Figure 1

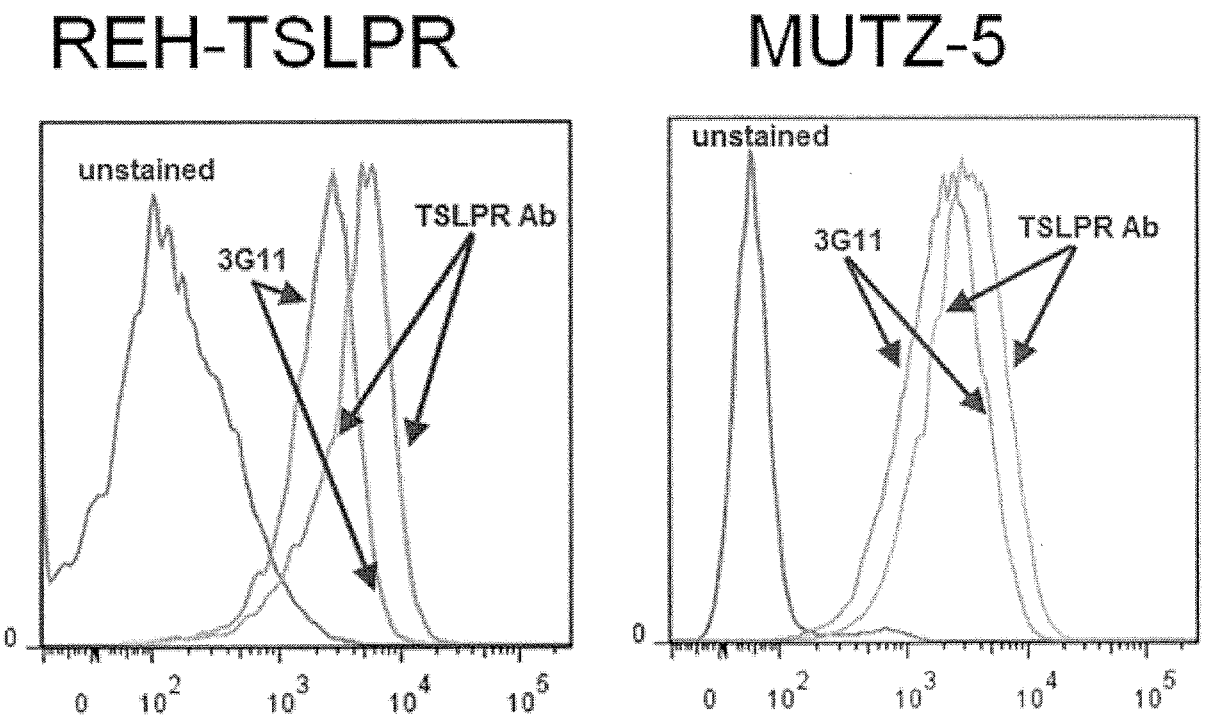
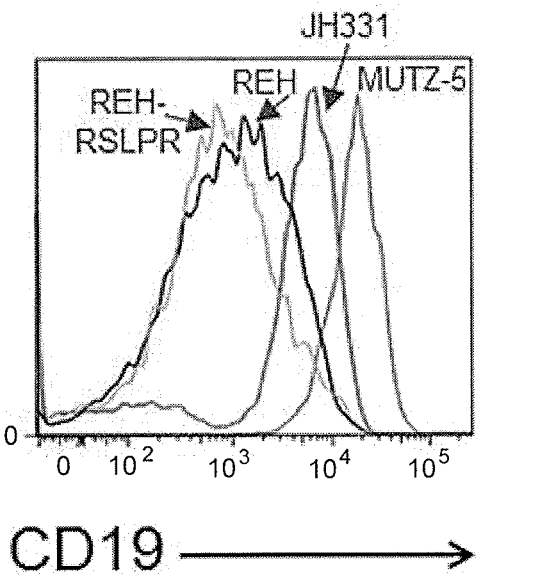
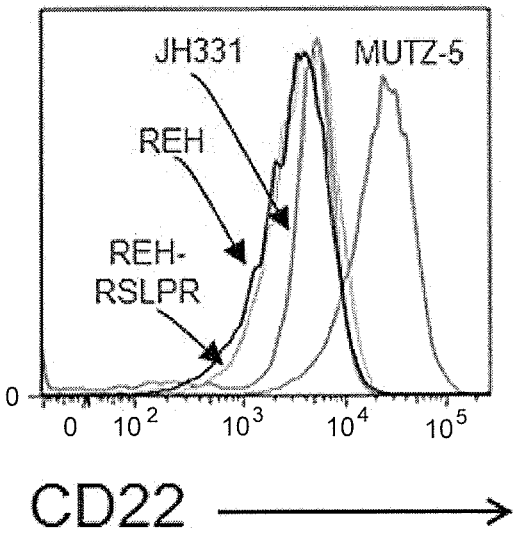

REH-TSLPR
unstained
3G11
TSLPR Ab
0
0
10^2
10^3
10^4
10^5
MUTZ-5
unstained
3G11
TSLPR Ab
0
0
10^2
10^3
10^4
10^5

Figure 2


JH331
REH
MUTZ-5
REH-
RSLPR
0
0
10^2
10^3
10^4
10^5
CD19


JH331
MUTZ-5
REH
REH-
RSLPR
0
0
10^2
10^3
10^4
10^5
CD22

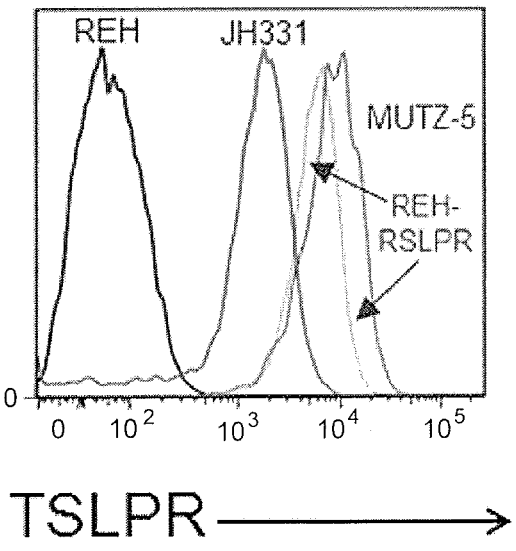

REH
JH331
MUTZ-5
REH-
RSLPR
0
0
10^2
10^3
10^4
10^5
TSLPR

Figure 3

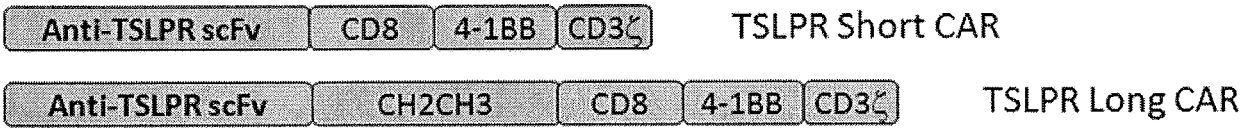
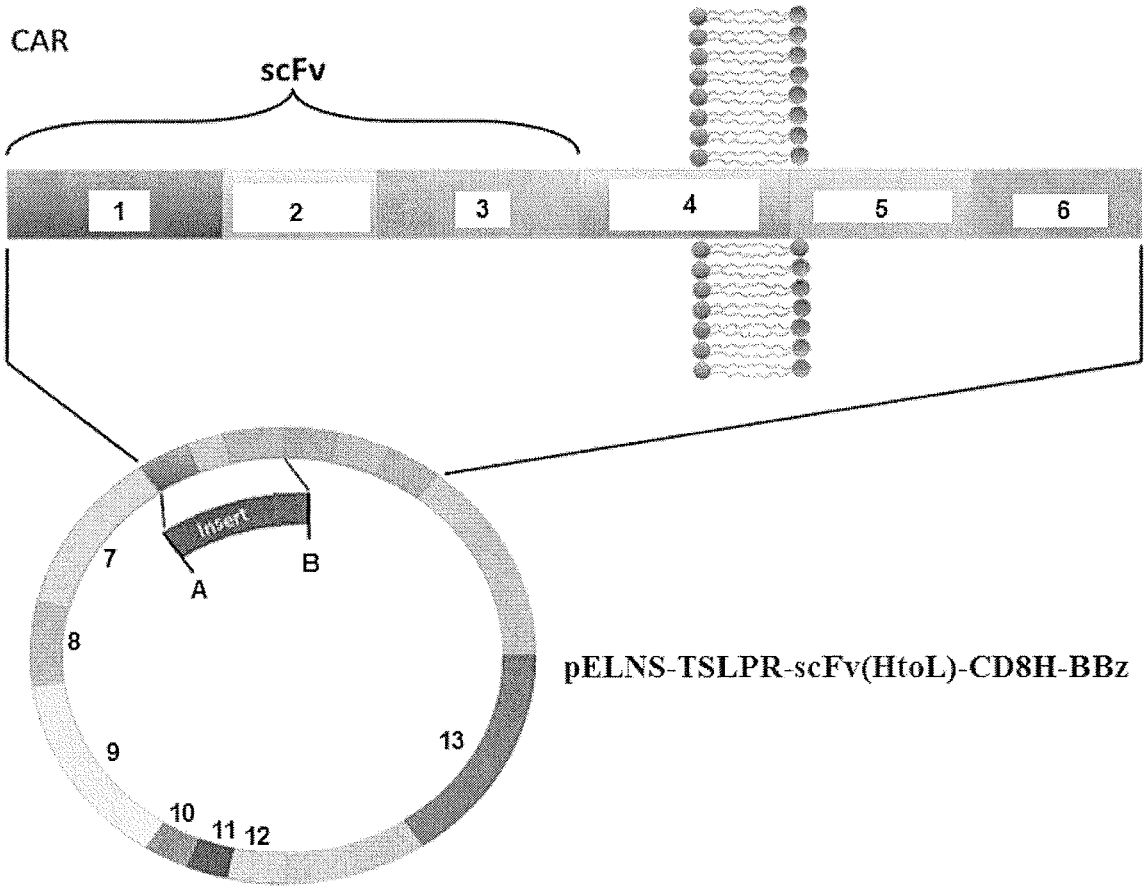

Anti-TSLPR scFv
CD8
4-1BB
CD3ζ
TSLPR Short CAR
Anti-TSLPR scFv
CH2CH3
CD8
4-1BB
CD3ζ
TSLPR Long CAR

Figure 4



1 V_H
2 Glycine-Serine Linker
3 V_L
4 CD8 α hinge + CD8 TM
5 41BB ICD
6 CD3ζ
7 EF1α Promoter
8 cPPT
9 Partial *gag* sequence
10 U5
11 R
12 RSV Promoter
13 β lactamase
A Nfe1
B BspE1

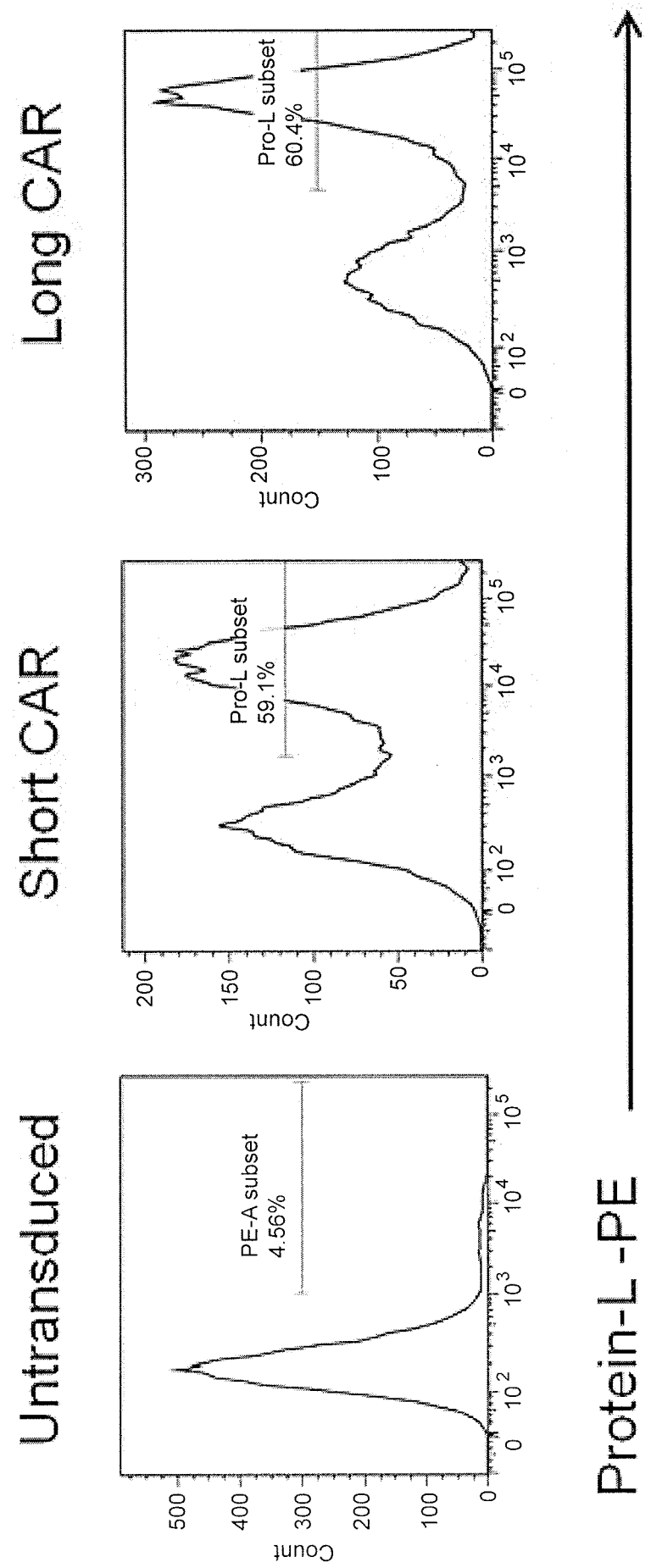

Untransduced
Short CAR
Long CAR
Count
PE-A subset
4.56%
Pro-L subset
59.1%
Pro-L subset
60.4%
Protein-L -PE

Figure 5A

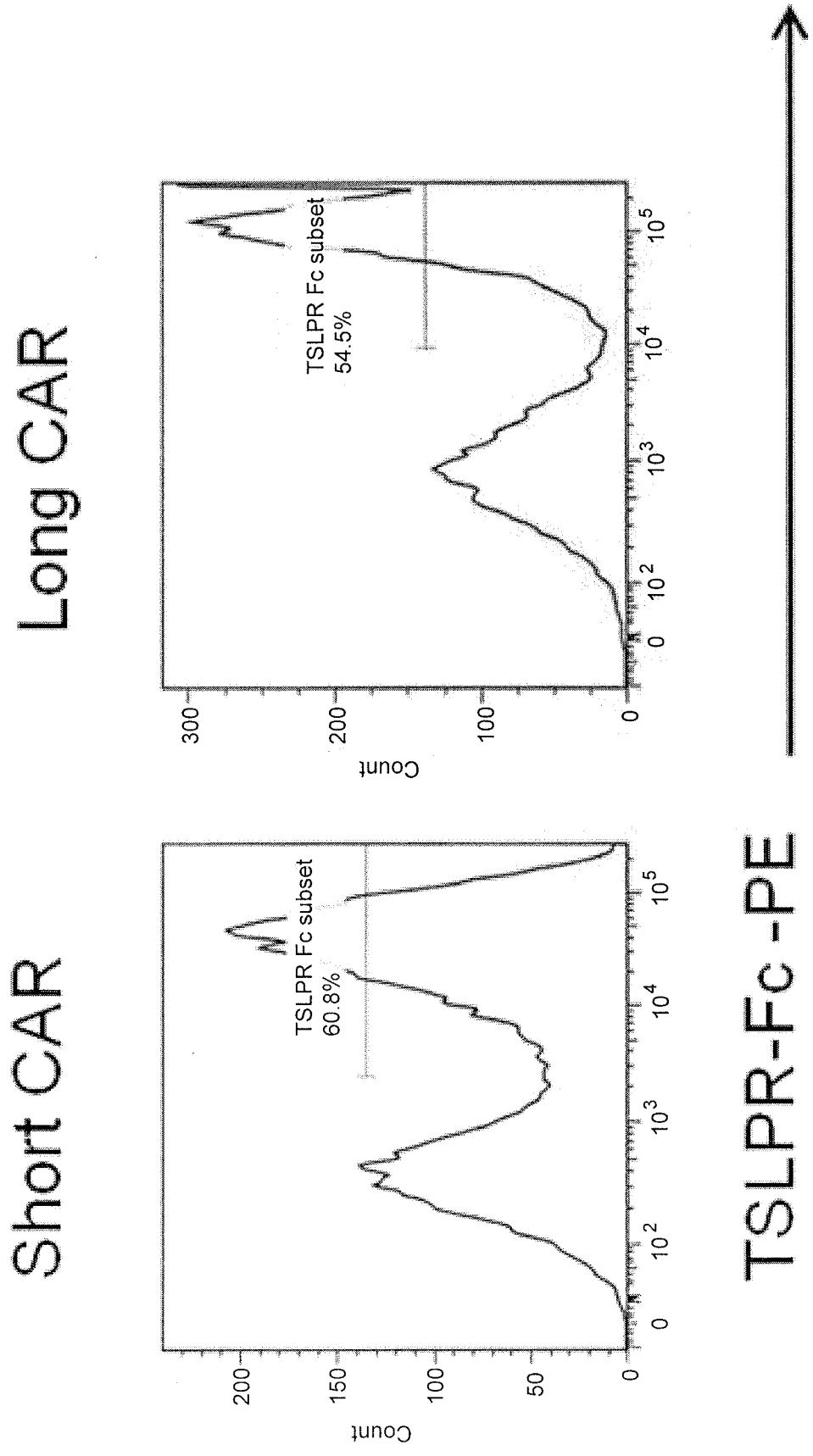

Short CAR
Long CAR
TSLPR Fc subset
60.8%
TSLPR Fc subset
54.5%
Count
TSLPR-Fc -PE

Figure 5B

Figure 6

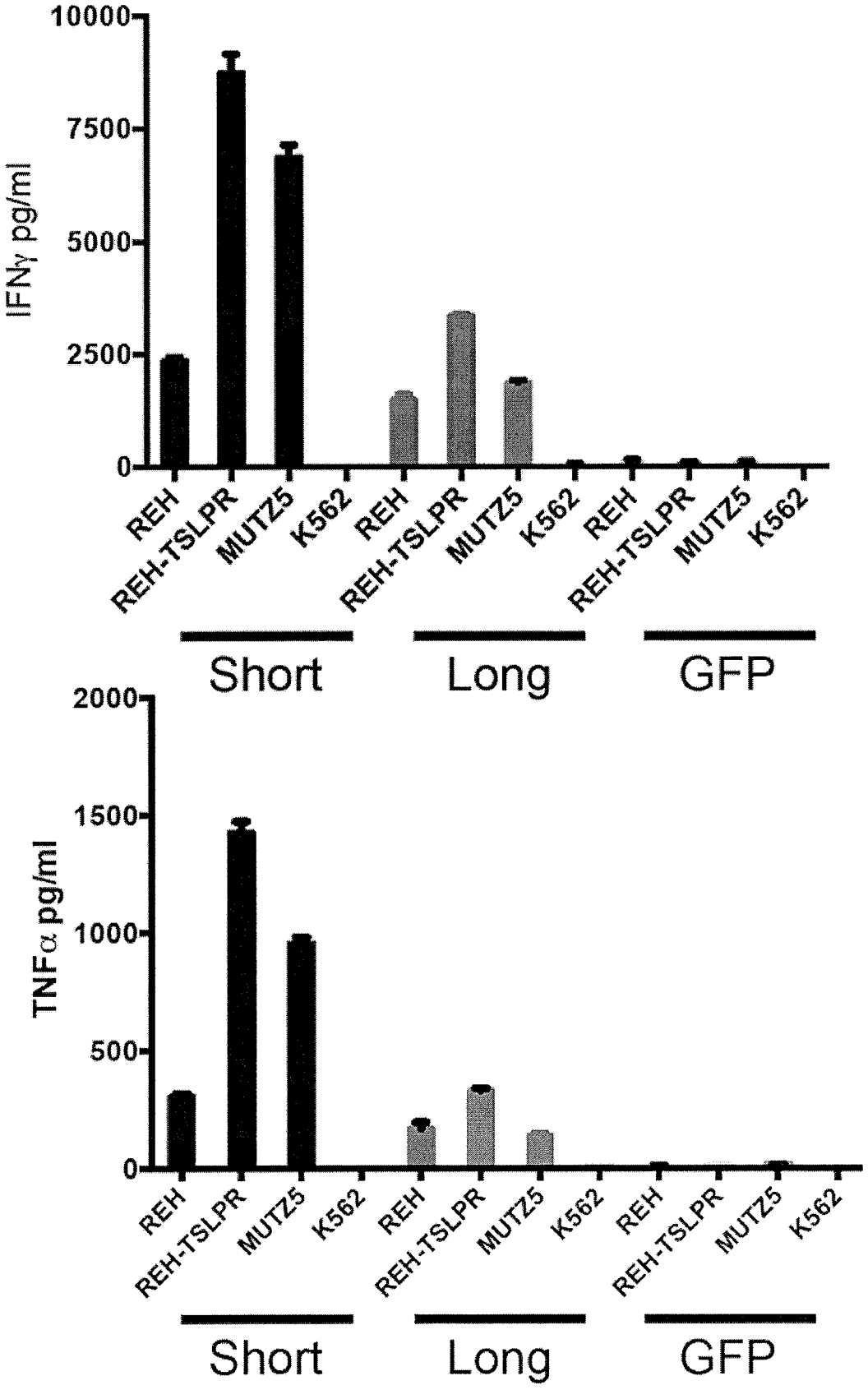

10000
7500
5000
2500
0
IFNγ pg/ml
REH
REH-TSLPR
MUTZ5
K562
REH
REH-TSLPR
MUTZ5
K562
REH
REH-TSLPR
MUTZ5
K562
Short
Long
GFP
2000
1500
1000
500
0
TNFα pg/ml
REH
REH-TSLPR
MUTZ5
K562
REH
REH-TSLPR
MUTZ5
K562
REH
REH-TSLPR
MUTZ5
K562
Short
Long
GFP

Figure 7A

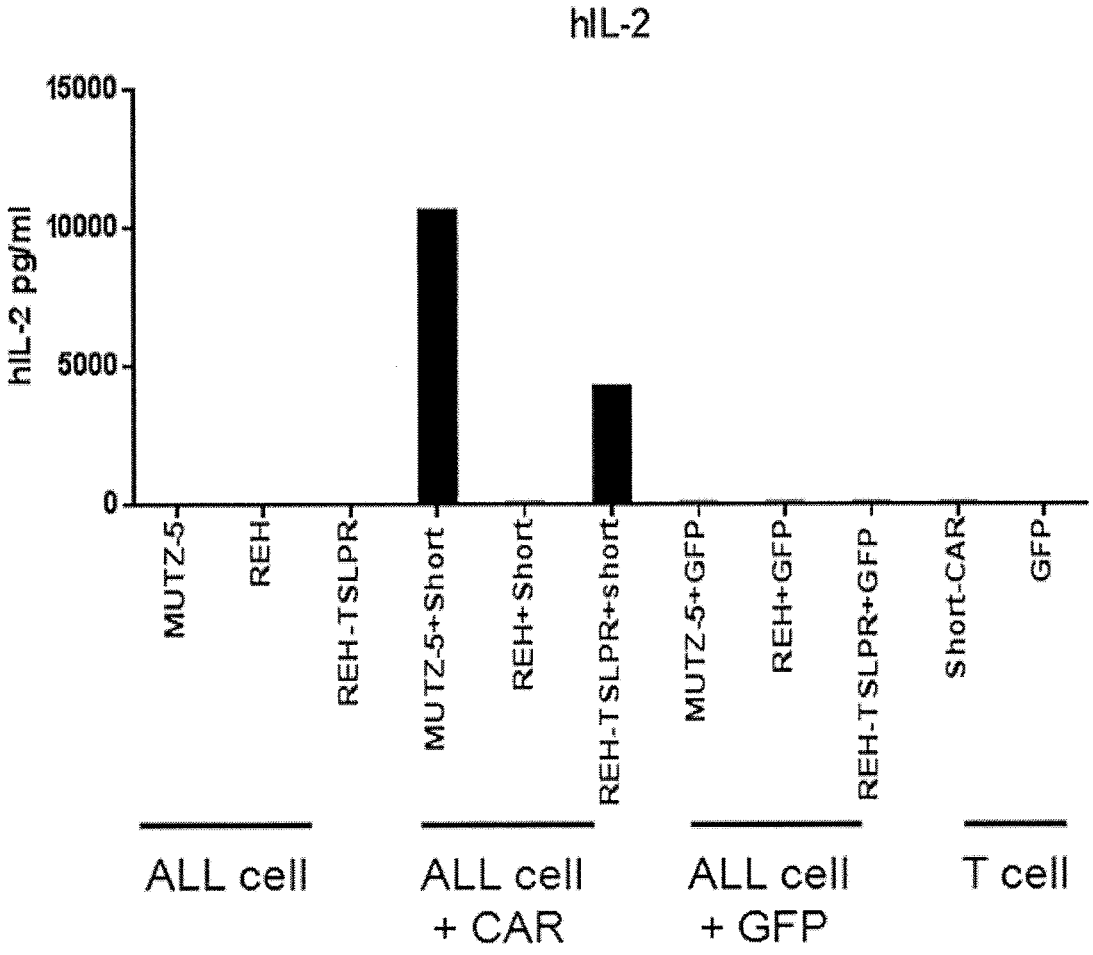

hIL-2
hIL-2 pg/ml
15000
10000
5000
0
MUTZ-5
REH
REH-TSLPR
MUTZ-5+Short
REH+Short
REH-TSLPR+short
MUTZ-5+GFP
REH+GFP
REH-TSLPR+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 7B

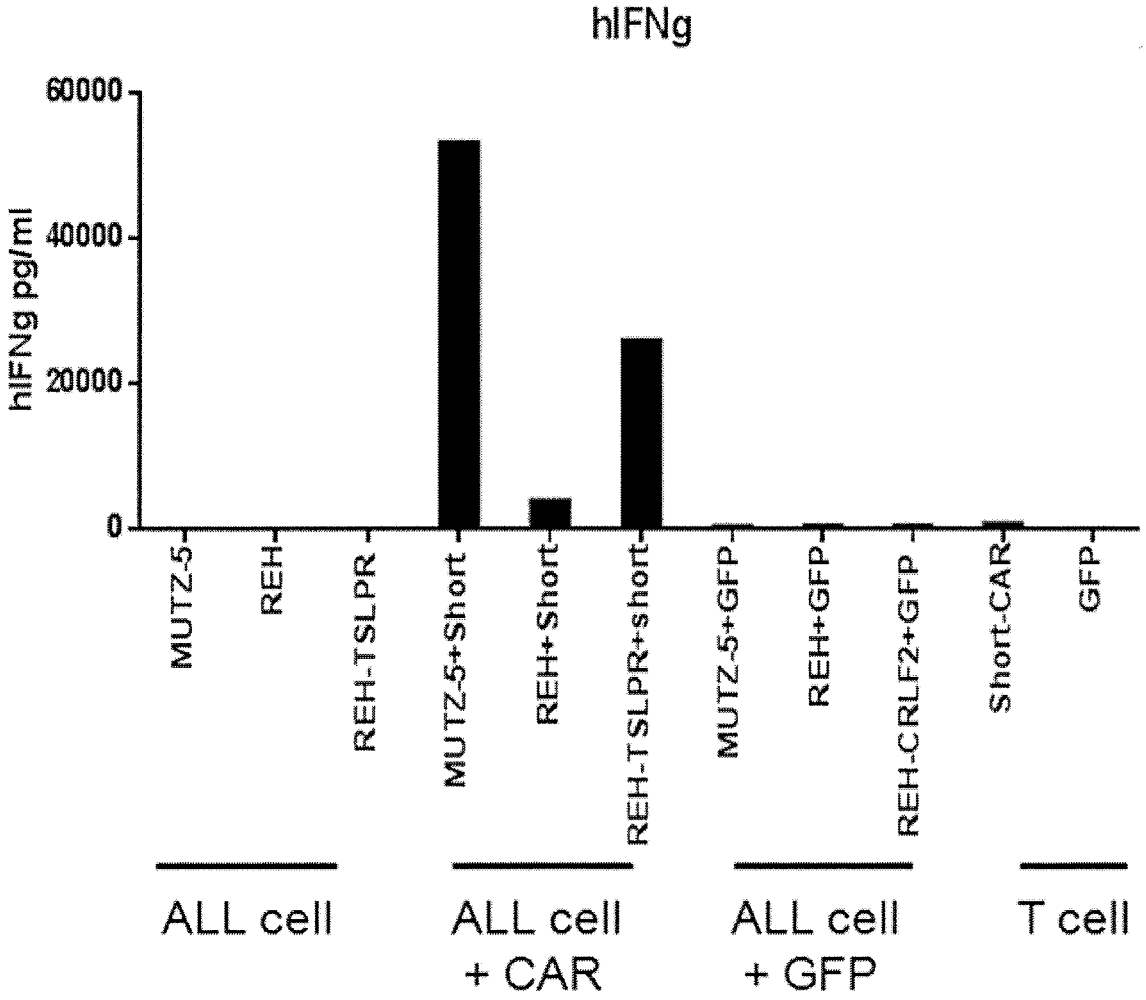

hIFNg
hIFNg pg/ml
60000
40000
20000
0
MUTZ-5
REH
REH-TSLPR
MUTZ-5+Short
REH+Short
REH-TSLPR+short
MUTZ-5+GFP
REH+GFP
REH-CRLF2+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 7C

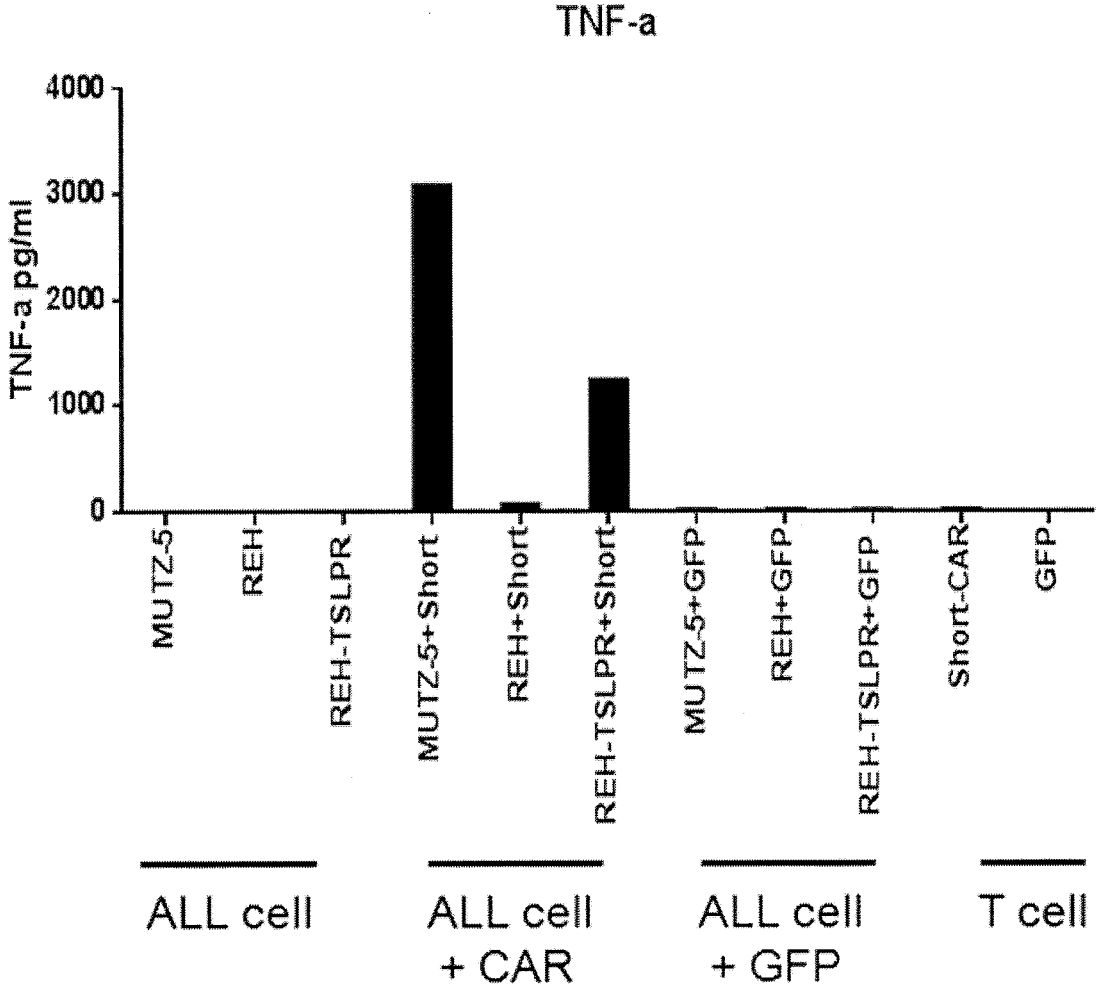

TNF-a
TNF-a pg/ml
4000
3000
2000
1000
0
MUTZ-5
REH
REH-TSLPR
MUTZ-5+Short
REH+Short
REH-TSLPR+Short
MUTZ-5+GFP
REH+GFP
REH-TSLPR+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 7D

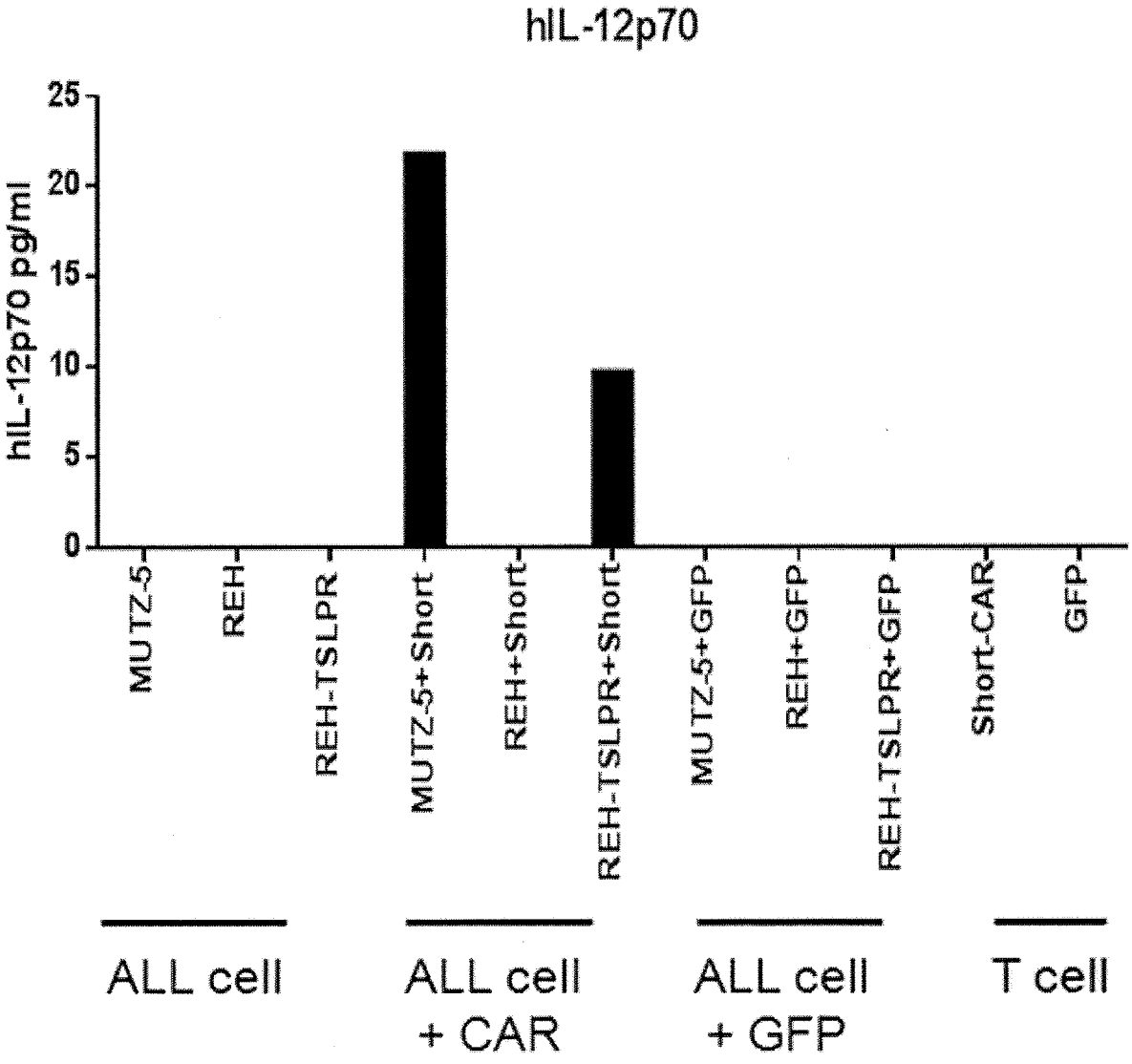

hIL-12p70
hIL-12p70 pg/ml
25
20
15
10
5
0
MUTZ-5
REH
REH-TSLPR
MUTZ-5+Short
REH+Short
REH-TSLPR+Short
MUTZ-5+GFP
REH+GFP
REH-TSLPR+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 7E

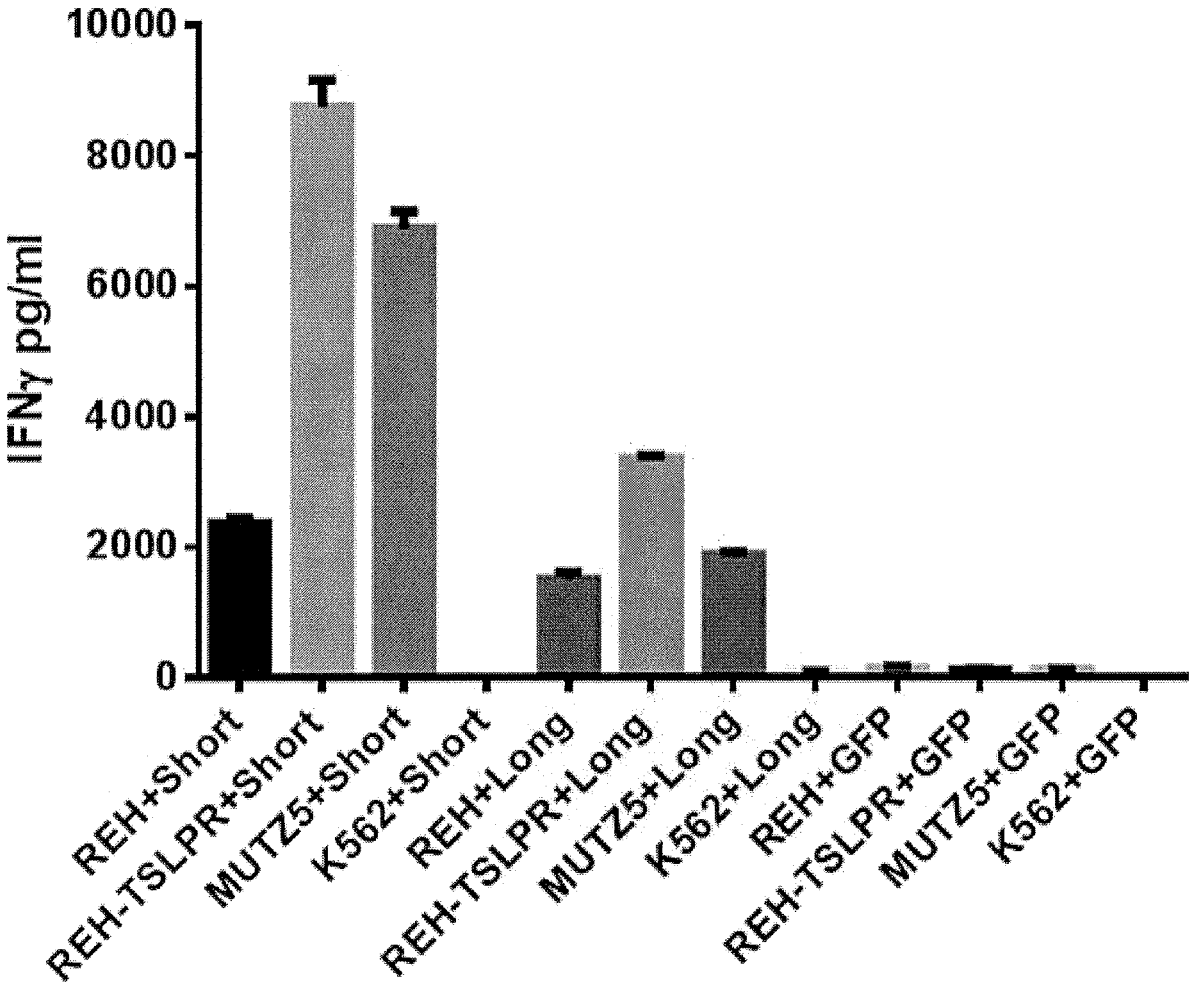

10000
8000
6000
4000
2000
0
IFNγ pg/ml
REH+Short
REH-TSLPR+Short
MUTZ5+Short
K562+Short
REH+Long
REH-TSLPR+Long
MUTZ5+Long
K562+Long
REH+GFP
REH-TSLPR+GFP
MUTZ5+GFP
K562+GFP

Figure 7F

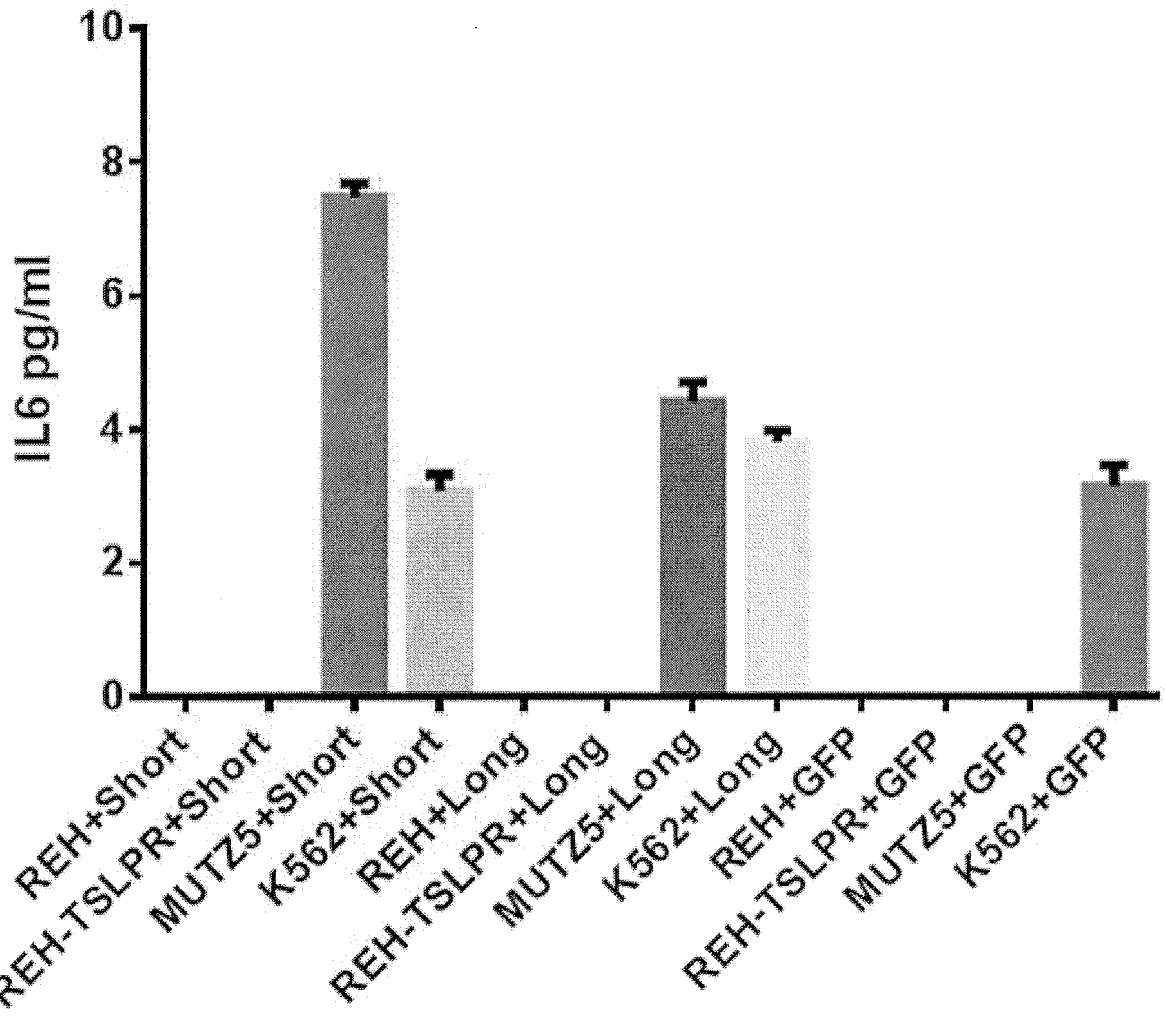

IL6 pg/ml
10
8
6
4
2
0
REH+Short
REH-TSLPR+Short
MUTZ5+Short
K562+Short
REH+Long
REH-TSLPR+Long
MUTZ5+Long
K562+Long
REH+GFP
REH-TSLPR+GFP
MUTZ5+GFP
K562+GFP

Figure 7G

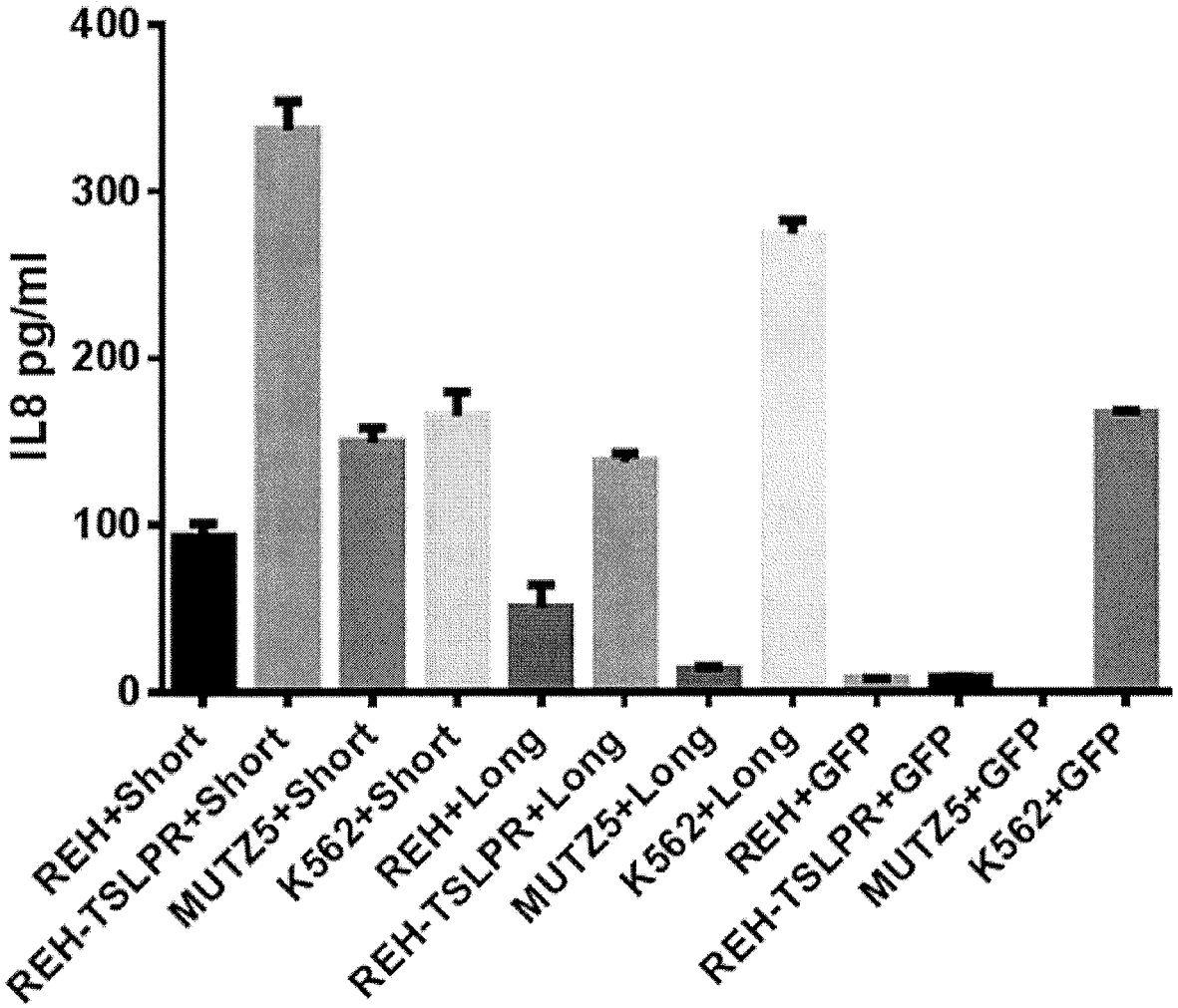

IL8 pg/ml
400
300
200
100
0
REH+Short
REH-TSLPR+Short
MUTZ5+Short
K562+Short
REH+Long
REH-TSLPR+Long
MUTZ5+Long
K562+Long
REH+GFP
REH-TSLPR+GFP
MUTZ5+GFP
K562+GFP

Figure 7H

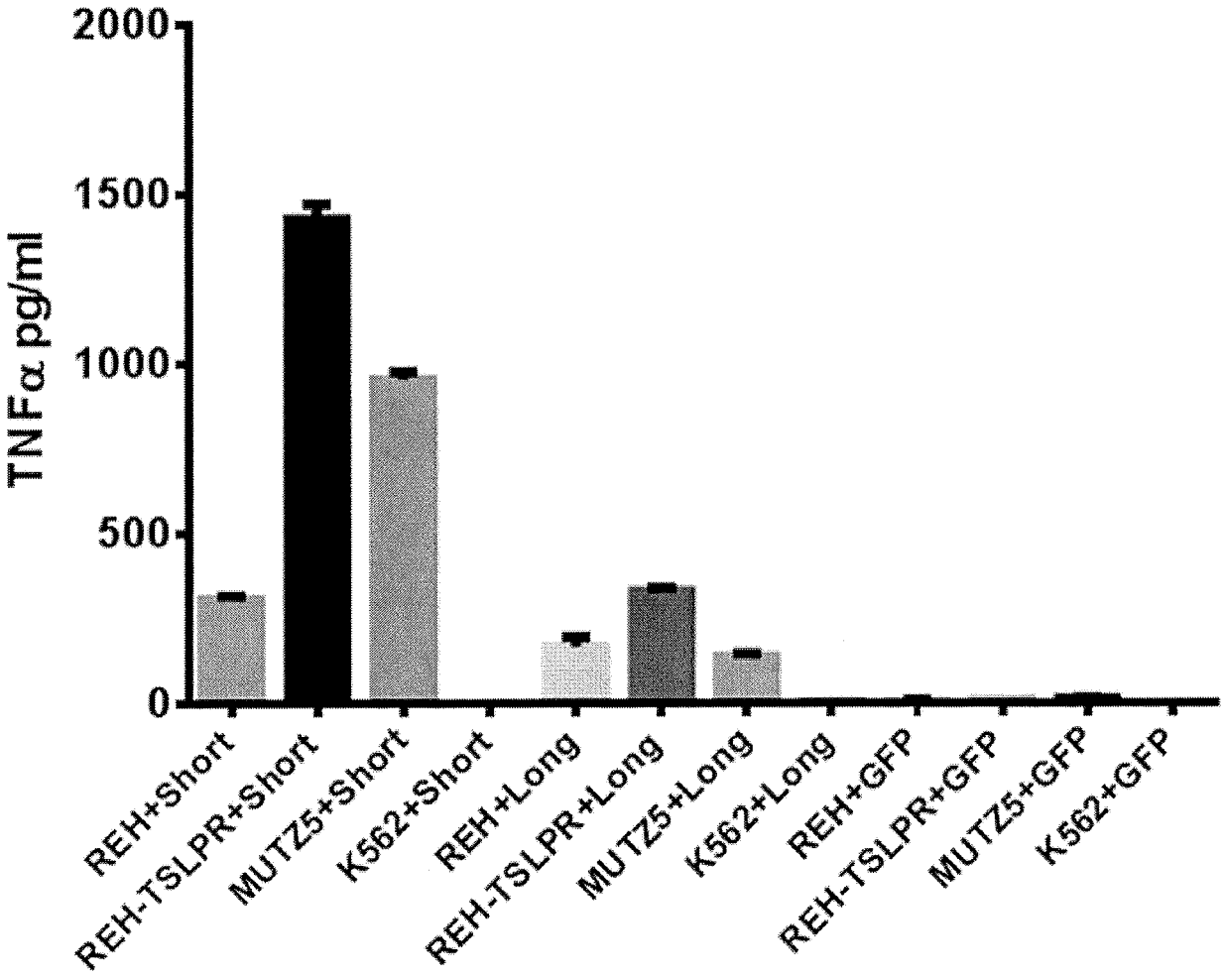

2000
1500
1000
500
0
TNFα pg/ml
REH+Short
REH-TSLPR+Short
MUTZ5+Short
K562+Short
REH+Long
REH-TSLPR+Long
MUTZ5+Long
K562+Long
REH+GFP
REH-TSLPR+GFP
MUTZ5+GFP
K562+GFP

Figure 8A

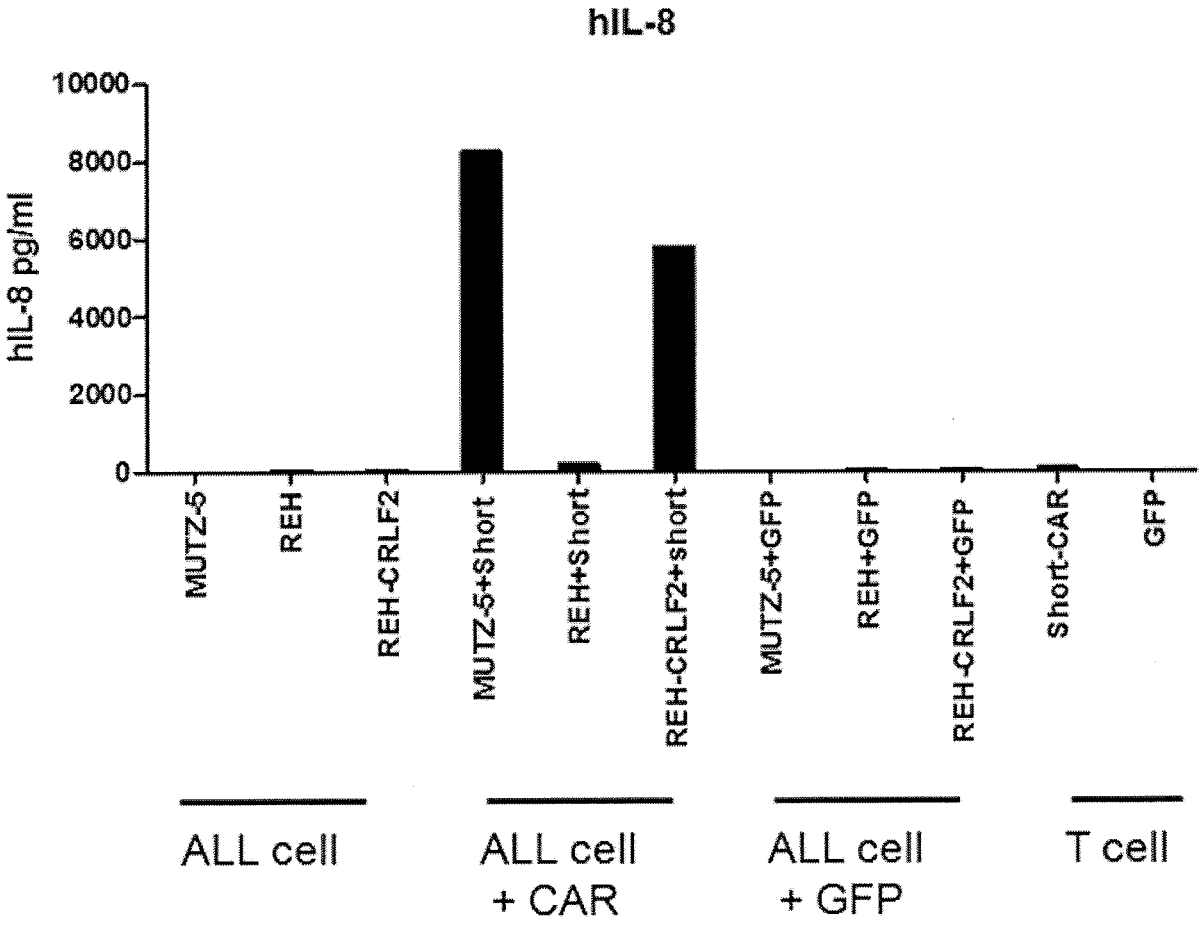

hIL-8
hIL-8 pg/ml
10000
8000
6000
4000
2000
0
MUTZ-5
REH
REH-CRLF2
MUTZ-5+Short
REH+Short
REH-CRLF2+short
MUTZ-5+GFP
REH+GFP
REH-CRLF2+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 8B

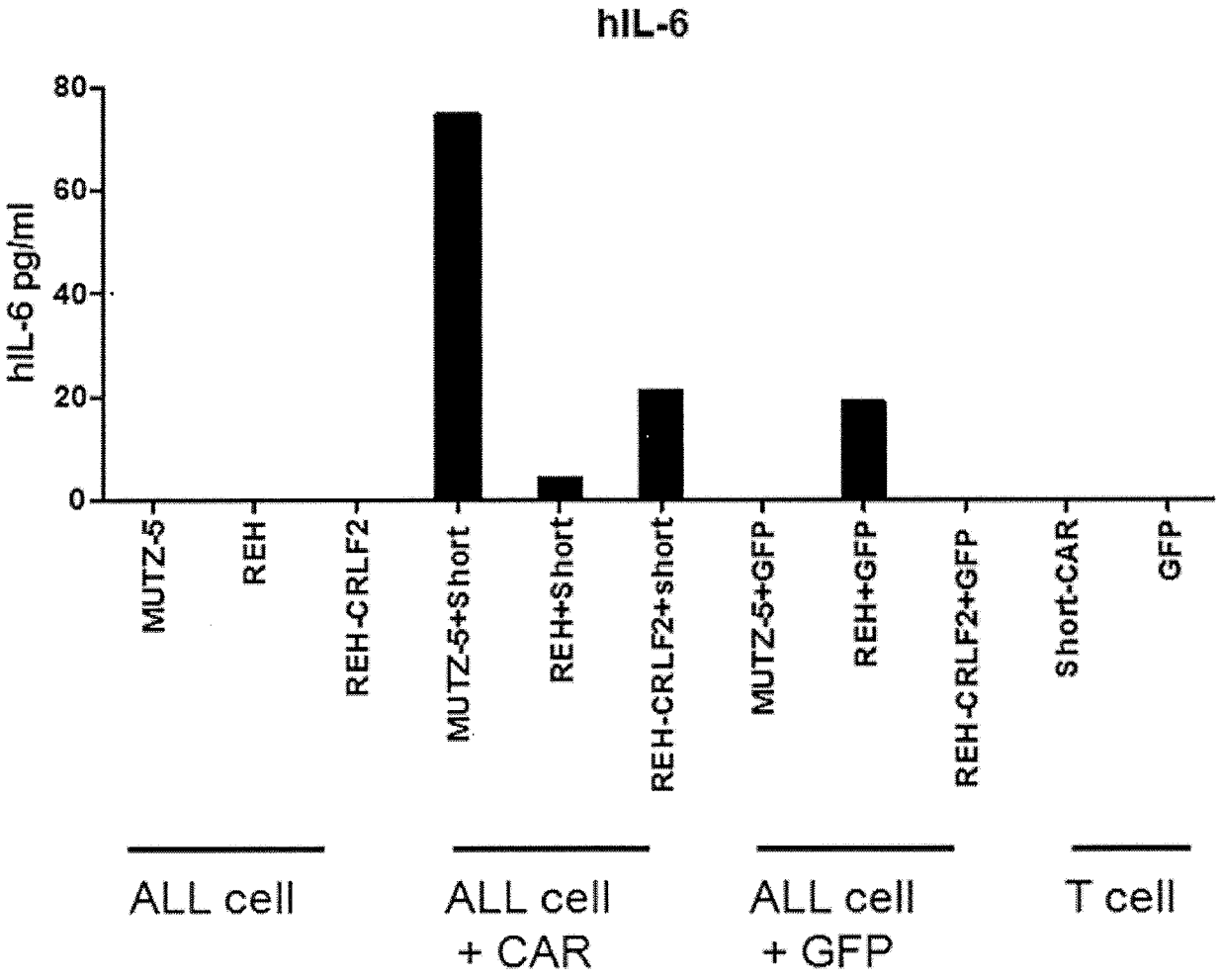

hIL-6
hIL-6 pg/ml
80
60
40
20
0
MUTZ-5
REH
REH-CRLF2
MUTZ-5+short
REH+short
REH-CRLF2+short
MUTZ-5+GFP
REH+GFP
REH-CRLF2+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 8C

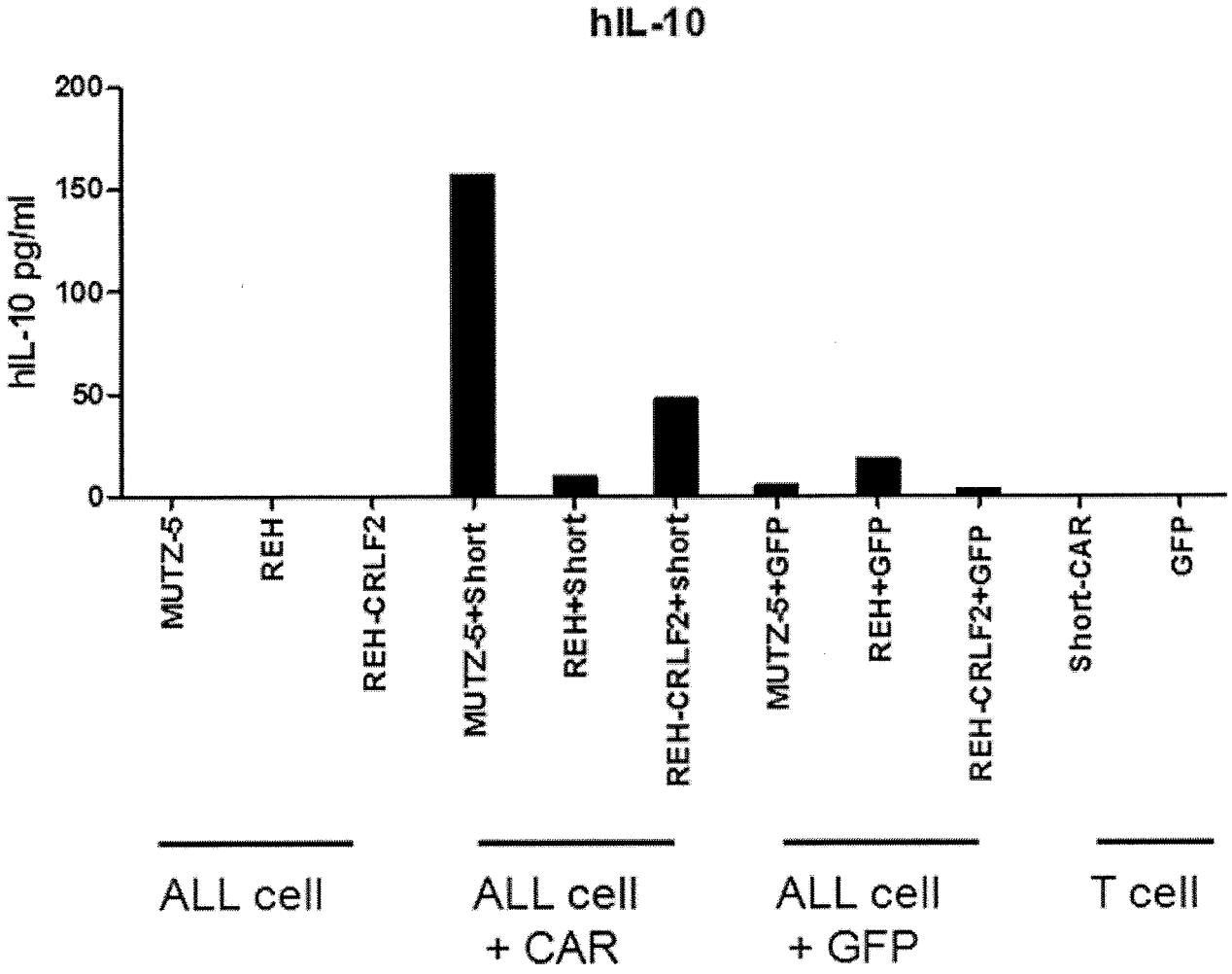

hIL-10
hIL-10 pg/ml
200
150
100
50
0
MUTZ-5
REH
REH-CRLF2
MUTZ-5+Short
REH+Short
REH-CRLF2+short
MUTZ-5+GFP
REH+GFP
REH-CRLF2+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 8D

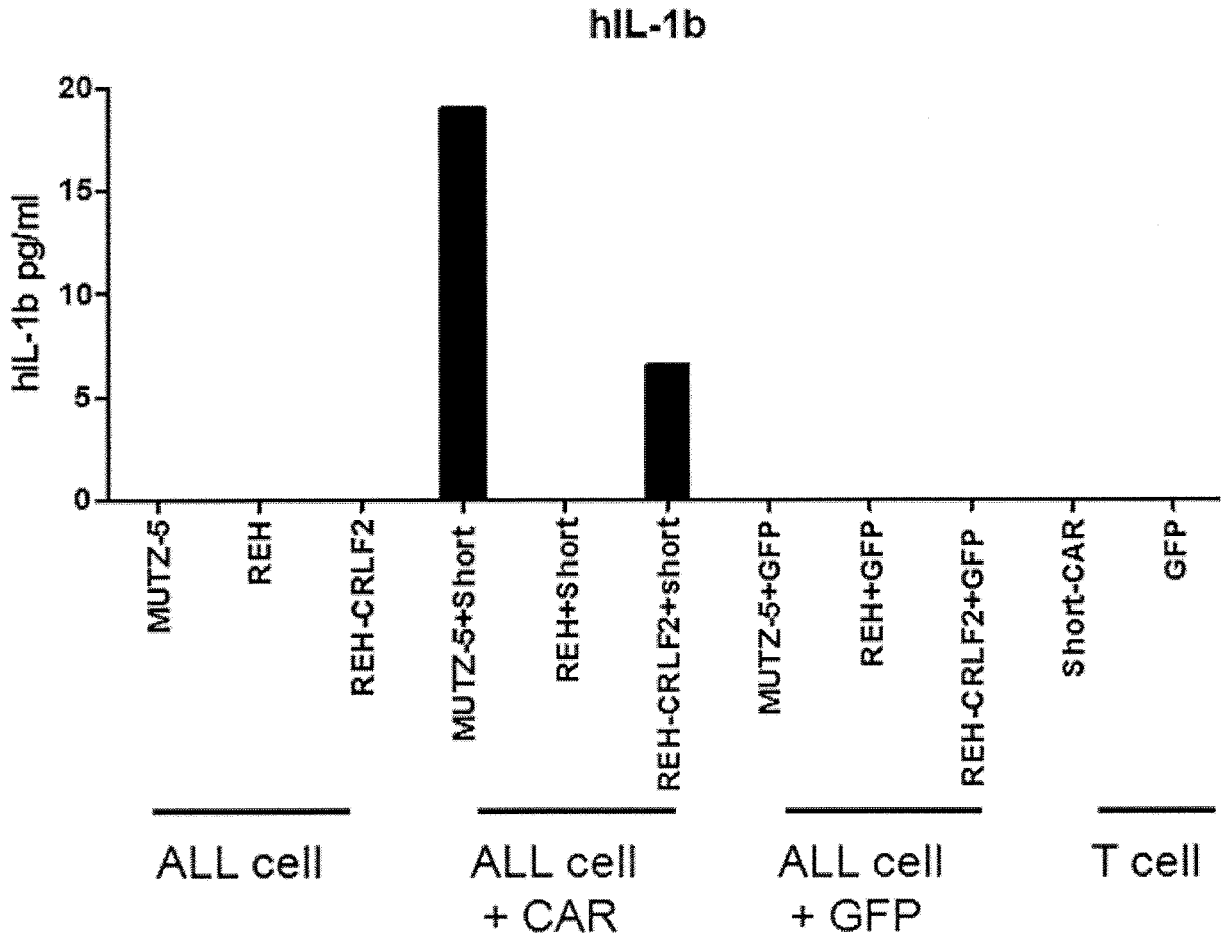

hIL-1b
hIL-1b pg/ml
20
15
10
5
0
MUTZ-5
REH
REH-CRLF2
MUTZ-5+Short
REH+Short
REH-CRLF2+short
MUTZ-5+GFP
REH+GFP
REH-CRLF2+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 8E

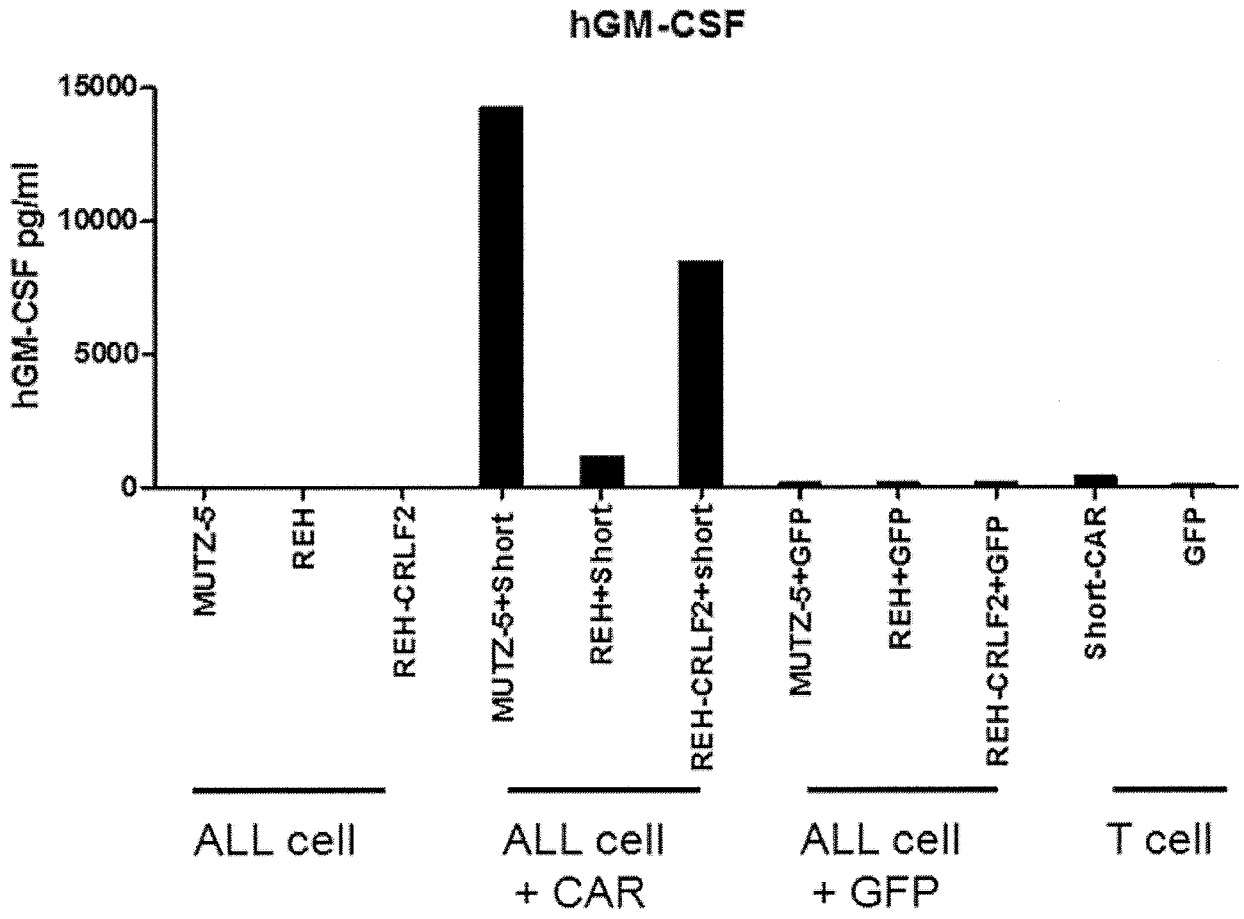

hGM-CSF
hGM-CSF pg/ml
15000
10000
5000
0
MUTZ-5
REH
REH-CRLF2
MUTZ-5+Short
REH+Short
REH-CRLF2+short
MUTZ-5+GFP
REH+GFP
REH-CRLF2+GFP
Short-CAR
GFP
ALL cell
ALL cell + CAR
ALL cell + GFP
T cell

Figure 9A

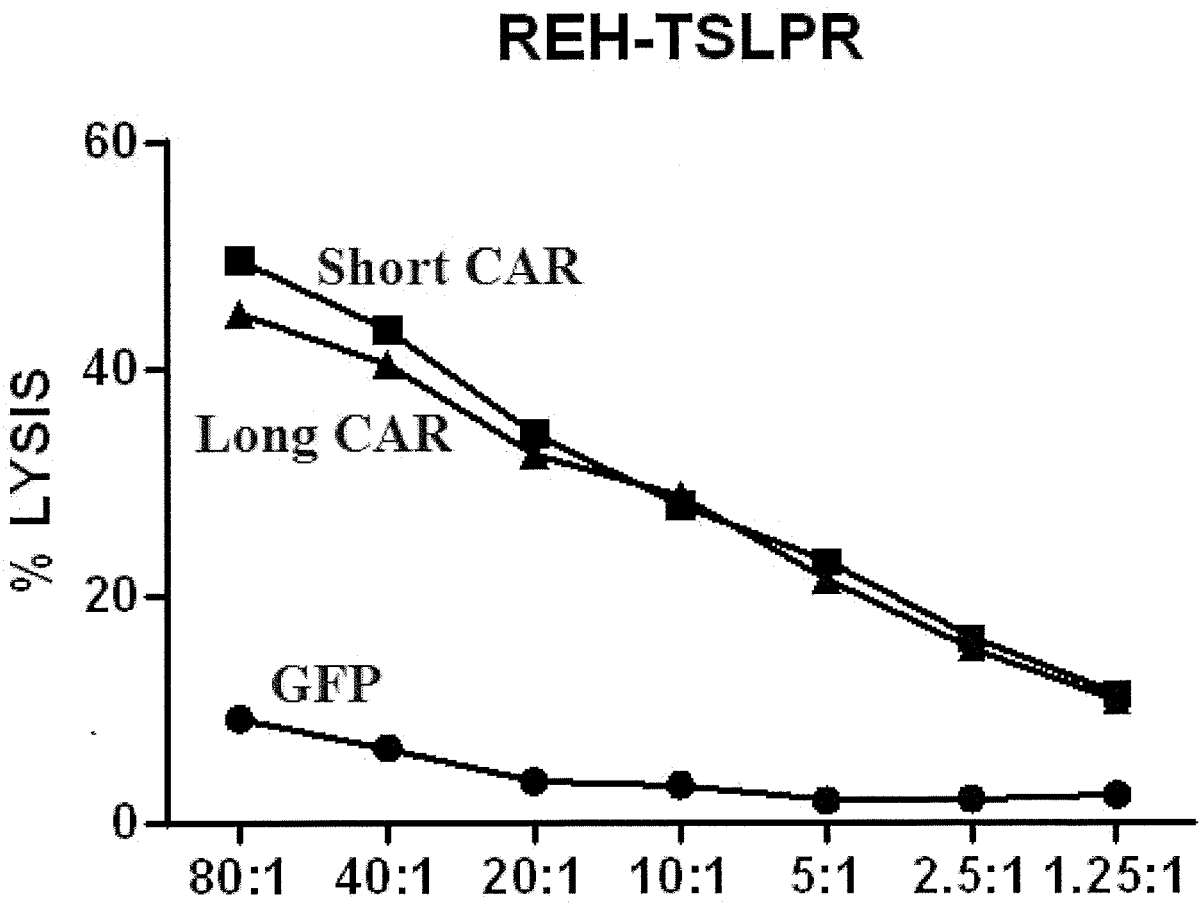

REH-TSLPR
% LYSIS
60
40
20
0
Short CAR
Long CAR
GFP
80:1
40:1
20:1
10:1
5:1
2.5:1
1.25:1

Figure 9B

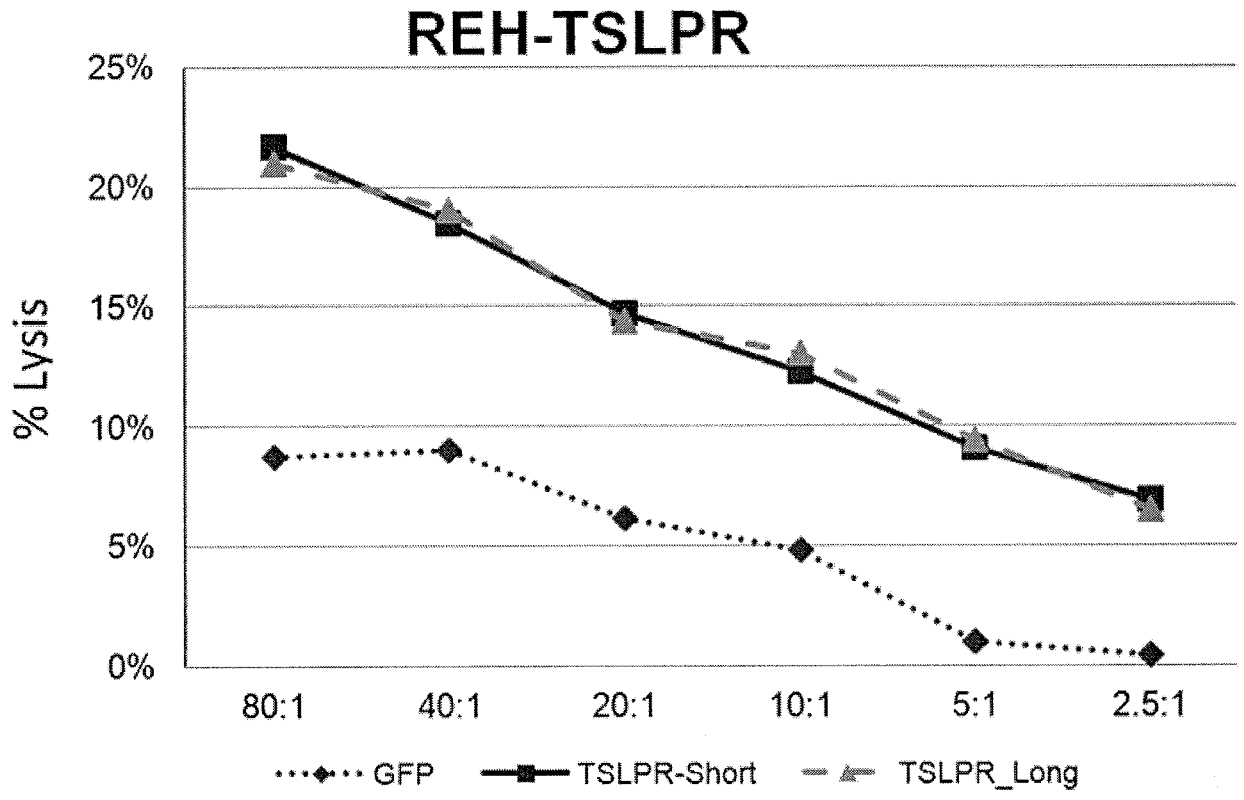

REH-TSLPR
25%
20%
15%
10%
5%
0%
% Lysis
80:1
40:1
20:1
10:1
5:1
2.5:1
GFP
TSLPR-Short
TSLPR_Long

Figure 9C

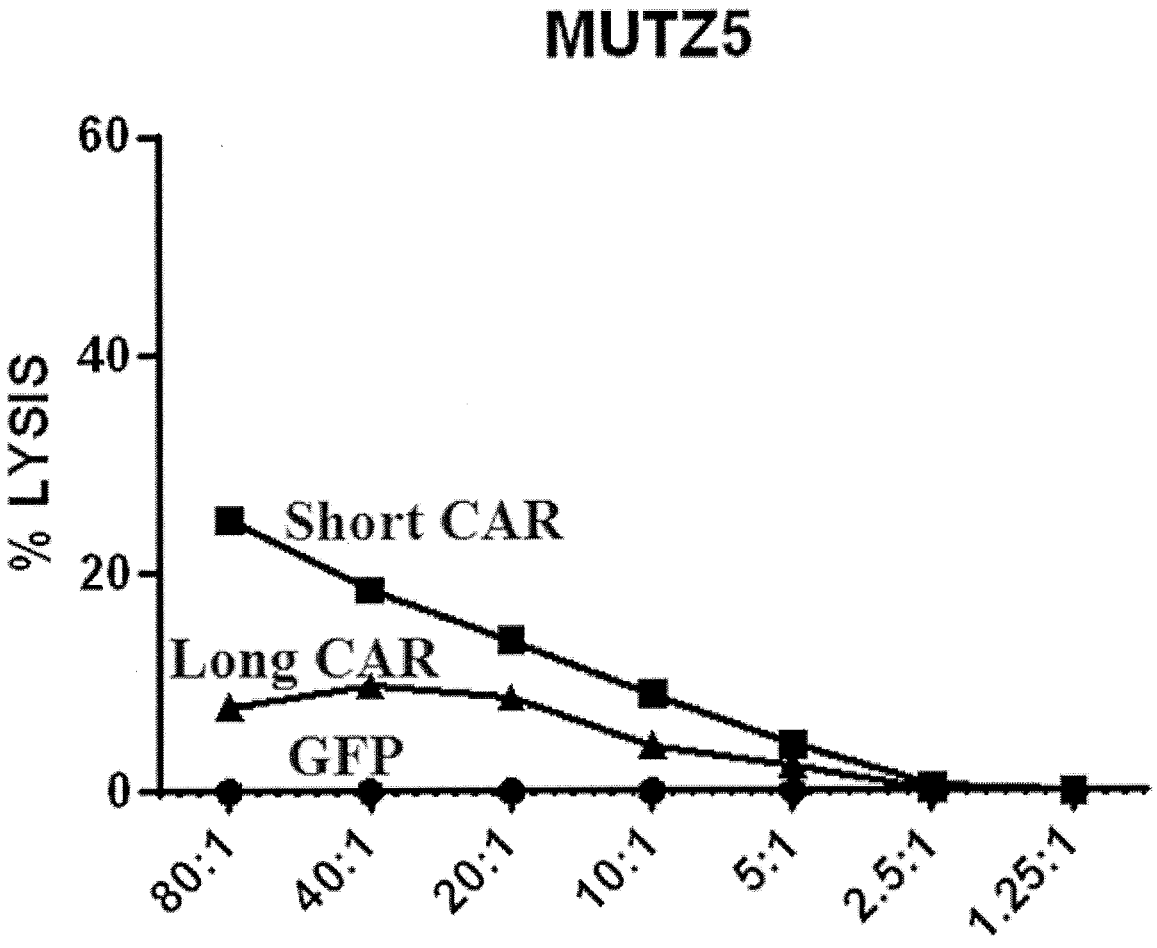

MUTZ5
% LYSIS
60
40
20
0
Short CAR
Long CAR
GFP
80:1
40:1
20:1
10:1
5:1
2.5:1
1.25:1

Figure 9D

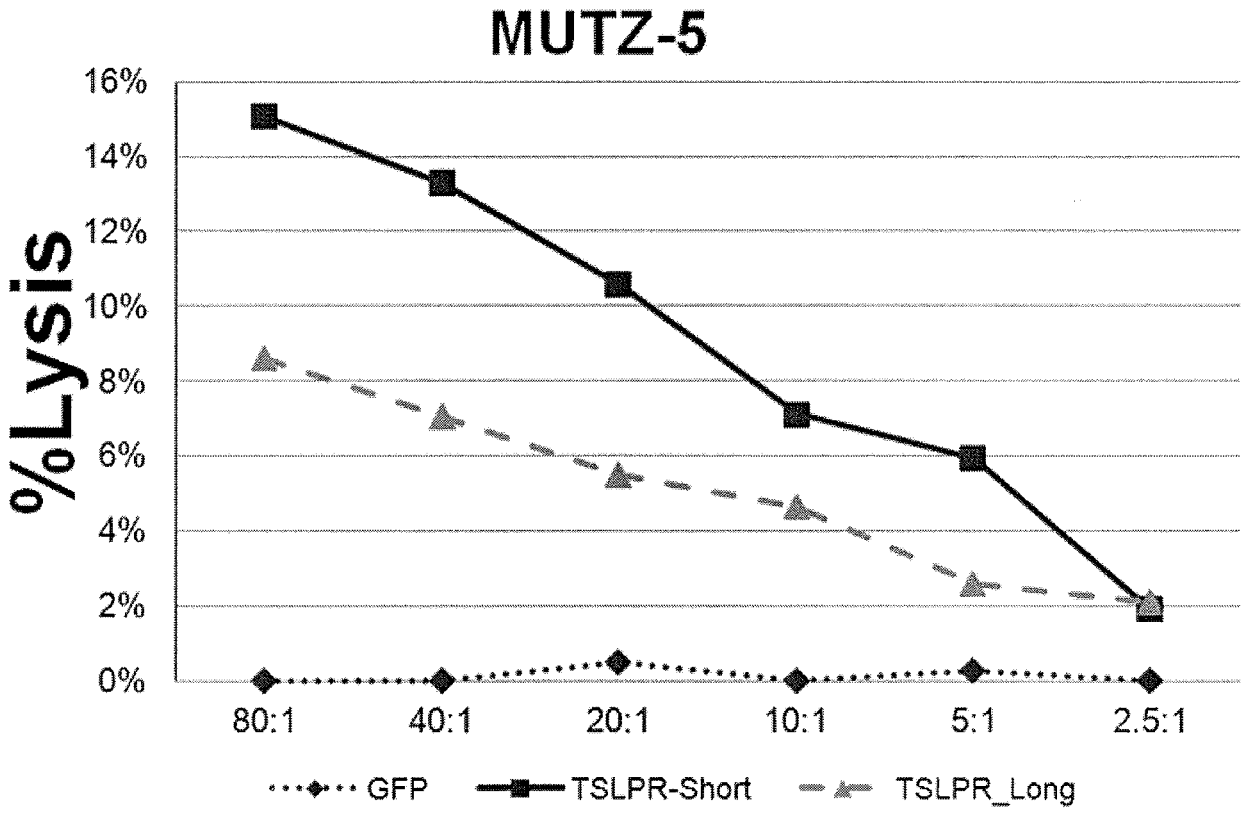

MUTZ-5
%Lysis
16%
14%
12%
10%
8%
6%
4%
2%
0%
80:1
40:1
20:1
10:1
5:1
2.5:1
GFP
TSLPR-Short
TSLPR_Long

Figure 10

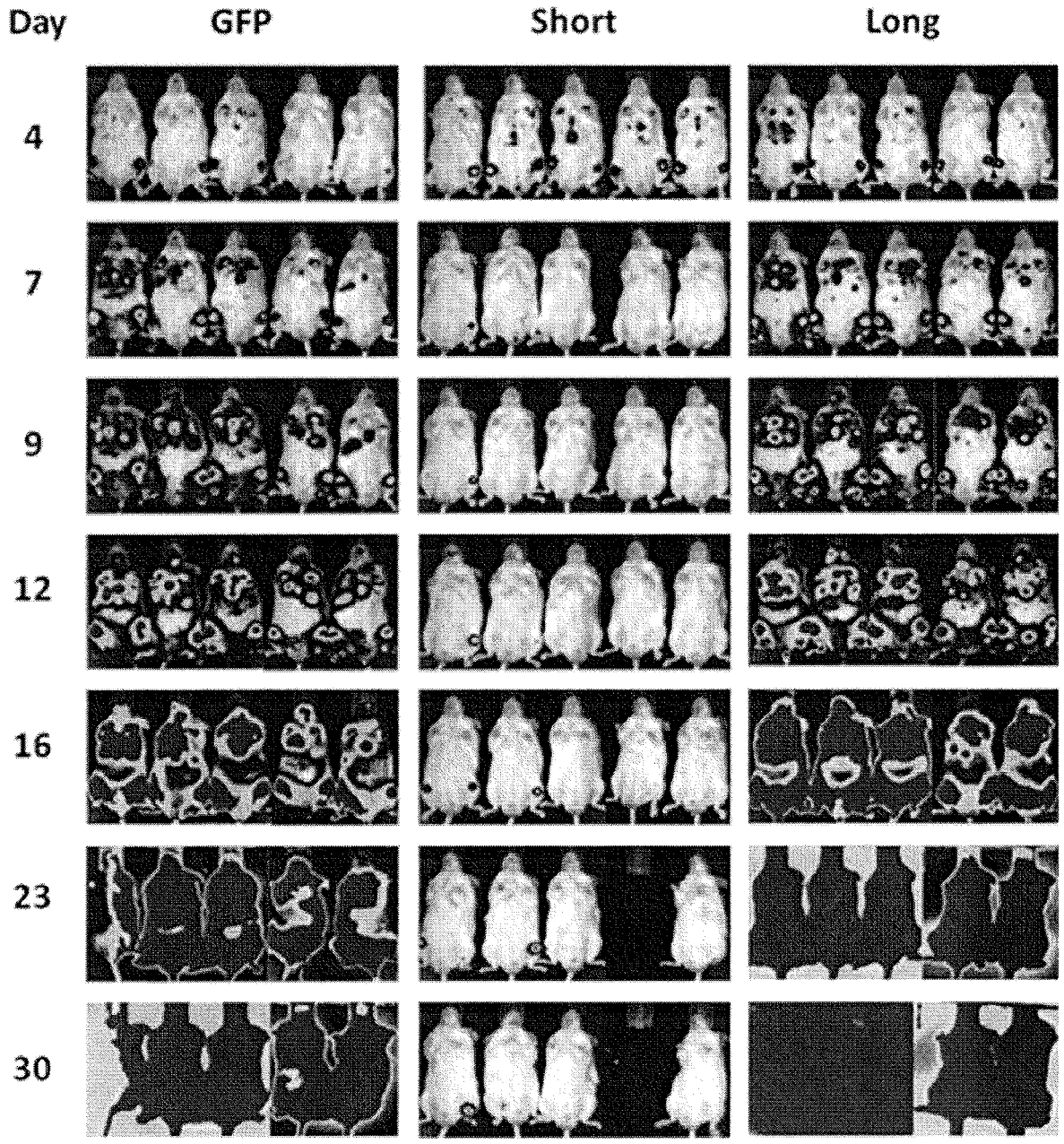


Figure 11

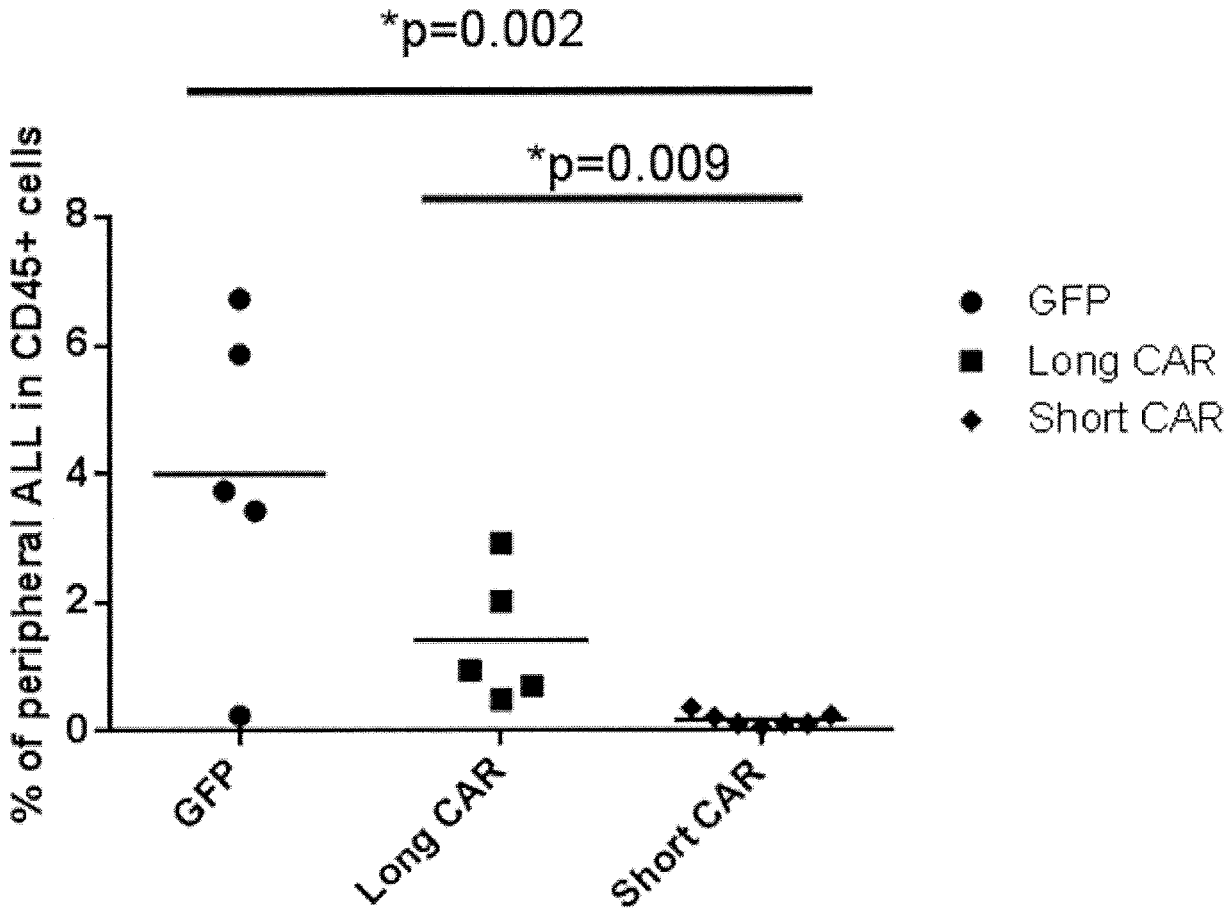

*p=0.002
*p=0.009
% of peripheral ALL in CD45+ cells
0
2
4
6
8
GFP
Long CAR
Short CAR
GFP
Long CAR
Short CAR

Figure 12A

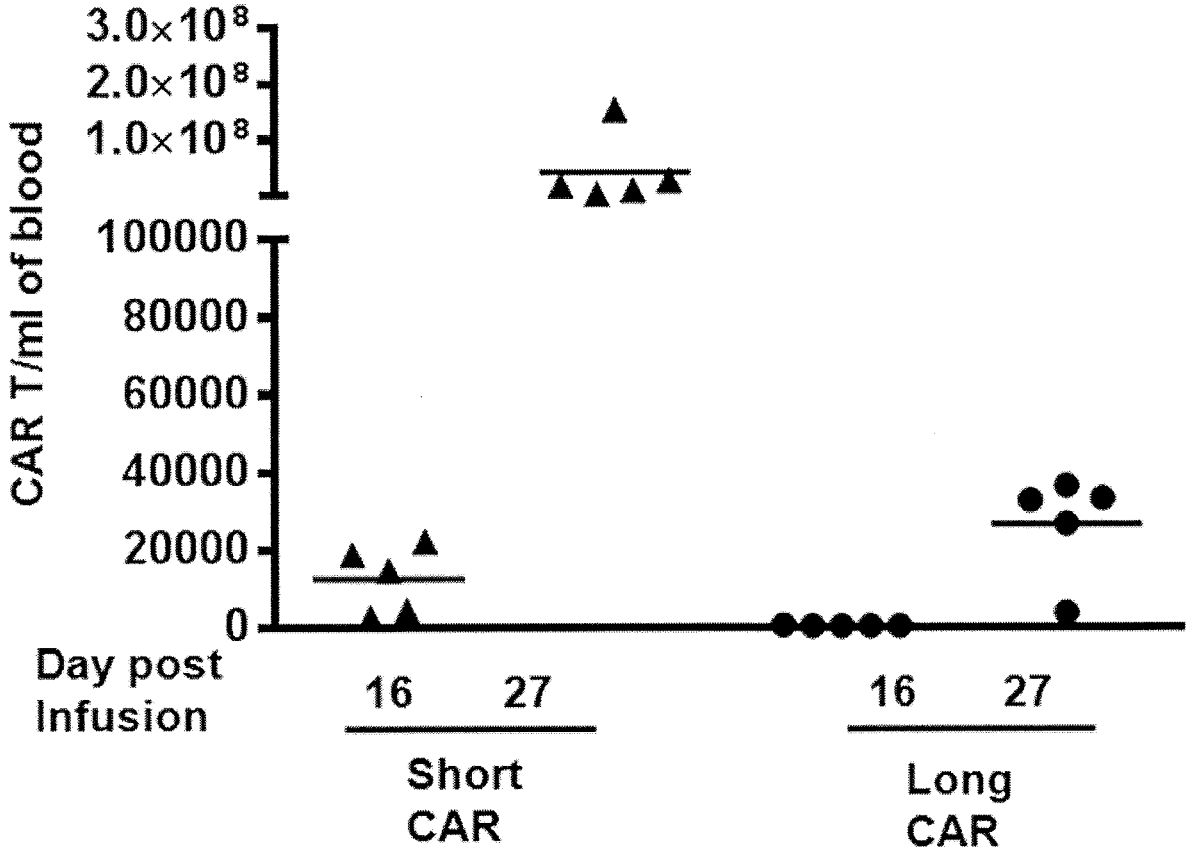

3.0×10⁸
2.0×10⁸
1.0×10⁸
100000
80000
60000
40000
20000
0
CAR T/ml of blood
Day post Infusion
16
27
16
27
Short CAR
Long CAR

Figure 12B

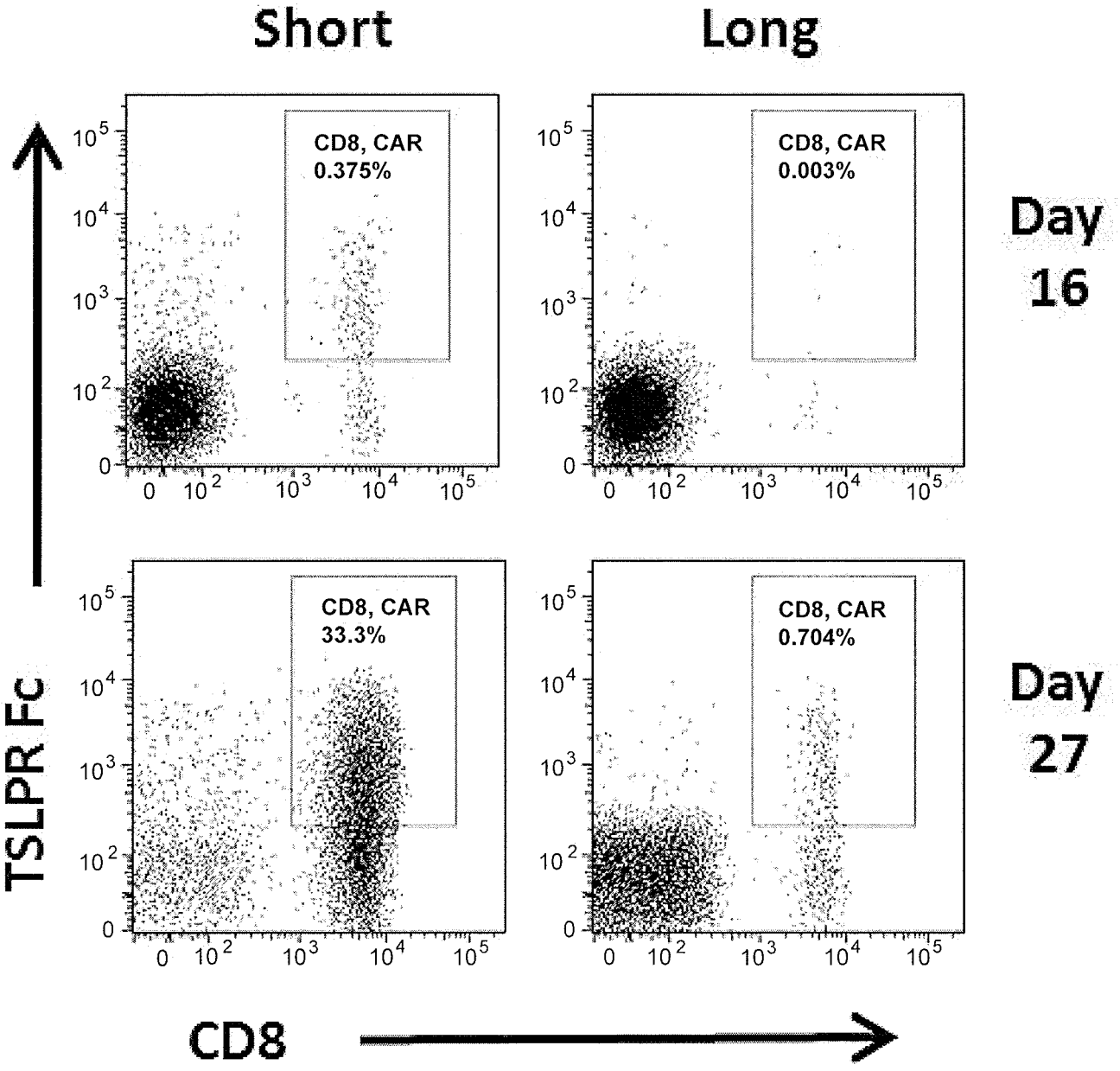

Short
Long
CD8, CAR
0.375%
CD8, CAR
0.003%
Day
16
CD8, CAR
33.3%
CD8, CAR
0.704%
Day
27
TSLPR Fc
CD8
0
10 2
10 3
10 4
10 5

Figure 13

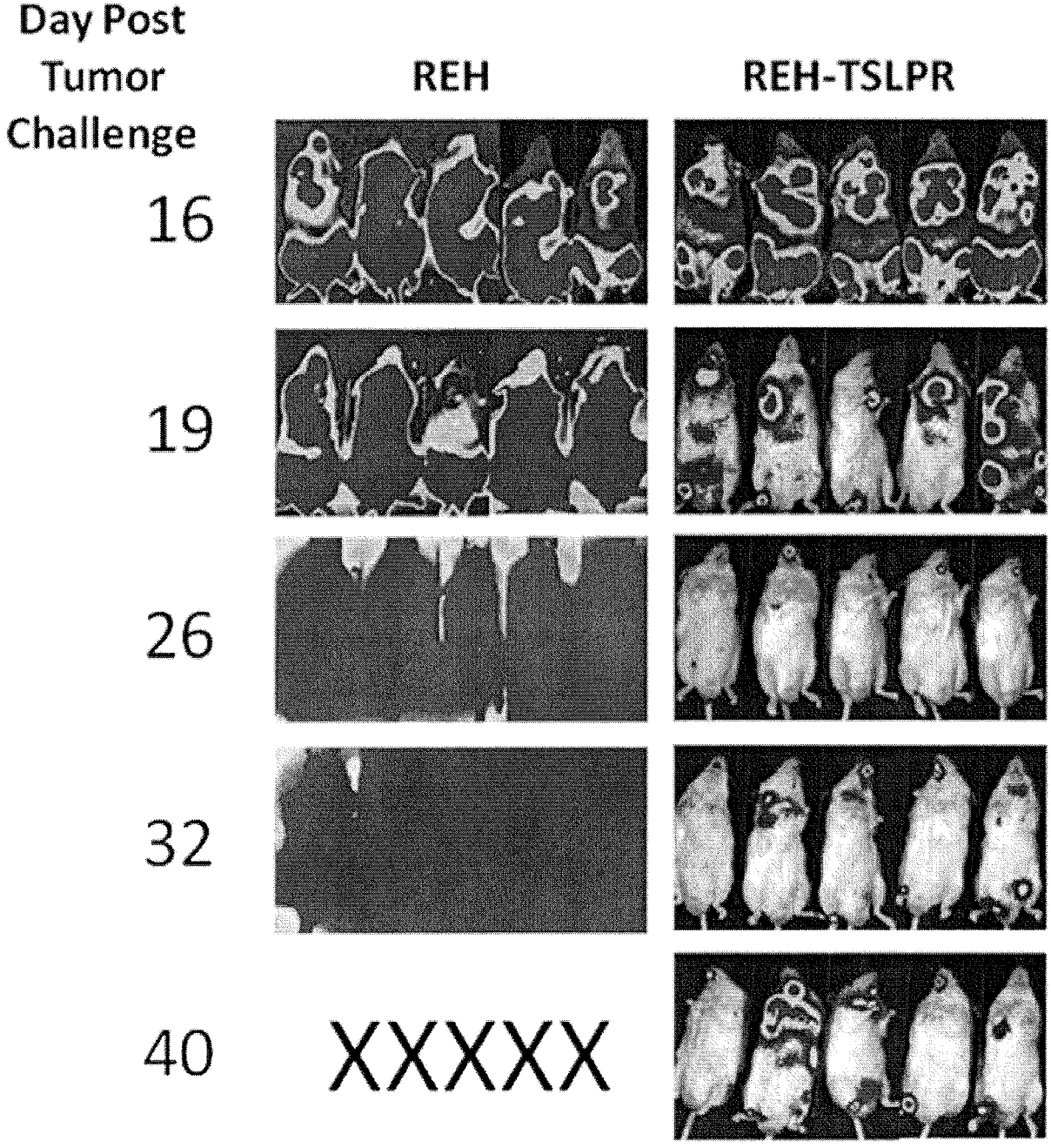


Figure 14

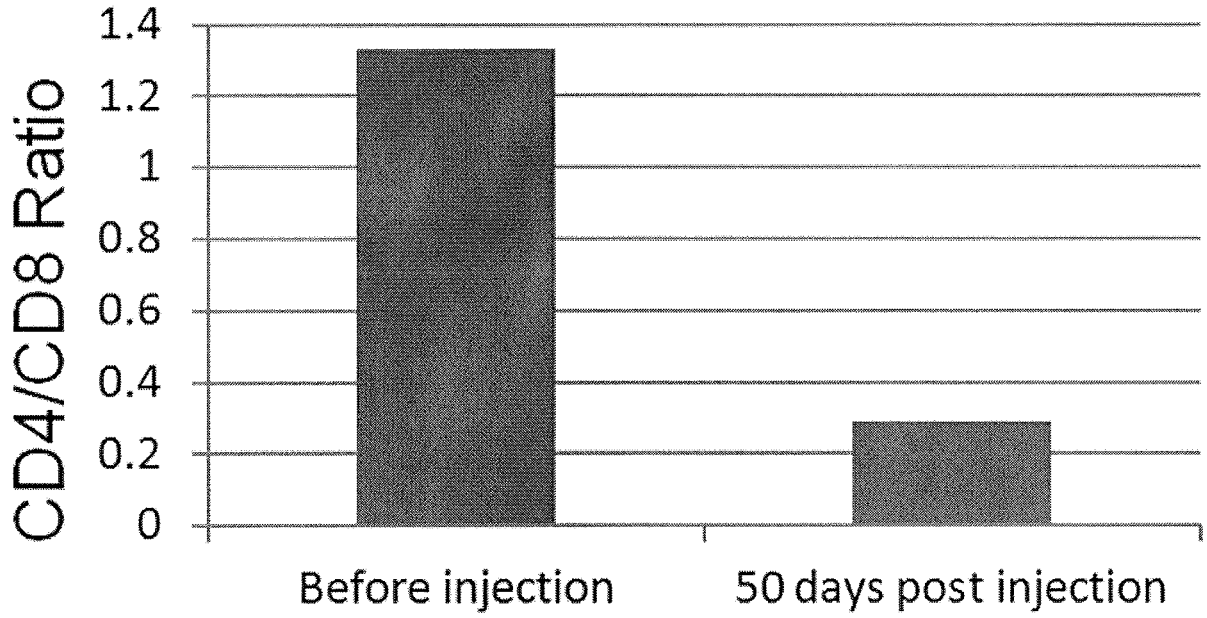

CD4/CD8 Ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Before injection
50 days post injection

Figure 15

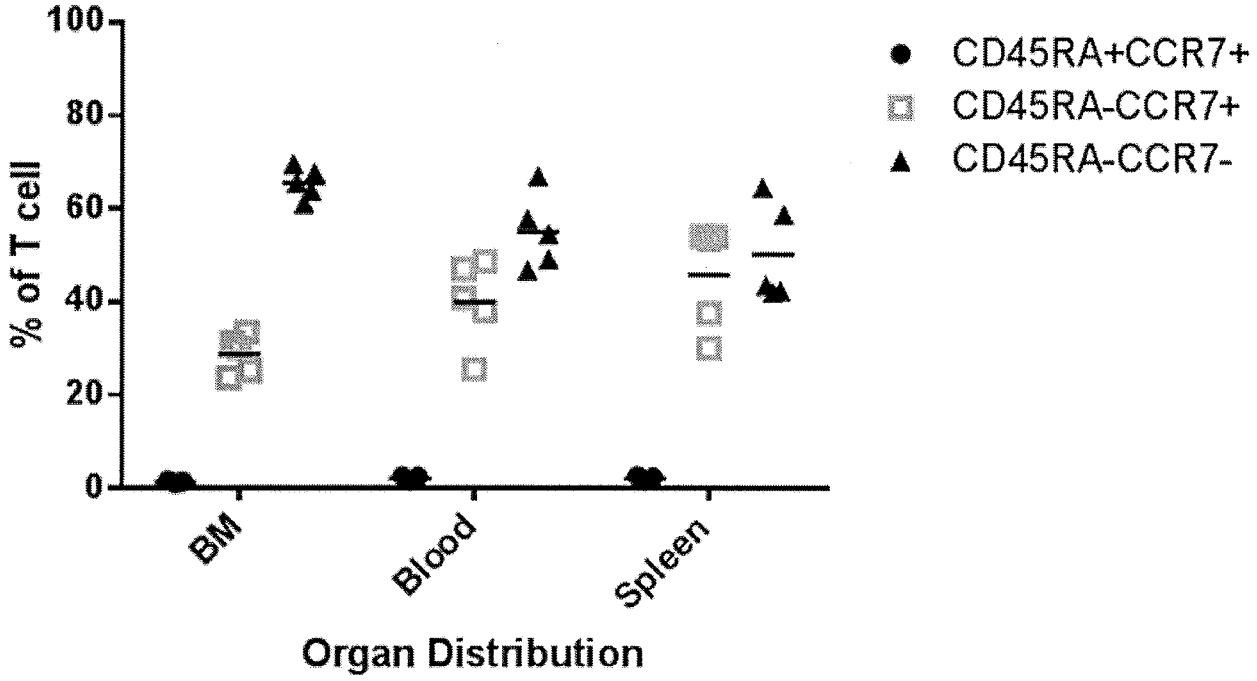

100
80
60
40
20
0
% of T cell
BM
Blood
Spleen
Organ Distribution
CD45RA+CCR7+
CD45RA-CCR7+
CD45RA-CCR7-

Figure 16A

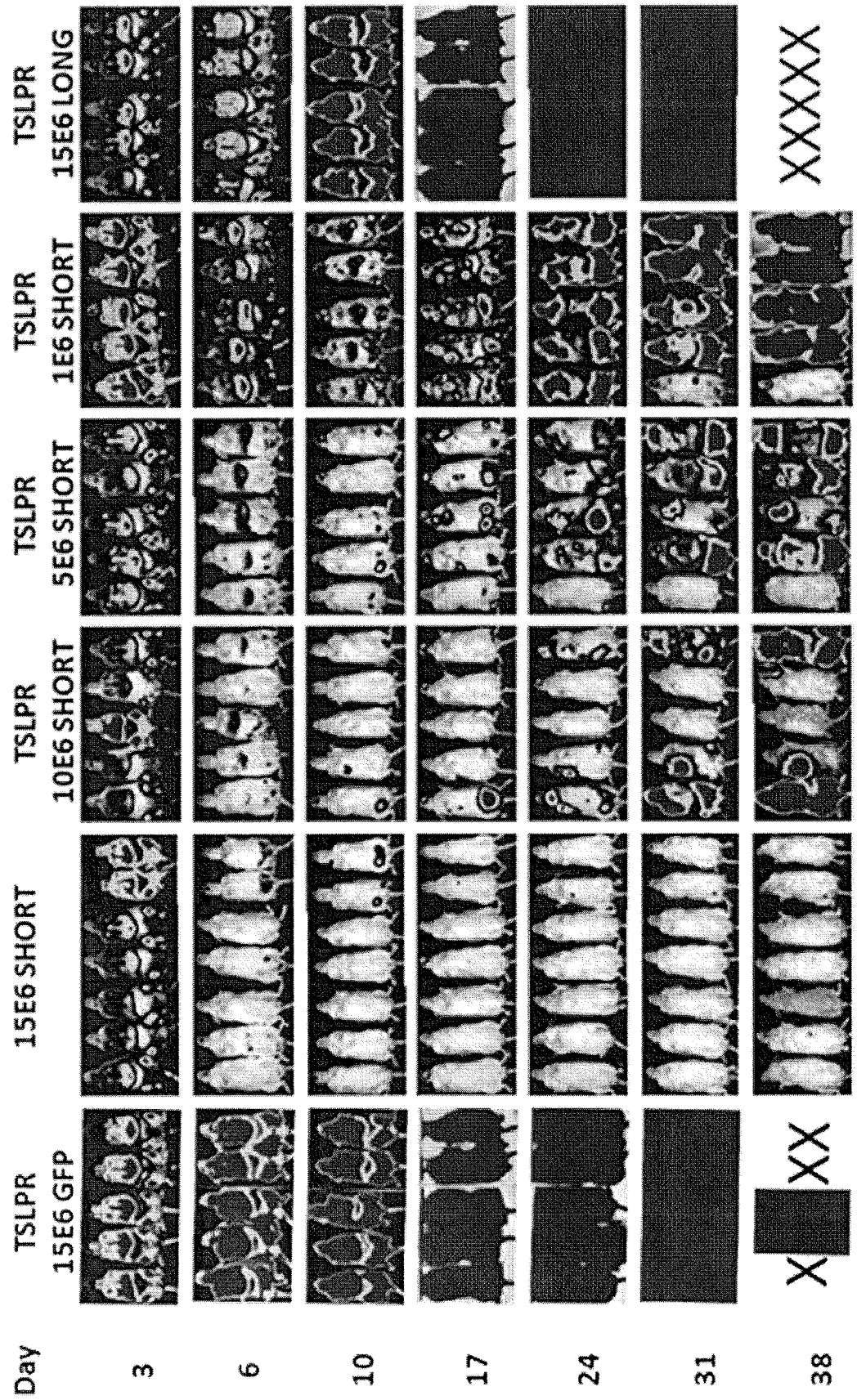

Day
3
6
10
17
24
31
38
5E6 REH-TSLPR 15E6 GFP
5E6 REH-TSLPR 15E6 SHORT
5E6 REH-TSLPR 10E6 SHORT
5E6 REH-TSLPR 5E6 SHORT
5E6 REH-TSLPR 1E6 SHORT
5E6 REH-TSLPR 15E6 LONG
X
XX
XXXXX

Figure 16B

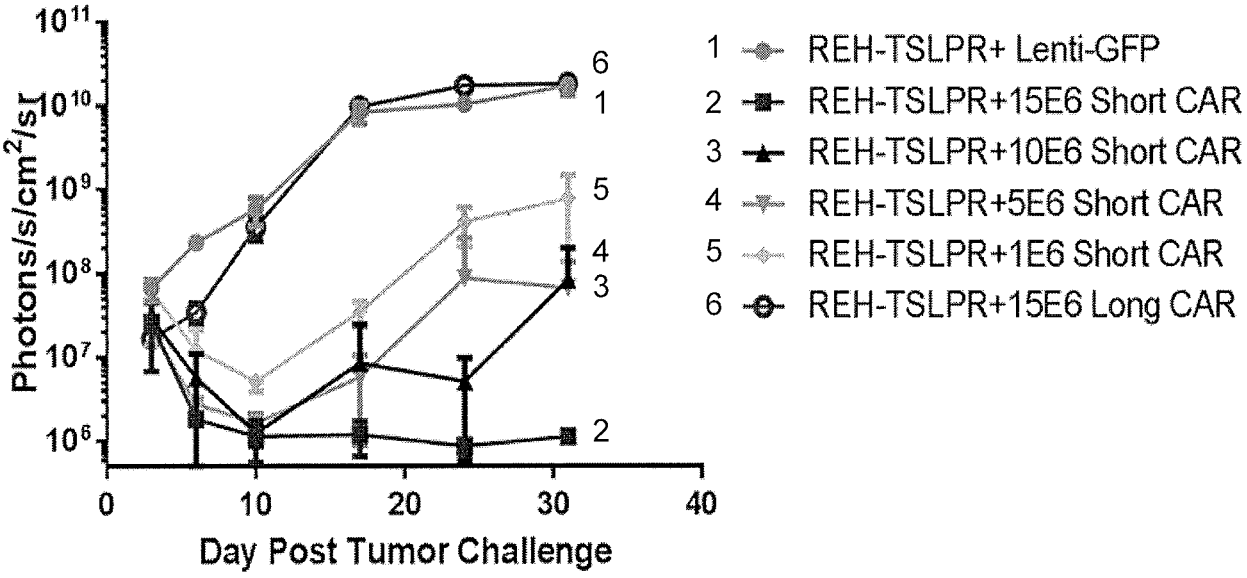

Photons/s/cm²/sr
10¹¹
10¹⁰
10⁹
10⁸
10⁷
10⁶
0
10
20
30
40
Day Post Tumor Challenge
6
1
5
4
3
2
1 REH-TSLPR+ Lenti-GFP
2 REH-TSLPR+15E6 Short CAR
3 REH-TSLPR+10E6 Short CAR
4 REH-TSLPR+5E6 Short CAR
5 REH-TSLPR+1E6 Short CAR
6 REH-TSLPR+15E6 Long CAR

Figure 16C

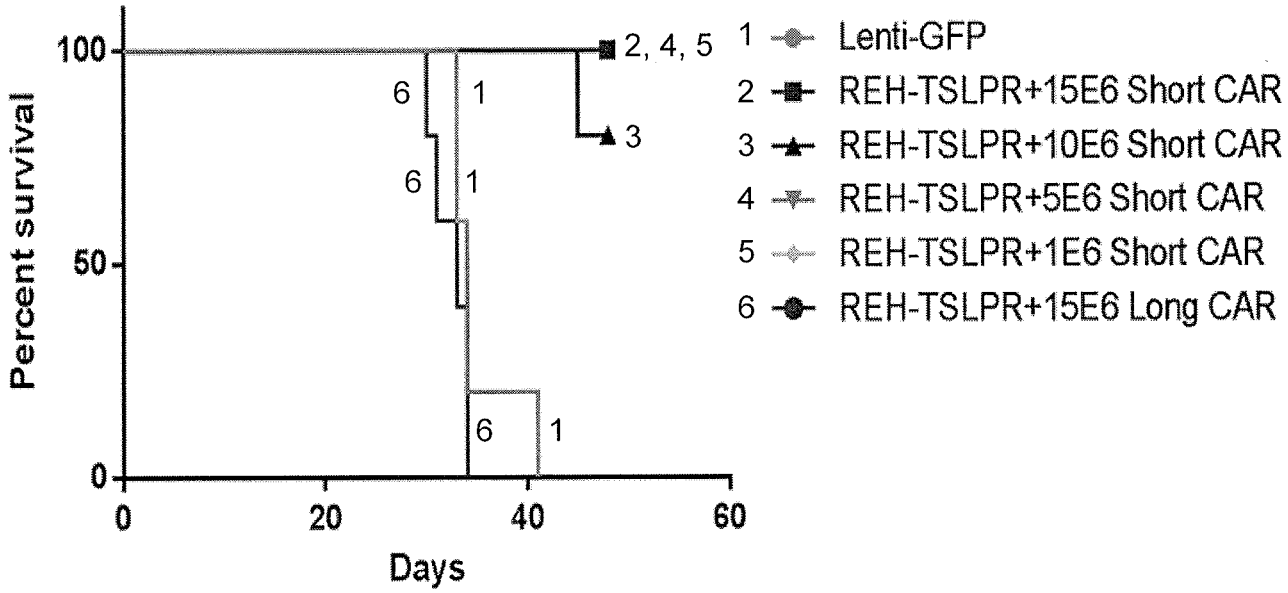

Percent survival
100
50
0
0
20
40
60
Days
2, 4, 5
3
6
1
6
1
6
1
1 Lenti-GFP
2 REH-TSLPR+15E6 Short CAR
3 REH-TSLPR+10E6 Short CAR
4 REH-TSLPR+5E6 Short CAR
5 REH-TSLPR+1E6 Short CAR
6 REH-TSLPR+15E6 Long CAR

Figure 17

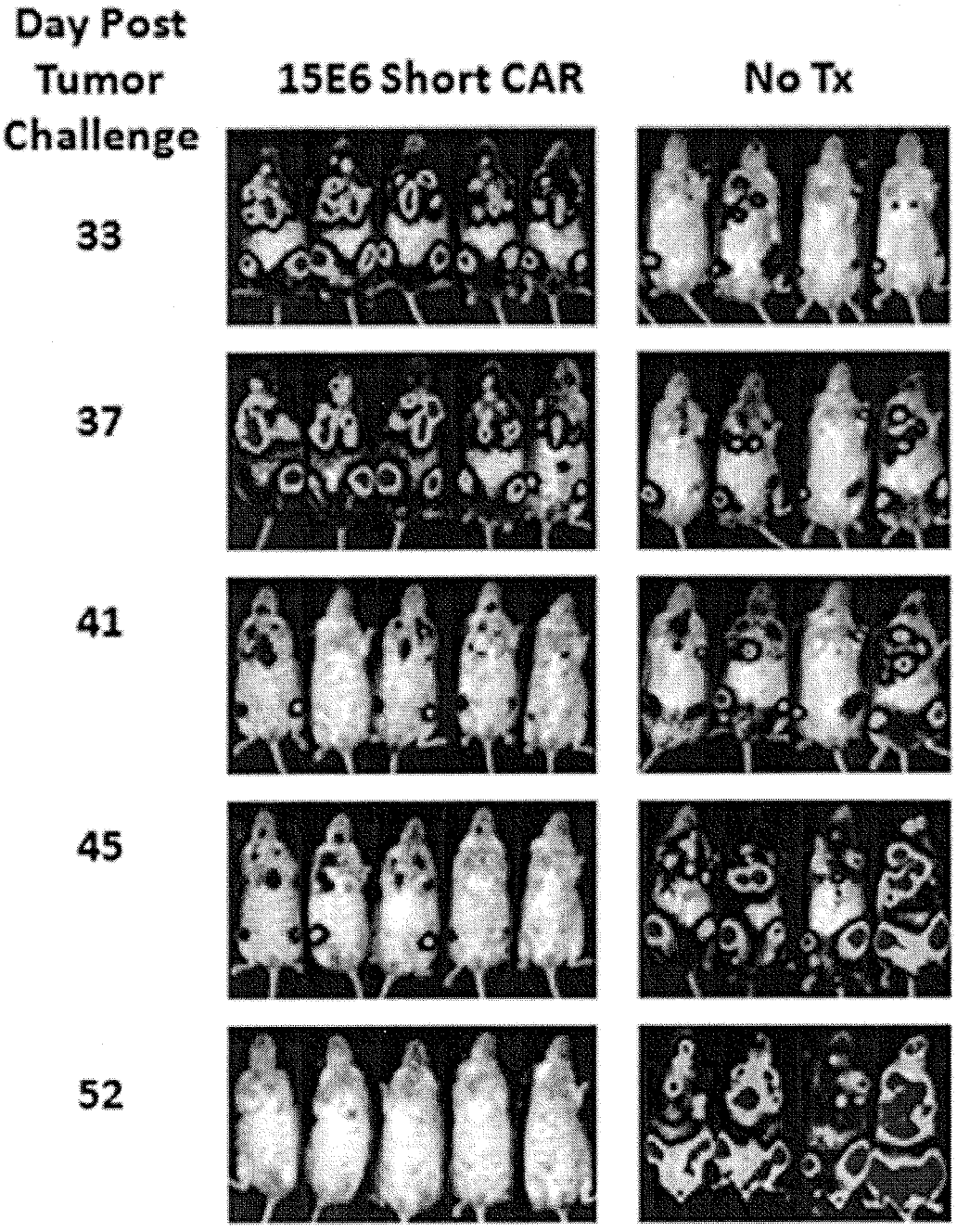


Figure 18A

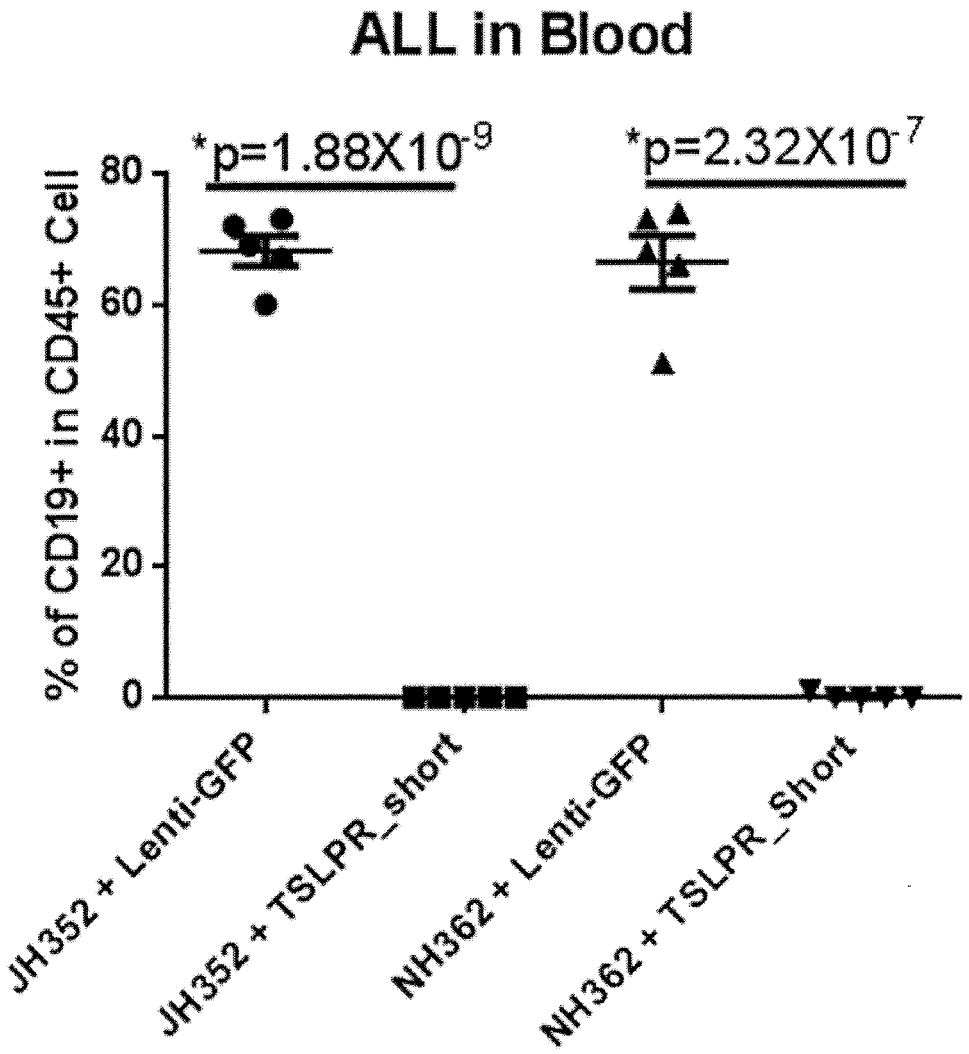

ALL in Blood
*p=1.88X10-9
*p=2.32X10-7
% of CD19+ in CD45+ Cell
80
60
40
20
0
JH352 + Lenti-GFP
JH352 + TSLPR_short
NH362 + Lenti-GFP
NH362 + TSLPR_Short

Figure 18B

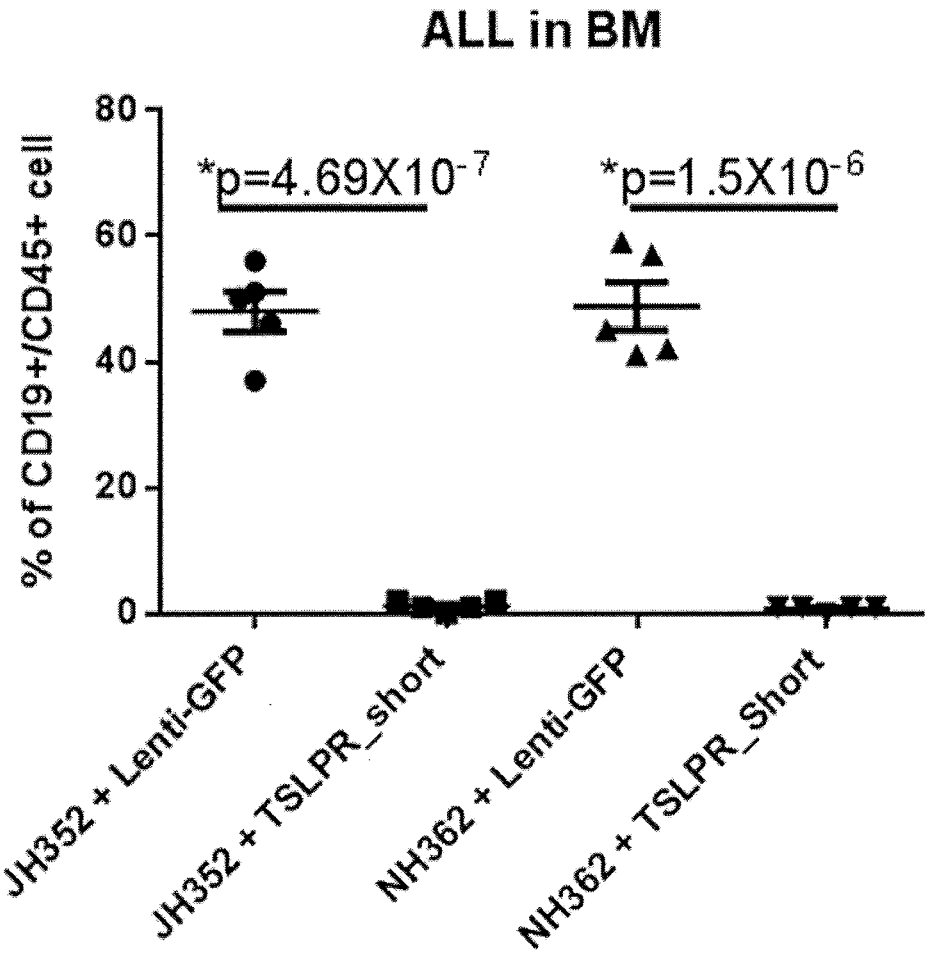

ALL in BM
% of CD19+/CD45+ cell
80
60
40
20
0
*p=4.69X10-7
*p=1.5X10-6
JH352 + Lenti-GFP
JH352 + TSLPR_short
NH362 + Lenti-GFP
NH362 + TSLPR_Short

Figure 19

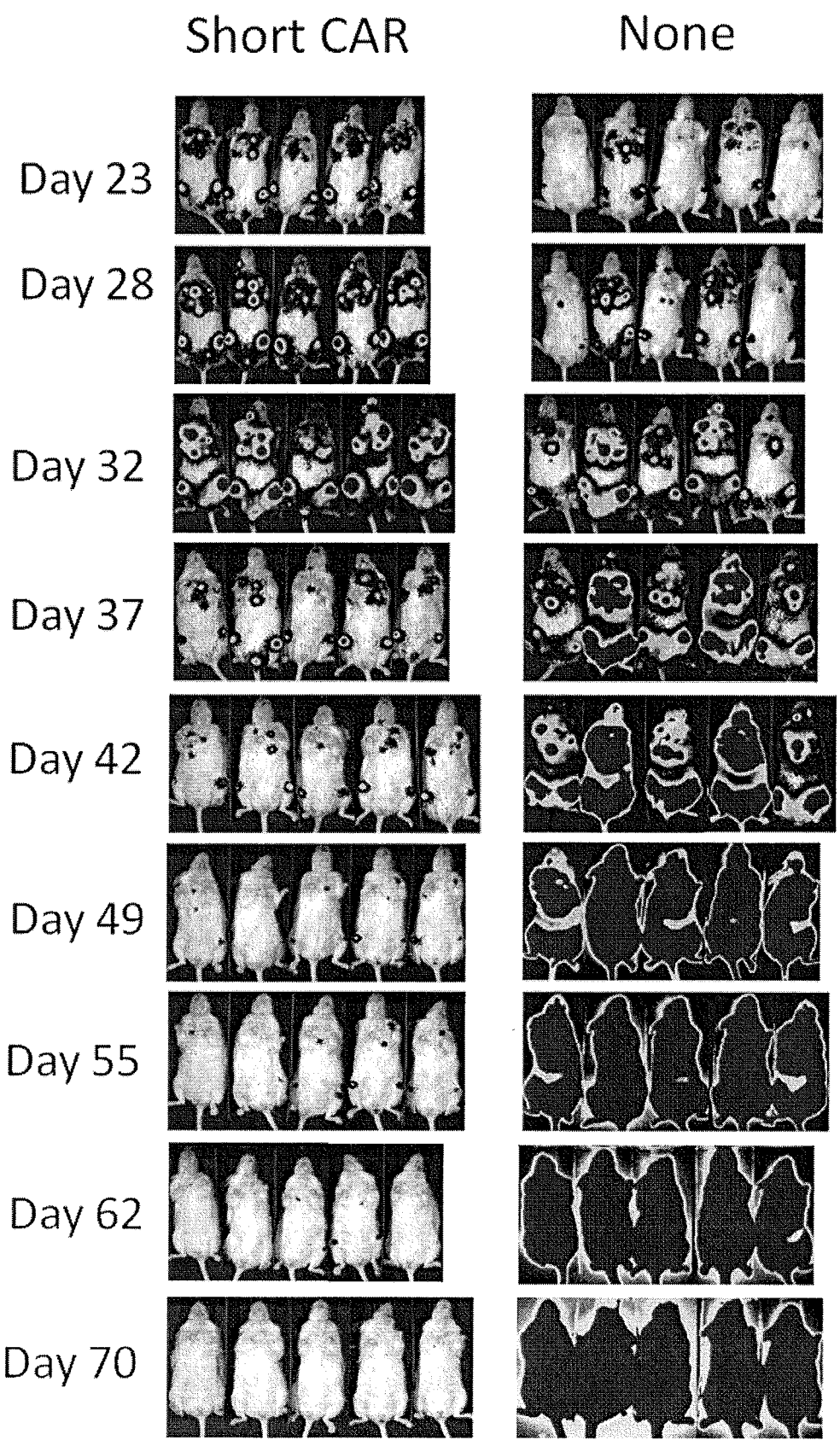

Short CAR
None
Day 23
Day 28
Day 32
Day 37
Day 42
Day 49
Day 55
Day 62
Day 70

Figure 20

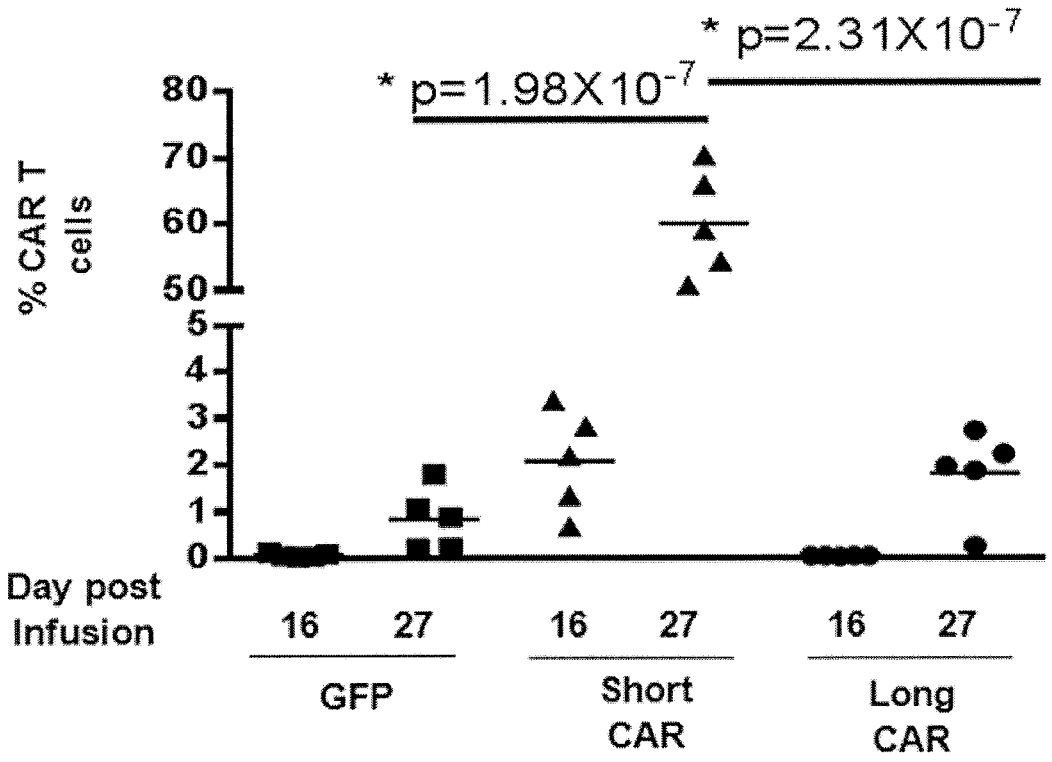

* p=2.31X10-7
* p=1.98X10-7
% CAR T cells
80
70
60
50
5
4
3
2
1
0
Day post Infusion
16
27
16
27
16
27
GFP
Short CAR
Long CAR

Figure 21

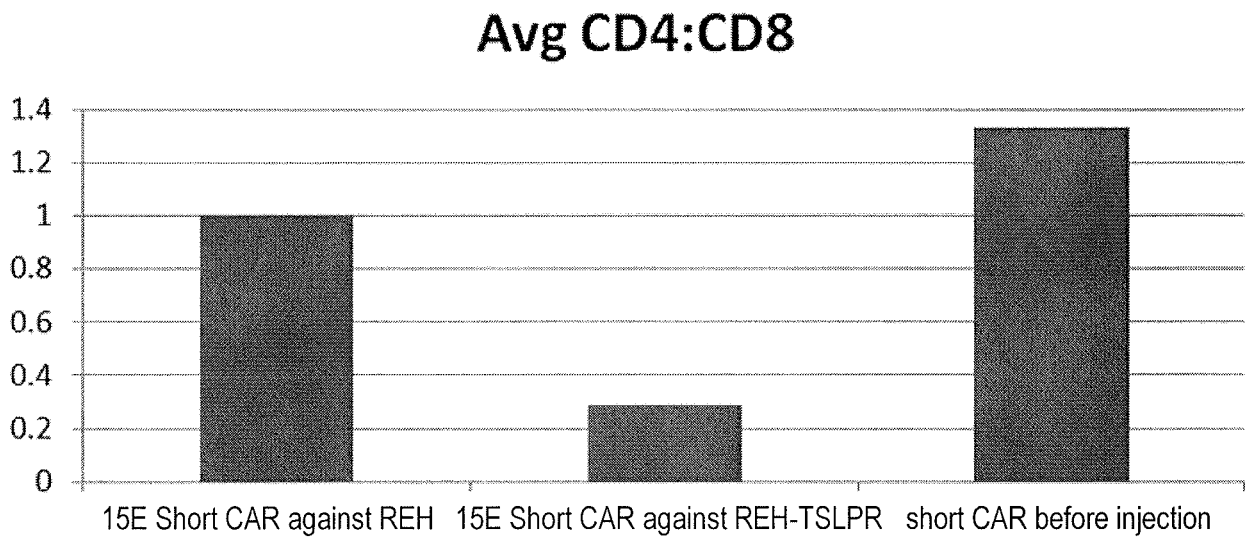

Avg CD4:CD8
1.4
1.2
1
0.8
0.6
0.4
0.2
0
15E Short CAR against REH
15E Short CAR against REH-TSLPR
short CAR before injection

Figure 22

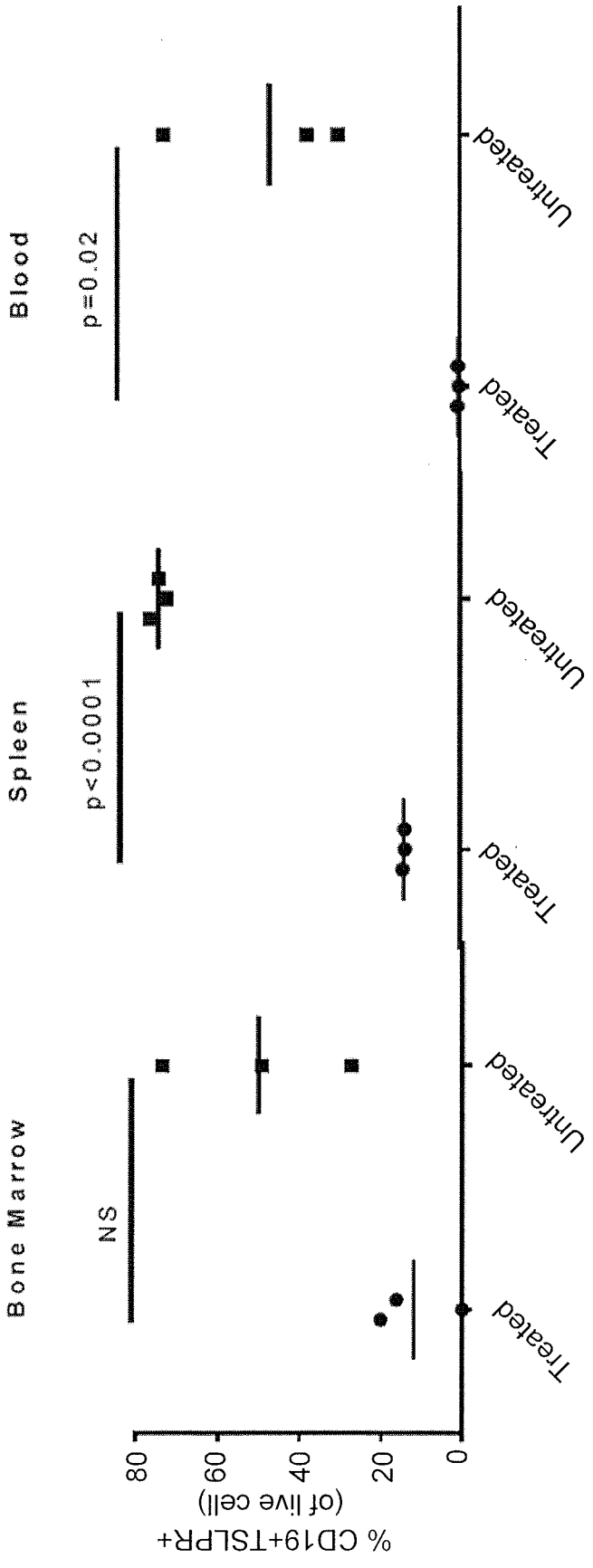

% CD19+TSLPR+
(of live cell)
0
20
40
60
80
Bone Marrow
NS
Spleen
p<0.0001
Blood
p=0.02
Untreated
Treated
Untreated
Treated
Untreated
Treated

Figure 23

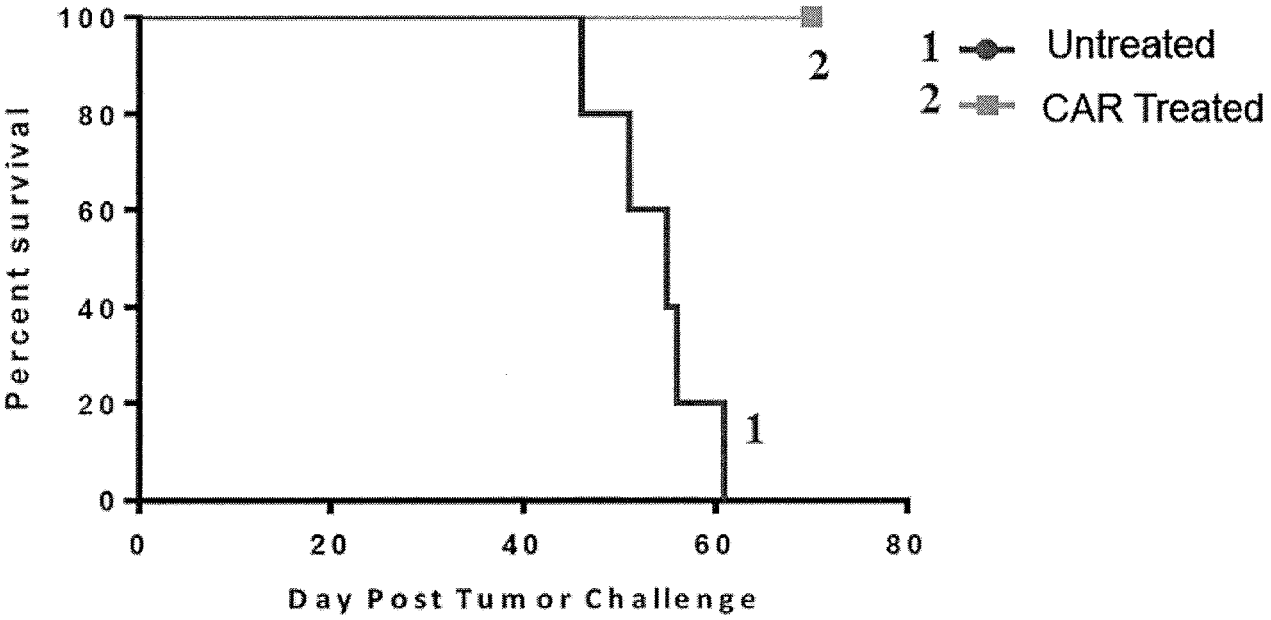

100
80
60
40
20
0
Percent survival
0
20
40
60
80
Day Post Tumor Challenge
1
2
1 Untreated
2 CAR Treated

Figure 24

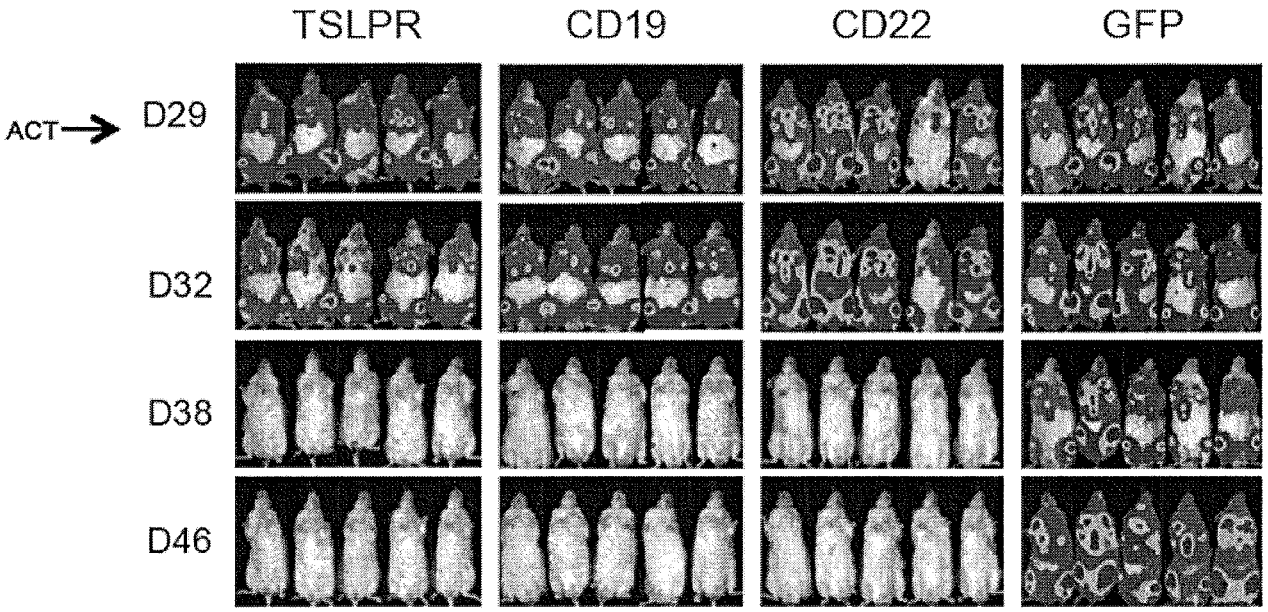


Figure 25A

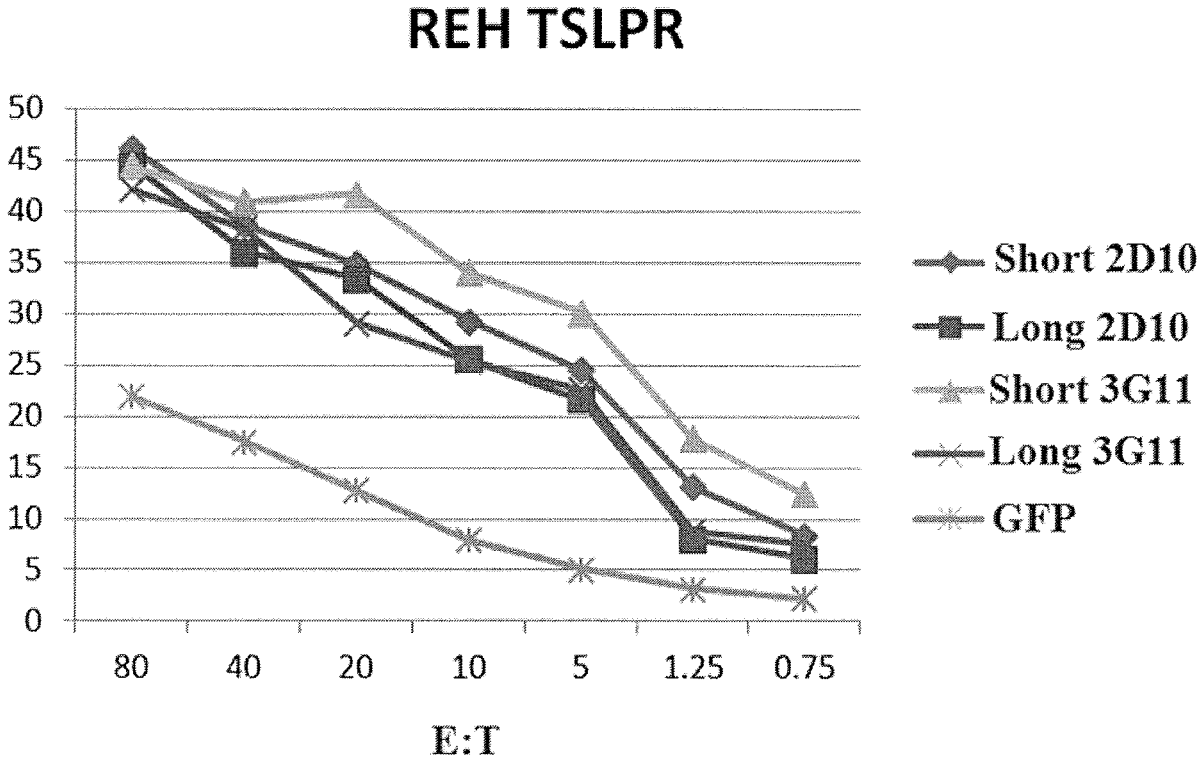

REH TSLPR
50
45
40
35
30
25
20
15
10
5
0
80
40
20
10
5
1.25
0.75
E:T
Short 2D10
Long 2D10
Short 3G11
Long 3G11
GFP

Figure 25B

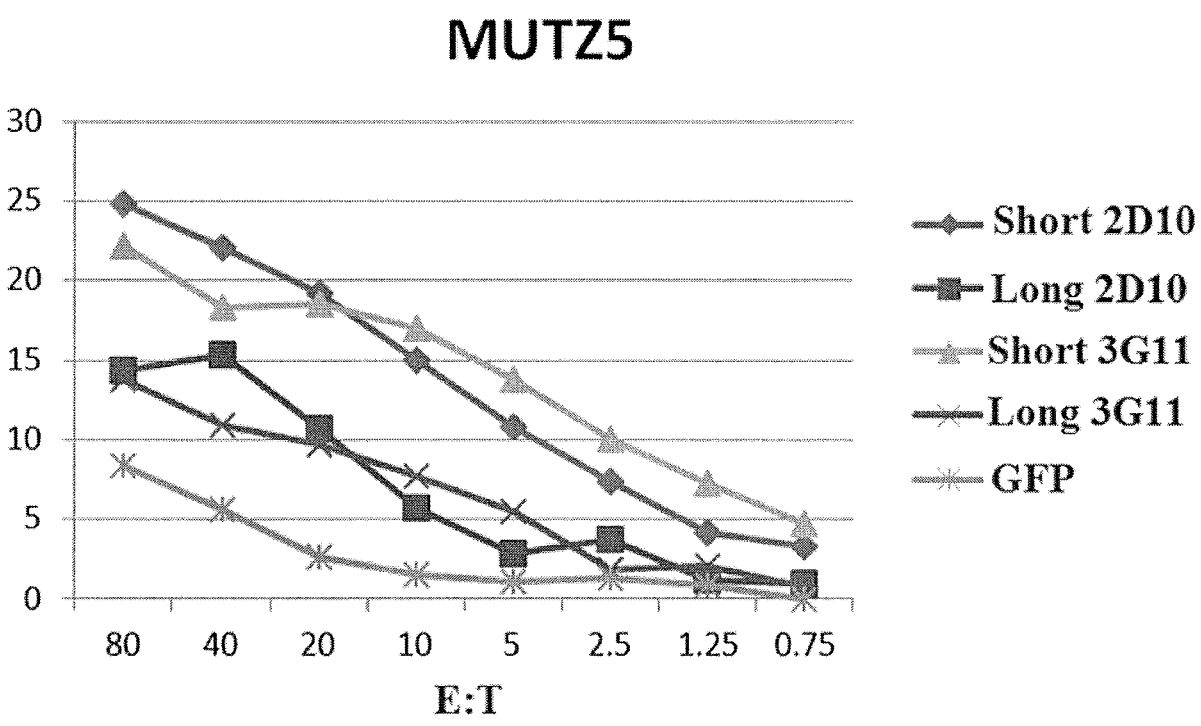

MUTZ5
30
25
20
15
10
5
0
80
40
20
10
5
2.5
1.25
0.75
E:T
Short 2D10
Long 2D10
Short 3G11
Long 3G11
GFP

Figure 25C

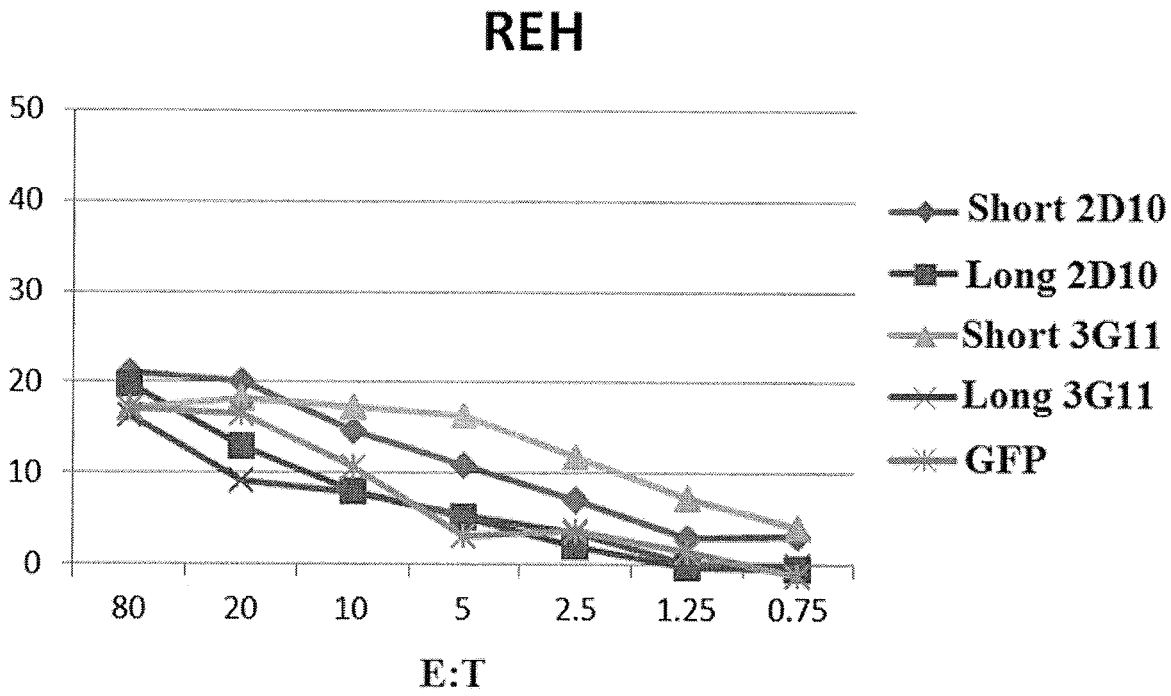
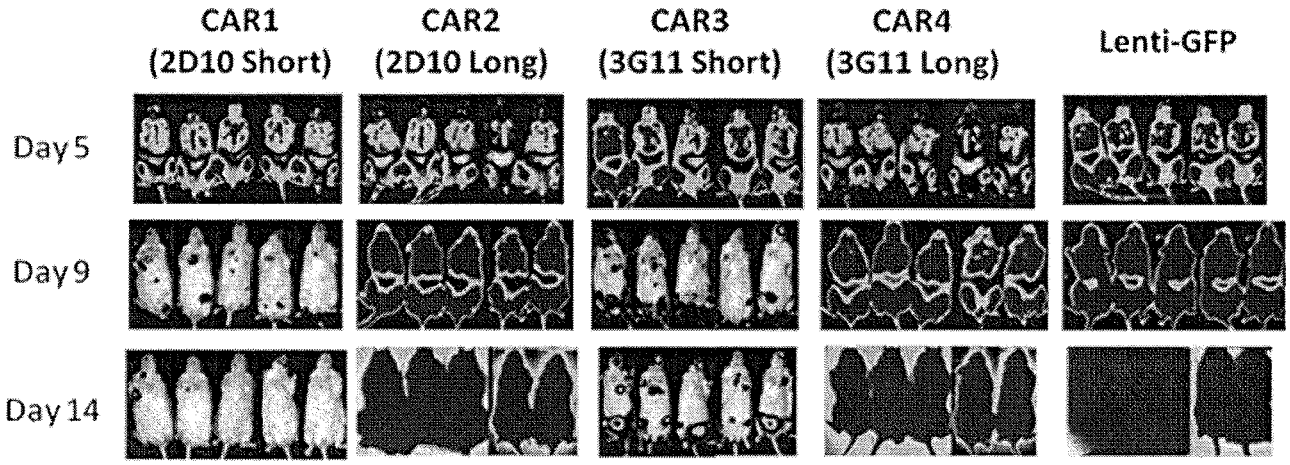

REH
50
40
30
20
10
0
80
20
10
5
2.5
1.25
0.75
E:T
Short 2D10
Long 2D10
Short 3G11
Long 3G11
GFP

Figure 26


CAR1
(2D10 Short)
CAR2
(2D10 Long)
CAR3
(3G11 Short)
CAR4
(3G11 Long)
Lenti-GFP
Day 5
Day 9
Day 14