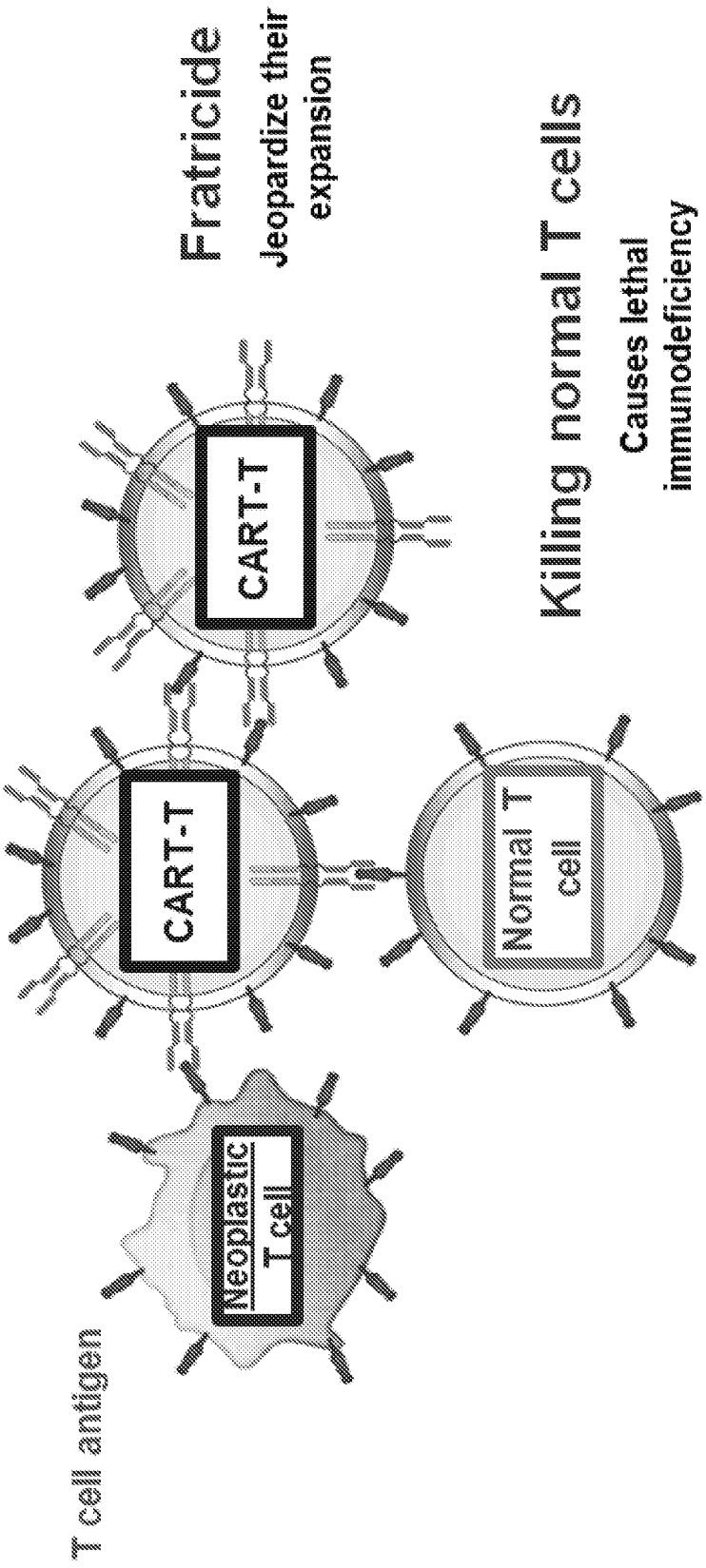

T cell antigen
Neoplastic T cell
CART-T
CART-T
Normal T cell
Killing normal T cells
Causes lethal immunodeficiency
Fratricide
Jeopardize their expansion

FIG. 1

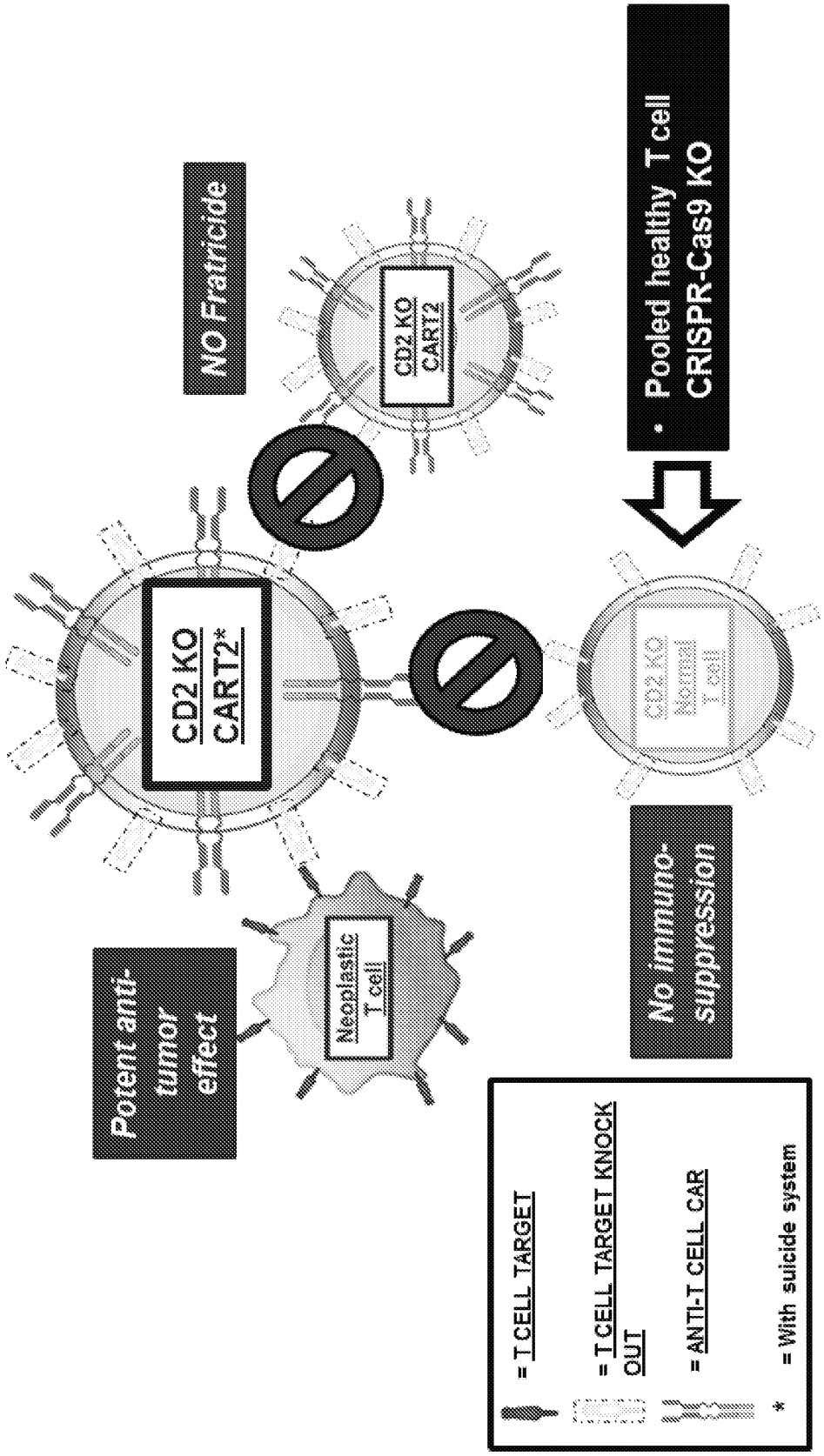

NO Fratricide
CD2 KO CART2
• Pooled healthy T cell CRISPR-Cas9 KO
CD2 KO CART2*
CD2 KO Normal T cell
Potent anti-tumor effect
Neoplastic T cell
No immuno-suppression
= T CELL TARGET
= T CELL TARGET KNOCK OUT
= ANTI-T CELL CAR
* = With suicide system

FIG. 2

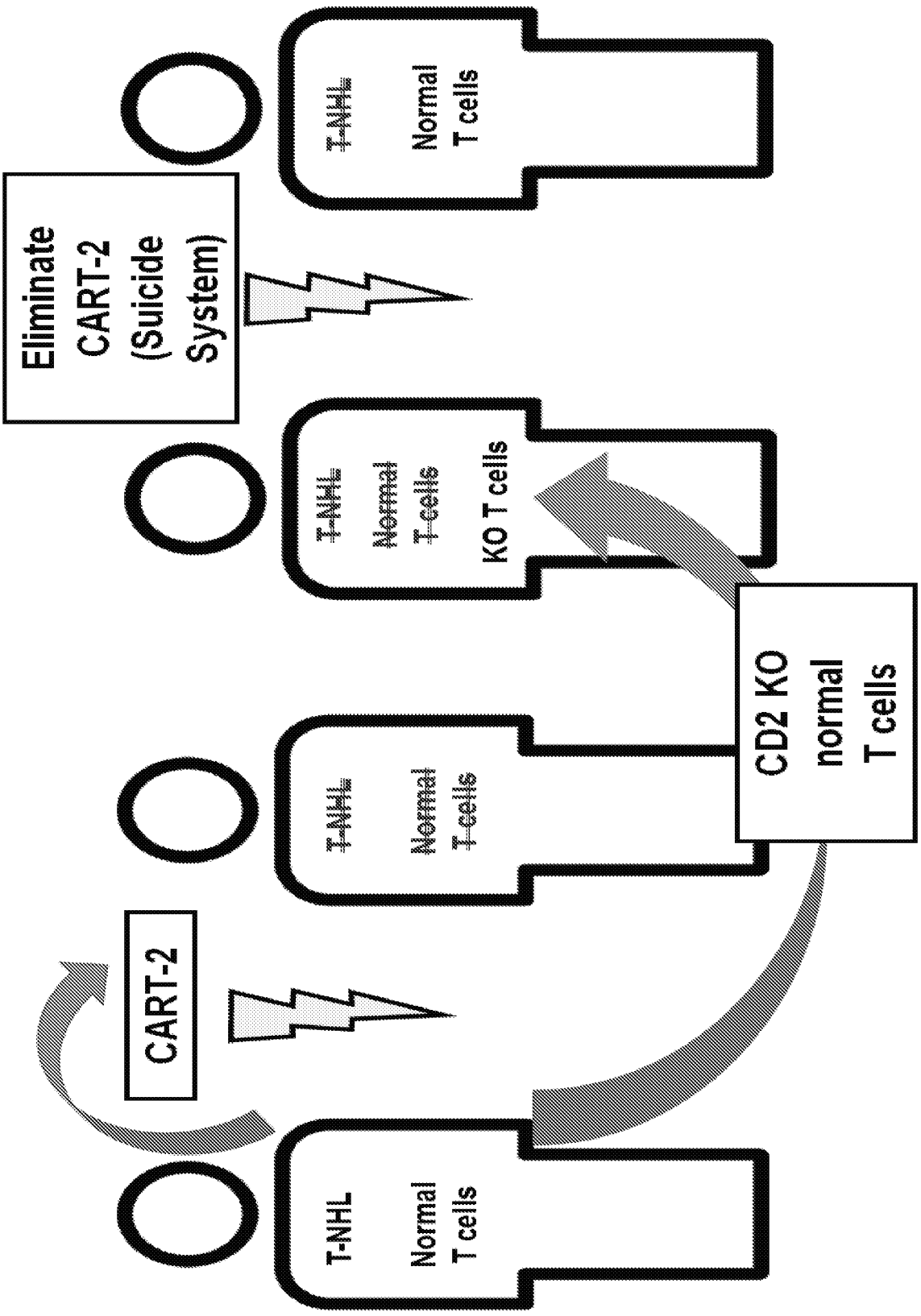

T-NHL
Normal
T cells
CART-2
T-NHL
Normal
T cells
CD2 KO
normal
T cells
T-NHL
Normal
T cells
KO T cells
Eliminate
CART-2
(Suicide
System)
T-NHL
Normal
T cells

FIG. 3

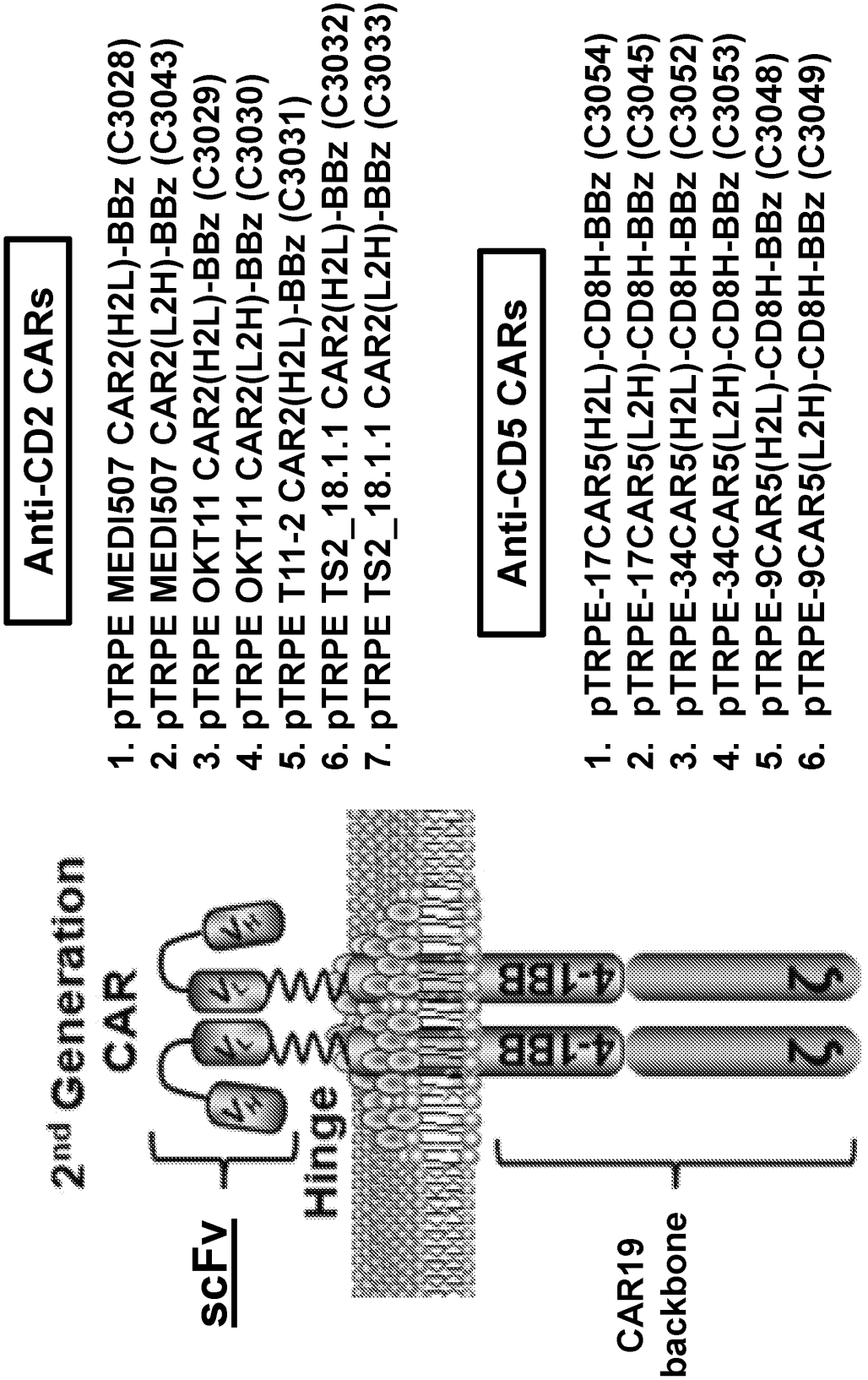

Anti-CD2 CARs
1. pTRPE MEDI507 CAR2(H2L)-BBz (C3028)
2. pTRPE MEDI507 CAR2(L2H)-BBz (C3043)
3. pTRPE OKT11 CAR2(H2L)-BBz (C3029)
4. pTRPE OKT11 CAR2(L2H)-BBz (C3030)
5. pTRPE T11-2 CAR2(H2L)-BBz (C3031)
6. pTRPE TS2_18.1.1 CAR2(H2L)-BBz (C3032)
7. pTRPE TS2_18.1.1 CAR2(L2H)-BBz (C3033)
Anti-CD5 CARs
1. pTRPE-17CAR5(H2L)-CD8H-BBz (C3054)
2. pTRPE-17CAR5(L2H)-CD8H-BBz (C3045)
3. pTRPE-34CAR5(H2L)-CD8H-BBz (C3052)
4. pTRPE-34CAR5(L2H)-CD8H-BBz (C3053)
5. pTRPE-9CAR5(H2L)-CD8H-BBz (C3048)
6. pTRPE-9CAR5(L2H)-CD8H-BBz (C3049)
2nd Generation CAR
scFv
Hinge
4-1BB
ζ
CAR19 backbone

FIG. 4

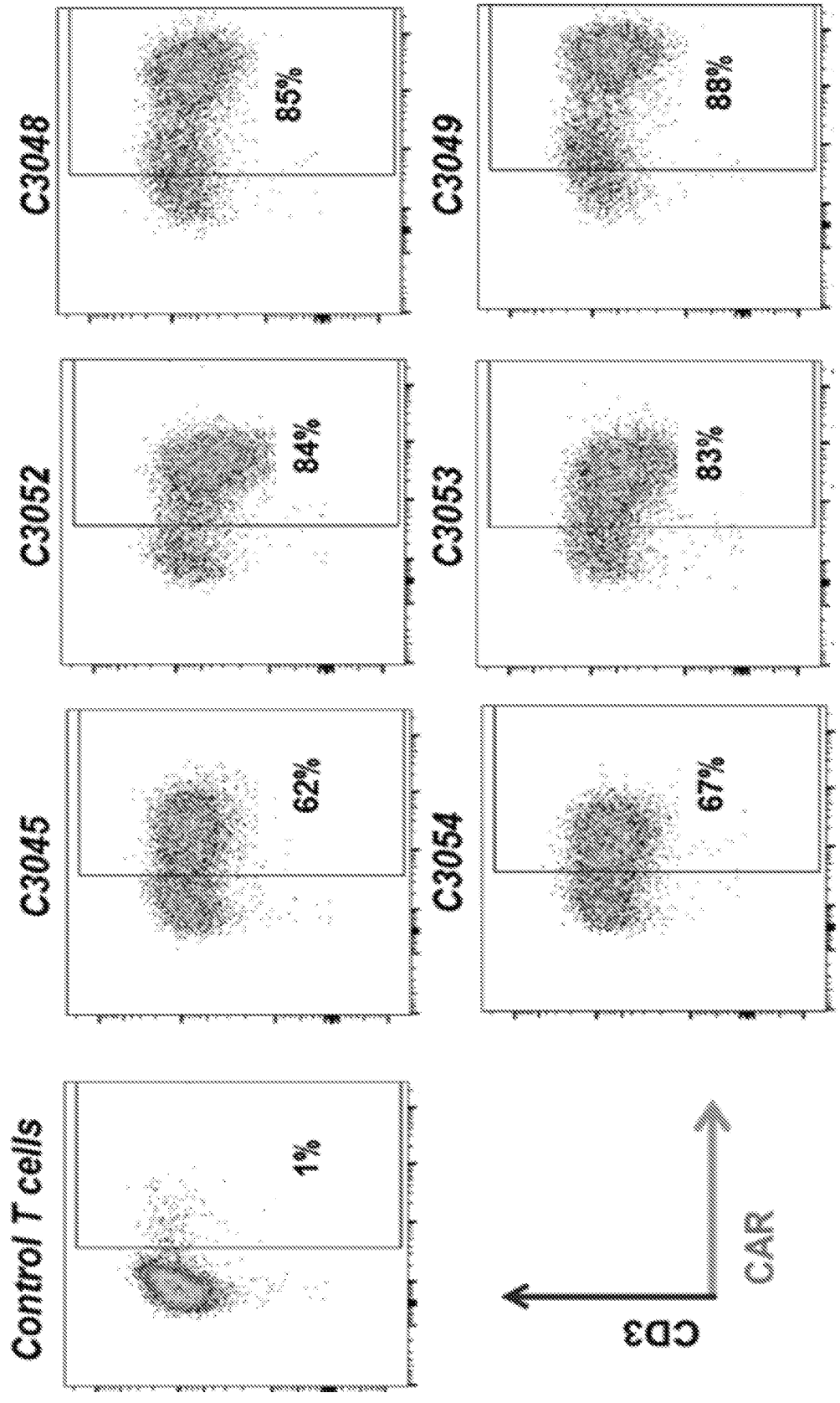

Control T cells
1%
scFv #17
(high affinity)
C3045
62%
C3054
67%
scFv #34
(med affinity)
C3052
84%
C3053
83%
scFv #9
(low affinity)
C3048
85%
C3049
88%
CD3
CAR

FIG. 5

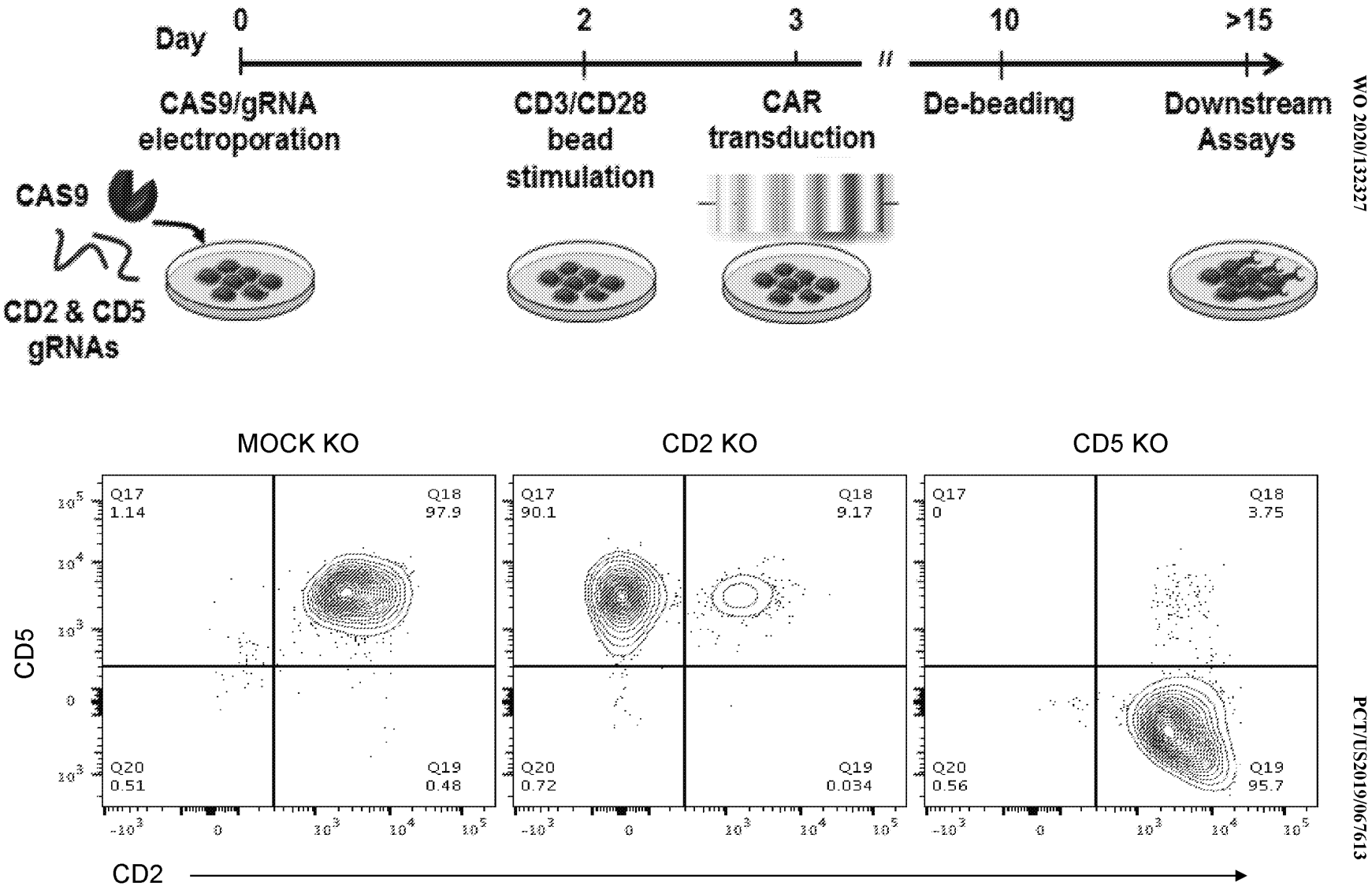

Day
0
2
3
10
>15
CAS9/gRNA electroporation
CD3/CD28 bead stimulation
CAR transduction
De-beading
Downstream Assays
CAS9
CD2 & CD5 gRNAs
MOCK KO
CD2 KO
CD5 KO
Q17 1.14
Q18 97.9
Q20 0.51
Q19 0.48
Q17 90.1
Q18 9.17
Q20 0.72
Q19 0.034
Q17 0
Q18 3.75
Q20 0.56
Q19 95.7
CD5
CD2

FIG. 6

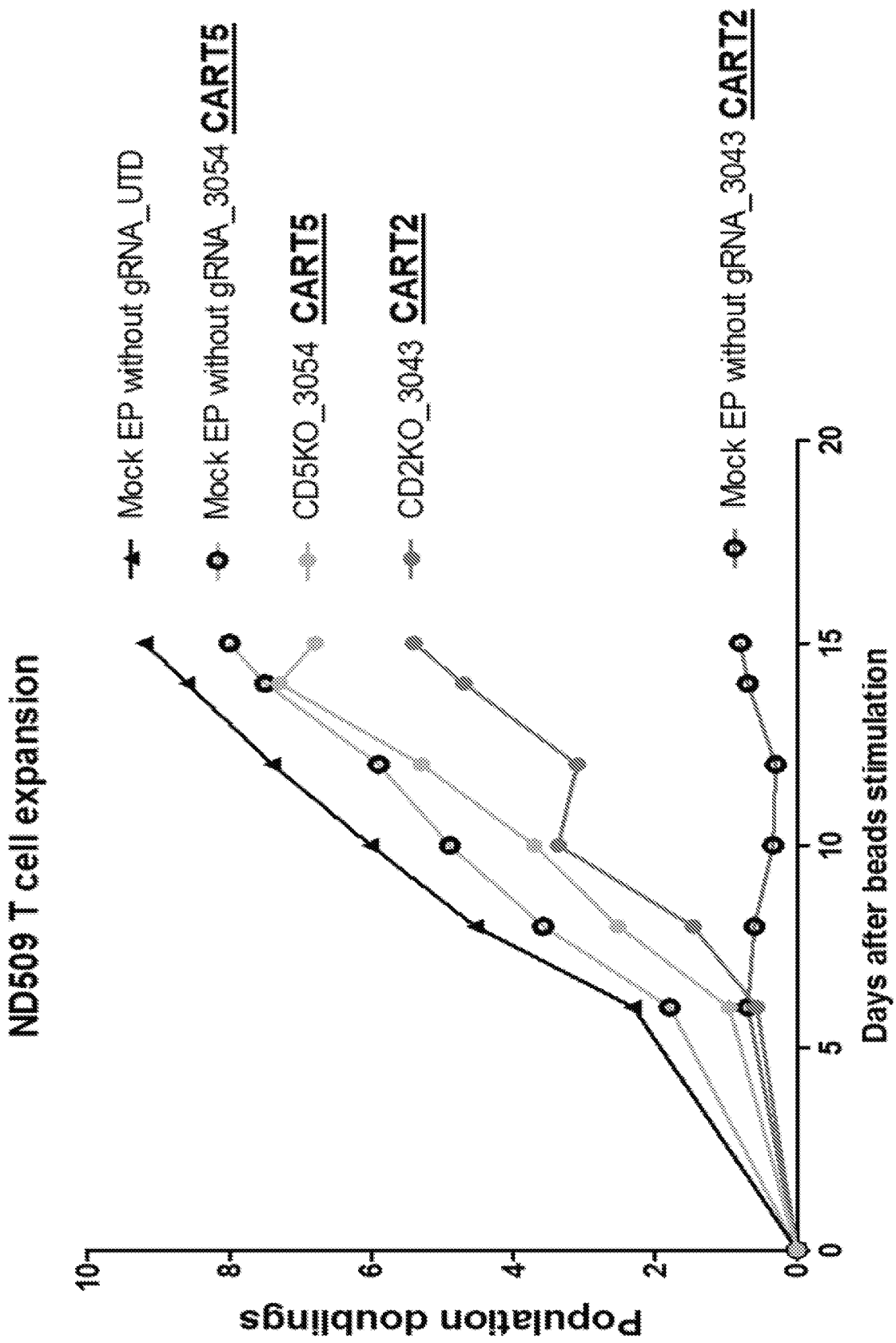

ND509 T cell expansion
Mock EP without gRNA_UTD
Mock EP without gRNA_3054 CART5
CD5KO_3054 CART5
CD2KO_3043 CART2
Mock EP without gRNA_3043 CART2
Population doublings
Days after beads stimulation
0
2
4
6
8
10
0
5
10
15
20

FIG. 7

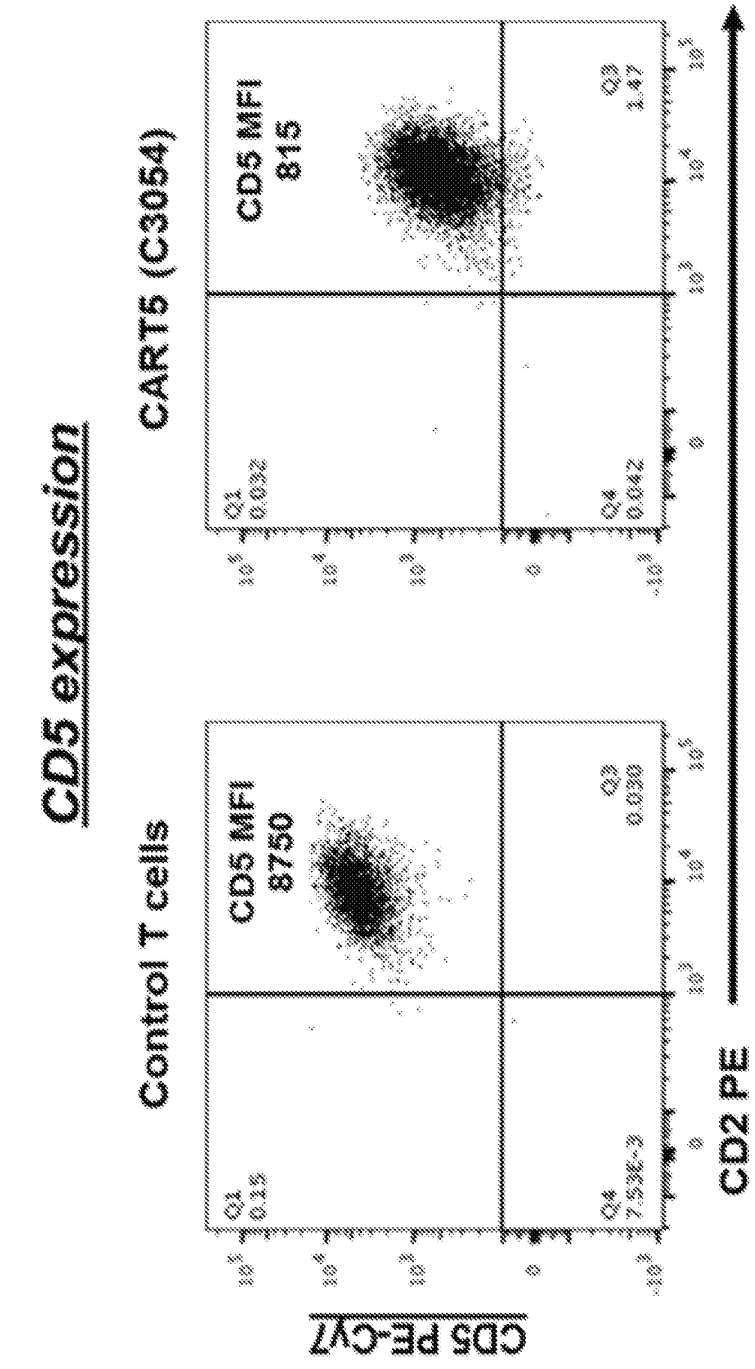

CD5 expression
Control T cells
CD5 MFI
8750
Q1
0.15
Q3
0.030
Q4
7.53E-3
CART5 (C3054)
CD5 MFI
815
Q1
0.032
Q3
1.47
Q4
0.042
CD5 PE-CY7
CD2 PE

FIG. 8

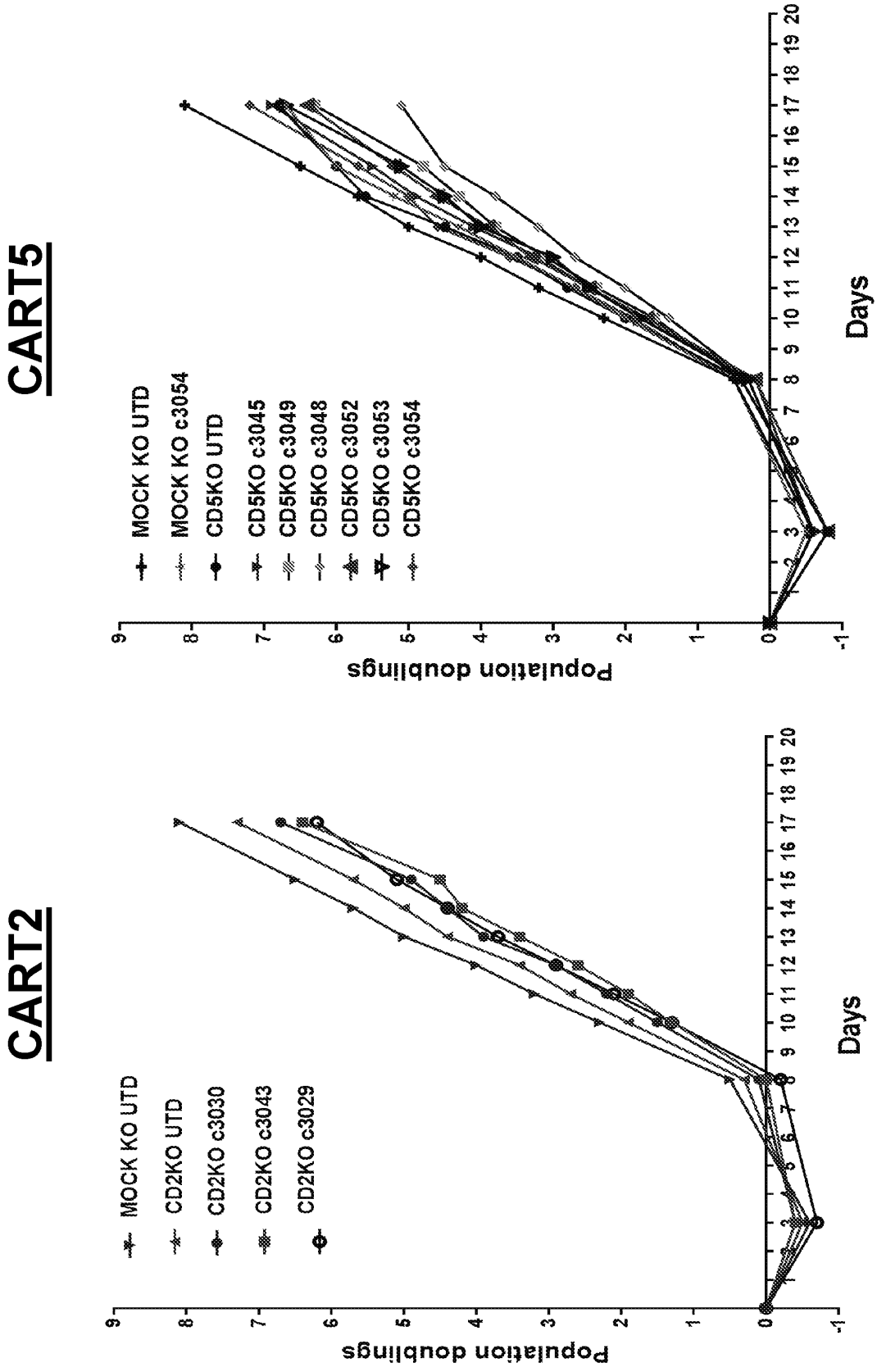

CART5
MOCK KO UTD
MOCK KO c3054
CD5KO UTD
CD5KO c3045
CD5KO c3049
CD5KO c3048
CD5KO c3052
CD5KO c3053
CD5KO c3054
Population doublings
Days
CART2
MOCK KO UTD
CD2KO UTD
CD2KO c3030
CD2KO c3043
CD2KO c3029
Population doublings
Days

FIG. 9

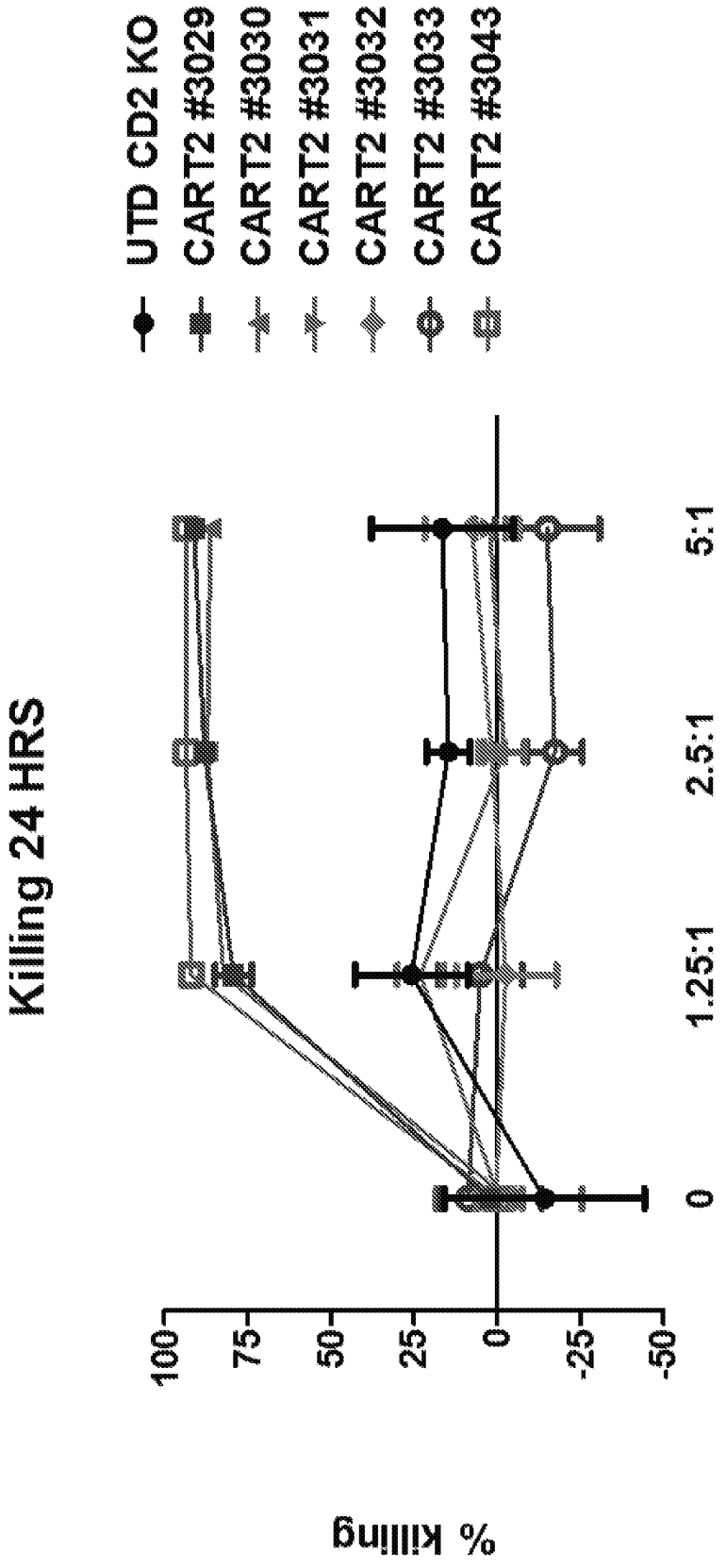

Killing 24 HRS
UTD CD2 KO
CART2 #3029
CART2 #3030
CART2 #3031
CART2 #3032
CART2 #3033
CART2 #3043
% killing
100
75
50
25
0
-25
-50
0
1.25:1
2.5:1
5:1
E:T RATIO

FIG. 10

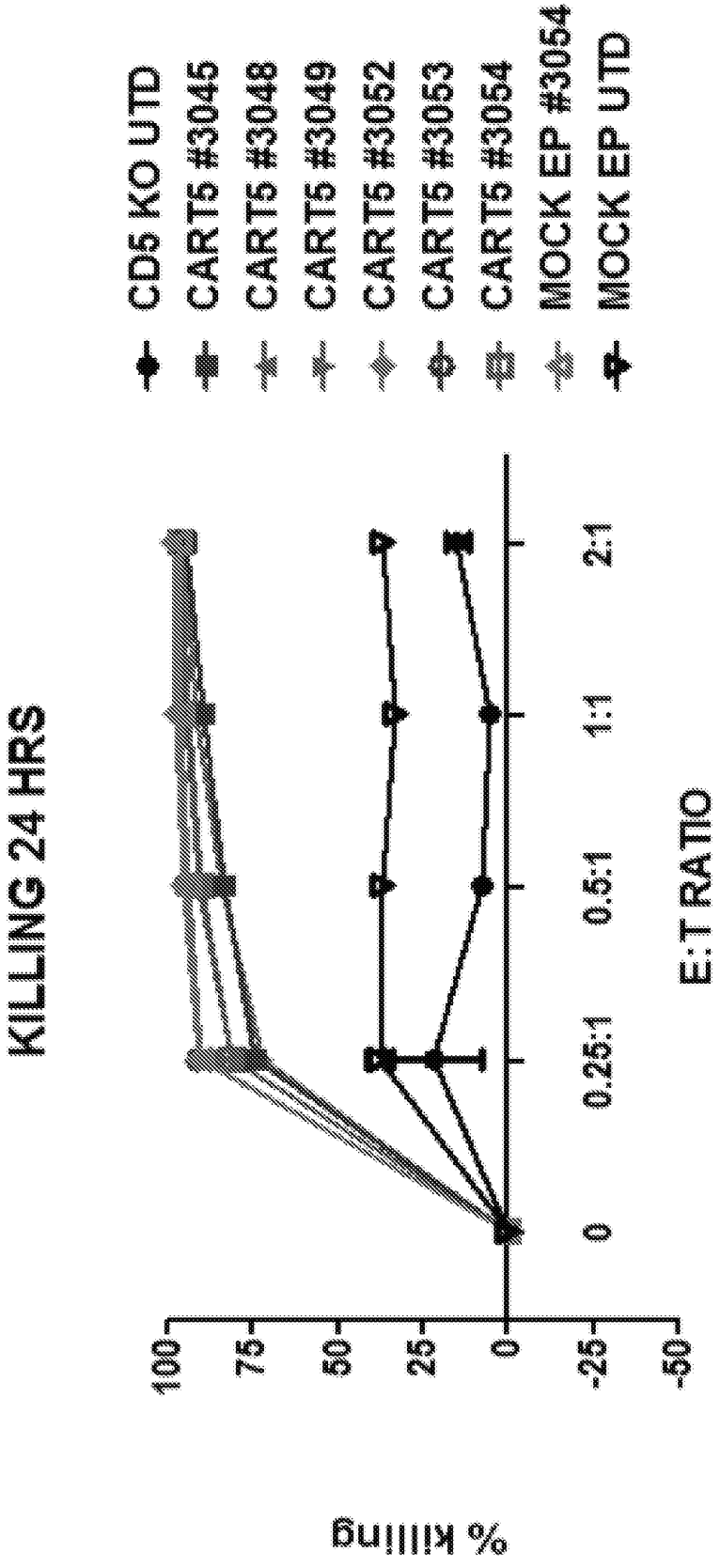

KILLING 24 HRS
CD5 KO UTD
CART5 #3045
CART5 #3048
CART5 #3049
CART5 #3052
CART5 #3053
CART5 #3054
MOCK EP #3054
MOCK EP UTD
100
75
50
25
0
-25
-50
0
0.25:1
0.5:1
1:1
2:1
E:T RATIO
% killing

FIG. 11

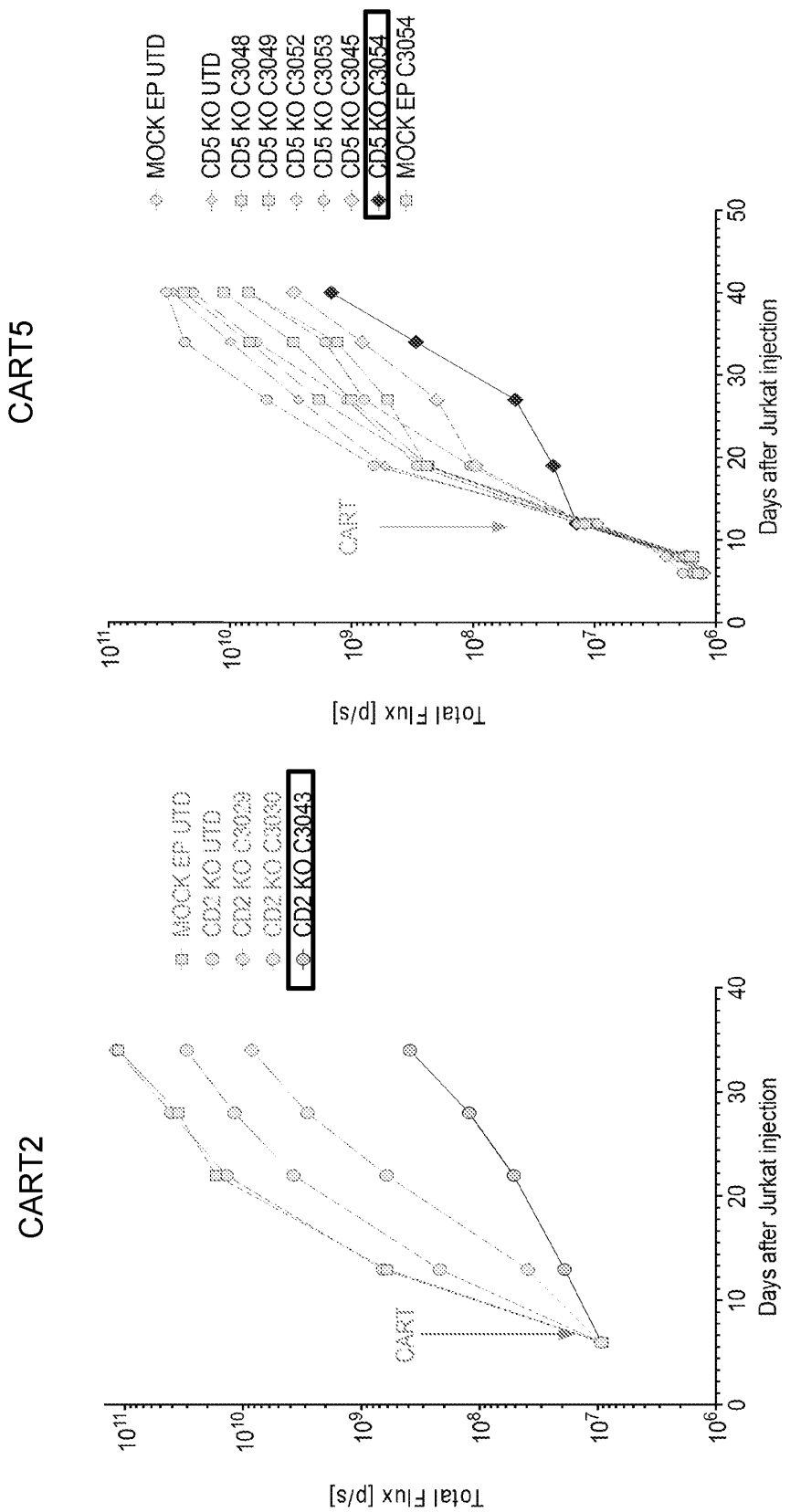

CART5
MOCK EP UTD
CD5 KO UTD
CD5 KO C3048
CD5 KO C3049
CD5 KO C3052
CD5 KO C3053
CD5 KO C3045
CD5 KO C3054
MOCK EP C3054
CART
Total Flux [p/s]
Days after Jurkat injection
CART2
MOCK EP UTD
CD2 KO UTD
CD2 KO C3029
CD2 KO C3030
CD2 KO C3043
CART
Total Flux [p/s]
Days after Jurkat injection

FIG. 12

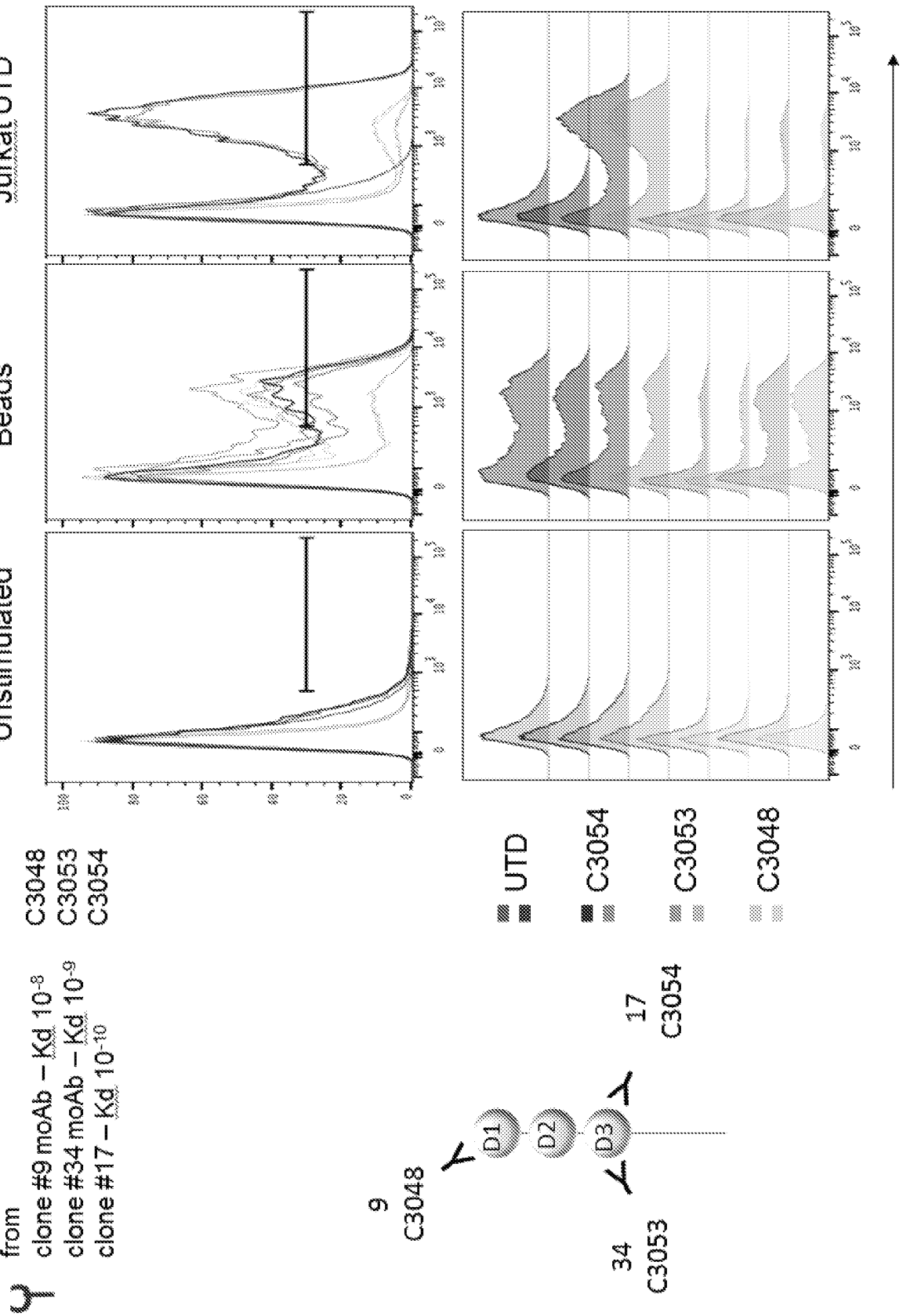

Unstimulated
Beads
Jurkat UTD
UTD
C3054
C3053
C3048
GFP
from
clone #9 moAb – Kd 10-8
clone #34 moAb – Kd 10-9
clone #17 – Kd 10-10
C3048
C3053
C3054
9
C3048
34
C3053
17
C3054
D1
D2
D3

FIG. 13

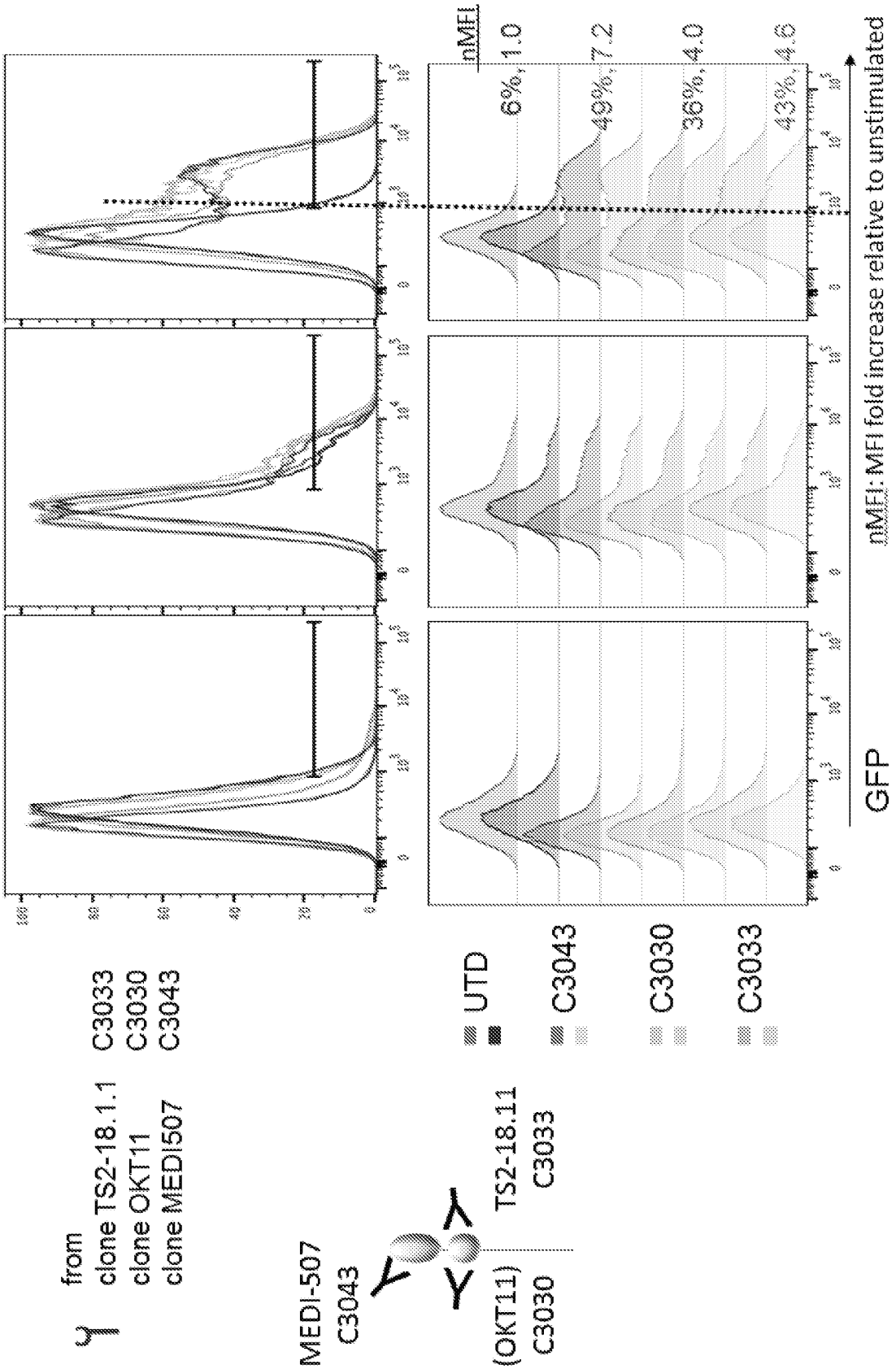

Unstimulated
Beads
Jurkat UTD
nMFI
6%, 1.0
49%, 7.2
36%, 4.0
43%, 4.6
GFP
nMFI: MFI fold increase relative to unstimulated
UTD
C3043
C3030
C3033
from
clone TS2-18.1.1 C3033
clone OKT11 C3030
clone MEDI507 C3043
MEDI-507
C3043
(OKT11)
C3030
TS2-18.11
C3033

FIG. 14

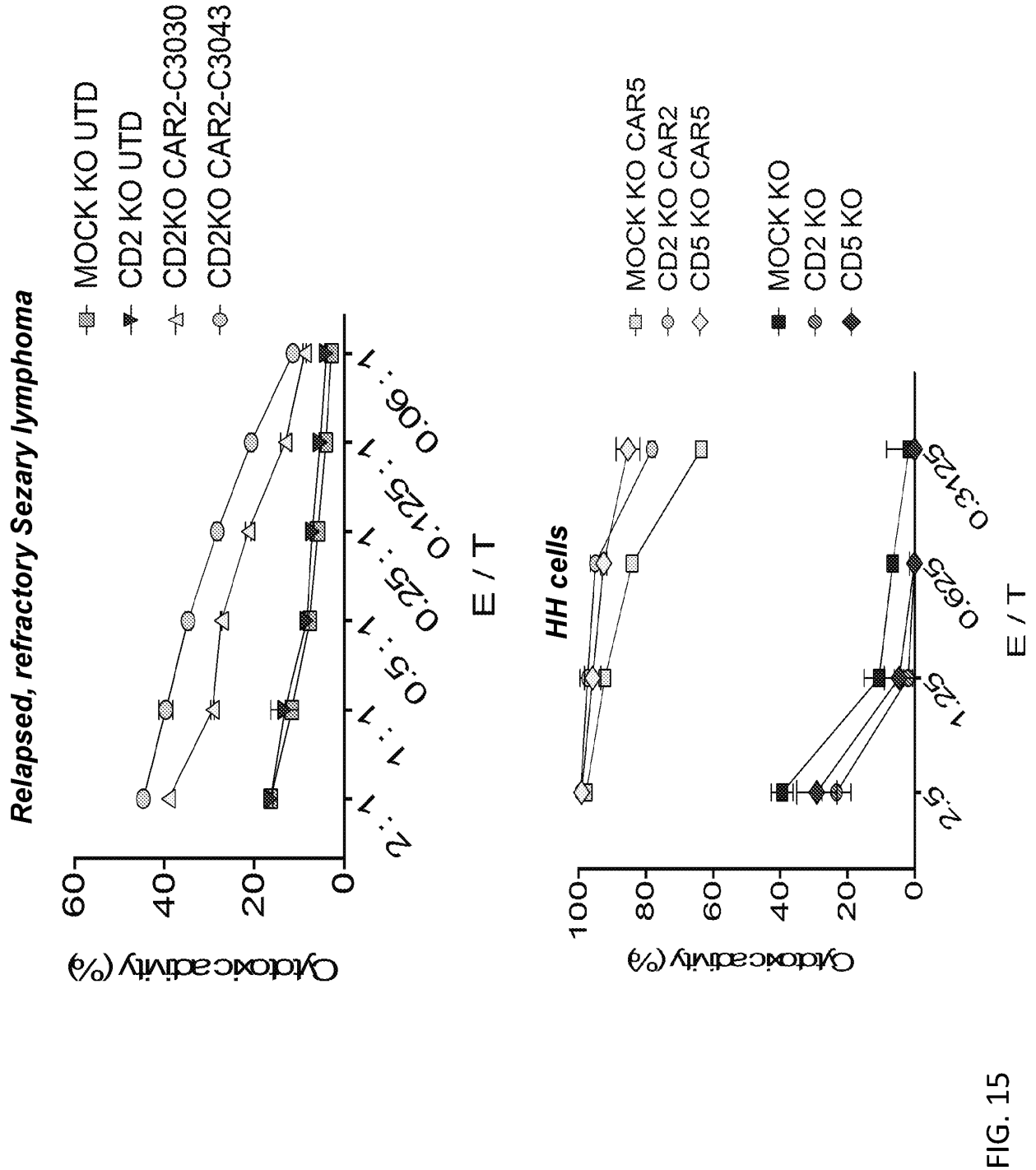

Relapsed, refractory Sezary lymphoma
MOCK KO UTD
CD2 KO UTD
CD2KO CAR2-C3030
CD2KO CAR2-C3043
Cytotoxic activity (%)
60
40
20
0
2 : 1
1 : 1
0.5 : 1
0.25 : 1
0.125 : 1
0.06 : 1
E / T
HH cells
MOCK KO CAR5
CD2 KO CAR2
CD5 KO CAR5
MOCK KO
CD2 KO
CD5 KO
Cytotoxic activity (%)
100
80
60
40
20
0
2.5
1.25
0.625
0.3125
E / T

FIG. 15

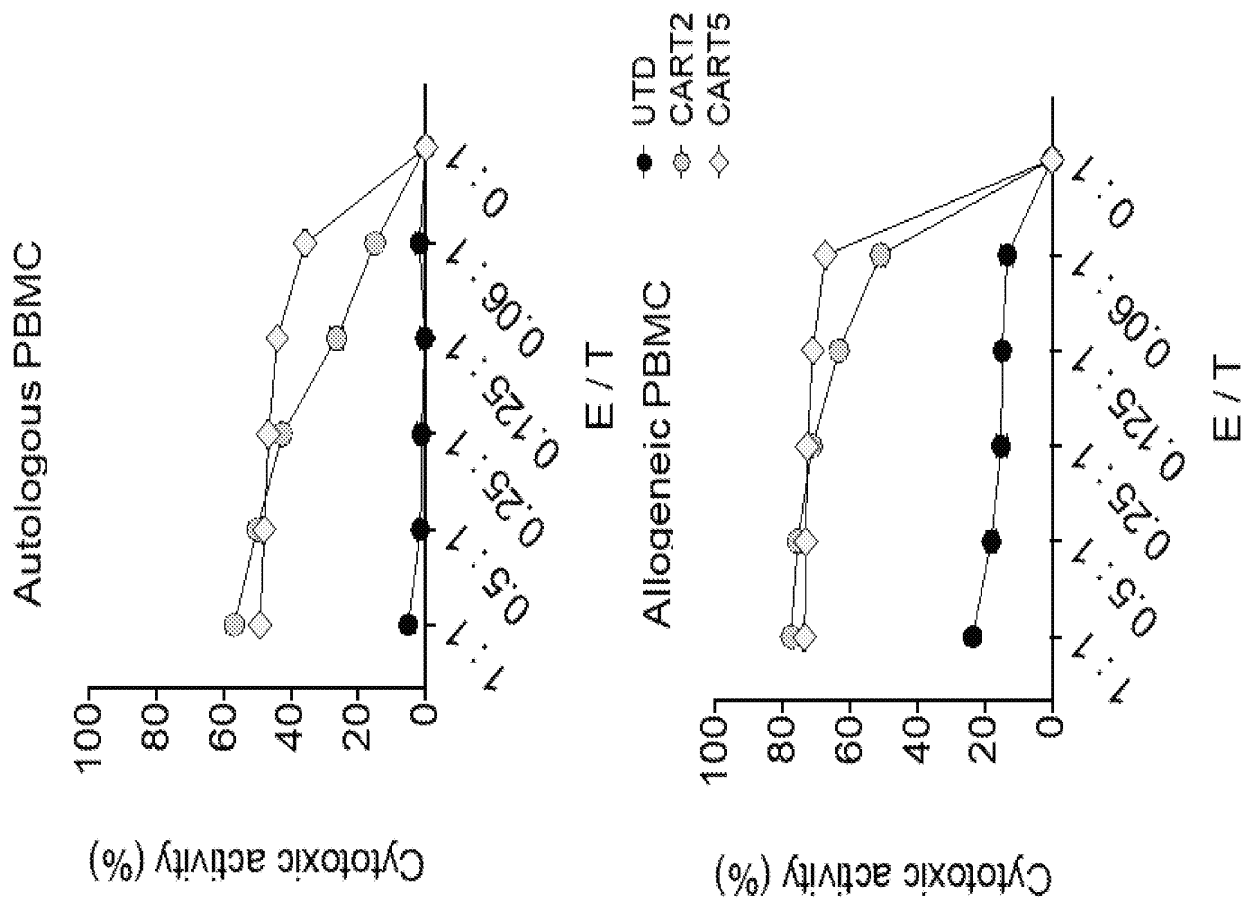

Autologous PBMC
Cytotoxic activity (%)
100
80
60
40
20
0
1 : 1
0.5 : 1
0.25 : 1
0.125 : 1
0.06 : 1
0 : 1
E / T
Allogeneic PBMC
UTD
CART2
CART5

FIG. 16

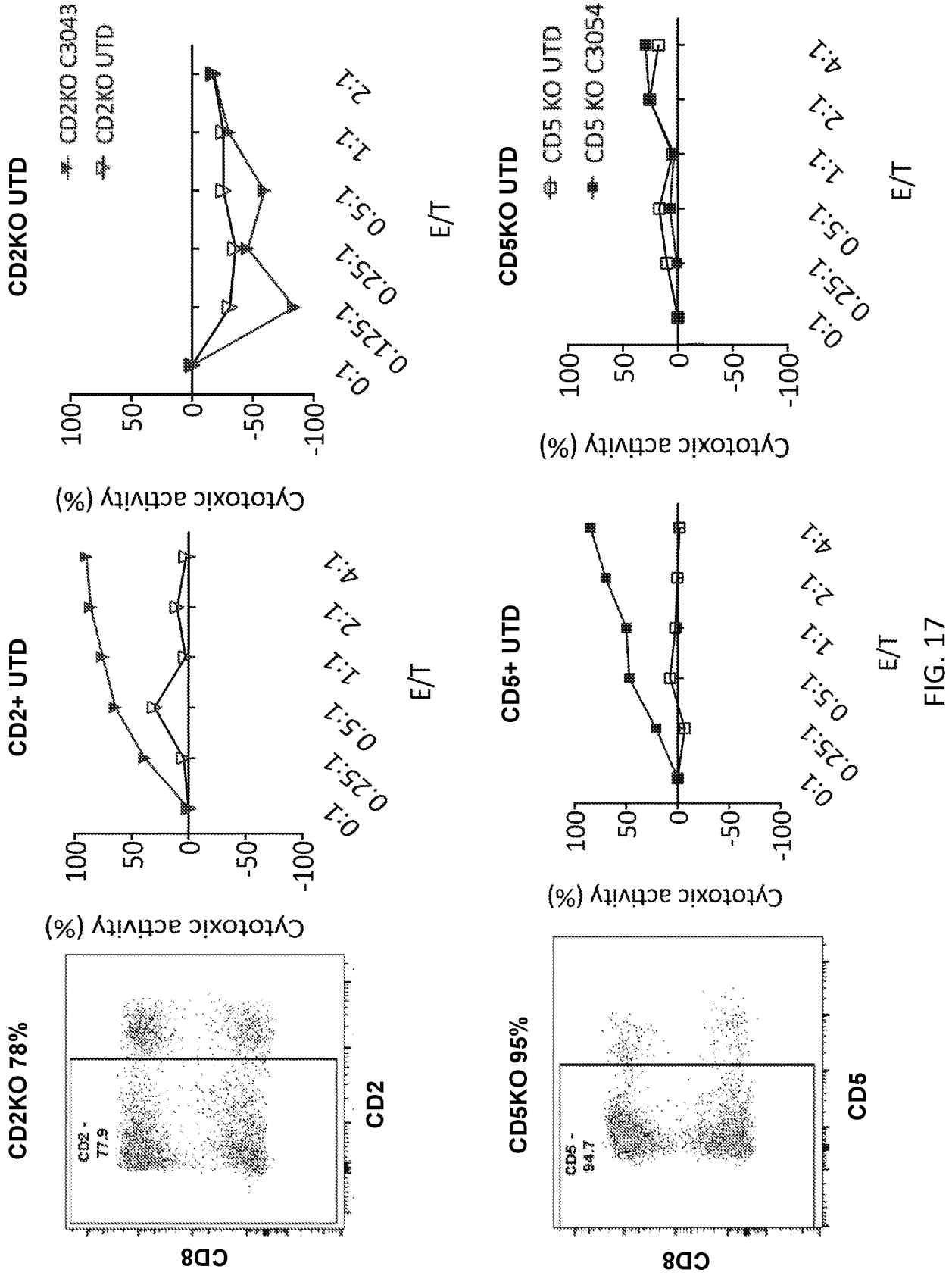

CD2KO 78%
CD2 -
77.9
CD8
CD2
CD2+ UTD
Cytotoxic activity (%)
100
50
0
-50
-100
0:1
0.25:1
0.5:1
1:1
2:1
4:1
E/T
CD2KO UTD
CD2KO C3043
CD2KO UTD
0:1
0.125:1
0.25:1
0.5:1
1:1
2:1
E/T
CD5KO 95%
CD5 -
94.7
CD8
CD5
CD5+ UTD
Cytotoxic activity (%)
E/T
CD5KO UTD
CD5 KO UTD
CD5 KO C3054
Cytotoxic activity (%)
E/T

FIG. 17

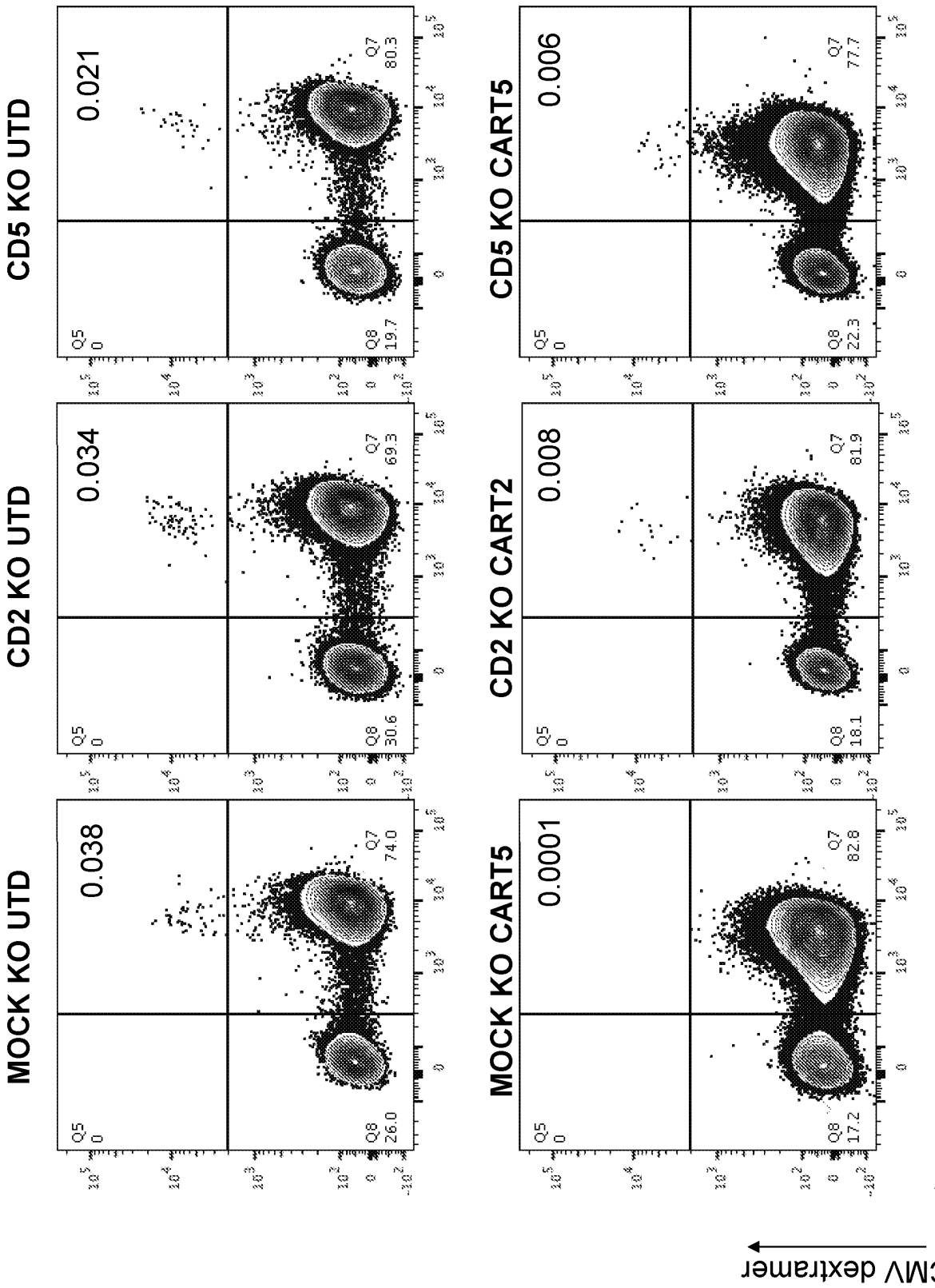

MOCK KO UTD
CD2 KO UTD
CD5 KO UTD
MOCK KO CART5
CD2 KO CART2
CD5 KO CART5
0.038
0.034
0.021
0.0001
0.008
0.006
Q5
0
Q8
26.0
Q7
74.0
Q8
30.6
Q7
69.3
Q8
19.7
Q7
80.3
Q8
17.2
Q7
82.8
Q8
18.1
Q7
81.9
Q8
22.3
Q7
77.7
CMV dextramer
CD8

FIG. 18

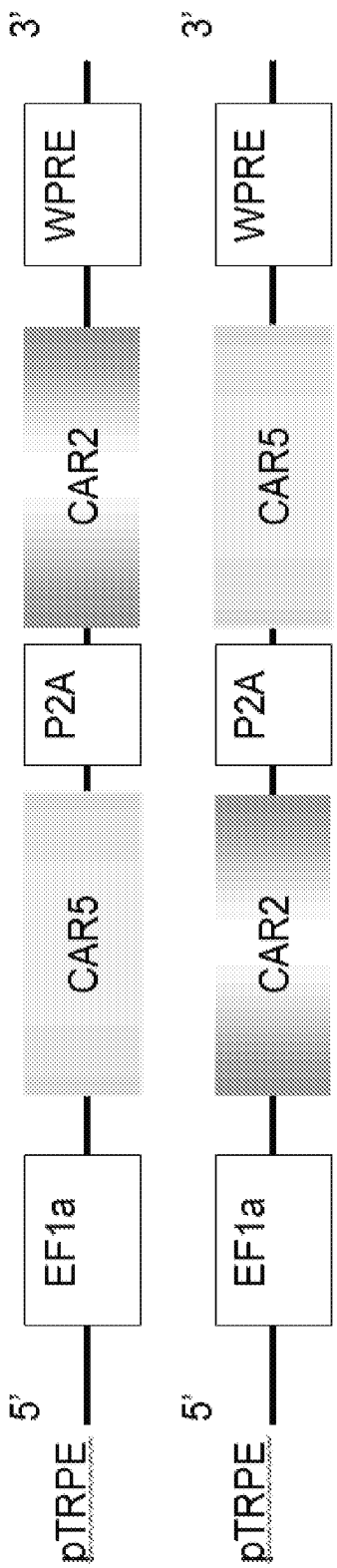

3'
WPRE
CAR2
P2A
CAR5
EF1a
5'
pTRPE
3'
WPRE
CAR5
P2A
CAR2
EF1a
5'
pTRPE

FIG. 19

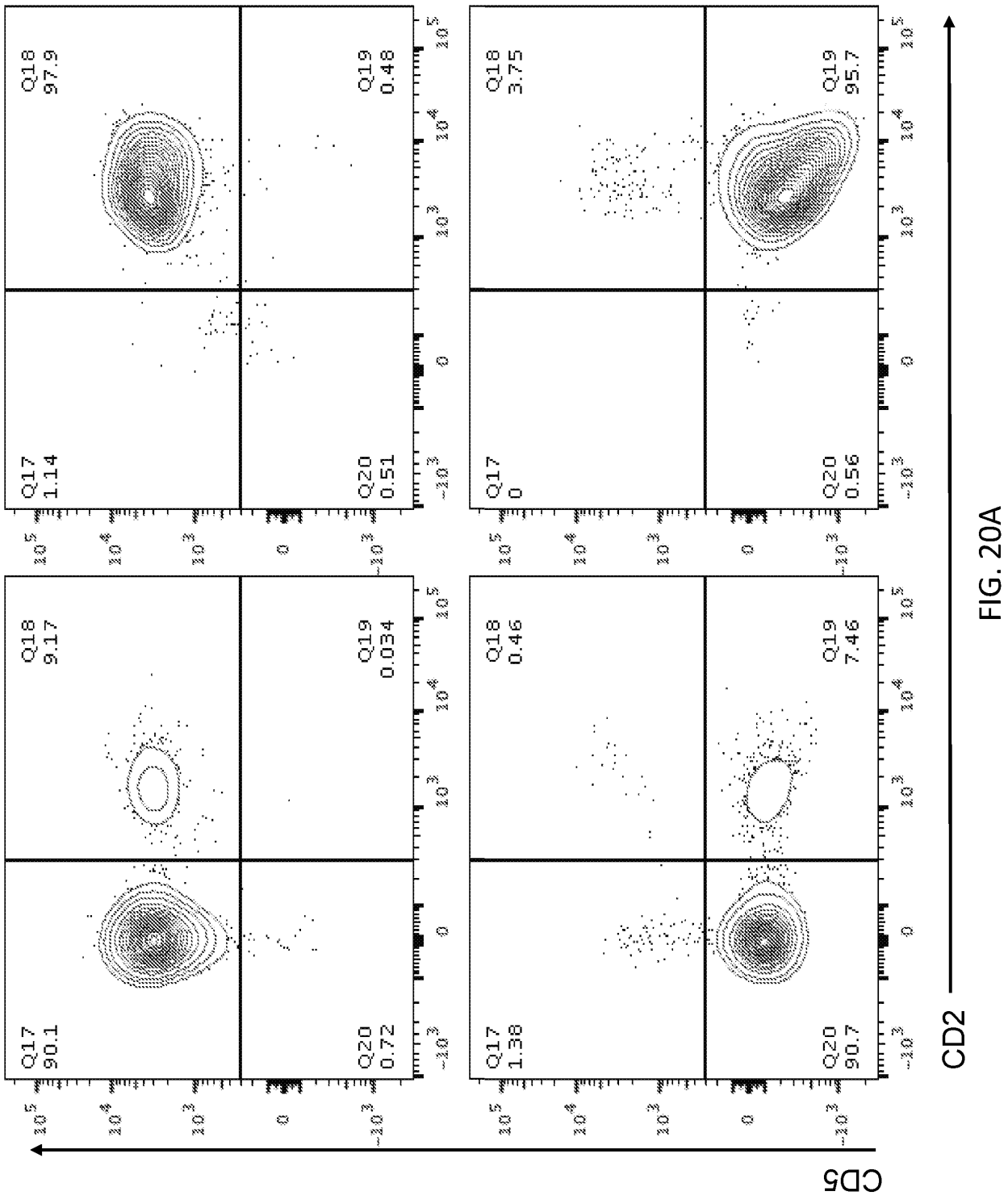

Q17
90.1
Q18
9.17
Q20
0.72
Q19
0.034
Q17
1.38
Q18
0.46
Q20
90.7
Q19
7.46
Q17
1.14
Q18
97.9
Q20
0.51
Q19
0.48
Q17
0
Q18
3.75
Q20
0.56
Q19
95.7
CD5
CD2

FIG. 20A

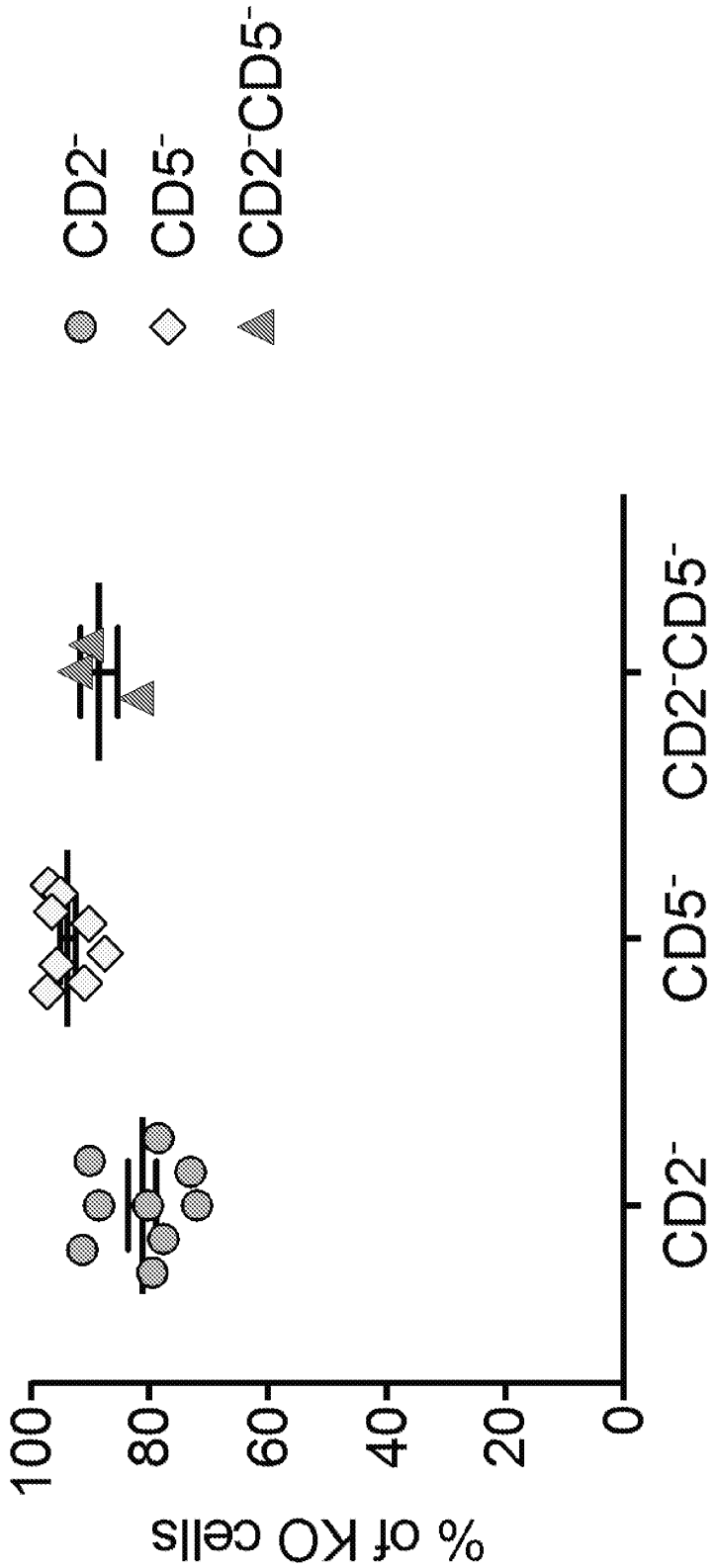


FIG. 20B

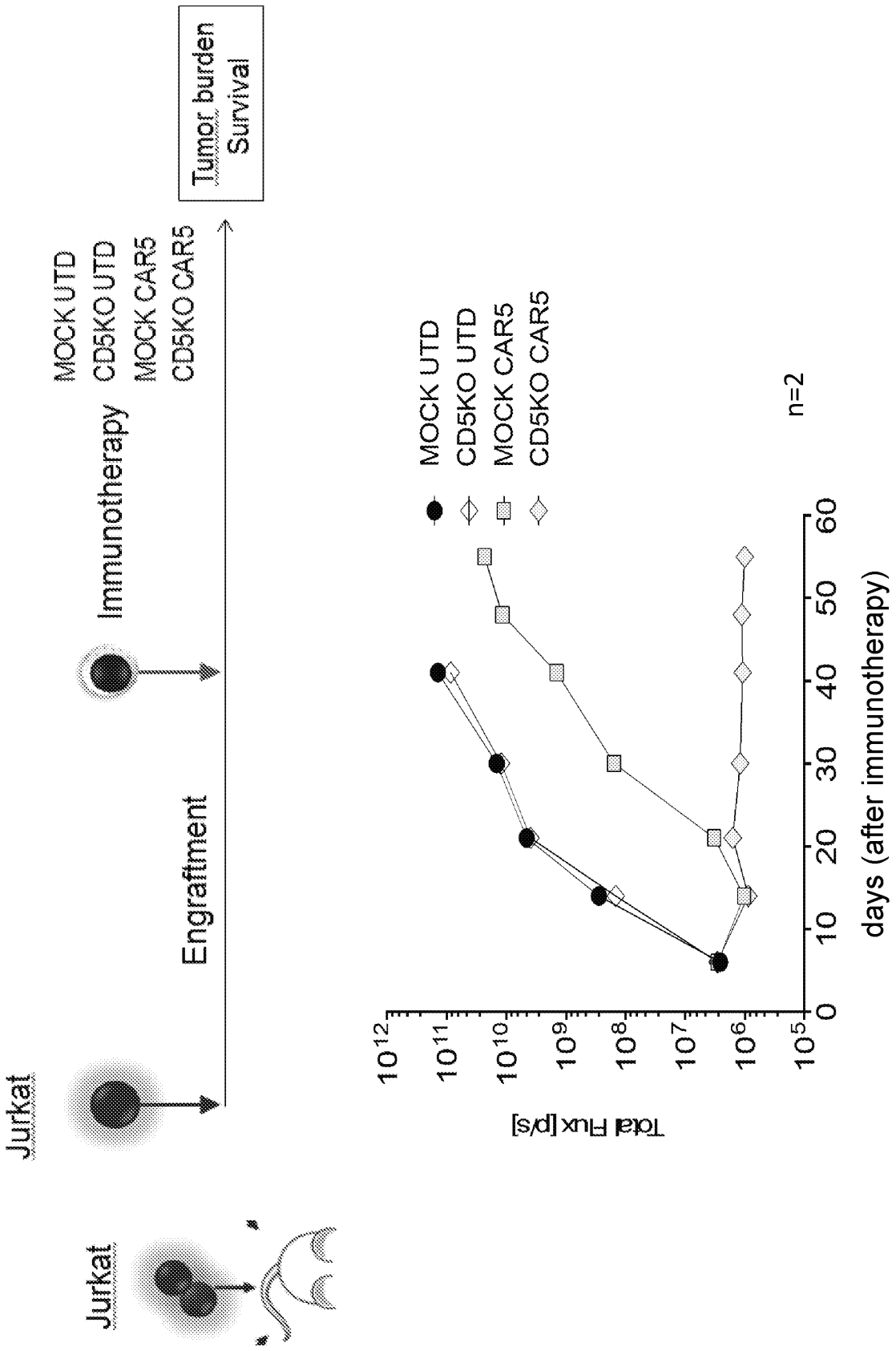

Jurkat
Jurkat
Engraftment
Immunotherapy
MOCK UTD
CD5KO UTD
MOCK CAR5
CD5KO CAR5
Tumor burden
Survival
MOCK UTD
CD5KO UTD
MOCK CAR5
CD5KO CAR5
n=2
Total Flux [p/s]
days (after immunotherapy)

FIG. 21

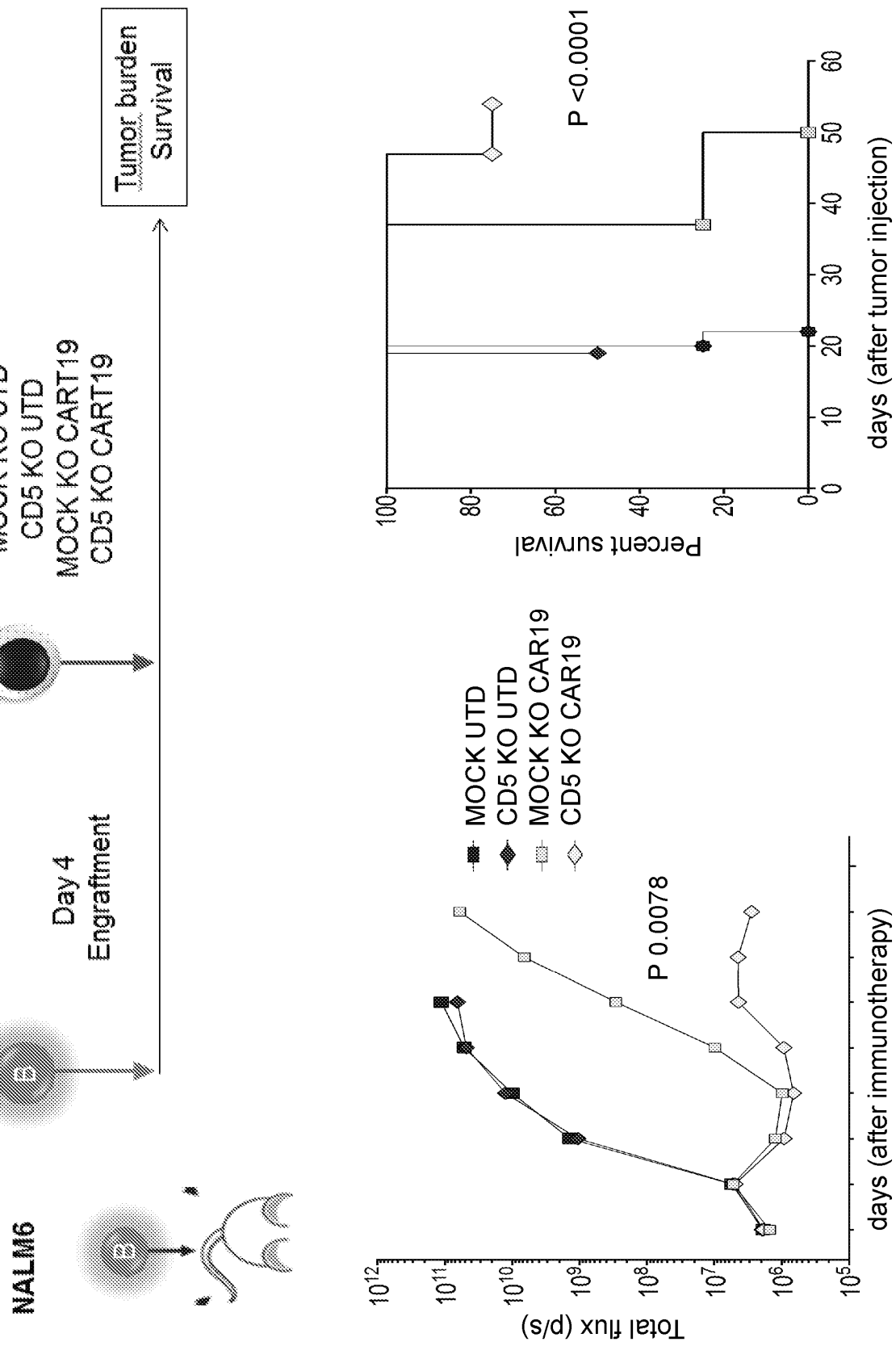

NALM6
Day 4
Engraftment
MOCK KO UTD
CD5 KO UTD
MOCK KO CART19
CD5 KO CART19
Tumor burden
Survival
Total flux (p/s)
days (after immunotherapy)
MOCK UTD
CD5 KO UTD
MOCK KO CAR19
CD5 KO CAR19
P 0.0078
Percent survival
days (after tumor injection)
P <0.0001

FIG. 22

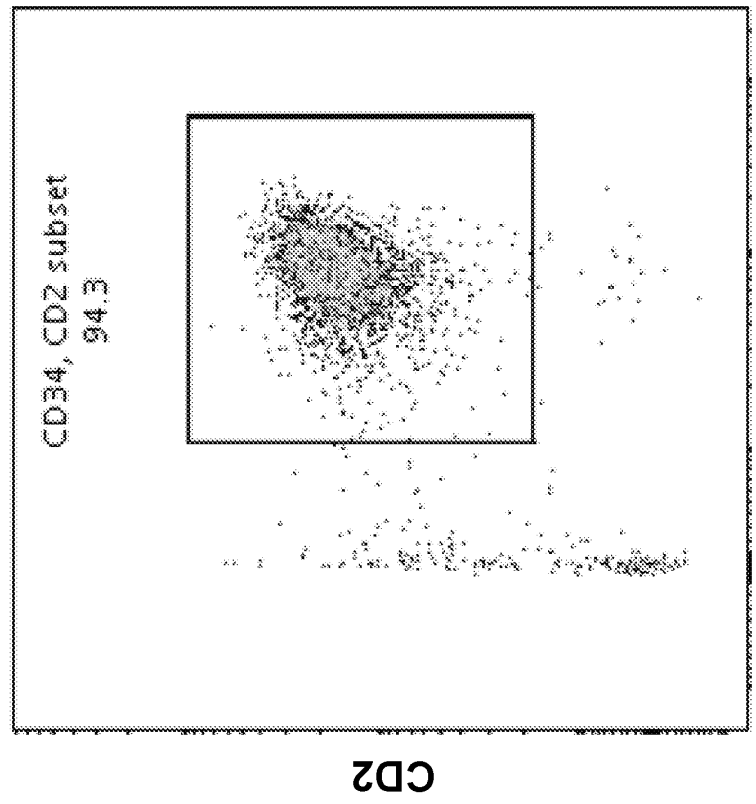


FIG. 23A

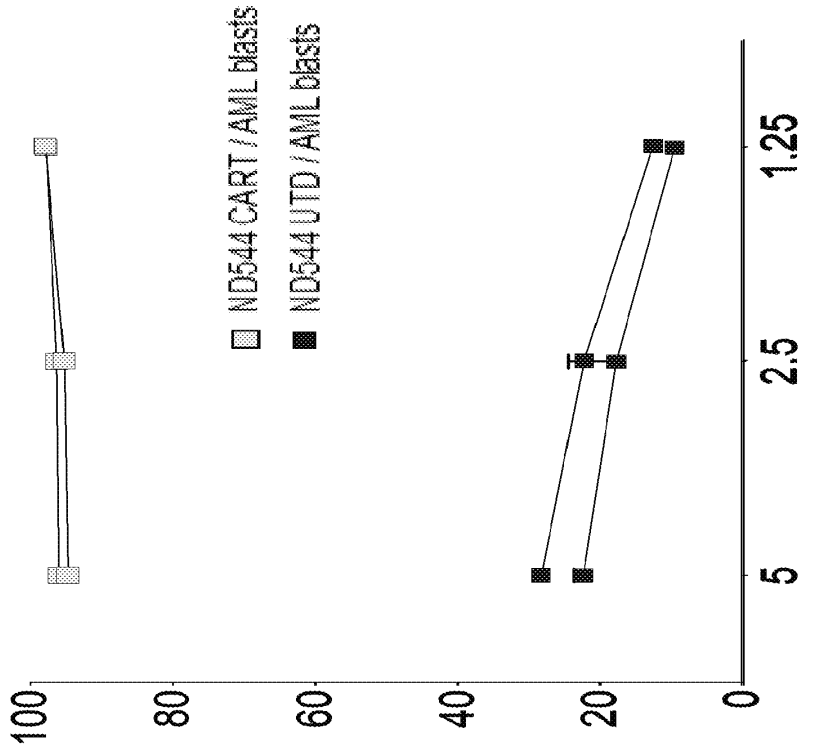


FIG. 23B

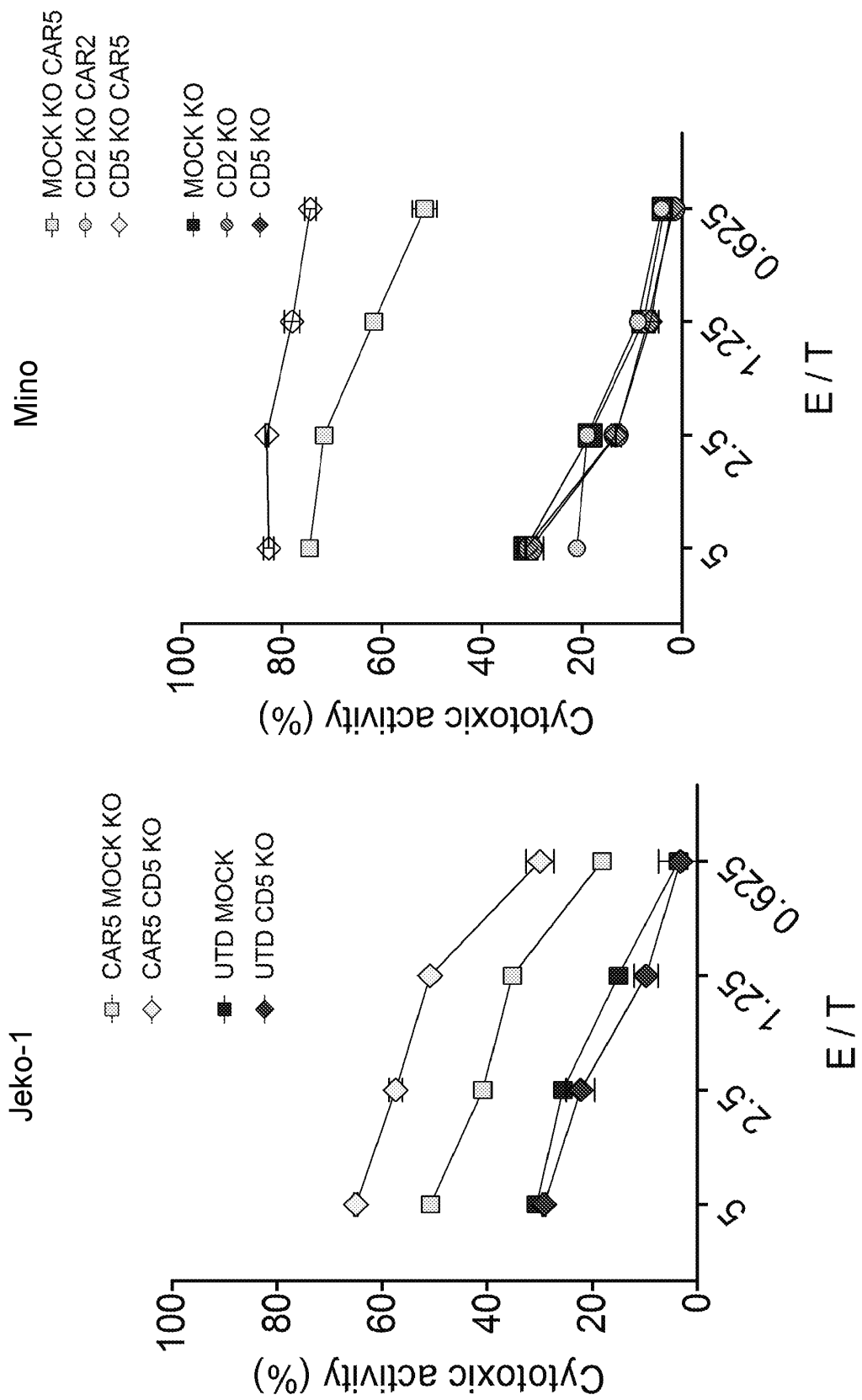
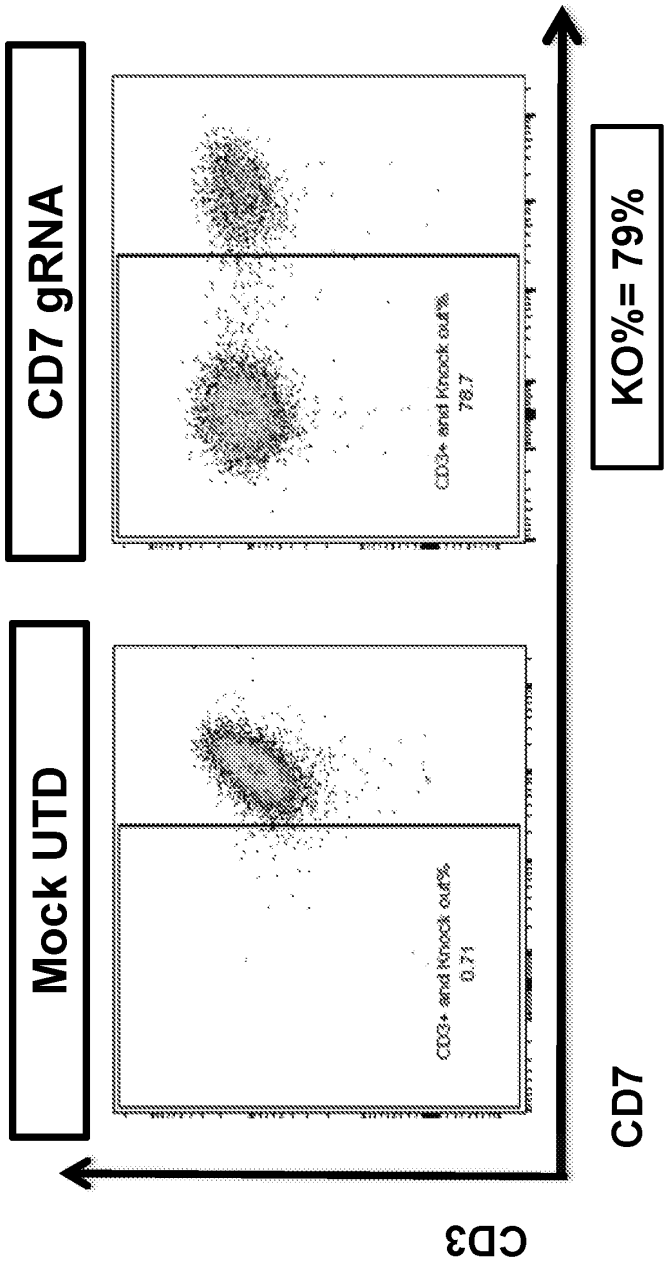

Jeko-1
Mino
Cytotoxic activity (%)
E / T
0.625
1.25
2.5
5
0
20
40
60
80
100
CAR5 MOCK KO
CAR5 CD5 KO
UTD MOCK
UTD CD5 KO
MOCK KO CAR5
CD2 KO CAR2
CD5 KO CAR5
MOCK KO
CD2 KO
CD5 KO

FIG. 24



Anti-CD7 CARs

1. pTRPE-CAR7(H2L)-CD8H-BBz TH-69 from WO2003051926A2 patent
2. pTRPE-CAR7(L2H)-CD8H-BBz TH-69 from WO2003051926A2 patent
3. pTRPE-CAR7(H2L1)-CD8H-BBz from hybridoma
4. pTRPE-CAR7(L12H)-CD8H-BBz from hybridoma
5. pTRPE-CAR7(H2L2)-CD8H-BBz from hybridoma
6. pTRPE-CAR7(L22H)-CD8H-BBz from hybridoma

FIG. 25

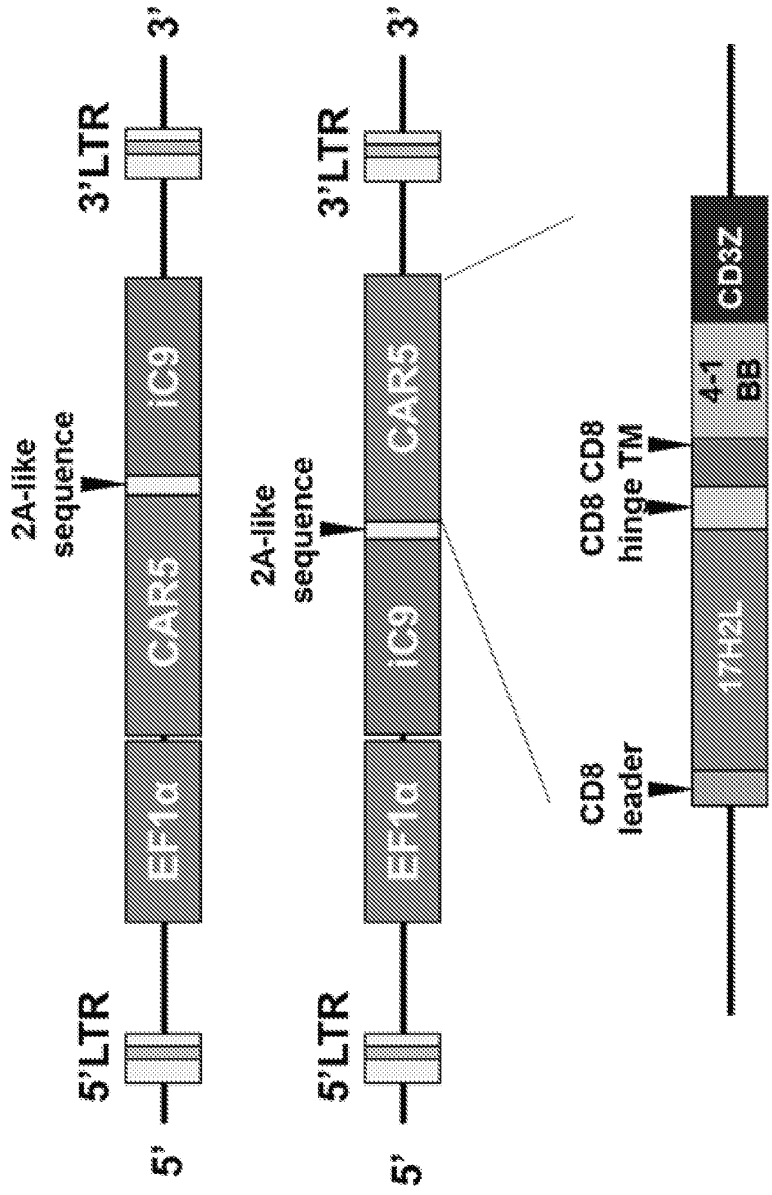

5'LTR
3'LTR
5'
3'
EF1α
CAR5
IC9
2A-like sequence
CD8 leader
CD8 hinge TM
4-1 BB
CD3Z

FIG. 26