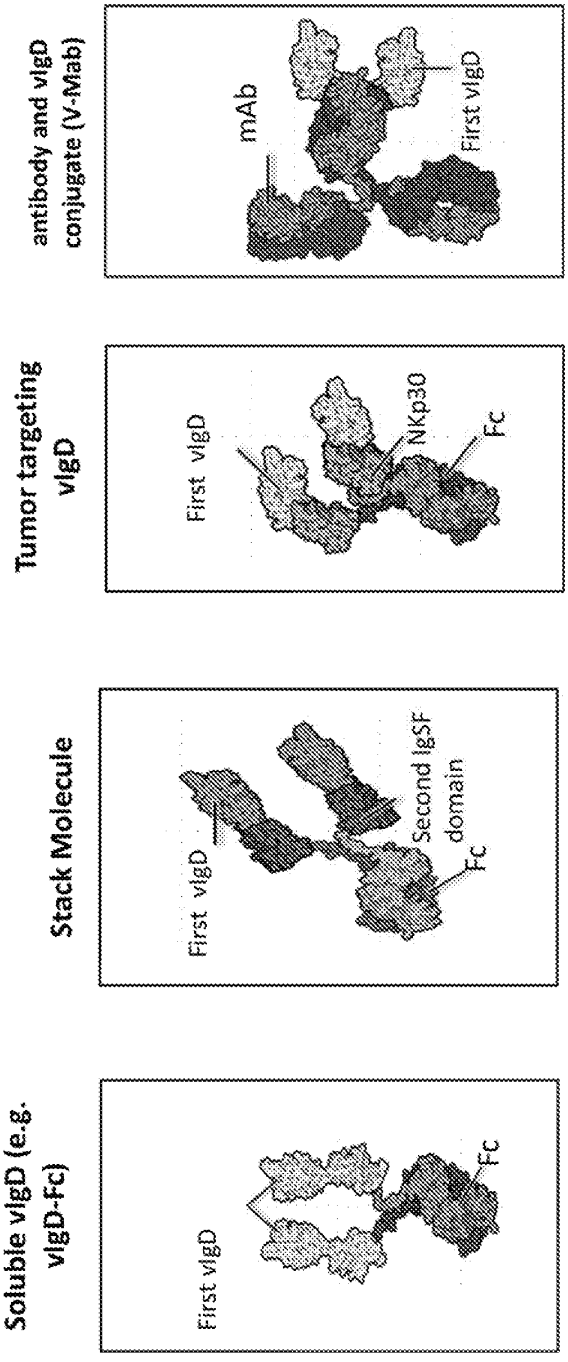
FIG. 1A


Soluble vIgD (e.g. vIgD-Fc)
First vIgD
Fc
Stack Molecule
First vIgD
Second IgSF domain
Fc
Tumor targeting vIgD
First vIgD
Nkp30
Fc
antibody and vIgD conjugate (V-Mab)
mAb
First vIgD

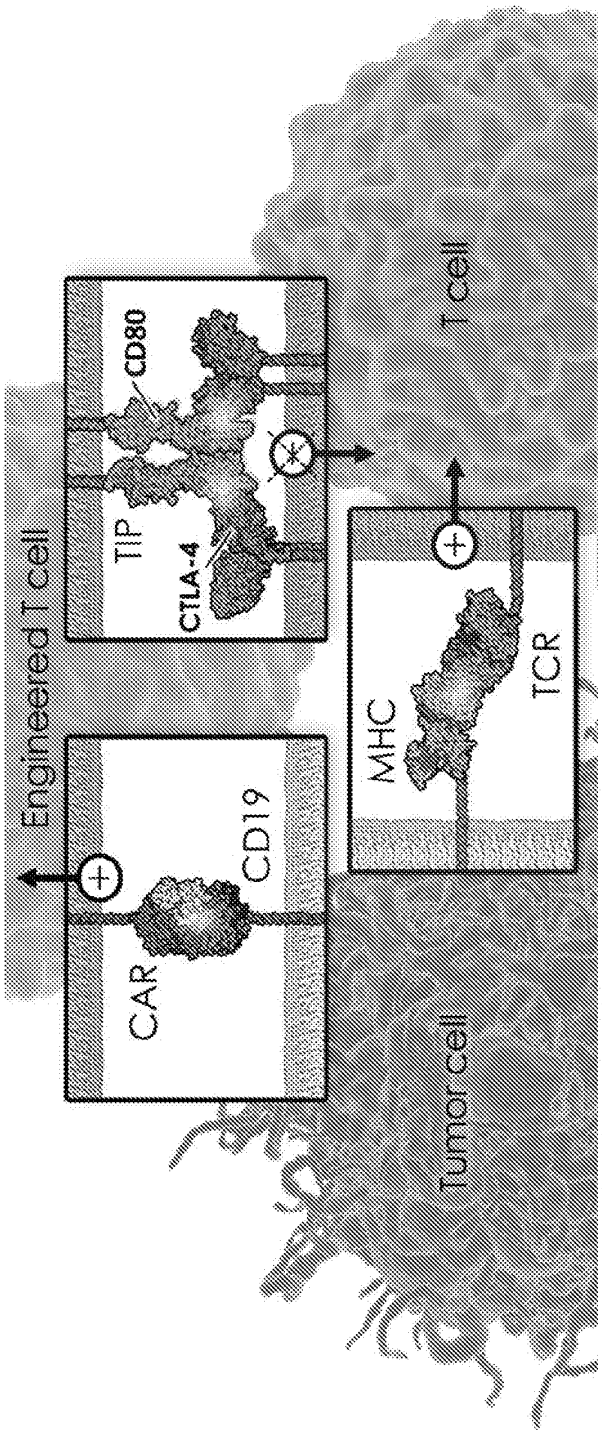

Engineered T cell
CAR
CD19
TIP
CD80
CTLA-4
MHC
TCR
T cell
Tumor cell

FIG. 1B

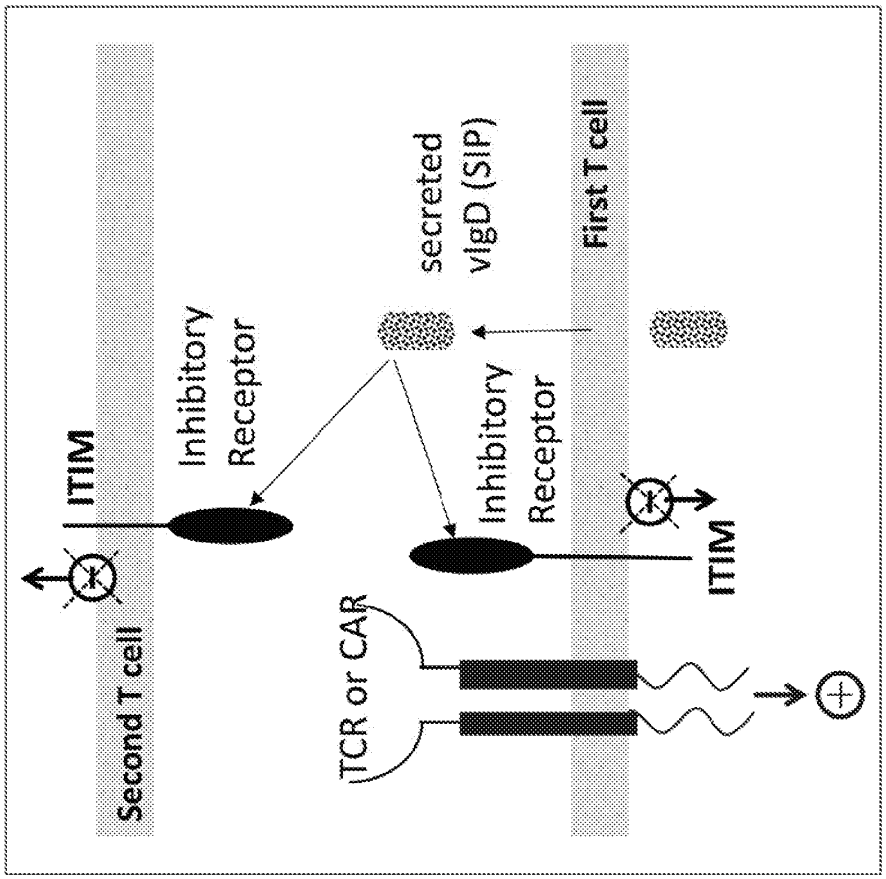

Second T cell
ITIM
Inhibitory Receptor
secreted vIgD (SIP)
First T cell
Inhibitory Receptor
ITIM
TCR or CAR

FIG. 1C

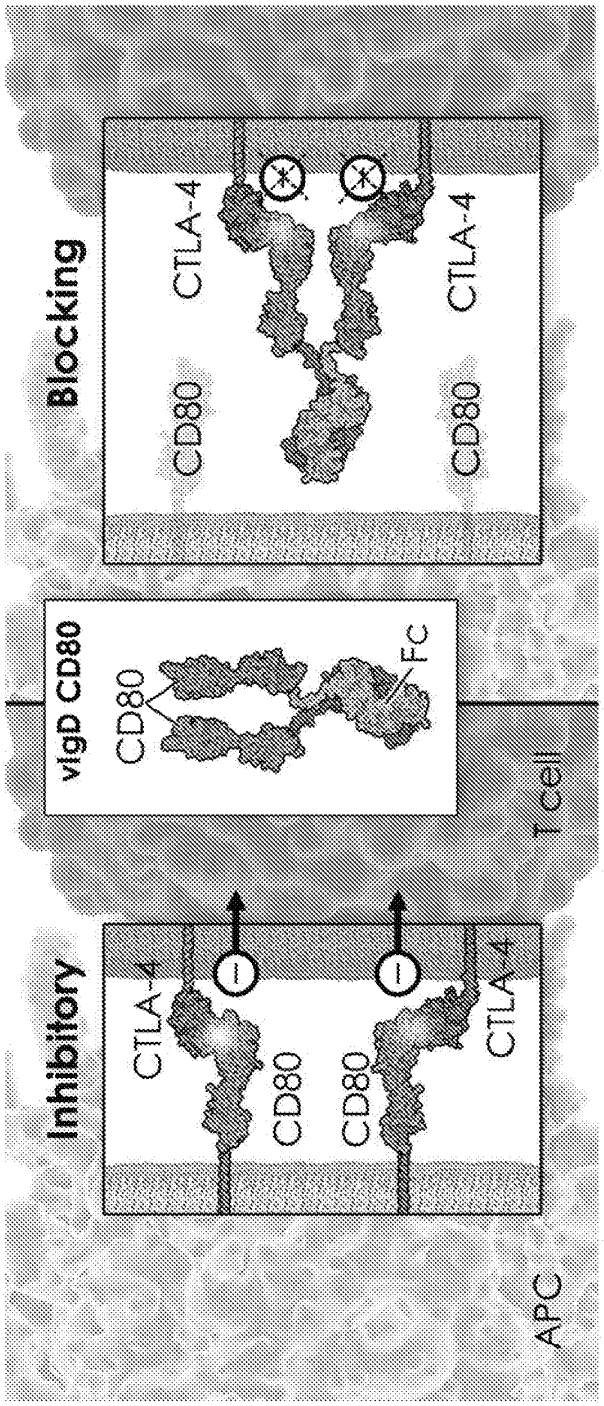
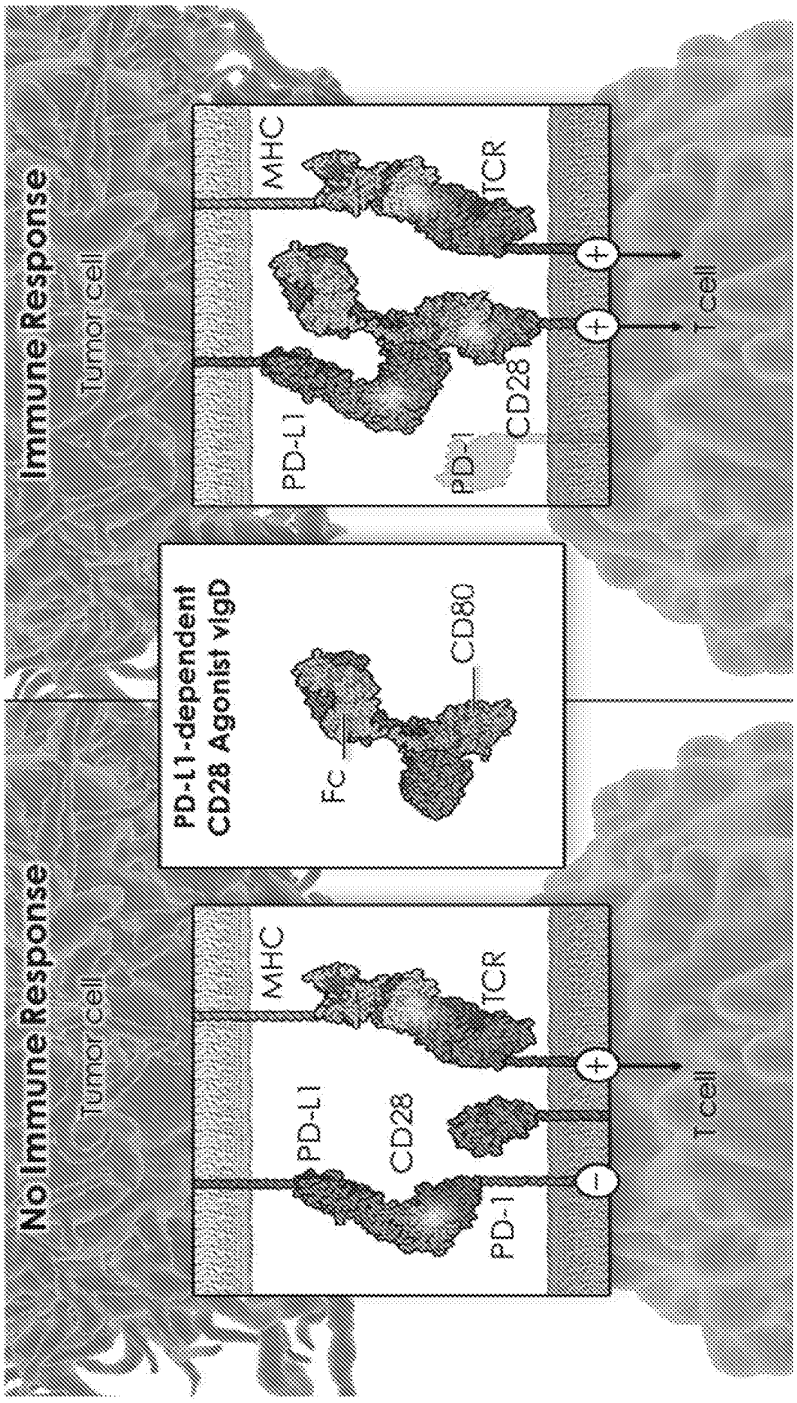

Blocking
CTLA-4
CTLA-4
CD80
CD80
vIgD CD80
CD80
Fc
T cell
Inhibitory
CTLA-4
CTLA-4
CD80
CD80
APC

FIG. 2

FIG. 3A


No Immune Response
Tumor cell
MHC
TCR
PD-L1
CD28
PD-1
T cell
PD-L1-dependent
CD28 Agonist vIgD
Fc
CD80
Immune Response
Tumor cell
MHC
TCR
PD-L1
PD-1
CD28
T cell

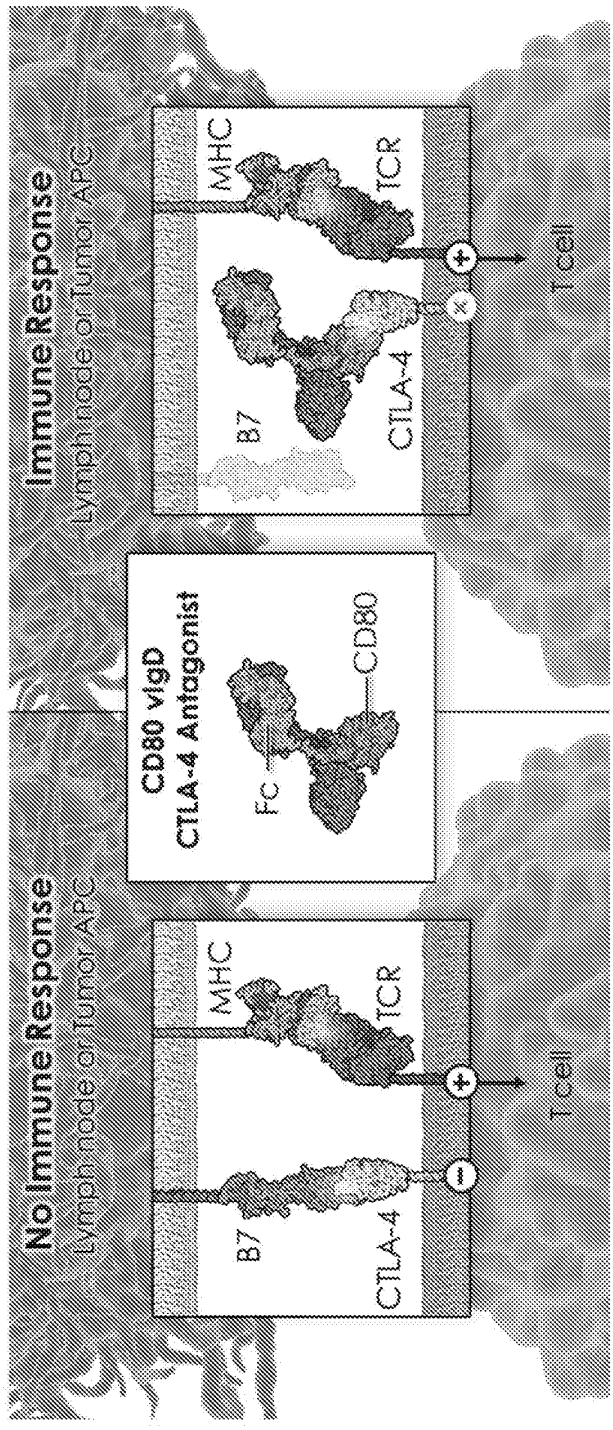

No Immune Response
Lymph node or Tumor APC
MHC
TCR
B7
CTLA-4
T cell
CD80 vIgD
CTLA-4 Antagonist
Fc
CD80
Immune Response
Lymph node or Tumor APC
MHC
TCR
B7
CTLA-4
T cell

FIG. 3B

FIG. 4

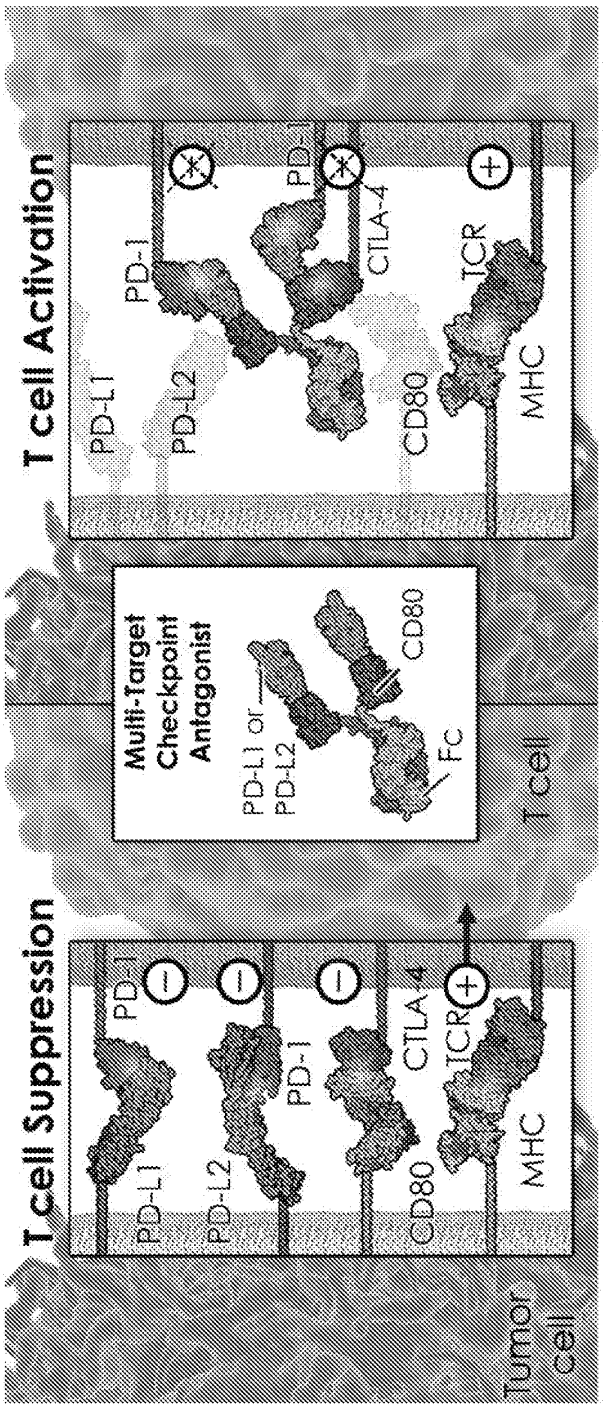

T cell Suppression
PD-L1
PD-1
PD-L2
PD-1
CD80
CTLA-4
TCR
MHC
Tumor cell
Multi-Target Checkpoint Antagonist
PD-L1 or PD-L2
CD80
Fc
T cell
T cell Activation
PD-L1
PD-1
PD-L2
PD-1
CTLA-4
CD80
TCR
MHC

FIG. 5

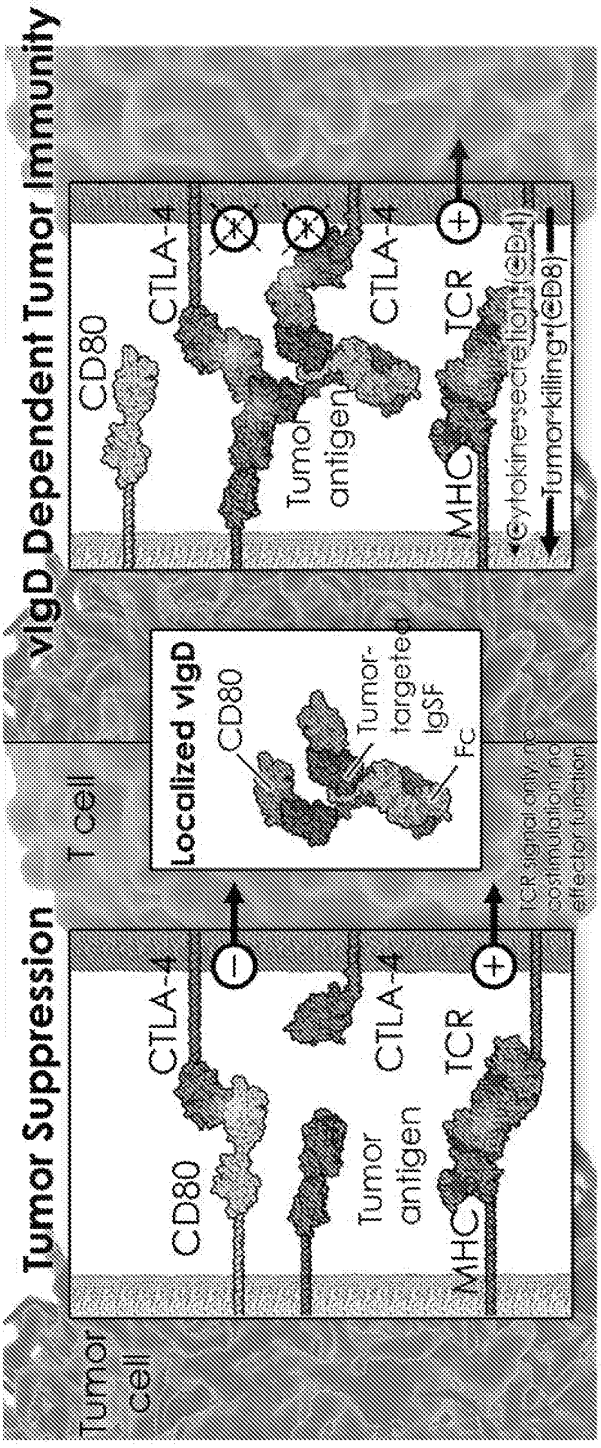
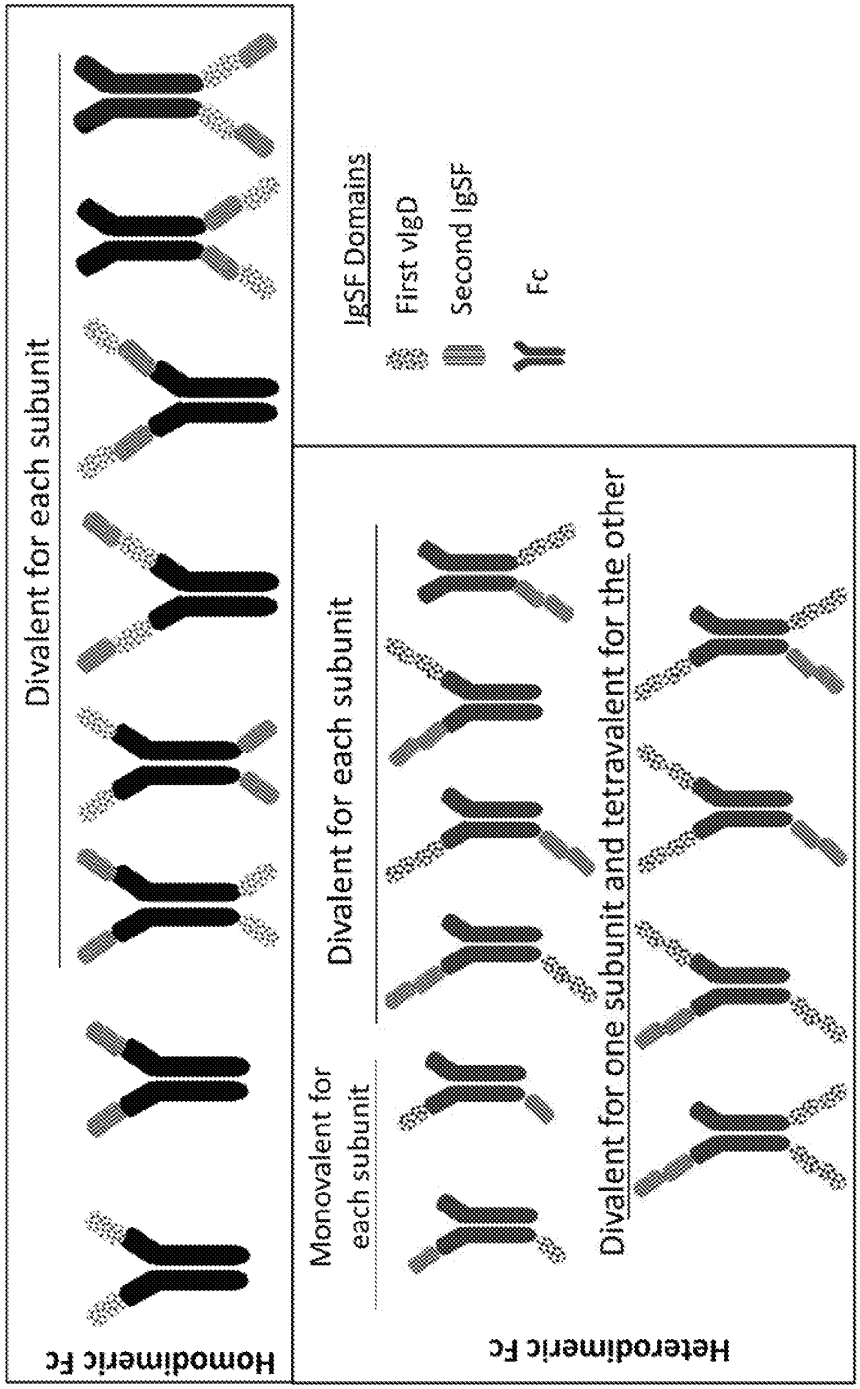

Tumor Suppression
vIgD Dependent Tumor Immunity
Tumor cell
T cell
CD80
CTLA-4
Tumor antigen
MHC
TCR
Localized vIgD
Tumor-targeted IgSF
Fc
TCR signal only or costimulation and effector function
cytokine secretion (CD4)
Tumor killing (CD8)

FIG. 6A


Homodimeric Fc
Divalent for each subunit
Heterodimeric Fc
Monovalent for each subunit
Divalent for each subunit
Divalent for one subunit and tetravalent for the other
IgSF Domains
First vIgD
Second IgSF
Fc

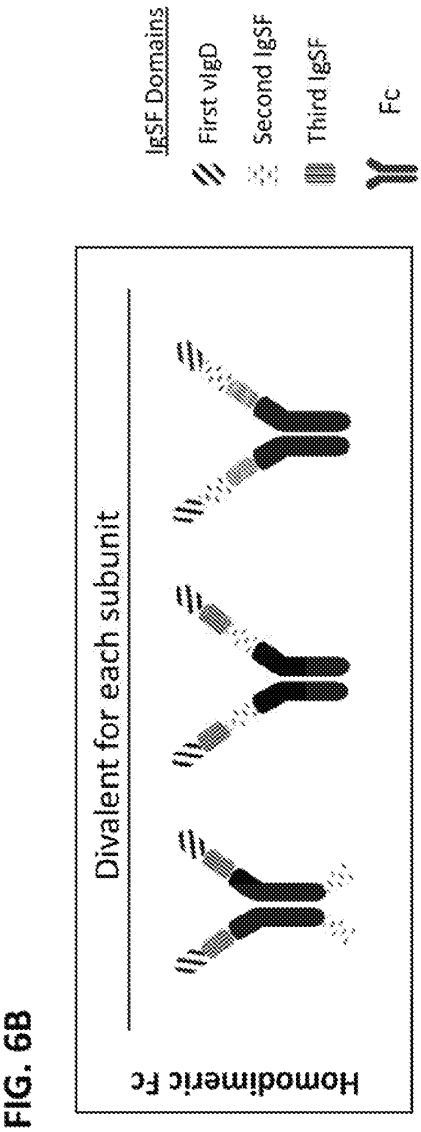
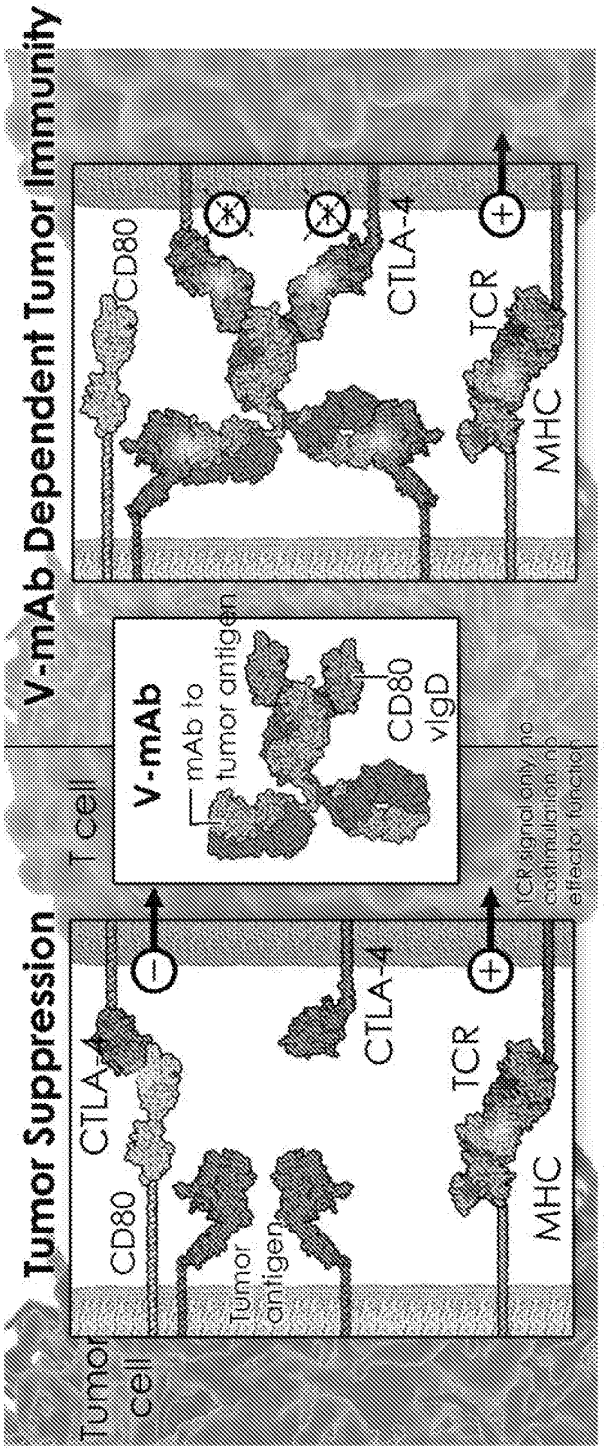

IgSF Domains
First vIgD
Second IgSF
Third IgSF
Fc
Homodimeric Fc
Divalent for each subunit

FIG. 6B

FIG. 7


Tumor Suppression
Tumor cell
CD80
CTLA-4
Tumor antigen
MHC
TCR
T cell
CTLA-4
TCR
MHC
V-mAb
mAb to tumor antigen
CD80 vIgD
TCR signal only, no costimulation, no effector function
V-mAb Dependent Tumor Immunity
CD80
CTLA-4
TCR
MHC

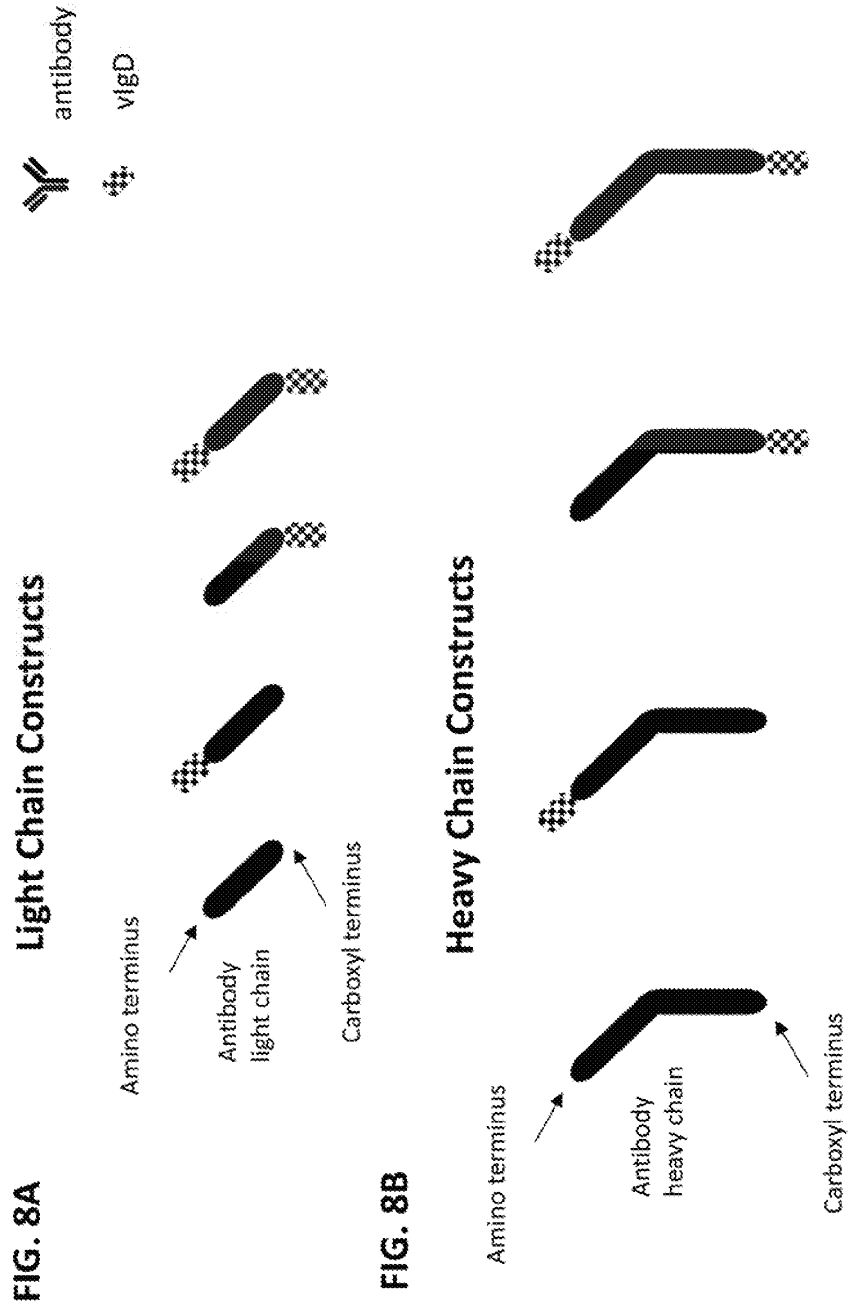

antibody
vIgD
Light Chain Constructs
Amino terminus
Antibody light chain
Carboxyl terminus
Heavy Chain Constructs
Amino terminus
Antibody heavy chain
Carboxyl terminus

FIG. 8A

FIG. 8B

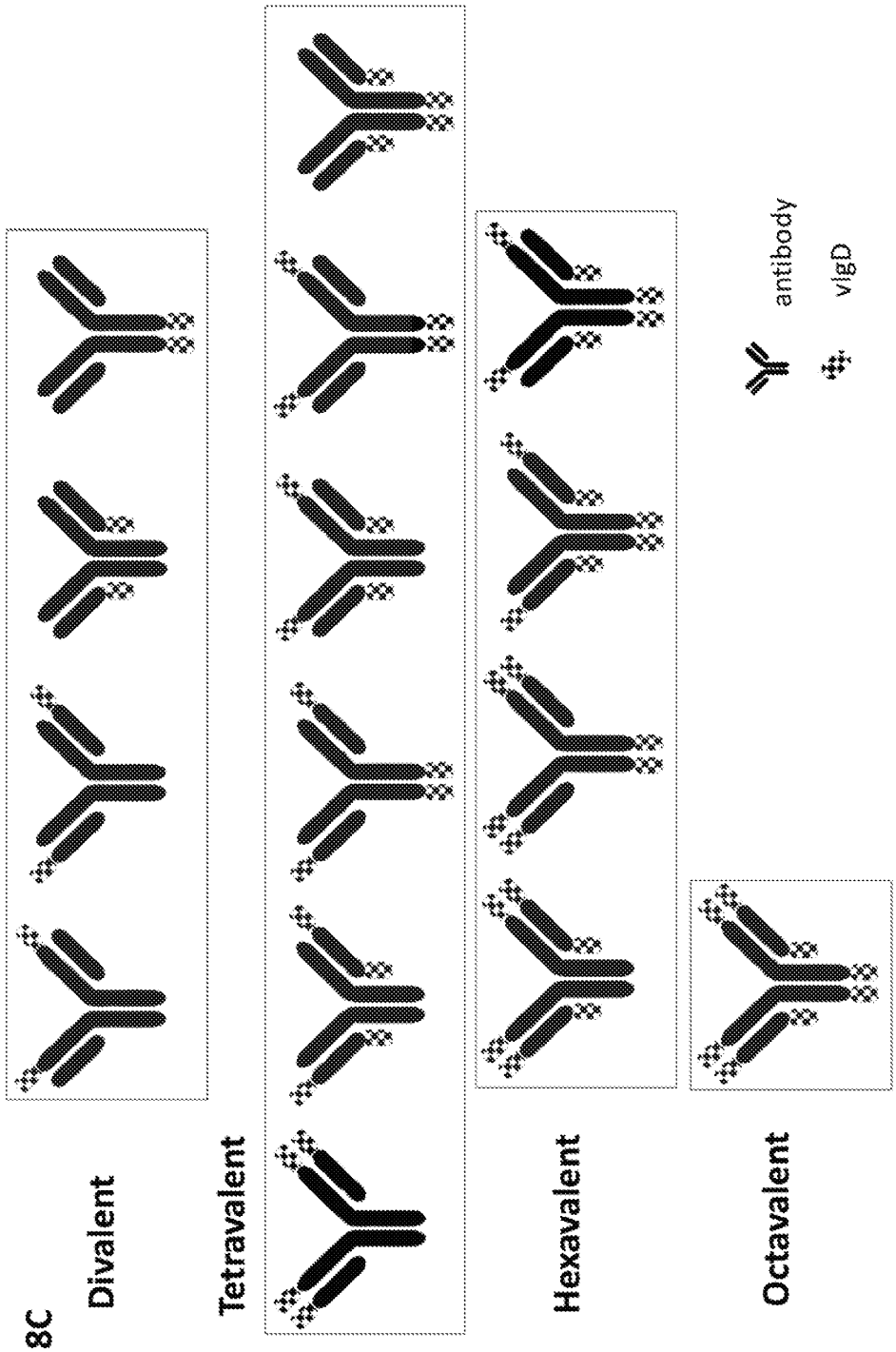

Divalent
Tetravalent
Hexavalent
Octavalent
antibody
vIgD

FIG. 8C

FIG. 9A

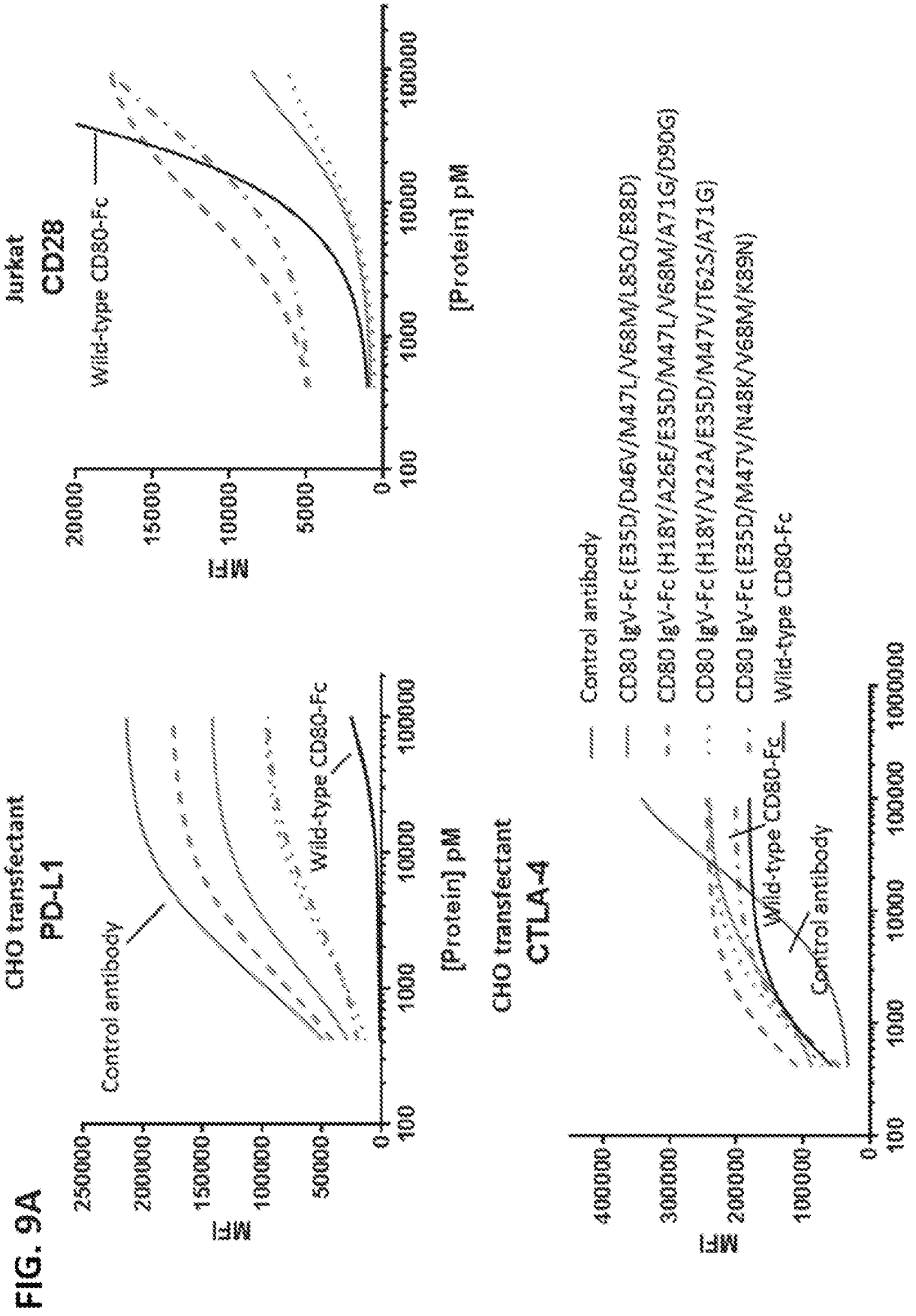

CHO transfectant
PD-L1
Control antibody
Wild-type CD80-Fc
MFI
[Protein] pM
Jurkat
CD28
Wild-type CD80-Fc
MFI
[Protein] pM
CHO transfectant
CTLA-4
Control antibody
CD80 IgV-Fc (E35D/D46V/M47L/V68M/L85Q/E88D)
CD80 IgV-Fc (H18Y/A26E/E35D/M47L/V68M/A71G/D90G)
CD80 IgV-Fc (H18Y/V22A/E35D/M47V/T62S/A71G)
CD80 IgV-Fc (E35D/M47V/N48K/V68M/K89N)
Wild-type CD80-Fc
Wild-type CD80-Fc
Control antibody
MFI
[Protein] pM

FIG. 9B

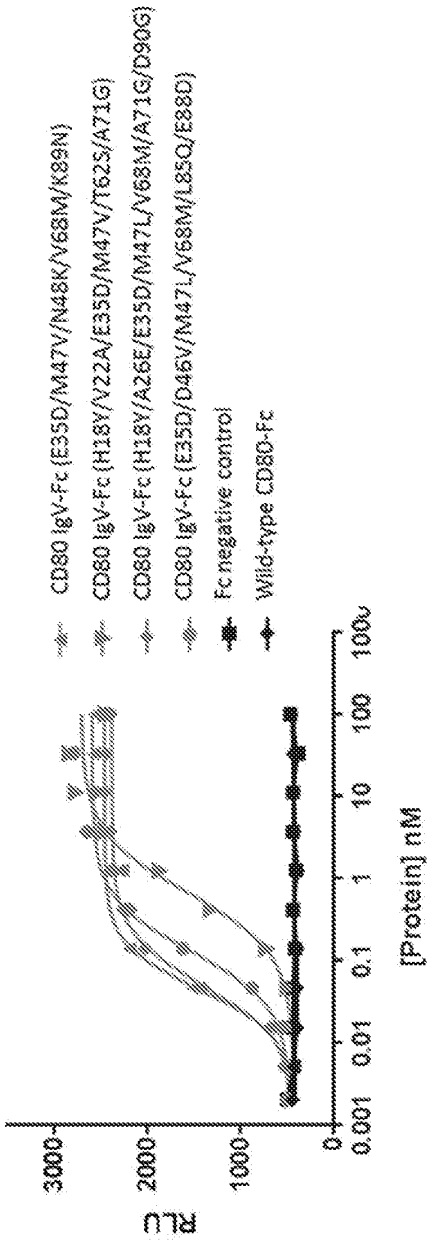

CD80 IgV-Fc (E35D/M47V/N48K/V68M/K89N)
CD80 IgV-Fc (H18Y/V22A/E35D/M47V/T62S/A71G)
CD80 IgV-Fc (H18Y/A26E/E35D/M47L/V68M/A71G/D90G)
CD80 IgV-Fc (E35D/D46V/M47L/V68M/I83Q/E88D)
Fc negative control
Wild-type CD80-Fc
RLU
3000
2000
1000
0
0.001
0.01
0.1
1
10
100
1000
[Protein] nM

FIG. 9C

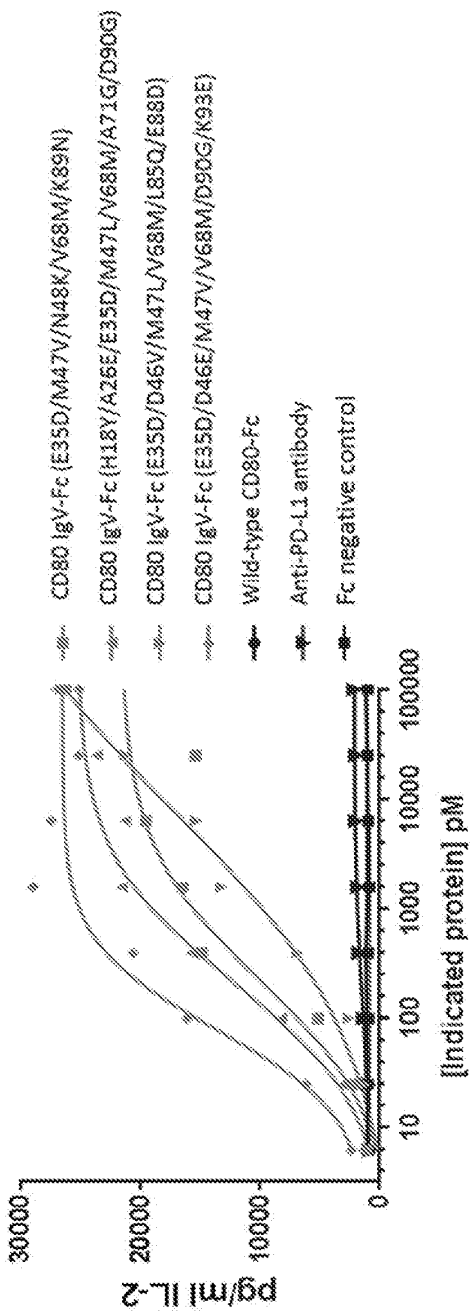

pg/ml IL-2
30000
20000
10000
0
10
100
1000
10000
100000
[Indicated protein] pM
CD80 IgV-Fc (E35D/M47V/N48K/V68M/K89N)
CD80 IgV-Fc (H18Y/A25E/E35D/M47L/V68M/A71G/D90G)
CD80 IgV-Fc (E35D/D46V/M47L/V68M/L85Q/E88D)
CD80 IgV-Fc (E35D/D46E/M47V/V68M/D90G/K93E)
Wild-type CD80-Fc
Anti-PD-L1 antibody
Fc negative control

FIG. 9D

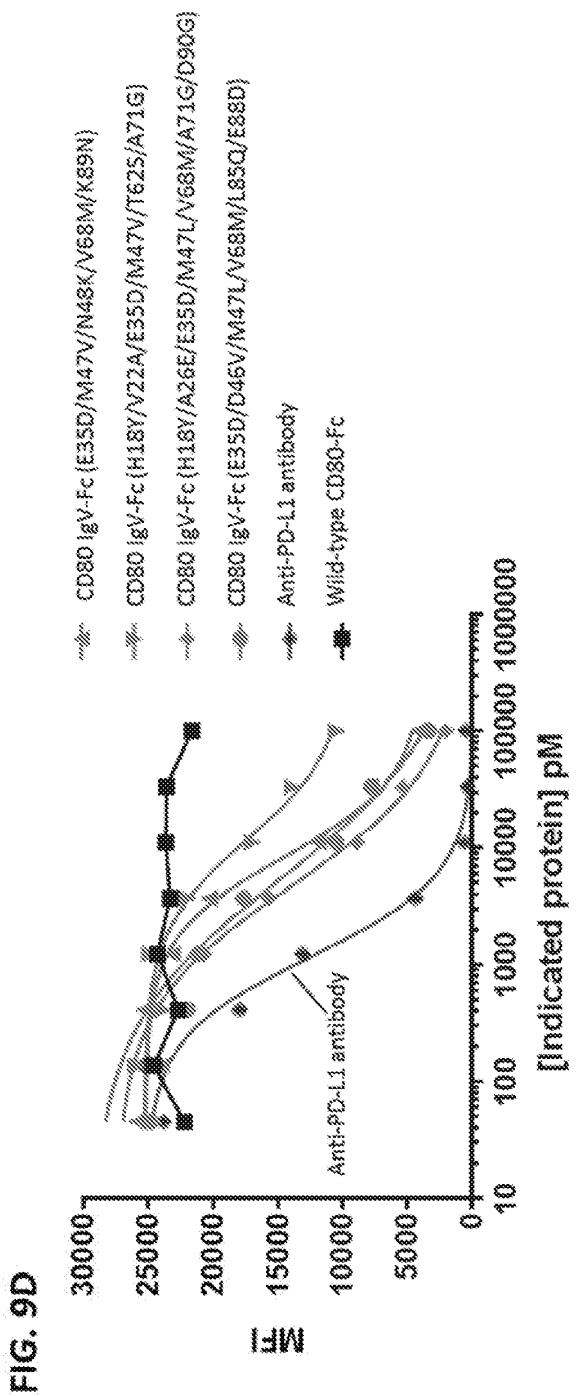
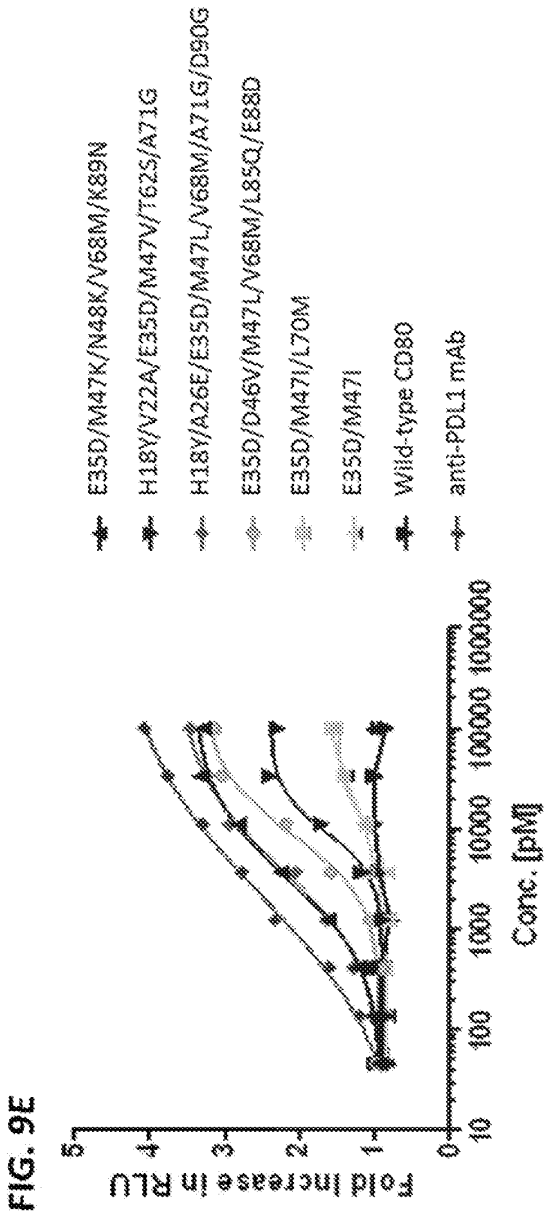

CD80 IgV-Fc (E35D/M47V/N48K/V68M/K89N)
CD80 IgV-Fc (H18Y/V22A/E35D/M47V/T62S/A71G)
CD80 IgV-Fc (H18Y/A26E/E35D/M47L/V68M/A71G/D90G)
CD80 IgV-Fc (E35D/D46V/M47L/V68M/L85Q/E88D)
Anti-PD-L1 antibody
Wild-type CD80-Fc
MFI
30000
25000
20000
15000
10000
5000
0
10
100
1000
10000
100000
1000000
[Indicated protein] pM
Anti-PD-L1 antibody

FIG. 9E


E35D/M47K/N48K/V68M/K89N
H18Y/V22A/E35D/M47V/T62S/A71G
H18Y/A26E/E35D/M47L/V68M/A71G/D90G
E35D/D46V/M47L/V68M/L85Q/E88D
E35D/M47I/L70M
E35D/M47I
Wild-type CD80
anti-PDL1 mAb
Fold increase in RLU
0
1
2
3
4
5
10
100
1000
10000
100000
1000000
Conc. [pM]

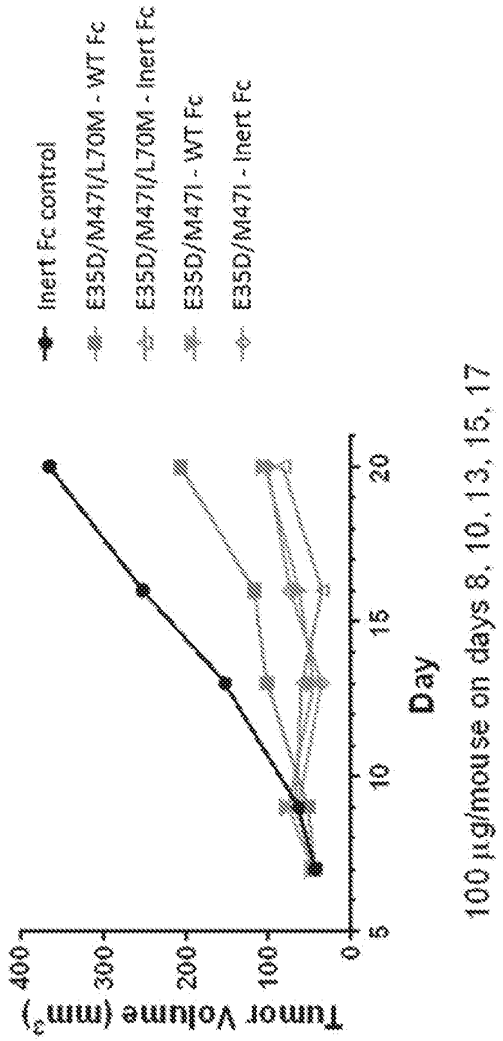

Inert Fc control
E35D/M471/L70M - WT Fc
E35D/M471/L70M - Inert Fc
E35D/M471 - WT Fc
E35D/M471 - Inert Fc
Tumor Volume (mm³)
400
300
200
100
0
5
10
15
20
Day
100 µg/mouse on days 8, 10, 13, 15, 17

FIG. 10

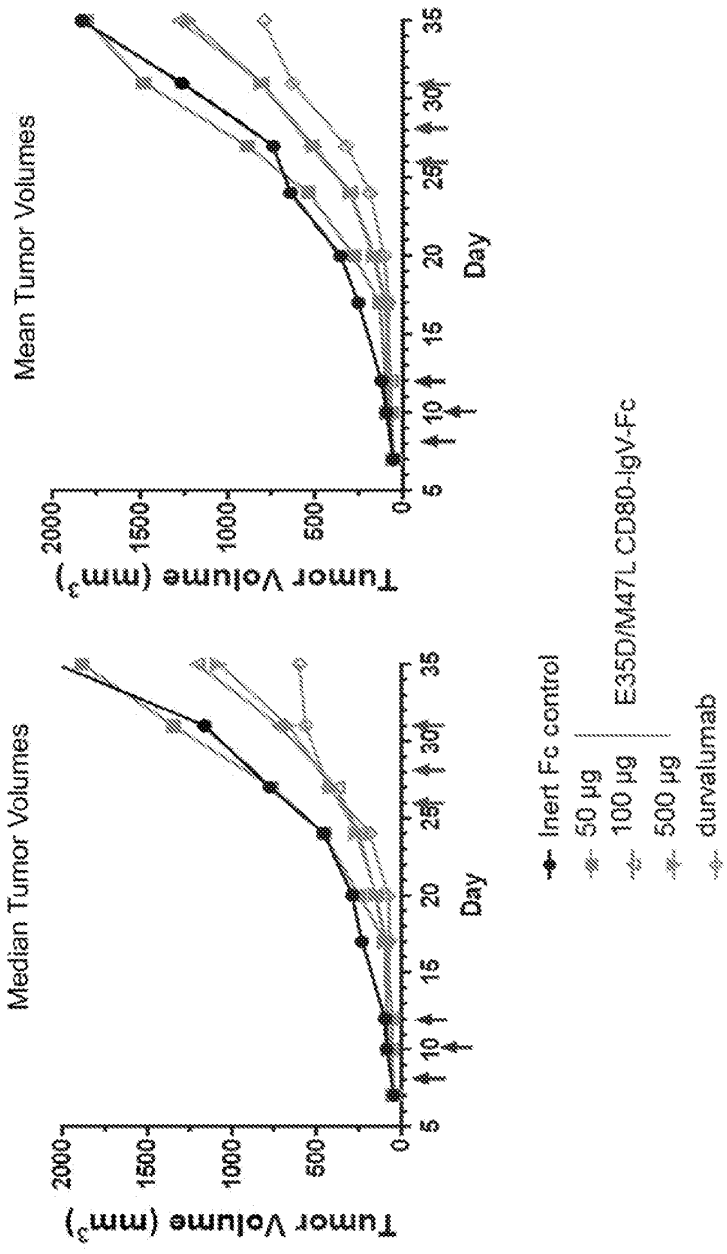
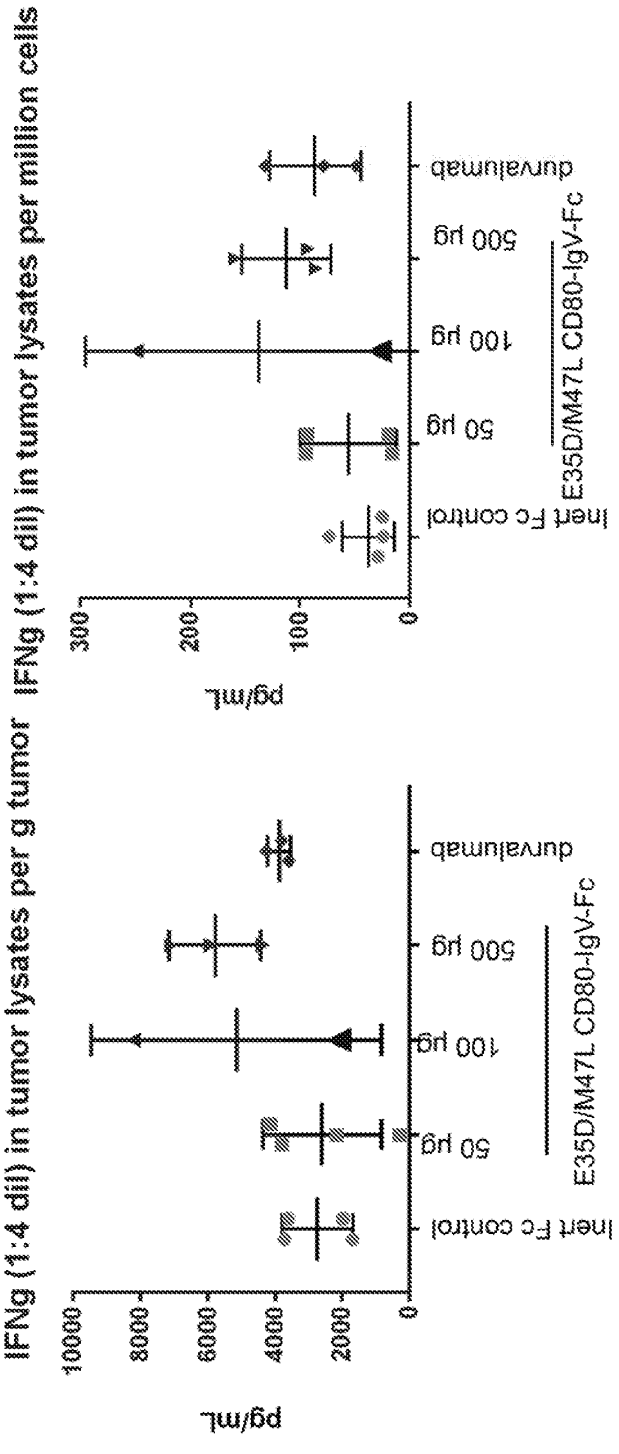

Median Tumor Volumes
Mean Tumor Volumes
Tumor Volume (mm³)
Day
0
500
1000
1500
2000
5
10
15
20
25
30
35
Inert Fc control
50 µg
100 µg
500 µg
E35D/M47L CD80-IgV-Fc
durvalumab

FIG. 11

FIG. 12


IFNg (1:4 dil) in tumor lysates per g tumor
pg/mL
10000
8000
6000
4000
2000
0
Inert Fc control
50 µg
100 µg
500 µg
durvalumab
E35D/M47L CD80-IgV-Fc
IFNg (1:4 dil) in tumor lysates per million cells
pg/mL
300
200
100
0
Inert Fc control
50 µg
100 µg
500 µg
durvalumab
E35D/M47L CD80-IgV-Fc

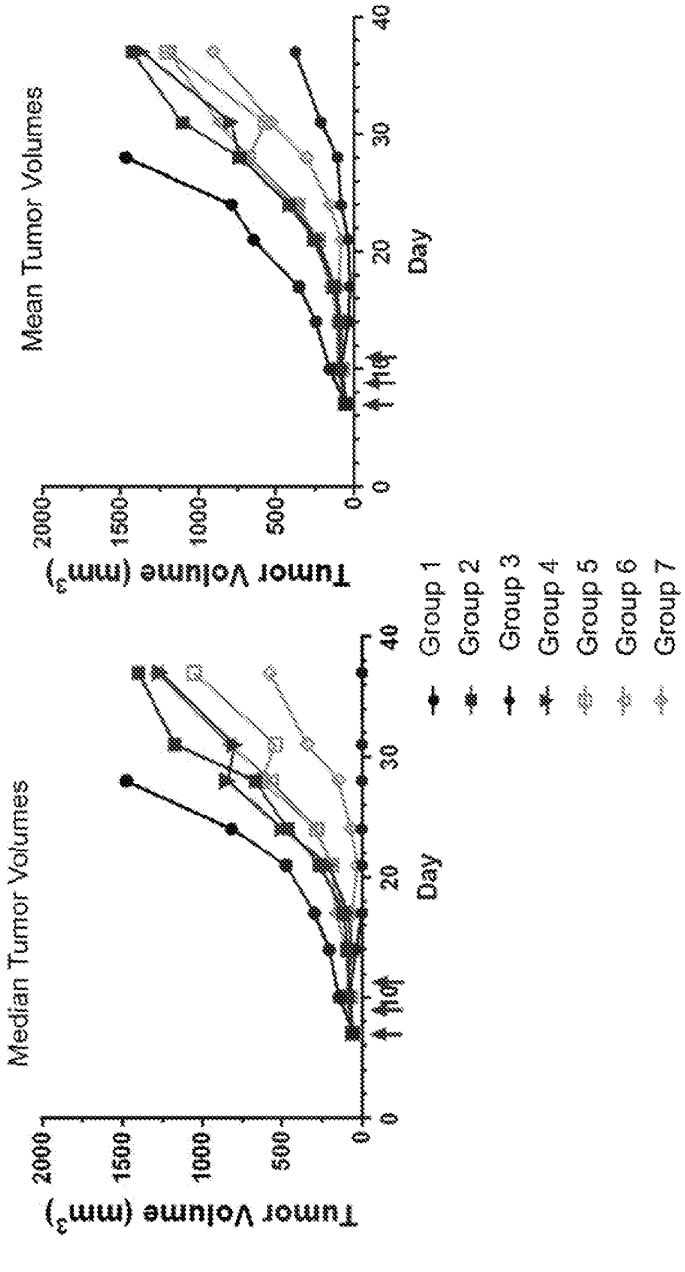
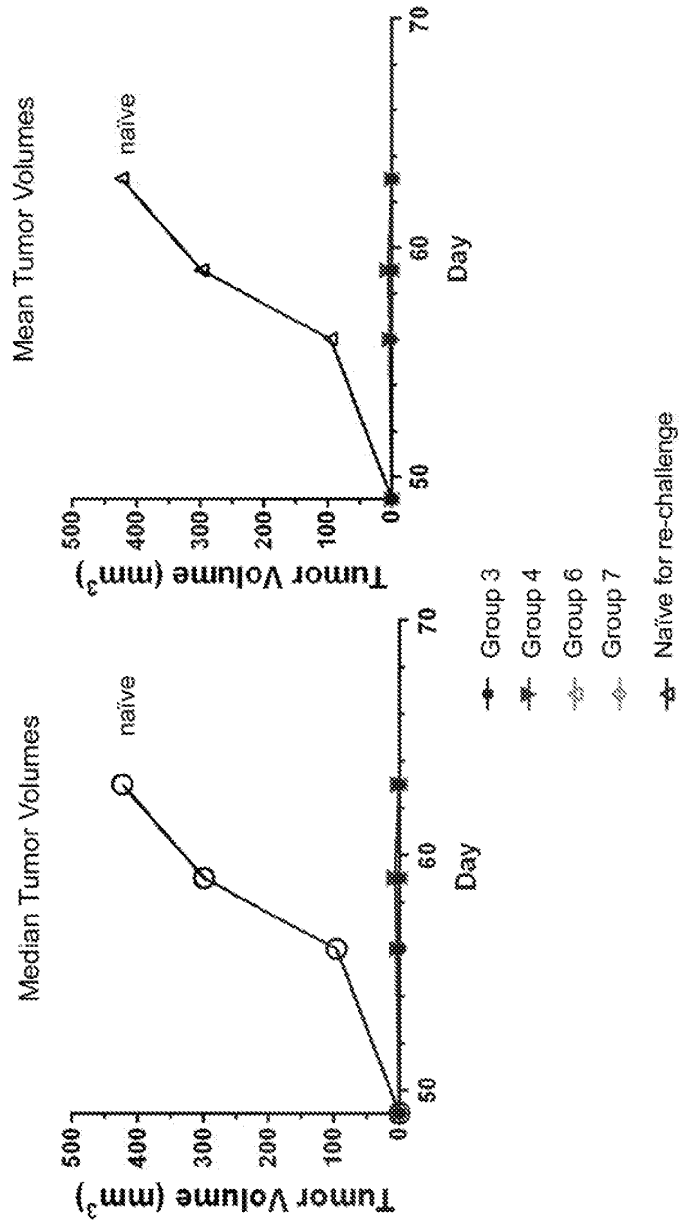

Median Tumor Volumes
Tumor Volume (mm³)
2000
1500
1000
500
0
0
10
20
30
40
Day
Mean Tumor Volumes
Tumor Volume (mm³)
2000
1500
1000
500
0
0
10
20
30
40
Day
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7

FIG. 13

FIG. 14


Median Tumor Volumes
Tumor Volume (mm³)
500
400
300
200
100
0
50
60
70
Day
naïve
Mean Tumor Volumes
Tumor Volume (mm³)
500
400
300
200
100
0
50
60
70
Day
naïve
Group 3
Group 4
Group 6
Group 7
Naïve for re-challenge

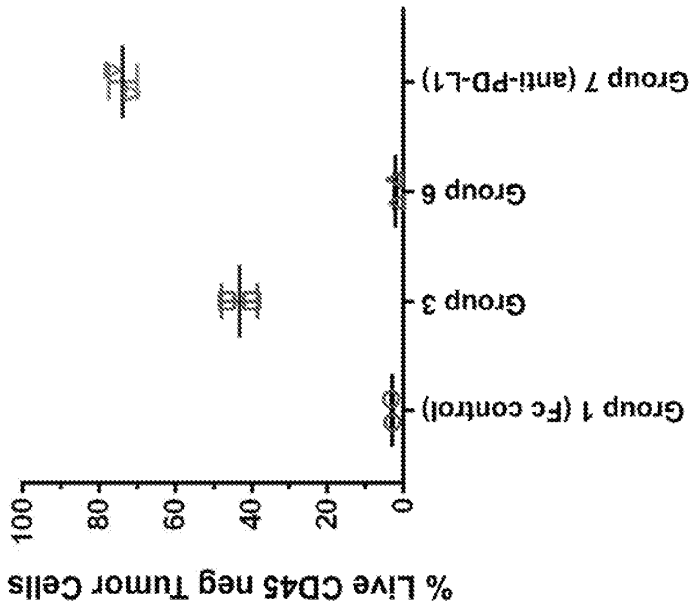

% Live CD45 neg Tumor Cells
100
80
60
40
20
0
Group 1 (Fc control)
Group 3
Group 6
Group 7 (anti-PD-L1)

FIG. 15

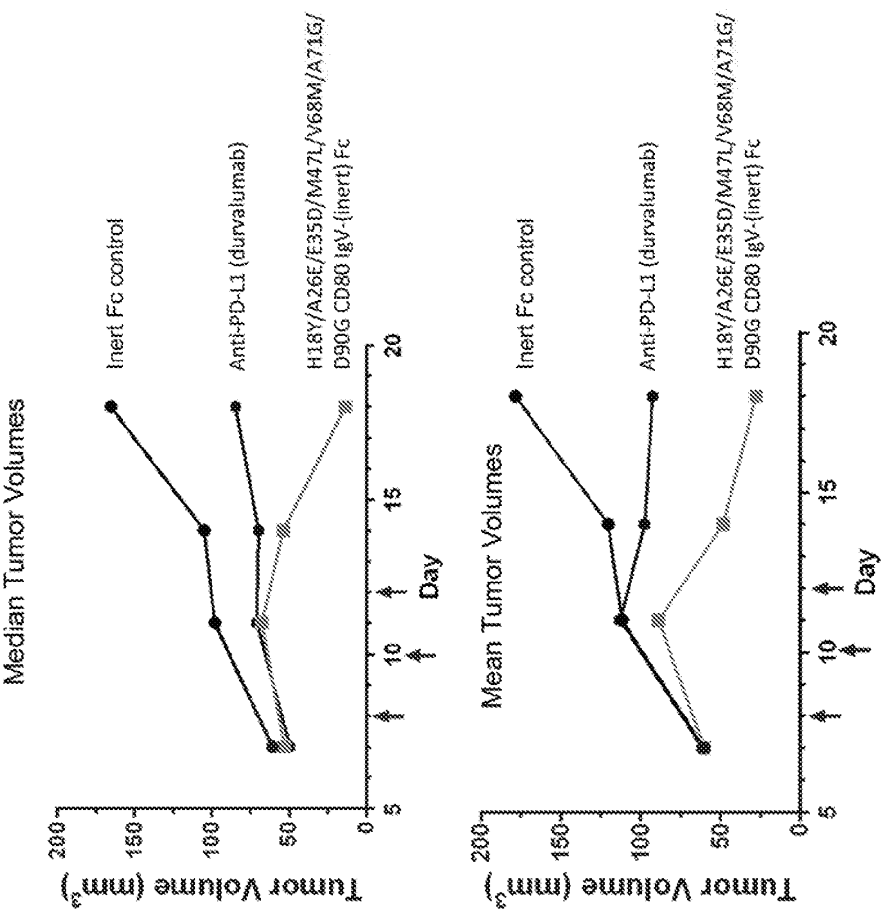

Median Tumor Volumes
Tumor Volume (mm³)
200
150
100
50
0
5
10
15
20
Day
Inert Fc control
Anti-PD-L1 (durvalumab)
H18Y/A26E/E35D/M47L/V68M/A71G/
D90G CD80 IgV-(inert) Fc
Mean Tumor Volumes
Tumor Volume (mm³)
200
150
100
50
0
5
10
15
20
Day
Inert Fc control
Anti-PD-L1 (durvalumab)
H18Y/A26E/E35D/M47L/V68M/A71G/
D90G CD80 IgV-(inert) Fc

FIG. 16

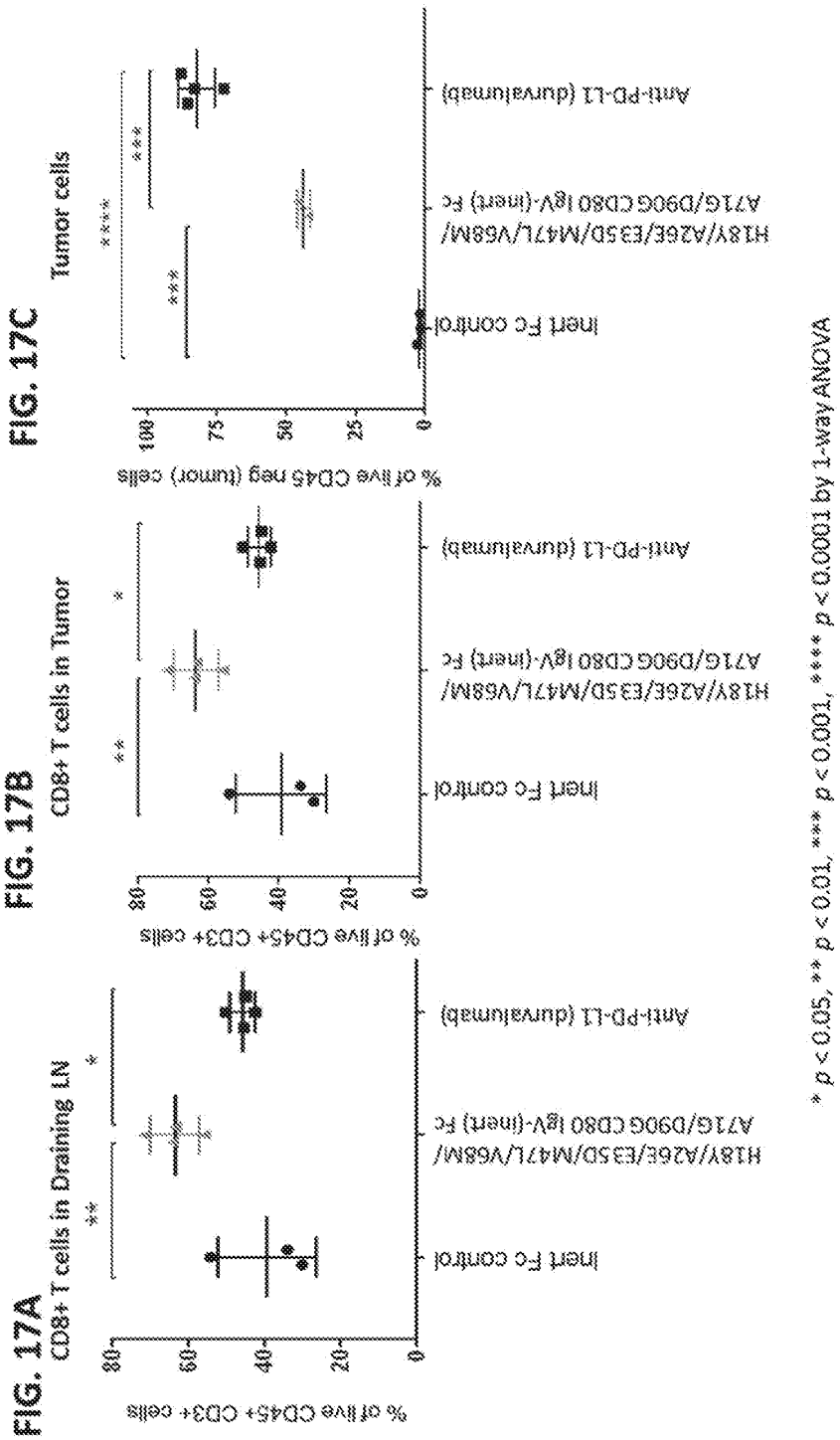


FIG. 17A
CD8+ T cells in Draining LN
% of live CD45+ CD3+ cells
0
20
40
60
80
**
*
Inert Fc control
H18Y/A26E/E35D/M47L/V68M/
A71G/D90G CD80 IgV-(inert) Fc
Anti-PD-L1 (durvalumab)
FIG. 17B
CD8+ T cells in Tumor
% of live CD45+ CD3+ cells
0
20
40
60
80
**
*
Inert Fc control
H18Y/A26E/E35D/M47L/V68M/
A71G/D90G CD80 IgV-(inert) Fc
Anti-PD-L1 (durvalumab)
FIG. 17C
Tumor cells
% of live CD45 neg (tumor) cells
0
25
50
75
100
****
***
***
Inert Fc control
H18Y/A26E/E35D/M47L/V68M/
A71G/D90G CD80 IgV-(inert) Fc
Anti-PD-L1 (durvalumab)
* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 by 1-way ANOVA

FIG. 18

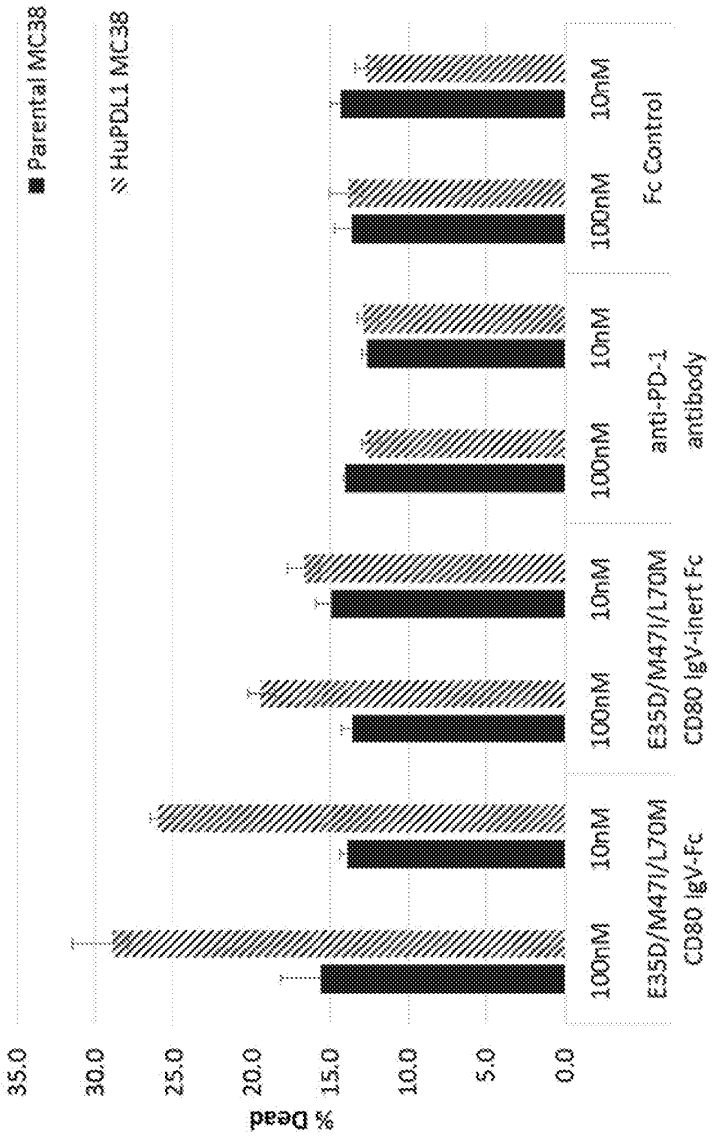

Parental MC38
HuPDL1 MC38
% Dead
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
100nM
10nM
E35D/M471/L70M
CD80 IgV-Fc
100nM
10nM
E35D/M471/L70M
CD80 IgV-inert Fc
100nM
10nM
anti-PD-1
antibody
100nM
10nM
Fc Control

FIG. 19A

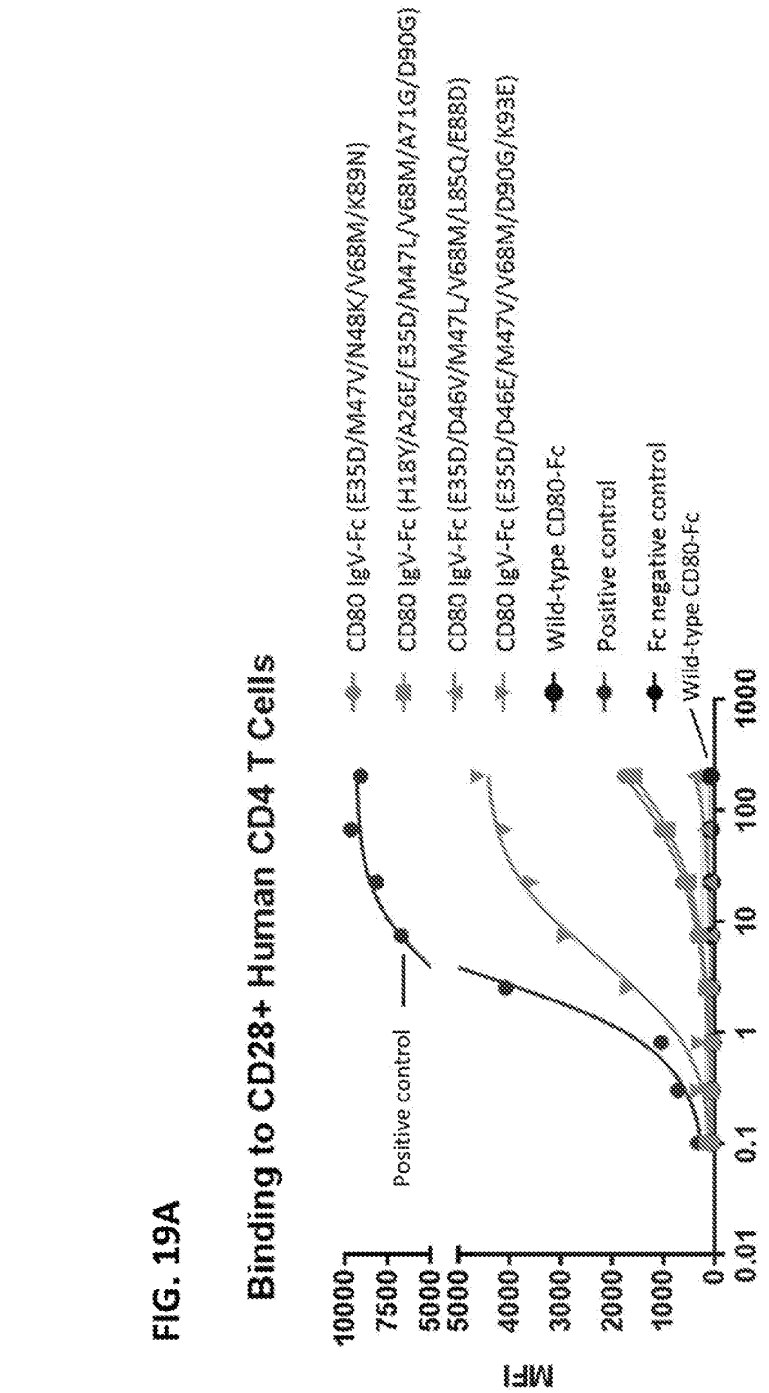

Binding to CD28+ Human CD4 T Cells
MFI
10000
7500
5000
5000
4000
3000
2000
1000
0
0.01
0.1
1
10
100
1000
[Indicated protein] nM
Positive control
CD80 IgV-Fc (E35D/M47V/N48K/V68M/K89N)
CD80 IgV-Fc (H18Y/A26E/E35D/M47L/V68M/A71G/D90G)
CD80 IgV-Fc (E35D/D46V/M47L/V68M/L85Q/E88D)
CD80 IgV-Fc (E35D/D46E/M47V/V68M/D90G/K93E)
Wild-type CD80-Fc
Positive control
Fc negative control
Wild-type CD80-Fc

FIG. 19B

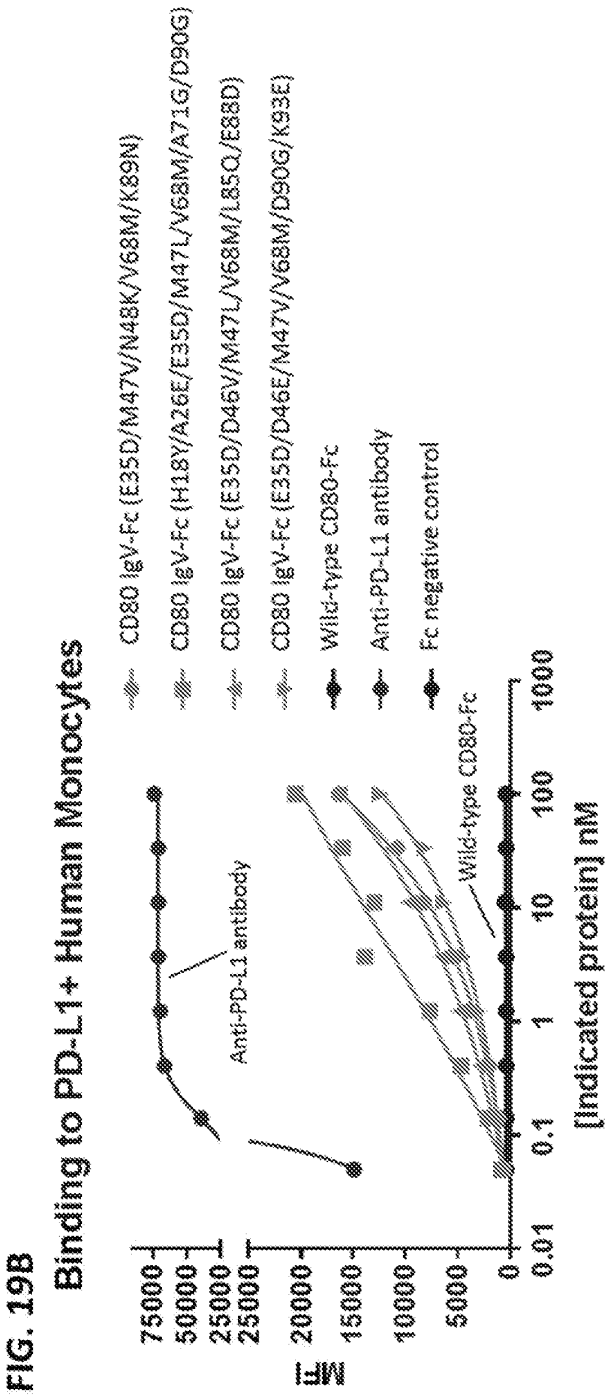

Binding to PD-L1+ Human Monocytes
CD80 IgV-Fc (E35D/M47V/N48K/V68M/K89N)
CD80 IgV-Fc (H18Y/A26E/E35D/M47L/V68M/A71G/D90G)
CD80 IgV-Fc (E35D/D46V/M47L/V68M/L85Q/E88D)
CD80 IgV-Fc (E35D/D46E/M47V/V68M/D90G/K93E)
Wild-type CD80-Fc
Anti-PD-L1 antibody
Fc negative control
MFI
75000
50000
25000
25000
20000
15000
10000
5000
0
0.01
0.1
1
10
100
1000
[Indicated protein] nM
Anti-PD-L1 antibody
Wild-type CD80-Fc

FIG. 20

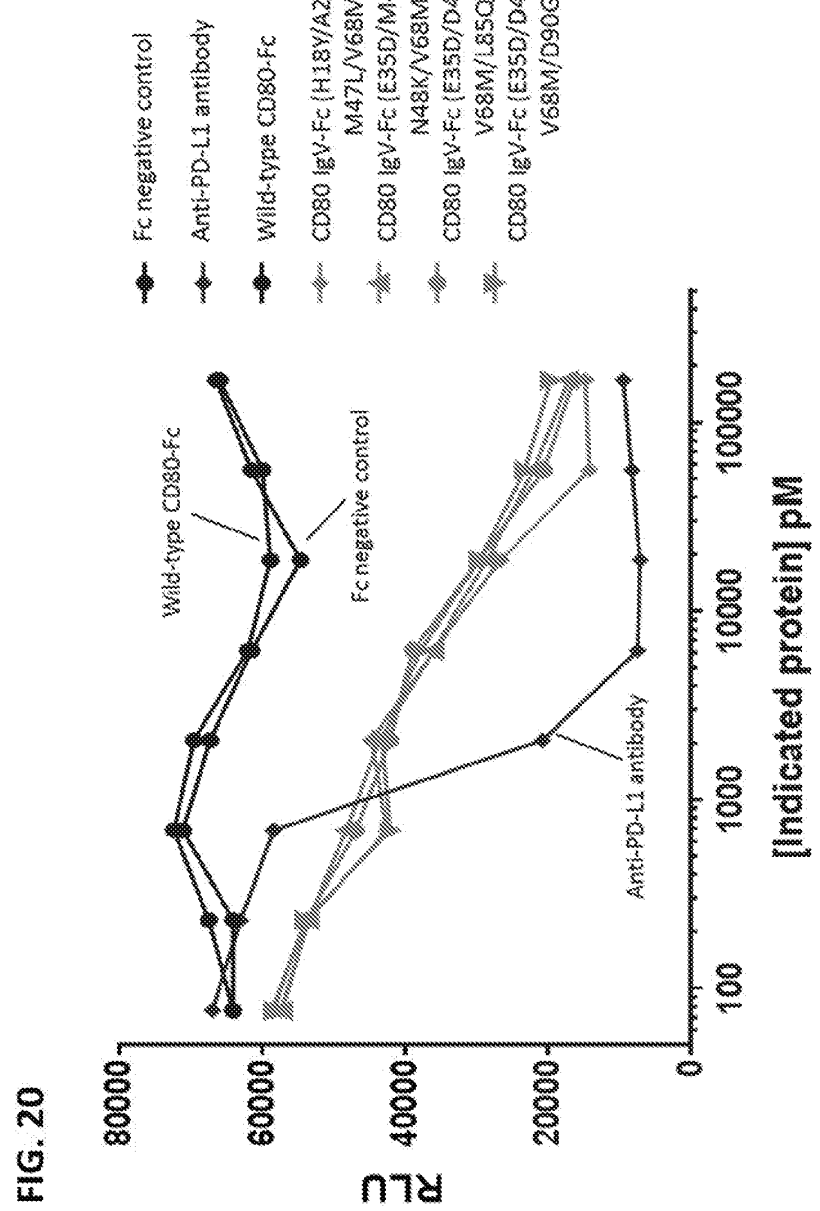
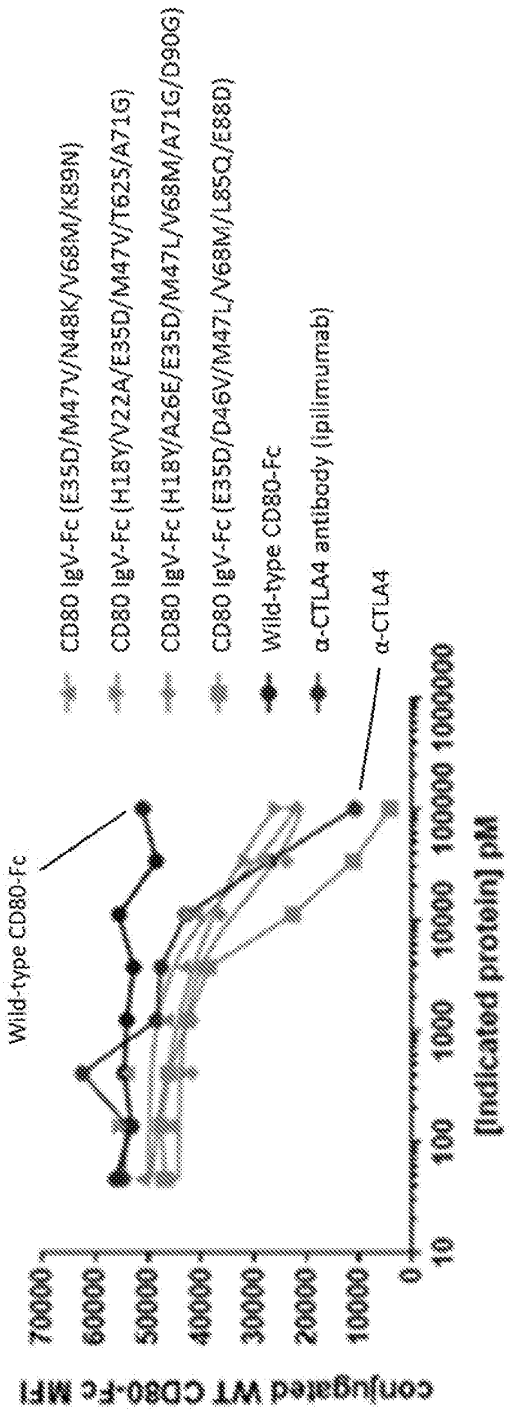

Fc negative control
Anti-PD-L1 antibody
Wild-type CD80-Fc
CD80 IgV-Fc (H18Y/A26E/E35D/M47L/V68M/A71G/D90G)
CD80 IgV-Fc (E35D/M47V/N48K/V68M/K89N)
CD80 IgV-Fc (E35D/D46V/M47L/V68M/L85Q/E88D)
CD80 IgV-Fc (E35D/D46E/M47V/V68M/D90G/K93E)
Wild-type CD80-Fc
Fc negative control
Anti-PD-L1 antibody
RLU
80000
60000
40000
20000
0
100
1000
10000
100000
[Indicated protein] pM

FIG. 21


conjugated WT CD80-Fc MFI
70000
60000
50000
40000
30000
20000
10000
0
10
100
1000
10000
100000
1000000
[Indicated protein] pM
Wild-type CD80-Fc
α-CTLA4
CD80 IgV-Fc (E35D/M47V/N48K/V68M/K89W)
CD80 IgV-Fc (H18Y/V22A/E35D/M47V/T62S/A71G)
CD80 IgV-Fc (H18Y/A26E/E35D/M47L/V68M/A71G/D90G)
CD80 IgV-Fc (E35D/D46V/M47L/V68M/L85Q/E88D)
Wild-type CD80-Fc
α-CTLA4 antibody (ipilimumab)