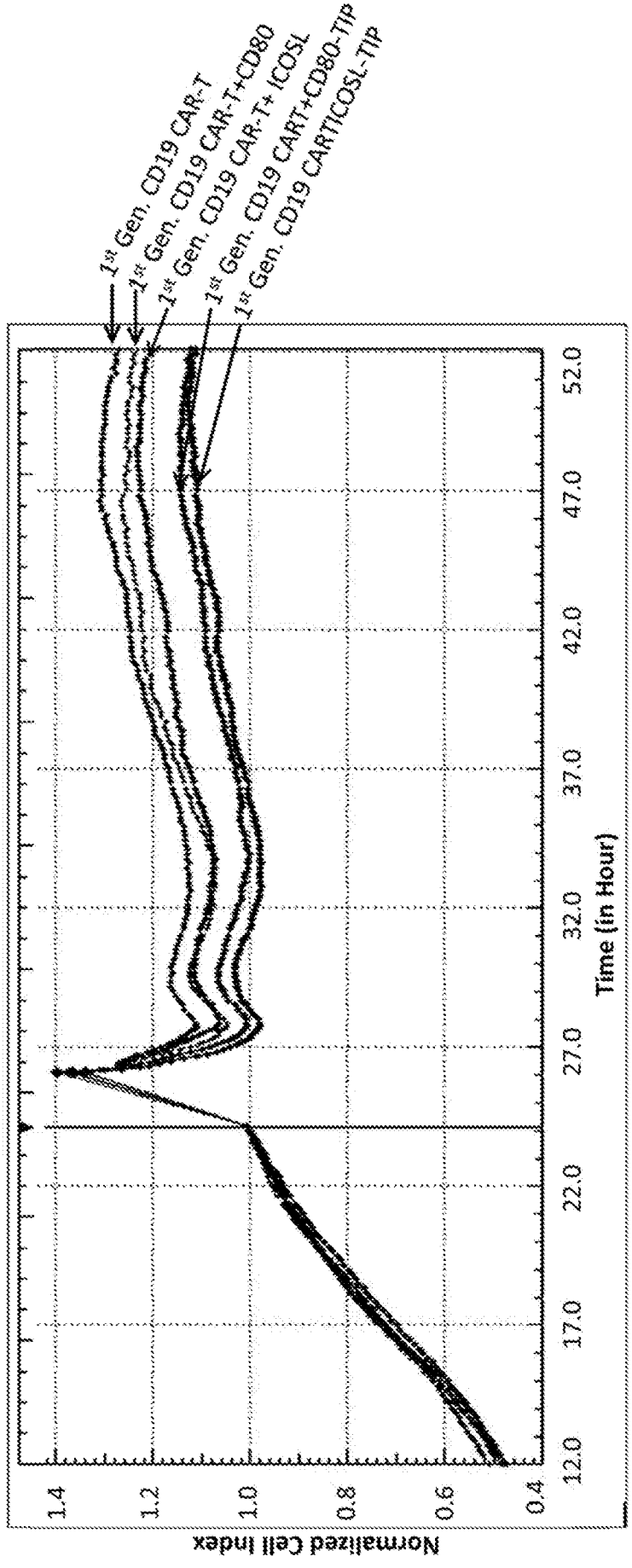

1st Gen. CD19 CAR-T
1st Gen. CD19 CAR-T+CD80
1st Gen. CD19 CAR-T+ ICOSL
1st Gen. CD19 CART+CD80-TIP
1st Gen. CD19 CARTICOSL-TIP
Normalized Cell Index
1.4
1.2
1.0
0.8
0.6
0.4
Time (in Hour)
12.0
17.0
22.0
27.0
32.0
37.0
42.0
47.0
52.0

FIG. 1

FIG. 2A

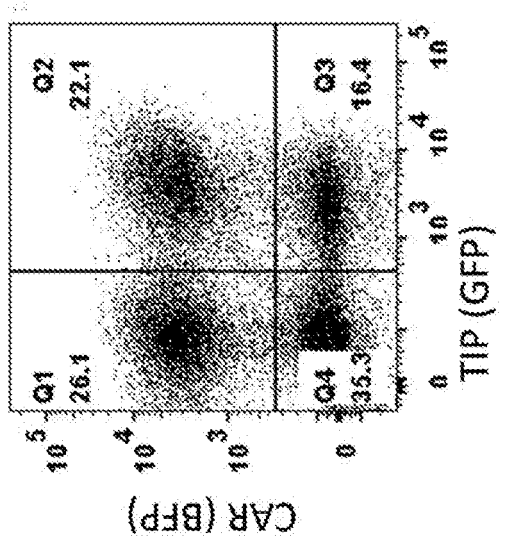
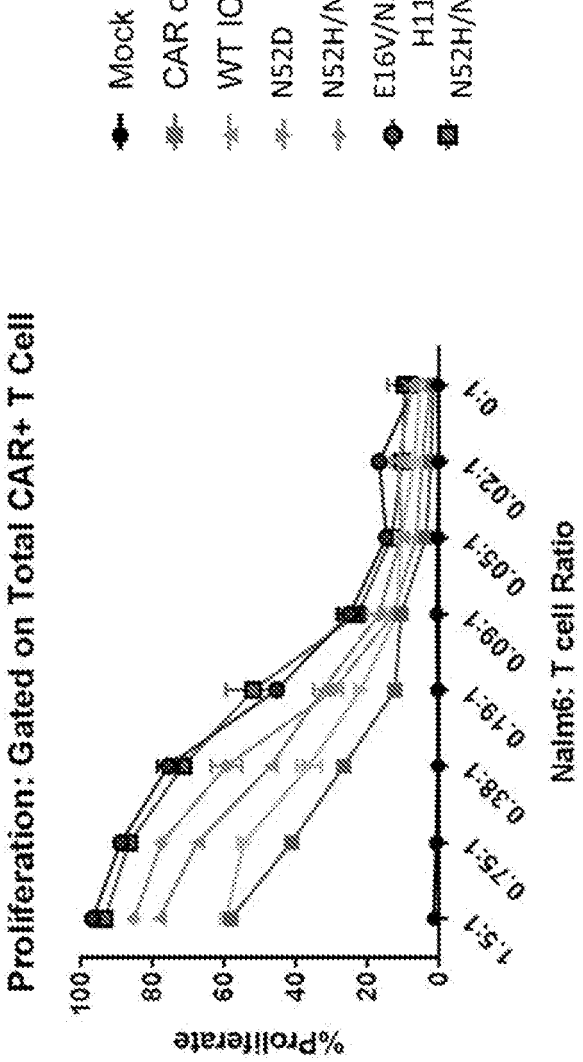

Q1
26.1
Q2
22.1
Q4
35.3
Q3
16.4
CAR (BFP)
TIP (GFP)

FIG. 2B


Proliferation: Gated on Total CAR+ T Cell
Mock
CAR only
WT ICOSL CAR
N52D
N52H/N57Y/Q100P
E16V/N52H/N57Y/Q100R/V110D/
H115R/Y152C/K156M/C198R
N52H/N57Y/Q100R
%Proliferate
Nalm6: T cell Ratio
1:5:1
0.75:1
0.38:1
0.19:1
0.09:1
0.05:1
0.02:1
0:1

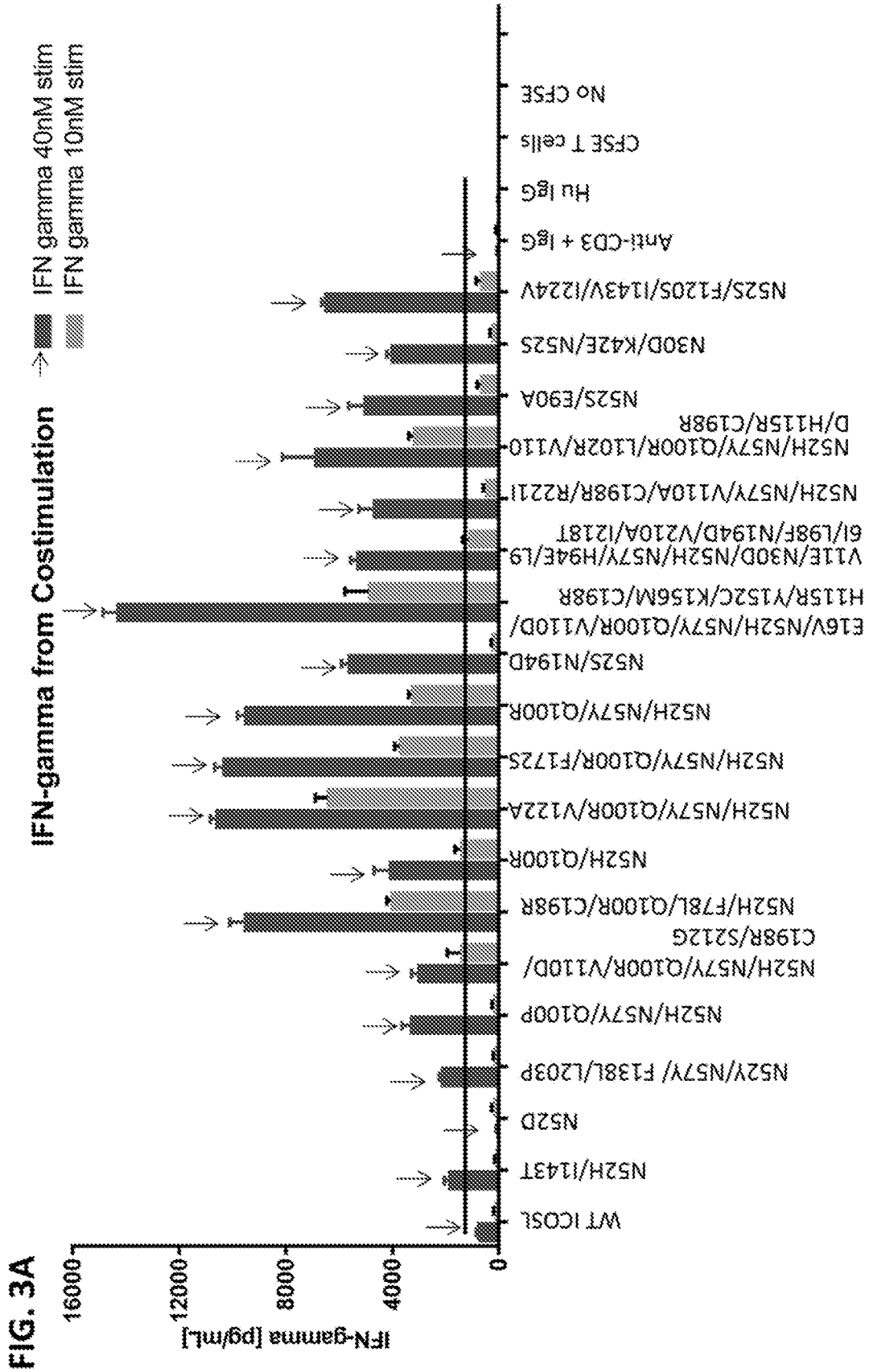

IFN-gamma from Costimulation
IFN gamma 40nM stim
IFN gamma 10nM stim
IFN-gamma [pg/mL]
16000
12000
8000
4000
0
WT ICOSL
N52H/I143T
N52D
N52Y/N57Y/ F138L/L203P
N52H/N57Y/Q100P
N52H/N57Y/Q100R/V110D/
C198R/S212G
N52H/F78L/Q100R/C198R
N52H/Q100R
N52H/N57Y/Q100R/V122A
N52H/N57Y/Q100R/F172S
N52H/N57Y/Q100R
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/
H115R/Y152C/K156M/C198R
V11E/N30D/N52H/N57Y/H94E/L9
6I/L98F/N194D/V210A/I218T
N52H/N57Y/V110A/C198R/R221I
N52H/N57Y/Q100R/L102R/V110
D/H115R/C198R
N52S/E90A
N30D/K42E/N52S
N52S/F120S/I143V/I224V
Anti-CD3 + IgG
Hu IgG
CFSE T cells
No CFSE

FIG. 3A

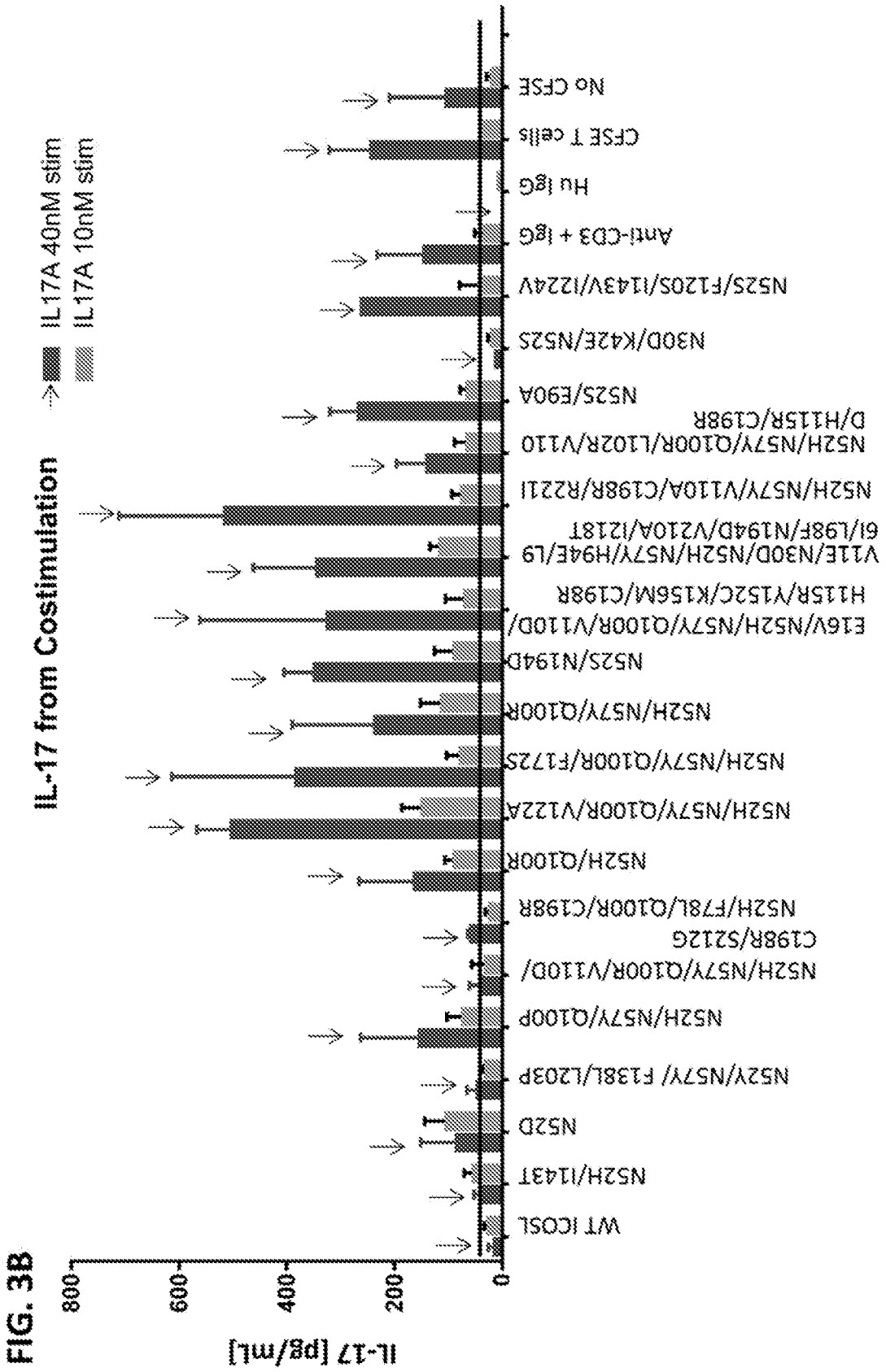

IL-17 from Costimulation
IL17A 40nM stim
IL17A 10nM stim
IL-17 [pg/mL]
0
200
400
600
800
WT ICOSL
N52H/I143T
N52D
N52Y/N57Y/ F138L/L203P
N52H/N57Y/Q100P
N52H/N57Y/Q100R/V110D/
C198R/S212G
N52H/F78L/Q100R/C198R
N52H/Q100R
N52H/N57Y/Q100R/V122A
N52H/N57Y/Q100R/F172S
N52H/N57Y/Q100R
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/
H115R/Y152C/K156M/C198R
V11E/N30D/N52H/N57Y/H94E/L9
6I/L98F/N194D/V210A/I218T
N52H/N57Y/V110A/C198R/R221
N52H/N57Y/Q100R/L102R/V110
D/H115R/C198R
N52S/E90A
N30D/K42E/N52S
N52S/F120S/I143V/I224V
Anti-CD3 + IgG
Hu IgG
CFSE T cells
No CFSE

FIG. 3B

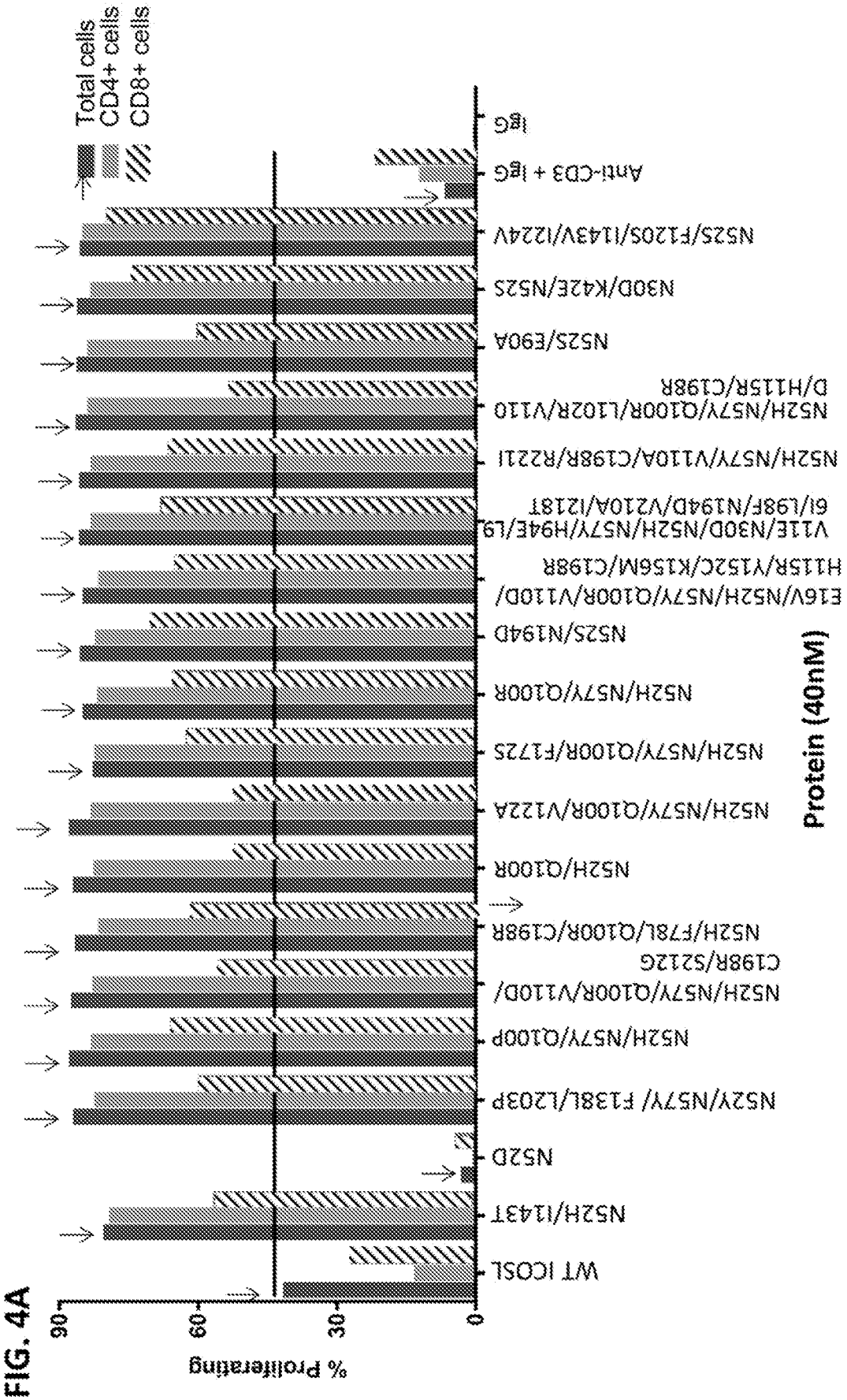

Total cells
CD4+ cells
CD8+ cells
% Proliferating
0
30
60
90
Protein (40nM)
WT ICOSL
N52H/I143T
N52D
N52Y/N57Y/ F138L/L203P
N52H/N57Y/Q100P
N52H/N57Y/Q100R/V110D/
C198R/S212G
N52H/F78L/Q100R/C198R
N52H/Q100R
N52H/N57Y/Q100R/V122A
N52H/N57Y/Q100R/F172S
N52H/N57Y/Q100R
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/
H115R/Y152C/K156M/C198R
V11E/N30D/N52H/N57Y/H94E/L9
6I/L98F/N194D/V210A/I218T
N52H/N57Y/V110A/C198R/R221I
N52H/N57Y/Q100R/L102R/V110
D/H115R/C198R
N52S/E90A
N30D/K42E/N52S
N52S/F120S/I143V/I224V
Anti-CD3 + IgG
IgG

FIG. 4A

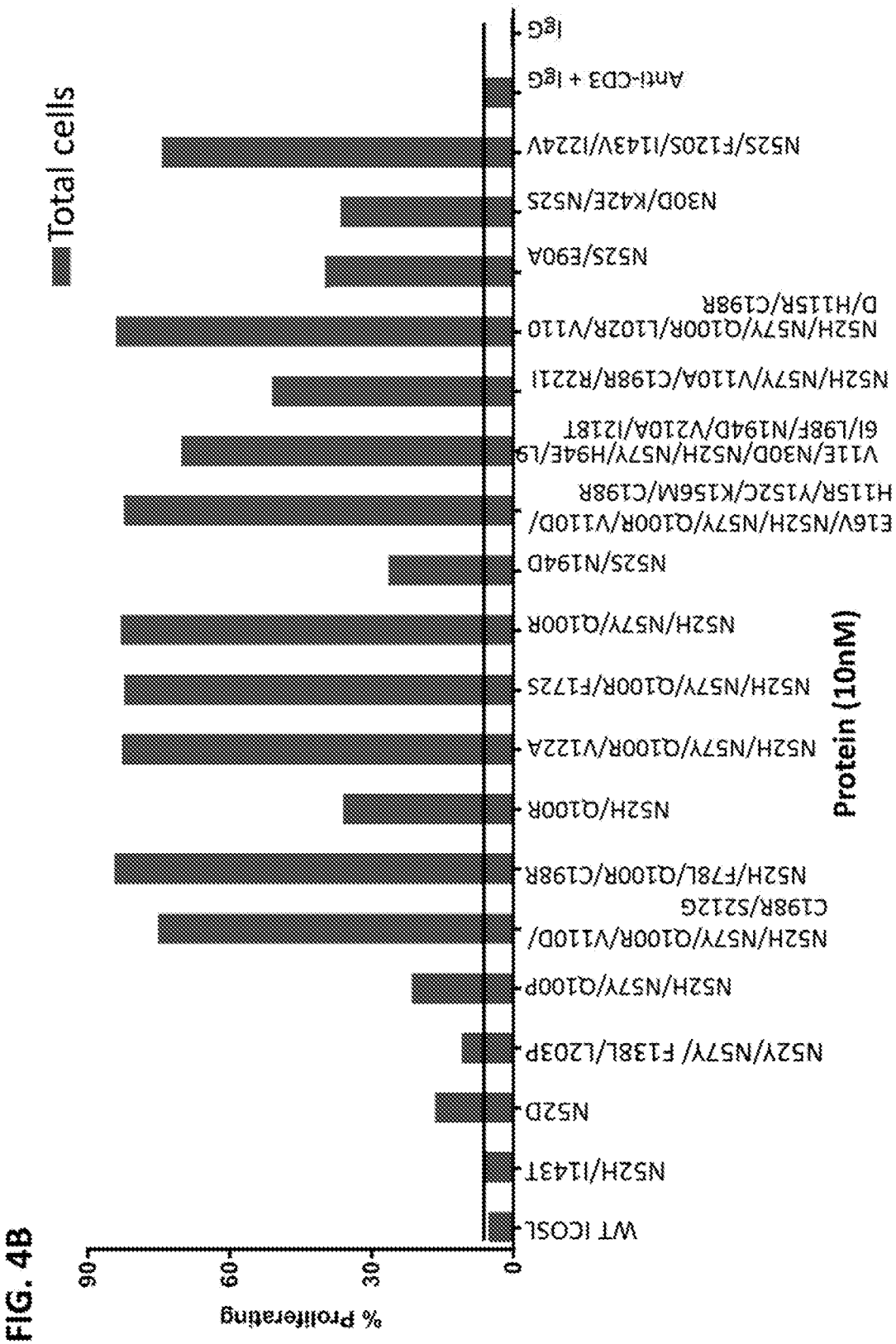

Total cells
% Proliferating
0
30
60
90
Protein (10nM)
WT ICOSL
N52H/I143T
N52D
N52Y/N57Y/ F138L/L203P
N52H/N57Y/Q100P
N52H/N57Y/Q100R/V110D/
C198R/S212G
N52H/F78L/Q100R/C198R
N52H/Q100R
N52H/N57Y/Q100R/V122A
N52H/N57Y/Q100R/F172S
N52H/N57Y/Q100R
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/
H115R/Y152C/K156M/C198R
V11E/N30D/N52H/N57Y/H94E/L9
6I/L98F/N194D/V210A/I218T
N52H/N57Y/V110A/C198R/R221I
N52H/N57Y/Q100R/L102R/V110
D/H115R/C198R
N52S/E90A
N30D/K42E/N52S
N52S/F120S/I143V/I224V
Anti-CD3 + IgG
IgG

FIG. 4B

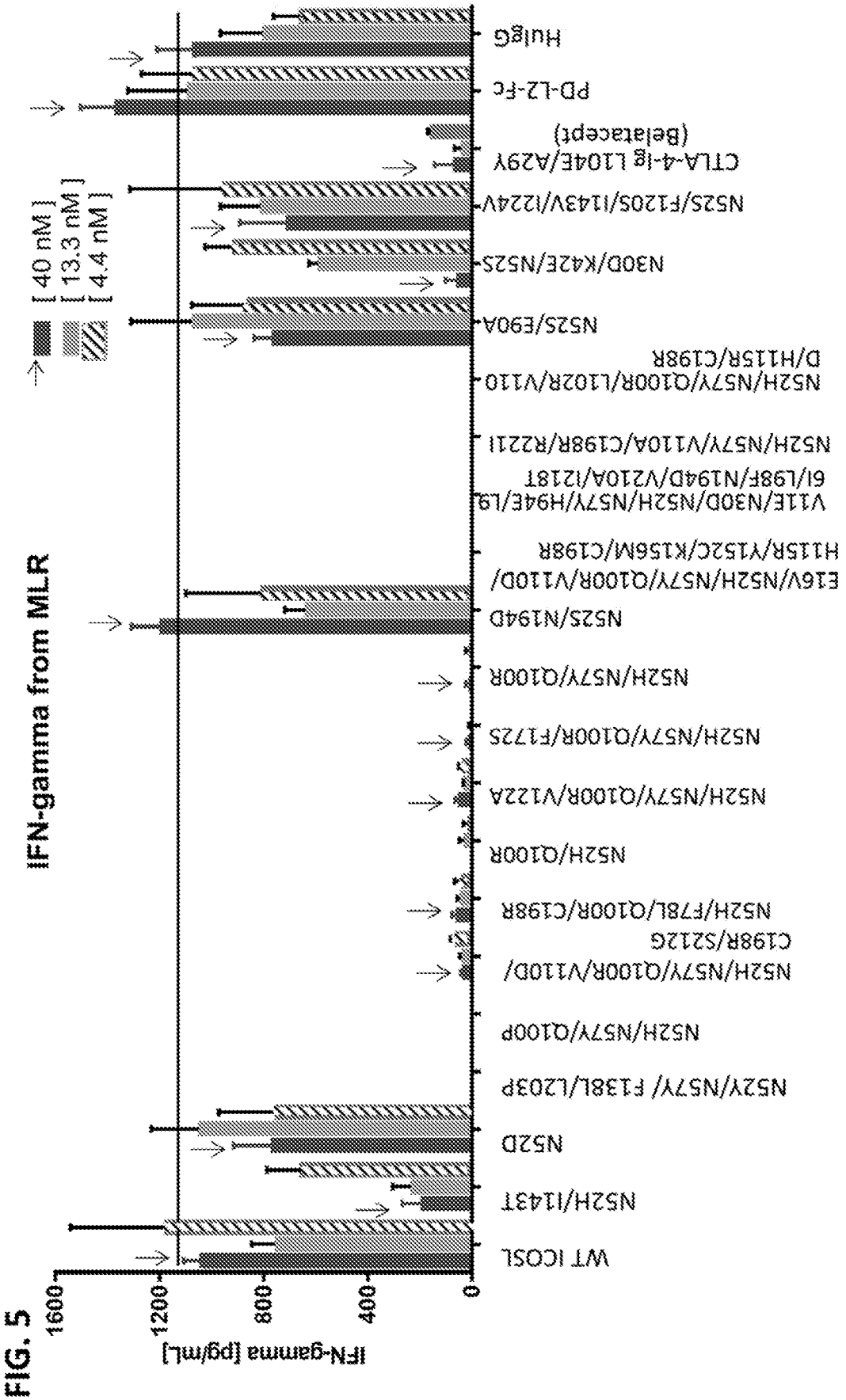

IFN-gamma from MLR
IFN-gamma [pg/mL]
1600
1200
800
400
0
[40 nM]
[13.3 nM]
[4.4 nM]
WT ICOSL
N52H/I143T
N52D
N52Y/N57Y/ F138L/L203P
N52H/N57Y/Q100P
N52H/N57Y/Q100R/V110D/
C198R/S212G
N52H/F78L/Q100R/C198R
N52H/Q100R
N52H/N57Y/Q100R/V122A
N52H/N57Y/Q100R/F172S
N52H/N57Y/Q100R
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/
H115R/Y152C/K156M/C198R
V11E/N30D/N52H/N57Y/H94E/L9
6I/L98F/N194D/V210A/I218T
N52H/N57Y/V110A/C198R/R221I
N52H/N57Y/Q100R/L102R/V110
D/H115R/C198R
N52S/E90A
N30D/K42E/N52S
N52S/F120S/I143V/I224V
CTLA-4-Ig L104E/A29Y
(Belatacept)
PD-L2-Fc
HuIgG

FIG. 5

FIG. 6A

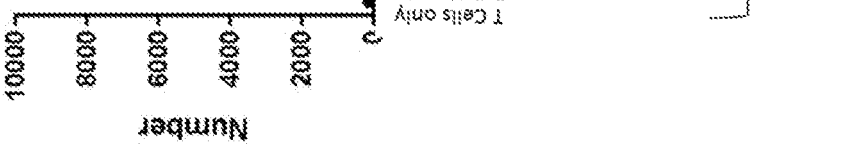

Divided B Cells
Number
10000
8000
6000
4000
2000
0
None
SEB
PWM
SEB+PWM
Stimulation
T Cells only
B Cells only
No Protein
B+T WT ICOSL ECD
B+T N52H/Q100R
B+T N52H/N57Y/Q100R/F172S
B+T N52H/N57Y/Q100P
B+T Belatacept

FIG. 6B

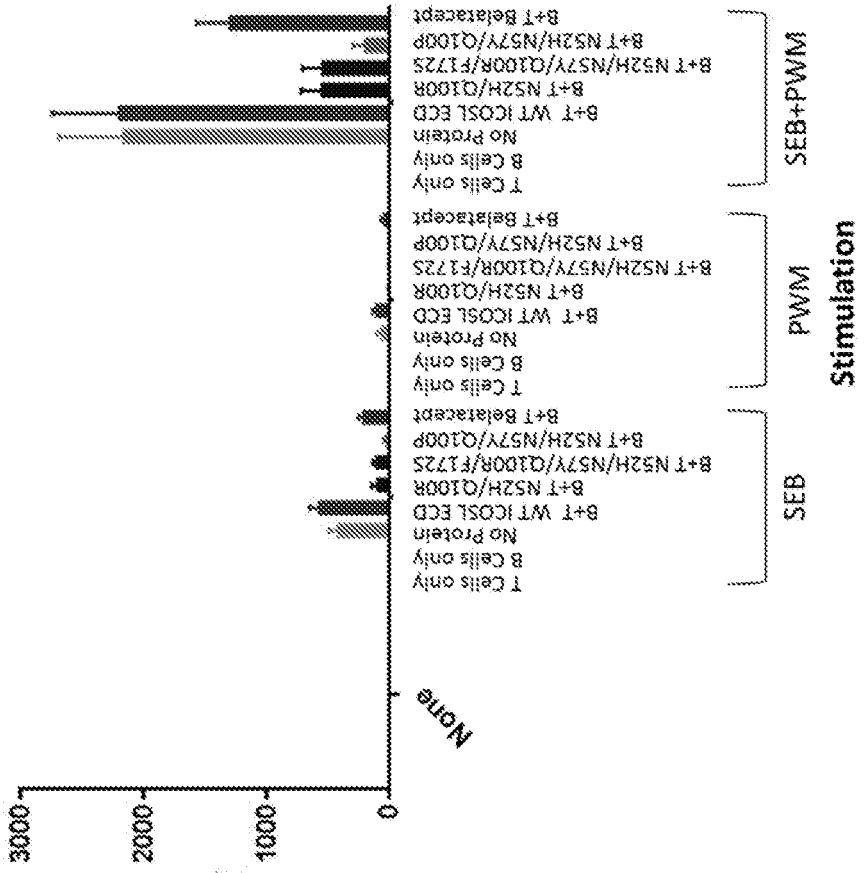

IL-5
pg/ml
3000
2000
1000
0
None
SEB
PWM
SEB+PWM
Stimulation
T Cells only
B Cells only
No Protein
B+T WT ICOSL ECD
B+T N52H/Q100R
B+T N52H/N57Y/Q100R/F172S
B+T N52H/N57Y/Q100P
B+T Belatacept

FIG. 6C

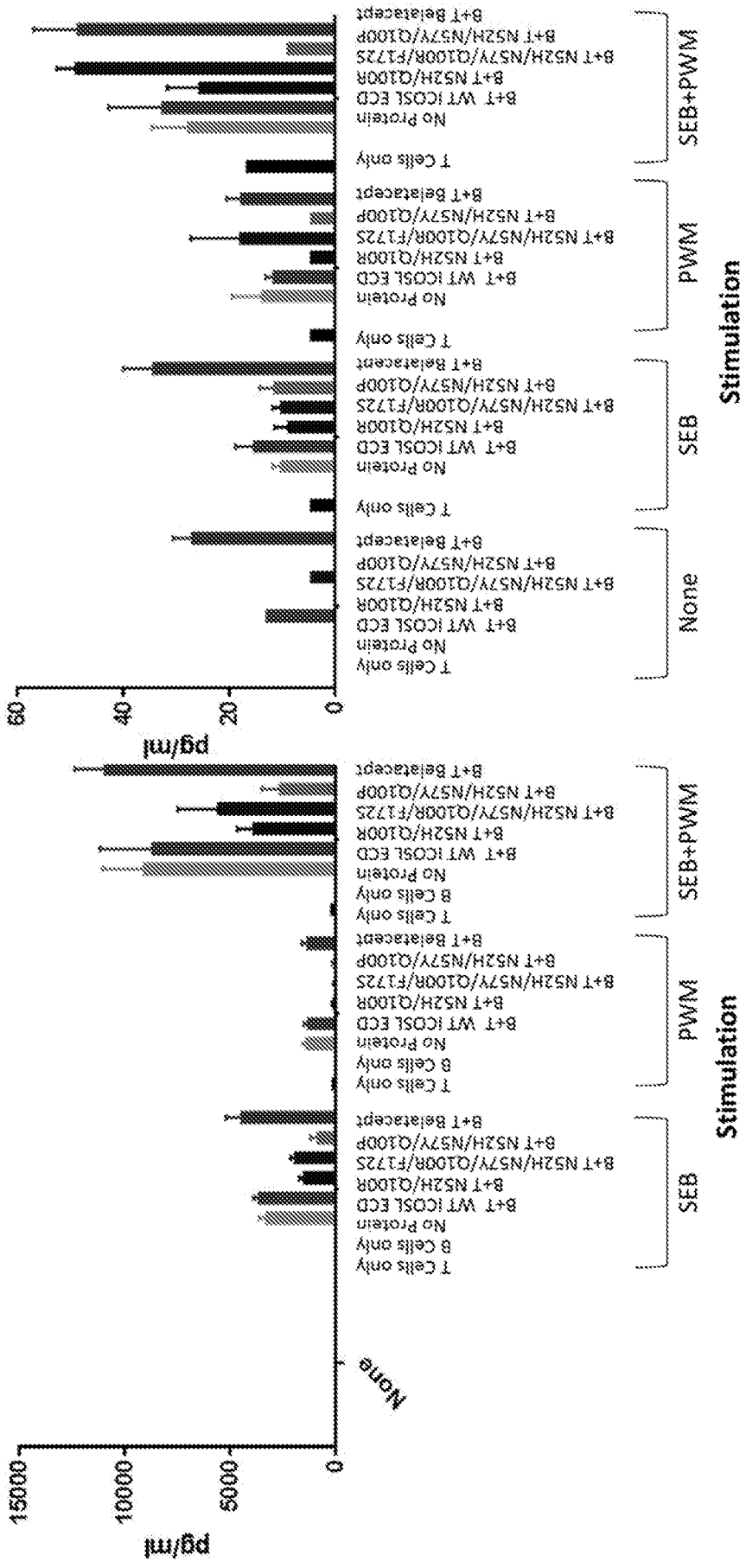

IL-13
pg/ml
15000
10000
5000
0
None
T Cells only
B Cells only
No Protein
B+T WT ICOSL ECD
B+T N52H/Q100R
B+T N52H/N57V/Q100R/F172S
B+T N52H/N57V/Q100P
B+T Belatacept
SEB
PWM
SEB+PWM
Stimulation

FIG. 6D

IL-21
pg/ml
60
40
20
0
T Cells only
No Protein
B+T WT ICOSL ECD
B+T N52H/Q100R
B+T N52H/N57V/Q100R/F172S
B+T N52H/N57V/Q100P
B+T Belatacept
None
SEB
PWM
SEB+PWM
Stimulation

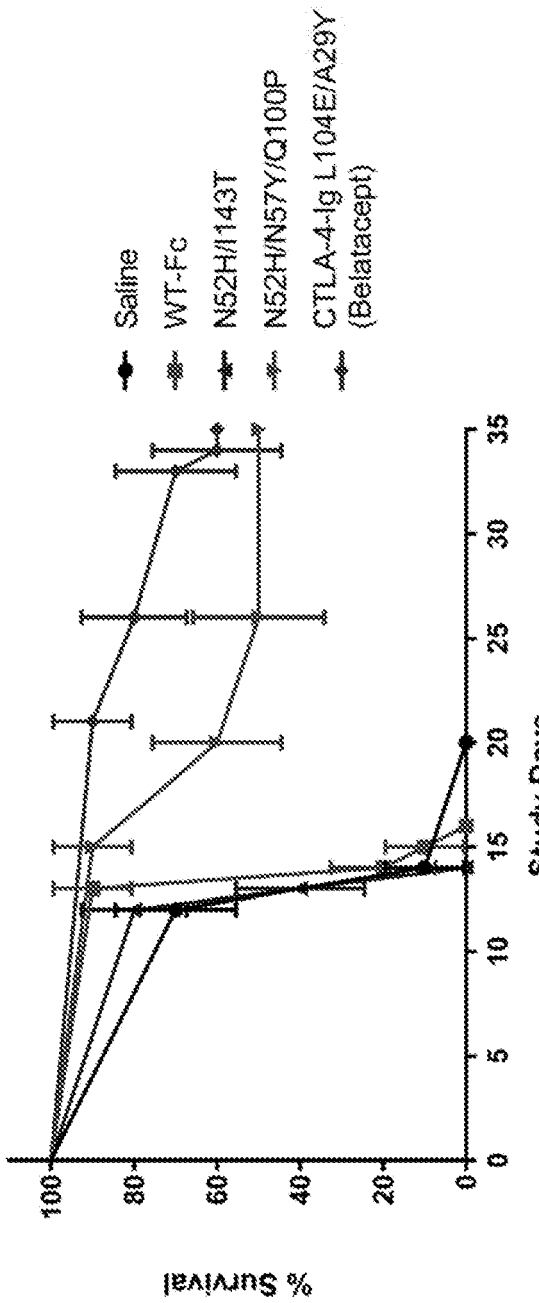

Survival in Hu-PBMC-NSG™♀GvHD
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y (Belatacept)
% Survival
Study Days
100
80
60
40
20
0
0
5
10
15
20
25
30
35

FIG. 7A

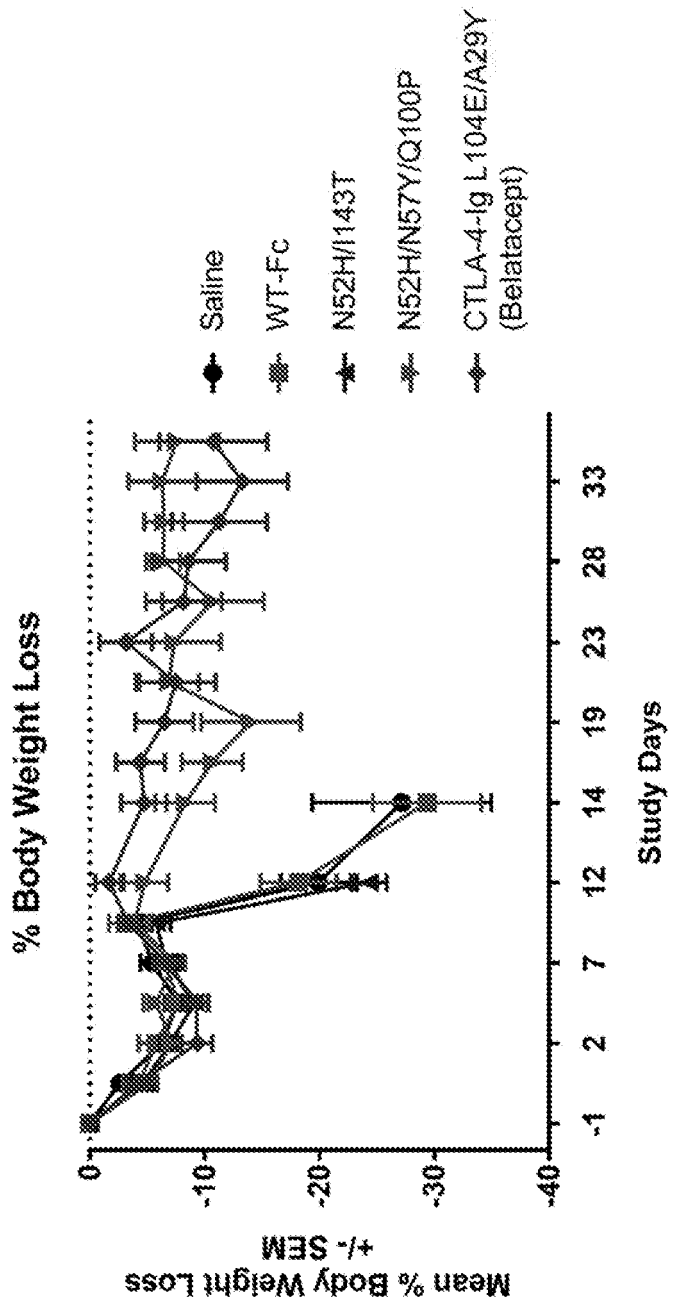

% Body Weight Loss
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y (Belatacept)
Mean % Body Weight Loss +/- SEM
Study Days
0
-10
-20
-30
-40
-1
2
7
12
14
19
23
28
33

FIG. 7B

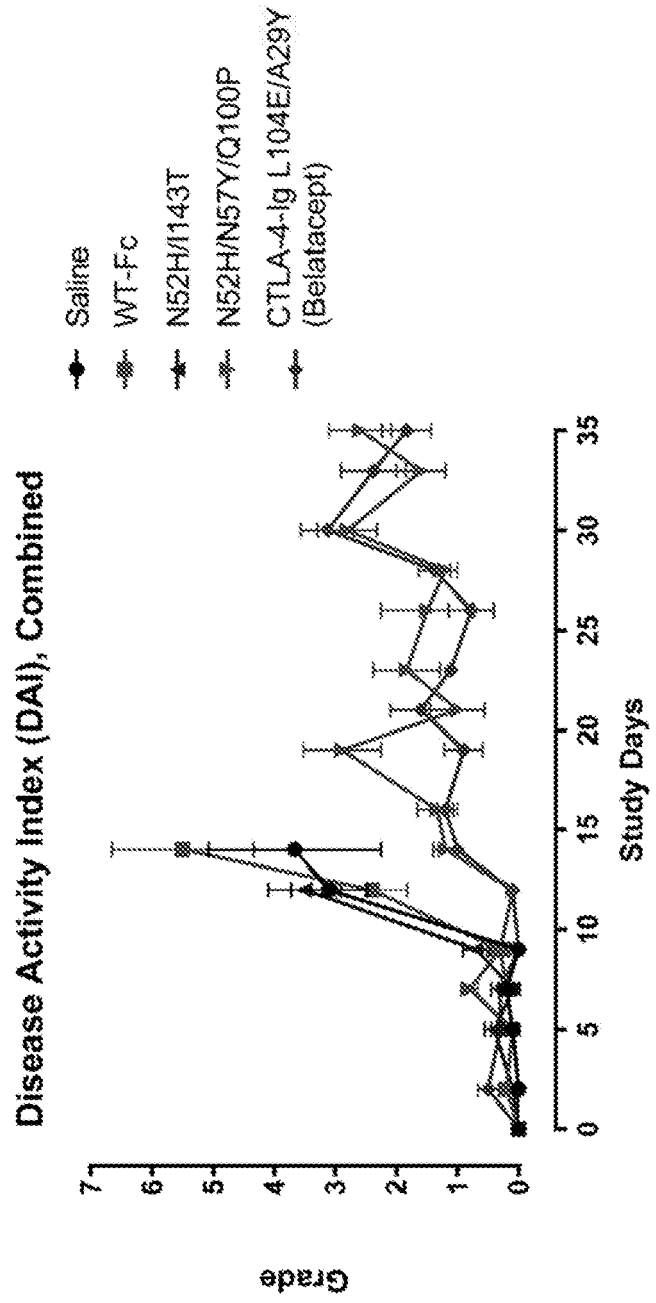

Disease Activity Index (DAI), Combined
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y
(Belatacept)
Grade
0
1
2
3
4
5
6
7
Study Days
0
5
10
15
20
25
30
35

FIG. 7C

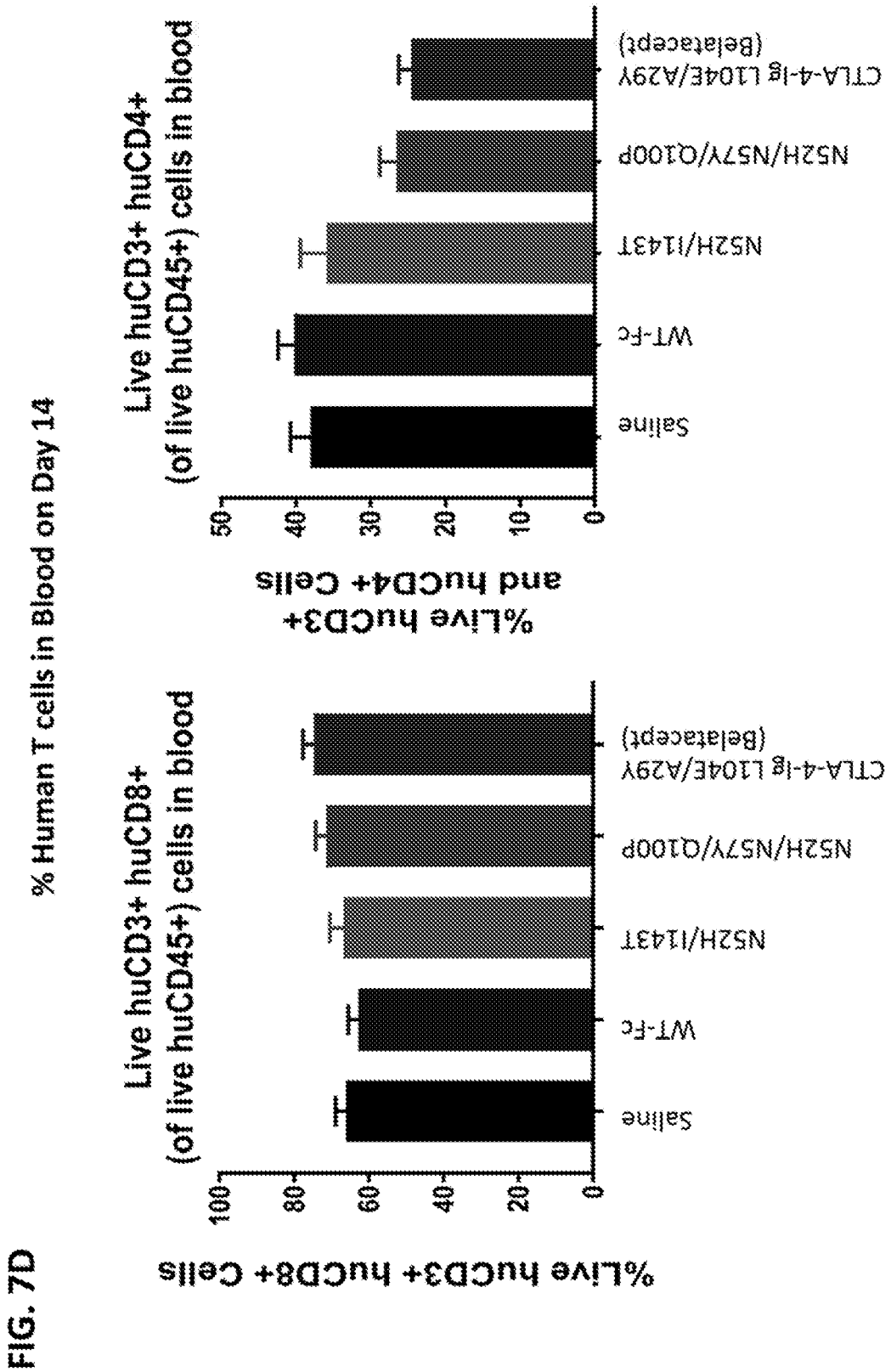
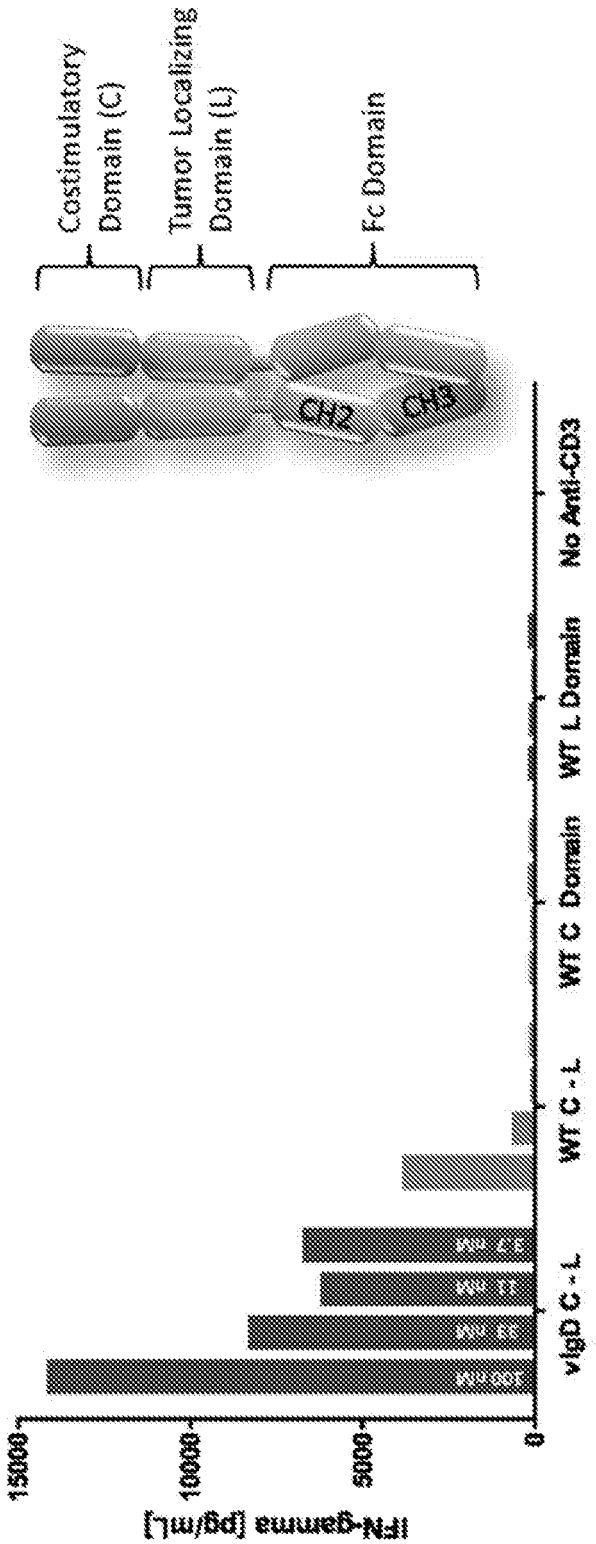

% Human T cells in Blood on Day 14
Live huCD3+ huCD8+ cells
(of live huCD45+) cells in blood
%Live huCD3+ huCD8+ Cells
100
80
60
40
20
0
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y
(Belatacept)
Live huCD3+ huCD4+
(of live huCD45+) cells in blood
%Live huCD3+
and huCD4+ Cells
50
40
30
20
10
0
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y
(Belatacept)

FIG. 7D

FIG. 8


Costimulatory Domain (C)
Tumor Localizing Domain (L)
Fc Domain
CH2
CH3
IFN-gamma [pg/mL]
15000
10000
5000
0
vIgD C - L
WT C - L
WT C Domain
WT L Domain
No Anti-CD3

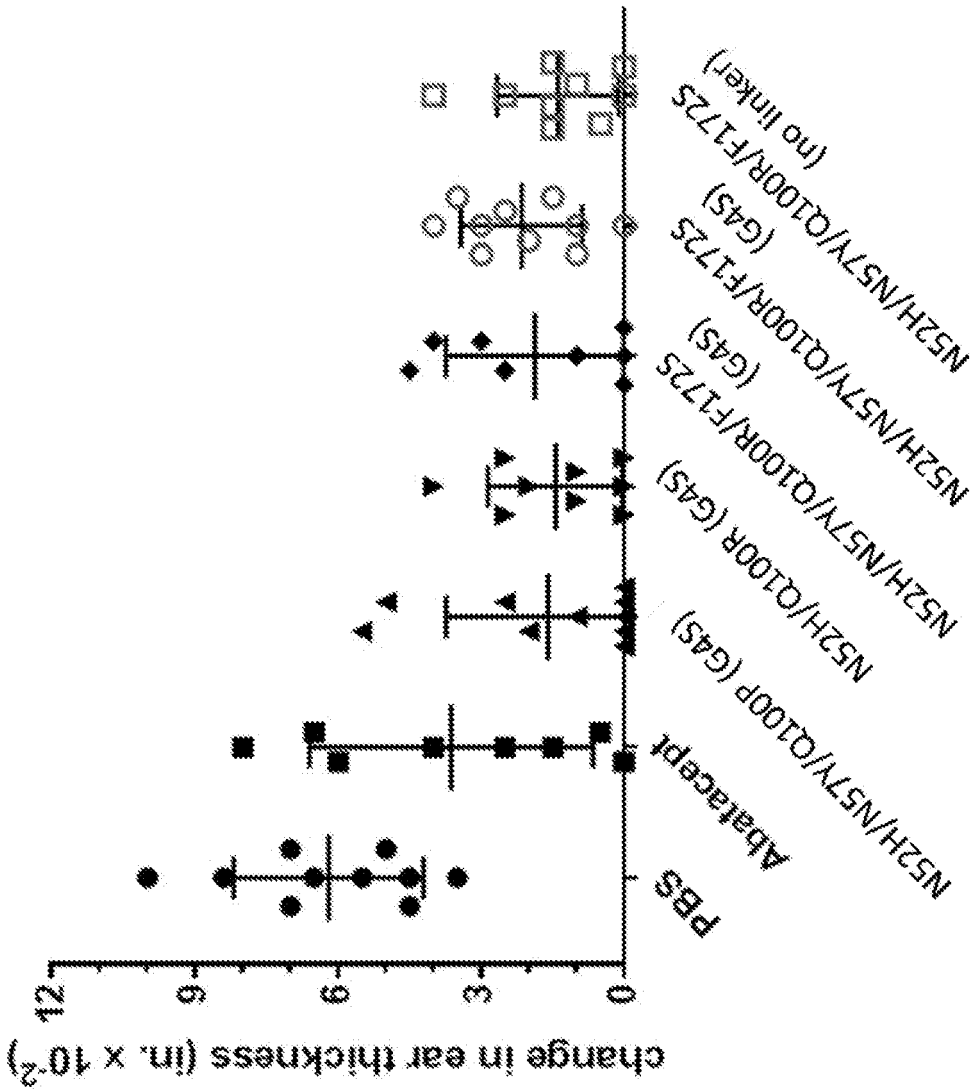

change in ear thickness (in. x 10^{-2})
12
9
6
3
0
PBS
Abatacept
N52H/N57Y/Q100P (GAS)
N52H/Q100R (GAS)
N52H/N57Y/Q100R/F172S (GAS)
N52H/N57Y/Q100R/F172S (GAS)
N52H/N57Y/Q100R/F172S (no linker)

FIG. 9

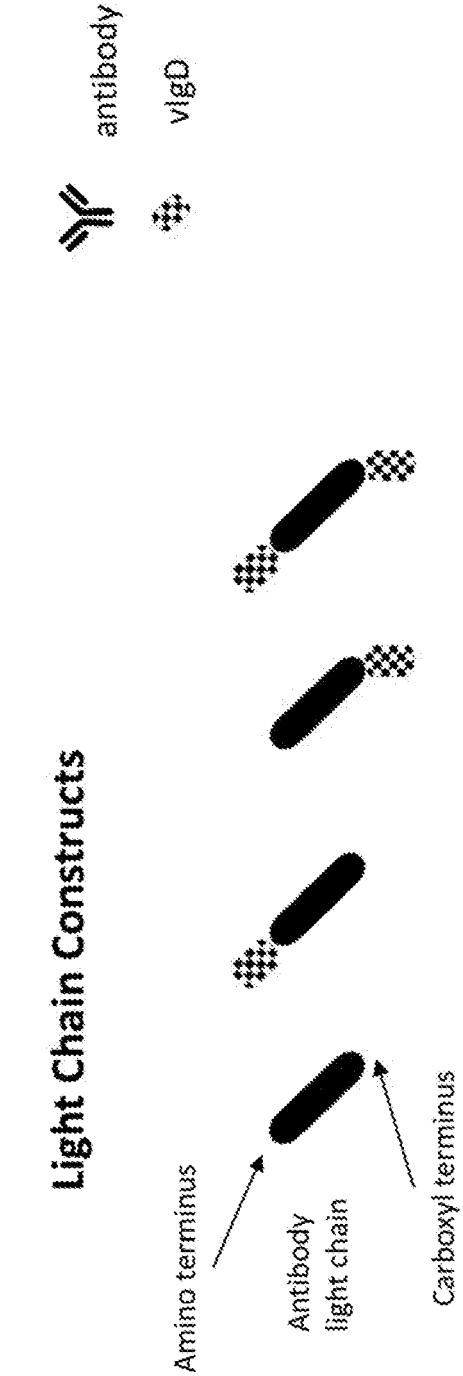


FIG. 10A

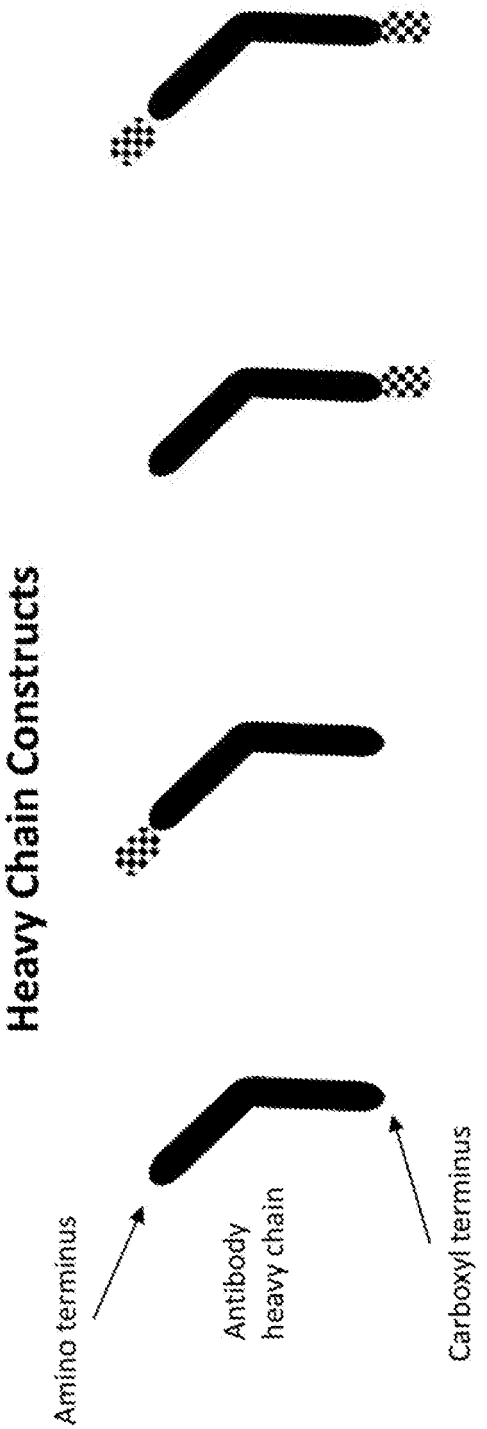


FIG. 10B

FIG. 10C

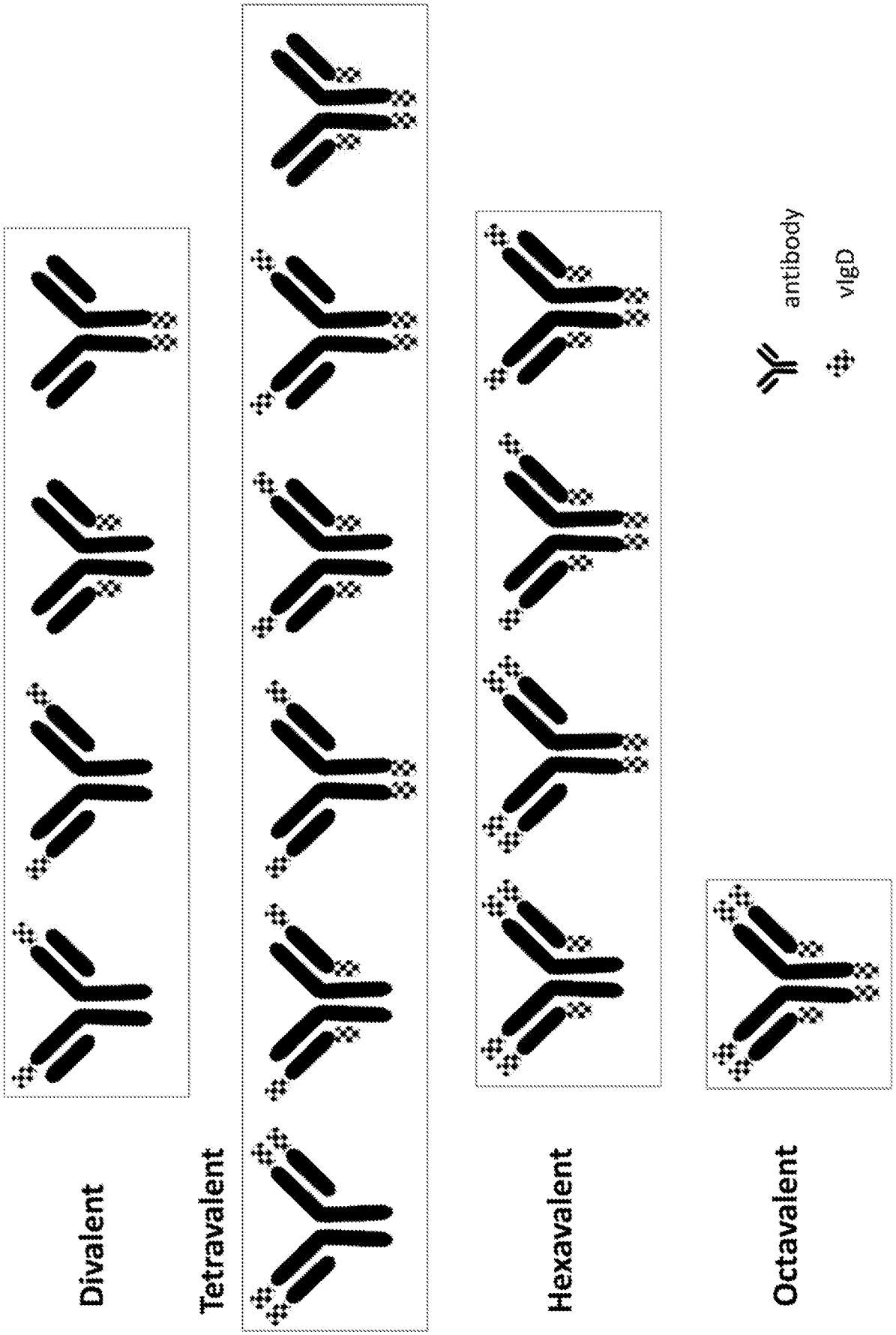

Divalent
Tetravalent
Hexavalent
Octavalent
antibody
vIgD

FIG. 11A

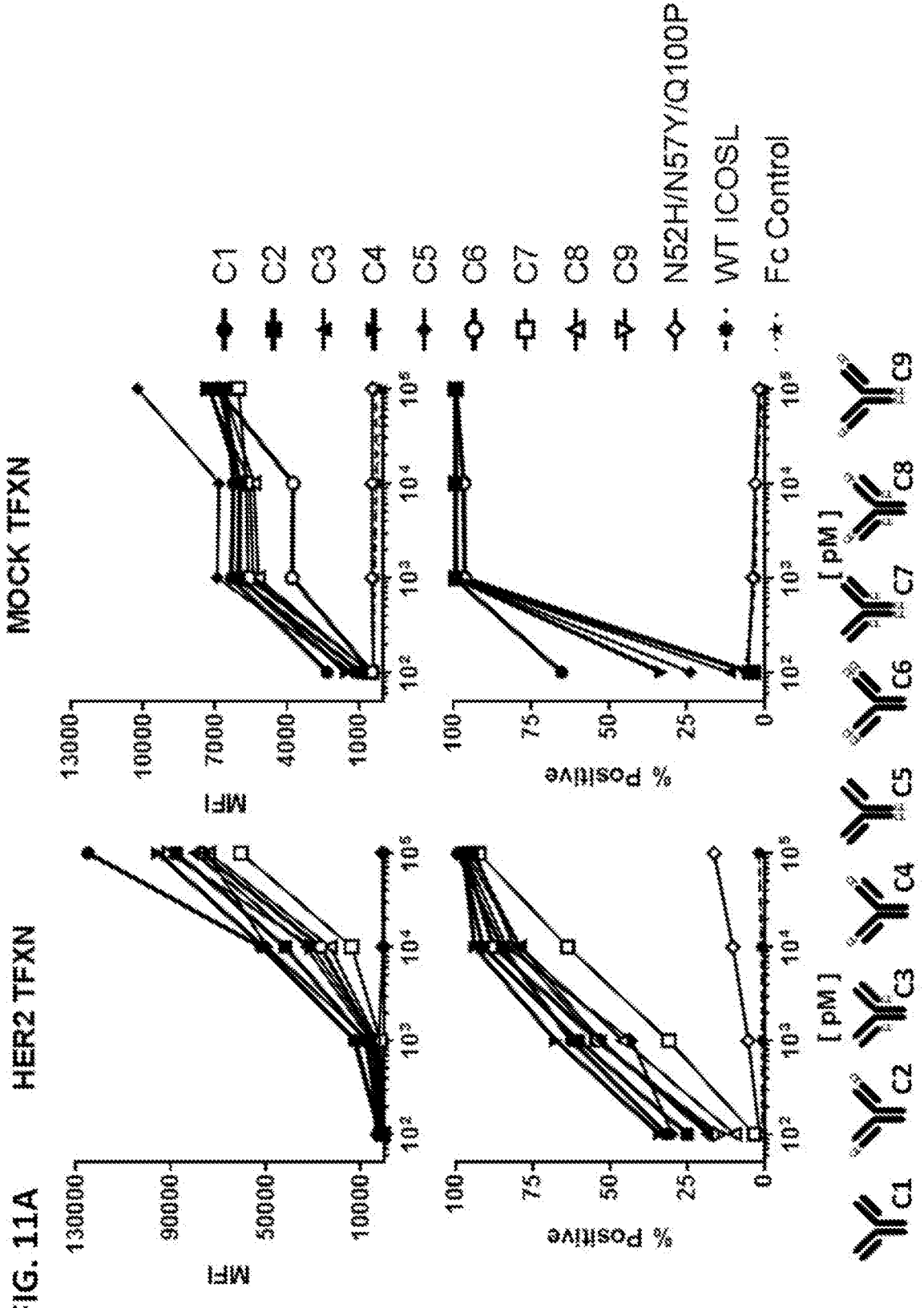

HER2 TFXN
MOCK TFXN
MFI
% Positive
[pM]
C1
C2
C3
C4
C5
C6
C7
C8
C9
N52H/N57Y/Q100P
WT ICOSL
Fc Control

FIG. 11B

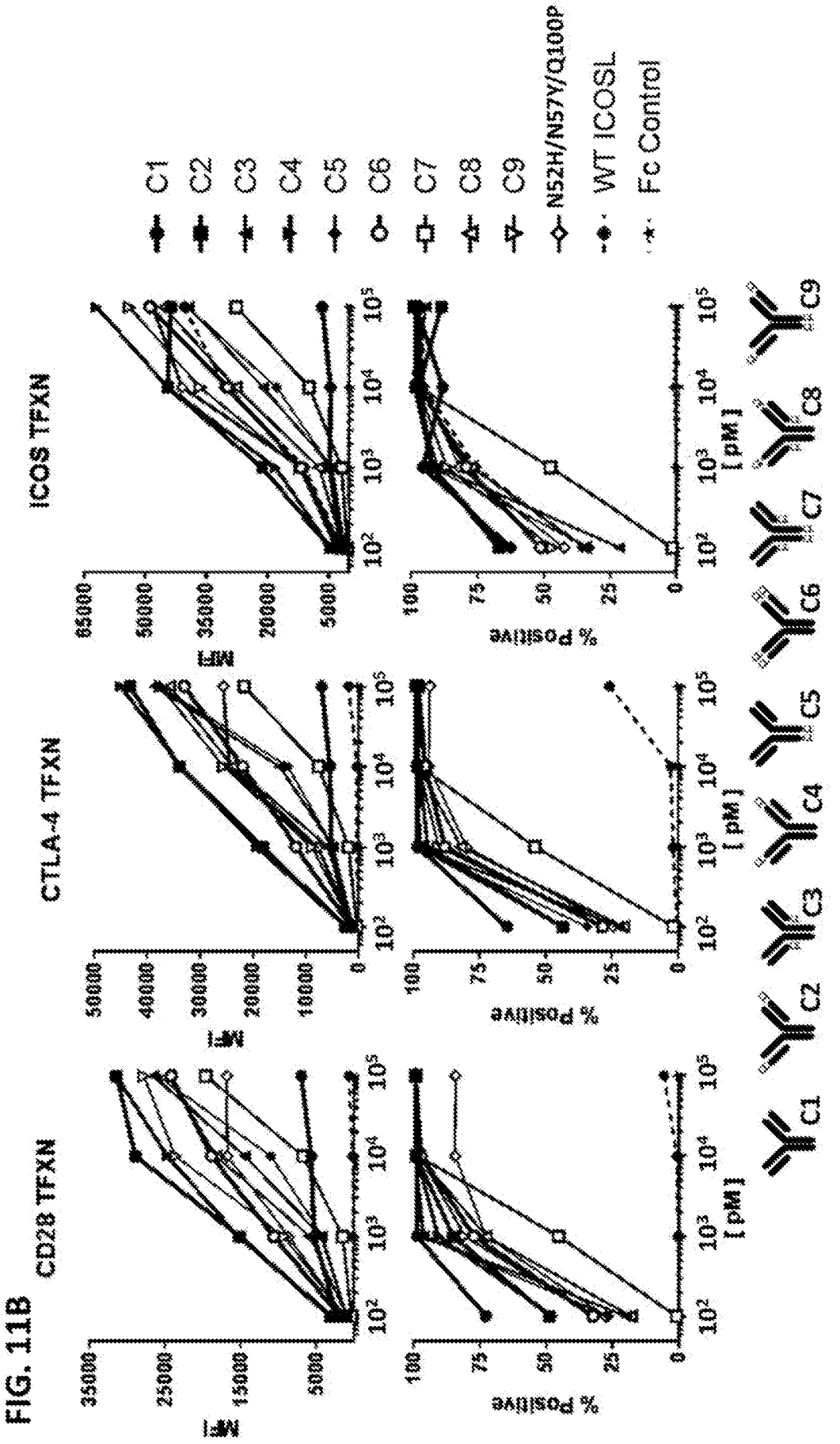

CD28 TFXN
CTLA-4 TFXN
ICOS TFXN
MFI
% Positive
[pM]
C1
C2
C3
C4
C5
C6
C7
C8
C9
N52H/N57Y/Q100P
WT ICOSL
Fc Control

FIG. 12

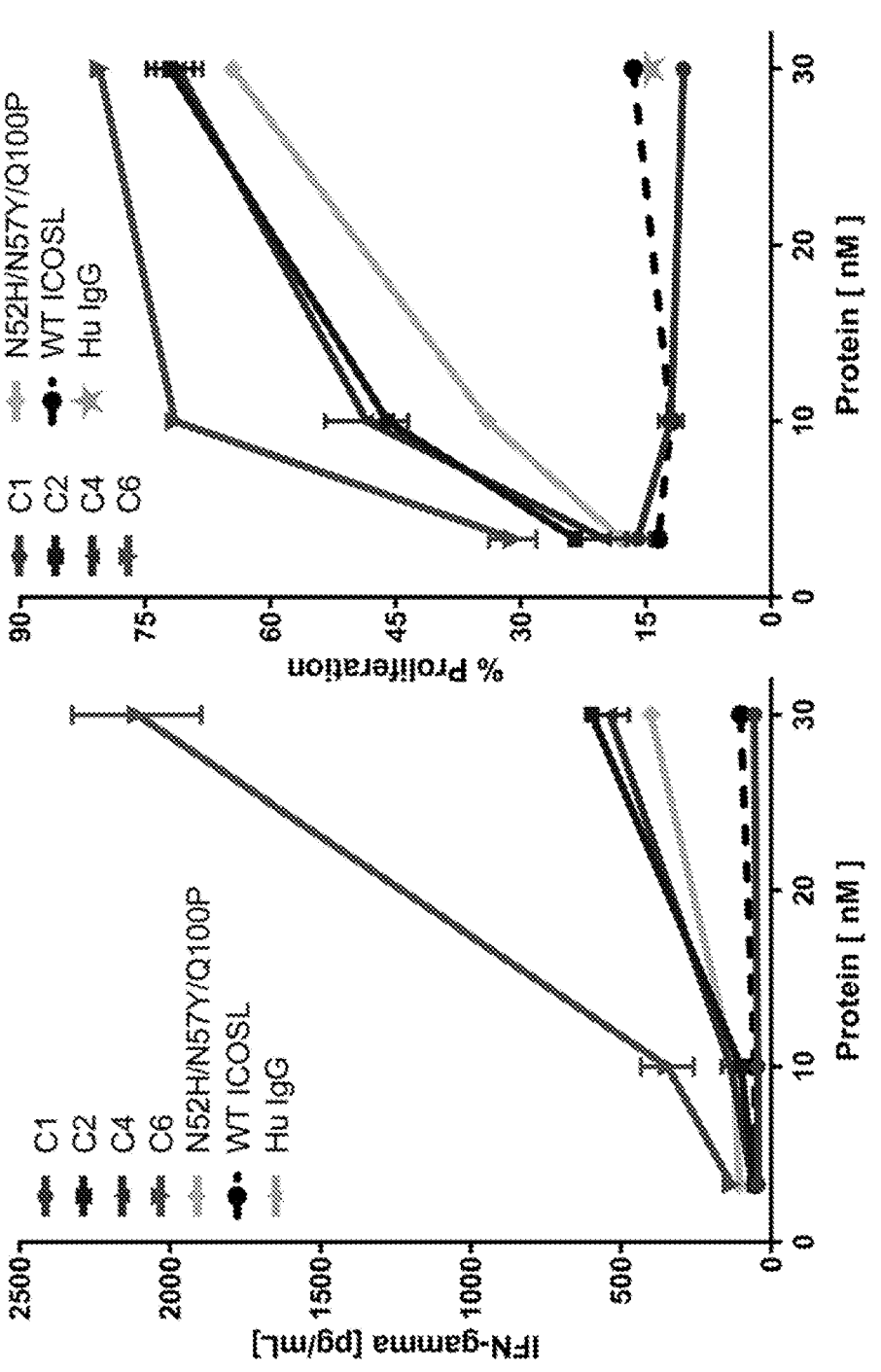

IgSF conjugate Costim Assay
IFN-gamma [pg/mL]
2500
2000
1500
1000
500
0
0
10
20
30
Protein [nM]
C1
C2
C4
C6
N52H/N57Y/Q100P
WT ICOSL
Hu IgG
IgSF conjugate Costim Proliferation
% Proliferation
90
75
60
45
30
15
0
0
10
20
30
Protein [nM]
C1
C2
C4
C6
N52H/N57Y/Q100P
WT ICOSL
Hu IgG

FIG. 13A

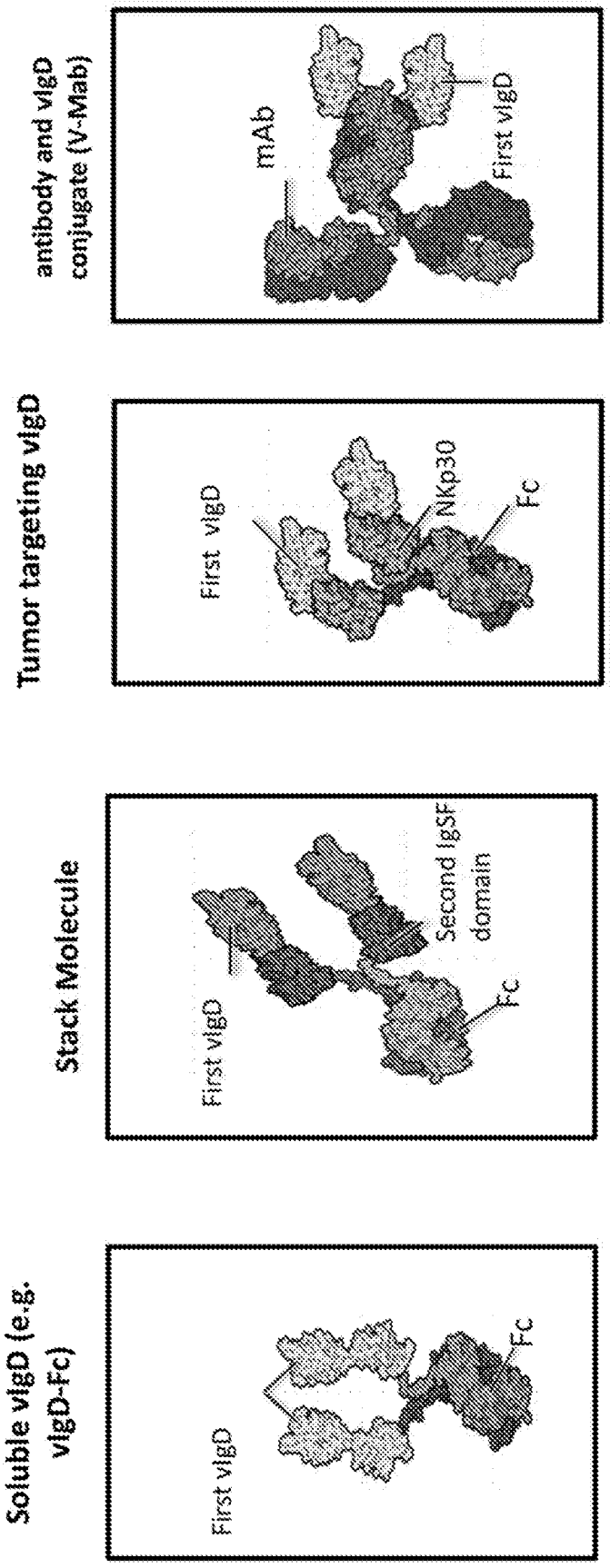
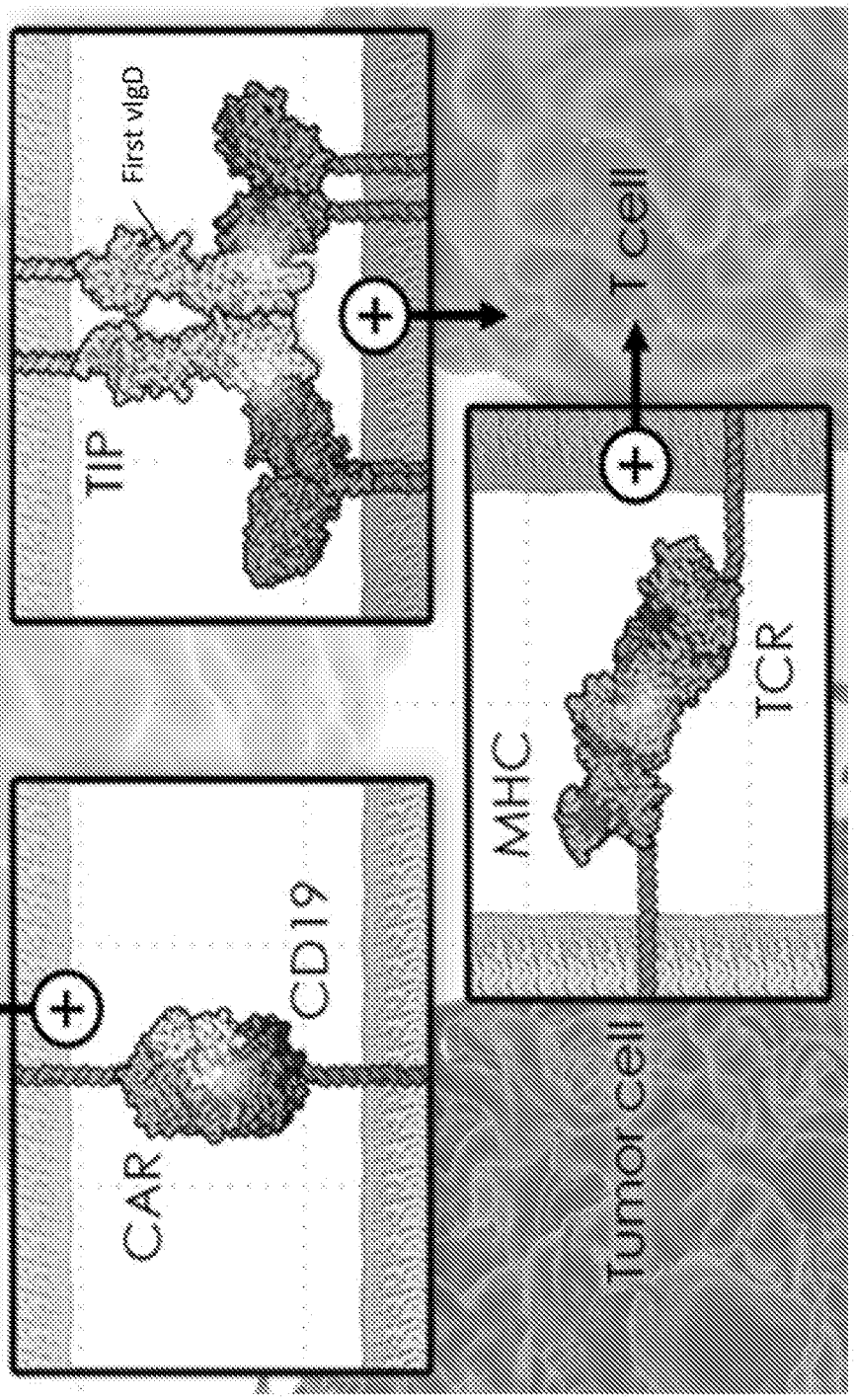

Soluble vIgD (e.g. vIgD-Fc)
First vIgD
Fc
Stack Molecule
First vIgD
Second IgSF domain
Fc
Tumor targeting vIgD
First vIgD
NKp30
Fc
antibody and vIgD conjugate (V-Mab)
mAb
First vIgD

FIG. 13B


TIP
Engineered T cell
CAR
CD19
TIP
First vIgD
MHC
TCR
T Cell
Tumor cell

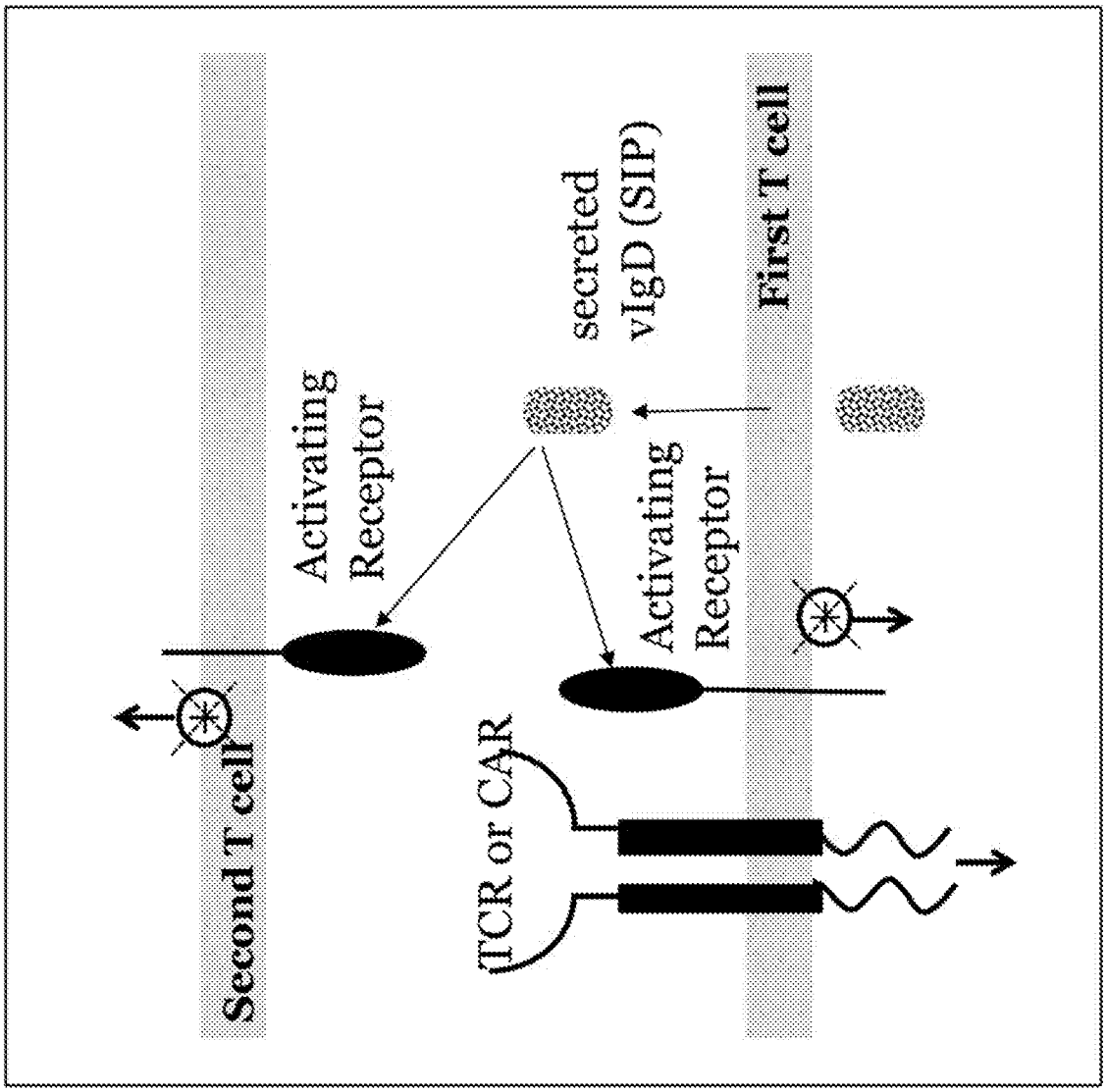

Second T cell
Activating Receptor
secreted vIgD (SIP)
First T cell
Activating Receptor
TCR or CAR

FIG. 13C

FIG. 14

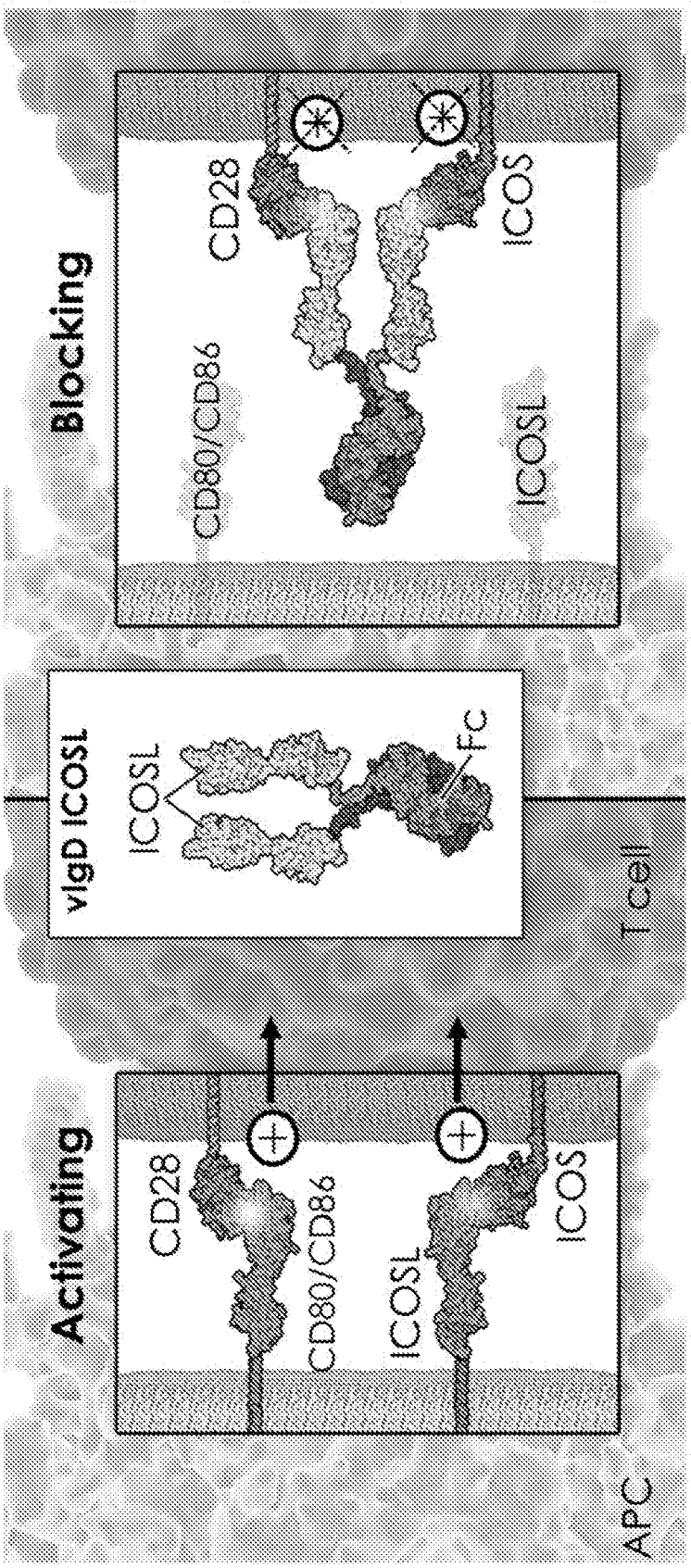

Activating
CD28
CD80/CD86
ICOSL
ICOS
APC
vIgD ICOSL
ICOSL
Fc
T cell
Blocking
CD80/CD86
CD28
ICOSL
ICOS

FIG. 15

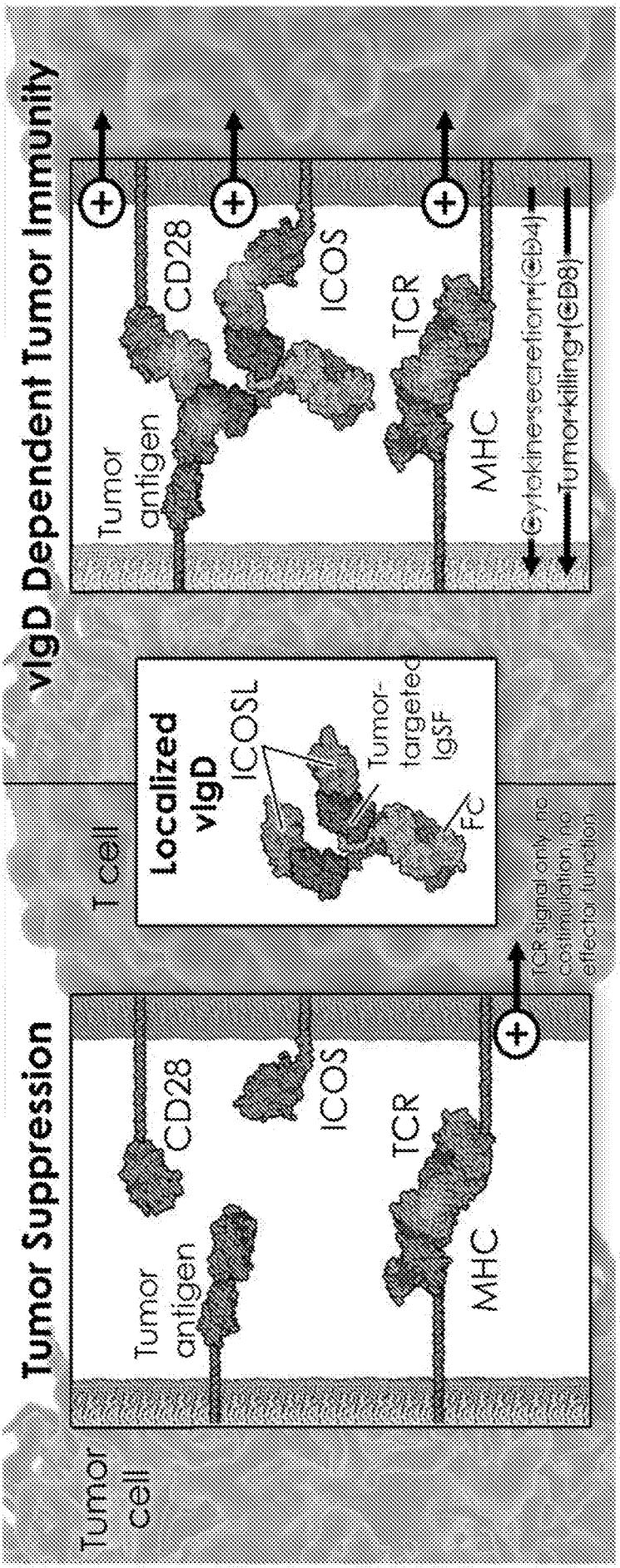

Tumor Suppression
vIgD Dependent Tumor Immunity
Tumor cell
T cell
Tumor antigen
CD28
ICOS
TCR
MHC
Localized vIgD
ICOSL
Tumor-targeted IgSF
Fc
TCR signal only, no costimulation, no effector function
+
Tumor antigen
CD28
ICOS
TCR
MHC
+
+
+
cytokine secretion (CD4)
Tumor killing (CD8)

FIG. 16

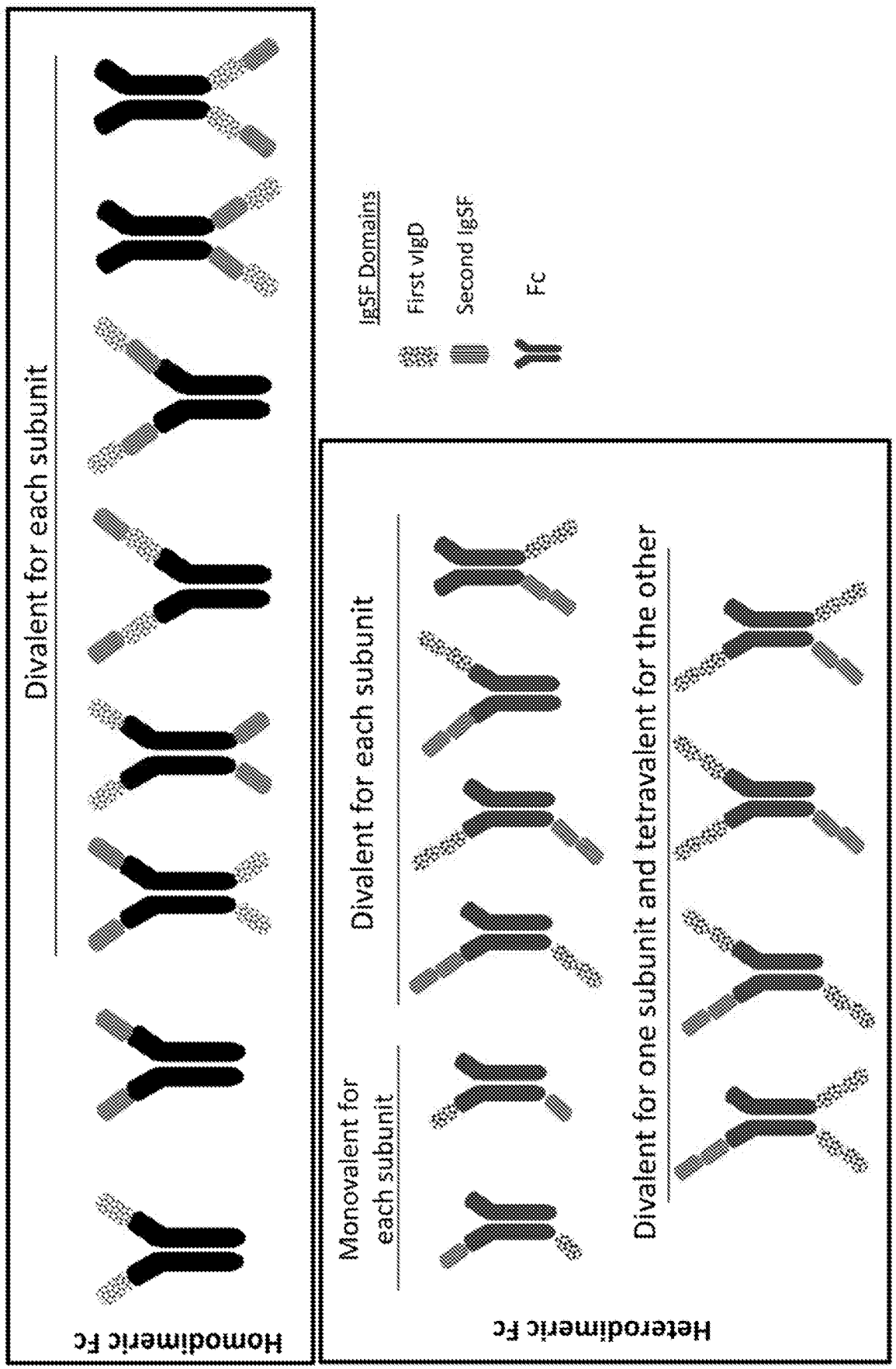
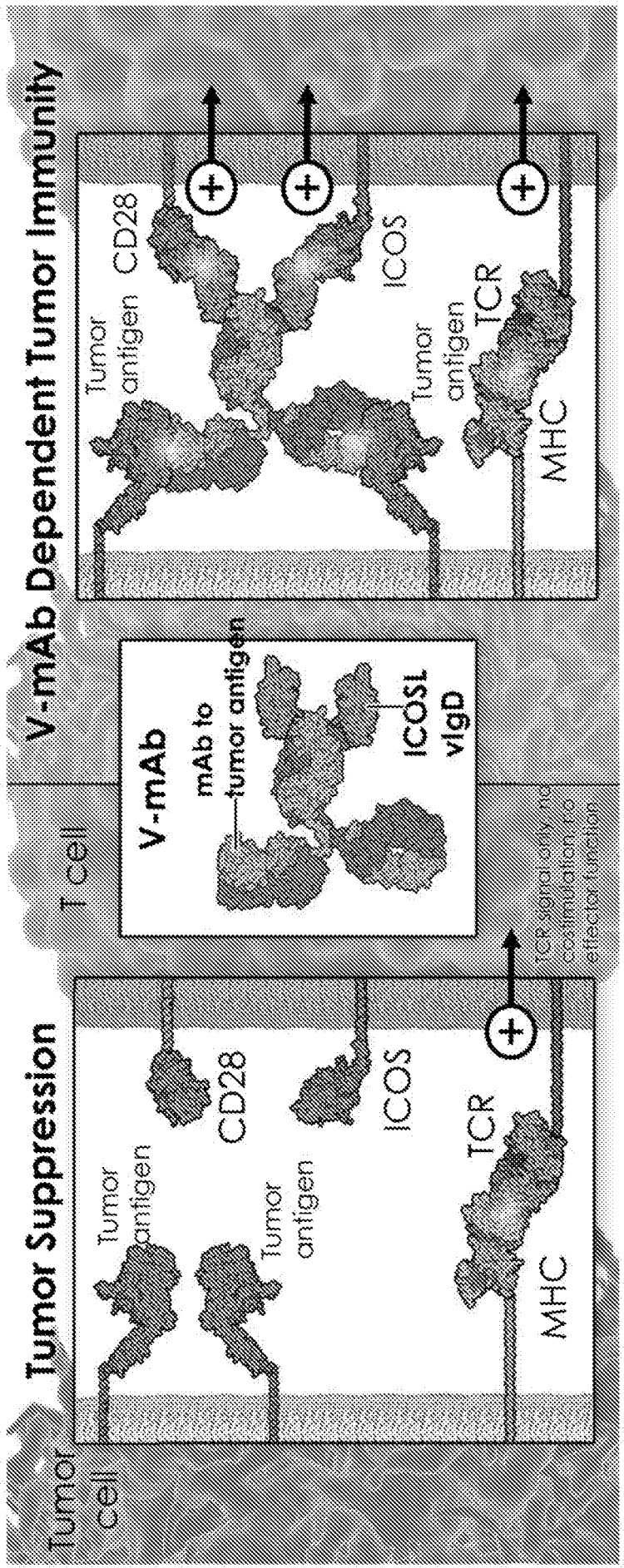

Homodimeric Fc
Divalent for each subunit
Heterodimeric Fc
Monovalent for each subunit
Divalent for each subunit
Divalent for one subunit and tetravalent for the other
IgSF Domains
First vIgD
Second IgSF
Fc

FIG. 17


Tumor Suppression
Tumor cell
Tumor antigen
Tumor antigen
CD28
ICOS
TCR
MHC
T Cell
TCR signaling and costimulation for effector function
V-mAb
mAb to tumor antigen
ICOSL vIgD
V-mAb Dependent Tumor Immunity
Tumor antigen
CD28
ICOS
Tumor antigen
TCR
MHC

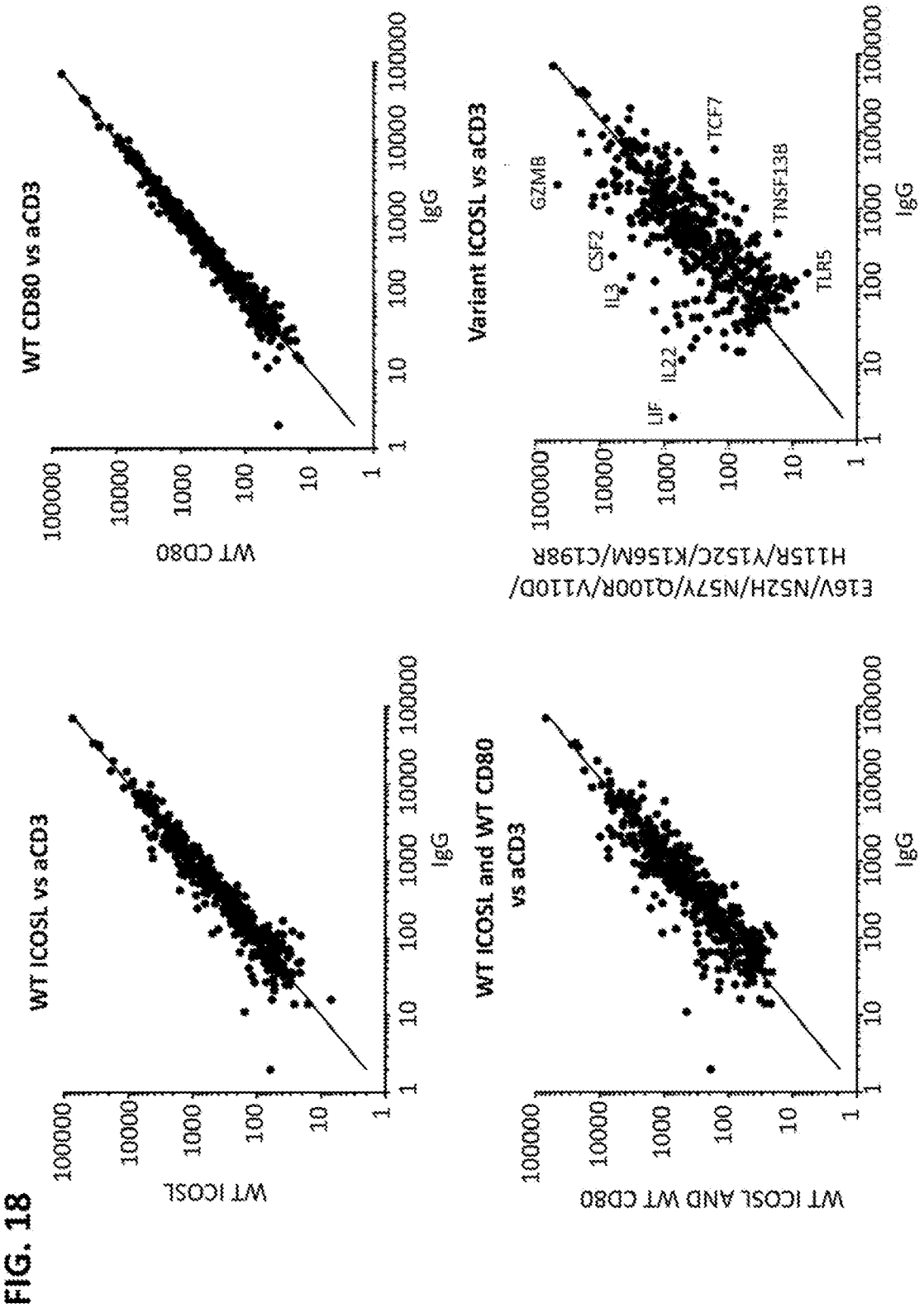

WT CD80 vs aCD3
WT CD80
IgG
Variant ICOSL vs aCD3
E16V/N52H/N57Y/Q100R/V110D/
H115R/Y152C/K156M/C198R
GZMB
CSF2
IL3
LIF
IL22
TCF7
TNSF13B
TLR5
WT ICOSL vs aCD3
WT ICOSL
WT ICOSL and WT CD80
vs aCD3
WT ICOSL AND WT CD80

FIG. 18

FIG. 19

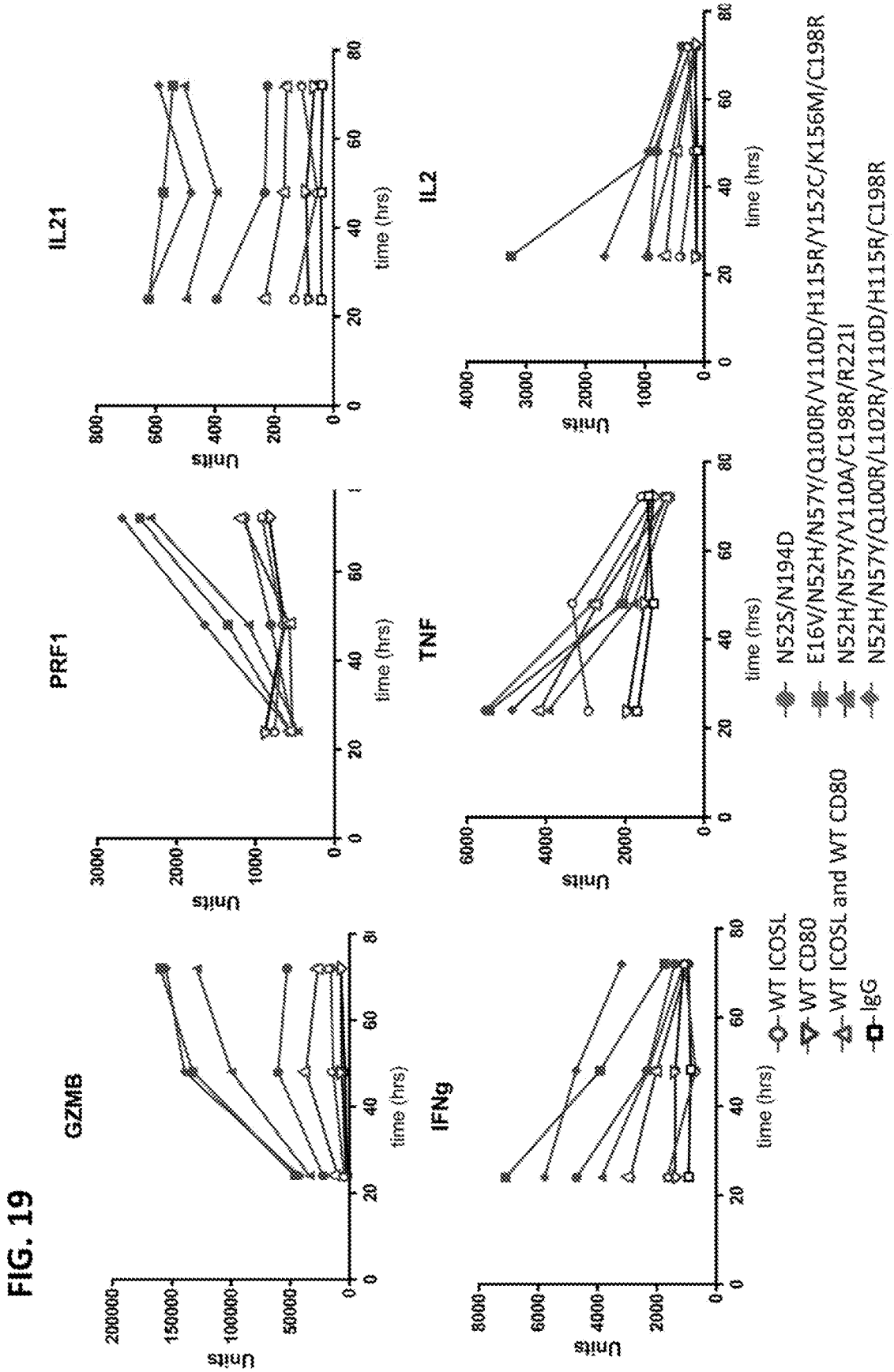

GZMB
PRF1
IL21
IFNg
TNF
IL2
Units
time (hrs)
WT ICOSL
WT CD80
WT ICOSL and WT CD80
IgG
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/H115R/Y152C/K156M/C198R
N52H/N57Y/V110A/C198R/R221I
N52H/N57Y/Q100R/L102R/V110D/H115R/C198R

FIG. 20A

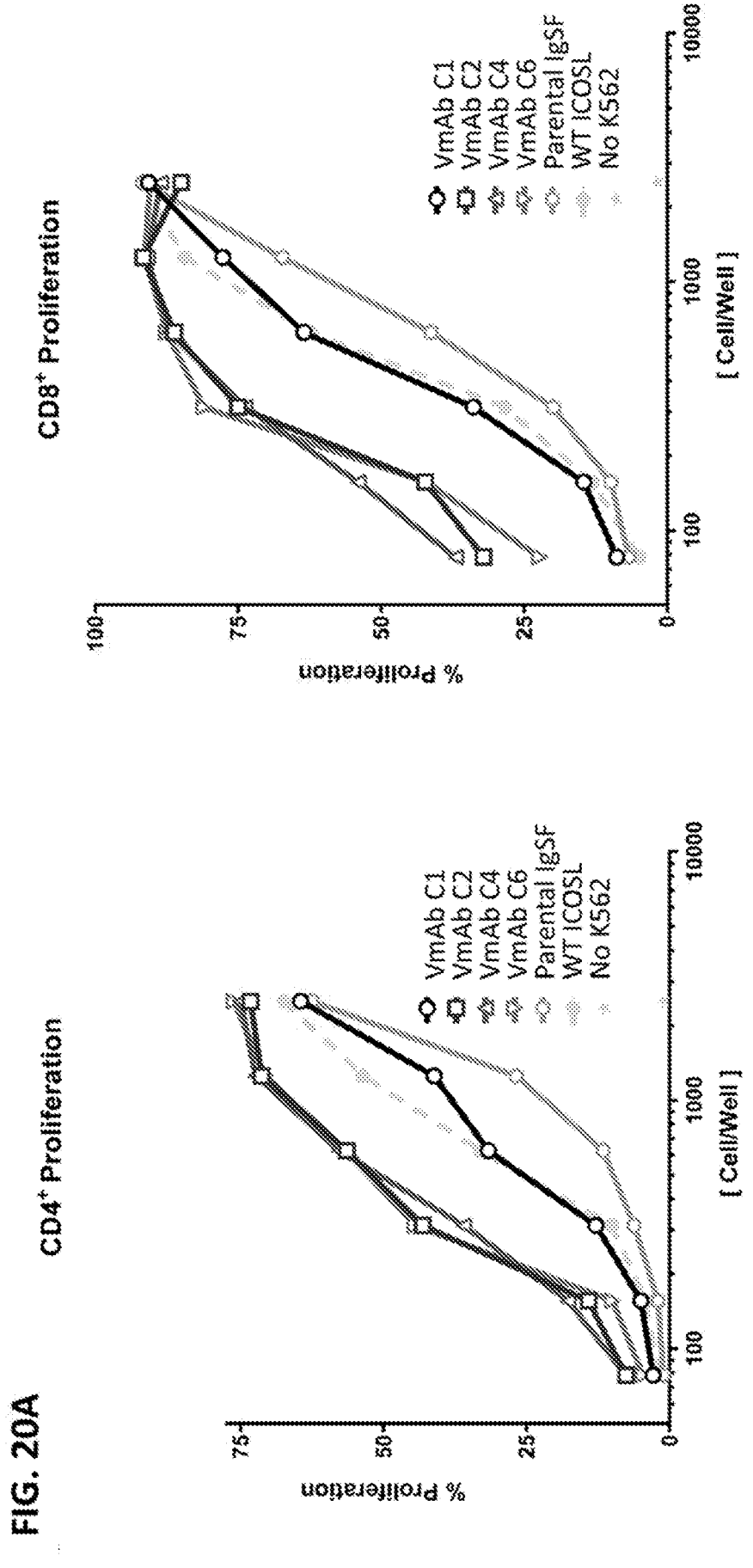
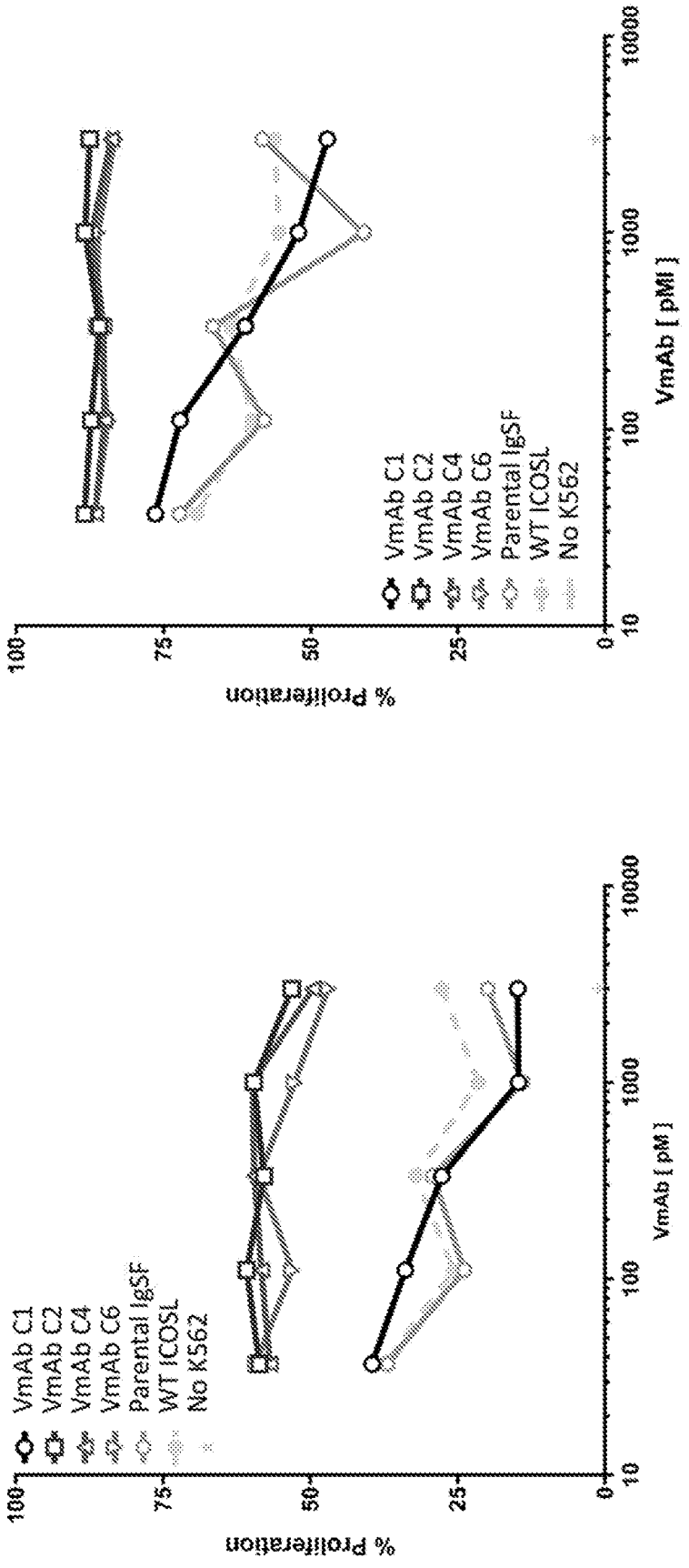

CD4+ Proliferation
% Proliferation
100
75
50
25
0
100
1000
10000
[Cell/Well]
VmAb C1
VmAb C2
VmAb C4
VmAb C6
Parental IgSF
WT ICOSL
No K562
CD8+ Proliferation
% Proliferation
100
75
50
25
0
100
1000
10000
[Cell/Well]
VmAb C1
VmAb C2
VmAb C4
VmAb C6
Parental IgSF
WT ICOSL
No K562

FIG. 20B


% Proliferation
100
75
50
25
0
10
100
1000
10000
VmAb [pM]
VmAb [pMl]
VmAb C1
VmAb C2
VmAb C4
VmAb C6
Parental IgSF
WT ICOSL
No K562