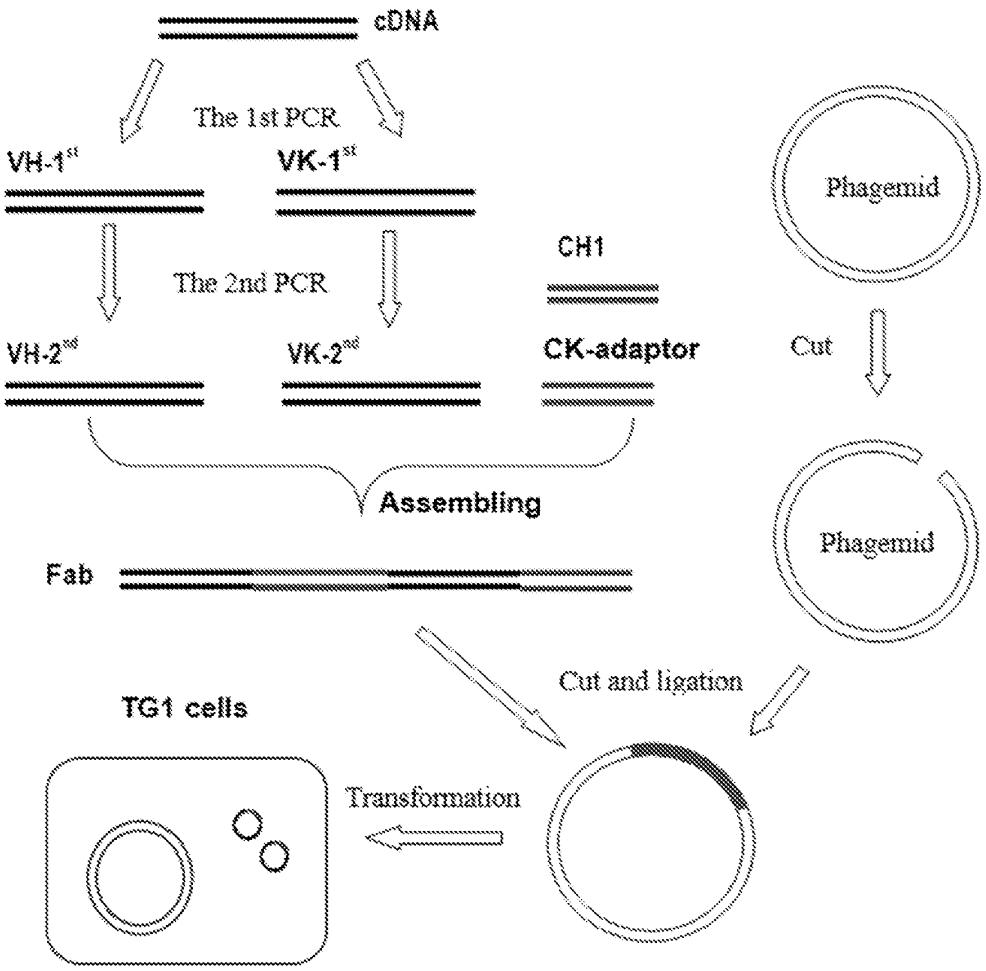

cDNA
The 1st PCR
VH-1st
VK-1st
The 2nd PCR
VH-2nd
VK-2nd
CH1
CK-adaptor
Assembling
Fab
Phagemid
Cut
Phagemid
Cut and ligation
TG1 cells
Transformation

FIG. 1

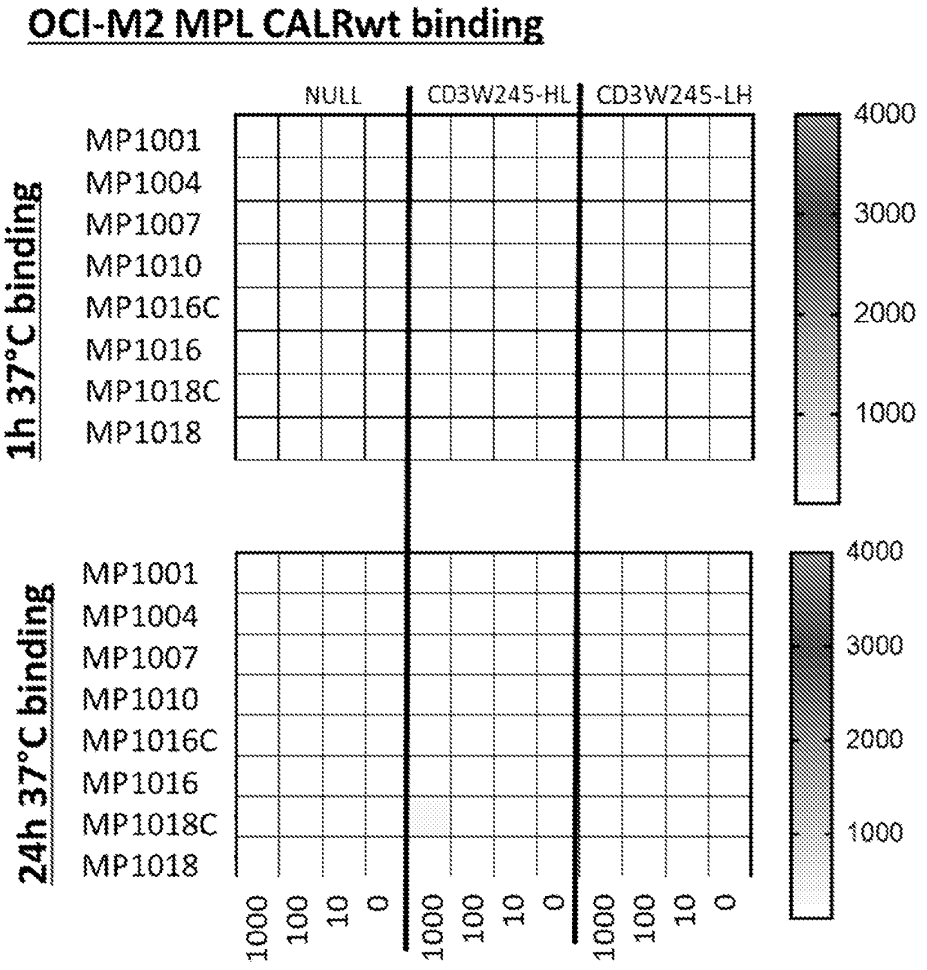

OCI-M2 MPL CALRwt binding
NULL
CD3W245-HL
CD3W245-LH
1h 37°C binding
24h 37°C binding
MP1001
MP1004
MP1007
MP1010
MP1016C
MP1016
MP1018C
MP1018
4000
3000
2000
1000
1000
100
10
0

FIG. 2A

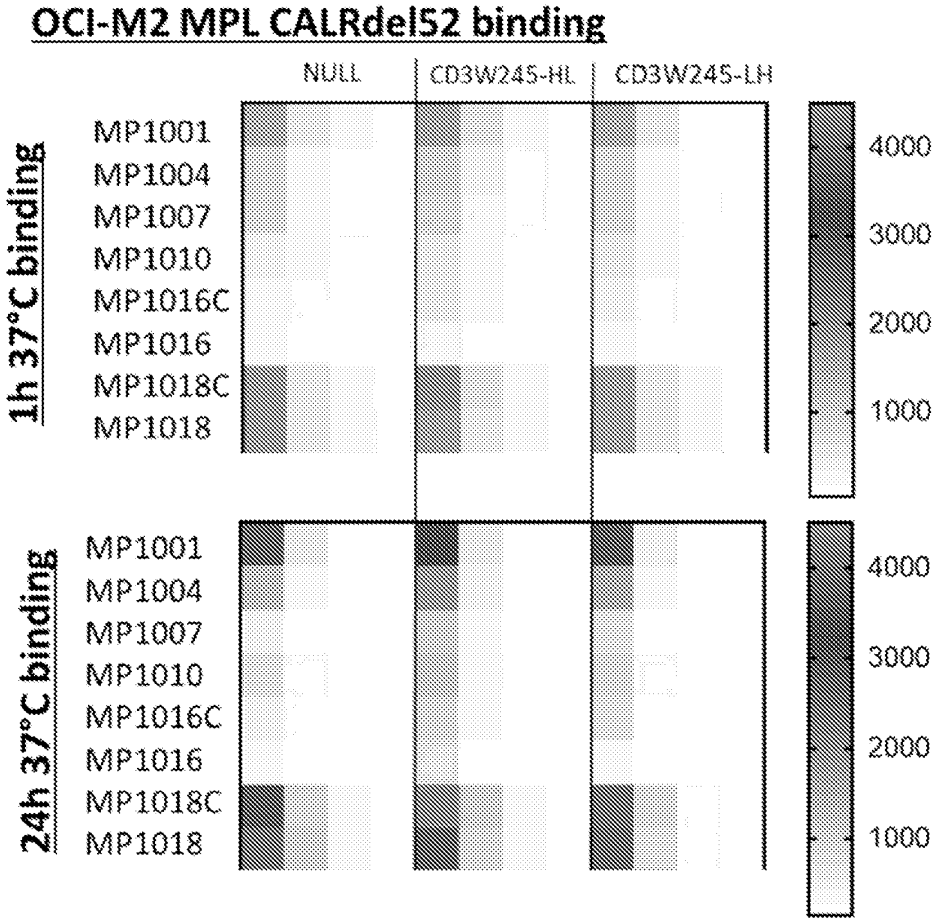

OCI-M2 MPL CALRdel52 binding
NULL
CD3W245-HL
CD3W245-LH
1h 37°C binding
MP1001
MP1004
MP1007
MP1010
MP1016C
MP1016
MP1018C
MP1018
4000
3000
2000
1000
24h 37°C binding
MP1001
MP1004
MP1007
MP1010
MP1016C
MP1016
MP1018C
MP1018
4000
3000
2000
1000

FIG. 2B

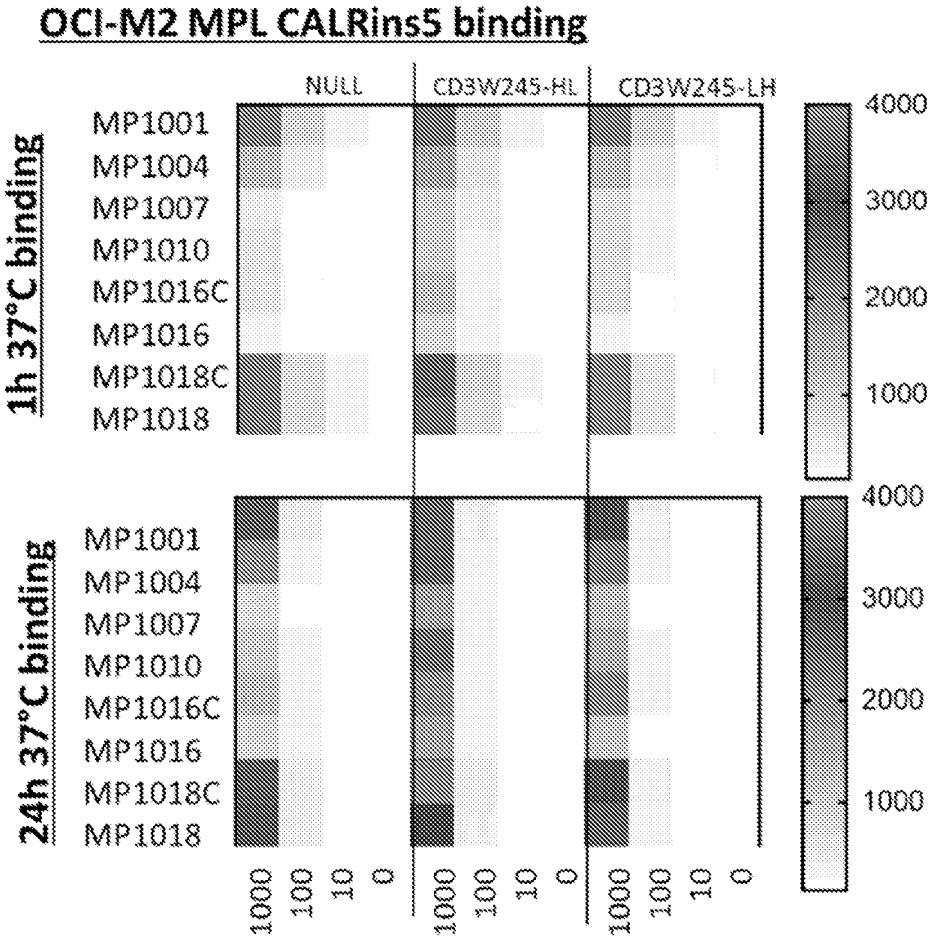
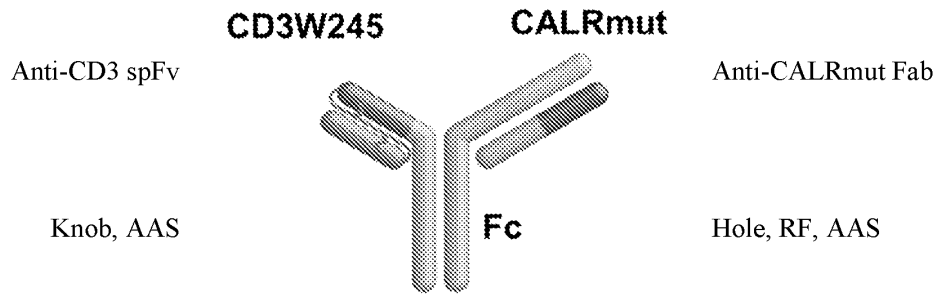

OCI-M2 MPL CALRins5 binding
NULL
CD3W245-HL
CD3W245-LH
1h 37°C binding
24h 37°C binding
MP1001
MP1004
MP1007
MP1010
MP1016C
MP1016
MP1018C
MP1018
4000
3000
2000
1000
1000
100
10
0

FIG. 2C



AAS (L234A, L235A, D265S); CALRmut, mutant calreticulin; CD, cluster of differentiation; Fab, fragment antigen-binding; Fc, fragment crystallizable; RF, H435R, Y436F; spFv, stapled single chain fragment variable.

FIG. 3

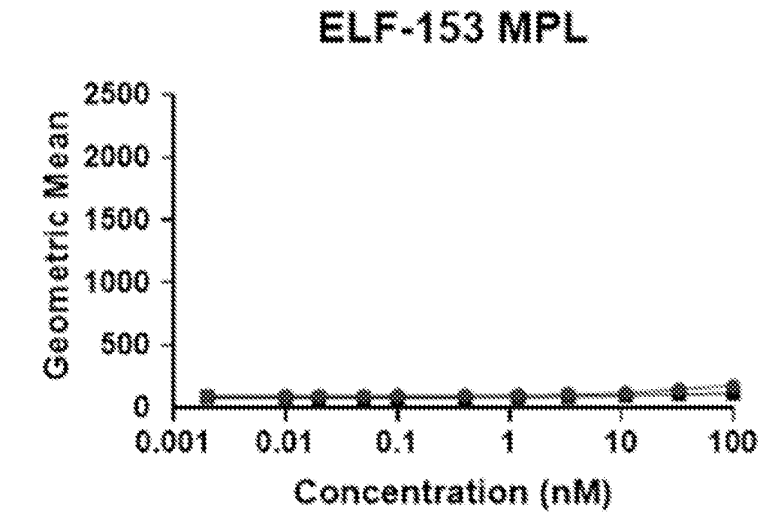

ELF-153 MPL
Geometric Mean
2500
2000
1500
1000
500
0
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4A

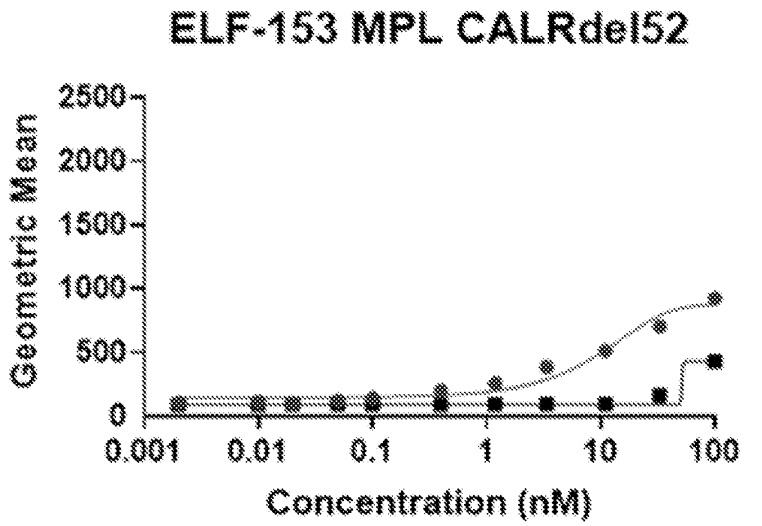

ELF-153 MPL CALRdel52
Geometric Mean
2500
2000
1500
1000
500
0
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4B

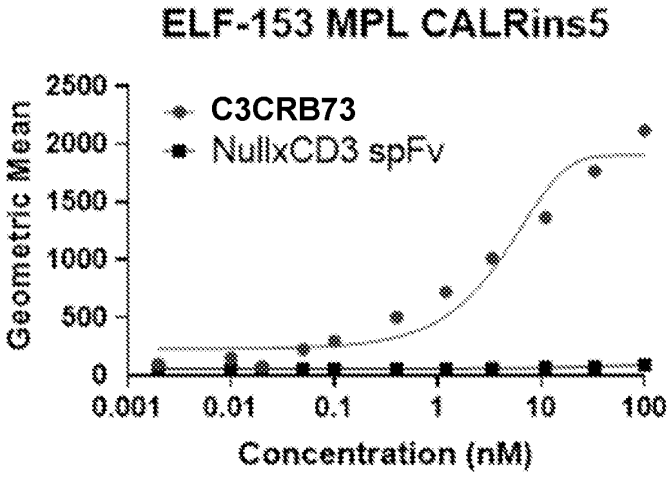

ELF-153 MPL CALRins5
C3CRB73
NullxCD3 spFv
Geometric Mean
2500
2000
1500
1000
500
0
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4C

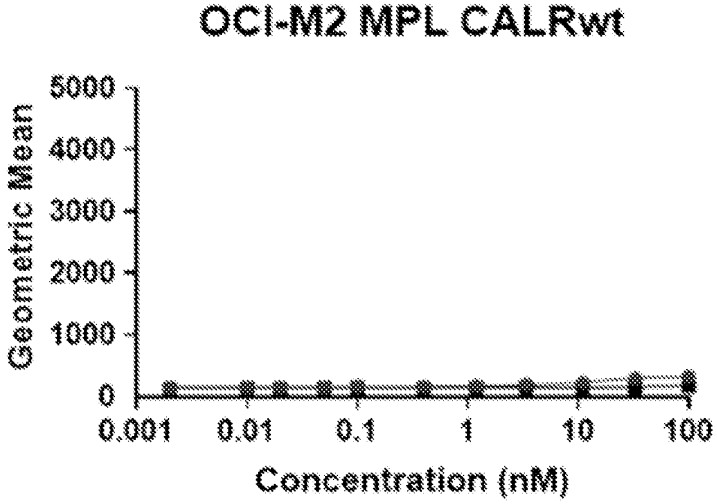

OCI-M2 MPL CALRwt
Geometric Mean
5000
4000
3000
2000
1000
0
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4D

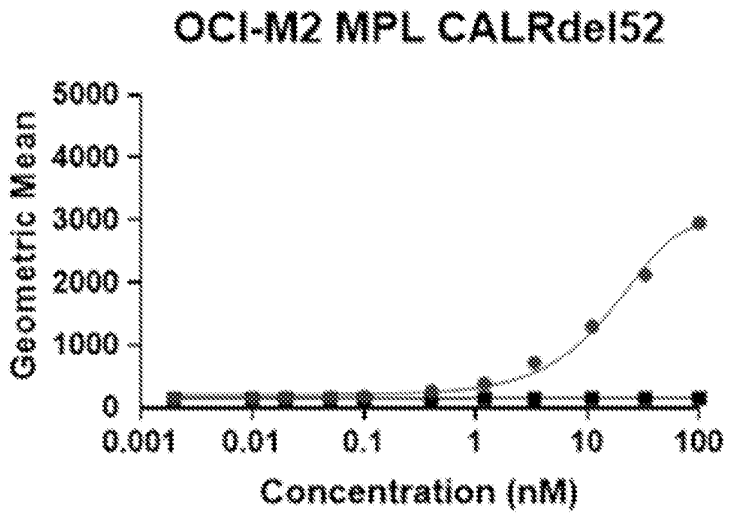

OCI-M2 MPL CALRdel52
Geometric Mean
5000
4000
3000
2000
1000
0
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4E

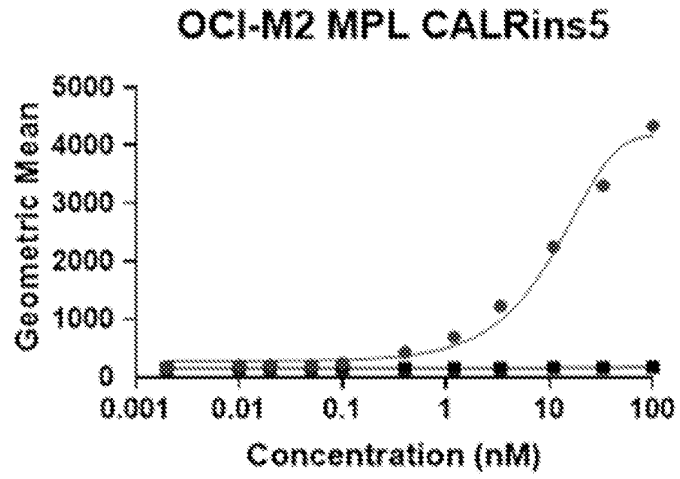

OCI-M2 MPL CALRins5
Geometric Mean
5000
4000
3000
2000
1000
0
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 4F

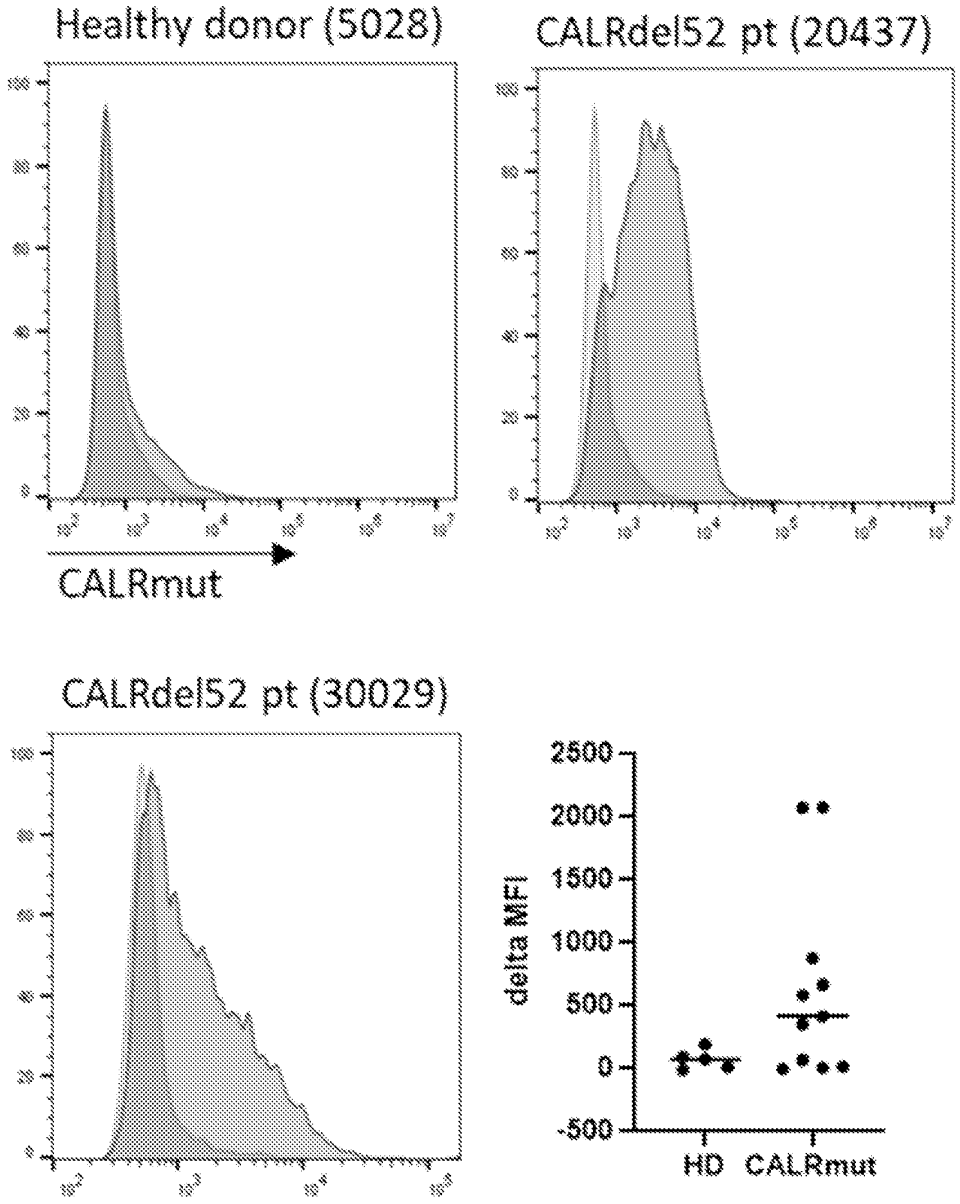

Healthy donor (5028)
CALRdel52 pt (20437)
CALRmut
CALRdel52 pt (30029)
delta MFI
2500
2000
1500
1000
500
0
-500
HD
CALRmut

FIG. 5

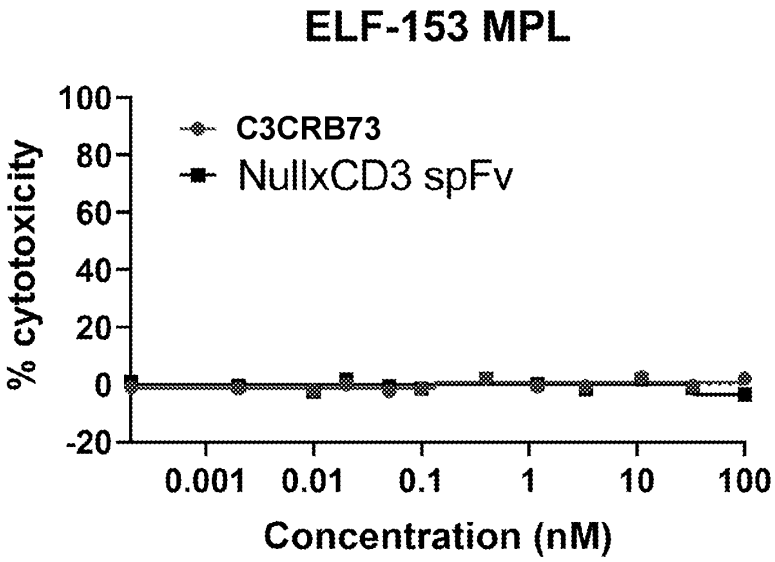

ELF-153 MPL
C3CRB73
NullxCD3 spFv
% cytotoxicity
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 6A

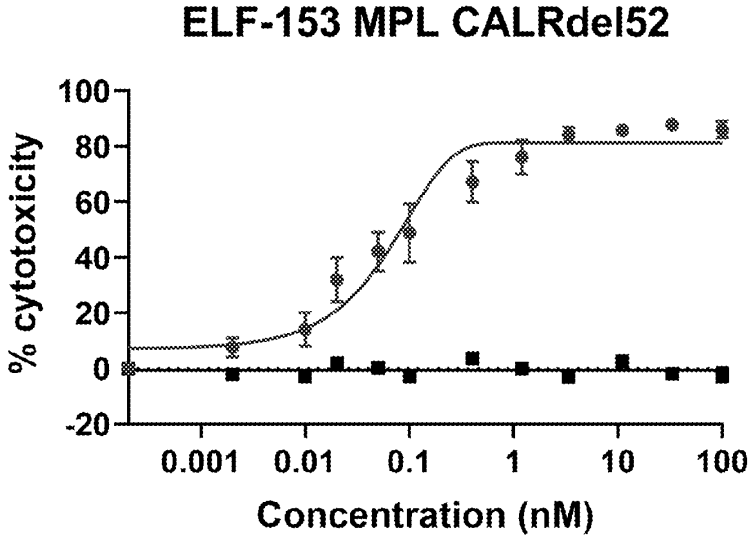

ELF-153 MPL CALRdel52
% cytotoxicity
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 6B

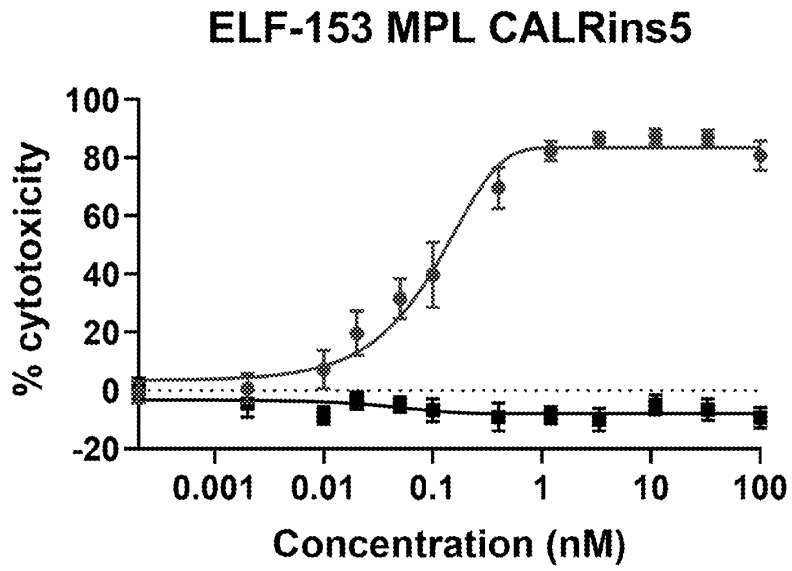

ELF-153 MPL CALRins5
% cytotoxicity
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 6C

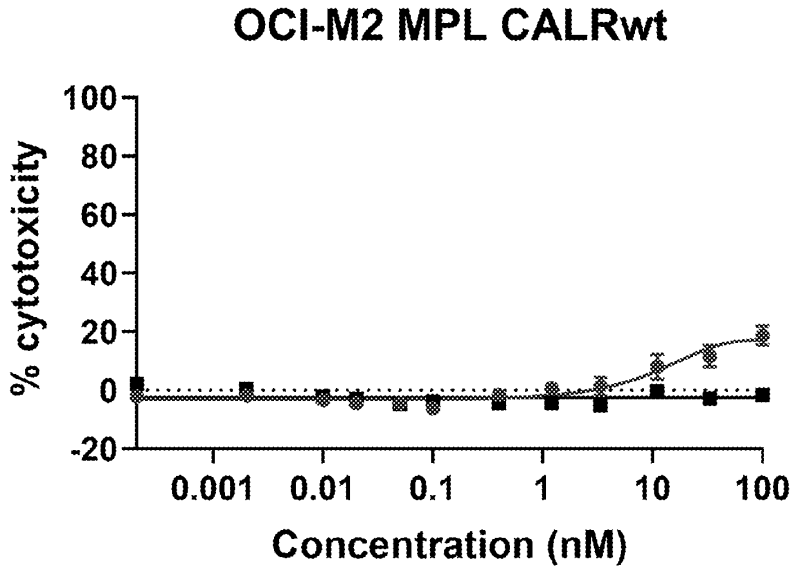

OCI-M2 MPL CALRwt
% cytotoxicity
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 6D

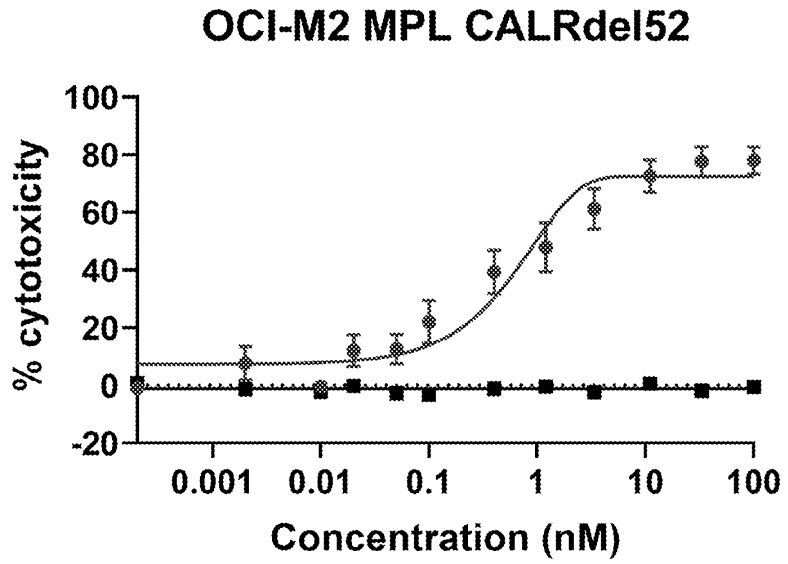

OCI-M2 MPL CALRdel52
% cytotoxicity
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 6E

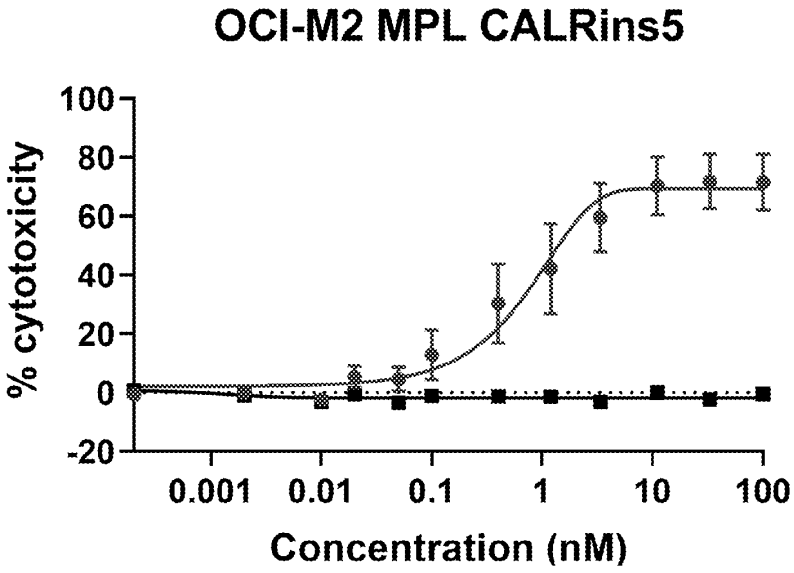

OCI-M2 MPL CALRins5
% cytotoxicity
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 6F

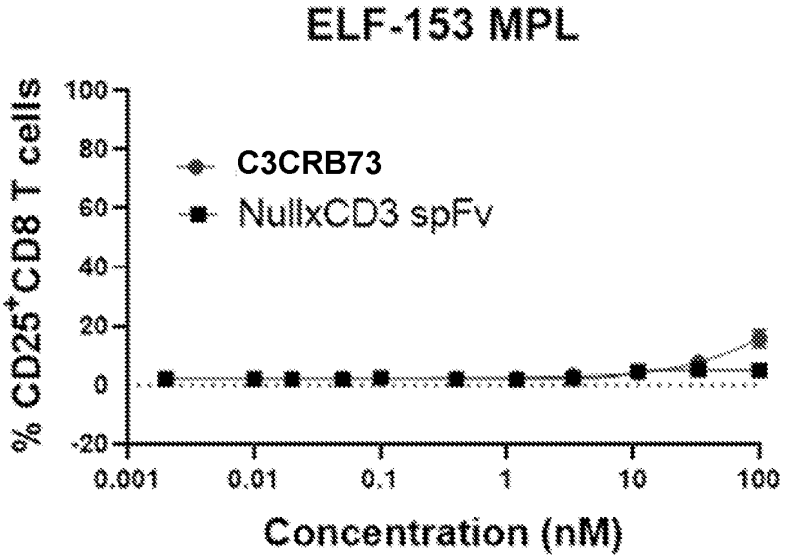

ELF-153 MPL
% CD25+CD8 T cells
100
80
60
40
20
0
-20
C3CRB73
NullxCD3 spFv
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 7A

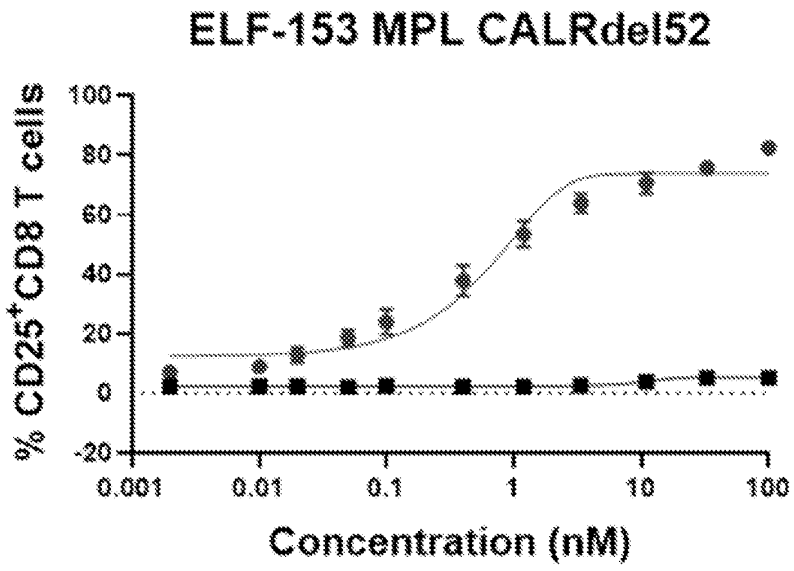

ELF-153 MPL CALRdel52
% CD25+CD8 T cells
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 7B

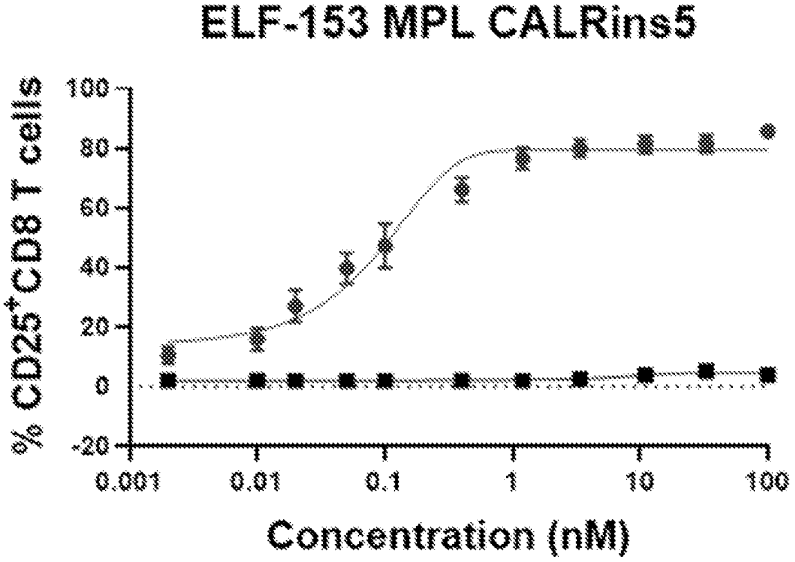

ELF-153 MPL CALRins5
% CD25+CD8 T cells
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 7C

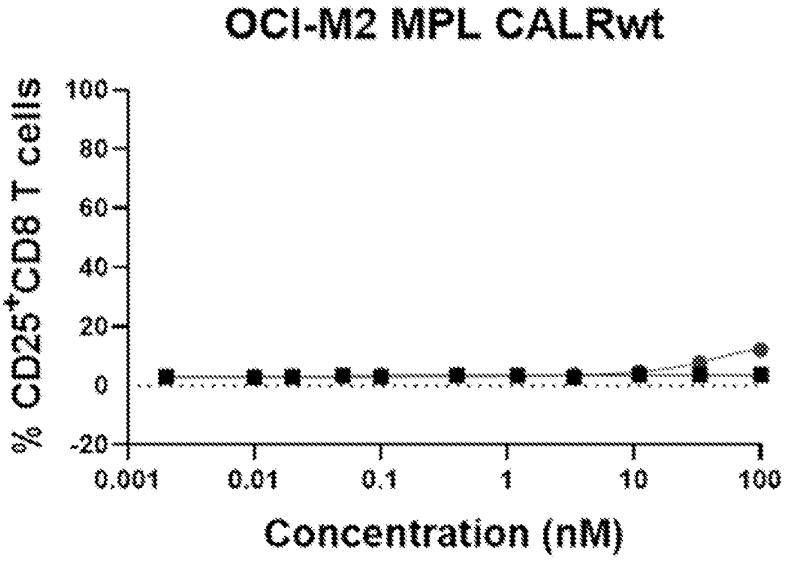

OCI-M2 MPL CALRwt
% CD25+CD8 T cells
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 7D

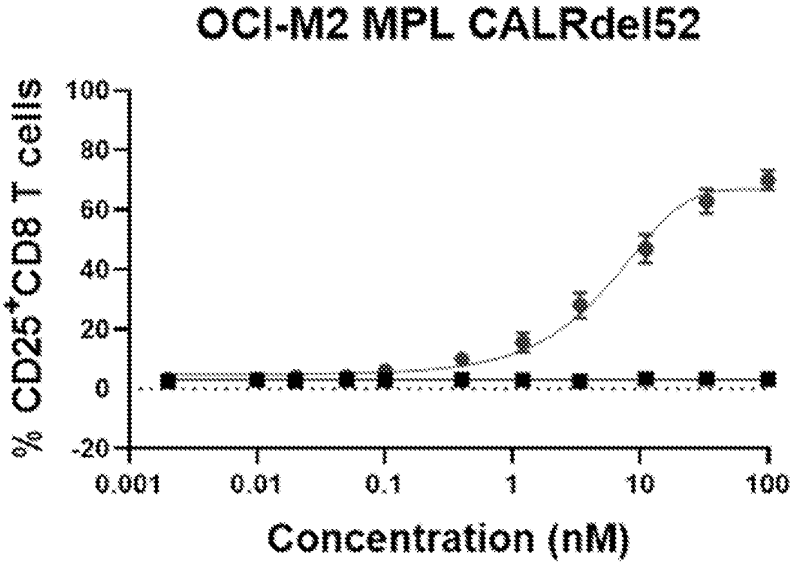

OCI-M2 MPL CALRdel52
% CD25+CD8 T cells
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 7E

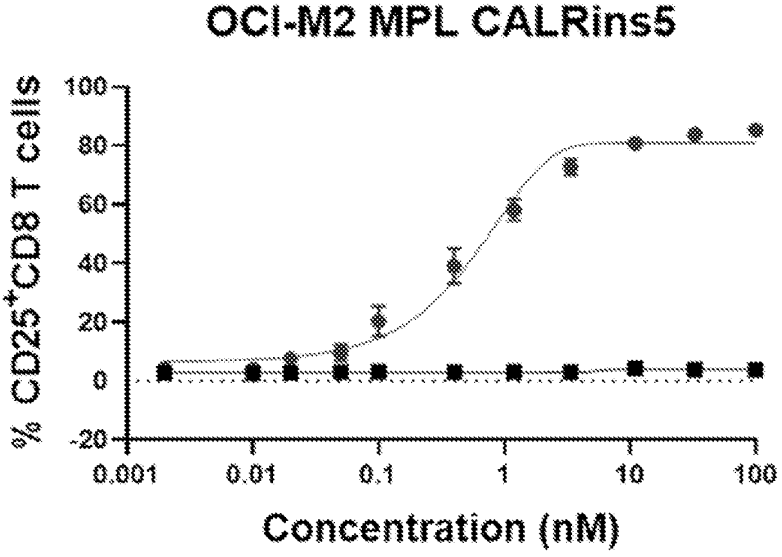

OCI-M2 MPL CALRins5
% CD25+CD8 T cells
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 7F

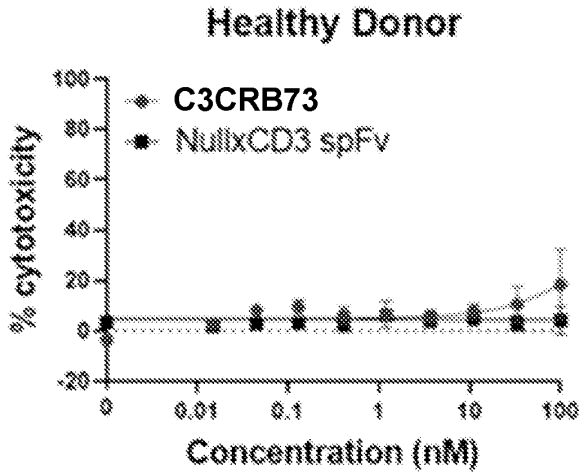

Healthy Donor
C3CRB73
NullxCD3 spFv
% cytotoxicity
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Concentration (nM)

FIG. 8A

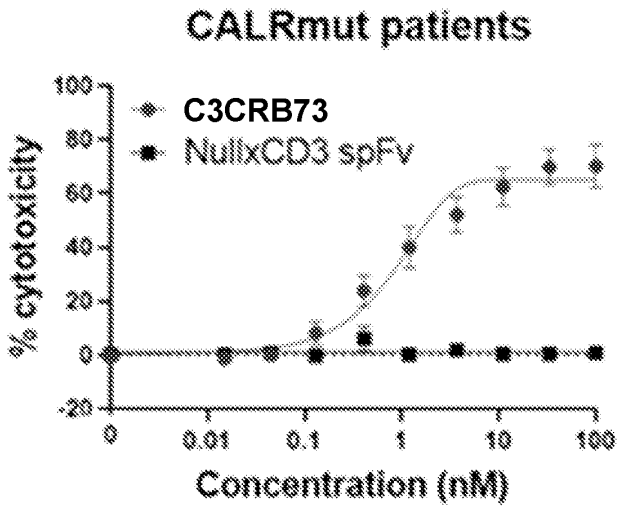

CALRmut patients
C3CRB73
NullxCD3 spFv
% cytotoxicity
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Concentration (nM)

FIG. 8B

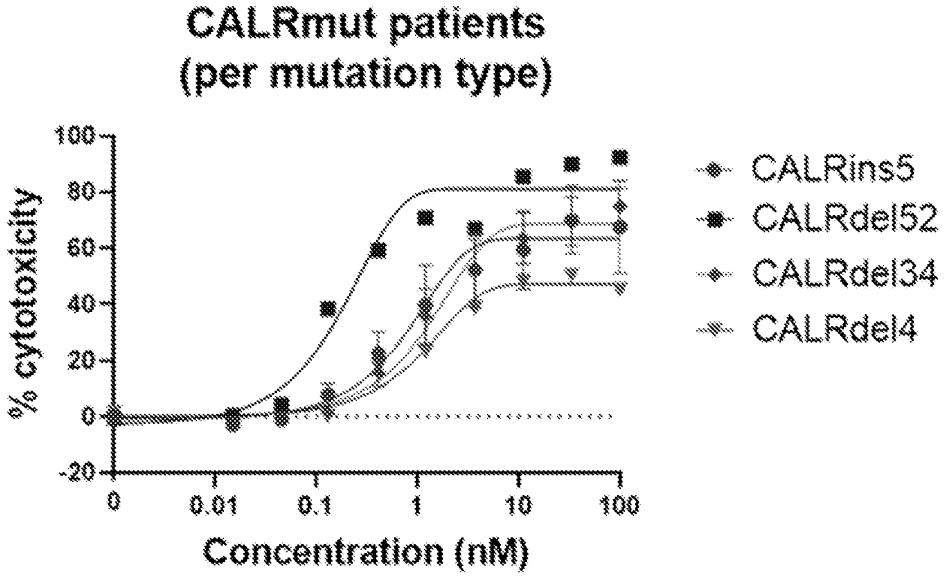

CALRmut patients
(per mutation type)
% cytotoxicity
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Concentration (nM)
CALRins5
CALRdel52
CALRdel34
CALRdel4

FIG. 8C

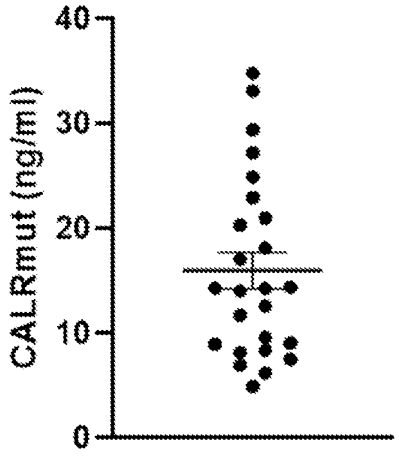


FIG. 9

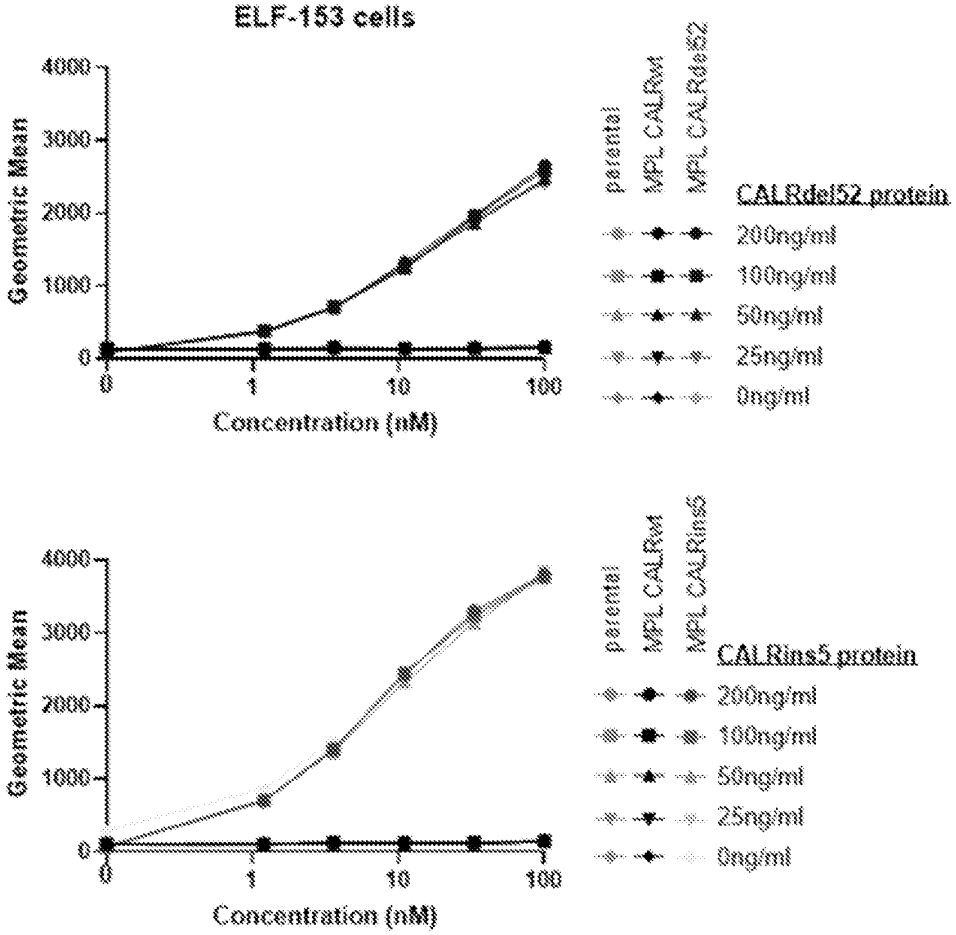

ELF-153 cells
Geometric Mean
4000
3000
2000
1000
0
0
1
10
100
Concentration (nM)
parental
MPL CALRwt
MPL CALRdel52
CALRdel52 protein
200ng/ml
100ng/ml
50ng/ml
25ng/ml
0ng/ml
Geometric Mean
4000
3000
2000
1000
0
0
1
10
100
Concentration (nM)
parental
MPL CALRwt
MPL CALRins5
CALRins5 protein
200ng/ml
100ng/ml
50ng/ml
25ng/ml
0ng/ml

FIG. 10A

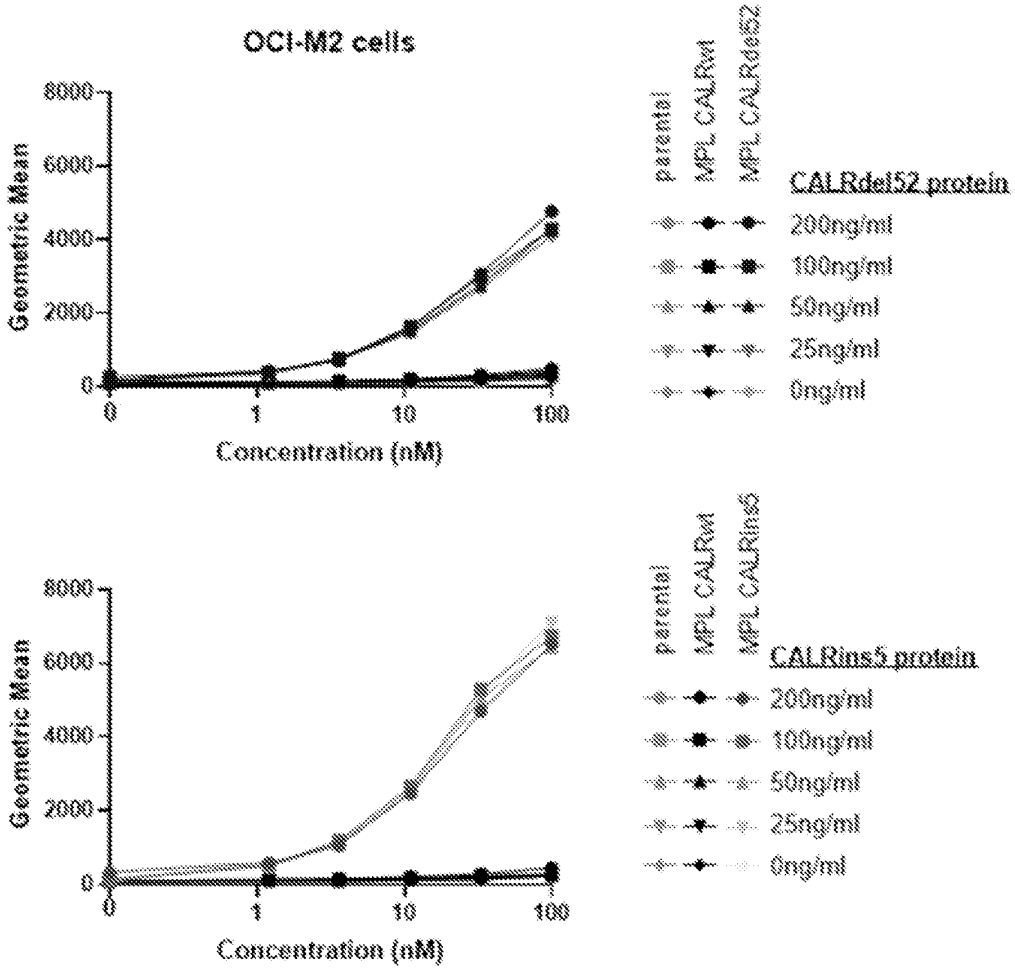

OCI-M2 cells
Geometric Mean
8000
6000
4000
2000
0
0
1
10
100
Concentration (nM)
parental
MPL CALRwt
MPL CALRdel52
CALRdel52 protein
200ng/ml
100ng/ml
50ng/ml
25ng/ml
0ng/ml
Geometric Mean
8000
6000
4000
2000
0
0
1
10
100
Concentration (nM)
parental
MPL CALRwt
MPL CALRins5
CALRins5 protein
200ng/ml
100ng/ml
50ng/ml
25ng/ml
0ng/ml

FIG. 10B

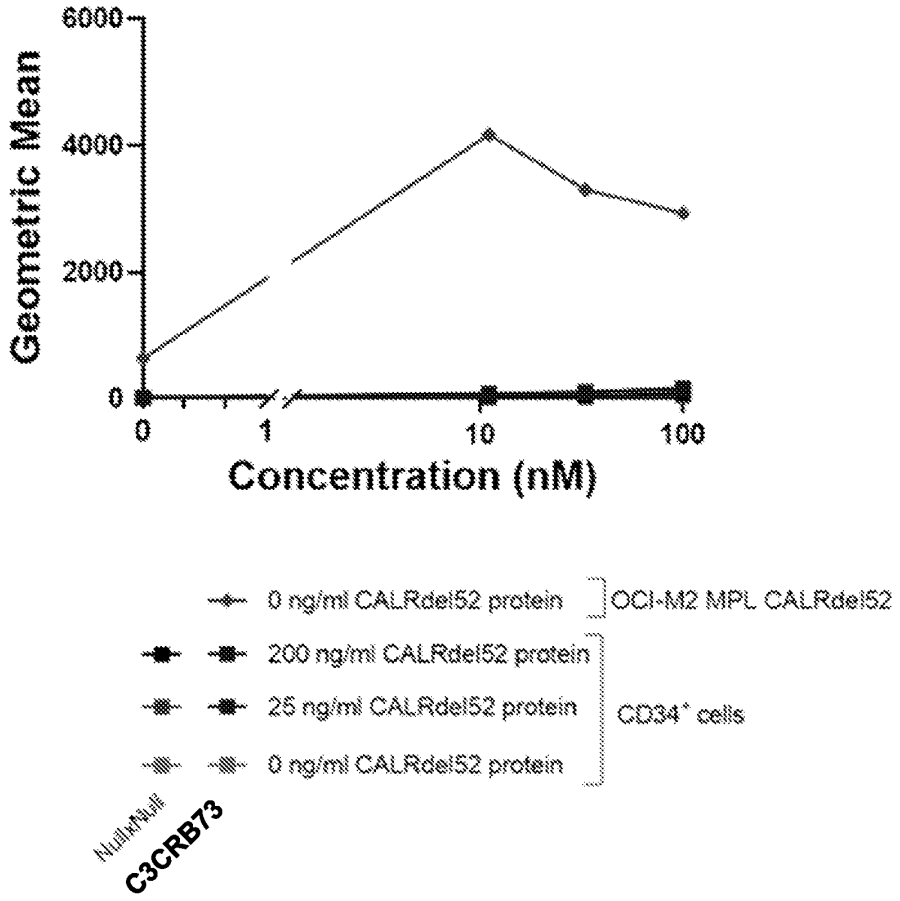

6000
4000
2000
0
Geometric Mean
0
1
10
100
Concentration (nM)
0 ng/ml CALRdel52 protein
OCI-M2 MPL CALRdel52
200 ng/ml CALRdel52 protein
25 ng/ml CALRdel52 protein
CD34+ cells
0 ng/ml CALRdel52 protein
NullMut
C3CRB73

FIG. 11A

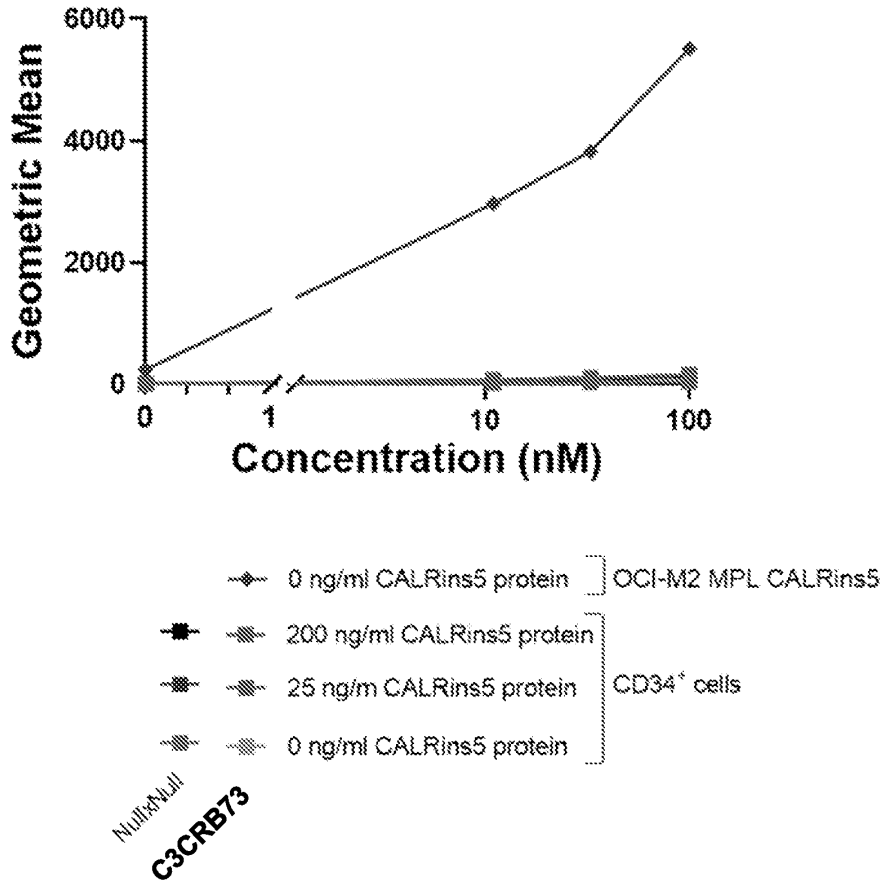

6000
4000
2000
0
Geometric Mean
0
1
10
100
Concentration (nM)
0 ng/ml CALRins5 protein
OCI-M2 MPL CALRins5
200 ng/ml CALRins5 protein
25 ng/m CALRins5 protein
CD34+ cells
0 ng/ml CALRins5 protein
NuMoNuJu
C3CRB73

FIG. 11B

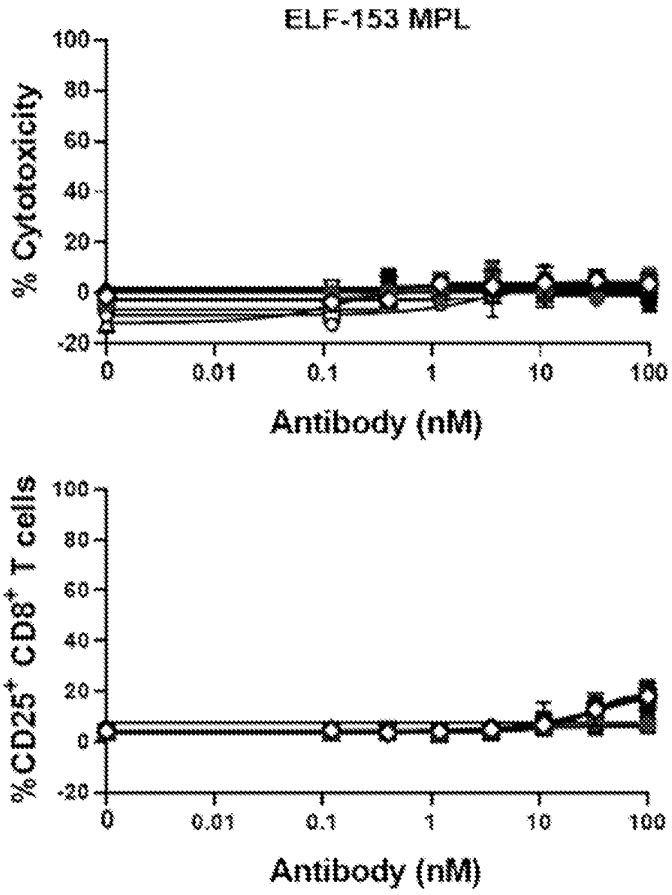

ELF-153 MPL
% Cytotoxicity
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Antibody (nM)
%CD25+ CD8+ T cells
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Antibody (nM)
NullxCD3
C3CRB73
200 ng/mL CALRdel52 protein
100 ng/mL CALRdel52 protein
50 ng/mL CALRdel52 protein
25 ng/mL CALRdel52 protein
0 ng/mL CALRdel52 protein

FIG. 12A

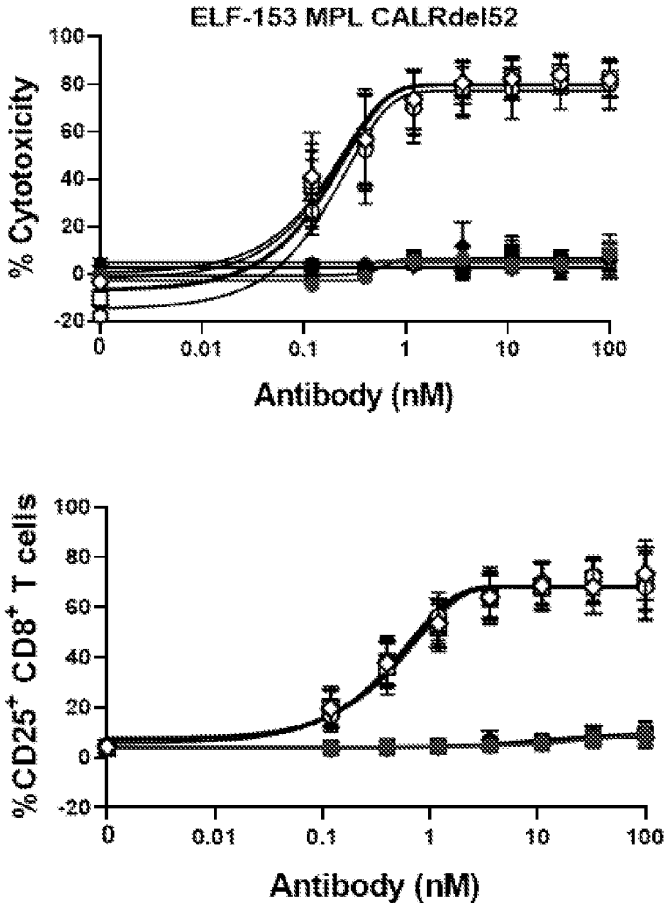

ELF-153 MPL CALRdel52
% Cytotoxicity
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Antibody (nM)
%CD25+ CD8+ T cells
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Antibody (nM)
NullxCD3
C3CRB73
200 ng/mL CALRdel52 protein
100 ng/mL CALRdel52 protein
50 ng/mL CALRdel52 protein
25 ng/mL CALRdel52 protein
0 ng/mL CALRdel52 protein

FIG. 12B

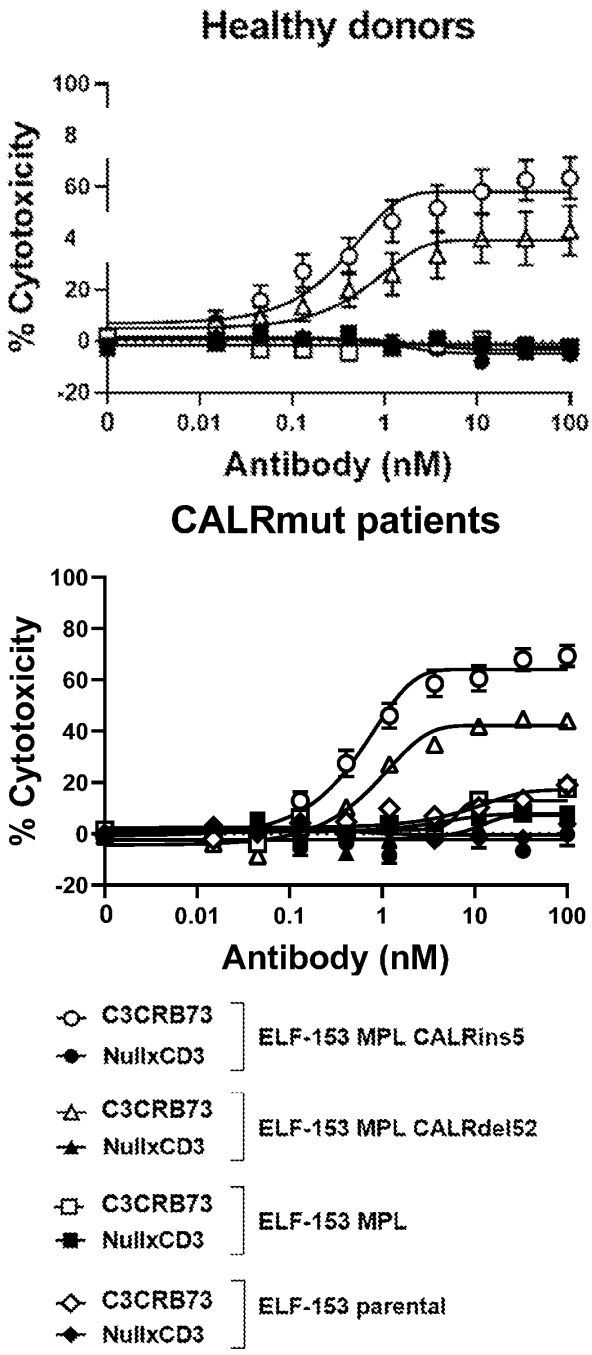

Healthy donors
% Cytotoxicity
100
8
60
4
20
0
-20
0
0.01
0.1
1
10
100
Antibody (nM)
CALRmut patients
% Cytotoxicity
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Antibody (nM)
C3CRB73
NullxCD3
ELF-153 MPL CALRins5
C3CRB73
NullxCD3
ELF-153 MPL CALRdel52
C3CRB73
NullxCD3
ELF-153 MPL
C3CRB73
NullxCD3
ELF-153 parental

FIG. 13

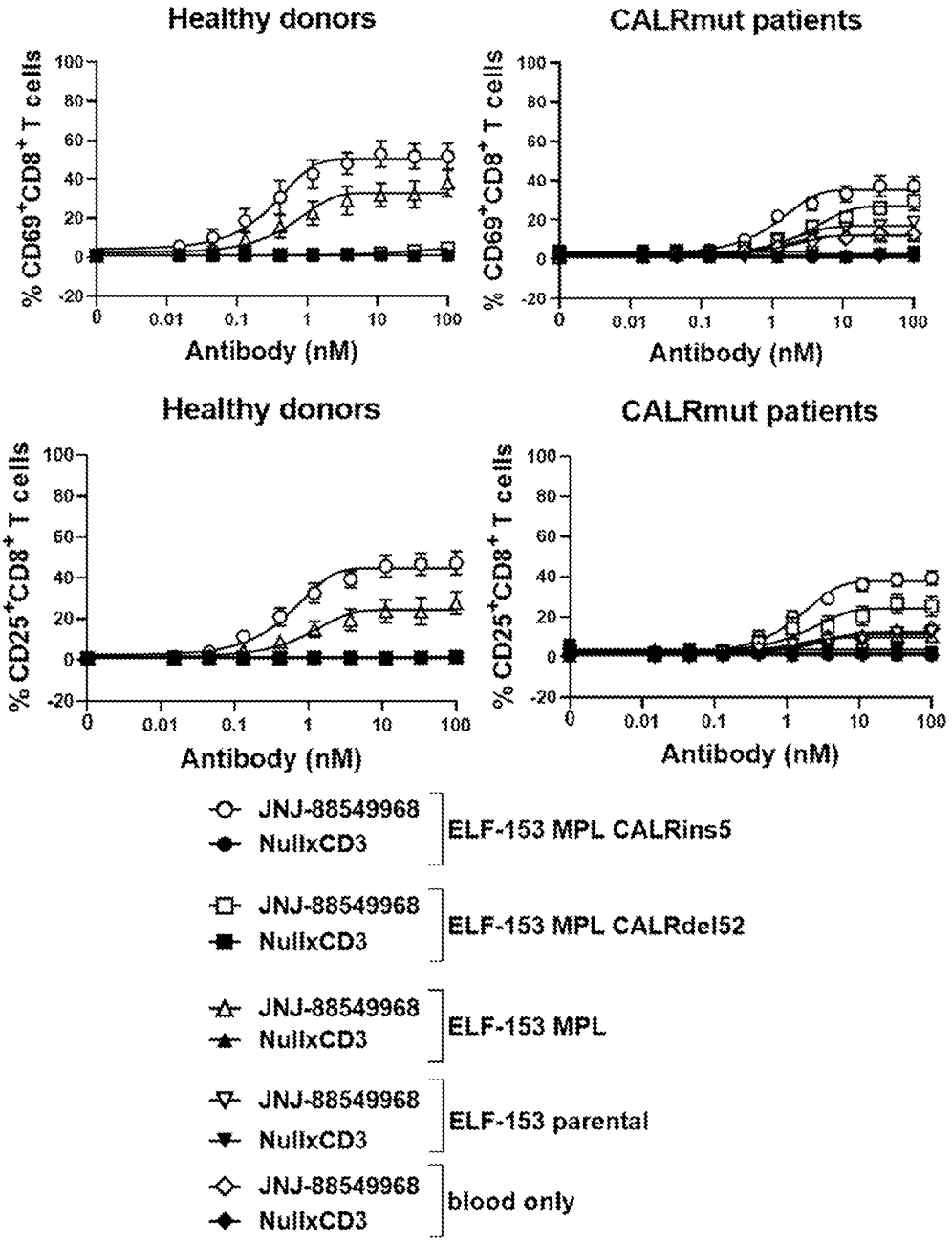

Healthy donors
CALRmut patients
% CD69+CD8+ T cells
% CD25+CD8+ T cells
Antibody (nM)
JNJ-88549968
NullxCD3
ELF-153 MPL CALRins5
ELF-153 MPL CALRdel52
ELF-153 MPL
ELF-153 parental
blood only

FIG. 14

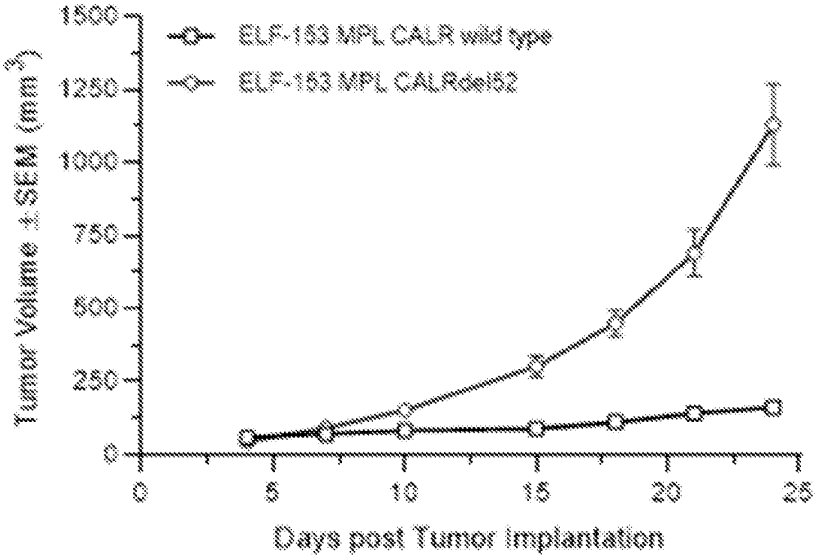


FIG. 15A

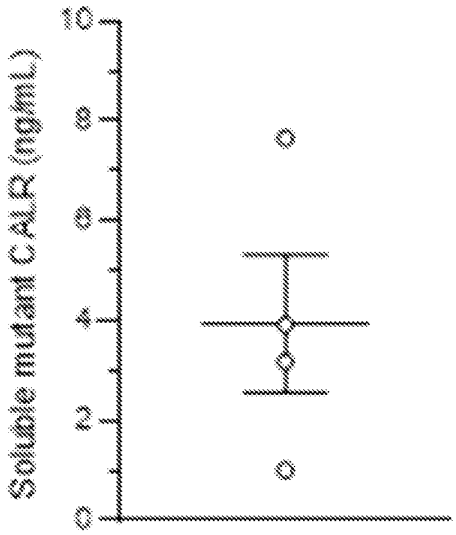


FIG. 15B

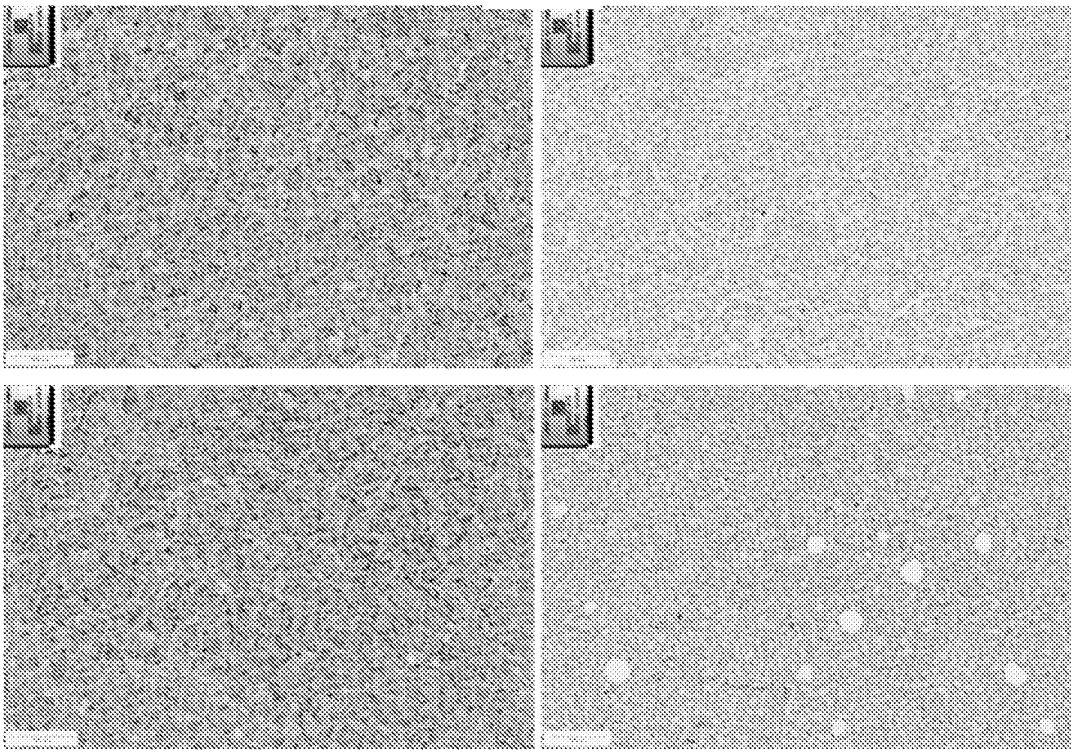


FIG. 16

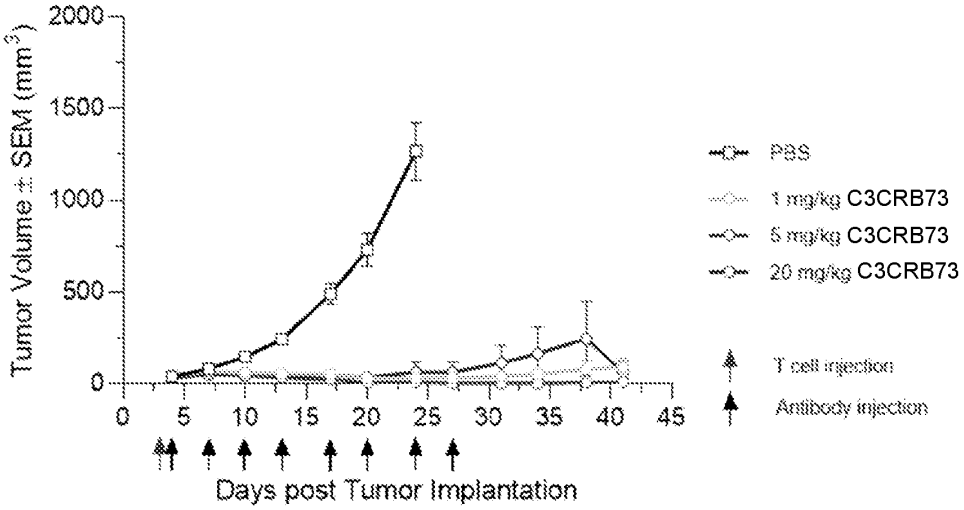

Tumor Volume ± SEM (mm3)
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
Days post Tumor Implantation
PBS
1 mg/kg C3CRB73
5 mg/kg C3CRB73
20 mg/kg C3CRB73
T cell injection
Antibody injection

FIG. 17

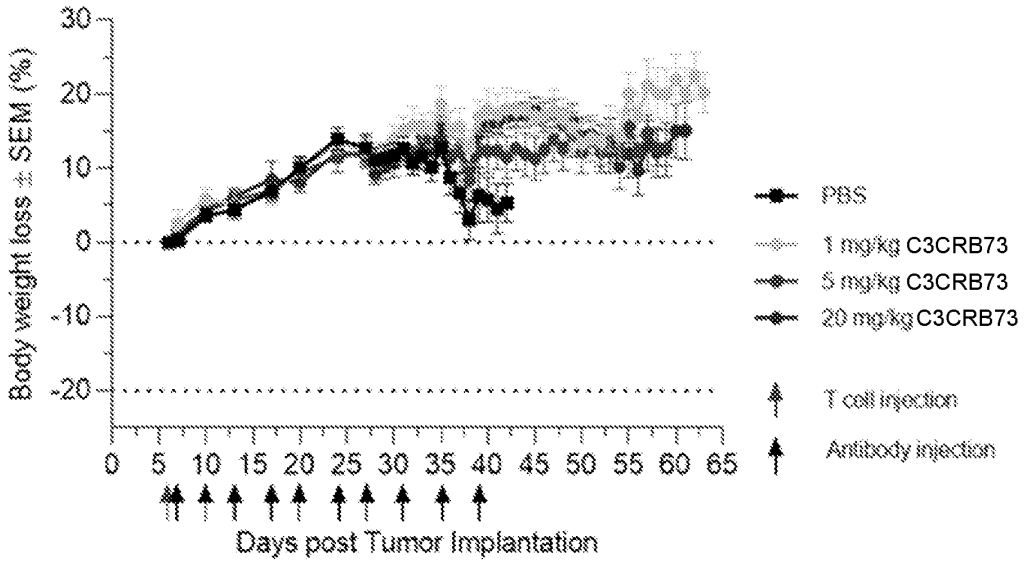


FIG. 18A

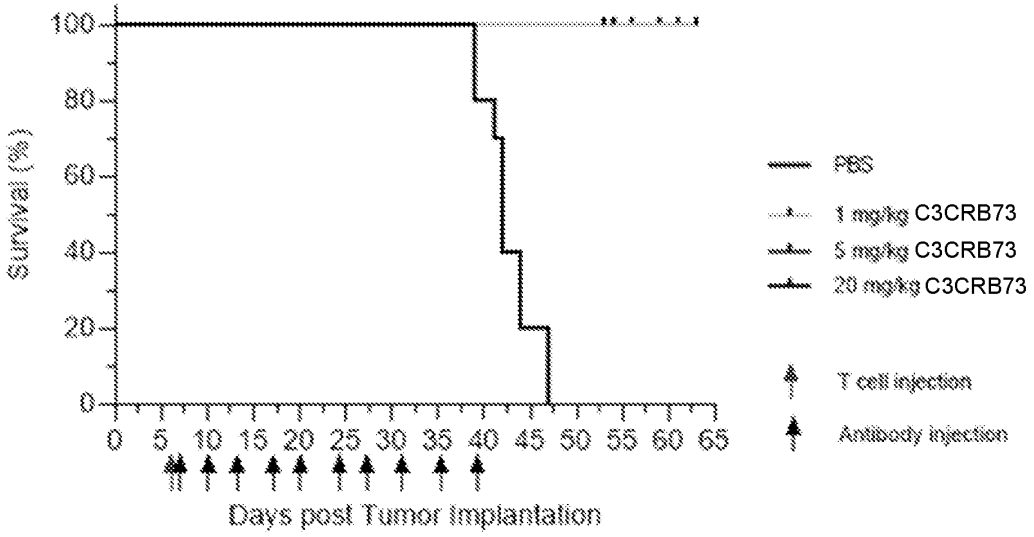


FIG. 18B

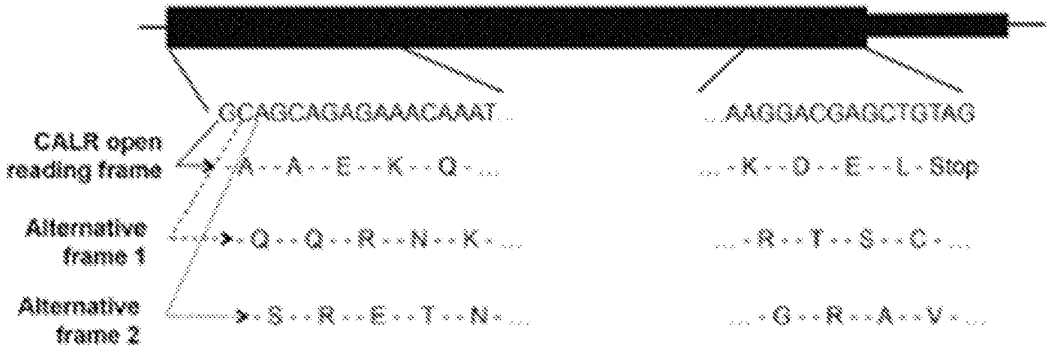


FIG. 19A

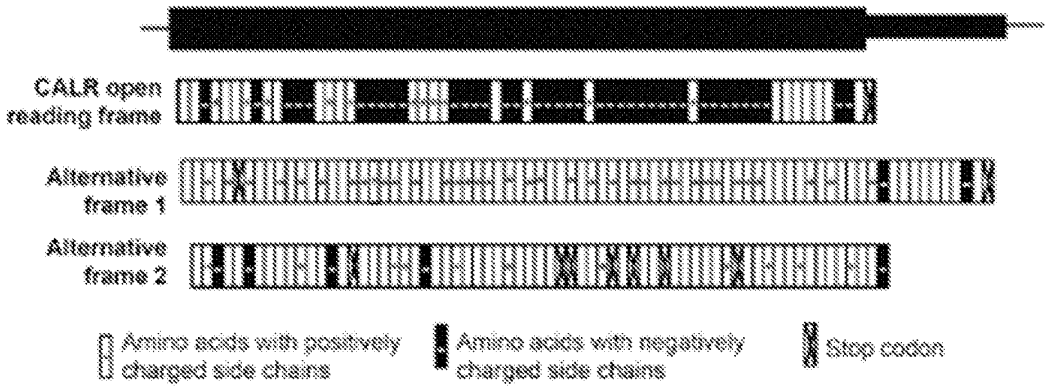


FIG. 19B

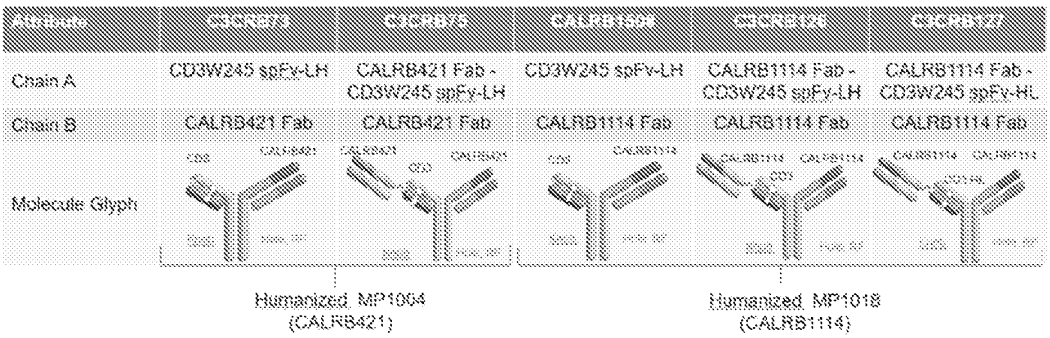

Antibody
CD3W245
CALRB421
CALRB1114
CALRB1114
CALRB1114
Chain A
CD3W245 spFv-LH
CALRB421 Fab - CD3W245 spFv-LH
CD3W245 spFv-LH
CALRB1114 Fab - CD3W245 spFv-LH
CALRB1114 Fab - CD3W245 spFv-HL
Chain B
CALRB421 Fab
CALRB421 Fab
CALRB1114 Fab
CALRB1114 Fab
CALRB1114 Fab
Molecule Glyph
Humanized MP1004 (CALRB421)
Humanized MP1018 (CALRB1114)

FIG. 20

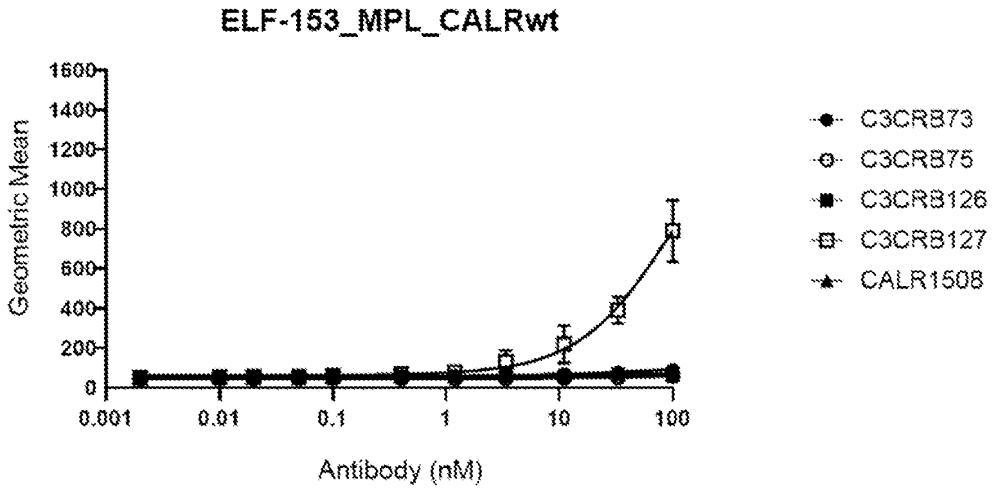


FIG. 21A

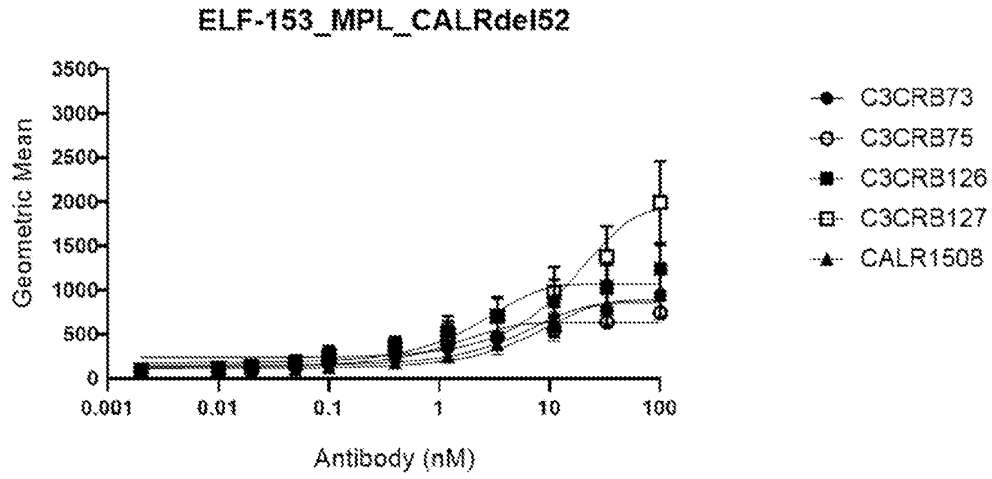


FIG. 21B

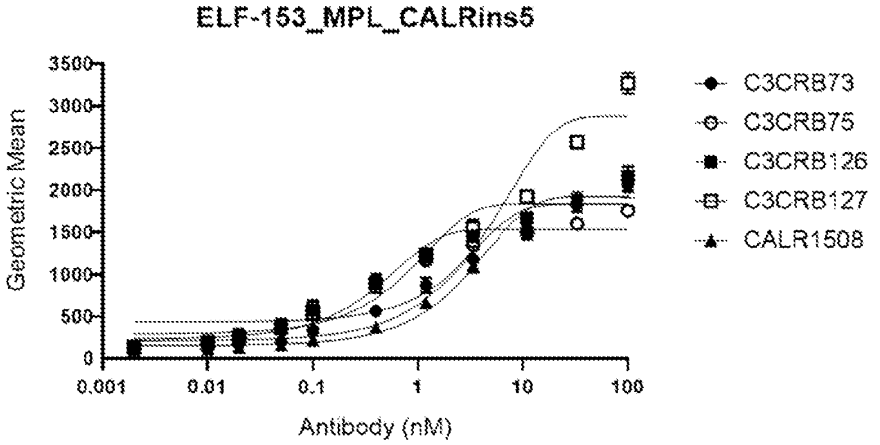


FIG. 21C

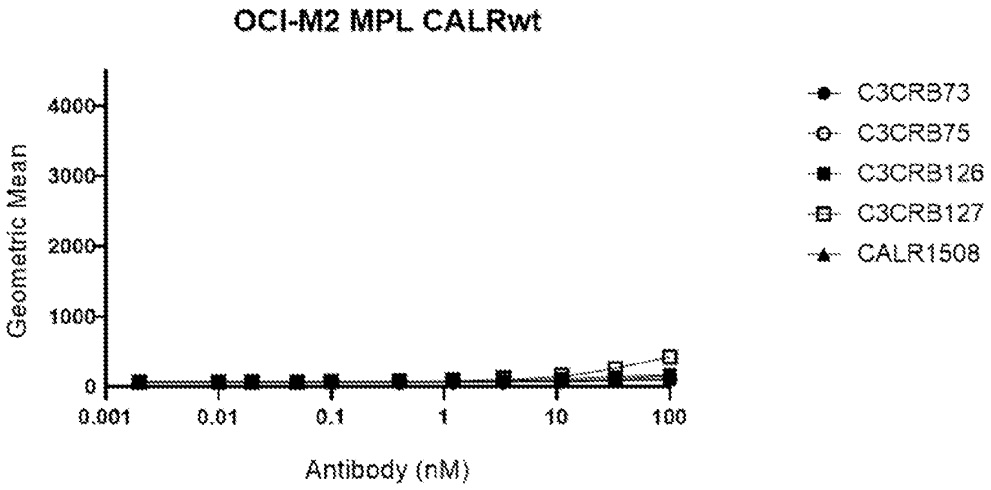


FIG. 21D

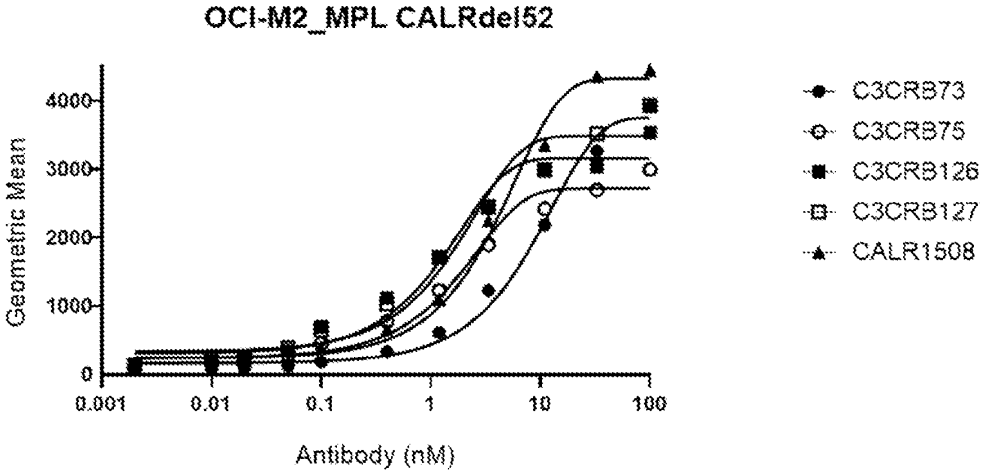


FIG. 21E

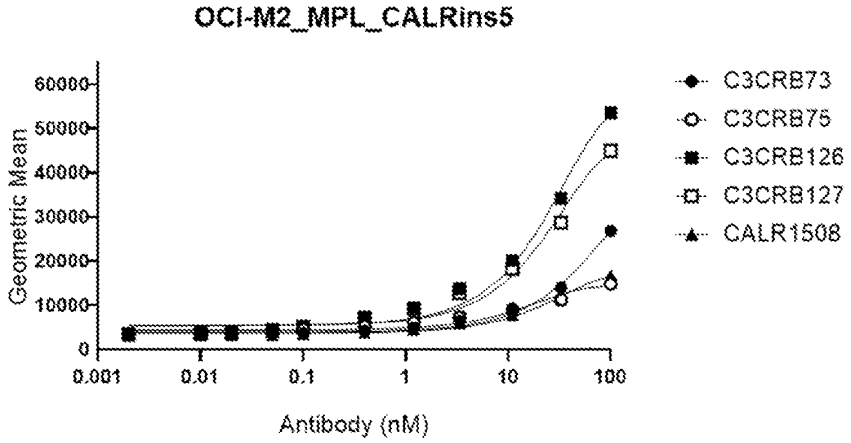


FIG. 21F

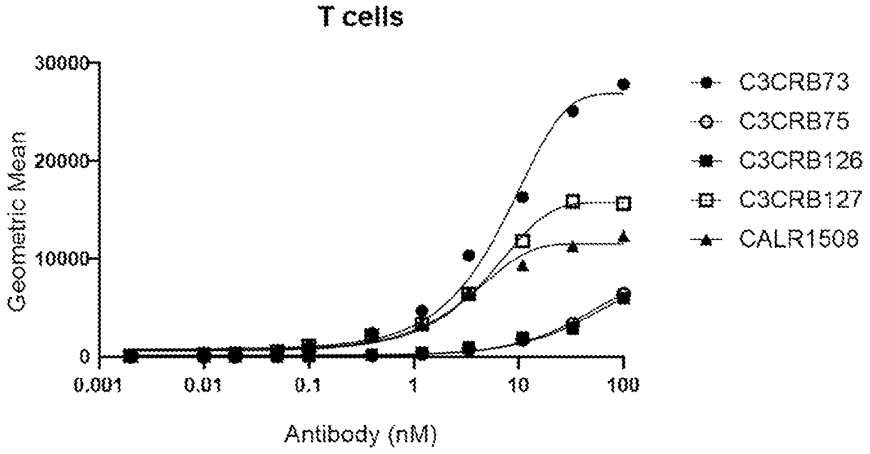

T cells
Geometric Mean
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
Antibody (nM)
C3CRB73
C3CRB75
C3CRB126
C3CRB127
CALR1508

FIG. 22

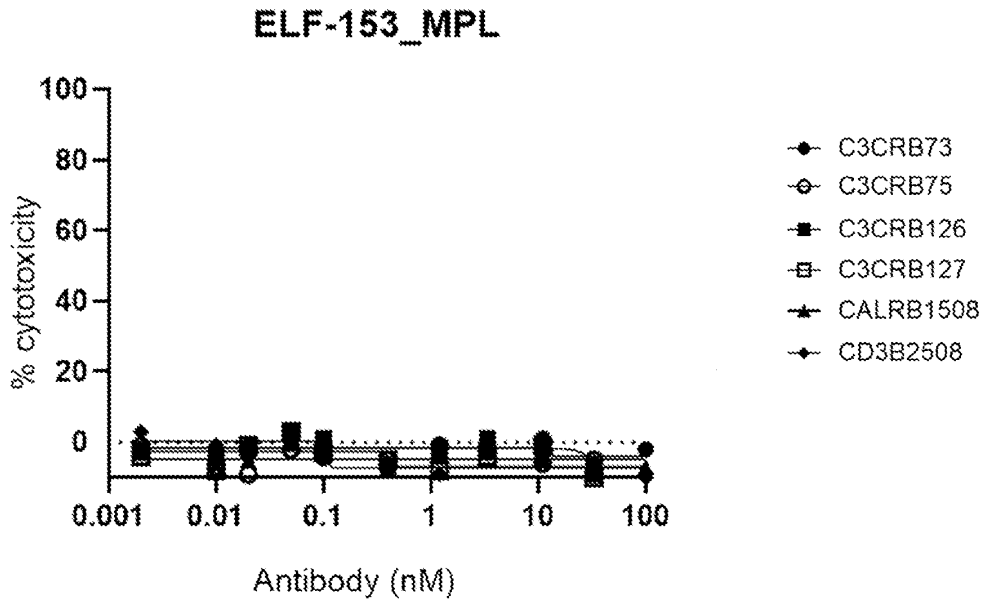


FIG. 23A

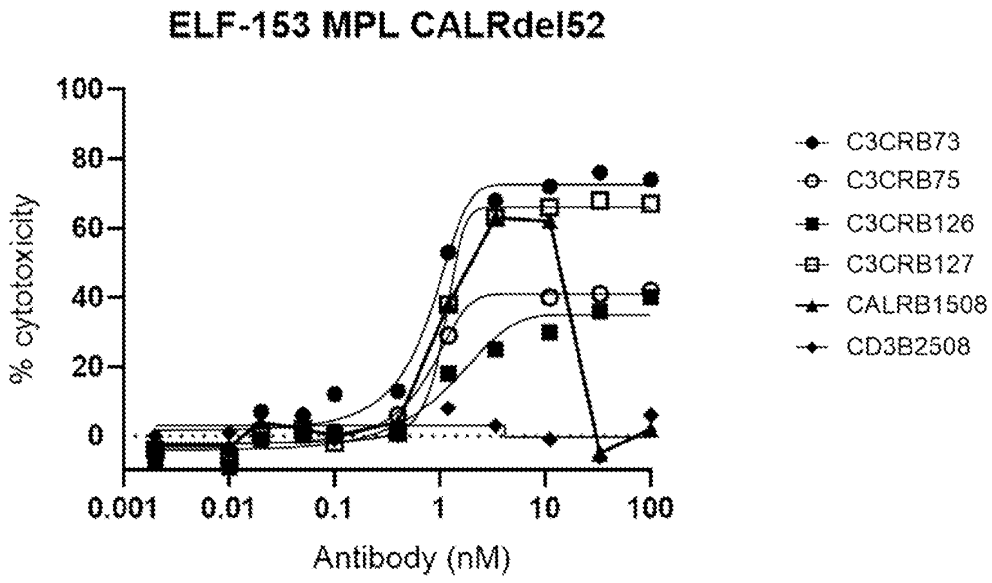


FIG. 23B

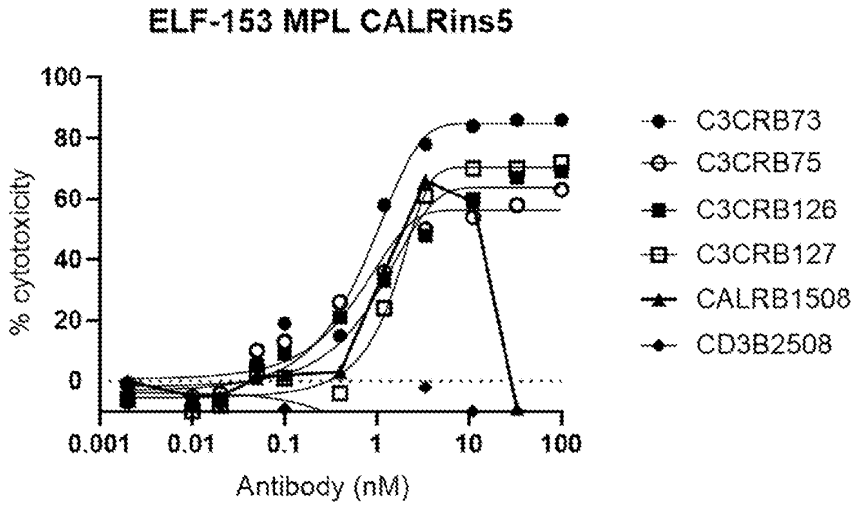


FIG. 23C

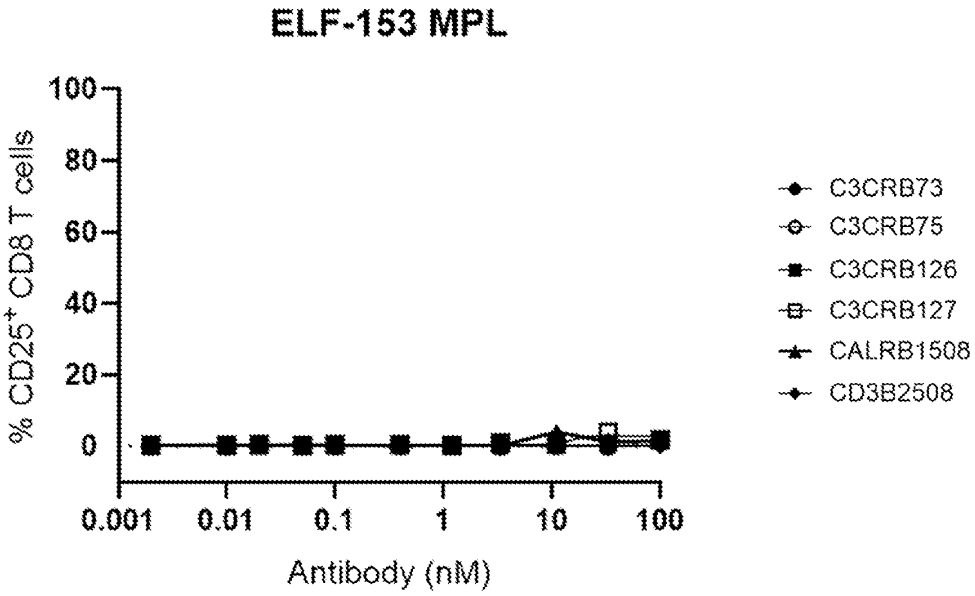


FIG. 23D

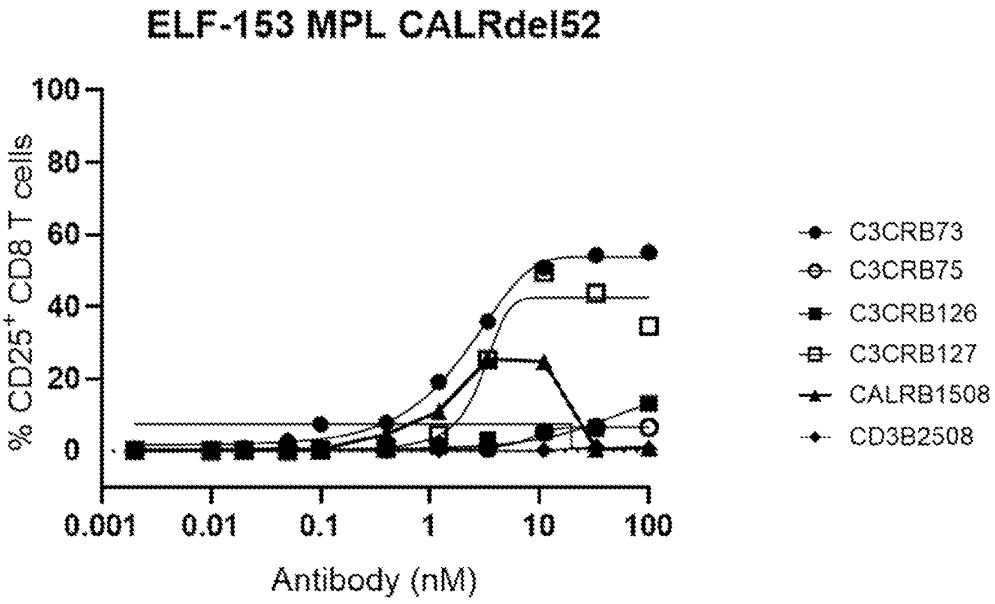


FIG. 23E

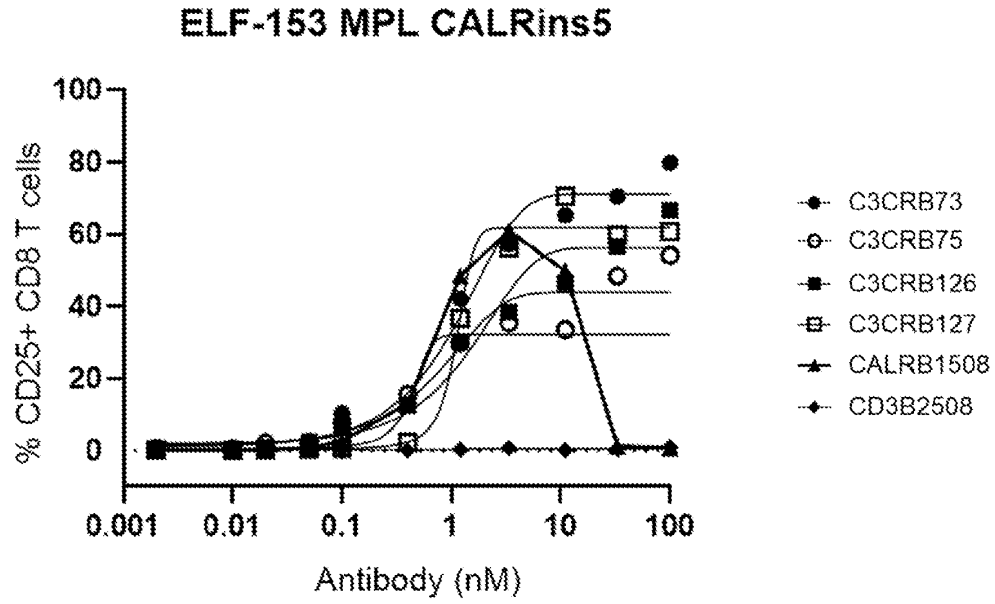


FIG. 23F

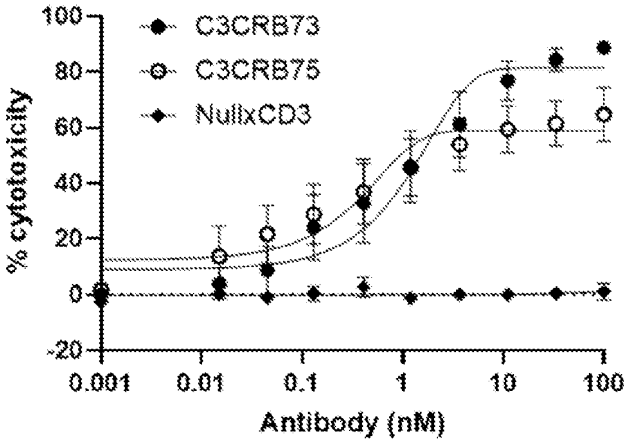


FIG. 24A

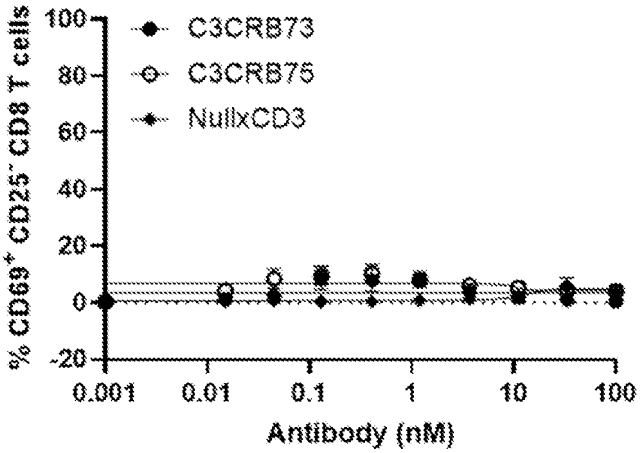


FIG. 24B

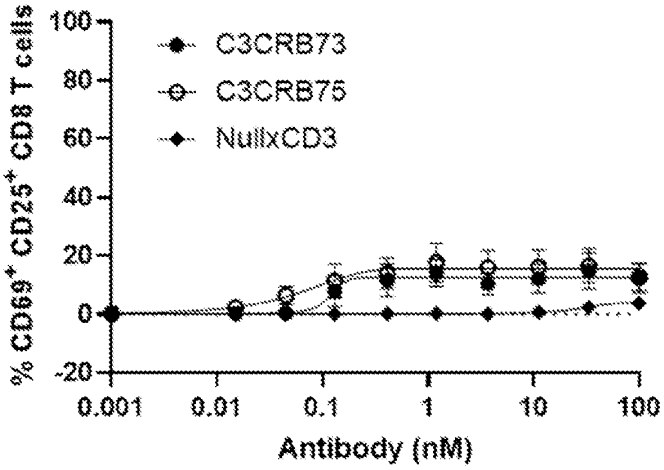


FIG. 24C

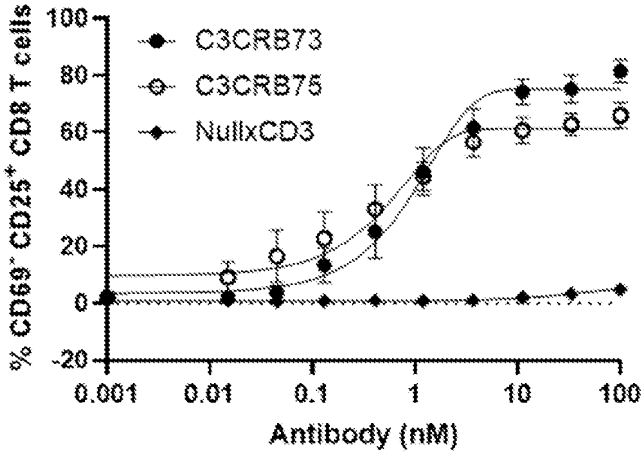


FIG. 24D

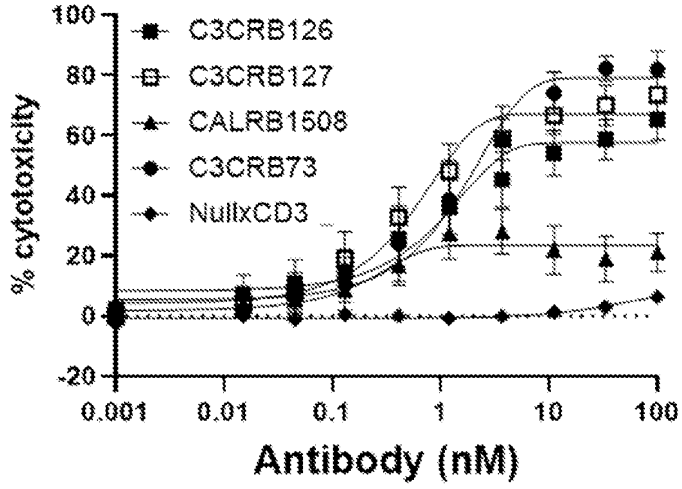


FIG. 24E

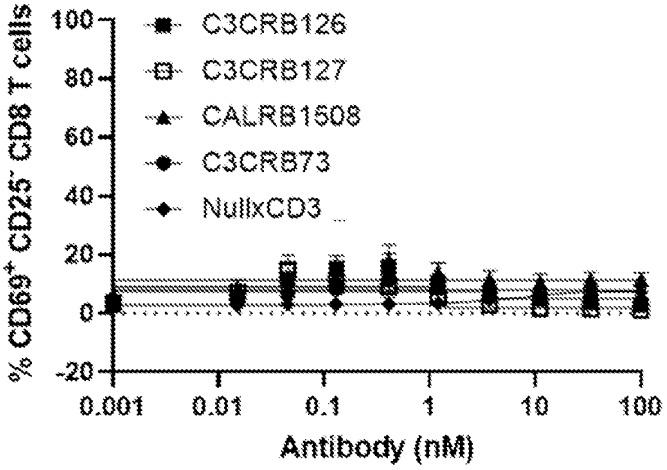


FIG. 24F

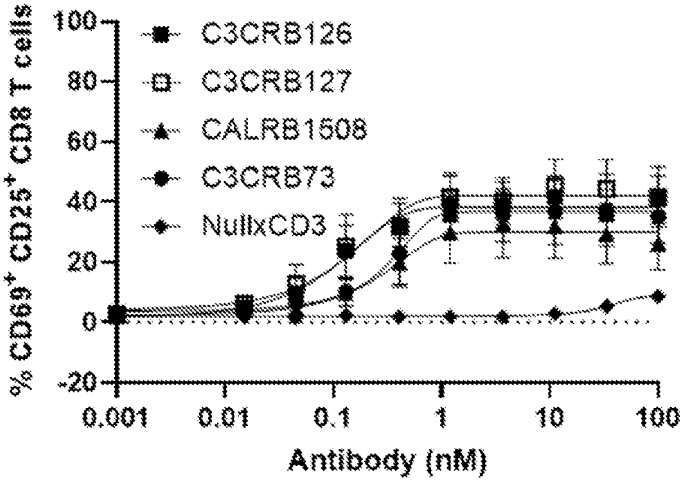


FIG. 24G

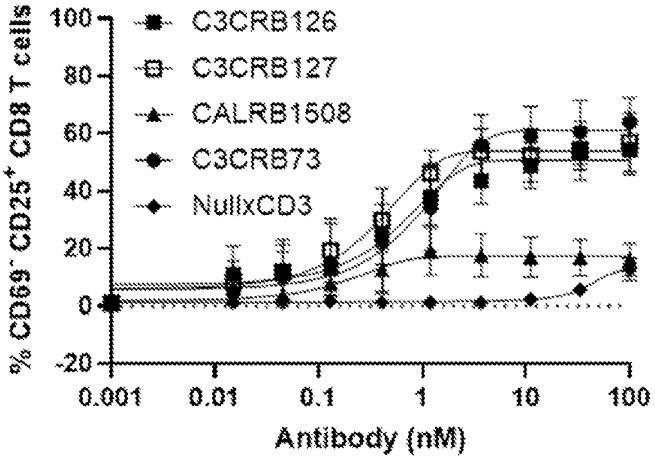


FIG. 24H

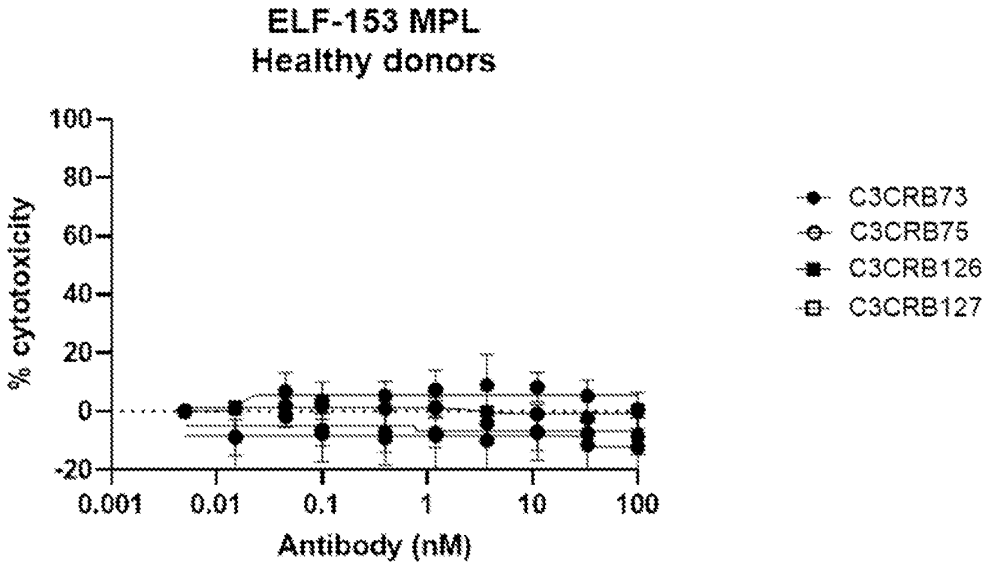


FIG. 25A

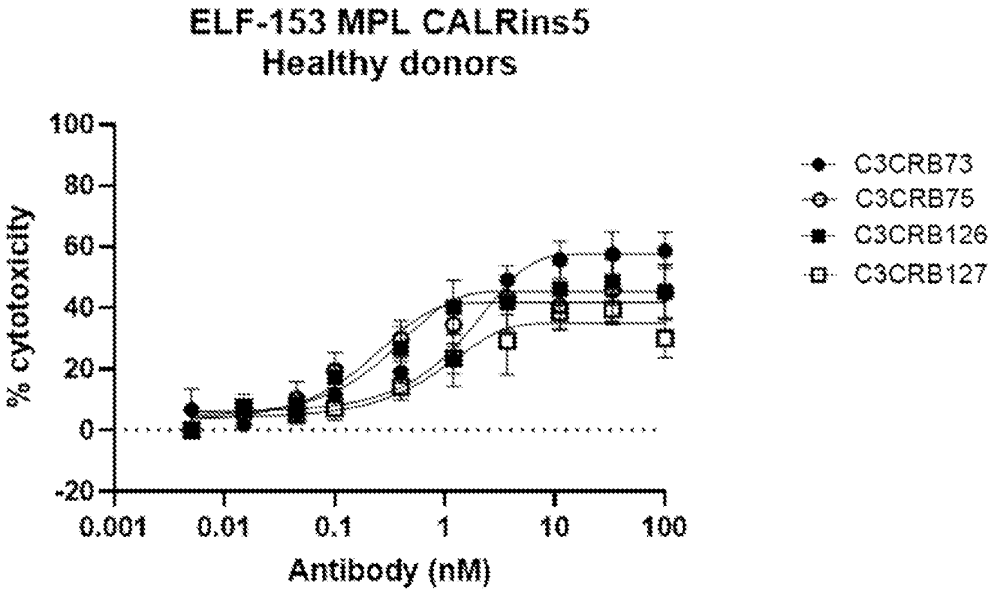


FIG. 25B

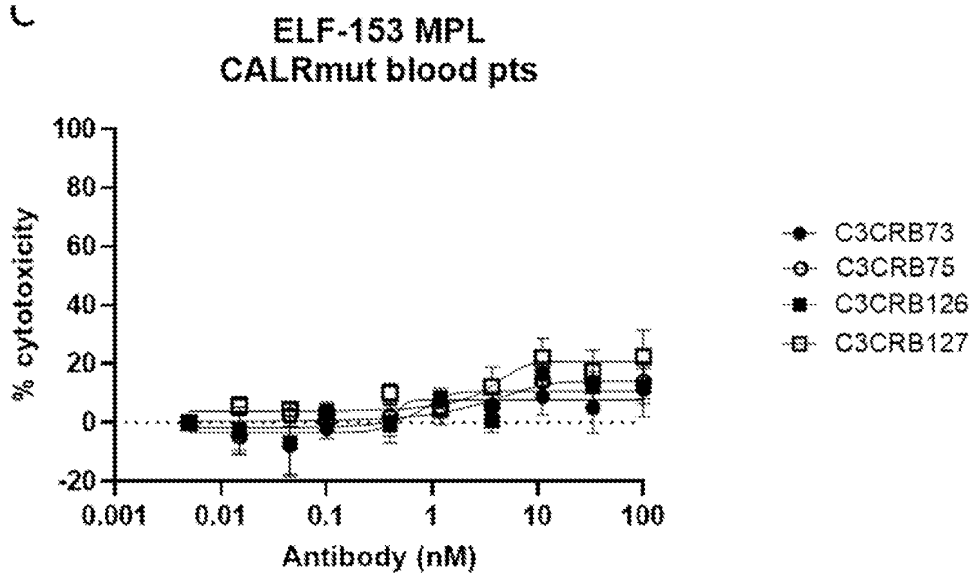


FIG. 25C

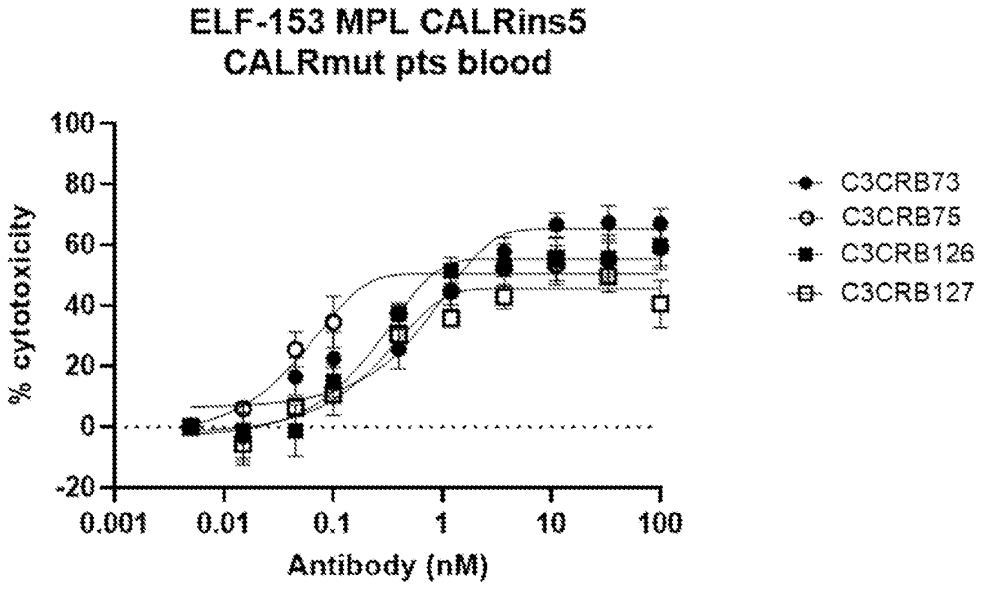


FIG. 25D

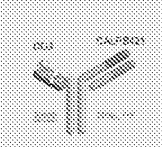
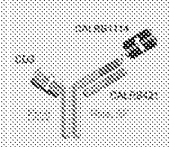
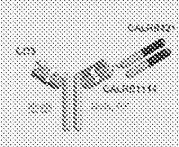
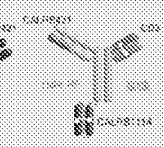
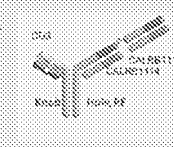
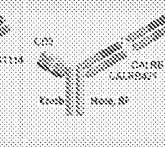
Name	C3CRB71	C3CRB133	C3CRB134	C3CRB435	C3CRB135	C3CRB137
Chain A	CD3 W245 spFv-LH	CD3 W245 spFv-LH	CD3 W245 spFv-LH	CALRB421 Fab CALRB1114 spFv-LH	CD3 W245 spFv-LH	CD3 W245 spFv-LH
Chain B	CALRB421 Fab	CALRB421 Fab CALRB1114 spF-LH	CALRB421 Fab (VR00061221) CALRB1114 spF-LH	CD3 W245 spFv-LH	2x CALRB1114 Fab	2x CALRB421 Fab
	scFv-Fc x HC bispecific antibody	scFv-Fc x scFV-HC multispecific antibody	scFv-Fc x scFV-HC multispecific antibody	HC-scFv x scFv-Fc multispecific antibody	scFv-Fc x **Tandem-Fab** HC bispecific antibody	scFv-Fc x **Tandem-Fab** HC bispecific antibody
Molecule glyph						

FIG. 26

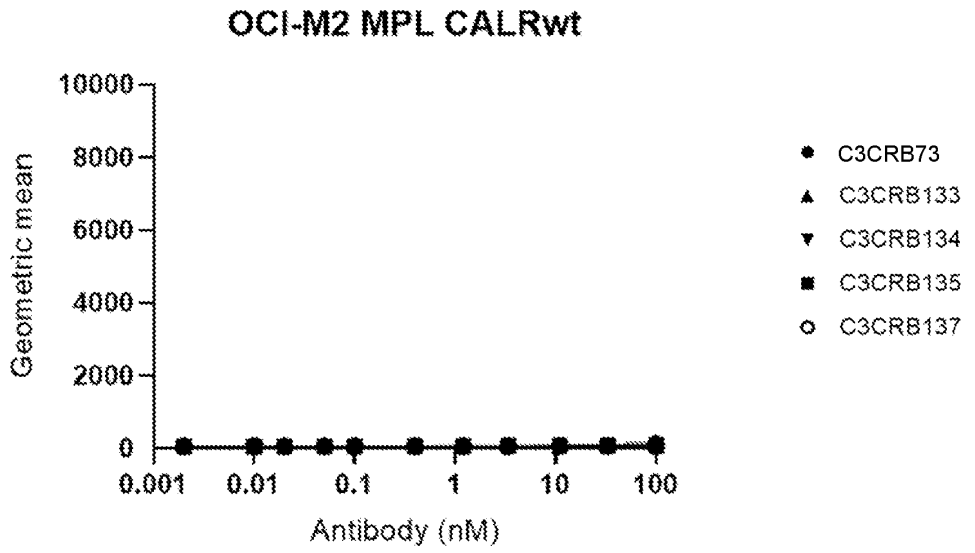


FIG. 27A

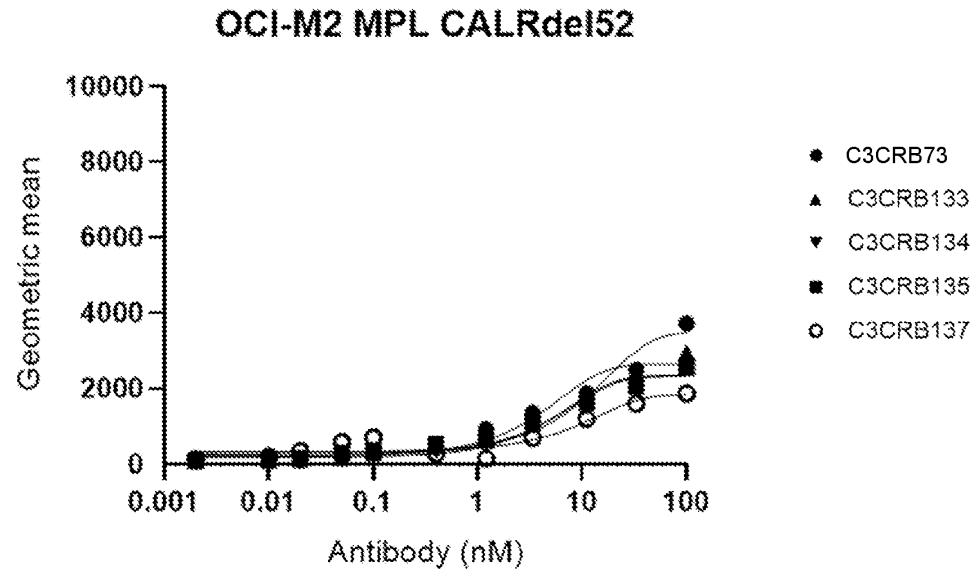


FIG. 27B

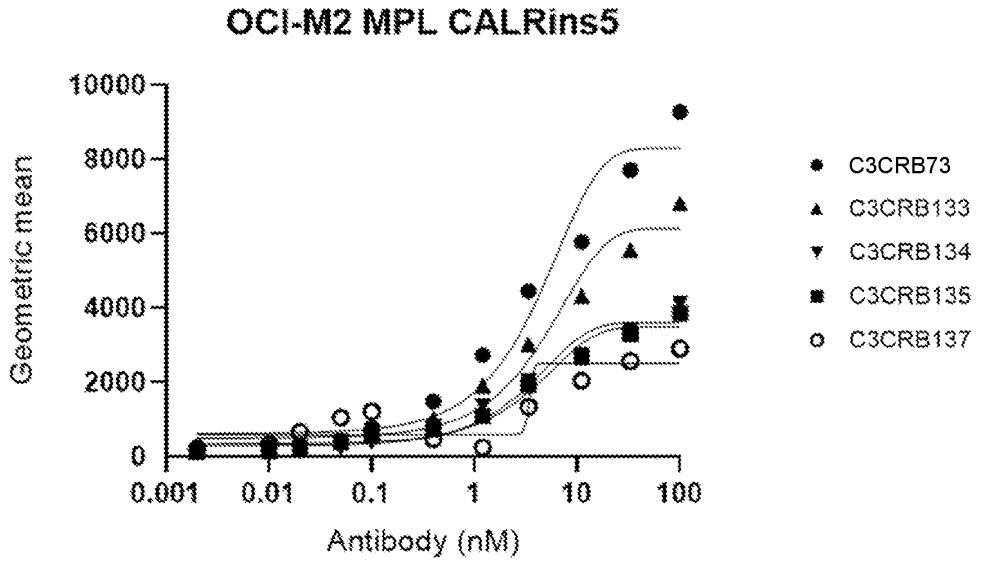


FIG. 27C

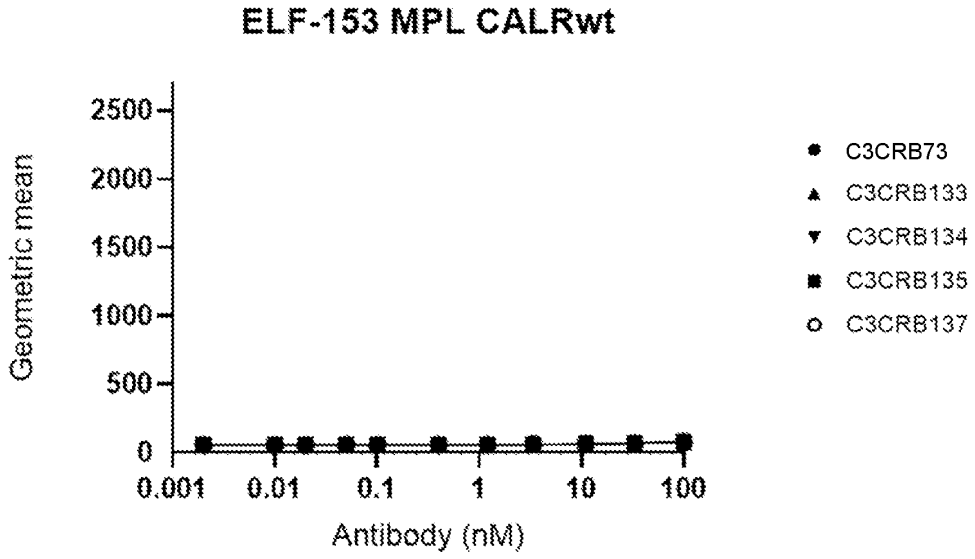


FIG. 27D

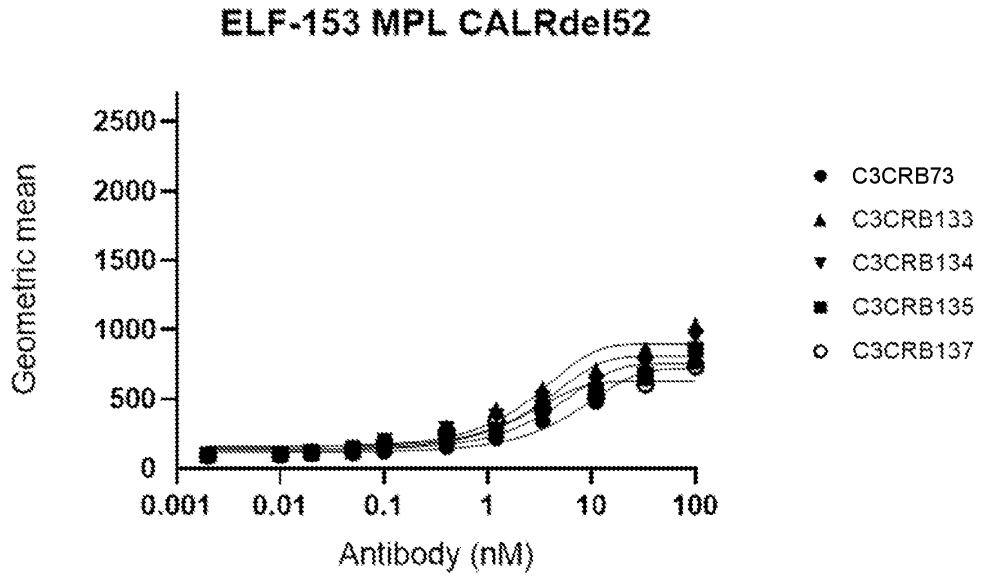


FIG. 27E

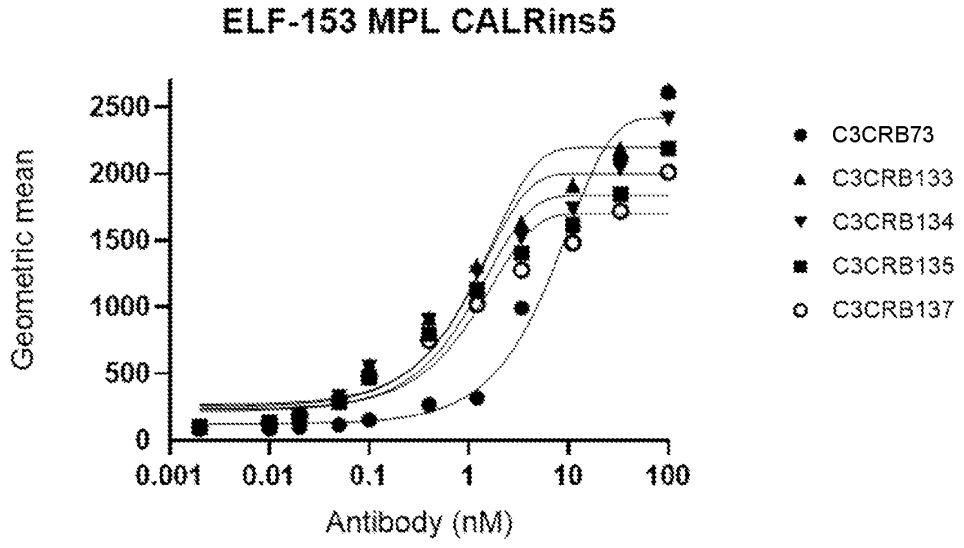


FIG. 27F

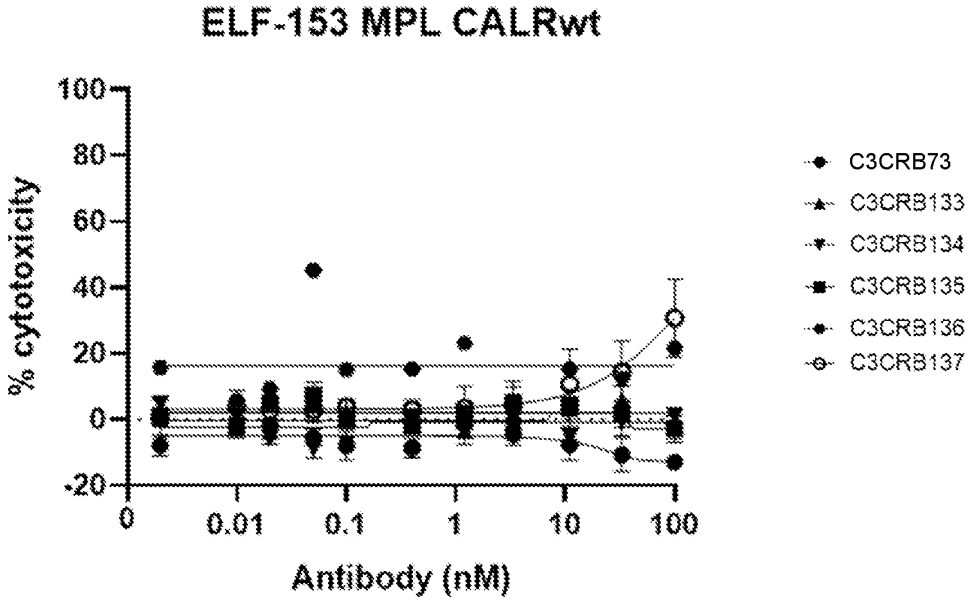


FIG. 28A

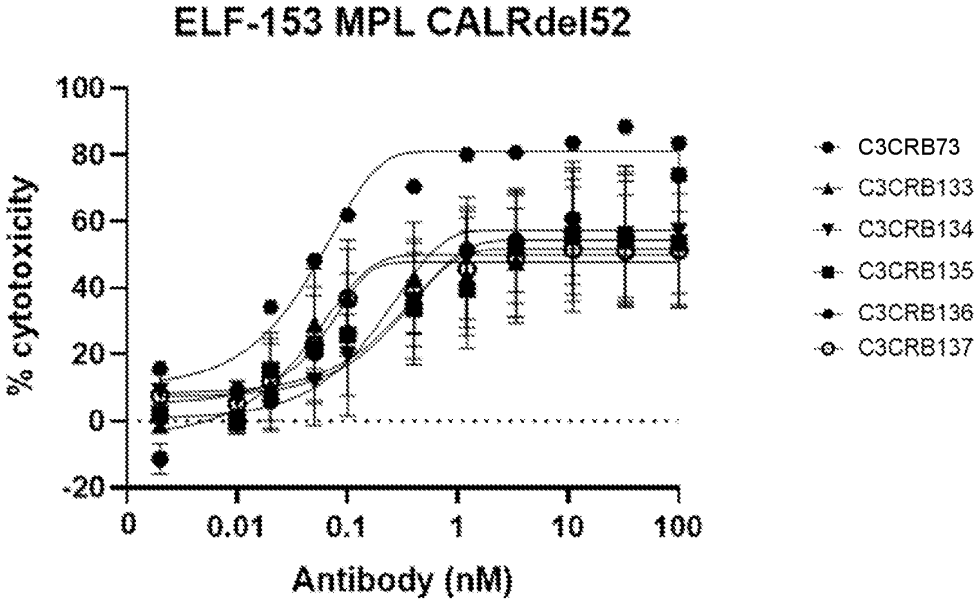


FIG. 28B

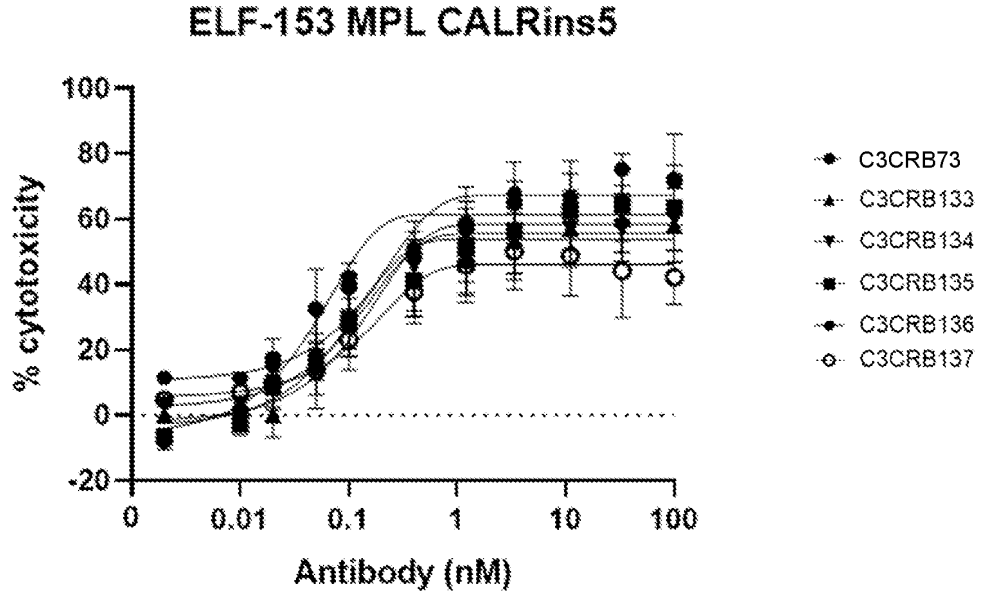


FIG. 28C

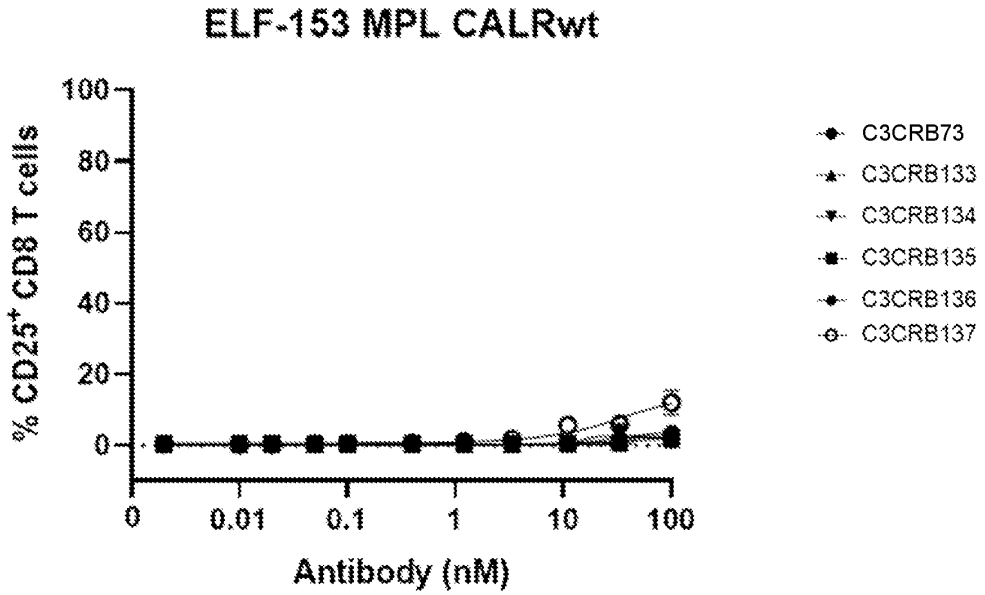


FIG. 28D

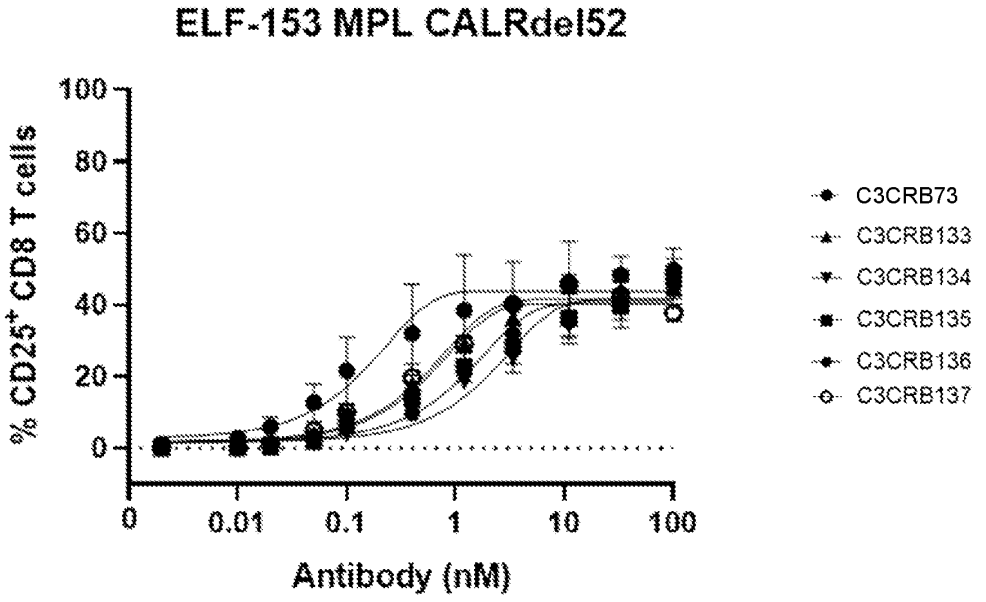


FIG. 28E

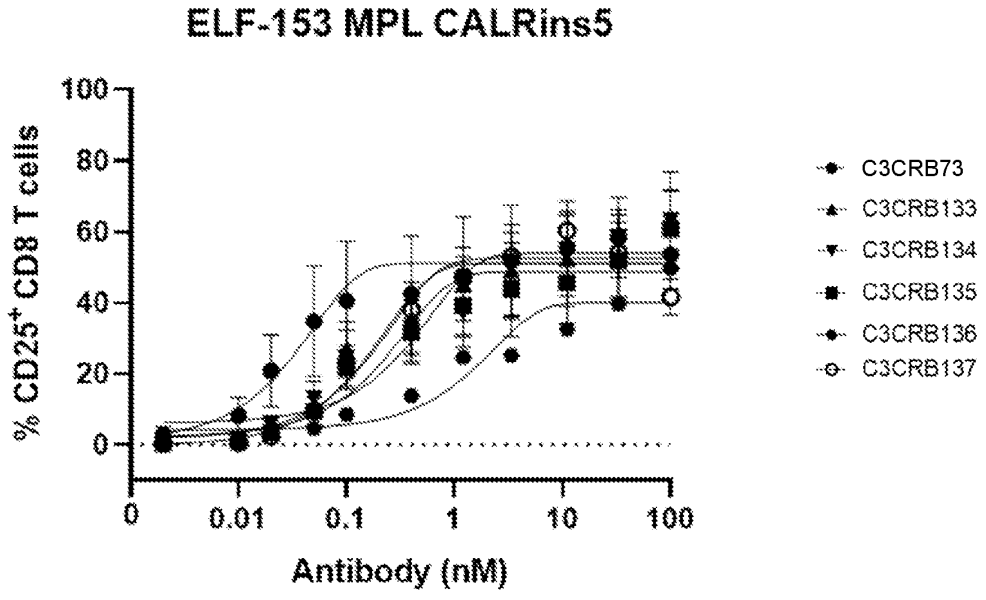


FIG. 28F

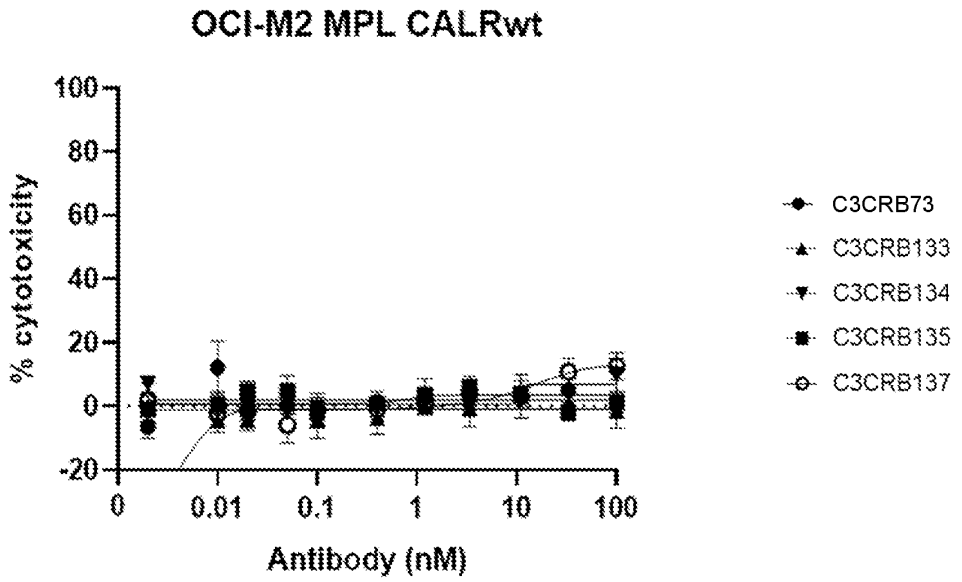


FIG. 29A

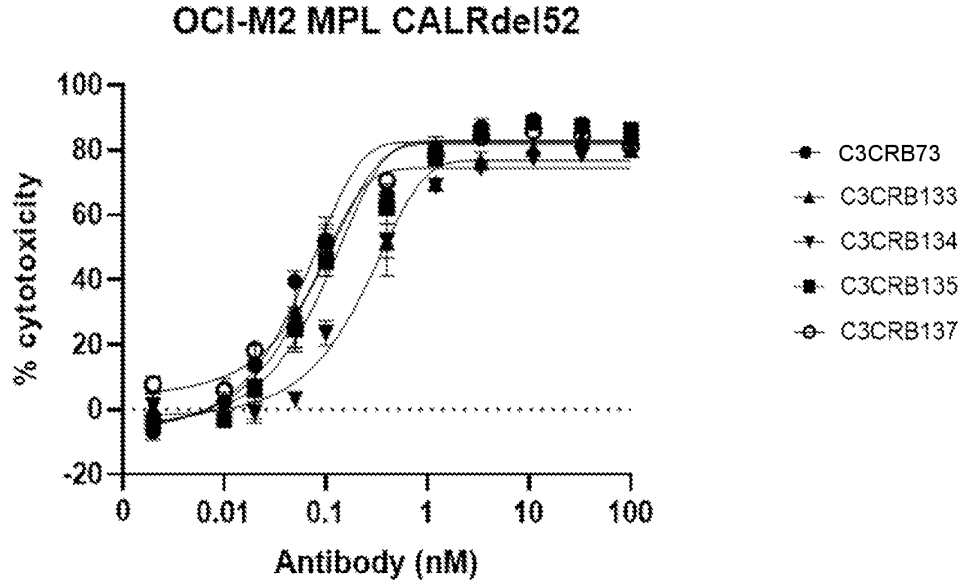


FIG. 29B

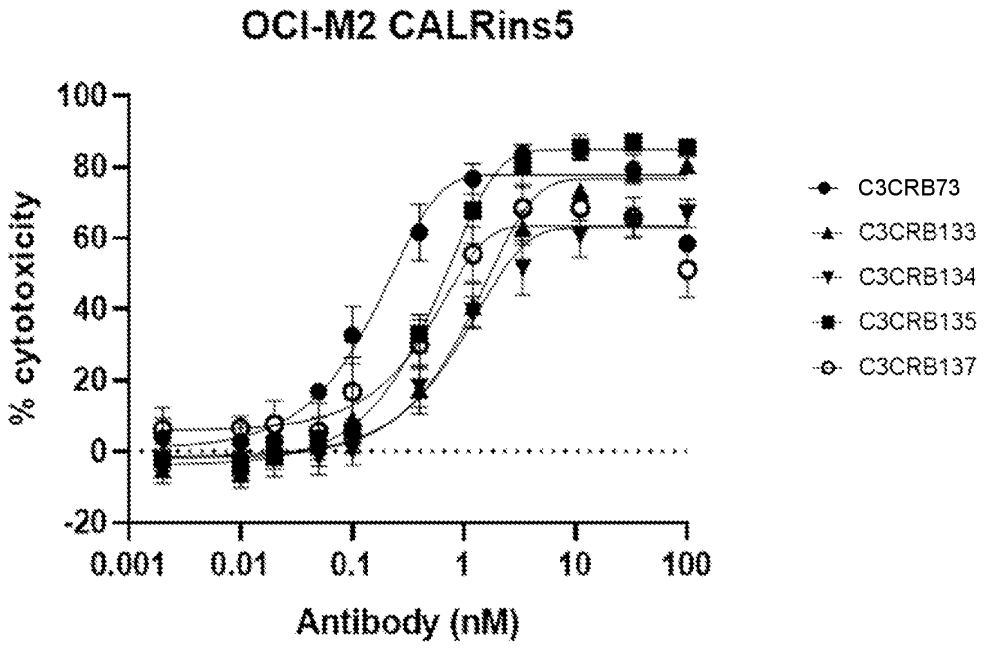


FIG. 29C

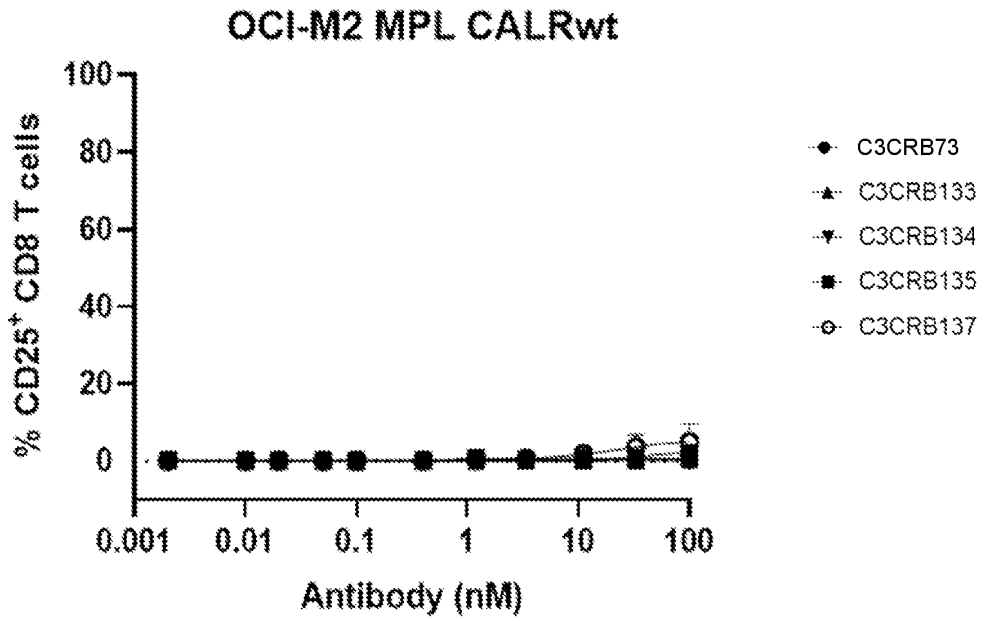


FIG. 29D

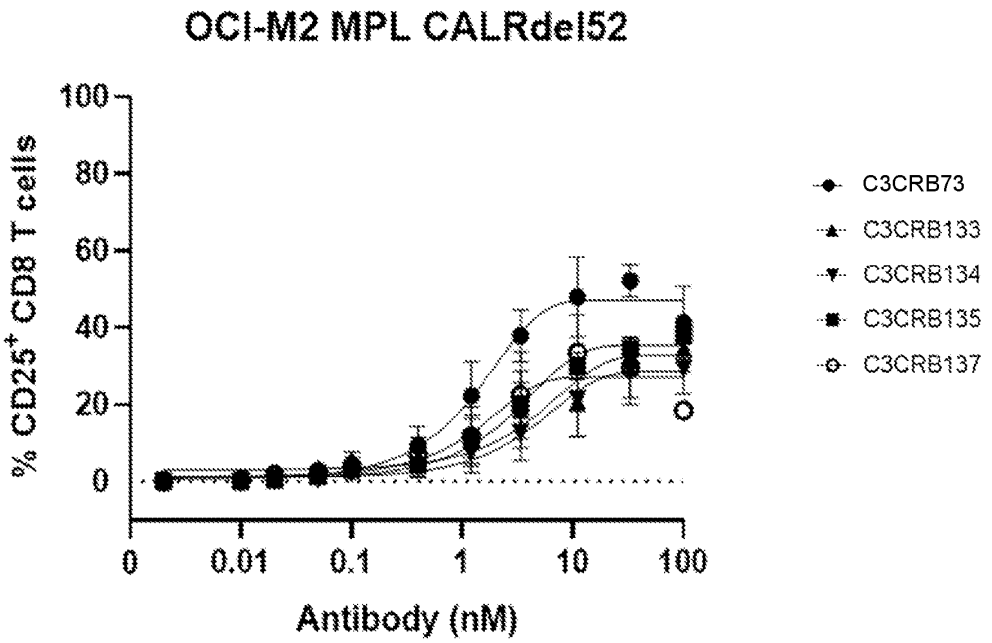


FIG. 29E

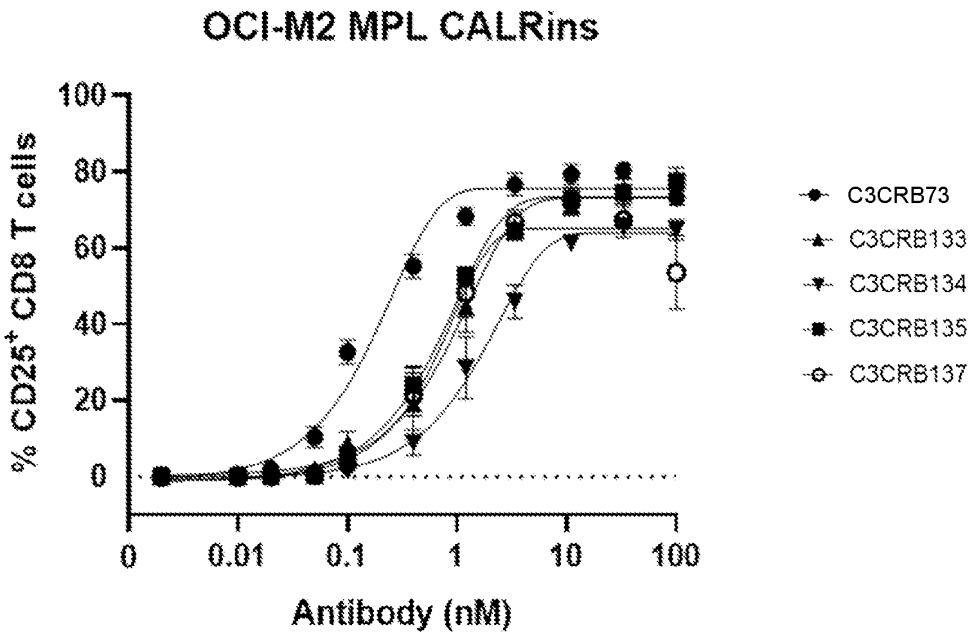


FIG. 29F

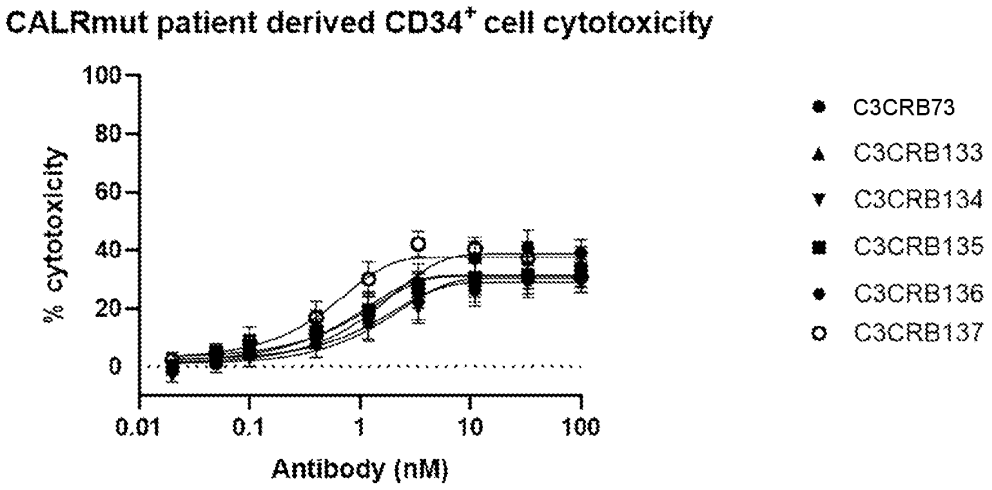


FIG. 30A

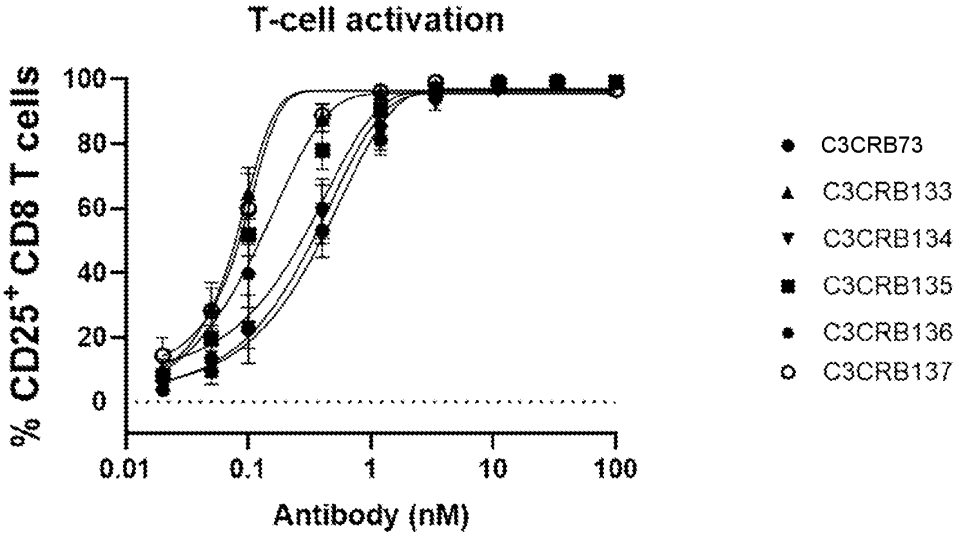


FIG. 30B