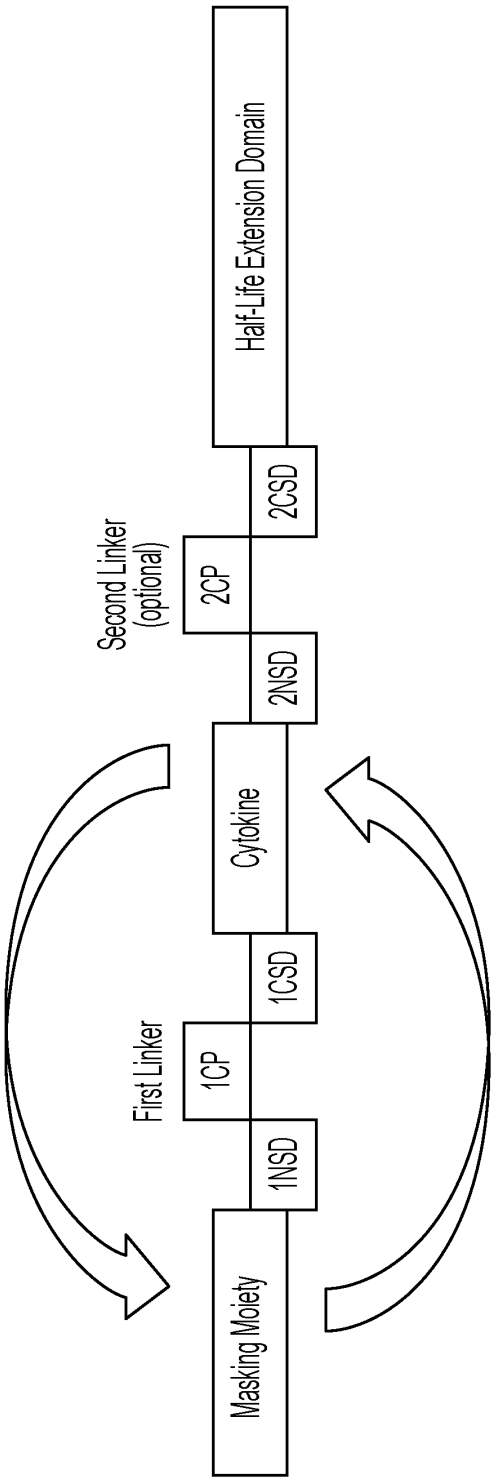

Half-Life Extension Domain
2CSD
Second Linker (optional)
2CP
2NSD
Cytokine
1CSD
First Linker
1CP
1NSD
Masking Moiety

FIG. 1

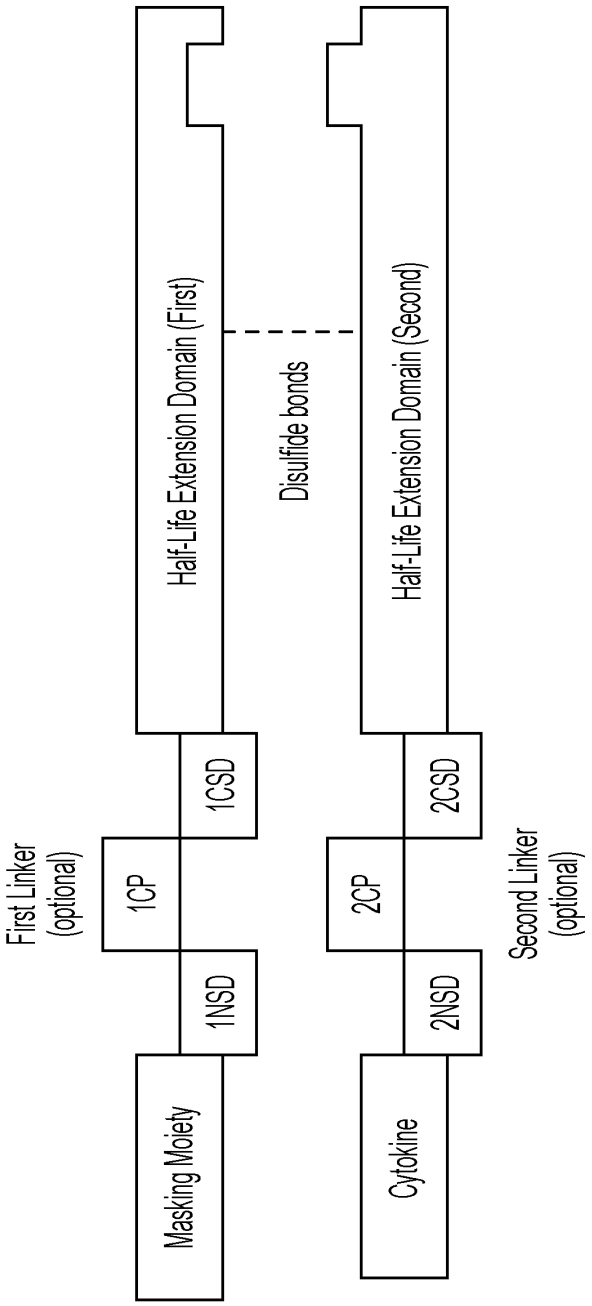

Masking Moiety
1NSD
1CP
First Linker (optional)
1CSD
Half-Life Extension Domain (First)
Disulfide bonds
Cytokine
2NSD
2CP
Second Linker (optional)
2CSD
Half-Life Extension Domain (Second)

FIG. 2

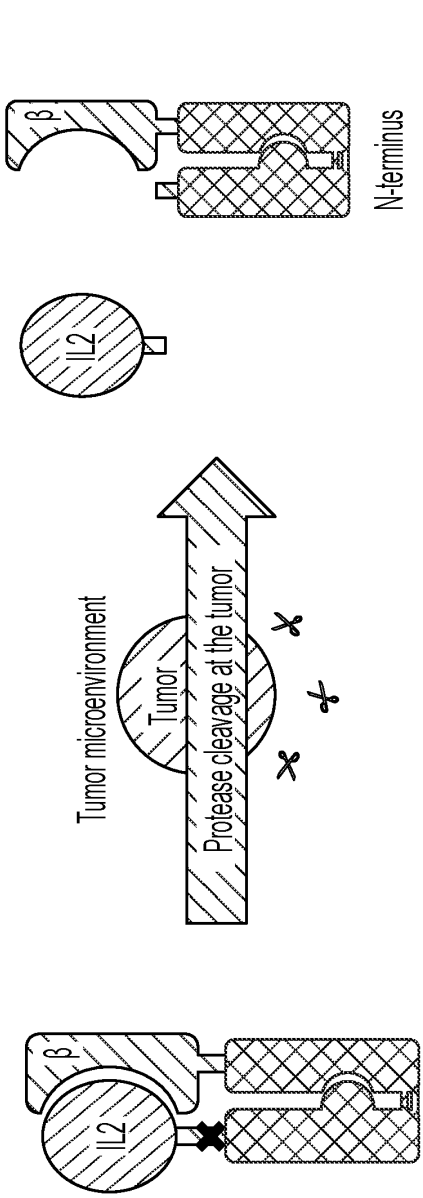

β
N-terminus
IL2
Tumor microenvironment
Tumor
Protease cleavage at the tumor
IL2
β
N-terminus

FIG. 3A

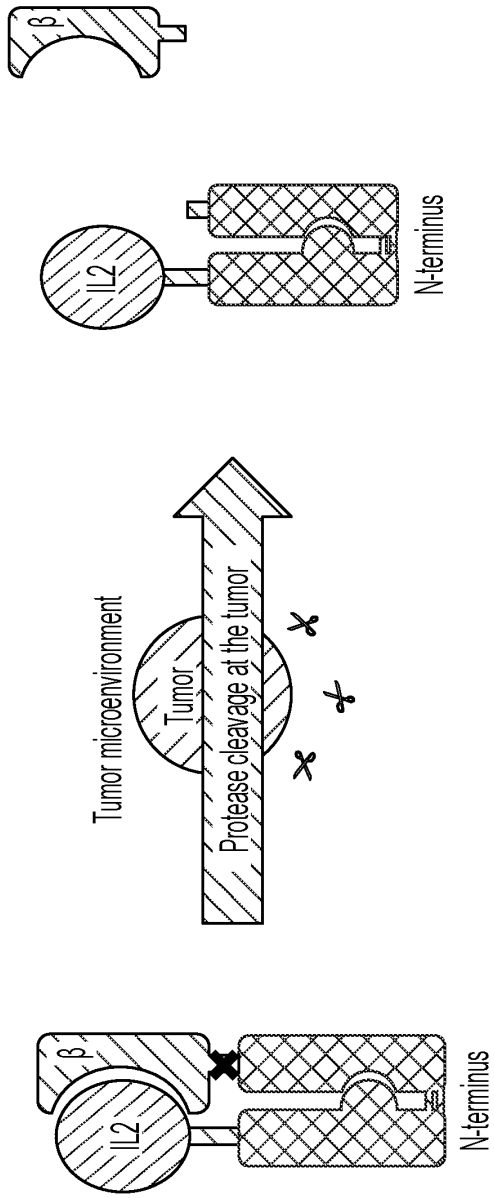

β
IL2
N-terminus
Tumor microenvironment
Tumor
Protease cleavage at the tumor
β
IL2
N-terminus

FIG. 3B

FIG. 4

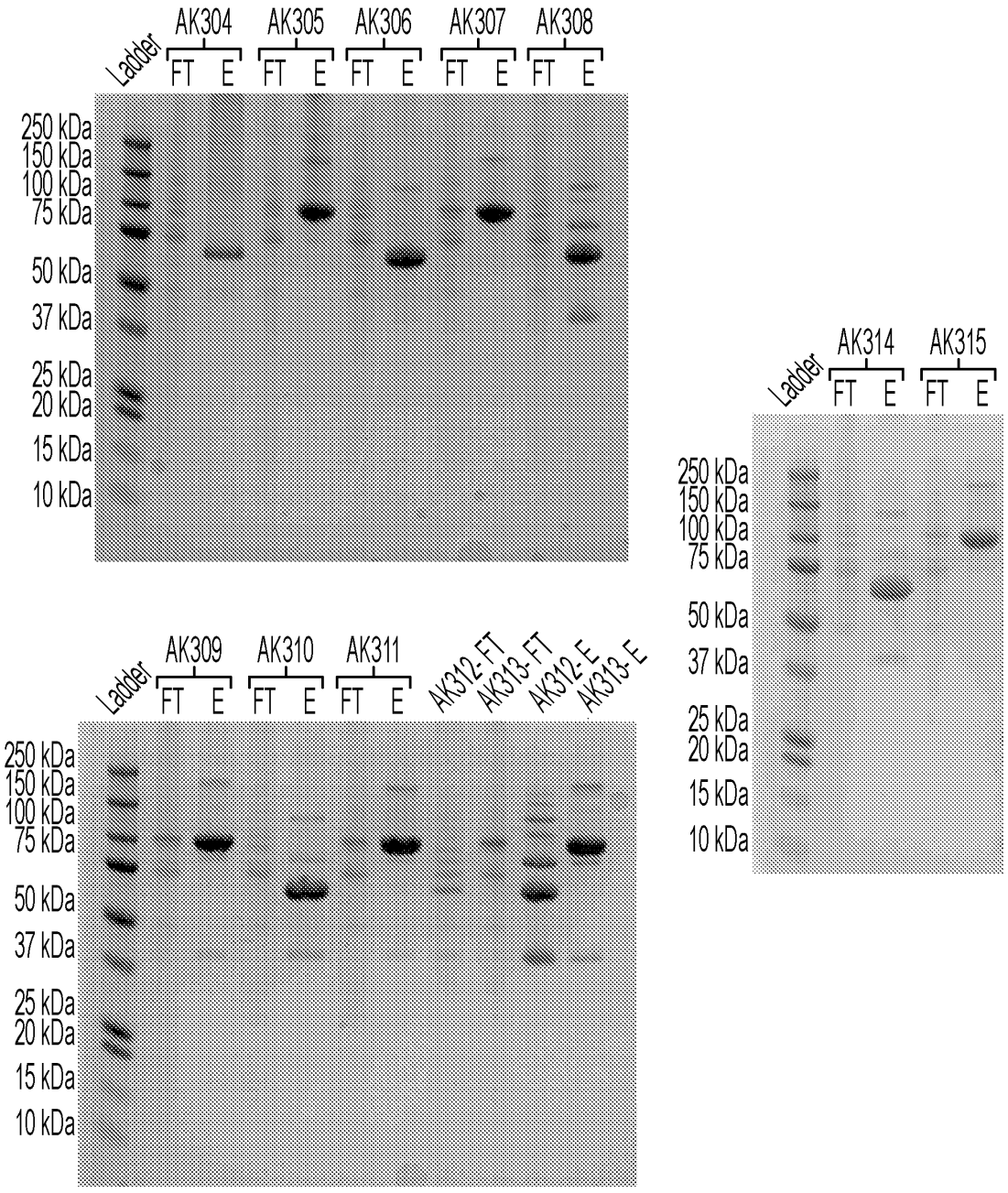

Ladder
AK304
AK305
AK306
AK307
AK308
FT E FT E FT E FT E FT E
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Ladder
AK309
AK310
AK311
AK312-FT
AK313-FT
AK312-E
AK313-E
FT E FT E FT E
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Ladder
AK314
AK315
FT E FT E
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa

FIG. 5A

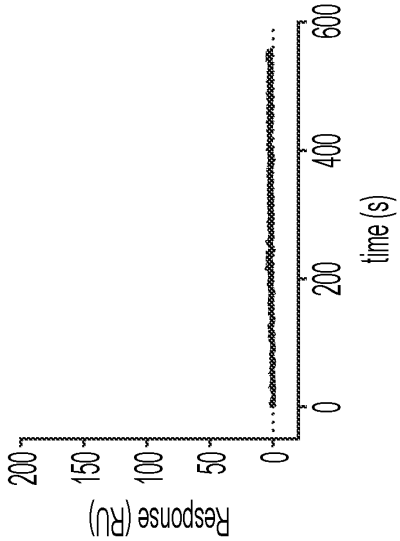


FIG. 5B

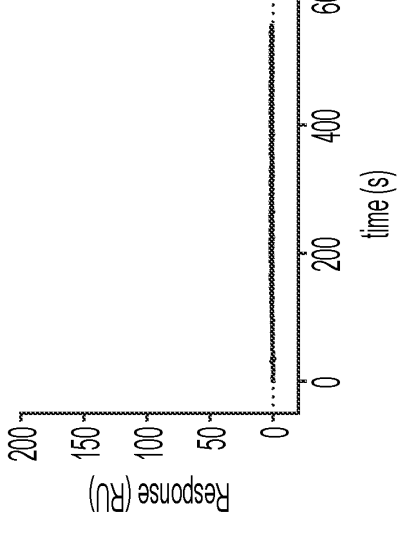


FIG. 5C

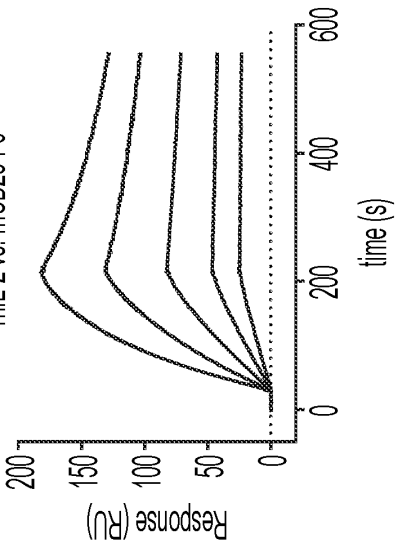


FIG. 5D

Sample	ka (1/(M*s))	kd (1/s)	KD	Chi2	U-value: kd (%)
AK168	ND	ND	ND	ND	ND
AK168 + MMP	ND	ND	ND	ND	ND
rhIL-2	8.30e+5	7.29e-4	0.878 nM	7.01	3.1

FIG. 6A

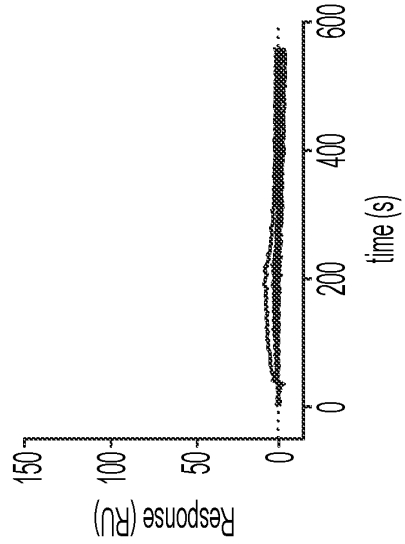


FIG. 6B

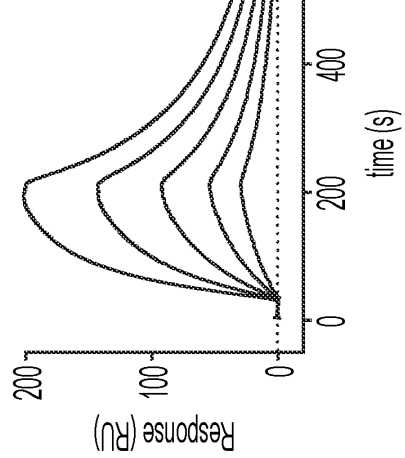


FIG. 6C

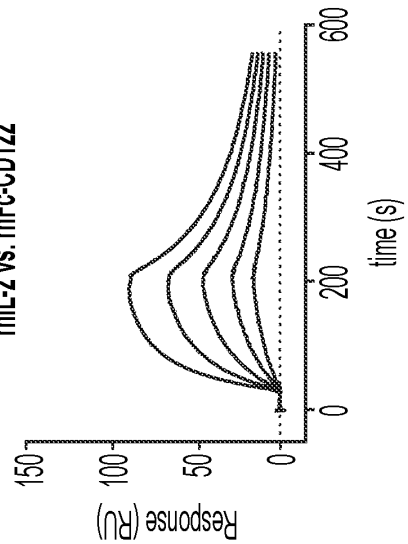


FIG. 6D

Sample	ka (1/(M*s))	kd (1/s)	KD	Chi2	U-value: kd (%)
AK111	ND	ND	ND	ND	ND
AK111 + MMP	2.64e+4	6.21e-3	235 nM	9.82	0.6
rhIL-2	4.17e+4	5.17e-3	124 nM	2.09	0.8

FIG. 7B

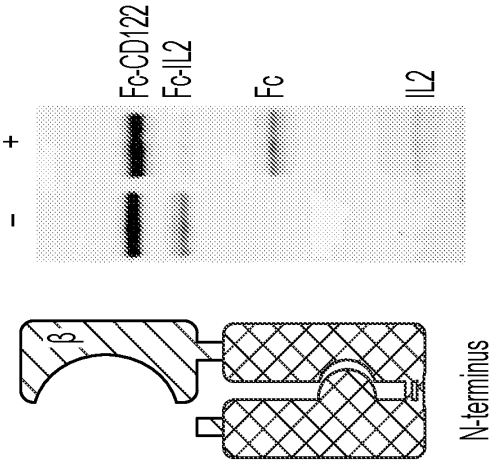
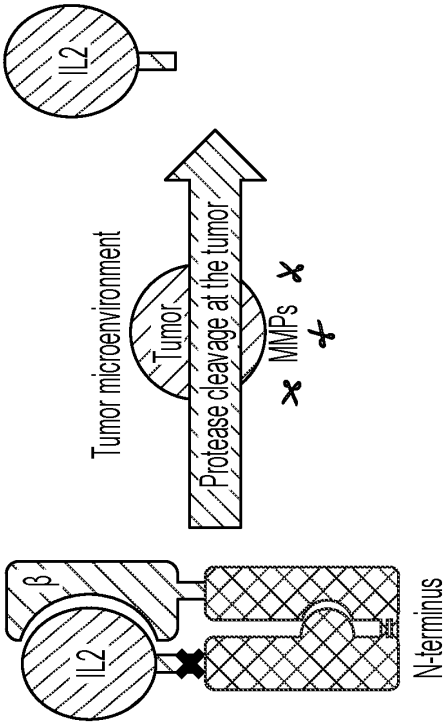

MMP10
+
-
Fc-CD122
Fc-IL2
Fc
IL2
β
N-terminus

FIG. 7A


IL2
Tumor microenvironment
Tumor
Protease cleavage at the tumor
MMPs
β
IL2
N-terminus

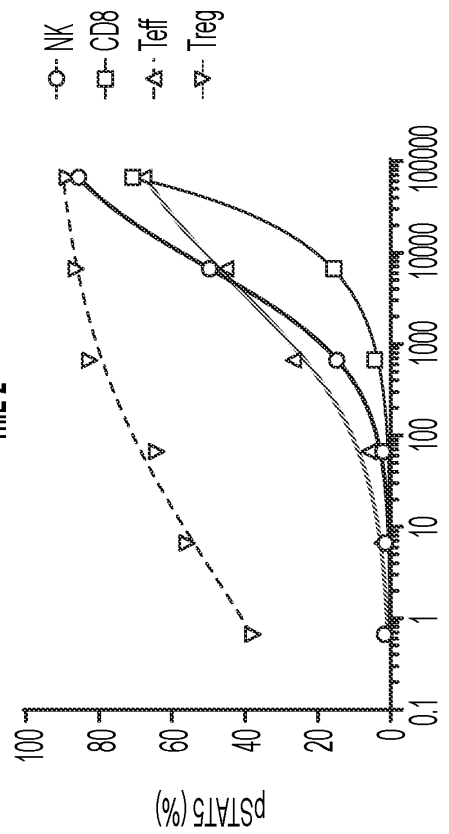

rhIL-2
NK
CD8
Teff
Treg
pSTAT5 (%)
0
20
40
60
80
100
0.1
1
10
100
1000
10000
100000

FIG. 8A

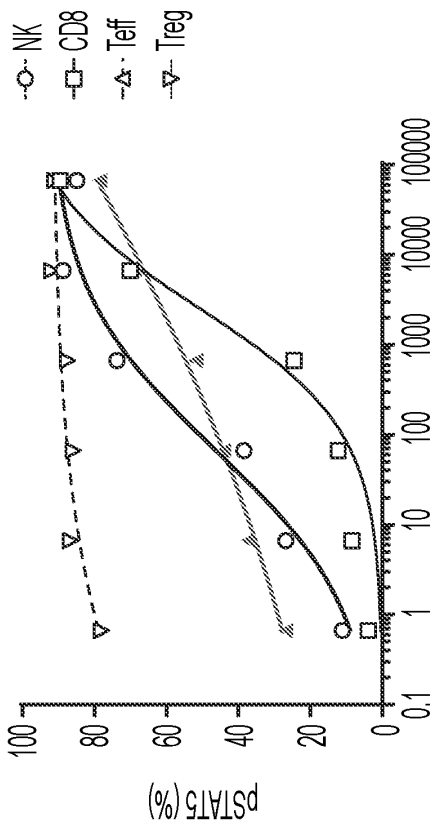

AK032
NK
CD8
Teff
Treg
pSTAT5 (%)
0
20
40
60
80
100
0.1
1
10
100
1000
10000
100000

FIG. 8B

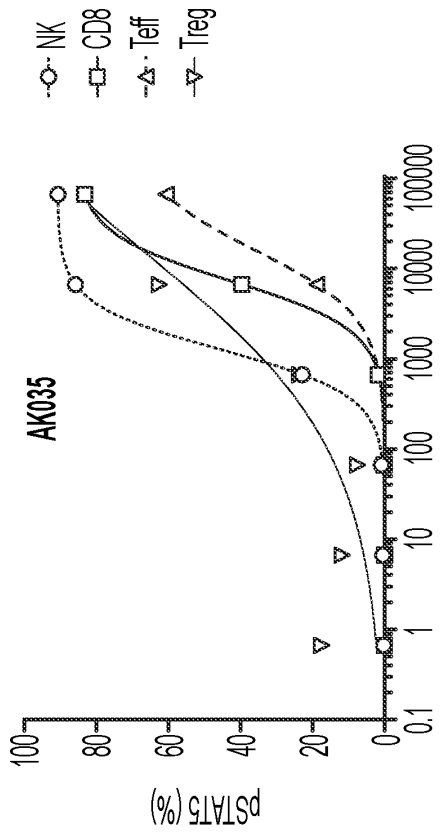

NK
CD8
Teff
Treg
AK035
pSTAT5 (%)
100
80
60
40
20
0
0.1
1
10
100
1000
10000
100000

FIG. 8C

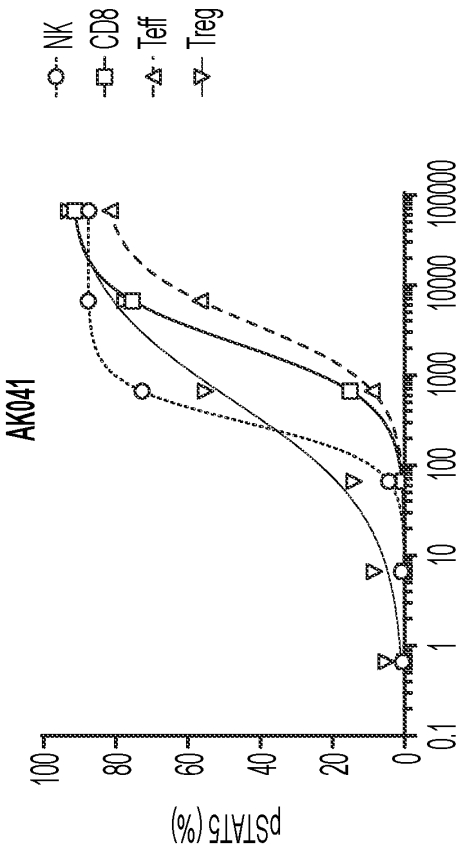

NK
CD8
Teff
Treg
AK041
pSTAT5 (%)
100
80
60
40
20
0
0.1
1
10
100
1000
10000
100000

FIG. 8D

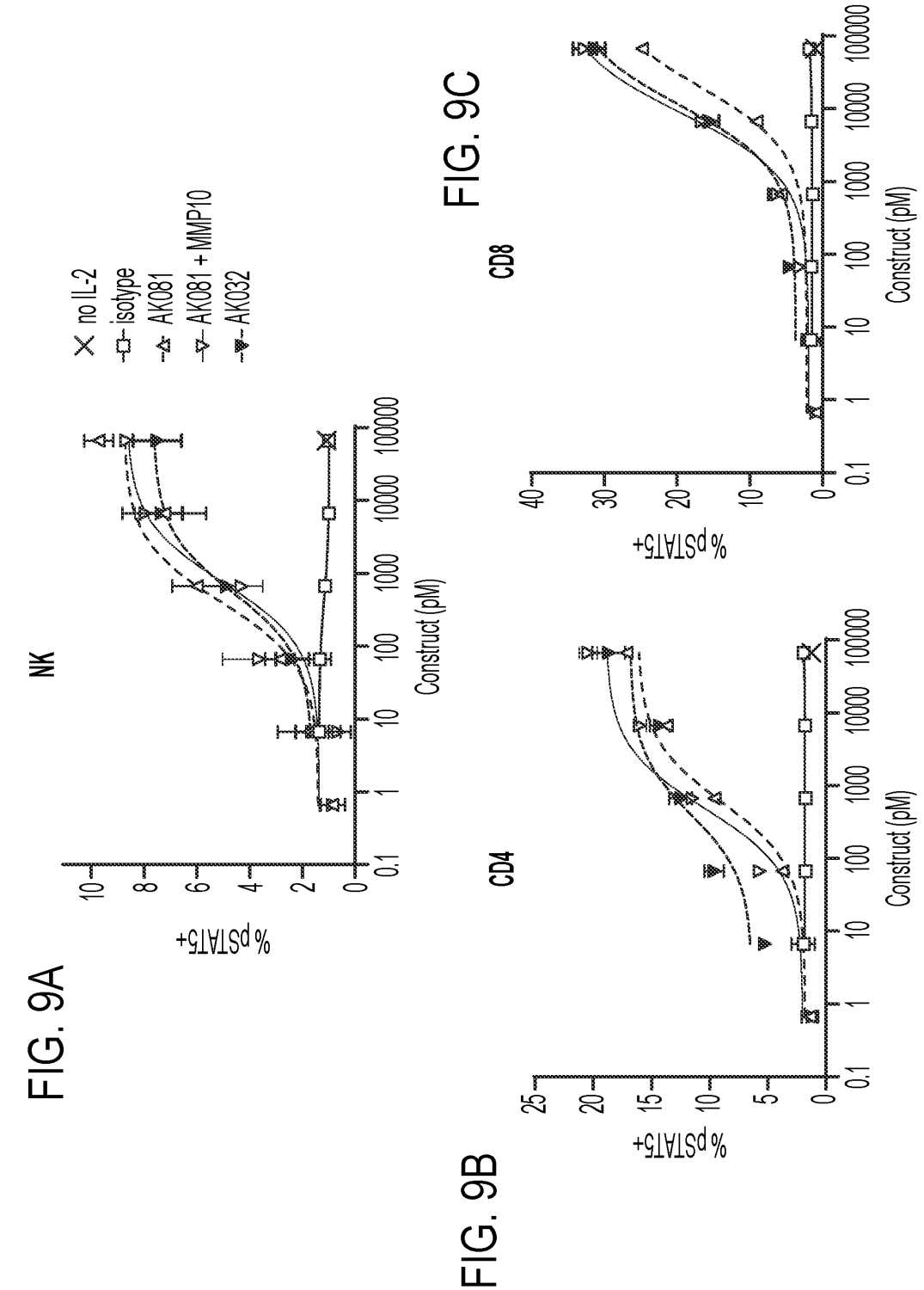


FIG. 9A

FIG. 9B

FIG. 9C

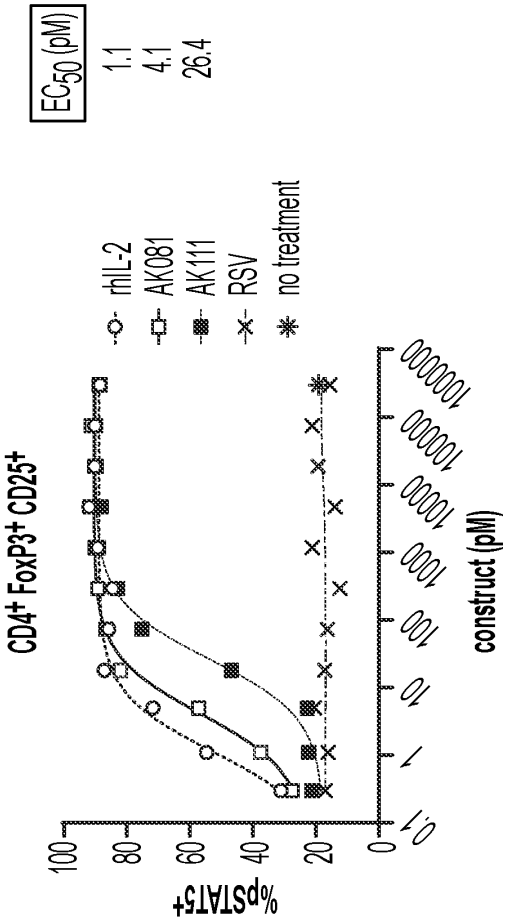


FIG. 10A

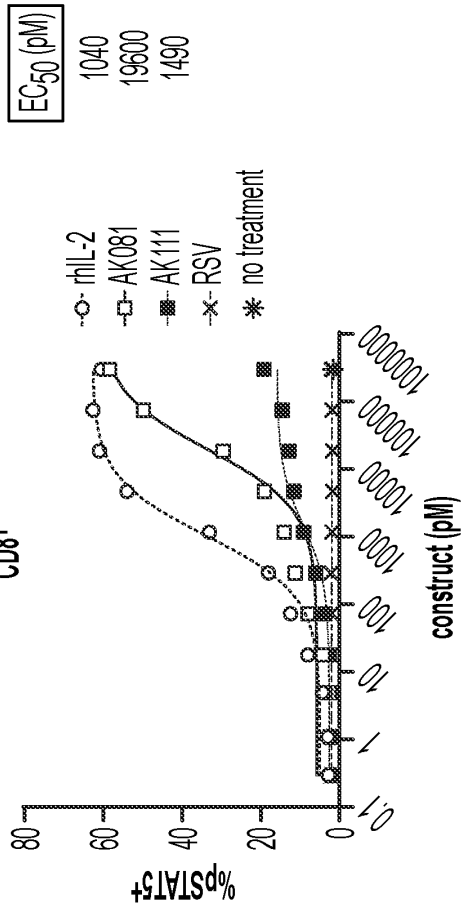


FIG. 10B

FIG. 10C

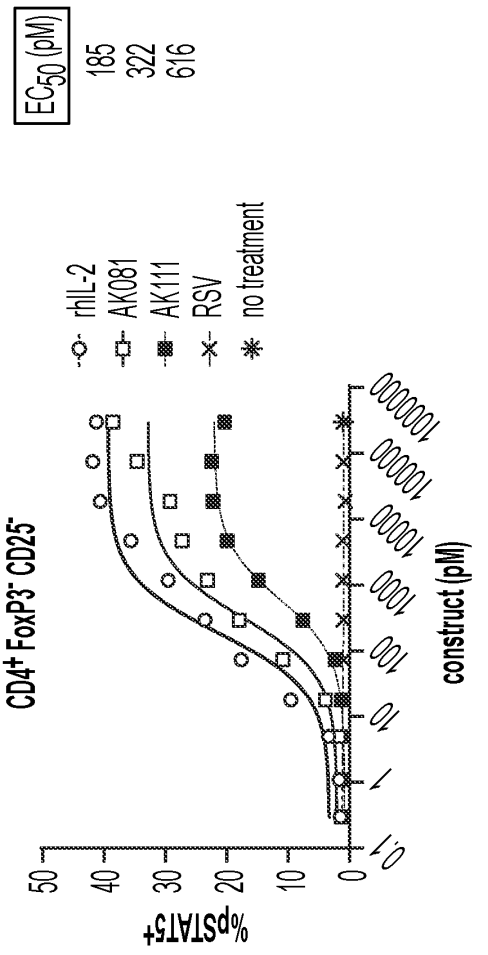


FIG. 10D

Construct	$FoxP3^+ T_{reg}$		$CD4^+ T_{help}$		$CD8^+ T_{cyto}$	
	EC_{50} (pM)	Fold-change	EC_{50} (pM)	Fold-change	EC_{50} (pM)	Fold-change
rhIL-2	1.1		1040		185	
AK081	4.1	3.7	19600	18.8	322	1.7
AK111	26.4	24.0	1490*	1.4*	616	3.3

** Prism was able to find a best-fit value, but was unable to calculate a complete confidence interval. This best-fit value should be interpreted with caution.*

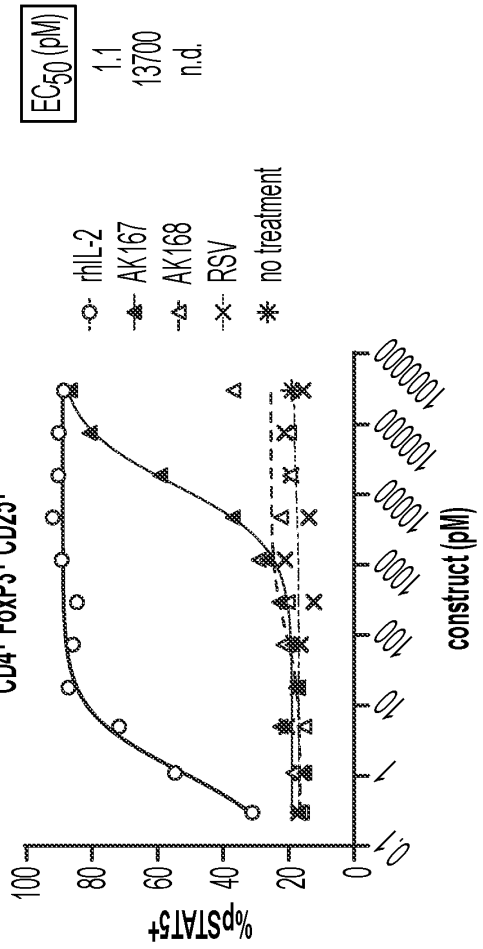


FIG. 11A

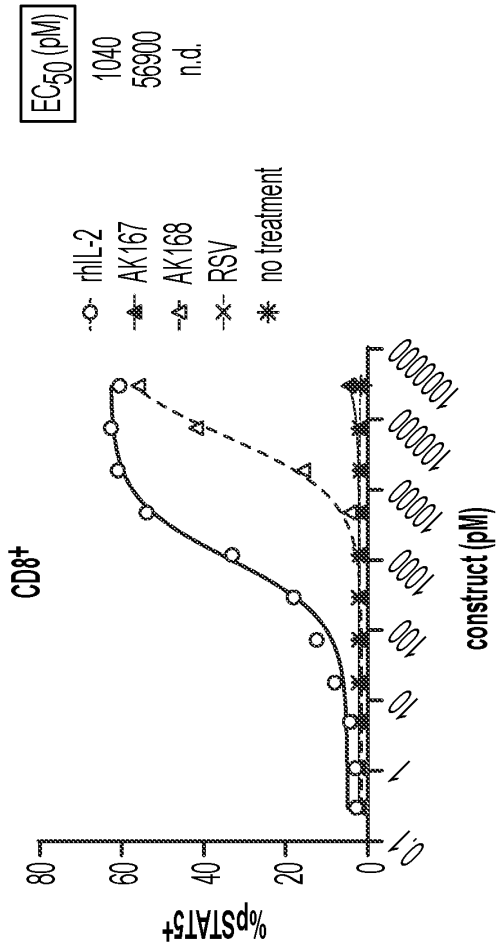


FIG. 11B

FIG. 11C

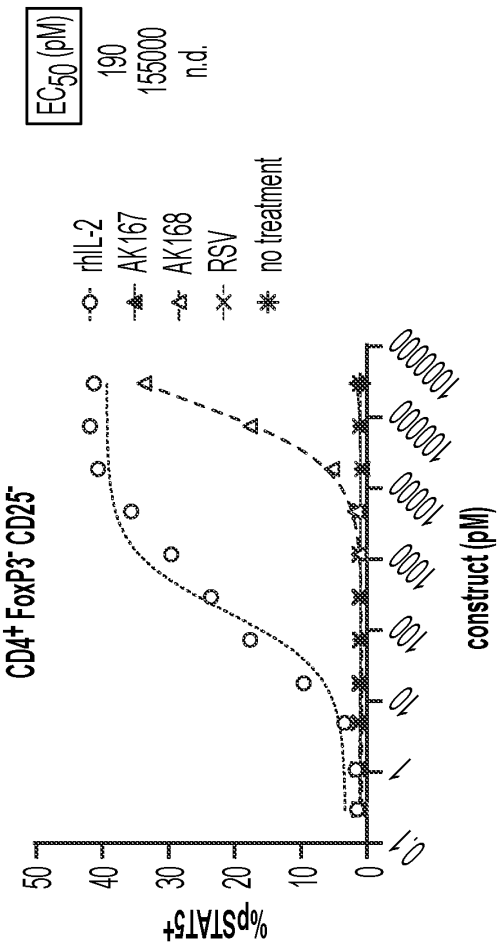


FIG. 11D

Construct	FoxP3+ Treg		CD4+ Thelp		CD8+ Tcyto	
	EC50 (pM)	*Fold-change*	*EC50 (pM)*	*Fold-change*	*EC50 (pM)*	*Fold-change*
rhIL-2	1.1		1040		190	
AK167	13700	12455	56900	55	155000	816
AK168	*n.d.*		*n.d.*		*n.d.*	

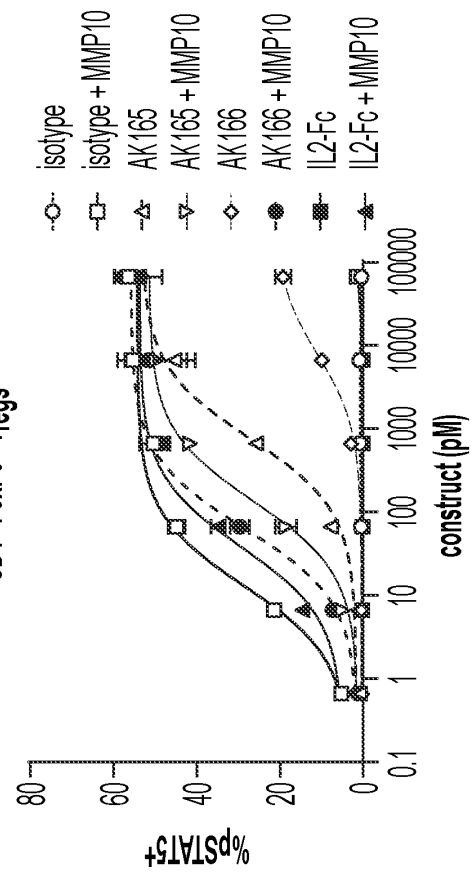


FIG. 12A

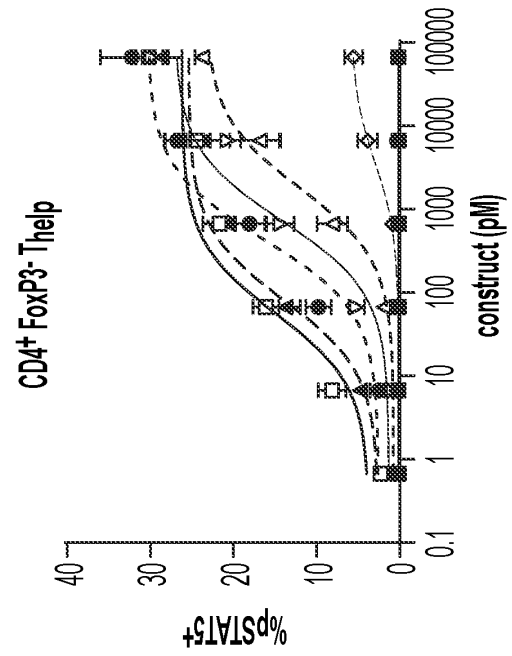


FIG. 12B

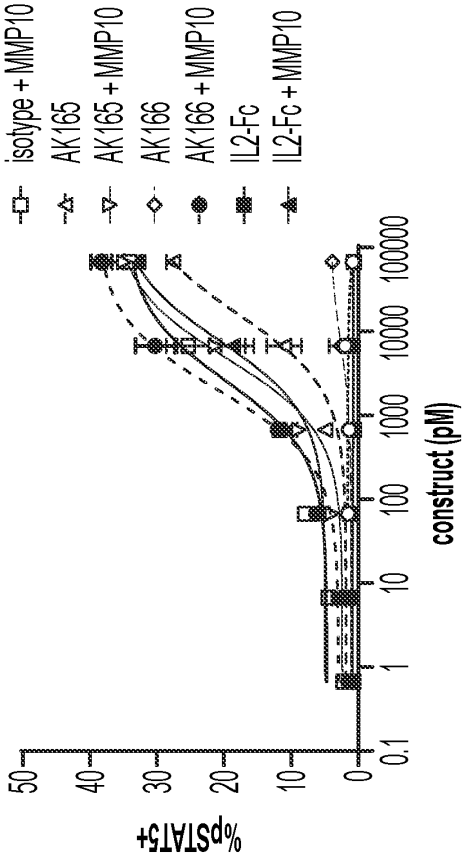


FIG. 12C

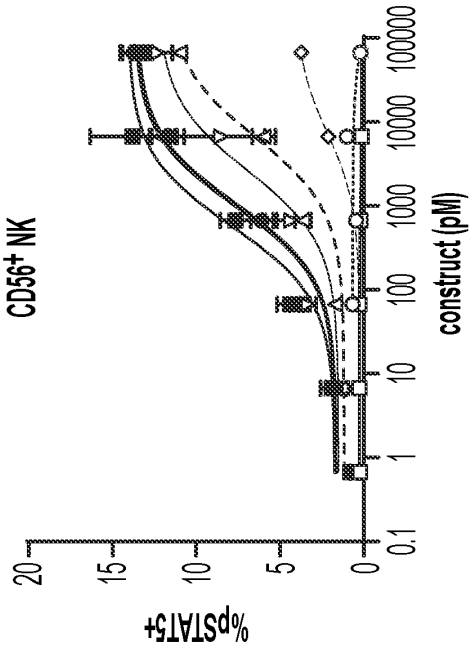


FIG. 12D

FIG. 13A

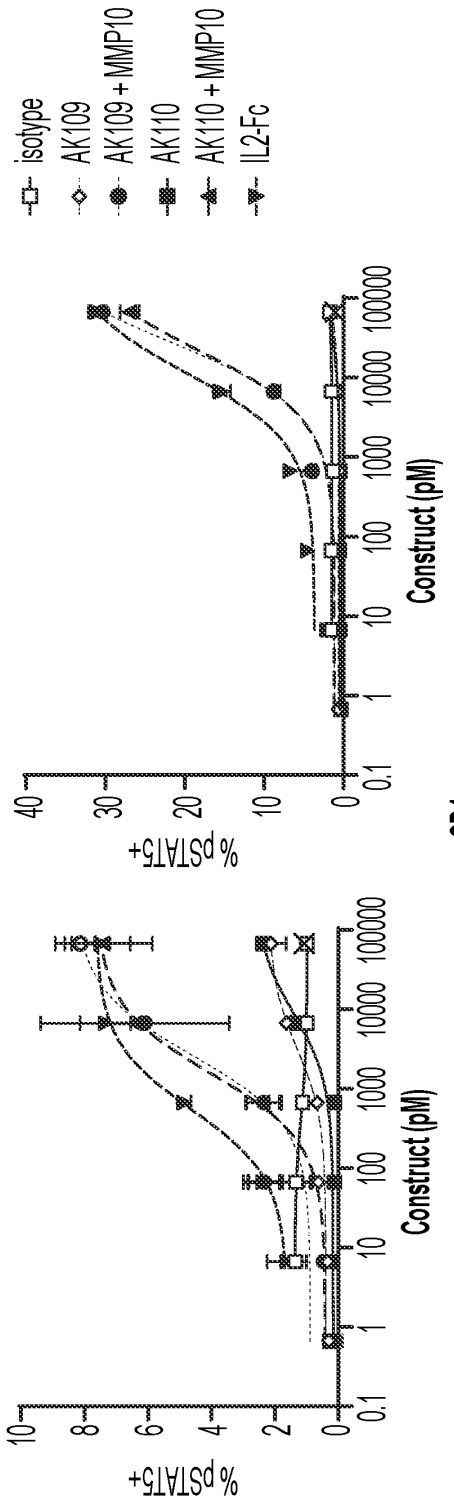
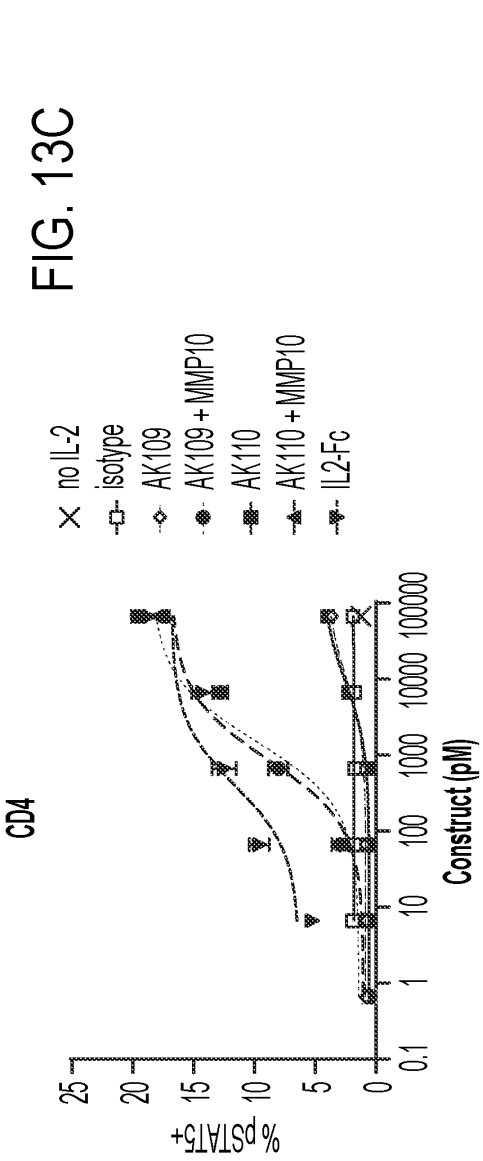


FIG. 13B

FIG. 13C



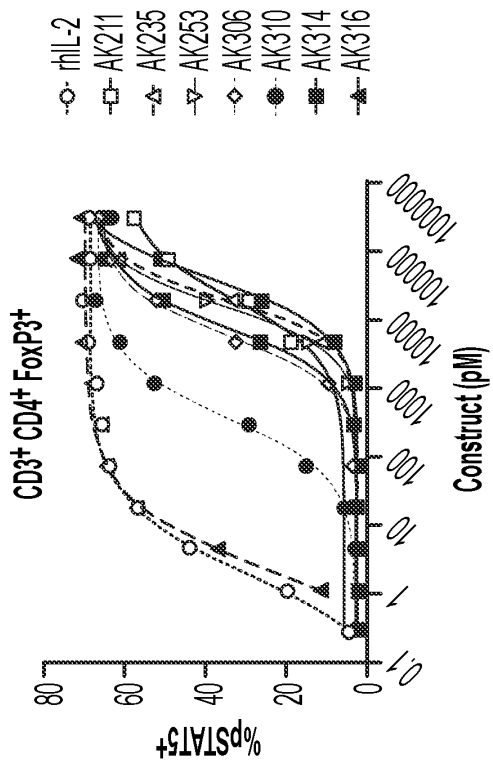


FIG. 14A

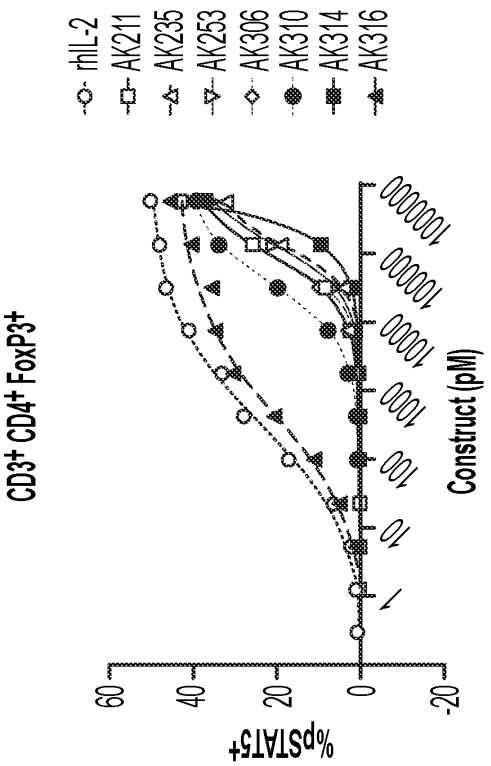


FIG. 14B

FIG. 14C

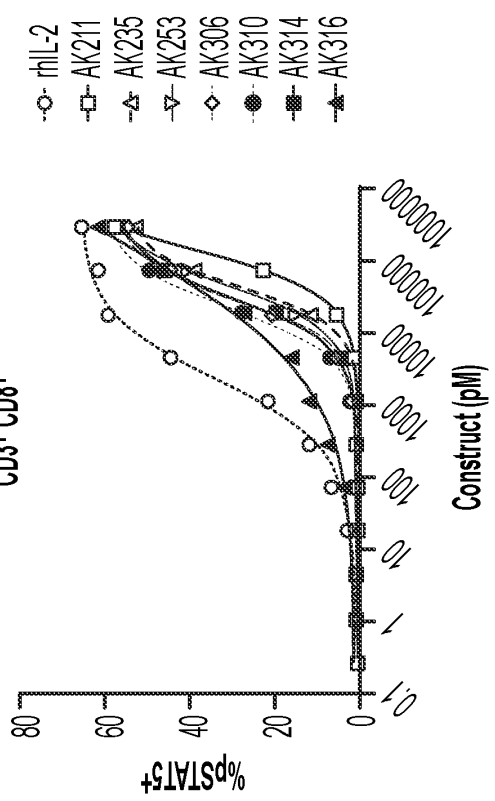


FIG. 14D

Construct	CD3+ CD4+ FoxP3+ EC_{50} (pM)	CD3+ CD4+ FoxP3- EC_{50} (pM)	CD3+ CD8+ EC_{50} (pM)
rhIL-2	1.94	247	2300
AK211	39500	*	164000
AK253	15400	75100	46400
AK235	19200	69500	44100
AK314	7250	49400	29500
AK306	5680	56300	28800
AK310	373	20800	22300
AK316	1.73	331	167000**

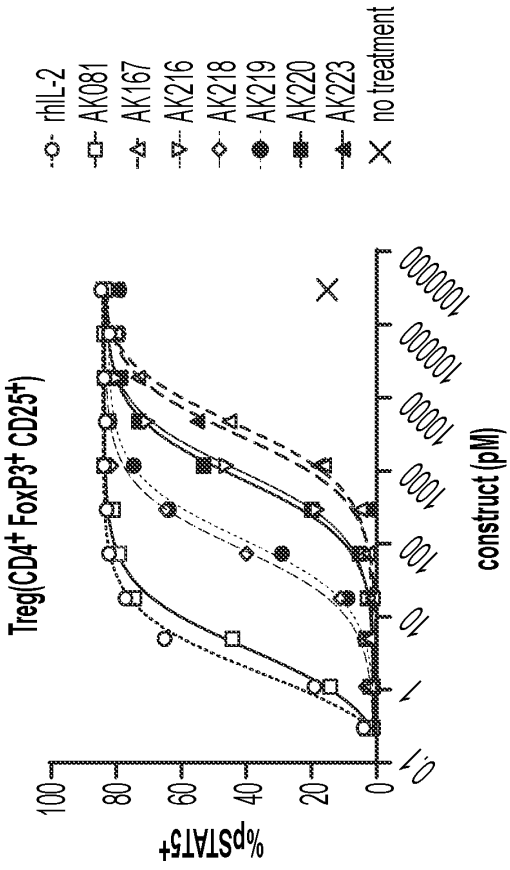


FIG. 15A

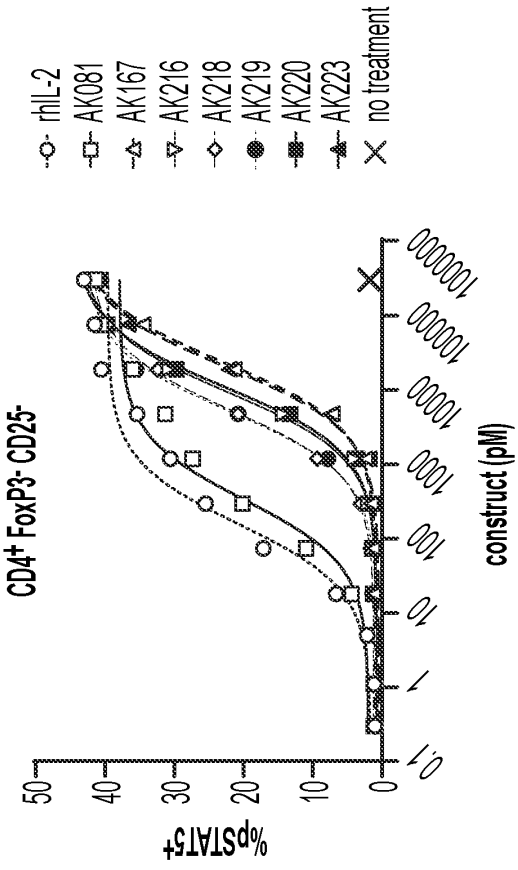


FIG. 15B

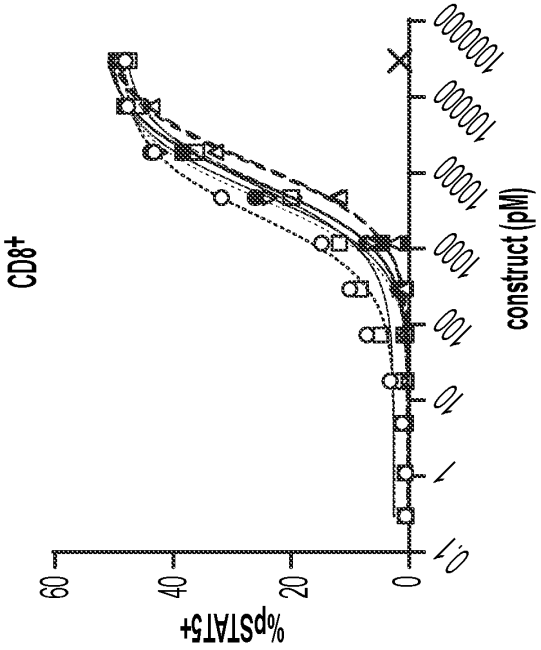


FIG. 15C

FIG. 15D

Construct	FoxP3+ T_{reg}	CD4+ T_{help}	CD8+ T_{cyto}
	EC_{50} (pM)	EC_{50} (pM)	EC_{50} (pM)
rhIL-2	1.67	154	2710
AK081	3.59	332	6700
AK167	4390	22900	13200
AK216	1020	9200	5490
AK218	86.7	5270	7550
AK219	117	5030	4570
AK220	782	10900	7650
AK223	3040	20200	12600

FIG. 16A

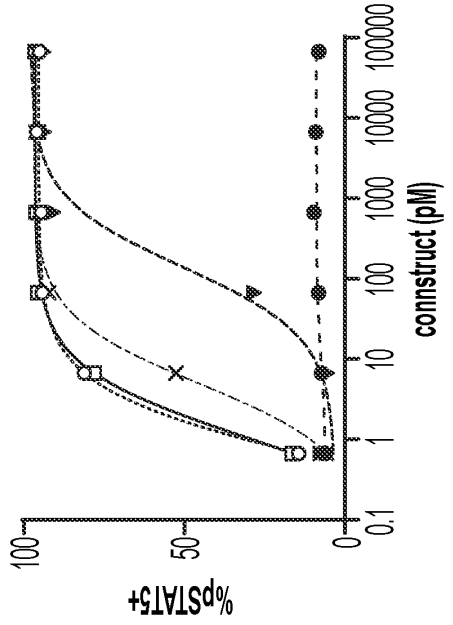


FIG. 16B

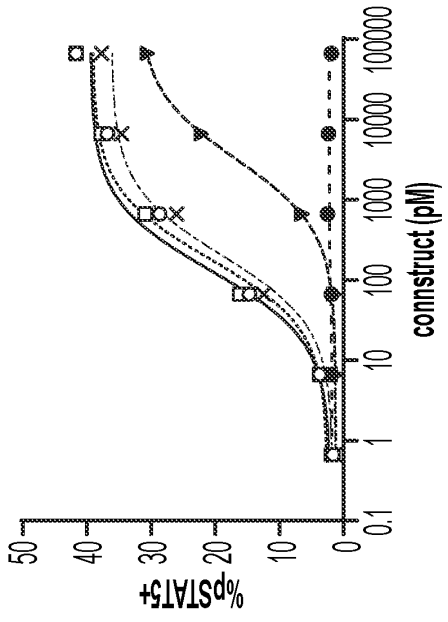


FIG. 16C

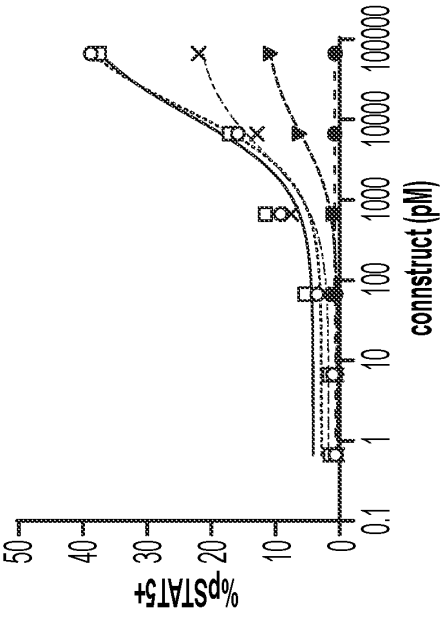


FIG. 17A

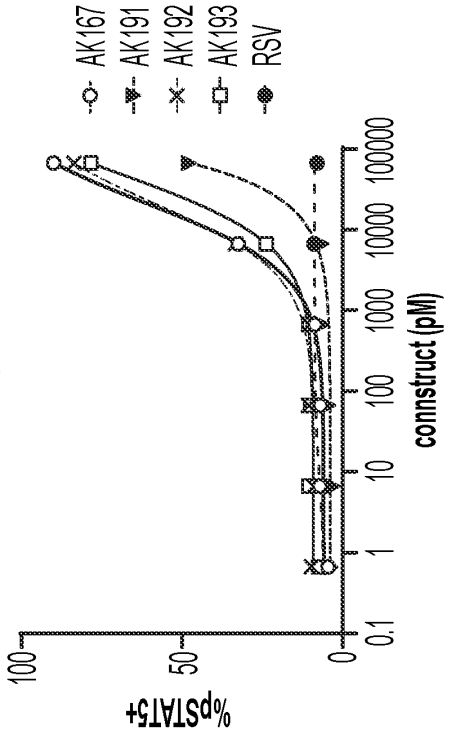


FIG. 17B

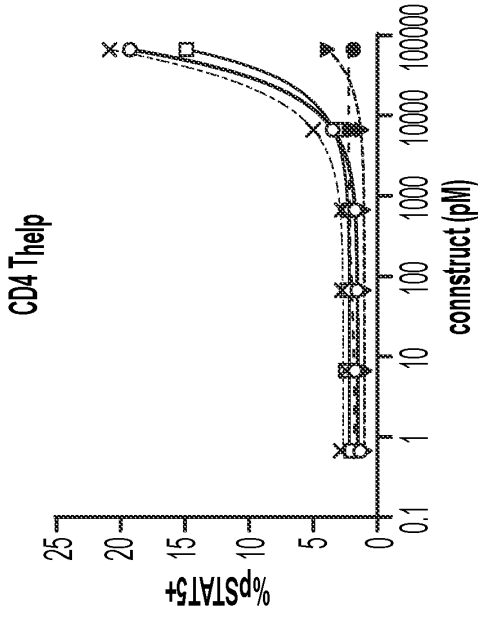


FIG. 17C

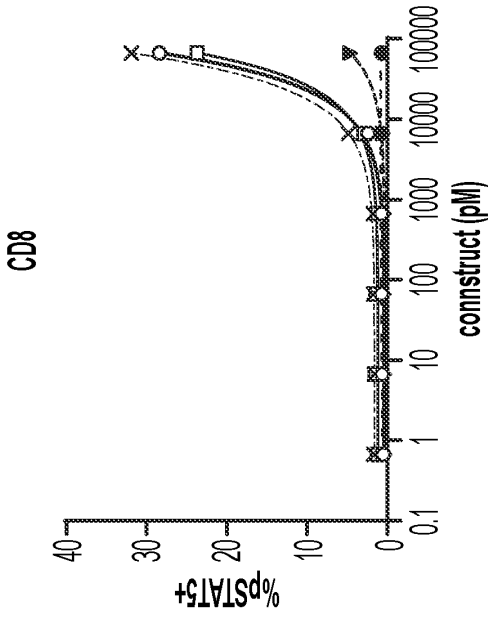


FIG. 18A

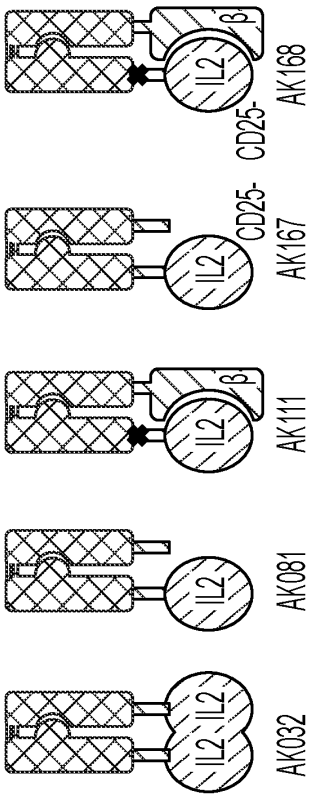


FIG. 18B

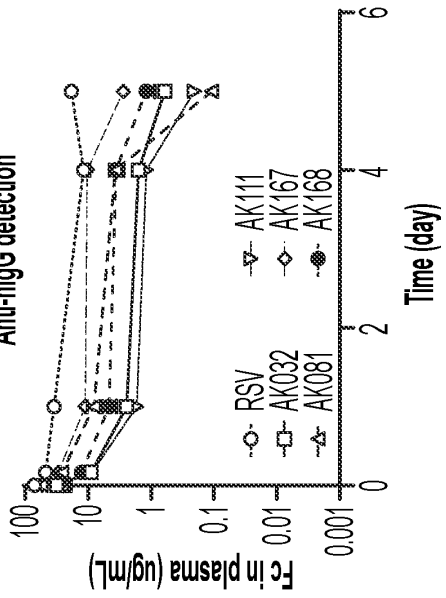


FIG. 18C

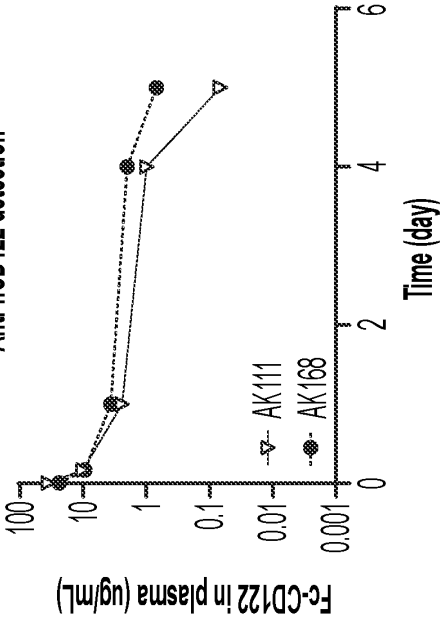


FIG. 18D

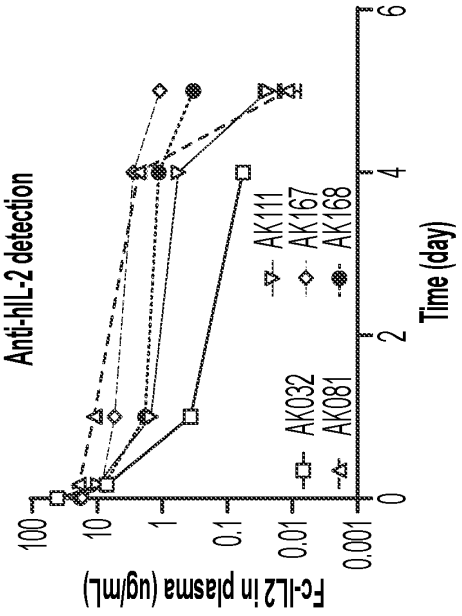


FIG. 19A

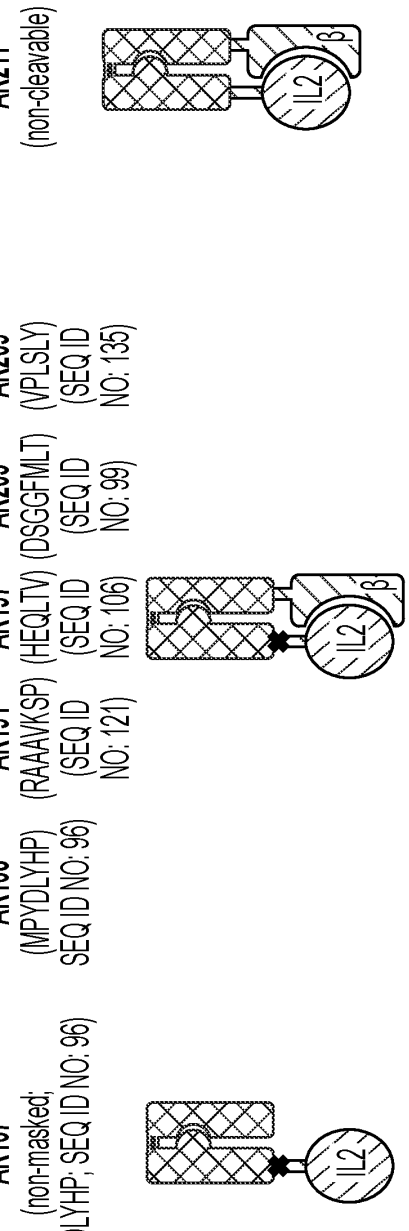
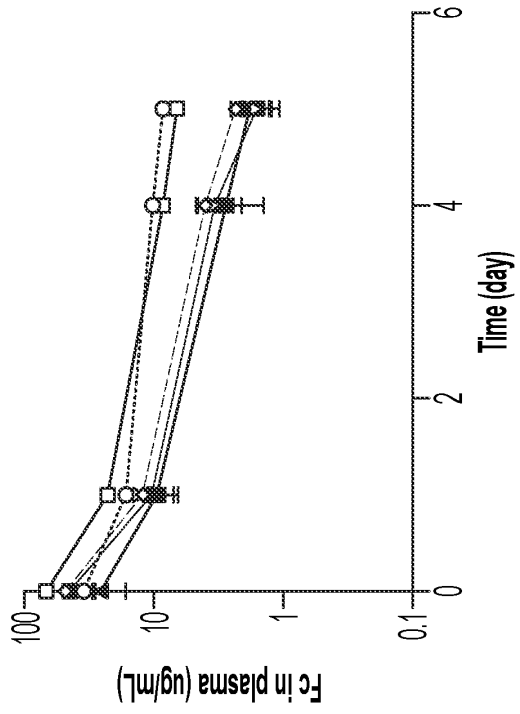


FIG. 19B



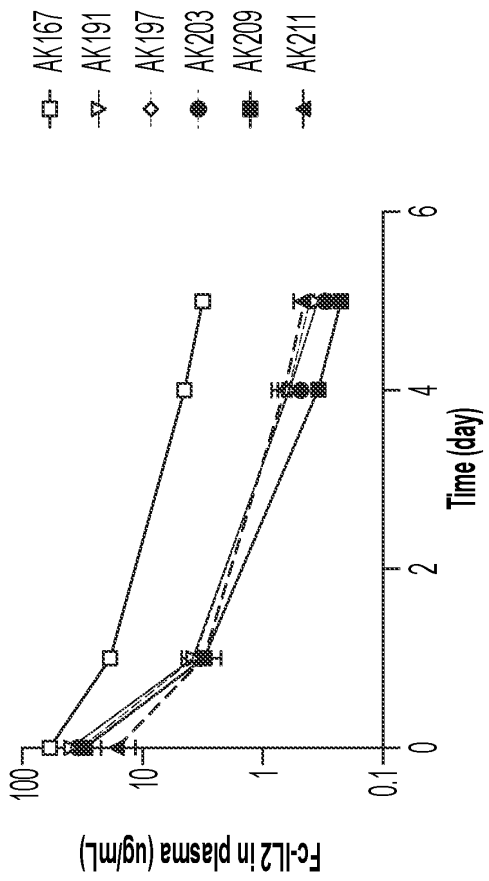


FIG. 19C

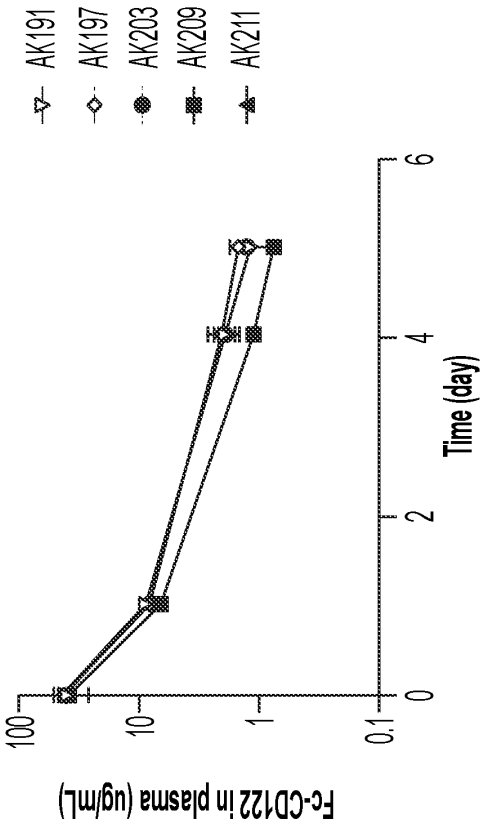


FIG. 19D

FIG. 20A

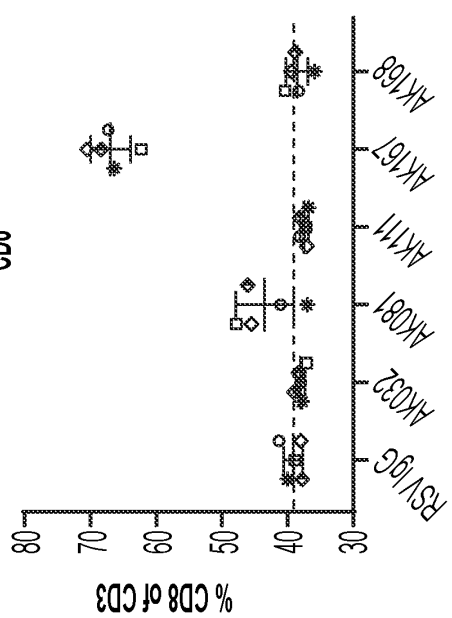


FIG. 20B

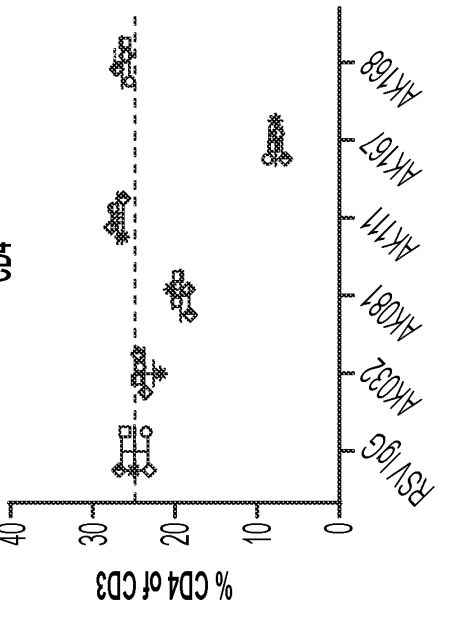


FIG. 20C

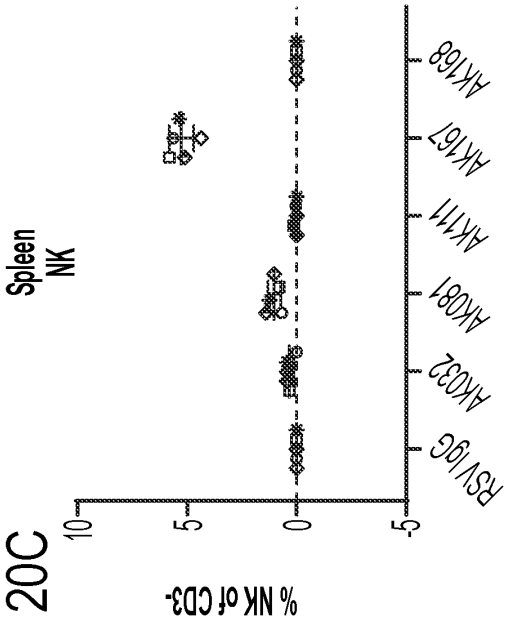


FIG. 20D

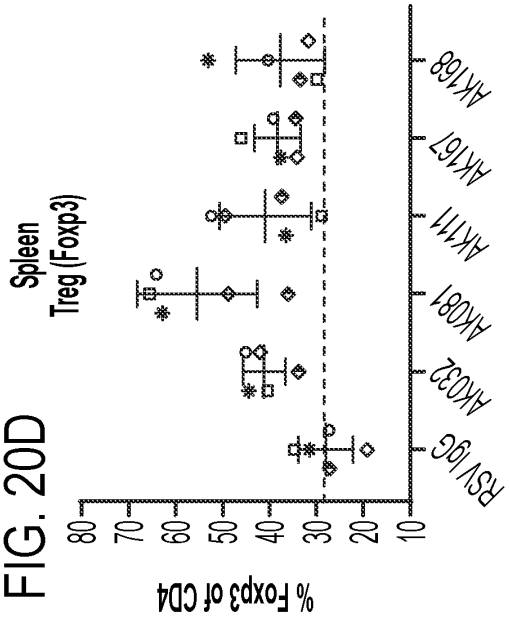


FIG. 20F

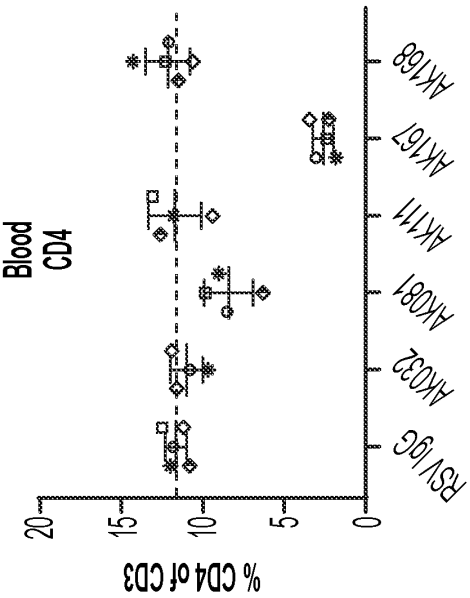


FIG. 20H

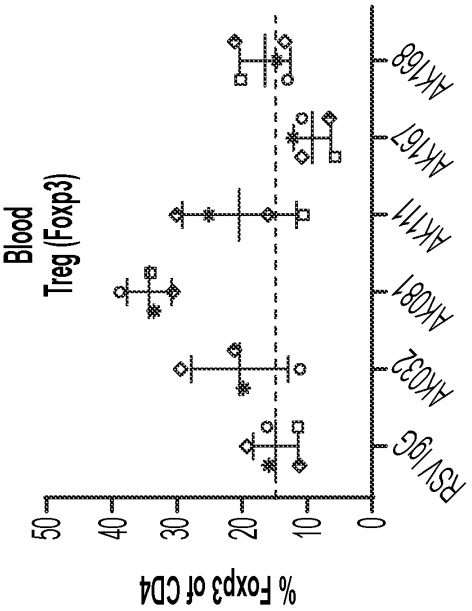


FIG. 20E

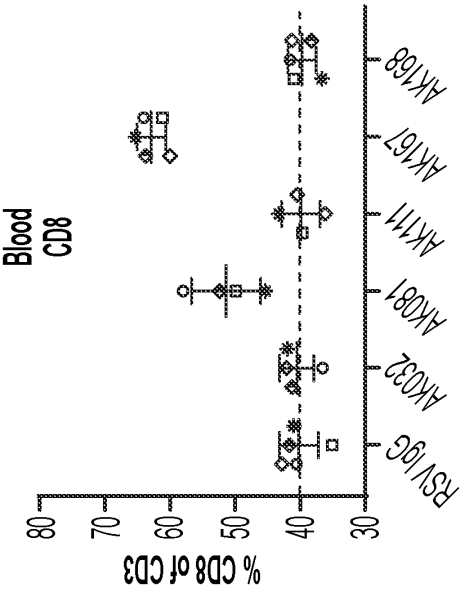


FIG. 20G

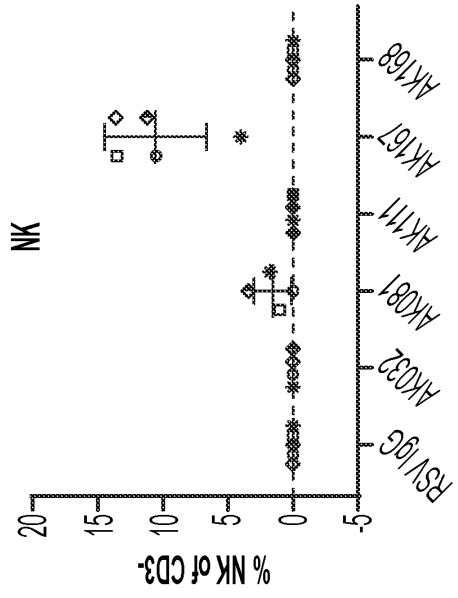


FIG. 20J

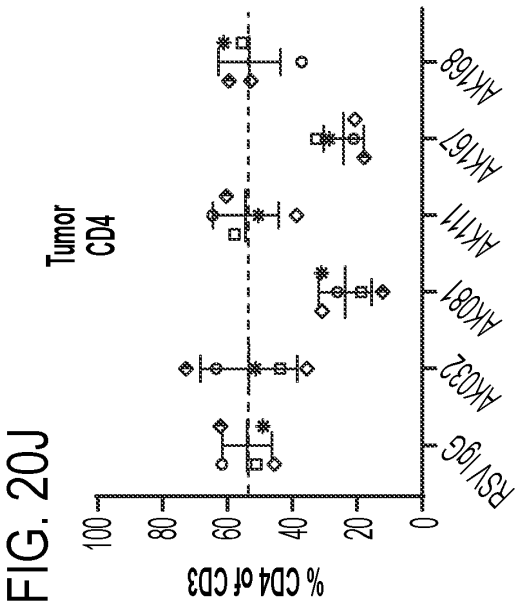


FIG. 20I

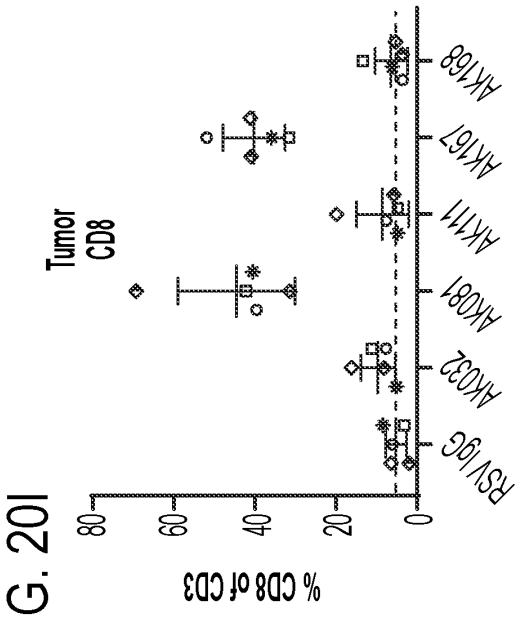


FIG. 20L

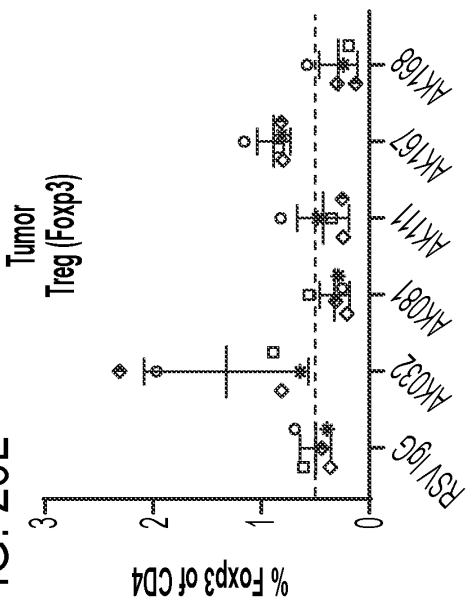


FIG. 20K

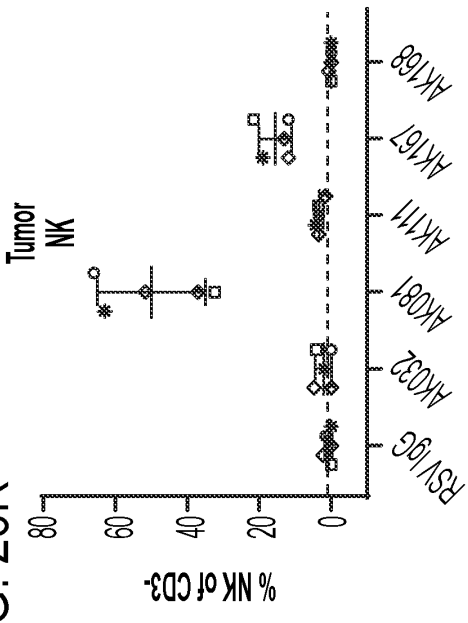


FIG. 21A

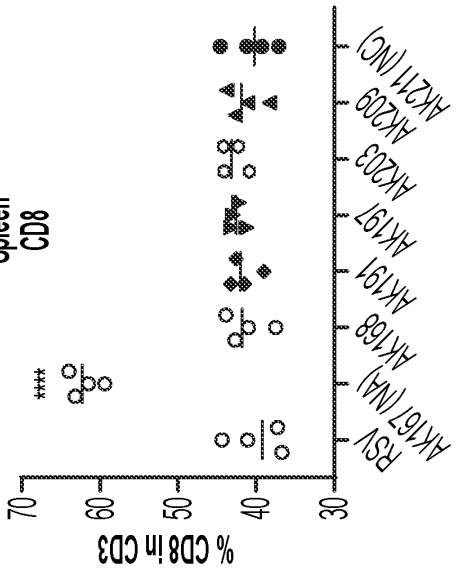


FIG. 21B

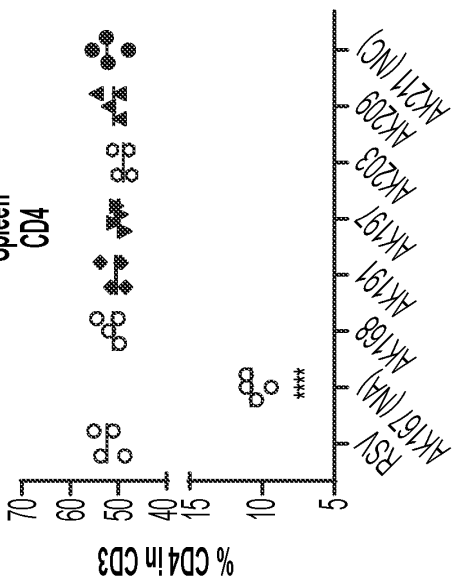


FIG. 21C

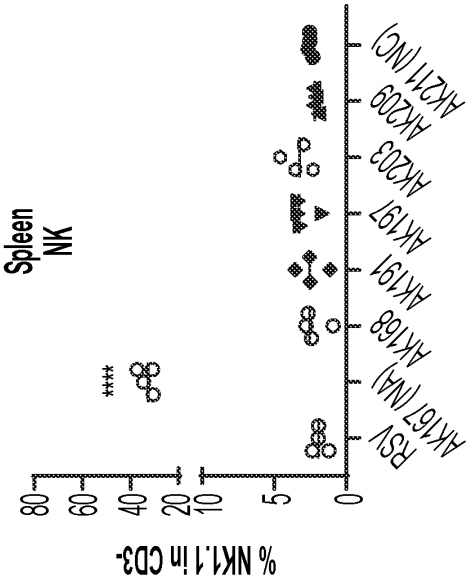
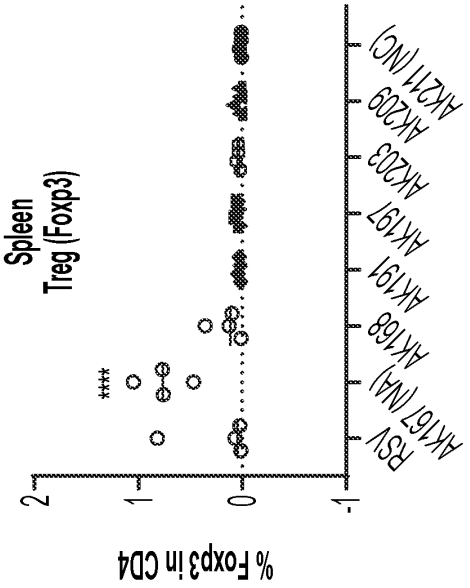


FIG. 21D



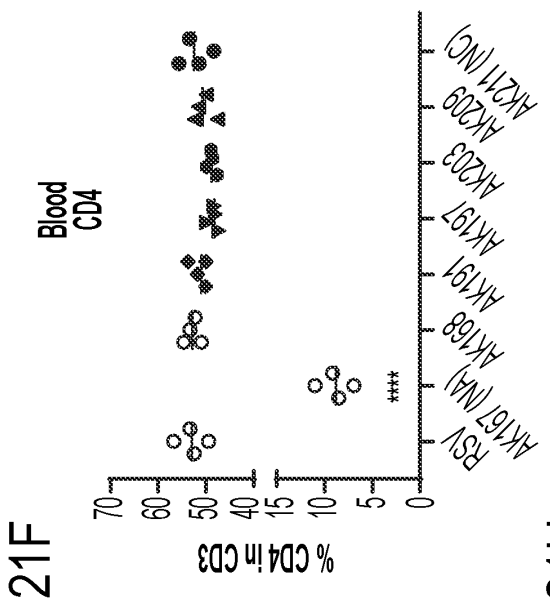


FIG. 21F

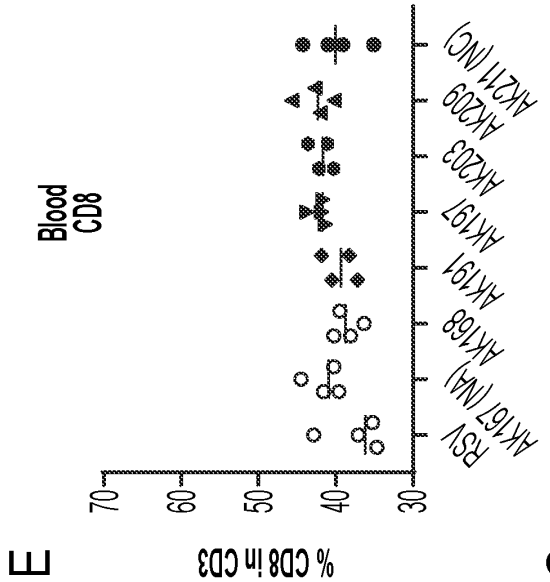


FIG. 21E

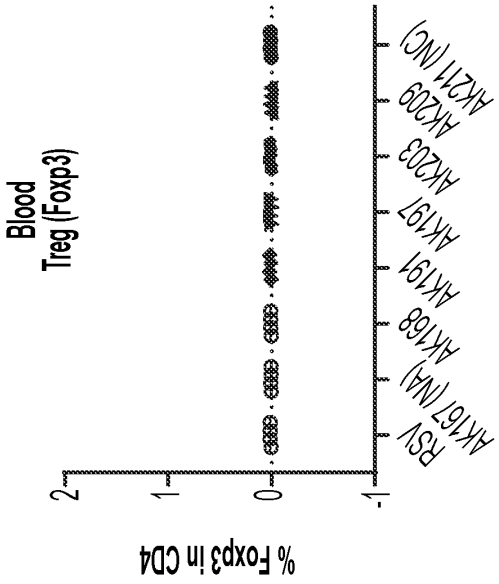


FIG. 21H

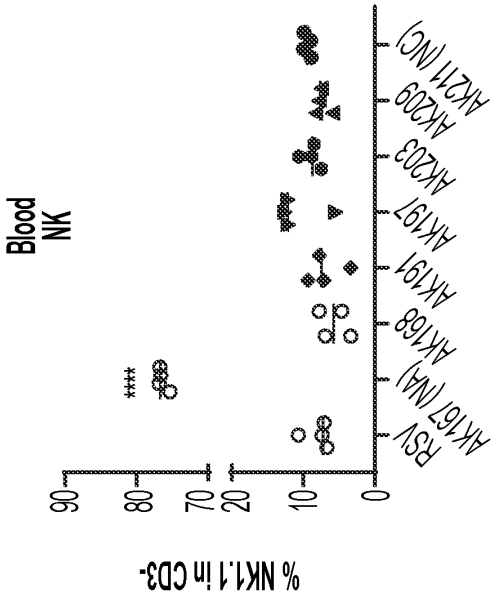


FIG. 21G

FIG. 21J

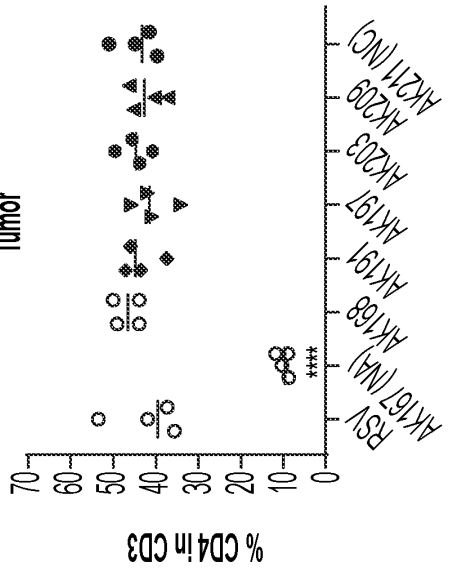


FIG. 21L

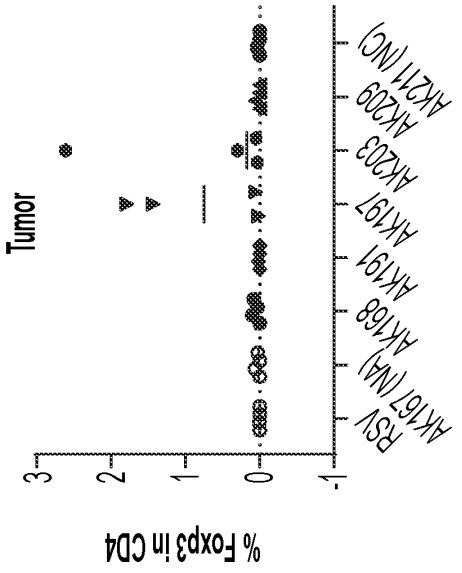


FIG. 21I

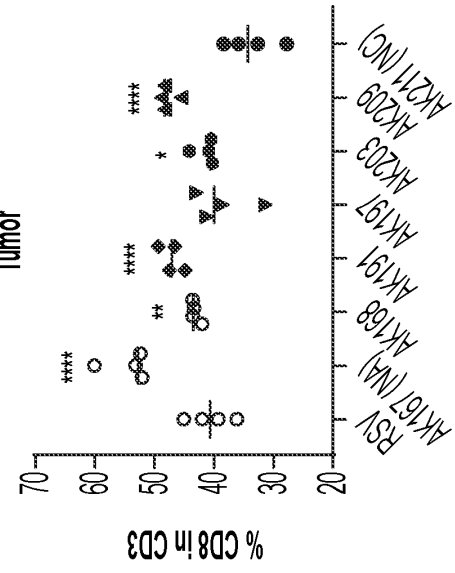
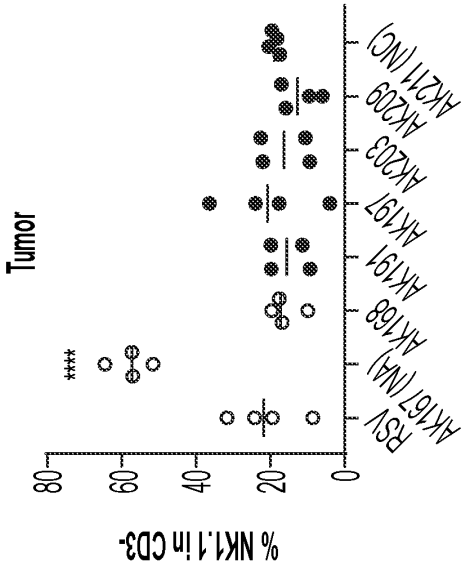


FIG. 21K



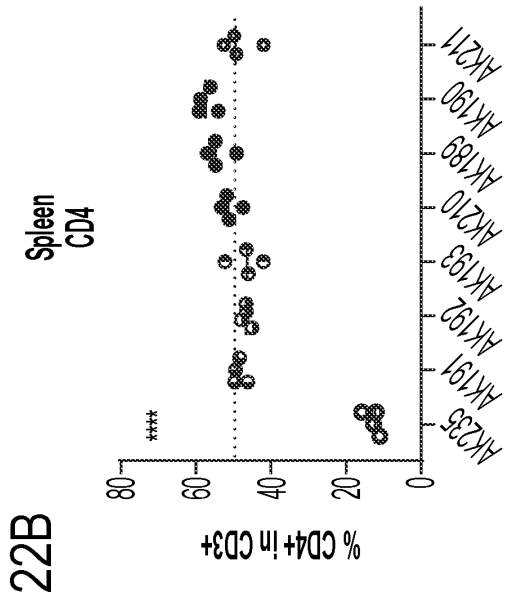


FIG. 22B

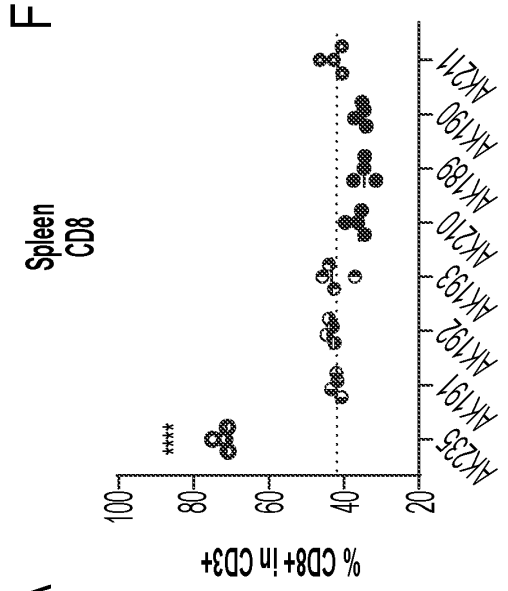


FIG. 22A

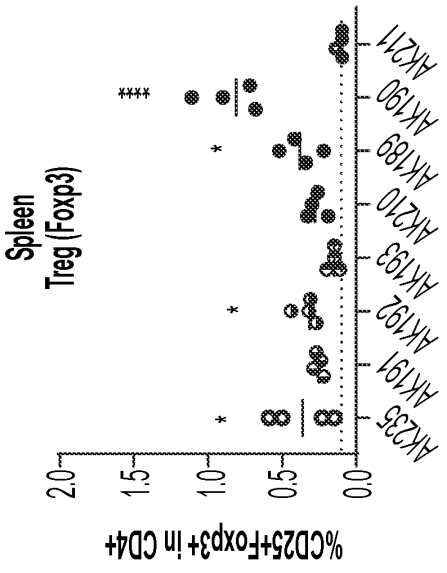


FIG. 22D

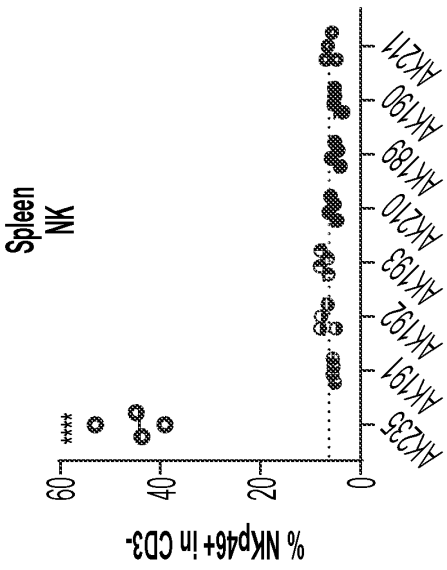


FIG. 22C

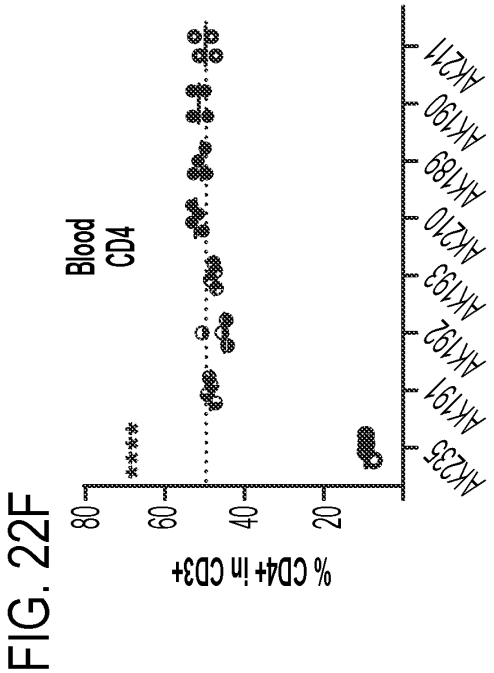


FIG. 22F

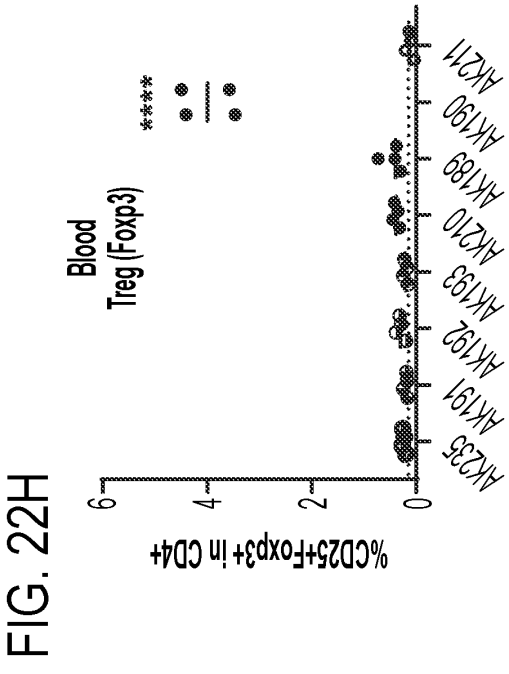


FIG. 22H

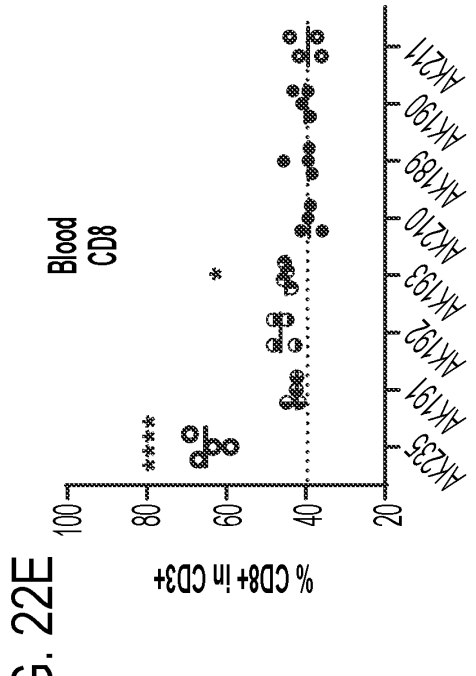


FIG. 22E

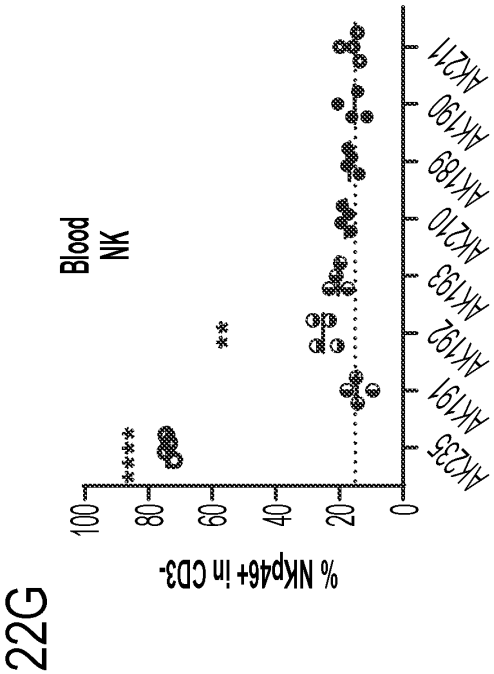


FIG. 22G

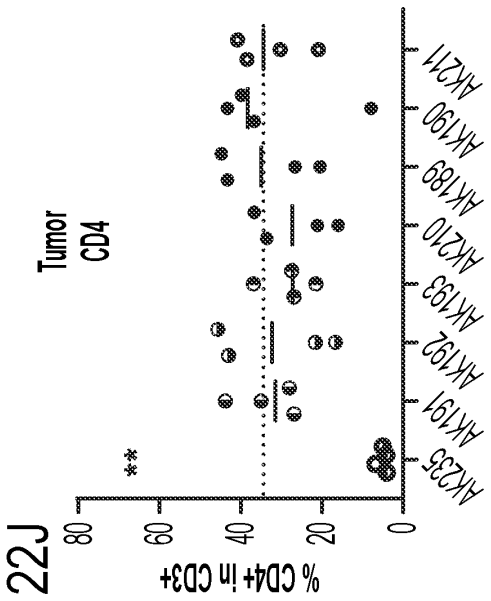


FIG. 22J

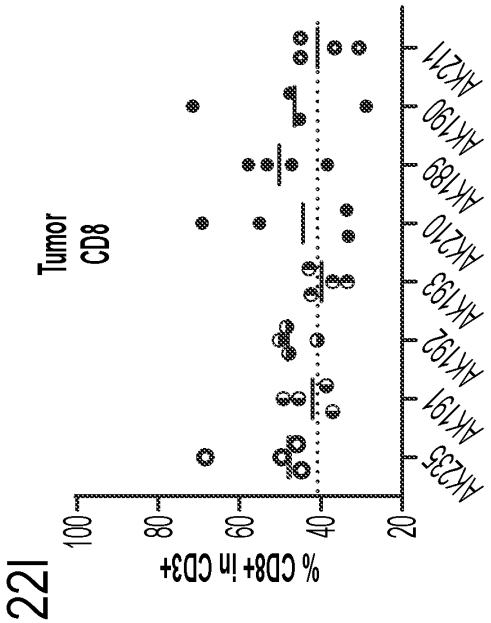


FIG. 22I

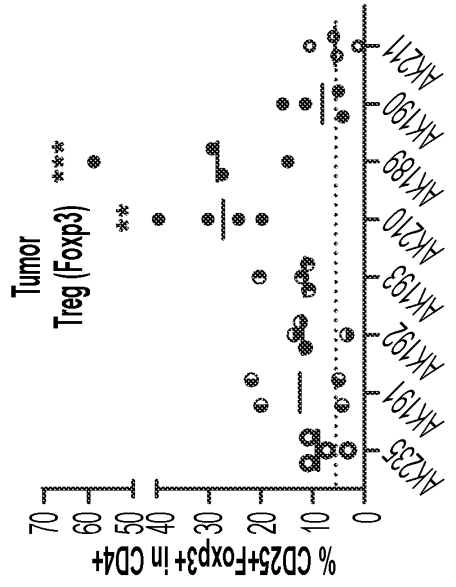


FIG. 22L

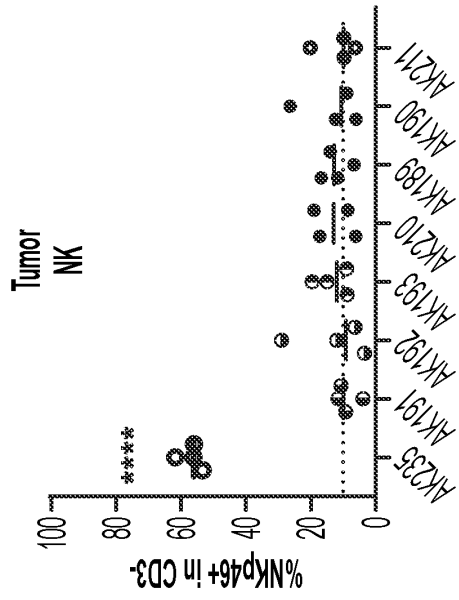


FIG. 22K

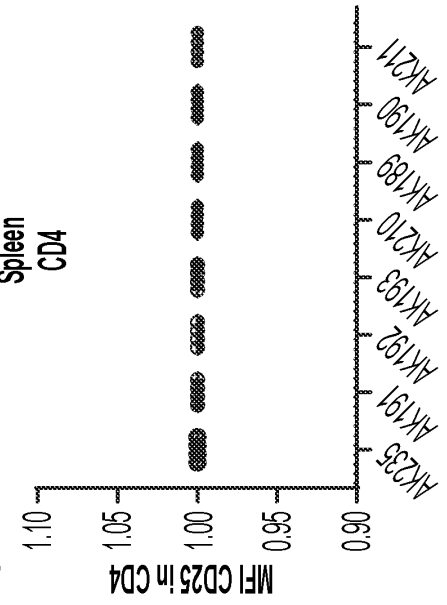


FIG. 23B

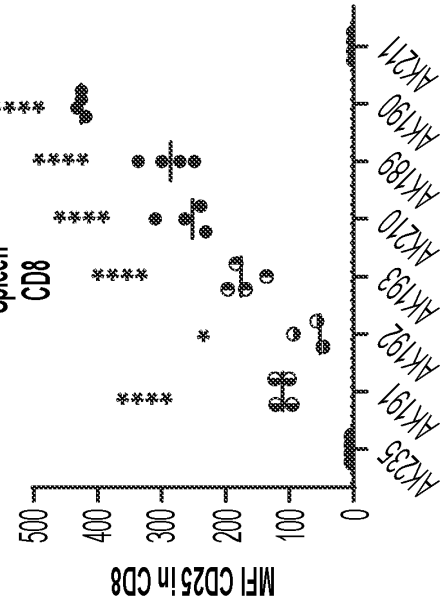


FIG. 23A

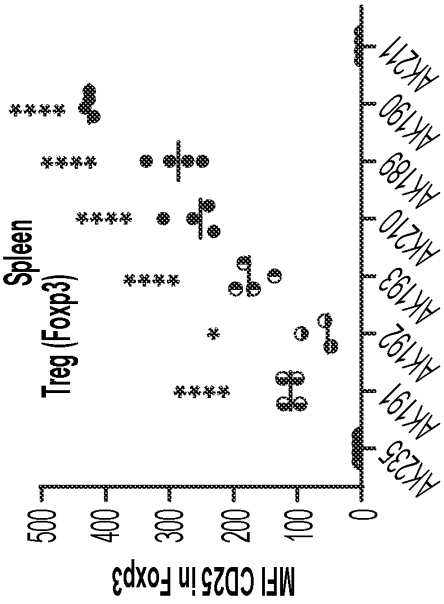


FIG. 23C

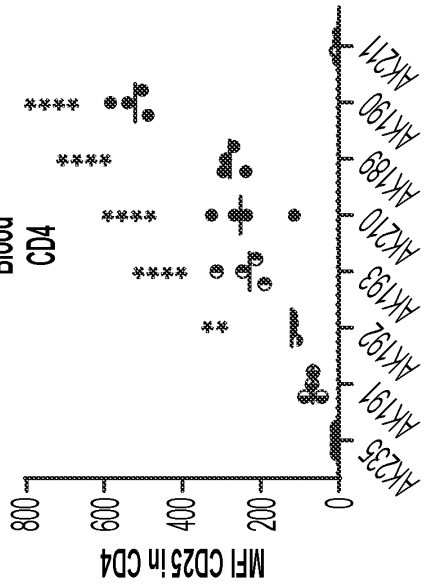


FIG. 23E

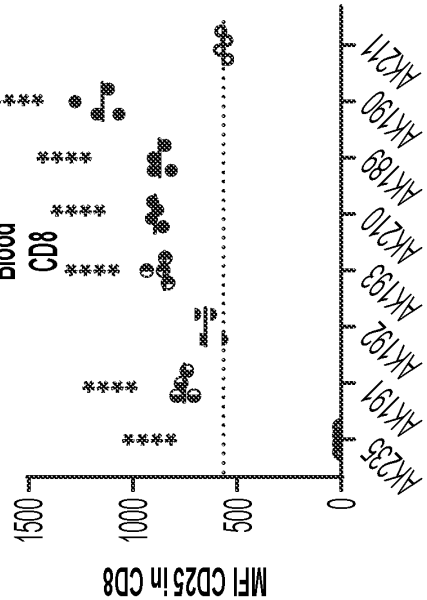


FIG. 23D

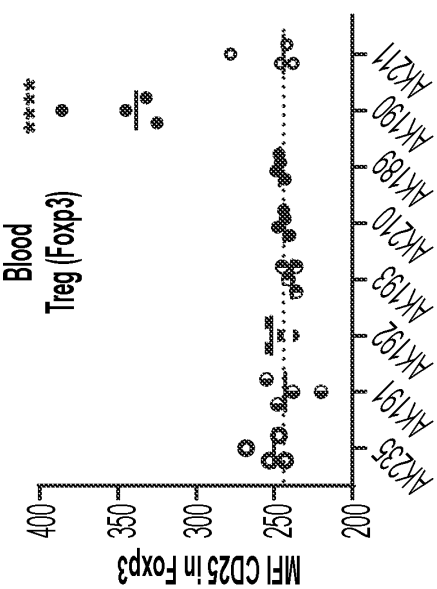


FIG. 23F

FIG. 23H

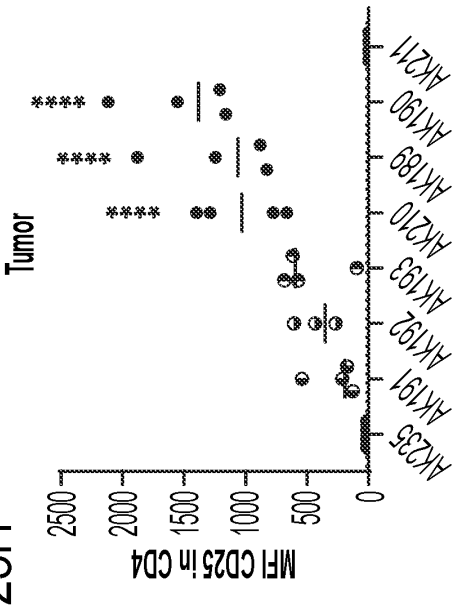


FIG. 23G

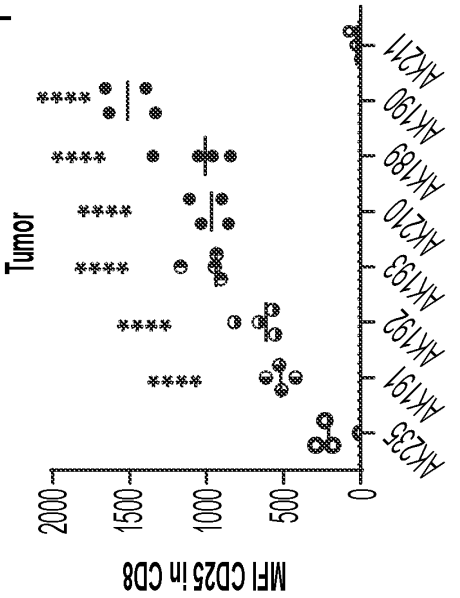
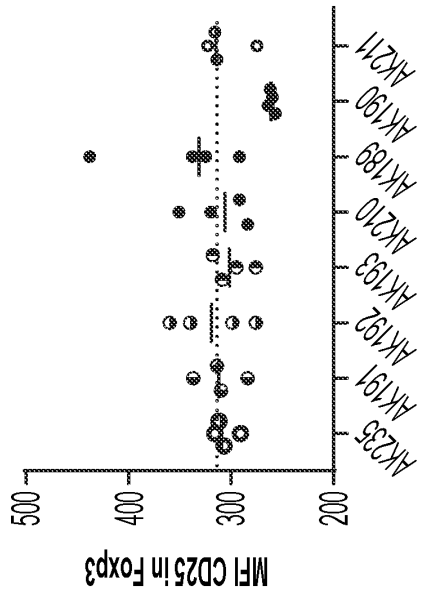


FIG. 23I



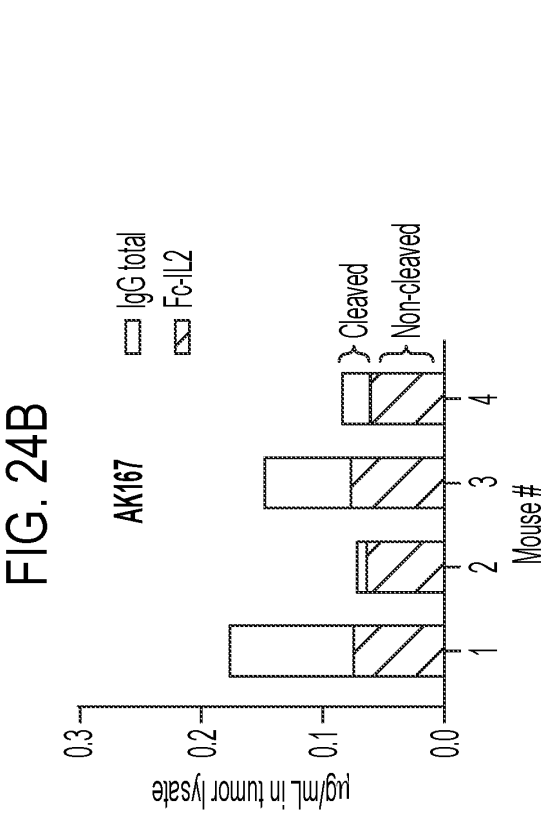


FIG. 24A

FIG. 24B

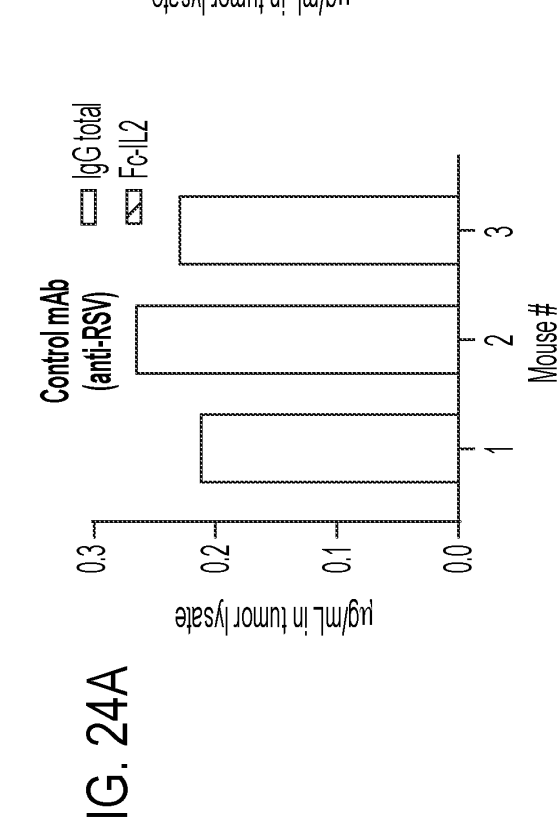


FIG. 24C

FIG. 24D

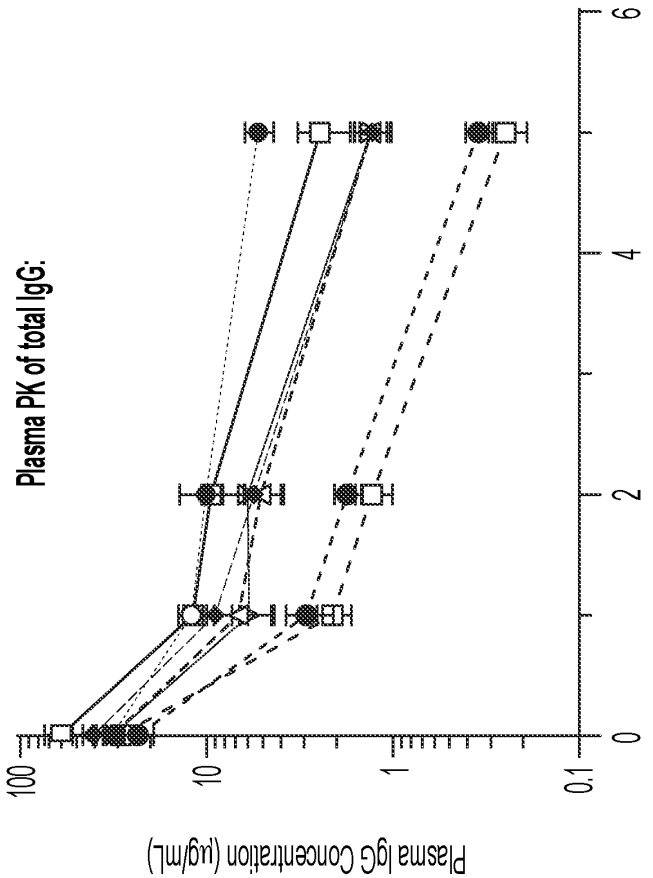

Plasma PK of total IgG:
Gr. 1: RSV
Gr. 2: AK167
AK167 Non-cleaved
Gr. 3: AK168
AK168 Non-cleaved
Gr. 4: AK209
AK209 Non-cleaved
Plasma IgG Concentration (μg/mL)
100
10
1
0.1
0
2
4
6
Time (day)

FIG. 24E

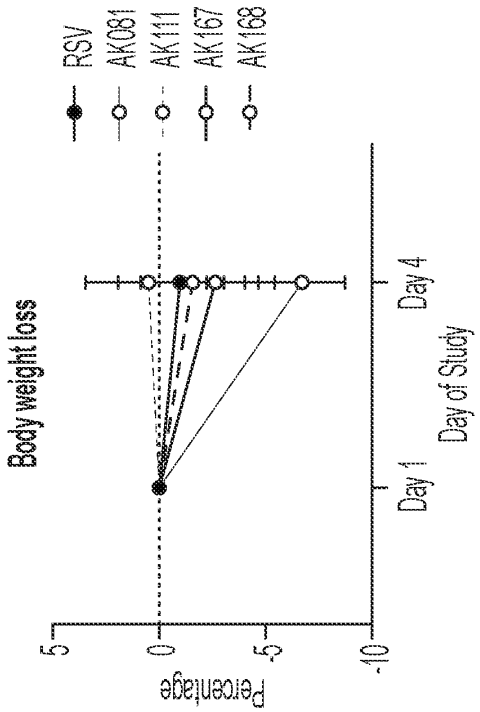


FIG. 25A

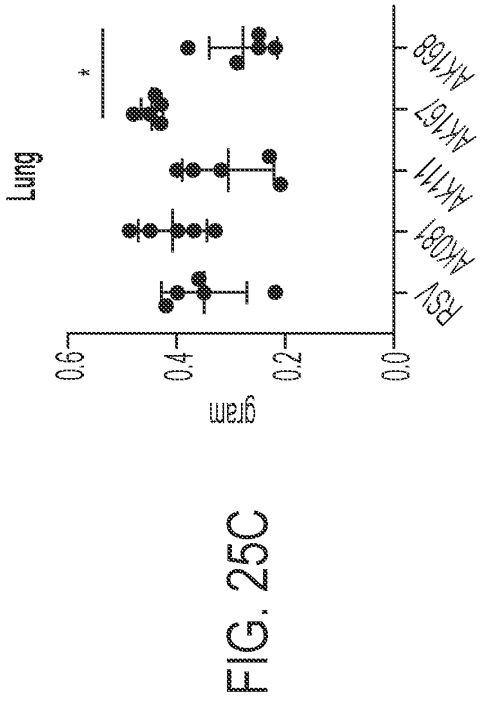


FIG. 25C

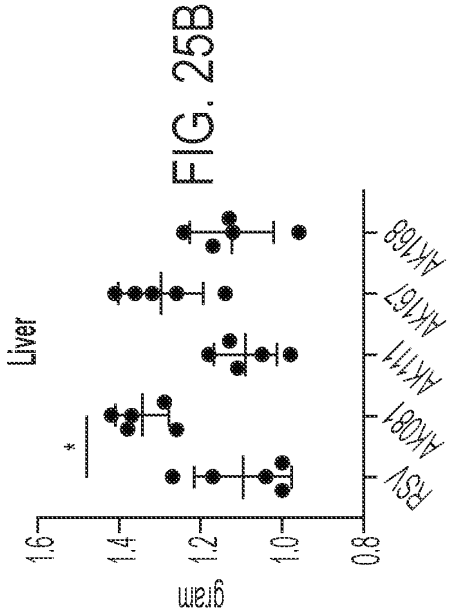


FIG. 25B

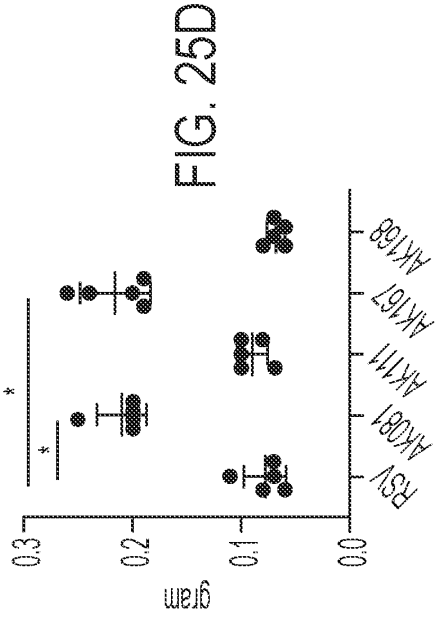


FIG. 25D

FIG. 26B

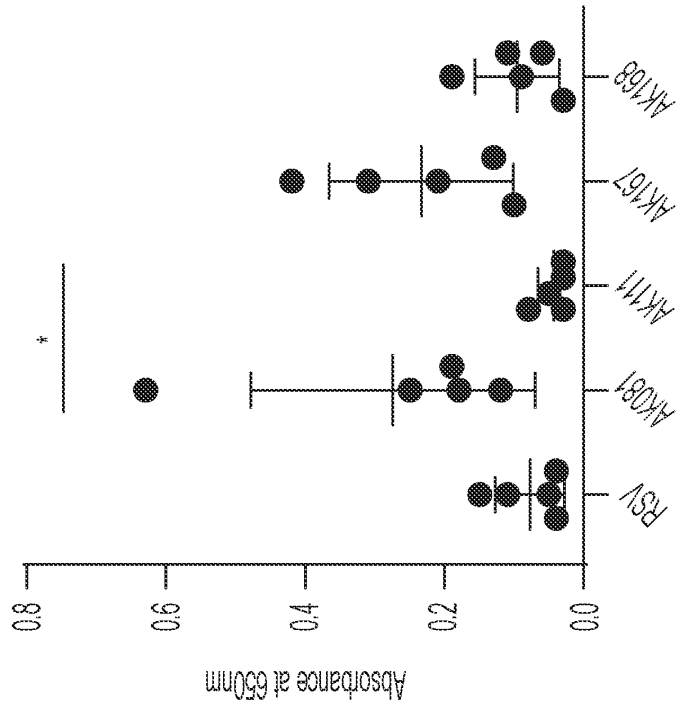


FIG. 26A

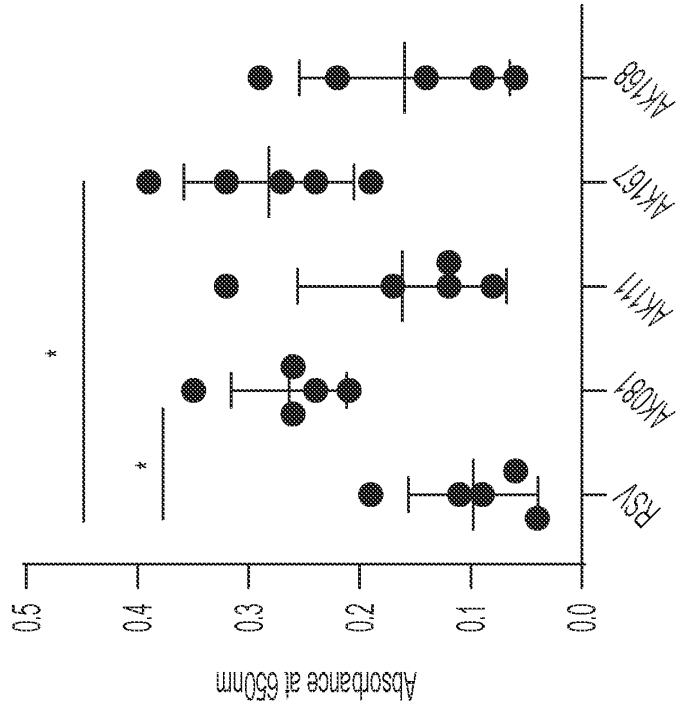


FIG. 27B

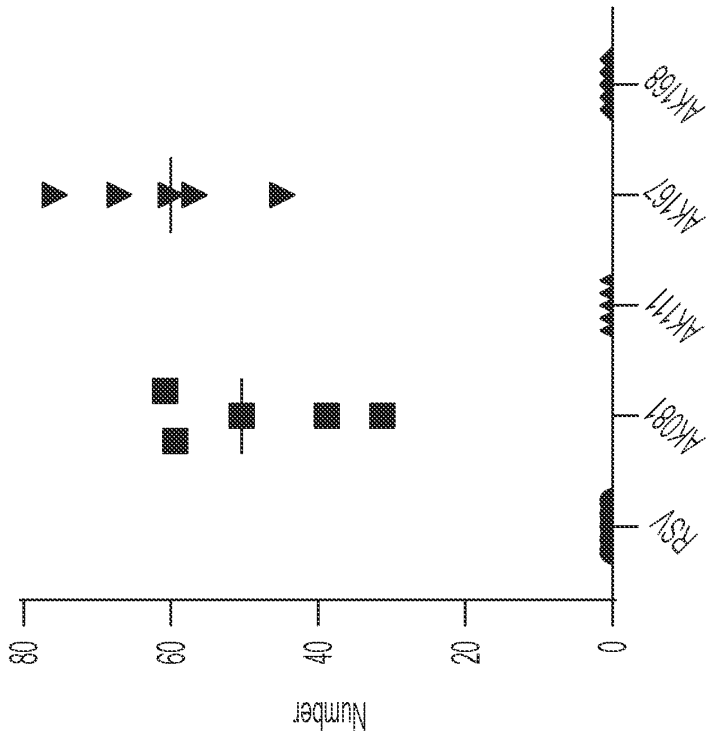
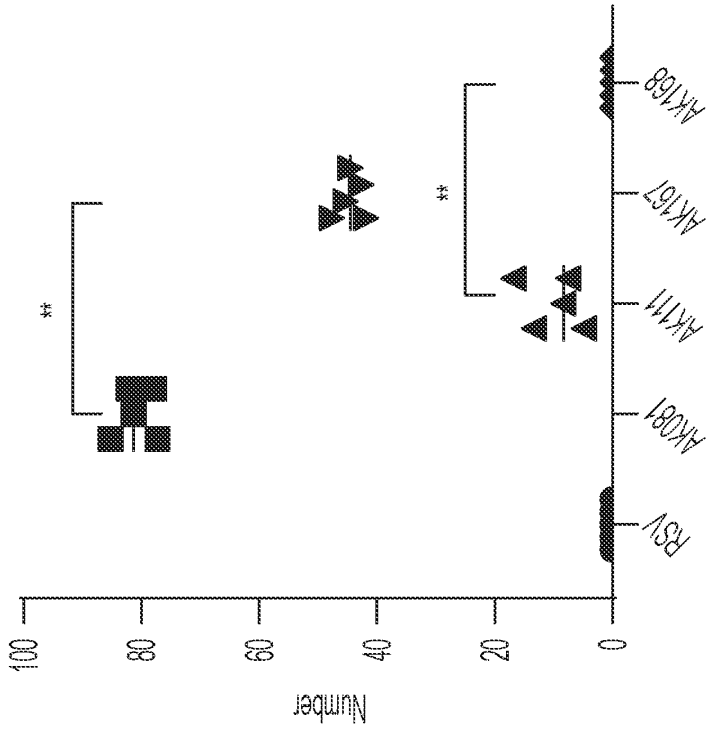


FIG. 27A



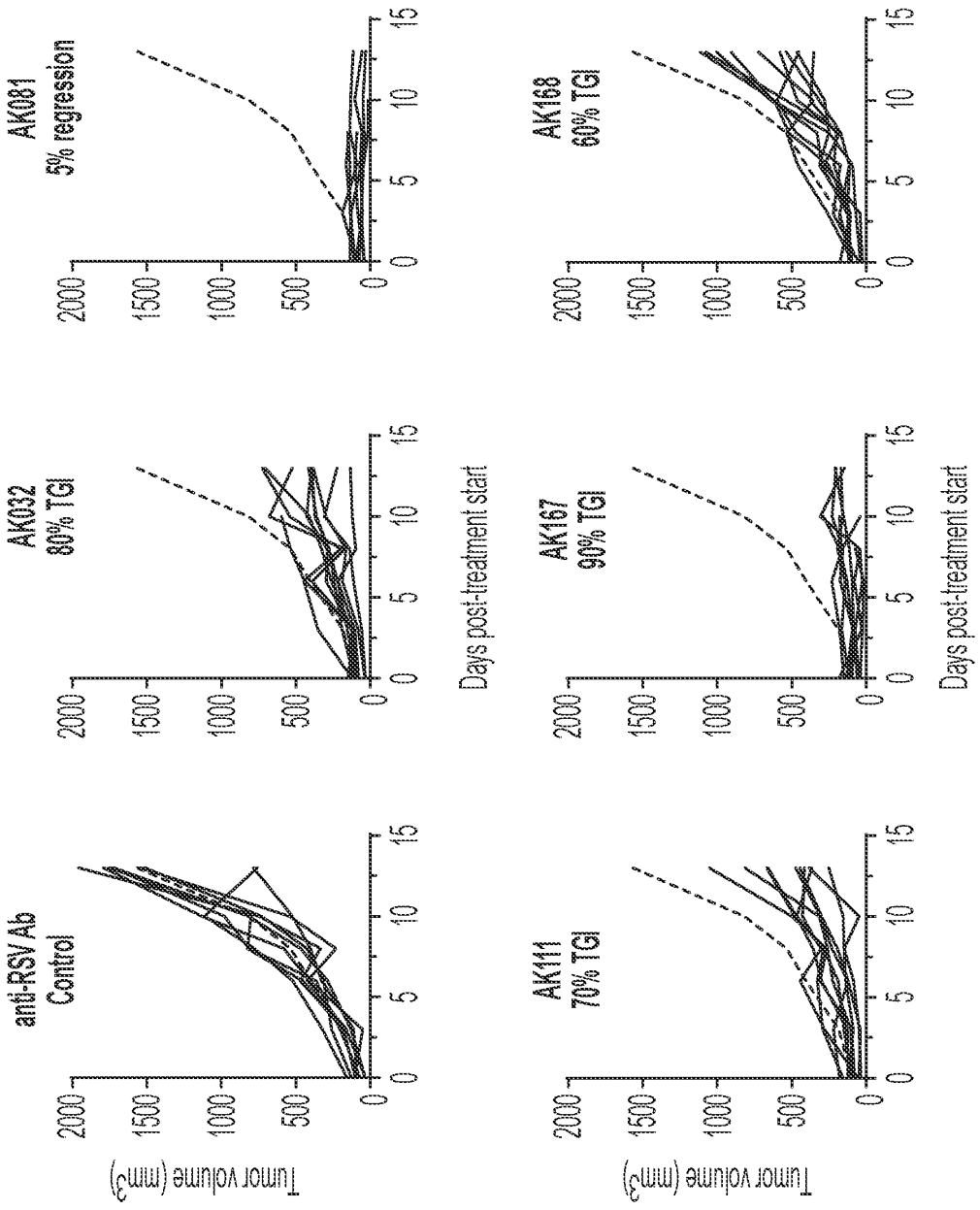

anti-RSV Ab
Control
AK032
80% TGI
AK081
5% regression
AK111
70% TGI
AK167
90% TGI
AK168
60% TGI
Tumor volume (mm3)
Days post-treatment start
0
500
1000
1500
2000
0
5
10
15

FIG. 28A

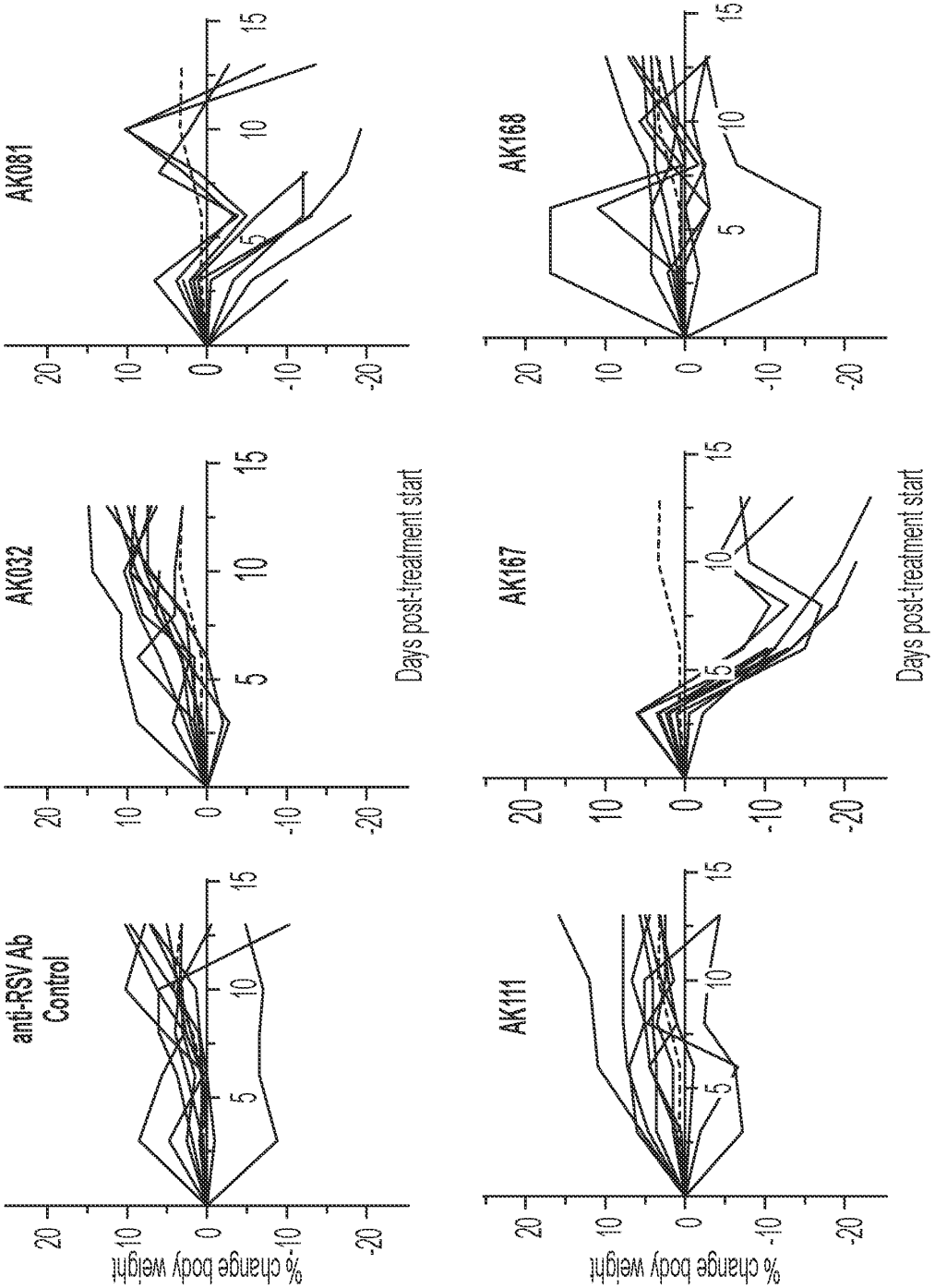

AK081
AK168
AK032
AK167
anti-RSV Ab Control
AK111
% change body weight
Days post-treatment start
20
10
0
-10
-20
5
10
15

FIG. 28B

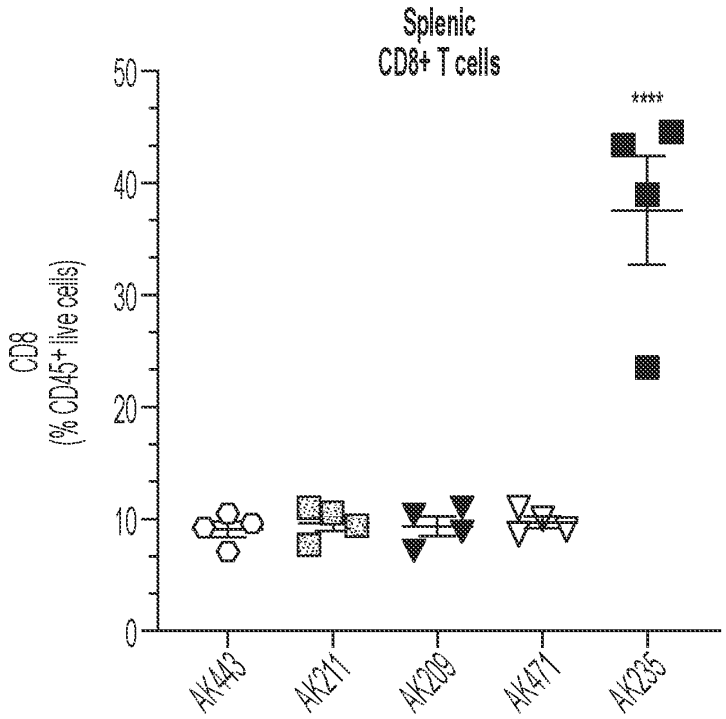


FIG. 29A

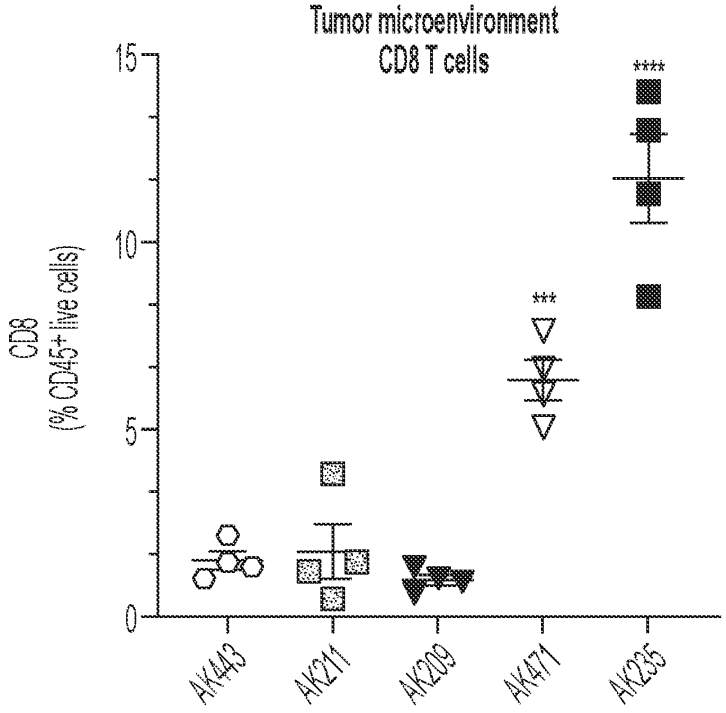


FIG. 29B

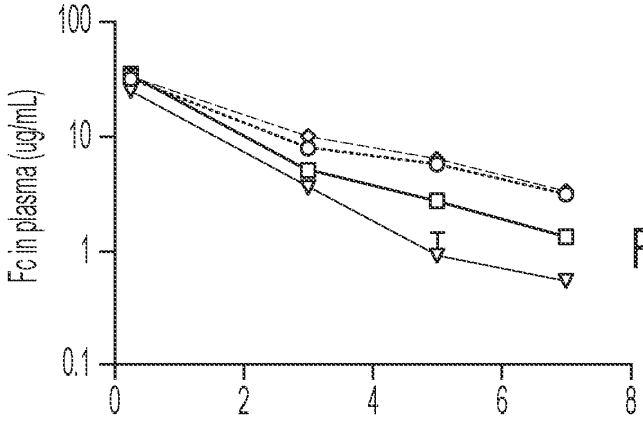


FIG. 30A

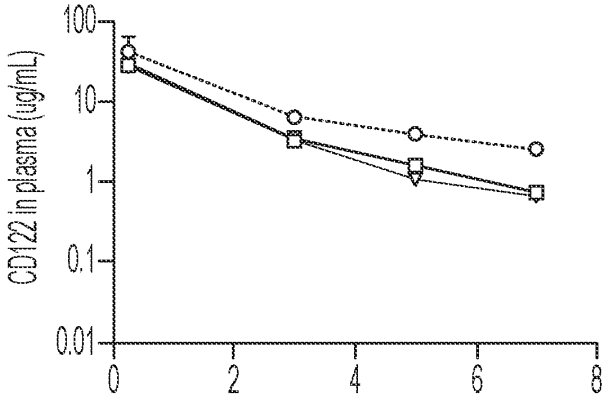


FIG. 30B

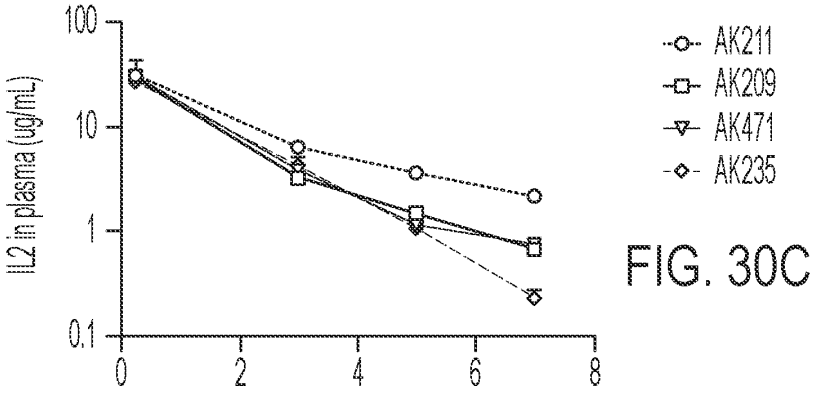


FIG. 30C

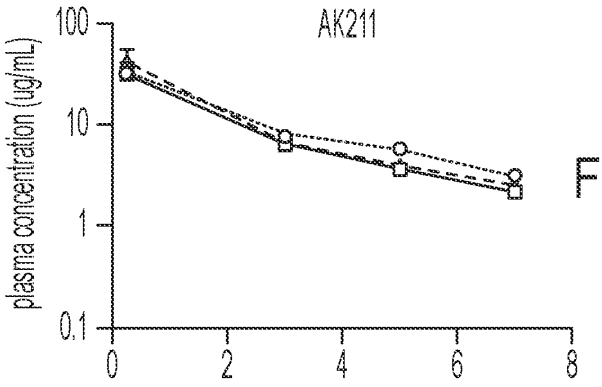


FIG. 31A

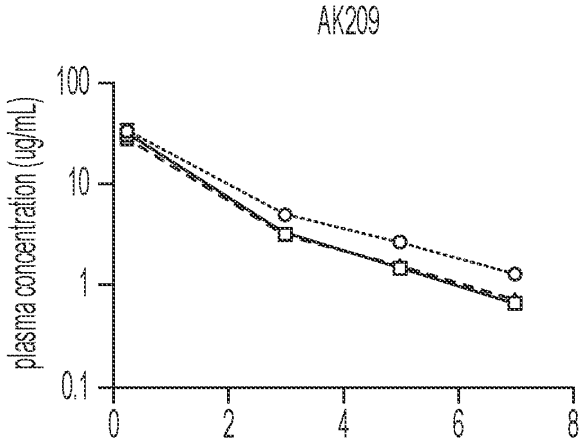


FIG. 31B

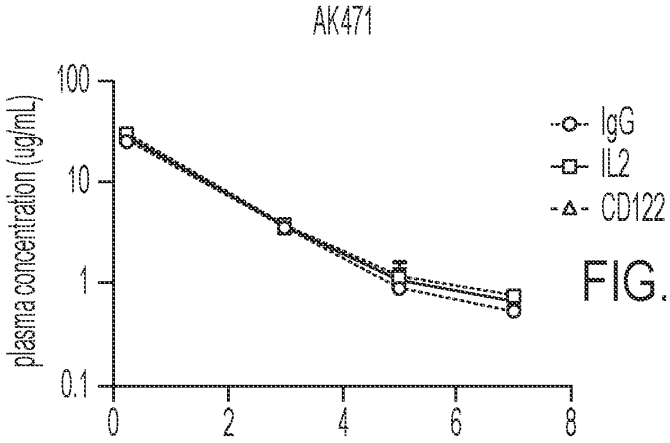


FIG. 31C

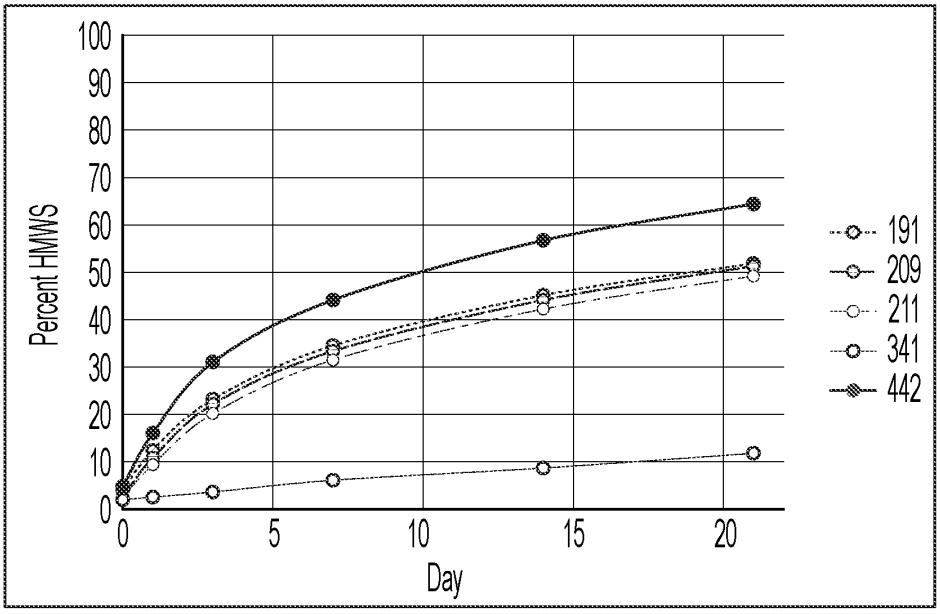


FIG. 32A

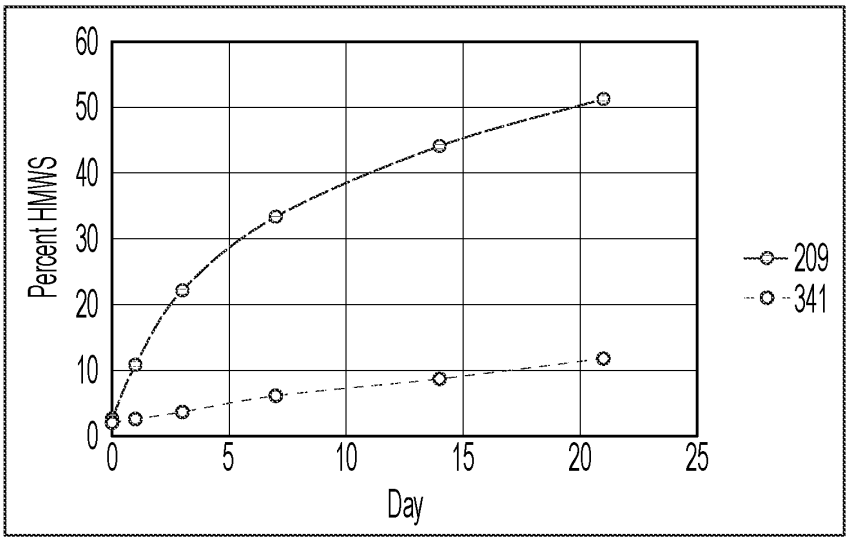


FIG. 32B

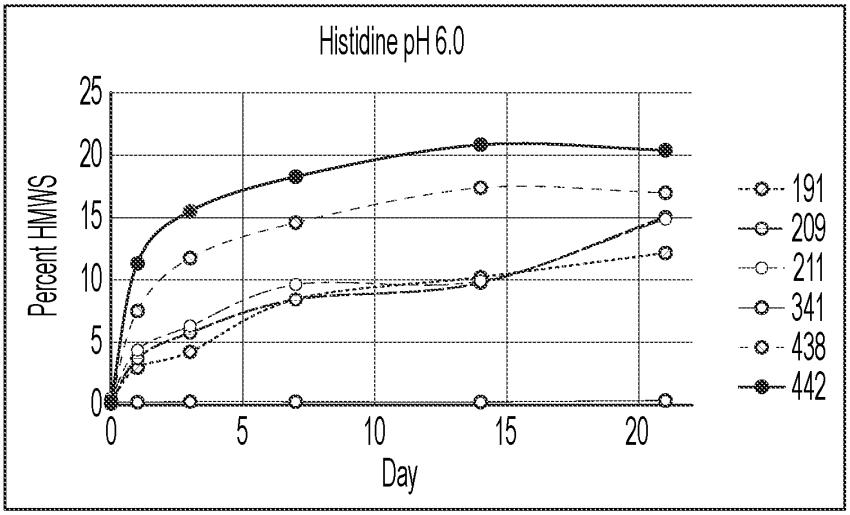


FIG. 33A

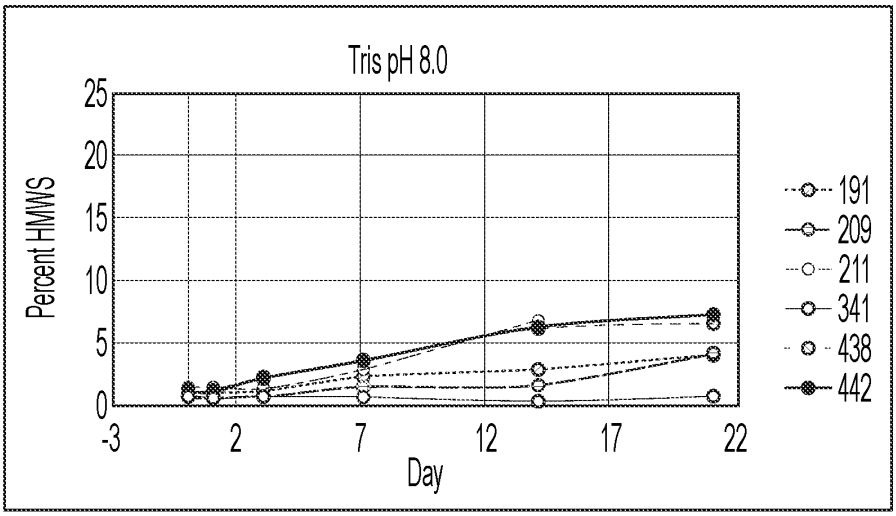


FIG. 33B

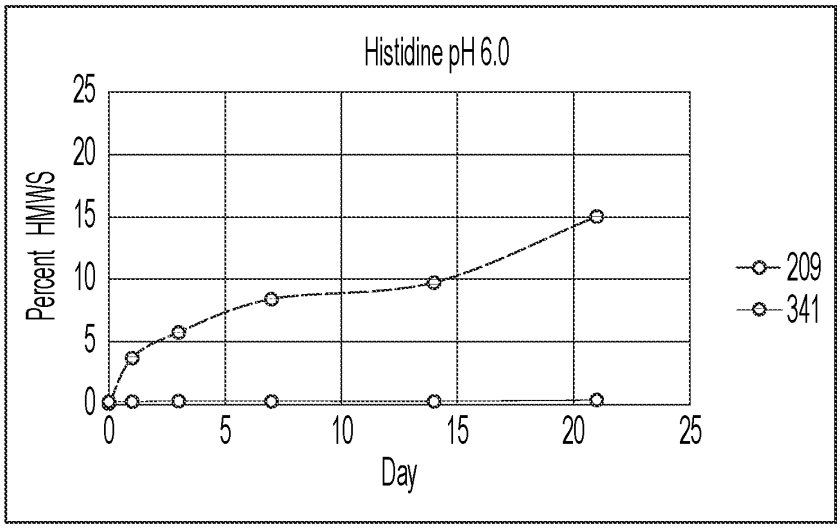


FIG. 33C

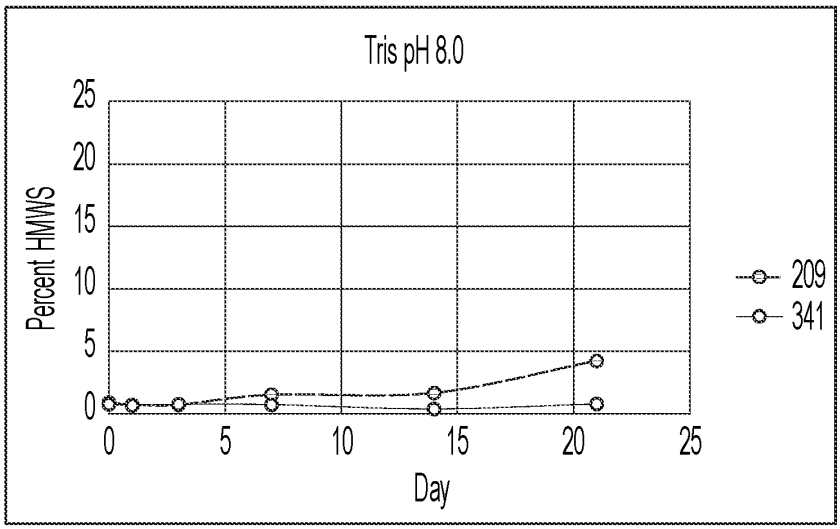


FIG. 33D

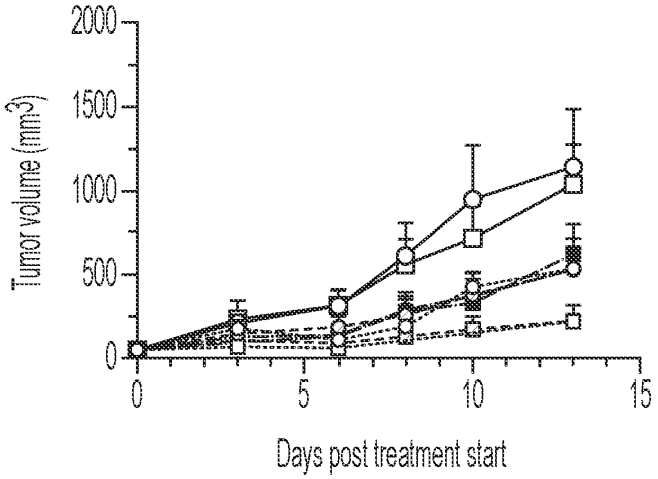


FIG. 34A

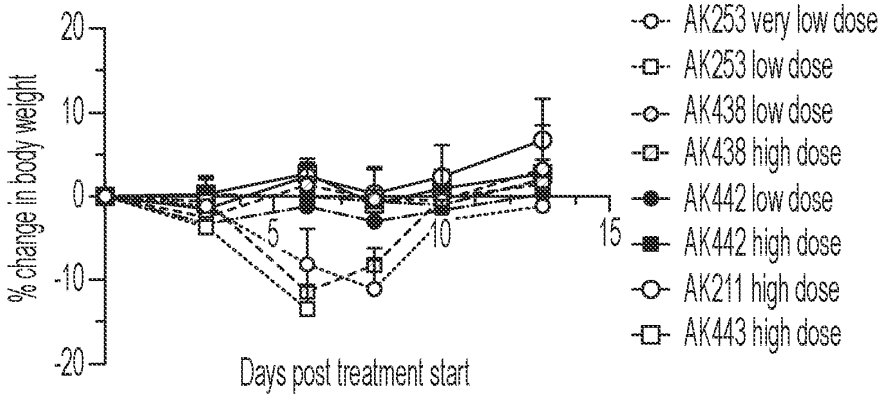


FIG. 34B

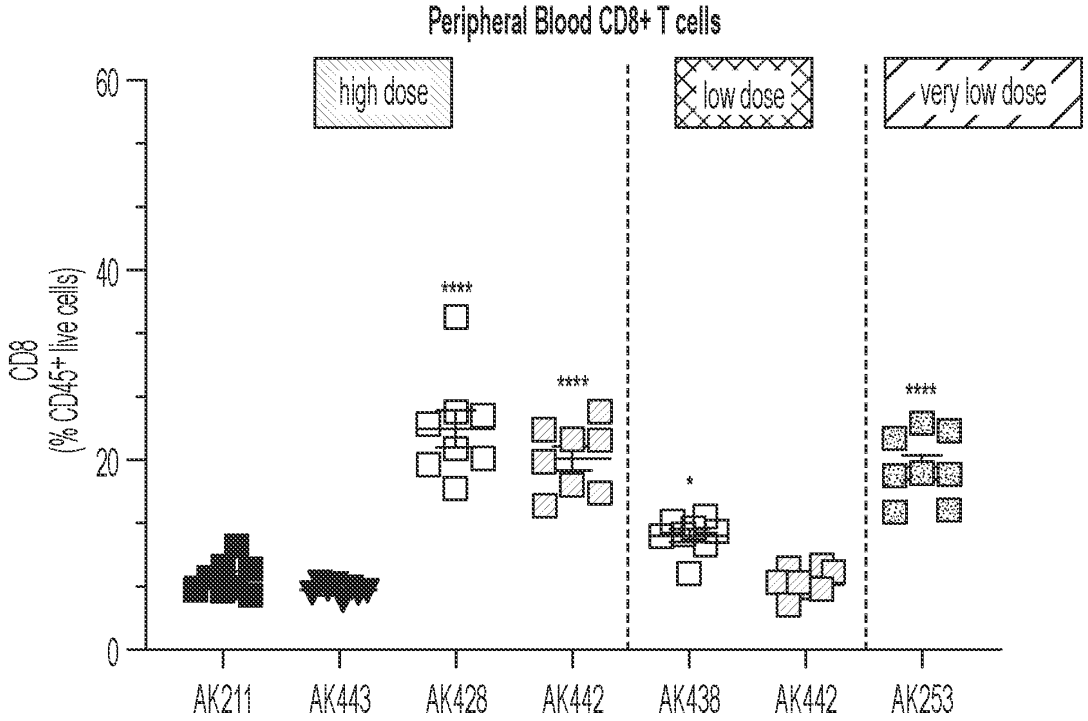


FIG. 35A

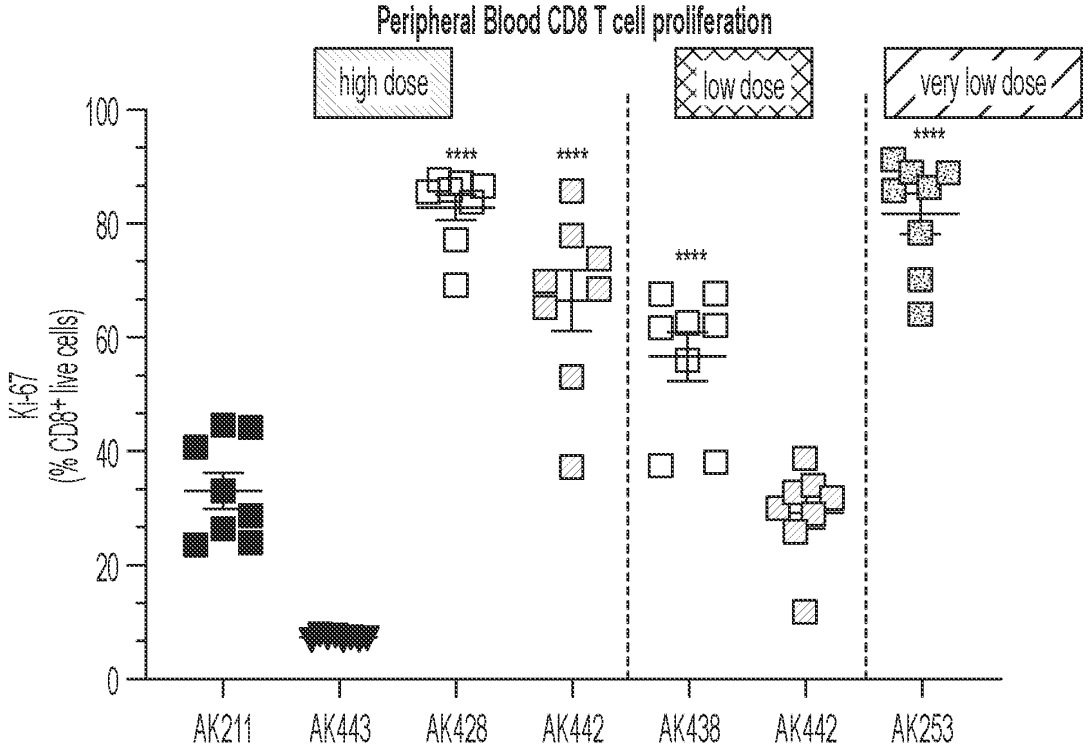


FIG. 35B

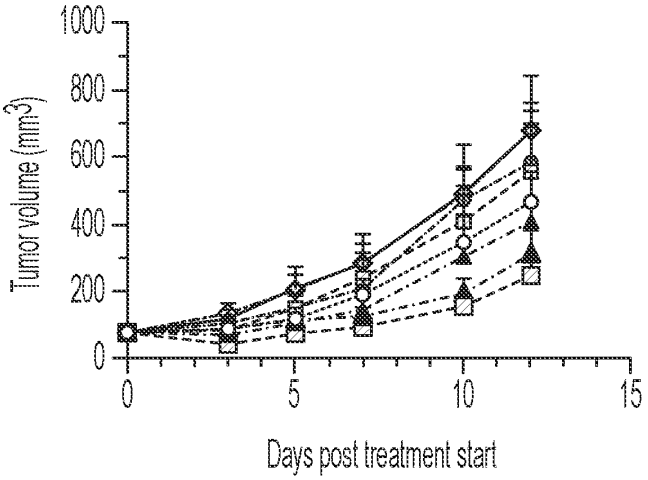


FIG. 36A

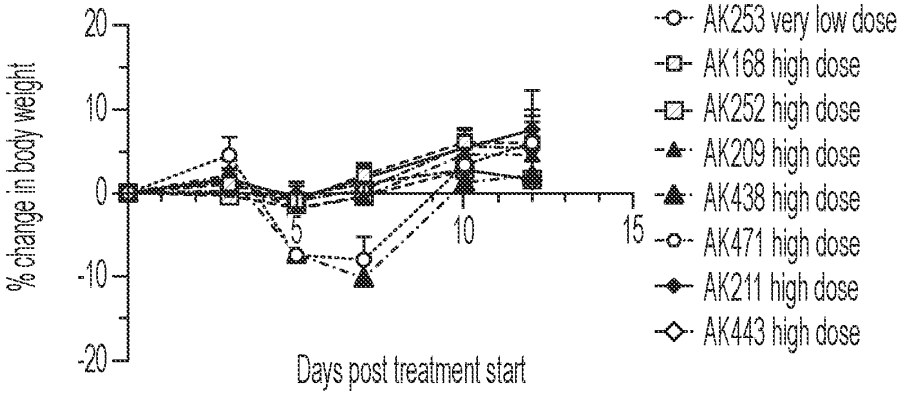


FIG. 36B

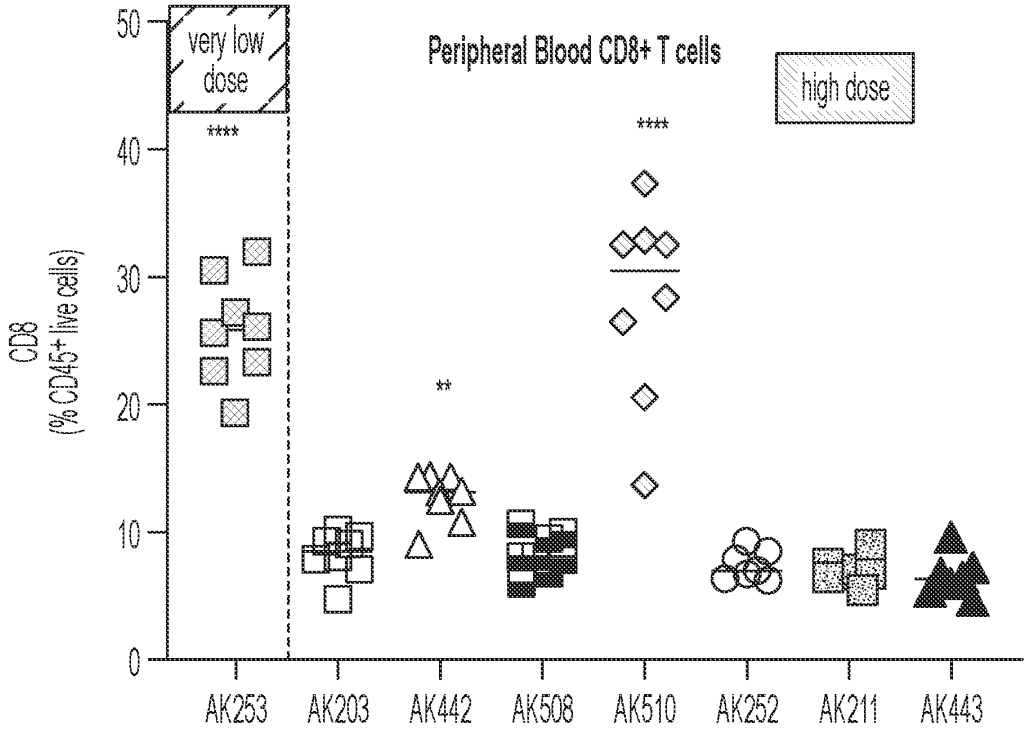


FIG. 37A

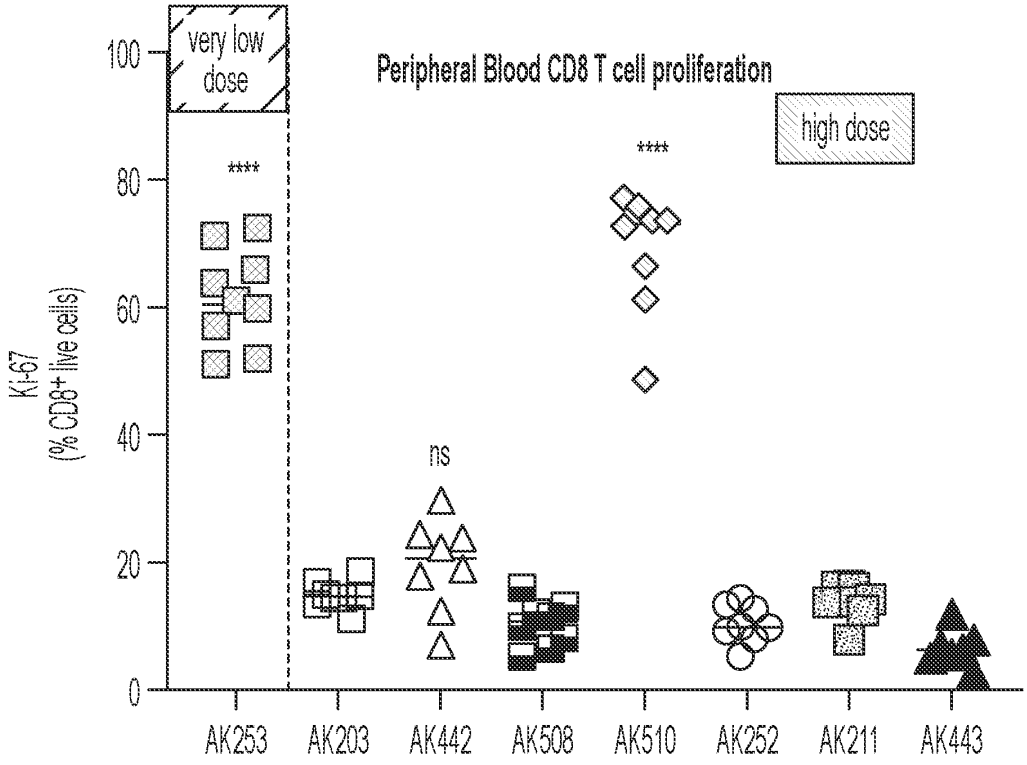


FIG. 37B

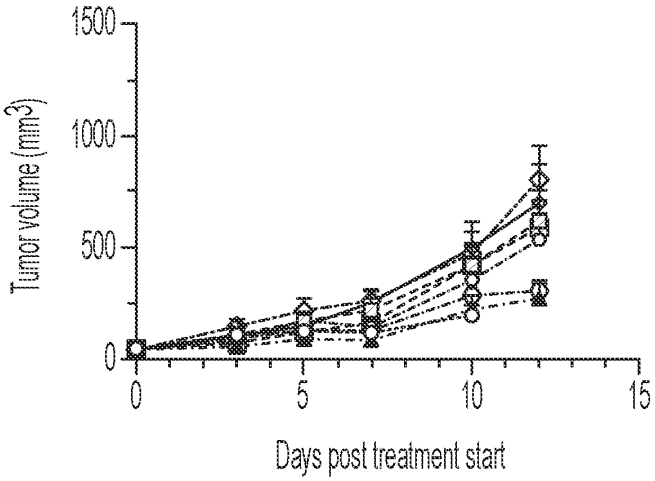


FIG. 38A

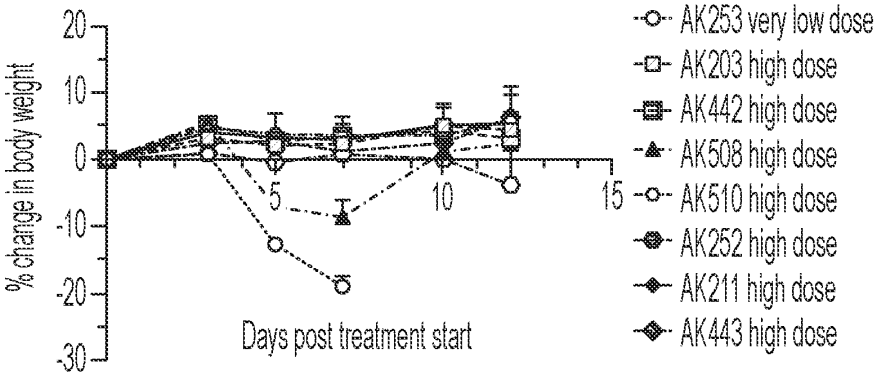


FIG. 38B

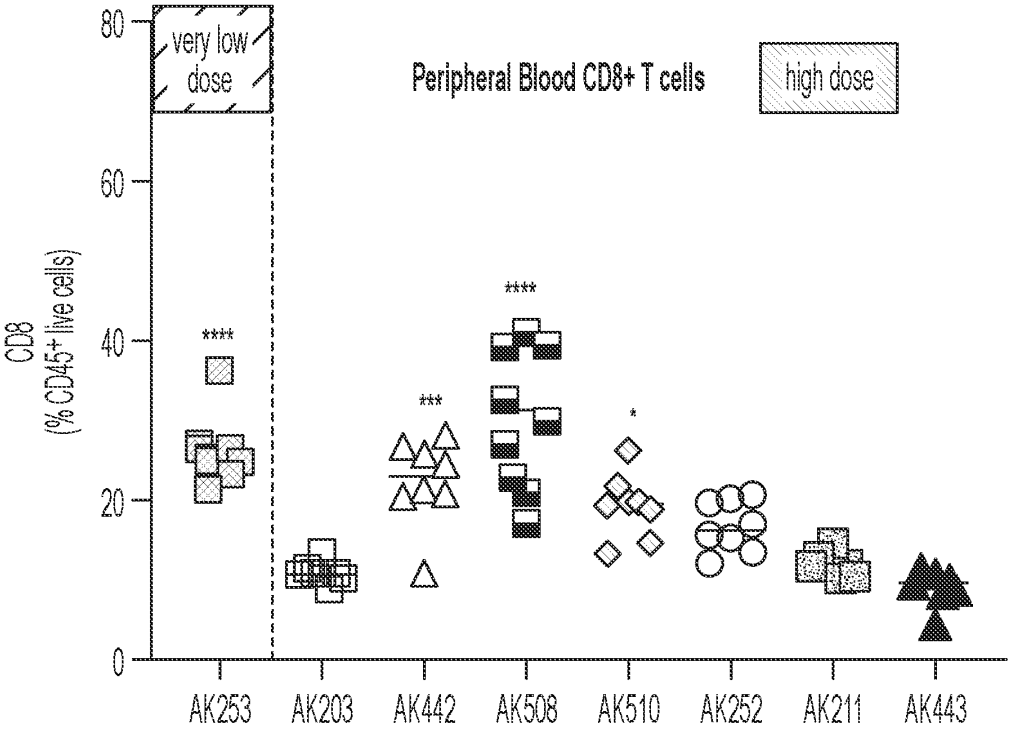


FIG. 39A

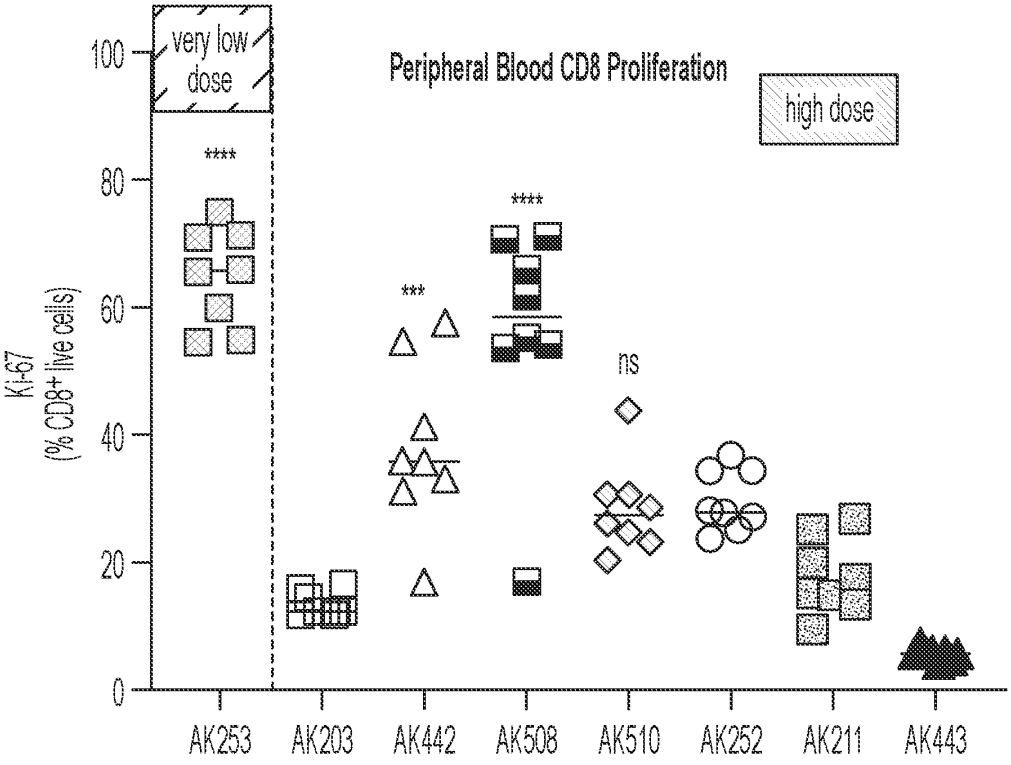


FIG. 39B

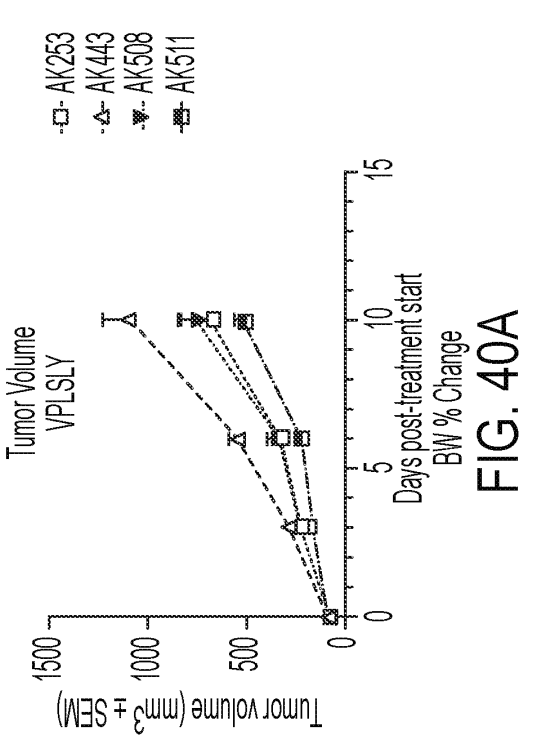


FIG. 40A

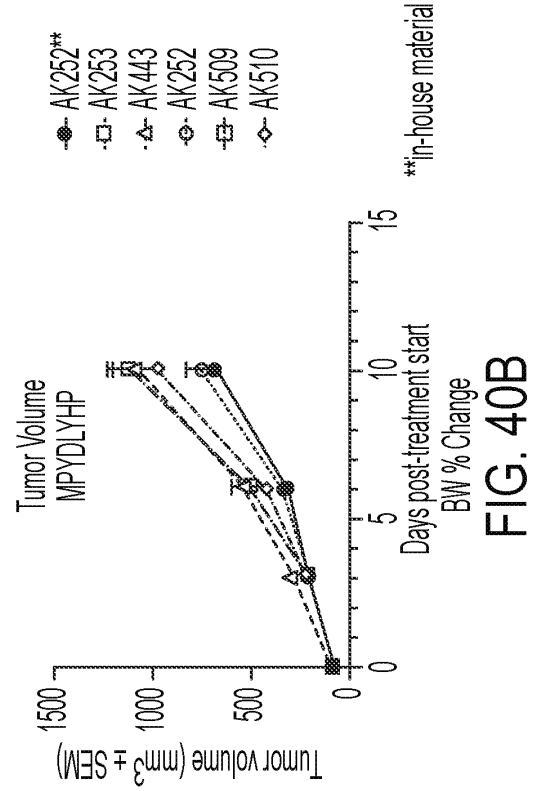


FIG. 40B

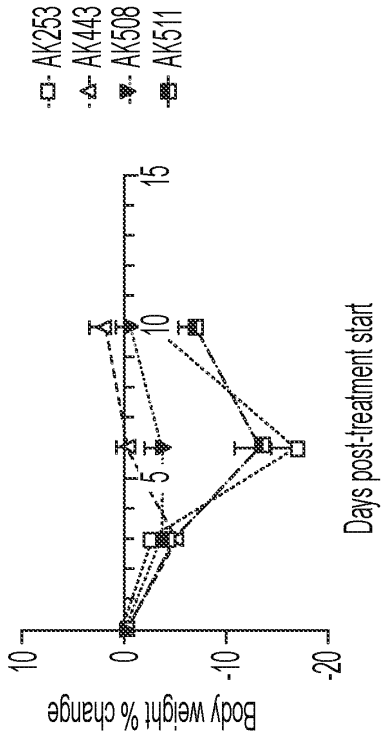


FIG. 40C

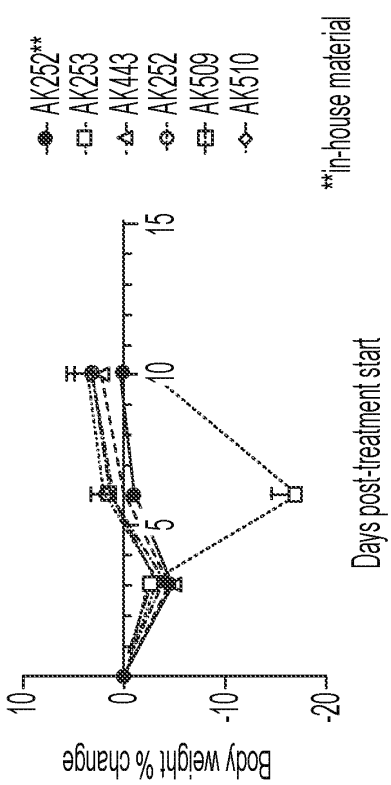


FIG. 40D

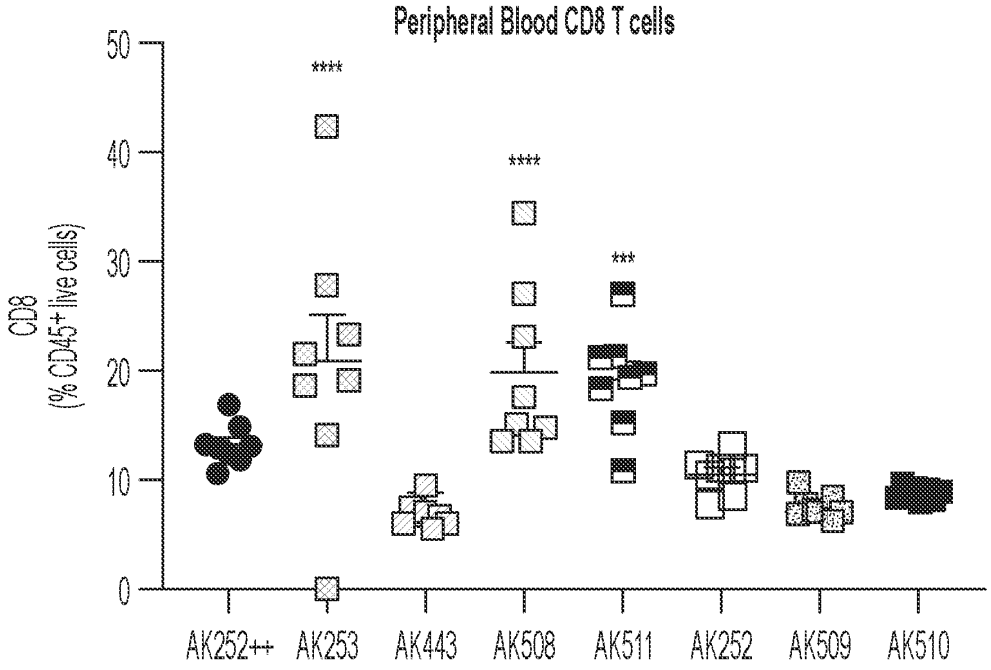


FIG. 41A

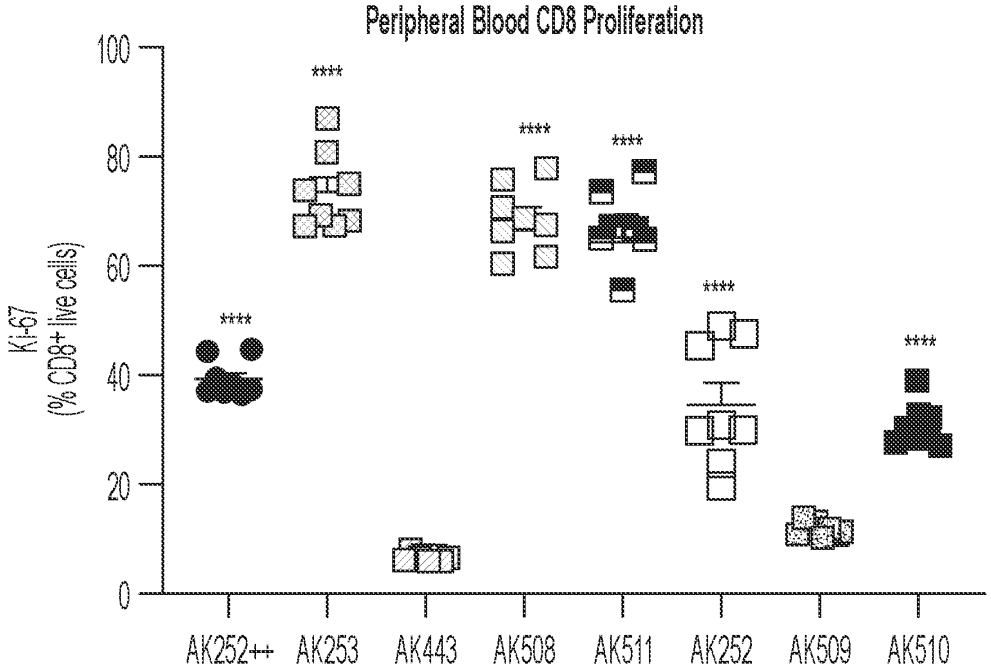


FIG. 41B

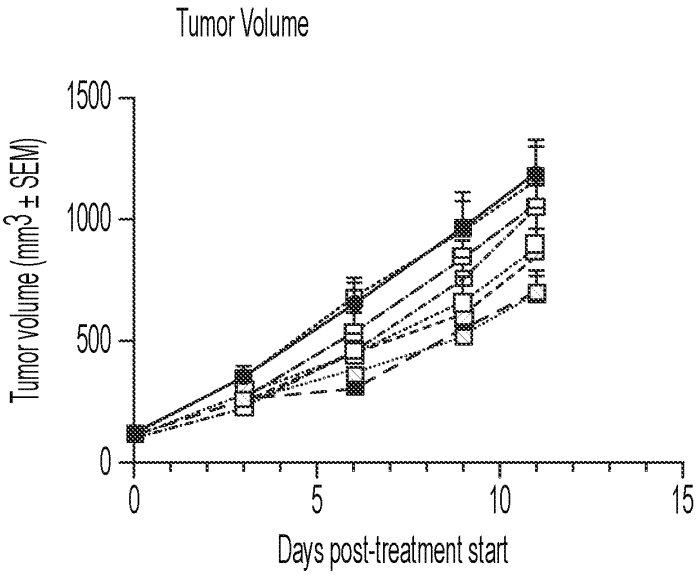


FIG. 42A

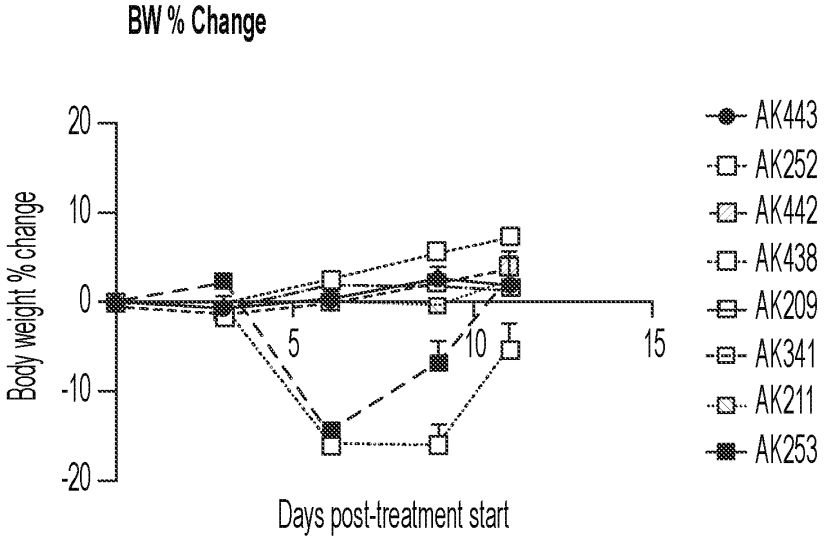


FIG. 42B

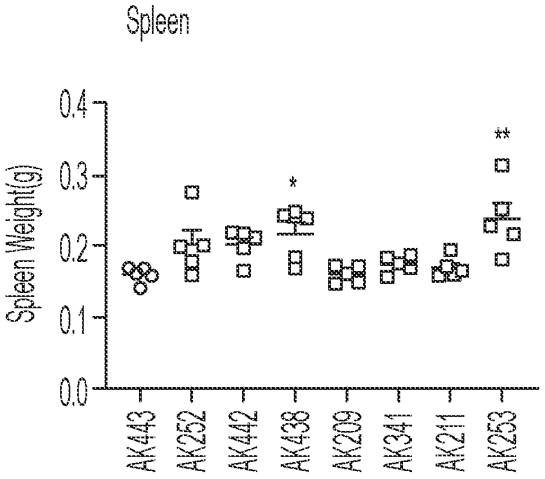


FIG. 43A

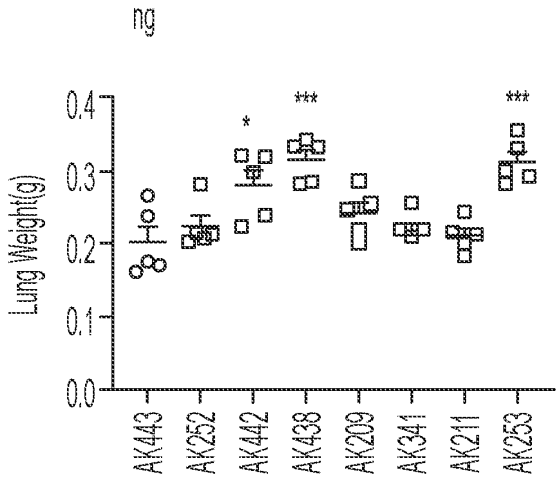


FIG. 43B

FIG. 44A

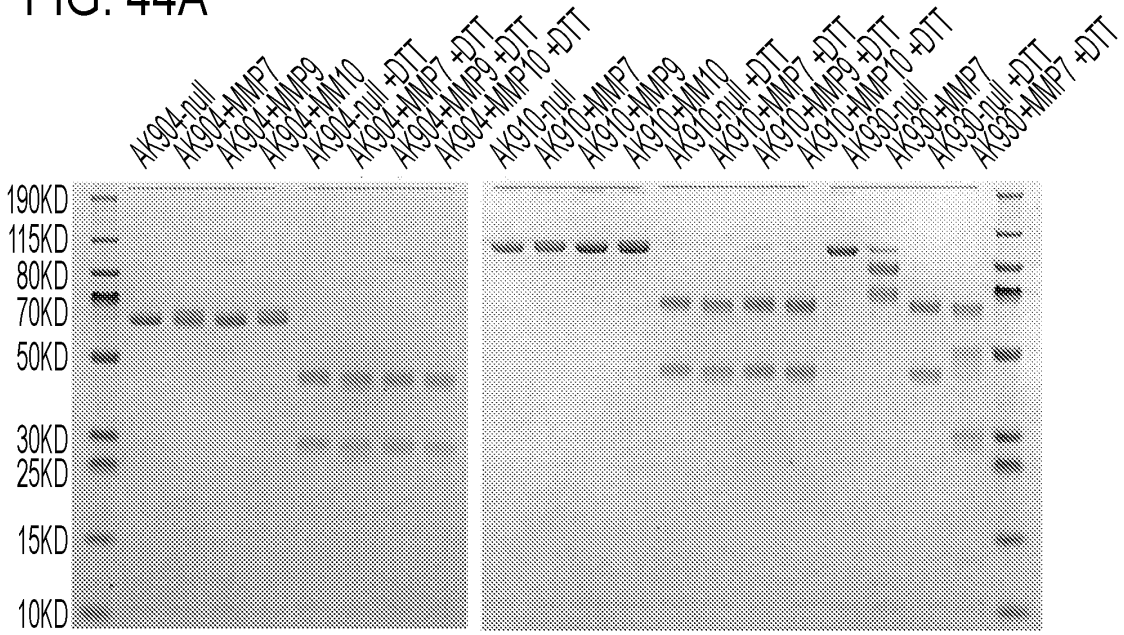


FIG. 44B

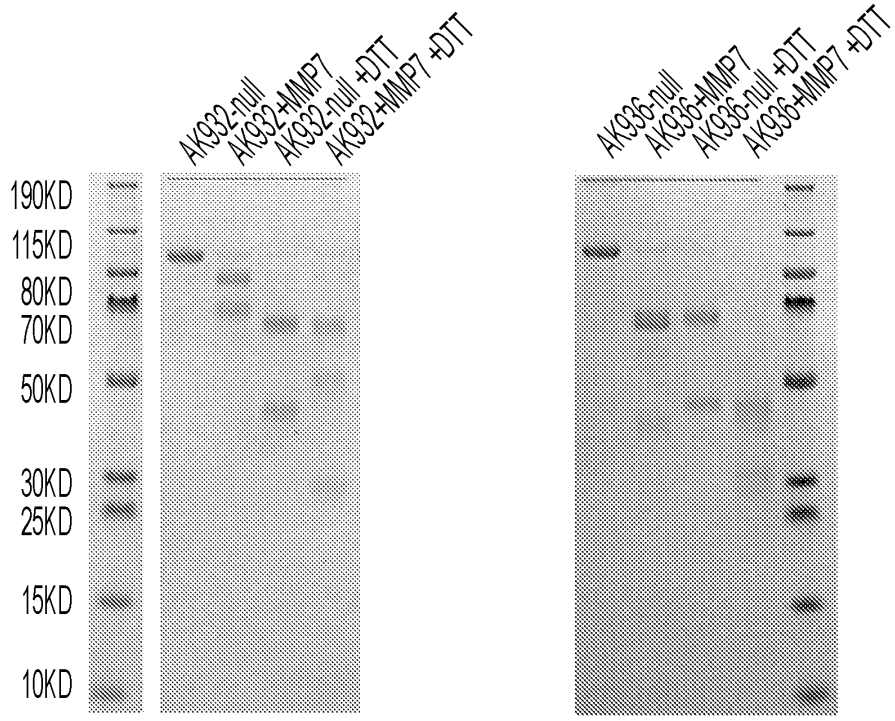


FIG. 44C

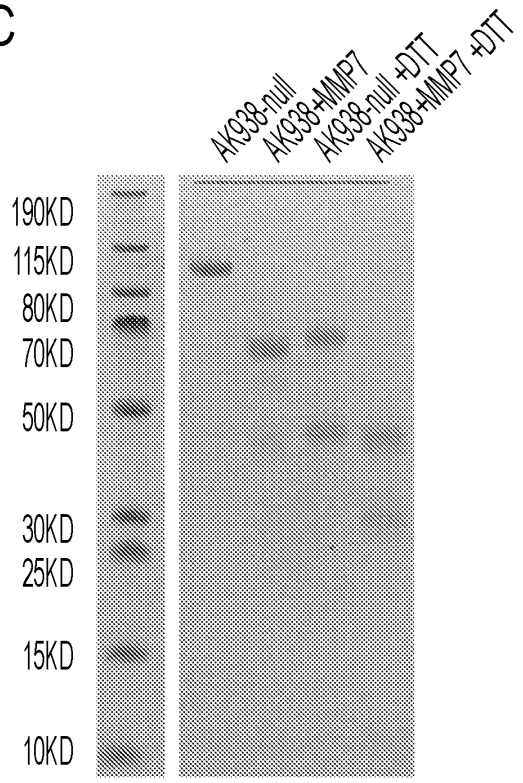
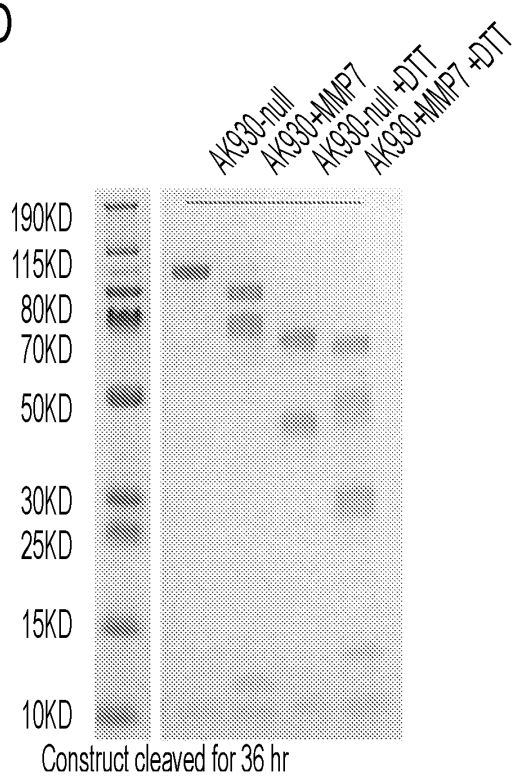


FIG. 44D



Construct cleaved for 36 hr

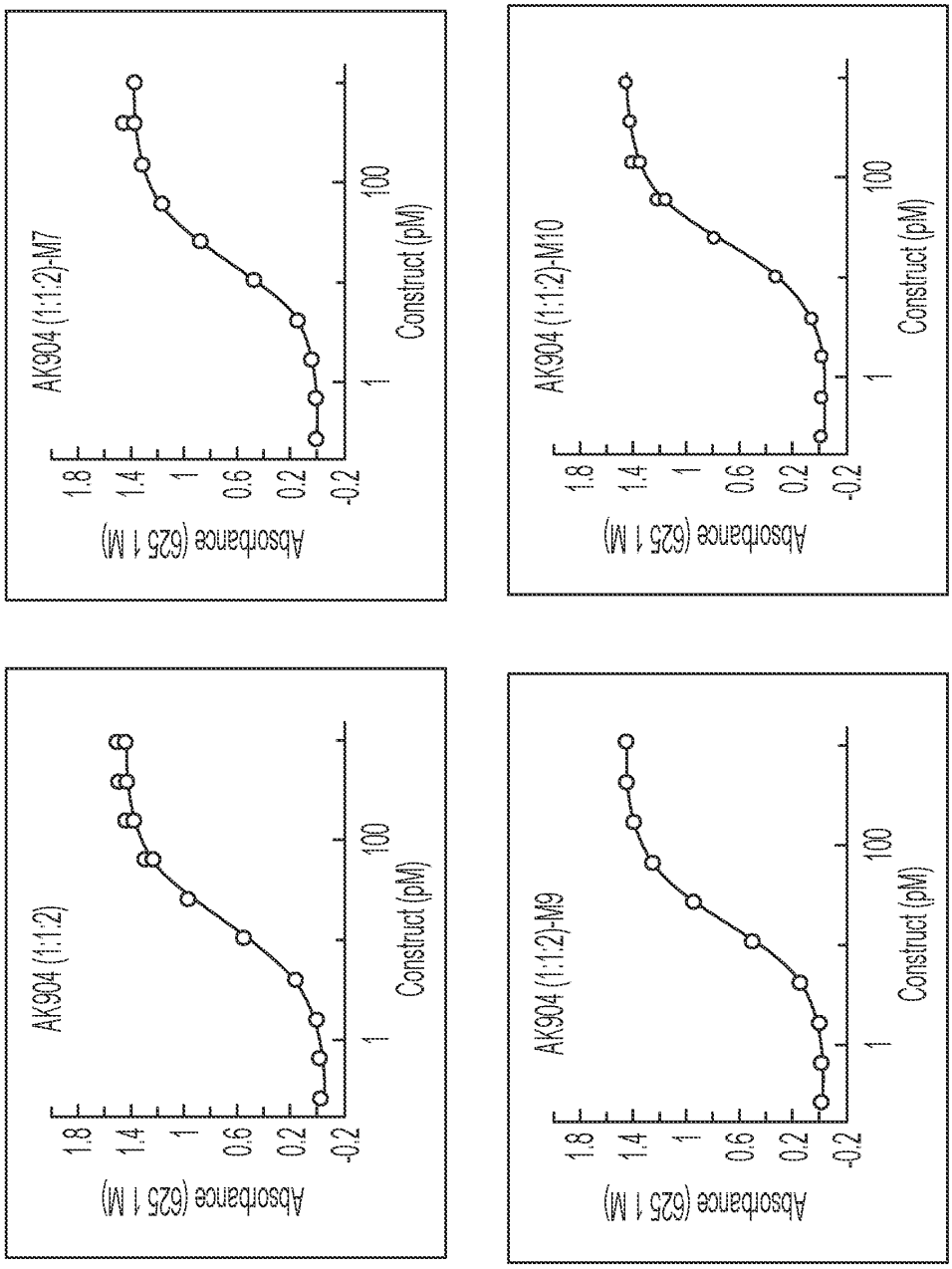


FIG. 45A (AK904)

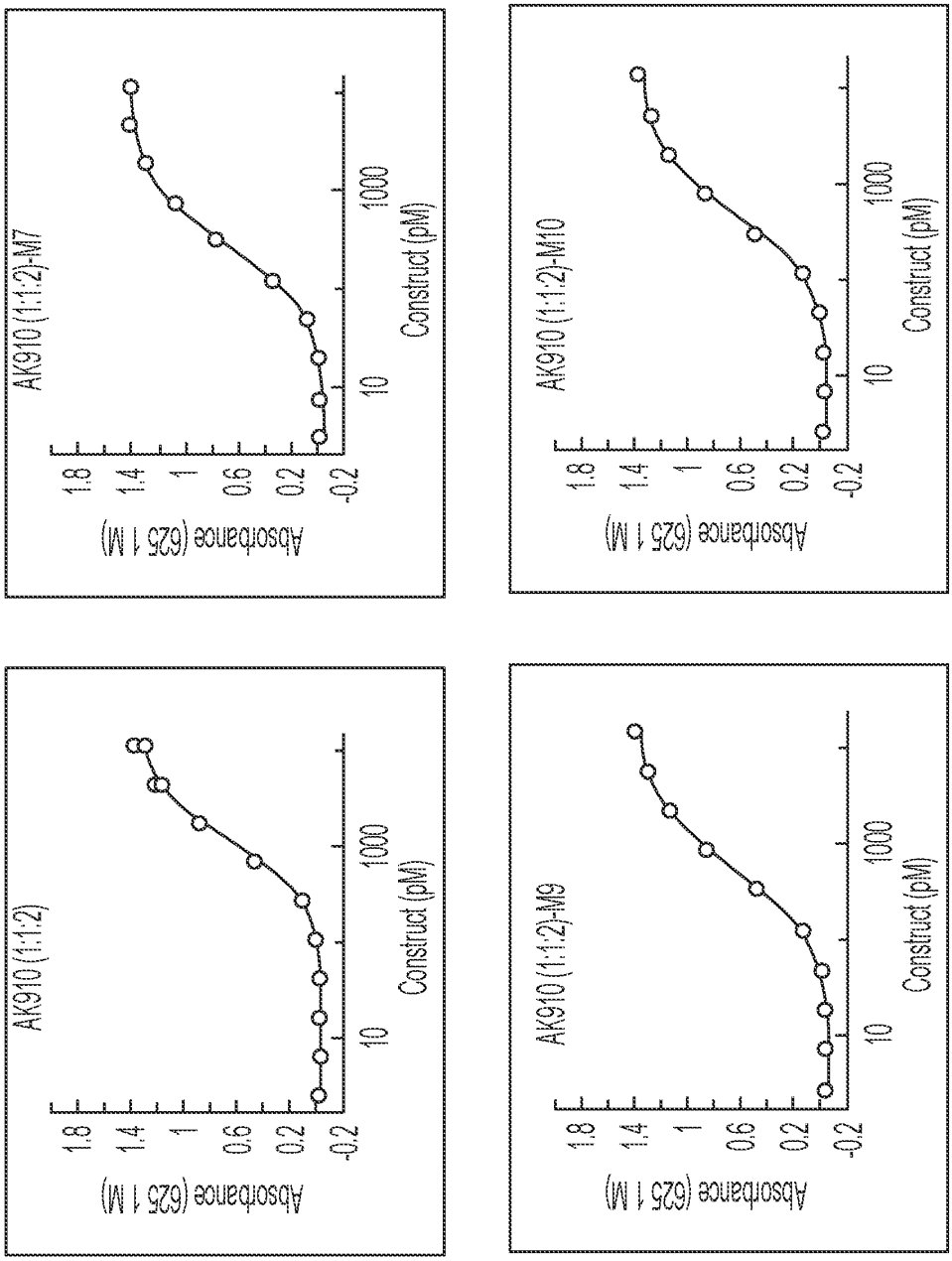


FIG. 45B (AK910)

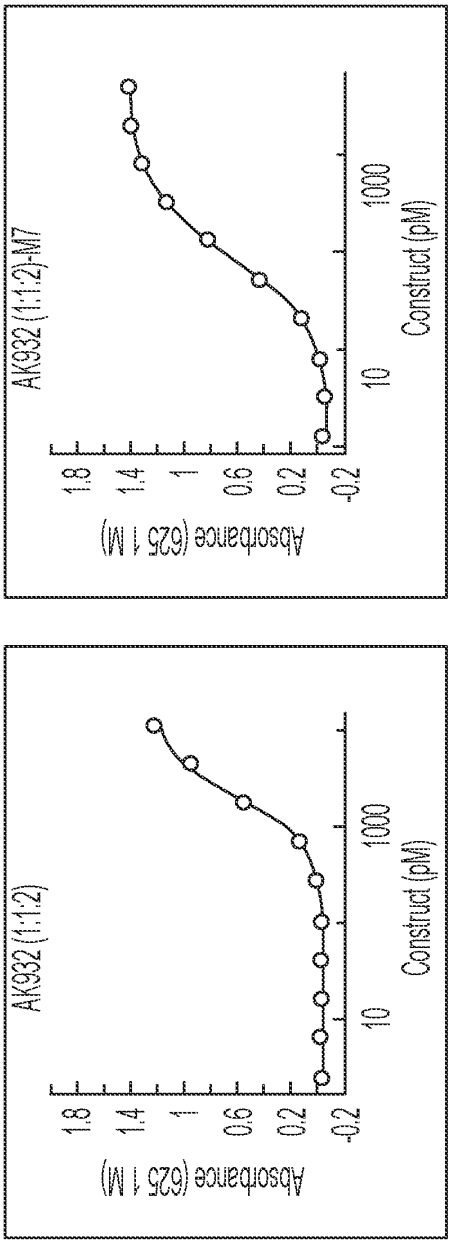
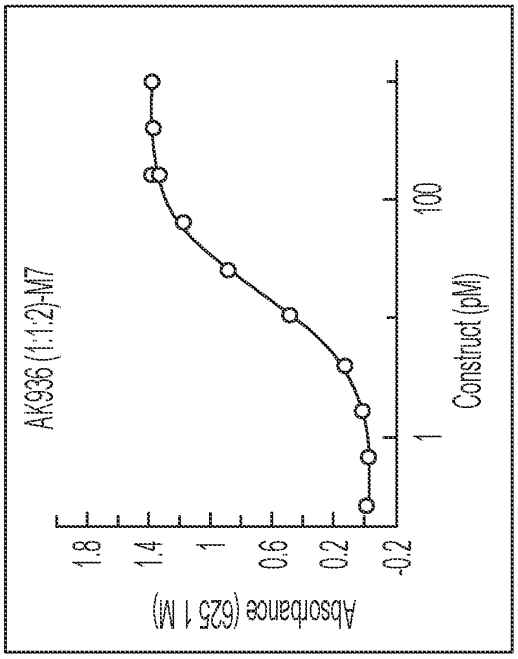


FIG. 45C (AK932)



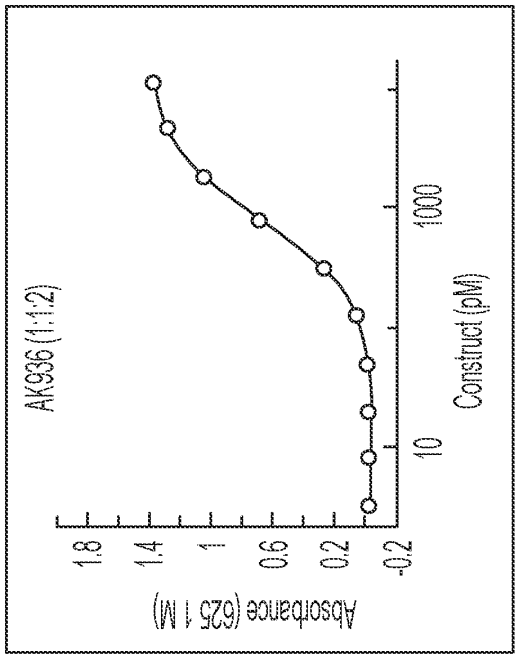
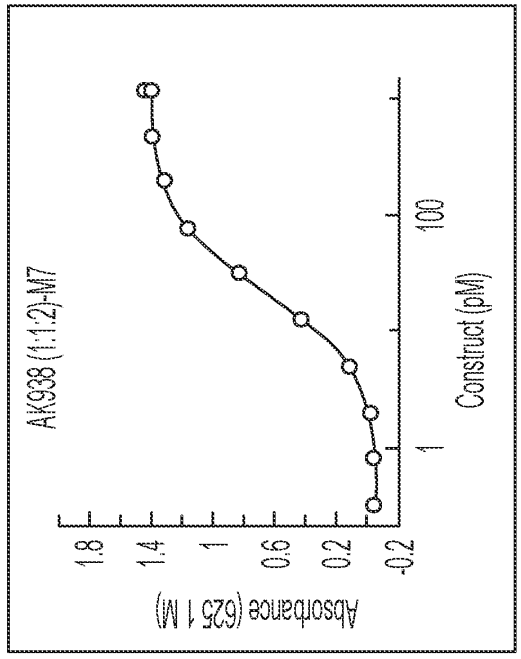


FIG. 45D (AK936)



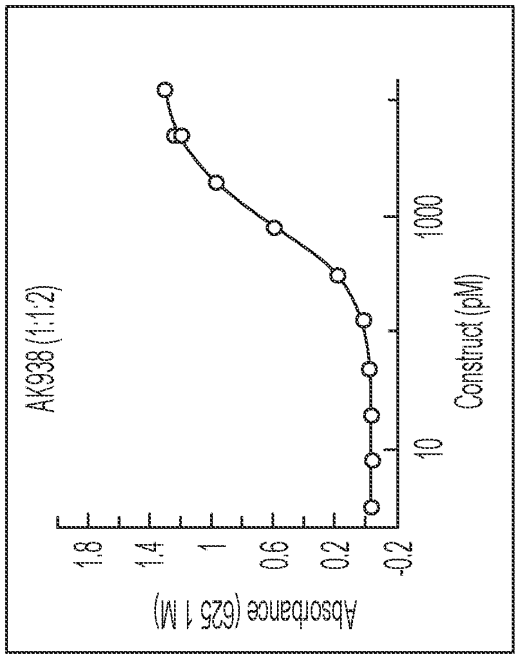
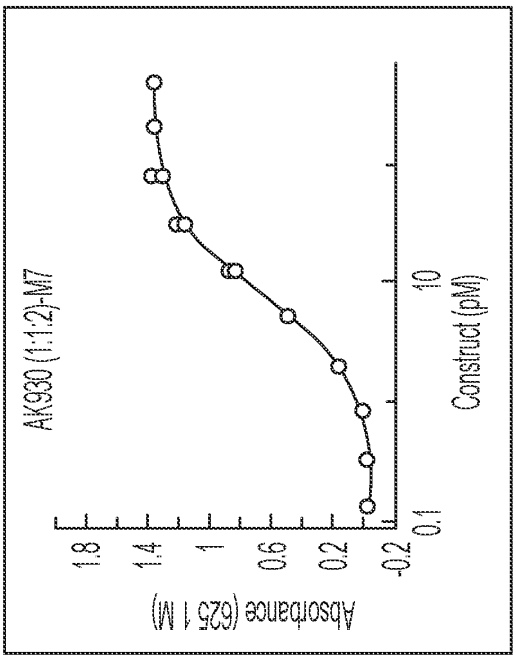


FIG. 45E (AK938)



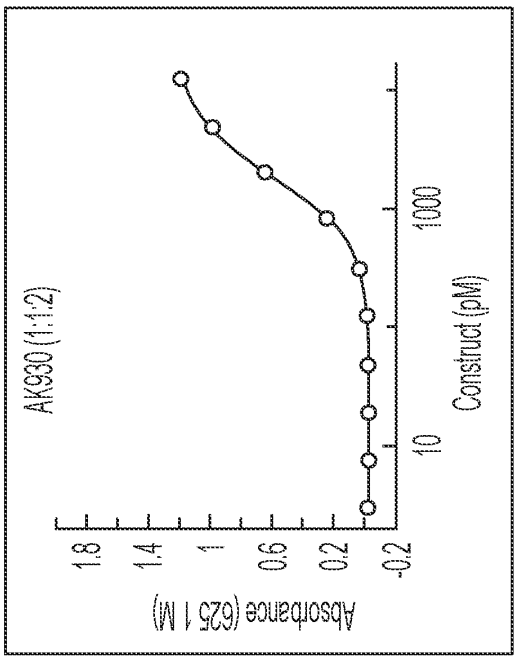


FIG. 45F (AK930)

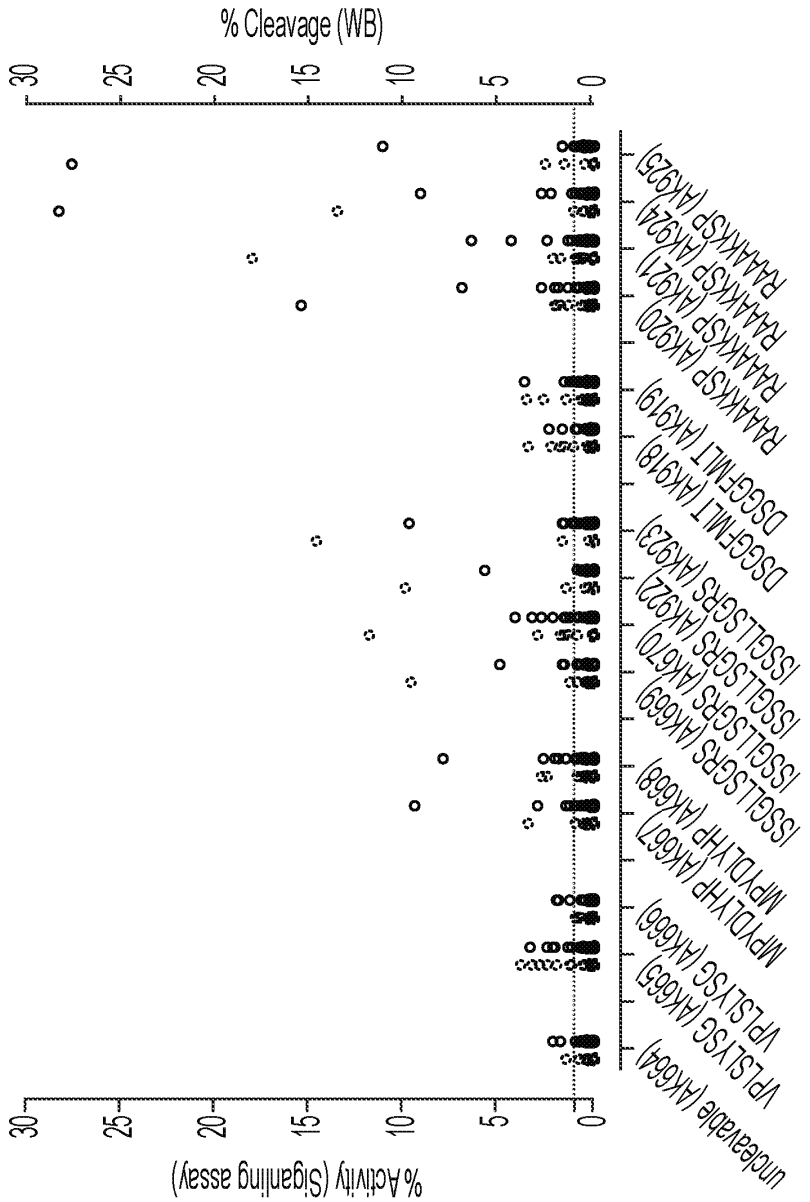

% Cleavage (WB)
30
25
20
15
10
5
0
% Activity (Signaling assay)
uncleavable (AK664)
VPLSLYSG (AK665)
VPLSLYSG (AK666)
MPYDLYHP (AK667)
MPYDLYHP (AK668)
ISSGLLSGRS (AK669)
ISSGLLSGRS (AK670)
ISSGLLSGRS (AK922)
DSGGFMLT (AK923)
DSGGFMLT (AK918)
DSGGFMLT (AK919)
RAAAKKSP (AK920)
RAAAKKSP (AK921)
RAAAKKSP (AK924)
RAAAKKSP (AK925)

FIG. 46

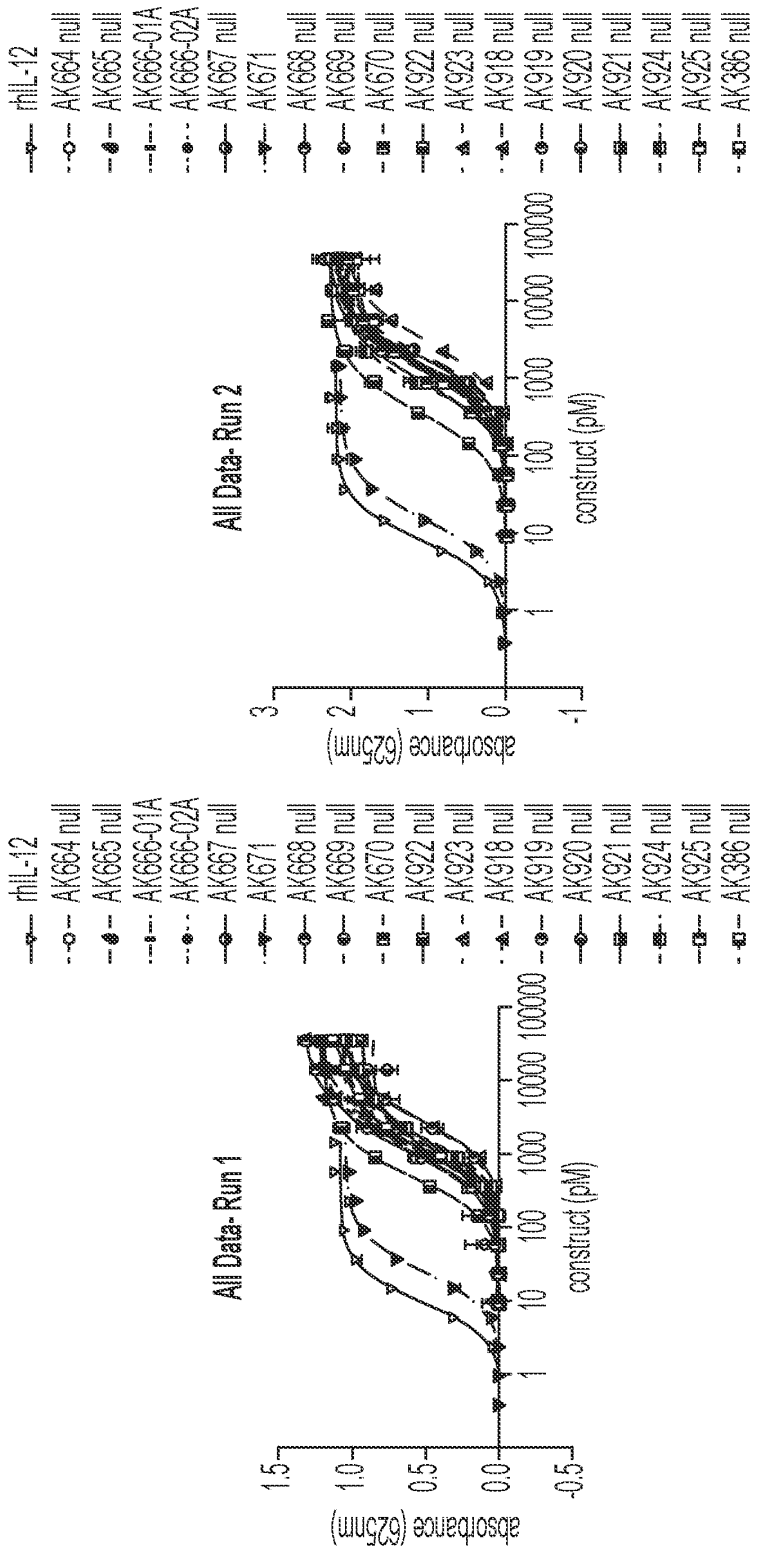

All Data- Run 1
All Data- Run 2
absorbance (625nm)
construct (pM)
rhIL-12
AK664 null
AK665 null
AK666-01A
AK666-02A
AK667 null
AK671
AK668 null
AK669 null
AK670 null
AK922 null
AK923 null
AK918 null
AK919 null
AK920 null
AK921 null
AK924 null
AK925 null
AK386 null

FIG. 47

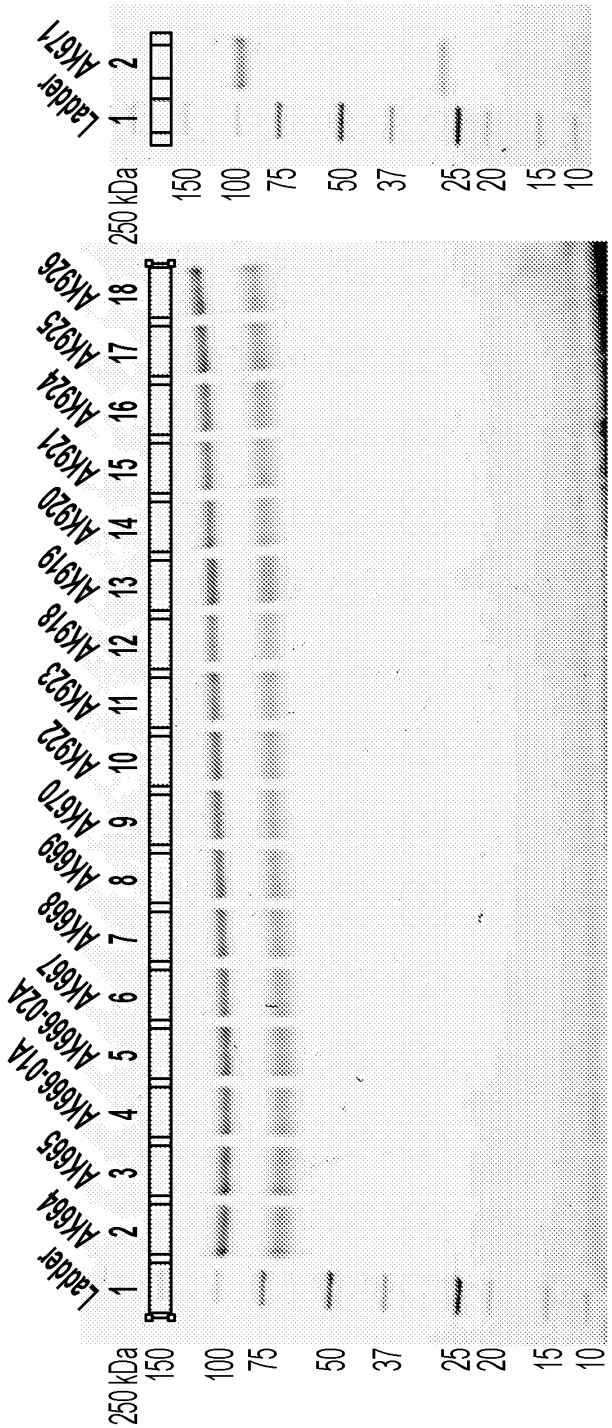

Ladder
AK671
1
2
250 kDa
150
100
75
50
37
25
20
15
10
Ladder
AK664
AK665
AK666-01A
AK666-02A
AK667
AK668
AK669
AK670
AK922
AK923
AK918
AK919
AK920
AK921
AK924
AK925
AK926
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
250 kDa
150
100
75
50
37
25
20
15
10

FIG. 48A

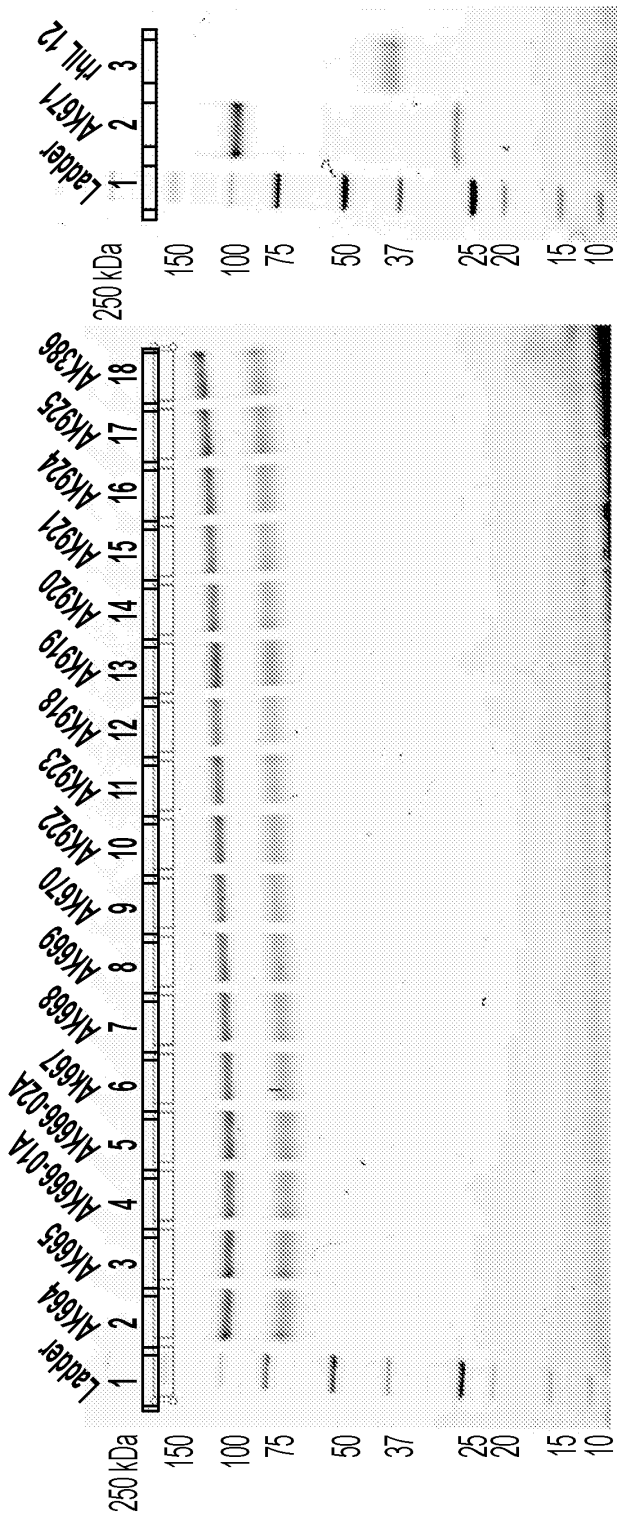

250 kDa
150
100
75
50
37
25
20
15
10
Ladder
AK664
AK665
AK666-01A
AK666-02A
AK667
AK668
AK669
AK670
AK922
AK923
AK918
AK919
AK920
AK921
AK924
AK925
AK386
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Ladder
AK671
rhIL-12
1
2
3

FIG. 48B

FIG. 49A

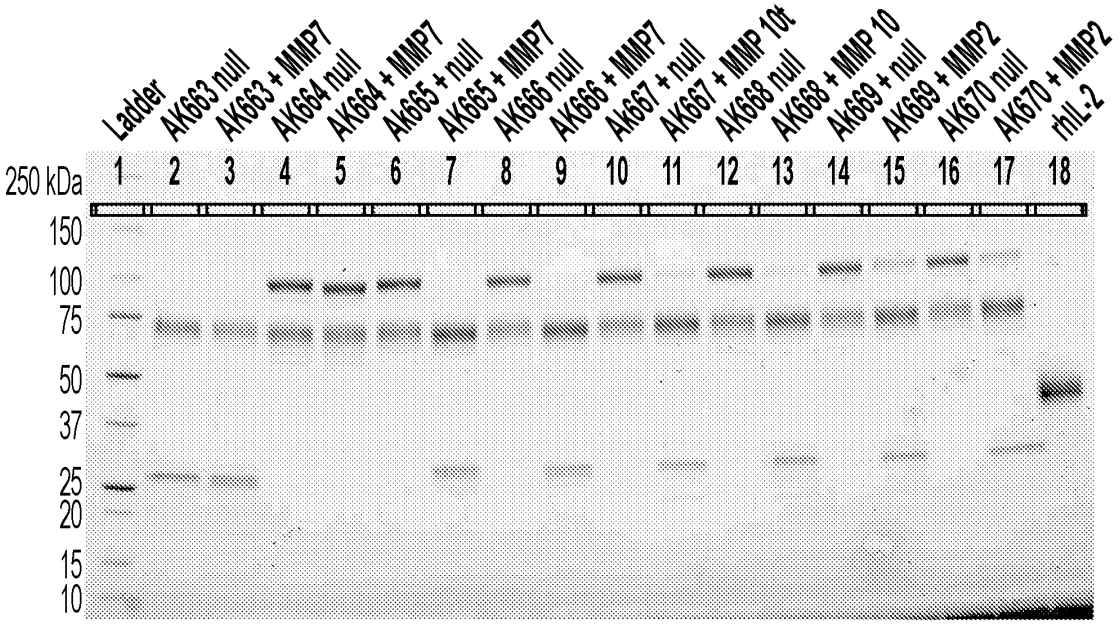


FIG. 49B

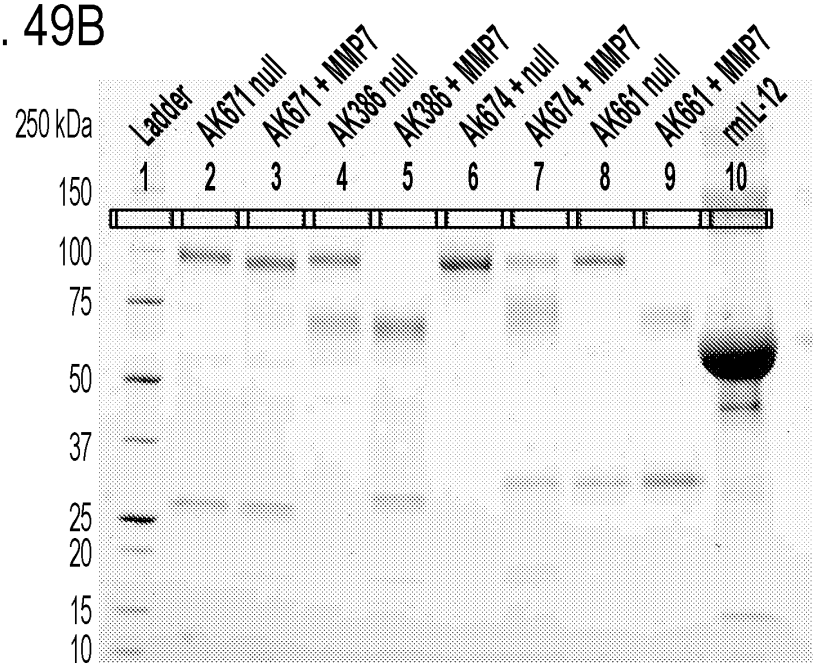
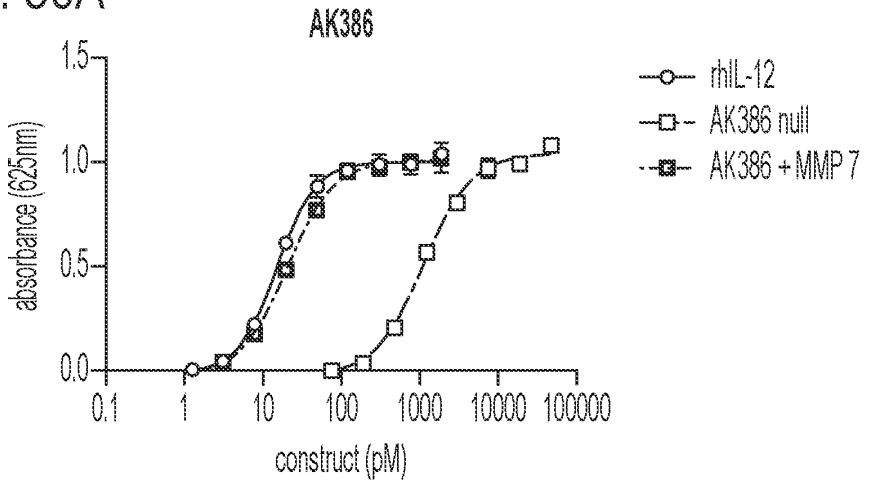
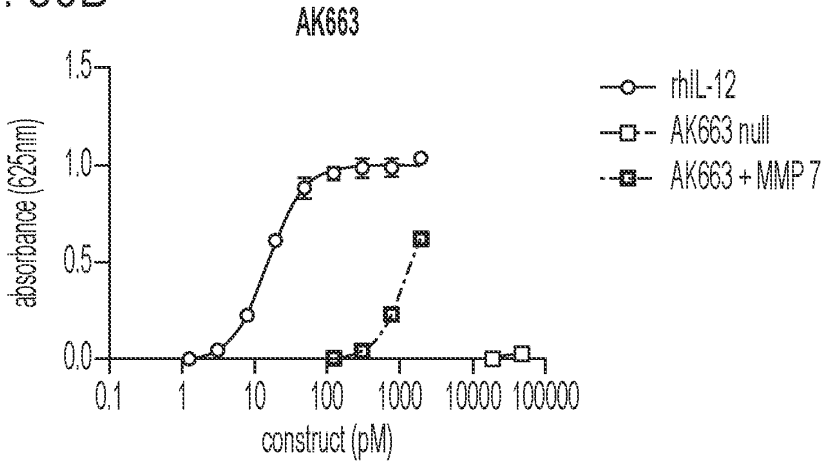


FIG. 50A



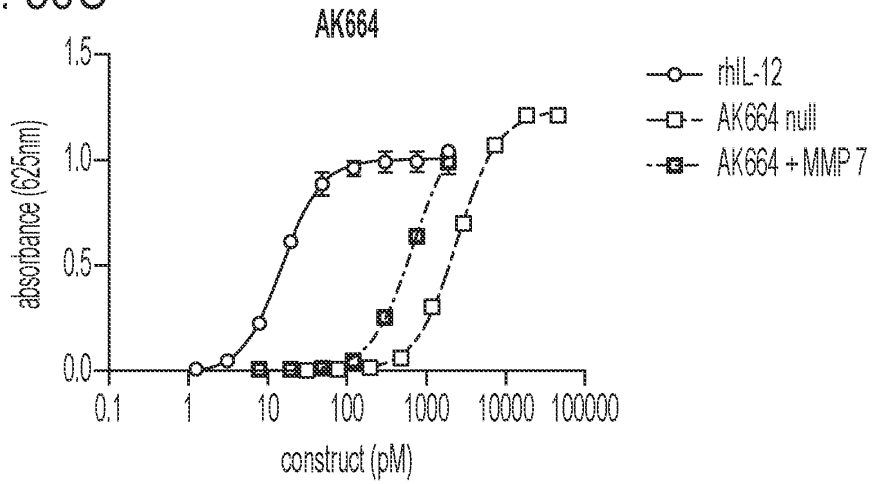
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK386 null	1123	74.2
AK386 + MMP 7	20.64	1.4

FIG. 50B



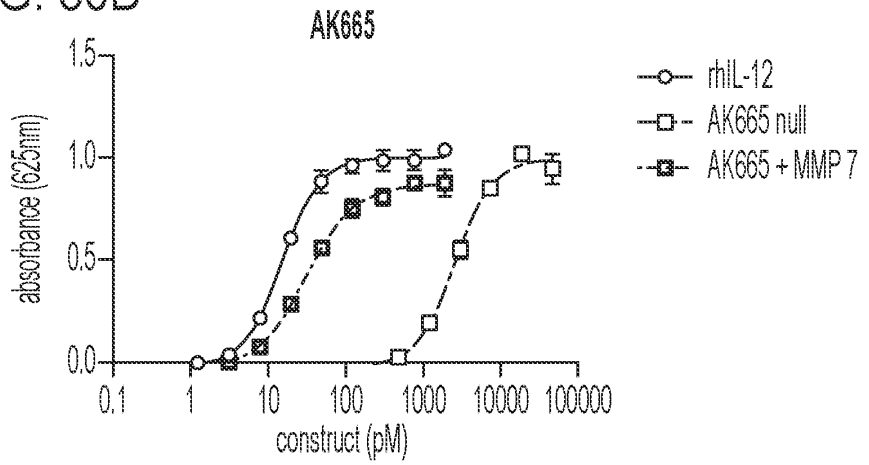
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK663 null	~ 21100	N/A
AK663 + MMP 7	1240	82.0

FIG. 50C



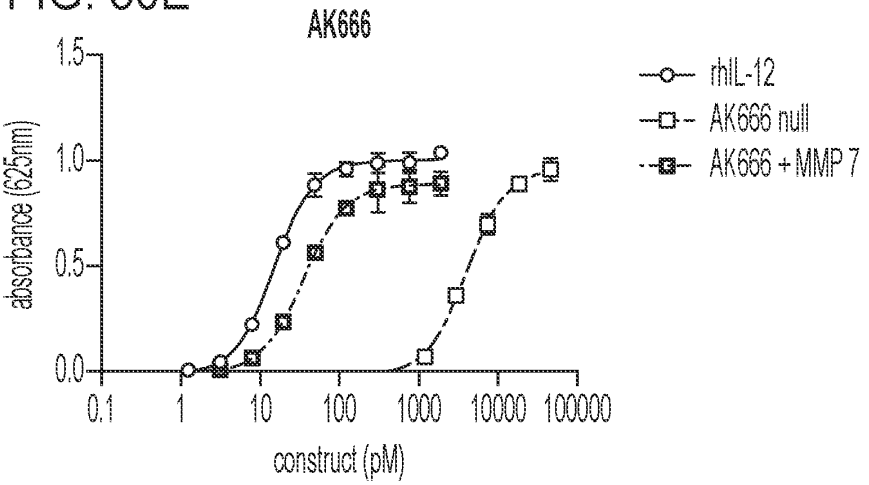
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK664 null	2485	164.2
AK664 + MMP 7	667.4	44.1

FIG. 50D



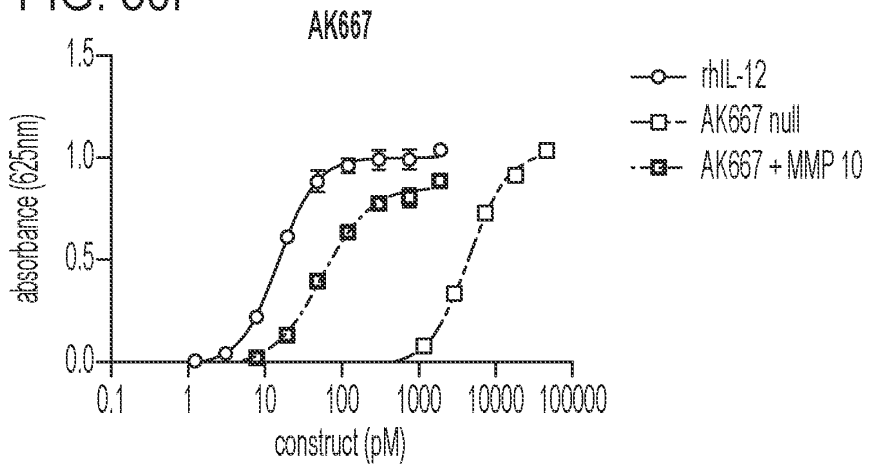
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK665 null	2555	168.9
AK665 + MMP 7	31.21	2.1

FIG. 50E



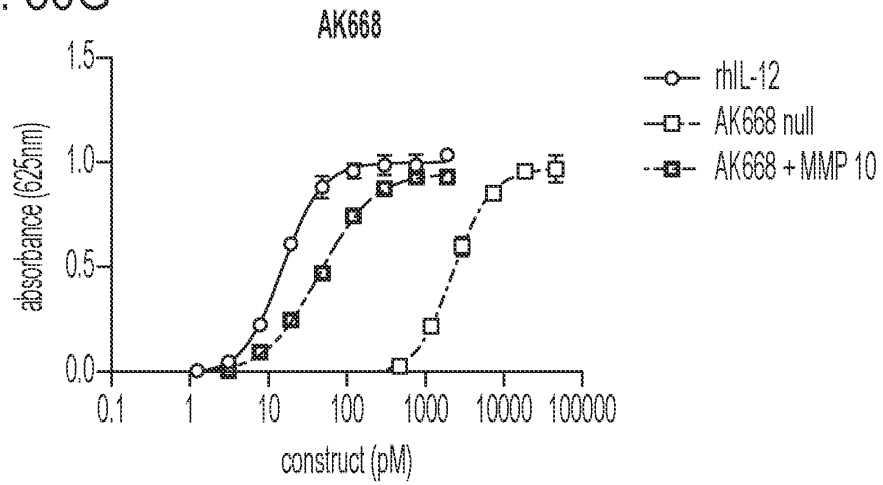
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK666 null	4061	268.4
AK666 + MMP 7	35.09	2.3

FIG. 50F



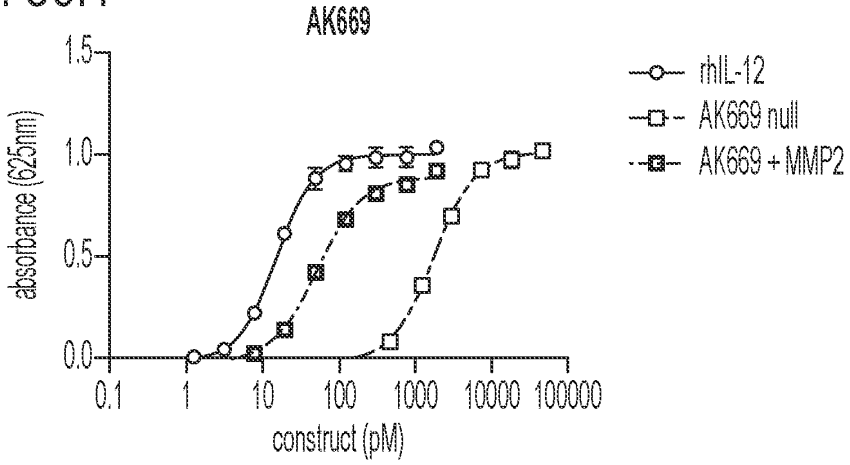
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK667 null	4497	297.2
AK667 + MMP 10	55.77	3.7

FIG. 50G



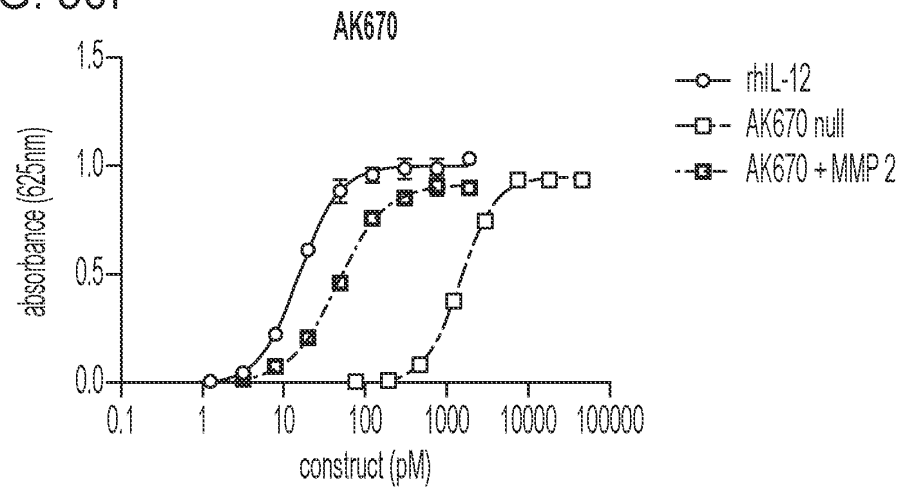
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK668 null	2293	151.6
AK668 + MMP 10	45.05	3.0

FIG. 50H



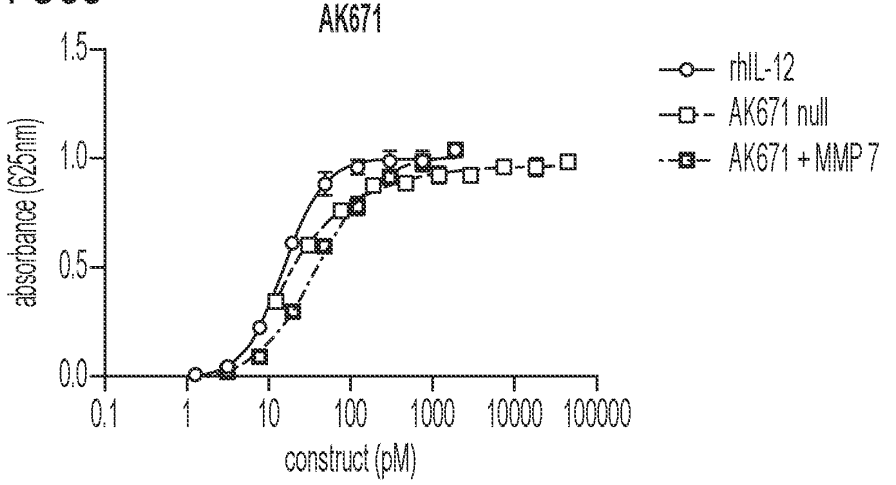
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK669 null	1751	115.7
AK669 + MMP2	52.7	3.5

FIG. 50I



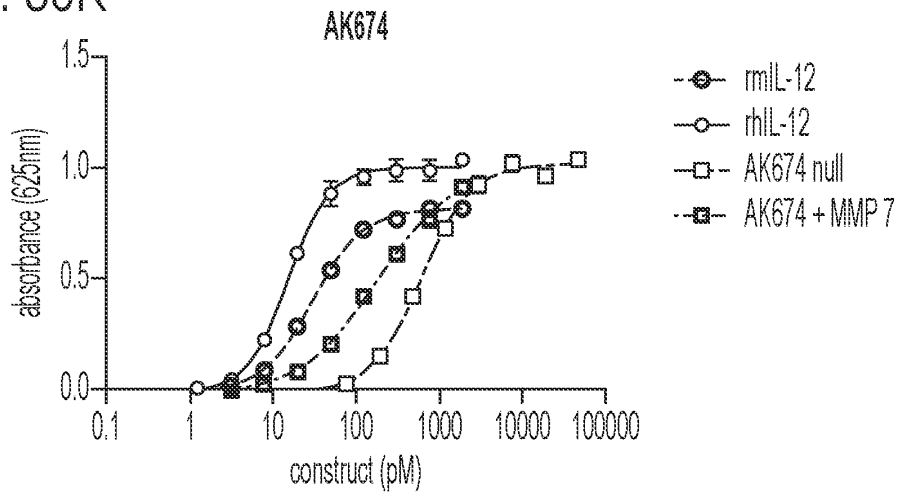
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK670 null	1478	97.7
AK670 + MMP 2	45.49	3.0

FIG. 50J



Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	15.13	
AK671 null	~ 0.0003067*	N/A
AK671 + MMP 7	38.44	2.5

FIG. 50K



Construct	EC50 (pM)	Fold-Aklusion
rmIL-12	29.82	
AK674 null	603.8	20.2
AK674 + MMP 7	177.2	5.9

FIG. 51A

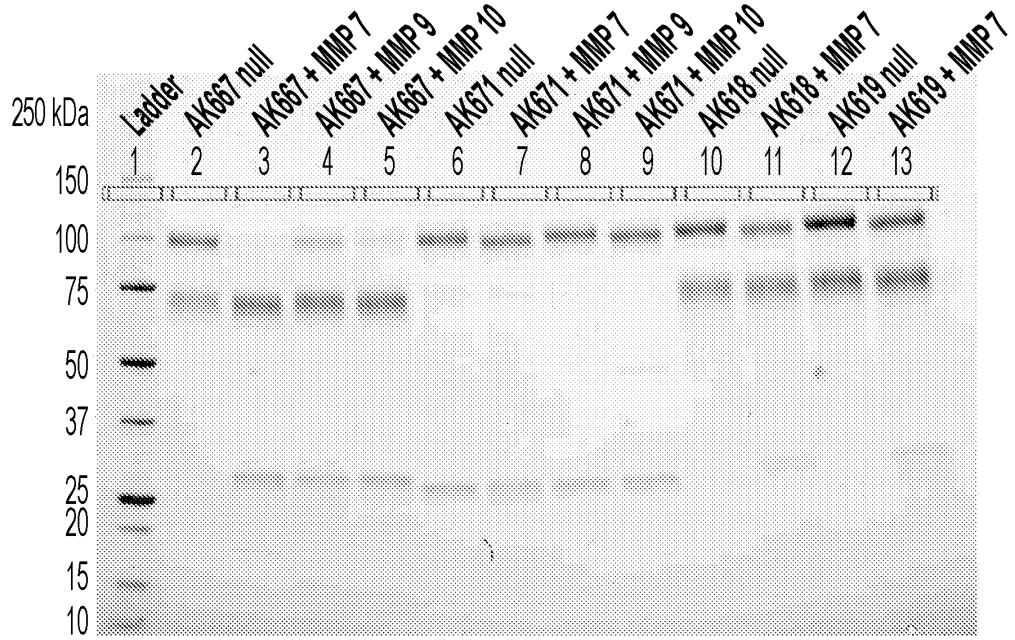


FIG. 51B

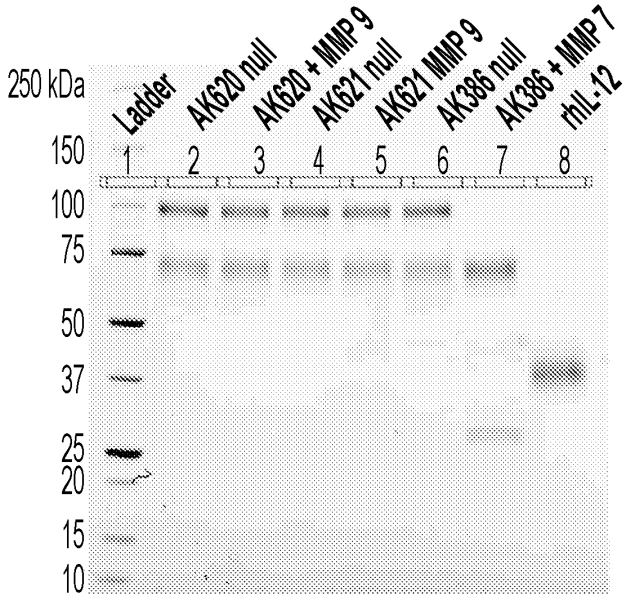
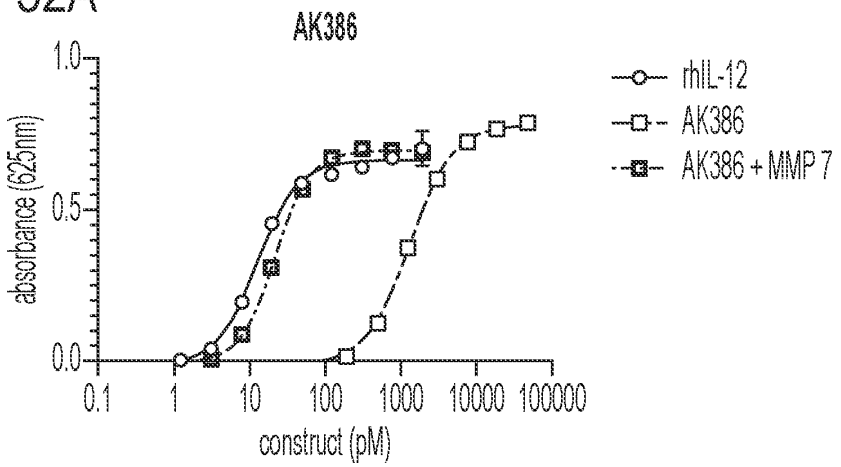
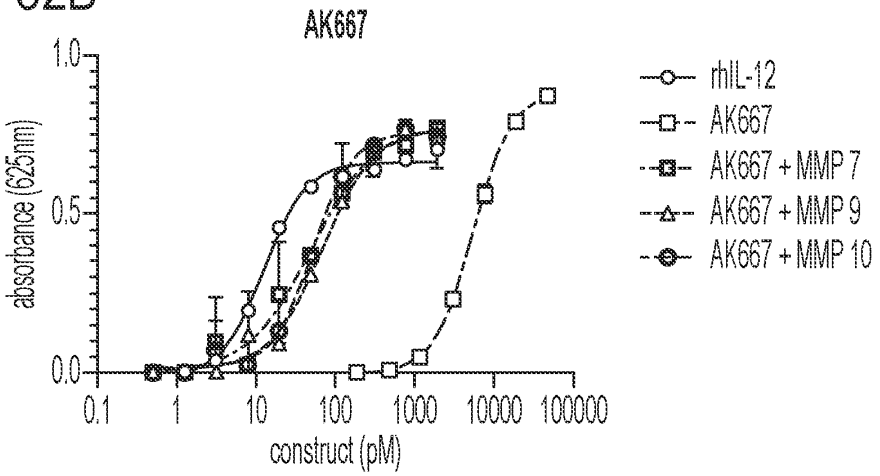


FIG. 52A



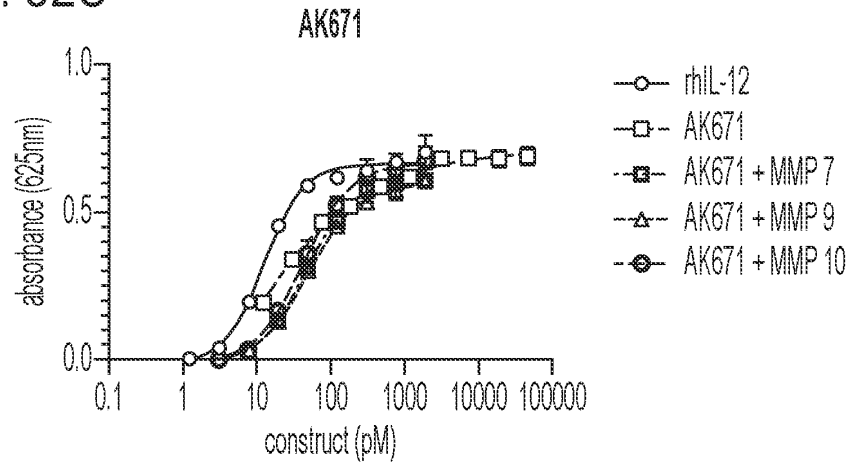
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	12.55	N/A
AK386	1294	103.1
AK386 + MMP 7	21.76	1.7

FIG. 52B



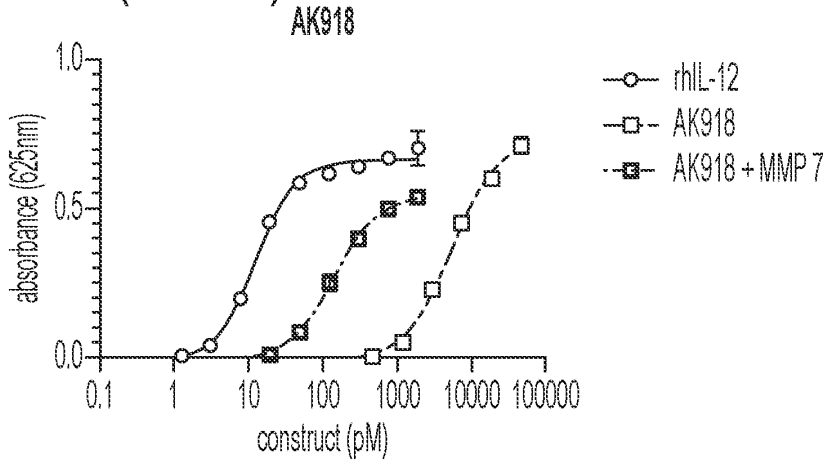
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	12.55	N/A
AK667	5412	431.2
AK667 + MMP 7	49.24	3.9
AK667 + MMP 9	67.88	5.4
AK667 + MMP 10	52.29	4.2

FIG. 52C



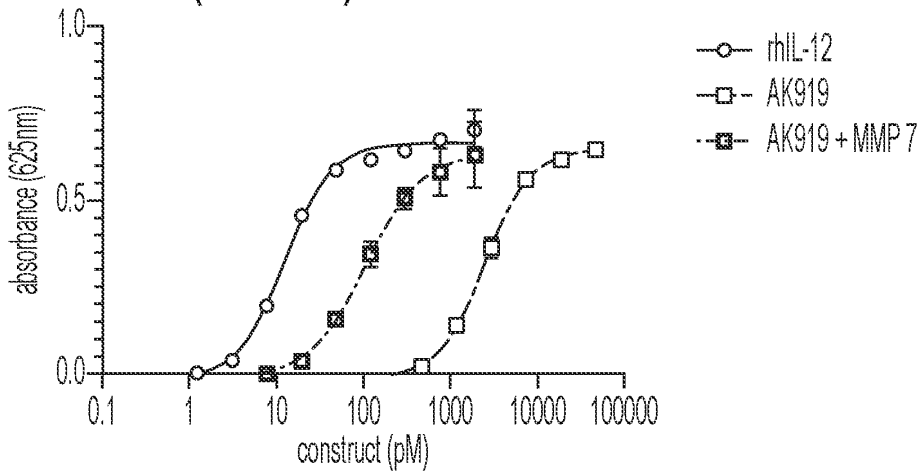
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	12.55	N/A
AK671	1.36*	0.1*
AK671 + MMP 7	46.04	3.7
AK671 + MMP 9	48.42	3.9
AK671 + MMP 10	42.47	3.4

FIG. 52D (AK918)



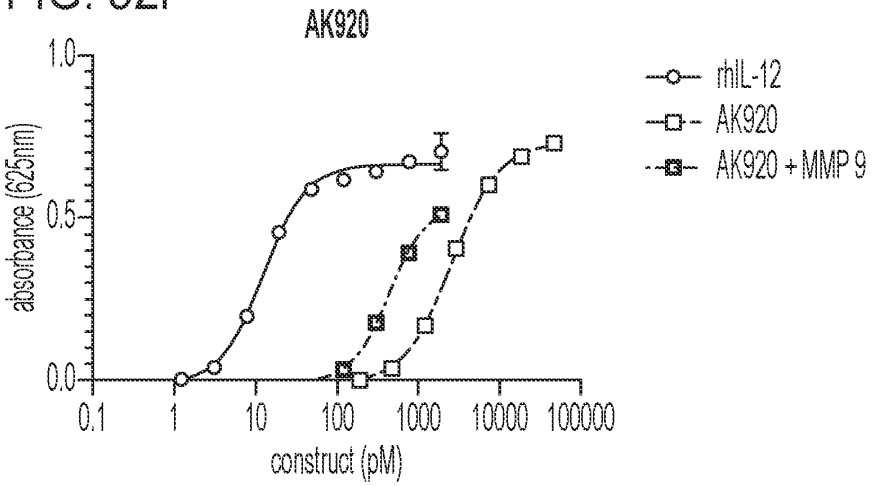
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	12.55	N/A
AK918	5372	428.0
AK918 + MMP 7	141	11.2

FIG. 52E (AK919)



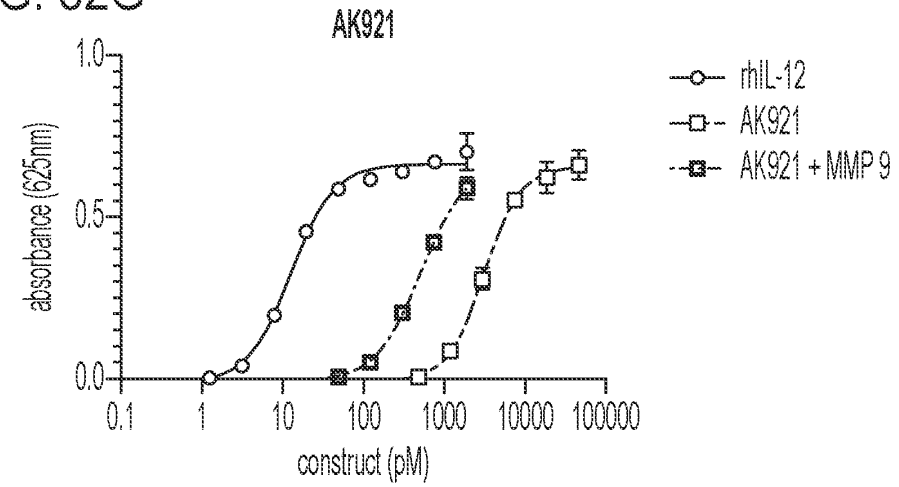
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	12.55	N/A
AK919	2506	199.7
AK919 + MMP 7	106.5	8.5

FIG. 52F



Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	12.55	N/A
AK920	2600	207.2
AK920 + MMP 9	442.9	35.3

FIG. 52G



Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	12.55	N/A
AK921	3119	248.5
AK921 + MMP 9	518.4	41.3

FIG. 53

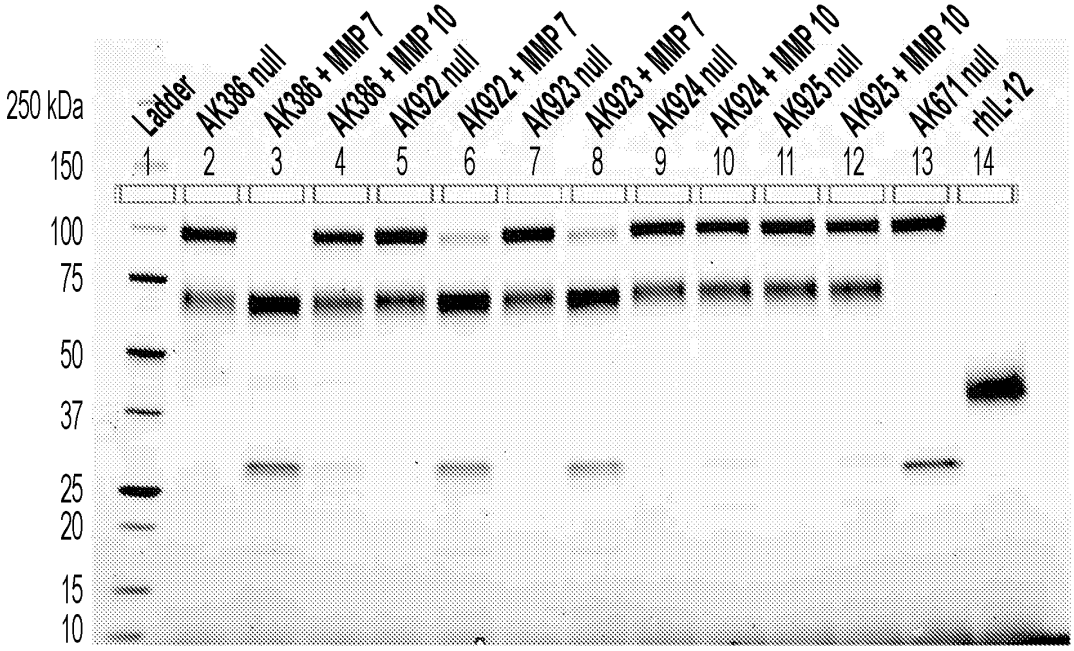
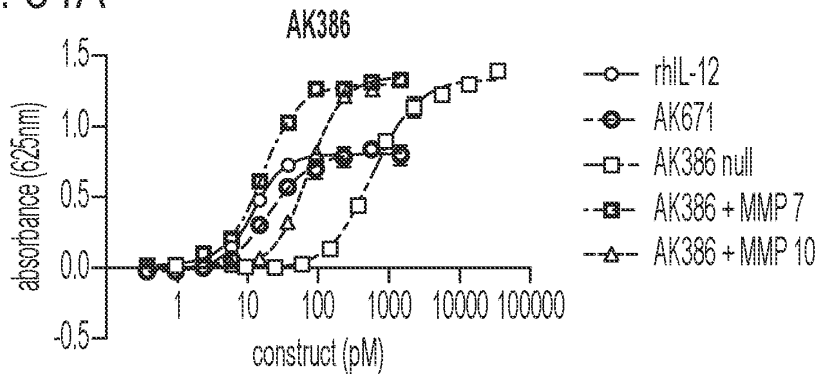
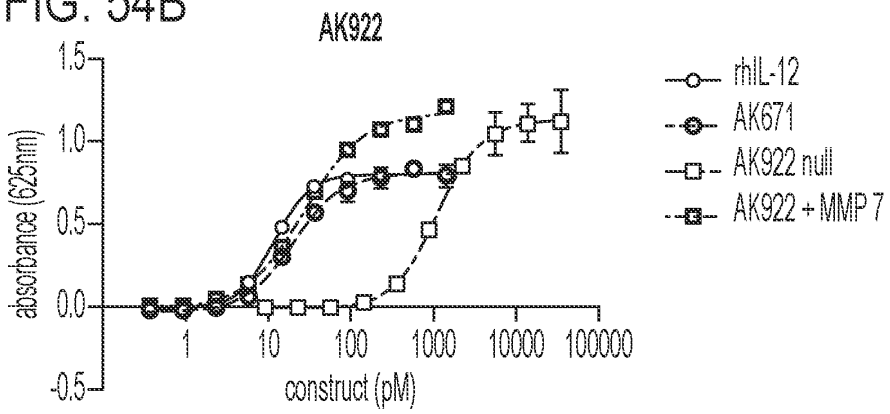


FIG. 54A



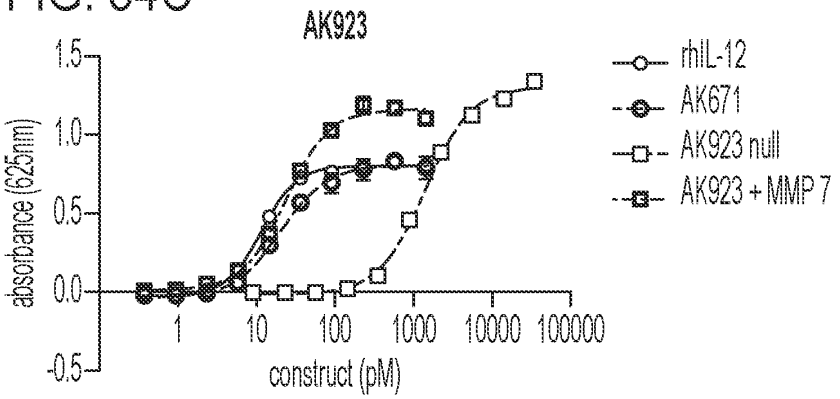
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	11.69	N/A
AK671	20.12	N/A
AK386 null	580.8	28.9
AK386 + MMP 7	16.09	0.8
AK386 + MMP 10	71.95	3.6

FIG. 54B



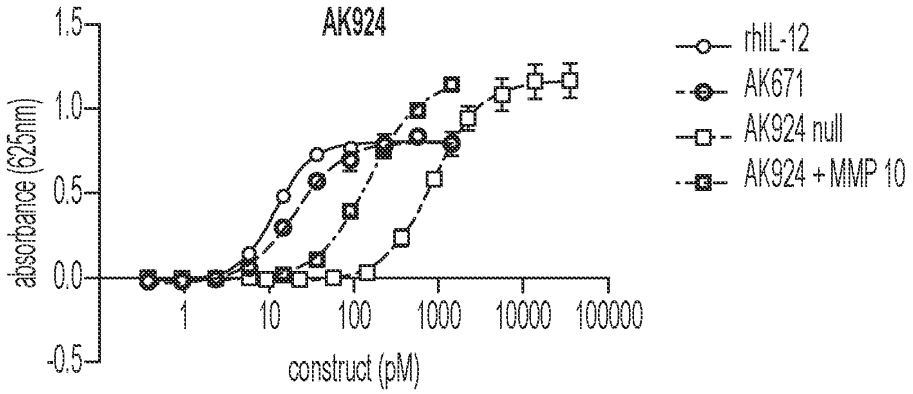
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	11.69	
AK671	20.12	
AK922 null	1102	54.8
AK922 + MMP 7	27.5	1.4

FIG. 54C



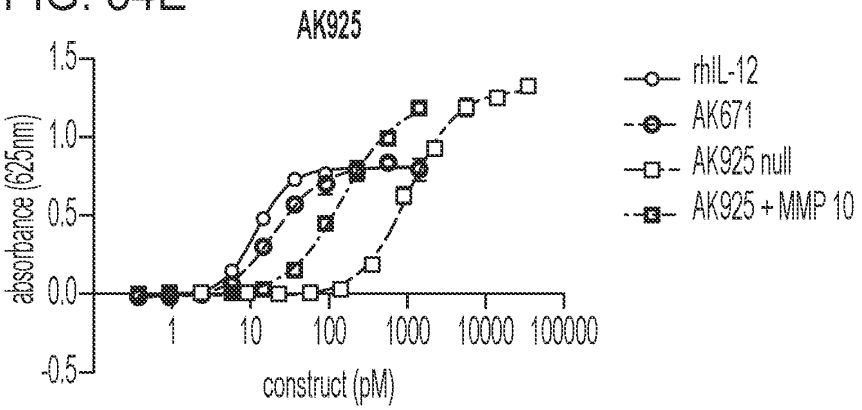
Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	11.69	
AK671	20.12	
AK923 null	1393	69.2
AK923 + MMP 7	23.84	1.2

FIG. 54D



Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	11.69	
AK671	20.12	
AK924 null	876.2	43.5
AK924 + MMP 10	151	7.5

FIG. 54E



Construct	EC50 (pM)	Fold-Aklusion
rhIL-12	11.69	
AK671	20.12	
AK925 null	1055	52.4
AK925 + MMP 10	149.5	7.4

FIG. 55A

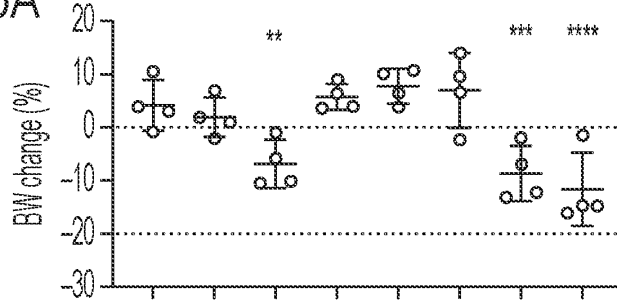


FIG. 55B

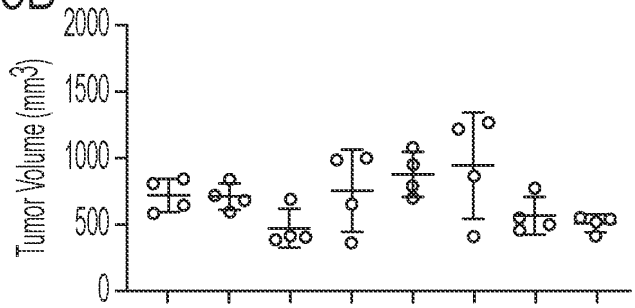


FIG. 55C

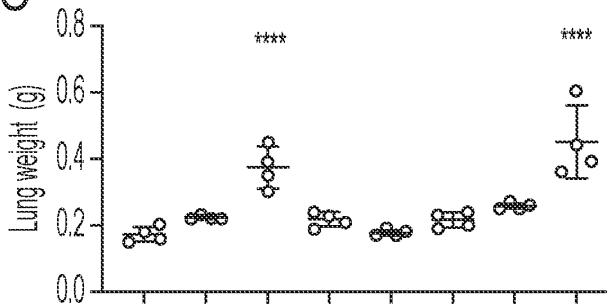


FIG. 55D

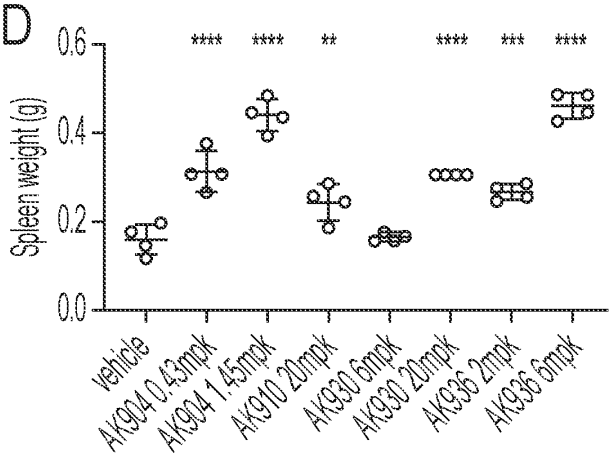


FIG. 56A

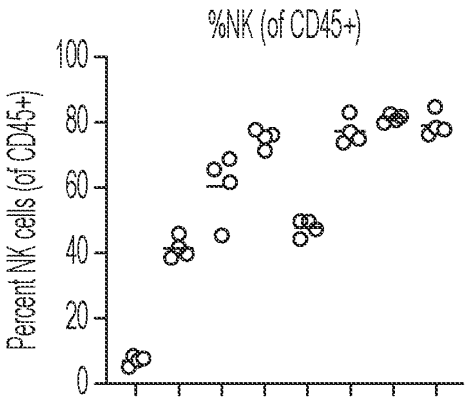


FIG. 56B

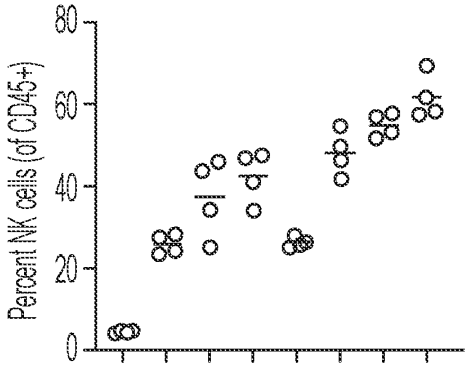


FIG. 56C

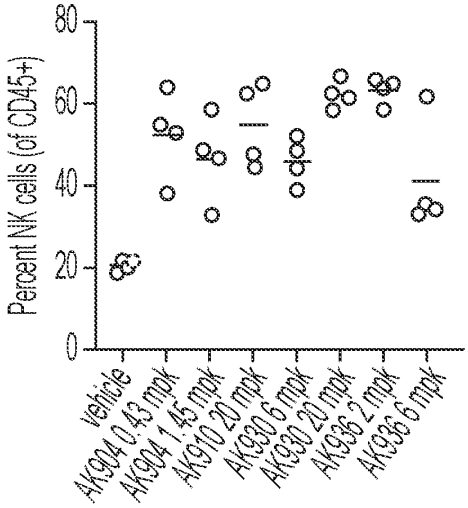


FIG. 57A

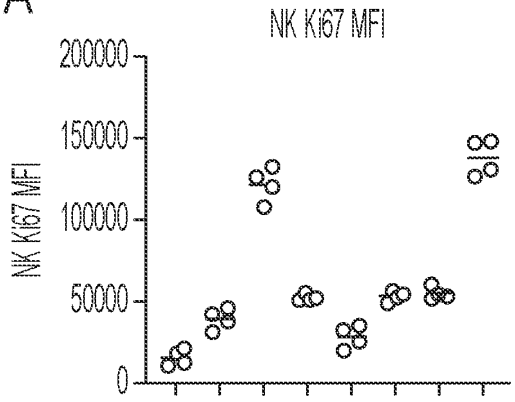


FIG. 57B

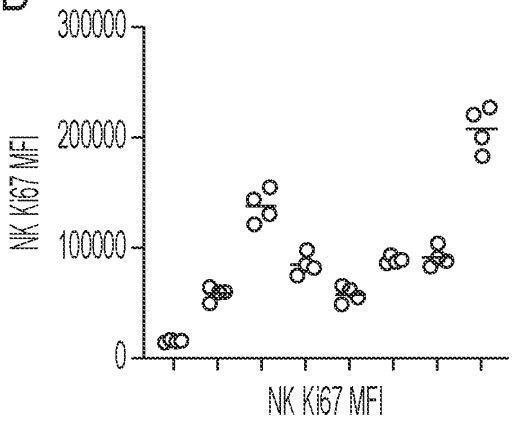


FIG. 57C

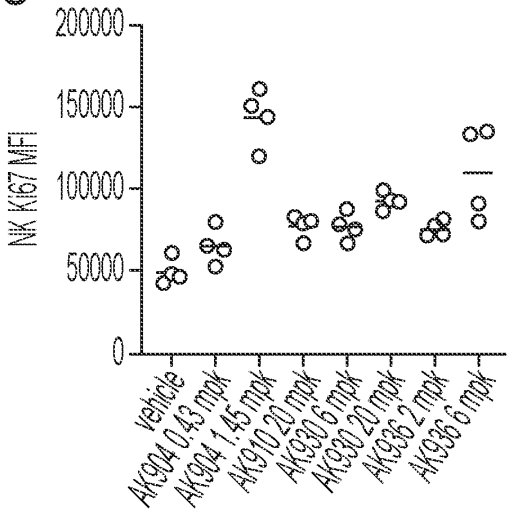


FIG. 58A

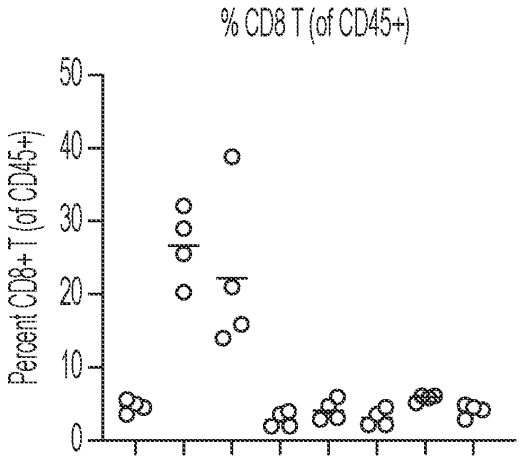


FIG. 58B

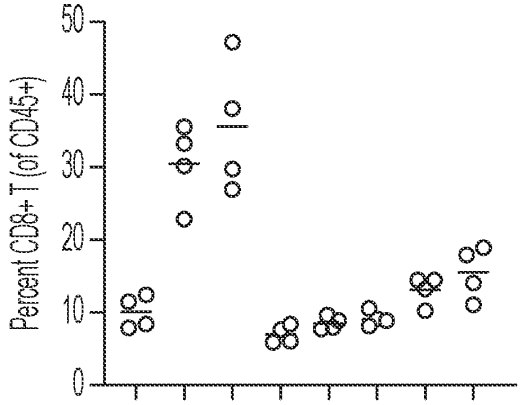


FIG. 58C

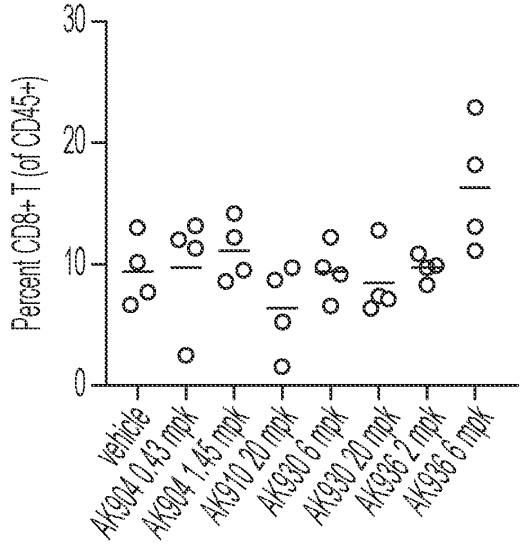


FIG. 59A

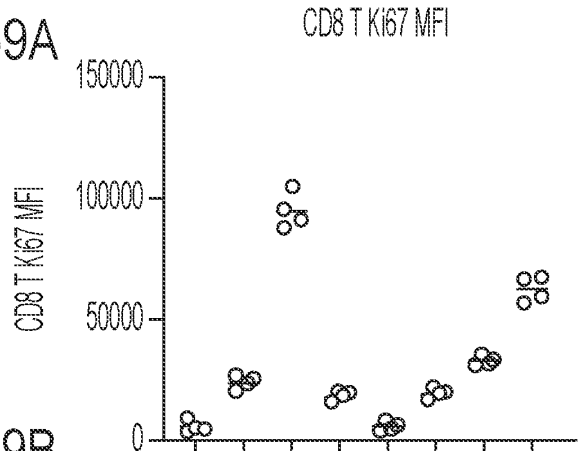


FIG. 59B

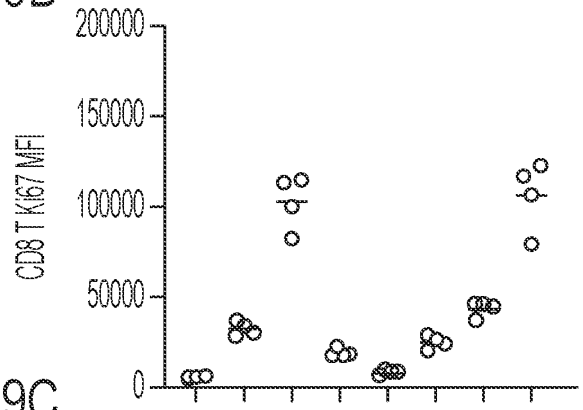


FIG. 59C

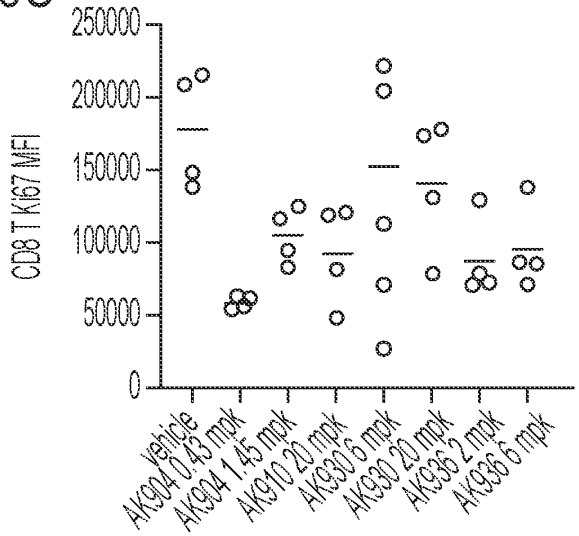


FIG. 60A

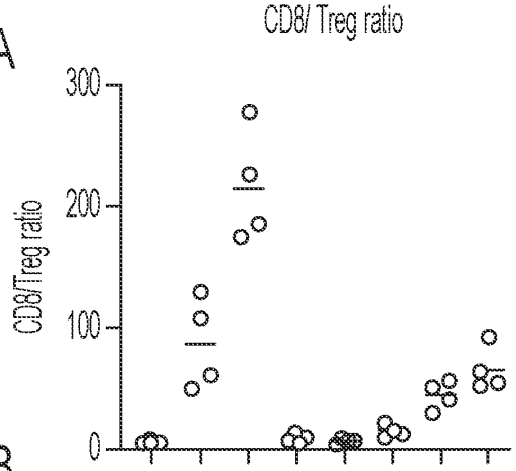


FIG. 60B

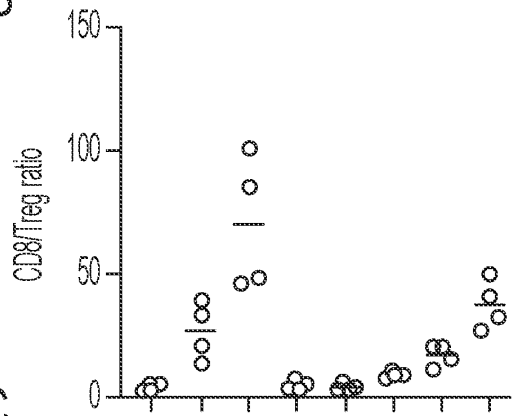


FIG. 60C

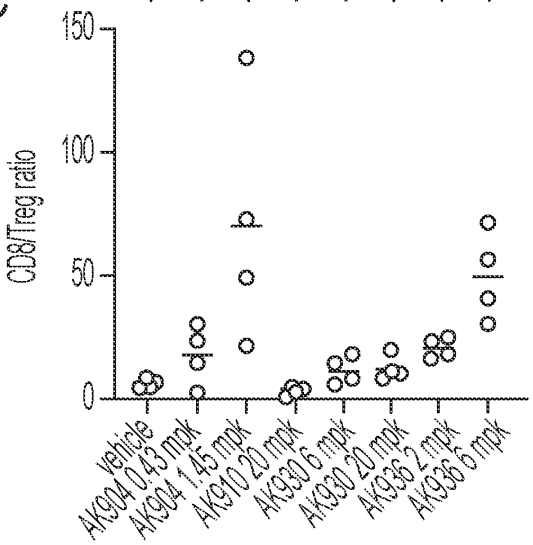


FIG. 61A

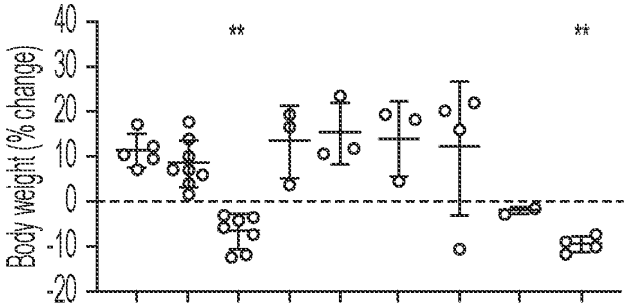


FIG. 61B

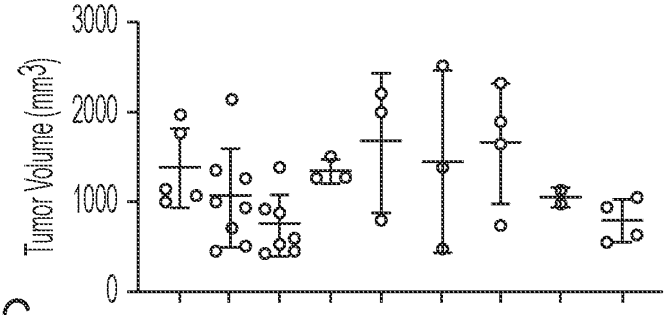


FIG. 61C

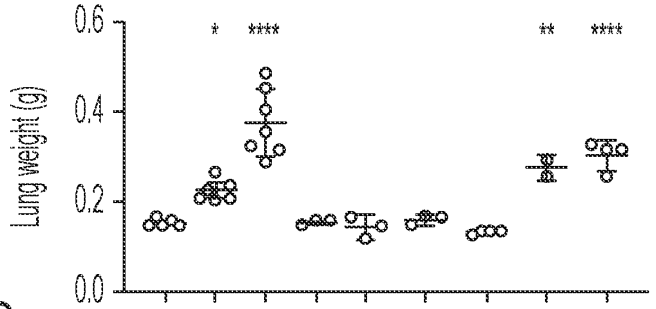


FIG. 61D

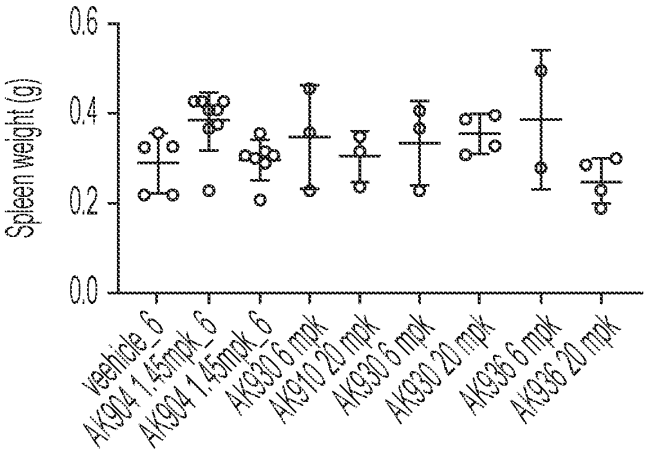


FIG. 62A

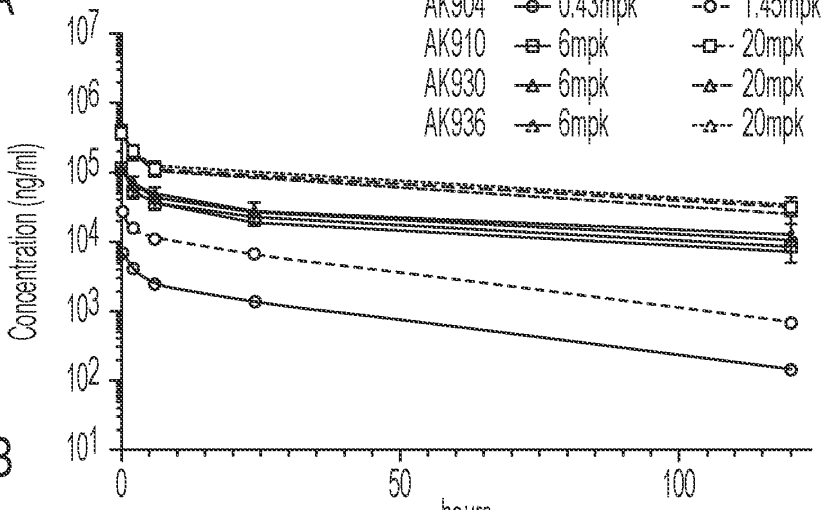


FIG. 62B

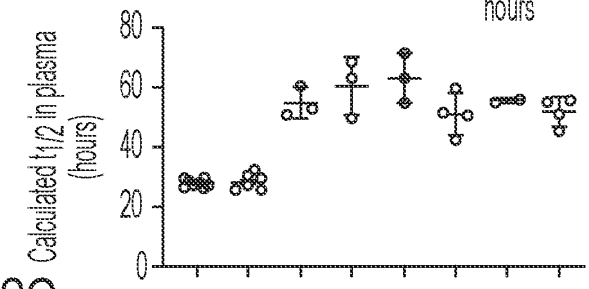


FIG. 62C

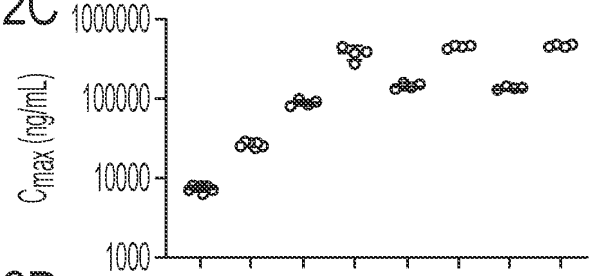


FIG. 62D

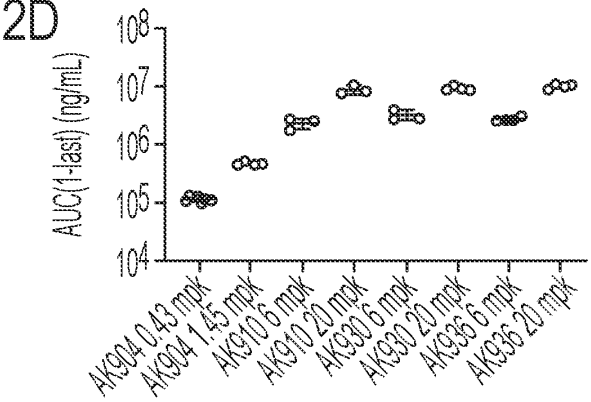


FIG. 63A

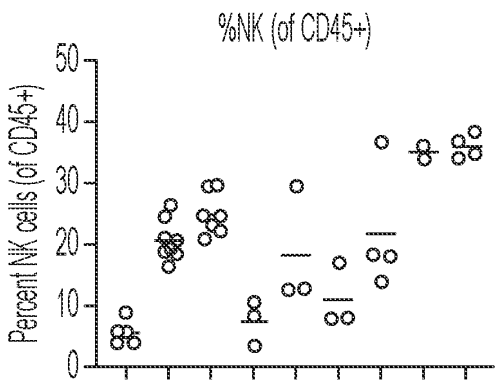


FIG. 63B

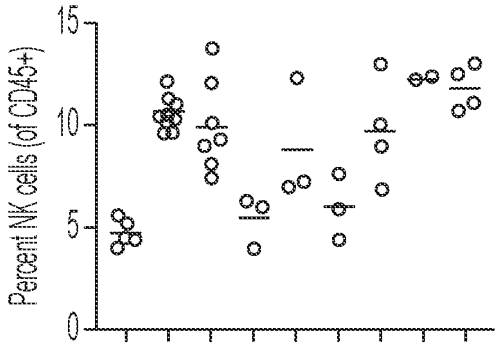


FIG. 63C

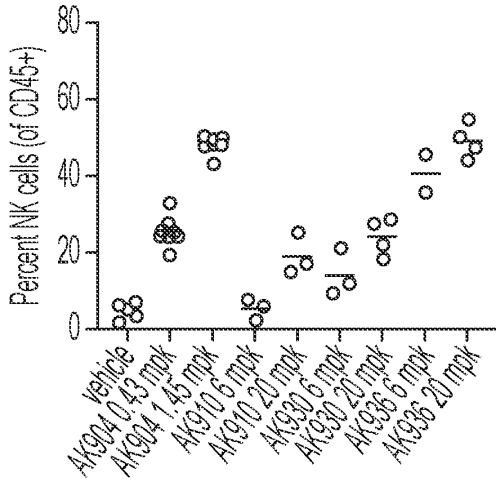


FIG. 64A

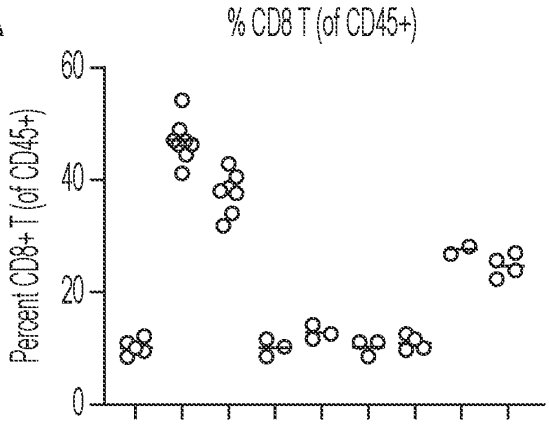


FIG. 64B

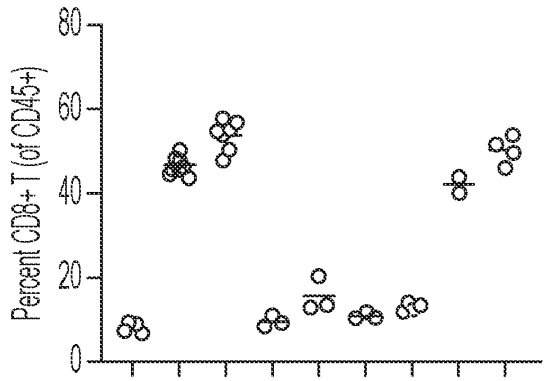


FIG. 64C

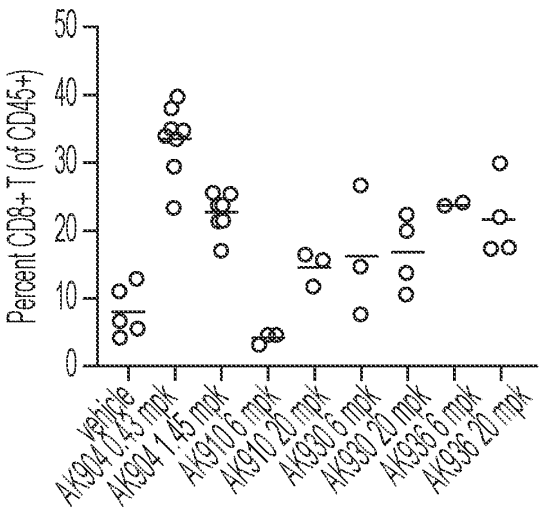


FIG. 65A

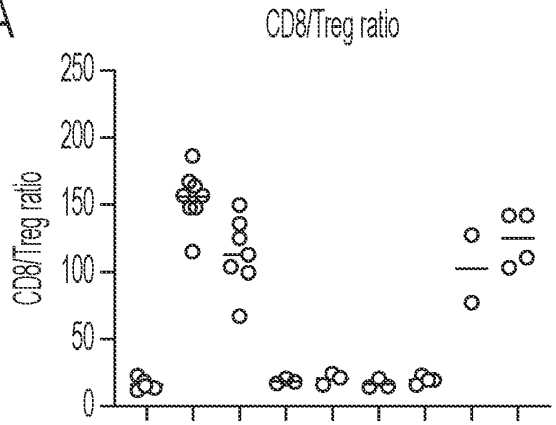


FIG. 65B

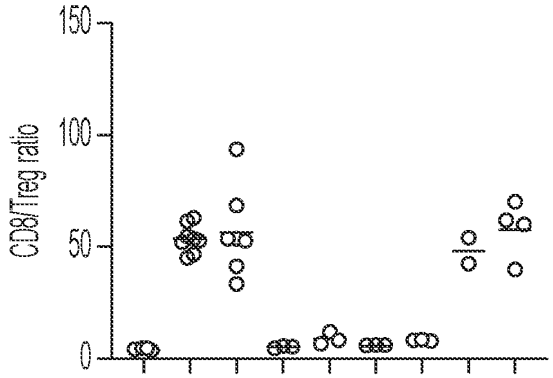
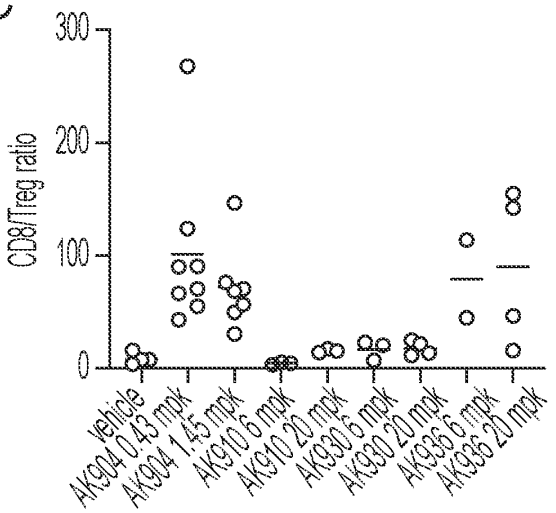


FIG. 65C



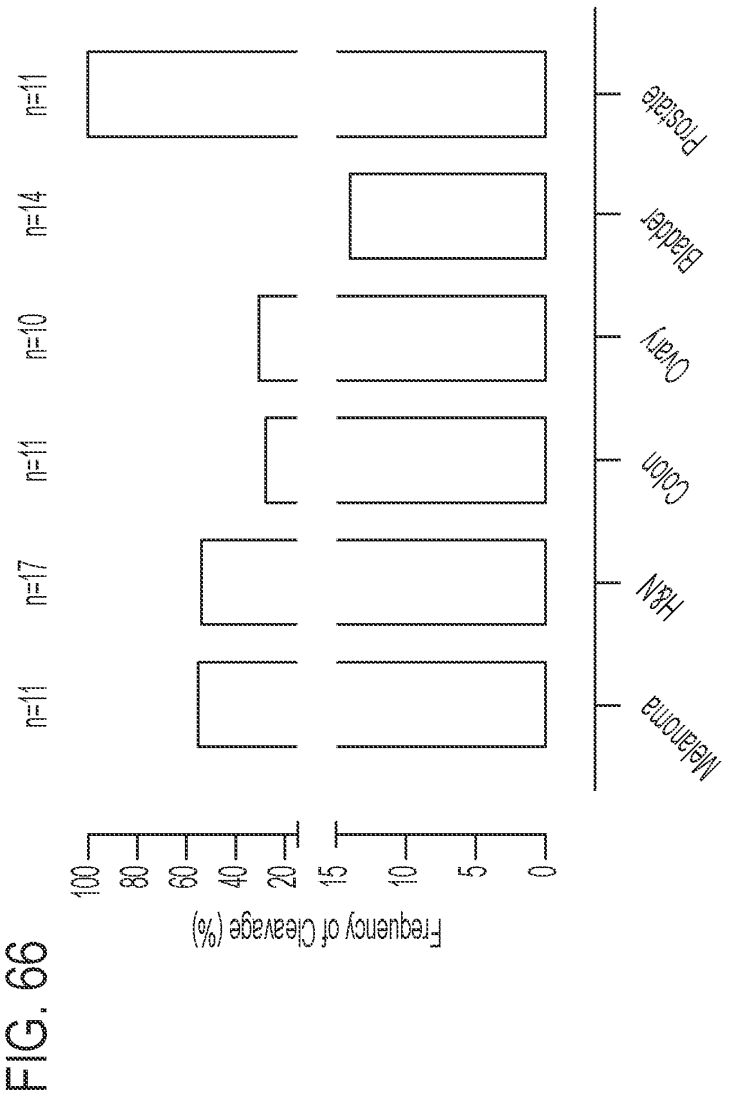
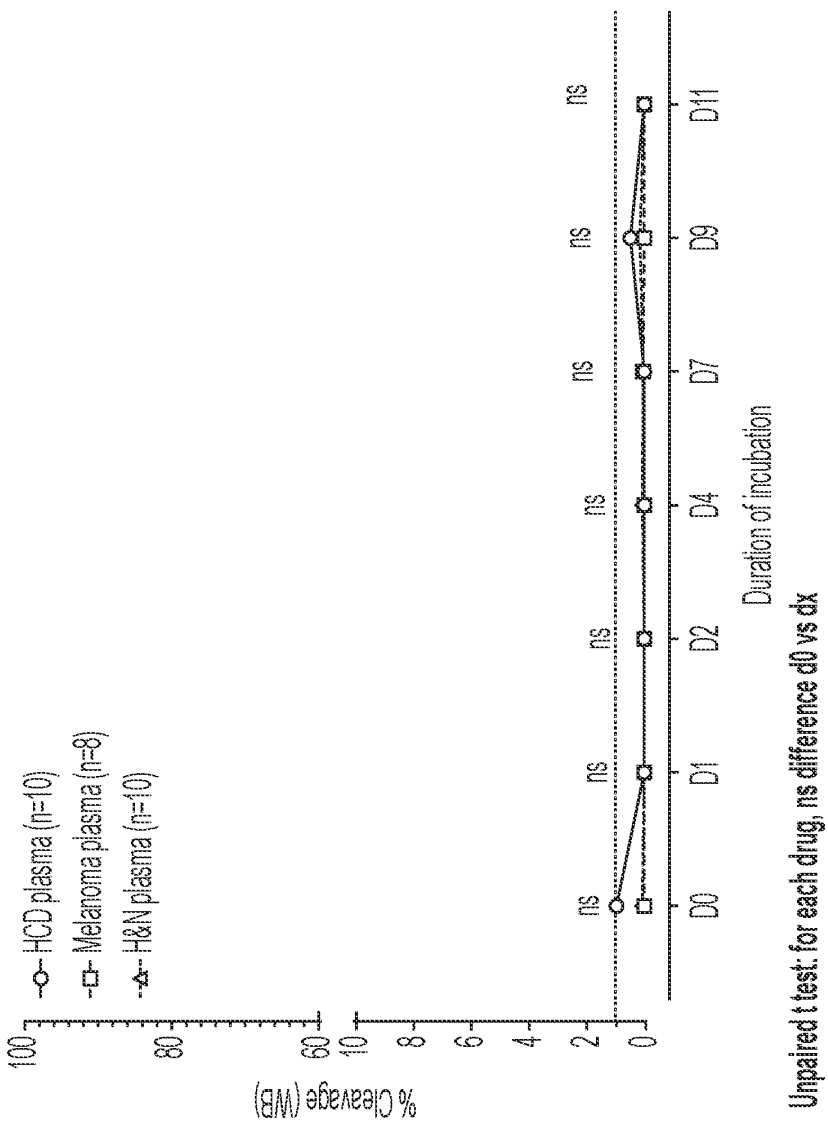

Melanoma
HeN
Colon
Ovary
Bladder
Prostate
n=11
n=17
n=11
n=10
n=14
n=11
Frequency of Cleavage (%)
100
80
60
40
20
15
10
5
0

FIG. 66

FIG. 67


HCD plasma (n=10)
Melanoma plasma (n=8)
H&N plasma (n=10)
% Cleavage (WB)
100
80
60
10
8
6
4
2
0
ns
D0
D1
D2
D4
D7
D9
D11
Duration of incubation
Unpaired t test: for each drug, ns difference d0 vs dx