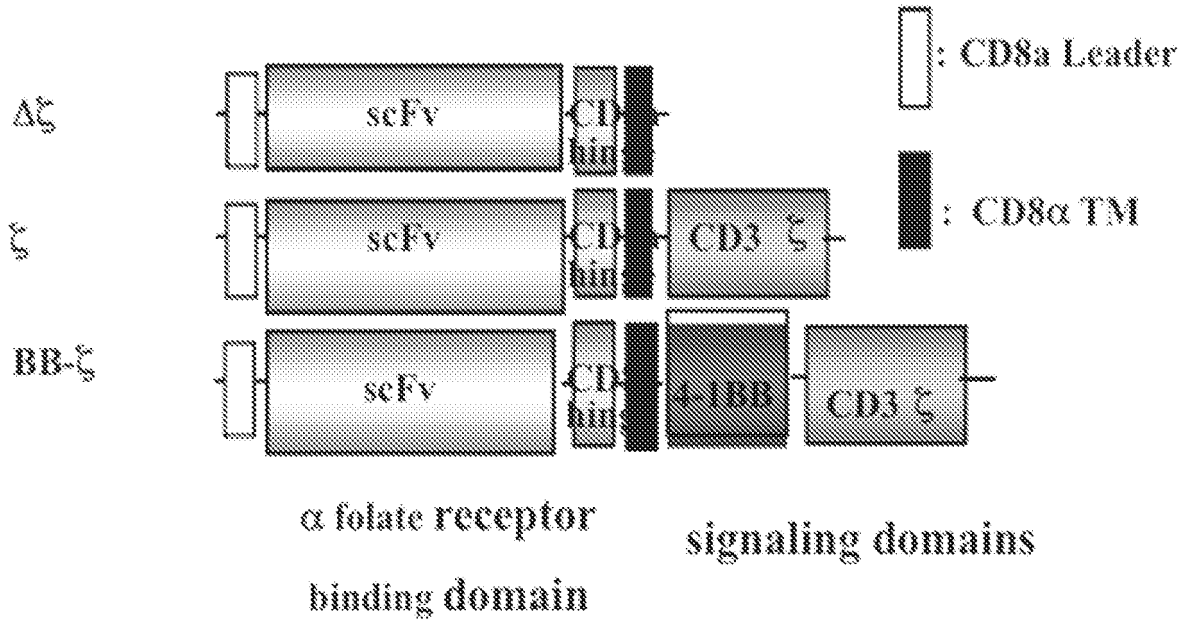


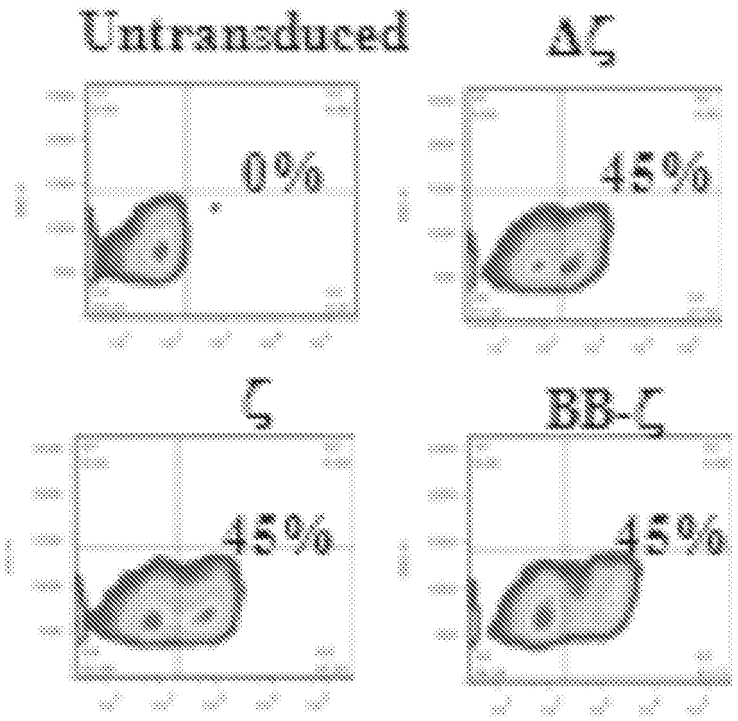


Figure 1
REPLACEMENT SHEET

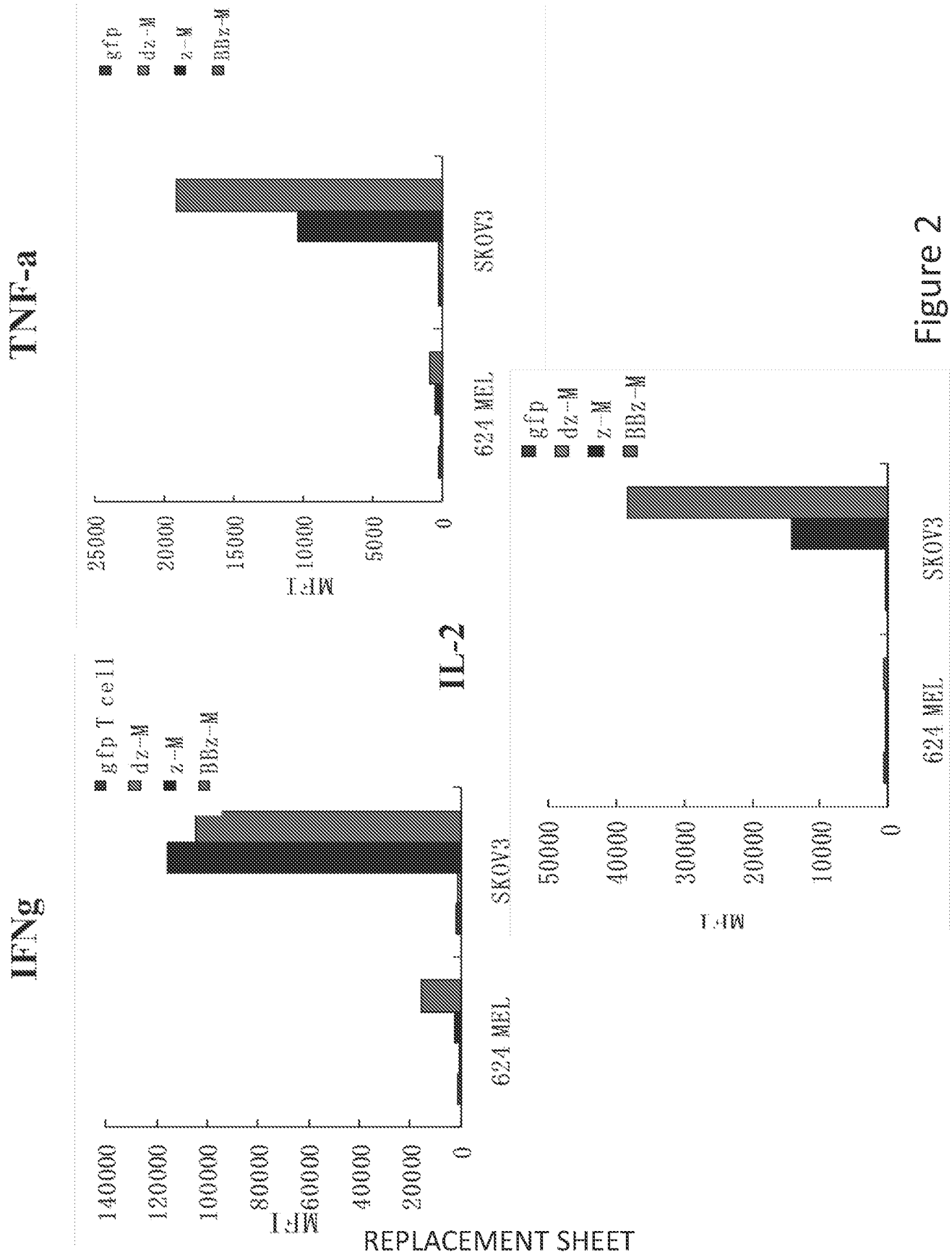

IFNg
gfp T cell
dz-M
z-M
BBz-M
MFI
140000
120000
100000
80000
60000
40000
20000
0
624 MEL
SKOV3
IL-2
gfp
dz-M
z-M
BBz-M
MFI
50000
40000
30000
20000
10000
0
624 MEL
SKOV3
TNF-a
gfp
dz-M
z-M
BBz-M
MFI
25000
20000
15000
10000
5000
0
624 MEL
SKOV3

Figure 2

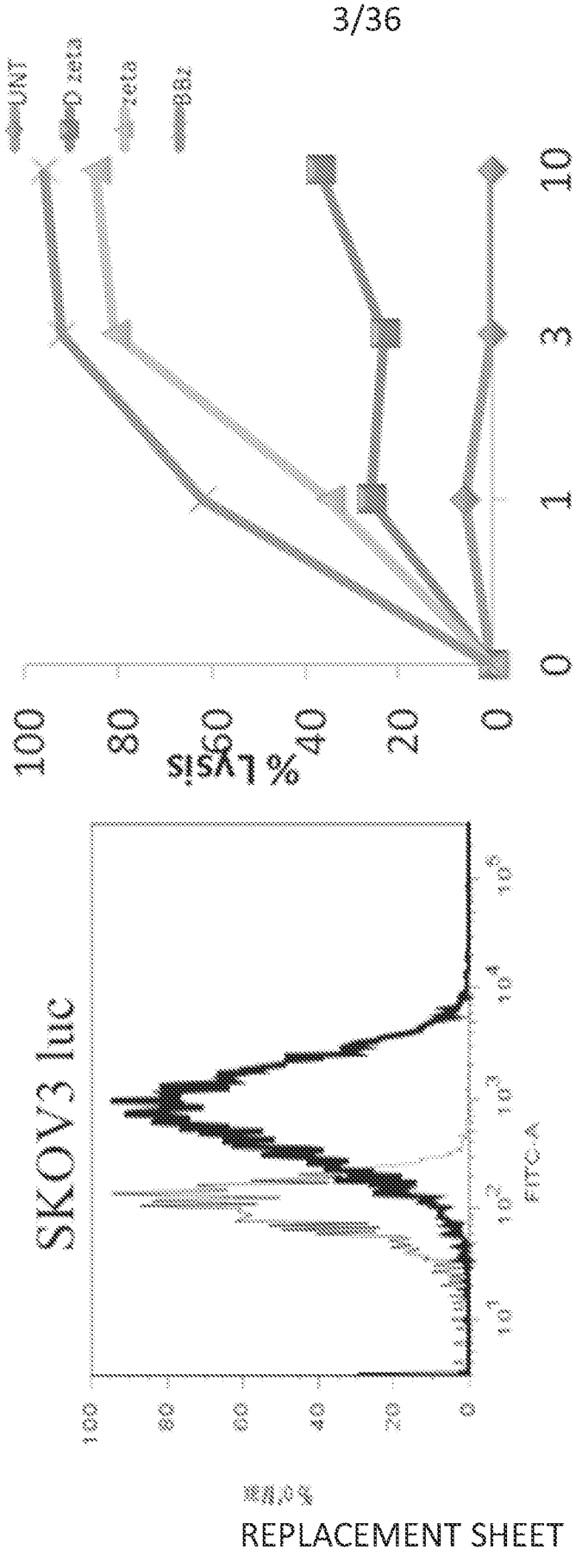

UNT
ζ zeta
ζ zeta
BBz
100
80
60
40
20
0
% Lysis
0
1
3
10
E:T ratio
SKOV3 luc
aFR expression
FITC-A

Figure 3

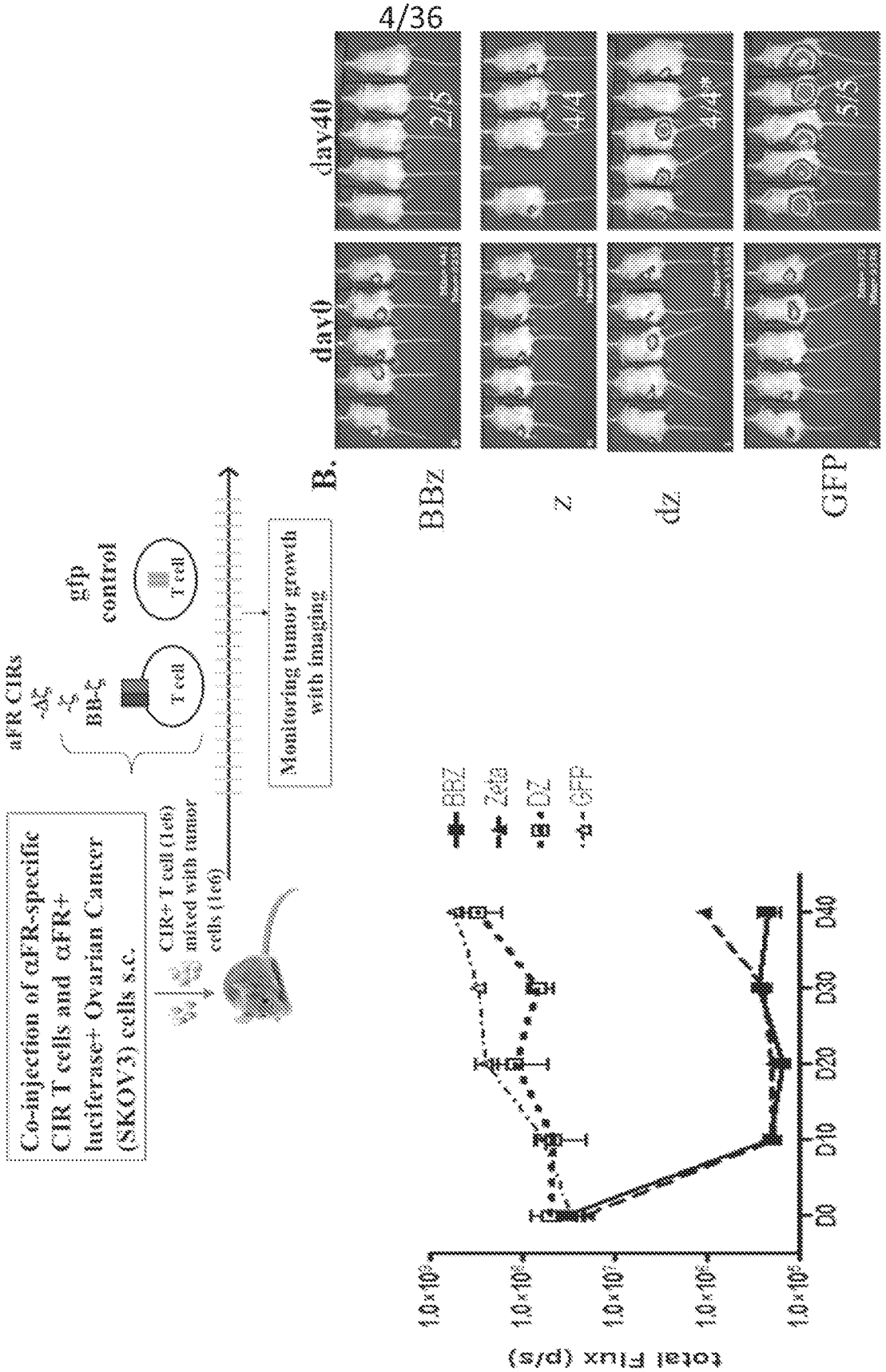
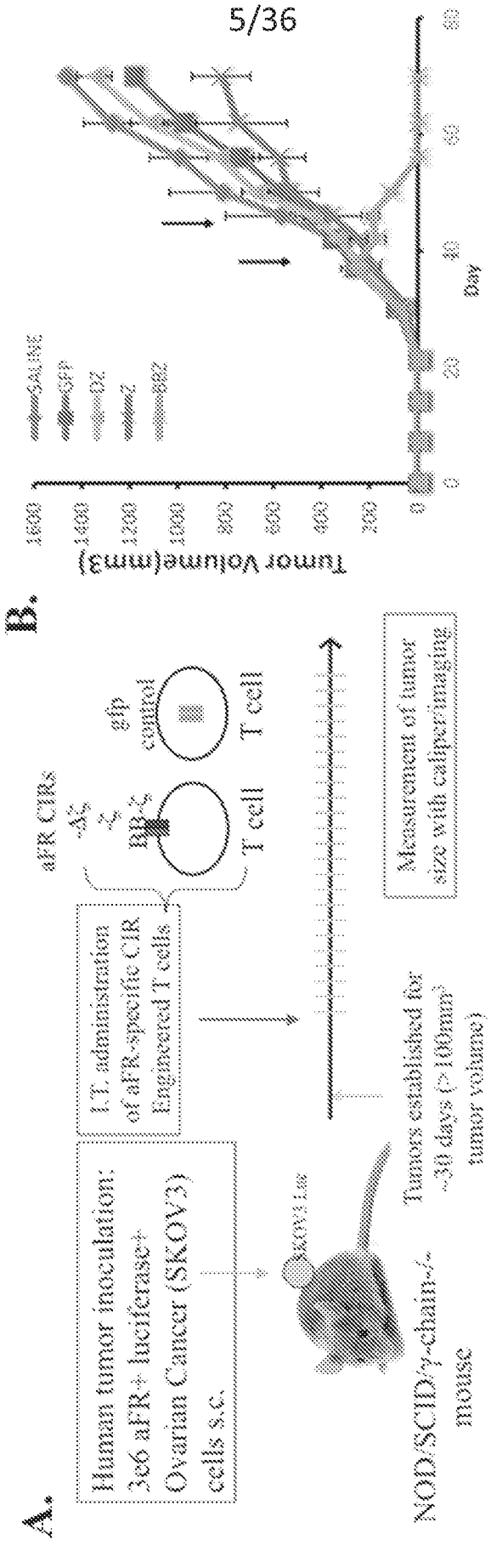

A.
Co-injection of αFR-specific CIR T cells and αFR+ luciferase+ Ovarian Cancer (SKOV3) cells s.c.
αFR CIRs
ζ
BB-ζ
gfp control
T cell
T cell
CIR+ T cell (1e6) mixed with tumor cells (1e6)
Monitoring tumor growth with imaging
BBZ
Zeta
DZ
GFP
total Flux (p/s)
1.0×10⁶
1.0×10⁷
1.0×10⁸
1.0×10⁹
D0
D10
D20
D30
D40
B.
day0
day40
BBz
z
dz
GFP
2.5
4.4
4.4
5.5

Figure 4



Figures 5A-5B

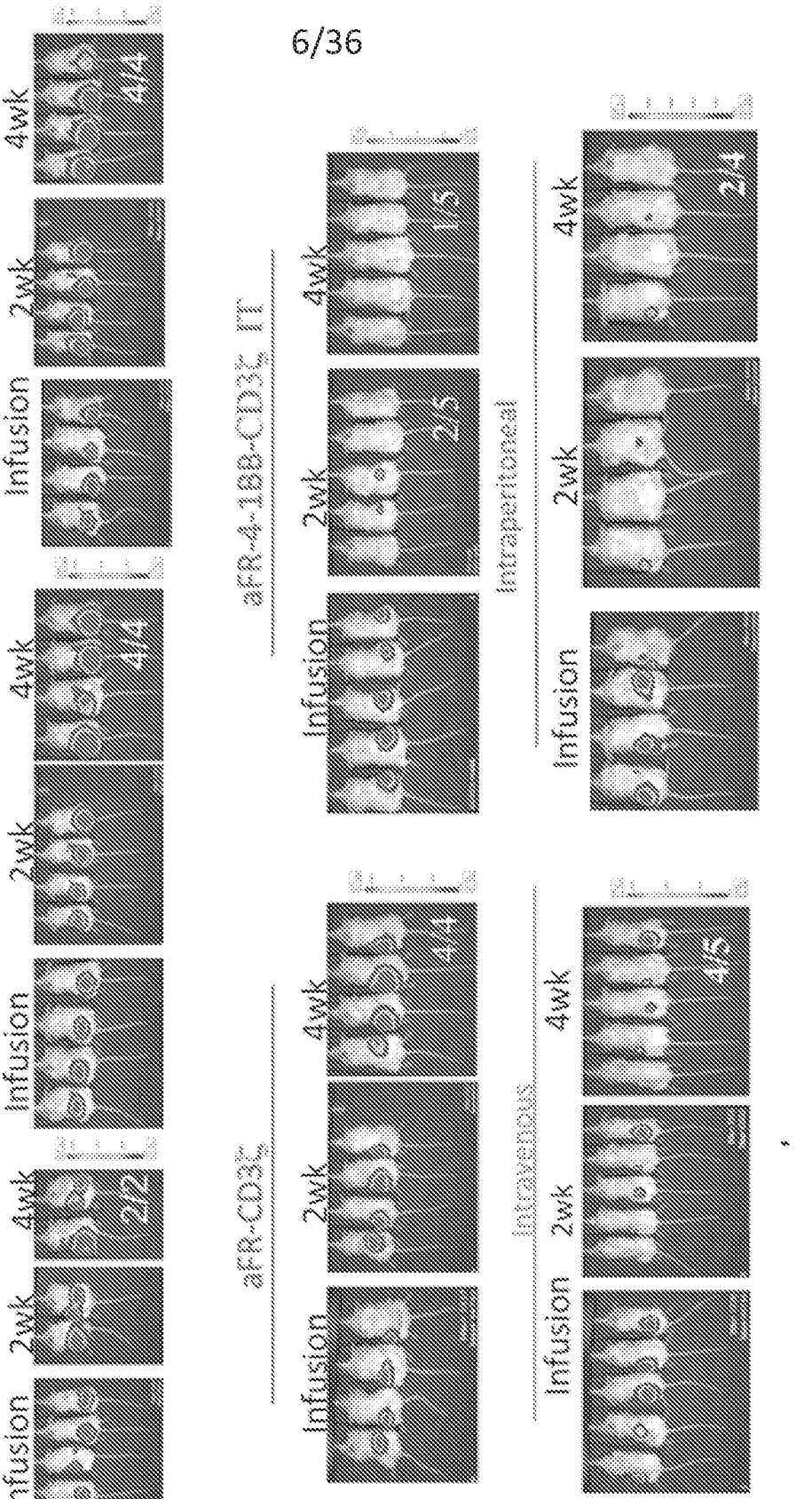
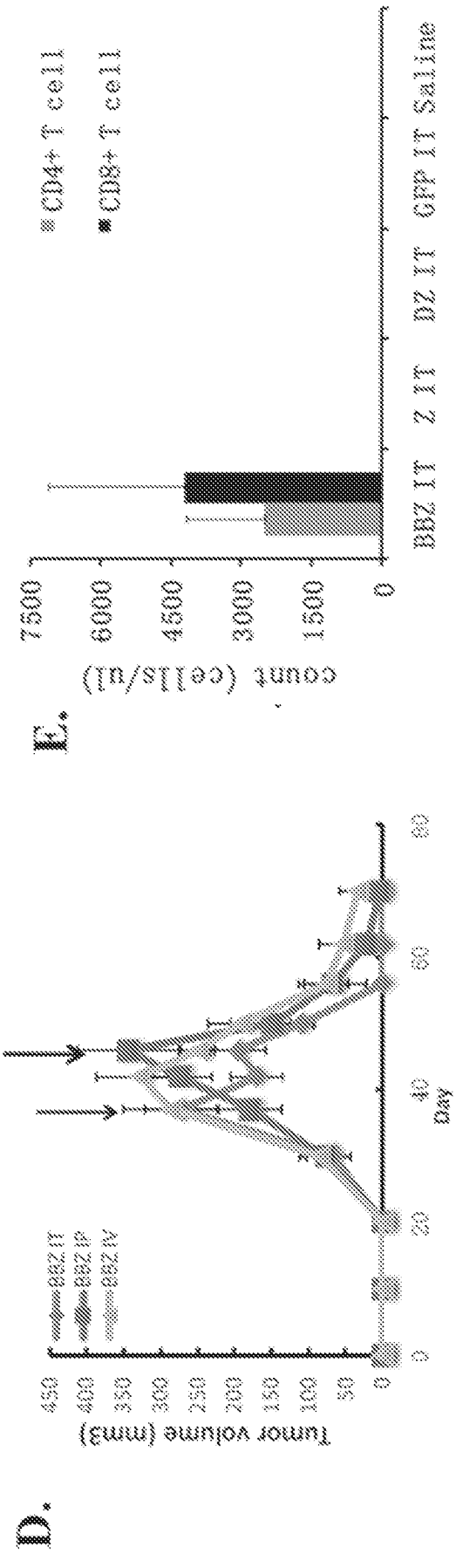

saline
gfp-T cells
aER-CD3ε
aFR-CD3ε
aFR-4-1BB-CD3ε IT
Infusion
2wk
4wk
Intravenous
Intraperitoneal
4/4
2/2
2/5
1/5
4/5
2/4

Figure 5C



Figures 5D-5E

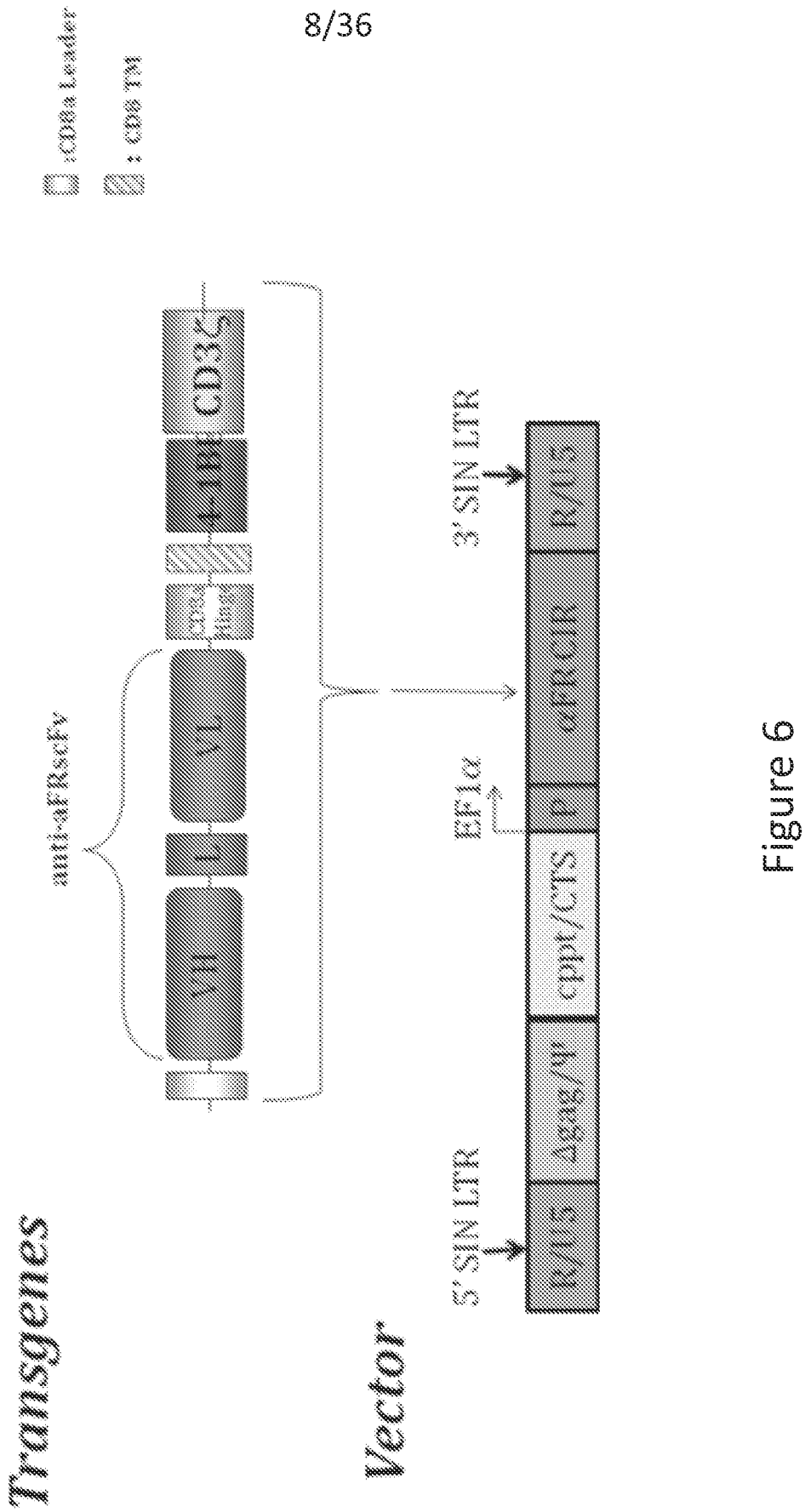


Figure 6

REPLACEMENT SHEET

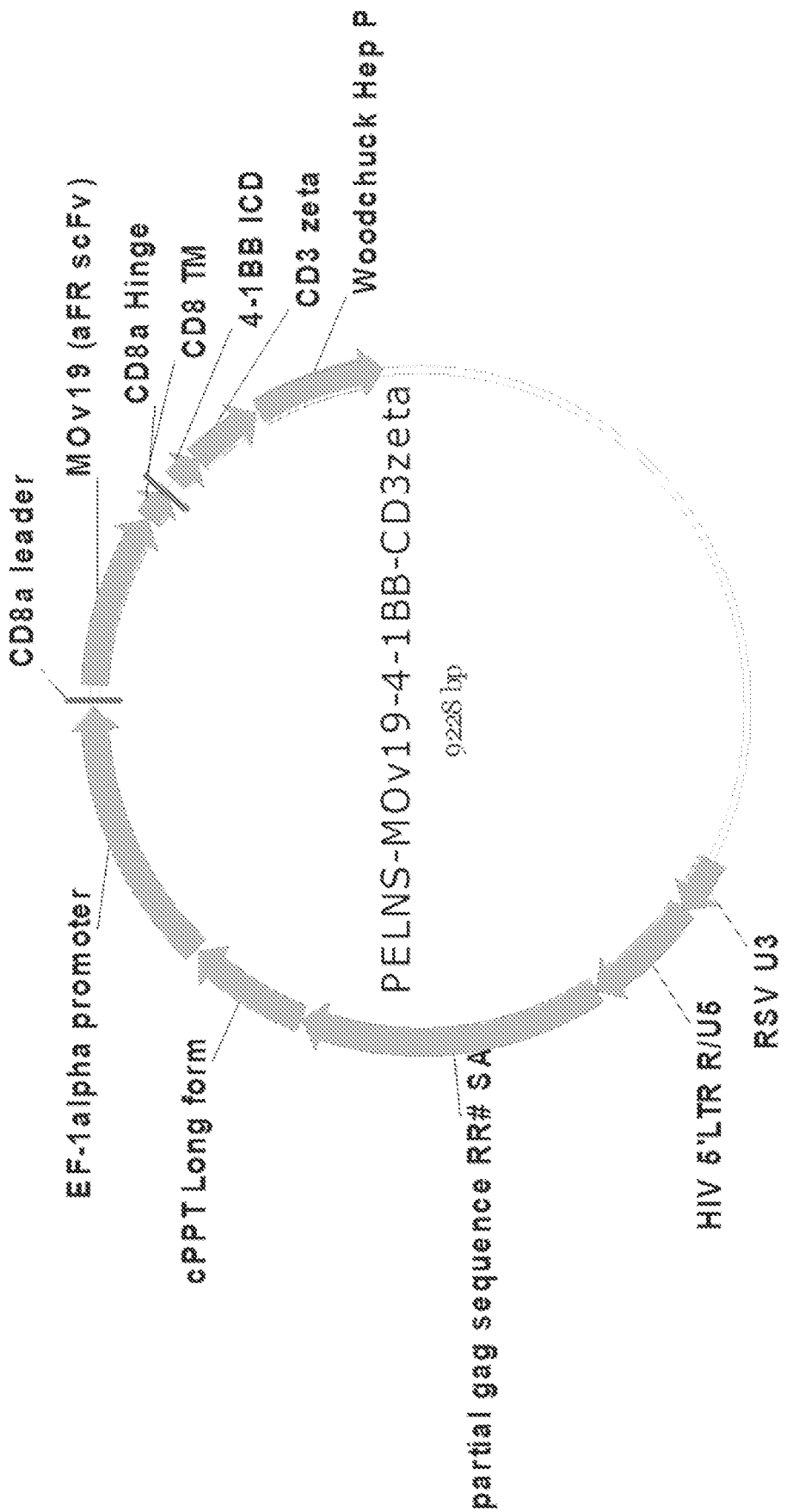
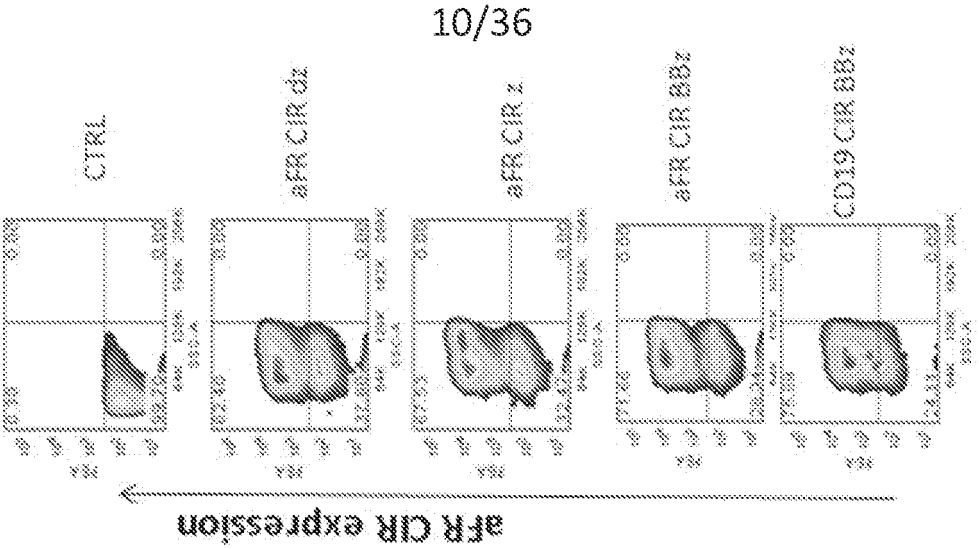

PELNS-MOv19-4-1BB-CD3zeta
9228 bp
CD8a leader
MOv19 (aFR scFv)
CD8a Hinge
CD8 TM
4-1BB ICD
CD3 zeta
Woodchuck Hep P
EF-1alpha promoter
cPPT Long form
partial gag sequence RR# SA
HIV 5'LTR R/U5
RSV U3

Figure 7


CTRL
aFR CIR dz
aFR CIR z
aFR CIR BBz
CD19 CIR BBz
aFR CIR expression

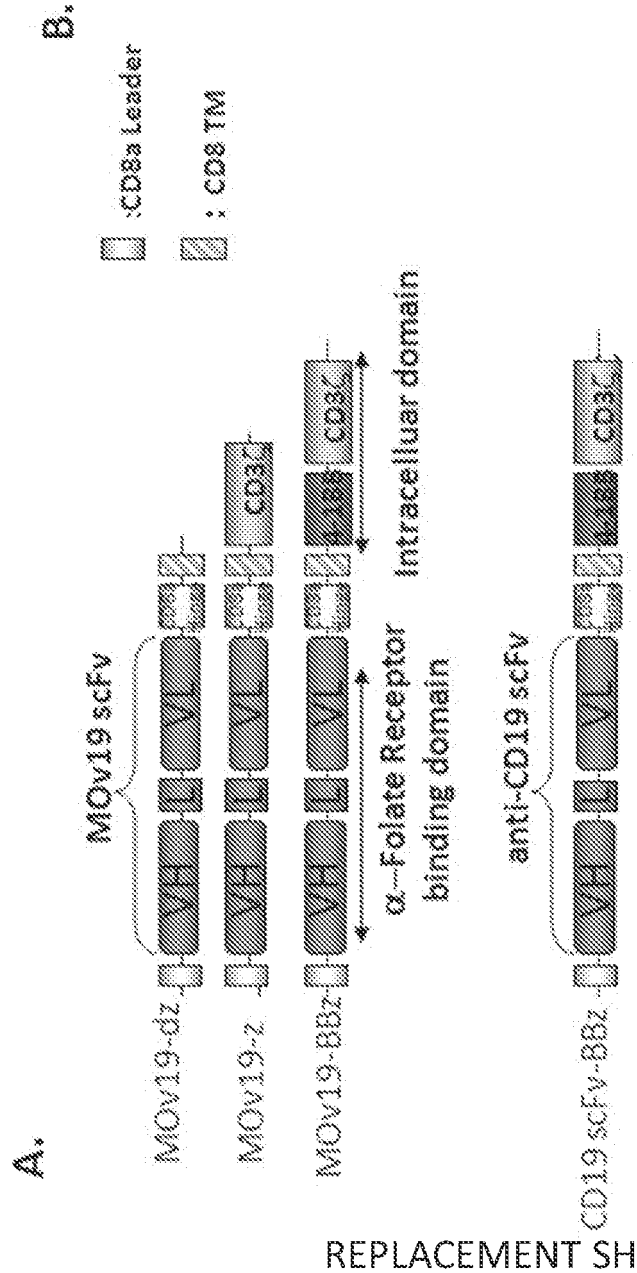

B.
: CD8a Leader
: CD8 TM
A.
MOv19 scFv
MOv19-dz
MOv19-z
MOv19-BBz
CD19 scFv-BBz
VH
L
VL
CD3ζ
4-1BB
α–Folate Receptor binding domain
Intracellular domain
anti-CD19 scFv

Figure 8

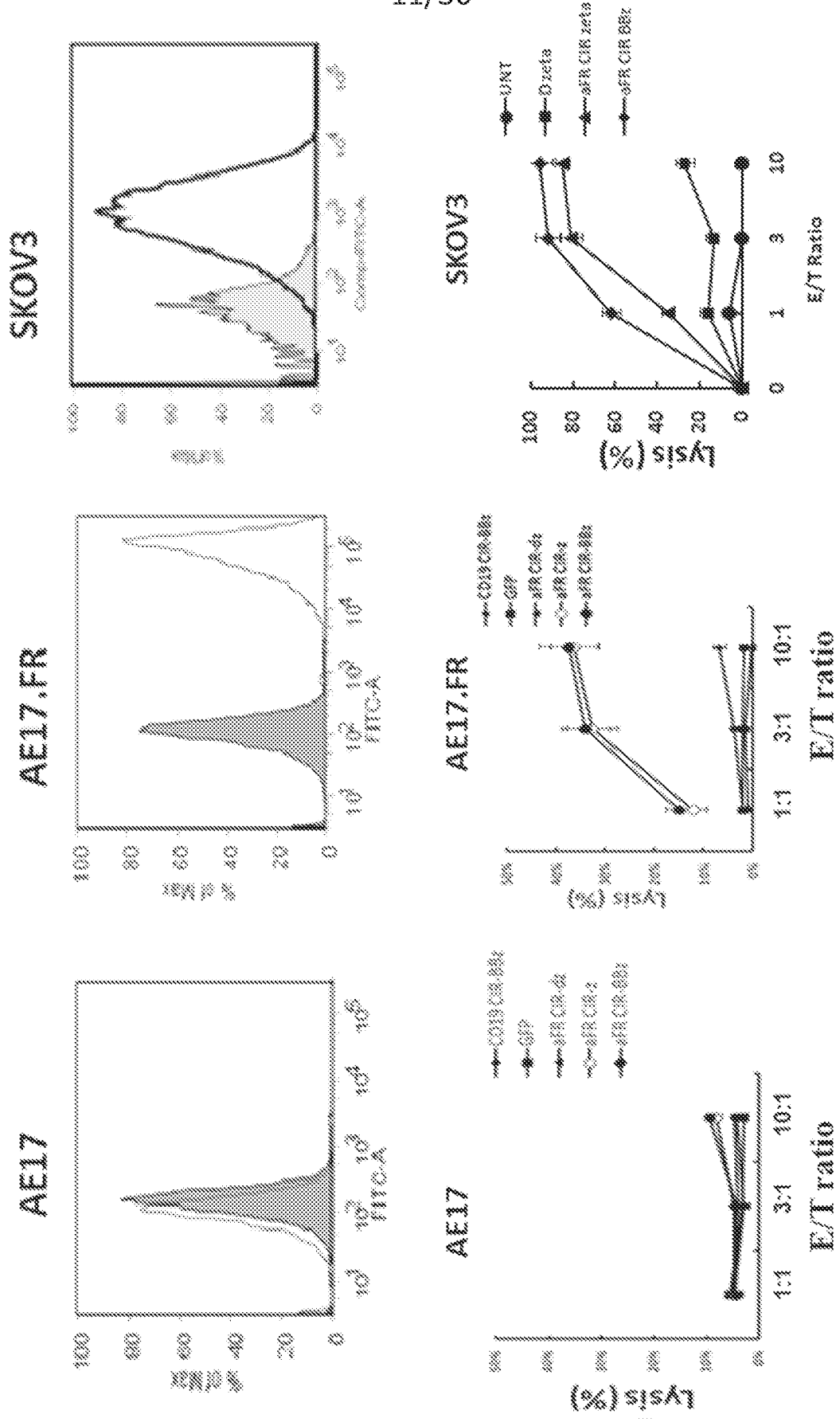

SKOV3
AE17.FR
AE17
FITC-A
Count
% of Max
Lysis (%)
E/T ratio
1:1
3:1
10:1
UNT
CD19 CAR-BBz
GFP
αFR CAR-ζ
αFR CAR-2
αFR CAR-BBz

Figure 9

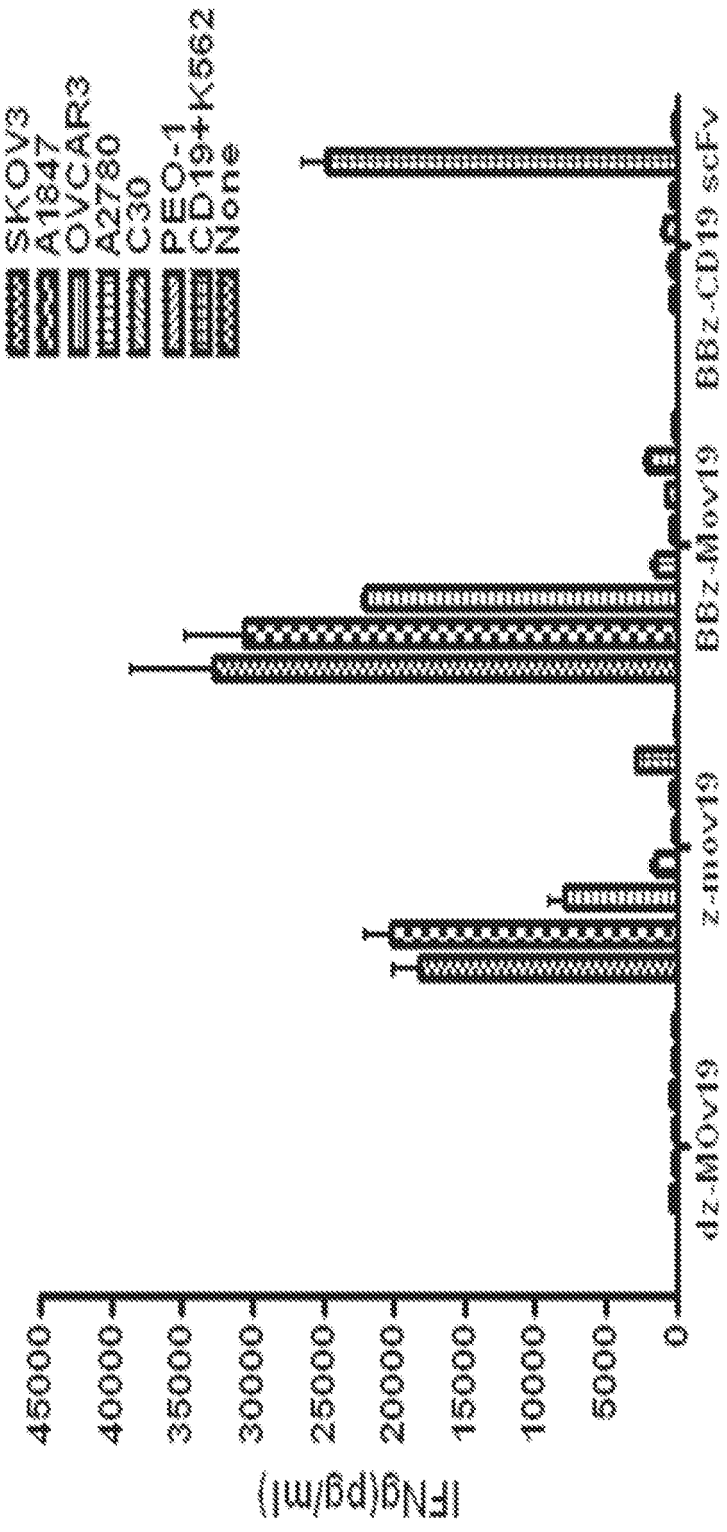


Figure 9 cont'd

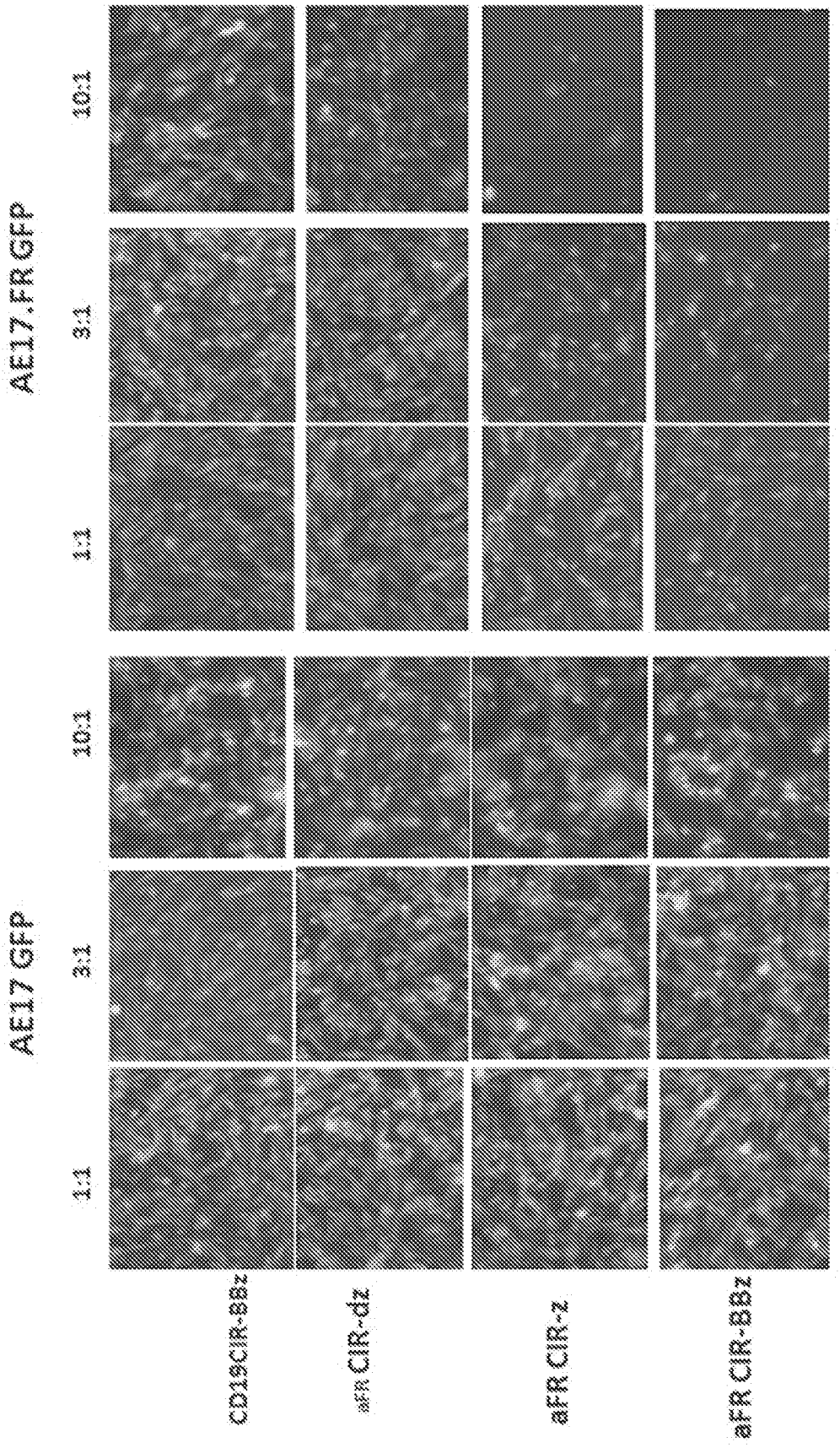

AE17 GFP
AE17.FR GFP
1:1
3:1
10:1
CD19CIR-BBz
αFR CIR-dz
aFR CIR-z
aFR CIR-BBz

Figure 10

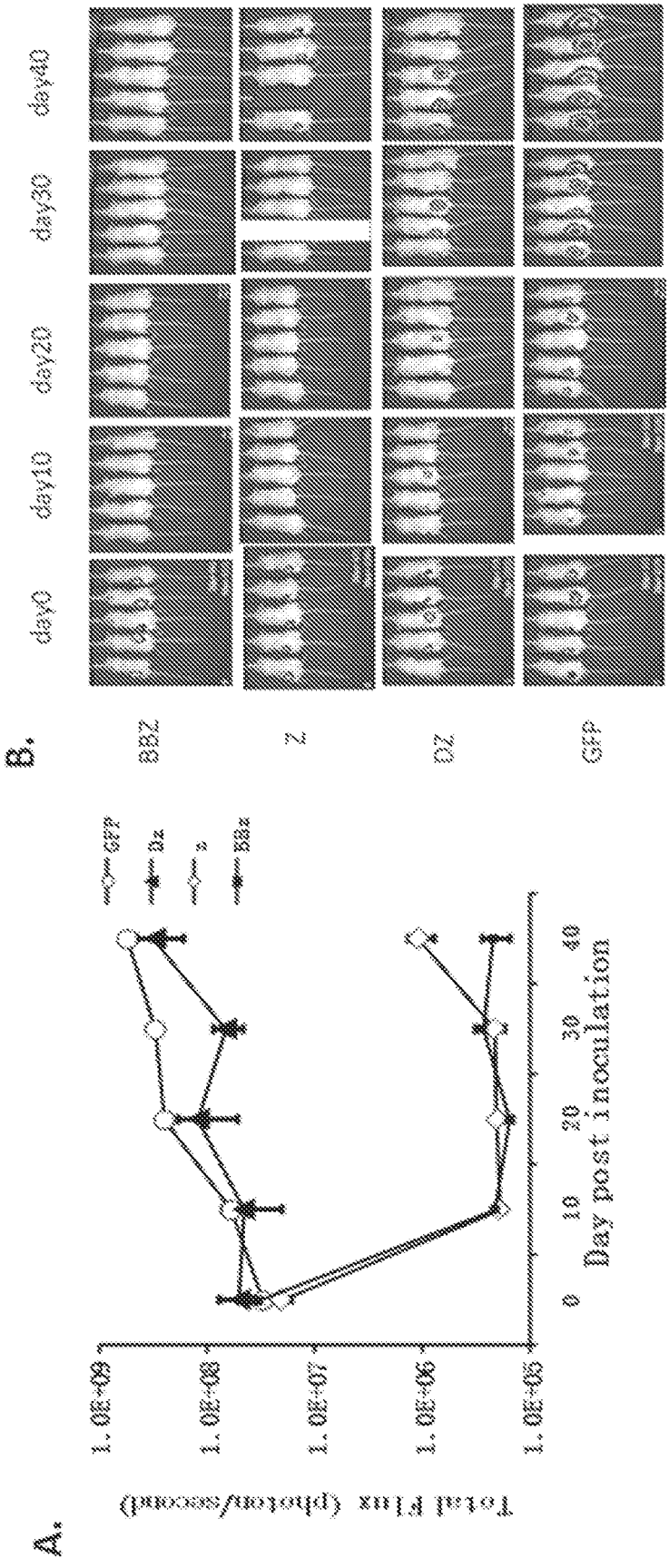
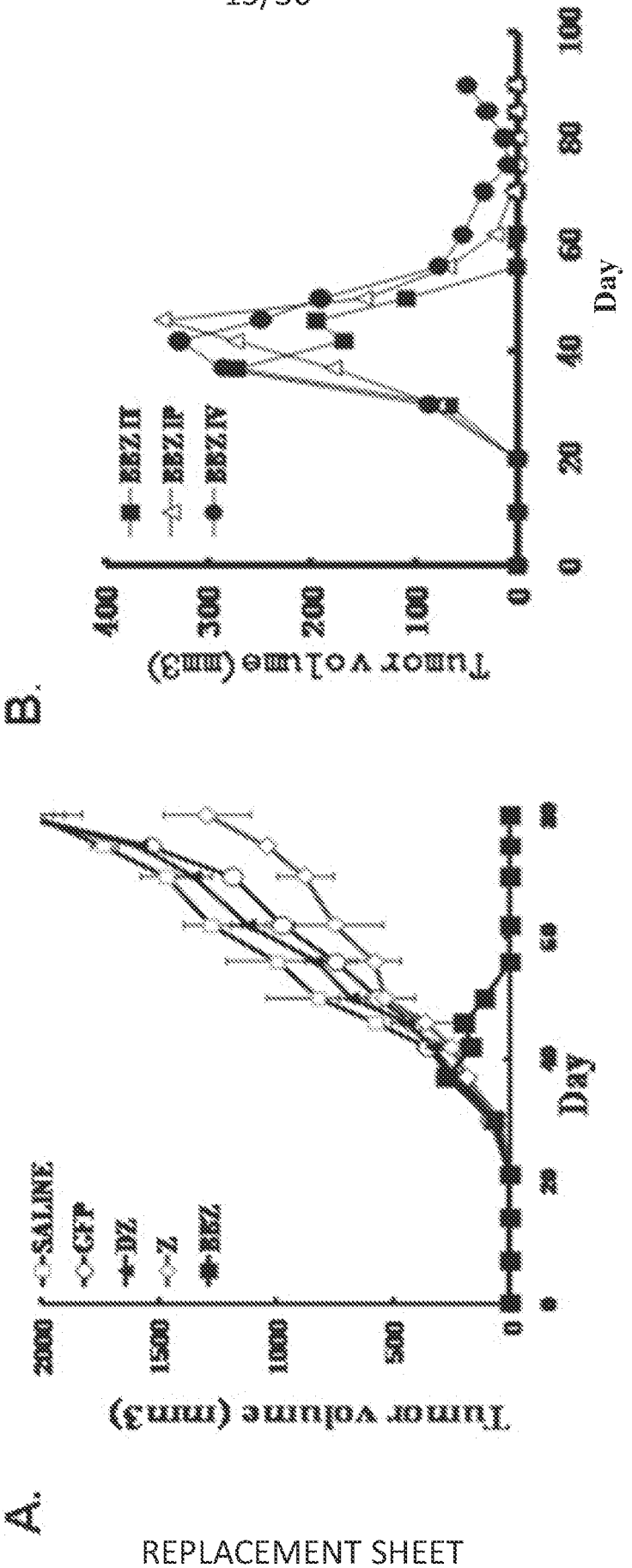
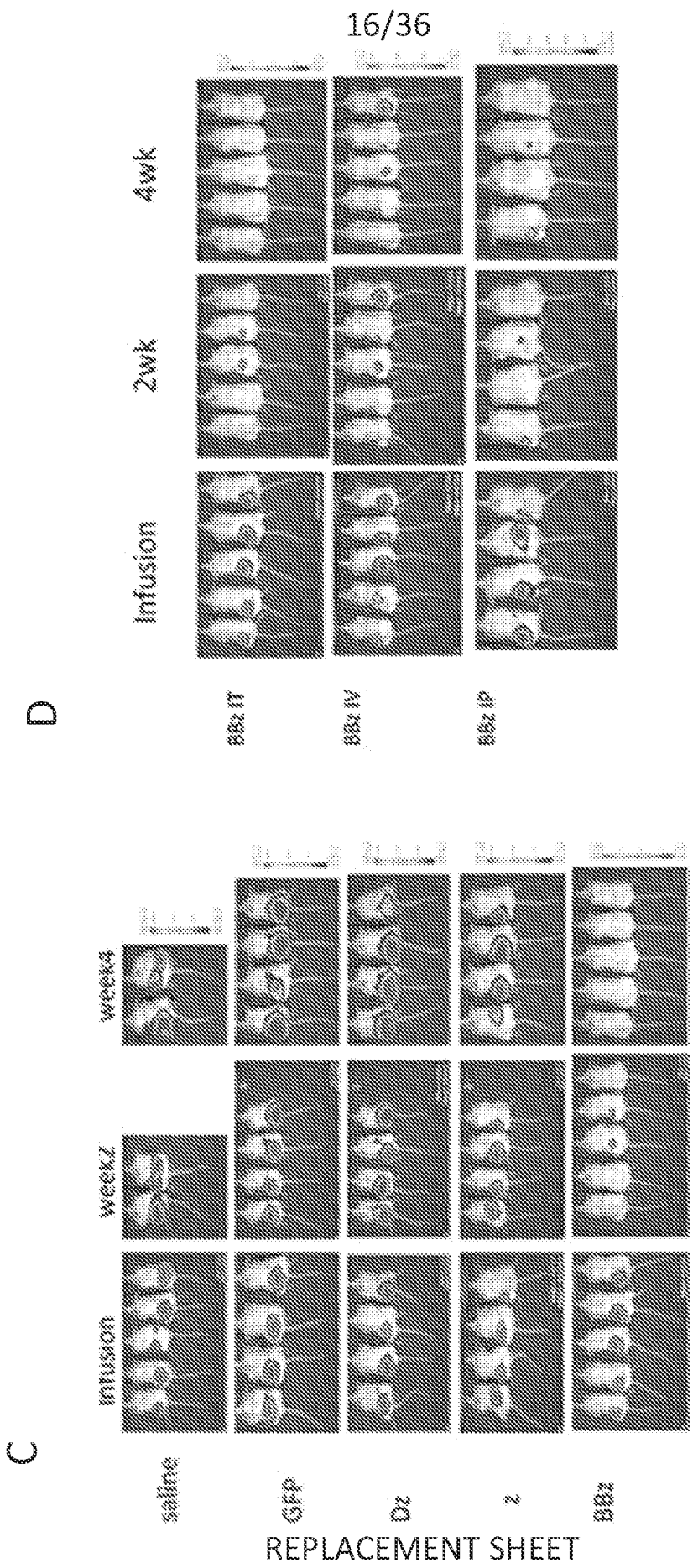

A.
Total Flux (photon/second)
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
0
10
20
30
40
Day post inoculation
GFP
BBa
B.
day0
day10
day20
day30
day40
BBZ
Z
OZ
GFP

Figure 11



Figures 12A-12B



Figures 12C-12D

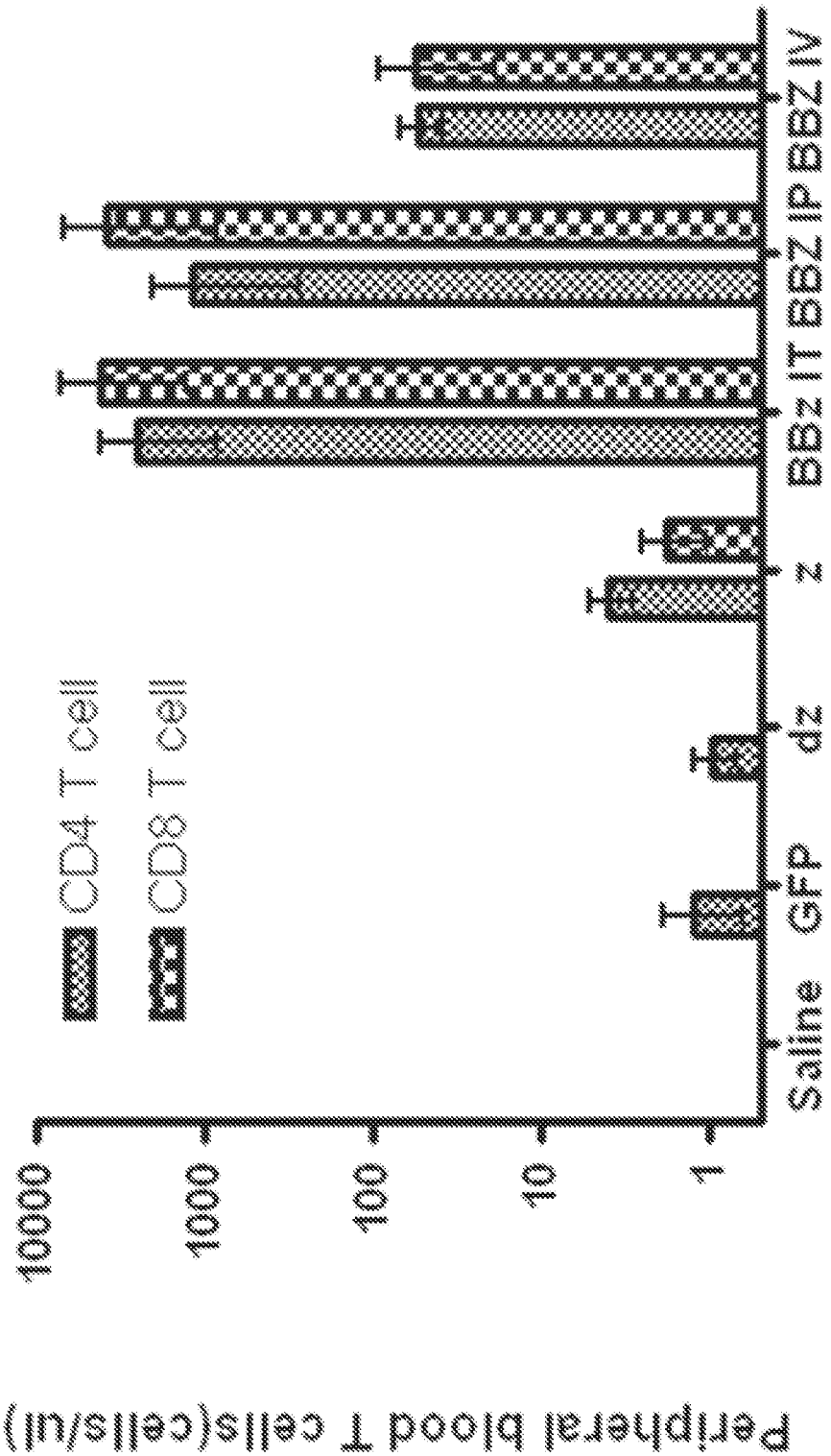

Peripheral blood T cells(cells/ul)
10000
1000
100
10
1
CD4 T cell
CD8 T cell
Saline
GFP
dz
z
BBz IT
BBZ IP
BBZ IV

Figure 13

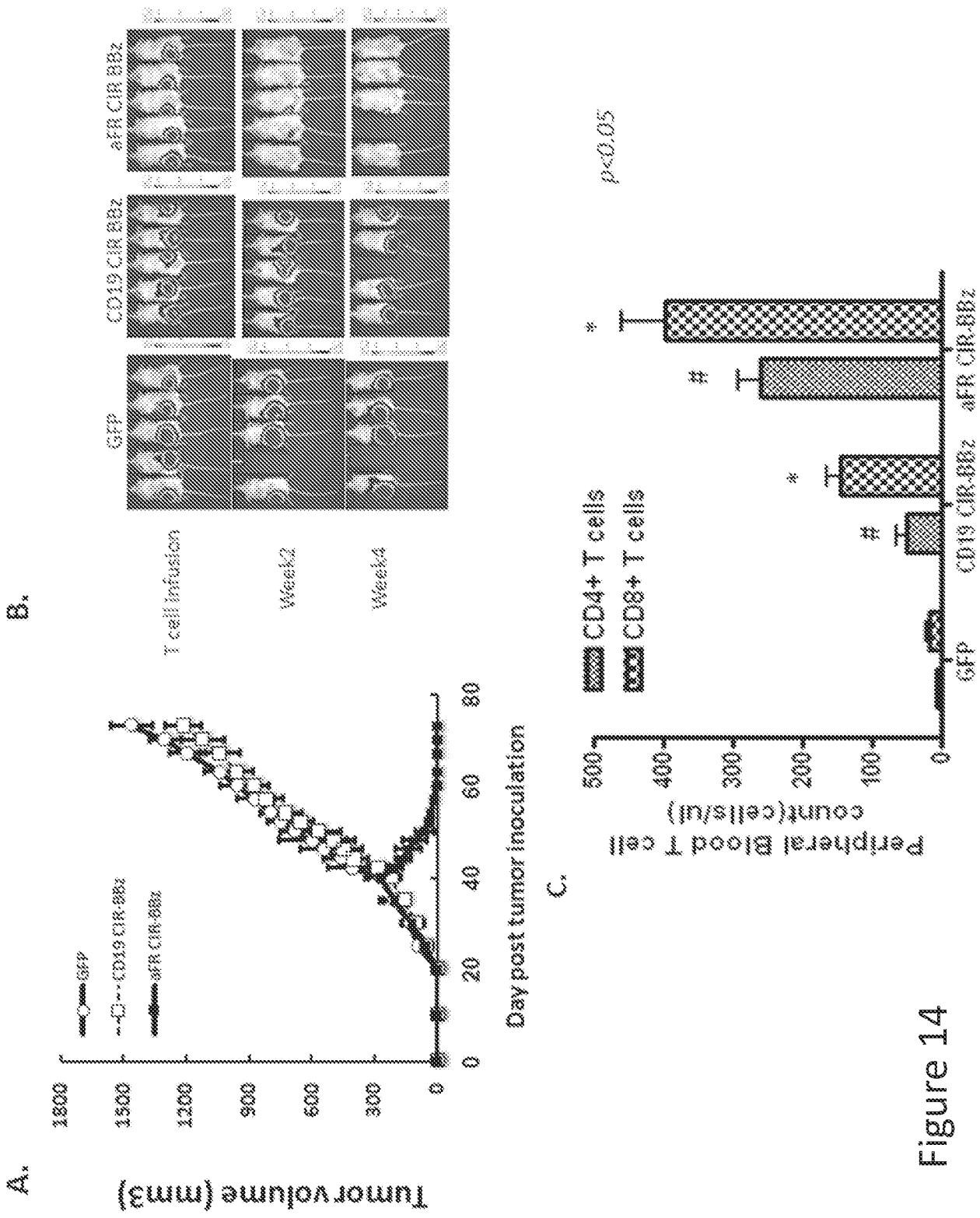

A.
Tumor volume (mm3)
1800
1500
1200
900
600
300
0
0
20
40
60
80
Day post tumor inoculation
GFP
CD19 CIR-BBz
aFR CIR-BBz
B.
T cell infusion
Week2
Week4
GFP
CD19 CIR BBz
aFR CIR BBz
C.
Peripheral Blood T cell count(cells/ul)
500
400
300
200
100
0
CD4+ T cells
CD8+ T cells
p<0.05
*
#
GFP
CD19 CIR-BBz
aFR CIR-BBz

Figure 14

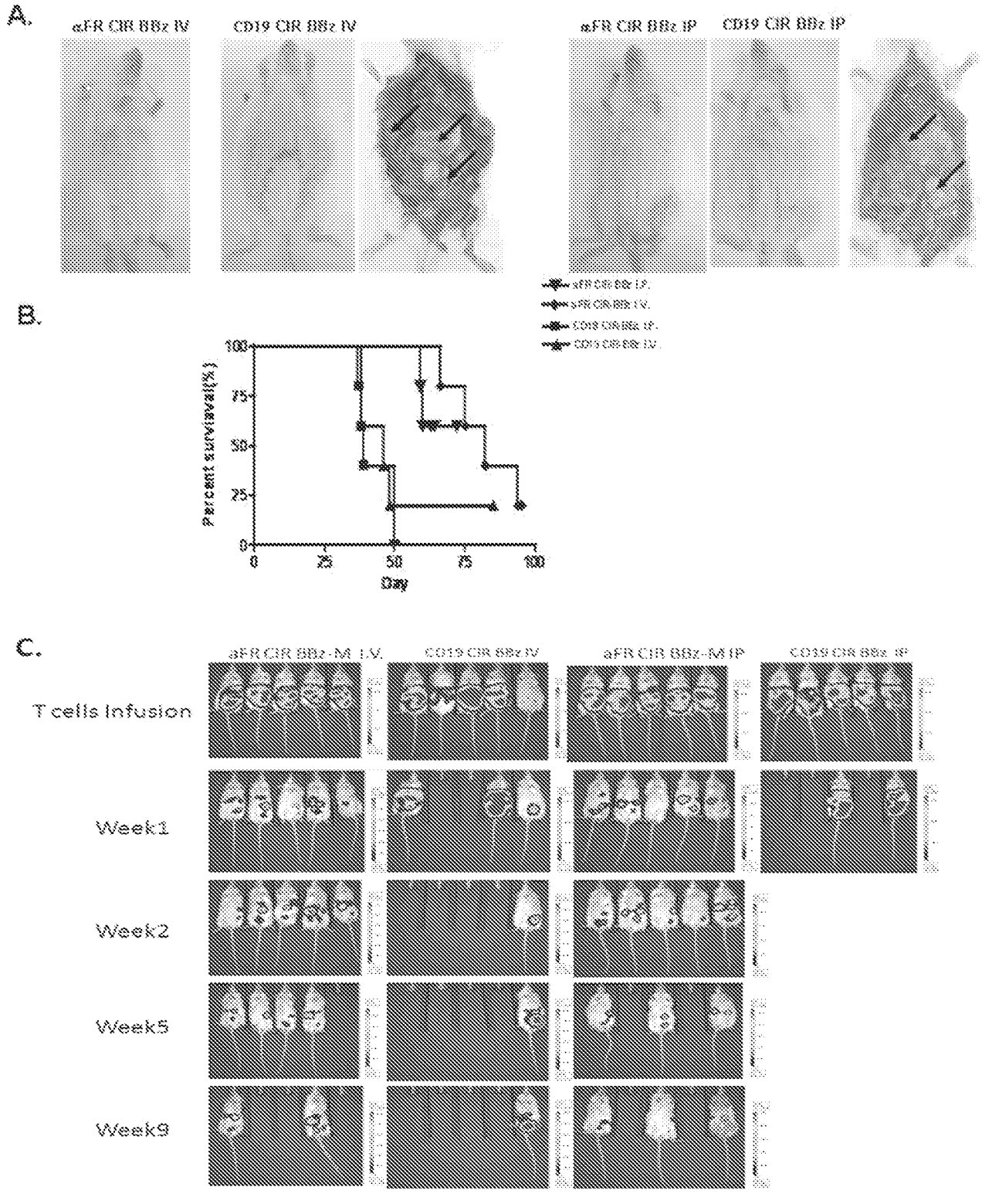


Figure 15

REPLACEMENT SHEET

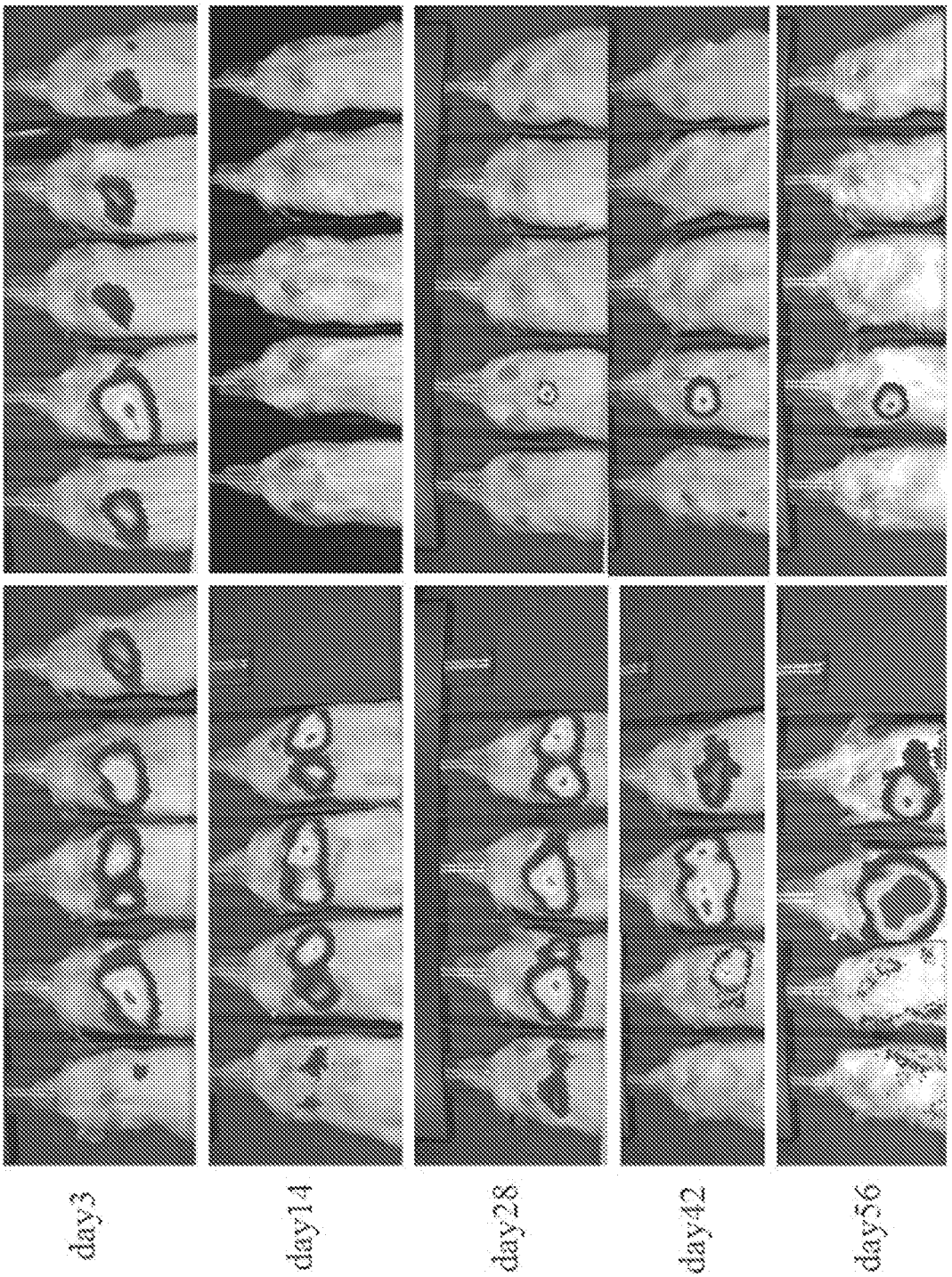


Figure 16

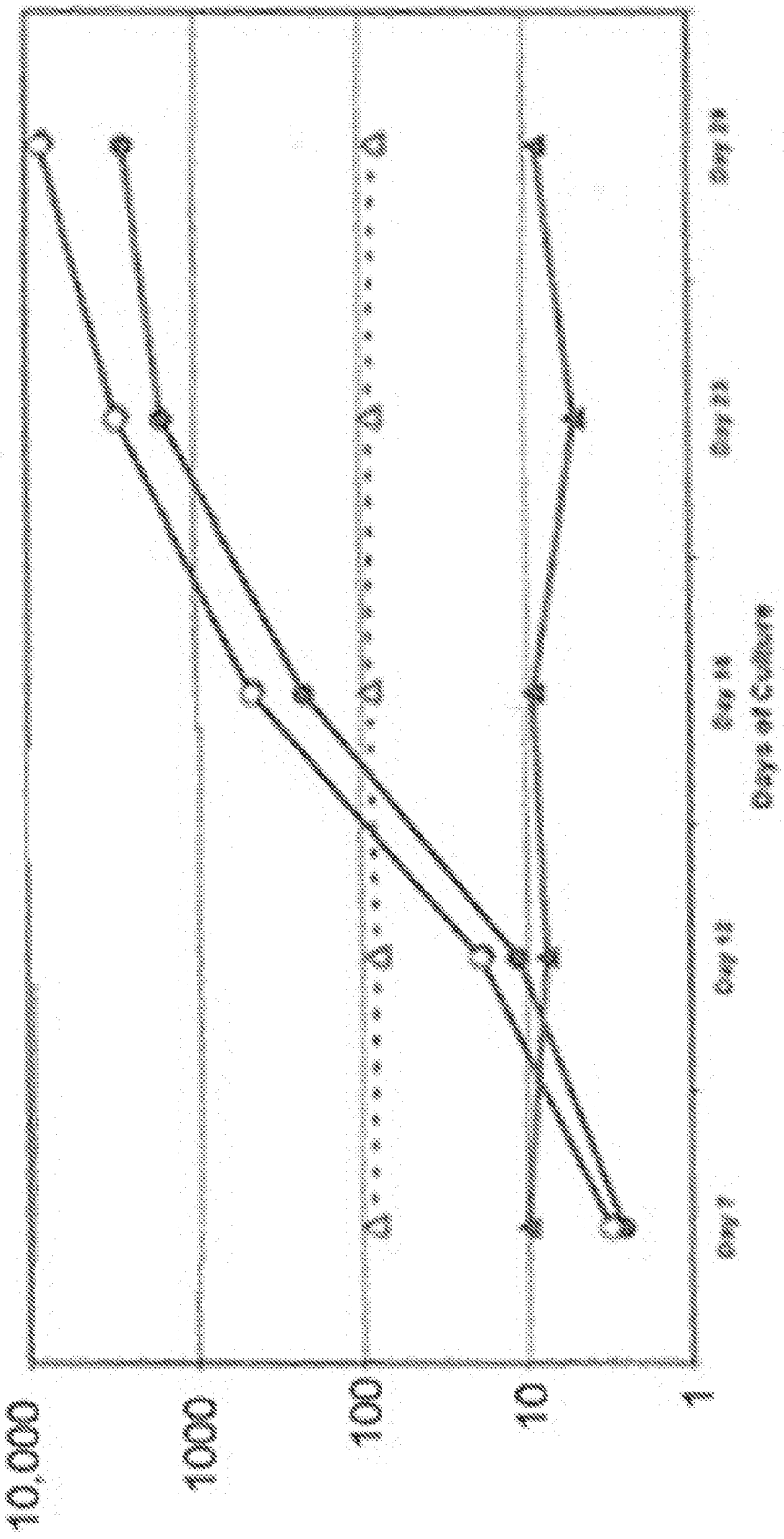
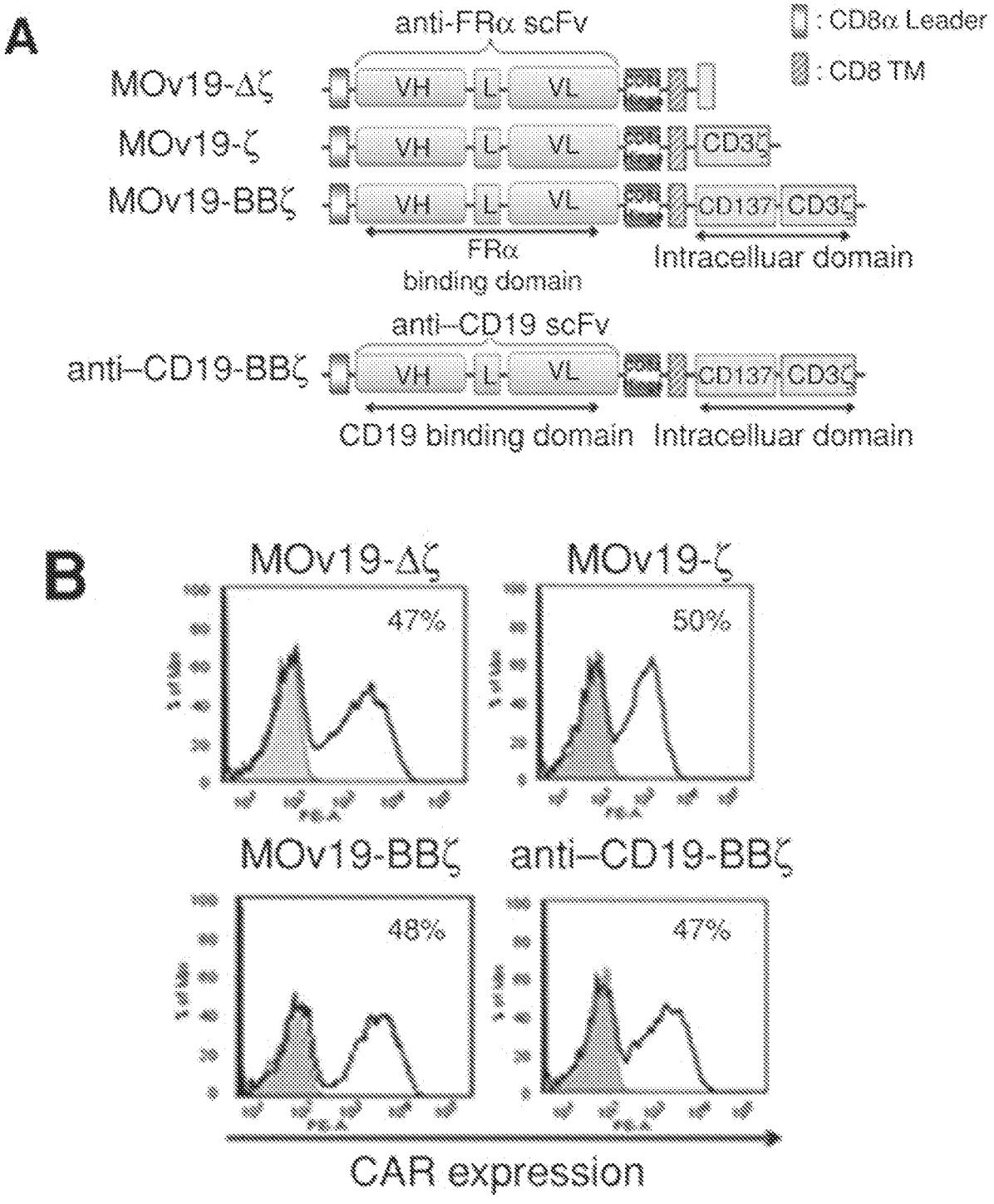

10,000
1000
100
10
1
Days at Culture
Day 21
Day 16
Day 11
Day 6
Day 1

Figure 17



Figures 18A-18B

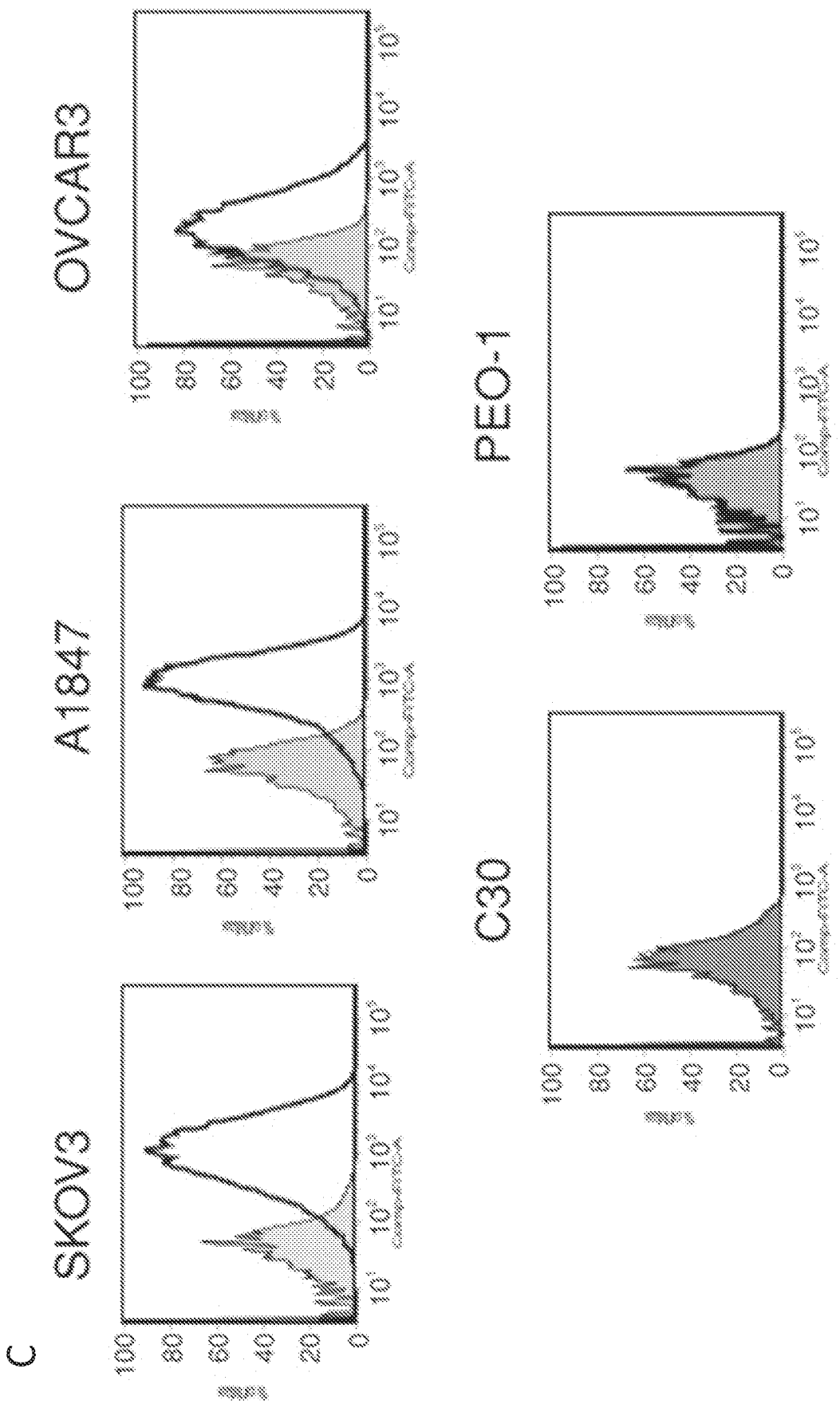
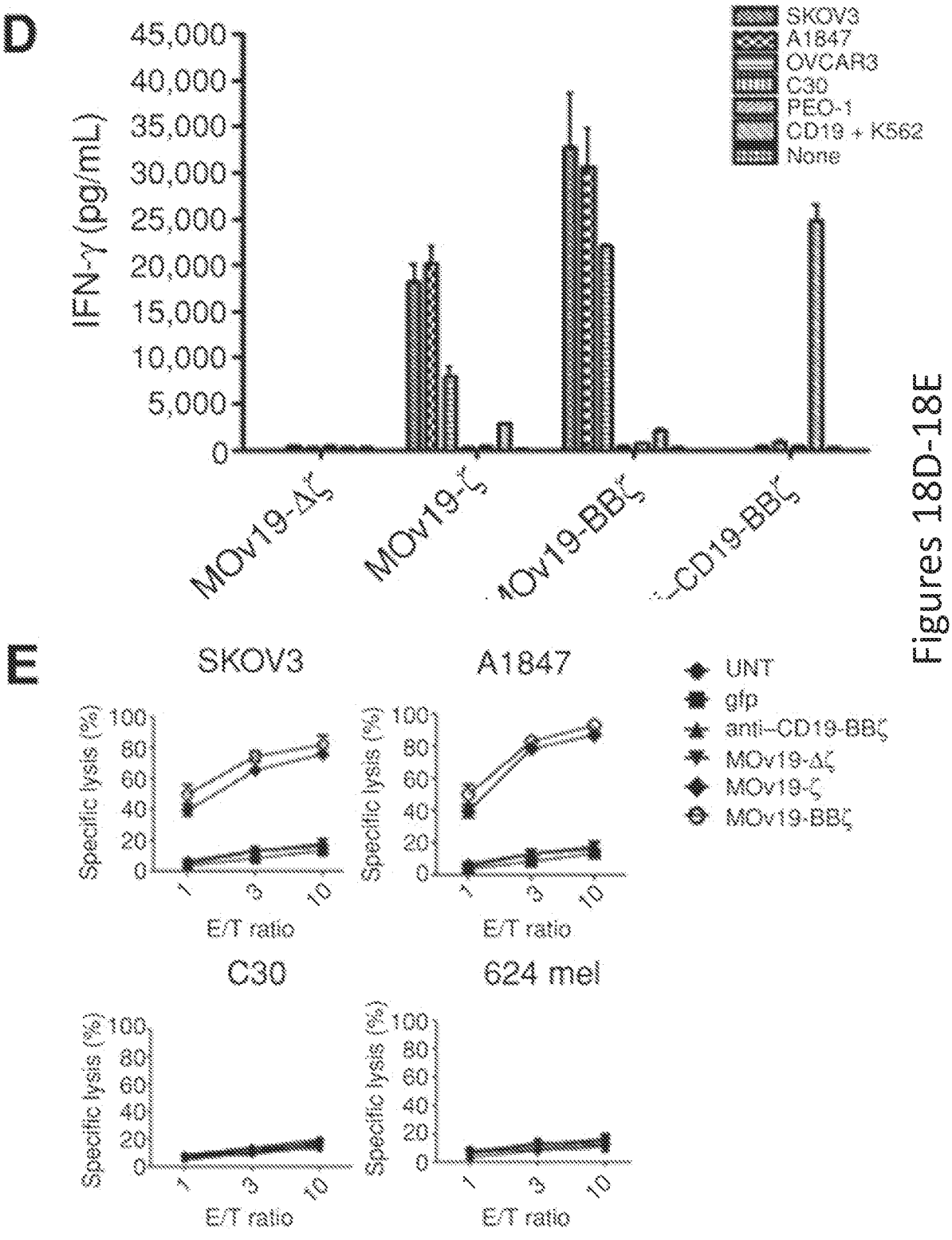

C
SKOV3
A1847
C30
PEO-1
OVCAR3
100
80
60
40
20
0
%
10^1
10^2
10^3
10^4
10^5

Figure 18C



Figures 18D-18E

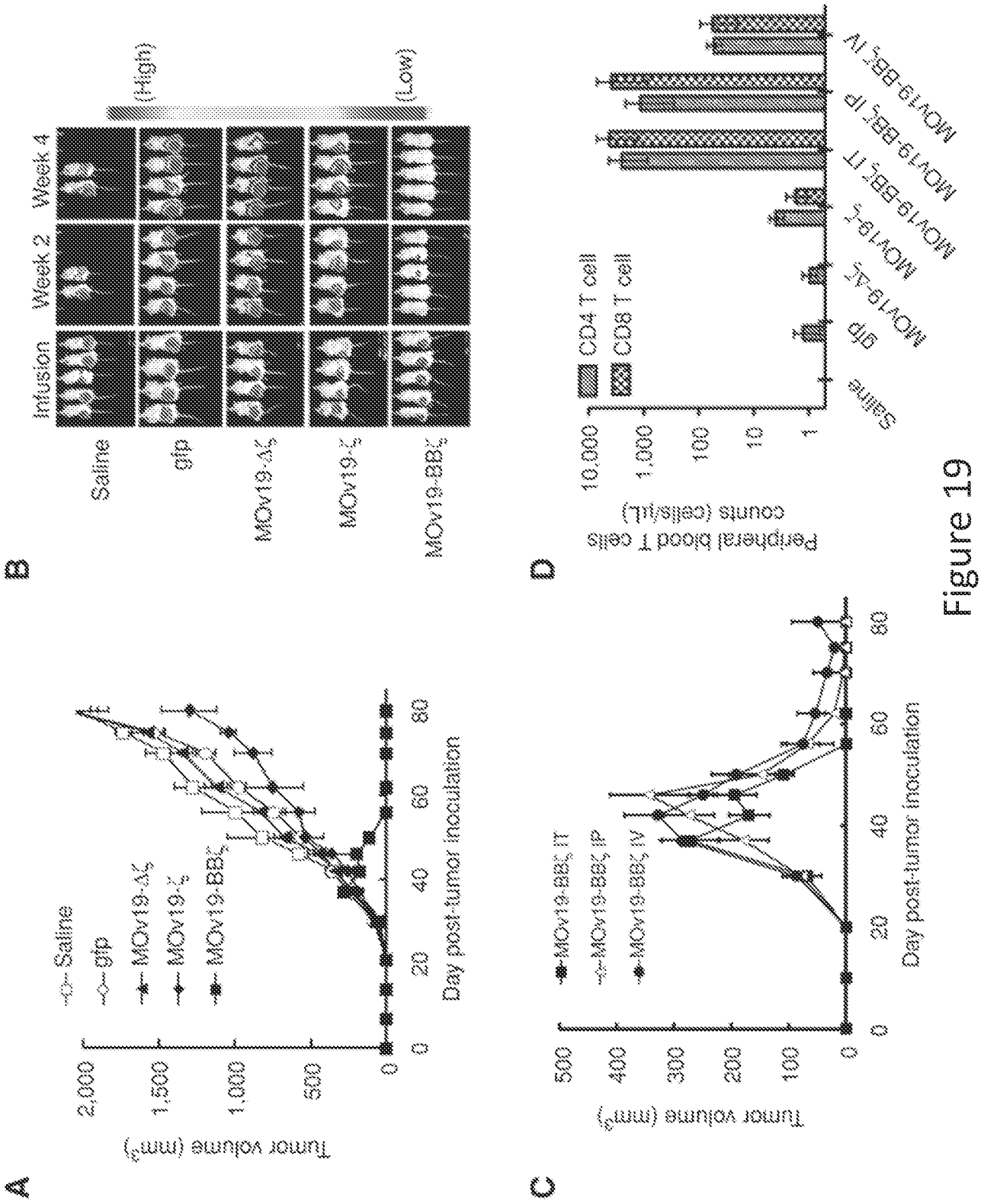

A
Tumor volume (mm³)
2,000
1,500
1,000
500
0
0
20
40
60
80
Day post-tumor inoculation
Saline
gfp
MOv19-ΔζCD4 T cell
MOv19-ζCD8 T cell
MOv19-BBζ
B
Infusion
Week 2
Week 4
(High)
(Low)
Saline
gfp
MOv19-Δζ
MOv19-ζ
MOv19-BBζ
C
Tumor volume (mm³)
500
400
300
200
100
0
0
20
40
60
80
Day post-tumor inoculation
MOv19-BBζ IT
MOv19-BBζ IP
MOv19-BBζ IV
D
Peripheral blood T cells counts (cells/μL)
10,000
1,000
100
10
1
Saline
gfp
MOv19-Δζ
MOv19-ζ
MOv19-BBζ IT
MOv19-BBζ IP
MOv19-BBζ IV

Figure 19

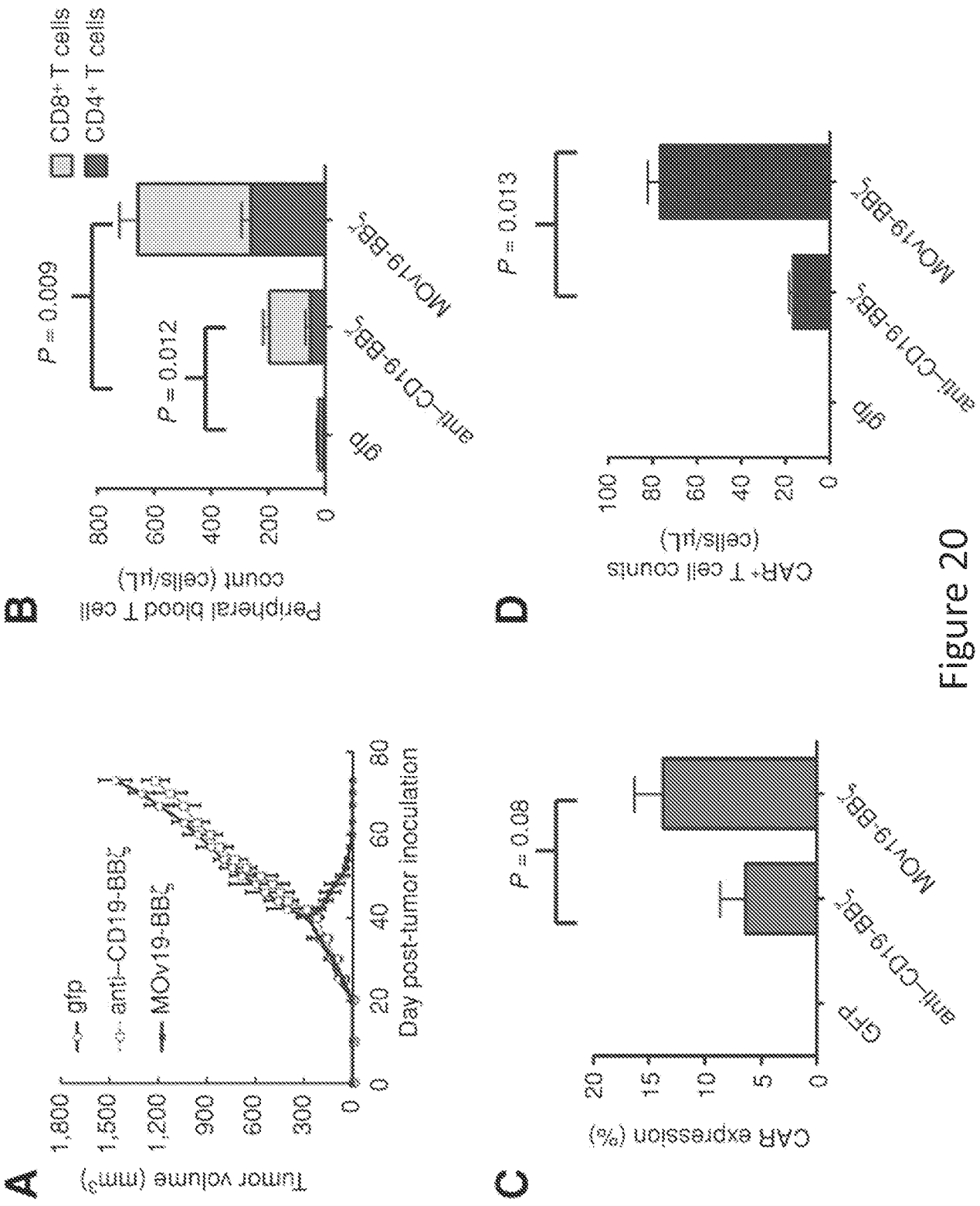

A
Tumor volume (mm³)
1,800
1,500
1,200
900
600
300
0
0
20
40
60
80
Day post-tumor inoculation
gfp
anti–CD19-BBζ
MOv19-BBζ
B
Peripheral blood T cell count (cells/μL)
800
600
400
200
0
P = 0.009
P = 0.012
CD8+ T cells
CD4+ T cells
gfp
anti–CD19-BBζ
MOv19-BBζ
C
CAR expression (%)
20
15
10
5
0
P = 0.08
GFP
anti–CD19-BBζ
MOv19-BBζ
D
CAR+ T cell counts (cells/μL)
100
80
60
40
20
0
P = 0.013
gfp
anti–CD19-BBζ
MOv19-BBζ

Figure 20

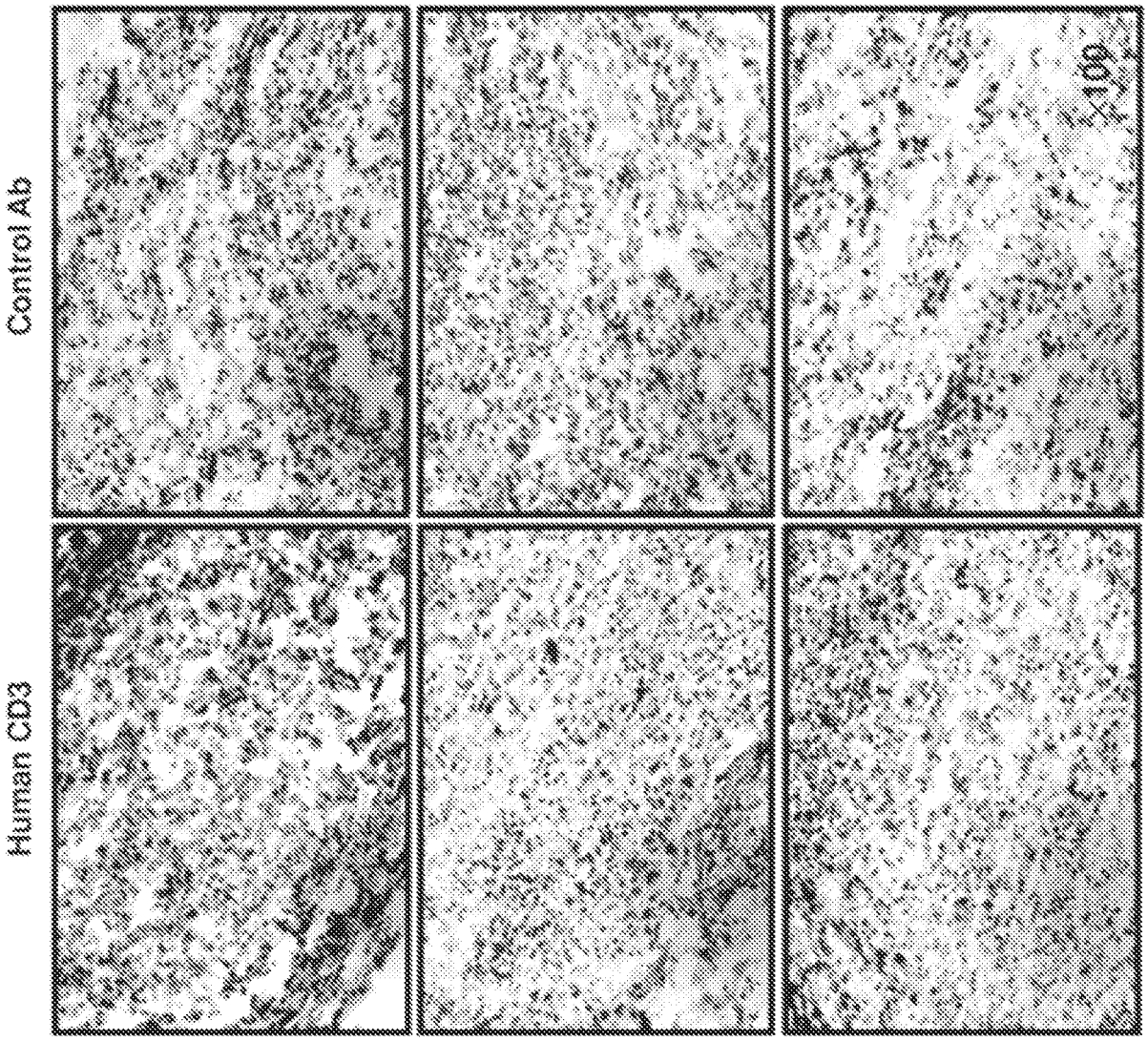


Figure 21

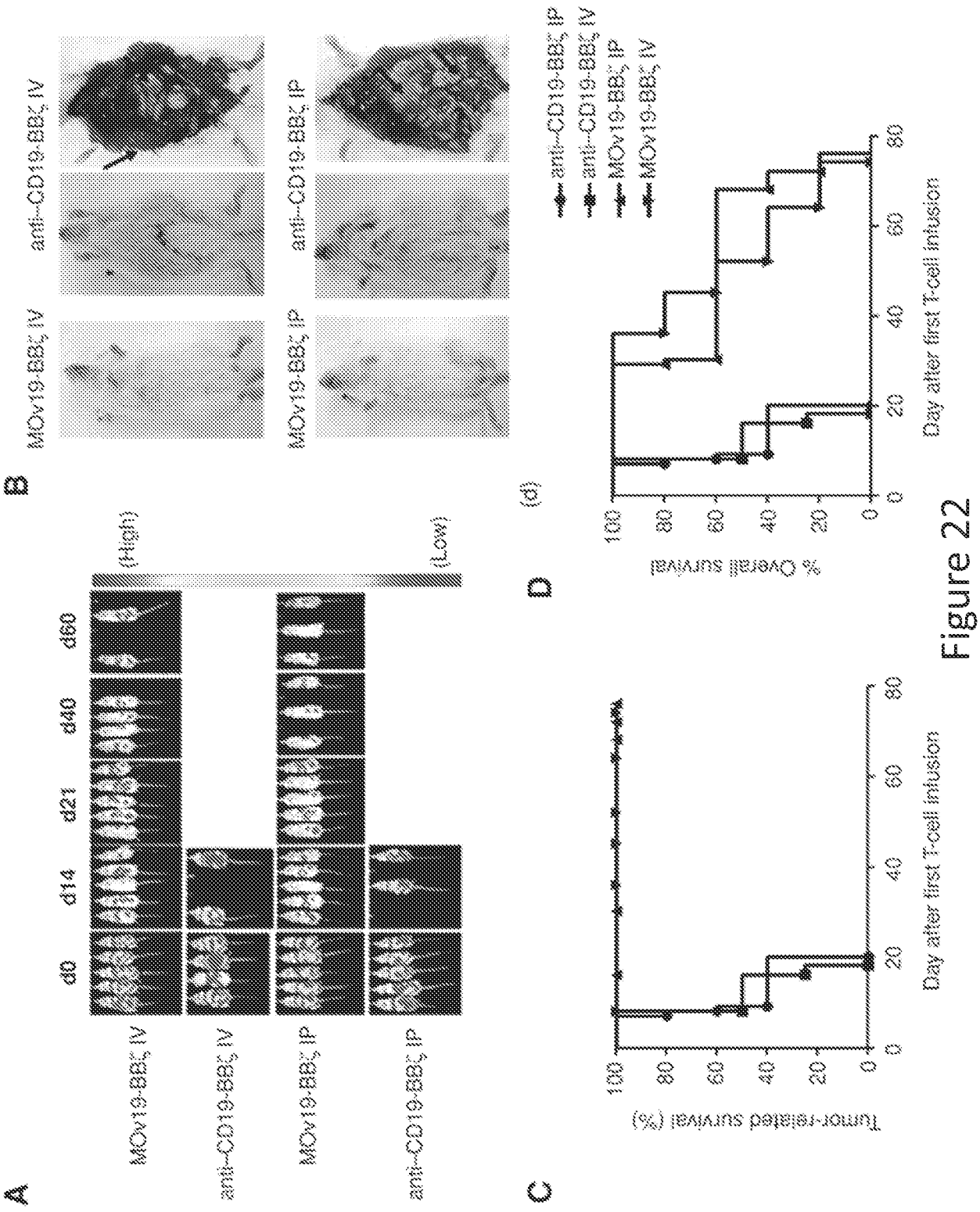

A
d0
d14
d21
d40
d60
(High)
(Low)
MOv19-BBζ IV
anti-CD19-BBζ IV
MOv19-BBζ IP
anti-CD19-BBζ IP
B
MOv19-BBζ IV
anti-CD19-BBζ IV
MOv19-BBζ IP
anti-CD19-BBζ IP
C
Tumor-related survival (%)
Day after first T-cell infusion
D
(d)
% Overall survival
Day after first T-cell infusion
anti-CD19-BBζ IP
anti-CD19-BBζ IV
MOv19-BBζ IP
MOv19-BBζ IV

Figure 22

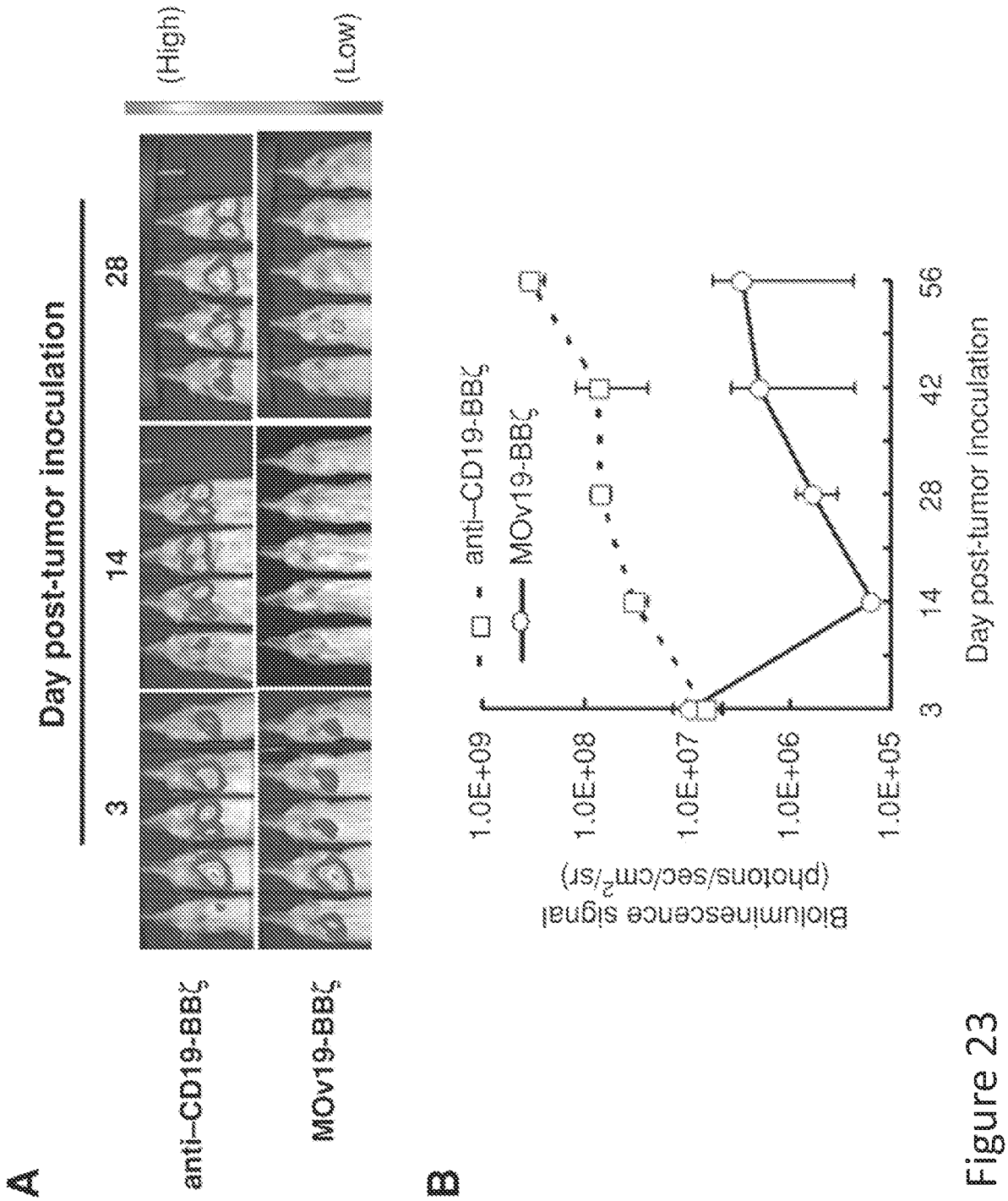

A
Day post-tumor inoculation
3
14
28
(High)
(Low)
anti–CD19-BBζ
MOv19-BBζ
B
anti–CD19-BBζ
MOv19-BBζ
Bioluminescence signal
(photons/sec/cm²/sr)
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
3
14
28
42
56
Day post-tumor inoculation

Figure 23

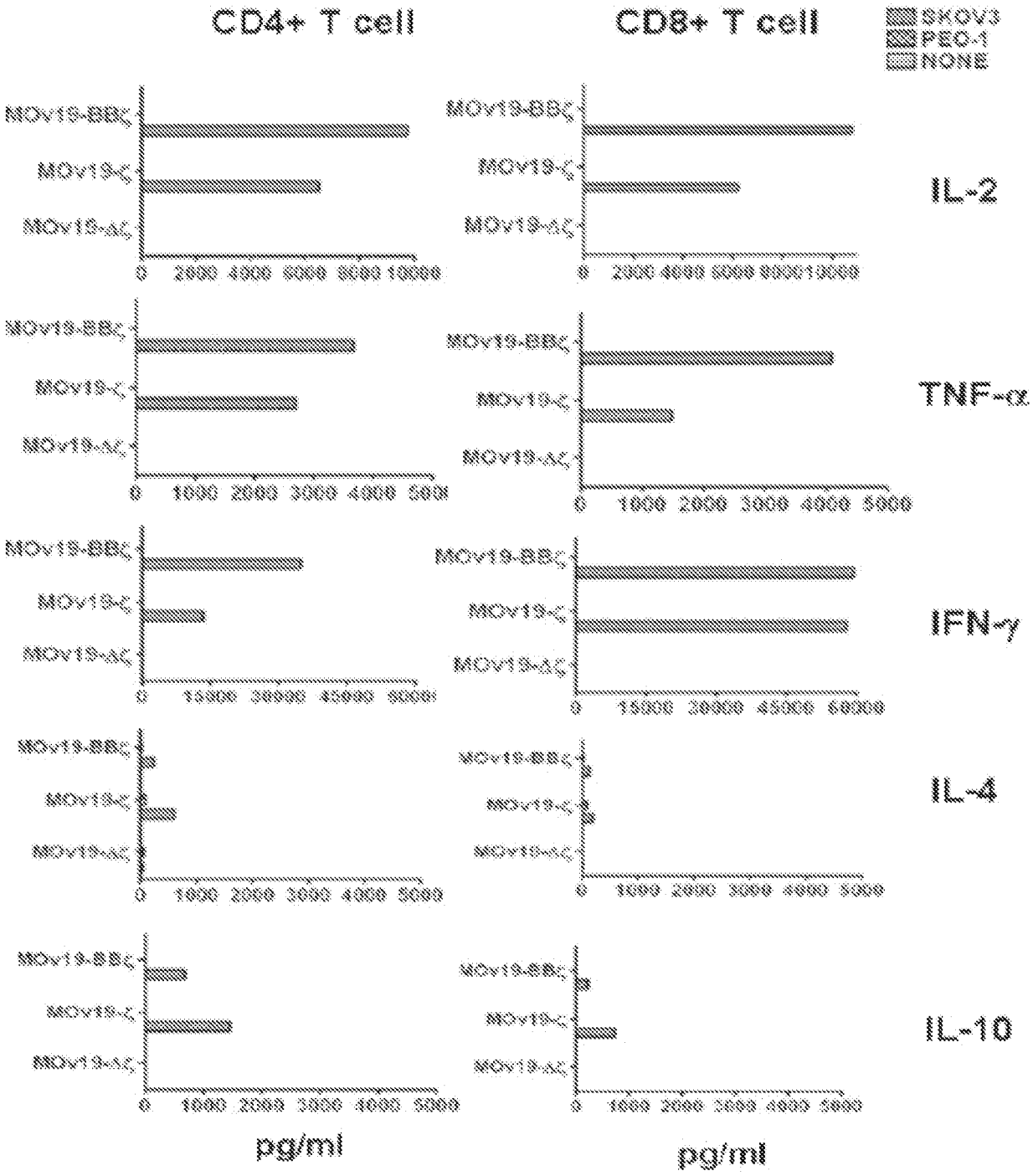


Figure 24

REPLACEMENT SHEET

SUBSTITUTE SHEET (RULE 26)

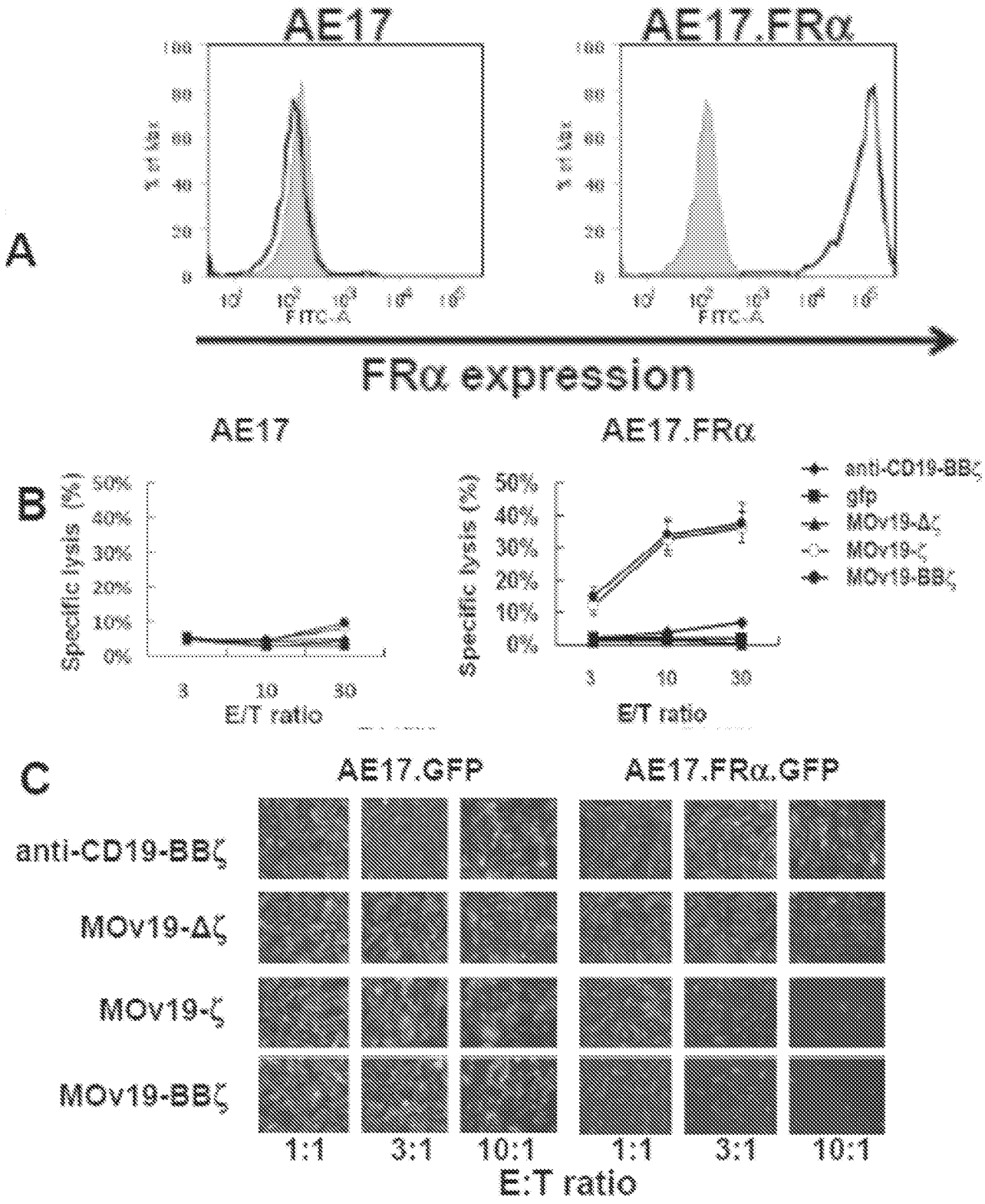


Figure 25

REPLACEMENT SHEET

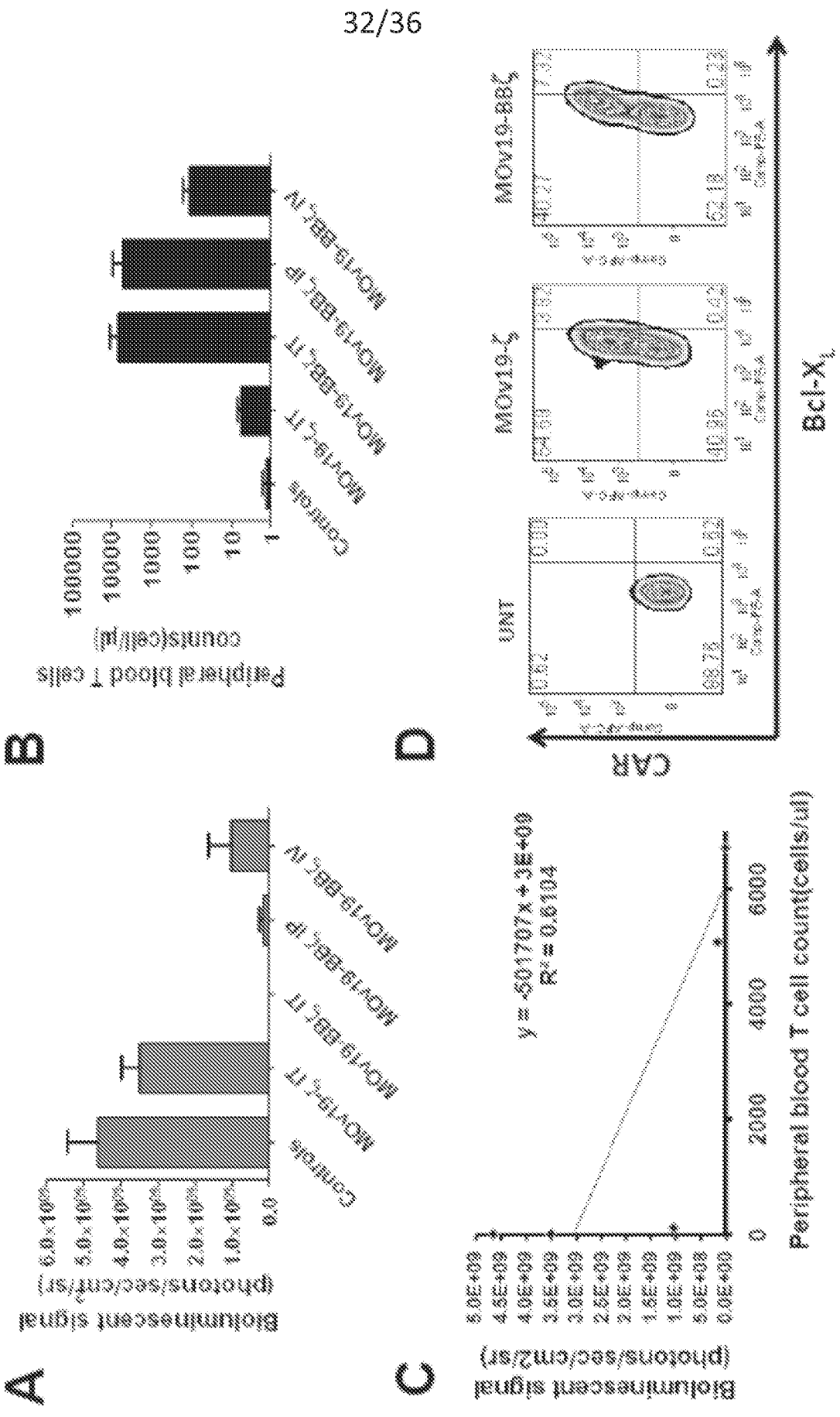

A
Bioluminescent signal (photons/sec/cm²/sr)
6.0×10⁶
5.0×10⁶
4.0×10⁶
3.0×10⁶
2.0×10⁶
1.0×10⁶
0.0
Controls
MOv19-ζ IT
MOv19-BBζ IT
MOv19-BBζ IP
MOv19-BBζ IV
B
Paliphteral blood T cells counts(cell/µl)
100000
10000
1000
100
10
1
Controls
MOv19-ζ IT
MOv19-BBζ IT
MOv19-BBζ IP
MOv19-BBζ IV
C
Bioluminescent signal (photons/sec/cm2/sr)
y = 501707x + 3E+09
R² = 0.6104
Peripheral blood T cell count(cells/ul)
0
2000
4000
6000
8.0E+09
4.5E+09
4.0E+09
3.5E+09
3.0E+09
2.5E+09
2.0E+09
1.5E+09
1.0E+09
5.0E+08
0.0E+00
D
UNT
MOv19-ζ
MOv19-BBζ
CAR
Bcl-X_L

Figure 26

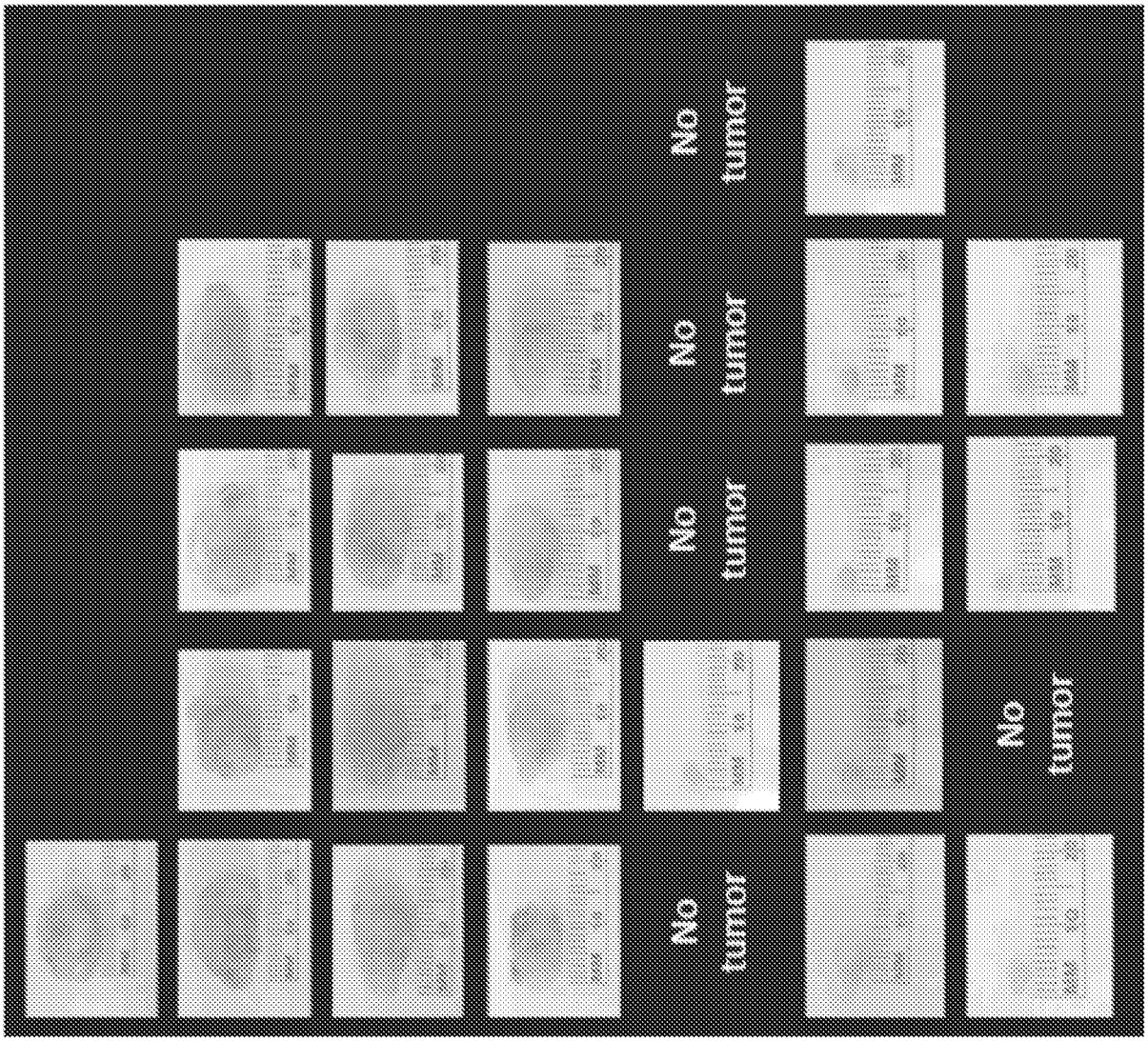

No tumor
No tumor
No tumor
No tumor
No tumor
Saline IT
gfp IT
MOv19-ζ IT
MOv19-BBζ IT
MOv19-BBζ IV
MOv19-BBζ IP

Figure 27

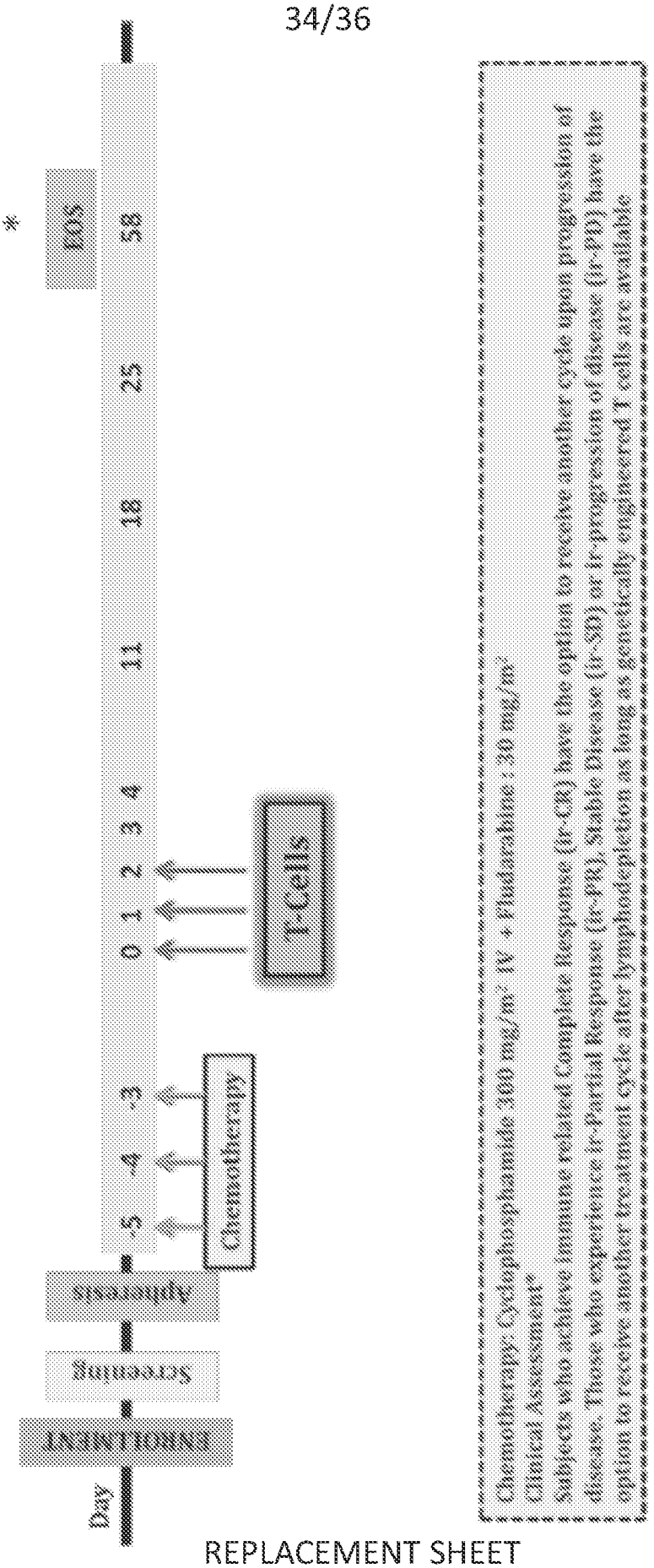

Day
-5 -4 -3 0 1 2 3 4 11 18 25 58
*
EOS
Leukapheresis
Screening
ENROLLMENT
Chemotherapy
T-Cells
Chemotherapy: Cyclophosphamide 300 mg/m² IV + Fludarabine : 30 mg/m²
Clinical Assessment*
Subjects who achieve Immune related Complete Response (ir-CR) have the option to receive another cycle upon progression of disease. Those who experience ir-Partial Response (ir-PR), Stable Disease (ir-SD) or ir-progression of disease (ir-PD) have the option to receive another treatment cycle after lymphodepletion as long as genetically engineered T cells are available

Figure 28

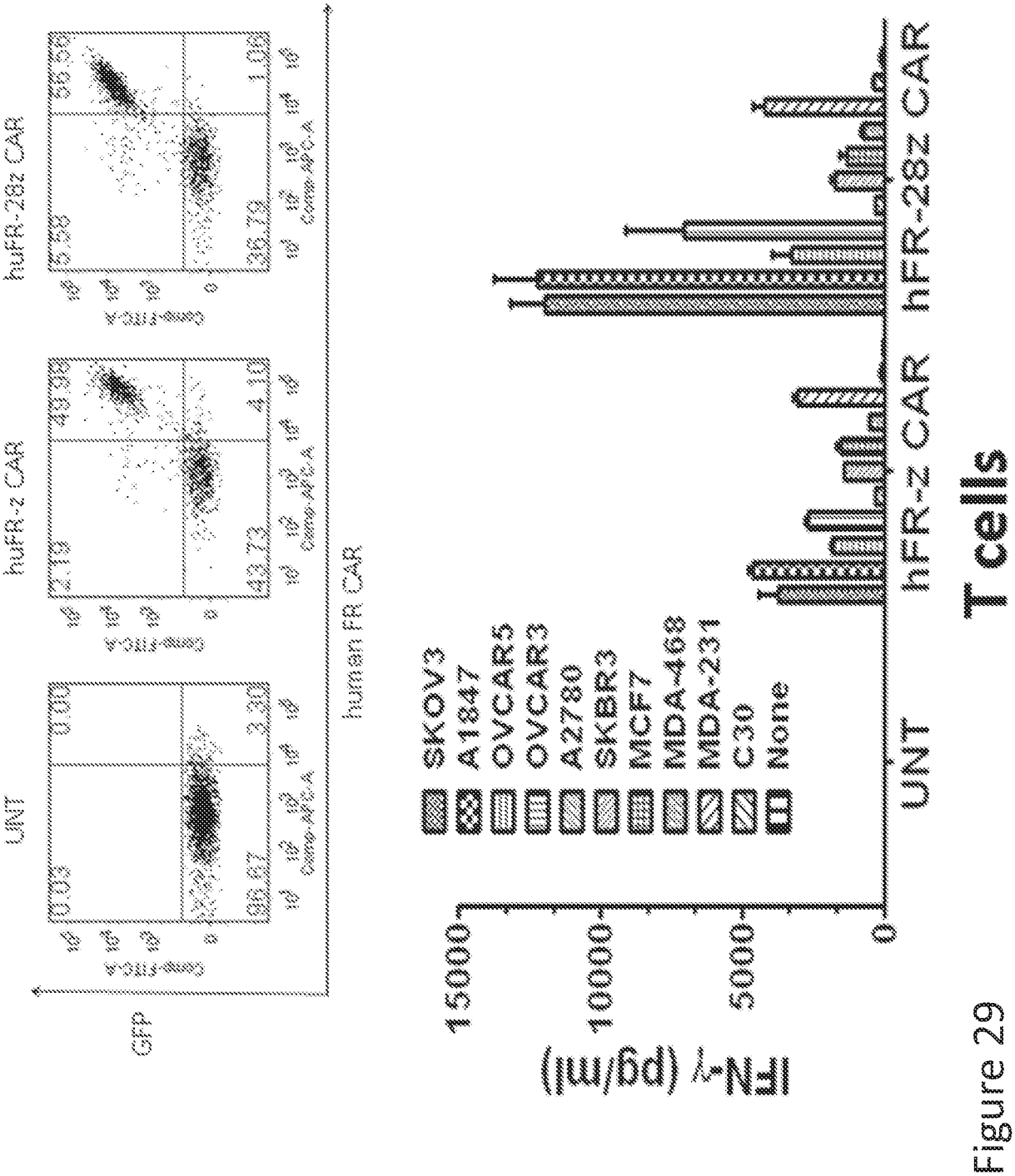

UNT
huFR-z CAR
huFR-28z CAR
human FR CAR
GFP
0.03
0.00
3.30
99.67
2.70
49.36
4.10
43.73
6.58
56.50
1.06
36.79
SKOV3
A1847
OVCAR5
OVCAR3
A2780
SKBR3
MCF7
MDA-468
MDA-231
C30
None
IFN-γ (pg/ml)
15000
10000
5000
0
UNT
hFR-z CAR
hFR-28z CAR
T cells

Figure 29

SEQ ID NO: 1	MOv19-4-1BB-CD3zeta CAR – DNA
SEQ ID NO: 2	CD8a leader – DNA
SEQ ID NO: 3	MOv19 scFv – DNA [FRα binding domain]
SEQ ID NO: 4	CD8α hinge – DNA
SEQ ID NO: 5	CD8α transmembrane – DNA
SEQ ID NO: 6	4-1BB Intracellular domain – DNA
SEQ ID NO: 7	CD3 zeta intracellular domain – DNA
SEQ ID NO: 8	LTR R & U5 regions
SEQ ID NO: 9	RSV Promoter
SEQ ID NO: 10	Partial gag
SEQ ID NO: 11	cPPT
SEQ ID NO: 12	EF1-a Promoter
SEQ ID NO: 13	MOv19-4-1BB-CD3zeta CAR amino acid
SEQ ID NO: 14	CD8a leader – amino acid
SEQ ID NO: 15	MOv19 scFv – amino acid [FRα binding domain]
SEQ ID NO: 16	CD8α hinge – amino acid
SEQ ID NO: 17	CD8α transmembrane – amino acid
SEQ ID NO: 18	4-1BB Intracellular domain – amino acid
SEQ ID NO: 19	CD3 zeta intracellular domain – amino acid
SEQ ID NO: 20	C4 scFv-4-1BB-CD3zeta CAR – DNA
SEQ ID NO: 21	C4 scFv – DNA [FRα binding domain]
SEQ ID NO: 22	C4 scFv-4-1BB-CD3zeta CAR – amino acid
SEQ ID NO: 23	C4 scFv – amino acid [FRα binding domain]

Figure 30