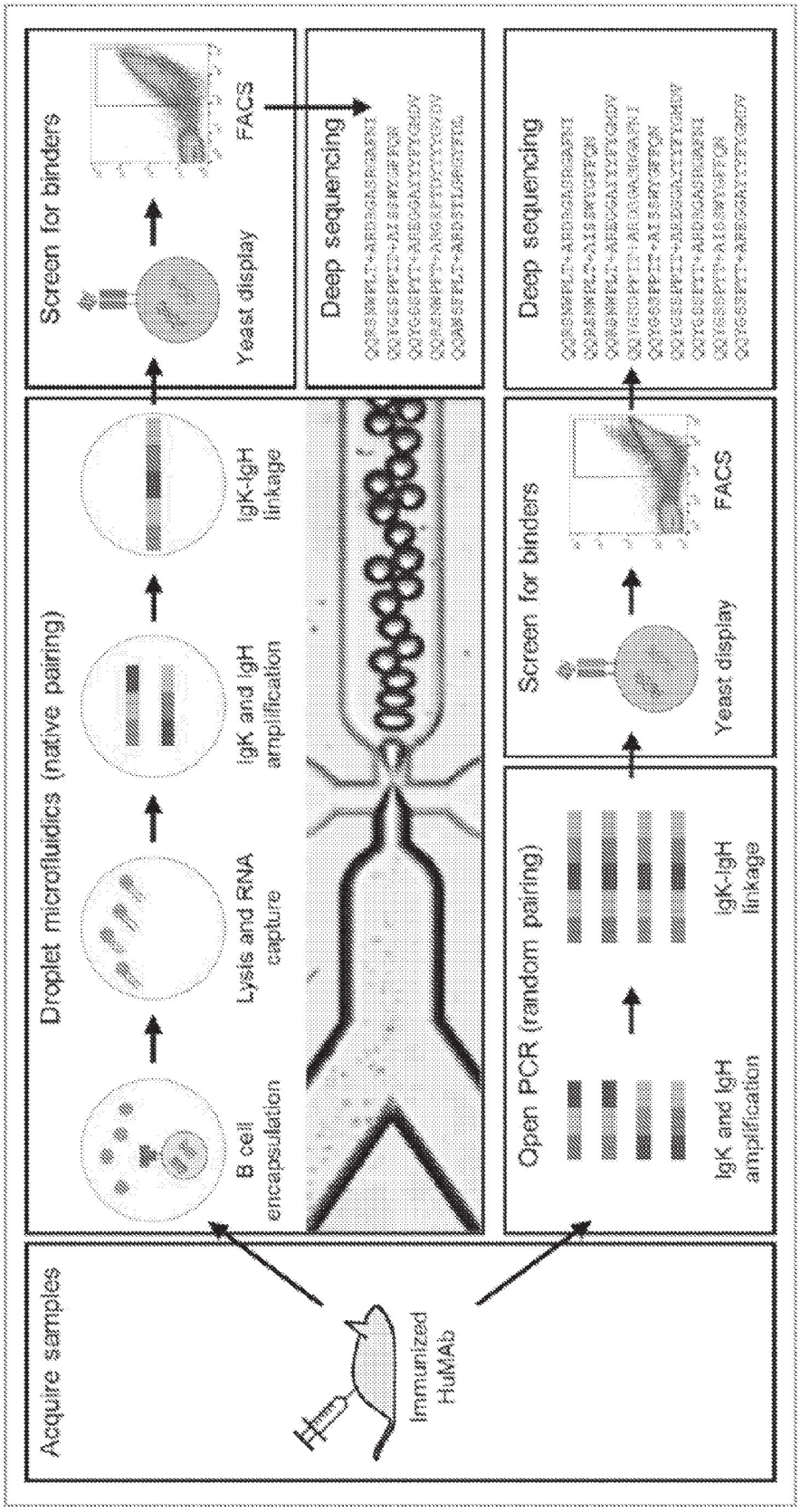
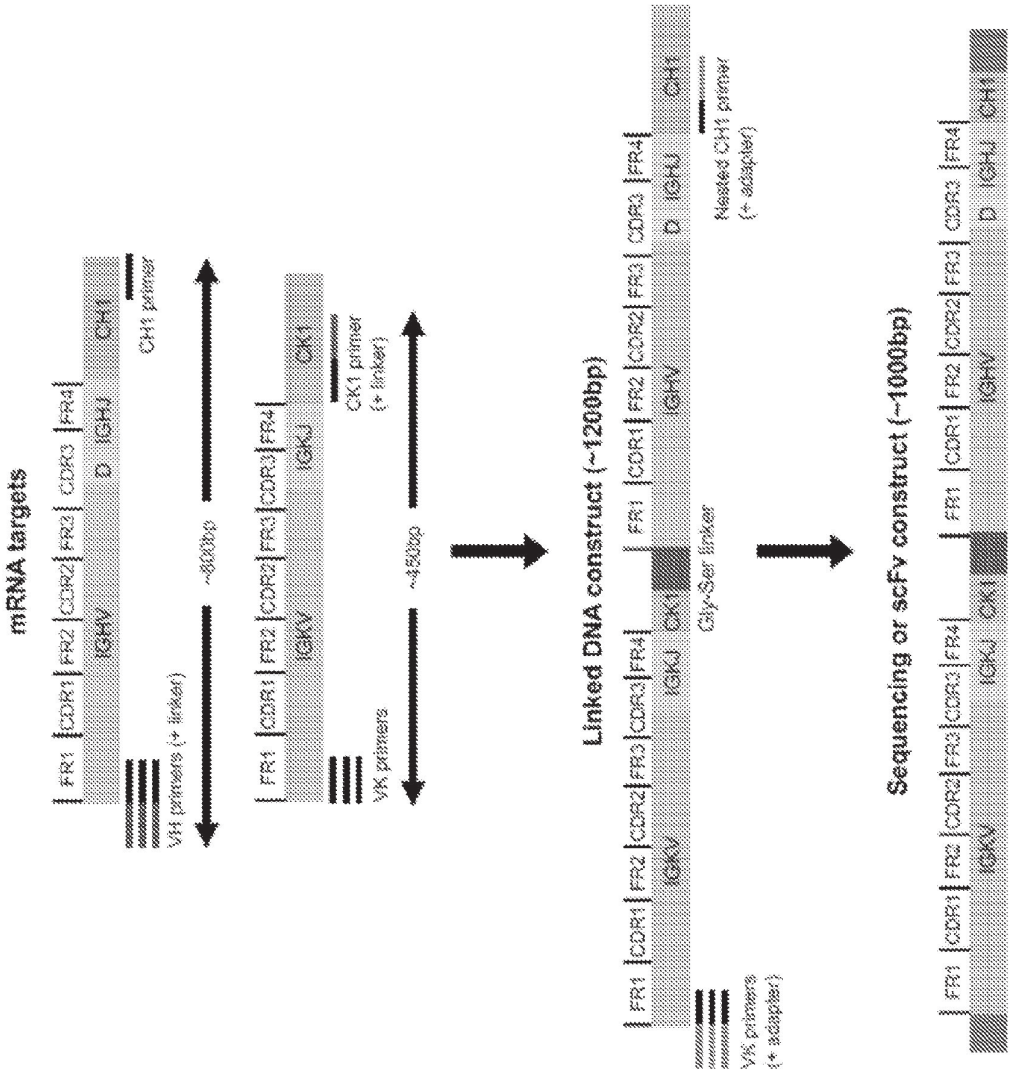

Acquire samples
Immunized HuMAb
Droplet microfluidics (native pairing)
B cell encapsulation
Lysis and RNA capture
IgK and IgH amplification
IgK-IgH linkage
Screen for binders
Yeast display
FACS
Deep sequencing
Open PCR (random pairing)
IgK and IgH amplification
IgK-IgH linkage
Screen for binders
Yeast display
FACS
Deep sequencing

FIG. 1

FIG. 2


mRNA targets
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
IGHV
D
IGHJ
CH1
CH1 primer
VH primers (+ linker)
~800bp
IGKV
IGKJ
CK1
CK1 primer
(+ linker)
VK primers
~450bp
Linked DNA construct (~1200bp)
Gly-Ser linker
VK primers
(+ adapter)
Nested CH1 primer
(+ adapter)
Sequencing or scFv construct (~1000bp)

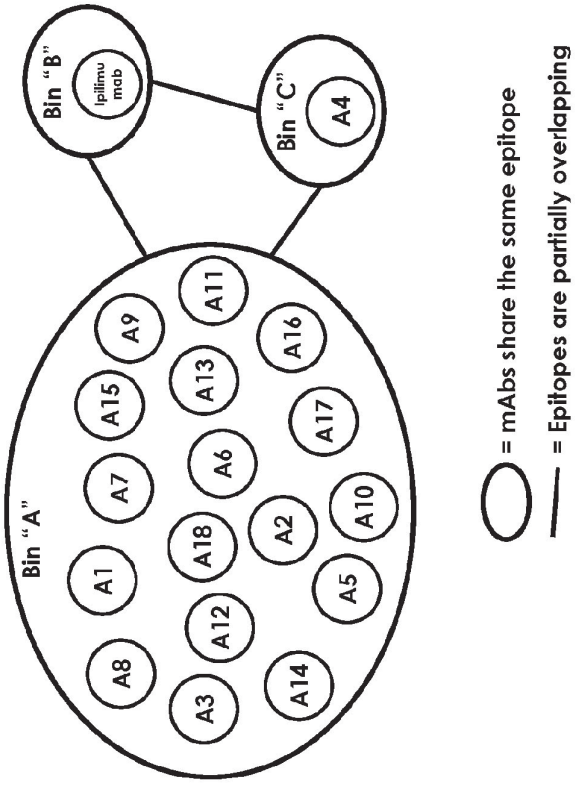

Bin "B"
Ipilimumab
Bin "C"
A4
Bin "A"
A1
A2
A3
A5
A6
A7
A8
A9
A10
A11
A12
A13
A14
A15
A16
A17
A18
= mAbs share the same epitope
= Epitopes are partially overlapping

FIG. 3

FIG. 4A

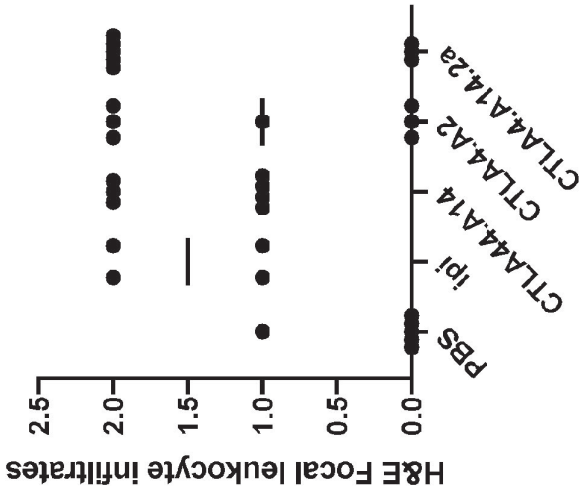


FIG. 4B

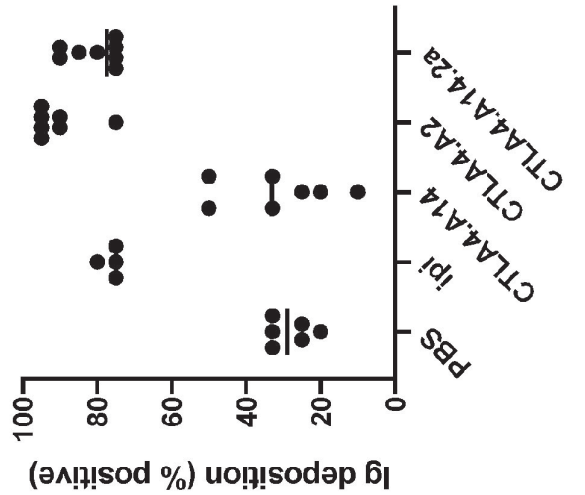


FIG. 4C

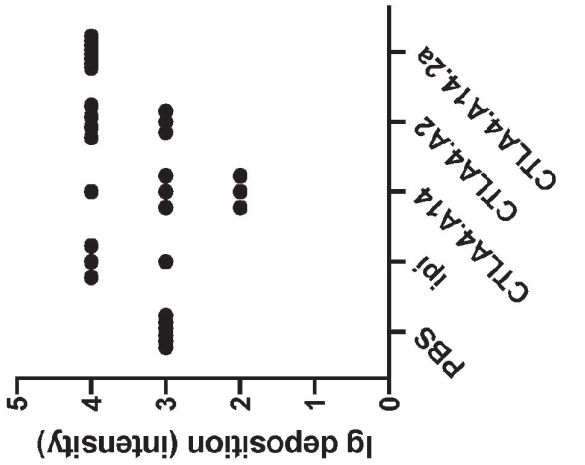


FIG. 4E

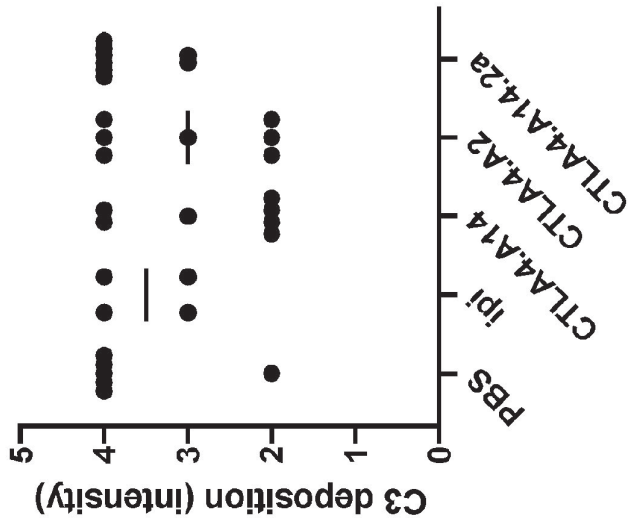
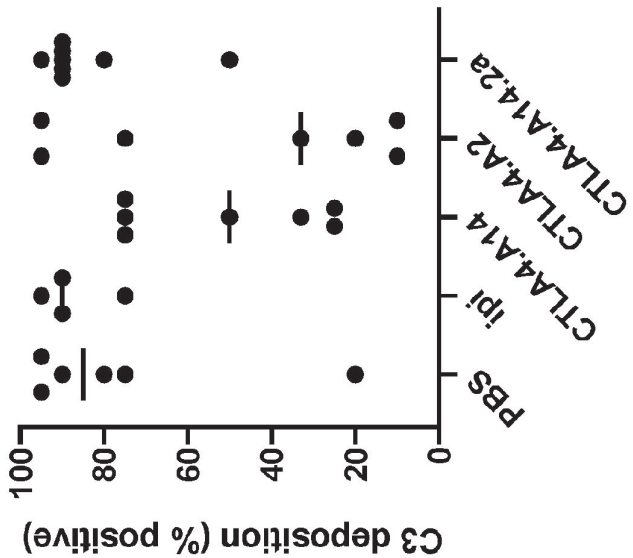

C3 deposition (intensity)
0
1
2
3
4
5
PBS
ipi
CTLA4.A14
CTLA4.A2
CTLA4.A14.2a

FIG. 4D


C3 deposition (% positive)
0
20
40
60
80
100
PBS
ipi
CTLA4.A14
CTLA4.A2
CTLA4.A14.2a

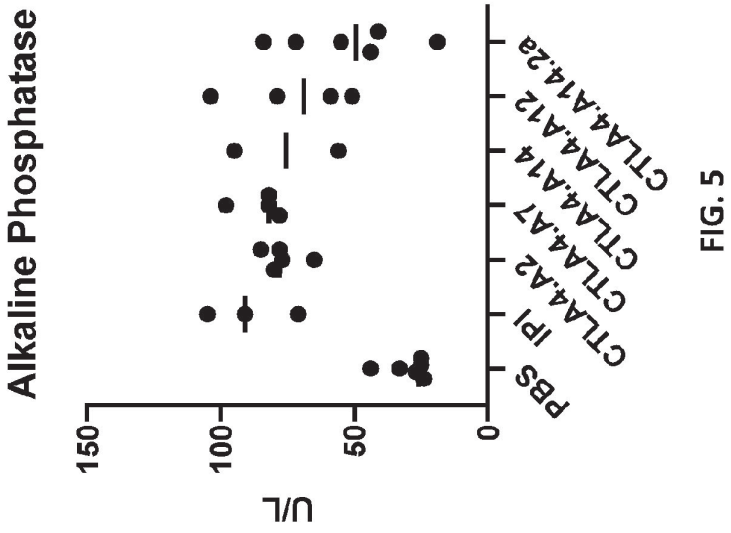

Alkaline Phosphatase
U/L
150
100
50
0
PBS
IPI
CTLA4.A2
CTLA4.A7
CTLA4.A14
CTLA4.A12
CTLA4.A14.2a

FIG. 5

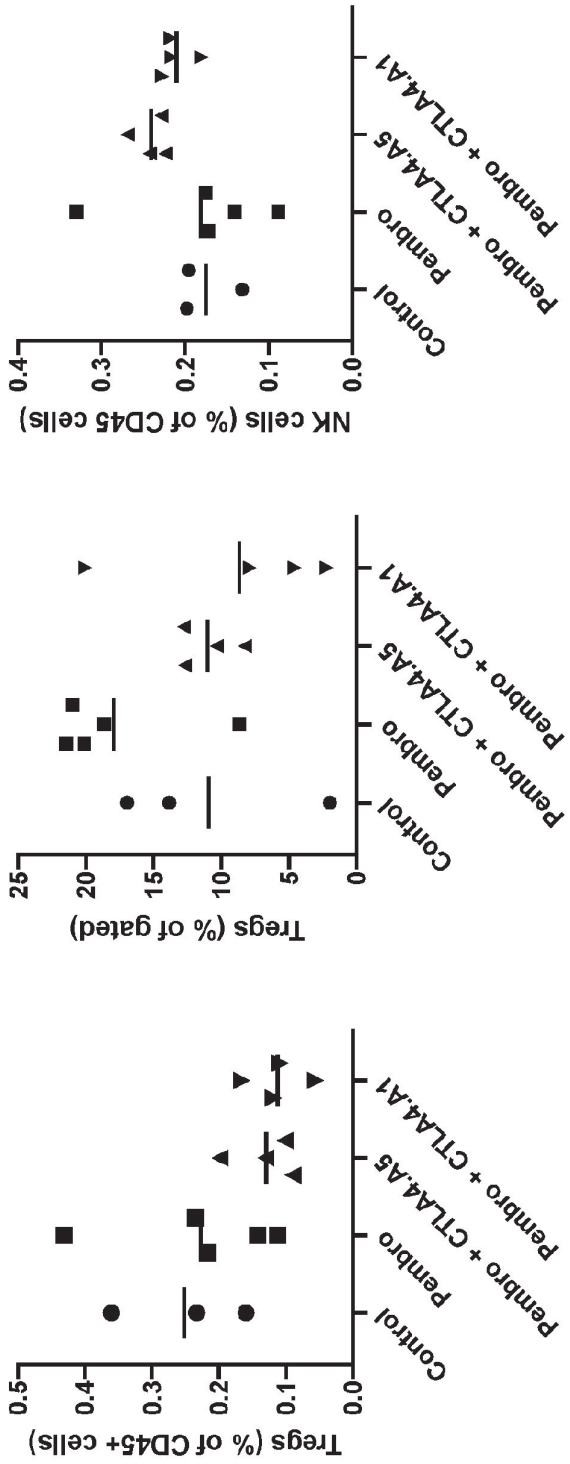

Tregs (% of CD45+ cells)
0.0
0.1
0.2
0.3
0.4
0.5
Control
Pembro
Pembro + CTLA4.A5
Pembro + CTLA4.A7
Tregs (% of gated)
0
5
10
15
20
25
Control
Pembro
Pembro + CTLA4.A5
Pembro + CTLA4.A7
NK cells (% of CD45 cells)
0.0
0.1
0.2
0.3
0.4
Control
Pembro
Pembro + CTLA4.A5
Pembro + CTLA4.A7

FIG. 6

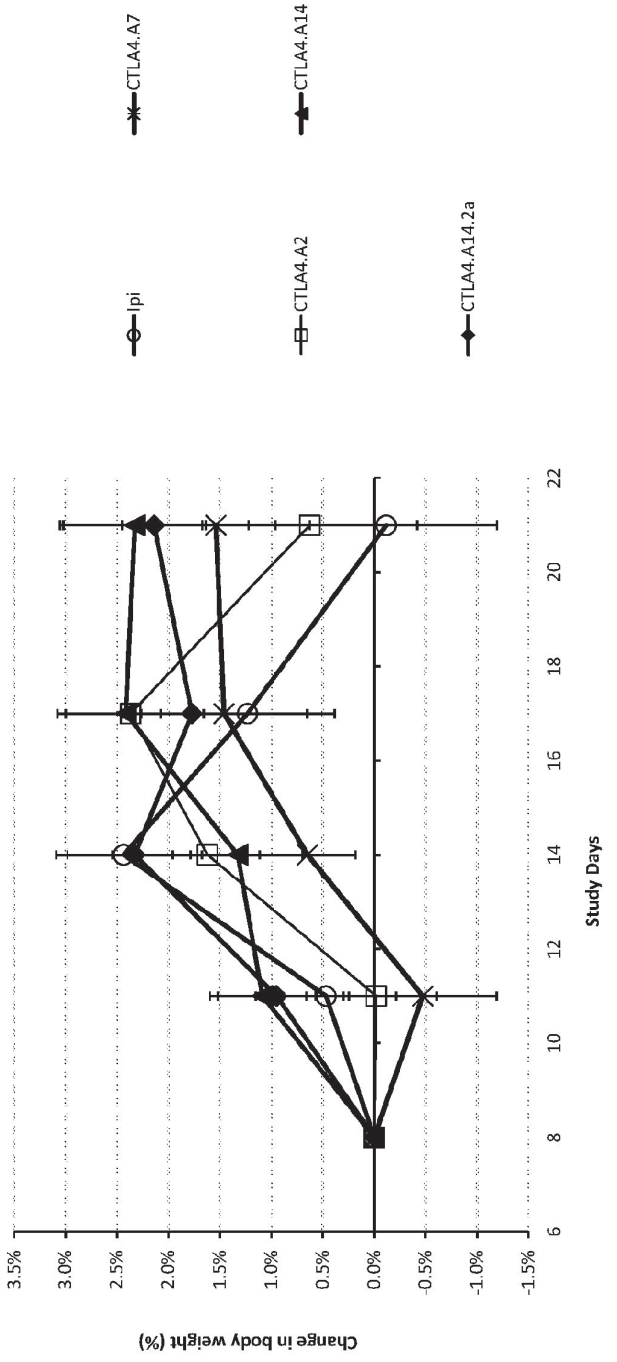

Ipi
CTLA4.A7
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a
Change in body weight (%)
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
-0.5%
-1.0%
-1.5%
6
8
10
12
14
16
18
20
22
Study Days

FIG. 7

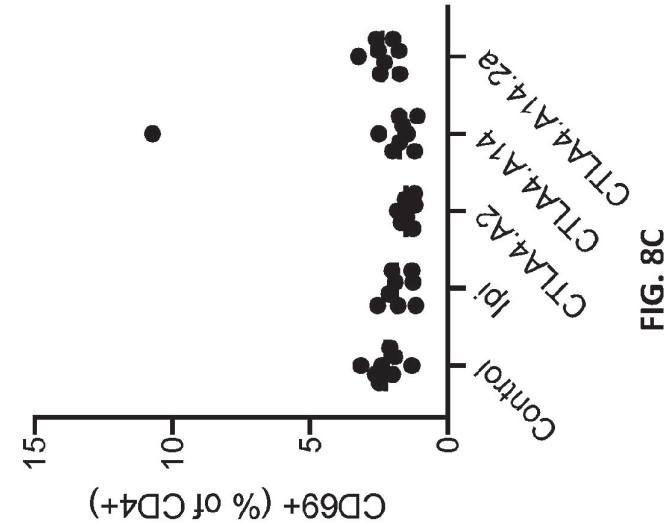


FIG. 8C

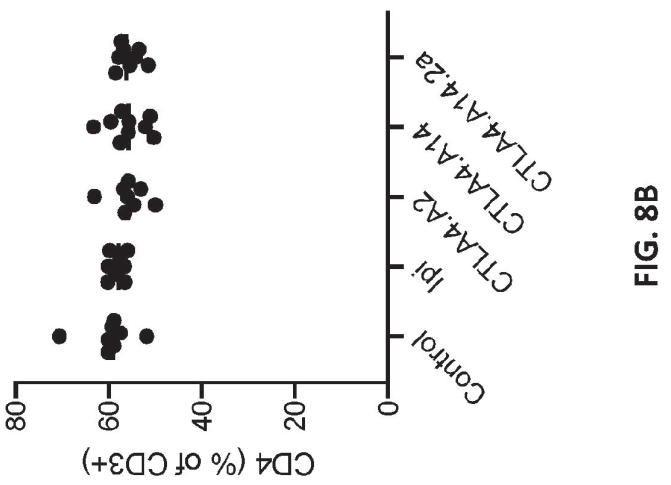


FIG. 8B

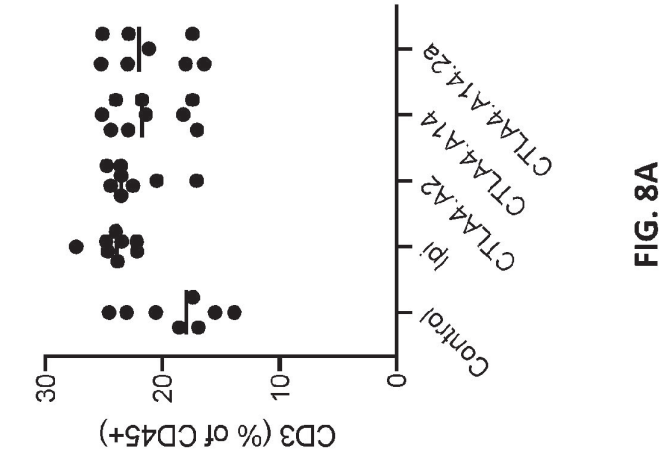


FIG. 8A

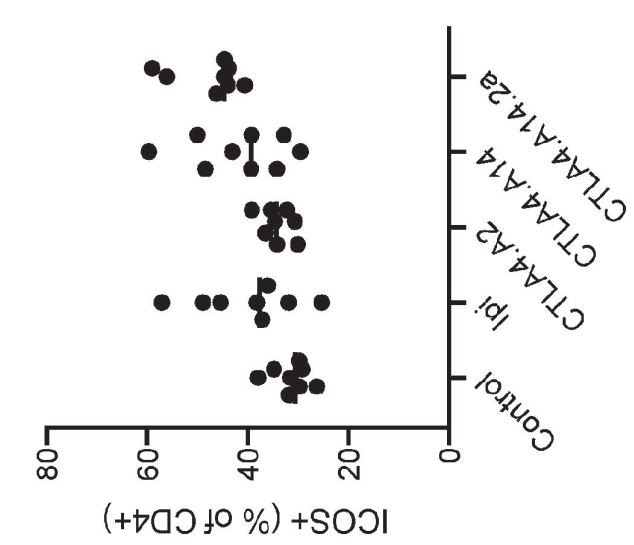


FIG. 8D

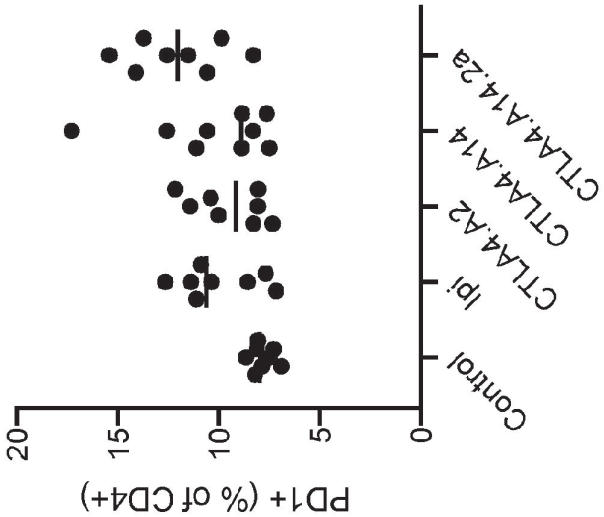


FIG. 8E

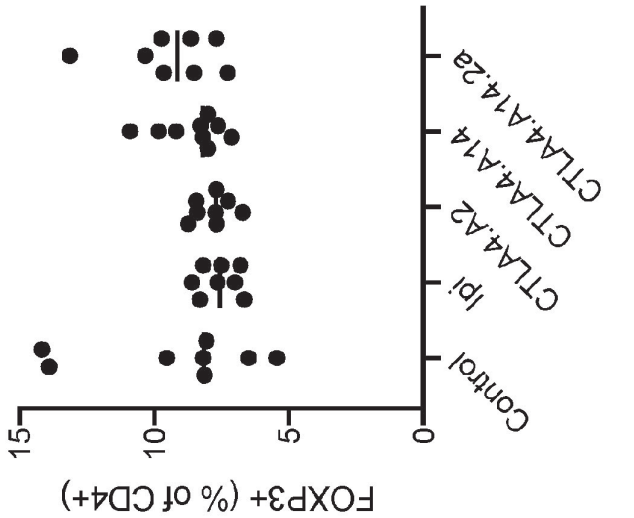


FIG. 8F

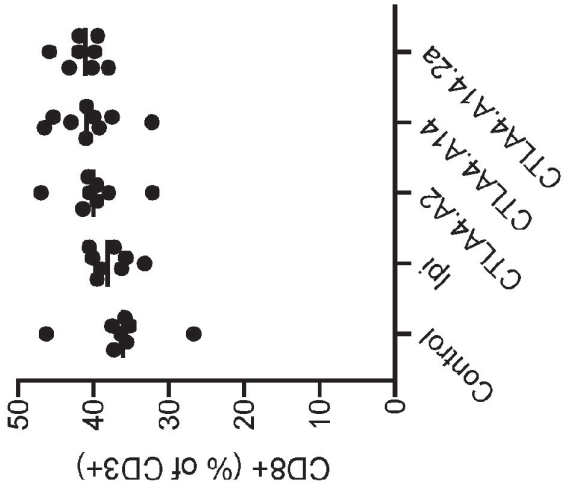

CD69+ (% of CD8+)
0
10
20
30
40
50
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9B

CD8+ (% of CD3+)
0
10
20
30
40
50
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9A

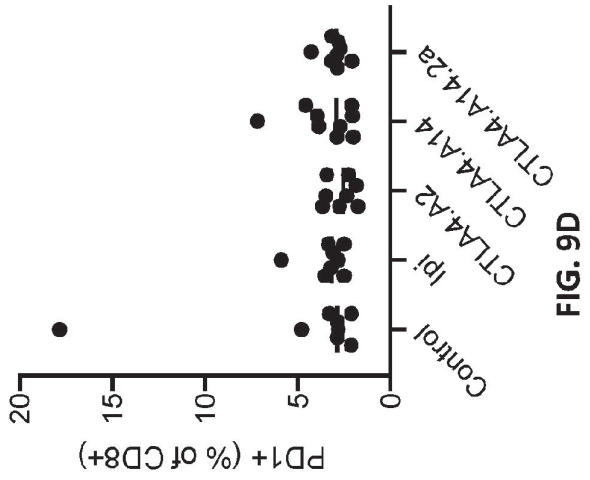

PD1+ (% of CD8+)
0
5
10
15
20
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9D

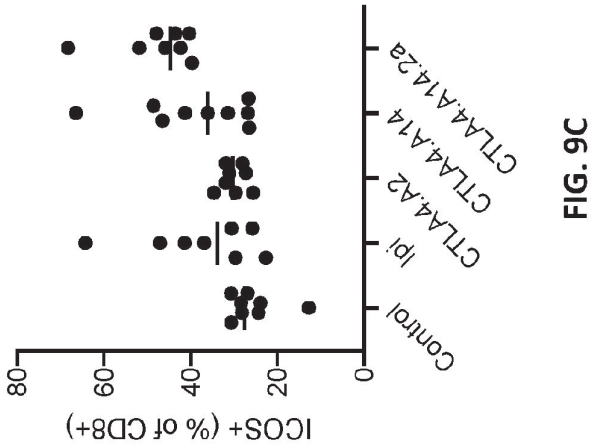

ICOS+ (% of CD8+)
0
20
40
60
80
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9C

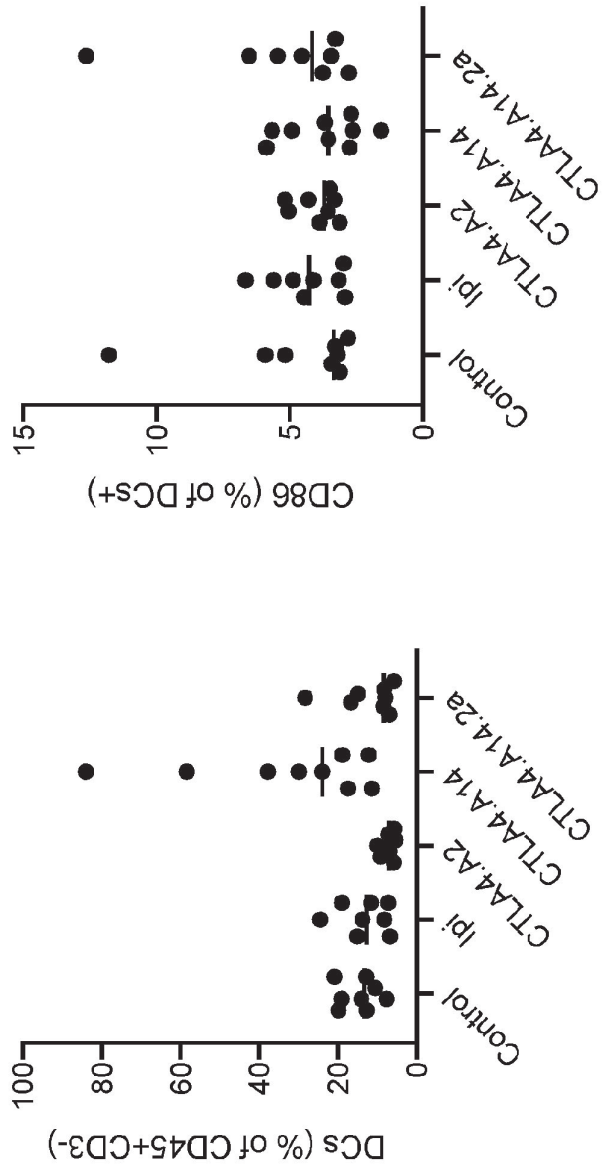

CD86 (% of DCs+)
15
10
5
0
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a
DCs (% of CD45+CD3-)
100
80
60
40
20
0
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 10

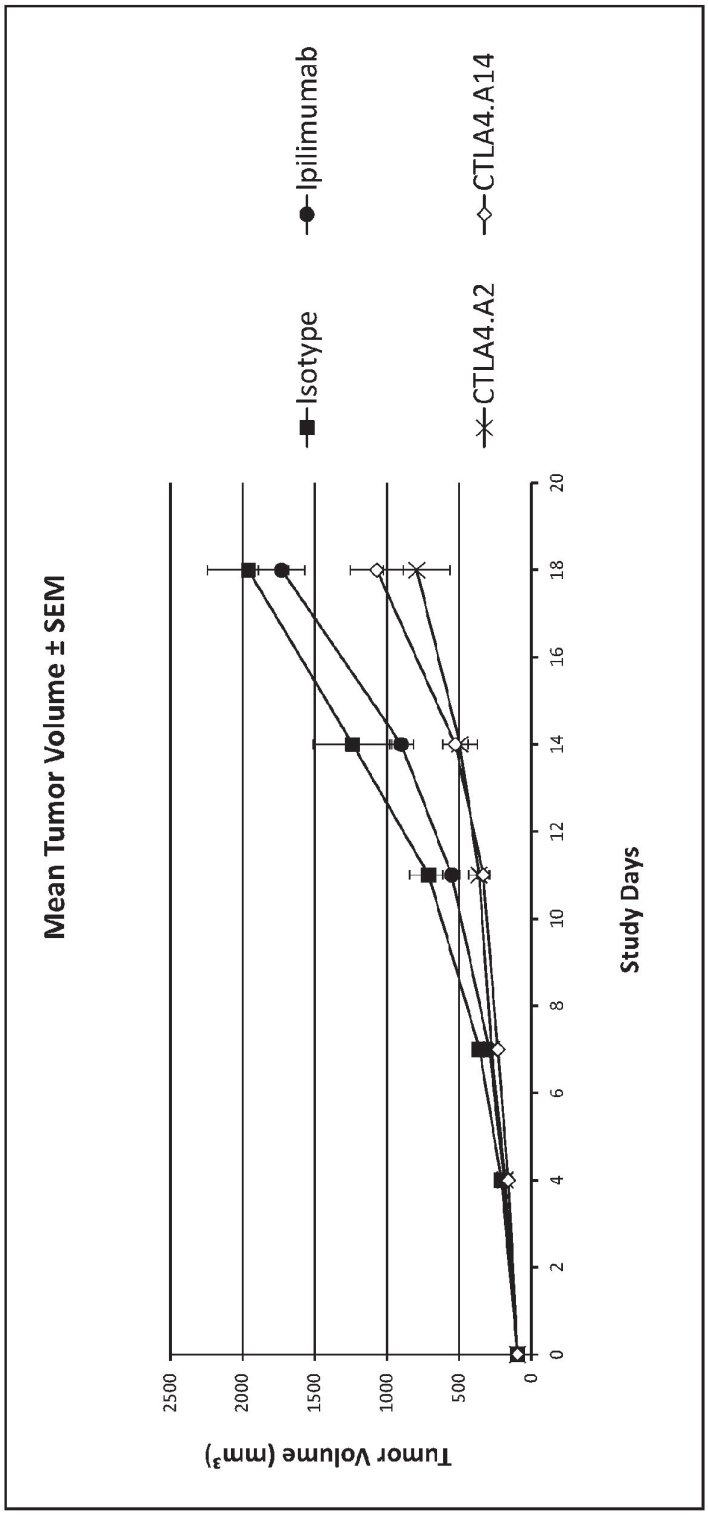

Mean Tumor Volume ± SEM
Isotype
Ipilimumab
CTLA4.A2
CTLA4.A14
Tumor Volume (mm³)
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
18
20
Study Days

FIG. 11

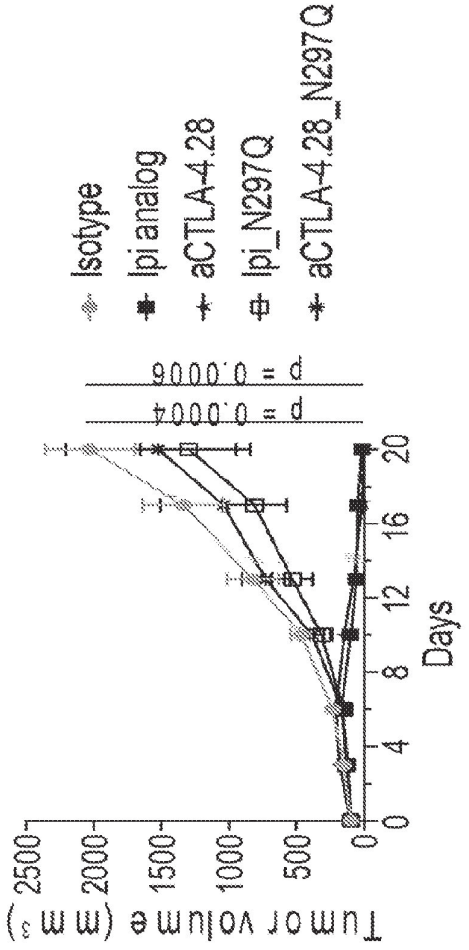


FIG. 12B

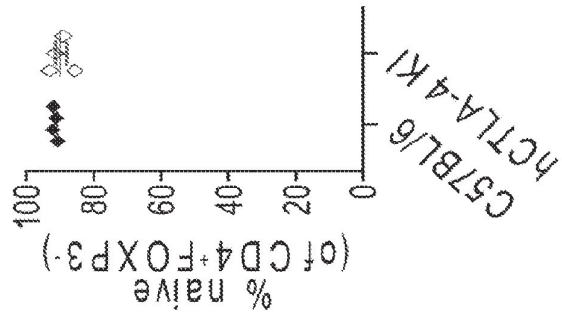


FIG. 12A

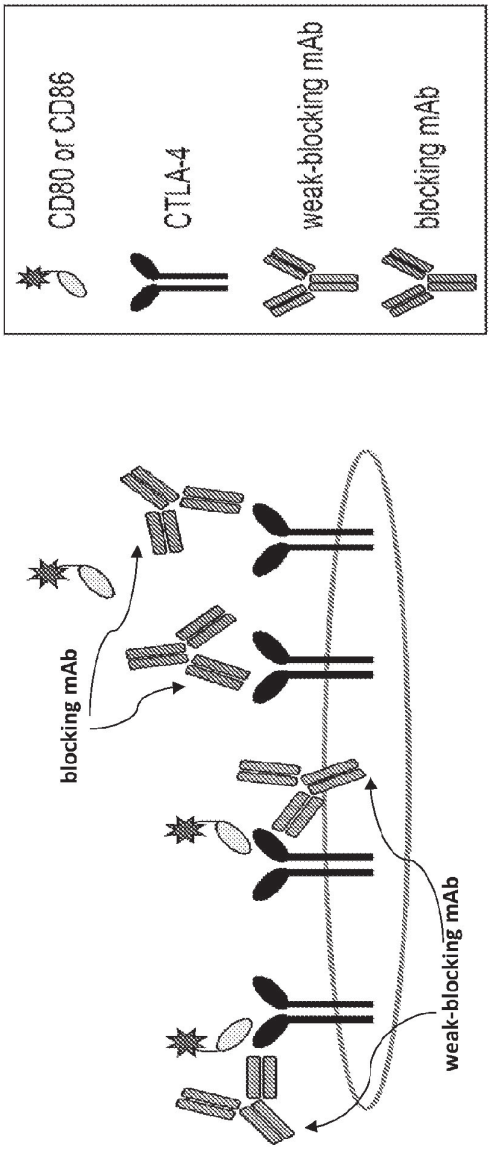

CD80 or CD86
CTLA-4
weak-blocking mAb
blocking mAb
blocking mAb
weak-blocking mAb

FIG. 13A

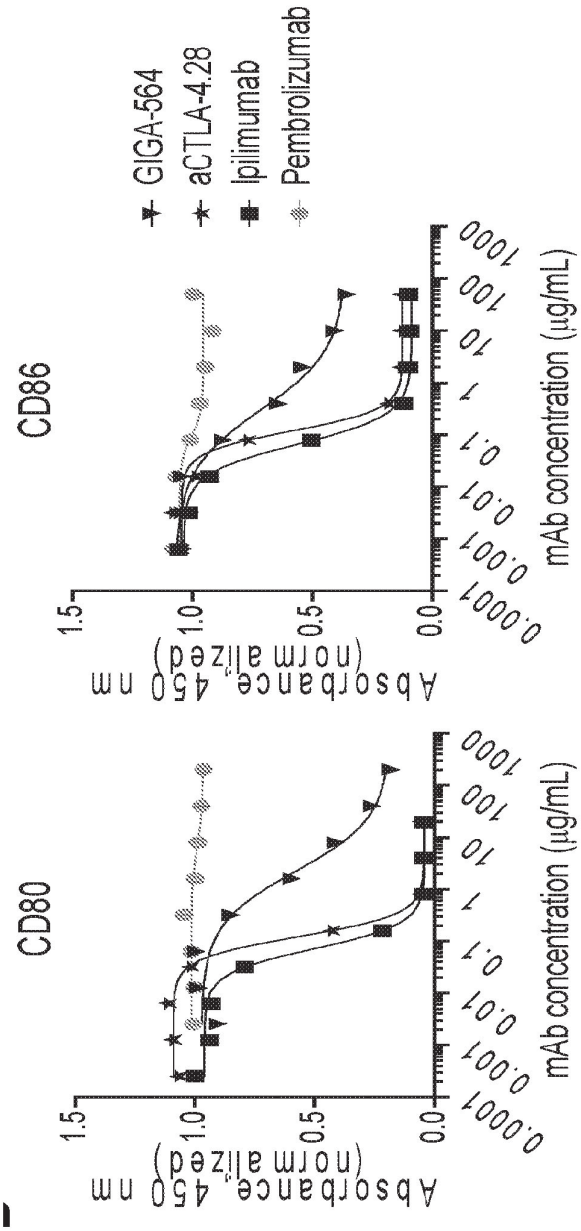

CD80
CD86
GIGA-564
aCTLA-4.28
Ipilimumab
Pembrolizumab
Absorbance, 450 nm (normalized)
mAb concentration (µg/mL)
0.0
0.5
1.0
1.5
0.0001
0.001
0.01
0.1
1
10
100
1000

FIG. 13B

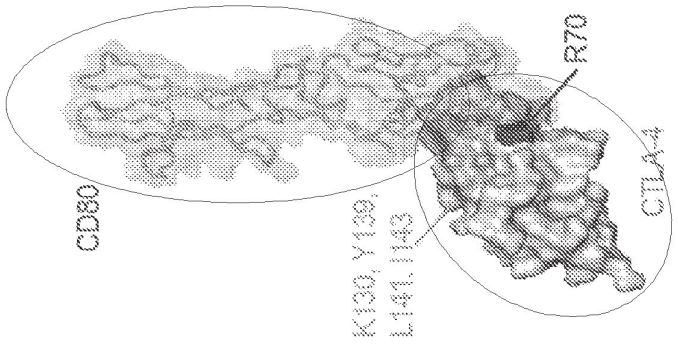


FIG. 13C

	GIGA-564	Ipilimumab	CD80	CD86
R70		X	X	X
K130	X	X	X	X
Y139	X	X	X	X
L141	X	X		
I143	X	X	X	X

FIG. 13D

FIG. 14A

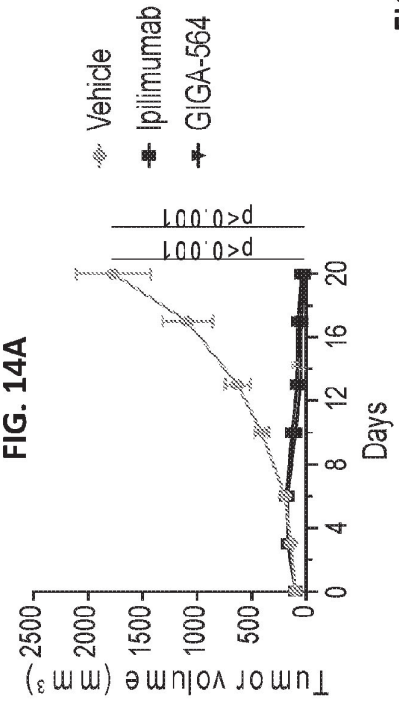


FIG. 14B

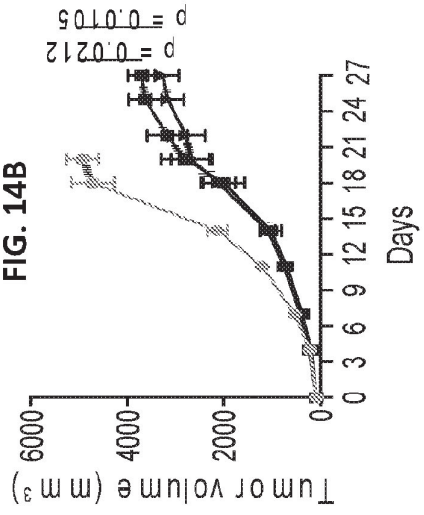
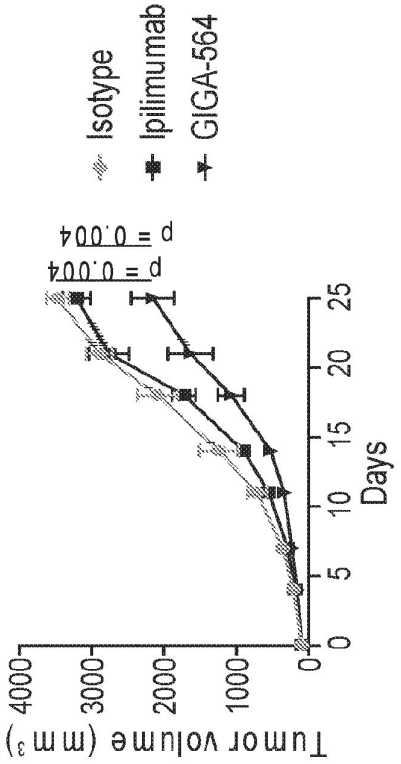


FIG. 14C



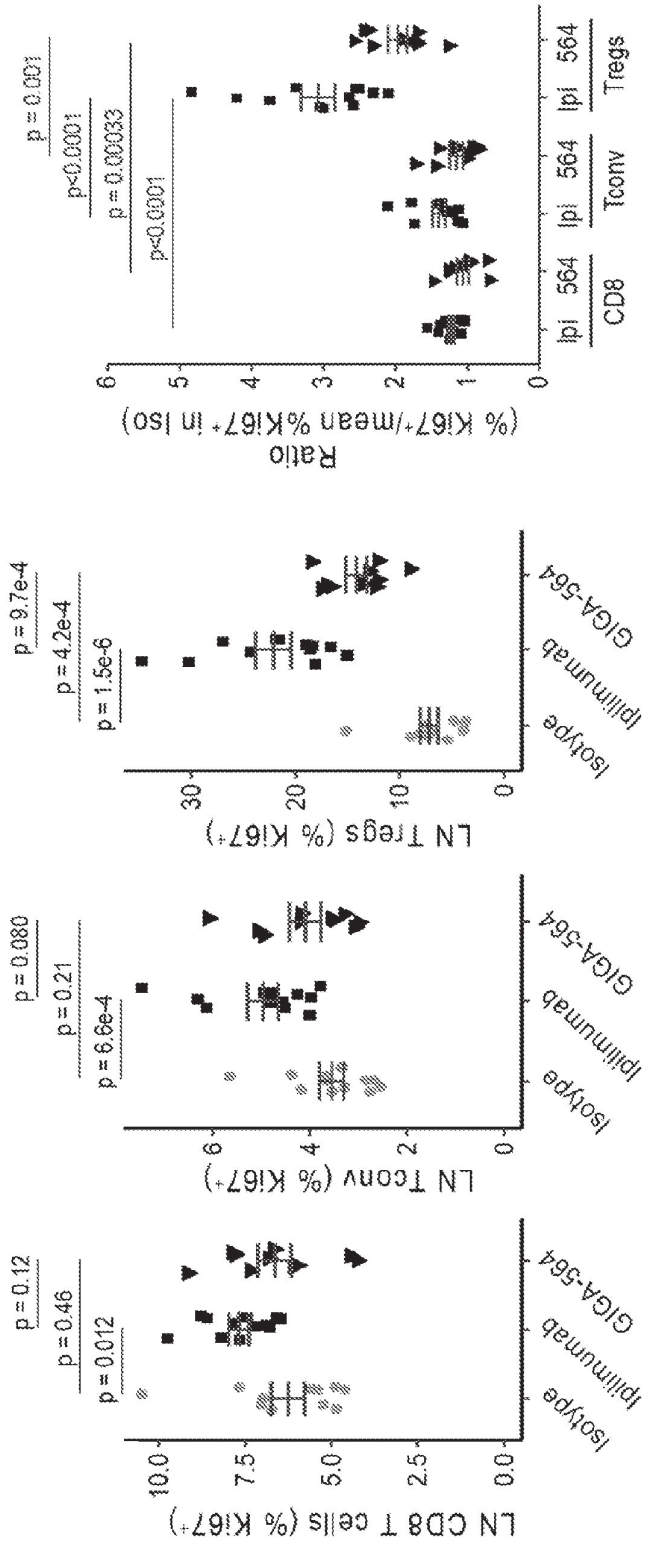

LN CD8 T cells (% Ki67+)
p = 0.12
p = 0.46
p = 0.012
LN Tconv (% Ki67+)
p = 0.080
p = 0.21
p = 6.6e-4
LN Tregs (% Ki67+)
p = 9.7e-4
p = 4.2e-4
p = 1.5e-6
Isotype
Ipilimumab
GIGA-564
Ratio
(% Ki67+/mean %Ki67+ in Iso)
p = 0.001
p<0.0001
p = 0.00033
p<0.0001
Ipi 564
CD8
Ipi 564
Tconv
Ipi 564
Tregs

FIG. 15A

FIG. 15B

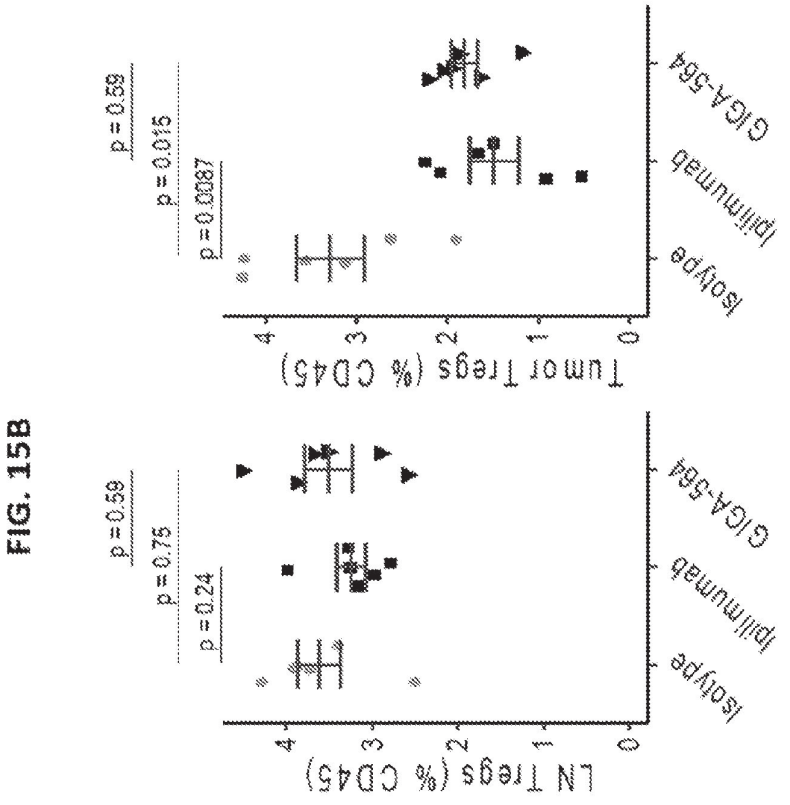
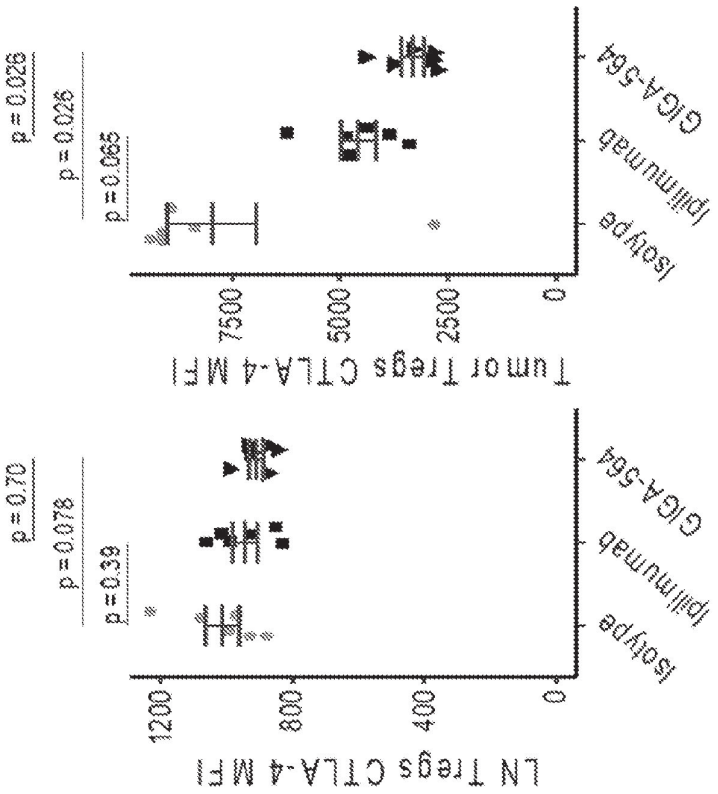


FIG. 15C



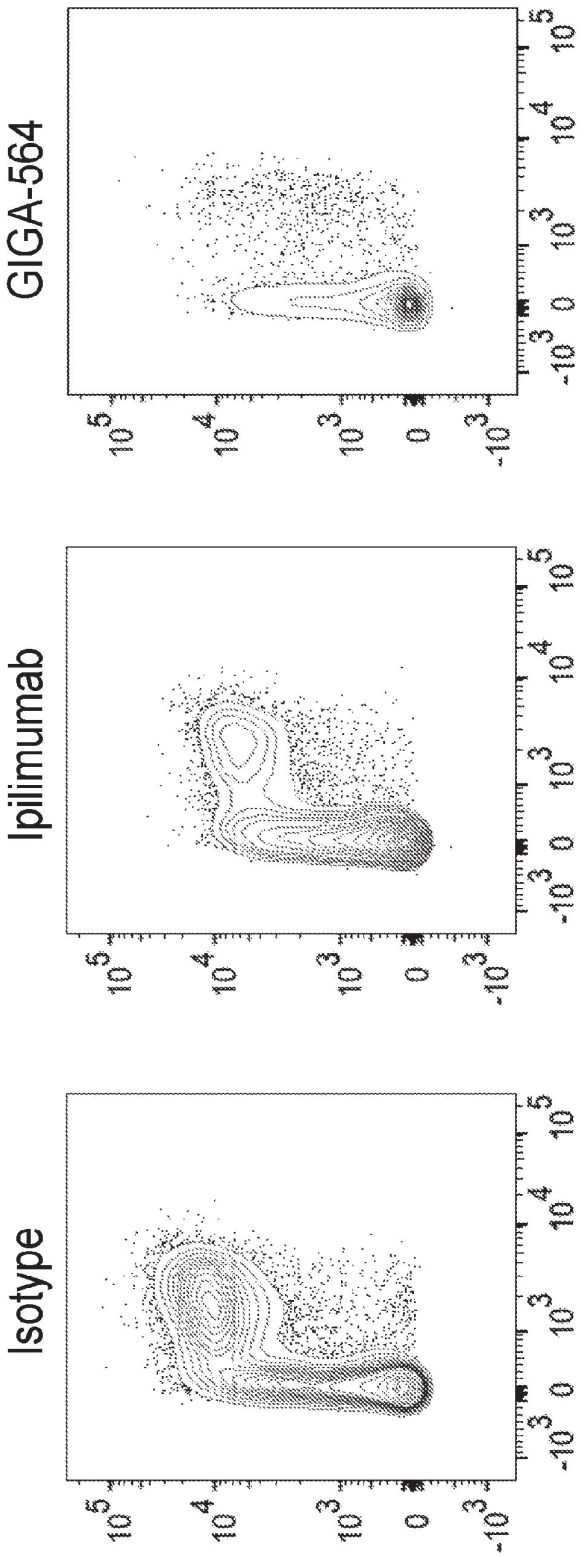


FIG. 15D

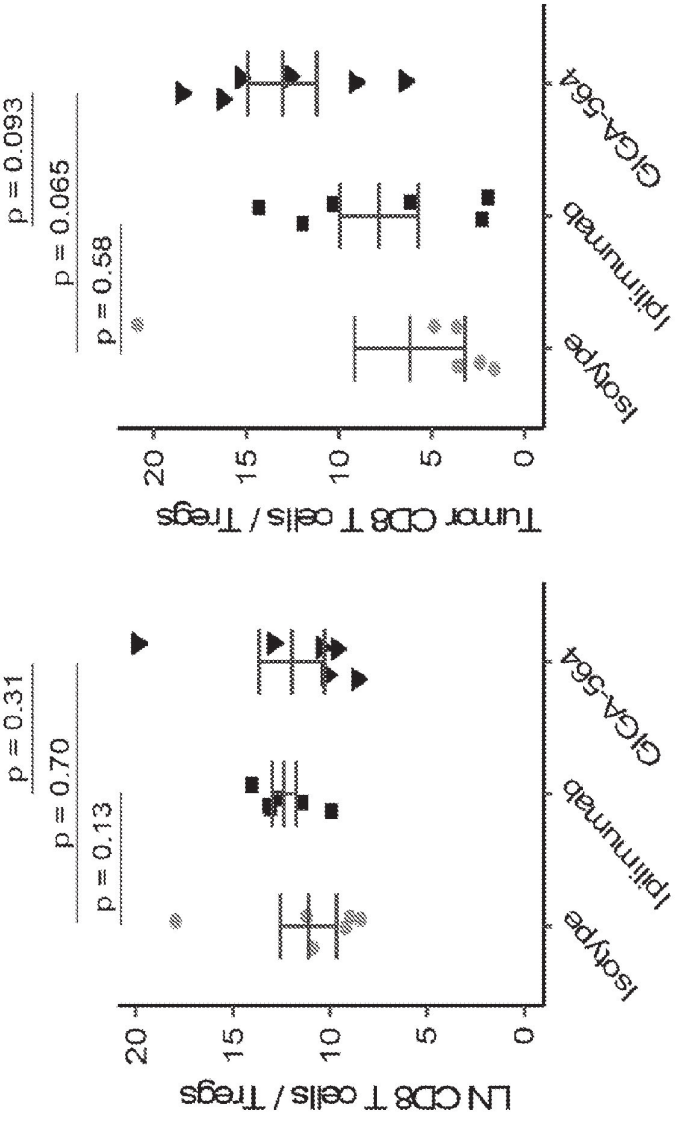

p = 0.093
p = 0.065
p = 0.58
Tumor CD8 T cells / Tregs
20
15
10
5
0
Isotype
Ipilimumab
GITR-56a
p = 0.31
p = 0.70
p = 0.13
LN CD8 T cells / Tregs
20
15
10
5
0
Isotype
Ipilimumab
GITR-56a

FIG. 15E

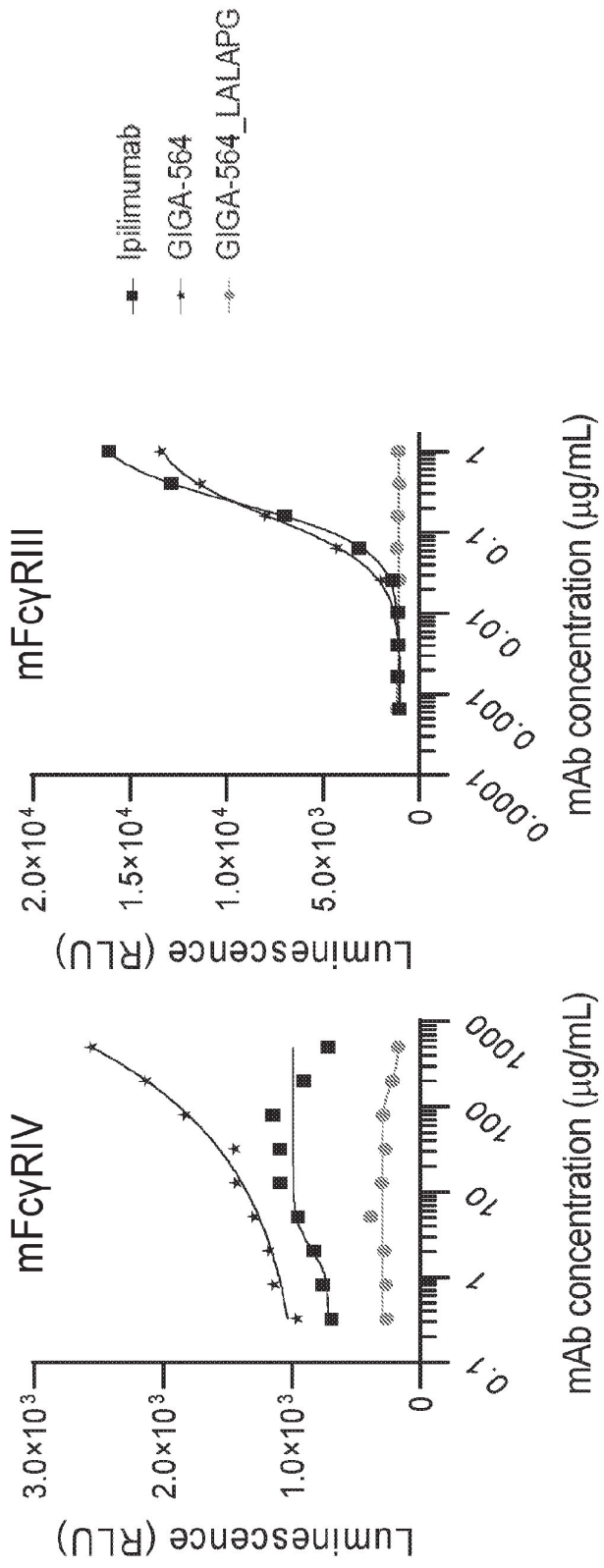

mFcγRIV
mFcγRIII
Luminescence (RLU)
mAb concentration (μg/mL)
Ipilimumab
GIGA-564
GIGA-564_LALAPG

FIG. 16A

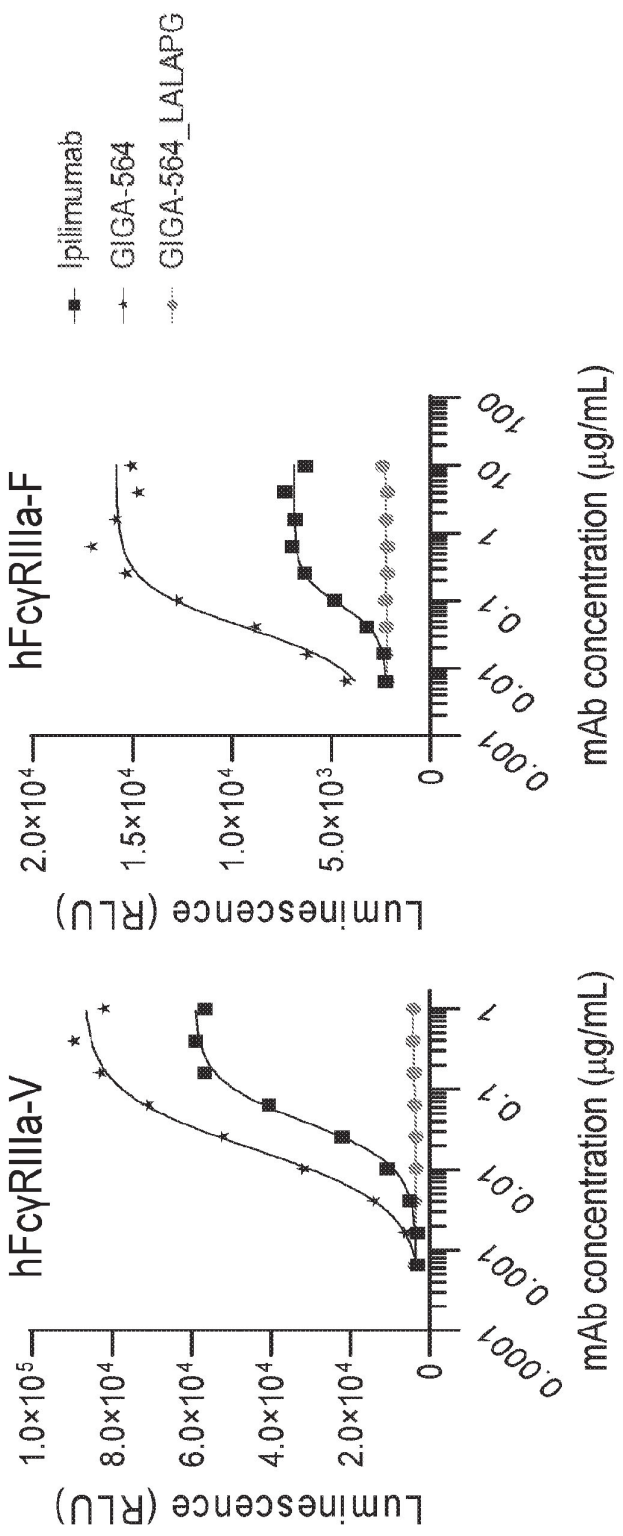

hFcγRIIIa-V
hFcγRIIIa-F
Luminescence (RLU)
mAb concentration (μg/mL)
Ipilimumab
GIGA-564
GIGA-564_LALAPG

FIG. 16B

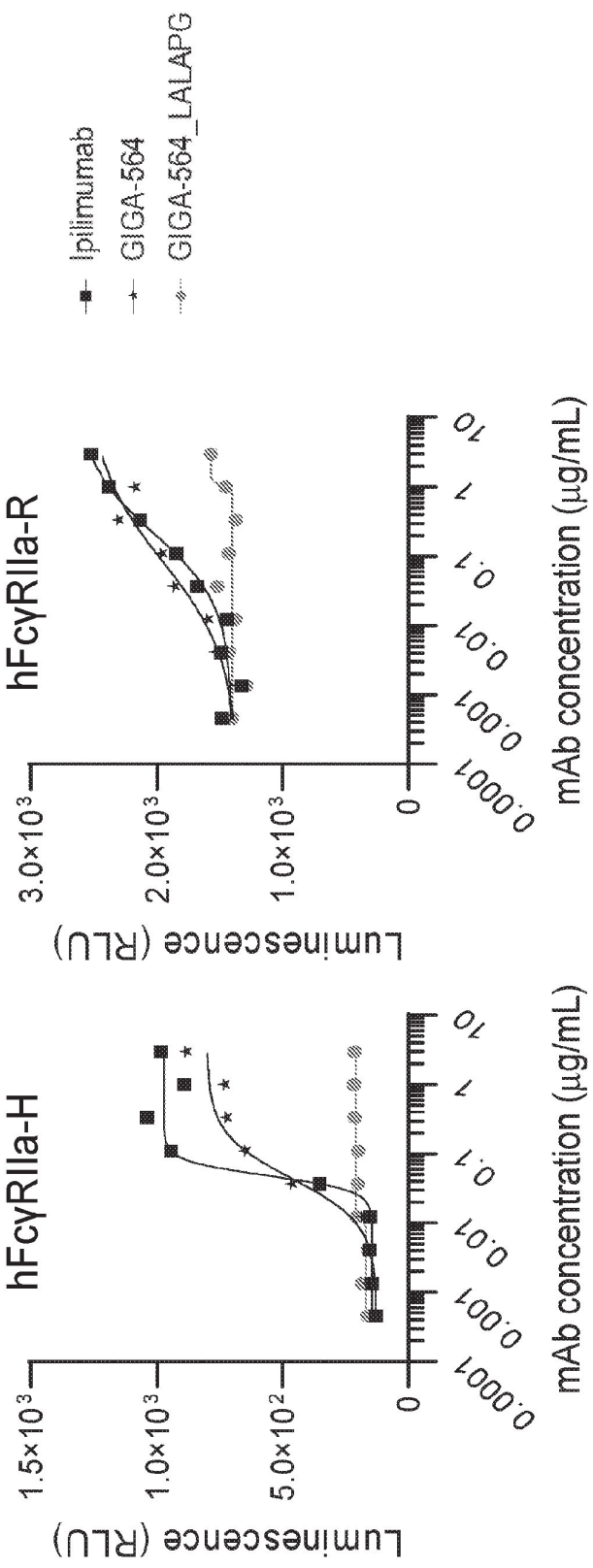

hFcγRIIa-H
hFcγRIIa-R
Luminescence (RLU)
1.5×10³
1.0×10³
5.0×10²
0
3.0×10³
2.0×10³
1.0×10³
0.0001
0.001
0.01
0.1
1
10
mAb concentration (μg/mL)
Ipilimumab
GIGA-564
GIGA-564_LALAPG

FIG. 16C

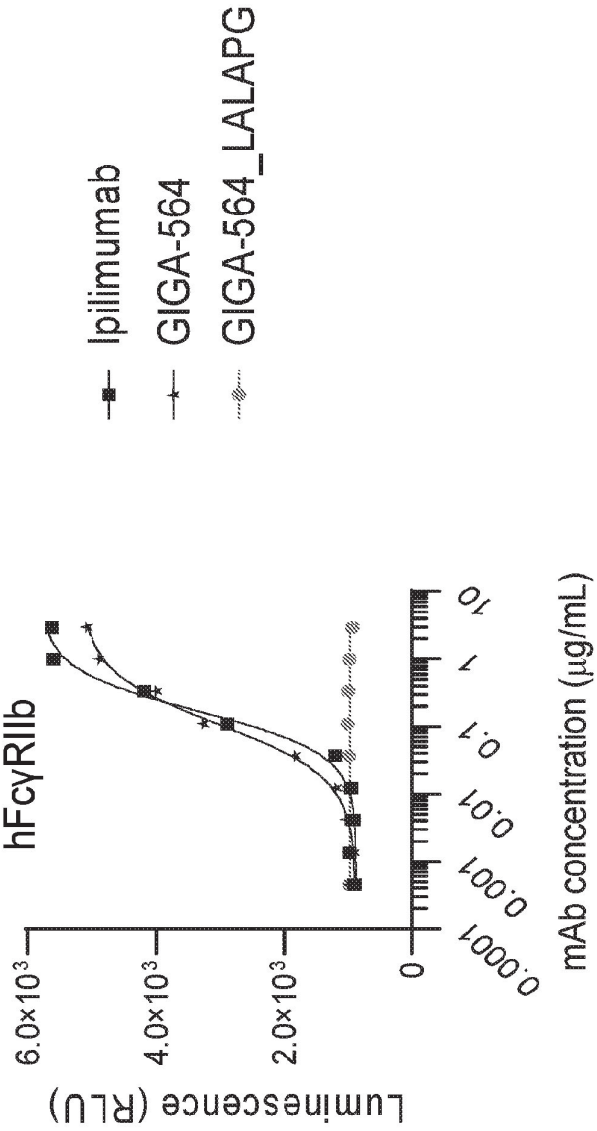

Ipilimumab
GIGA-564
GIGA-564_LALAPG
hFcγRIIb
Luminescence (RLU)
6.0×10³
4.0×10³
2.0×10³
0
0.0001
0.001
0.01
0.1
1
10
mAb concentration (μg/mL)

FIG. 16D

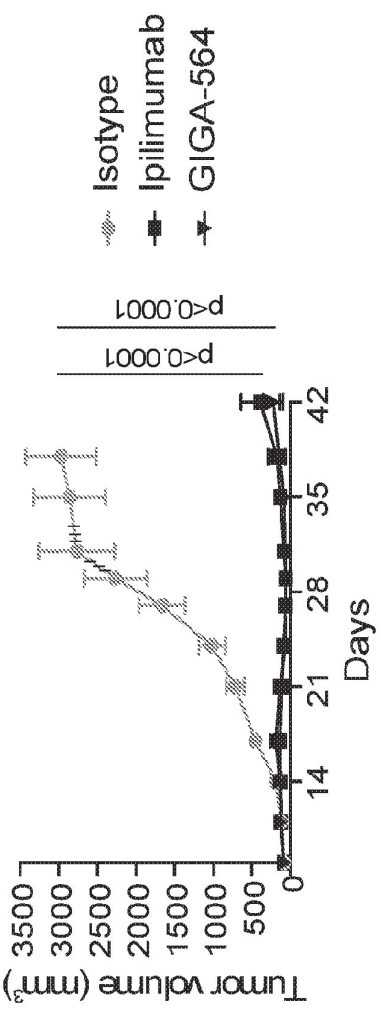


FIG. 17A

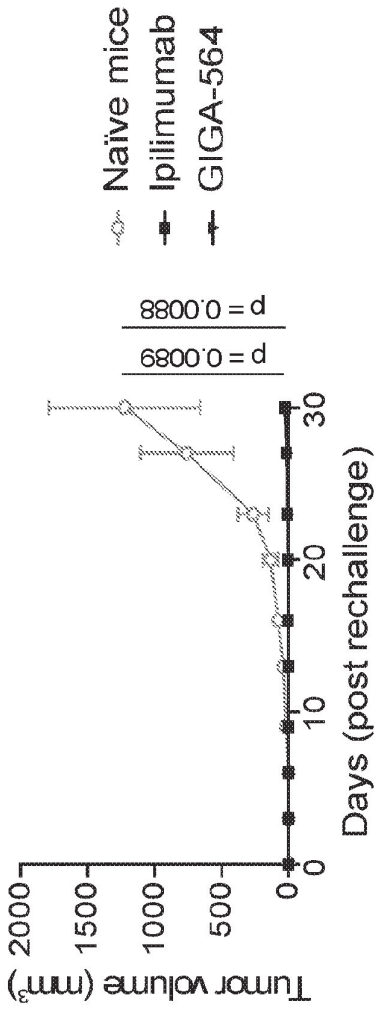


FIG. 17B

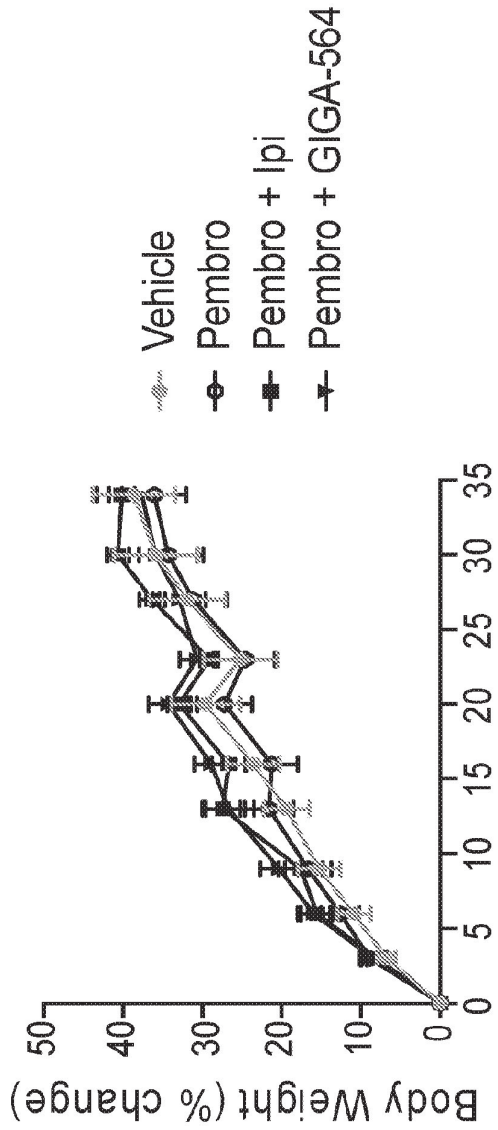

Vehicle
Pembro
Pembro + Ipi
Pembro + GIGA-564
Body Weight (% change)
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Days

FIG. 18A

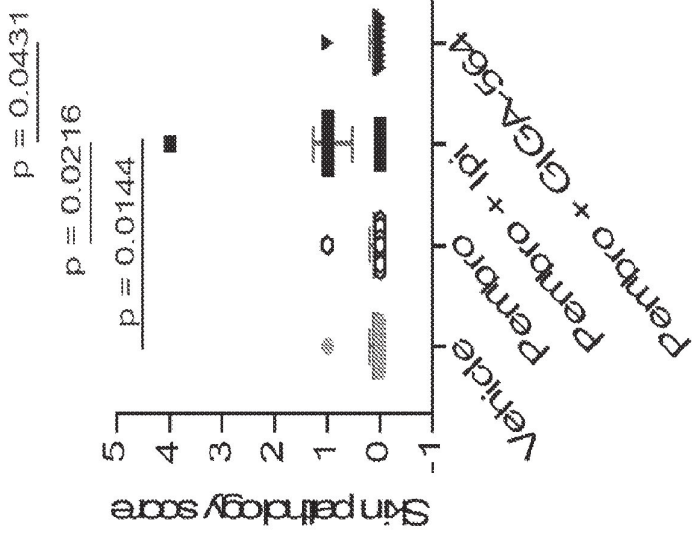


FIG. 18B

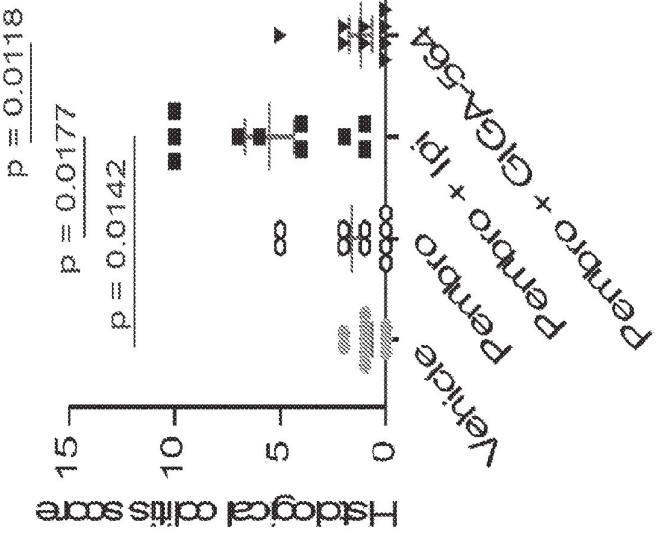
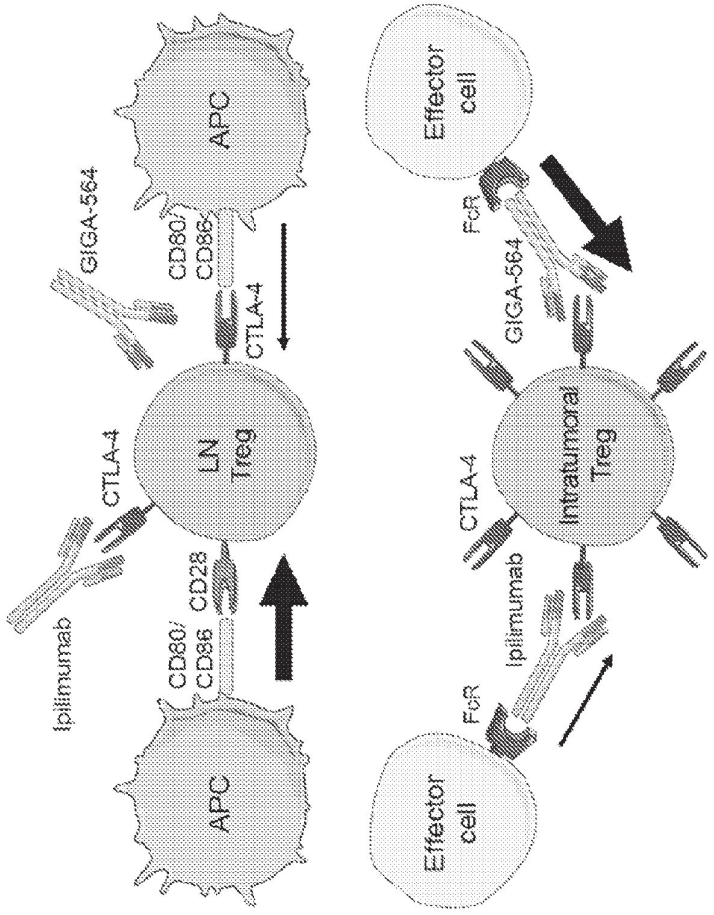


FIG. 18C



Ipilimumab:
- Strongly blocks CTLA-4 interaction with CD80/CD86
- ↑↑↑ LN Treg proliferation
- Weaker intratumoral Treg killing

GIGA-564:
- Weakly blocks CTLA-4 interaction with CD80/CD86
- Stronger FcR signaling
- More efficient intratumoral Treg killing

FIG. 19

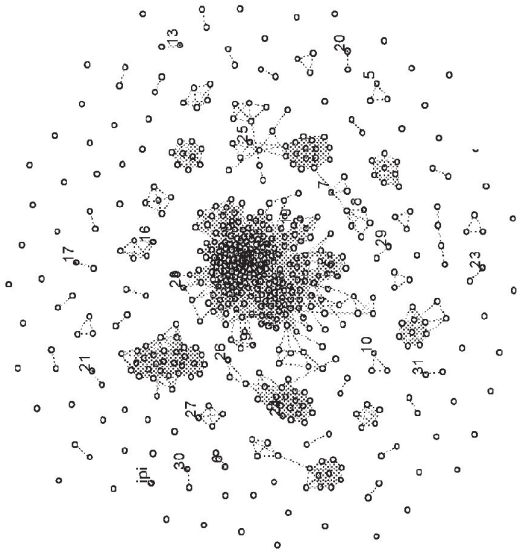


FIG. 20A

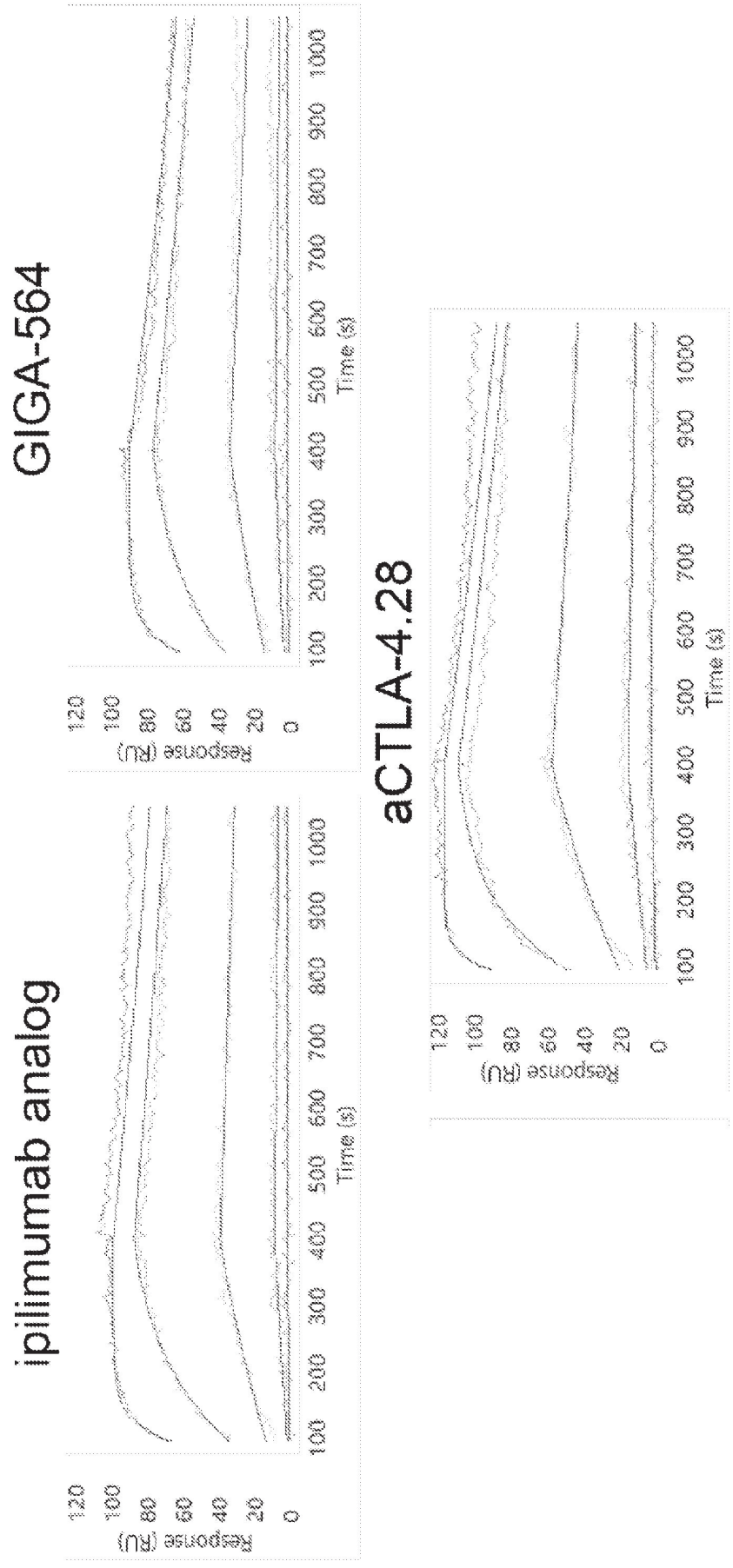

ipilimumab analog
GIGA-564
aCTLA-4.28
Response (RU)
Time (s)
0
20
40
60
80
100
120
100
200
300
400
500
600
700
800
900
1000

FIG. 20B

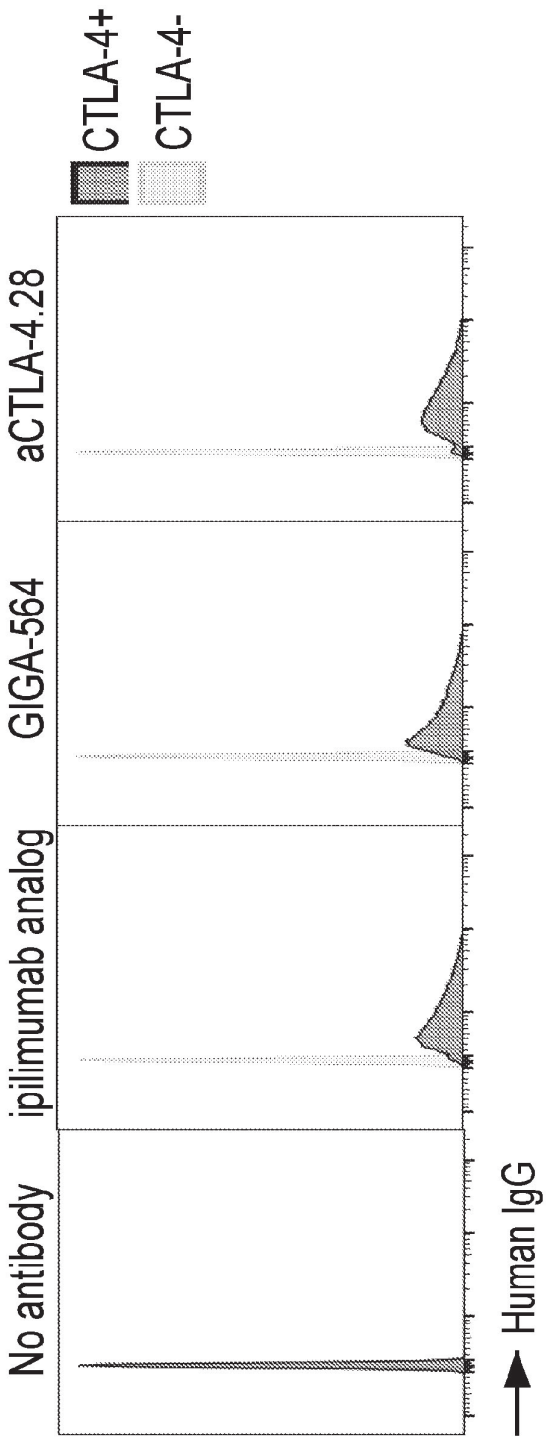

CTLA-4+
CTLA-4-
aCTLA-4.28
GIGA-564
ipilimumab analog
No antibody
Human IgG

FIG. 20C

FIG. 20D

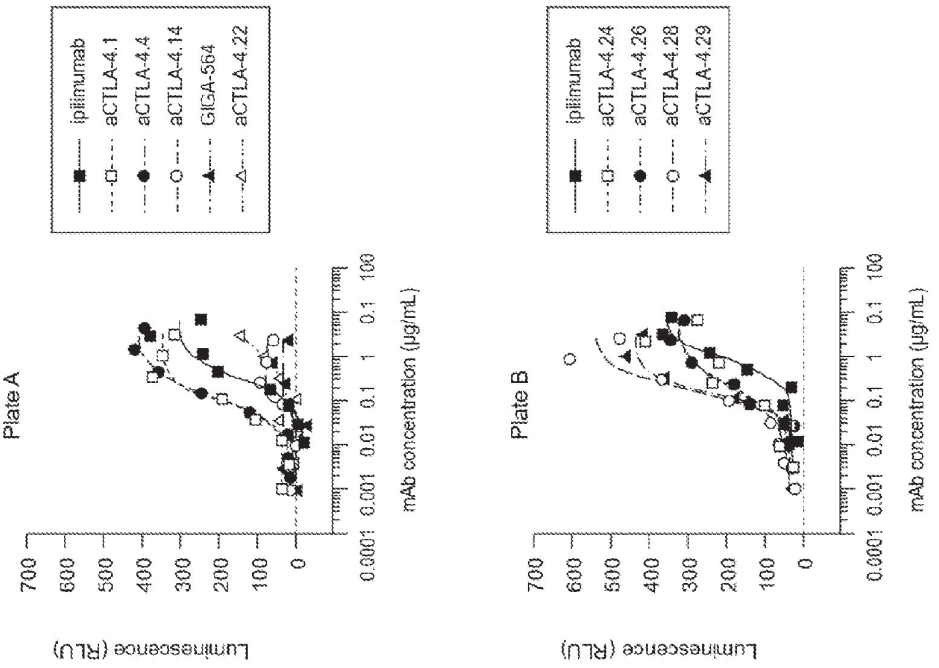
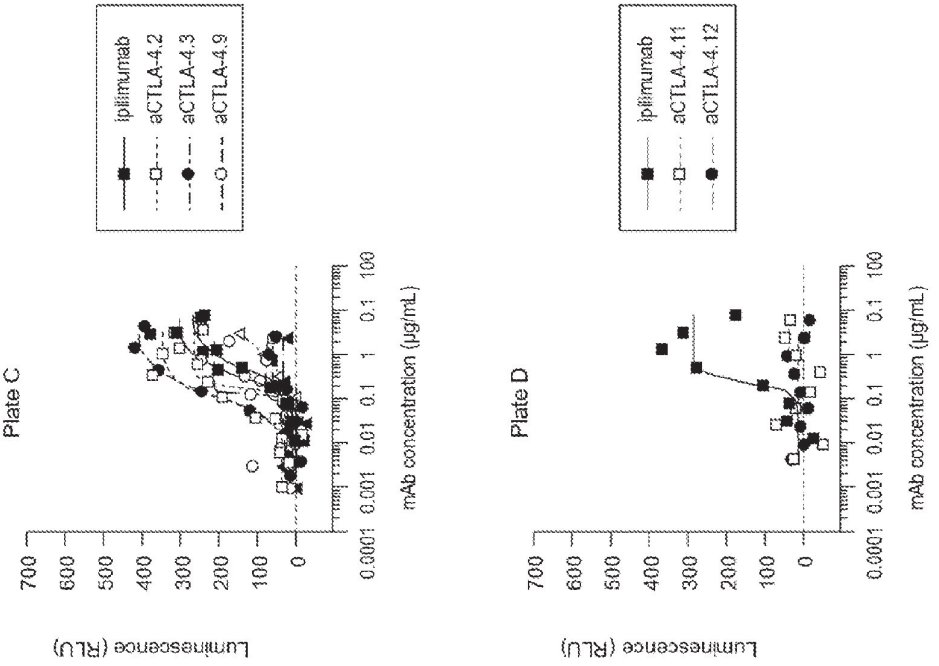

Plate A
Luminescence (RLU)
700
600
500
400
300
200
100
0
0.0001 0.001 0.01 0.1 1 10 100
mAb concentration (µg/mL)
ipilimumab
aCTLA-4.1
aCTLA-4.4
aCTLA-4.14
GIGA-564
aCTLA-4.22
Plate B
Luminescence (RLU)
700
600
500
400
300
200
100
0
0.0001 0.001 0.01 0.1 1 10 100
mAb concentration (µg/mL)
ipilimumab
aCTLA-4.24
aCTLA-4.26
aCTLA-4.28
aCTLA-4.29

FIG. 20D (Cont.)


Plate C
Luminescence (RLU)
700
600
500
400
300
200
100
0
0.0001 0.001 0.01 0.1 1 10 100
mAb concentration (µg/mL)
ipilimumab
aCTLA-4.2
aCTLA-4.3
aCTLA-4.9
Plate D
Luminescence (RLU)
700
600
500
400
300
200
100
0
0.0001 0.001 0.01 0.1 1 10 100
mAb concentration (µg/mL)
ipilimumab
aCTLA-4.11
aCTLA-4.12

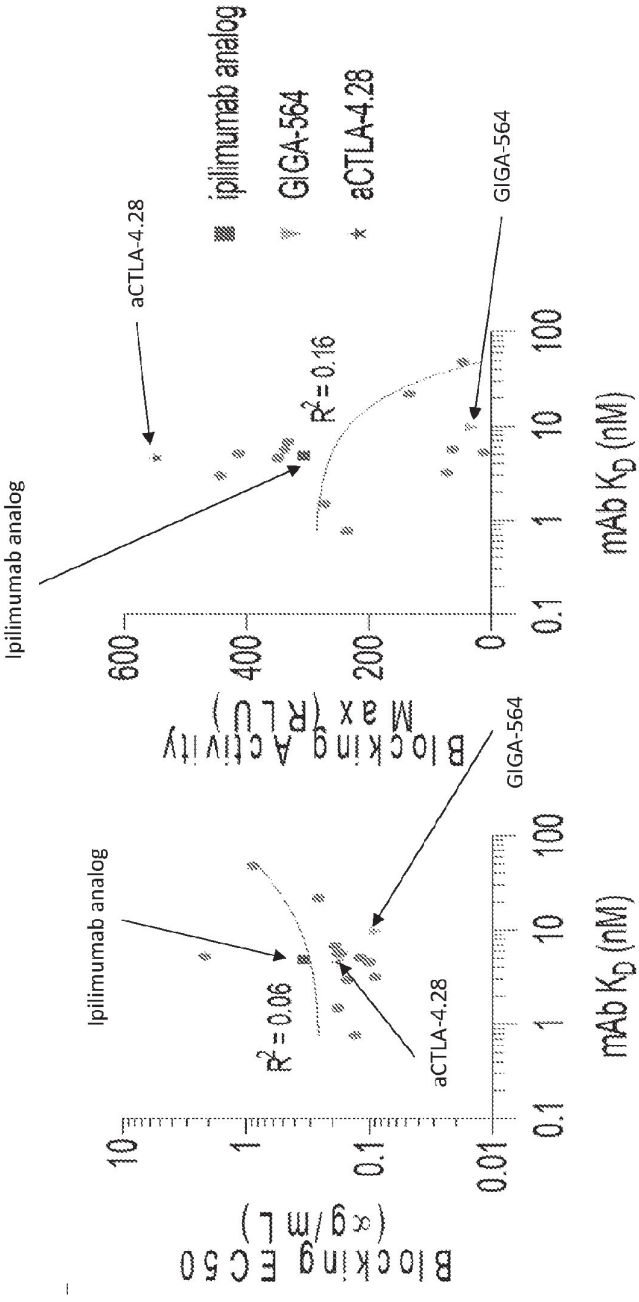

Ipilimumab analog
aCTLA-4.28
Ipilimumab analog
GIGA-564
aCTLA-4.28
R^2 = 0.16
Blocking Activity Max (RLU)
600
400
200
0
0.1
1
10
100
mAb K_D (nM)
GIGA-564
Ipilimumab analog
R^2 = 0.06
aCTLA-4.28
Blocking EC50 (µg/mL)
10
1
0.1
0.01
0.1
1
10
100
mAb K_D (nM)

FIG. 20E

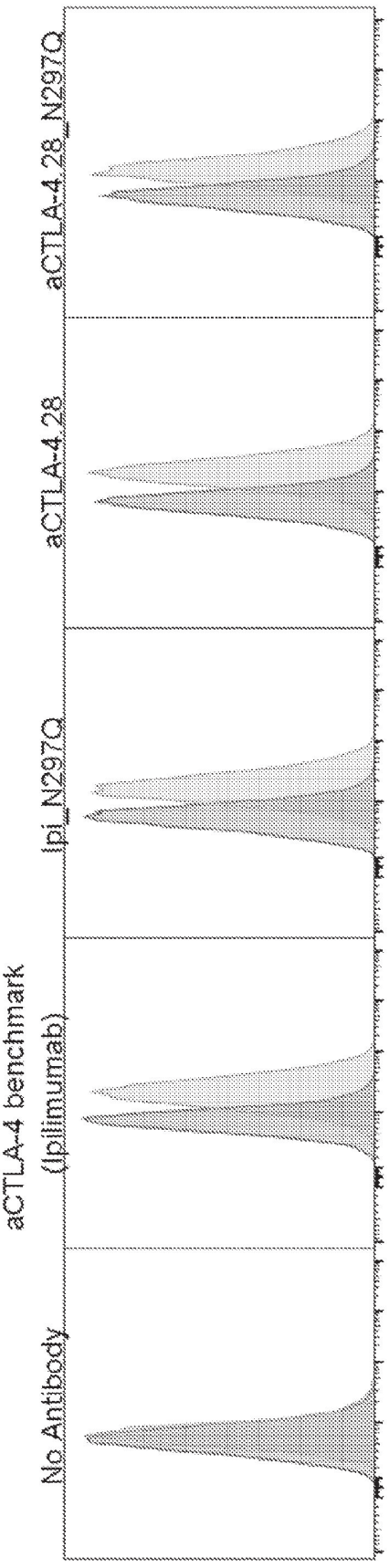

No Antibody
aCTLA-4 benchmark (Ipilimumab)
Ipi_N297Q
aCTLA-4.28
aCTLA-4.28_N297Q

FIG. 21

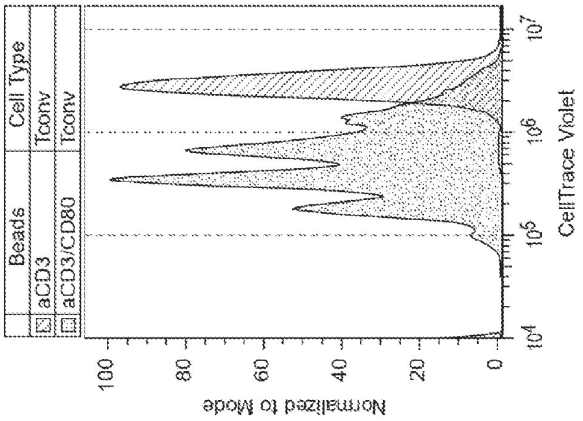


FIG. 22B

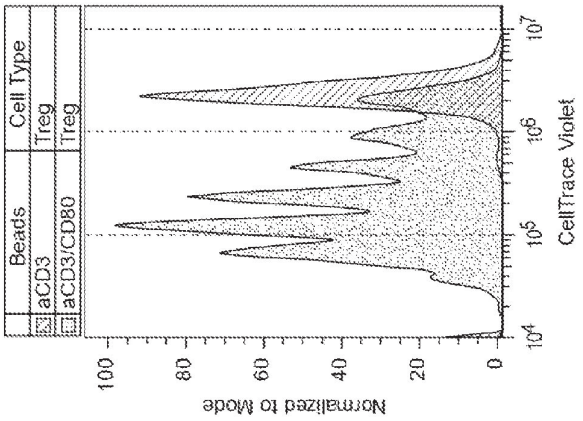


FIG. 22A

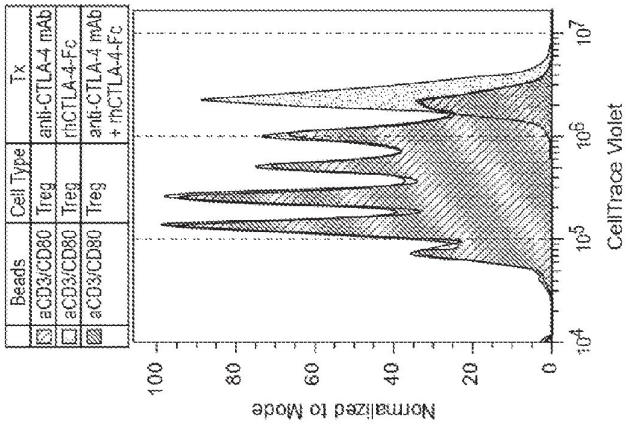


FIG. 22C

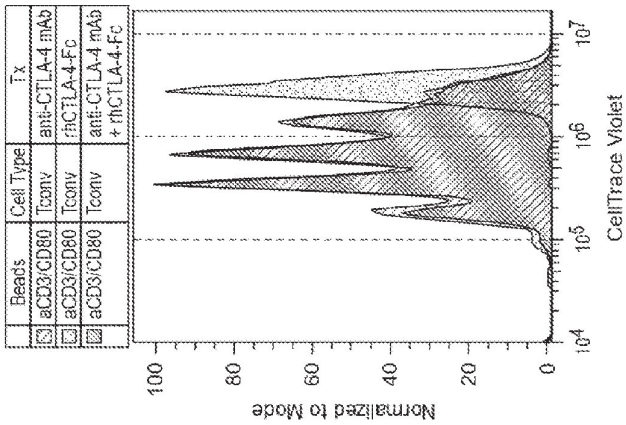


FIG. 22D

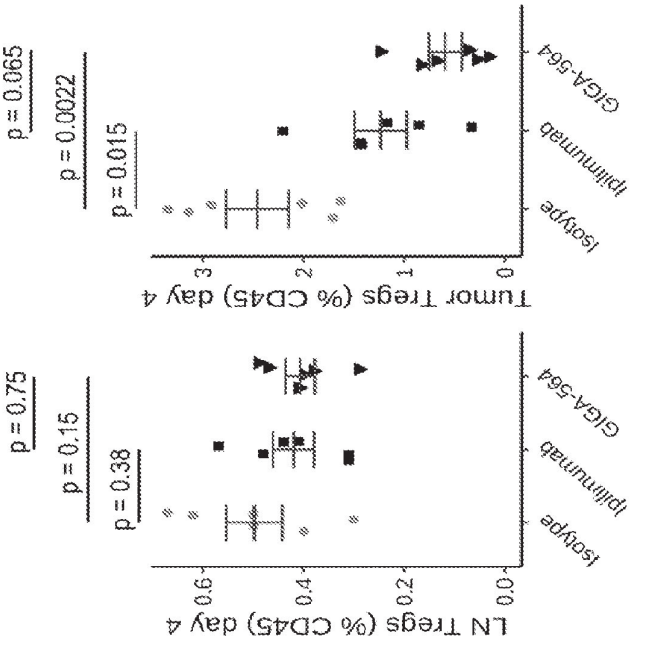


FIG. 23A

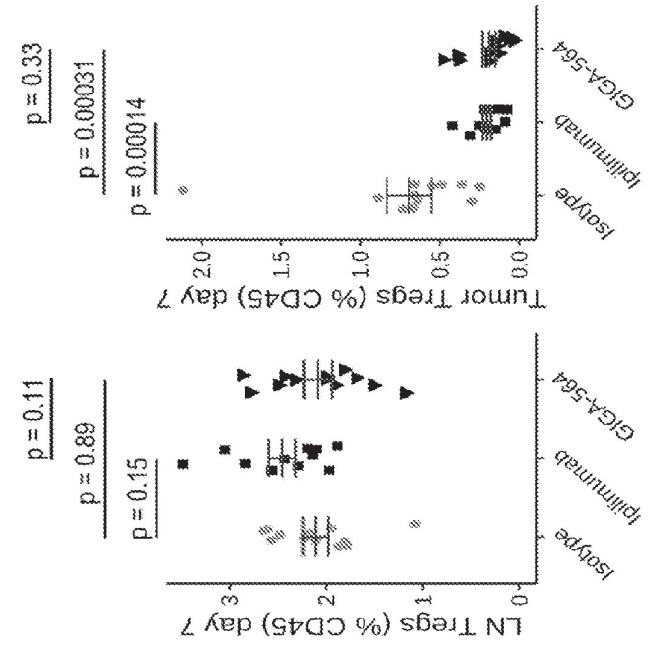


FIG. 23B

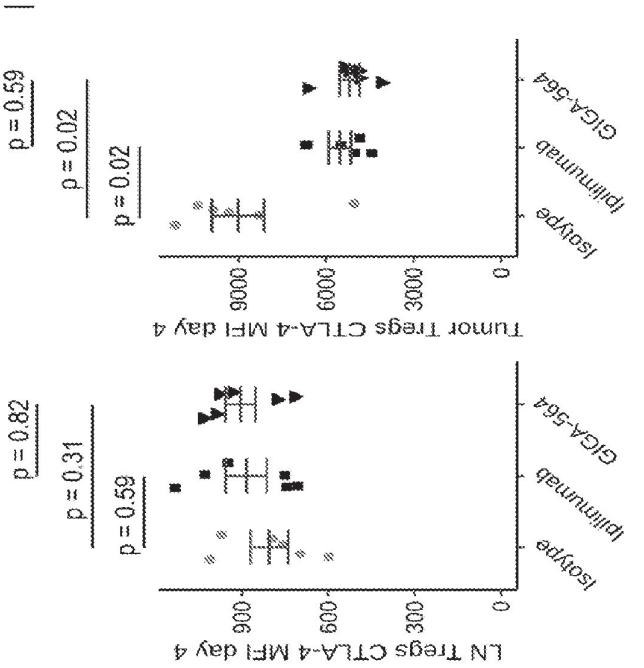


FIG. 23C

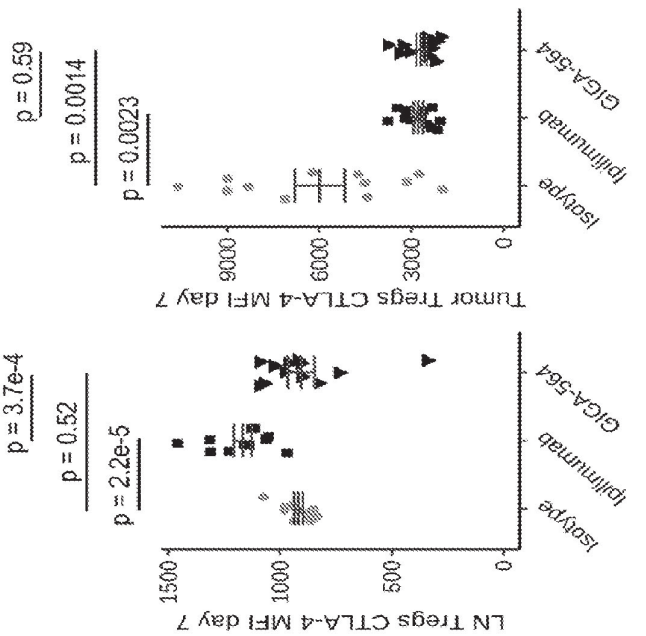


FIG. 23D

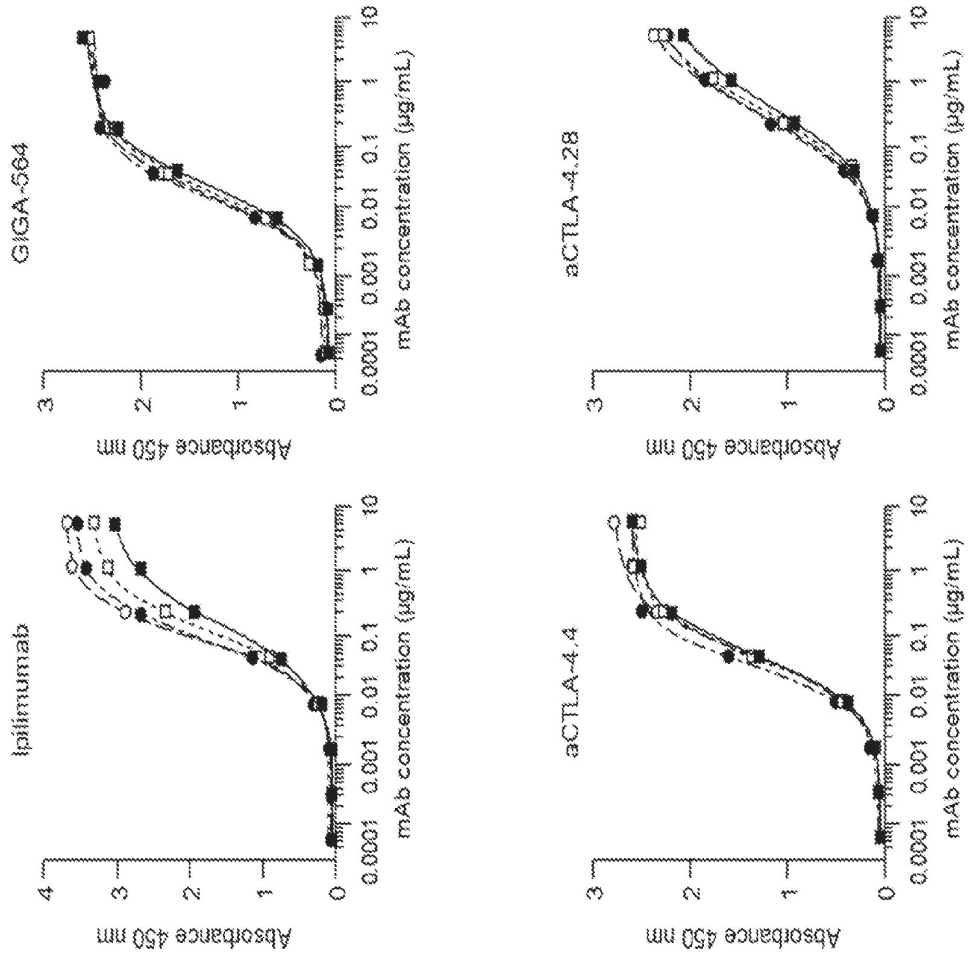

Ipilimumab
GIGA-564
aCTLA-4.4
aCTLA-4.28
Absorbance 450 nm
mAb concentration (µg/mL)
0.0001 0.001 0.01 0.1 1 10
pH 4.0
pH 5.0
pH 6.0
pH 7.0

FIG. 24A

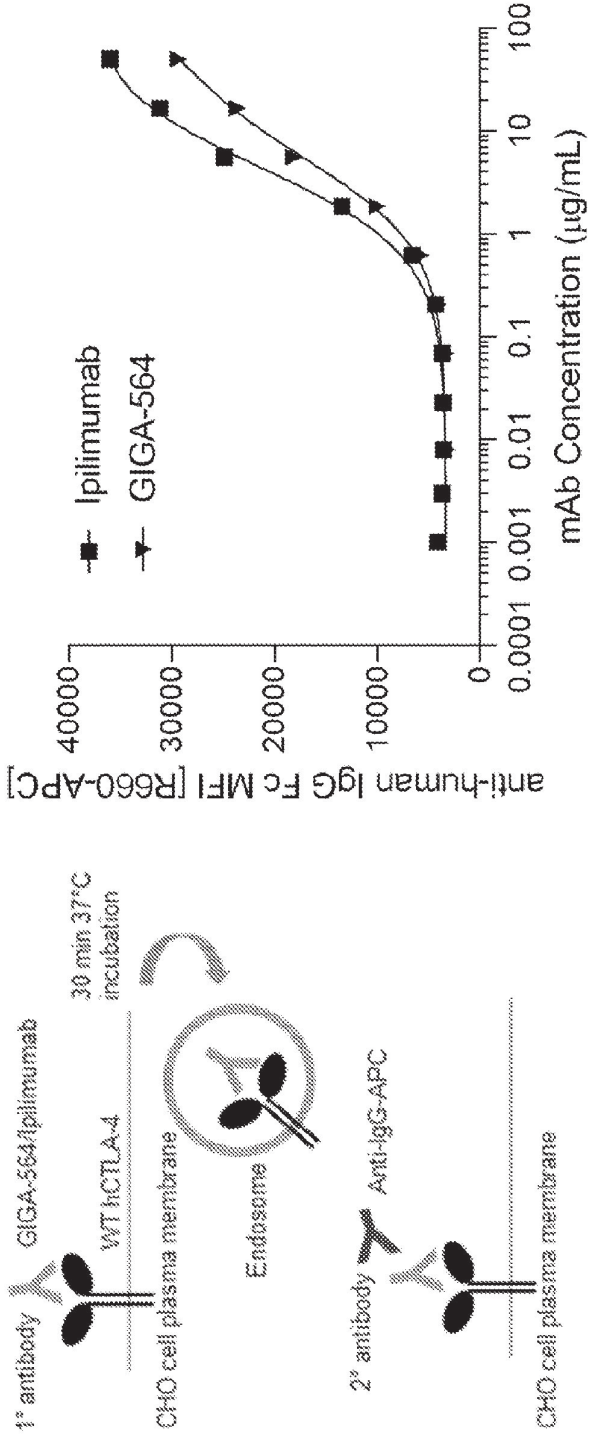

anti-human IgG Fc MFI [R660-APC]
40000
30000
20000
10000
0
Ipilimumab
GIGA-564
0.0001 0.001 0.01 0.1 1 10 100
mAb Concentration (µg/mL)
1° antibody
GIGA-564/Ipilimumab
WT hCTLA-4
CHO cell plasma membrane
30 min 37°C incubation
Endosome
2° antibody
Anti-IgG-APC
CHO cell plasma membrane

FIG. 24B

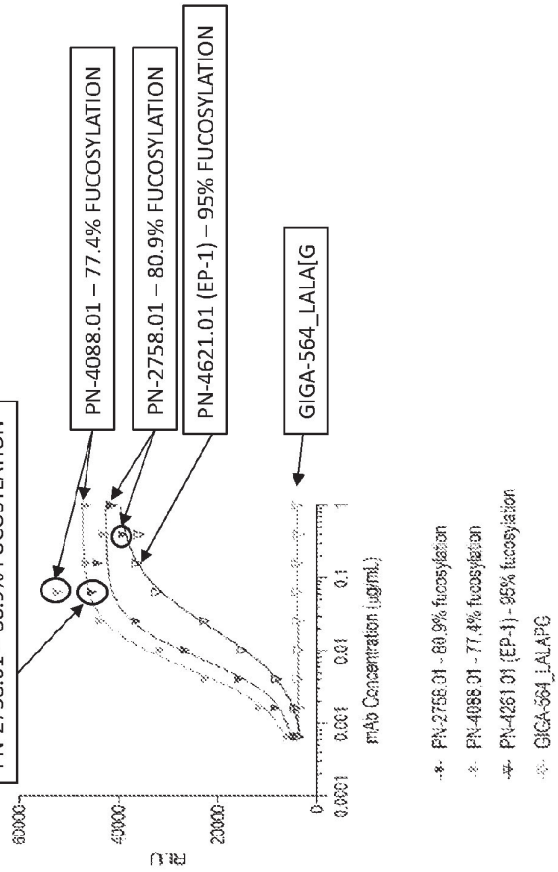


FIG. 24D

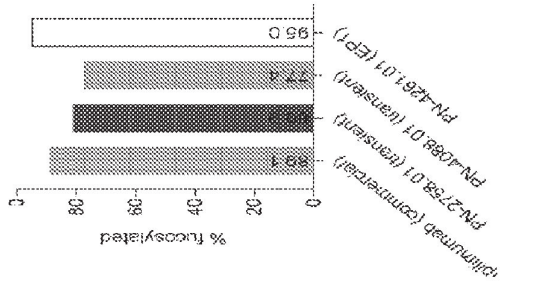


FIG. 24C

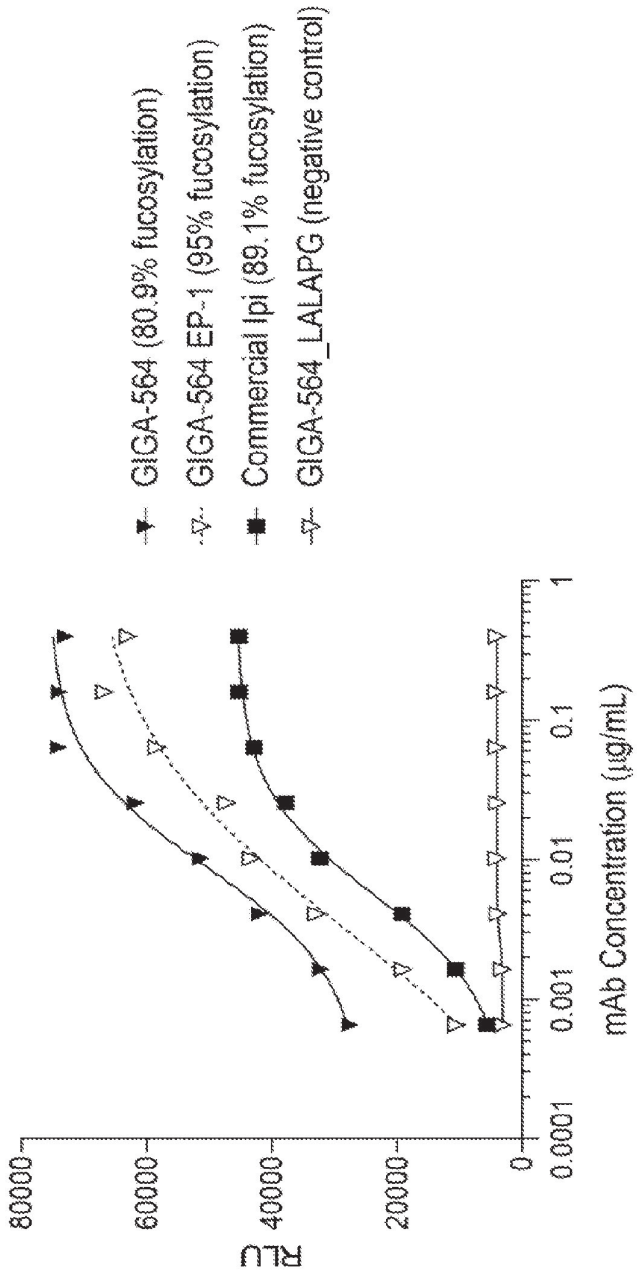

GIGA-564_LALAPG
GIGA-564 (80.9% fucosylation)
GIGA-564 EP-1 (95% fucosylation)
Commercial Ipi (89.1% fucosylation)
GIGA-564_LALAPG (negative control)
RLU
80000
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
mAb Concentration (μg/mL)

FIG. 24E

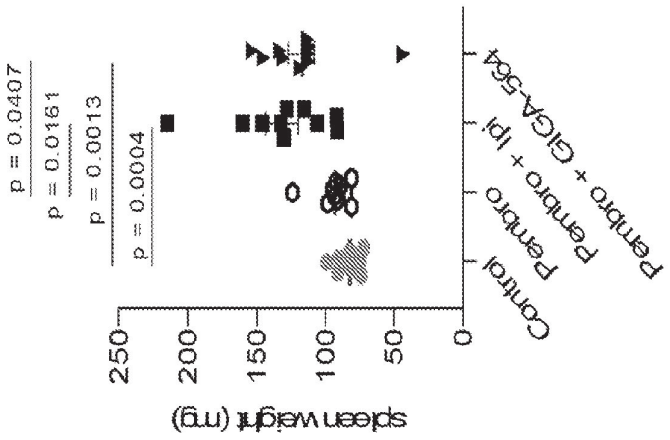


FIG. 25B

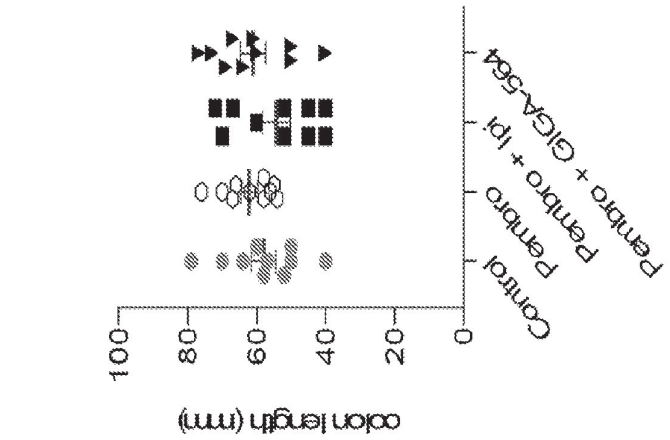


FIG. 25A

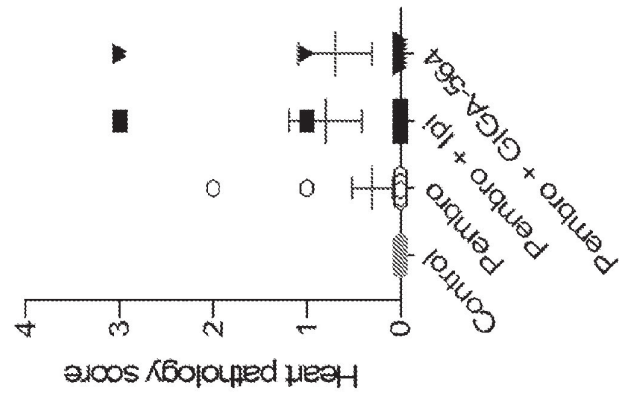


FIG. 25D

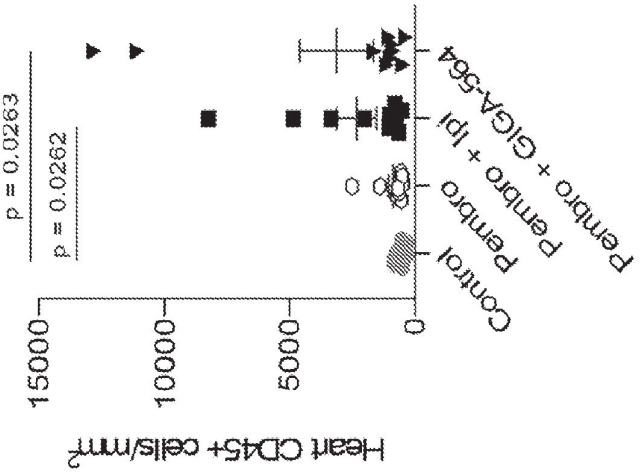


FIG. 25C

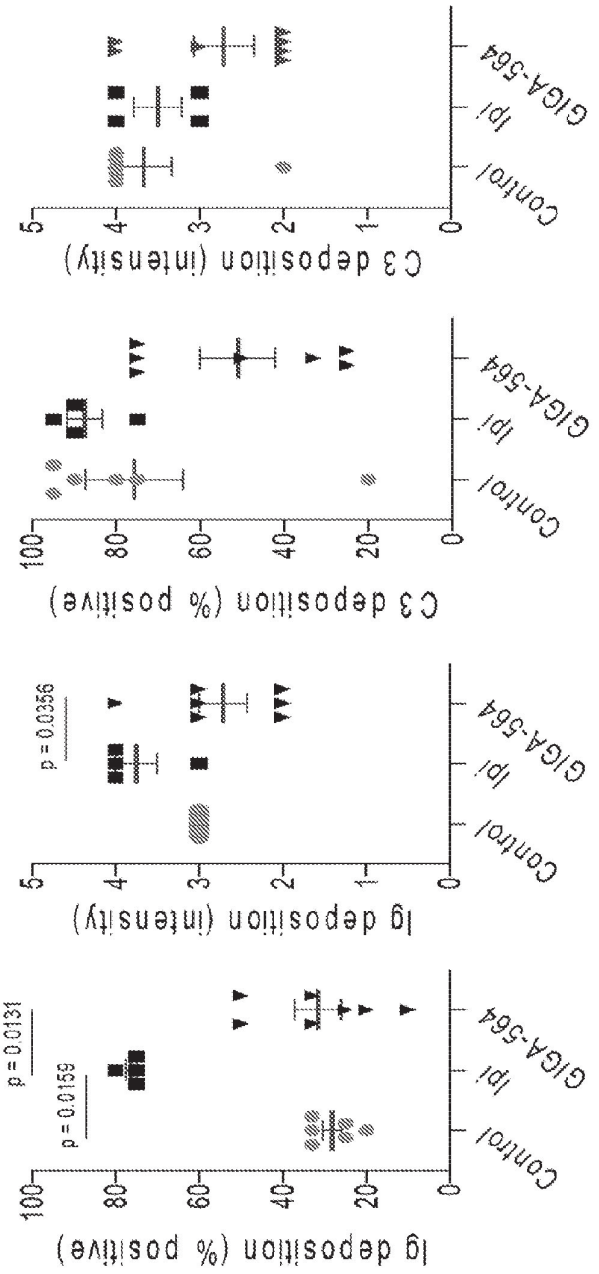

Ig deposition (% positive)
p = 0.0131
p = 0.0159
Ig deposition (intensity)
p = 0.0356
C3 deposition (% positive)
C3 deposition (intensity)
Control
IgI
GIGA-564

FIG. 25E

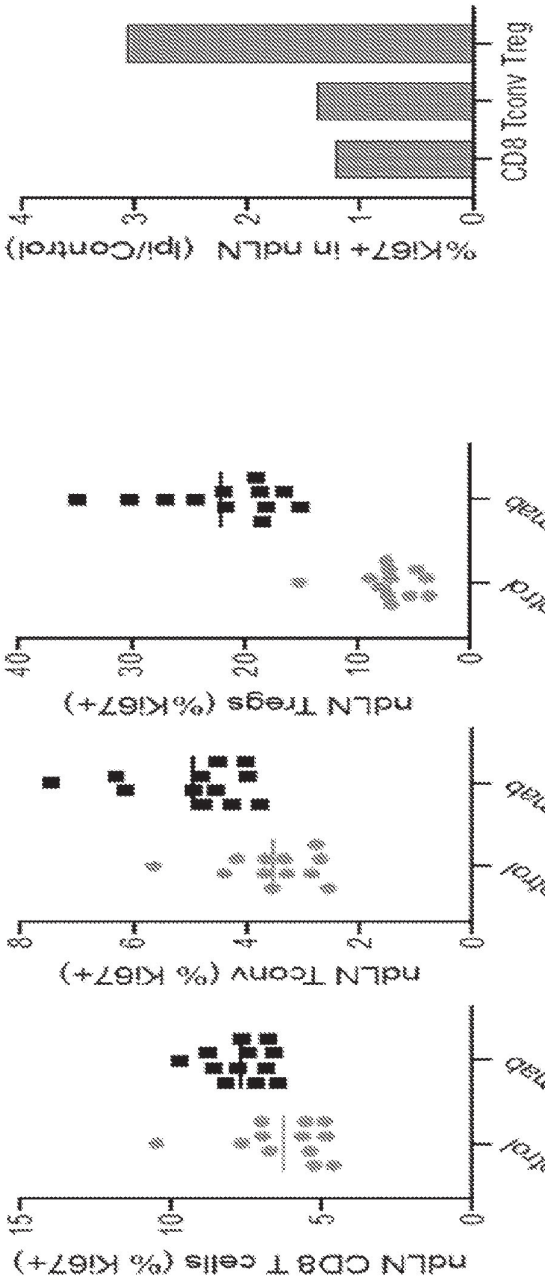

%Ki67+ in ndLN (Ipi/Control)
0
1
2
3
4
CD8 Tconv Treg
ndLN CD8 T cells (% Ki67+)
0
5
10
15
Control
Ipilimumab
ndLN Tconv (% Ki67+)
0
2
4
6
8
Control
Ipilimumab
ndLN Tregs (% Ki67+)
0
10
20
30
40
Control
Ipilimumab

FIG. 26

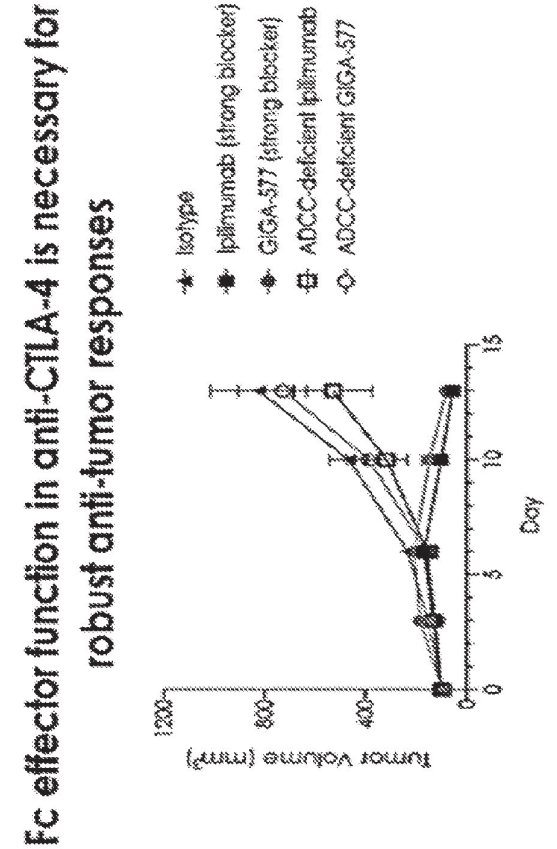

Fc effector function in anti-CTLA-4 is necessary for robust anti-tumor responses
Isotype
Ipilimumab (strong blocker)
GIGA-577 (strong blocker)
ADCC-deficient Ipilimumab
ADCC-deficient GIGA-577
Tumor Volume (mm³)
1200
800
400
0
0
5
10
15
Day

FIG. 27

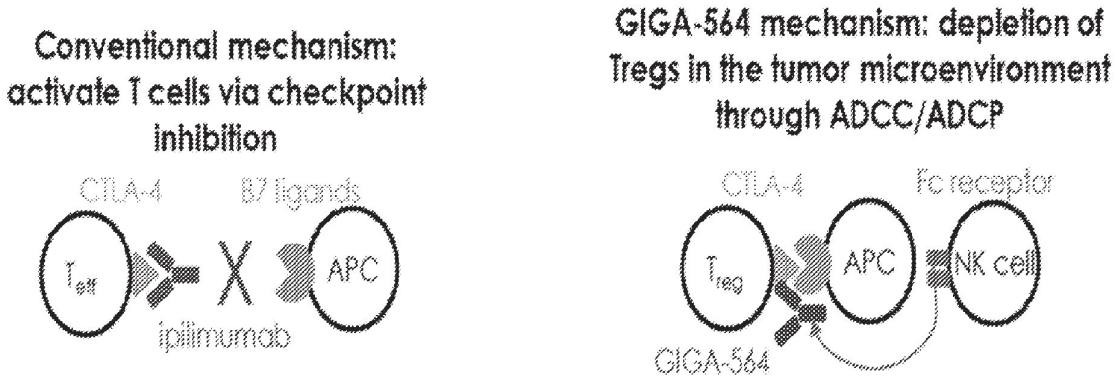

Conventional mechanism:
activate T cells via checkpoint
inhibition
CTLA-4
B7 ligands
T_{eff}
APC
ipilimumab
GIGA-564 mechanism: depletion of
Tregs in the tumor microenvironment
through ADCC/ADCP
CTLA-4
Fc receptor
T_{reg}
APC
NK cell
GIGA-564

FIG. 28

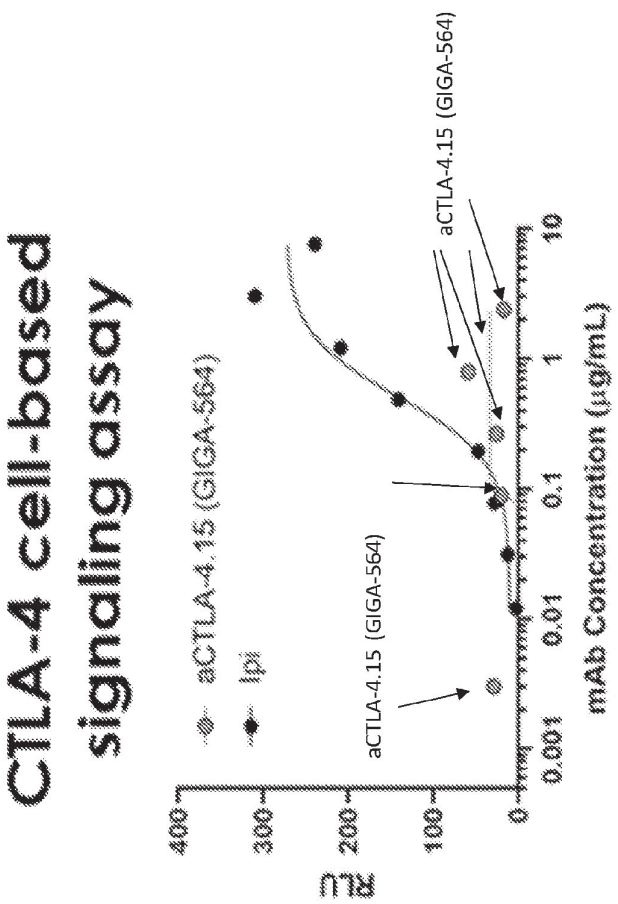

CTLA-4 cell-based signaling assay
aCTLA-4.15 (GIGA-564)
Ipi
aCTLA-4.15 (GIGA-564)
aCTLA-4.15 (GIGA-564)
RLU
400
300
200
100
0
0.001
0.01
0.1
1
10
mAb Concentration (µg/mL)

FIG. 29

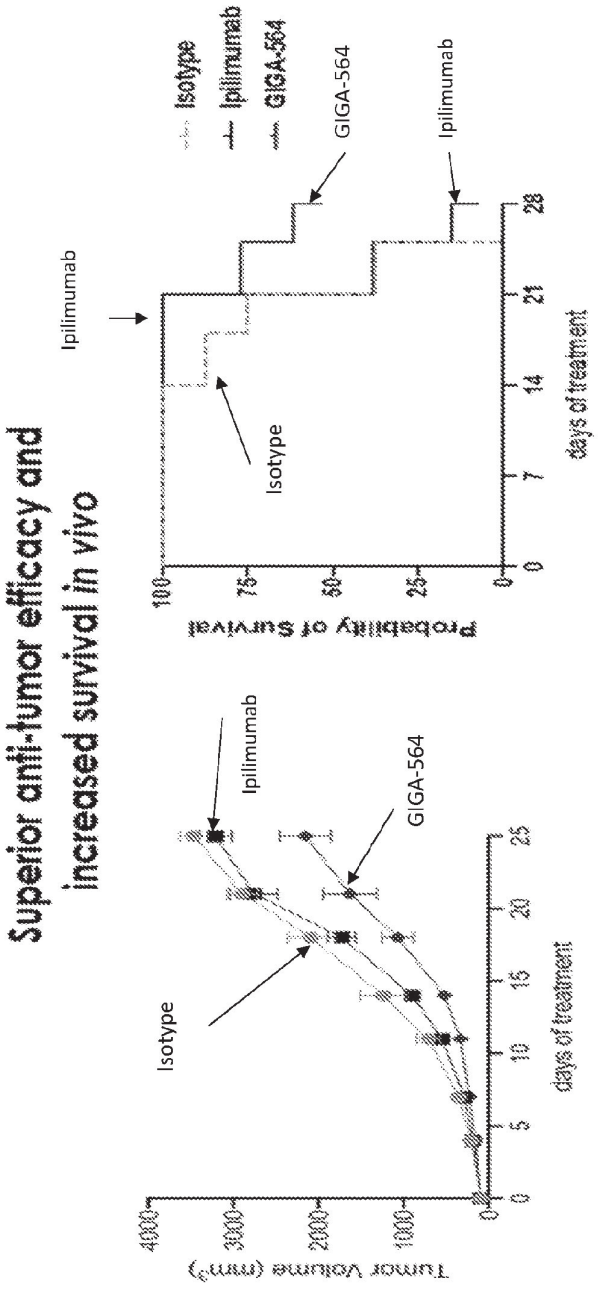

Superior anti-tumor efficacy and increased survival in vivo
Ipilimumab
Isotype
Ipilimumab
GIGA-564
Isotype
GIGA-564
Ipilimumab
Tumor Volume (mm³)
4000
3000
2000
1000
0
0
5
10
15
20
25
days of treatment
Probability of Survival
100
75
50
25
0
0
7
14
21
28
days of treatment

FIG. 30

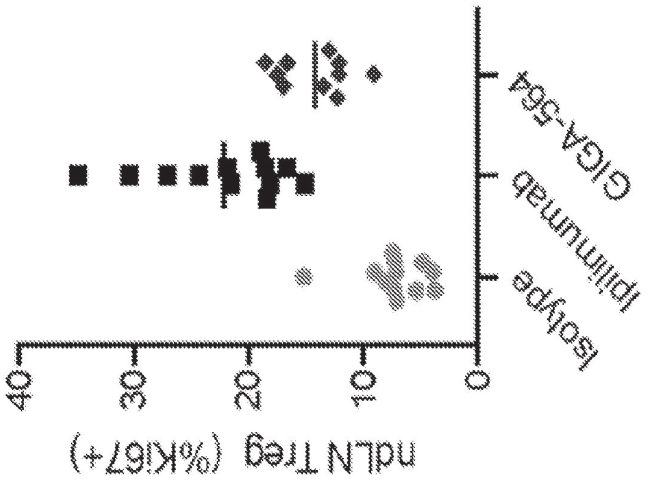


FIG. 31

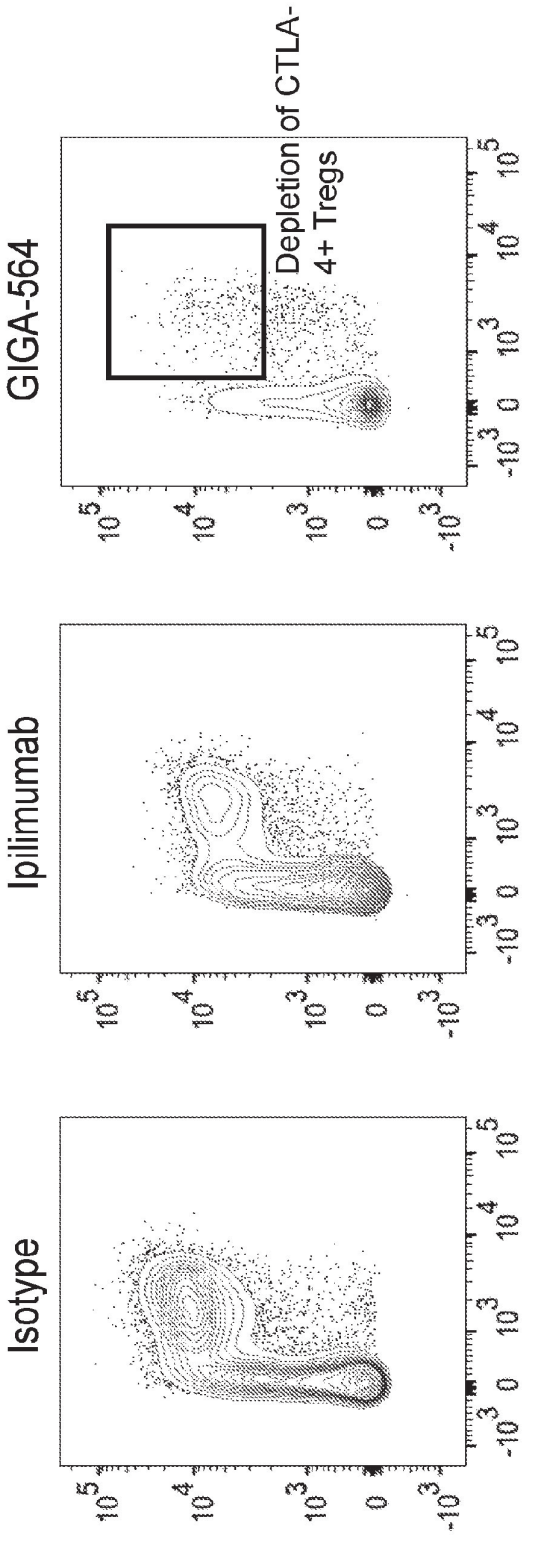


FIG. 32

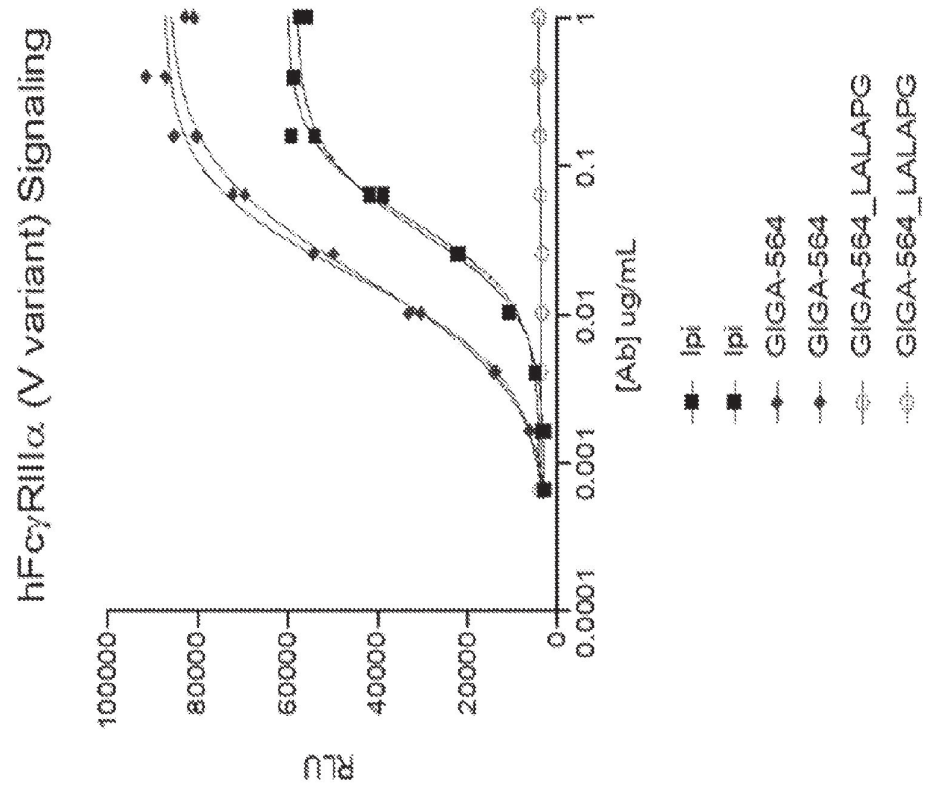
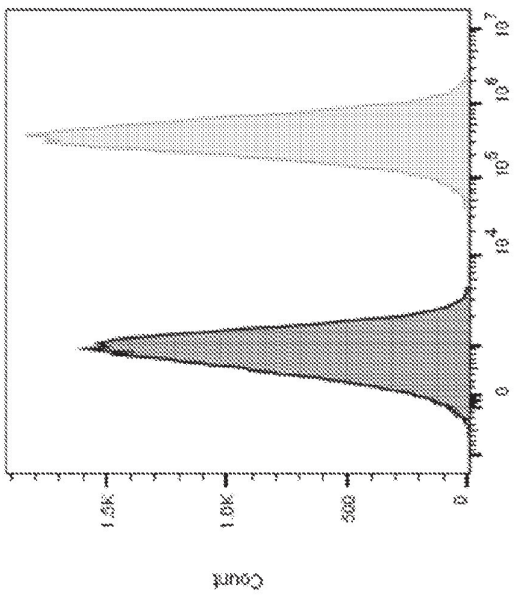
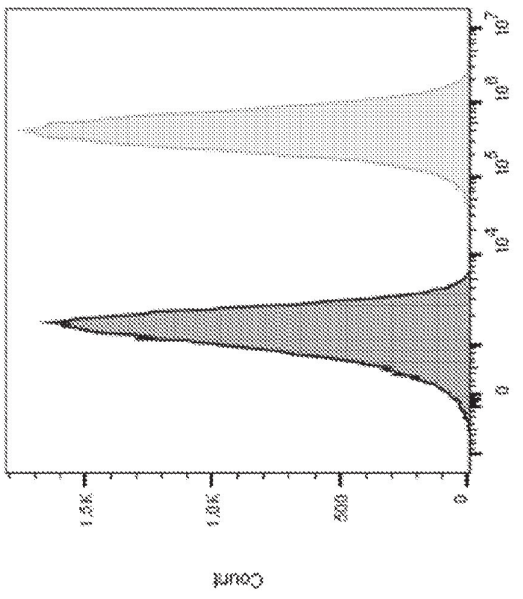

hFcγRIIIα (V variant) Signaling
RLU
100000
80000
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
[Ab] ug/mL
Ipi
Ipi
GIGA-564
GIGA-564
GIGA-564_LALAPG
GIGA-564_LALAPG

FIG. 33

FIG. 34

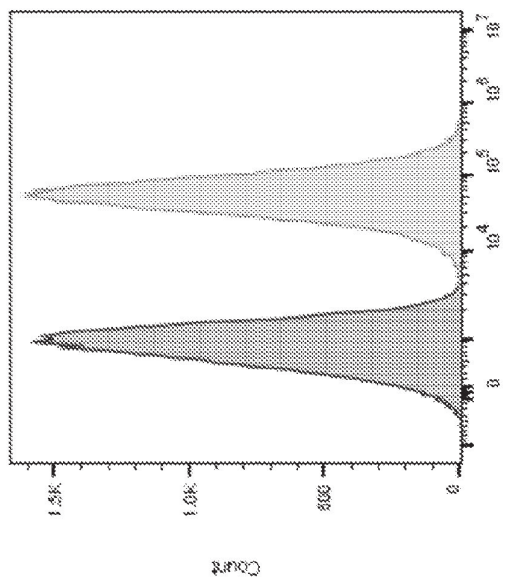


	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Week-B5.fcs	Live Cells	hCTL4-4	aCTLA-4 (L3D10)	47640
	01-Week-A5.fcs	Live Cells	Parental	aCTLA-4 (L3D10)	46550

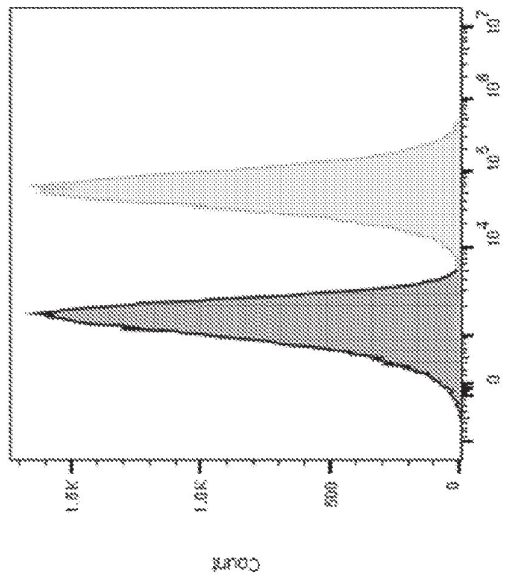


	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Week-B4.fcs	Live Cells	hCTL4-4	aCTLA-4 (BND)	47039
	01-Week-A4.fcs	Live Cells	Parental	aCTLA-4 (BND)	46560

FIG. 34 (CONTINUED)



	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Web-C5.fcs	Live Cells	cyno CTLA4	aCTLA4 (L3D10)	45597
	01-Web-A5.fcs	Live Cells	Parental	aCTLA4 (L3D10)	46940



	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Web-C4.fcs	Live Cells	cyno CTLA4	aCTLA4 (BNI3)	47098
	01-Web-A4.fcs	Live Cells	Parental	aCTLA4 (BNI3)	46890

FIG. 34 (CONTINUED)

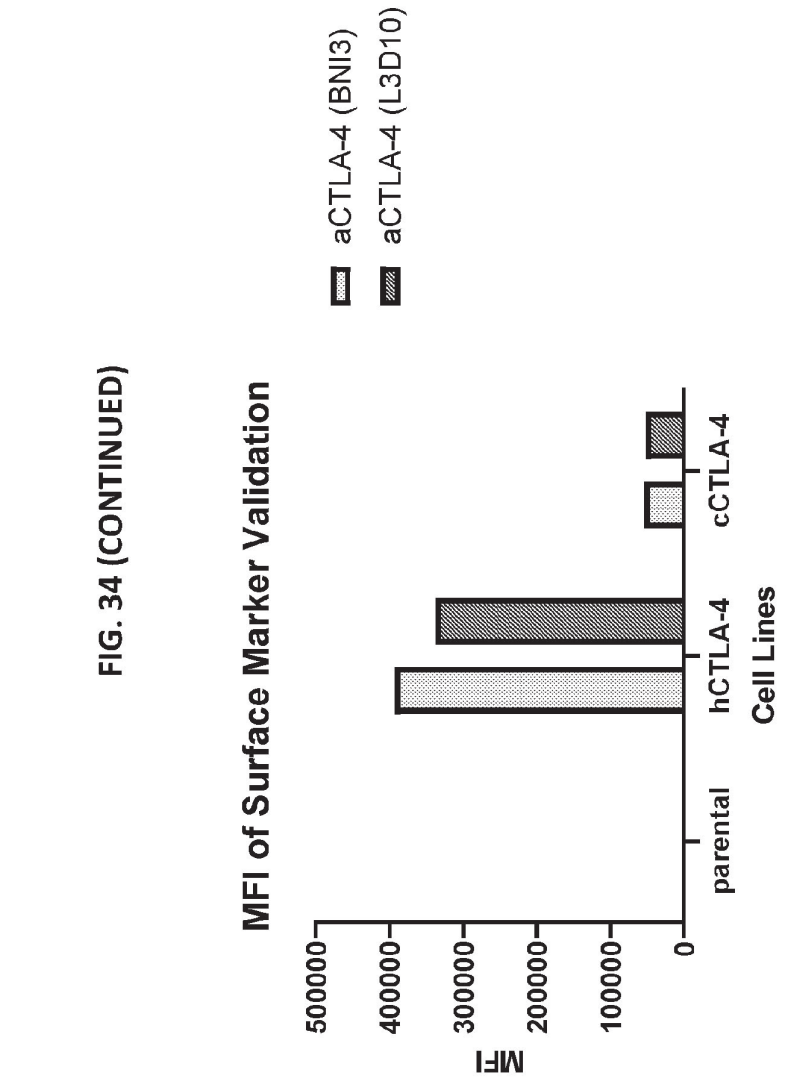
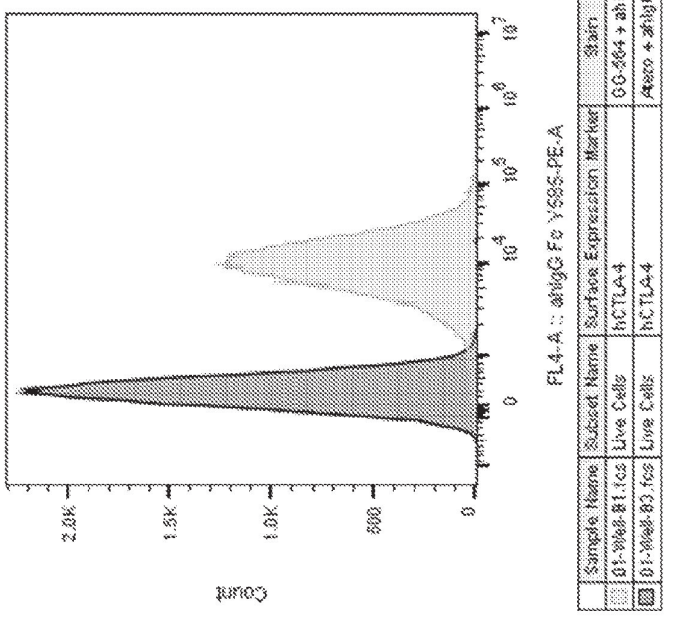
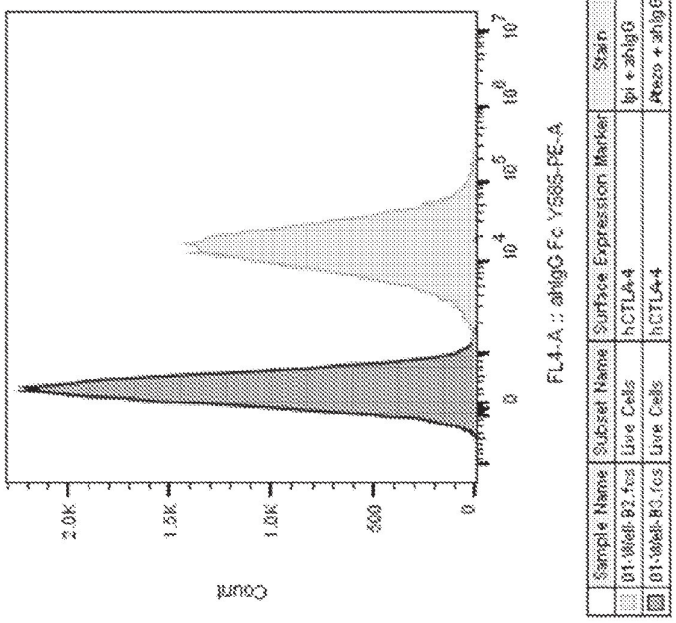


FIG. 35



Sample Name	Subset Name	Surface Expression Marker	Stain	Count
01-Web-B1.fcs	Live Cells	hCTLA4	GG-864 + ahIgG	46616
01-Web-B3.fcs	Live Cells	hCTLA4	Aeso + ahIgG	46841



Sample Name	Subset Name	Surface Expression Marker	Stain	Count
01-Web-B2.fcs	Live Cells	hCTLA4	Ipi + ahIgG	46639
01-Web-B3.fcs	Live Cells	hCTLA4	Aeso + ahIgG	46841

FIG. 35 (CONTINUED)

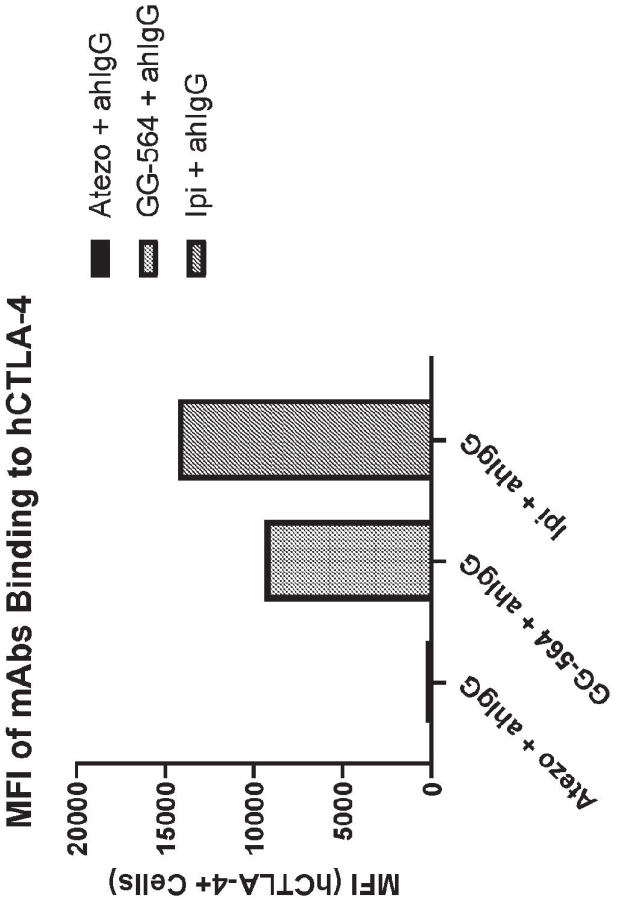
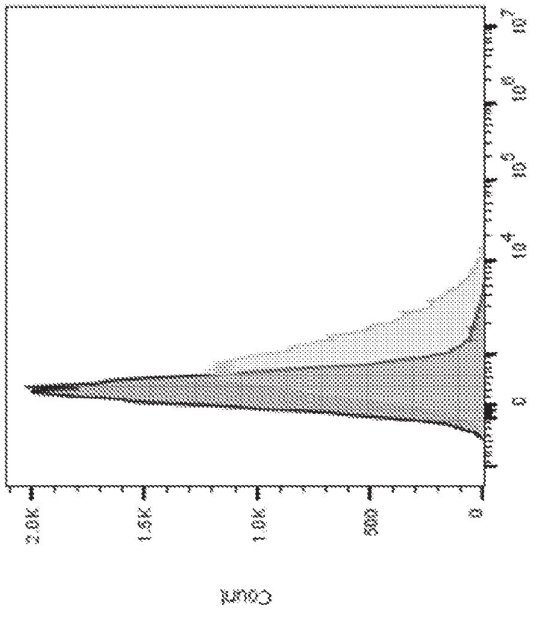
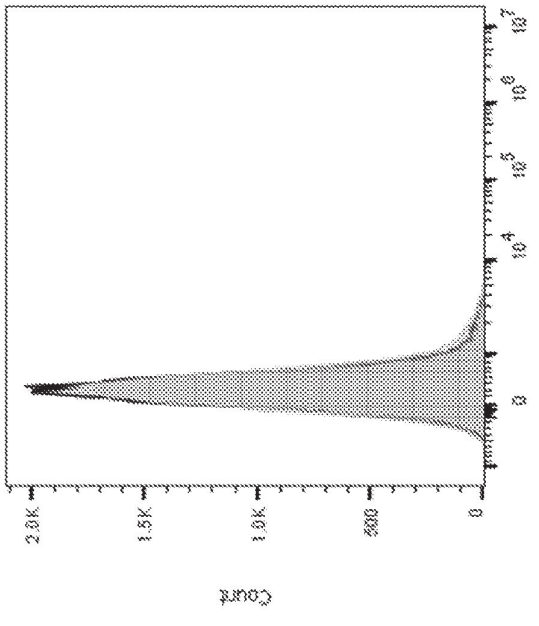


FIG. 35 (CONTINUED)



	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Week-C1.fcs	Live Cells	cyno CTLA4	GD-564 + ahIgG	46949
	01-Week-C3.fcs	Live Cells	cyno CTLA4	Ateza + ahIgG	45566



	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Week-C2.fcs	Live Cells	cyno CTLA4	Ipi + ahIgG	46894
	01-Week-C4.fcs	Live Cells	cyno CTLA4	Ateza + ahIgG	45565

FIG. 35 (CONTINUED)

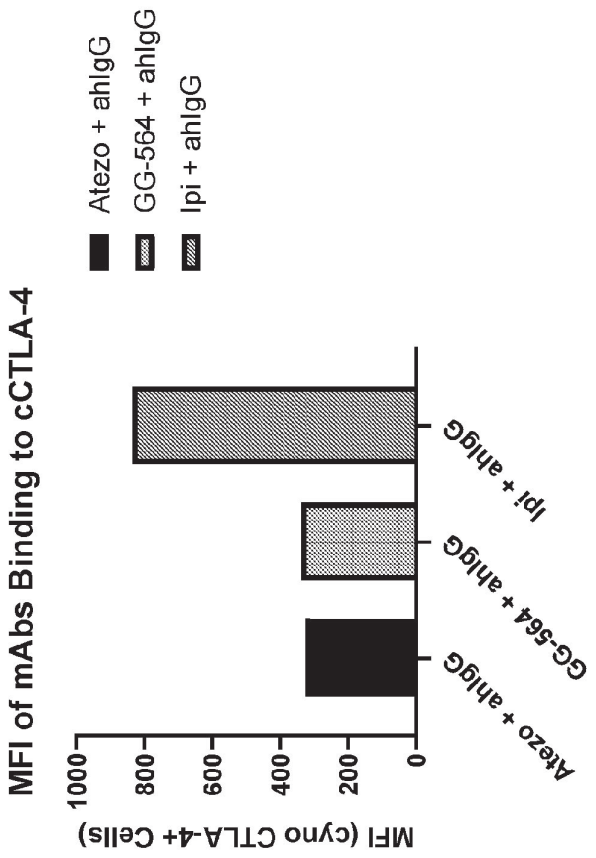


FIG. 36A

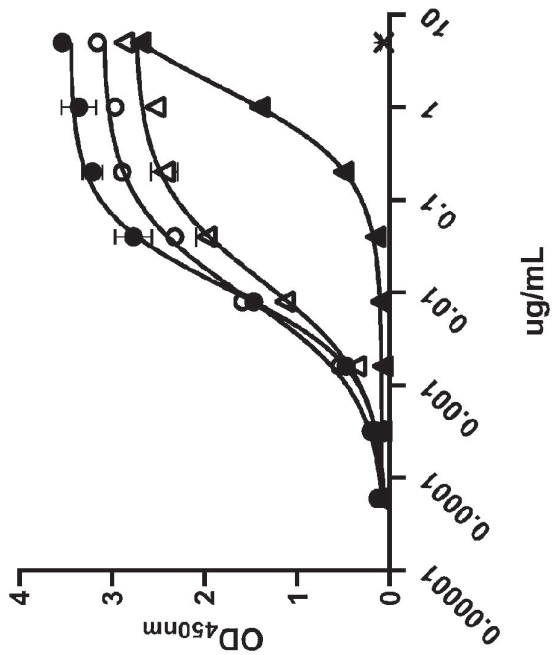

Ipilimumab - R&D rcmCTLA-4 Fc
GG-564 - R&D rcmCTLA-4 Fc
Atezolizumab - R&D rcmCTLA-4 Fc
Ipilimumab - R&D rhCTLA-4 Fc
GG-564 - R&D rhCTLA-4 Fc
Atezolizumab - R&D rhCTLA-4 Fc
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 36B

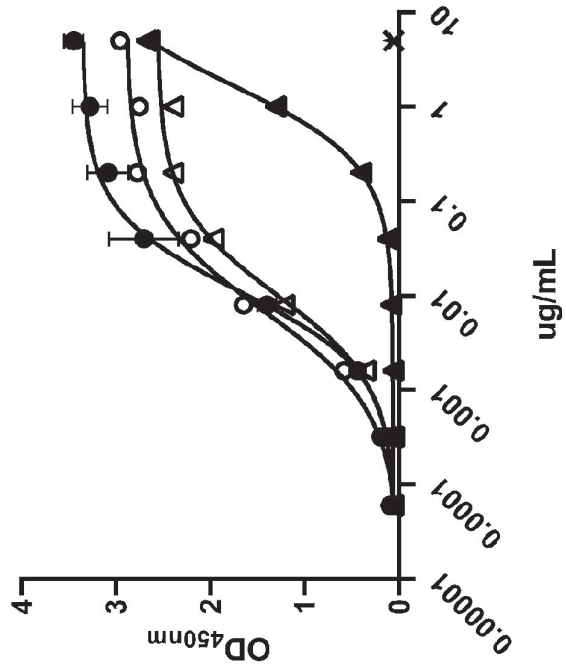

Ipilimumab - Sino rcmCTLA-4 Fc
GG-564 - Sino rcmCTLA-4 Fc
Atezolizumab - Sino rcmCTLA-4 Fc
Ipilimumab - R&D rhCTLA-4 Fc
GG-564 - R&D rhCTLA-4 Fc
Atezolizumab - R&D rhCTLA-4 Fc
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 36C

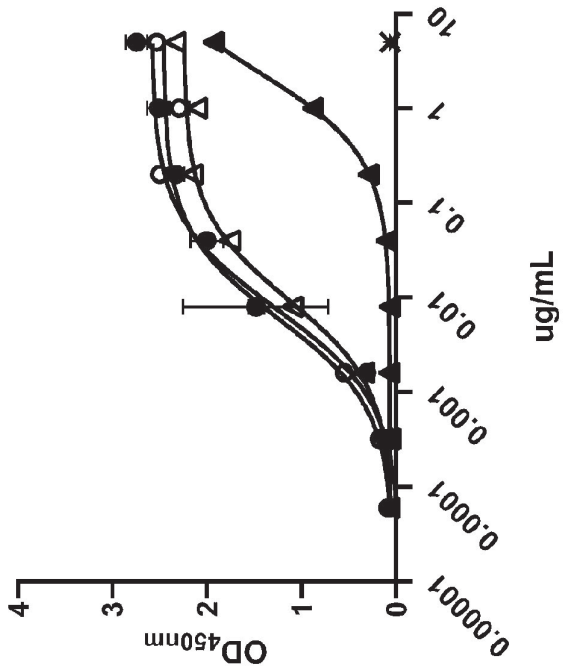

Ipilimumab - Acro rcmCTLA-4 Fc
GG-564 - Acro rcmCTLA-4 Fc
Atezolizumab - Acro rcmCTLA-4 Fc
Ipilimumab - R&D rhCTLA-4 Fc
GG-564 - R&D rhCTLA-4 Fc
Atezolizumab - R&D rhCTLA-4 Fc
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 36D

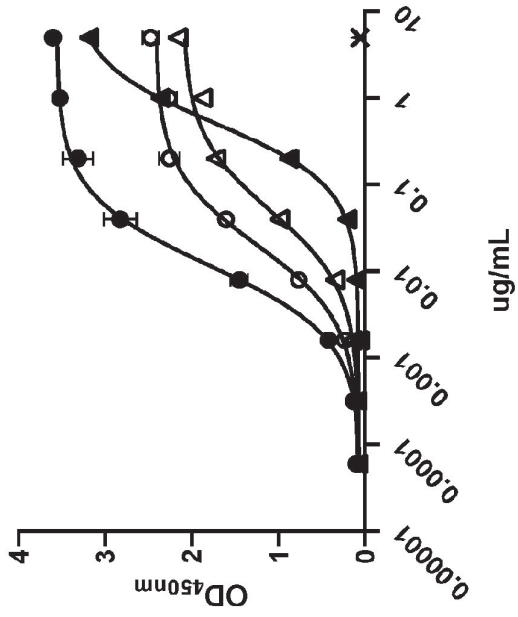

Ipilimumab - Sino rcmCTLA-4 His
GG-564 - Sino rcmCTLA-4 His
Atezolizumab - Sino rcmCTLA-4 His
Ipilimumab - Acro rhCTLA-4 His
GG-564 - Acro rhCTLA-4 His
Atezolizumab - Acro rhCTLA-4 His
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 36E

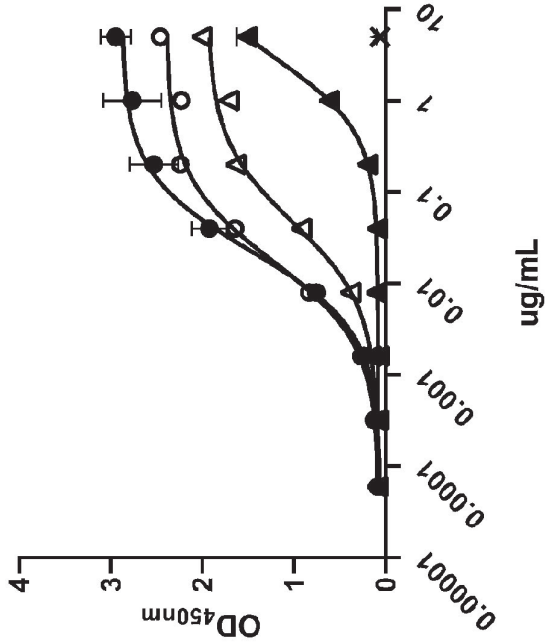

Ipilimumab - Acro rcmCTLA-4 His
GG-564 - Acro rcmCTLA-4 His
Atezolizumab - Acro rcmCTLA-4 His
Ipilimumab - Acro rhCTLA-4 His
GG-564 - Acro rhCTLA-4 His
Atezolizumab - Acro rhCTLA-4 His
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 37

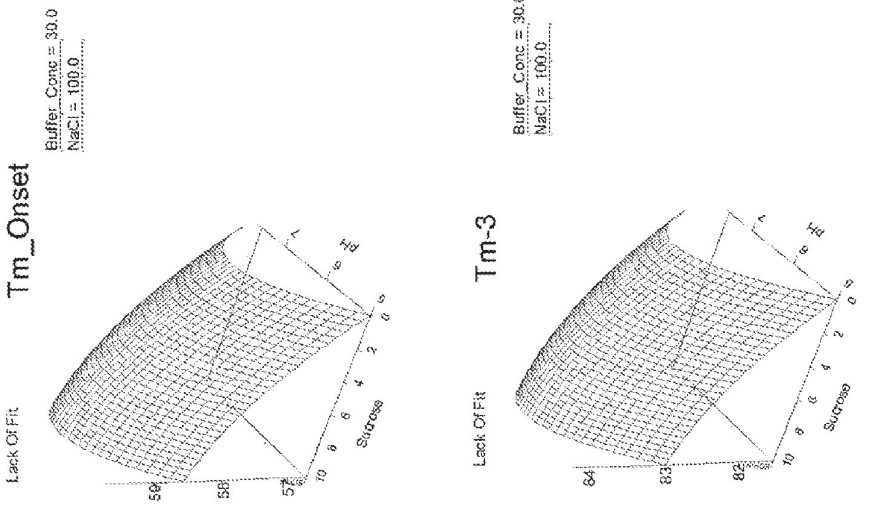
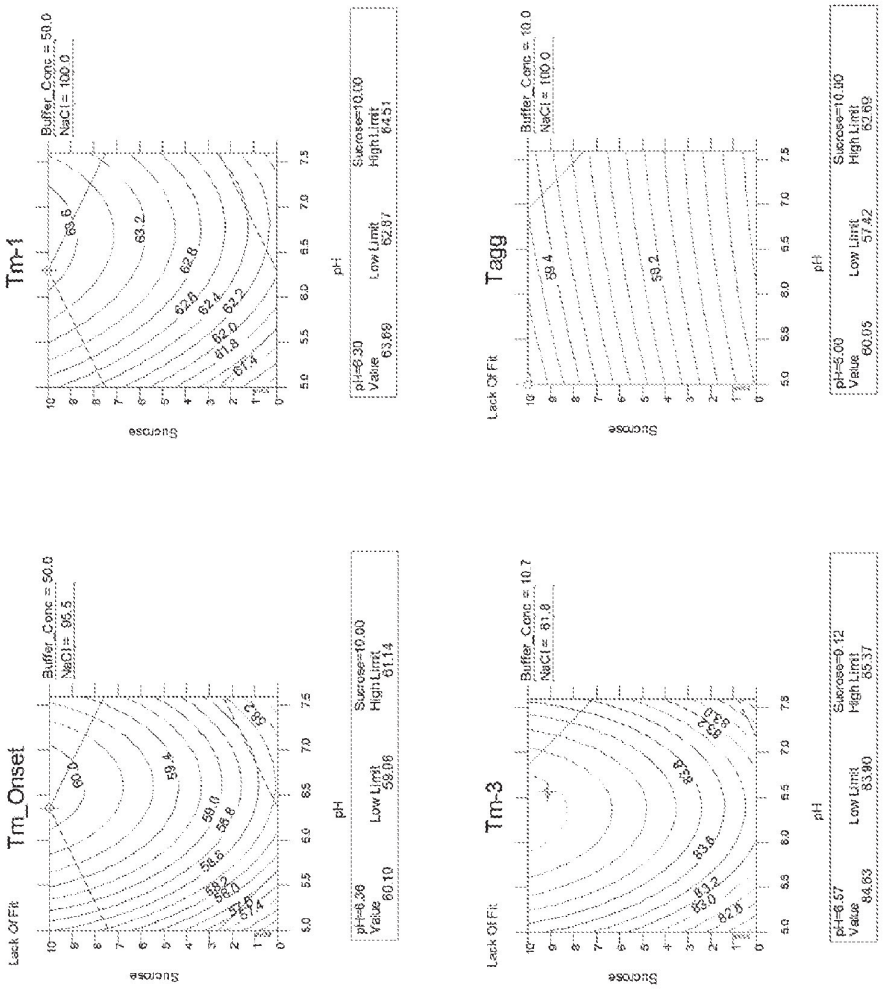

Tm_Onset
Buffer_Conc = 30.0
NaCl = 100.0
Lack Of Fit
Sucrose
pH
Tm-1
Buffer_Conc = 30.0
NaCl = 100.0
Sucrose
pH
Tagg
Buffer_Conc = 30.0
NaCl = 100.0
Lack Of Fit
Sucrose
pH
Tm-3
Buffer_Conc = 30.0
NaCl = 100.0
Lack Of Fit
Sucrose
pH

FIG. 38


Tm_Onset
Lack Of Fit
Buffer_Conc = 50.0
NaCl = 55.5
Sucrose
pH
pH=6.38
Value 60.10
Low Limit 58.06
Sucrose=10.00
High Limit 61.14
Tm-1
Buffer_Conc = 50.0
NaCl = 100.0
pH=6.33
Value 63.69
Low Limit 62.97
Sucrose=10.00
High Limit 64.51
Tm-3
Lack Of Fit
Buffer_Conc = 10.7
NaCl = 61.8
pH=6.57
Value 84.63
Low Limit 83.90
Sucrose=9.12
High Limit 85.37
Tagg
Lack Of Fit
Buffer_Conc = 10.0
NaCl = 110.0
pH=6.00
Value 60.05
Low Limit 57.42
Sucrose=10.00
High Limit 62.69

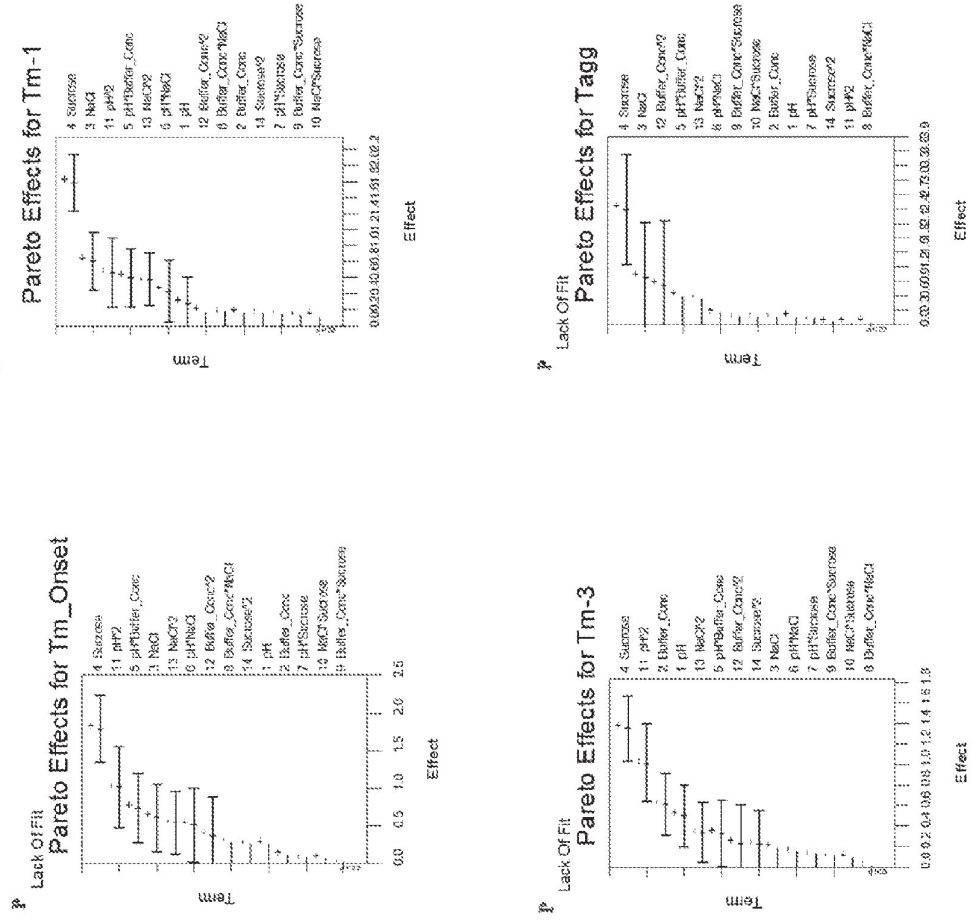
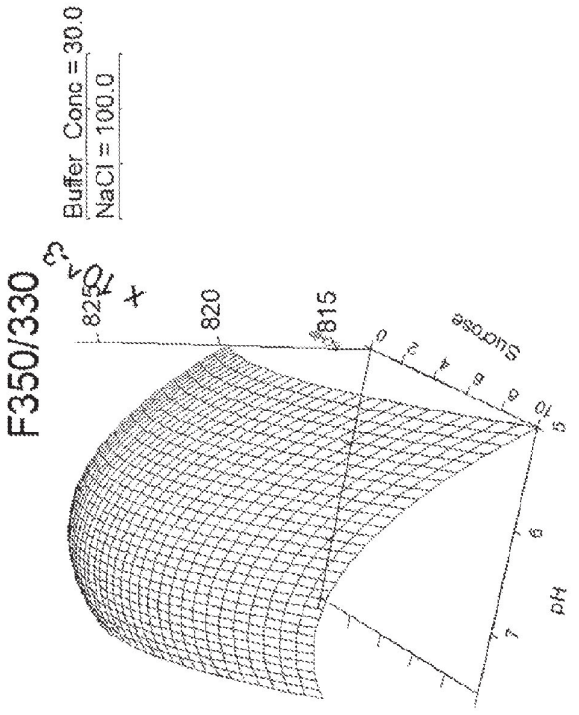

Lack Of Fit
Pareto Effects for Tm_Onset
Term
Effect
Pareto Effects for Tm-1
Pareto Effects for Tagg
Pareto Effects for Tm-3

FIG. 39


F350/330
Buffer Conc = 30.0
NaCl = 100.0
x 10^3
825
820
815
Sucrose
pH

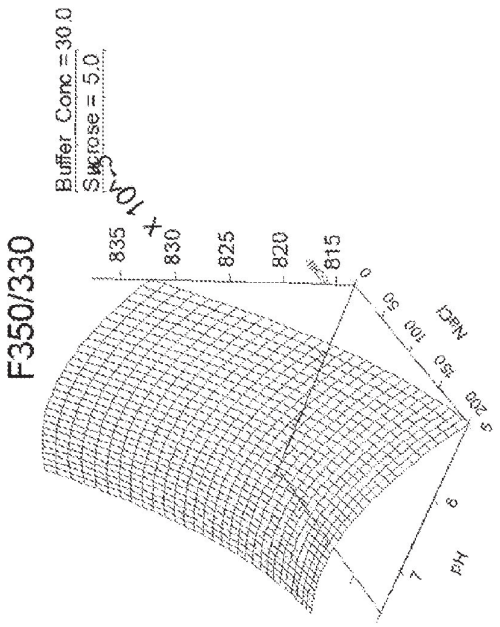

F350/330
Buffer Conc = 30.0
Sucrose = 5.0
x 10^3
835
830
825
820
815
NaCl
pH

FIG. 40

FIG. 40 (CONTINUED)

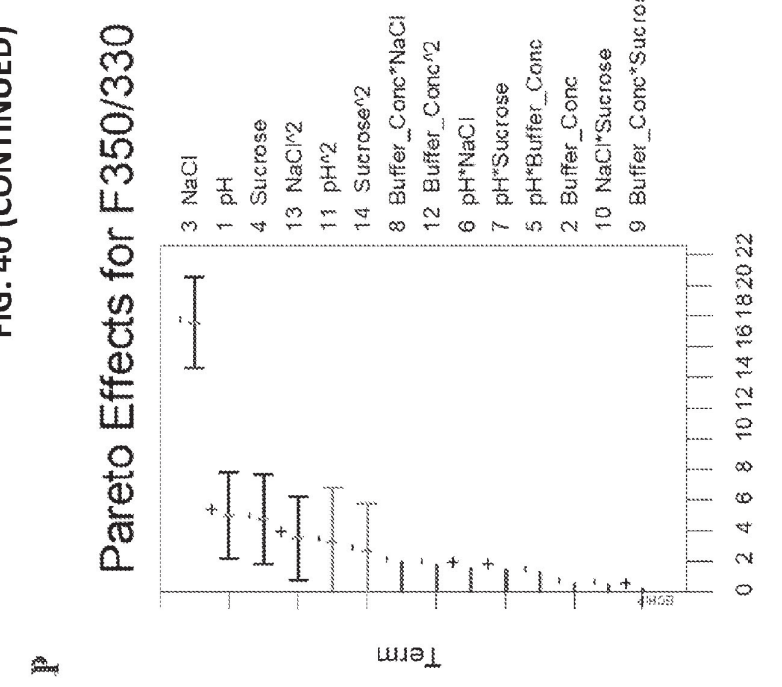


FIG. 41

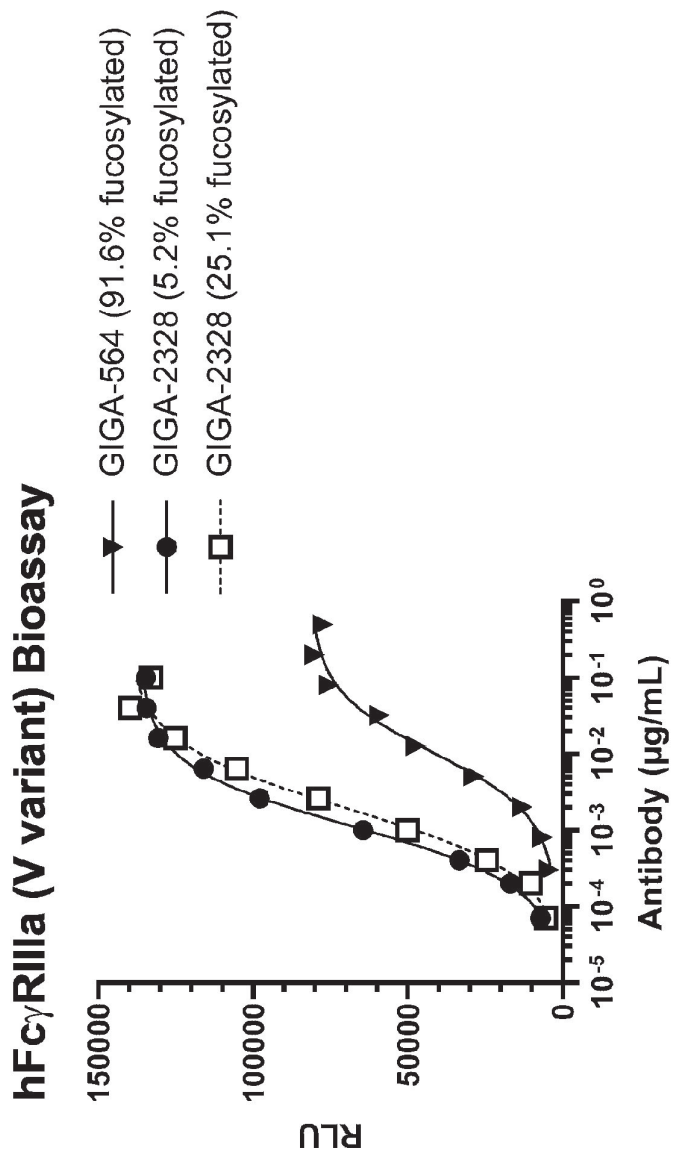

hFcγRIIIa (V variant) Bioassay
GIGA-564 (91.6% fucosylated)
GIGA-2328 (5.2% fucosylated)
GIGA-2328 (25.1% fucosylated)
RLU
150000
100000
50000
0
10-5 10-4 10-3 10-2 10-1 100
Antibody (µg/mL)

FIG. 42A

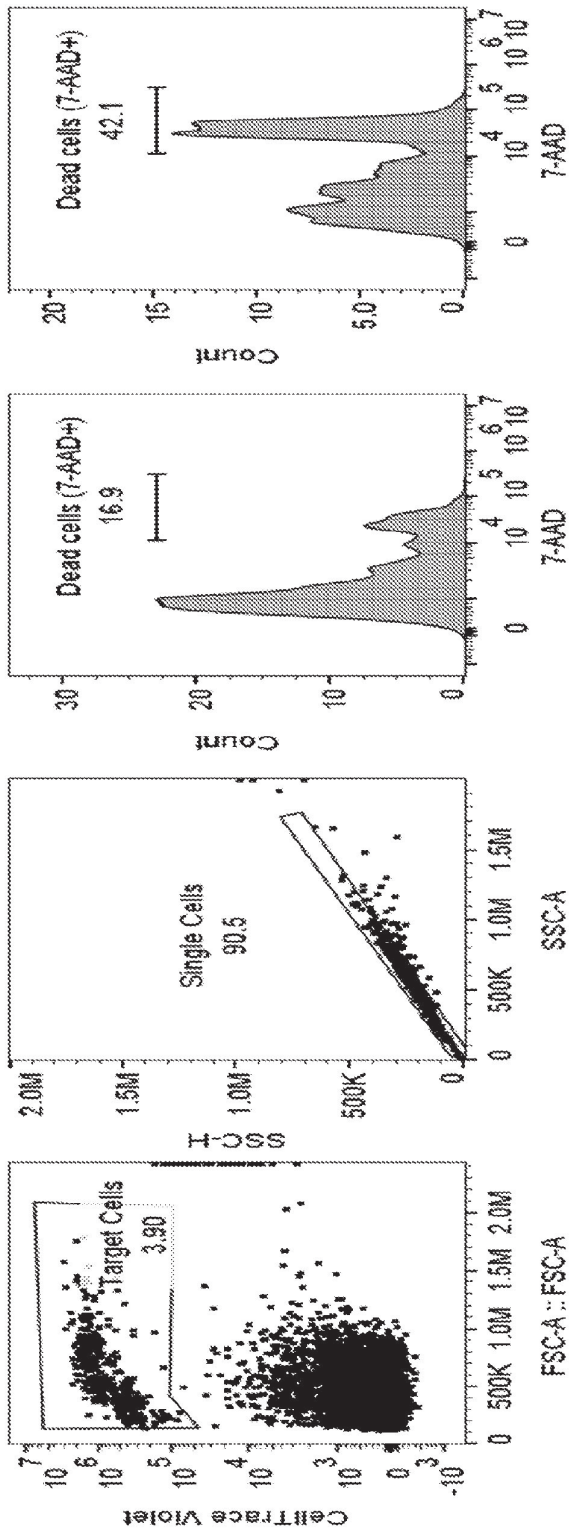

CellTrace Violet
FSC-A :: FSC-A
Target Cells
3.90
SSC-H
SSC-A
Single Cells
90.5
Count
Dead cells (7-AAD+)
16.9
7-AAD
Count
Dead cells (7-AAD+)
42.1
7-AAD

FIG. 42B

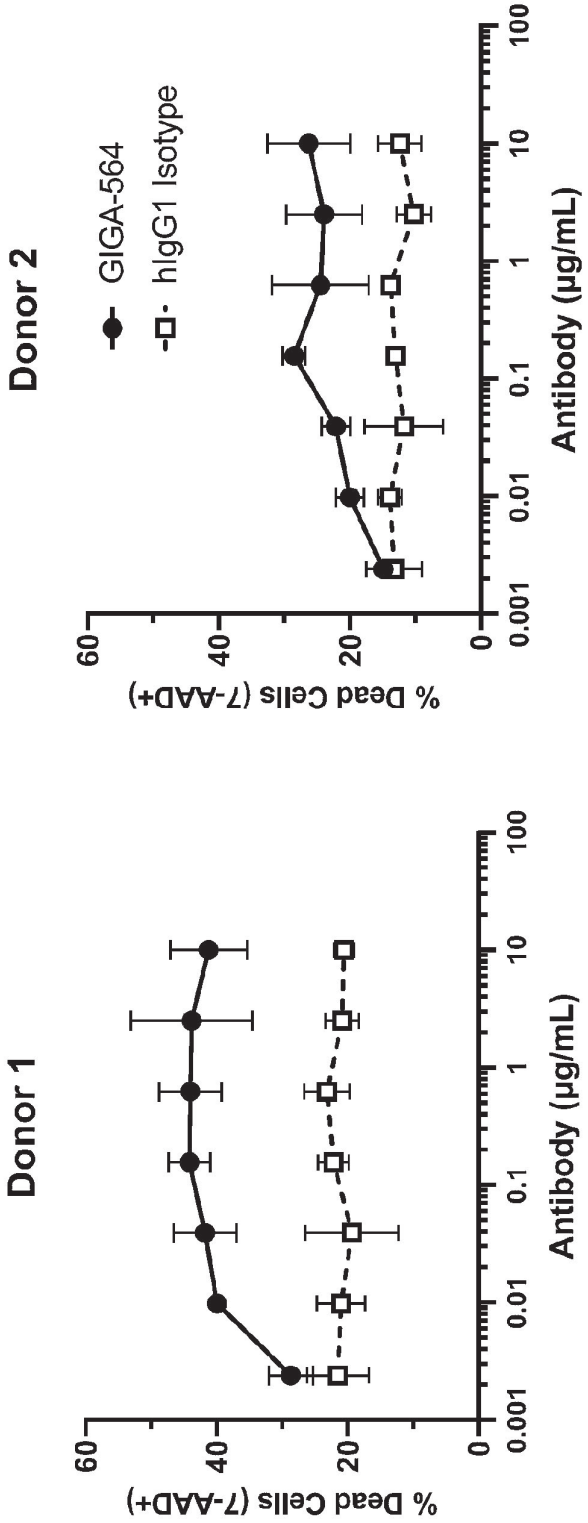

Donor 1
Donor 2
GIGA-564
hIgG1 Isotype
% Dead Cells (7-AAD+)
Antibody (µg/mL)
0
20
40
60
0.001
0.01
0.1
1
10
100

FIG. 43A

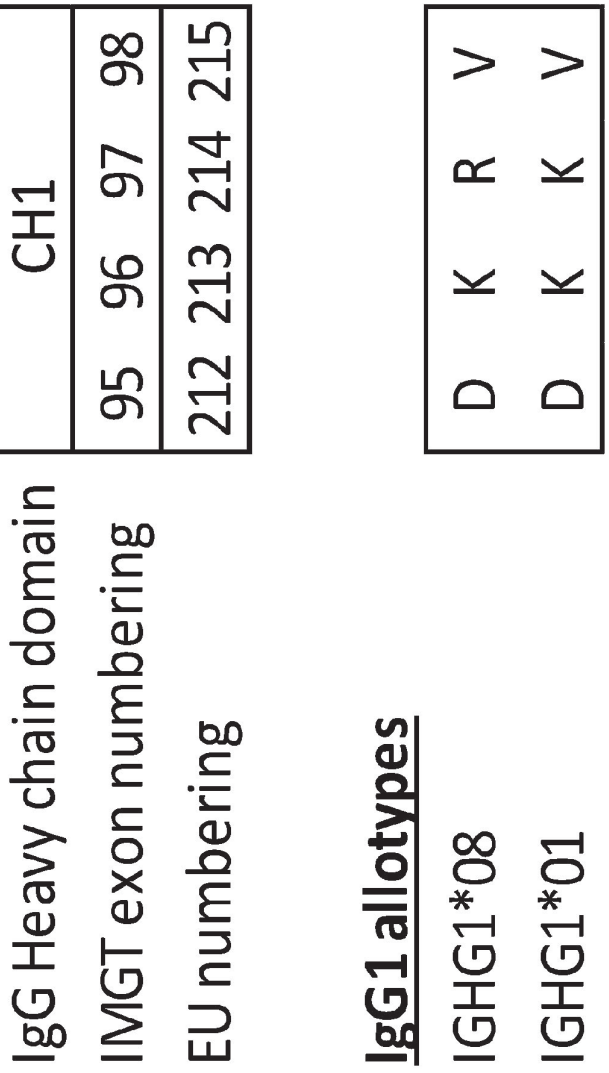
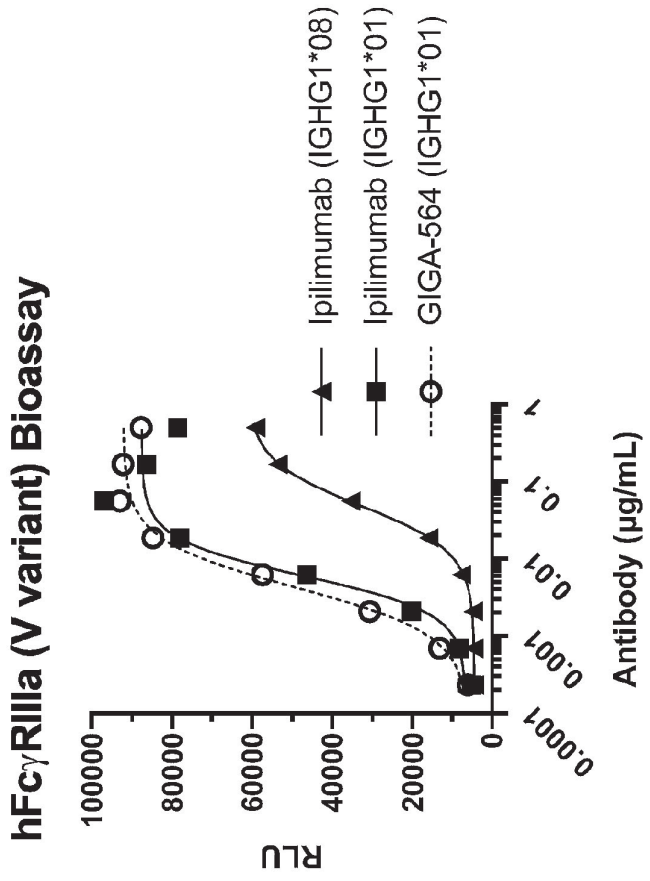

IgG Heavy chain domain
IMGT exon numbering
EU numbering
CH1
95 96 97 98
212 213 214 215
IgG1 allotypes
IGHG1*08
D K R V
IGHG1*01
D K K V

FIG. 43B


hFcγRIIIa (V variant) Bioassay
RLU
100000
80000
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
Antibody (µg/mL)
Ipilimumab (IGHG1*08)
Ipilimumab (IGHG1*01)
GIGA-564 (IGHG1*01)

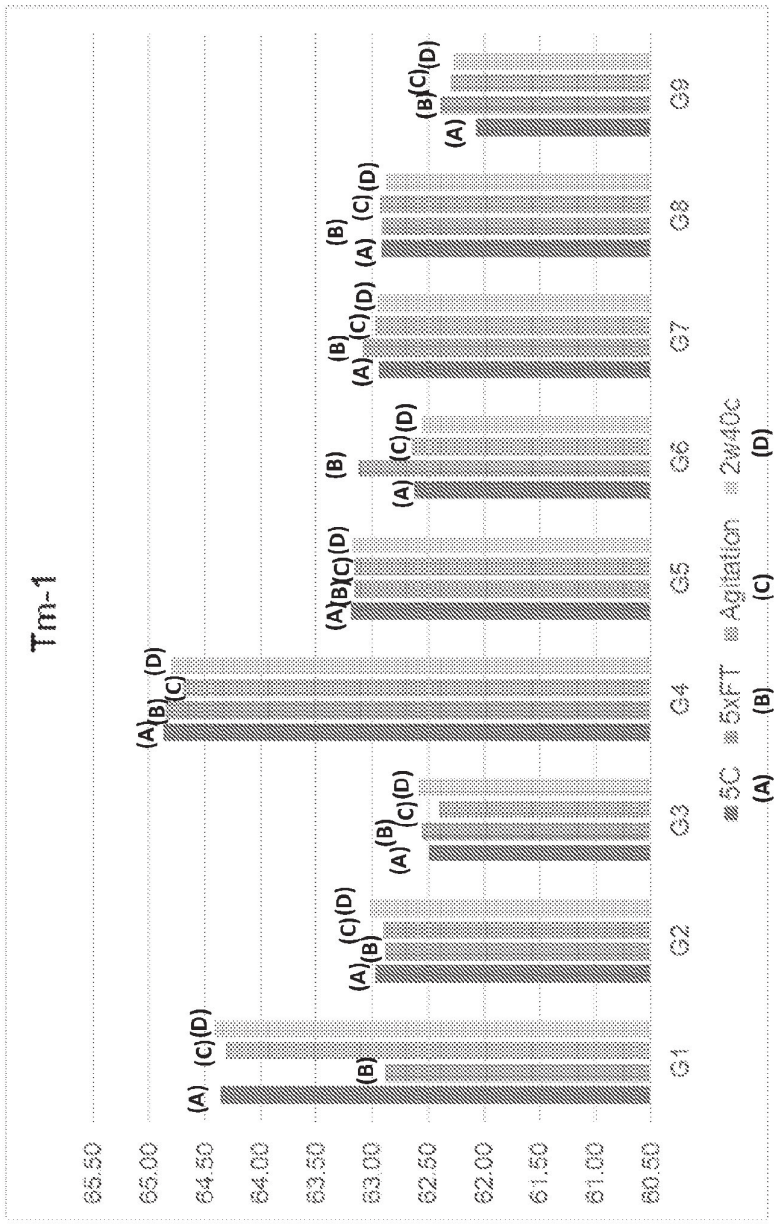

Tm-1
65.50
65.00
64.50
64.00
63.50
63.00
62.50
62.00
61.50
61.00
60.50
G1
G2
G3
G4
G5
G6
G7
G8
G9
5C
5xFT
Agitation
2w40c
(A)
(B)
(C)
(D)

FIG. 44A

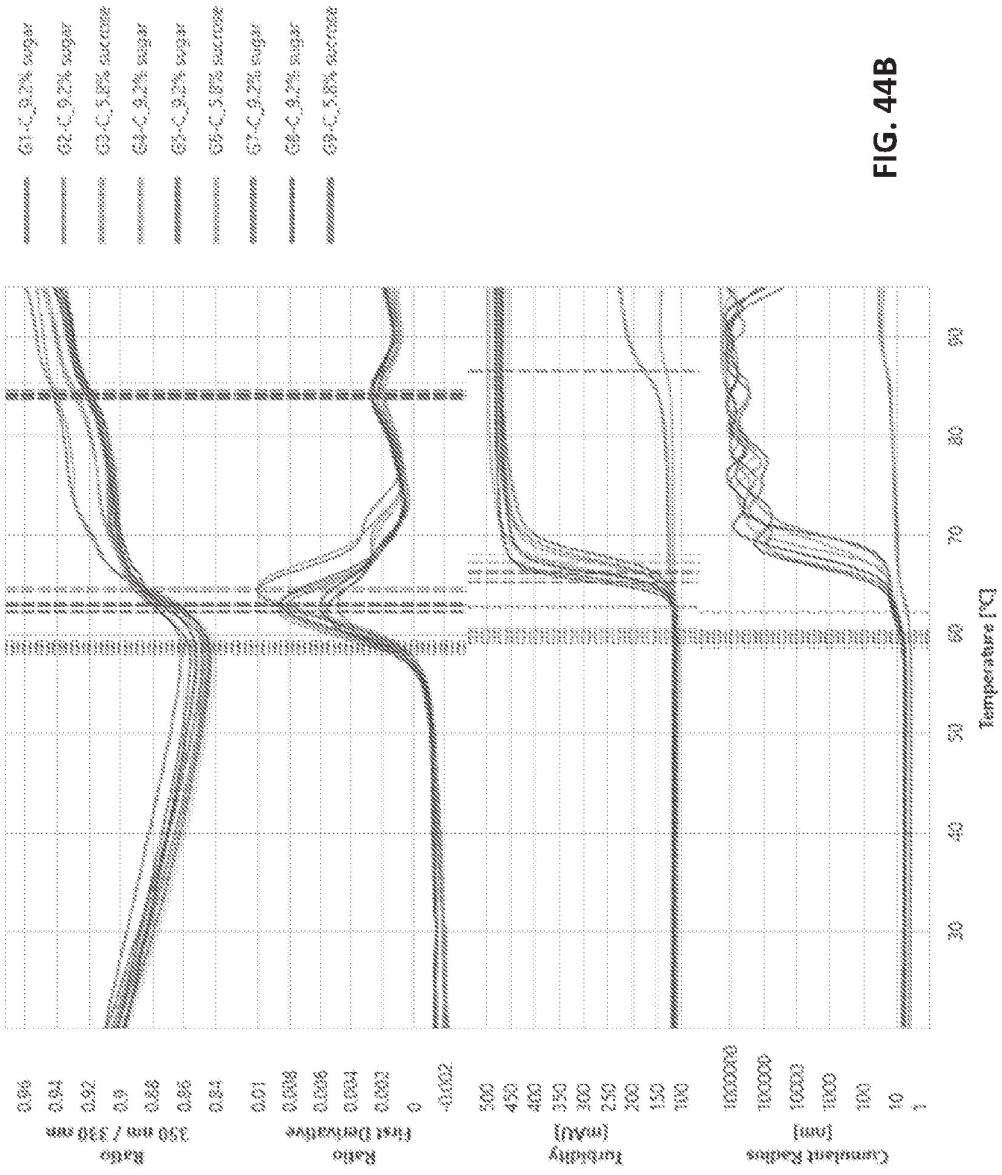

01-C_9.2% sugar
02-C_9.2% sugar
03-C_5.8% sucrose
04-C_9.2% sugar
05-C_9.2% sugar
06-C_5.8% sucrose
07-C_9.2% sugar
08-C_9.2% sugar
09-C_5.8% sucrose
Ratio 350 nm / 330 nm
Ratio first Derivative
Turbidity [mAU]
Unmolar Radius [nm]
Temperature [°C]

FIG. 44B

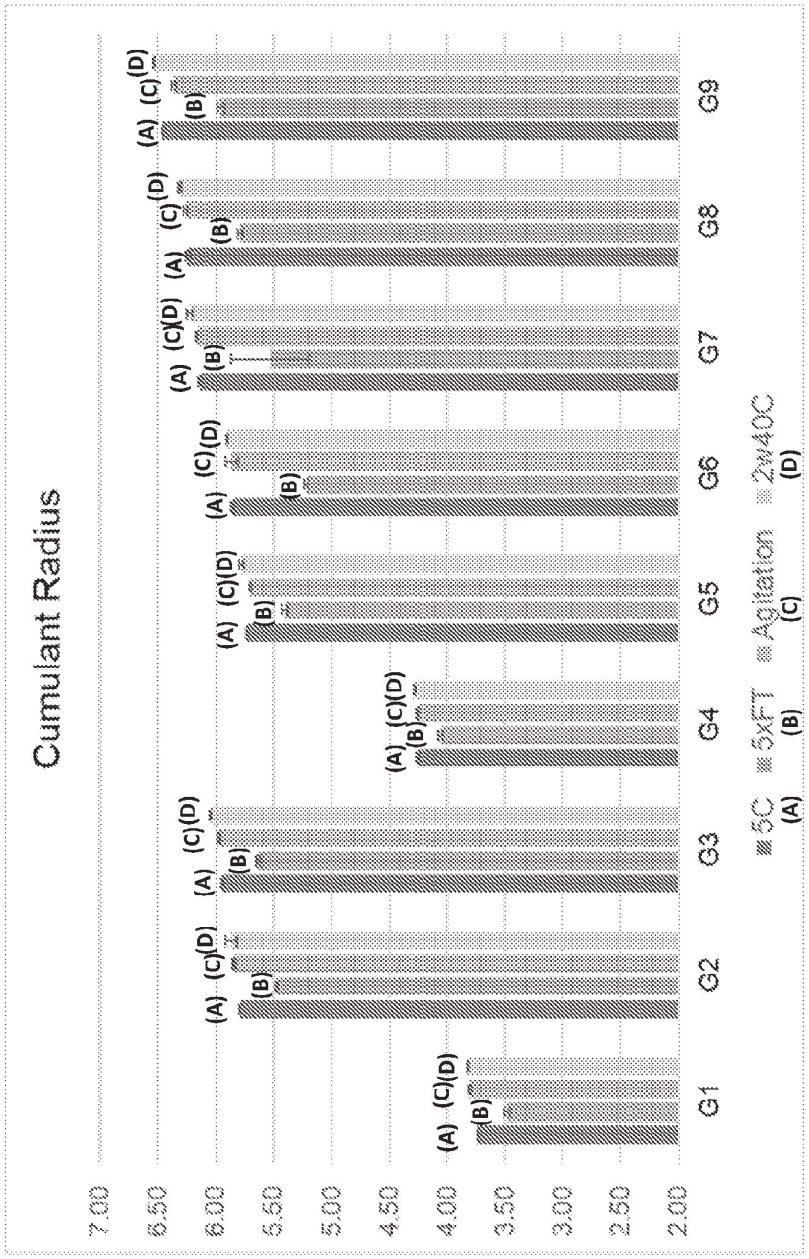

Cumulant Radius
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
G1
G2
G3
G4
G5
G6
G7
G8
G9
5C (A)
5xFT (B)
Agitation (C)
2w40C (D)

FIG. 45

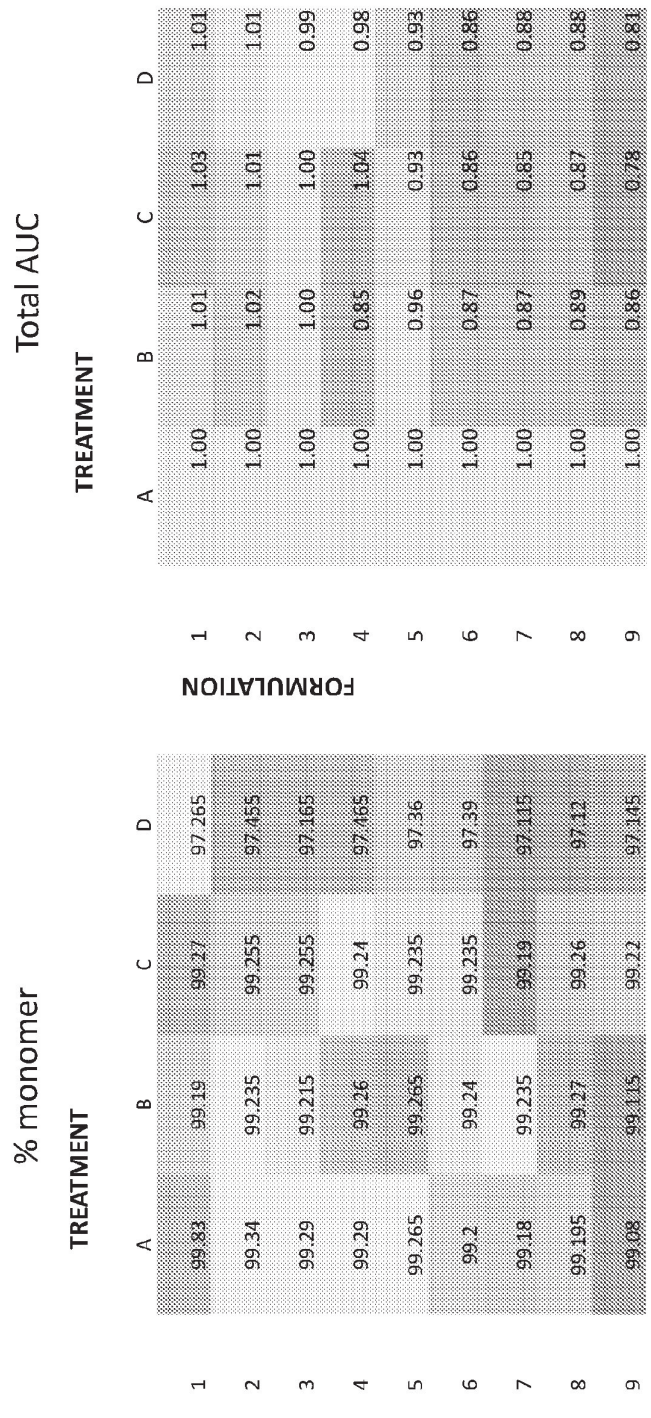


FIG. 46A

FIG. 46B

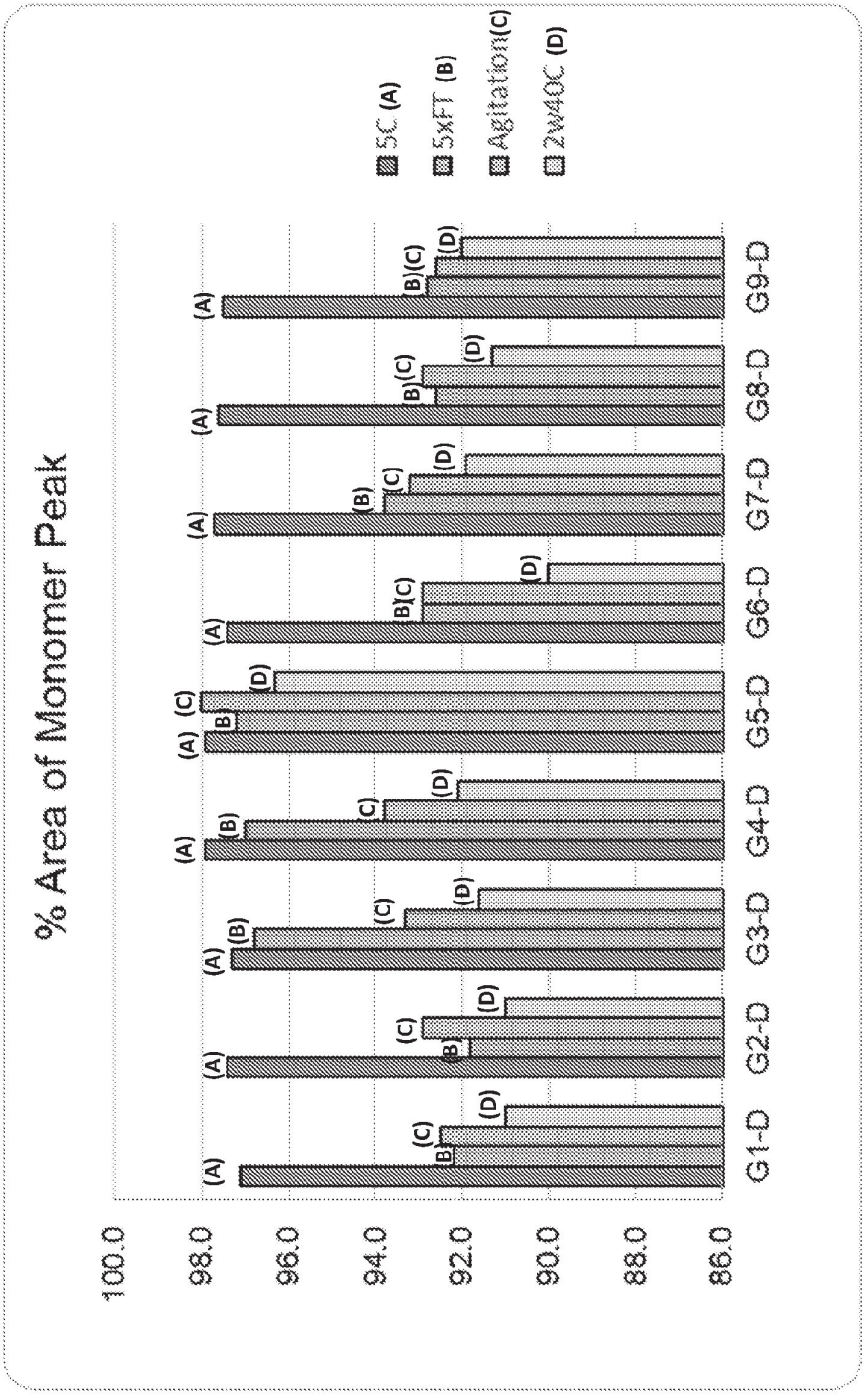

% Area of Monomer Peak
100.0
98.0
96.0
94.0
92.0
90.0
88.0
86.0
G1-D
G2-D
G3-D
G4-D
G5-D
G6-D
G7-D
G8-D
G9-D
5C (A)
5xFT (B)
Agitation(C)
2w40C (D)

FIG. 47A

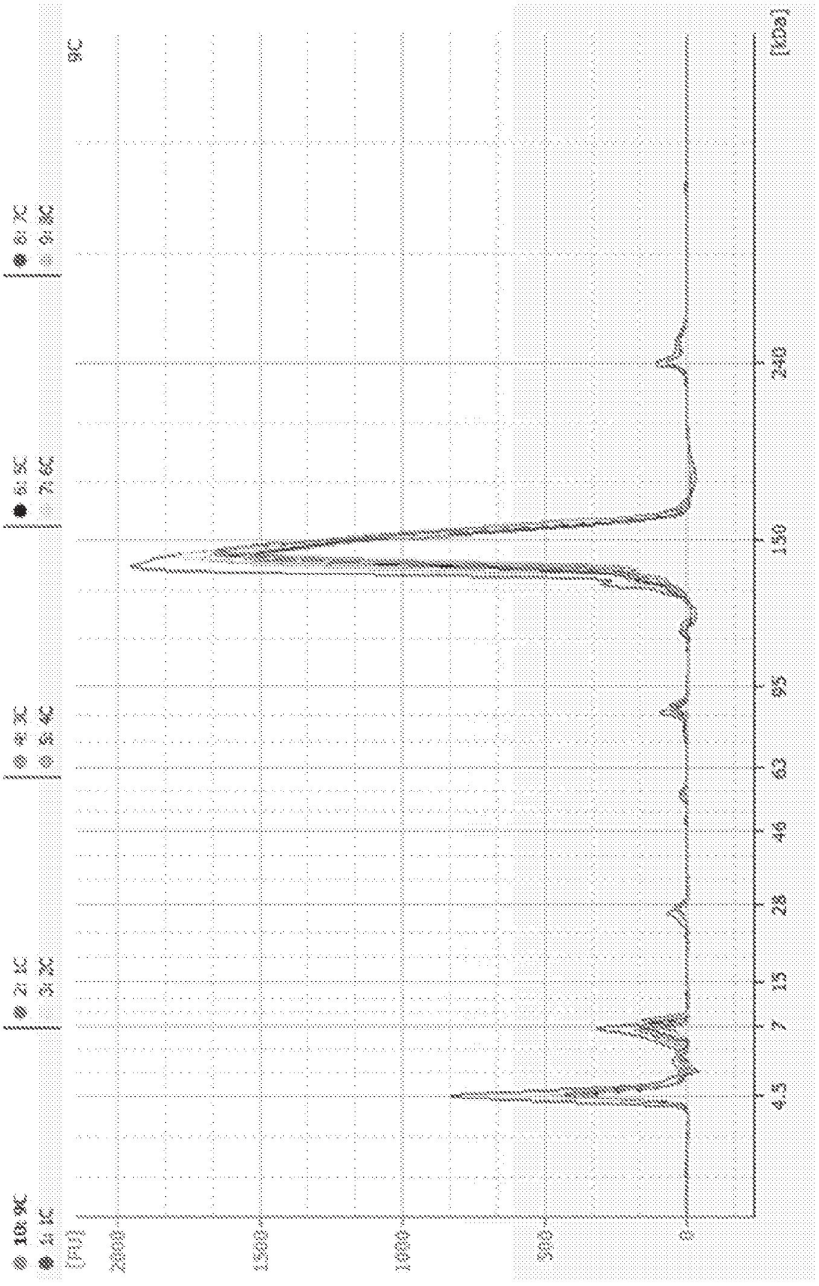
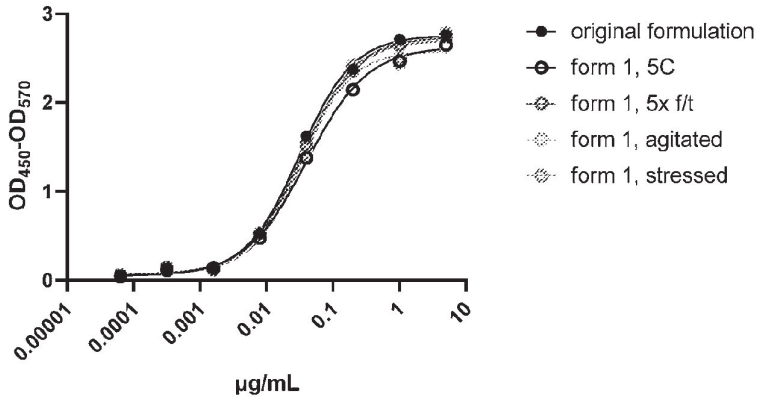

10: 9C
4: 1C
2: 2C
3: 2C
4: 3C
5: 4C
6: 5C
7: 6C
8: 7C
9: 8C
[FU]
2000
1500
1000
500
0
4.5
7
15
28
46
63
95
130
240
[kDa]

FIG. 47B

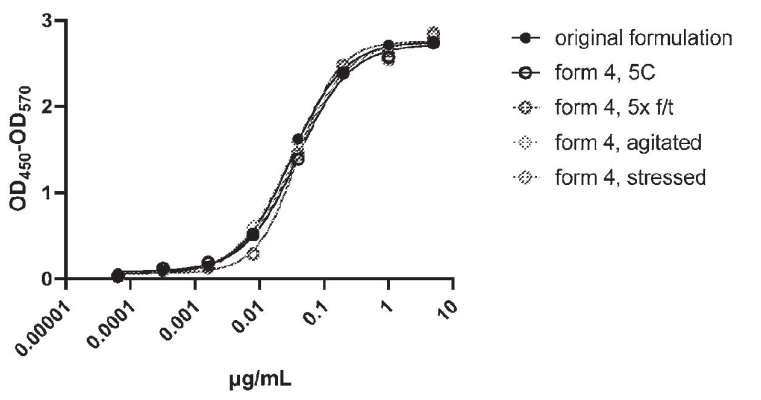
formulation 1
6.5 pH, 20 mM histidine, 270 mM sucrose, 0.02% PS-20



	form 1, 5C	form 1, 5x f/t	form 1, agitated	form 1, stressed
EC50	0.04022	0.03438	0.03219	0.03676

FIG. 48A

formulation 4
6.0 pH, 20 mM histidine, 270 mM sucrose, 0.02% PS-20



	form 4, 5C	form 4, 5x f/t	form 4, agitated	form 4, stressed
EC50	0.03876	0.03962	0.02998	0.03603

FIG. 48B

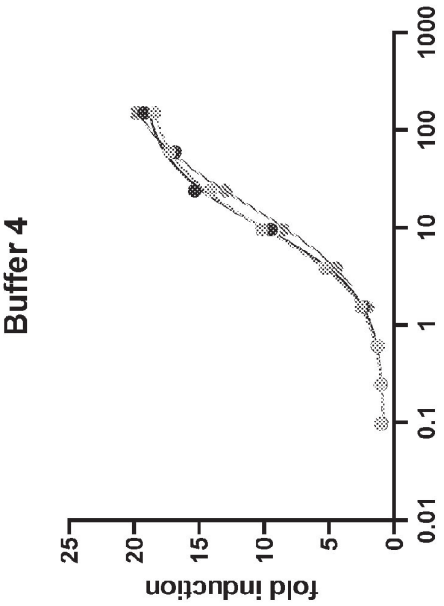


FIG. 49B

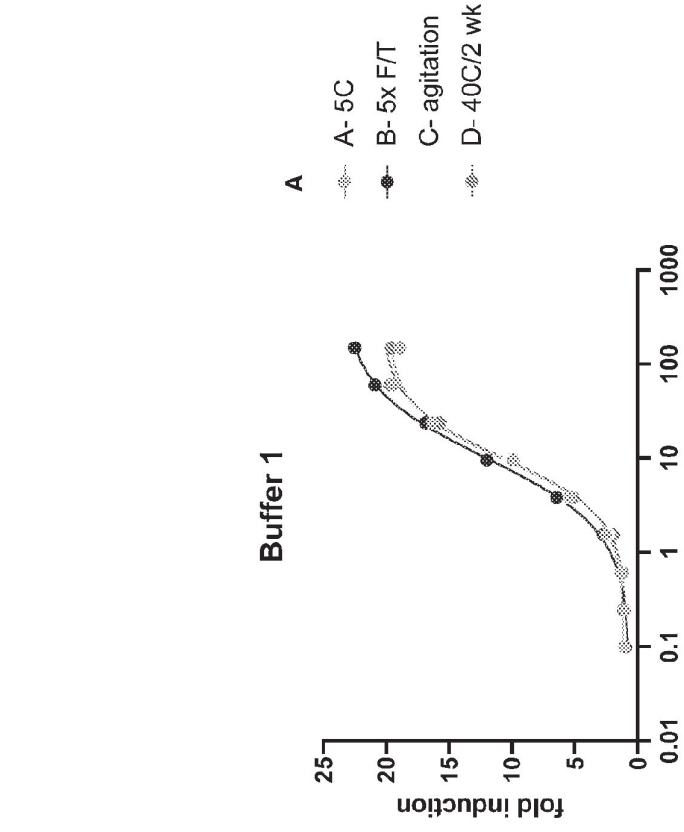


FIG. 49A

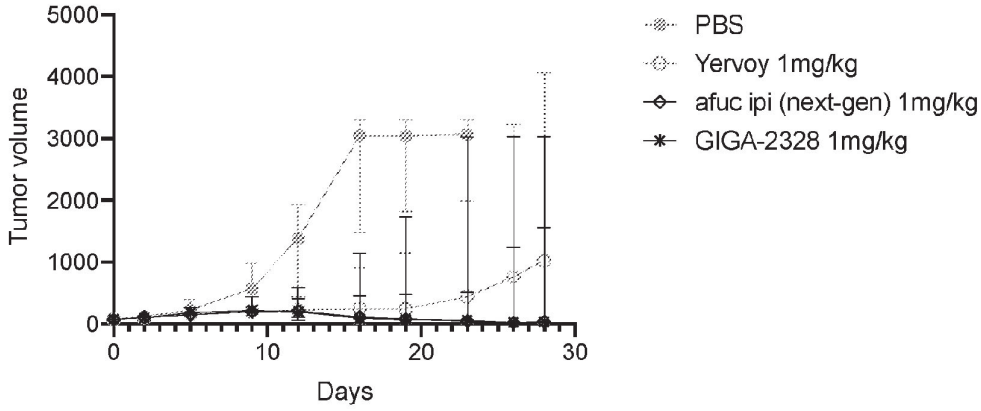


FIG. 50A

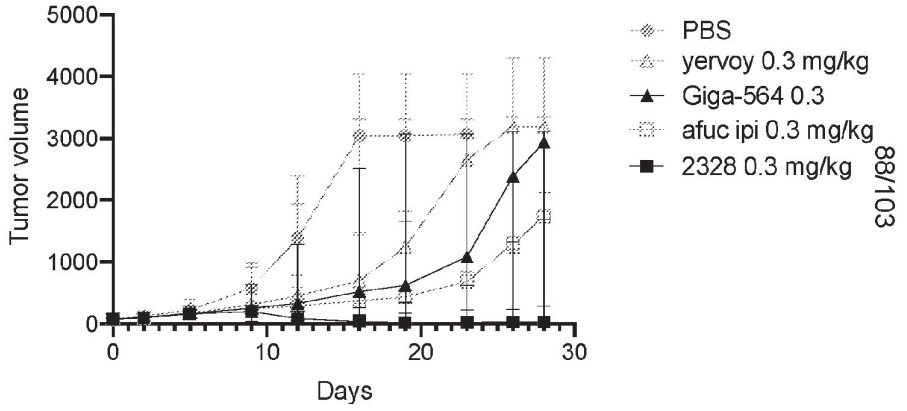


FIG. 50B

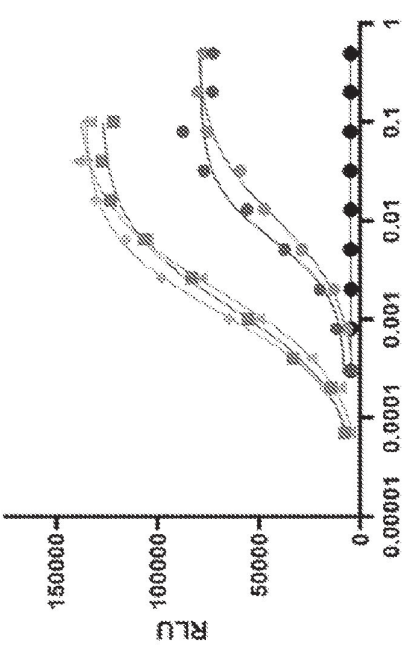


FIG. 51A

FIG. 51B

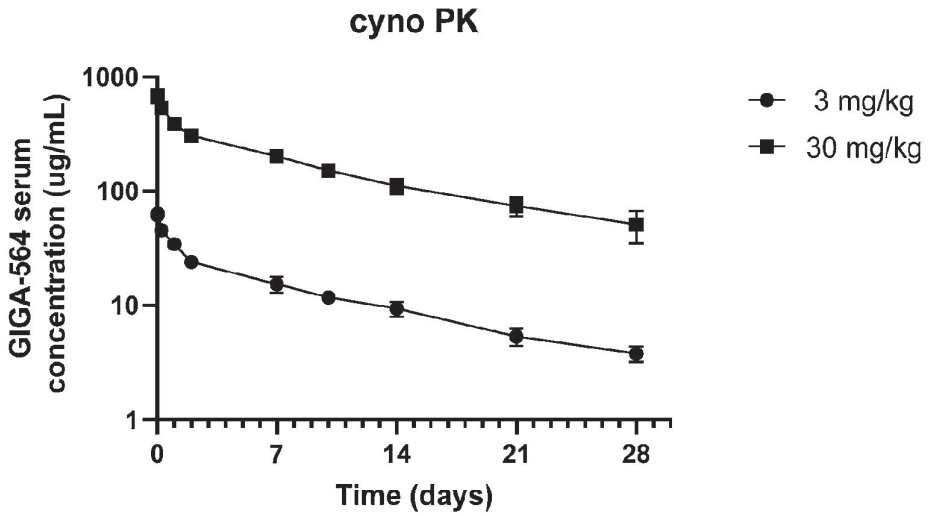


FIG. 52A

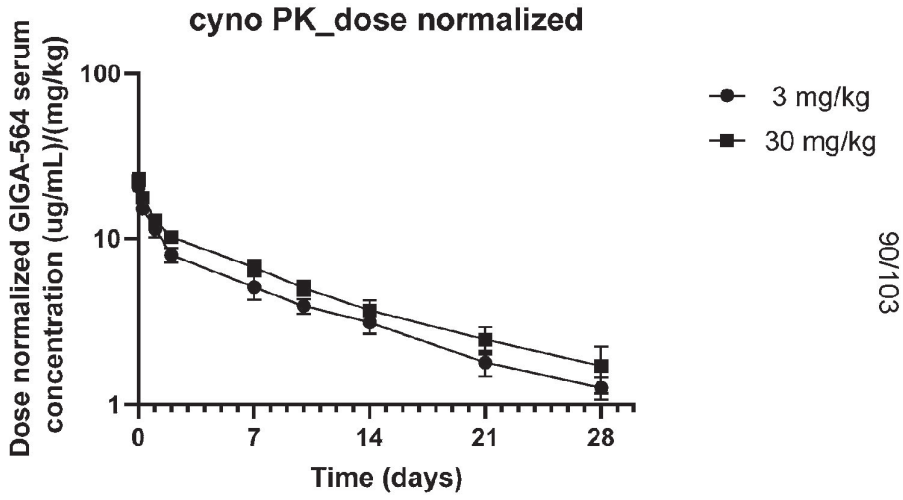


FIG. 52B

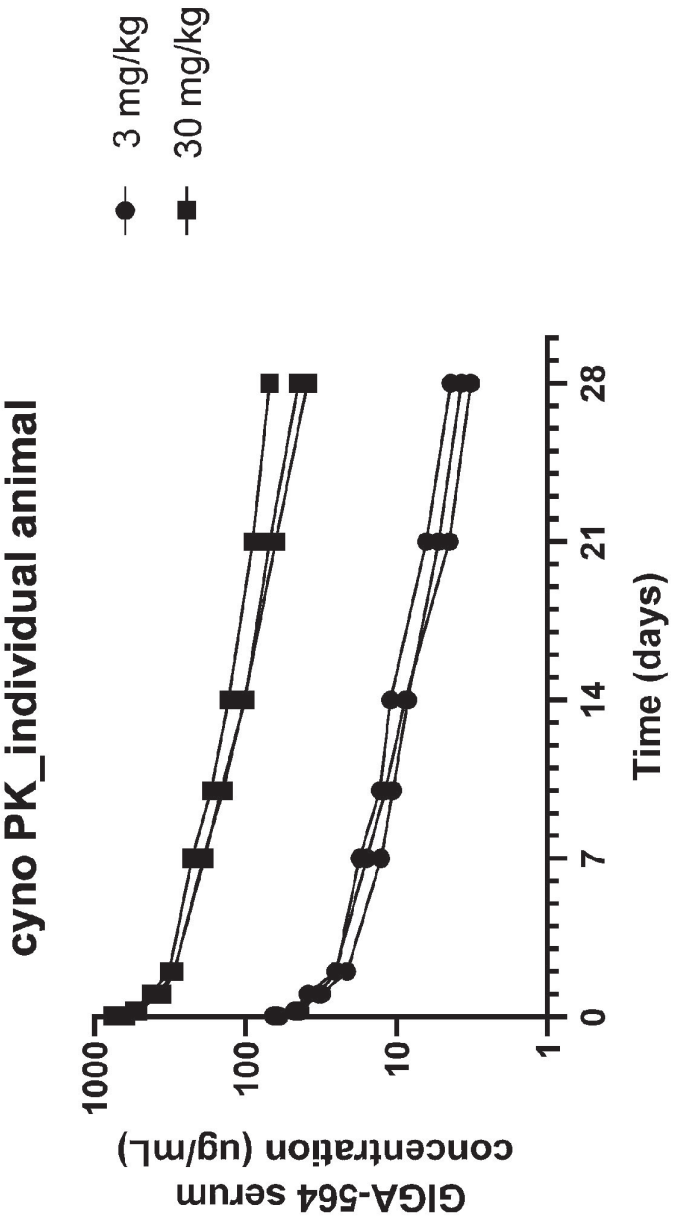

cyno PK_individual animal
3 mg/kg
30 mg/kg
GIGA-564 serum concentration (ug/mL)
1000
100
10
1
0
7
14
21
28
Time (days)

FIG. 53

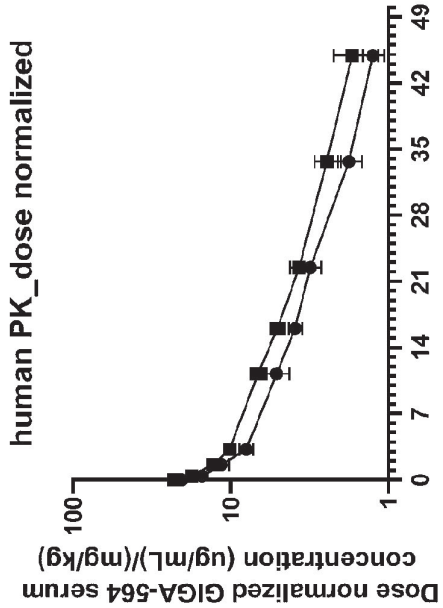


FIG. 54B

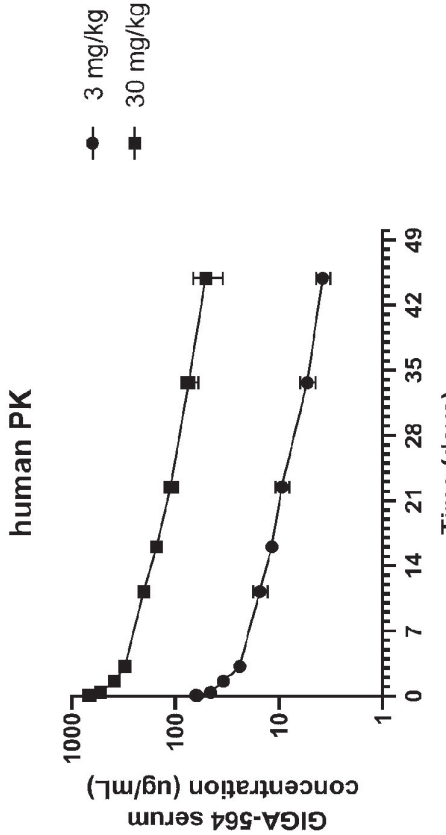


FIG. 54A

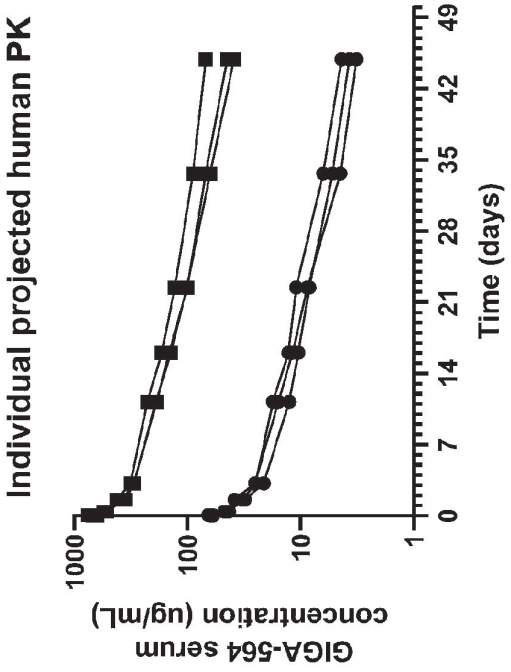


FIG. 55A

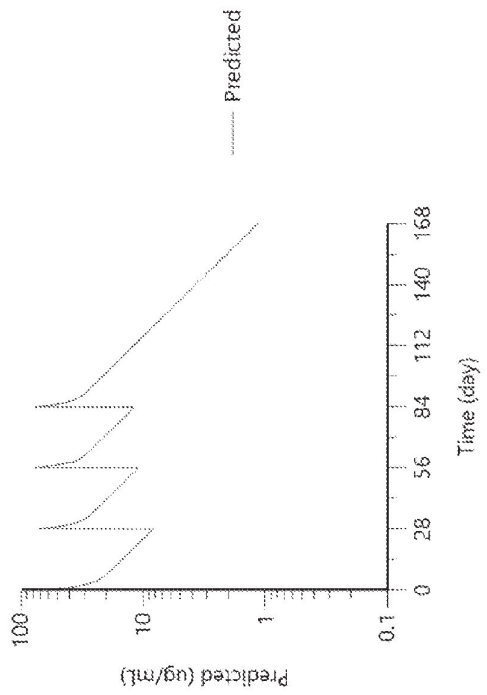


FIG. 55B

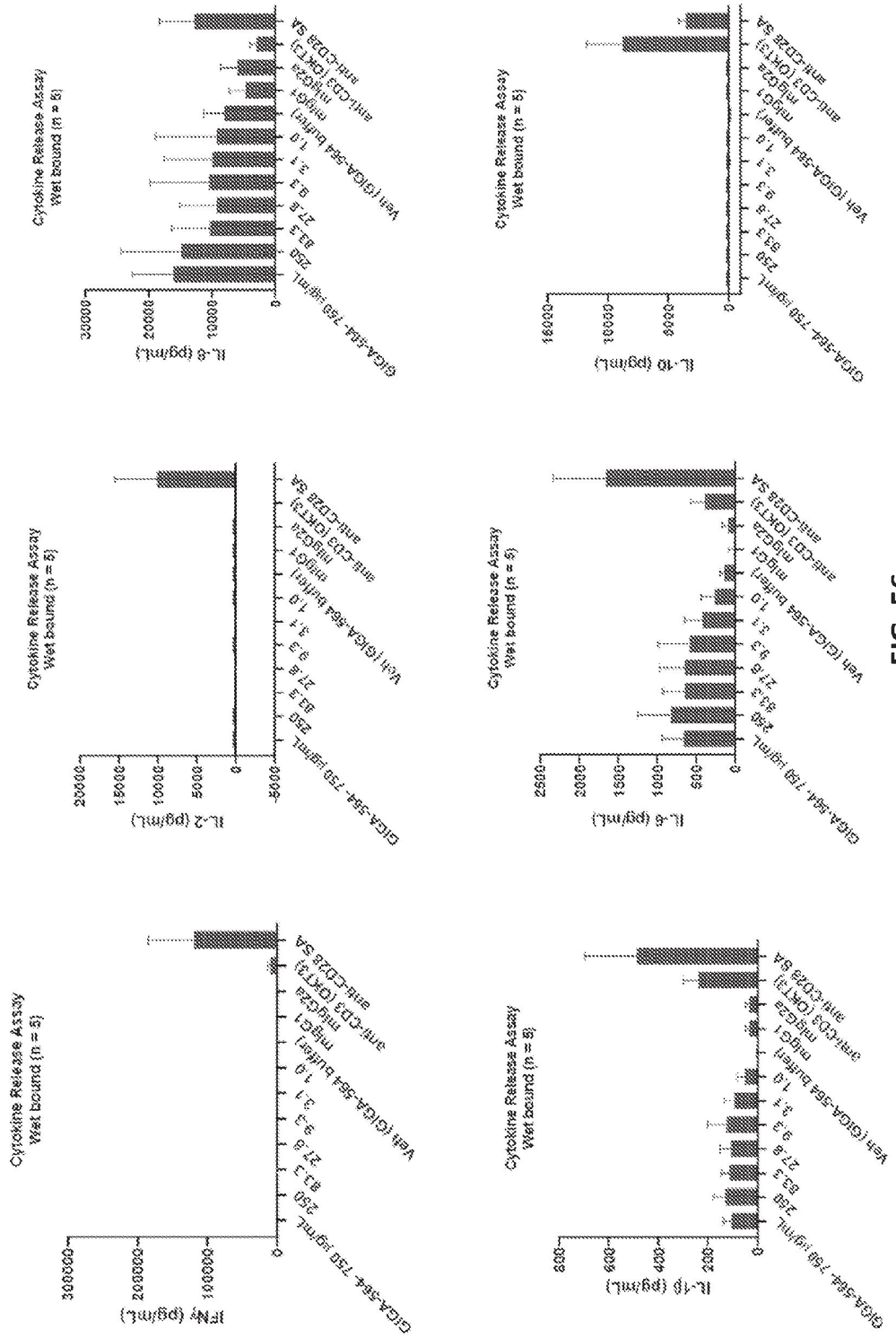
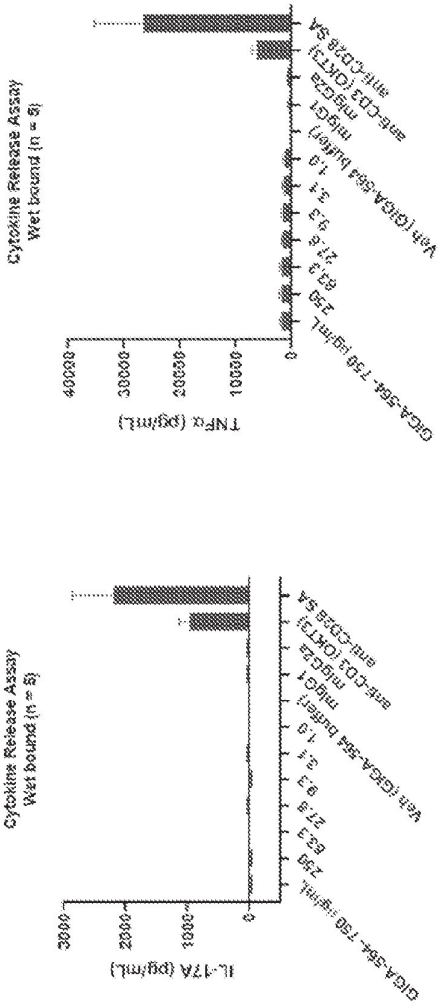

Cytokine Release Assay
Wet bound (n = 5)
IFNγ (pg/mL)
IL-2 (pg/mL)
IL-6 (pg/mL)
IL-8 (pg/mL)
IL-10 (pg/mL)
IL-1β (pg/mL)
GIGA-564- 750 µg/mL
GIGA-564- 250 µg/mL
GIGA-564- 83.3
GIGA-564- 27.8
GIGA-564- 9.3
GIGA-564- 3.1
GIGA-564- 1.0
Veh (GIGA-564 buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA

FIG. 56


Cytokine Release Assay
Wet bound (n = 5)
TNFα (pg/mL)
40000
30000
20000
10000
0
GIGA-564- 750 ug/mL
250
83.3
27.8
9.3
3.1
1.0
Veh (GIGA-564 buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA

Cytokine Release Assay
Wet bound (n = 5)
IL-17A (pg/mL)
3000
2000
1000
0
GIGA-564- 750 ug/mL
250
83.3
27.8
9.3
3.1
1.0
Veh (GIGA-564 buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA

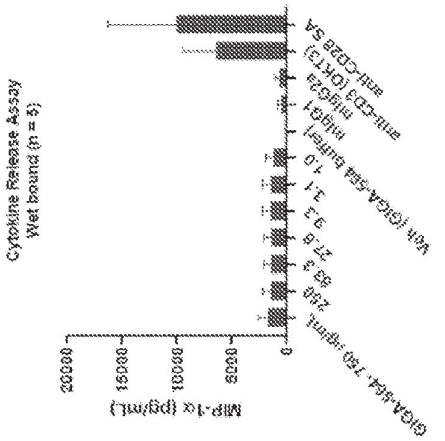

Cytokine Release Assay
Wet bound (n = 5)
MIP-1α (pg/mL)
20000
15000
10000
5000
0
GIGA-564- 750 ug/mL
250
83.3
27.8
9.3
3.1
1.0
Veh (GIGA-564 buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA

FIG. 56 (cont'd)

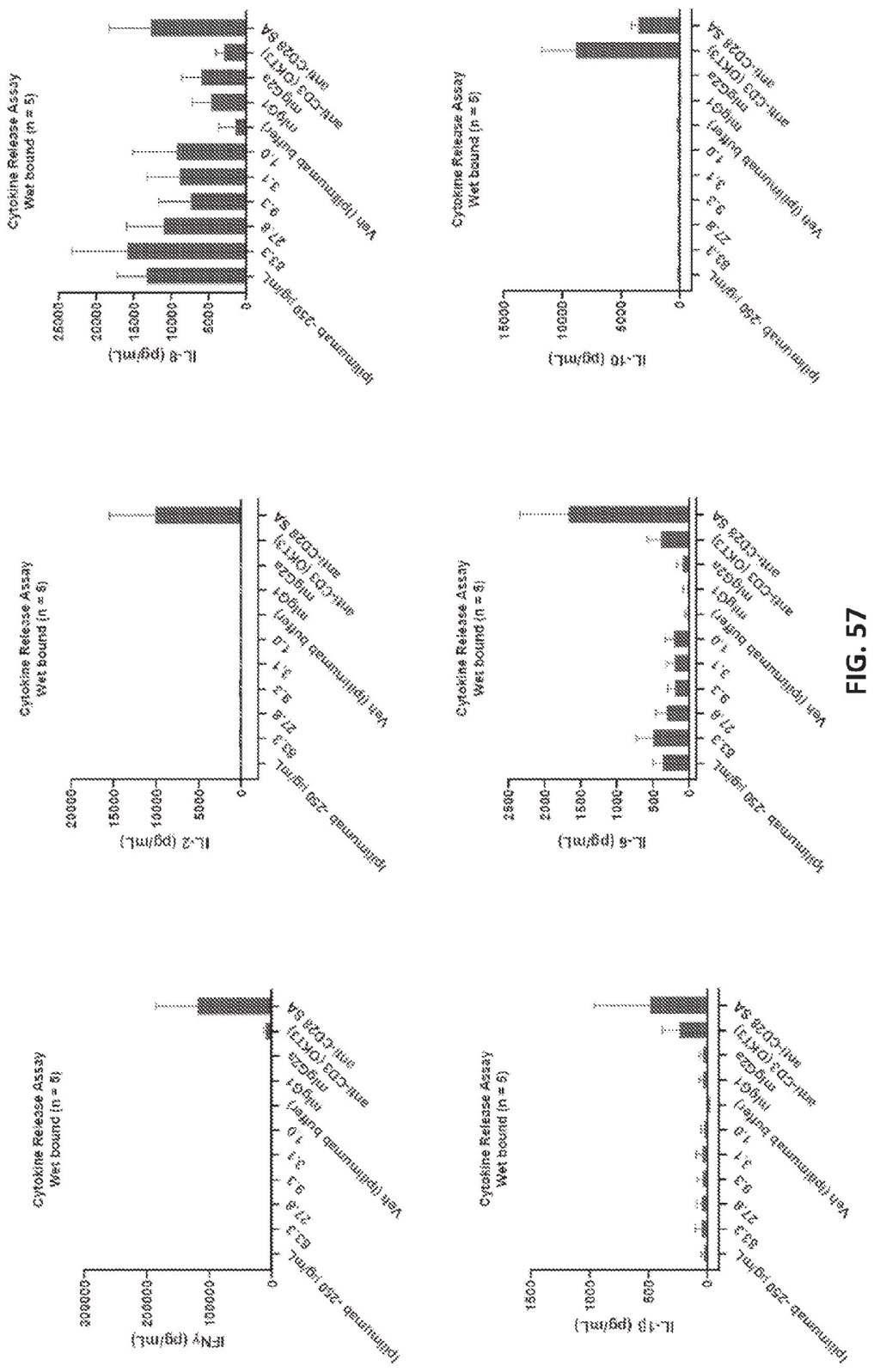

Cytokine Release Assay
Wet bound (n = 6)
IL-8 (pg/mL)
IL-10 (pg/mL)
IL-2 (pg/mL)
IL-6 (pg/mL)
IFNγ (pg/mL)
IL-13 (pg/mL)
Ipilimumab -250 µg/mL
63.3
27.8
9.3
3.1
1.0
Vehicle (Ipilimumab buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA

FIG. 57

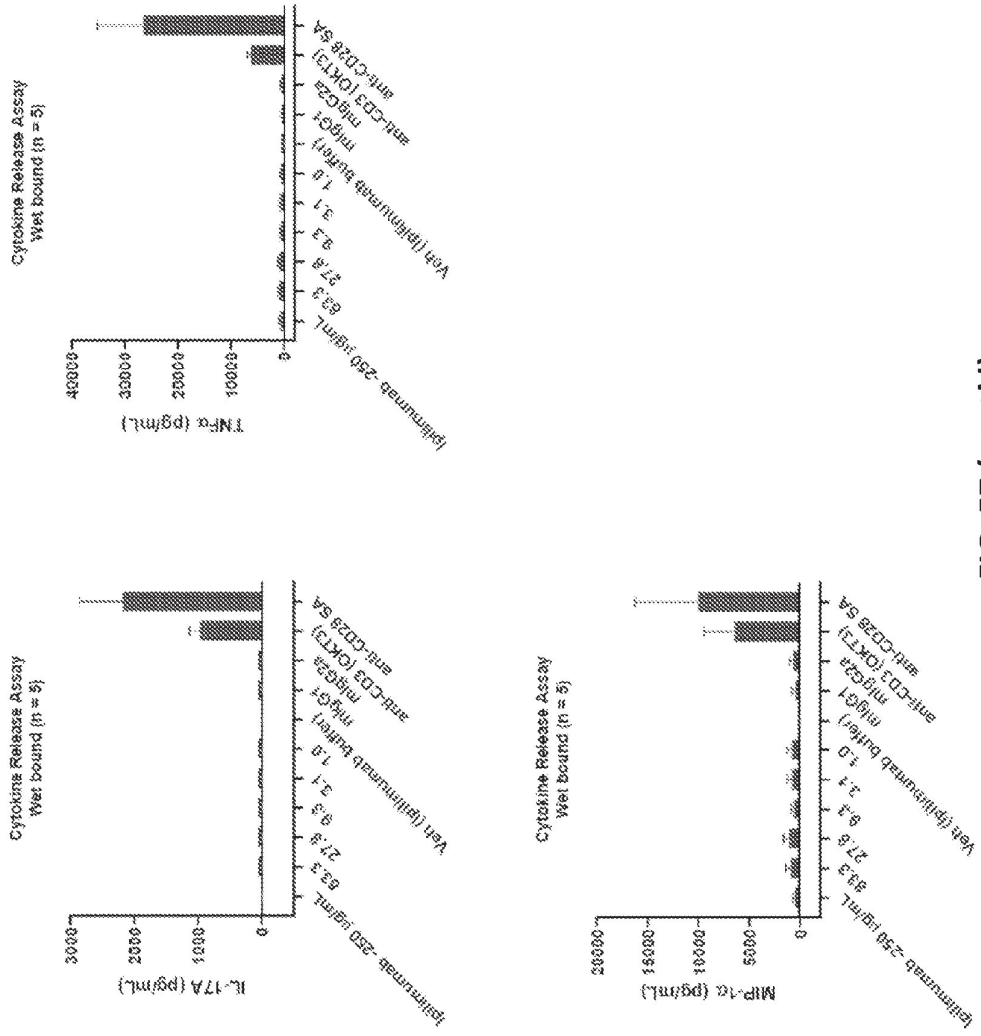

Cytokine Release Assay
Wet bound (n = 5)
TNFα (pg/mL)
40000
30000
20000
10000
0
Ipilimumab -250 µg/mL
63.3
27.8
9.3
3.1
1.0
Veh (pHumanab buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA
Cytokine Release Assay
Wet bound (n = 5)
IL-17A (pg/mL)
3000
2000
1000
0
Ipilimumab -250 µg/mL
63.3
27.8
9.3
3.1
1.0
Veh (pHumanab buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA
Cytokine Release Assay
Wet Bound (n = 5)
MIP-1α (pg/mL)
20000
15000
10000
5000
0
Ipilimumab -250 µg/mL
63.3
27.8
9.3
3.1
1.0
Veh (pHumanab buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA

FIG. 57 (cont'd)

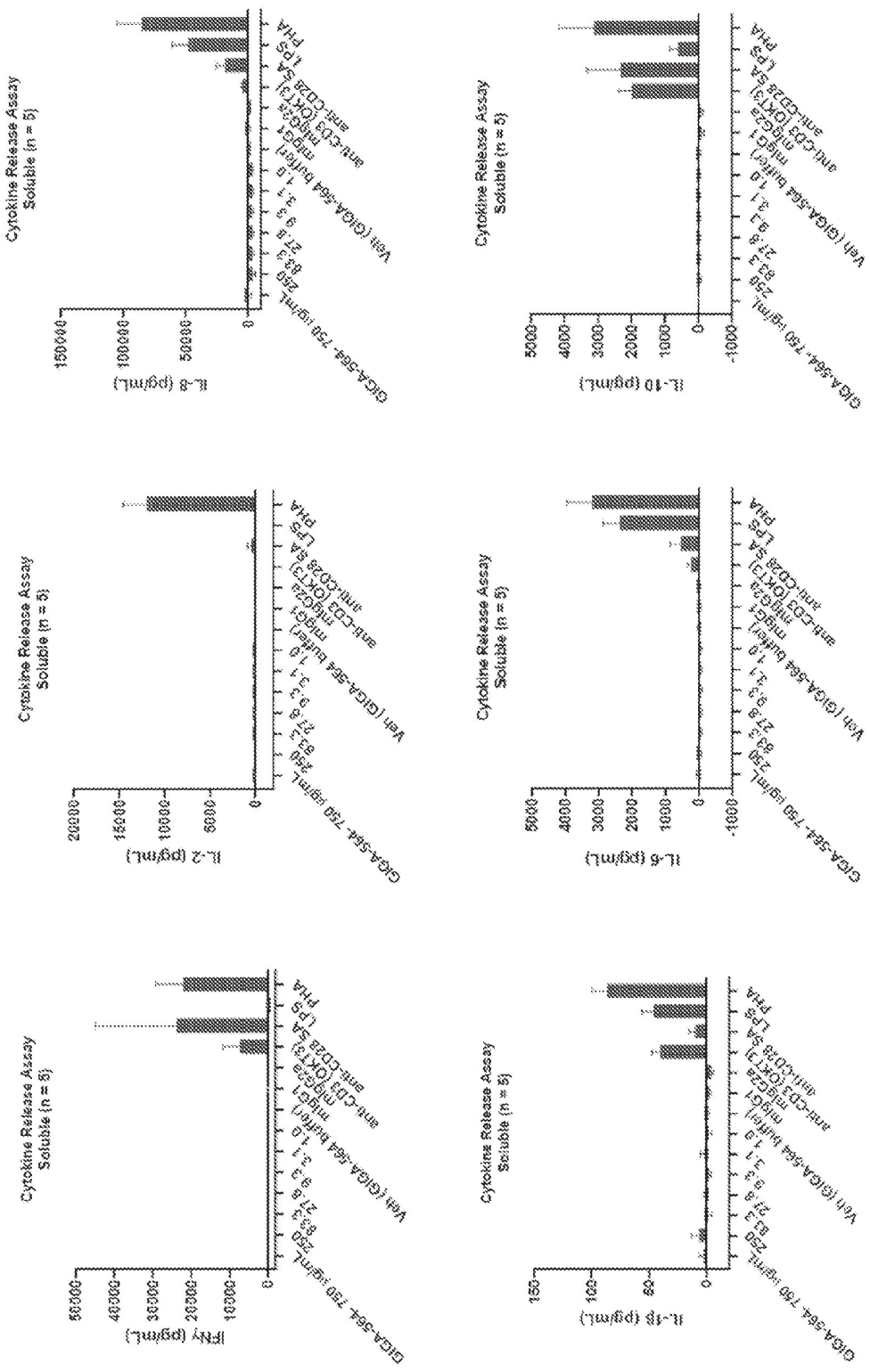

Cytokine Release Assay
Soluble (n = 5)
IFNγ (pg/mL)
IL-2 (pg/mL)
IL-8 (pg/mL)
IL-6 (pg/mL)
IL-10 (pg/mL)
IL-1β (pg/mL)
GIGA-564- 750 ug/mL
Veh (GIGA-564 buffer)
anti-CD3 (OKT3)
anti-CD28 SA
LPS
PHA

FIG. 58

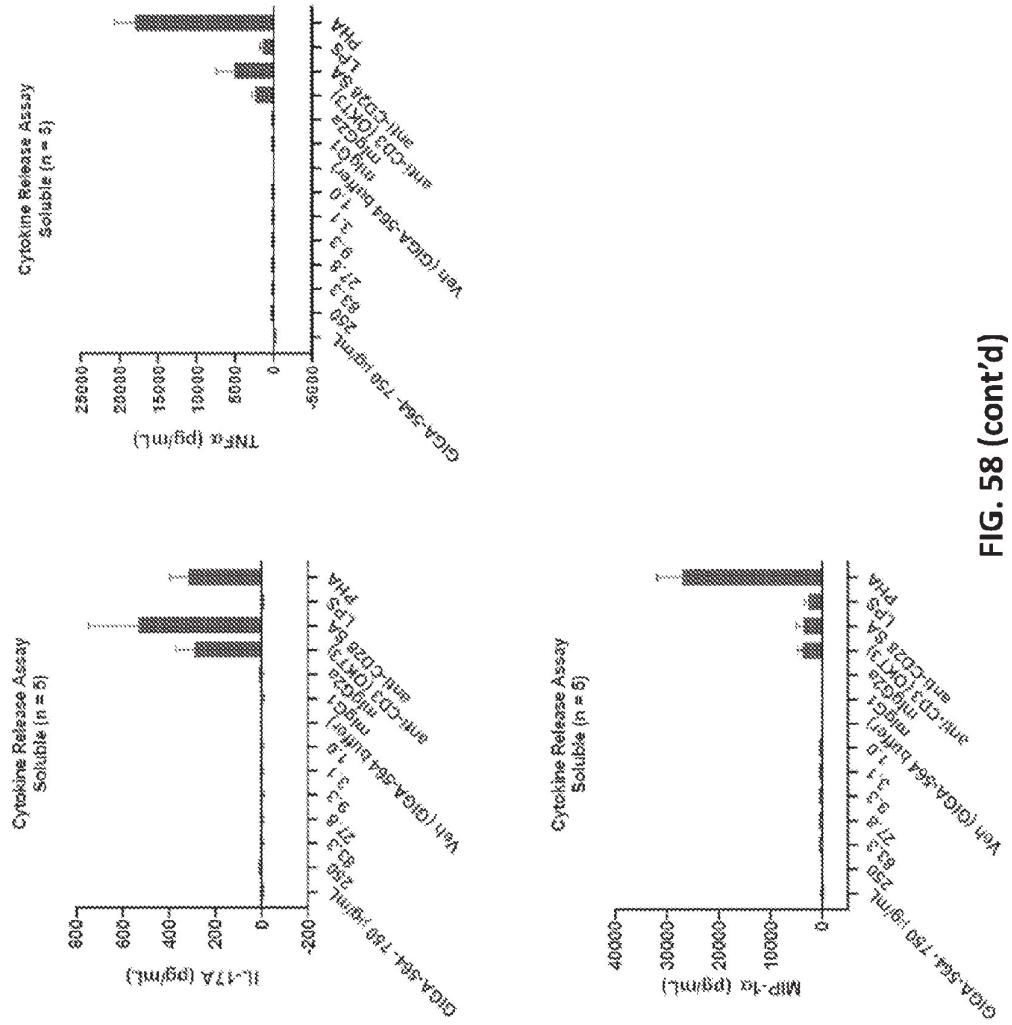

Cytokine Release Assay
Soluble (n = 6)
IL-17A (pg/mL)
600
400
200
0
-200
GIGA-564- 750 ng/mL
250
83.3
27.8
9.3
3.1
1.0
Veh (GIGA-564 buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3) 5A
anti-CD28 5A
LPS
PHA
Cytokine Release Assay
Soluble (n = 6)
TNFα (pg/mL)
25000
20000
15000
10000
5000
0
-5000
GIGA-564- 750 ng/mL
250
83.3
27.8
9.3
3.1
1.0
Veh (GIGA-564 buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3) 5A
anti-CD28 5A
LPS
PHA
Cytokine Release Assay
Soluble (n = 6)
MIP-1α (pg/mL)
40000
30000
20000
10000
0
GIGA-564- 750 ng/mL
250
83.3
27.8
9.3
3.1
1.0
Veh (GIGA-564 buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3) 5A
anti-CD28 5A
LPS
PHA

FIG. 58 (cont'd)

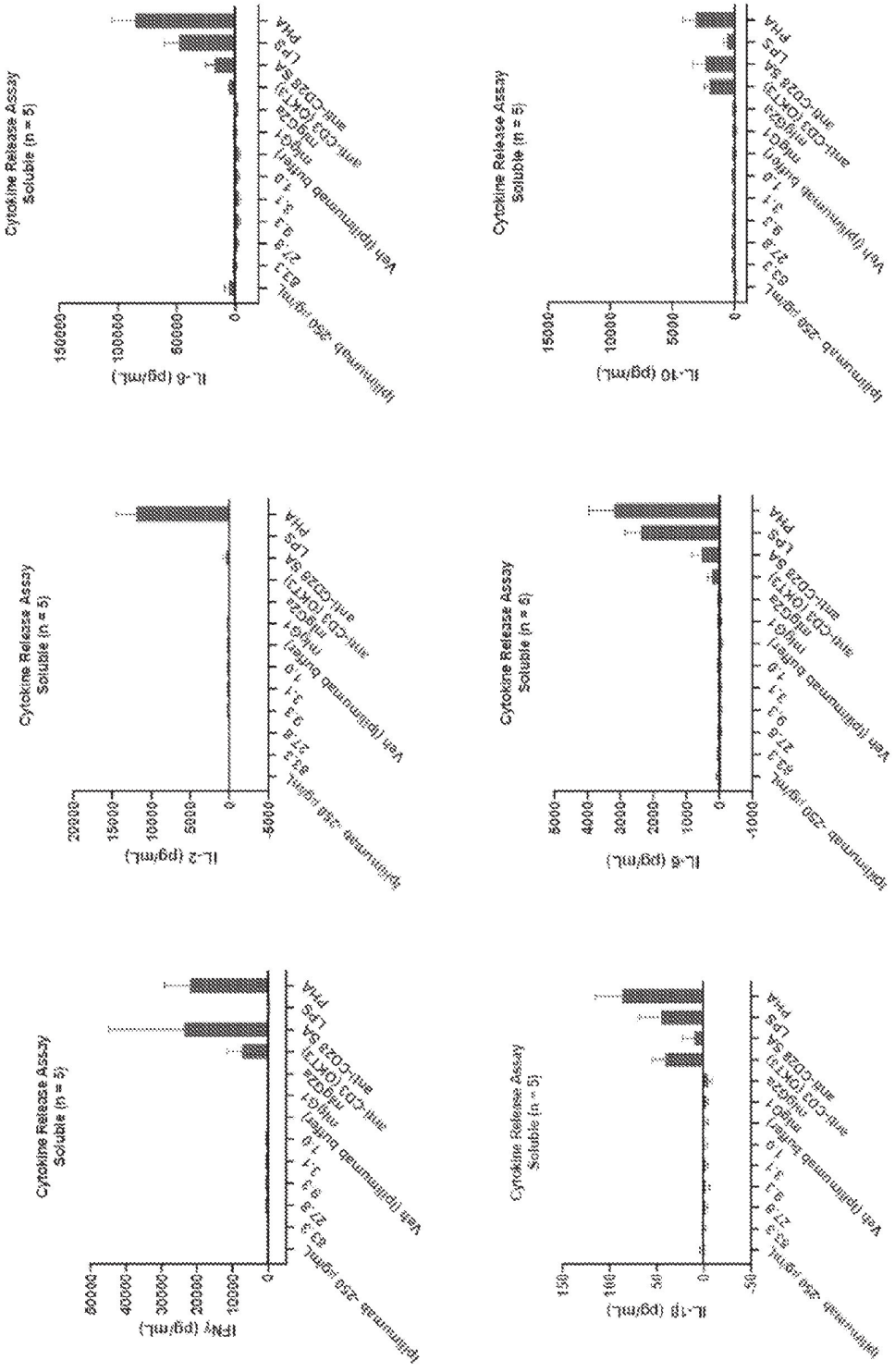

Cytokine Release Assay
Soluble (n = 5)
IFNγ (pg/mL)
IL-2 (pg/mL)
IL-6 (pg/mL)
IL-10 (pg/mL)
IL-1β (pg/mL)
Ipilimumab -250 ug/mL
Vehicle (Ipilimumab buffer)
anti-CD3 (OKT3) mIgG1
anti-CD3 (OKT3) mIgG2a
anti-CD28 SA
LPS
PHA

FIG. 59

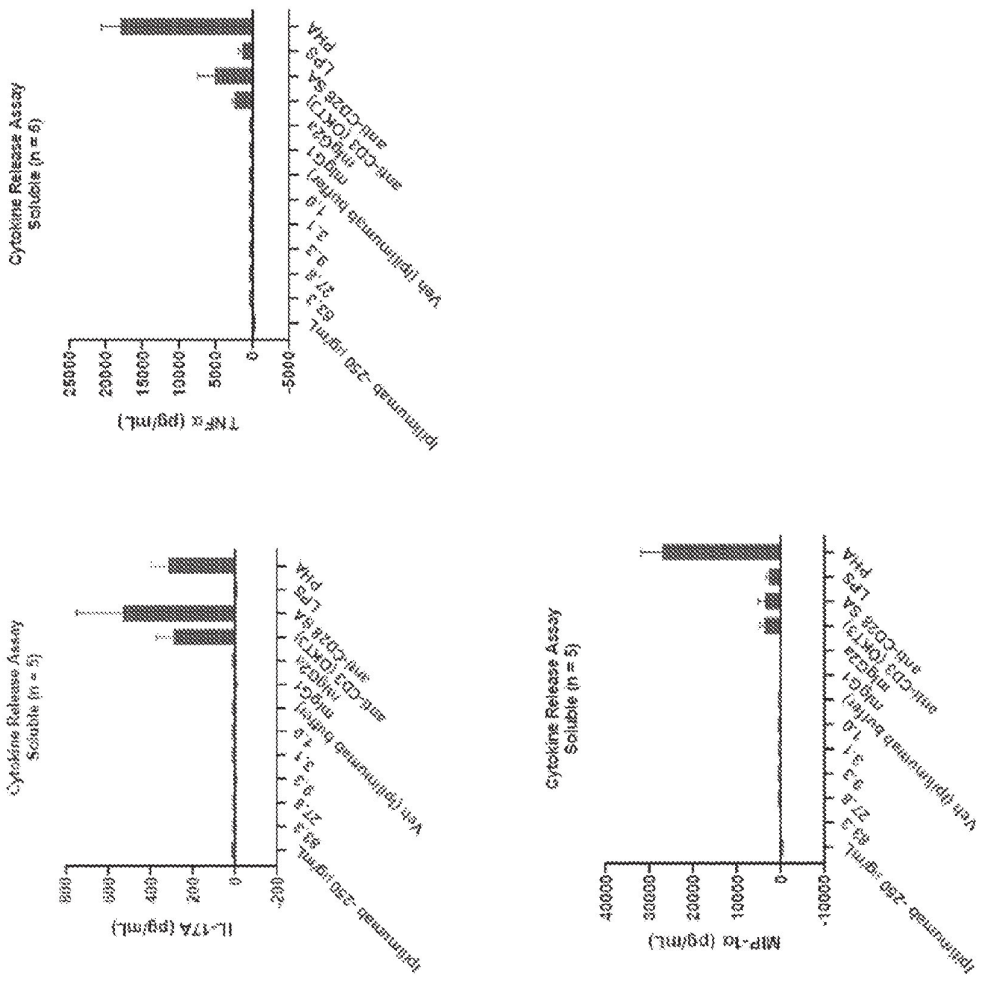

Cytokine Release Assay
Soluble (n = 5)
TNFα (pg/mL)
25000
20000
15000
10000
5000
0
-5000
Ipilimumab -250 µg/mL
63.3
21.8
9.3
3.1
1.0
Vehi Ipilimumab buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA
LPS
PHA
Cytokine Release Assay
Soluble (n = 5)
IL-17A (pg/mL)
800
600
400
200
0
-200
Ipilimumab -250 µg/mL
63.3
21.8
9.3
3.1
1.0
Vehi Ipilimumab buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA
LPS
PHA
Cytokine Release Assay
Soluble (n = 5)
MIP-1α (pg/mL)
40000
30000
20000
10000
0
-10000
Ipilimumab -250 µg/mL
63.3
21.8
9.3
3.1
1.0
Vehi Ipilimumab buffer)
mIgG1
mIgG2a
anti-CD3 (OKT3)
anti-CD28 SA
LPS
PHA

FIG. 59 (cont'd)

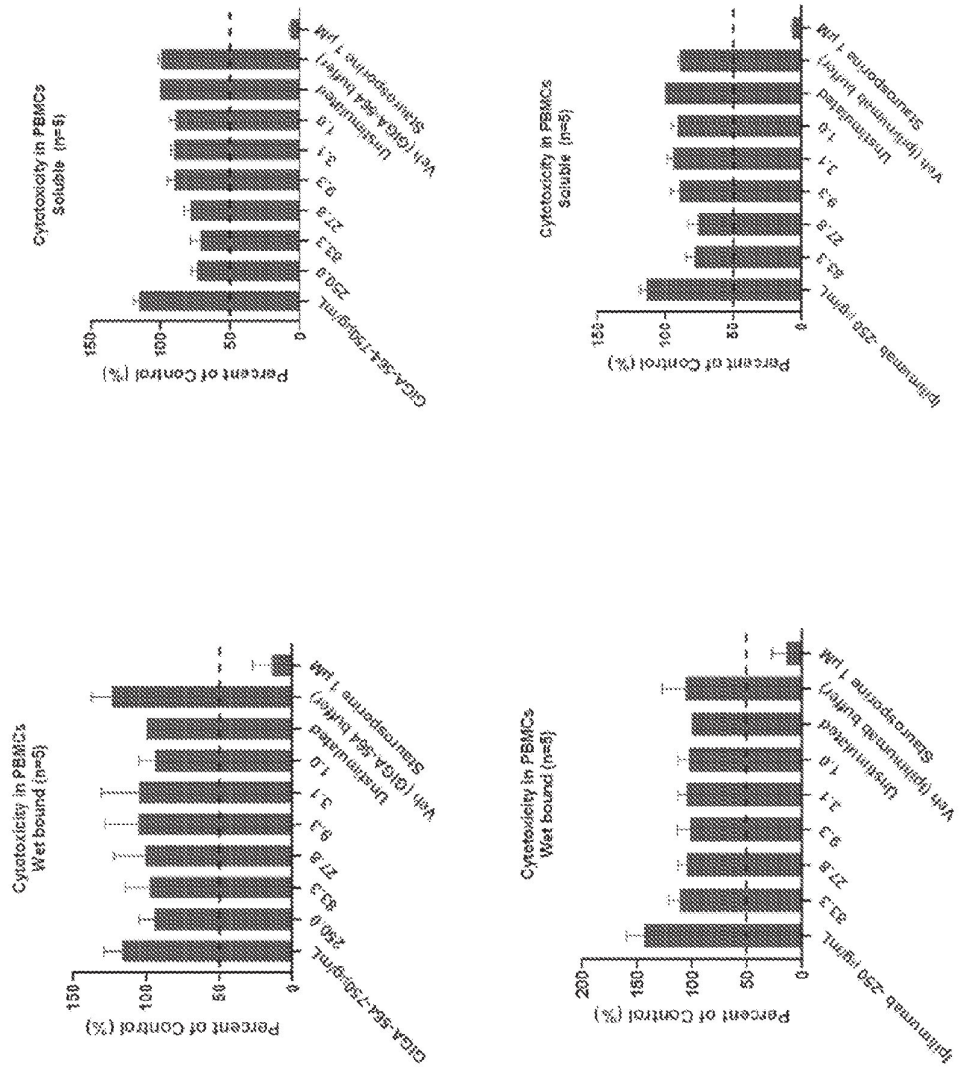

Cytotoxicity in PBMCs
Soluble (n=5)
Percent of Control (%)
150
100
50
0
GIGA-564-750ug/mL
250.0
83.3
27.8
9.3
3.1
1.0
Unstimulated
Veh (GIGA-564 buffer)
Staurosporine 1 µM
Cytotoxicity in PBMCs
Soluble (n=5)
Percent of Control (%)
150
100
50
0
Ibrutinib -250 ng/mL
83.3
27.8
9.3
3.1
1.0
Unstimulated
Veh (Ibrutinib buffer)
Staurosporine 1 µM
Cytotoxicity in PBMCs
Wet bound (n=5)
Percent of Control (%)
150
100
50
0
GIGA-564-750ug/mL
250.0
83.3
27.8
9.3
3.1
1.0
Unstimulated
Veh (GIGA-564 buffer)
Staurosporine 1 µM
Cytotoxicity in PBMCs
Wet bound (n=5)
Percent of Control (%)
200
150
100
50
0
Ibrutinib -250 ng/mL
83.3
27.8
9.3
3.1
1.0
Unstimulated
Veh (Ibrutinib buffer)
Staurosporine 1 µM

FIG. 60

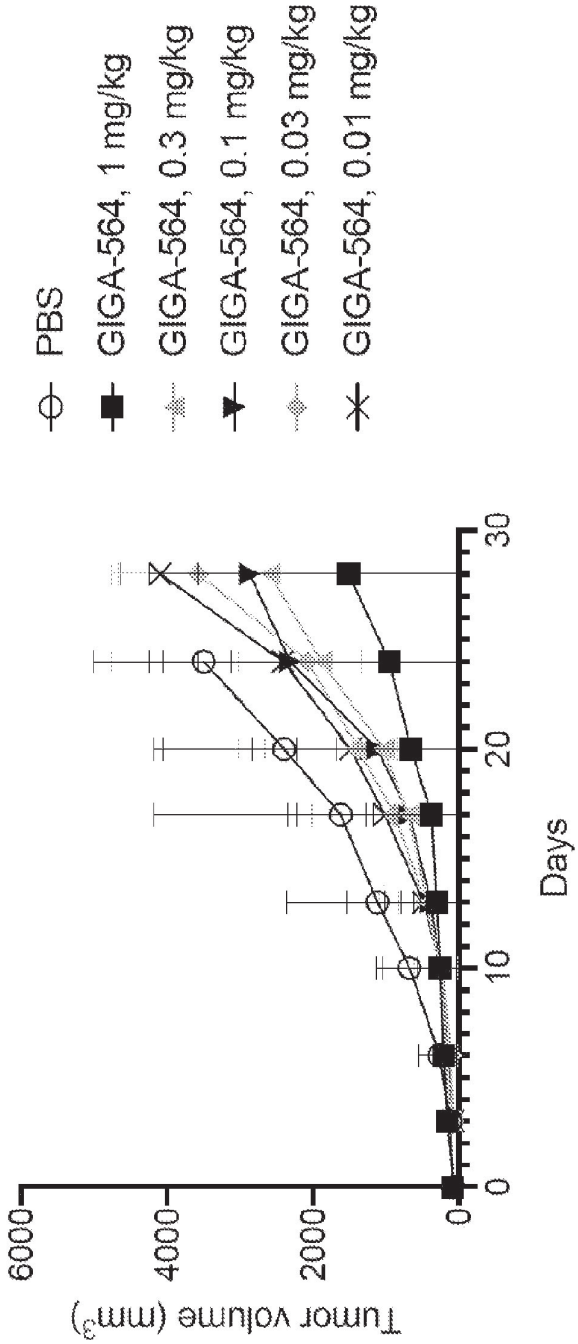

PBS
GIGA-564, 1 mg/kg
GIGA-564, 0.3 mg/kg
GIGA-564, 0.1 mg/kg
GIGA-564, 0.03 mg/kg
GIGA-564, 0.01 mg/kg
Tumor volume (mm3)
6000
4000
2000
0
0
10
20
30
Days

FIG. 61