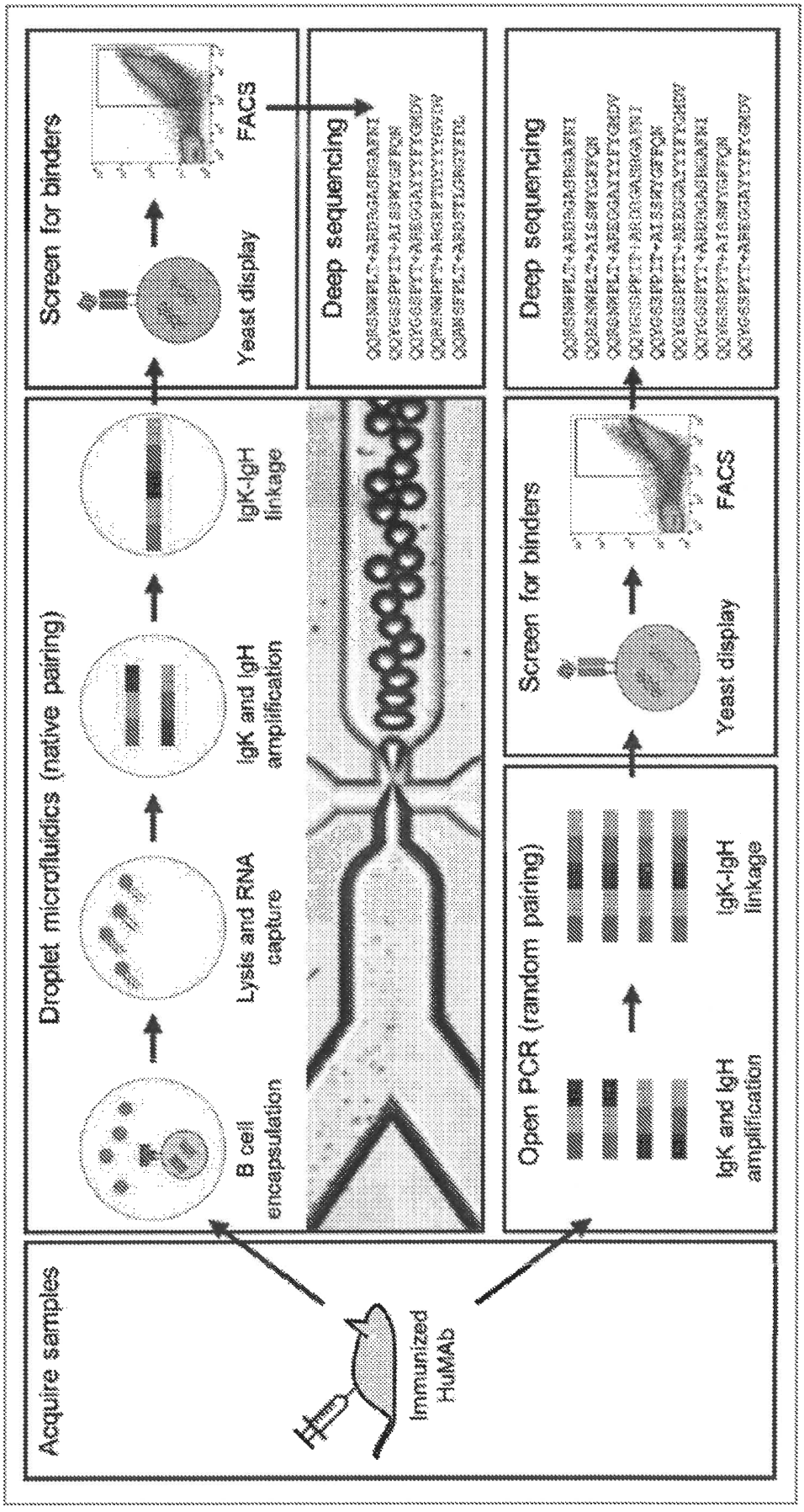
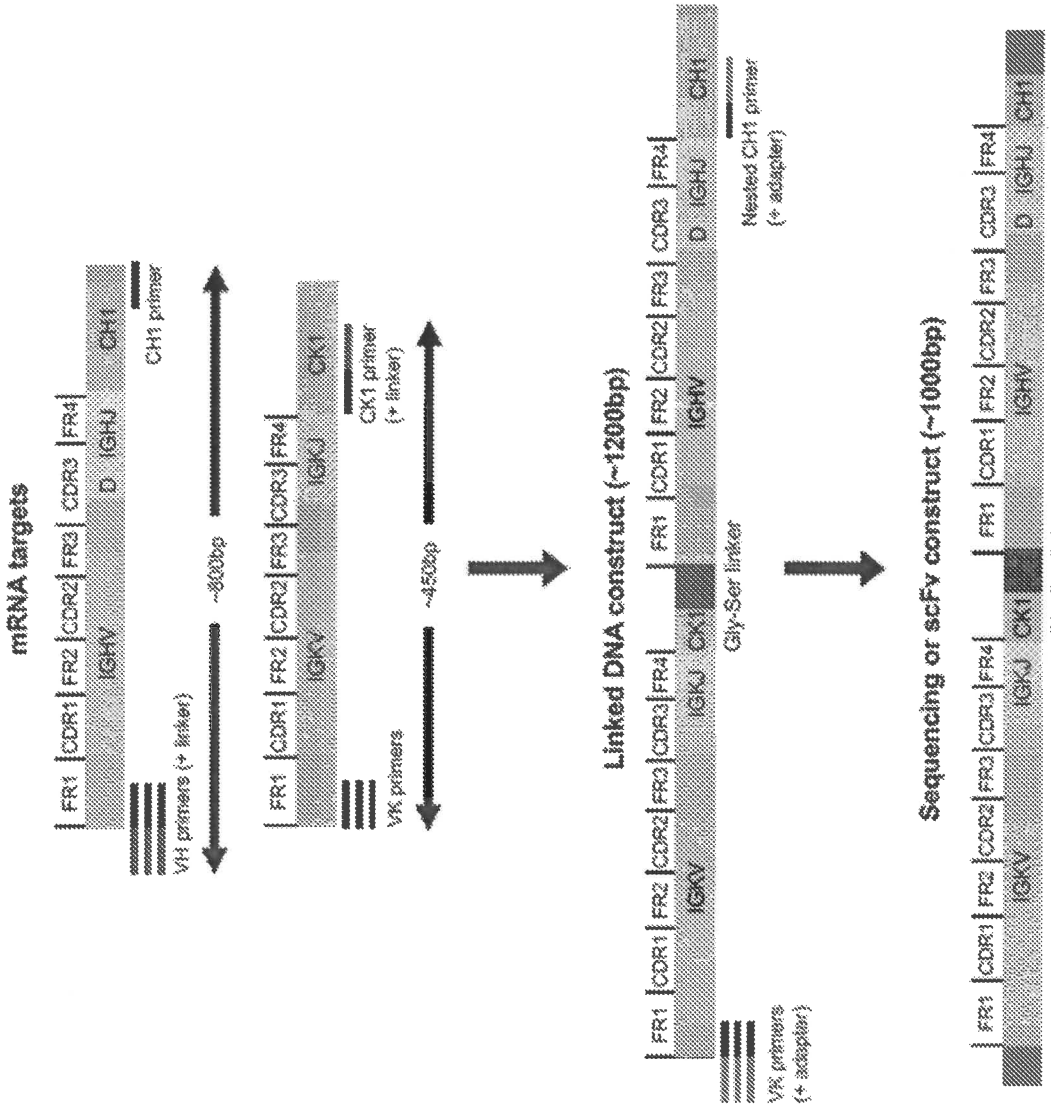

Acquire samples
Immunized HuMAb
Droplet microfluidics (native pairing)
B cell encapsulation
Lysis and RNA capture
IgK and IgH amplification
IgK-IgH linkage
Screen for binders
Yeast display
FACS
Deep sequencing
Open PCR (random pairing)
IgK and IgH amplification
IgK-IgH linkage
Screen for binders
Yeast 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
Nested CH1 primer (+ adapter)
VK primers (+ adapter)
Sequencing or scFv construct (~1000bp)

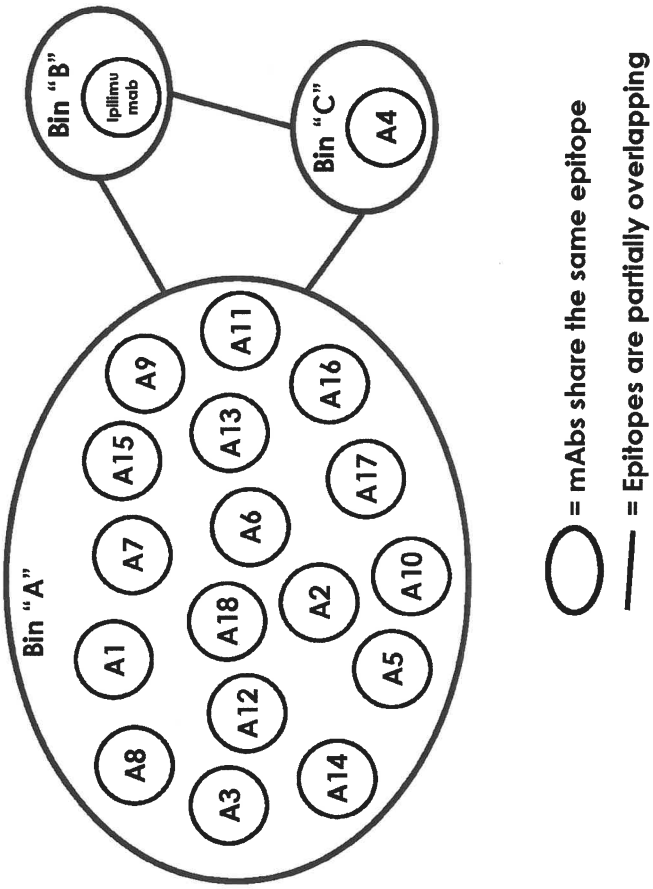

Bin "A"
A1
A7
A15
A9
A11
A13
A16
A6
A17
A18
A2
A10
A5
A12
A8
A3
A14
Bin "B"
Ipilimumab
Bin "C"
A4
= mAbs share the same epitope
= Epitopes are partially overlapping

FIG. 3

FIG. 4A

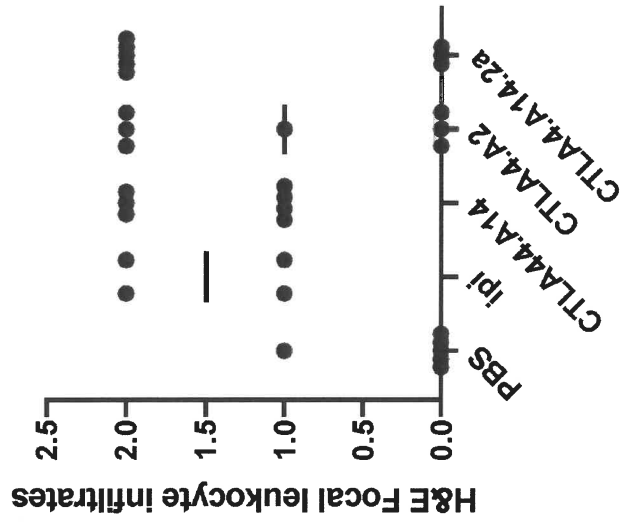


FIG. 4B

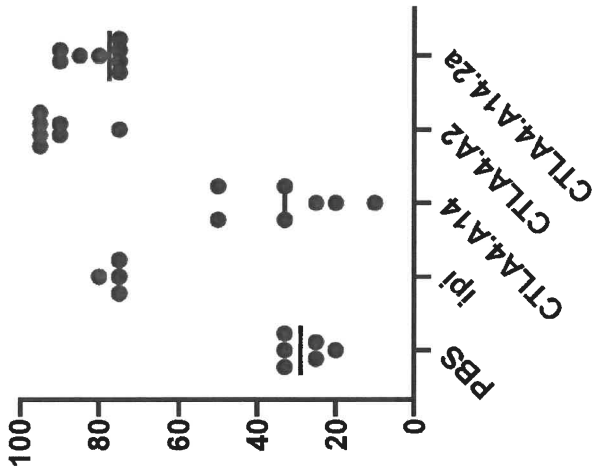


FIG. 4C

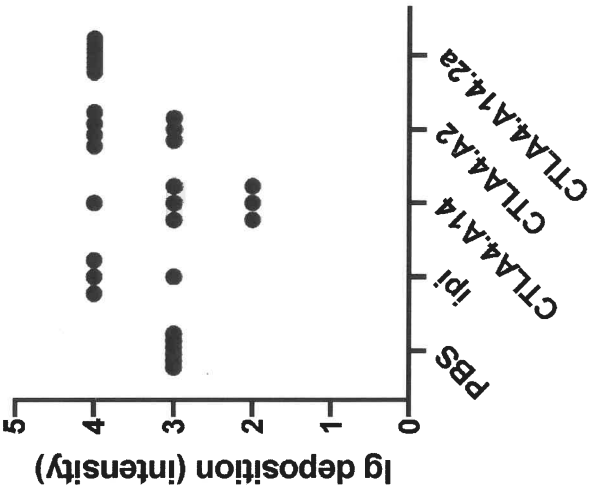


FIG. 4E

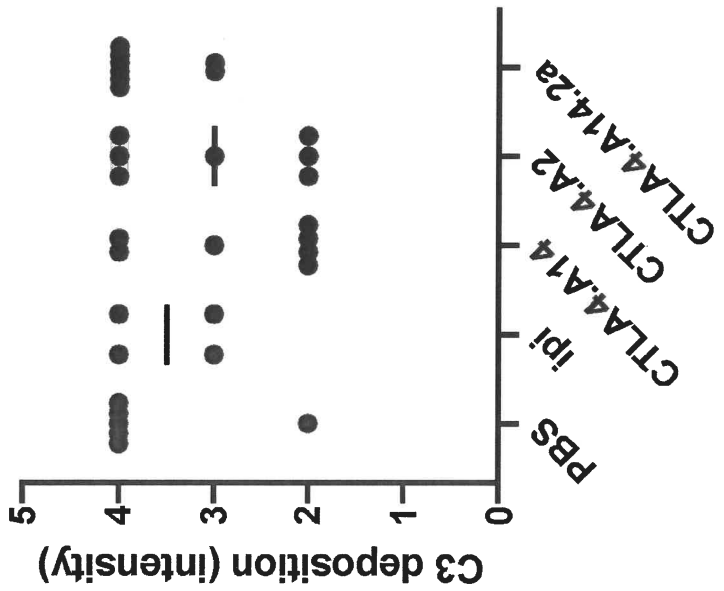
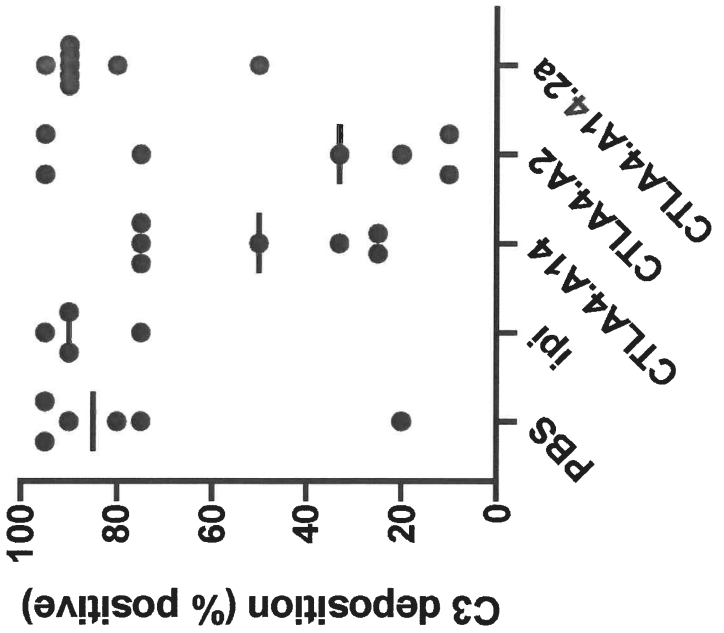

C3 deposition (intensity)
0
1
2
3
4
5
PBS
ipi
CTLA4.A14
CTLA4.A2
CTLA4.A14.2a

FIG. 4D


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

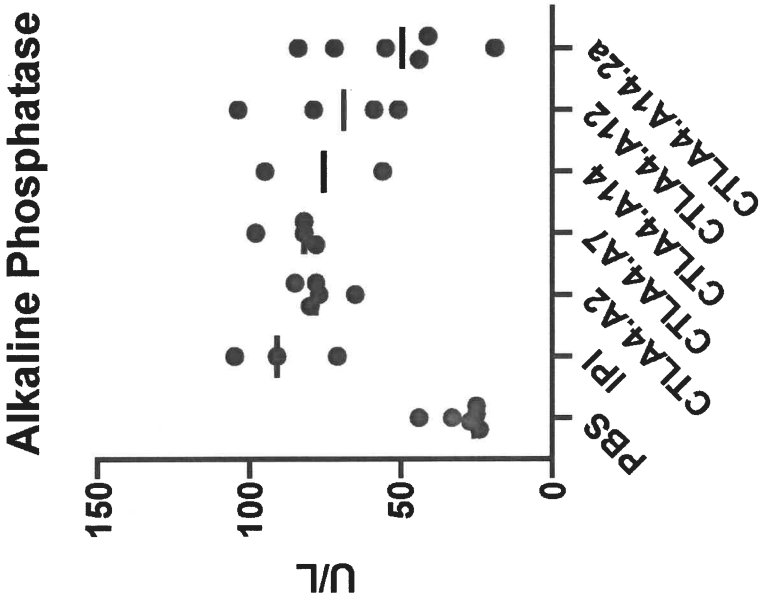

Alkaline Phosphatase
U/L
150
100
50
0
PBS
Ipi
CTLA4.A2
CTLA4.A7
CTLA4.A14
CTLA4.A12
CTLA4.A14.2a

FIG. 5

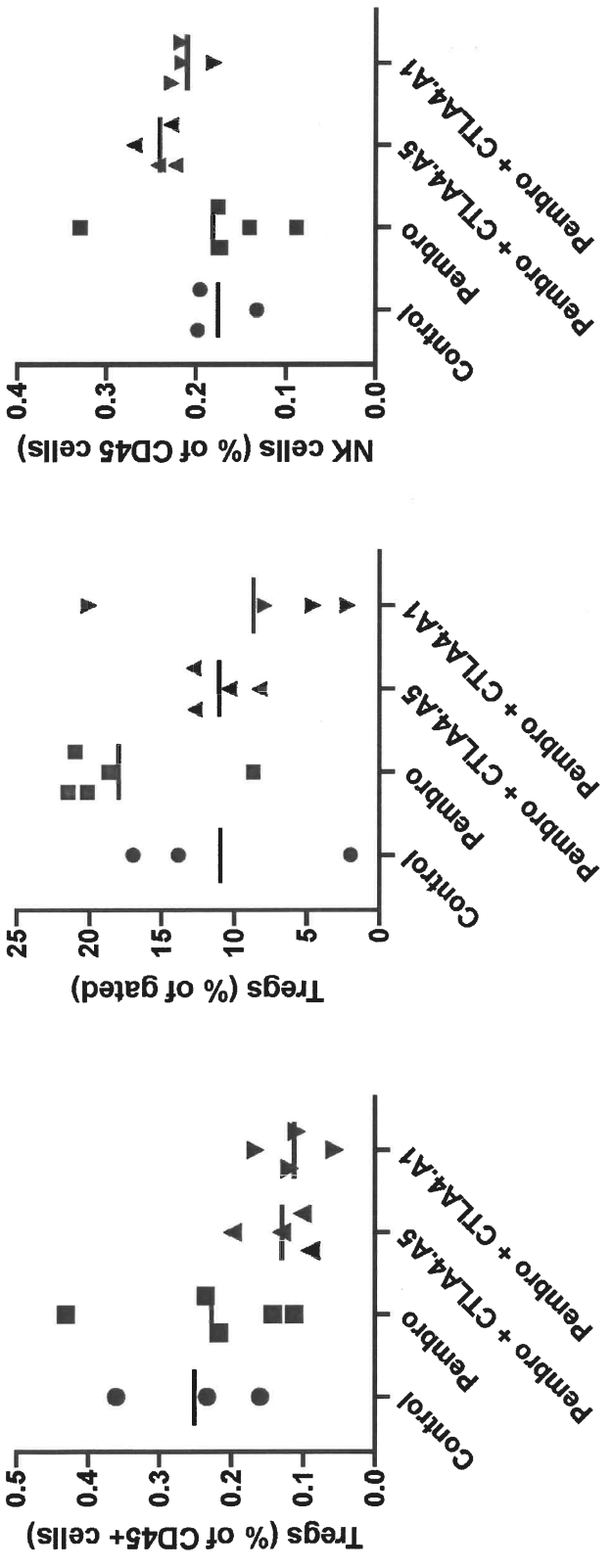

Tregs (% of CD45+ cells)
0.0
0.1
0.2
0.3
0.4
0.5
Control
Pembro
Pembro + CTLA4.A5
Pembro + CTLA4.A1
Tregs (% of gated)
0
5
10
15
20
25
Control
Pembro
Pembro + CTLA4.A5
Pembro + CTLA4.A1
NK cells (% of CD45 cells)
0.0
0.1
0.2
0.3
0.4
Control
Pembro
Pembro + CTLA4.A5
Pembro + CTLA4.A1

FIG. 6

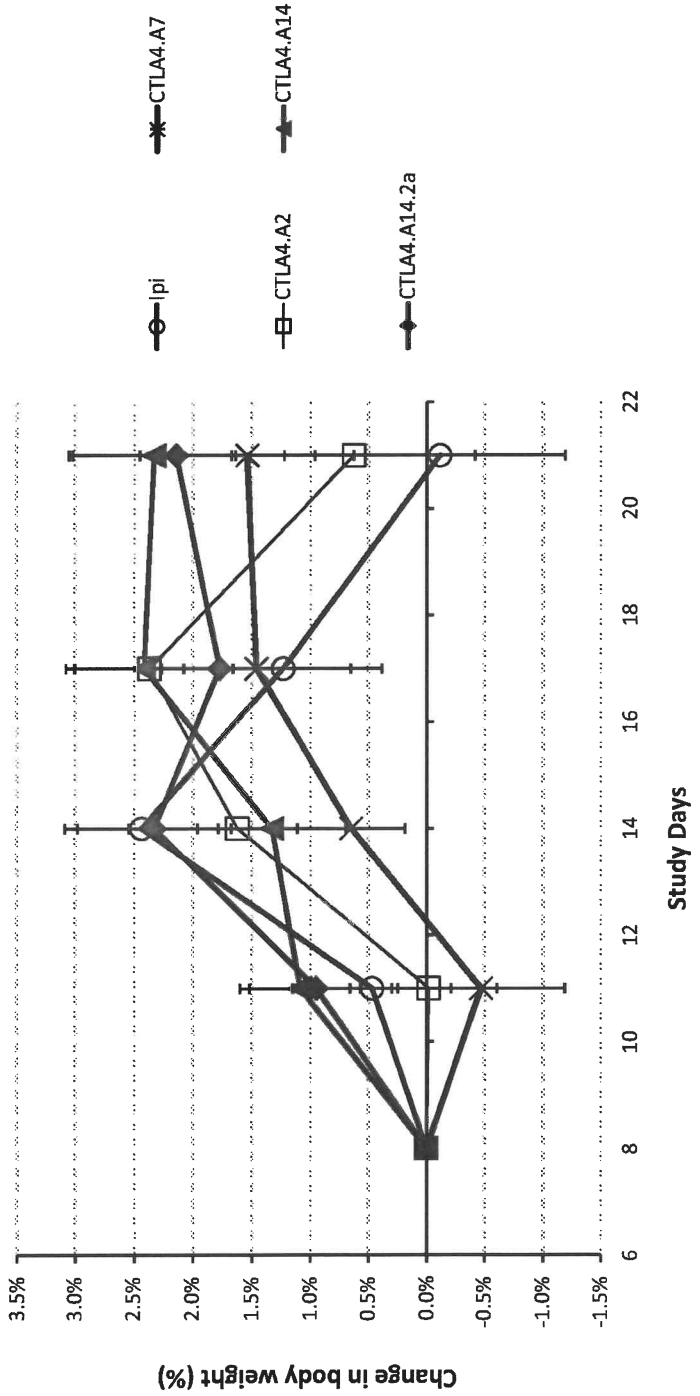

Ipi
CTLA4.A7
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a
Change in body weight (%)
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
-0.5%
-1.0%
-1.5%
6
8
10
12
14
16
18
20
22
Study Days

FIG. 7

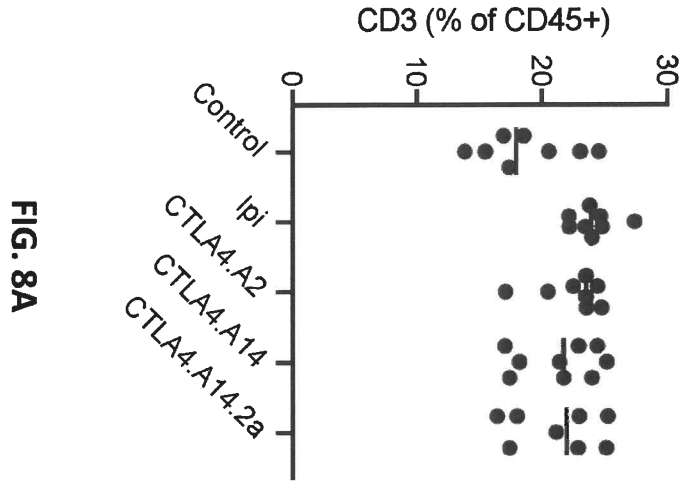


FIG. 8A

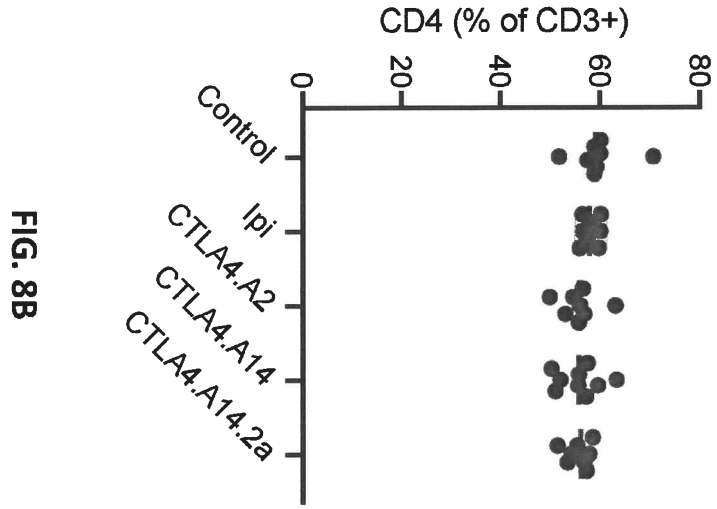


FIG. 8B

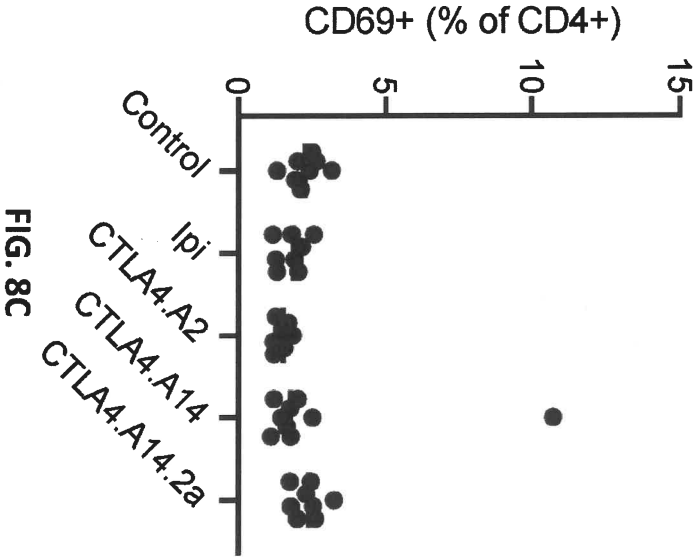


FIG. 8C

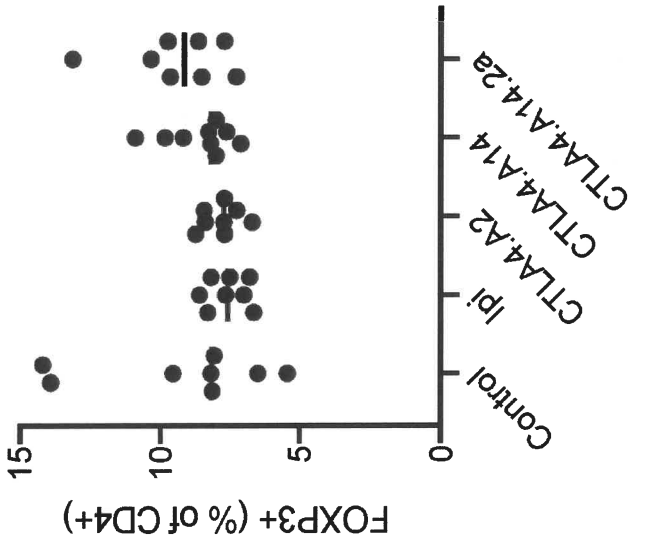


FIG. 8F

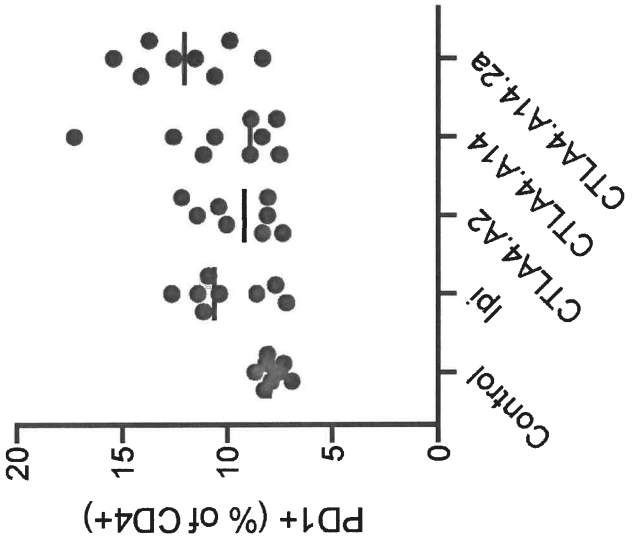


FIG. 8E

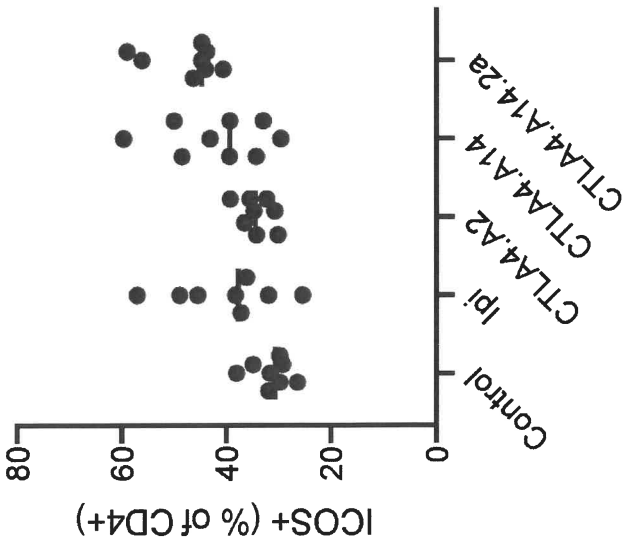


FIG. 8D

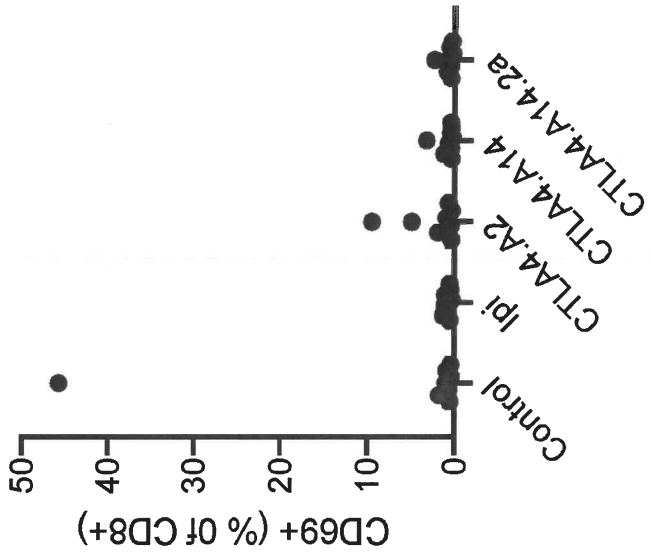

CD69+ (% of CD8+)
0
10
20
30
40
50
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9B

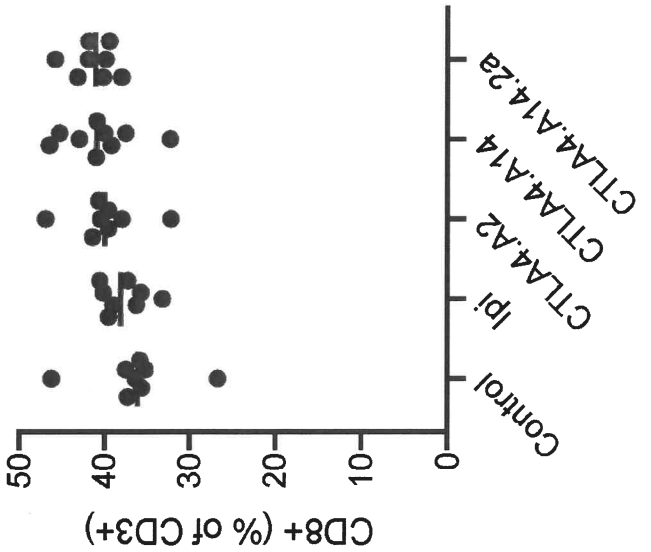

CD8+ (% of CD3+)
0
10
20
30
40
50
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9A

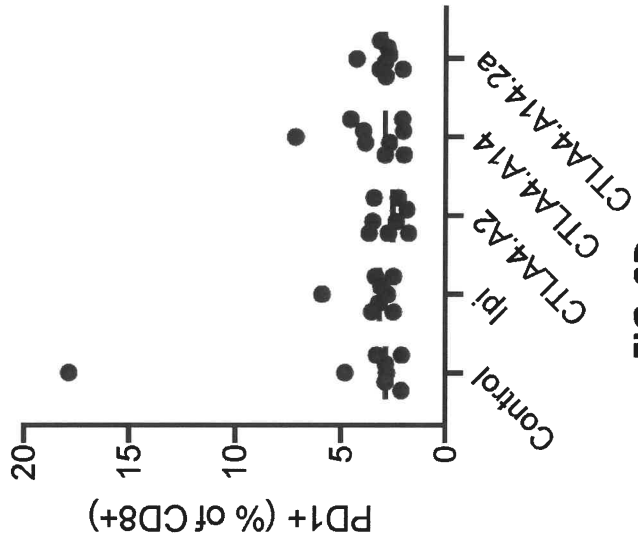

PD1+ (% of CD8+)
0
5
10
15
20
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9D

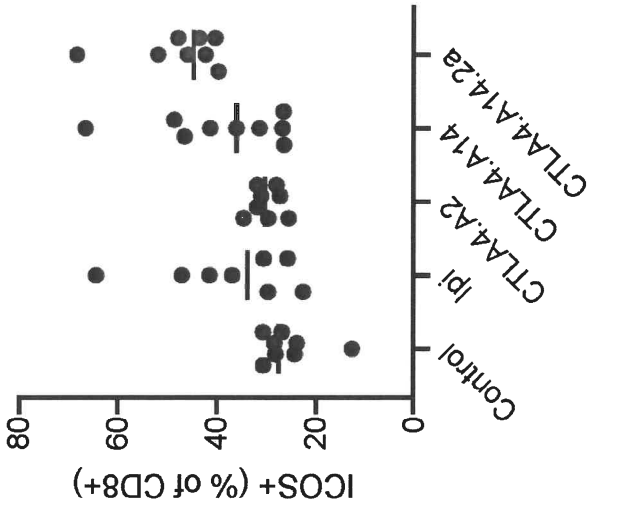

ICOS+ (% of CD8+)
0
20
40
60
80
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9C

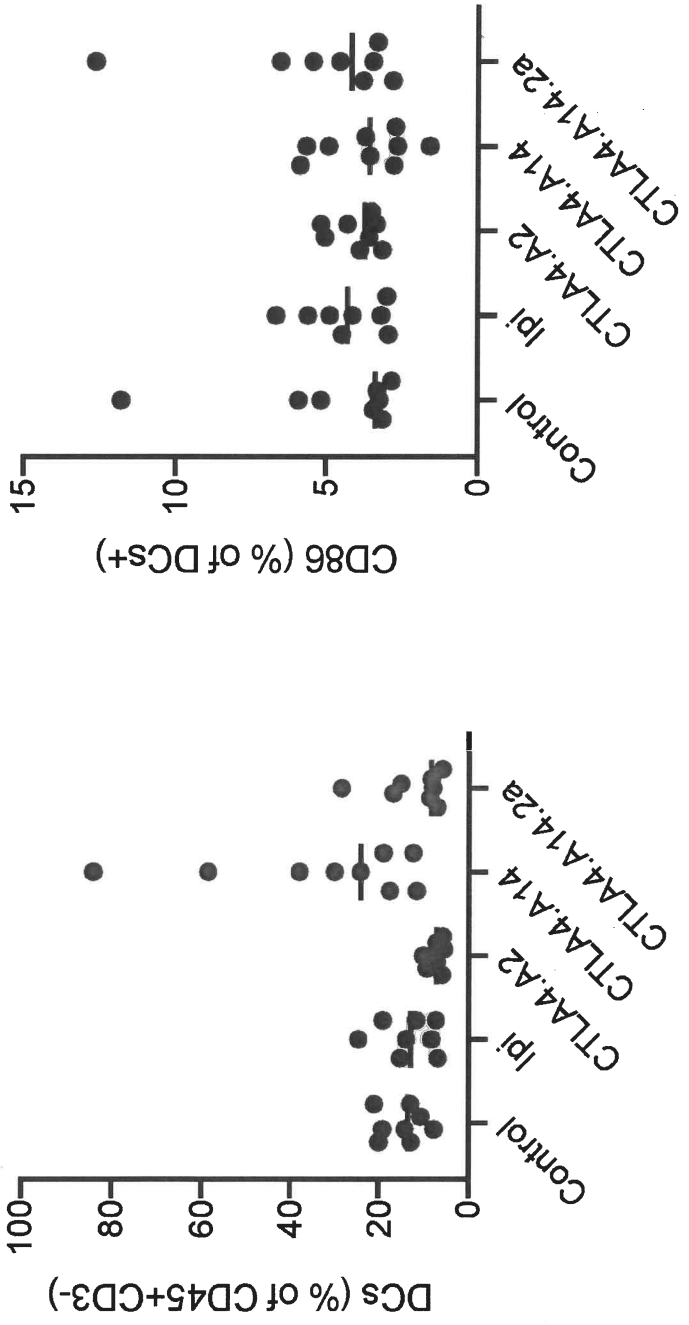

DCs (% of CD45+CD3-)
100
80
60
40
20
0
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a
CD86 (% of DCs+)
15
10
5
0
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 10

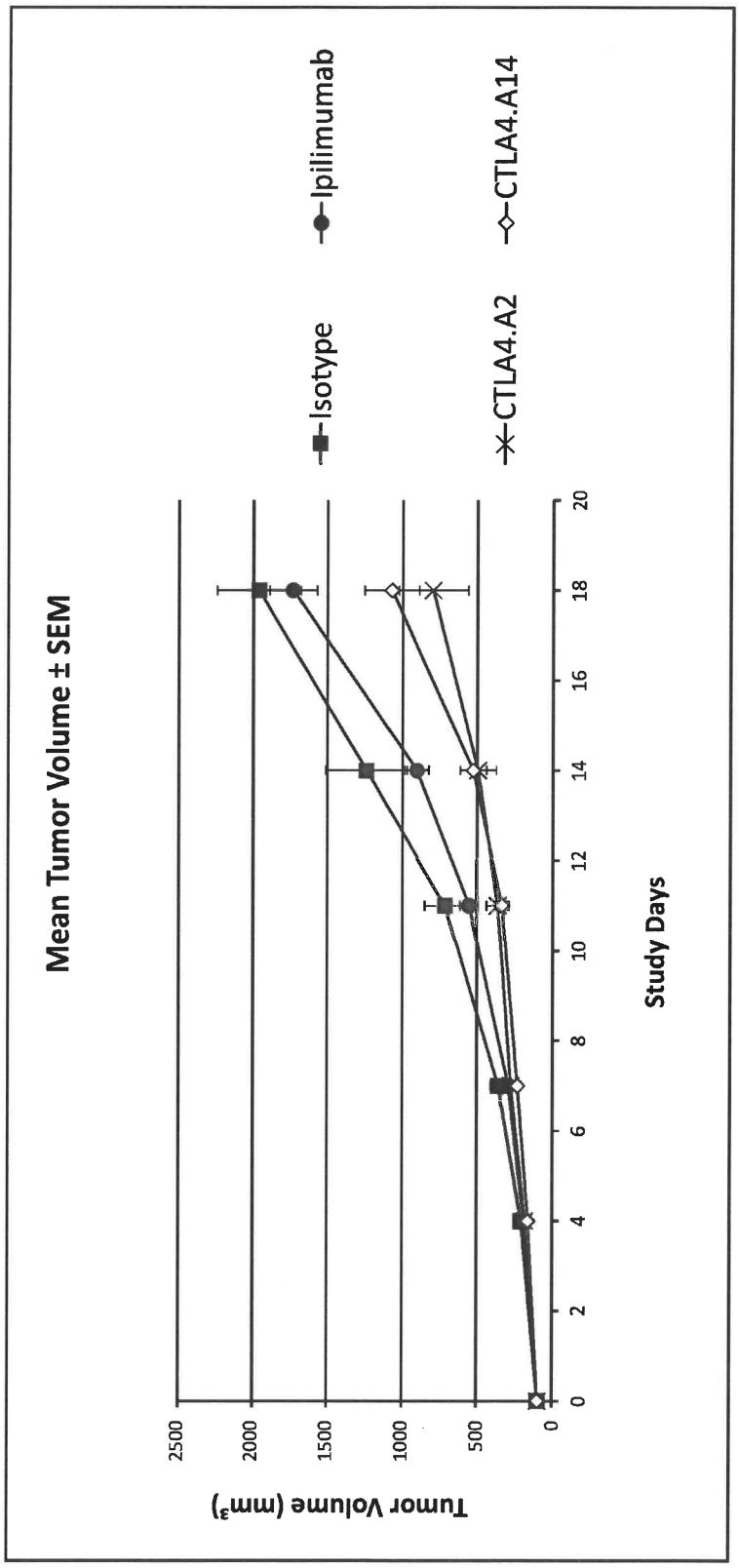

Mean Tumor Volume ± SEM
Isotype
Ipilimumab
CTLA4.A2
CTLA4.A14
Tumor Volume (mm³)
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
18
20
Study Days

FIG. 11

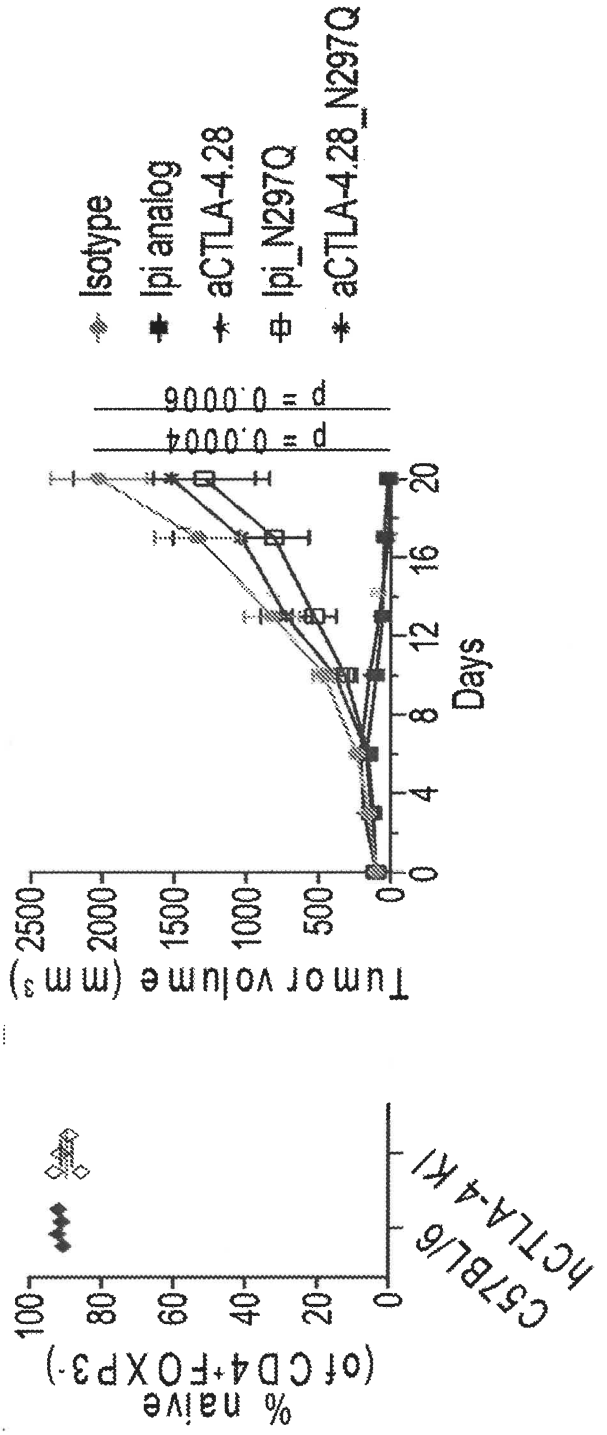


FIG. 12B

FIG. 12A

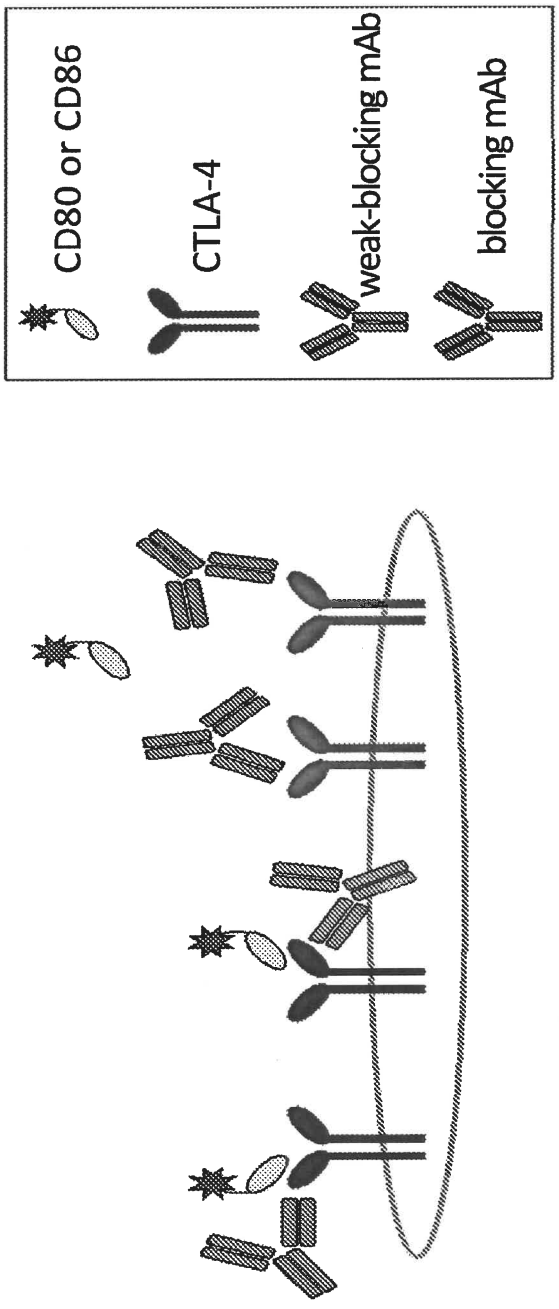


FIG. 13A

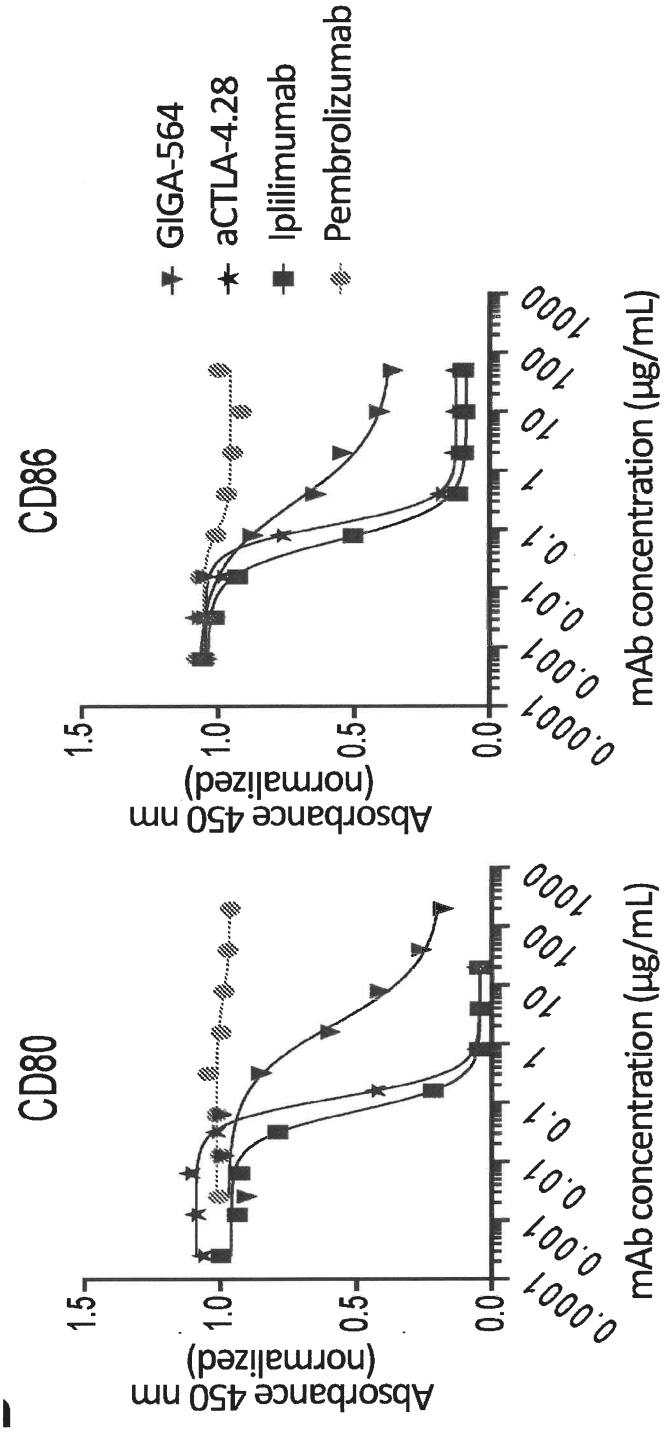

CD80
CD86
GIGA-564
aCTLA-4.28
Iplilimumab
Pembrolizumab
Absorbance 450 nm (normalized)
mAb concentration (µg/mL)
1.5
1.0
0.5
0.0
0.0001
0.001
0.01
0.1
1
10
100
1000

FIG. 13B

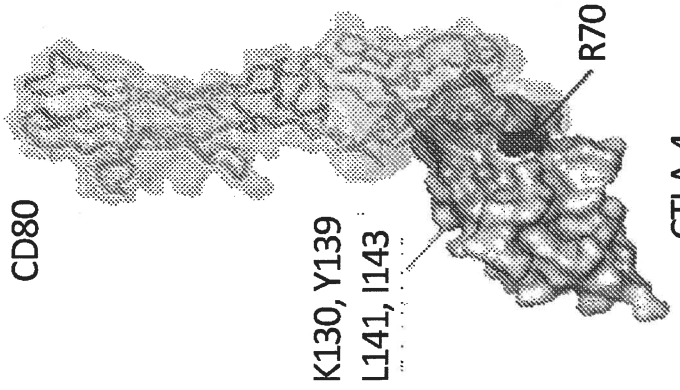


FIG. 13C

	GIGA-564	Iplilimumab	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

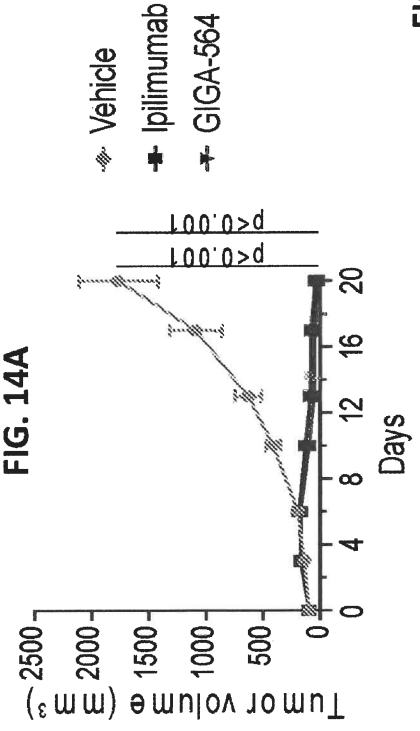


FIG. 14B

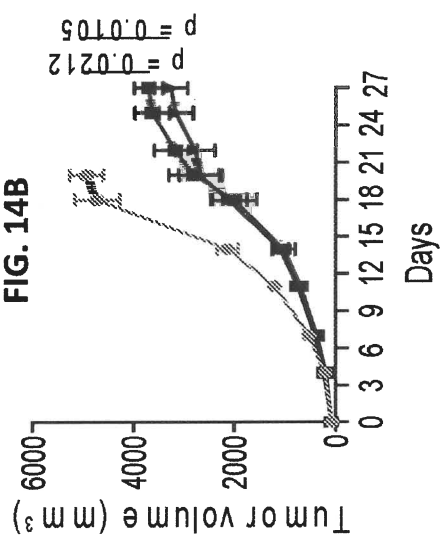
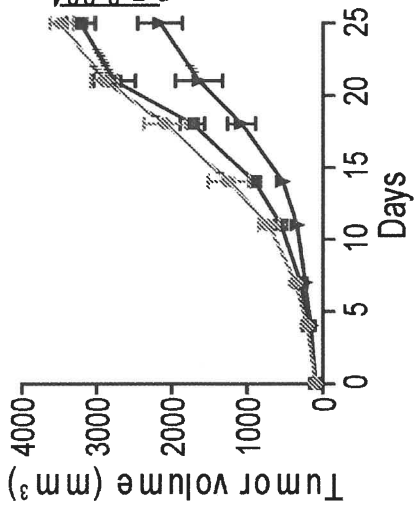


FIG. 14C



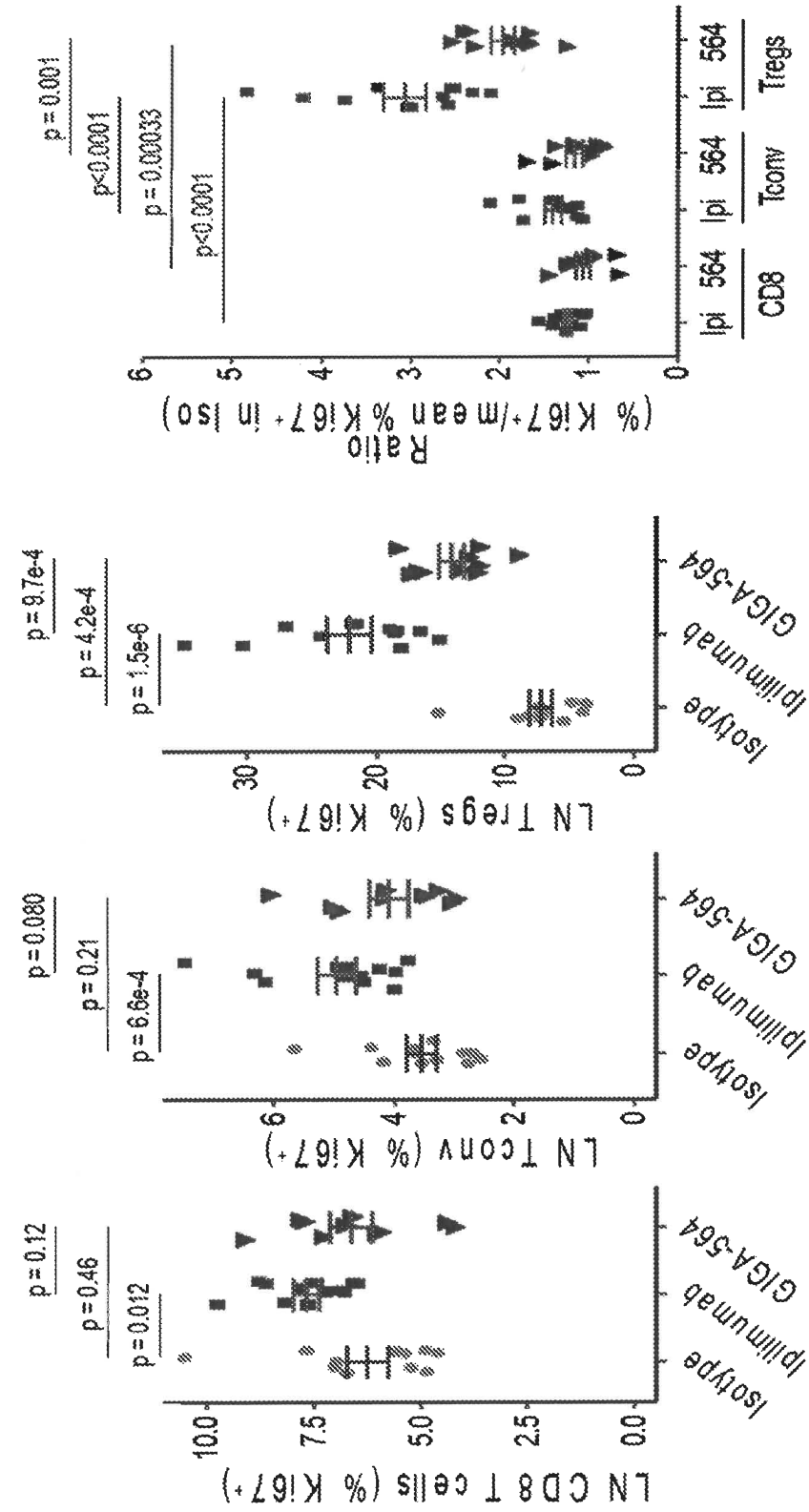

LN CD8 T cells (% Ki67+)
p = 0.12
p = 0.46
p = 0.012
LN Tconv (% Ki67+)
p = 0.080
p = 0.21
p = 6.6e-4
LN Tregs (% Ki67+)
p = 9.7e-4
p = 4.2e-4
p = 1.5e-6
Isotype
Ipilimumab
GIGA-564
Ratio
(% Ki67+/mean % Ki67+ in iso)
p = 0.001
p<0.0001
p = 0.00033
p<0.0001
Ipi 564
Ipi 564
Ipi 564
CD8
Tconv
Tregs

FIG. 15A

FIG. 15B

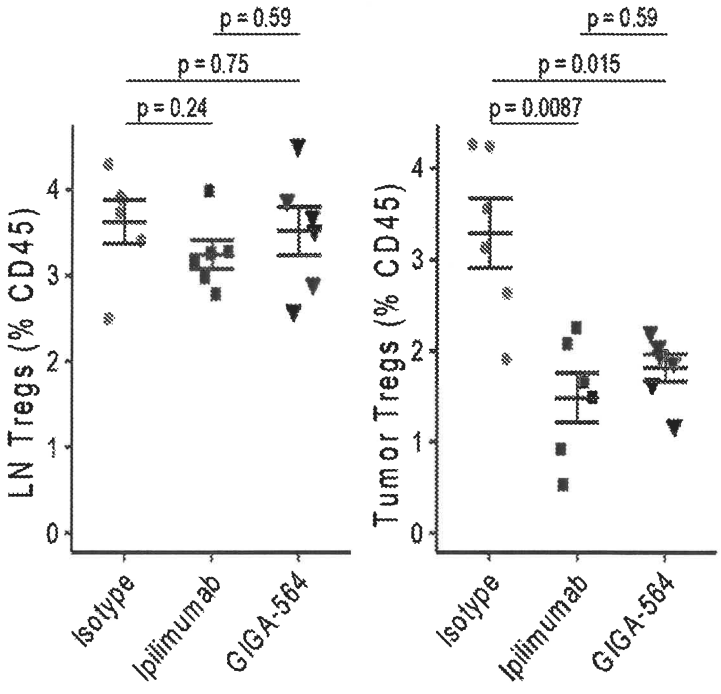
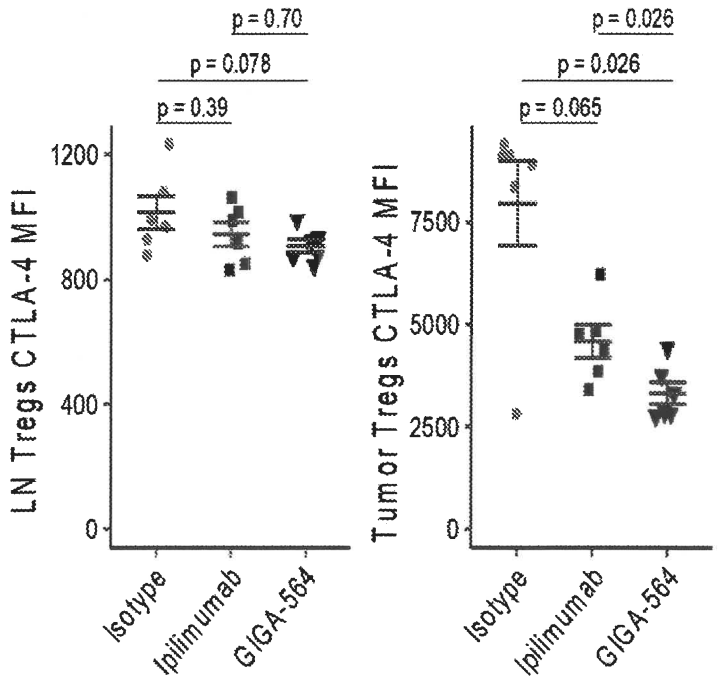


FIG. 15C



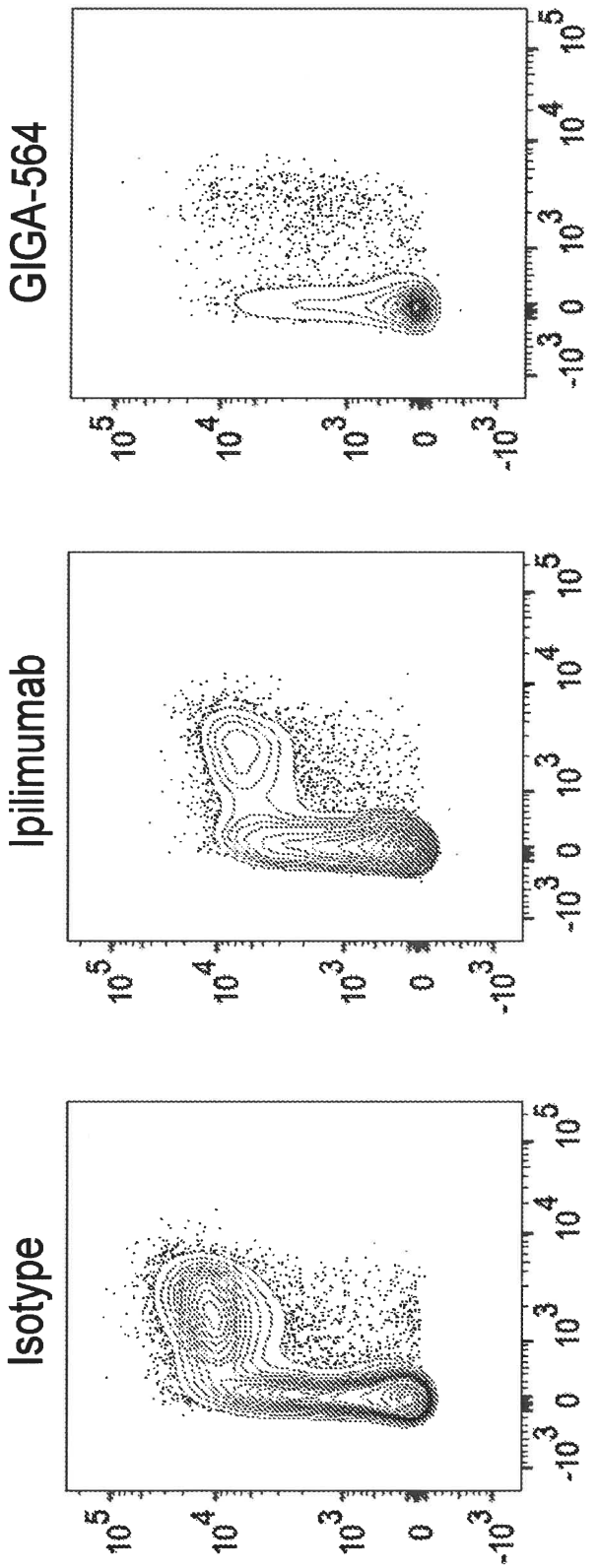


FIG. 15D

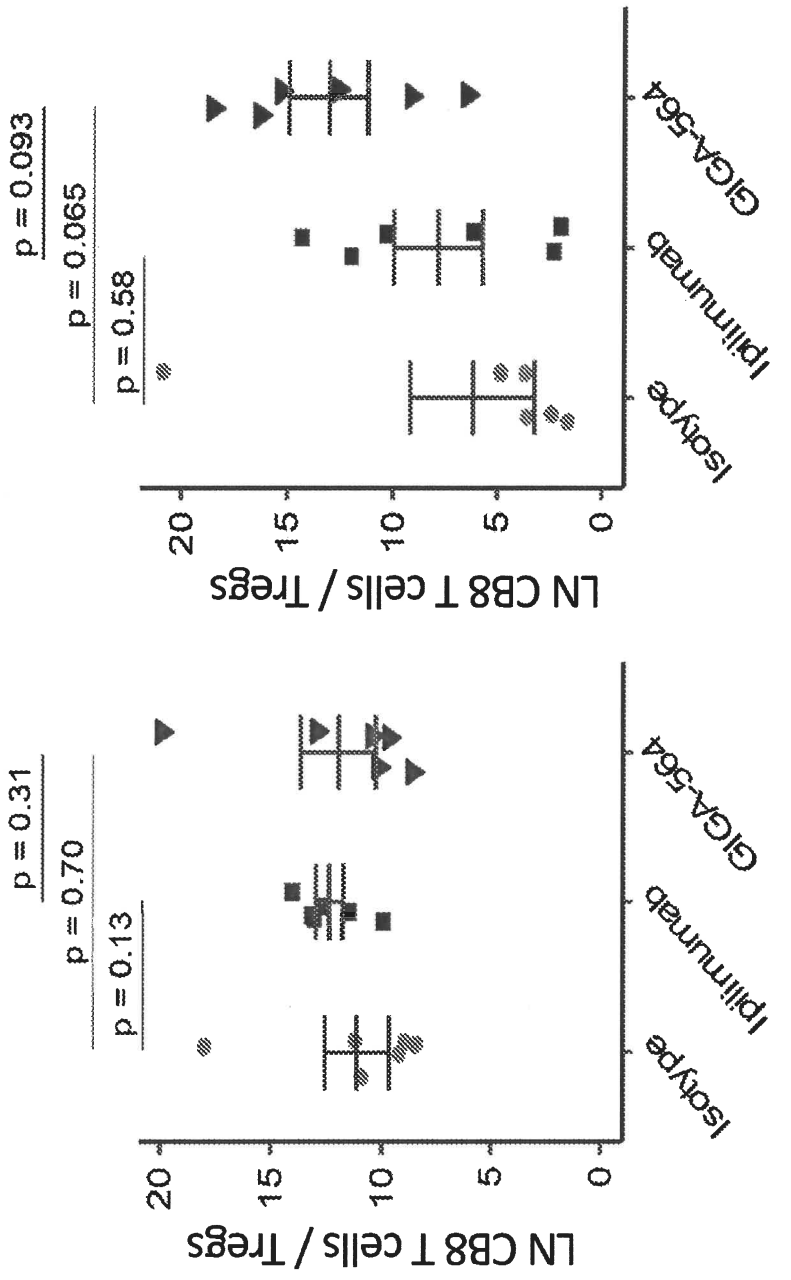

LN CB8 T cells / Tregs
20
15
10
5
0
p = 0.093
p = 0.065
p = 0.58
p = 0.31
p = 0.70
p = 0.13
Isotype
Ipilimumab
GIGA-564

FIG. 15E

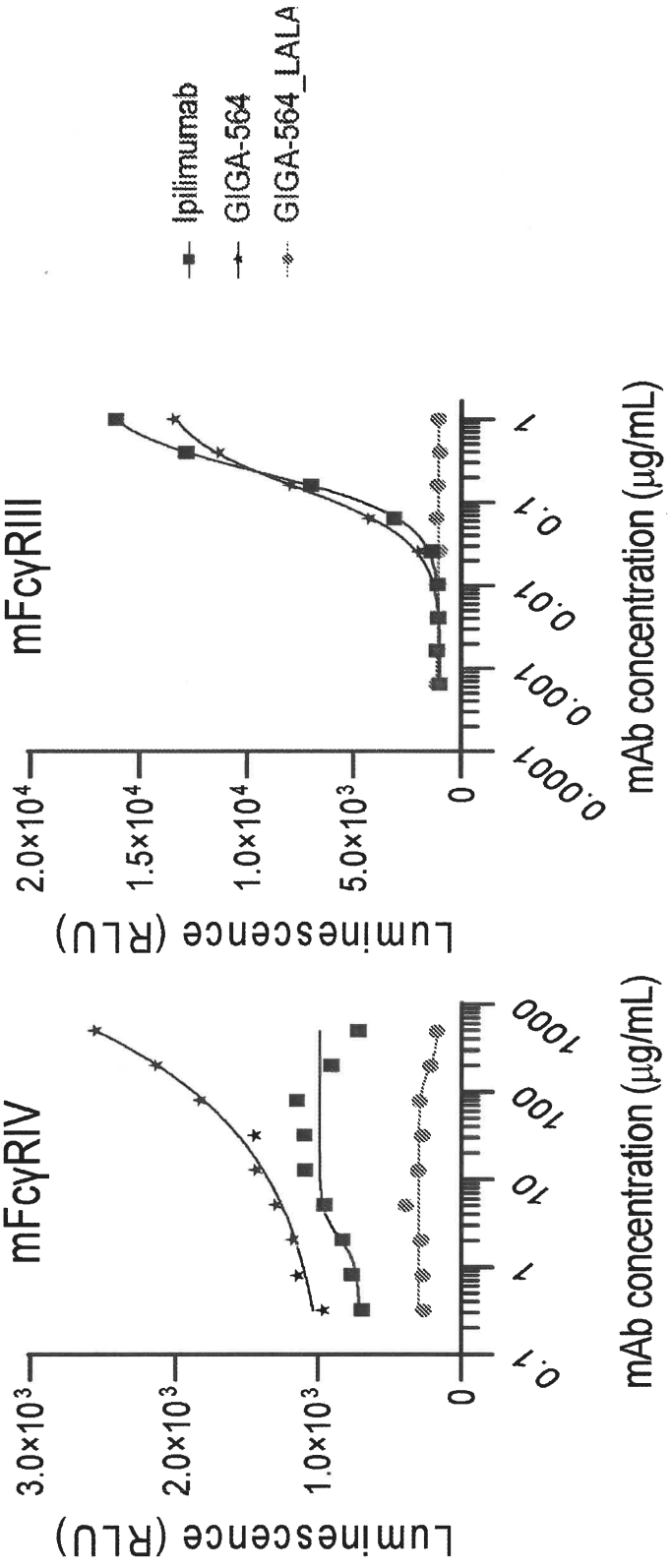

Ipilimumab
GIGA-564
GIGA-564_LALA
mFcγRIII
Luminescence (RLU)
2.0×10^4
1.5×10^4
1.0×10^4
5.0×10^3
0
mAb concentration (μg/mL)
1
0.1
0.01
0.001
0.0001
mFcγRIV
Luminescence (RLU)
3.0×10^3
2.0×10^3
1.0×10^3
0
mAb concentration (μg/mL)
0.1
1
10
100
1000

FIG. 16A

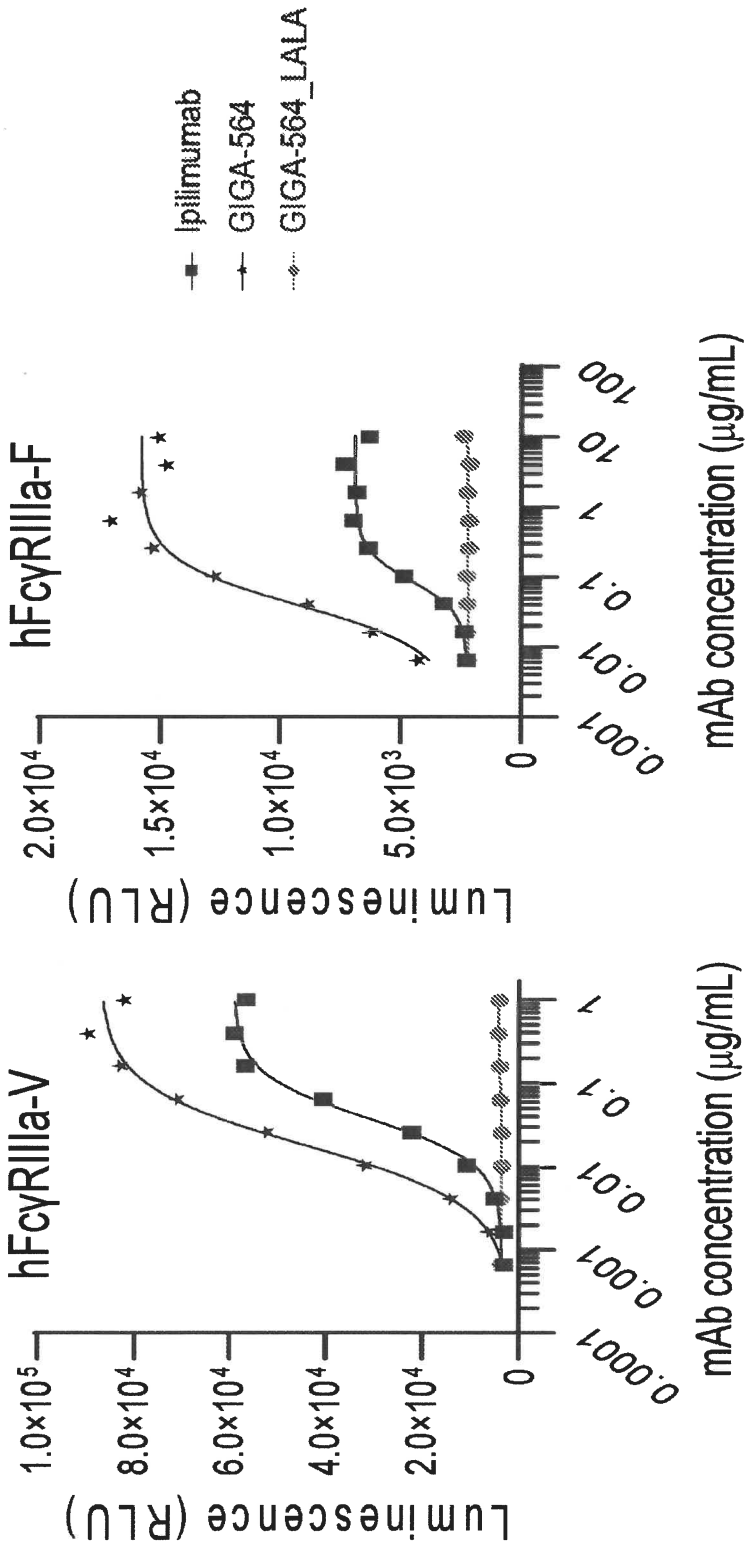

hFcγRIIIa-V
hFcγRIIIa-F
Luminescence (RLU)
mAb concentration (μg/mL)
Ipilimumab
GIGA-564
GIGA-564_LALA

FIG. 16B

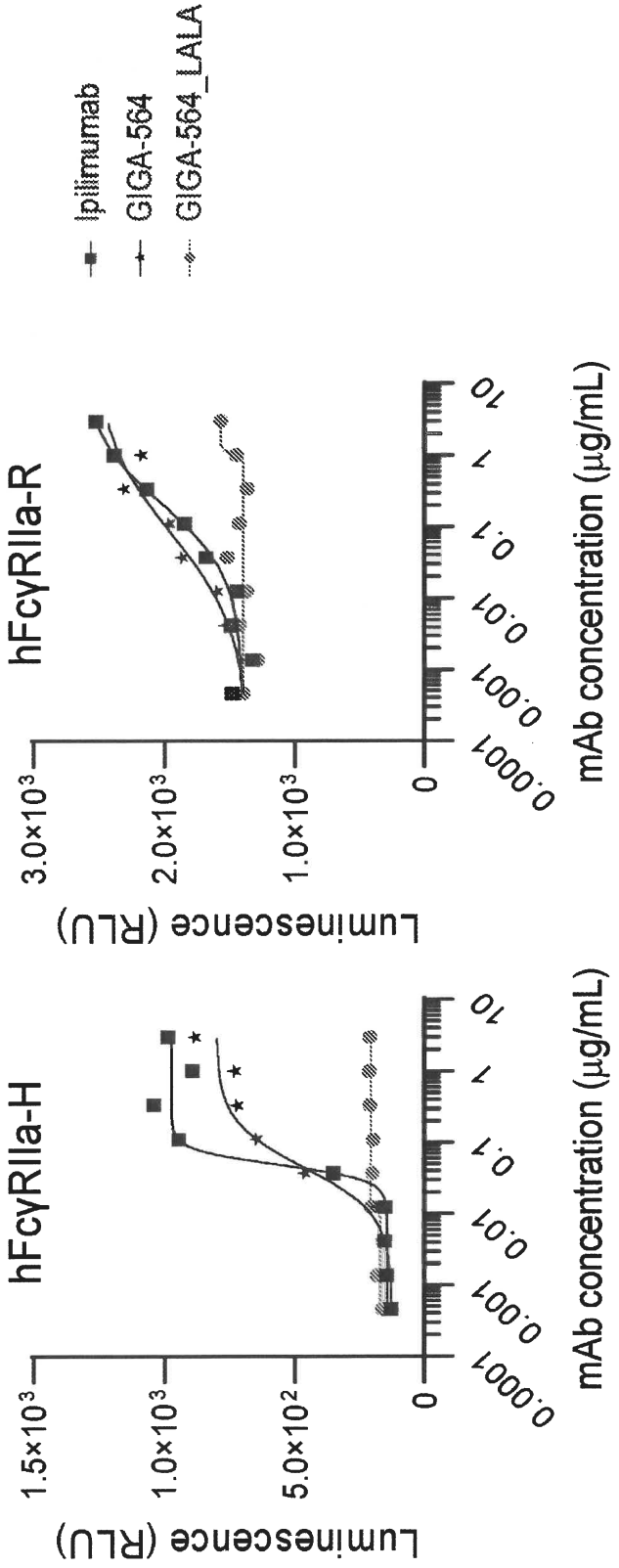

hFcγRIIa-H
hFcγRIIa-R
Luminescence (RLU)
1.5×10³
1.0×10³
5.0×10²
3.0×10³
2.0×10³
1.0×10³
0
0.0001
0.001
0.01
0.1
1
10
mAb concentration (μg/mL)
Ipilimumab
GIGA-564
GIGA-564_LALA

FIG. 16C

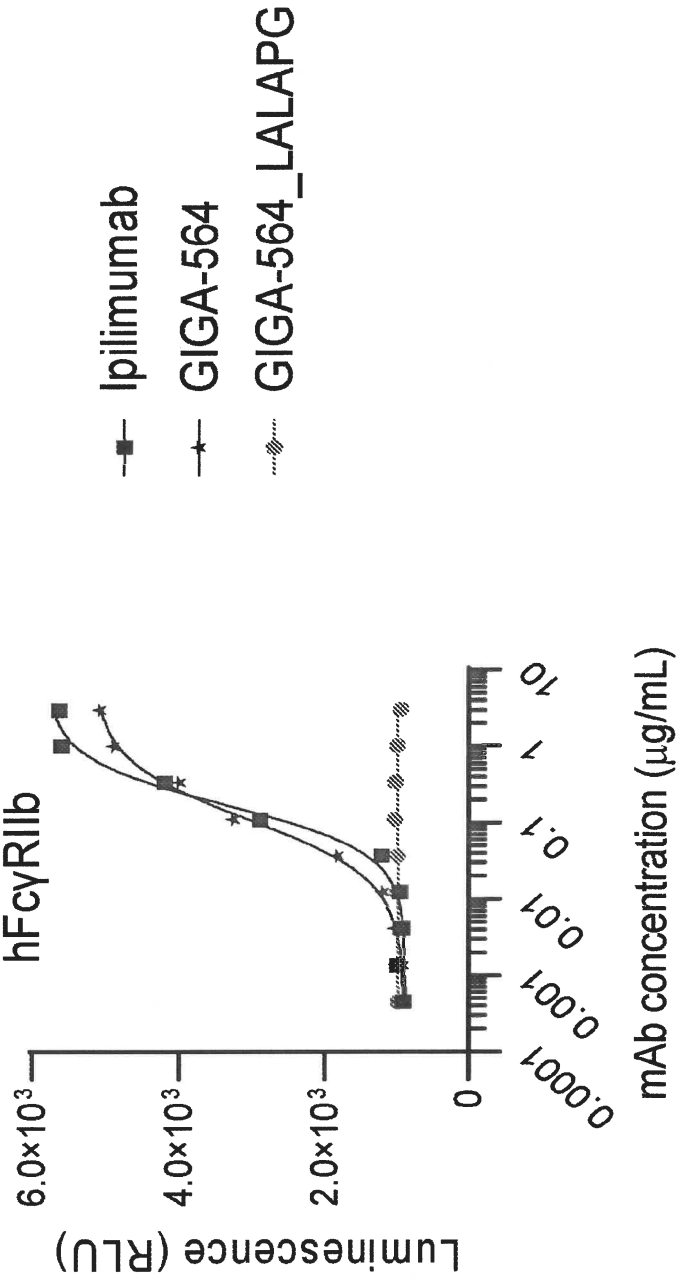

Ipilimumab
GIGA-564
GIGA-564_LALAPG
hFcγRIIb
Luminescence (RLU)
6.0x10³
4.0x10³
2.0x10³
0
0.0001
0.001
0.01
0.1
1
10
mAb concentration (μg/mL)

FIG. 16D

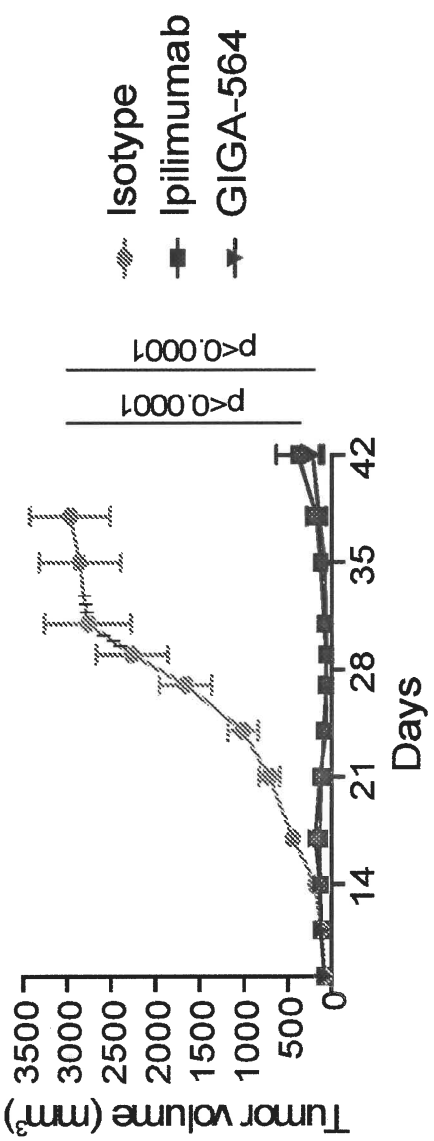


FIG. 17A

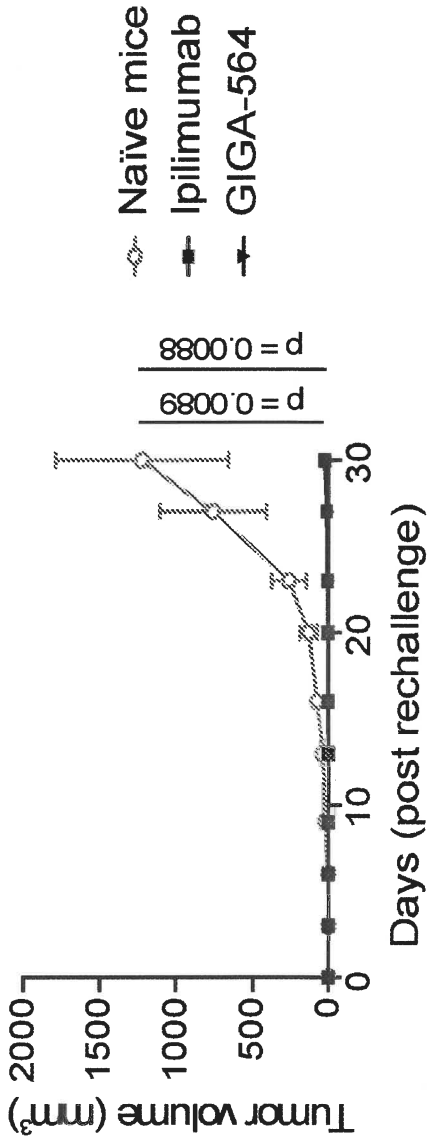


FIG. 17B

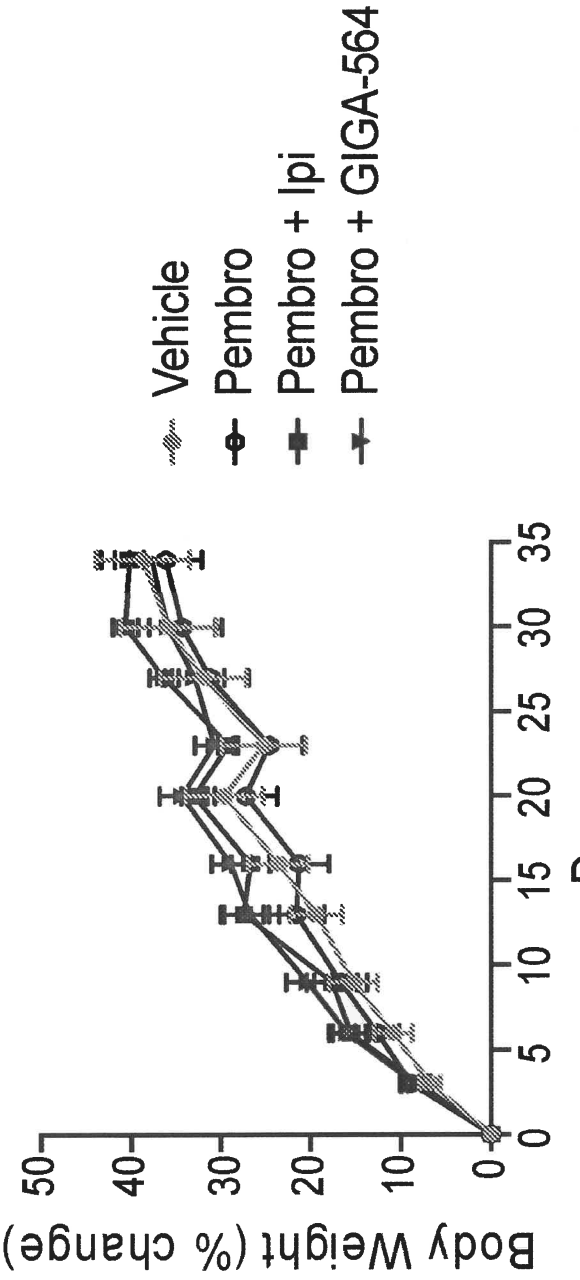

Vehicle
Pembro
Pembro + Ipi
Pembro + GIGA-564
Body Weight (% change)
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Days

FIG. 18A

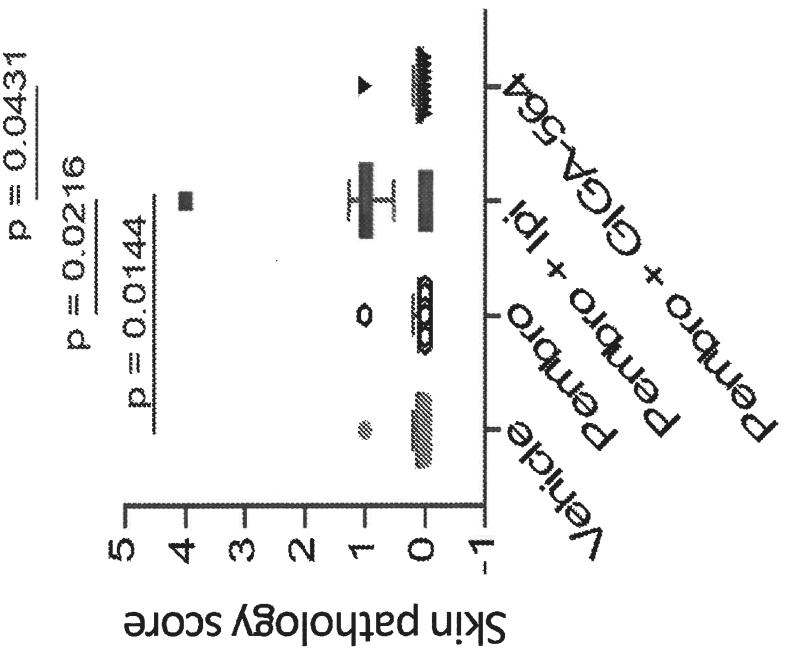


FIG. 18B

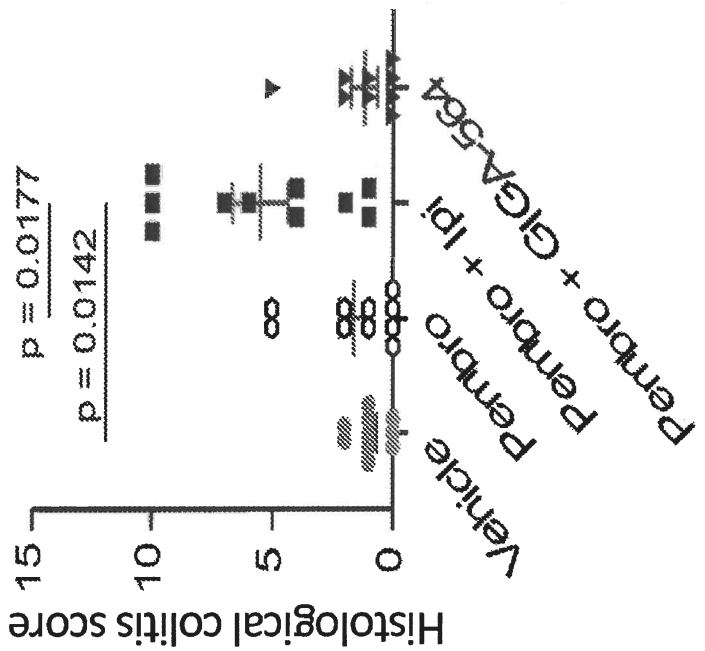
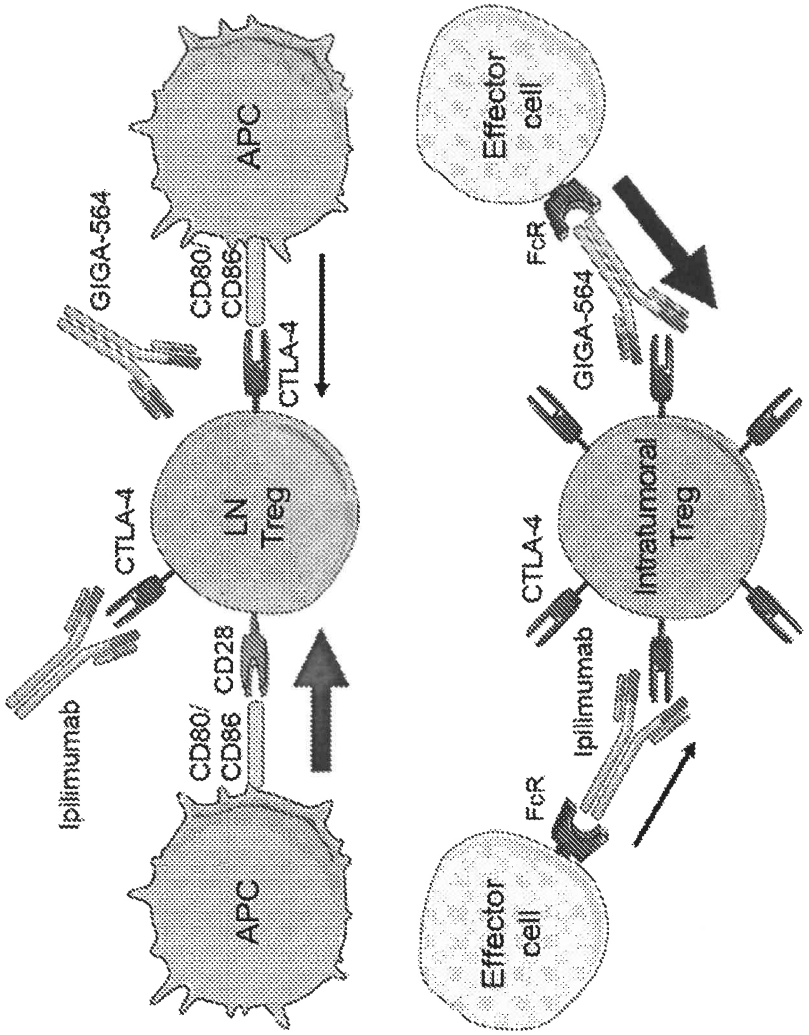


FIG. 18C



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

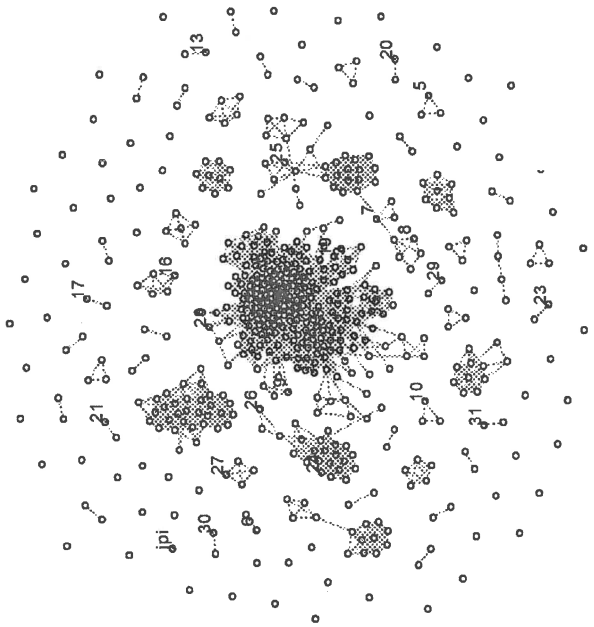


FIG. 20A

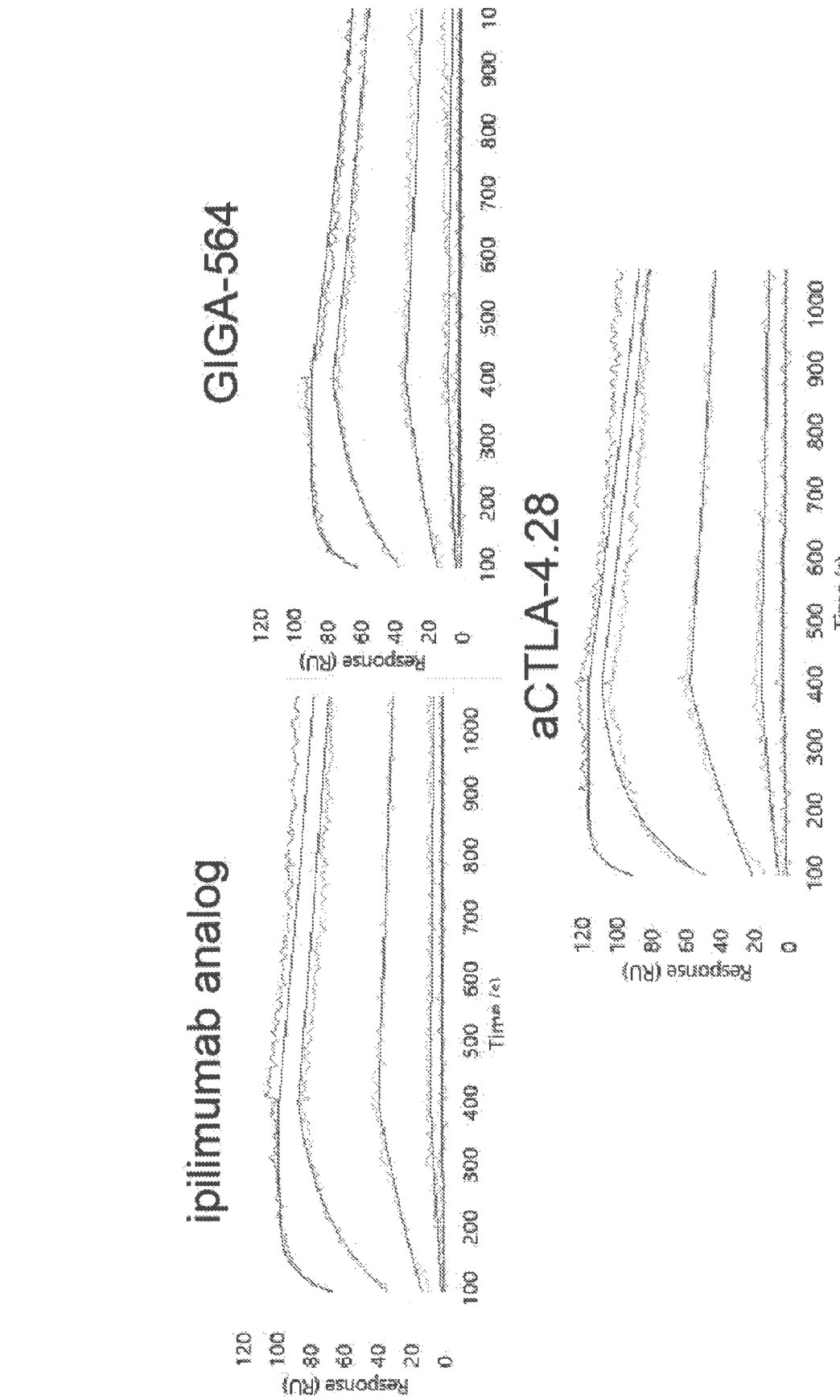

ipilimumab analog
GIGA-564
aCTLA-4.28
Response (RU)
Time (s)
0
20
40
60
80
100
120
100
200
300
400
500
600
700
800
900
1000

FIG. 20B

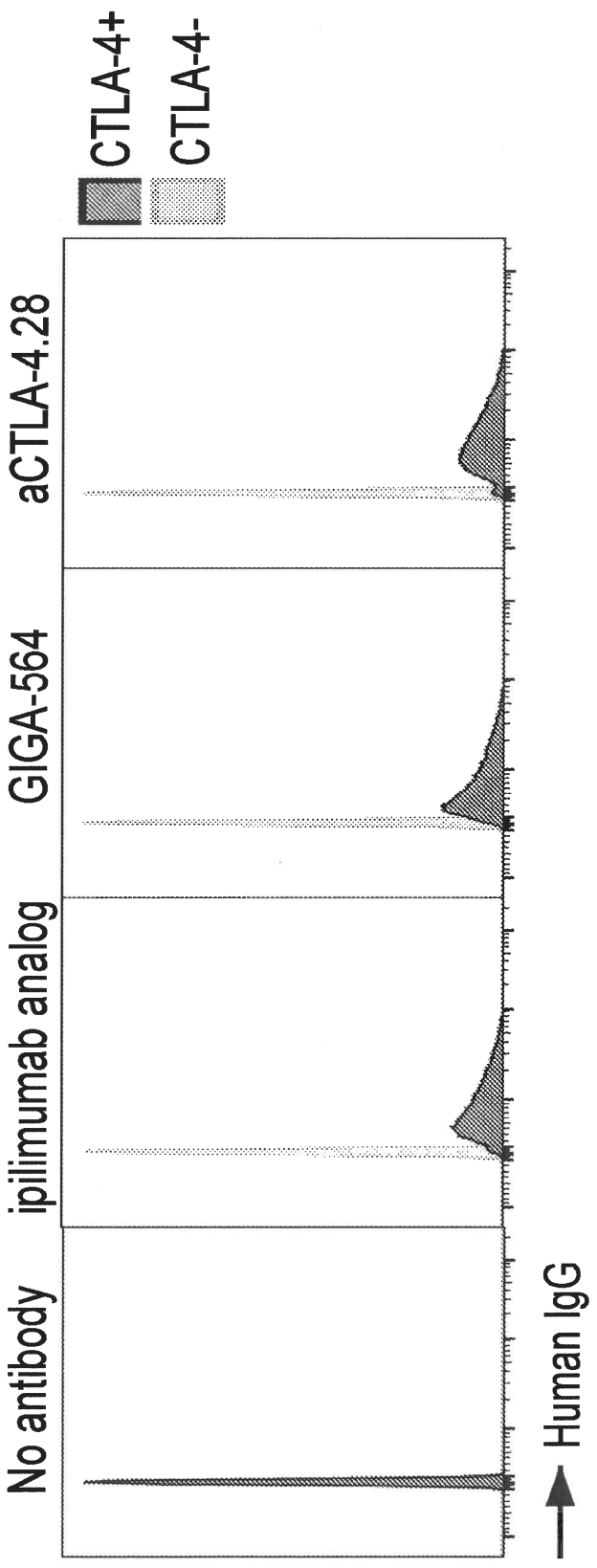

CTLA-4+
CTLA-4-
aCTLA-4.28
GIGA-564
ipilimumab analog
No antibody
Human IgG

FIG. 20C

FIG. 20D

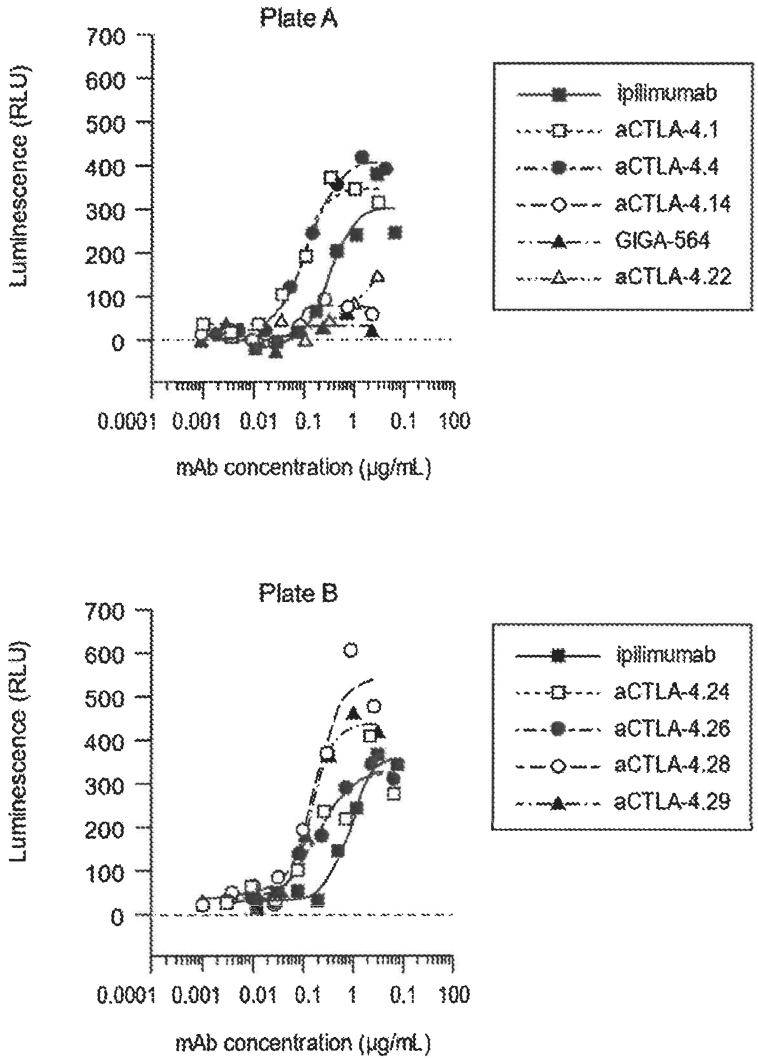


Plate A
Luminescence (RLU)
700
600
500
400
300
200
100
0
0.0001 0.001 0.01 0.1 1 0.1 100
mAb concentration (µg/mL)
ipilimumab
aCTLA-4.1
aCTLA-4.4
aCTLA-4.14
GIGA-564
aCTLA-4.22
Plate B
Luminescence (RLU)
700
600
500
400
300
200
100
0
0.0001 0.001 0.01 0.1 1 0.1 100
mAb concentration (µg/mL)
ipilimumab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

FIG. 20D (Cont.)

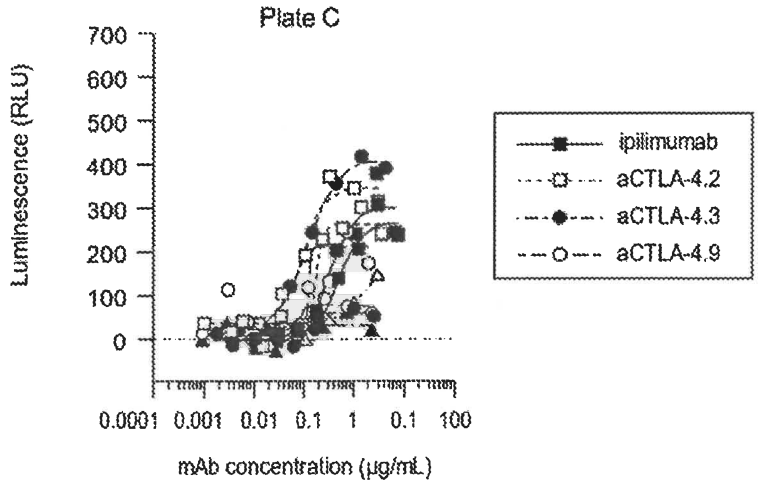


Plate C
Luminescence (RLU)
700
600
500
400
300
200
100
0
0.0001 0.001 0.01 0.1 1 0.1 100
mAb concentration (µg/mL)
ipilimumab
aCTLA-4.2
aCTLA-4.3
aCTLA-4.9

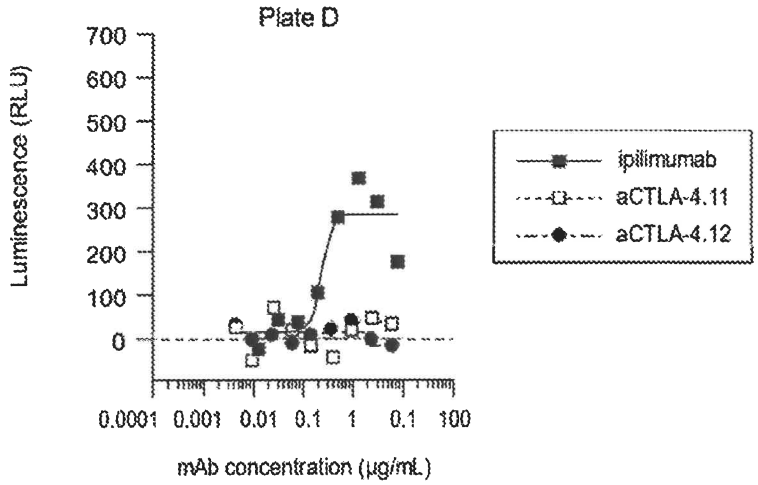


Plate D
Luminescence (RLU)
700
600
500
400
300
200
100
0
0.0001 0.001 0.01 0.1 1 0.1 100
mAb concentration (µg/mL)
ipilimumab
aCTLA-4.11
aCTLA-4.12

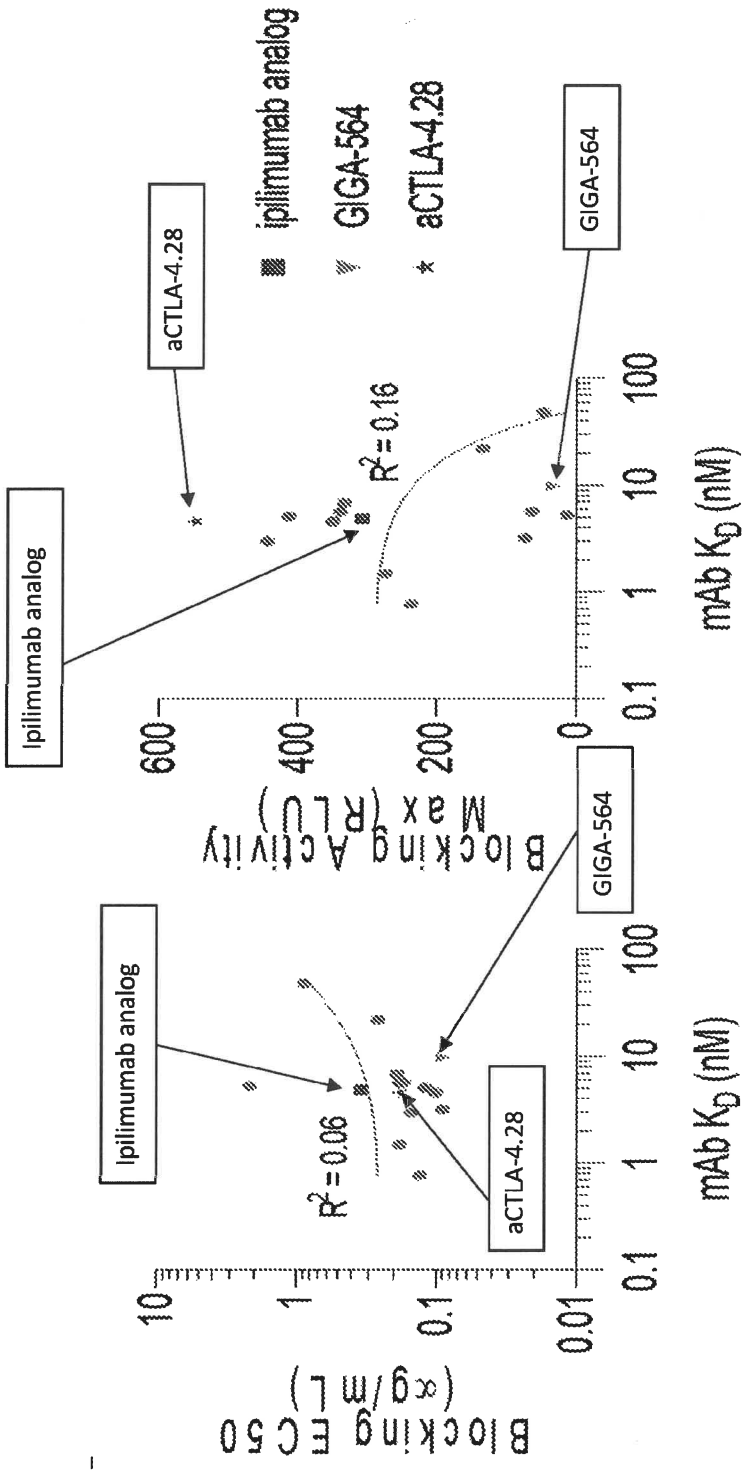

Ipilimumab analog
aCTLA-4.28
GIGA-564
$R^2 = 0.06$
$R^2 = 0.16$
Blocking EC50 (αg/mL)
Blocking Activity Max (RLU)
mAb K_D (nM)
ipilimumab analog
GIGA-564
aCTLA-4.28

FIG. 20E

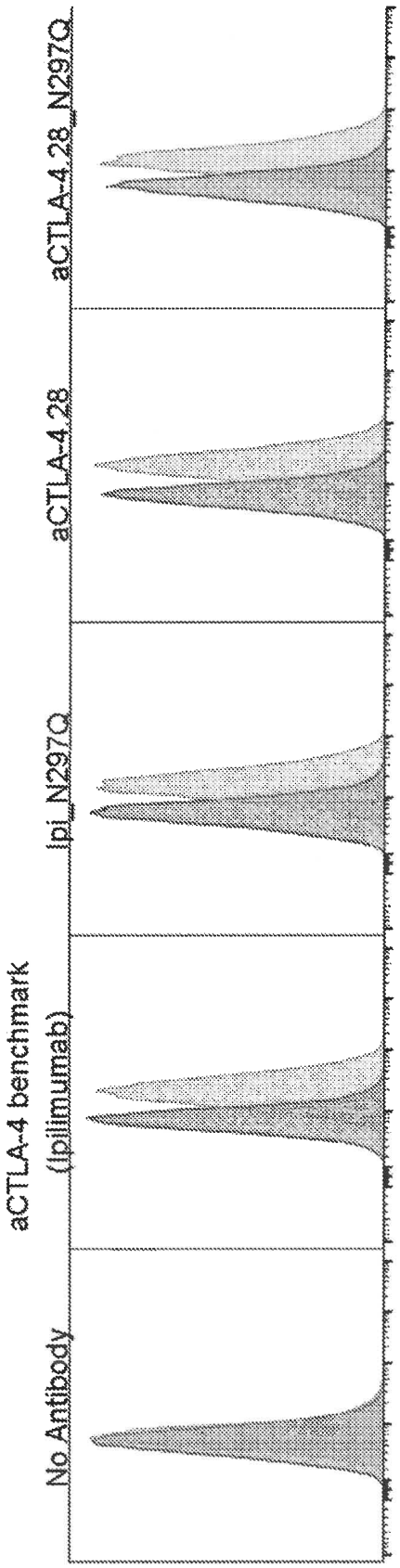

No Antibody
aCTLA-4 benchmark (Ipilimumab)
Ipi_N297Q
aCTLA-4.28
aCTLA-4.28_N297Q

FIG. 21

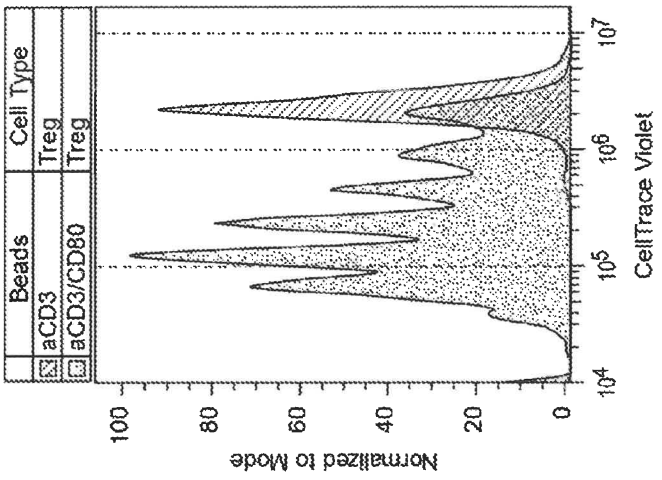


FIG. 22B

FIG. 22A

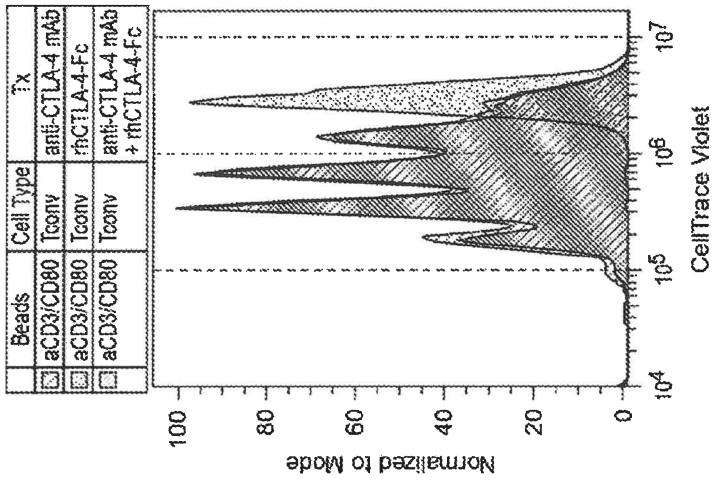


FIG. 22C

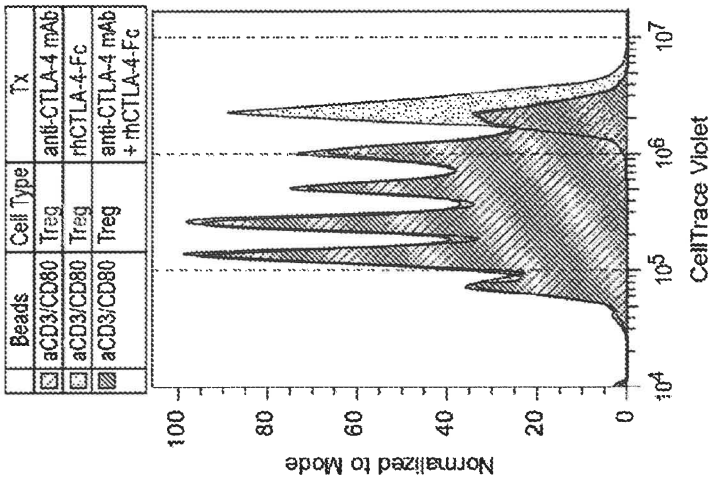


FIG. 22D

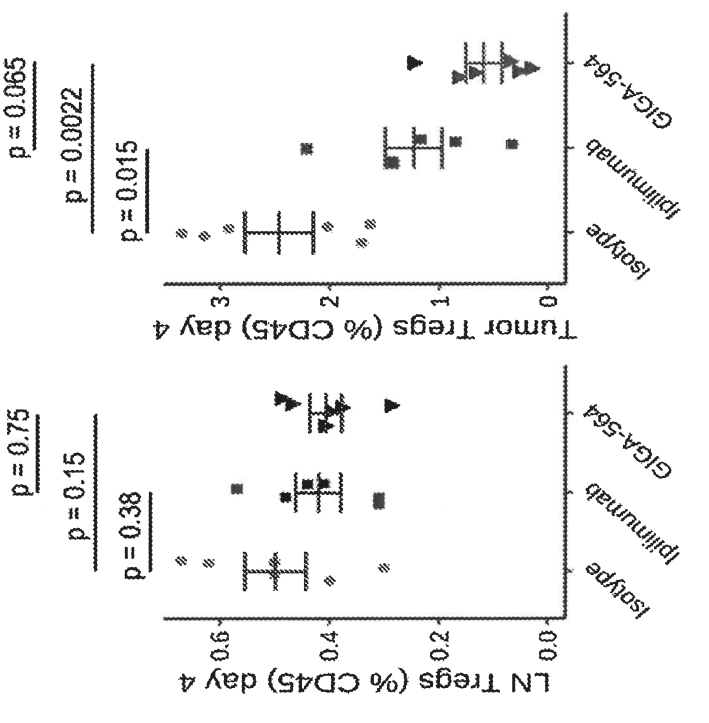


FIG. 23A

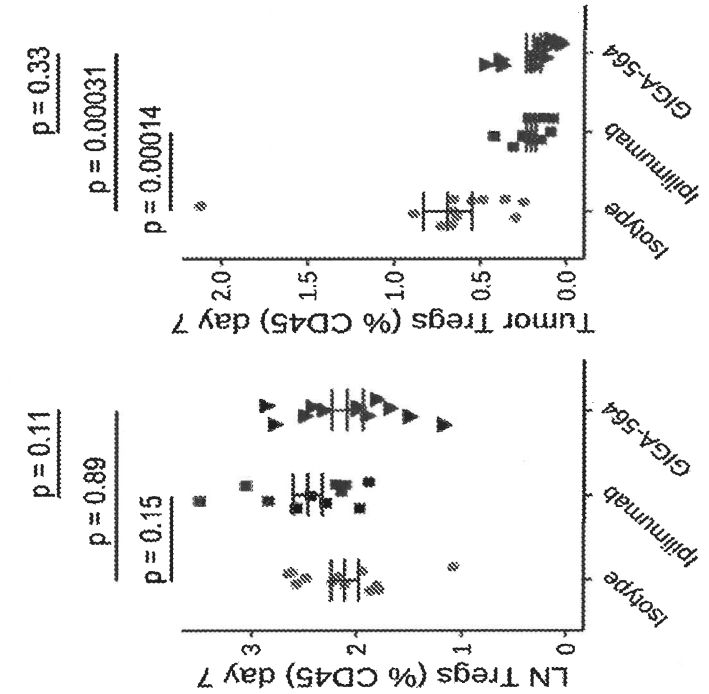


FIG. 23B

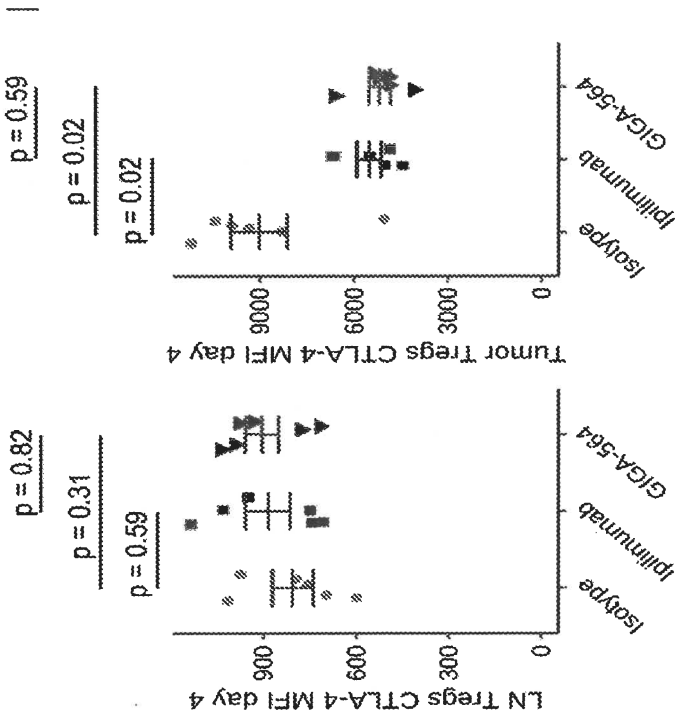


FIG. 23C

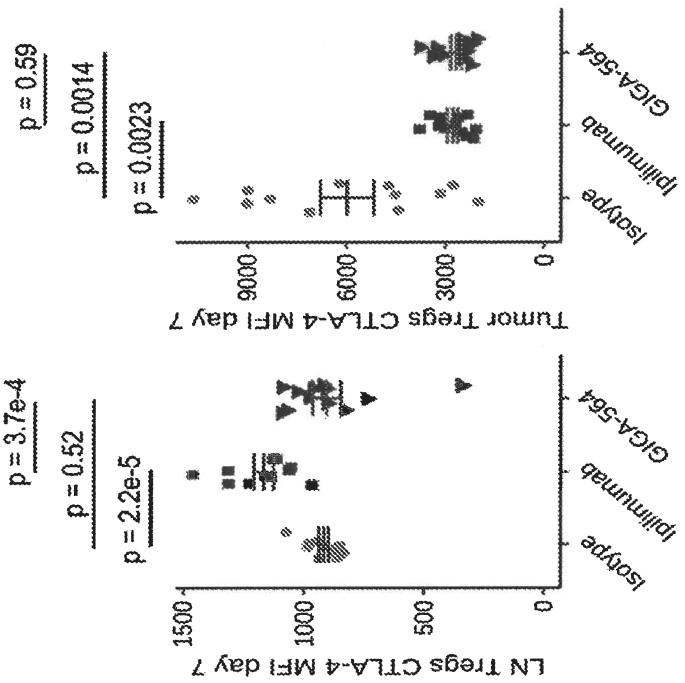
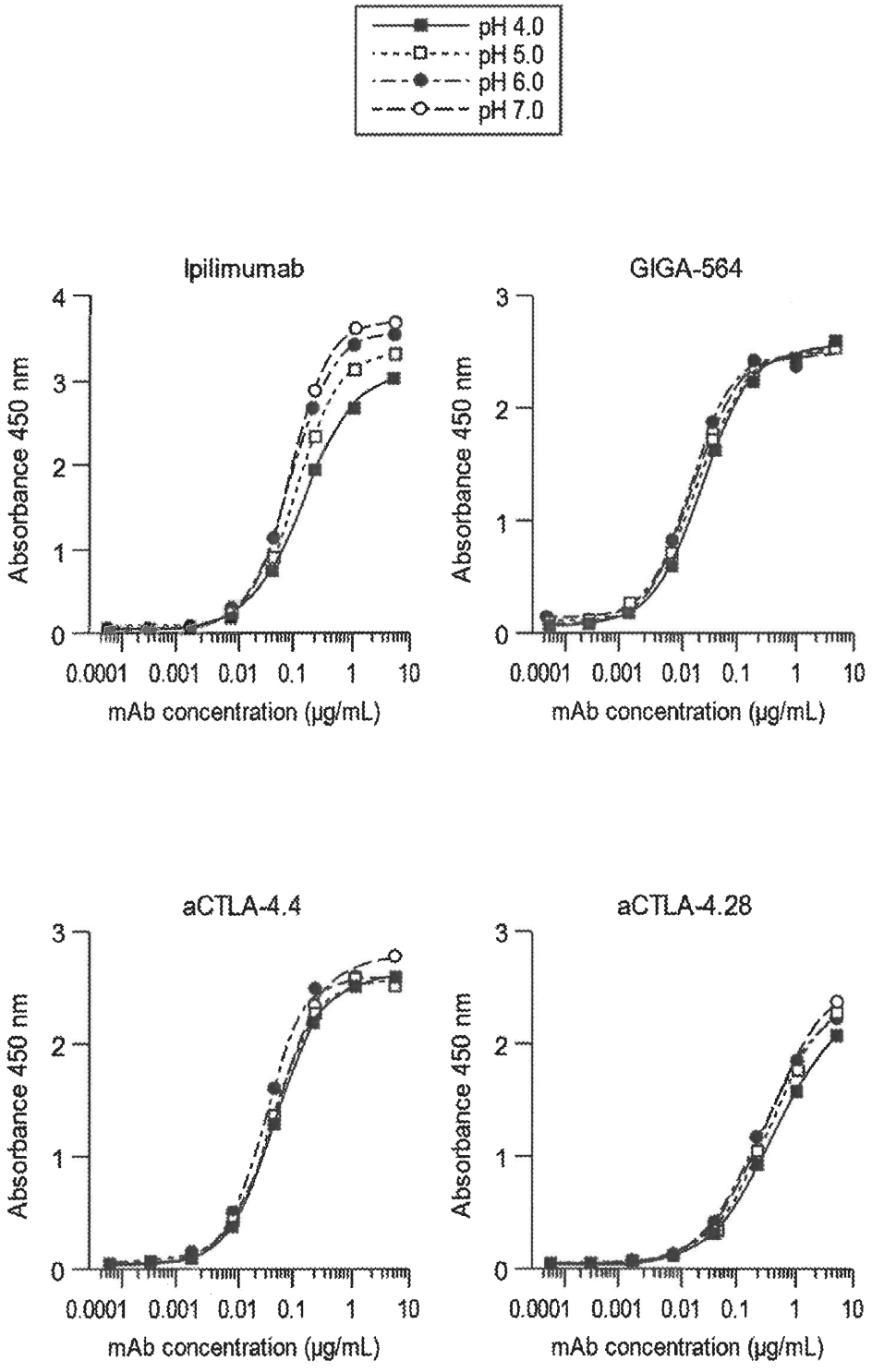


FIG. 23D

FIG. 24A


pH 4.0
pH 5.0
pH 6.0
pH 7.0
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
0
1
2
3
4

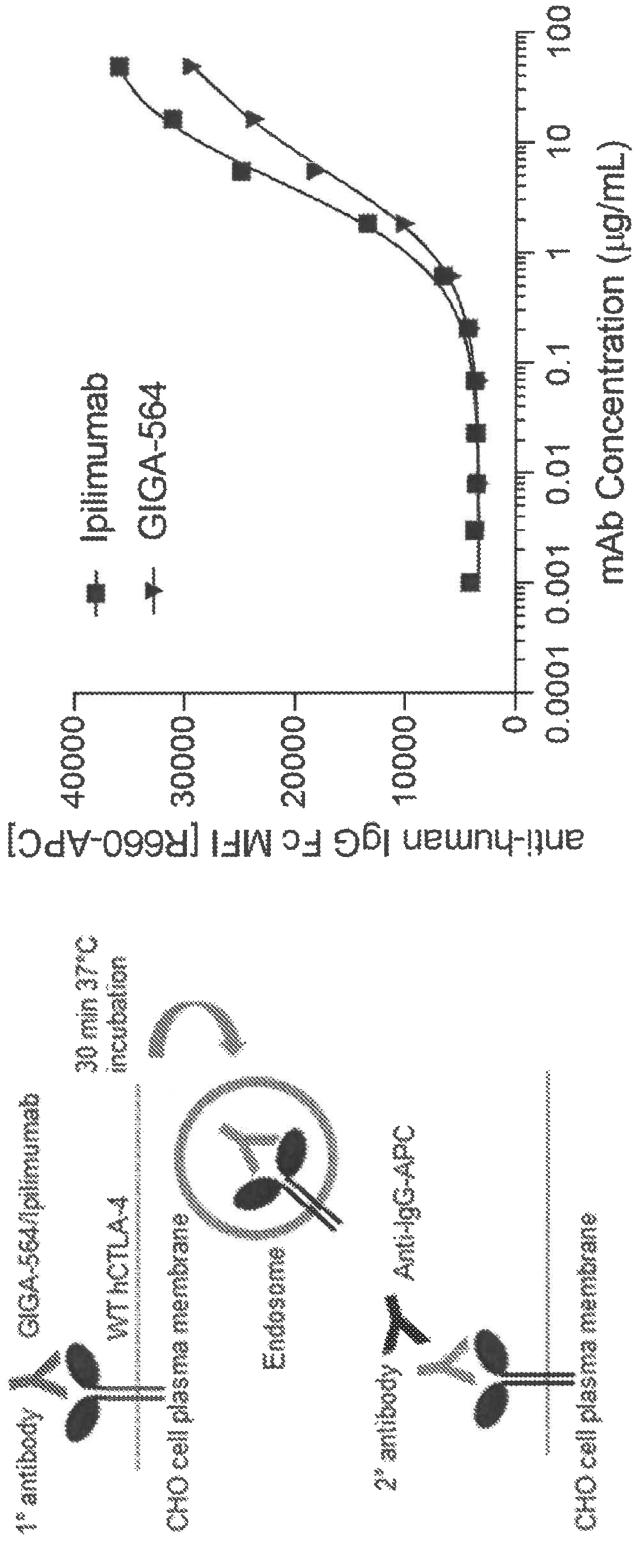

anti-human IgG Fc MFI [R660-APC]
40000
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
100
mAb Concentration (µg/mL)
Ipilimumab
GIGA-564
1° antibody
GIGA-564/ipilimumab
WT hCTLA-4
CHO cell plasma membrane
30 min 37°C incubation
Endosome
2° antibody
Anti-IgG-APC
CHO cell plasma membrane

FIG. 24B

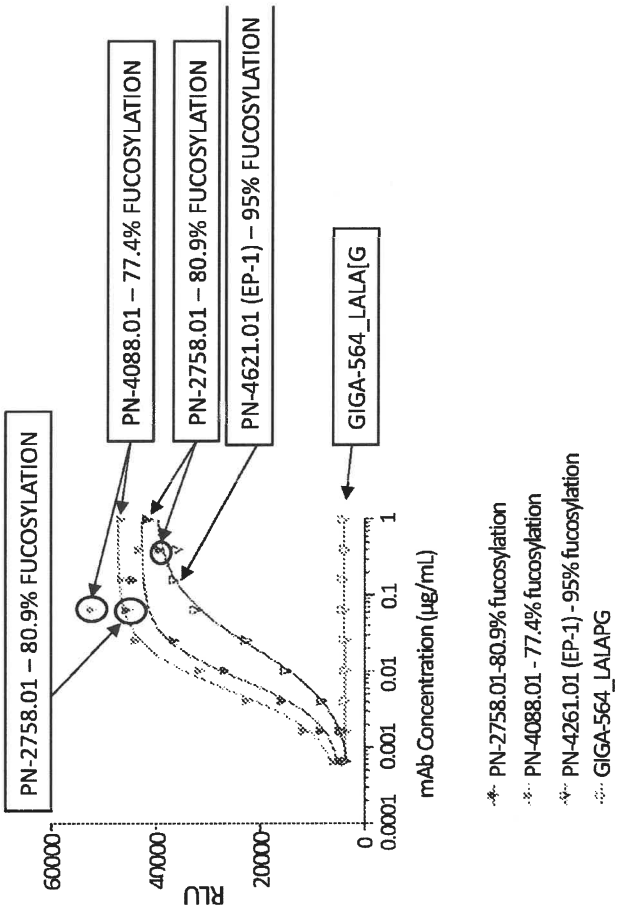


FIG. 24D

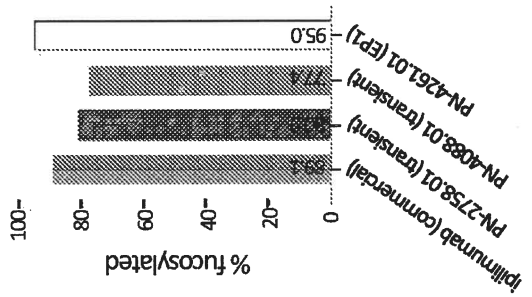


FIG. 24C

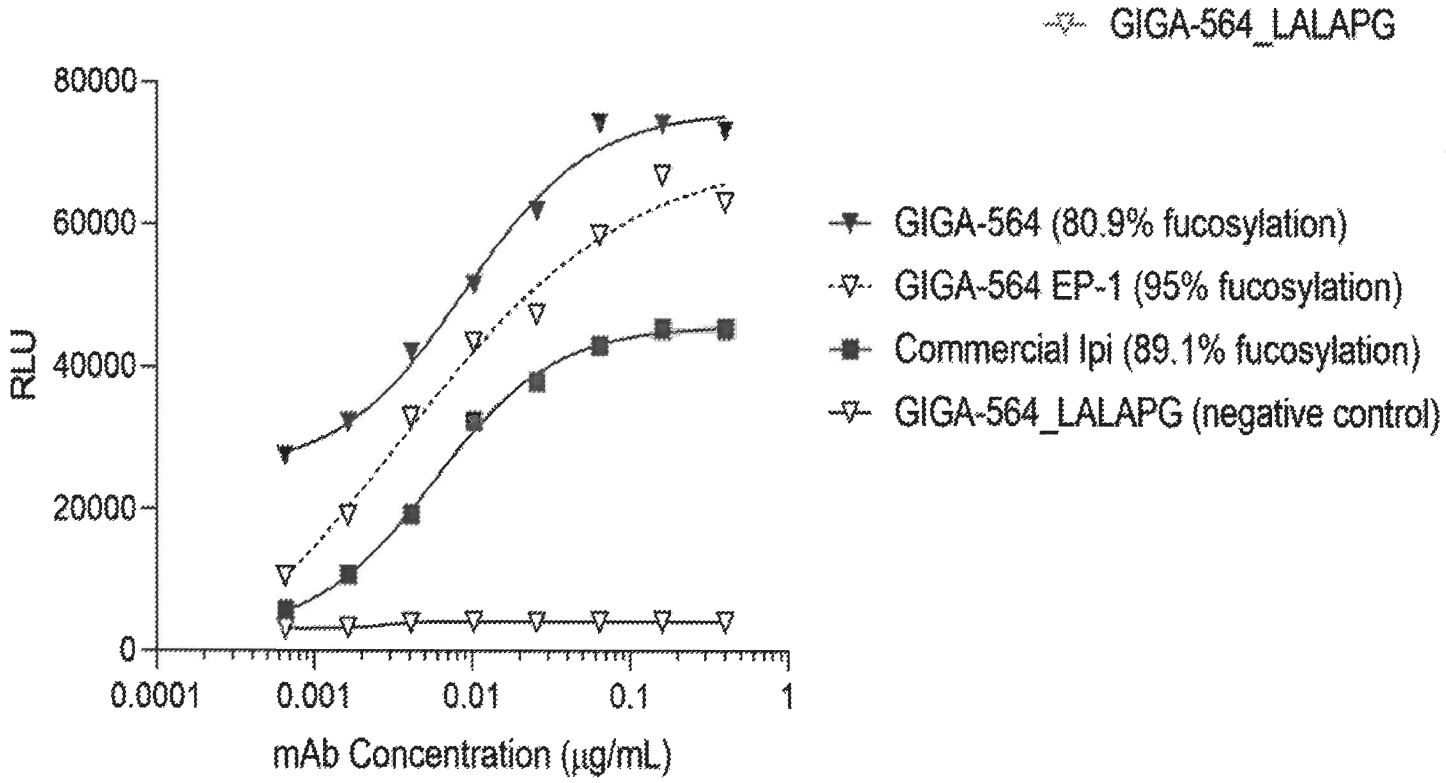

GIGA-564_LALAPG
80000
60000
40000
20000
0
RLU
0.0001
0.001
0.01
0.1
1
mAb Concentration (μg/mL)
GIGA-564 (80.9% fucosylation)
GIGA-564 EP-1 (95% fucosylation)
Commercial Ipi (89.1% fucosylation)
GIGA-564_LALAPG (negative control)

FIG. 24E



FIG. 25A

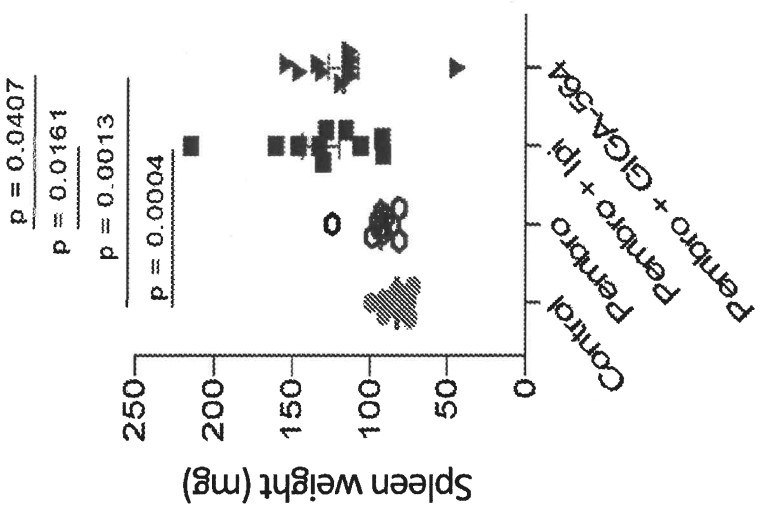


FIG. 25B

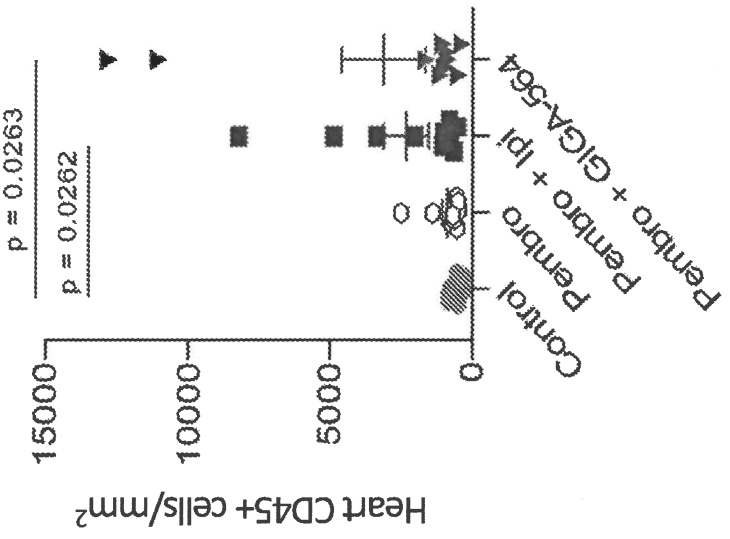


FIG. 25C

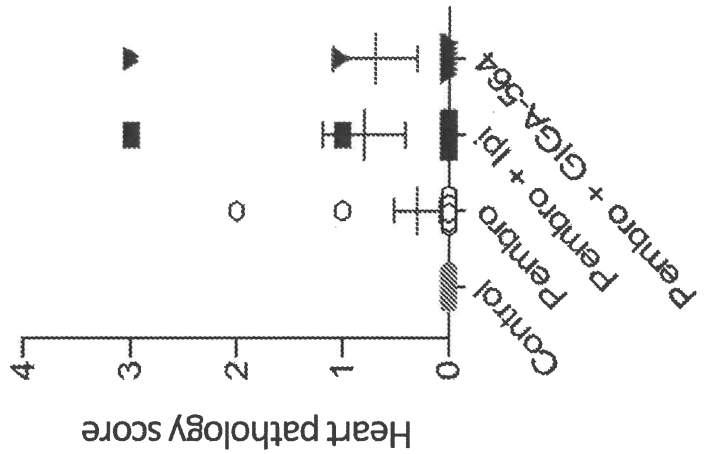


FIG. 25D

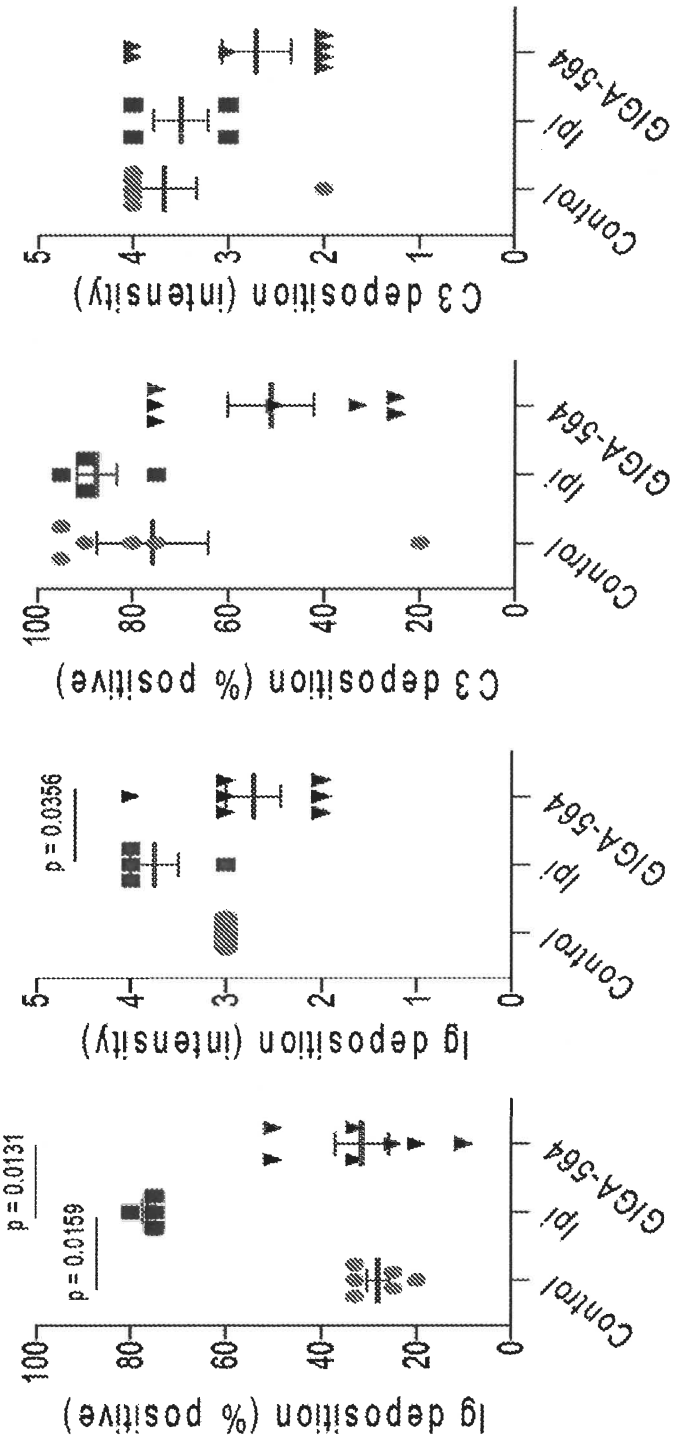

Ig deposition (% positive)
p = 0.0131
p = 0.0159
Ig deposition (intensity)
p = 0.0356
C3 deposition (% positive)
C3 deposition (intensity)
Control
Ipi
GIGA-564

FIG. 25E

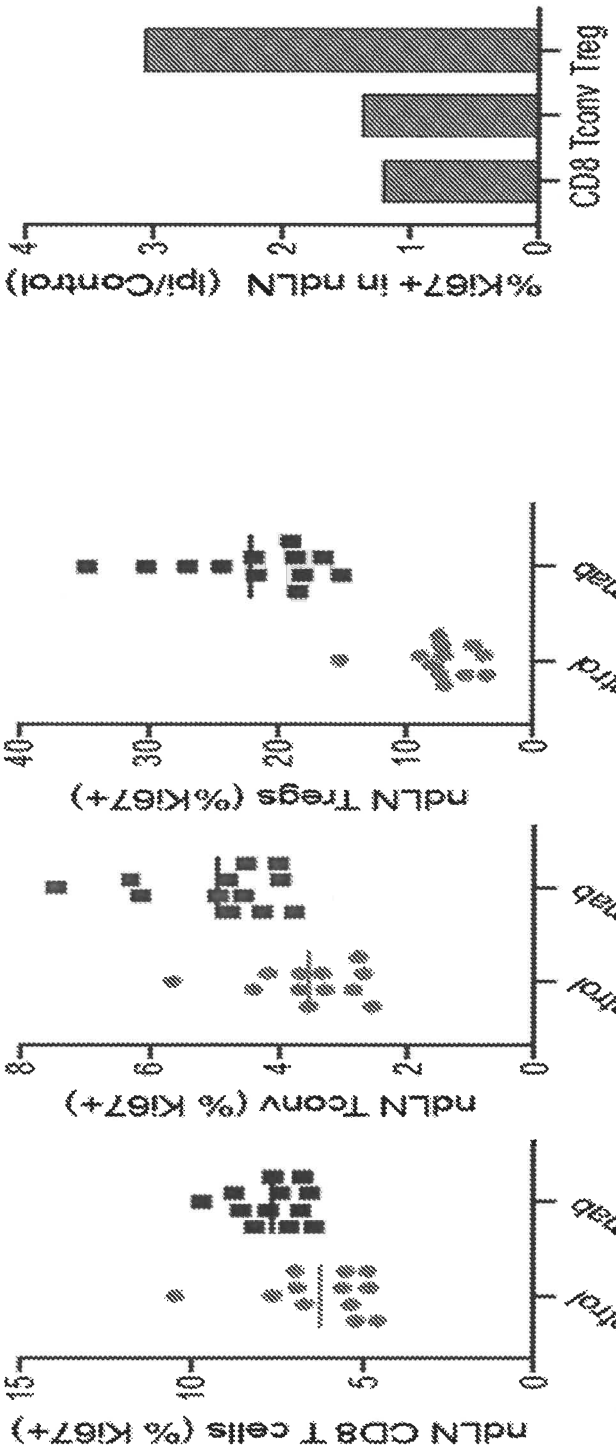

%Ki67+ in ndLN (Ipi/Control)
CD8 Tconv Treg
ndLN CD8 T cells (% Ki67+)
ndLN Tconv (% Ki67+)
ndLN Tregs (% Ki67+)
Control
Ipilimumab

FIG. 26

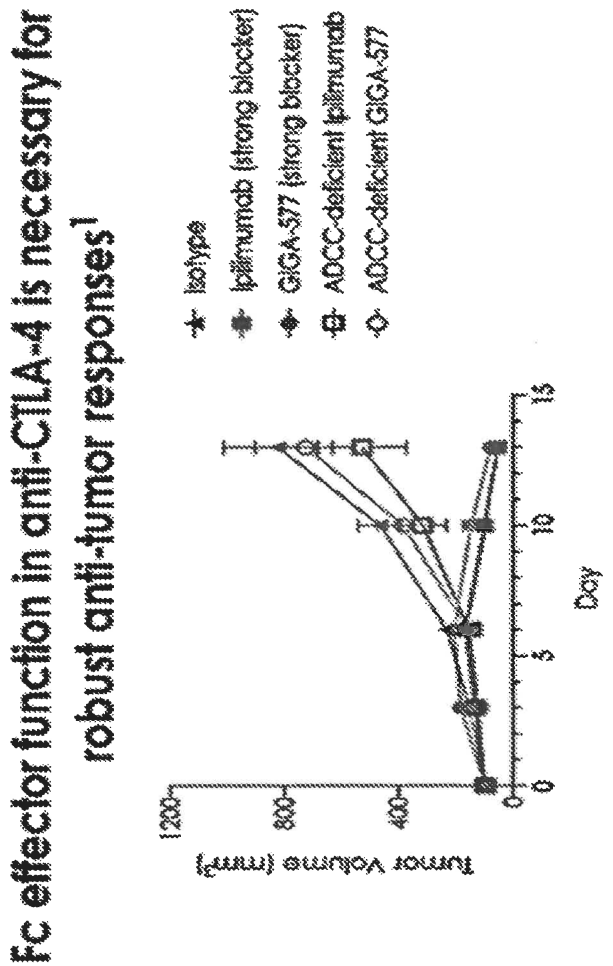

Fc effector function in anti-CTLA-4 is necessary for robust anti-tumor responses[1]
Isotype
Ipilimumab (strong blocker)
GIGA-577 (strong blocker)
ADCC-deficient Ipilimumab
ADCC-deficient GIGA-577
Tumor Volume (mm³)
1200
800
400
0
0
5
10
15
Day

FIG. 27

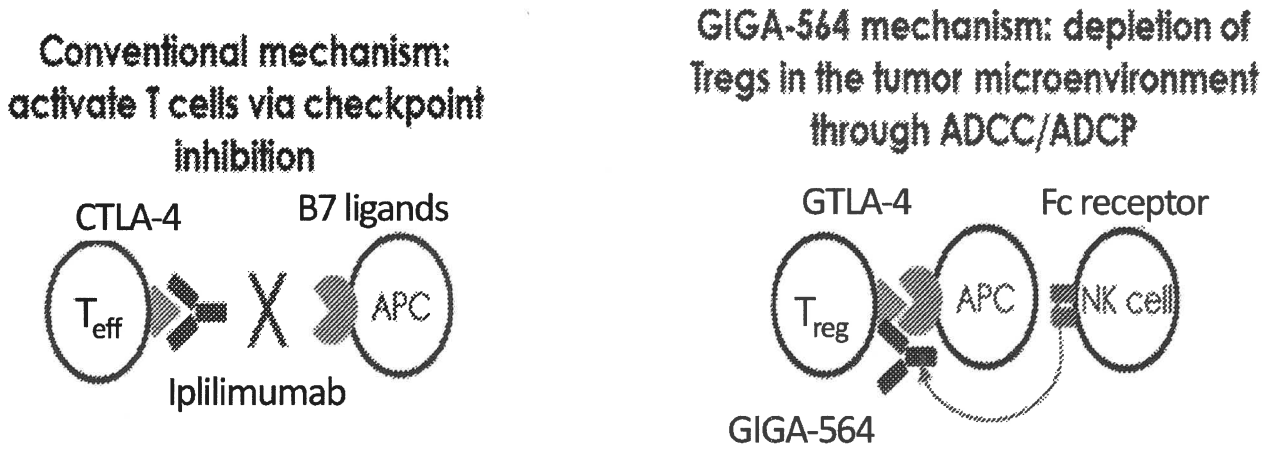

Conventional mechanism:
activate T cells via checkpoint
inhibition
CTLA-4
B7 ligands
T_{eff}
APC
Iplilimumab
GIGA-564 mechanism: depletion of
Tregs in the tumor microenvironment
through ADCC/ADCP
GTLA-4
Fc receptor
T_{reg}
APC
NK cell
GIGA-564

FIG. 28

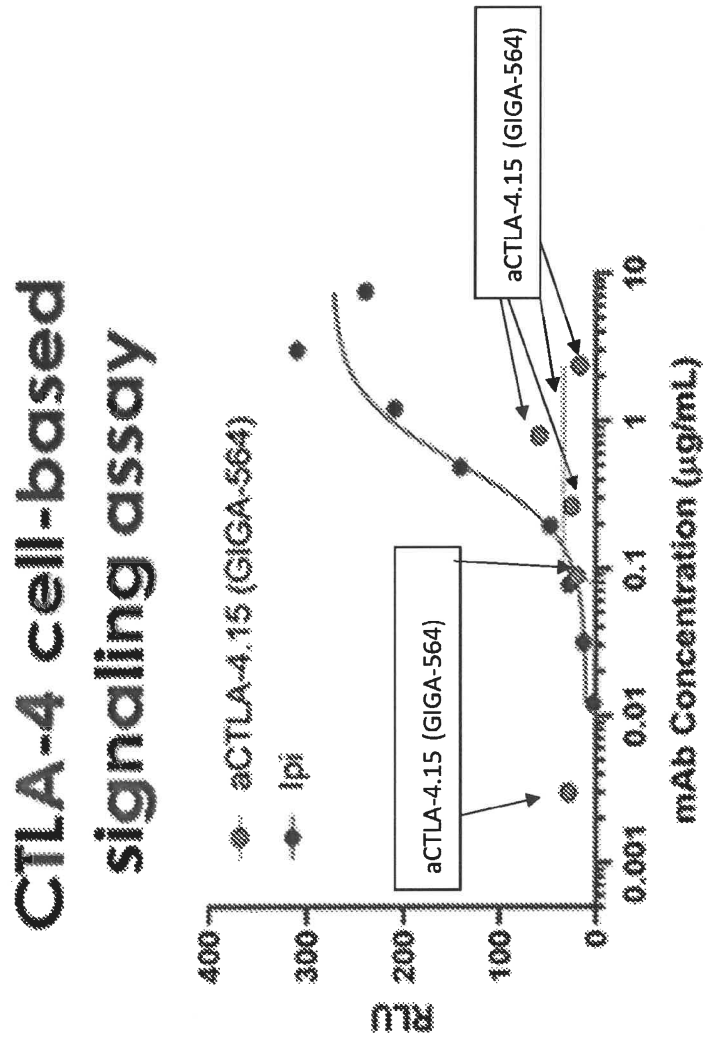

CTLA-4 cell-based signaling assay
aCTLA-4.15 (GIGA-564)
Ipi
RLU
400
300
200
100
0
aCTLA-4.15 (GIGA-564)
aCTLA-4.15 (GIGA-564)
0.001
0.01
0.1
1
10
mAb Concentration (µg/mL)

FIG. 29

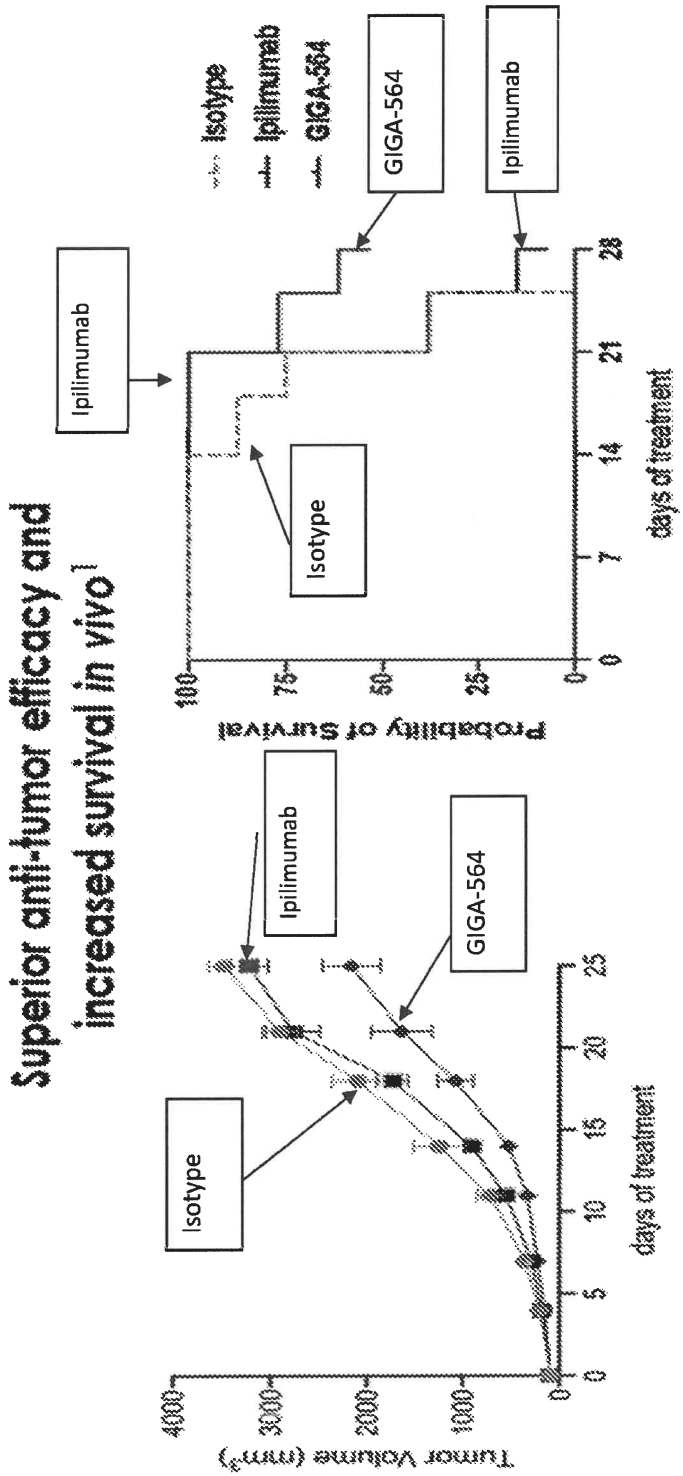

Superior anti-tumor efficacy and increased survival in vivo[1]
Isotype
Ipilimumab
GIGA-564
Tumor Volume (mm³)
4000
3000
2000
1000
0
0
5
10
15
20
25
days of treatment
Probability of Survival
100
75
50
25
0
0
7
14
21
28
days of treatment

FIG. 30

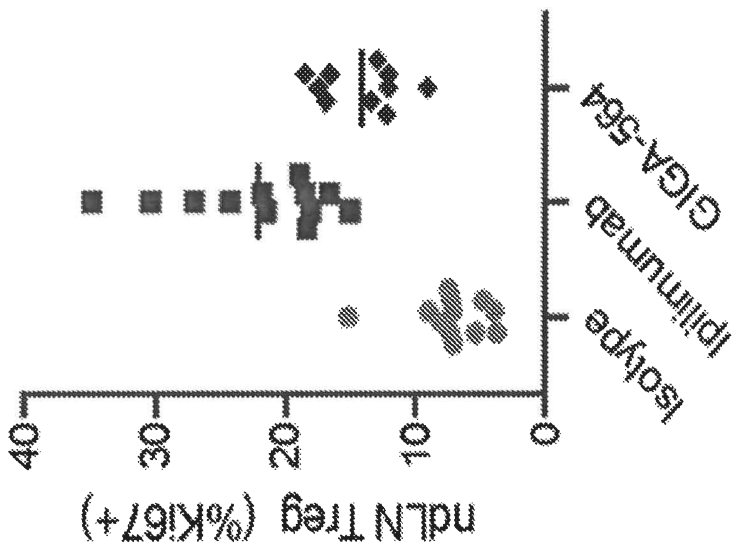


FIG. 31

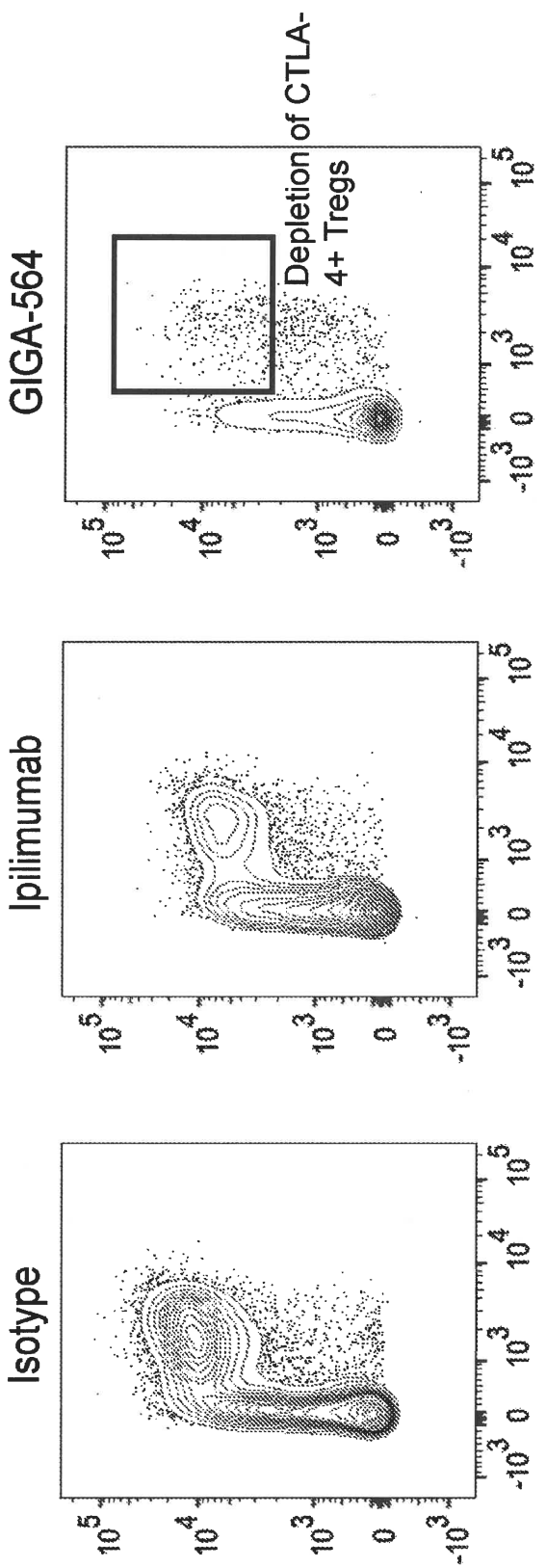


FIG. 32

FIG. 33

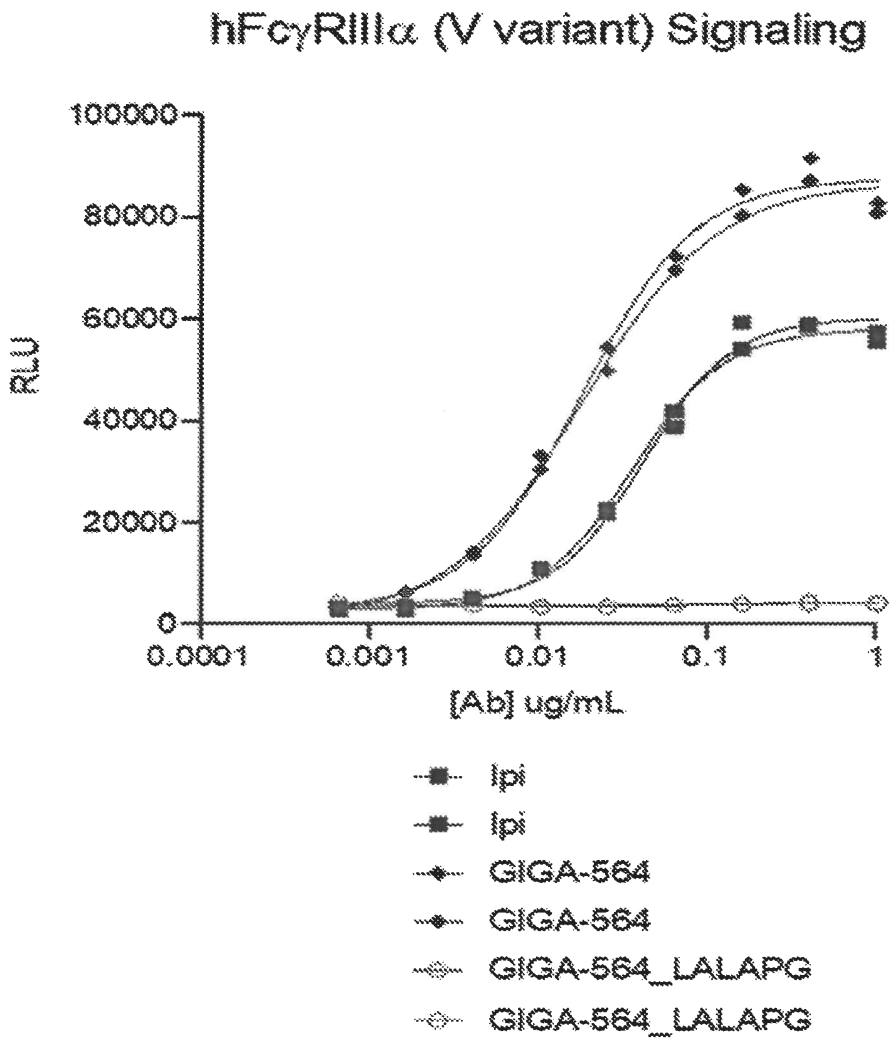
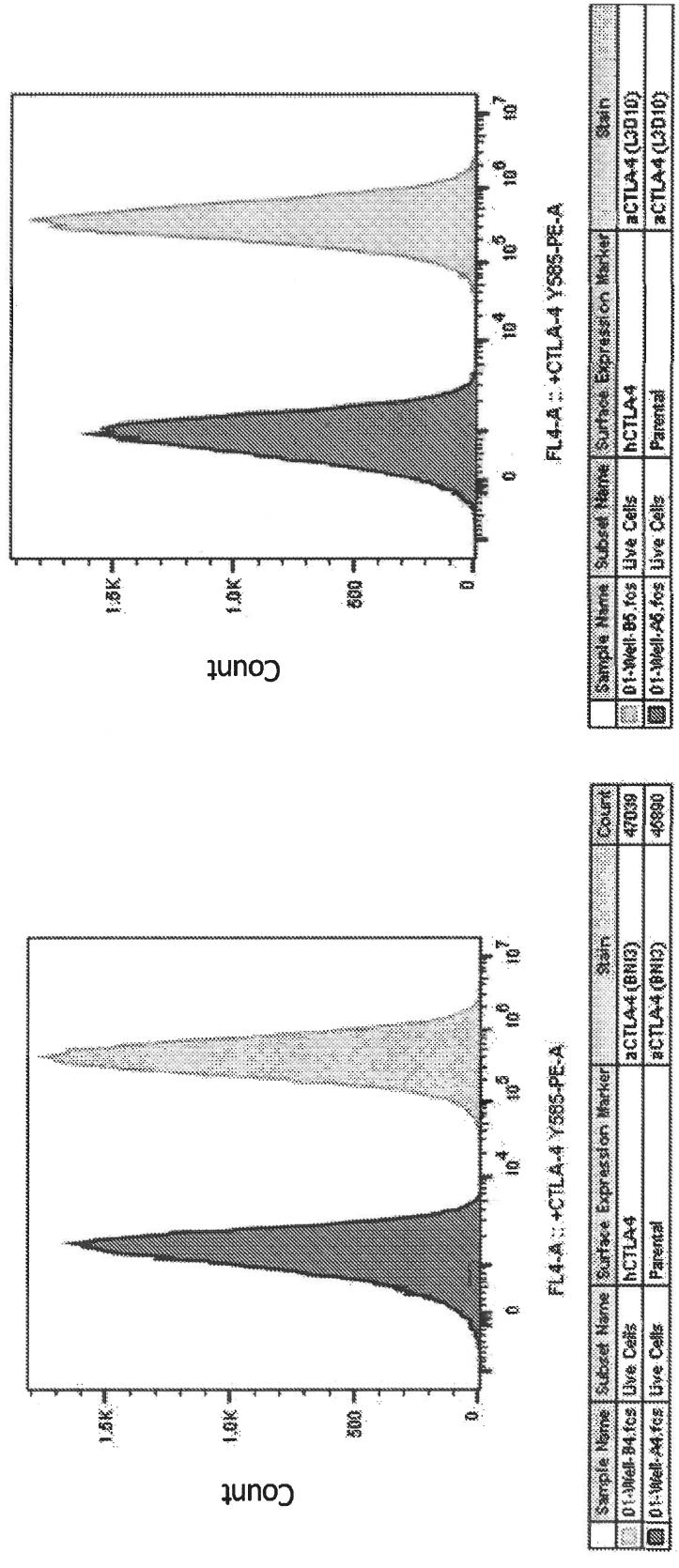

hFcγRIIIα (V variant) Signaling
RLU
100000
80000
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
[Ab] ug/mL
Ipi
Ipi
GIGA-564
GIGA-564
GIGA-564_LALAPG
GIGA-564_LALAPG

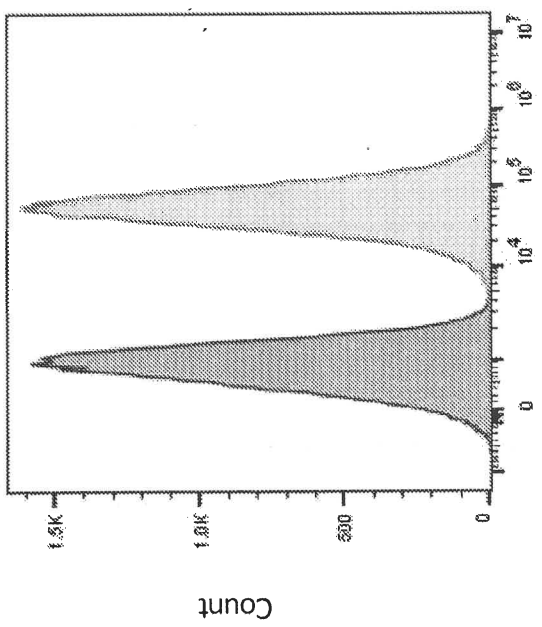
FIG. 34



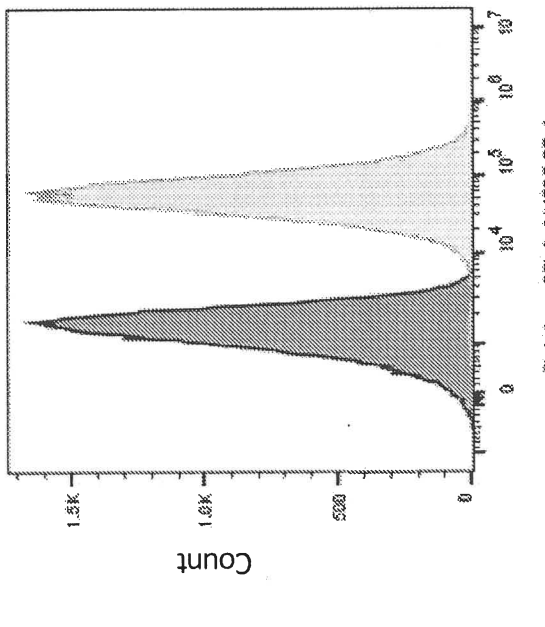
Sample Name	Subset Name	Surface Expression Marker	Stain	Count
01-Well-B4.fcs	Live Cells	hCTLA4	aCTLA4 (BNI3)	47039
01-Well-A4.fcs	Live Cells	Parental	aCTLA4 (BNI3)	46890

Sample Name	Subset Name	Surface Expression Marker	Stain
01-Well-B5.fcs	Live Cells	hCTLA4	aCTLA4 (L3D10)
01-Well-A5.fcs	Live Cells	Parental	aCTLA4 (L3D10)

FIG. 34 (CONTINUED)



	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Well-C5.fcs	Live Cells	cyno CTLA-4	aCTLA4 (BM3)	47996
	01-Well-A6.fcs	Live Cells	Parental	aCTLA4 (BM3)	48280



	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Well-C5.fcs	Live Cells	cyno CTLA-4	aCTLA4 (L3D10)	46587
	01-Well-A6.fcs	Live Cells	Parental	aCTLA4 (L3D10)	46680

FIG. 34 (CONTINUED)

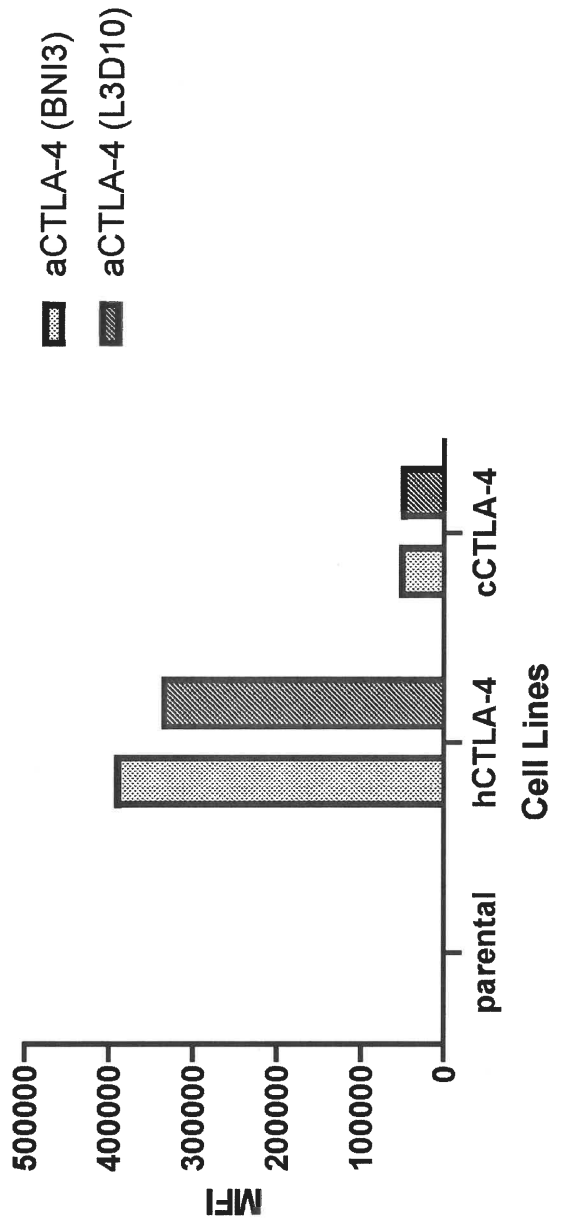


FIG. 35

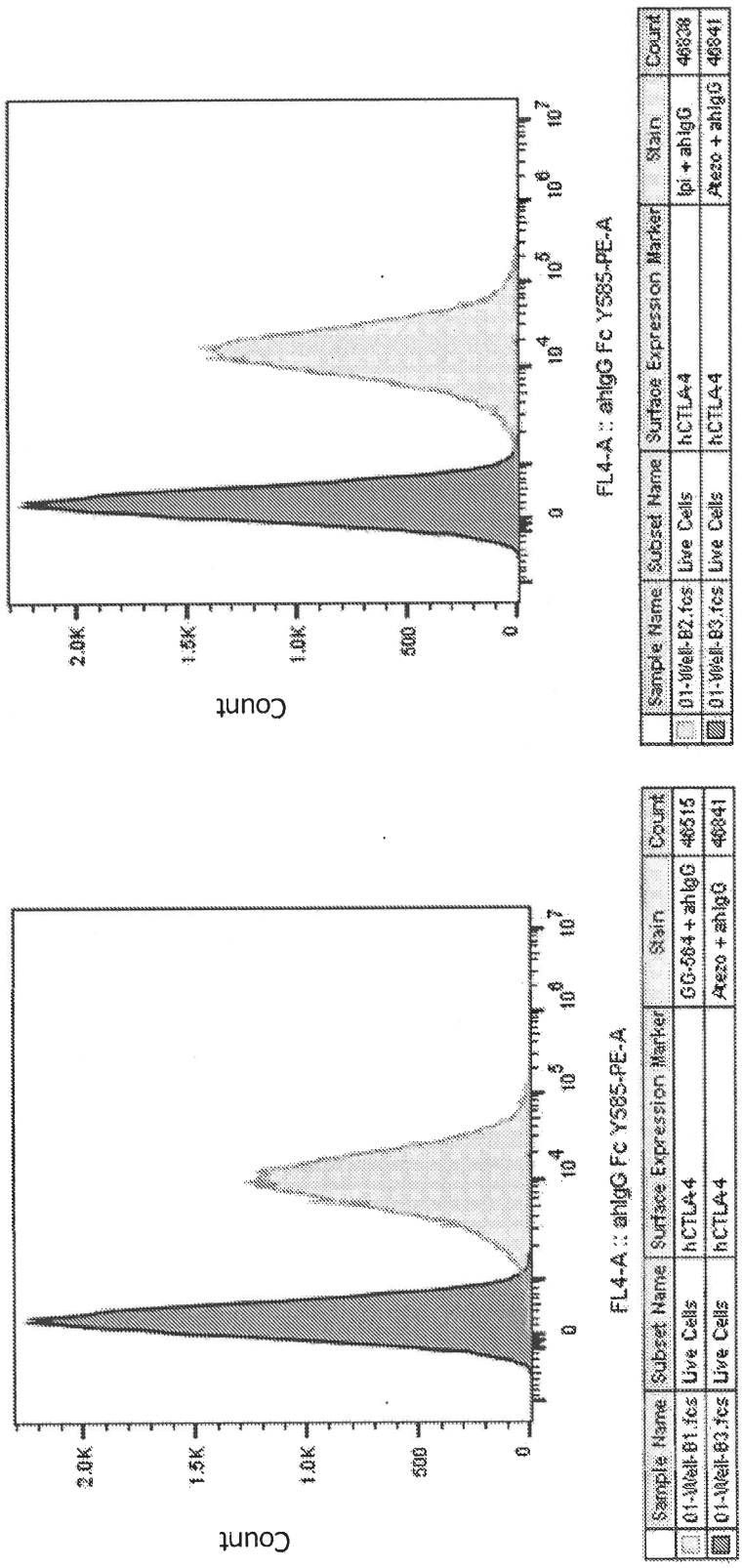

Count
2.0K
1.5K
1.0K
500
0
FL4-A :: ahIgG Fc Y585-PE-A
Sample Name | Subset Name | Surface Expression Marker | Stain | Count
01-Well-B1.fcs | Live Cells | hCTLA-4 | GG-564 + ahIgG | 46515
01-Well-B3.fcs | Live Cells | hCTLA-4 | Atezo + ahIgG | 46841
Count
2.0K
1.5K
1.0K
500
0
FL4-A :: ahIgG Fc Y585-PE-A
Sample Name | Subset Name | Surface Expression Marker | Stain | Count
01-Well-B2.fcs | Live Cells | hCTLA-4 | Ipi + ahIgG | 46838
01-Well-B3.fcs | Live Cells | hCTLA-4 | Atezo + ahIgG | 46841

FIG. 35 (CONTINUED)

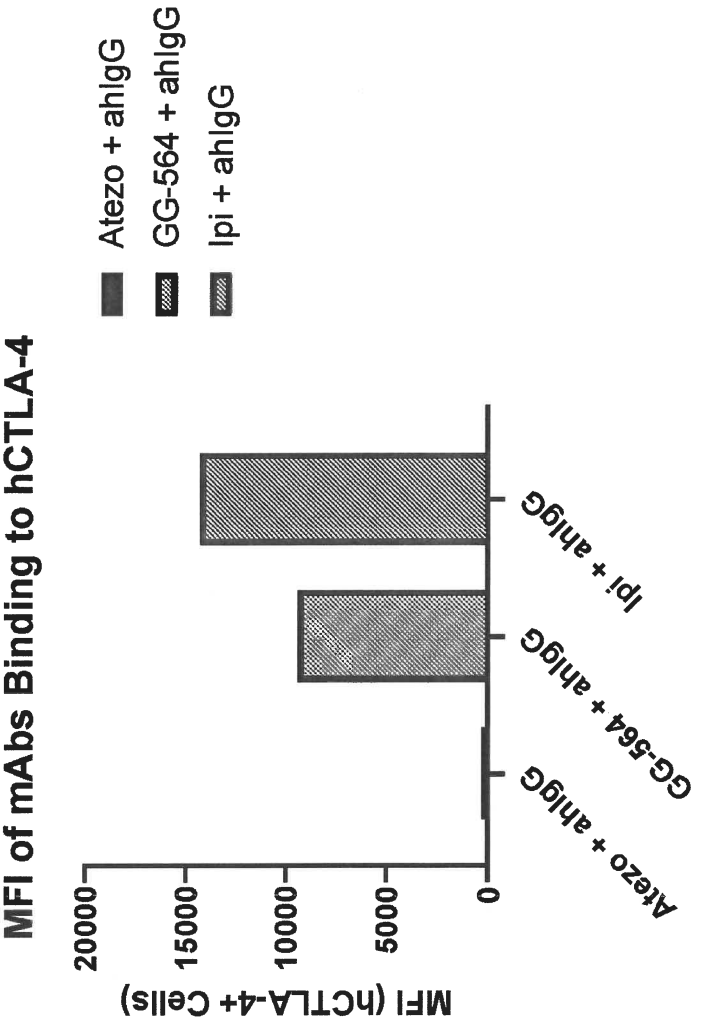
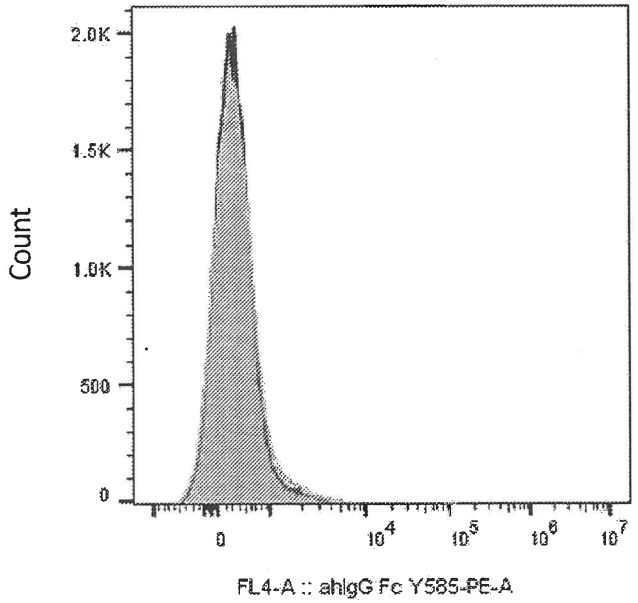
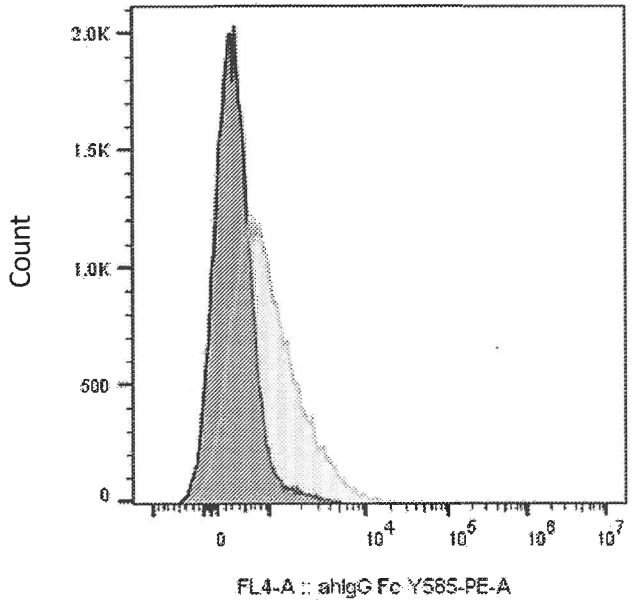


FIG. 35 (CONTINUED)



	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Well-C1.fcs	Live Cells	cyno CTLA4	GG-564 + ahIgG	45849
	01-Well-C3.fcs	Live Cells	cyno CTLA4	Atezo + ahIgG	46586



	Sample Name	Subset Name	Surface Expression Marker	Stain	Count
	01-Well-C2.fcs	Live Cells	cyno CTLA4	Ipi + ahIgG	46099
	01-Well-C3.fcs	Live Cells	cyno CTLA4	Atezo + ahIgG	45566

FIG. 35 (CONTINUED)

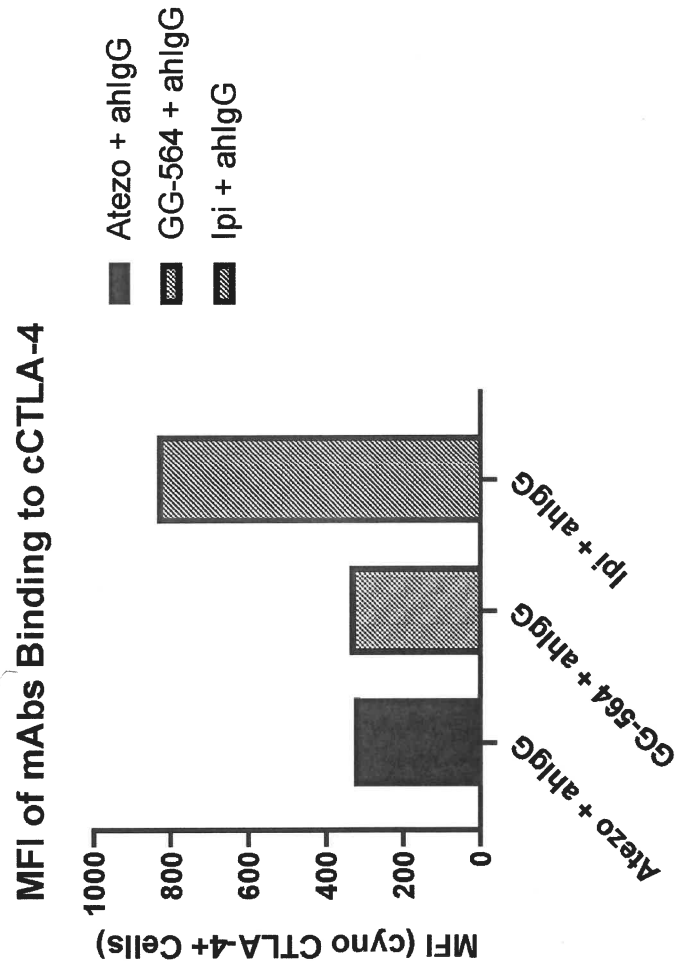


FIG. 36A

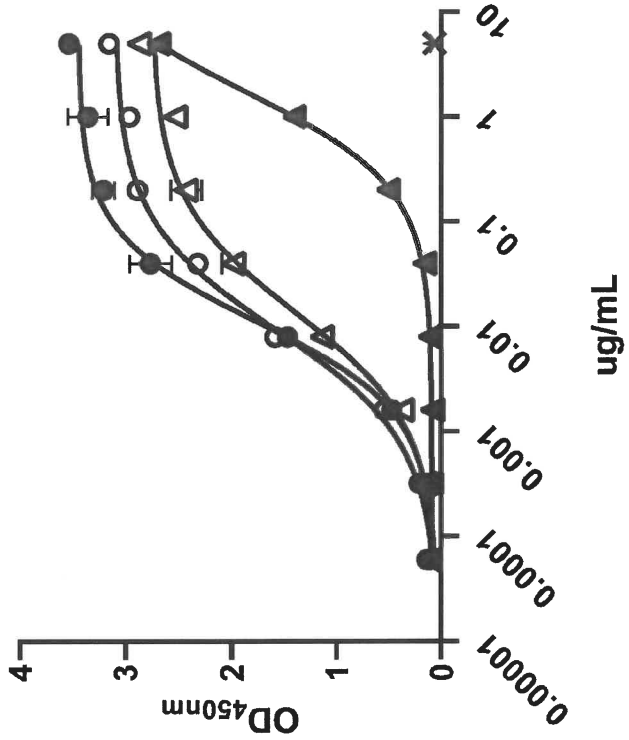

Ipilimumab - R&D rcmCTLA-4 Fc
GG-564 - R&D rcmCTLA-4 Fc
Atezolizumab - R&D rcmCTLA-4 Fc
Ipilimumab - R&D rhCTLA-4 Fc
GG-564 - R&D rhCTLA-4 Fc
Atezolizumab - R&D rhCTLA-4 Fc
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 36B

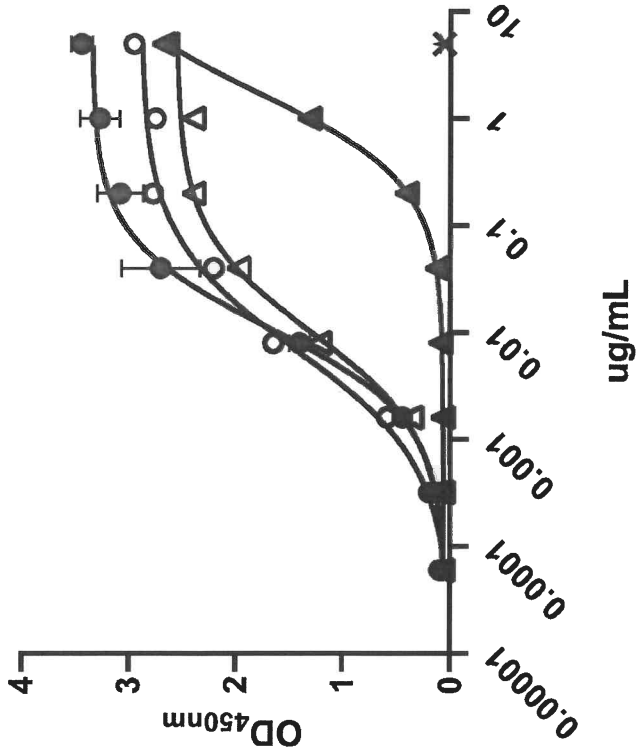

Ipilimumab - Sino rcmCTLA-4 Fc
GG-564 - Sino rcmCTLA-4 Fc
Atezolizumab - Sino rcmCTLA-4 Fc
Ipilimumab - R&D rhCTLA-4 Fc
GG-564 - R&D rhCTLA-4 Fc
Atezolizumab - R&D rhCTLA-4 Fc
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 36C

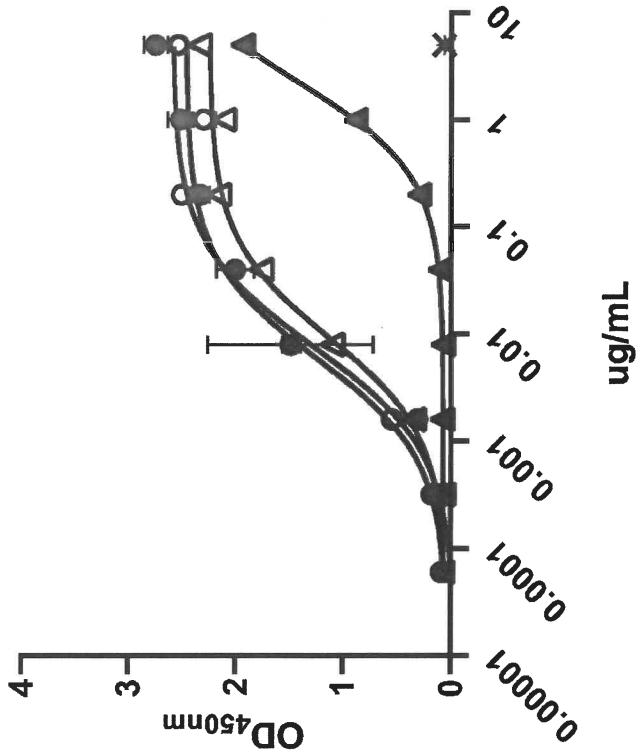

Ipilimumab - Acro rcmCTLA-4 Fc
GG-564 - Acro rcmCTLA-4 Fc
Atezolizumab - Acro rcmCTLA-4 Fc
Ipilimumab - R&D rhCTLA-4 Fc
GG-564 - R&D rhCTLA-4 Fc
Atezolizumab - R&D rhCTLA-4 Fc
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 36D

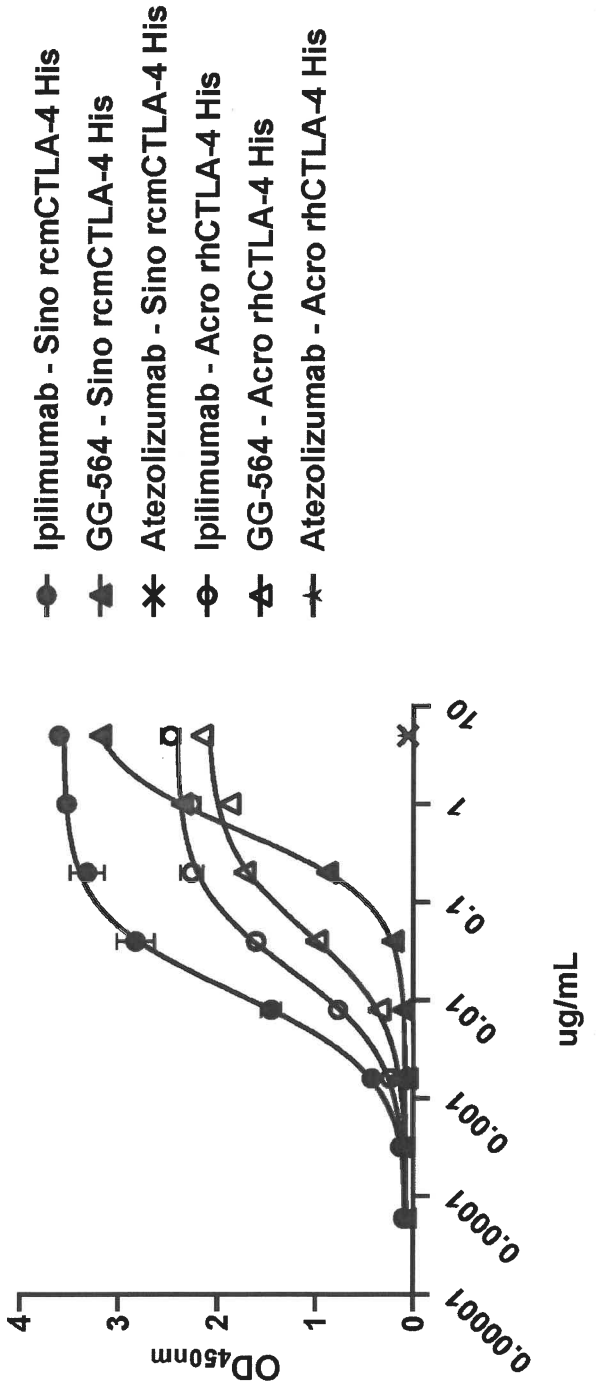

Ipilimumab - Sino rcmCTLA-4 His
GG-564 - Sino rcmCTLA-4 His
Atezolizumab - Sino rcmCTLA-4 His
Ipilimumab - Acro rhCTLA-4 His
GG-564 - Acro rhCTLA-4 His
Atezolizumab - Acro rhCTLA-4 His
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 36E

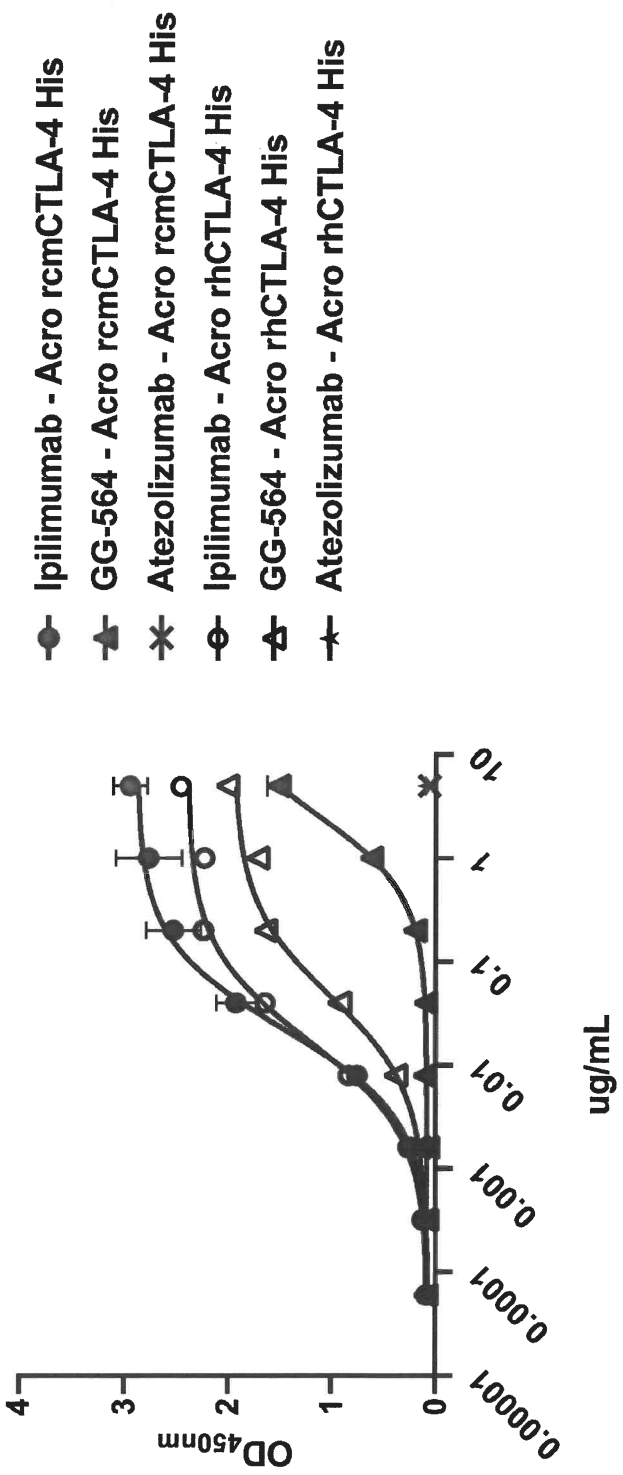

Ipilimumab - Acro rcmCTLA-4 His
GG-564 - Acro rcmCTLA-4 His
Atezolizumab - Acro rcmCTLA-4 His
Ipilimumab - Acro rhCTLA-4 His
GG-564 - Acro rhCTLA-4 His
Atezolizumab - Acro rhCTLA-4 His
OD450nm
0
1
2
3
4
0.00001
0.0001
0.001
0.01
0.1
1
10
ug/mL

FIG. 37

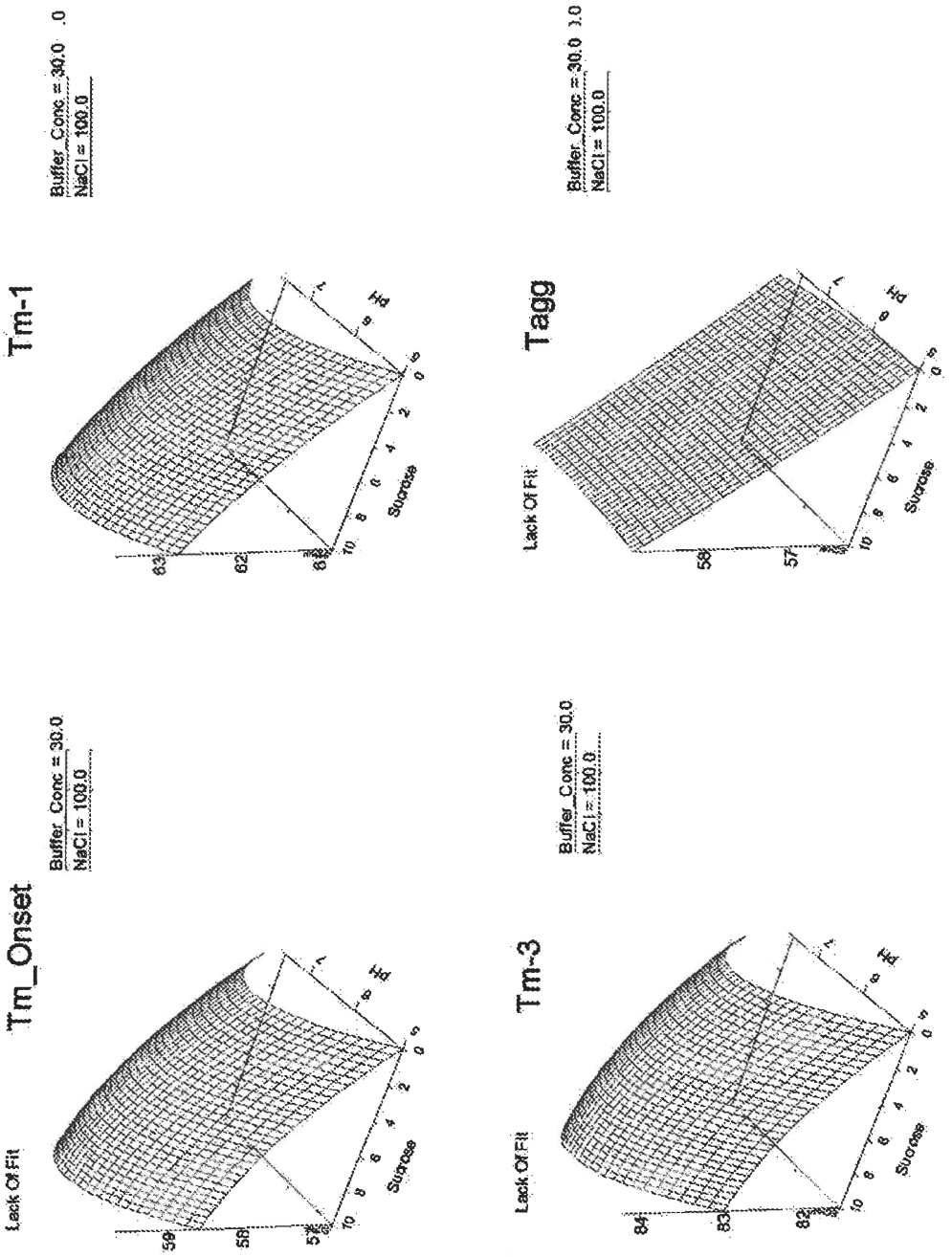

Tm_Onset
Buffer_Conc = 30.0
NaCl = 100.0
Lack Of Fit
Sucrose
Tm-1
Buffer_Conc = 30.0
NaCl = 100.0
Tagg
Buffer_Conc = 30.0
NaCl = 100.0
Lack Of Fit
Tm-3
Buffer_Conc = 30.0
NaCl = 100.0
Lack Of Fit

FIG. 38

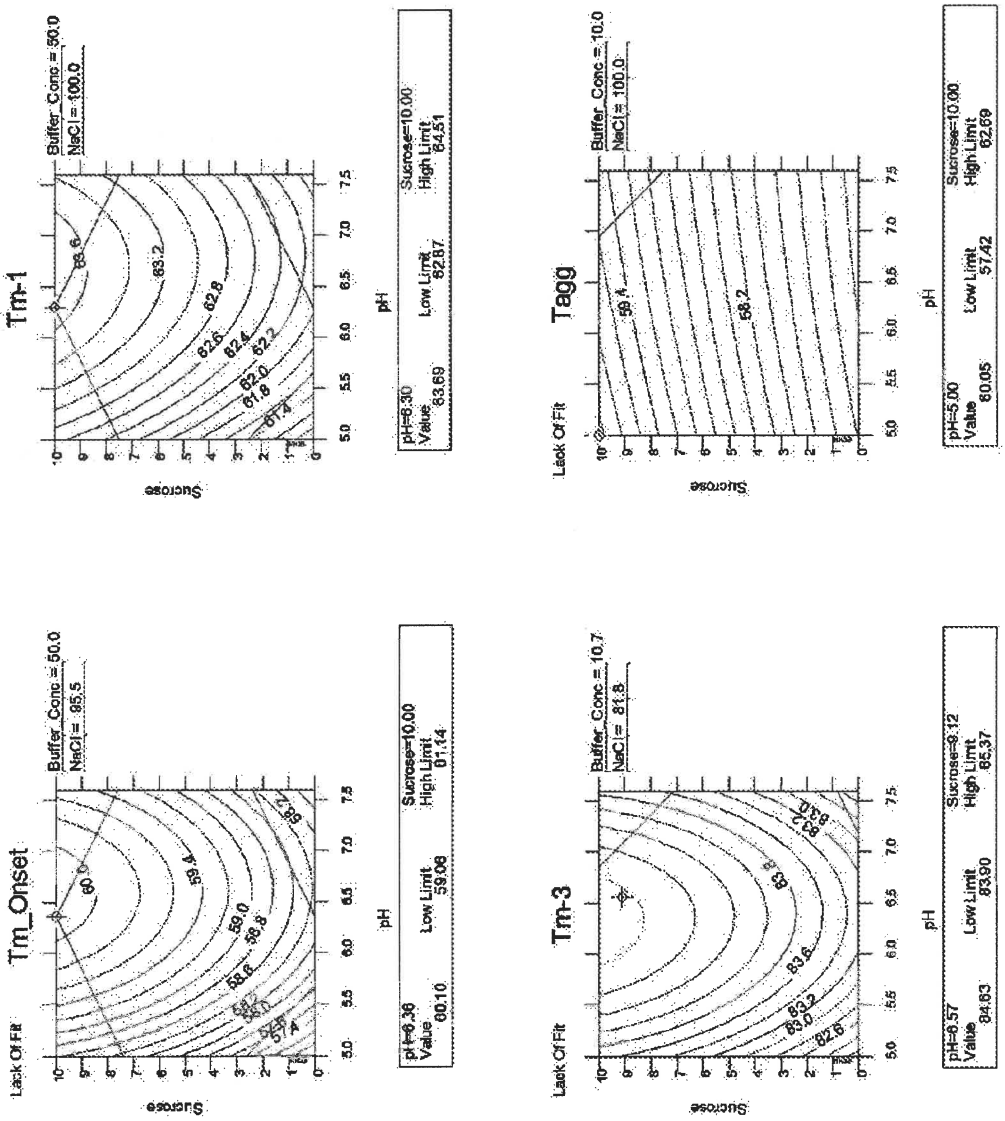
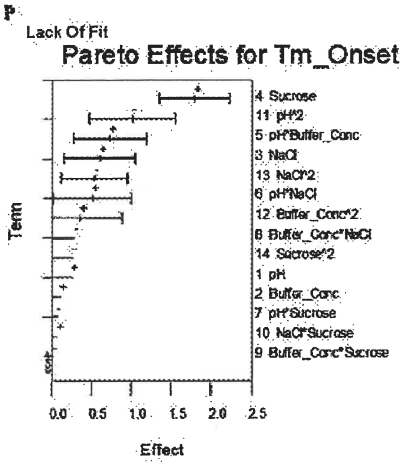
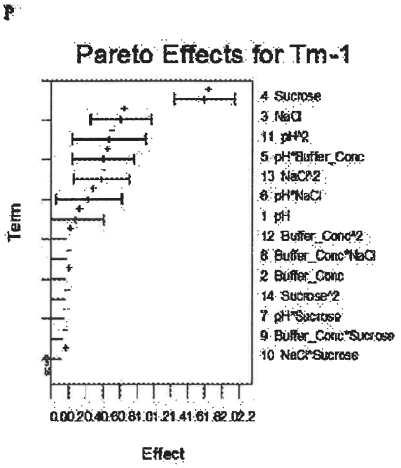
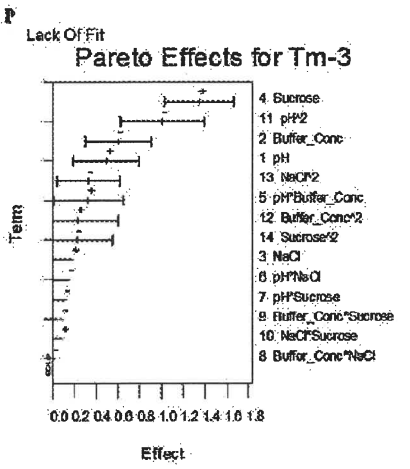

Tm-1
Buffer_Conc = 50.0
NaCl = 100.0
Sucrose
pH
pH=6.30
Value 83.69
Low Limit 82.87
Sucrose=10.00
High Limit 84.51
Tagg
Buffer_Conc = 10.0
NaCl = 100.0
pH=5.00
Value 60.05
Low Limit 57.42
Sucrose=10.00
High Limit 62.69
Tm_Onset
Buffer_Conc = 50.0
NaCl = 85.5
pH=6.30
Value 60.10
Low Limit 59.08
Sucrose=10.00
High Limit 61.14
Tm-3
Buffer_Conc = 10.7
NaCl = 81.8
pH=6.57
Value 84.63
Low Limit 83.90
Sucrose=9.12
High Limit 85.37

FIG. 39


P
Lack Of Fit
Pareto Effects for Tm_Onset
Term
4 Sucrose
11 pH^2
5 pH*Buffer_Conc
3 NaCl
13 NaCl^2
6 pH*NaCl
12 Buffer_Conc^2
8 Buffer_Conc*NaCl
14 Sucrose^2
1 pH
2 Buffer_Conc
7 pH*Sucrose
10 NaCl*Sucrose
9 Buffer_Conc*Sucrose
0.0 0.5 1.0 1.5 2.0 2.5
Effect


P
Pareto Effects for Tm-1
Term
4 Sucrose
3 NaCl
11 pH^2
5 pH*Buffer_Conc
13 NaCl^2
6 pH*NaCl
1 pH
12 Buffer_Conc^2
8 Buffer_Conc*NaCl
2 Buffer_Conc
14 Sucrose^2
7 pH*Sucrose
9 Buffer_Conc*Sucrose
10 NaCl*Sucrose
Effect


P
Lack Of Fit
Pareto Effects for Tm-3
Term
4 Sucrose
11 pH^2
2 Buffer_Conc
1 pH
13 NaCl^2
5 pH*Buffer_Conc
12 Buffer_Conc^2
14 Sucrose^2
3 NaCl
6 pH*NaCl
7 pH*Sucrose
9 Buffer_Conc*Sucrose
10 NaCl*Sucrose
8 Buffer_Conc*NaCl
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
Effect

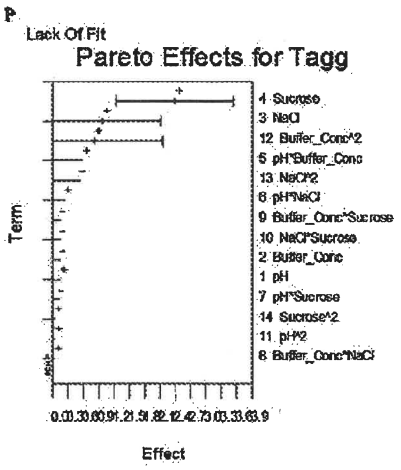
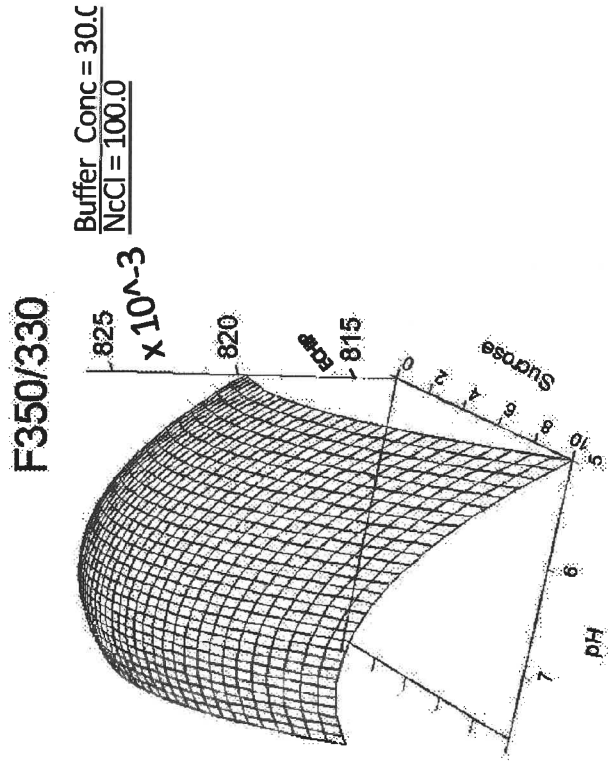

P
Lack Of Fit
Pareto Effects for Tagg
Term
4 Sucrose
3 NaCl
12 Buffer_Conc^2
5 pH*Buffer_Conc
13 NaCl^2
6 pH*NaCl
9 Buffer_Conc*Sucrose
10 NaCl*Sucrose
2 Buffer_Conc
1 pH
7 pH*Sucrose
14 Sucrose^2
11 pH^2
8 Buffer_Conc*NaCl
Effect

FIG. 40


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

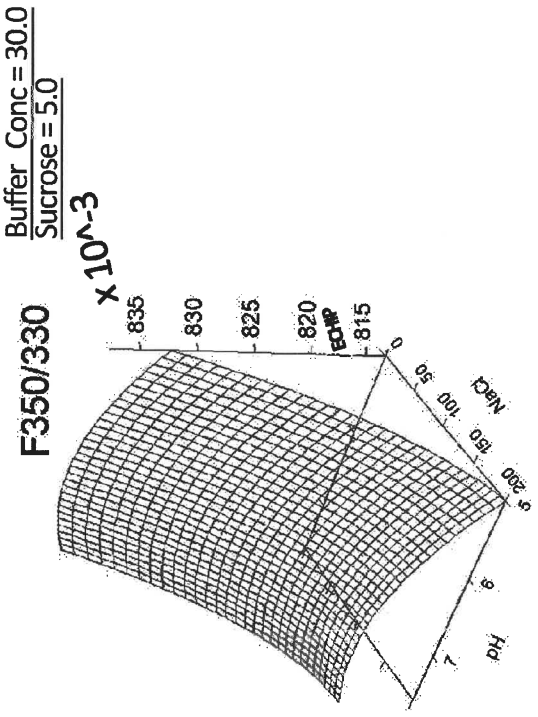

F350/330
Buffer Conc = 30.0
Sucrose = 5.0
x 10^-3
835
830
825
820
815
NaCl
pH

FIG. 40 (CONTINUED)

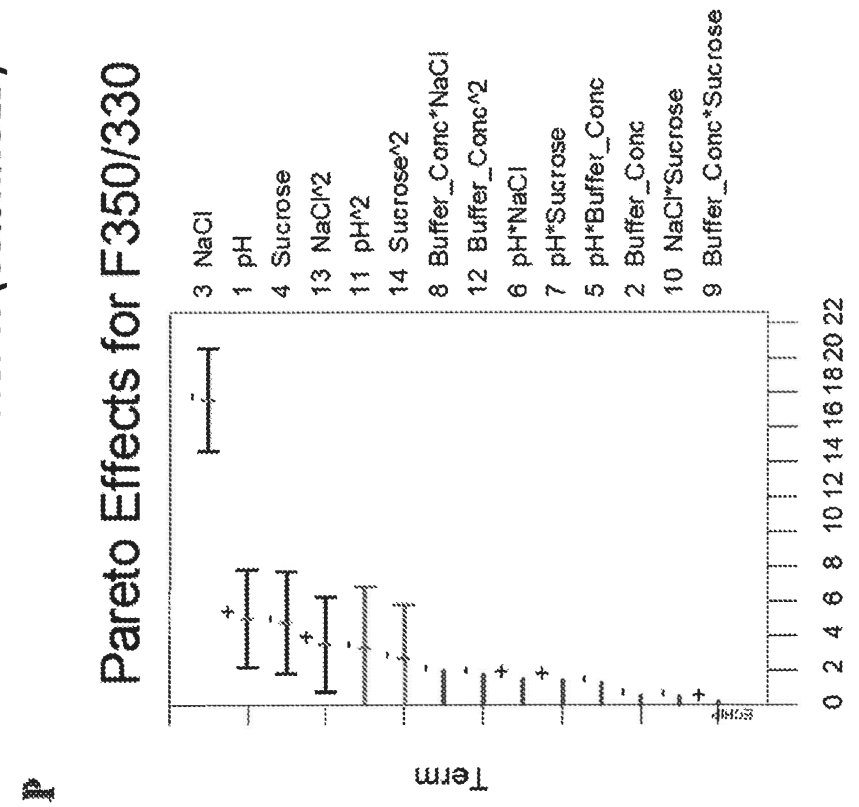


FIG. 41

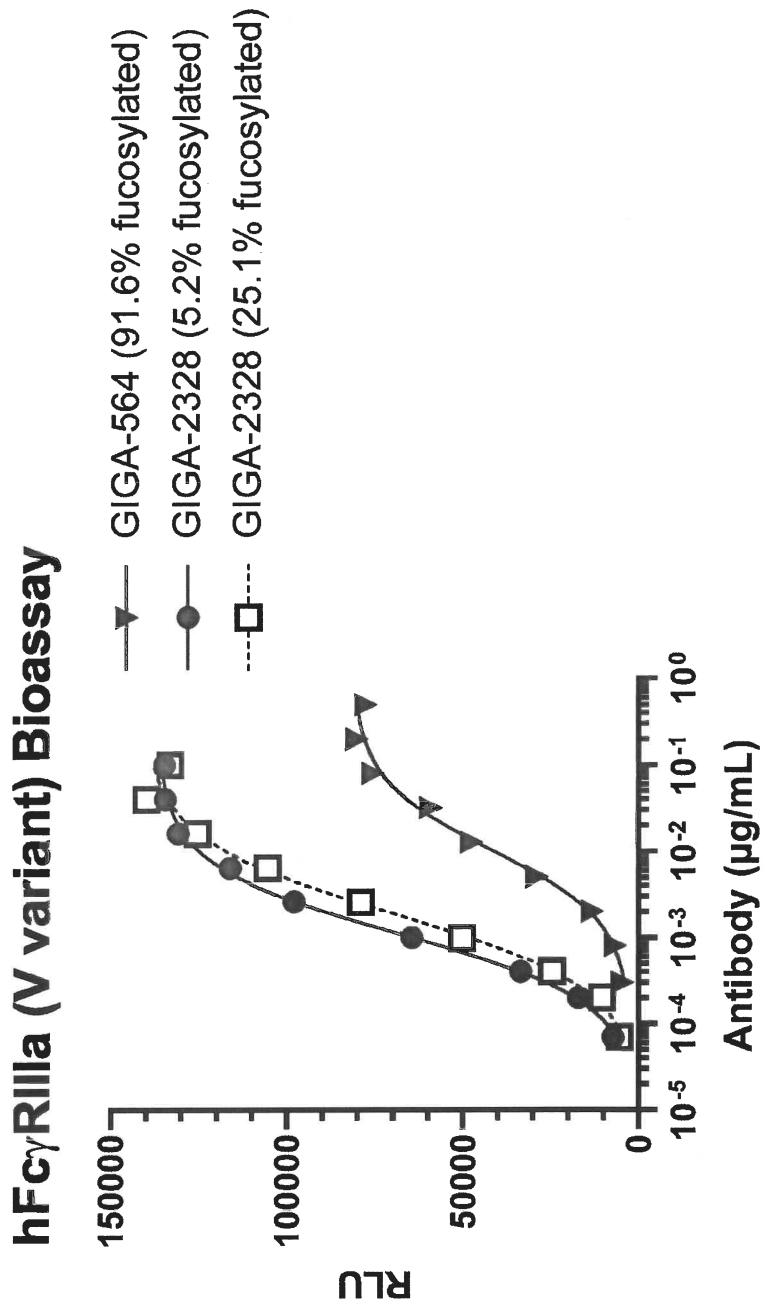

hFcγRIIIa (V variant) Bioassay
GIGA-564 (91.6% fucosylated)
GIGA-2328 (5.2% fucosylated)
GIGA-2328 (25.1% fucosylated)
RLU
150000
100000
50000
0
10-5 10-4 10-3 10-2 10-1 100
Antibody (µg/mL)

FIG. 42A

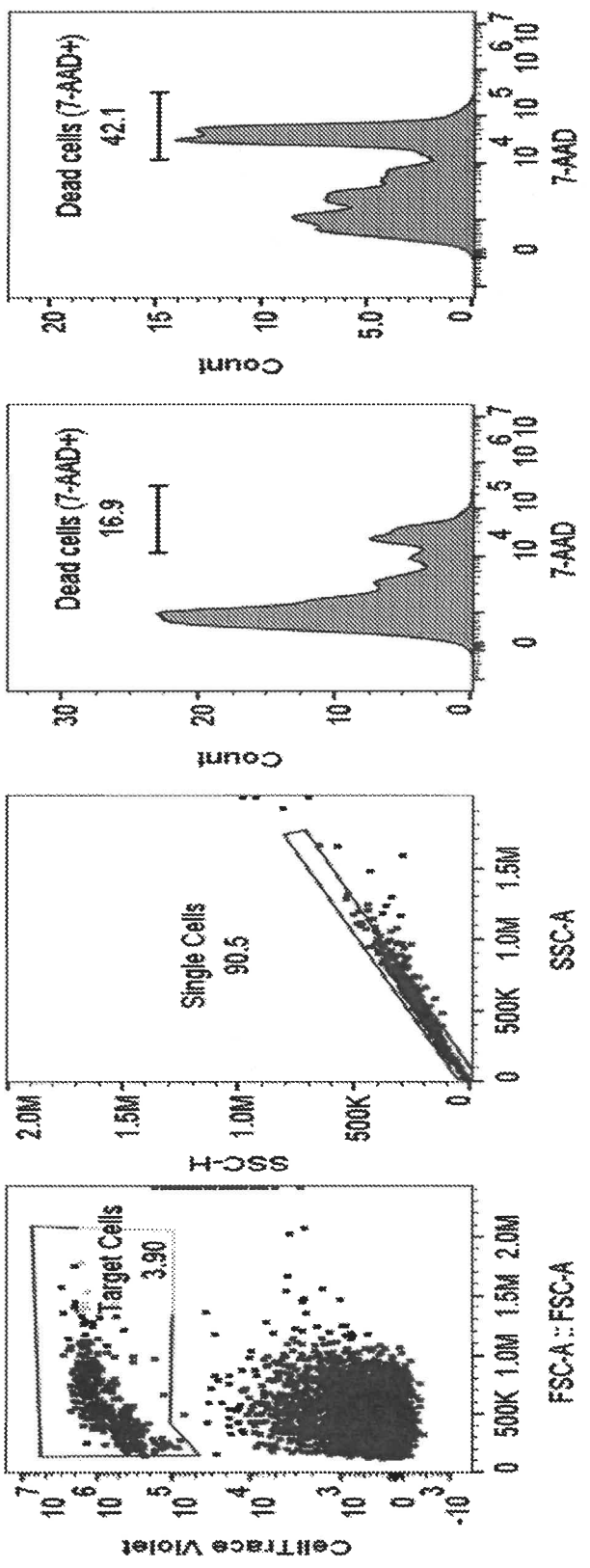

CellTrace Violet
FSC-A :: FSC-A
Target Cells
3.90
SSC-H
SSC-A
Single Cells
90.5
Count
7-AAD
Dead cells (7-AAD+)
16.9
Count
7-AAD
Dead cells (7-AAD+)
42.1

FIG. 42B

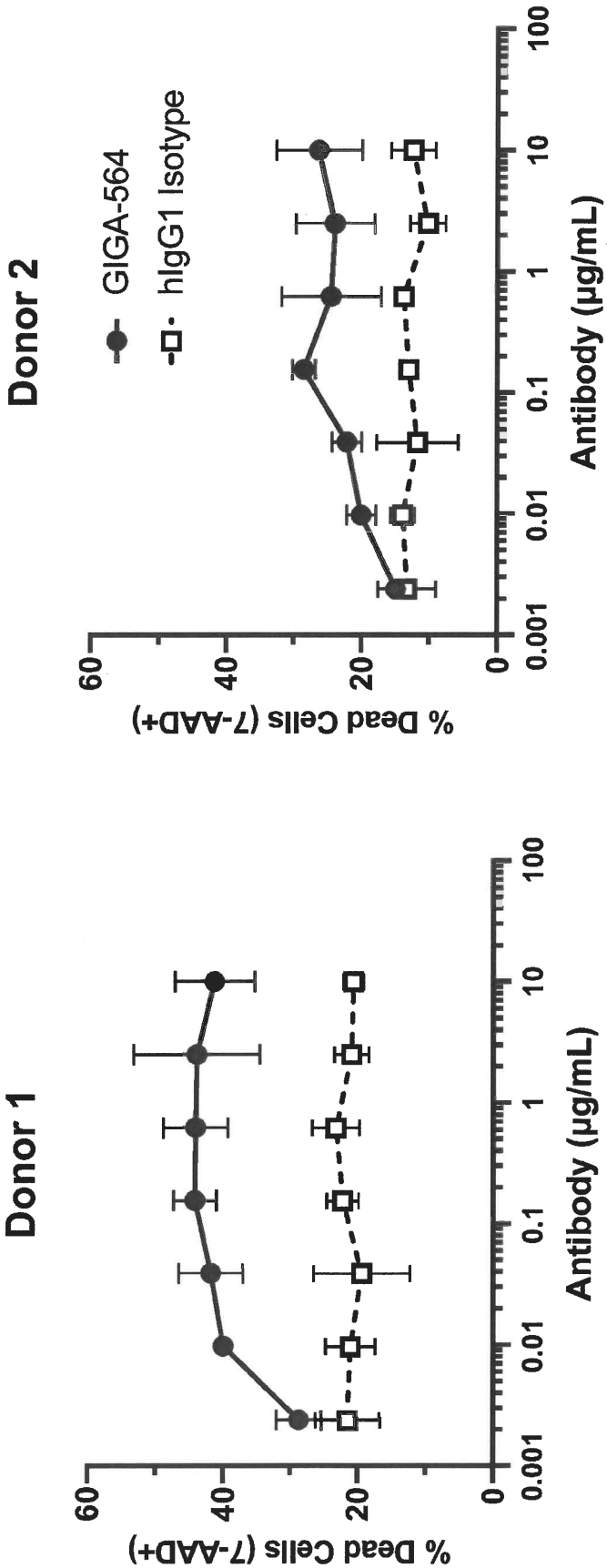

Donor 1
Donor 2
GIGA-564
hIgG1 Isotype
% Dead Cells (7-AAD+)
Antibody (µg/mL)
0
20
40
60
0.001
0.01
0.1
1
10
100

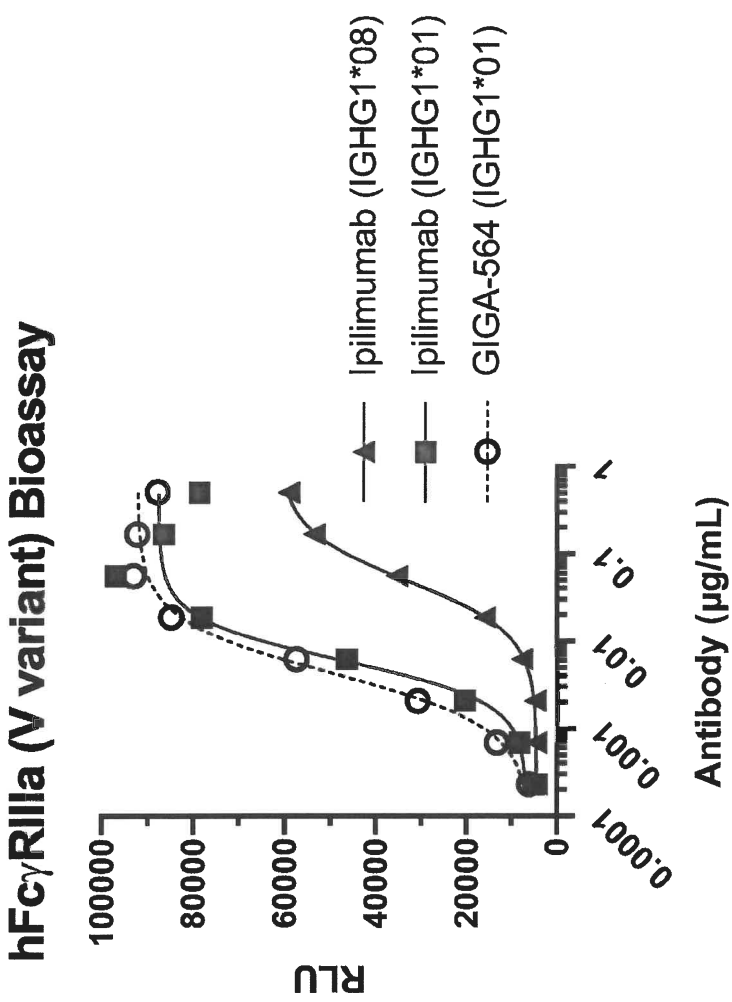
FIG. 43A

IgG Heavy chain domain	CH1			
IMGT exon numbering	95	96	97	98
EU numbering	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
Ipilimumab (IGHG1*08)
Ipilimumab (IGHG1*01)
GIGA-564 (IGHG1*01)
0.0001
0.001
0.01
0.1
1
Antibody (µg/mL)