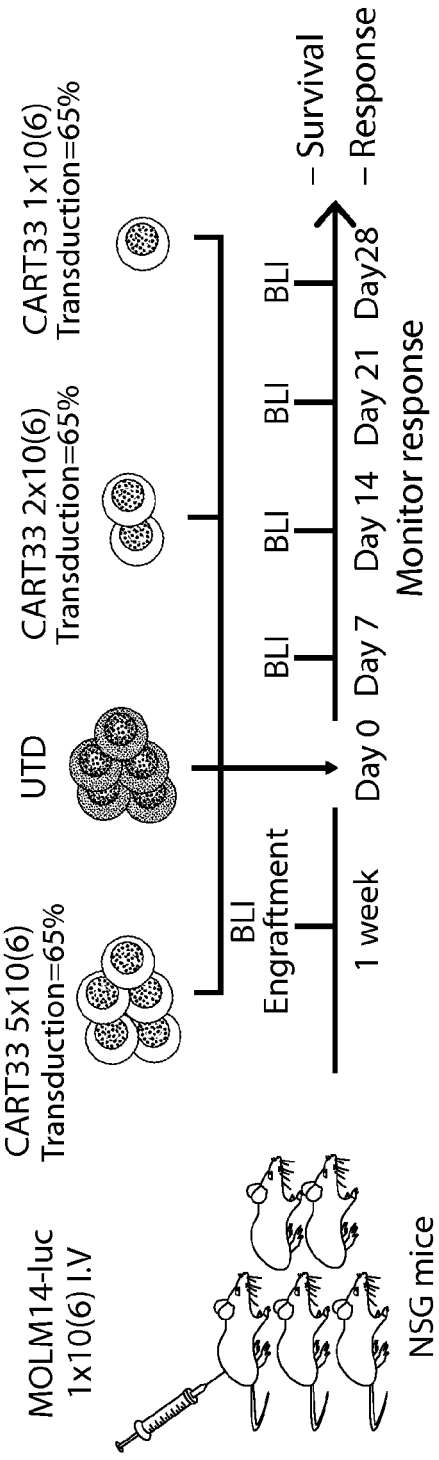

MOLM14-luc
1x10(6) I.V
NSG mice
1 week
BLI
Engraftment
CART33 5x10(6)
Transduction=65%
UTD
CART33 2x10(6)
Transduction=65%
CART33 1x10(6)
Transduction=65%
Day 0
Day 7
Day 14
Day 21
Day28
BLI
BLI
BLI
BLI
Monitor response
– Survival
– Response

FIG. 43A

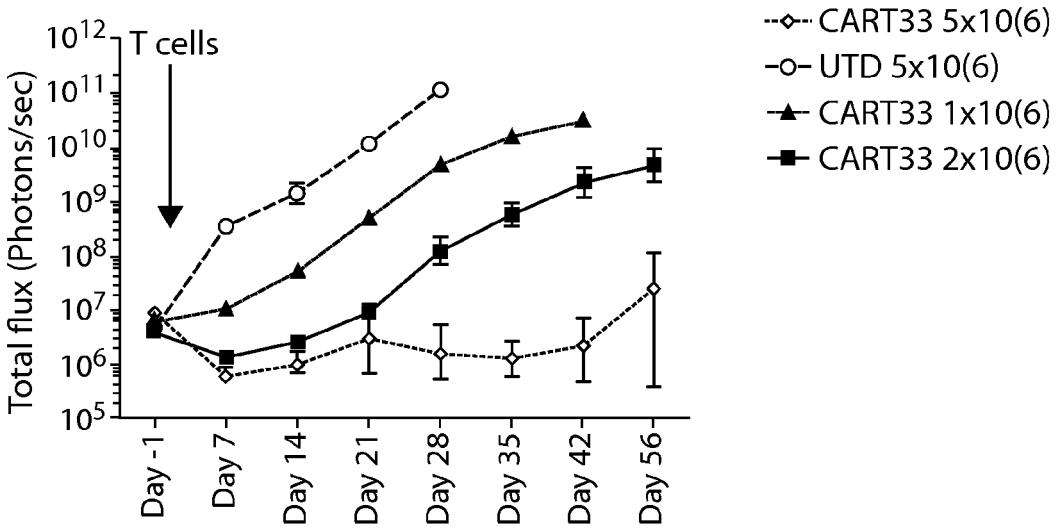


FIG. 43B

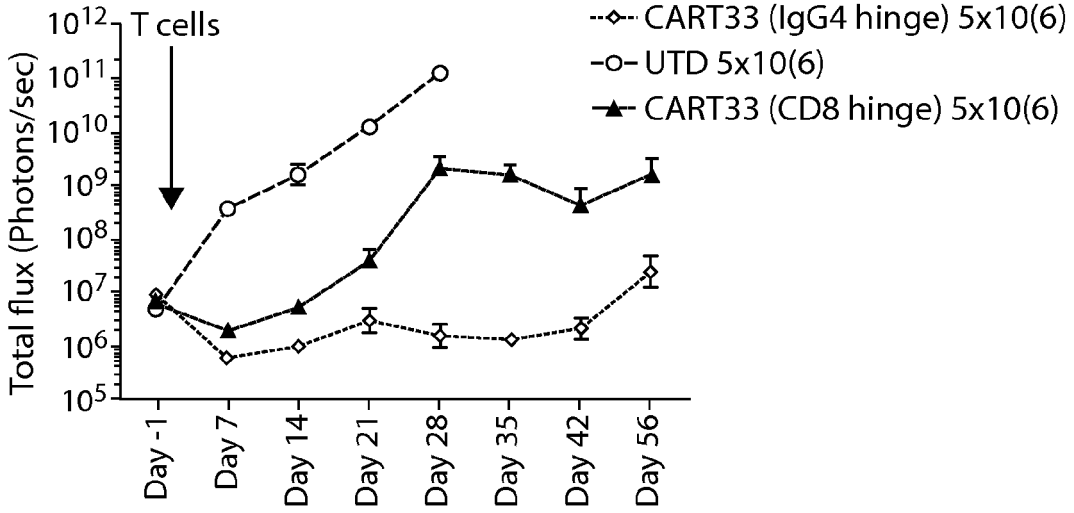


FIG. 44

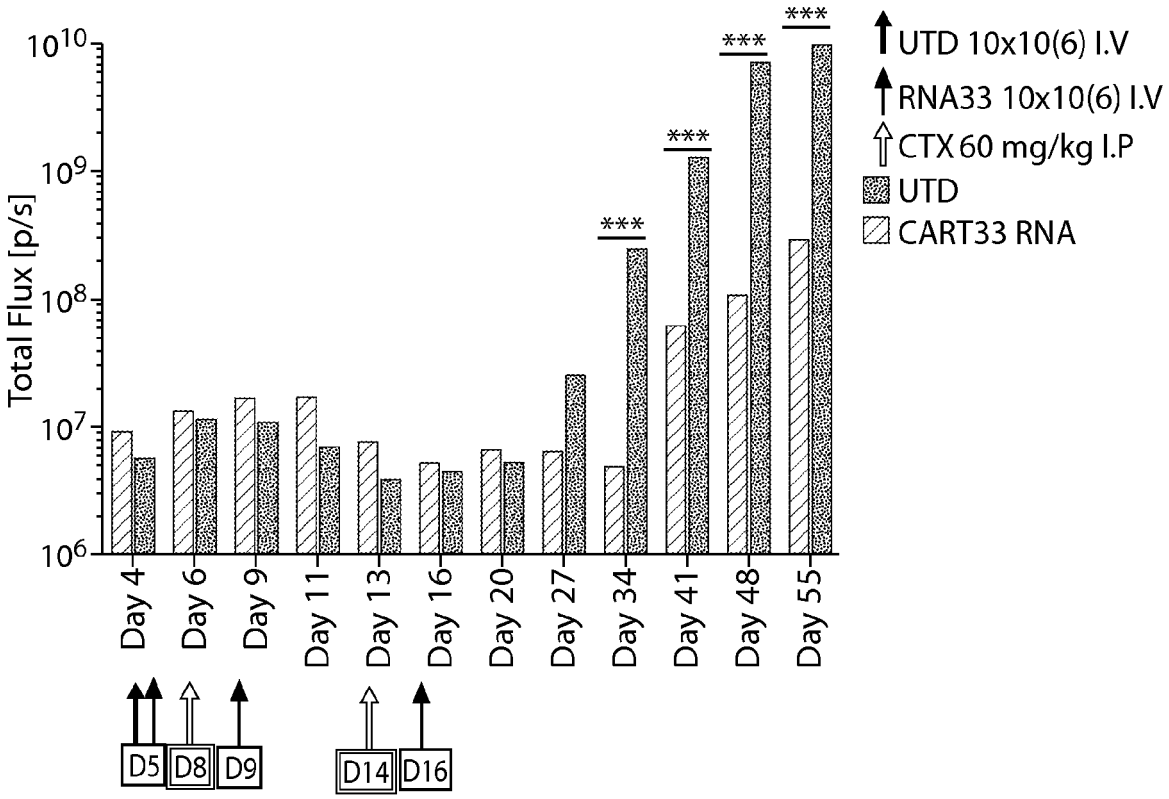

Total Flux [p/s]
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
***
***
***
***
Day 4
Day 6
Day 9
Day 11
Day 13
Day 16
Day 20
Day 27
Day 34
Day 41
Day 48
Day 55
D5
D8
D9
D14
D16
Days since MOLM14 (1x10(6) I.V) injection
UTD 10x10(6) I.V
RNA33 10x10(6) I.V
CTX 60 mg/kg I.P
UTD
CART33 RNA

FIG. 45

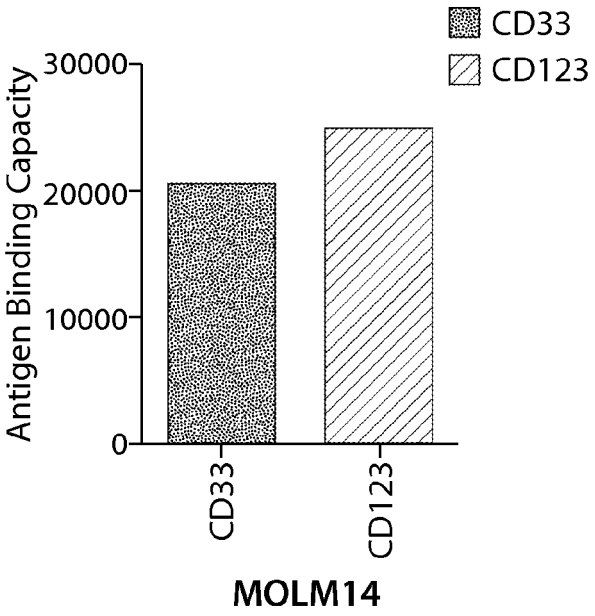


FIG. 46A

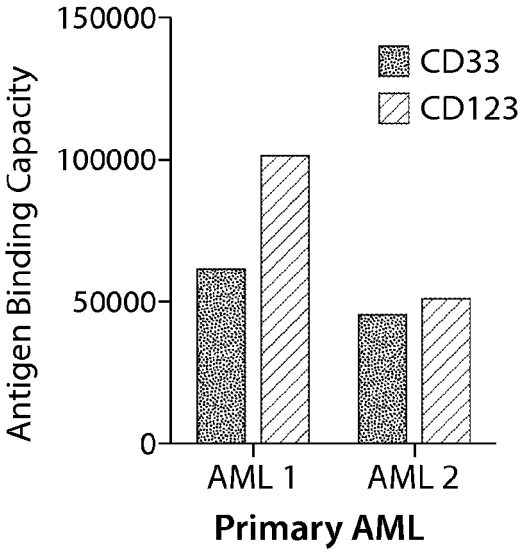


FIG. 46B

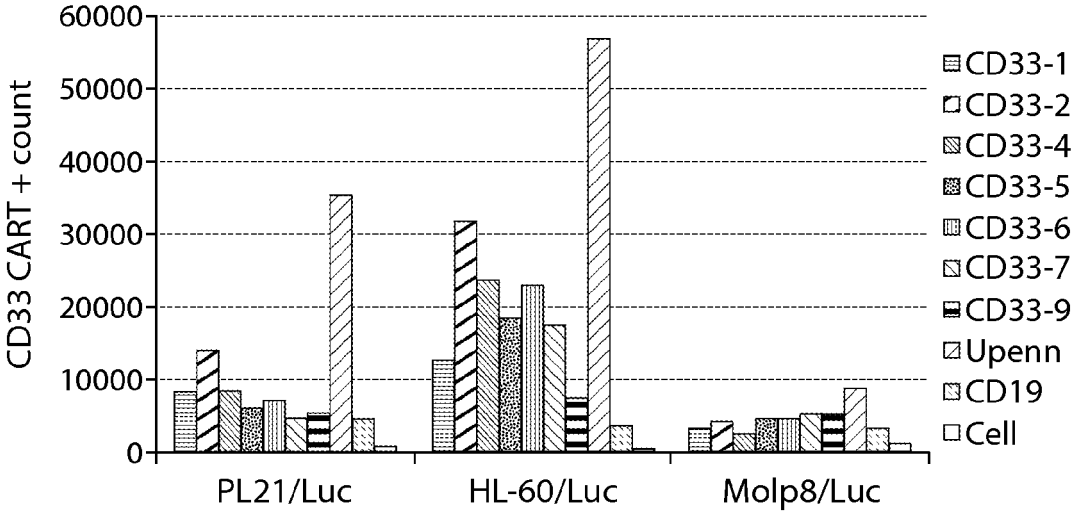

60000
50000
40000
30000
20000
10000
0
CD33 CART + count
PL21/Luc
HL-60/Luc
Molp8/Luc
CD33-1
CD33-2
CD33-4
CD33-5
CD33-6
CD33-7
CD33-9
Upenn
CD19
Cell

FIG. 47

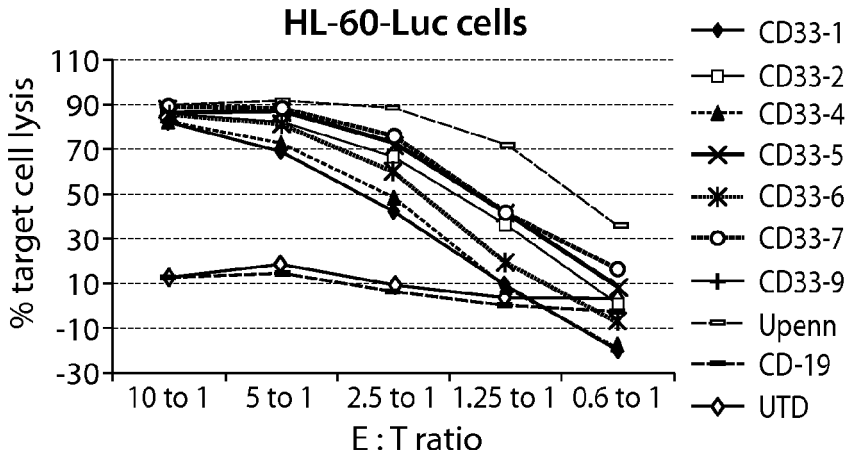


FIG. 48A

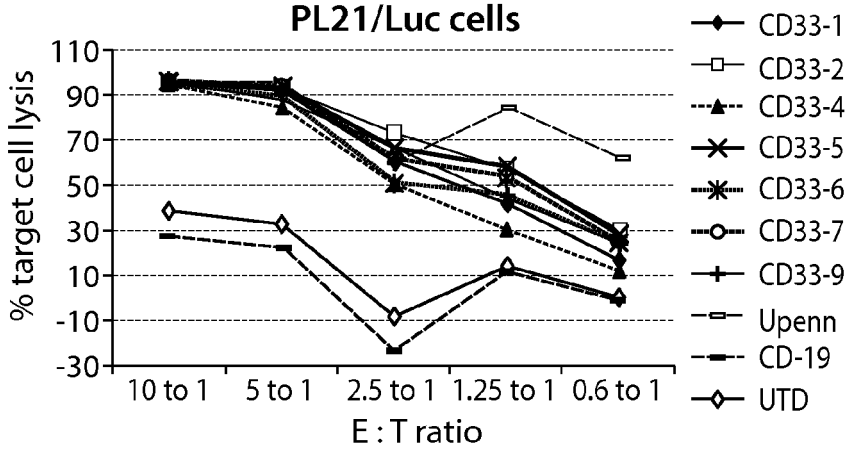


FIG. 48B

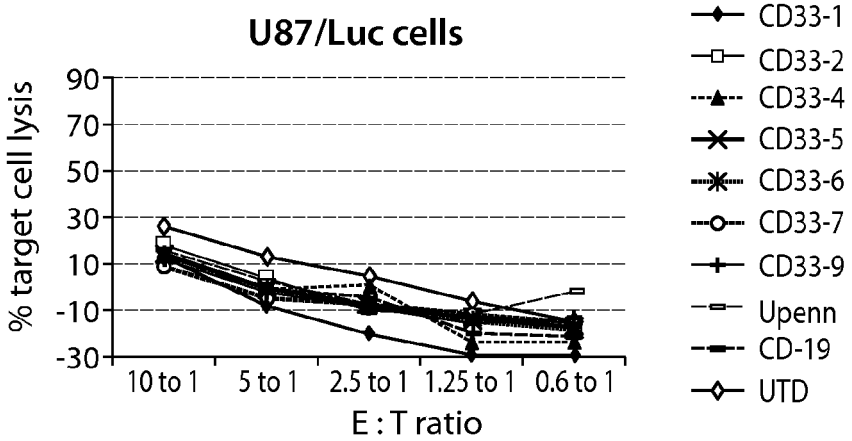


FIG. 48C

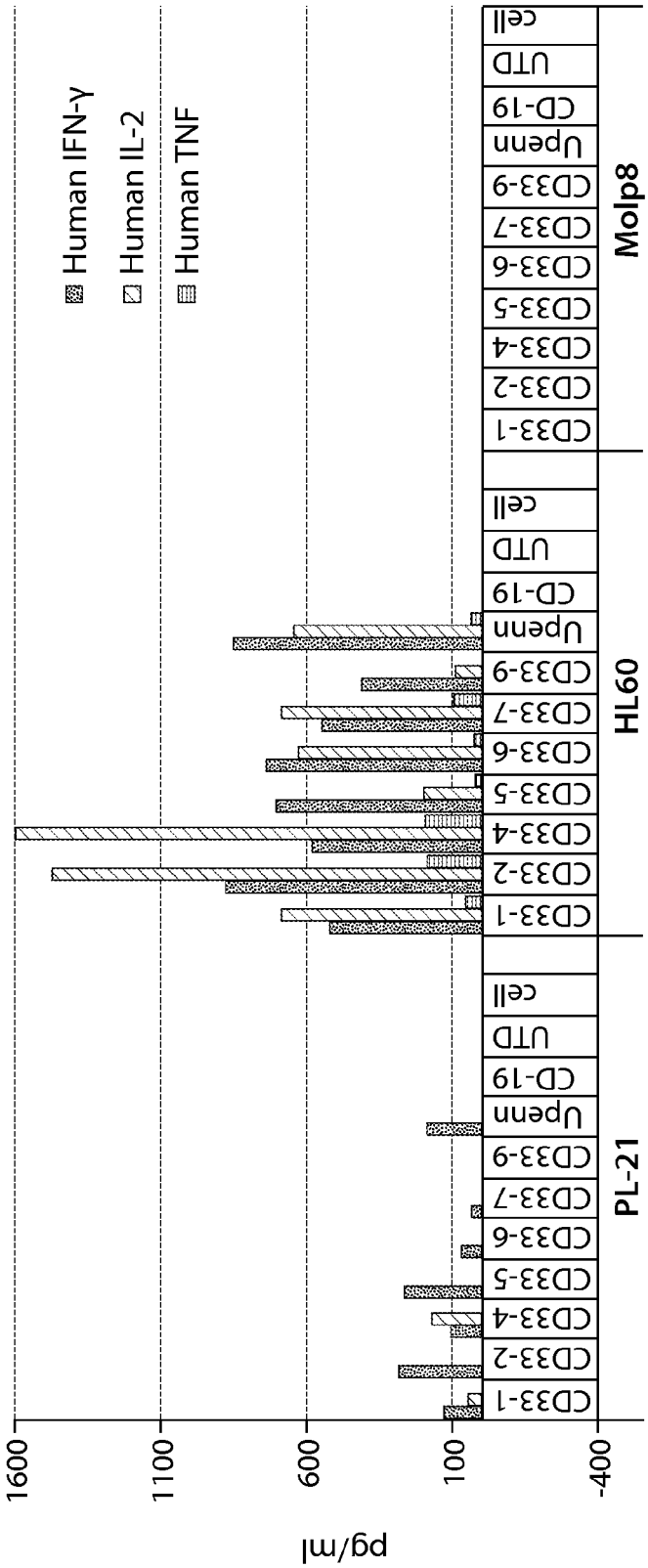
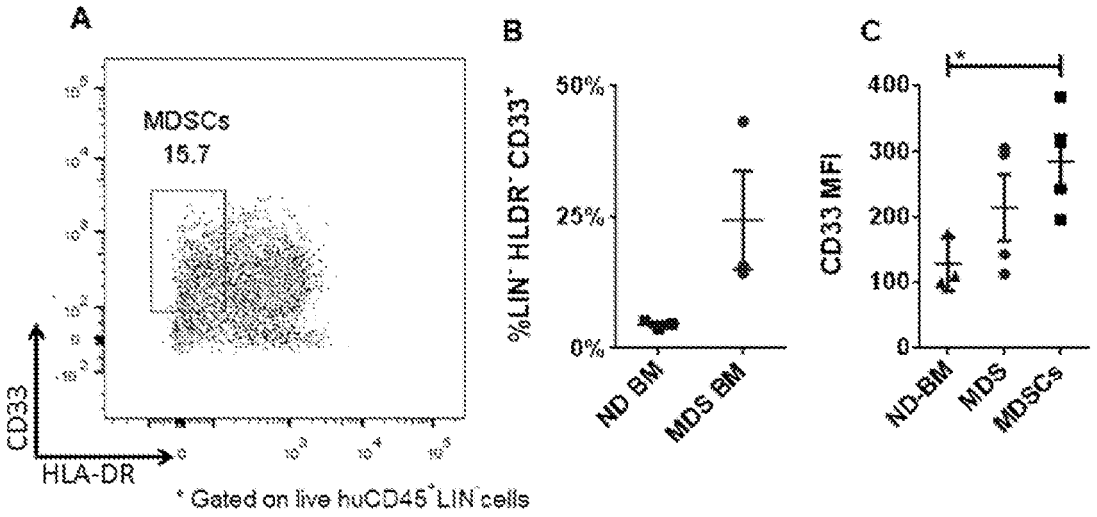

Human IFN-γ
Human IL-2
Human TNF
pg/ml
1600
1100
600
100
-400
PL-21
HL60
Molp8
CD33-1
CD33-2
CD33-4
CD33-5
CD33-6
CD33-7
CD33-9
Upenn
CD-19
UTD
cell

FIG. 49

FIG. 50


A
MDSCs
15.7
CD33
HLA-DR
* Gated on live huCD45+LIN- cells
B
%LIN- HLDR+ CD33+
50%
25%
0%
ND BM
MDS BM
C
CD33 MFI
400
300
200
100
0
*
ND-BM
MDS
MDSCs

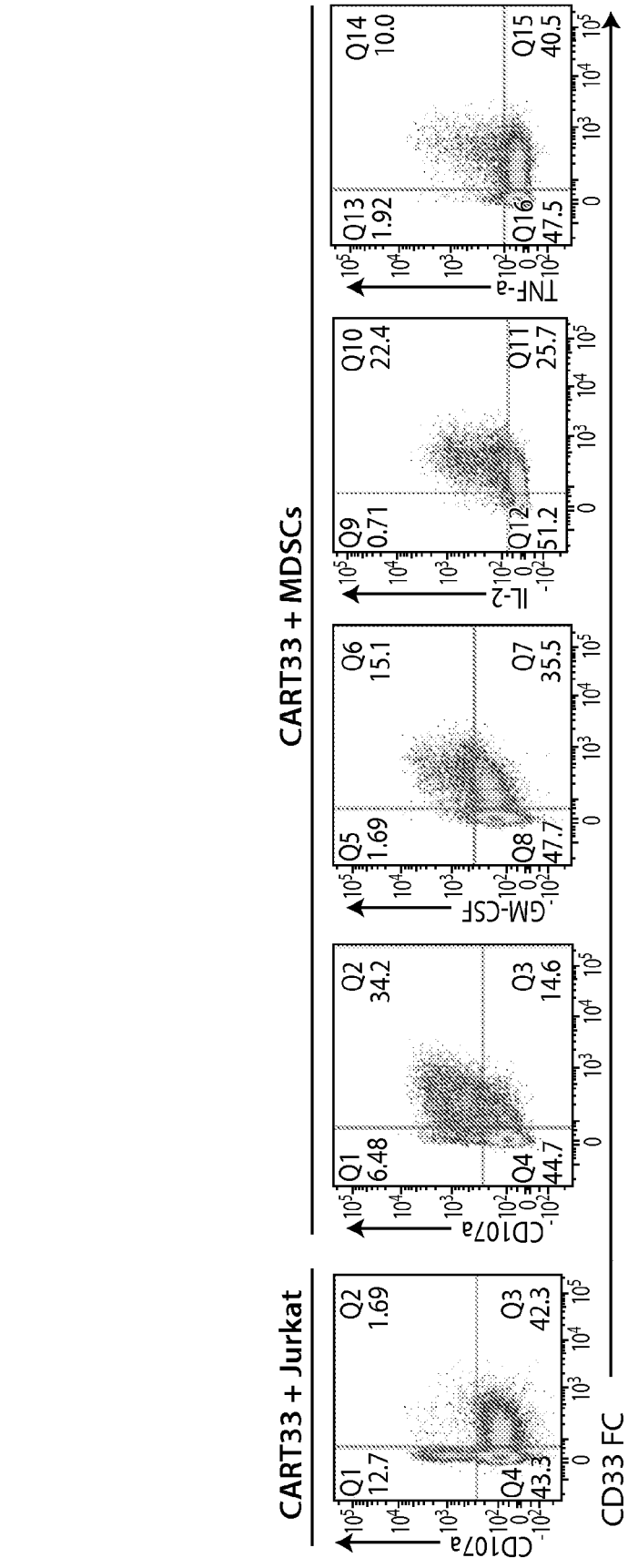

CART33 + Jurkat
CART33 + MDSCs
CD107a
GM-CSF
IL-2
TNF-α
CD33 FC
* Gated on live CD3+ cells
Q1 12.7
Q2 1.69
Q3 42.3
Q4 43.3
Q1 6.48
Q2 34.2
Q3 14.6
Q4 44.7
Q5 1.69
Q6 15.1
Q7 35.5
Q8 47.7
Q9 0.71
Q10 22.4
Q11 25.7
Q12 51.2
Q13 1.92
Q14 10.0
Q15 40.5
Q16 47.5

FIG. 51A

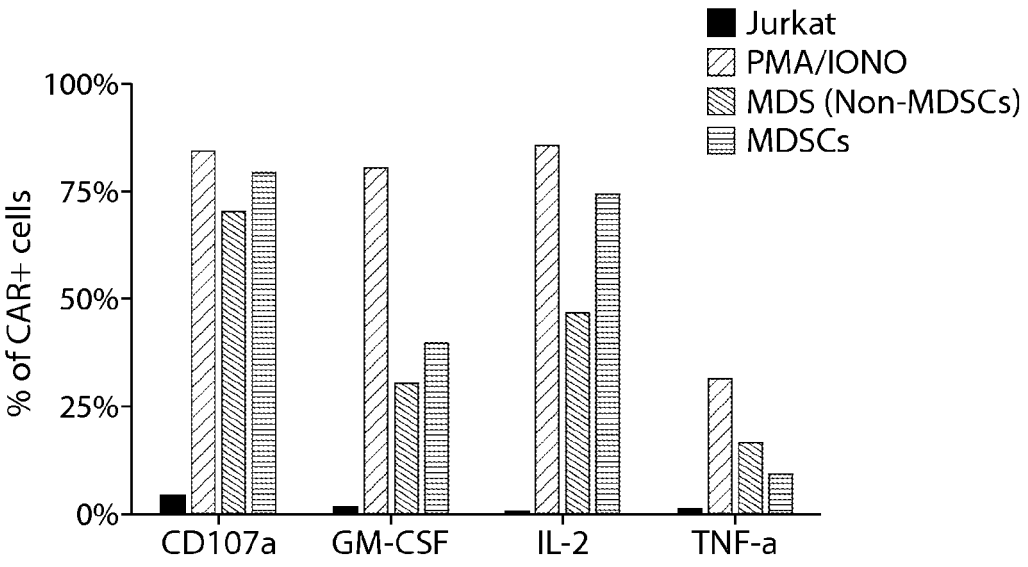

Jurkat
PMA/IONO
MDS (Non-MDSCs)
MDSCs
100%
75%
50%
25%
0%
% of CAR+ cells
CD107a
GM-CSF
IL-2
TNF-a

FIG. 51B

Vector constructs

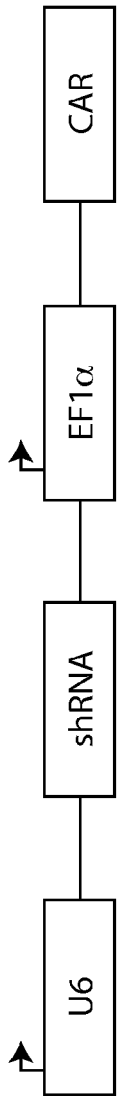


FIG. 52A

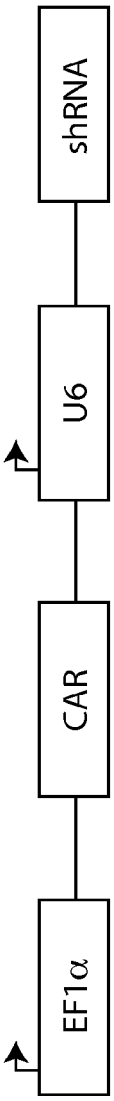


FIG. 52B

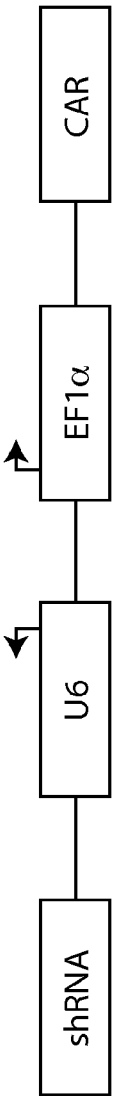


FIG. 52C

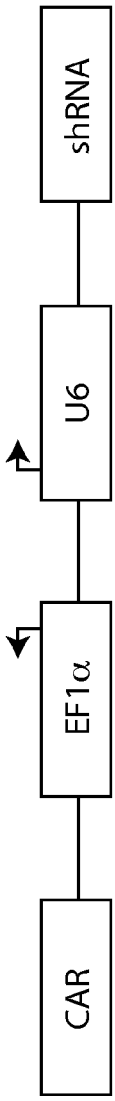


FIG. 52D

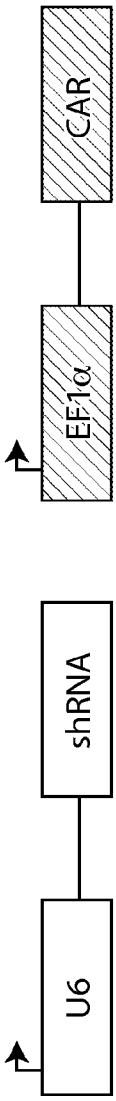


FIG. 52E

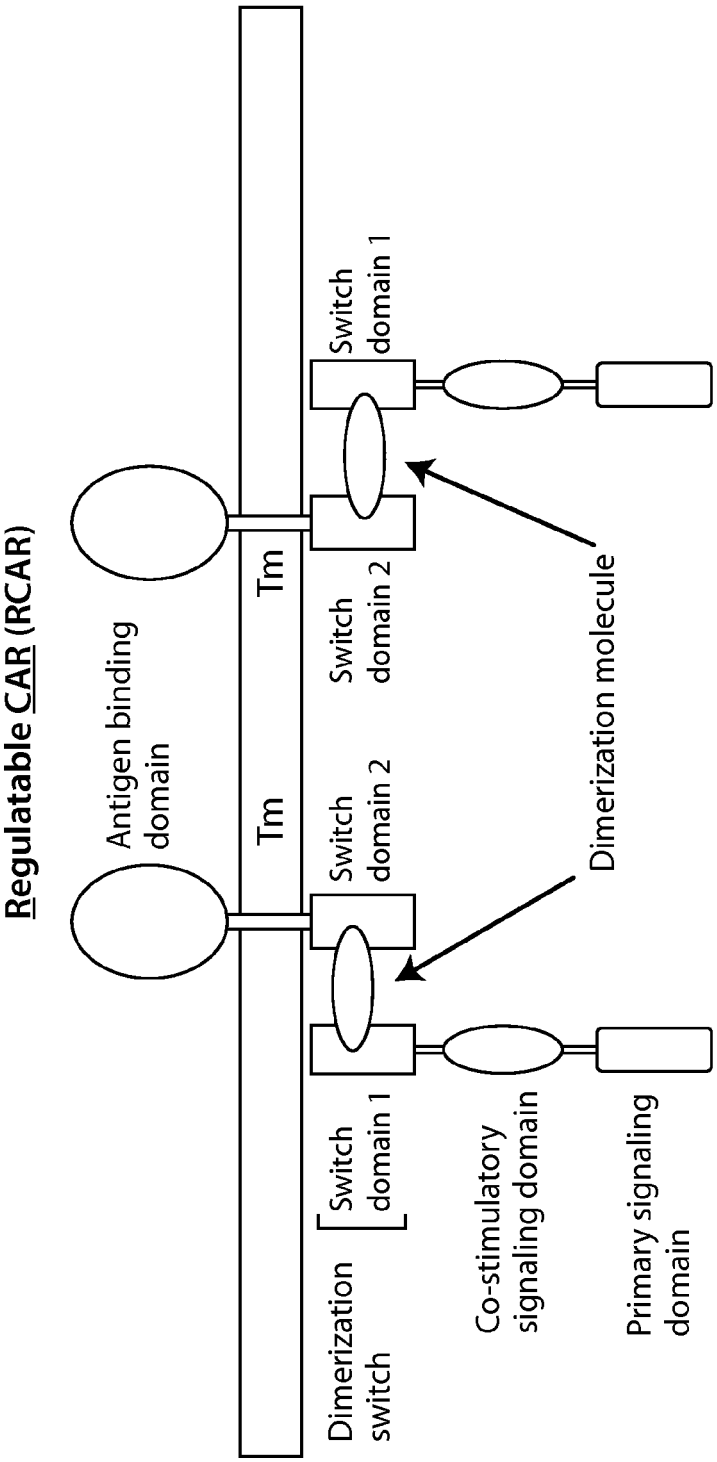

Regulatable CAR (RCAR)
Antigen binding domain
Tm
Tm
Switch domain 1
Switch domain 2
Switch domain 2
Dimerization molecule
Switch domain 1
Dimerization switch
Co-stimulatory signaling domain
Primary signaling domain

Fig. 53

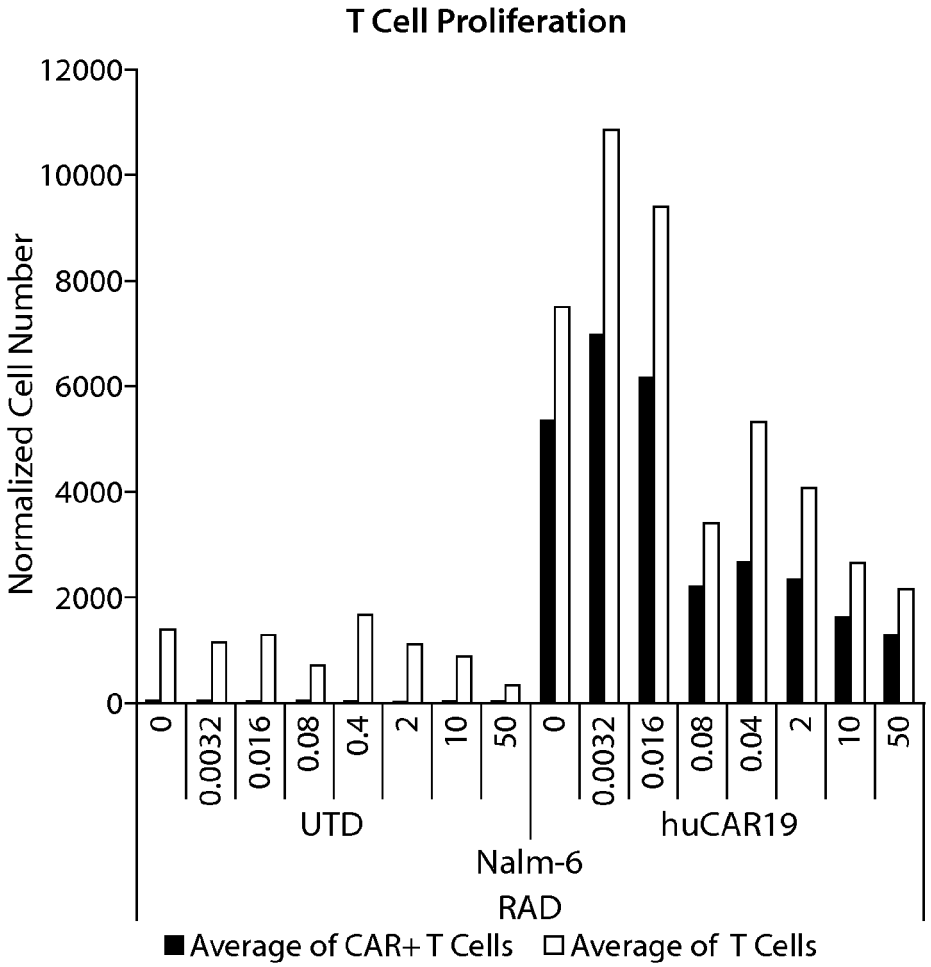

T Cell Proliferation
Normalized Cell Number
12000
10000
8000
6000
4000
2000
0
0
0.0032
0.016
0.08
0.4
2
10
50
0
0.0032
0.016
0.08
0.04
2
10
50
UTD
huCAR19
Nalm-6
RAD
Average of CAR+ T Cells
Average of T Cells

FIG. 54

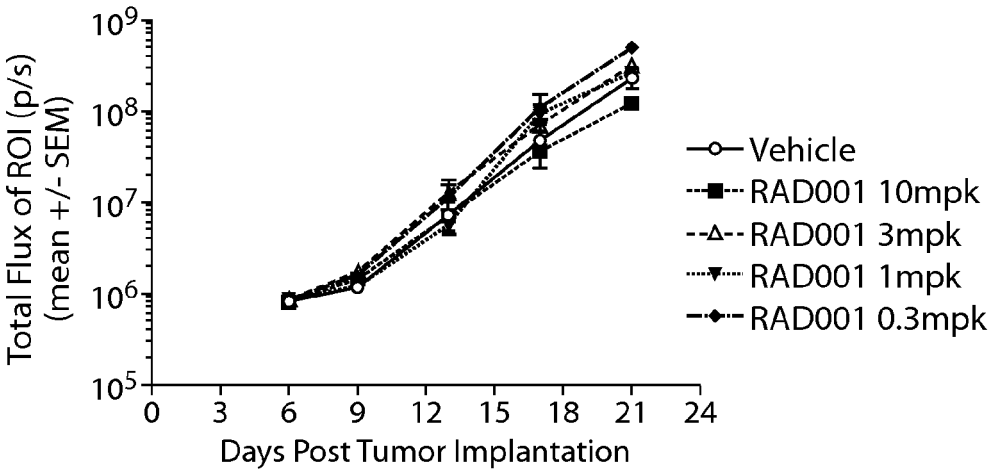

Total Flux of ROI (p/s)
(mean +/- SEM)
10^9
10^8
10^7
10^6
10^5
0
3
6
9
12
15
18
21
24
Days Post Tumor Implantation
Vehicle
RAD001 10mpk
RAD001 3mpk
RAD001 1mpk
RAD001 0.3mpk

FIG. 55

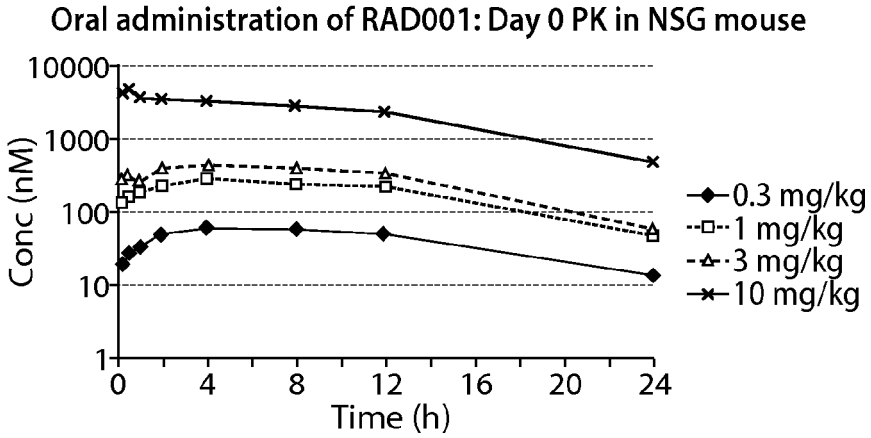


FIG. 56A

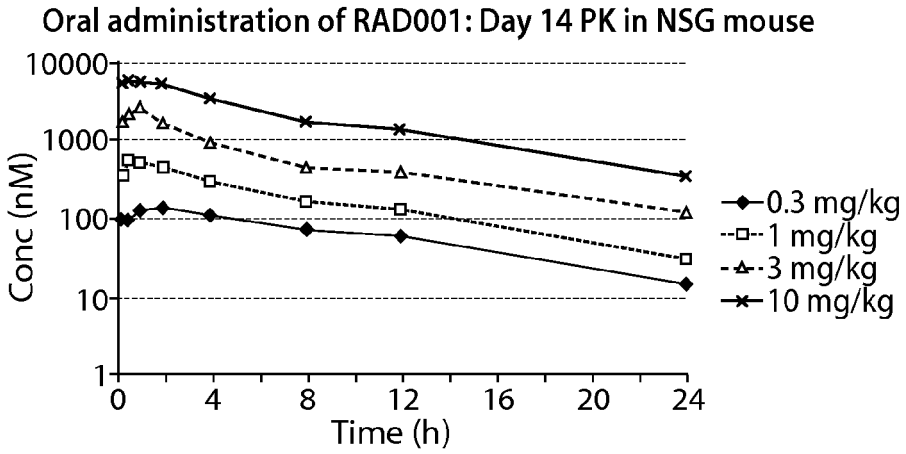


FIG. 56B

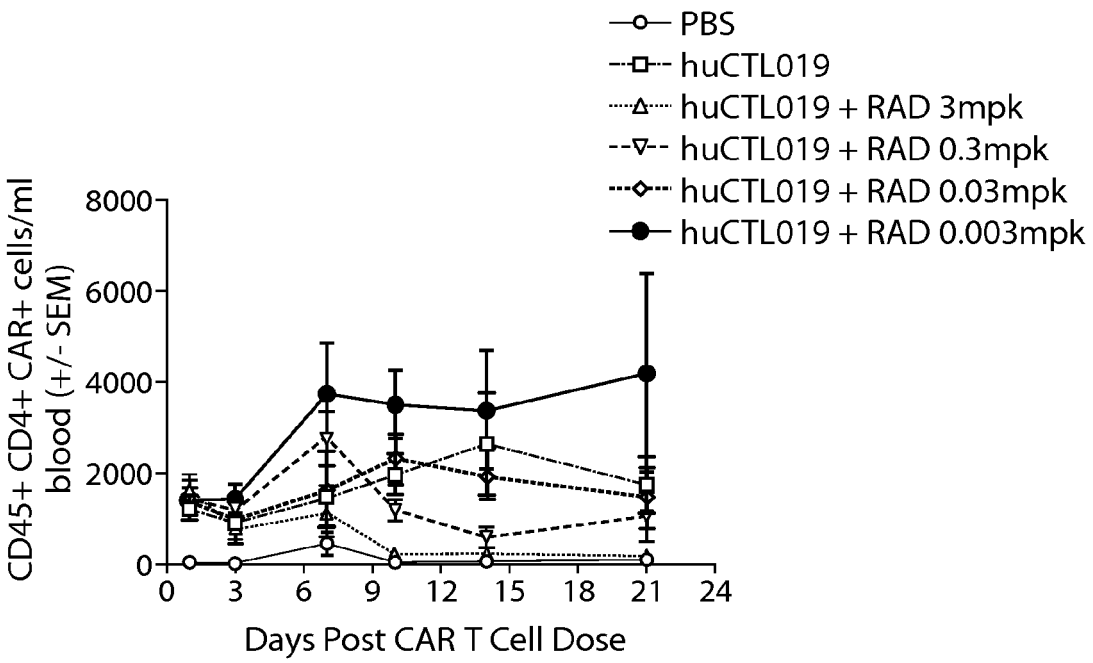


FIG. 57A

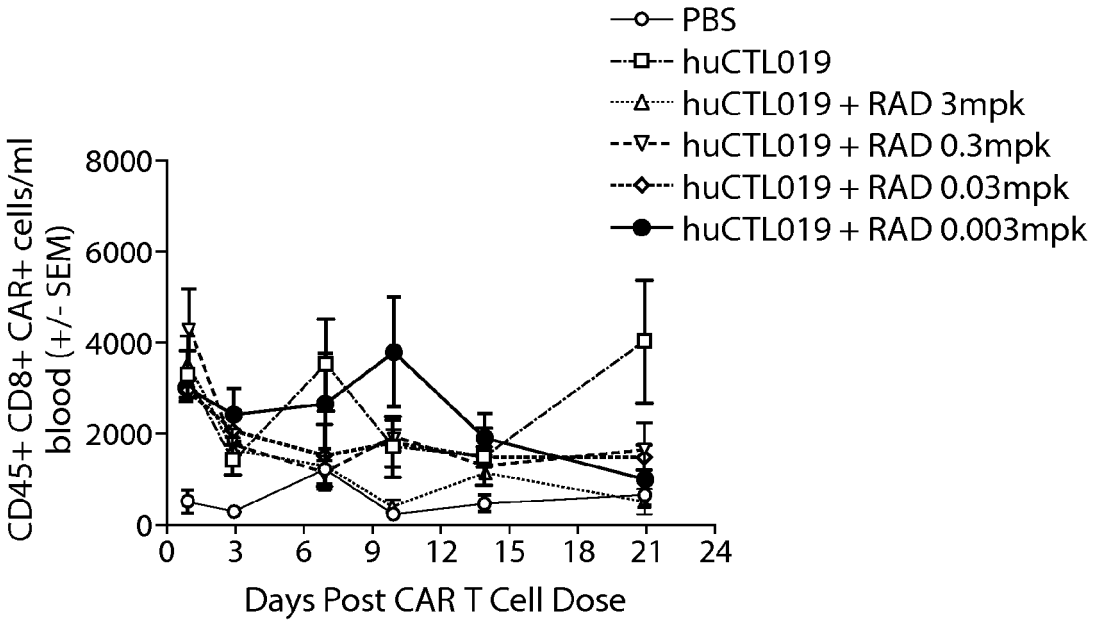


FIG. 57B

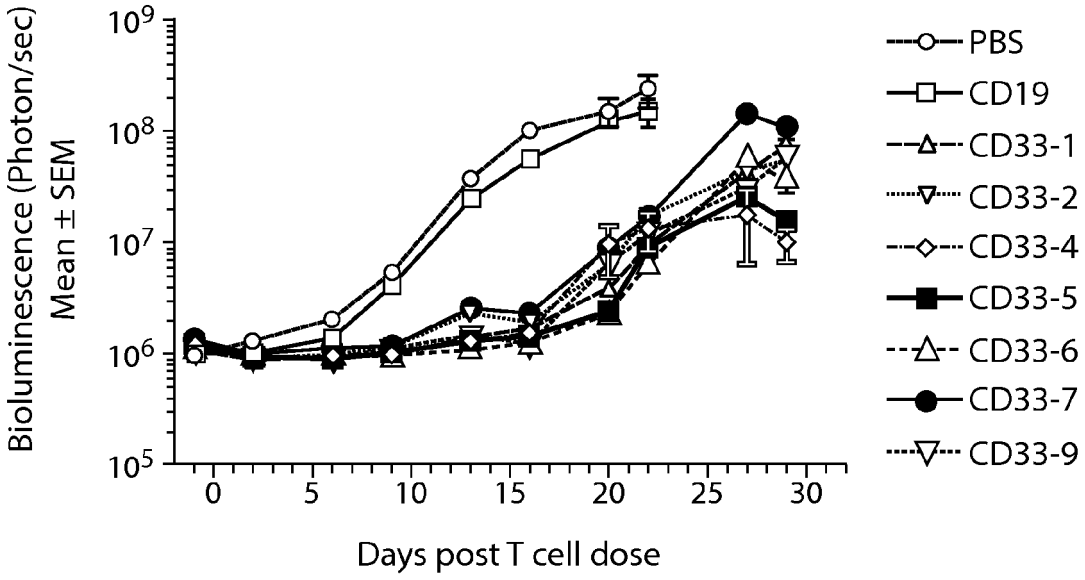

Bioluminescence (Photon/sec)
Mean ± SEM
10^9
10^8
10^7
10^6
10^5
0
5
10
15
20
25
30
Days post T cell dose
PBS
CD19
CD33-1
CD33-2
CD33-4
CD33-5
CD33-6
CD33-7
CD33-9

FIG. 58