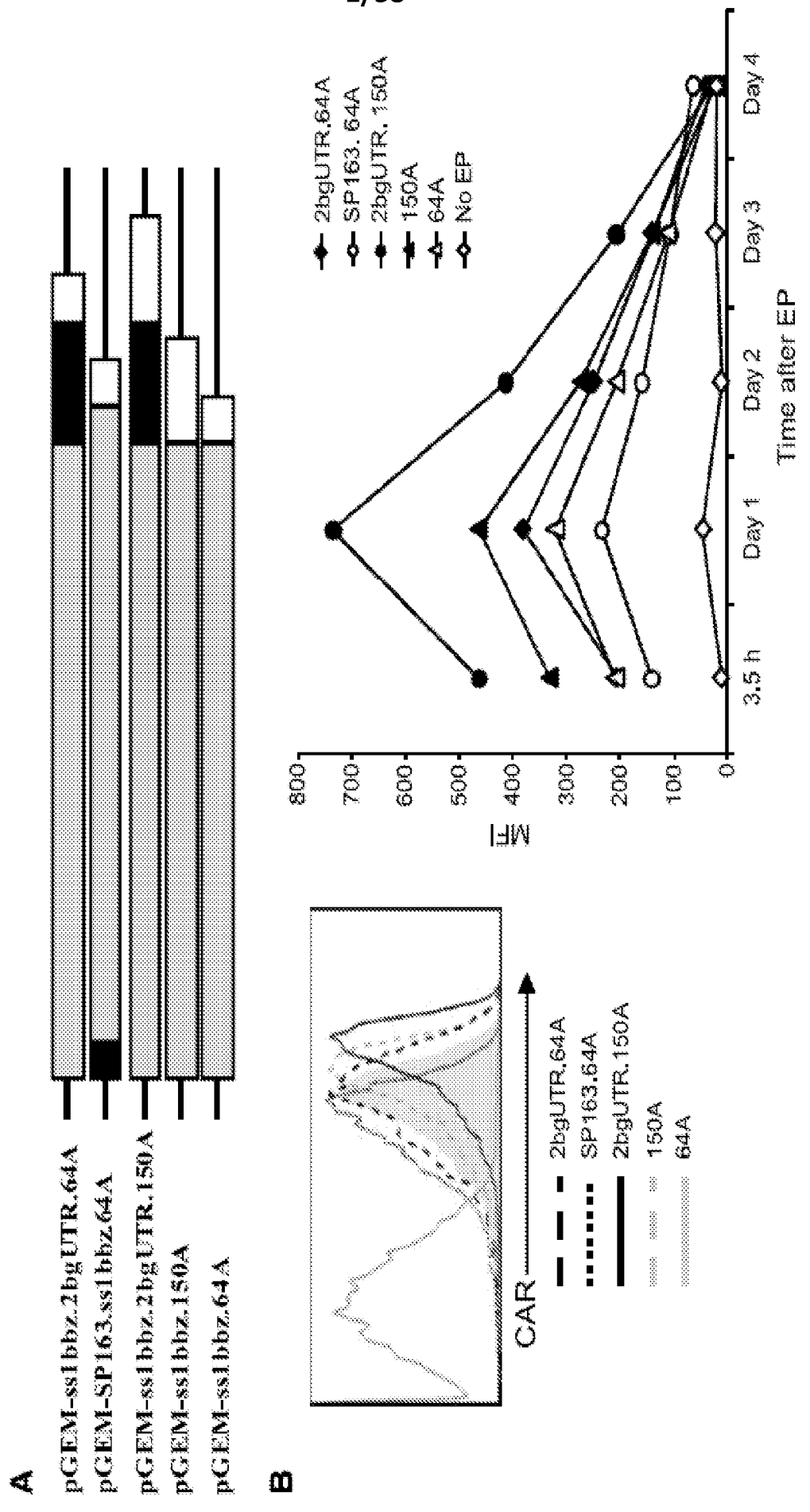

A
pGEM-ss1bbz.2bgUTR.64A
pGEM-SP163.ss1bbz.64A
pGEM-ss1bbz.2bgUTR.150A
pGEM-ss1bbz.150A
pGEM-ss1bbz.64A
B
CAR
2bgUTR.64A
SP163.64A
2bgUTR.150A
150A
64A
MFI
800
700
600
500
400
300
200
100
0
3.5 h
Day 1
Day 2
Day 3
Day 4
Time after EP
2bgUTR.64A
SP163. 64A
2bgUTR. 150A
150A
64A
No EP

Figure 1

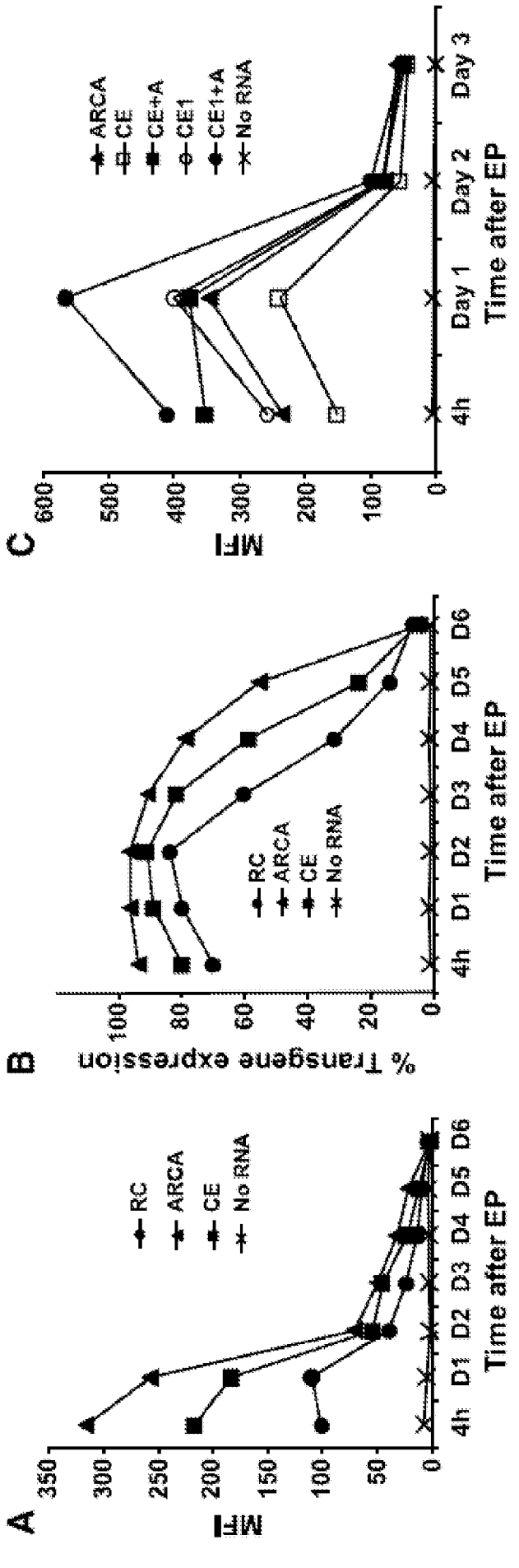
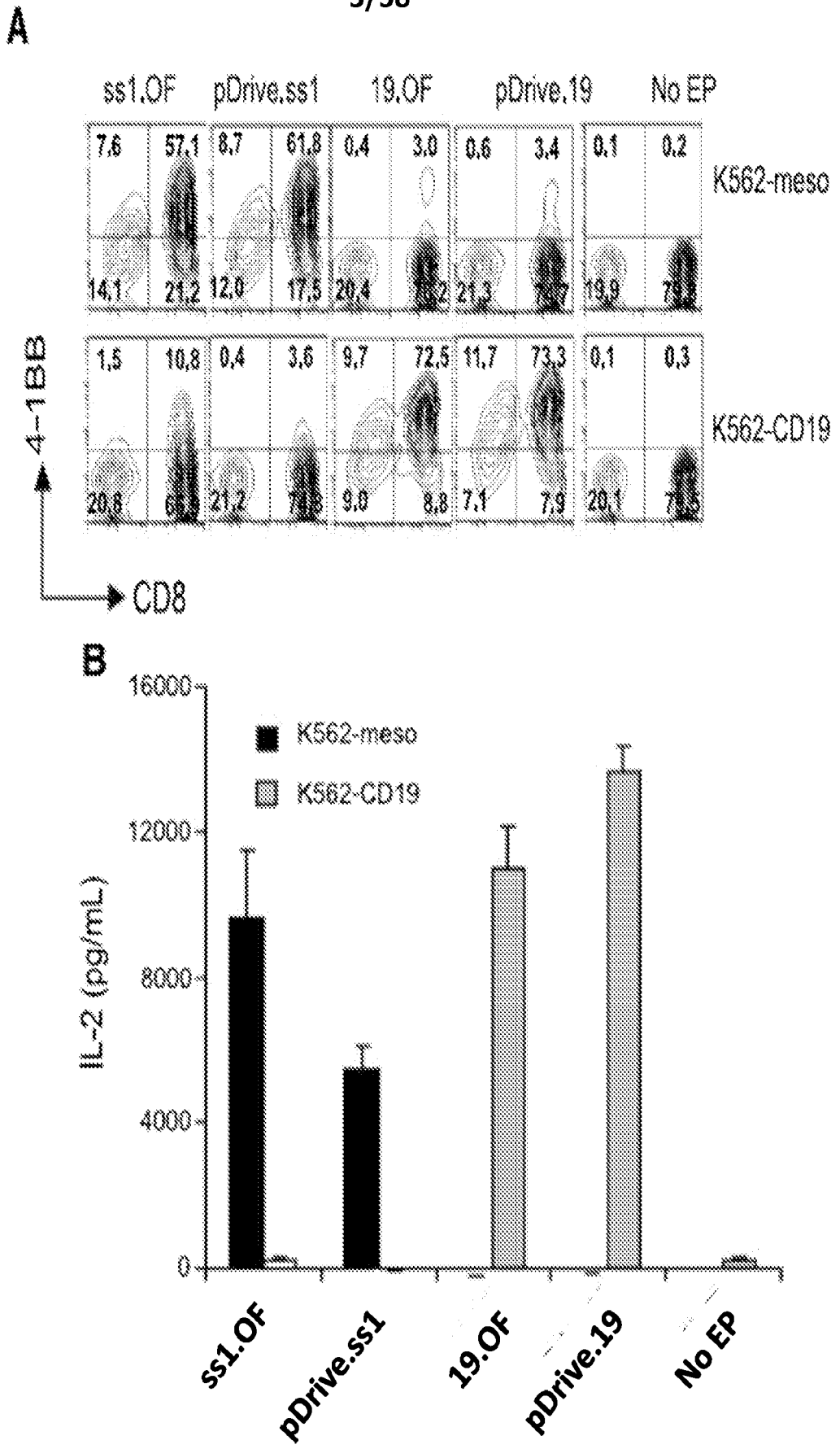
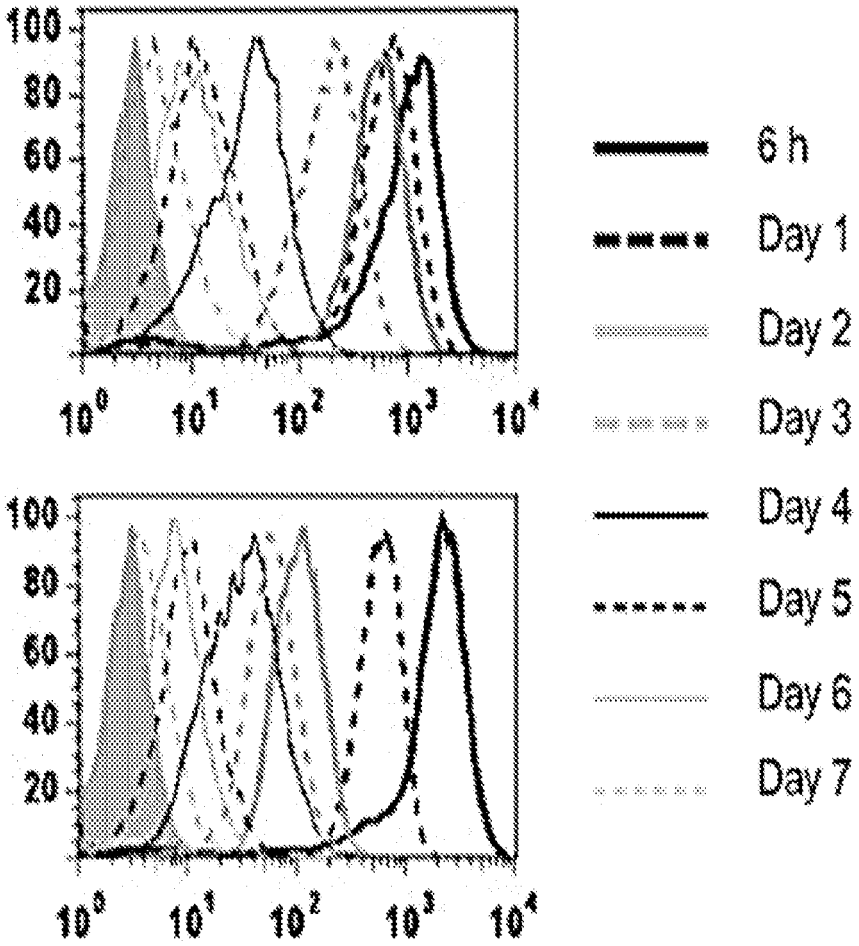

A
MFI
Time after EP
4h D1 D2 D3 D4 D5 D6
RC
ARCA
CE
No RNA
B
% Transgene expression
Time after EP
4h D1 D2 D3 D4 D5 D6
RC
ARCA
CE
No RNA
C
MFI
Time after EP
4h Day 1 Day 2 Day 3
ARCA
CE
CE+A
CE1
CE1+A
No RNA

Figure 2

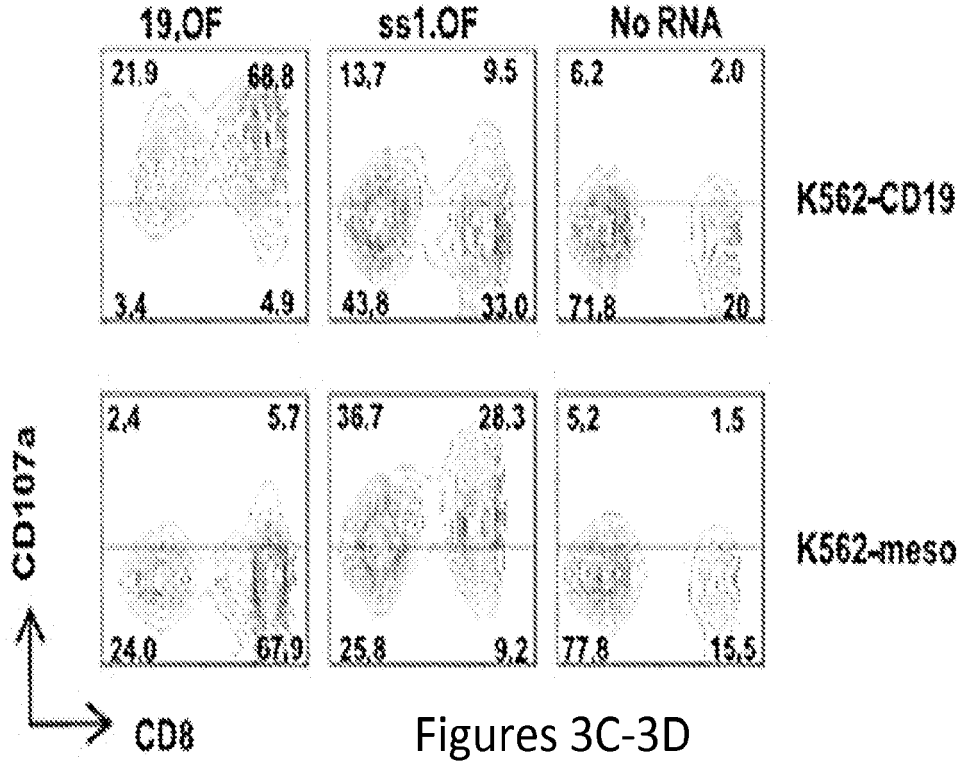


Figures 3A-3B

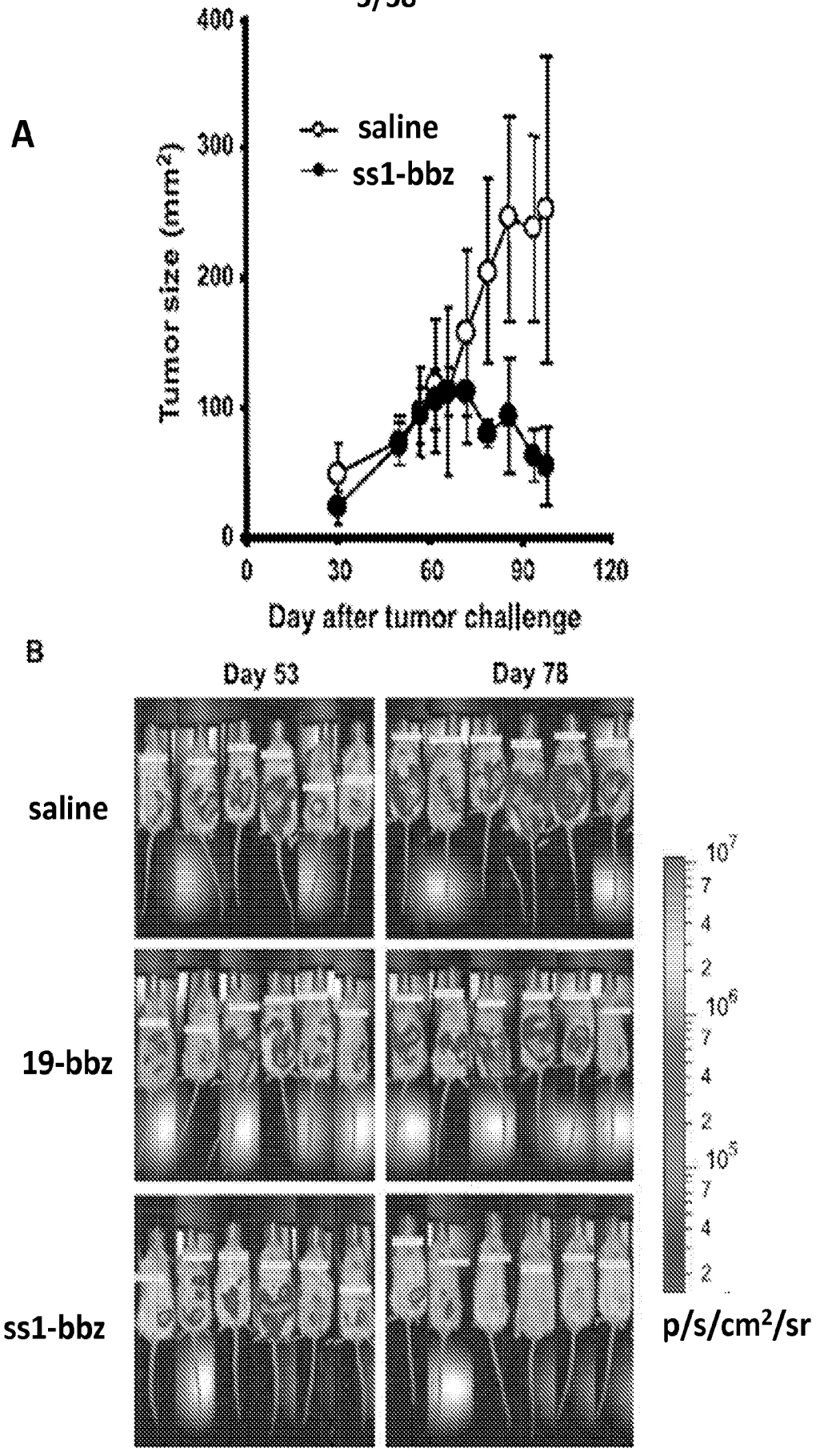
C



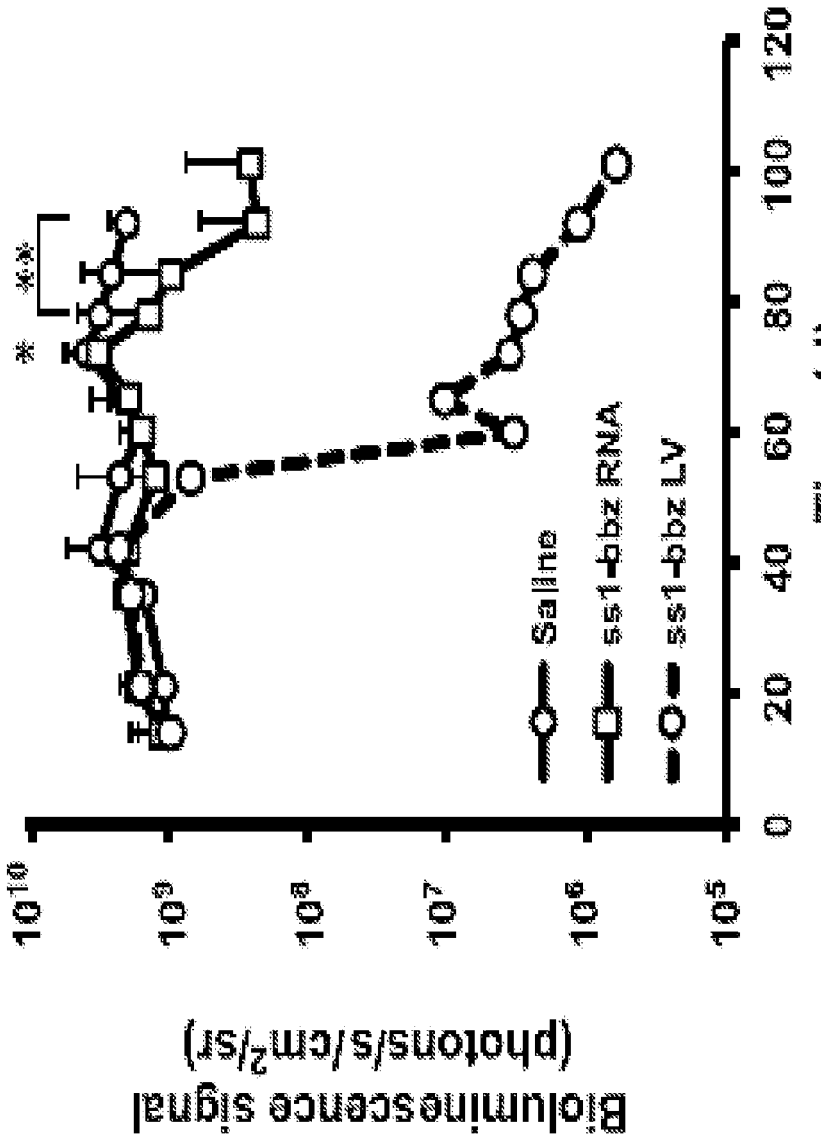
D



Figures 3C-3D



Figures 4A-4B


Bioluminescence signal
(photons/s/cm²/sr)
10¹⁰
10⁹
10⁸
10⁷
10⁶
10⁵
0
20
40
60
80
100
120
Time (d)
Saline
ss1-bbz RNA
ss1-bbz LV
*
***

C

Figure 4C

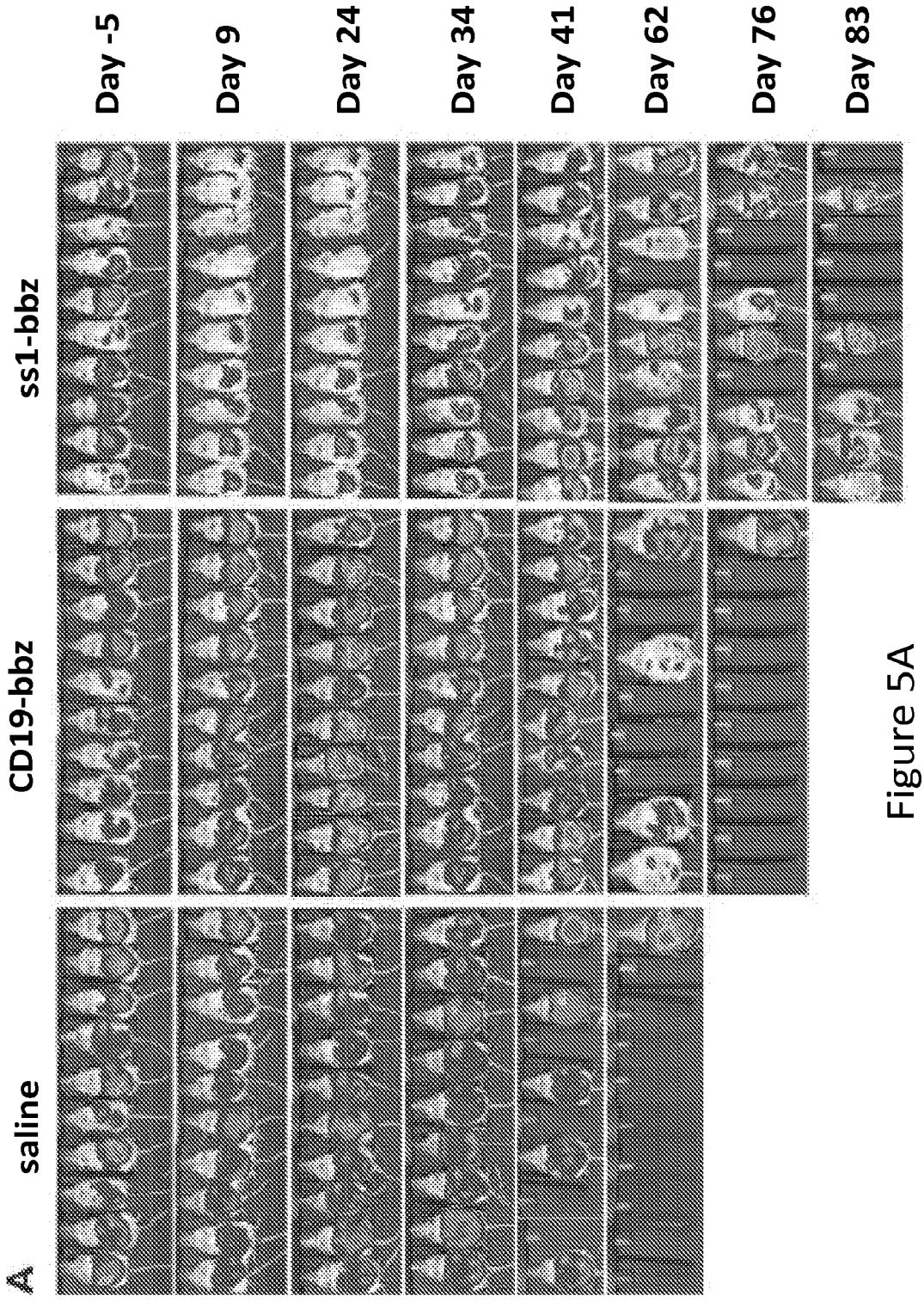
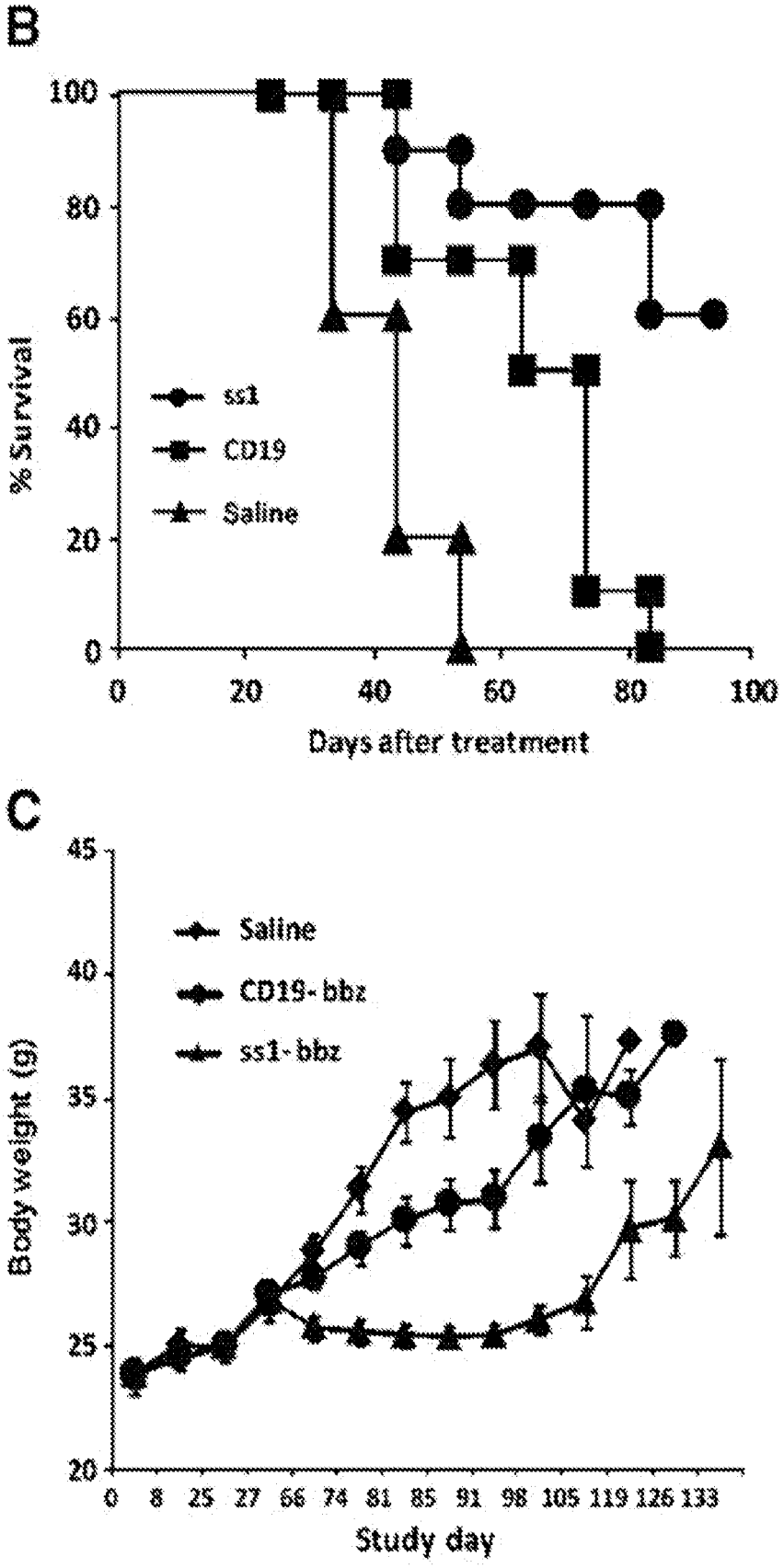

A
saline
CD19-bbz
ss1-bbz
Day -5
Day 9
Day 24
Day 34
Day 41
Day 62
Day 76
Day 83

Figure 5A



Figures 5B-5C

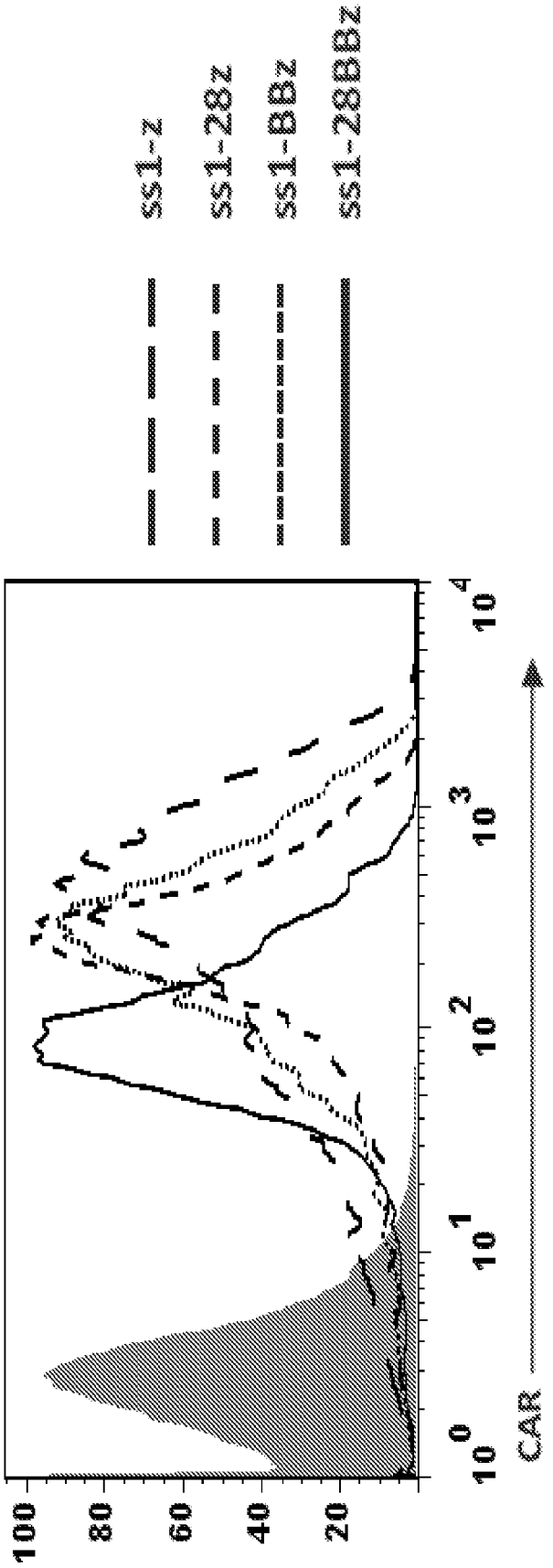

ss1-z
ss1-28z
ss1-BBz
ss1-28BBz
100
80
60
40
20
10^0
10^1
10^2
10^3
10^4
CAR

Figure 6

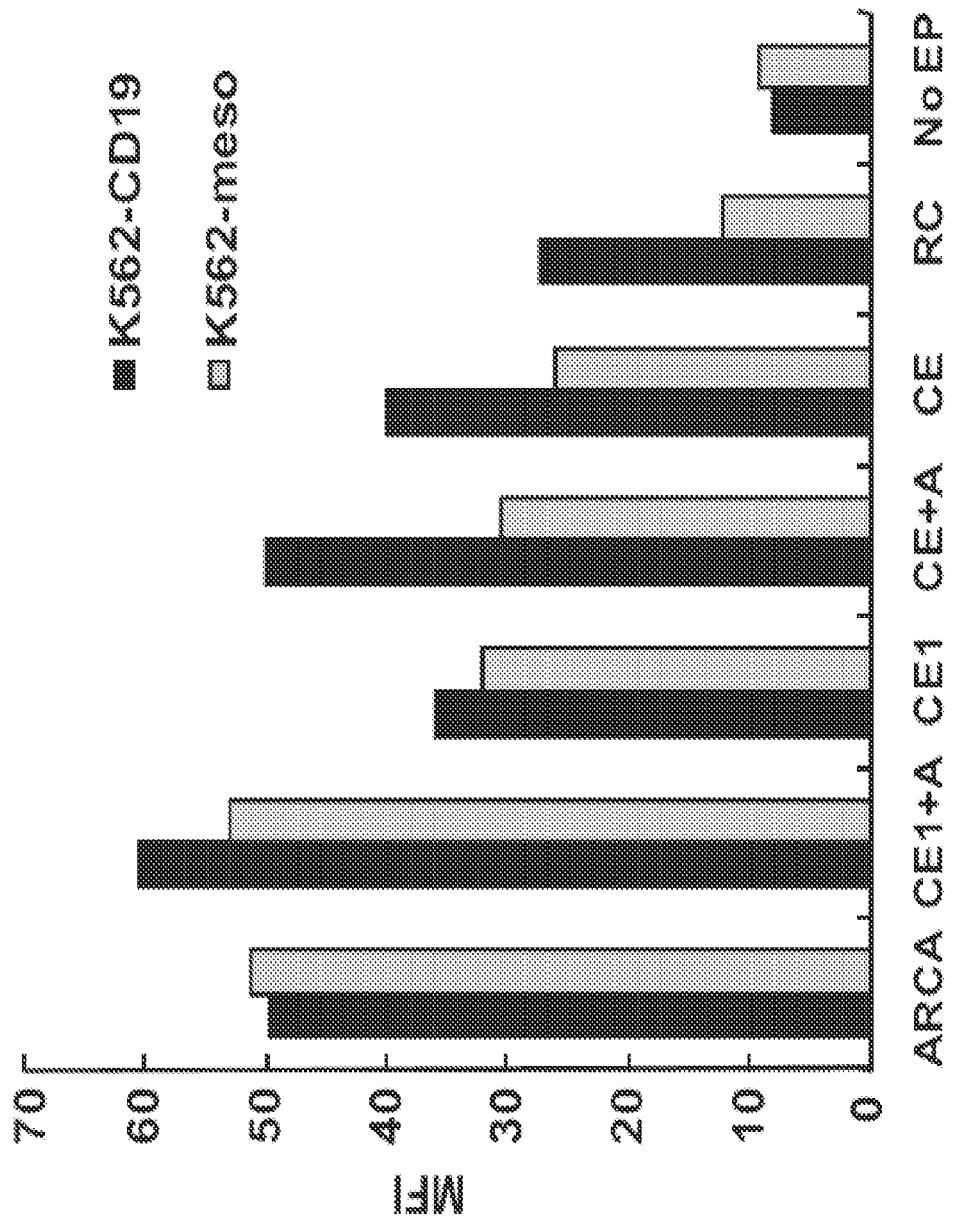

K562-CD19
K562-meso
MFI
70
60
50
40
30
20
10
0
ARCA
CE1+A
CE1
CE+A
CE
RC
No EP

Figure 7

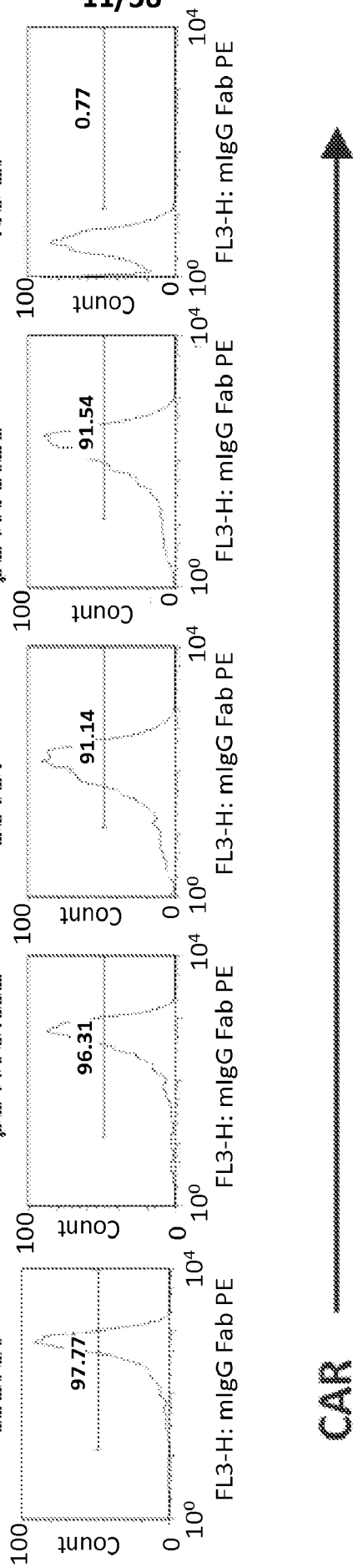

ss1.OF
pDrive.ss1
19.OF
pDrive.19
No EP
97.77
96.31
91.14
91.54
0.77
Count
FL3-H: mIgG Fab PE
CAR

Figure 8

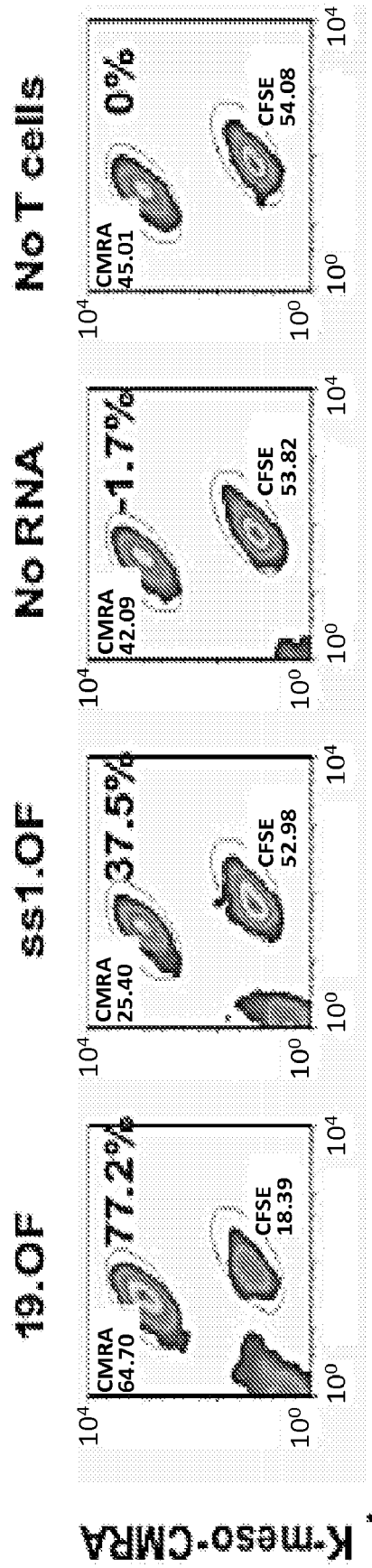

19.OF
ss1.OF
No RNA
No T cells
K-meso-CMRA
KCD19-CFSE
CMRA 64.70
77.2%
CFSE 18.39
CMRA 25.40
37.5%
CFSE 52.98
CMRA 42.09
1.7%
CFSE 53.82
CMRA 45.01
0%
CFSE 54.08
10^4
10^0

Figure 9

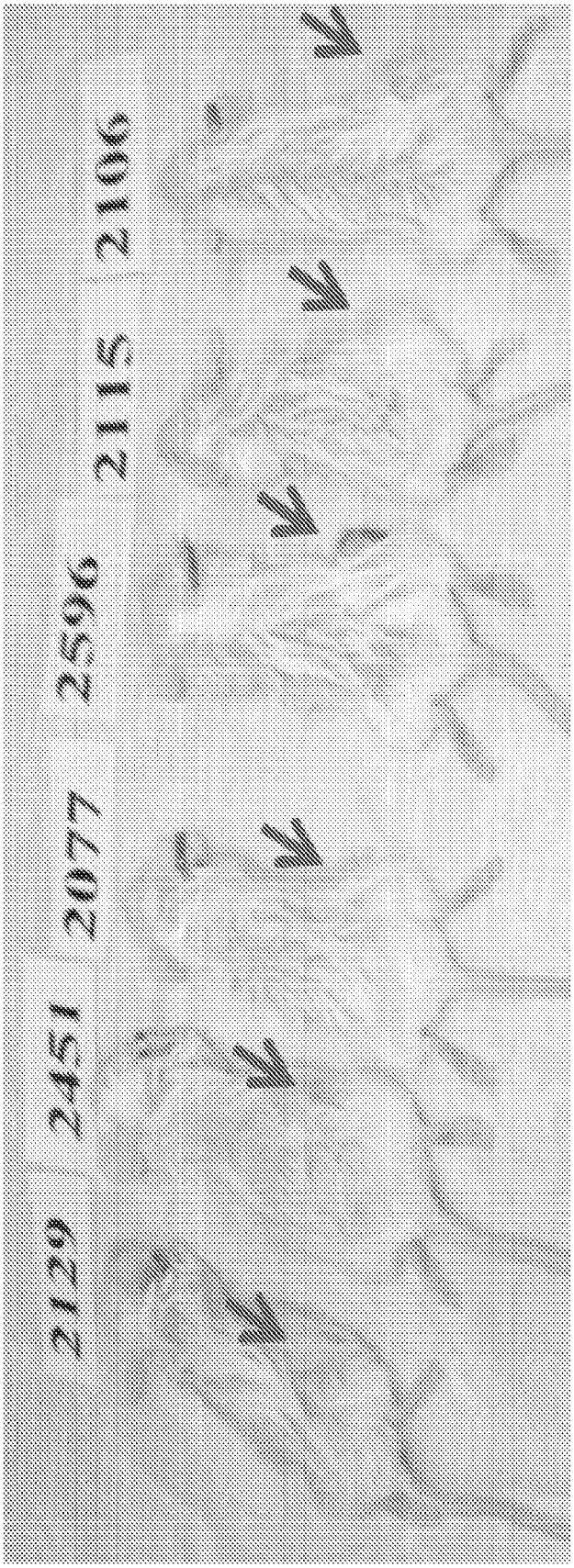


Figure 10

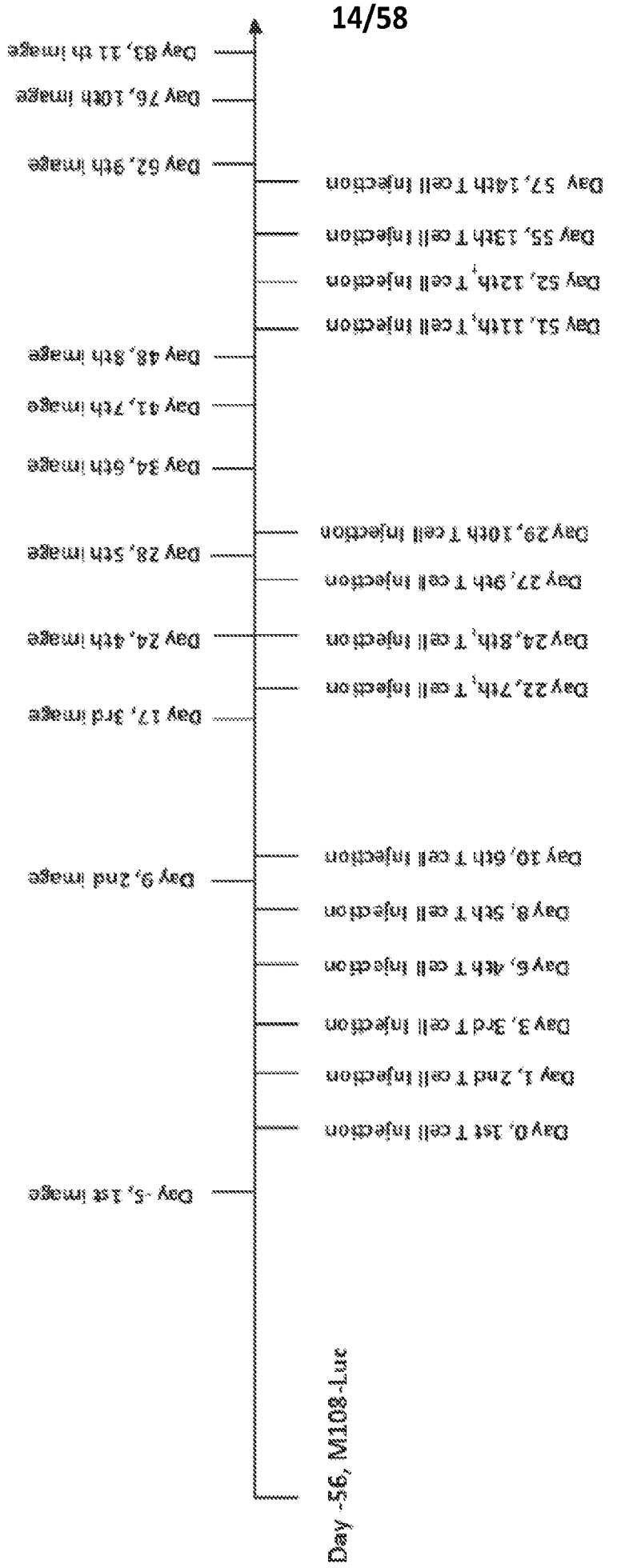
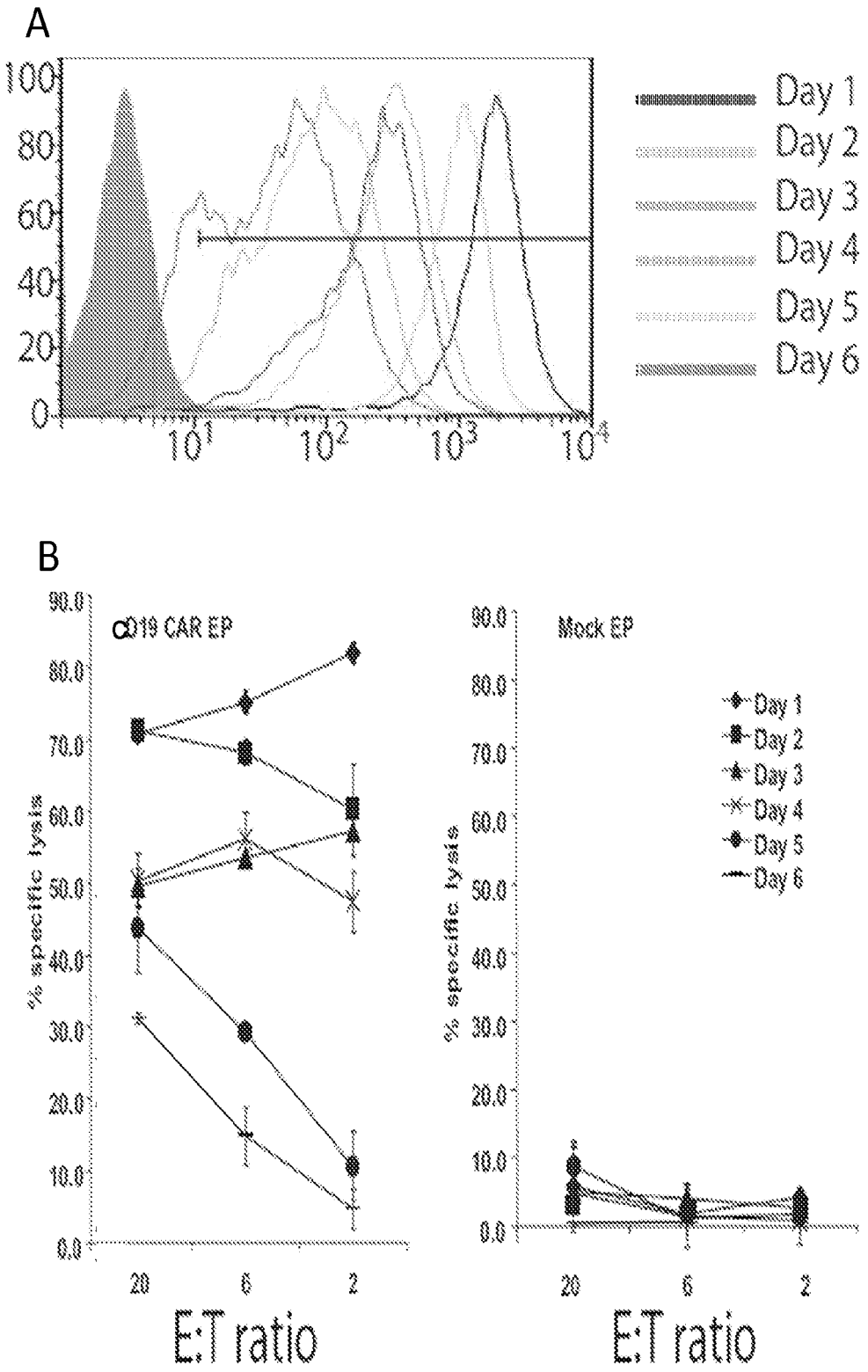
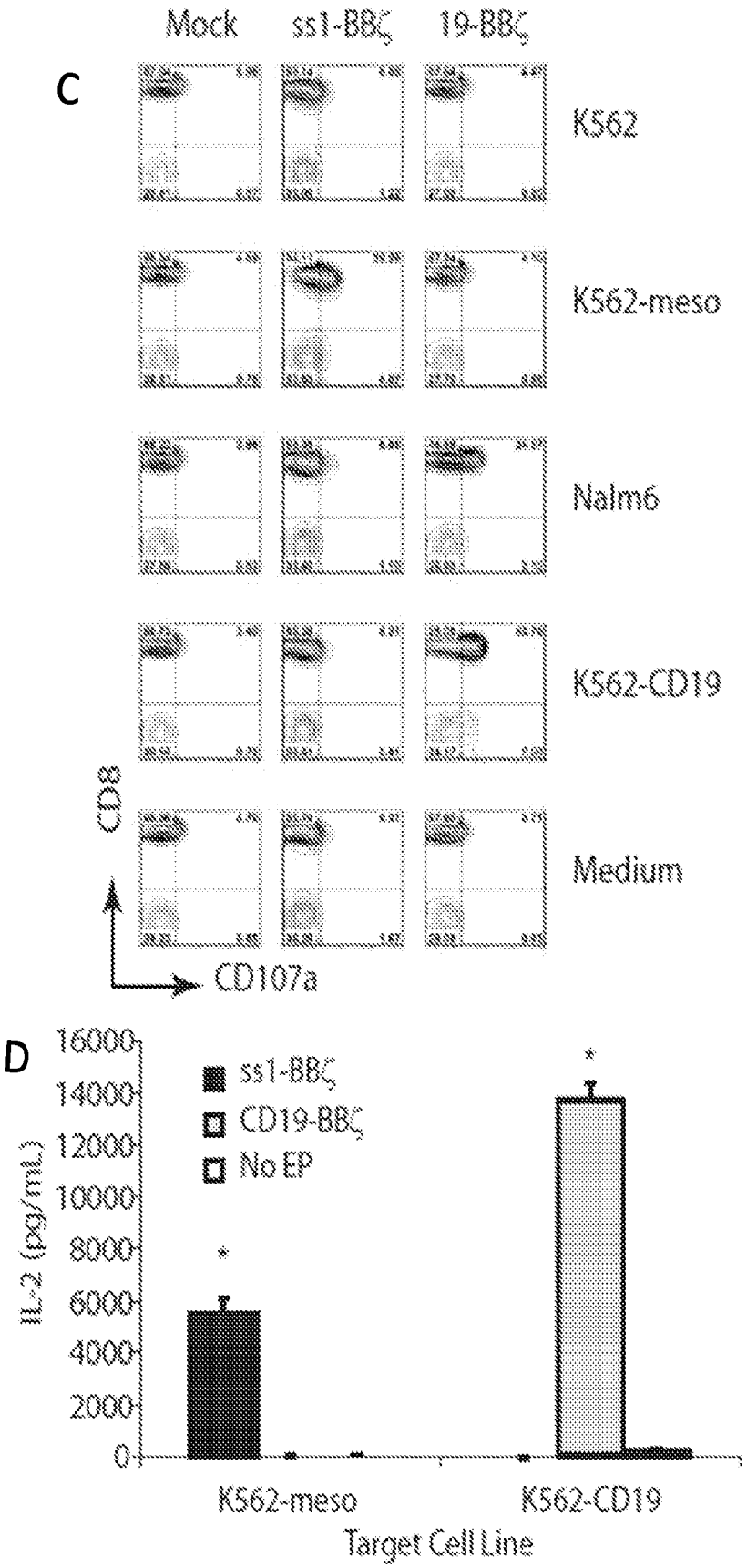

Day -36, M108-Luc
Day -5, 1st image
Day 0, 1st T cell injection
Day 1, 2nd T cell injection
Day 3, 3rd T cell injection
Day 6, 4th T cell injection
Day 8, 5th T cell injection
Day 9, 2nd image
Day 10, 6th T cell injection
Day 17, 3rd image
Day 22, 7th, T cell injection
Day 24, 8th, T cell injection
Day 24, 4th image
Day 27, 9th T cell injection
Day 28, 5th image
Day 29, 10th T cell injection
Day 34, 6th image
Day 41, 7th image
Day 48, 8th image
Day 51, 11th, T cell injection
Day 52, 12th, T cell injection
Day 55, 13th T cell injection
Day 57, 14th T cell injection
Day 62, 9th image
Day 76, 10th image
Day 83, 11 th image

Figure 11



Figures 12A-12B



Figures 12C-12D

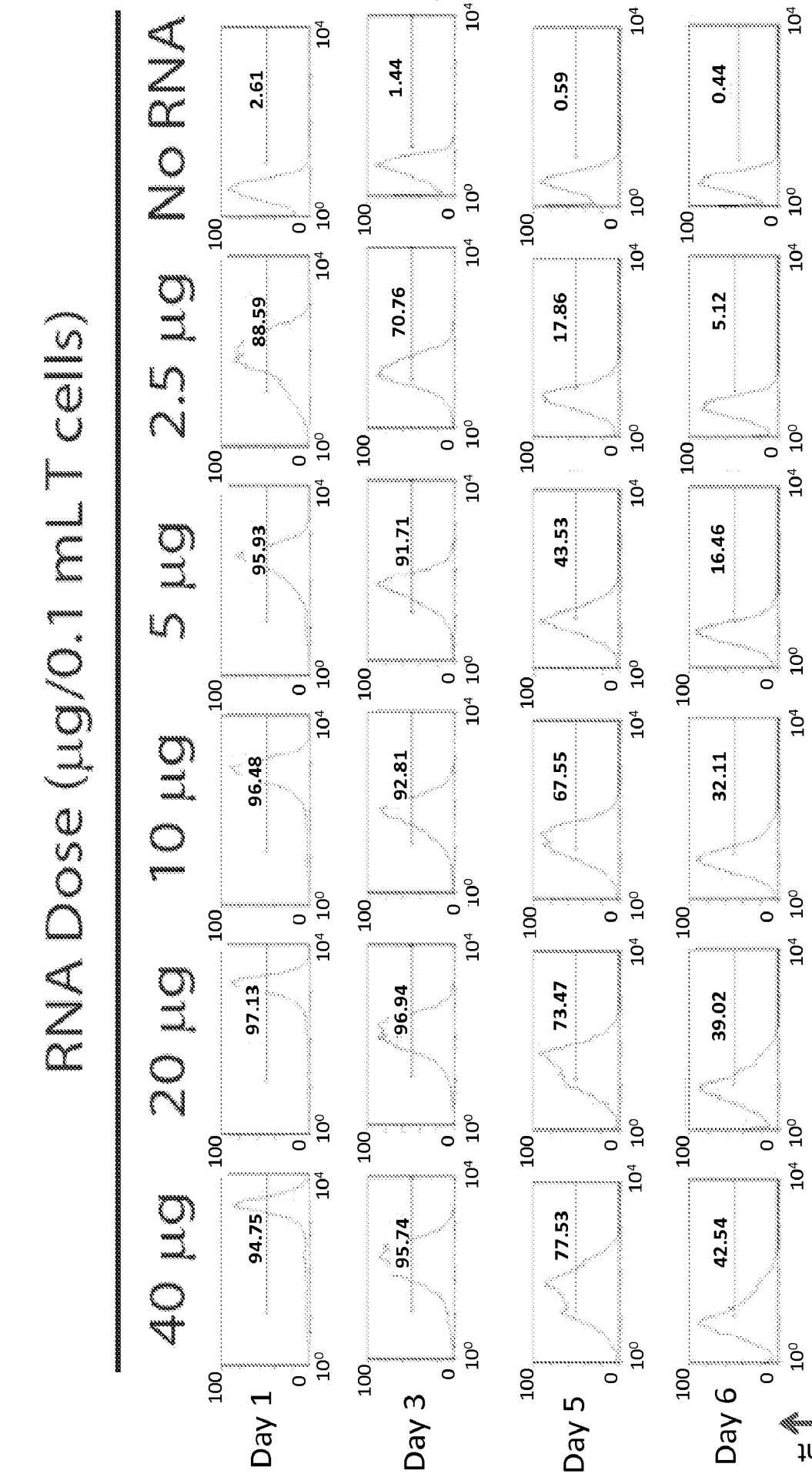
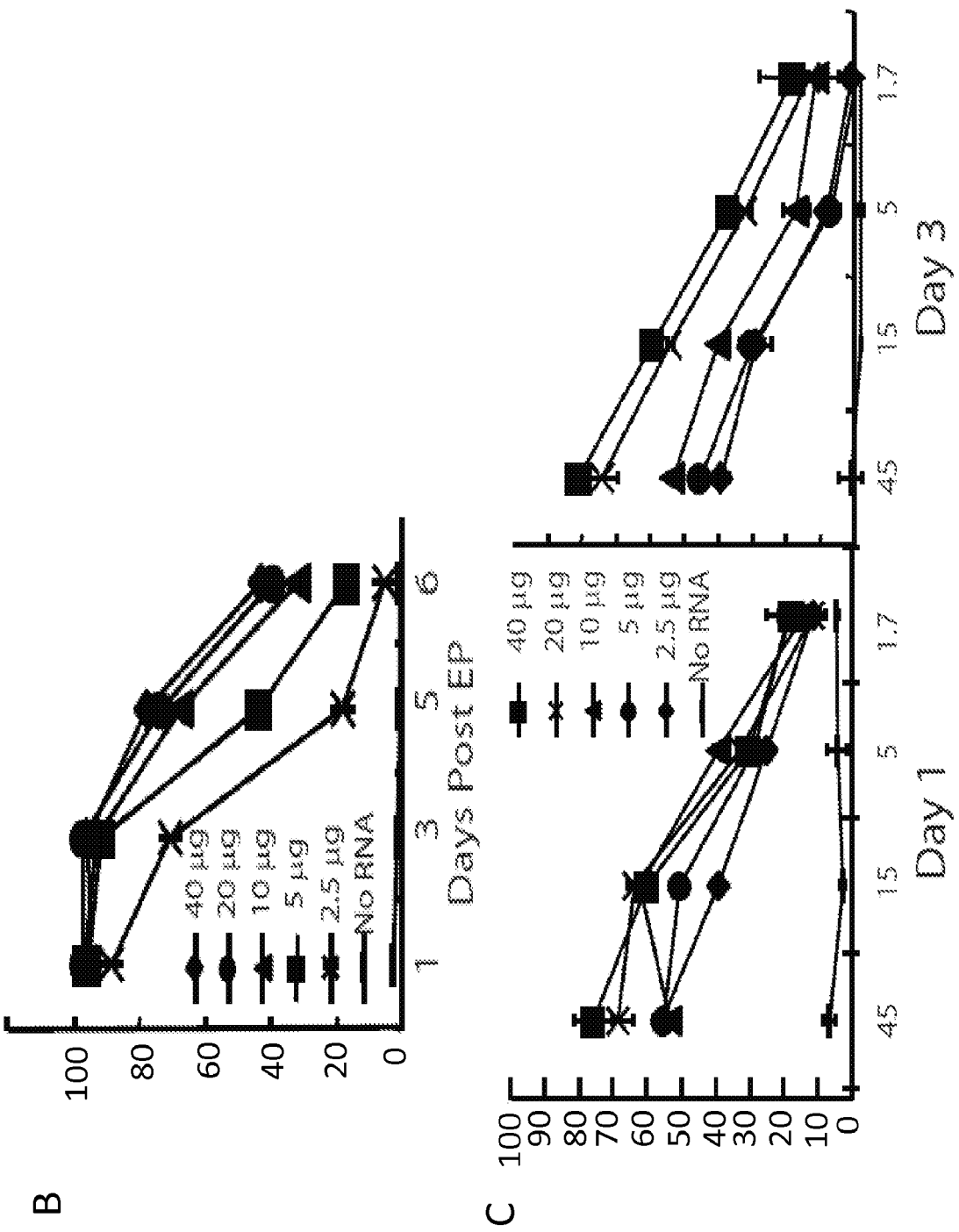

RNA Dose (µg/0.1 mL T cells)
40 µg
20 µg
10 µg
5 µg
2.5 µg
No RNA
Day 1
Day 3
Day 5
Day 6
94.75
97.13
96.48
95.93
88.59
2.61
95.74
96.94
92.81
91.71
70.76
1.44
77.53
73.47
67.55
43.53
17.86
0.59
42.54
39.02
32.11
16.46
5.12
0.44
Count
FL3-H: mIgG Fab PE

Figure 13A



Figures 13B-13C

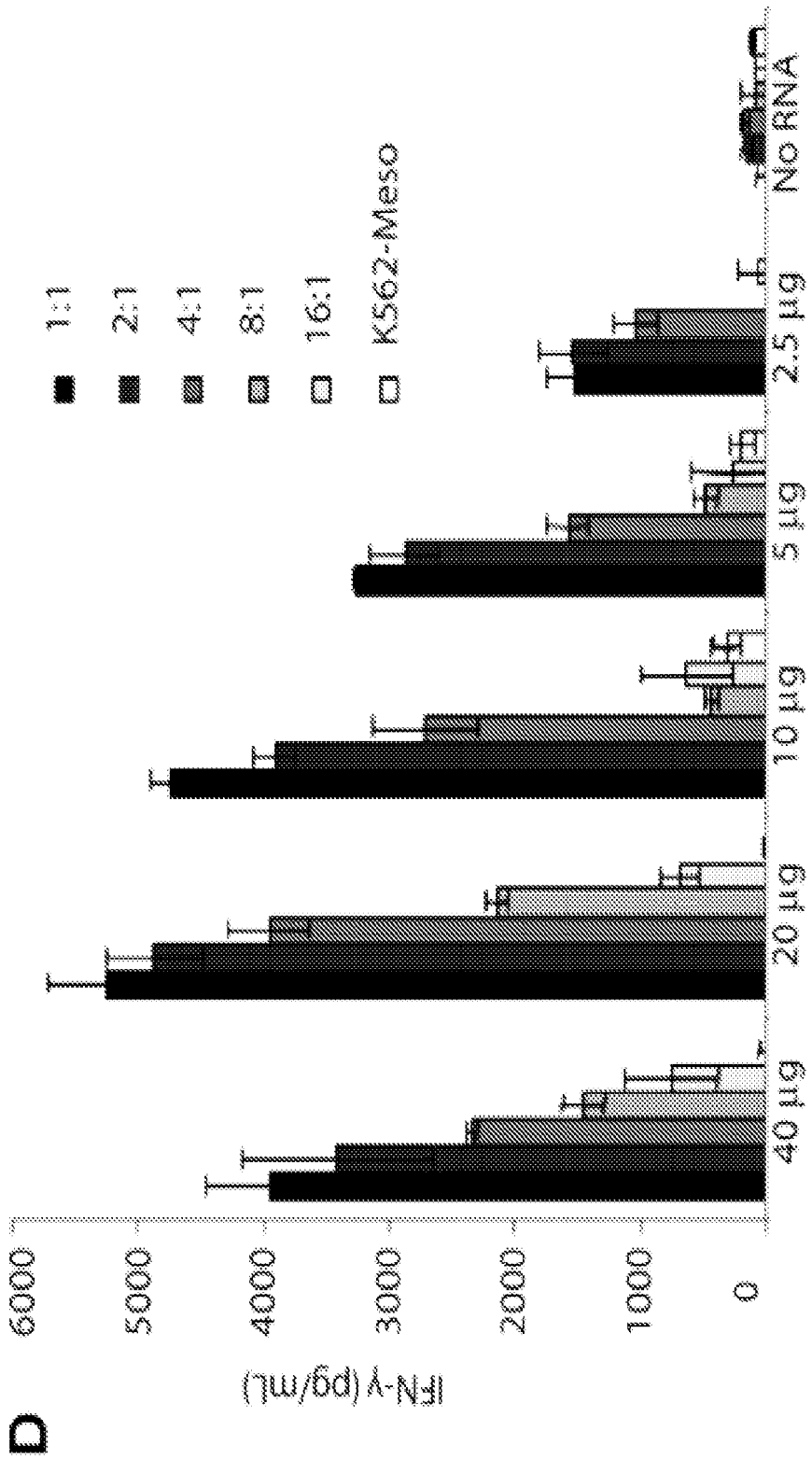

D
IFN-γ (pg/mL)
6000
5000
4000
3000
2000
1000
0
40 µg
20 µg
10 µg
5 µg
2.5 µg
No RNA
1:1
2:1
4:1
8:1
16:1
K562-Meso

Figure 13D

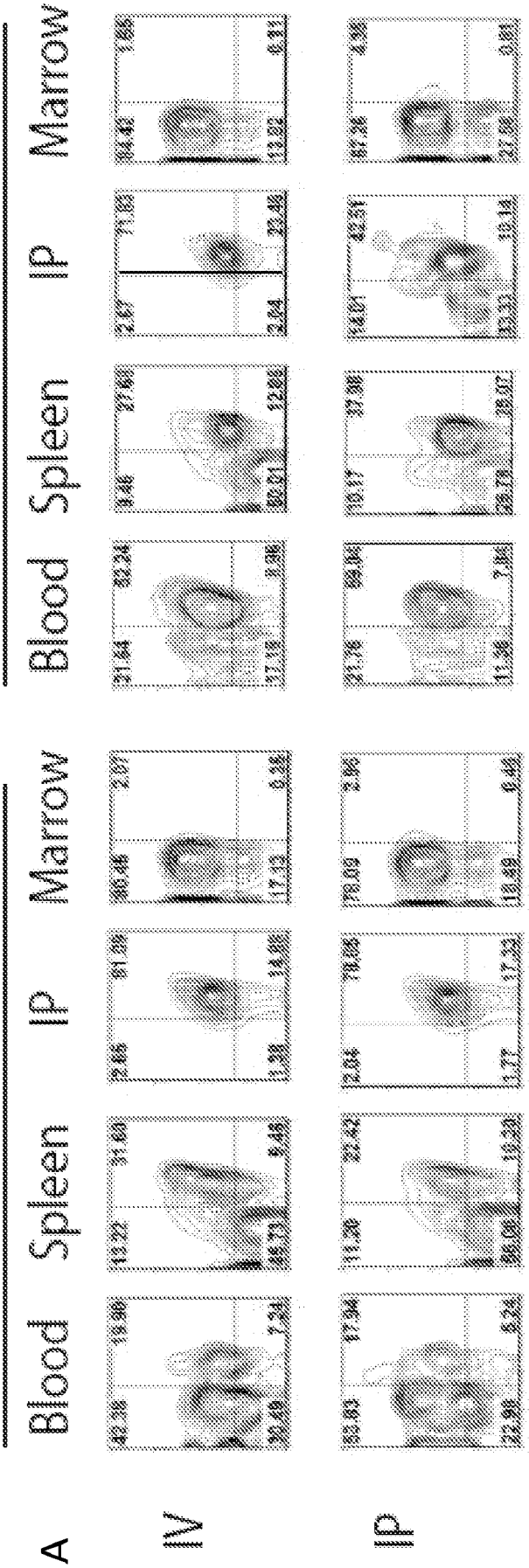

A
IV
IP
CD19-BBz
meso-BBz
Blood
Spleen
IP
Marrow
Blood
Spleen
IP
Marrow
CD19-BBz
meso-BBz
no RNA
In vitro cultured PBLs
hCD3

Figure 14A

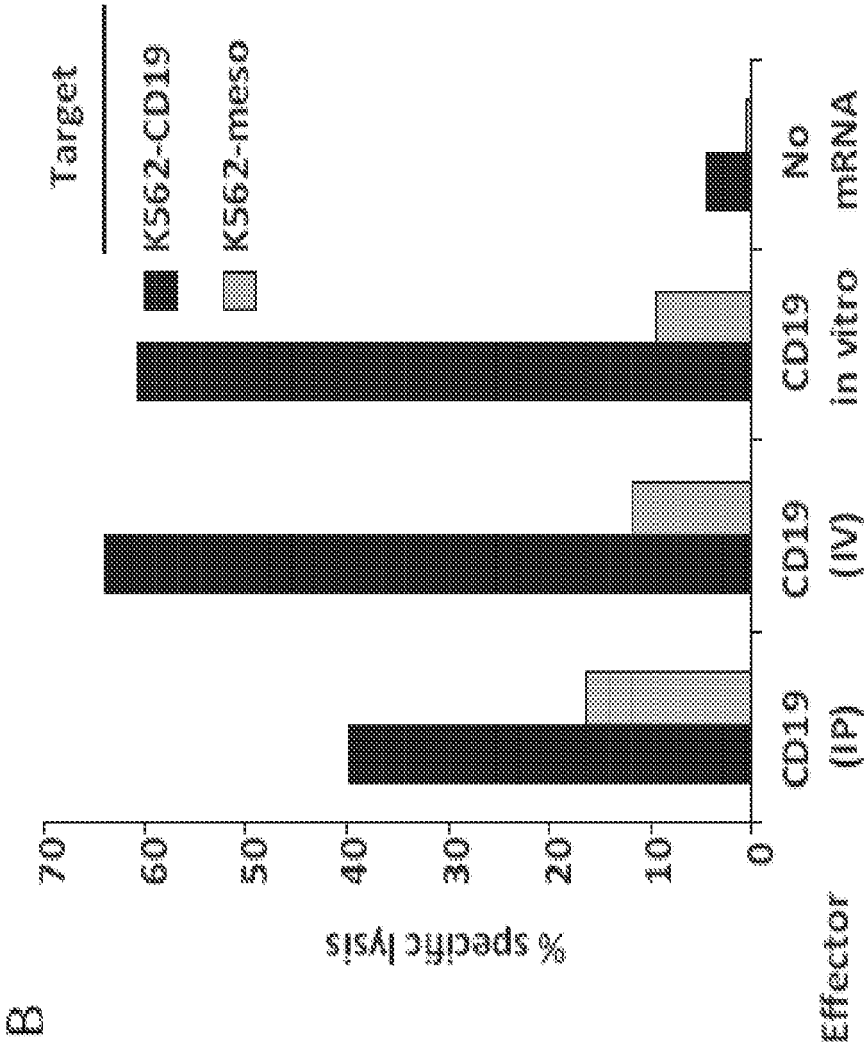

B
% specific lysis
70
60
50
40
30
20
10
0
Target
K562-CD19
K562-meso
CD19 (IP)
CD19 (IV)
CD19 in vitro
No mRNA
Effector

Figure 14B

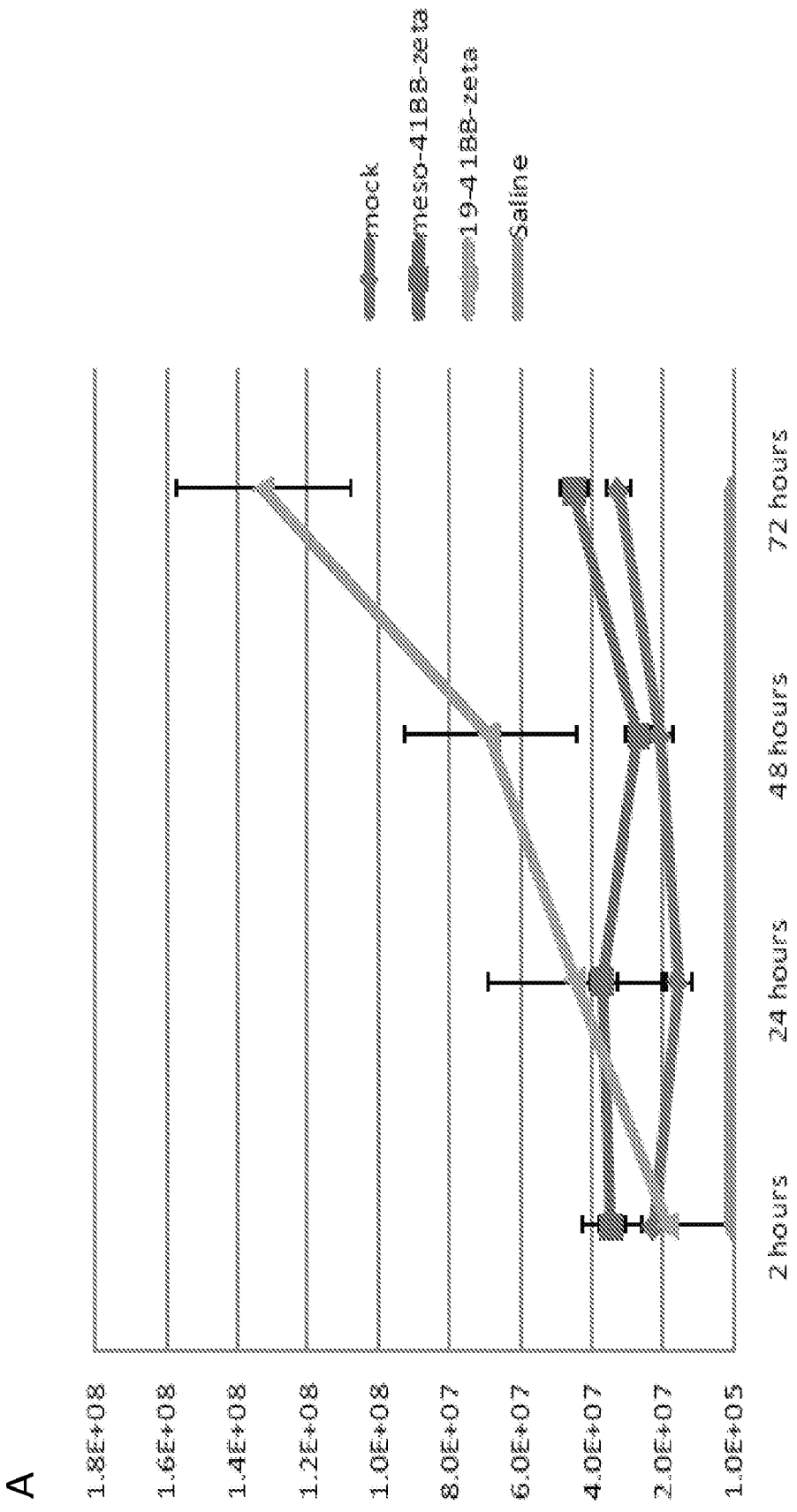

A
1.8E+08
1.6E+08
1.4E+08
1.2E+08
1.0E+08
8.0E+07
6.0E+07
4.0E+07
2.0E+07
1.0E+05
2 hours
24 hours
48 hours
72 hours
mock
meso-4188-zeta
19-4188-zeta
Saline

Figure 15A

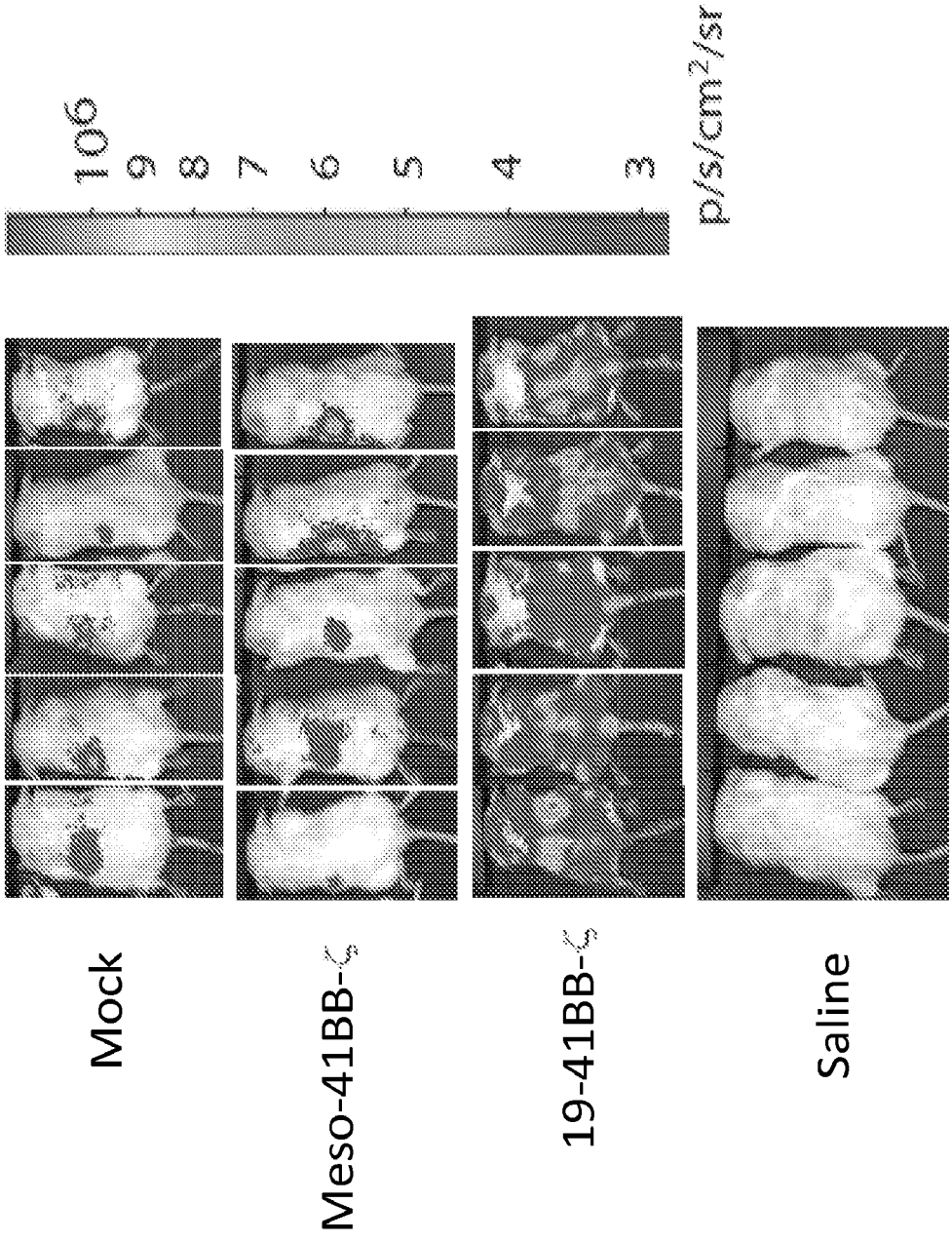


Figure 15B

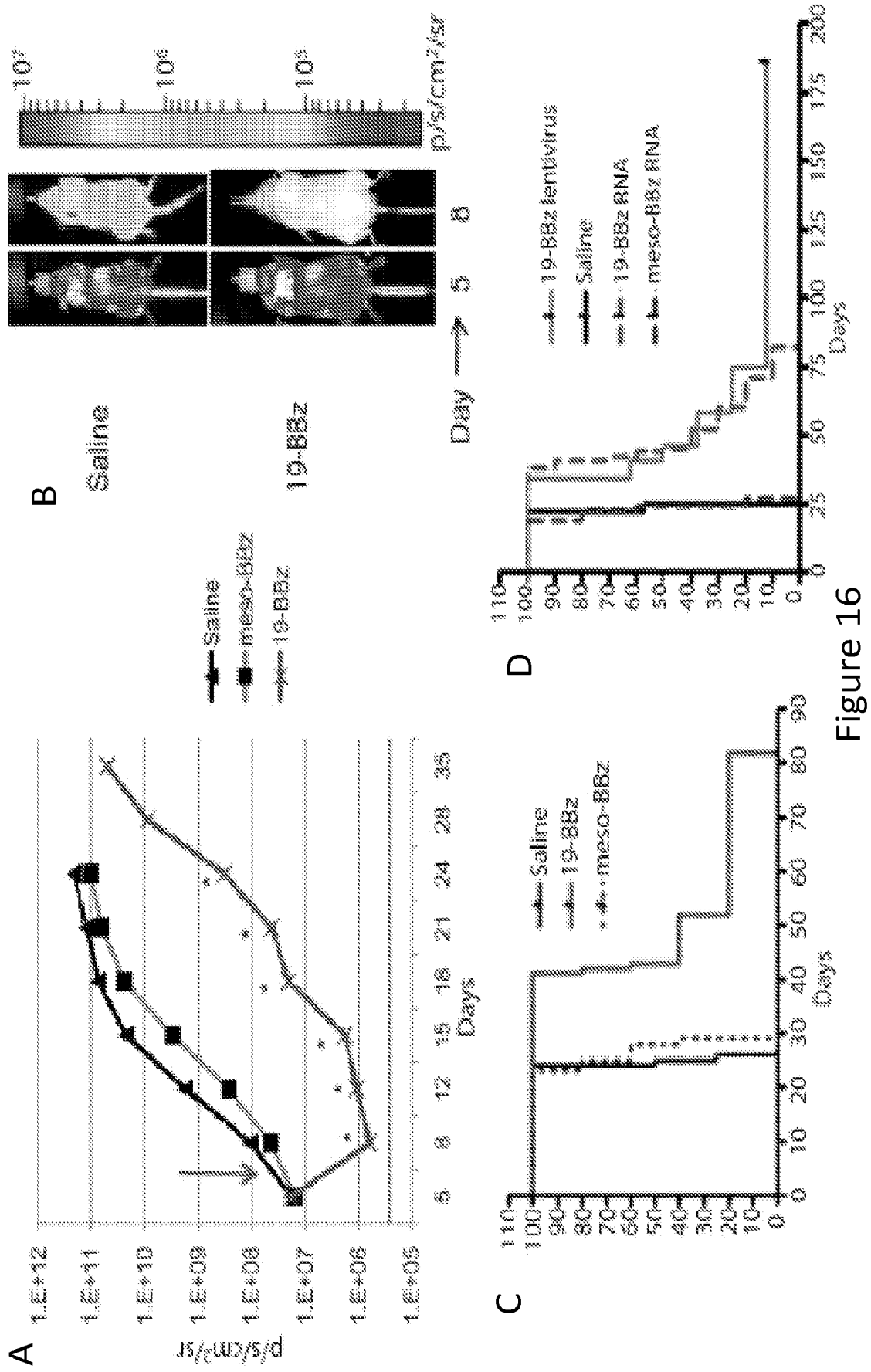

A
p/s/cm²/sr
Days
Saline
meso-BBz
19-BBz
B
Saline
19-BBz
Day
p/s/cm²/sr
C
Saline
19-BBz
meso-BBz
Days
D
19-BBz lentivirus
Saline
19-BBz RNA
meso-BBz RNA
Days

Figure 16

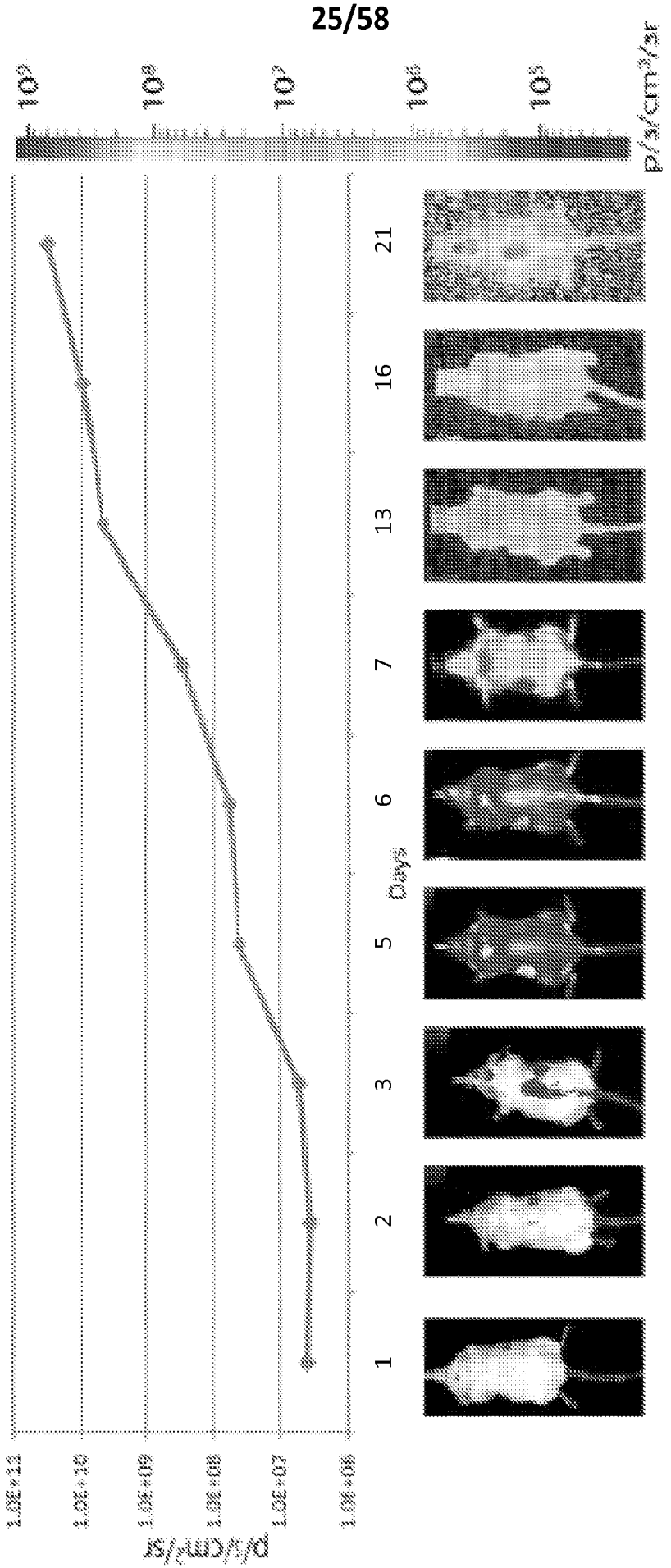

p/s/cm²/sr
10⁹
10⁸
10⁷
10⁶
10⁵
Days
1
2
3
5
6
7
13
16
21
p/s/cm²/sr
1.0E+11
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06

Figure 17

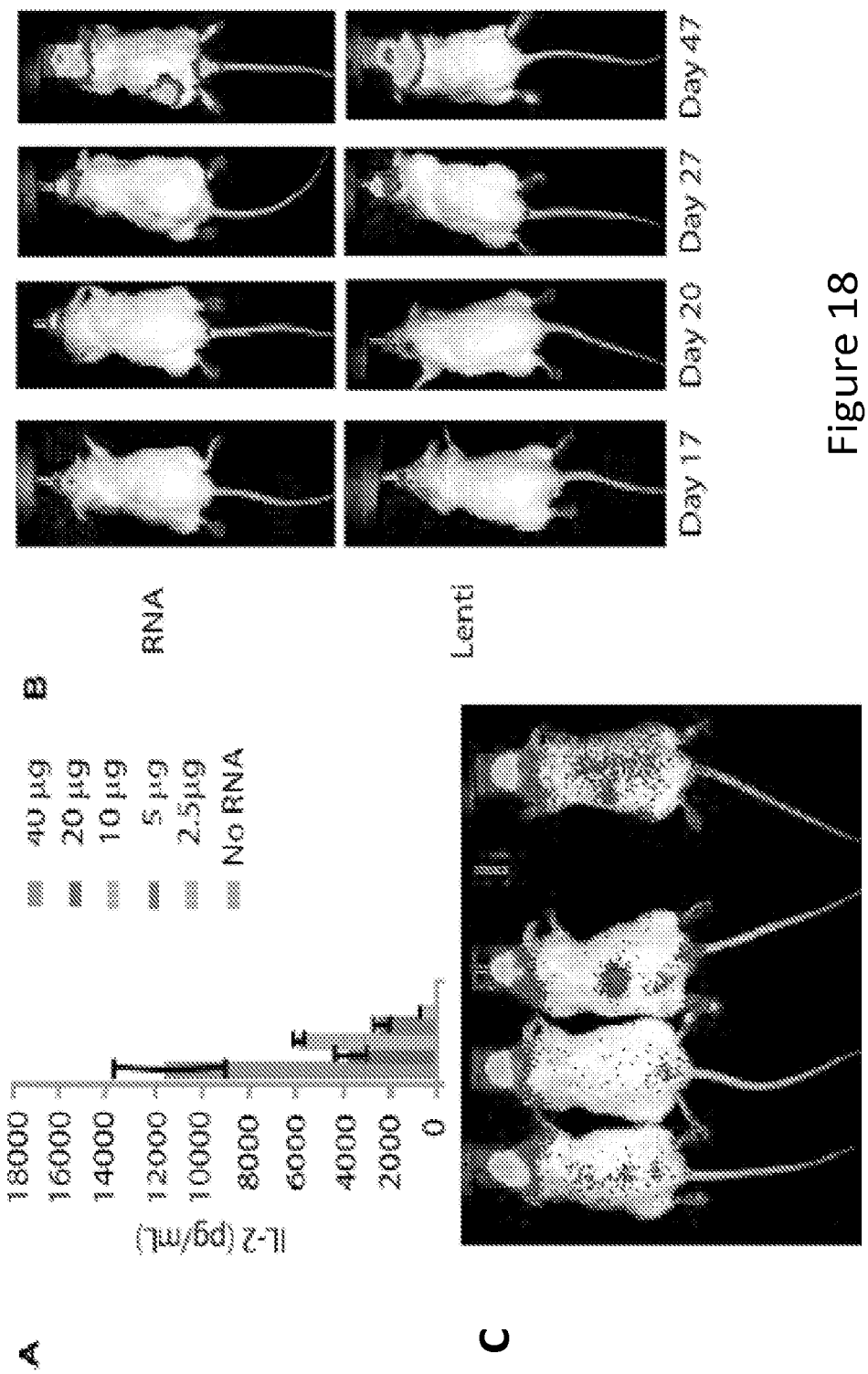
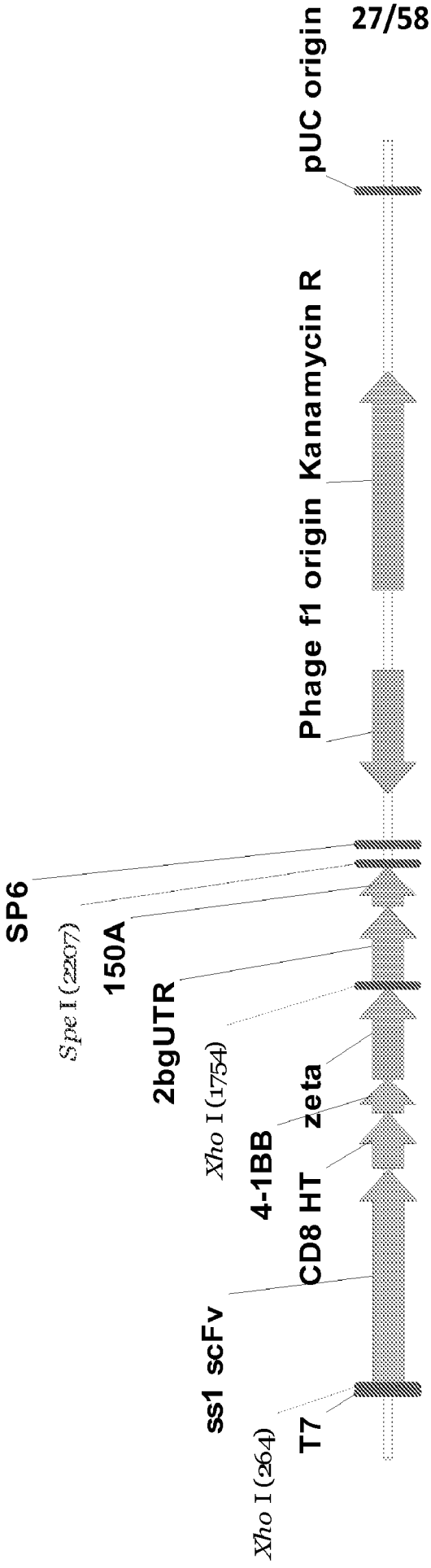

A
B
C
RNA
Lenti
Day 17
Day 20
Day 27
Day 47
40 µg
20 µg
10 µg
5 µg
2.5µg
No RNA
IL-2 (pg/mL)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0

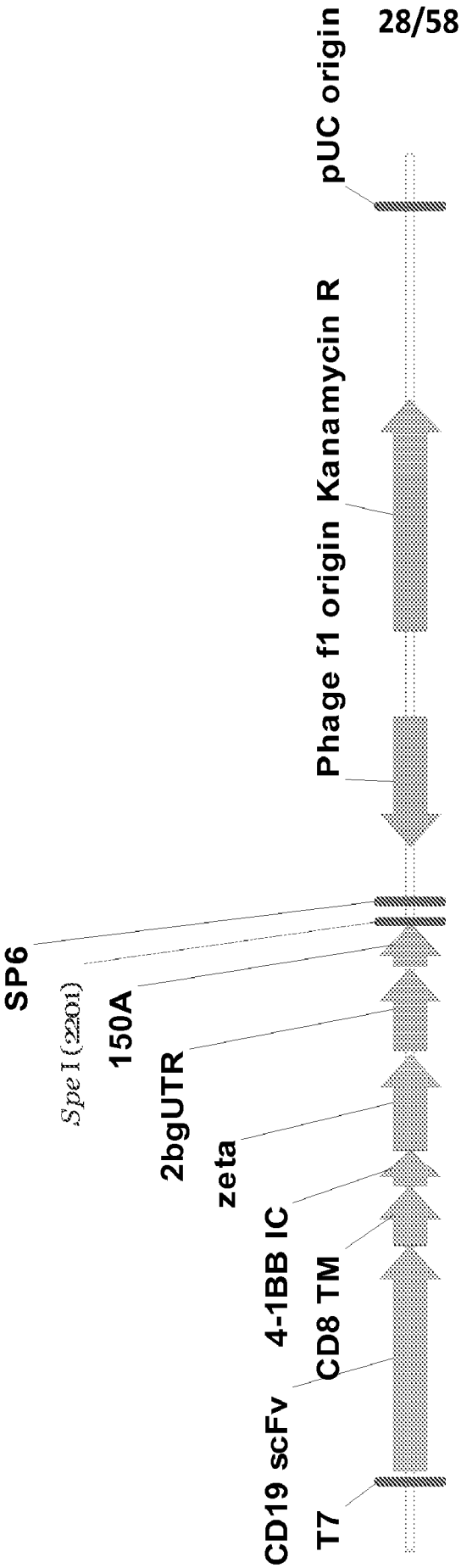
Figure 18



pD-A. ss1.OF.2bg.150A

4893 bp

Figure 19



pD-A.19.OF.2bg.150A.

4888 bp

Figure 20

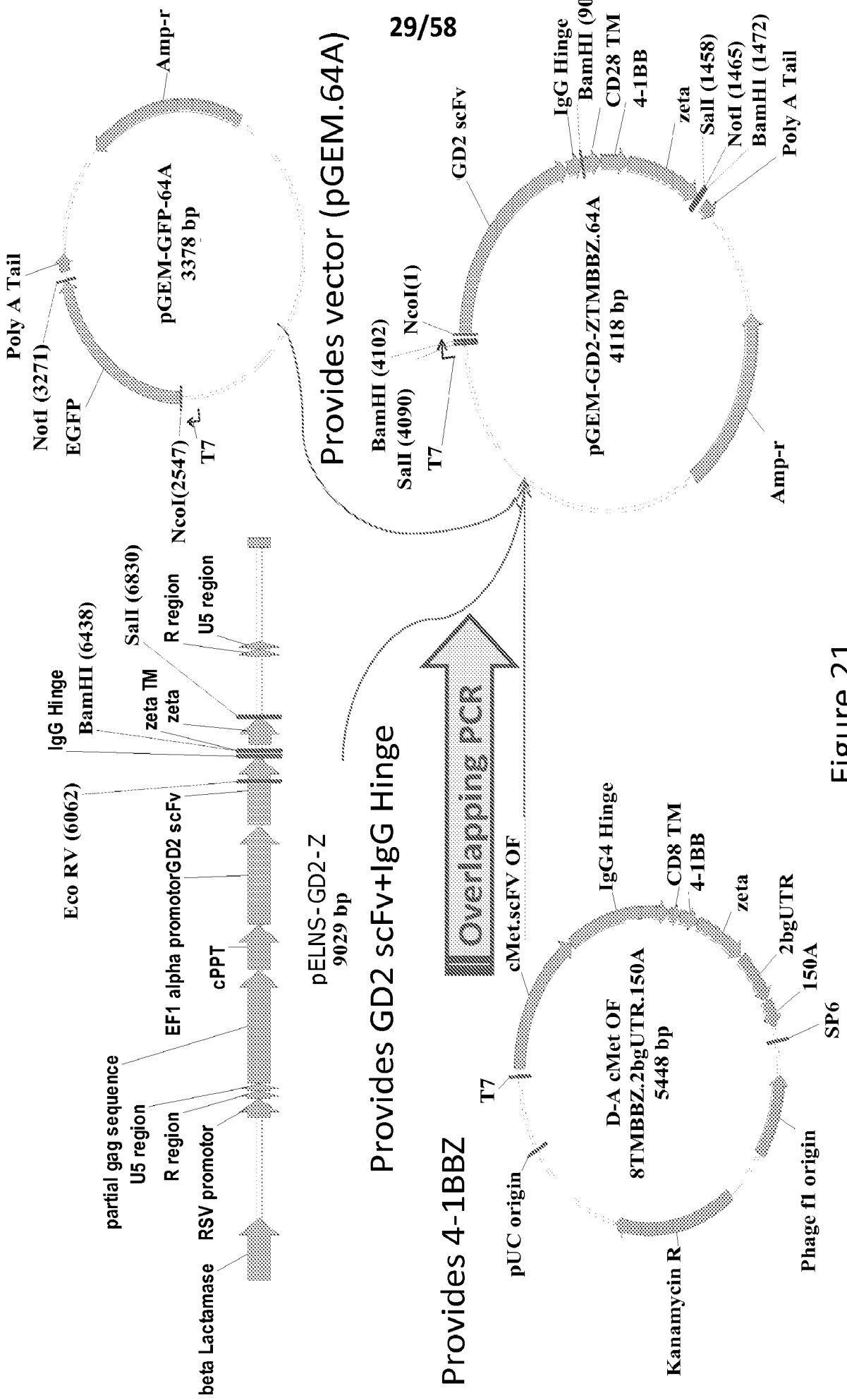

Provides vector (pGEM.64A)
pGEM-GFP-64A
3378 bp
Amp-r
Poly A Tail
NotI (3271)
EGFP
NcoI(2547)
T7
pGEM-GD2-ZTMBBZ.64A
4118 bp
GD2 scFv
NcoI(1)
BamHI (4102)
SalI (4090)
T7
IgG Hinge
BamHI (903
CD28 TM
4-1BB
zeta
SalI (1458)
NotI (1465)
BamHI (1472)
Poly A Tail
Amp-r
Provides GD2 scFv+IgG Hinge
pELNS-GD2-Z
9029 bp
Eco RV (6062)
partial gag sequence
U5 region
R region
RSV promotor
beta Lactamase
EF1 alpha promotor
GD2 scFv
cPPT
IgG Hinge
BamHI (6438)
zeta TM
zeta
SalI (6830)
R region
U5 region
Overlapping PCR
cMet.scFV OF
Provides 4-1BBZ
D-A cMet OF
8TMBBZ.2bgUTR.150A
5448 bp
T7
pUC origin
Kanamycin R
IgG4 Hinge
CD8 TM
4-1BB
zeta
2bgUTR
150A
SP6
Phage f1 origin

Figure 21

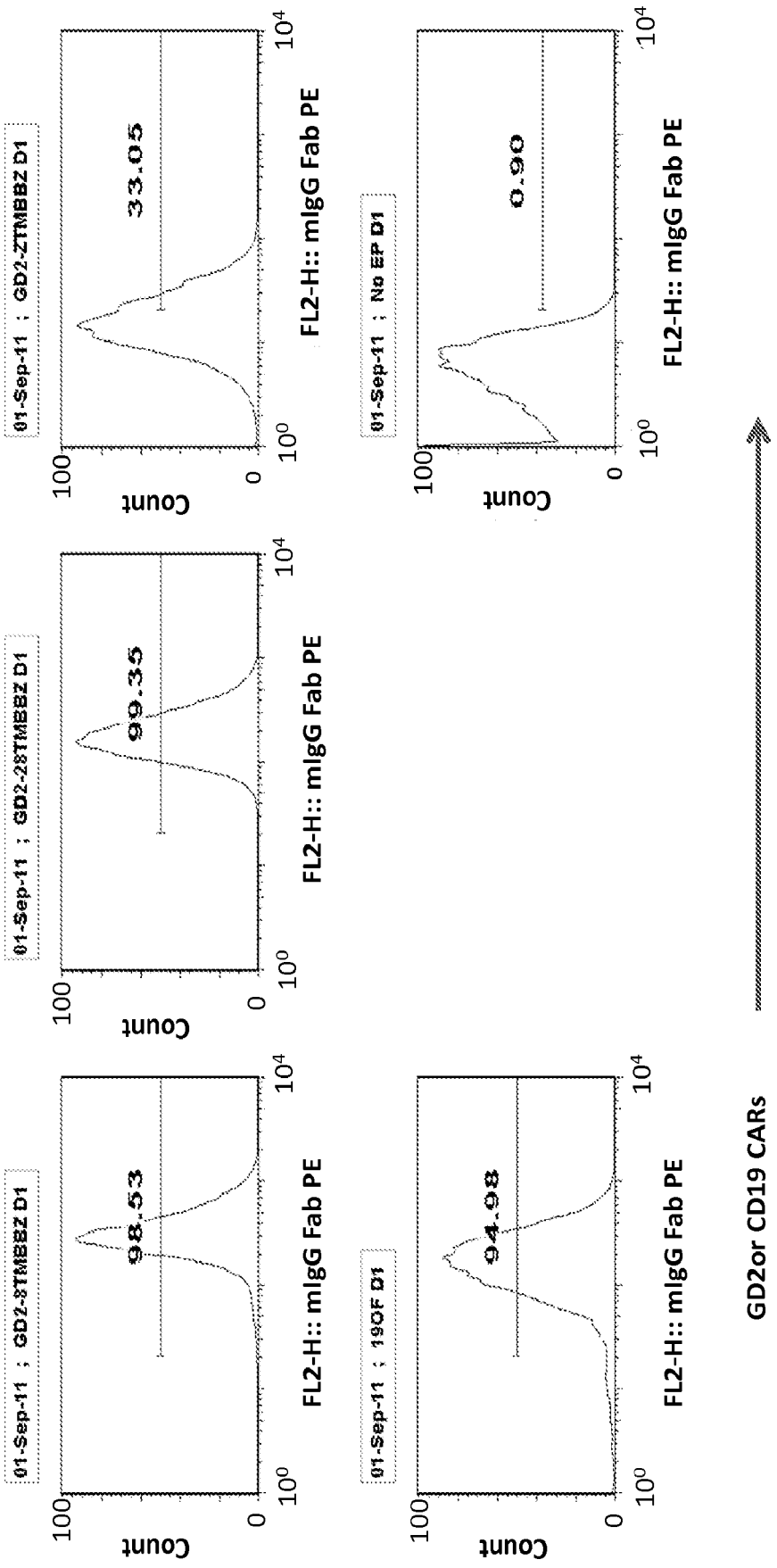

01-Sep-11 ; GD2-ZTMBBZ D1
33.05
01-Sep-11 ; No EP D1
0.90
01-Sep-11 ; GD2-28TMBBZ D1
99.35
01-Sep-11 ; GD2-8TMBBZ D1
98.53
01-Sep-11 ; 19OF D1
94.98
Count
FL2-H:: mIgG Fab PE
GD2or CD19 CARs

Figure 22

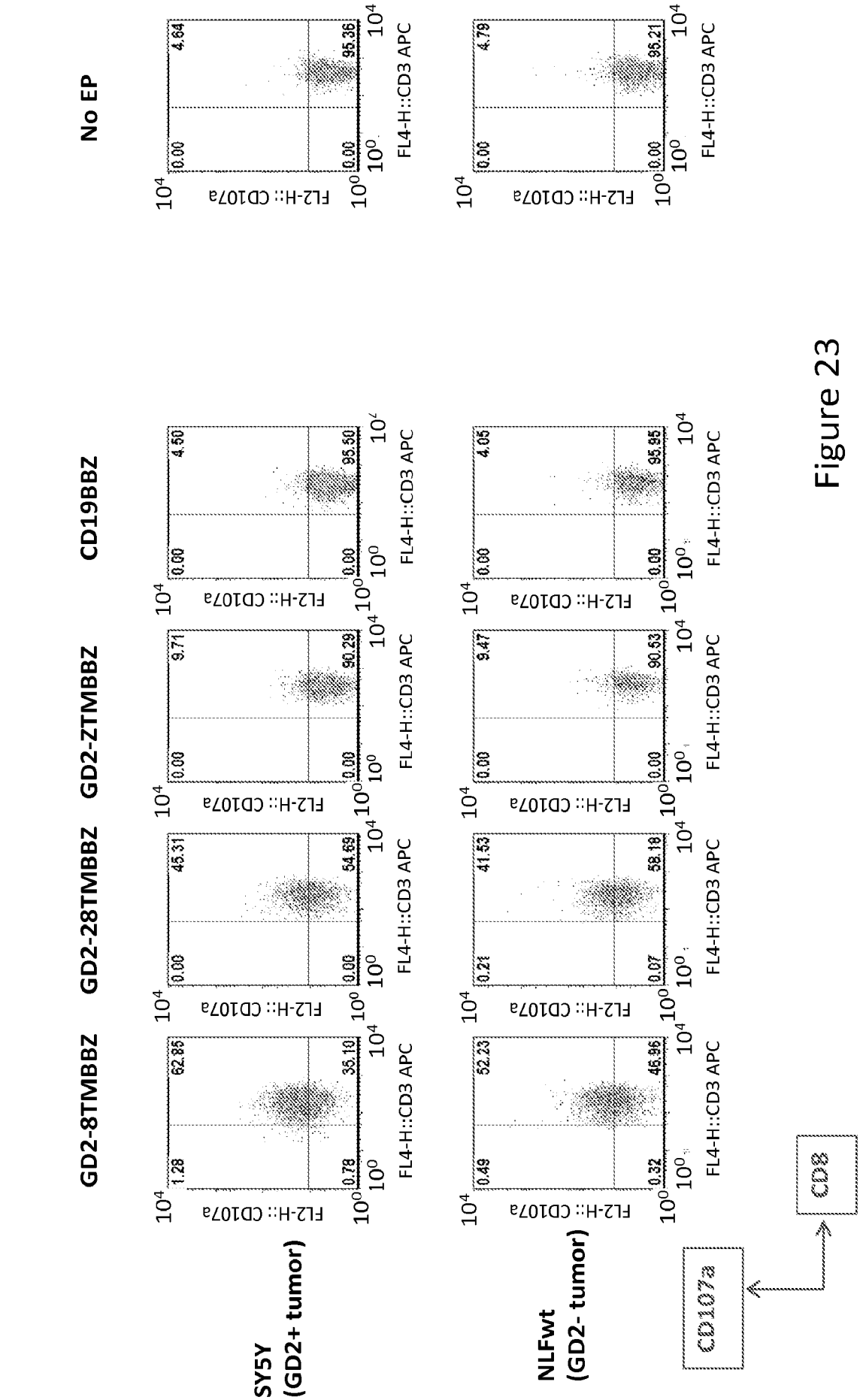

GD2-8TMBBZ
GD2-28TMBBZ
GD2-ZTMBBZ
CD19BBZ
No EP
SY5Y
(GD2+ tumor)
NLFwt
(GD2- tumor)
FL2-H:: CD107a
FL4-H::CD3 APC
CD107a
CD8

Figure 23

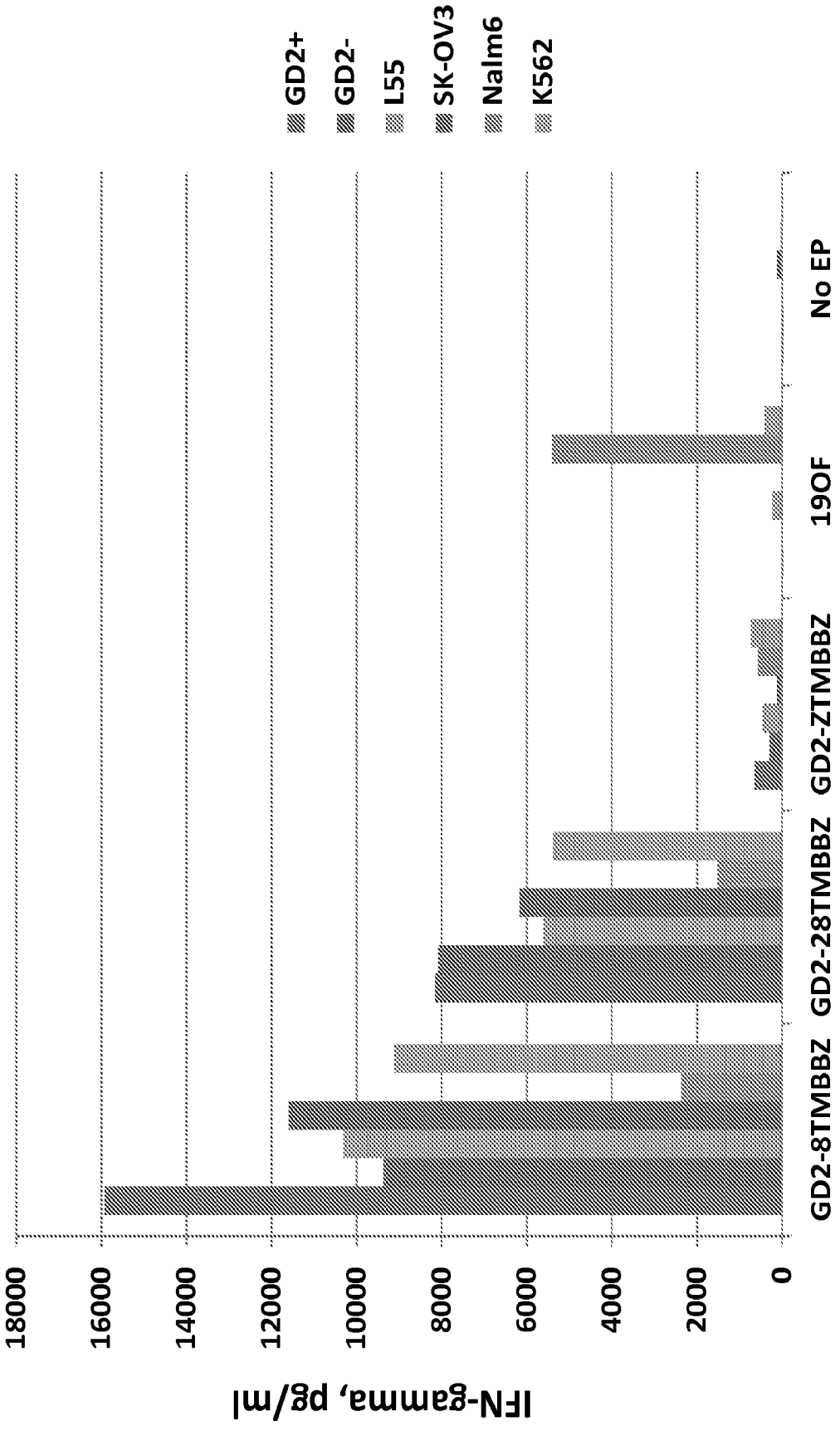

GD2+
GD2-
L55
SK-OV3
Nalm6
K562
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
IFN-gamma, pg/ml
GD2-8TMBBZ
GD2-28TMBBZ
GD2-ZTMBBZ
19OF
No EP
RNA CAR electroporated T cells

Figure 24

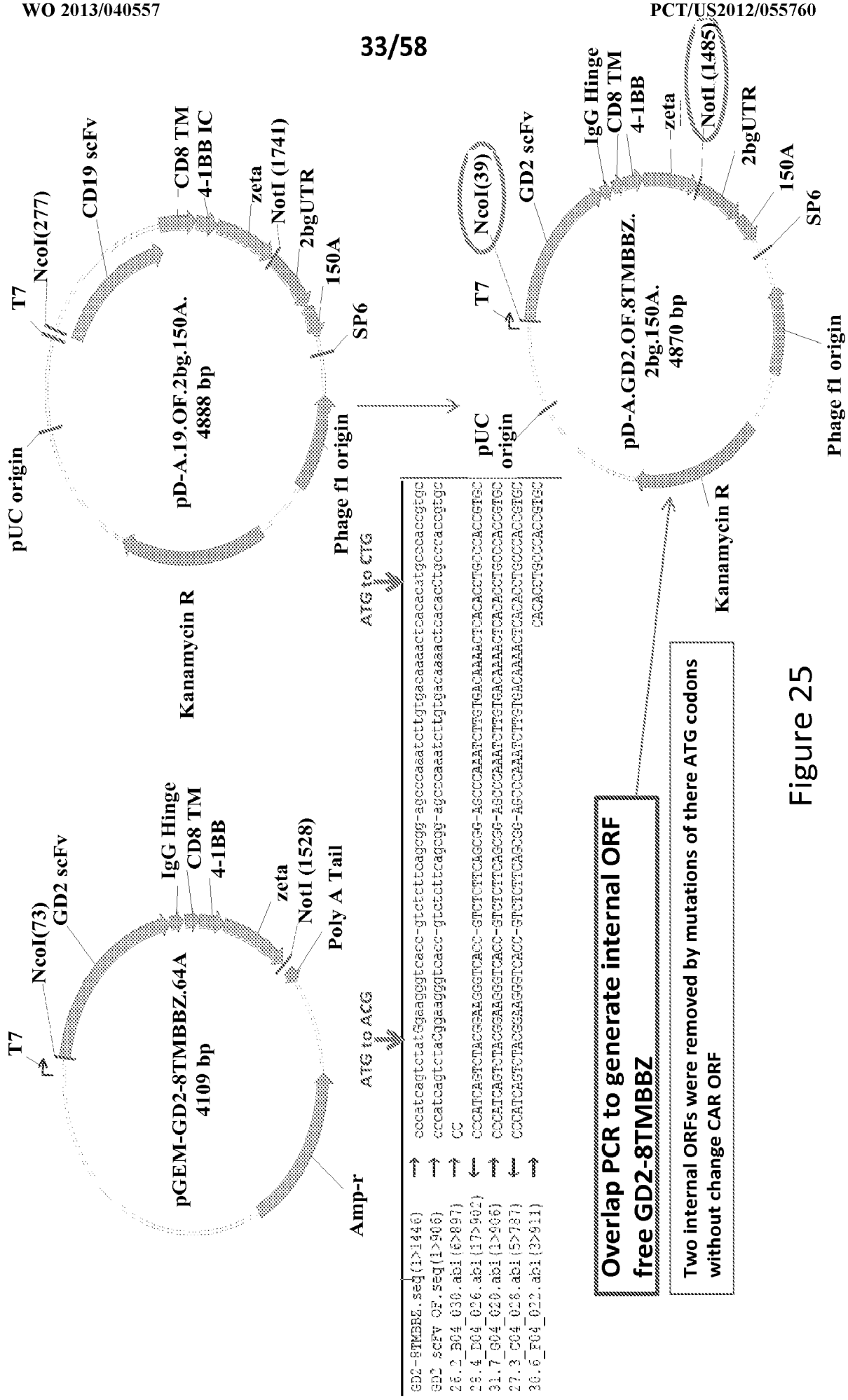
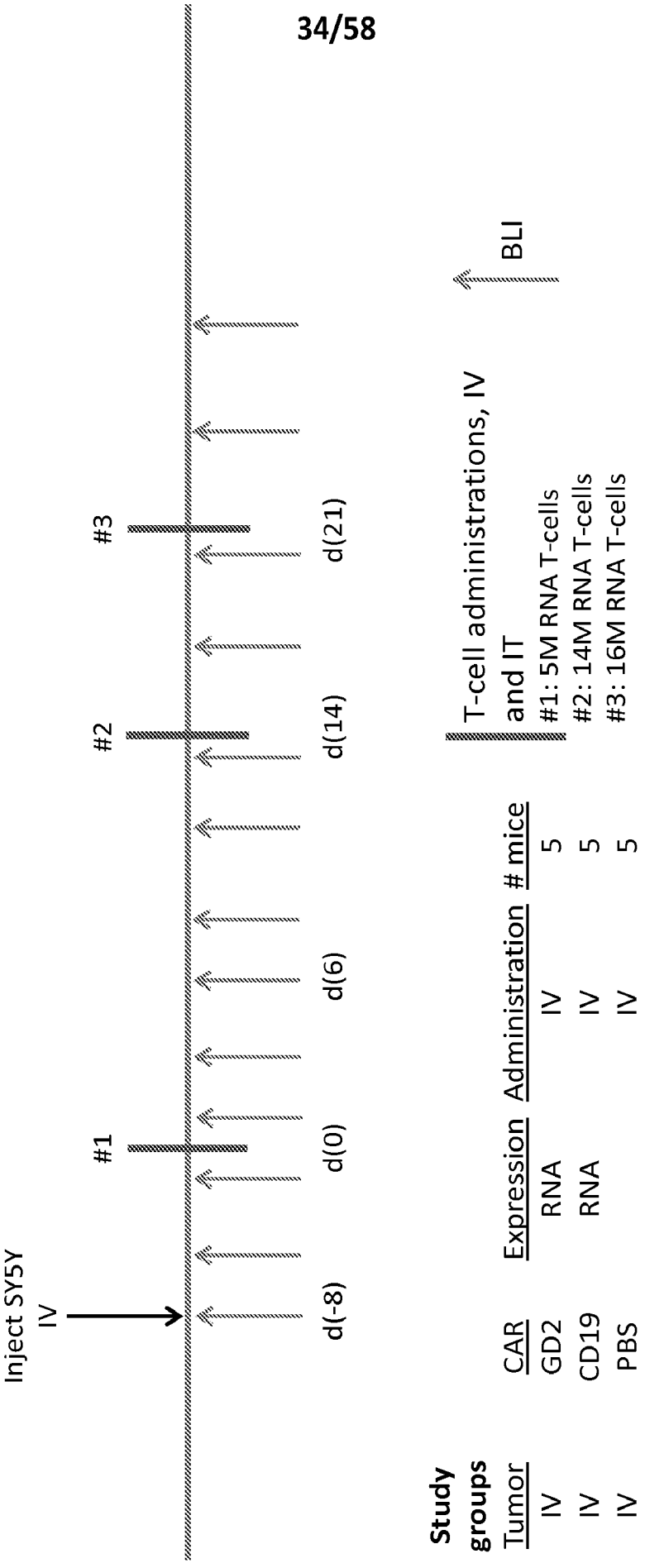

T7
NcoI(277)
CD19 scFv
CD8 TM
4-1BB IC
zeta
NotI (1741)
2bgUTR
150A
SP6
pUC origin
pD-A.19.OF.2bg.150A.
4888 bp
Kanamycin R
Phage f1 origin
ATG to CTG
T7
NcoI(39)
GD2 scFv
IgG Hinge
CD8 TM
4-1BB
zeta
NotI (1485)
2bgUTR
150A
SP6
pUC origin
pD-A.GD2.OF.8TMBBZ.
2bg.150A.
4870 bp
Kanamycin R
Phage f1 origin
T7
NcoI(73)
GD2 scFv
IgG Hinge
CD8 TM
4-1BB
zeta
NotI (1528)
Poly A Tail
pGEM-GD2-8TMBBZ.64A
4109 bp
Amp-r
ATG to ACG
GD2-8TMBBZ.seq(1>1446)
GD2 scFv OF.seq(1>906)
26.2_B04_030.ab1(6>897)
28.4_D04_026.ab1(17>902)
31.7_G04_020.ab1(1>906)
27.3_C04_028.ab1(5>787)
30.6_F04_022.ab1(3>911)
Overlap PCR to generate internal ORF free GD2-8TMBBZ
Two internal ORFs were removed by mutations of there ATG codons without change CAR ORF

Figure 25



Study groups

Tumor	CAR	Expression	Administration	# mice
IV	GD2	RNA	IV	5
IV	CD19	RNA	IV	5
IV	PBS		IV	5

Figure 26

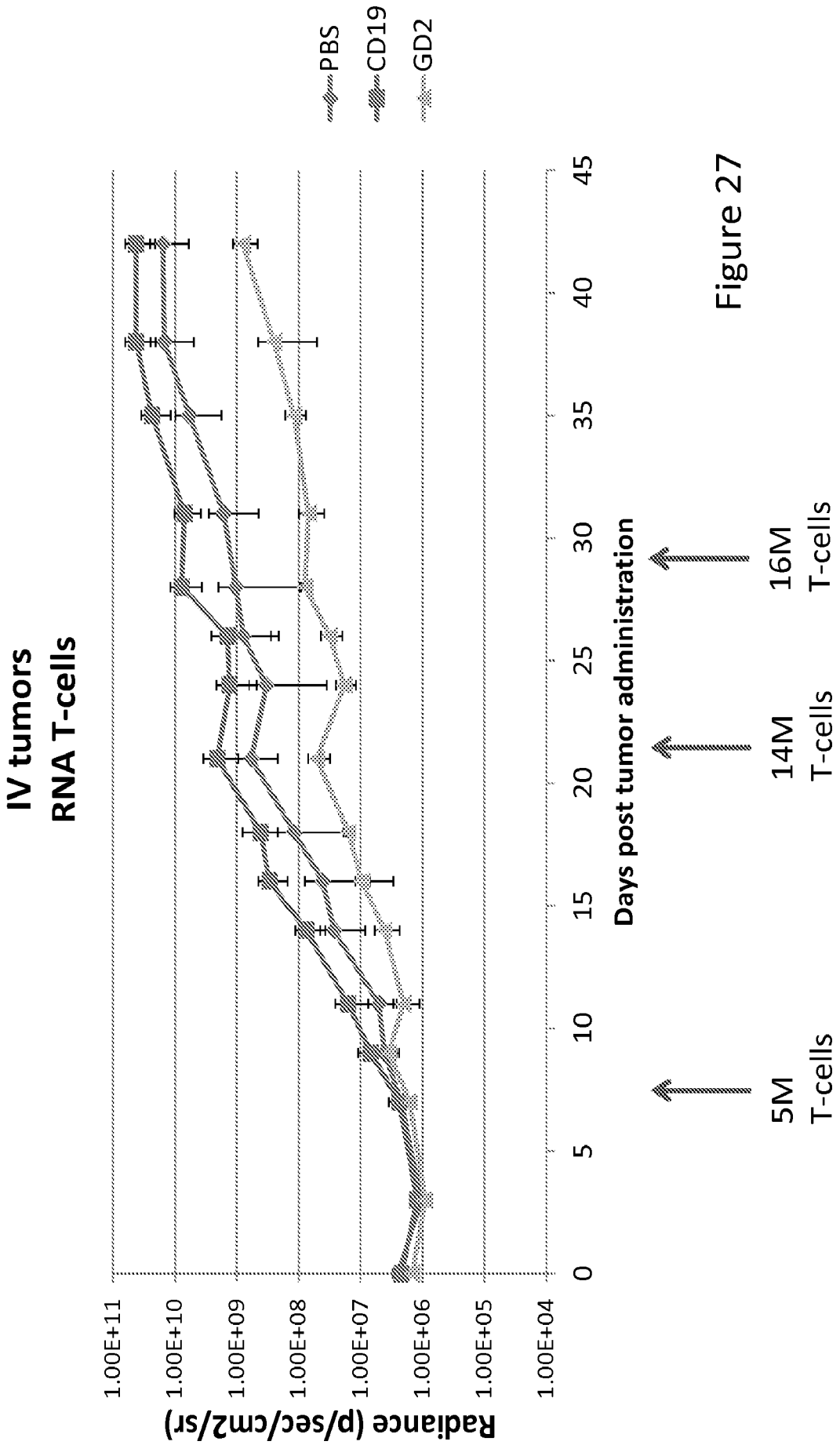

IV tumors
RNA T-cells
PBS
CD19
GD2
Radiance (p/sec/cm2/sr)
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
0
5
10
15
20
25
30
35
40
45
Days post tumor administration
5M T-cells
14M T-cells
16M T-cells

Figure 27

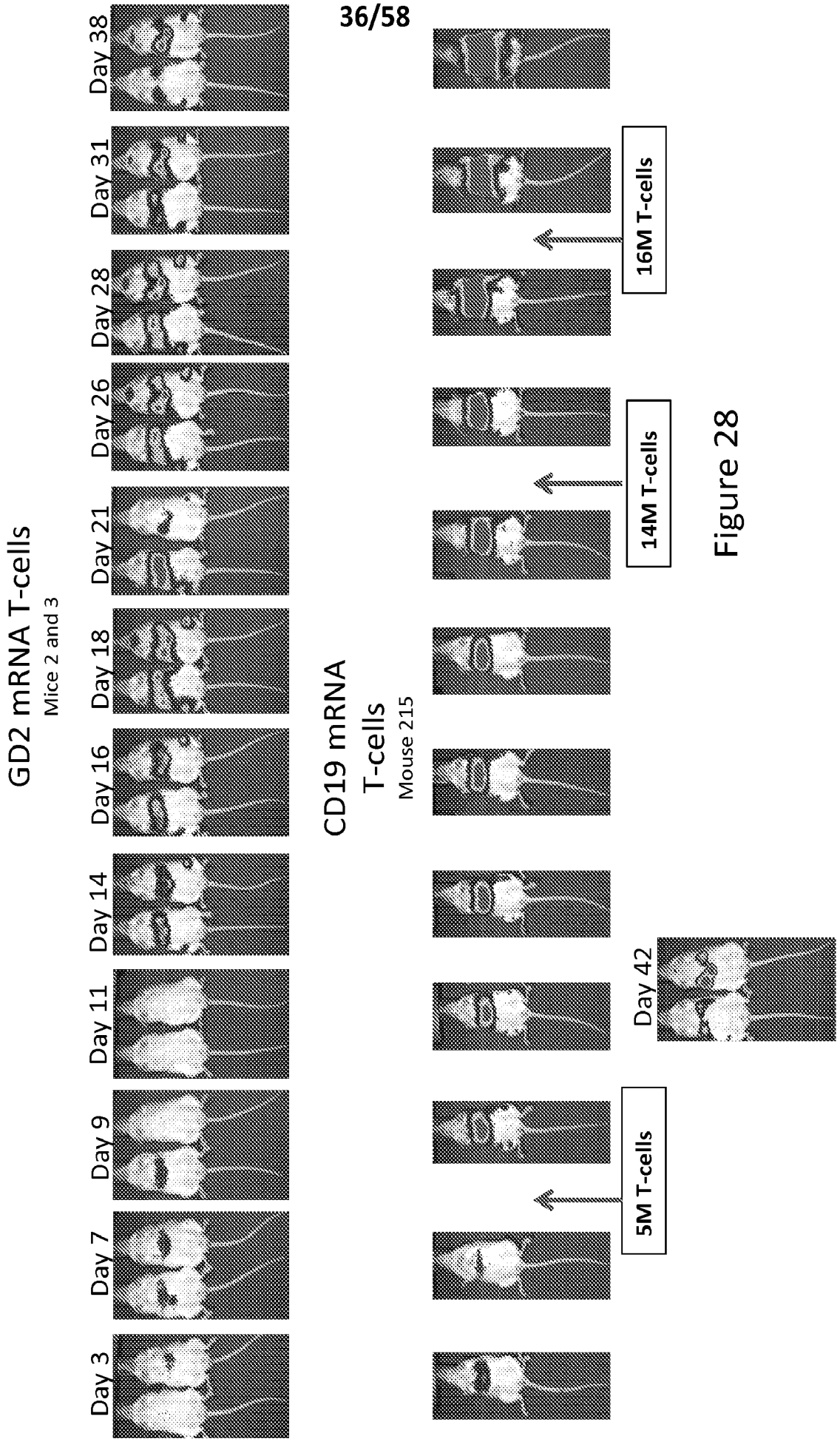

GD2 mRNA T-cells
Mice 2 and 3
Day 3
Day 7
Day 9
Day 11
Day 14
Day 16
Day 18
Day 21
Day 26
Day 28
Day 31
Day 38
CD19 mRNA
T-cells
Mouse 215
5M T-cells
14M T-cells
16M T-cells
Day 42

Figure 28

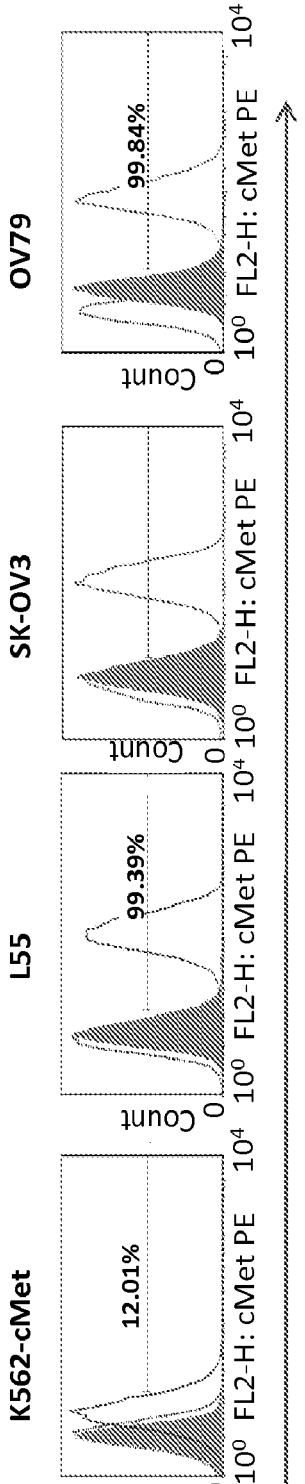

K562-CD19
K562-cMet
L55
SK-OV3
OV79
2.58%
12.01%
99.39%
99.84%
Count
FL2-H: cMet PE
cMet
IgG1 Isotype
No Ab
cMet Ab

Figure 29

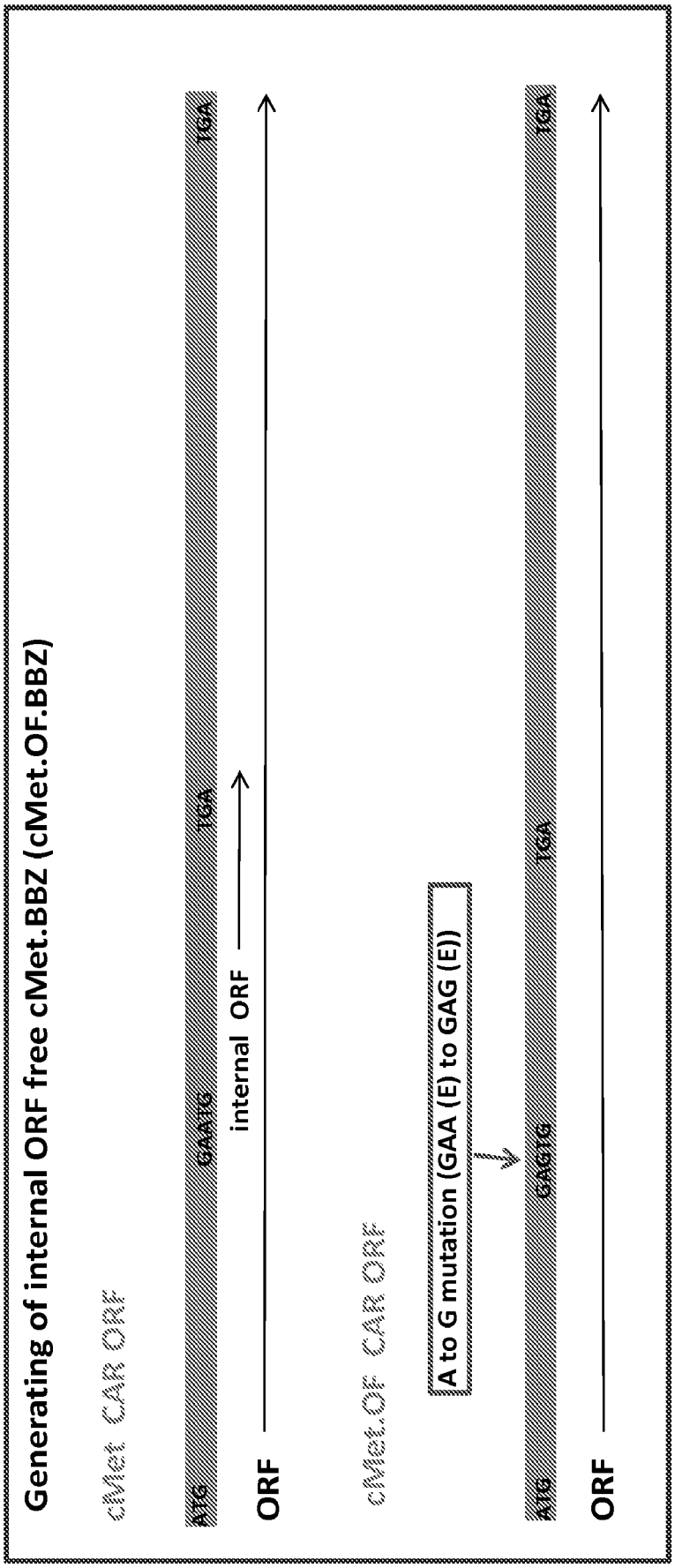

Generating of internal ORF free cMet.BBZ (cMet.OF.BBZ)
cMet CAR ORF
ATG
GAATG
TGA
TGA
internal ORF
ORF
cMet.OF CAR ORF
A to G mutation (GAA (E) to GAG (E))
ATG
GAGTG
TGA
TGA
ORF

Figure 30

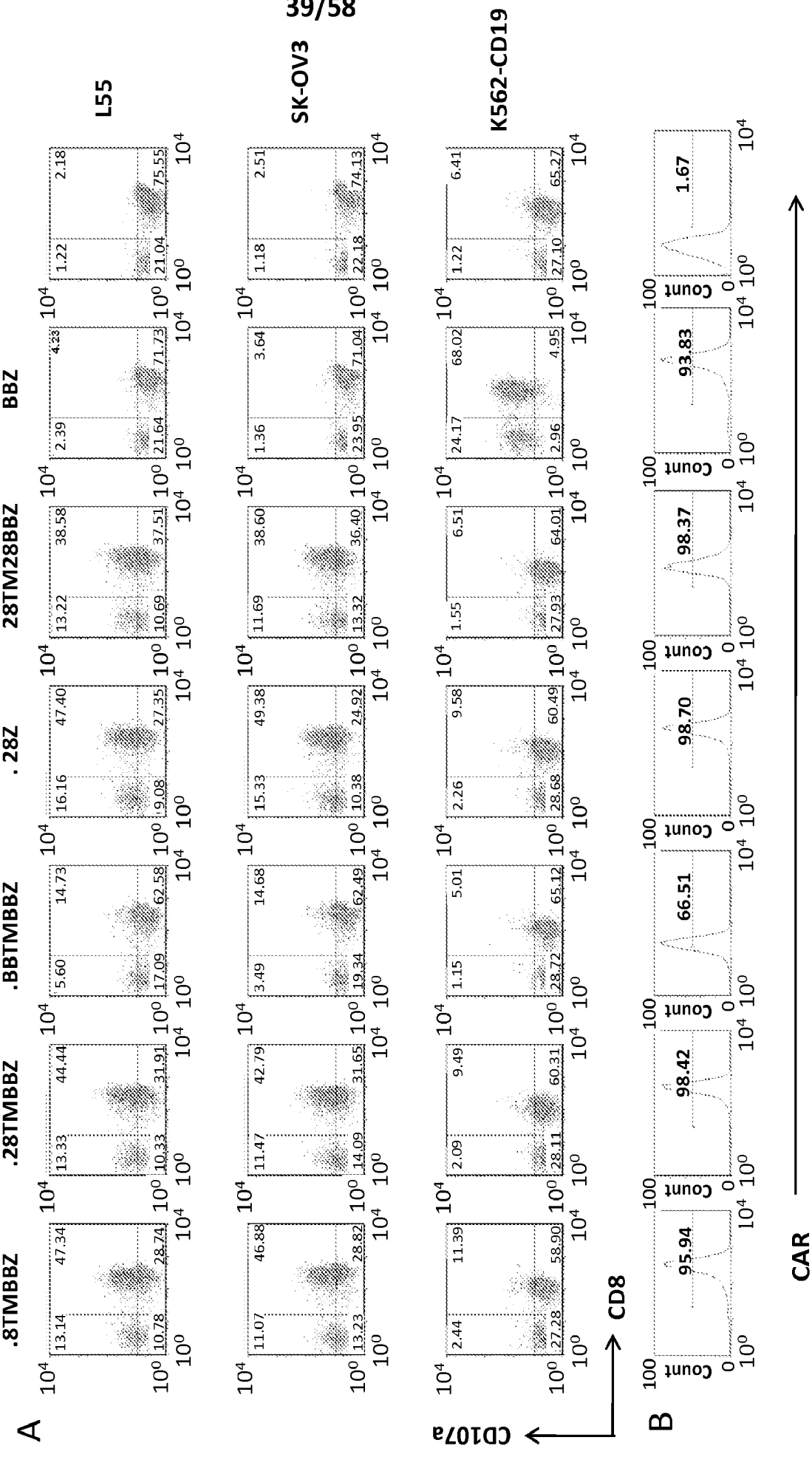
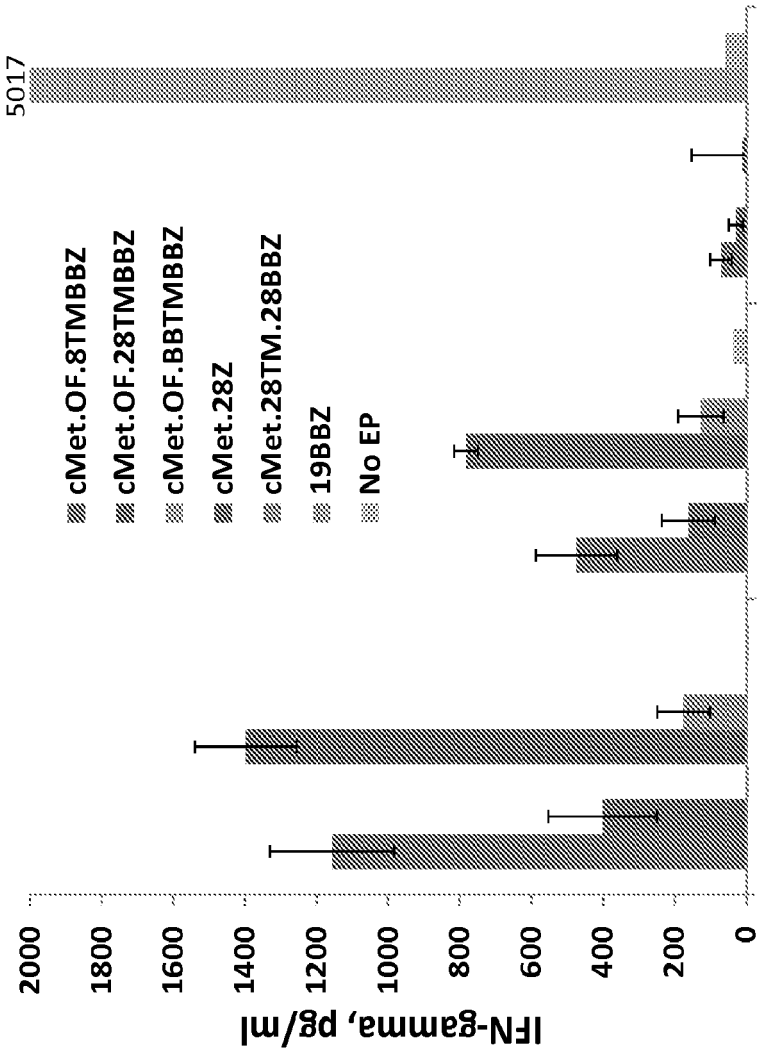

A
B
L55
SK-OV3
K562-CD19
cMetOF .8TMBBZ
cMetOF .28TMBBZ
cMetOF .BBTMBBZ
cMet . 28Z
cMet 28TM28BBZ
CD19.OF BBZ
No EP
CD107a
CD8
CAR
Count
95.94
98.42
66.51
98.70
98.37
93.83
1.67

Figure 31


A
IFN-gamma, pg/ml
2000
1800
1600
1400
1200
1000
800
600
400
200
0
5017
cMet.OF.8TMBBZ
cMet.OF.28TMBBZ
cMet.OF.BBTMBBZ
cMet.28Z
cMet.28TM.28BBZ
19BBZ
No EP
L55
SK-OV3
K562-CD19
Tumor lines

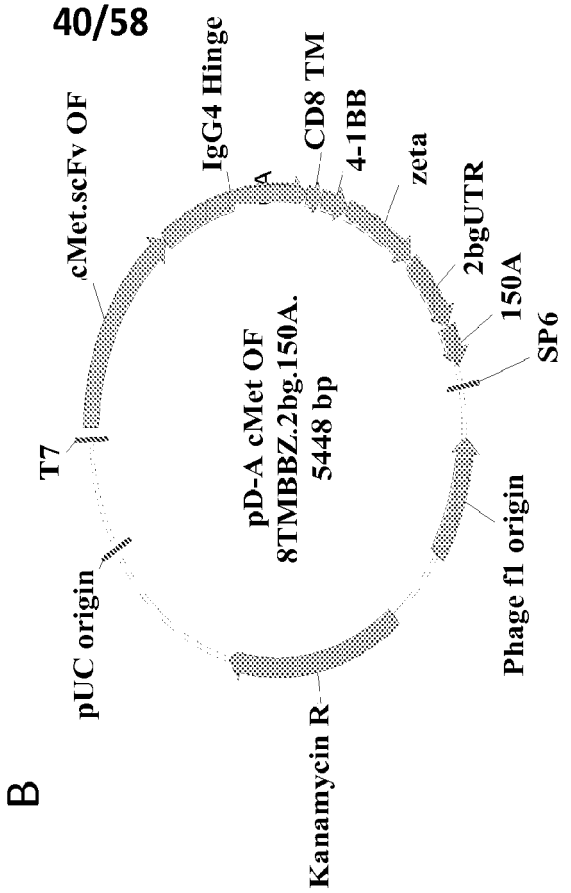

B
pD-A cMet OF
8TMBBZ.2bg.150A.
5448 bp
T7
cMet.scFv OF
IgG4 Hinge
CD8 TM
4-1BB
zeta
2bgUTR
150A
SP6
Phage f1 origin
Kanamycin R
pUC origin

Figure 32

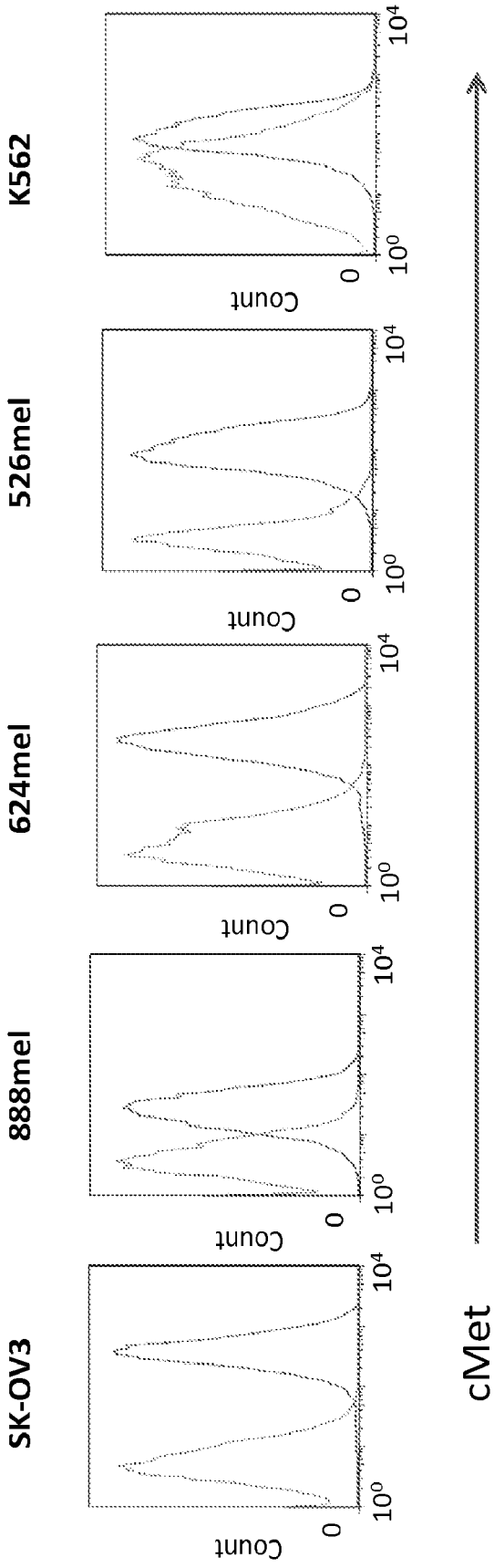


Figure 33

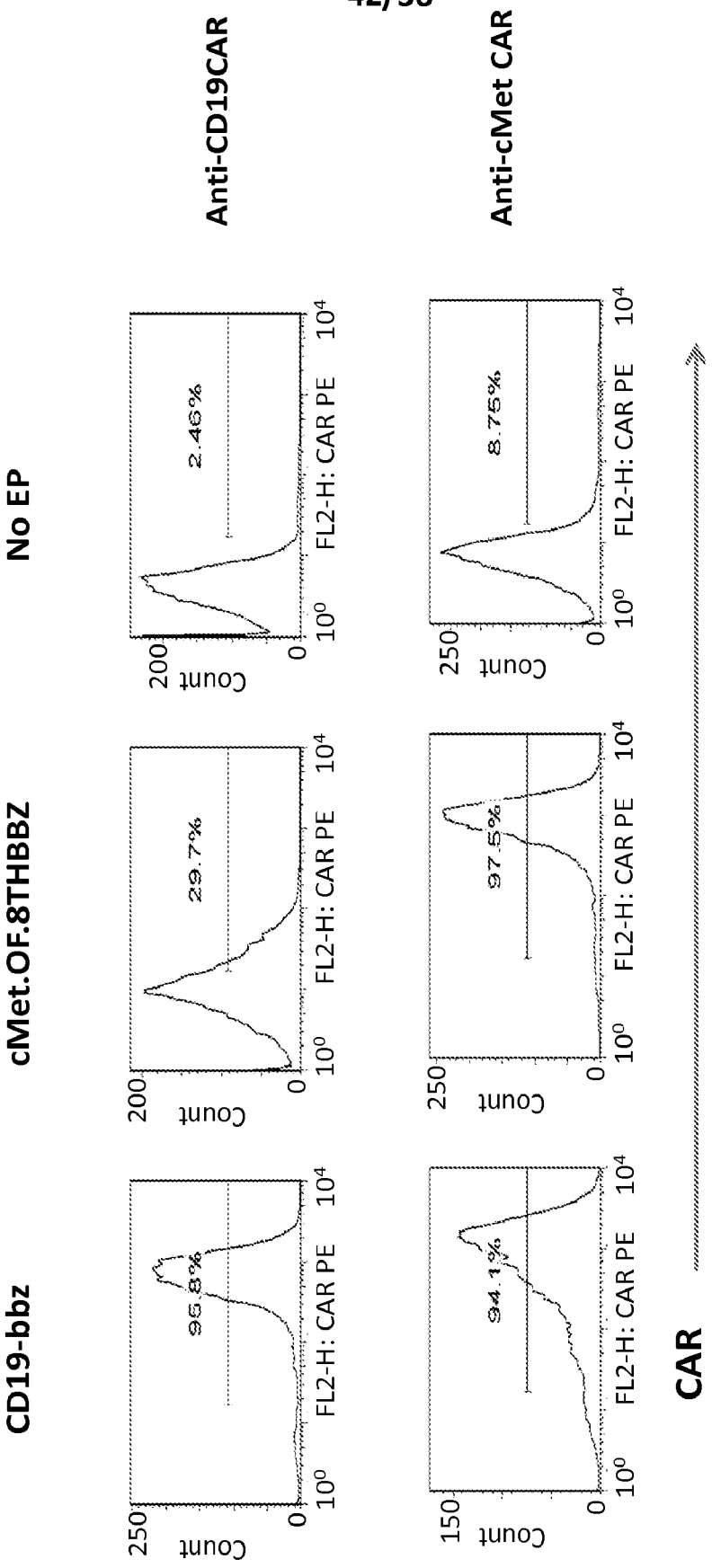

Anti-CD19CAR
Anti-cMet CAR
No EP
cMet.OF.8THBBZ
CD19-bbz
CAR
2.46%
8.75%
29.7%
97.5%
95.8%
94.1%
Count
FL2-H: CAR PE

Figure 34

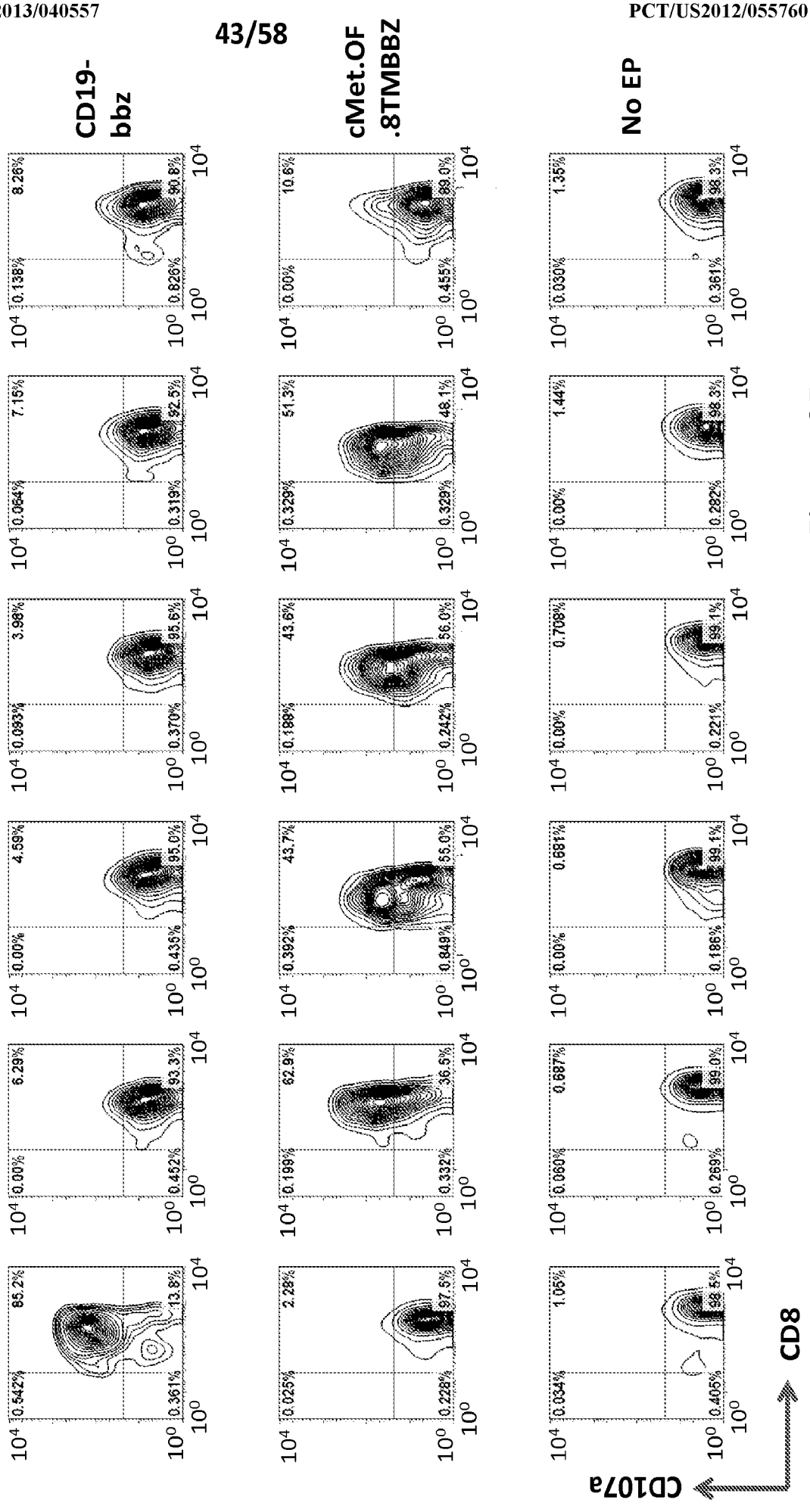

Nalm6
SK-OV3
888mel
624mel
526mel
K562
CD19-bbz
cMet.OF.8TMBBZ
No EP
CD107a
CD8

Figure 35

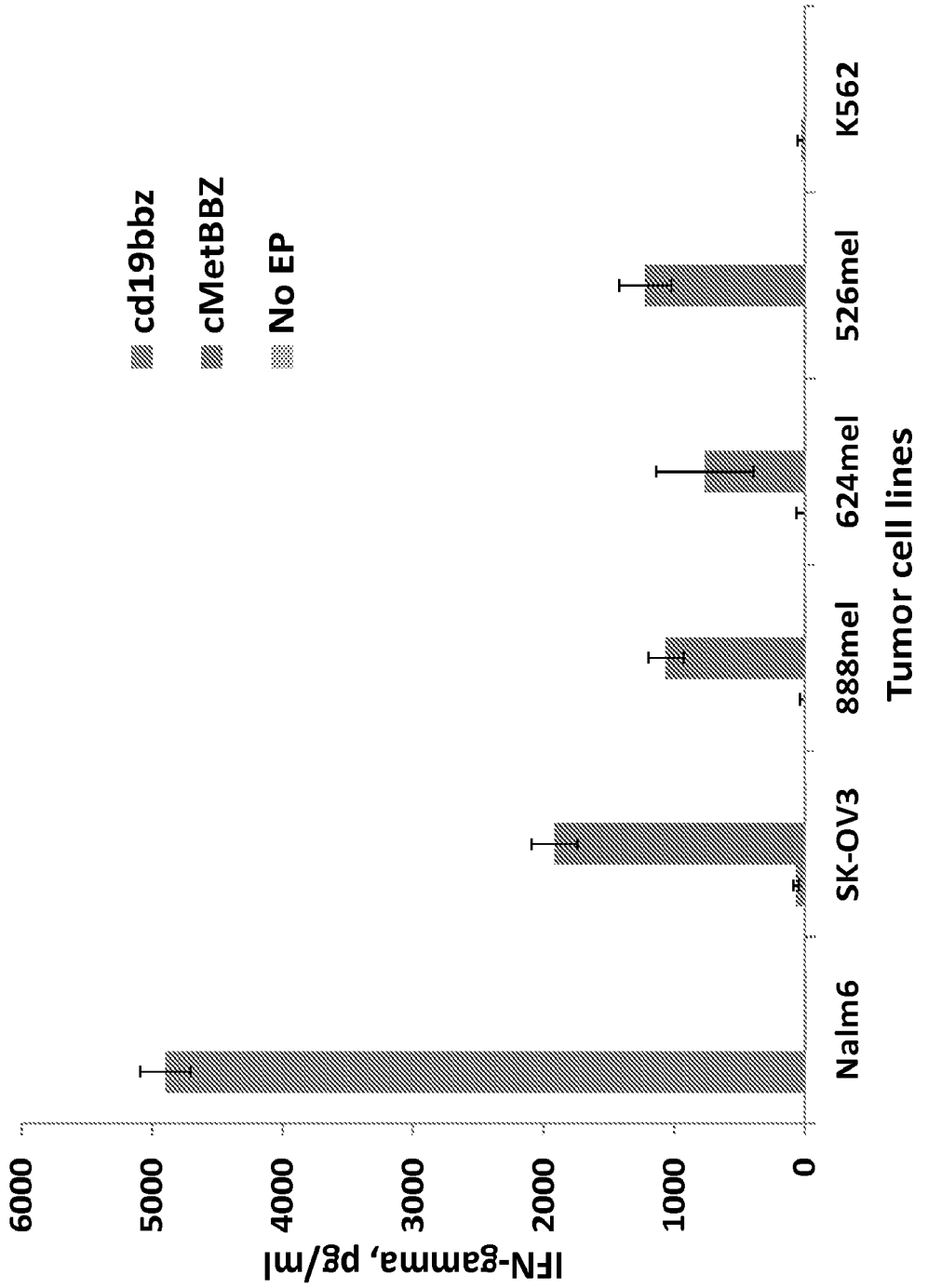

cd19bbz
cMetBBZ
No EP
IFN-gamma, pg/ml
6000
5000
4000
3000
2000
1000
0
Nalm6
SK-OV3
888mel
624mel
526mel
K562
Tumor cell lines

Figure 36

SKOV3 epithelial ovarian carcinoma cells (wild type cells express c-MET)

Group	Mice (#)	Tumor Cells s.c.	Treatment i.t. Injection T cells
cMET CAR + Cytoxan	9	SKOV3-Luc	cMET CAR + Cytoxan
CD19CAR + Cytoxan	9	SKOV3-Luc	CD19 CAR + Cytoxan
PBS	7	SKOV3-Luc	PBS

Experimental design

Establish vascularized flank xenograft tumors with SkOV3/luc tumors on day 0 in female NSG mice (n=7 to 9 mice per group)

Treatment beginning at week 6, 7, 9 and 11: 1x10e7 CAR cells injected IT
Cytoxan i.p. injection 24 hour before treatment at weeks 7, 9 and 11

Endpoints: survival and tumor burden (bioluminescence)

Figure 37

SUBSTITUTE SHEET (RULE 26)

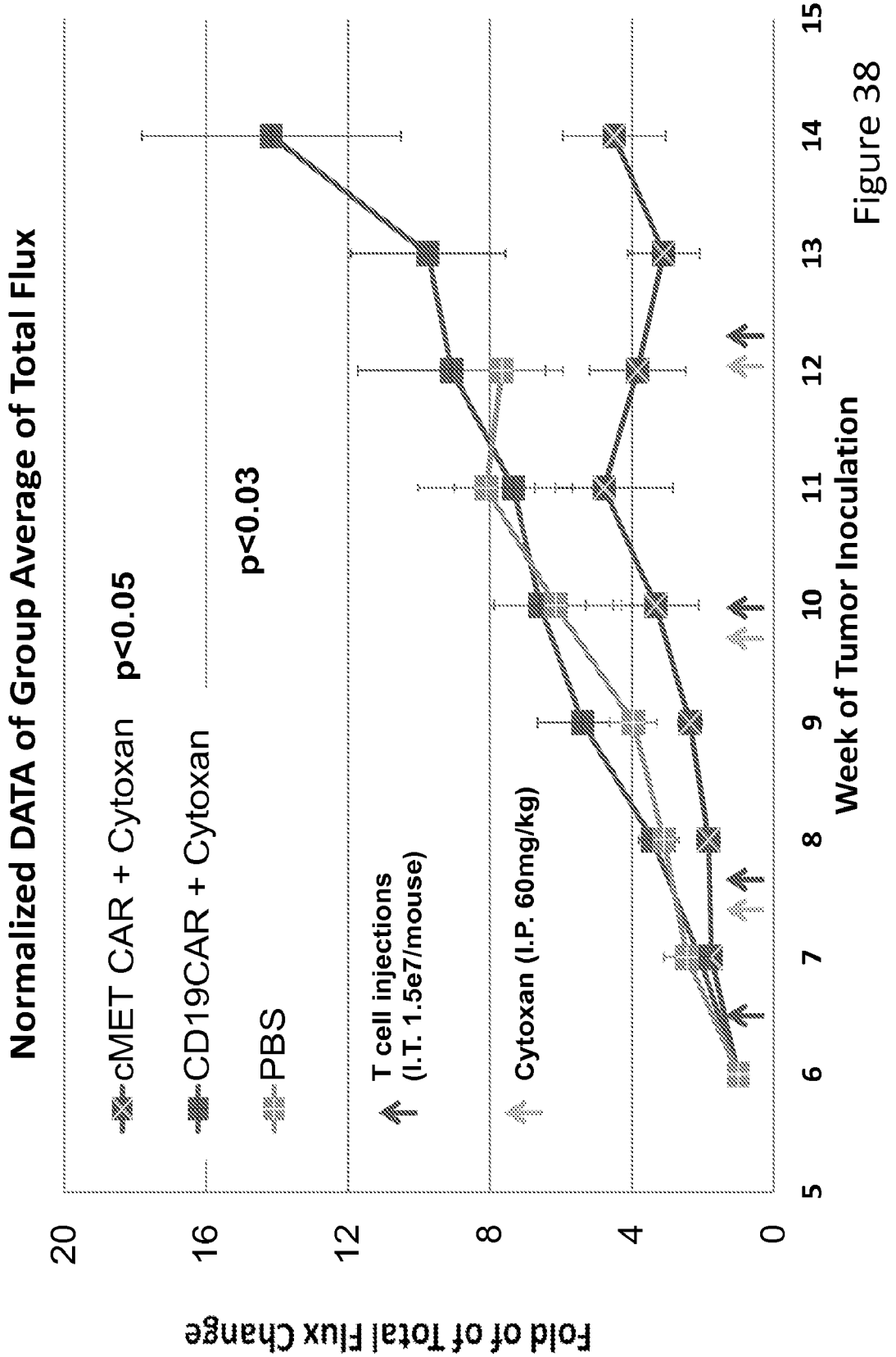

Normalized DATA of Group Average of Total Flux
cMET CAR + Cytoxan p<0.05
CD19CAR + Cytoxan
p<0.03
PBS
T cell injections
(I.T. 1.5e7/mouse)
Cytoxan (I.P. 60mg/kg)
Fold of of Total Flux Change
Week of Tumor Inoculation
0
4
8
12
16
20
5
6
7
8
9
10
11
12
13
14
15

Figure 38

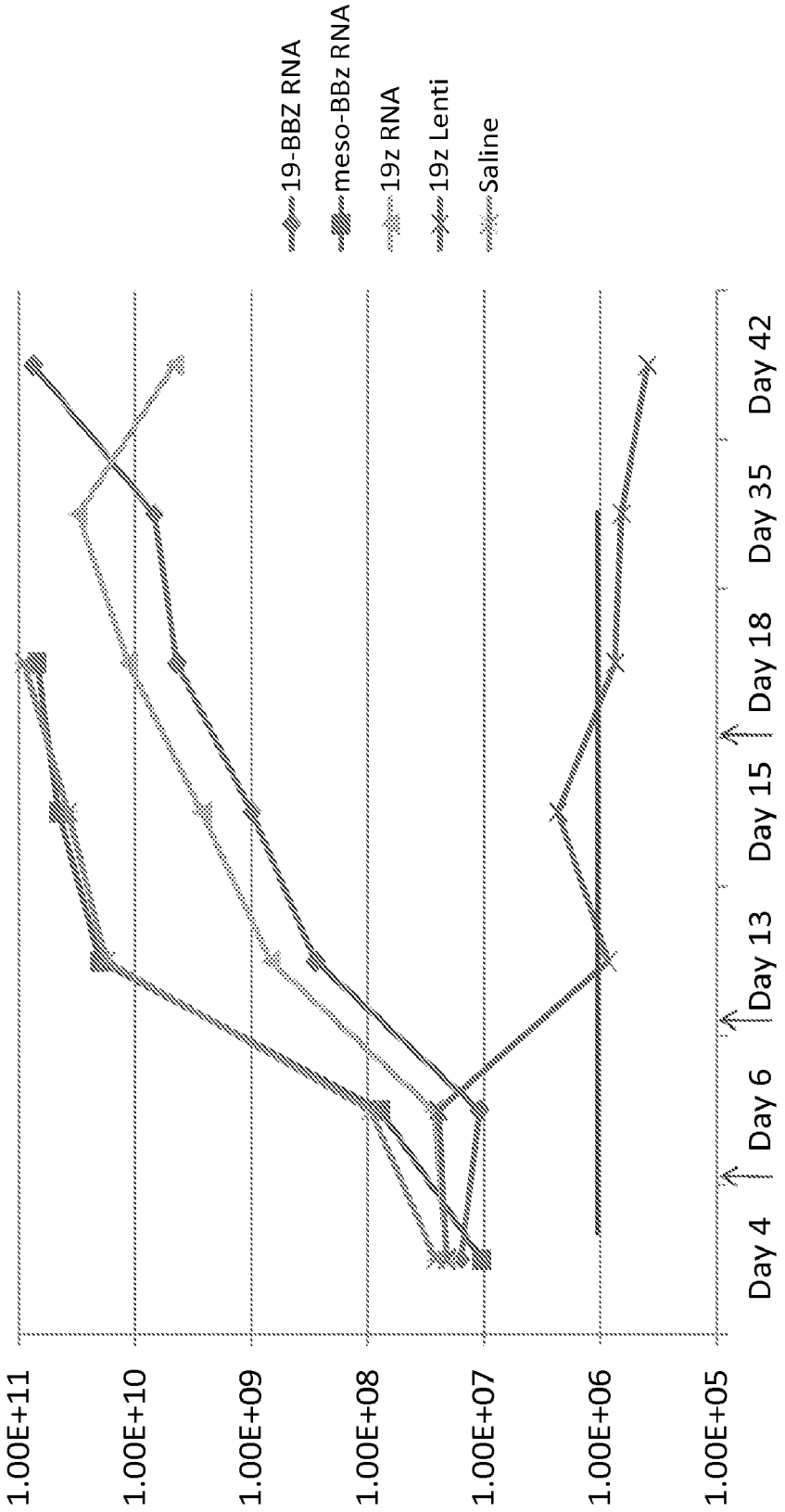

19-BBZ RNA
meso-BBz RNA
19z RNA
19z Lenti
Saline
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
Day 4
Day 6
Day 13
Day 15
Day 18
Day 35
Day 42

Figure 39

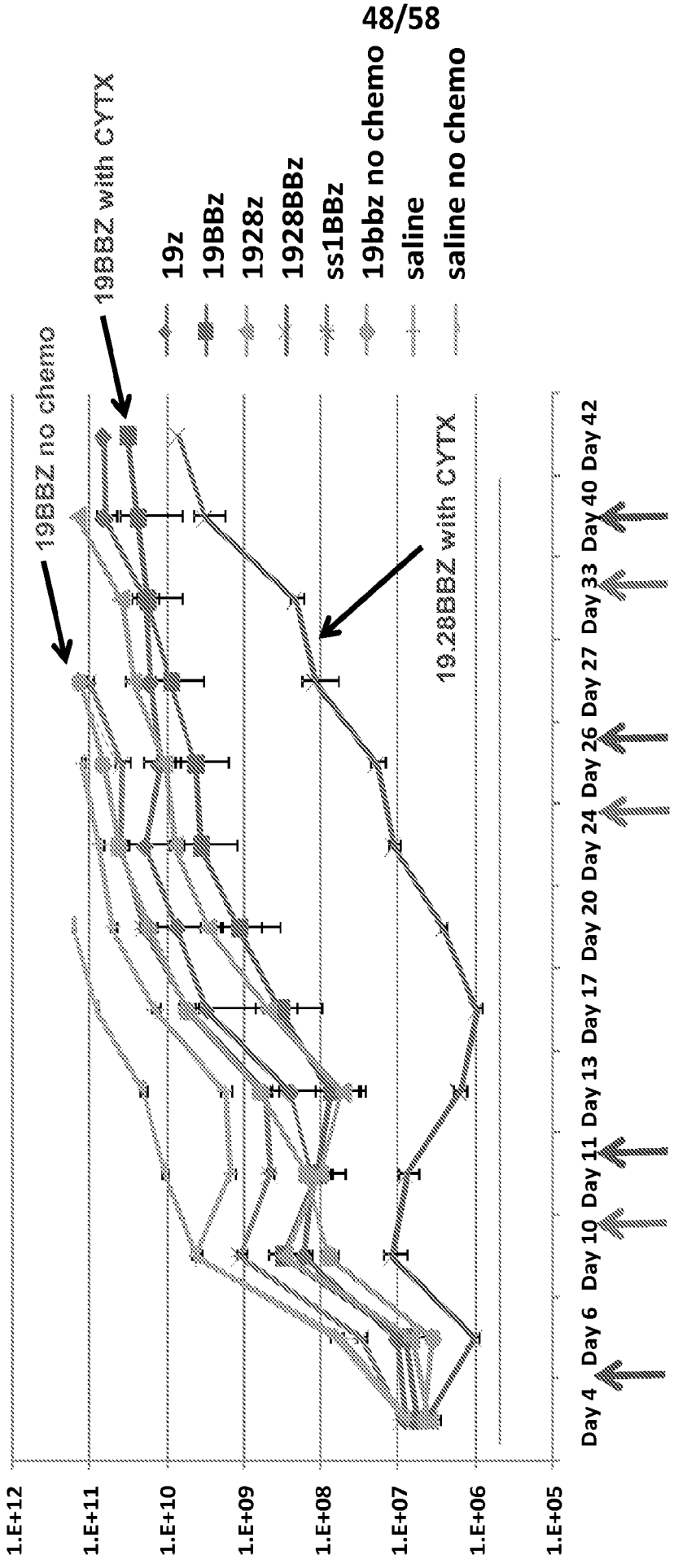

19BBZ no chemo
19BBZ with CYTX
19.28BBZ with CYTX
19z
19BBz
1928z
1928BBz
ss1BBz
19bbz no chemo
saline
saline no chemo
1.E+12
1.E+11
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
Day 4
Day 6
Day 10
Day 11
Day 13
Day 17
Day 20
Day 24
Day 26
Day 27
Day 33
Day 40
Day 42
10⁷ CAR T cells
80 mg/kg CYTX
50 mg/kg CYTX

Figure 40

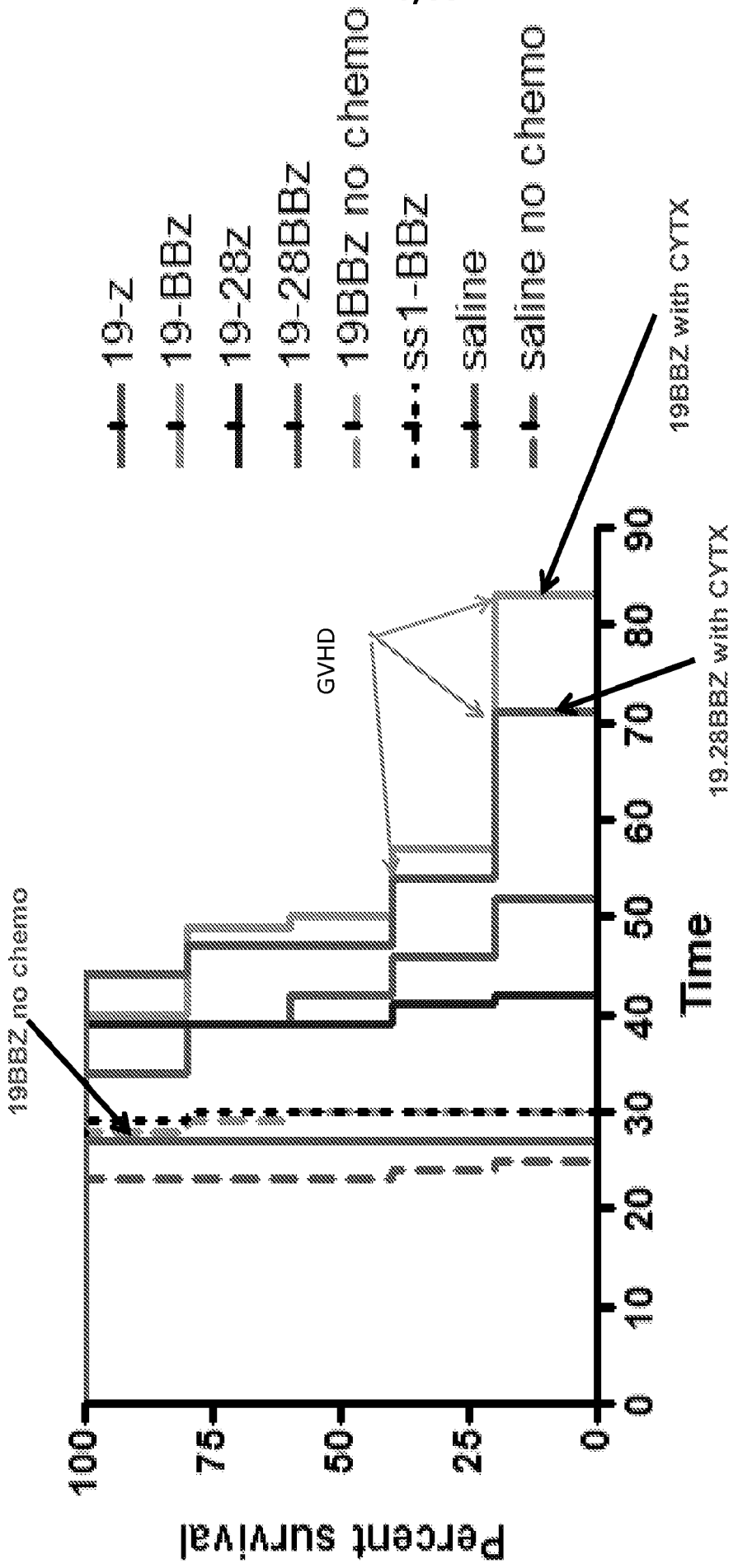

19-z
19-BBz
19-28z
19-28BBz
19BBz no chemo
ss1-BBz
saline
saline no chemo
19BBZ with CYTX
19.28BBZ with CYTX
19BBZ no chemo
GVHD
Percent survival
Time
100
75
50
25
0
0
10
20
30
40
50
60
70
80
90

Figure 41

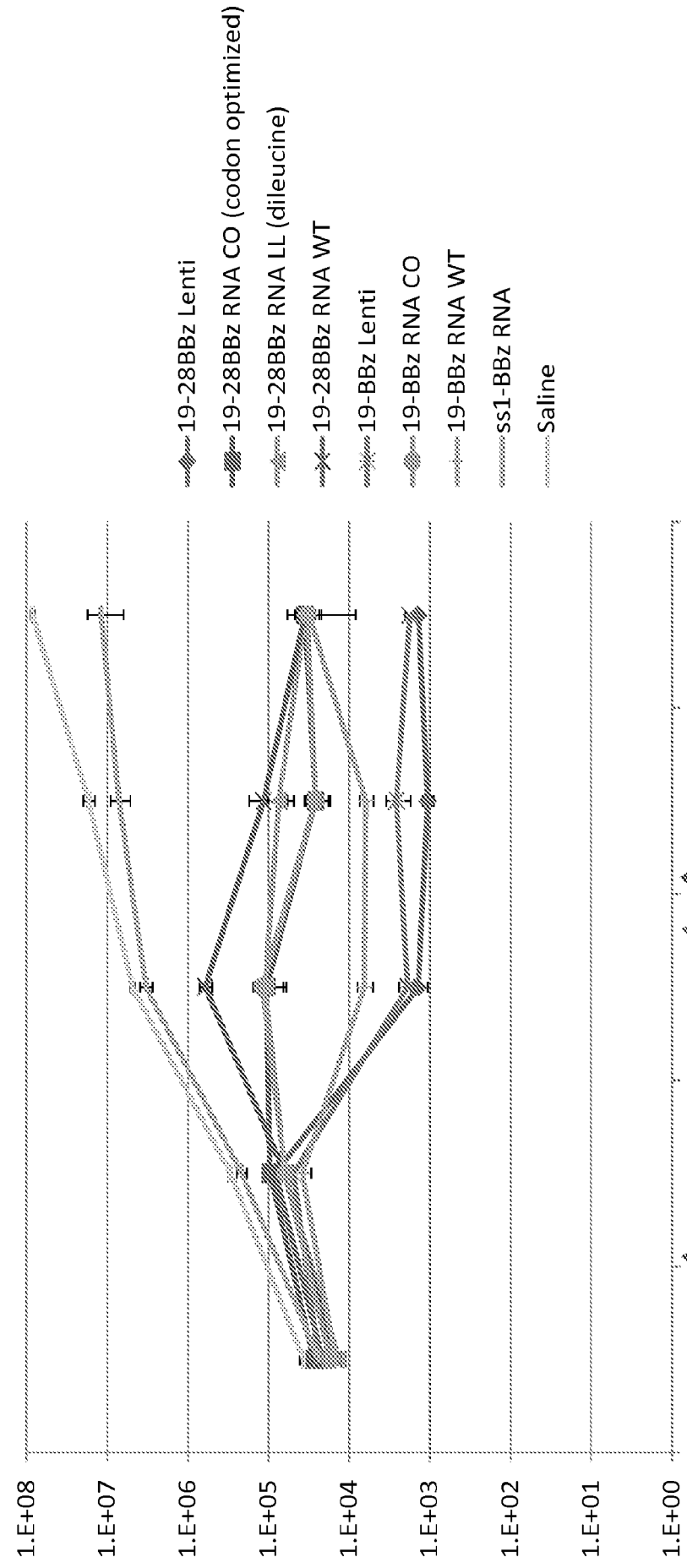

19-28BBz Lenti
19-28BBz RNA CO (codon optimized)
19-28BBz RNA LL (dileucine)
19-28BBz RNA WT
19-BBz Lenti
19-BBz RNA CO
19-BBz RNA WT
ss1-BBz RNA
Saline
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Day 4
Day 6
Day 10
Day 15
Day 21
10^7 T cells (RNA and Lentivirus)
60 mg/kg Cytoxan and 24 hours later 10^7 T cells (RNA ONLY)

Figure 42

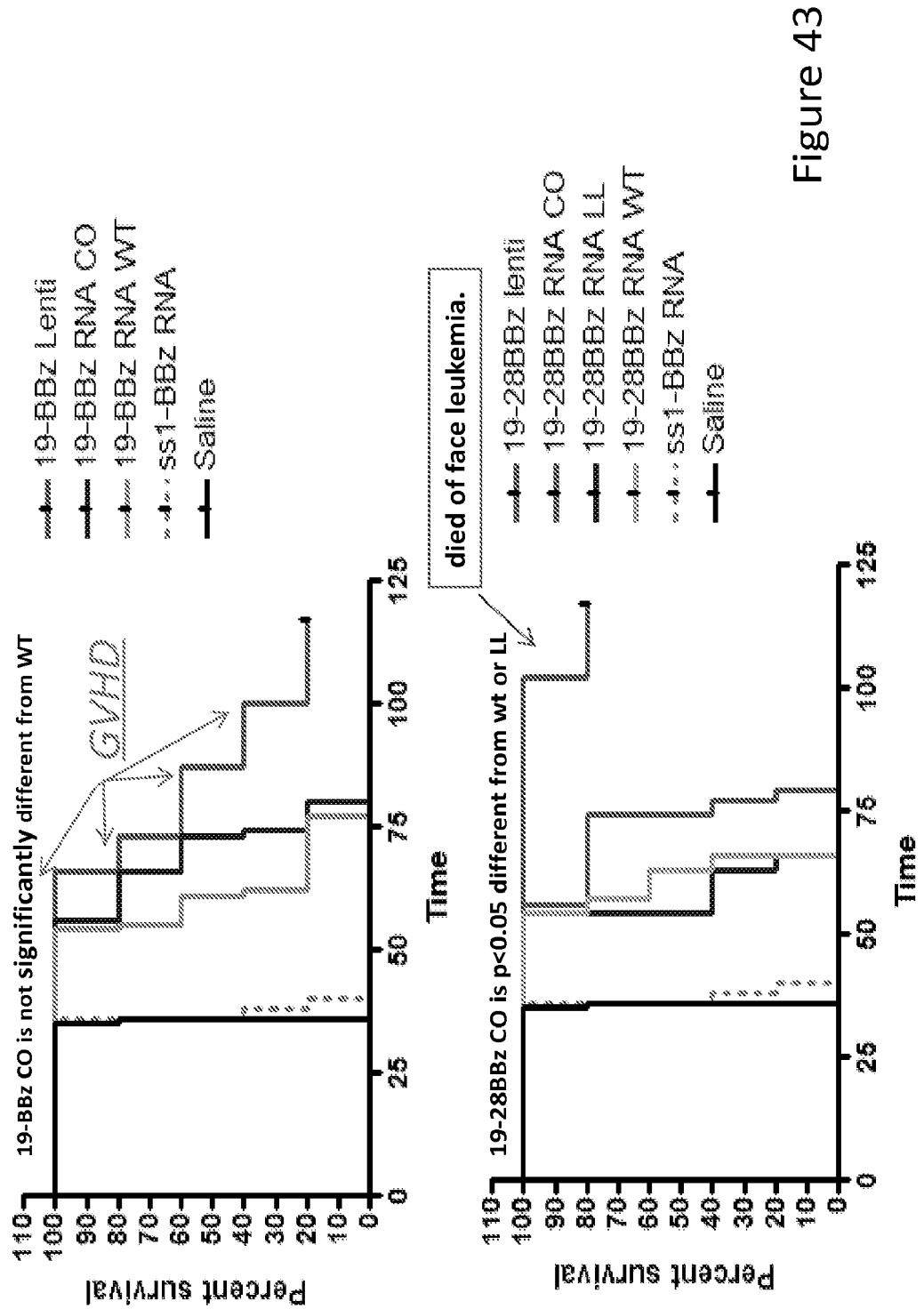

19-BBz Lenti
19-BBz RNA CO
19-BBz RNA WT
ss1-BBz RNA
Saline
GVHD
19-BBz CO is not significantly different from WT
Percent survival
Time
0
25
50
75
100
125
0
10
20
30
40
50
60
70
80
90
100
110
died of face leukemia.
19-28BBz lenti
19-28BBz RNA CO
19-28BBz RNA LL
19-28BBz RNA WT
ss1-BBz RNA
Saline
19-28BBz CO is p<0.05 different from wt or LL
Percent survival
Time

Figure 43

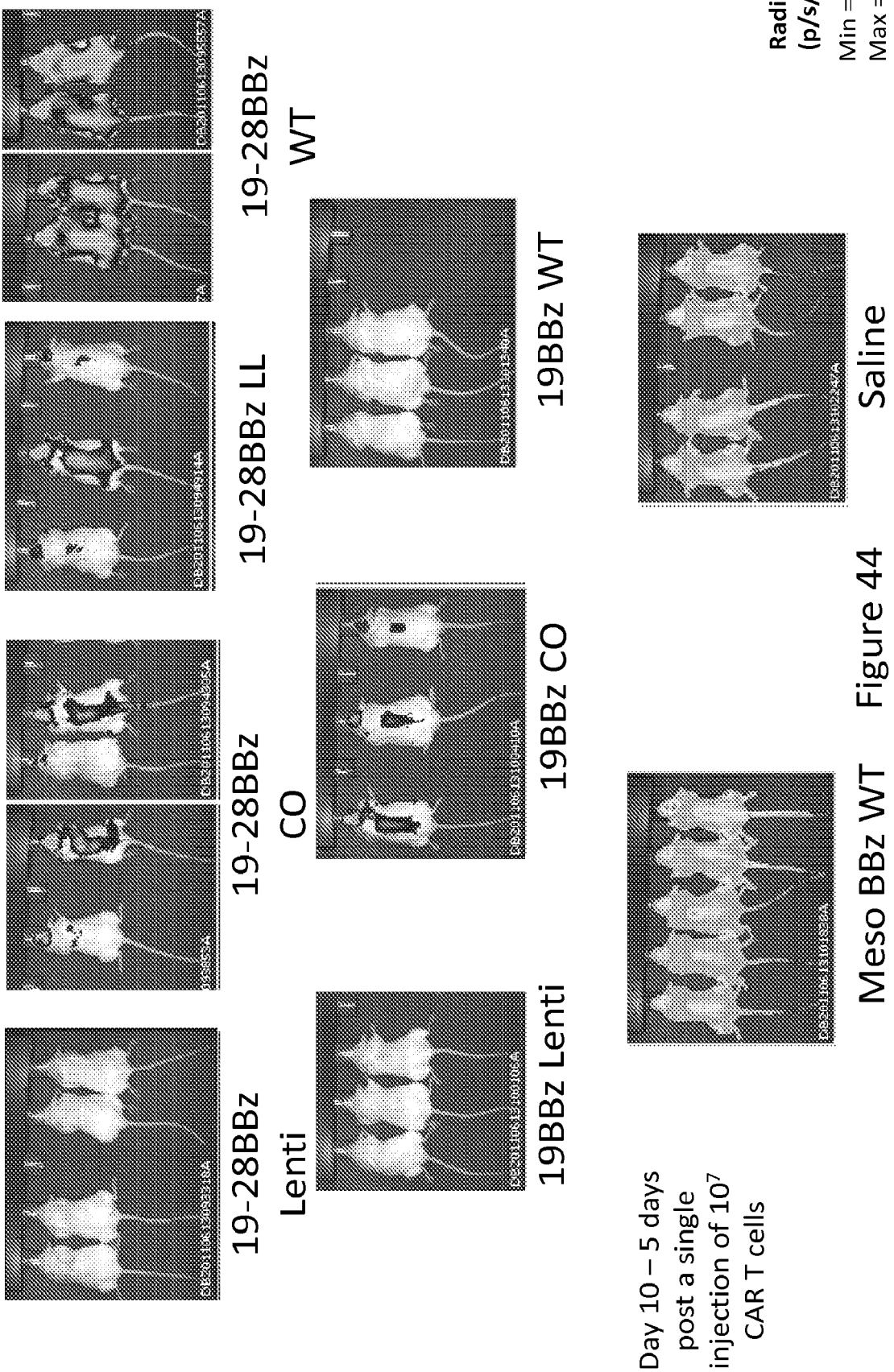

Radiance (p/s/cm²/sr)
Min = 2x10⁵
Max = 5.6x10⁷
19-28BBz Lenti
19-28BBz CO
19-28BBz LL
19-28BBz WT
19BBz Lenti
19BBz CO
19BBz WT
Saline
Meso BBz WT
Day 10 – 5 days post a single injection of 10⁷ CAR T cells

Figure 44

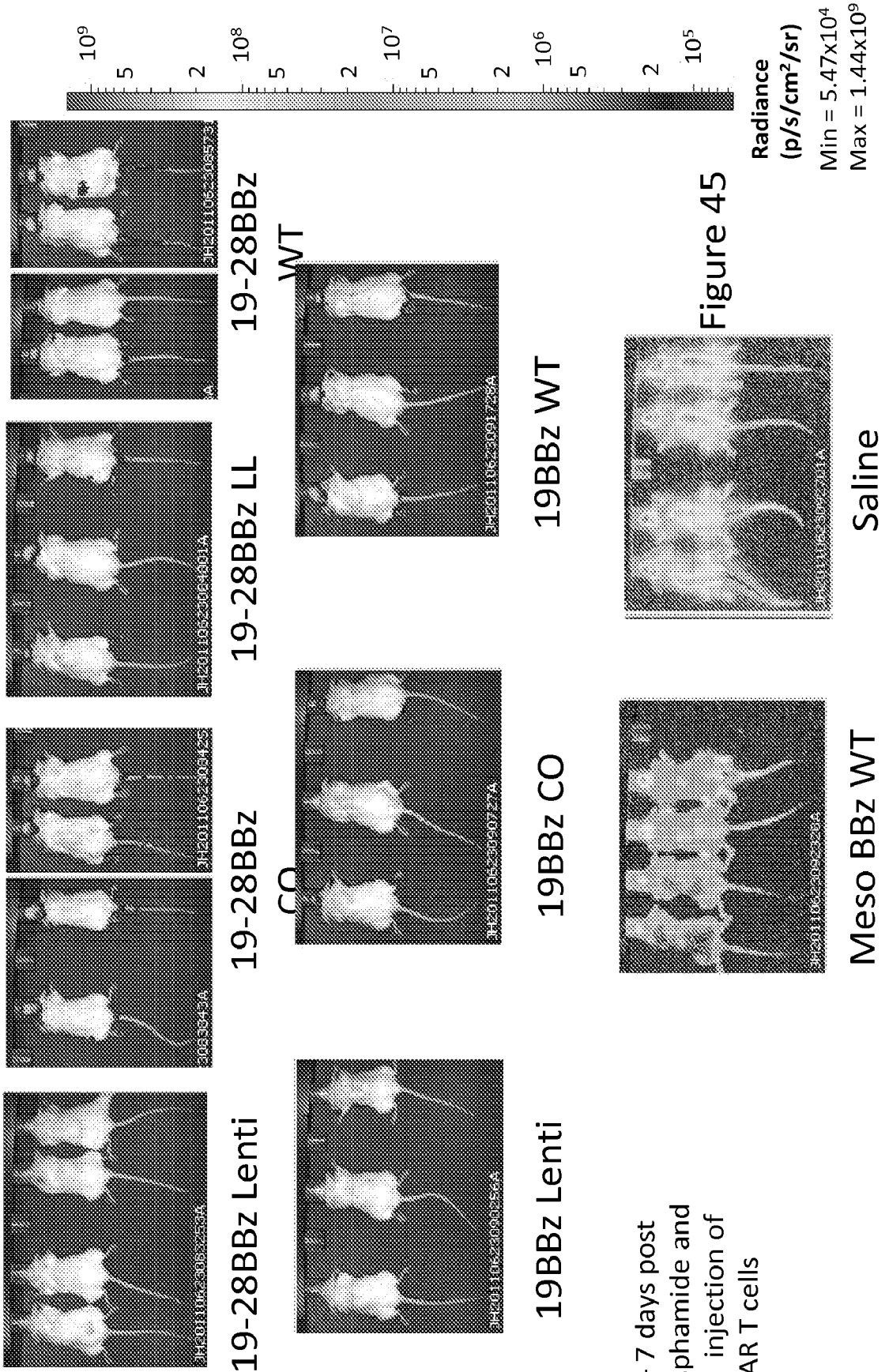

Radiance (p/s/cm²/sr)
Min = 5.47x10⁴
Max = 1.44x10⁹
19-28BBz Lenti
19-28BBz CO
19-28BBz LL
19-28BBz WT
19BBz Lenti
19BBz CO
19BBz WT
Meso BBz WT
Saline
Day 21 – 7 days post cyclophosphamide and a second injection of 10⁷ CAR T cells

Figure 45

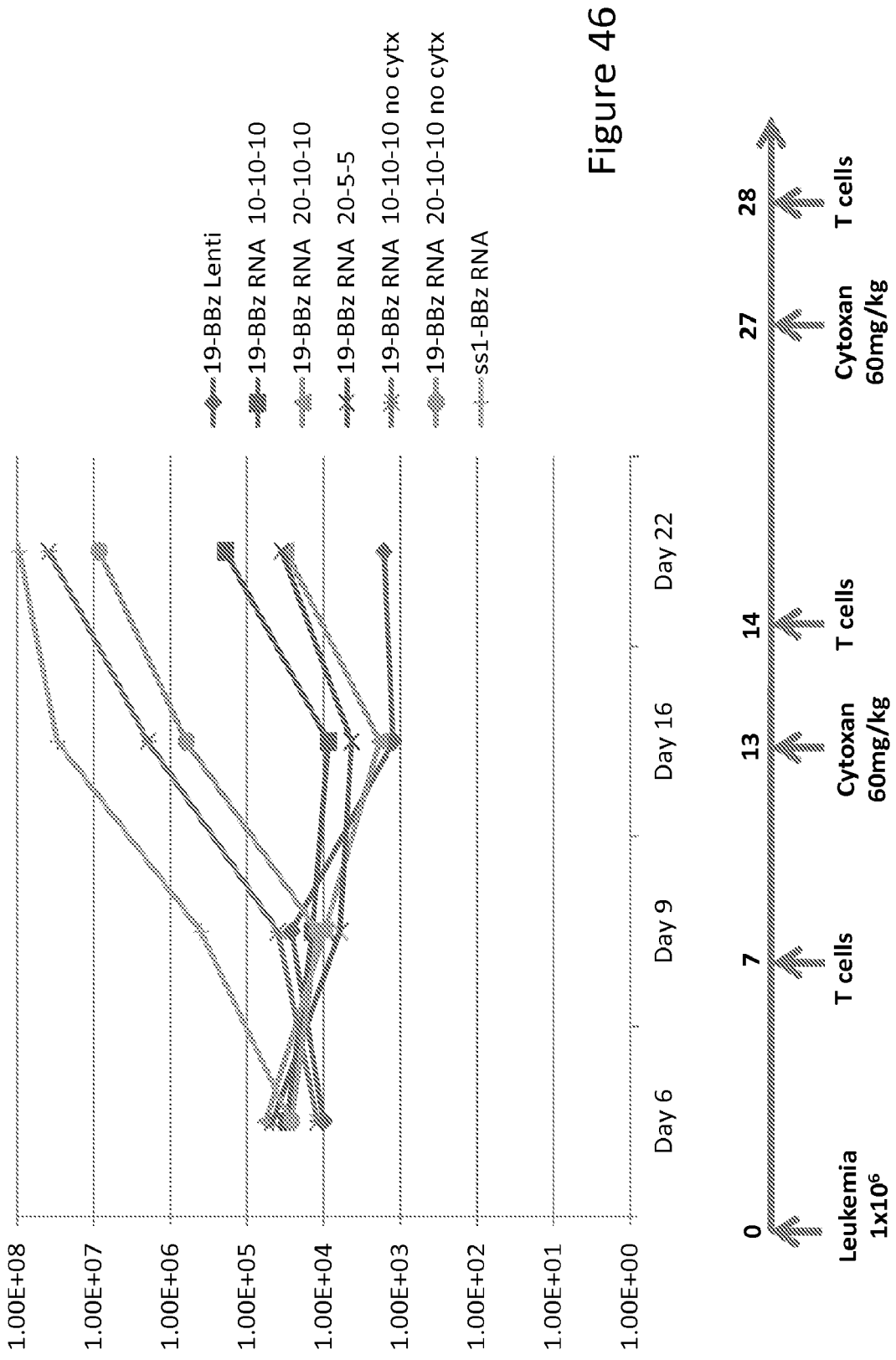

19-BBz Lenti
19-BBz RNA 10-10-10
19-BBz RNA 20-10-10
19-BBz RNA 20-5-5
19-BBz RNA 10-10-10 no cytx
19-BBz RNA 20-10-10 no cytx
ss1-BBz RNA
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Day 6
Day 9
Day 16
Day 22
0
7
13
14
27
28
Leukemia 1x10⁶
T cells
Cytoxan 60mg/kg
T cells
Cytoxan 60mg/kg
T cells

Figure 46

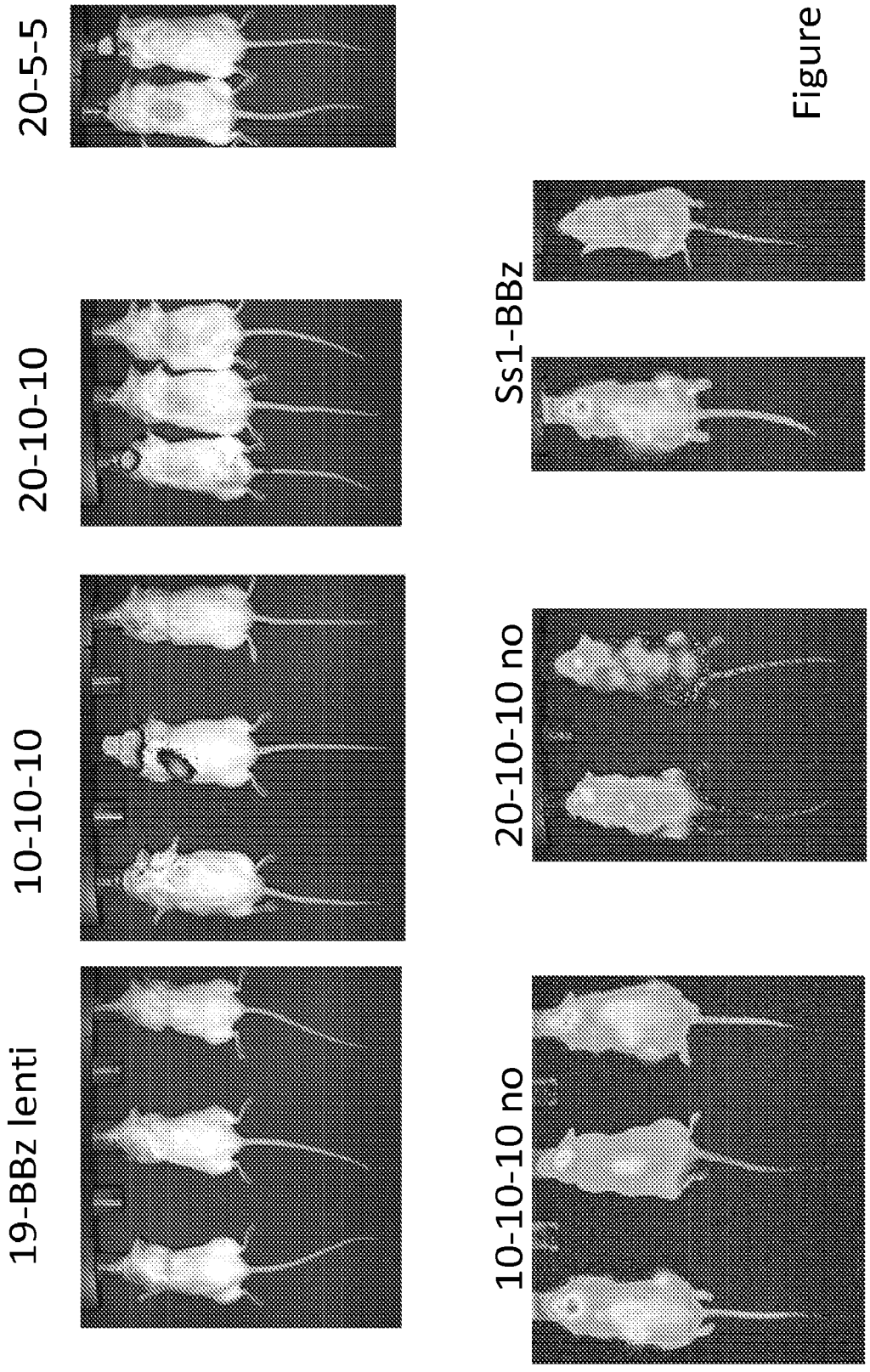


Figure 47

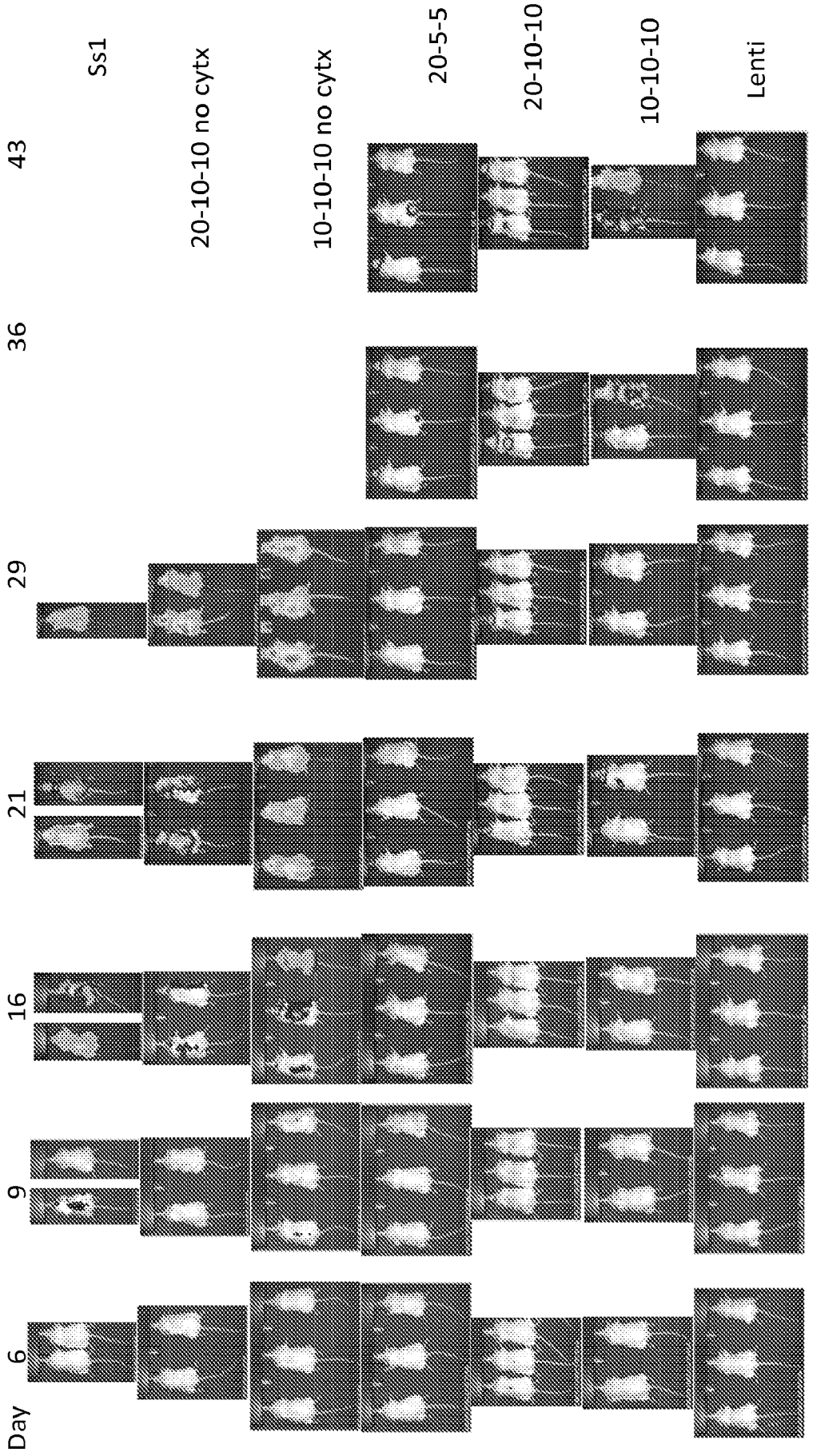

Day
6
9
16
21
29
36
43
Ss1
20-10-10 no cytx
10-10-10 no cytx
20-5-5
20-10-10
10-10-10
Lenti

Figure 48

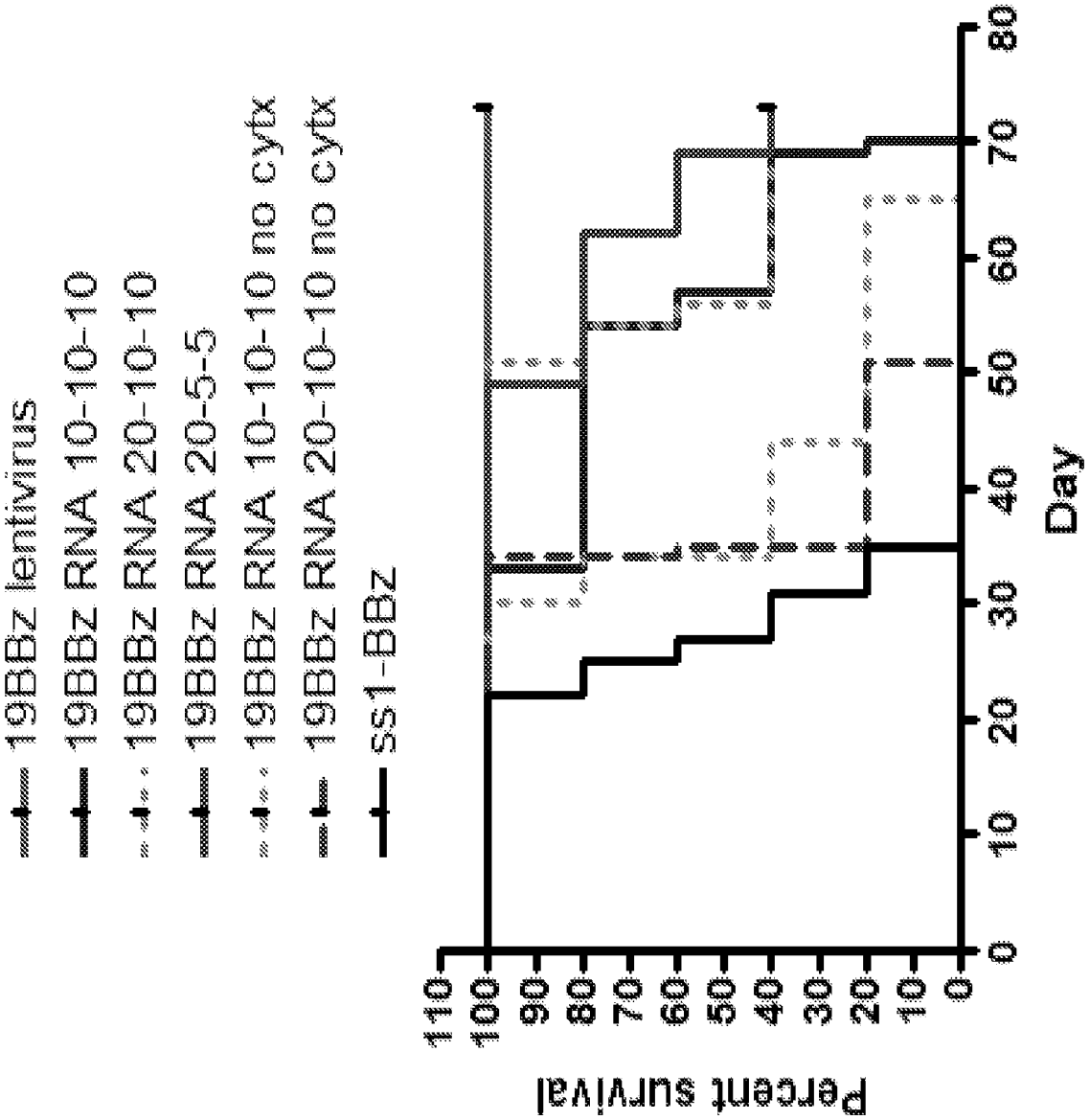

19BBz lentivirus
19BBz RNA 10-10-10
19BBz RNA 20-10-10
19BBz RNA 20-5-5
19BBz RNA 10-10-10 no cytx
19BBz RNA 20-10-10 no cytx
ss1-BBz
Percent survival
0
10
20
30
40
50
60
70
80
90
100
110
Day
0
10
20
30
40
50
60
70
80

Figure 49

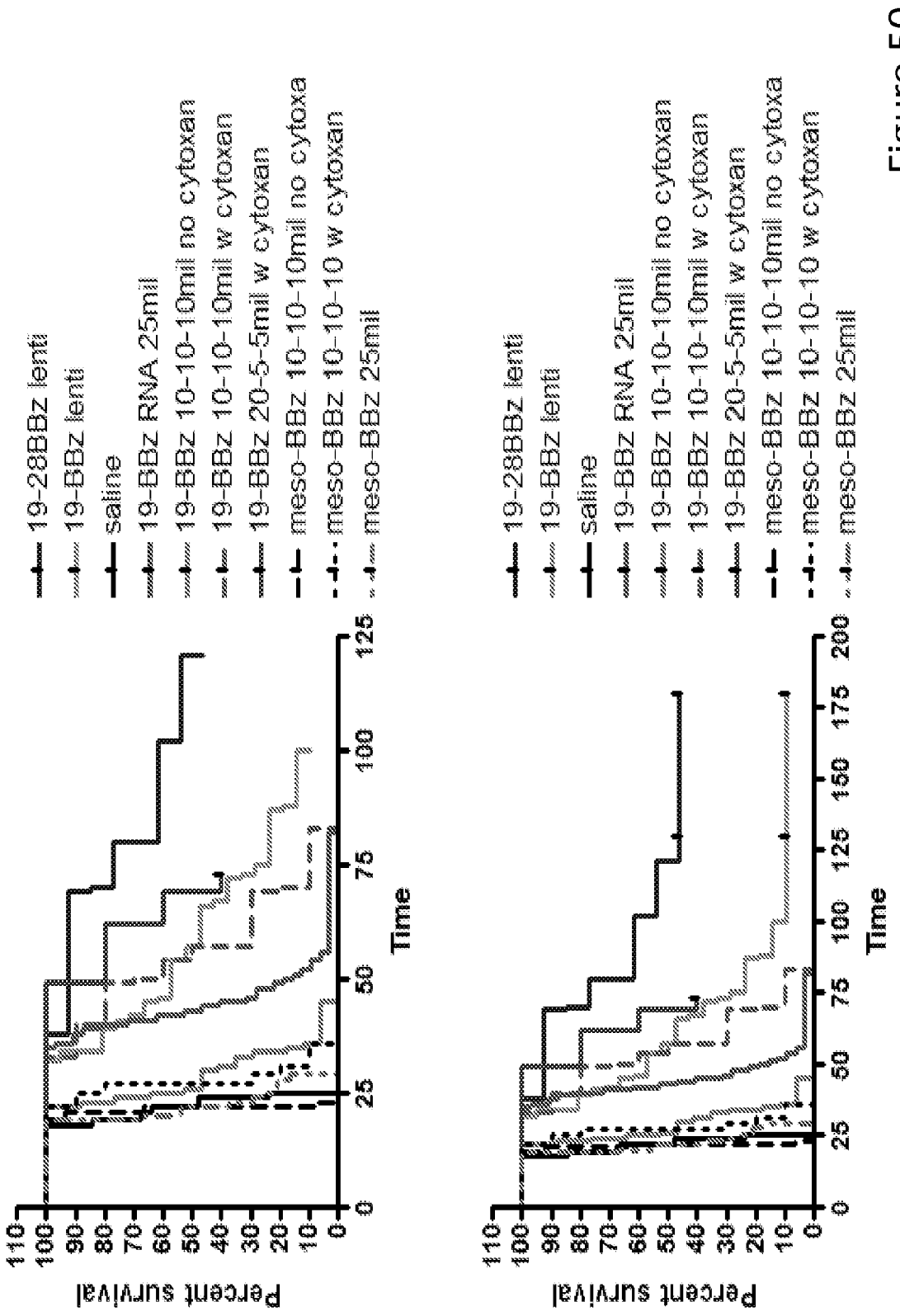

19-28BBz lenti
19-BBz lenti
saline
19-BBz RNA 25mil
19-BBz 10-10-10mil no cytoxan
19-BBz 10-10-10mil w cytoxan
19-BBz 20-5-5mil w cytoxan
meso-BBz 10-10-10mil no cytoxa
meso-BBz 10-10-10 w cytoxan
meso-BBz 25mil
Percent surVival
Time
0
10
20
30
40
50
60
70
80
90
100
110
0
25
50
75
100
125
150
175
200

Figure 50