Figure 1A

Surface Plasmon Resonance (K_D)

	Human		Cynomolgus		Mouse	Rat	
	CTLA-4		CTLA-4		CTLA-4	CTLA-4	
	dimer	monomer	dimer	monomer	dimer	dimer	monomer
AGEN1884	0.6 nM	0.8 nM	6.2 nM	1.6 nM	9.9 nM	No binding	No binding
Reference Antibody (IgG1)	0.54 nM	1.2 nM	1.2 nM	0.75 nM	1.2 nM	26 nM	22 nM

Figure 1B

CTLA-4 Expressing Cells

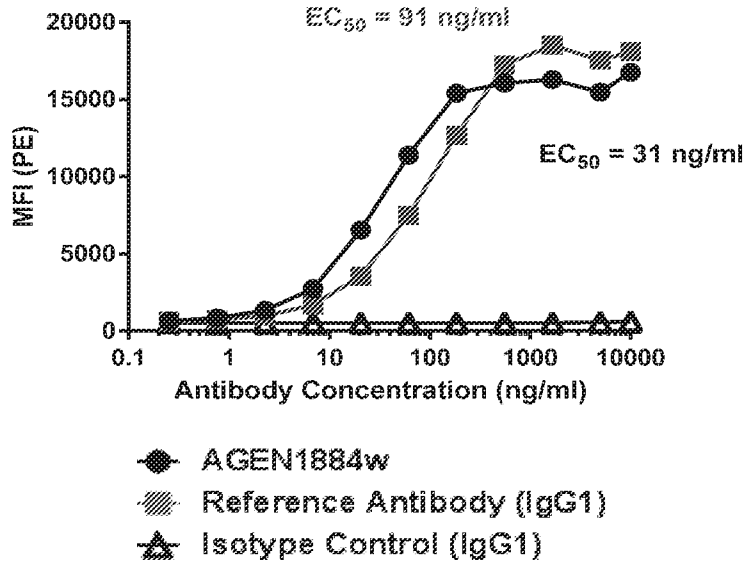


Figure 1C

CTLA-4 Selectivity Assay

	Conc. in ng/ml	rhCTLA-4 Fc (R&D)	rcCTLA-4 Fc (Sino)	rhCD28 Fc (R&D)	rhICOS Fc (R&D)	rhBTLA Fc (Sino)	rhPD-1 Fc (R&D)	rcPD-1 Fc (4AB)
AGEN1884	1000	22729	16557	45	43	87	15	32
	100	20859	12676	48	47	115	25	41
	10	7820	4096	77	60	95	32	46
Reference Antibody (IgG1)	1000	20845	21580	52	46	97	24	40
	100	19131	19810	55	53	87	28	41
	10	6825	7245	65	57	92	35	41
Isotype Control (IgG1)	1000	79	154	57	34	73	31	49
	100	75	166	67	67	123	39	45
	10	88	175	52	48	91	31	30

Figure 1D

Relative Binding Affinity

	AGEN1884	Reference Antibody (IgG1)	Isotype Control (IgG1)
rhCTLA-4	+++	+++	—
rcCTLA-4	+++	+++	—
rhCD28	—	—	—
rhICOS	—	—	—
rhBTLA	—	—	—
rhPD-1	—	—	—
rcPD-1	—	—	—

Figure 2A

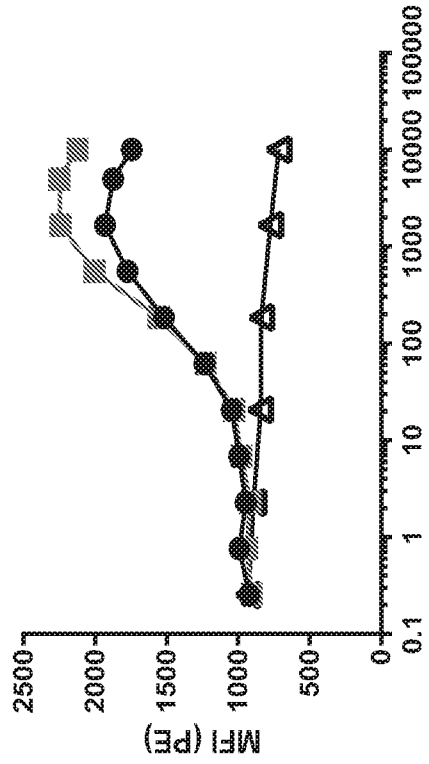


Figure 2B

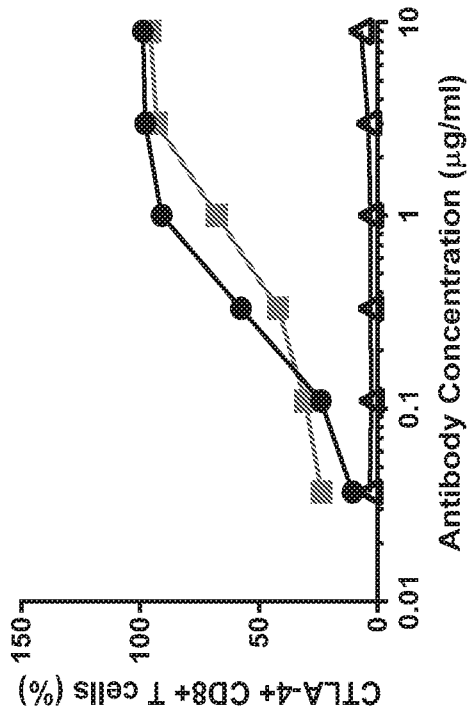


Figure 2C

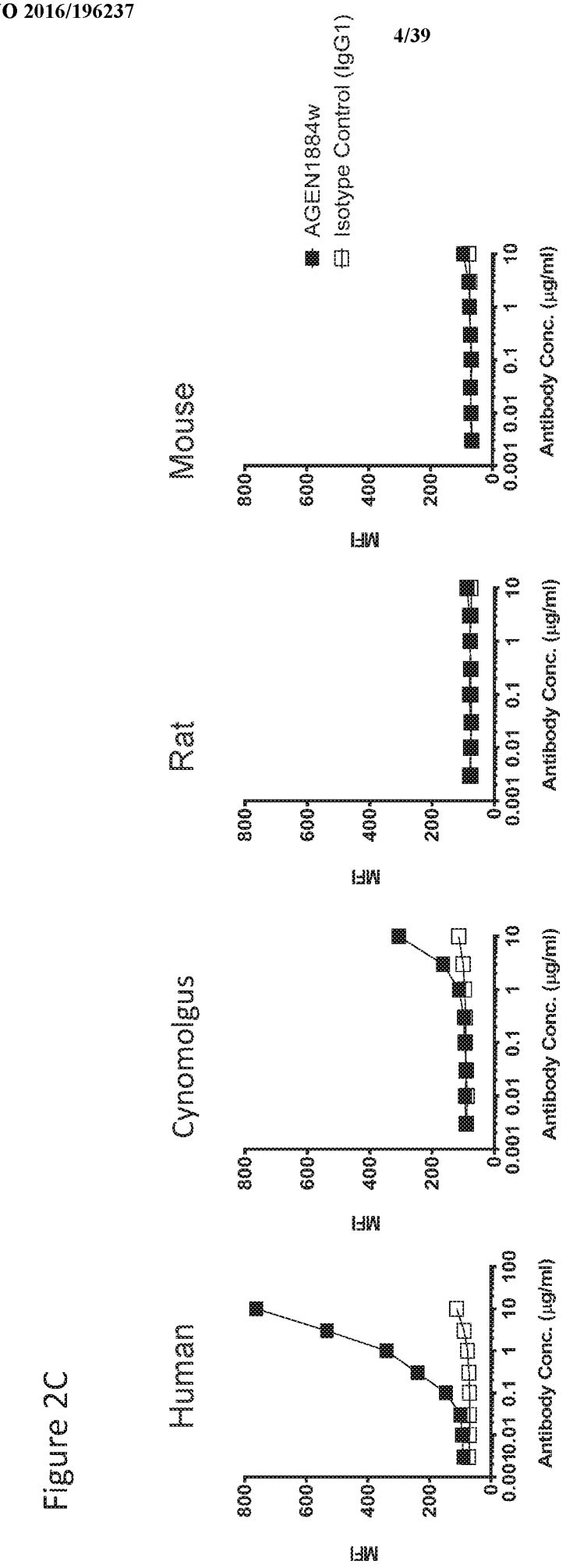

Human
Cynomolgus
Rat
Mouse
MF
Antibody Conc. (µg/ml)
AGEN1884w
Isotype Control (IgG1)

Figure 3A

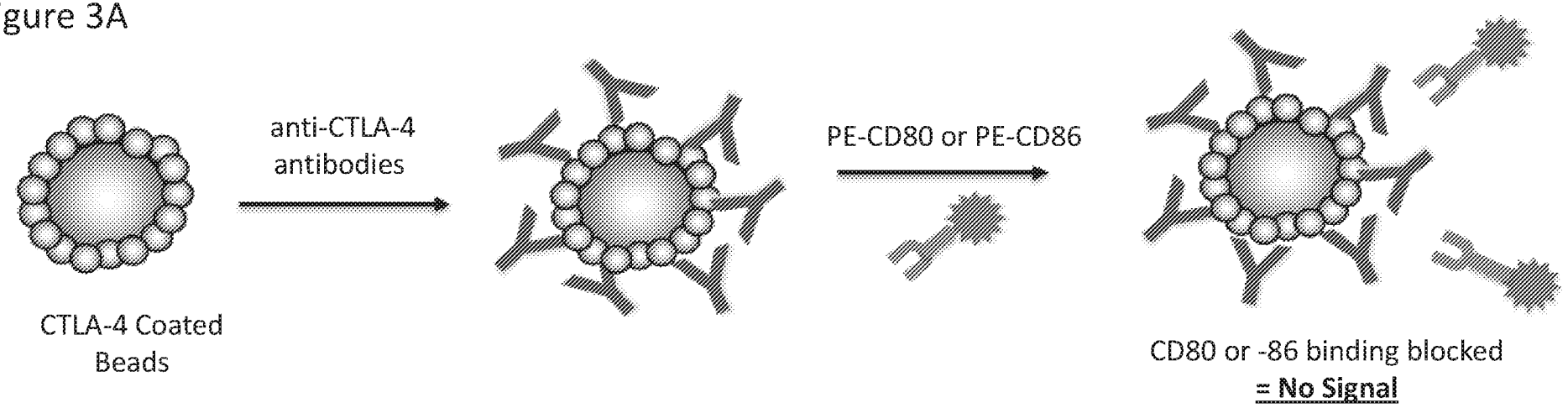


Figure 3B

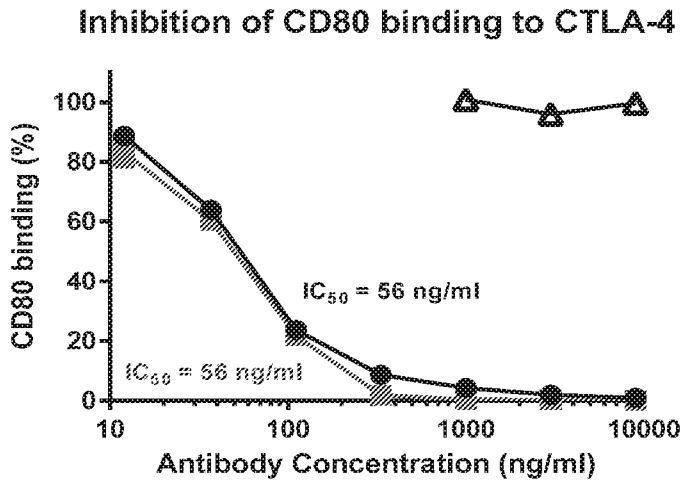
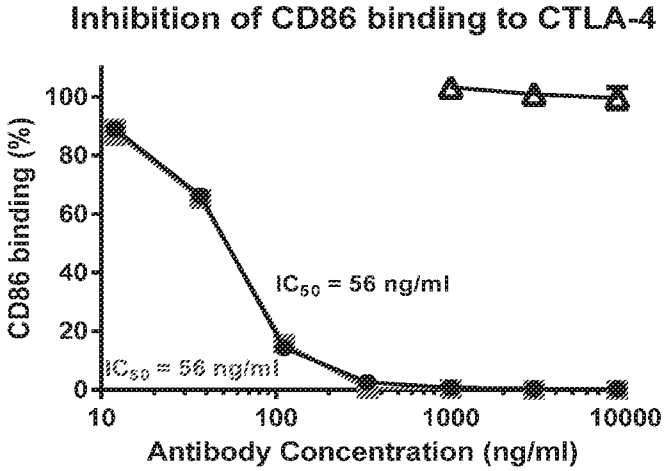


Figure 3C



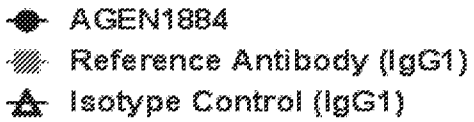


Figure 3D

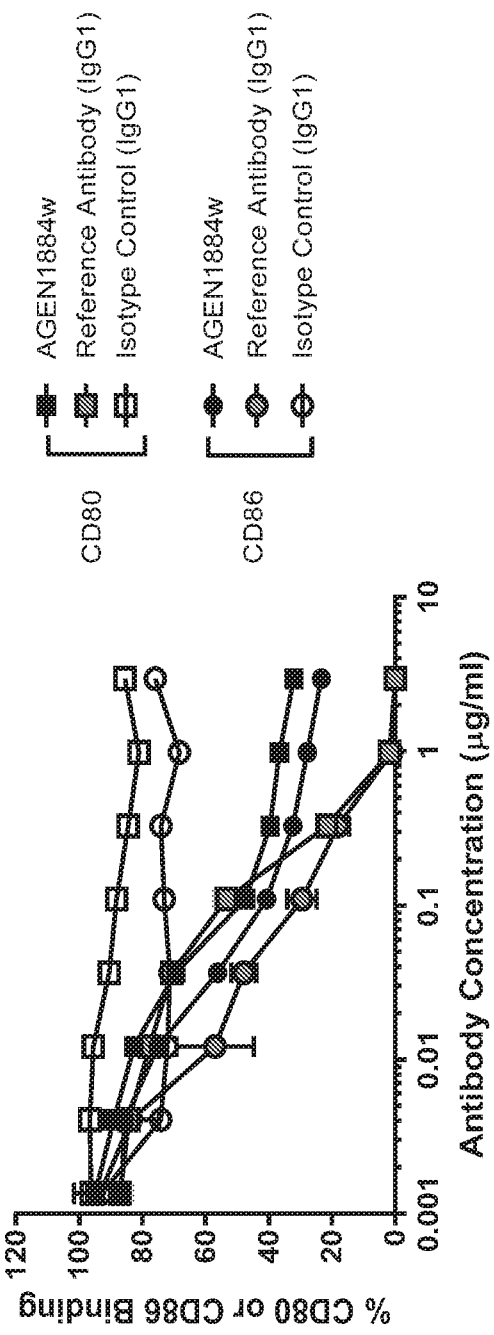
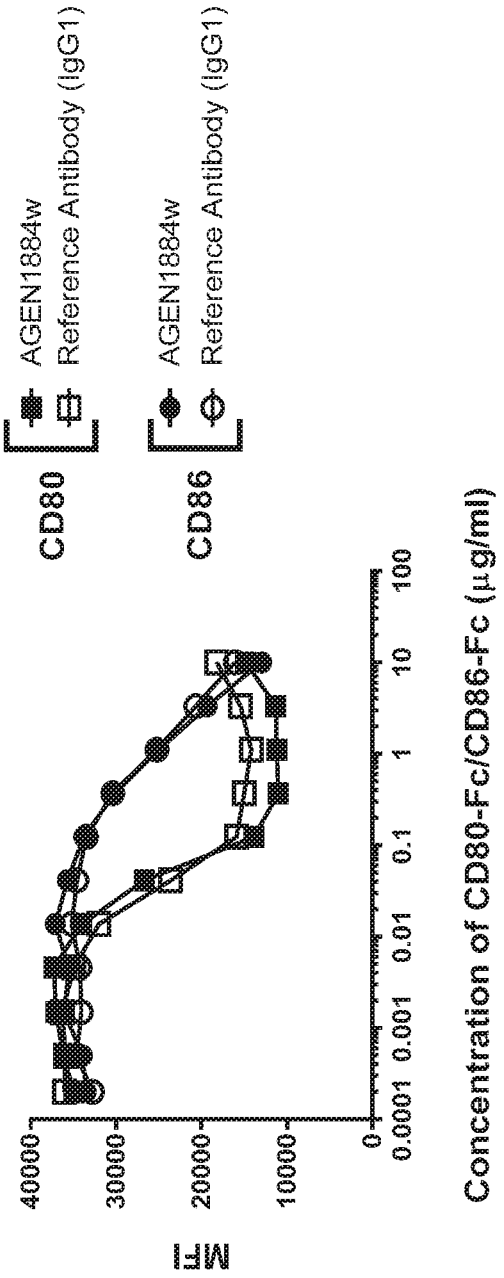


Figure 3E



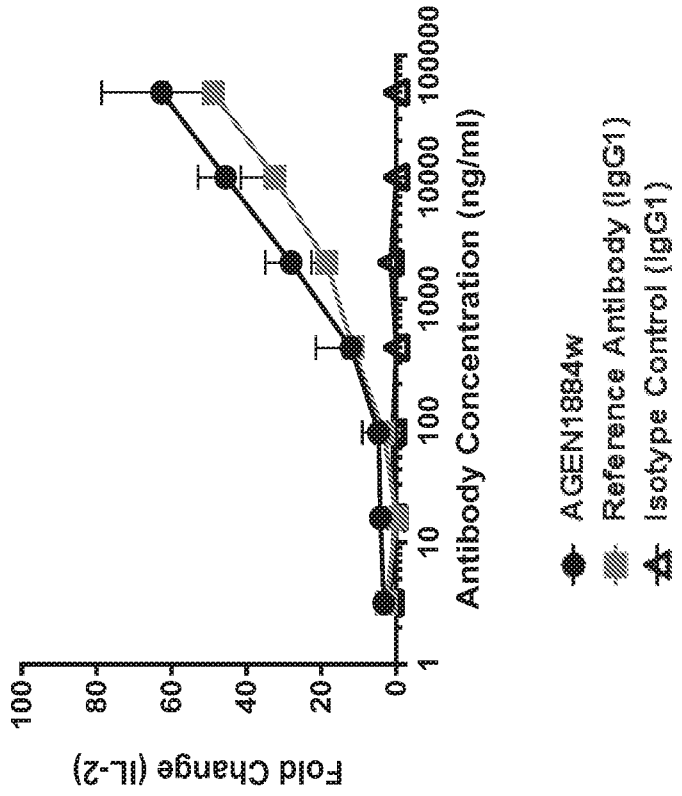

Enhanced T cell function
Fold Change (IL-2)
0
20
40
60
80
100
1
10
100
1000
10000
100000
Antibody Concentration (ng/ml)
AGEN1884w
Reference Antibody (IgG1)
Isotype Control (IgG1)

Figure 4A

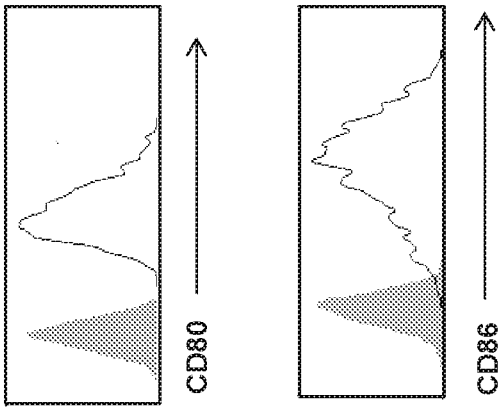


Figure 4B

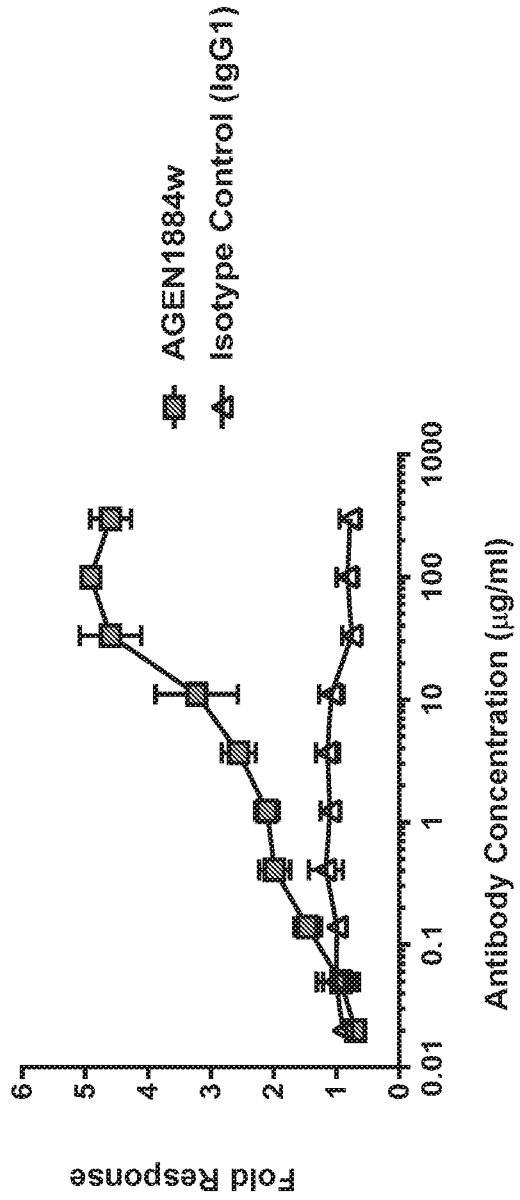


Figure 4C

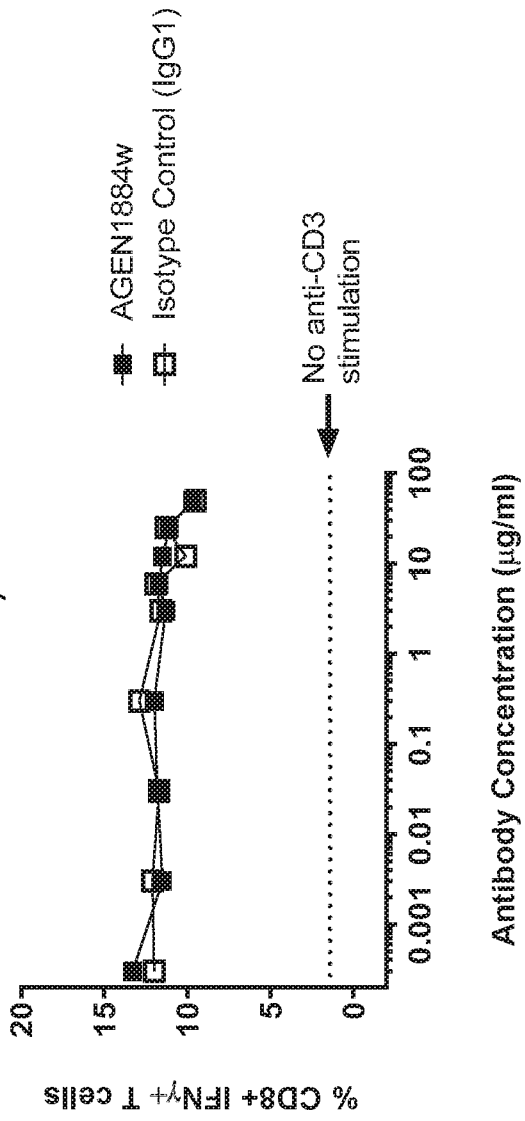


Figure 4D

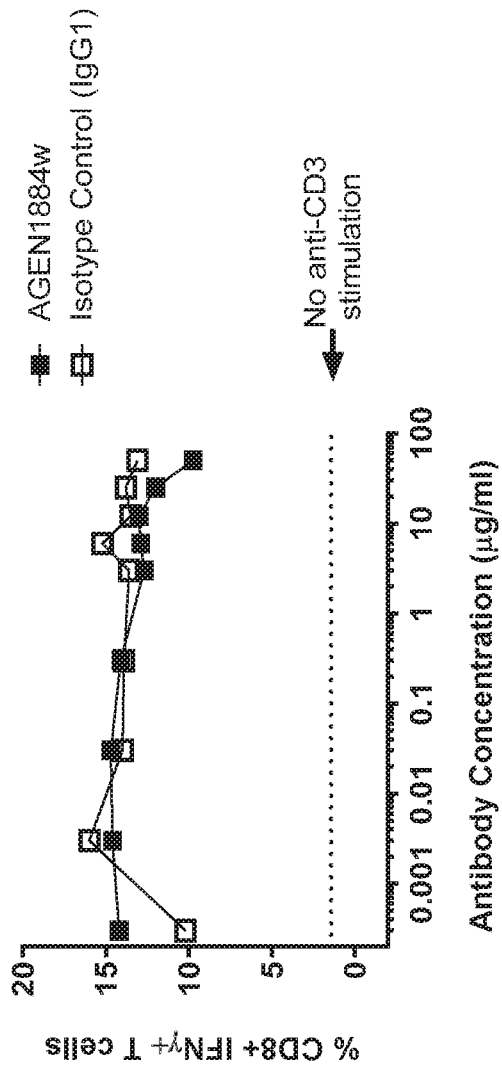


Figure 4E

Figure 4F

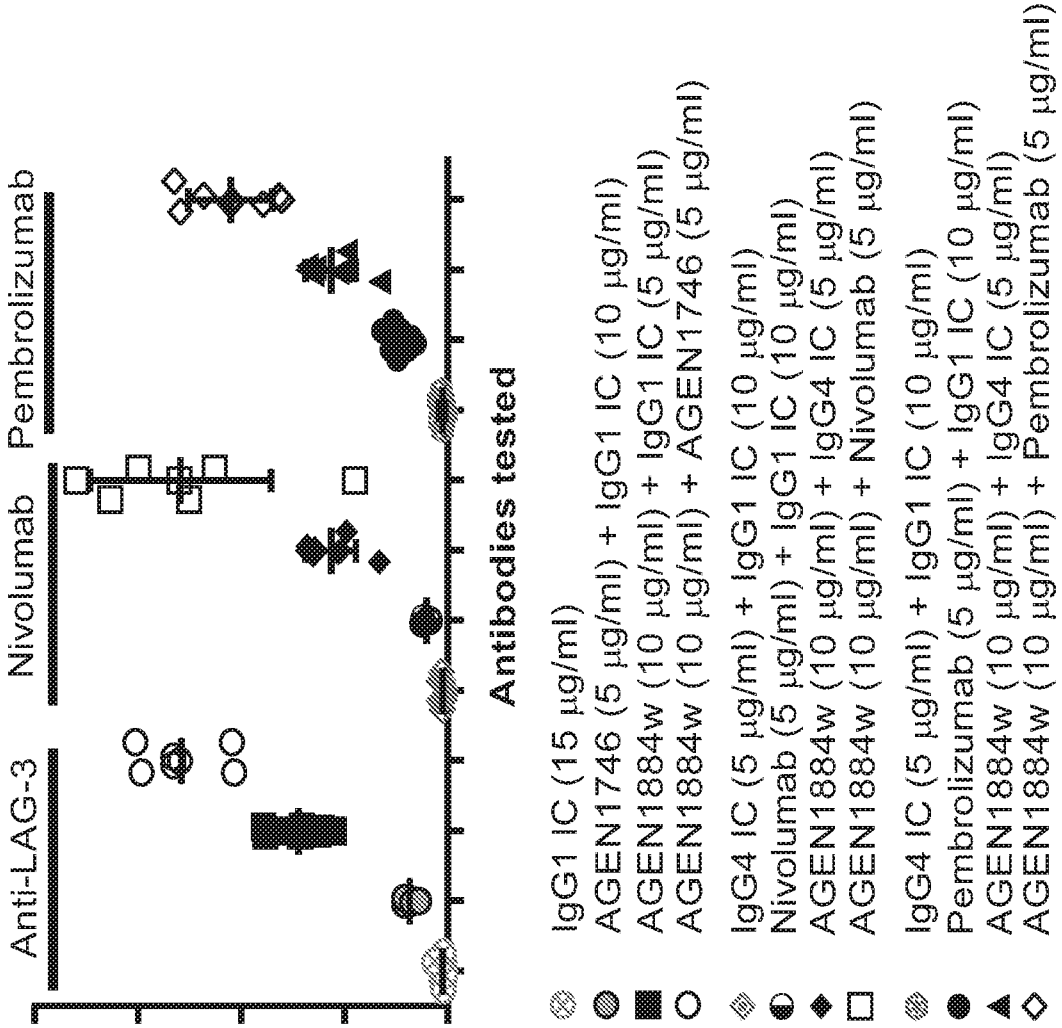

Anti-LAG-3
Nivolumab
Pembrolizumab
IL-2 (pg/ml)
2000
1500
1000
500
0
Antibodies tested
IgG1 IC (15 µg/ml)
AGEN1746 (5 µg/ml) + IgG1 IC (10 µg/ml)
AGEN1884w (10 µg/ml) + IgG1 IC (5 µg/ml)
AGEN1884w (10 µg/ml) + AGEN1746 (5 µg/ml)
IgG4 IC (5 µg/ml) + IgG1 IC (10 µg/ml)
Nivolumab (5 µg/ml) + IgG1 IC (10 µg/ml)
AGEN1884w (10 µg/ml) + IgG4 IC (5 µg/ml)
AGEN1884w (10 µg/ml) + Nivolumab (5 µg/ml)
IgG4 IC (5 µg/ml) + IgG1 IC (10 µg/ml)
Pembrolizumab (5 µg/ml) + IgG1 IC (10 µg/ml)
AGEN1884w (10 µg/ml) + IgG4 IC (5 µg/ml)
AGEN1884w (10 µg/ml) + Pembrolizumab (5 µg/ml)

Figure 5A

Surface Plasmon Resonance (K_D)

	Human		Cynomolgus	
	CTLA-4		CTLA-4	
	dimer	monomer	dimer	monomer
AGEN2041w	1.7 nM	2.0 nM	4.4 nM	2.1 nM
Reference Antibody (IgG1)	0.5 nM	1.2 nM	1.2 nM	0.8 nM
Reference Antibody (IgG2)	1.5 nM	1.6 nM	3.1 nM	1.5 nM

Figure 5B

CTLA-4 expressing cells

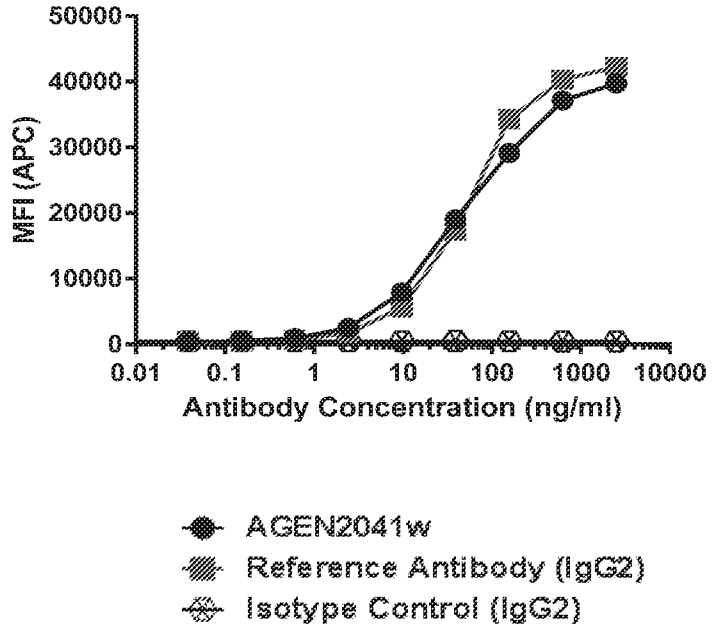


Figure 5C

Inhibition of CD80 and CD86 binding to CTLA-4

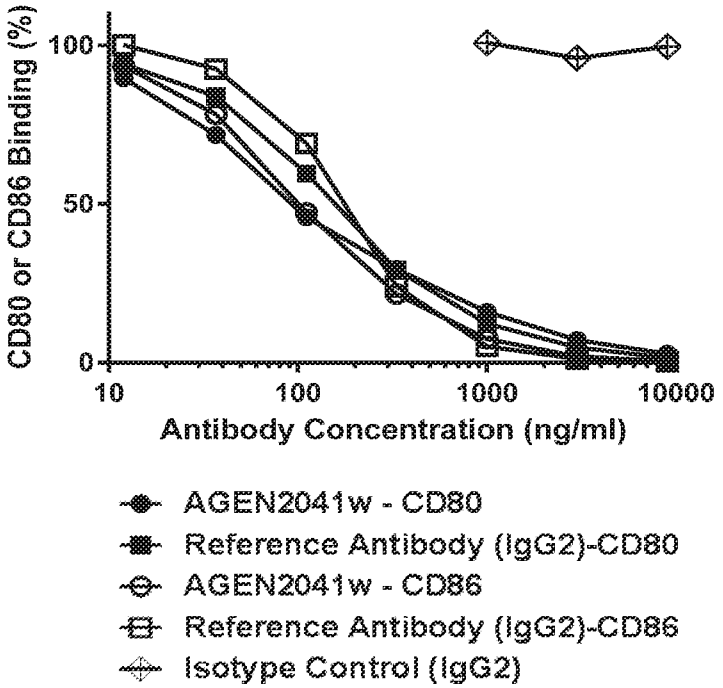


Figure 5D

CTLA-4 Selectivity Assay

	Conc. in ng/ml	rhCTLA-4 Fc (R&D)	rcCTLA-4 Fc (Sino)	rhCD28 Fc (R&D)	rhICOS Fc (R&D)	rhBTLA Fc (Sino)	rhPD-1 Fc (R&D)	rcPD-1 Fc (4AB)
AGEN2041w	1000	16693	13885	58	48	95	30	31
	100	15890	9949	42	43	104	19	27
	10	7737	3684	73	55	89	32	29
Reference Antibody (IgG2)	1000	17288	16442	58	49	106	33	39
	100	13932	13721	62	47	102	43	50
	10	4846	5143	71	59	111	36	60
Isotype Control (IgG2)	1000	79	158	64	41	93	36	41
	100	83	184	52	57	88	94	96
	10	80	187	55	58	99	79	70

Figure 5E

Relative Binding Affinity

	AGEN2041w	Reference Antibody (IgG2)	Isotype Control (IgG2)
rhCTLA-4	+++	+++	—
rcCTLA-4	+++	+++	—
rhCD28	—	—	—
rhICOS	—	—	—
rhBTLA	—	—	—
rhPD-1	—	—	—
rcPD-1	—	—	—

Figure 6A

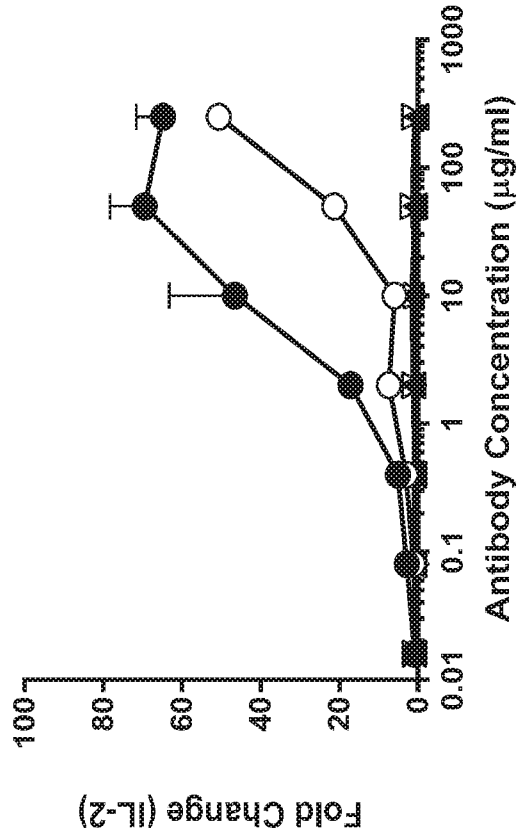


Figure 6B

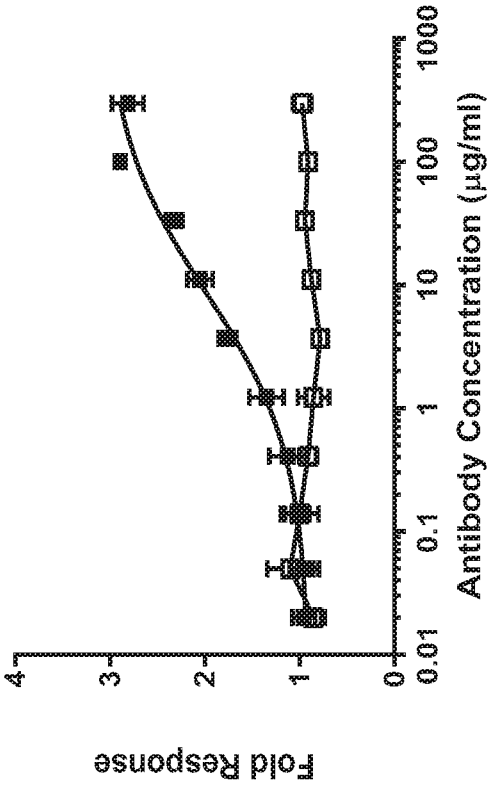


Figure 7A

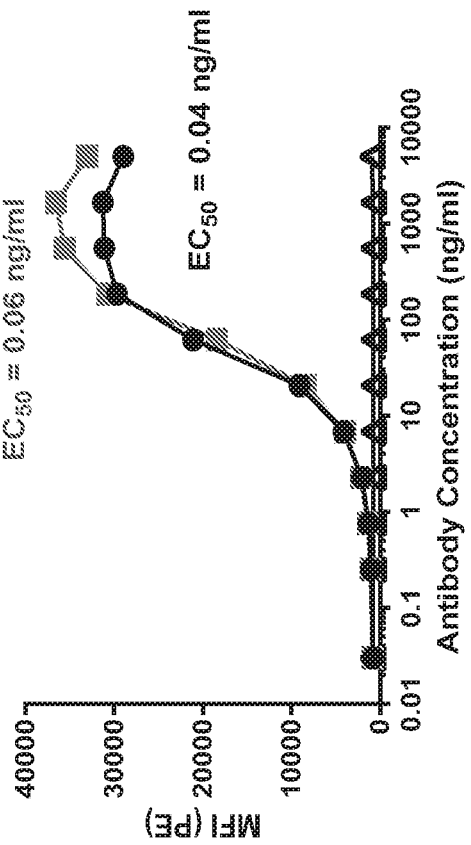
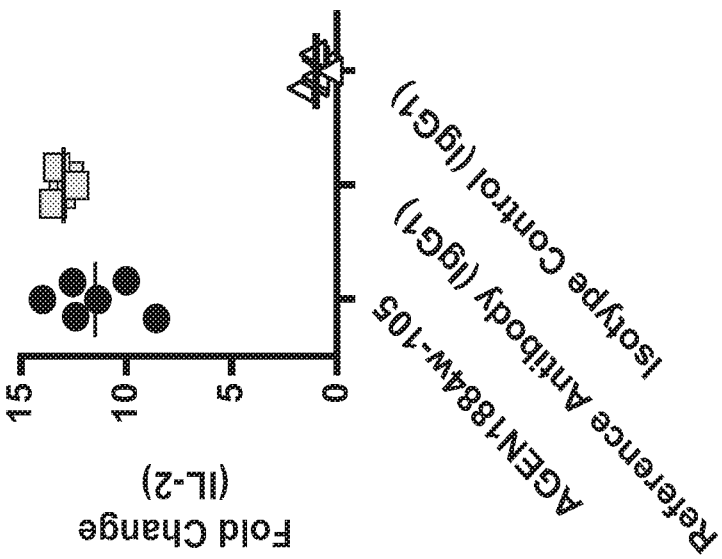


Figure 7B



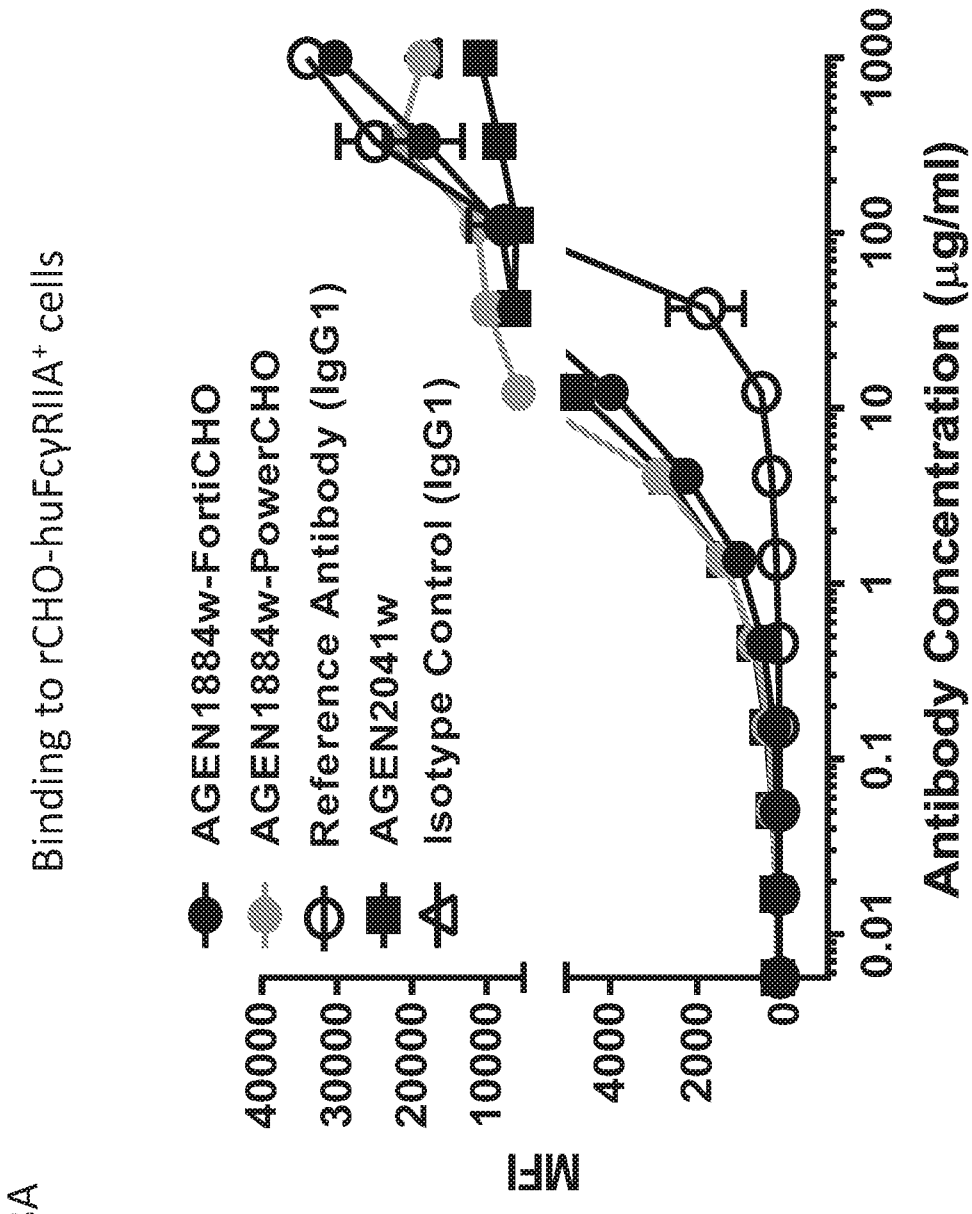

Binding to rCHO-huFcγRIIA+ cells
AGEN1884w-FortiCHO
AGEN1884w-PowerCHO
Reference Antibody (IgG1)
AGEN2041w
Isotype Control (IgG1)
MFI
40000
30000
20000
10000
4000
2000
0
0.01
0.1
1
10
100
1000
Antibody Concentration (μg/ml)

Figure 8A

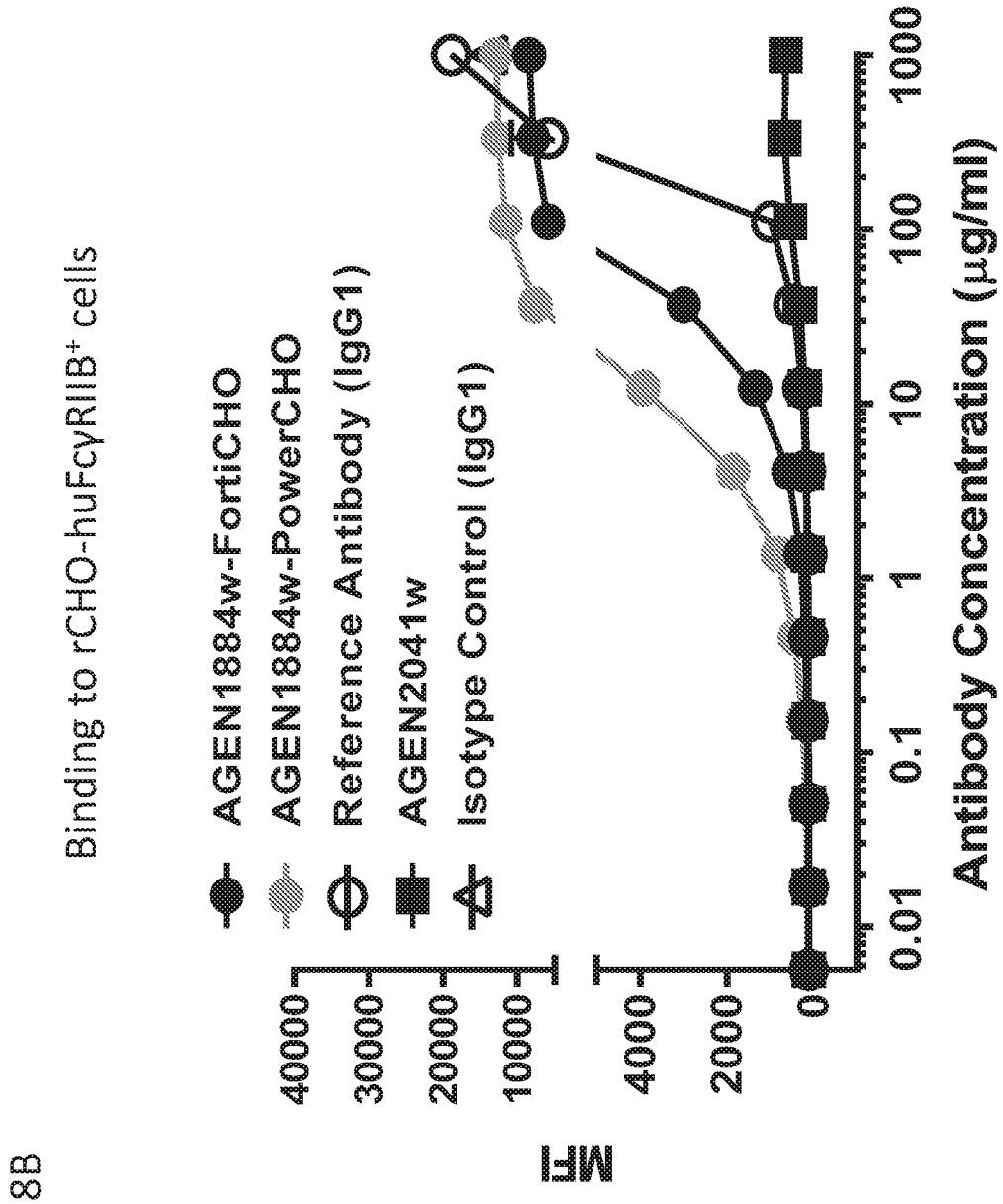

Binding to rCHO-huFcγRIIB+ cells
AGEN1884w-FortiCHO
AGEN1884w-PowerCHO
Reference Antibody (IgG1)
AGEN2041w
Isotype Control (IgG1)
MFI
40000
30000
20000
10000
4000
2000
0
0.01
0.1
1
10
100
1000
Antibody Concentration (μg/ml)

Figure 8B

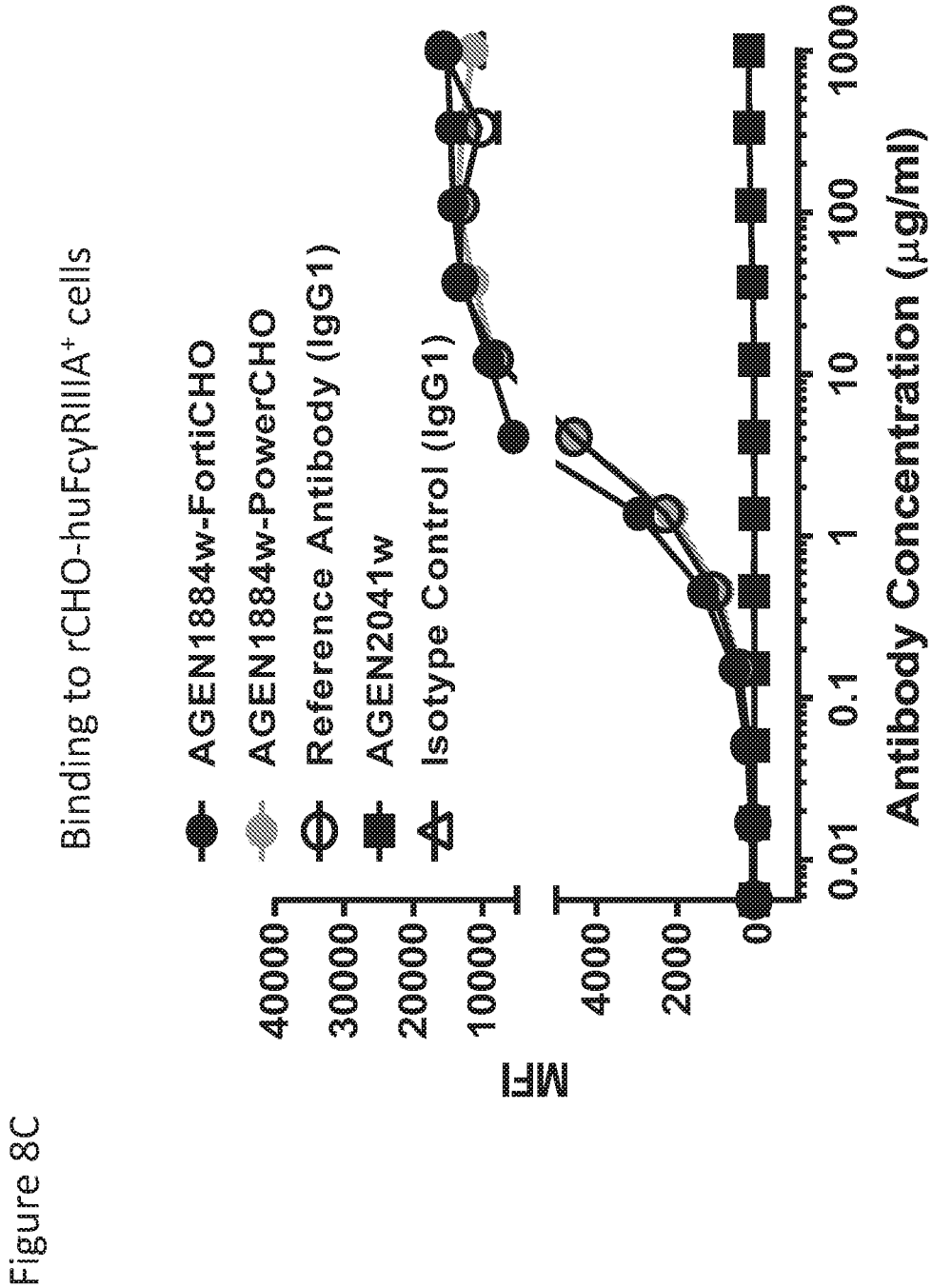

Binding to rCHO-huFcγRIIIA+ cells
AGEN1884w-FortiCHO
AGEN1884w-PowerCHO
Reference Antibody (IgG1)
AGEN2041w
Isotype Control (IgG1)
MFI
40000
30000
20000
10000
4000
2000
0
0.01
0.1
1
10
100
1000
Antibody Concentration (μg/ml)

Figure 8C

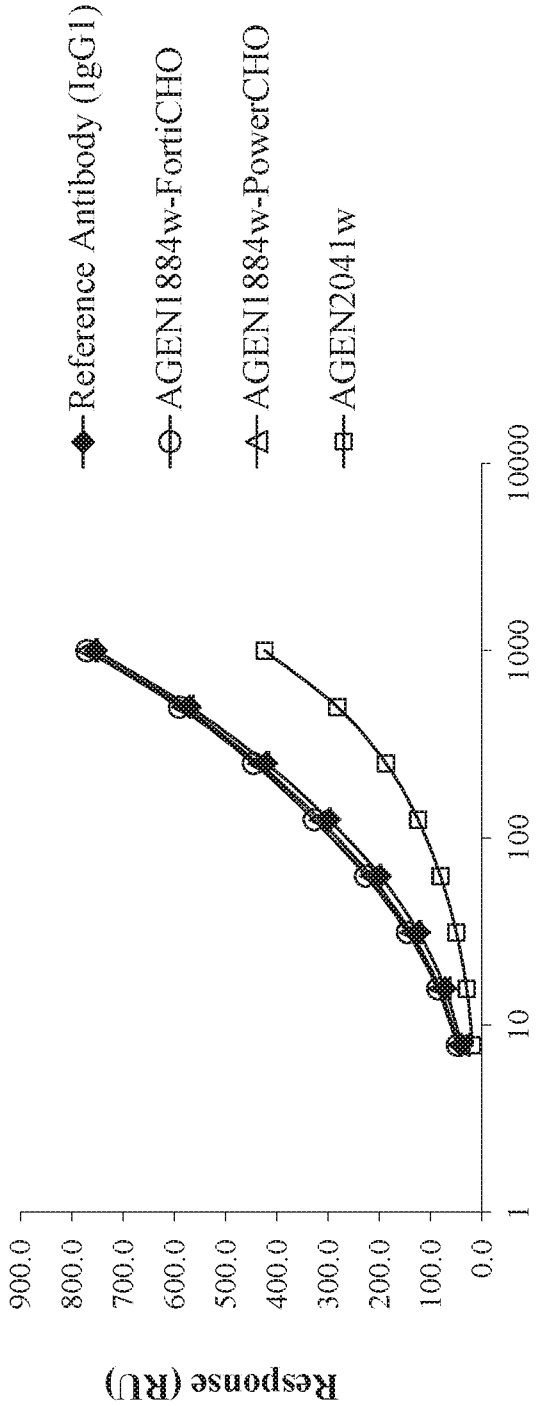


Figure 9A

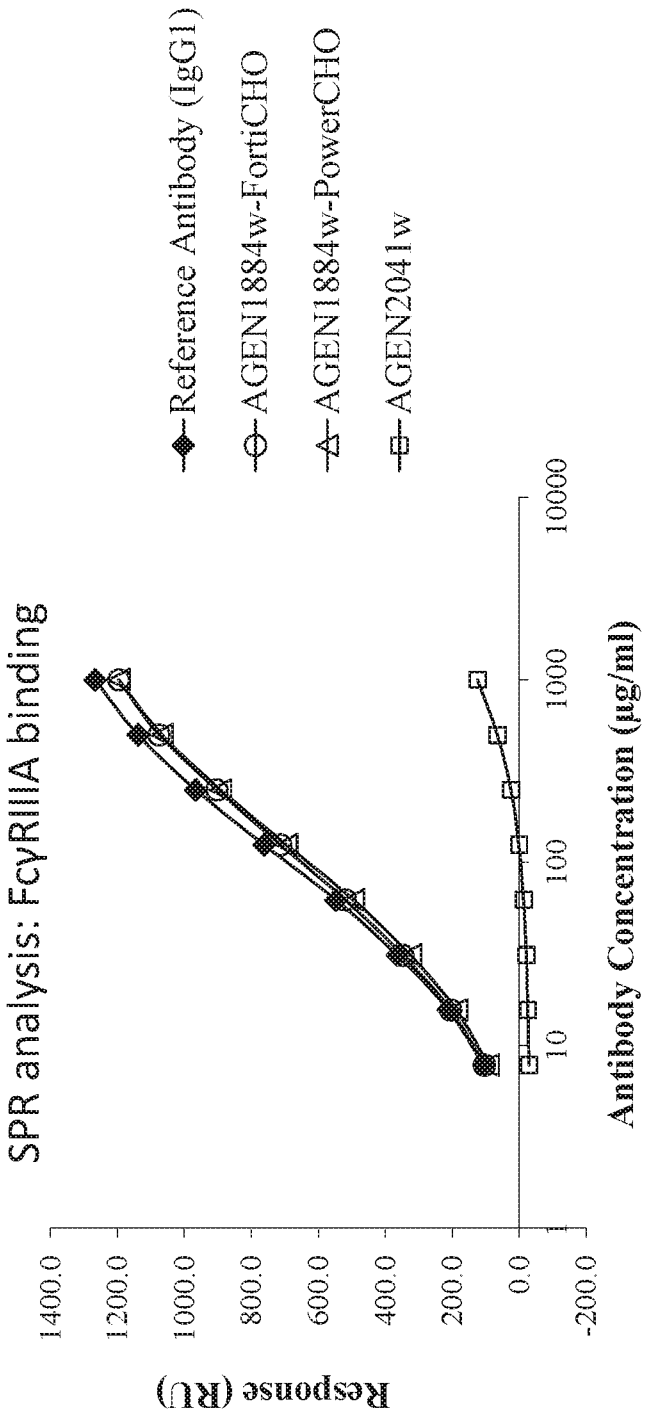


Figure 9B

Figure 10A

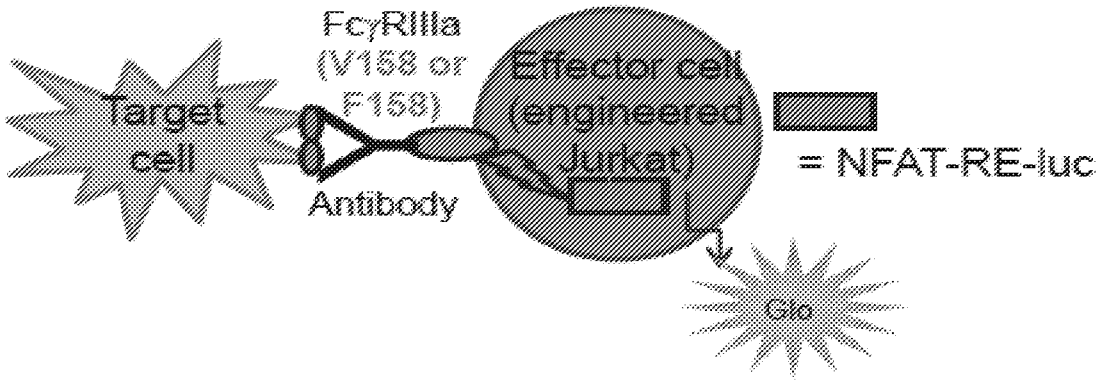


Figure 10B

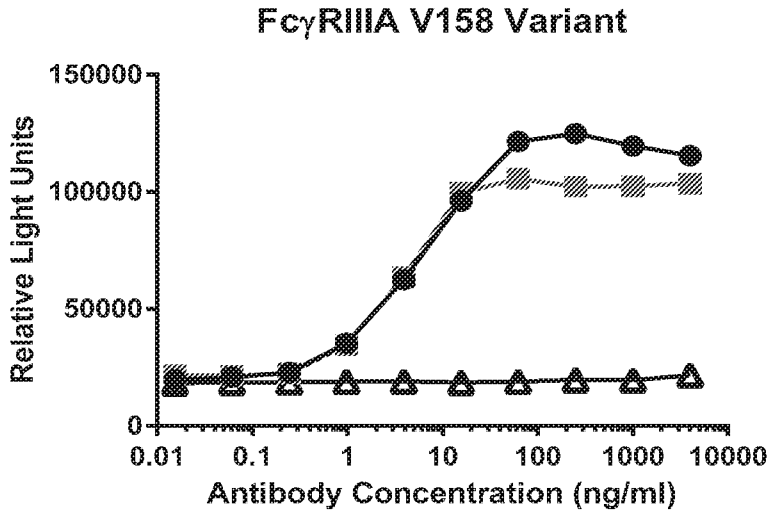
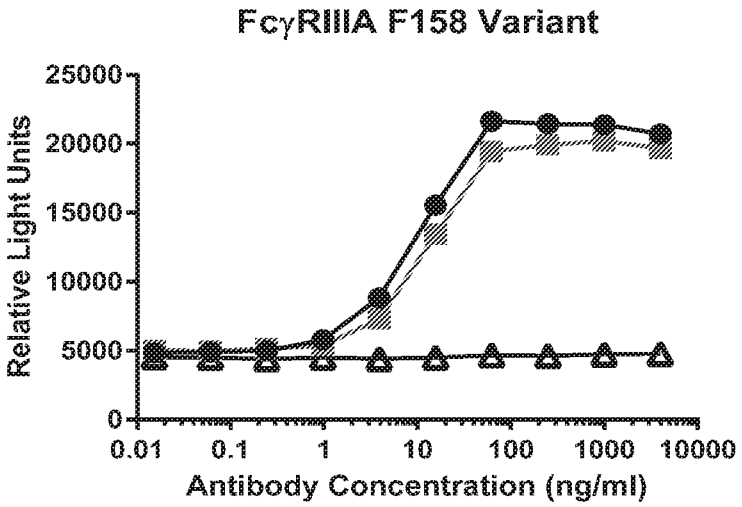


Figure 10C



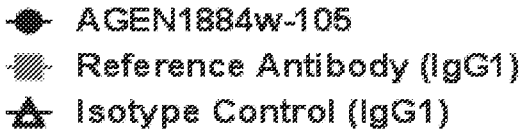


Figure 10D

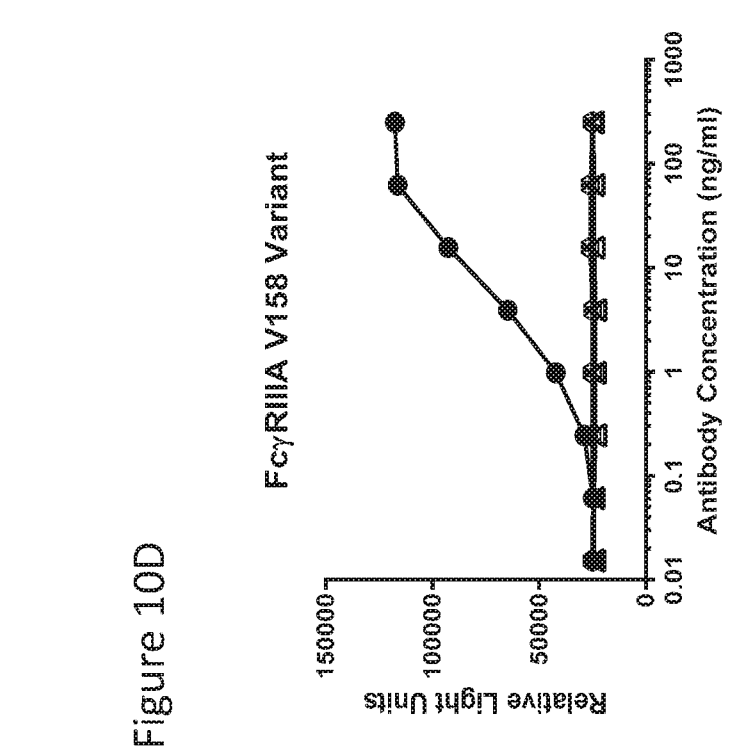


Figure 10E

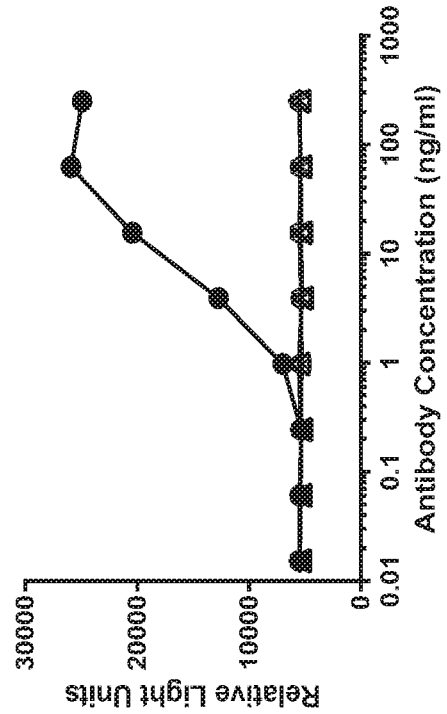


Figure 10F

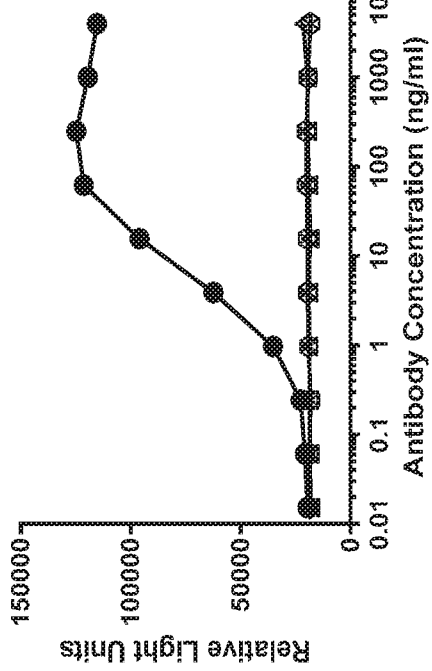
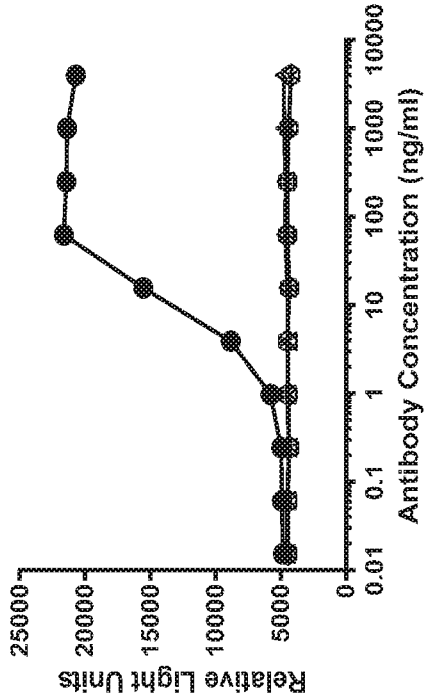
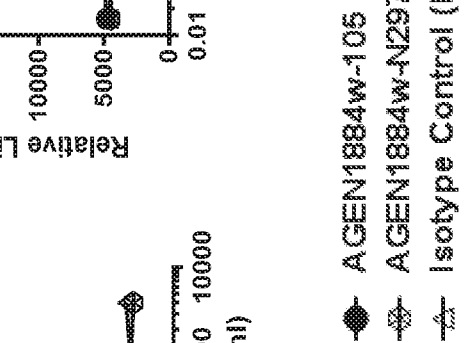


Figure 10G





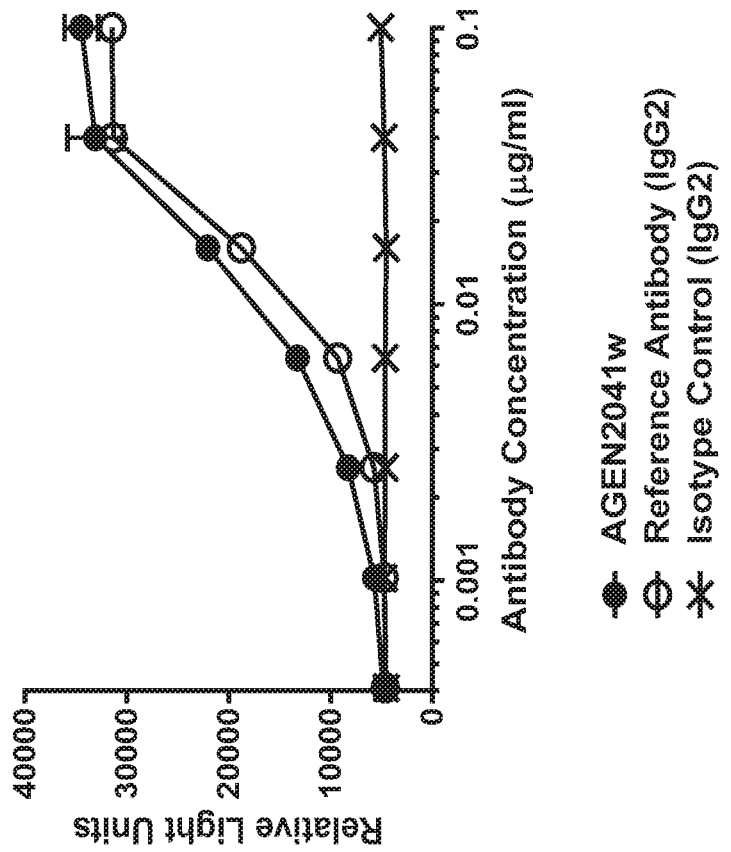

FcγRIIA H131 Variant
Relative Light Units
40000
30000
20000
10000
0
0.001
0.01
0.1
Antibody Concentration (μg/ml)
AGEN2041w
Reference Antibody (IgG2)
Isotype Control (IgG2)

Figure 11

Figure 12A

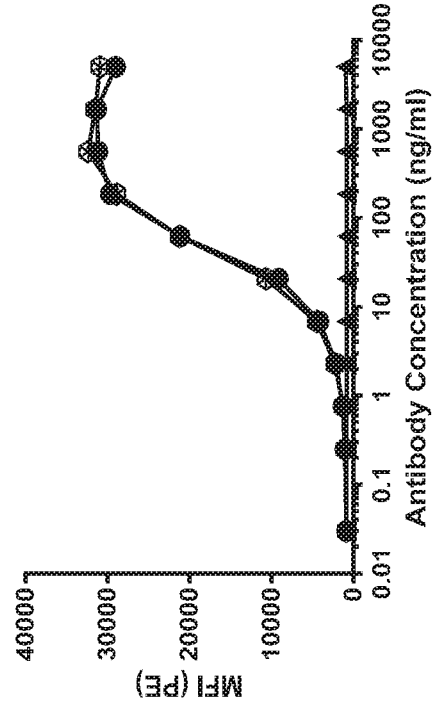


Figure 12B

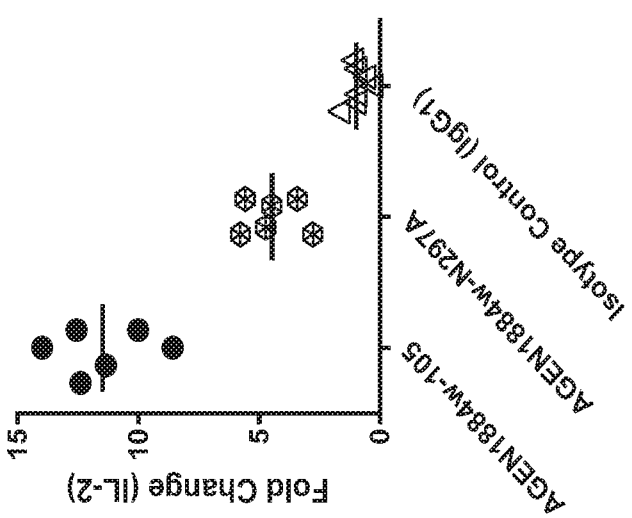


Figure 13A

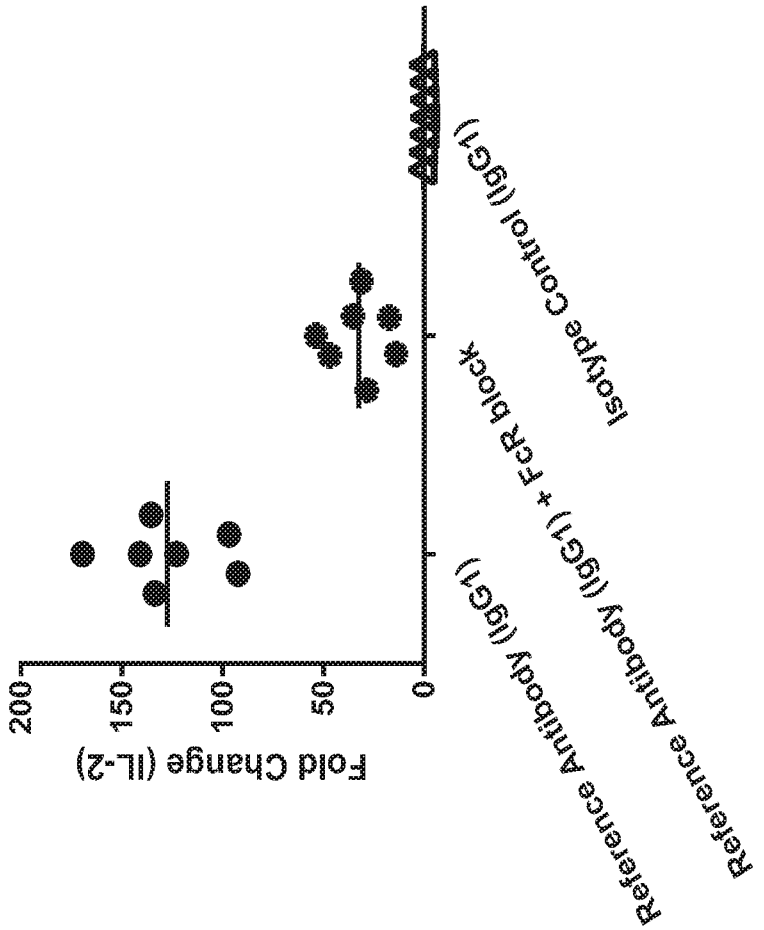
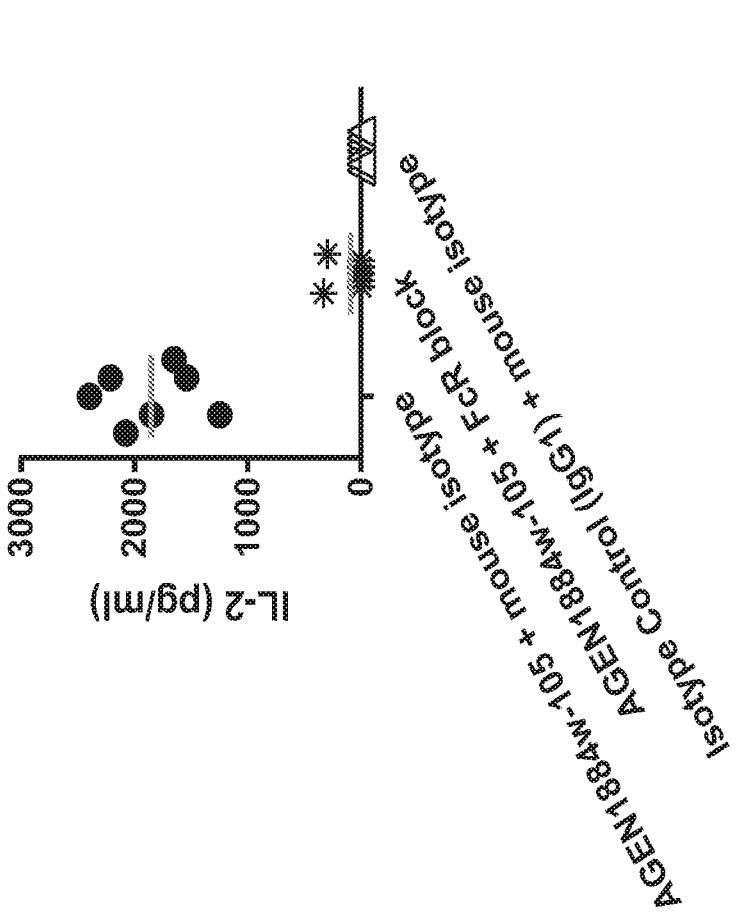


Figure 13B



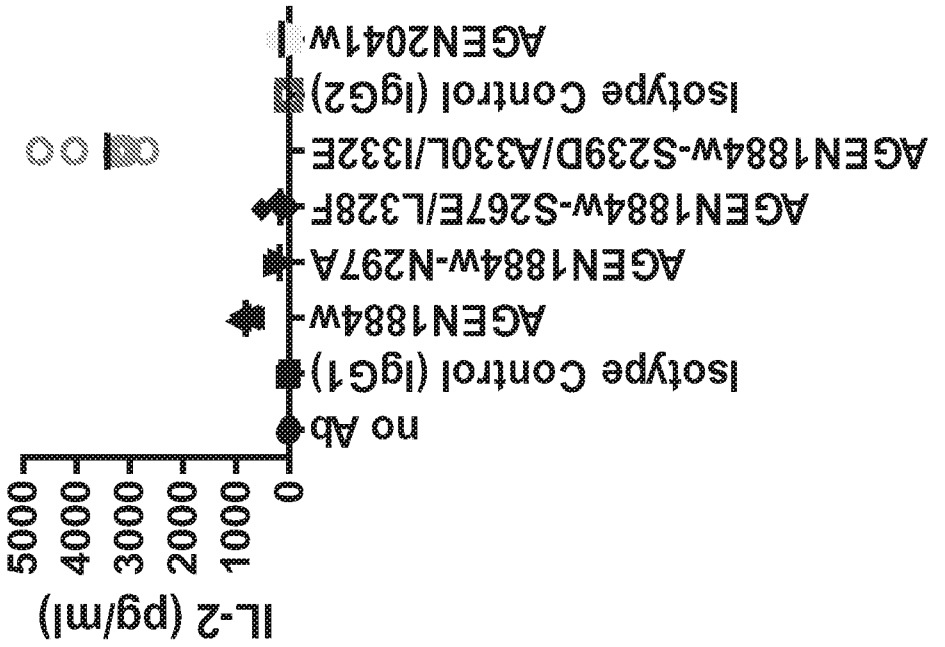

IL-2 (pg/ml)
5000
4000
3000
2000
1000
0
no Ab
Isotype Control (IgG1)
AGEN1884w
AGEN1884w-N297A
AGEN1884w-S267E/L328F
AGEN1884w-S239D/A330L/I332E
Isotype Control (IgG2)
AGEN2041w

Figure 14

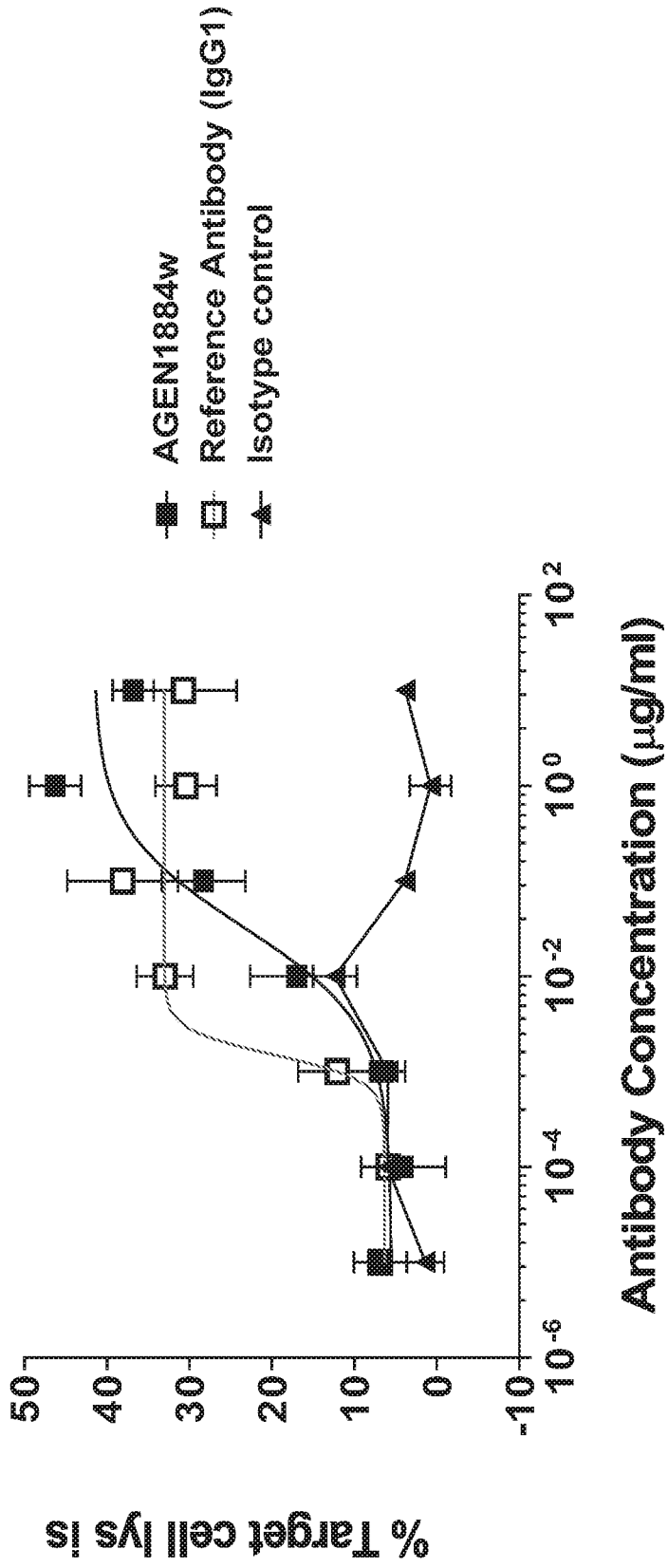
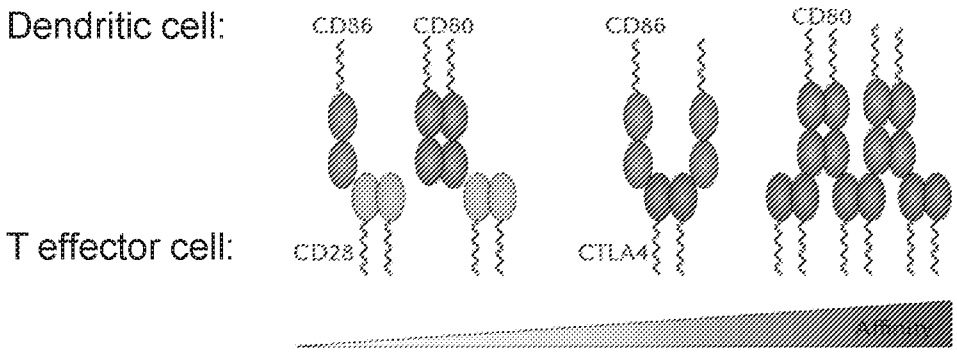

AGEN1884w
Reference Antibody (IgG1)
Isotype control
% Target cell lysis
50
40
30
20
10
0
-10
10-6
10-4
10-2
100
102
Antibody Concentration (µg/ml)

Figure 15

Figure 16



Walker and Sansom Nat. Rev. Immunology 2011

CTLA-4-Ig	K_D (nM)
CD80-Fc-His*	0.3 nM
CD86-Fc-His*	3.6 nM
Reference Antibody (IgG1)	0.5 nM
AGEN1884	0.6 nM

**Modified from: Xu et al., JI 2012*

Figure 17A

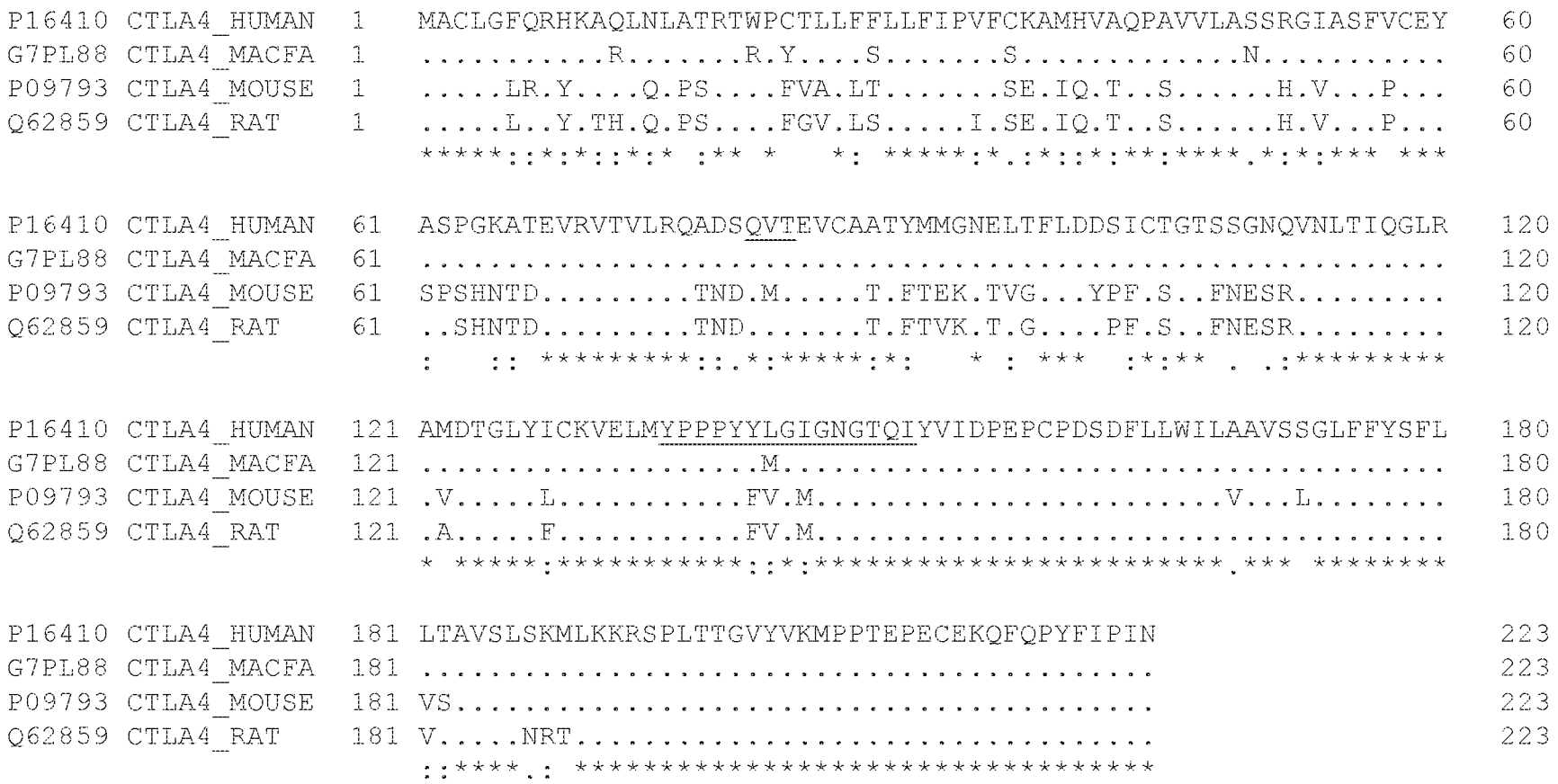

P16410 CTLA4_HUMAN 1 MACLGFQRHKAQLNLATRTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIASFVCEY 60
G7PL88 CTLA4_MACFA 1 .....R.....R.Y....S.....S.....N..... 60
P09793 CTLA4_MOUSE 1 .....LR.Y....Q.PS....FVA.LT.....SE.IQ.T..S.....H.V...P... 60
Q62859 CTLA4_RAT 1 .....L..Y.TH.Q.PS....FGV.LS.....I.SE.IQ.T..S.....H.V...P... 60
P16410 CTLA4_HUMAN 61 ASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLR 120
G7PL88 CTLA4_MACFA 61 120
P09793 CTLA4_MOUSE 61 SPSHNTD.....TND.M.....T.FTEK.TVG...YPF.S..FNESR..... 120
Q62859 CTLA4_RAT 61 ..SHNTD.....TND.....T.FTVK.T.G....PF.S..FNESR..... 120
P16410 CTLA4_HUMAN 121 AMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDFLLWILAAVSSGLFFYSFL 180
G7PL88 CTLA4_MACFA 121 .....M..... 180
P09793 CTLA4_MOUSE 121 .V.....L.....FV.M.....V...L..... 180
Q62859 CTLA4_RAT 121 .A.....F.....FV.M..... 180
P16410 CTLA4_HUMAN 181 LTAVSLSKMLKKRSPLTTGVYVKMPPTEPECEKQFQPYFIPIN 223
G7PL88 CTLA4_MACFA 181 223
P09793 CTLA4_MOUSE 181 VS..... 223
Q62859 CTLA4_RAT 181 V.....NRT..... 223

Figure 17B

SP|P16410|CTLA4_HUMAN MACLGFQRHKAQLNLATRTWPCTLLFFLLFIP--VFC-------KAMHVAQPAVVLA--- 48
TR|G7PL88|G7PL88_MACFA MACLGFQRHKARLNLATRTRPYTLLFSLLFIP--VFS-------KAMHVAQPAVVLA--- 48
SP|P10747|CD28_HUMAN ----------MLRLLLALN--LFPS-IQVTGNKILVKQSP-MLV--- 30
SP|Q9Y6W8|ICOS_HUMAN ----------MKSGLWY---------FF--LFCLRIKVLTGEINGSANYEMFI--- 32
SP|Q7Z6A9|BTLA_HUMAN ----------MKTLPAMLGTGKLFWV---FFLIPYLDIWNIHGKESCDVQLYIK 41
SP|Q15116|PDCD1_HUMAN ----------MQIPQAPWPVVWAVLQLGWRPGWFLDSPD-RPWNPPTFSPALLVV--- 44
SP|P16410|CTLA4_HUMAN -------SSRGIASFVCEYASPGKA---TEVRVTVLRQADSQVTEVCAATYMMGNE--LT 96
TR|G7PL88|G7PL88_MACFA -------NSRGIASFVCEYASPGKA---TEVRVTVLRQADSQVTEVCAATYMMGNE--LT 96
SP|P10747|CD28_HUMAN -------AYDNAVNLSCKYSYNLFS---REFRASLHKGLDSA-VEVCVVYGNYSQQLQVY 79
SP|Q9Y6W8|ICOS_HUMAN -------FHNGGVQILCKYPD--IV---QQFKMQLLKGGQ----ILCDLTKTKGSGNTVS 76
SP|Q7Z6A9|BTLA_HUMAN RQSEHSILAGDPFELECPVKYCANRPHVTWCKLN---GT------TCVKLEDRQ------ 86
SP|Q15116|PDCD1_HUMAN -------TEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDK----LAAFPEDR------- 86
SP|P16410|CTLA4_HUMAN FLDDSICT----GTSSGNQVNLTI---QGLRAMDTGLYICKVELMYPPPYYLGIG----- 144
TR|G7PL88|G7PL88_MACFA FLDDSICT----GTSSGNQVNLTI---QGLRAMDTGLYICKVELMYPPPYYMGIG----- 144
SP|P10747|CD28_HUMAN SKTGFNCD----GKLGNESVTFYL---QNLYVNQTDIYFCKIEVMYPPPYLDNEK----- 127
SP|Q9Y6W8|ICOS_HUMAN IKSLKFCH----SQLSNNSVSFFL---YNLDHSHANYYFCNLSIFDPPPFKVTLT----- 124
SP|Q7Z6A9|BTLA_HUMAN ----------TSWKEEKNISFFILHFEPVLPNDNGSYRCSANFQSNL---IE------- 125
SP|Q15116|PDCD1_HUMAN SQPGQDCRFRVTQLPNGRDFHMSV---VRARRNDSGTYLCGAISLAPKAQIKESLRAELR 143

Figure 17C

```
SP|P16410|CTLA4_HUMAN  -NGTQIYVIDP-----EPCPDS-----DFLLWILAAVSSGLFFYSFLLTAVSL--S-KML 190
TR|G7PL88|G7PL88_MACFA -NGTQIYVIDP-----EPCPDS-----DFLLWILAAVSSGLFFYSFLLTAVSL--S-KML 190
SP|P10747|CD28_HUMAN   SNGTIIHVKGK-----HLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFI--IFWVR 180
SP|Q9Y6W8|ICOS_HUMAN   --GGYLHIYES-----QLCCQL-------KFWLPIGCAA---FVVVCILGCIL--ICWLT 165
SP|Q7Z6A9|BTLA_HUMAN   SHSTTLYVTDVKSASERPSKDEMASR-PWLLYRLLPLGG----LPLLITTCFCLFCCLRR 180
SP|Q15116|PDCD1_HUMAN  VTERRAEVPTA-----HPSPSPRPAG-QFQTLVVGVVGGLLGSLVLLVWVLAV--ICSRA 195
                              :         . . .                  ..       . :

SP|P16410|CTLA4_HUMAN  KKRSP----LTTGVYVKMPPTE-----PEC-EKQFQPYFIPIN----------------- 223
TR|G7PL88|G7PL88_MACFA KKRSP----LTTGVYVKMPPTE-----PEC-EKQFQPYFIPIN----------------- 223
SP|P10747|CD28_HUMAN   SKRSR----LLHSDYMNMTPRR-----PGPTRKHYQPYAPPRD--FAAYRS---------- 220
SP|Q9Y6W8|ICOS_HUMAN   KKKYSSSVHDPNGEYMFMRAVN-----TAKKSRLTDVTL---------------------- 199
SP|Q7Z6A9|BTLA_HUMAN   HQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCFRMQEGS 240
SP|Q15116|PDCD1_HUMAN  ARG--------TIG-------ARR------TGQP-LKEDPSAVPVF--SVDYGELDFQWREKT 234
                        :           .         .              .

SP|P16410|CTLA4_HUMAN  ------------------------------------------------------------
TR|G7PL88|G7PL88_MACFA ------------------------------------------------------------
SP|P10747|CD28_HUMAN   ------------------------------------------------------------
SP|Q9Y6W8|ICOS_HUMAN   ------------------------------------------------------------
SP|Q7Z6A9|BTLA_HUMAN   EVYSNPCLEENKP--GIVYASLNHSVIGPNSRLARNVKEAPTEYASI------CVRS- 289
SP|Q15116|PDCD1_HUMAN  PEPPVPCVPEQTEYATIVFP----SGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL 288
```

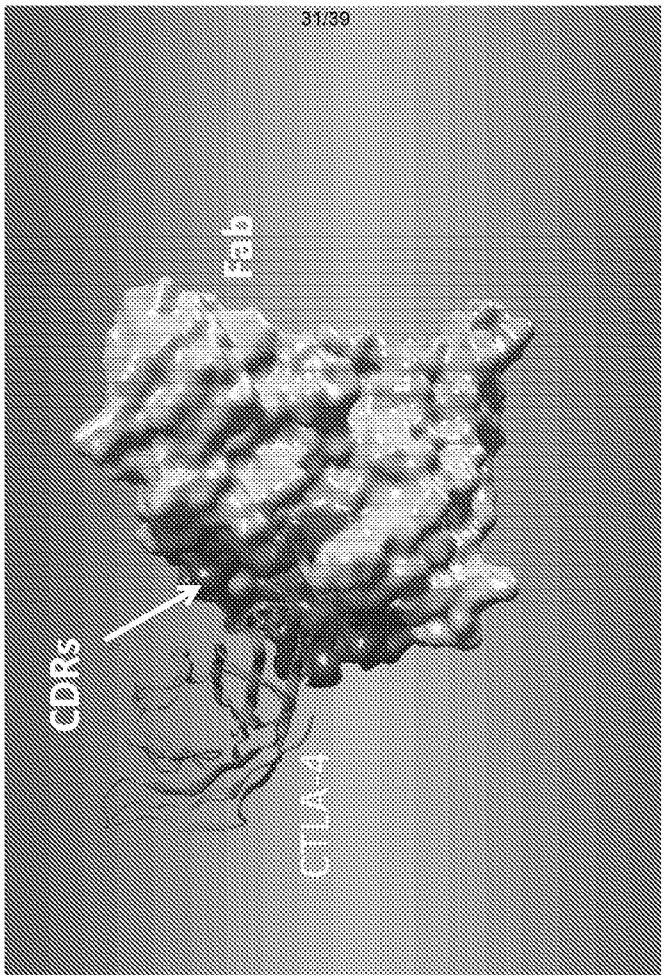


Figure 17E

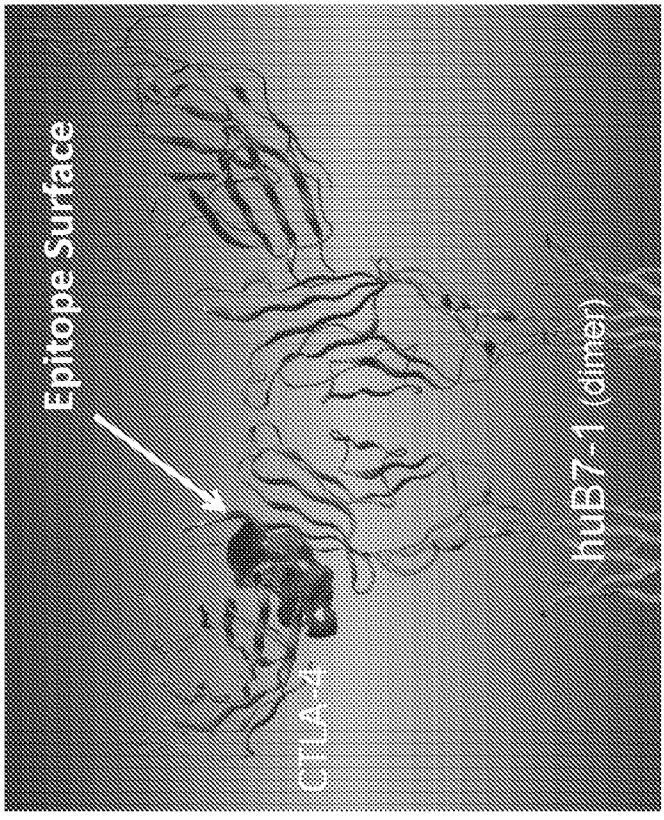


Figure 17D

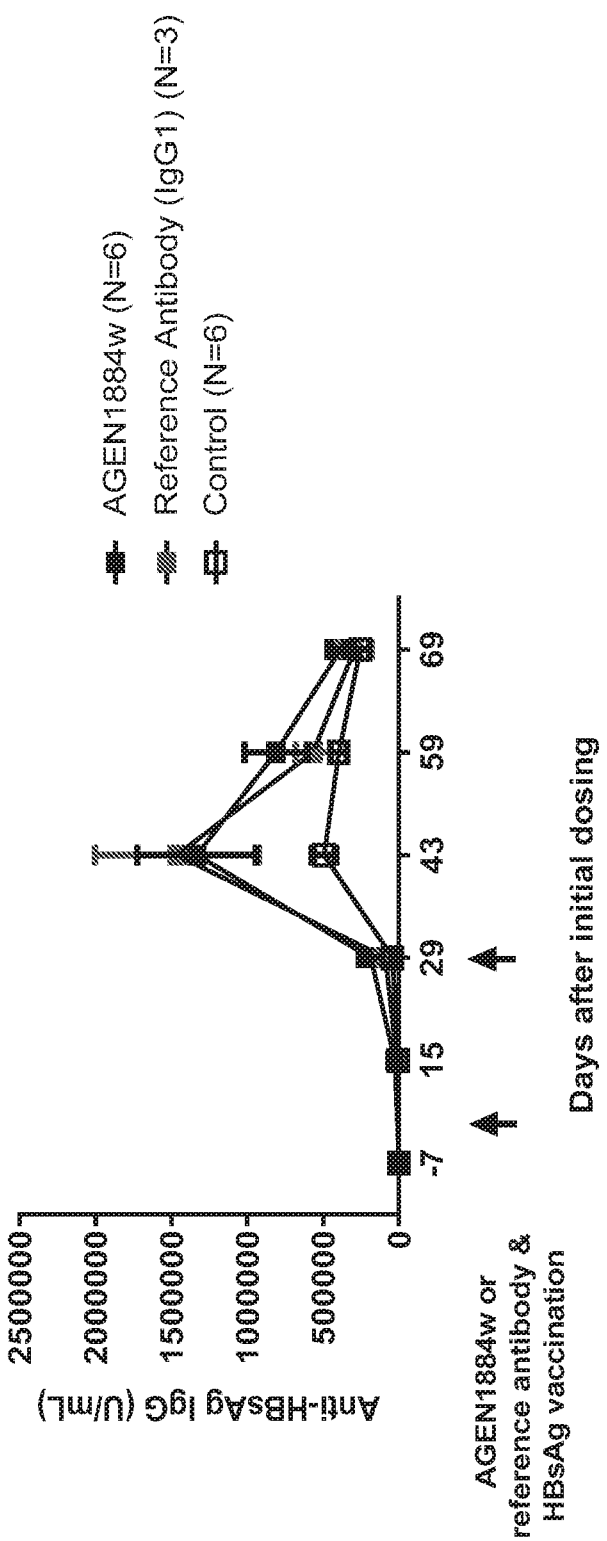


Figure 18A

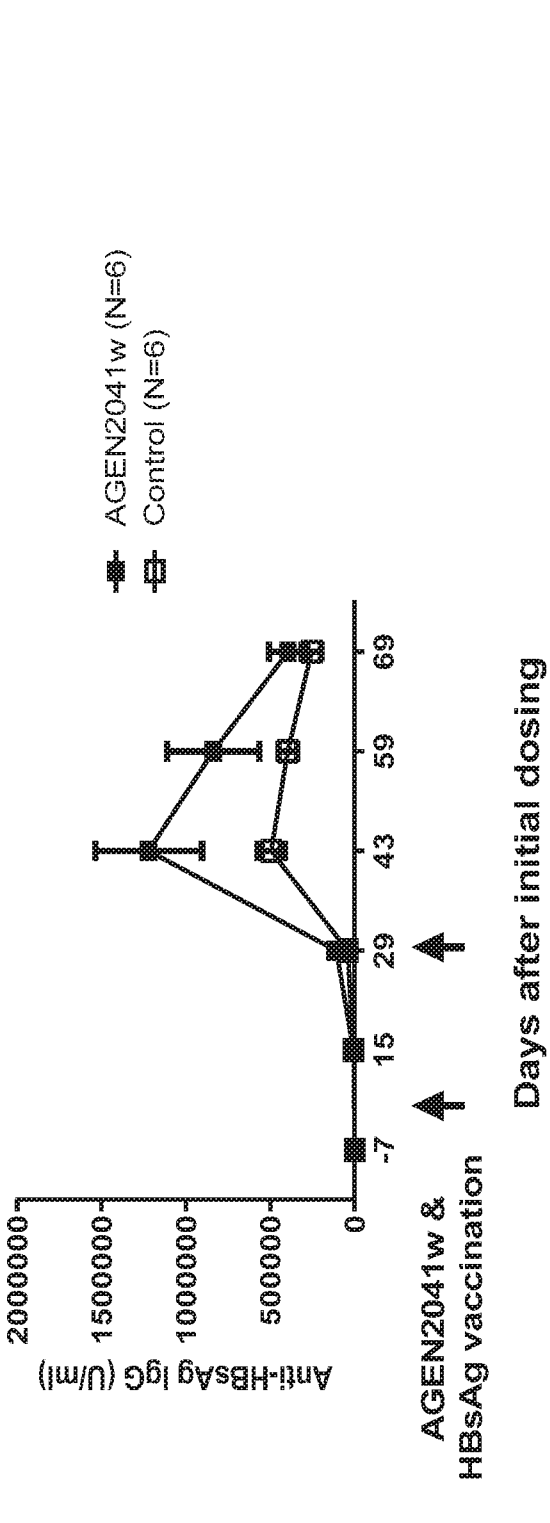


Figure 18B

Figure 19A

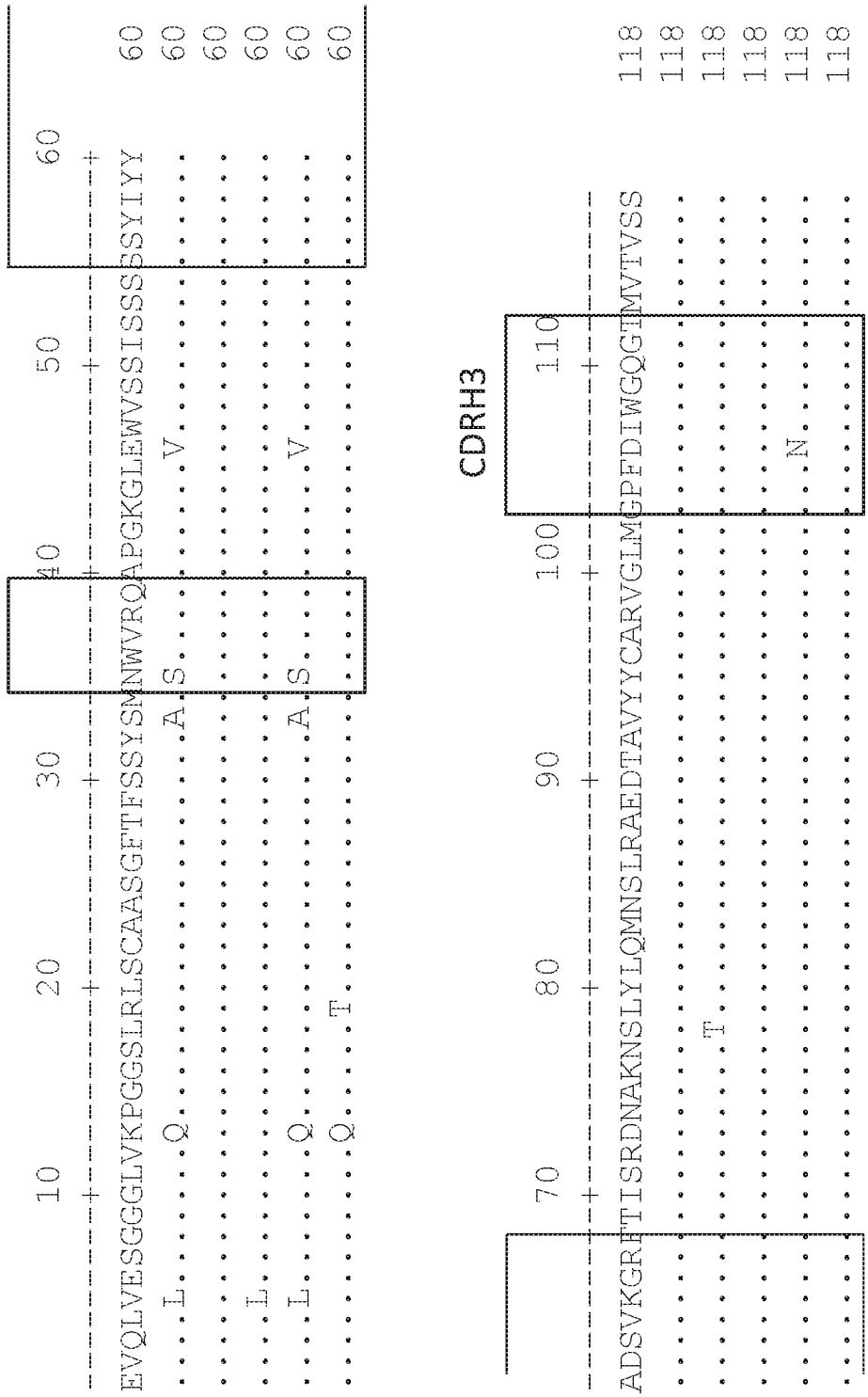

CDRH1
CDRH2
CDRH3
10 20 30 40 50 60
70 80 90 100 110
BADD411-2354 EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYY 60
BADD412-2356 ....L.....Q.....A.S.....V..... 60
BADD412-2357 60
BADD412-2358 ....L.....A.S.....V..... 60
BADD412-2359 ....L.....Q.....A.S.....V..... 60
BADD412-2360 .....Q.....T..... 60
BADD411-2354 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLMGPFDIWGQGTMVTVSS 118
BADD412-2356 118
BADD412-2357 .....T..... 118
BADD412-2358 118
BADD412-2359 .....N..... 118
BADD412-2360 118

Figure 19B

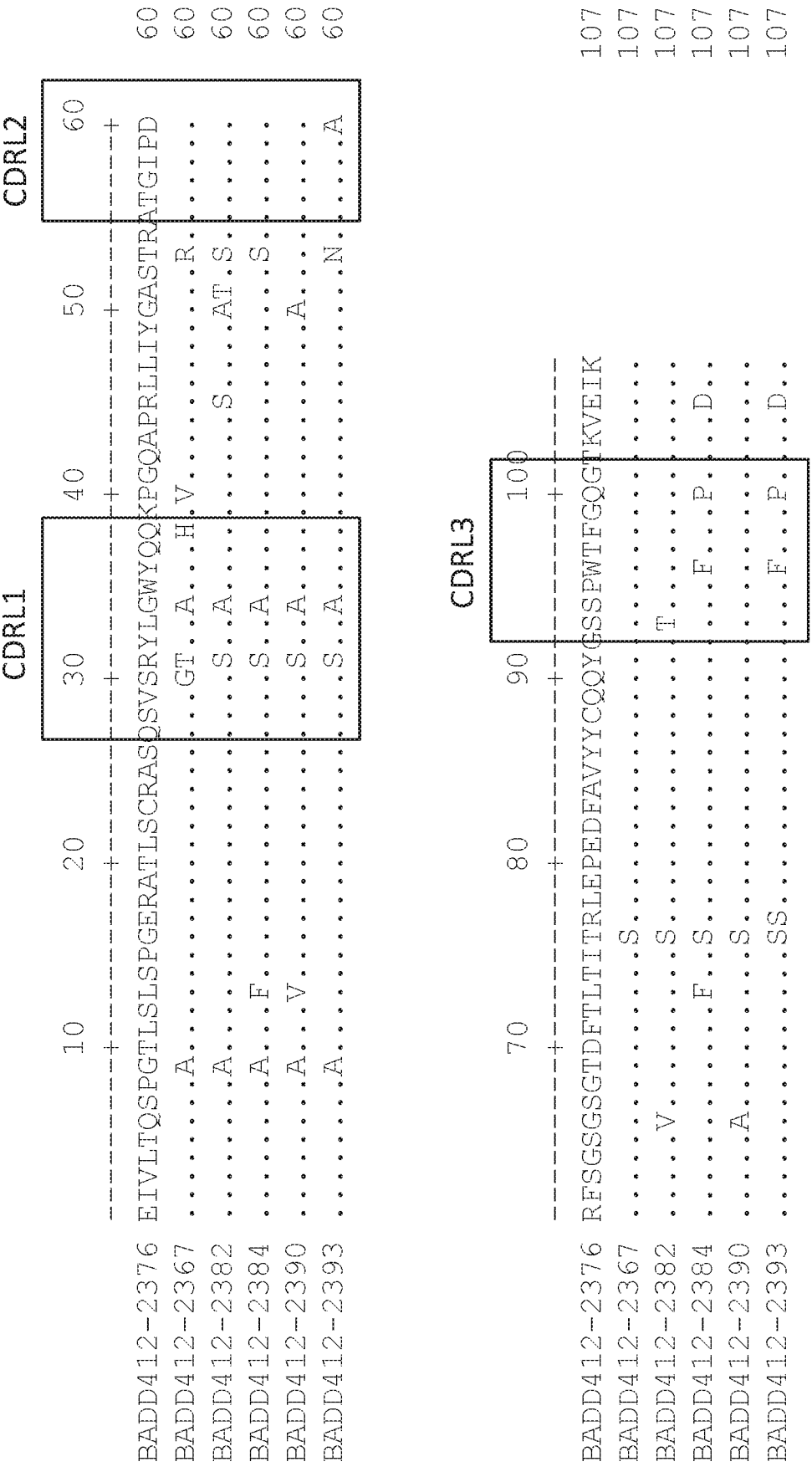

CDRL1
CDRL2
CDRL3
10 20 30 40 50 60
70 80 90 100
BADD412-2376 EIVLTQSPGTLSLSPGERATLSCRASQSVSRYLGWYQQKPGQAPRLLIYGASTRATGIPD 60
BADD412-2367 .....A.....GT..A...H.V.....R..... 60
BADD412-2382 .....A.....S..A.....S.....AT.S..... 60
BADD412-2384 .....A...F.....S..A.....S..... 60
BADD412-2390 .....A...V.....S..A.....A..... 60
BADD412-2393 .....A.....S..A.....N.....A
BADD412-2376 RFSGSGSGTDFTLTITRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK 107
BADD412-2367 .....S..... 107
BADD412-2382 .....V.....S.....T..... 107
BADD412-2384 .....F..S.....F...P...D.. 107
BADD412-2390 .....A.....S..... 107
BADD412-2393 .....SS.....F...P...D.. 107

Figure 20A

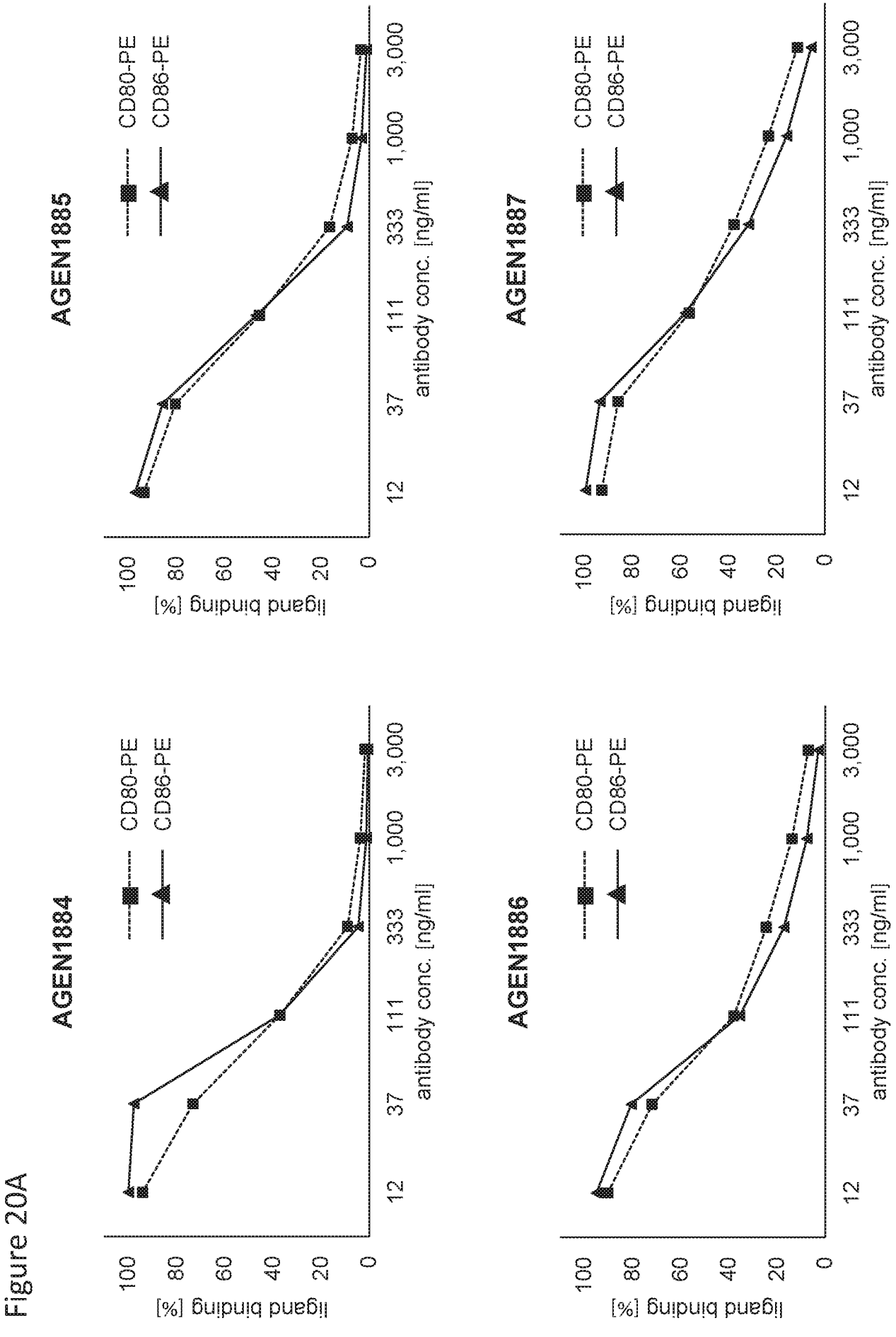

AGEN1884
AGEN1885
AGEN1886
AGEN1887
CD80-PE
CD86-PE
ligand binding [%]
antibody conc. [ng/ml]
0
20
40
60
80
100
12
37
111
333
1,000
3,000

Figure 20B

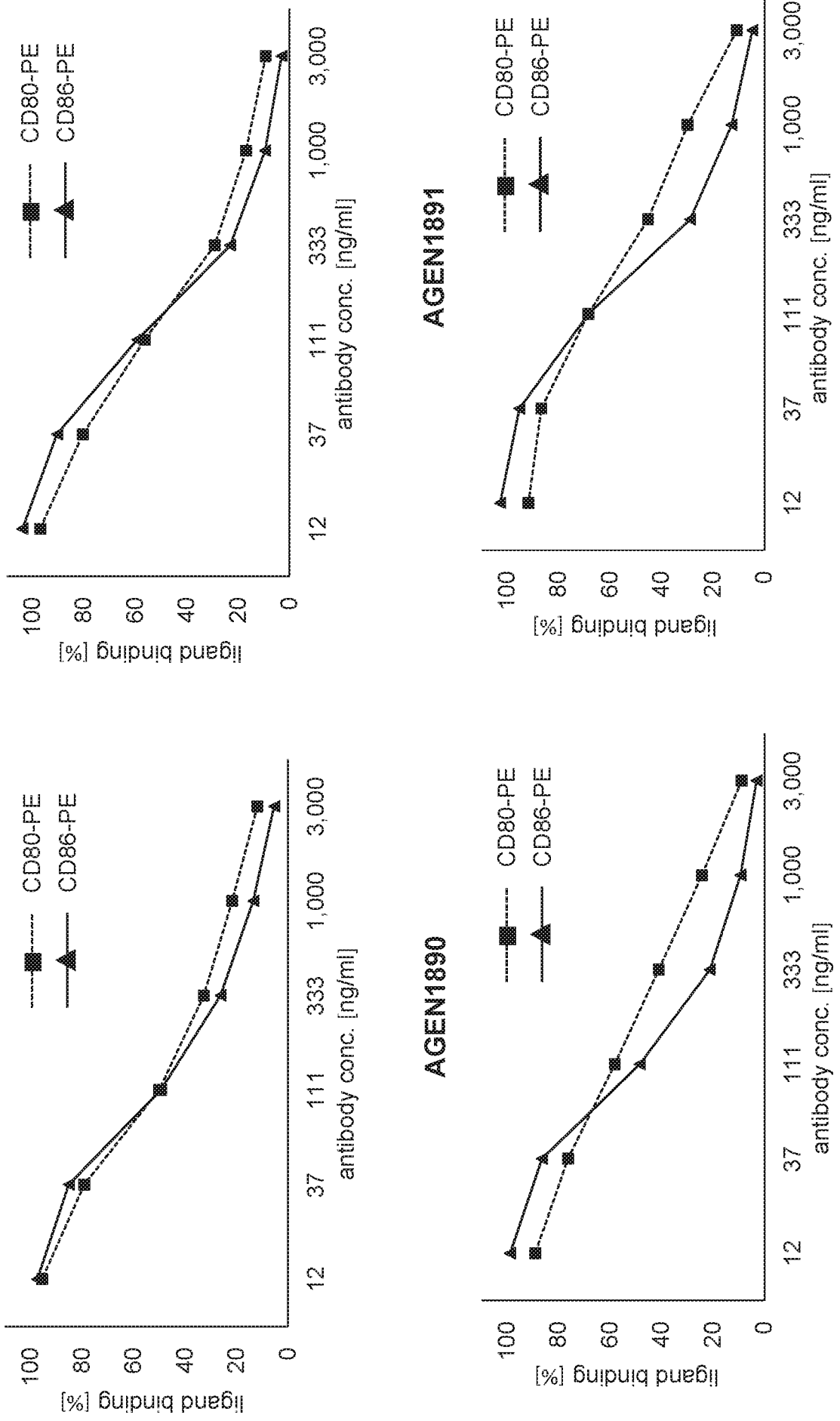

AGEN1888
AGEN1889
AGEN1890
AGEN1891
CD80-PE
CD86-PE
ligand binding [%]
antibody conc. [ng/ml]
0
20
40
60
80
100
12
37
111
333
1,000
3,000

Figure 20C

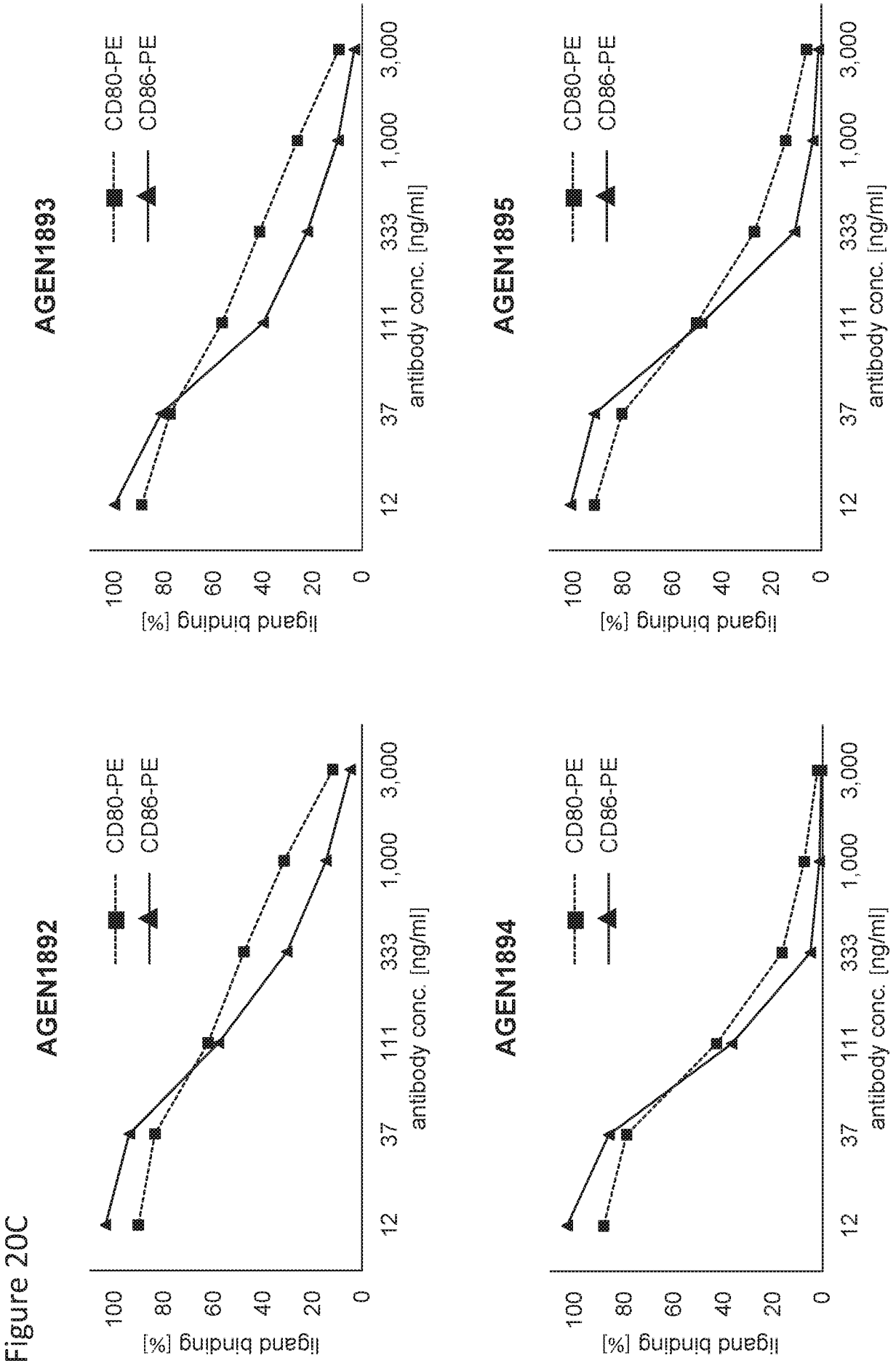
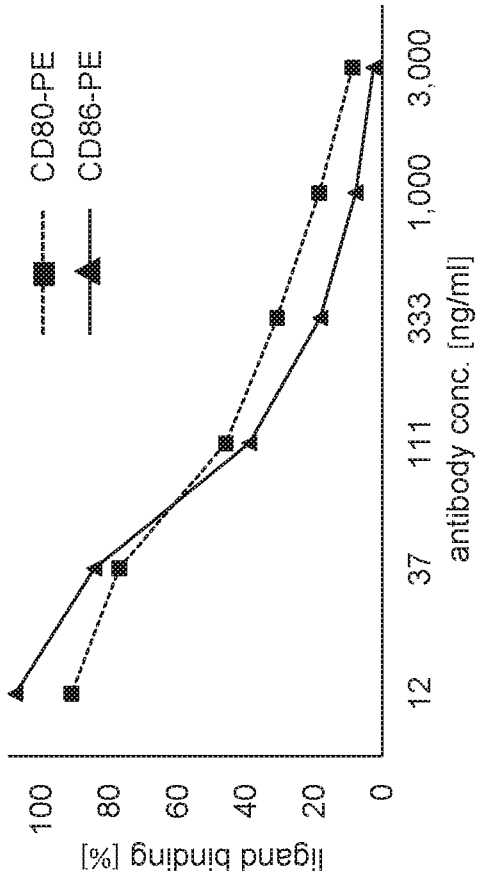
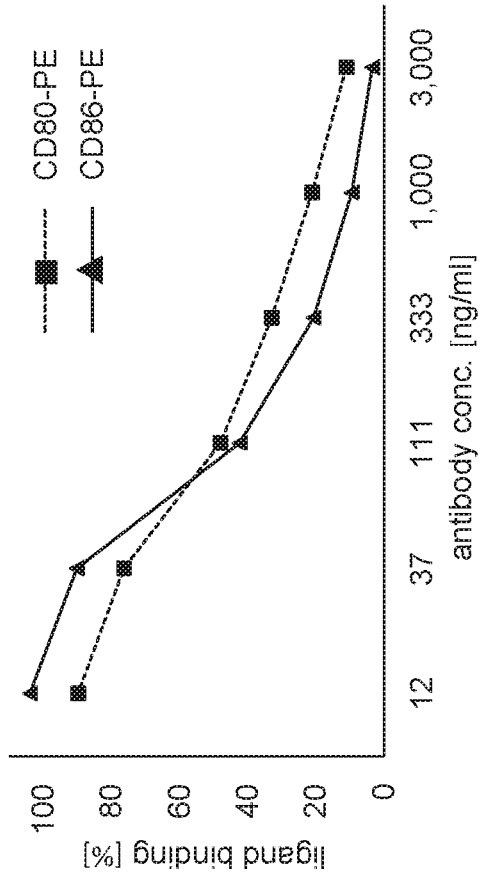
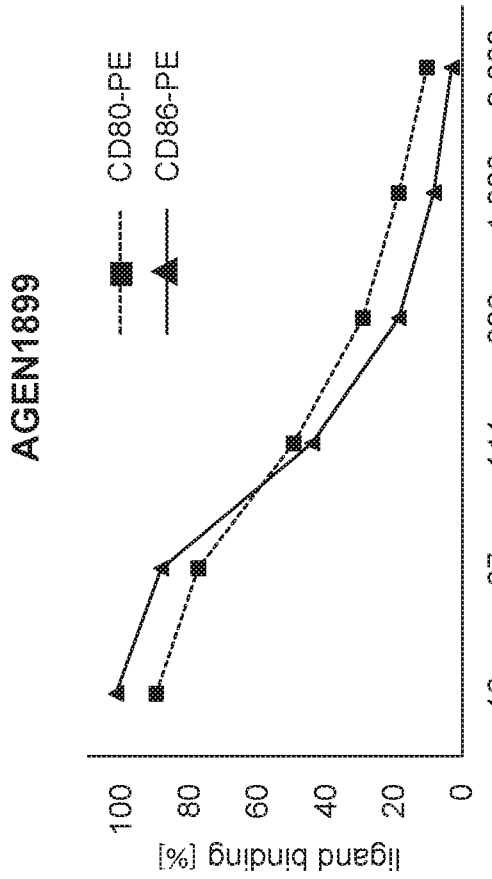

AGEN1892
AGEN1893
AGEN1894
AGEN1895
CD80-PE
CD86-PE
ligand binding [%]
antibody conc. [ng/ml]
0
20
40
60
80
100
12
37
111
333
1,000
3,000

Figure 20D


AGEN1897
CD80-PE
CD86-PE
ligand binding [%]
100
80
60
40
20
0
12
37
111
333
1,000
3,000
antibody conc. [ng/ml]


AGEN1896
CD80-PE
CD86-PE
ligand binding [%]
100
80
60
40
20
0
12
37
111
333
1,000
3,000
antibody conc. [ng/ml]


AGEN1899
CD80-PE
CD86-PE
ligand binding [%]
100
80
60
40
20
0
12
37
111
333
1,000
3,000
antibody conc. [ng/ml]

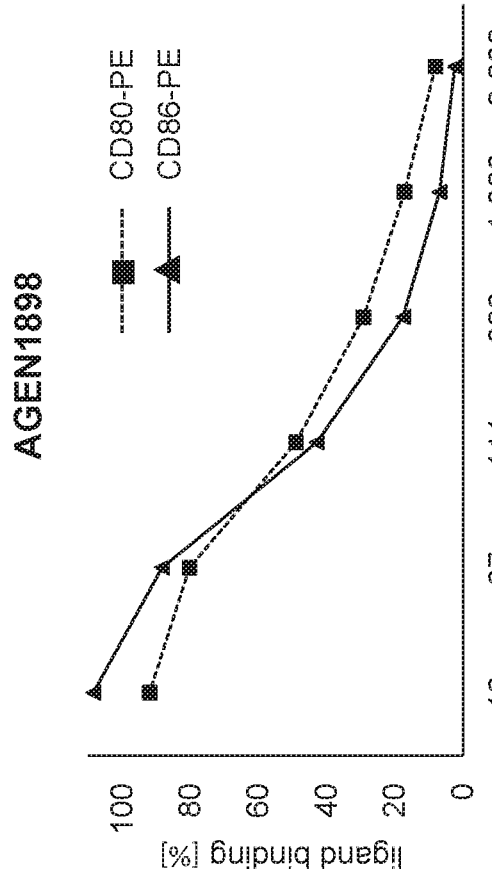
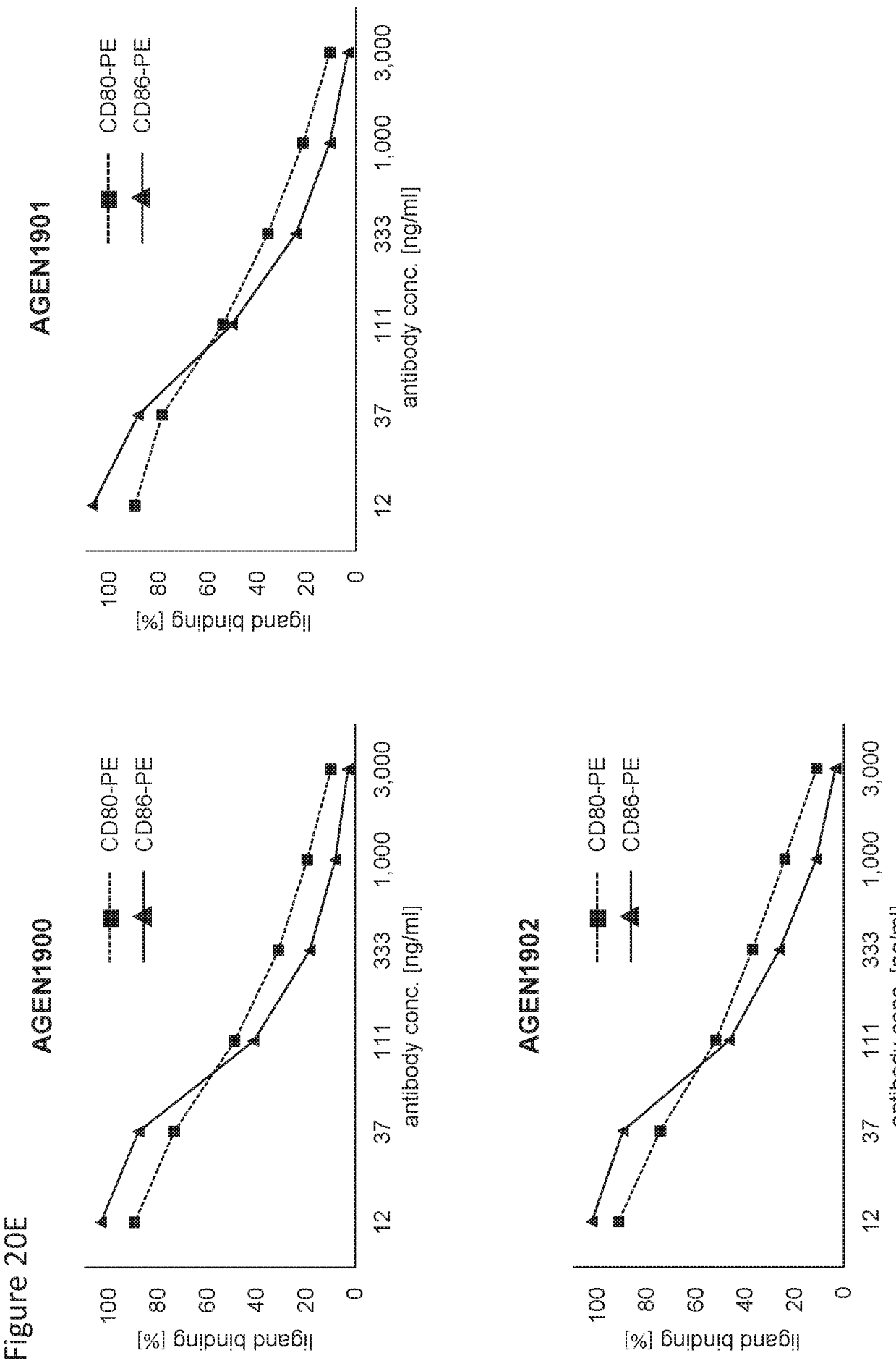

AGEN1898
CD80-PE
CD86-PE
ligand binding [%]
100
80
60
40
20
0
12
37
111
333
1,000
3,000
antibody conc. [ng/ml]

Figure 20E


AGEN1900
AGEN1901
AGEN1902
CD80-PE
CD86-PE
ligand binding [%]
antibody conc. [ng/ml]
0
20
40
60
80
100
12
37
111
333
1,000
3,000