Figure 1



LTR, long terminal repeat promoter; SD, SA, splice donor and acceptor; Ψ, packaging element; black box, signal peptide; IRES, internal ribosomal entry site; GLuc, Gaussia luciferase; CD8, the human CD8 transmembrane domain

Figure 2

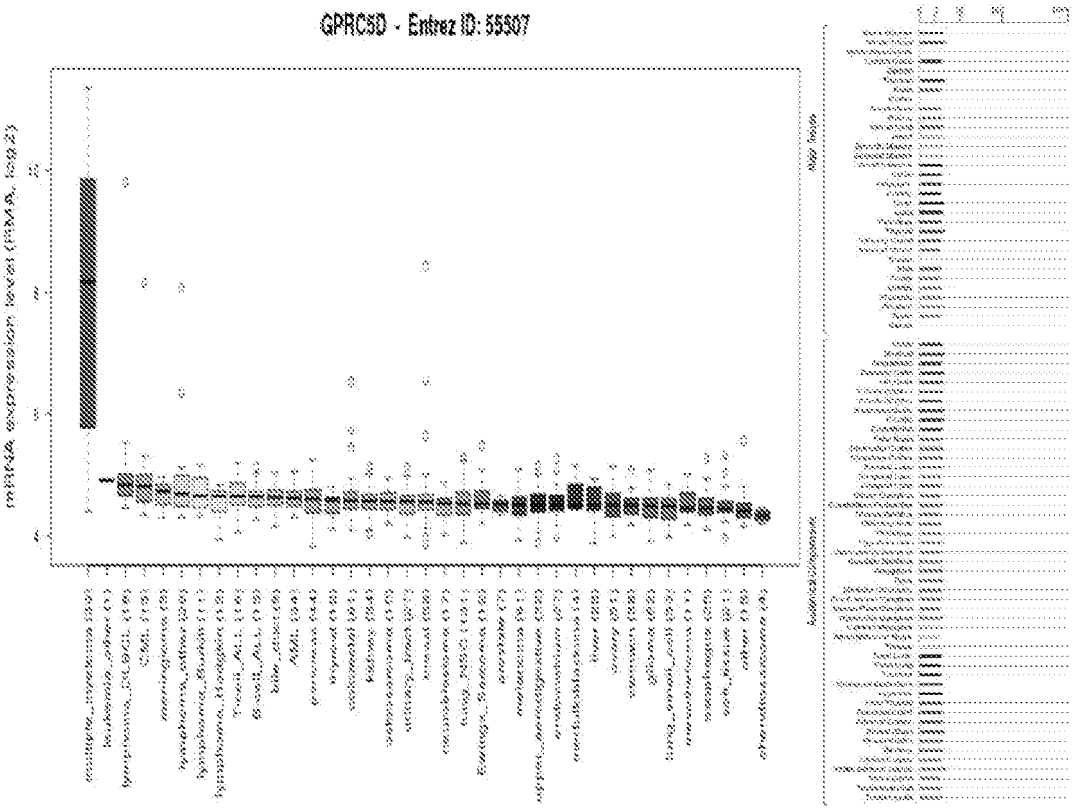


Figure 3

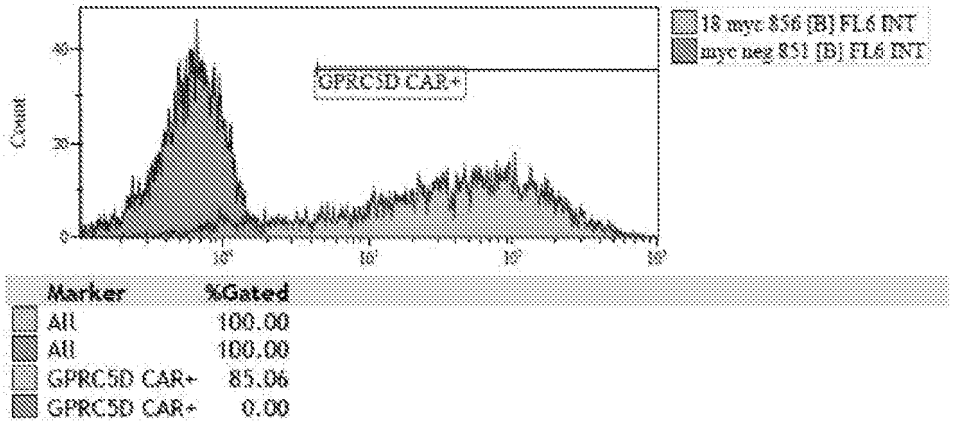

18 myc 8.56 [B] FL6 INT
myc neg 8.51 [B] FL6 INT
GPRC5D CAR+
Count
Marker %Gated
All 100.00
All 100.00
GPRC5D CAR+ 85.06
GPRC5D CAR+ 0.00

Figure 4

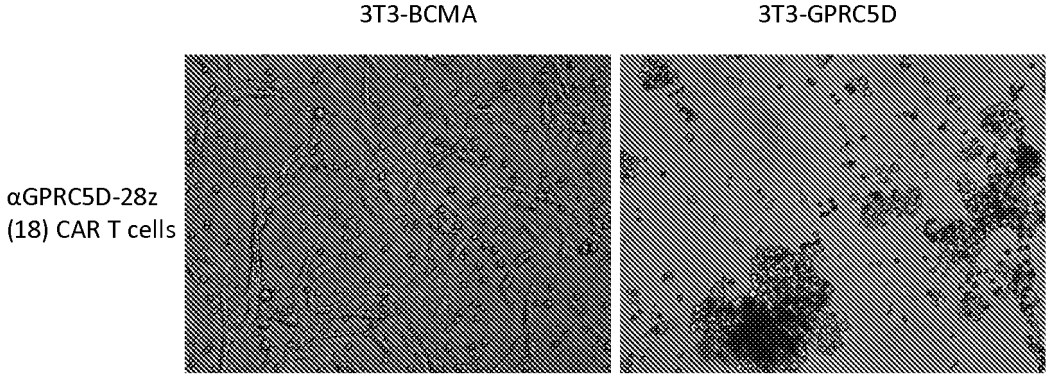


Figure 5

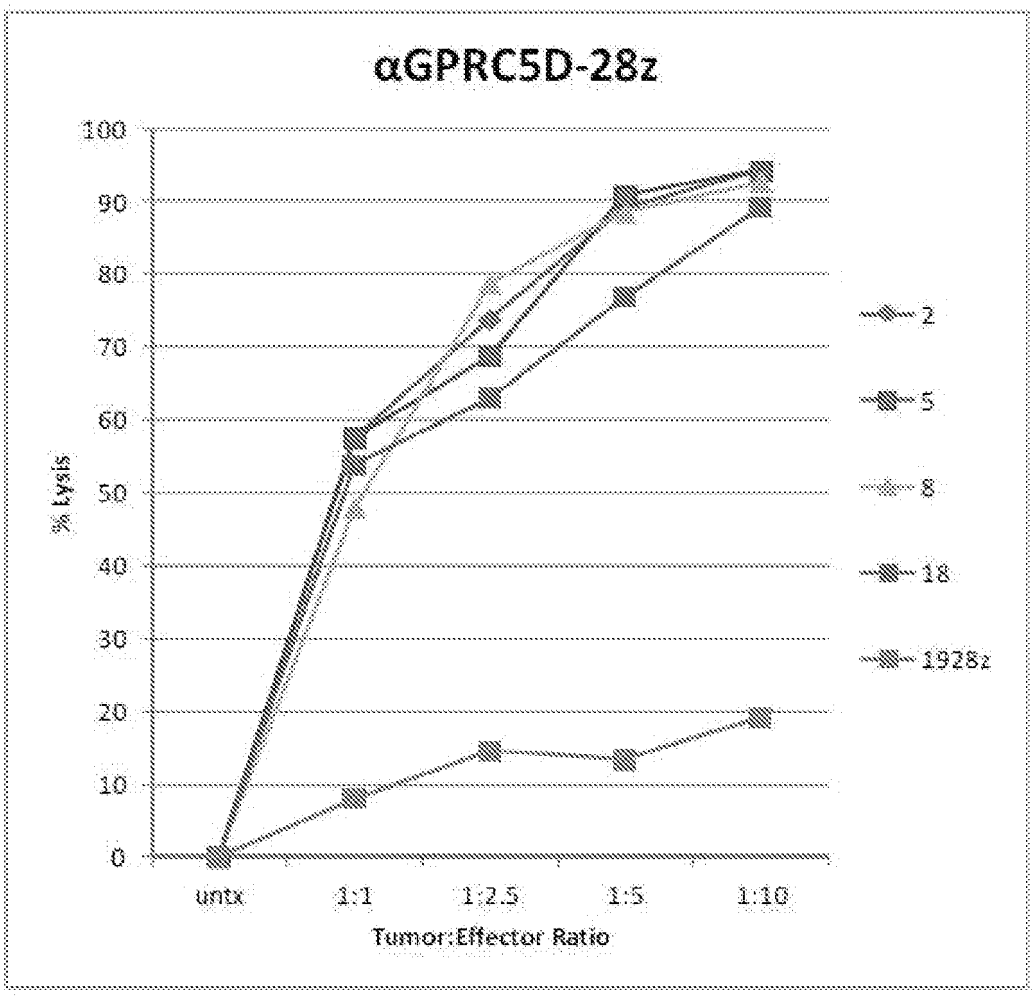

αGPRC5D-28z
100
90
80
70
60
50
40
30
20
10
0
% Lysis
untx
1:1
1:2.5
1:5
1:10
Tumor:Effector Ratio
2
5
8
18
1928z

Figure 6



Figure 7

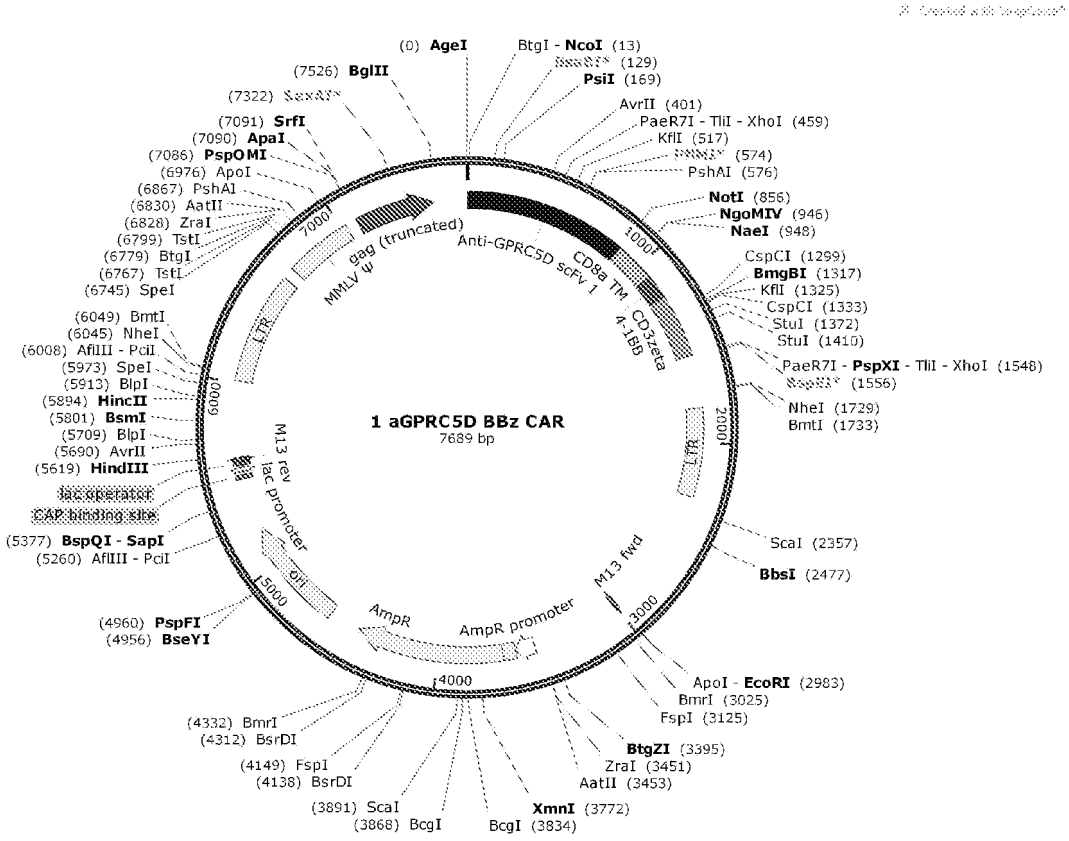

1 aGPRC5D BBz CAR
7689 bp
(0) AgeI
BtgI - NcoI (13)
PsiI (169)
AvrII (401)
PaeR7I - TliI - XhoI (459)
KflI (517)
PshAI (576)
NotI (856)
NgoMIV (946)
NaeI (948)
CspCI (1299)
BmgBI (1317)
KflI (1325)
CspCI (1333)
StuI (1372)
StuI (1410)
PaeR7I - PspXI - TliI - XhoI (1548)
NheI (1729)
BmtI (1733)
ScaI (2357)
BbsI (2477)
ApoI - EcoRI (2983)
BmrI (3025)
FspI (3125)
BtgZI (3395)
ZraI (3451)
AatII (3453)
XmnI (3772)
BcgI (3834)
(3868) BcgI
(3891) ScaI
(4138) BsrDI
(4149) FspI
(4312) BsrDI
(4332) BmrI
(4956) BseYI
(4960) PspFI
(5260) AflIII - PciI
(5377) BspQI - SapI
CAP binding site
lac operator
(5619) HindIII
(5690) AvrII
(5709) BlpI
(5801) BsmI
(5894) HincII
(5913) BlpI
(5973) SpeI
(6008) AflIII - PciI
(6045) NheI
(6049) BmtI
(6745) SpeI
(6767) TstI
(6779) BtgI
(6799) TstI
(6828) ZraI
(6830) AatII
(6867) PshAI
(6976) ApoI
(7086) PspOMI
(7090) ApaI
(7091) SrfI
(7322) SexAI
(7526) BglII
gag (truncated)
MMLV Ψ
LTR
M13 rev
lac promoter
ori
AmpR
AmpR promoter
M13 fwd
LTR
Anti-GPRC5D scFv 1
CD8a TM
4-1BB
CD3zeta
1000
2000
3000
4000
5000
6000
7000

Figure 8

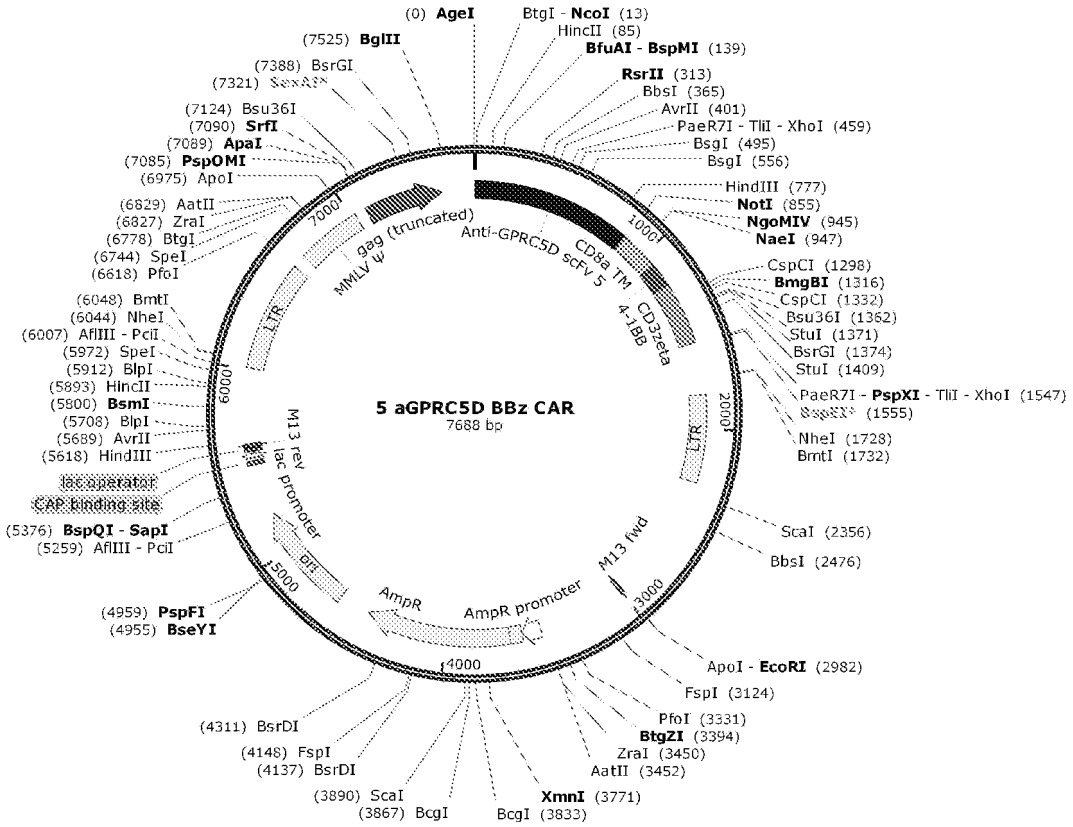

5 aGPRC5D BBz CAR
7688 bp
(0) AgeI
BtgI - NcoI (13)
HincII (85)
BfuAI - BspMI (139)
RsrII (313)
BbsI (365)
AvrII (401)
PaeR7I - TliI - XhoI (459)
BsgI (495)
BsgI (556)
HindIII (777)
NotI (855)
NgoMIV (945)
NaeI (947)
CspCI (1298)
BmgBI (1316)
CspCI (1332)
Bsu36I (1362)
StuI (1371)
BsrGI (1374)
StuI (1409)
PaeR7I - PspXI - TliI - XhoI (1547)
NheI (1728)
BmtI (1732)
ScaI (2356)
BbsI (2476)
ApoI - EcoRI (2982)
FspI (3124)
PfoI (3331)
BtgZI (3394)
ZraI (3450)
AatII (3452)
XmnI (3771)
BcgI (3833)
(3867) BcgI
(3890) ScaI
(4137) BsrDI
(4148) FspI
(4311) BsrDI
(4955) BseYI
(4959) PspFI
(5259) AflII - PciI
(5376) BspQI - SapI
lac operator
CAP binding site
(5618) HindIII
(5689) AvrII
(5708) BlpI
(5800) BsmI
(5893) HincII
(5912) BlpI
(5972) SpeI
(6007) AflII - PciI
(6044) NheI
(6048) BmtI
(6618) PfoI
(6744) SpeI
(6778) BtgI
(6827) ZraI
(6829) AatII
(7085) PspOMI
(7089) ApaI
(7090) SrfI
(7124) Bsu36I
(7321) SexAI
(7388) BsrGI
(7525) BglII
MMLV Ψ
gag (truncated)
Anti-GPRC5D scFv 5
CD8a TM
4-1BB
CD3zeta
LTR
LTR
M13 rev
lac promoter
ori
AmpR
AmpR promoter
M13 fwd
1000
2000
3000
4000
5000
6000
7000

Figure 9

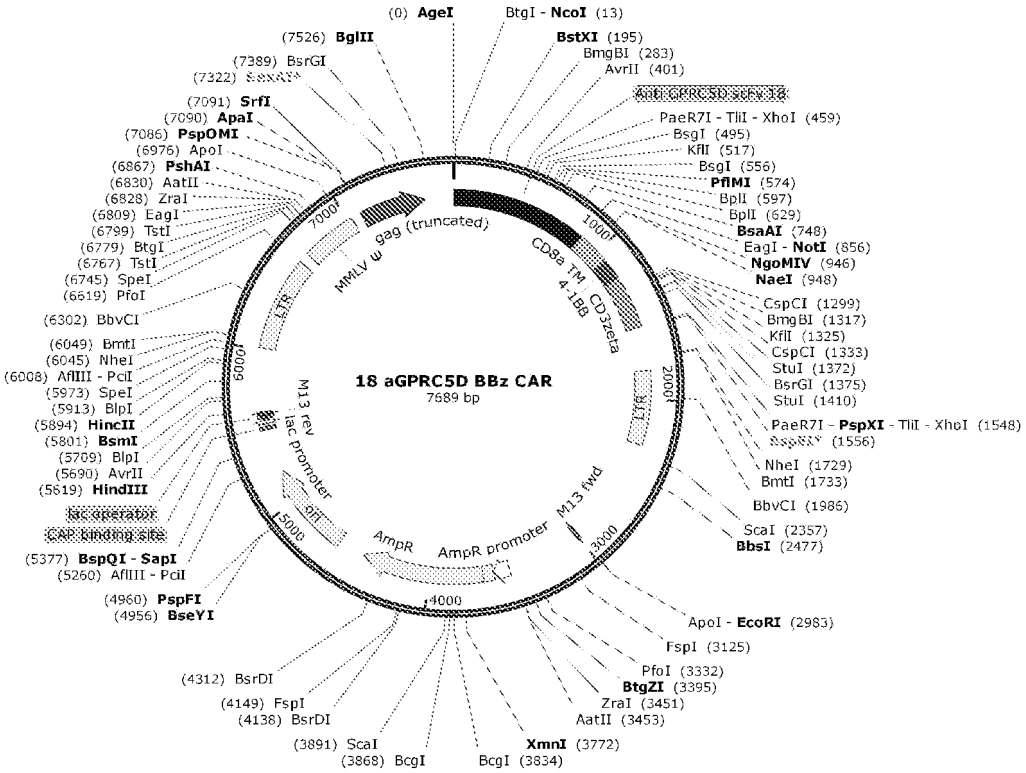

18 aGPRC5D BBz CAR
7689 bp
MMLV ψ
gag (truncated)
CD8a TM
4-1BB
CD3zeta
LTR
M13 rev
lac promoter
ori
AmpR
AmpR promoter
M13 fwd
(0) AgeI
BtgI - NcoI (13)
BstXI (195)
BmgBI (283)
AvrII (401)
PaeR7I - TliI - XhoI (459)
BsgI (495)
KflI (517)
BsgI (556)
PflMI (574)
BplI (597)
BplI (629)
BsaAI (748)
EagI - NotI (856)
NgoMIV (946)
NaeI (948)
CspCI (1299)
BmgBI (1317)
KflI (1325)
CspCI (1333)
StuI (1372)
BsrGI (1375)
StuI (1410)
PaeR7I - PspXI - TliI - XhoI (1548)
NheI (1729)
BmtI (1733)
BbvCI (1986)
ScaI (2357)
BbsI (2477)
ApoI - EcoRI (2983)
FspI (3125)
PfoI (3332)
BtgZI (3395)
ZraI (3451)
AatII (3453)
XmnI (3772)
BcgI (3834)
(3868) BcgI
(3891) ScaI
(4138) BsrDI
(4149) FspI
(4312) BsrDI
(4956) BseYI
(4960) PspFI
(5260) AflIII - PciI
(5377) BspQI - SapI
(5619) HindIII
(5690) AvrII
(5709) BlpI
(5801) BsmI
(5894) HincII
(5913) BlpI
(5973) SpeI
(6008) AflIII - PciI
(6045) NheI
(6049) BmtI
(6302) BbvCI
(6619) PfoI
(6745) SpeI
(6767) TstI
(6779) BtgI
(6799) TstI
(5809) EagI
(6828) ZraI
(6830) AatII
(6867) PshAI
(6976) ApoI
(7086) PspOMI
(7090) ApaI
(7091) SrfI
(7322) BssSI
(7389) BsrGI
(7526) BglII
1000
2000
3000
4000
5000
6000
7000

Figure 10

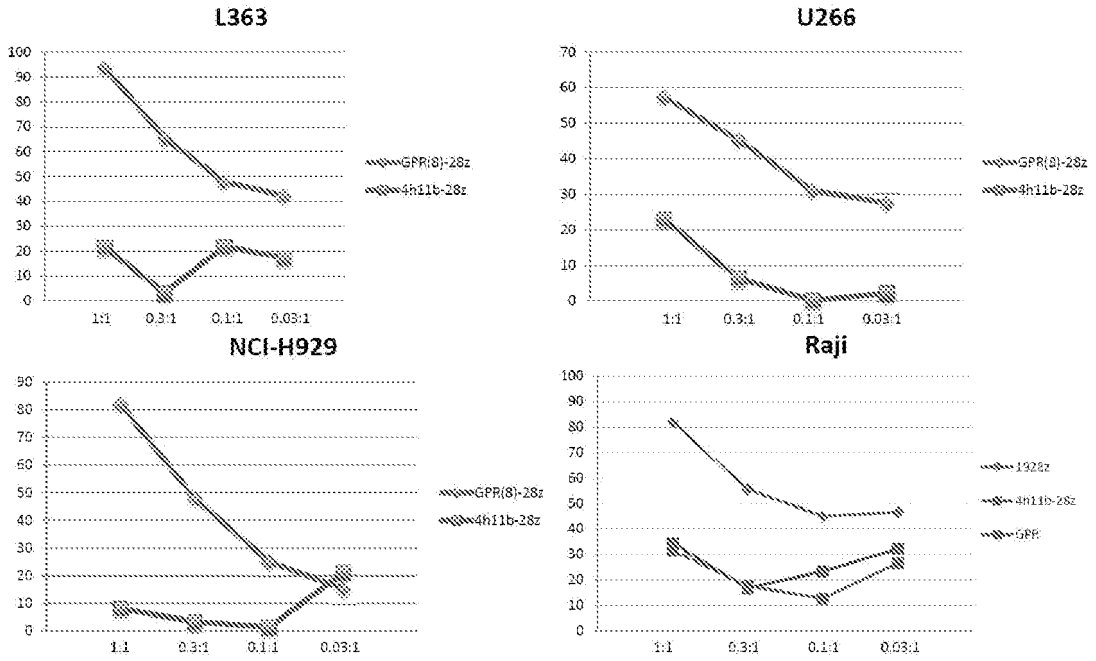
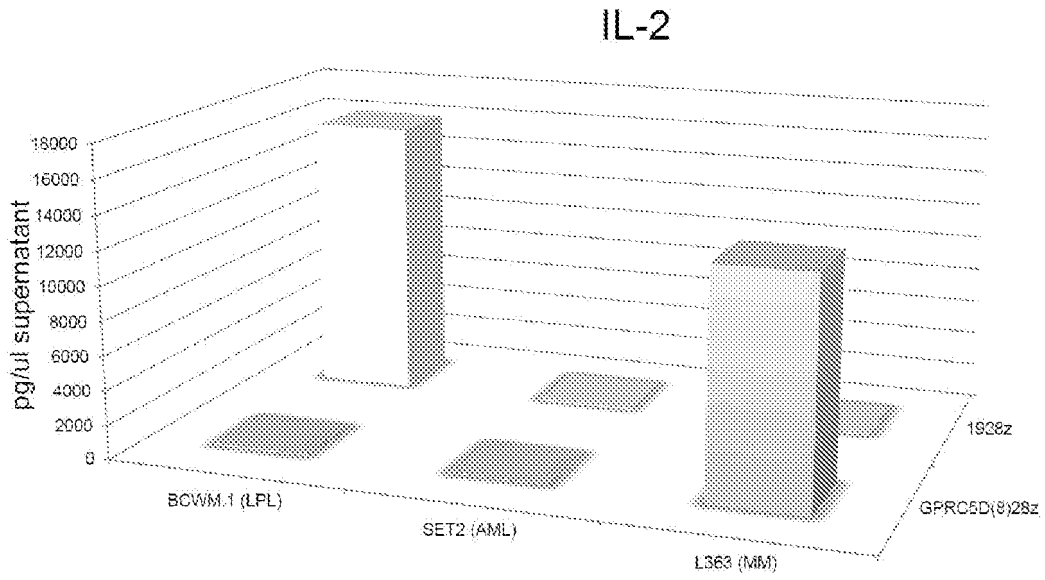

L363
U266
NCI-H929
Raji
GPR(8)-28z
4h11b-28z
1928z
GPR
1:1
0.3:1
0.1:1
0.03:1

Figure 11



IFNg, IL-6, TNFa, sCD40L, GM-CSF

Figure 12

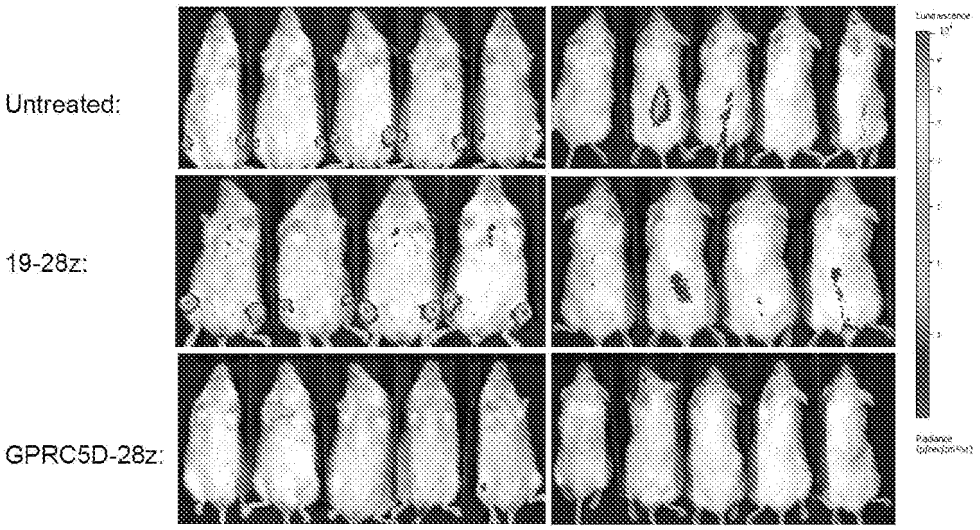


Figure 13A

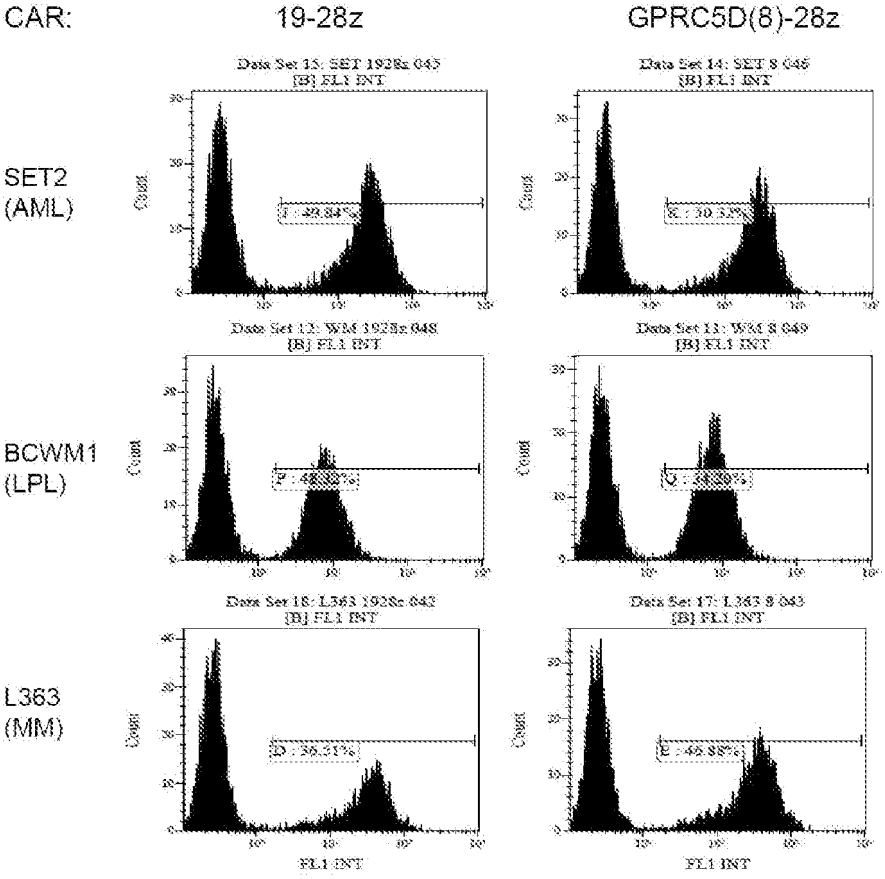

CAR:
19-28z
GPRC5D(8)-28z
SET2
(AML)
BCWM1
(LPL)
L363
(MM)
Count
FL1 INT

Figure 13B

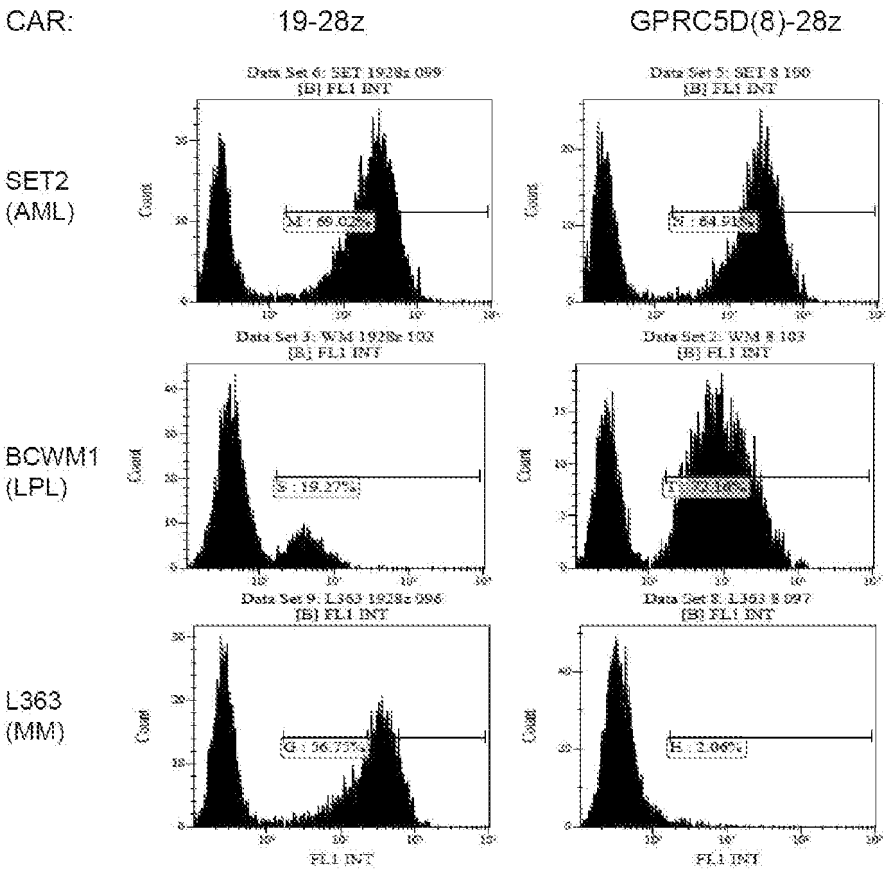

CAR:
19-28z
GPRC5D(8)-28z
SET2
(AML)
BCWM1
(LPL)
L363
(MM)
Count
FL1 INT

Figure 14

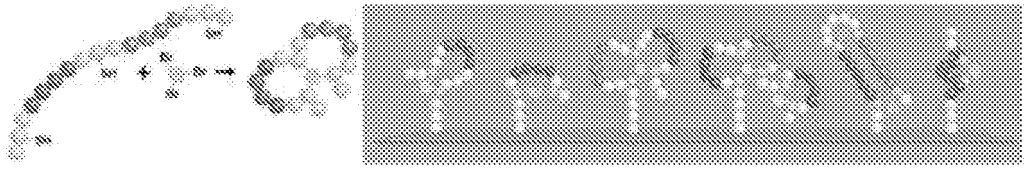


Figure 15

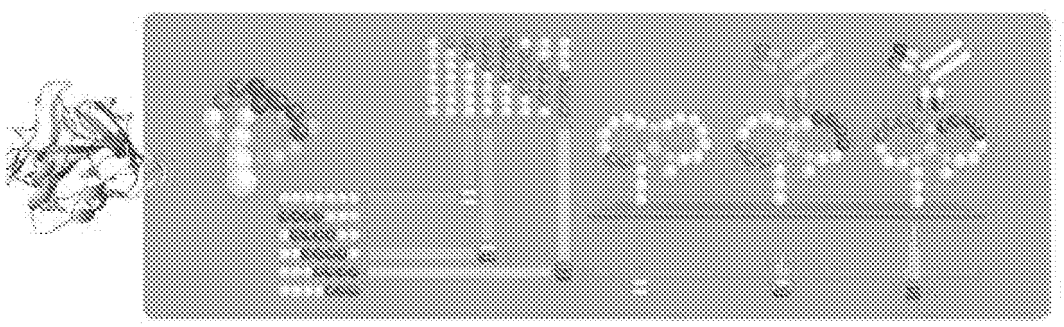
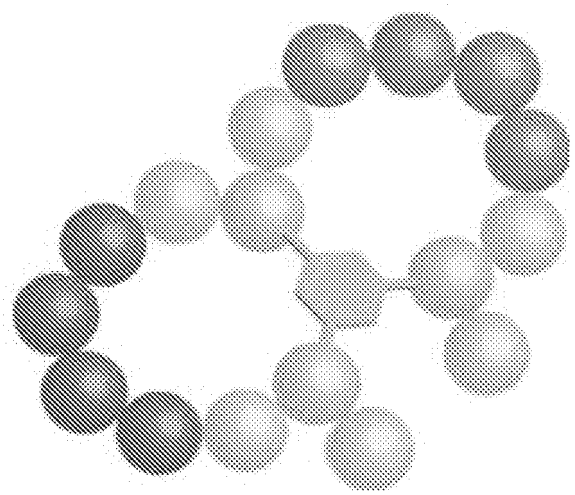


Figure 16



Figures 17A-17D

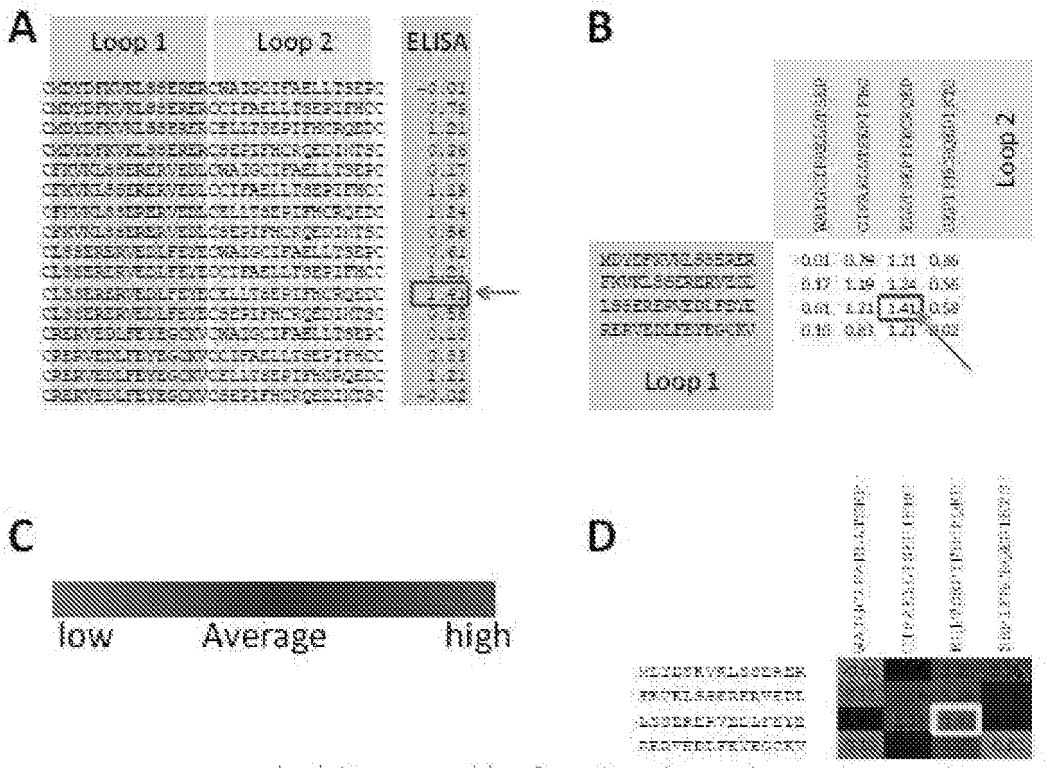


Figure 18

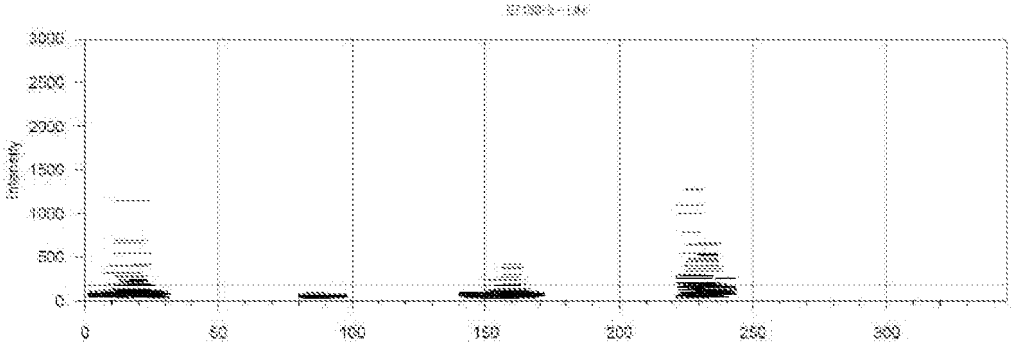


Figure 19

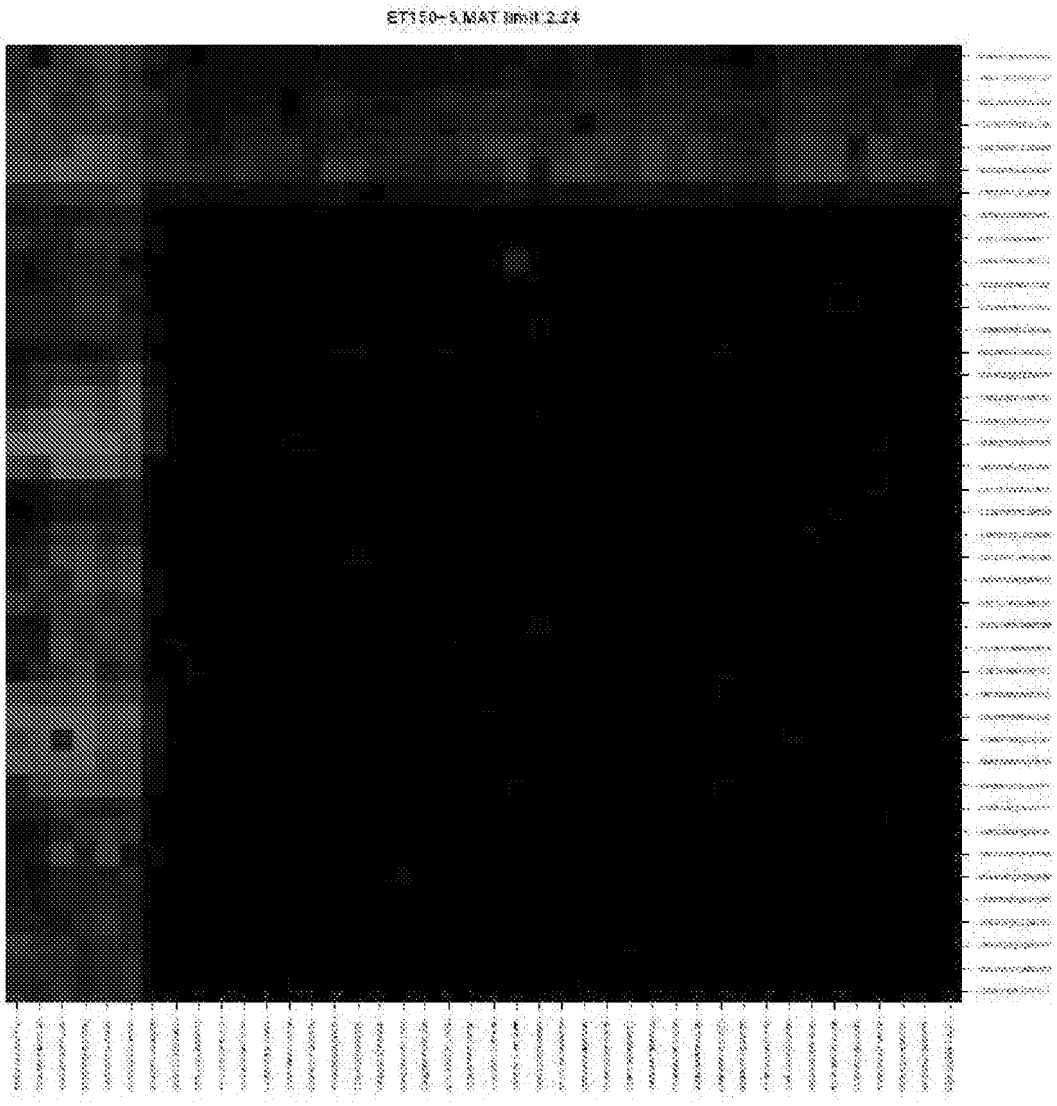


Figure 20

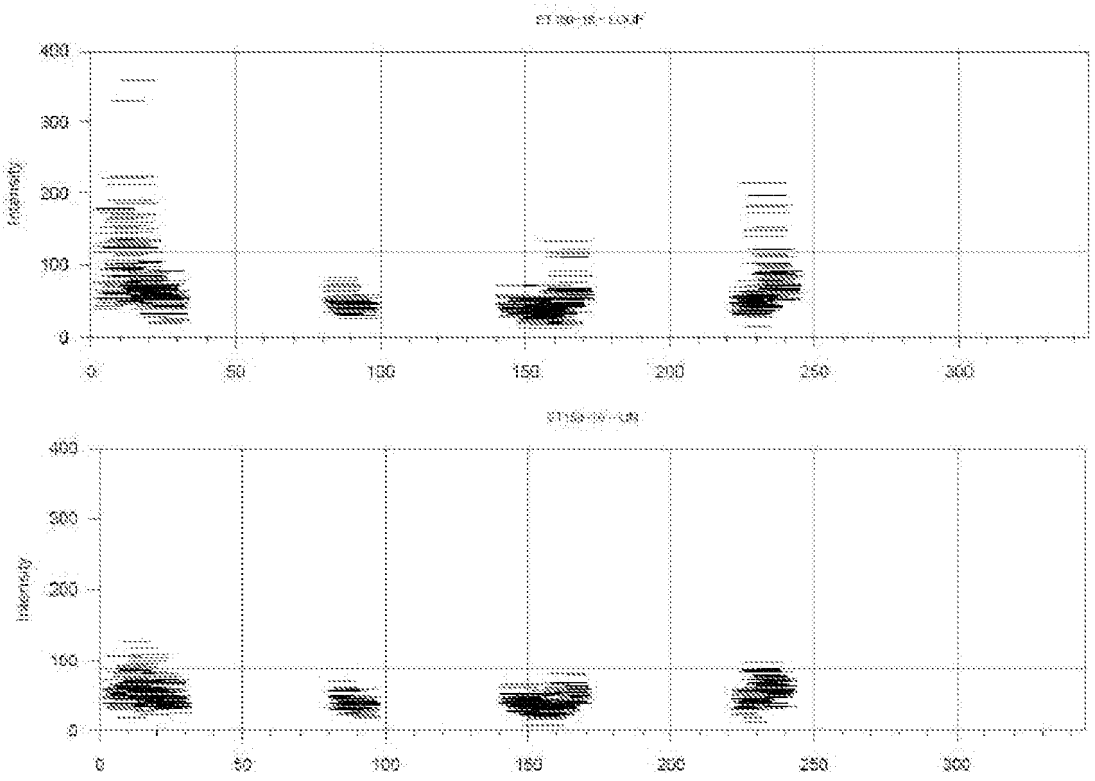


Figure 21

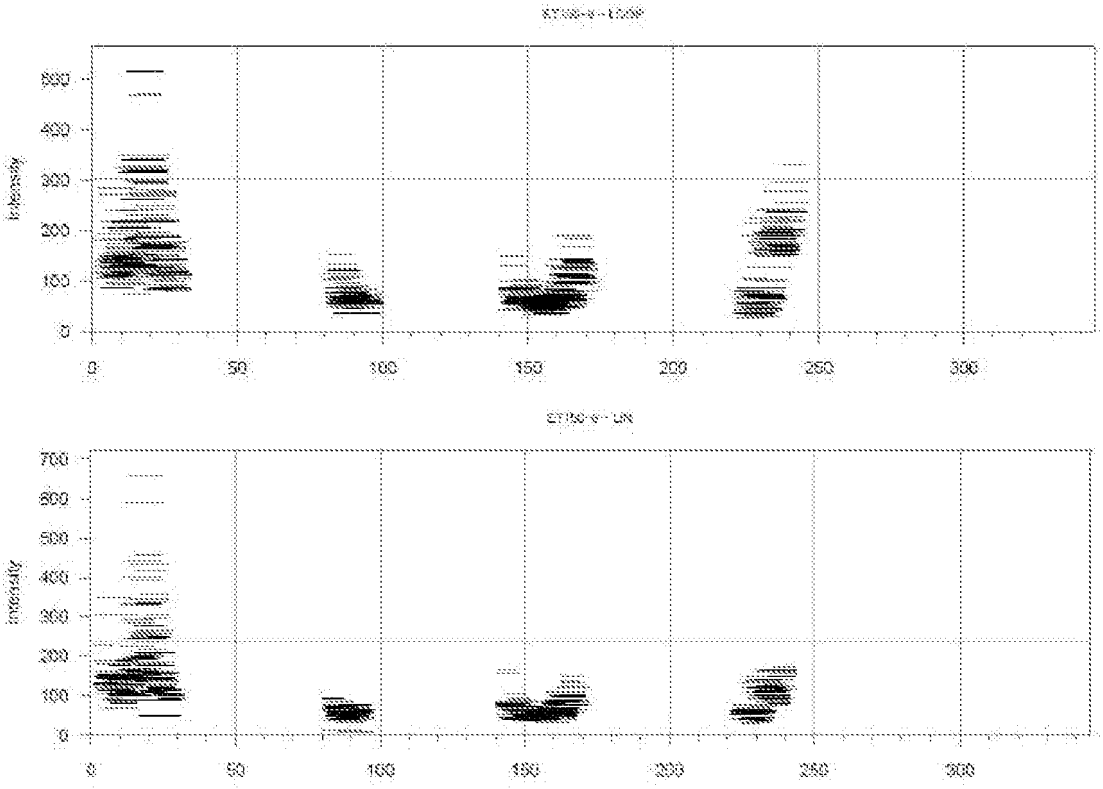


Figure 22

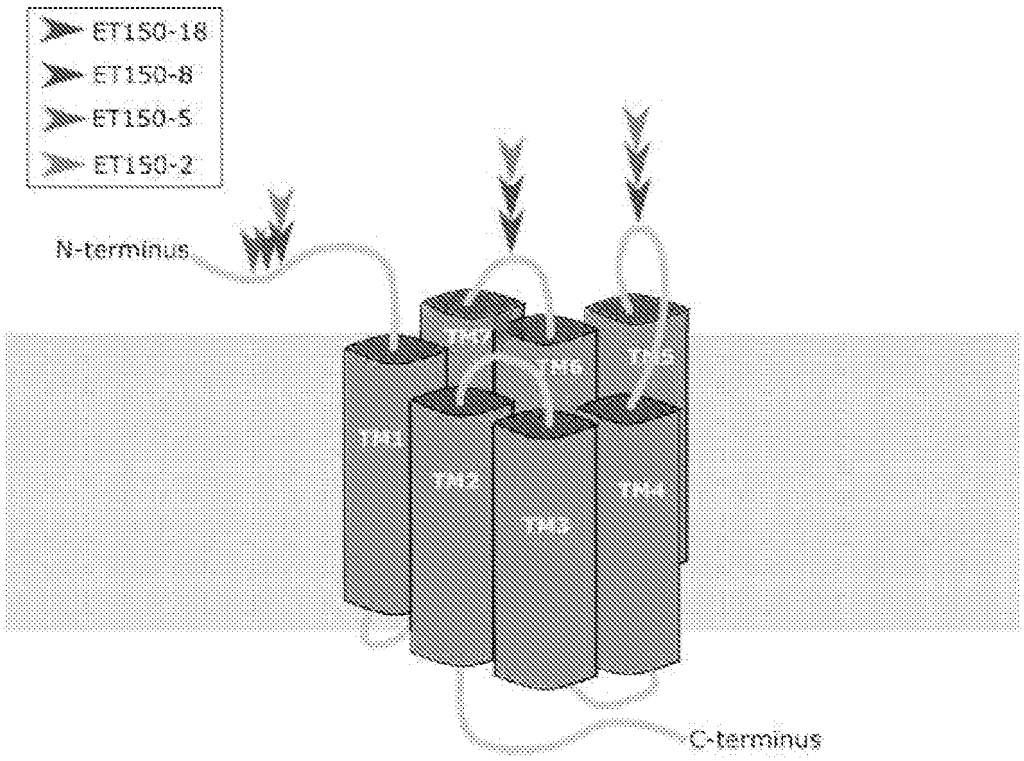

ET150-18
ET150-8
ET150-5
ET150-2
N-terminus
TM1
TM2
TM3
TM4
C-terminus

Figure 23

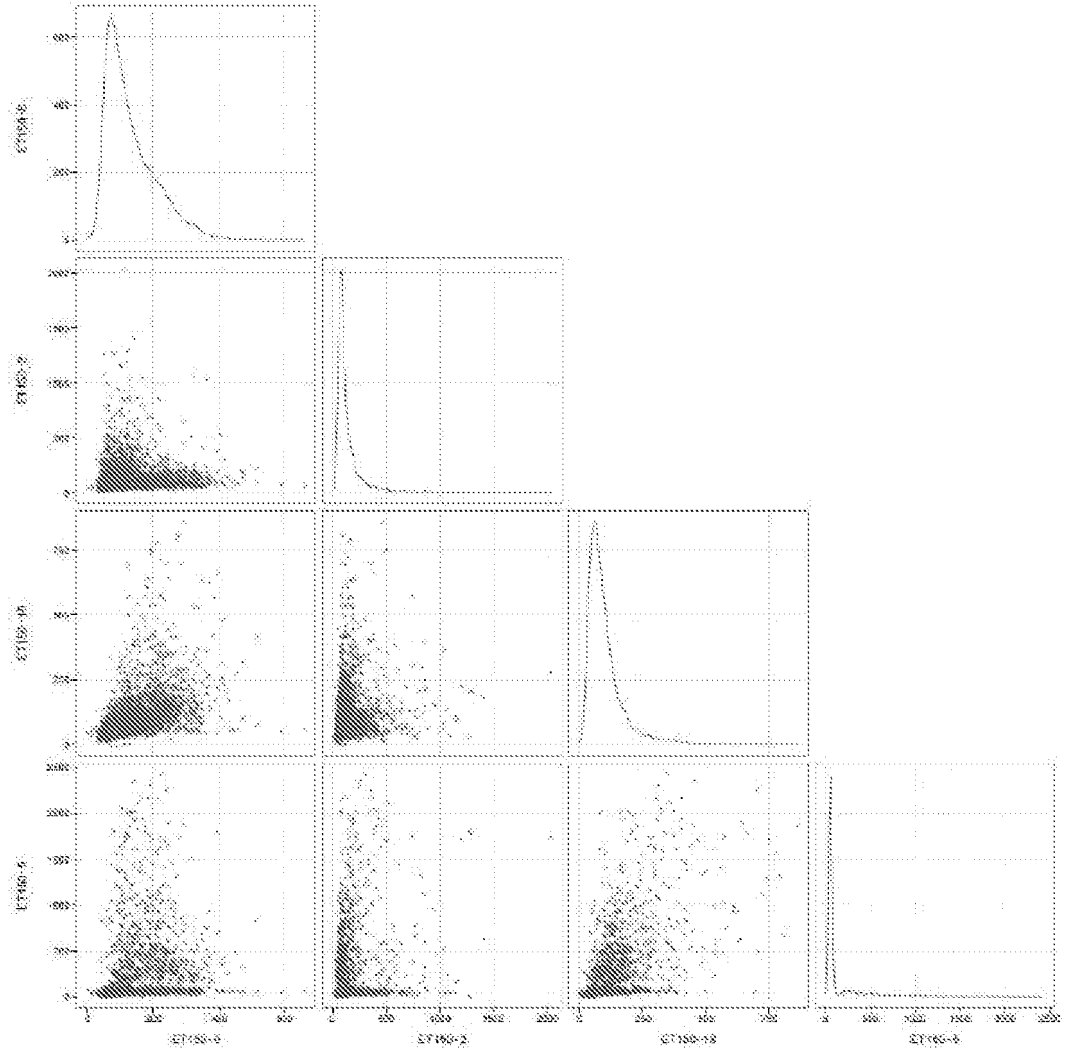


Figure 24

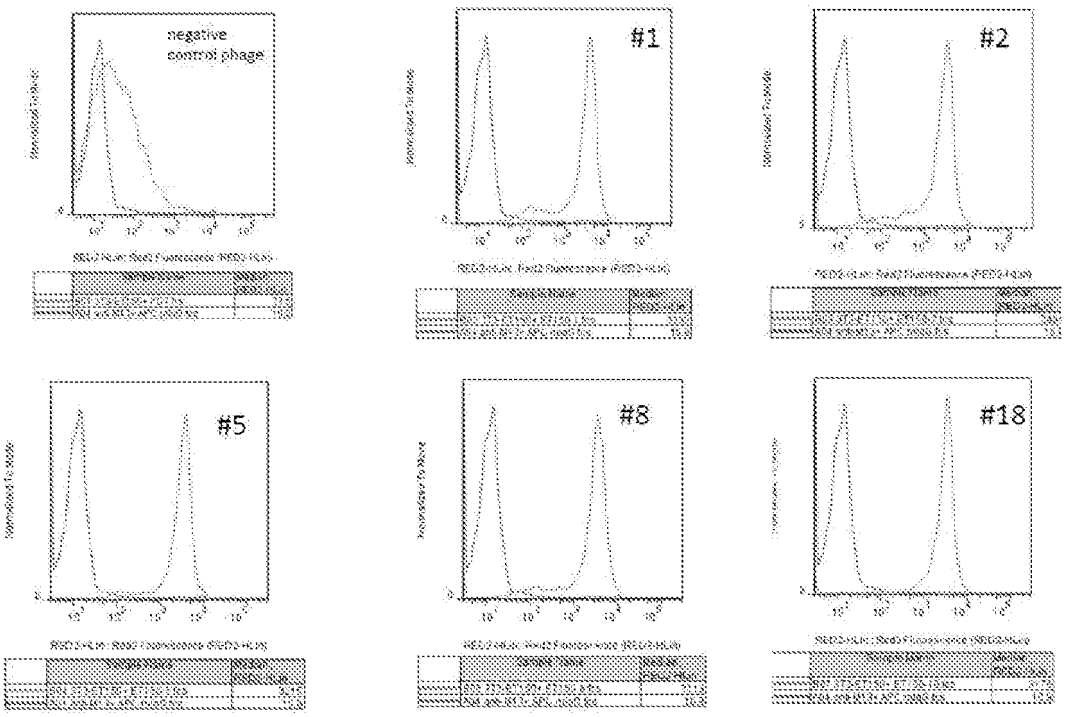


Figure 25

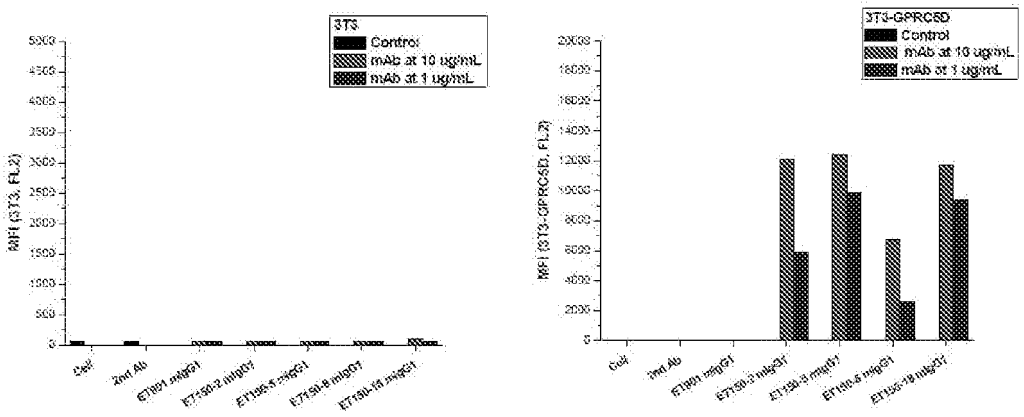

3T3
Control
mAb at 10 ug/mL
mAb at 1 ug/mL
MFI (3T3, FL2)
Cell
2nd Ab
3T3-GPRC5D
Control
mAb at 10 ug/mL
mAb at 1 ug/mL
MFI (3T3-GPRC5D, FL2)
Cell
2nd Ab

Figure 26

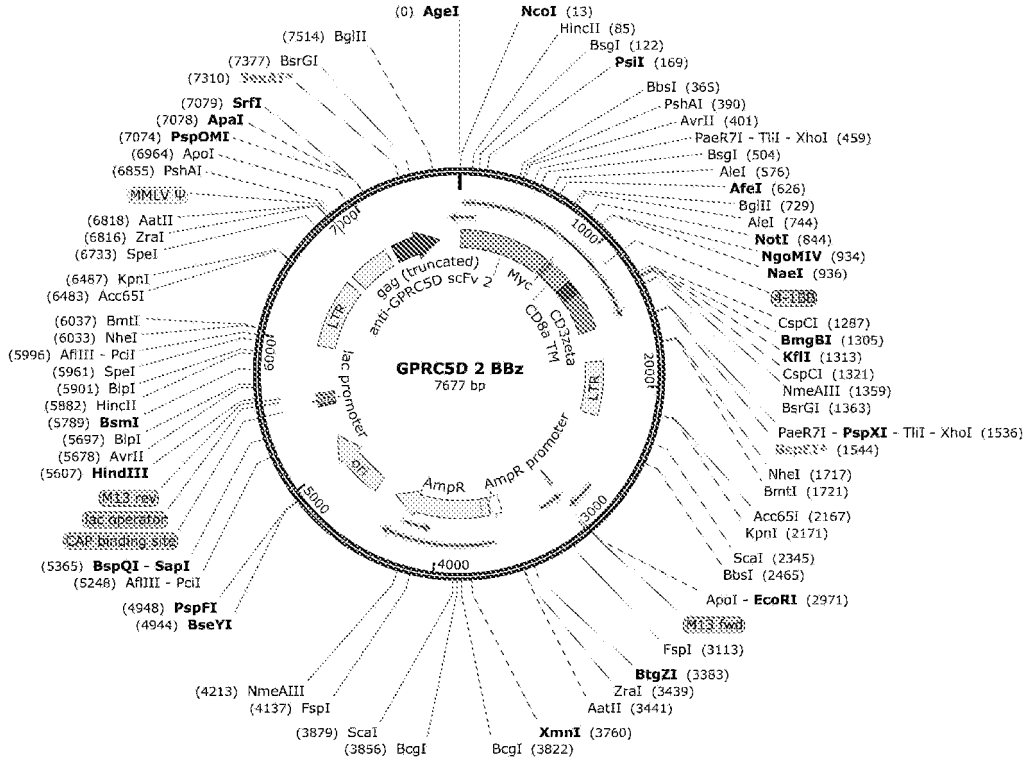
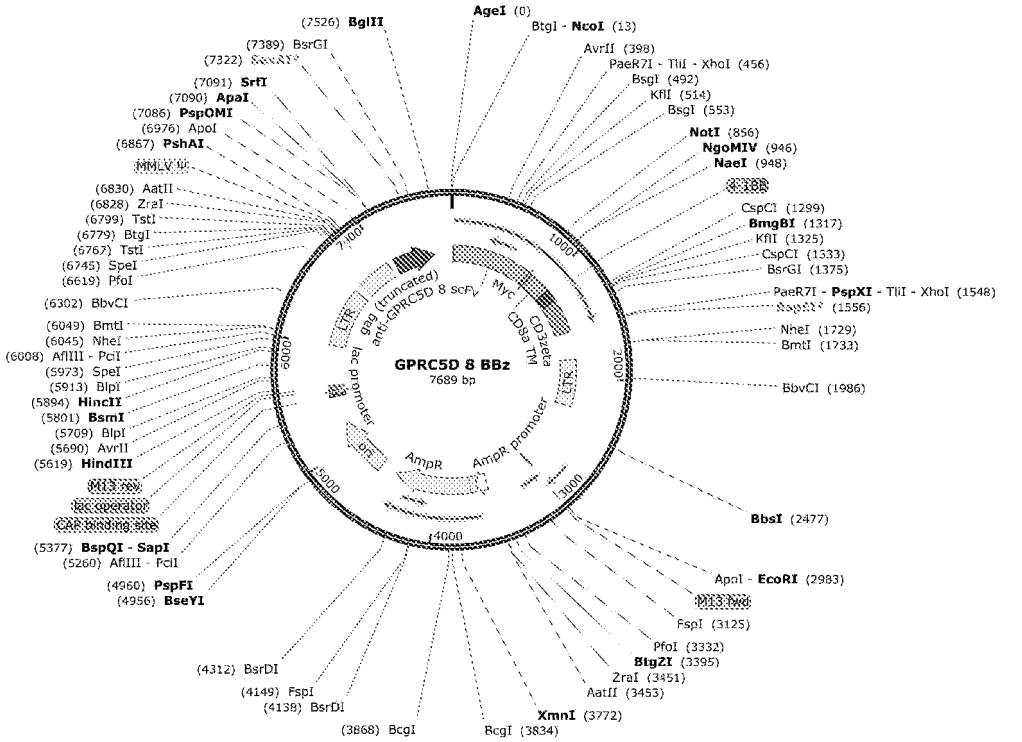

GPRC5D 2 BBz
7677 bp
anti-GPRC5D scFv 2
Myc
CD3zeta
CD8a TM
LTR
LTR
lac promoter
ori
AmpR
AmpR promoter
gag (truncated)
1000
2000
3000
4000
5000
6000
7000
(0) AgeI
NcoI (13)
HincII (85)
BsgI (122)
PsiI (169)
BbsI (365)
PshAI (390)
AvrII (401)
PaeR7I - TliI - XhoI (459)
BsgI (504)
AleI (576)
AfeI (626)
BglII (729)
AleI (744)
NotI (844)
NgoMIV (934)
NaeI (936)
CspCI (1287)
BmgBI (1305)
KflI (1313)
CspCI (1321)
NmeAIII (1359)
BsrGI (1363)
PaeR7I - PspXI - TliI - XhoI (1536)
NheI (1717)
BmtI (1721)
Acc65I (2167)
KpnI (2171)
ScaI (2345)
BbsI (2465)
ApoI - EcoRI (2971)
FspI (3113)
BtgZI (3383)
ZraI (3439)
AatII (3441)
XmnI (3760)
BcgI (3822)
(3879) ScaI
(3856) BcgI
(4213) NmeAIII
(4137) FspI
(4948) PspFI
(4944) BseYI
(5365) BspQI - SapI
(5248) AflIII - PciI
M13 rev
lac operator
CAP binding site
(5607) HindIII
(5678) AvrII
(5697) BlpI
(5789) BsmI
(5882) HincII
(5901) BlpI
(5961) SpeI
(5996) AflIII - PciI
(6033) NheI
(6037) BmtI
(6483) Acc65I
(6487) KpnI
(6733) SpeI
(6816) ZraI
(6818) AatII
MMLV Ψ
(6855) PshAI
(6964) ApoI
(7074) PspOMI
(7078) ApaI
(7079) SrfI
(7310)
(7377) BsrGI
(7514) BglII

Figure 27


GPRC5D 8 BBz
7689 bp
AgeI (0)
BtgI - NcoI (13)
AvrII (398)
PaeR7I - TliI - XhoI (456)
BsgI (492)
KflI (514)
BsgI (553)
NotI (856)
NgoMIV (946)
NaeI (948)
CspCI (1299)
BmgBI (1317)
KflI (1325)
CspCI (1333)
BsrGI (1375)
PaeR7I - PspXI - TliI - XhoI (1548)
NheI (1729)
BmtI (1733)
BbvCI (1986)
BbsI (2477)
ApaI - EcoRI (2983)
FspI (3125)
PfoI (3332)
BtgZI (3395)
ZraI (3451)
AatII (3453)
XmnI (3772)
BcgI (3834)
(3868) BcgI
(4138) BsrDI
(4149) FspI
(4312) BsrDI
(4956) BseYI
(4960) PspFI
(5260) AflIII - PciI
(5377) BspQI - SapI
(5619) HindIII
(5690) AvrII
(5709) BlpI
(5801) BsmI
(5894) HincII
(5913) BlpI
(5973) SpeI
(6008) AflIII - PciI
(6045) NheI
(6049) BmtI
(6302) BbvCI
(6619) PfoI
(6745) SpeI
(6767) TstI
(6779) BtgI
(6799) TstI
(6828) ZraI
(6830) AatII
(6867) PshAI
(6976) ApoI
(7086) PspOMI
(7090) ApaI
(7091) SrfI
(7322) SexAI
(7389) BsrGI
(7526) BglII
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
LTR
gag (truncated)
anti-GPRC5D 8 scFv
MYC
CD8a TM
CD3zeta
LTR
lac promoter
ori
AmpR
AmpR promoter
M13 rev
lac operator
CAR binding site
M13 fwd