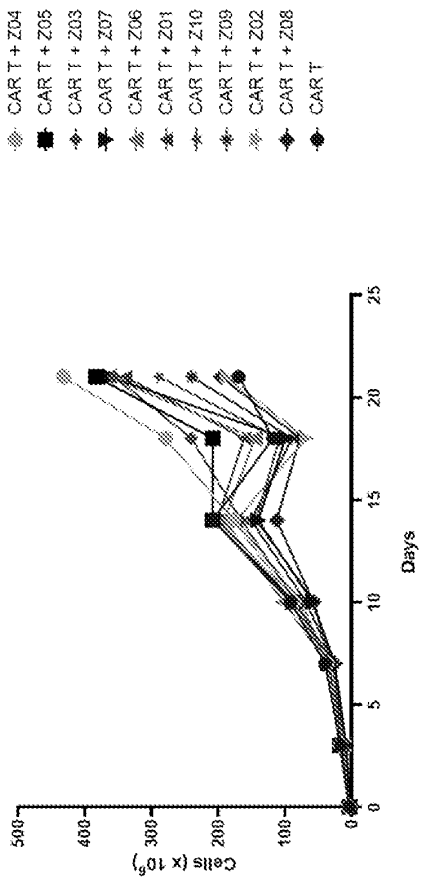

CAR T + Z04
CAR T + Z05
CAR T + Z03
CAR T + Z07
CAR T + Z06
CAR T + Z01
CAR T + Z10
CAR T + Z09
CAR T + Z02
CAR T + Z08
CAR T
Cells (x 10^9)
Days
0
100
200
300
400
500
0
5
10
15
20
25

FIG. 1A

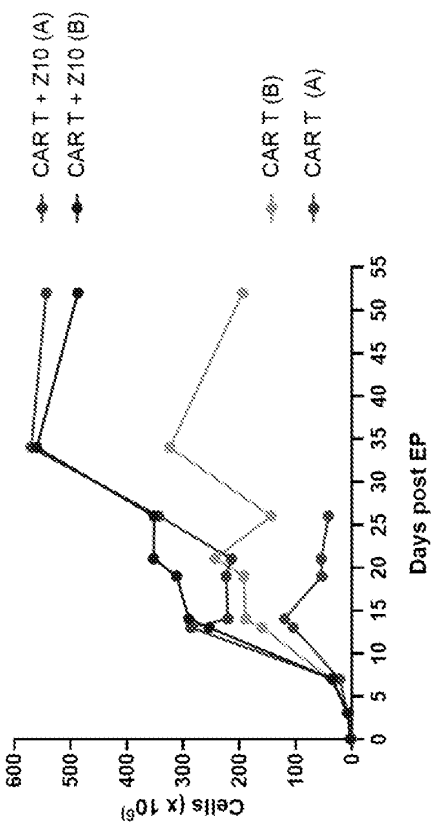

CAR T + Z10 (A)
CAR T + Z10 (B)
CAR T (B)
CAR T (A)
Cells (x 10^9)
Days post EP
0
100
200
300
400
500
600
0
5
10
15
20
25
30
35
40
45
50
55

FIG. 1B

FIG. 2A

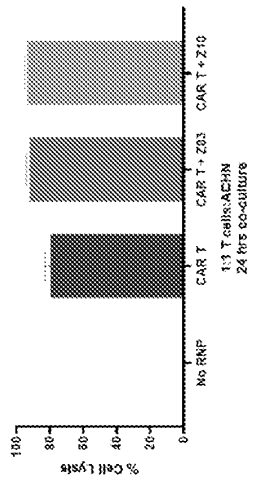


FIG. 2B

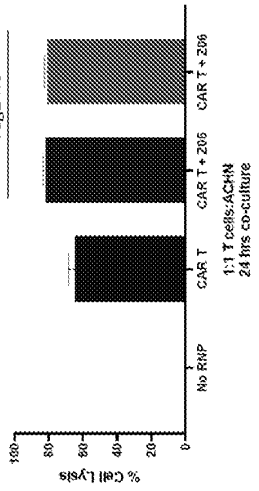


FIG. 2C

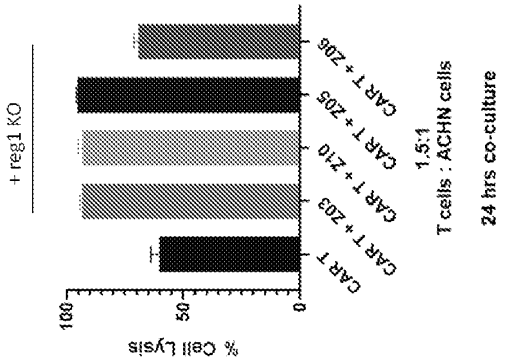


FIG. 2D

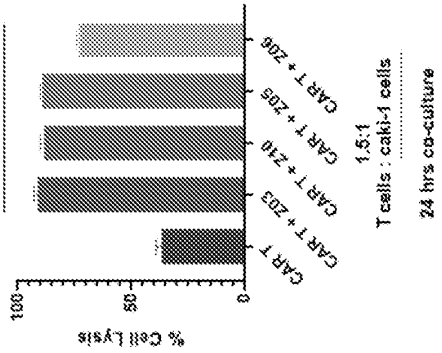


FIG. 2E

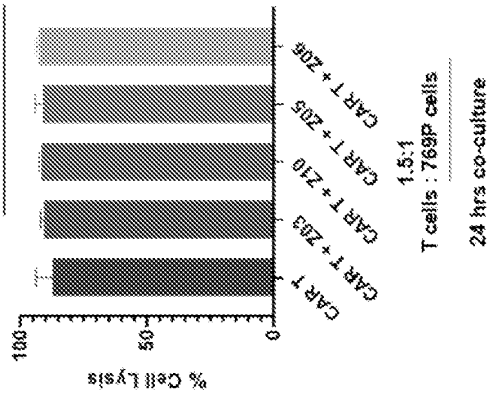


FIG. 3A

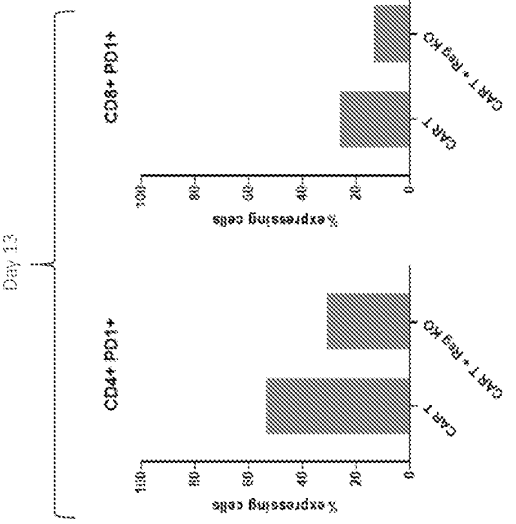


FIG. 3B

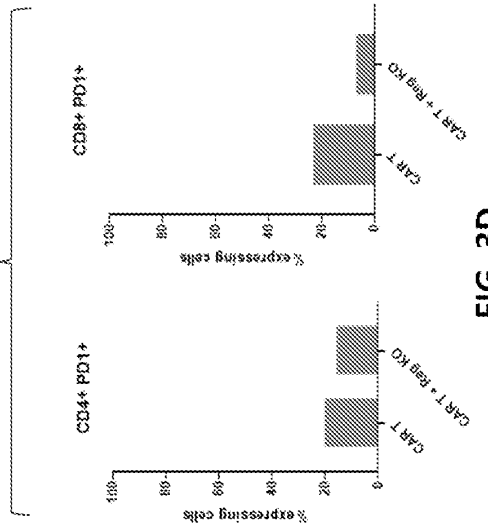


FIG. 3C

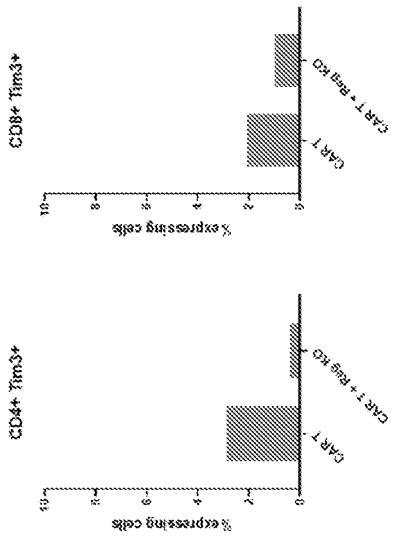


FIG. 3D

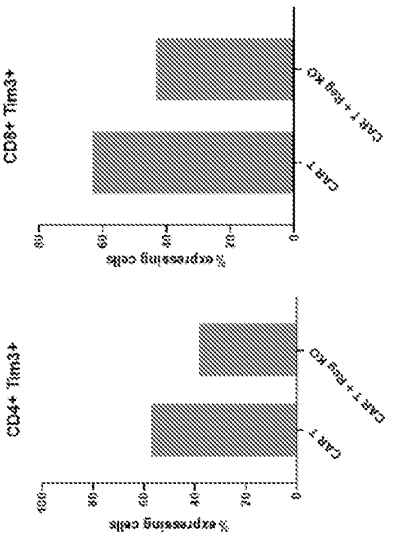


FIG. 4

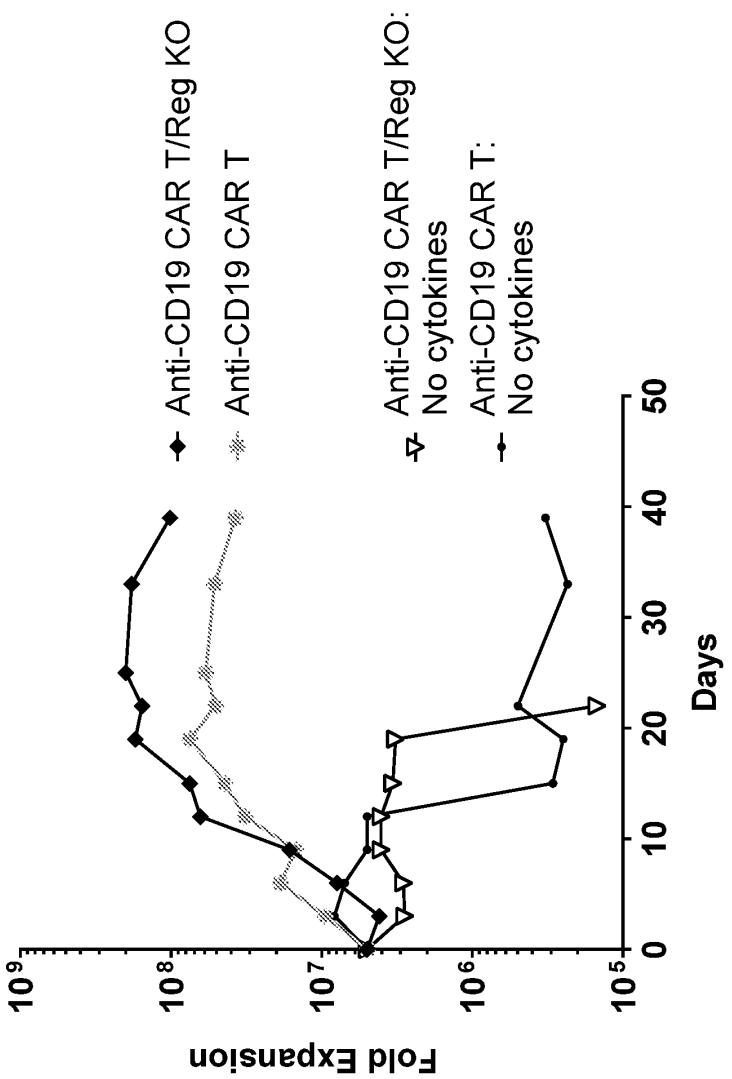

Anti-CD19 CAR T/Reg KO
Anti-CD19 CAR T
Anti-CD19 CAR T/Reg KO:
No cytokines
Anti-CD19 CAR T:
No cytokines
Fold Expansion
Days
10^9
10^8
10^7
10^6
10^5
0
10
20
30
40
50

FIG. 5A

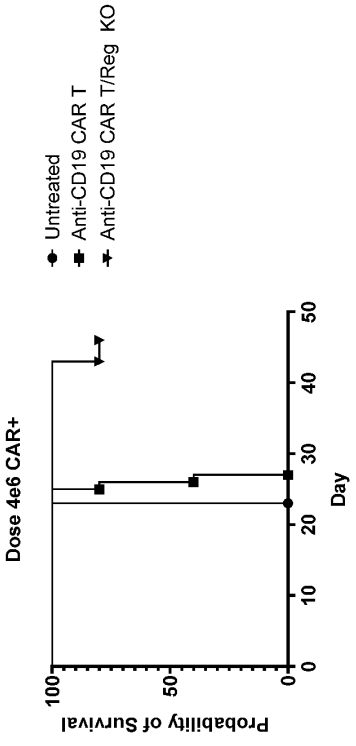


FIG. 5B

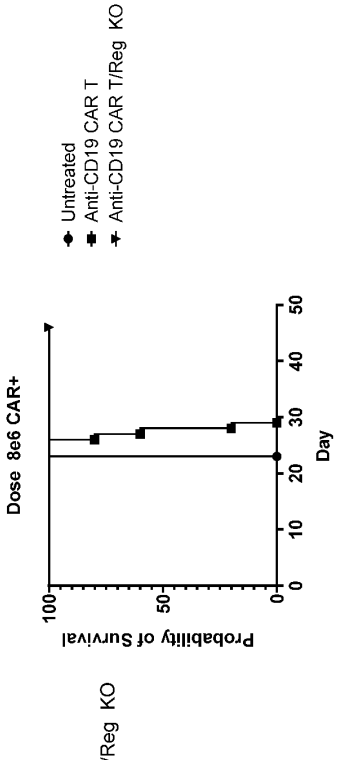


FIG. 5C

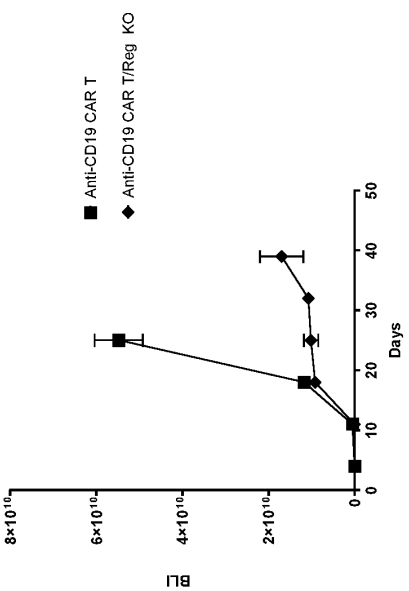


FIG. 5D

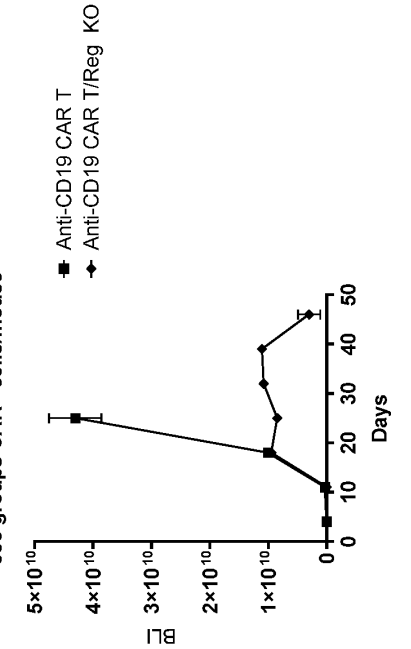


FIG. 6A

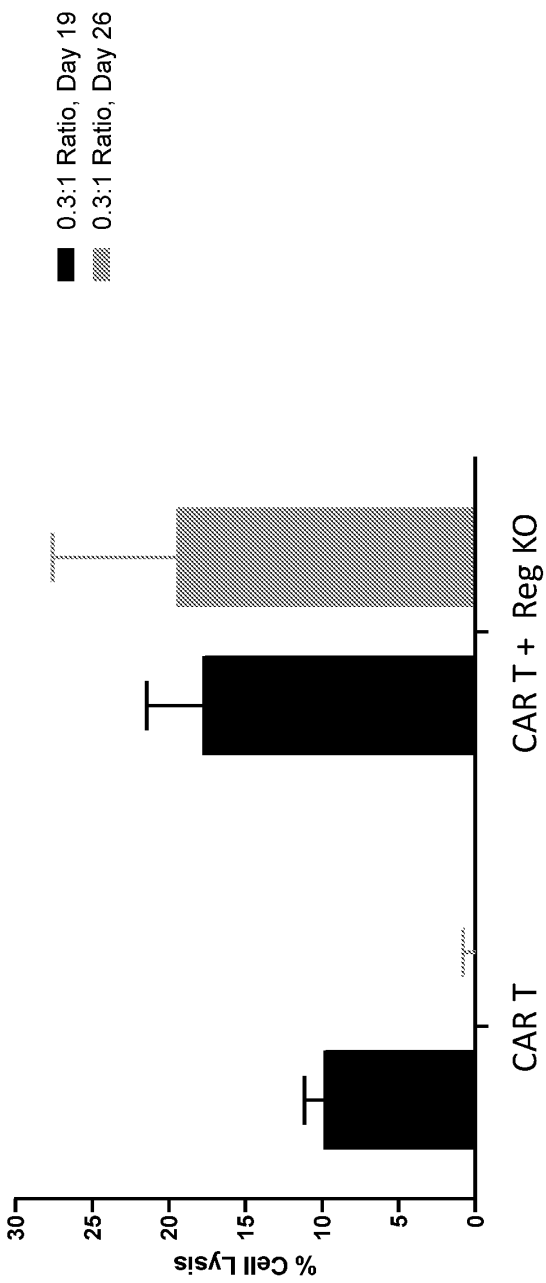
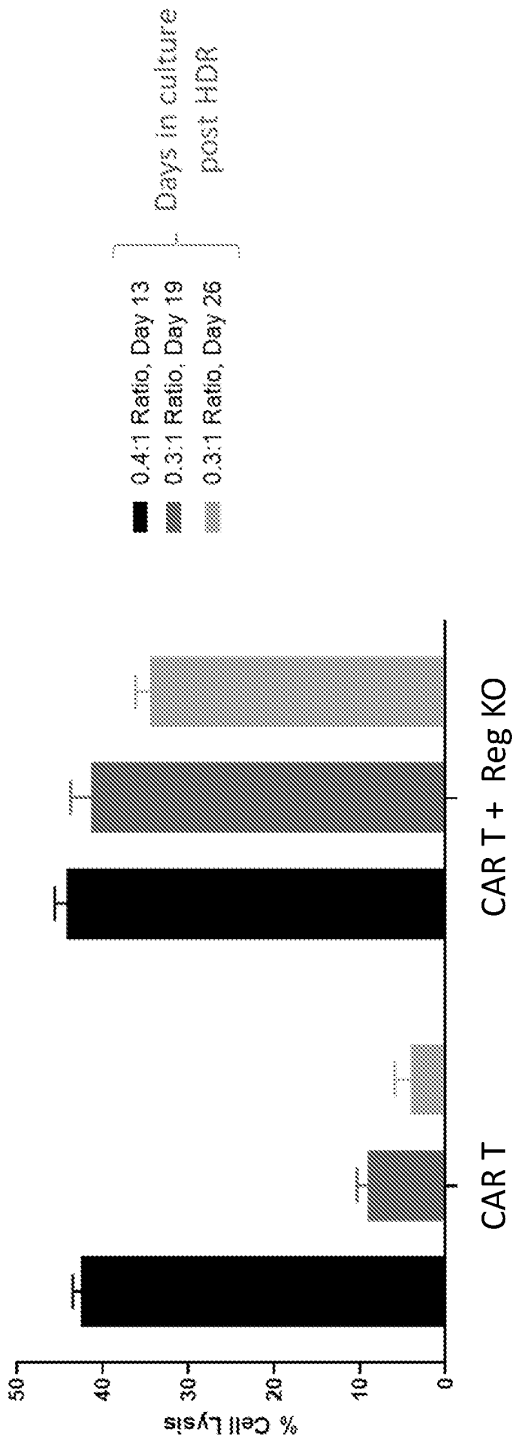

0.3:1 Ratio, Day 19
0.3:1 Ratio, Day 26
% Cell Lysis
0
5
10
15
20
25
30
CAR T
CAR T + Reg KO

FIG. 6B


Days in culture post HDR
0.4:1 Ratio, Day 13
0.3:1 Ratio, Day 19
0.3:1 Ratio, Day 26
% Cell Lysis
0
10
20
30
40
50
CAR T
CAR T + Reg KO

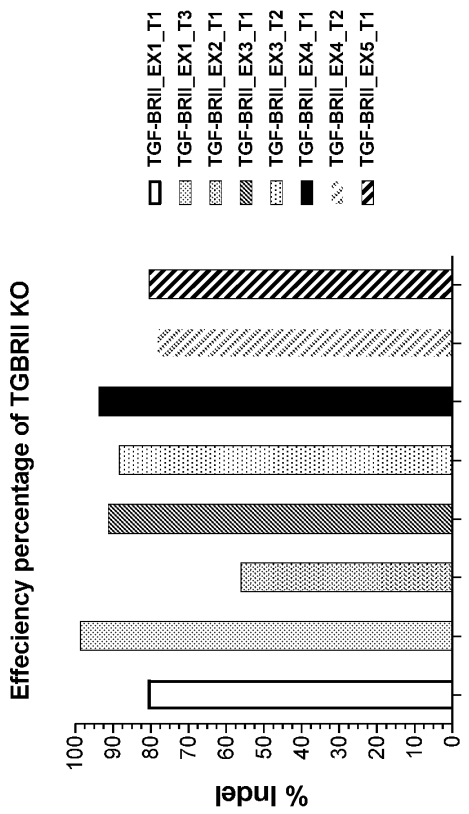

Effeciency percentage of TGBRII KO
% Indel
0
10
20
30
40
50
60
70
80
90
100
TGF-BRII_EX1_T1
TGF-BRII_EX1_T3
TGF-BRII_EX2_T1
TGF-BRII_EX3_T1
TGF-BRII_EX3_T2
TGF-BRII_EX4_T1
TGF-BRII_EX4_T2
TGF-BRII_EX5_T1

FIG. 7A

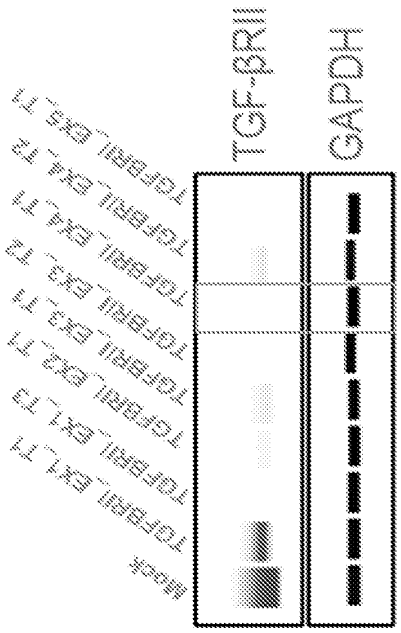

Blank
TGFBRII_EX1_T1
TGFBRII_EX1_T3
TGFBRII_EX2_T1
TGFBRII_EX3_T1
TGFBRII_EX3_T2
TGFBRII_EX4_T1
TGFBRII_EX4_T2
TGFBRII_EX5_T1
TGF-βRII
GAPDH

FIG. 7B

FIG. 8A

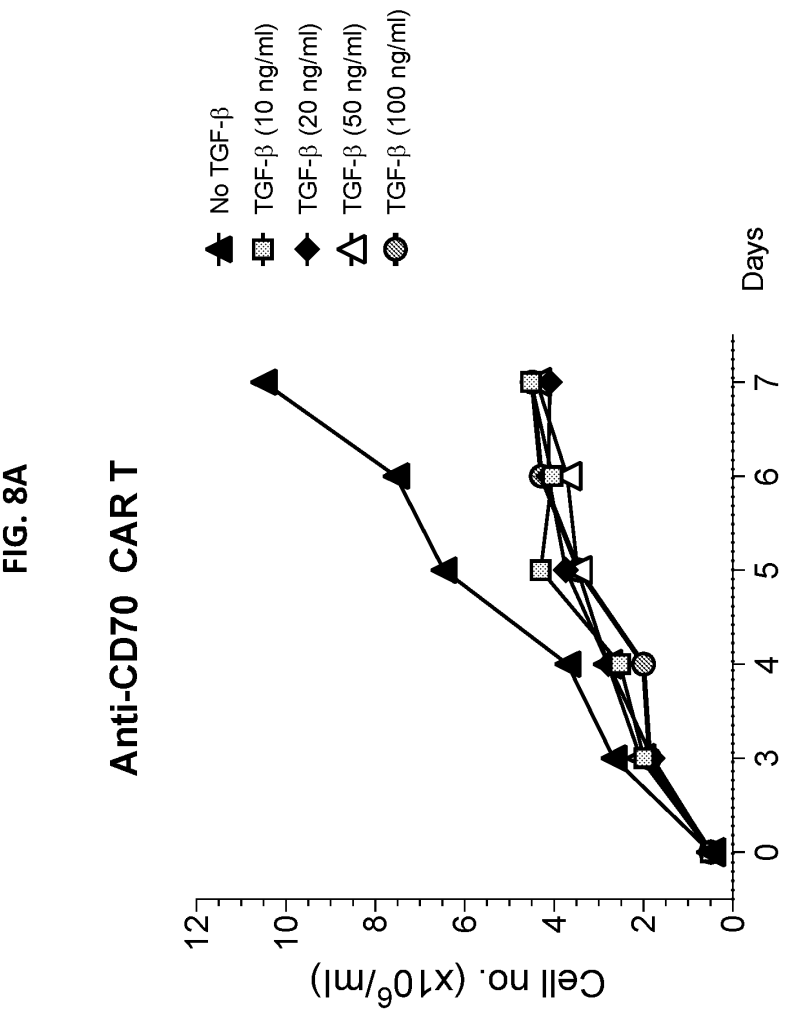

Anti-CD70 CAR T
No TGF-β
TGF-β (10 ng/ml)
TGF-β (20 ng/ml)
TGF-β (50 ng/ml)
TGF-β (100 ng/ml)
Cell no. (x10^6/ml)
Days
0
2
4
6
8
10
12
0
3
4
5
6
7

FIG. 8B

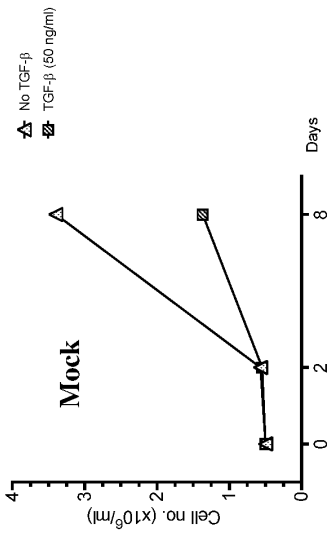


FIG. 8C

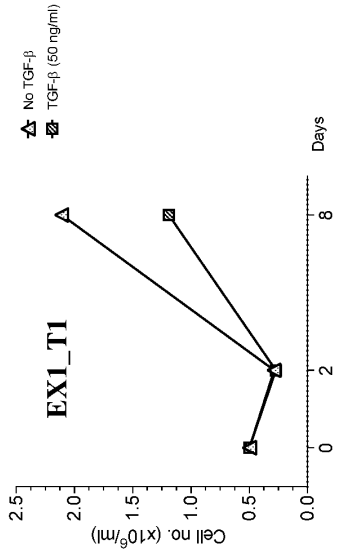


FIG. 8D

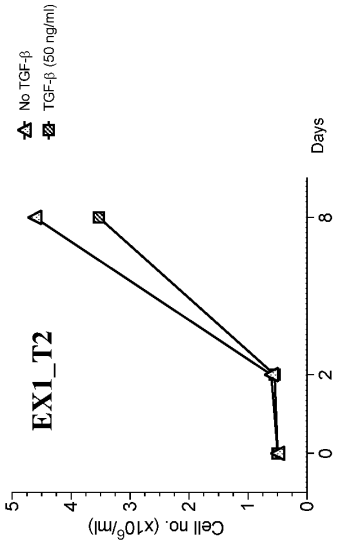


FIG. 8E

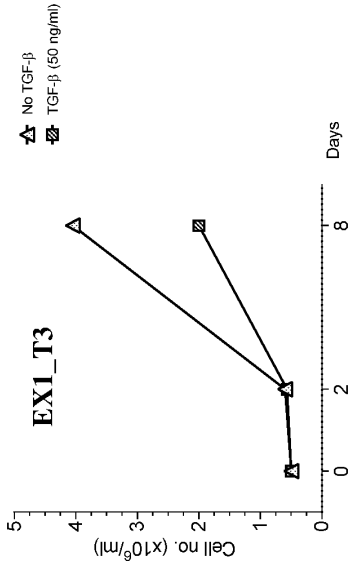


FIG. 8F

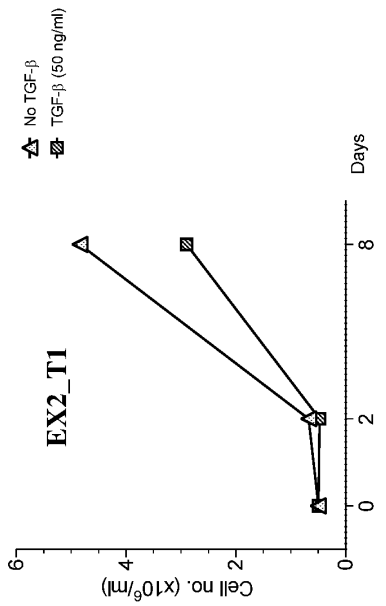


FIG. 8G

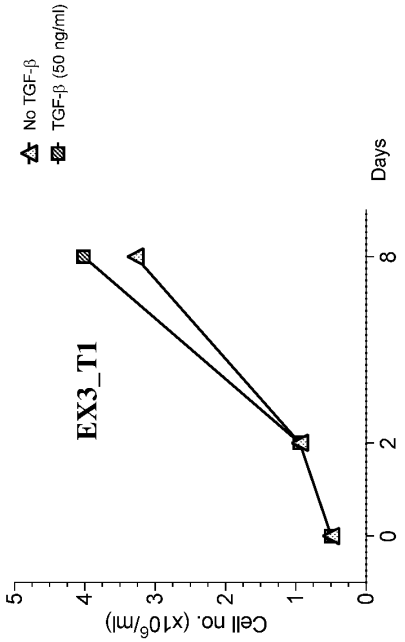


FIG. 8H

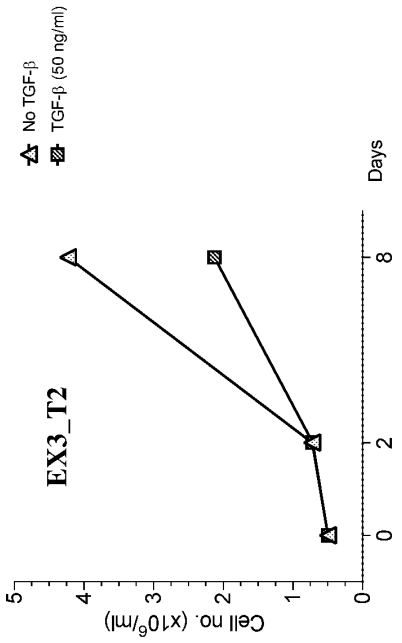


FIG. 8I

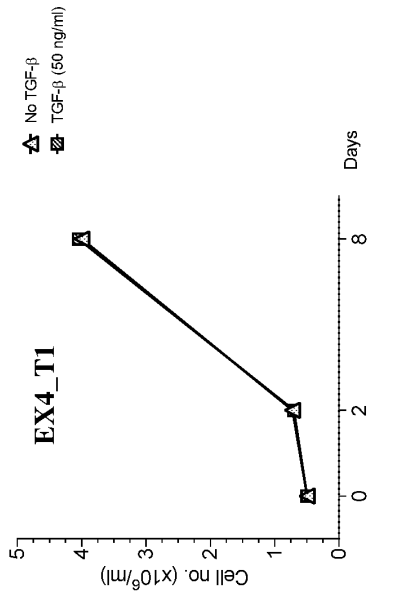


FIG. 8K

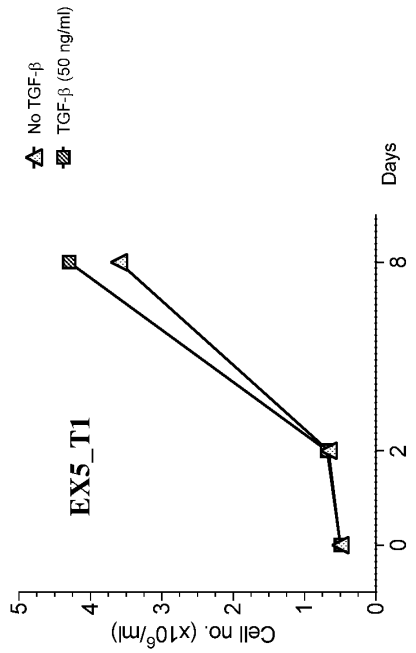

No TGF-β
TGF-β (50 ng/ml)
EX5_T1
Cell no. (x10^6/ml)
Days
0
1
2
3
4
5
0
2
8

FIG. 8J

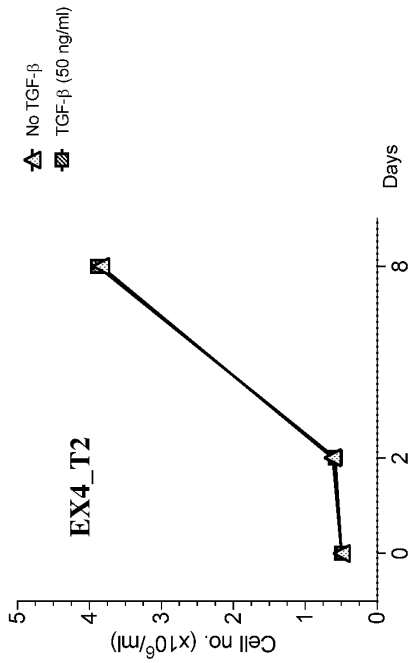

No TGF-β
TGF-β (50 ng/ml)
EX4_T2
Cell no. (x10^6/ml)
Days
0
1
2
3
4
5
0
2
8

FIG. 9

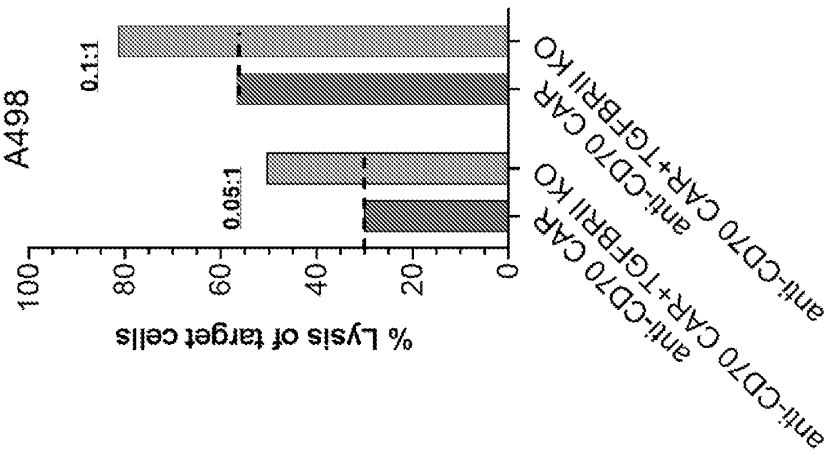

A498
0.1:1
0.05:1
% Lysis of target cells
100
80
60
40
20
0
anti-CD70 CAR
anti-CD70 CAR+TGFBRII KO
anti-CD70 CAR
anti-CD70 CAR+TGFBRII KO

FIG. 10A

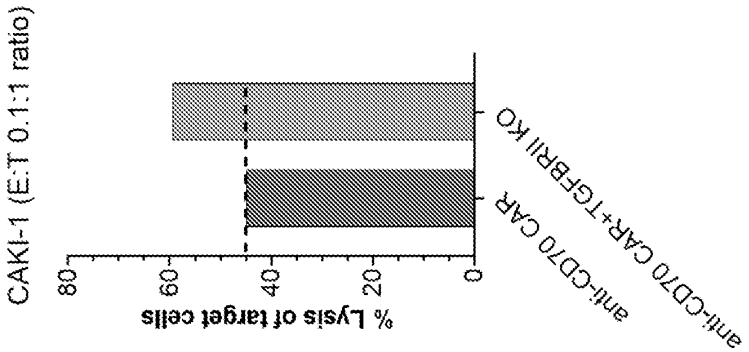


FIG. 10B

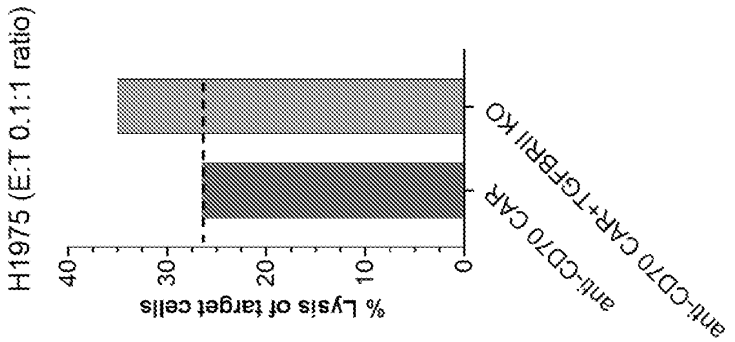


FIG. 10C

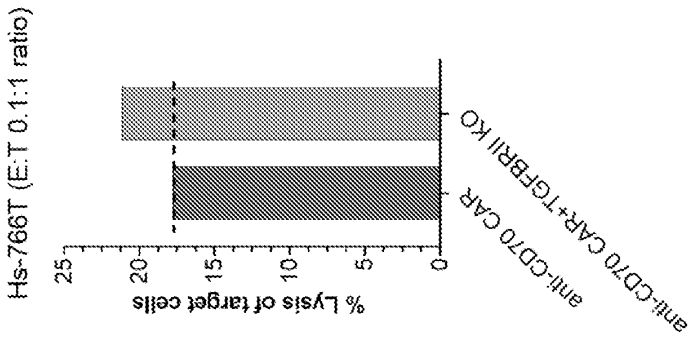


FIG. 10E

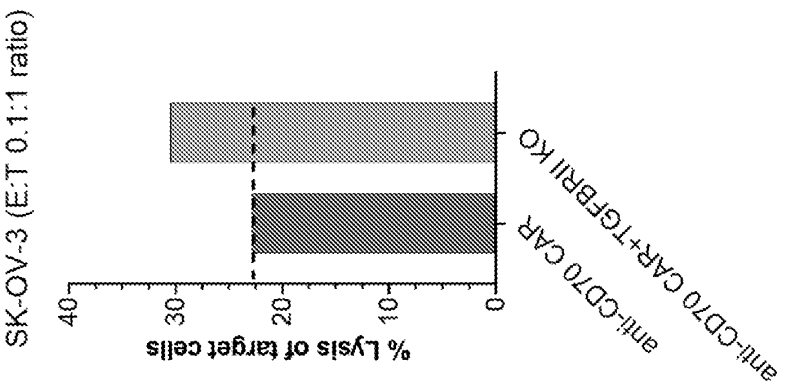


FIG. 10D

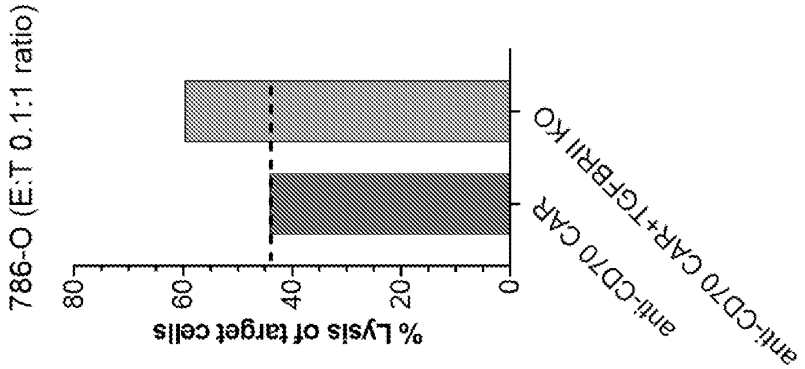


FIG. 11

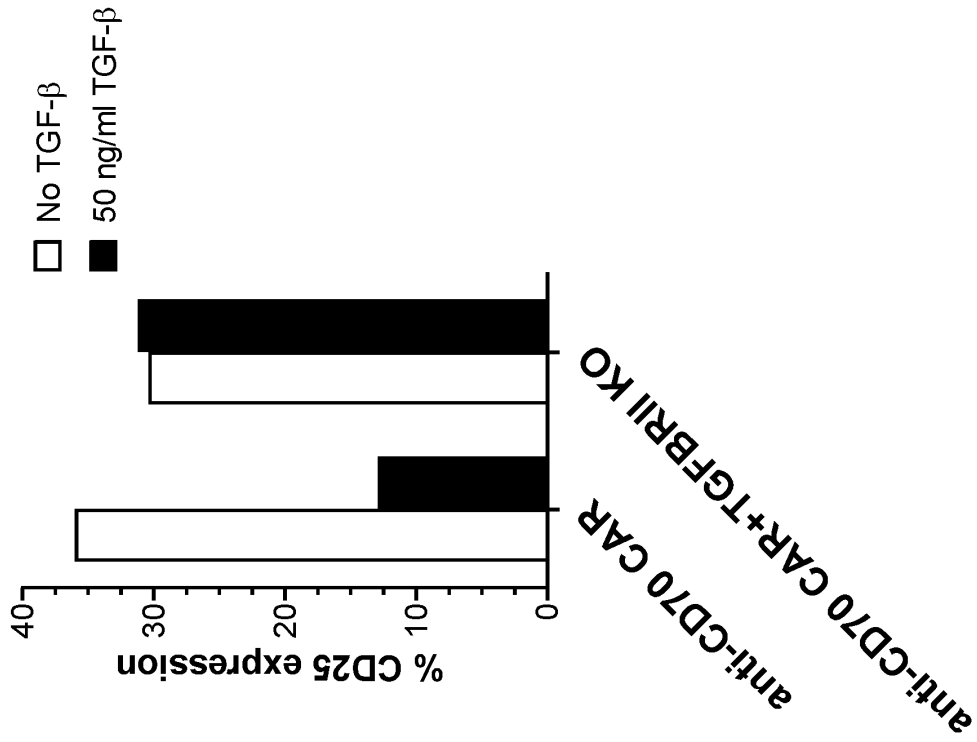

No TGF-β
50 ng/ml TGF-β
% CD25 expression
0
10
20
30
40
anti-CD70 CAR
anti-CD70 CAR+TGFBRII KO

FIG. 12

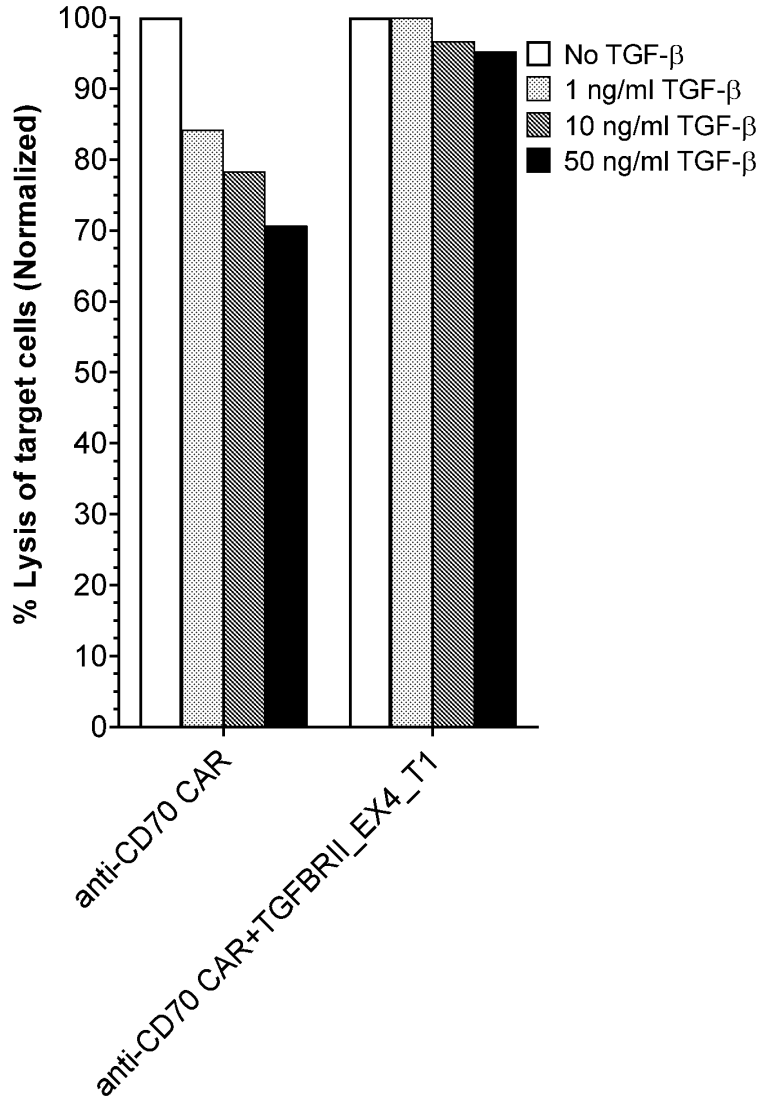

% Lysis of target cells (Normalized)
100
90
80
70
60
50
40
30
20
10
0
No TGF-β
1 ng/ml TGF-β
10 ng/ml TGF-β
50 ng/ml TGF-β
anti-CD70 CAR
anti-CD70 CAR+TGFBRII_EX4_T1

FIG. 13A

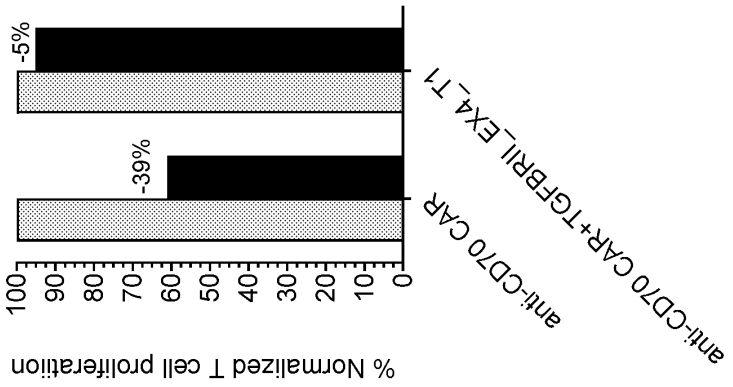


FIG. 13B

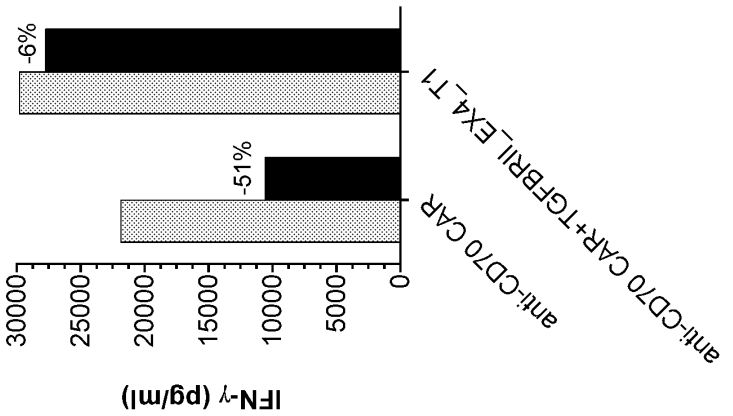


FIG. 13C

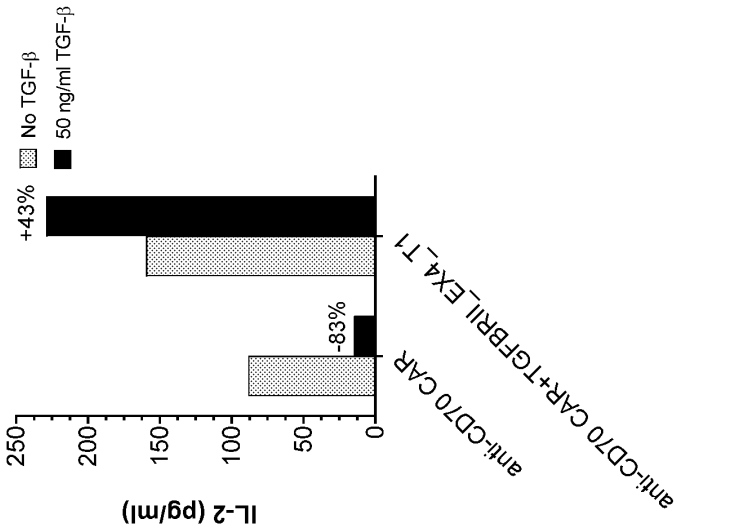
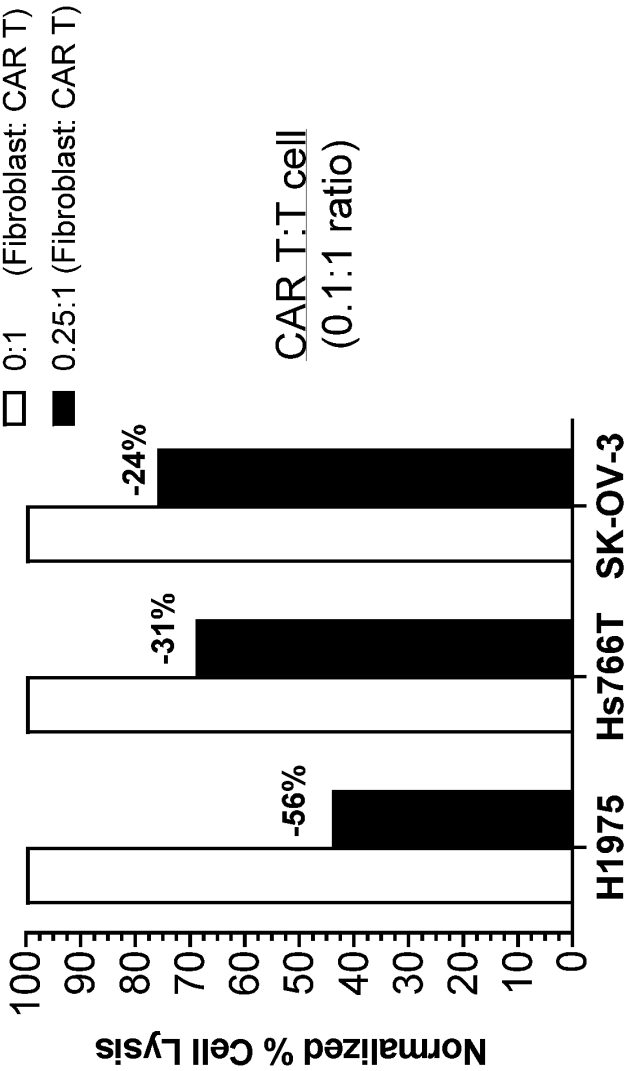


FIG. 14


0:1 (Fibroblast: CAR T)
0.25:1 (Fibroblast: CAR T)
CAR T:T cell
(0.1:1 ratio)
Normalized % Cell Lysis
100
90
80
70
60
50
40
30
20
10
0
-56%
-31%
-24%
H1975
Hs766T
SK-OV-3

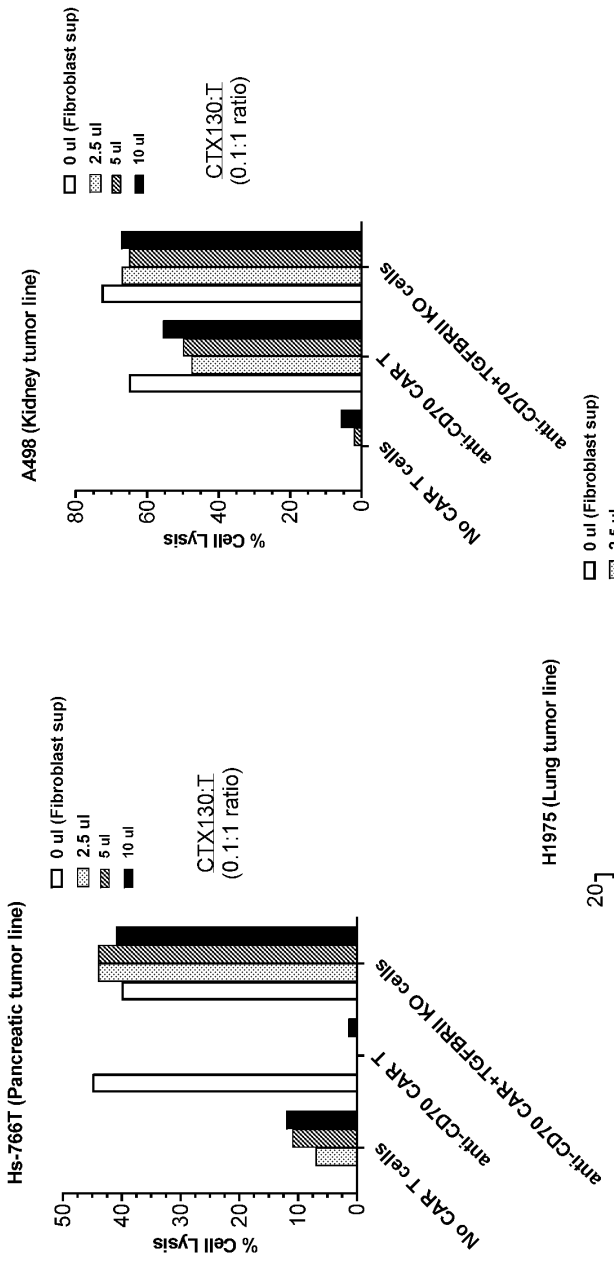


FIG. 15A

FIG. 15B

FIG. 15C

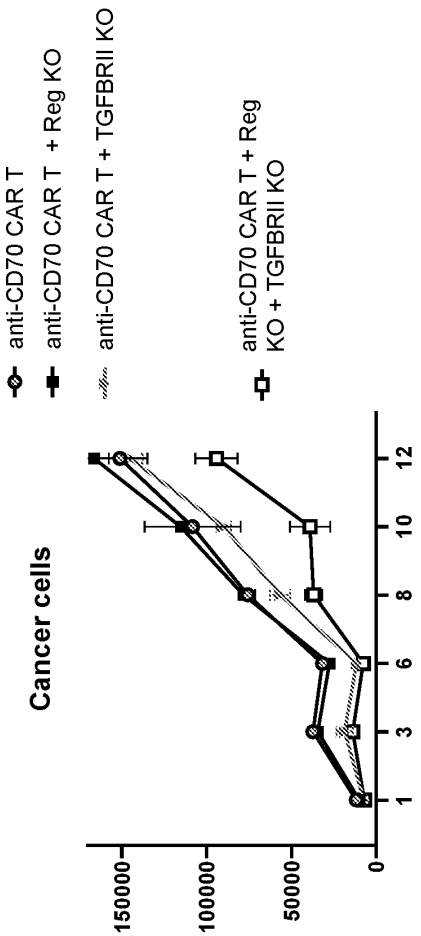


FIG. 16A

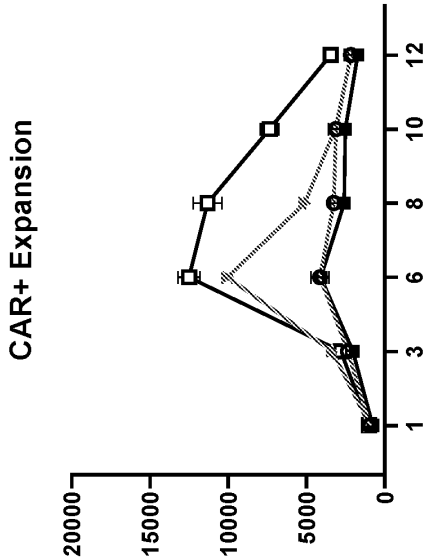


FIG. 16B

FIG. 17A

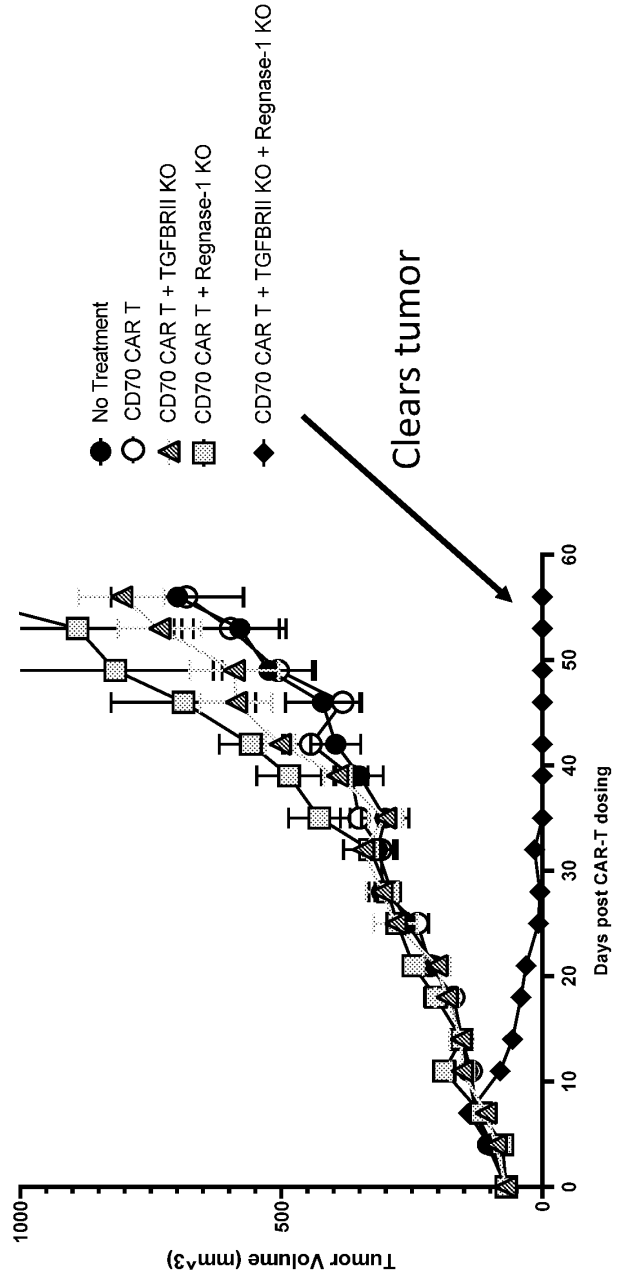

No Treatment
CD70 CAR T
CD70 CAR T + TGFBRII KO
CD70 CAR T + Regnase-1 KO
CD70 CAR T + TGFBRII KO + Regnase-1 KO
Clears tumor
Tumor Volume (mm^3)
1000
500
0
0
10
20
30
40
50
60
Days post CAR-T dosing

FIG. 17B

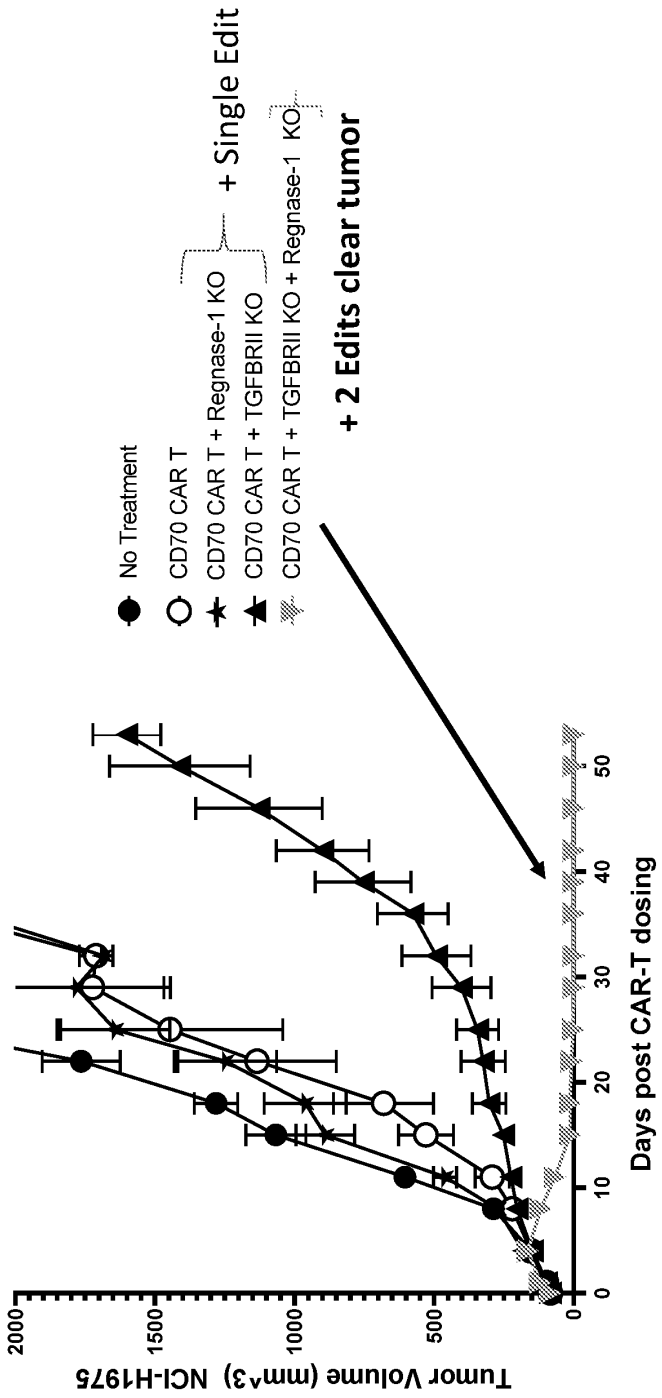

Tumor Volume (mm^3) NCI-H1975
2000
1500
1000
500
0
0
10
20
30
40
50
Days post CAR-T dosing
No Treatment
CD70 CAR T
CD70 CAR T + Regnase-1 KO
CD70 CAR T + TGFBRII KO
CD70 CAR T + TGFBRII KO + Regnase-1 KO
+ Single Edit
+ 2 Edits clear tumor

FIG. 18A

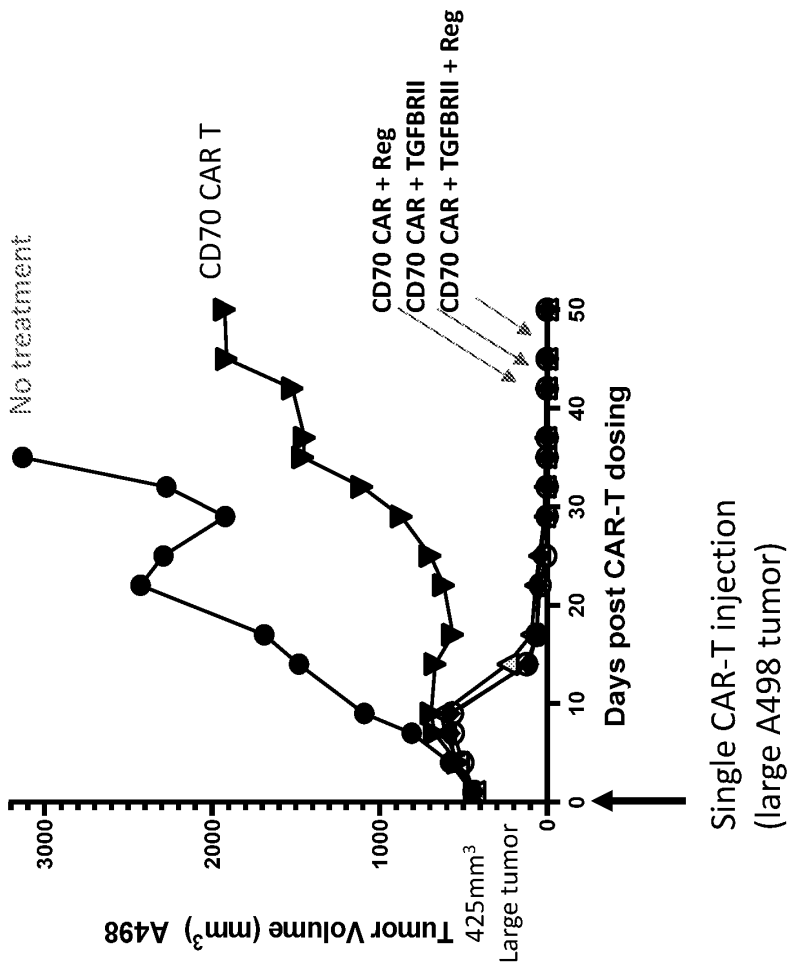

No treatment
CD70 CAR T
CD70 CAR + Reg
CD70 CAR + TGFBRII
CD70 CAR + TGFBRII + Reg
Tumor Volume (mm³) A498
3000
2000
1000
0
425mm³
Large tumor
0
10
20
30
40
50
Days post CAR-T dosing
Single CAR-T injection
(large A498 tumor)

FIG. 18B

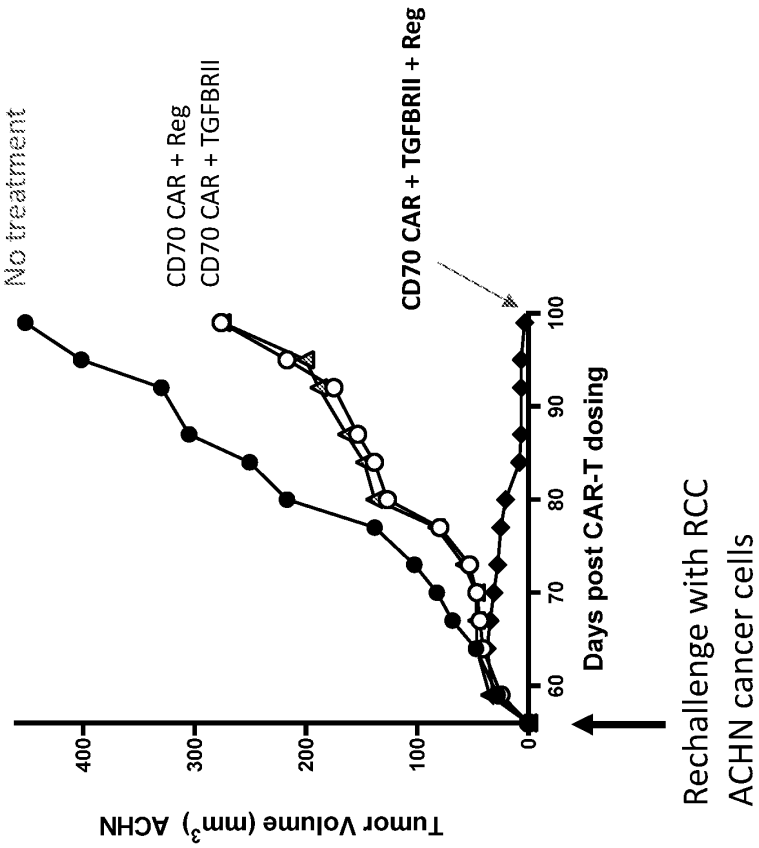

No treatment
CD70 CAR + Reg
CD70 CAR + TGFBRII
CD70 CAR + TGFBRII + Reg
Tumor Volume (mm3) ACHN
0
100
200
300
400
60
70
80
90
100
Days post CAR-T dosing
Rechallenge with RCC
ACHN cancer cells

FIG. 19A

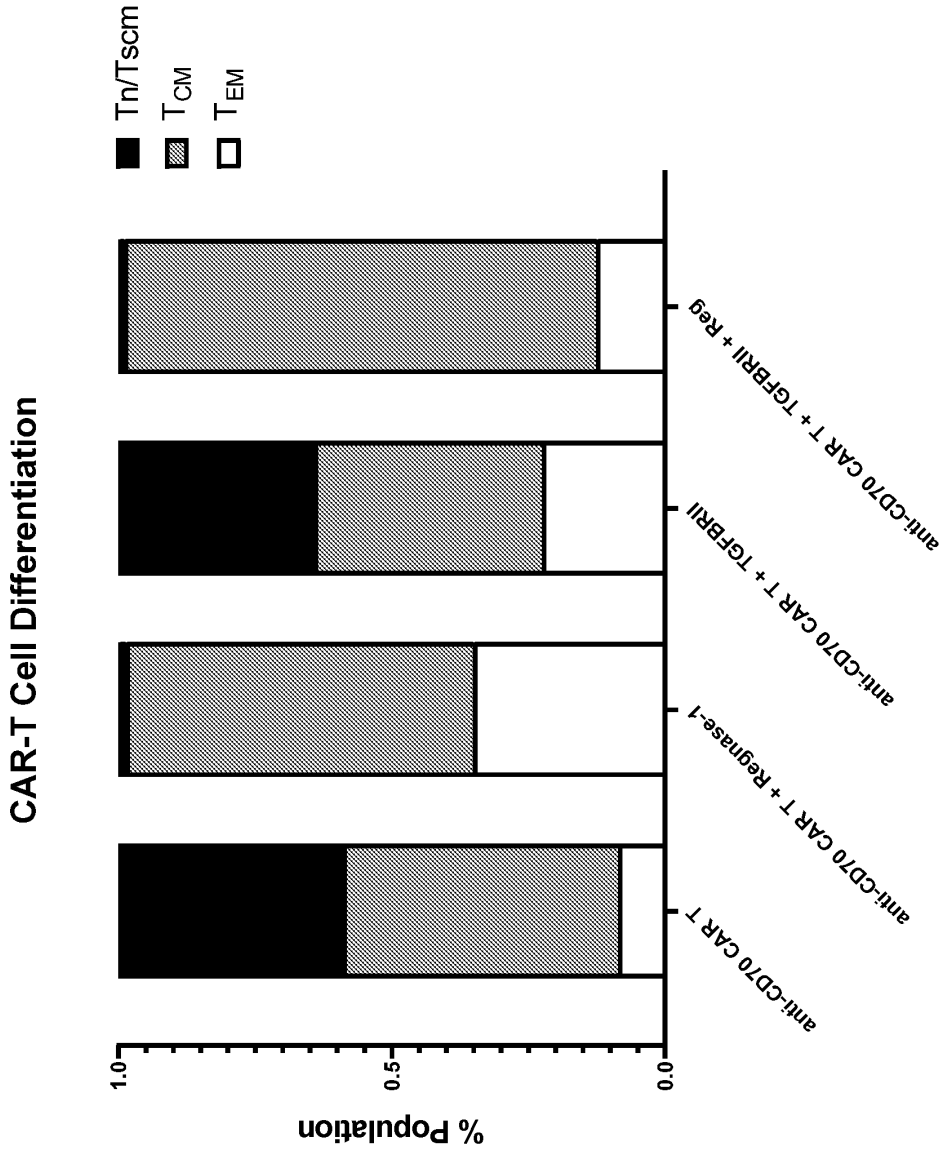
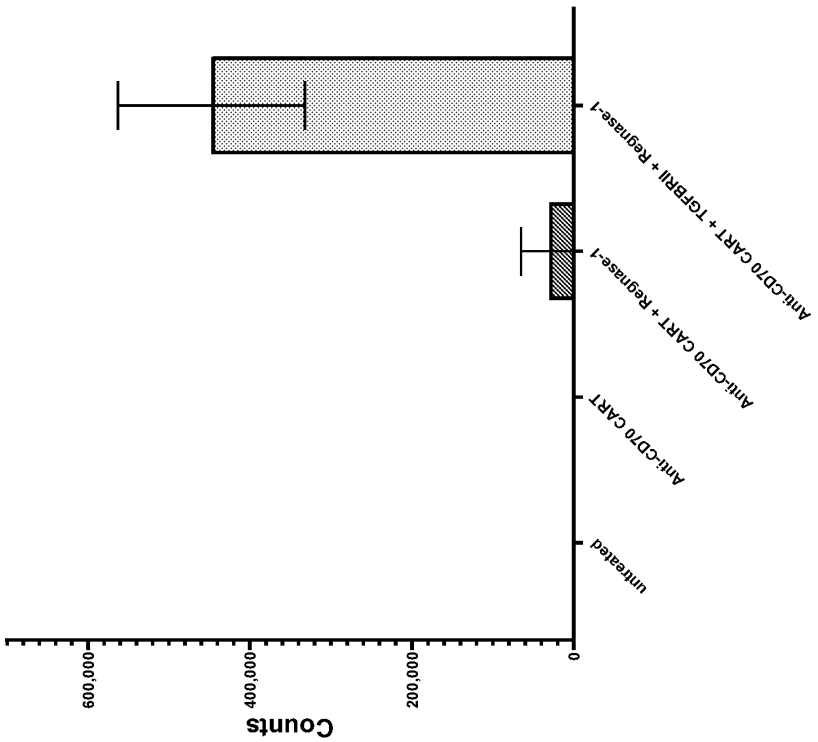

CAR-T Cell Differentiation
Tn/Tscm
TCM
TEM
% Population
1.0
0.5
0.0
anti-CD70 CAR T
anti-CD70 CAR T + Regnase-1
anti-CD70 CAR T + TGFBRII
anti-CD70 CAR T + TGFBRII + Reg

FIG. 19B


CAR+ Cells in 100ul blood
Counts
600,000
400,000
200,000
0
untreated
Anti-CD70 CAR T
Anti-CD70 CAR T + Regnase-1
Anti-CD70 CAR T + TGFBRII + Regnase-1

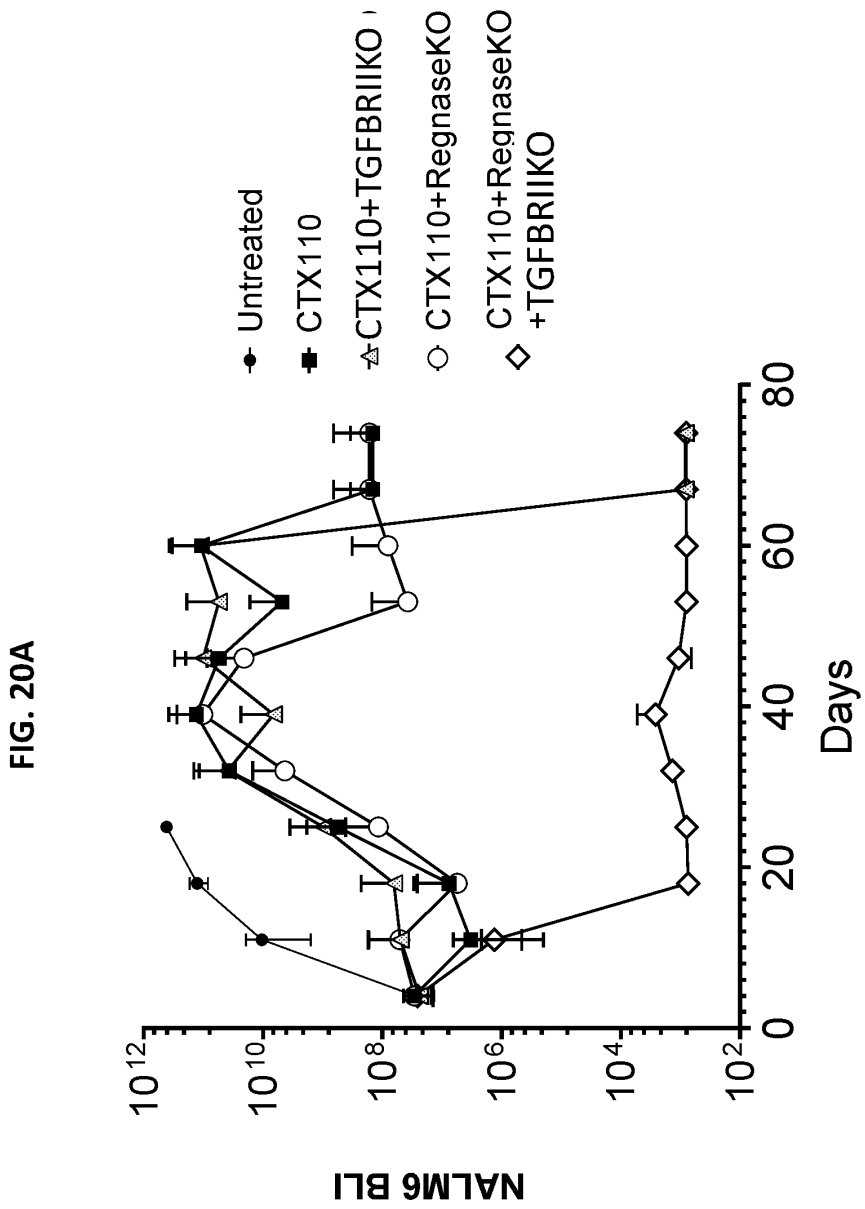

Untreated
CTX110
CTX110+TGFBRIIKO
CTX110+RegnaseKO
CTX110+RegnaseKO +TGFBRIIKO
NALM6 BLI
Days
0
20
40
60
80
10^{12}
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}

FIG. 20A

FIG. 20B

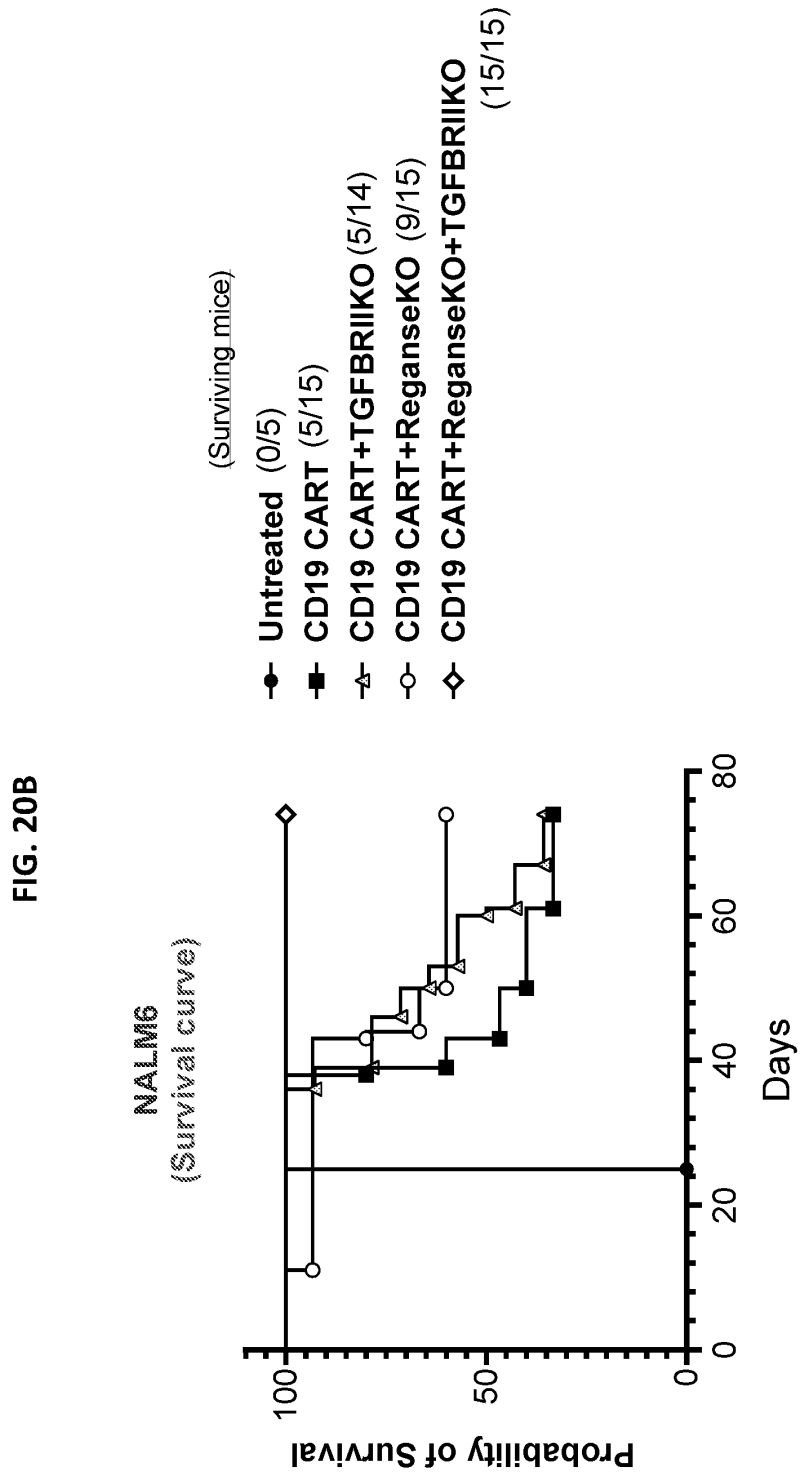

NALM6
(Survival curve)
Probability of Survival
100
50
0
0
20
40
60
80
Days
(Surviving mice)
Untreated (0/5)
CD19 CART (5/15)
CD19 CART+TGFBRIIKO (5/14)
CD19 CART+ReganseKO (9/15)
CD19 CART+ReganseKO+TGFBRIIKO (15/15)

FIG. 21

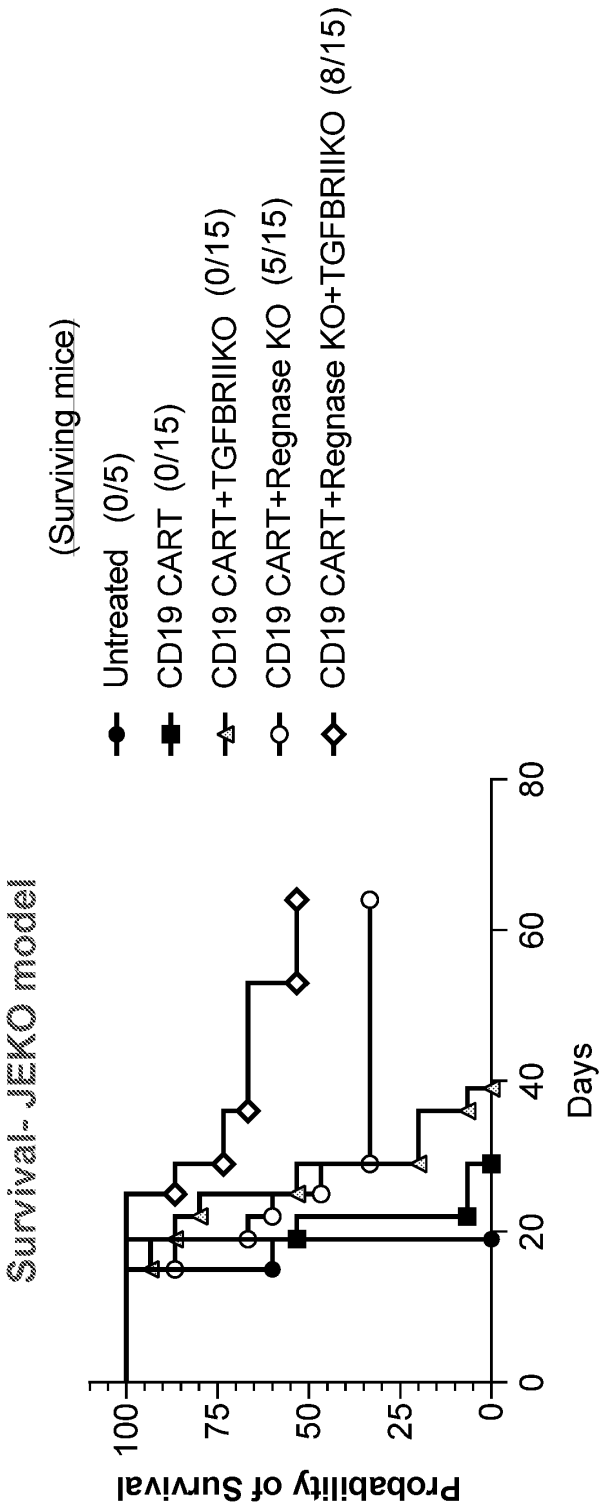

Survival- JEKO model
(Surviving mice)
Untreated (0/5)
CD19 CART (0/15)
CD19 CART+TGFBRIIKO (0/15)
CD19 CART+Regnase KO (5/15)
CD19 CART+Regnase KO+TGFBRIIKO (8/15)
Probability of Survival
100
75
50
25
0
0
20
40
60
80
Days

FIG. 22A

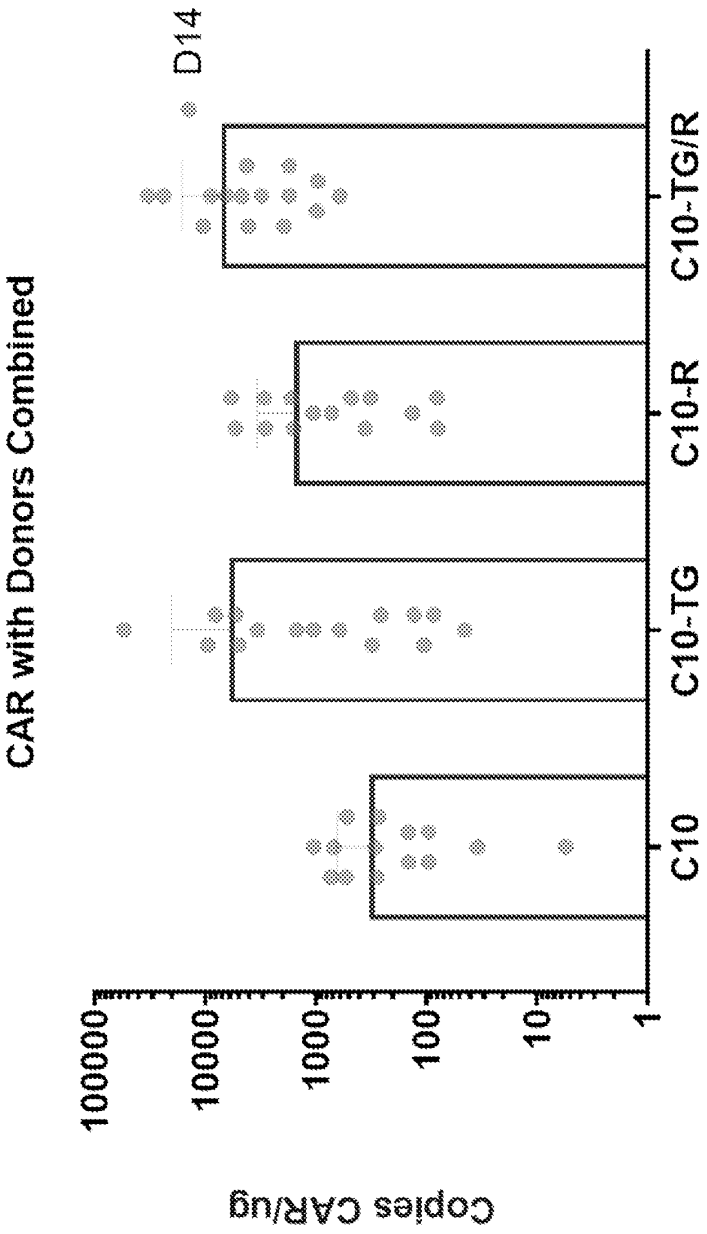

CAR with Donors Combined
D14
Copies CAR/ug
100000
10000
1000
100
10
1
C10
C10-TG
C10-R
C10-TG/R

FIG. 22B

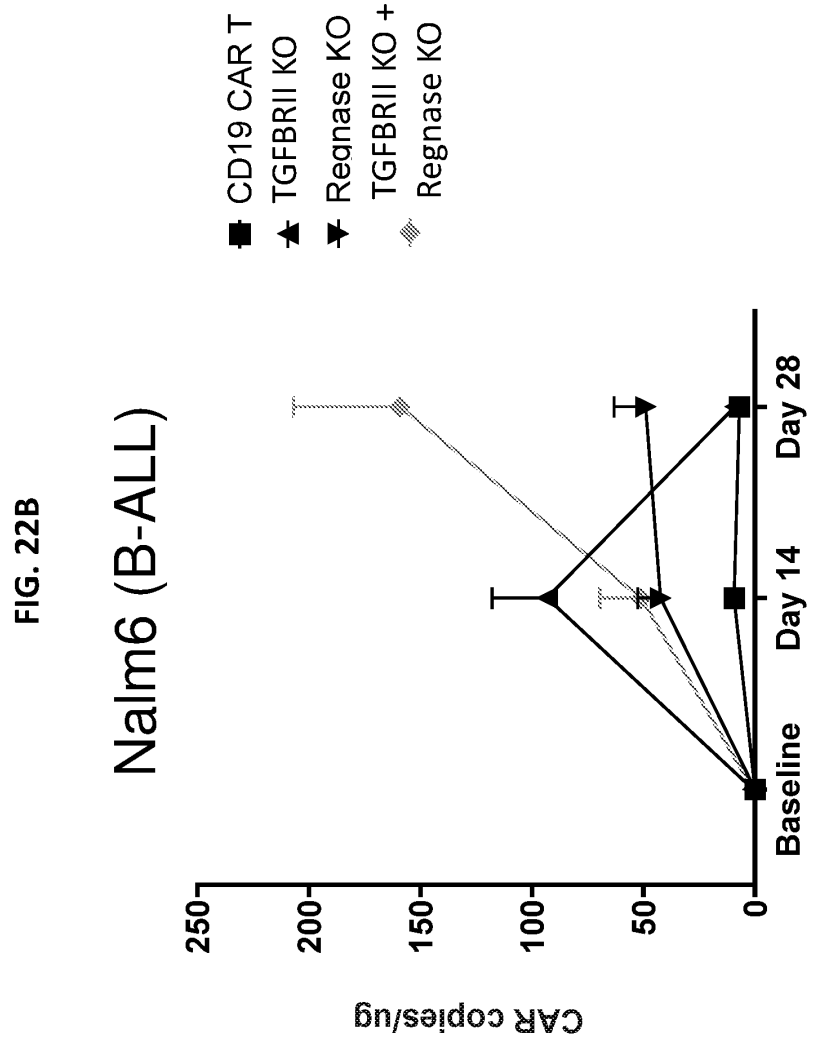

Nalm6 (B-ALL)
CD19 CAR T
TGFBRII KO
Regnase KO
TGFBRII KO + Regnase KO
CAR copies/ug
250
200
150
100
50
0
Baseline
Day 14
Day 28

FIG. 23B

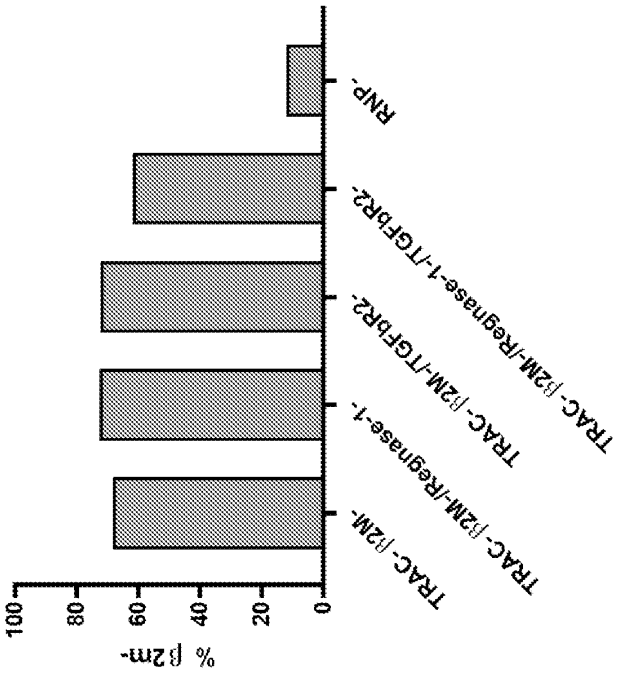


FIG. 23A

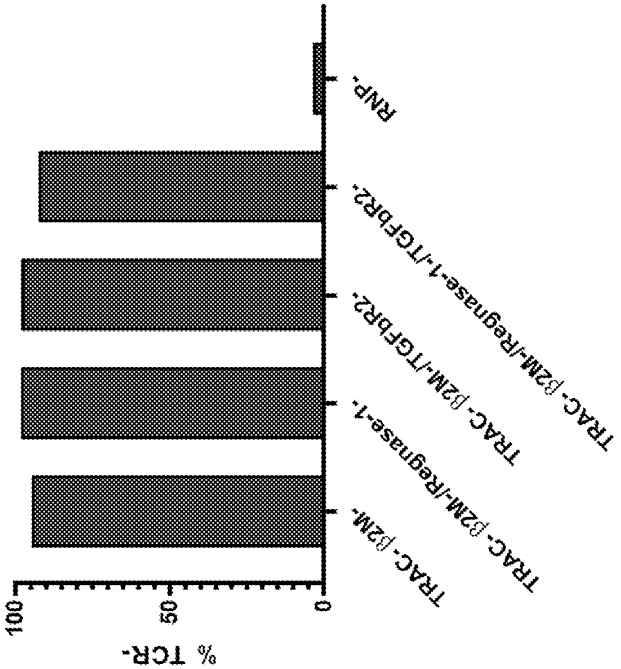


FIG. 23C

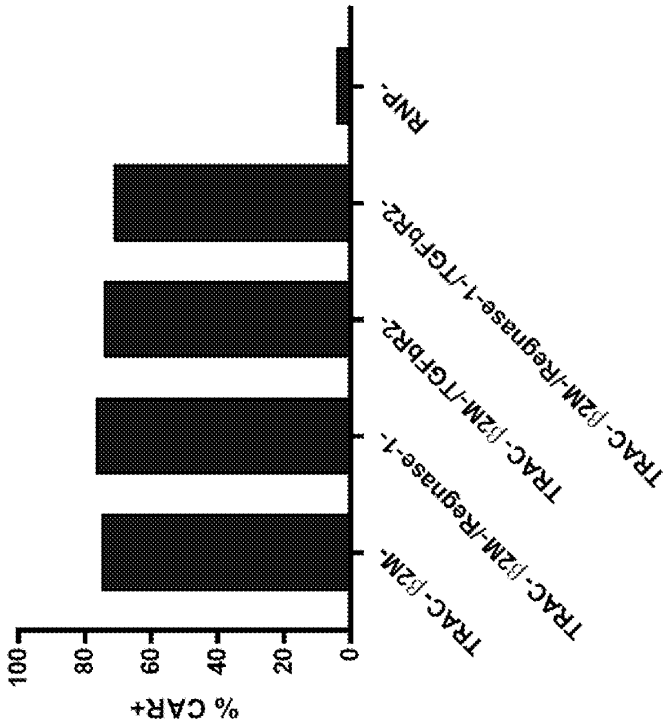

Day 10 CAR+
% CAR+
100
80
60
40
20
0
TRAC- β2M-
TRAC- β2M-/Regnase-1-
TRAC- β2M-/TGFBR2-
TRAC- β2M-/Regnase-1-/TGFBR2-
RNP-

FIG. 23D

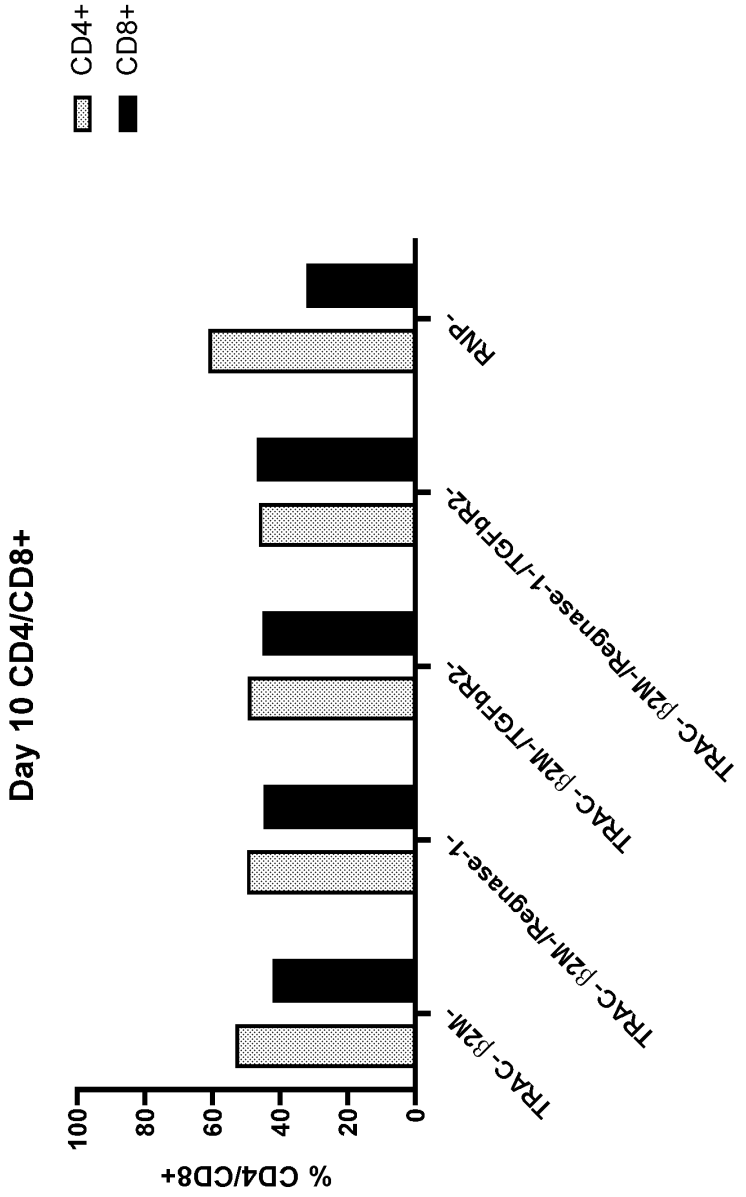

Day 10 CD4/CD8+
CD4+
CD8+
% CD4/CD8+
100
80
60
40
20
0
TRAC- β2M-
TRAC- β2M-/Regnase-1-
TRAC- β2M-/TGFbR2-
TRAC- β2M-/Regnase-1-/TGFbR2-
RNP-

FIG. 24A

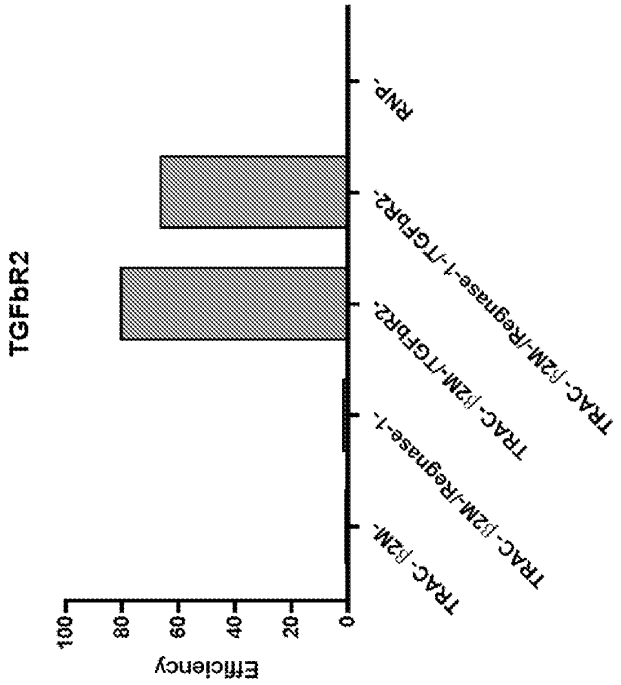


FIG. 24B

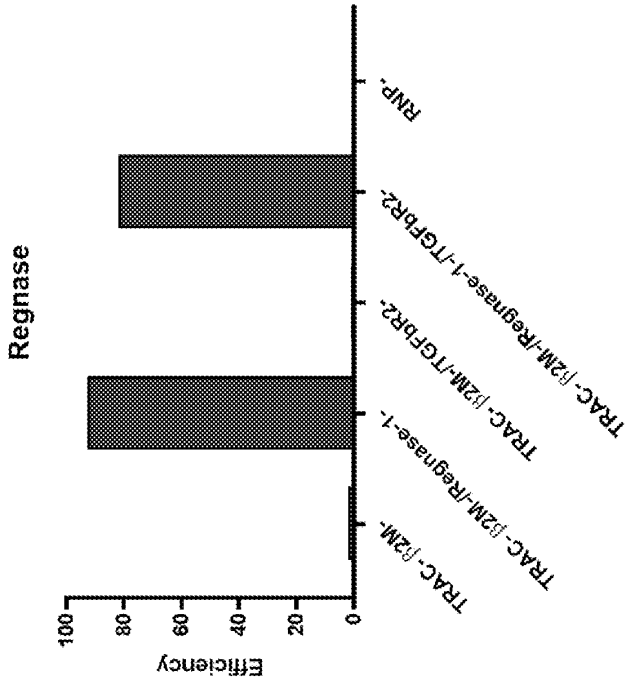


FIG. 25A

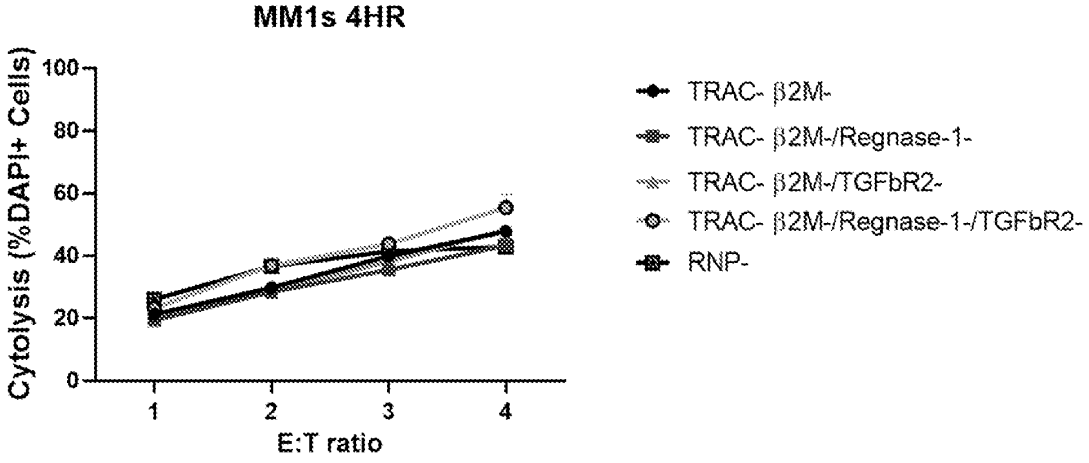


FIG. 25B

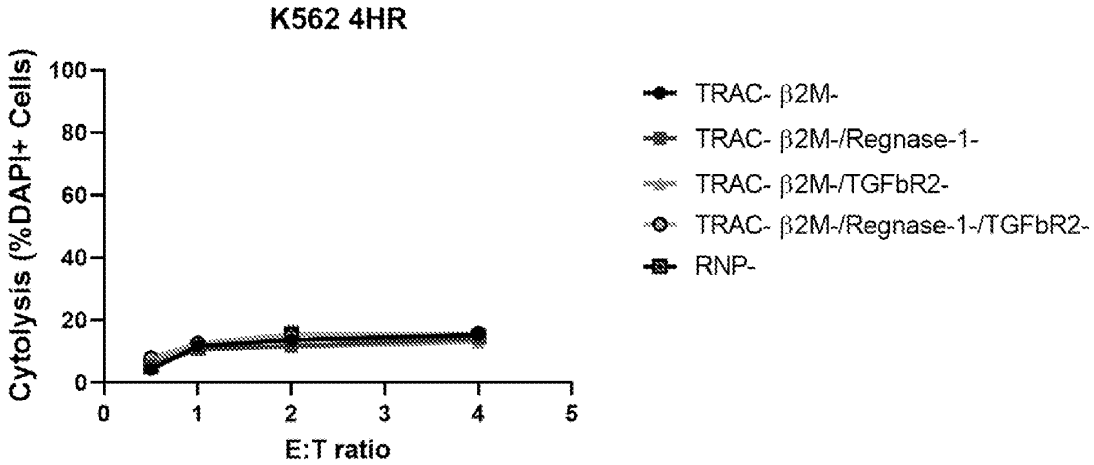


FIG. 25C

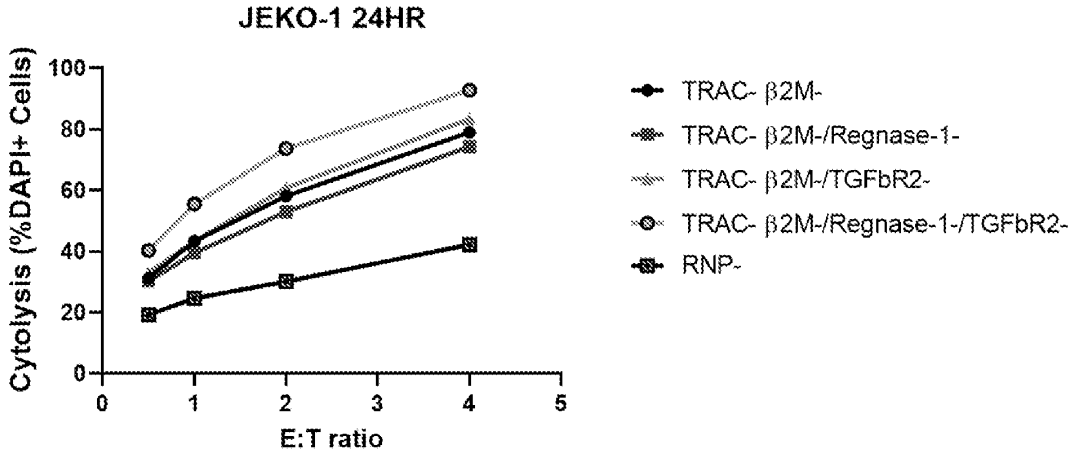


FIG. 25D

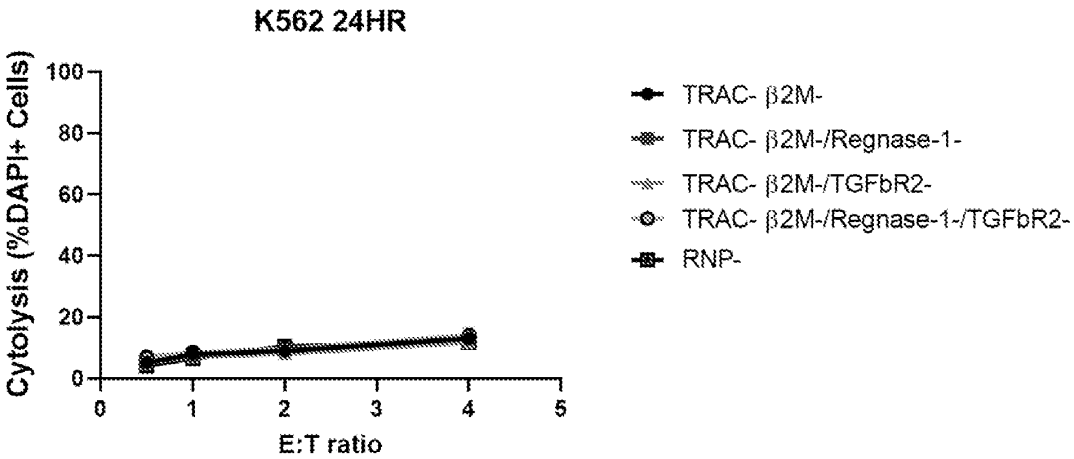


FIG. 26A

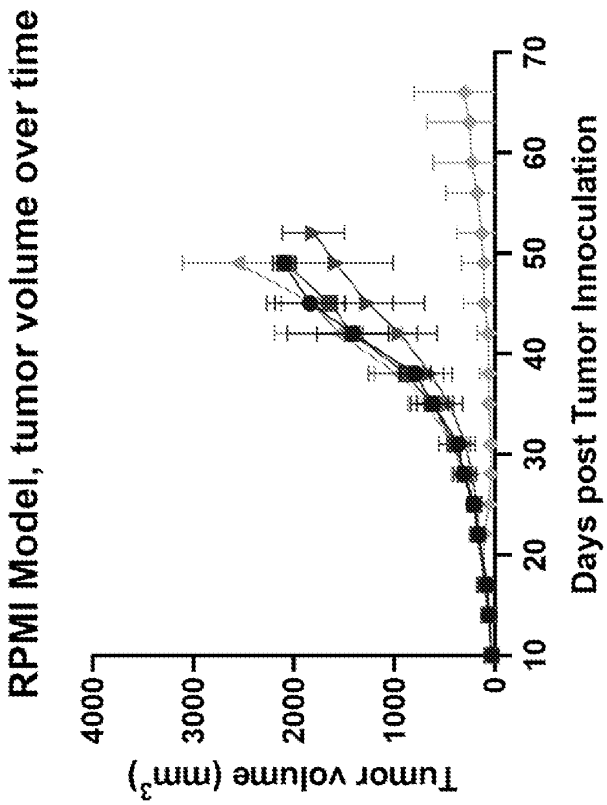

RPMI Model, tumor volume over time
No Treatment
TRAC-/β2M-
TRAC-/β2M-/Regnase-
TRAC-/β2M-/TGFbR2-
TRAC-/β2M-/TGFbR2-/Regnase-
Tumor volume (mm3)
4000
3000
2000
1000
0
10
20
30
40
50
60
70
Days post Tumor Innoculation

FIG. 26B

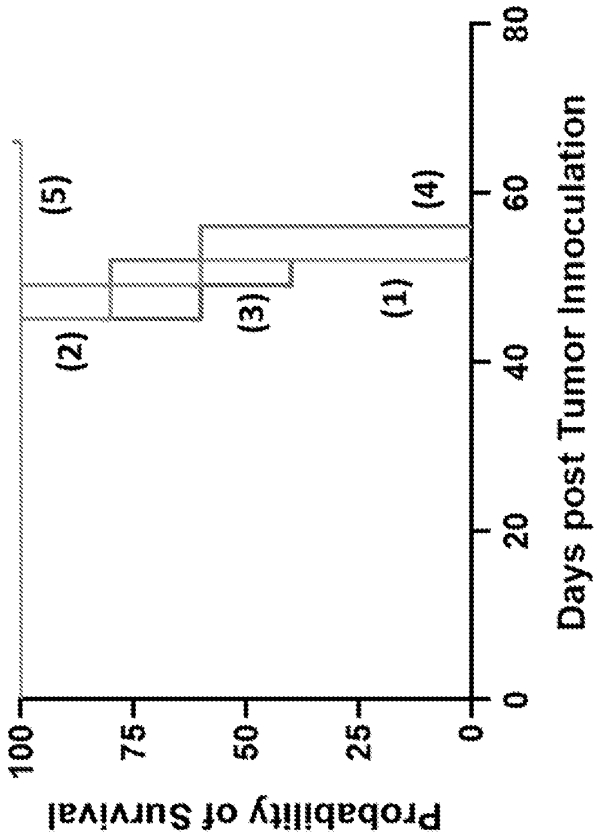

RPMI Model, survival over time
Probability of Survival
100
75
50
25
0
0
20
40
60
80
Days post Tumor Innoculation
(1) No Treatment
(2) TRAC-/β2M-
(3) TRAC-/β2M-/Regnase-
(4) TRAC-/β2M-/TGFbR2-
(5) TRAC-/β2M-/TGFbR2-/ Regnase-

FIG. 26C

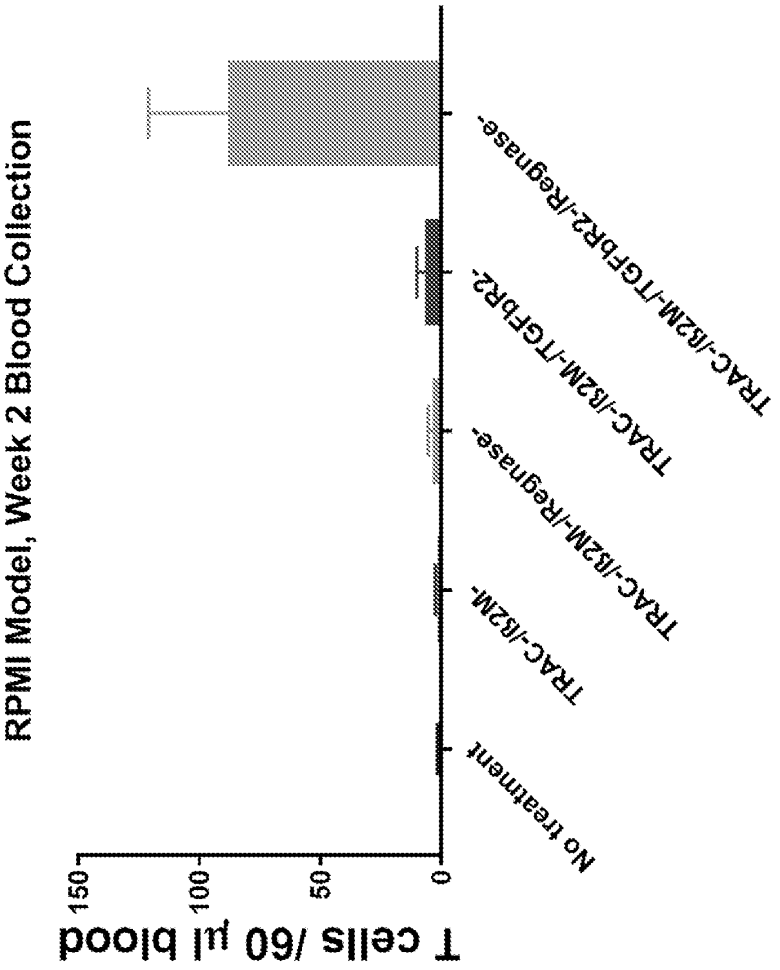

RPMI Model, Week 2 Blood Collection
T cells /60 µl blood
150
100
50
0
No treatment
TRAC-/B2M-
TRAC-/B2M-/Regnase-
TRAC-/B2M-/TGFBR2-
TRAC-/B2M-/TGFBR2-/Regnase-

FIG. 27A

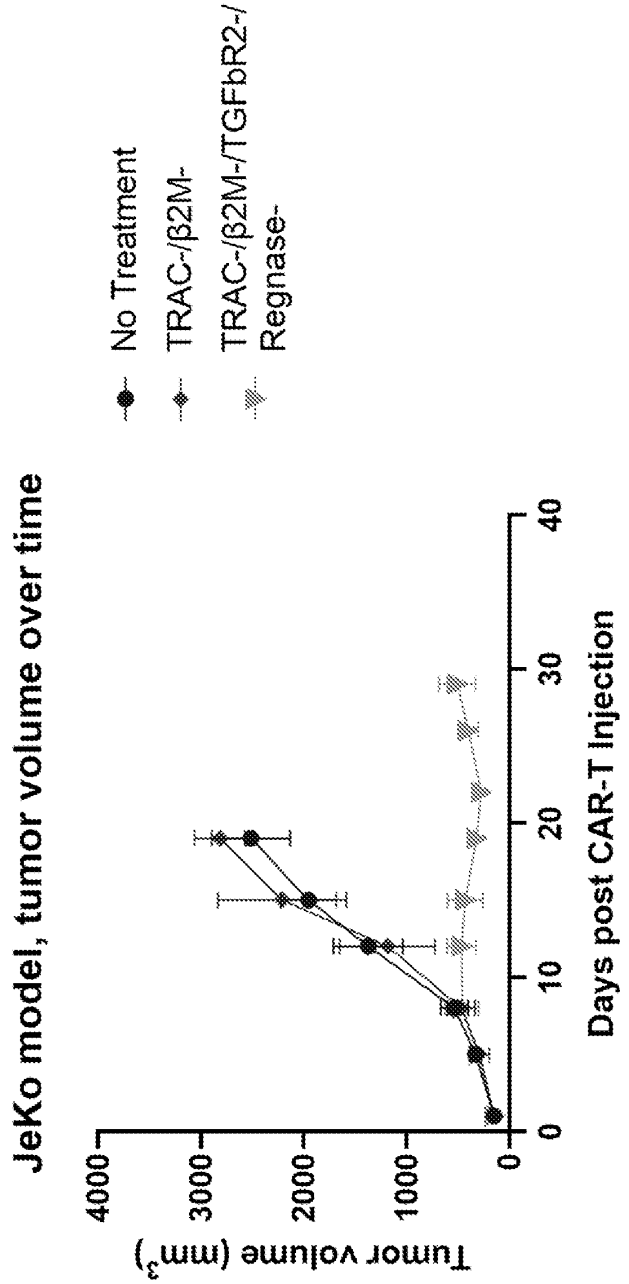
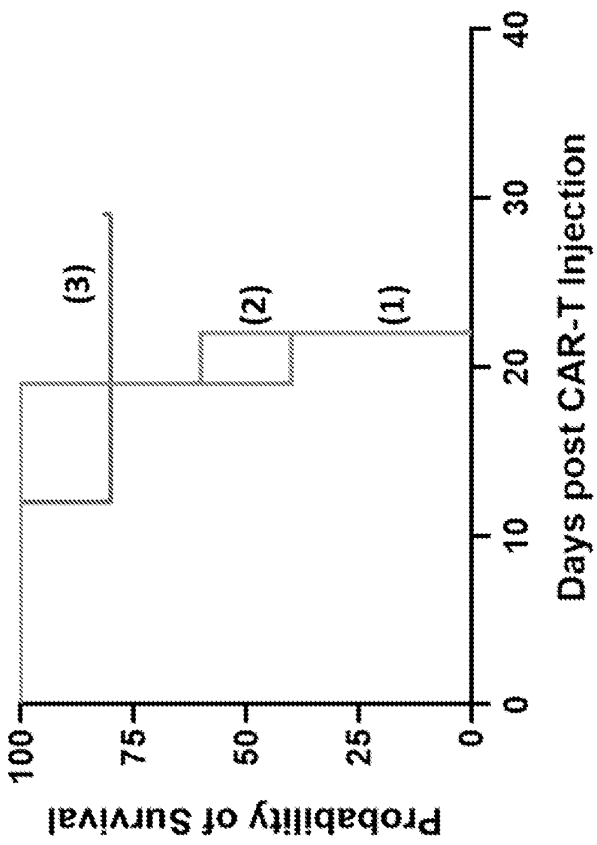

JeKo model, tumor volume over time
No Treatment
TRAC-/β2M-
TRAC-/β2M-/TGFbR2-/ Regnase-
Tumor volume (mm³)
4000
3000
2000
1000
0
0
10
20
30
40
Days post CAR-T Injection

FIG. 27B


JeKo model, mouse survival over time
(1) No Treatment
(2) TRAC-/β2M-
(3) TRAC-/β2M-/TGFbR2-/ Regnase-
Probability of Survival
100
75
50
25
0
0
10
20
30
40
Days post CAR-T Injection
(1)
(2)
(3)

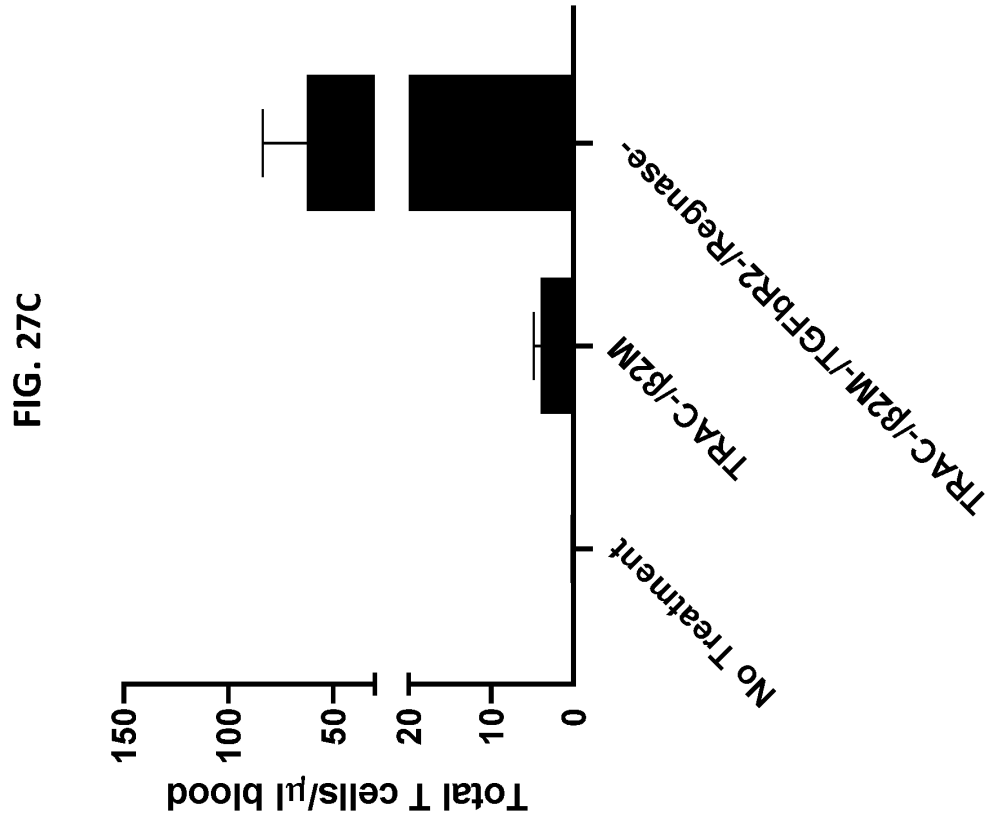

Total T cells/µl blood
150
100
50
20
10
0
No Treatment
TRAC-/B2M
TRAC-/B2M-/TGFbR2-/Regnase-

FIG. 27C

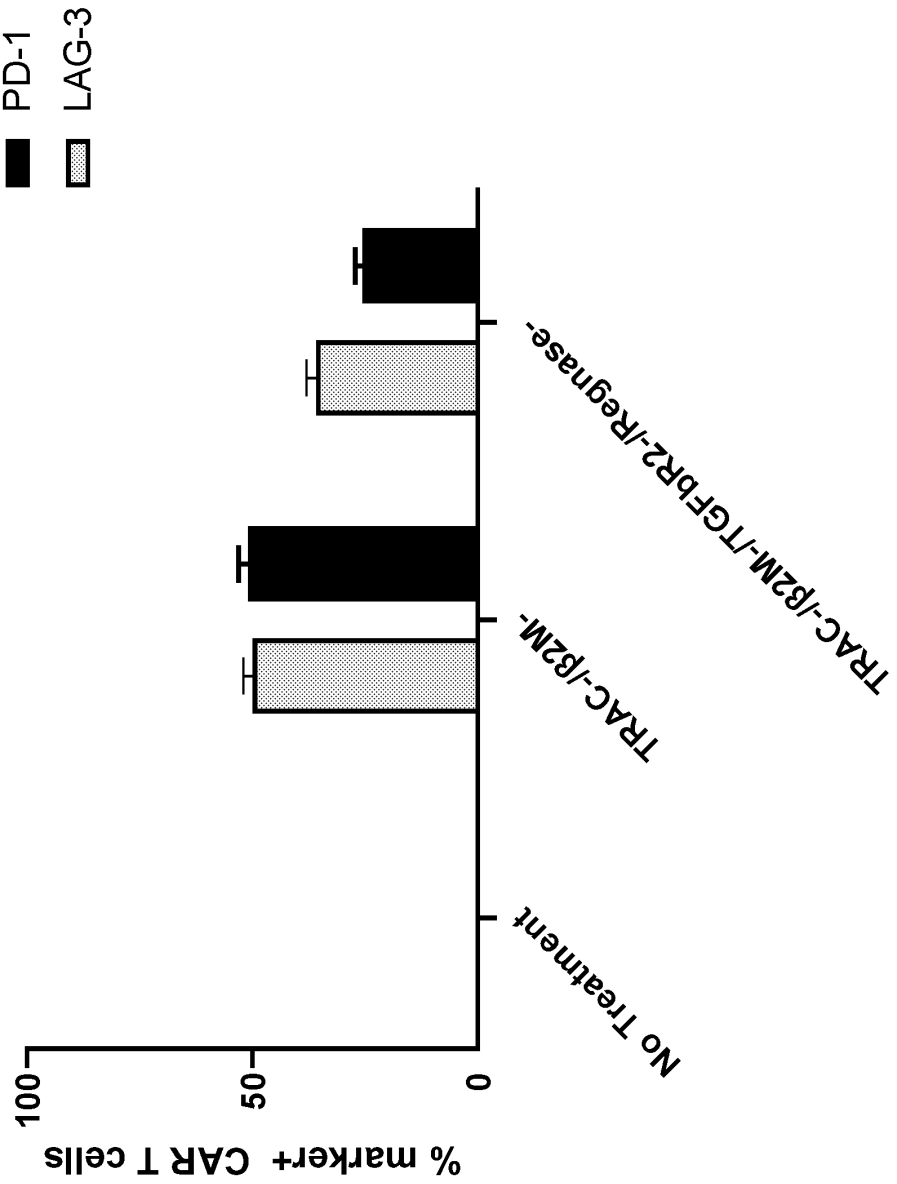

PD-1
LAG-3
% marker+ CAR T cells
100
50
0
No Treatment
TRAC-/β2M-
TRAC-/β2M-/TGFbR2-/Regnase-

FIG. 27D

FIG. 27E

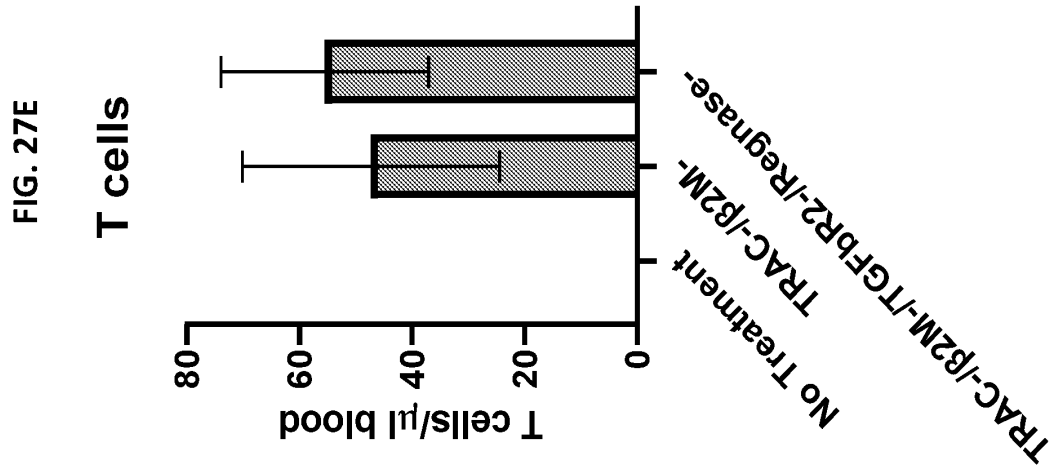

T cells
T cells/μl blood
0
20
40
60
80
No Treatment
TRAC-/β2M-
TRAC-/β2M-/TGFbR2-/Regnase-

FIG. 27F

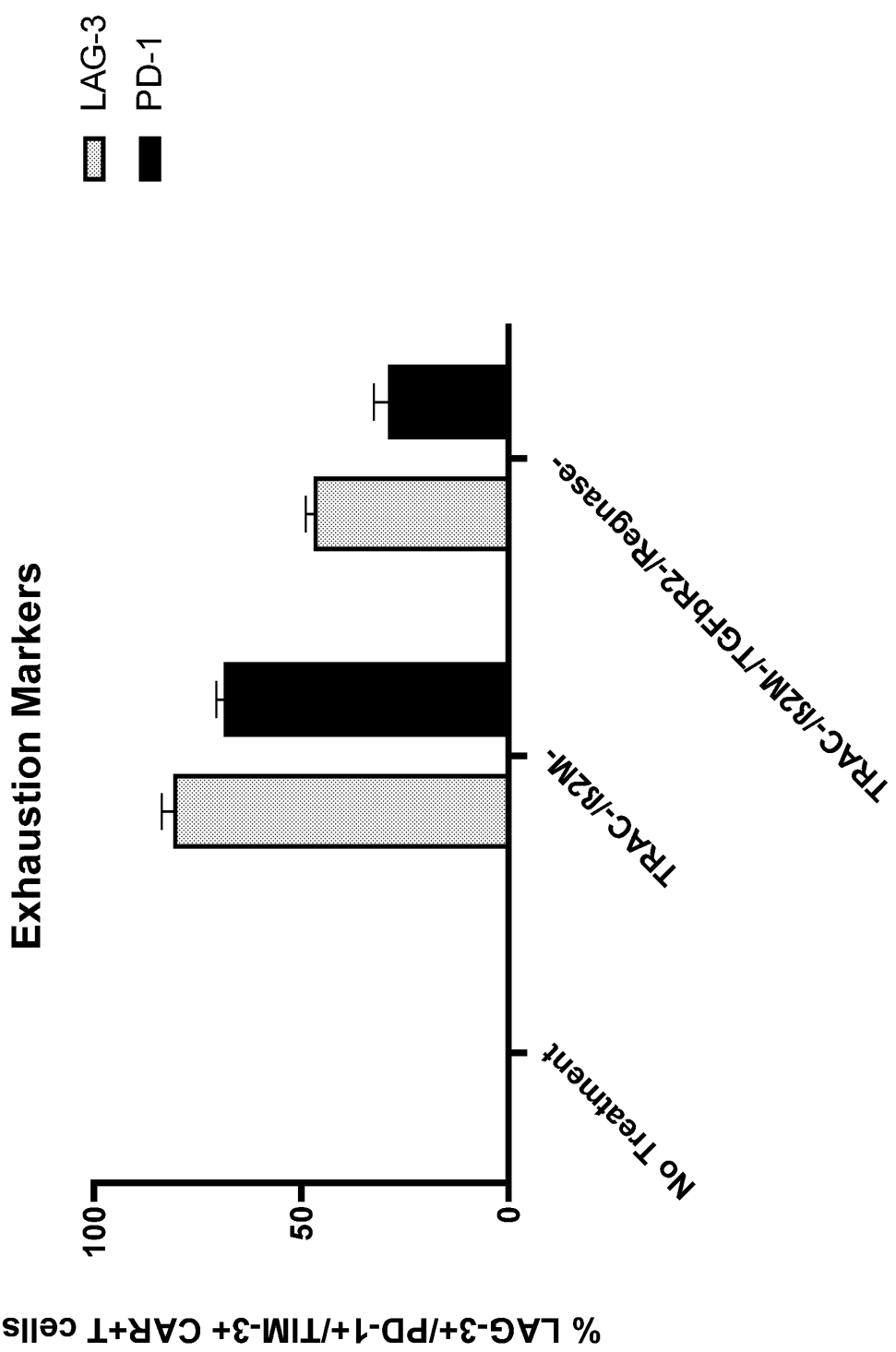

LAG-3
PD-1
Exhaustion Markers
% LAG-3+/PD-1+/TIM-3+ CAR+T cells
100
50
0
No Treatment
TRAC-/B2M-
TRAC-/B2M-/TGFbR2-/Regnase-

FIG. 28

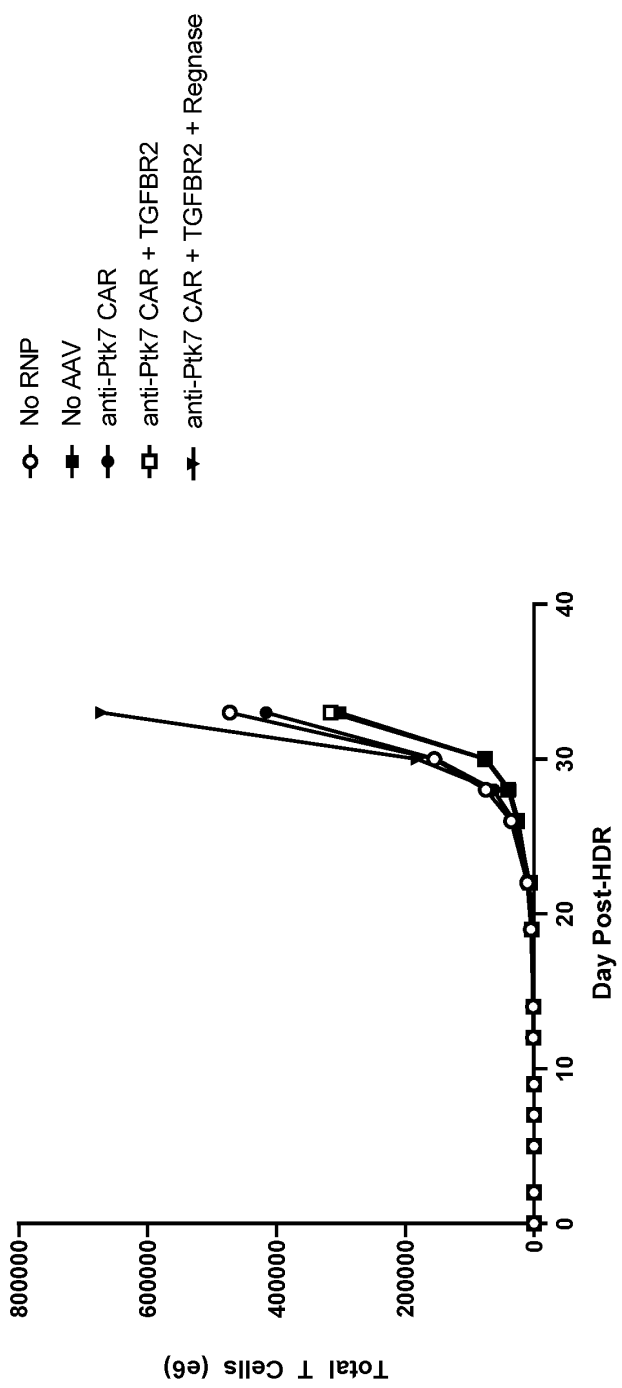

No RNP
No AAV
anti-Ptk7 CAR
anti-Ptk7 CAR + TGFBR2
anti-Ptk7 CAR + TGFBR2 + Regnase
Total T Cells (e6)
800000
600000
400000
200000
0
0
10
20
30
40
Day Post-HDR

FIG. 29A

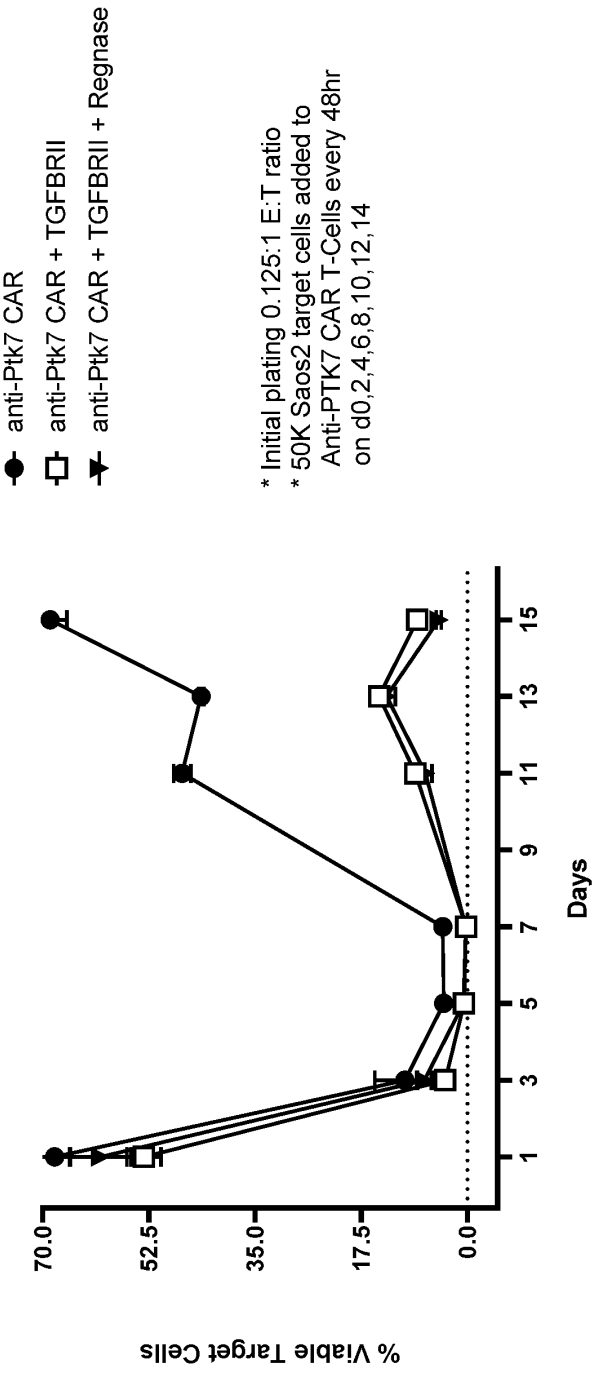

anti-Ptk7 CAR
anti-Ptk7 CAR + TGFBRII
anti-Ptk7 CAR + TGFBRII + Regnase
* Initial plating 0.125:1 E:T ratio
* 50K Saos2 target cells added to Anti-PTK7 CAR T-Cells every 48hr on d0,2,4,6,8,10,12,14
% Viable Target Cells
70.0
52.5
35.0
17.5
0.0
1
3
5
7
9
11
13
15
Days

FIG. 29B

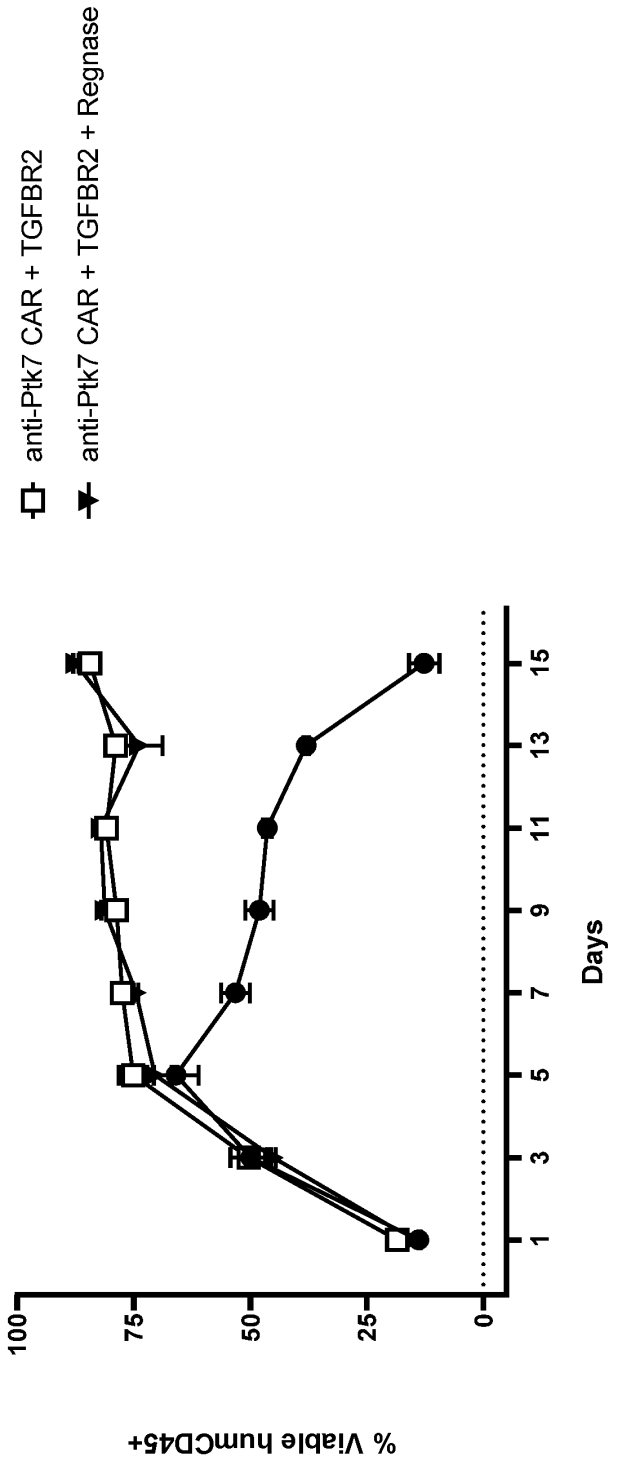

anti-Ptk7 CAR
anti-Ptk7 CAR + TGFBR2
anti-Ptk7 CAR + TGFBR2 + Regnase
% Viable humCD45+
100
75
50
25
0
1
3
5
7
9
11
13
15
Days

FIG. 29C

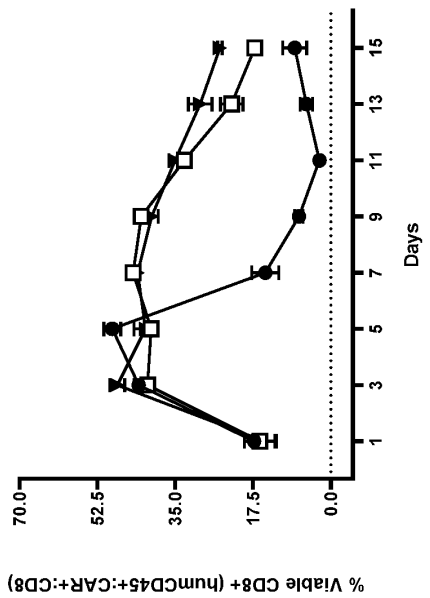


FIG. 29D

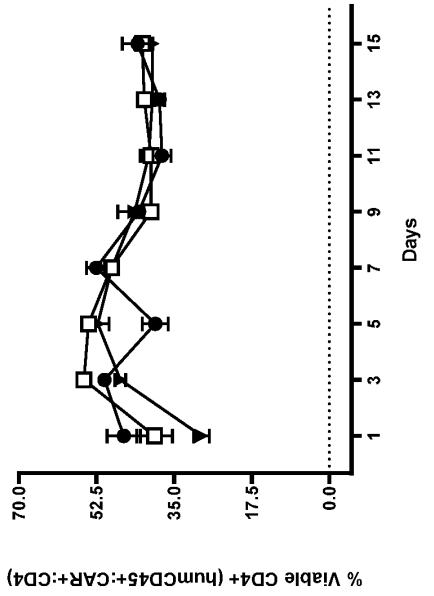


FIG. 30A

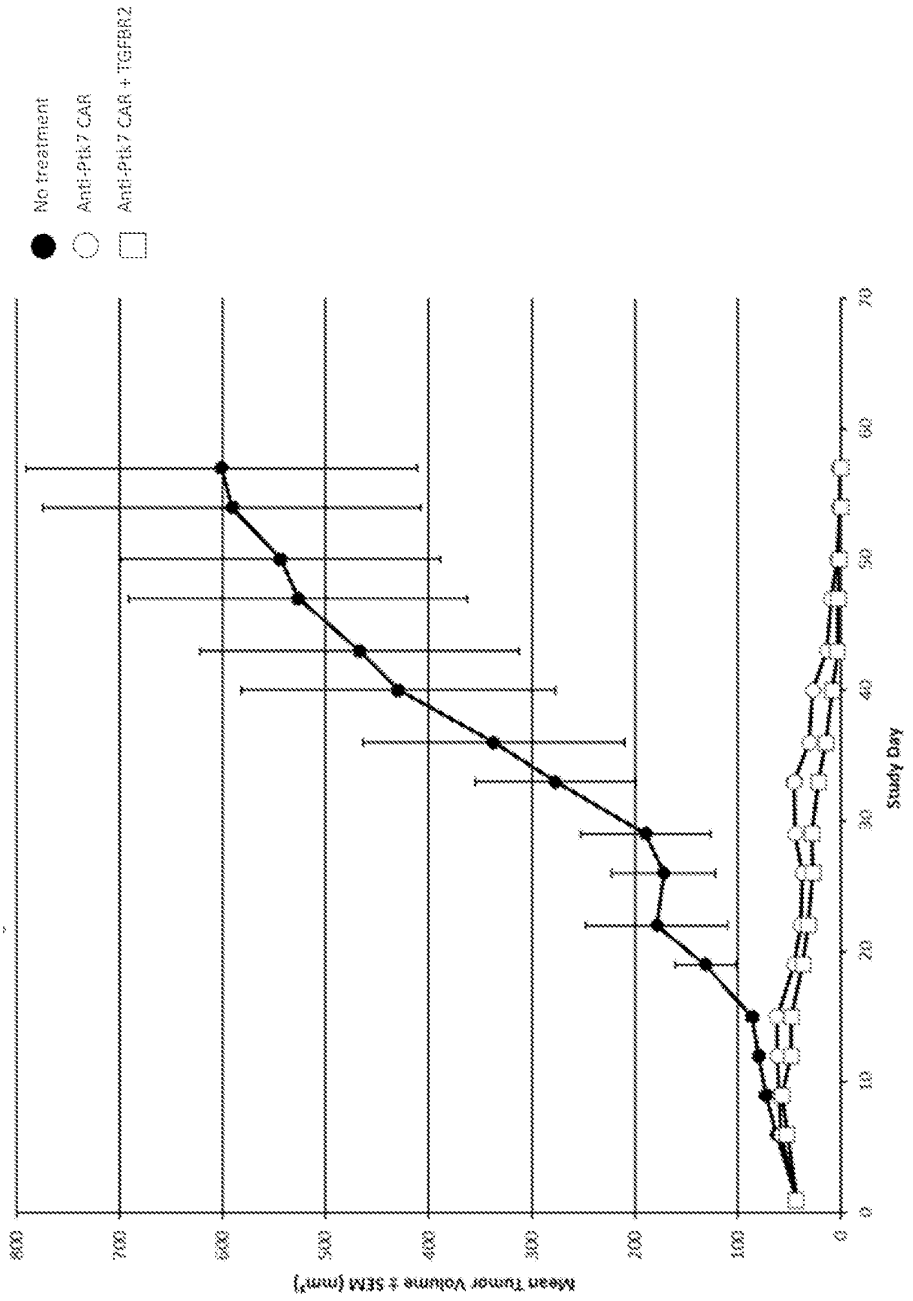

No treatment
Anti-Ptk7 CAR
Anti-Ptk7 CAR + TGFBR2
Mean Tumor Volume ± SEM (mm³)
Study Day
0
100
200
300
400
500
600
700
800
0
10
20
30
40
50
60
70

FIG. 30B

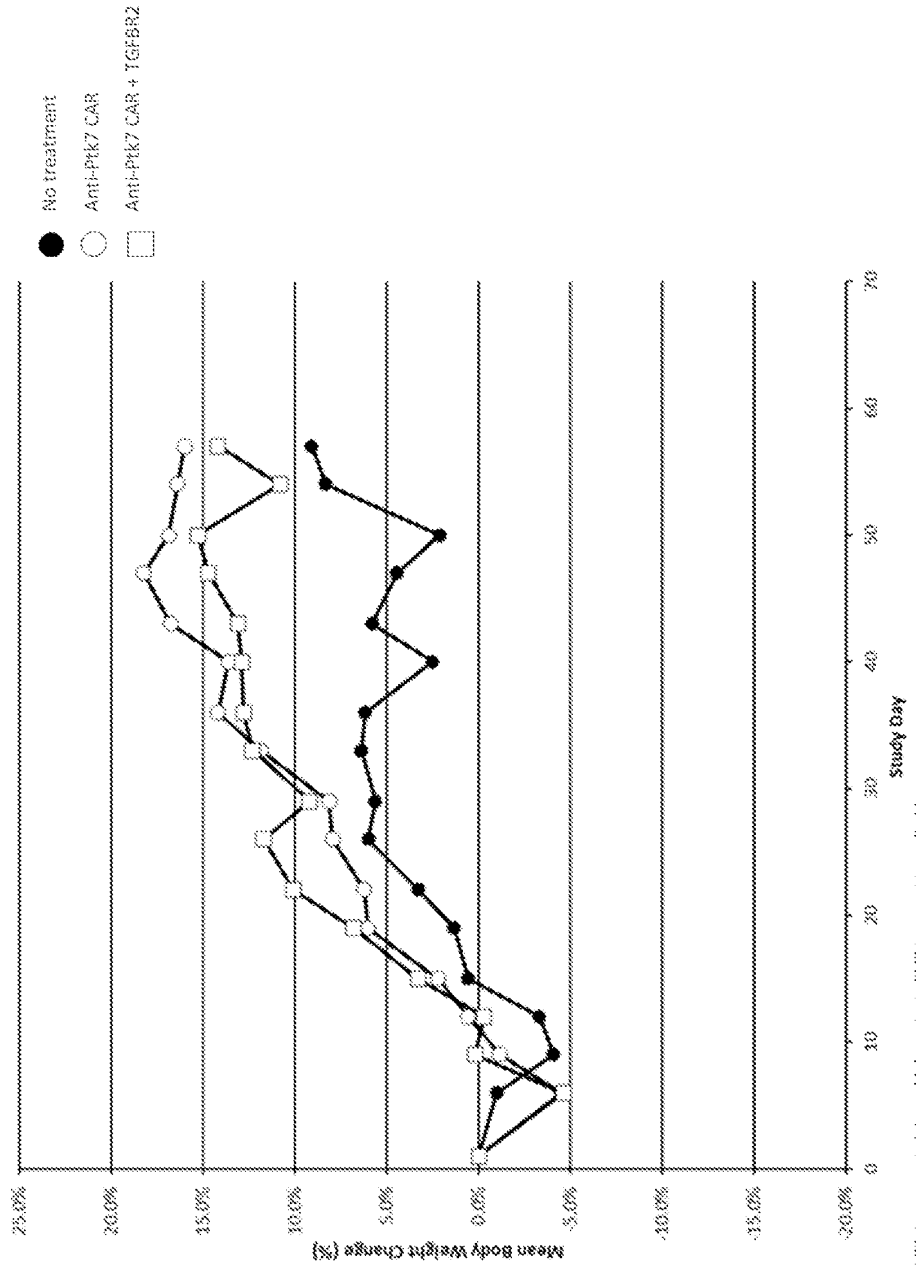

No treatment
Anti-Ptk7 CAR
Anti-Ptk7 CAR + TGFBR2
Mean Body Weight Change (%)
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
-5.0%
-10.0%
-15.0%
-20.0%
0
10
20
30
40
50
60
70
Study Days

FIG. 31A

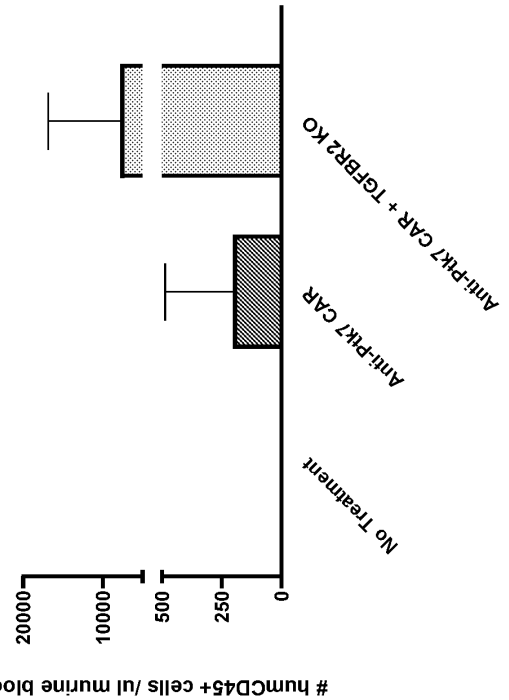
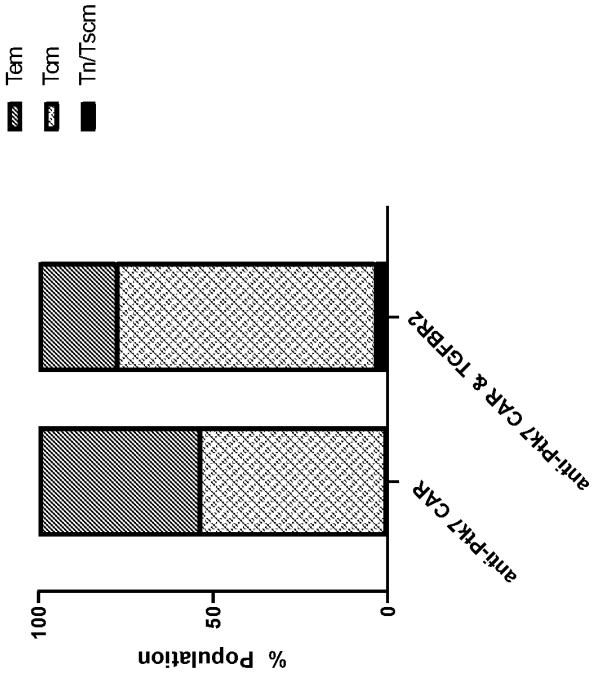



FIG. 31B