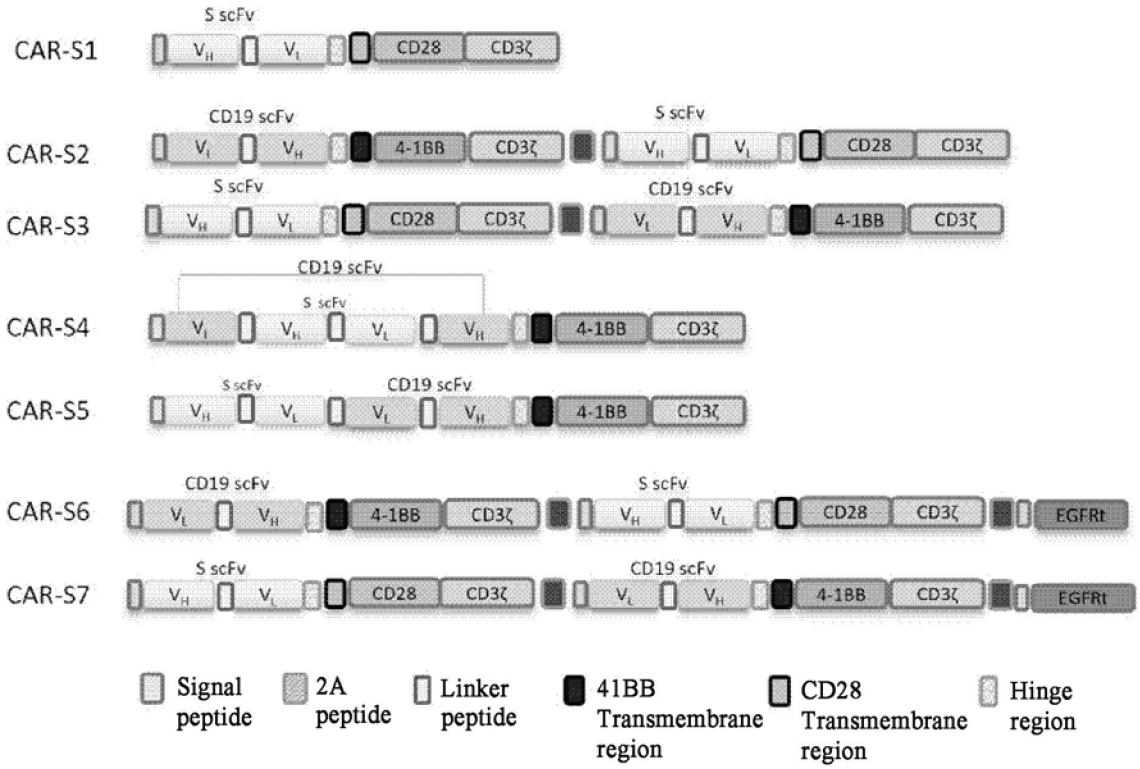
CAR-S1
S scFv
VH
VL
CD28
CD3ζ
CAR-S2
CD19 scFv
VL
VH
4-1BB
CD3ζ
S scFv
VH
VL
CD28
CD3ζ
CAR-S3
S scFv
VH
VL
CD28
CD3ζ
CD19 scFv
VL
VH
4-1BB
CD3ζ
CAR-S4
CD19 scFv
S scFv
VL
VH
VL
VH
4-1BB
CD3ζ
CAR-S5
S scFv
VH
VL
CD19 scFv
VL
VH
4-1BB
CD3ζ
CAR-S6
CD19 scFv
VL
VH
4-1BB
CD3ζ
S scFv
VH
VL
CD28
CD3ζ
EGFRt
CAR-S7
S scFv
VH
VL
CD28
CD3ζ
CD19 scFv
VL
VH
4-1BB
CD3ζ
EGFRt
Signal peptide
2A peptide
Linker peptide
41BB Transmembrane region
CD28 Transmembrane region
Hinge region

Fig. 1

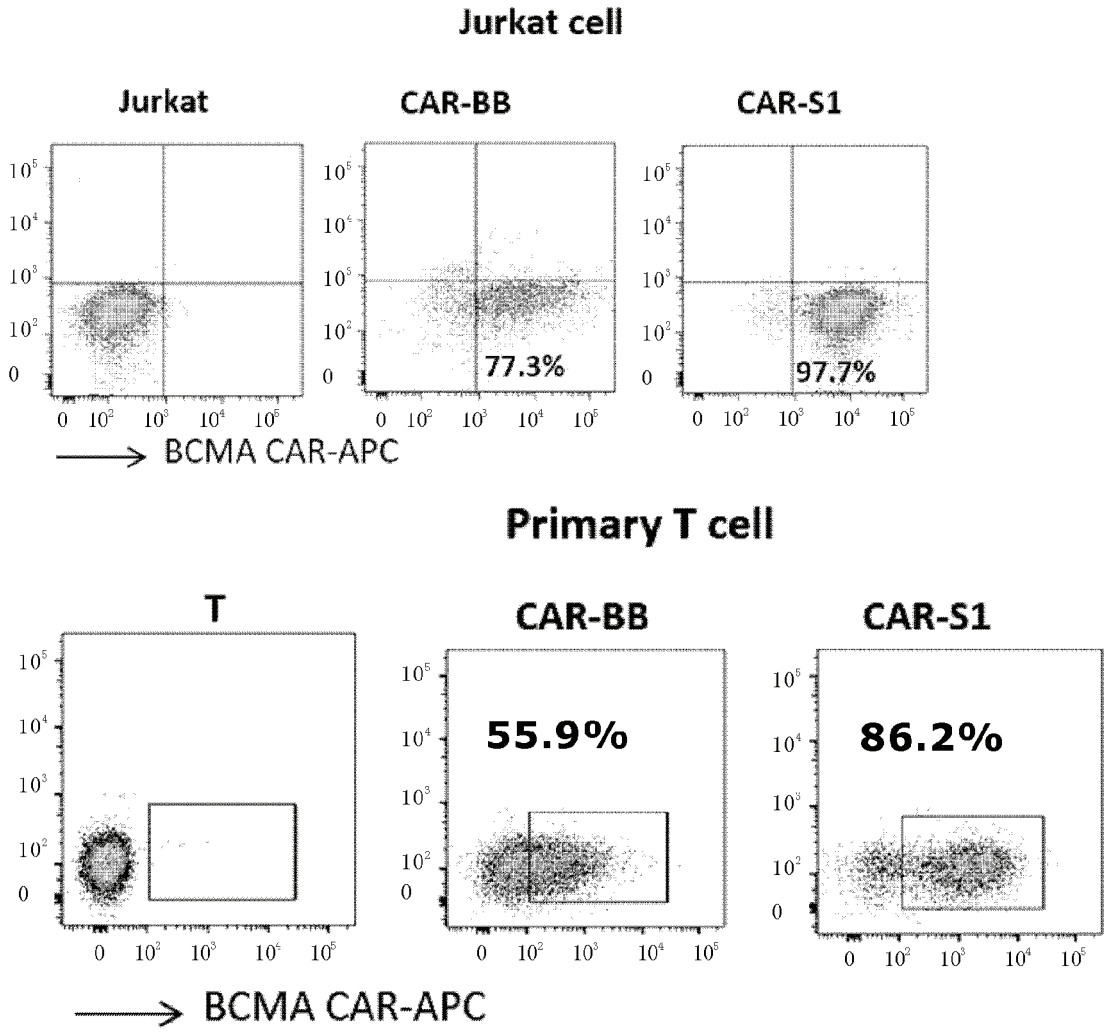
Jurkat cell
Jurkat
CAR-BB
CAR-S1
77.3%
97.7%
BCMA CAR-APC
Primary T cell
T
CAR-BB
CAR-S1
55.9%
86.2%
BCMA CAR-APC

Fig. 2

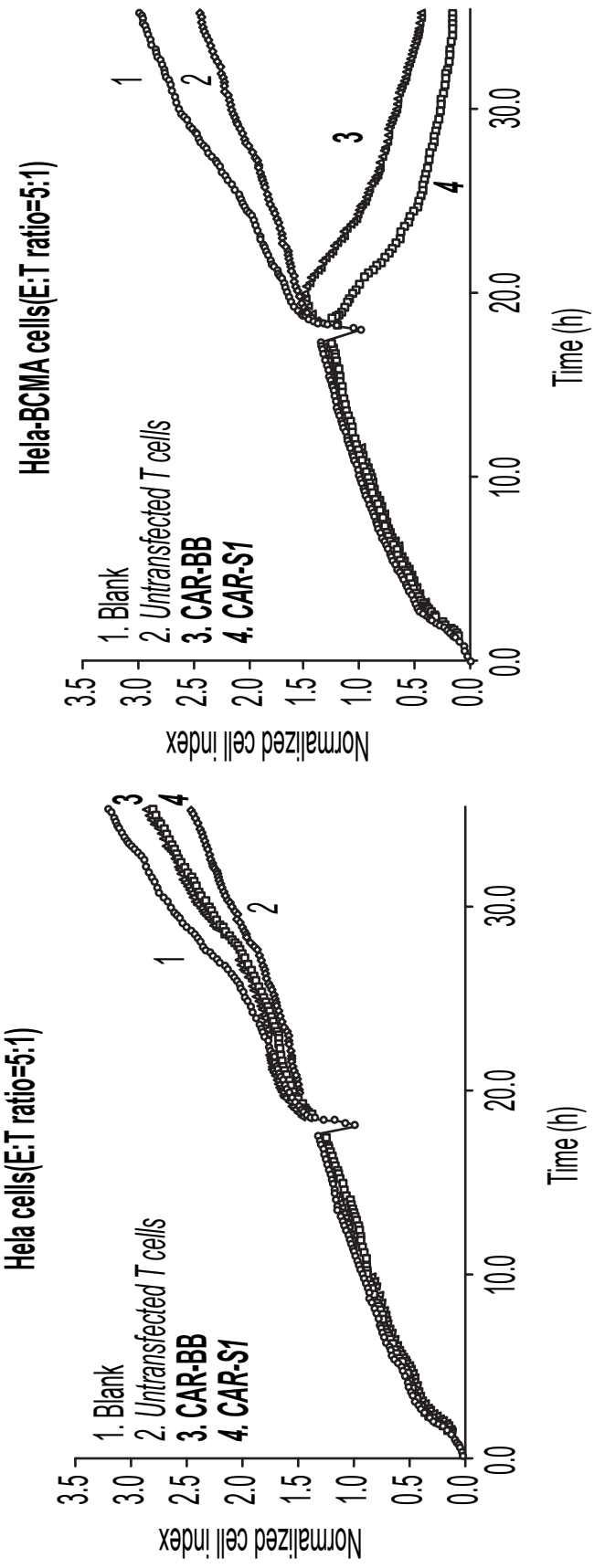
Hela cells(E:T ratio=5:1)
1. Blank
2. Untransfected T cells
3. CAR-BB
4. CAR-S1
Normalized cell index
Time (h)
Hela-BCMA cells(E:T ratio=5:1)
1. Blank
2. Untransfected T cells
3. CAR-BB
4. CAR-S1
Normalized cell index
Time (h)

Fig. 3

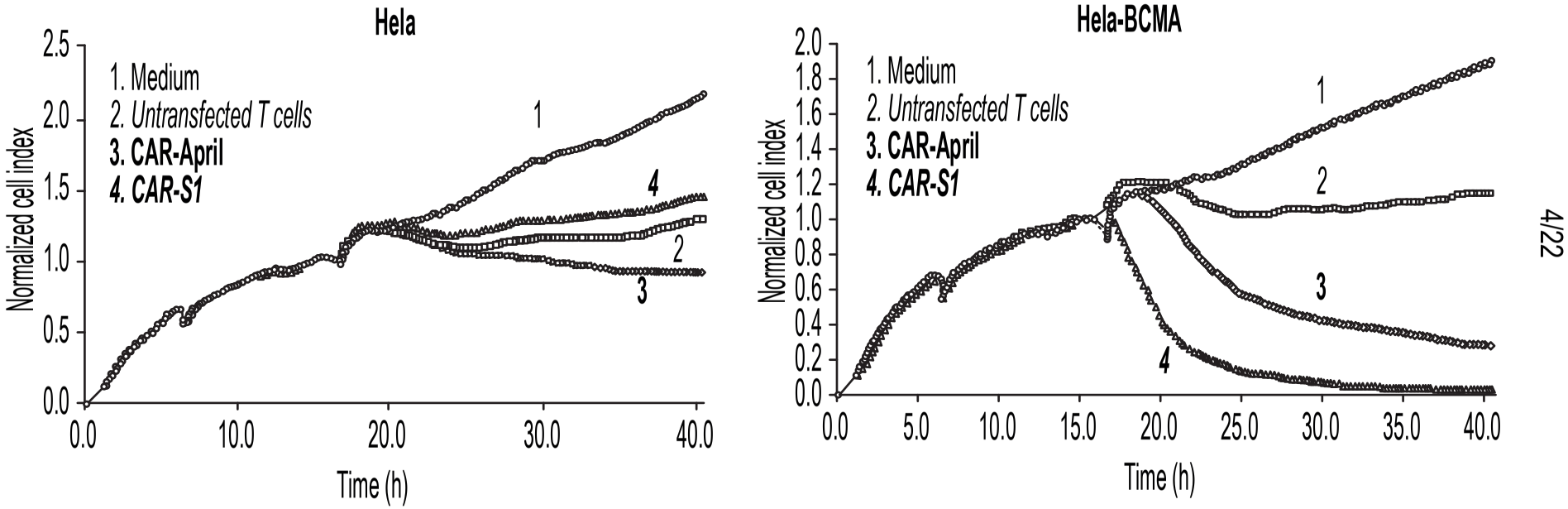

Fig. 3, Continued

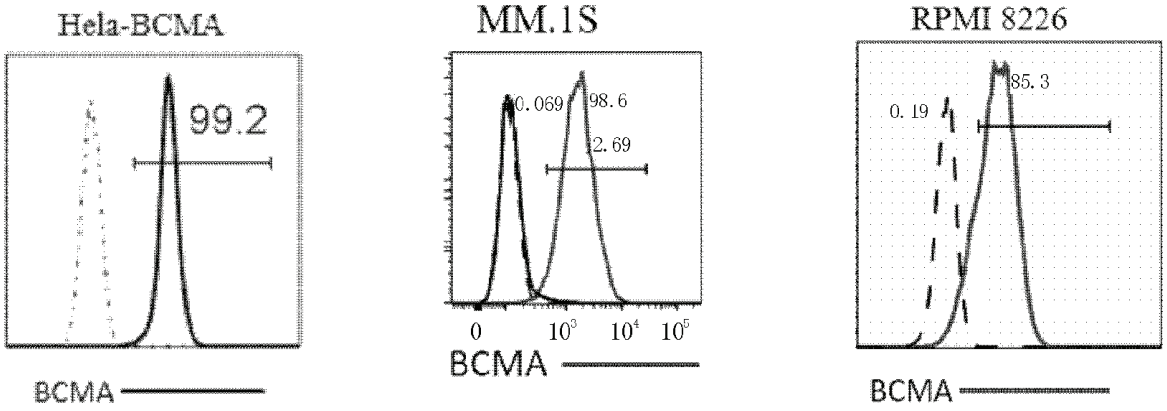
Hela-BCMA
99.2
BCMA
MM.1S
0.069
98.6
2.69
0
10^3
10^4
10^5
BCMA
RPMI 8226
0.19
85.3
BCMA

Fig. 4

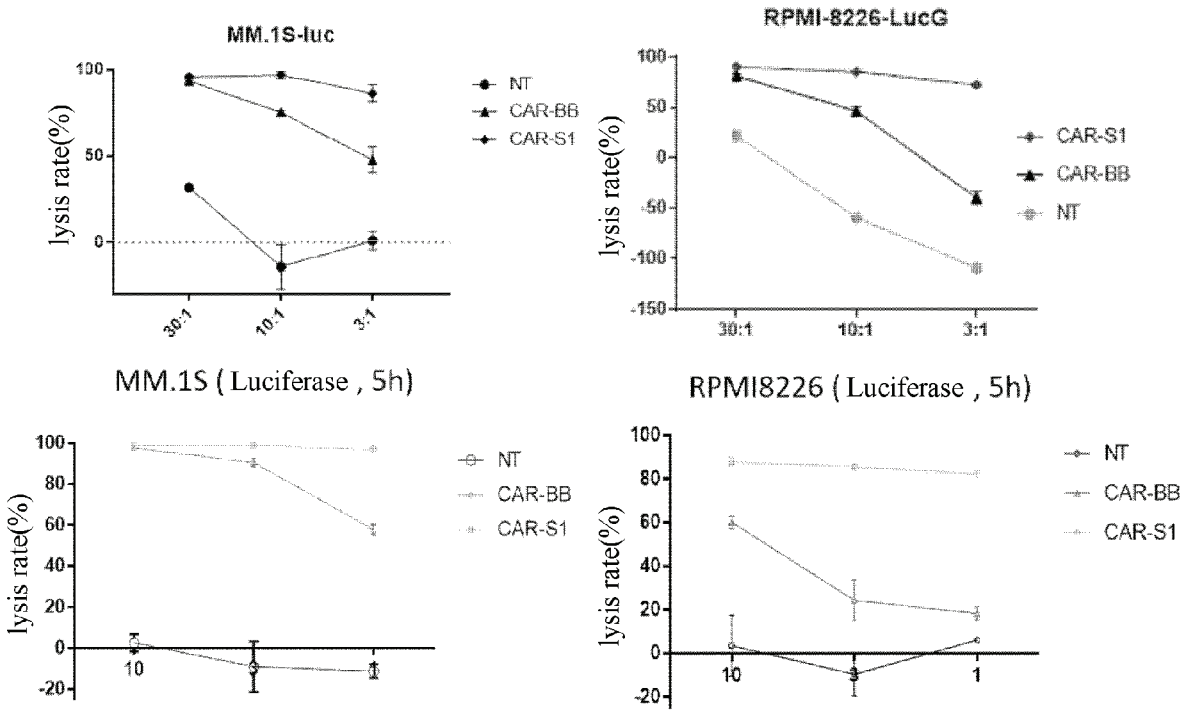
MM.1S-luc
lysis rate(%)
100
50
0
30:1
10:1
3:1
NT
CAR-BB
CAR-S1
RPMI-8226-LucG
lysis rate(%)
100
50
0
-50
-100
-150
30:1
10:1
3:1
CAR-S1
CAR-BB
NT
MM.1S (Luciferase , 5h)
lysis rate(%)
100
80
60
40
20
0
-20
10
NT
CAR-BB
CAR-S1
RPMI8226 (Luciferase , 5h)
lysis rate(%)
100
80
60
40
20
0
-20
10
1
NT
CAR-BB
CAR-S1

Fig. 5

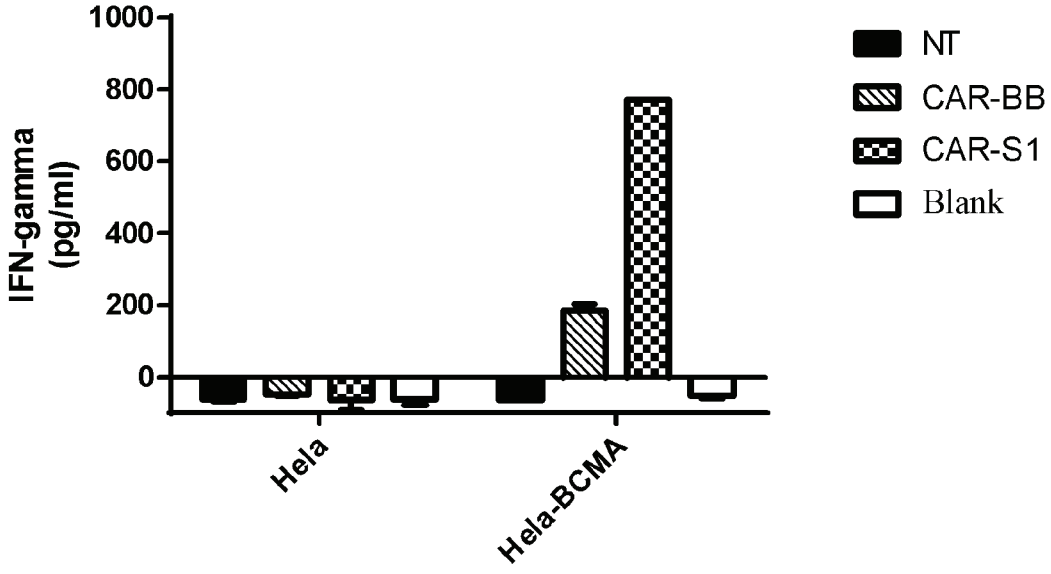
IFN-gamma (pg/ml)
1000
800
600
400
200
0
Hela
Hela-BCMA
NT
CAR-BB
CAR-S1
Blank

Fig. 6

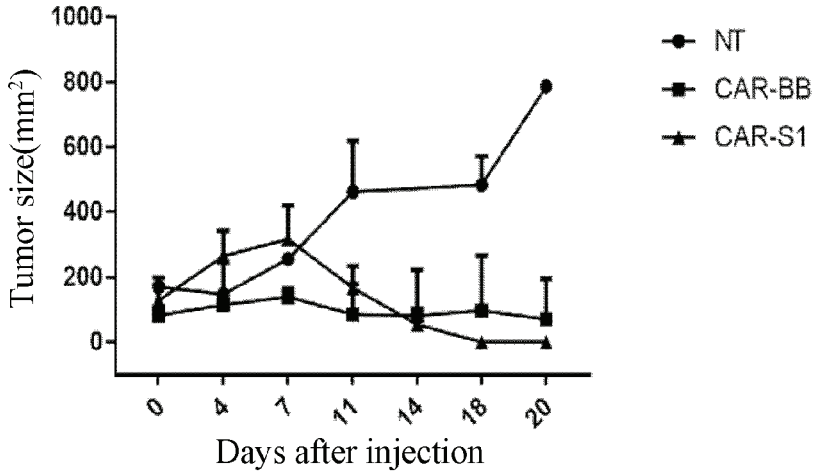
1000
800
600
400
200
0
Tumor size(mm²)
0
4
7
11
14
18
20
Days after injection
NT
CAR-BB
CAR-S1

Fig. 7

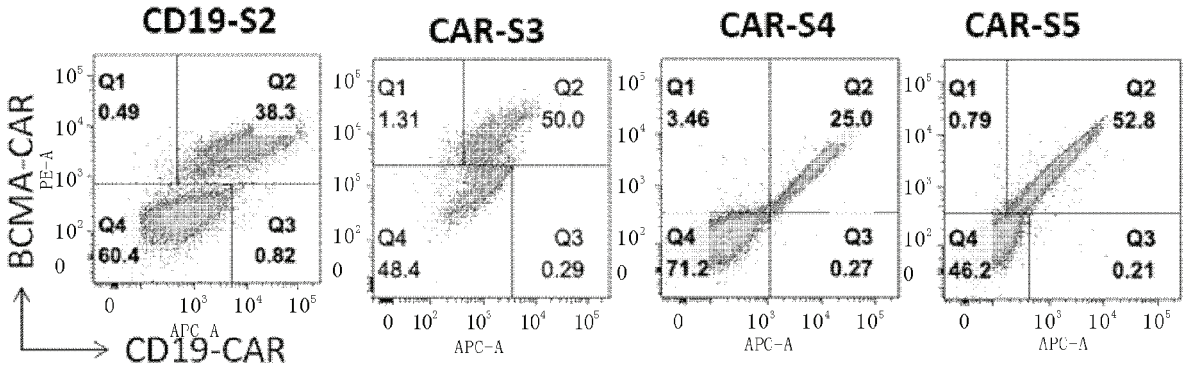
CD19-S2
CAR-S3
CAR-S4
CAR-S5
BCMA-CAR
CD19-CAR
PE-A
APC-A
Q1
0.49
Q2
38.3
Q4
60.4
Q3
0.82
Q1
1.31
Q2
50.0
Q4
48.4
Q3
0.29
Q1
3.46
Q2
25.0
Q4
71.2
Q3
0.27
Q1
0.79
Q2
52.8
Q4
46.2
Q3
0.21

Fig. 8

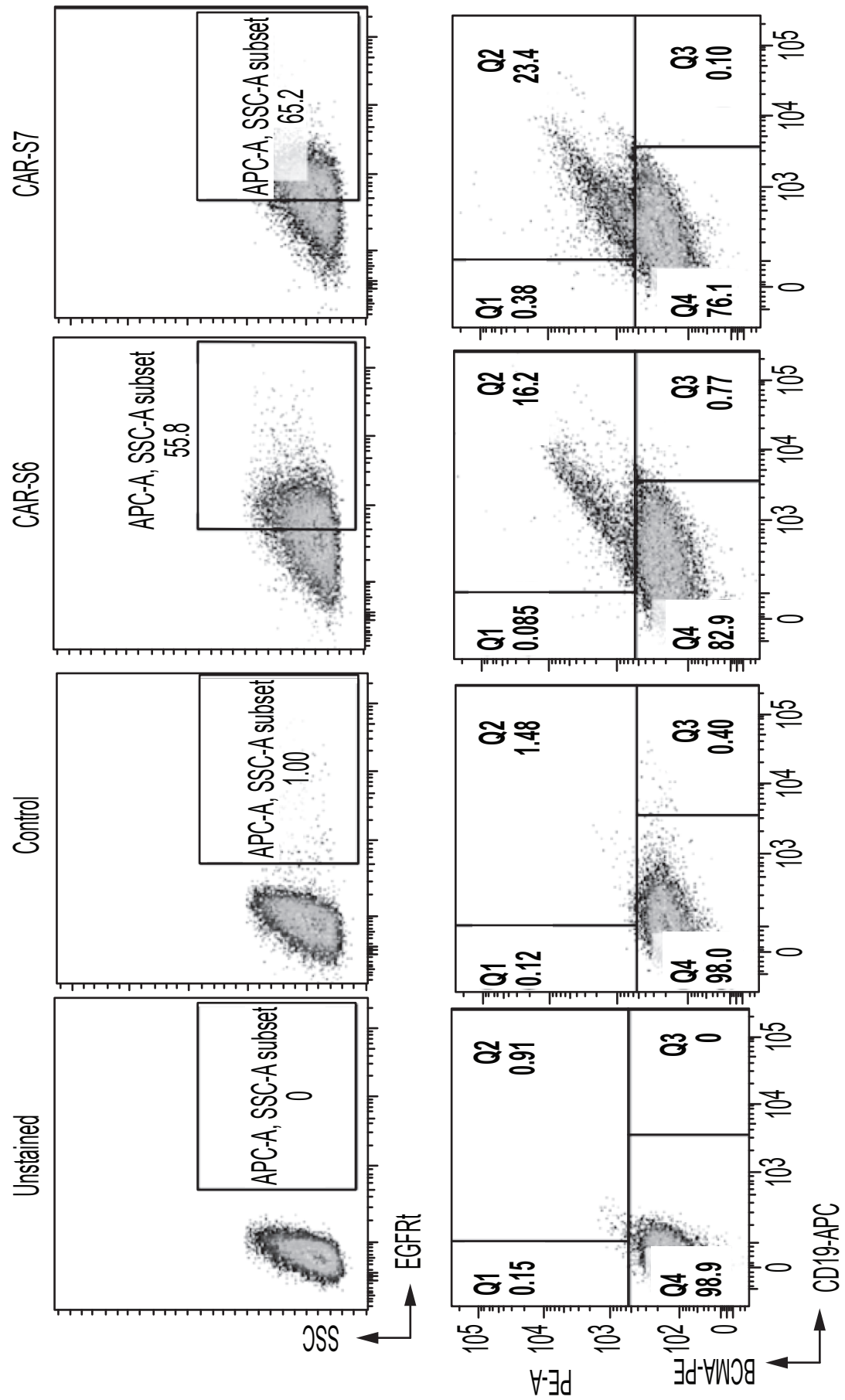
Unstained
Control
CAR-S6
CAR-S7
APC-A, SSC-A subset
0
APC-A, SSC-A subset
1.00
APC-A, SSC-A subset
55.8
APC-A, SSC-A subset
65.2
SSC
EGFRt
Q1
0.15
Q2
0.91
Q3
0
Q4
98.9
Q1
0.12
Q2
1.48
Q3
0.40
Q4
98.0
Q1
0.085
Q2
16.2
Q3
0.77
Q4
82.9
Q1
0.38
Q2
23.4
Q3
0.10
Q4
76.1
PE-A
BCMA-PE
CD19-APC
0
10³
10⁴
10⁵

Fig. 9

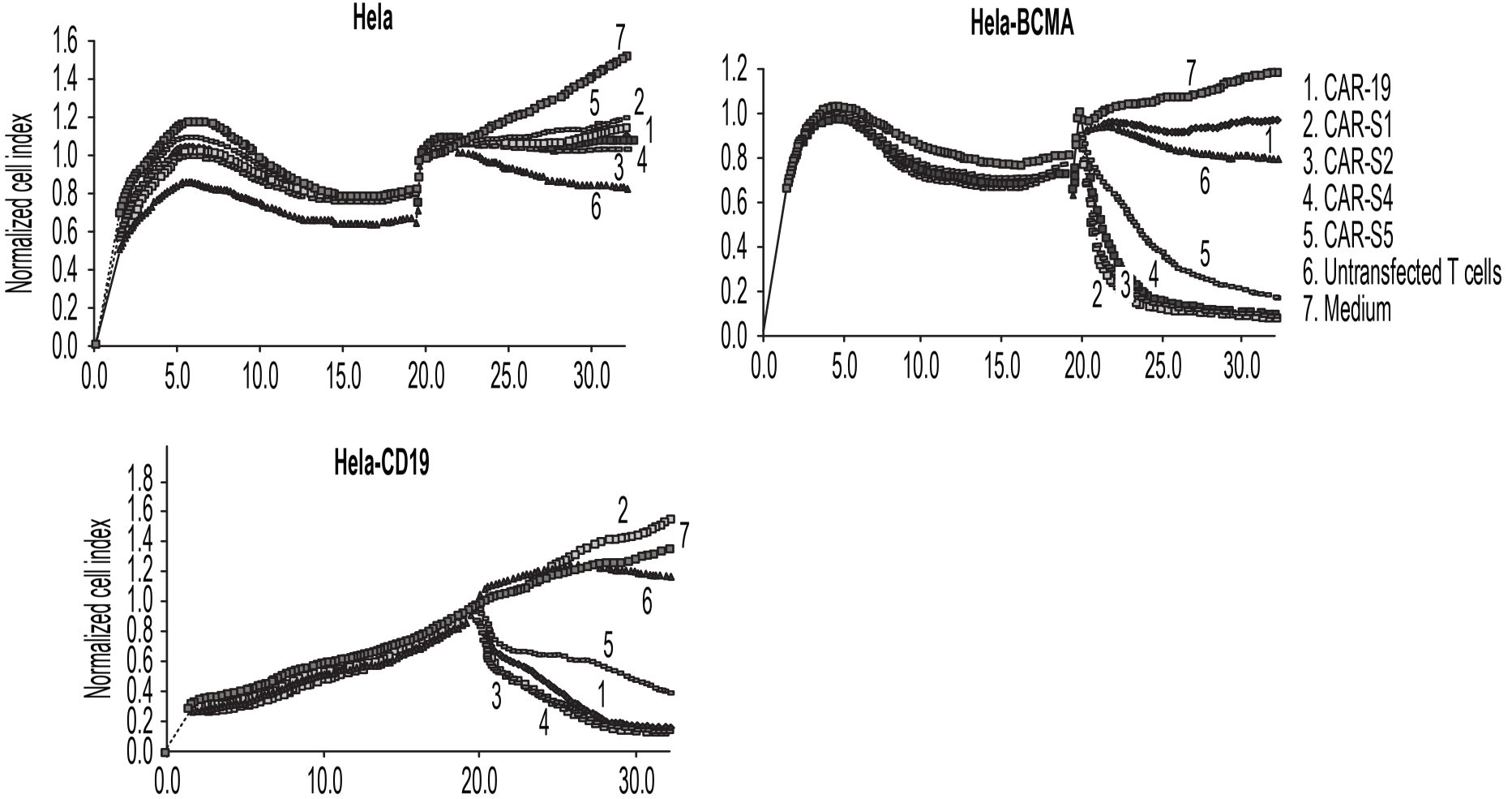
Hela
Normalized cell index
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
7
5
2
1
3
4
6
Hela-BCMA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
7
1
6
5
2
3
4
1. CAR-19
2. CAR-S1
3. CAR-S2
4. CAR-S4
5. CAR-S5
6. Untransfected T cells
7. Medium
Hela-CD19
Normalized cell index
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
10.0
20.0
30.0
2
7
6
5
3
1
4

Fig. 10

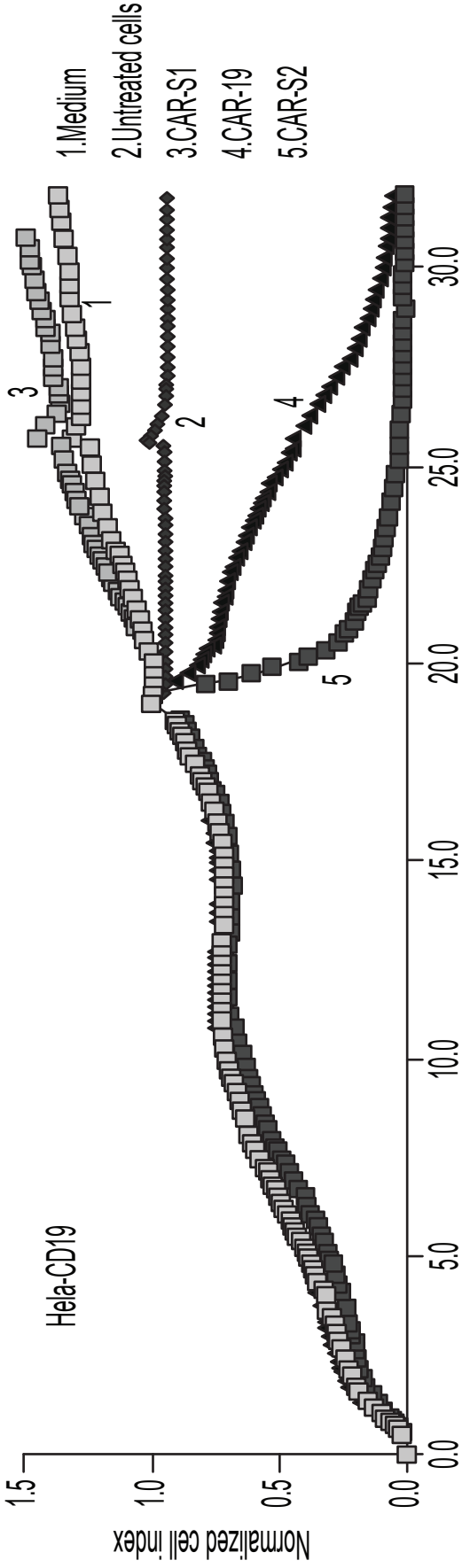

Fig. 10, Continued

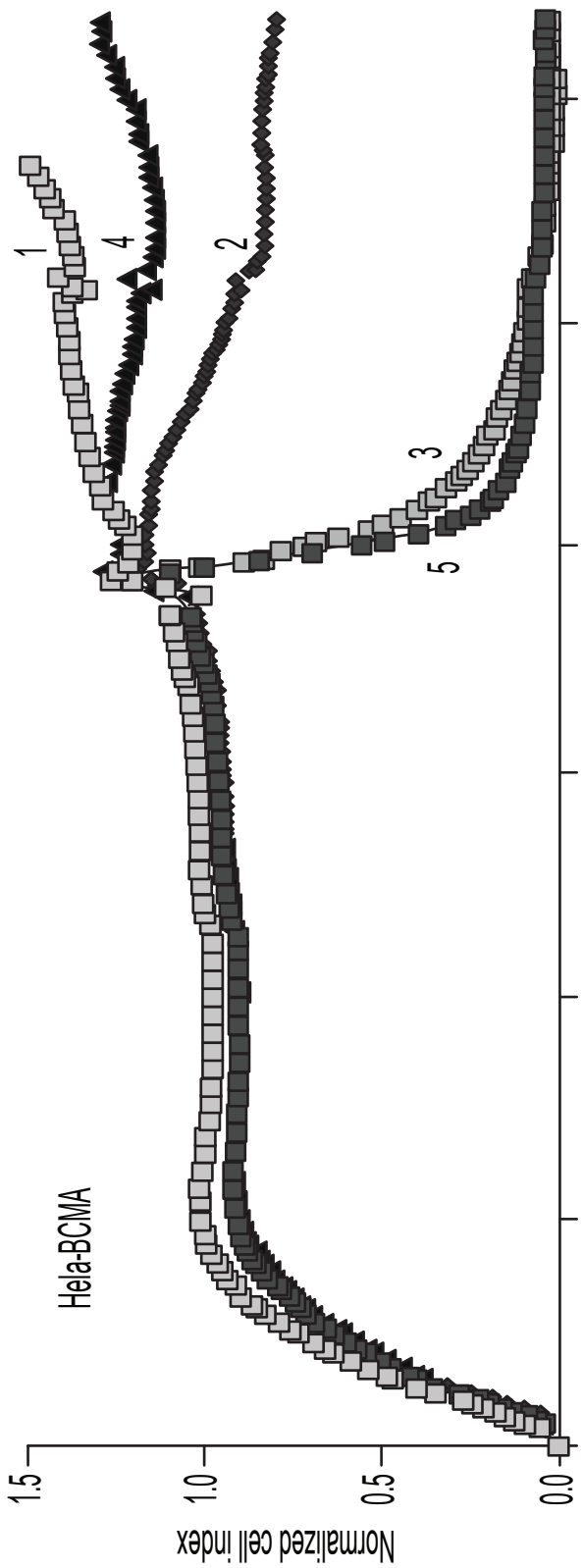

Fig. 10, Continued

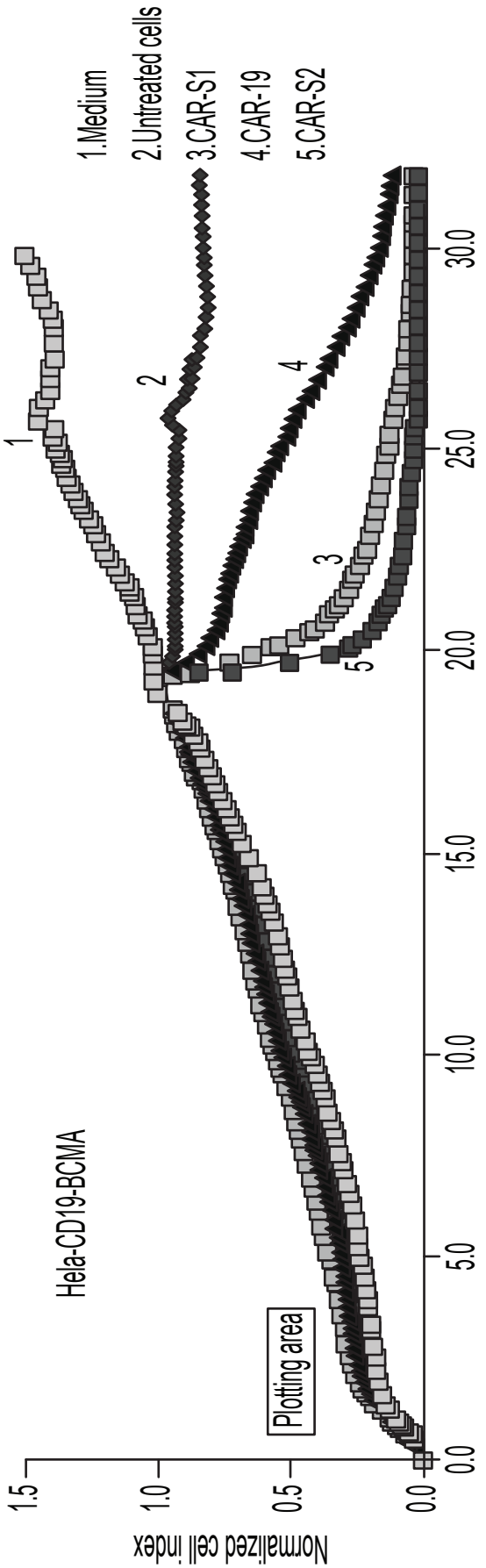

Fig. 10, Continued

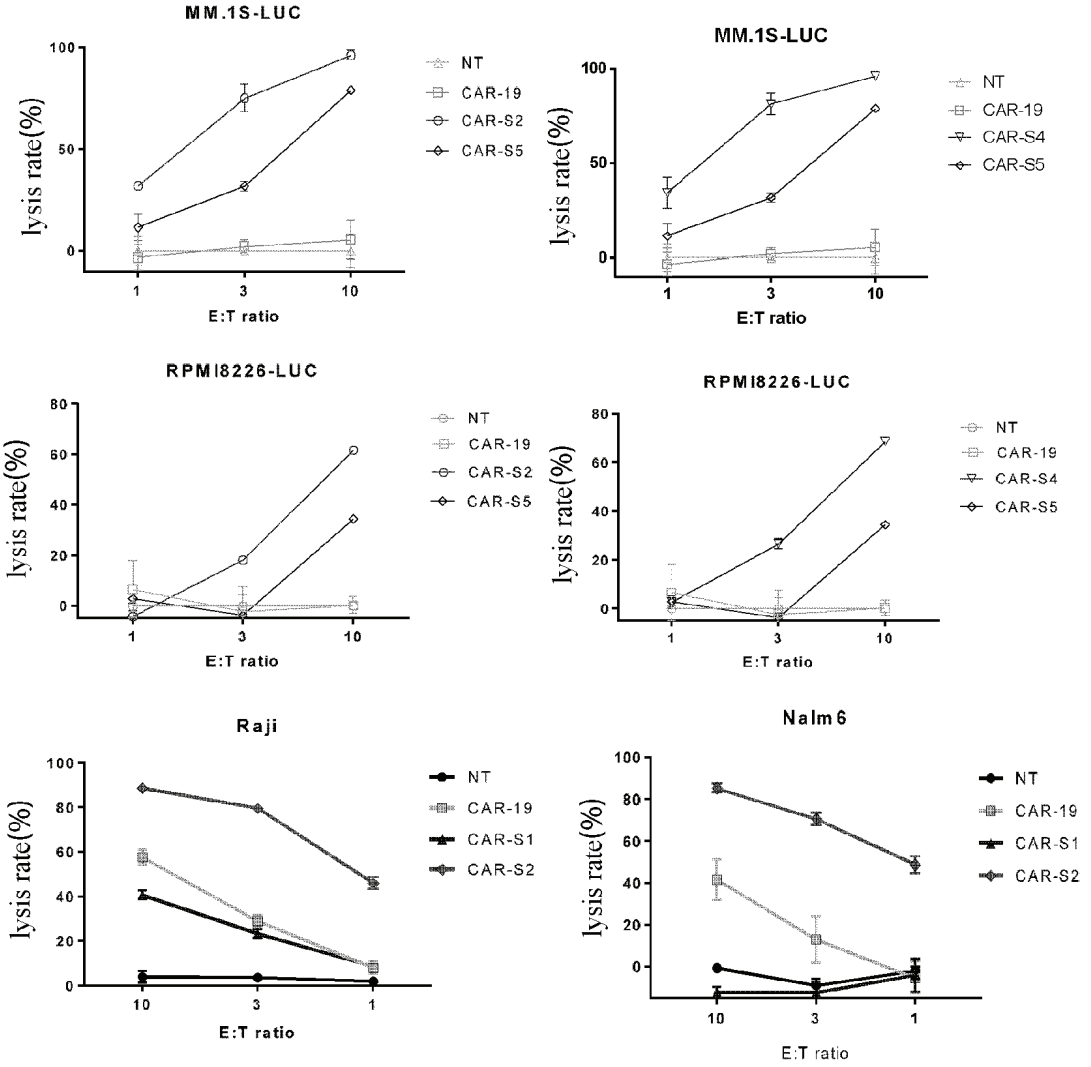
MM.1S-LUC
lysis rate(%)
E:T ratio
NT
CAR-19
CAR-S2
CAR-S5
MM.1S-LUC
lysis rate(%)
E:T ratio
NT
CAR-19
CAR-S4
CAR-S5
RPMI8226-LUC
lysis rate(%)
E:T ratio
NT
CAR-19
CAR-S2
CAR-S5
RPMI8226-LUC
lysis rate(%)
E:T ratio
NT
CAR-19
CAR-S4
CAR-S5
Raji
lysis rate(%)
E:T ratio
NT
CAR-19
CAR-S1
CAR-S2
Nalm6
lysis rate(%)
E:T ratio
NT
CAR-19
CAR-S1
CAR-S2

Fig. 11

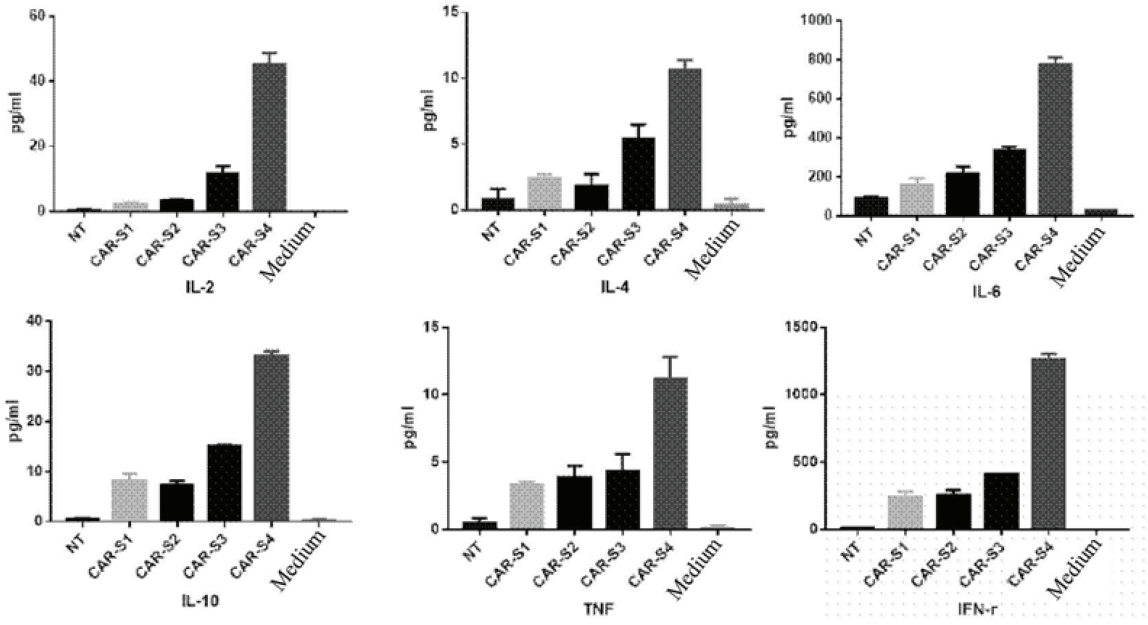
pg/ml
NT
CAR-S1
CAR-S2
CAR-S3
CAR-S4
Medium
IL-2
IL-4
IL-6
IL-10
TNF
IFN-r

Fig. 12

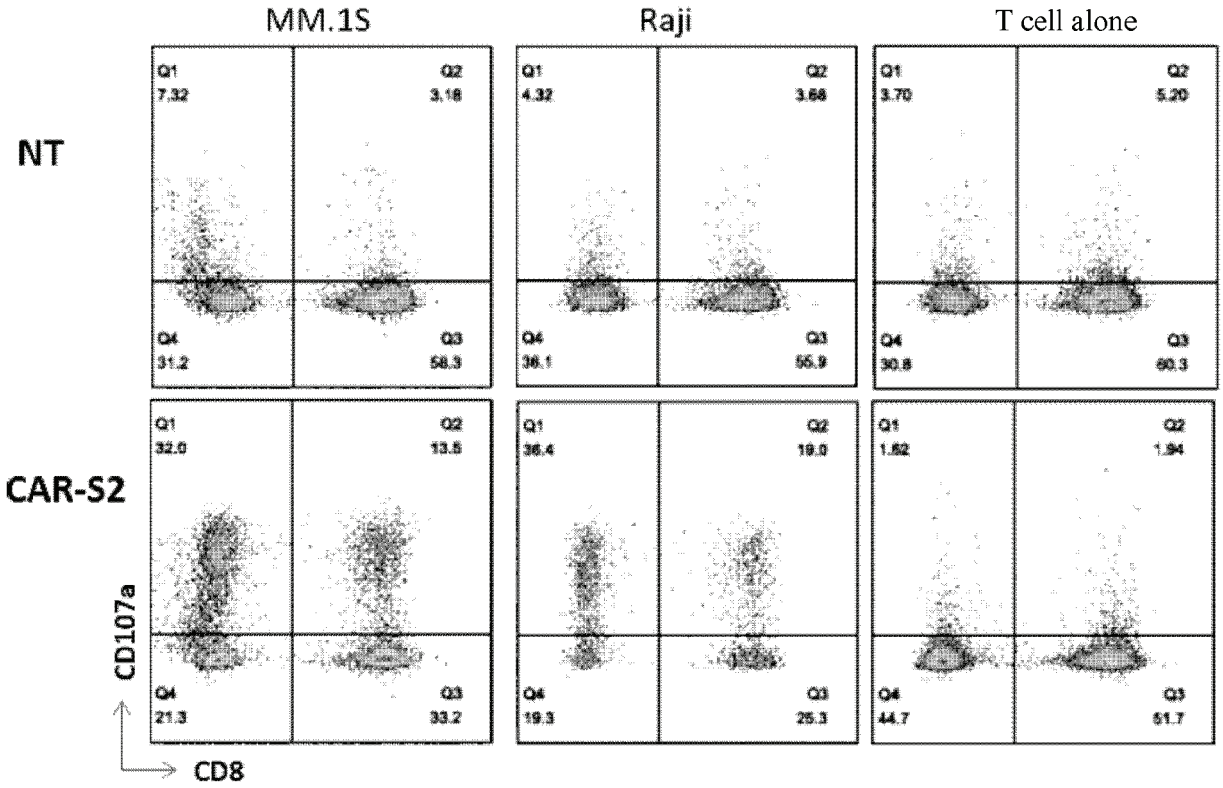
MM.1S
Raji
T cell alone
NT
CAR-S2
Q1
7.32
Q2
3.18
Q4
31.2
Q3
58.3
Q1
4.32
Q2
3.68
Q4
36.1
Q3
55.9
Q1
3.70
Q2
5.20
Q4
30.8
Q3
60.3
Q1
32.0
Q2
13.5
Q4
21.3
Q3
33.2
Q1
36.4
Q2
19.0
Q4
19.3
Q3
25.3
Q1
1.62
Q2
1.94
Q4
44.7
Q3
51.7
CD107a
CD8

Fig. 13

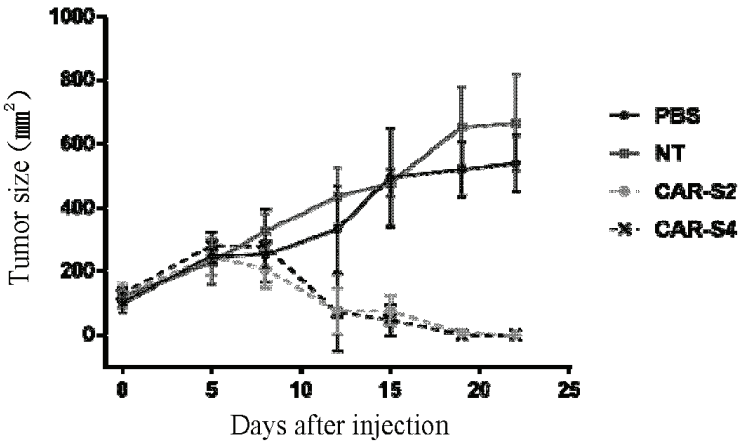
1000
800
600
400
200
0
Tumor size (mm²)
0
5
10
15
20
25
Days after injection
PBS
NT
CAR-S2
CAR-S4

Fig. 14

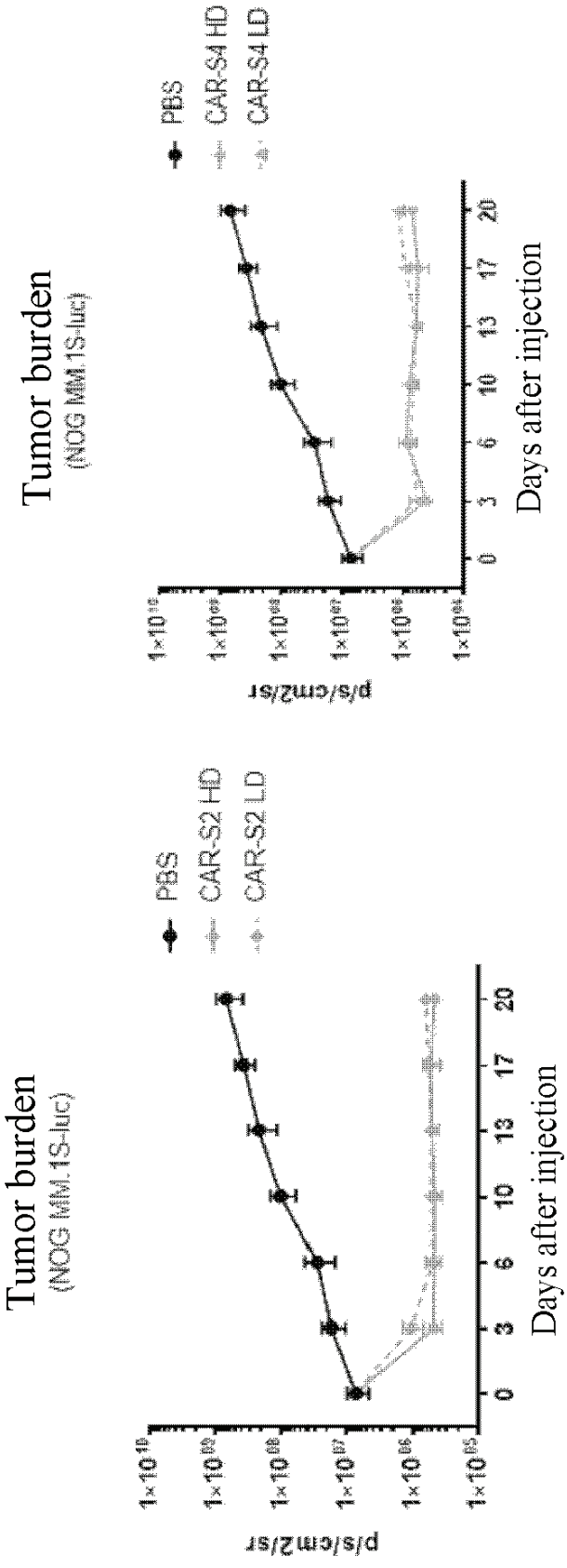
Tumor burden
(NOG MM.1S-luc)
PBS
CAR-S2 HD
CAR-S2 LD
p/s/cm2/sr
Days after injection
Tumor burden
(NOG MM.1S-luc)
PBS
CAR-S4 HD
CAR-S4 LD
p/s/cm2/sr
Days after injection

Fig. 15

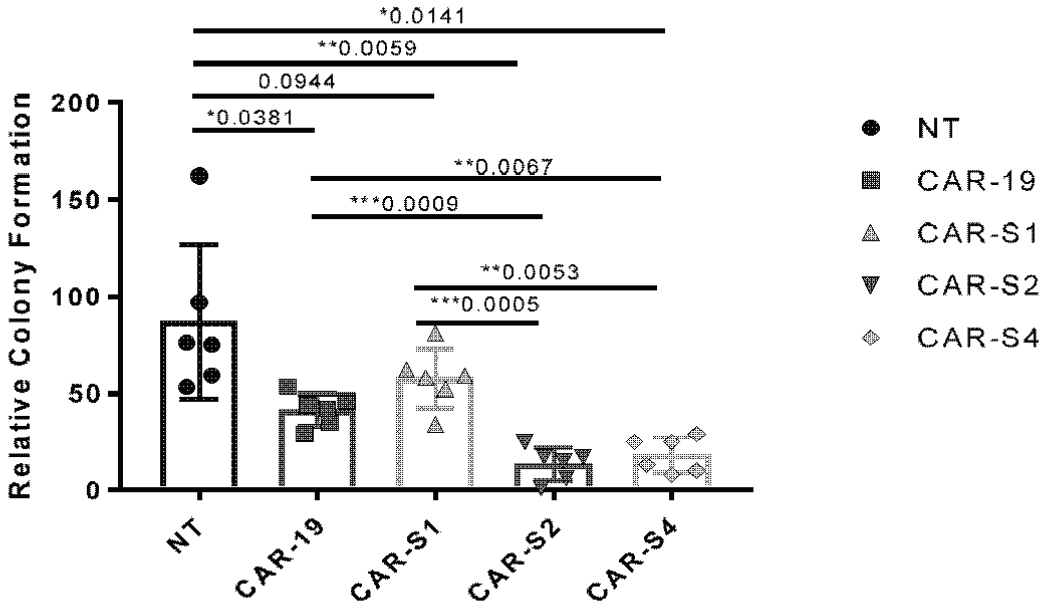
*0.0141
**0.0059
0.0944
*0.0381
**0.0067
***0.0009
**0.0053
***0.0005
Relative Colony Formation
200
150
100
50
0
NT
CAR-19
CAR-S1
CAR-S2
CAR-S4
NT
CAR-19
CAR-S1
CAR-S2
CAR-S4

Fig. 16

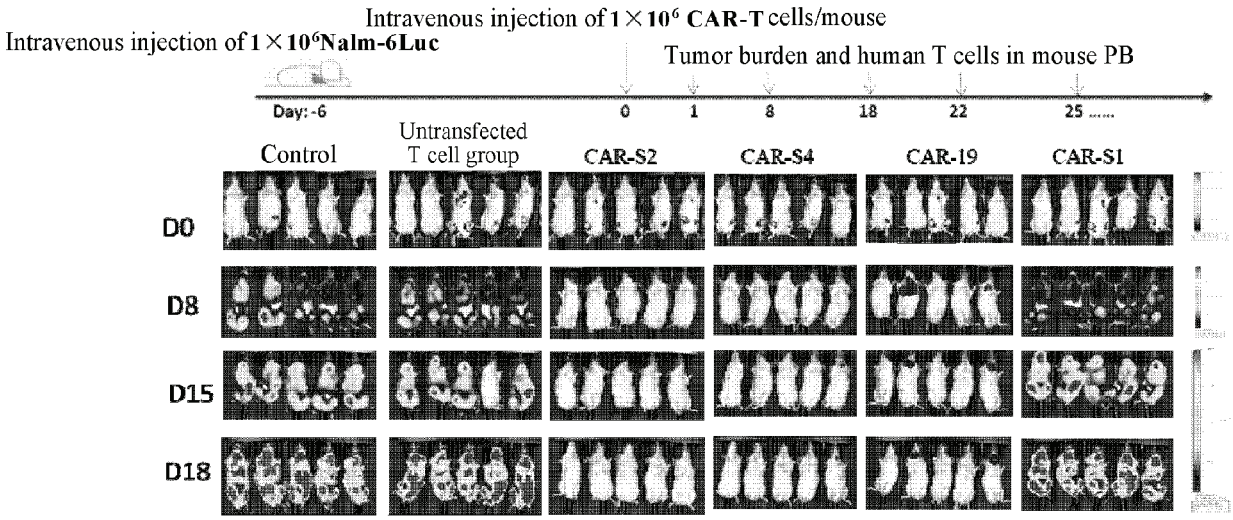
Intravenous injection of 1×10⁶ CAR-T cells/mouse
Intravenous injection of 1×10⁶Nalm-6Luc
Tumor burden and human T cells in mouse PB
Day: -6
0
1
8
18
22
25 .....
Control
Untransfected T cell group
CAR-S2
CAR-S4
CAR-19
CAR-S1
D0
D8
D15
D18

Fig. 17

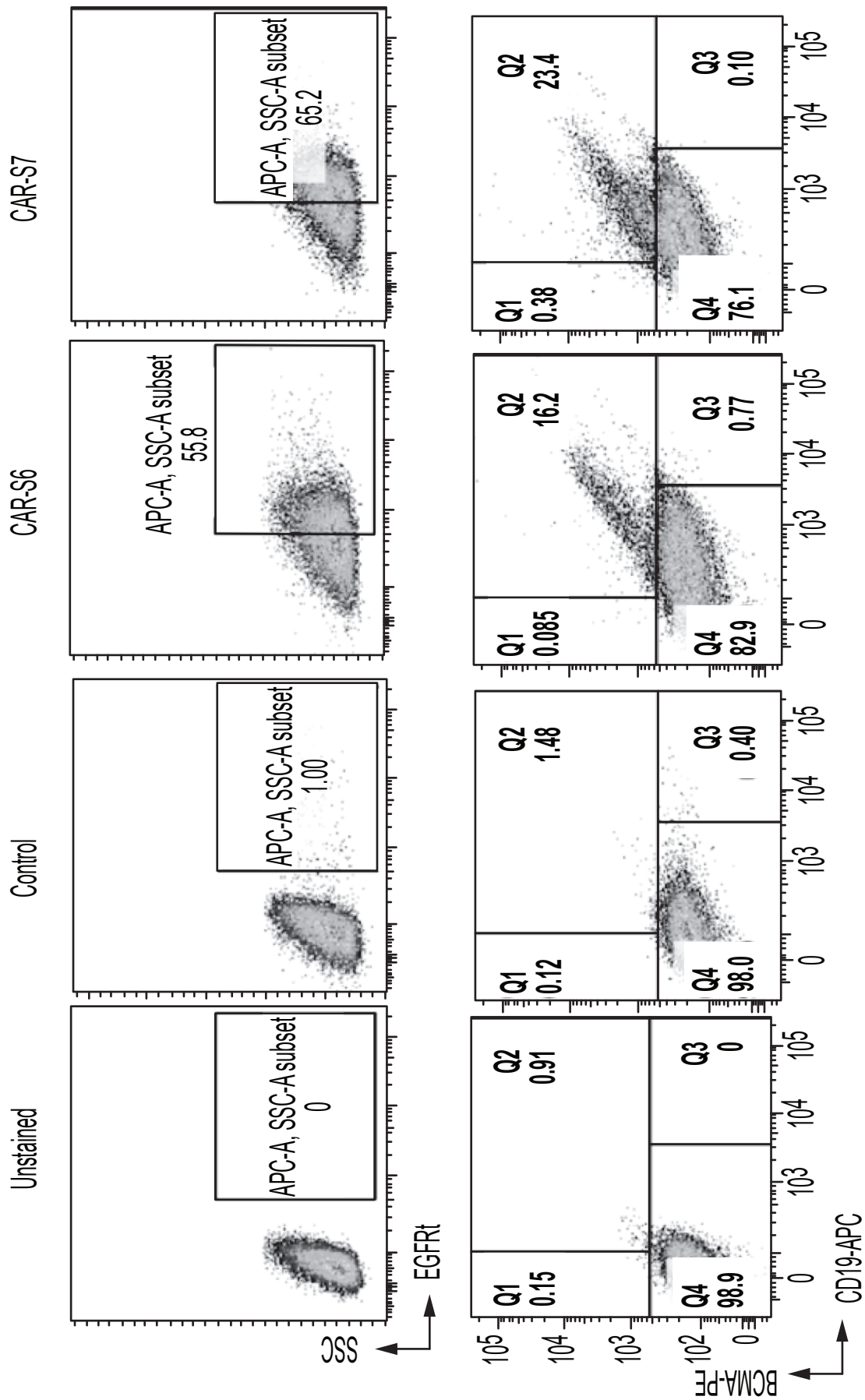
Unstained
Control
CAR-S6
CAR-S7
APC-A, SSC-A subset
0
APC-A, SSC-A subset
1.00
APC-A, SSC-A subset
55.8
APC-A, SSC-A subset
65.2
SSC
EGFRt
Q1
0.15
Q2
0.91
Q3
0
Q4
98.9
Q1
0.12
Q2
1.48
Q3
0.40
Q4
98.0
Q1
0.085
Q2
16.2
Q3
0.77
Q4
82.9
Q1
0.38
Q2
23.4
Q3
0.10
Q4
76.1
BCMA-PE
CD19-APC
0
10^2
10^3
10^4
10^5

Fig. 18

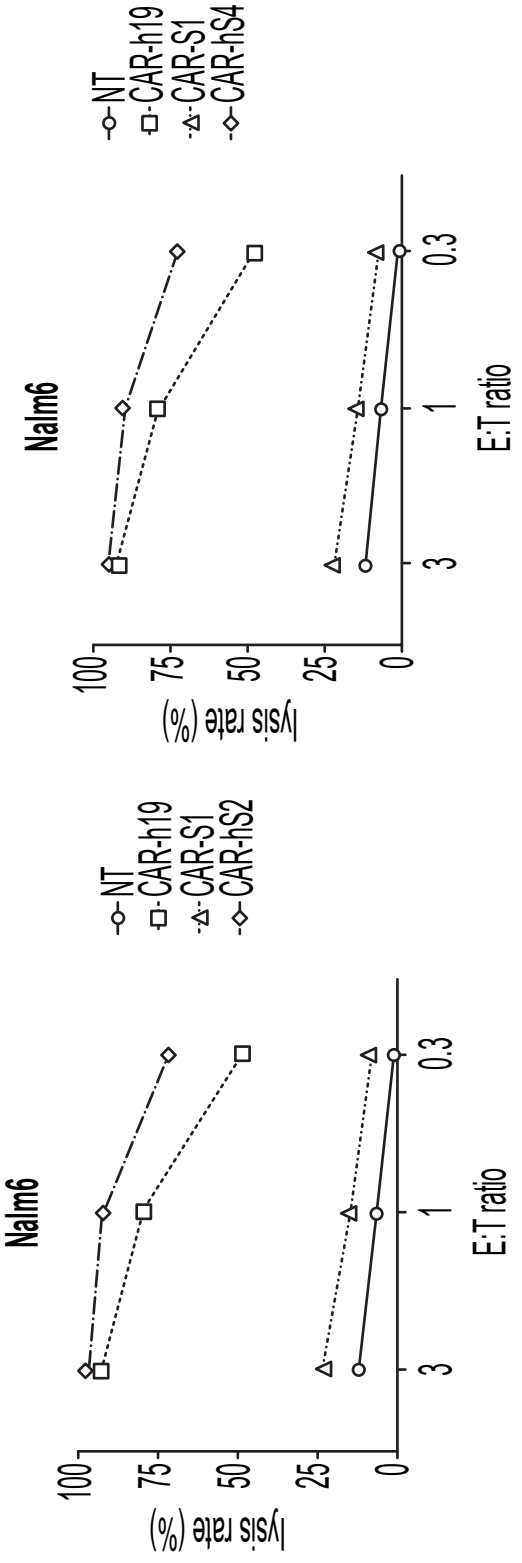
Nalm6
Lysis rate (%)
100
75
50
25
0
3
1
0.3
E:T ratio
NT
CAR-h19
CAR-S1
CAR-hS2
Nalm6
Lysis rate (%)
100
75
50
25
0
3
1
0.3
E:T ratio
NT
CAR-h19
CAR-S1
CAR-hS4

Fig. 19

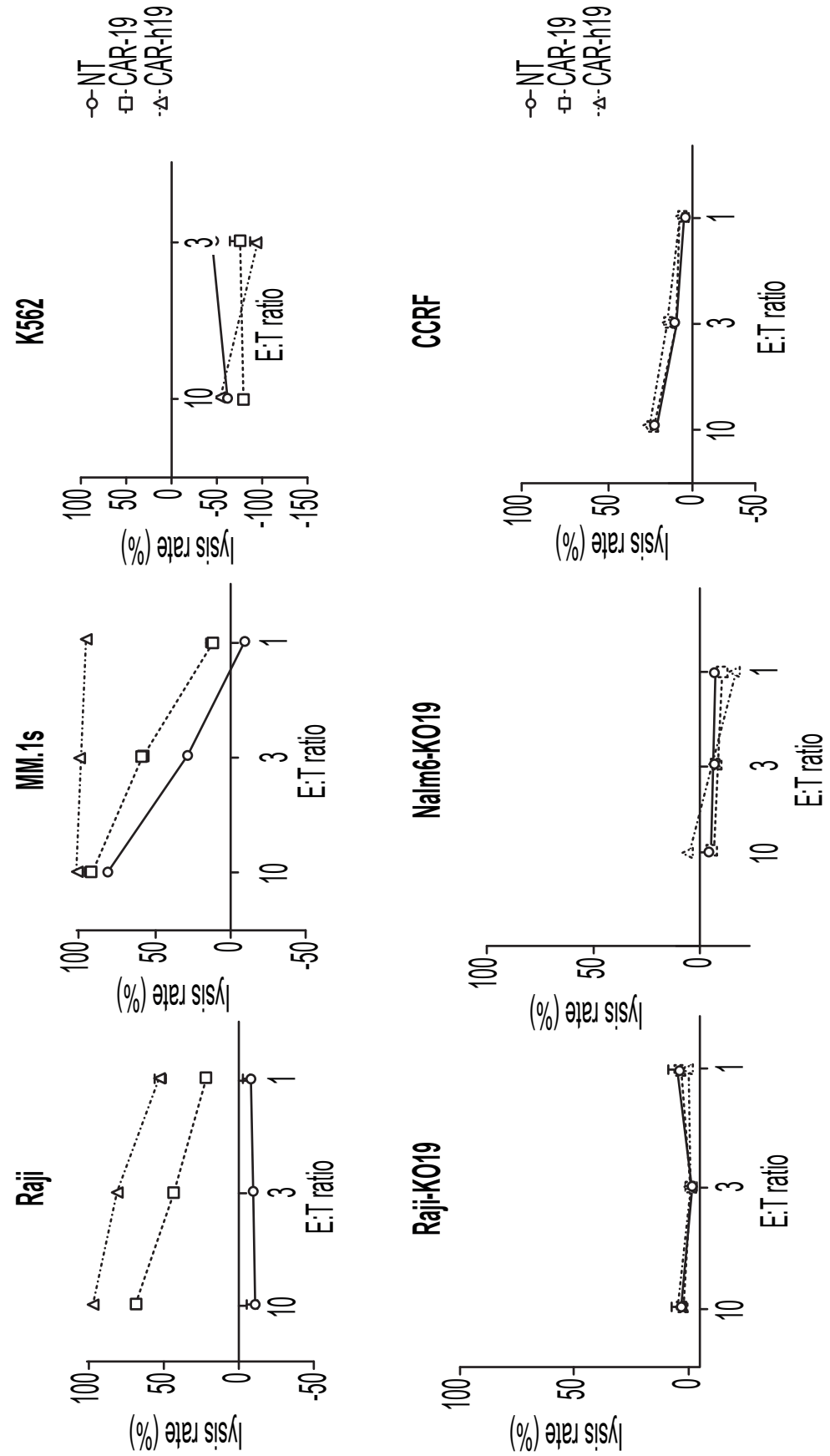
Raji
MM.1s
K562
Raji-KO19
Nalm6-KO19
CCRF
NT
CAR-19
CAR-h19
lysis rate (%)
E:T ratio
100
50
0
-50
-100
-150
10
3
1

Fig. 20

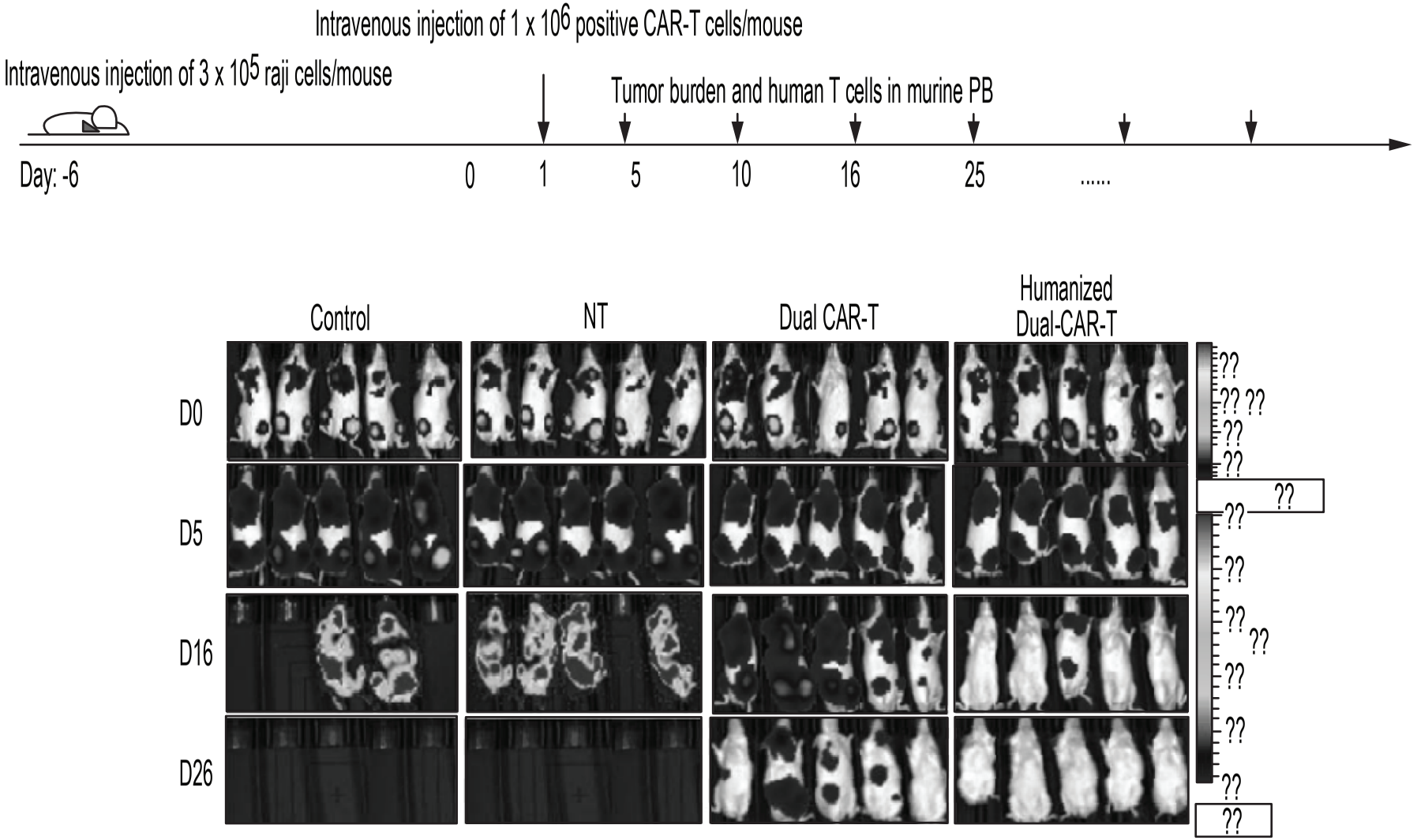
Intravenous injection of 1 x 10^6 positive CAR-T cells/mouse
Intravenous injection of 3 x 10^5 raji cells/mouse
Tumor burden and human T cells in murine PB
Day: -6
0
1
5
10
16
25
.....
Control
NT
Dual CAR-T
Humanized Dual-CAR-T
D0
D5
D16
D26
??
??
??
??
??
??
??
??
??
??
??
??
??

Fig. 21

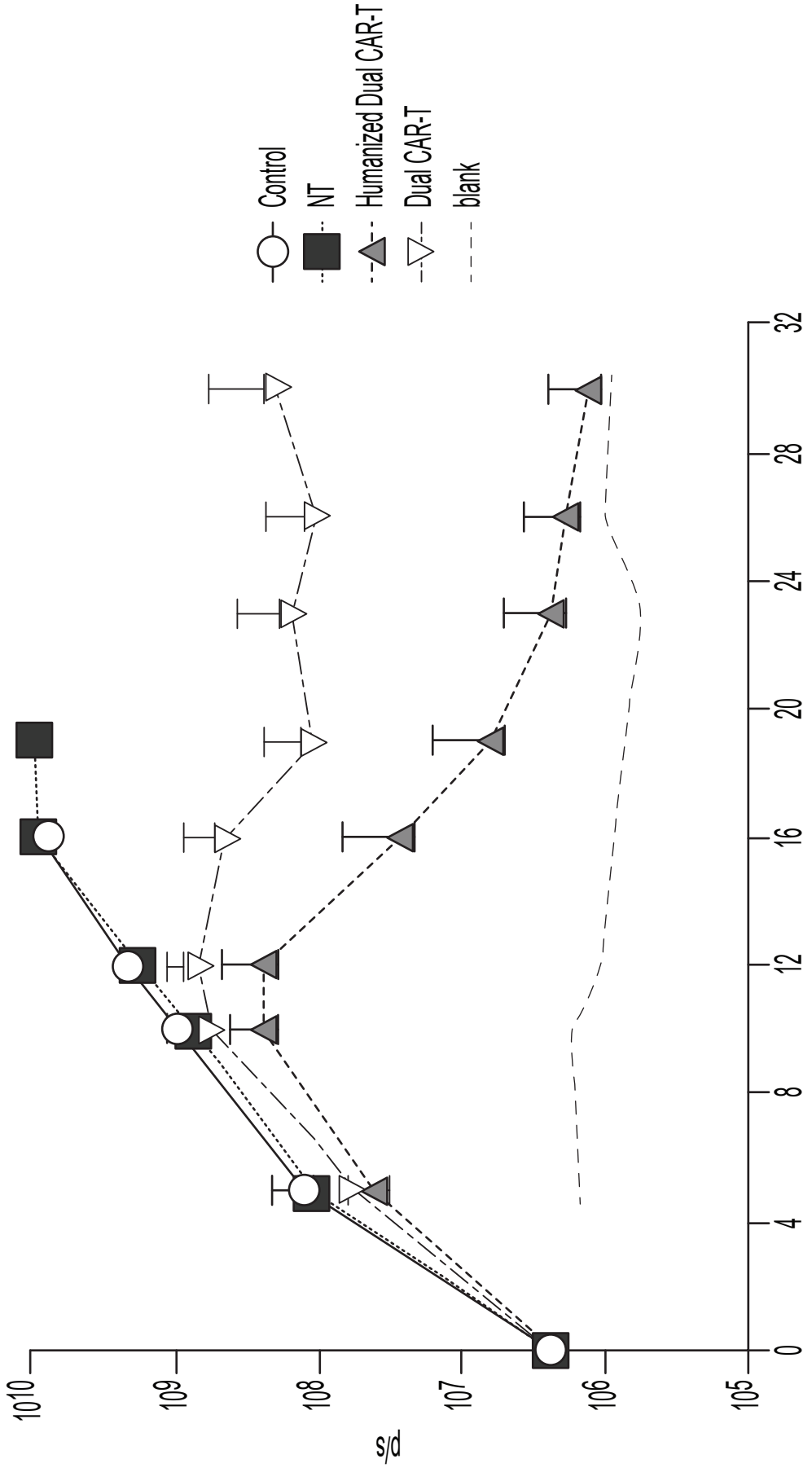

Fig. 21, Continued

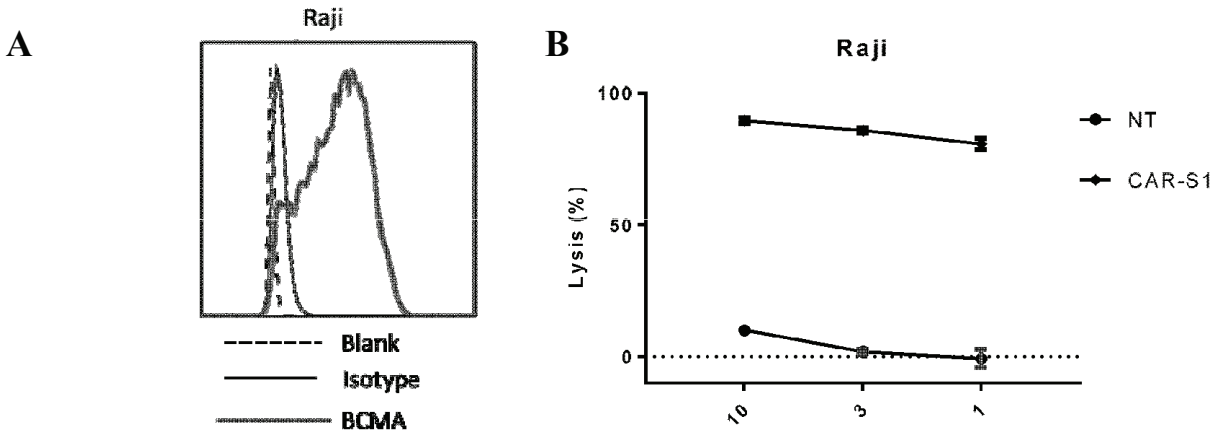

Fig. 22