Figure 1

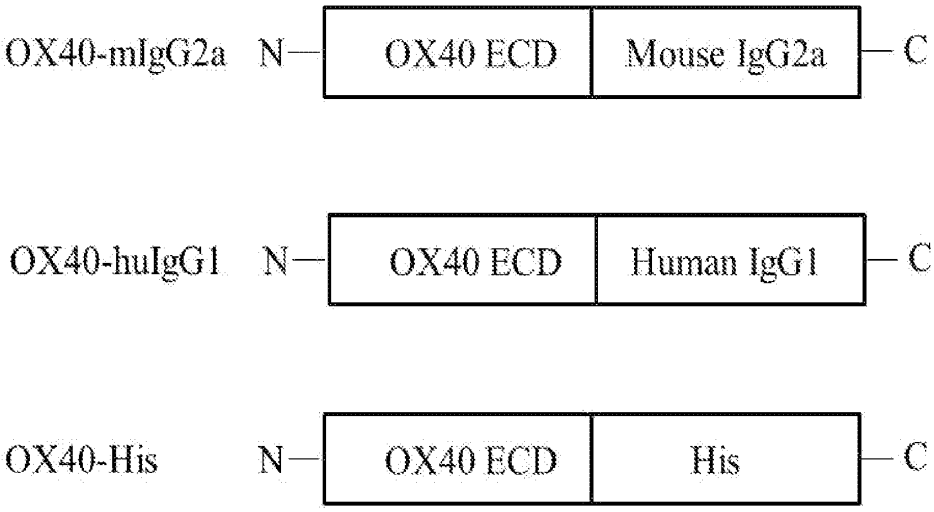

OX40-mIgG2a
N
OX40 ECD
Mouse IgG2a
C
OX40-huIgG1
N
OX40 ECD
Human IgG1
C
OX40-His
N
OX40 ECD
His
C

Figure 2

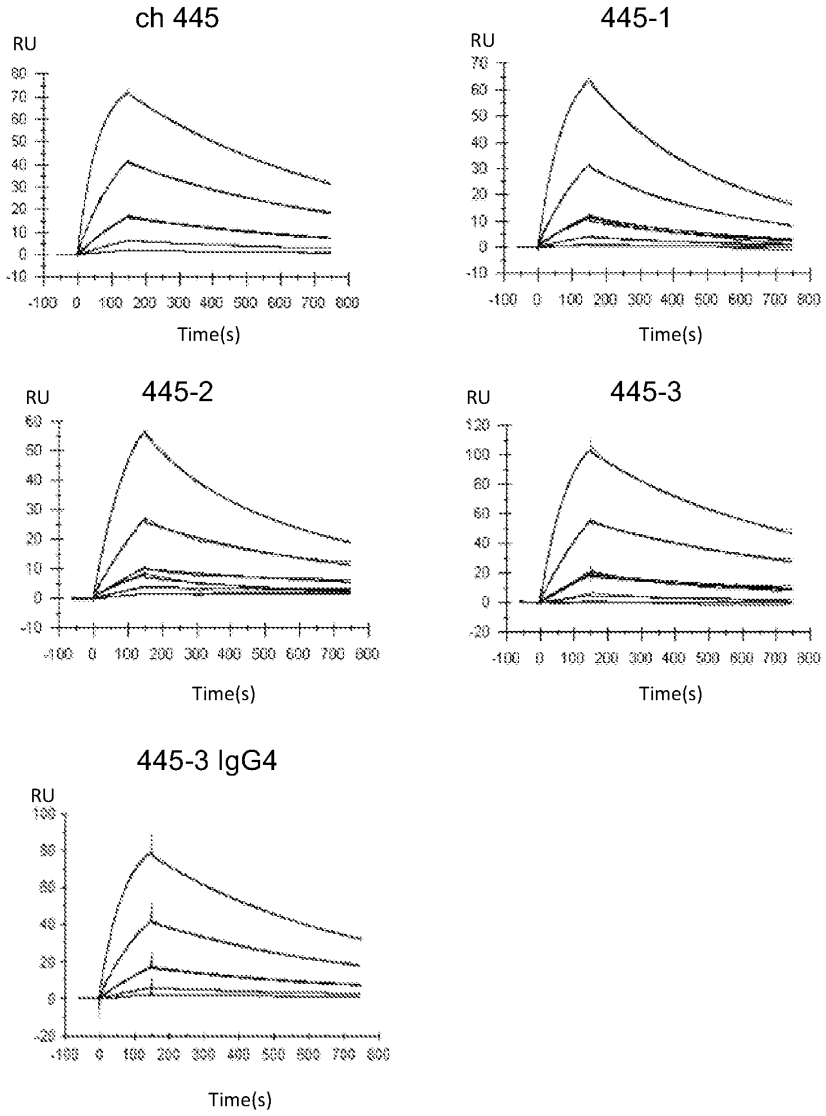

ch 445
RU
Time(s)
445-1
RU
Time(s)
445-2
RU
Time(s)
445-3
RU
Time(s)
445-3 IgG4
RU
Time(s)

Figure 3

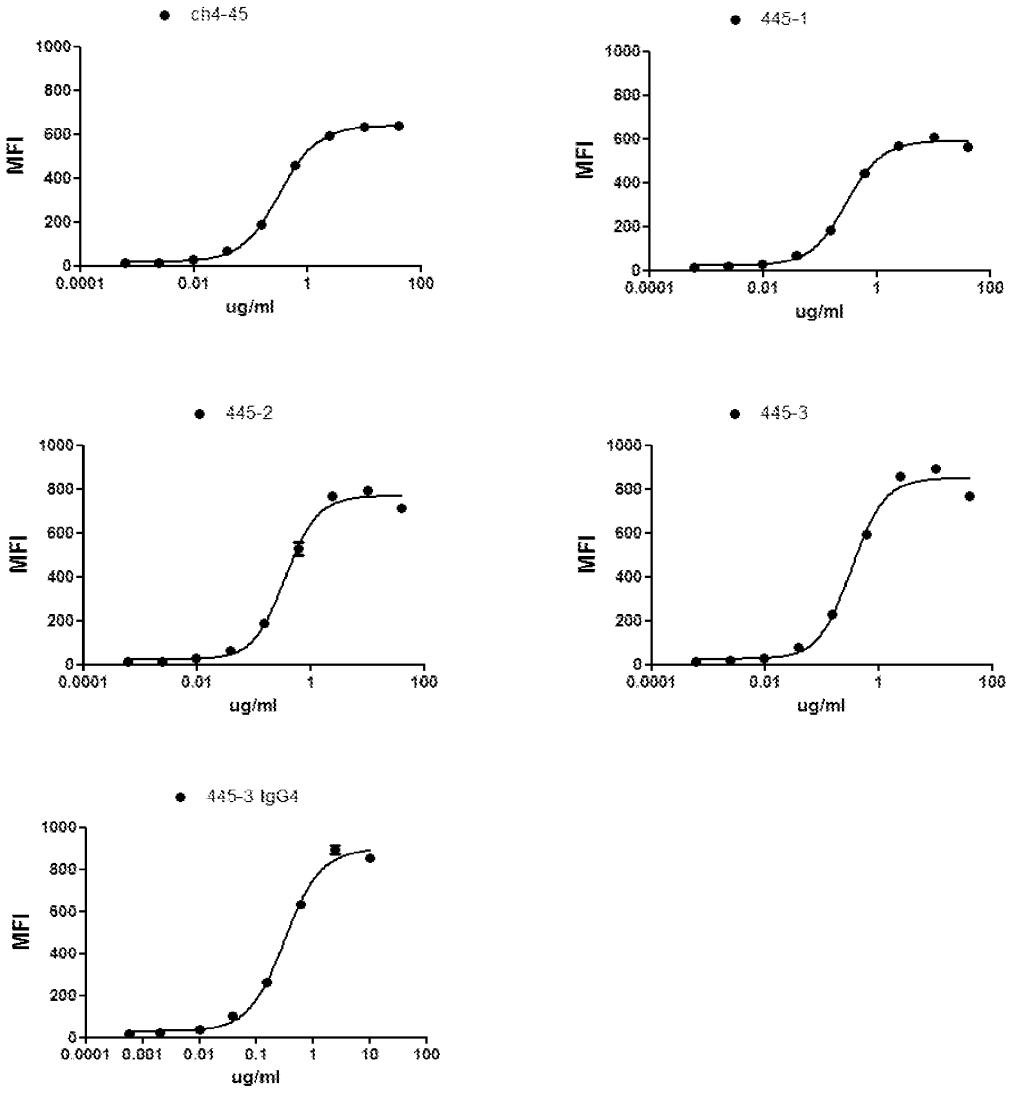

ch4-45
445-1
445-2
445-3
445-3 IgG4
MFI
ug/ml
0
200
400
600
800
1000
0.0001
0.001
0.01
0.1
1
10
100

Figure 4

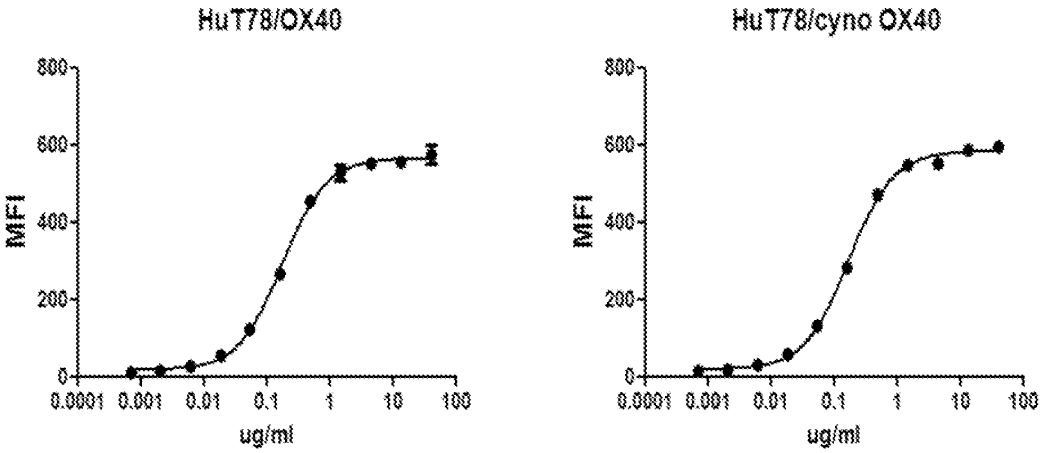

HuT78/OX40
MFI
800
600
400
200
0
0.0001 0.001 0.01 0.1 1 10 100
ug/ml
HuT78/cyno OX40
MFI
800
600
400
200
0
0.0001 0.001 0.01 0.1 1 10 100
ug/ml

Figure 5

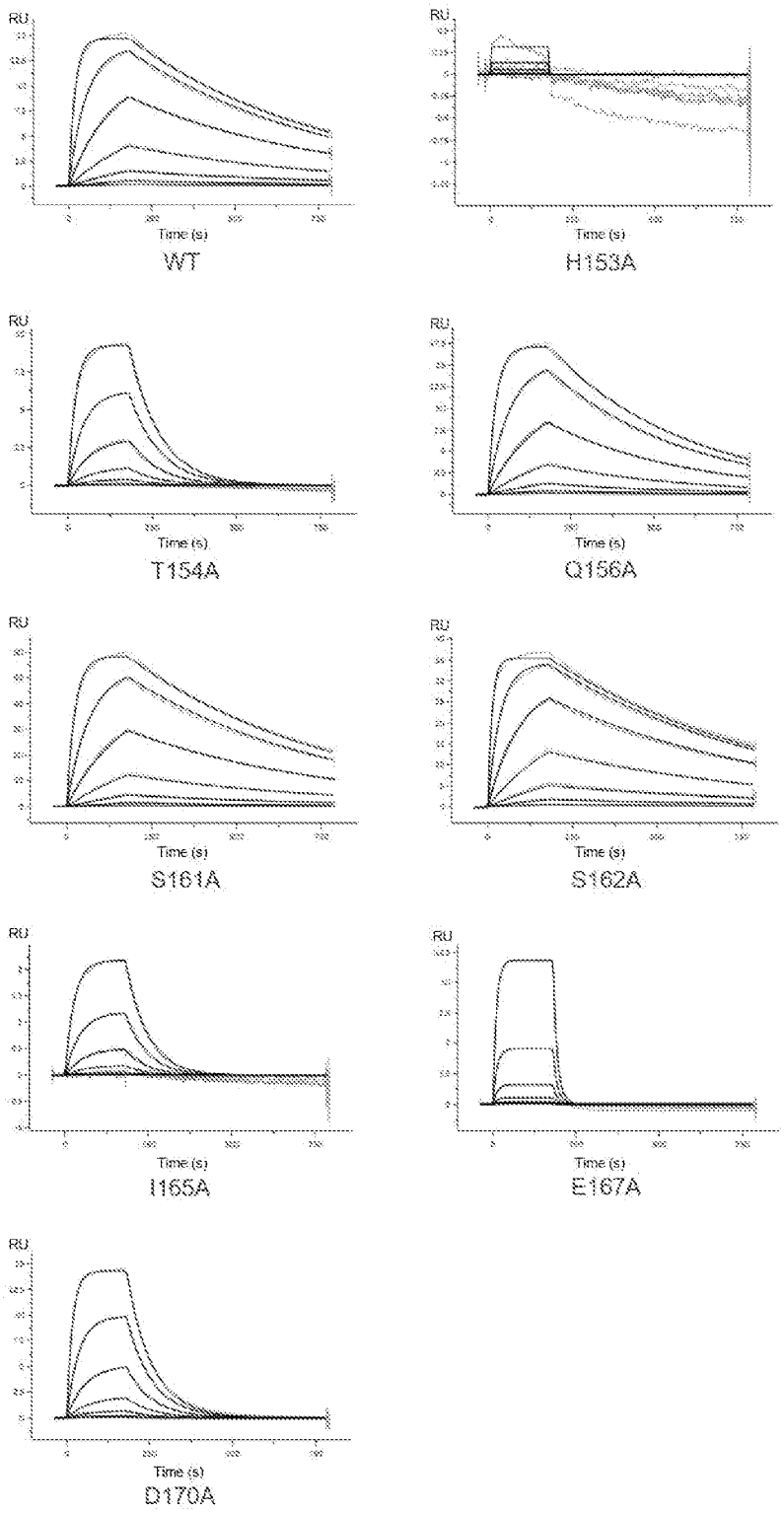


Figure 6

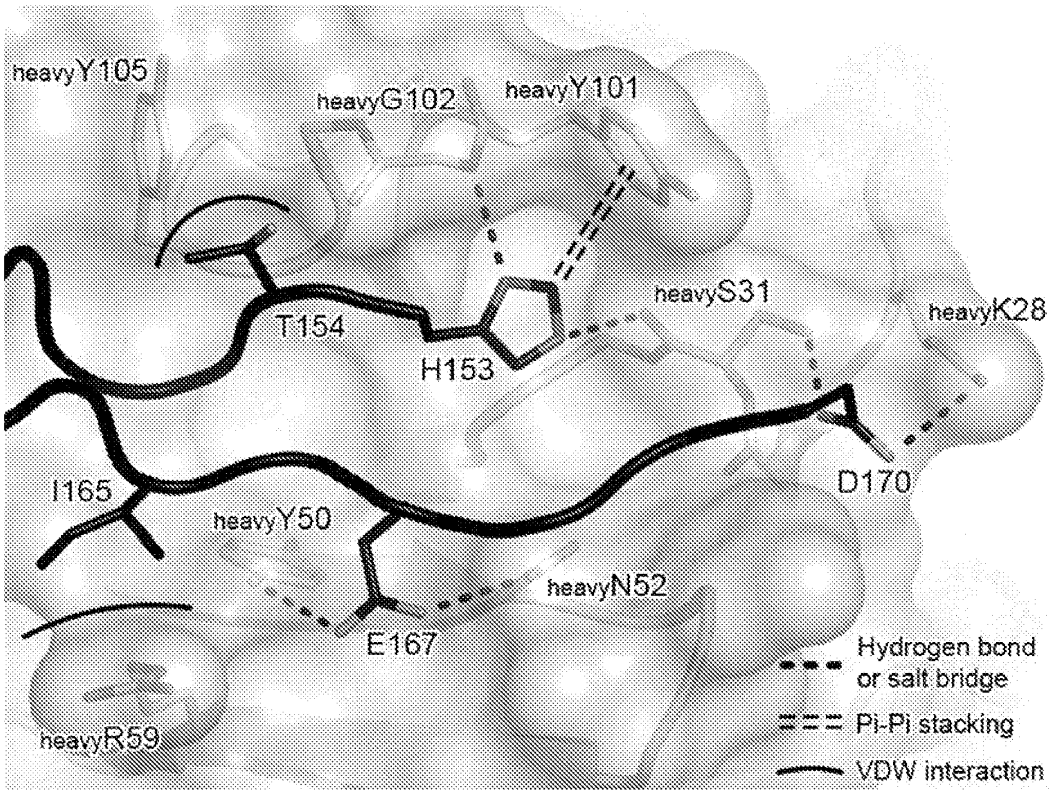

heavyY105
heavyG102
heavyY101
heavyS31
heavyK28
T154
H153
I165
heavyY50
D170
heavyN52
E167
heavyR59
Hydrogen bond or salt bridge
Pi-Pi stacking
VDW interaction

Figure 7

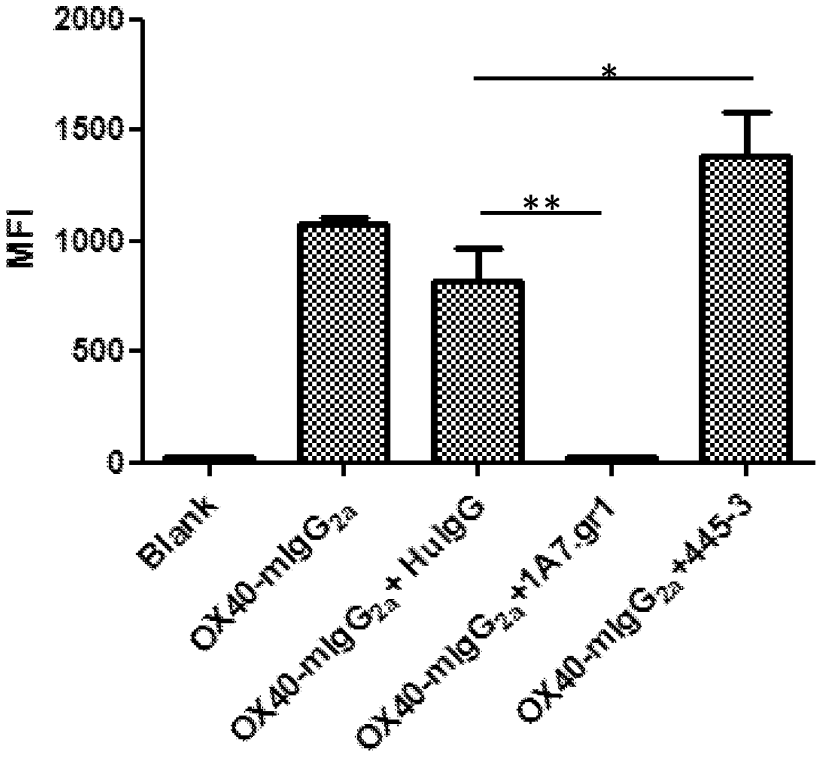

2000
1500
1000
500
0
MFI
*
**
Blank
OX40-mIgG2a
OX40-mIgG2a + HuIgG
OX40-mIgG2a +1A7.gr1
OX40-mIgG2a +445-3

Figure 8

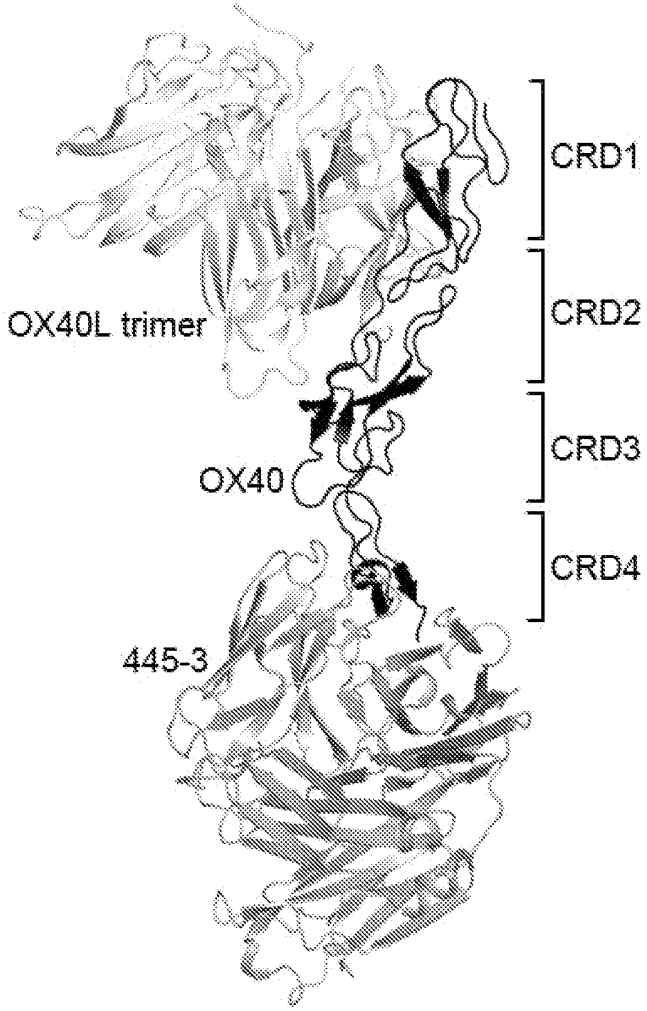


Figure 9A-B

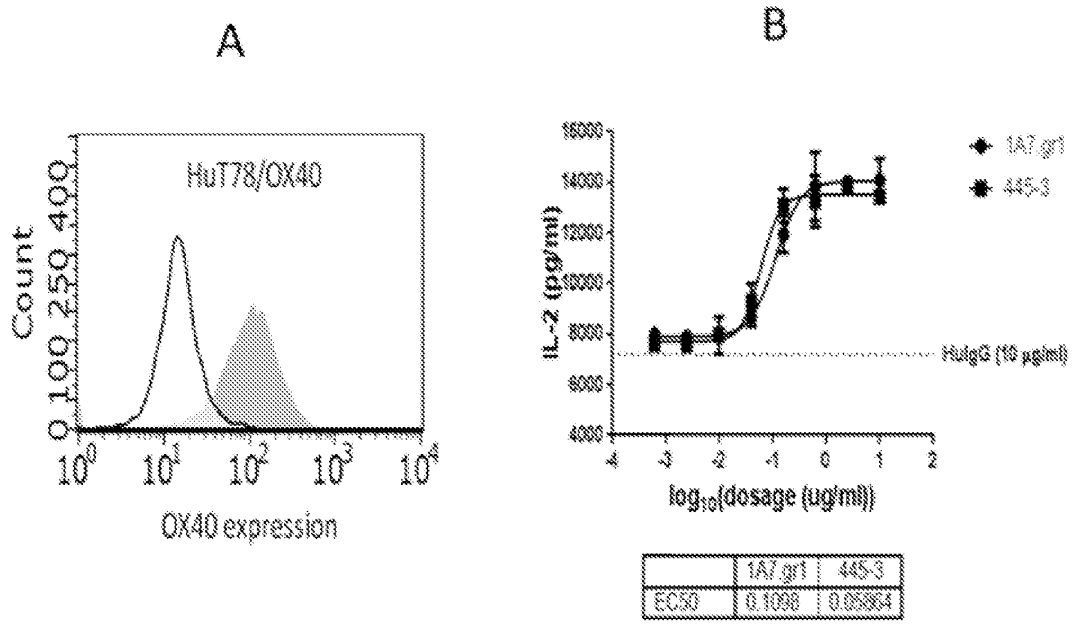

A
HuT78/OX40
Count
0 100 250 400
10^0 10^1 10^2 10^3 10^4
OX40 expression
B
IL-2 (pg/ml)
1600
1400
1200
1000
800
600
400
1A7 gr1
445-3
HuIgG (10 μg/ml)
-4 -3 -2 -1 0 1 2
log_{10}(dosage (ug/ml))
1A7 gr1 445-3
EC50 0.1006 0.0534

Figure 10

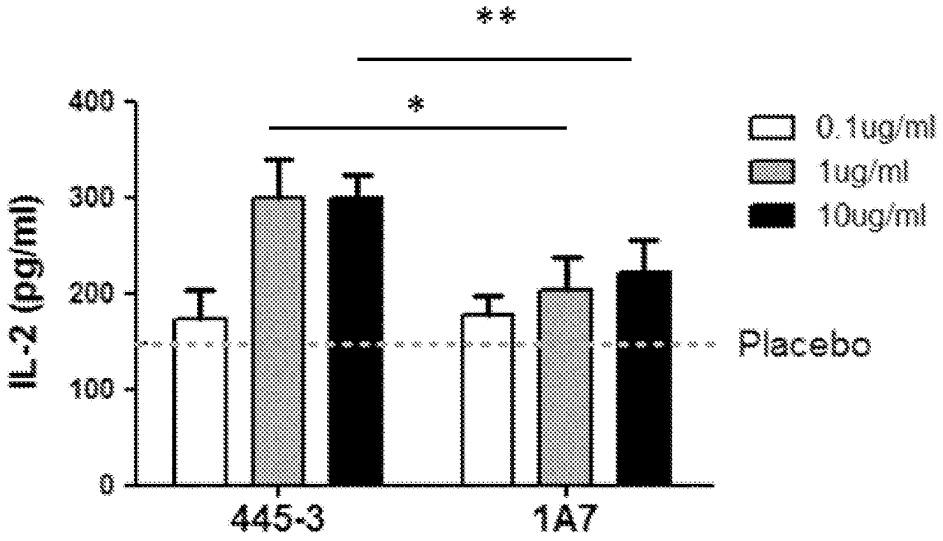
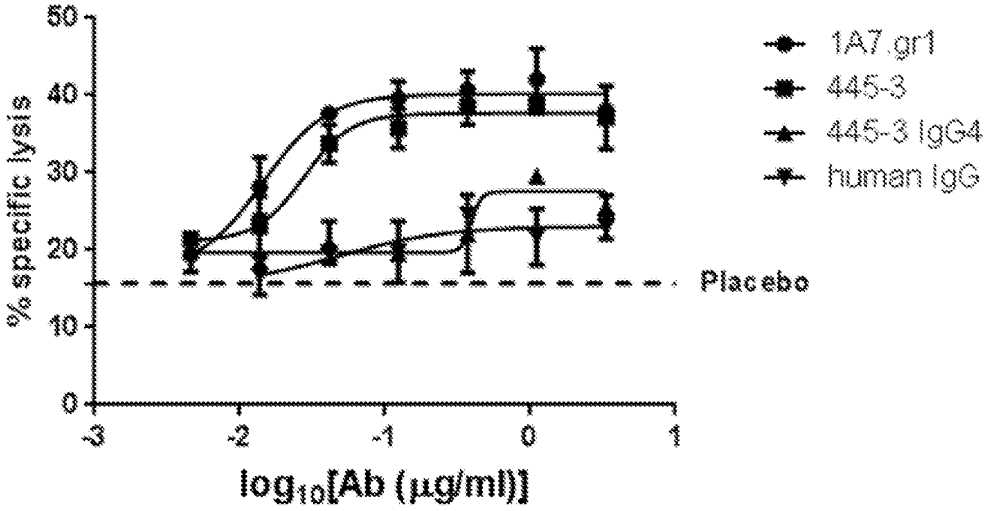

**
*
400
300
200
100
0
IL-2 (pg/ml)
0.1ug/ml
1ug/ml
10ug/ml
Placebo
445-3
1A7

Figure 11



	1A7.gr1	445-3
EC50	0.01441	0.02754

Figure 12A-12C

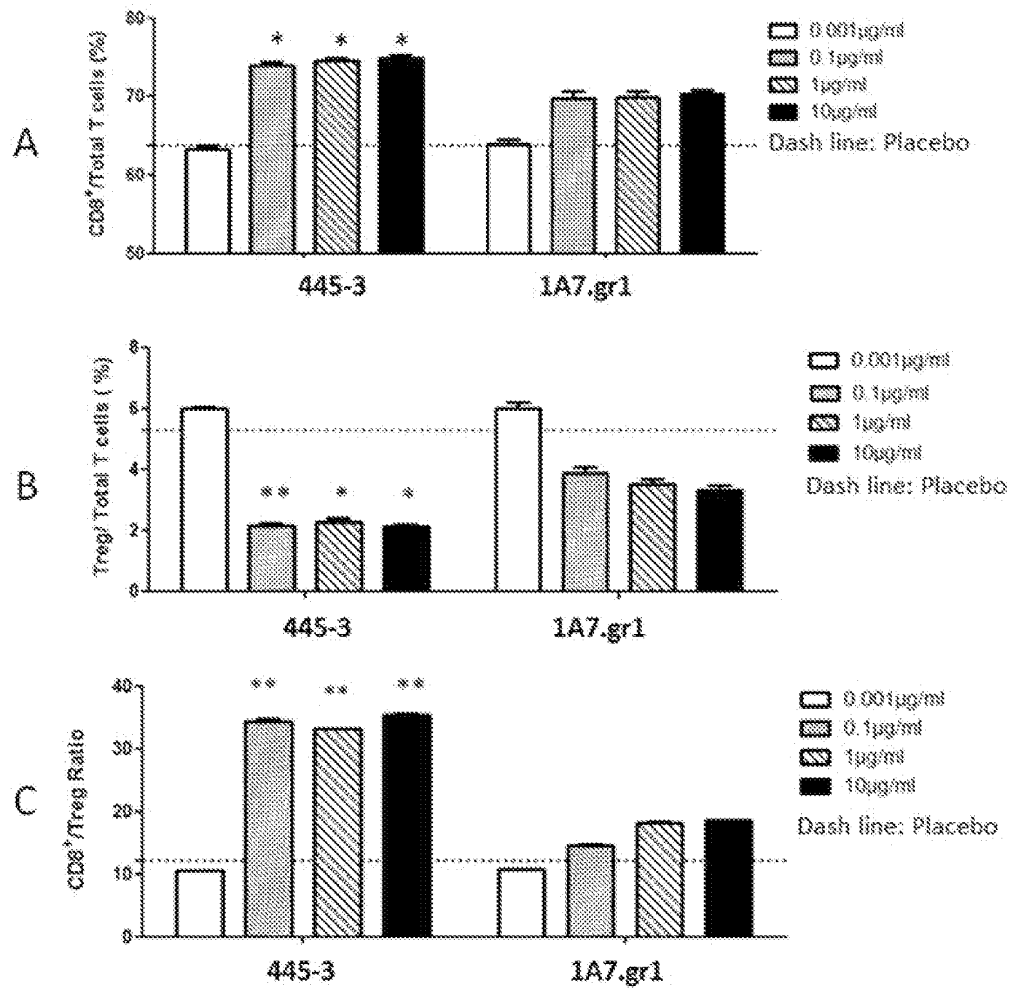


Figure 13A

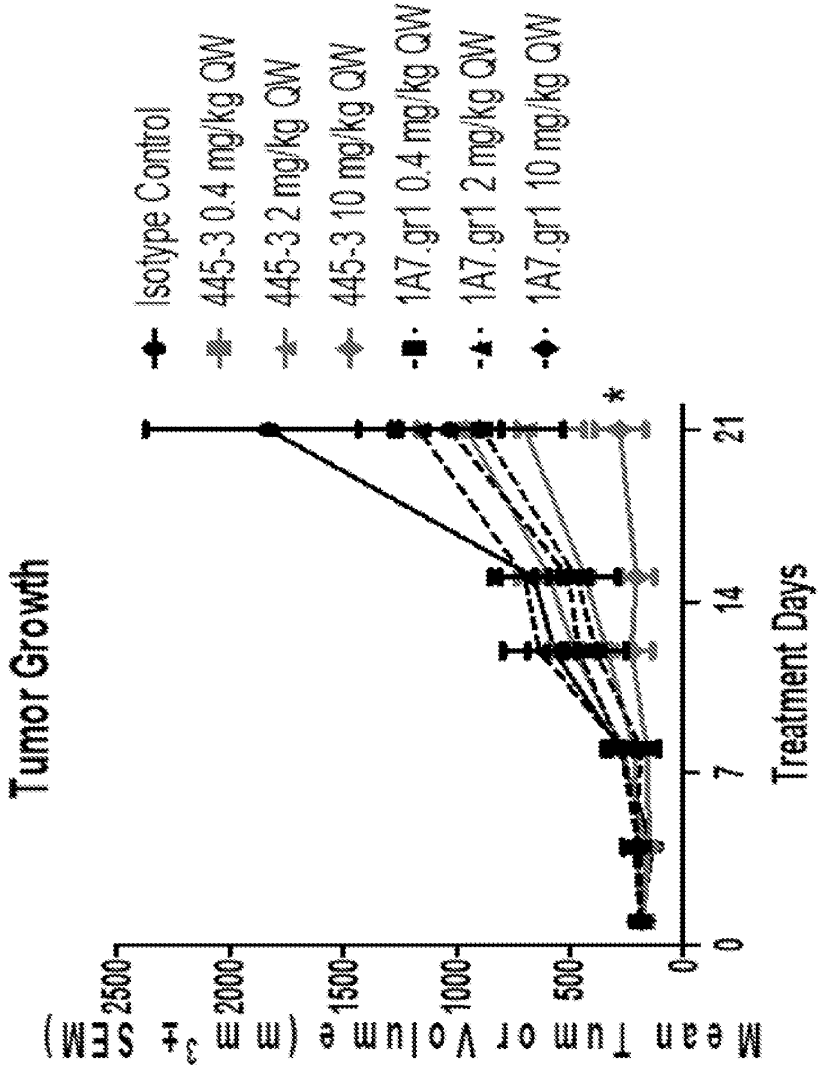

Tumor Growth
Isotype Control
445-3 0.4 mg/kg QW
445-3 2 mg/kg QW
445-3 10 mg/kg QW
1A7.gr1 0.4 mg/kg QW
1A7.gr1 2 mg/kg QW
1A7.gr1 10 mg/kg QW
*
Mean Tumor Volume (mm3 ± SEM)
2500
2000
1500
1000
500
0
0
7
14
21
Treatment Days

Figure 13B

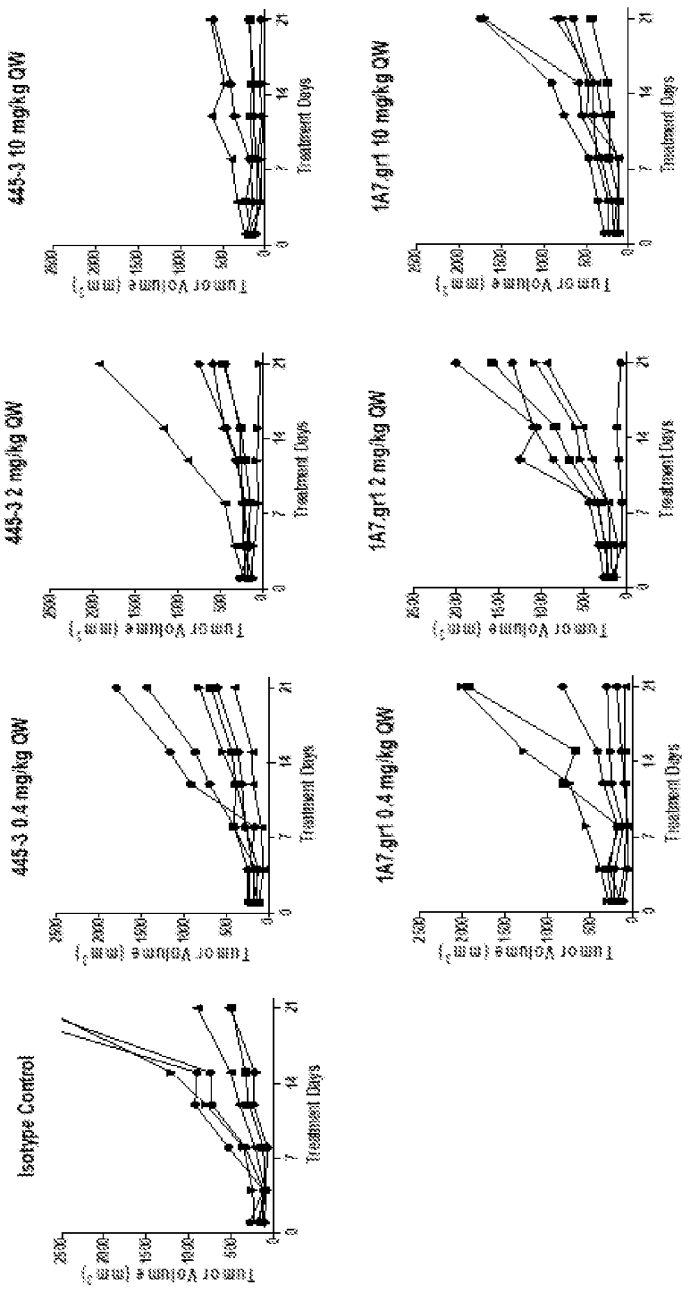

445-3 10 mg/kg QW
1A7.gr1 10 mg/kg QW
445-3 2 mg/kg QW
1A7.gr1 2 mg/kg QW
445-3 0.4 mg/kg QW
1A7.gr1 0.4 mg/kg QW
Isotype Control
Tumor Volume (mm³)
Treatment Days

Figure 14A

Heavy chain		Light chain		Co-transfection with heavy chain and light chain	FACS		Biacore		
ID	CDR/FR	ID	CDR/FR	Ab ID	EC_{50}(ng/ml)	Top(MFI)	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	K_D (M)
445-1VH	-	445-1VL	-	445-1	336	500	2.37E+05	3.89E-03	1.64E-08
445-1VH-M48I	FR2	445-1VL	-	445-2(M48I)-1 (445-1 with M48I mutation on VH)	353	480	2.30E+05	3.56E-03	1.55E-08
445-1VH	-	445-1VL-S24G	CDR1	445-1-2(S24G) (445-1 with S24G mutation on VL)	307	470	2.22E+05	3.60E-03	1.62E-08
445-1VH	-	445-1VL-S10T/E81D/D70E	FR1/FR3	445-1-2(S10T/E81D/D70E) (445-1 with S10T, E81D and D70E mutations on VL)	280	510	2.28E+05	3.40E-03	1.49E-08

Figure 14B

Heavy chain		Light chain		Co-transfection with heavy chain and light chain	FACS		Biacore		
ID	CDR/FR	ID	CDR/FR	Ab ID	EC_{50}(ng/ml)	Top(MFI)	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	K_D (M)
445-2VH	-	445-2VL	-	445-2	397	760	2.06E+05	2.64E-03	1.28E-08
445-2VH-Q62E-Q65K	CDR2	445-2VL-S24R	CDR1	445-3(Q62E/Q65K)-3(S24R) (445-2 with Q62E and Q65K mutations on VH and S24R mutation on VL)	318	790	1.87E+05	2.32E-03	1.24E-08
445-2VH-Q62E	CDR2	445-2VL-S24R	CDR1	445-3(Q62E)-3(S24R) (445-2 with Q62E mutation on VH and S24R mutation on VL)	363	760	1.94E+05	2.27E-03	1.17E-08
445-2VH-K28T	FR1	445-2VL-S24R	CDR1	445-3(K28T)-3(S24R) (445-2 with K28T mutation on VH and S24R mutation on VL)	660	750	4.44E+05	5.74E-03	1.29E-08
445-2VH-Y27G	FR1	445-2VL-S24R	CDR1	445-3(Y27G)-3(S24R) (445-2 with Y27G mutation on VH and S24R mutation on VL)	755	630	6.25E+04	1.35E-03	2.15E-08
445-2VH-T30S	FR1	445-2VL-S24R	CDR1	445-3(T30S)-3(S24R) (445-2 with T30S mutation on VH and S24R mutation on VL)	405	800	2.91E+05	3.81E-03	1.31E-08
445-3VH	-	445-3VL	-	445-3	462	990	1.74E+05	1.48E-03	8.51E-09
445-2VH-A61N-K28R	CDR2/FR1	445-3VL	-	445-3(A61N-K28R)-3 (445-2 VH with A61N and K28R mutations and 445-3 VL)	369	1080	1.57E+05	1.15E-03	7.35E-09
445-2VH-A61N-K63R	CDR2	445-3VL	-	445-3(A61N-K63R)-3 (445-2 VH with A61N and K63R mutations and 445-3 VL)	483	1100	1.61E+05	1.55E-03	9.65E-09
445-3VH	-	445-2VL-G41D-K42G-S24R-K93R	FR2/CDR1/CDR3	445-3-3(G41D-K42G-S24R-K93R) (445-3 VH and 445-2 VL with G41D, K42G, S24R and K93R mutations)	337	1050	2.12E+05	1.68E-03	7.90E-09