FIG. 1A

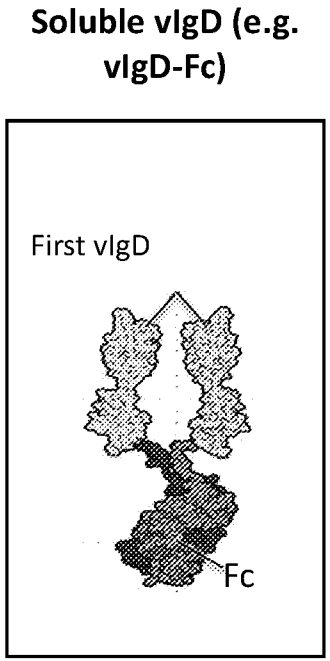


FIG. 1B

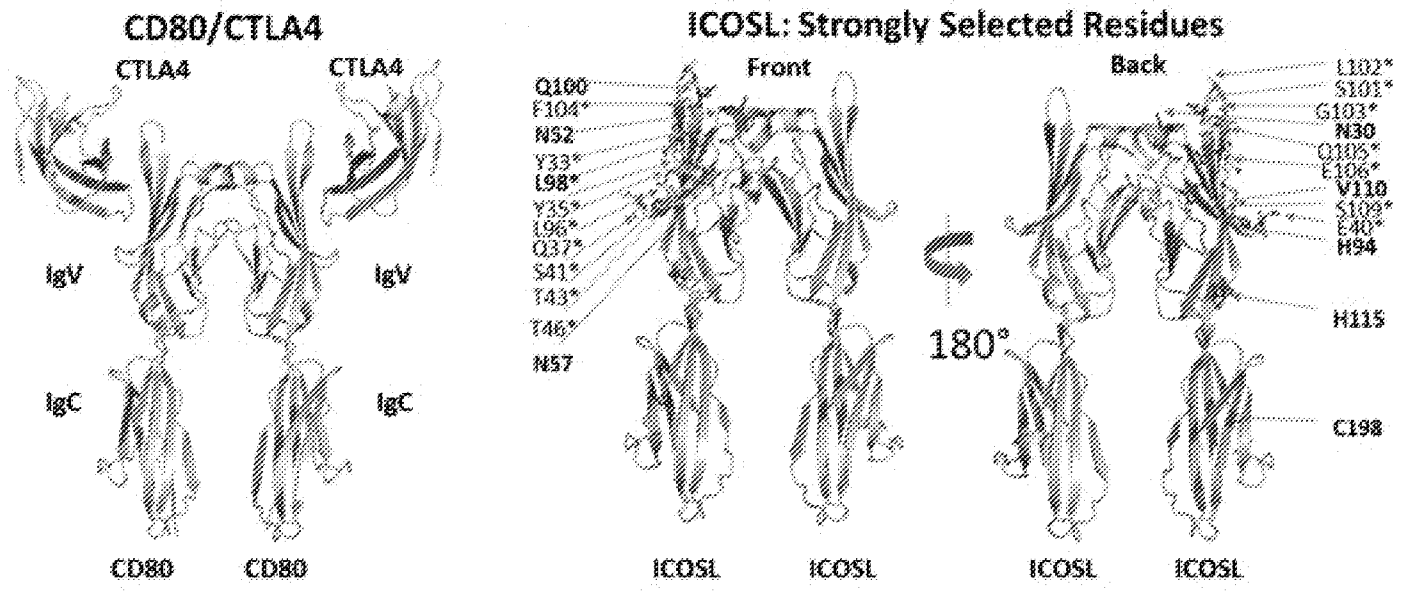

CD80/CTLA4
CTLA4
CTLA4
IgV
IgV
IgC
IgC
CD80
CD80
ICOSL: Strongly Selected Residues
Front
Back
Q100
F104*
N52
Y33*
L98*
Y35*
L96*
Q37*
S41*
T43*
T46*
N57
L102*
S101*
G103*
N30
Q105*
E106*
V110
S109*
E40*
H94
H115
C198
180°
ICOSL
ICOSL
ICOSL
ICOSL

FIG. 2A

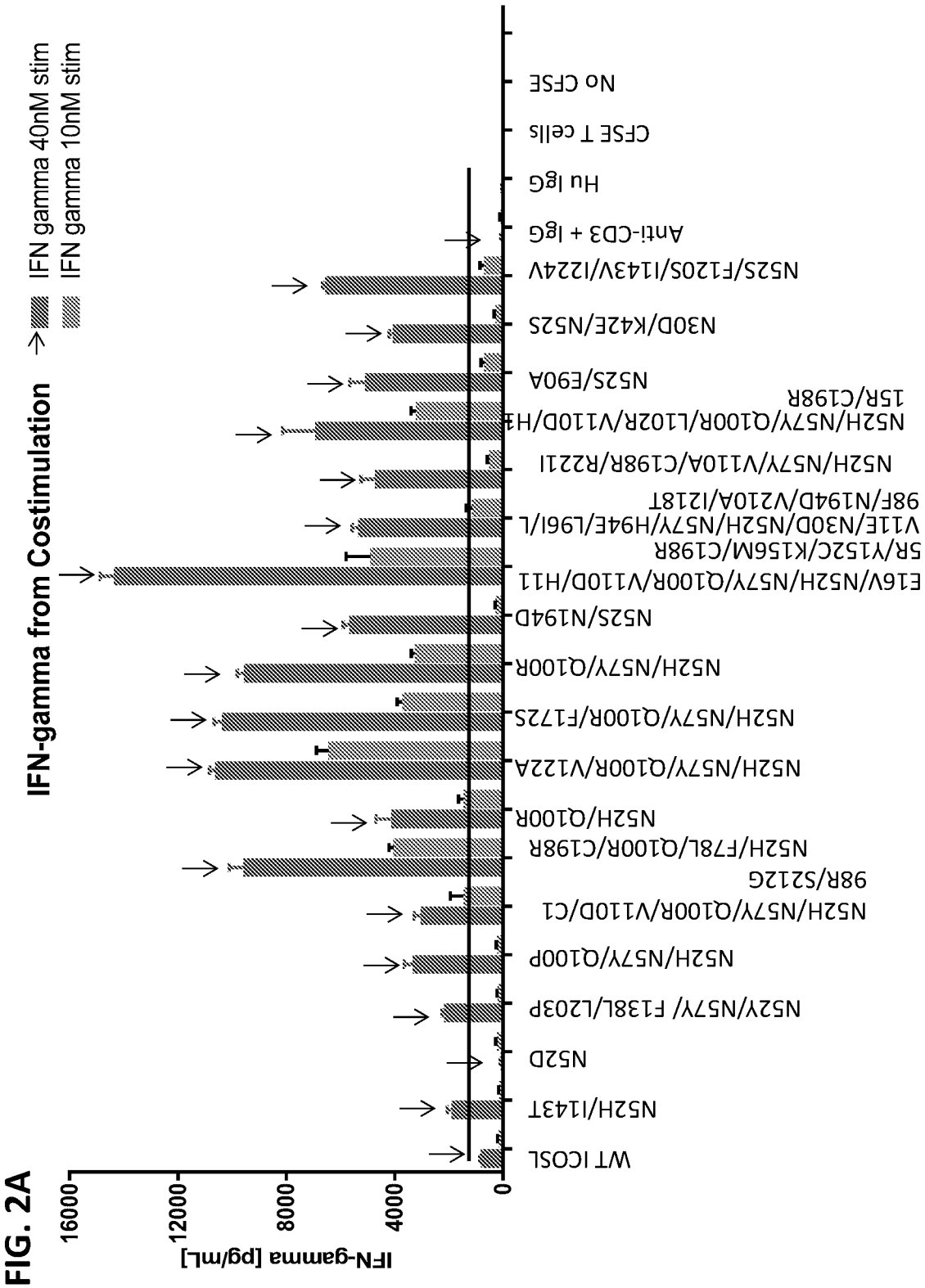
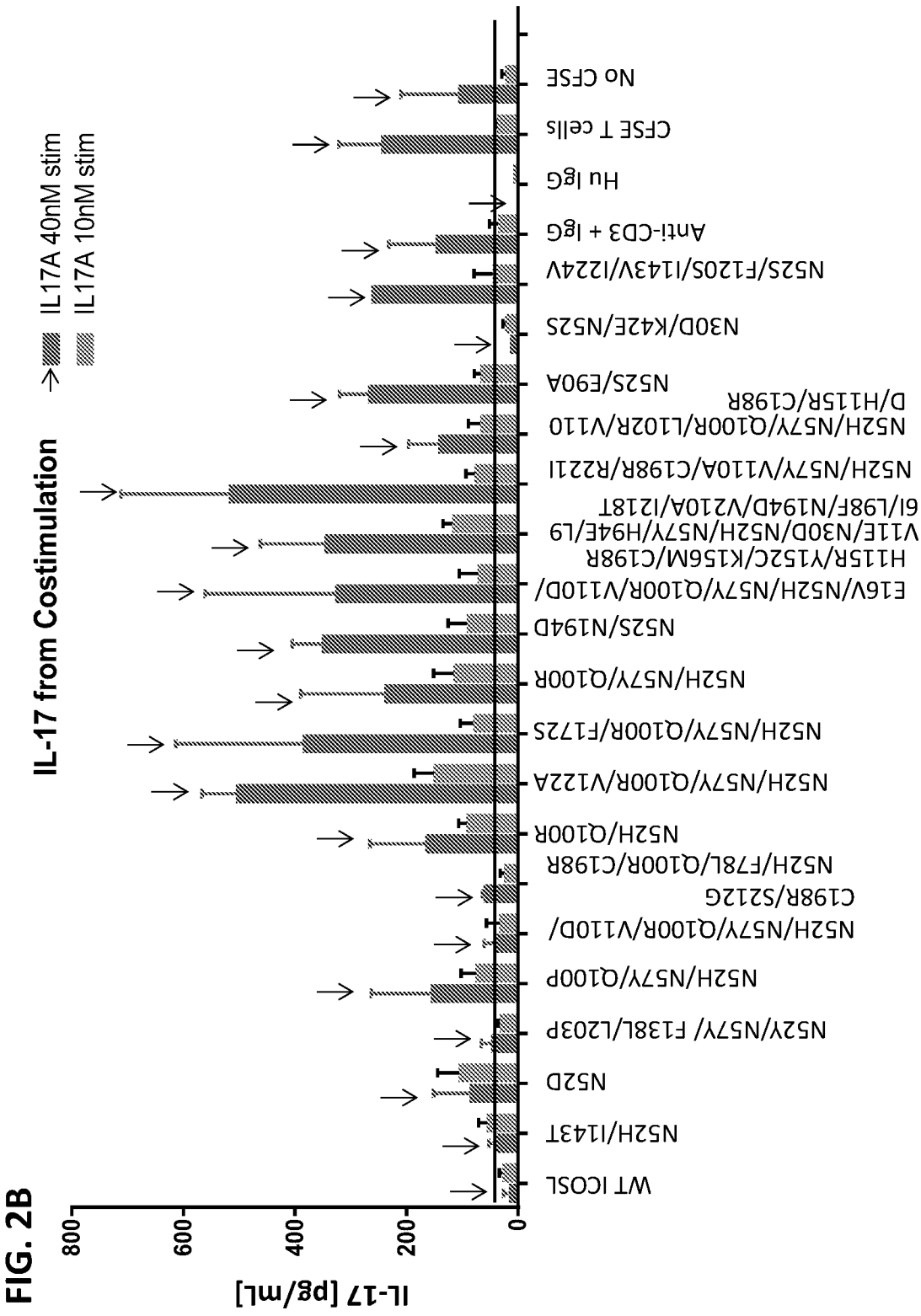

IFN-gamma from Costimulation
IFN gamma 40nM stim
IFN gamma 10nM stim
IFN-gamma [pg/mL]
16000
12000
8000
4000
0
WT ICOSL
N52H/I143T
N52D
N52Y/N57Y/ F138L/L203P
N52H/N57Y/Q100P
N52H/N57Y/Q100R/V110D/C198R/S212G
N52H/F78L/Q100R/C198R
N52H/Q100R
N52H/N57Y/Q100R/V122A
N52H/N57Y/Q100R/F172S
N52H/N57Y/Q100R
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/H115R/Y152C/K156M/C198R
V11E/N30D/N52H/N57Y/H94E/L96I/L98F/N194D/V210A/I218T
N52H/N57Y/V110A/C198R/R221I
N52H/N57Y/Q100R/L102R/V110D/H115R/C198R
N52S/E90A
N30D/K42E/N52S
N52S/F120S/I143V/I224V
Anti-CD3 + IgG
Hu IgG
CFSE T cells
No CFSE

FIG. 2B


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/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

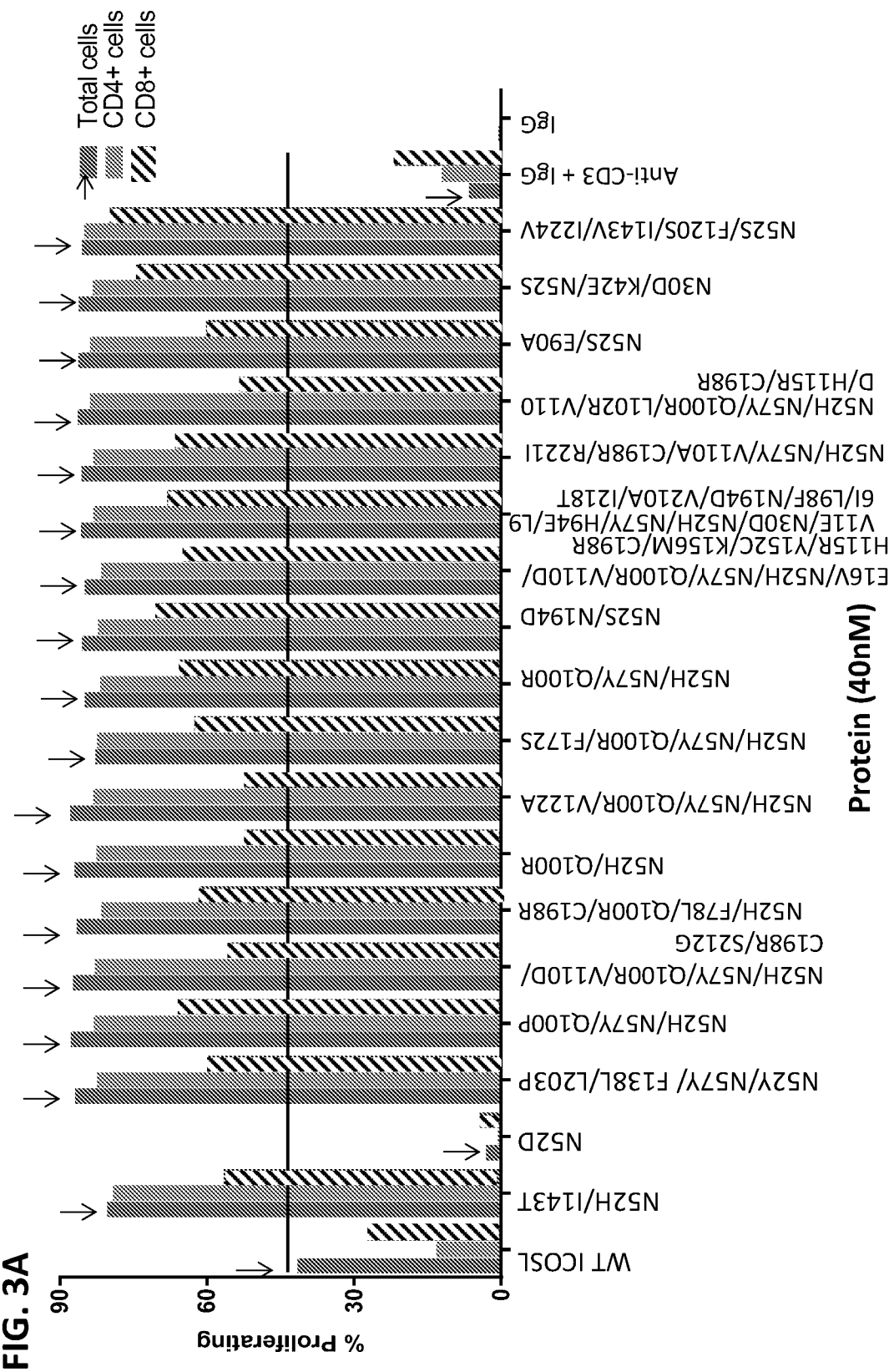
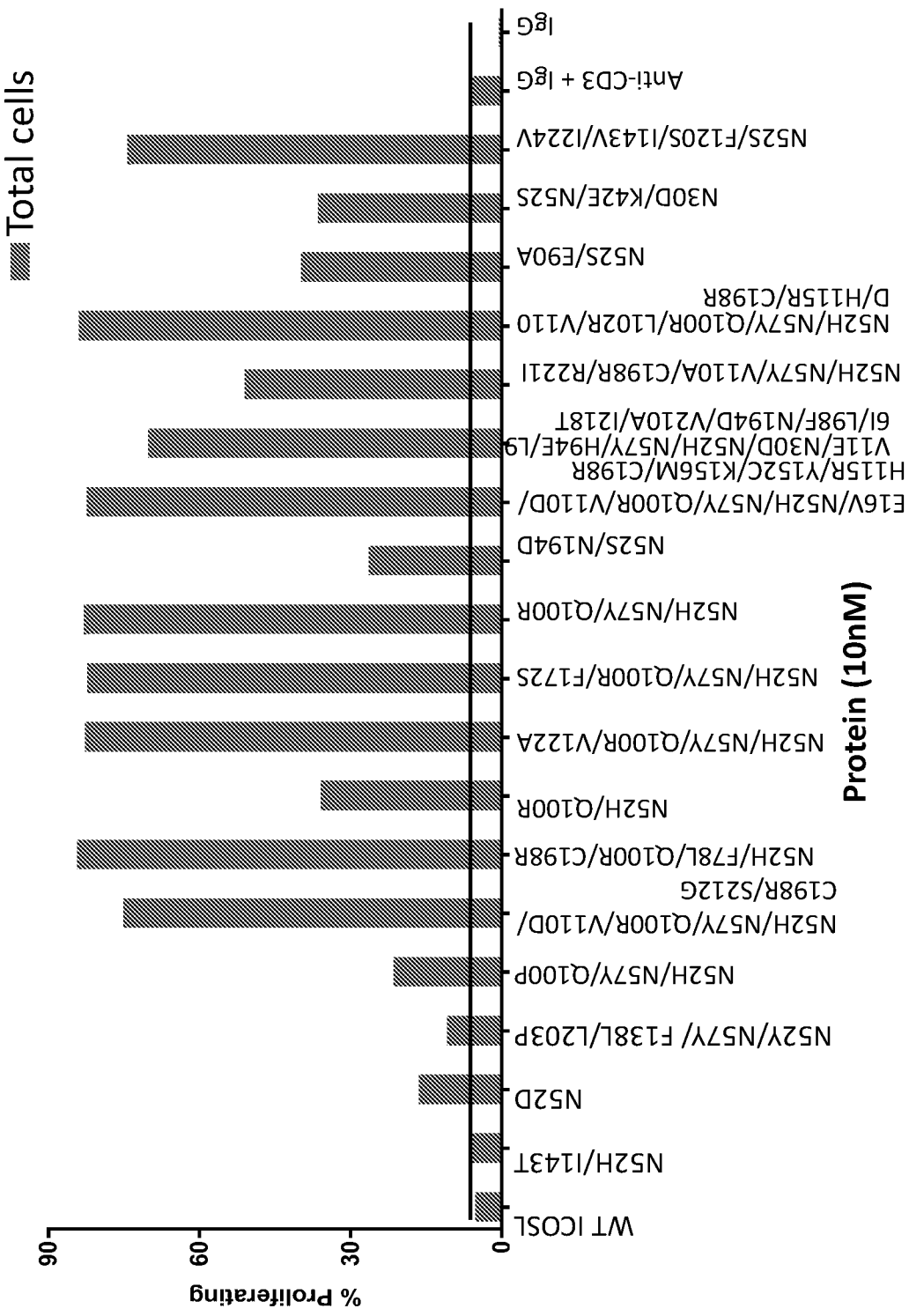


FIG. 3A
% Proliferating
0
30
60
90
Total cells
CD4+ cells
CD8+ cells
WT ICOSL
N52H/I143T
N52D
N52Y/N57Y/ F138L/L203P
N52H/N57Y/Q100P
N52H/N57Y/Q100R/V110D/
C198R/S212G
N52H/F78L/Q100R/C198R
N52H/Q100R
N52H/N57Y/Q100R/V122A
N52H/N57Y/Q100R/F172S
N52H/N57Y/Q100R
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/
H115R/Y152C/K156M/C198R
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
Protein (40nM)

FIG. 3B


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

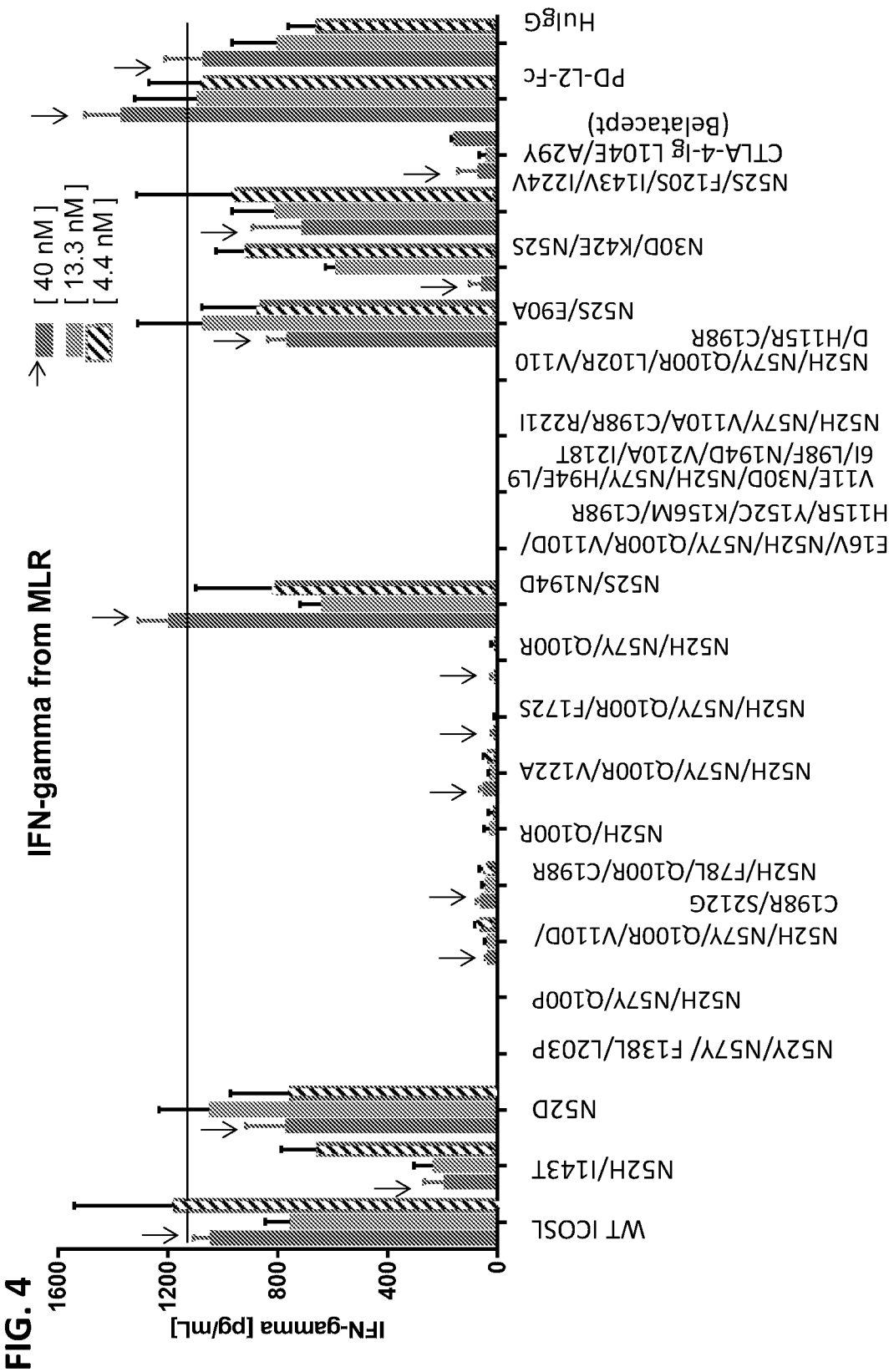


FIG. 4
IFN-gamma from MLR
IFN-gamma [pg/mL]
1600
1200
800
400
0
[40 nM]
[13.3 nM]
[4.4 nM]
WT ICOSL
N52H/I143T
N52D
N52Y/N57Y/ F138L/L203P
N52H/N57Y/Q100P
N52H/N57Y/Q100R/V110D/
C198R/S212G
N52H/F78L/Q100R/C198R
N52H/Q100R
N52H/N57Y/Q100R/V122A
N52H/N57Y/Q100R/F172S
N52H/N57Y/Q100R
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/
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. 5A

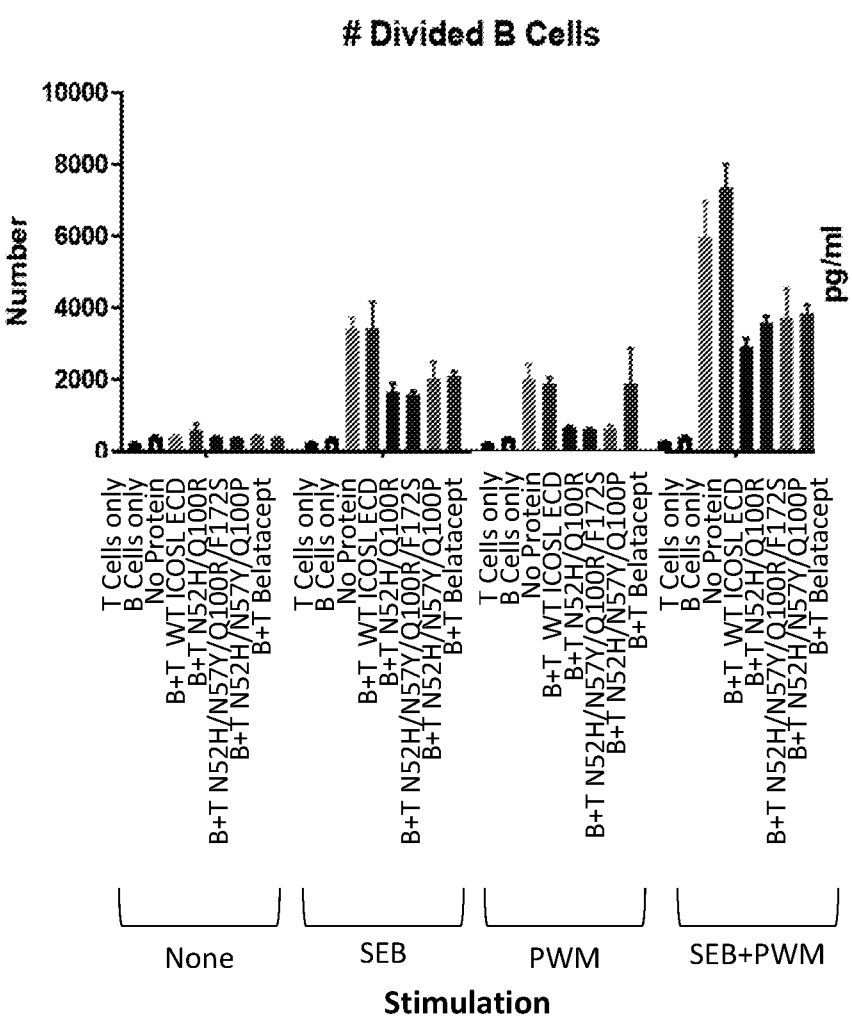

Divided B Cells
Number
10000
8000
6000
4000
2000
0
T Cells only
B Cells only
No Protein
B+T WT ICOSL ECD
B+T N52H/Q100R
B+T N52H/N57Y/Q100R/F172S
B+T N52H/N57Y/Q100P
B+T Belatacept
None
SEB
PWM
SEB+PWM
Stimulation

FIG. 5B

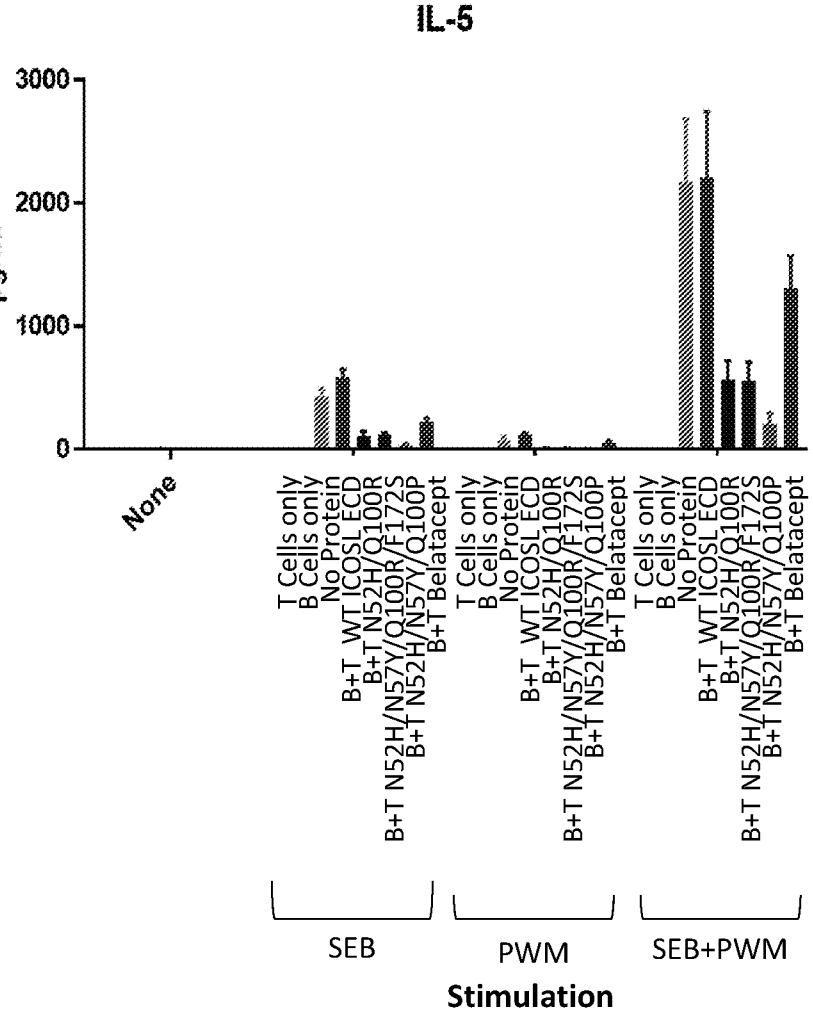

IL-5
pg/ml
3000
2000
1000
0
None
T Cells only
B Cells only
No Protein
B+T WT ICOSL ECD
B+T N52H/Q100R
B+T N52H/N57Y/Q100R/F172S
B+T N52H/N57Y/Q100P
B+T Belatacept
SEB
PWM
SEB+PWM
Stimulation

FIG. 5C

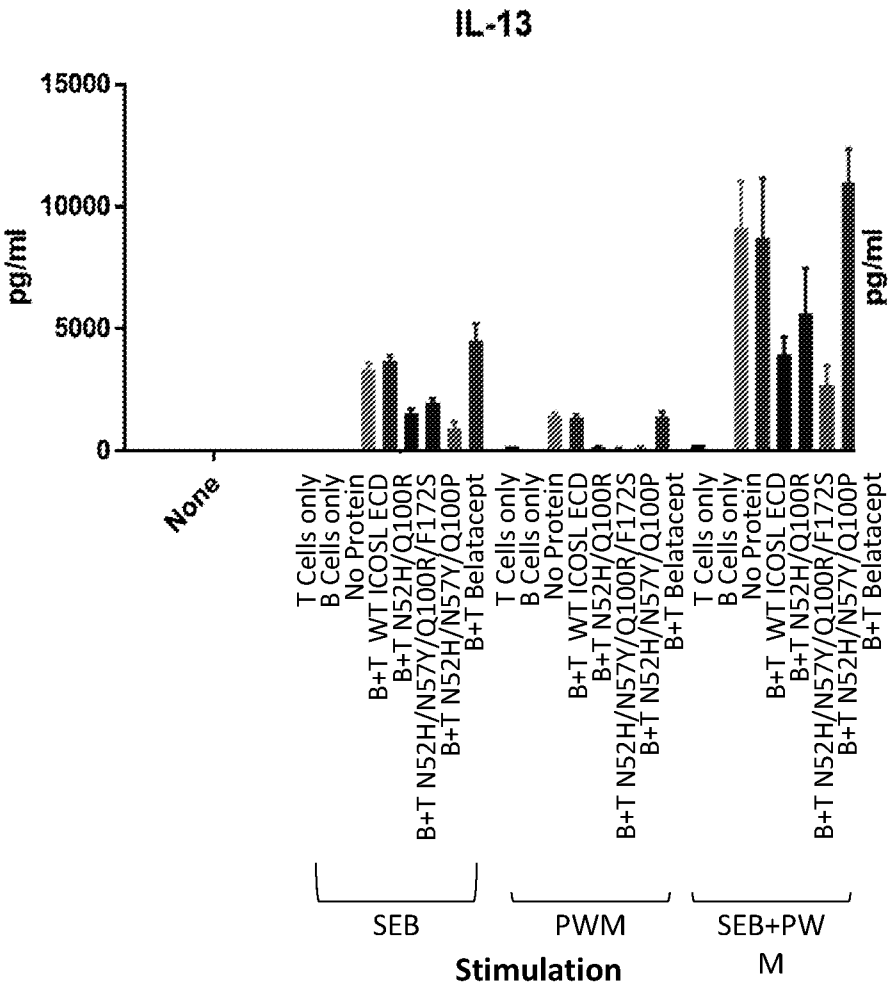

IL-13
pg/ml
15000
10000
5000
0
None
T Cells only
B Cells only
No Protein
B+T WT ICOSL ECD
B+T N52H/Q100R
B+T N52H/N57Y/Q100R/F172S
B+T N52H/N57Y/Q100P
B+T Belatacept
SEB
PWM
SEB+PWM
Stimulation

FIG. 5D

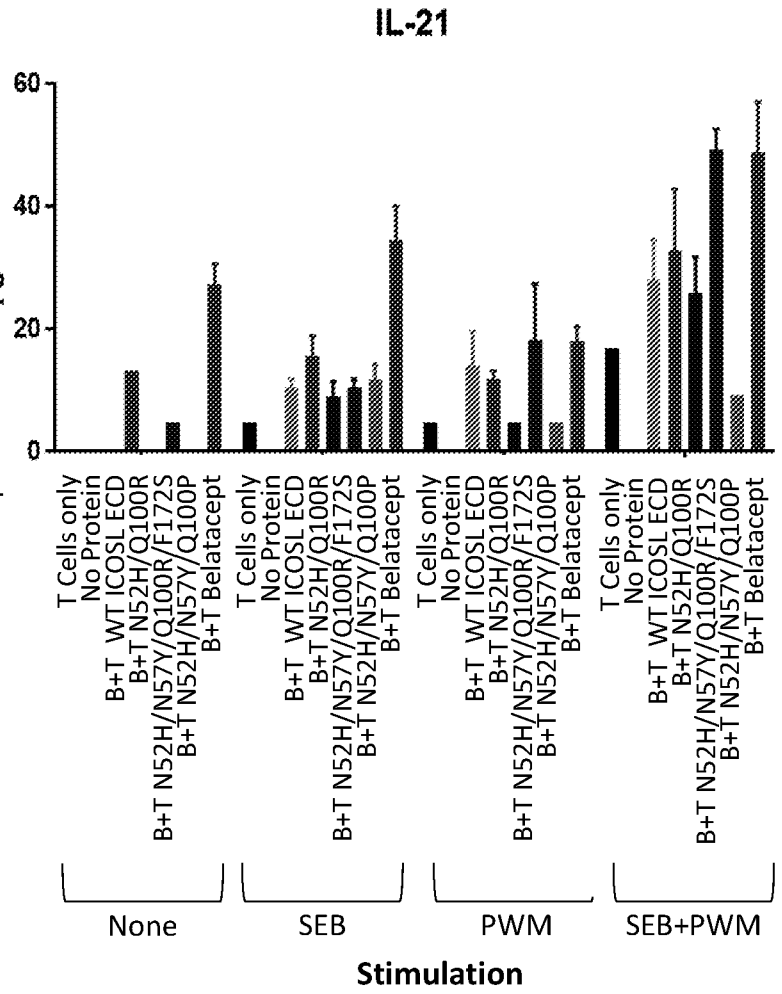

IL-21
pg/ml
60
40
20
0
T Cells only
No Protein
B+T WT ICOSL ECD
B+T N52H/Q100R
B+T N52H/N57Y/Q100R/F172S
B+T N52H/N57Y/Q100P
B+T Belatacept
None
SEB
PWM
SEB+PWM
Stimulation

FIG. 6A

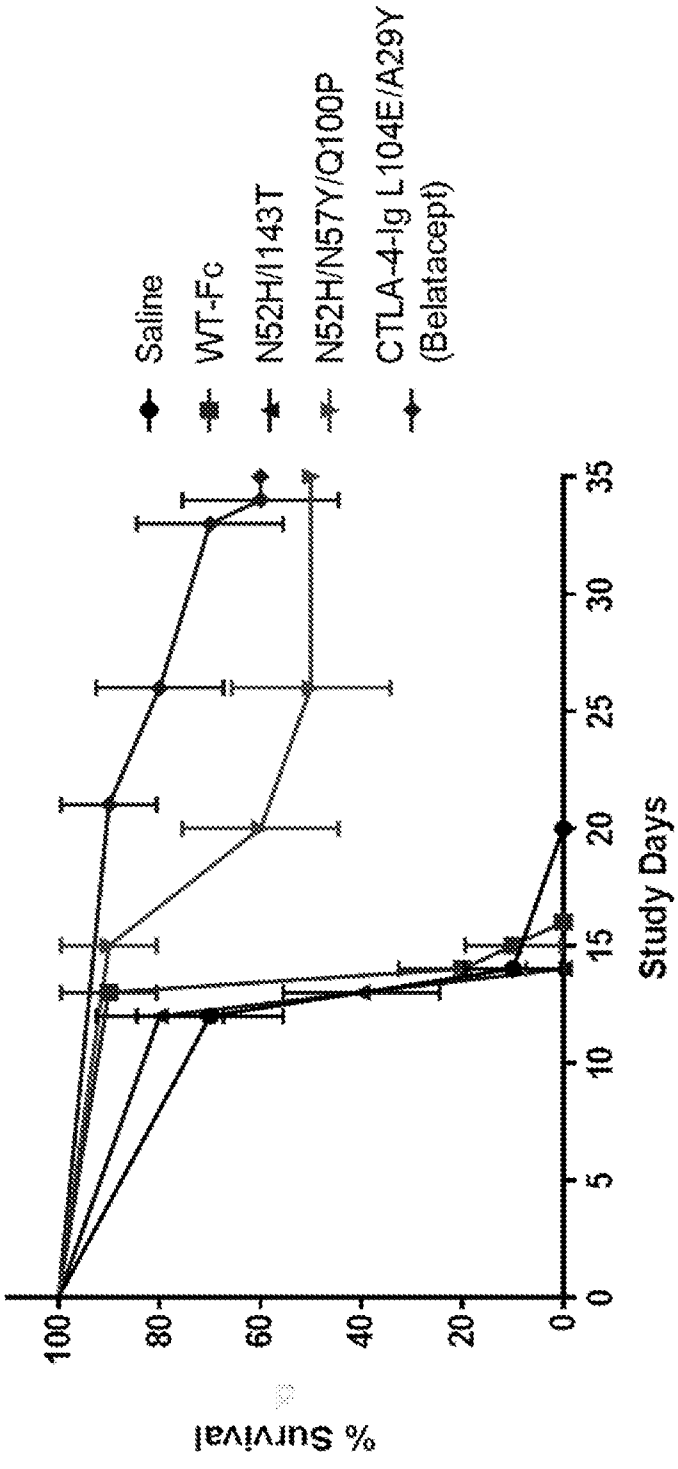

Survival in Hu-PBMC-NSG™♀ GvHD
% Survival
Study Days
0
20
40
60
80
100
0
5
10
15
20
25
30
35
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y
(Belatacept)

FIG. 6B

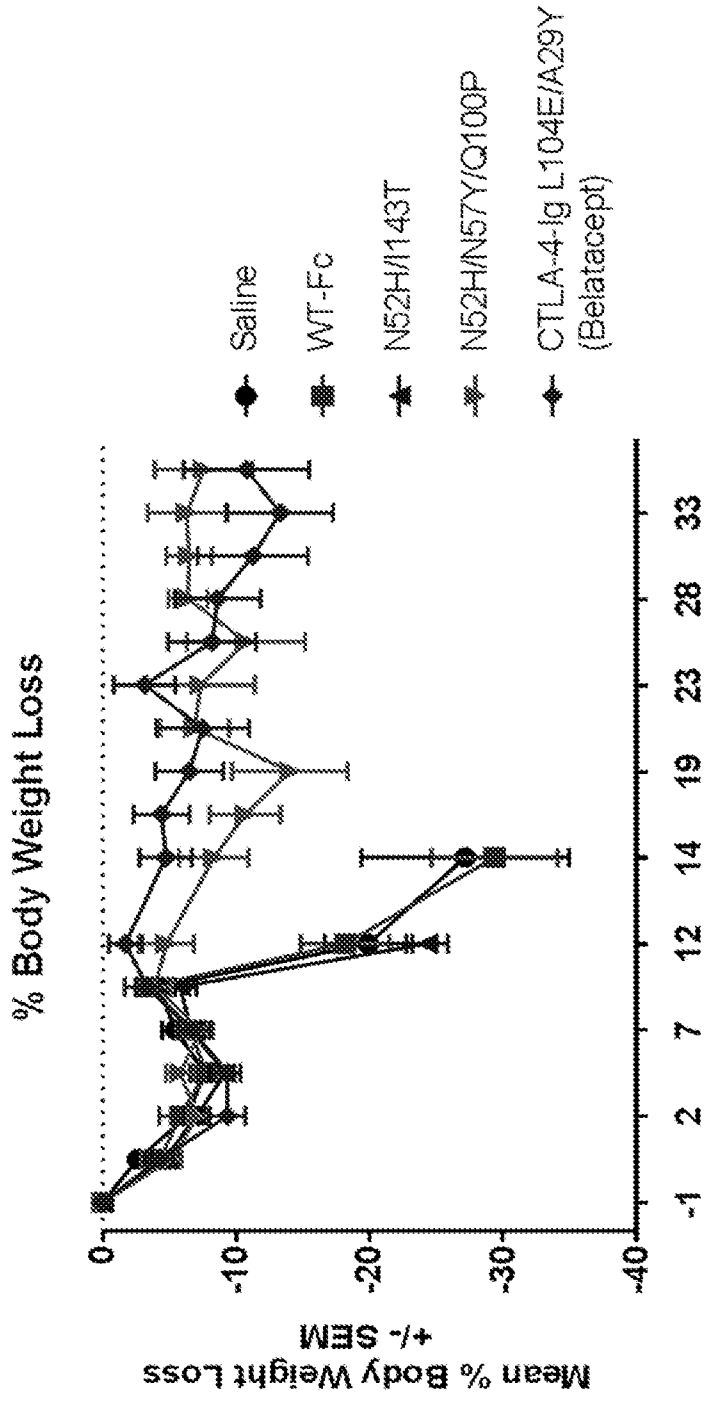

% Body Weight Loss
Mean % Body Weight Loss
+/- SEM
0
-10
-20
-30
-40
-1
2
7
12
14
19
23
28
33
Study Days
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y
(Belatacept)

FIG. 6C

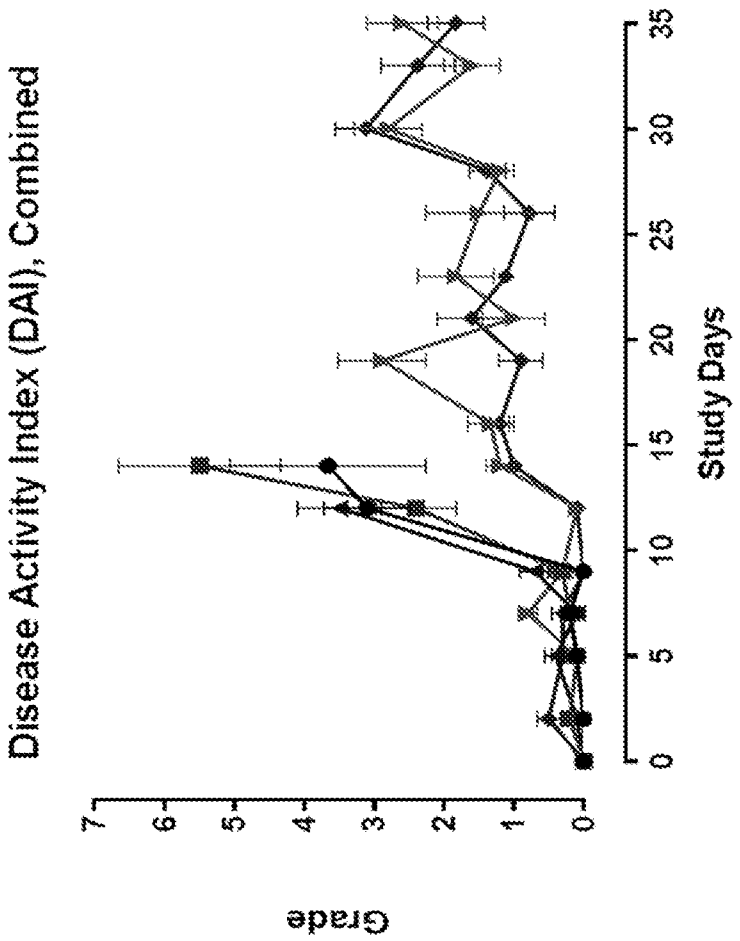

Disease Activity Index (DAI), Combined
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y
(Belatacept)
Grade
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30
35
Study Days

FIG. 6D

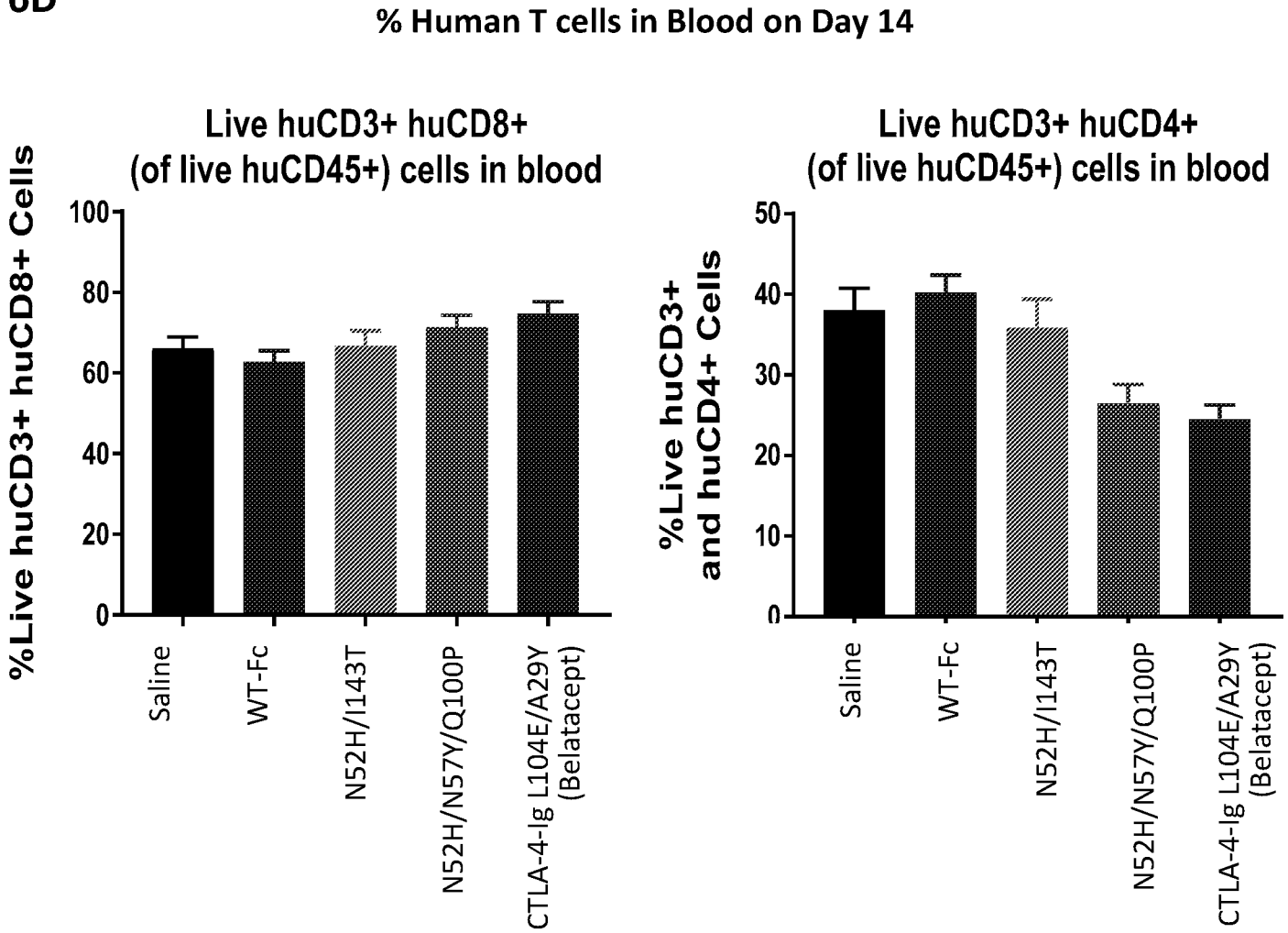

% Human T cells in Blood on Day 14
Live huCD3+ huCD8+
(of live huCD45+) cells in blood
%Live huCD3+ huCD8+ Cells
100
80
60
40
20
0
Saline
WT-Fc
N52H/I143T
N52H/N57Y/Q100P
CTLA-4-Ig L104E/A29Y
(Belatacept)
Live huCD3+ huCD4+
(of live huCD45+) cells in blood
%Live huCD3+
and huCD4+ Cells
50
40
30
20
10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

FIG. 6E

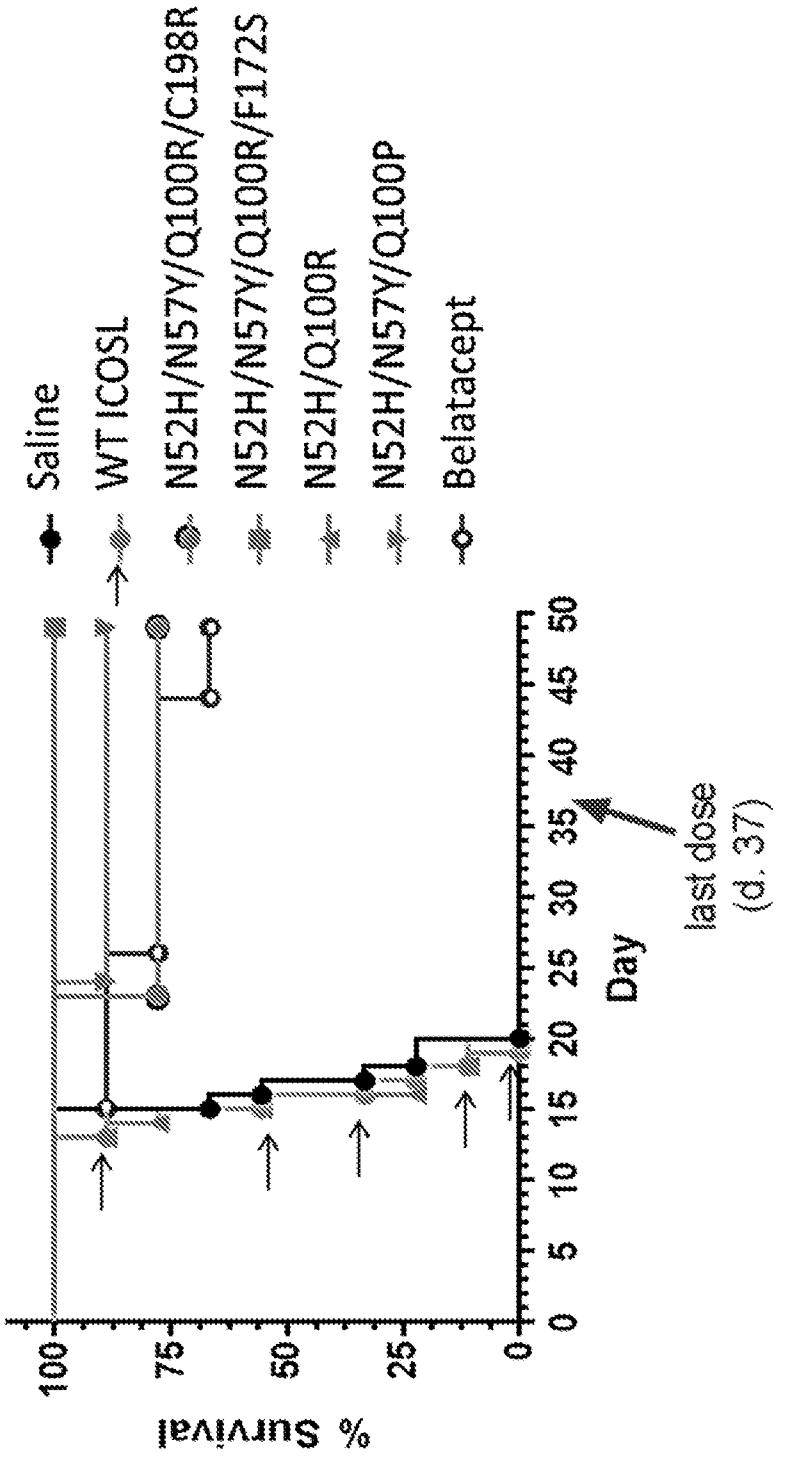

HuPBMC-->NSG GVHD
% Survival
100
75
50
25
0
0
5
10
15
20
25
30
35
40
45
50
Day
last dose
(d. 37)
Saline
WT ICOSL
N52H/N57Y/Q100R/C198R
N52H/N57Y/Q100R/F172S
N52H/Q100R
N52H/N57Y/Q100P
Belatacept

FIG. 6F

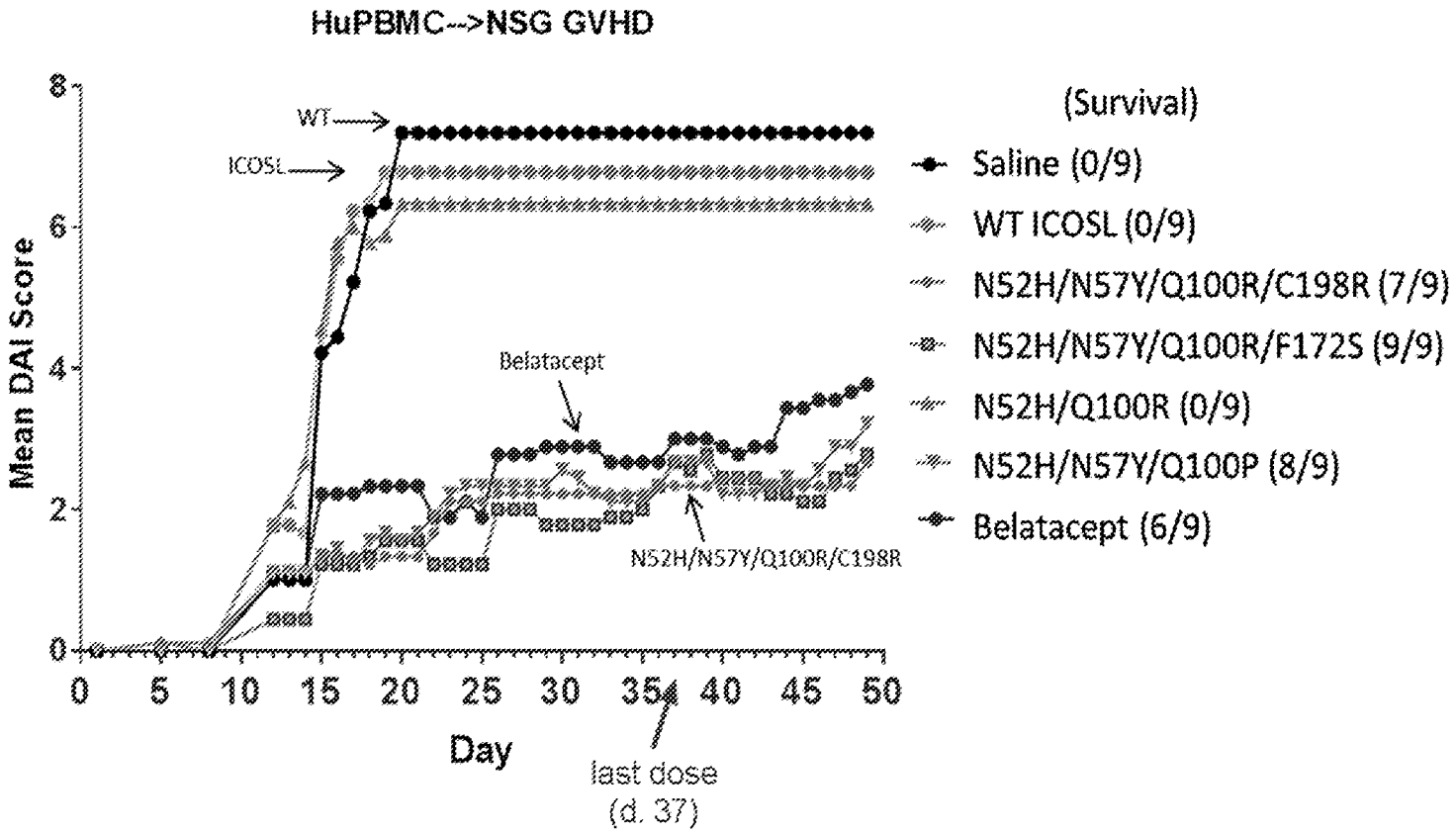

HuPBMC-->NSG GVHD
Mean DAI Score
0
2
4
6
8
0
5
10
15
20
25
30
35
40
45
50
Day
WT
ICOSL
Belatacept
N52H/N57Y/Q100R/C198R
last dose
(d. 37)
(Survival)
Saline (0/9)
WT ICOSL (0/9)
N52H/N57Y/Q100R/C198R (7/9)
N52H/N57Y/Q100R/F172S (9/9)
N52H/Q100R (0/9)
N52H/N57Y/Q100P (8/9)
Belatacept (6/9)

FIG. 7

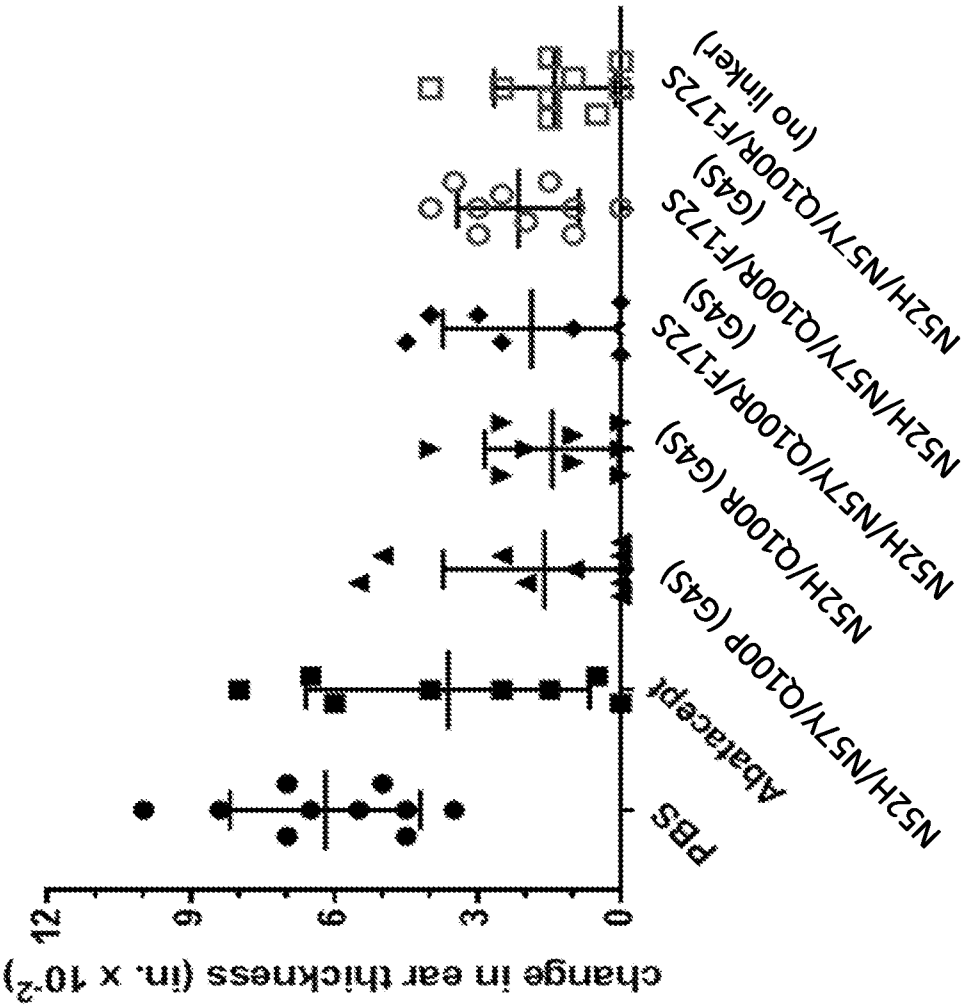

change in ear thickness (in. x 10^{-2})
12
9
6
3
0
PBS
Abatacept
N52H/N57Y/Q100P (G4S)
N52H/Q100R (G4S)
N52H/N57Y/Q100R/F172S (G4S)
N52H/N57Y/Q100R/F172S (G4S)
N52H/N57Y/Q100R/F172S (no linker)

FIG. 8

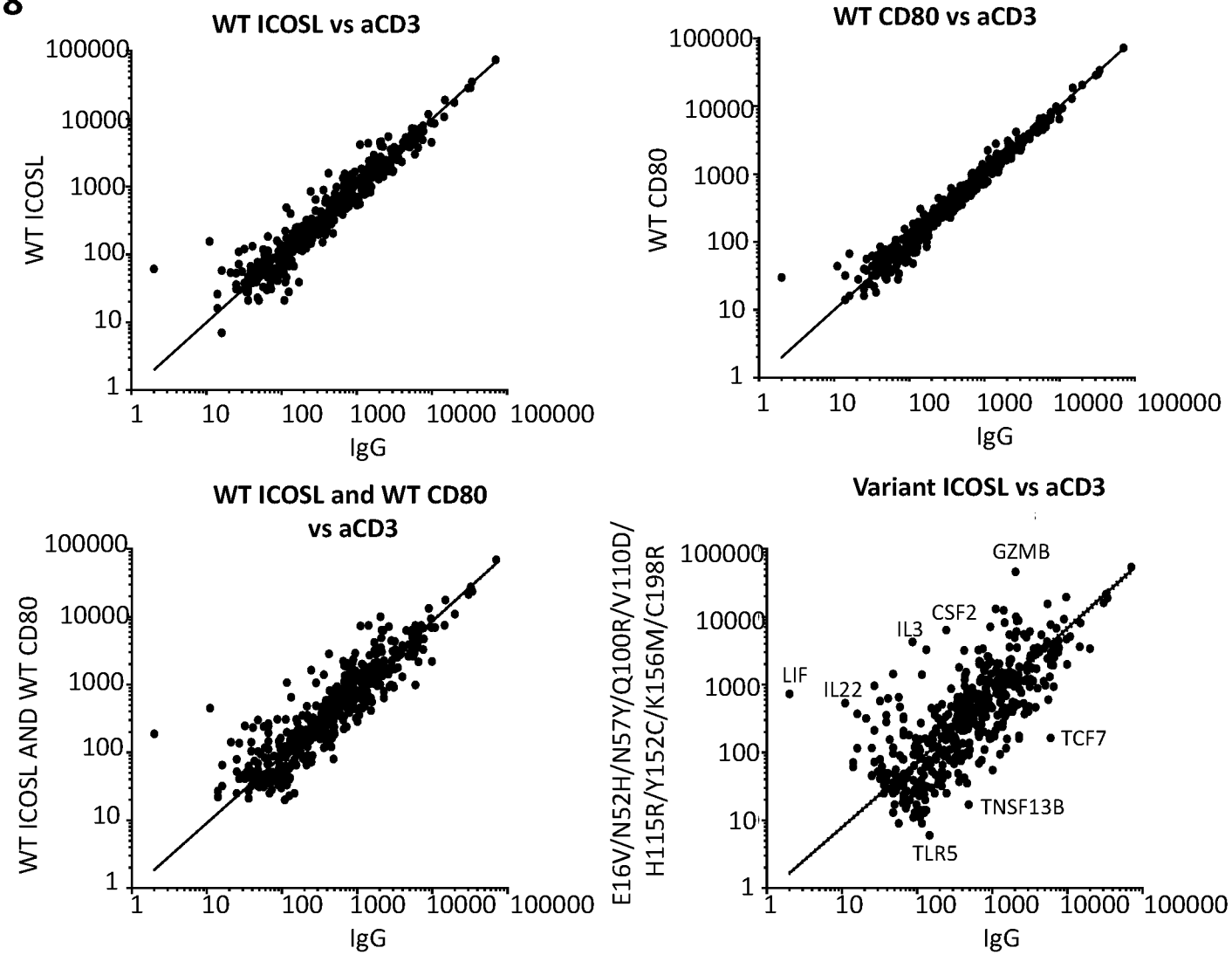

WT ICOSL vs aCD3
WT ICOSL
IgG
WT CD80 vs aCD3
WT CD80
IgG
WT ICOSL and WT CD80 vs aCD3
WT ICOSL AND WT CD80
IgG
Variant ICOSL vs aCD3
E16V/N52H/N57Y/Q100R/V110D/ H115R/Y152C/K156M/C198R
IgG
GZMB
CSF2
IL3
LIF
IL22
TCF7
TNSF13B
TLR5
1
10
100
1000
10000
100000

FIG. 9

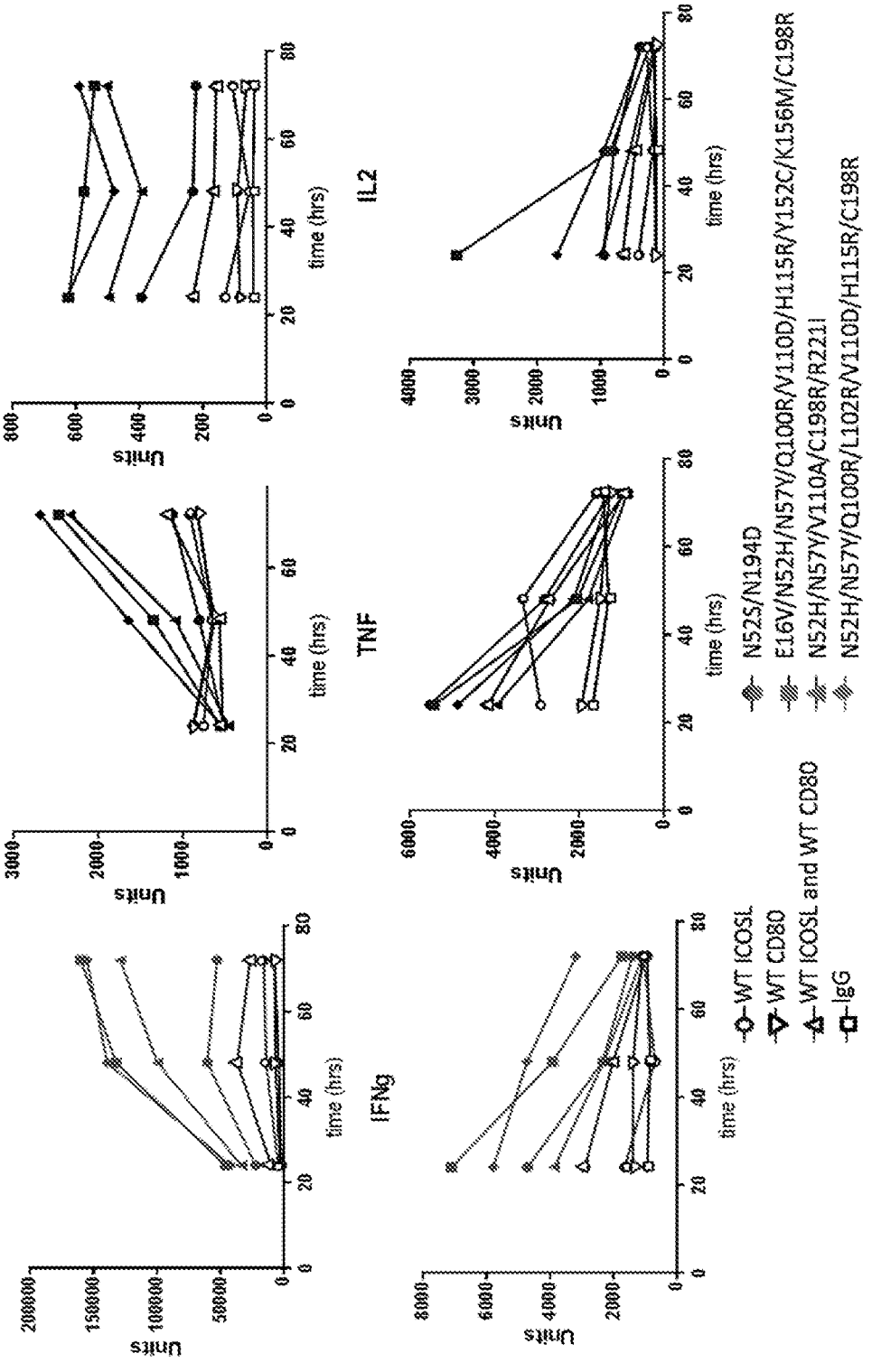

GZMB
PRF1
IL21
IFNg
TNF
IL2
Units
time (hrs)
WT ICOSL
WT CD80
WT ICOSL and WT CD80
IgG
N52S/N194D
E16V/N52H/N57Y/Q100R/V110D/H115R/Y152C/K156M/C198R
N52H/N57Y/V110A/C198R/R221I
N52H/N57Y/Q100R/L102R/V110D/H115R/C198R

FIG. 10A

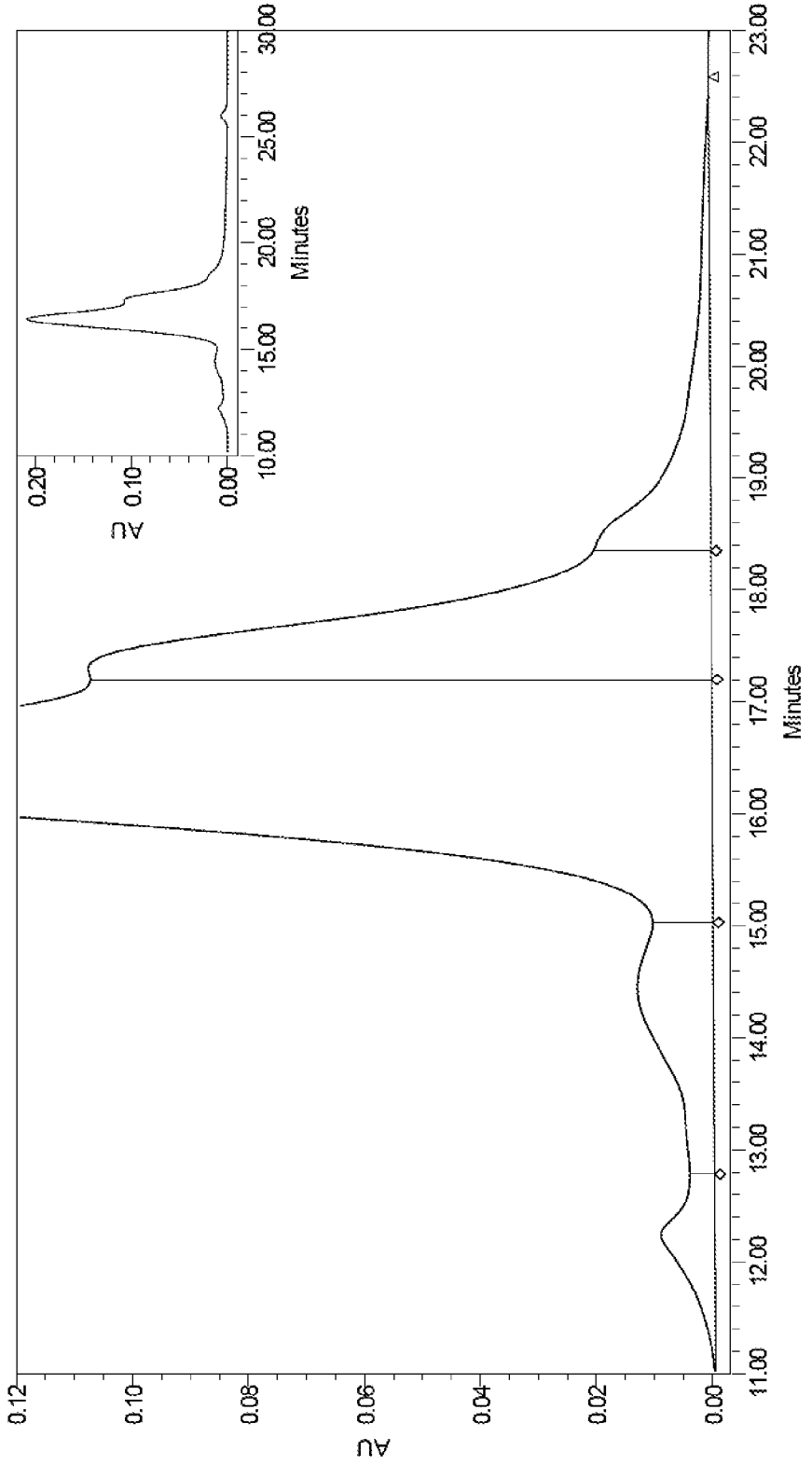

ICOSL variant ECD (SEQ ID NO: 1) with N52H/N57Y/Q100R/F172S
AU
Minutes
0.12
0.10
0.08
0.06
0.04
0.02
0.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
21.00
22.00
23.00
0.20
0.10
0.00
10.00
15.00
20.00
25.00
30.00

FIG. 10B

ICOSL variant truncation #4 (SEQ ID NO: 7) with N52H/N57Y/Q100R/F172S

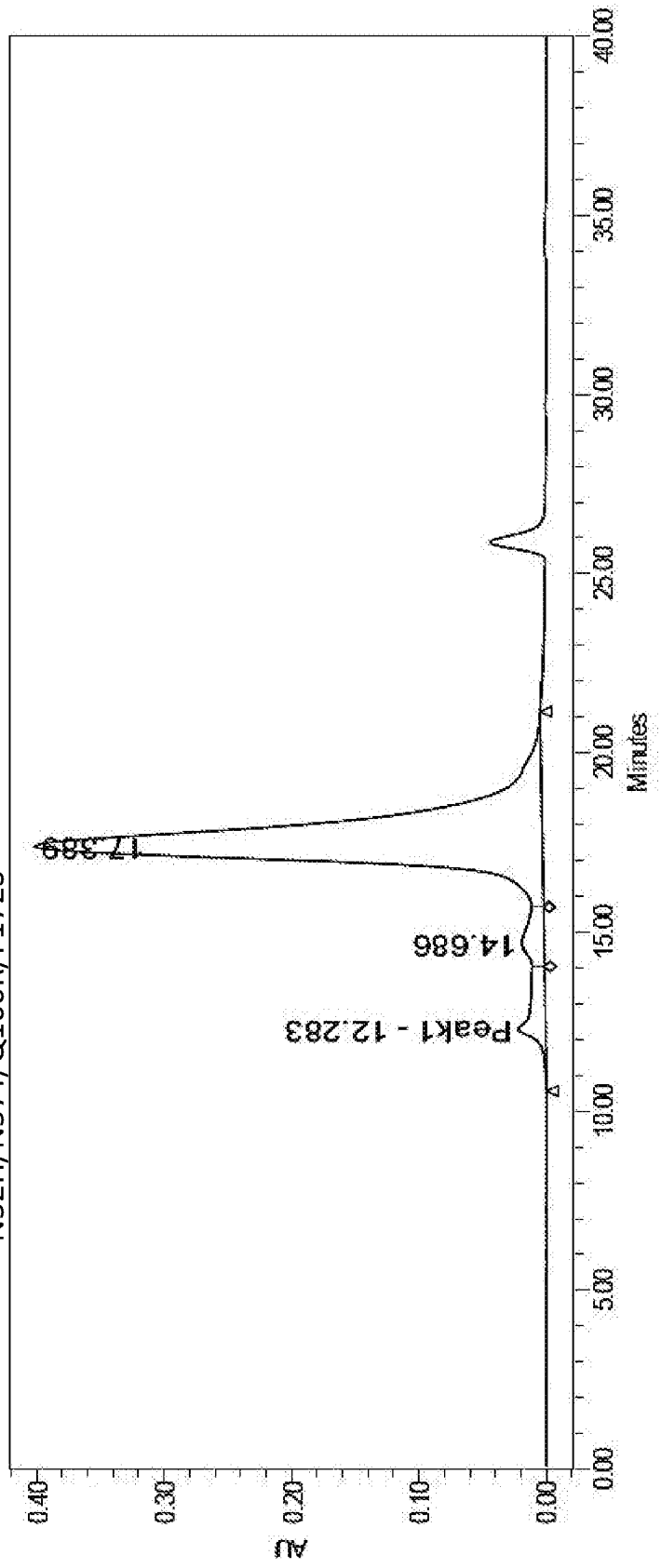


FIG. 10C

ICOSL variant truncation #5 (SEQ ID NO: 8) with N52H/N57Y/Q100R/F172S

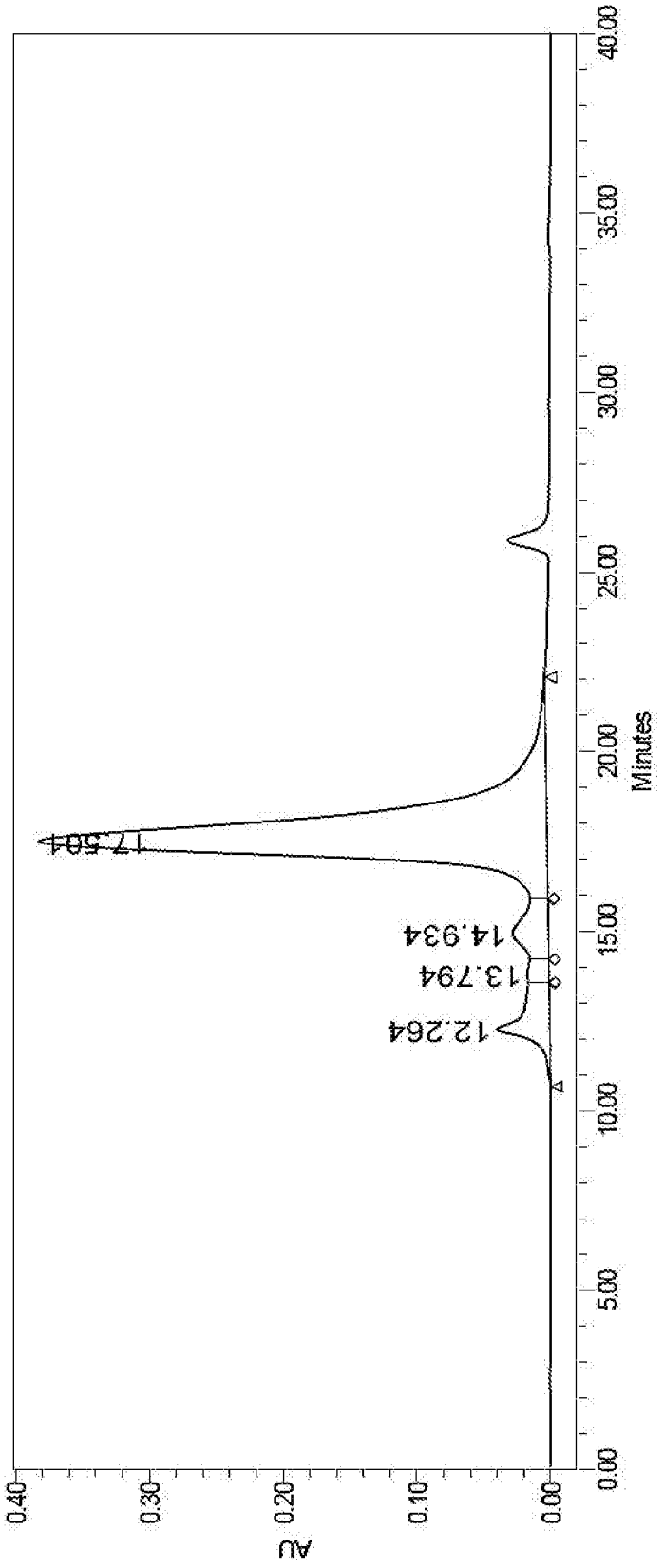


FIG. 10D

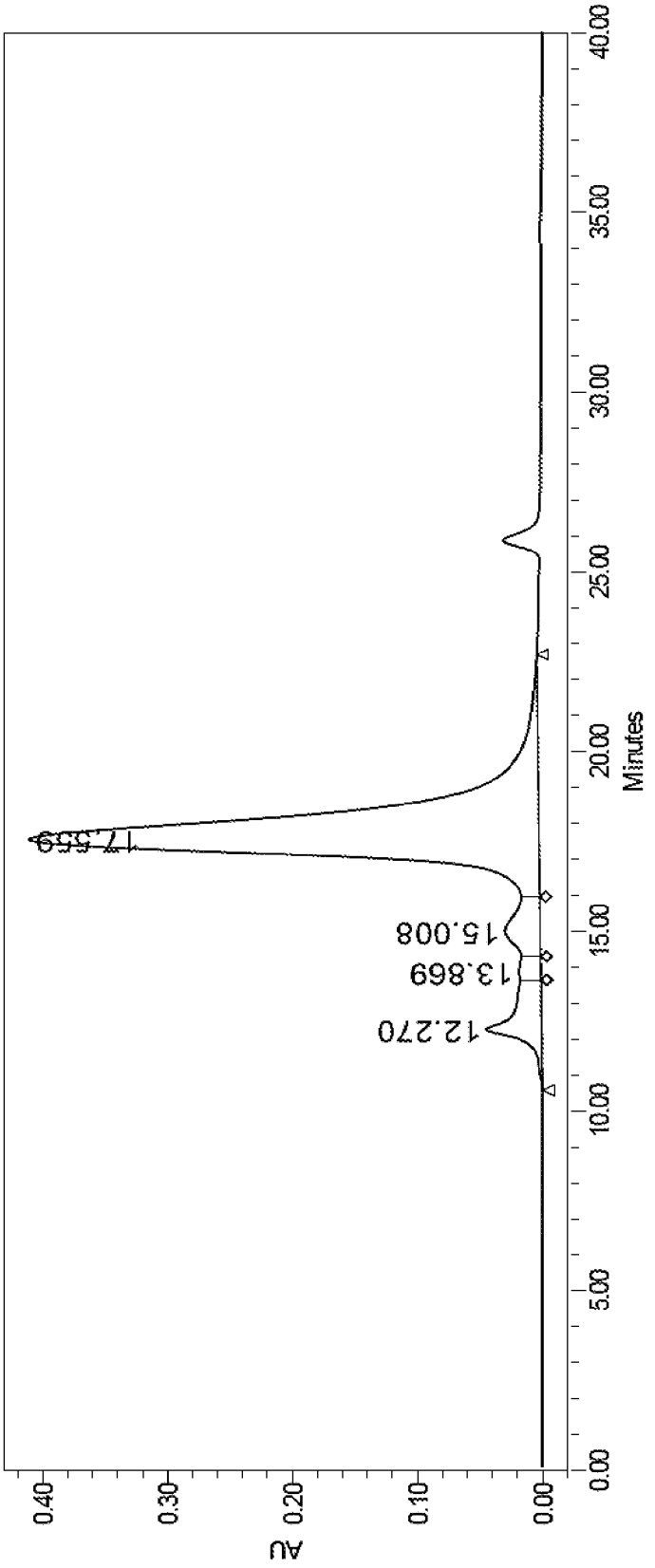

ICOSL variant truncation #6 (SEQ ID NO: 9) with N52H/N57Y/Q100R/F172S
AU
0.00
0.10
0.20
0.30
0.40
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Minutes
17.559
15.008
13.869
12.270

FIG. 10E

ICOSL variant truncation #8 with mutations N207G/L208G (SEQ ID NO: 28)
and with N52H/N57Y/Q100R/F172S

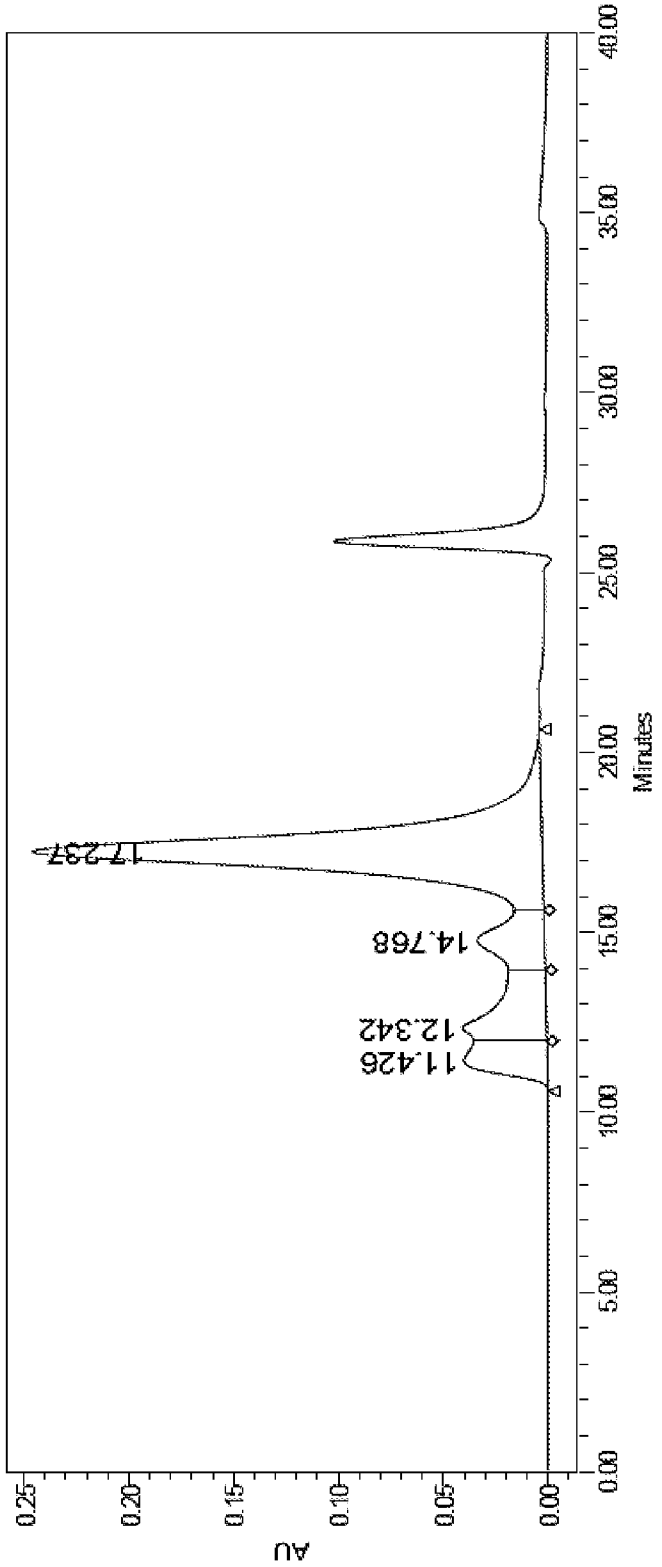


FIG. 10F

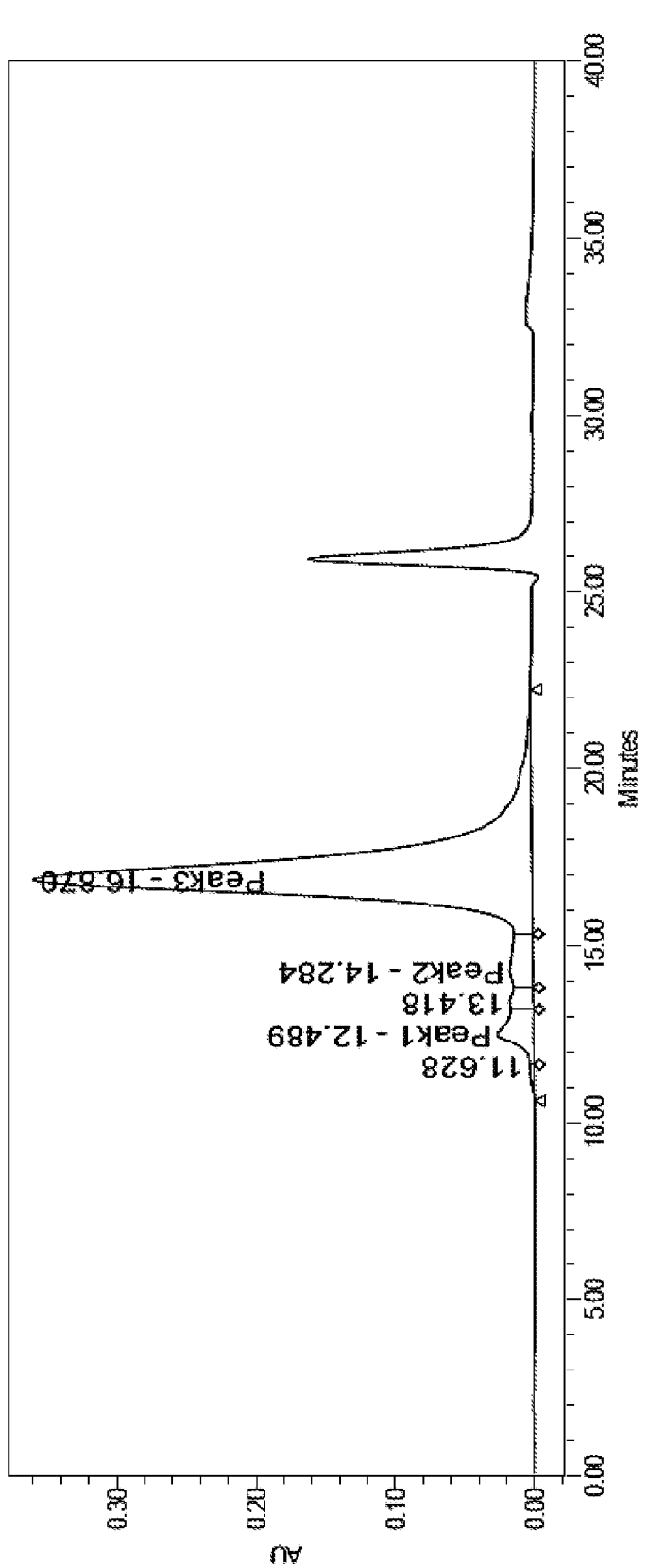

ICOSL variant ECD with mutations N207G/L208G (SEQ ID NO: 33) with N52H/N57Y/Q100R/F172S
Peak3 - 16.870
Peak2 - 14.284
13.418
Peak1 - 12.489
11.628
AU
0.30
0.20
0.10
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Minutes

FIG. 10G

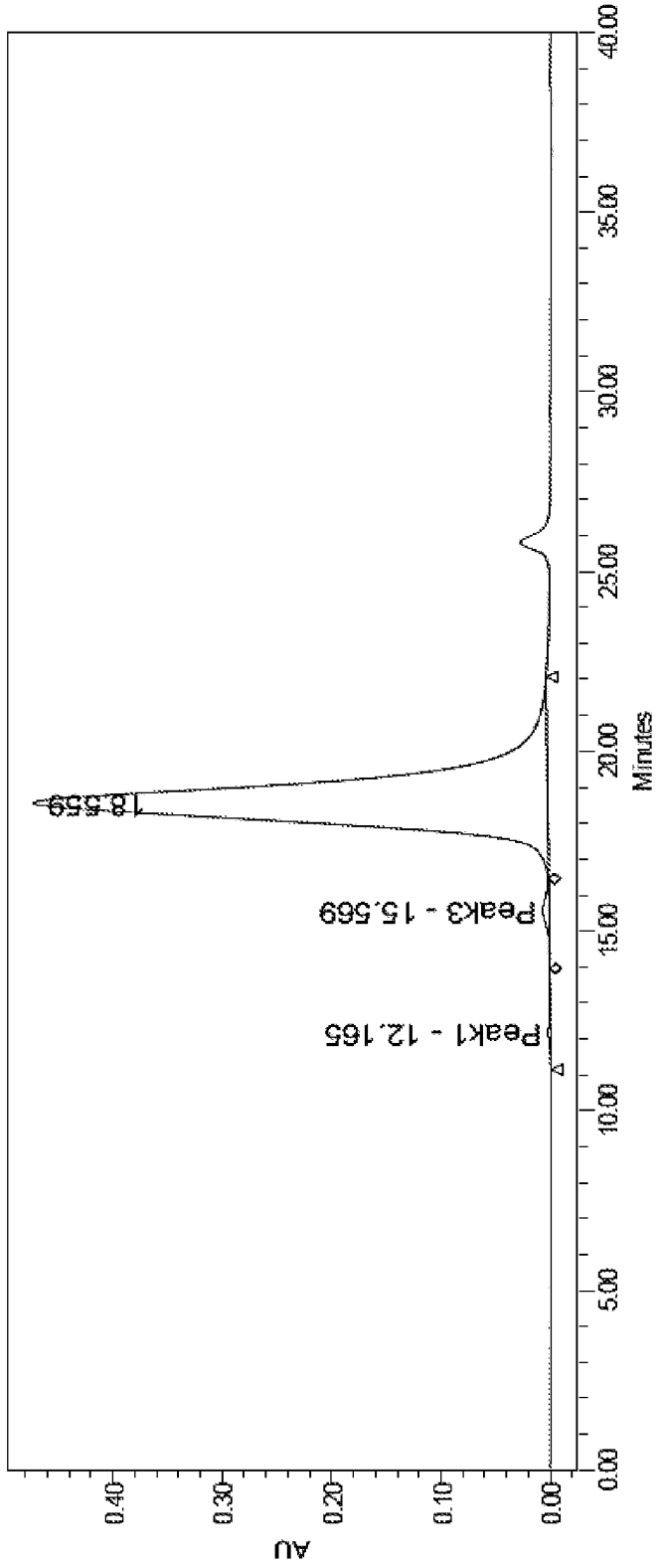

ICOSL variant IgV (SEQ ID NO: 3) with N52H/N57Y/Q100R/F172S
AU
0.00
0.10
0.20
0.30
0.40
Minutes
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Peak1 - 12.165
Peak3 - 15.569
18.559

FIG. 10H

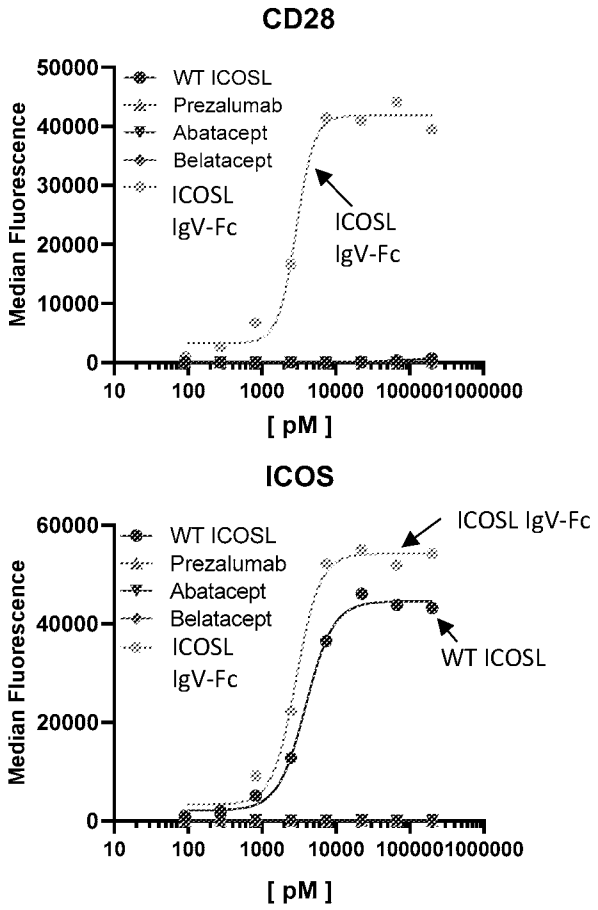


FIG. 10I

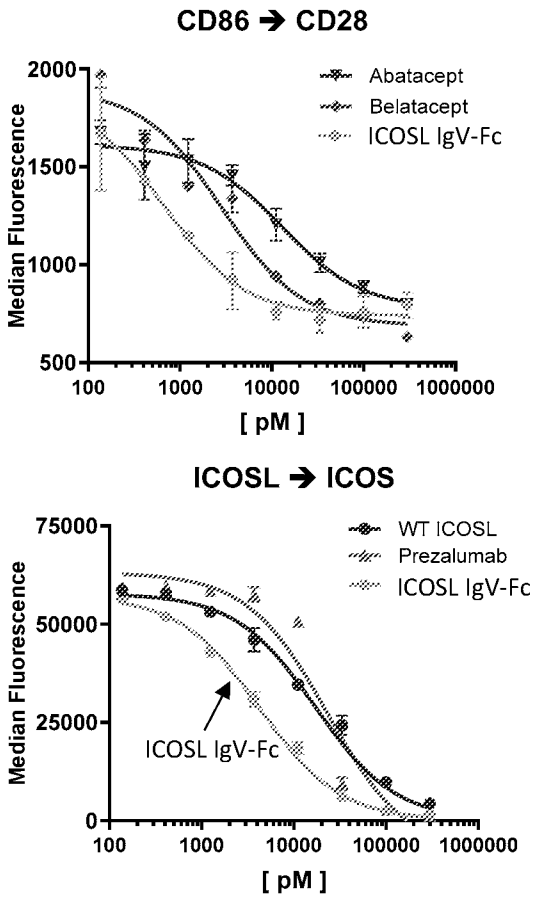


FIG. 10J

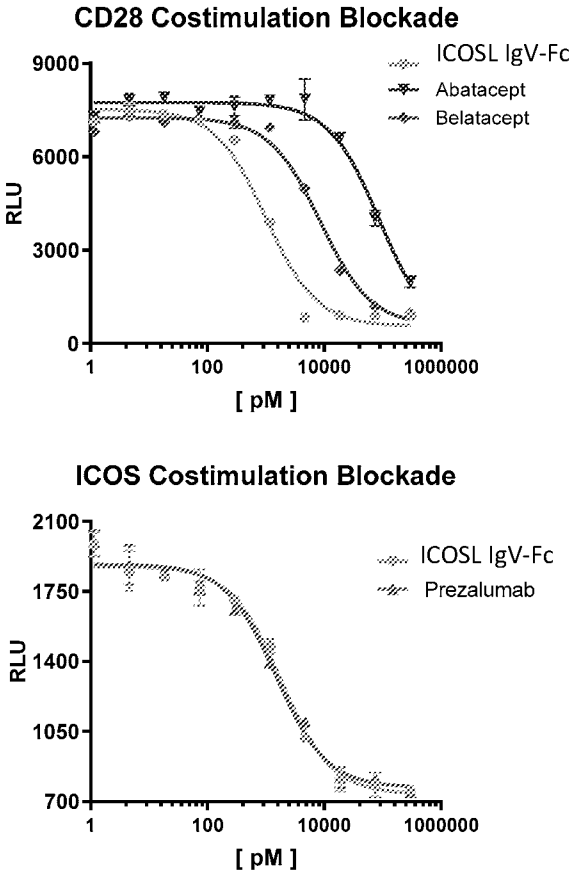


FIG. 11A

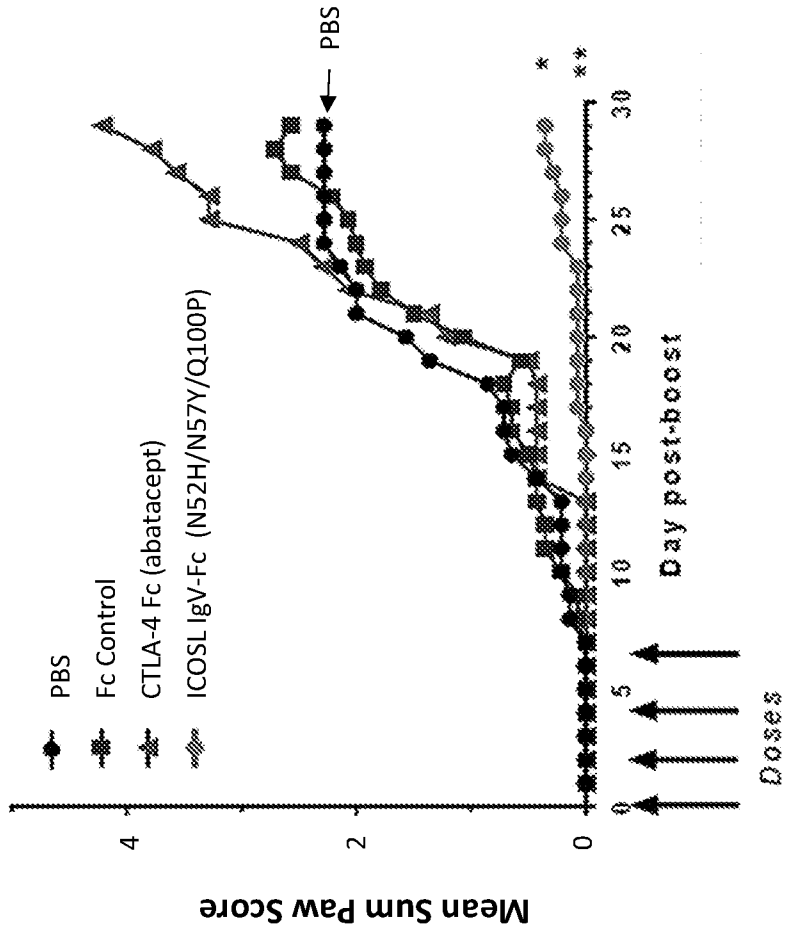

CIA Paw Scores Over Time
PBS
Fc Control
CTLA-4 Fc (abatacept)
ICOSL IgV-Fc (N52H/N57Y/Q100P)
Mean Sum Paw Score
0
2
4
0
5
10
15
20
25
30
Day post-boost
Doses
PBS
*
**

FIG. 11B

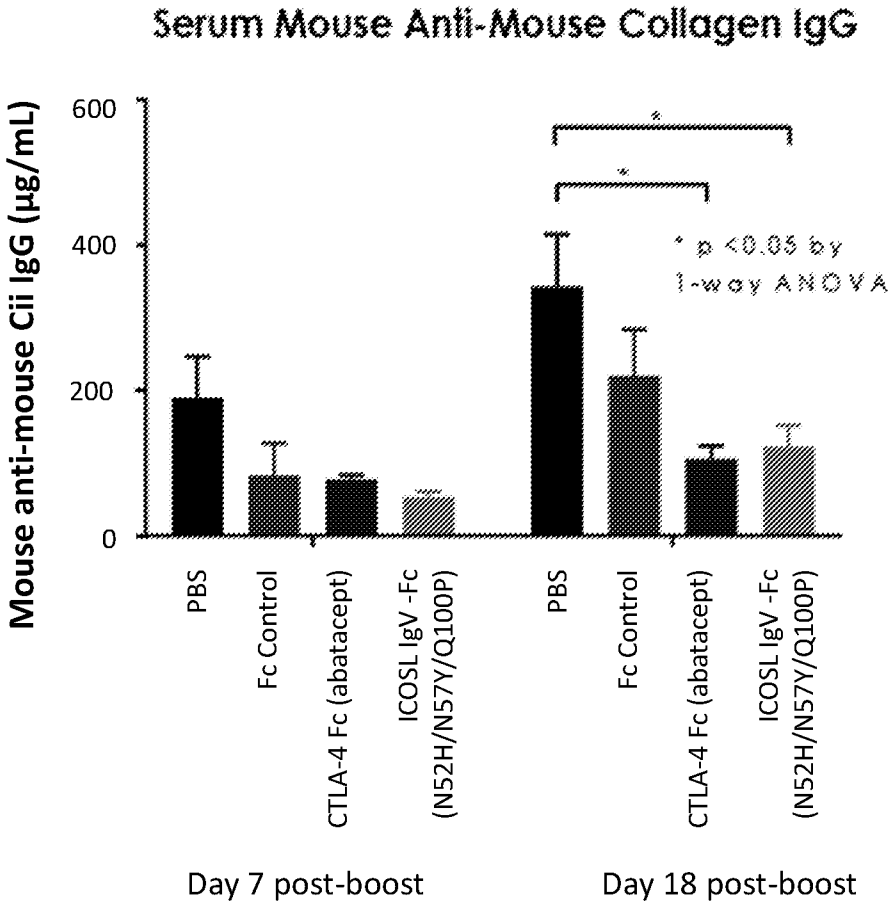

Serum Mouse Anti-Mouse Collagen IgG
Mouse anti-mouse Cii IgG (µg/mL)
600
400
200
0
* p <0.05 by 1-way ANOVA
PBS
Fc Control
CTLA-4 Fc (abatacept)
ICOSL IgV -Fc (N52H/N57Y/Q100P)
PBS
Fc Control
CTLA-4 Fc (abatacept)
ICOSL IgV -Fc (N52H/N57Y/Q100P)
Day 7 post-boost
Day 18 post-boost

FIG. 11C

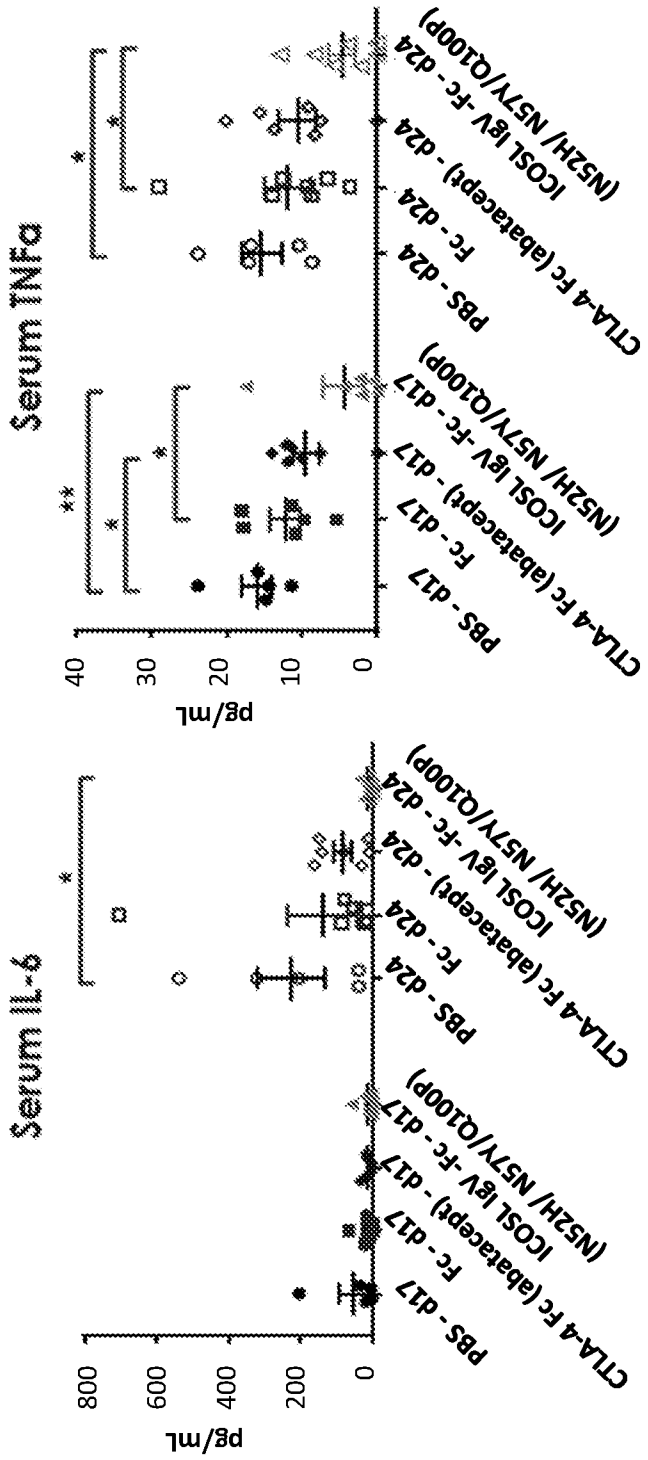

Serum IL-6
pg/mL
0
200
400
600
800
PBS - d17
Fc - d17
ICOSL IgV -Fc - d17
CTLA-4 Fc (abatacept) - d17
(N52H/ N57V/Q100P)
PBS - d24
Fc - d24
ICOSL IgV -Fc - d24
CTLA-4 Fc (abatacept) - d24
(N52H/ N57V/Q100P)
Serum TNFa
pg/mL
0
10
20
30
40
PBS - d17
Fc - d17
ICOSL IgV -Fc - d17
CTLA-4 Fc (abatacept) - d17
(N52H/ N57V/Q100P)
PBS - d24
Fc - d24
ICOSL IgV -Fc - d24
CTLA-4 Fc (abatacept) - d24
(N52H/ N57V/Q100P)

FIG. 11D

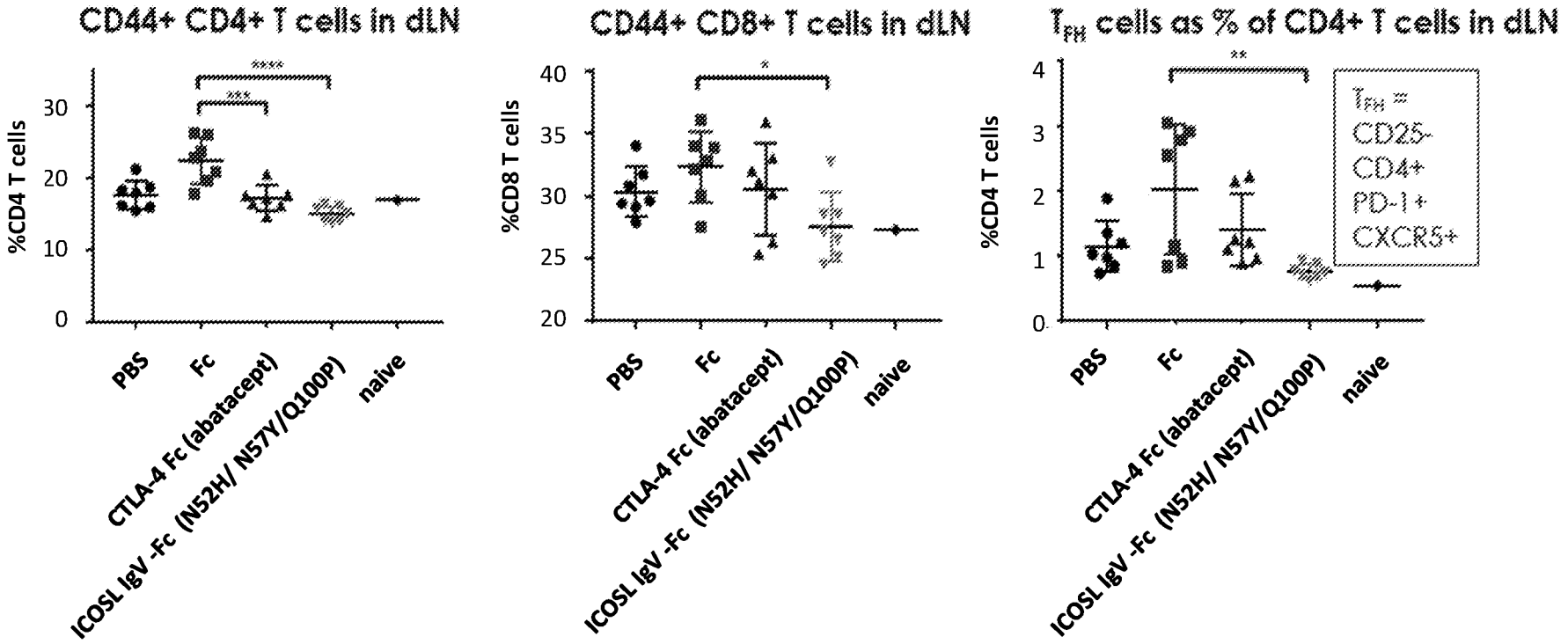

CD44+ CD4+ T cells in dLN
%CD4 T cells
0
10
20
30
****
***
PBS
Fc
CTLA-4 Fc (abatacept)
ICOSL IgV -Fc (N52H/ N57Y/Q100P)
naive
CD44+ CD8+ T cells in dLN
%CD8 T cells
20
25
30
35
40
*
PBS
Fc
CTLA-4 Fc (abatacept)
ICOSL IgV -Fc (N52H/ N57Y/Q100P)
naive
T_FH cells as % of CD4+ T cells in dLN
%CD4 T cells
0
1
2
3
4
**
T_FH = CD25- CD4+ PD-1+ CXCR5+
PBS
Fc
CTLA-4 Fc (abatacept)
ICOSL IgV -Fc (N52H/ N57Y/Q100P)
naive

FIG. 11E

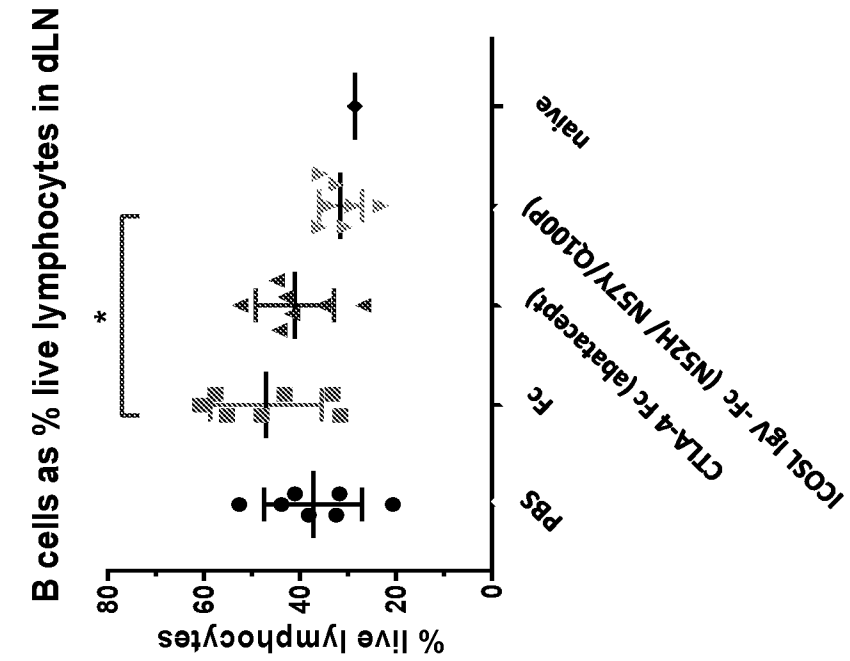

B cells as % live lymphocytes in dLN
% live lymphocytes
0
20
40
60
80
*
PBS
Fc
CTLA-4 Fc (abatacept)
ICOSL IgV -Fc (N52H/ N57Y/Q100P)
naive

FIG. 12A

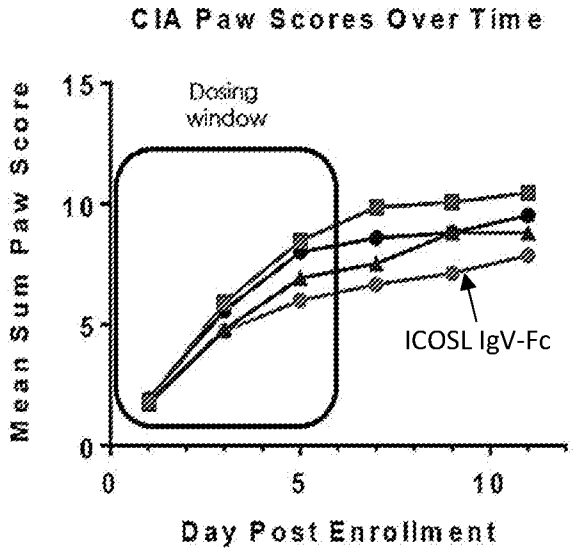
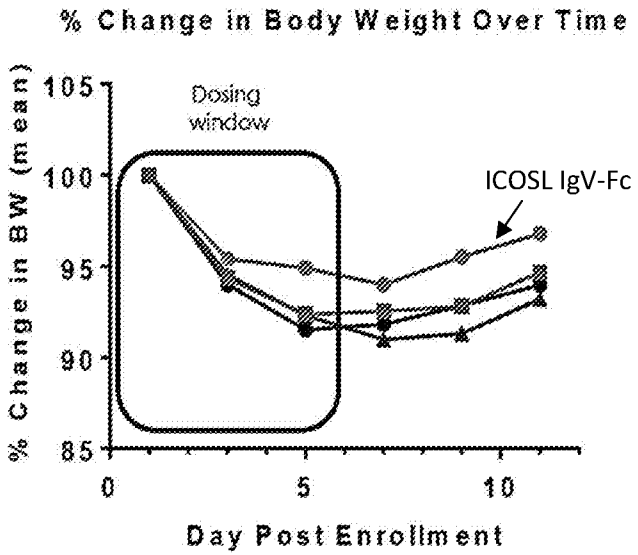


FIG. 12B



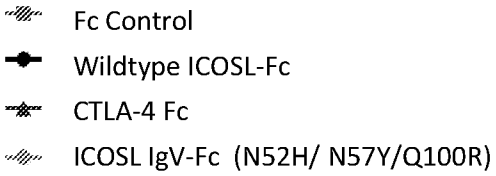


FIG. 12C

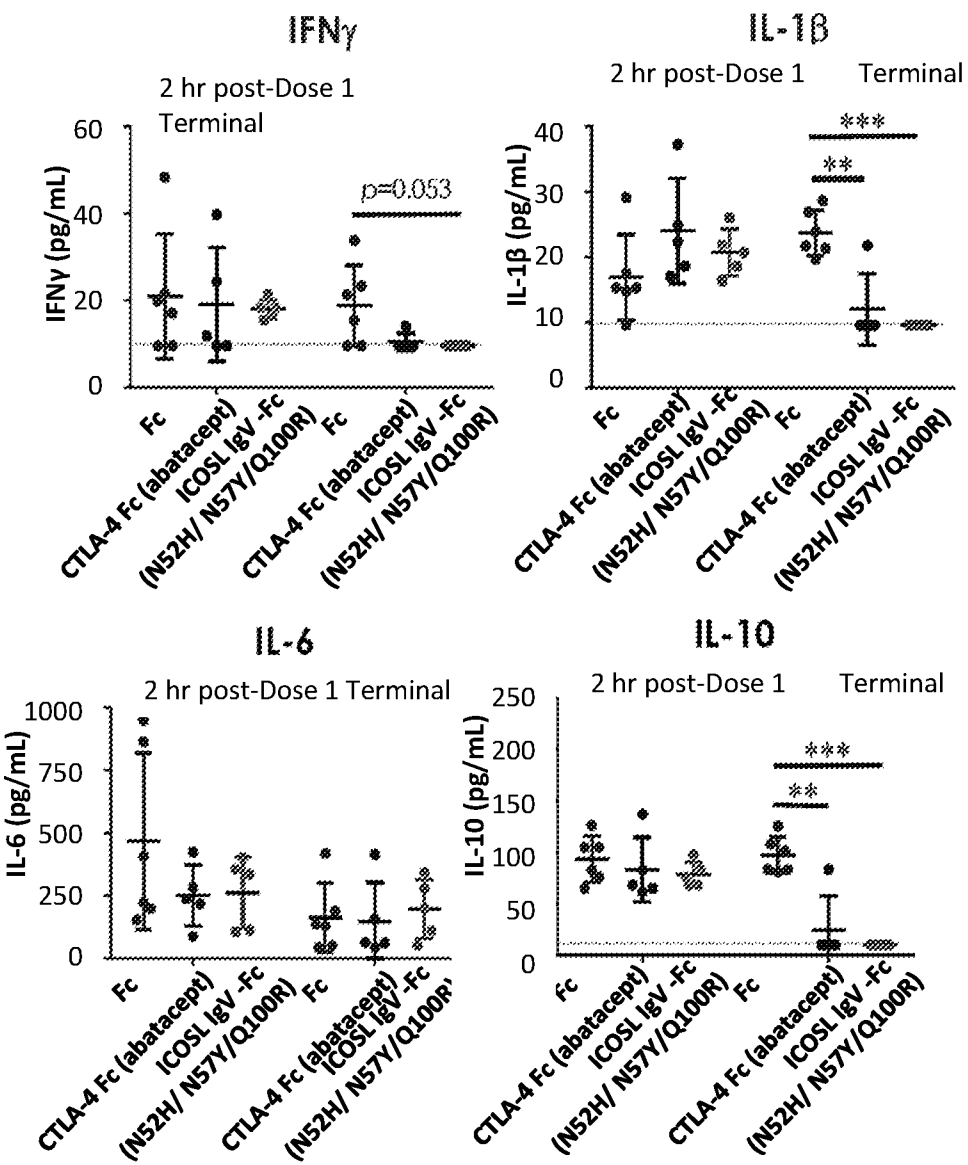

IFNγ
2 hr post-Dose 1
Terminal
IFNγ (pg/mL)
p=0.053
IL-1β
2 hr post-Dose 1
Terminal
IL-1β (pg/mL)
***
**
IL-6
2 hr post-Dose 1 Terminal
IL-6 (pg/mL)
IL-10
2 hr post-Dose 1
Terminal
IL-10 (pg/mL)
Fc
CTLA-4 Fc (abatacept)
ICOSL IgV -Fc
(N52H/ N57Y/Q100R)

FIG. 12D

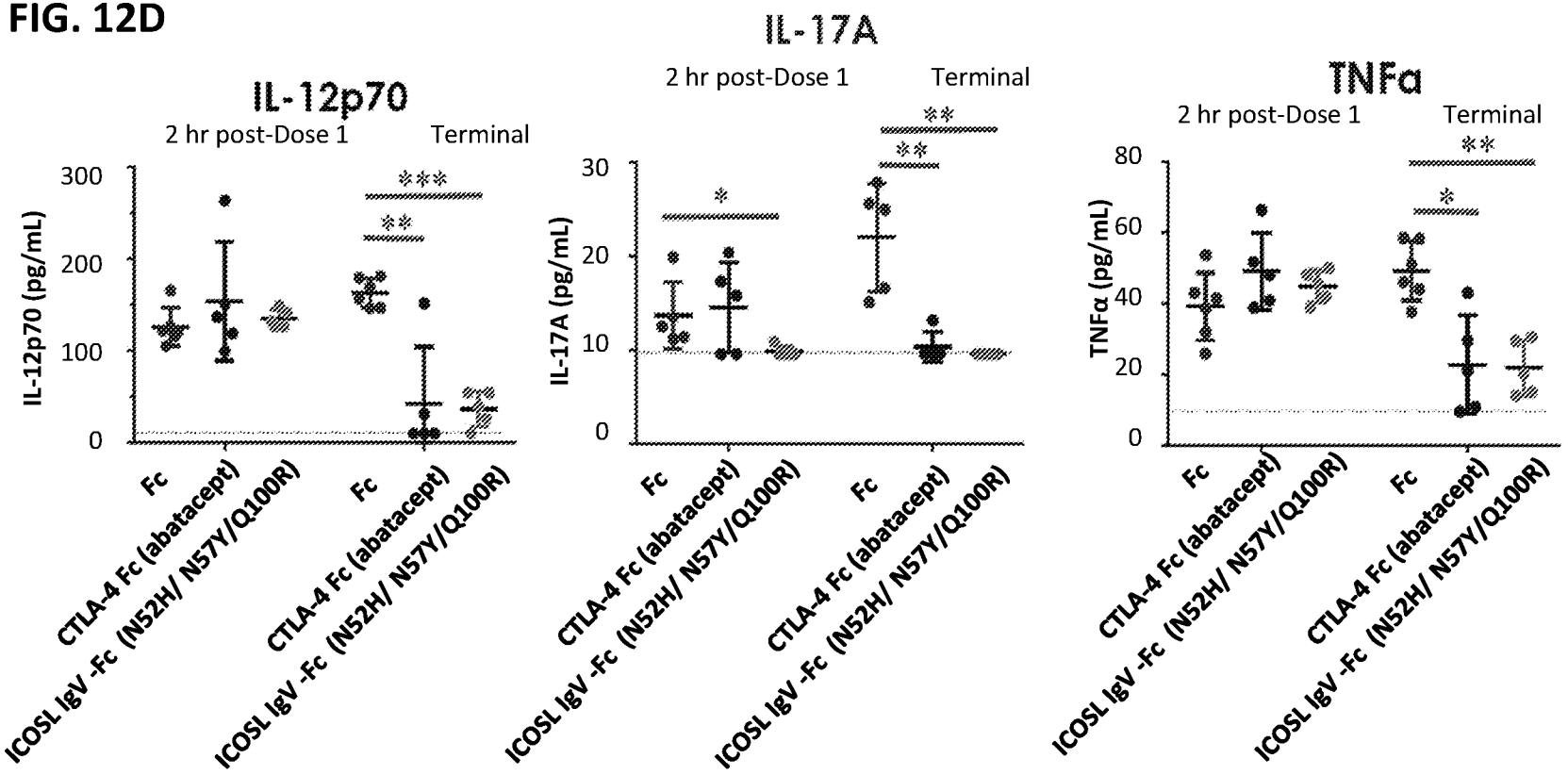

IL-12p70
2 hr post-Dose 1
Terminal
IL-12p70 (pg/mL)
300
200
100
0
***
**
IL-17A
2 hr post-Dose 1
Terminal
IL-17A (pg/mL)
30
20
10
0
*
**
**
TNFa
2 hr post-Dose 1
Terminal
TNFα (pg/mL)
80
60
40
20
0
**
*
Fc
CTLA-4 Fc (abatacept)
ICOSL IgV -Fc (N52H/ N57Y/Q100R)

FIG. 13A

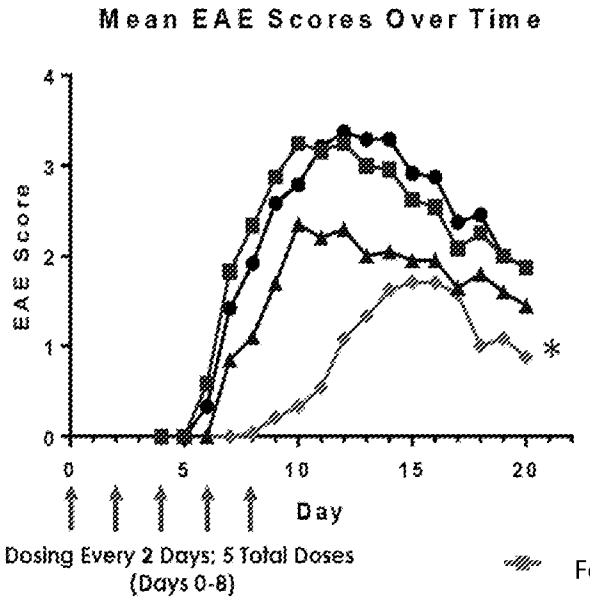
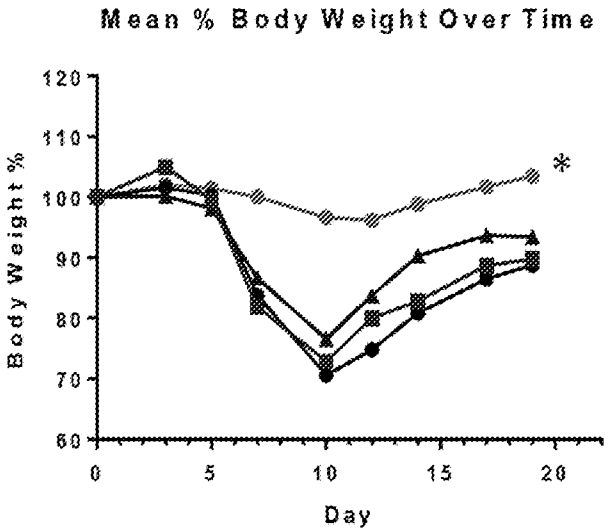


FIG. 13B



Fc Control
Wildtype ICOSL-Fc
CTLA-4 Fc (Abatacept)
ICOSL IgV-Fc (N52H/ N57Y/Q100R)

FIG. 13C

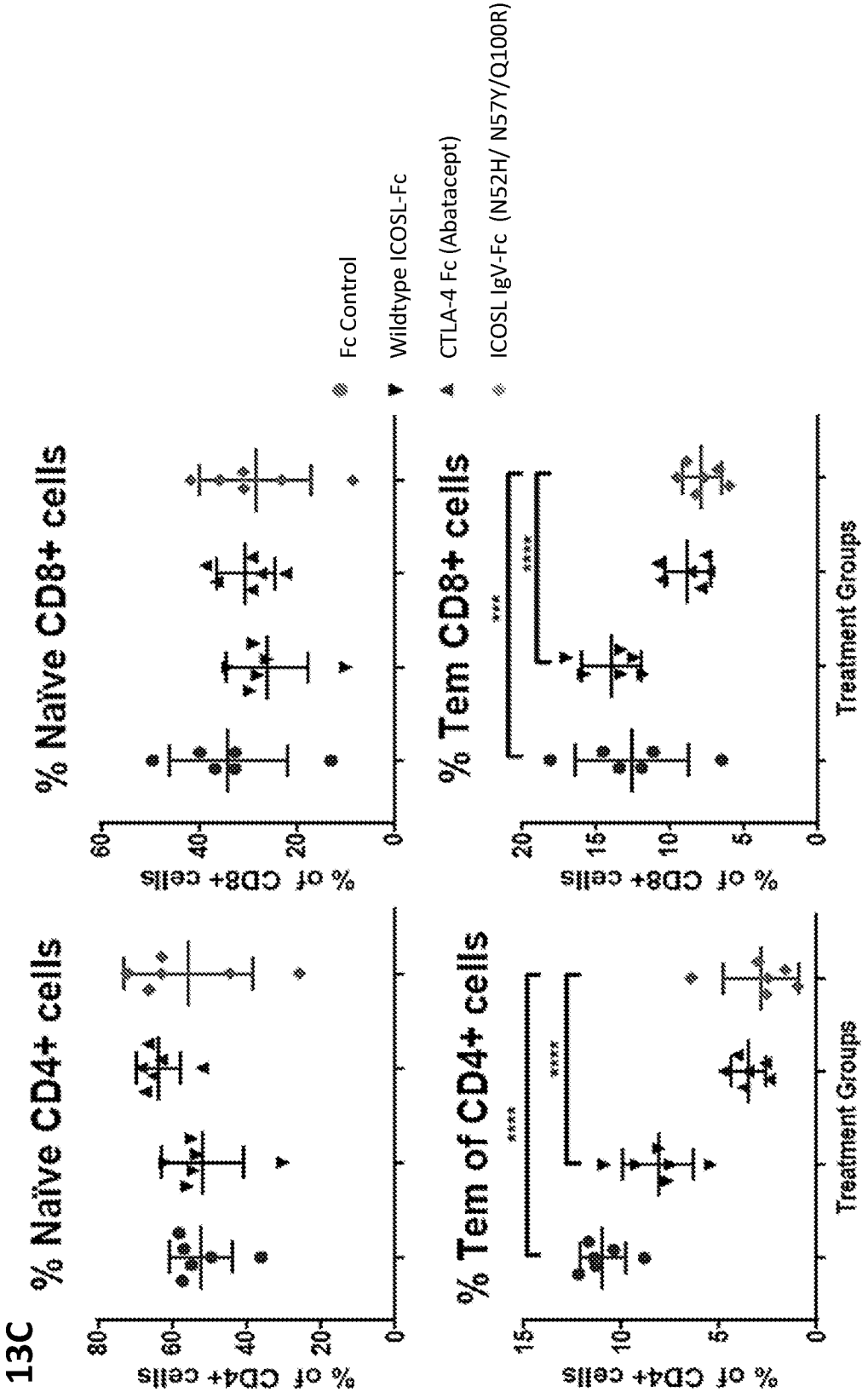

% Naïve CD4+ cells
% Naïve CD8+ cells
% Tem of CD4+ cells
% Tem CD8+ cells
% of CD4+ cells
% of CD8+ cells
Treatment Groups
Fc Control
Wildtype ICOSL-Fc
CTLA-4 Fc (Abatacept)
ICOSL IgV-Fc (N52H/ N57Y/Q100R)

FIG. 13D

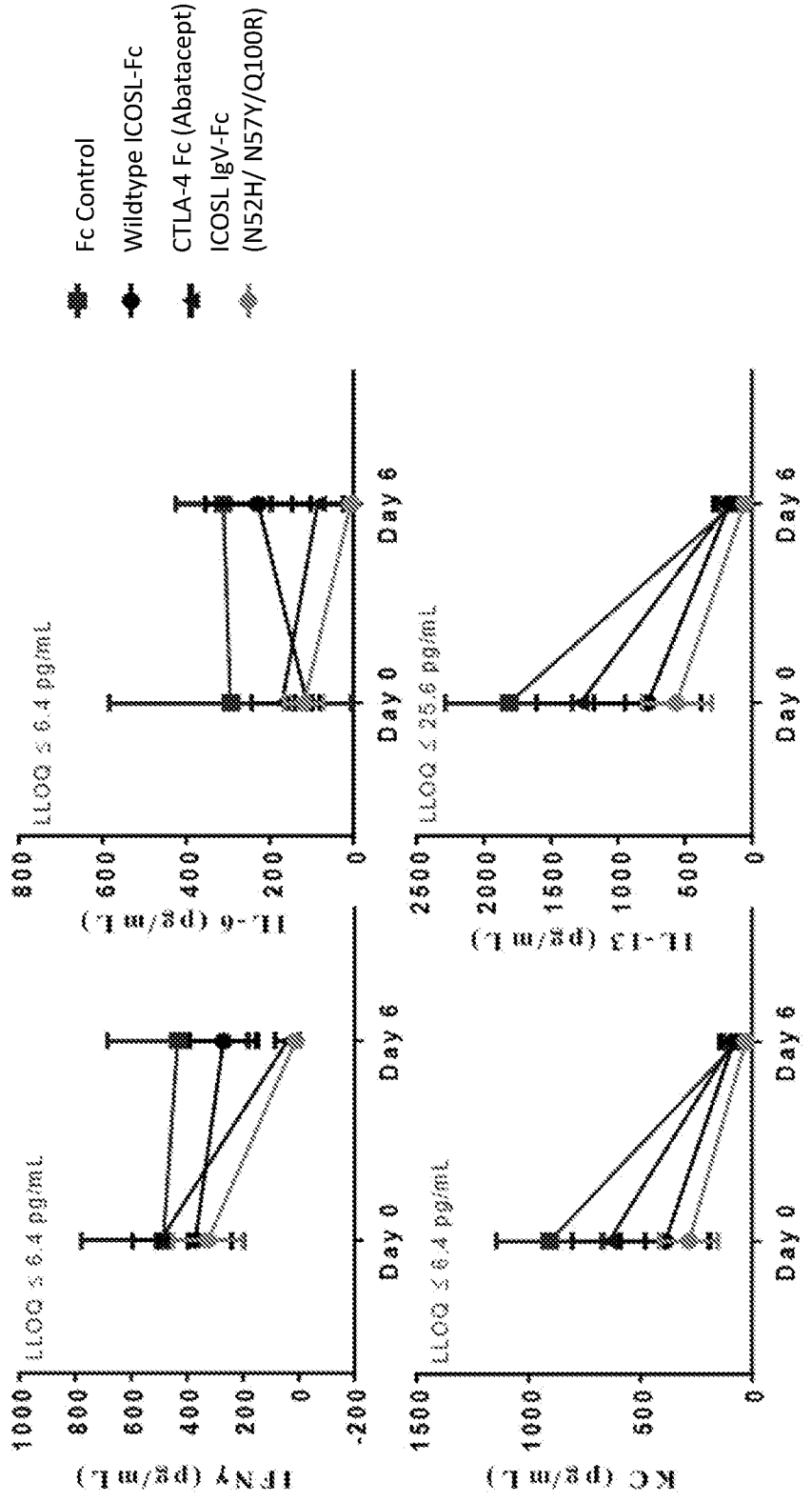

Fc Control
Wildtype ICOSL-Fc
CTLA-4 Fc (Abatacept)
ICOSL IgV-Fc
(N52H/ N57Y/Q100R)
IL-6 (pg/mL)
IL-13 (pg/mL)
IFNγ (pg/mL)
KC (pg/mL)
LLOQ ≤ 6.4 pg/mL
LLOQ ≤ 25.6 pg/mL
Day 0
Day 6

FIG. 14A

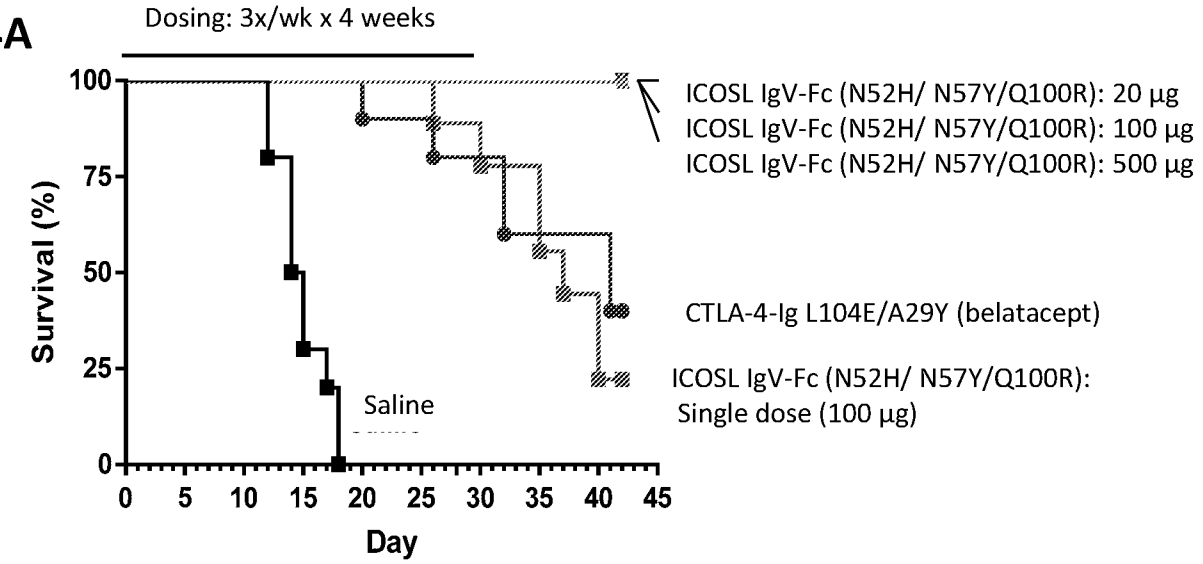


FIG. 14B

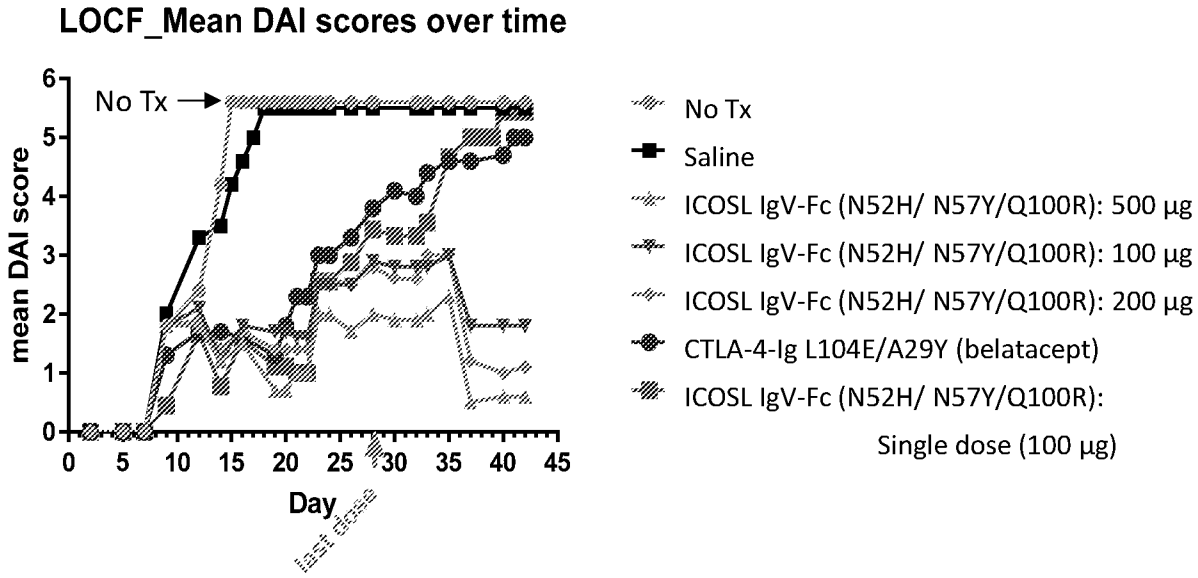


FIG. 15A

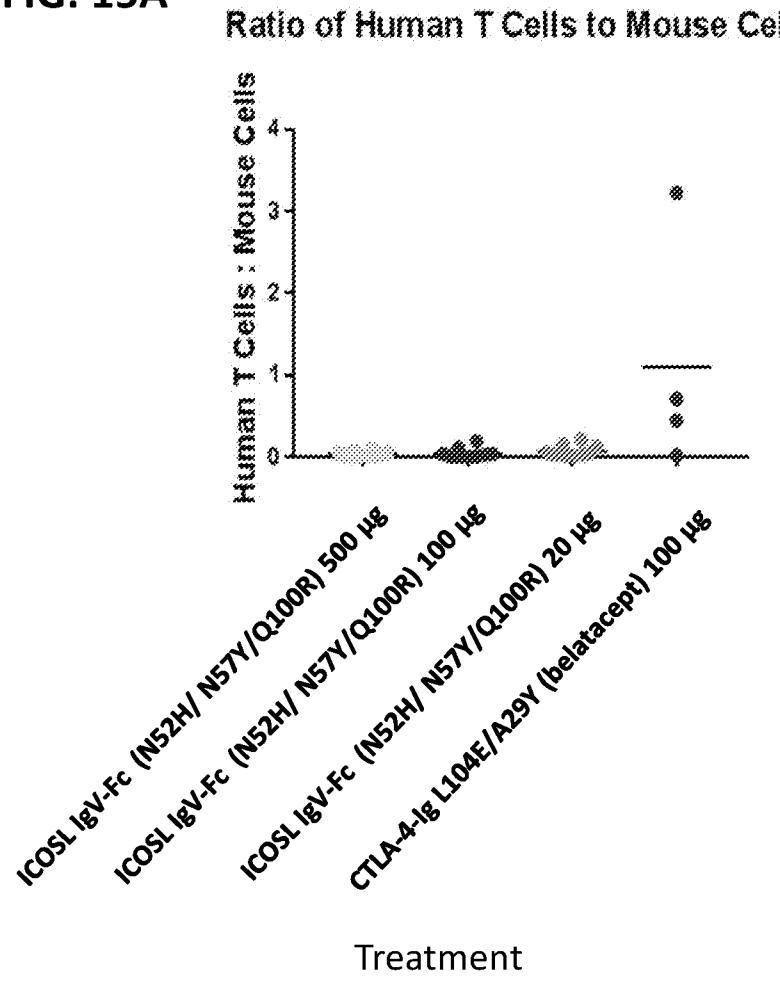


FIG. 15B

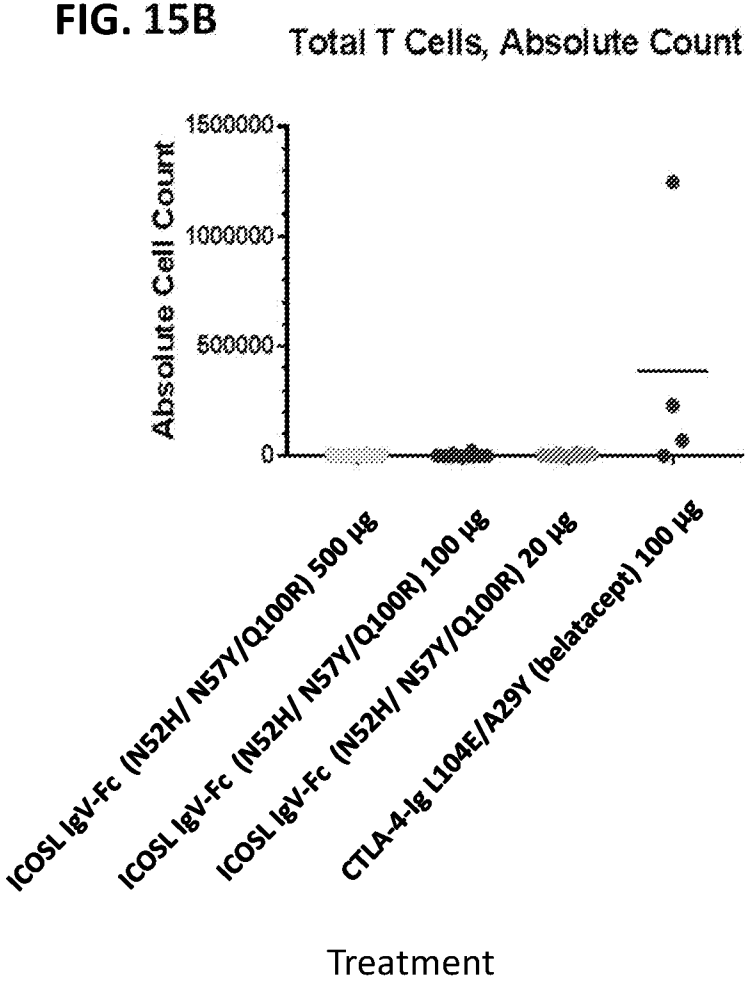


FIG. 15C

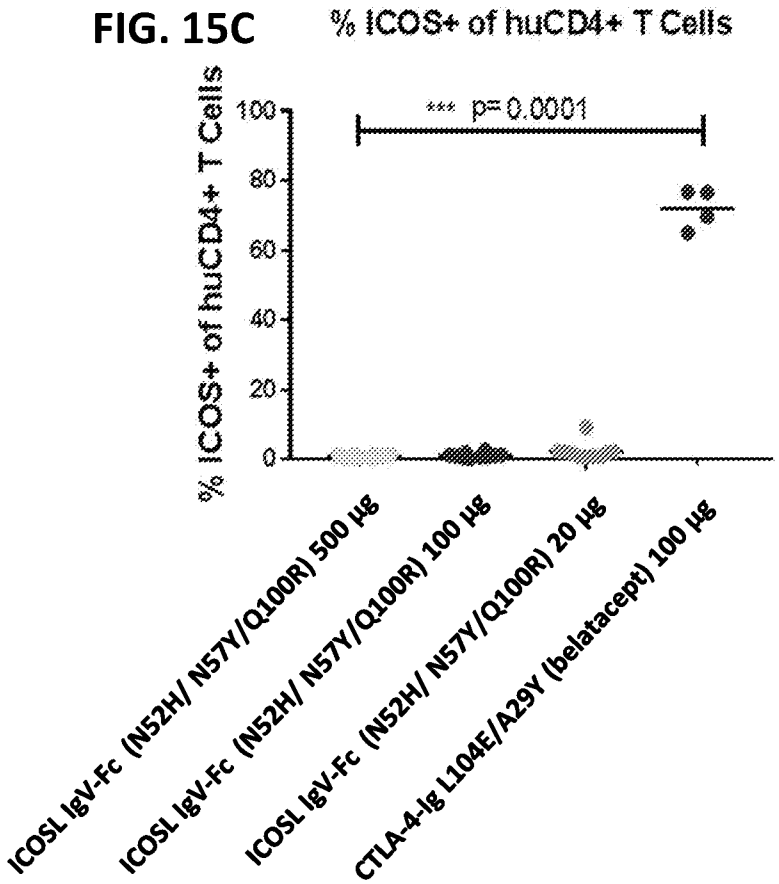


FIG. 15D

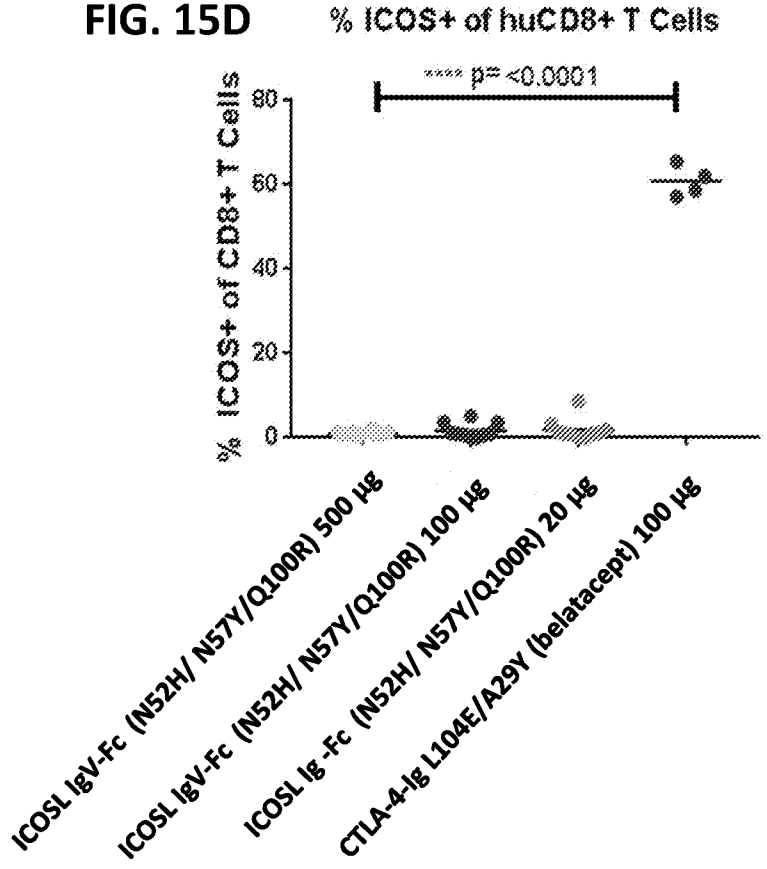


FIG. 15E

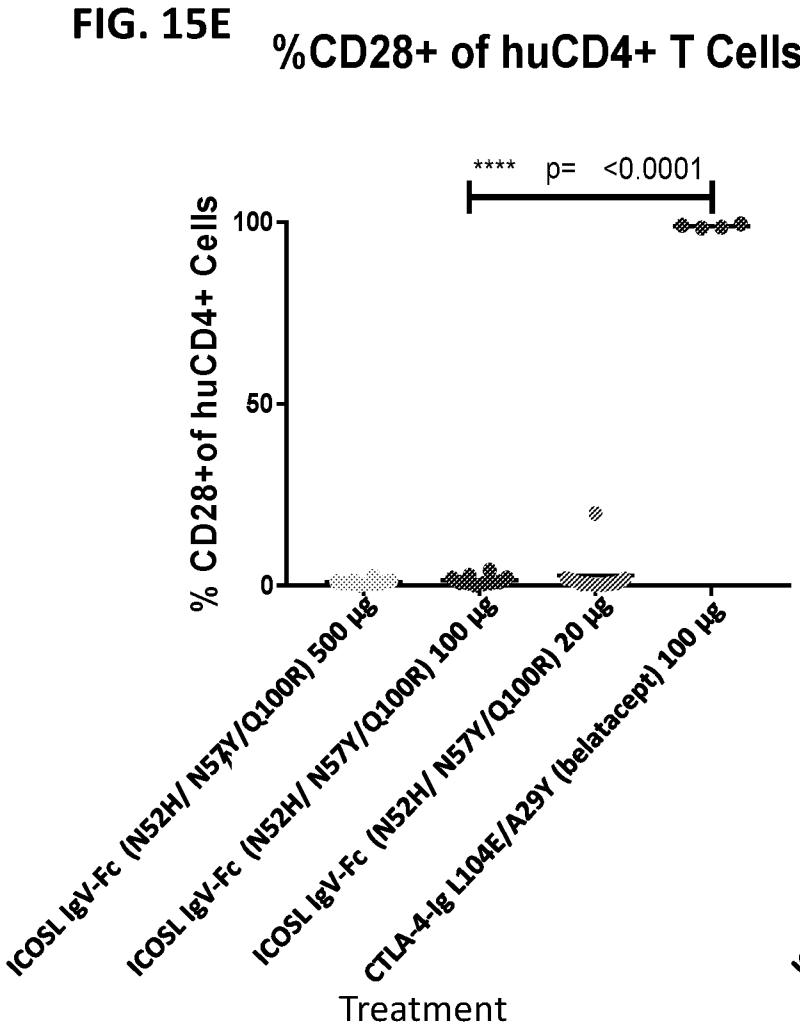


FIG. 15F

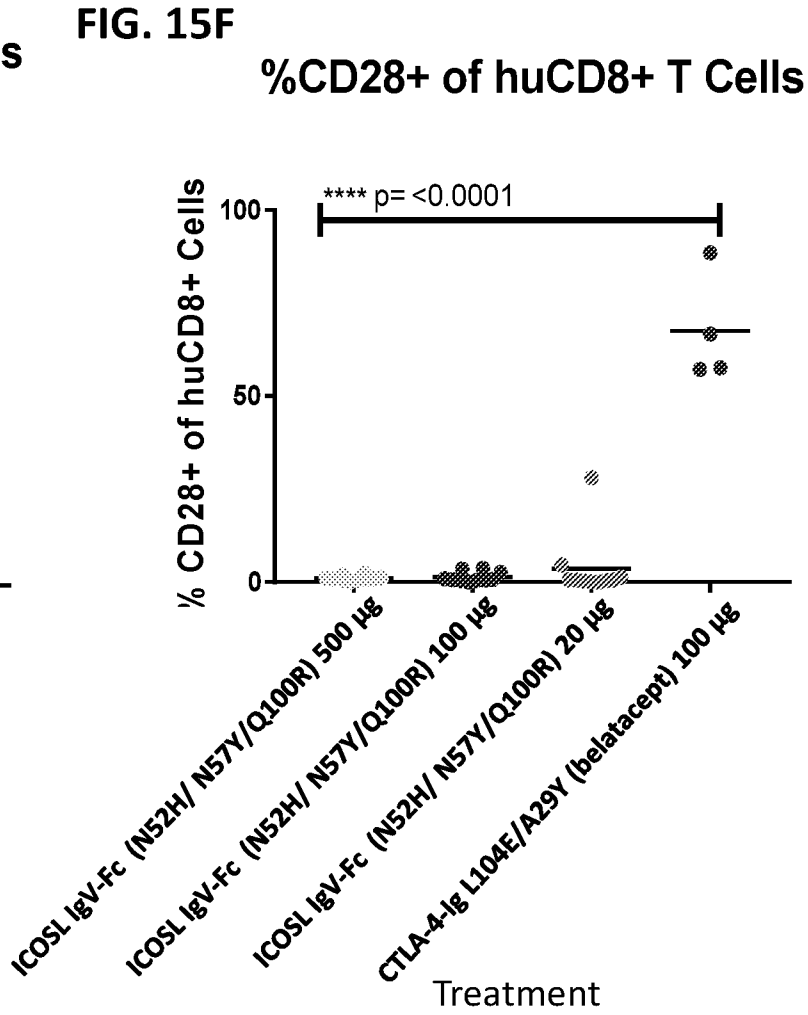


FIG. 16A

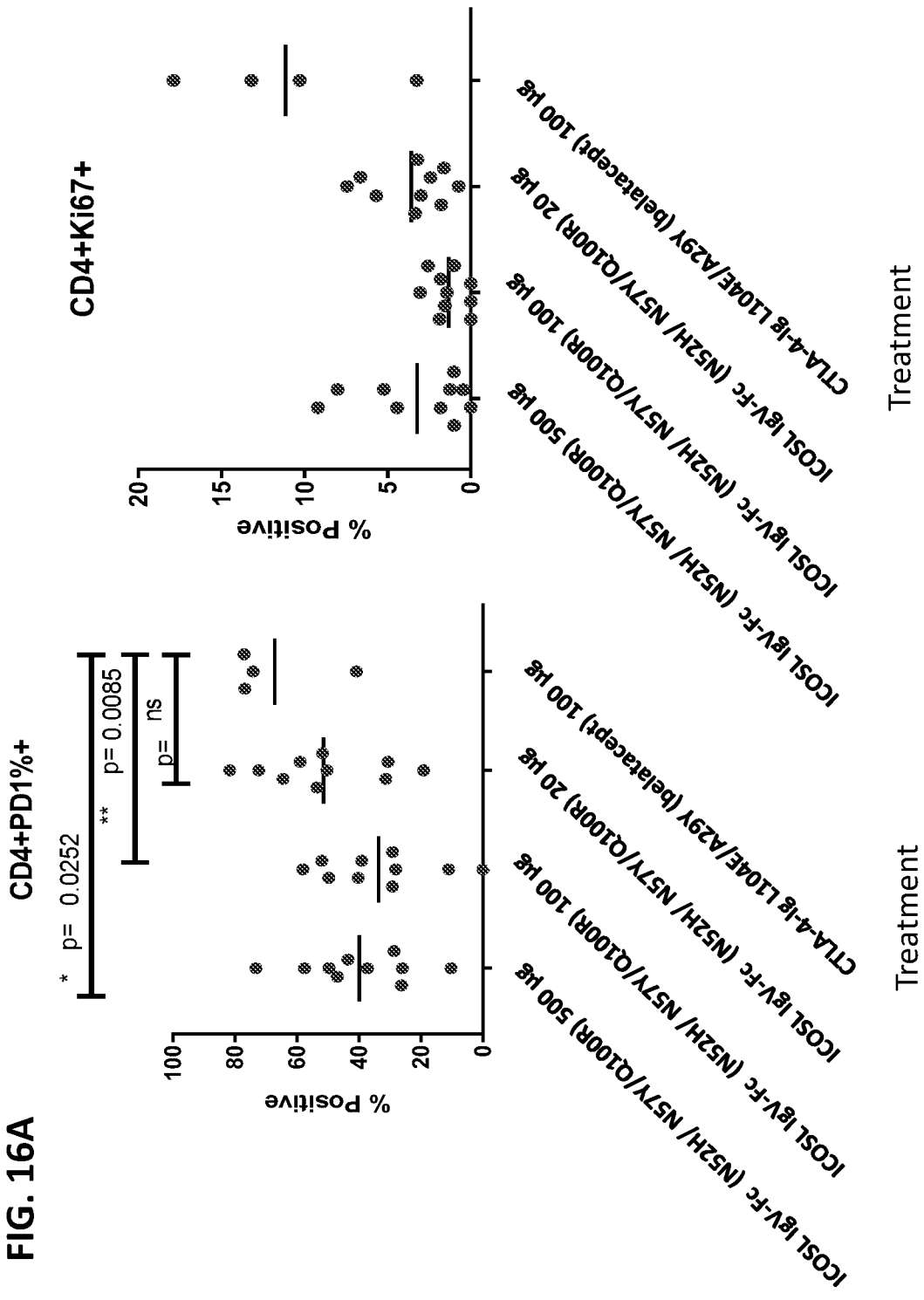

CD4+PD1%+
* p= 0.0252
** p= 0.0085
p= ns
% Positive
0
20
40
60
80
100
ICOSL IgV-Fc (N52H/ N57V/Q100R) 500 ug
ICOSL IgV-Fc (N52H/ N57V/Q100R) 100 ug
ICOSL IgV-Fc (N52H/ N57V/Q100R) 20 ug
CTLA-4-IgL104E/A29Y (belatacept) 100 ug
Treatment
CD4+Ki67+
% Positive
0
5
10
15
20
ICOSL IgV-Fc (N52H/ N57V/Q100R) 500 ug
ICOSL IgV-Fc (N52H/ N57V/Q100R) 100 ug
ICOSL IgV-Fc (N52H/ N57V/Q100R) 20 ug
CTLA-4-IgL104E/A29Y (belatacept) 100 ug
Treatment

FIG. 16B

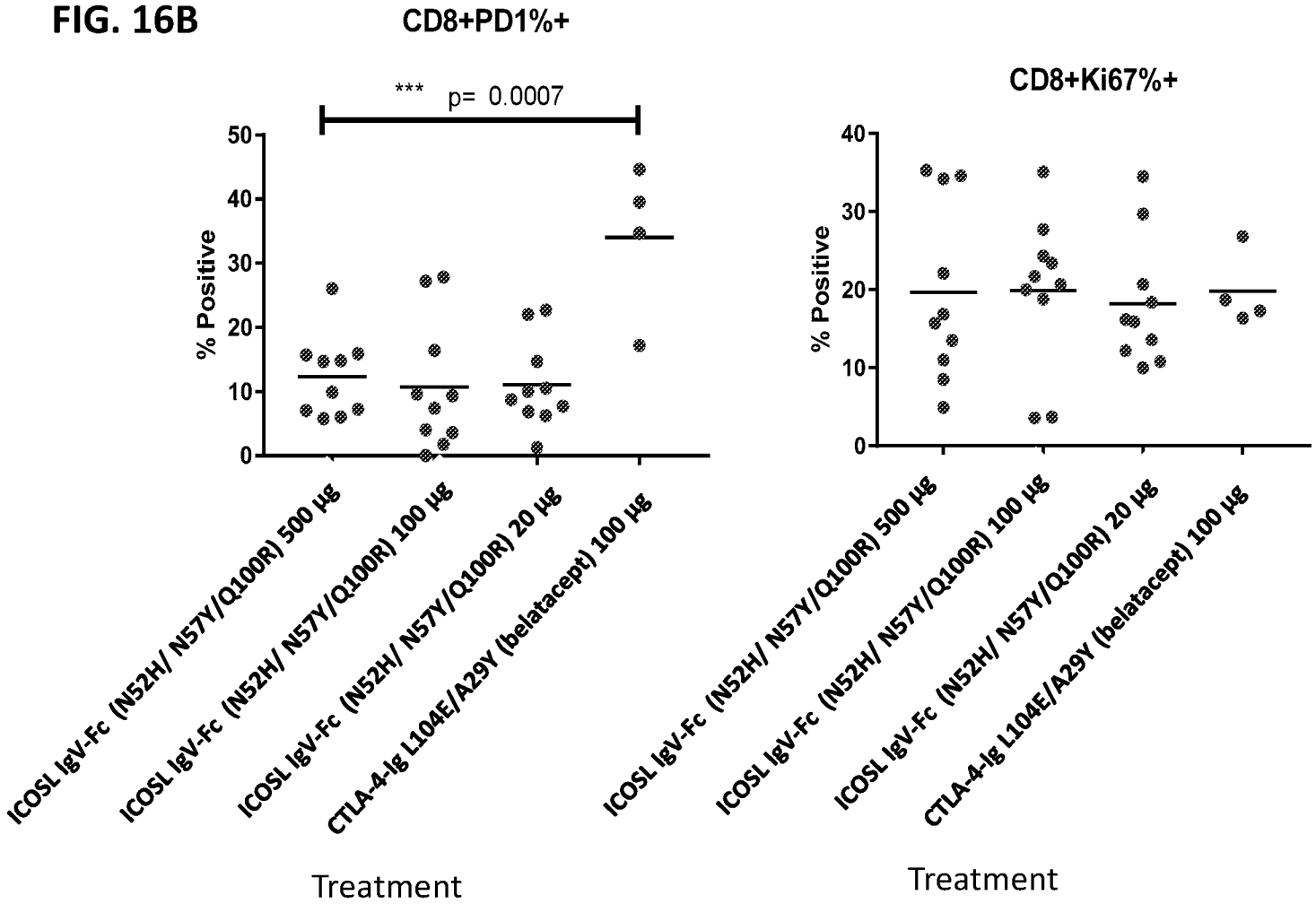

CD8+PD1%+
*** p= 0.0007
% Positive
50
40
30
20
10
0
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 500 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 100 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 20 µg
CTLA-4-Ig L104E/A29Y (belatacept) 100 µg
Treatment
CD8+Ki67%+
% Positive
40
30
20
10
0
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 500 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 100 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 20 µg
CTLA-4-Ig L104E/A29Y (belatacept) 100 µg
Treatment

FIG. 16C

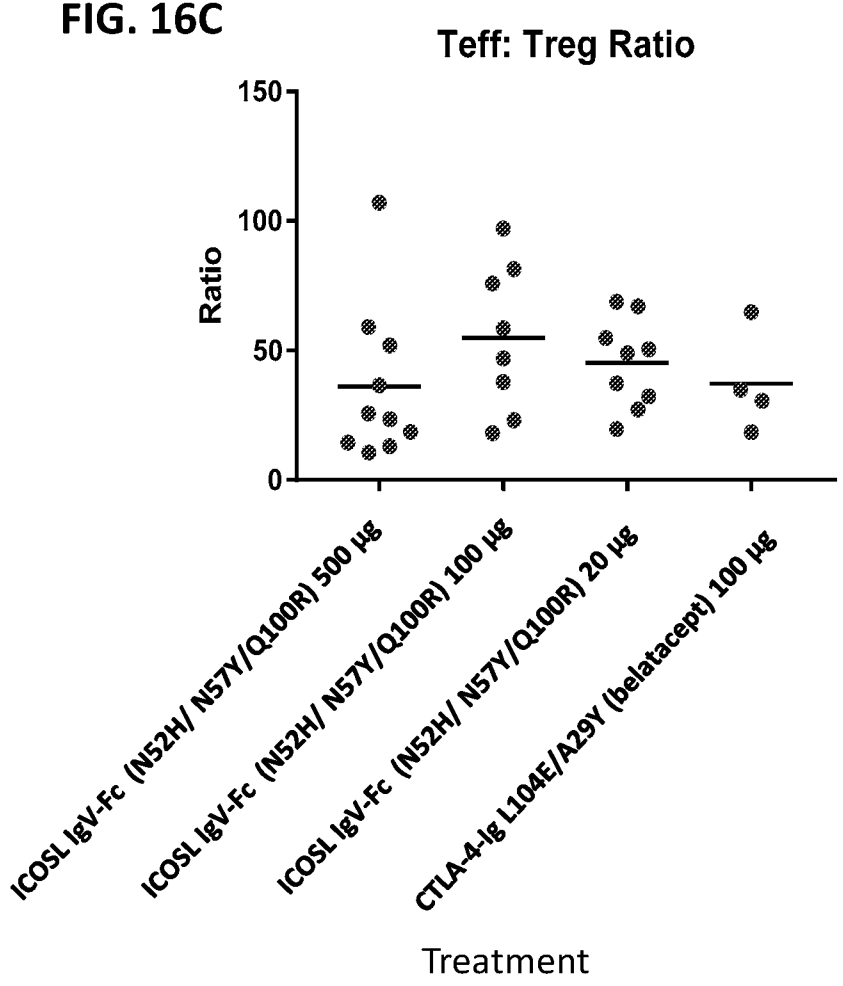

Teff: Treg Ratio
150
100
50
0
Ratio
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 500 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 100 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 20 µg
CTLA-4-Ig L104E/A29Y (belatacept) 100 µg
Treatment

FIG. 17A

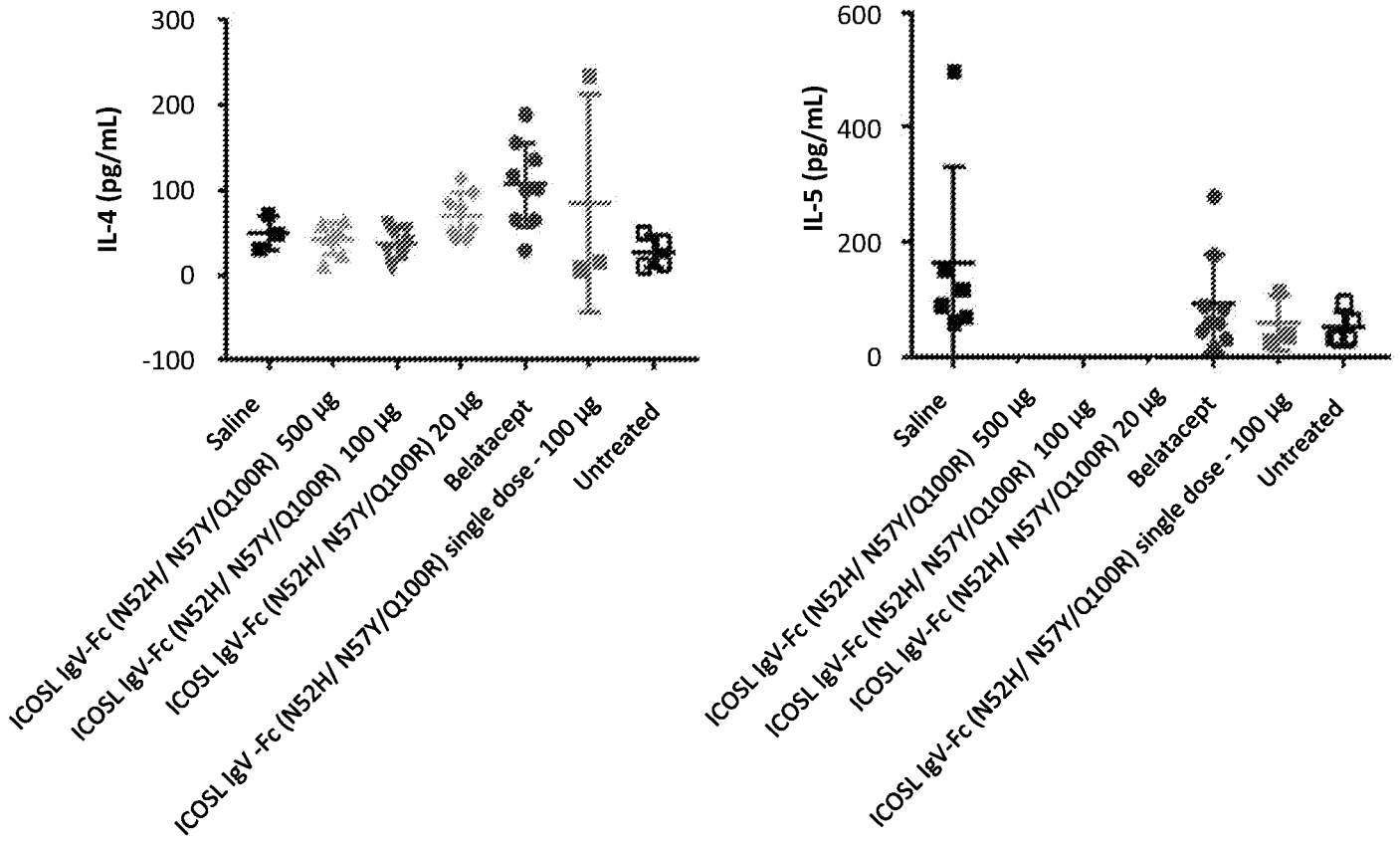

IL-4 (pg/mL)
300
200
100
0
-100
IL-5 (pg/mL)
600
400
200
0
Saline
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 500 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 100 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 20 µg
Belatacept
ICOSL IgV -Fc (N52H/ N57Y/Q100R) single dose - 100 µg
Untreated

FIG. 17B

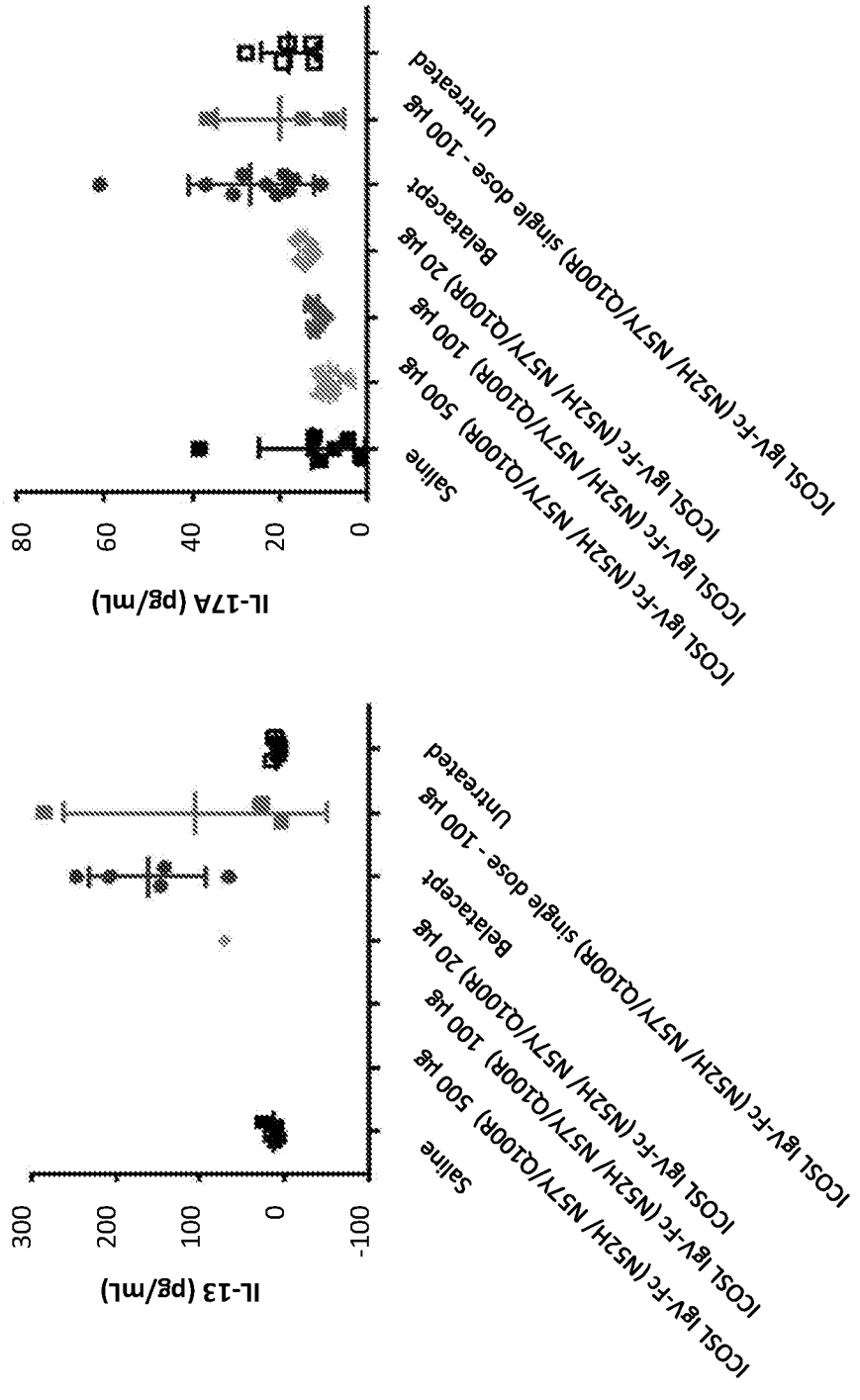

IL-17A (pg/mL)
0
20
40
60
80
IL-13 (pg/mL)
-100
0
100
200
300
Saline
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 500 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 100 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 20 µg
Belatacept
ICOSL IgV-Fc (N52H/ N57Y/Q100R) single dose - 100 µg
Untreated

FIG. 17C

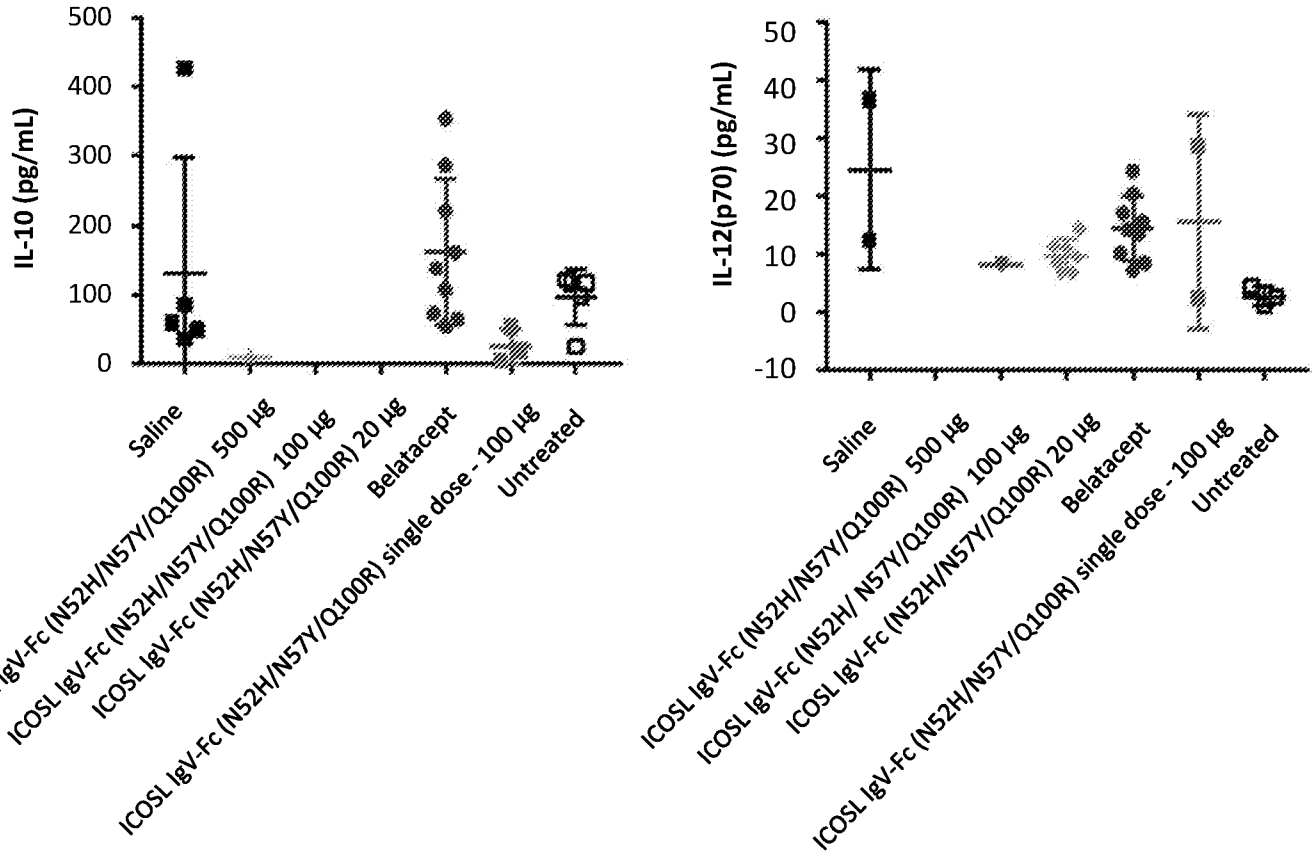

IL-10 (pg/mL)
500
400
300
200
100
0
Saline
ICOSL IgV-Fc (N52H/N57Y/Q100R) 500 µg
ICOSL IgV-Fc (N52H/N57Y/Q100R) 100 µg
ICOSL IgV-Fc (N52H/N57Y/Q100R) 20 µg
Belatacept
ICOSL IgV-Fc (N52H/N57Y/Q100R) single dose - 100 µg
Untreated
IL-12(p70) (pg/mL)
50
40
30
20
10
0
-10
Saline
ICOSL IgV-Fc (N52H/N57Y/Q100R) 500 µg
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 100 µg
ICOSL IgV-Fc (N52H/N57Y/Q100R) 20 µg
Belatacept
ICOSL IgV-Fc (N52H/N57Y/Q100R) single dose - 100 µg
Untreated

FIG. 17D

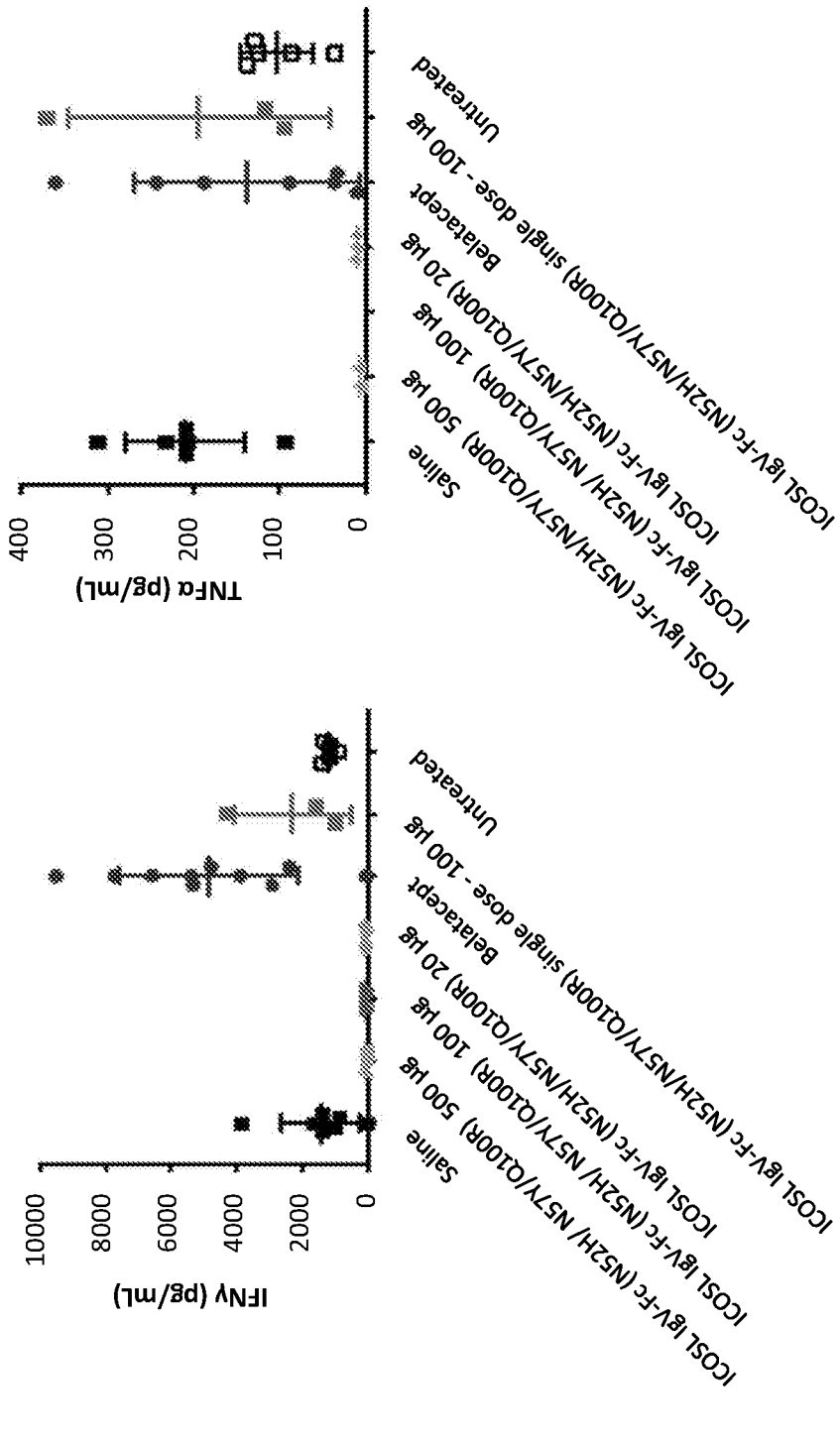
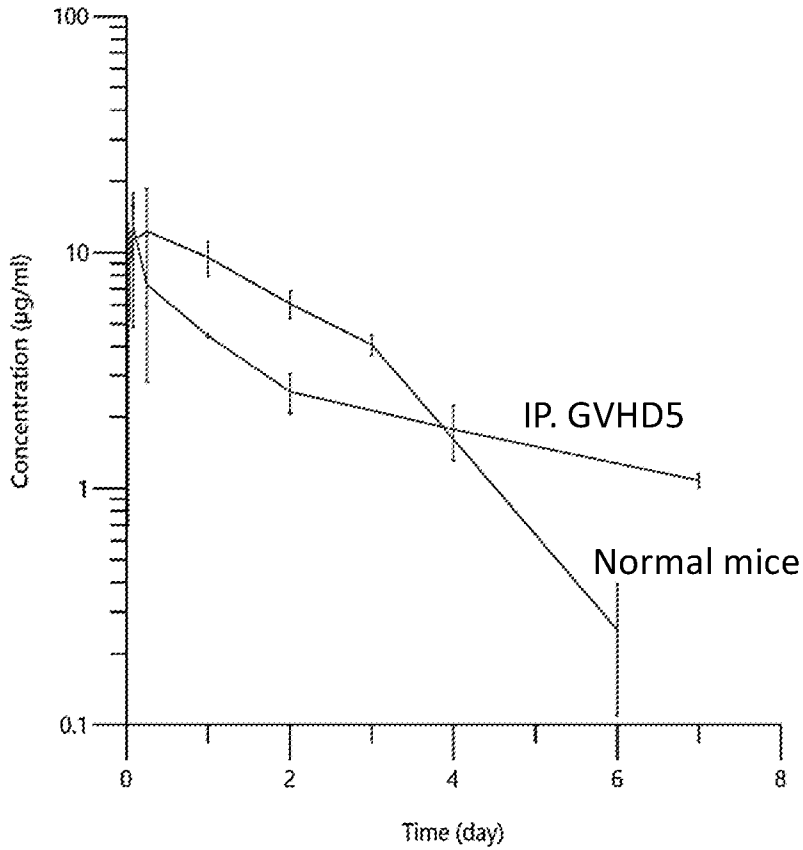

IFNγ (pg/mL)
0
2000
4000
6000
8000
10000
TNFα (pg/mL)
0
100
200
300
400
Saline
ICOSL IgV-Fc (N52H/ N57Y/Q100R) 500 µg
ICOSL IgV-Fc (N52H/N57Y/Q100R) 100 µg
ICOSL IgV-Fc (N52H/N57Y/Q100R) 20 µg
Belatacept
ICOSL IgV-Fc (N52H/N57Y/Q100R) single dose - 100 µg
Untreated

FIG. 17E



Study	Dose (μg)	Cmax (μg/mL)	T1/2 (day)	AUC (day*μg/mL)
GVHD5	100	12.94	4.02	18.97
Normal Mice	100	12.30	0.84	30.04

FIG. 18A

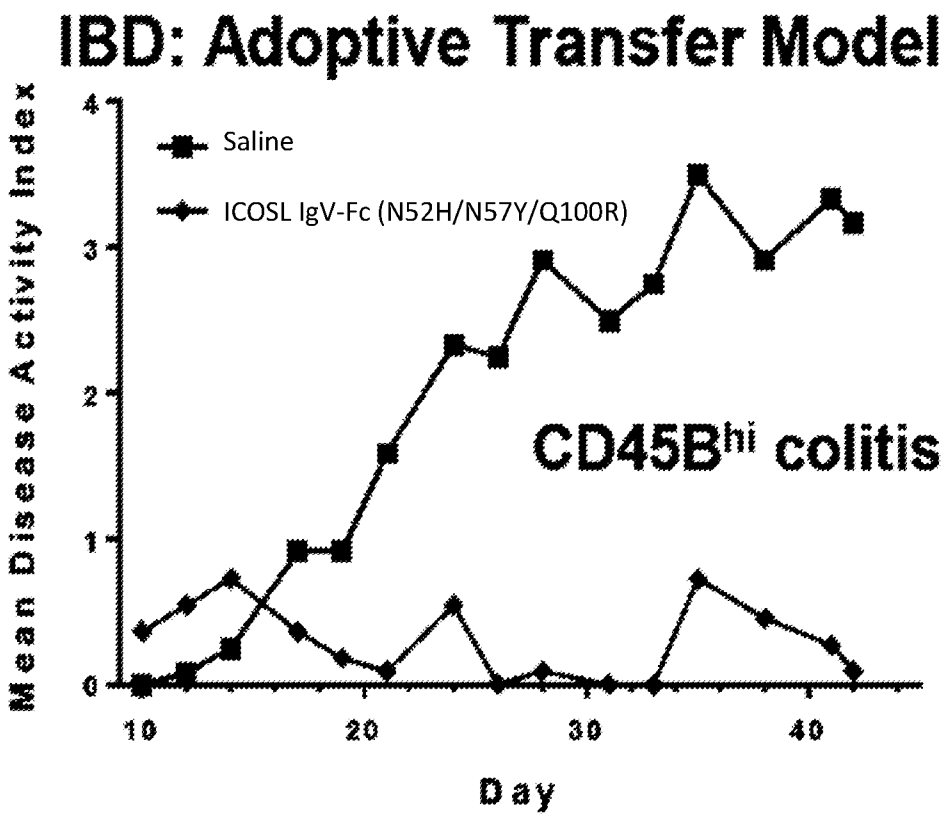

IBD: Adoptive Transfer Model
Mean Disease Activity Index
Saline
ICOSL IgV-Fc (N52H/N57Y/Q100R)
CD45B^hi colitis
0
1
2
3
4
10
20
30
40
Day

FIG. 18B

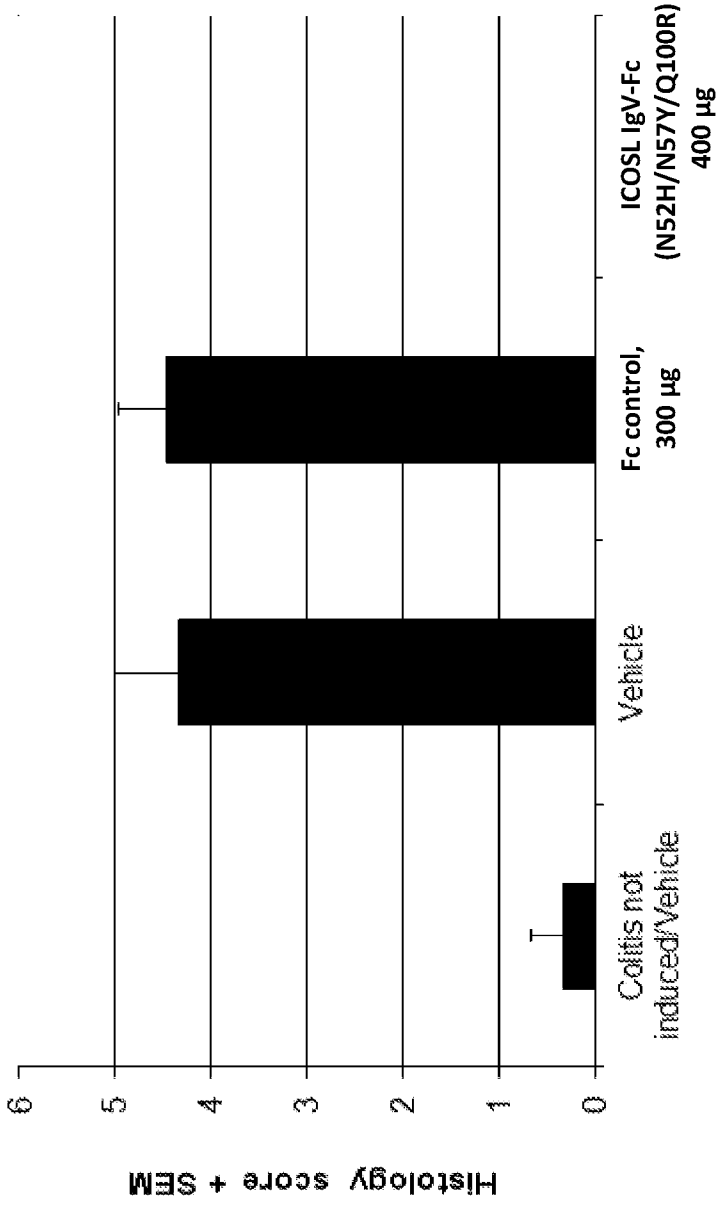

Histology score + SEM
0
1
2
3
4
5
6
Colitis not induced/Vehicle
Vehicle
Fc control, 300 µg
ICOSL IgV-Fc (N52H/N57Y/Q100R) 400 µg

FIG. 19A

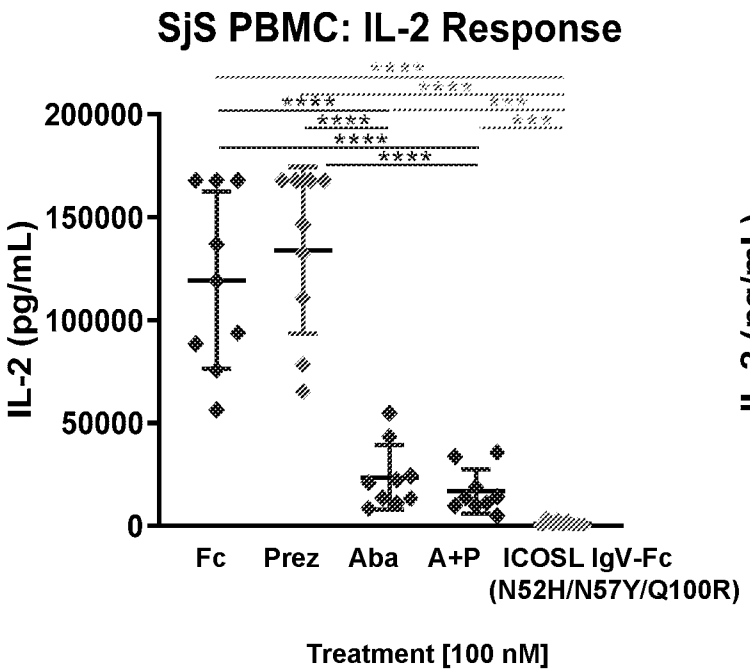


FIG. 19B

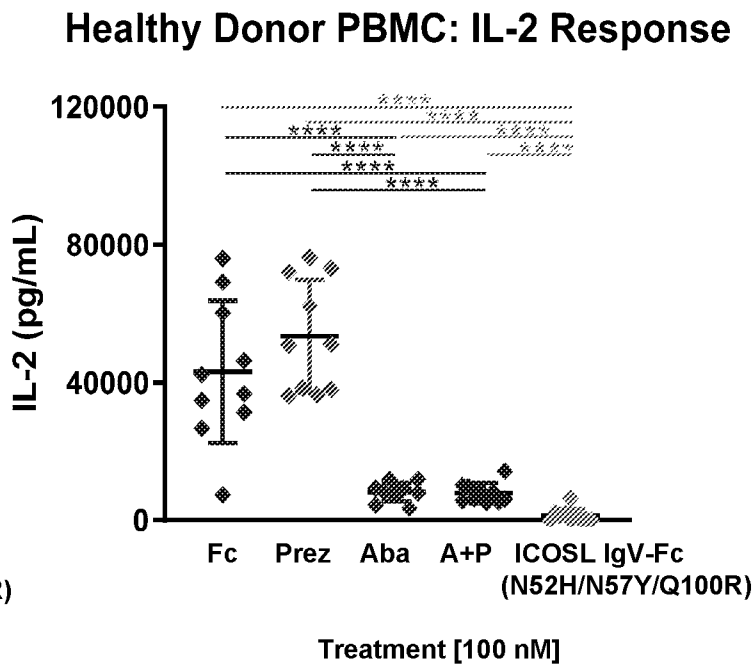


FIG. 19C

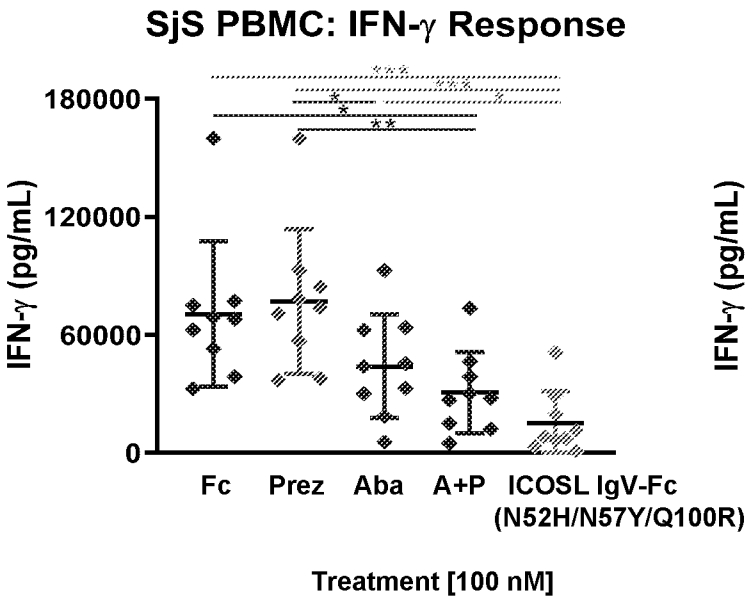


FIG. 19D

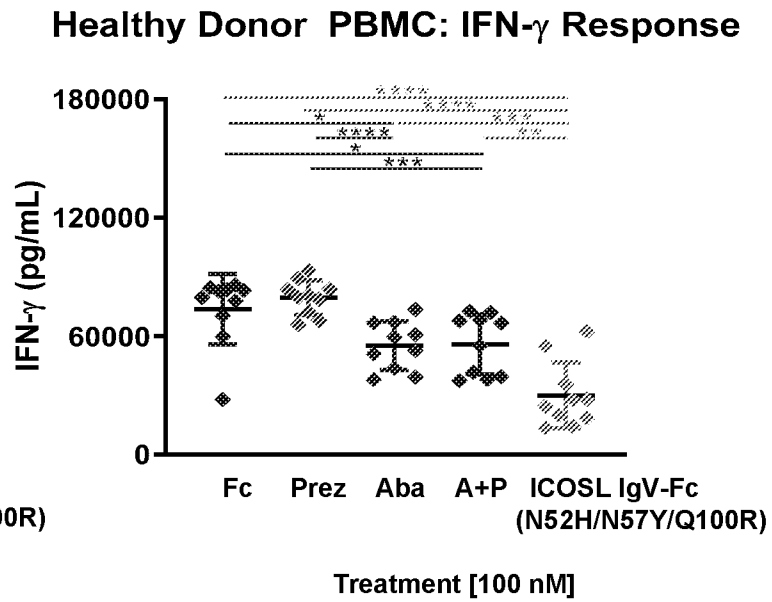


FIG. 19E

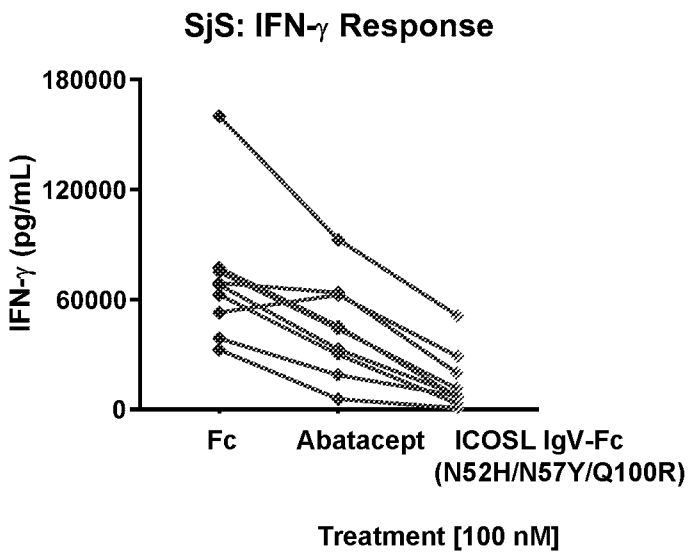


FIG. 19F

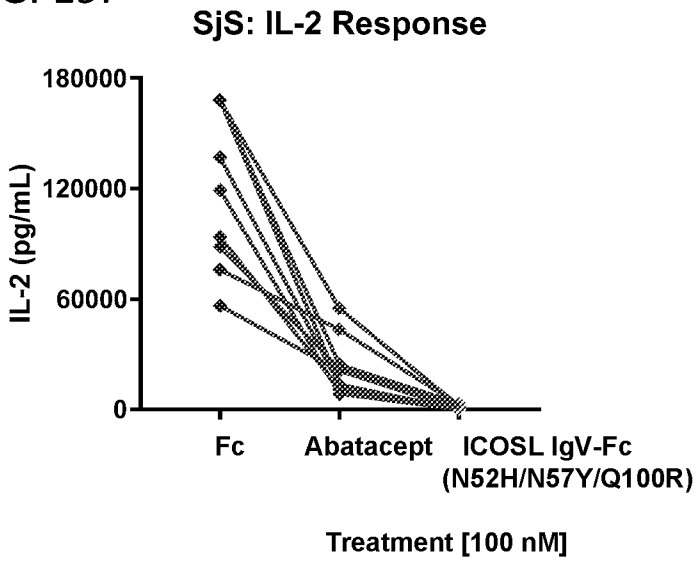


FIG. 19G

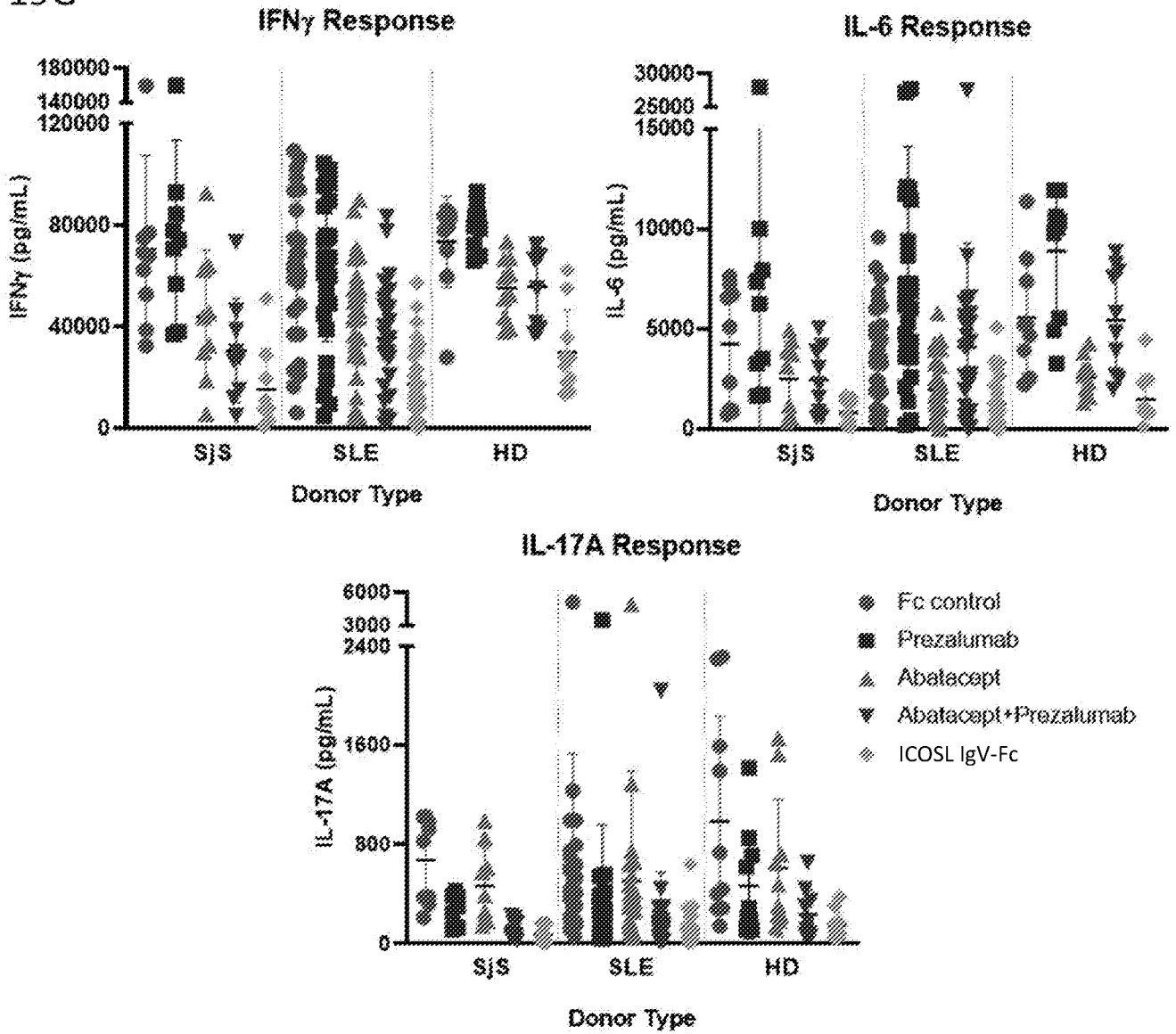

IFNγ Response
IFNγ (pg/mL)
180000
140000
120000
80000
40000
0
SjS
SLE
HD
Donor Type
IL-6 Response
IL-6 (pg/mL)
30000
25000
15000
10000
5000
0
SjS
SLE
HD
Donor Type
IL-17A Response
IL-17A (pg/mL)
6000
3000
2400
1600
800
0
SjS
SLE
HD
Donor Type
Fc control
Prezalumab
Abatacept
Abatacept+Prezalumab
ICOSL IgV-Fc

FIG. 19H

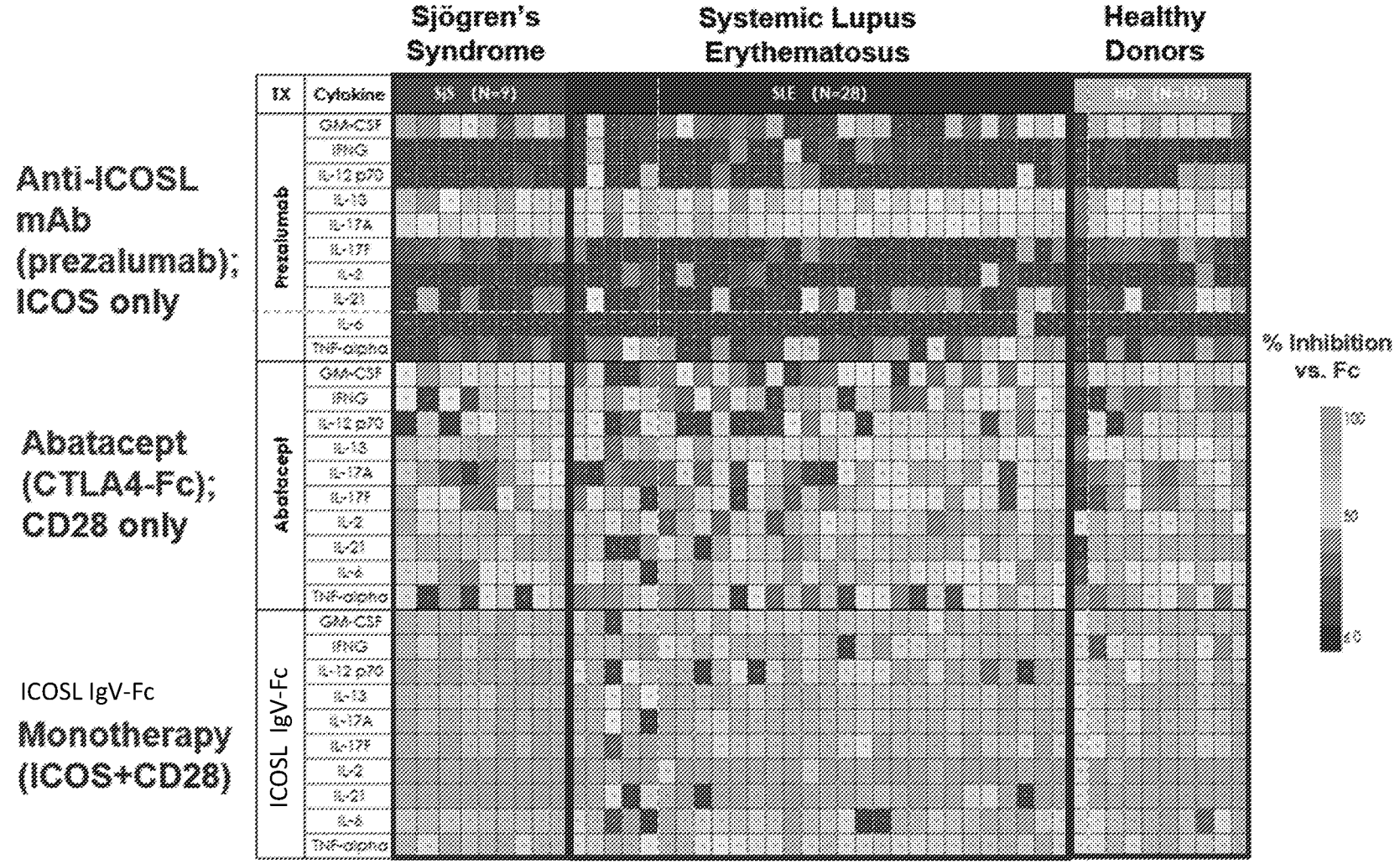

Sjögren's Syndrome
Systemic Lupus Erythematosus
Healthy Donors
Tx
Cytokine
SS (N=4)
SLE (N=20)
HD (N=10)
Prezalumab
Abatacept
ICOSL IgV-Fc
GM-CSF
IFNG
IL-12 p70
IL-13
IL-17A
IL-17F
IL-2
IL-21
IL-6
TNF-alpha
% Inhibition vs. Fc
100
50
0
Anti-ICOSL mAb (prezalumab); ICOS only
Abatacept (CTLA4-Fc); CD28 only
ICOSL IgV-Fc Monotherapy (ICOS+CD28)

FIG. 19I

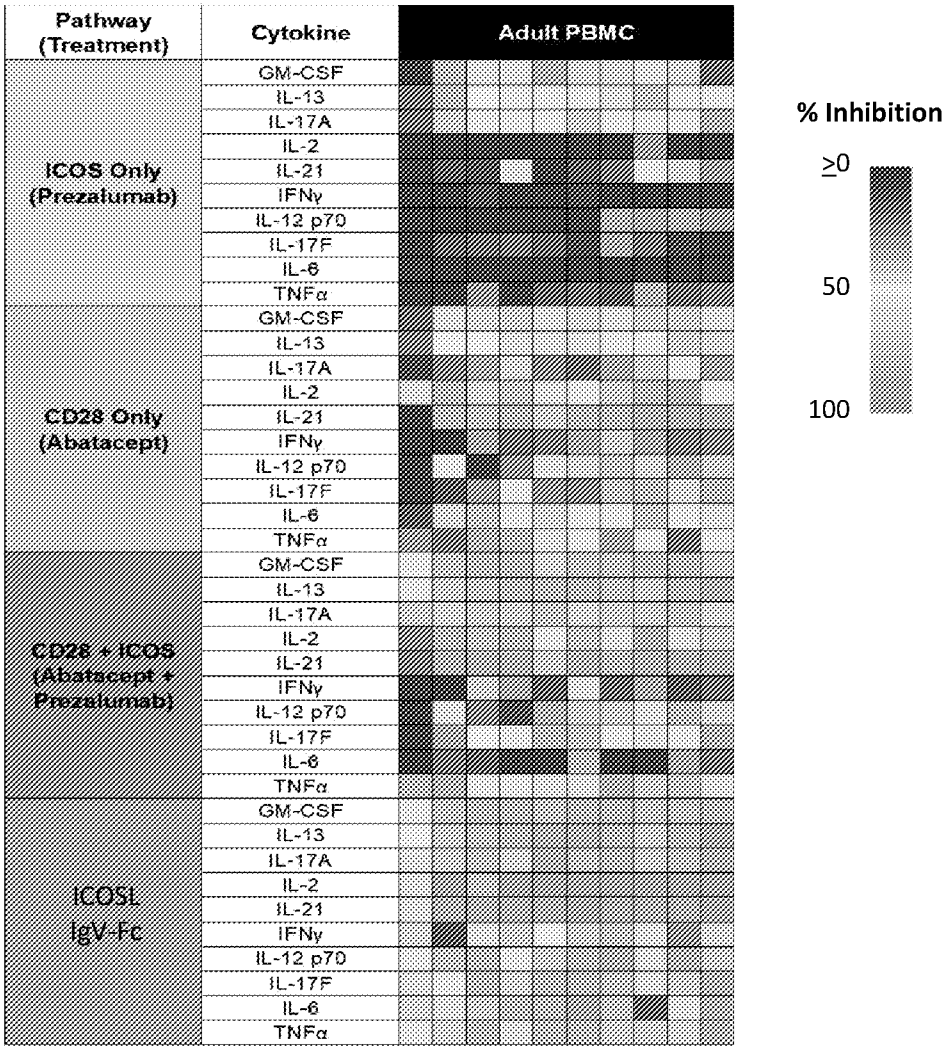

Pathway (Treatment)
Cytokine
Adult PBMC
ICOS Only (Prezalumab)
CD28 Only (Abatacept)
CD28 + ICOS (Abatacept + Prezalumab)
ICOSL IgV-Fc
GM-CSF
IL-13
IL-17A
IL-2
IL-21
IFNγ
IL-12 p70
IL-17F
IL-6
TNFα
% Inhibition
≥0
50
100

FIG. 19J

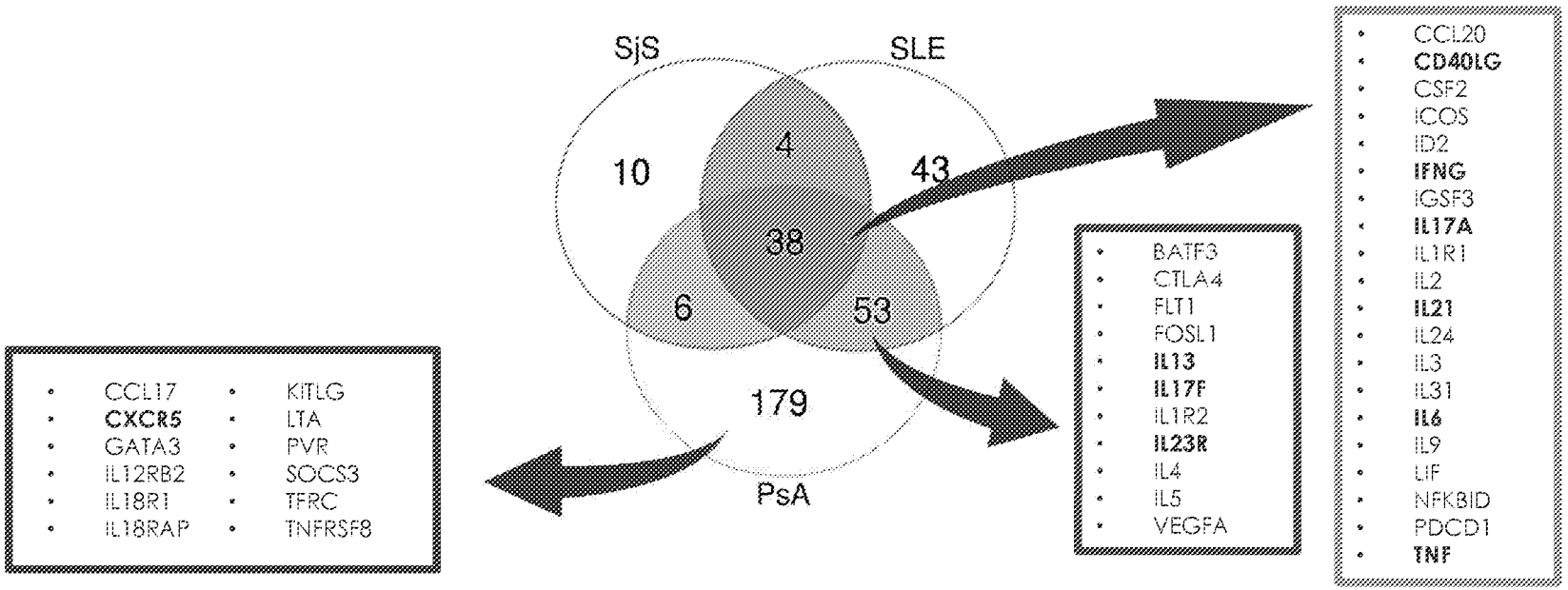

SjS
SLE
PsA
10
4
43
38
6
53
179
CCL17
CXCR5
GATA3
IL12RB2
IL18R1
IL18RAP
KITLG
LTA
PVR
SOCS3
TFRC
TNFRSF8
BATF3
CTLA4
FLT1
FOSL1
IL13
IL17F
IL1R2
IL23R
IL4
IL5
VEGFA
CCL20
CD40LG
CSF2
ICOS
ID2
IFNG
IGSF3
IL17A
IL1R1
IL2
IL21
IL24
IL3
IL31
IL6
IL9
LIF
NFKBID
PDCD1
TNF

FIG. 19K

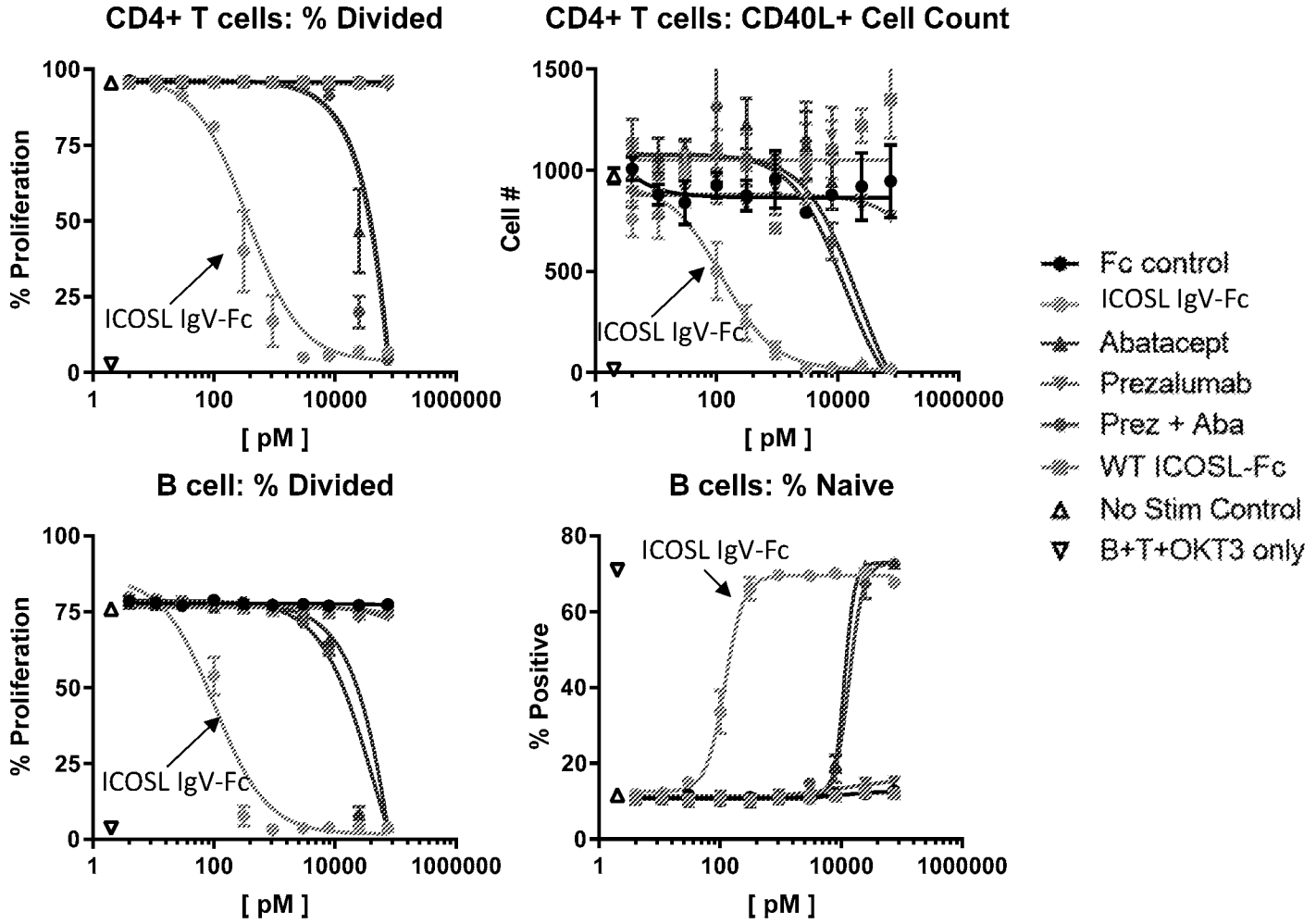

CD4+ T cells: % Divided
% Proliferation
100
75
50
25
0
ICOSL IgV-Fc
1
100
10000
1000000
[pM]
CD4+ T cells: CD40L+ Cell Count
Cell #
1500
1000
500
0
ICOSL IgV-Fc
1
100
10000
1000000
[pM]
B cell: % Divided
% Proliferation
100
75
50
25
0
ICOSL IgV-Fc
1
100
10000
1000000
[pM]
B cells: % Naive
% Positive
80
60
40
20
0
ICOSL IgV-Fc
1
100
10000
1000000
[pM]
Fc control
ICOSL IgV-Fc
Abatacept
Prezalumab
Prez + Aba
WT ICOSL-Fc
No Stim Control
B+T+OKT3 only

FIG. 19L

Parameter	D#	B-cell	IC50s (pM) Fc1.1	WT ICOSL	Preza	Aba	Aba + Prez	Bela	ICOSL IgV-Fc
T cell % Prolif	A	Naive	ND	ND	>75000	>75000	>75000		
	B	Naive	ND		ND				
	D	Total							
	E	Total	ND		ND	ND	ND		
T cell Recovery	A	Naive	ND	>75000	ND				
	B	Naive	ND						
	D	Total							
	E	Total	ND		ND				
CD40L+ T cell Recovery	A	Naive	ND	ND	ND				
	B	Naive							
	D	Total							
	E	Total	ND		ND	ND	ND		
B cell % Prolif	A	Naive	ND	>75000	ND	>75000			
	B	Naive	ND		ND				
	D	Total							
	E	Total	ND		ND				
B cell % Naive	A	Naive							
	B	Naive	ND						
	D	Total							
	E	Total	ND						
B cell % Activated (Non-Class-Switched)	A	Naive	ND						
	B	Naive	ND						
	D	Total							
	E	Total	ND						

(ND) Not Determined (-) Not assayed

Worse ... Better

FIG. 19M

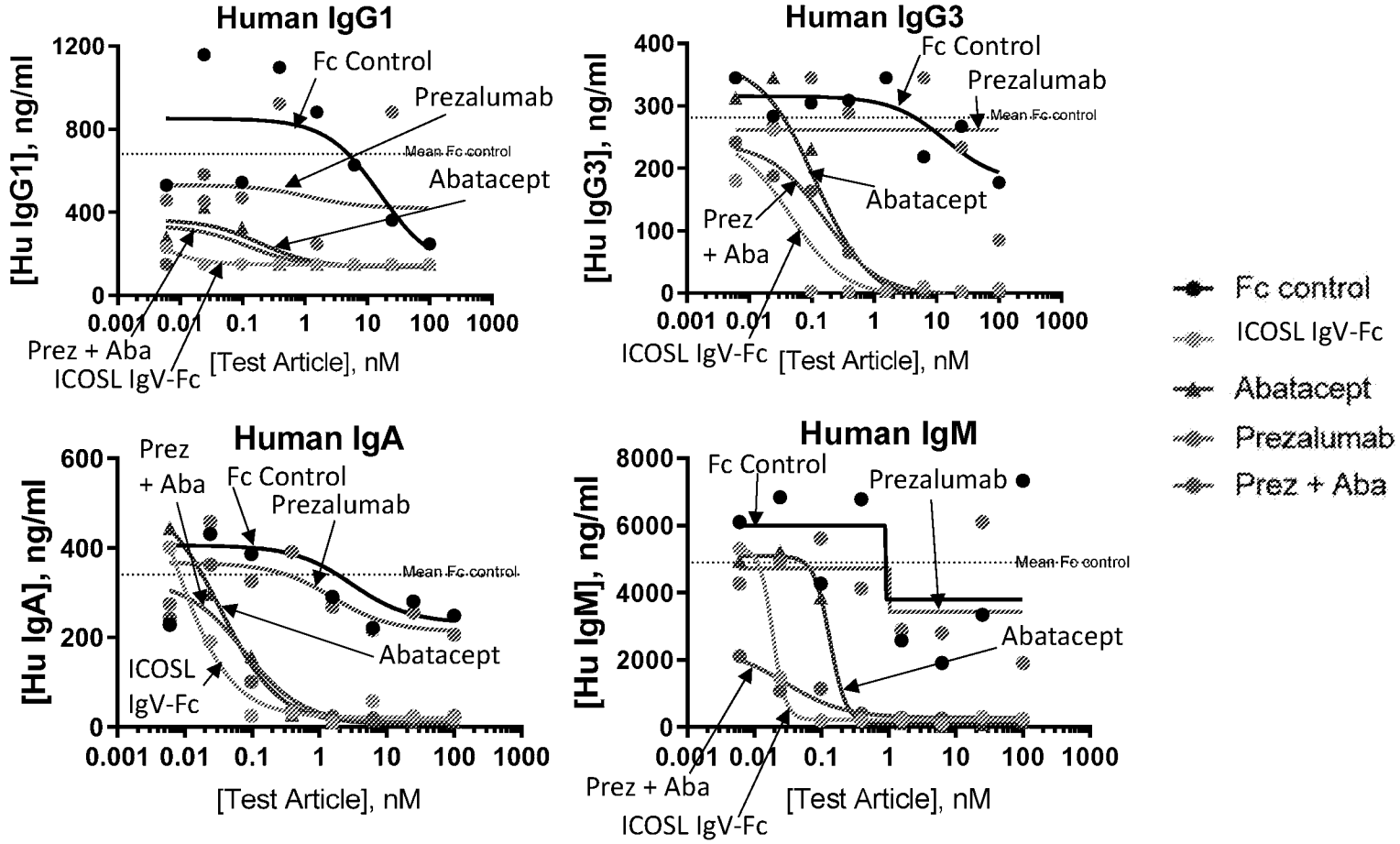

Human IgG1
[Hu IgG1], ng/ml
1200
800
400
0
Fc Control
Prezalumab
Mean Fc control
Abatacept
Prez + Aba
ICOSL IgV-Fc
0.001 0.01 0.1 1 10 100 1000
[Test Article], nM
Human IgG3
[Hu IgG3], ng/ml
400
300
200
100
0
Fc Control
Prezalumab
Mean Fc control
Abatacept
Prez
+ Aba
ICOSL IgV-Fc
0.001 0.01 0.1 1 10 100 1000
[Test Article], nM
Human IgA
[Hu IgA], ng/ml
600
400
200
0
Prez
+ Aba
Fc Control
Prezalumab
Mean Fc control
Abatacept
ICOSL
IgV-Fc
0.001 0.01 0.1 1 10 100 1000
[Test Article], nM
Human IgM
[Hu IgM], ng/ml
8000
6000
4000
2000
0
Fc Control
Prezalumab
Mean Fc control
Abatacept
Prez + Aba
ICOSL IgV-Fc
0.001 0.01 0.1 1 10 100 1000
[Test Article], nM
Fc control
ICOSL IgV-Fc
Abatacept
Prezalumab
Prez + Aba

FIG. 19N

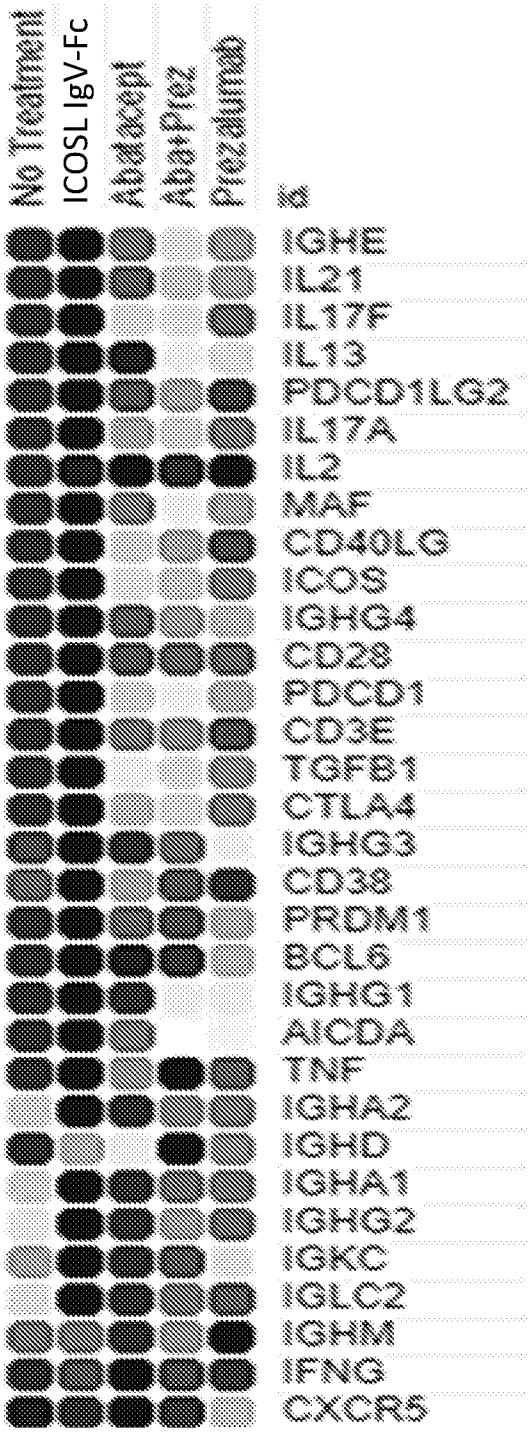

No Treatment
ICOSL IgV-Fc
Abatacept
Abat+Prez
Prezalumab
Id
IGHE
IL21
IL17F
IL13
PDCD1LG2
IL17A
IL2
MAF
CD40LG
ICOS
IGHG4
CD28
PDCD1
CD3E
TGFB1
CTLA4
IGHG3
CD38
PRDM1
BCL6
IGHG1
AICDA
TNF
IGHA2
IGHD
IGHA1
IGHG2
IGKC
IGLC2
IGHM
IFNG
CXCR5

FIG. 20A

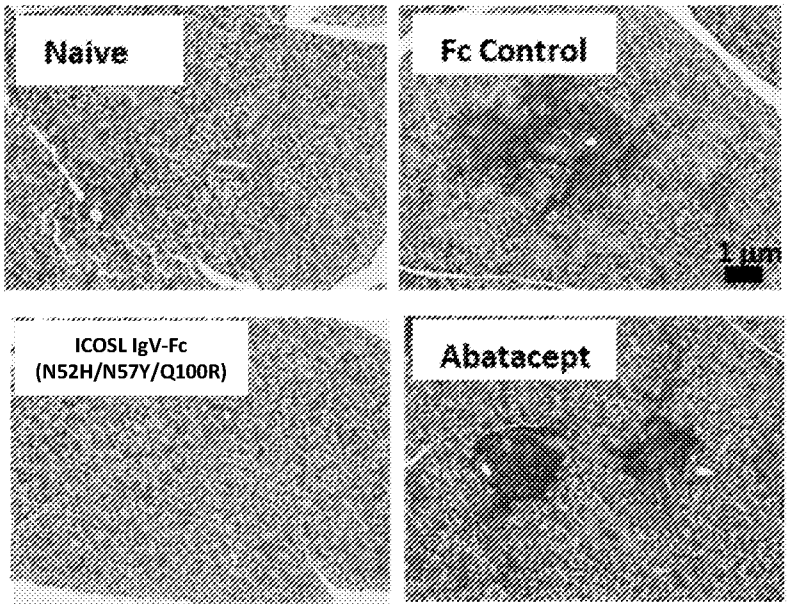


FIG. 20B

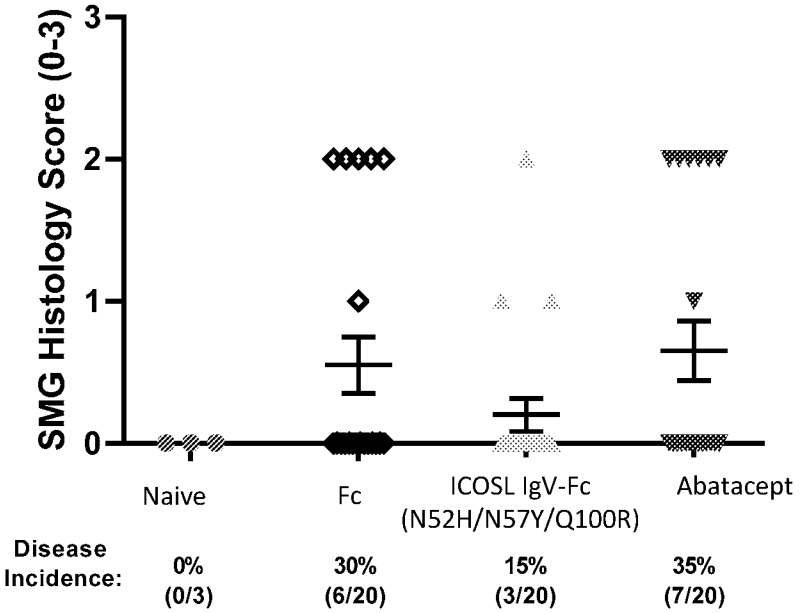


FIG. 20C

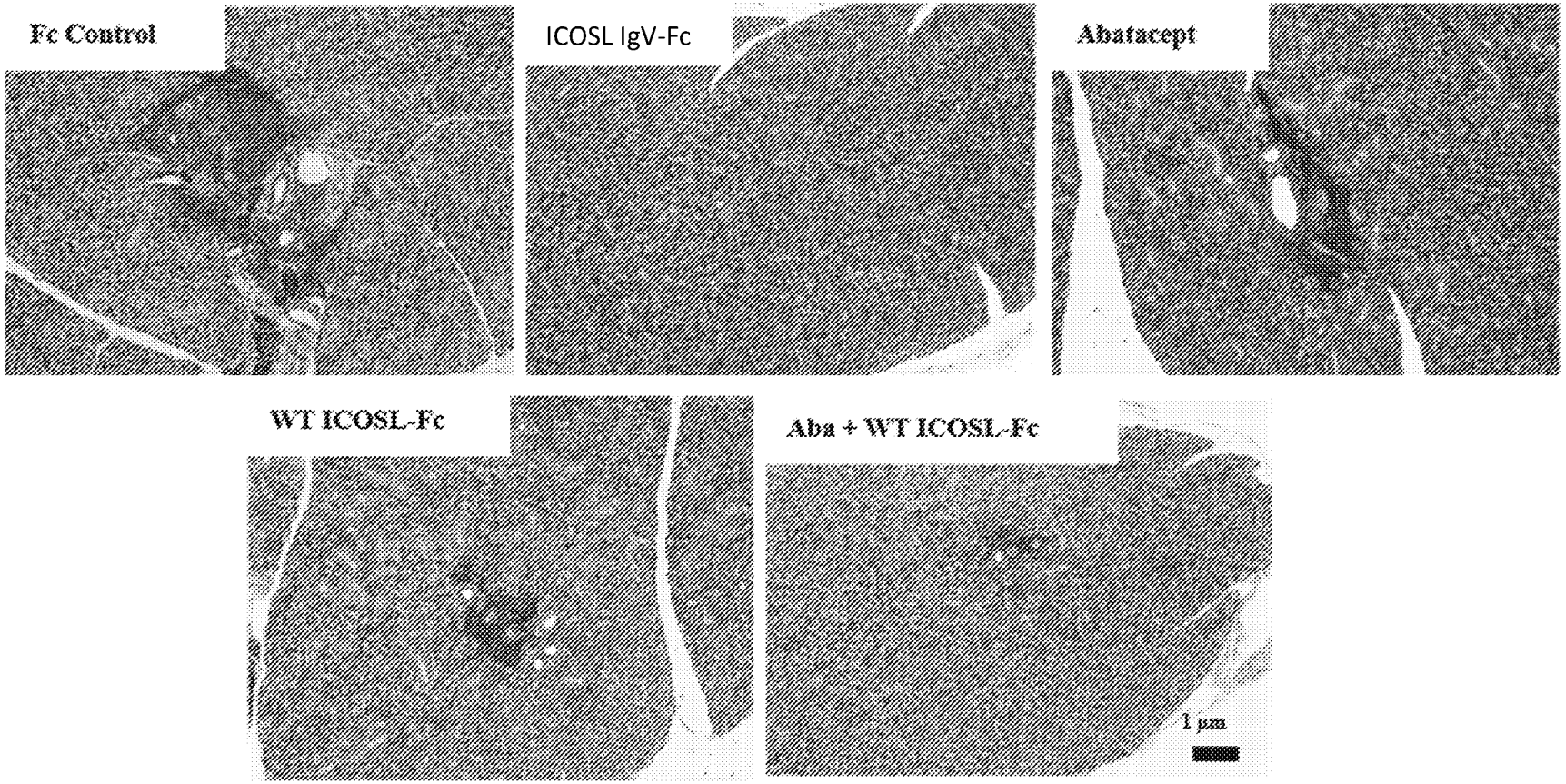

Fc Control
ICOSL IgV-Fc
Abatacept
WT ICOSL-Fc
Aba + WT ICOSL-Fc
1 μm

FIG. 20D

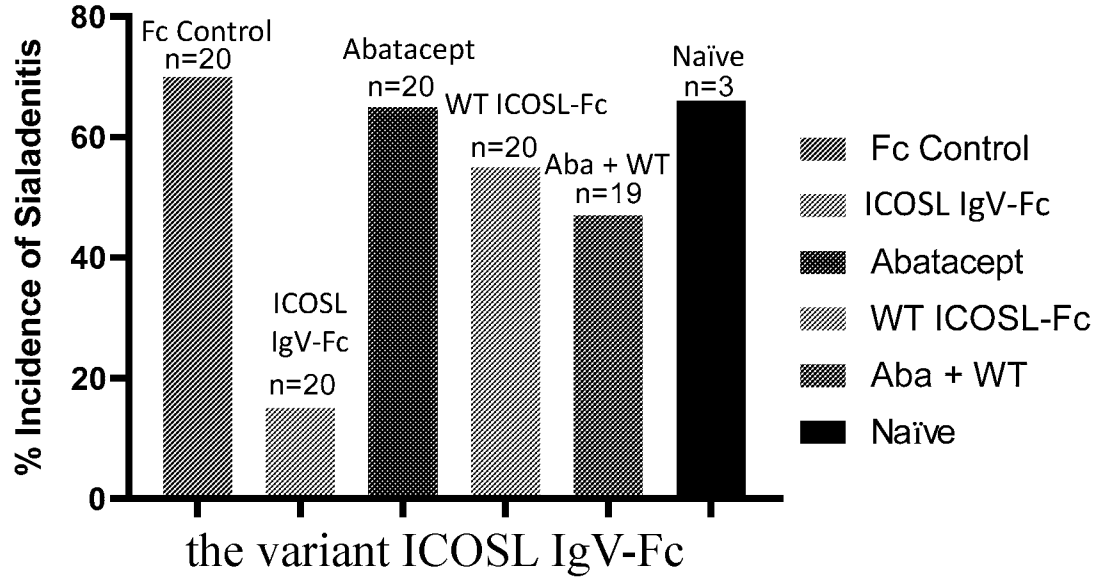


FIG. 20E

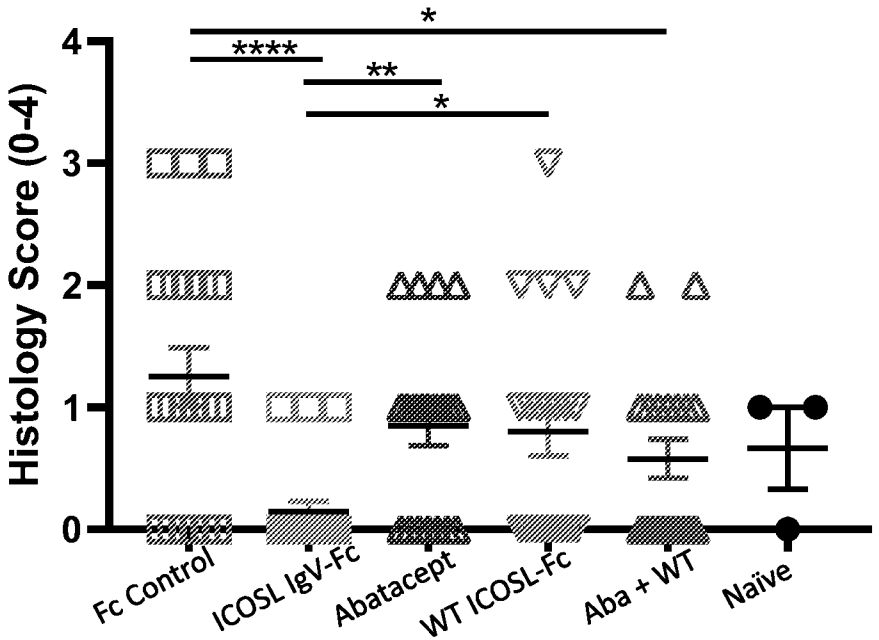
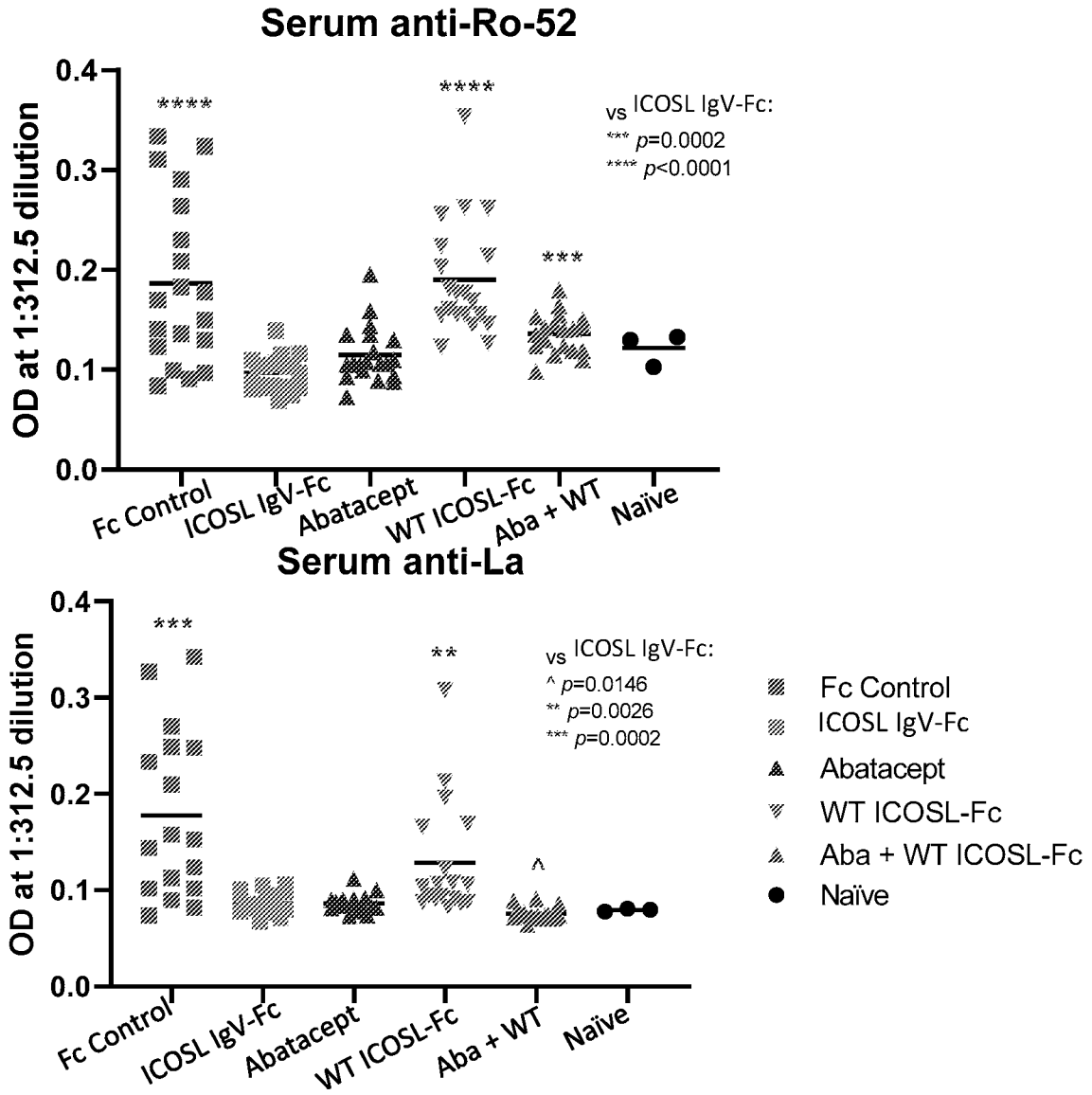


FIG. 20F


Serum anti-Ro-52
OD at 1:312.5 dilution
0.4
0.3
0.2
0.1
0.0
****
****
***
vs ICOSL IgV-Fc:
*** p=0.0002
**** p<0.0001
Fc Control
ICOSL IgV-Fc
Abatacept
WT ICOSL-Fc
Aba + WT
Naïve
Serum anti-La
OD at 1:312.5 dilution
0.4
0.3
0.2
0.1
0.0
***
**
^
vs ICOSL IgV-Fc:
^ p=0.0146
** p=0.0026
*** p=0.0002
Fc Control
ICOSL IgV-Fc
Abatacept
WT ICOSL-Fc
Aba + WT
Naïve
Fc Control
ICOSL IgV-Fc
Abatacept
WT ICOSL-Fc
Aba + WT ICOSL-Fc
Naïve

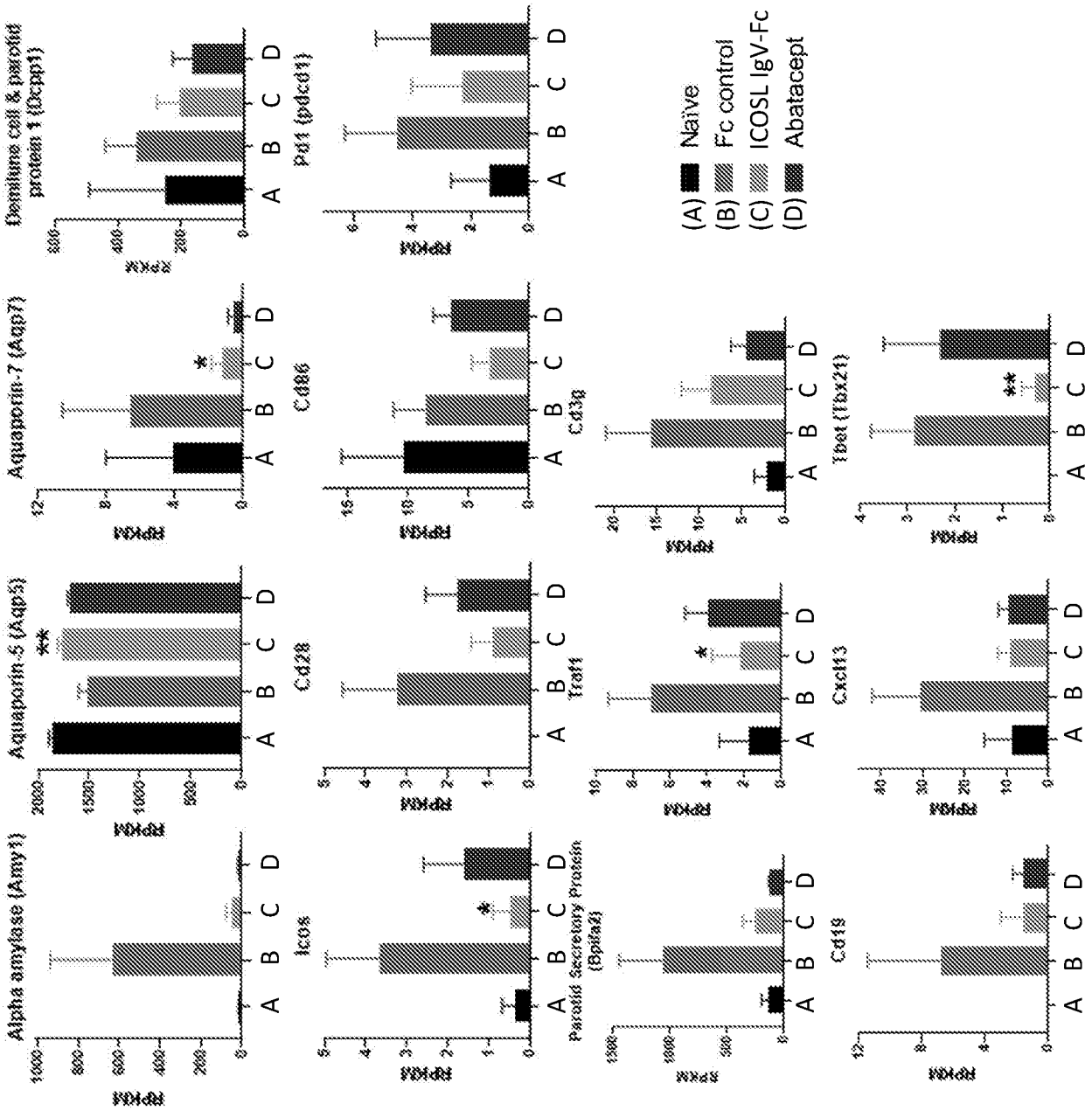

Alpha amylase (Amy1)
Aquaporin-5 (Aqp5)
Aquaporin-7 (Aqp7)
Dermiuna cell & parotid protein 1 (Dcpp1)
Icos
Cd28
Traf1
Pd1 (pdc01)
Parotid Secretory Protein (Bpifa2)
Cd86
Cd3g
Tbet (Tbx21)
Cd19
Cxcl13
RPKM
A B C D
(A) Naive
(B) Fc control
(C) ICOSL IgV-Fc
(D) Abatacept

FIG. 20G

FIG. 20H

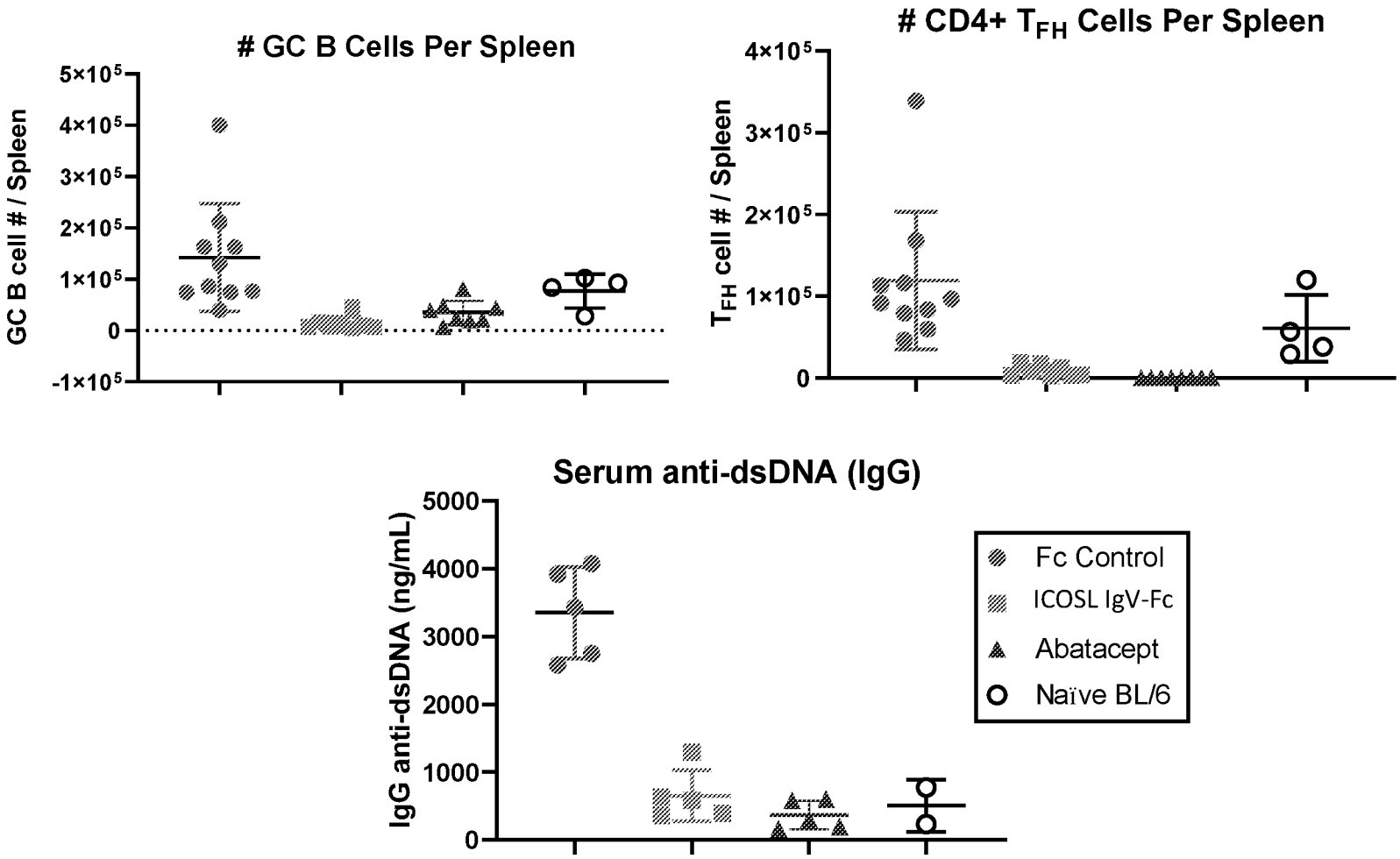

GC B Cells Per Spleen
GC B cell # / Spleen
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
0
-1×10^5
CD4+ T_FH Cells Per Spleen
T_FH cell # / Spleen
4×10^5
3×10^5
2×10^5
1×10^5
0
Serum anti-dsDNA (IgG)
IgG anti-dsDNA (ng/mL)
5000
4000
3000
2000
1000
0
Fc Control
ICOSL IgV-Fc
Abatacept
Naïve BL/6

FIG. 21A

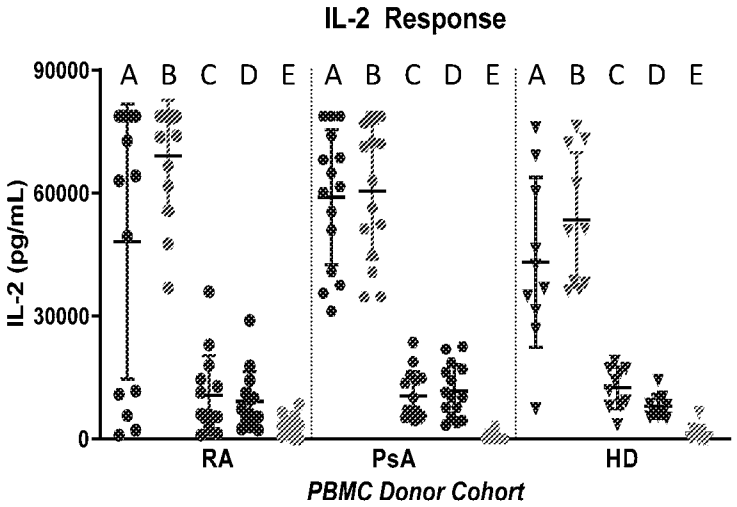
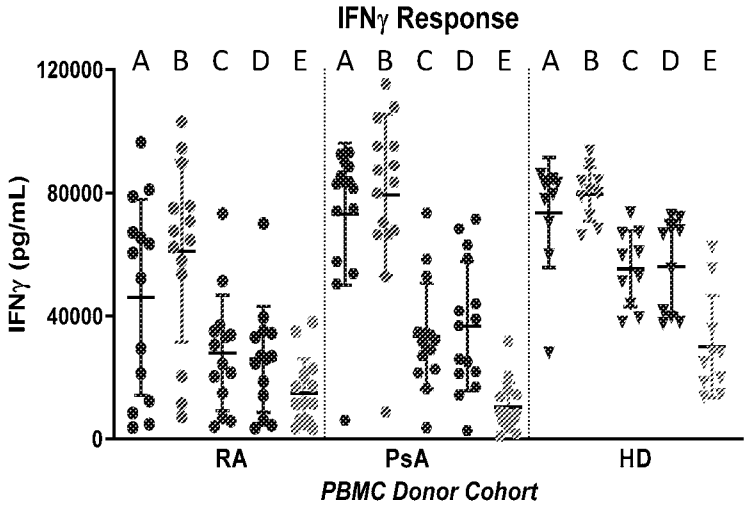


FIG. 21B



(A) Fc Control
(B) Prezalumab
(C) Abatacept
(D) Abatacept + Prezalumab
(E) ICOSL IgV-Fc (N52H/N57Y/Q100R)

FIG. 21C

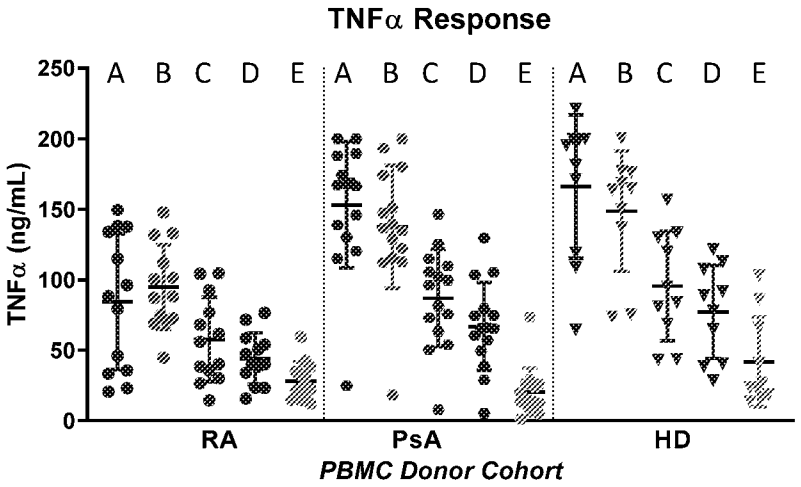
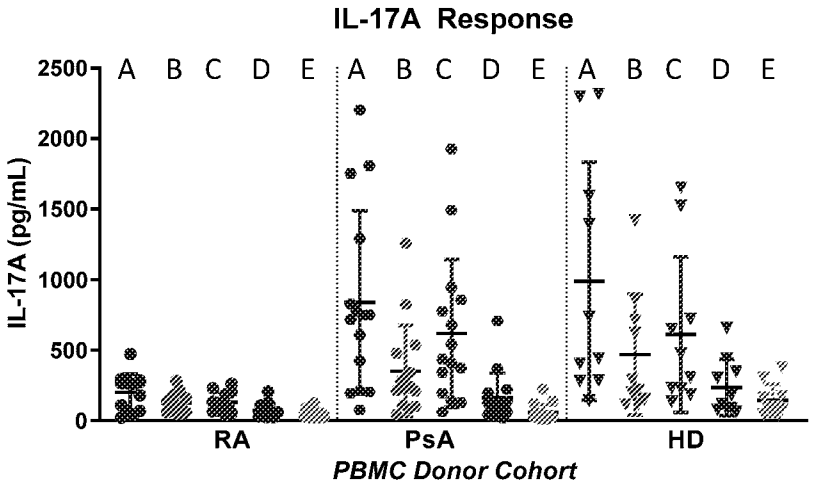


FIG. 21D



(A) Fc Control
(B) Prezalumab
(C) Abatacept
(D) Abatacept + Prezalumab
(E) ICOSL IgV-Fc (N52H/N57Y/Q100R)

FIG. 21E

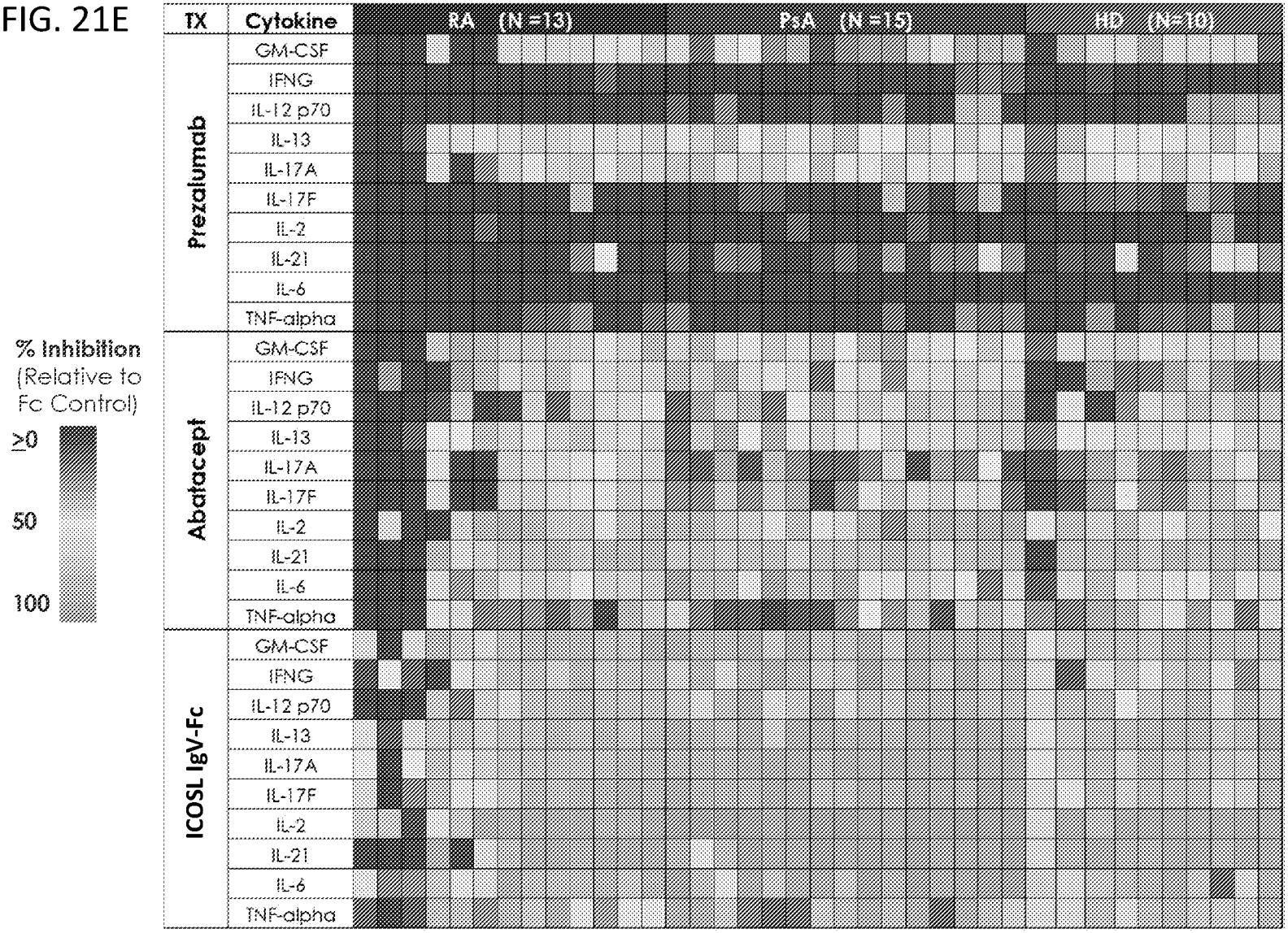

TX
Cytokine
RA (N=13)
PsA (N=15)
HD (N=10)
Prezalumab
Abatacept
ICOSL IgV-Fc
GM-CSF
IFNG
IL-12 p70
IL-13
IL-17A
IL-17F
IL-2
IL-21
IL-6
TNF-alpha
% Inhibition
(Relative to
Fc Control)
≥0
50
100

FIG. 22A

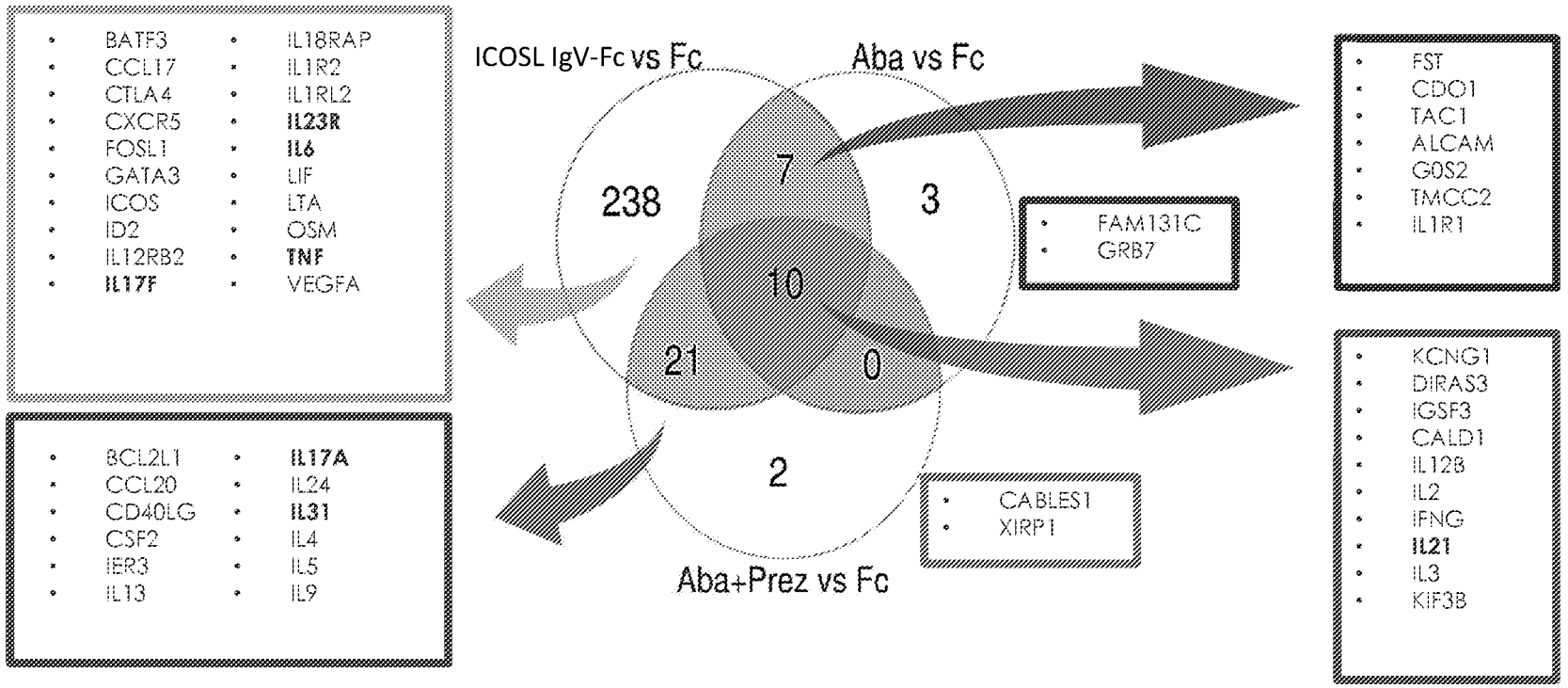

BATF3
CCL17
CTLA4
CXCR5
FOSL1
GATA3
ICOS
ID2
IL12RB2
IL17F
IL18RAP
IL1R2
IL1RL2
IL23R
IL6
LIF
LTA
OSM
TNF
VEGFA
BCL2L1
CCL20
CD40LG
CSF2
IER3
IL13
IL17A
IL24
IL31
IL4
IL5
IL9
ICOSL IgV-Fc vs Fc
Aba vs Fc
Aba+Prez vs Fc
238
7
3
10
21
0
2
FAM131C
GRB7
CABLES1
XIRP1
FST
CDO1
TAC1
ALCAM
G0S2
TMCC2
IL1R1
KCNG1
DIRAS3
IGSF3
CALD1
IL12B
IL2
IFNG
IL21
IL3
KIF3B

FIG. 22B

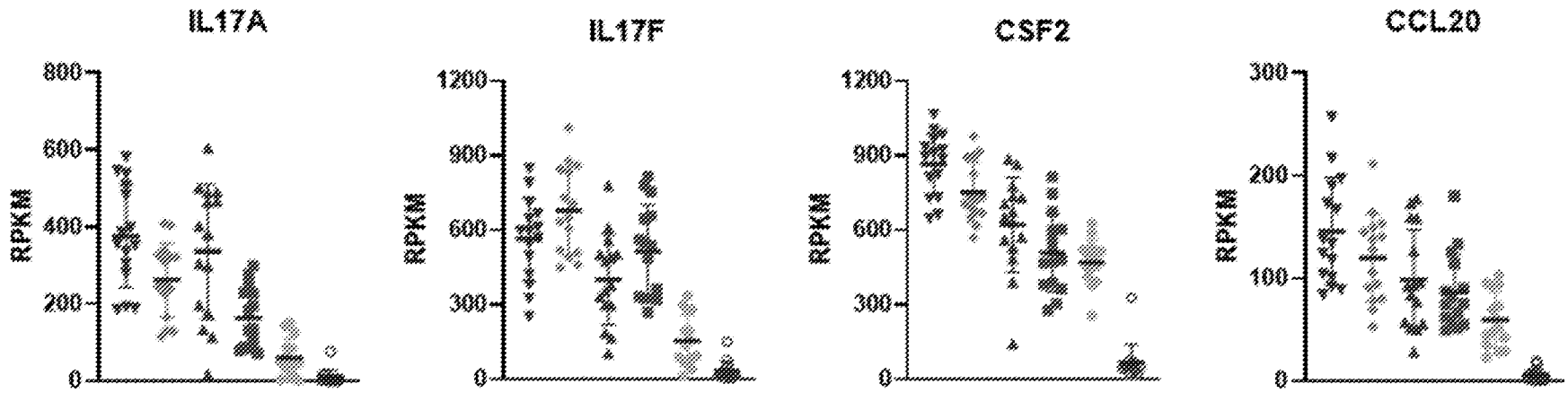


FIG. 22C

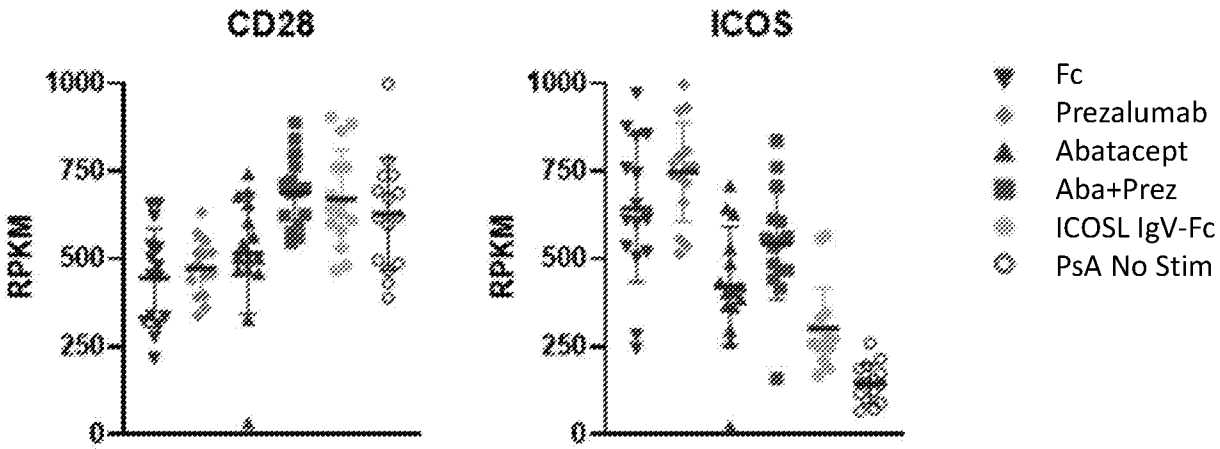


FIG. 22D

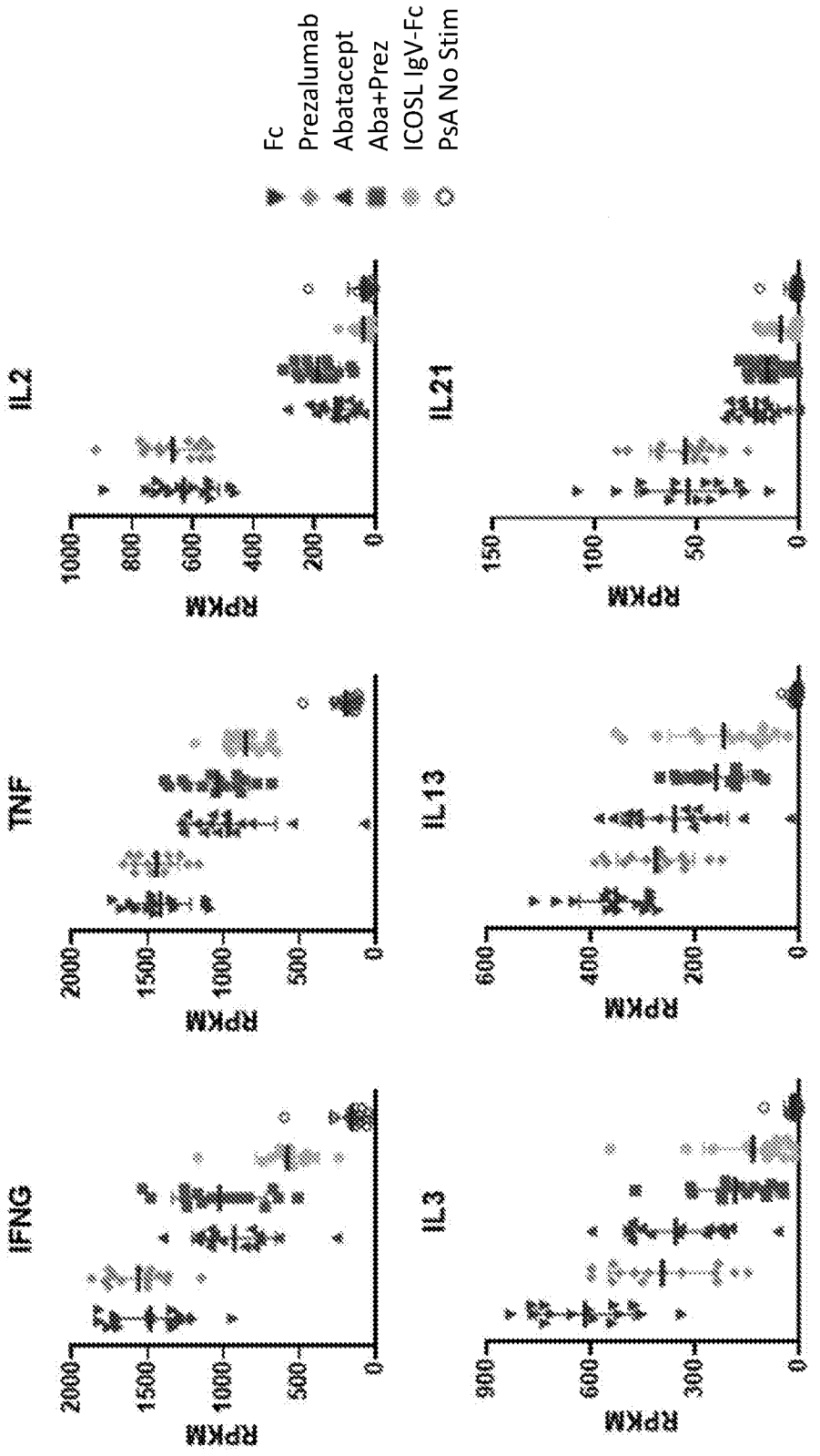

IFNG
TNF
IL2
IL3
IL13
IL21
RPKM
Fc
Prezalumab
Abatacept
Aba+Prez
ICOSL IgV-Fc
PsA No Stim

FIG. 23A

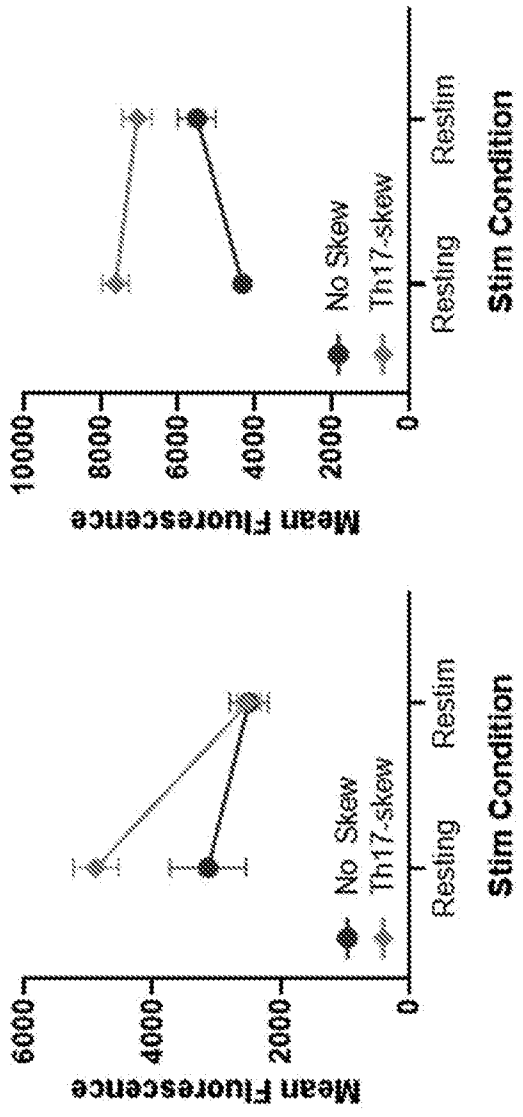

ICOS
Mean Fluorescence
10000
8000
6000
4000
2000
0
Resting
Restim
Stim Condition
No Skew
Th17-skew
CD28
Mean Fluorescence
6000
4000
2000
0
Resting
Restim
Stim Condition
No Skew
Th17-skew

FIG. 23B

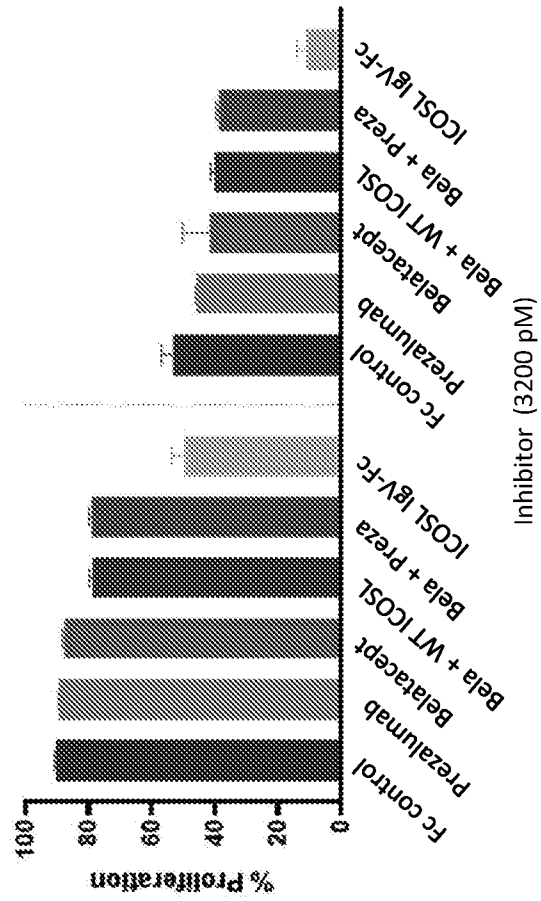


FIG. 23C

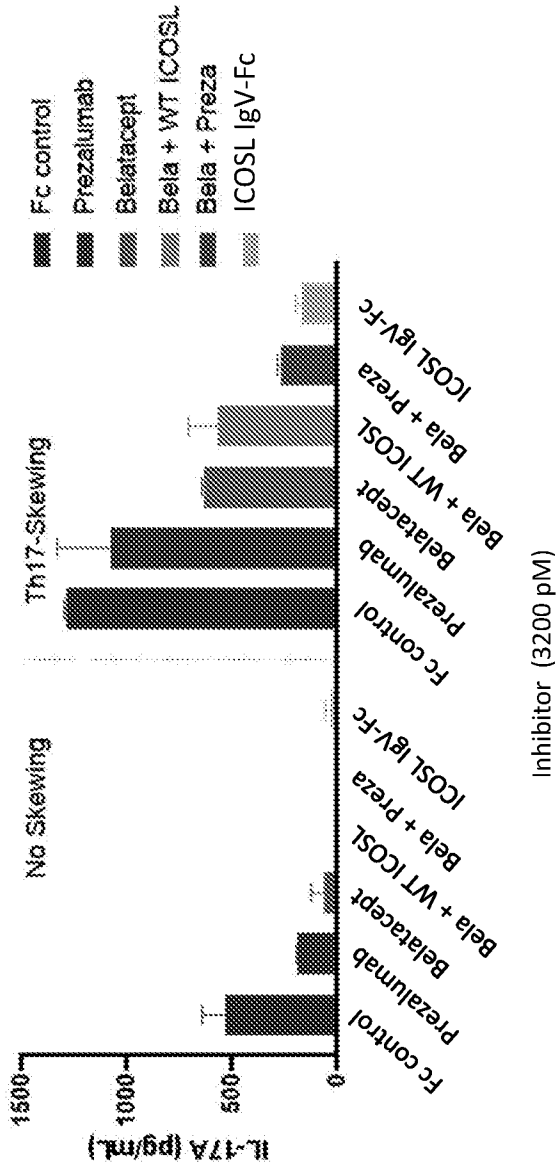
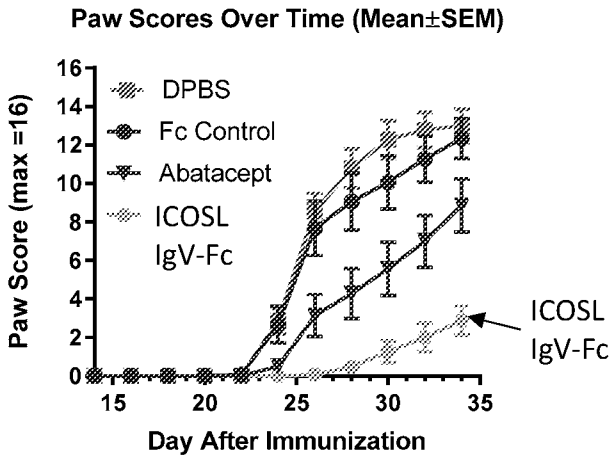


FIG. 24A



Paw Scores Over Time (P values)

	DPBS	Fc Control	Abatacept	ICOSL IgV-Fc
DPBS	-	-	0.0006	<0.0001
Fc Control	-	-	0.0172	<0.0001
Abatacept	-	-	-	0.0016
ICOSL IgV-Fc	-	-	-	-

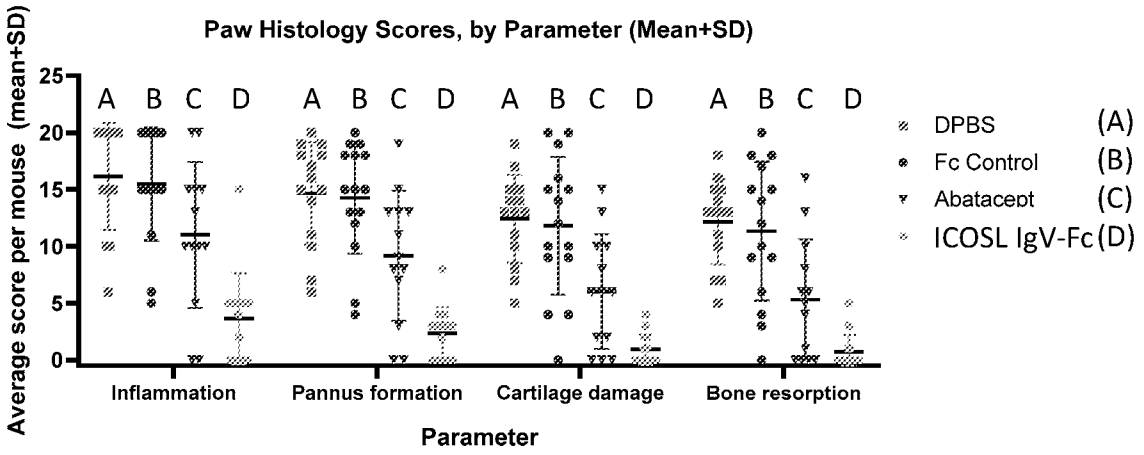


FIG. 24B

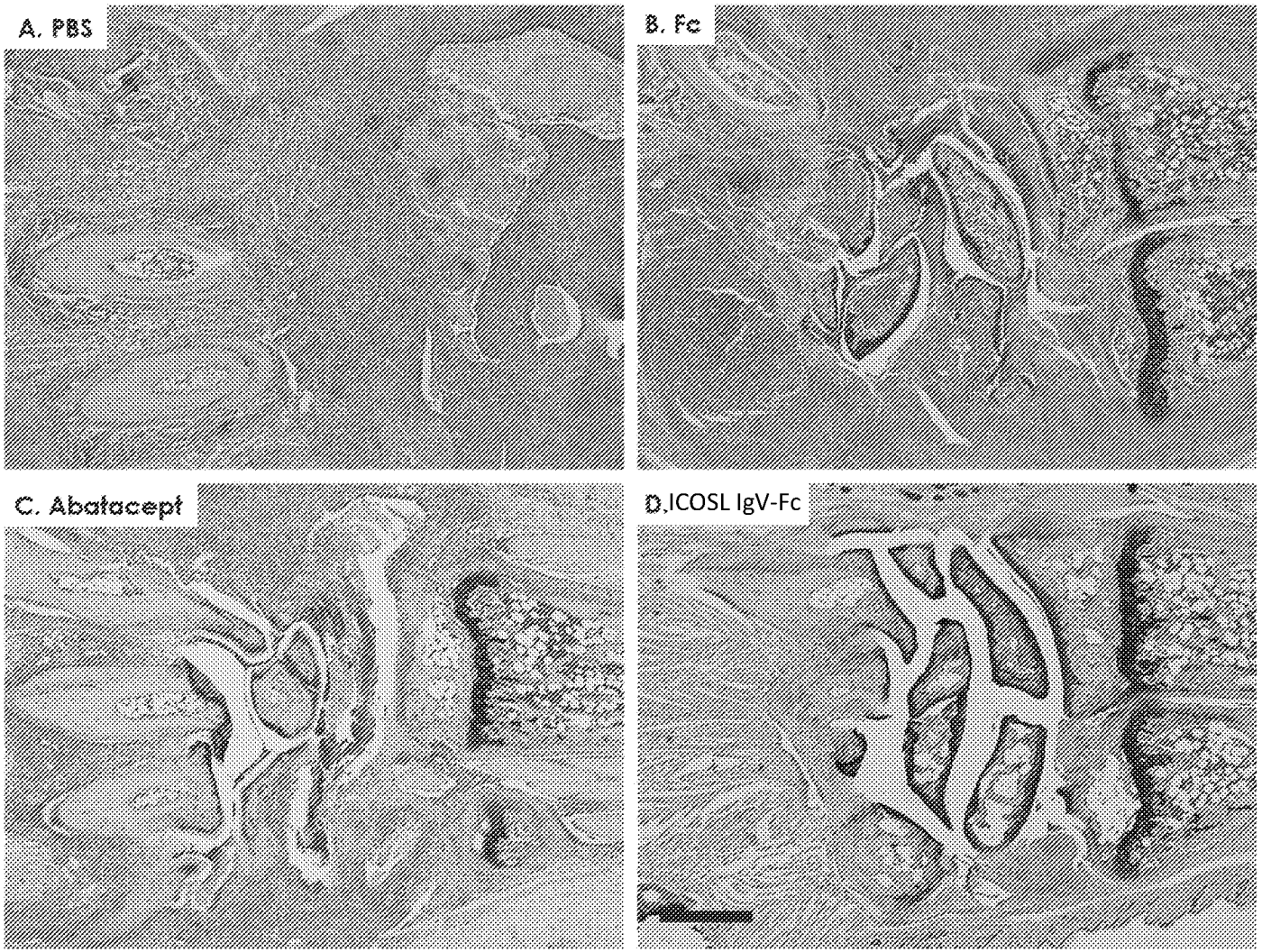


FIG. 24C

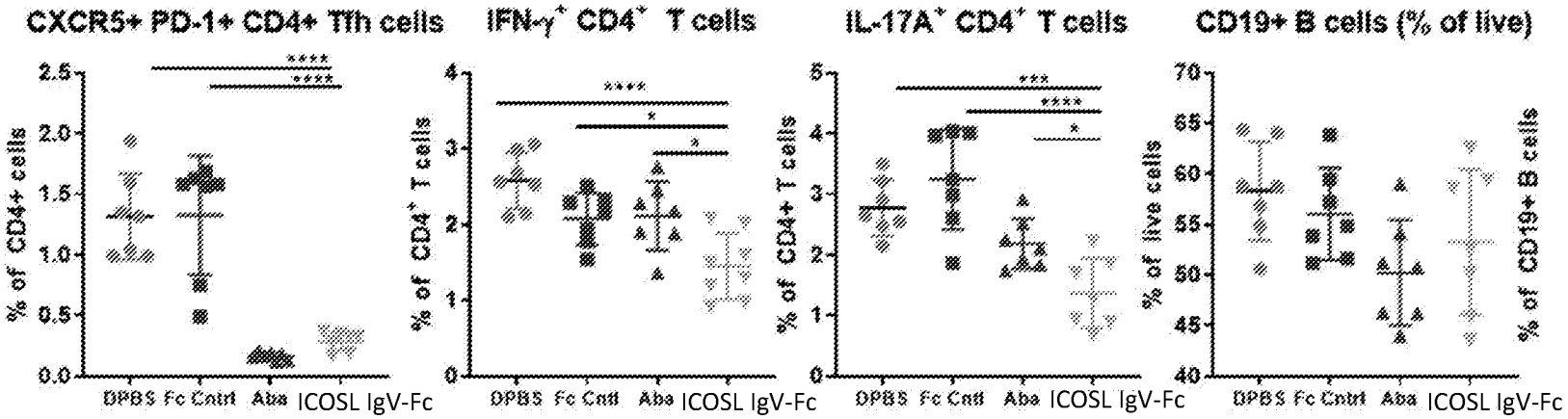


FIG. 24D

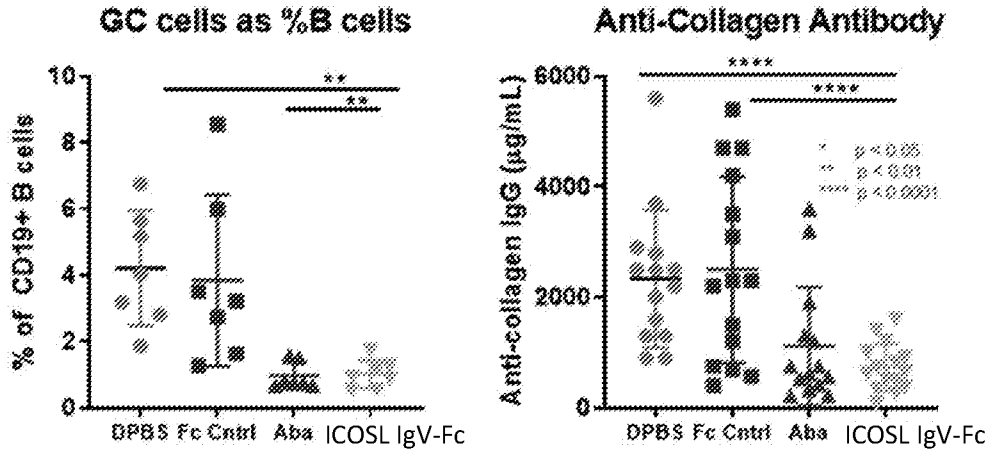


FIG. 24E

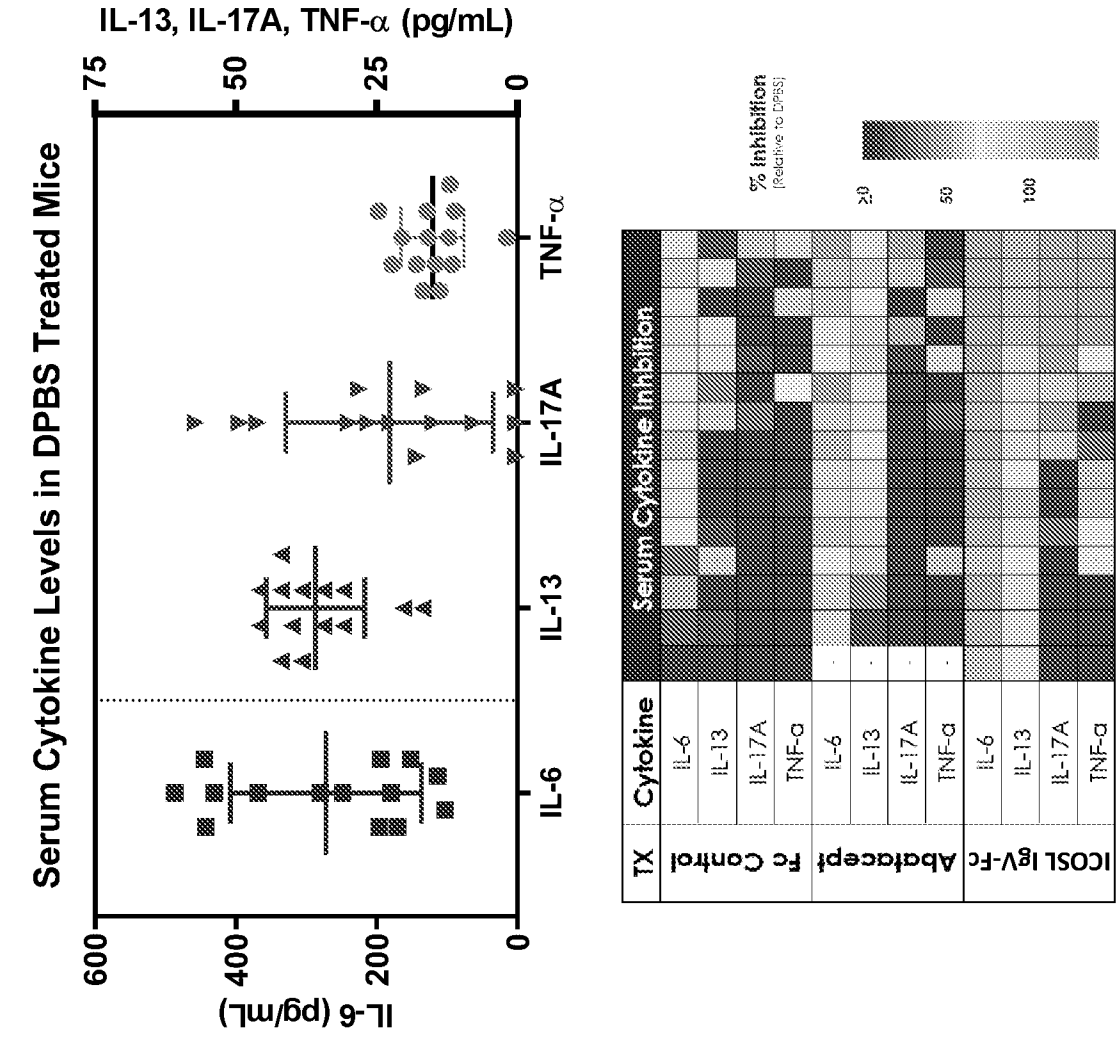

Serum Cytokine Levels in DPBS Treated Mice
IL-13, IL-17A, TNF-α (pg/mL)
IL-6 (pg/mL)
600
400
200
0
75
50
25
0
IL-6
IL-13
IL-17A
TNF-α
Serum Cytokine Inhibition
TX
Cytokine
Fc Control
Abatacept
ICOSL IgV-Fc
IL-6
IL-13
IL-17A
TNF-α
% Inhibition
(Relative to DPBS)
≥0
50
100

FIG. 25A

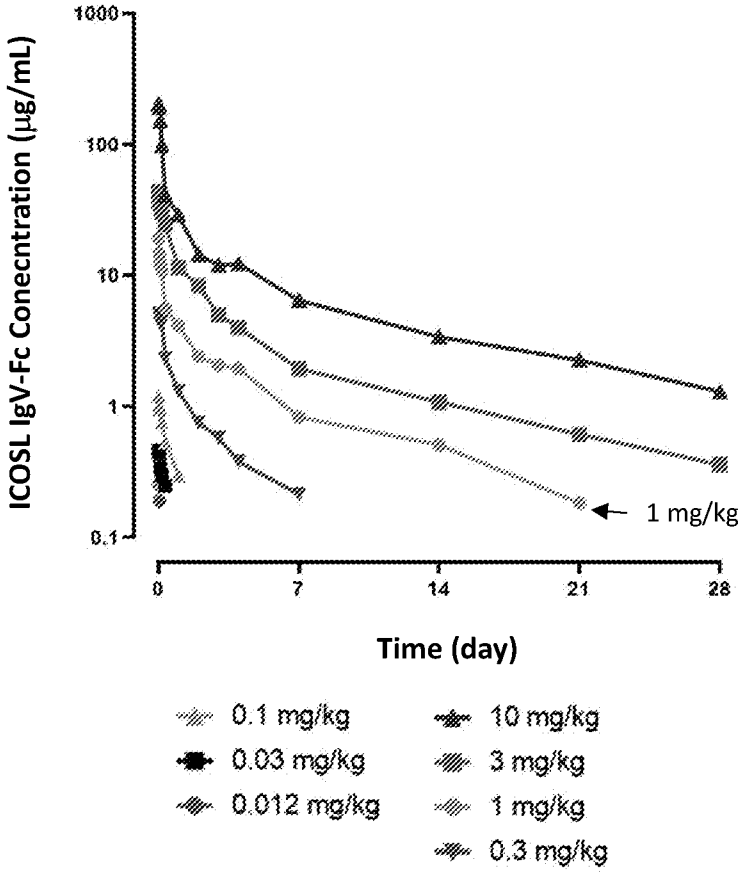


FIG. 25B

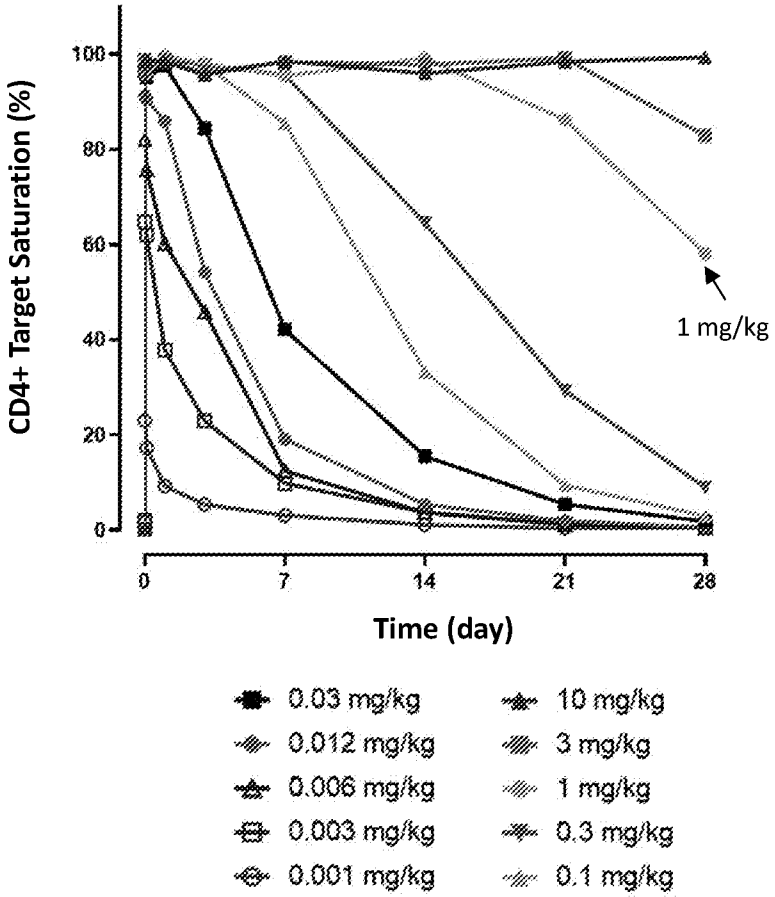


FIG. 26A

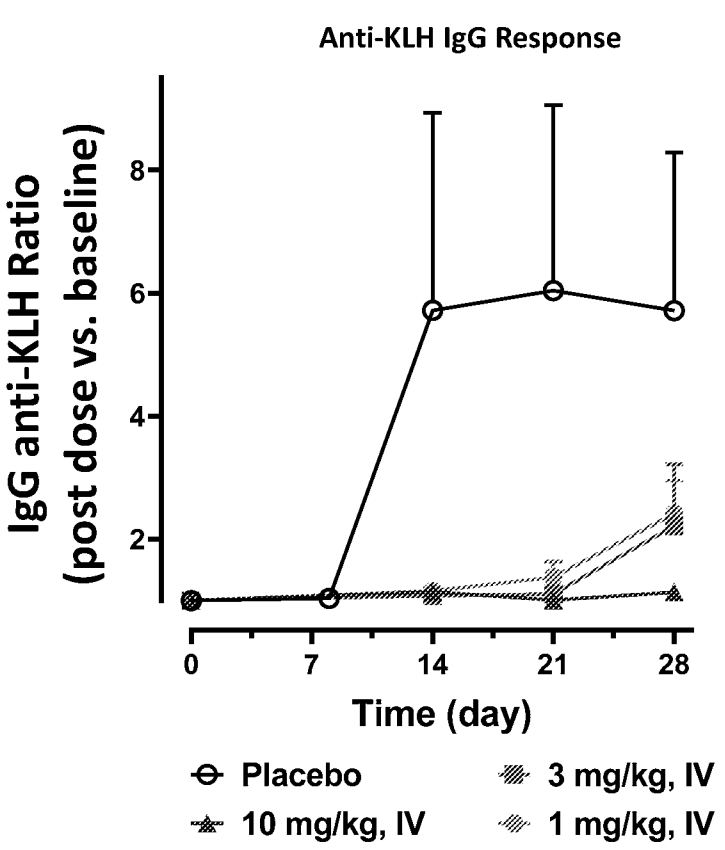


FIG. 26B

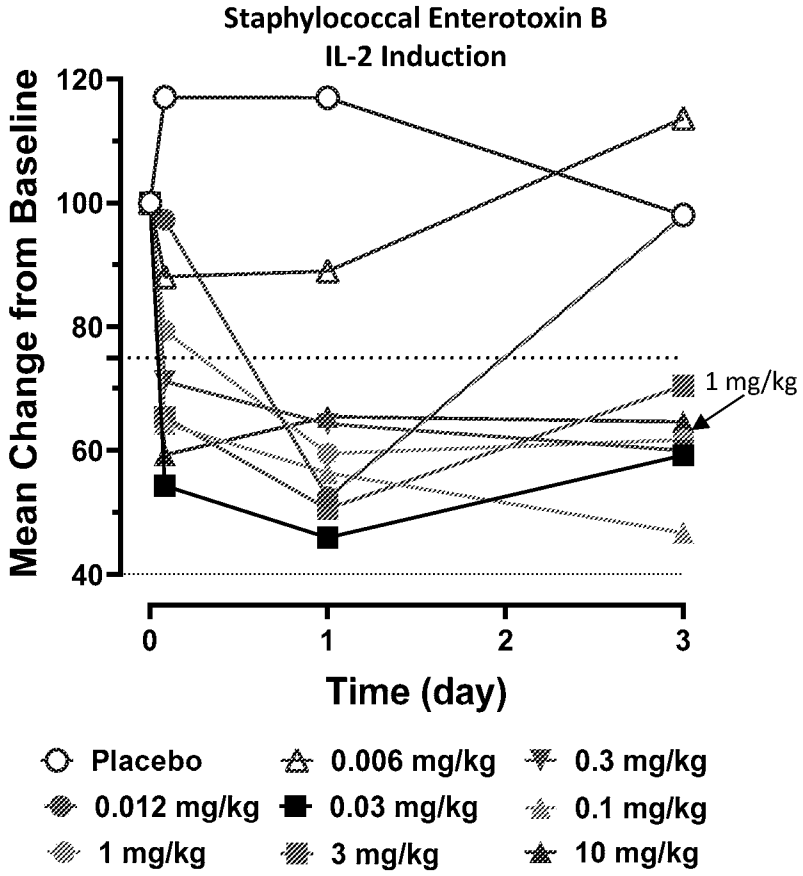


FIG. 27A

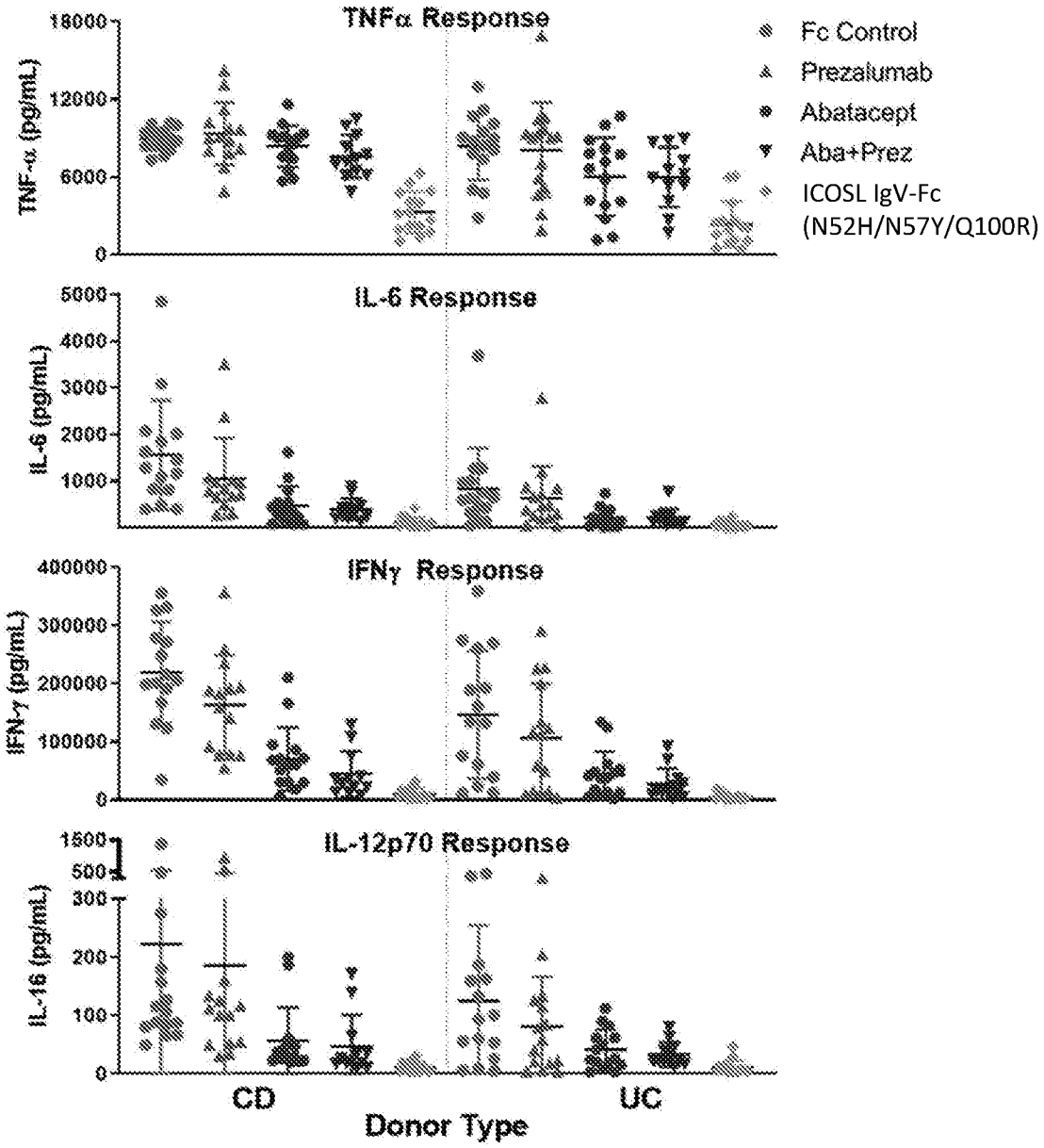

TNFα Response
TNF-α (pg/mL)
18000
12000
6000
0
IL-6 Response
IL-6 (pg/mL)
5000
4000
3000
2000
1000
IFNγ Response
IFN-γ (pg/mL)
400000
300000
200000
100000
0
IL-12p70 Response
IL-16 (pg/mL)
1500
500
300
200
100
0
CD
UC
Donor Type
Fc Control
Prezalumab
Abatacept
Aba+Prez
ICOSL IgV-Fc
(N52H/N57Y/Q100R)

FIG. 27B

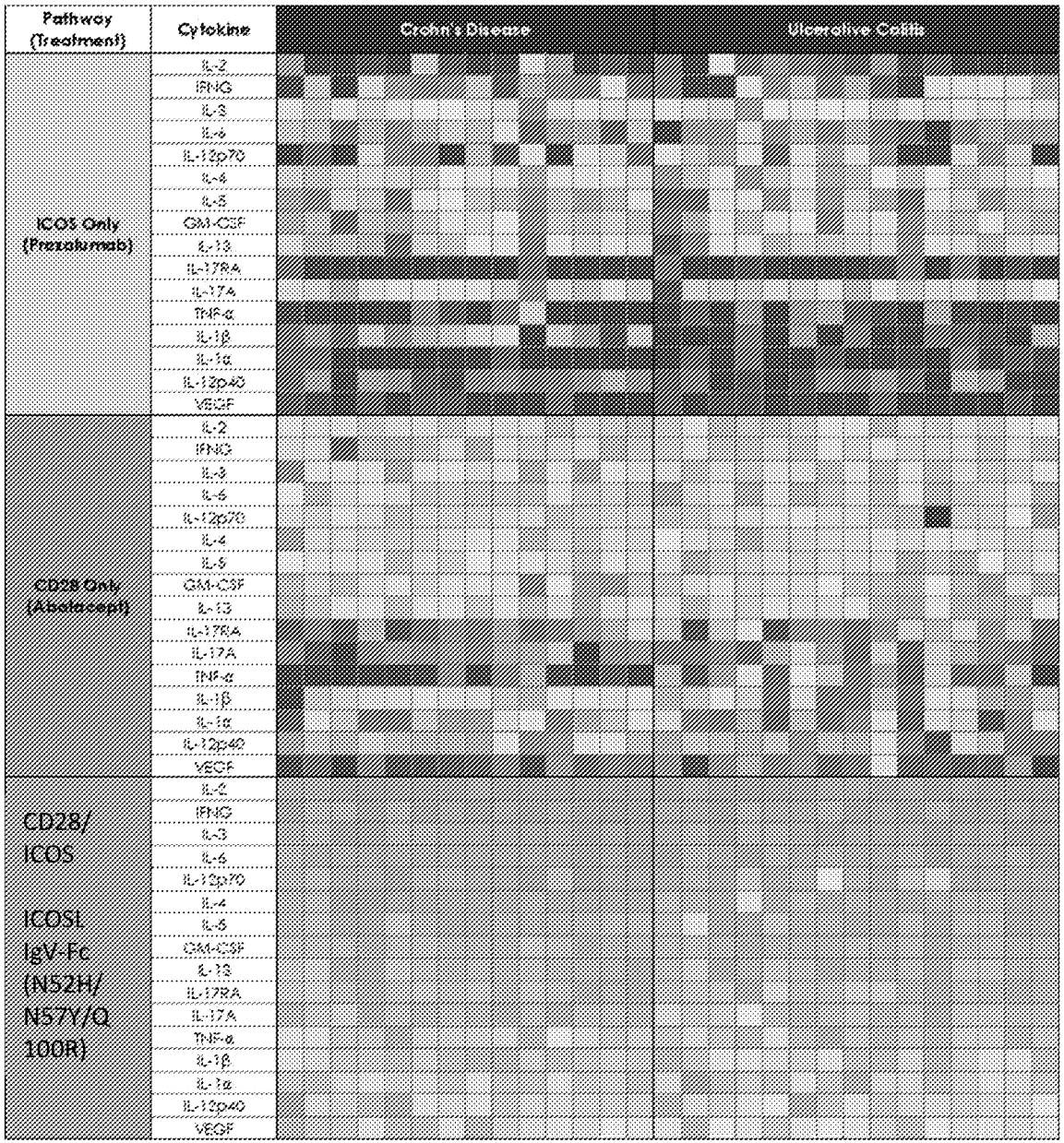

Pathway (Treatment)
Cytokine
Crohn's Disease
Ulcerative Colitis
ICOS Only (Frezalumab)
CD28 Only (Abatacept)
CD28/ ICOS
ICOSL IgV-Fc (N52H/ N57Y/Q 100R)
IL-2
IFNG
IL-3
IL-6
IL-12p70
IL-4
IL-5
GM-CSF
IL-13
IL-17RA
IL-17A
TNF-α
IL-1β
IL-1α
IL-12p40
VEGF

FIG. 28A

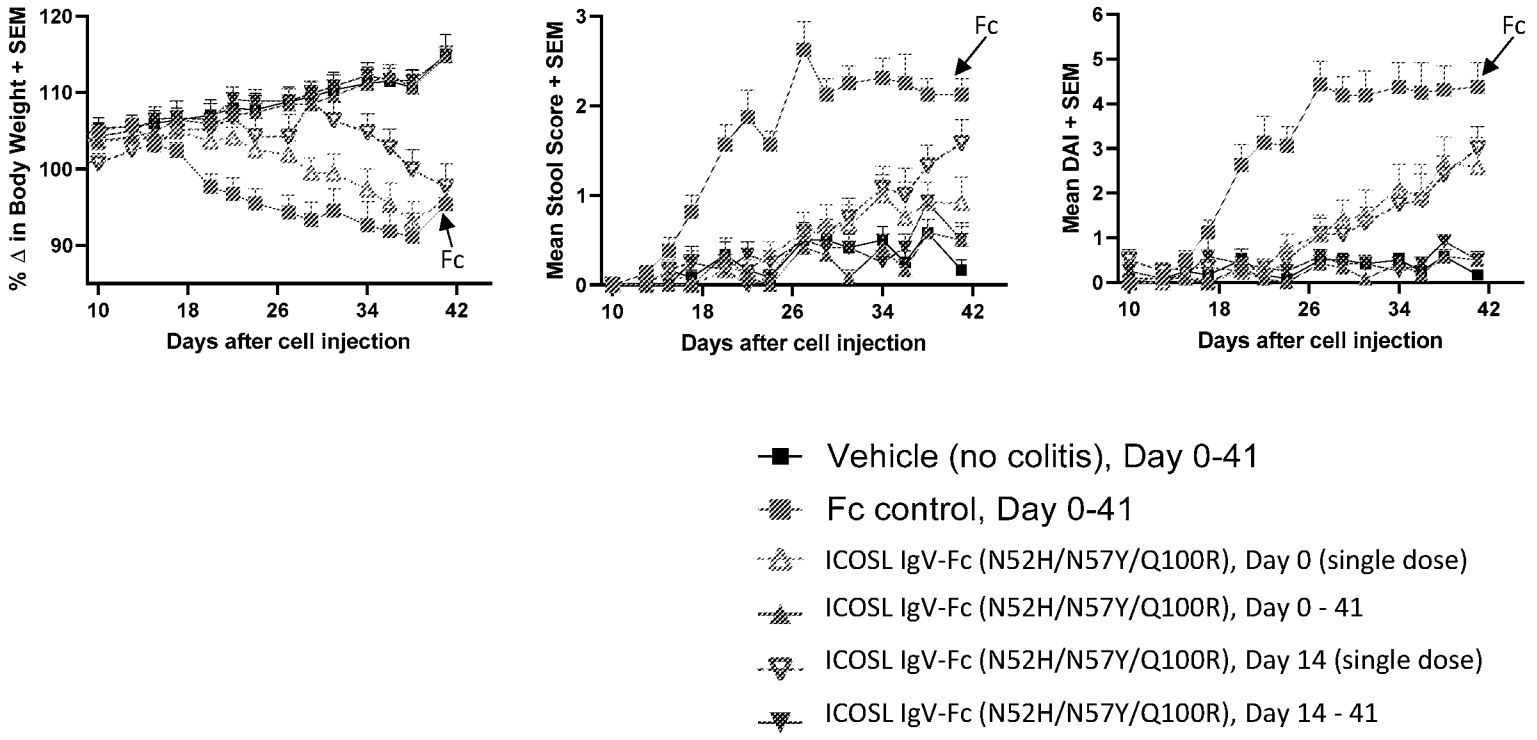

% Δ in Body Weight + SEM
120
110
100
90
10
18
26
34
42
Days after cell injection
Fc
Mean Stool Score + SEM
3
2
1
0
10
18
26
34
42
Days after cell injection
Fc
Mean DAI + SEM
6
5
4
3
2
1
0
10
18
26
34
42
Days after cell injection
Fc
Vehicle (no colitis), Day 0-41
Fc control, Day 0-41
ICOSL IgV-Fc (N52H/N57Y/Q100R), Day 0 (single dose)
ICOSL IgV-Fc (N52H/N57Y/Q100R), Day 0 - 41
ICOSL IgV-Fc (N52H/N57Y/Q100R), Day 14 (single dose)
ICOSL IgV-Fc (N52H/N57Y/Q100R), Day 14 - 41

FIG. 28B

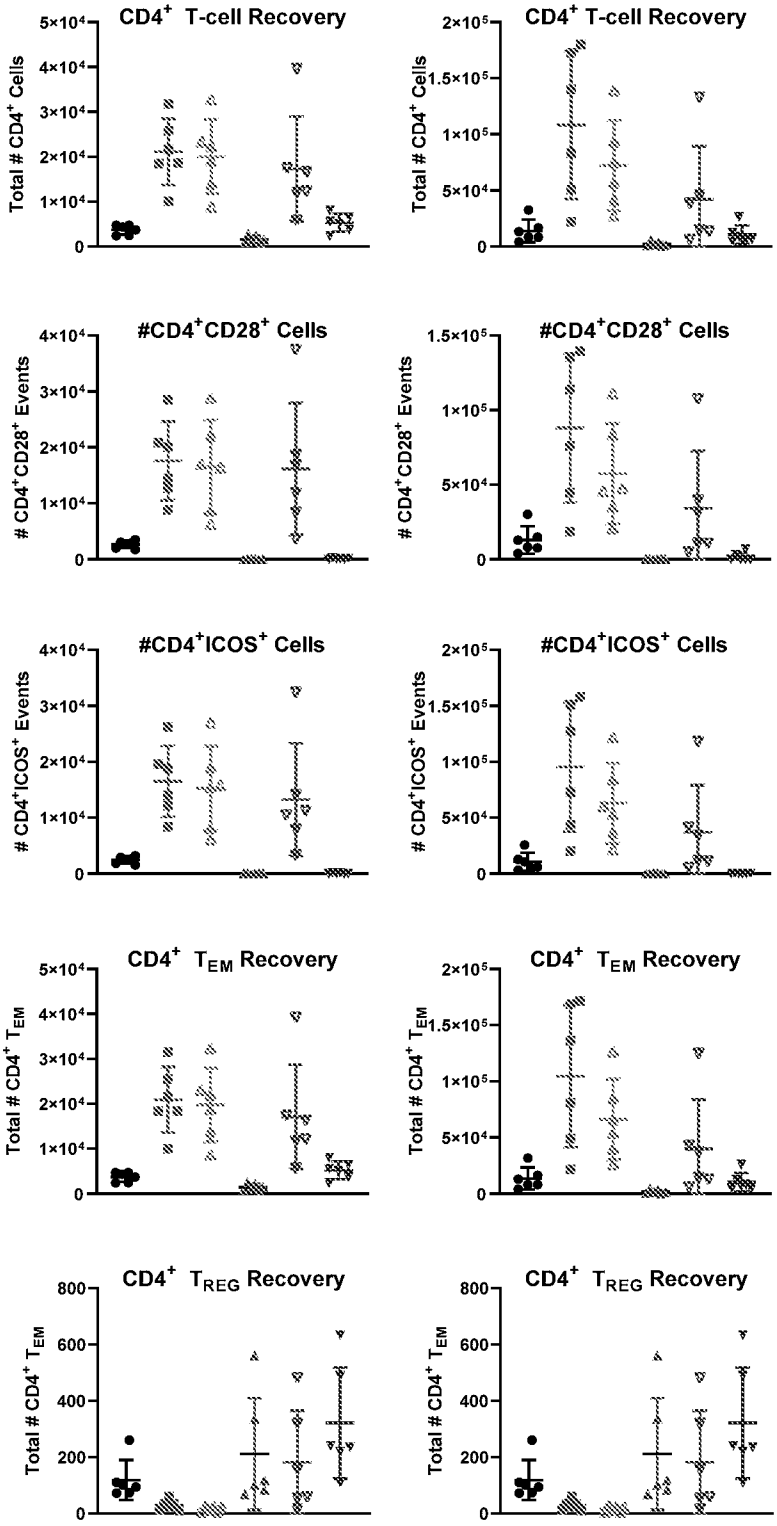

CD4+ T-cell Recovery
Total # CD4+ Cells
5×10⁴
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
CD4+ T-cell Recovery
Total # CD4+ Cells
2×10⁵
1.5×10⁵
1×10⁵
5×10⁴
0
#CD4+CD28+ Cells
CD4+CD28+ Events
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
#CD4+CD28+ Cells
CD4+CD28+ Events
1.5×10⁵
1×10⁵
5×10⁴
0
#CD4+ICOS+ Cells
CD4+ICOS+ Events
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
#CD4+ICOS+ Cells
CD4+ICOS+ Events
2×10⁵
1.5×10⁵
1×10⁵
5×10⁴
0
CD4+ TEM Recovery
Total # CD4+ TEM
5×10⁴
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
CD4+ TEM Recovery
Total # CD4+ TEM
2×10⁵
1.5×10⁵
1×10⁵
5×10⁴
0
CD4+ TREG Recovery
Total # CD4+ TEM
800
600
400
200
0
CD4+ TREG Recovery
Total # CD4+ TEM
800
600
400
200
0

FIG. 28C

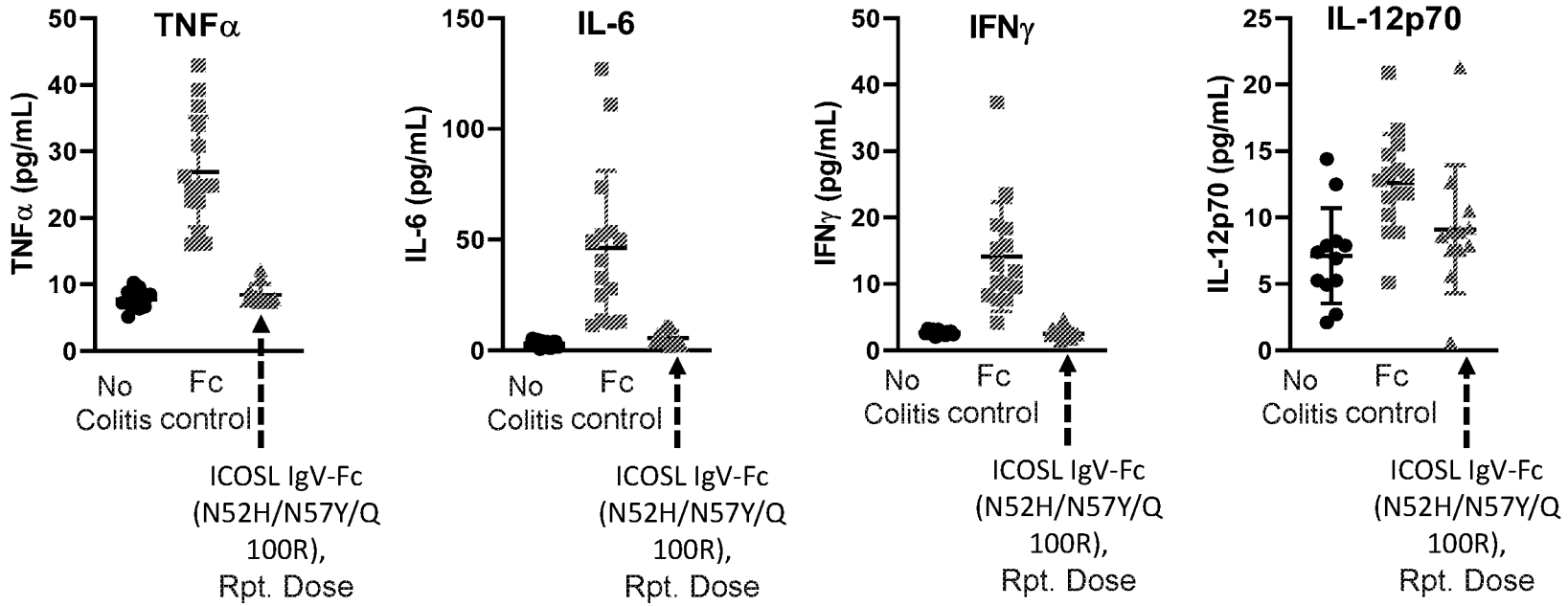

TNFα
TNFα (pg/mL)
50
40
30
20
10
0
No
Fc
Colitis control
ICOSL IgV-Fc
(N52H/N57Y/Q
100R),
Rpt. Dose
IL-6
IL-6 (pg/mL)
150
100
50
0
No
Fc
Colitis control
ICOSL IgV-Fc
(N52H/N57Y/Q
100R),
Rpt. Dose
IFNγ
IFNγ (pg/mL)
50
40
30
20
10
0
No
Fc
Colitis control
ICOSL IgV-Fc
(N52H/N57Y/Q
100R),
Rpt. Dose
IL-12p70
IL-12p70 (pg/mL)
25
20
15
10
5
0
No
Fc
Colitis control
ICOSL IgV-Fc
(N52H/N57Y/Q
100R),
Rpt. Dose

FIG. 28D

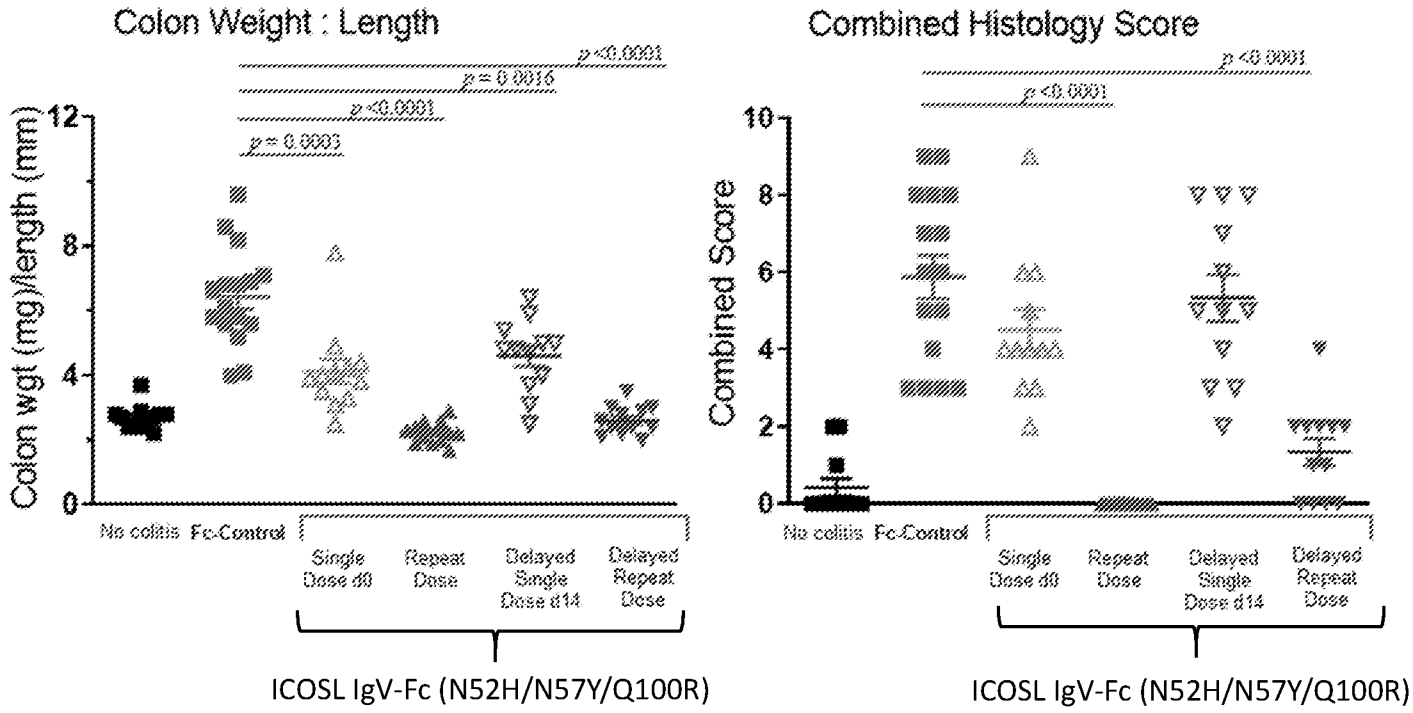
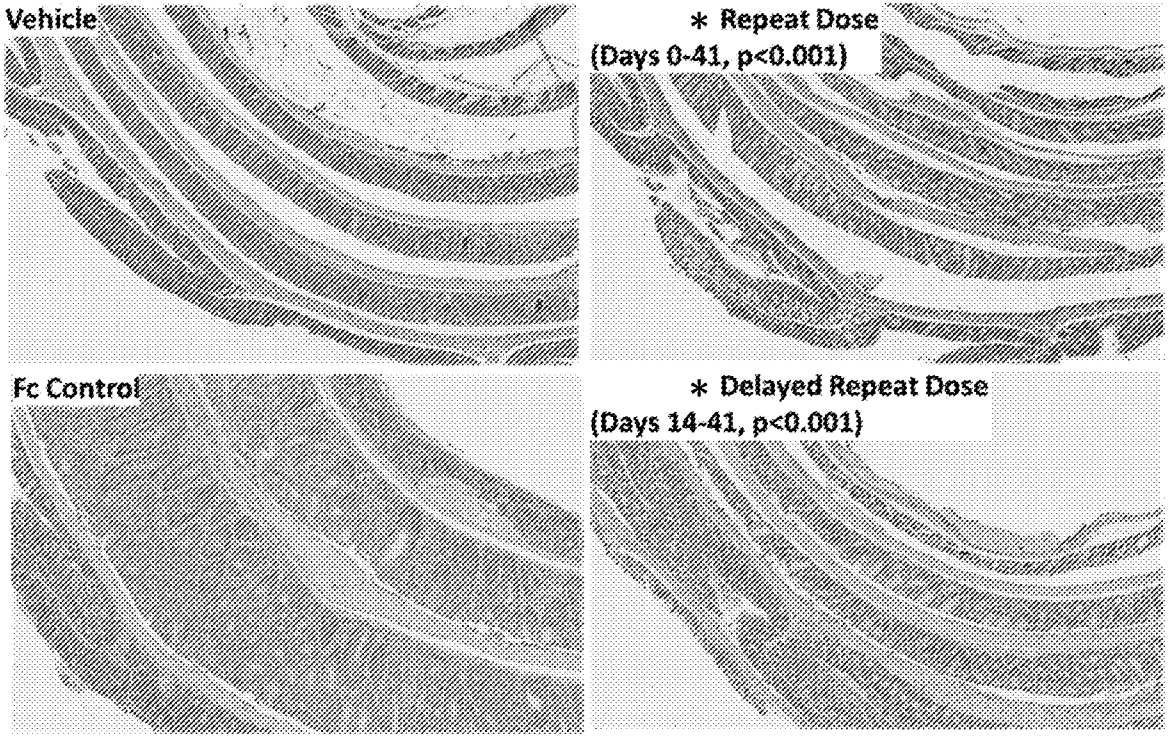
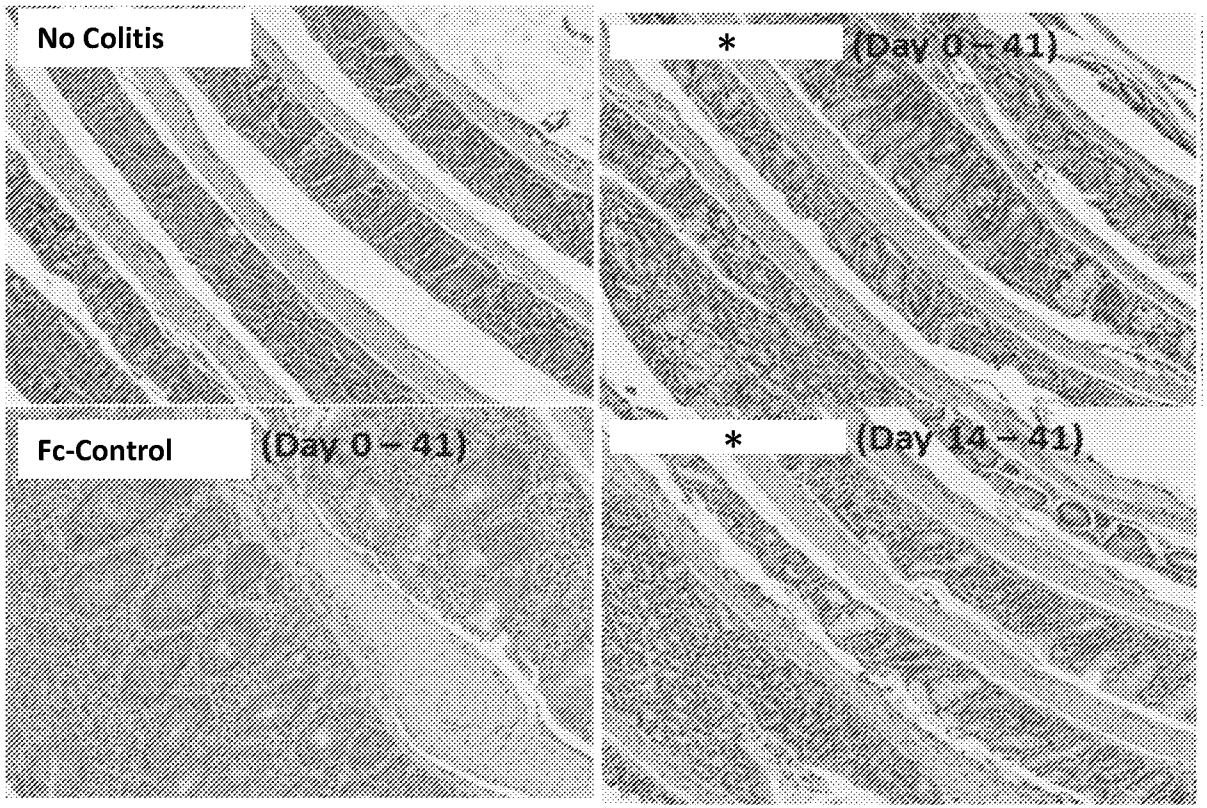

Colon Weight : Length
Colon wgt (mg)/length (mm)
p <0.0001
p = 0.0016
p <0.0001
p = 0.0003
No colitis
Fc-Control
Single Dose d0
Repeat Dose
Delayed Single Dose d14
Delayed Repeat Dose
ICOSL IgV-Fc (N52H/N57Y/Q100R)
Combined Histology Score
Combined Score
p <0.0001
p <0.0001
No colitis
Fc-Control
Single Dose d0
Repeat Dose
Delayed Single Dose d14
Delayed Repeat Dose
ICOSL IgV-Fc (N52H/N57Y/Q100R)

FIG. 28E



* ICOSL IgV-Fc
(N52H/N57Y/Q100R)

FIG. 28F



* ICOSL IgV-Fc
(N52H/N57Y/Q100R)

FIG. 29A

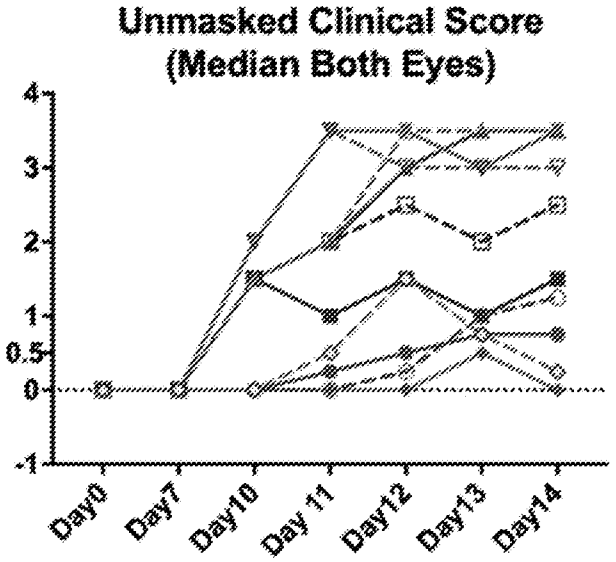


FIG. 29B

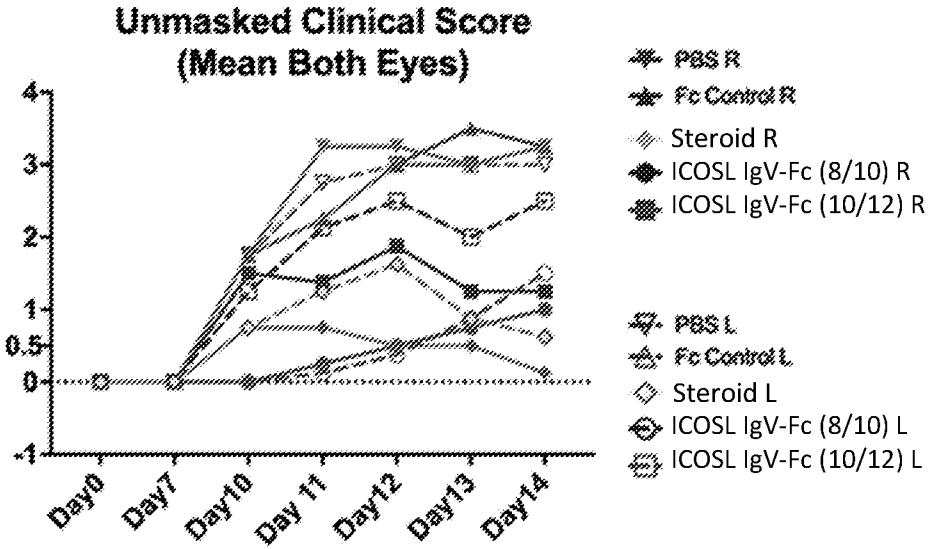


FIG. 29C

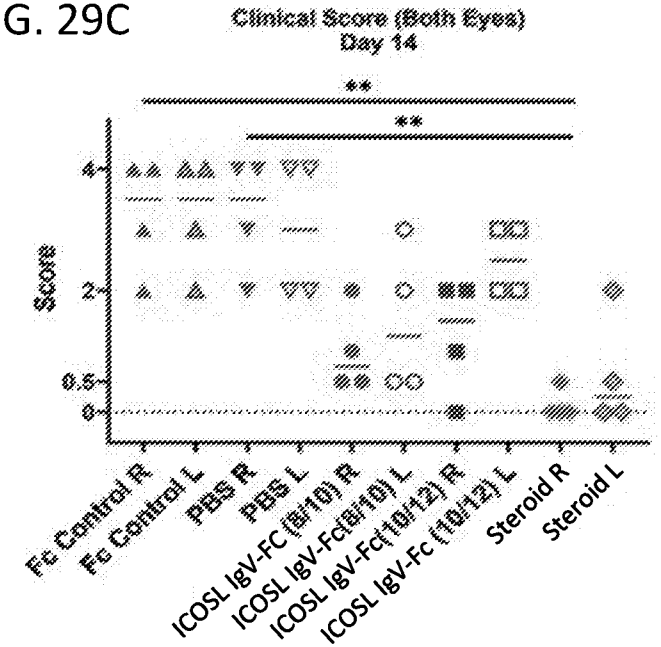


FIG. 29D

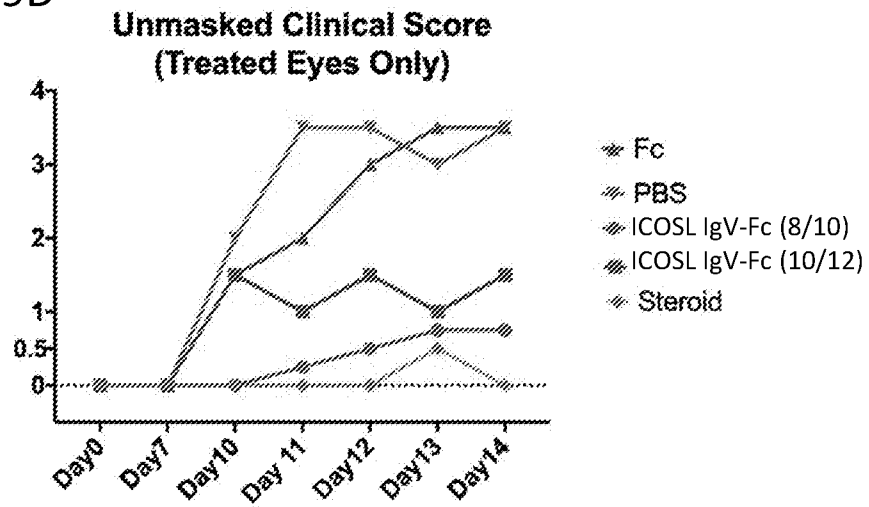


FIG. 29E

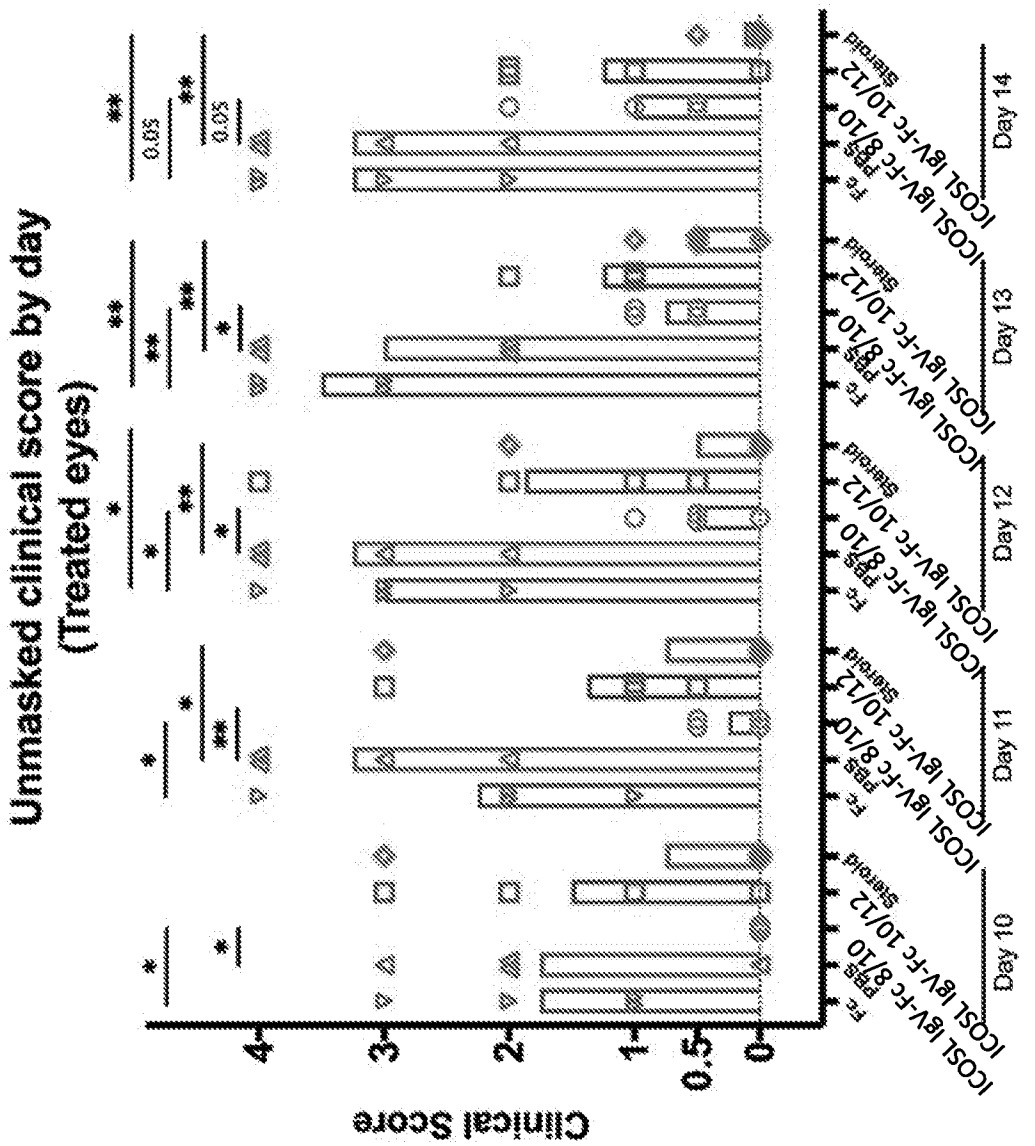

Unmasked clinical score by day
(Treated eyes)
Clinical Score
4
3
2
1
0.5
0
0.05
0.05
0.05
Day 10
Day 11
Day 12
Day 13
Day 14
Fc
PBS
ICOSL IgV-Fc 8/10
ICOSL IgV-Fc 10/12
Steroid

FIG. 30A

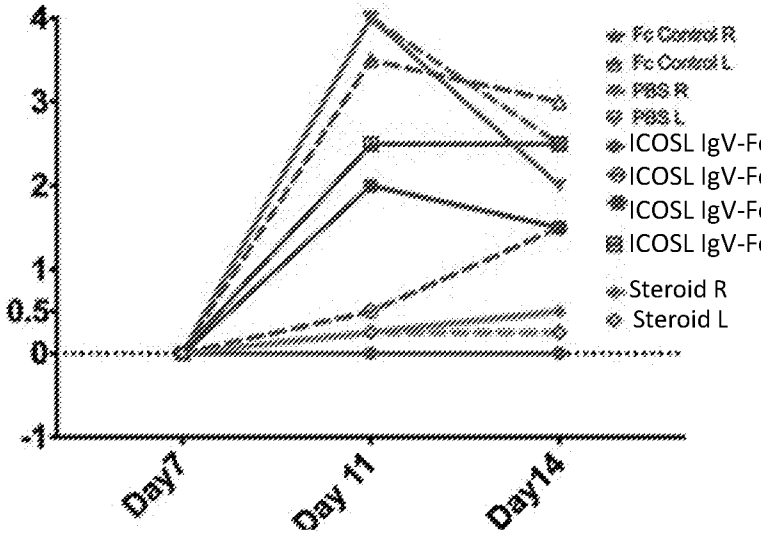


FIG. 30B

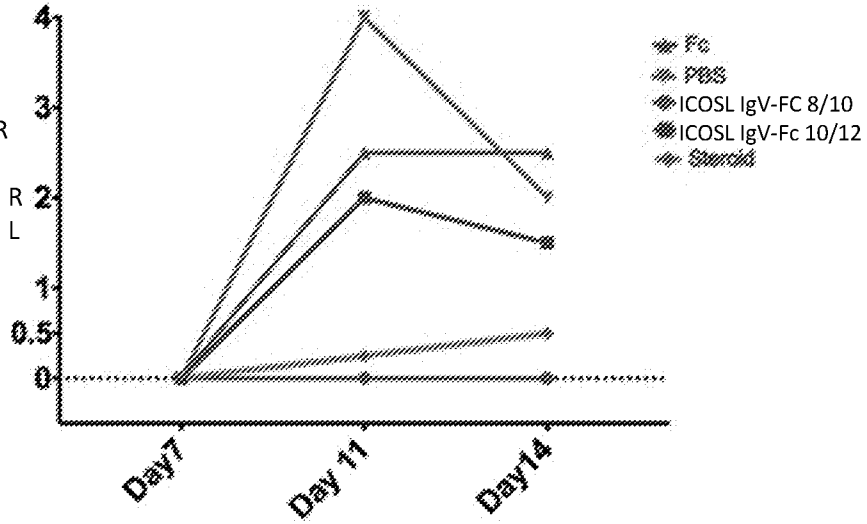


FIG. 30C

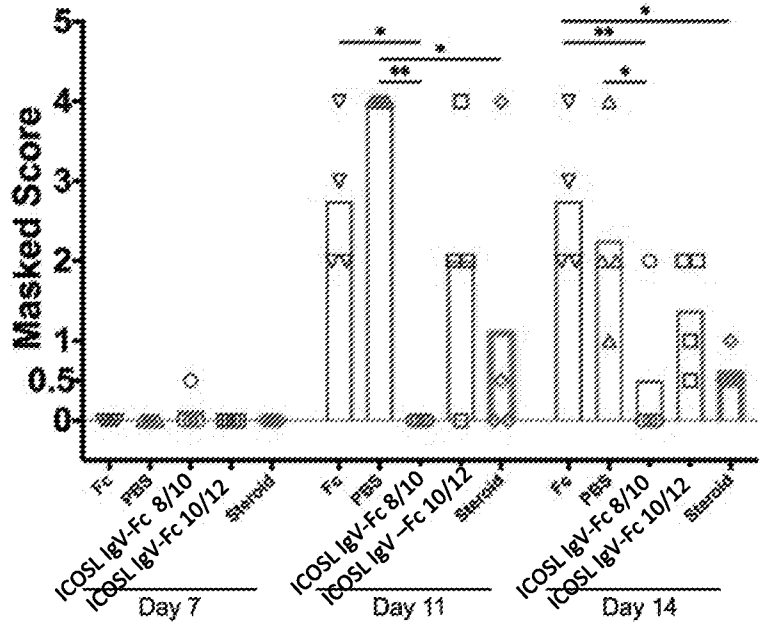


FIG. 31

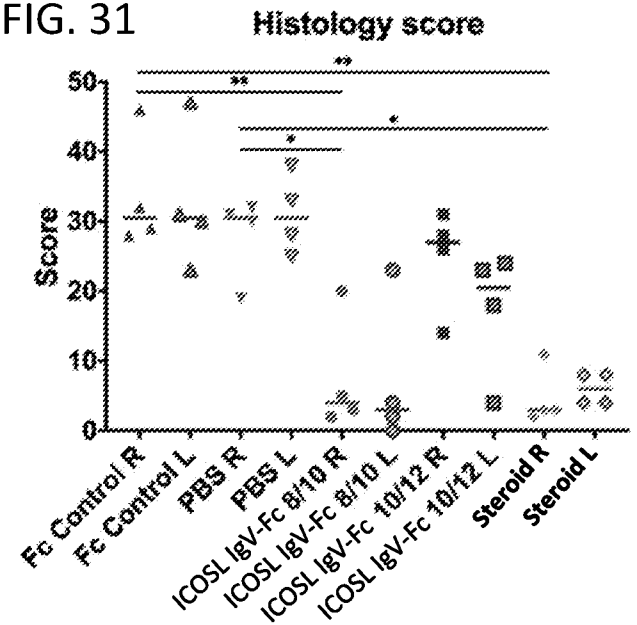


FIG. 32

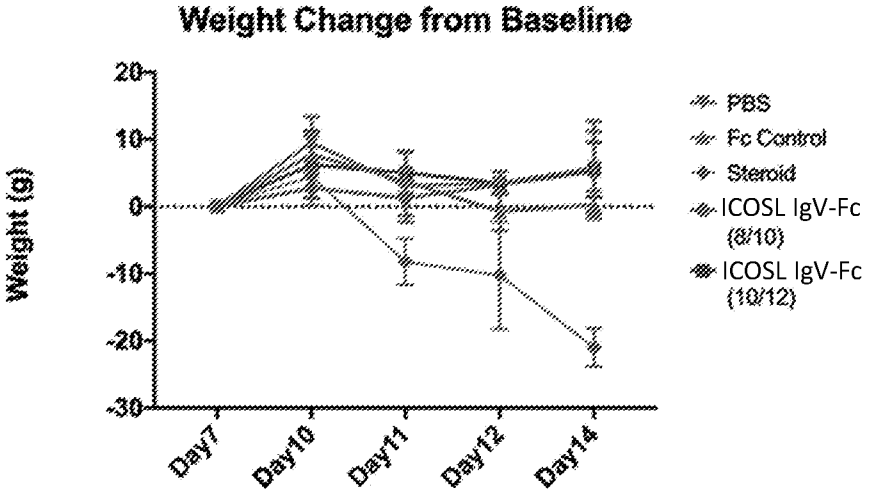


FIG. 33

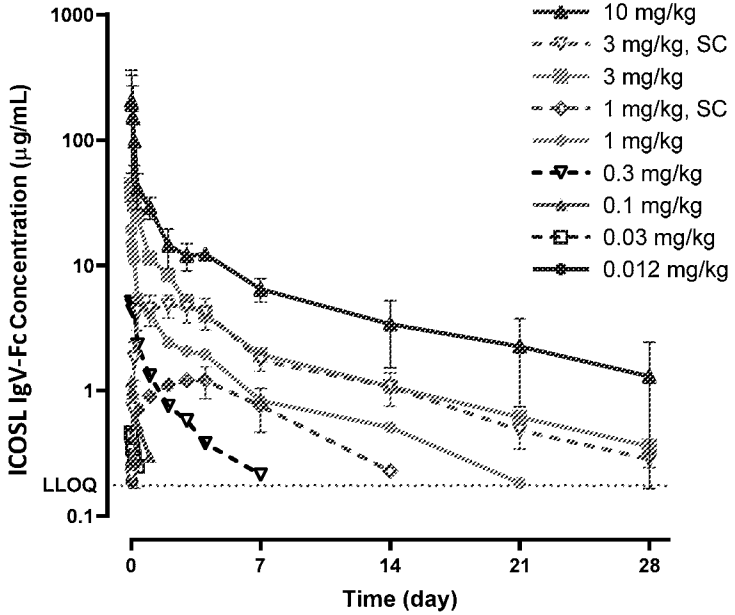


FIG. 34

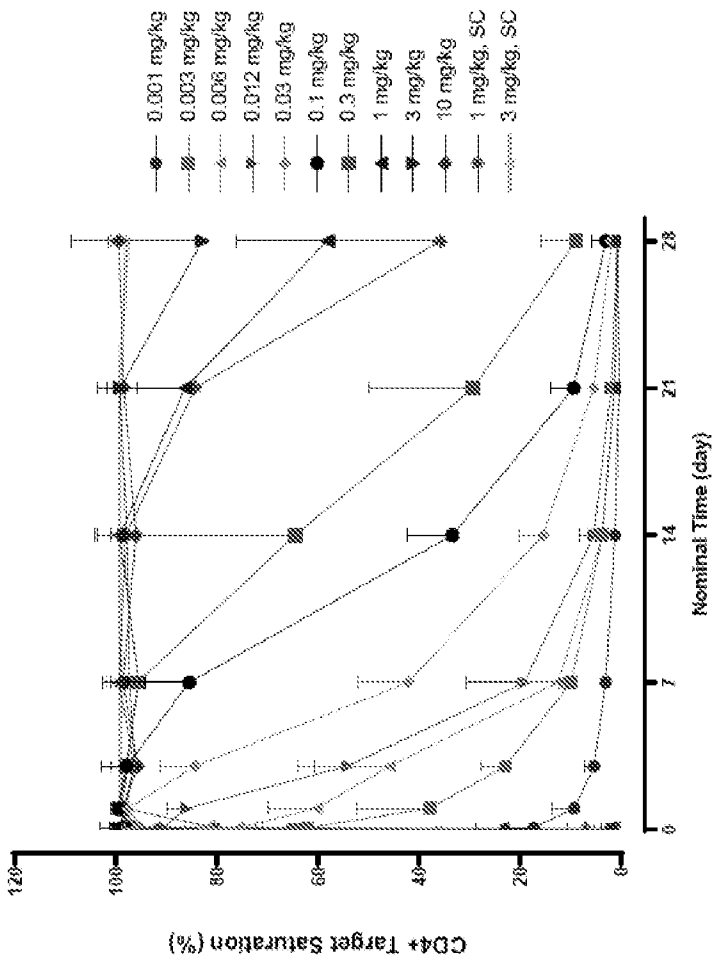

0.001 mg/kg
0.003 mg/kg
0.006 mg/kg
0.012 mg/kg
0.03 mg/kg
0.1 mg/kg
0.3 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
1 mg/kg, SC
3 mg/kg, SC
CD4+ Target Saturation (%)
120
100
80
60
40
20
0
0
7
14
21
28
Nominal Time (day)

FIG. 35

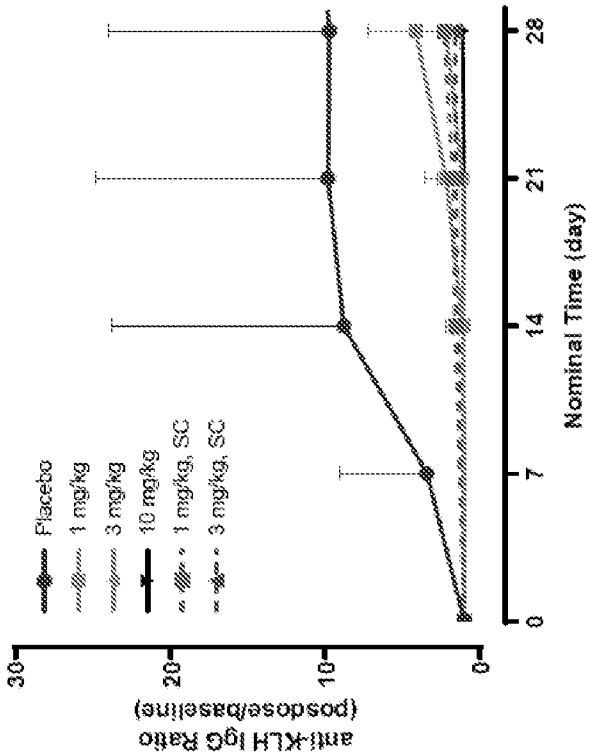

Placebo
1 mg/kg
3 mg/kg
10 mg/kg
1 mg/kg, SC
3 mg/kg, SC
anti-KLH IgG Ratio
(posdose/baseline)
30
20
10
0
0
7
14
21
28
Nominal Time (day)

FIG. 36

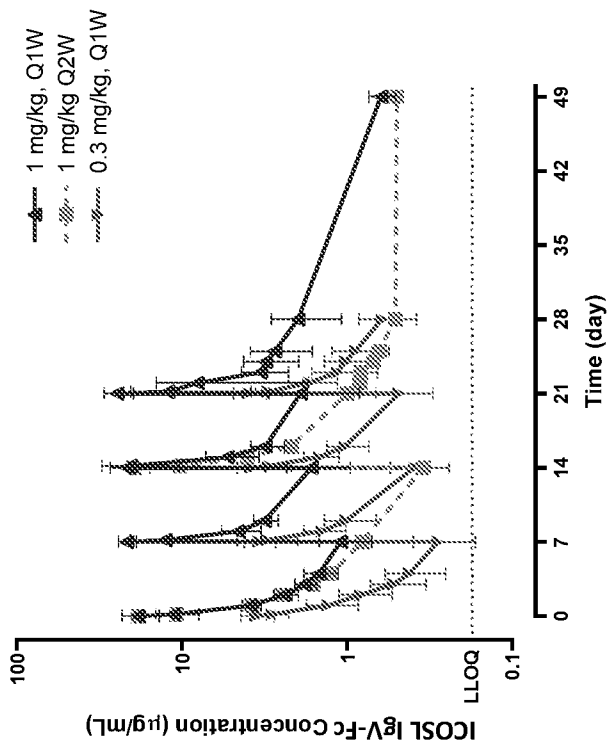

1 mg/kg, Q1W
1 mg/kg Q2W
0.3 mg/kg, Q1W
ICOSL IgV-Fc Concentration (μg/mL)
100
10
1
0.1
LLOQ
0
7
14
21
28
35
42
49
Time (day)

FIG. 37

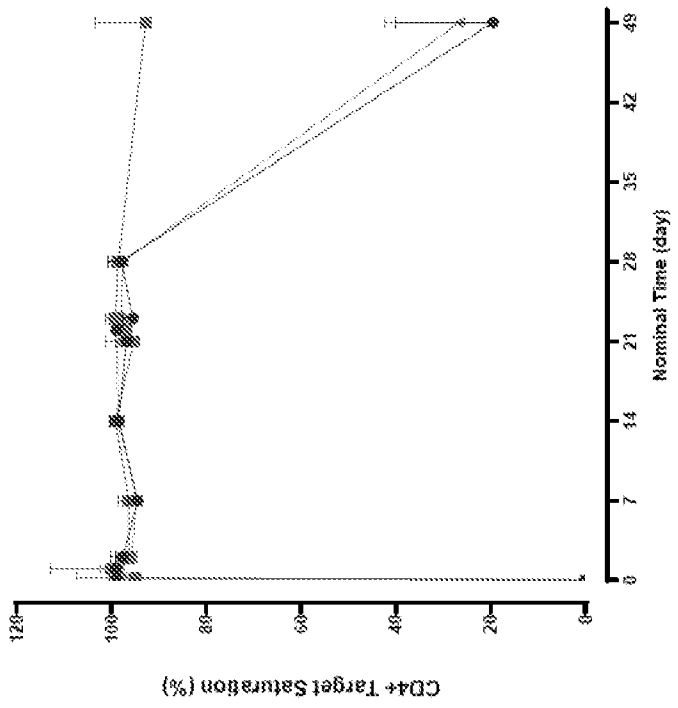
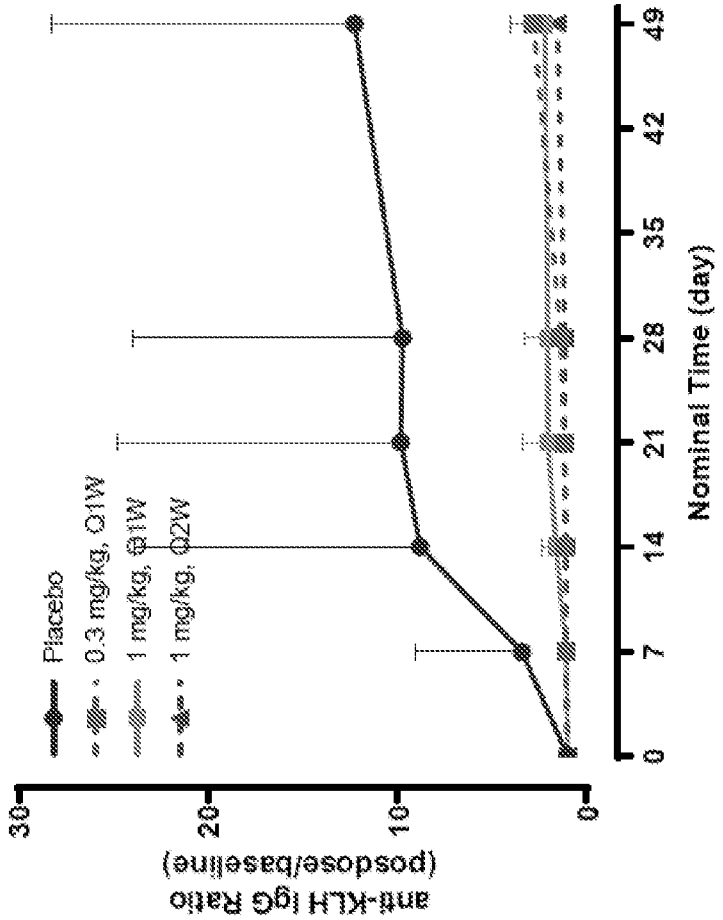

0.3 mg/kg,Q1W
1 mg/kg, Q1W
1 mg/kg, Q2W
CD4+ Target Saturation (%)
120
100
80
60
40
20
0
0
7
14
21
28
35
42
49
Nominal Time (day)

FIG. 38


anti-KLH IgG Ratio
(postdose/baseline)
30
20
10
0
Placebo
0.3 mg/kg, Q1W
1 mg/kg, Q1W
1 mg/kg, Q2W
0
7
14
21
28
35
42
49
Nominal Time (day)