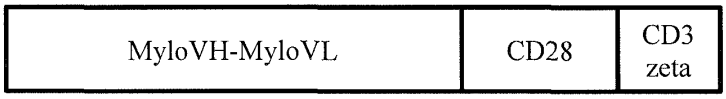
Figure 1A

CD33Mylo-BBZ CAR (CD33.1-BBz CAR)

CD33Mylo-CD28Z CAR (CD33.1-CD28z CAR)



CD33M195-BBZ CAR (CD33.3-BBz CAR)

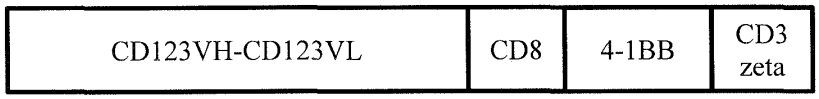
CD33M195-CD28Z CAR (CD33.3-CD28z CAR)

CD33Hu195-BBZ CAR (CD33.2-BBz CAR)

CD33Hu195-CD28Z CAR (CD33.2-CD28z CAR)

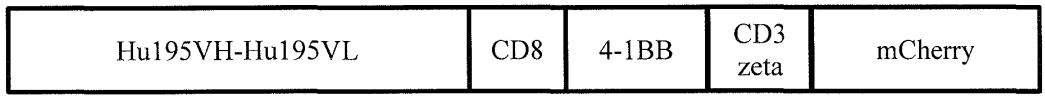
Figure 1B

CD123-BBZ CAR



CD123-CD28Z CAR

CD33.2-BBz-mCherry CAR



CD33.2-CD28z-mCherry CAR

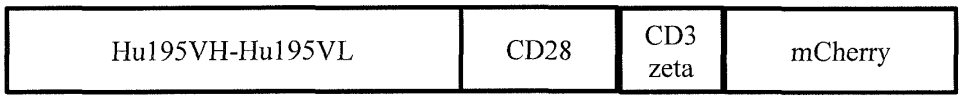
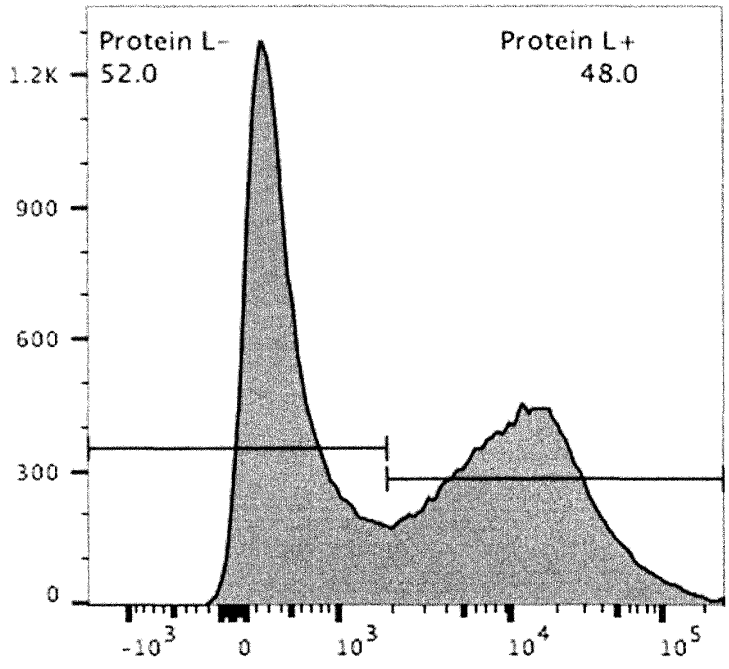
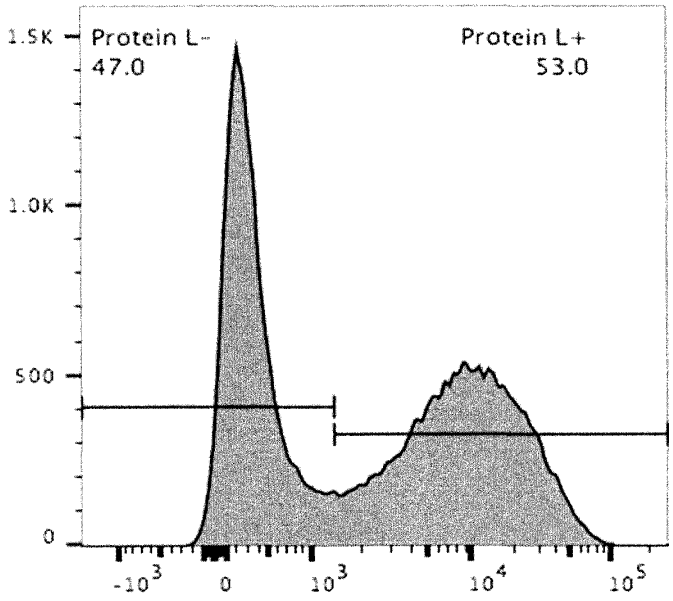


Figure 2A



CD33.1-BBz

Figure 2B



CD33.1-28z

Figure 2C

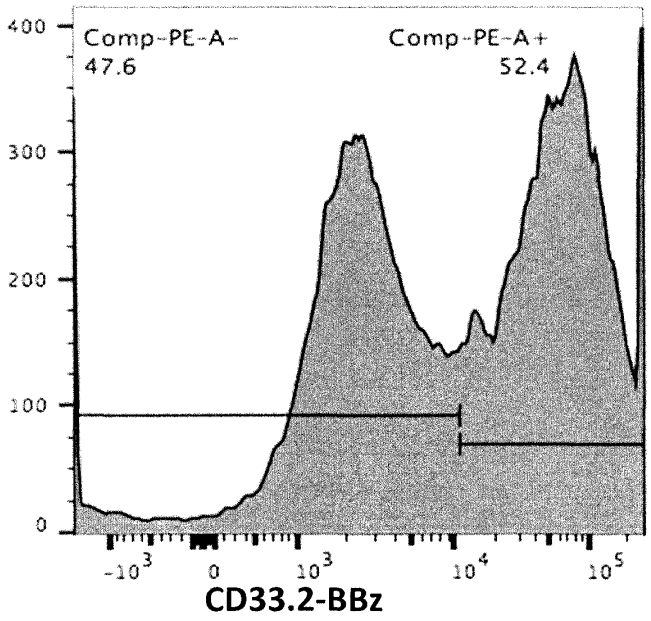

Comp-PE-A-
47.6
Comp-PE-A+
52.4
400
300
200
100
0
-10^3
0
10^3
10^4
10^5
CD33.2-BBz

Figure 2D

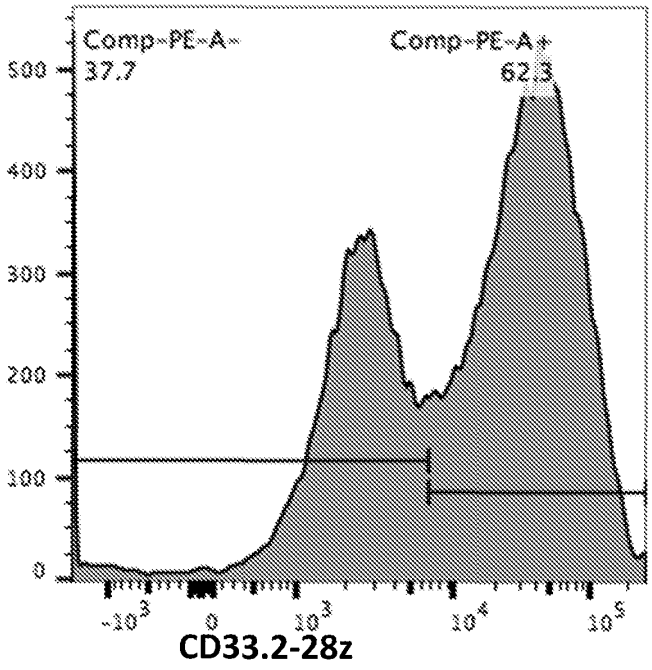

Comp-PE-A-
37.7
Comp-PE-A+
62.3
500
400
300
200
100
0
-10^3
0
10^3
10^4
10^5
CD33.2-28z

Figure 2E

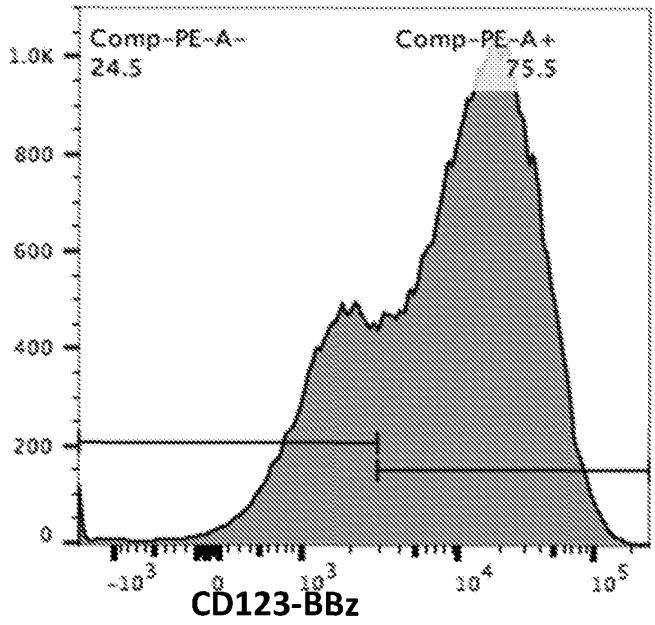

Comp-PE-A-
24.5
Comp-PE-A+
75.5
1.0K
800
600
400
200
0
-10^3
0
10^3
10^4
10^5
CD123-BBz

Figure 2F

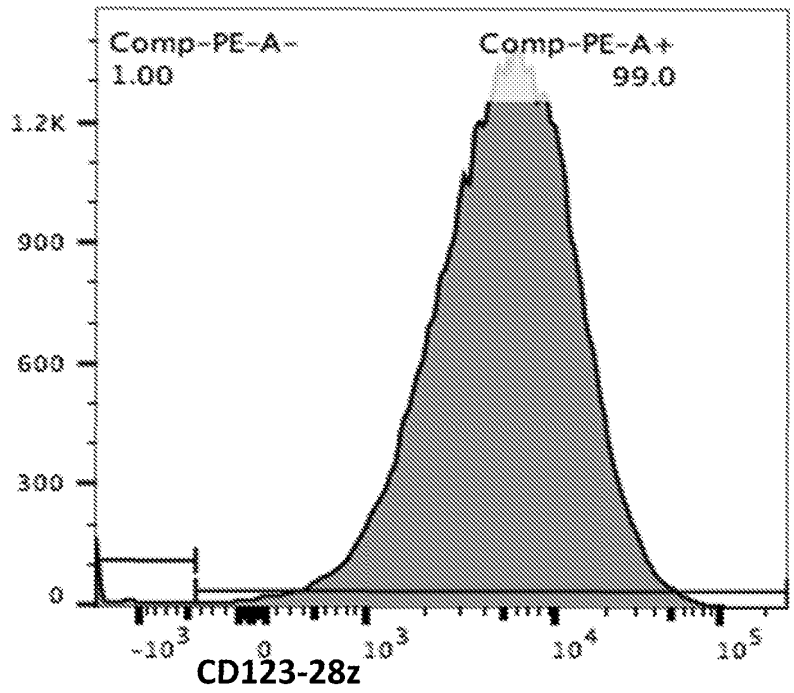

Comp-PE-A-
1.00
Comp-PE-A+
99.0
1.2K
900
600
300
0
-10^3
0
10^3
10^4
10^5
CD123-28z

Figure 3A

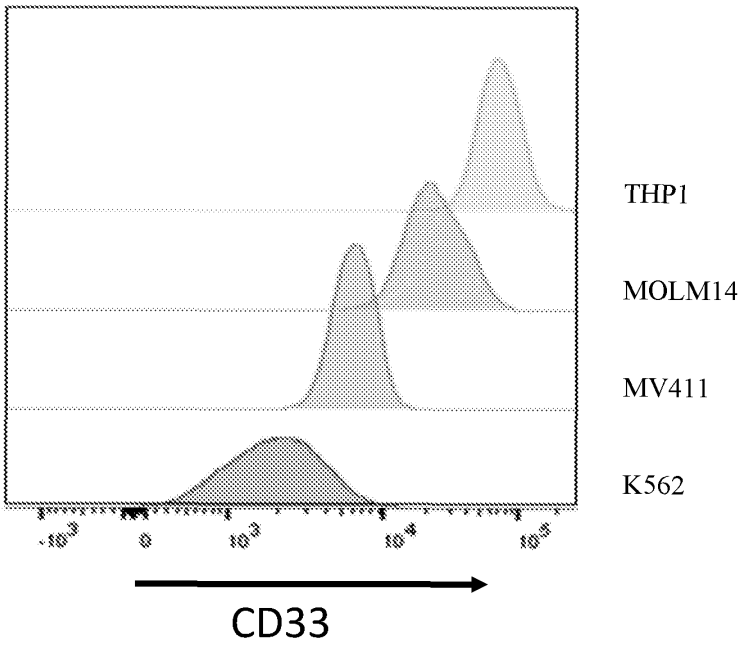


Figure 3B

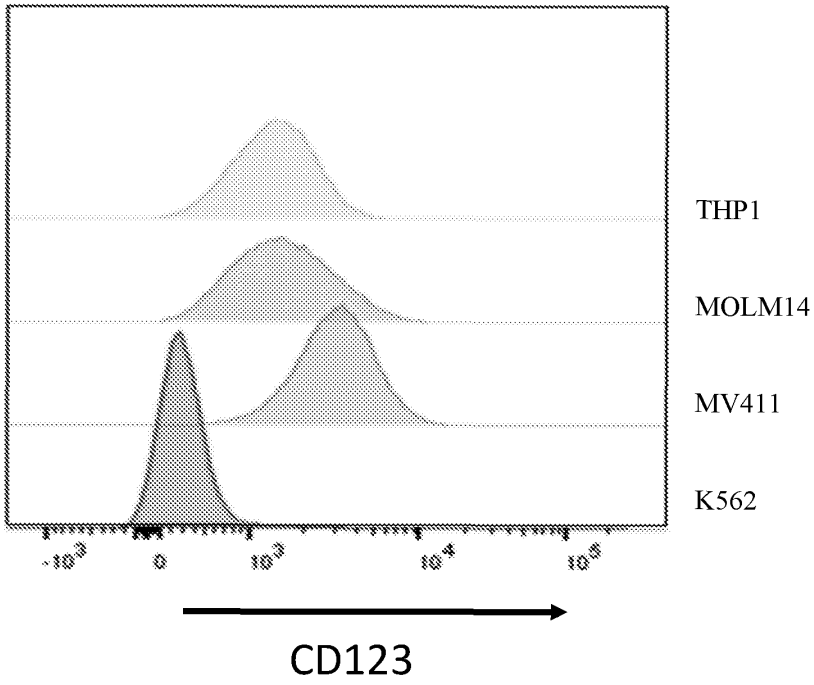


Figure 4A

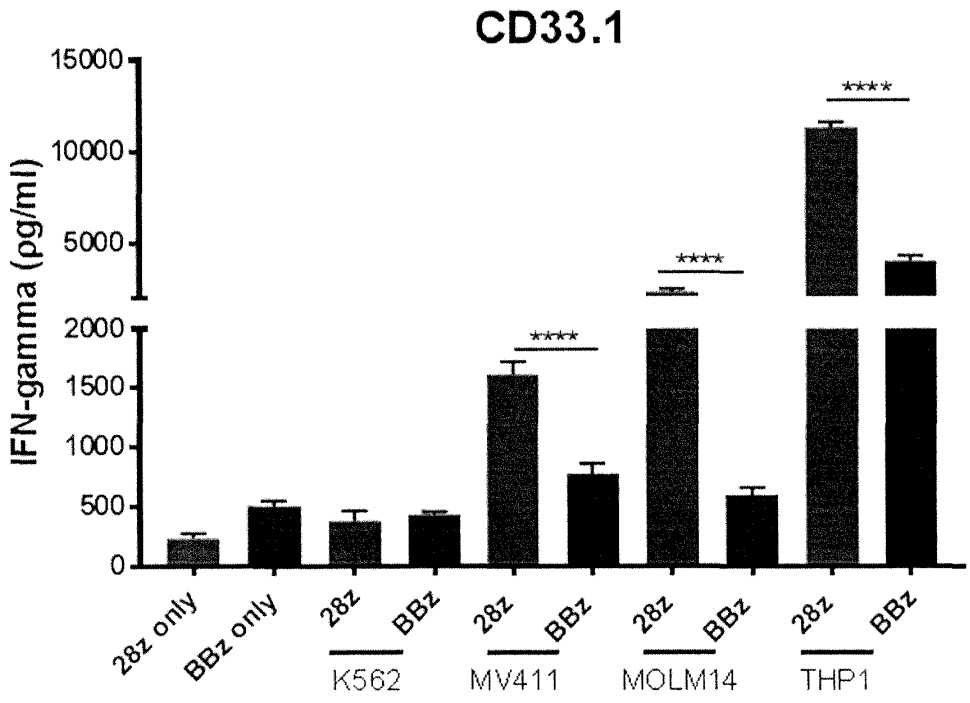

CD33.1
IFN-gamma (pg/ml)
15000
10000
5000
2000
1500
1000
500
0
****
****
****
28z only
BBz only
28z
BBz
28z
BBz
28z
BBz
28z
BBz
K562
MV411
MOLM14
THP1

Figure 4B

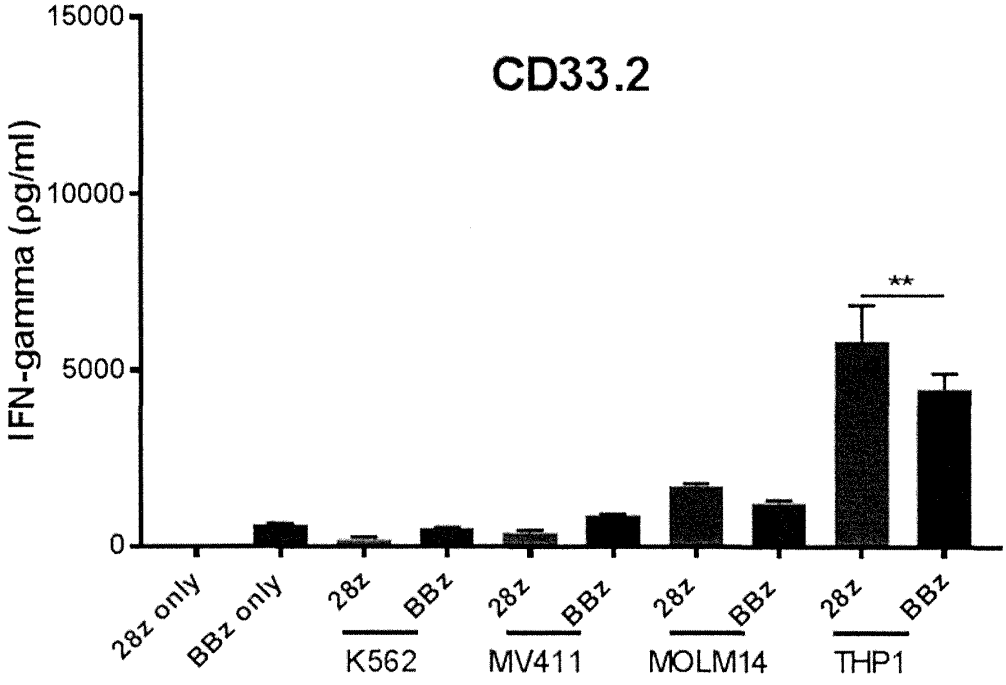

CD33.2
IFN-gamma (pg/ml)
15000
10000
5000
0
**
28z only
BBz only
28z
BBz
K562
MV411
MOLM14
THP1

Figure 4C

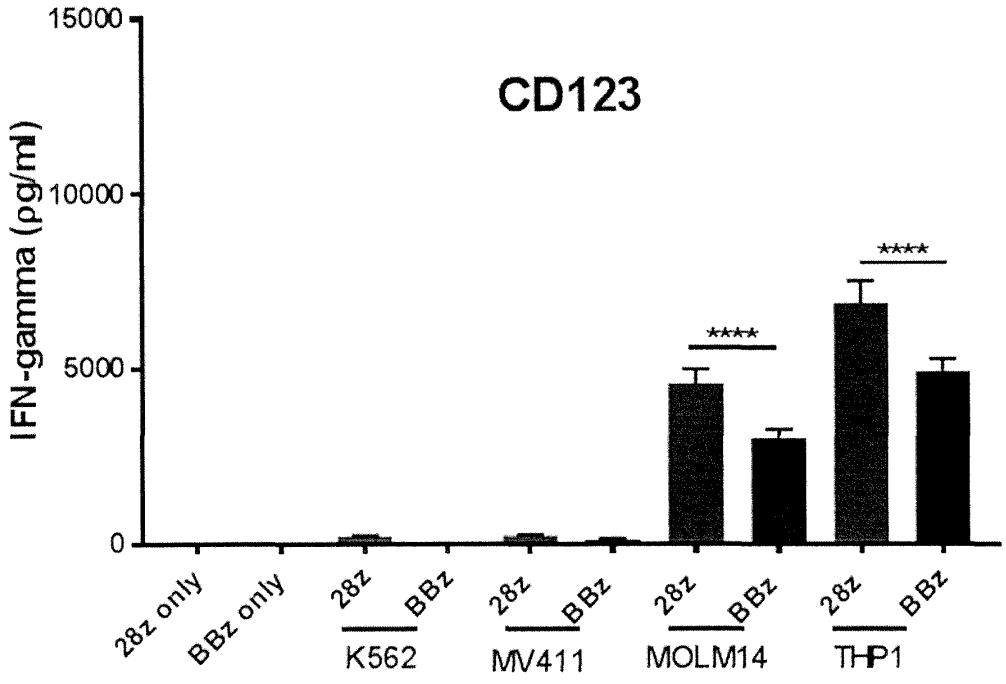

CD123
IFN-gamma (pg/ml)
15000
10000
5000
0
****
****
28z only
BBz only
28z
BBz
K562
28z
BBz
MV411
28z
BBz
MOLM14
28z
BBz
THP1

Figure 4D

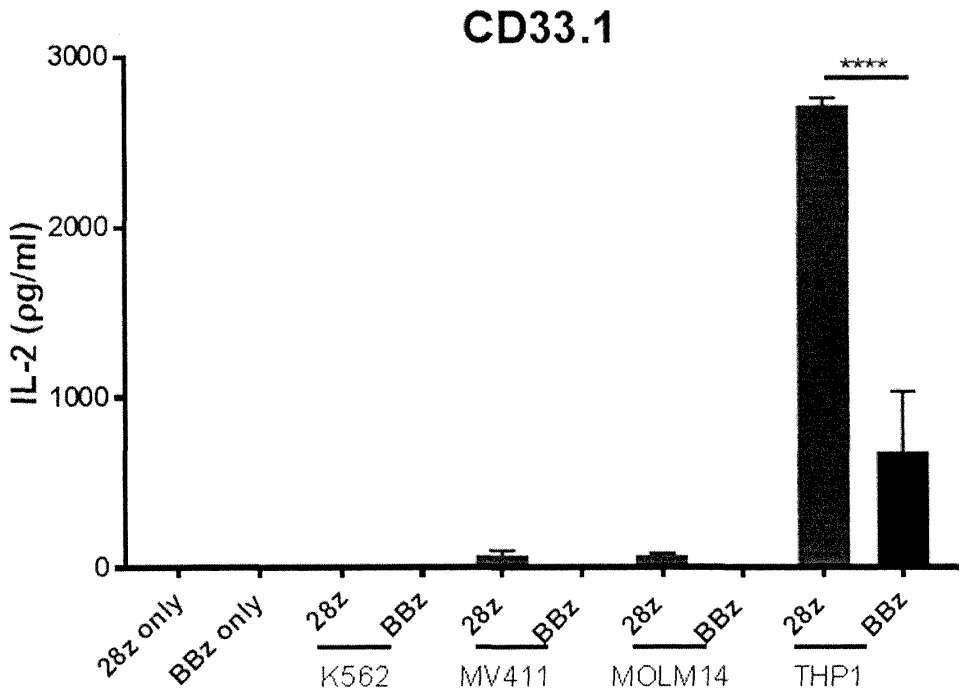

CD33.1
IL-2 (pg/ml)
3000
2000
1000
0
****
28z only
BBz only
28z
BBz
K562
MV411
MOLM14
THP1

Figure 4E

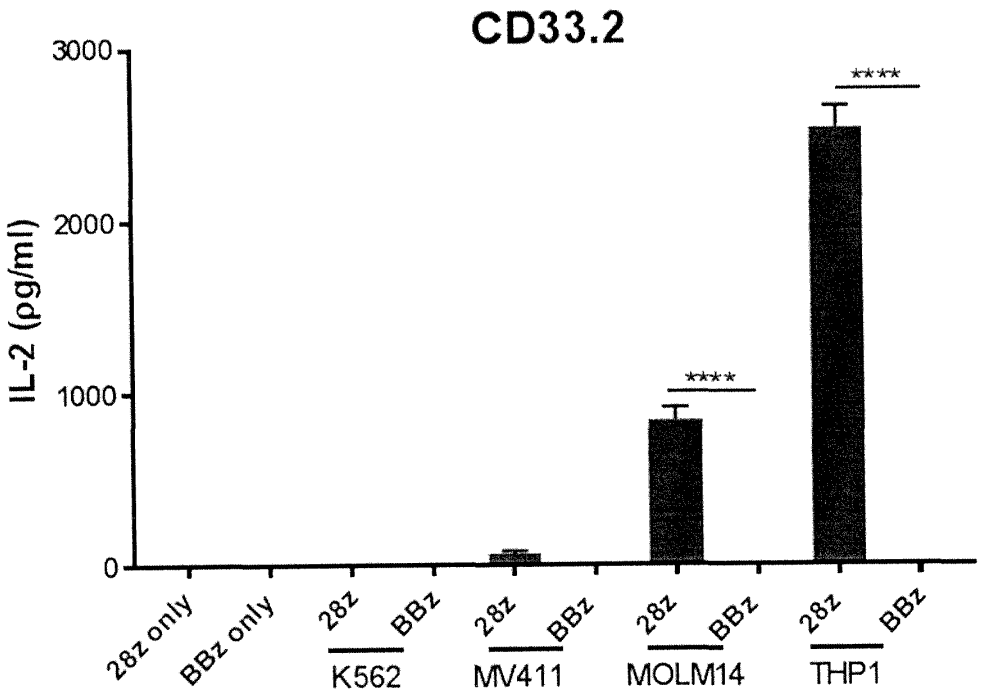

CD33.2
IL-2 (pg/ml)
3000
2000
1000
0
****
****
28z only
BBz only
28z
BBz
K562
28z
BBz
MV411
28z
BBz
MOLM14
28z
BBz
THP1

Figure 4F

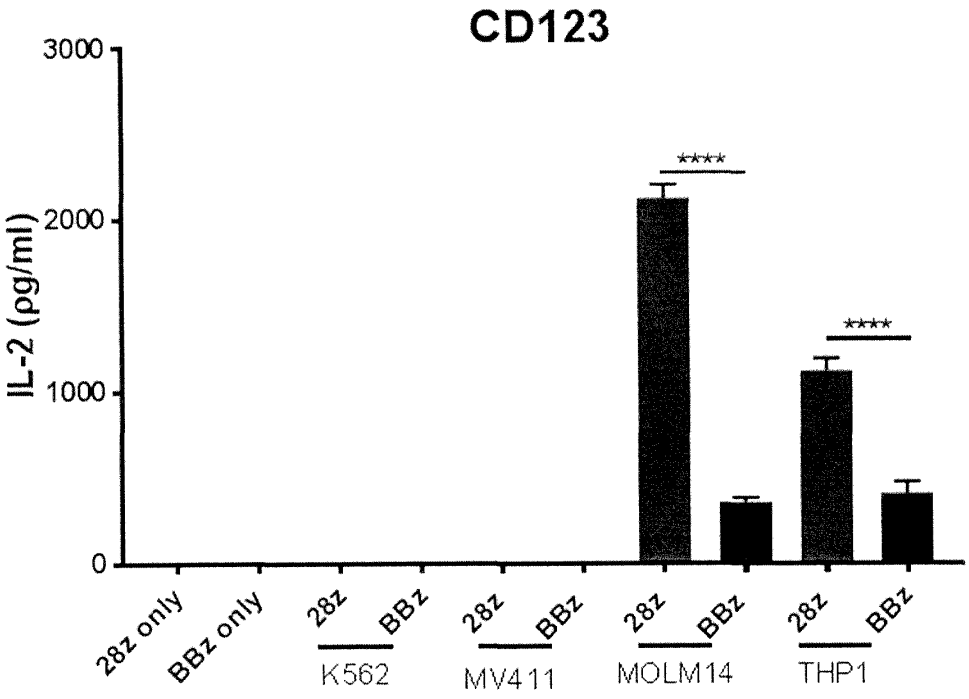

CD123
IL-2 (pg/ml)
3000
2000
1000
0
****
****
28z only
BBz only
28z
BBz
K562
28z
BBz
MV411
28z
BBz
MOLM14
28z
BBz
THP1

Figure 5A

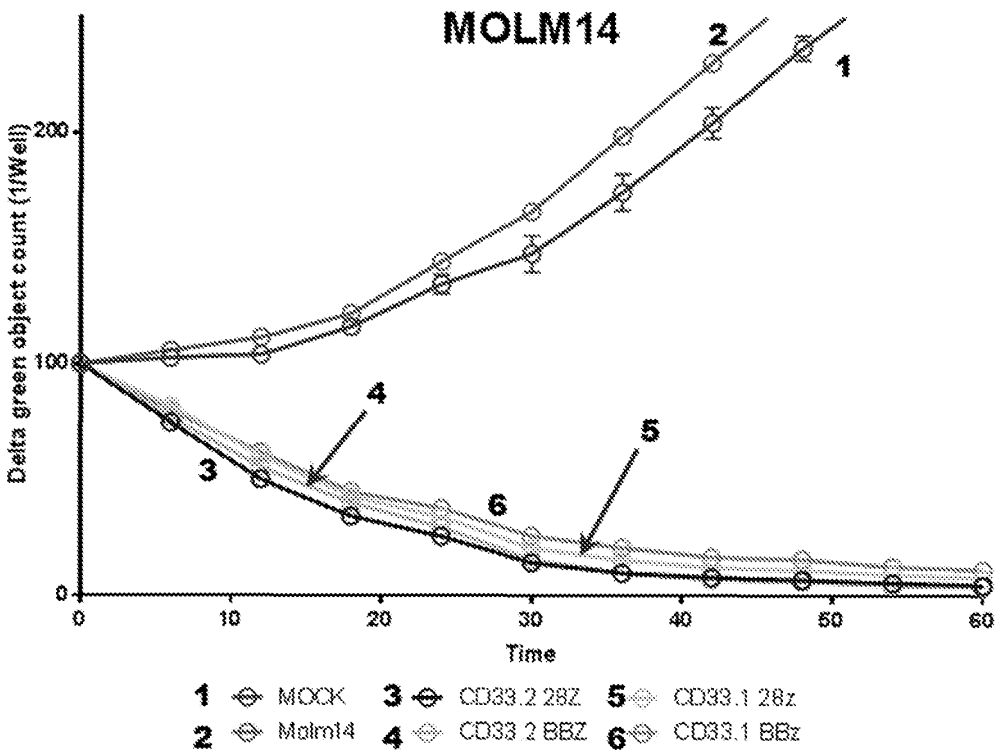

MOLM14
Delta green object count (1/Well)
200
100
0
0
10
20
30
40
50
60
Time
1
2
3
4
5
6
1 MOCK
2 Molm14
3 CD33.2 28Z
4 CD33.2 BBZ
5 CD33.1 28z
6 CD33.1 BBz

Figure 5B

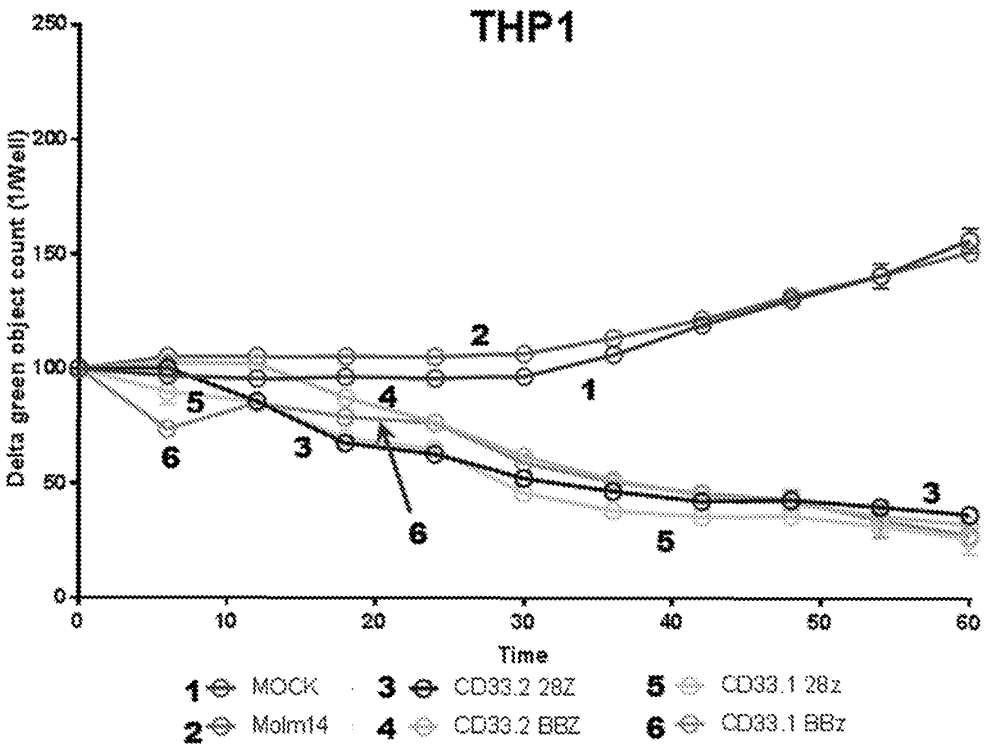

THP1
Delta green object count (1/Well)
250
200
150
100
50
0
0
10
20
30
40
50
60
Time
1 MOCK
2 Molm14
3 CD33.2 28Z
4 CD33.2 BBZ
5 CD33.1 28z
6 CD33.1 BBz

Figure 5C

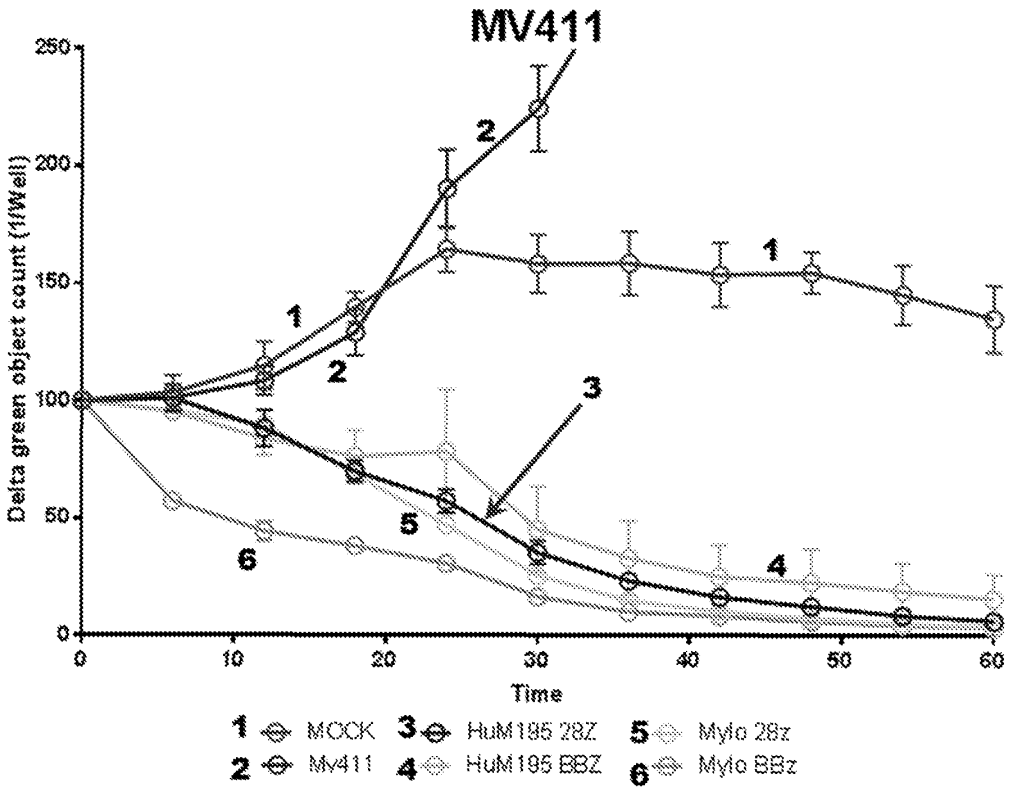

MV411
Delta green object count (1/Well)
250
200
150
100
50
0
0
10
20
30
40
50
60
Time
1
2
3
4
5
6
1 MOCK
2 Mv411
3 HuM195 28Z
4 HuM195 BBZ
5 Mylo 28z
6 Mylo BBz

Figure 5D

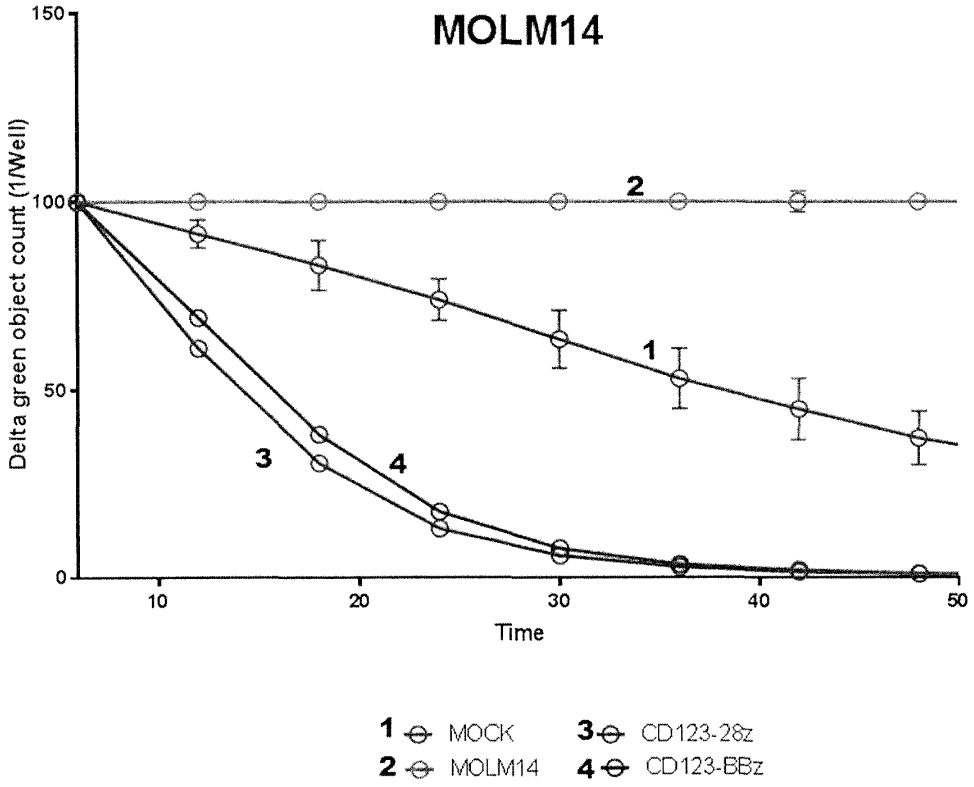

MOLM14
Delta green object count (1/Well)
150
100
50
0
10
20
30
40
50
Time
1
2
3
4
1 MOCK
2 MOLM14
3 CD123-28z
4 CD123-BBz

Figure 5E

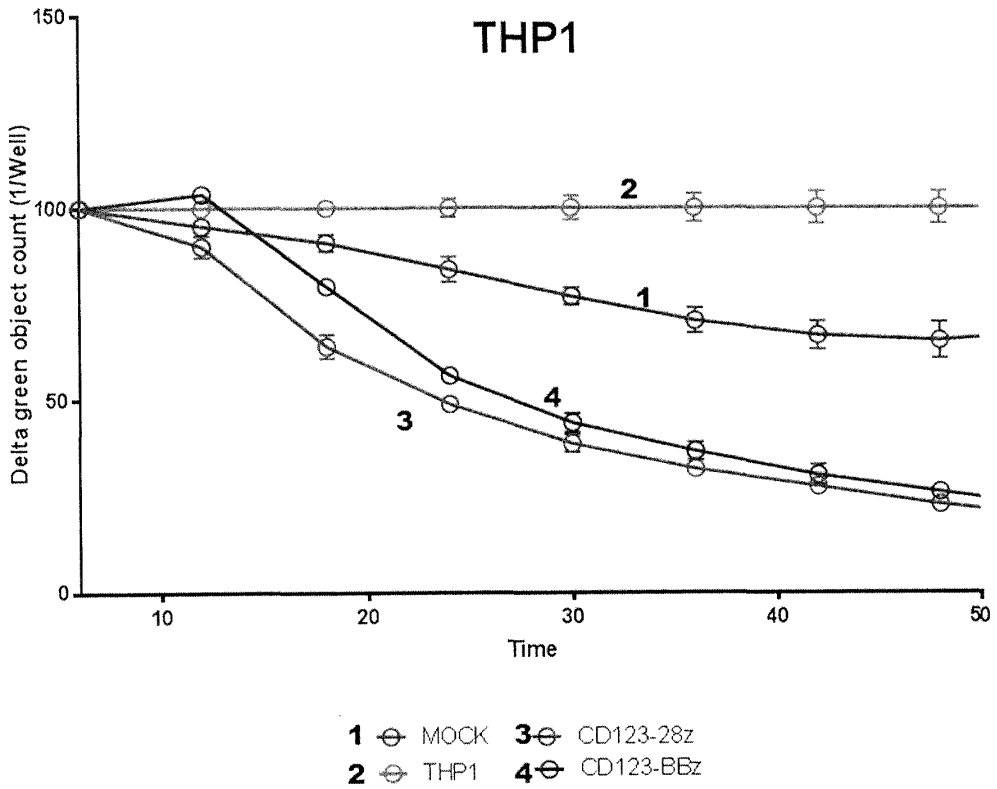

THP1
Delta green object count (1/Well)
150
100
50
0
10
20
30
40
50
Time
1
2
3
4
1 MOCK
2 THP1
3 CD123-28z
4 CD123-BBz

Figure 6A

Day 0 Injected 1E6 THP1
Day 15: Inject 6E6 CARs

Figure 6B

Day 0 : Injected 1E6 THP1
Day 11: ADT 6E6 CAR

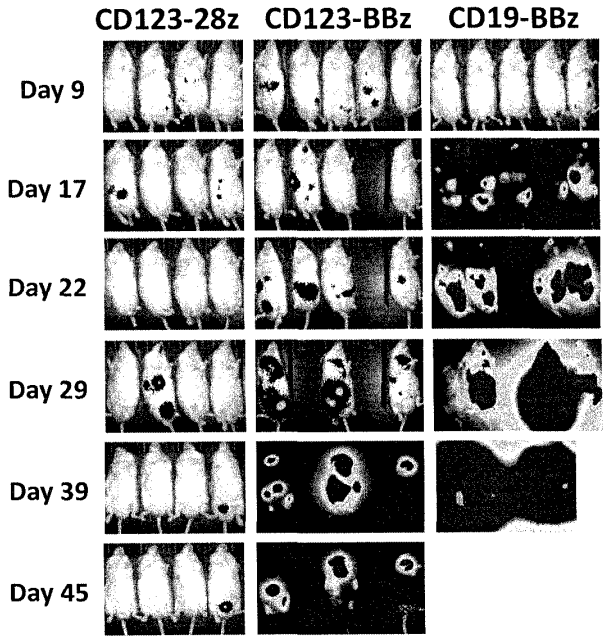


Figure 6C

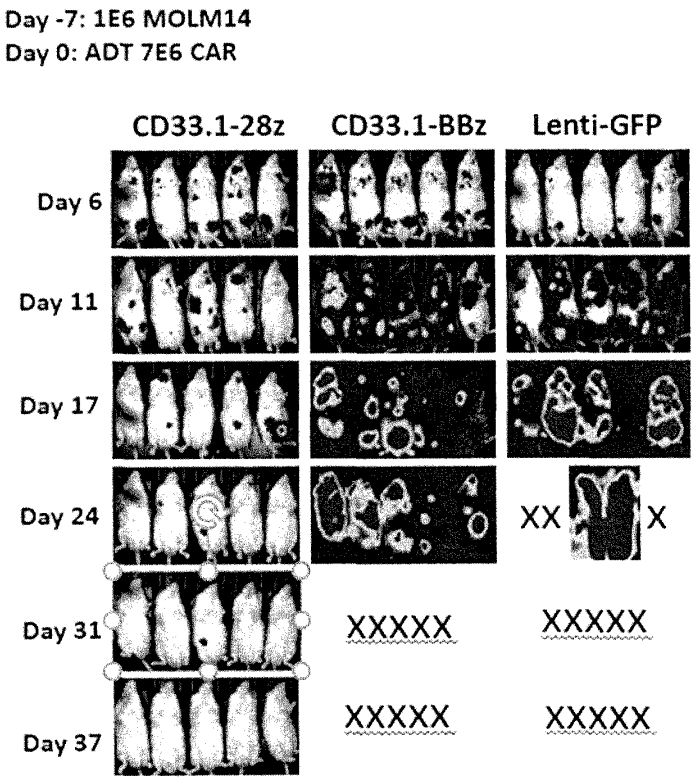

Day -7: 1E6 MOLM14
Day 0: ADT 7E6 CAR
CD33.1-28z
CD33.1-BBz
Lenti-GFP
Day 6
Day 11
Day 17
Day 24
Day 31
Day 37
XX
X
XXXXX
XXXXX
XXXXX
XXXXX

Figure 6D

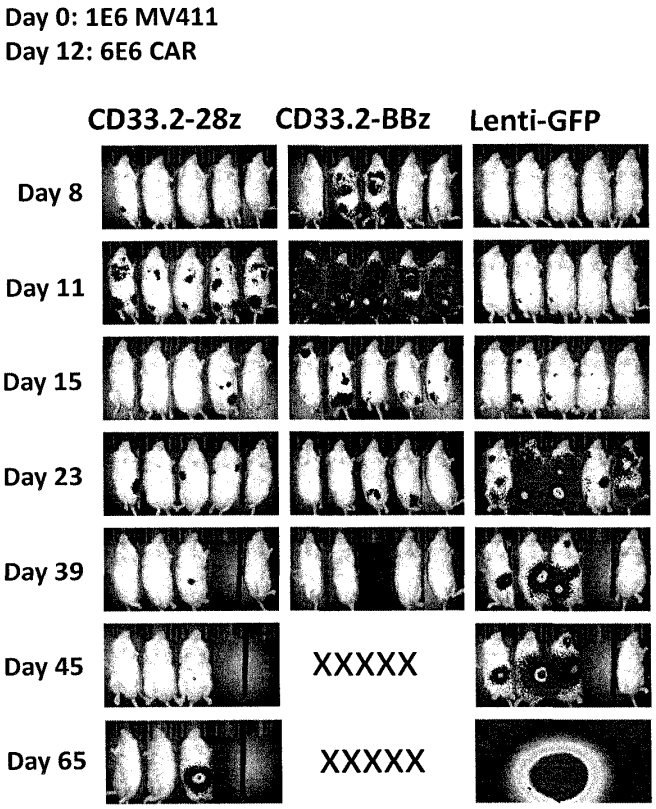

Day 0: 1E6 MV411
Day 12: 6E6 CAR
CD33.2-28z
CD33.2-BBz
Lenti-GFP
Day 8
Day 11
Day 15
Day 23
Day 39
Day 45
Day 65
XXXXX
XXXXX

Figure 6E

Day -7: 1E6 MOLM14
Day 0: ADT CAR

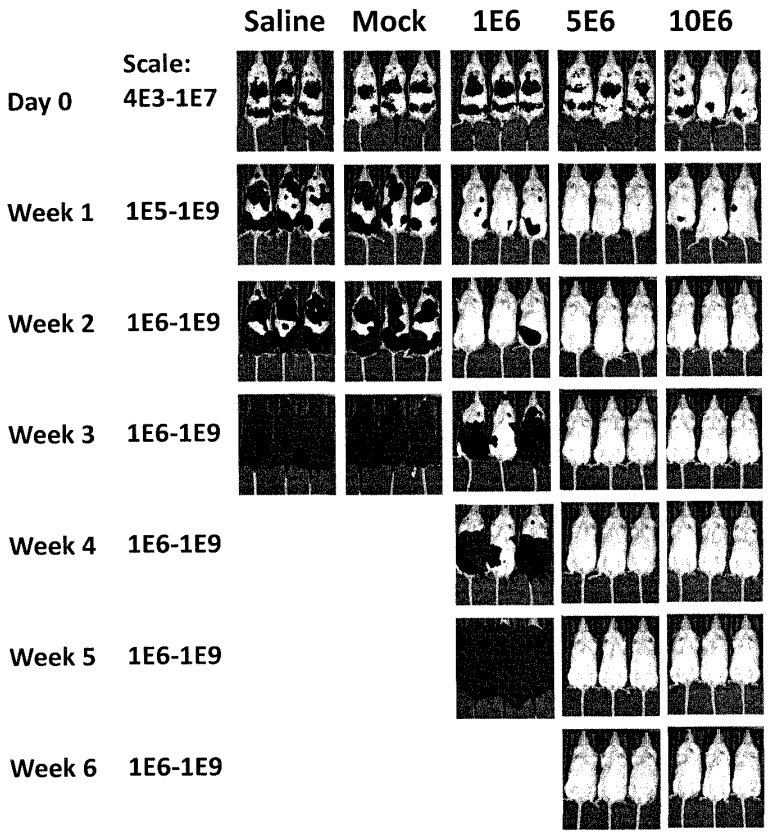


Figure 7A

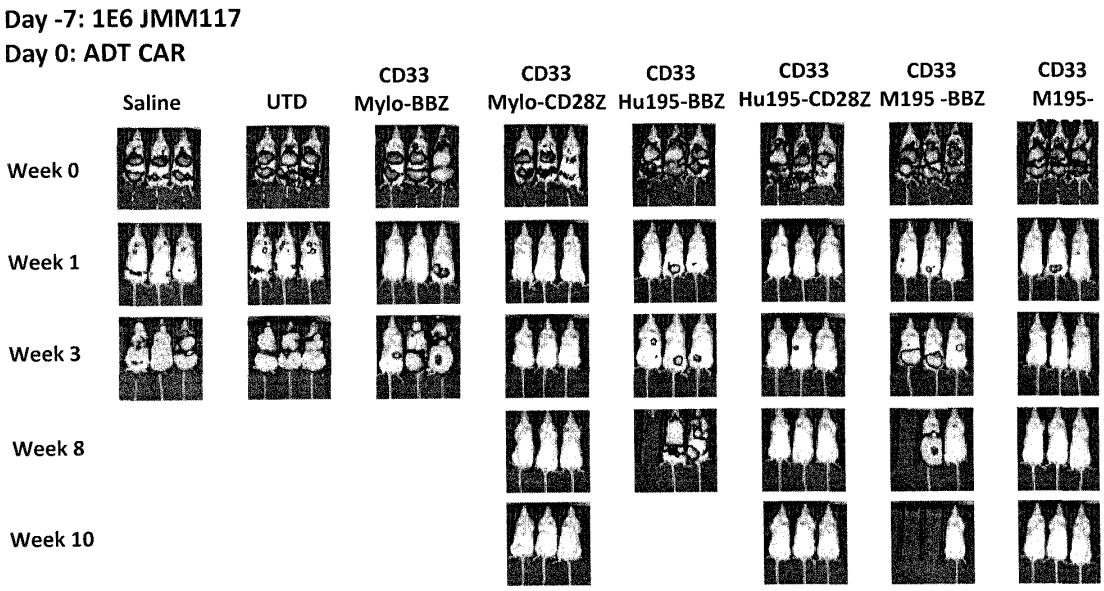

Day -7: 1E6 JMM117
Day 0: ADT CAR
Saline
UTD
CD33 Mylo-BBZ
CD33 Mylo-CD28Z
CD33 Hu195-BBZ
CD33 Hu195-CD28Z
CD33 M195 -BBZ
CD33 M195-
Week 0
Week 1
Week 3
Week 8
Week 10

Figure 7B

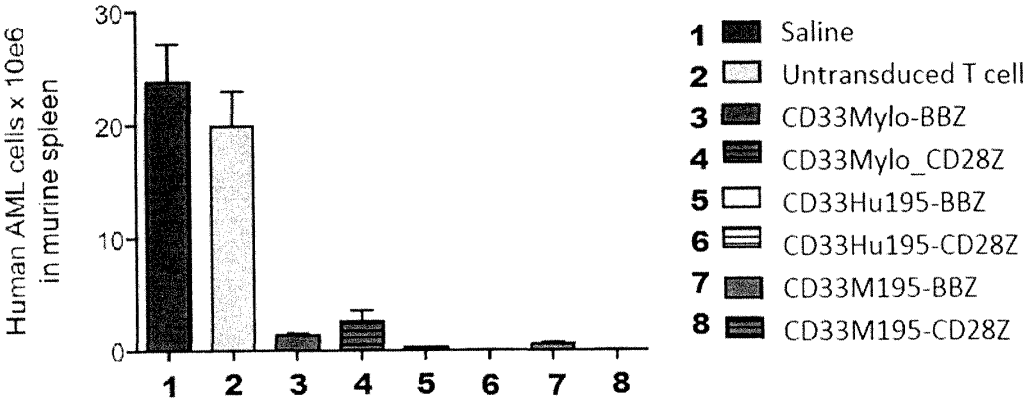

Human AML cells x 10e6 in murine spleen
30
20
10
0
1
2
3
4
5
6
7
8
1 Saline
2 Untransduced T cell
3 CD33Mylo-BBZ
4 CD33Mylo_CD28Z
5 CD33Hu195-BBZ
6 CD33Hu195-CD28Z
7 CD33M195-BBZ
8 CD33M195-CD28Z

Figure 7C

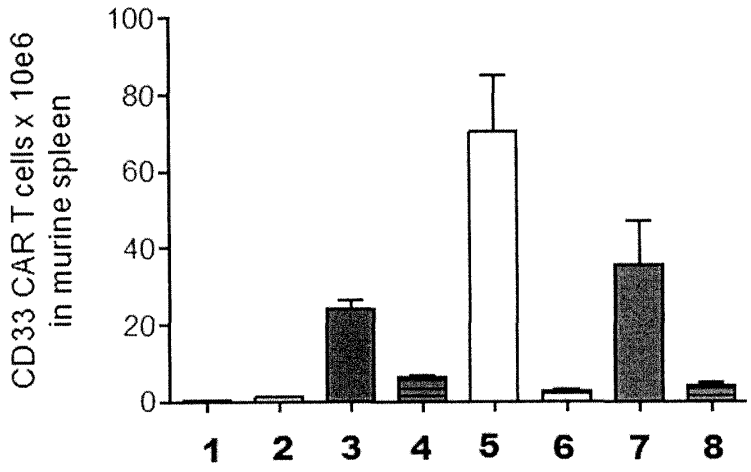

CD33 CAR T cells x 10e6
in murine spleen
100
80
60
40
20
0
1
2
3
4
5
6
7
8

Figure 8

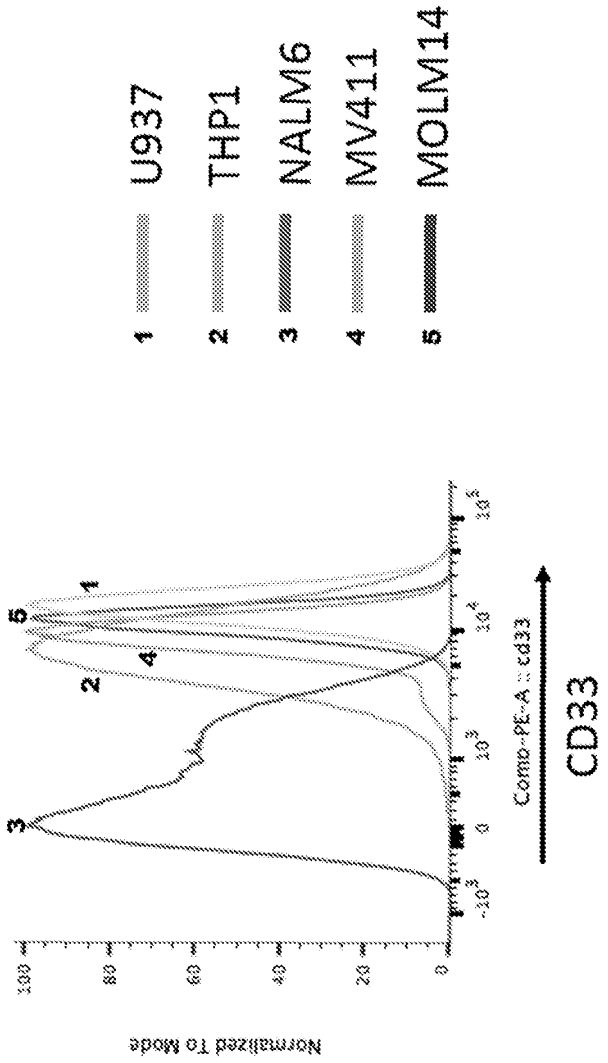

U937
THP1
NALM6
MV411
MOLM14
1
2
3
4
5
Normalized To Mode
Comp-PE-A :: cd33
CD33

Figure 9A

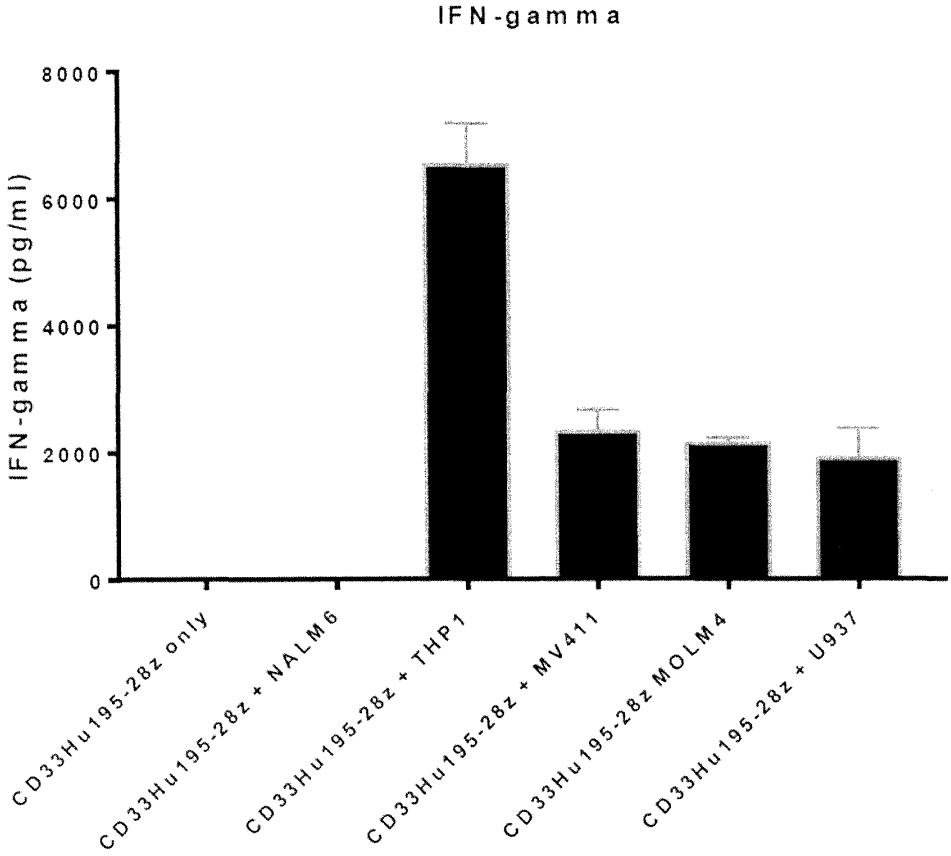

IFN-gamma
IFN-gamma (pg/ml)
8000
6000
4000
2000
0
CD33Hu195-28z only
CD33Hu195-28z + NALM6
CD33Hu195-28z + THP1
CD33Hu195-28z + MV411
CD33Hu195-28z MOLM4
CD33Hu195-28z + U937

Figure 9B

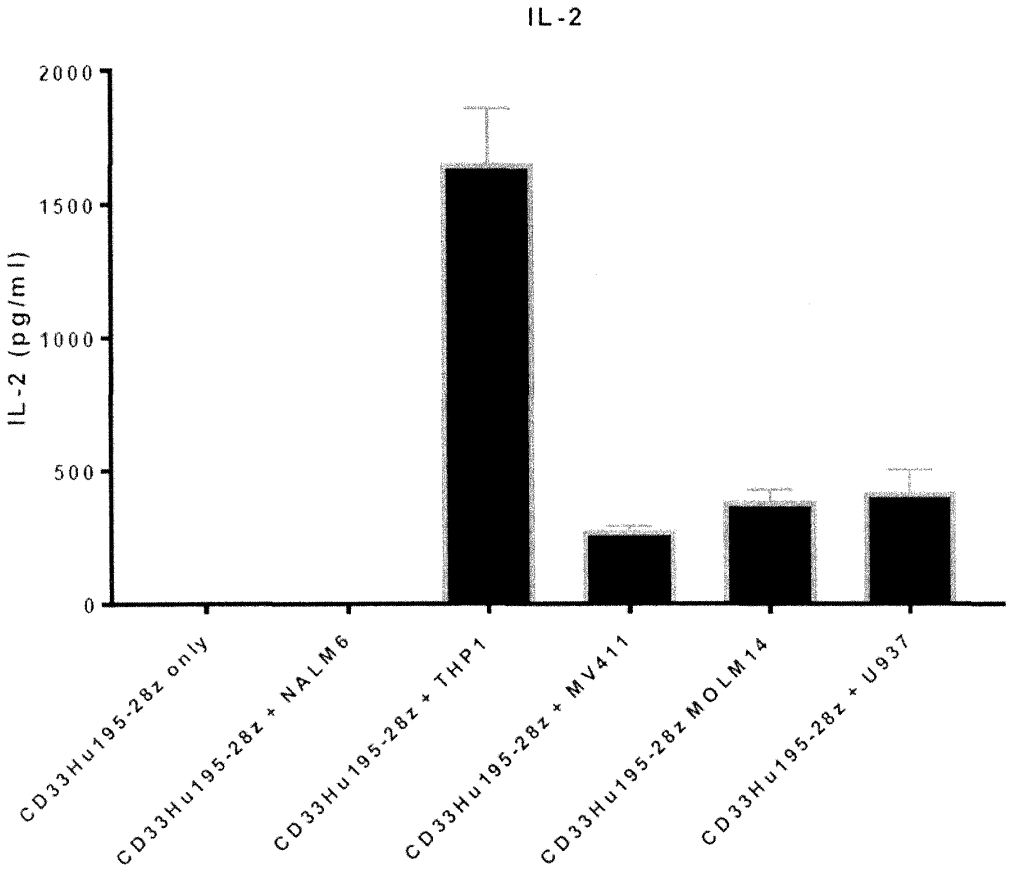

IL-2
2000
1500
1000
500
0
IL-2 (pg/ml)
CD33Hu195-28z only
CD33Hu195-28z + NALM6
CD33Hu195-28z + THP1
CD33Hu195-28z + MV411
CD33Hu195-28z MOLM14
CD33Hu195-28z + U937

Figure 10

Day -7: 1E6 MOLM14
Day 0: ADT CAR

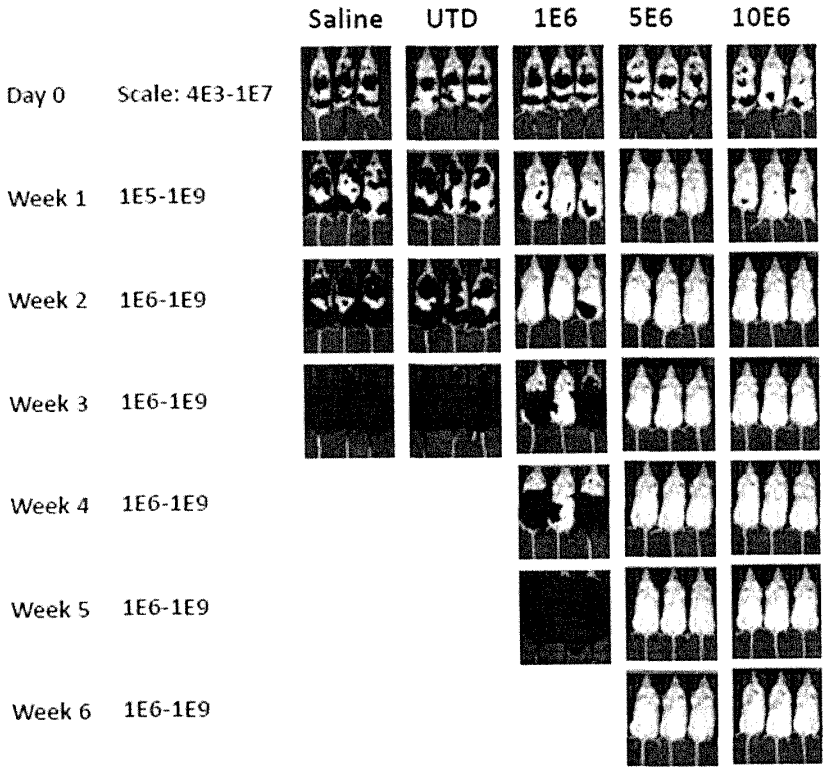


Figure 11A

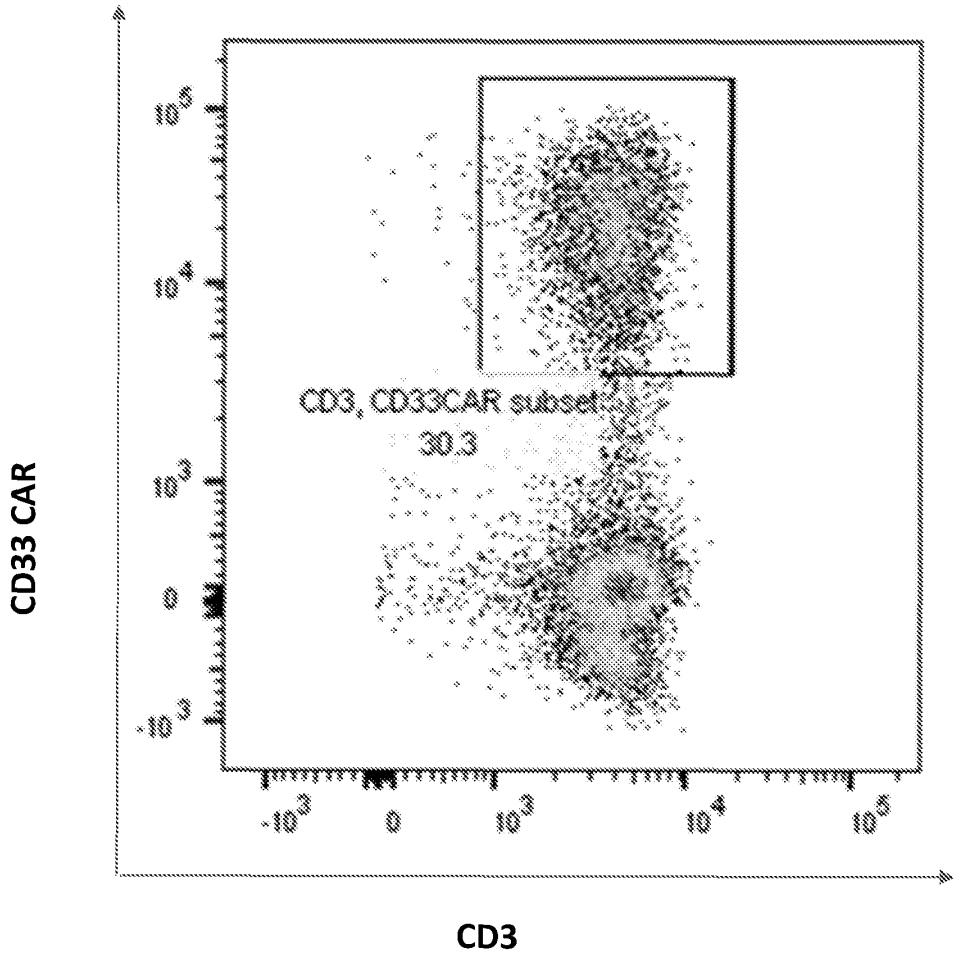

CD33 CAR
CD3
CD3, CD33CAR subset
30.3
10^5
10^4
10^3
0
-10^3
-10^3
0
10^3
10^4
10^5

Figure 11B

Day 0: IV 1E6 MOLM14-GL
Day 3: ADT 5E6 CAR+ T cell

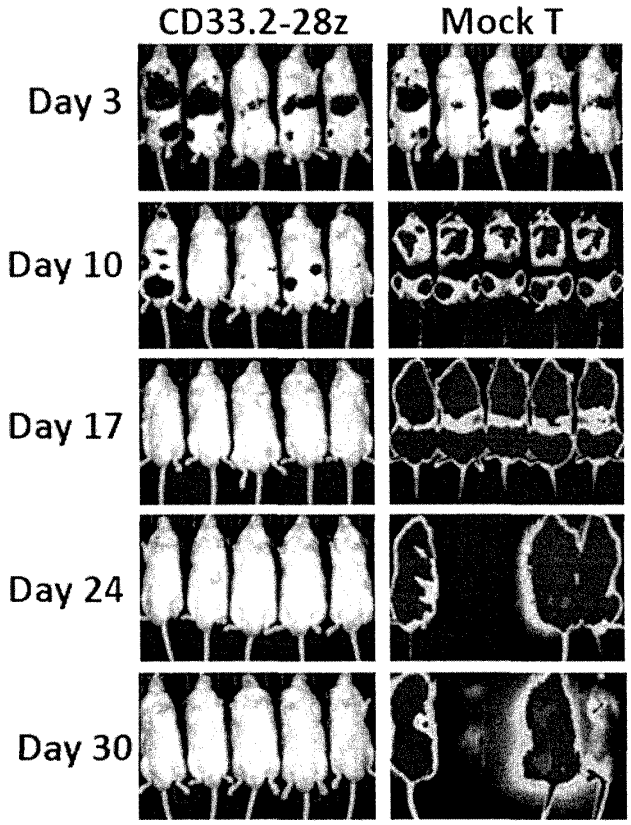


Figure 12A

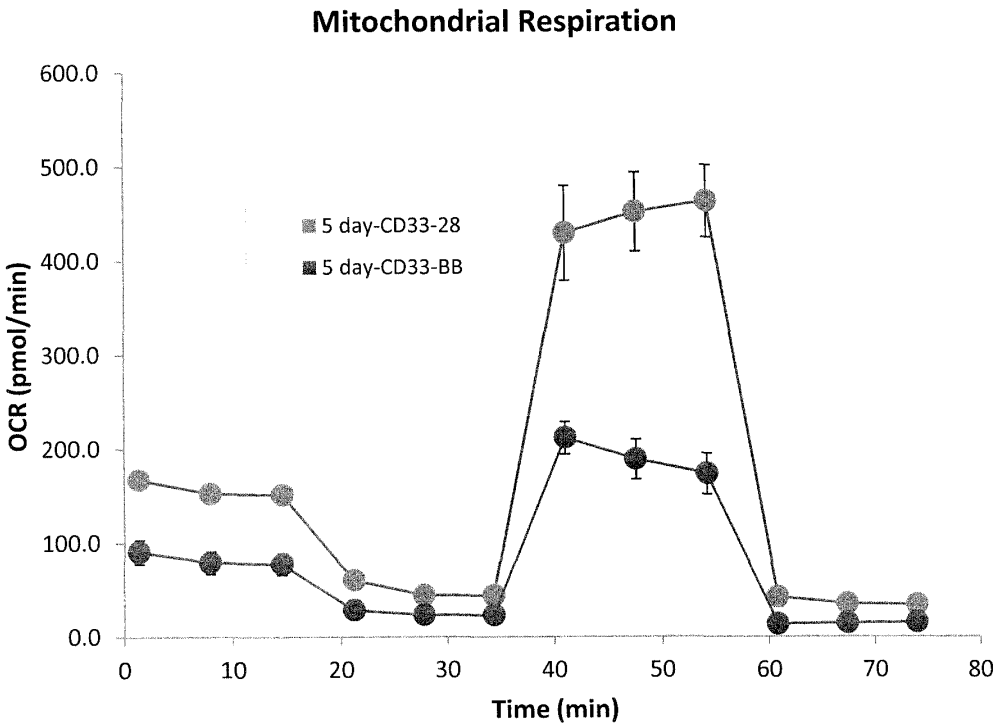

Mitochondrial Respiration
600.0
500.0
400.0
300.0
200.0
100.0
0.0
OCR (pmol/min)
5 day-CD33-28
5 day-CD33-BB
0
10
20
30
40
50
60
70
80
Time (min)

Figure 12B

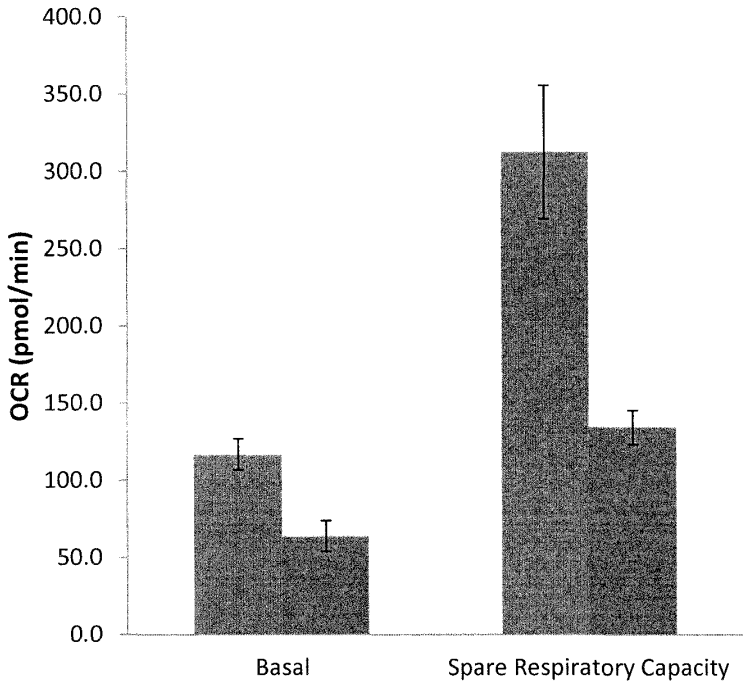

400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
OCR (pmol/min)
Basal
Spare Respiratory Capacity

Figure 12C

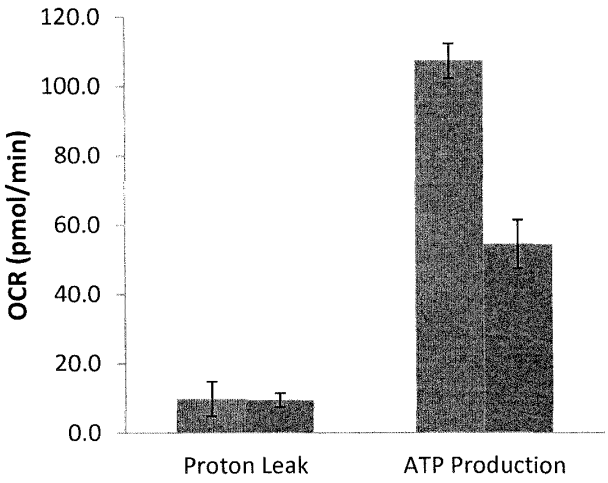

120.0
100.0
80.0
60.0
40.0
20.0
0.0
OCR (pmol/min)
Proton Leak
ATP Production

Figure 12D

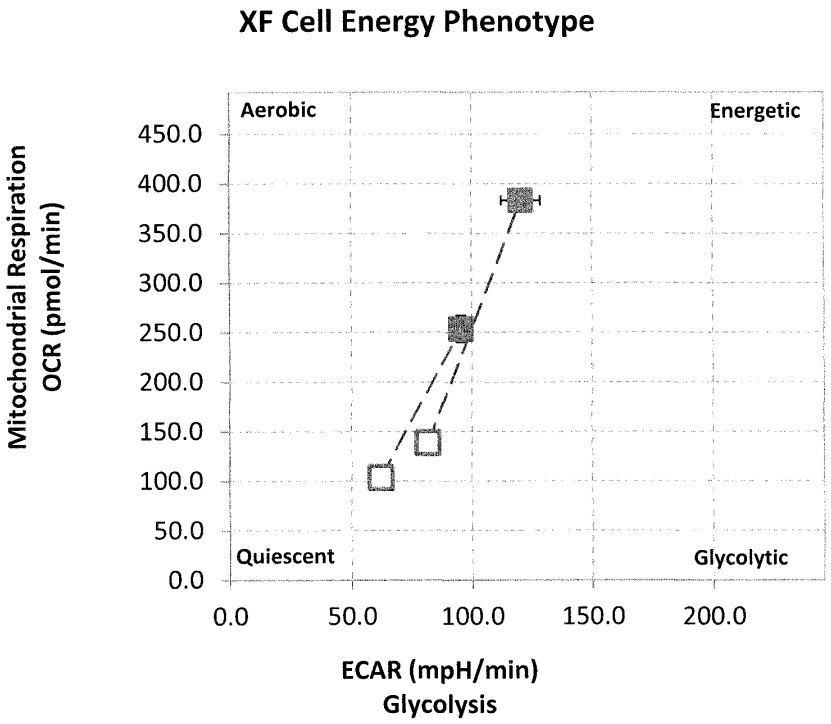

XF Cell Energy Phenotype
Aerobic
Energetic
Quiescent
Glycolytic
Mitochondrial Respiration
OCR (pmol/min)
450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.0
50.0
100.0
150.0
200.0
ECAR (mpH/min)
Glycolysis

Figure 12E

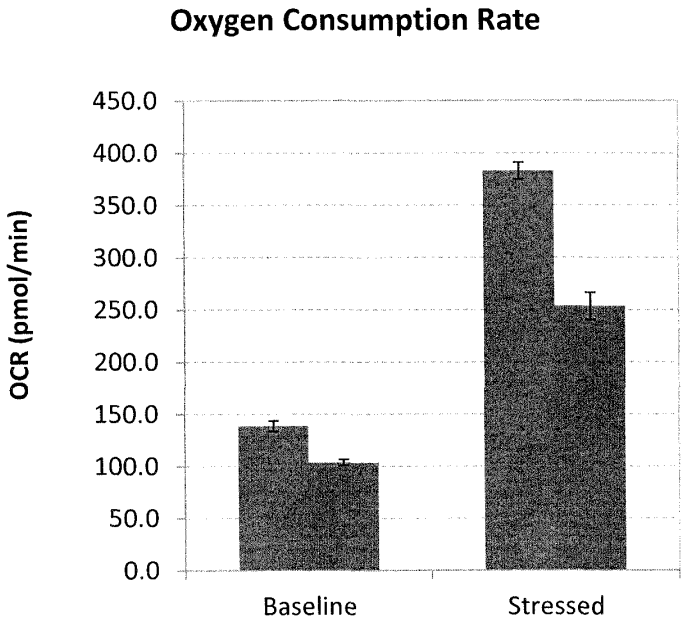

Oxygen Consumption Rate
OCR (pmol/min)
450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Baseline
Stressed

Figure 12F

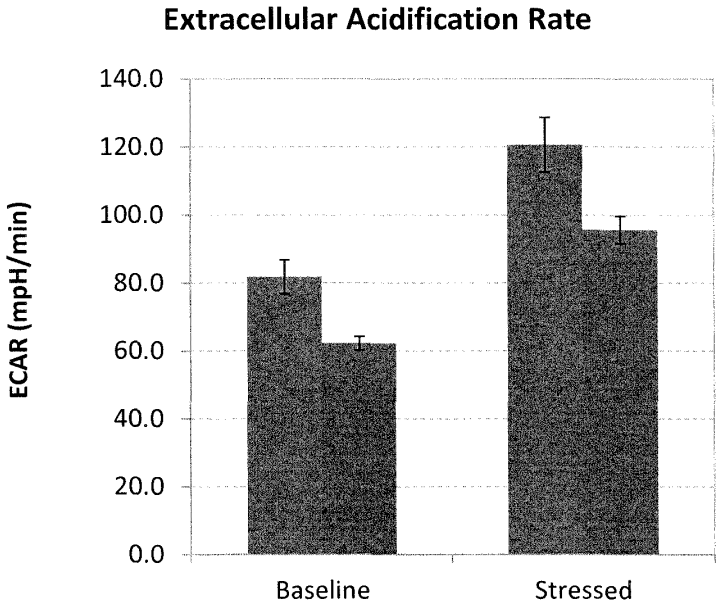
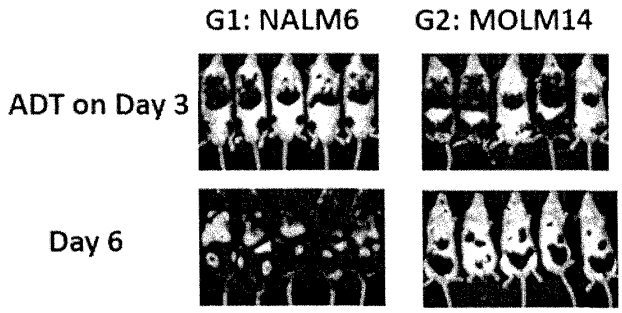

Extracellular Acidification Rate
ECAR (mpH/min)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Baseline
Stressed

Figure 13

Day 0: IV 1E6 NAML6-GL or MOLM14-GL
Day 3: ADT 3.5E6 CAR$^+$ T cell