Figure 1A

Figure 1B

Figure 1C

Figure 1D

Figure 1E

Figure 1F

Figure 1G

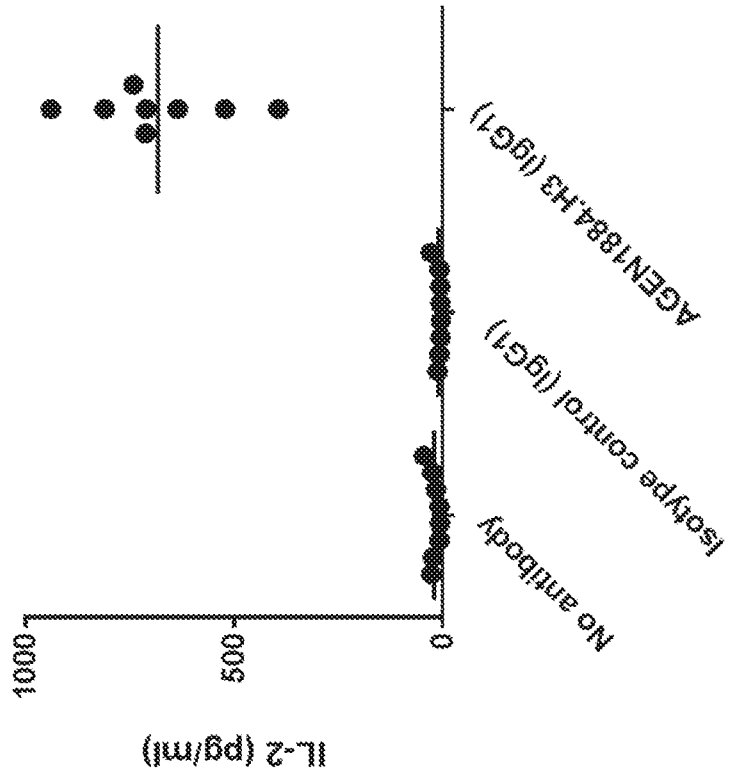

SEA assay
IL-2 (pg/ml)
1000
500
0
No antibody
Isotype control (IgG1)
AGEN1884.H3 (IgG1)

Figure 2

Figure 3

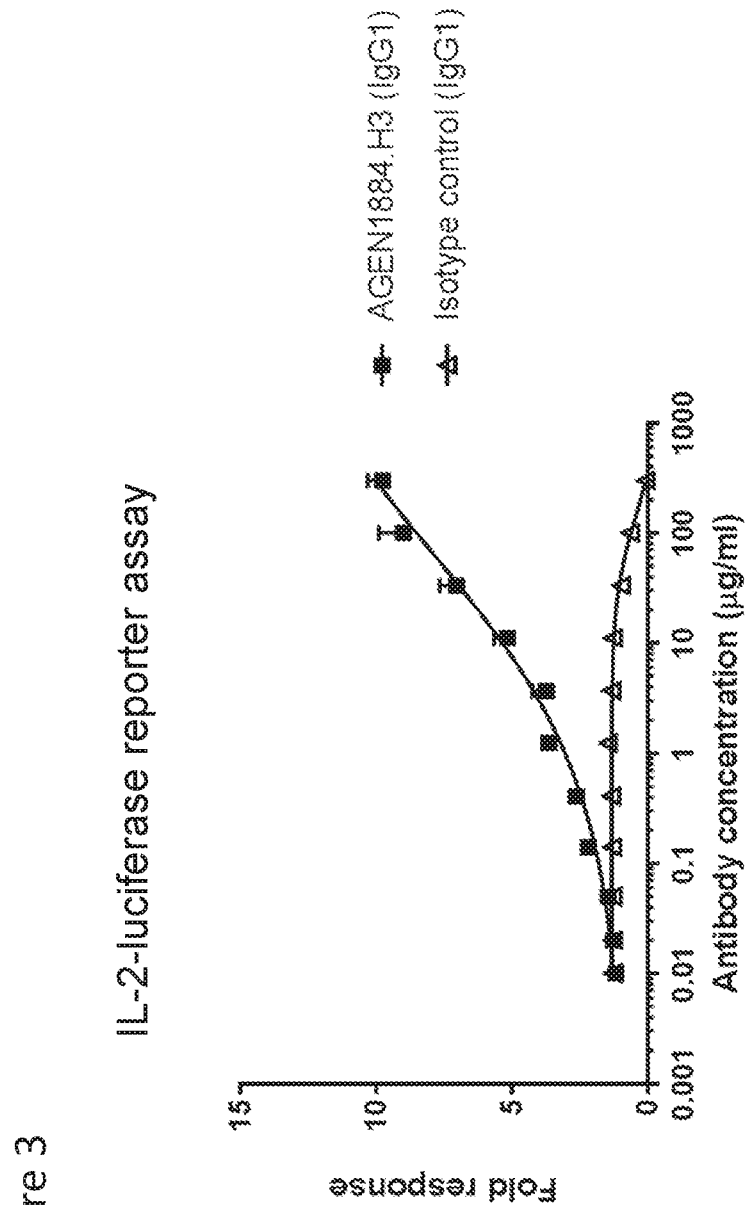

IL-2-luciferase reporter assay
AGEN1884.H3 (IgG1)
Isotype control (IgG1)
Fold response
15
10
5
0
0.001
0.01
0.1
1
10
100
1000
Antibody concentration (µg/ml)

Figure 4

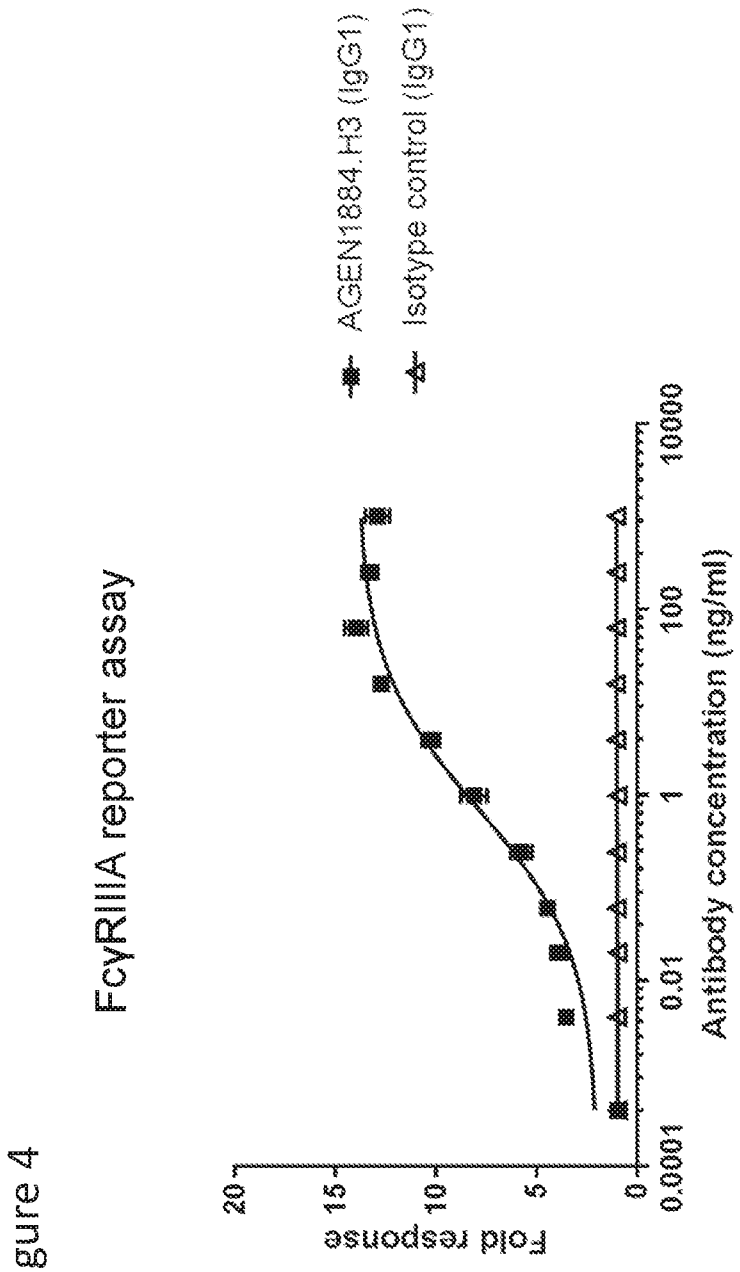
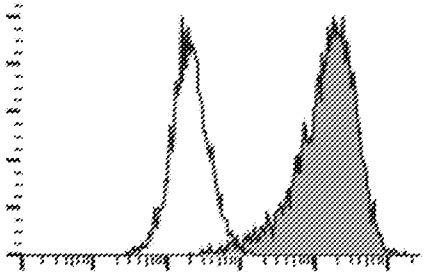
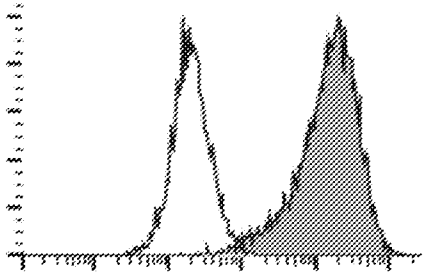

FcγRIIIA reporter assay
AGEN1884.H3 (IgG1)
Isotype control (IgG1)
Fold response
0
5
10
15
20
0.0001
0.01
1
100
10000
Antibody concentration (ng/ml)

Figure 5A



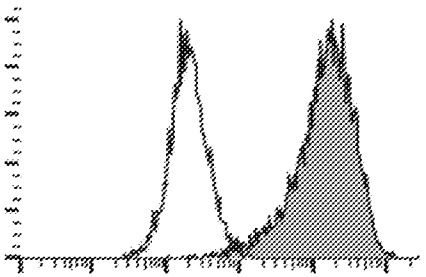
☐ Isotype (IgG1)
☐ AGEN1884.H3 (IgG1)

Figure 5B



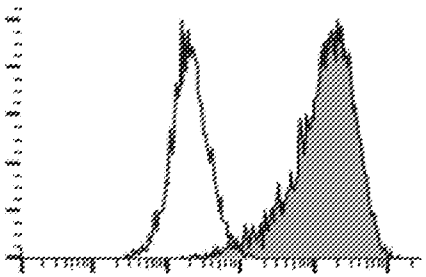
☐ Isotype (IgG1)
☐ AGEN1884.H3 (IgG1 S239D/I332E)

Figure 5C



☐ Isotype (IgG1)
☐ AGEN1884.H3 (IgG1 S239D/A330L/I332E)

Figure 5D



☐ Isotype (IgG1)
☐ AGEN1884.H3 (IgG1 L235V/F243L/R292P/Y300L/P396L)

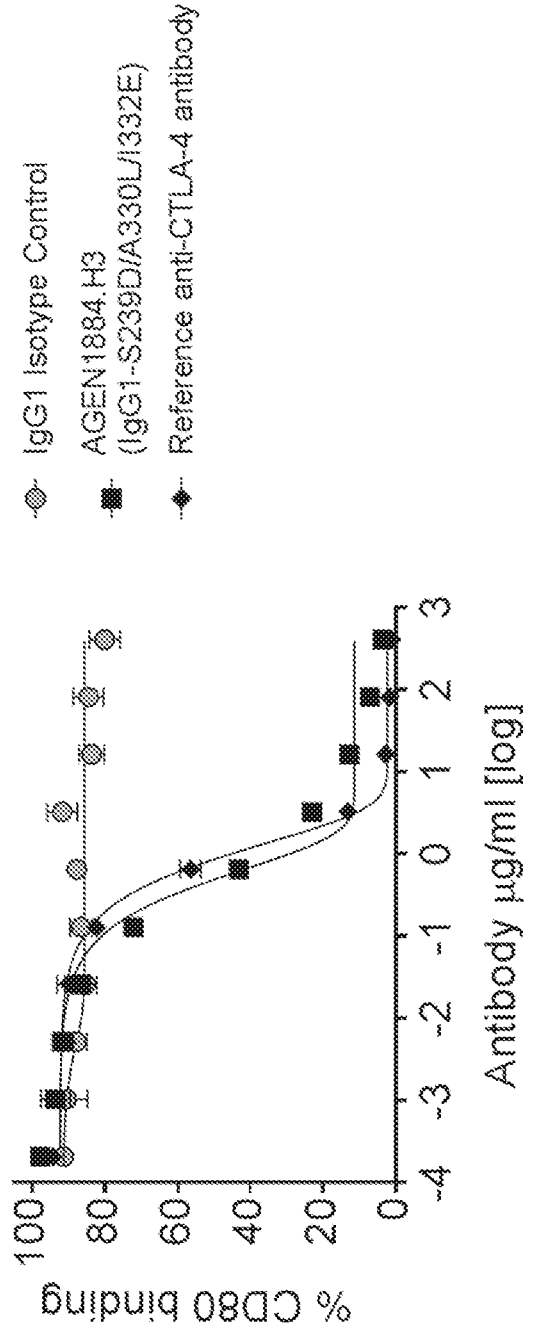


Figure 6A

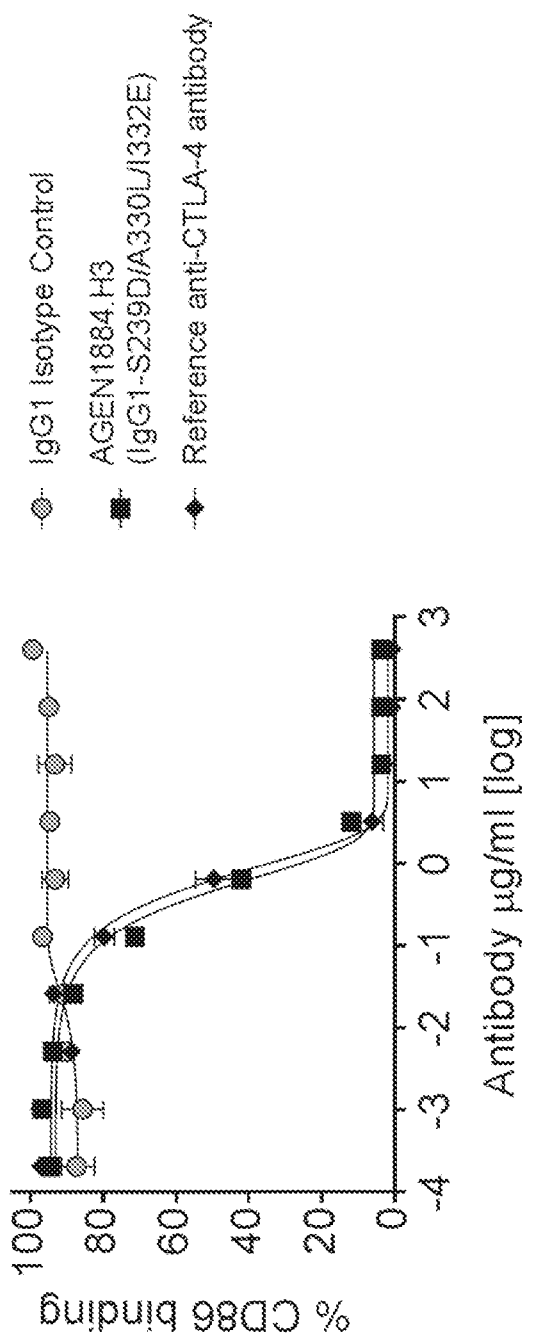


Figure 6B

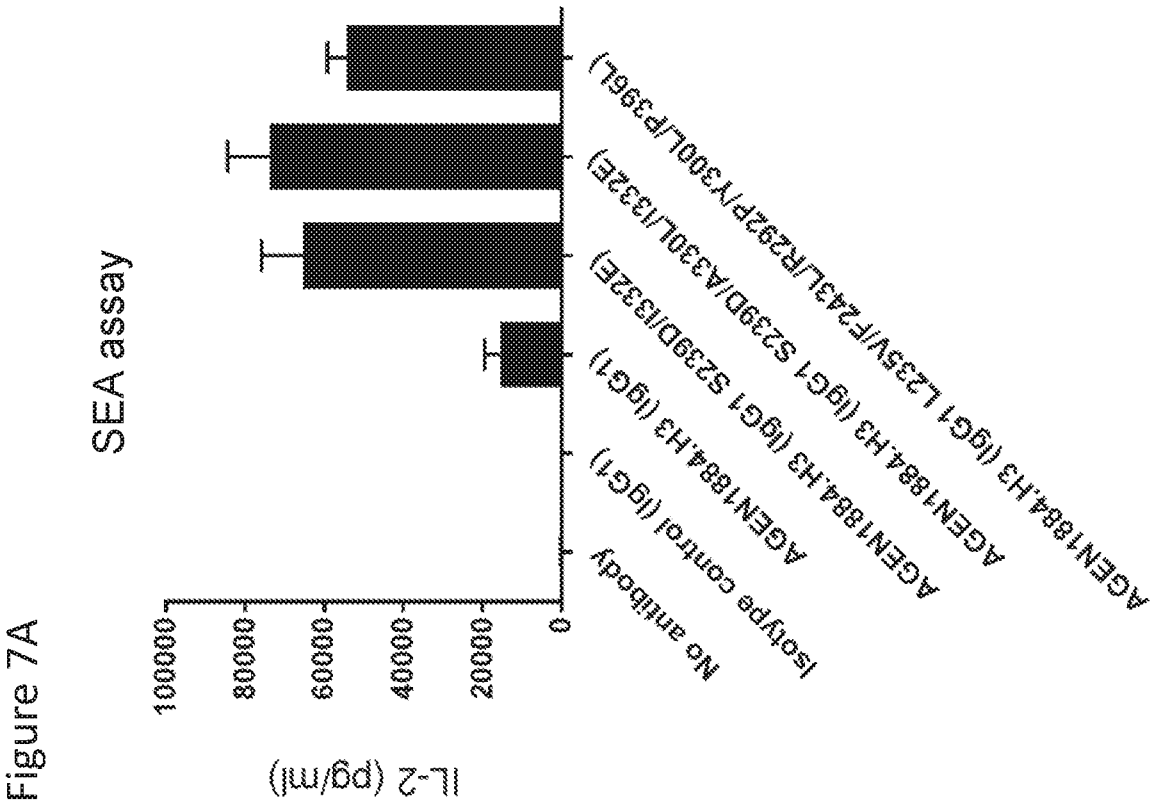

SEA assay
IL-2 (pg/ml)
100000
80000
60000
40000
20000
0
No antibody
Isotype control (IgG1)
AGEN1884.H3 (IgG1)
AGEN1884.H3 (IgG1 S239D/I332E)
AGEN1884.H3 (IgG1 S239D/A330L/I332E)
AGEN1884.H3 (IgG1 L235V/F243L/R292P/Y300L/P396L)

Figure 7A

Figure 7B

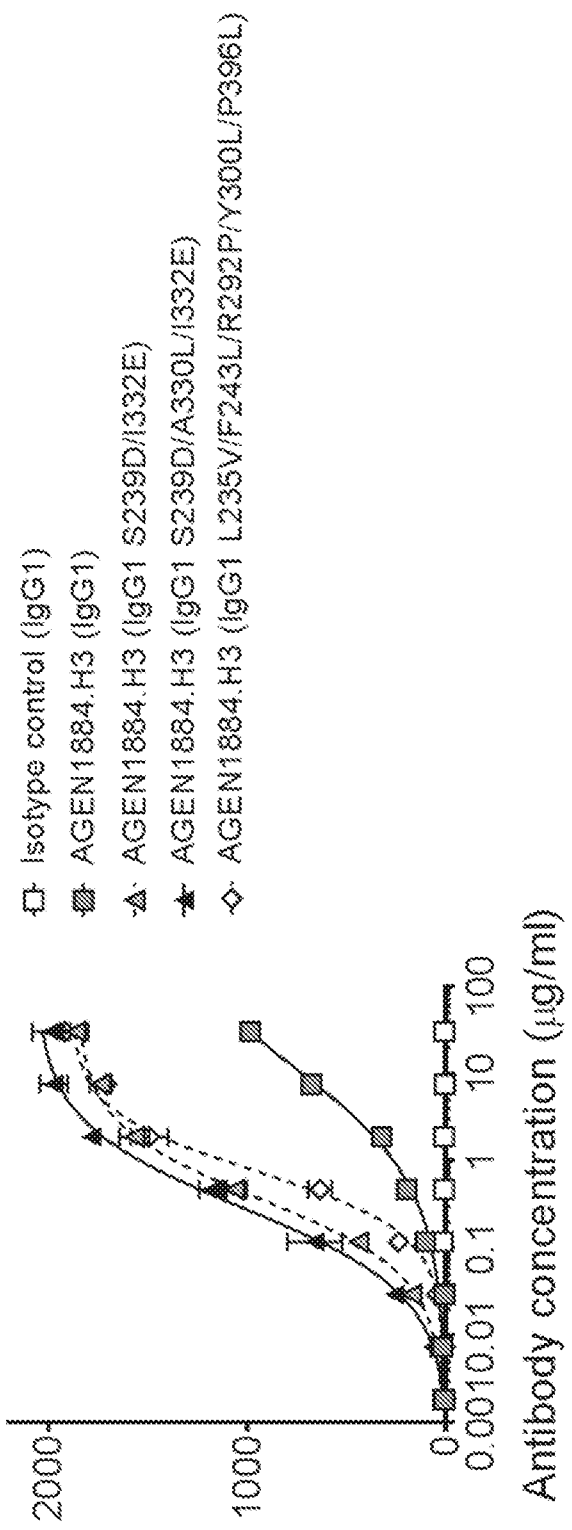


Figure 7C

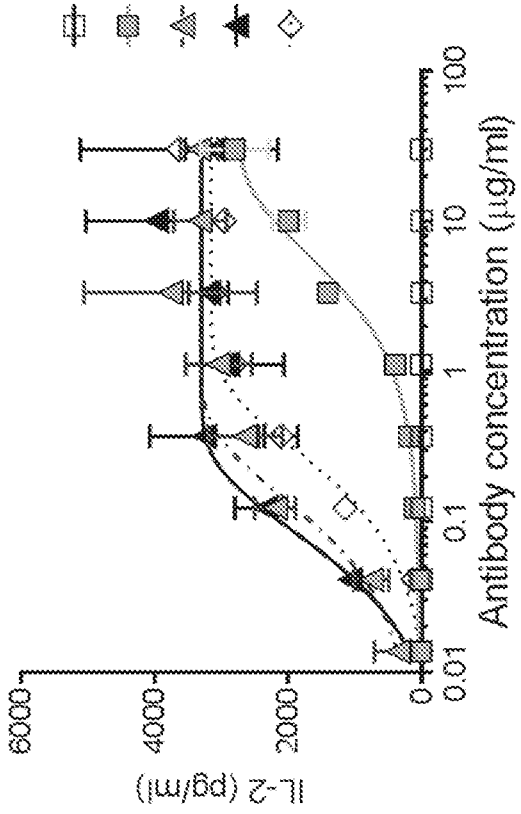


Figure 8A

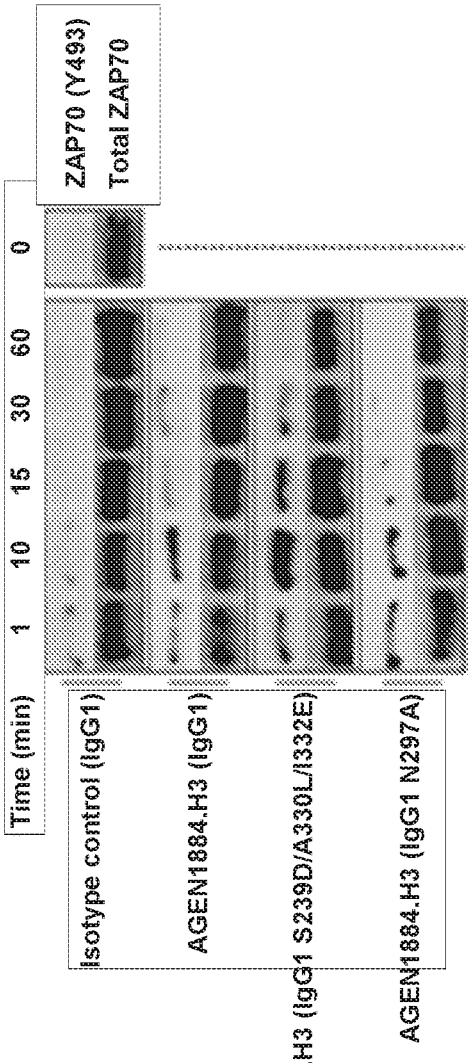


Figure 8B

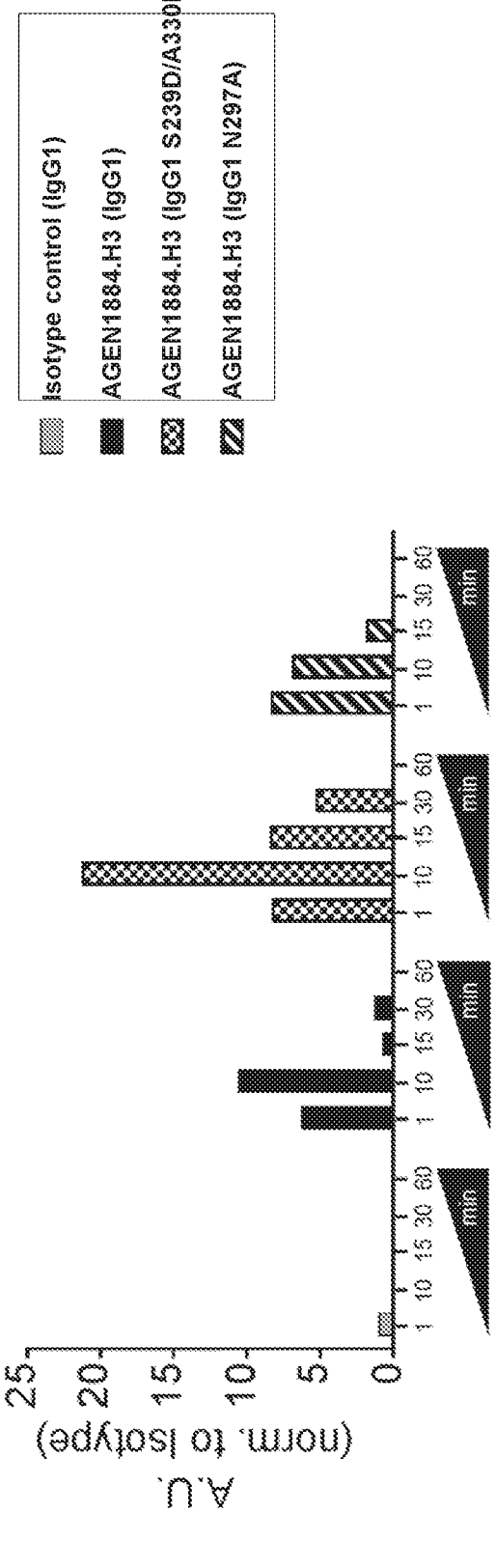


Figure 9A

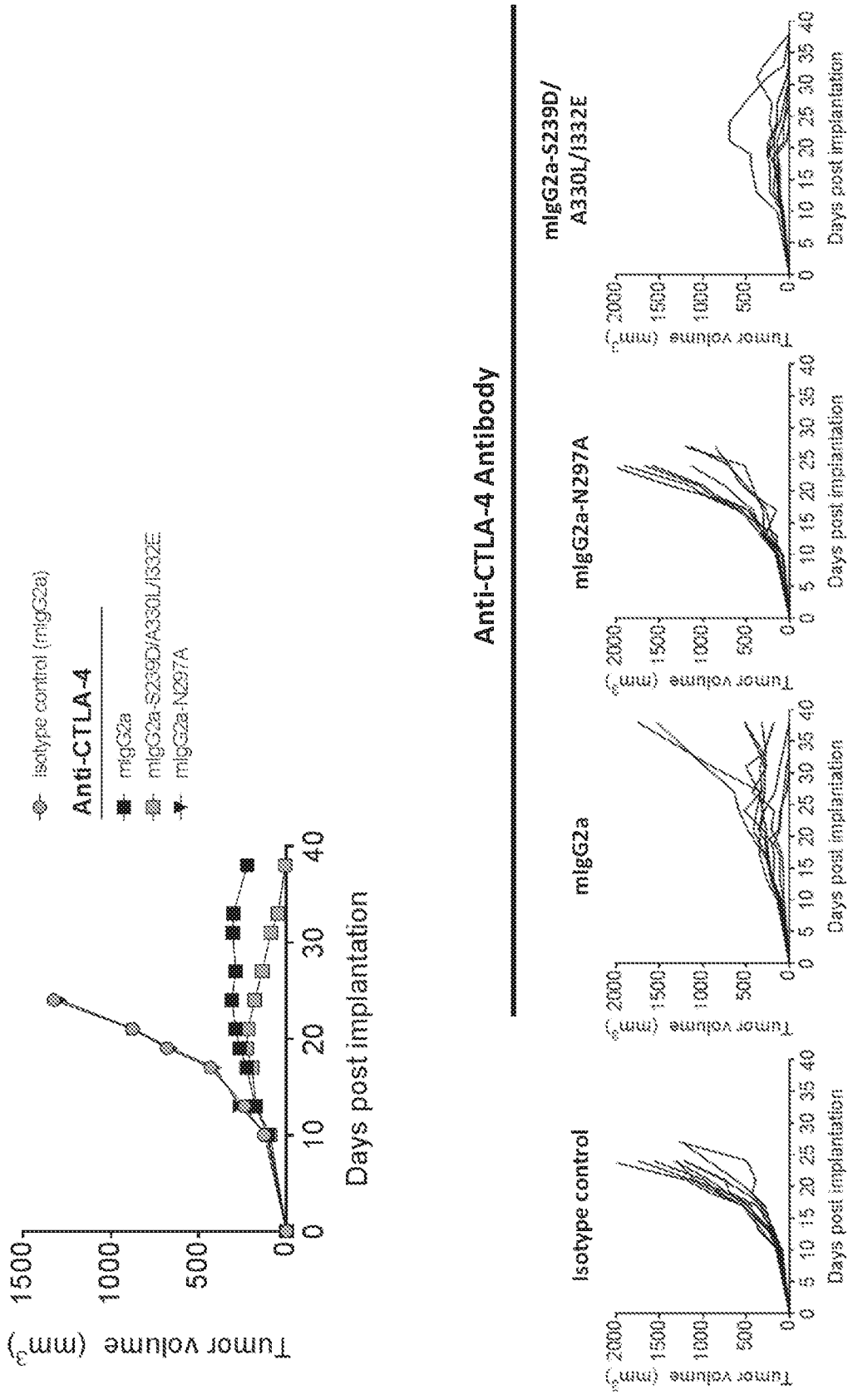

Tumor volume (mm³)
1500
1000
500
0
0
10
20
30
40
Days post implantation
isotype control (mIgG2a)
Anti-CTLA-4
mIgG2a
mIgG2a-S239D/A330L/I332E
mIgG2a-N297A
Anti-CTLA-4 Antibody
Isotype control
mIgG2a
mIgG2a-N297A
mIgG2a-S239D/
A330L/I332E
2000
1500
1000
500
0
0 5 10 15 20 25 30 35 40
Days post implantation

Figure 9B

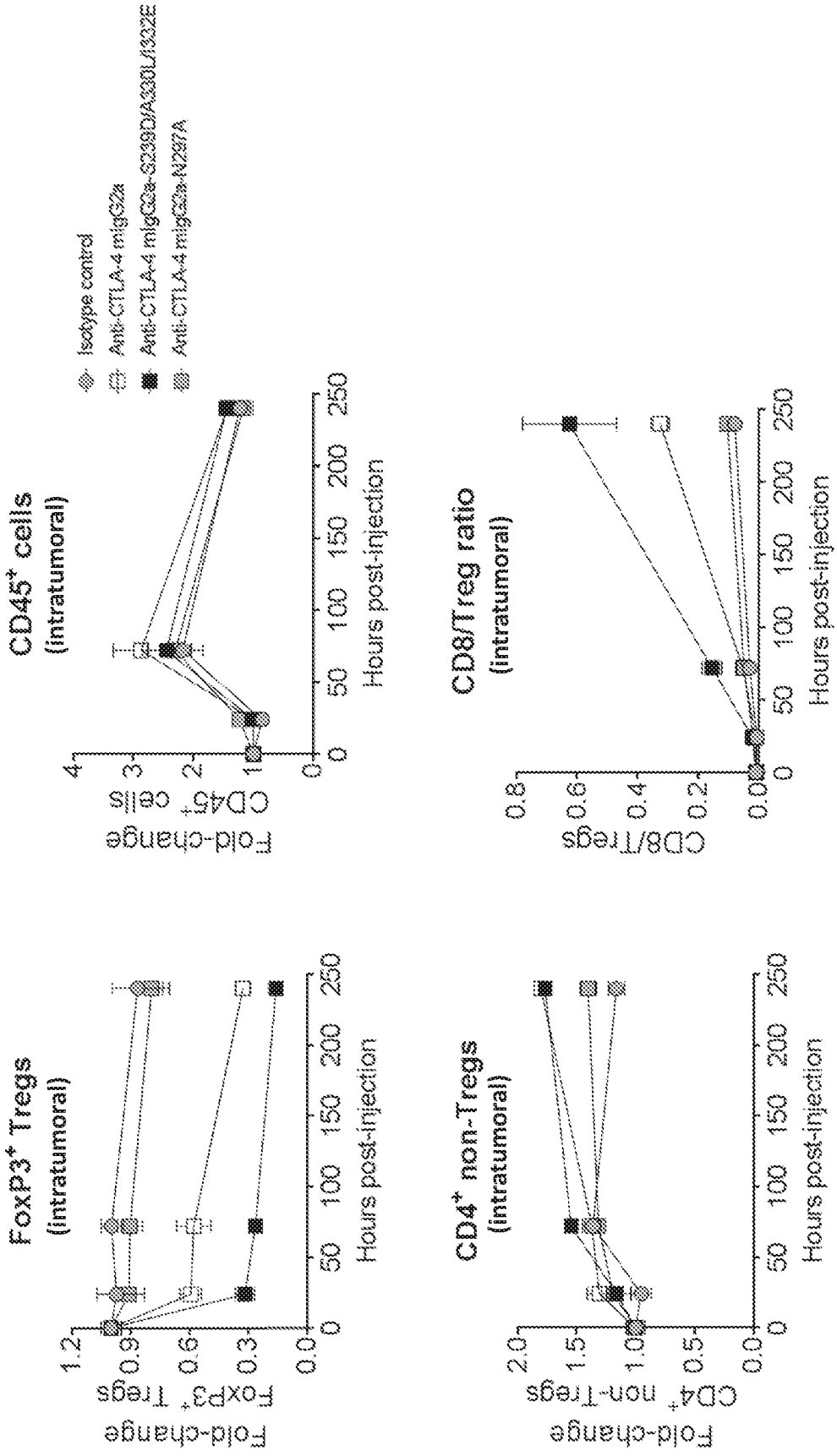

FoxP3+ Tregs
(intratumoral)
Fold-change
FoxP3+ Tregs
Hours post-injection
CD45+ cells
(intratumoral)
Fold-change
CD45+ cells
Hours post-injection
Isotype control
Anti-CTLA-4 mIgG2a
Anti-CTLA-4 mIgG2a-S239D/A330L/I332E
Anti-CTLA-4 mIgG2a-N297A
CD4+ non-Tregs
(intratumoral)
Fold-change
CD4+ non-Tregs
Hours post-injection
CD8/Treg ratio
(intratumoral)
CD8/Tregs
Hours post-injection

Figure 9C

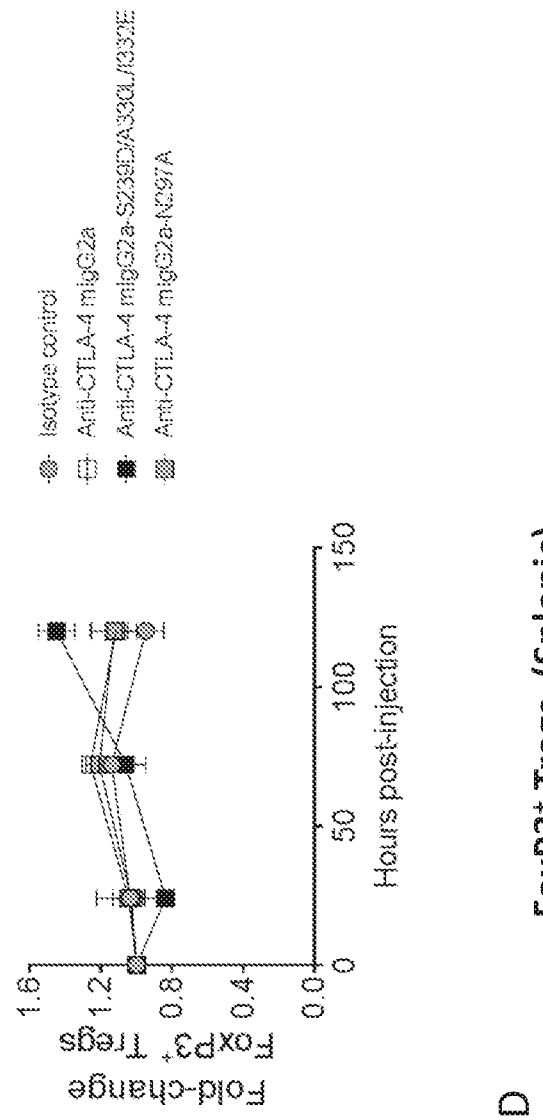


Figure 9D

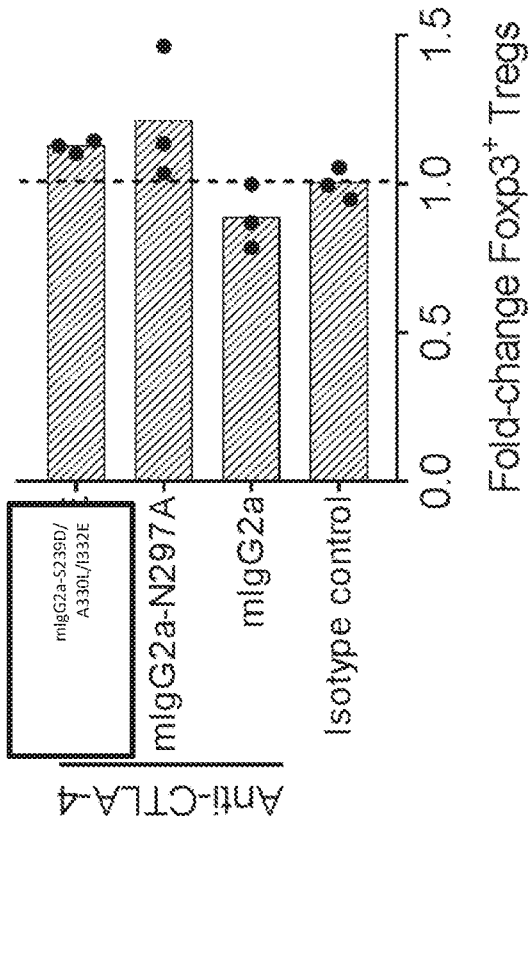
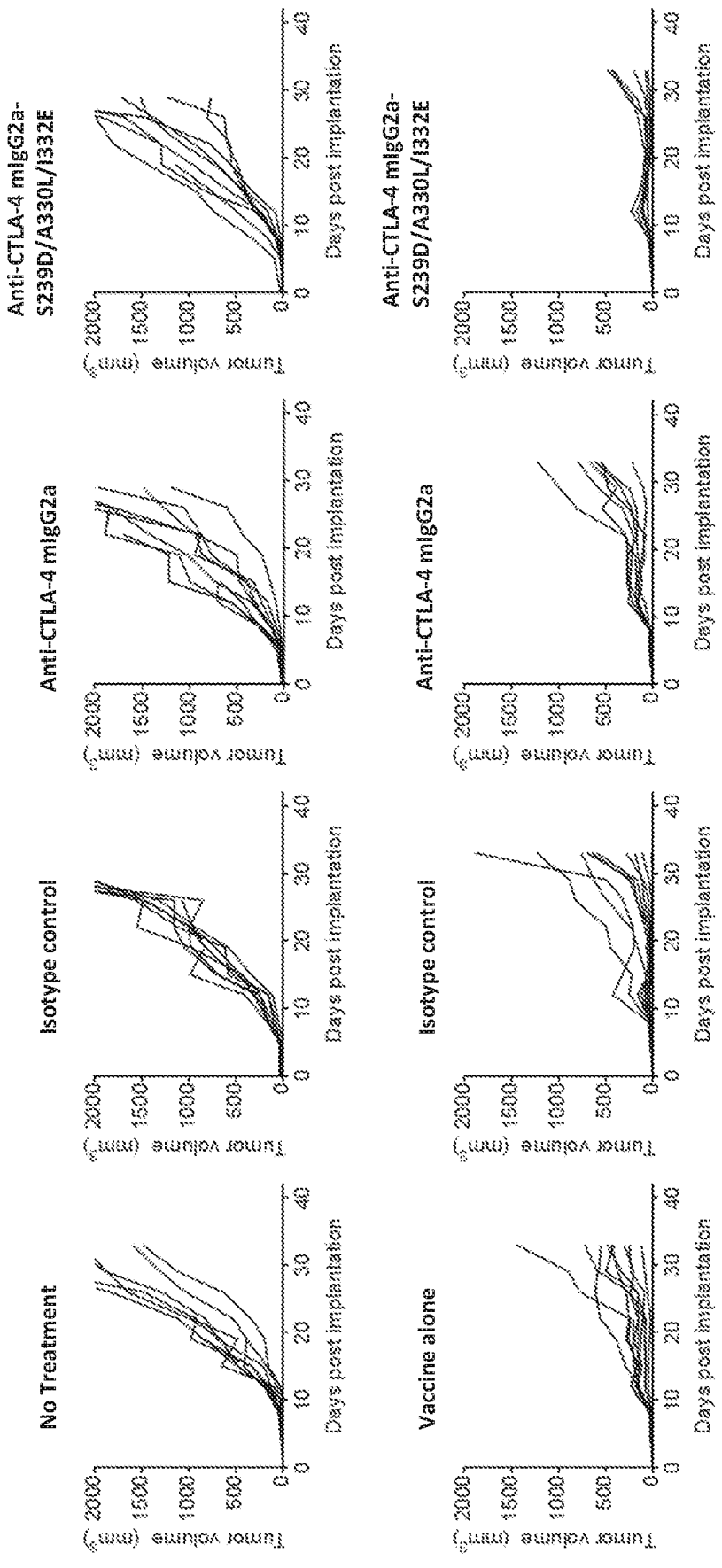


Figure 10


No Vaccine
+ Vaccine
No Treatment
Isotype control
Anti-CTLA-4 mIgG2a
Anti-CTLA-4 mIgG2a-S239D/A330L/I332E
Vaccine alone
Tumor volume (mm³)
Days post implantation
0
500
1000
1500
2000
10
20
30
40

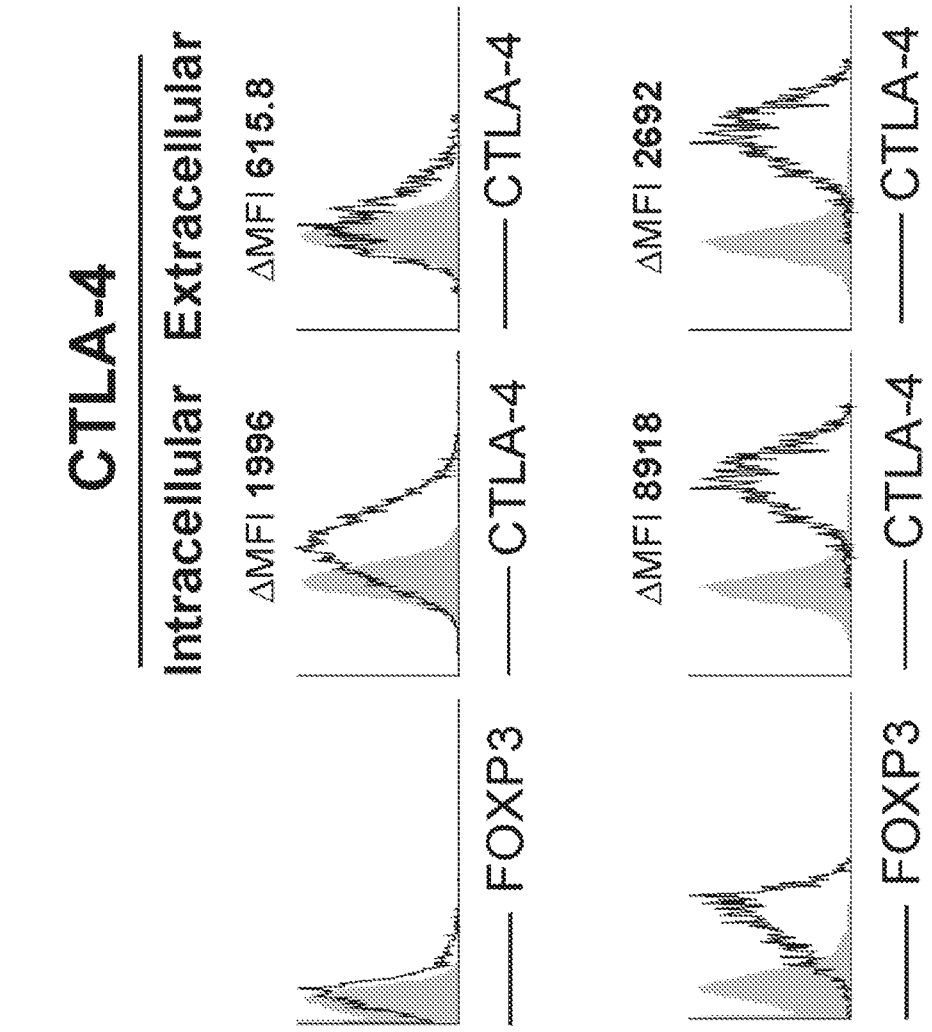

CTLA-4
Intracellular
Extracellular
ΔMFI 1996
ΔMFI 615.8
FOXP3
CTLA-4
CTLA-4
Activated Teff
ΔMFI 8918
ΔMFI 2692
FOXP3
CTLA-4
CTLA-4
Activated Treg

Figure 11A

Figure 11B

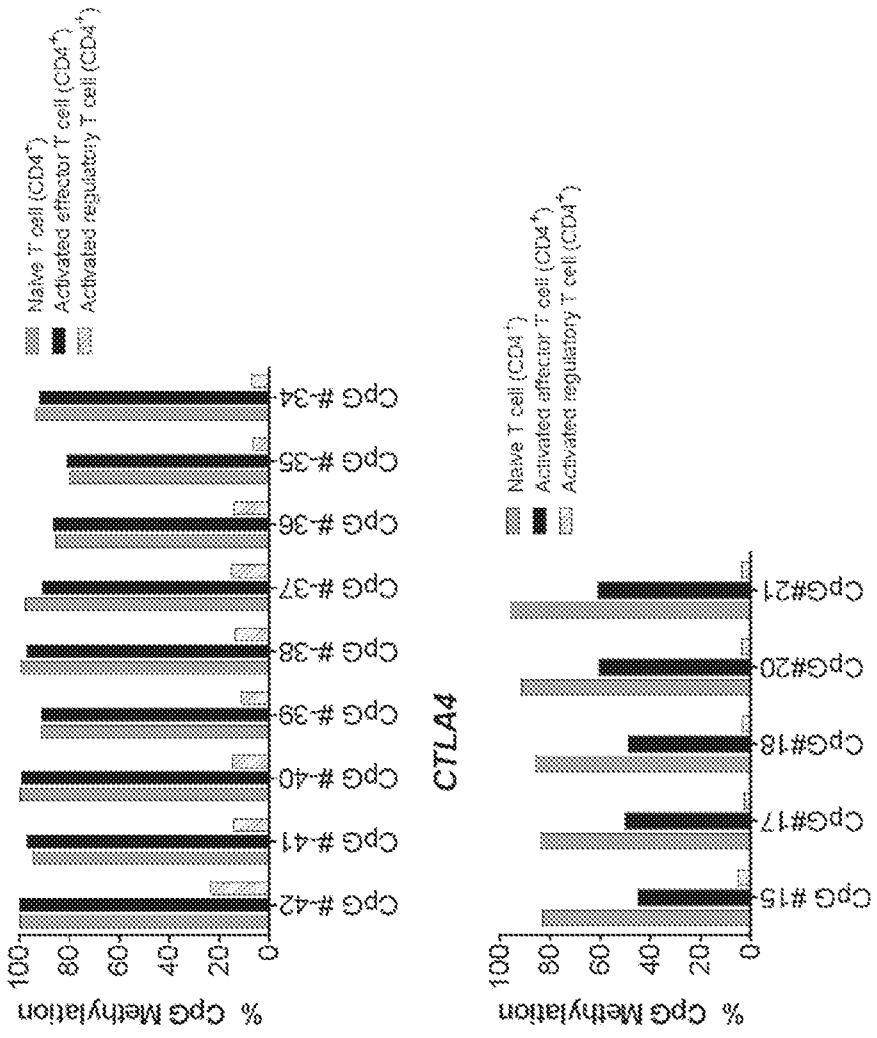

FOXP3
Naive T cell (CD4+)
Activated effector T cell (CD4+)
Activated regulatory T cell (CD4+)
% CpG Methylation
0
20
40
60
80
100
CpG #-42
CpG #-41
CpG #-40
CpG #-39
CpG #-38
CpG #-37
CpG #-36
CpG #-35
CpG #-34
CTLA4
Naive T cell (CD4+)
Activated effector T cell (CD4+)
Activated regulatory T cell (CD4+)
% CpG Methylation
0
20
40
60
80
100
CpG #15
CpG#17
CpG#18
CpG#20
CpG#21

Figure 12A

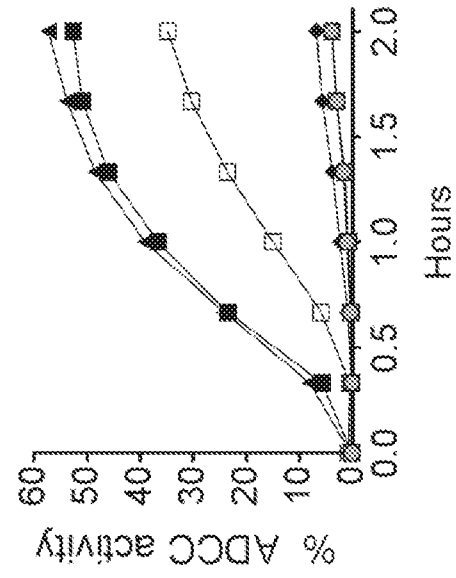


Figure 12B

Figure 13A

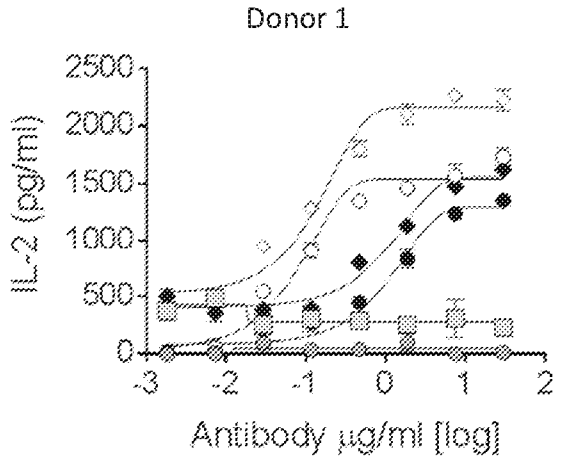
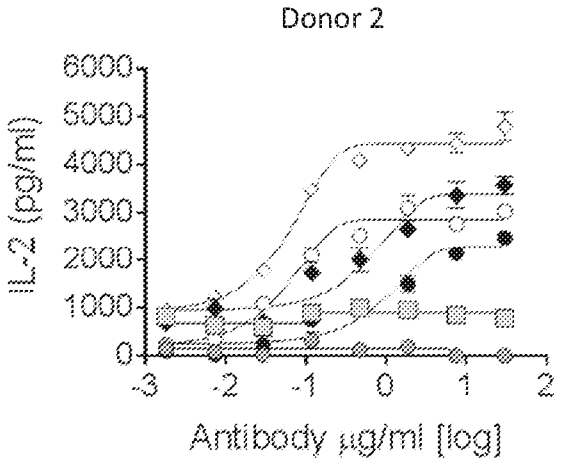


Figure 13C



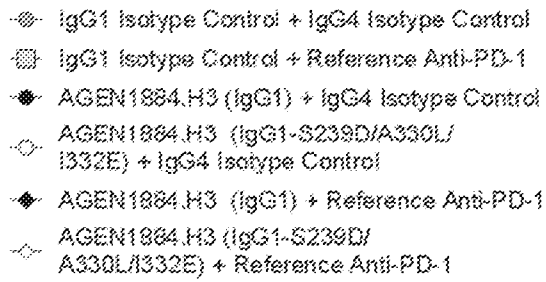


Figure 13B

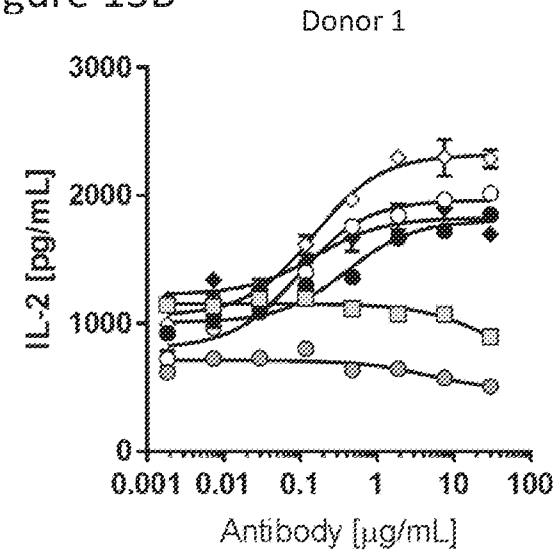


Figure 13D

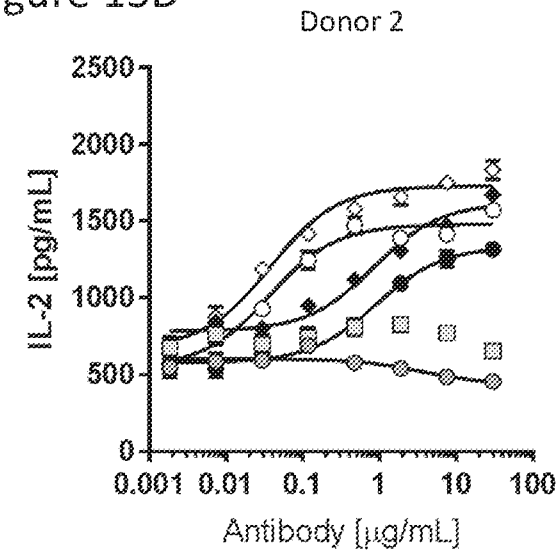


Figure 14A

```
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 14B

```
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 14C

```
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
```