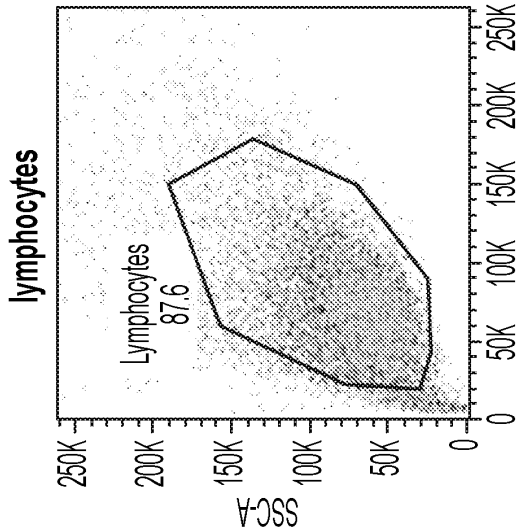


FIG. 1A

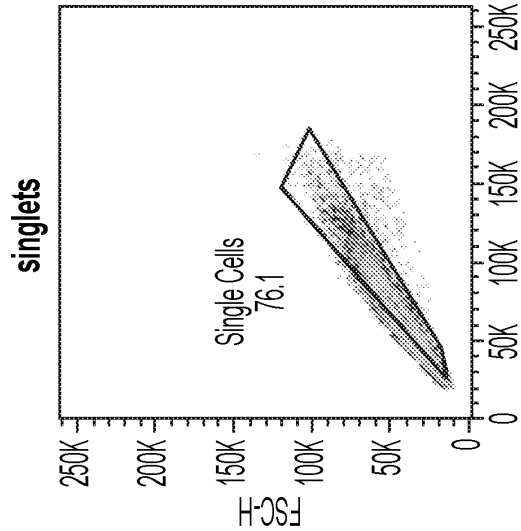


FIG. 1B

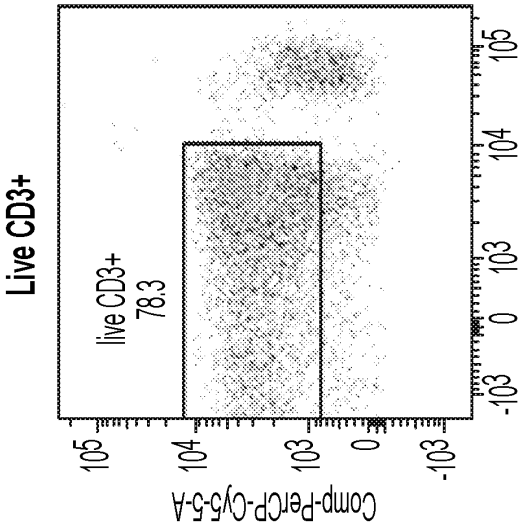


FIG. 1C

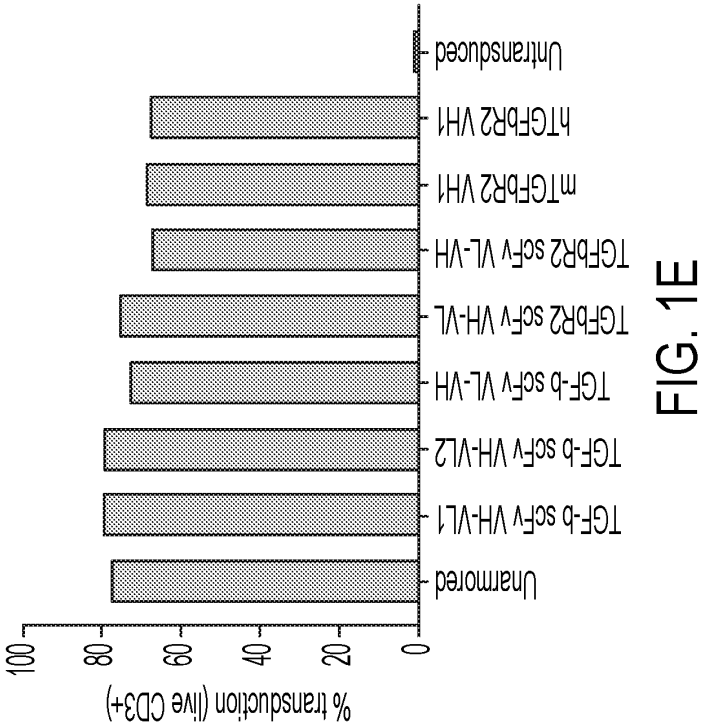

% transduction (live CD3+)
100
80
60
40
20
0
Unarmored
TGF-b scFv VH-VL1
TGF-b scFv VH-VL2
TGF-b scFv VL-VH
TGFbR2 scFv VH-VL
TGFbR2 scFv VL-VH
mTGFbR2 VH1
hTGFbR2 VH1
Untransduced

FIG. 1E

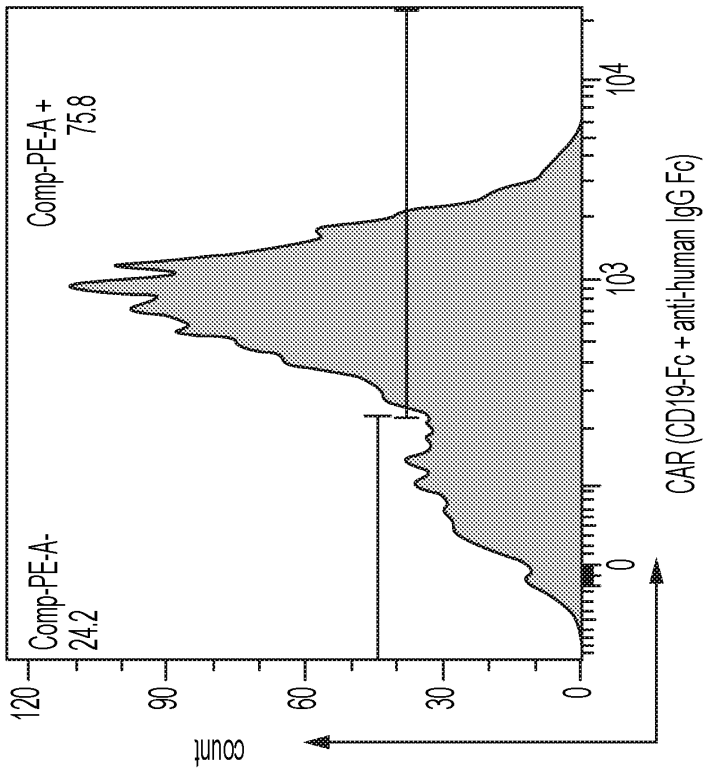

count
120
90
60
30
0
Comp-PE-A-
24.2
Comp-PE-A +
75.8
0
10^3
10^4
CAR (CD19-Fc + anti-human IgG Fc)

FIG. 1D

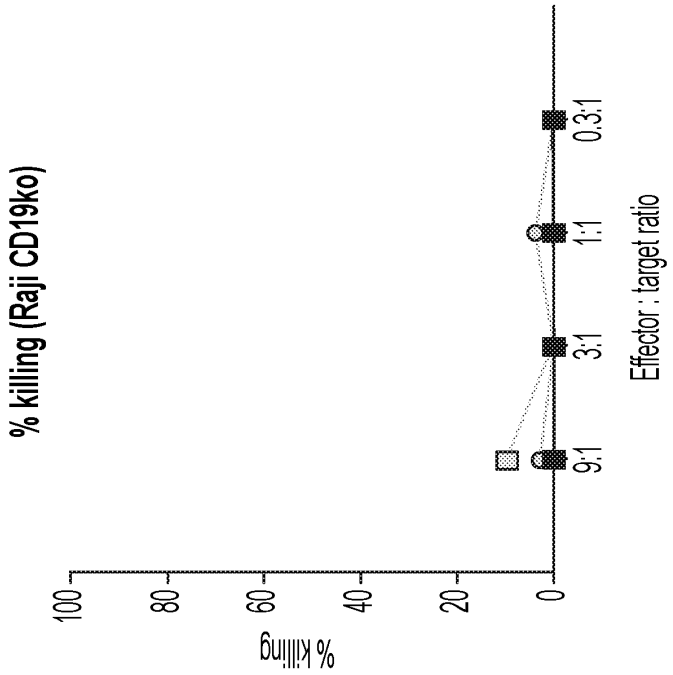

% killing (Raji CD19ko)
% killing
100
80
60
40
20
0
9:1
3:1
1:1
0.3:1
Effector : target ratio

FIG. 2B

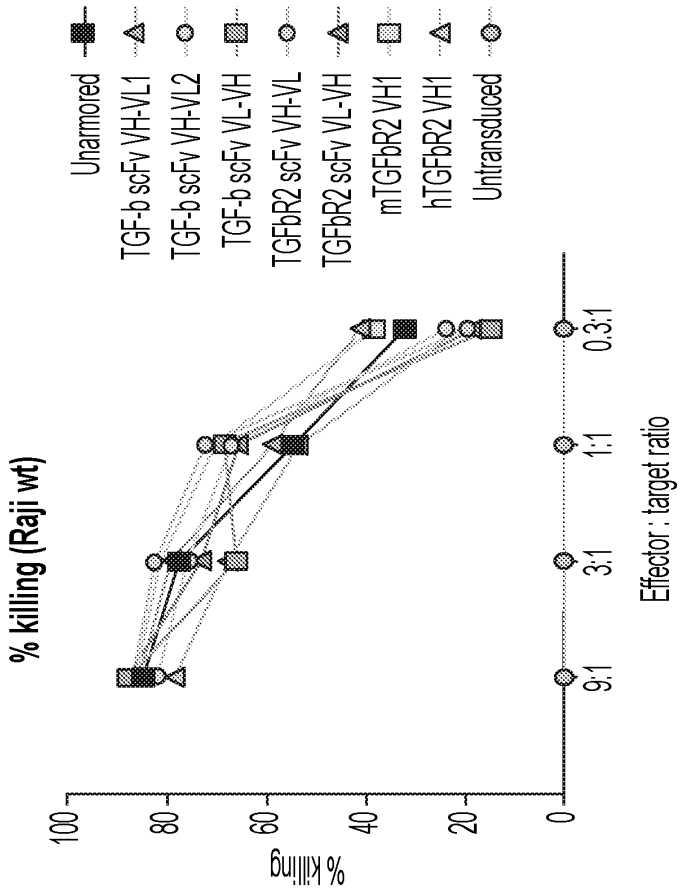

% killing (Raji wt)
% killing
100
80
60
40
20
0
9:1
3:1
1:1
0.3:1
Effector : target ratio
Unarmored
TGF-b scFv VH-VL1
TGF-b scFv VH-VL2
TGF-b scFv VL-VH
TGFbR2 scFv VH-VL
TGFbR2 scFv VL-VH
mTGFbR2 VH1
hTGFbR2 VH1
Untransduced

FIG. 2A

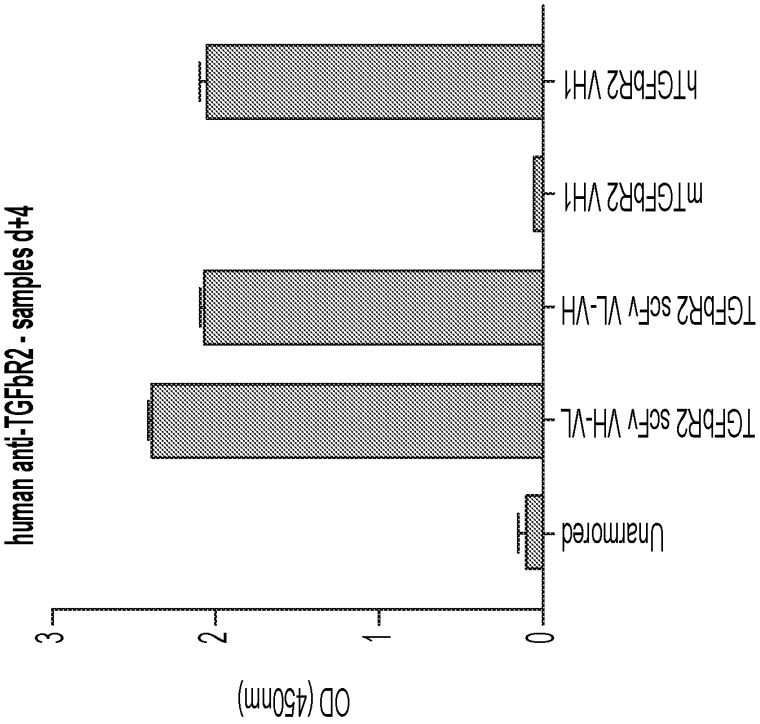

human anti-TGFbR2 - samples d+4
OD (450nm)
3
2
1
0
Unarmored
TGFbR2 scFv VH-VL
TGFbR2 scFv VL-VH
mTGFbR2 VH1
hTGFbR2 VH1

FIG. 3B

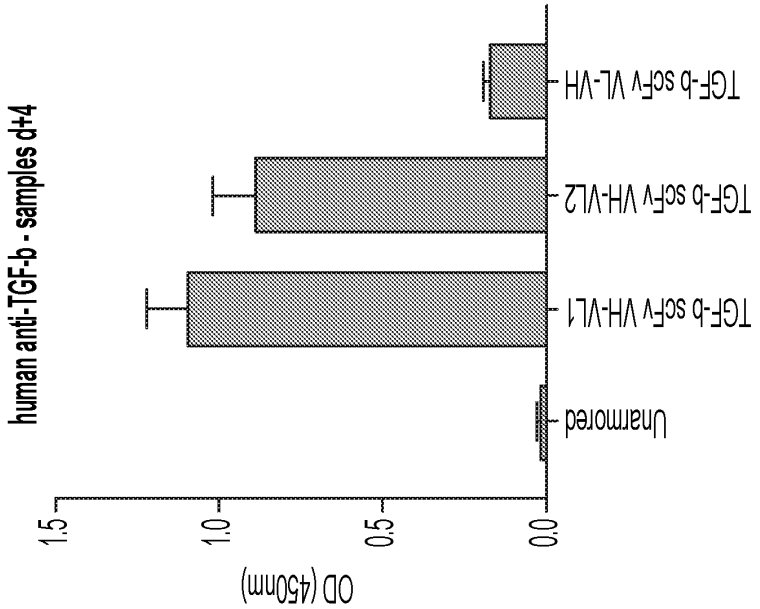

human anti-TGF-b - samples d+4
OD (450nm)
1.5
1.0
0.5
0.0
Unarmored
TGF-b scFv VH-VL1
TGF-b scFv VH-VL2
TGF-b scFv VL-VH

FIG. 3A

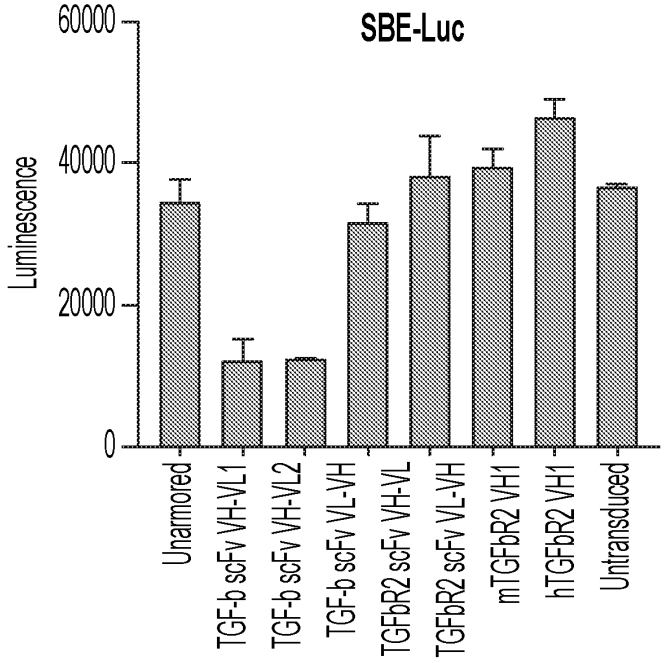

SBE-Luc
Luminescence
60000
40000
20000
0
Unarmored
TGF-b scFv VH-VL1
TGF-b scFv VH-VL2
TGF-b scFv VL-VH
TGFbR2 scFv VH-VL
TGFbR2 scFv VL-VH
mTGFbR2 VH1
hTGFbR2 VH1
Untransduced

FIG. 4

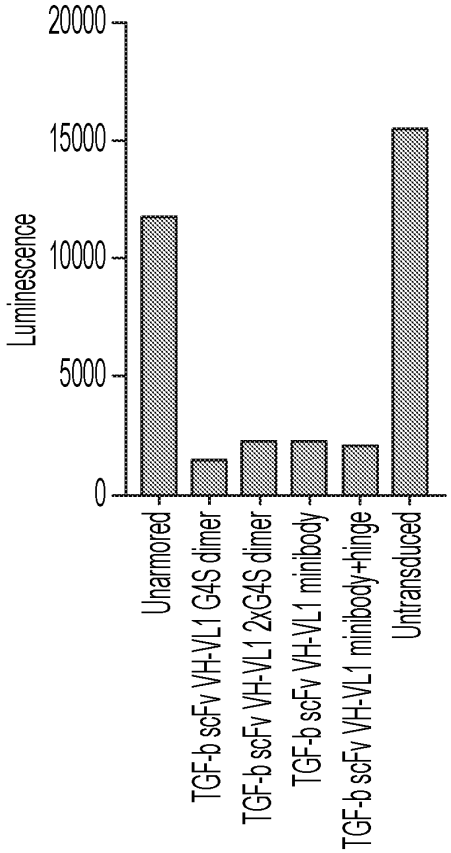

Luminescence
20000
15000
10000
5000
0
Unarmored
TGF-b scFv VH-VL1 G4S dimer
TGF-b scFv VH-VL1 2xG4S dimer
TGF-b scFv VH-VL1 minibody
TGF-b scFv VH-VL1 minibody+hinge
Untransduced

FIG. 5A

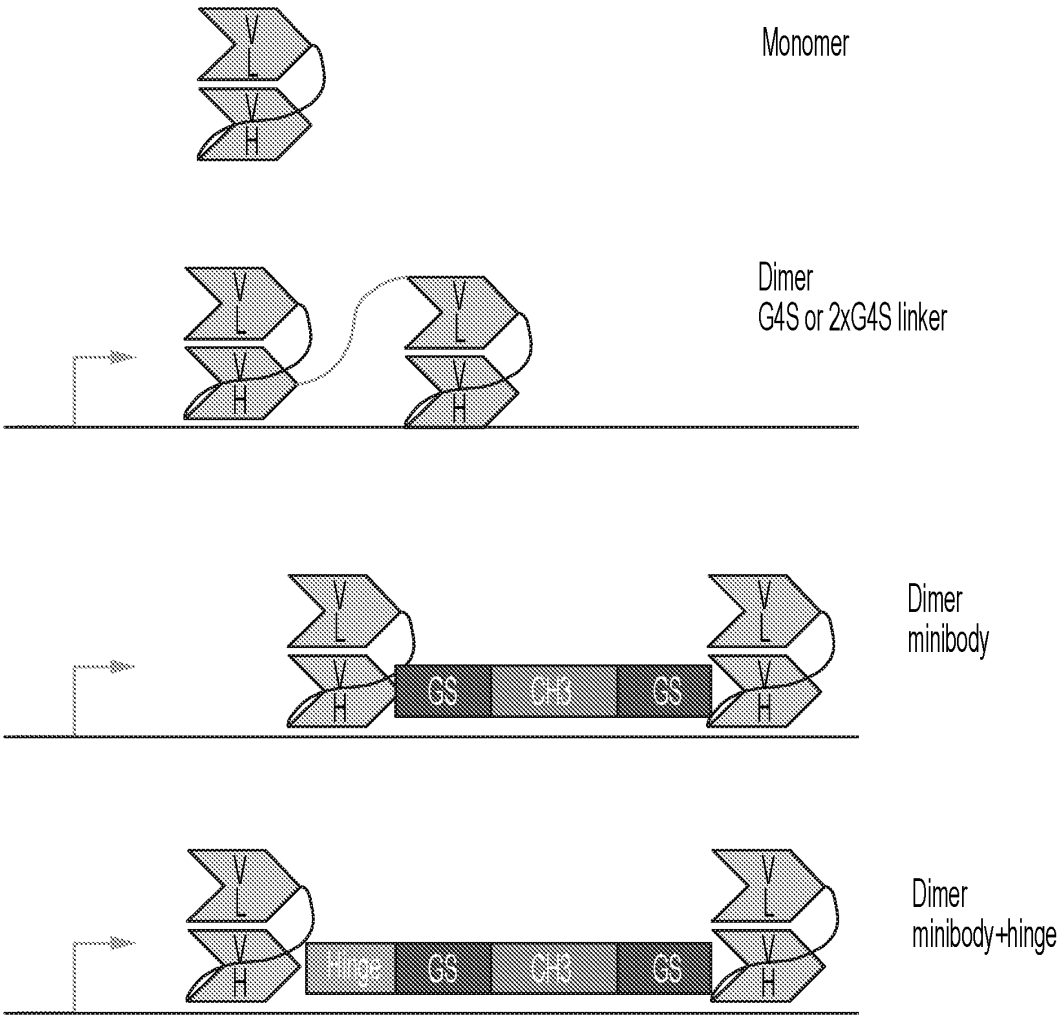

Monomer
Dimer
G4S or 2xG4S linker
Dimer
minibody
Dimer
minibody+hinge
VL
VH
GS
CH3
Hinge

FIG. 5B

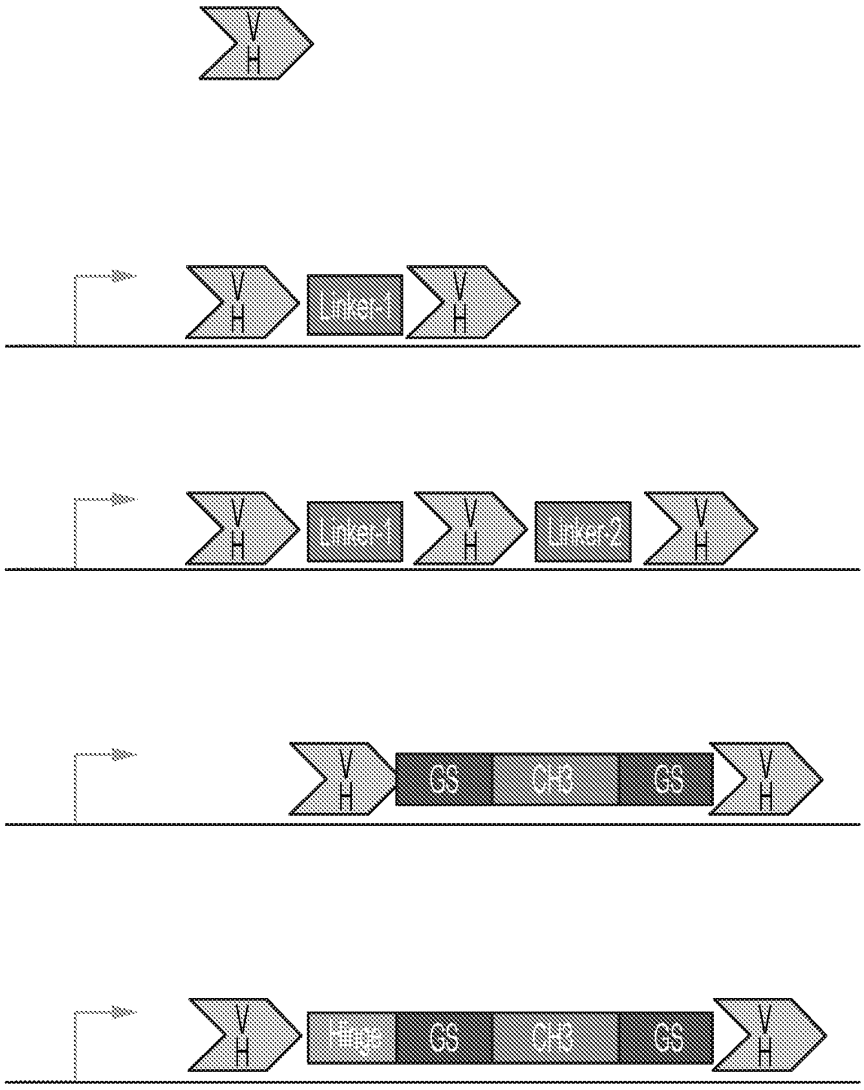

VH
VH
Linker-1
VH
VH
Linker-1
VH
Linker-2
VH
VH
GS
CH3
GS
VH
VH
Hinge
GS
CH3
GS
VH

FIG. 5C

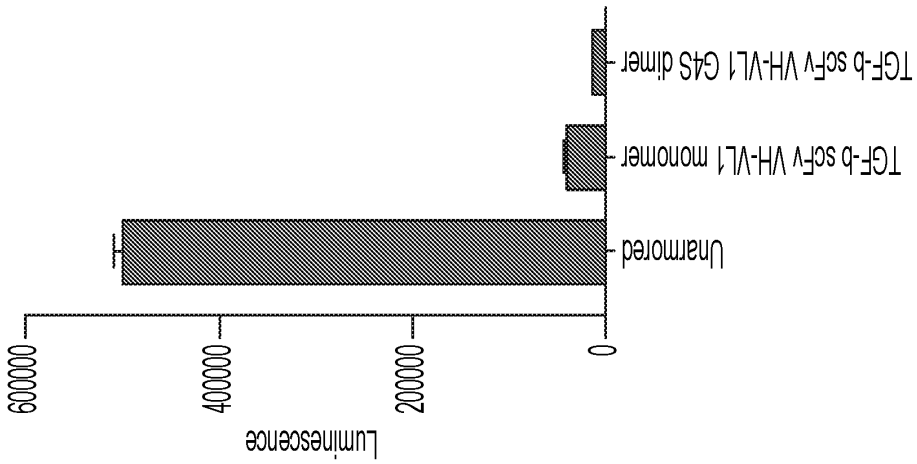

Luminescence
600000
400000
200000
0
Unarmored
TGF-b scFv VH-VL1 monomer
TGF-b scFv VH-VL1 G4S dimer

FIG. 6A

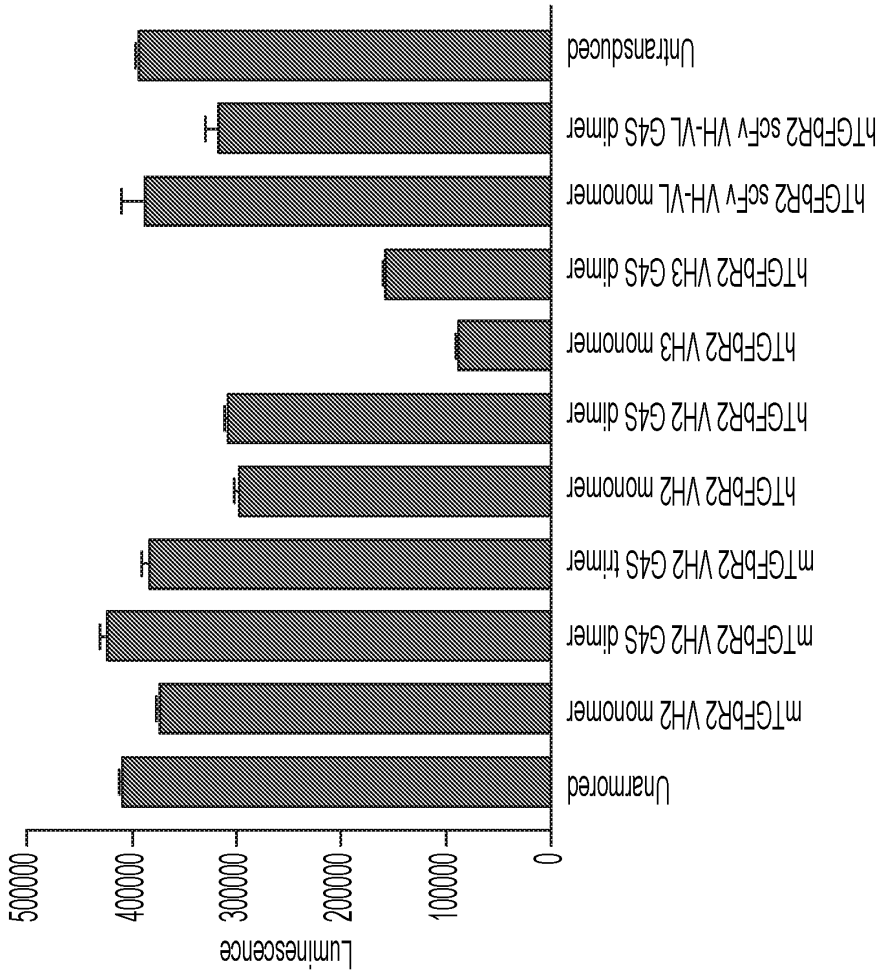

Luminescence
500000
400000
300000
200000
100000
0
Unarmored
mTGFbR2 VH2 monomer
mTGFbR2 VH2 G4S dimer
mTGFbR2 VH2 G4S trimer
hTGFbR2 VH2 monomer
hTGFbR2 VH2 G4S dimer
hTGFbR2 VH3 monomer
hTGFbR2 VH3 G4S dimer
hTGFbR2 scFv VH-VL monomer
hTGFbR2 scFv VH-VL G4S dimer
Untransduced

FIG. 6B

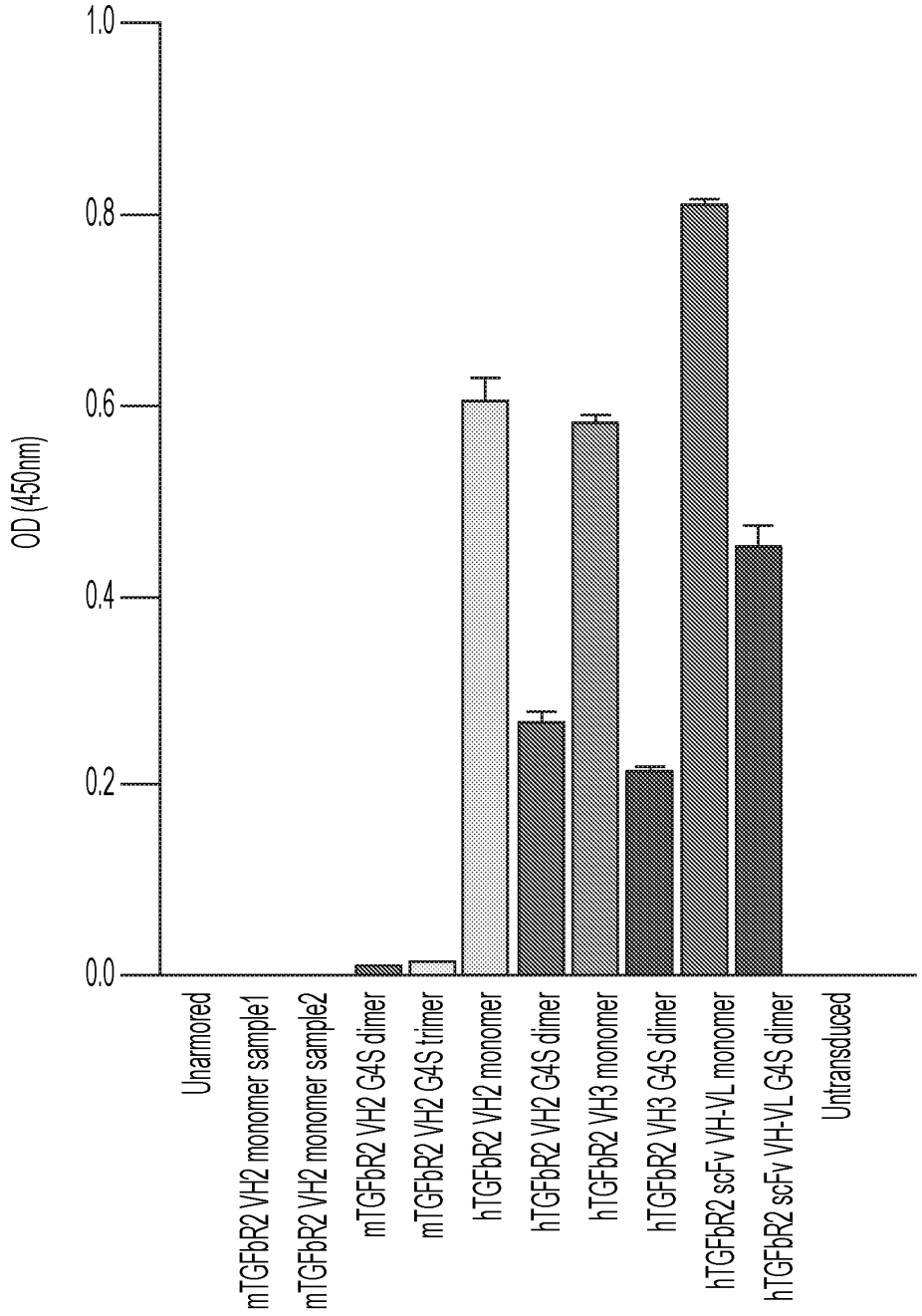

OD (450nm)
1.0
0.8
0.6
0.4
0.2
0.0
Unarmored
mTGFbR2 VH2 monomer sample1
mTGFbR2 VH2 monomer sample2
mTGFbR2 VH2 G4S dimer
mTGFbR2 VH2 G4S trimer
hTGFbR2 VH2 monomer
hTGFbR2 VH2 G4S dimer
hTGFbR2 VH3 monomer
hTGFbR2 VH3 G4S dimer
hTGFbR2 scFv VH-VL monomer
hTGFbR2 scFv VH-VL G4S dimer
Untransduced

FIG. 7A

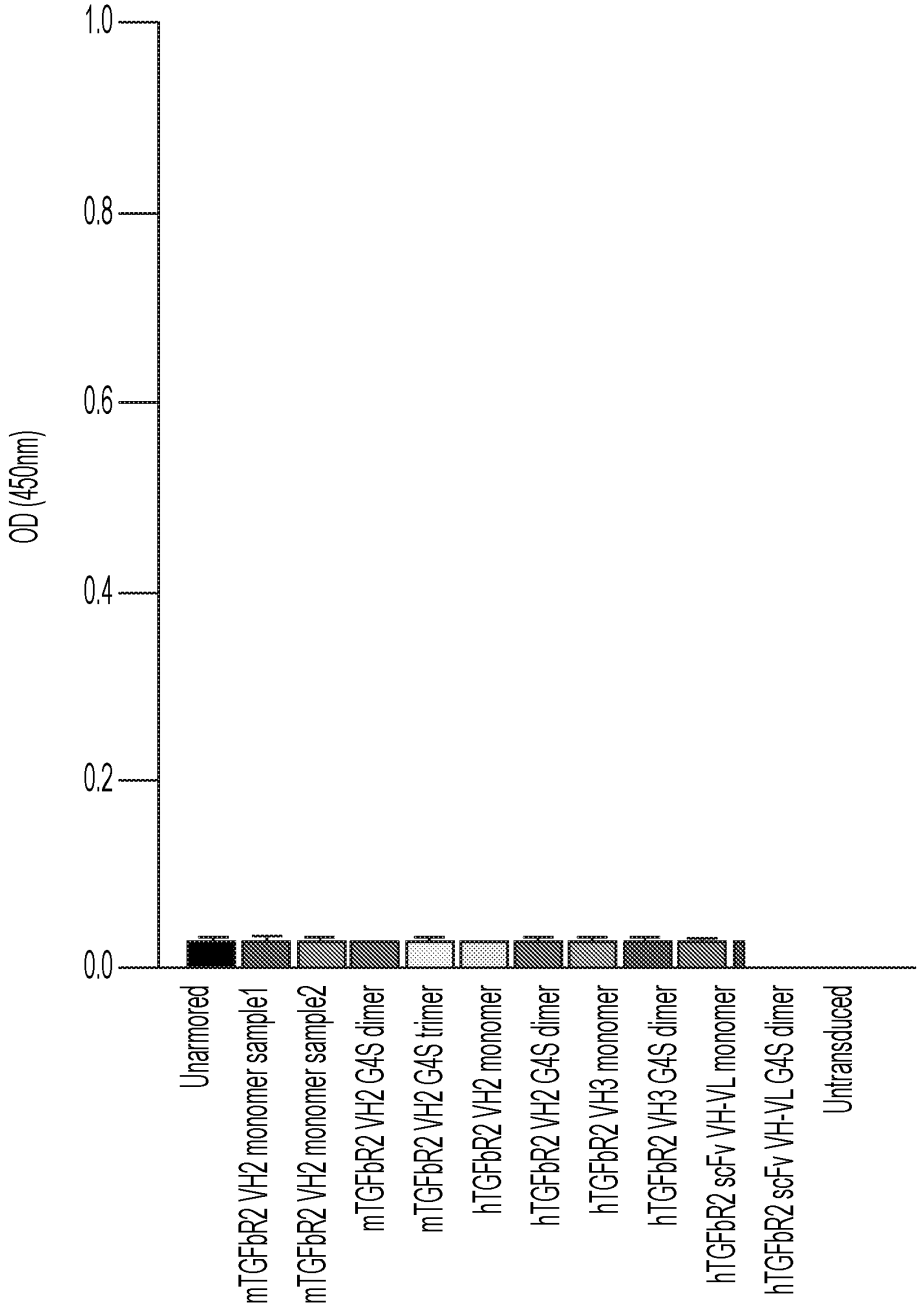

OD (450nm)
1.0
0.8
0.6
0.4
0.2
0.0
Unarmored
mTGFbR2 VH2 monomer sample1
mTGFbR2 VH2 monomer sample2
mTGFbR2 VH2 G4S dimer
mTGFbR2 VH2 G4S trimer
hTGFbR2 VH2 monomer
hTGFbR2 VH2 G4S dimer
hTGFbR2 VH3 monomer
hTGFbR2 VH3 G4S dimer
hTGFbR2 scFv VH-VL monomer
hTGFbR2 scFv VH-VL G4S dimer
Untransduced

FIG. 7B

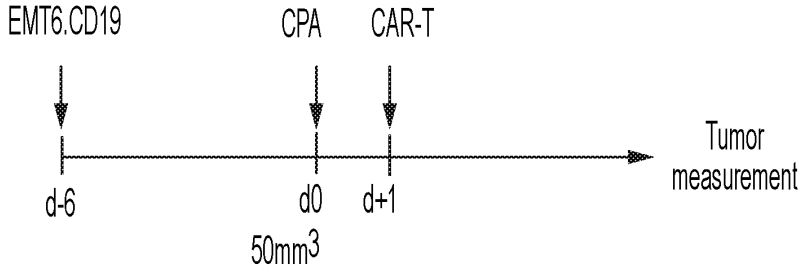


FIG. 8A

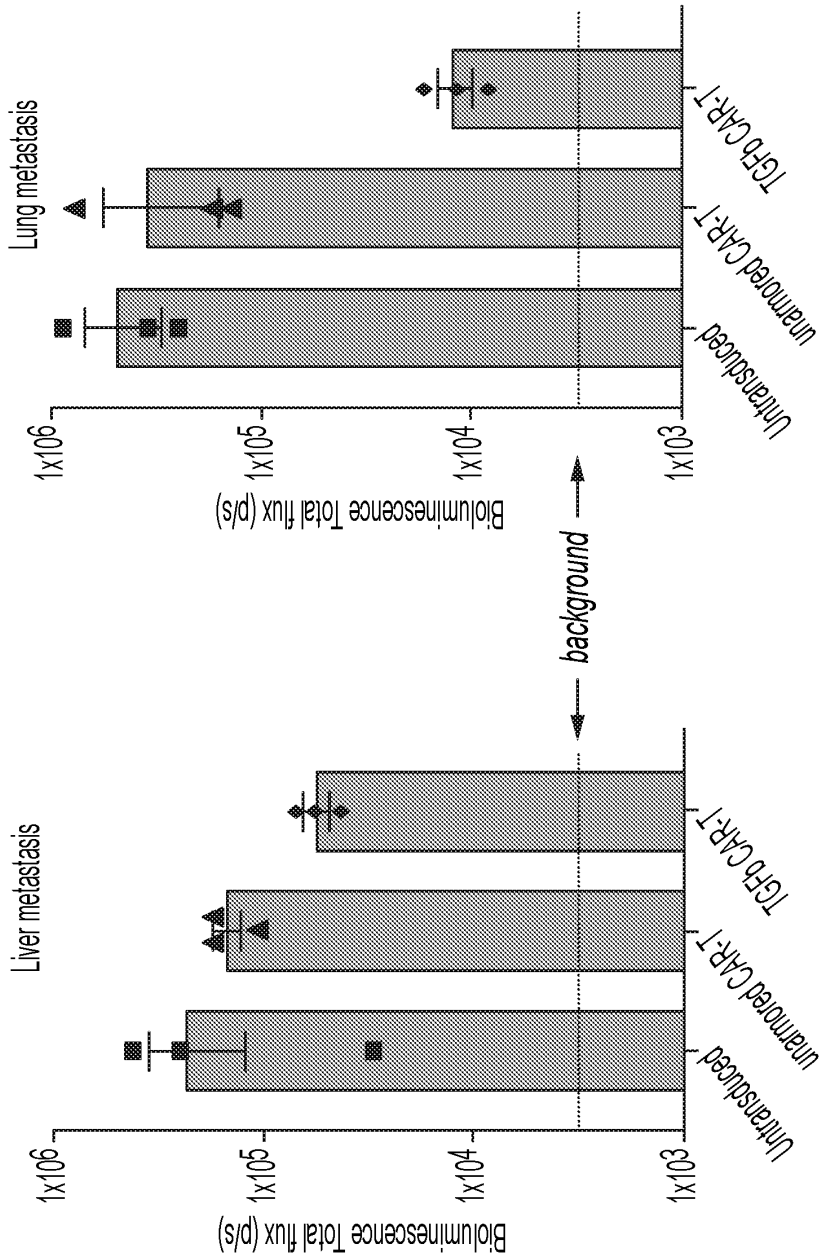

Liver metastasis
Bioluminescence Total flux (p/s)
1x10^6
1x10^5
1x10^4
1x10^3
Untransduced
unarmored CAR-T
TGFb CAR-T
background
Lung metastasis
Bioluminescence Total flux (p/s)
1x10^6
1x10^5
1x10^4
1x10^3
Untransduced
unarmored CAR-T
TGFb CAR-T

FIG. 8B

FIG. 8C

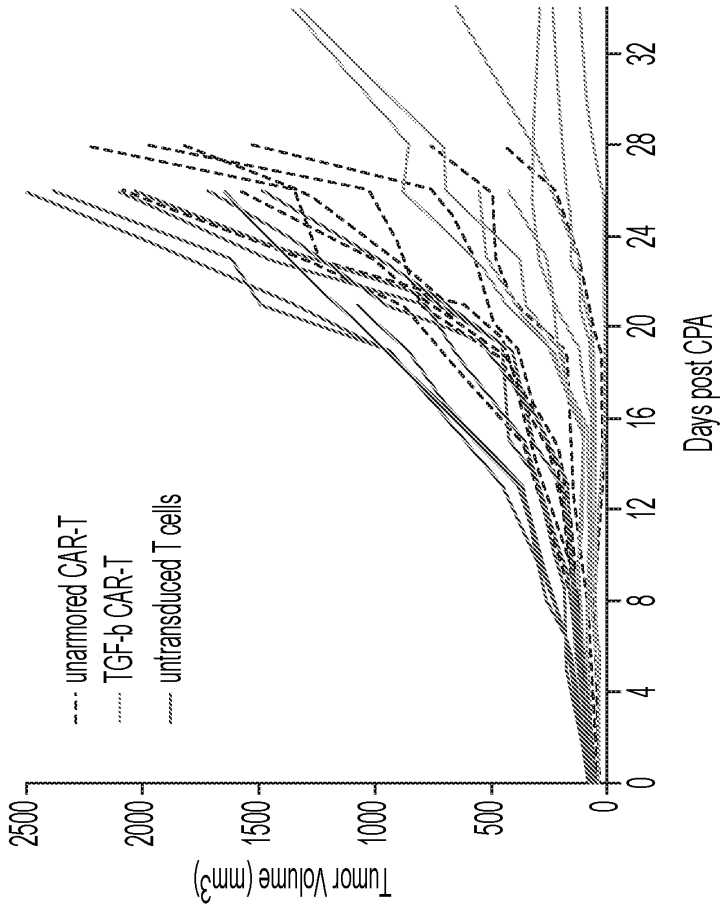

unarmored CAR-T
TGF-b CAR-T
untransduced T cells
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
4
8
12
16
20
24
28
32
Days post CPA

FIG. 8D

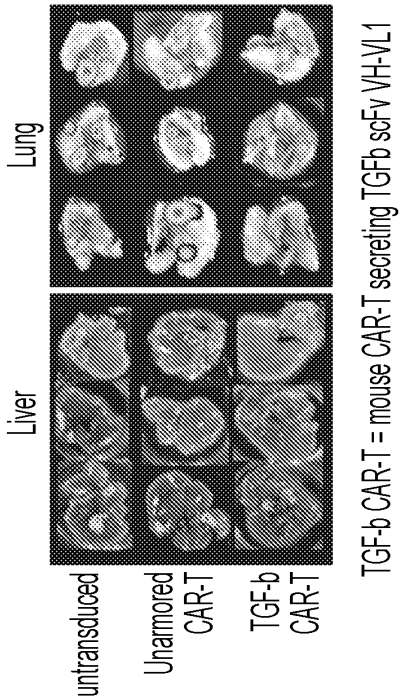

Liver
Lung
untransduced
Unarmored CAR-T
TGF-b CAR-T
TGF-b CAR-T = mouse CAR-T secreting TGFb scFv VH-VL1

FIG. 8E

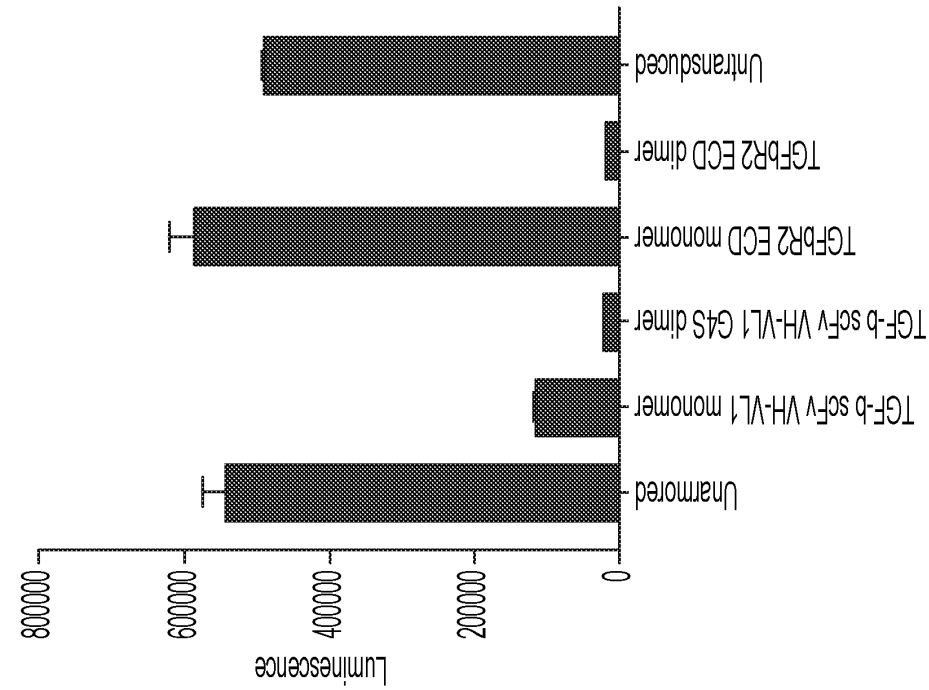

Luminescence
800000
600000
400000
200000
0
Unarmored
TGF-b scFv VH-VL1 monomer
TGF-b scFv VH-VL1 G4S dimer
TGFbR2 ECD monomer
TGFbR2 ECD dimer
Untransduced

FIG. 9A

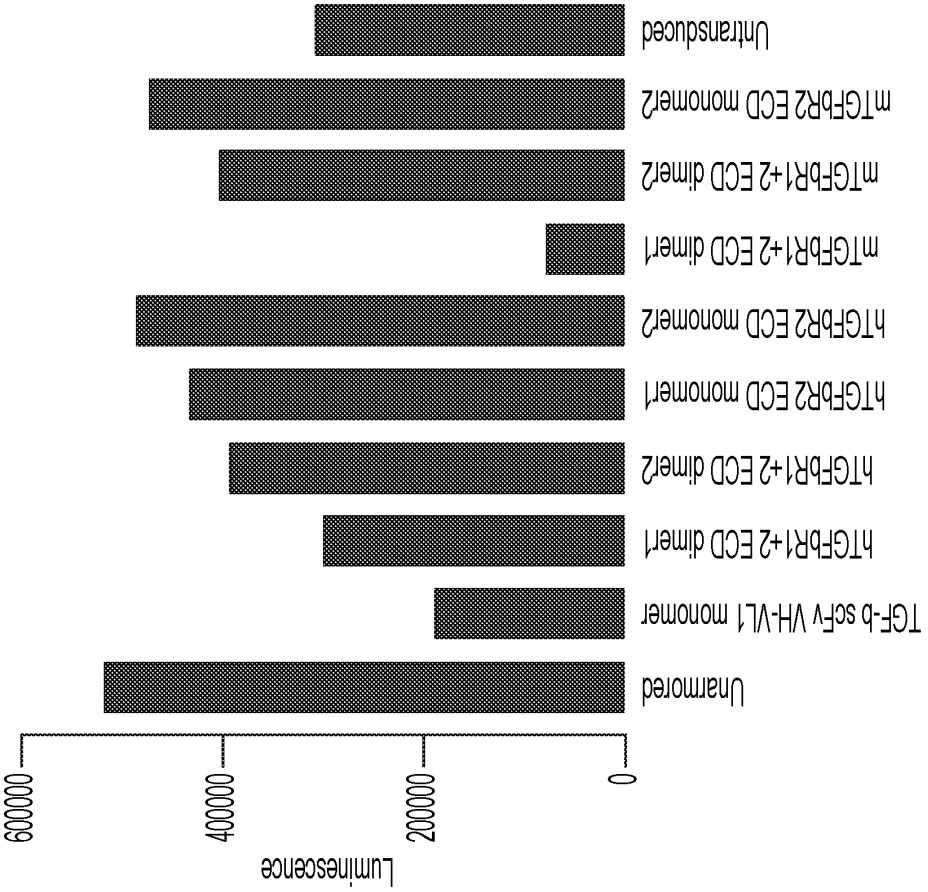

Luminescence
600000
400000
200000
0
Unarmored
TGF-b scFv VH-VL1 monomer
hTGFbR1+2 ECD dimer1
hTGFbR1+2 ECD dimer2
hTGFbR2 ECD monomer1
hTGFbR2 ECD monomer2
mTGFbR1+2 ECD dimer1
mTGFbR1+2 ECD dimer2
mTGFbR2 ECD monomer2
Untransduced

FIG. 9B

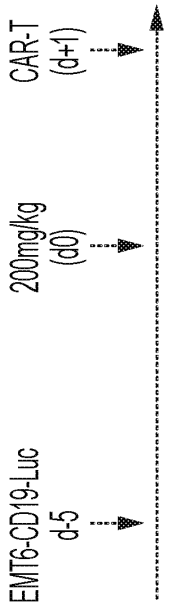


FIG. 10A

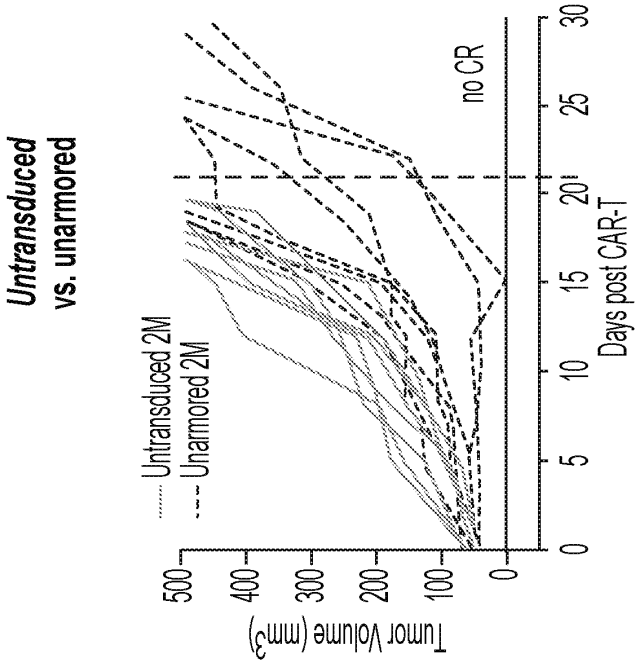


FIG. 10B

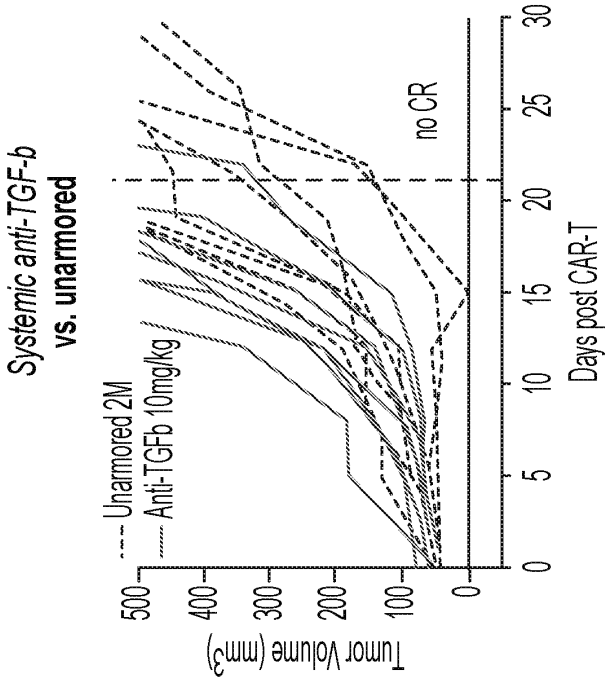
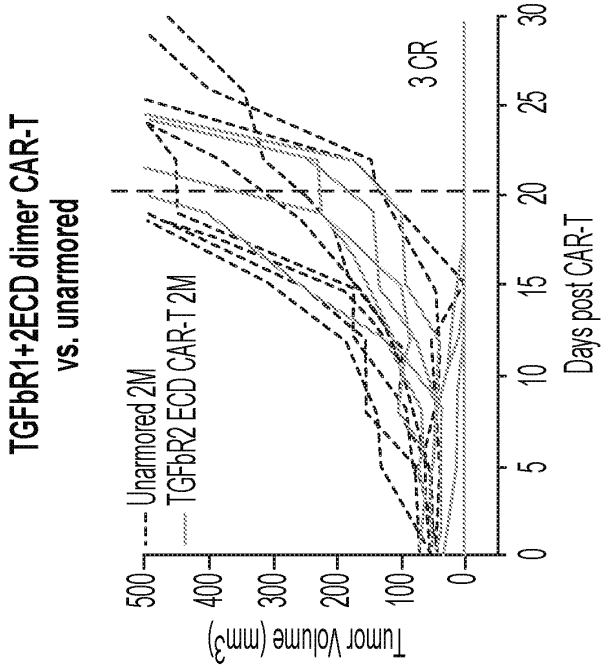


FIG. 10D



CR = complete response

FIG. 10C

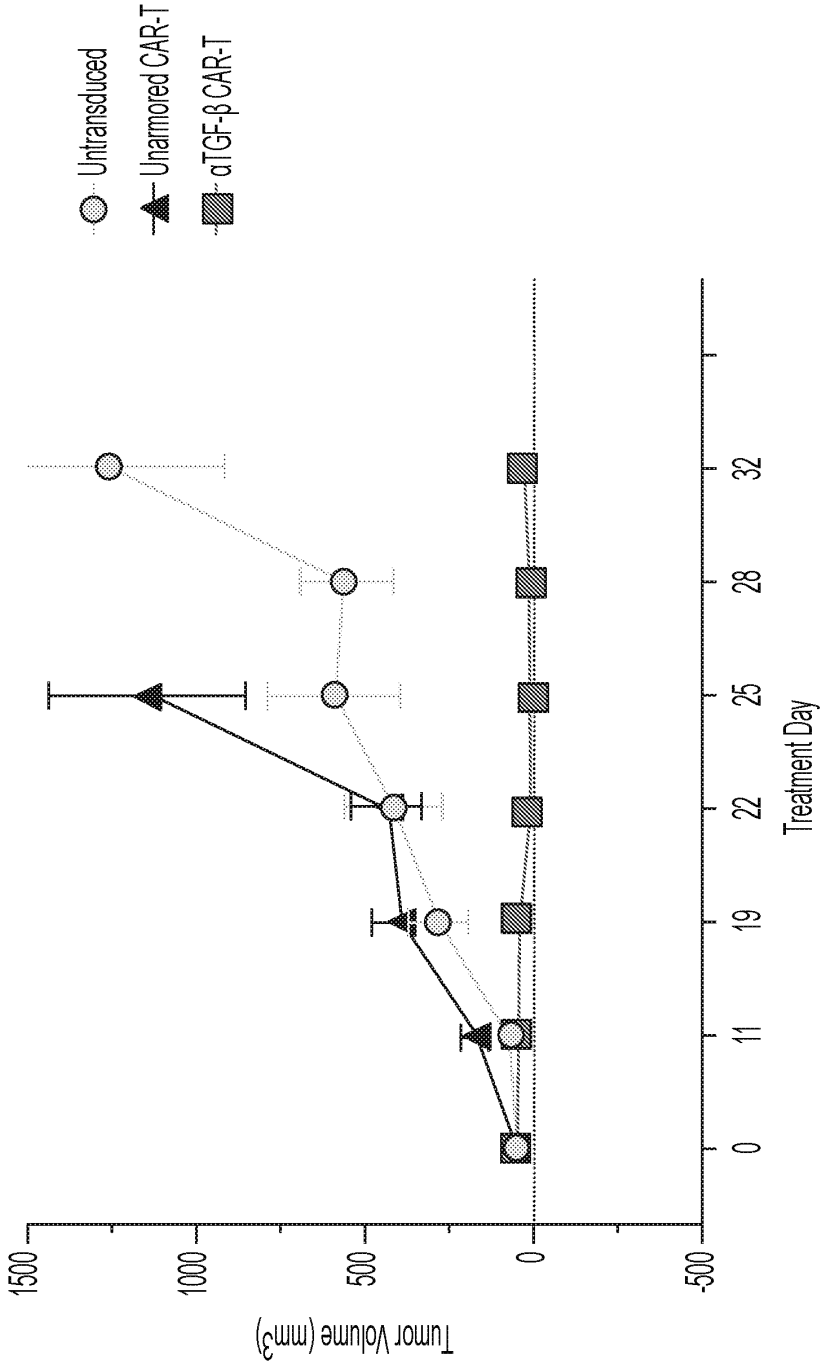

Untransduced
Unarmored CAR-T
αTGF-β CAR-T
Tumor Volume (mm3)
1500
1000
500
0
-500
0
11
19
22
25
28
32
Treatment Day

FIG. 11

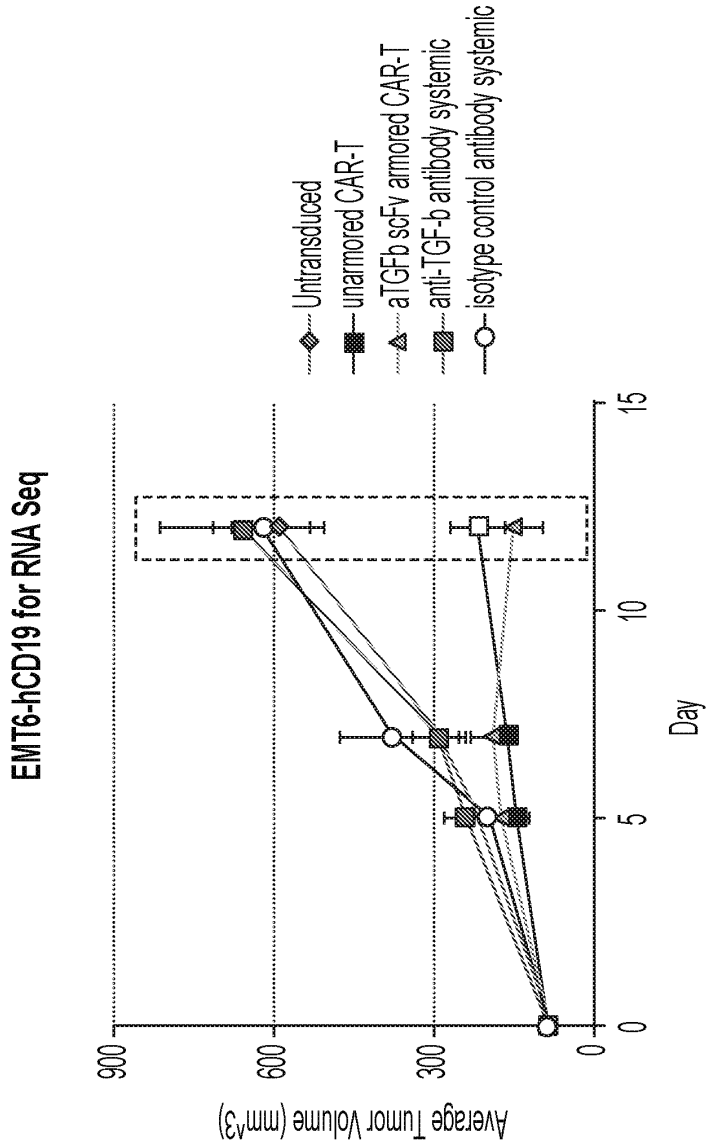

EMT6-hCD19 for RNA Seq
Untransduced
unarmored CAR-T
aTGFb scFv armored CAR-T
anti-TGF-b antibody systemic
isotype control antibody systemic
Average Tumor Volume (mm^3)
900
600
300
0
0
5
10
15
Day

FIG. 12

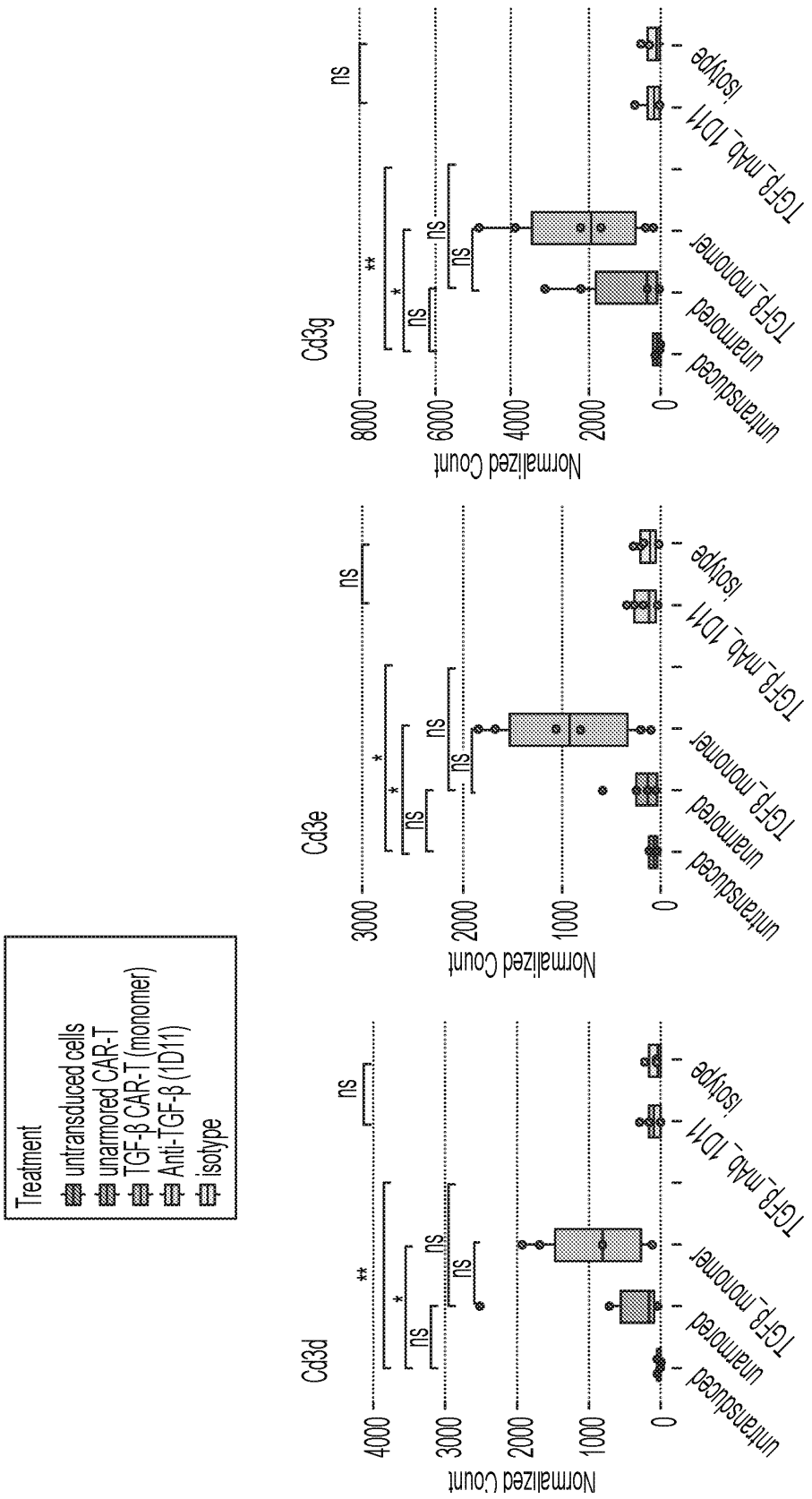

Treatment
untransduced cells
unarmored CAR-T
TGF-β CAR-T (monomer)
Anti-TGF-β (1D11)
isotype
Cd3d
Cd3e
Cd3g
Normalized Count
untransduced
unarmored
TGFβ_monomer
TGFβ_mAb_1D11
isotype
ns
*
**

FIG. 13

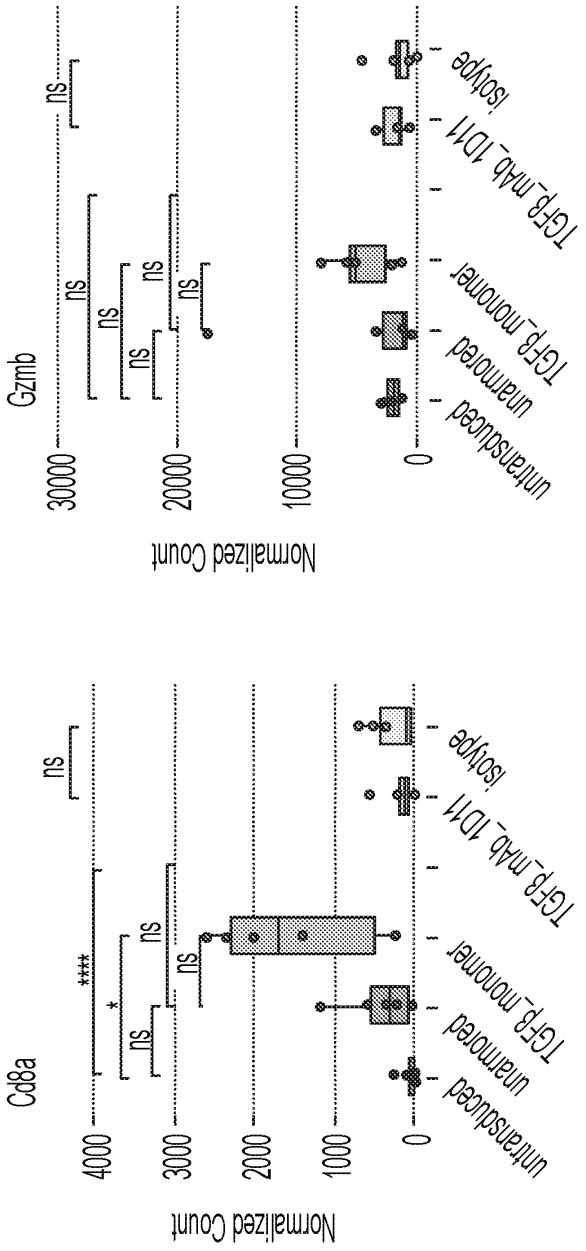


FIG. 13
CONTINUED

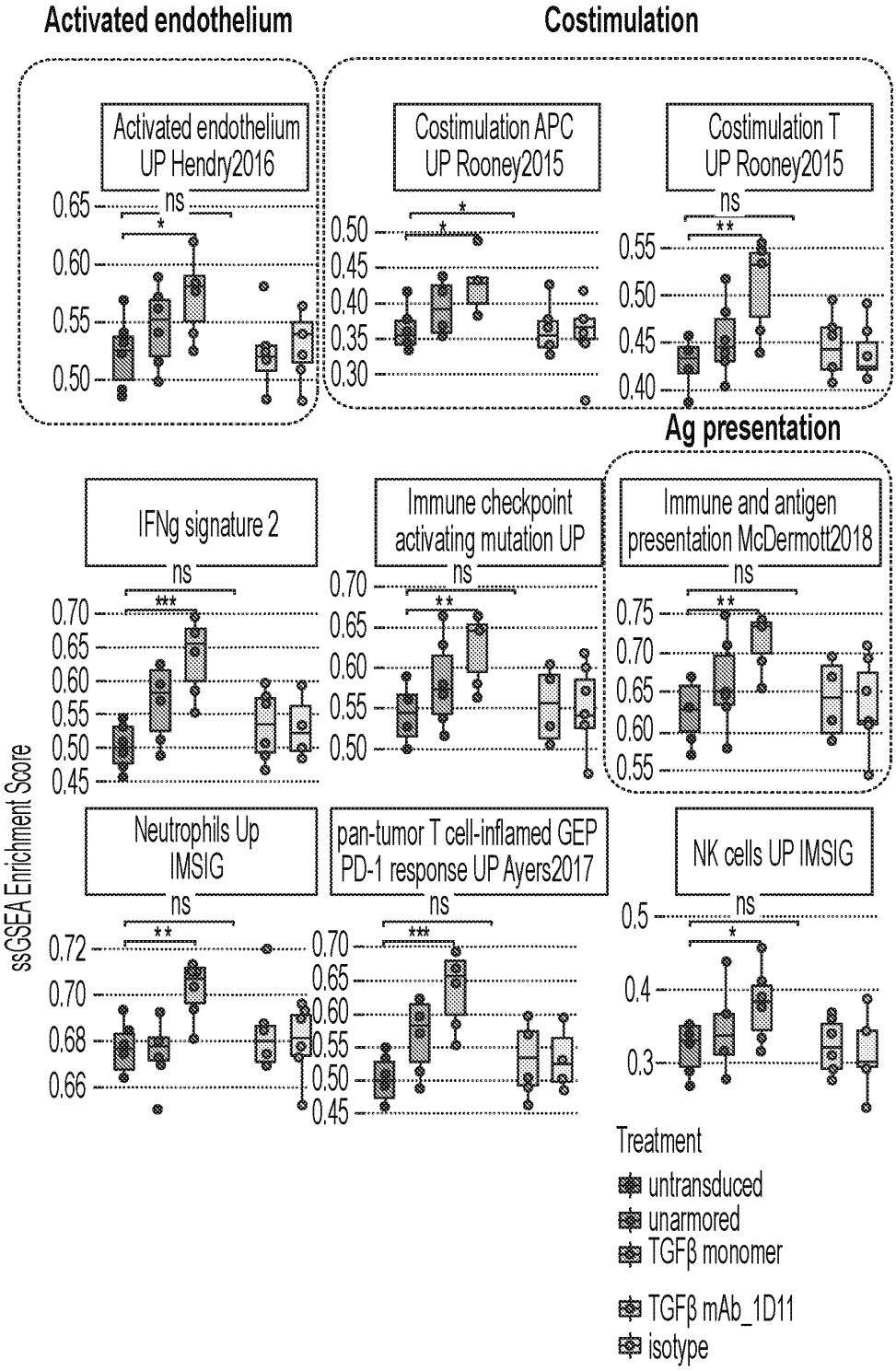

Activated endothelium
Costimulation
Activated endothelium UP Hendry2016
Costimulation APC UP Rooney2015
Costimulation T UP Rooney2015
Ag presentation
IFNg signature 2
Immune checkpoint activating mutation UP
Immune and antigen presentation McDermott2018
Neutrophils Up IMSIG
pan-tumor T cell-inflamed GEP PD-1 response UP Ayers2017
NK cells UP IMSIG
ssGSEA Enrichment Score
ns
*
**
***
Treatment
untransduced
unarmored
TGFβ monomer
TGFβ mAb_1D11
isotype

FIG. 14

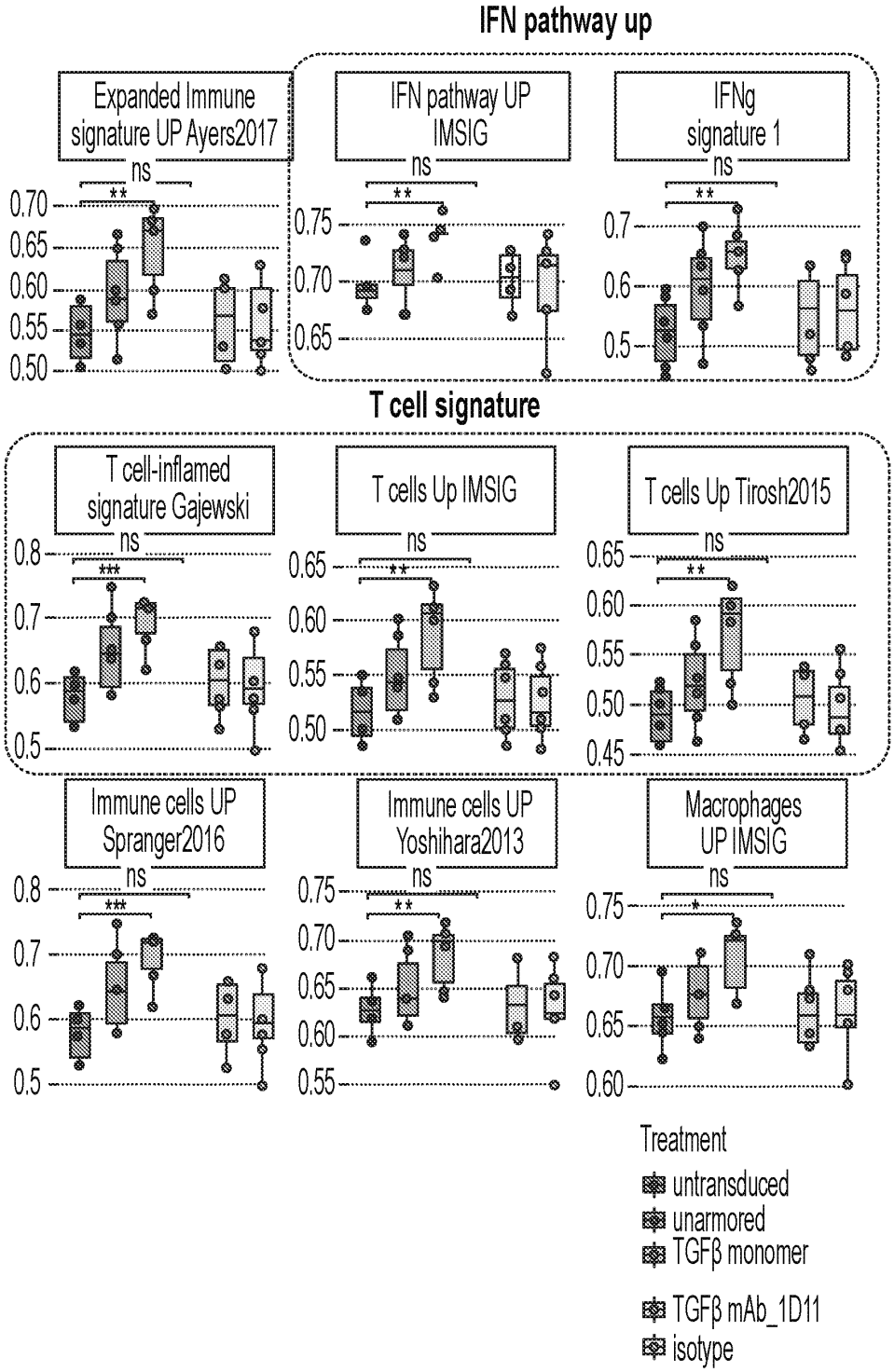


FIG. 14
CONTINUED

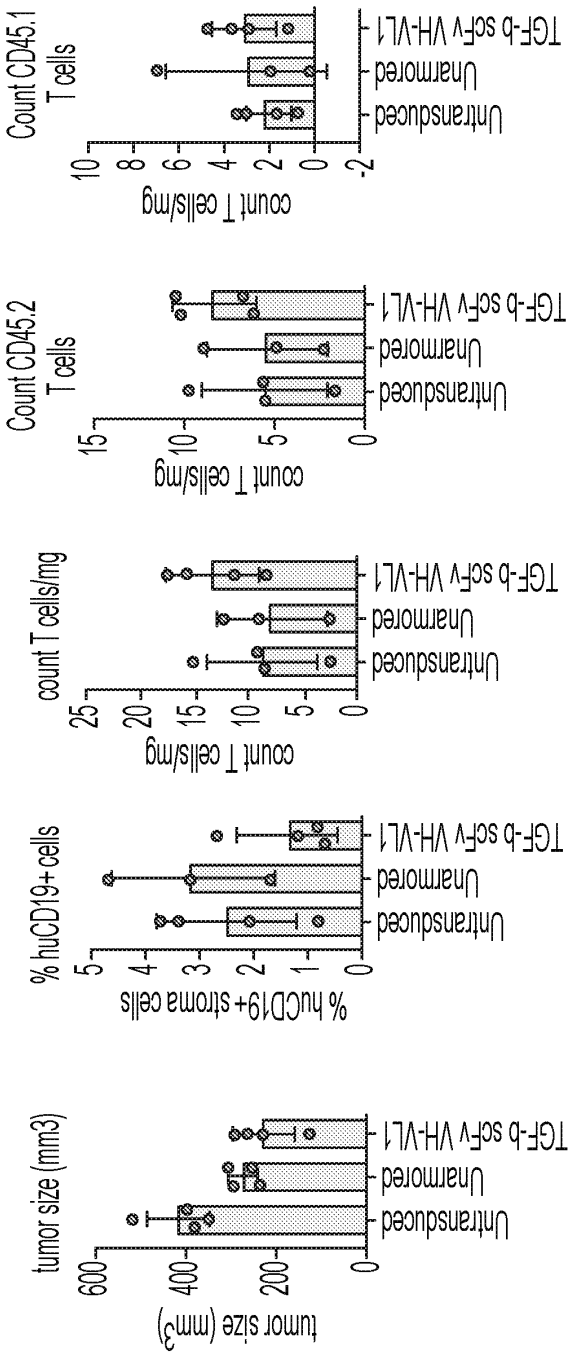

tumor size (mm3)
tumor size (mm³)
600
400
200
0
Untransduced
Unarmored
TGF-b scFv VH-VL1
% huCD19+ cells
% huCD19+ stroma cells
5
4
3
2
1
0
count T cells/mg
count T cells/mg
25
20
15
10
5
0
Count CD45.2 T cells
count T cells/mg
15
10
5
0
Count CD45.1 T cells
count T cells/mg
10
8
6
4
2
0
-2

FIG. 15

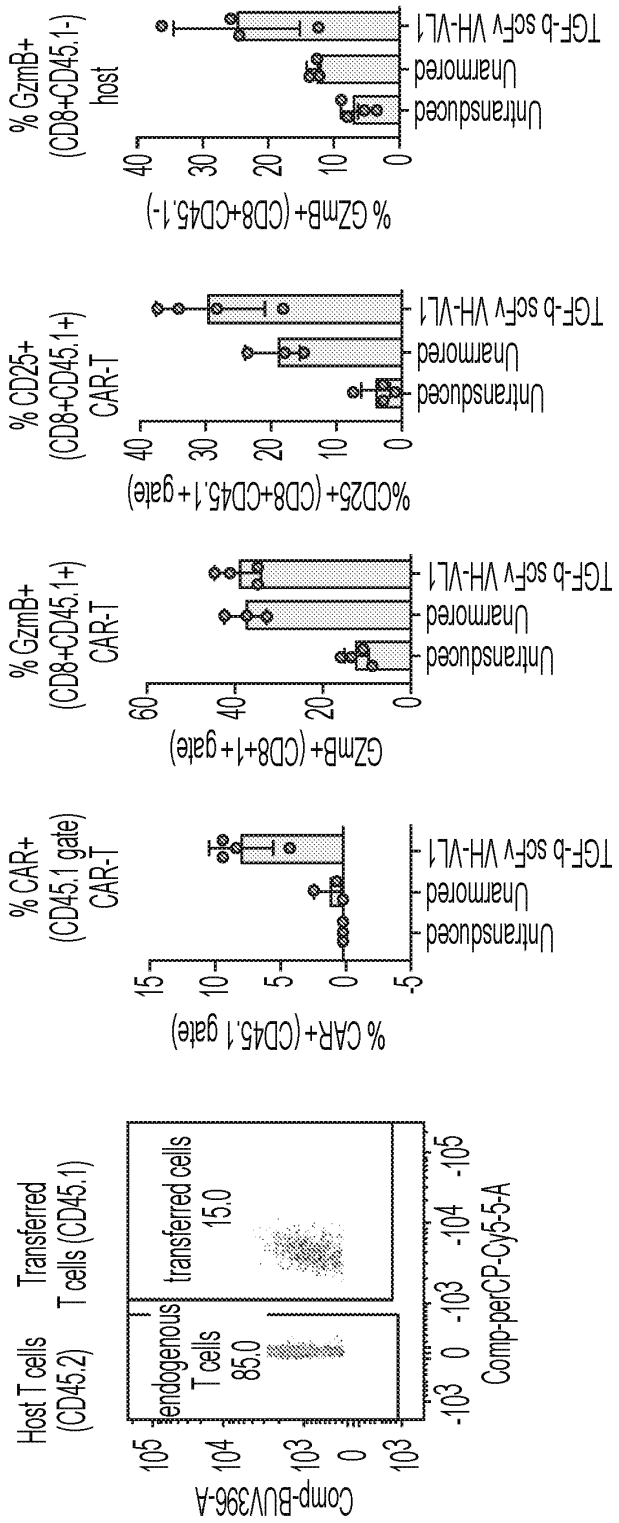


FIG. 15
CONTINUED

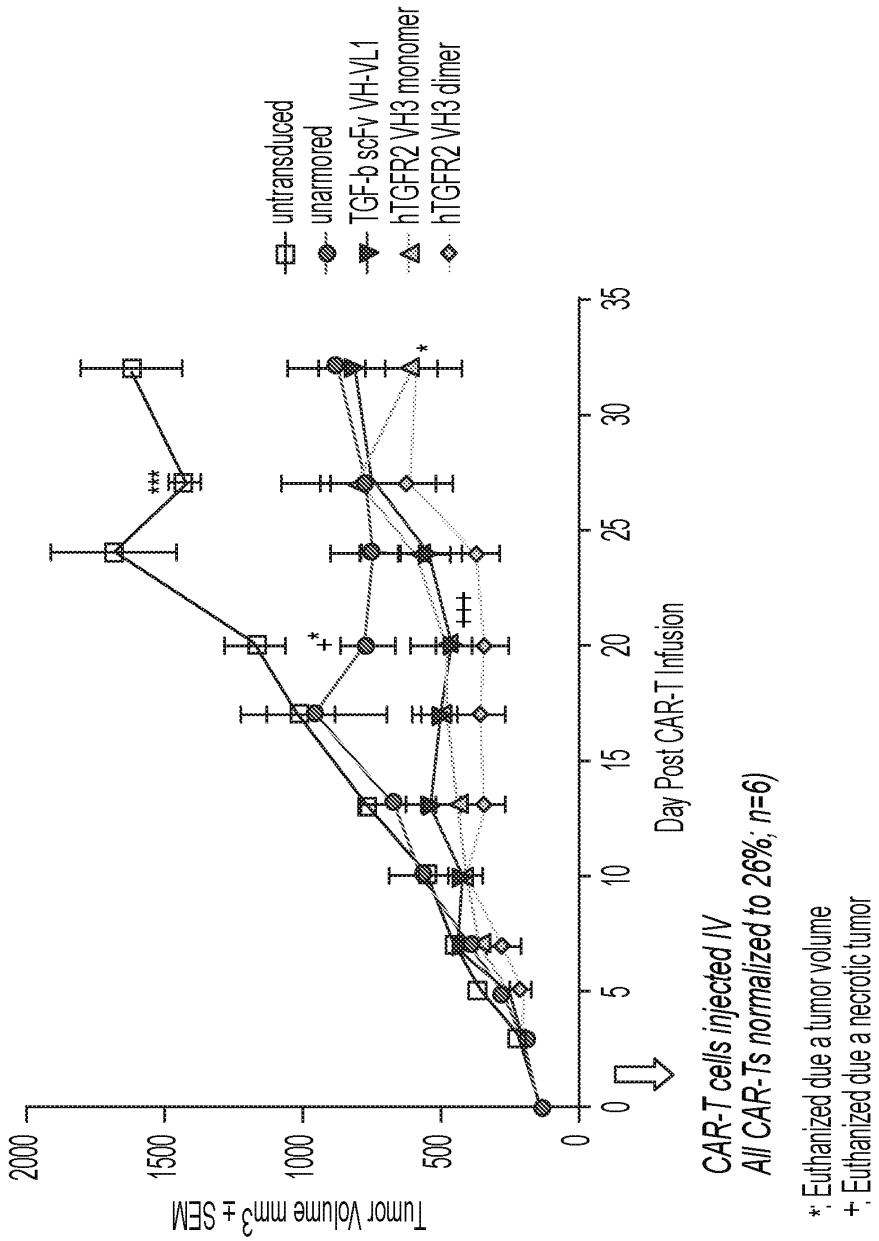

Tumor Volume mm3 ± SEM
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
Day Post CAR-T Infusion
untransduced
unarmored
TGF-b scFv VH-VL1
hTGFR2 VH3 monomer
hTGFR2 VH3 dimer
CAR-T cells injected IV
All CAR-Ts normalized to 26%; n=6)
*: Euthanized due a tumor volume
+: Euthanized due a necrotic tumor

FIG. 16A

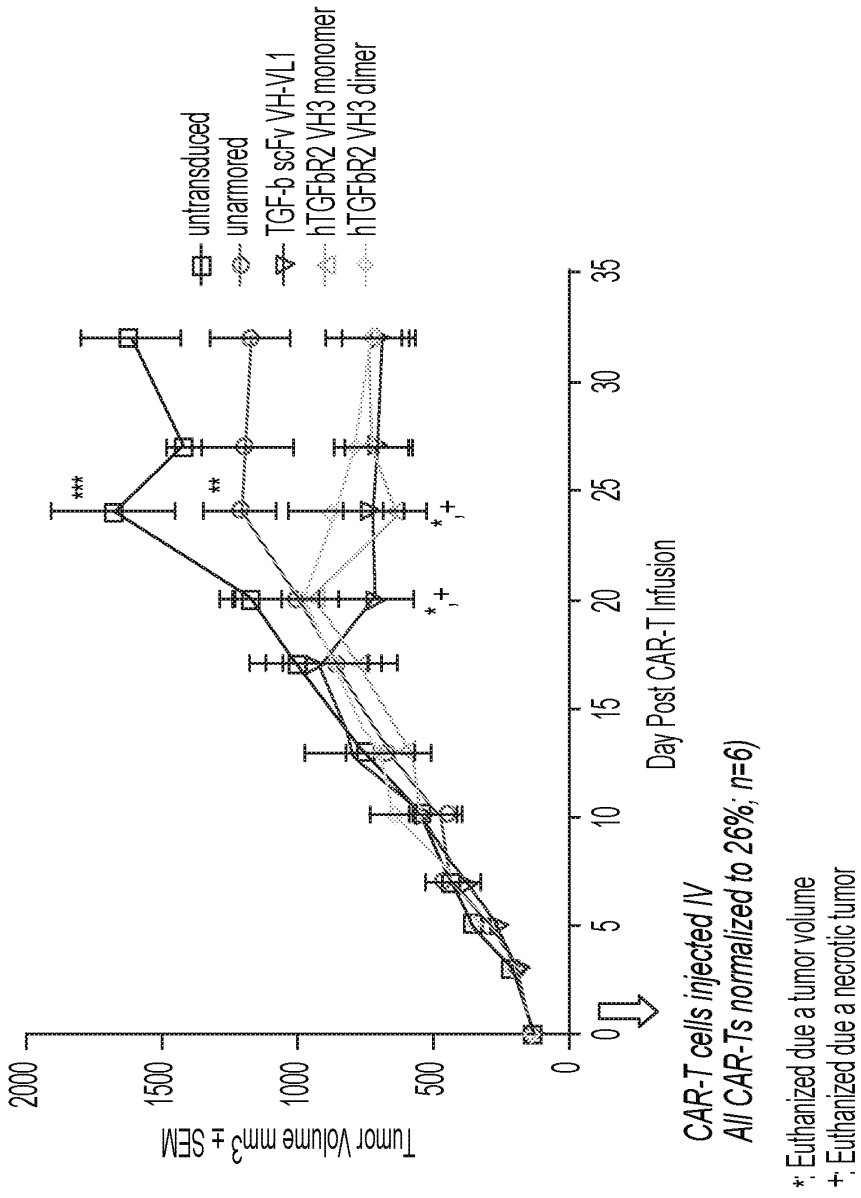

untransduced
unarmored
TGF-b scFv VH-VL1
hTGFbR2 VH3 monomer
hTGFbR2 VH3 dimer
Tumor Volume mm3 ± SEM
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
Day Post CAR-T Infusion
CAR-T cells injected IV
All CAR-Ts normalized to 26%; n=6)
*: Euthanized due a tumor volume
+: Euthanized due a necrotic tumor

FIG. 16B

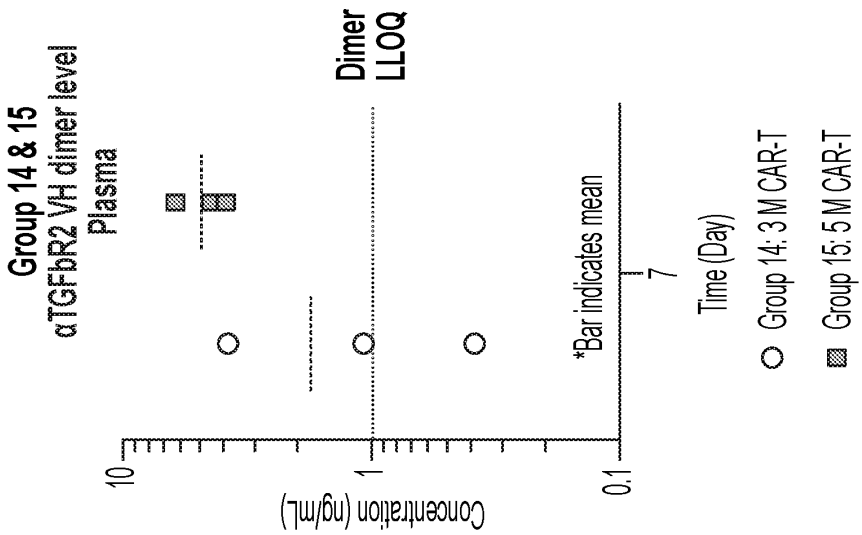


FIG. 17B

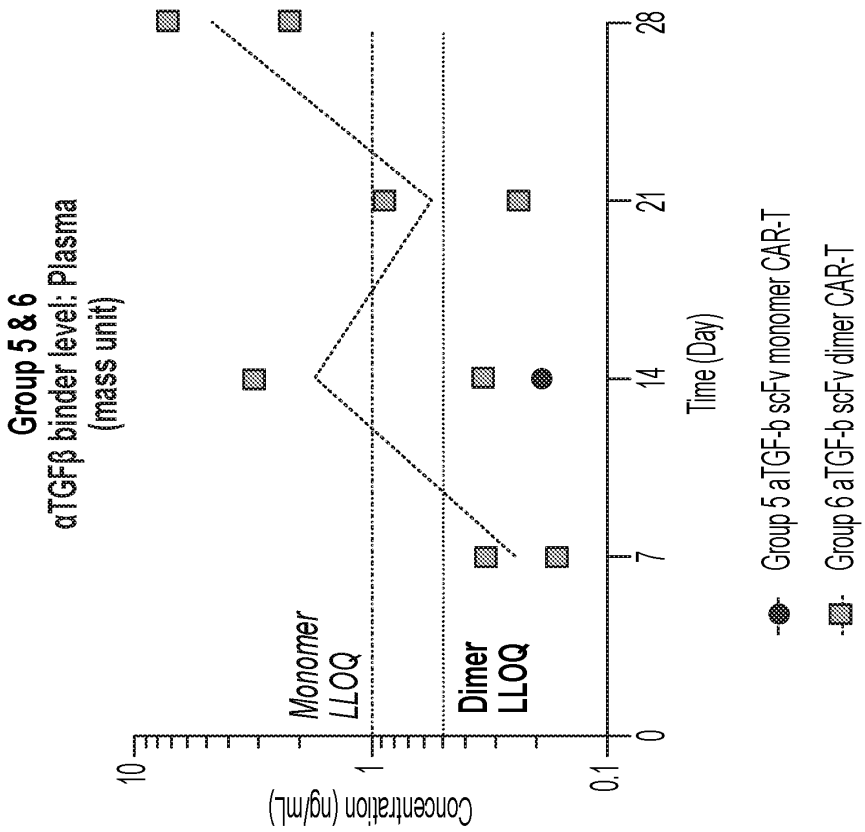


FIG. 17A

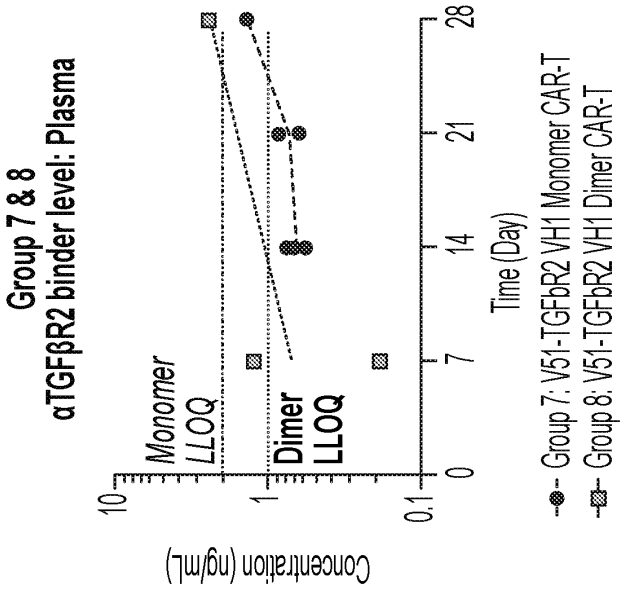


FIG. 17C

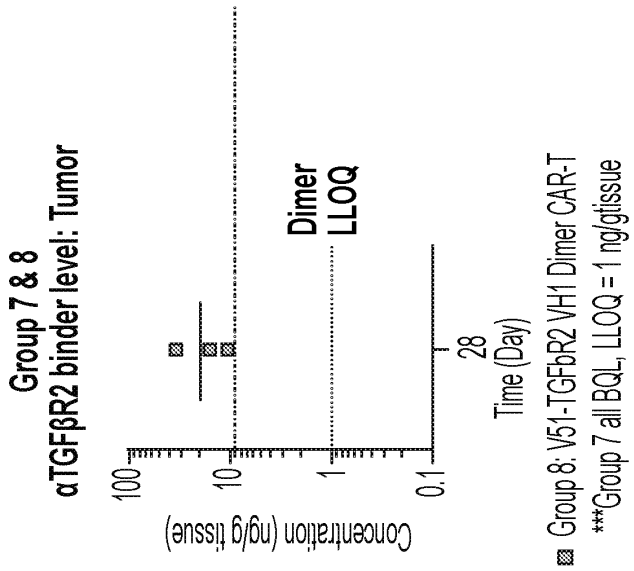


FIG. 17D

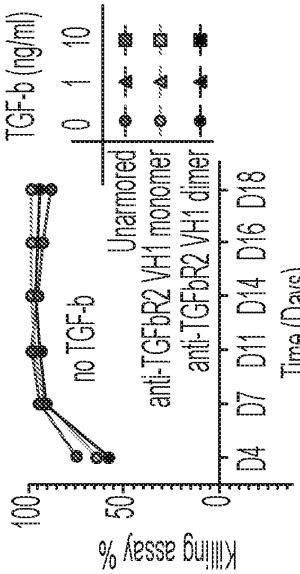


FIG. 18A

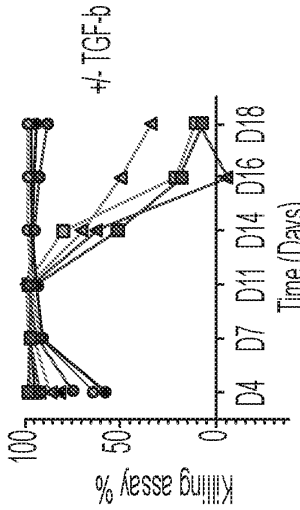


FIG. 18B

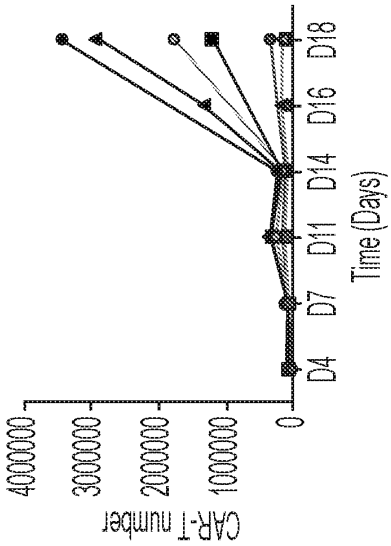


FIG. 18C

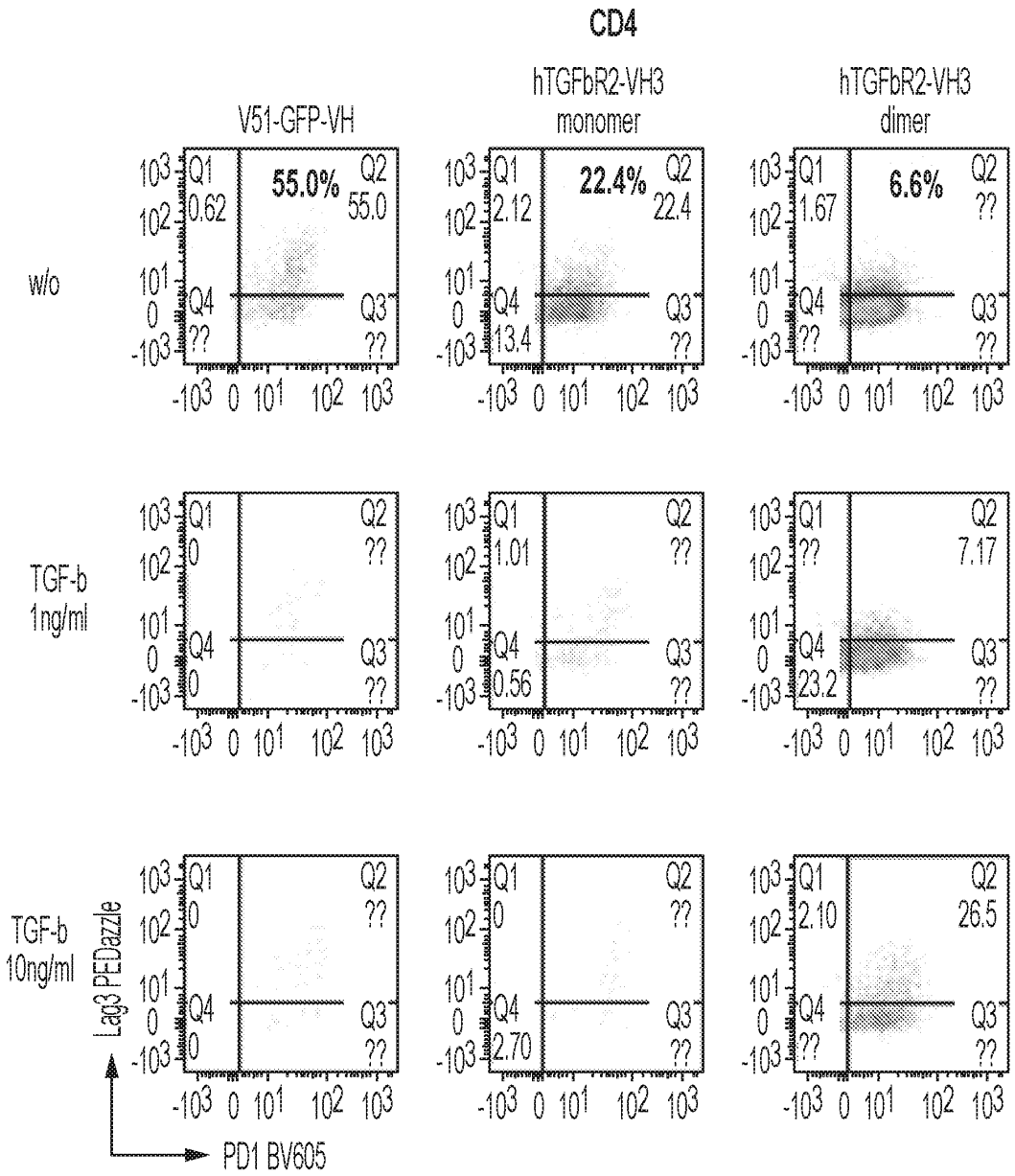

CD4
V51-GFP-VH
hTGFbR2-VH3 monomer
hTGFbR2-VH3 dimer
w/o
TGF-b 1ng/ml
TGF-b 10ng/ml
Q1 0.62
55.0%
Q2 55.0
Q4 ??
Q3 ??
Q1 2.12
22.4%
Q2 22.4
Q4 13.4
Q3 ??
Q1 1.67
6.6%
Q2 ??
Q4 ??
Q3 ??
Q1 0
Q2 ??
Q4 0
Q3 ??
Q1 1.01
Q2 ??
Q4 0.56
Q3 ??
Q1 ??
Q2 7.17
Q4 23.2
Q3 ??
Q1 0
Q2 ??
Q4 0
Q3 ??
Q1 0
Q2 ??
Q4 2.70
Q3 ??
Q1 2.10
Q2 26.5
Q4 ??
Q3 ??
Lag3 PEDazzle
PD1 BV605

FIG. 19

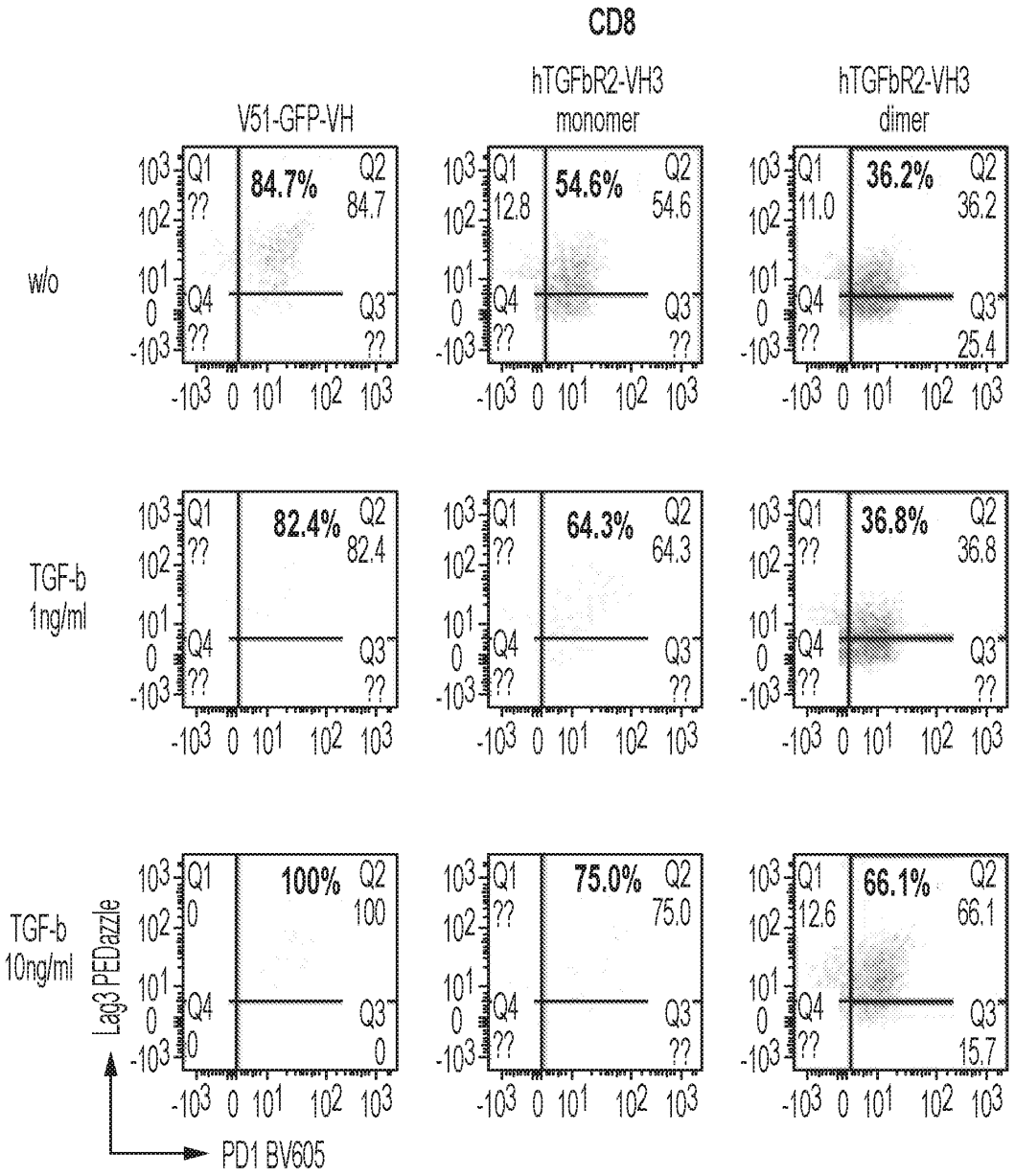


FIG. 19
CONTINUED

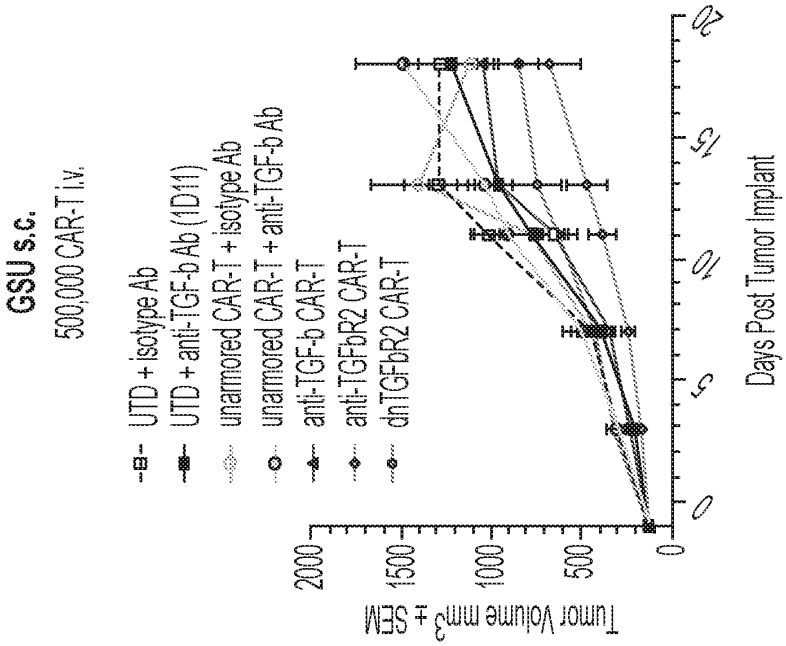


FIG. 20A

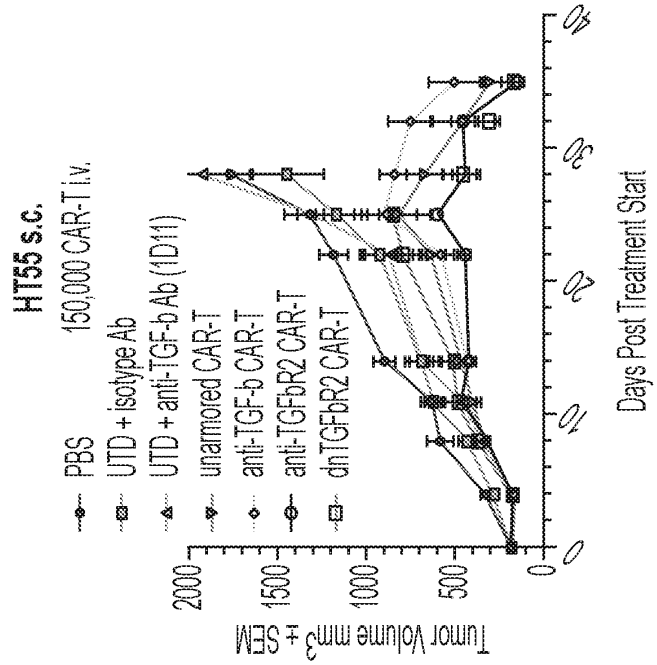


FIG. 20B

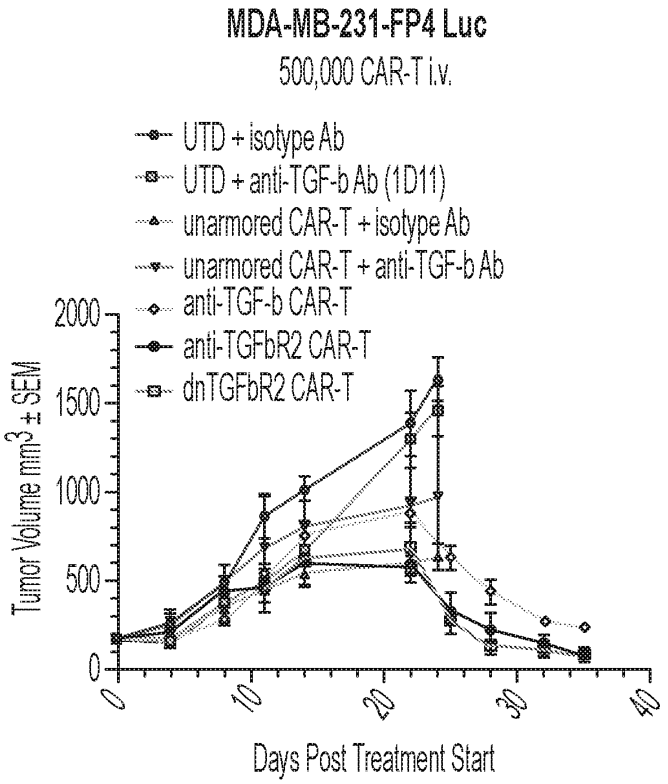

MDA-MB-231-FP4 Luc
500,000 CAR-T i.v.
UTD + isotype Ab
UTD + anti-TGF-b Ab (1D11)
unarmored CAR-T + isotype Ab
unarmored CAR-T + anti-TGF-b Ab
anti-TGF-b CAR-T
anti-TGFbR2 CAR-T
dnTGFbR2 CAR-T
Tumor Volume mm3 ± SEM
2000
1500
1000
500
0
0
10
20
30
40
Days Post Treatment Start

FIG. 20C

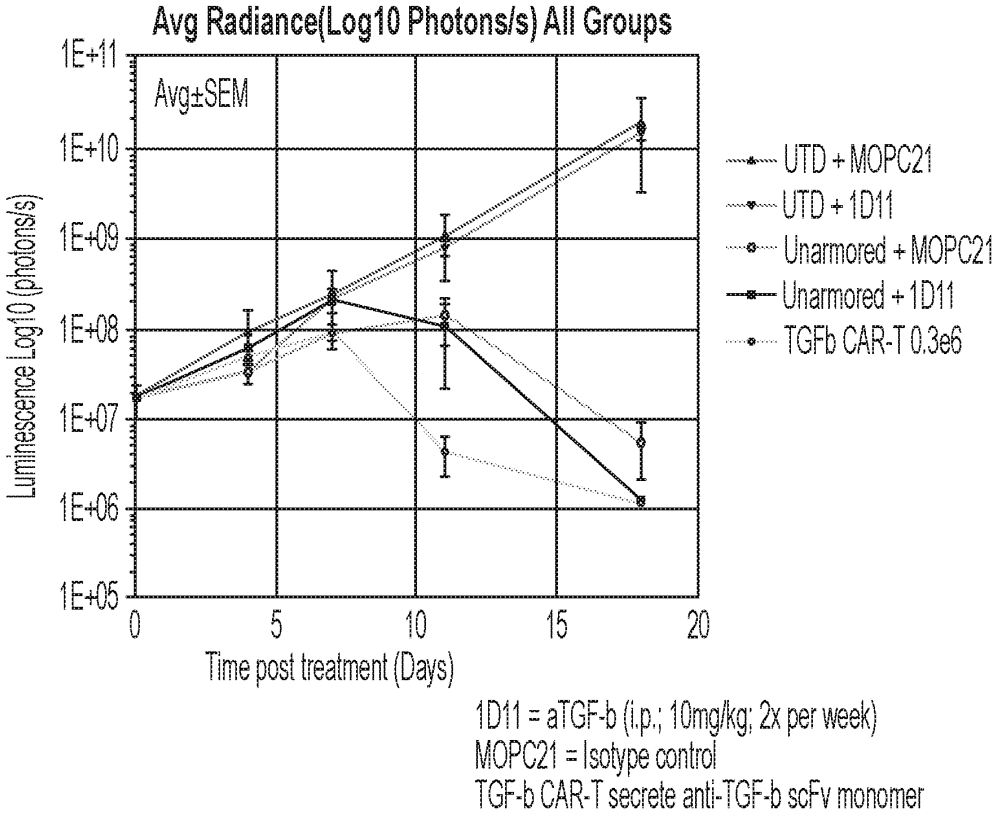


FIG. 21A

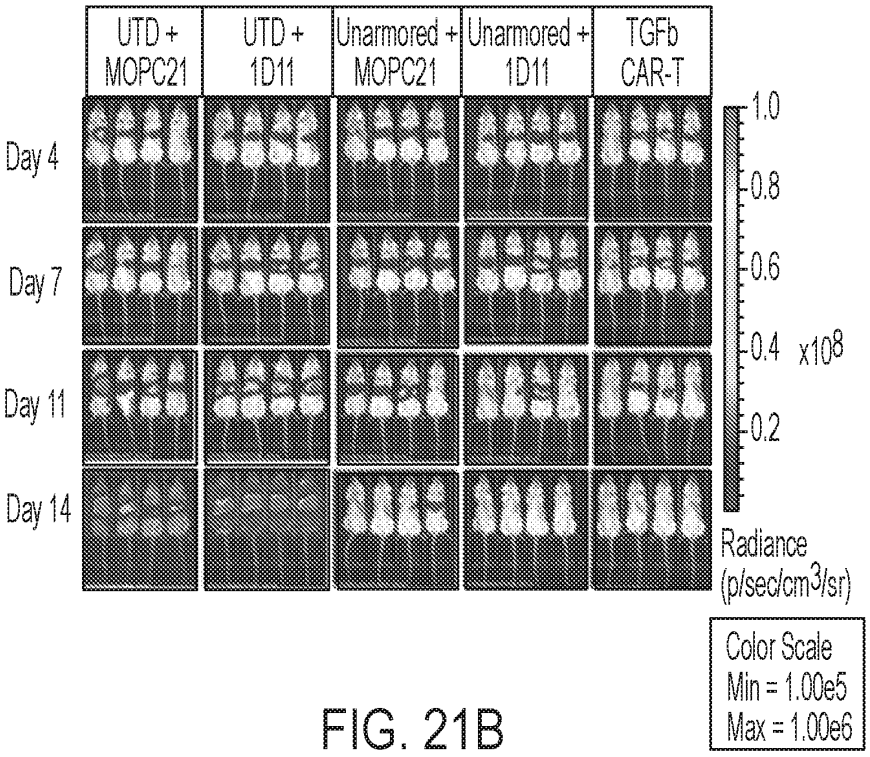


FIG. 21B

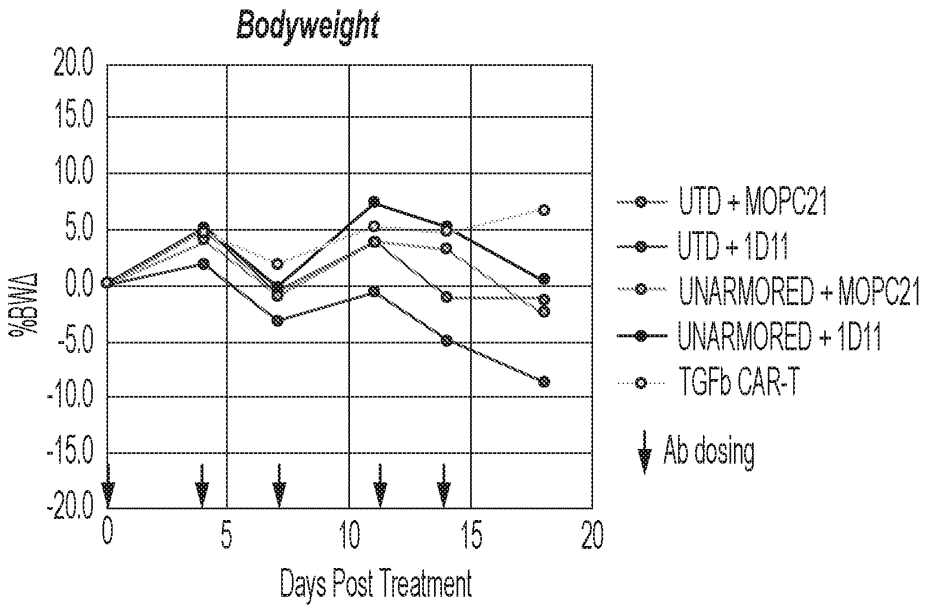

Bodyweight
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
-20.0
%BWΔ
0
5
10
15
20
Days Post Treatment
UTD + MOPC21
UTD + 1D11
UNARMORED + MOPC21
UNARMORED + 1D11
TGFb CAR-T
Ab dosing

FIG. 21C

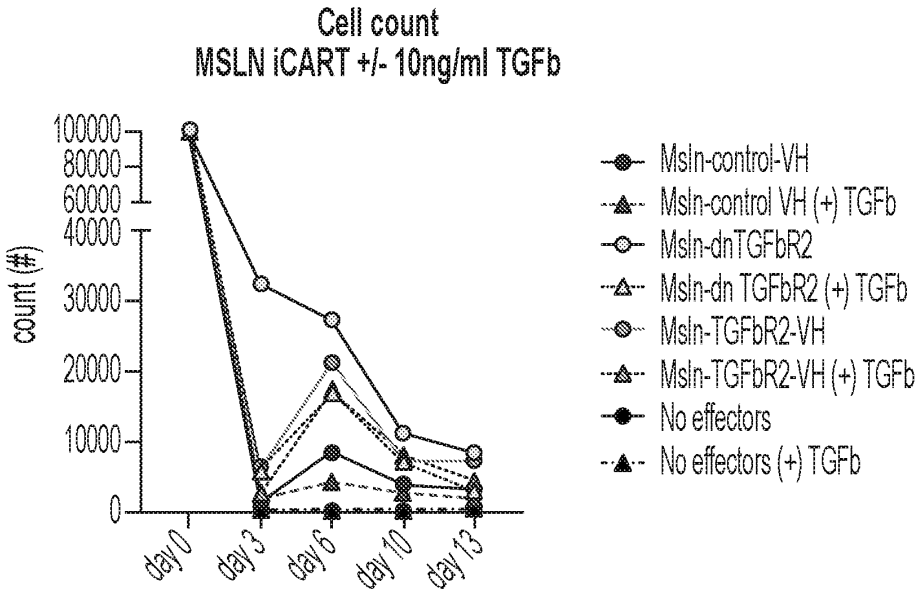


FIG. 22A

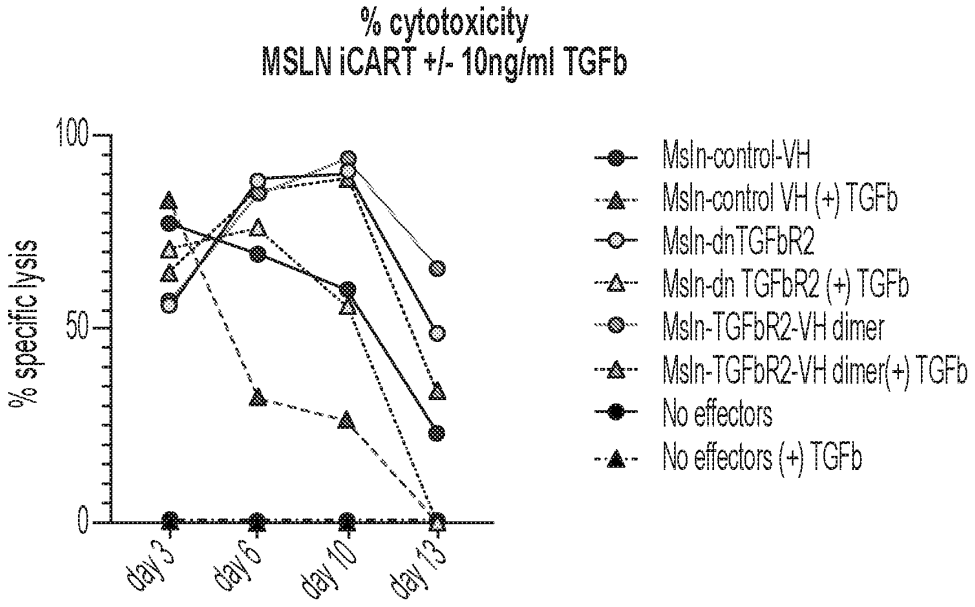


FIG. 22B