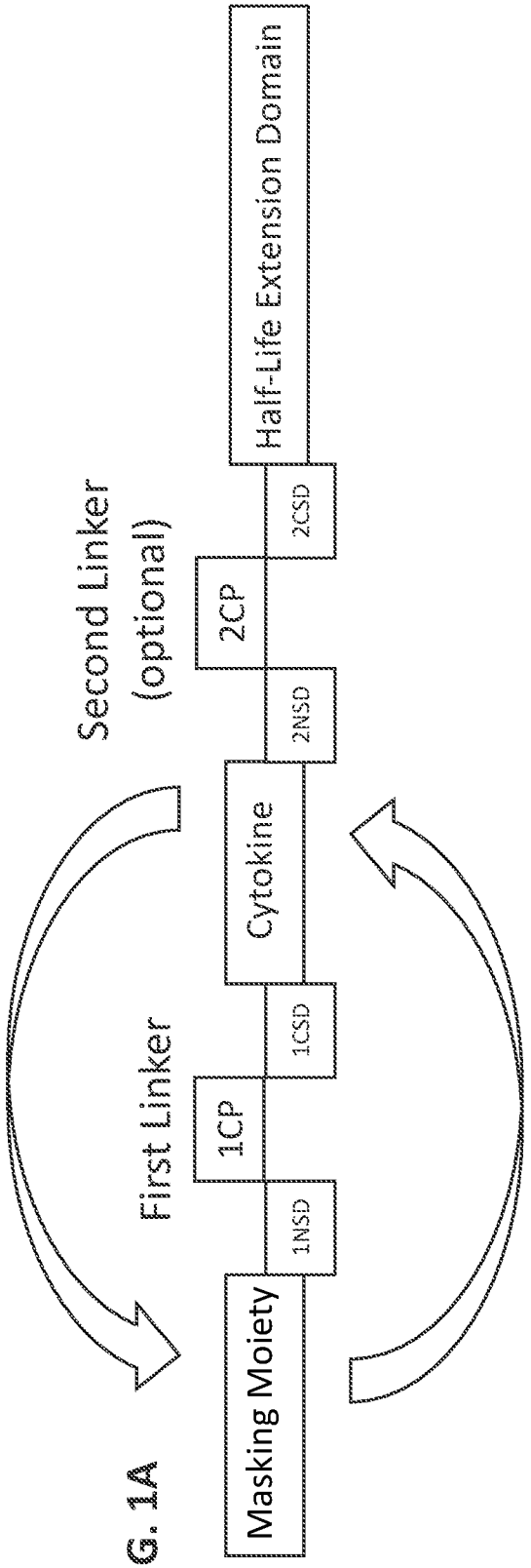

Second Linker
(optional)
Half-Life Extension Domain
2CP
2CSD
Cytokine
2NSD
First Linker
1CP
1CSD
Masking Moiety
1NSD

FIG. 1A

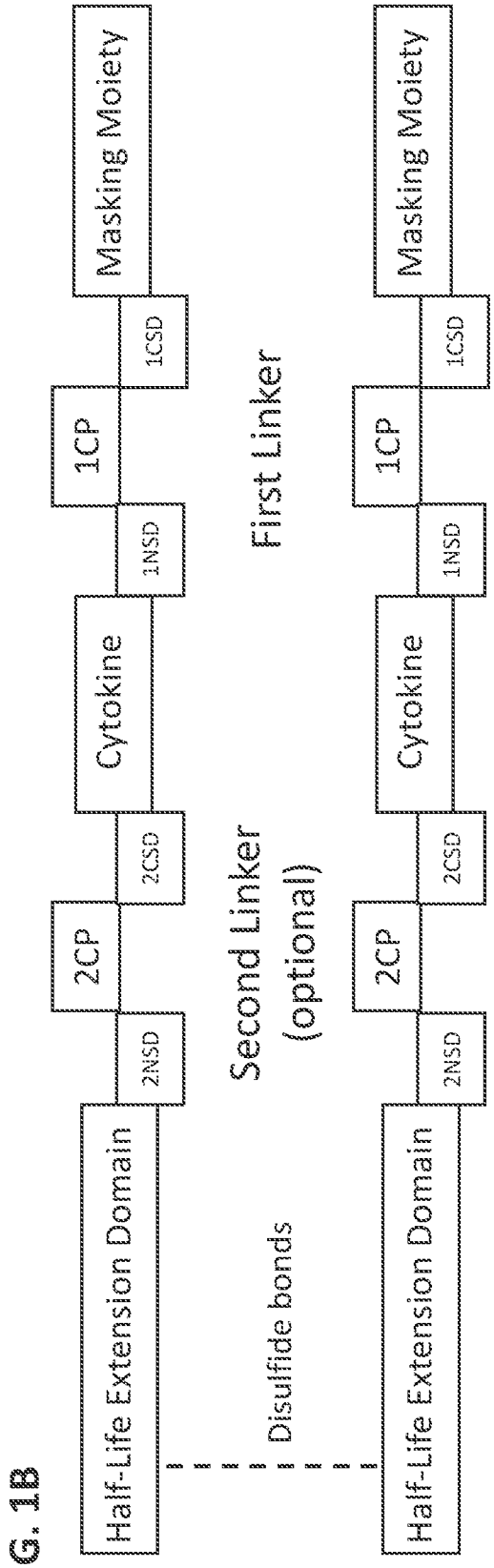

Masking Moiety
1CSD
1CP
1NSD
Cytokine
2CSD
2CP
2NSD
Half-Life Extension Domain
First Linker
Second Linker
(optional)
Disulfide bonds

FIG. 1B

FIG. 2A

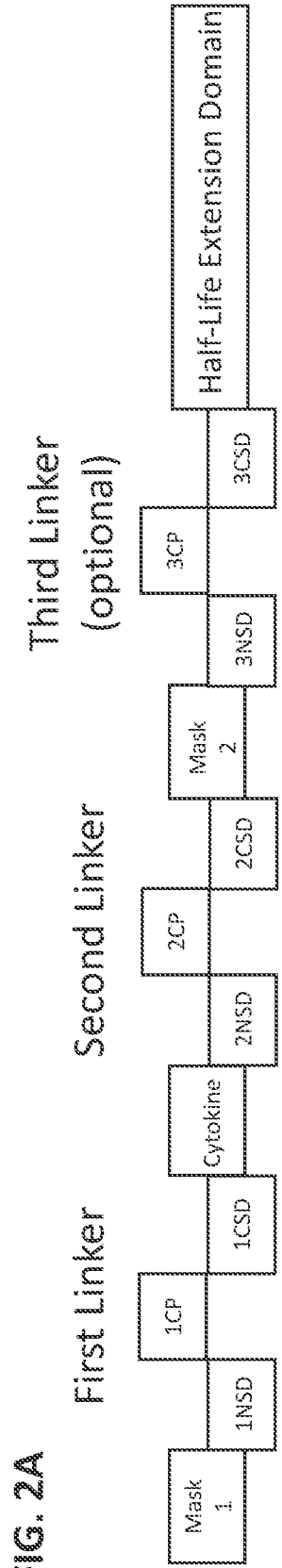

Mask 1
1NSD
1CP
1CSD
Cytokine
2NSD
2CP
2CSD
Mask 2
3NSD
3CP
3CSD
Half-Life Extension Domain
First Linker
Second Linker
Third Linker (optional)

FIG. 2B

Mask 1
1NSD
1CP
1CSD
Cytokine
2NSD
2CP
2CSD
Mask 2
3NSD
3CP
3CSD
Half-Life Extension Domain
First Linker
Second Linker
Third Linker (optional)
Disulfide bonds

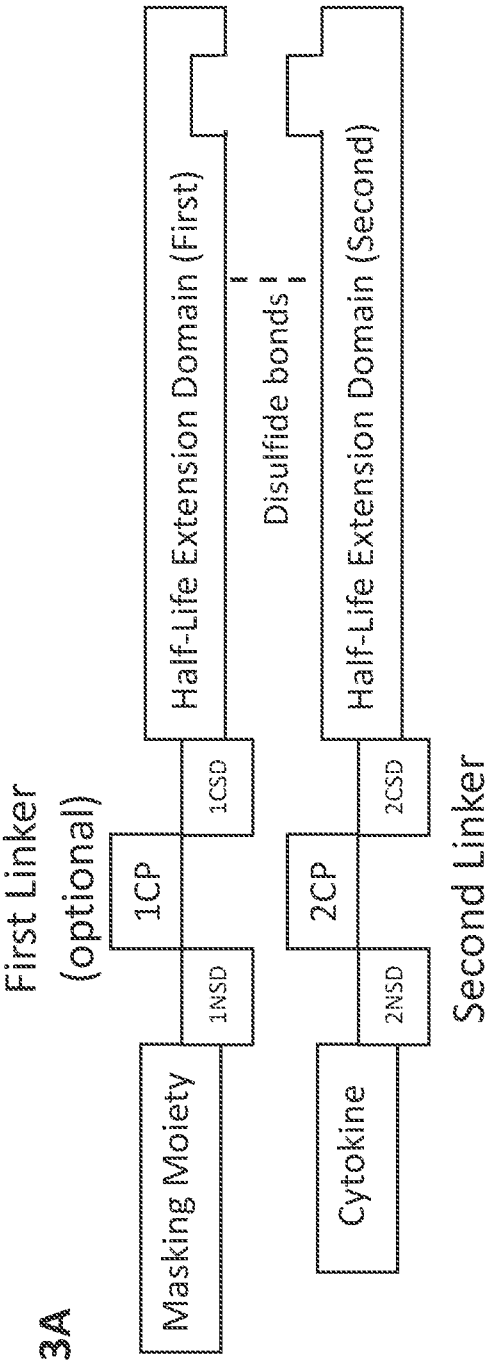

First Linker
(optional)
1CP
1CSD
Masking Moiety
1NSD
Half-Life Extension Domain (First)
Disulfide bonds
2CP
2CSD
Cytokine
2NSD
Half-Life Extension Domain (Second)
Second Linker
(optional)

FIG. 3A

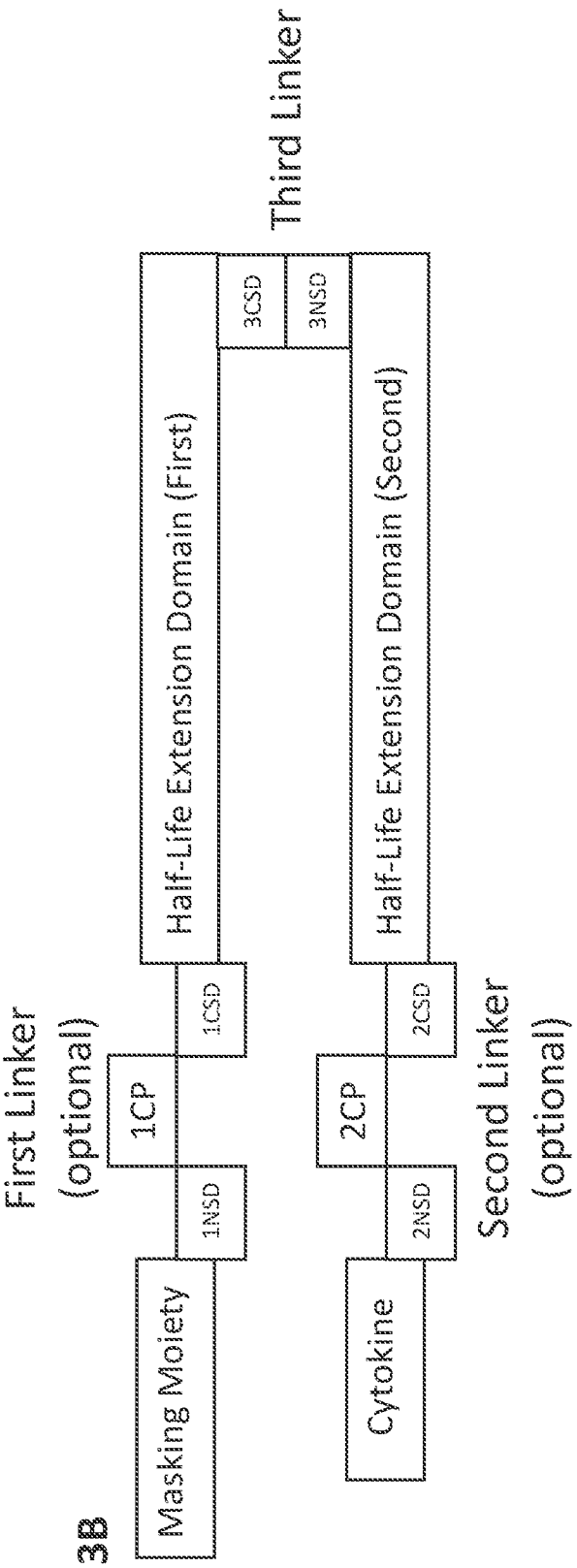

First Linker
(optional)
1CP
1CSD
Masking Moiety
1NSD
Half-Life Extension Domain (First)
Third Linker
3CSD
3NSD
2CP
2CSD
Cytokine
2NSD
Half-Life Extension Domain (Second)
Second Linker
(optional)

FIG. 3B

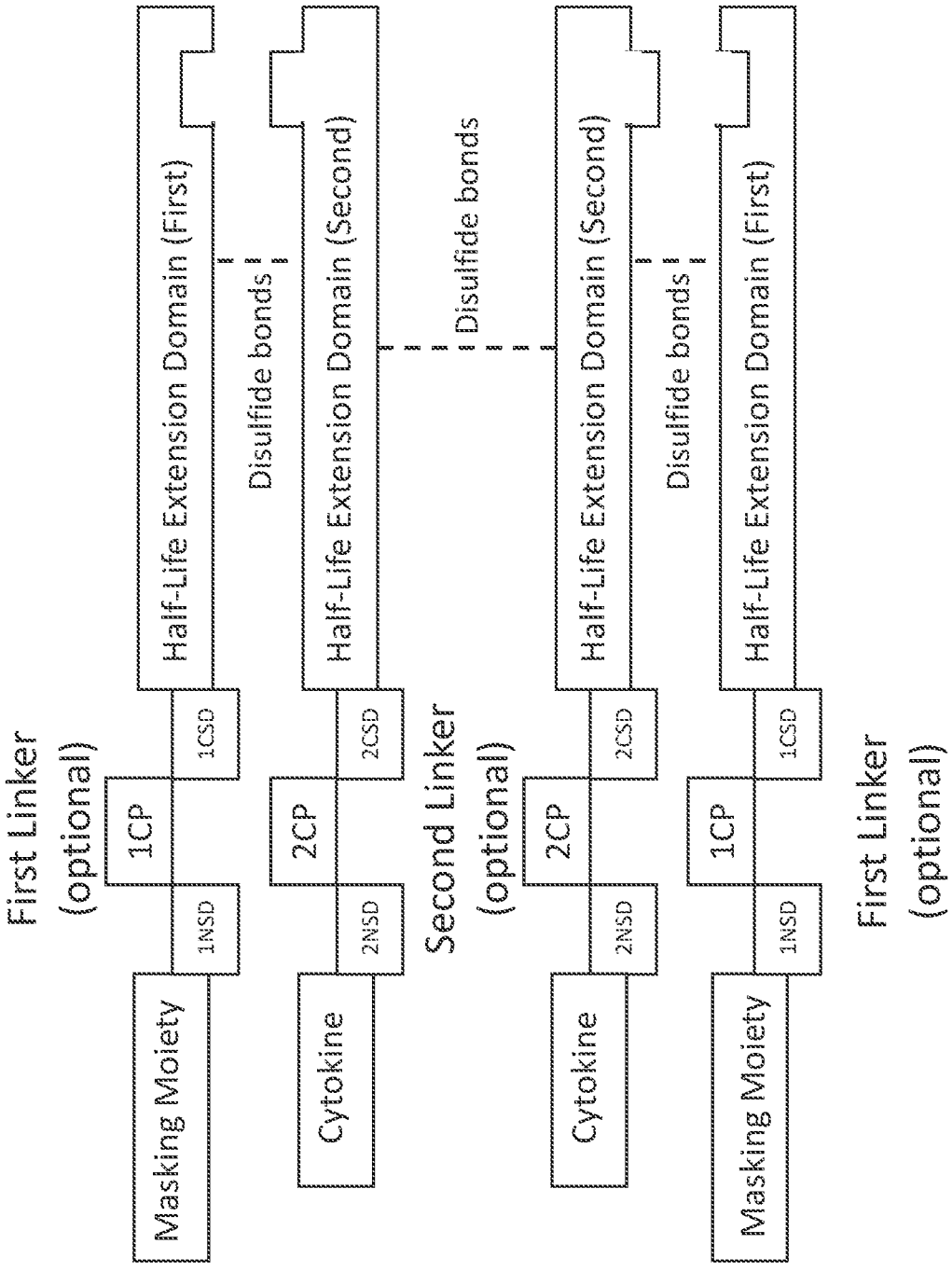

Masking Moiety
1NSD
1CP
1CSD
Half-Life Extension Domain (First)
First Linker (optional)
Disulfide bonds
Cytokine
2NSD
2CP
2CSD
Half-Life Extension Domain (Second)
Second Linker (optional)
Disulfide bonds
Cytokine
2NSD
2CP
2CSD
Half-Life Extension Domain (Second)
Disulfide bonds
Masking Moiety
1NSD
1CP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

Figure 4

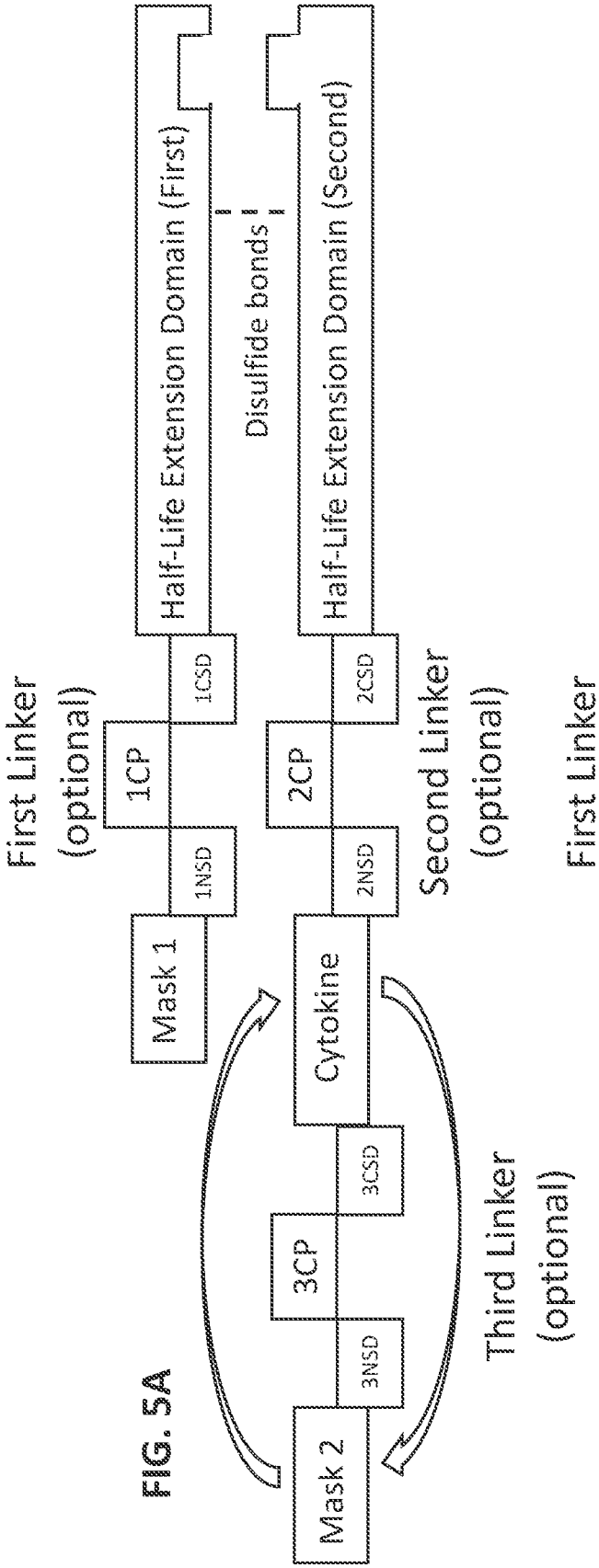

First Linker
(optional)
Mask 1
1NSD
1CP
1CSD
Half-Life Extension Domain (First)
Disulfide bonds
Half-Life Extension Domain (Second)
2CSD
2CP
2NSD
Second Linker
(optional)
Cytokine
3CSD
3CP
3NSD
Mask 2
Third Linker
(optional)

FIG. 5A

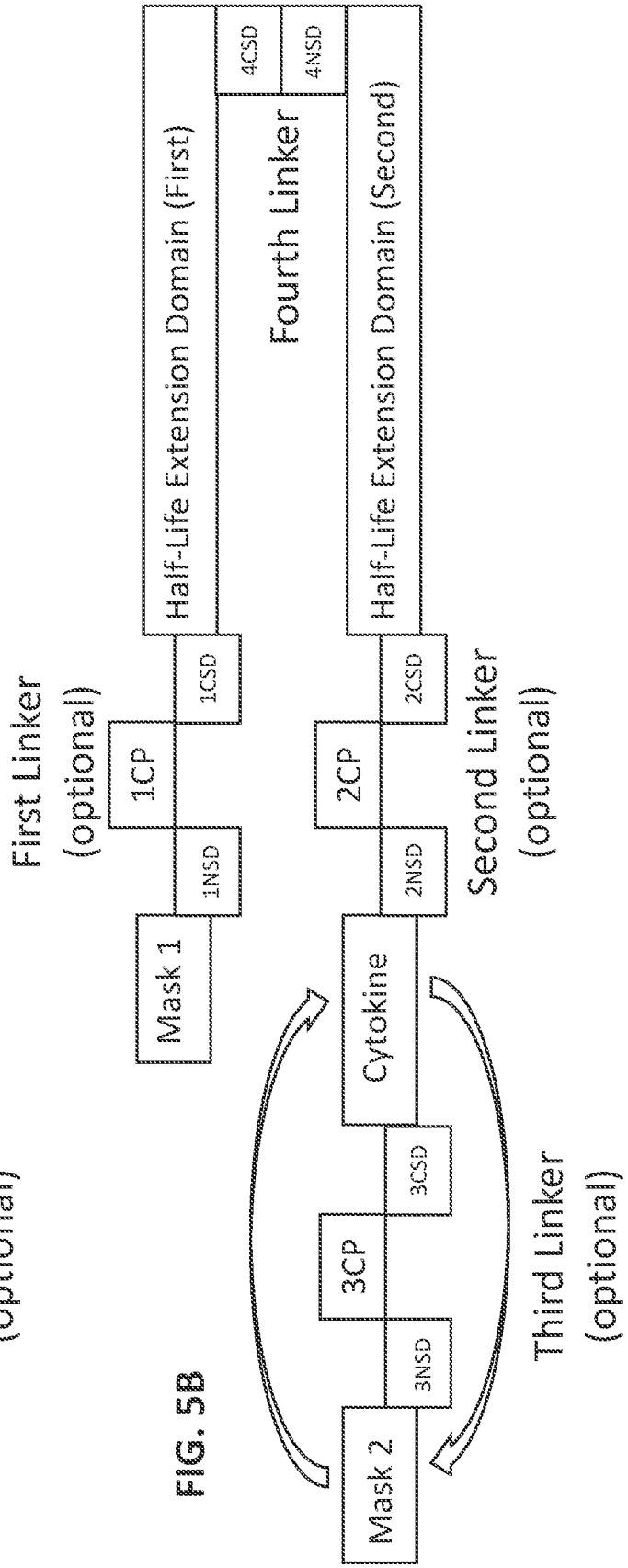

First Linker
(optional)
Mask 1
1NSD
1CP
1CSD
Half-Life Extension Domain (First)
4CSD
Fourth Linker
4NSD
Half-Life Extension Domain (Second)
2CSD
2CP
2NSD
Second Linker
(optional)
Cytokine
3CSD
3CP
3NSD
Mask 2
Third Linker
(optional)

FIG. 5B

Figure 6

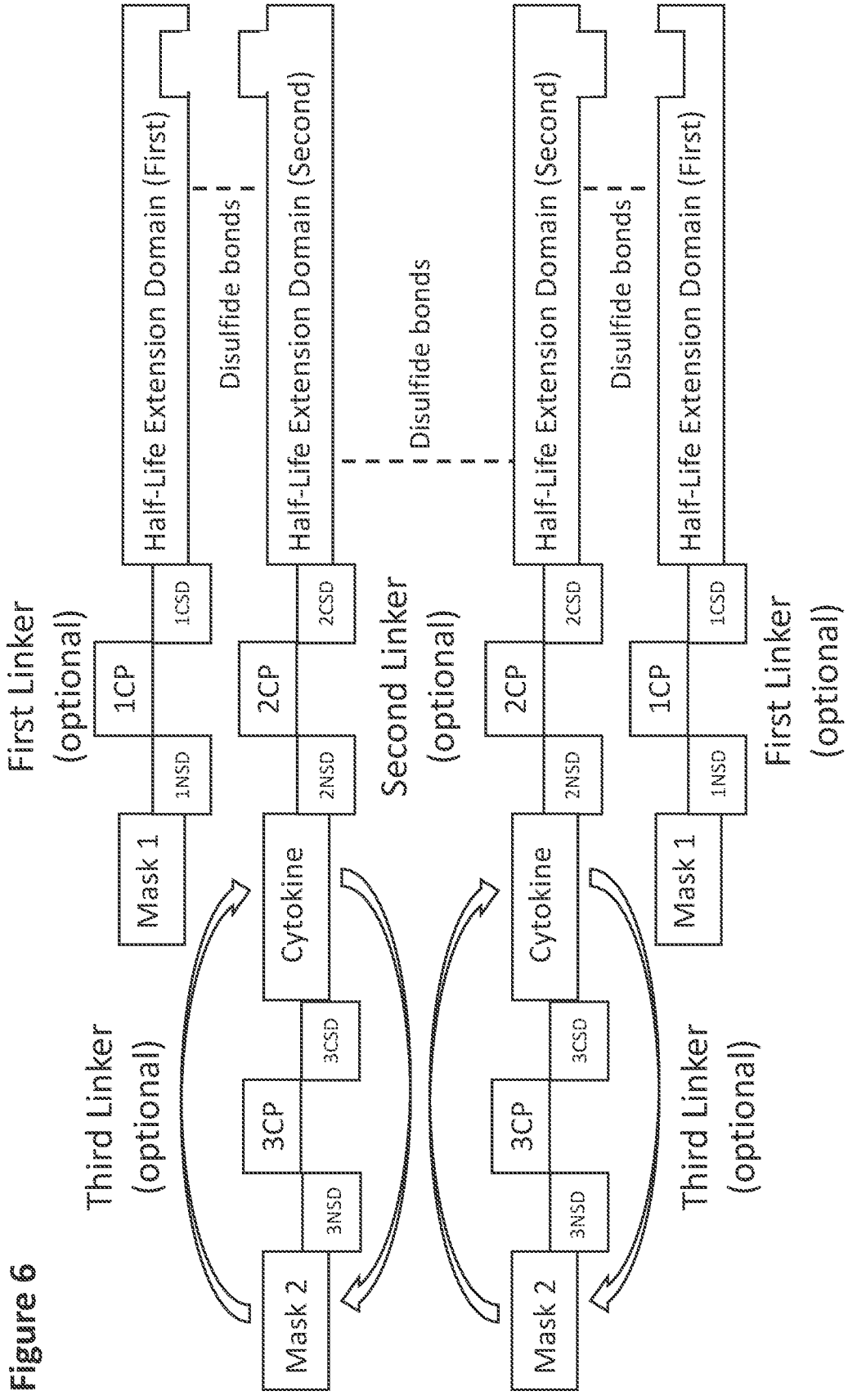

Half-Life Extension Domain (First)
Disulfide bonds
Half-Life Extension Domain (Second)
Disulfide bonds
Half-Life Extension Domain (Second)
Disulfide bonds
Half-Life Extension Domain (First)
First Linker (optional)
1CP
1CSD
1NSD
Mask 1
2CP
2CSD
2NSD
Cytokine
3CP
3CSD
3NSD
Mask 2
Second Linker (optional)
Third Linker (optional)
Third Linker (optional)
First Linker (optional)

FIG. 7A

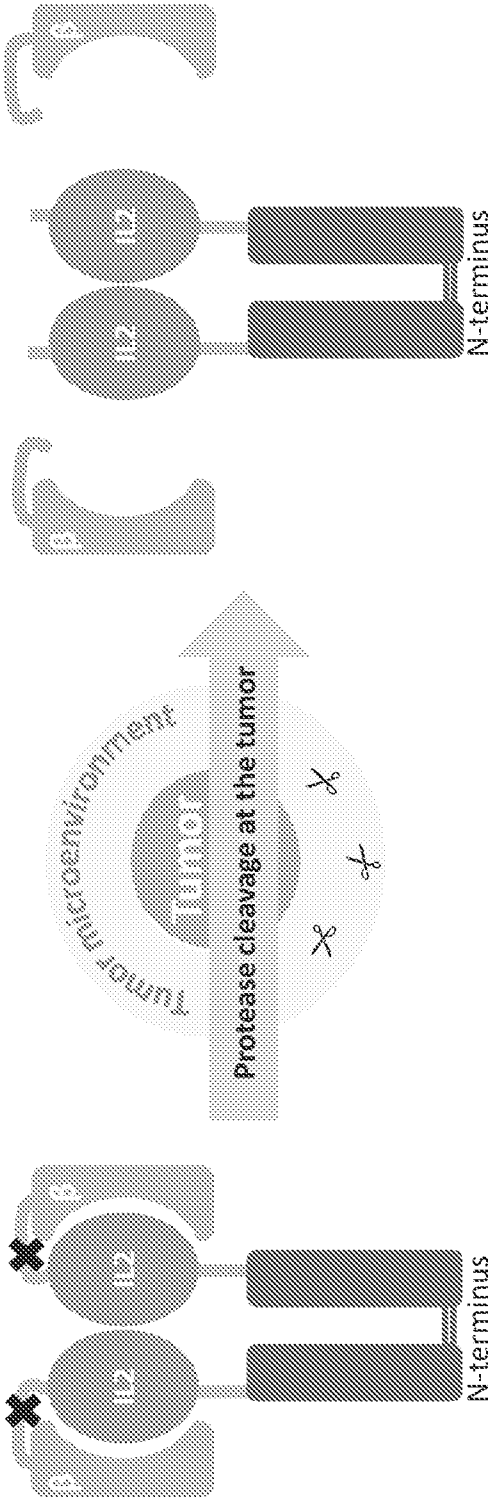

Protease cleavage at the tumor
Tumor microenvironment
Tumor
N-terminus

FIG. 7B

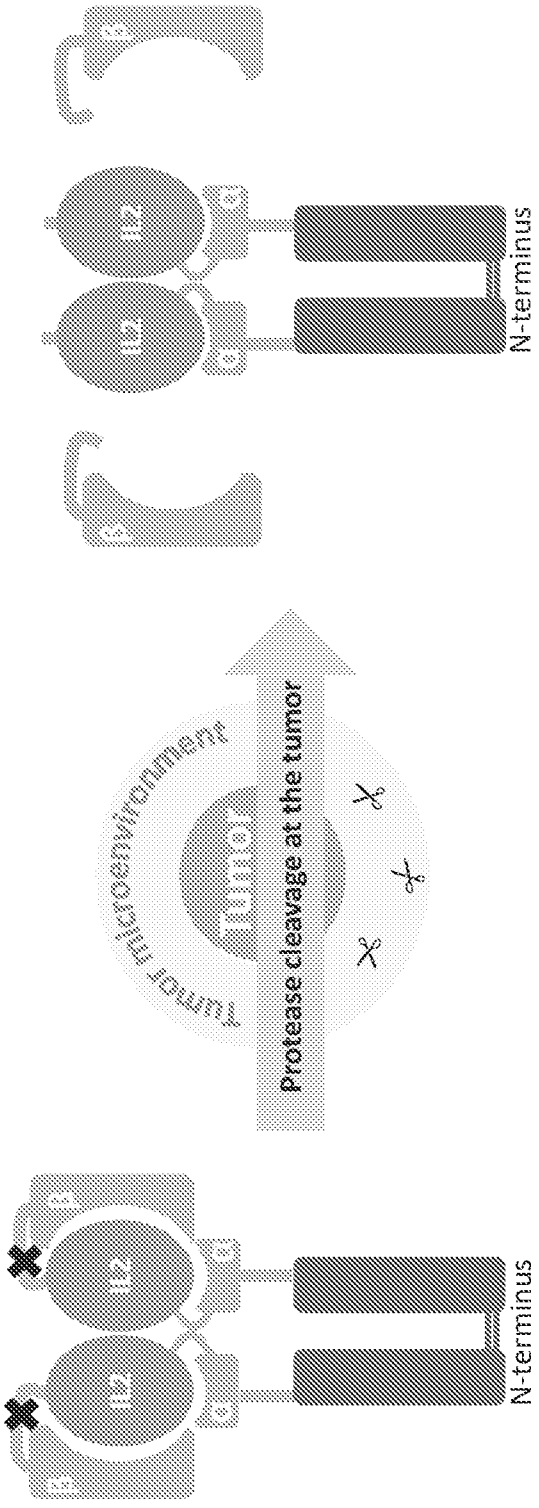

Protease cleavage at the tumor
Tumor microenvironment
Tumor
N-terminus

FIG. 7C

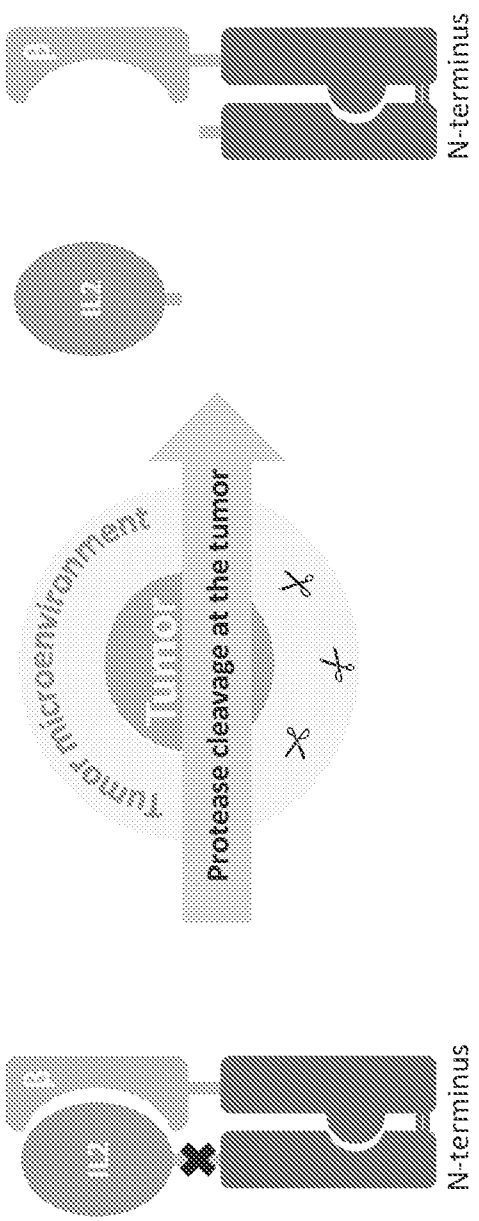
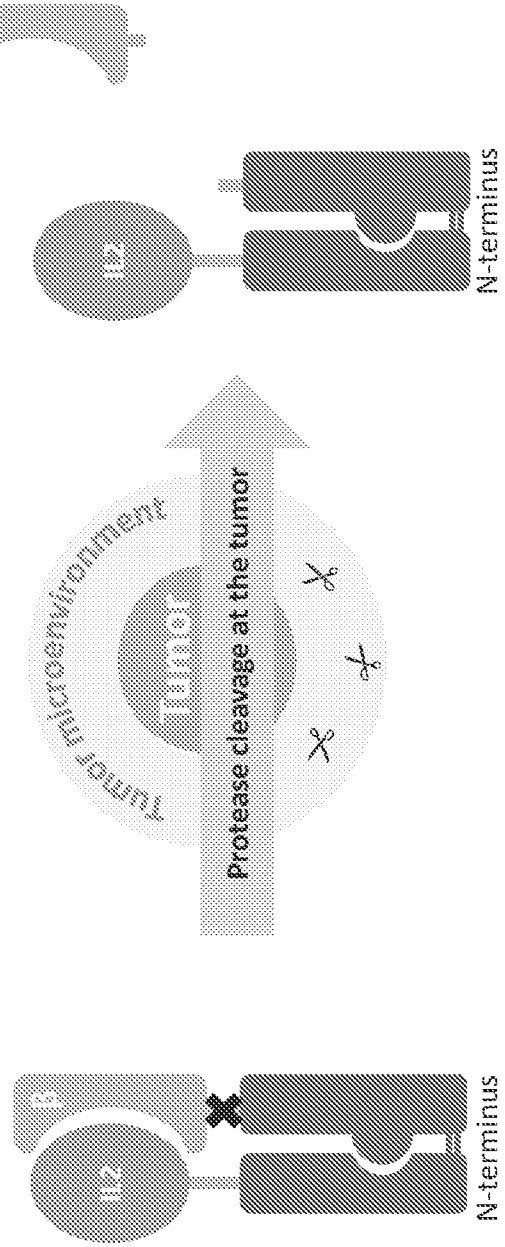

T1
T2
N-terminus
T1
T2
N-terminus
Protease cleavage at the tumor
Tumor microenvironment
Tumor

FIG. 7D


T1
T2
N-terminus
T1
T2
N-terminus
Protease cleavage at the tumor
Tumor microenvironment
Tumor

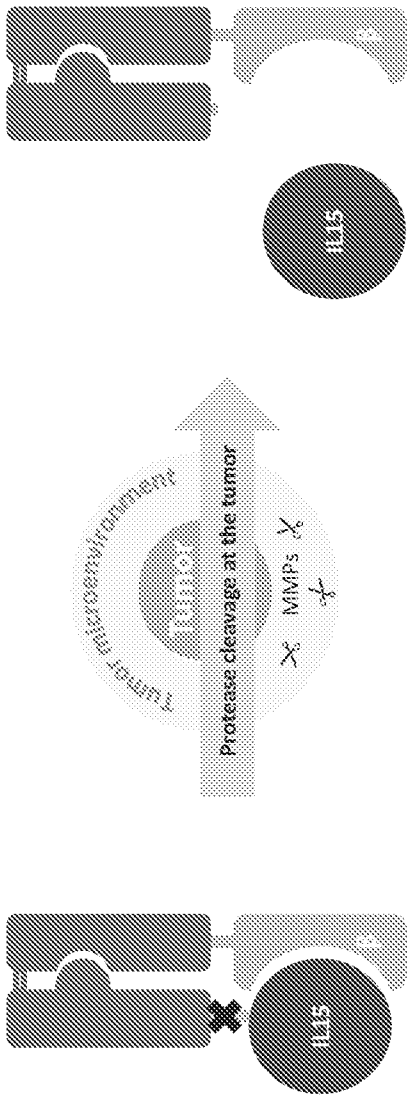

IL15
Protease cleavage at the tumor
Tumor microenvironment
Tumor
MMPs
IL15

FIG. 7E

Figure 8

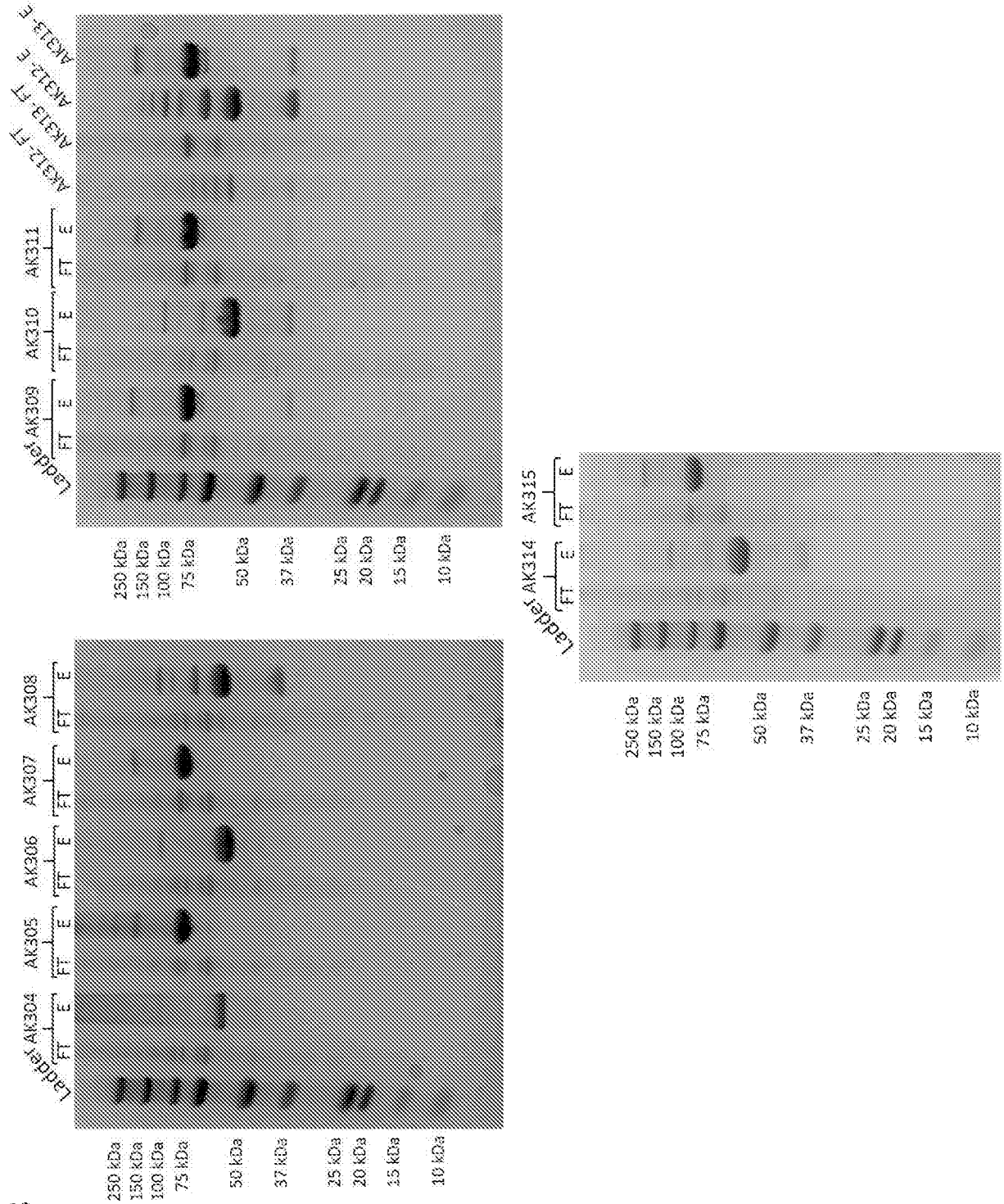

Ladder
AK304
FT
E
AK305
FT
E
AK306
FT
E
AK307
FT
E
AK308
FT
E
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Ladder
AK309
FT
E
AK310
FT
E
AK311
FT
E
AK312-FT
AK313-FT
AK312-E
AK313-E
Ladder
AK314
FT
E
AK315
FT
E

FIG. 9A

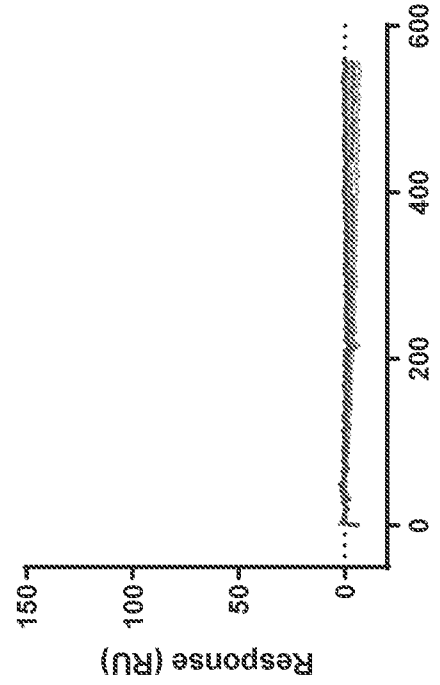


FIG. 9B

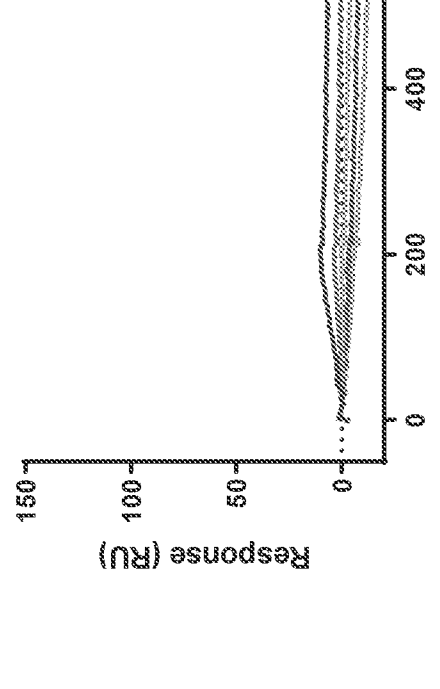


FIG. 9C

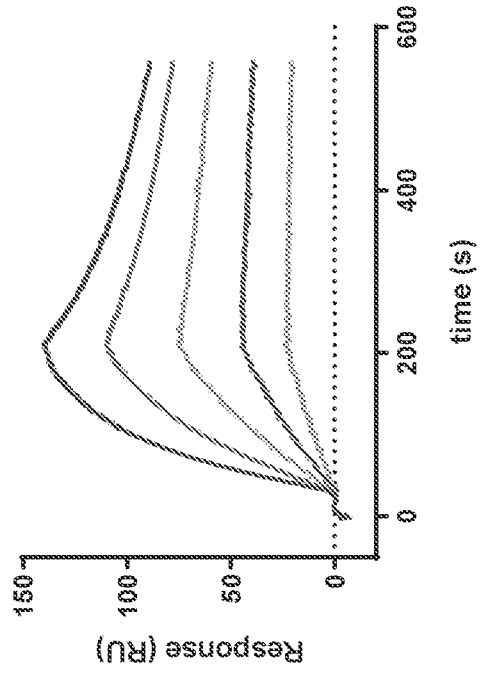


FIG. 9D

Sample	ka (1/(M*s))	kd (1/s)	KD	Chi2	U-value: kd (%)
AK215	ND	ND	ND	ND	ND
AK216	ND	ND	ND	ND	ND
rhIL2	8.61e+5	1.04e-3	1.21 nM	4.86	1.2

FIG. 10A

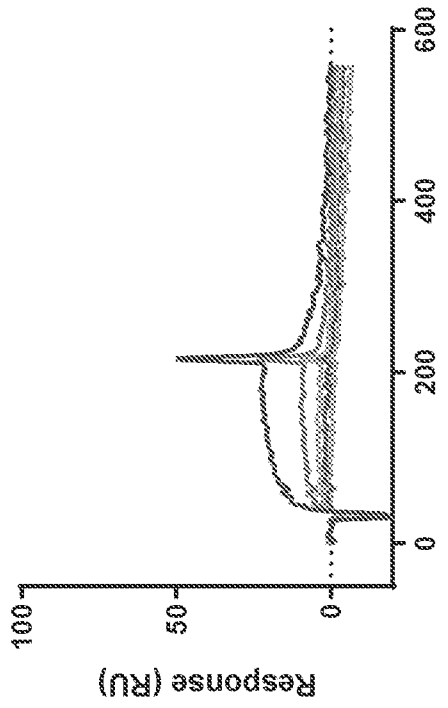


FIG. 10B

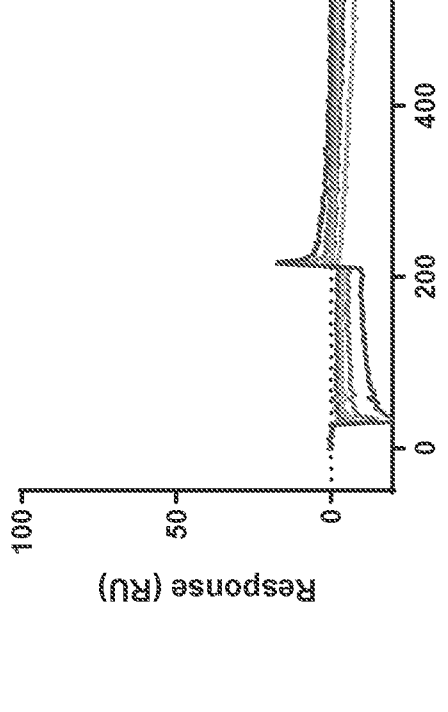


FIG. 10C

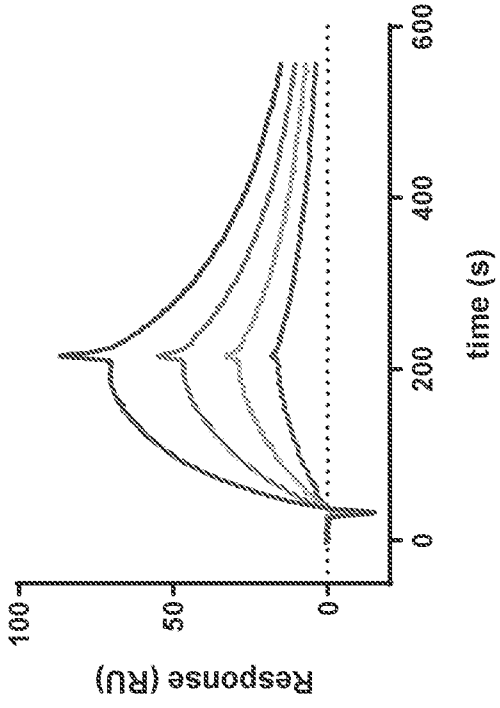


FIG. 10D

Sample	ka (1/(M*s))	kd (1/s)	KD	Chi2	U-value: kd (%)
AK216	ND	ND	ND	ND	ND
AK218	ND	ND	ND	ND	ND
rhIL2	3.95e+4	4.83e-3	122 nM	2.06	1.00

FIG. 11A

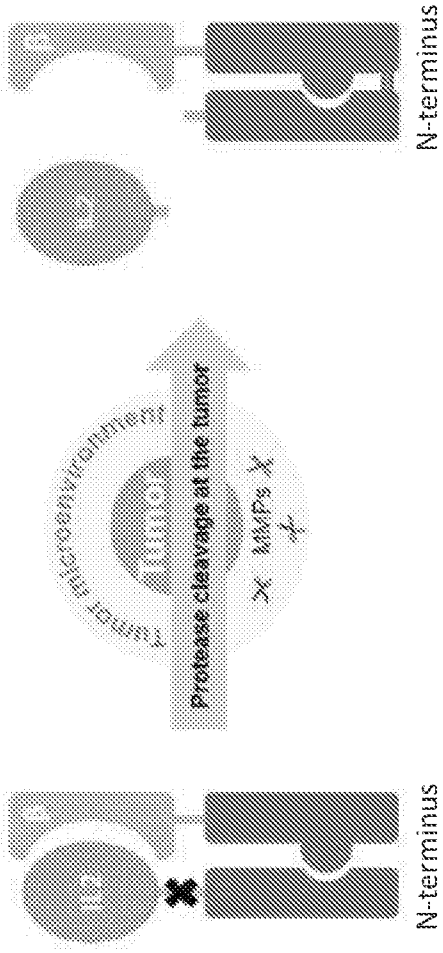
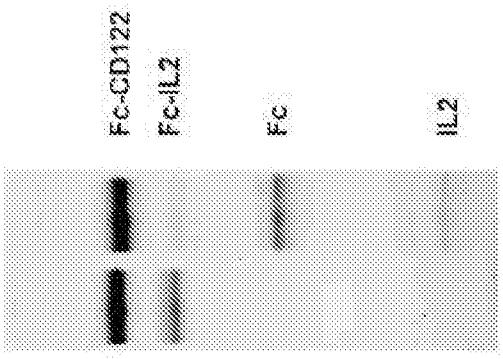


FIG. 11B



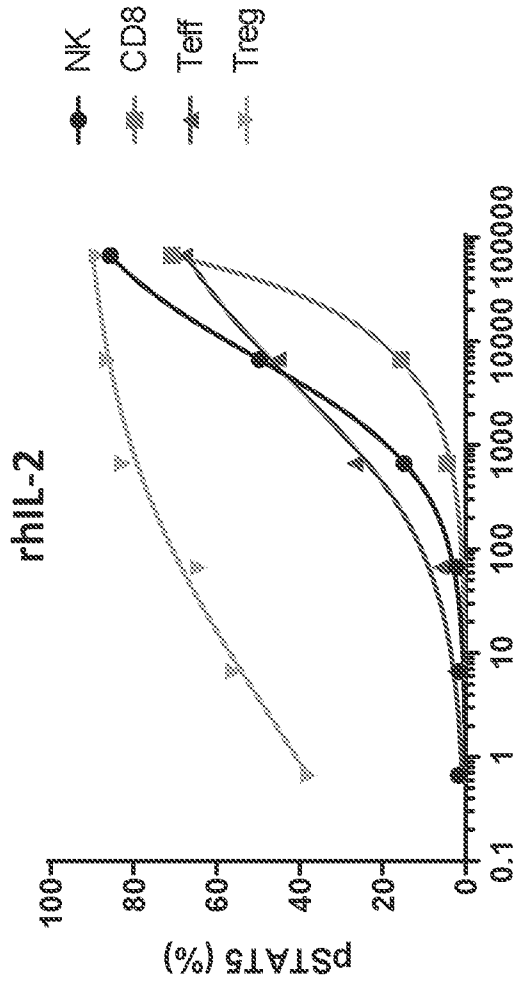


FIG. 12A

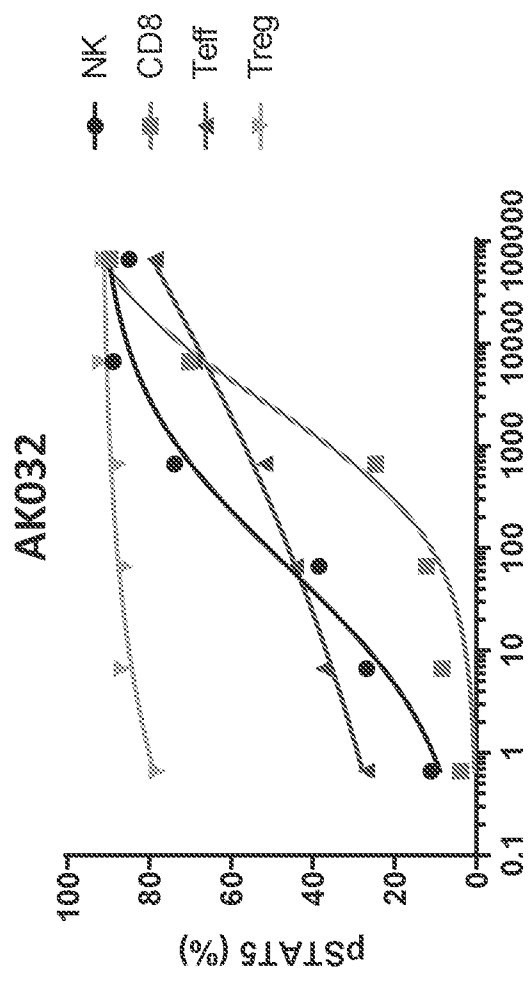


FIG. 12B

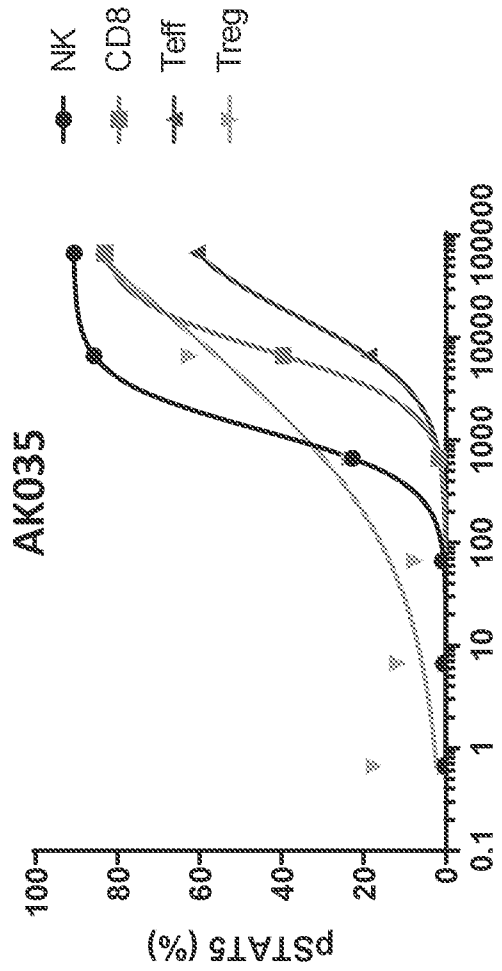


FIG. 12C

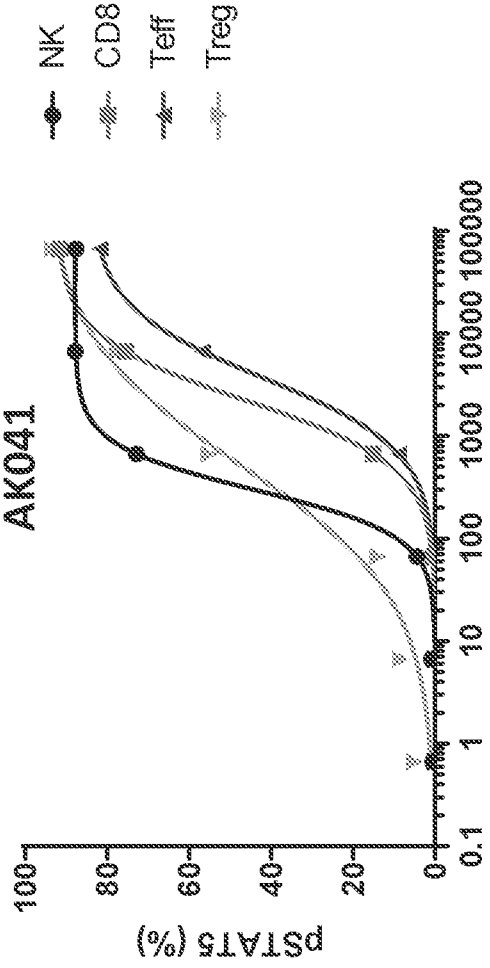


FIG. 12D

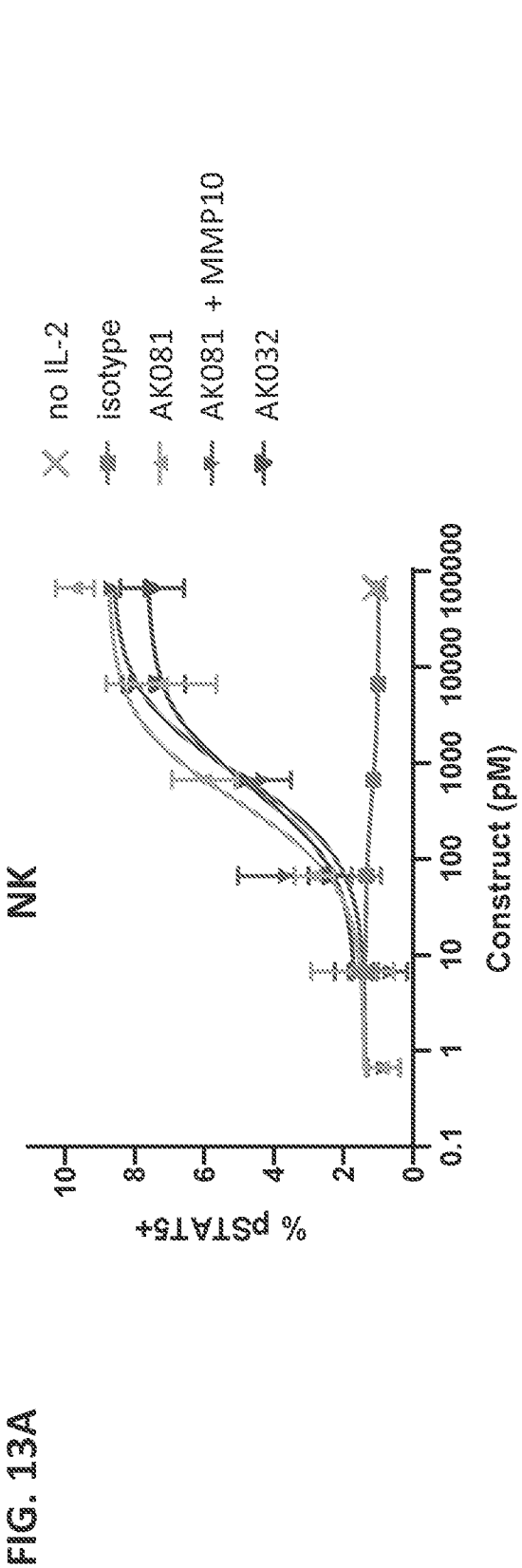


FIG. 13A

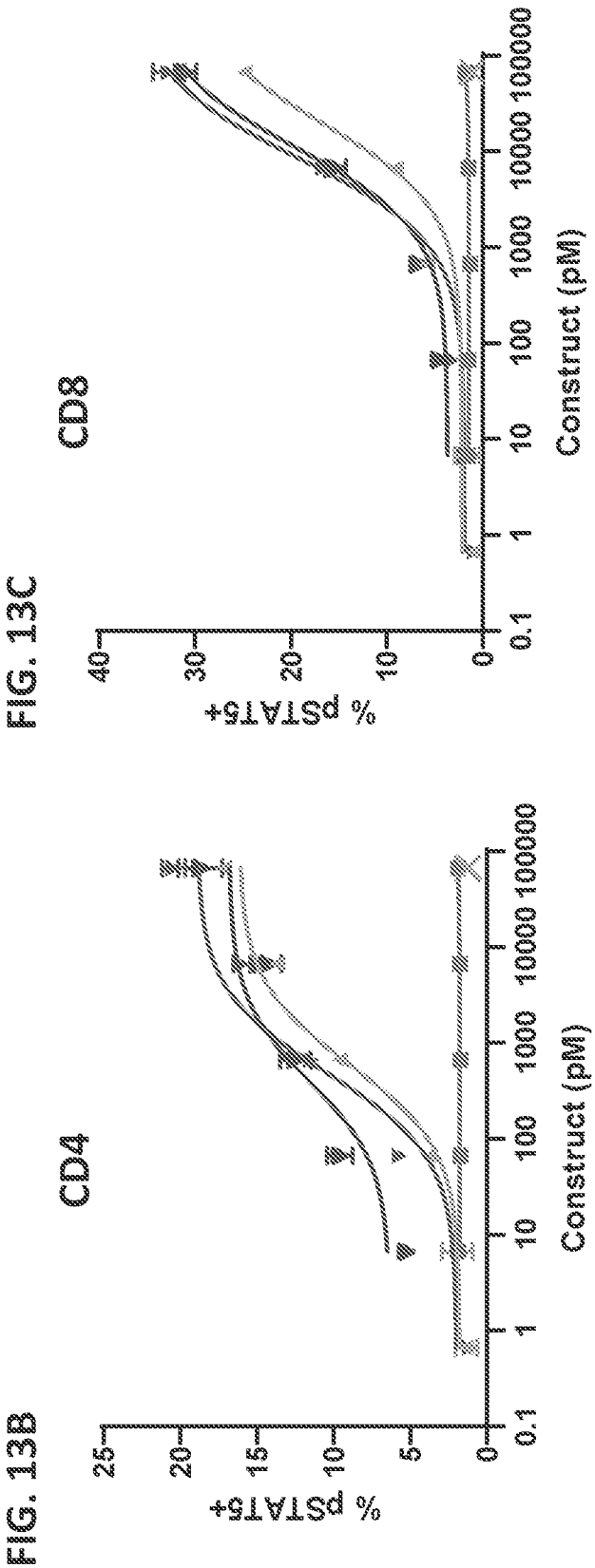


FIG. 13C

FIG. 13B

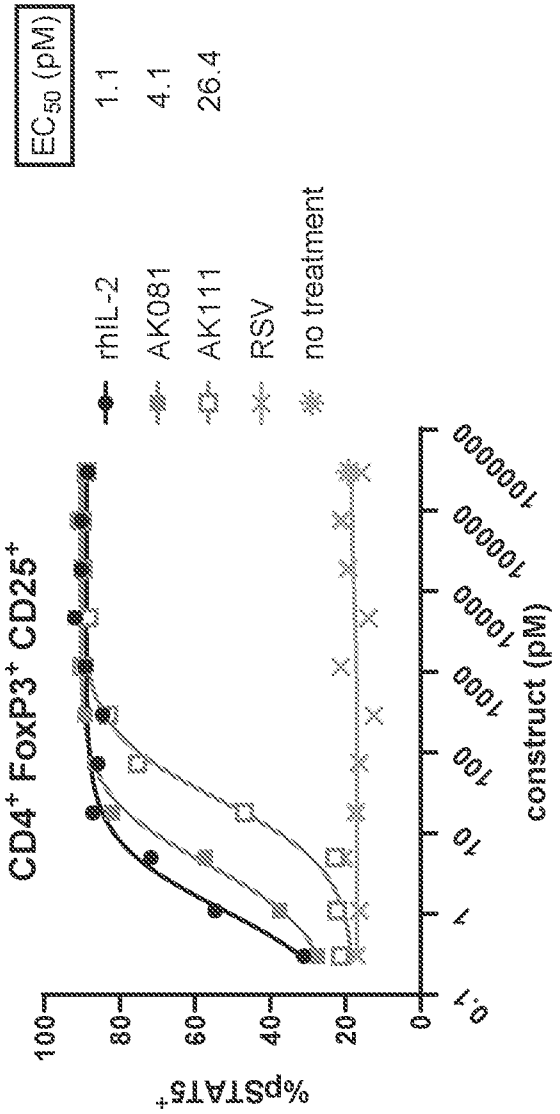


FIG. 14A

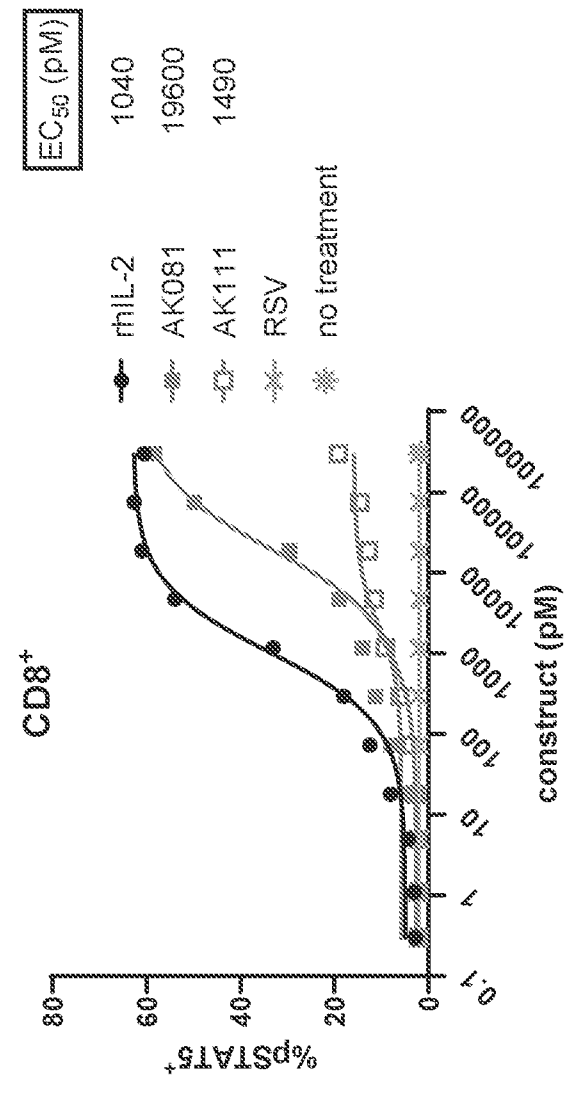


FIG. 14B

FIG. 14C

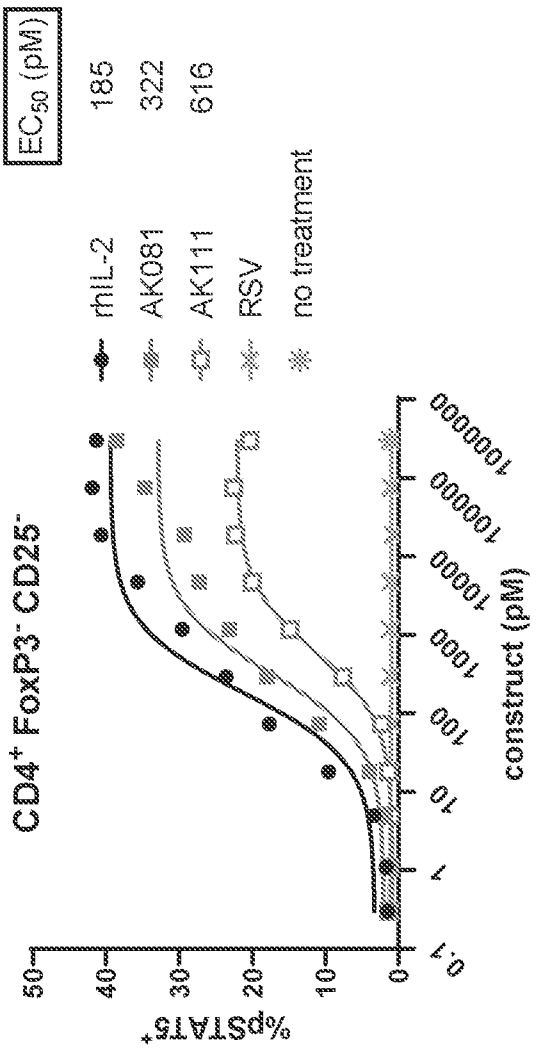


FIG. 14D

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.*

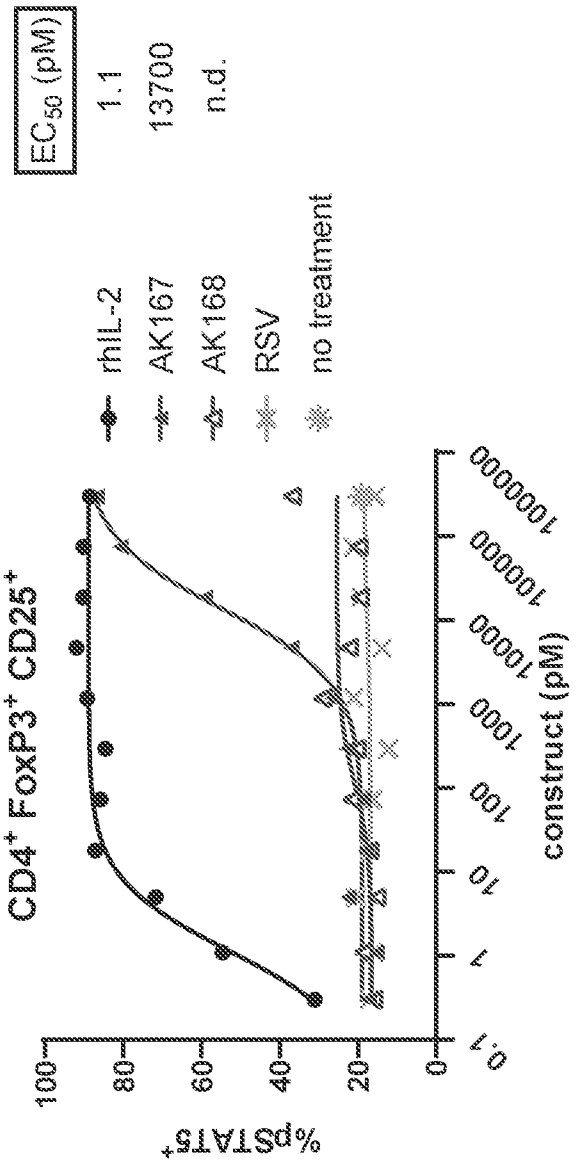


FIG. 15A

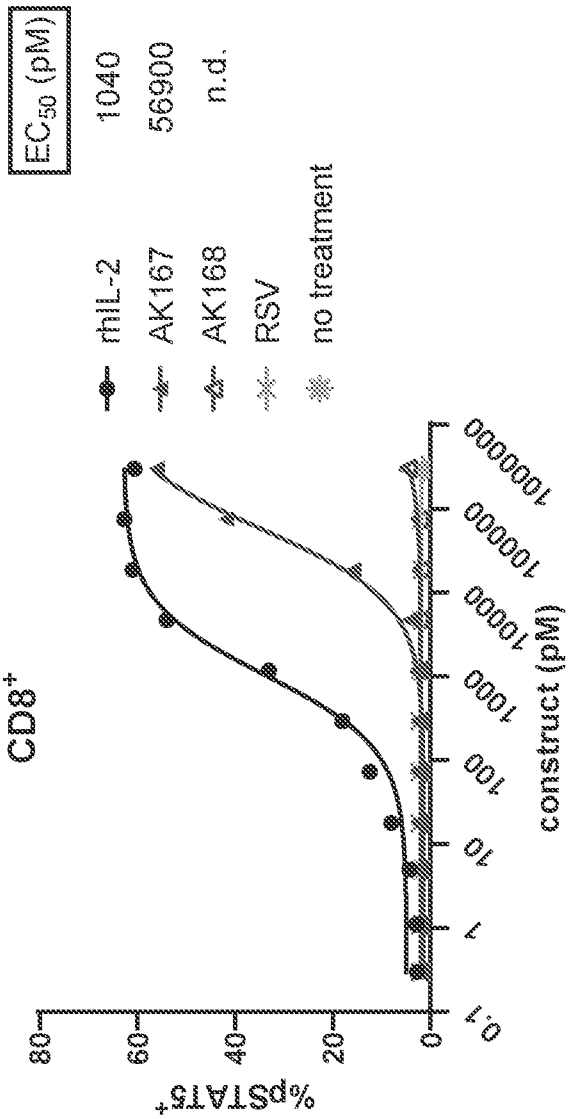


FIG. 15B

FIG. 15C

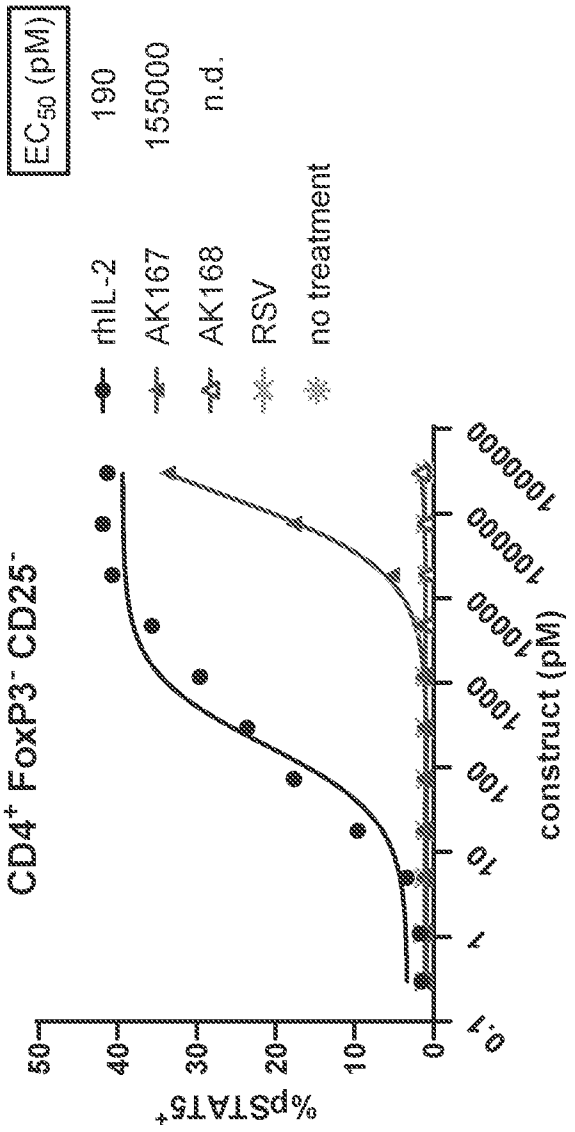


FIG. 15D

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		190	
AK167	13700	12455	56900	55	155000	816
AK168	*n.d.*		*n.d.*		*n.d.*	

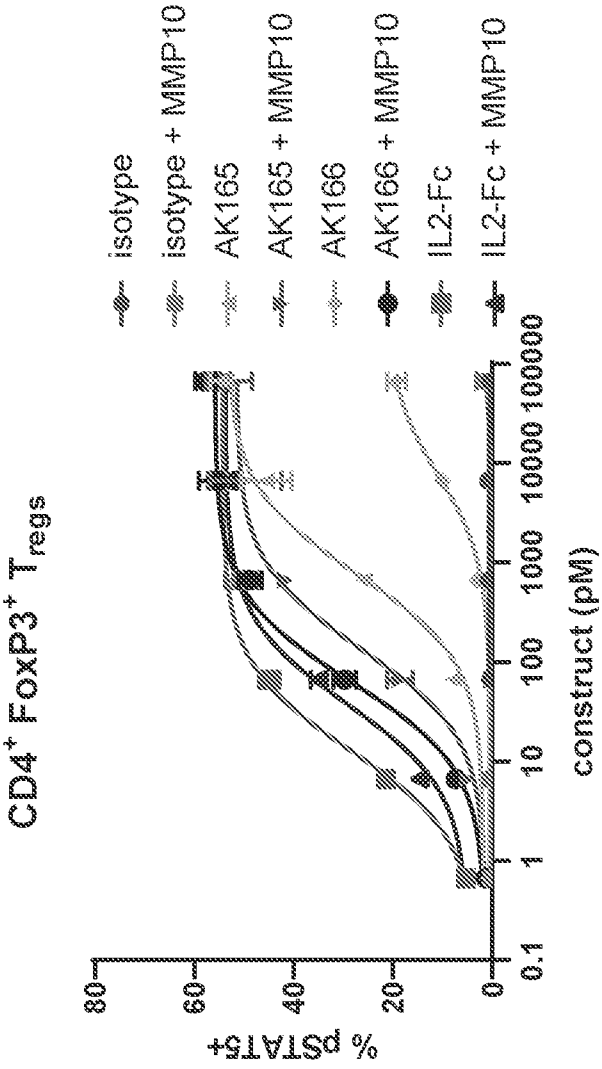


FIG. 16A

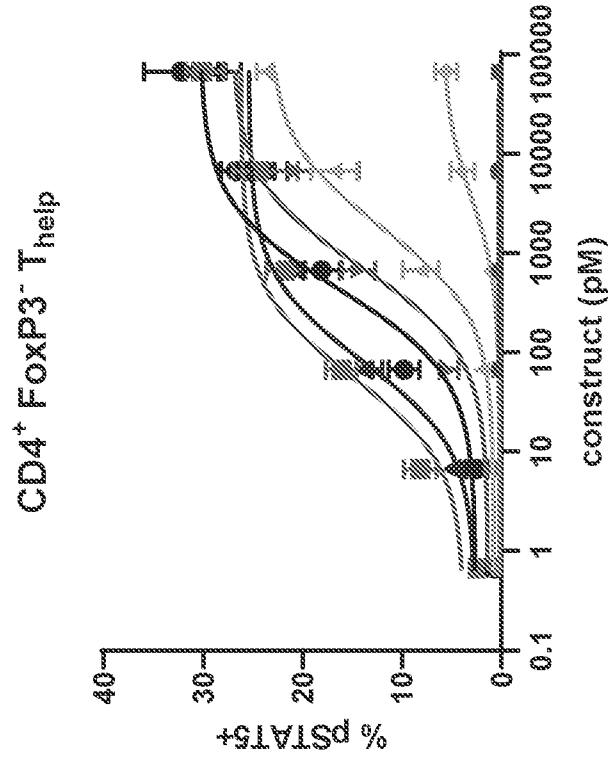


FIG. 16B

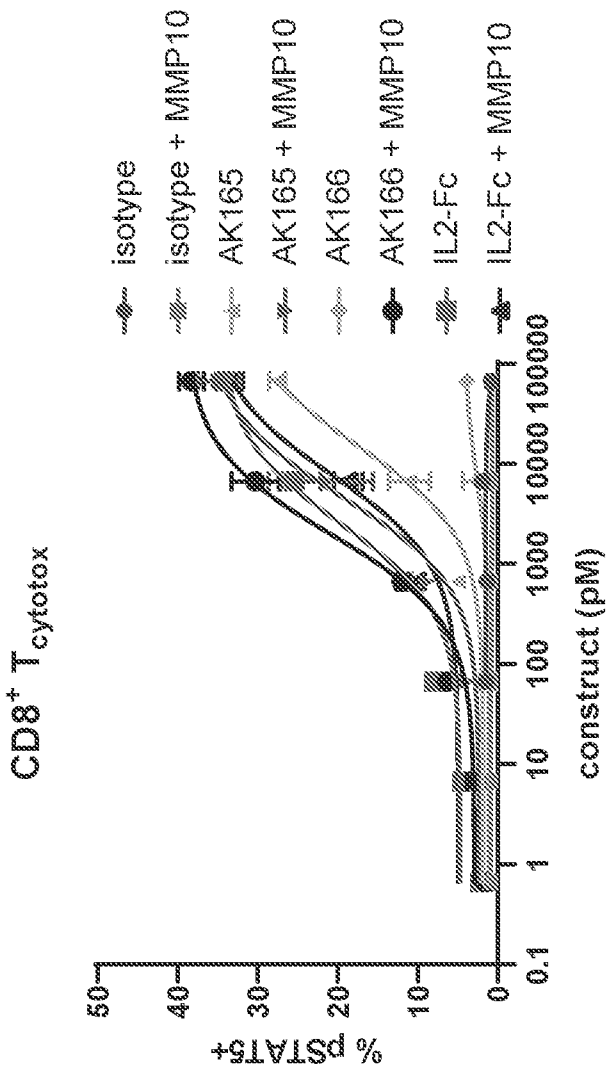


FIG. 16C

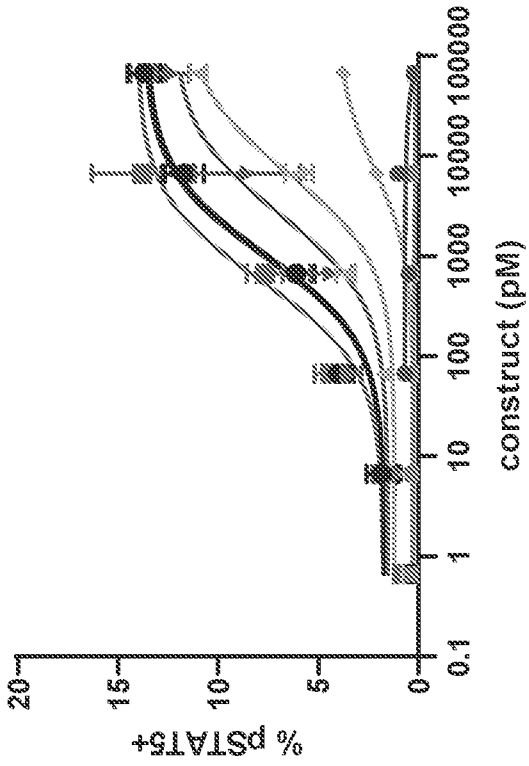


FIG. 16D

FIG. 17A

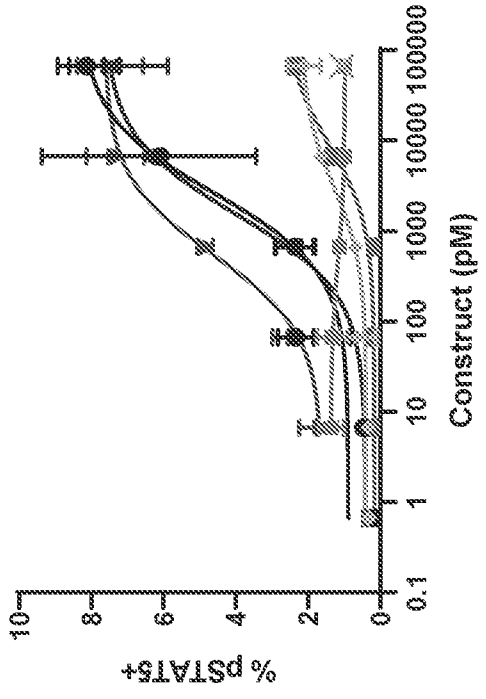


FIG. 17B

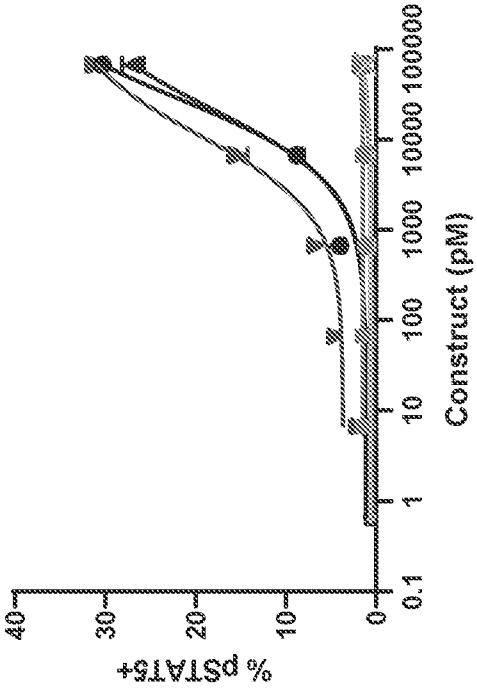
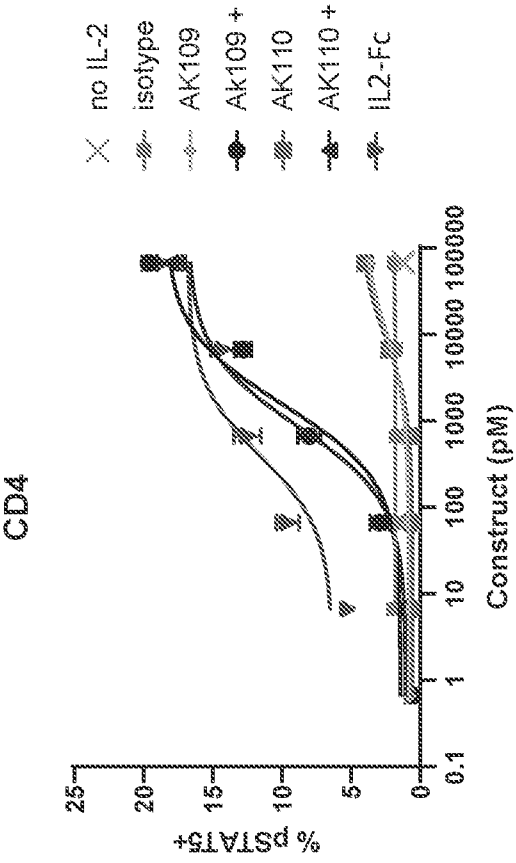


FIG. 17C



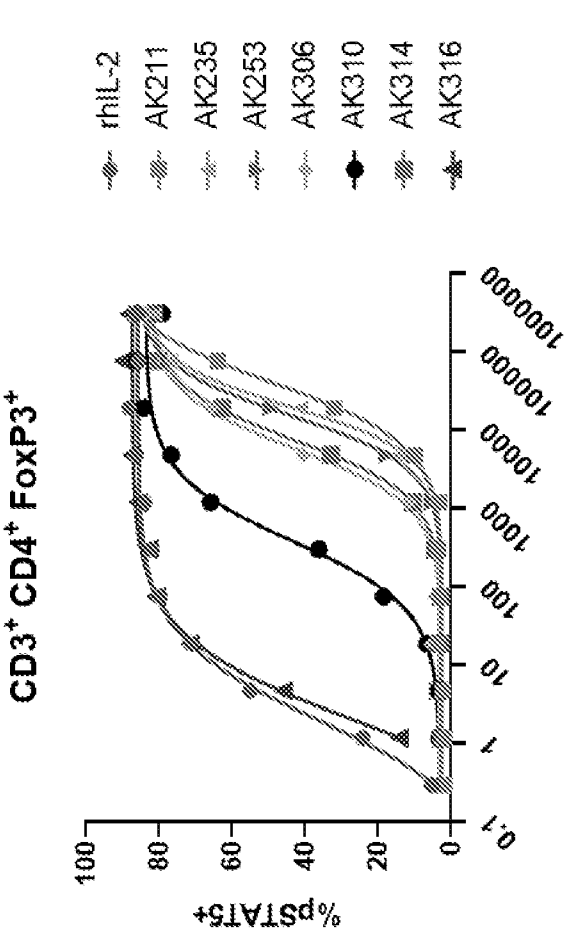


FIG. 18A

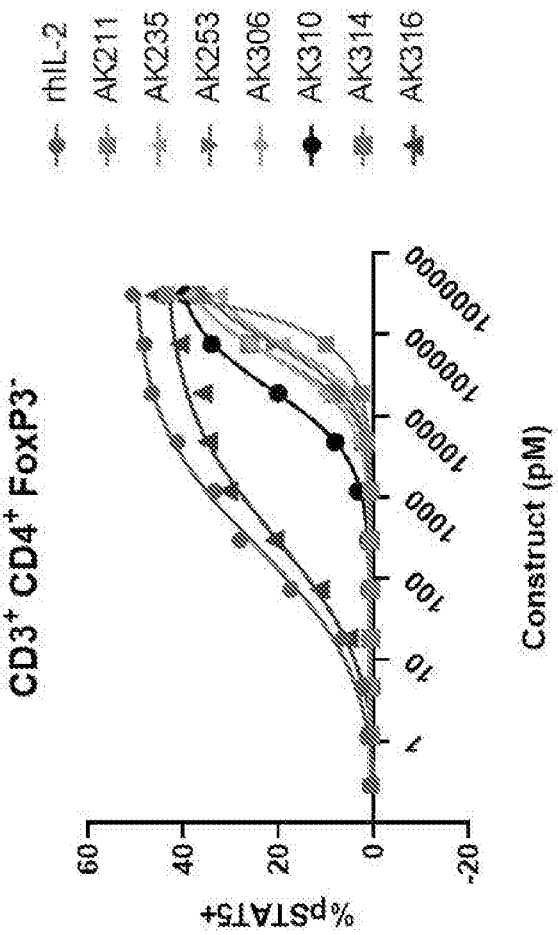


FIG. 18B

FIG. 18C

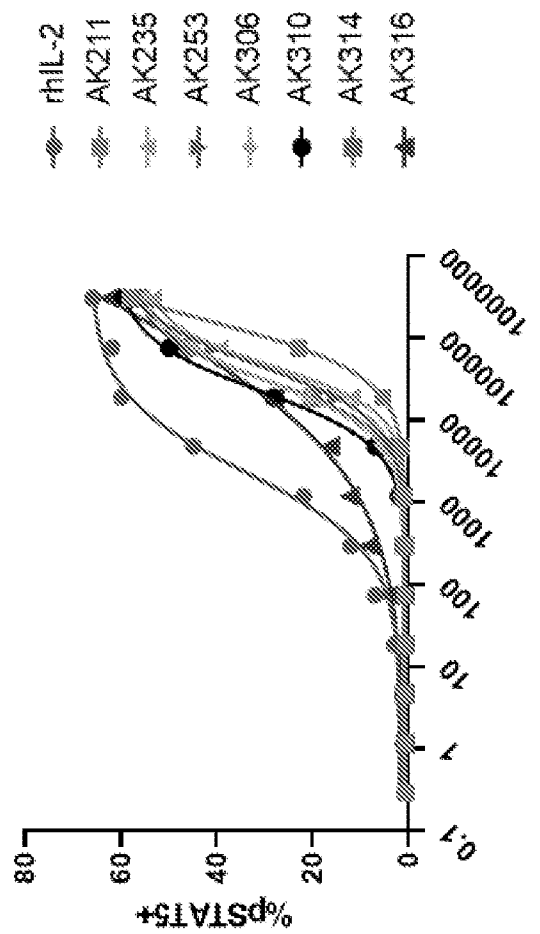


FIG. 18D

	CD3+ CD4+ FoxP3+	CD3+ CD4+ FoxP3-	CD3+ CD8+
Construct	EC_{50} (pM)	EC_{50} (pM)	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**

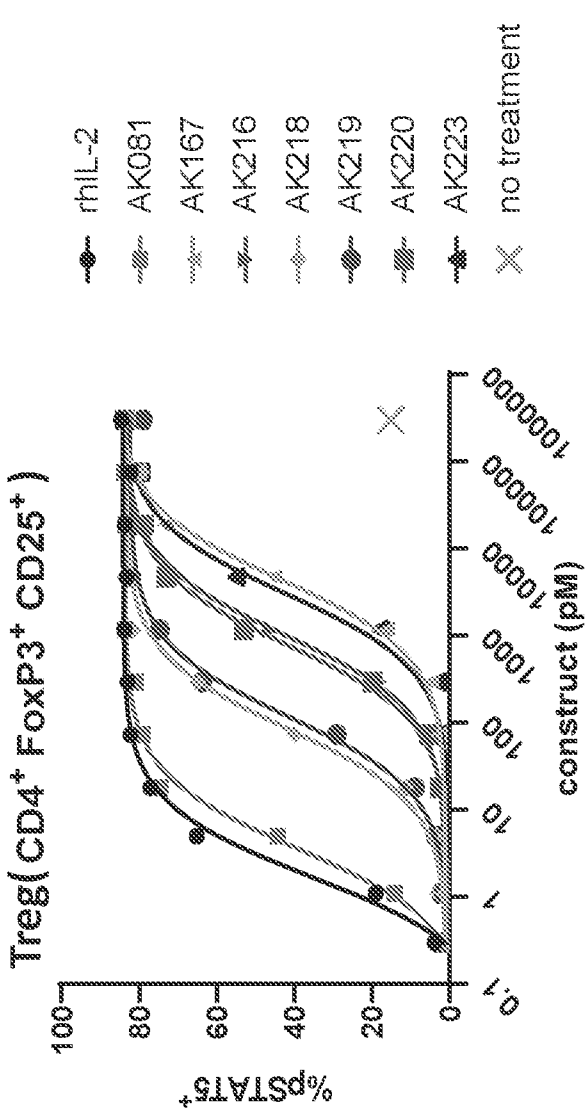


FIG. 19A

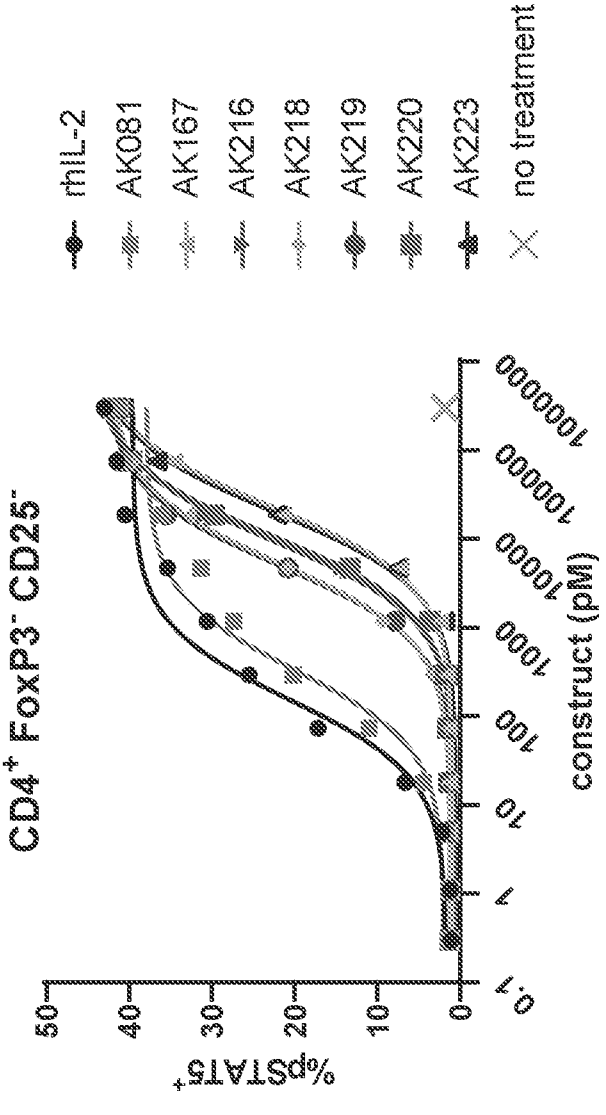


FIG. 19B

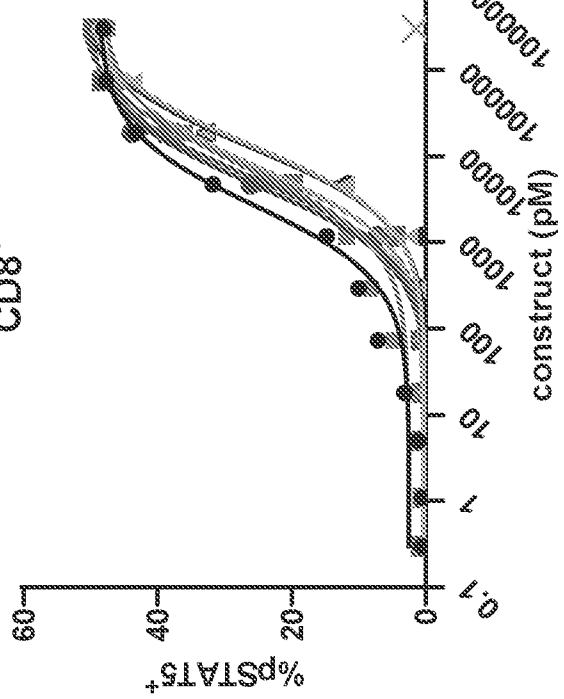


FIG. 19C

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. 19D

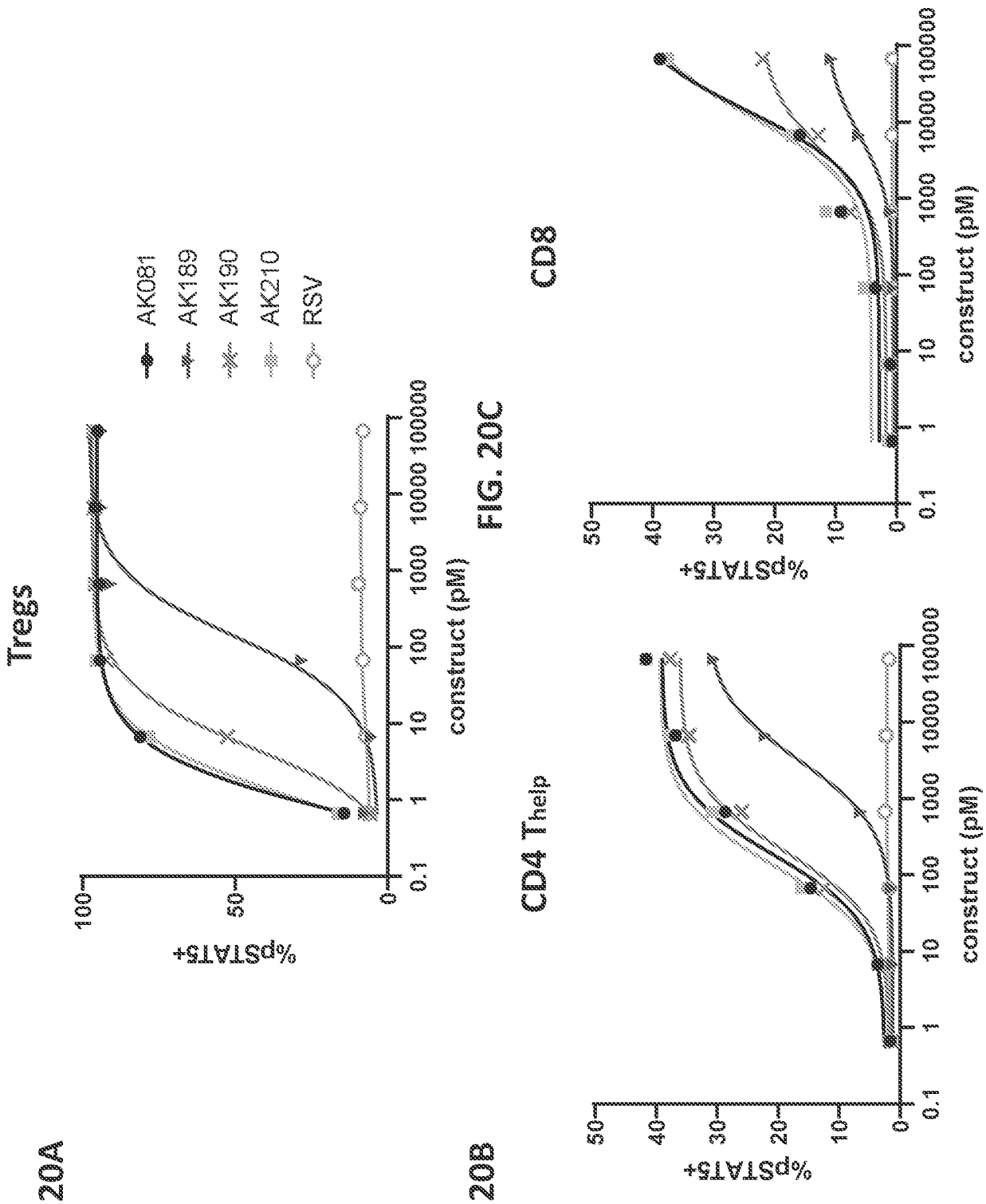


FIG. 20A

FIG. 20B

FIG. 20C

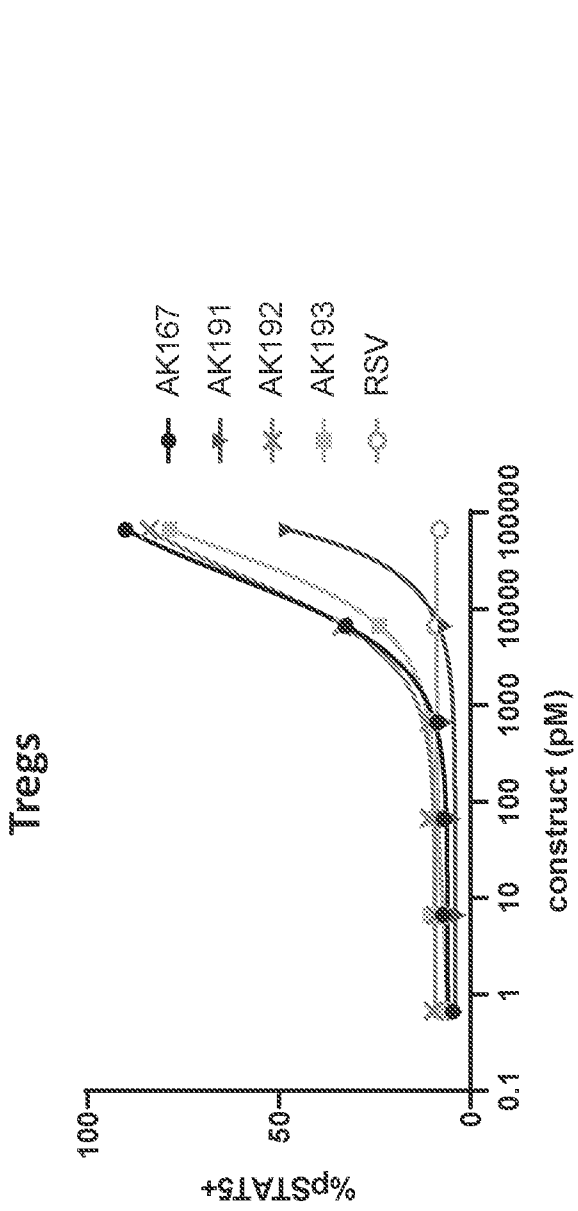


FIG. 21A

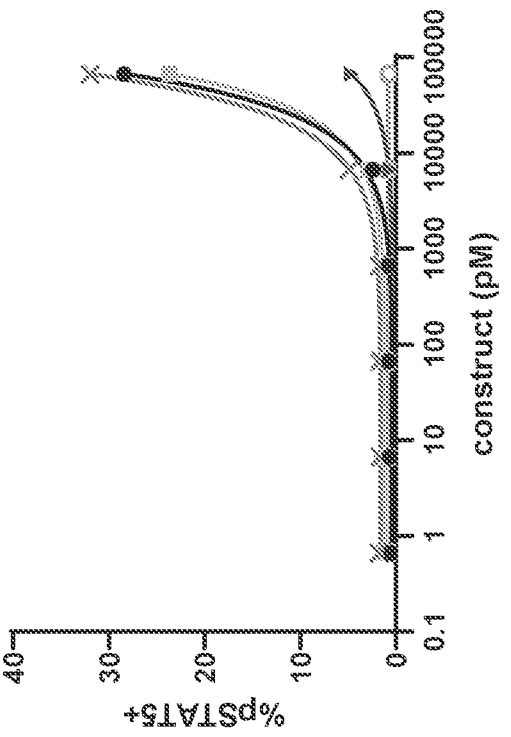


FIG. 21C

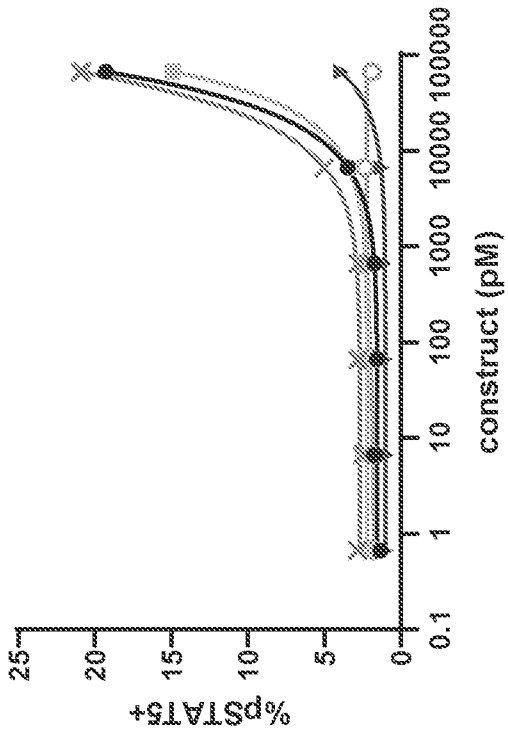


FIG. 21B

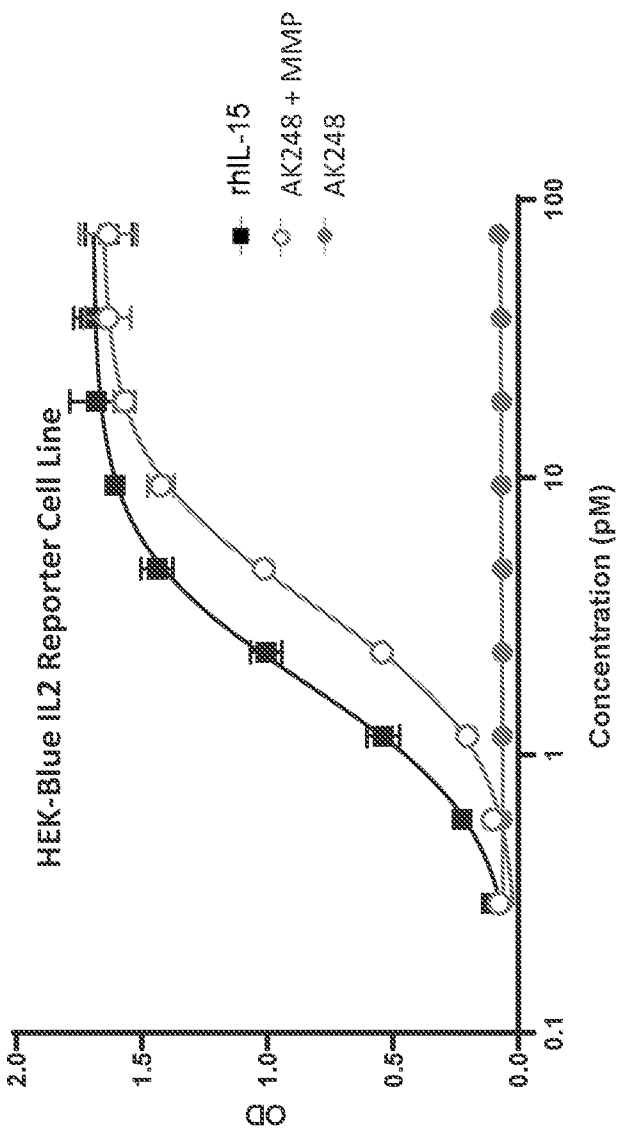


FIG. 22A

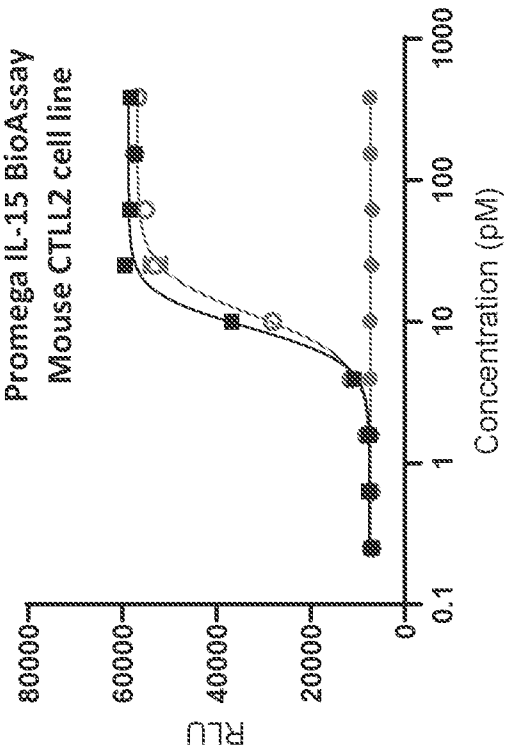


FIG. 22B

FIG. 23A

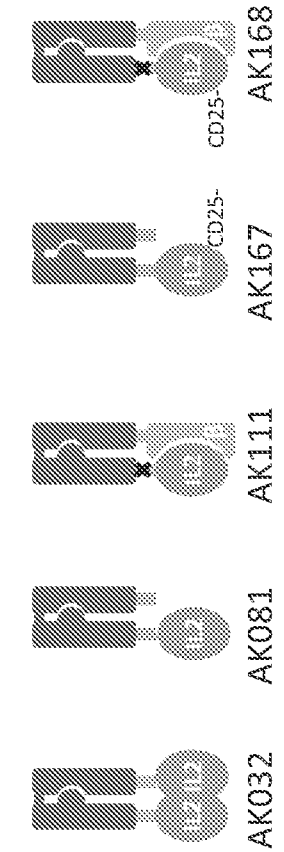


FIG. 23B

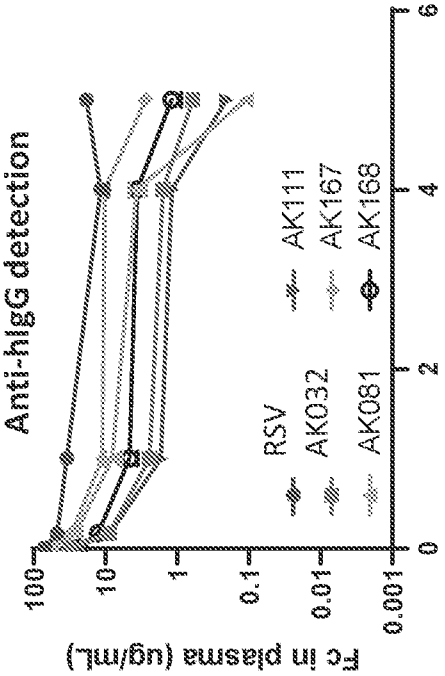


FIG. 23C

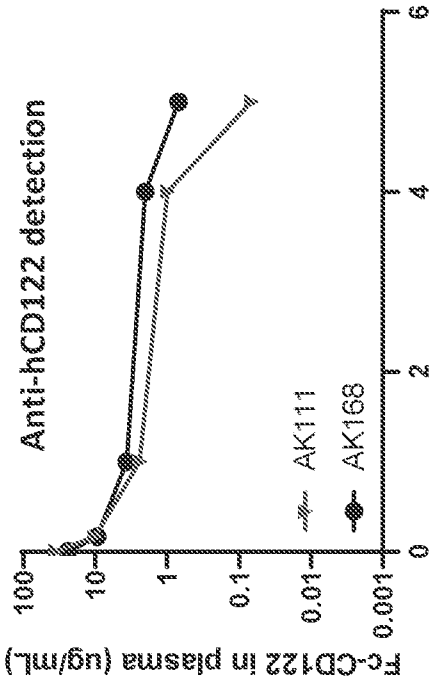


FIG. 23D

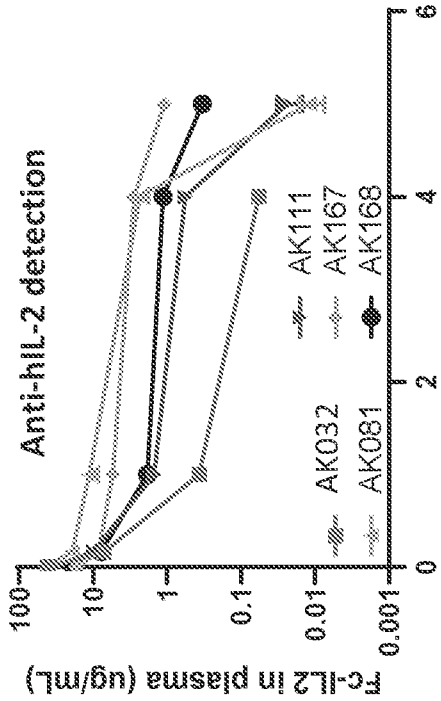


FIG. 24A

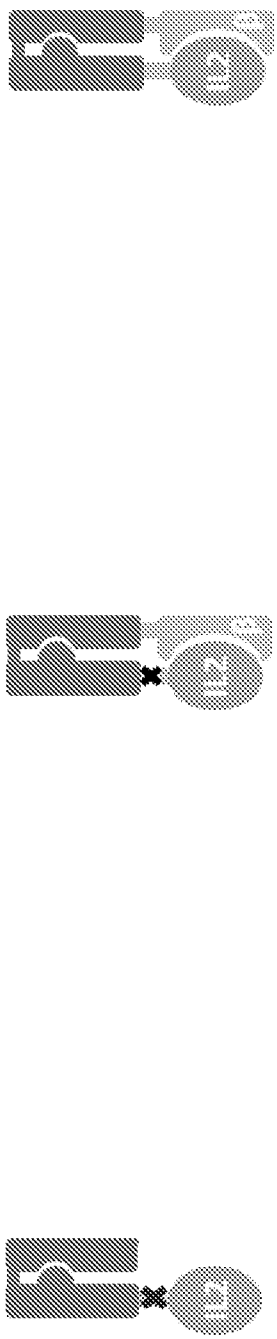
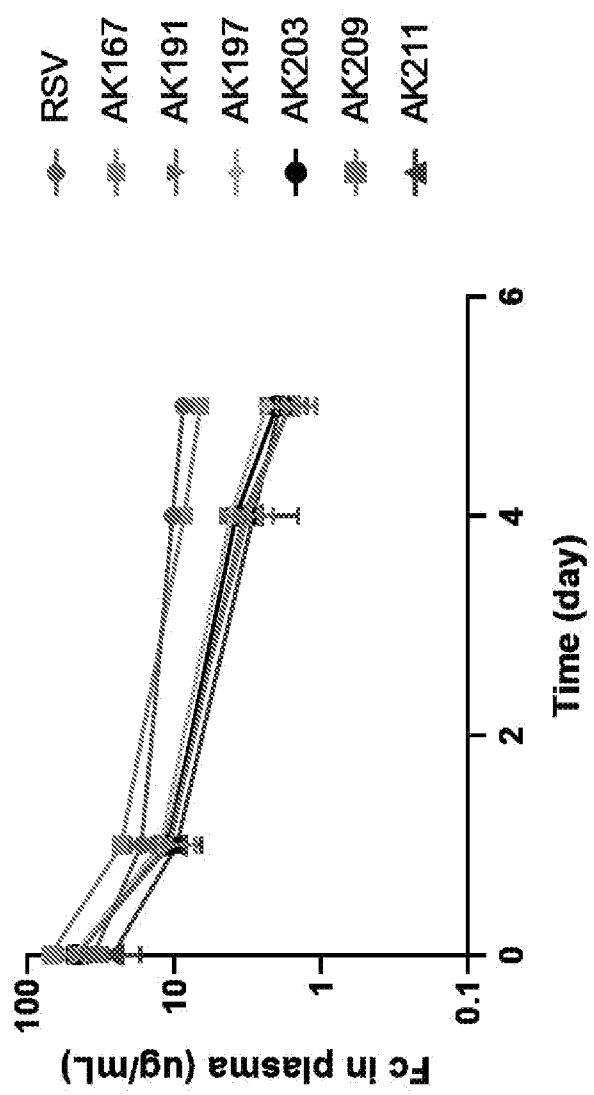


FIG. 24B



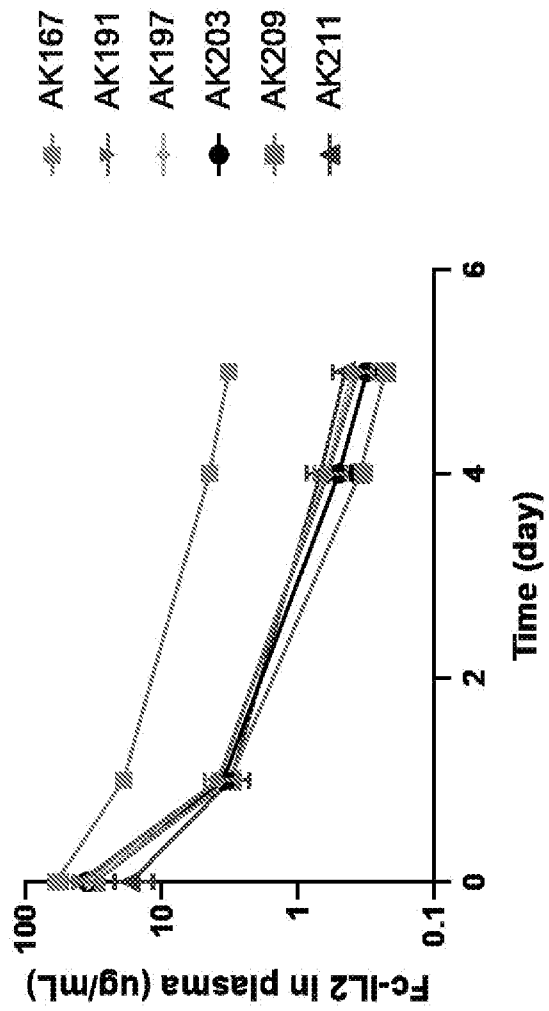


FIG. 24C

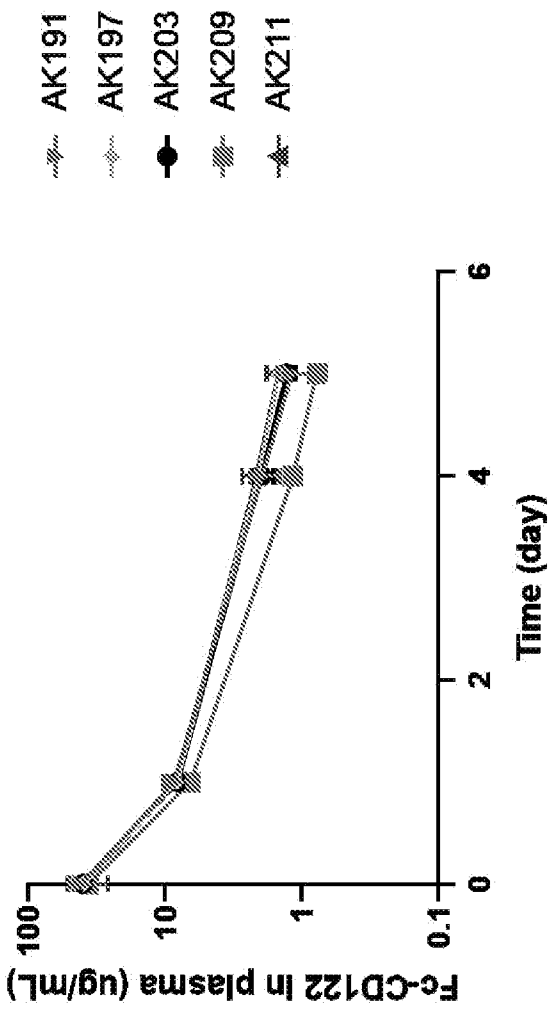


FIG. 24D

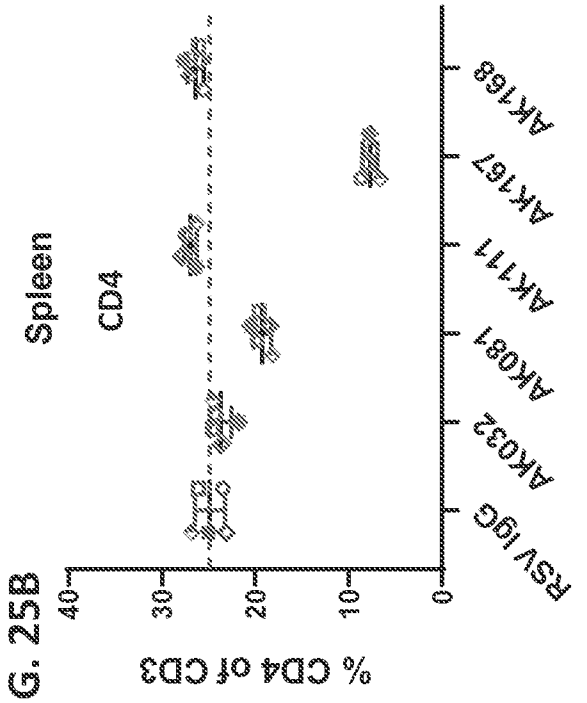


FIG. 25B

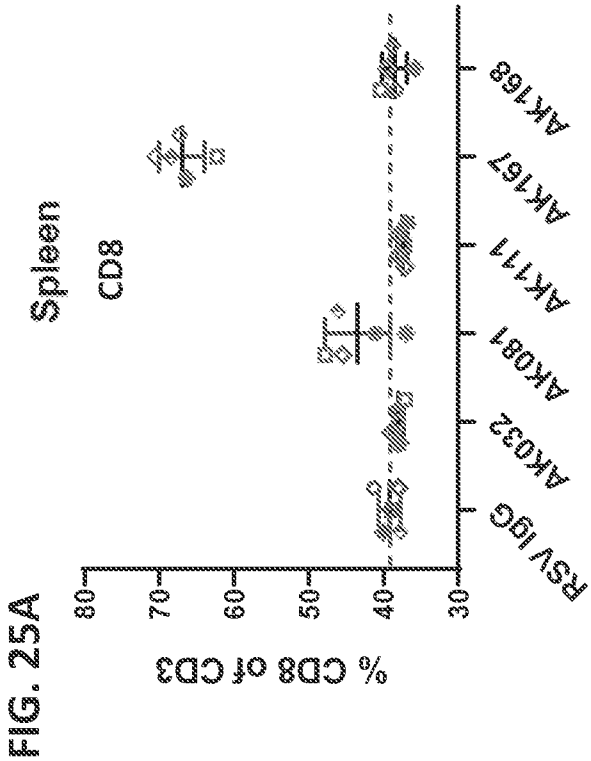


FIG. 25A

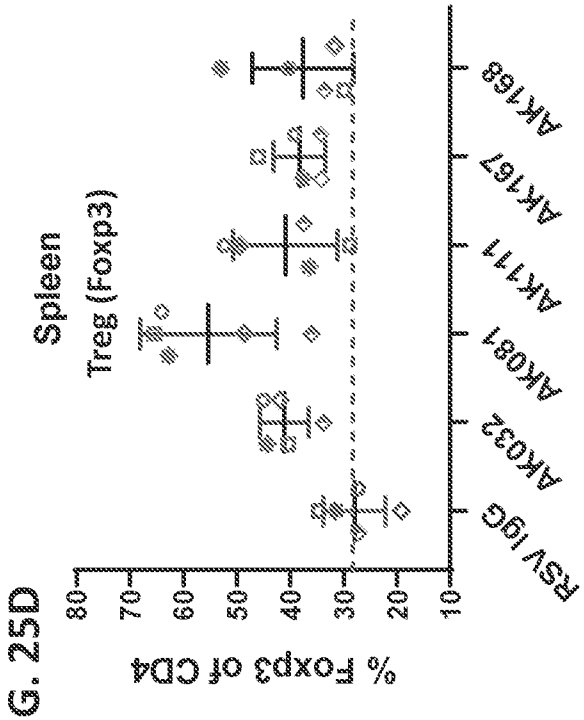


FIG. 25D

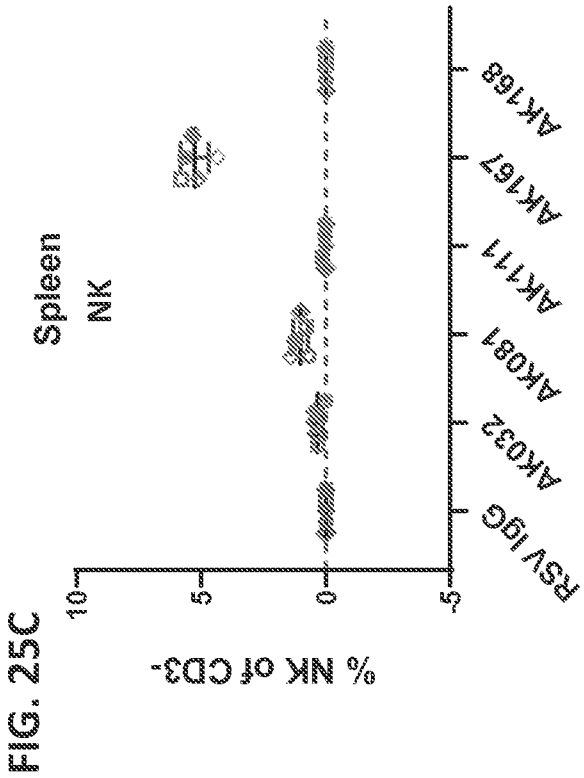


FIG. 25C

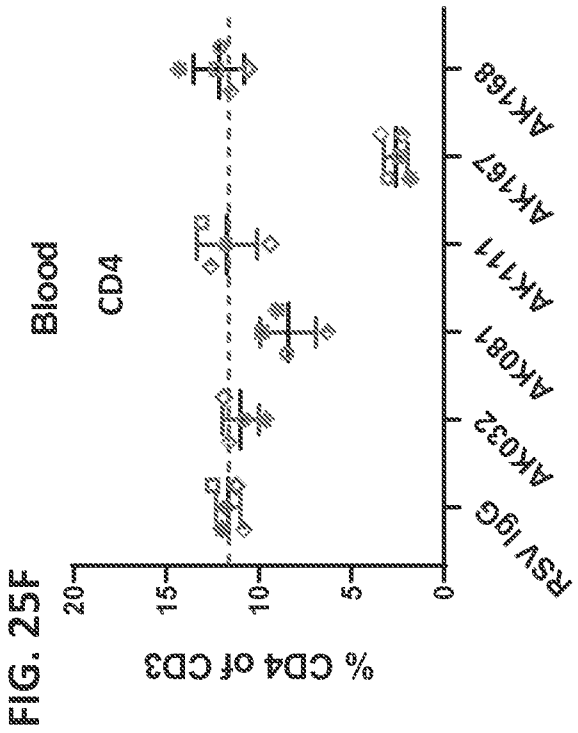


FIG. 25F

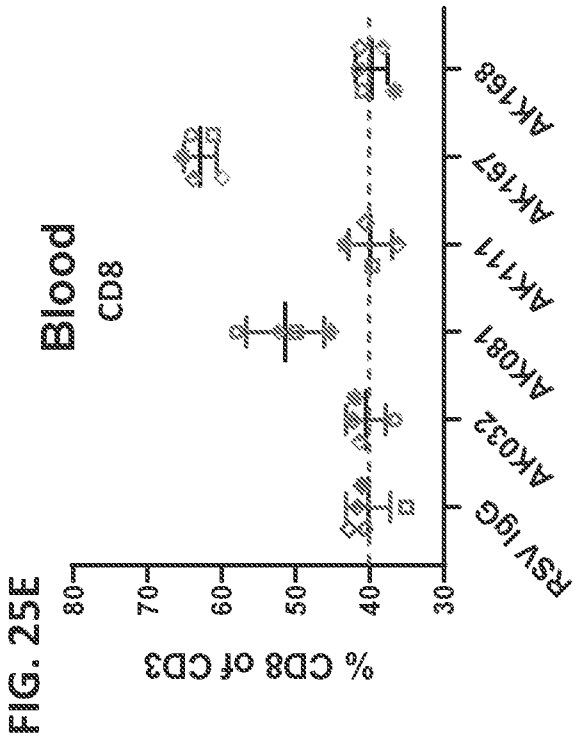


FIG. 25E

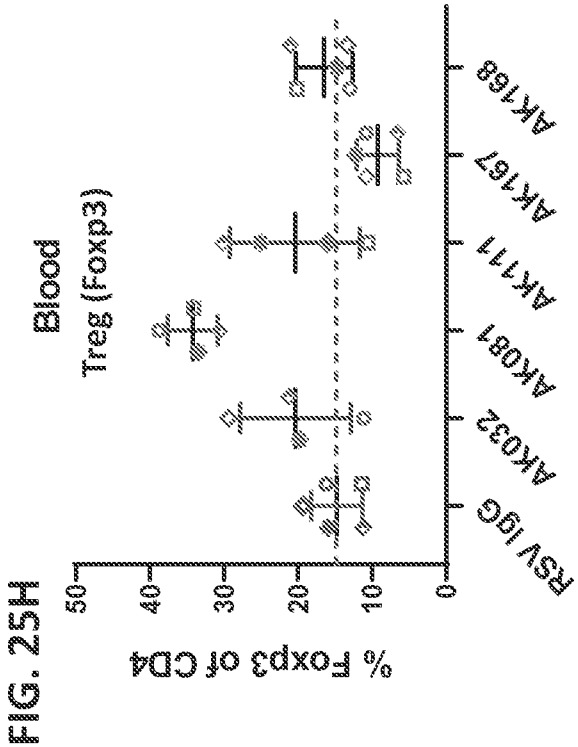


FIG. 25H

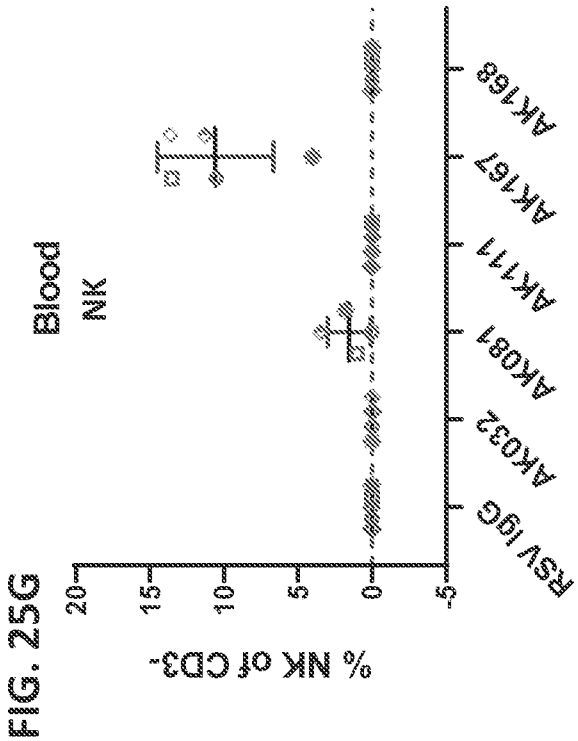


FIG. 25G

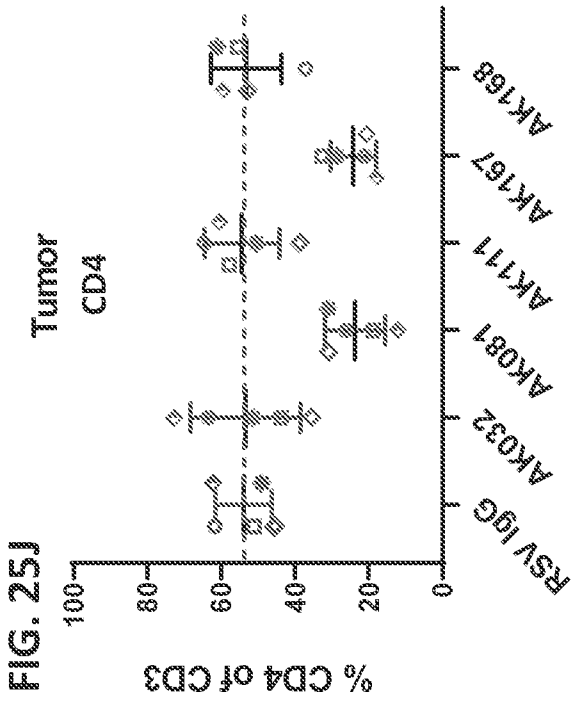


FIG. 25J

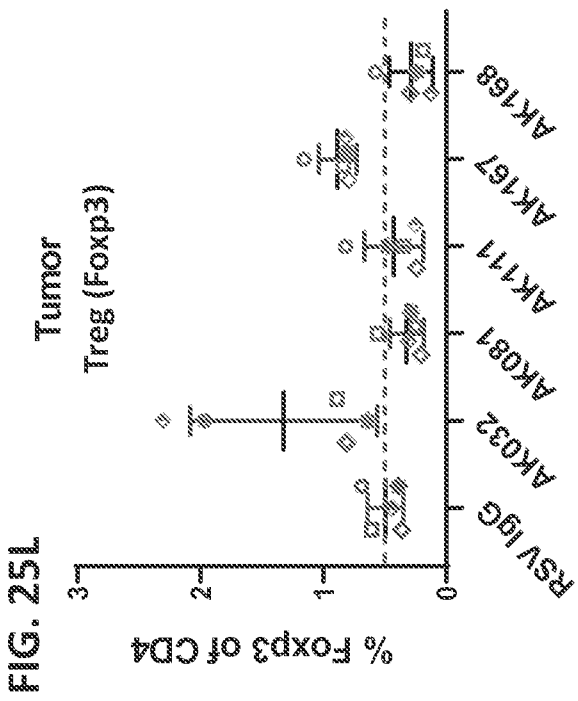


FIG. 25L

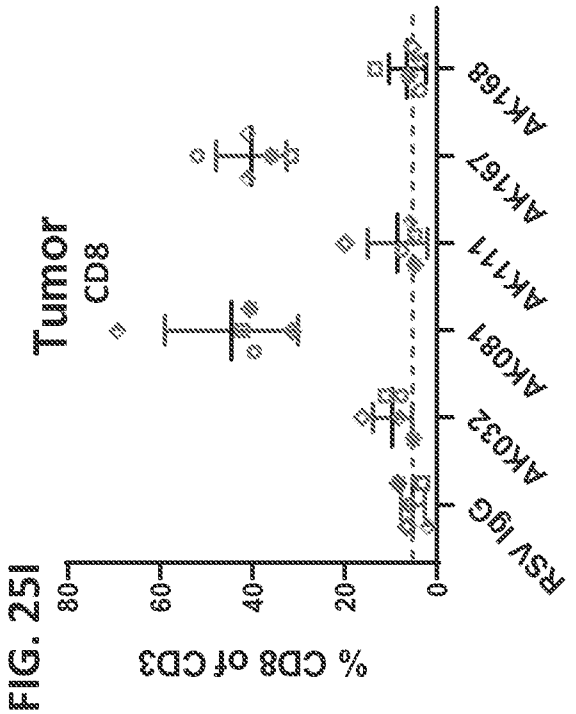


FIG. 25I

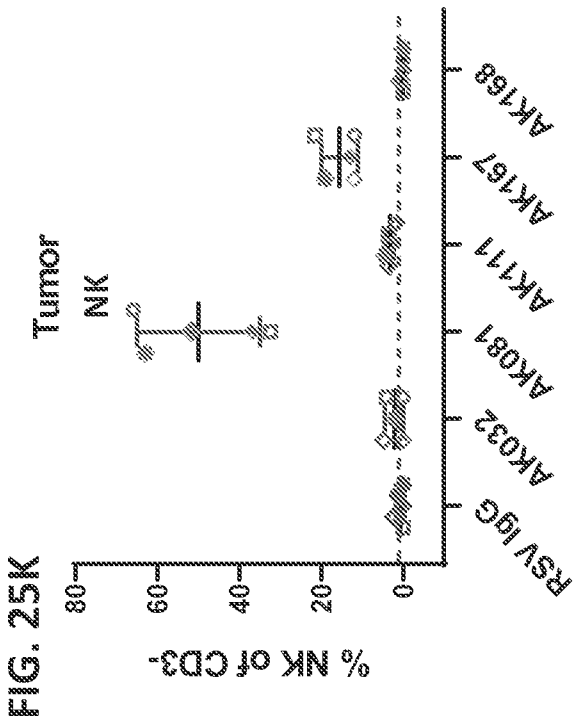


FIG. 25K

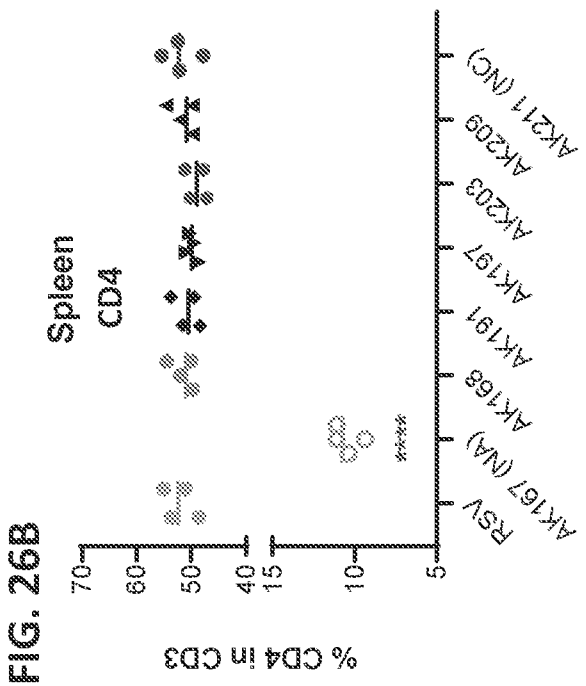


FIG. 26B

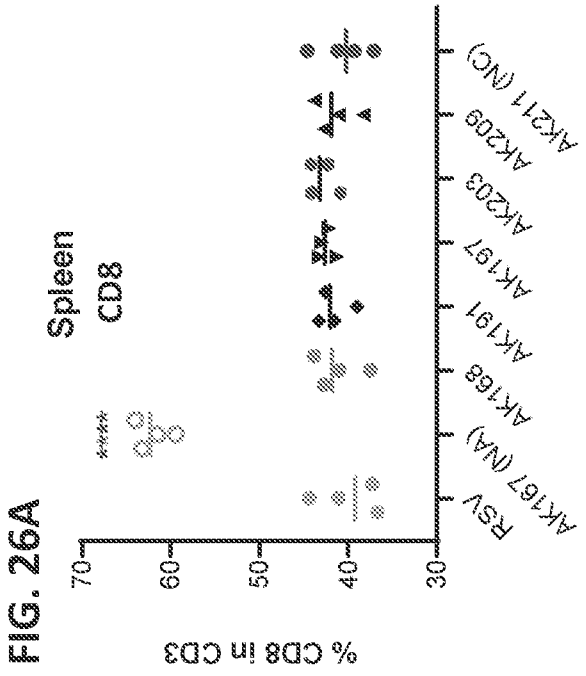


FIG. 26A

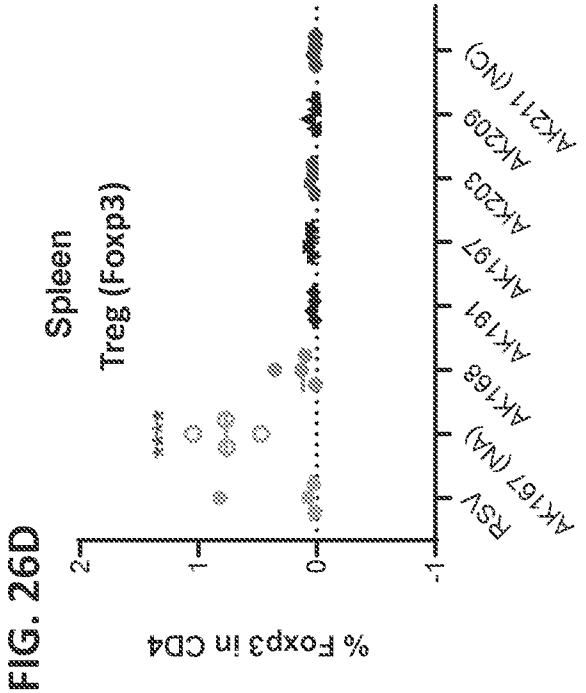


FIG. 26D

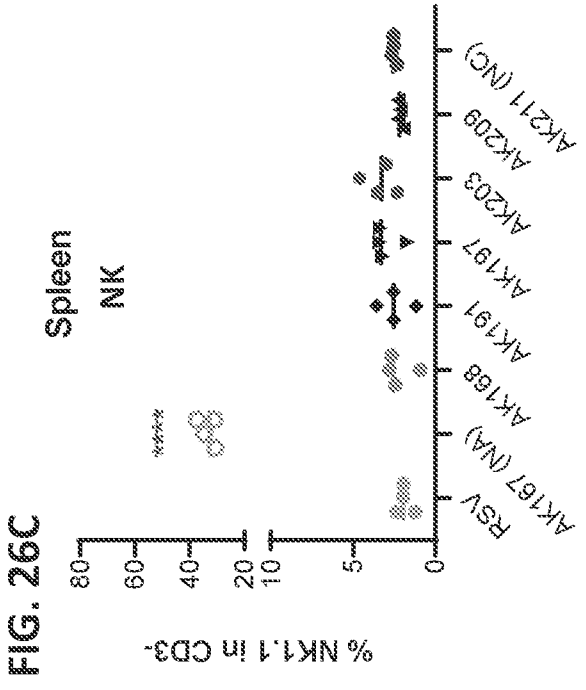


FIG. 26C

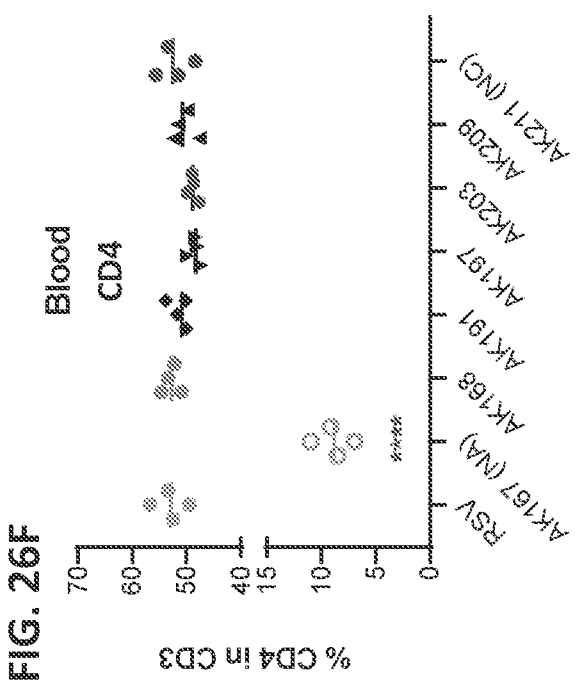


FIG. 26F

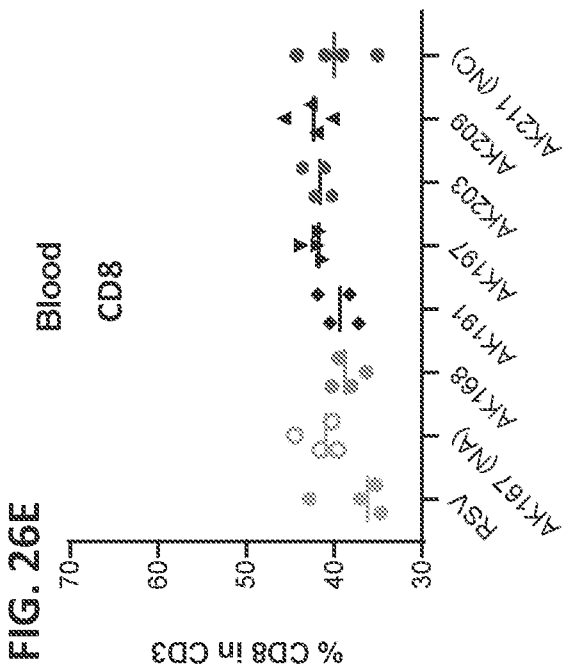


FIG. 26E

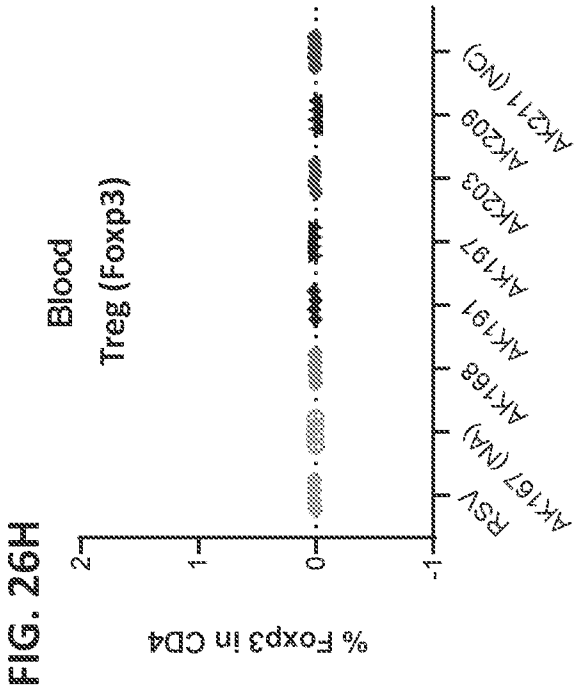


FIG. 26H

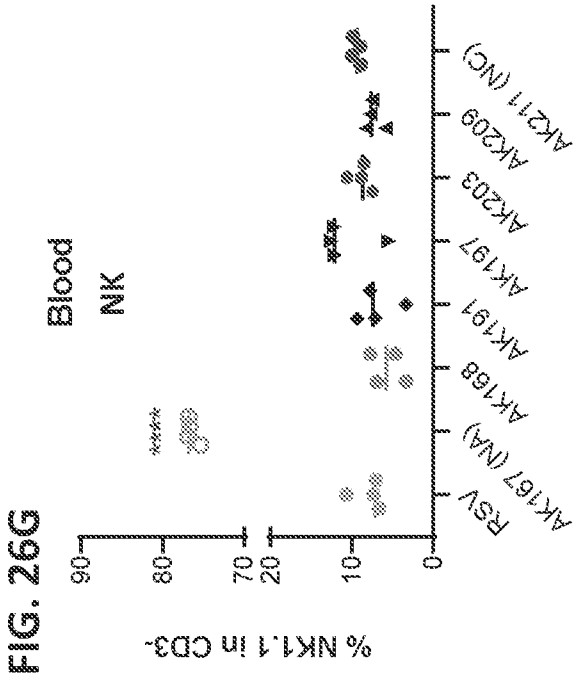


FIG. 26G

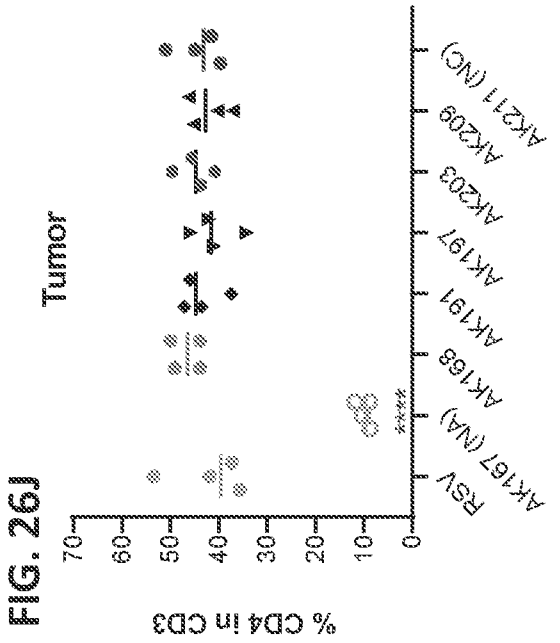


FIG. 26J

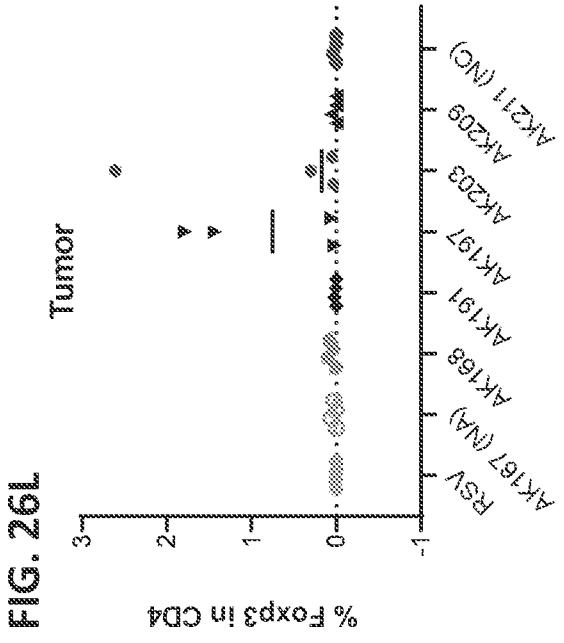


FIG. 26L

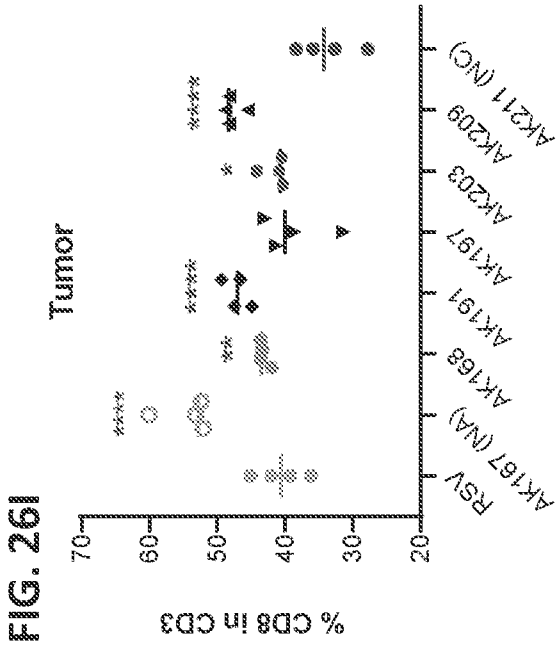


FIG. 26I

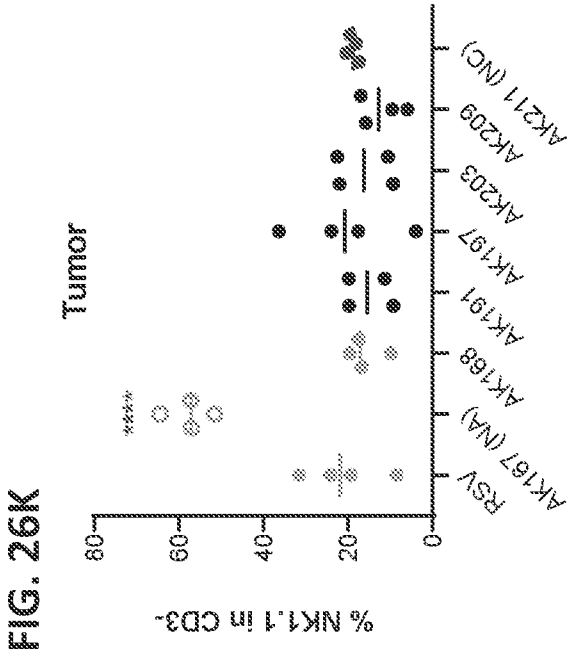


FIG. 26K

FIG. 27A

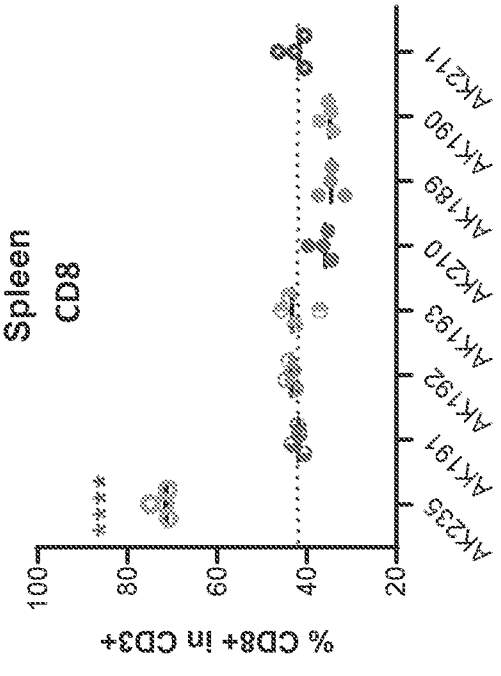


FIG. 27B

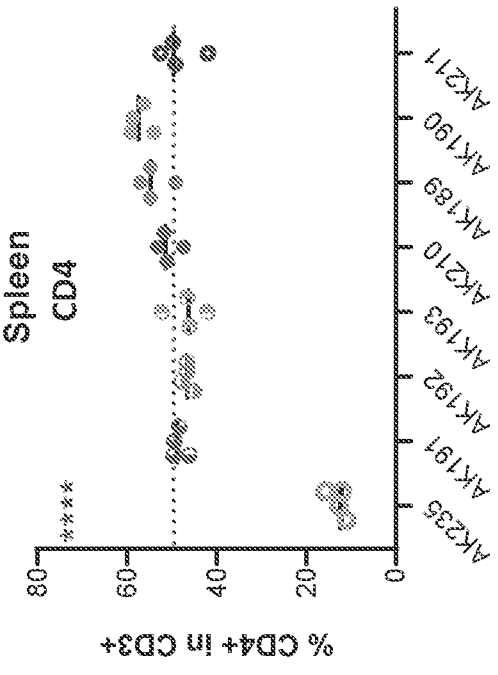


FIG. 27C

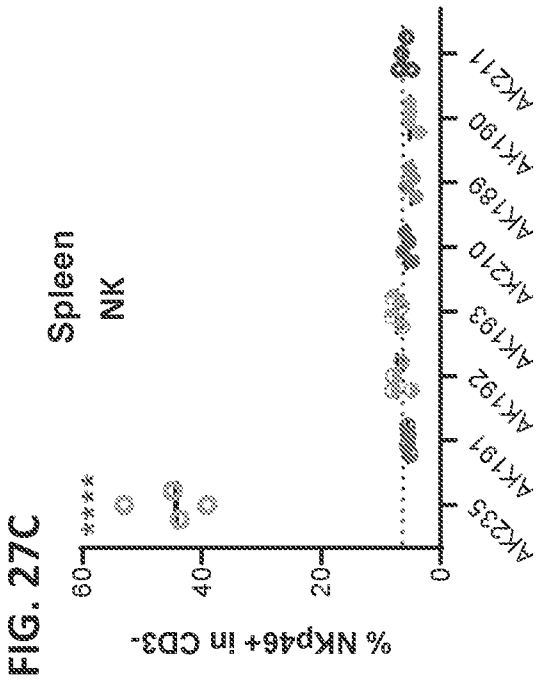
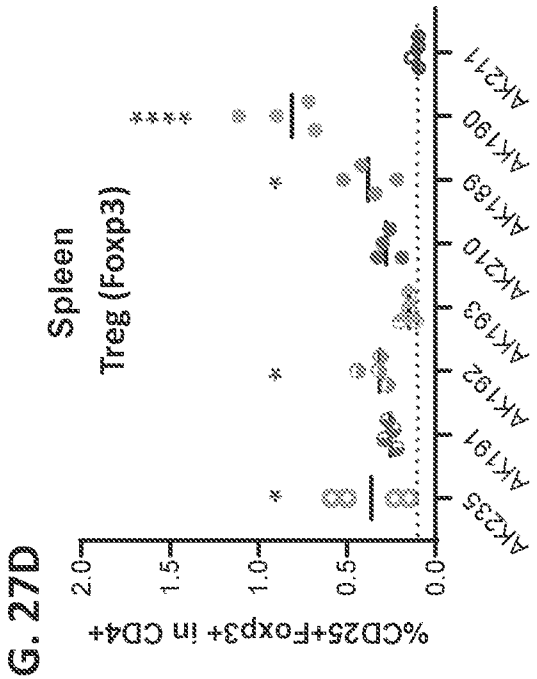


FIG. 27D



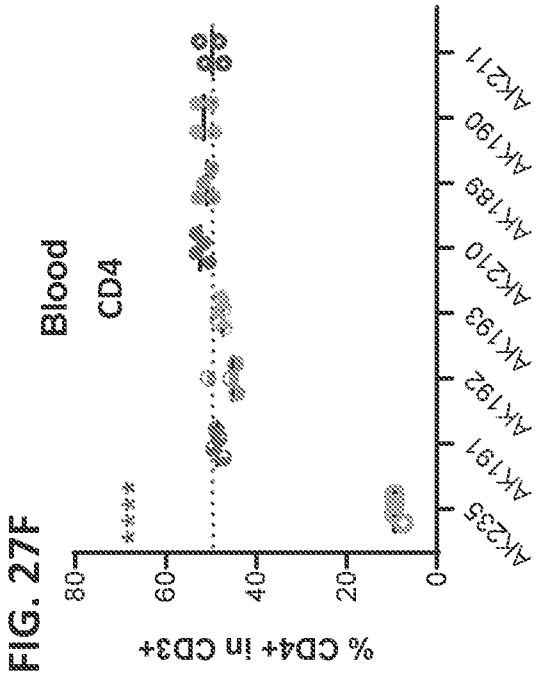


FIG. 27F

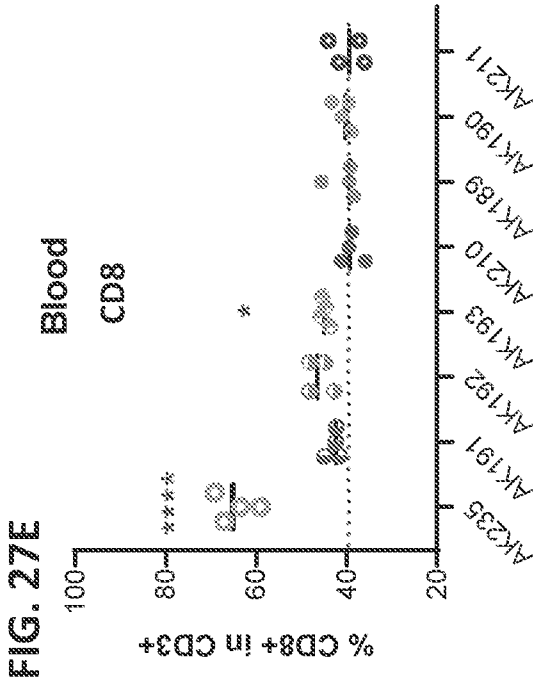


FIG. 27E

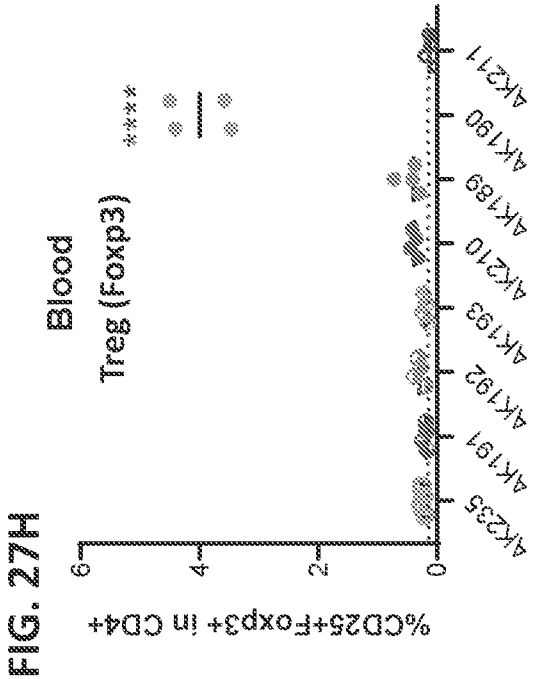


FIG. 27H

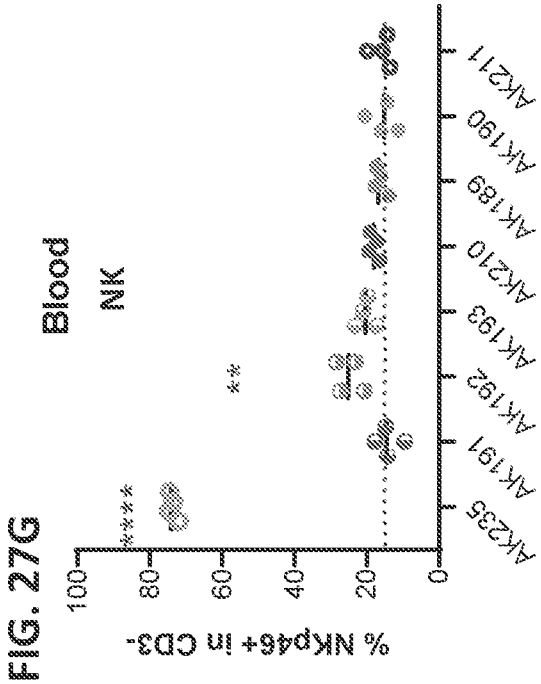


FIG. 27G

FIG. 27I

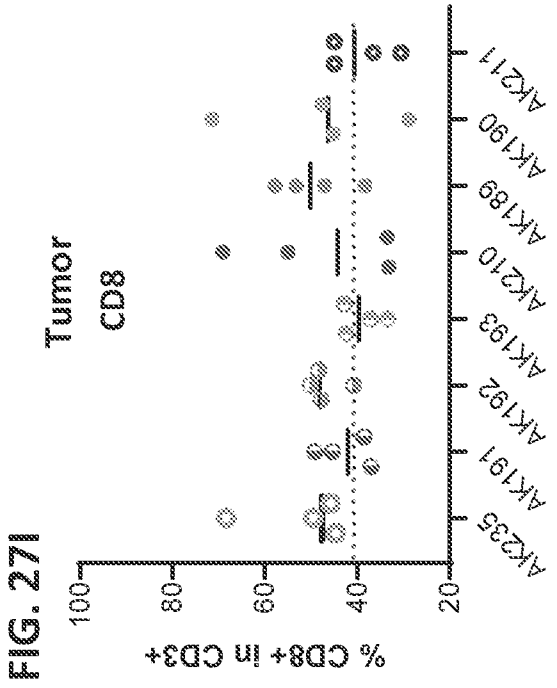


FIG. 27J

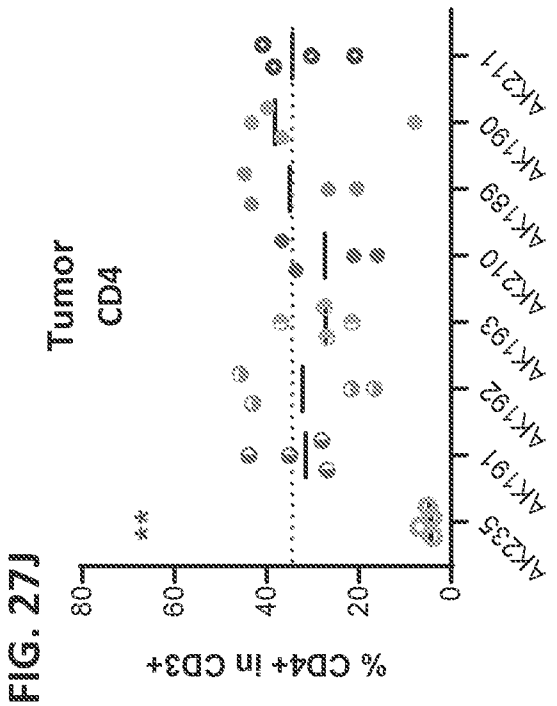


FIG. 27K

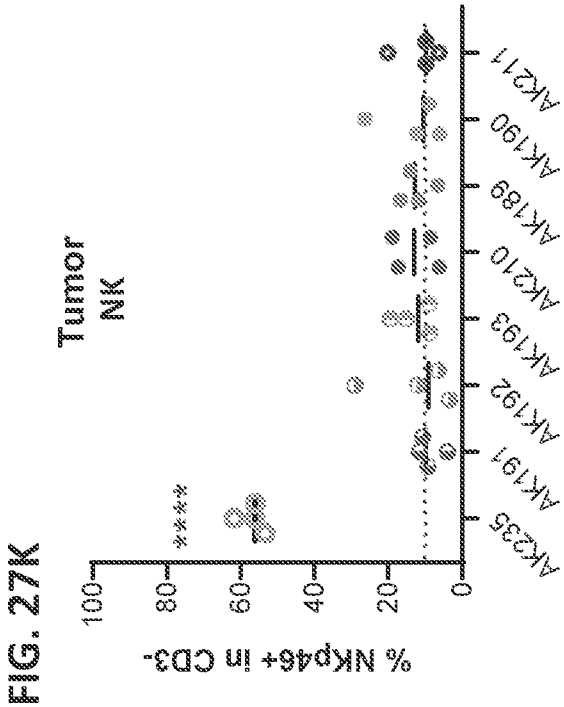
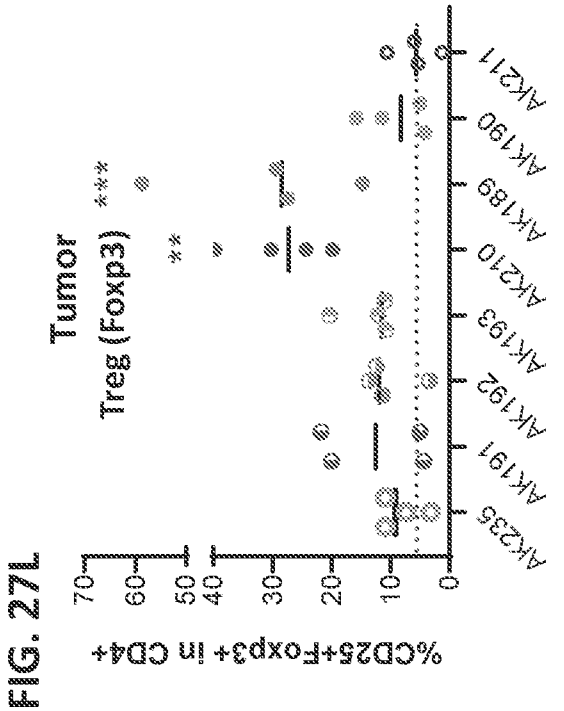


FIG. 27L



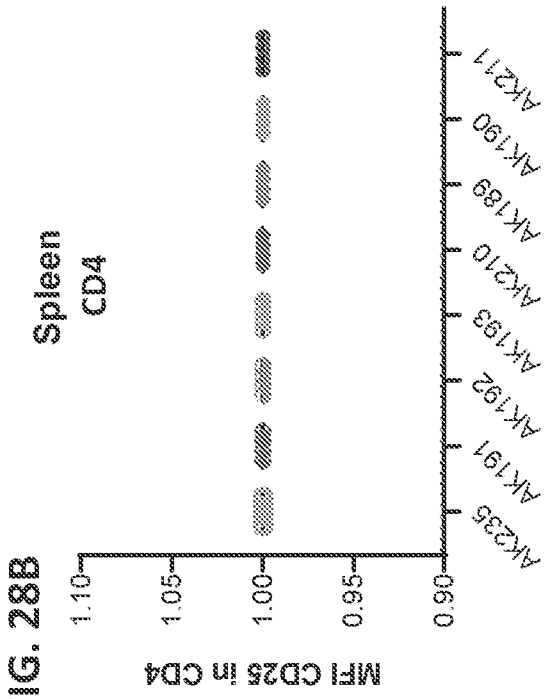


FIG. 28B

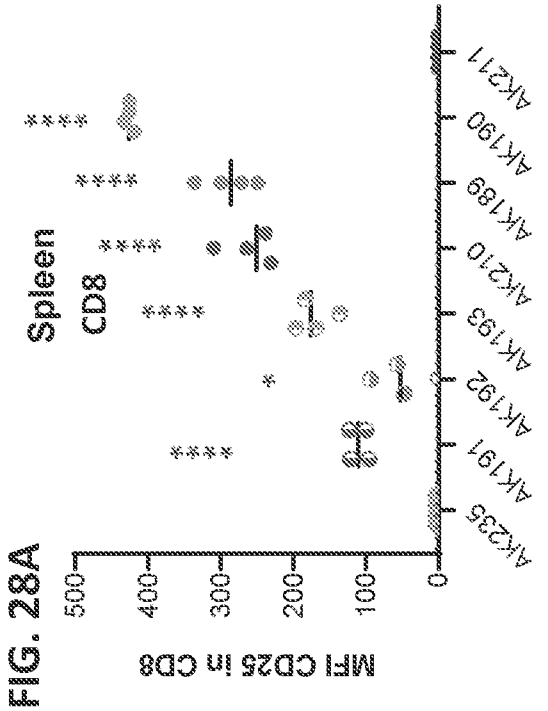


FIG. 28A

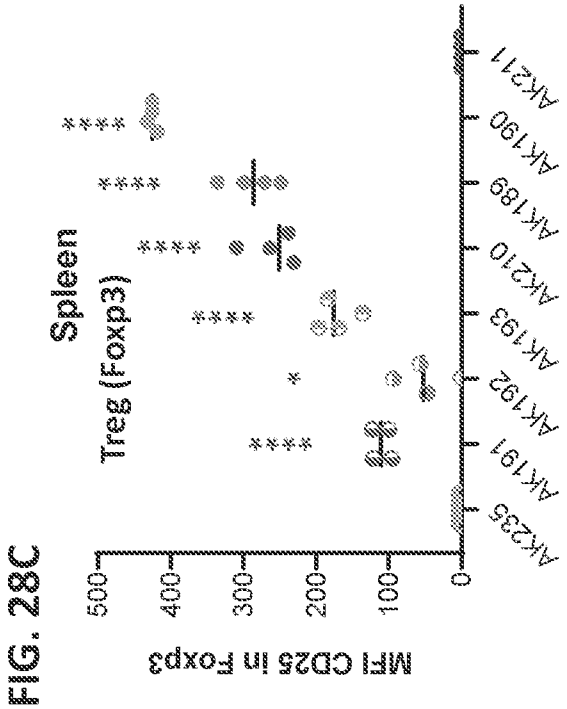


FIG. 28C

FIG. 28D

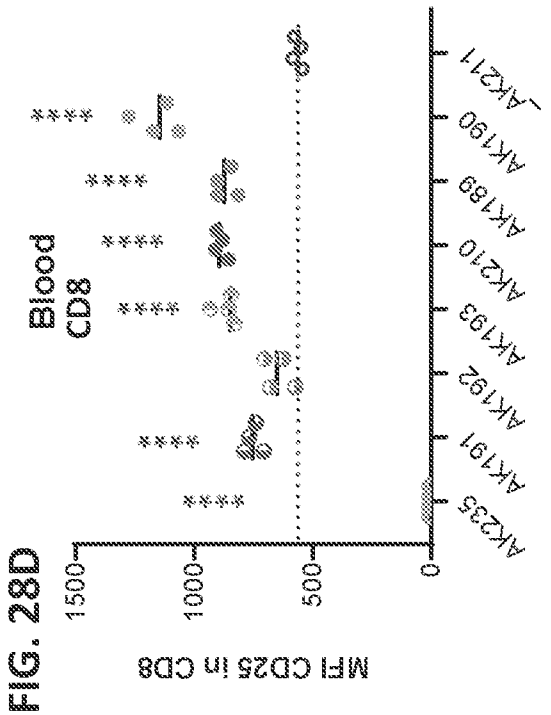


FIG. 28E

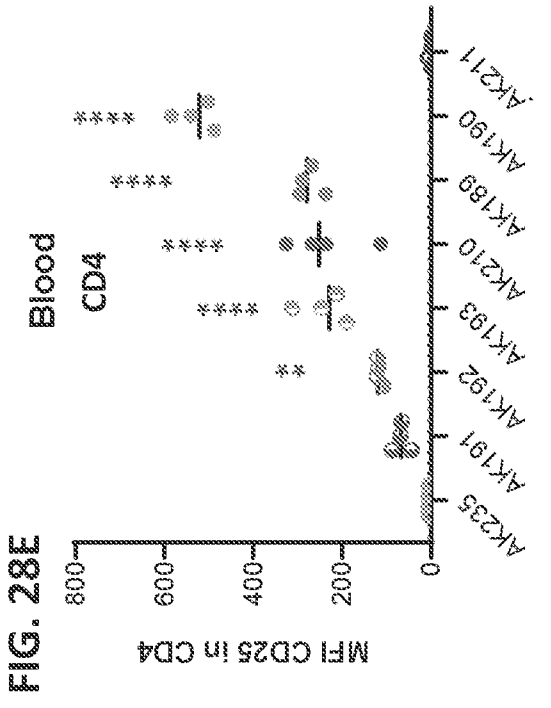
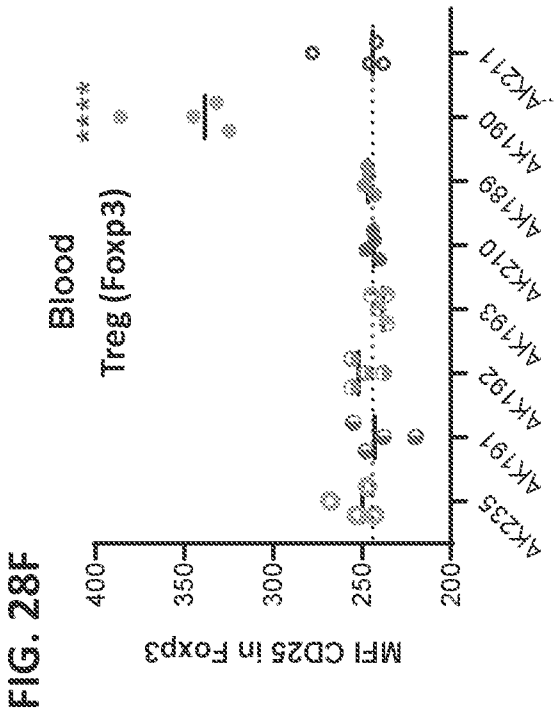


FIG. 28F



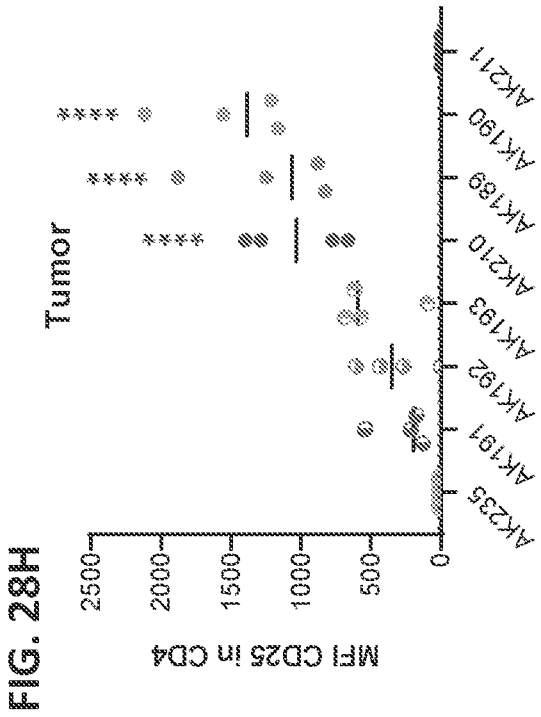


FIG. 28H

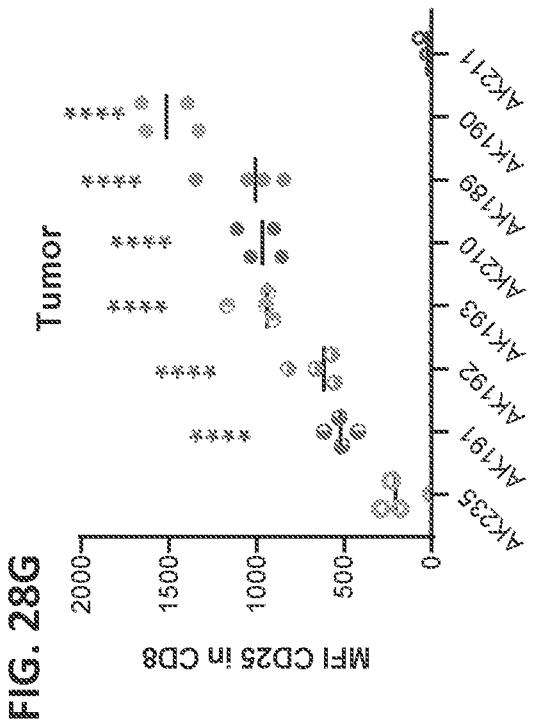


FIG. 28G

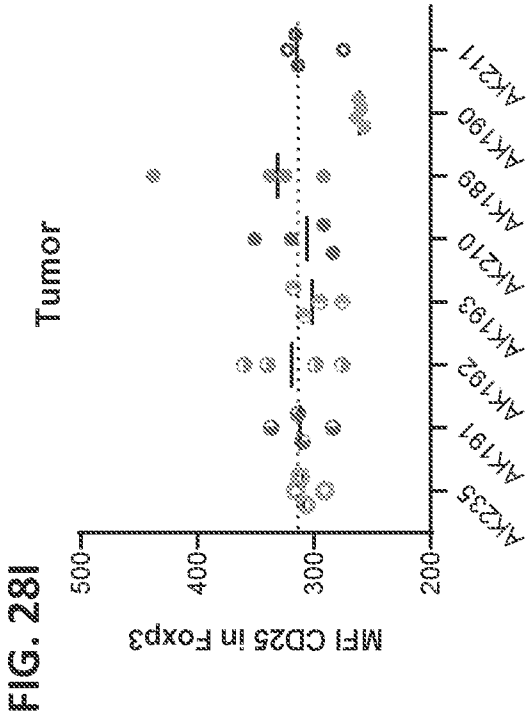


FIG. 28I

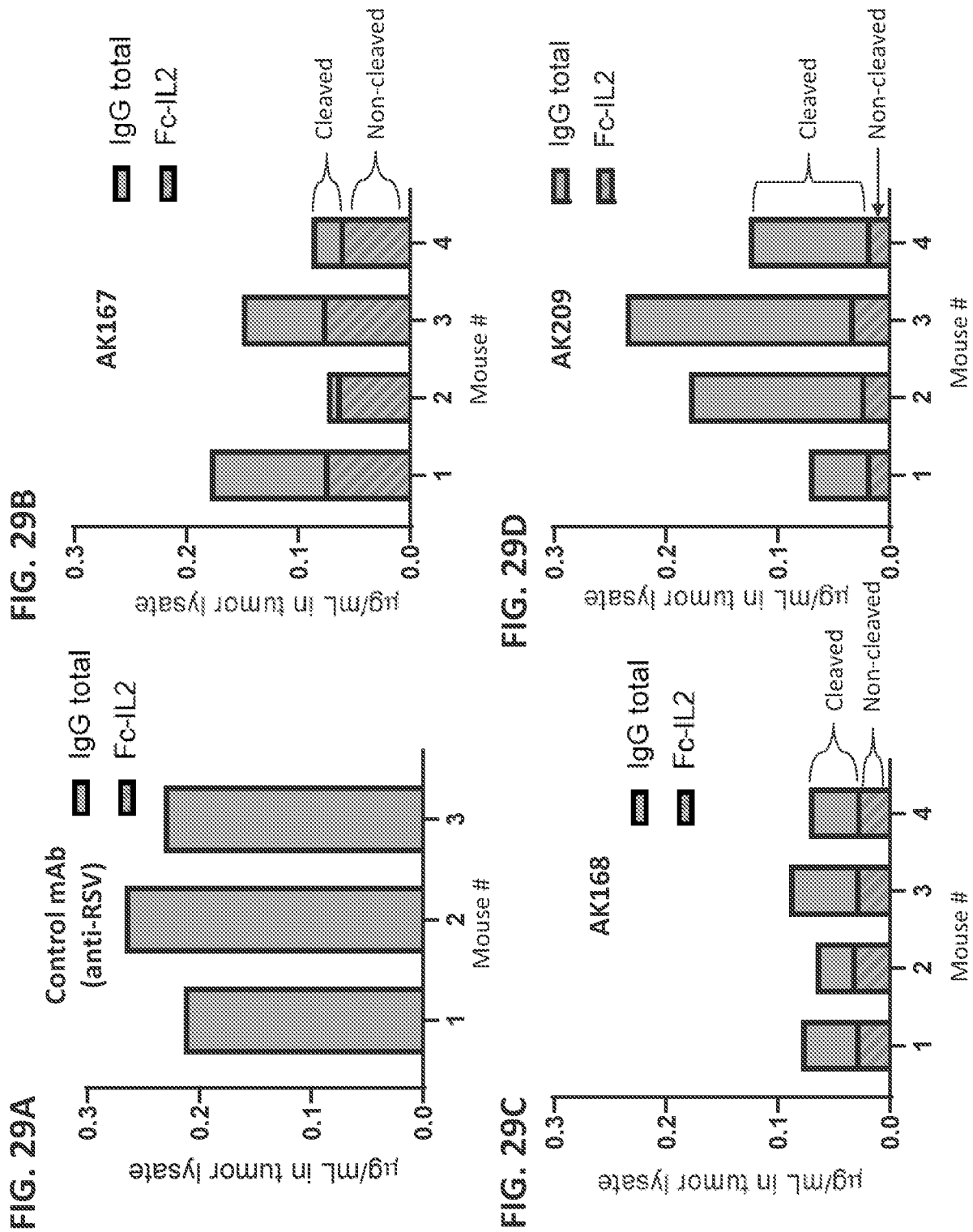


FIG. 29A
Control mAb (anti-RSV)
IgG total
Fc-IL2
μg/mL in tumor lysate
0.0
0.1
0.2
0.3
1
2
3
Mouse #
FIG. 29B
AK167
IgG total
Fc-IL2
Cleaved
Non-cleaved
μg/mL in tumor lysate
0.0
0.1
0.2
0.3
1
2
3
4
Mouse #
FIG. 29C
AK168
IgG total
Fc-IL2
Cleaved
Non-cleaved
μg/mL in tumor lysate
0.0
0.1
0.2
0.3
1
2
3
4
Mouse #
FIG. 29D
AK209
IgG total
Fc-IL2
Cleaved
Non-cleaved
μg/mL in tumor lysate
0.0
0.1
0.2
0.3
1
2
3
4
Mouse #

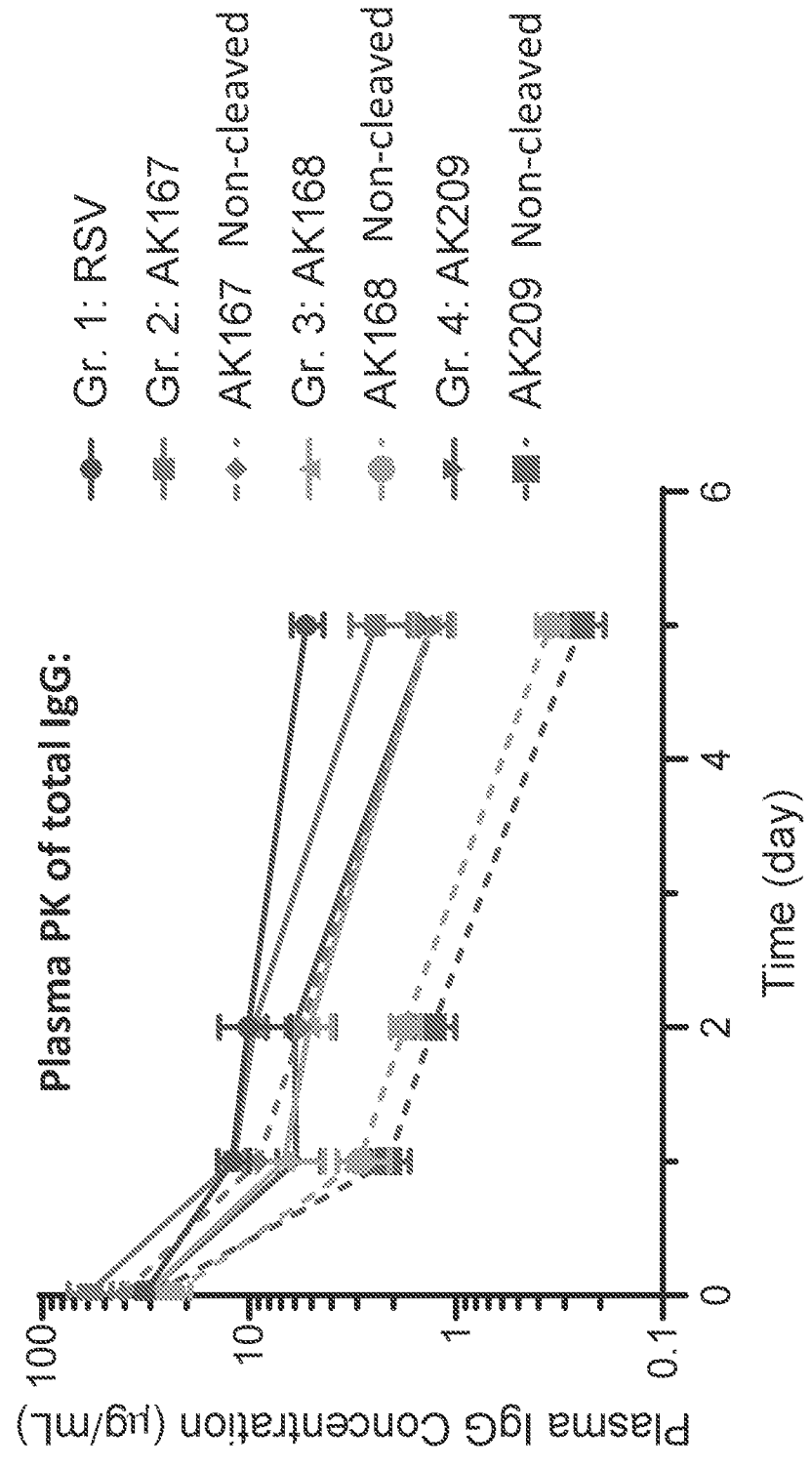

Plasma PK of total IgG:
Gr. 1: RSV
Gr. 2: AK167
AK167 Non-cleaved
Gr. 3: AK168
AK168 Non-cleaved
Gr. 4: AK209
AK209 Non-cleaved
Plasma IgG Concentration (μg/mL)
100
10
1
0.1
0
2
4
6
Time (day)

FIG. 29E

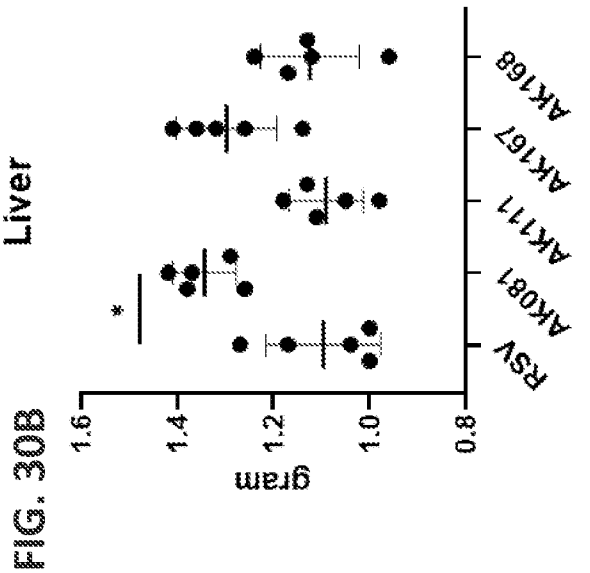


FIG. 30B

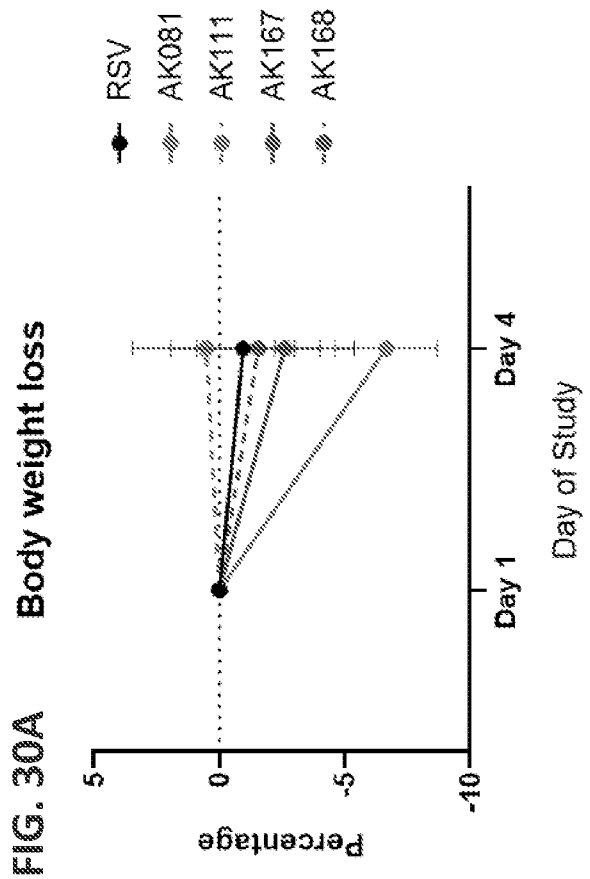


FIG. 30A

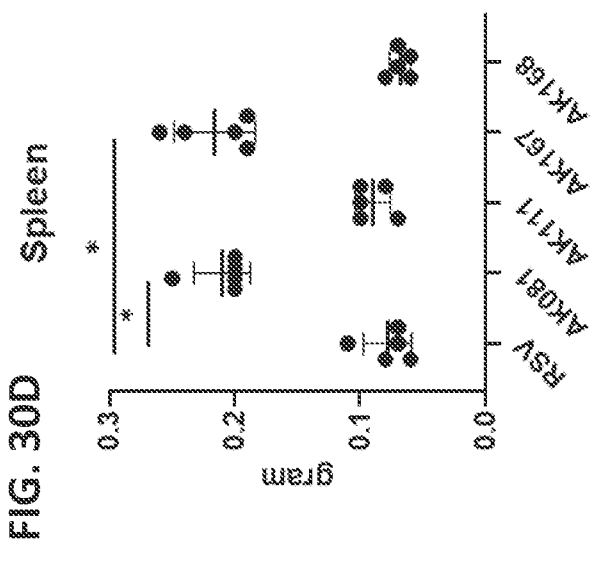


FIG. 30D

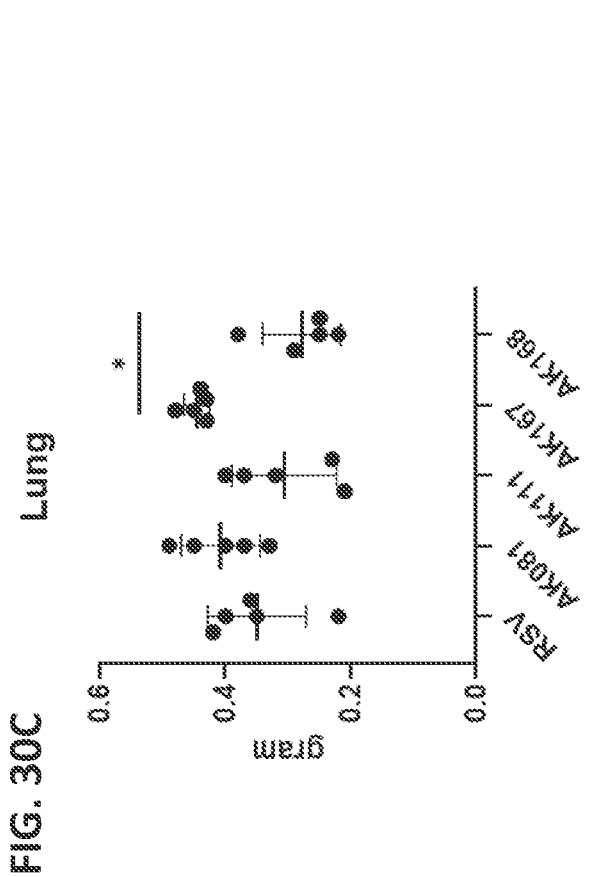


FIG. 30C

FIG. 31B

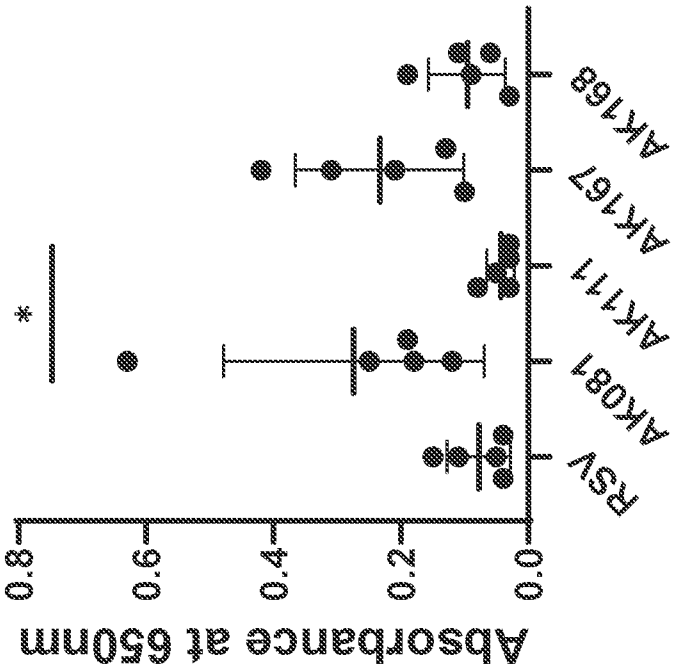


FIG. 31A

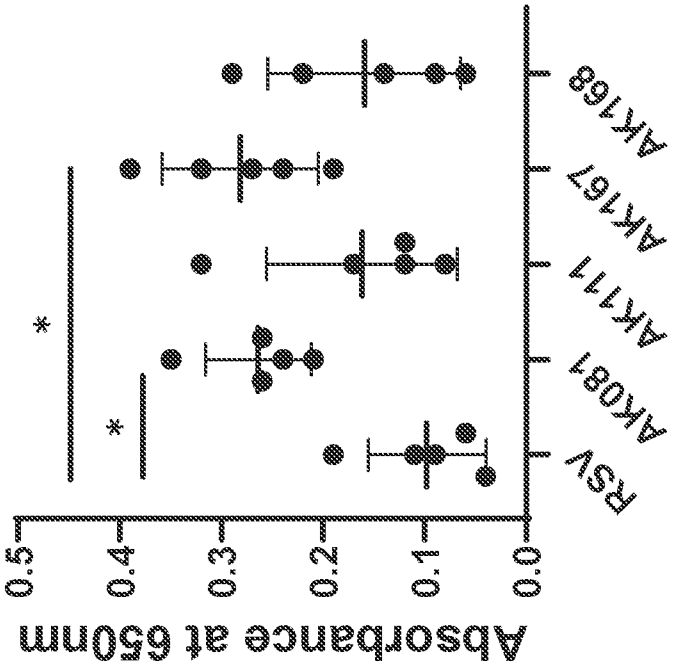


FIG. 32A

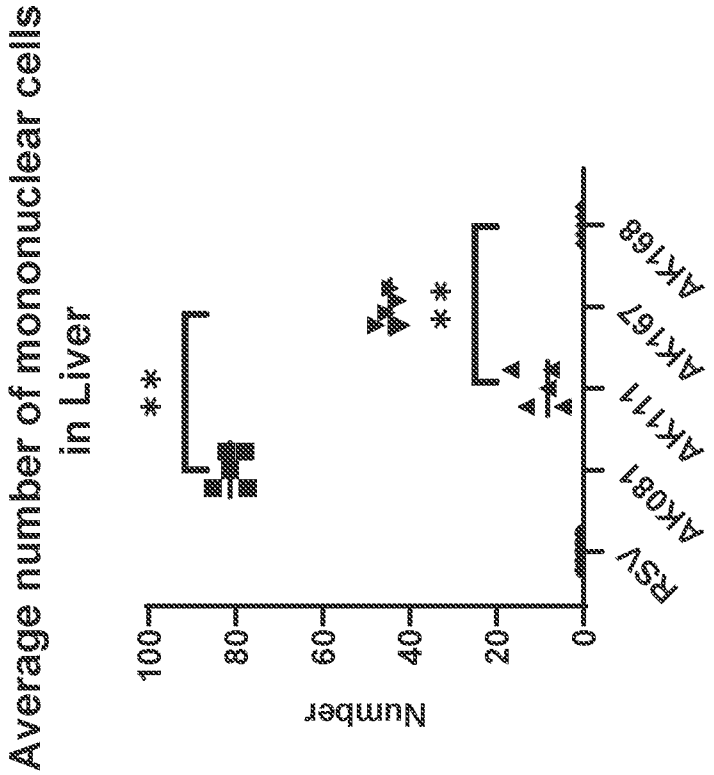


FIG. 32B

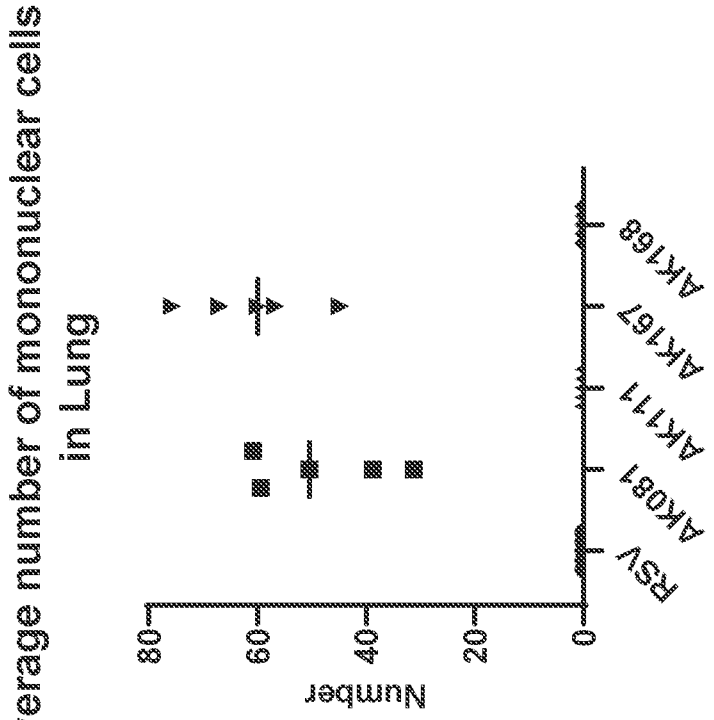
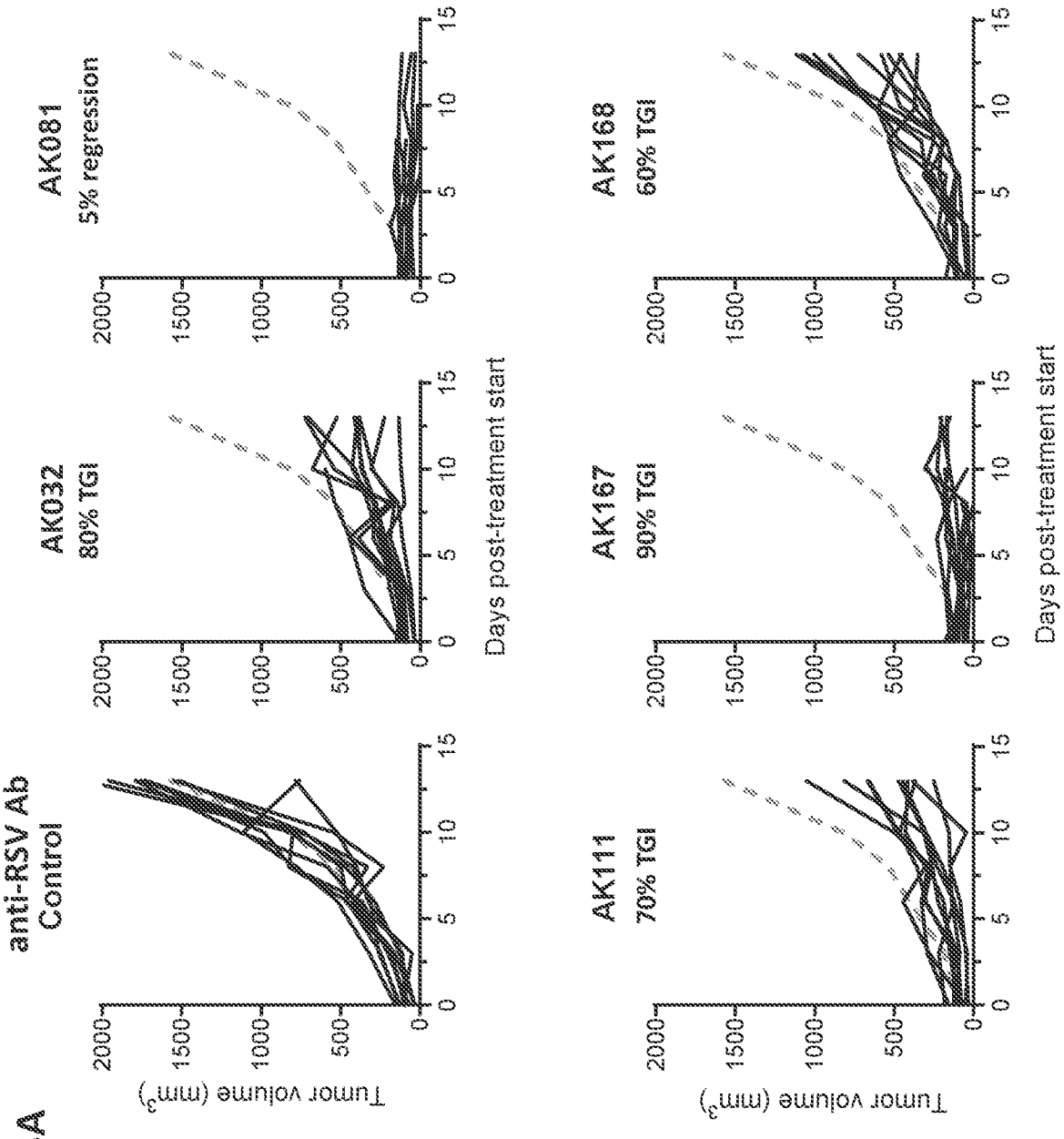


FIG. 33A


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

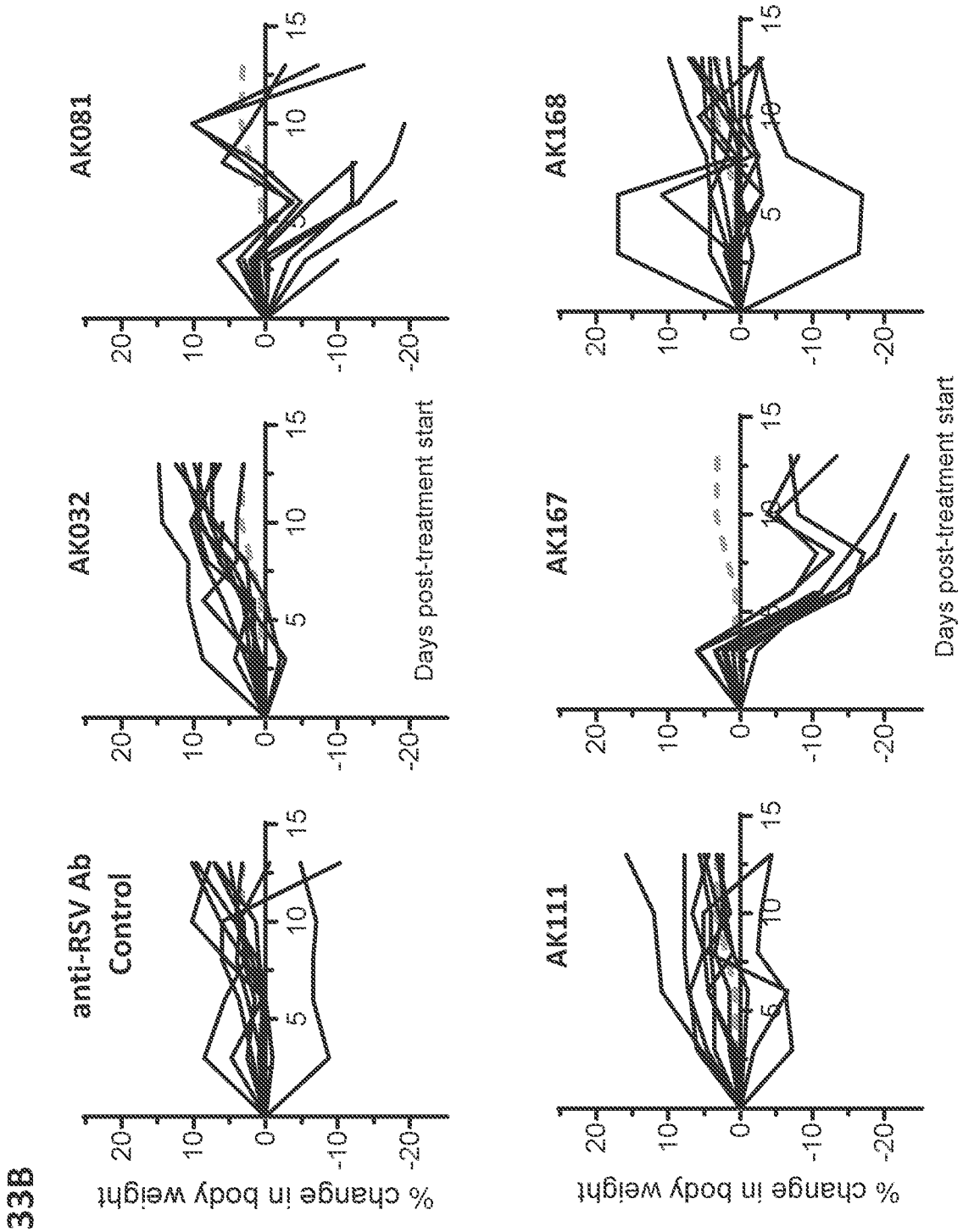

anti-RSV Ab Control
AK032
AK081
AK111
AK167
AK168
% change in body weight
Days post-treatment start
20
10
0
-10
-20
5
10
15

FIG. 33B