FIG. 1A

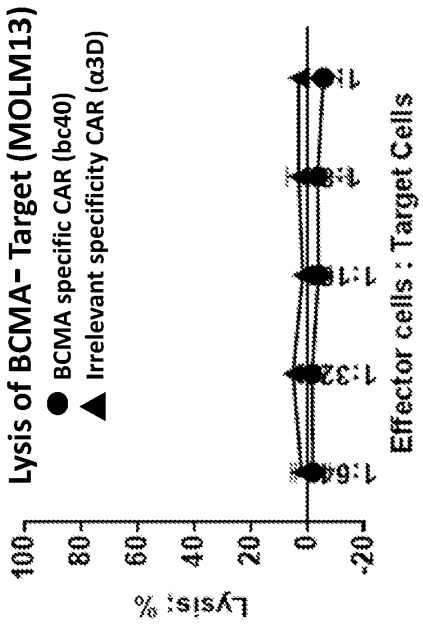


FIG. 1B

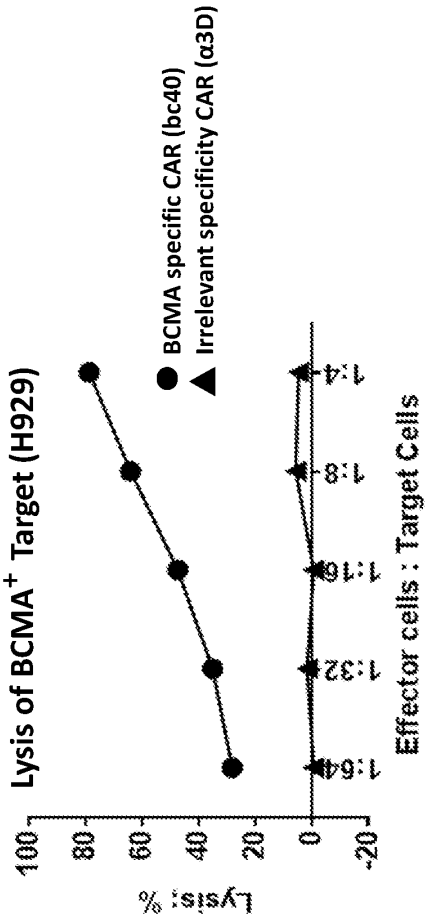


FIG. 1C

FIG. 1D

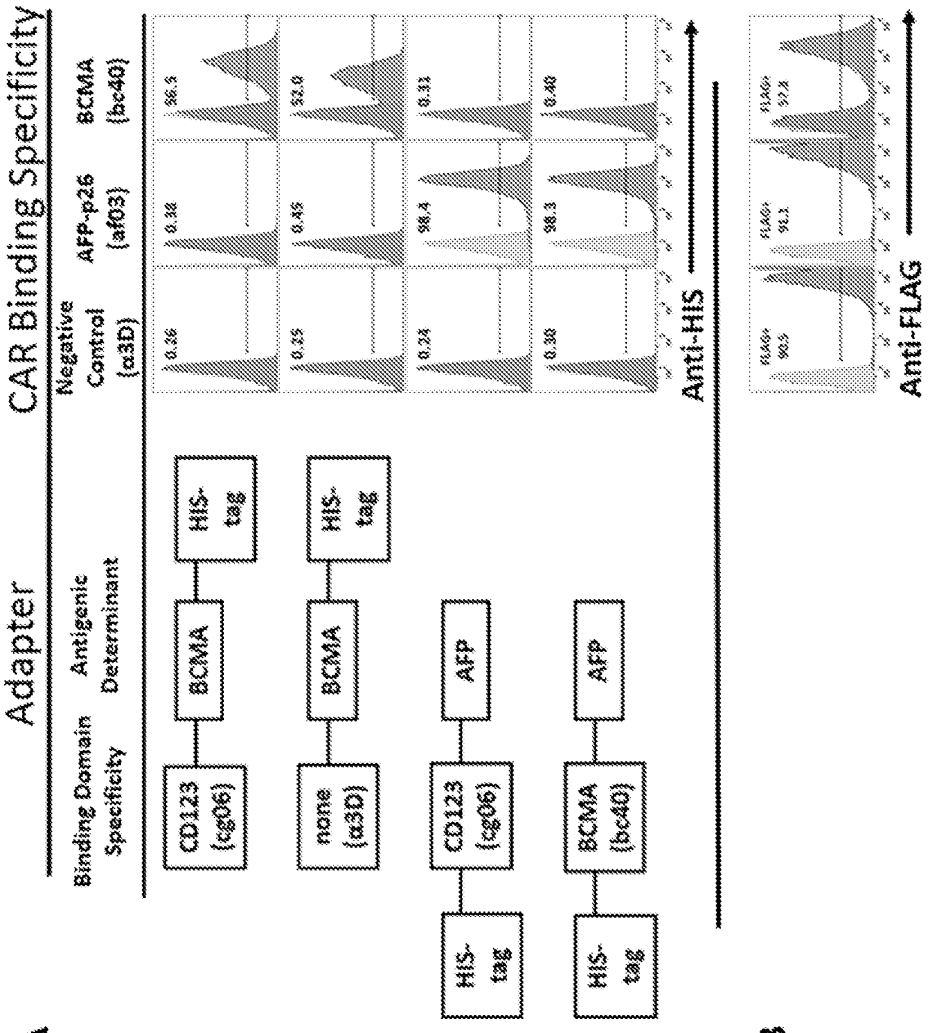

CAR Binding Specificity
Adapter
Binding Domain Specificity
Antigenic Determinant
Negative Control (α3D)
AFP-p26 (af03)
BCMA (bc40)
CD123 (cg06)
BCMA
HIS-tag
none (α3D)
BCMA
HIS-tag
CD123 (cg06)
AFP
HIS-tag
BCMA (bc40)
AFP
HIS-tag
0.26
0.38
96.5
0.25
0.45
92.0
0.24
98.4
0.31
0.38
98.3
0.40
Anti-HIS
FLAG+ 90.5
FLAG+ 91.1
FLAG+ 97.8
Anti-FLAG

FIG. 2A

FIG. 2B

FIG. 3A

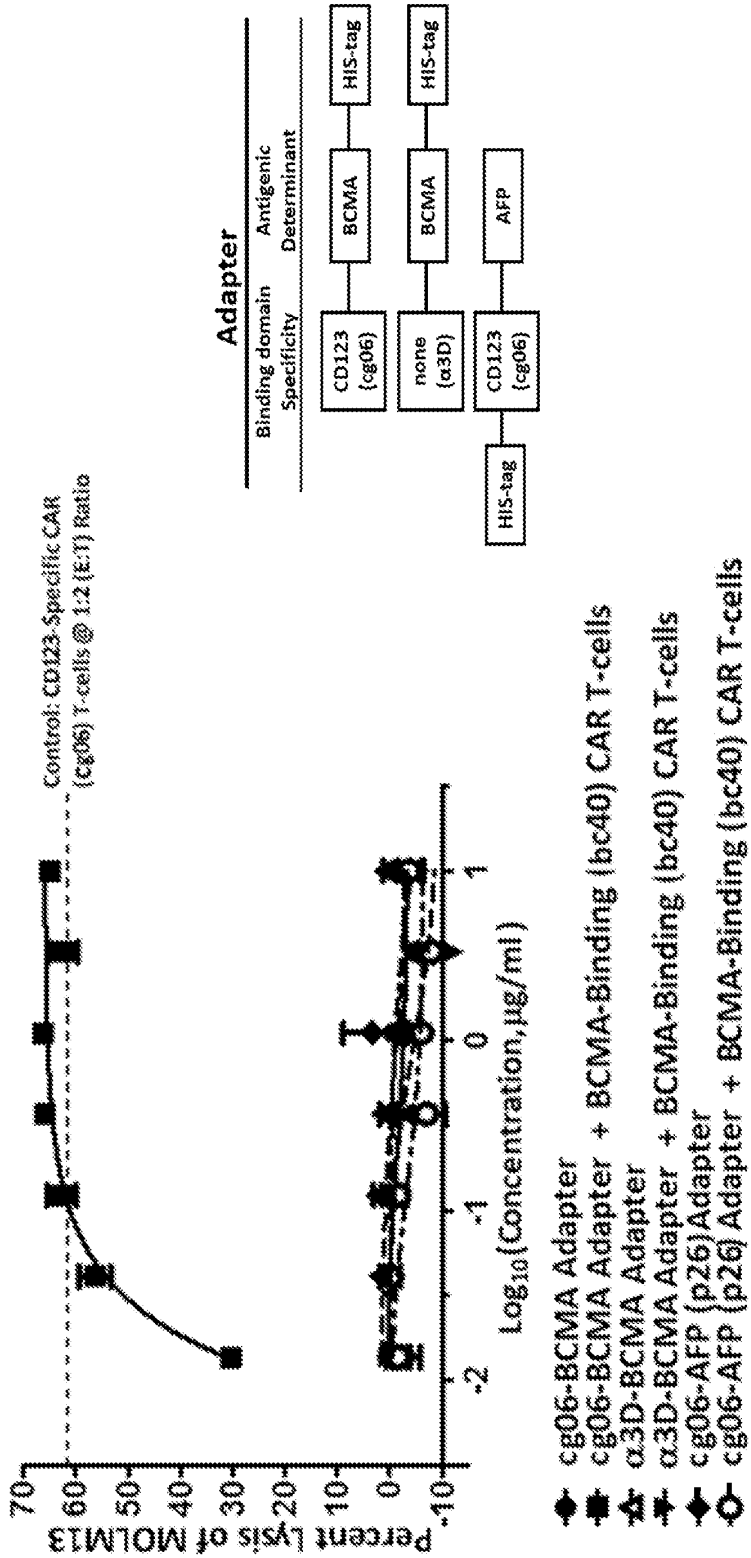

Lysis of CD123+ Target (MOLM13)
Control: CD123-Specific CAR (cg06) T-cells @ 1:2 (E:T) Ratio
Percent Lysis of MOLM13
70
60
50
40
30
20
10
0
-10
-2
-1
0
1
Log10(Concentration, µg/ml)
Adapter
Binding domain Specificity
Antigenic Determinant
CD123 (cg06)
BCMA
HIS-tag
none (α3D)
BCMA
HIS-tag
HIS-tag
CD123 (cg06)
AFP
cg06-BCMA Adapter
cg06-BCMA Adapter + BCMA-Binding (bc40) CAR T-cells
α3D-BCMA Adapter
α3D-BCMA Adapter + BCMA-Binding (bc40) CAR T-cells
cg06-AFP (p26)Adapter
cg06-AFP (p26) Adapter + BCMA-Binding (bc40) CAR T-cells

FIG. 3B

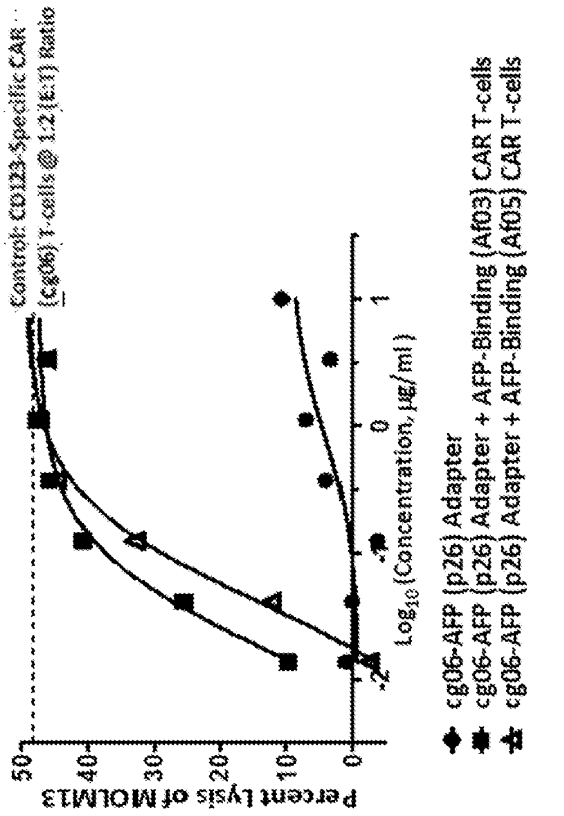
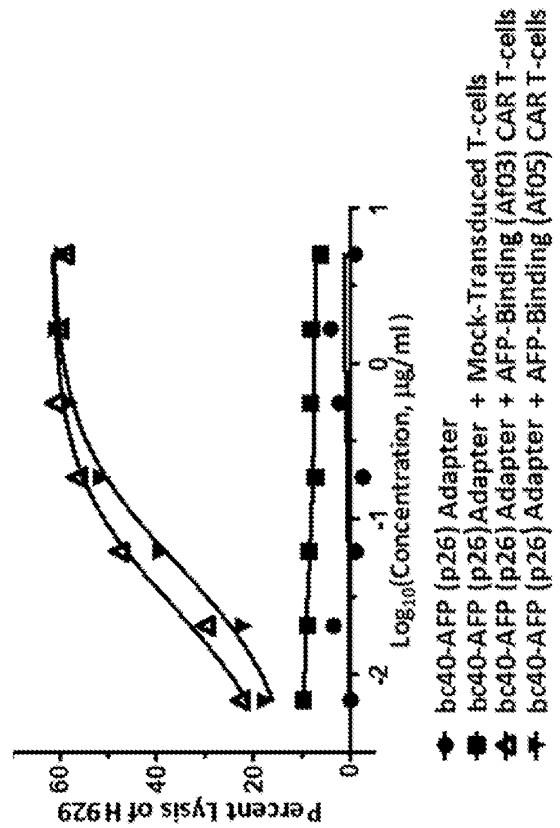

Lysis of CD123+ Target (MOLM13)
Control CD123-Specific CAR (cg06) T-cells @ 1:2 (E:T) Ratio
Percent Lysis of MOLM13
Log10 (Concentration, µg/ml)
cg06-AFP (p26) Adapter
cg06-AFP (p26) Adapter + AFP-Binding (Af03) CAR T-cells
cg06-AFP (p26) Adapter + AFP-Binding (Af05) CAR T-cells

FIG. 3C


Lysis of BCMA+ Target (H929)
Percent Lysis of H929
Log10 (Concentration, µg/ml)
bc40-AFP (p26) Adapter
bc40-AFP (p26) Adapter + Mock-Transduced T-cells
bc40-AFP (p26) Adapter + AFP-Binding (Af03) CAR T-cells
bc40-AFP (p26) Adapter + AFP-Binding (Af05) CAR T-cells

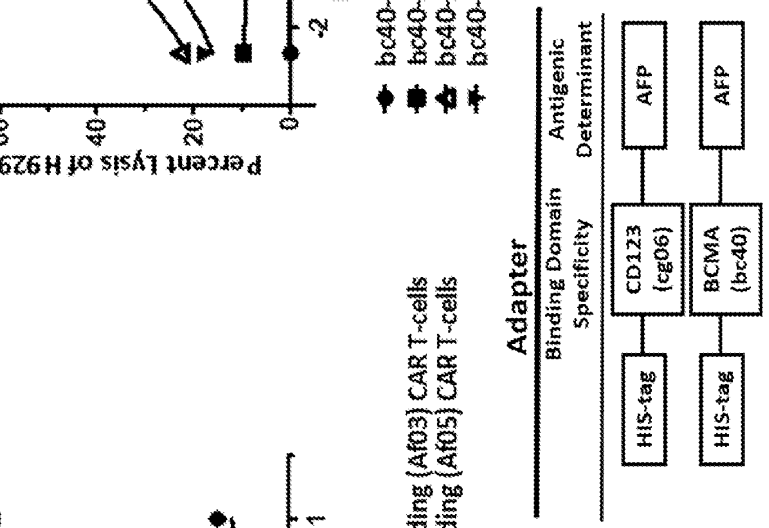

Adapter
Binding Domain
Specificity
Antigenic Determinant
HIS-tag
CD123 (cg06)
AFP
HIS-tag
BCMA (bc40)
AFP

FIG. 4A

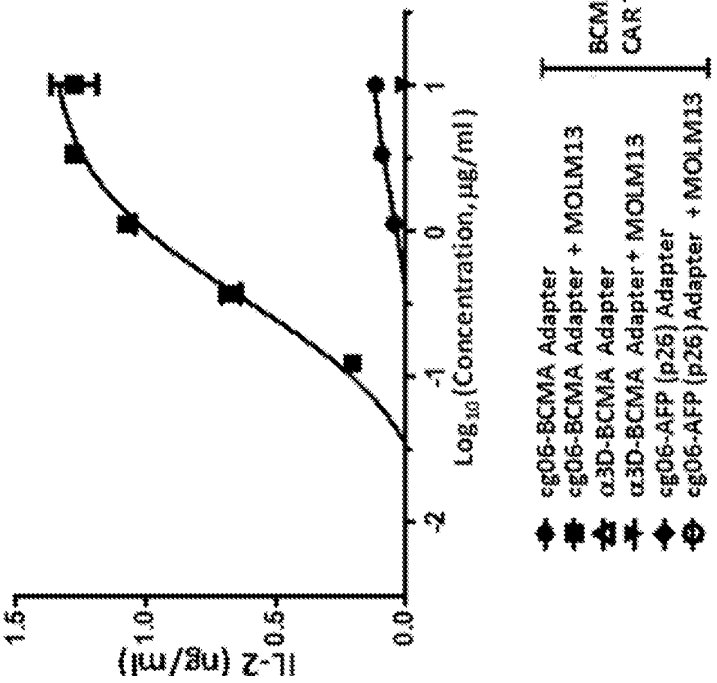
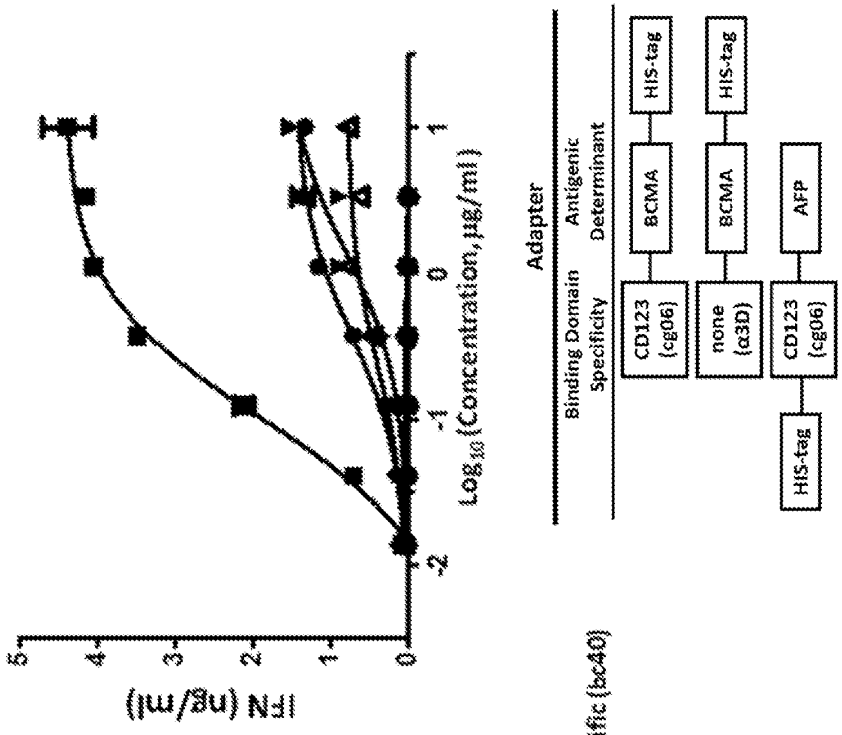

IL-2 (ng/ml)
1.5
1.0
0.5
0.0
-2
-1
0
1
Log10(Concentration, µg/ml)
cg06-BCMA Adapter
cg06-BCMA Adapter + MOLM13
α3D-BCMA Adapter
α3D-BCMA Adapter + MOLM13
cg06-AFP (p26) Adapter
cg06-AFP (p26) Adapter + MOLM13
BCMA-Specific (bc40) CAR T-cells

FIG. 4B


IFN (ng/ml)
5
4
3
2
1
0
-2
-1
0
1
Log10(Concentration, µg/ml)
Adapter
Binding Domain Specificity
Antigenic Determinant
CD123 (cg06)
BCMA
HIS-tag
none (α3D)
BCMA
HIS-tag
CD123 (cg06)
AFP
HIS-tag

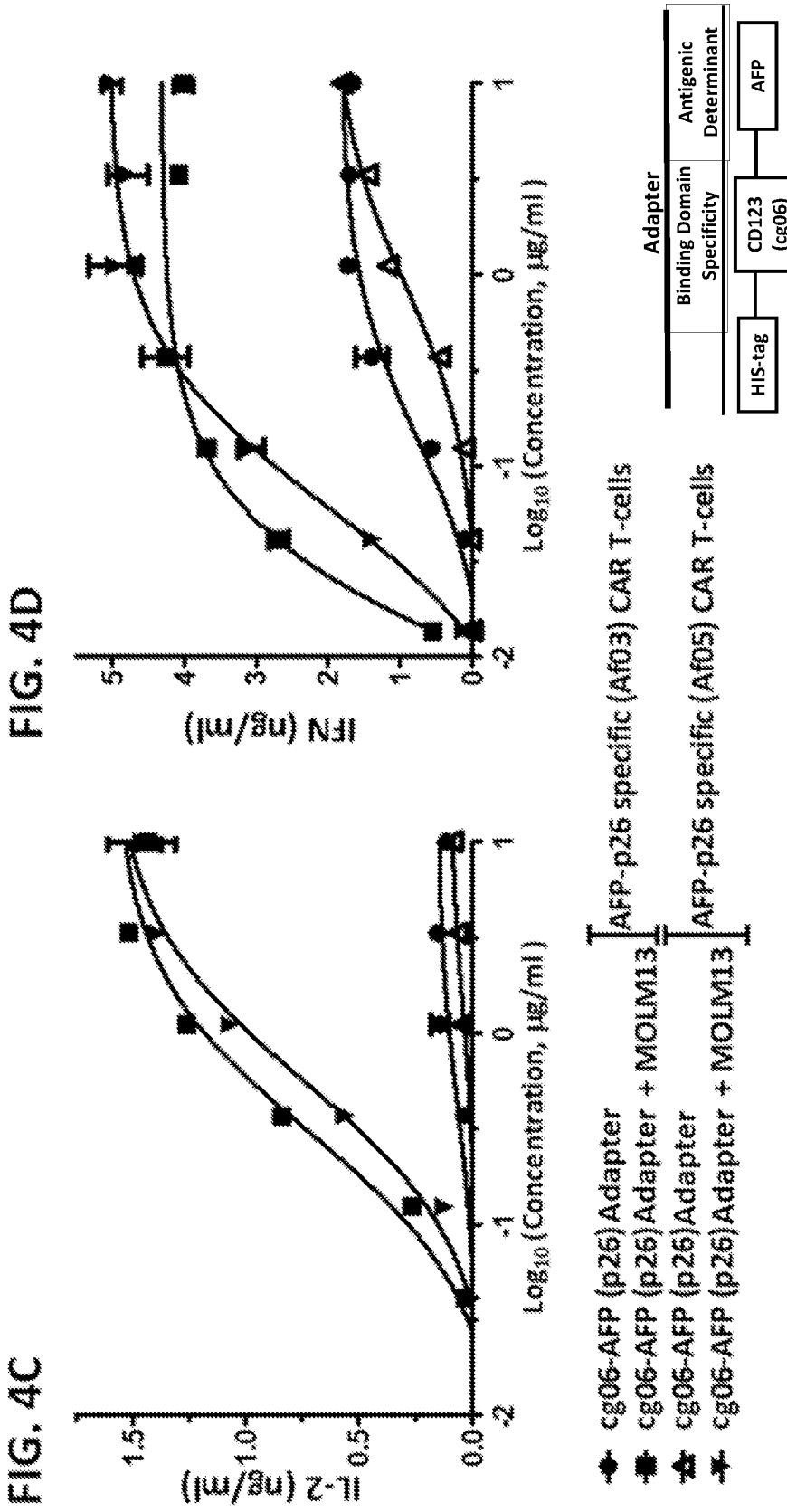

FIG. 4C
IL-2 (ng/ml)
1.5
1.0
0.5
0.0
-2
-1
0
1
Log10 (Concentration, µg/ml)
FIG. 4D
IFN (ng/ml)
5
4
3
2
1
0
-2
-1
0
1
Log10 (Concentration, µg/ml)
cg06-AFP (p26) Adapter
cg06-AFP (p26) Adapter + MOLM13
cg06-AFP (p26) Adapter
cg06-AFP (p26) Adapter + MOLM13
AFP-p26 specific (Af03) CAR T-cells
AFP-p26 specific (Af05) CAR T-cells
Adapter
Binding Domain Specificity
Antigenic Determinant
HIS-tag
CD123 (cg06)
AFP

FIG. 5

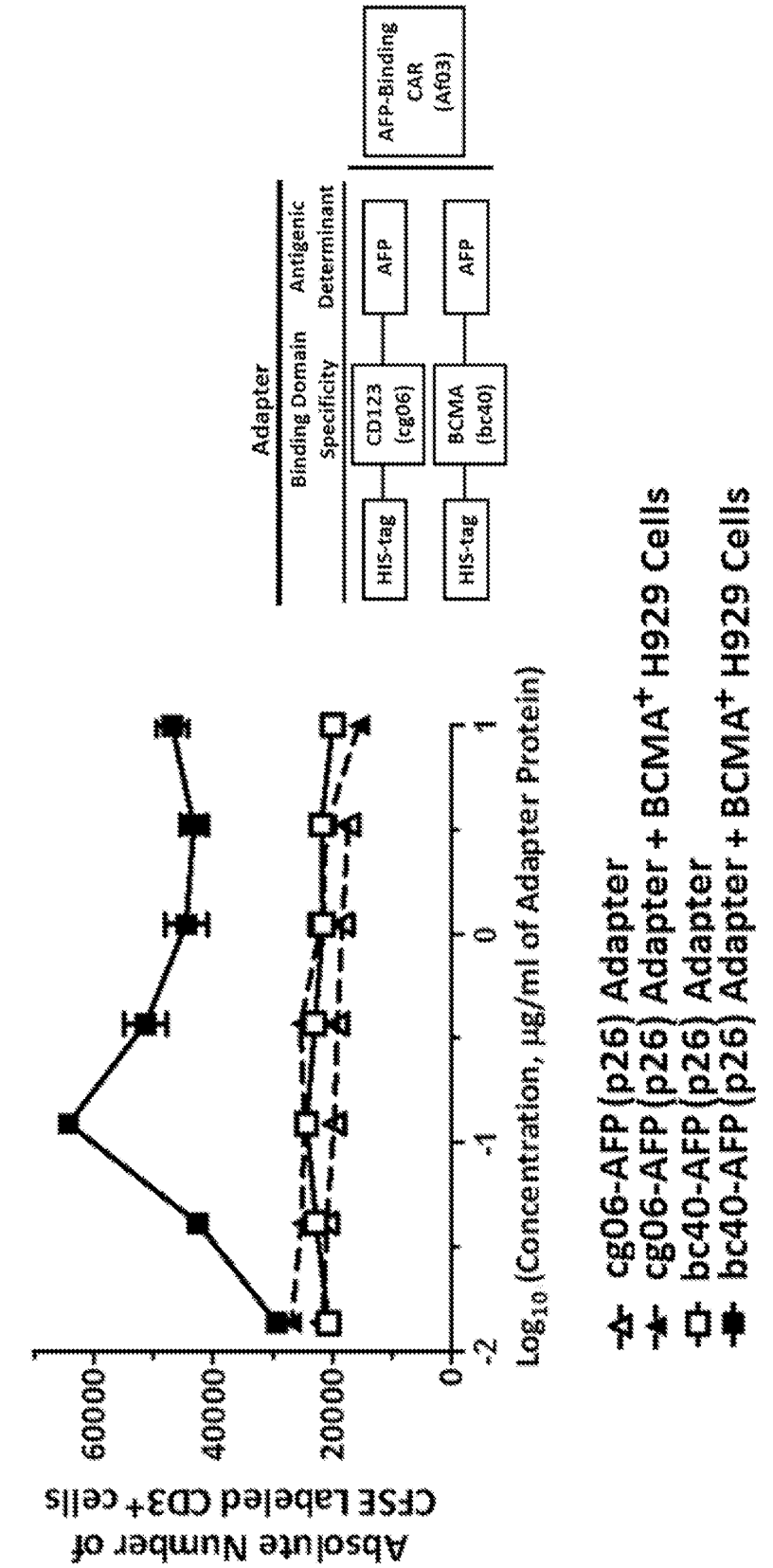

Proliferation Assay with Adapter Proteins at 72 Hours
Absolute Number of CFSE Labeled CD3+ cells
60000
40000
20000
0
-2
-1
0
1
Log10 (Concentration, µg/ml of Adapter Protein)
cg06-AFP (p26) Adapter
cg06-AFP (p26) Adapter + BCMA+ H929 Cells
bc40-AFP (p26) Adapter
bc40-AFP (p26) Adapter + BCMA+ H929 Cells
Adapter
Binding Domain Specificity
Antigenic Determinant
HIS-tag
CD123 (cg06)
AFP
HIS-tag
BCMA (bc40)
AFP
AFP-Binding CAR (Af03)

FIG. 6A

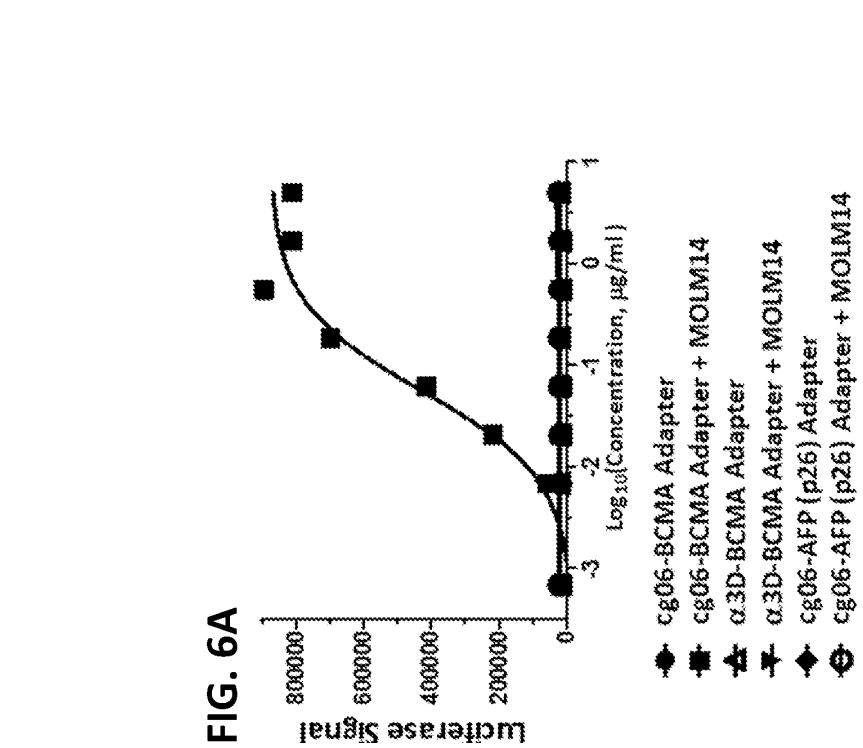

Luciferase Signal
800000
600000
400000
200000
0
-3
-2
-1
0
1
Log10(Concentration, µg/ml)
cg06-BCMA Adapter
cg06-BCMA Adapter + MOLM14
α3D-BCMA Adapter
α3D-BCMA Adapter + MOLM14
cg06-AFP (p26) Adapter
cg06-AFP (p26) Adapter + MOLM14
Adapter
Binding Domain Specificity
Antigenic Determinant
CD123 (cg06)
BCMA
HIS-tag
none (α3D)
BCMA
HIS-tag
CD123 (cg06)
AFP
HIS-tag
BCMA-Specific (bc40) CAR Expressing Jurkat Reporter Cells

FIG. 6B

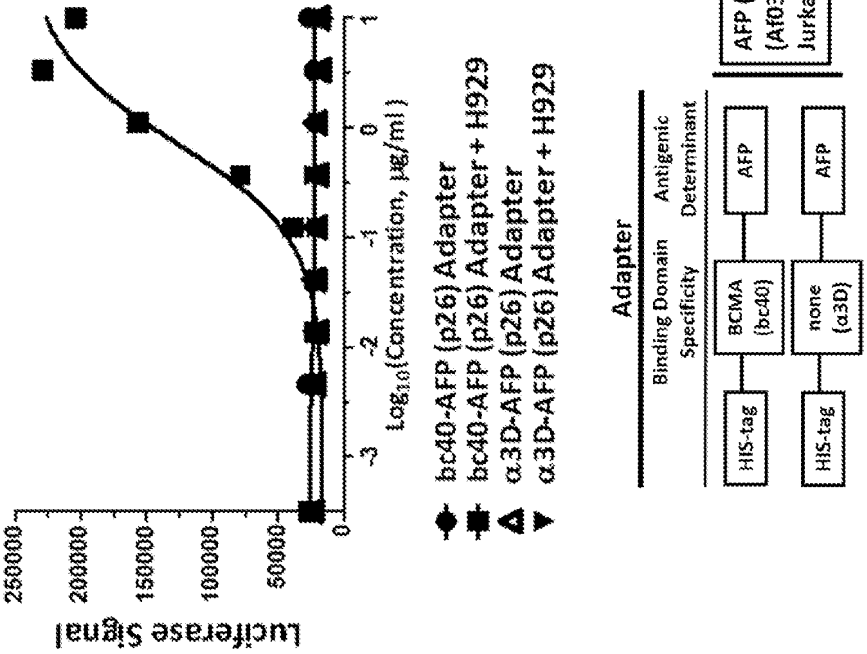

Luciferase Signal
250000
200000
150000
100000
50000
0
-3
-2
-1
0
1
Log10(Concentration, µg/ml)
bc40-AFP (p26) Adapter
bc40-AFP (p26) Adapter + H929
α3D-AFP (p26) Adapter
α3D-AFP (p26) Adapter + H929
Adapter
Binding Domain Specificity
Antigenic Determinant
HIS-tag
BCMA (bc40)
AFP
HIS-tag
none (α3D)
AFP
AFP (p26)-Specific (Af03) CAR Expressing Jurkat Reporter Cells

FIG. 7A

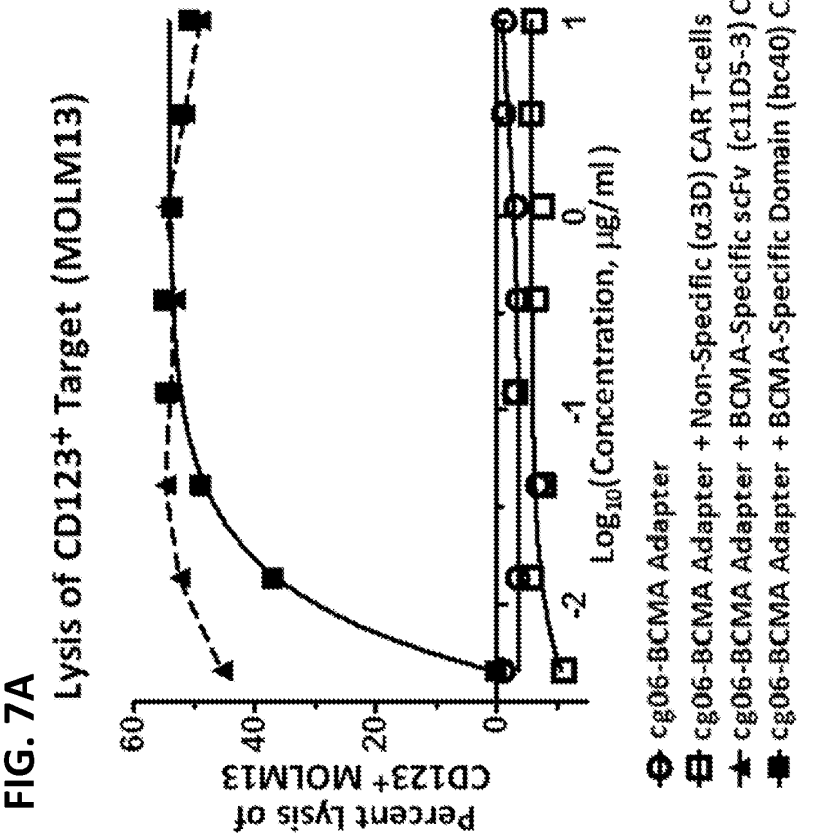

Lysis of CD123+ Target (MOLM13)
Percent Lysis of CD123+ MOLM13
60
40
20
0
-2
-1
0
1
Log10(Concentration, µg/ml)
cg06-BCMA Adapter
cg06-BCMA Adapter + Non-Specific (α3D) CAR T-cells
cg06-BCMA Adapter + BCMA-Specific scFv (c11D5-3) CAR T-cells
cg06-BCMA Adapter + BCMA-Specific Domain (bc40) CAR T-cells

FIG. 7B

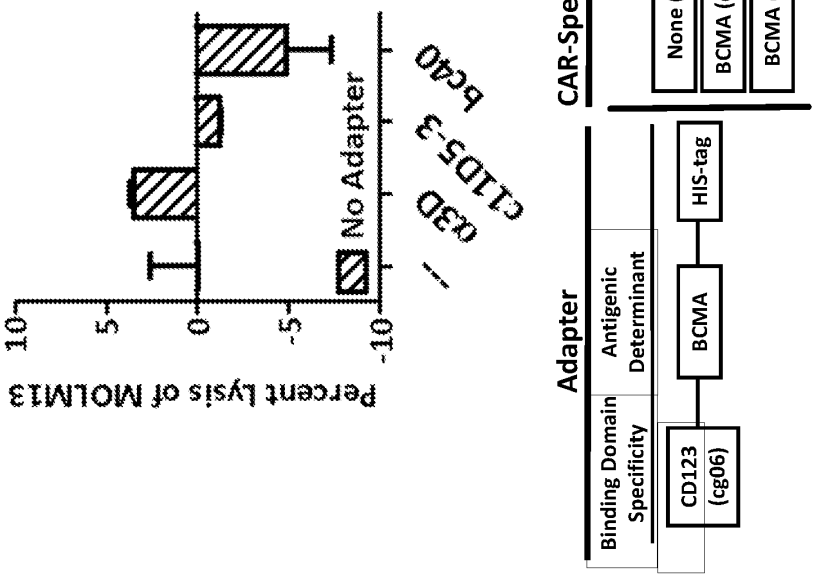

Percent Lysis of MOLM13
10
5
0
-5
-10
No Adapter
α3D
c11D5-3
bc40
Adapter
Binding Domain Specificity
Antigenic Determinant
CD123 (cg06)
BCMA
HIS-tag
CAR-Specificity
None (α3D)
BCMA (c11D5-3)
BCMA (bc40)

FIG. 8A

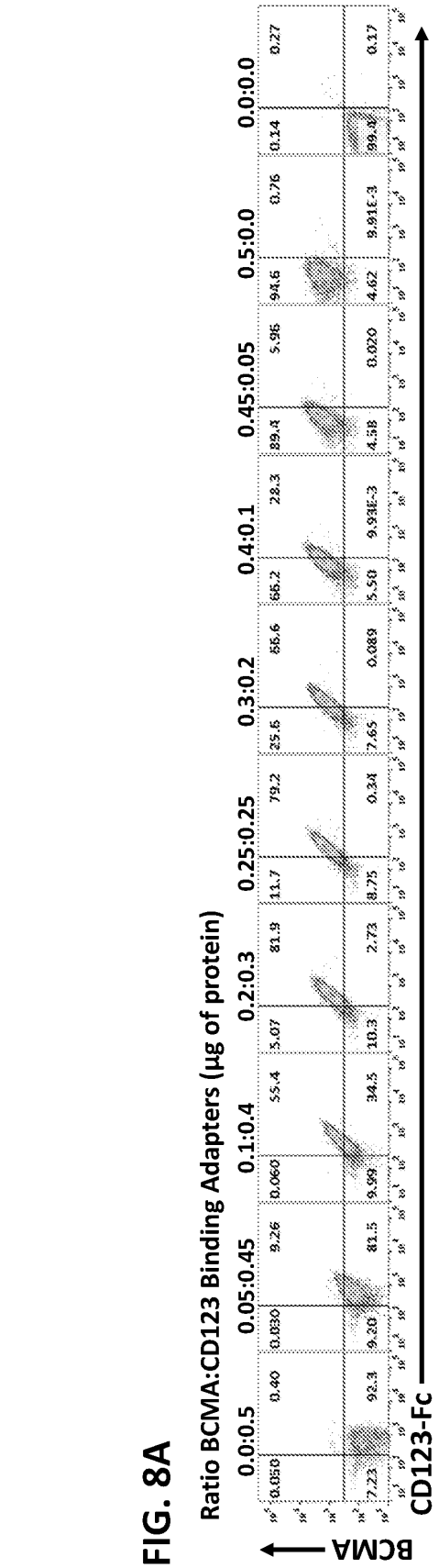

Ratio BCMA:CD123 Binding Adapters (µg of protein)
0.0:0.5
0.05:0.45
0.1:0.4
0.2:0.3
0.25:0.25
0.3:0.2
0.4:0.1
0.45:0.05
0.5:0.0
0.0:0.0
BCMA
CD123-Fc

FIG. 8B

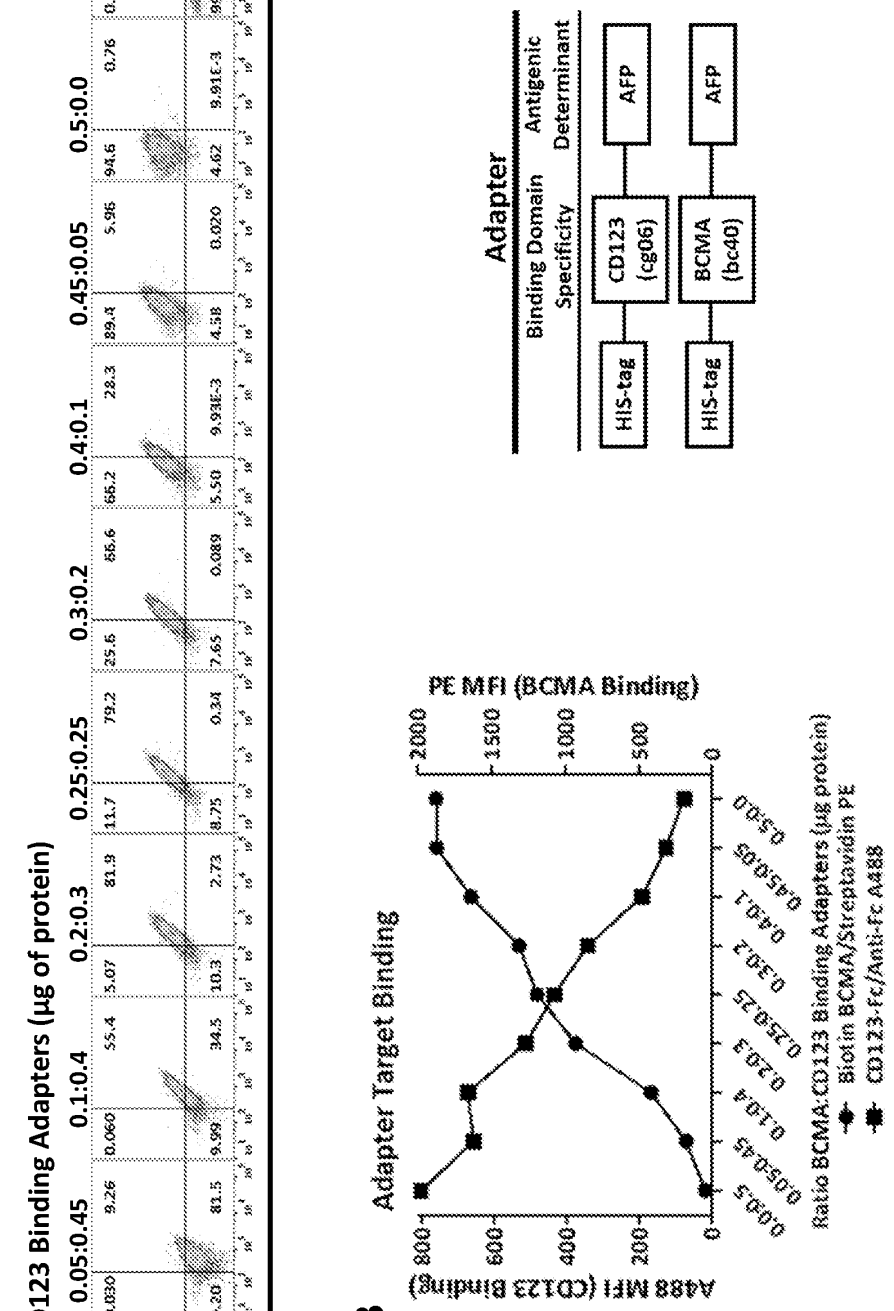

Adapter
Binding Domain Specificity
Antigenic Determinant
HIS-tag
CD123 (cg06)
AFP
HIS-tag
BCMA (bc40)
AFP
Adapter Target Binding
PE MFI (BCMA Binding)
A488 MFI (CD123 Binding)
Ratio BCMA:CD123 Binding Adapters (µg protein)
Biotin BCMA/Streptavidin PE
CD123-Fc/Anti-Fc A488

FIG. 9A

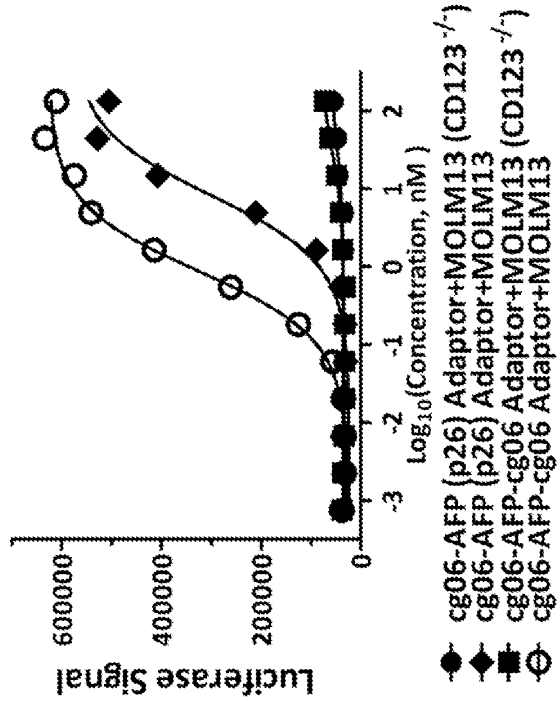

Luciferase Signal
600000
400000
200000
0
-3
-2
-1
0
1
2
Log10(Concentration, nM)
cg06-AFP (p26) Adaptor+MOLM13 (CD123 -/-)
cg06-AFP (p26) Adaptor+MOLM13
cg06-AFP-cg06 Adaptor+MOLM13 (CD123 -/-)
cg06-AFP-cg06 Adaptor+MOLM13
Adapter
Binding Domain Specificity
Antigenic Determinant
Binding Domain Specificity
HIS-tag
CD123 (cg06)
AFP
CD123 (cg06)
HIS-tag
CD123 (cg06)
AFP
CD123 (cg06)

FIG. 9B

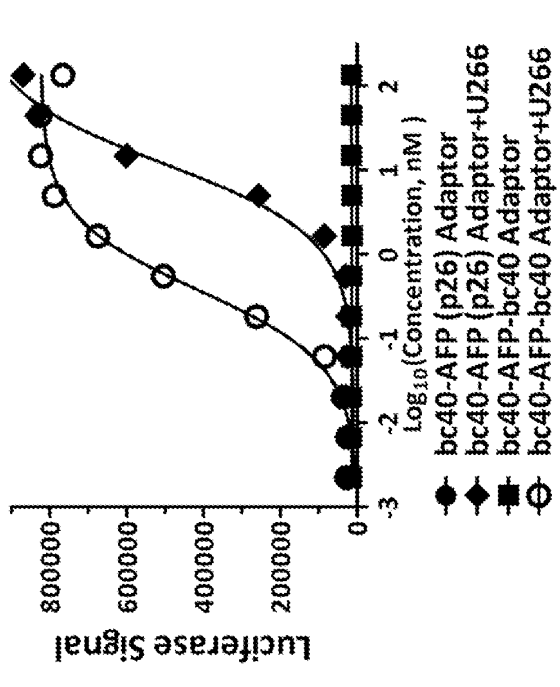

Luciferase Signal
800000
600000
400000
200000
0
-3
-2
-1
0
1
2
Log10(Concentration, nM)
bc40-AFP (p26) Adaptor
bc40-AFP (p26) Adaptor+U266
bc40-AFP-bc40 Adaptor
bc40-AFP-bc40 Adaptor+U266
Adapter
Binding Domain Specificity
Antigenic Determinant
Binding Domain Specificity
HIS-tag
BCMA (bc40)
AFP
BCMA (bc40)
HIS-tag
BCMA (bc40)
AFP
BCMA (bc40)

FIG. 10

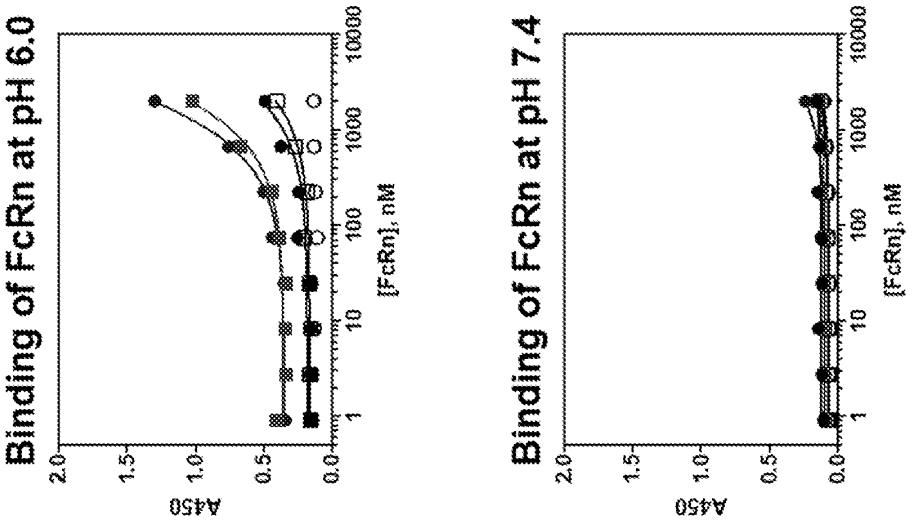

Binding of FcRn at pH 6.0
Binding of FcRn at pH 7.4
A450
[FcRn], nM
Well Coating
BC40-P26 SEQ ID NO: 1085
BC98-P26(Q26-V229)-BC98 SEQ ID NO: 2032
HSA
BC40 SEQ ID NO: 197 with his-tag
No Coating

FIG. 11A

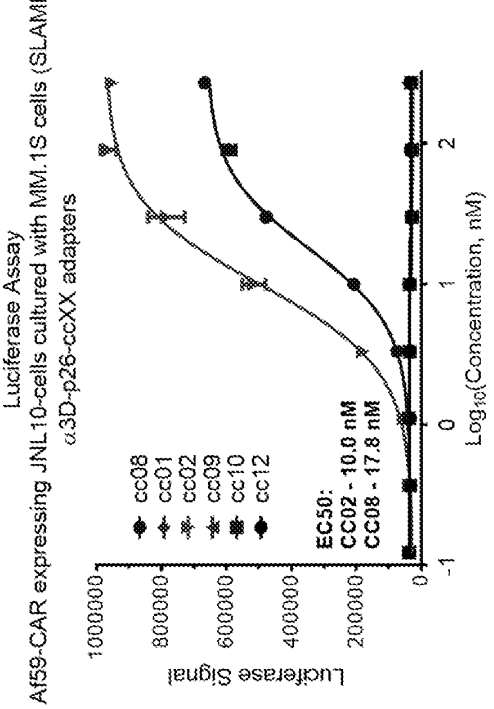


FIG. 11B

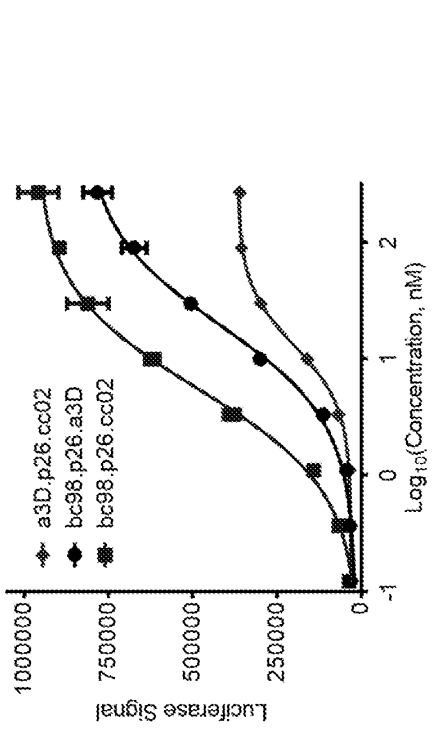


FIG. 11C

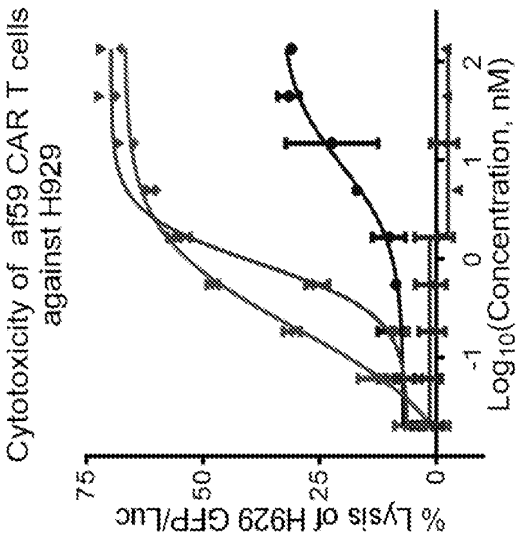


FIG. 11D

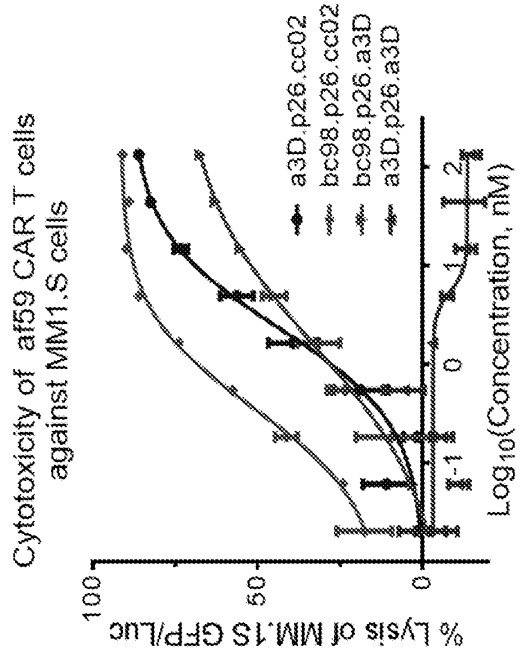


FIG. 12A

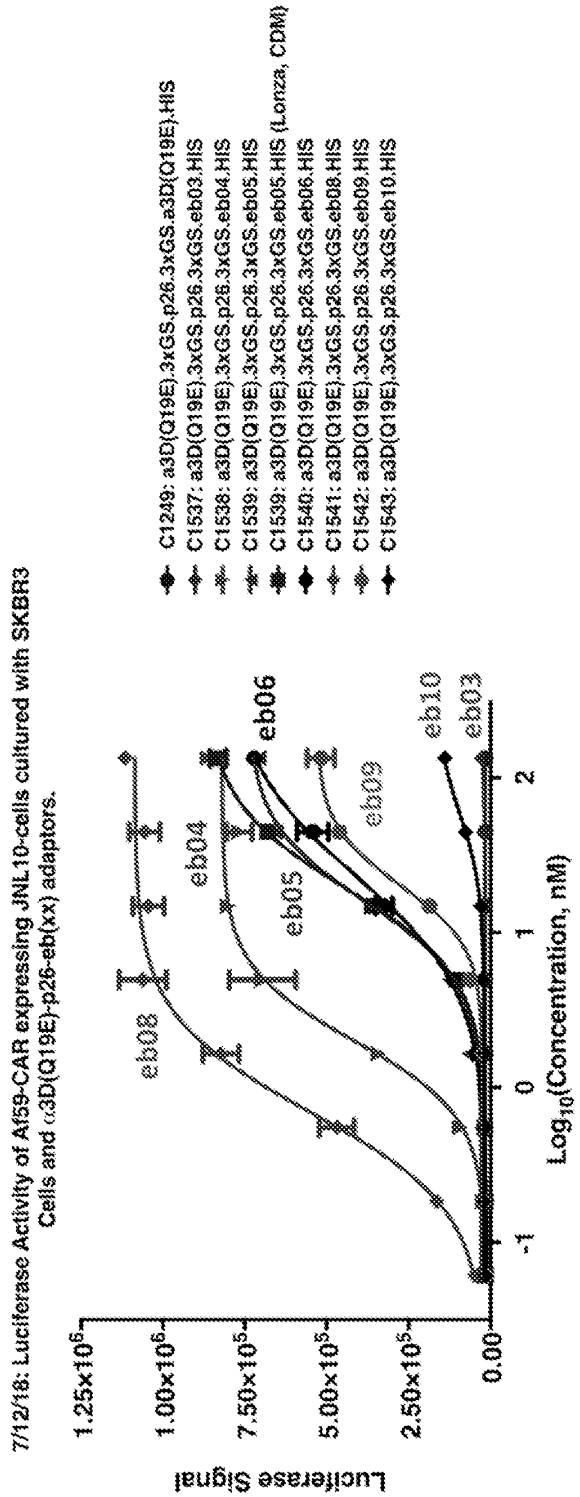

7/12/18: Luciferase Activity of A159-CAR expressing JNL10-cells cultured with SKBR3 Cells and α3D(Q19E)-p26-eb(xx) adaptors.
Luciferase Signal
1.25x10^6
1.00x10^6
7.50x10^5
5.00x10^5
2.50x10^5
0.00
Log10(Concentration, nM)
-1
0
1
2
eb08
eb04
eb06
eb05
eb09
eb10
eb03
C1249: a3D(Q19E).3xGS.p26.3xGS.a3D(Q19E).HIS
C1537: a3D(Q19E).3xGS.p26.3xGS.eb03.HIS
C1538: a3D(Q19E).3xGS.p26.3xGS.eb04.HIS
C1539: a3D(Q19E).3xGS.p26.3xGS.eb05.HIS
C1539: a3D(Q19E).3xGS.p26.3xGS.eb05.HIS (Lonza, CDM)
C1540: a3D(Q19E).3xGS.p26.3xGS.eb06.HIS
C1541: a3D(Q19E).3xGS.p26.3xGS.eb08.HIS
C1542: a3D(Q19E).3xGS.p26.3xGS.eb09.HIS
C1543: a3D(Q19E).3xGS.p26.3xGS.eb10.HIS

FIG. 12B

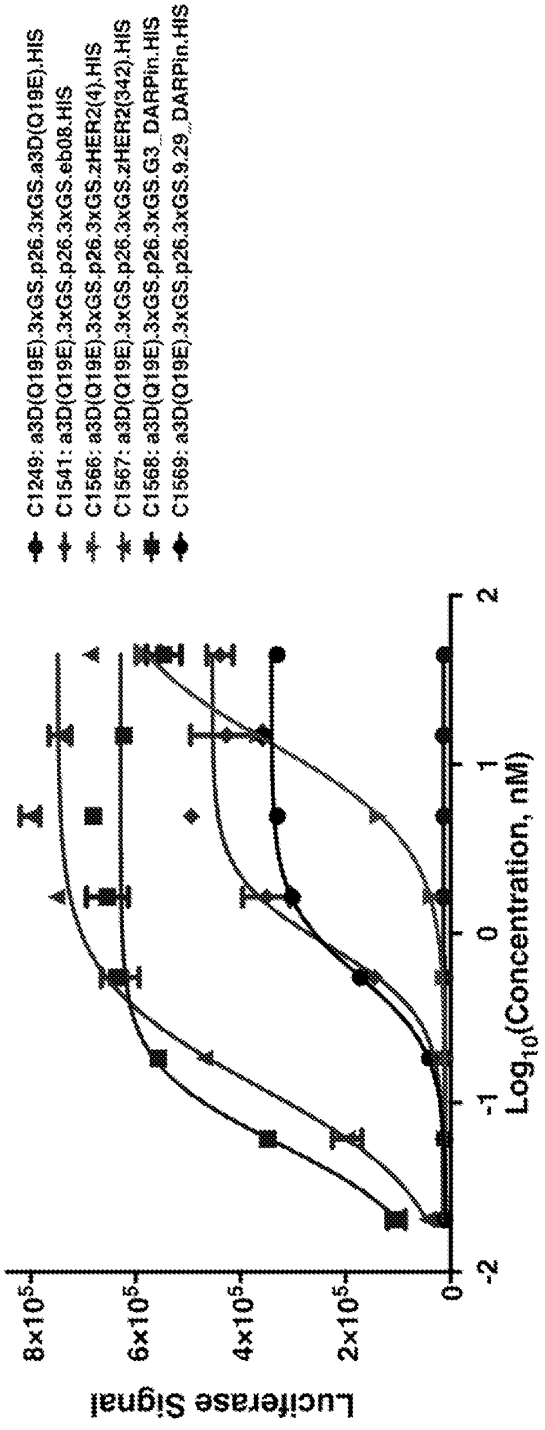
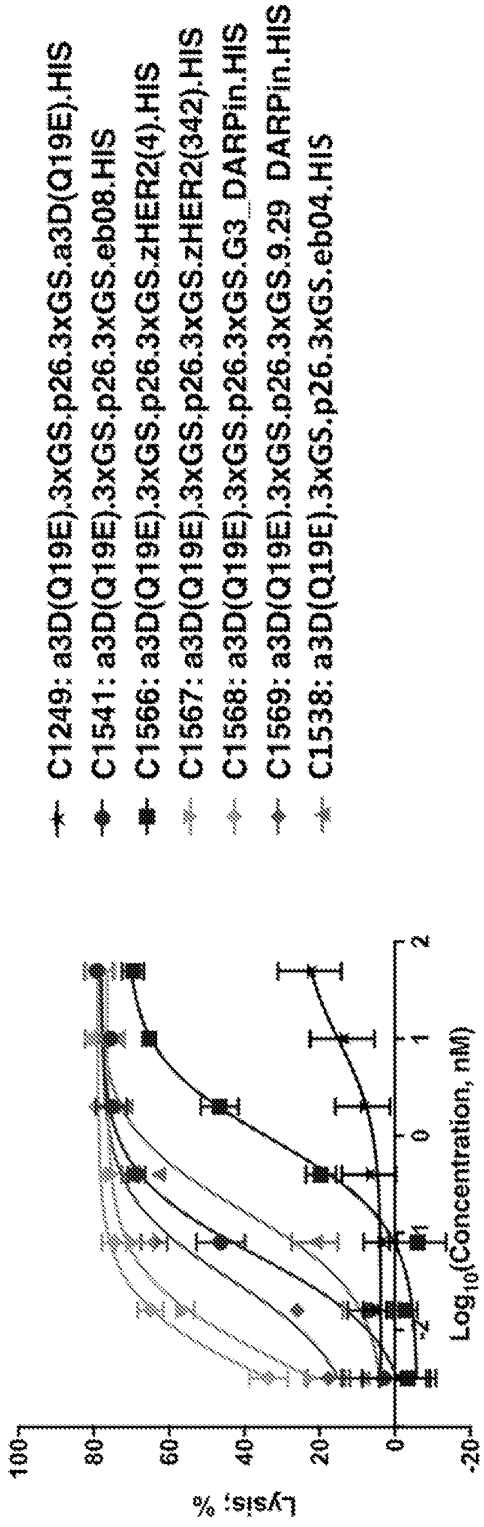

C1249: a3D(Q19E).3xGS.p26.3xGS.a3D(Q19E).HIS
C1541: a3D(Q19E).3xGS.p26.3xGS.eb08.HIS
C1566: a3D(Q19E).3xGS.p26.3xGS.zHER2(4).HIS
C1567: a3D(Q19E).3xGS.p26.3xGS.zHER2(342).HIS
C1568: a3D(Q19E).3xGS.p26.3xGS.G3_DARPin.HIS
C1569: a3D(Q19E).3xGS.p26.3xGS.9.29_DARPin.HIS
Luciferase Signal
8x10^5
6x10^5
4x10^5
2x10^5
0
-2
-1
0
1
2
Log10(Concentration, nM)

FIG. 12C


C1249: a3D(Q19E).3xGS.p26.3xGS.a3D(Q19E).HIS
C1541: a3D(Q19E).3xGS.p26.3xGS.eb08.HIS
C1566: a3D(Q19E).3xGS.p26.3xGS.zHER2(4).HIS
C1567: a3D(Q19E).3xGS.p26.3xGS.zHER2(342).HIS
C1568: a3D(Q19E).3xGS.p26.3xGS.G3_DARPin.HIS
C1569: a3D(Q19E).3xGS.p26.3xGS.9.29 DARPin.HIS
C1538: a3D(Q19E).3xGS.p26.3xGS.eb04.HIS
Lysis; %
100
80
60
40
20
0
-20
-2
-1
0
1
2
Log10(Concentration, nM)

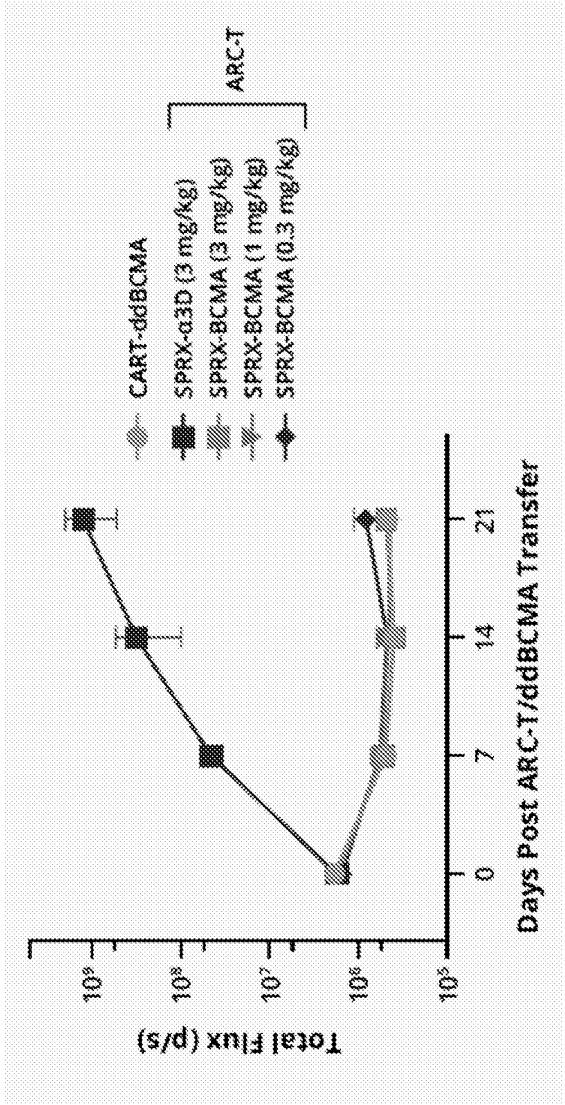

ARC-T
CART-ddBCMA
SPRX-α3D (3 mg/kg)
SPRX-BCMA (3 mg/kg)
SPRX-BCMA (1 mg/kg)
SPRX-BCMA (0.3 mg/kg)
Total Flux (p/s)
10^9
10^8
10^7
10^6
10^5
0
7
14
21
Days Post ARC-T/ddBCMA Transfer

FIG. 13

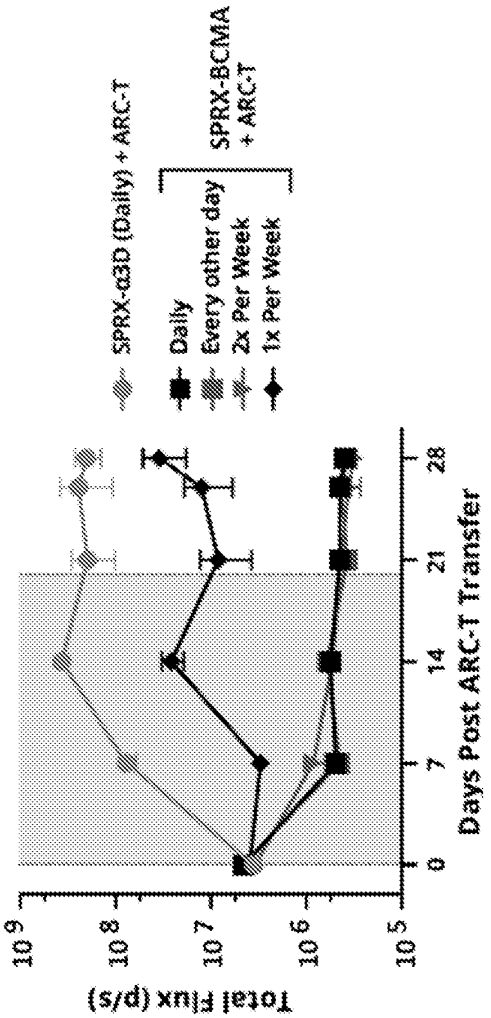

SPRX-α3D (Daily) + ARC-T
Daily
Every other day
2x Per Week
1x Per Week
SPRX-BCMA + ARC-T
Total Flux (p/s)
10 9
10 8
10 7
10 6
10 5
0
7
14
21
28
Days Post ARC-T Transfer

FIG. 14

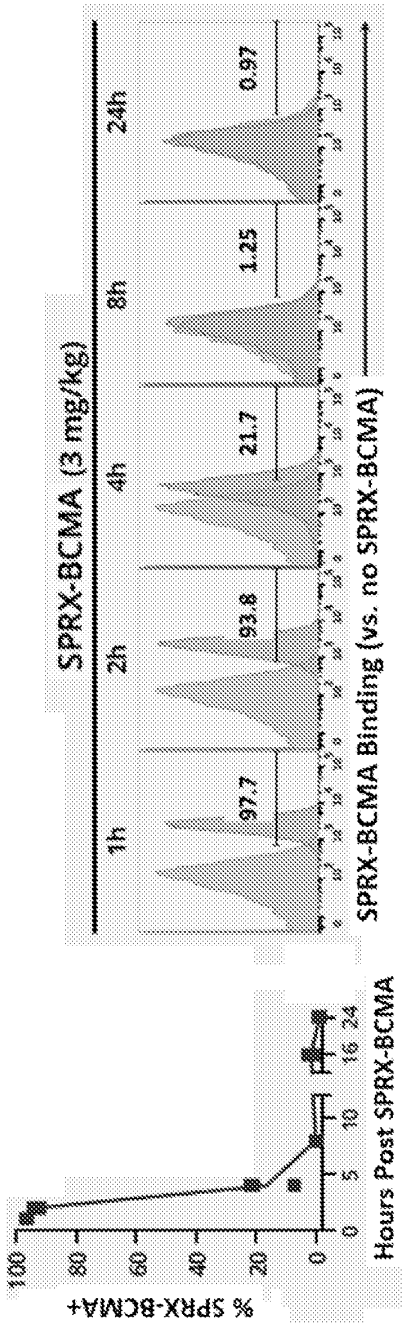

SPRX-BCMA (3 mg/kg)
1h
2h
4h
8h
24h
97.7
93.8
21.7
1.25
0.97
SPRX-BCMA Binding (vs. no SPRX-BCMA)
% SPRX-BCMA+
100
80
60
40
20
0
0
5
10
16
24
Hours Post SPRX-BCMA

FIG. 15