

Fig. 1A

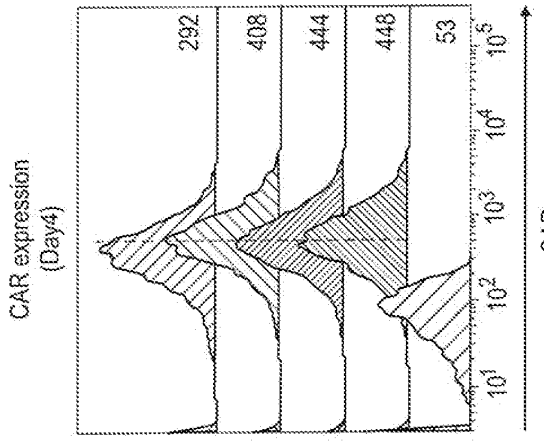


Fig. 1C

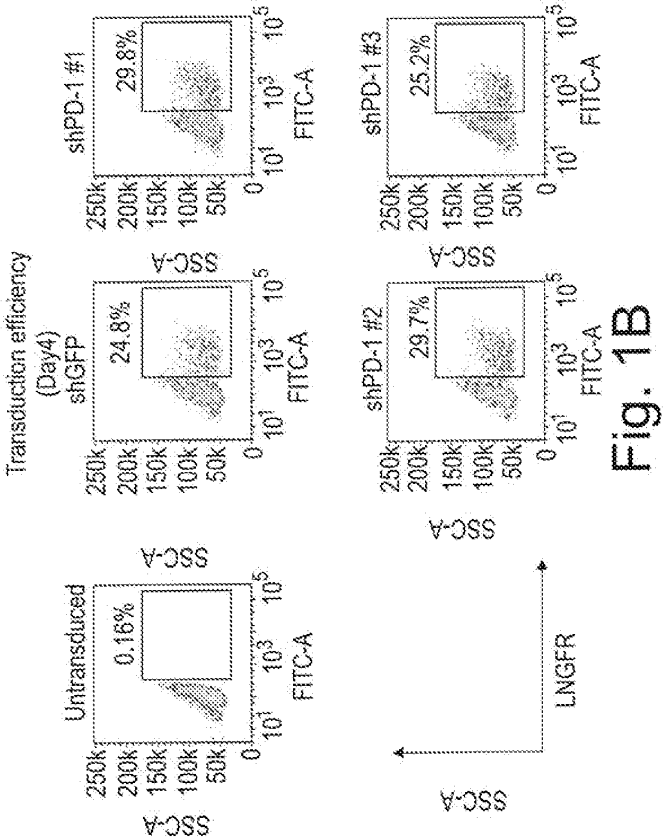


Fig. 1B

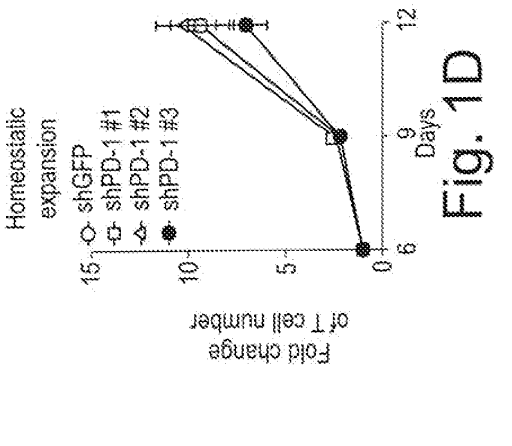


Fig. 1D

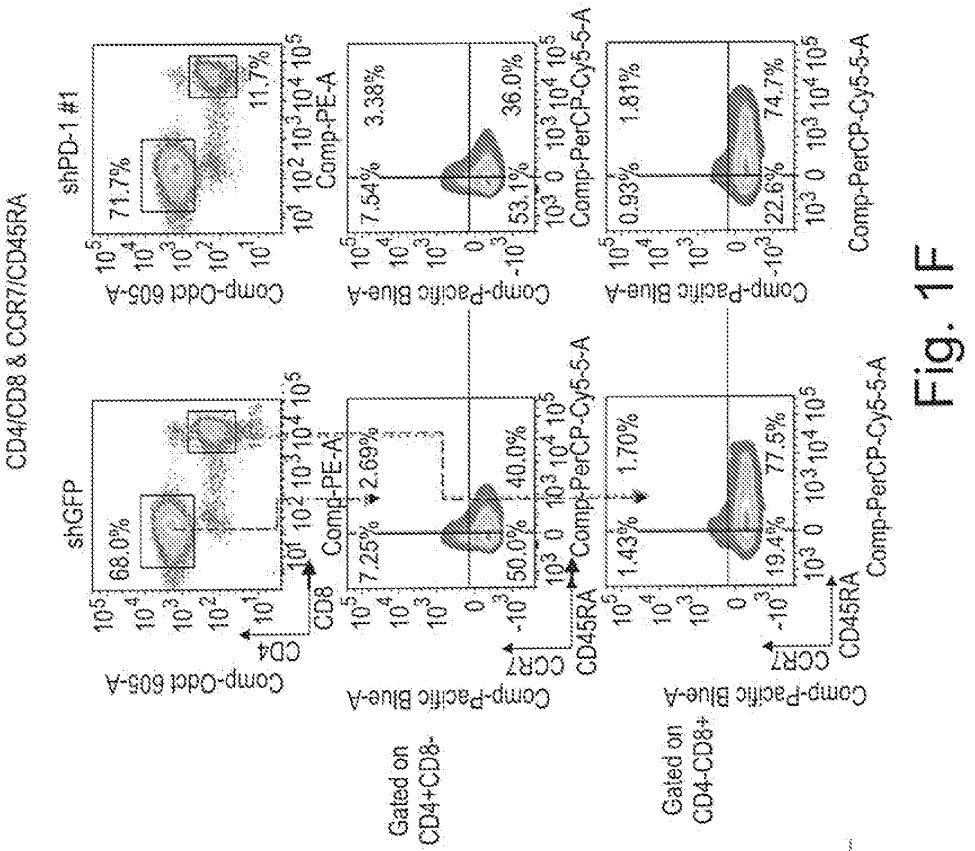


Fig. 1F

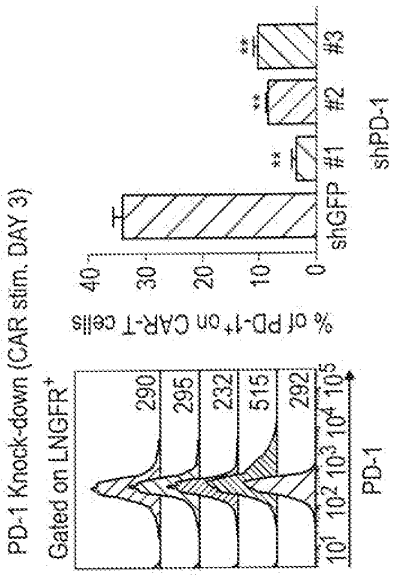


Fig. 1E

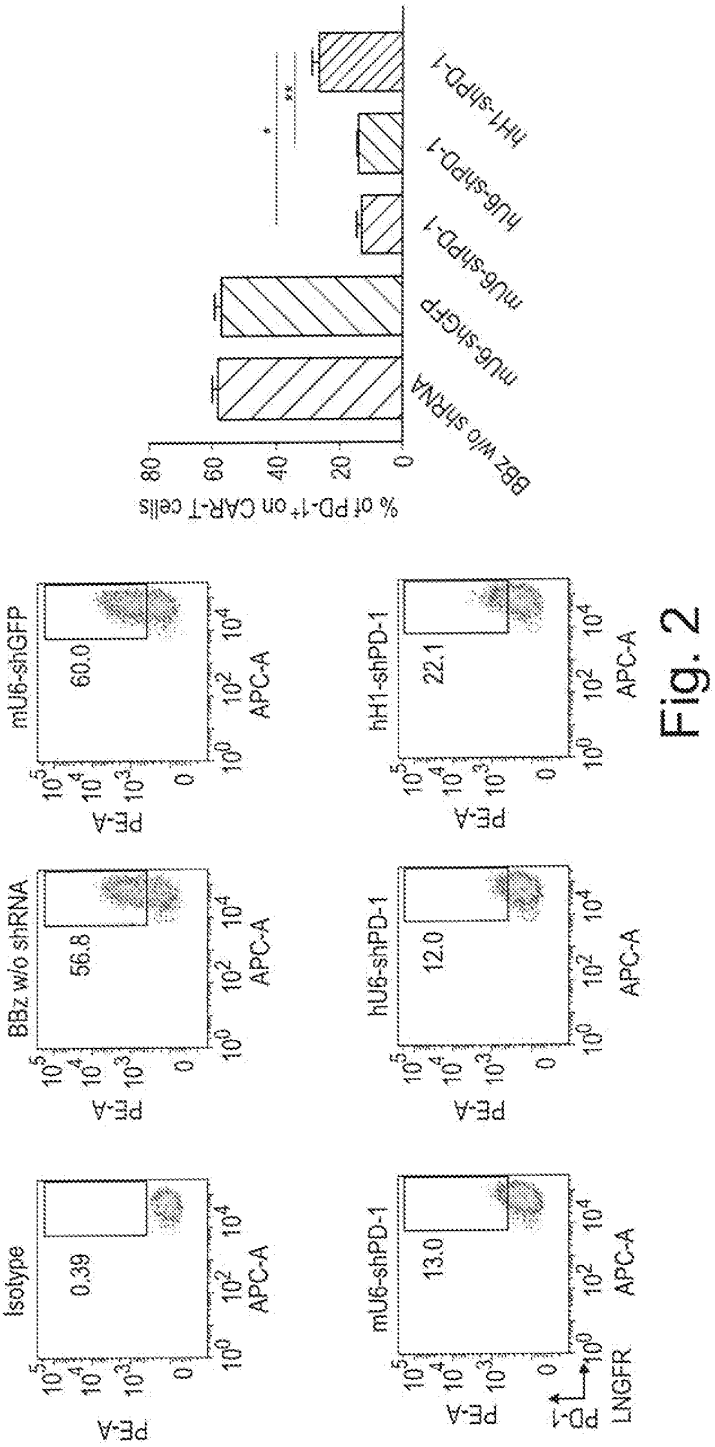

% of PD-1+ on CAR-T cells
0
20
40
60
80
BBz w/o shRNA
mU6-shGFP
mU6-shPD-1
hU6-shPD-1
hH1-shPD-1
*
**
Isotype
0.39
BBz w/o shRNA
56.8
mU6-shGFP
60.0
hU6-shPD-1
12.0
hH1-shPD-1
22.1
mU6-shPD-1
13.0
PE-A
APC-A
PD-1
LNGFR

Fig. 2

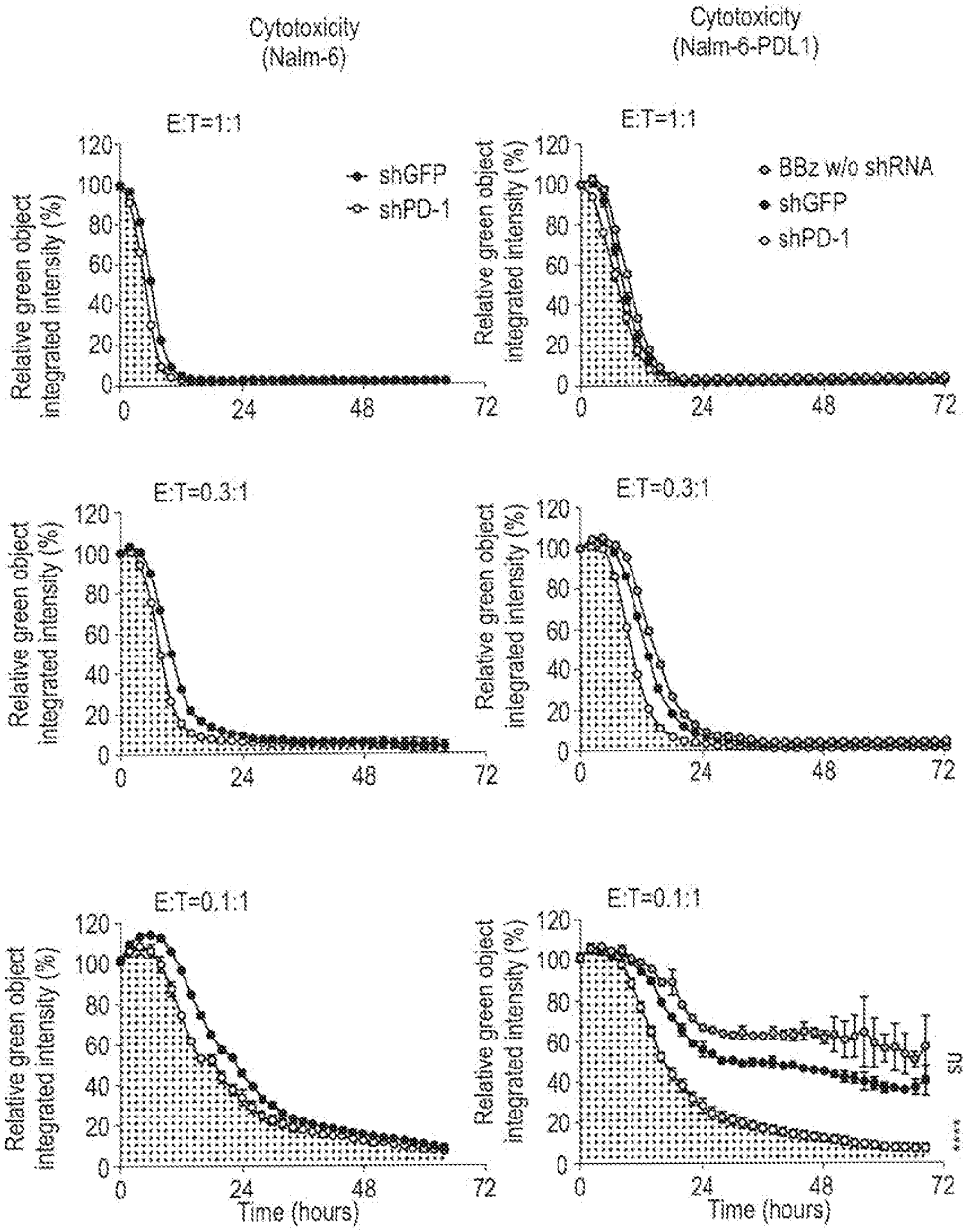

Cytotoxicity
(Nalm-6)
Cytotoxicity
(Nalm-6-PDL1)
E:T=1:1
E:T=0.3:1
E:T=0.1:1
Relative green object integrated intensity (%)
shGFP
shPD-1
BBz w/o shRNA
ns
****
Time (hours)

Fig. 3A

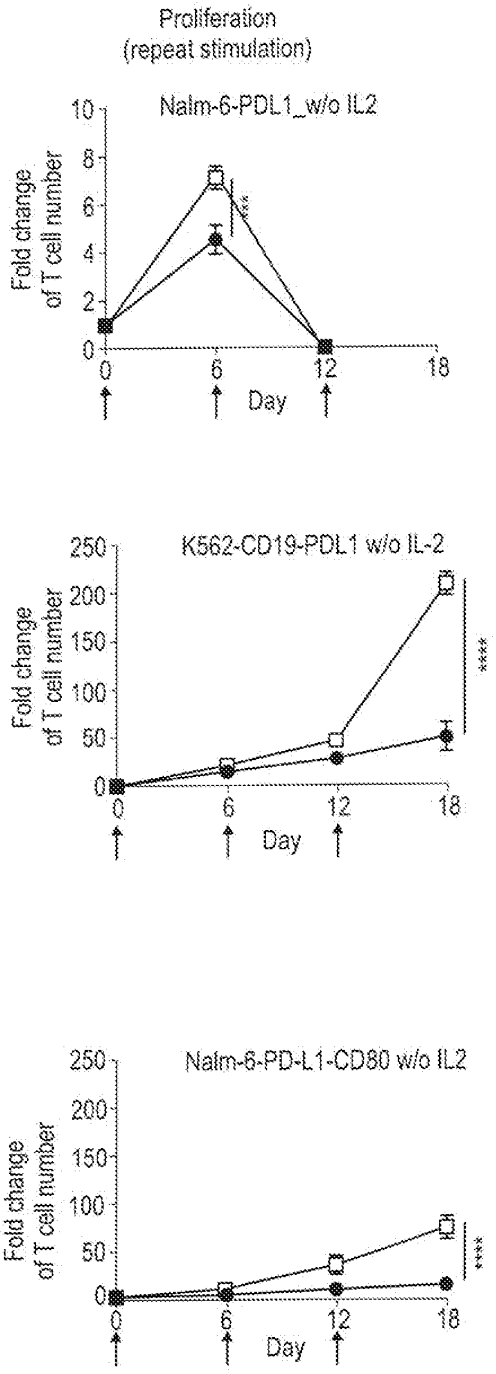

Proliferation
(repeat stimulation)
Nalm-6-PDL1_w/o IL2
Fold change of T cell number
Day
K562-CD19-PDL1 w/o IL-2
Nalm-6-PD-L1-CD80 w/o IL2

Fig. 3B

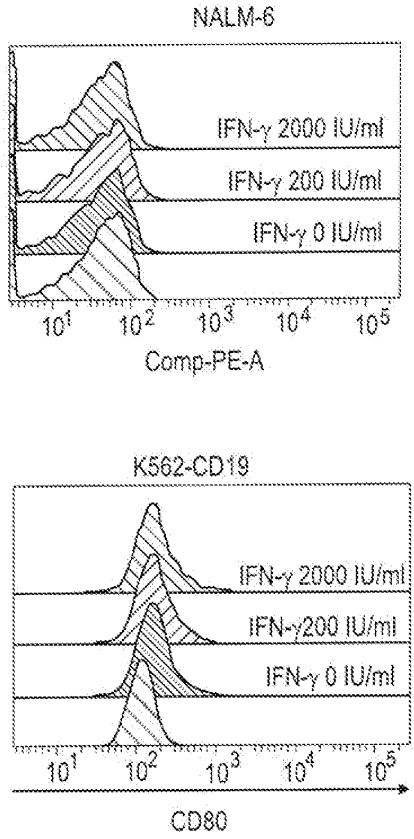

NALM-6
IFN-γ 2000 IU/ml
IFN-γ 200 IU/ml
IFN-γ 0 IU/ml
Comp-PE-A
K562-CD19
IFN-γ 2000 IU/ml
IFN-γ200 IU/ml
IFN-γ 0 IU/ml
CD80

Fig. 3C

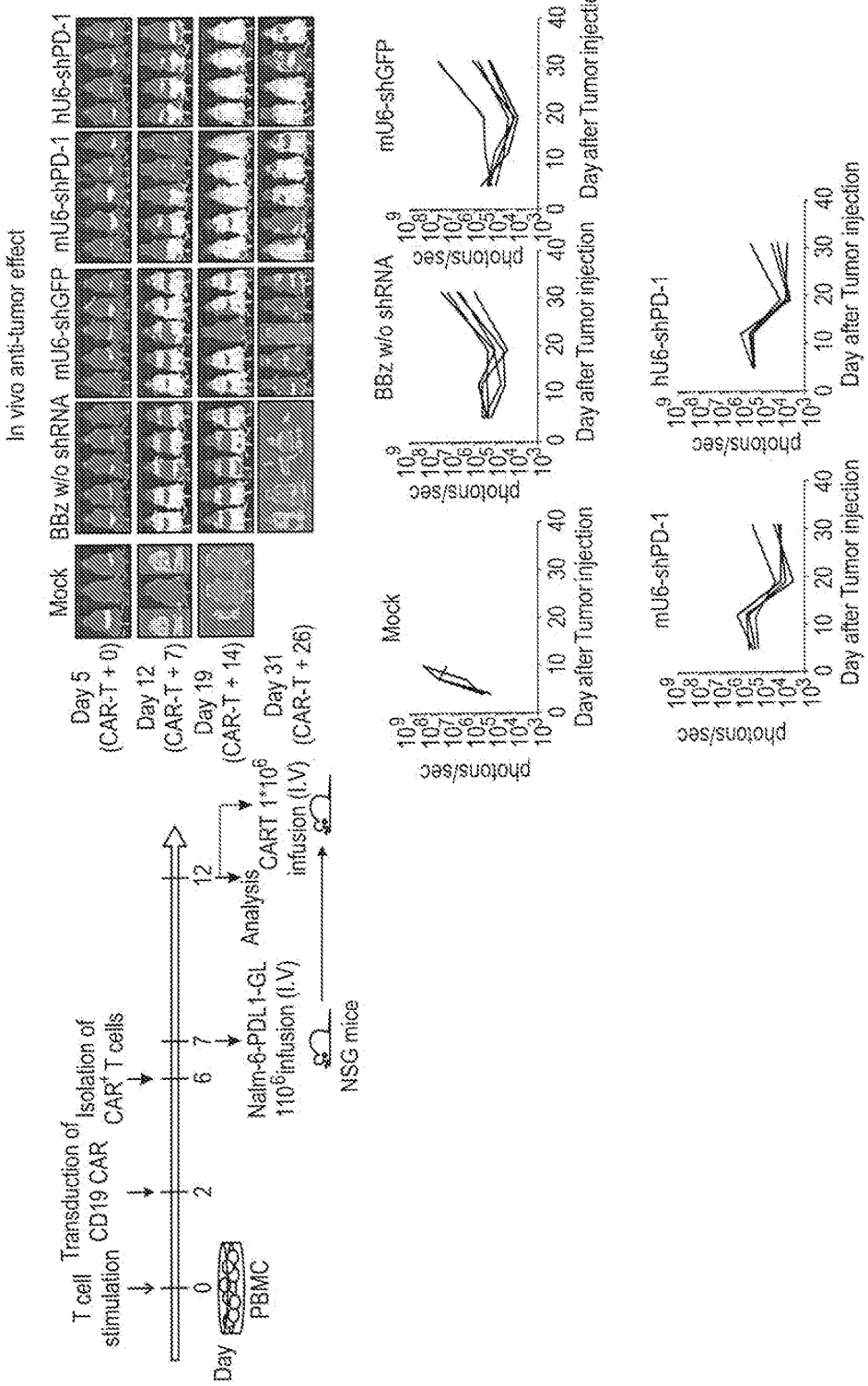

In vivo anti-tumor effect
Mock
BBz w/o shRNA
mU6-shGFP
mU6-shPD-1
hU6-shPD-1
Day 5 (CAR-T + 0)
Day 12 (CAR-T + 7)
Day 19 (CAR-T + 14)
Day 31 (CAR-T + 26)
T cell stimulation
Transduction of CD19 CAR
Isolation of CAR+ T cells
PBMC
Nalm-6-PDL1-GL 1*10^6 infusion (I.V)
Analysis
CART 1*10^6 infusion (I.V)
NSG mice
Day
photons/sec
Day after Tumor injection

Fig. 4

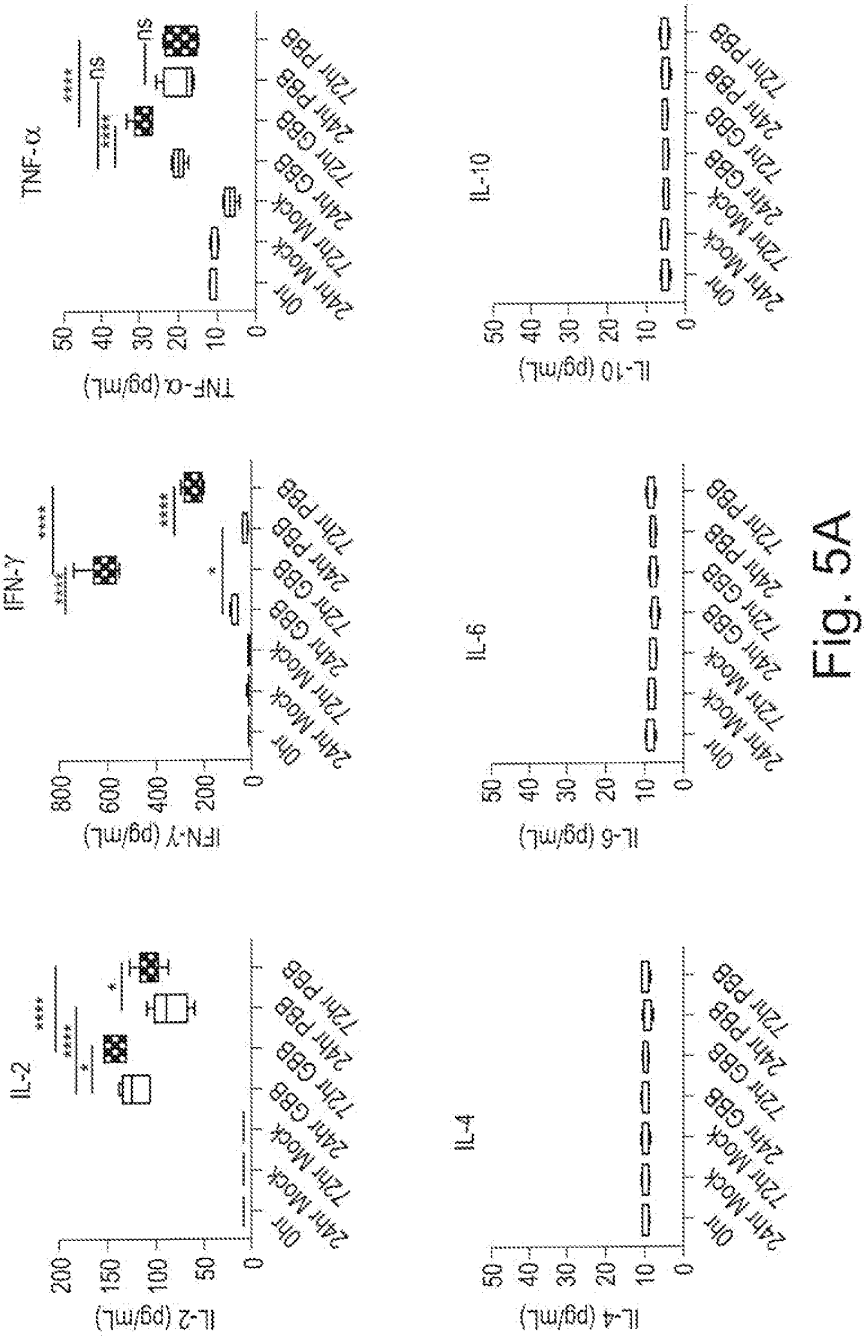

TNF-α
TNF-α (pg/mL)
IL-10
IL-10 (pg/mL)
IFN-γ
IFN-γ (pg/mL)
IL-6
IL-6 (pg/mL)
IL-2
IL-2 (pg/mL)
IL-4
IL-4 (pg/mL)
0hr
2ahr Mock
12hr Mock
24hr Mock
12hr GBB
24hr GBB
12hr PBB
24hr PBB
ns
****
*

Fig. 5A

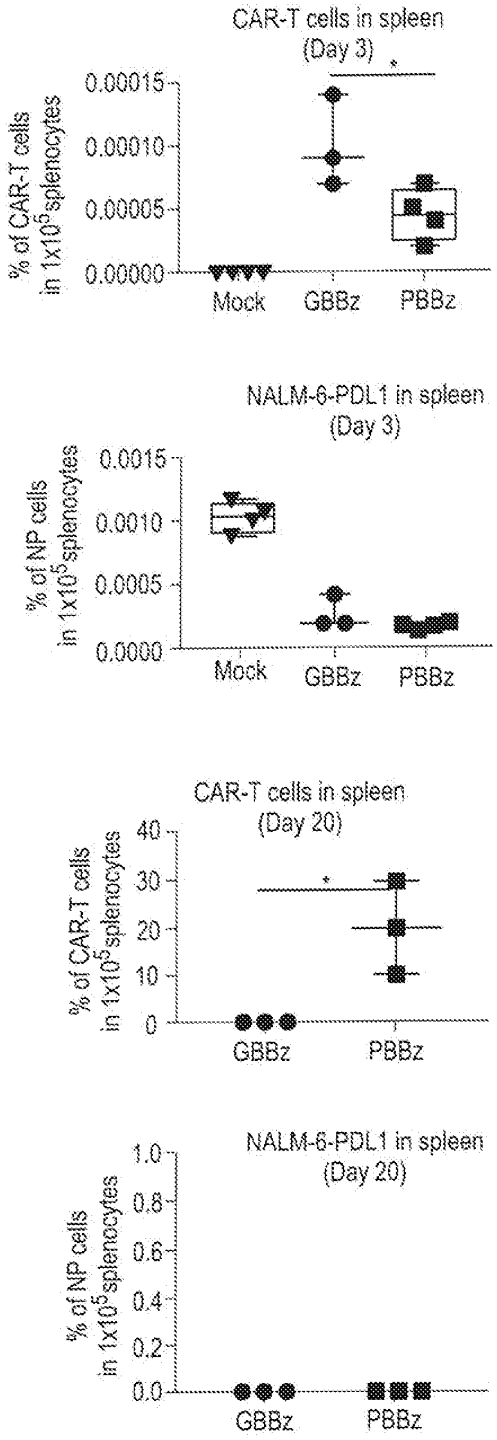

CAR-T cells in spleen
(Day 3)
% of CAR-T cells
in 1x10^5 splenocytes
0.00015
0.00010
0.00005
0.00000
Mock
GBBz
PBBz
NALM-6-PDL1 in spleen
(Day 3)
% of NP cells
in 1x10^5 splenocytes
0.0015
0.0010
0.0005
0.0000
Mock
GBBz
PBBz
CAR-T cells in spleen
(Day 20)
% of CAR-T cells
in 1x10^5 splenocytes
40
30
20
10
0
GBBz
PBBz
NALM-6-PDL1 in spleen
(Day 20)
% of NP cells
in 1x10^5 splenocytes
1.0
0.8
0.6
0.4
0.2
0.0
GBBz
PBBz

Fig. 5B

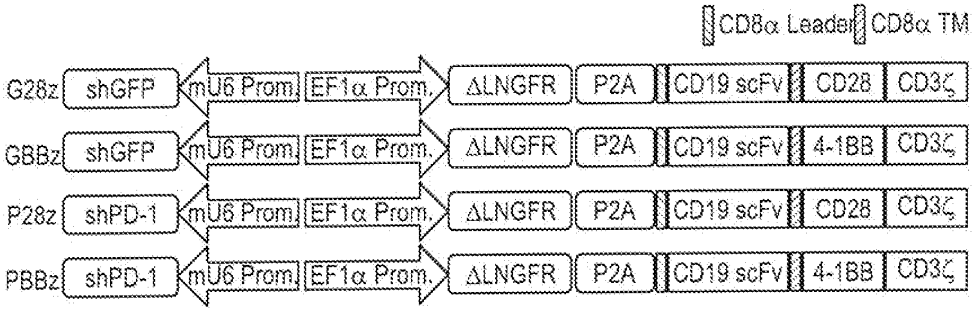

CD8α Leader
CD8α TM
G28z
shGFP
mU6 Prom.
EF1α Prom.
ΔLNGFR
P2A
CD19 scFv
CD28
CD3ζ
GBBz
shGFP
mU6 Prom.
EF1α Prom.
ΔLNGFR
P2A
CD19 scFv
4-1BB
CD3ζ
P28z
shPD-1
mU6 Prom.
EF1α Prom.
ΔLNGFR
P2A
CD19 scFv
CD28
CD3ζ
PBBz
shPD-1
mU6 Prom.
EF1α Prom.
ΔLNGFR
P2A
CD19 scFv
4-1BB
CD3ζ

Fig. 6A

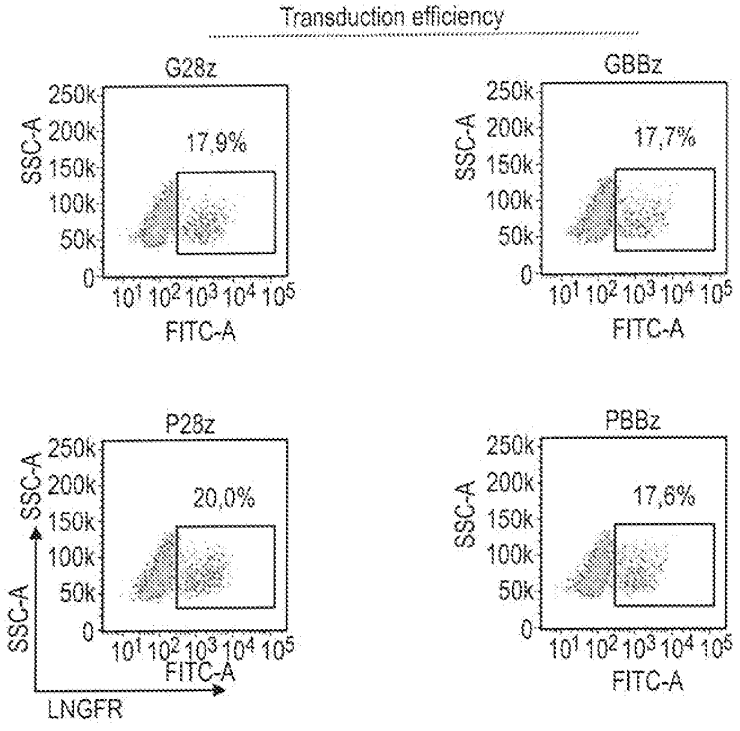

Transduction efficiency
G28z
17,9%
GBBz
17,7%
P28z
20,0%
PBBz
17,6%
SSC-A
FITC-A
LNGFR
250k
200k
150k
100k
50k
0
10^1 10^2 10^3 10^4 10^5

Fig. 6B

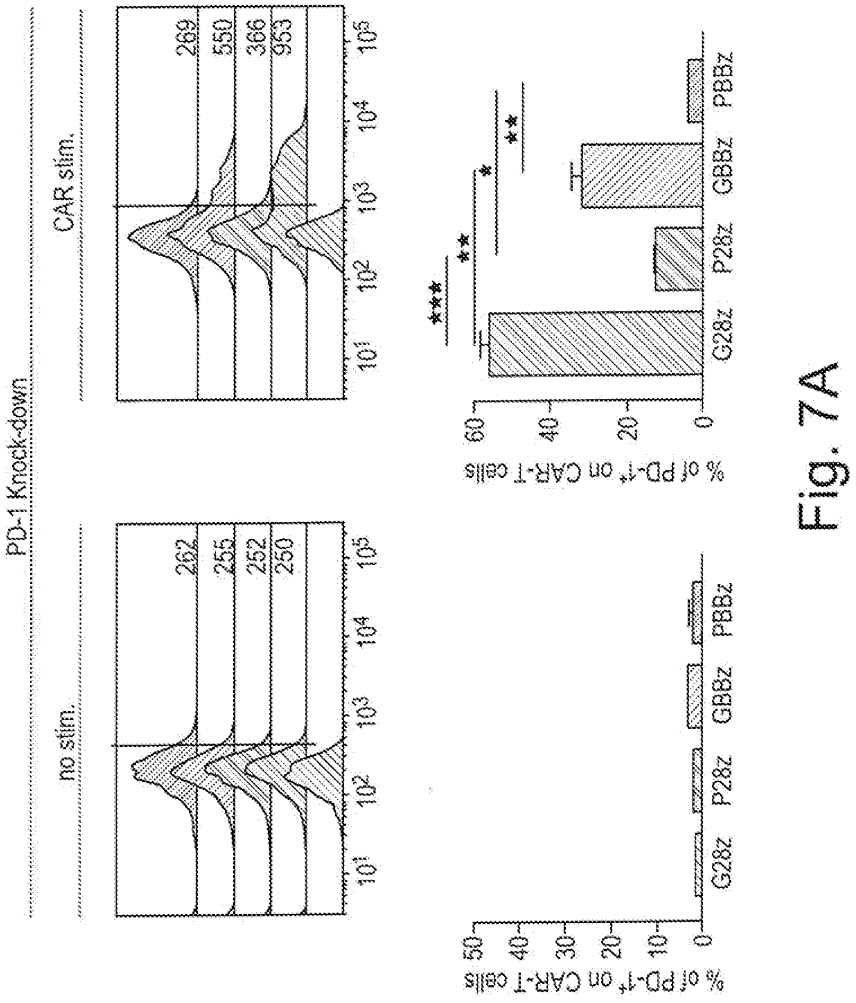

PD-1 Knock-down
no stim.
CAR stim.
262
255
252
250
269
550
366
953
10^1
10^2
10^3
10^4
10^5
% of PD-1+ on CAR-T cells
50
40
30
20
10
0
G28z
P28z
GBBz
PBBz
***
**
*
**
60
40
20
0

Fig. 7A

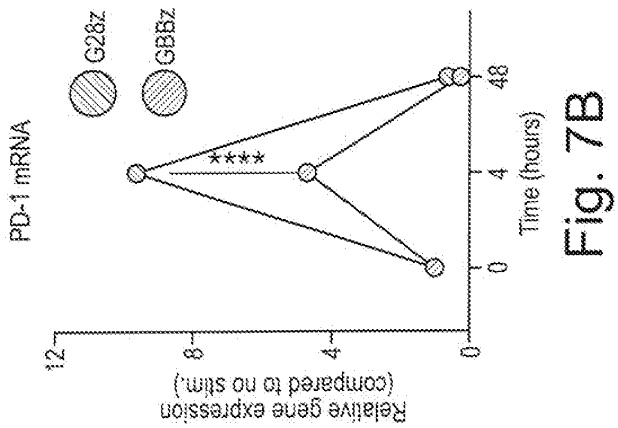

PD-1 mRNA
G28z
GBBz
****
Relative gene expression
(compared to no stim.)
12
8
4
0
0
4
48
Time (hours)

Fig. 7B

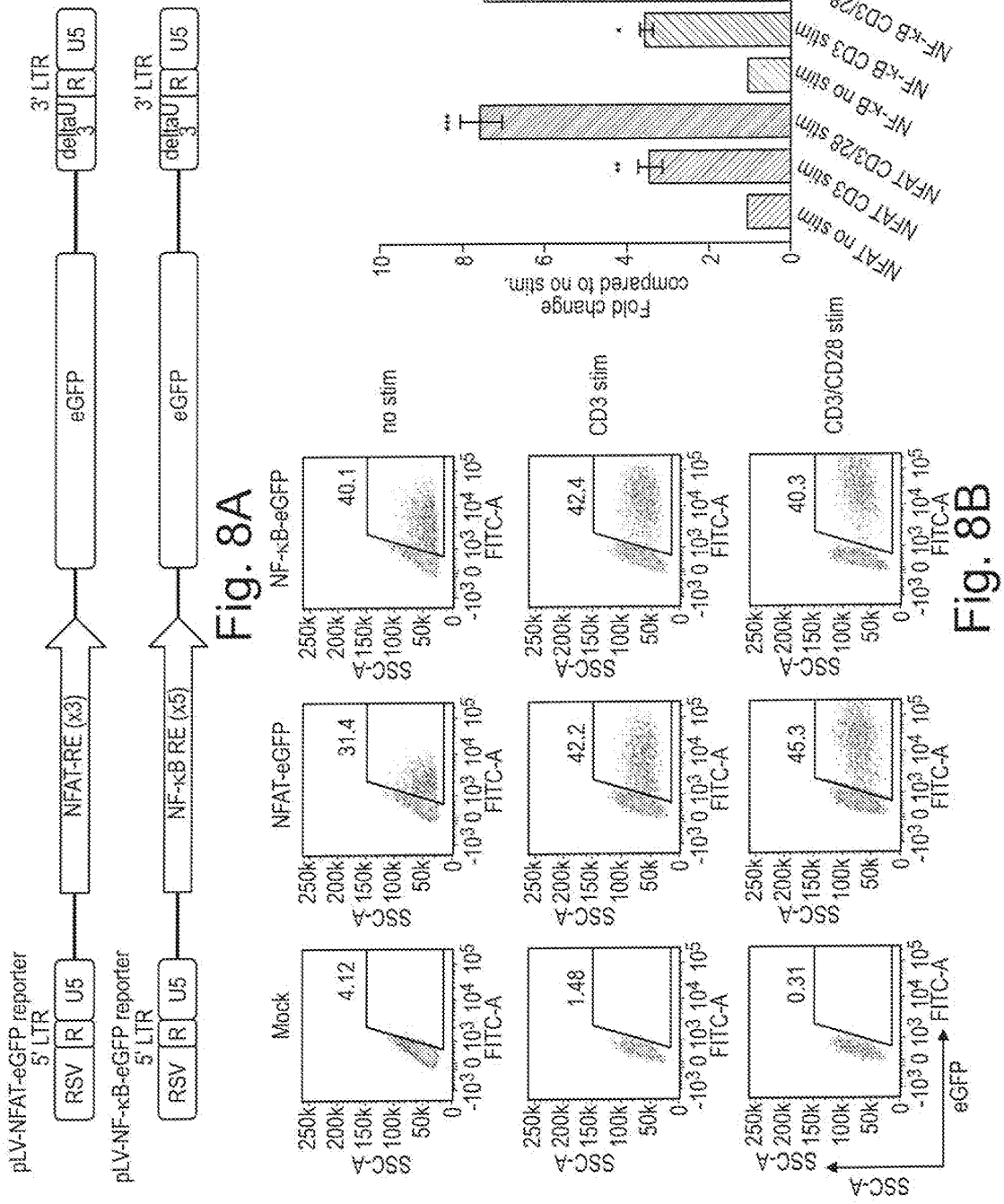

pLV-NFAT-eGFP reporter
5' LTR
RSV
R
U5
NFAT-RE (x3)
eGFP
3' LTR
delta U3
R
U5
pLV-NF-κB-eGFP reporter
5' LTR
RSV
R
U5
NF-κB RE (x5)
eGFP
3' LTR
delta U3
R
U5

Fig. 8A

Fold change compared to no stim.
NFAT no stim
NFAT CD3 stim
NFAT CD3/28 stim
NF-κB no stim
NF-κB CD3 stim
NF-κB CD3/28 stim
no stim
CD3 stim
CD3/CD28 stim
NF-κB-eGFP
NFAT-eGFP
Mock
4.12
40.1
31.4
42.4
42.2
40.3
45.3
1.48
0.31
SSC-A
FITC-A
eGFP

Fig. 8B

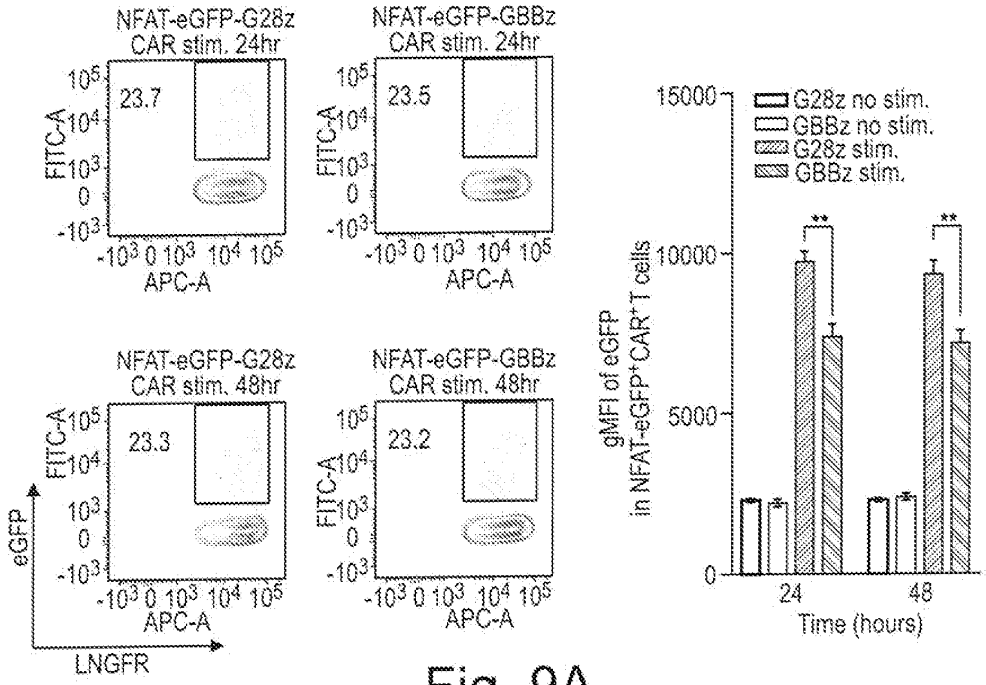

NFAT-eGFP-G28z
CAR stim. 24hr
23.7
NFAT-eGFP-GBBz
CAR stim. 24hr
23.5
NFAT-eGFP-G28z
CAR stim. 48hr
23.3
NFAT-eGFP-GBBz
CAR stim. 48hr
23.2
FITC-A
APC-A
eGFP
LNGFR
G28z no stim.
GBBz no stim.
G28z stim.
GBBz stim.
gMFI of eGFP
in NFAT-eGFP+CAR+T cells
Time (hours)

Fig. 9A

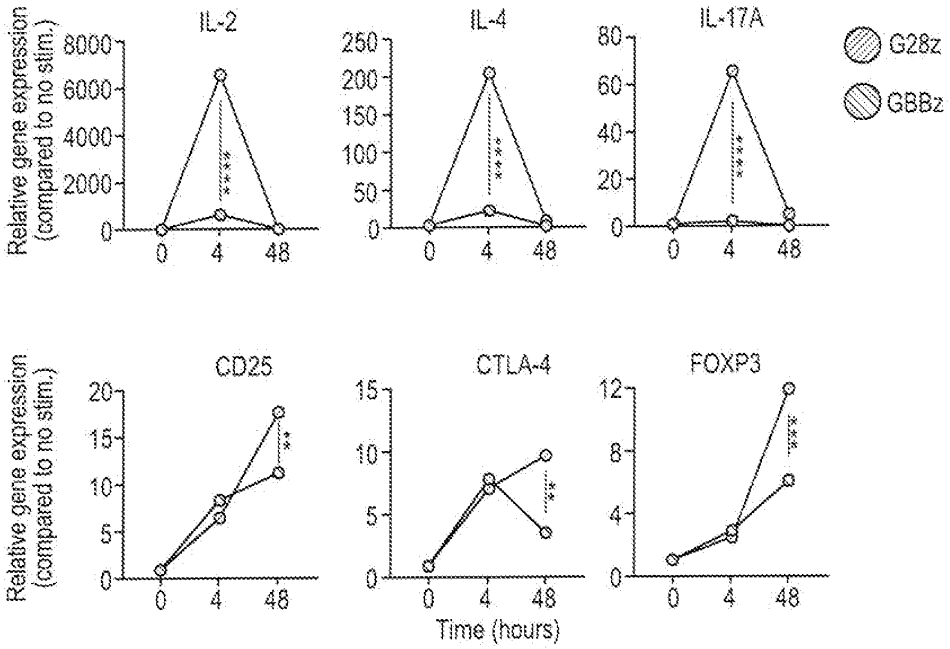

IL-2
IL-4
IL-17A
CD25
CTLA-4
FOXP3
Relative gene expression
(compared to no stim.)
G28z
GBBz
Time (hours)

Fig. 9B

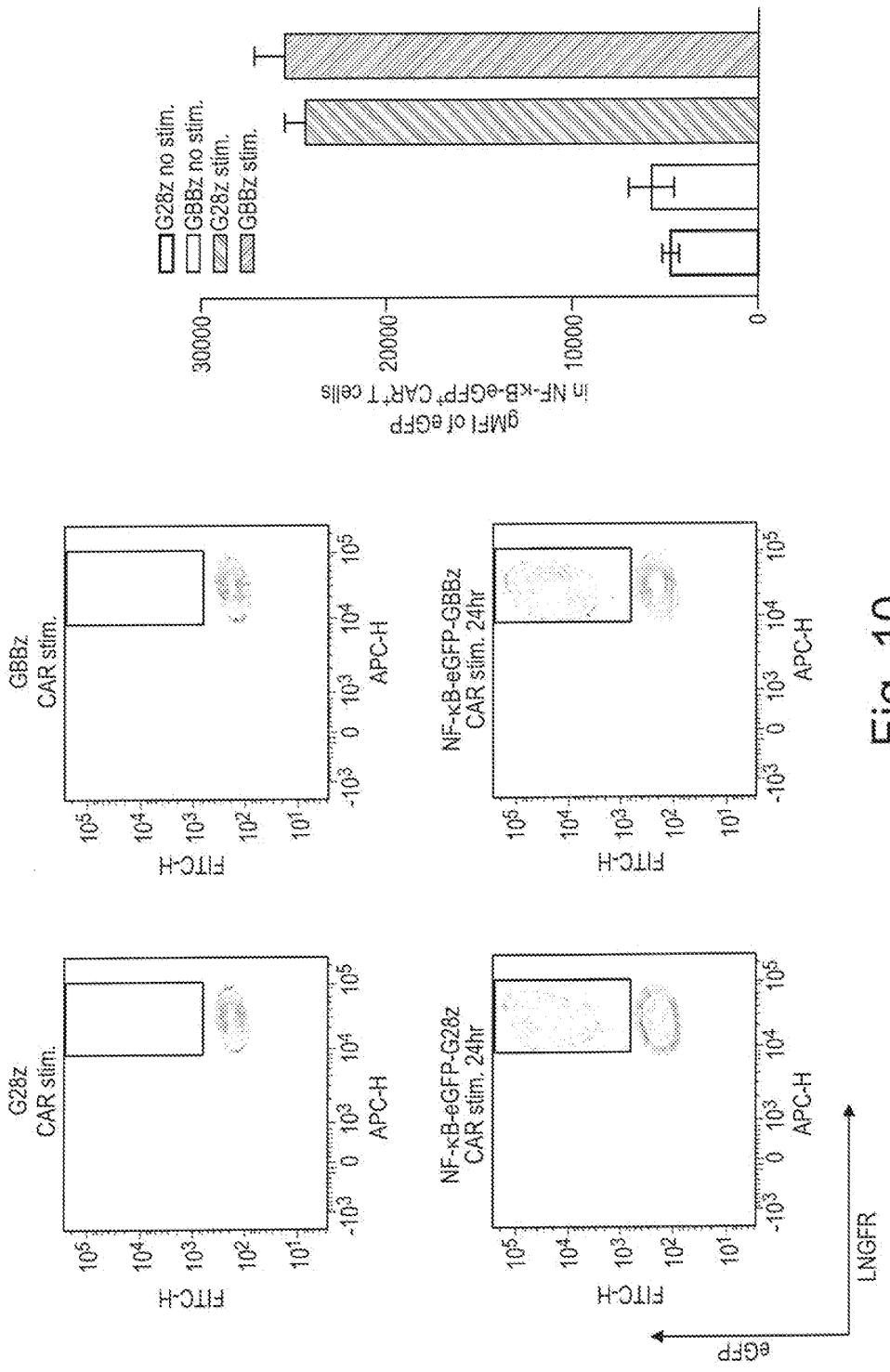

gMFI of eGFP
in NF-κB-eGFP+ CAR+T cells
30000
20000
10000
0
G28z no stim.
GBBz no stim.
G28z stim.
GBBz stim.
GBBz
CAR stim.
G28z
CAR stim.
NF-κB-eGFP-GBBz
CAR stim. 24hr
NF-κB-eGFP-G28z
CAR stim. 24hr
FITC-H
APC-H
eGFP
LNGFR

Fig. 10

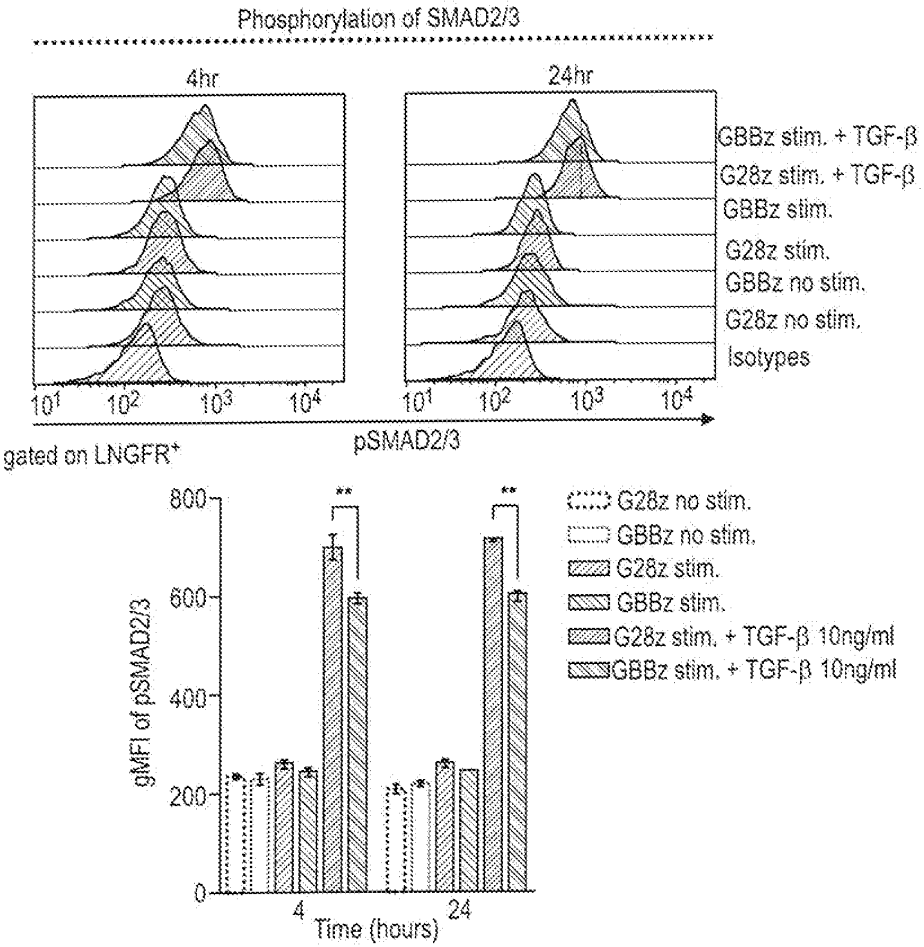

Phosphorylation of SMAD2/3
4hr
24hr
GBBz stim. + TGF-β
G28z stim. + TGF-β
GBBz stim.
G28z stim.
GBBz no stim.
G28z no stim.
Isotypes
10^1 10^2 10^3 10^4
gated on LNGFR+
pSMAD2/3
800
600
400
200
0
gMFI of pSMAD2/3
**
**
4
24
Time (hours)
G28z no stim.
GBBz no stim.
G28z stim.
GBBz stim.
G28z stim. + TGF-β 10ng/ml
GBBz stim. + TGF-β 10ng/ml

Fig. 11A

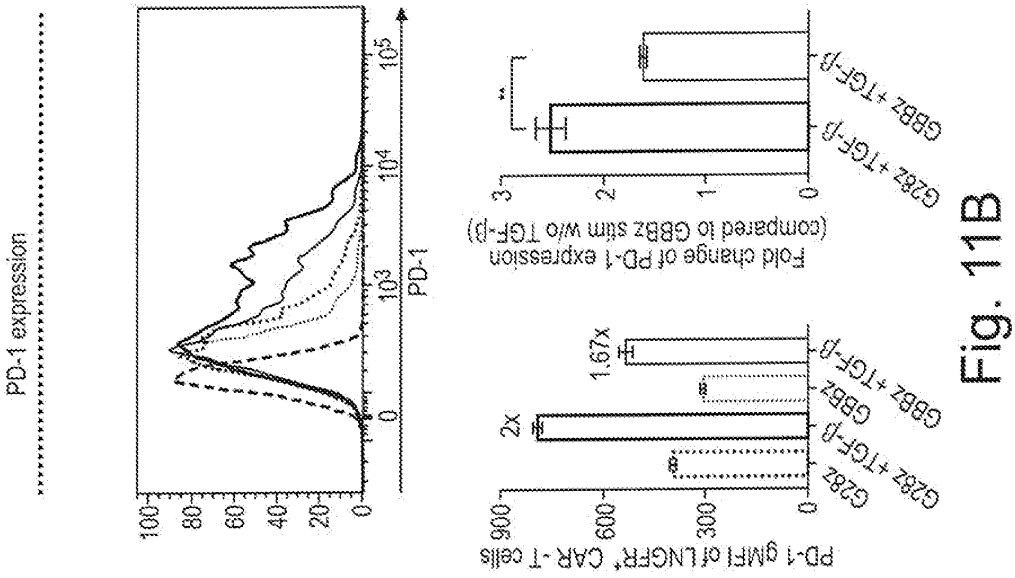


Fig. 11B

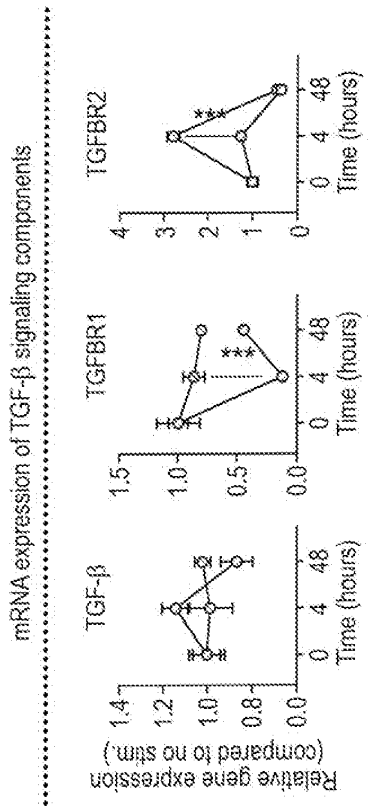


Fig. 11C

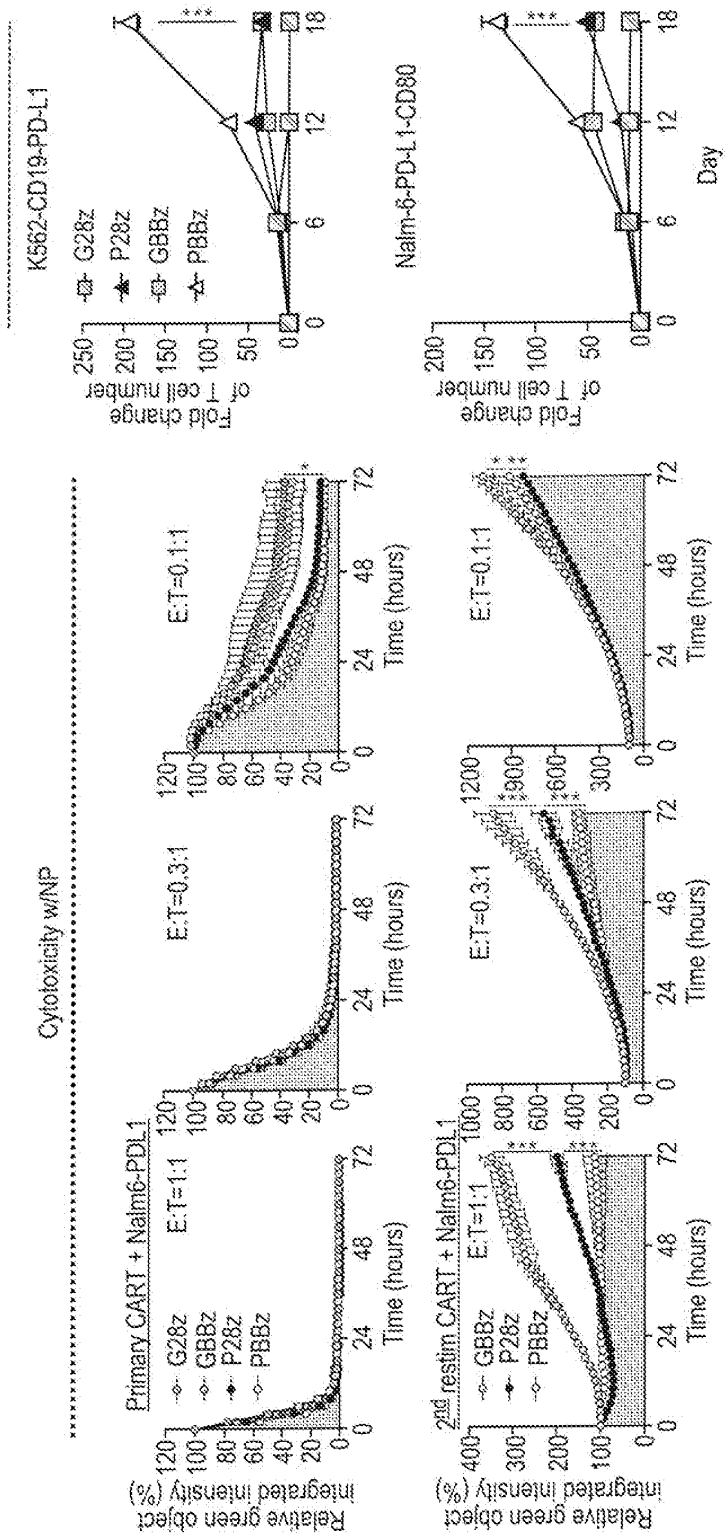


Fig. 12A

Fig. 12B

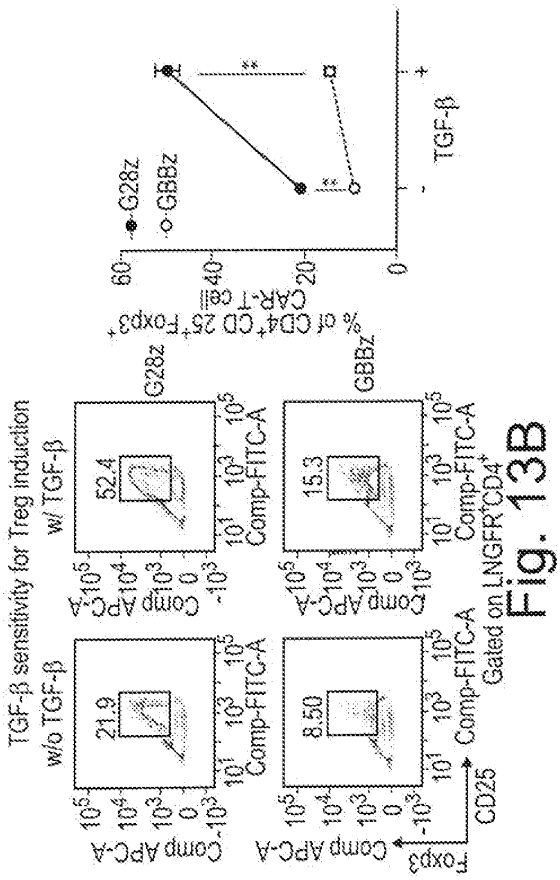


Fig. 13B

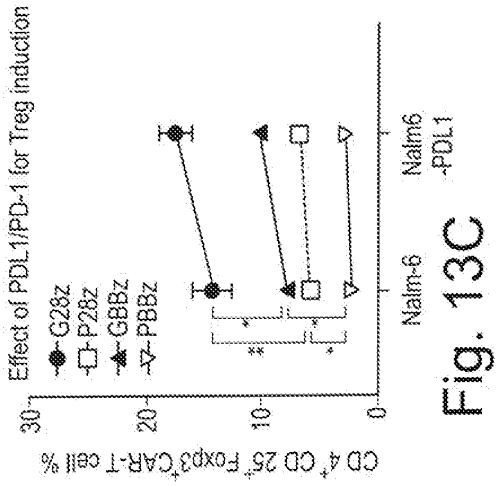


Fig. 13C

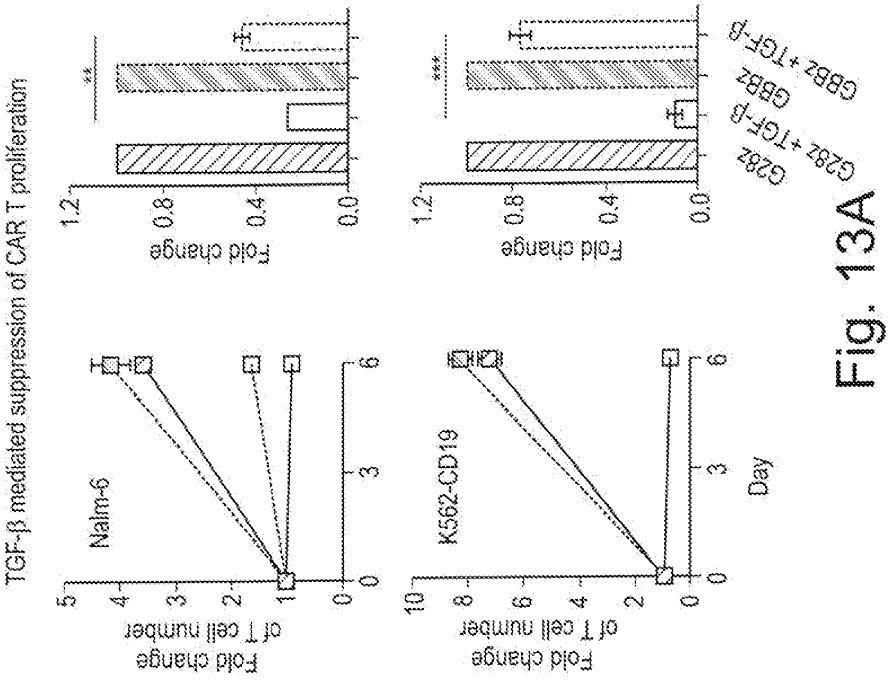


Fig. 13A

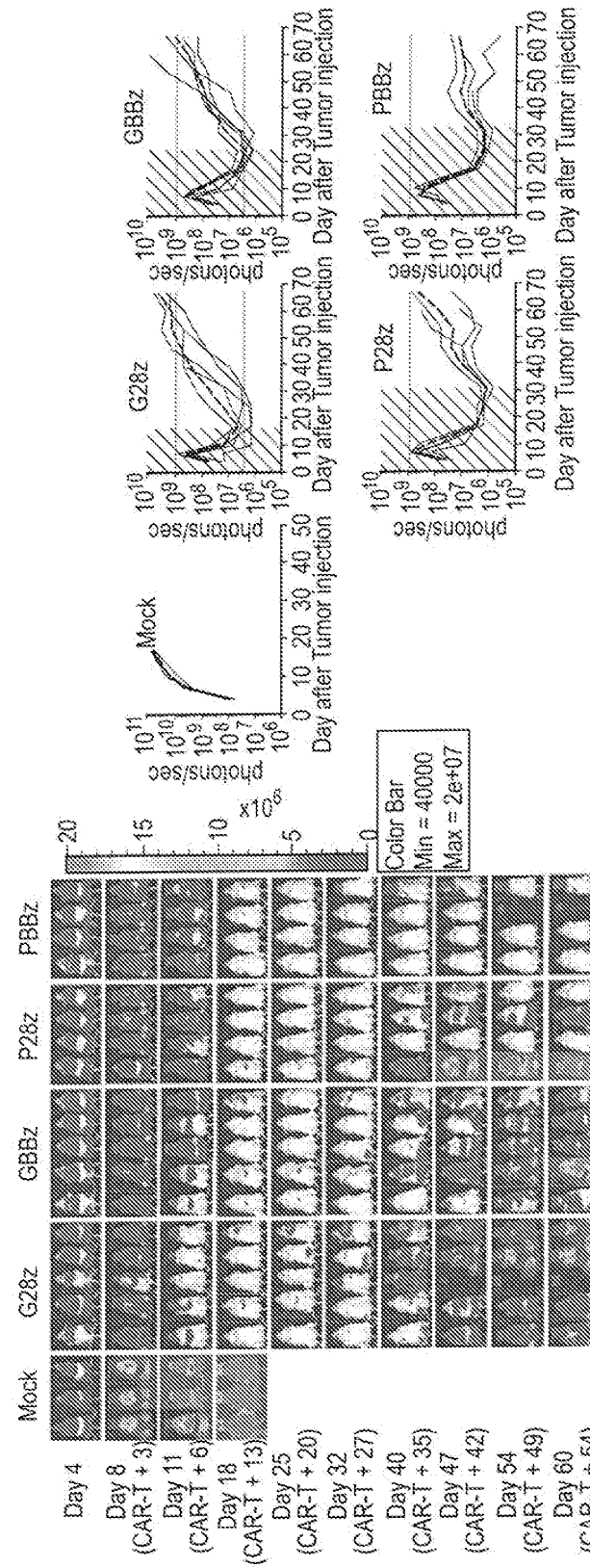

Mock
G28z
GBBz
P28z
PBBz
Day 4
Day 8 (CAR-T + 3)
Day 11 (CAR-T + 6)
Day 18 (CAR-T + 13)
Day 25 (CAR-T + 20)
Day 32 (CAR-T + 27)
Day 40 (CAR-T + 35)
Day 47 (CAR-T + 42)
Day 54 (CAR-T + 49)
Day 60 (CAR-T + 54)
x10^6
Color Bar
Min = 40000
Max = 2e+07
photons/sec
Day after Tumor injection

Fig. 14

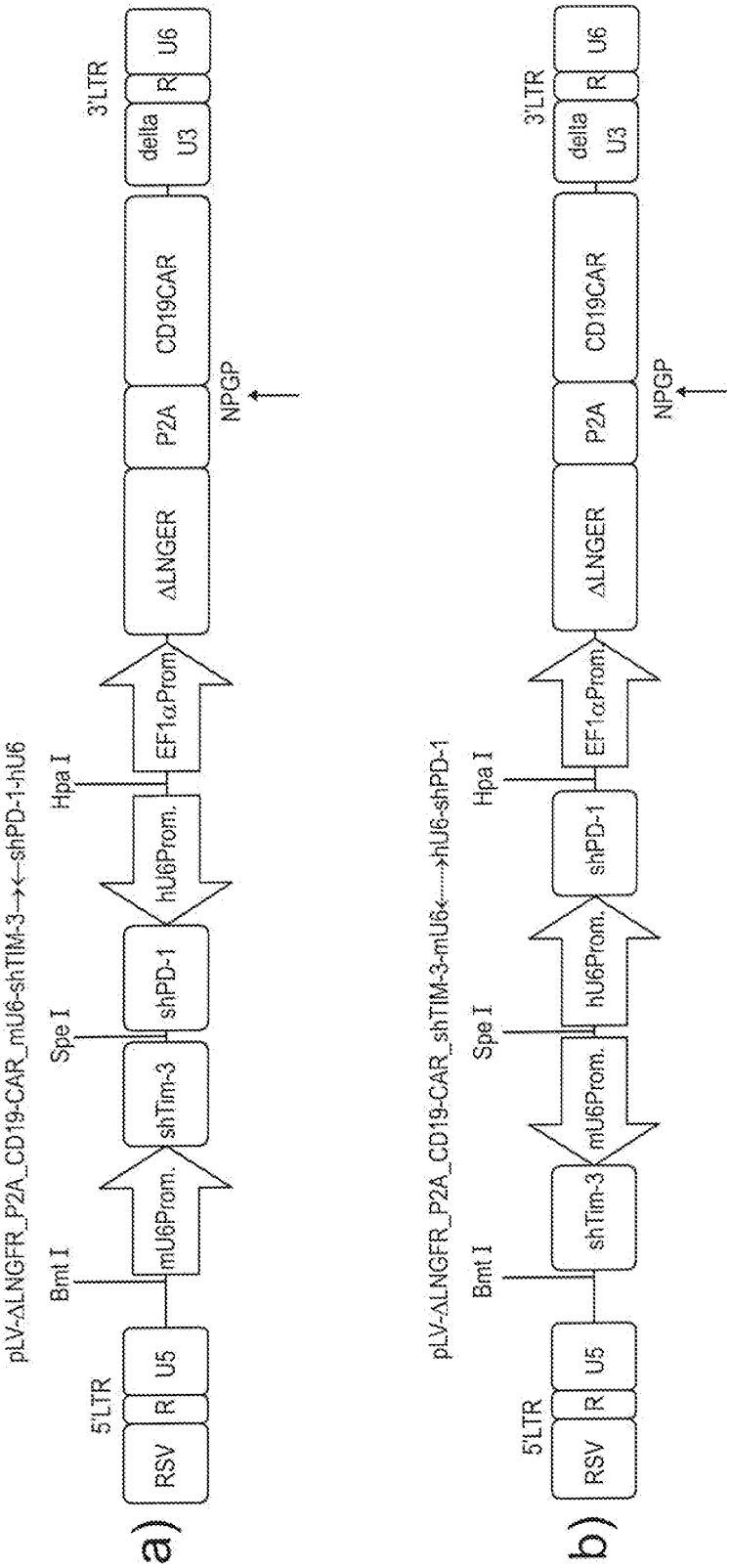

a)
pLV-ΔLNGFR_P2A_CD19-CAR_mU6-shTIM-3→←shPD-1-hU6
5'LTR
RSV
R
U5
Bmt I
mU6Prom.
shTim-3
Spe I
shPD-1
hU6Prom.
Hpa I
EF1αProm.
ΔLNGER
P2A
CD19CAR
NPGP
3'LTR
delta U3
R
U6
b)
pLV-ΔLNGFR_P2A_CD19-CAR_shTIM-3-mU6←→hU6-shPD-1
5'LTR
RSV
R
U5
Bmt I
shTim-3
Spe I
mU6Prom.
hU6Prom.
shPD-1
Hpa I
EF1αProm.
ΔLNGER
P2A
CD19CAR
NPGP
3'LTR
delta U3
R
U6

Fig. 15

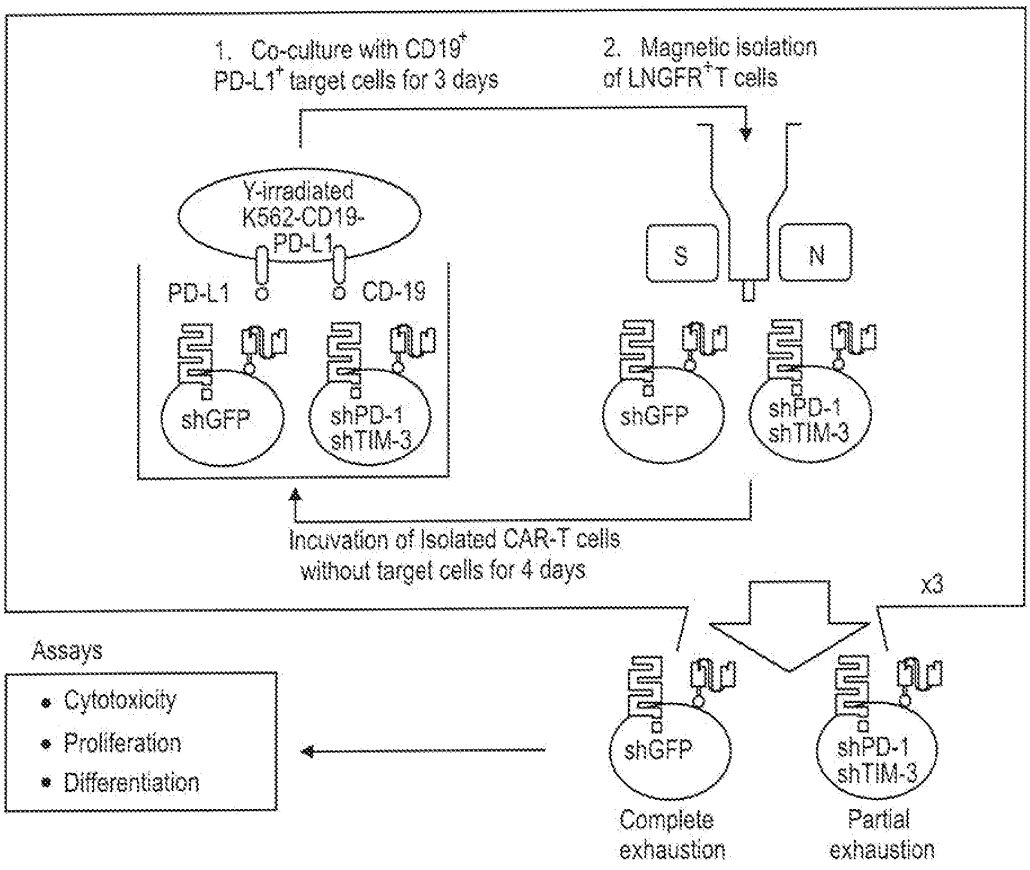

1. Co-culture with CD19+ PD-L1+ target cells for 3 days
2. Magnetic isolation of LNGFR+T cells
Y-irradiated K562-CD19-PD-L1
PD-L1
CD-19
shGFP
shPD-1 shTIM-3
S
N
shGFP
shPD-1 shTIM-3
Incuvation of Isolated CAR-T cells without target cells for 4 days
x3
Assays
Cytotoxicity
Proliferation
Differentiation
shGFP
shPD-1 shTIM-3
Complete exhaustion
Partial exhaustion

Fig. 16

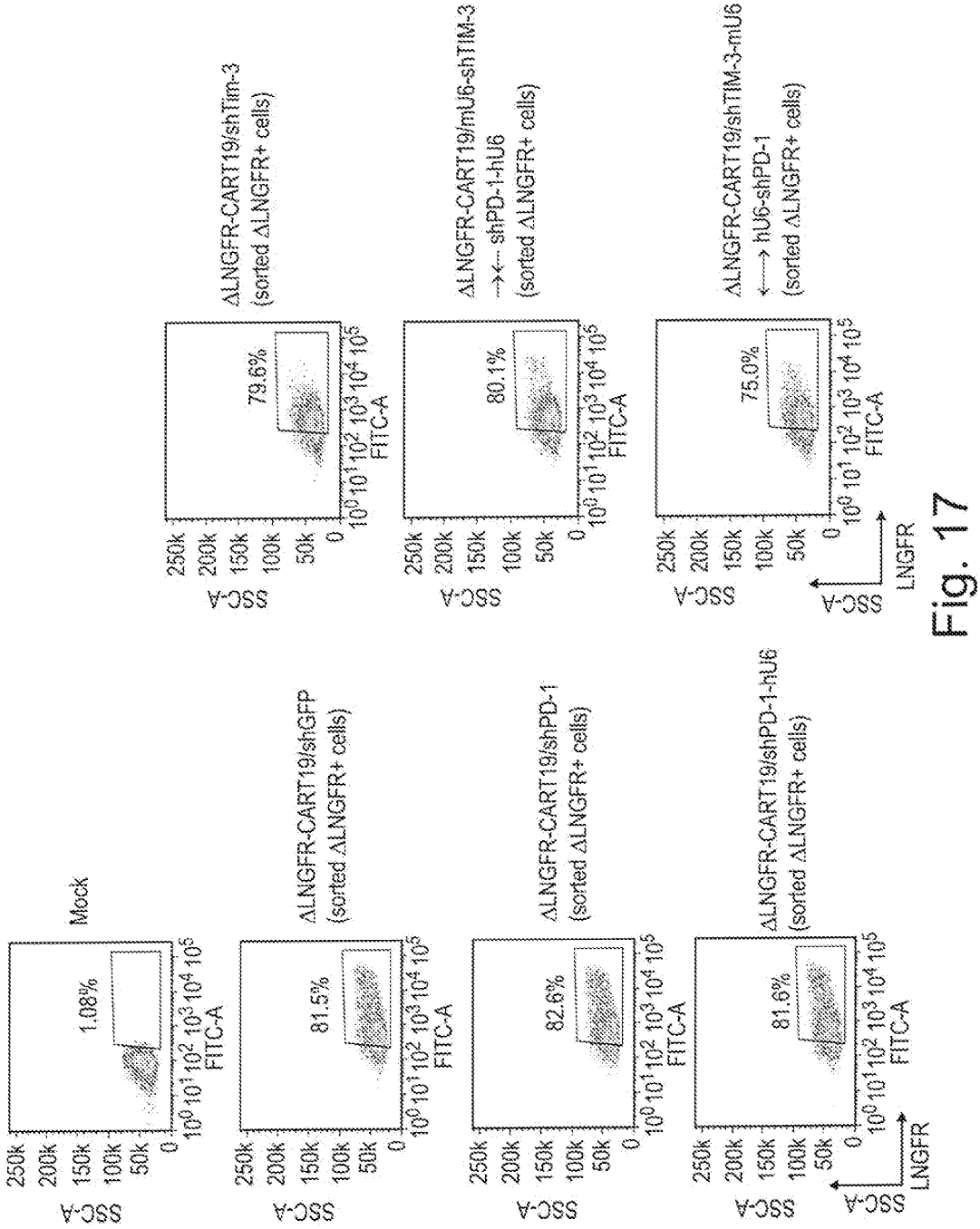

Mock
1.08%
ΔLNGFR-CART19/shGFP
(sorted ΔLNGFR+ cells)
81.5%
ΔLNGFR-CART19/shPD-1
(sorted ΔLNGFR+ cells)
82.6%
ΔLNGFR-CART19/shPD-1-hU6
(sorted ΔLNGFR+ cells)
81.6%
ΔLNGFR-CART19/shTim-3
(sorted ΔLNGFR+ cells)
79.6%
ΔLNGFR-CART19/mU6-shTIM-3
→← shPD-1-hU6
(sorted ΔLNGFR+ cells)
80.1%
ΔLNGFR-CART19/shTIM-3-mU6
←→ hU6-shPD-1
(sorted ΔLNGFR+ cells)
75.0%
SSC-A
FITC-A
LNGFR

Fig. 17

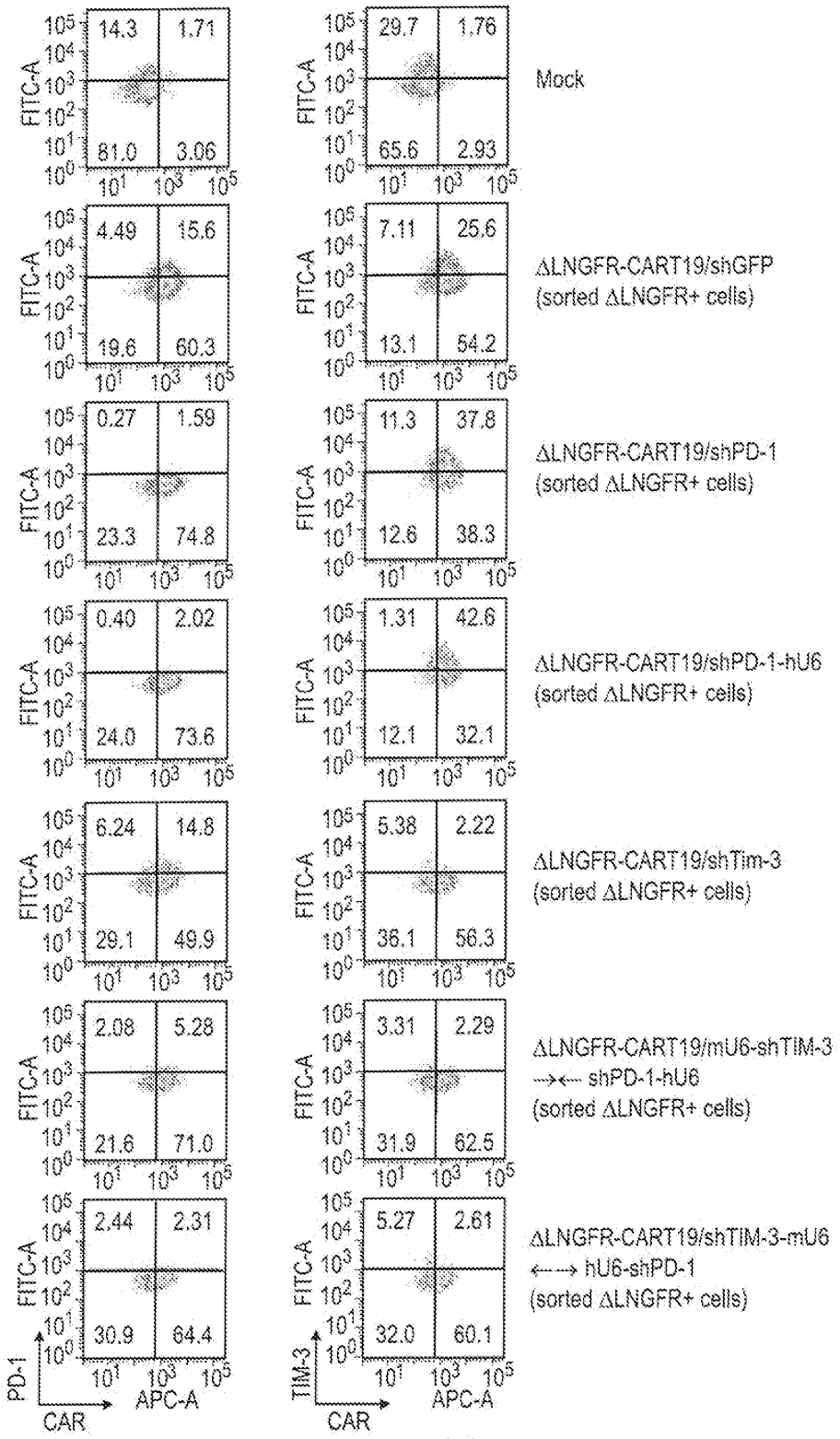

14.3
1.71
81.0
3.06
29.7
1.76
65.6
2.93
Mock
4.49
15.6
19.6
60.3
7.11
25.6
13.1
54.2
ΔLNGFR-CART19/shGFP
(sorted ΔLNGFR+ cells)
0.27
1.59
23.3
74.8
11.3
37.8
12.6
38.3
ΔLNGFR-CART19/shPD-1
(sorted ΔLNGFR+ cells)
0.40
2.02
24.0
73.6
1.31
42.6
12.1
32.1
ΔLNGFR-CART19/shPD-1-hU6
(sorted ΔLNGFR+ cells)
6.24
14.8
29.1
49.9
5.38
2.22
36.1
56.3
ΔLNGFR-CART19/shTim-3
(sorted ΔLNGFR+ cells)
2.08
5.28
21.6
71.0
3.31
2.29
31.9
62.5
ΔLNGFR-CART19/mU6-shTIM-3
→←shPD-1-hU6
(sorted ΔLNGFR+ cells)
2.44
2.31
30.9
64.4
5.27
2.61
32.0
60.1
ΔLNGFR-CART19/shTIM-3-mU6
←→hU6-shPD-1
(sorted ΔLNGFR+ cells)
FITC-A
PD-1
TIM-3
CAR
APC-A

Fig. 18A

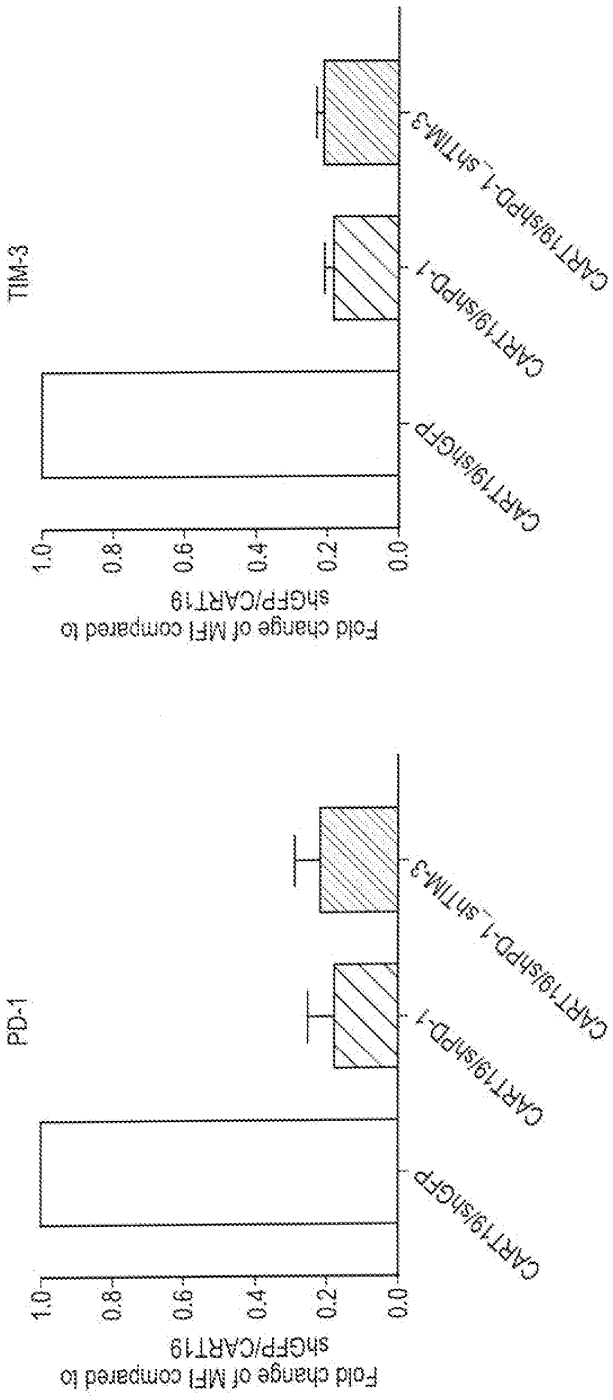

TIM-3
Fold change of MFI compared to shGFP/CART19
1.0
0.8
0.6
0.4
0.2
0.0
CART19/shGFP
CART19/shPD-1
CART19/shPD-1_shTIM-3
PD-1
Fold change of MFI compared to shGFP/CART19
1.0
0.8
0.6
0.4
0.2
0.0
CART19/shGFP
CART19/shPD-1
CART19/shPD-1_shTIM-3

Fig. 18B

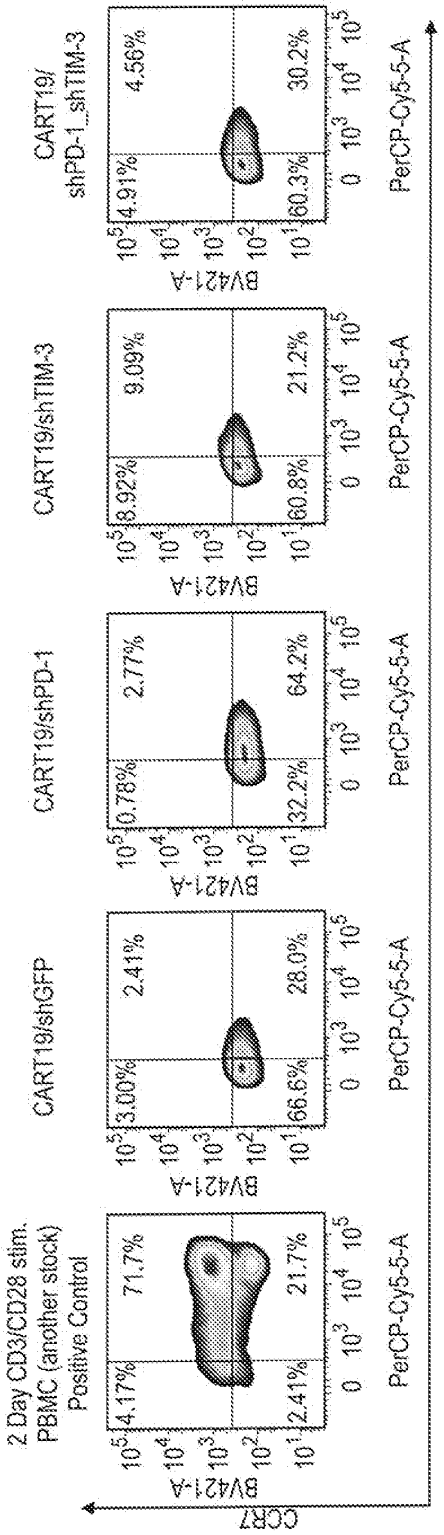

2 Day CD3/CD28 stim.
PBMC (another stock)
Positive Control
CART19/shGFP
CART19/shPD-1
CART19/shTIM-3
CART19/
shPD-1_shTIM-3
CCR7
CD45RA
BV421-A
PerCP-Cy5-5-A
4.17%
71.7%
2.41%
3.00%
2.41%
28.0%
0.78%
2.77%
32.2%
64.2%
8.92%
9.09%
60.8%
21.2%
4.91%
4.56%
60.3%
30.2%

Fig. 19

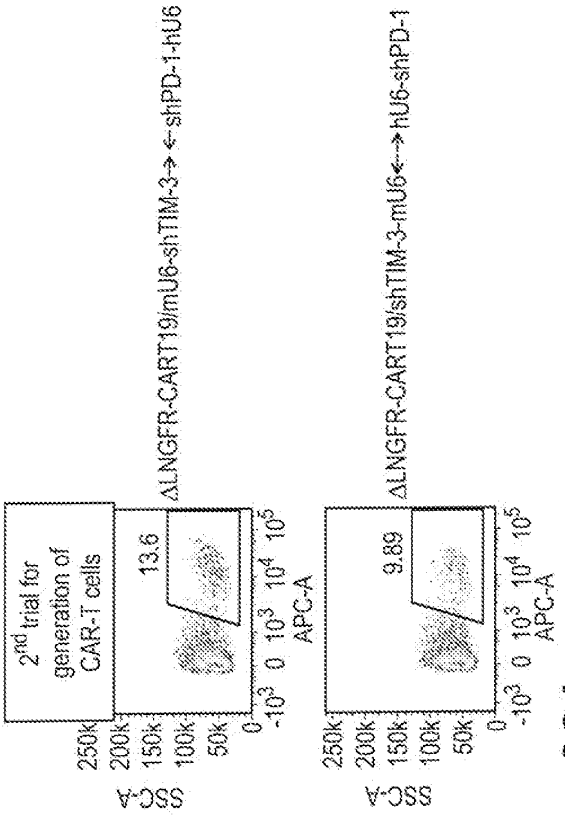


Fig. 20A

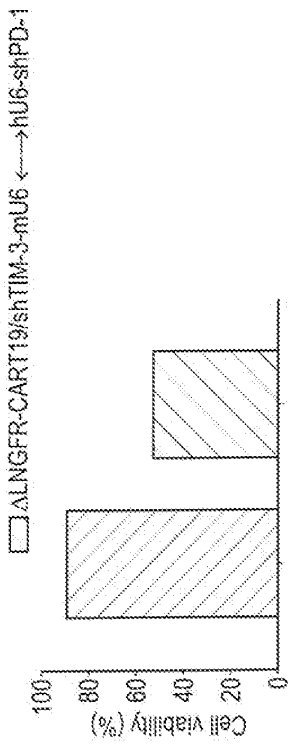


Fig. 20C

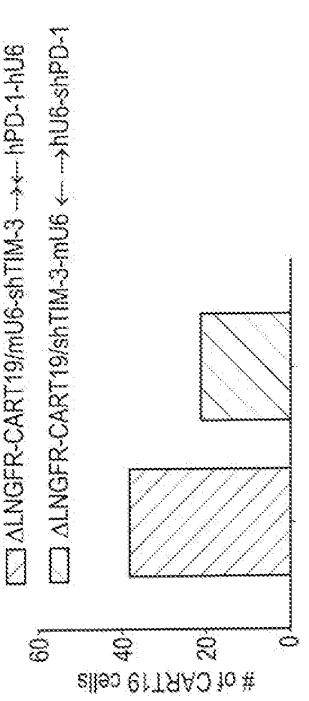


Fig. 20B

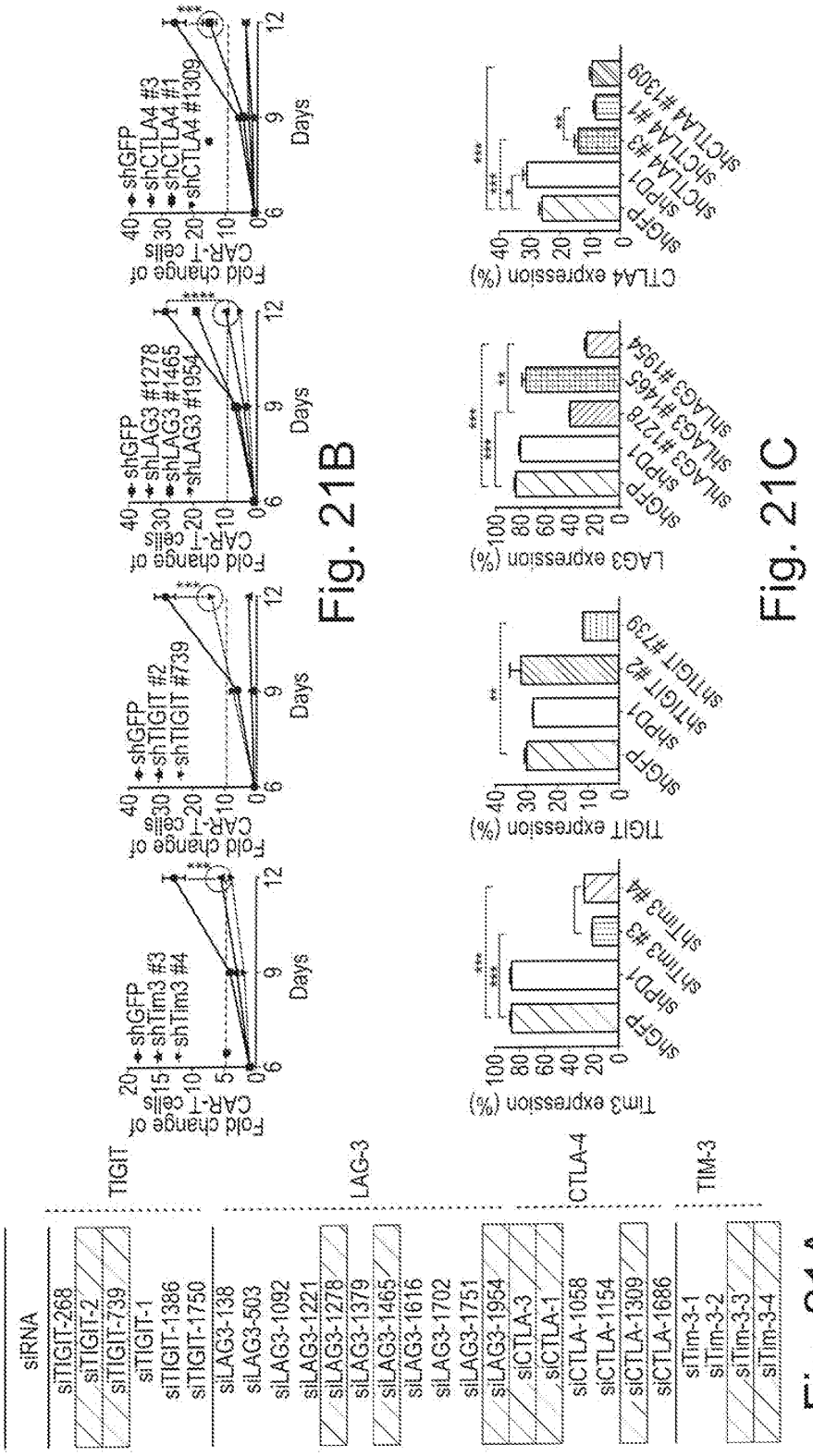


Fig. 21A

Fig. 21B

Fig. 21C

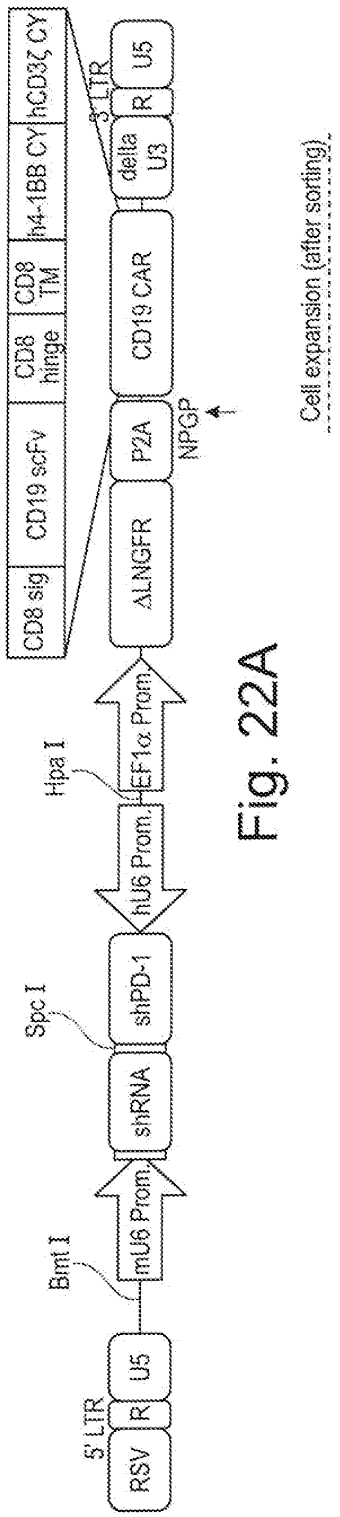


Fig. 22A

Fig. 22B

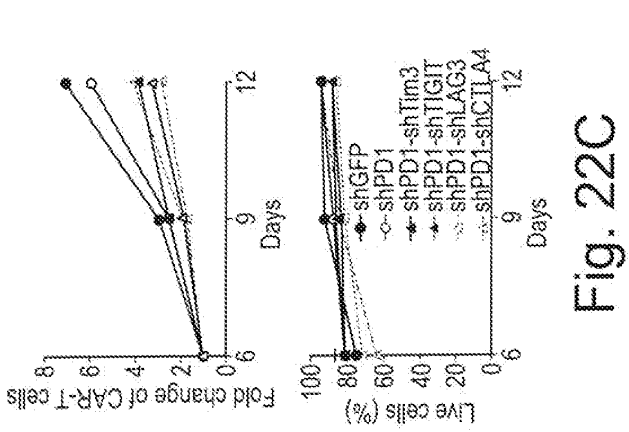


Fig. 22C

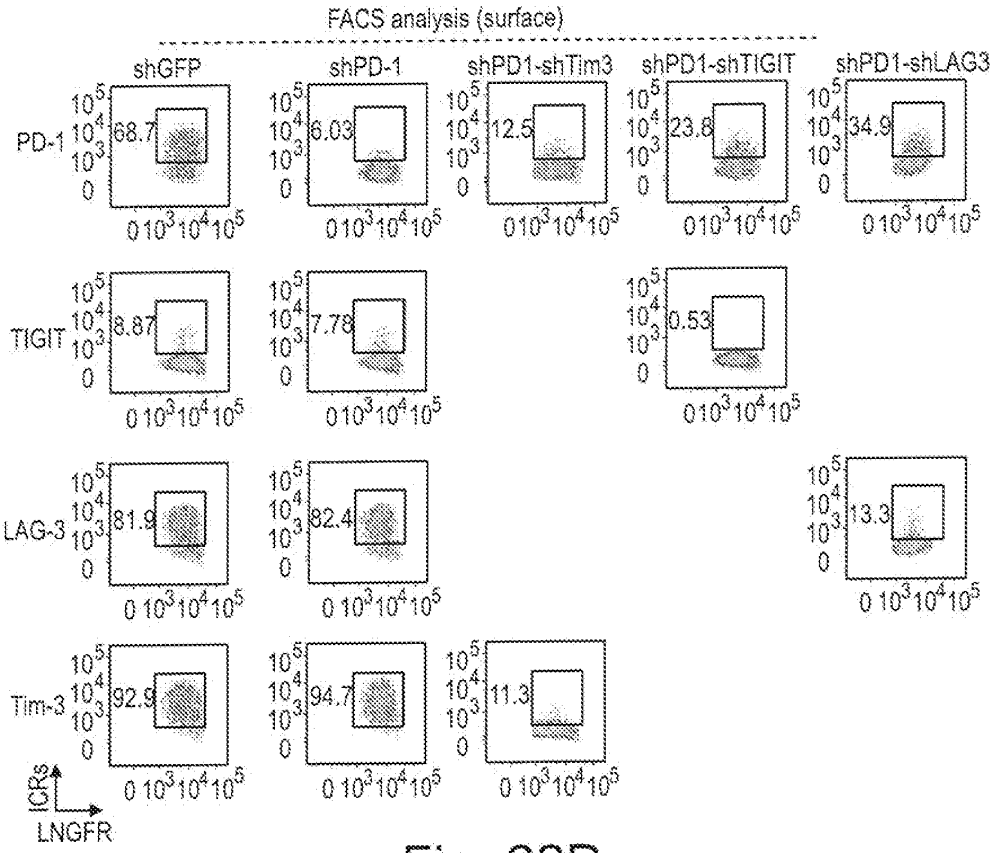


Fig. 22D

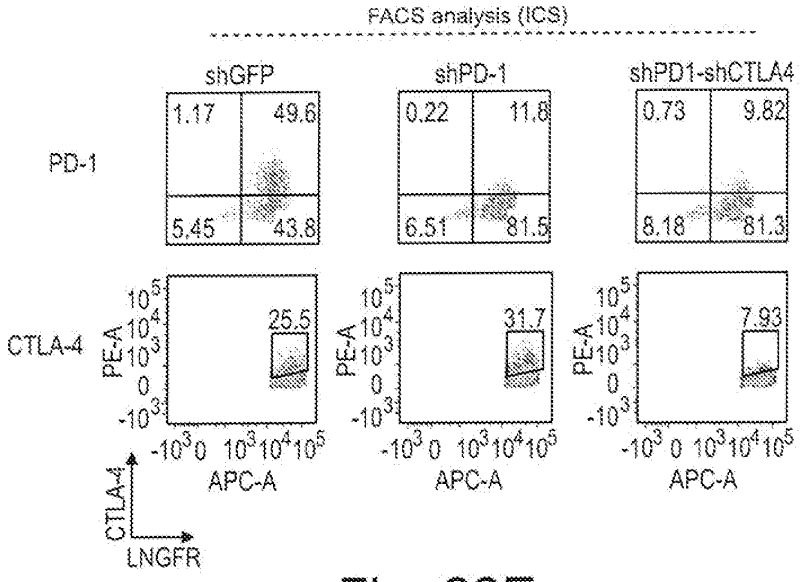


Fig. 22E

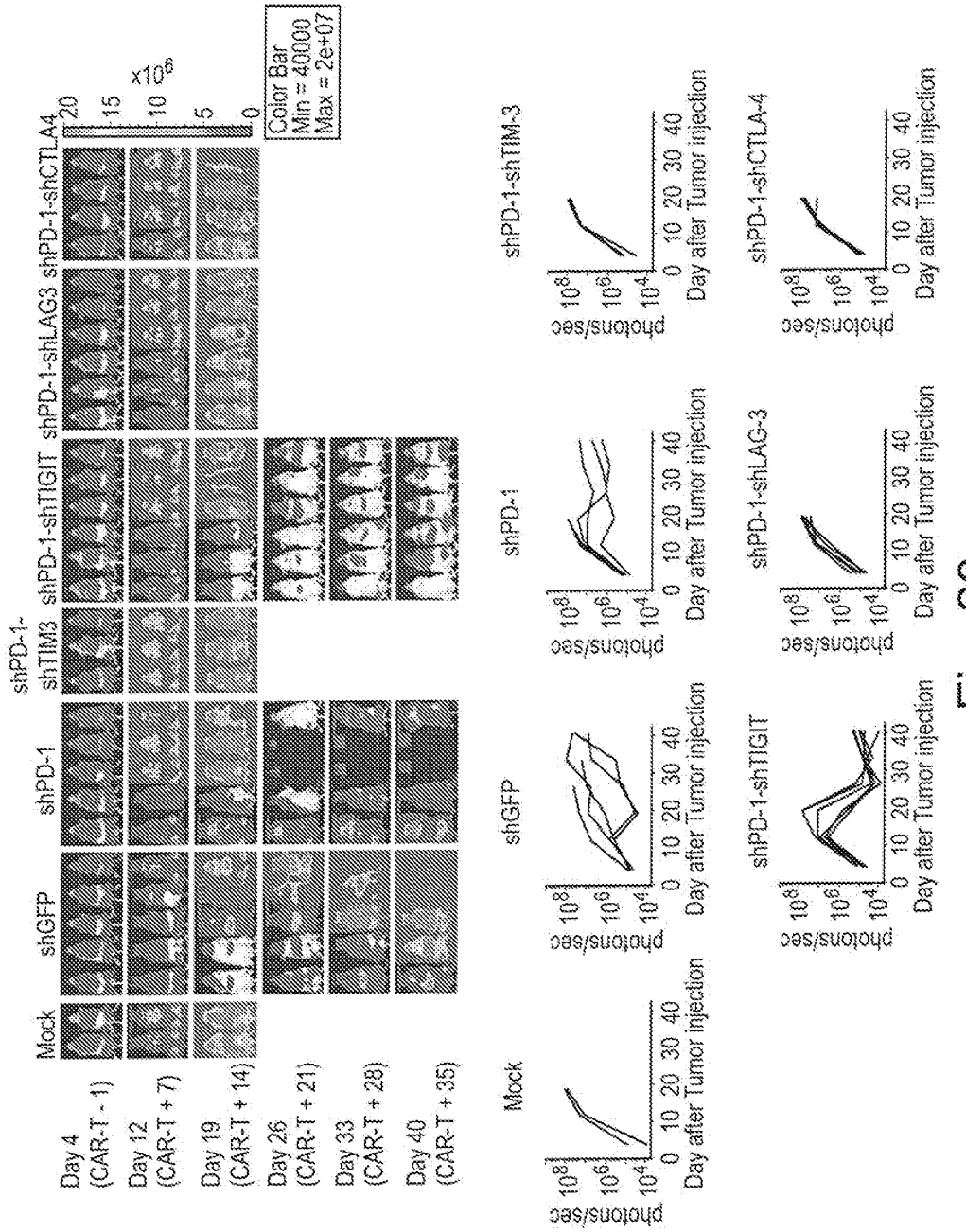

Mock
shGFP
shPD-1
shPD-1-shTIM3
shPD-1-shTIGIT
shPD-1-shLAG3
shPD-1-shCTLA4
Day 4 (CAR-T - 1)
Day 12 (CAR-T + 7)
Day 19 (CAR-T + 14)
Day 26 (CAR-T + 21)
Day 33 (CAR-T + 28)
Day 40 (CAR-T + 35)
x10^6
20
15
10
5
0
Color Bar
Min = 40000
Max = 2e+07
Mock
shGFP
shPD-1
shPD-1-shTIM-3
shPD-1-shTIGIT
shPD-1-shLAG-3
shPD-1-shCTLA-4
photons/sec
Day after Tumor injection

Fig. 23

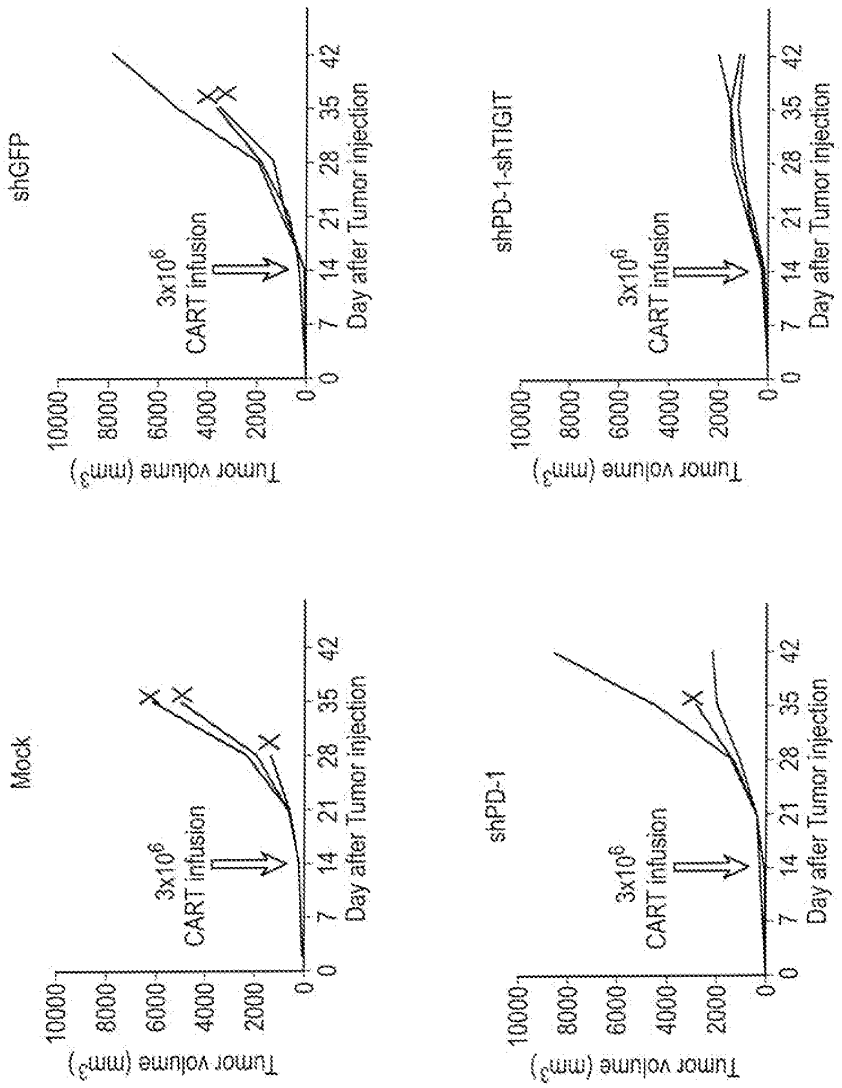

shGFP
shPD-1-shTIGIT
Mock
shPD-1
Tumor volume (mm³)
10000
8000
6000
4000
2000
0
0 7 14 21 28 35 42
Day after Tumor injection
3x10⁶
CART infusion

Fig. 24