FIG. 1B

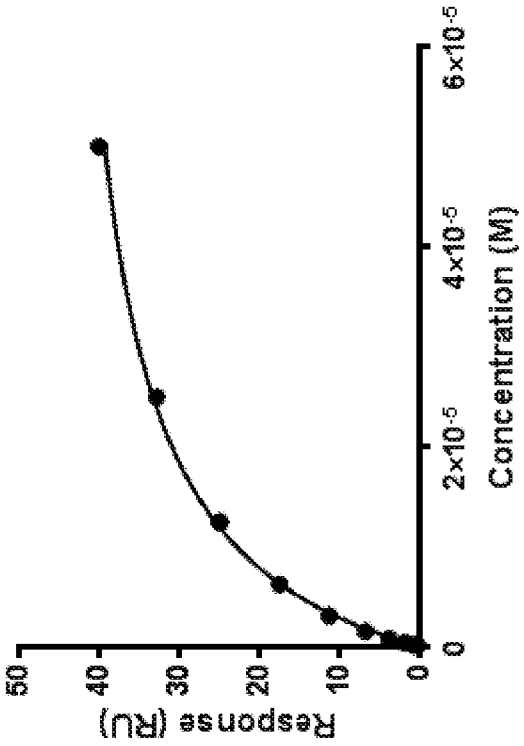

Response (RU)
50
40
30
20
10
0
0
2×10-5
4×10-5
6×10-5
Concentration (M)

FIG. 1A

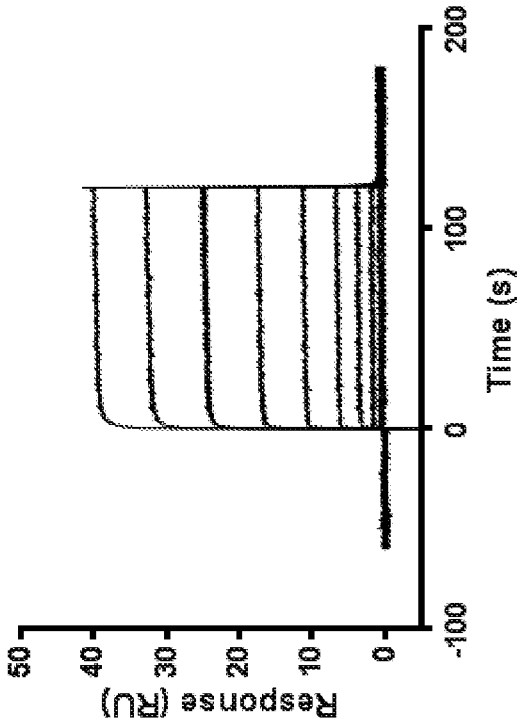

Response (RU)
50
40
30
20
10
0
-100
0
100
200
Time (s)

FIG. 2A

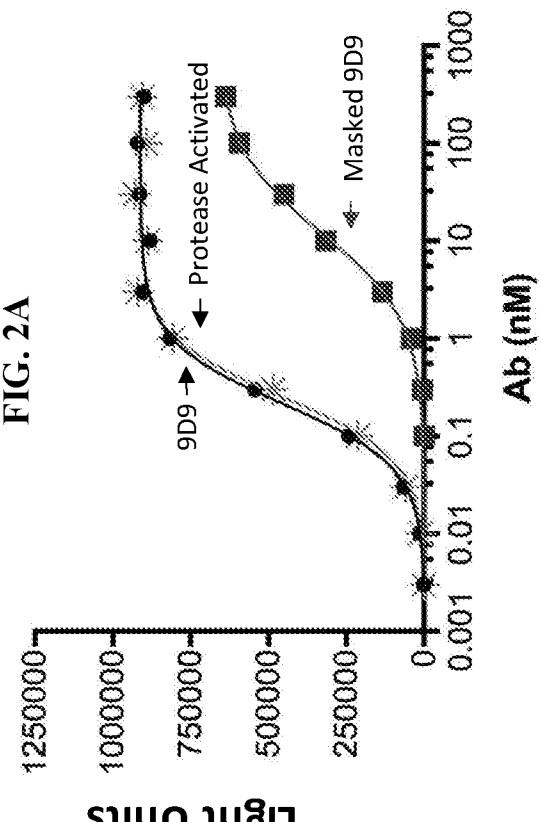


FIG. 2B

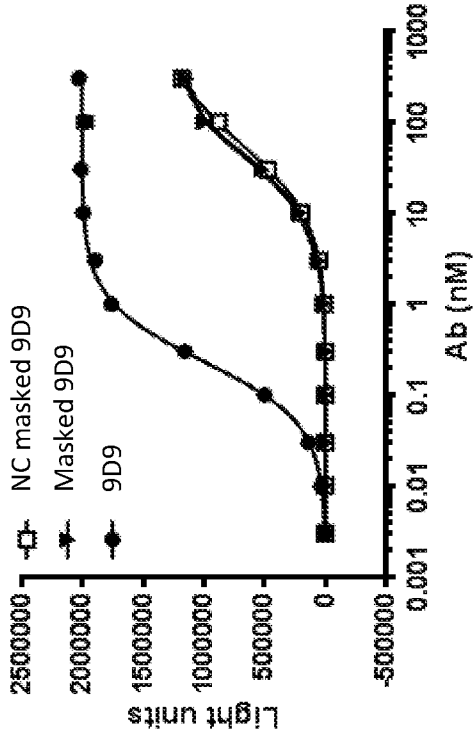


FIG. 2C

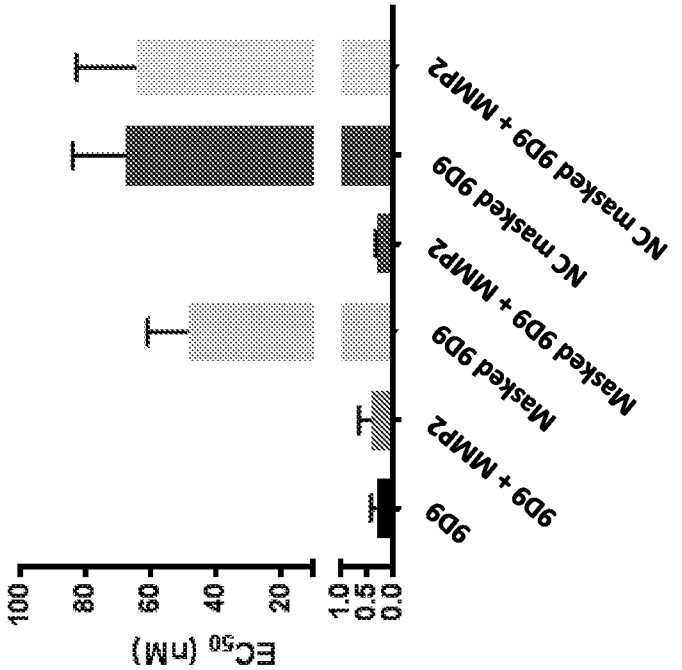


FIG. 3A

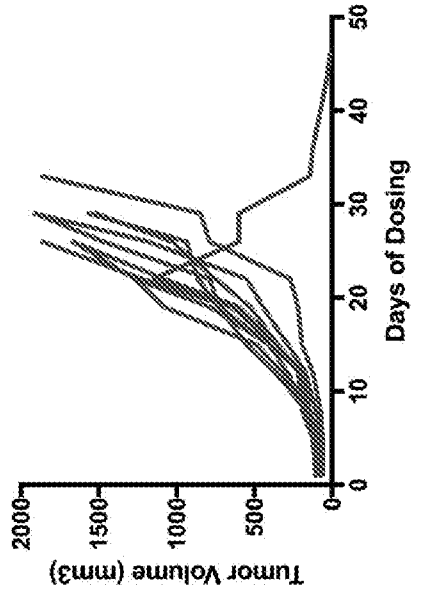


FIG. 3B

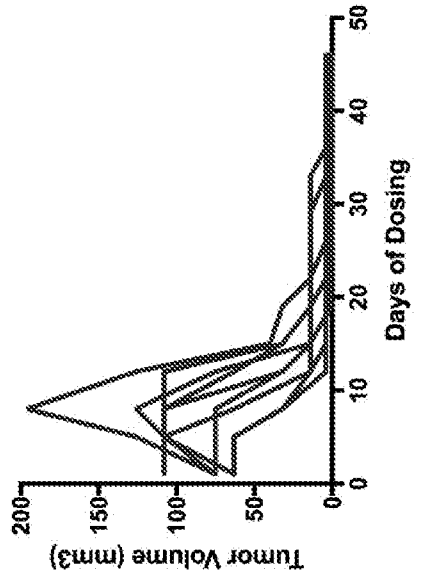


FIG. 3C

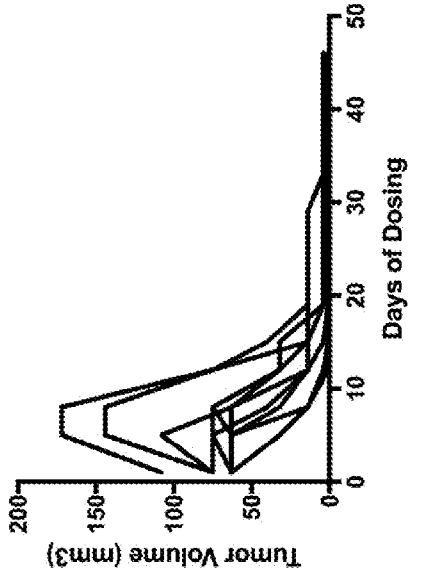


FIG. 3D

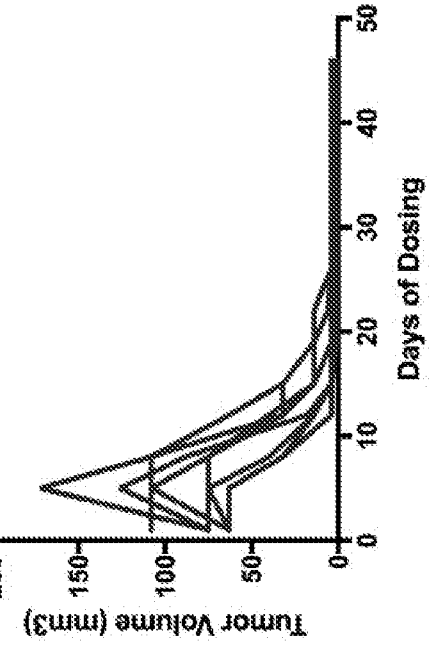


FIG. 4

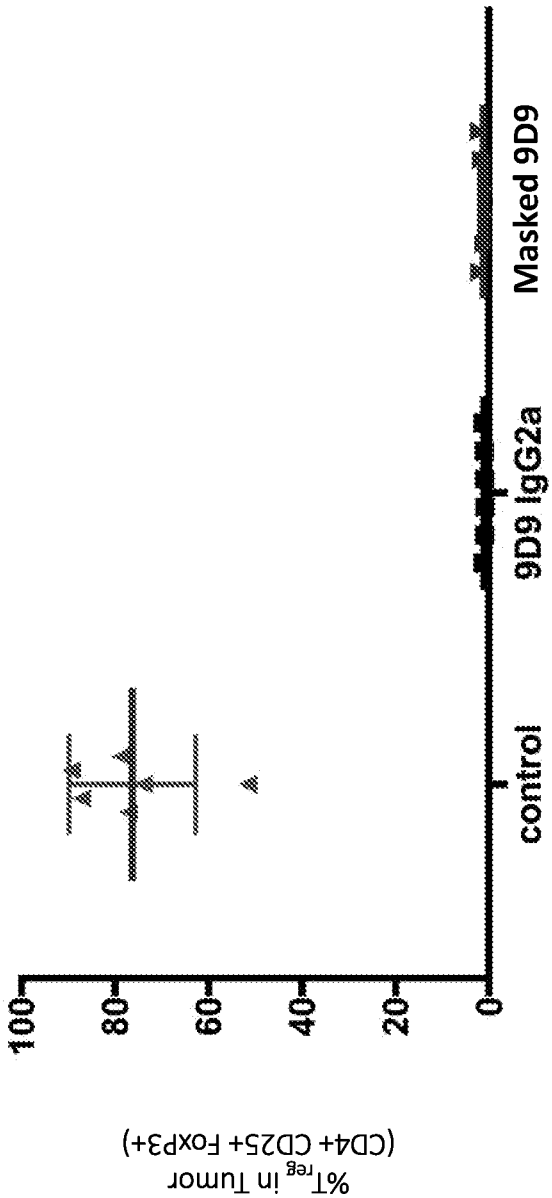

%T$_{reg}$ in Tumor
(CD4+ CD25+ FoxP3+)
100
80
60
40
20
0
control
9D9 IgG2a
Masked 9D9

FIG. 5A

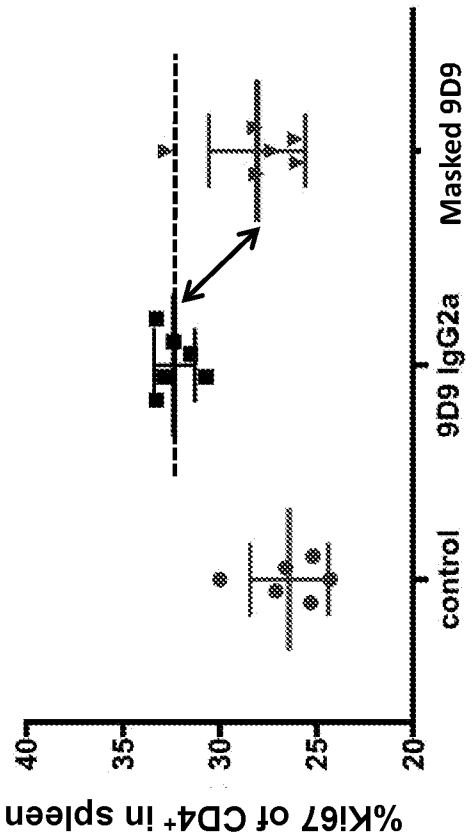

%Ki67 of CD4+ in spleen
40
35
30
25
20
control
9D9 IgG2a
Masked 9D9

FIG. 5B

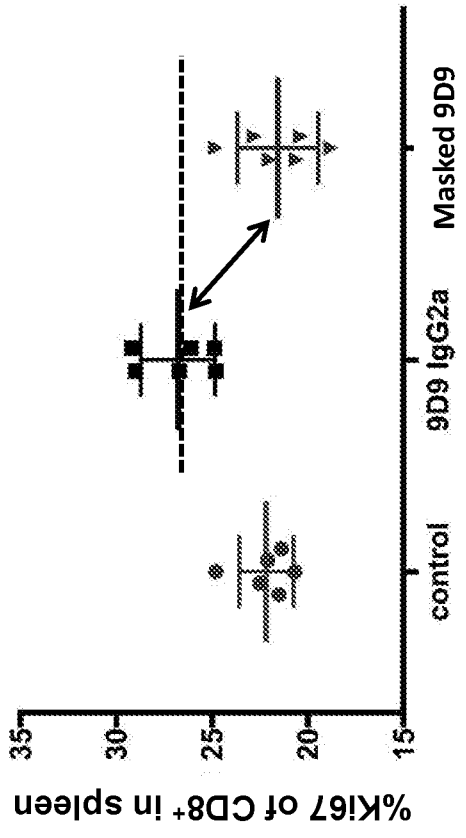

%Ki67 of CD8+ in spleen
35
30
25
20
15
control
9D9 IgG2a
Masked 9D9

FIG. 6A

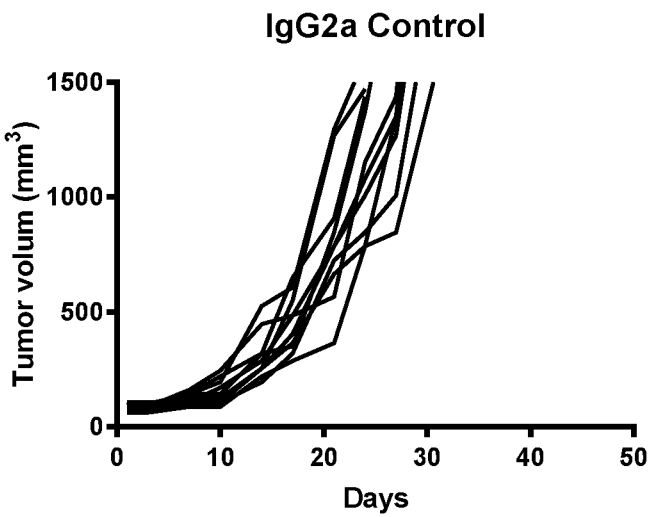


FIG. 6B

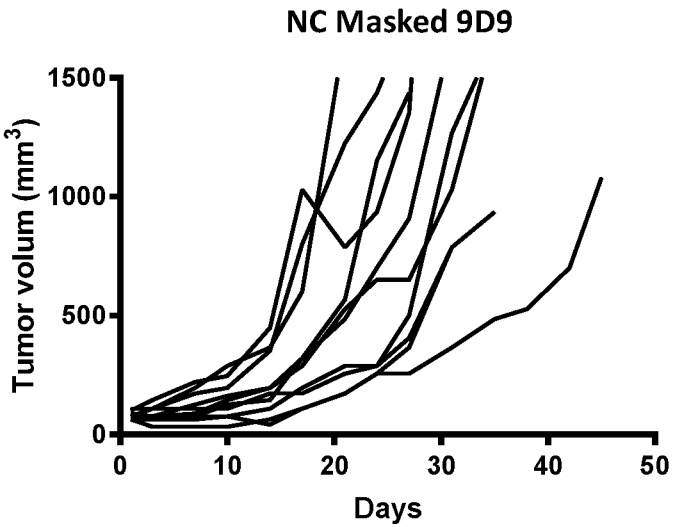


FIG. 6C

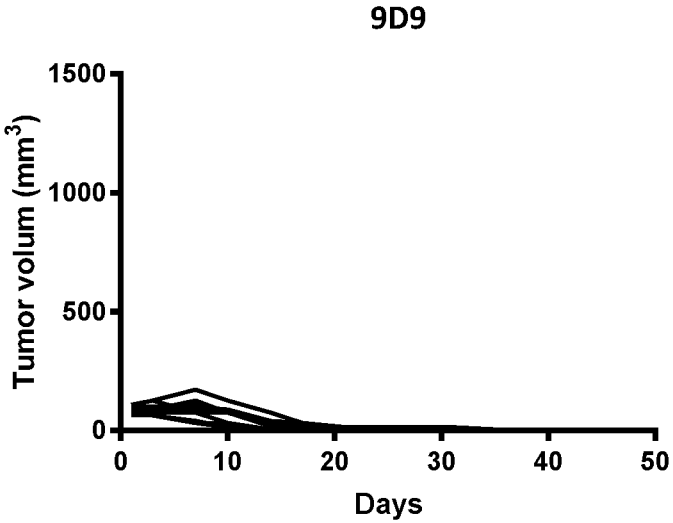


FIG. 6D

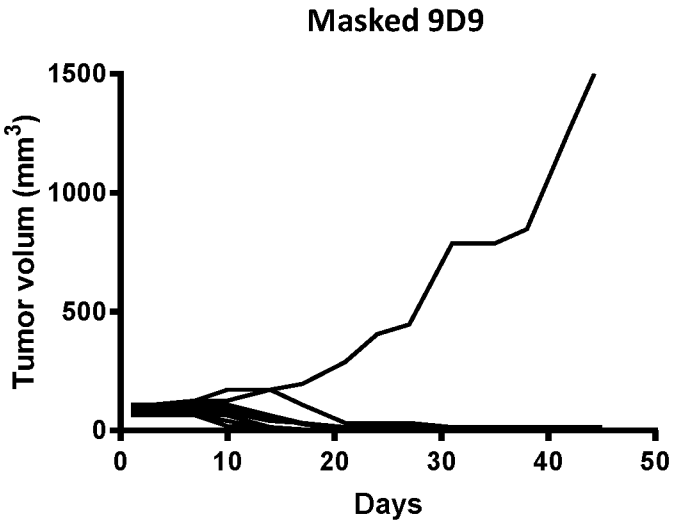


FIG. 7A

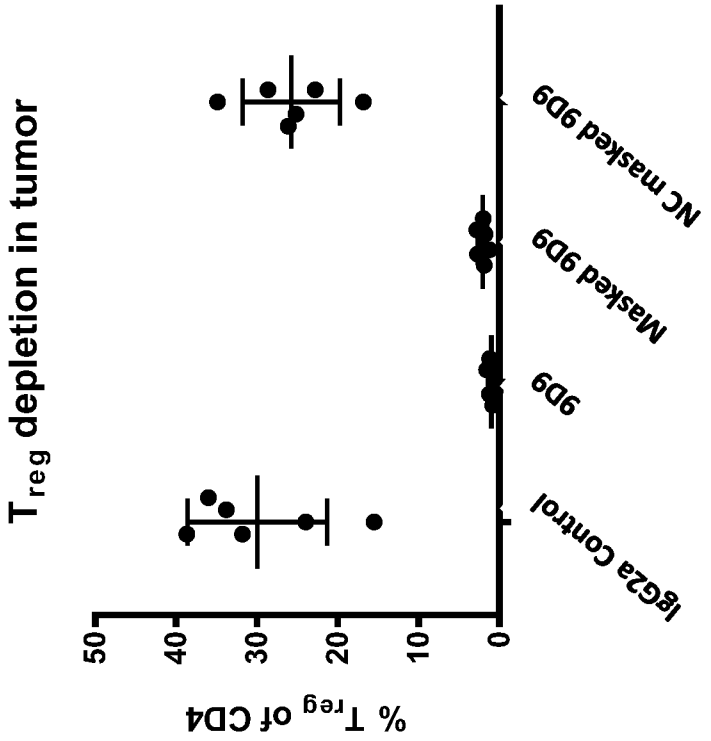

T_{reg} depletion in tumor
% T_{reg} of CD4
0
10
20
30
40
50
IgG2a Control
9D9
Masked 9D9
NC masked 9D9

FIG. 7B

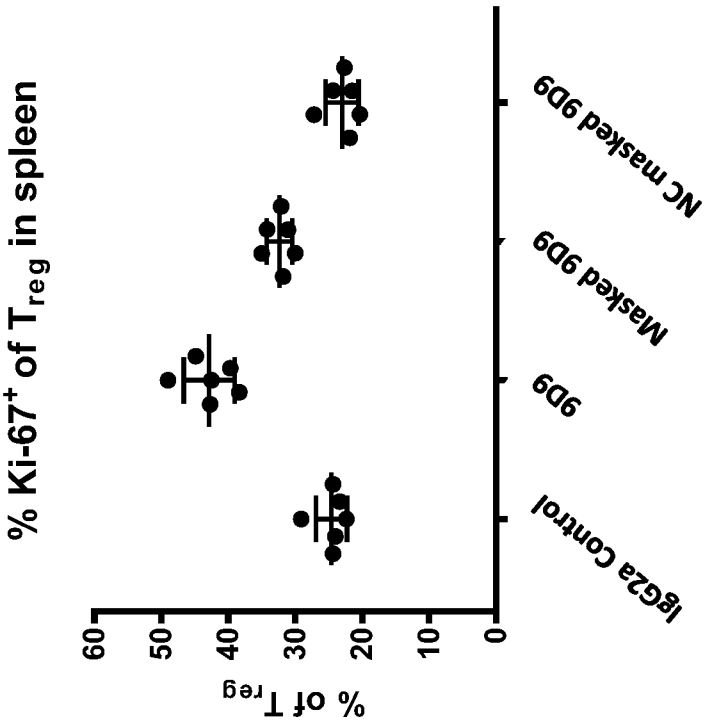

% Ki-67^{+} of T_{reg} in spleen
% of T_{reg}
0
10
20
30
40
50
60
IgG2a Control
9D9
Masked 9D9
NC masked 9D9

FIG. 8

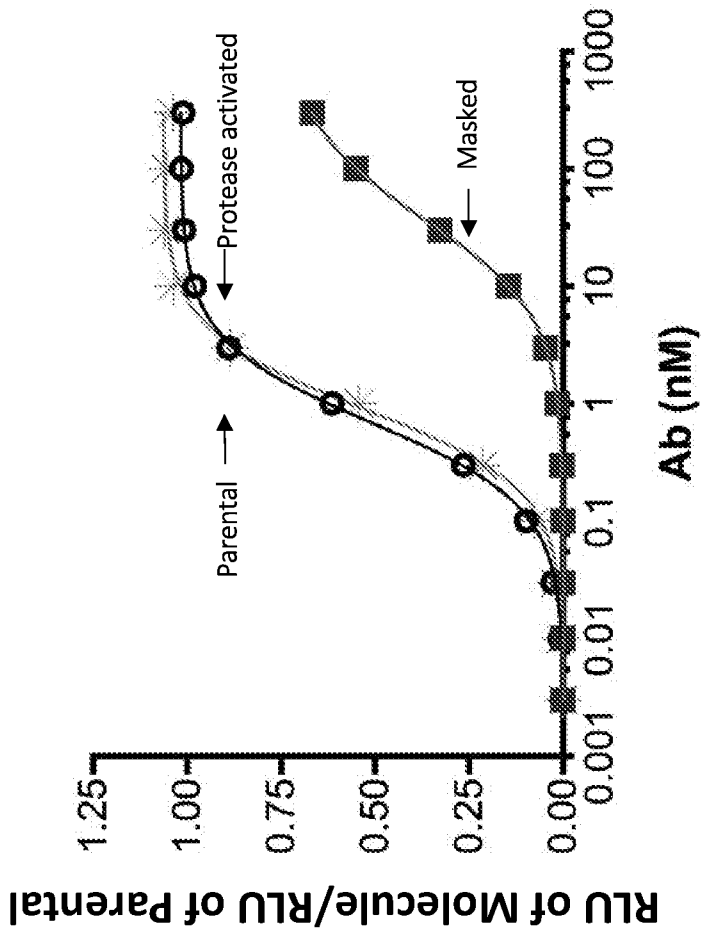

RLU of Molecule/RLU of Parental
1.25
1.00
0.75
0.50
0.25
0.00
0.001
0.01
0.1
1
10
100
1000
Ab (nM)
Parental
Protease activated
Masked

FIG. 9A

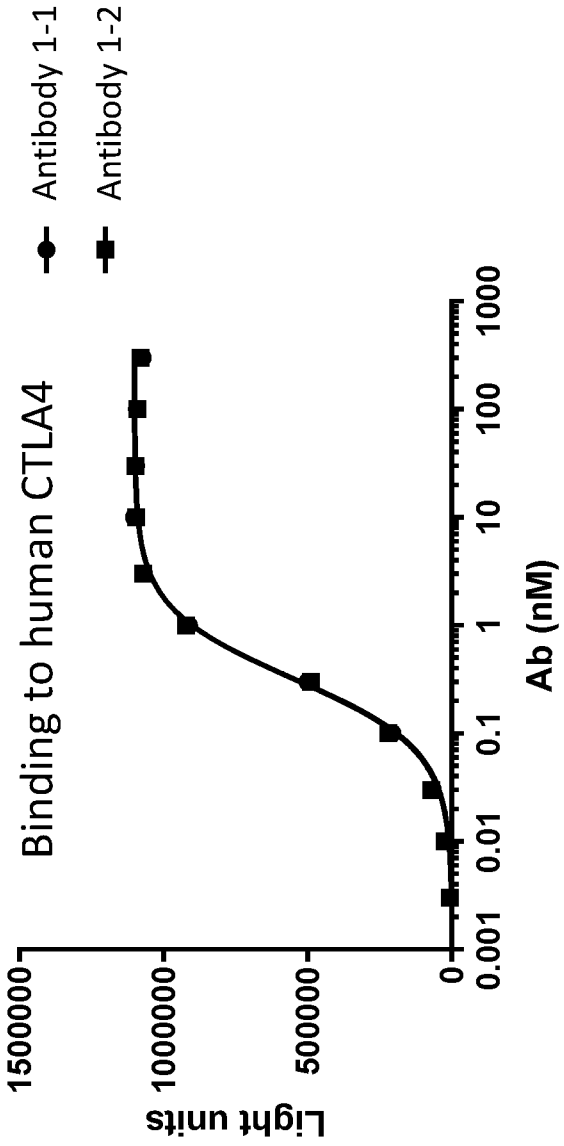

Binding to human CTLA4
Antibody 1-1
Antibody 1-2
Light units
1500000
1000000
500000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 9B

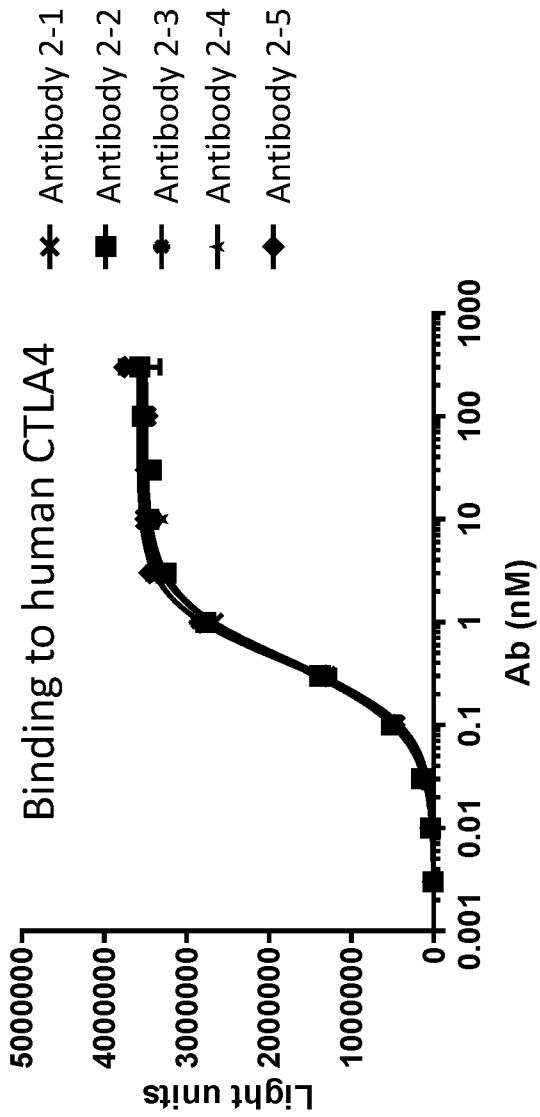

Binding to human CTLA4
Antibody 2-1
Antibody 2-2
Antibody 2-3
Antibody 2-4
Antibody 2-5
Light units
5000000
4000000
3000000
2000000
1000000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 10A

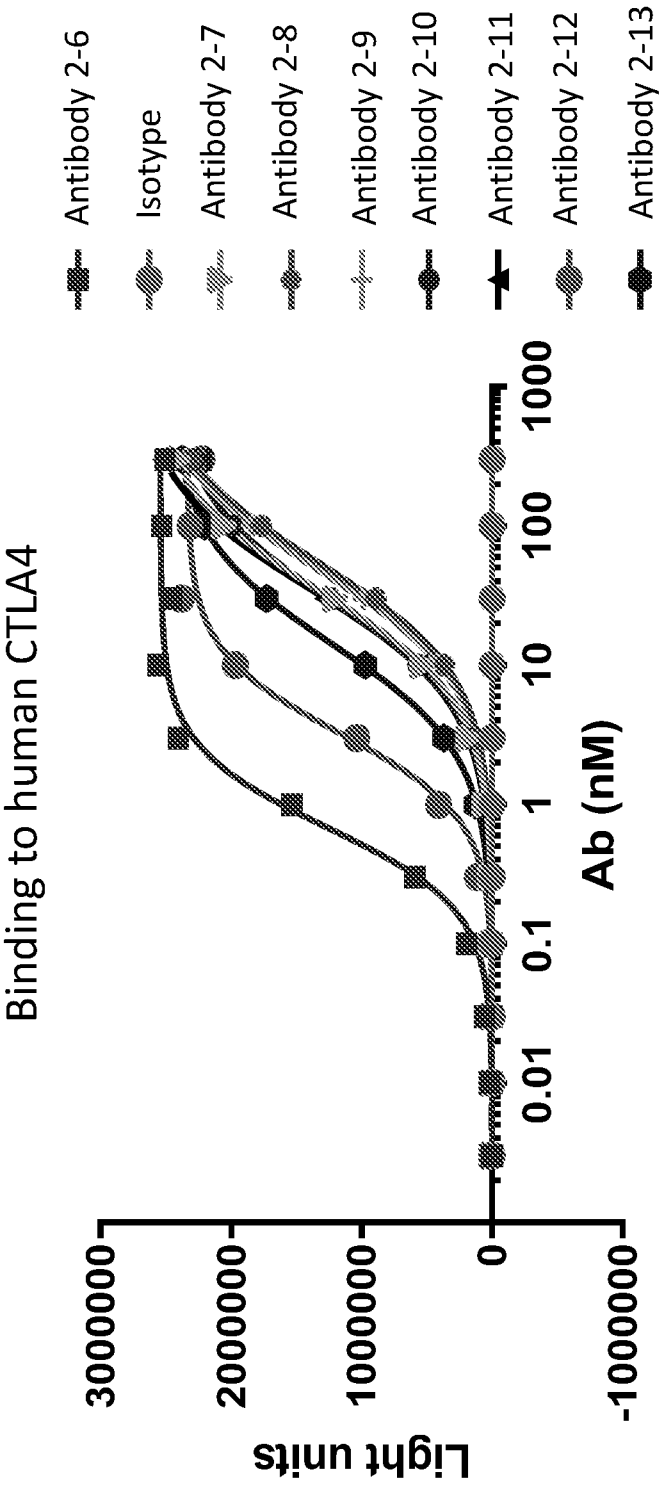

Binding to human CTLA4
Light units
3000000
2000000
1000000
0
-1000000
0.01
0.1
1
10
100
1000
Ab (nM)
Antibody 2-6
Isotype
Antibody 2-7
Antibody 2-8
Antibody 2-9
Antibody 2-10
Antibody 2-11
Antibody 2-12
Antibody 2-13

FIG. 10B

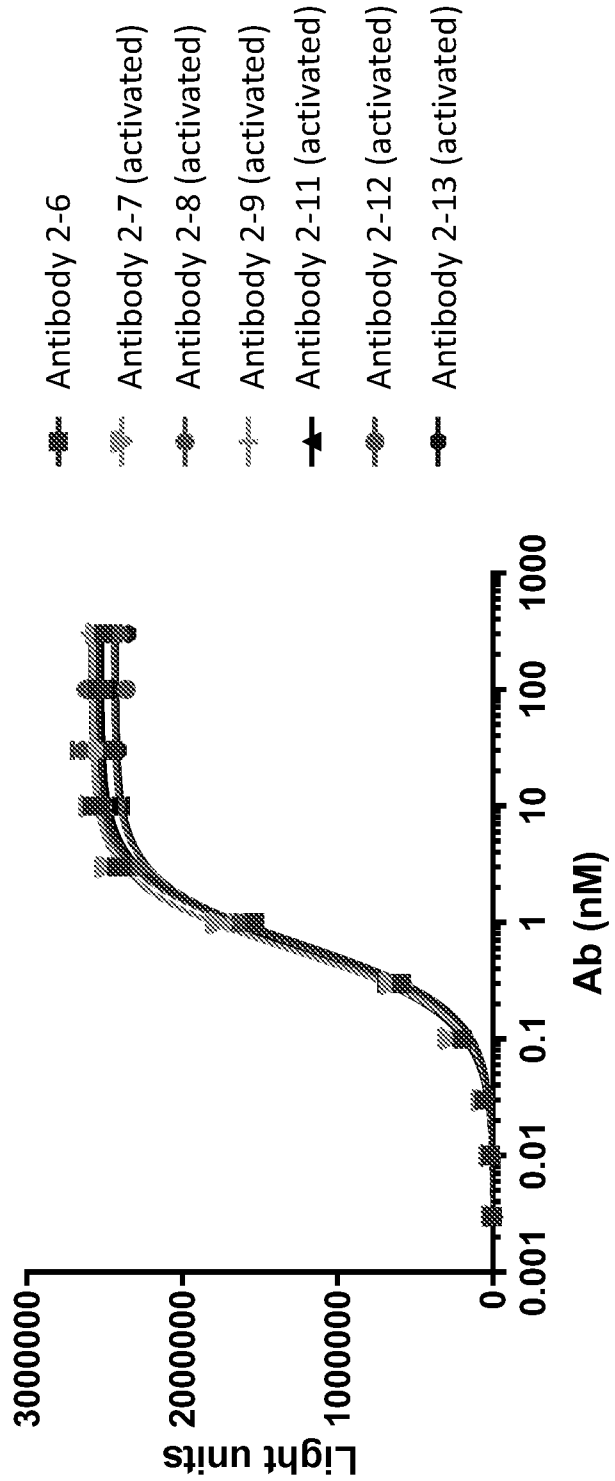

Binding to human CTLA4
Light units
3000000
2000000
1000000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)
Antibody 2-6
Antibody 2-7 (activated)
Antibody 2-8 (activated)
Antibody 2-9 (activated)
Antibody 2-11 (activated)
Antibody 2-12 (activated)
Antibody 2-13 (activated)

FIG. 11A Antibody 2-14
Cleavable Peptide : VPLSLY (SEQ ID NO: 86)

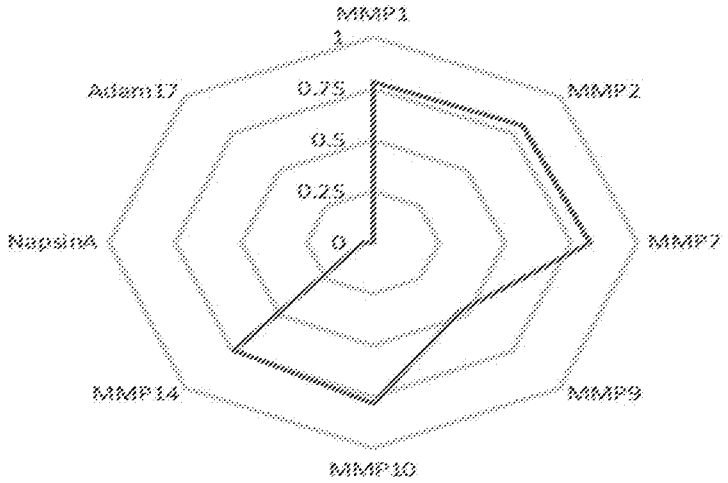


FIG. 11B Antibody 2-18
Cleavable Peptide: MPYDLYHP (SEQ ID NO: 47)

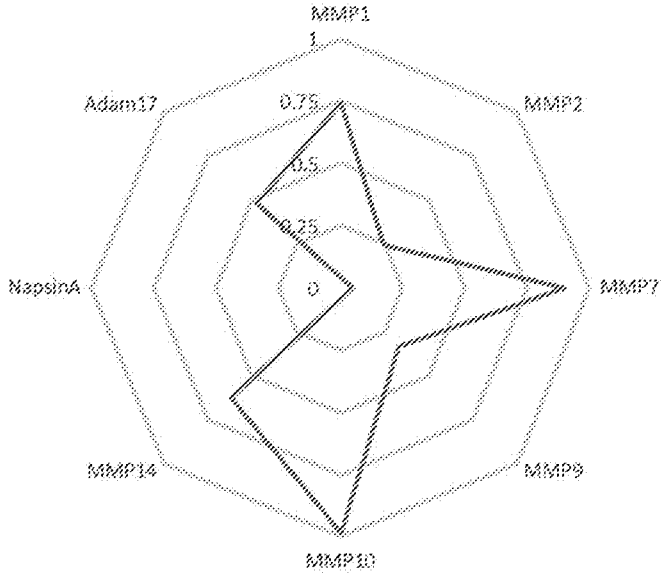


FIG. 11C Antibody 2-16
Cleavable Peptide: DSGGFMLT (SEQ ID NO: 50)

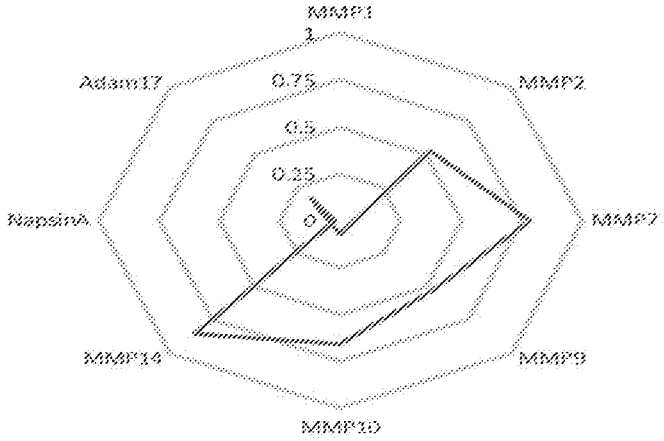


FIG. 11D Antibody 2-17
Cleavable Peptide: GGIGQLTA (SEQ ID NO: 48)

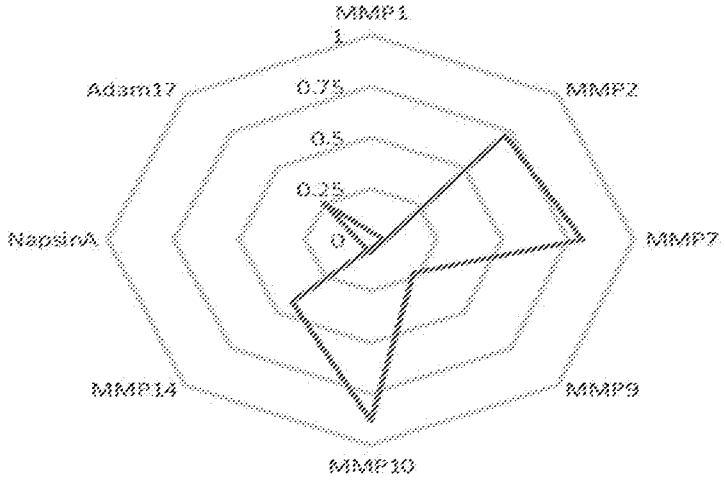


FIG. 11E Antibody 2-20
Cleavable Peptide: RAAAVKSP (SEQ ID NO: 72)

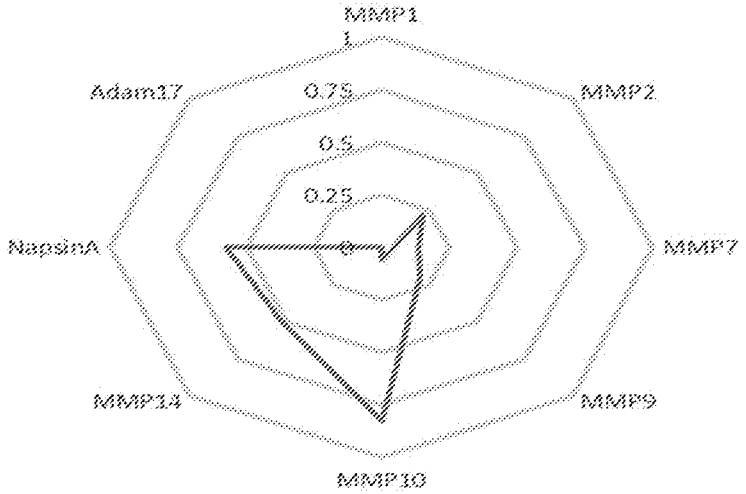


FIG. 11F Antibody 2-19
Cleavable Peptide: HEQLTV (SEQ ID NO: 57)

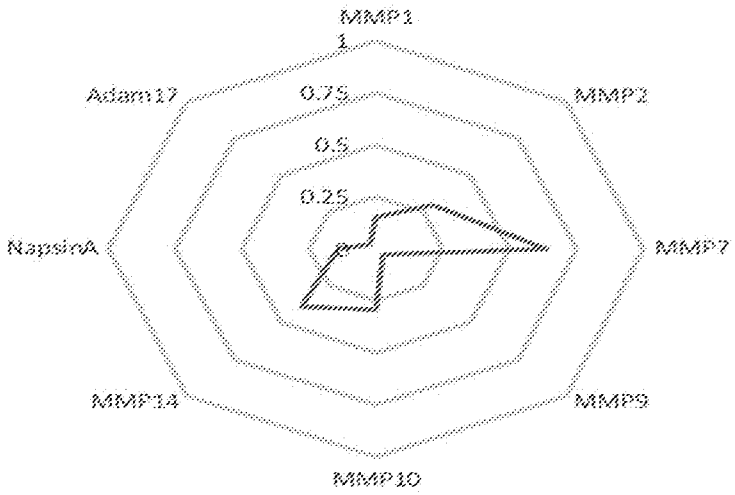


FIG. 11G Antibody 2-21
Cleavable Peptide: TSVLMAAP (SEQ ID NO: 51)

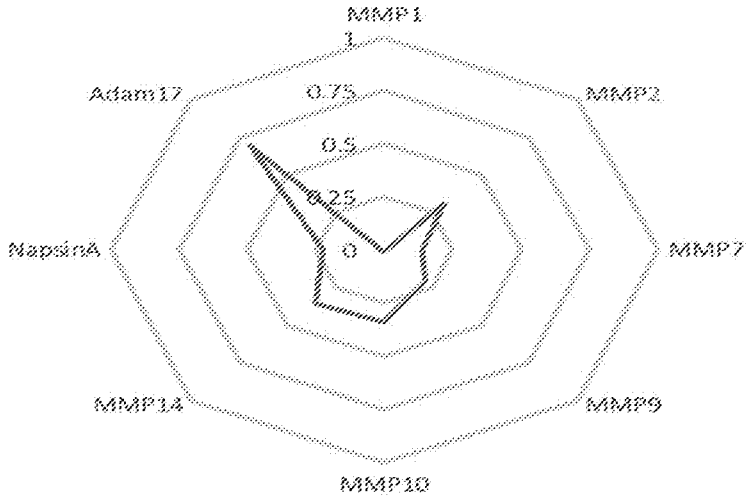


FIG. 11H Antibody 2-15
(Non)-Cleavable Peptide: GSGGSG (SEQ ID NO: 414)

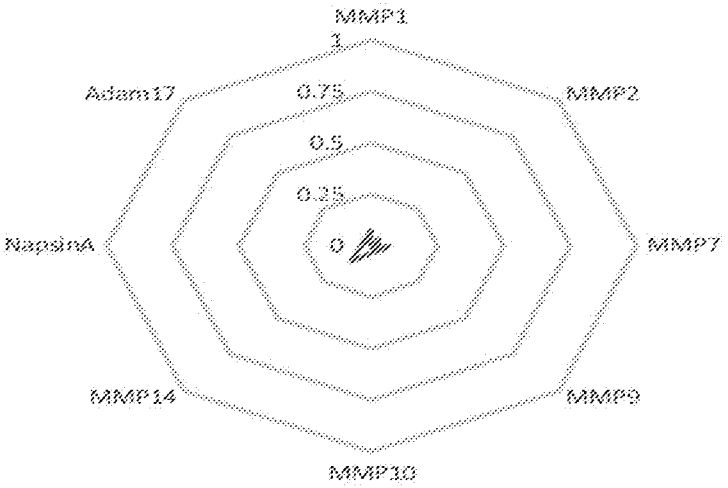


FIG. 11I

Antibody 2-14
Cleavable Peptide : VPLSLY (SEQ ID NO: 86)

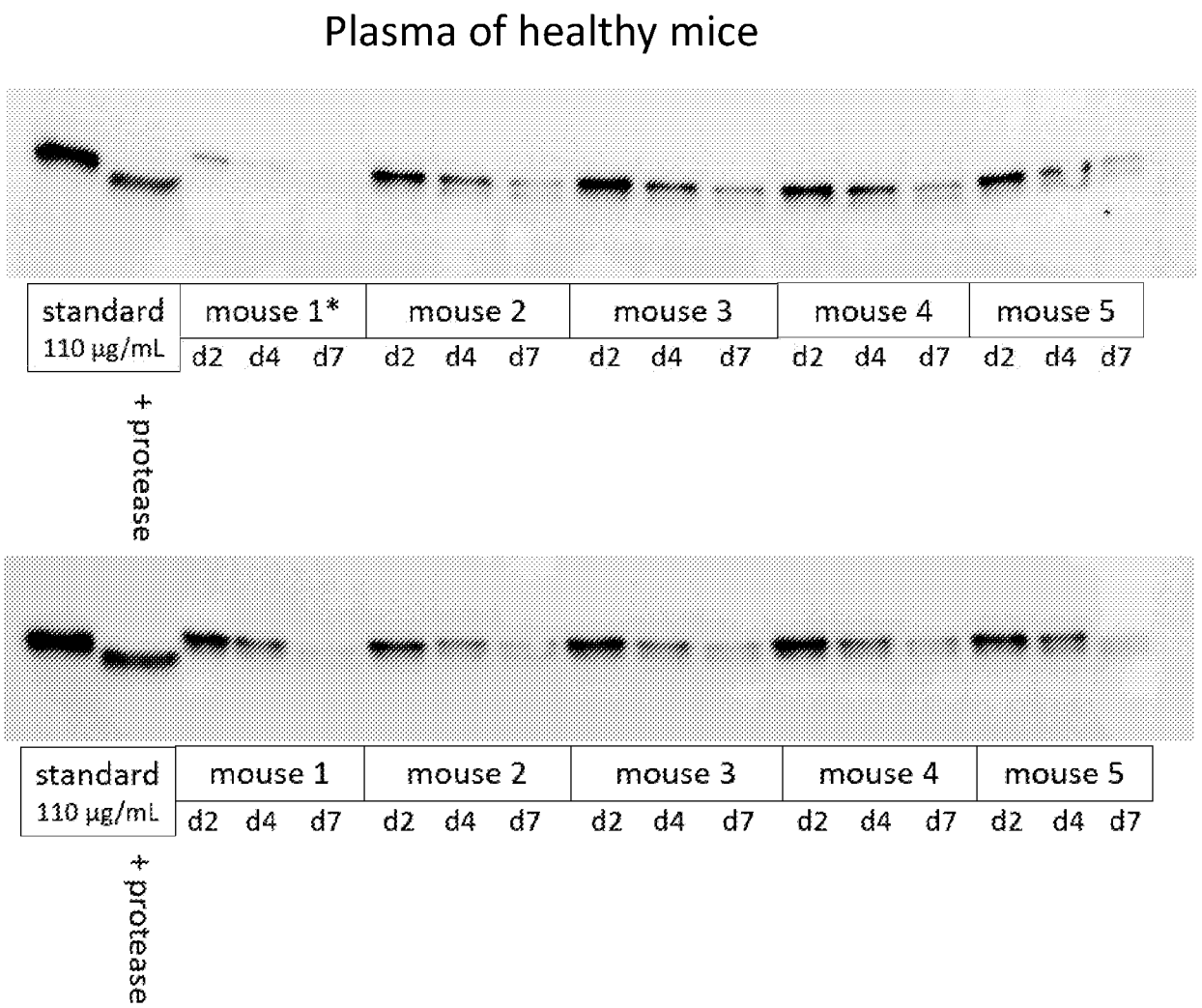


FIG. 11J

Antibody 2-16
Cleavable Peptide: DSGGFMLT (SEQ ID NO: 50)

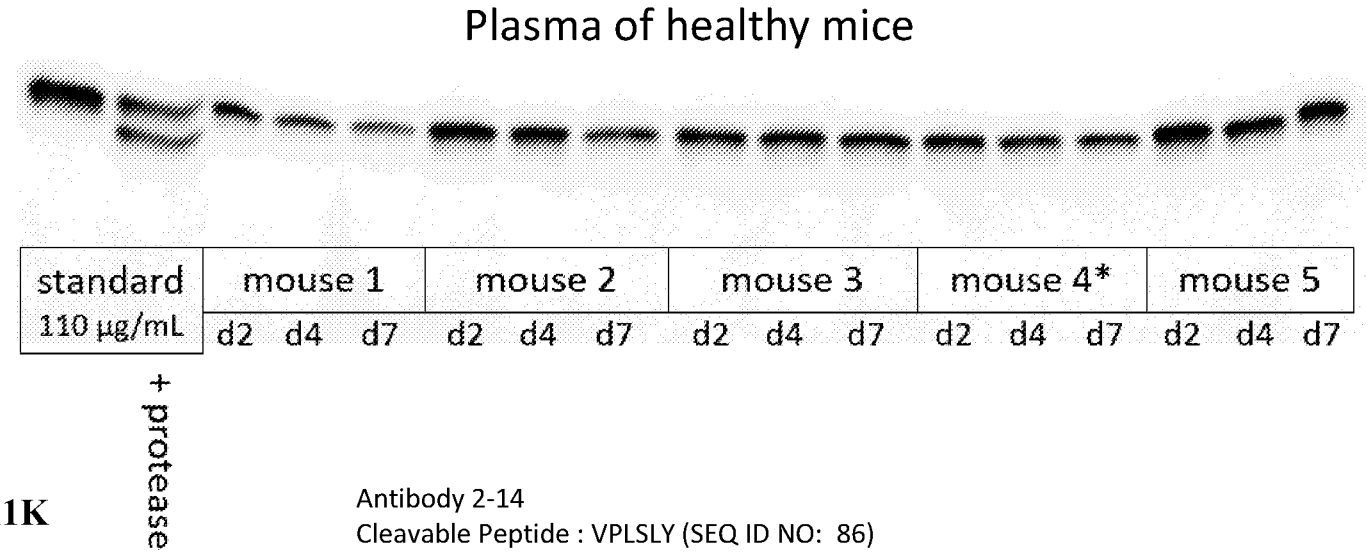


FIG. 11K

Antibody 2-14
Cleavable Peptide : VPLSLY (SEQ ID NO: 86)

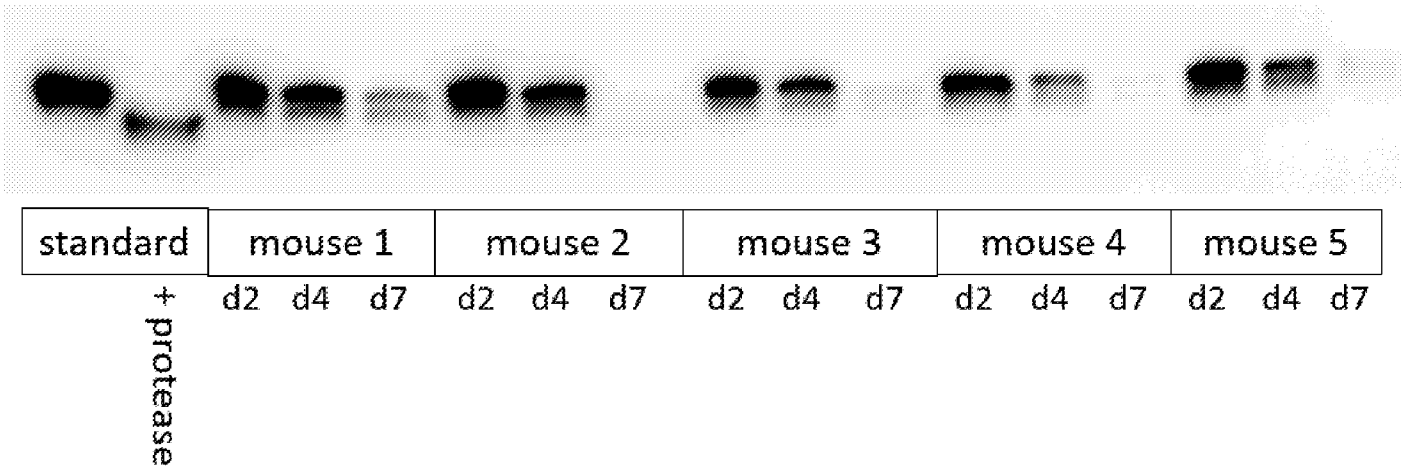


FIG. 11L

Antibody 2-15
(Non)-Cleavable Peptide: GSGGSG (SEQ ID NO: 414)

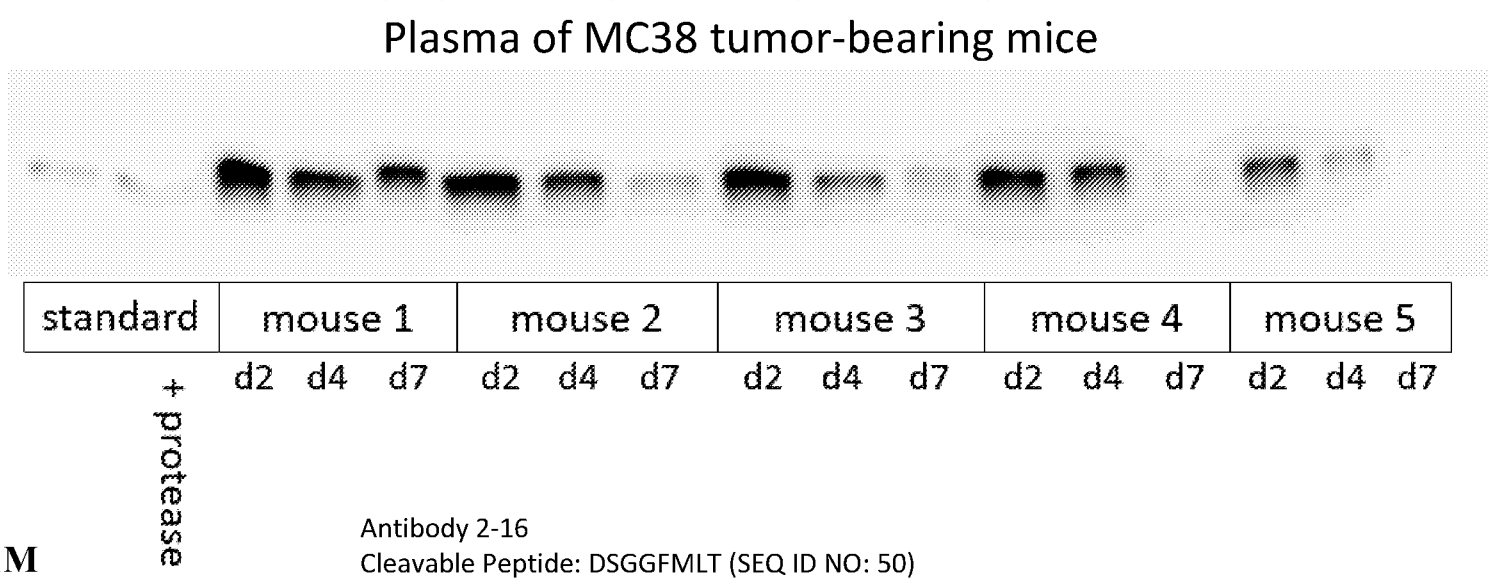


FIG. 11M

Antibody 2-16
Cleavable Peptide: DSGGFMLT (SEQ ID NO: 50)

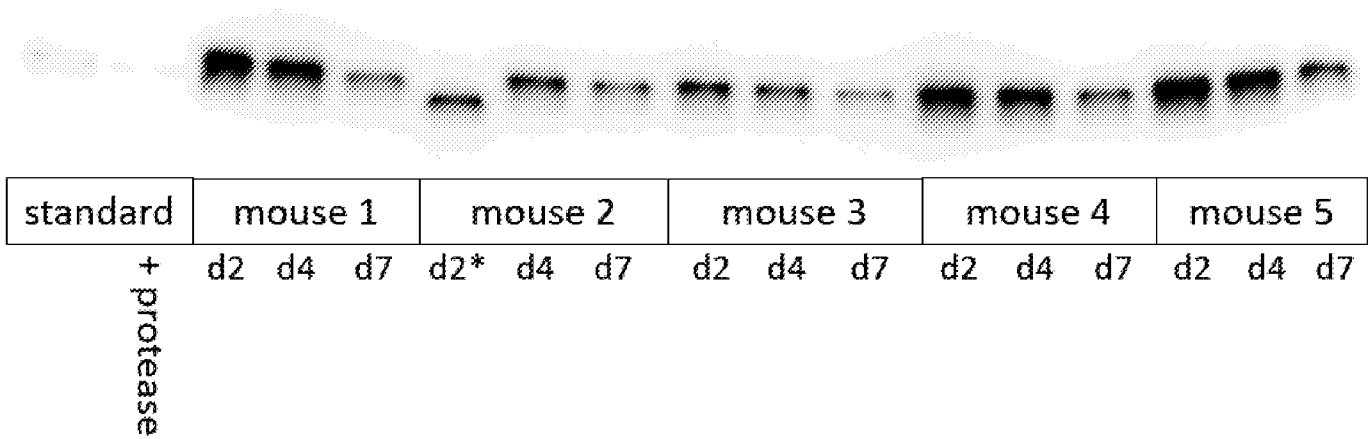


FIG. 12A

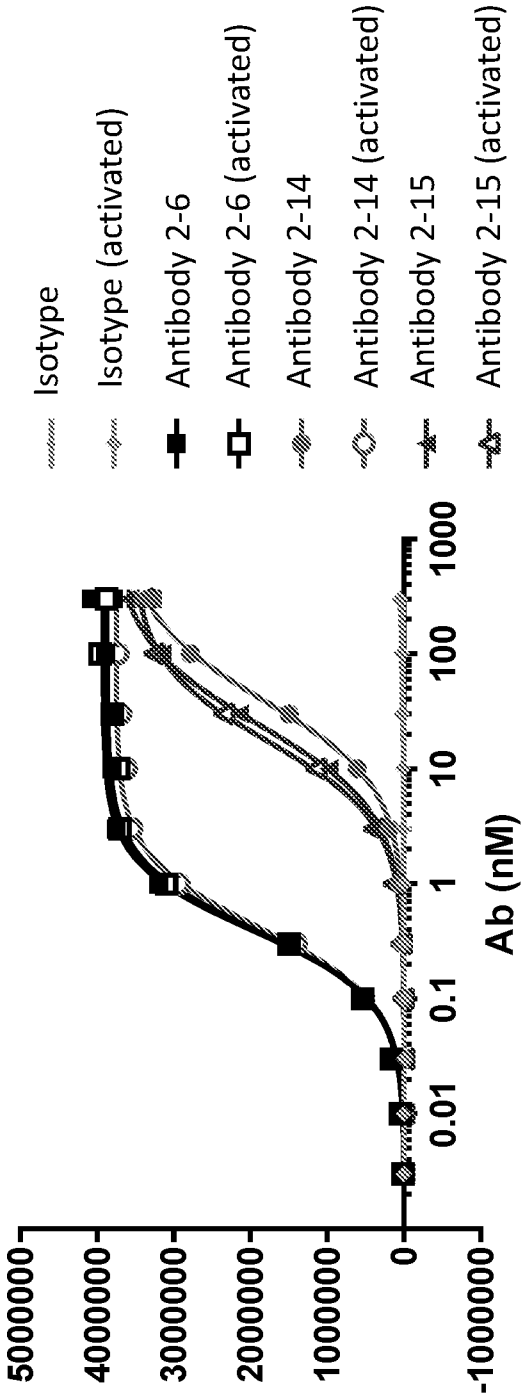


FIG. 12B

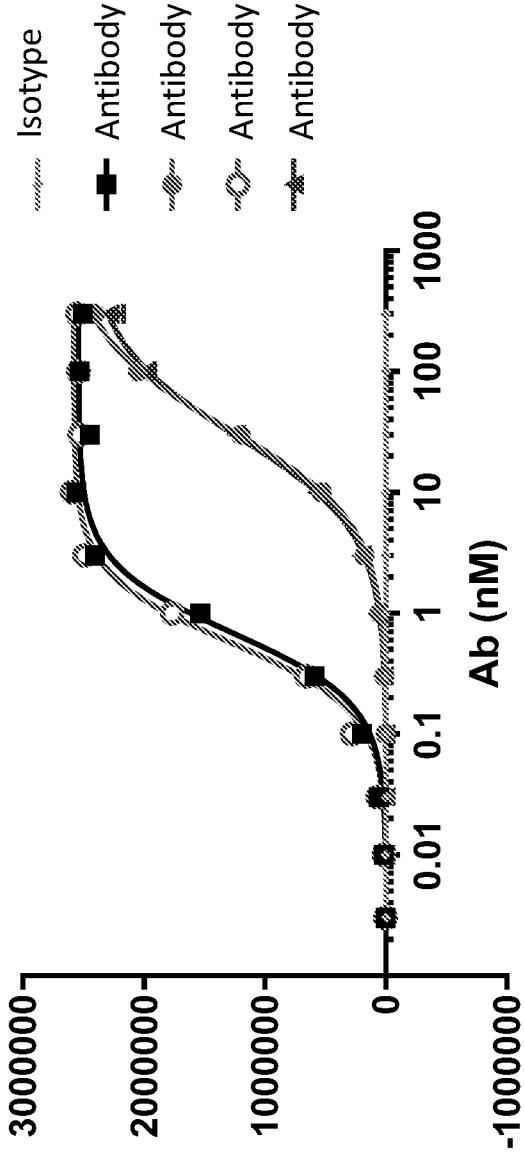


FIG. 13A

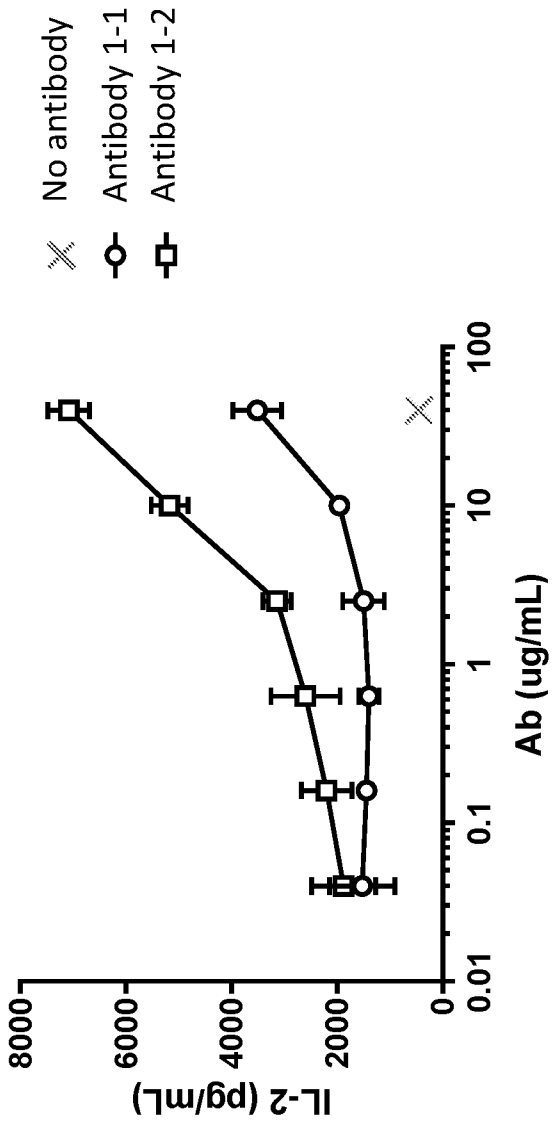


FIG. 13B

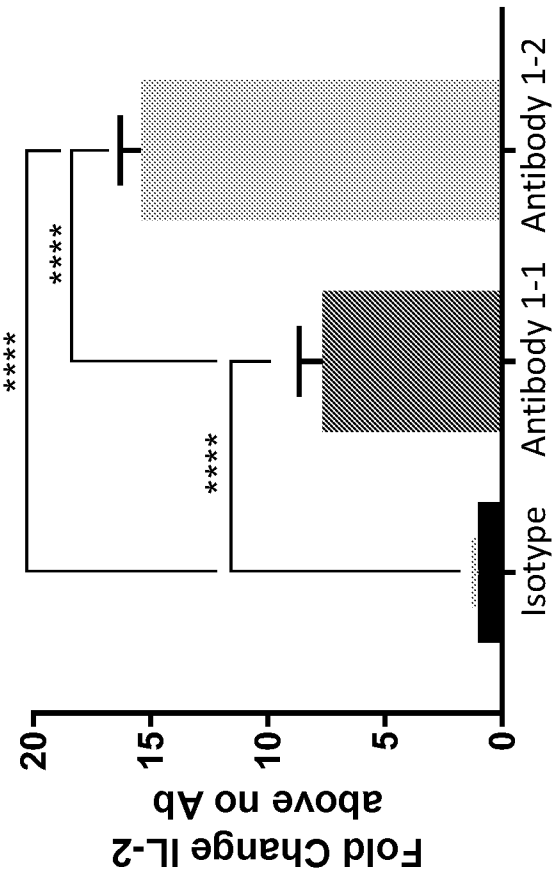


FIG. 13C

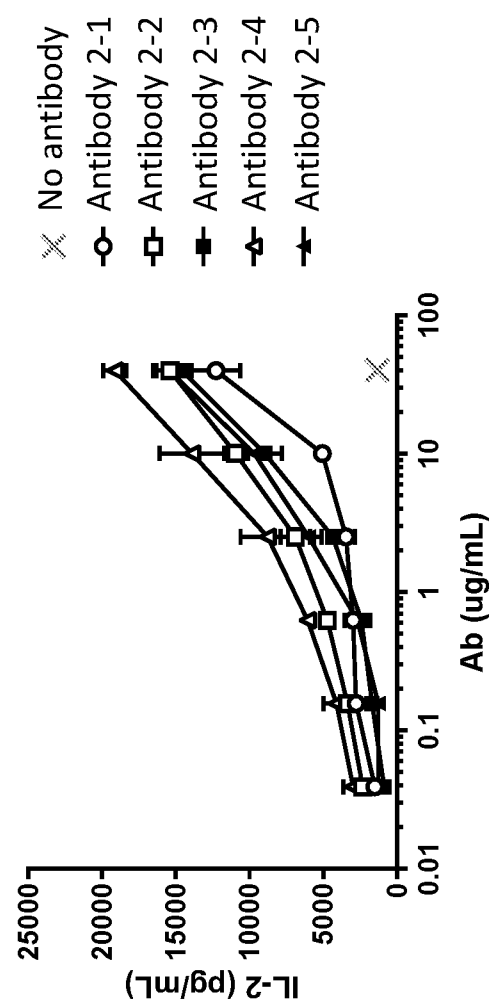


FIG. 13D

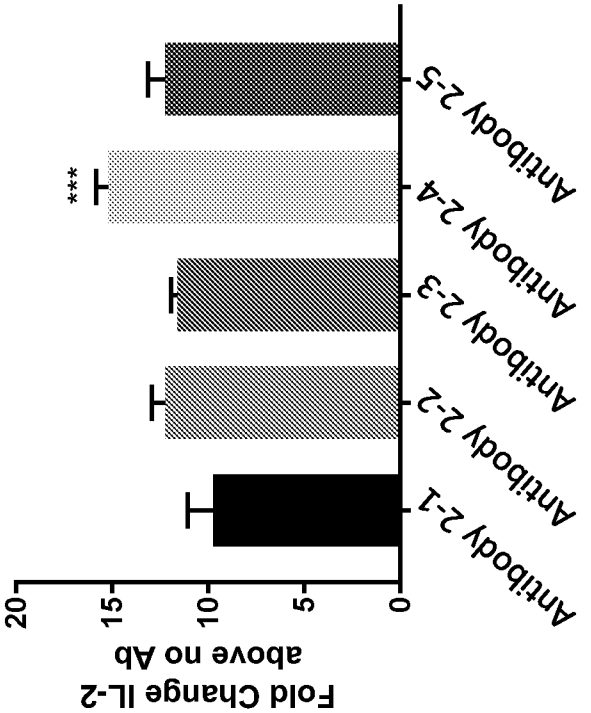
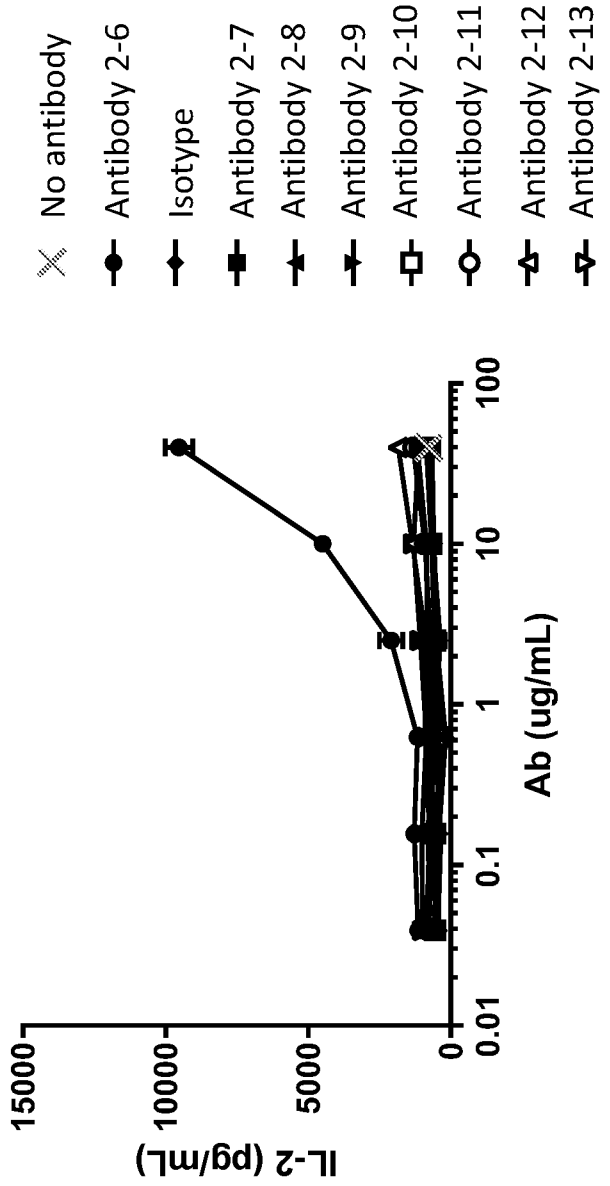


FIG. 14A


No antibody
Antibody 2-6
Isotype
Antibody 2-7
Antibody 2-8
Antibody 2-9
Antibody 2-10
Antibody 2-11
Antibody 2-12
Antibody 2-13
IL-2 (pg/mL)
15000
10000
5000
0
0.01
0.1
1
10
100
Ab (ug/mL)

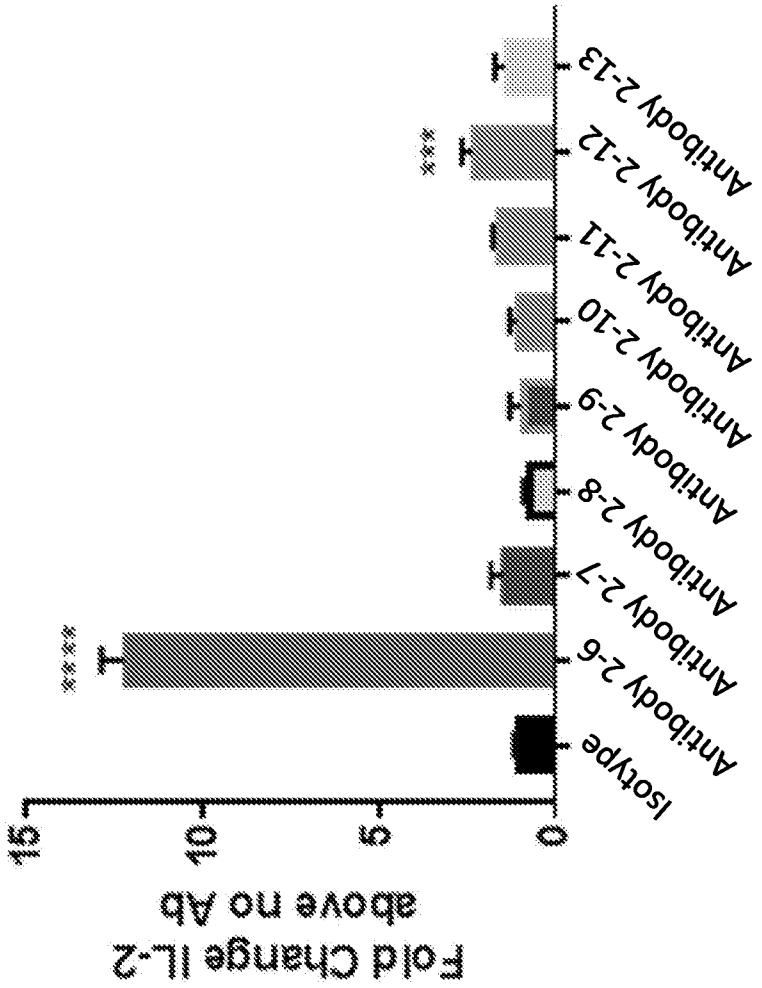

Fold Change IL-2
above no Ab
15
10
5
0
****
***
Isotype
Antibody 2-6
Antibody 2-7
Antibody 2-8
Antibody 2-9
Antibody 2-10
Antibody 2-11
Antibody 2-12
Antibody 2-13

FIG. 14B

FIG. 14C

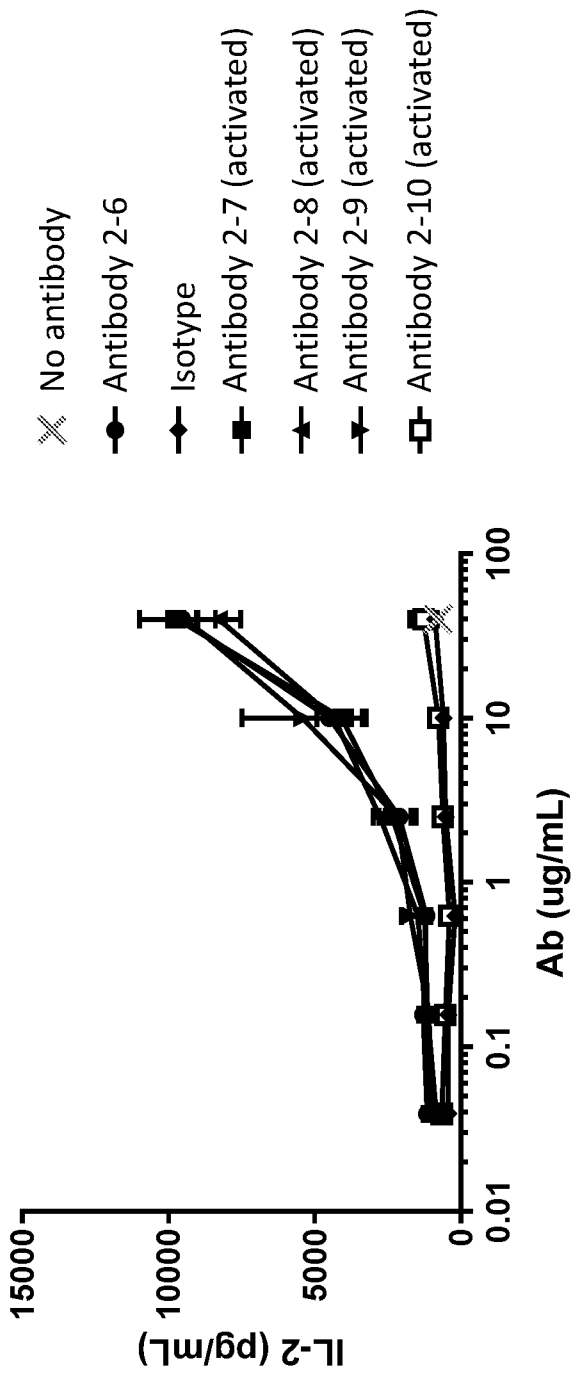

No antibody
Antibody 2-6
Isotype
Antibody 2-7 (activated)
Antibody 2-8 (activated)
Antibody 2-9 (activated)
Antibody 2-10 (activated)
IL-2 (pg/mL)
15000
10000
5000
0
0.01
0.1
1
10
100
Ab (ug/mL)

FIG. 14D

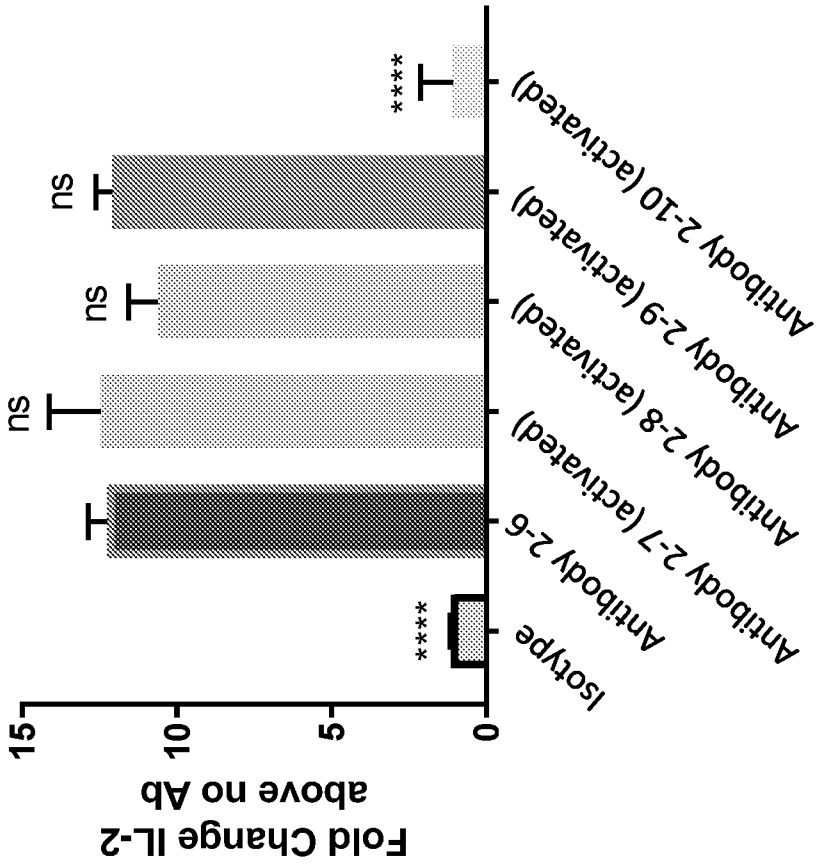

Fold Change IL-2
above no Ab
15
10
5
0
****
ns
ns
ns
****
Isotype
Antibody 2-6
Antibody 2-7 (activated)
Antibody 2-8 (activated)
Antibody 2-9 (activated)
Antibody 2-10 (activated)

FIG. 15A

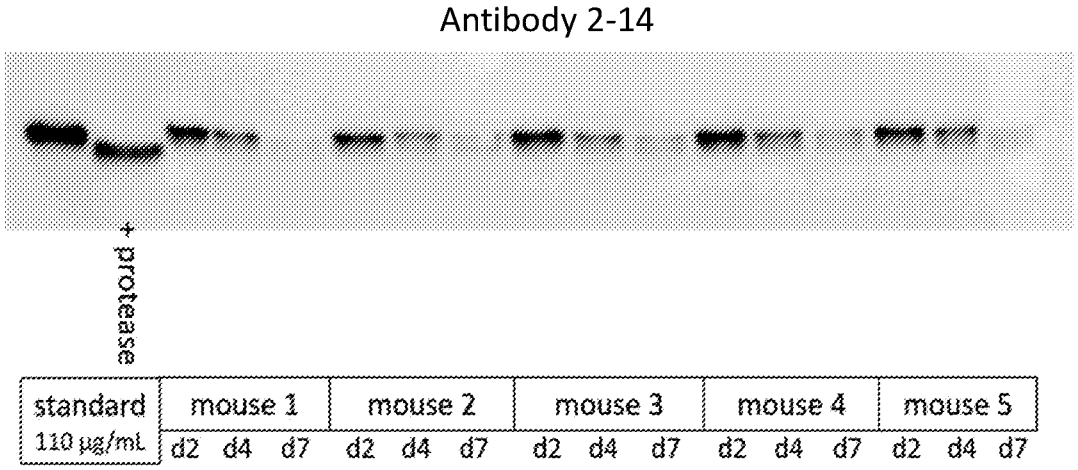


FIG. 15B

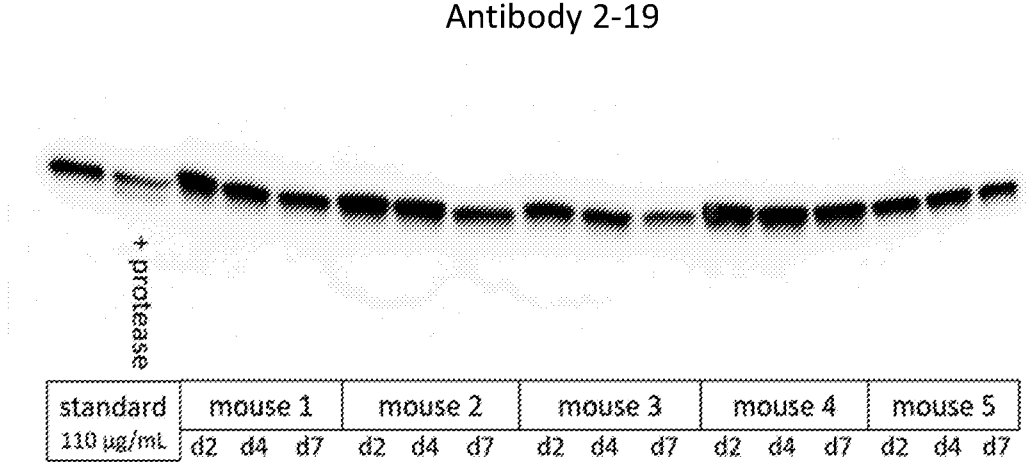


FIG. 15C

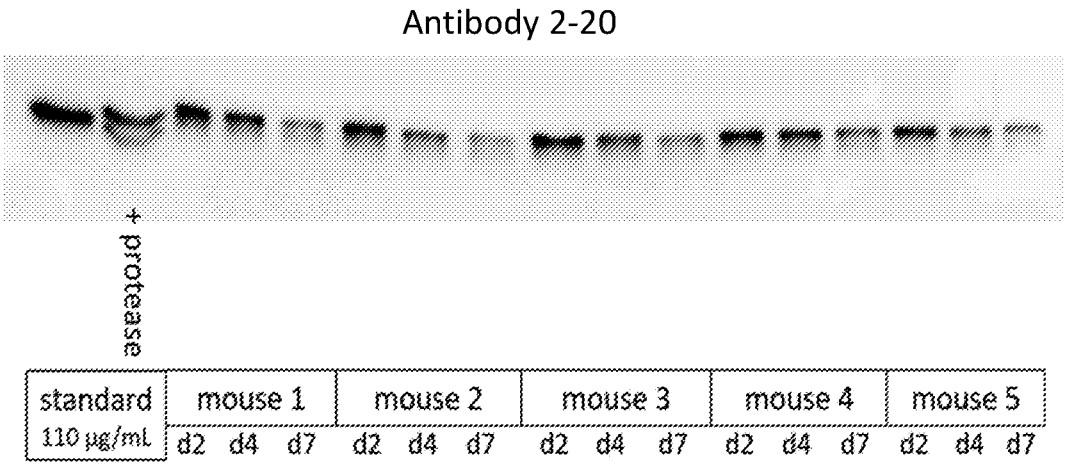


FIG. 15D

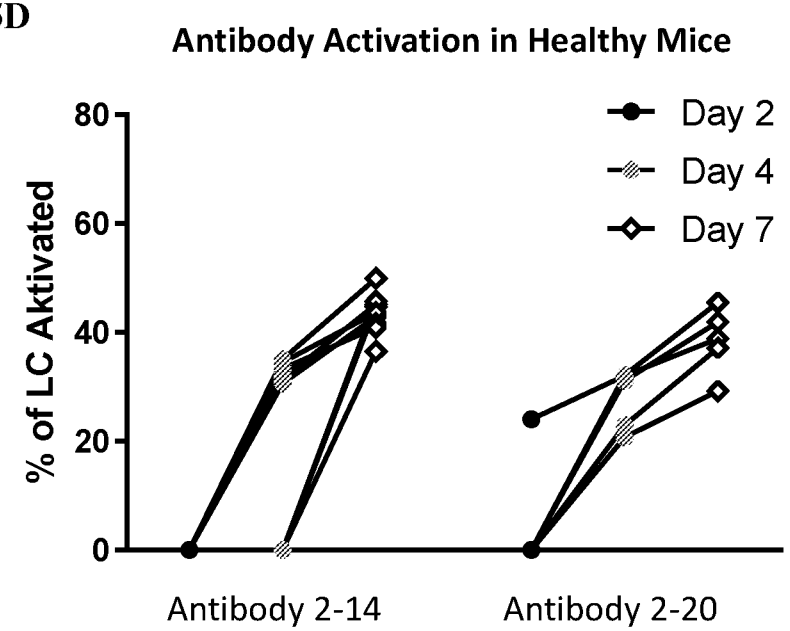


FIG. 16A

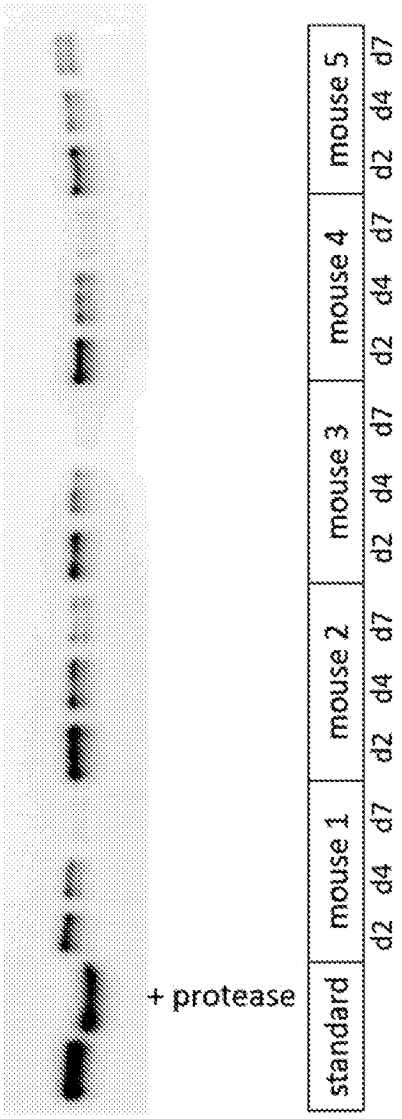


FIG. 16B

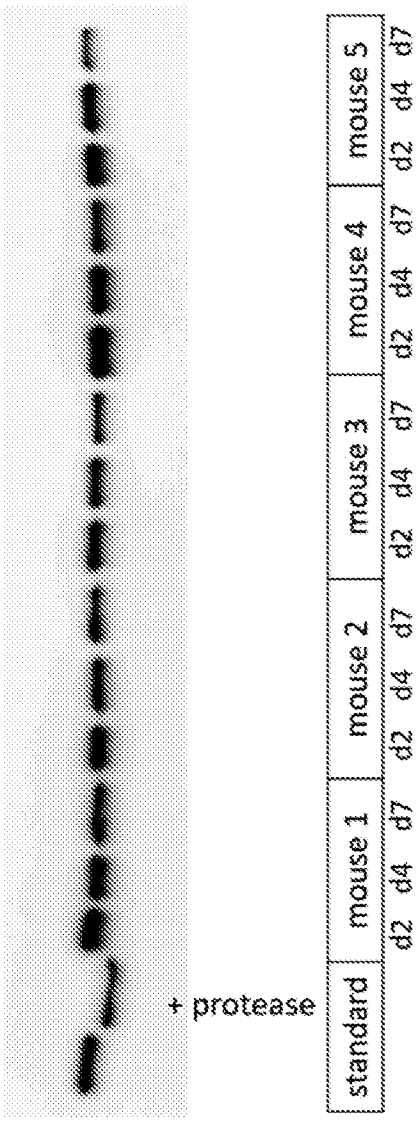


FIG. 16C

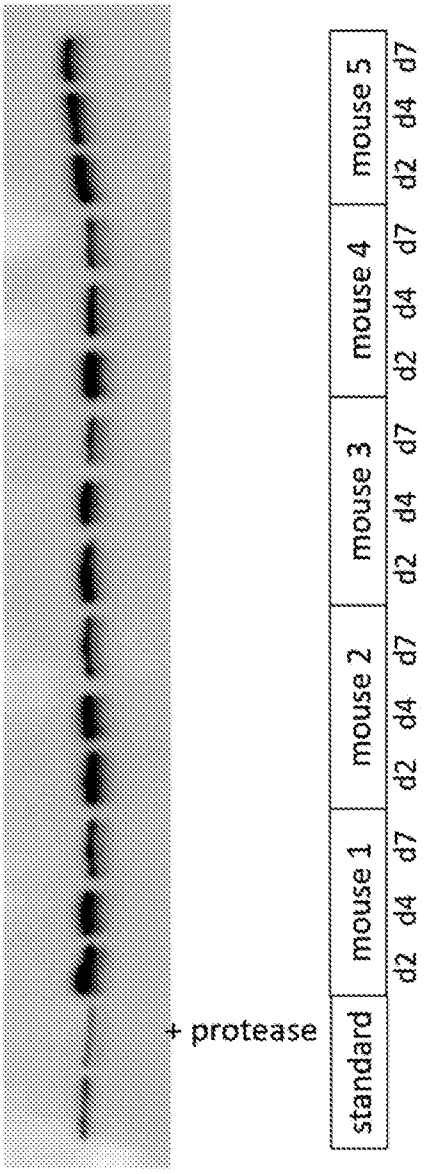


FIG. 16D

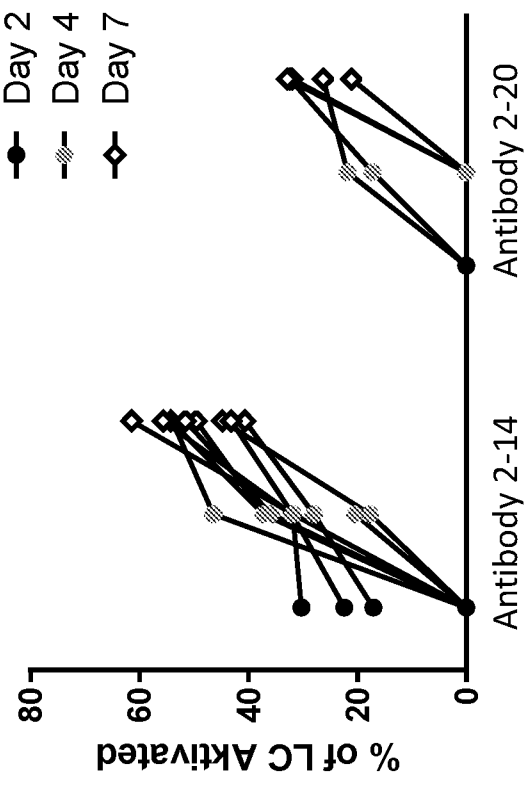


FIG. 17

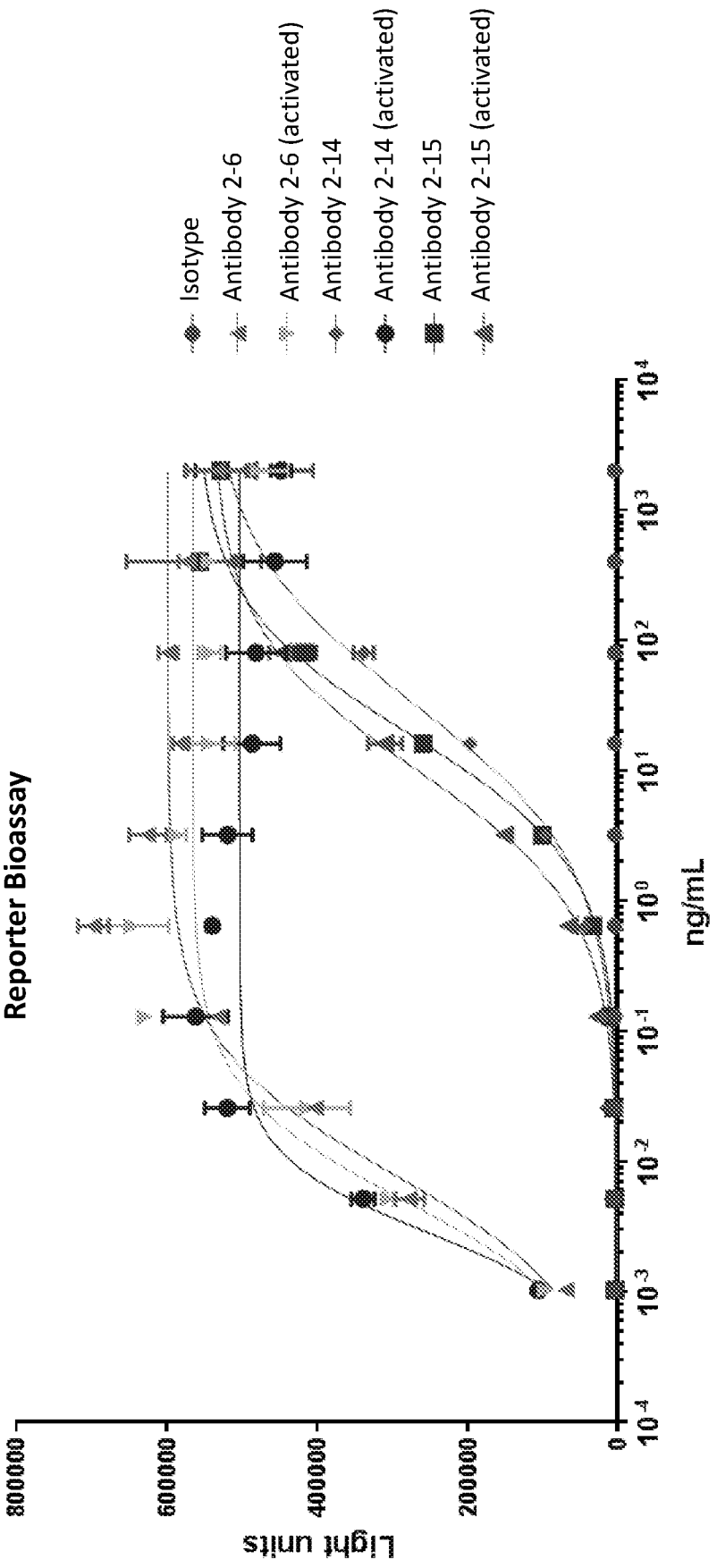
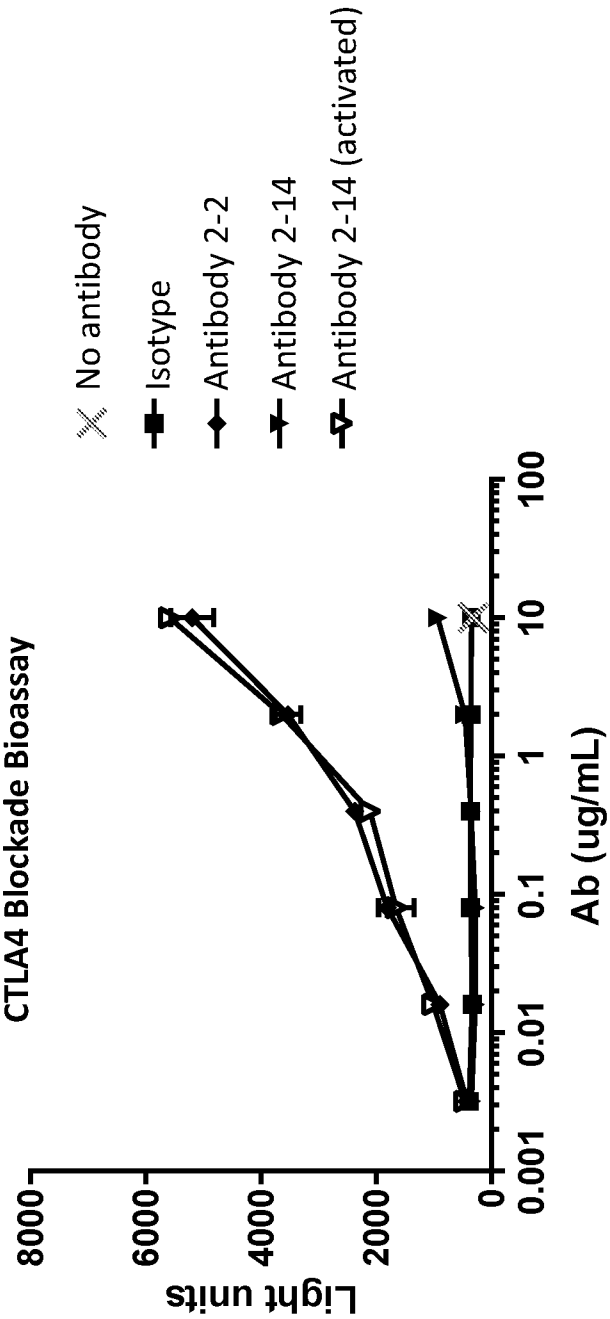

Reporter Bioassay
Light units
800000
600000
400000
200000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
ng/mL
Isotype
Antibody 2-6
Antibody 2-6 (activated)
Antibody 2-14
Antibody 2-14 (activated)
Antibody 2-15
Antibody 2-15 (activated)

FIG. 18A


CTLA4 Blockade Bioassay
No antibody
Isotype
Antibody 2-2
Antibody 2-14
Antibody 2-14 (activated)
Light units
8000
6000
4000
2000
0
0.001
0.01
0.1
1
10
100
Ab (ug/mL)

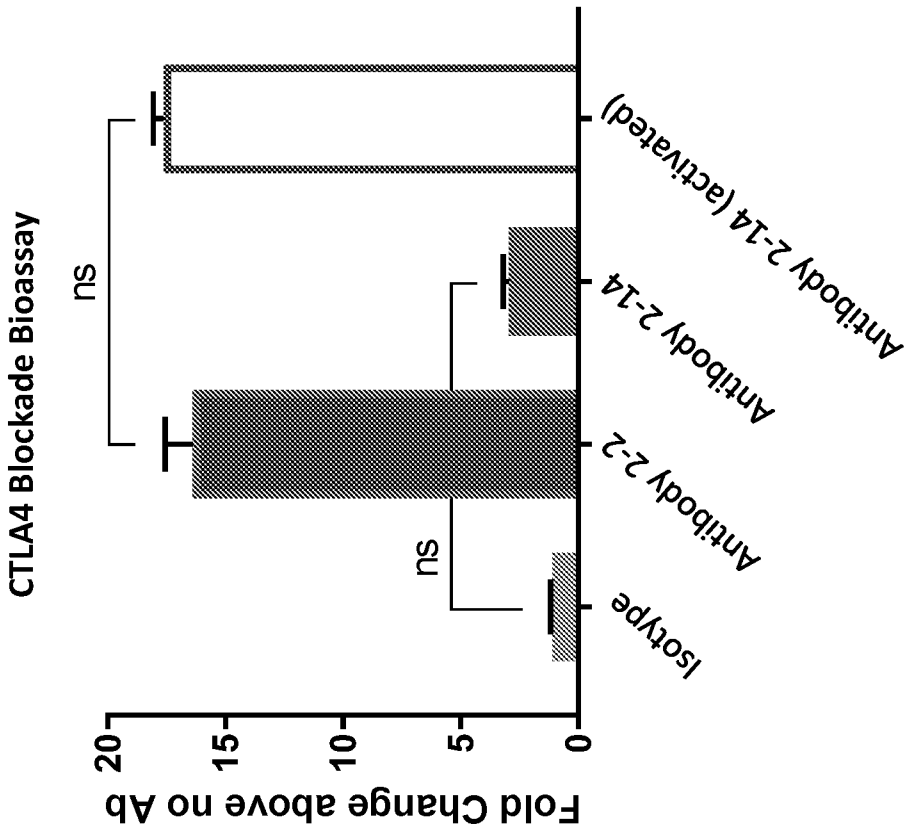

CTLA4 Blockade Bioassay
Fold Change above no Ab
0
5
10
15
20
ns
ns
Isotype
Antibody 2-2
Antibody 2-14
Antibody 2-14 (activated)

FIG. 18B

FIG. 19A

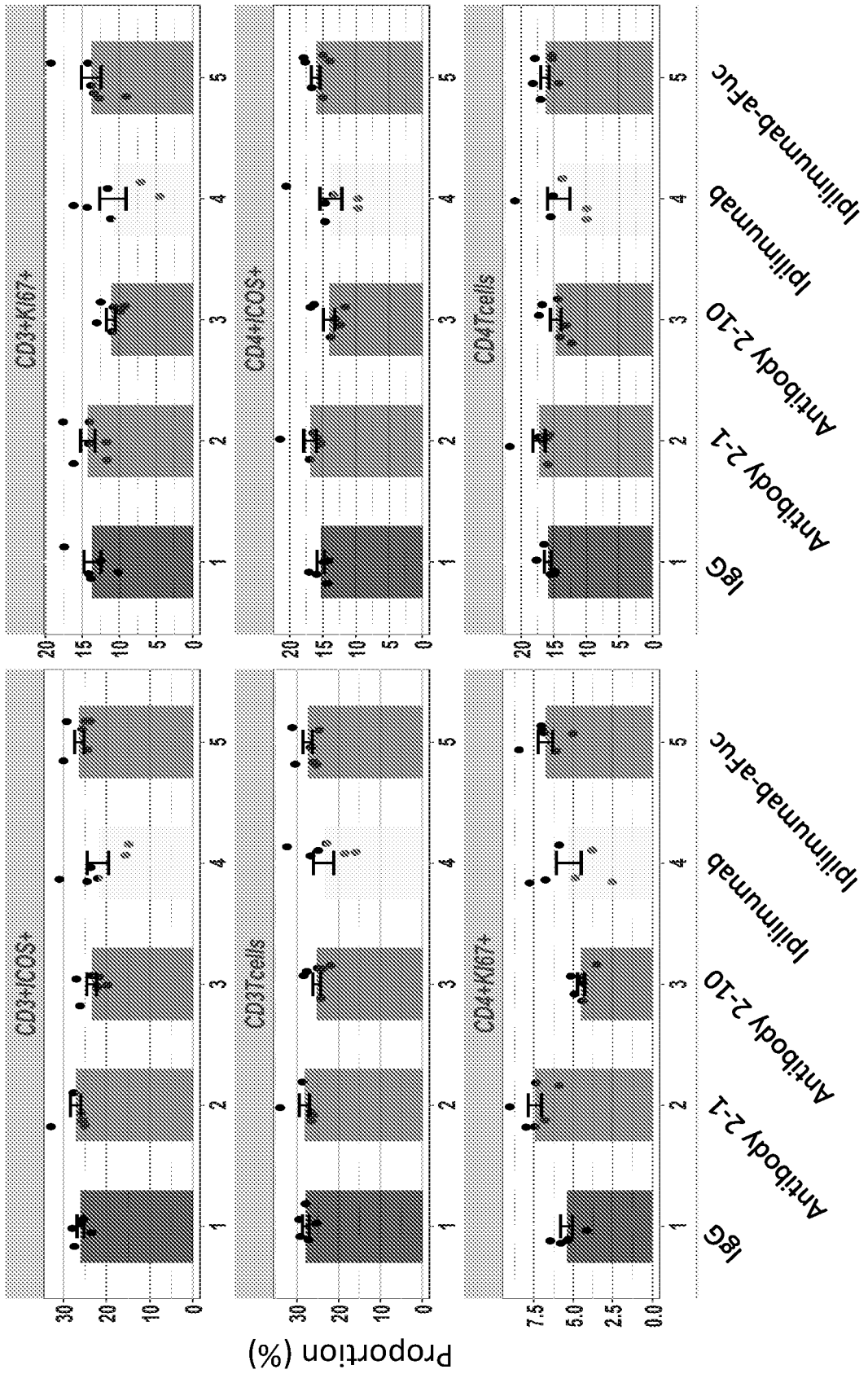

Splenic CD45+ Cells
Proportion (%)
CD3+KI67+
CD3+ICOS+
CD4+ICOS+
CD3Tcells
CD4Tcells
CD4+KI67+
IgG
Antibody 2-1
Antibody 2-10
Ipilimumab
Ipilimumab-aFuc

FIG. 19B

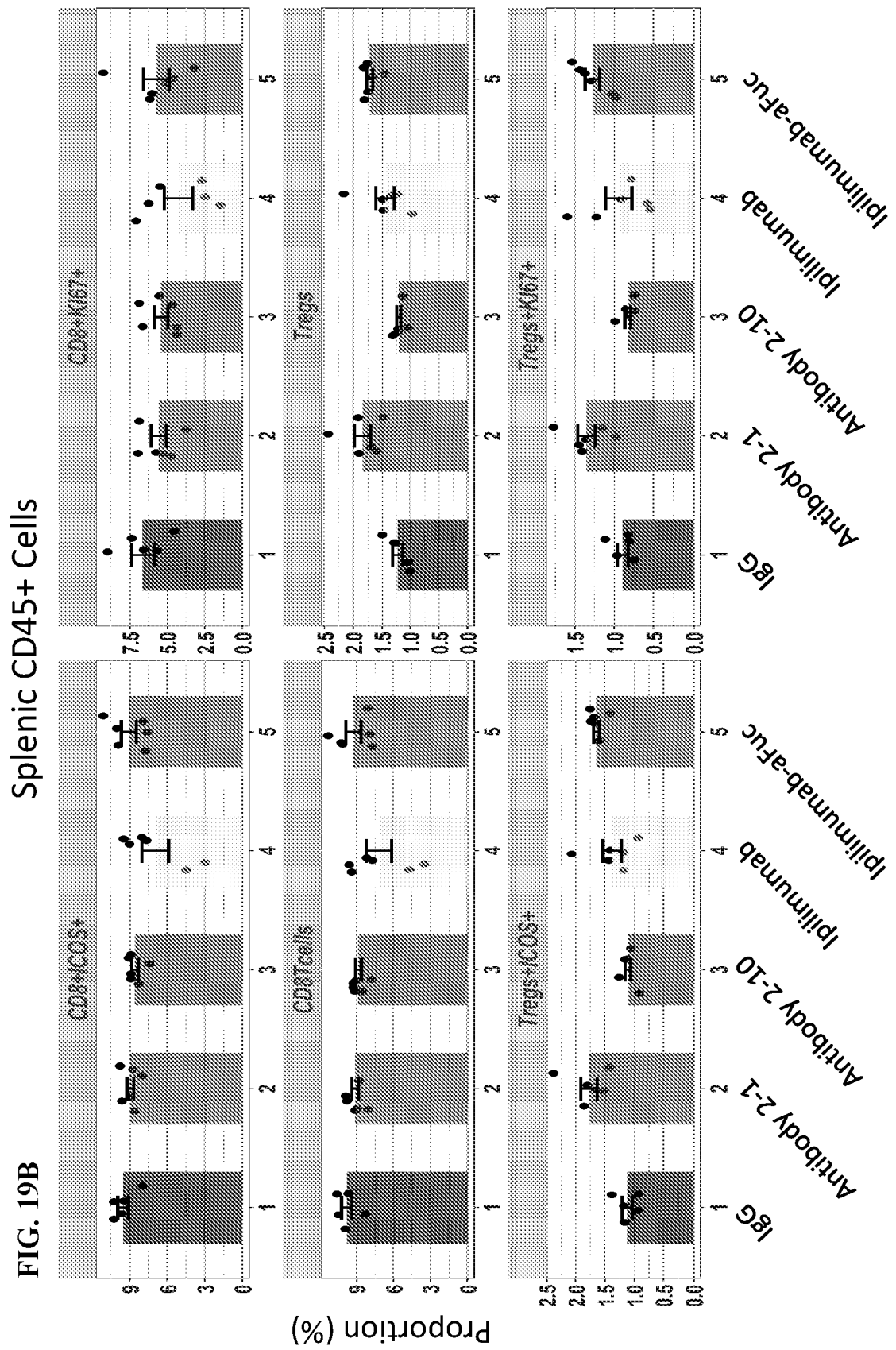

Splenic CD45+ Cells
CD8+ICOS+
CD8+Ki67+
CD8Tcells
Tregs
Tregs+ICOS+
Tregs+Ki67+
Proportion (%)
IgG
Antibody 2-1
Antibody 2-10
Ipilimumab
Ipilimumab-aFuc

FIG. 19C

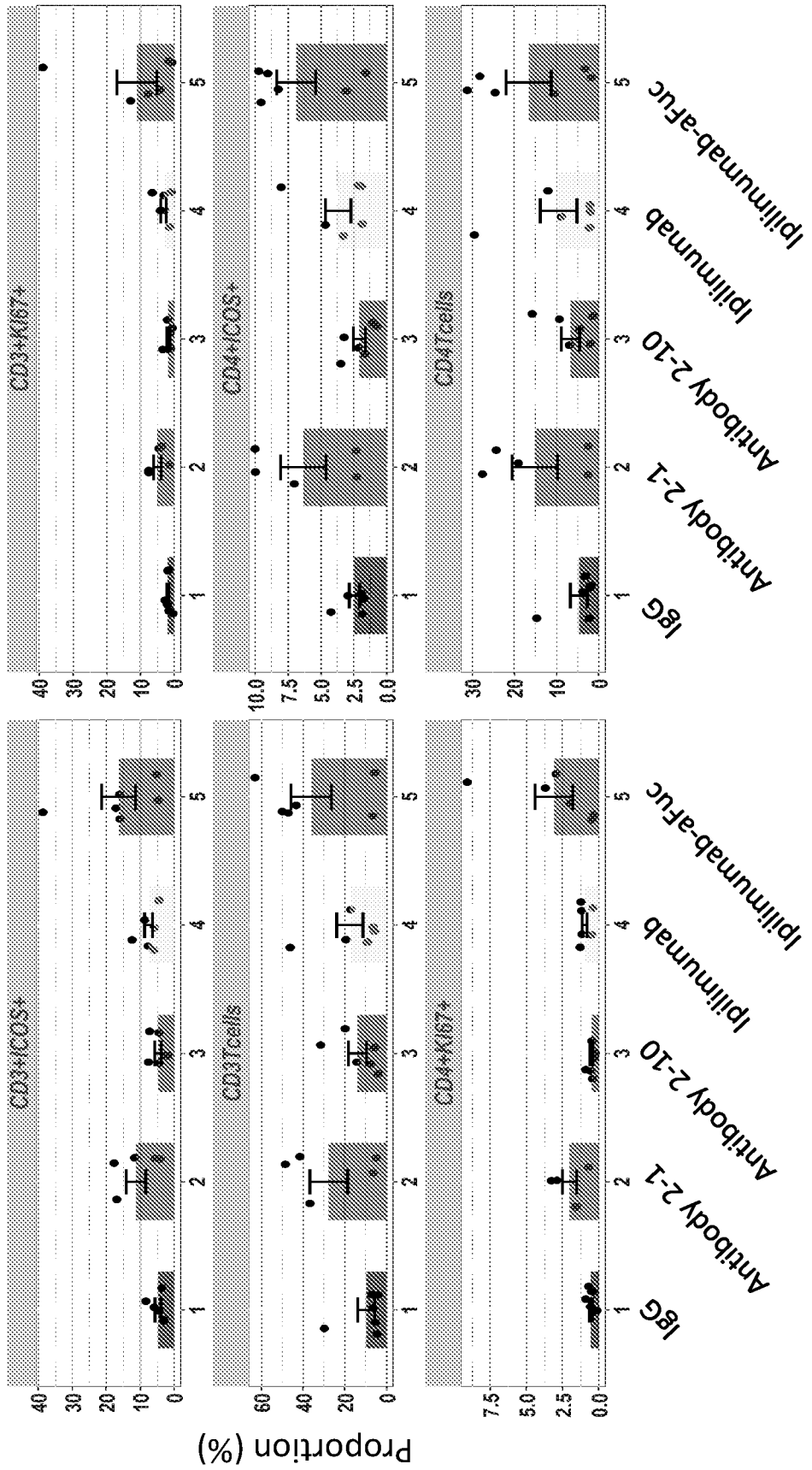

Intratumoral CD45+ Cells
Proportion (%)
CD3+ICOS+
CD3+KI67+
CD3Tcells
CD4+ICOS+
CD4+KI67+
CD4Tcells
IgG
Antibody 2-1
Antibody 2-10
Ipilimumab
Ipilimumab-aFuc

FIG. 19D

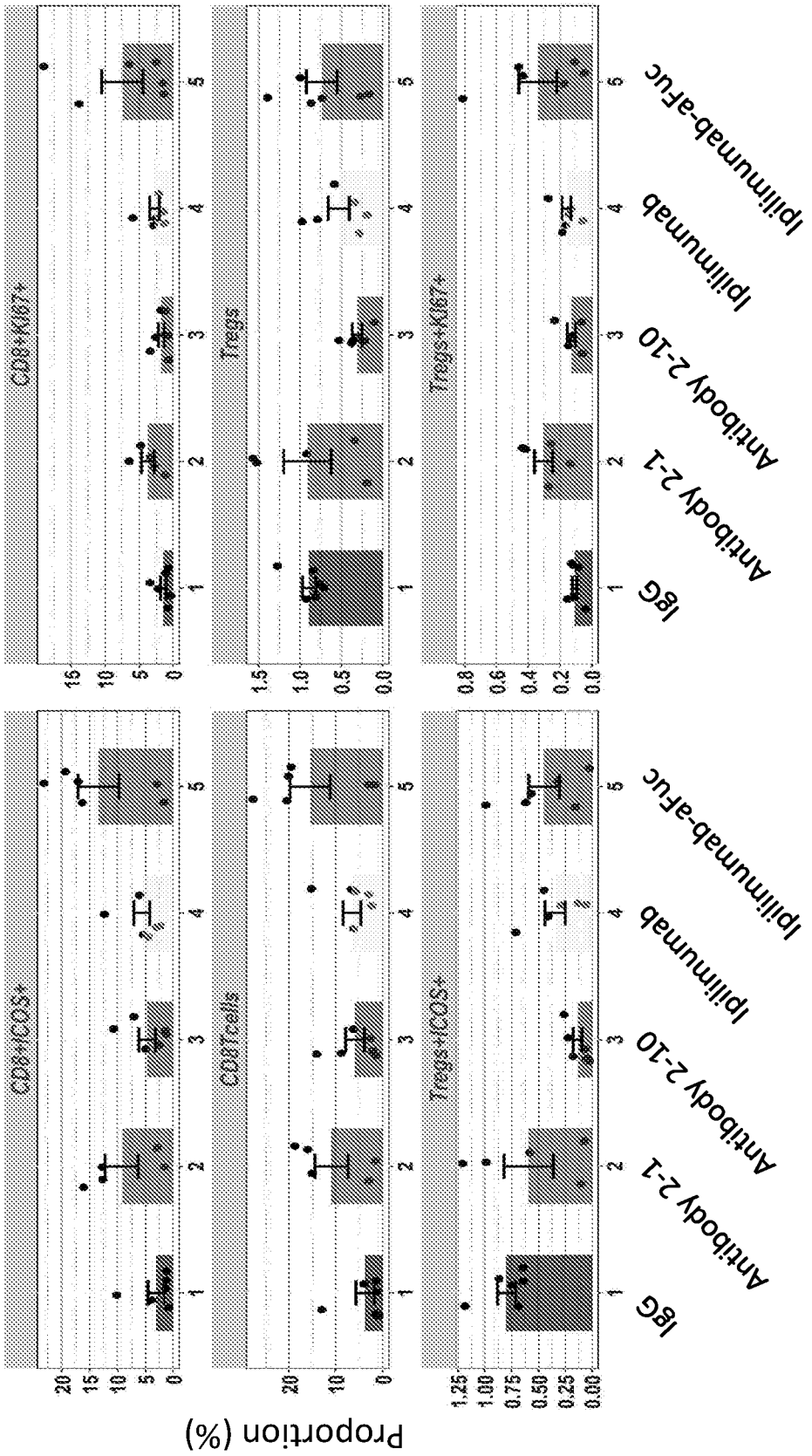

Intratumoral CD45+ Cells
Proportion (%)
CD8+ICOS+
CD8Tcells
Tregs+ICOS+
CD8+Ki67+
Tregs
Tregs+Ki67+
IgG
Antibody 2-1
Antibody 2-10
Ipilimumab
Ipilimumab-aFuc

FIG. 19E Splenic

Key for statistics and bar graph:

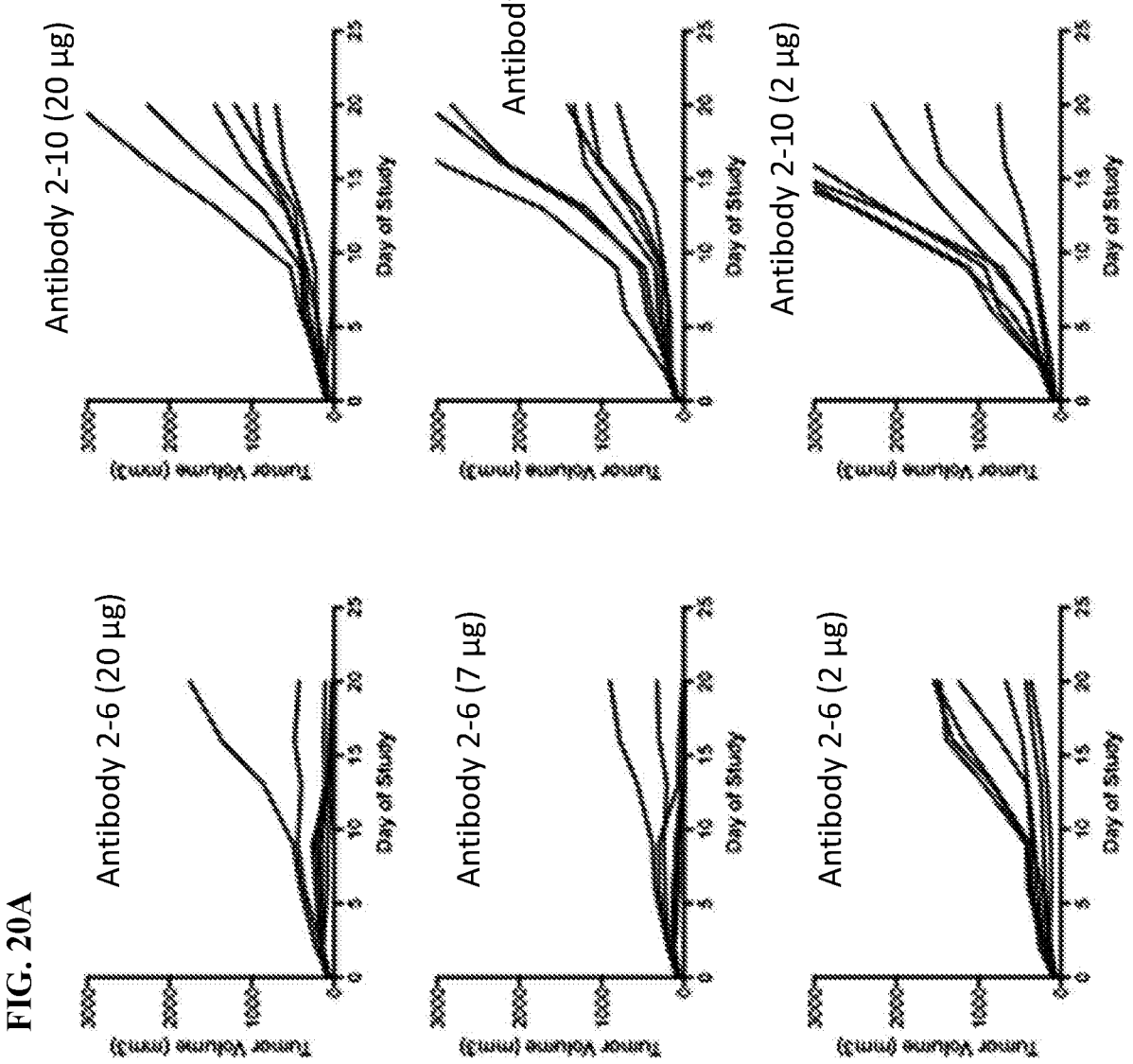
	Bartlett	NOVA/Krusk	1 VS 2	1 VS 3	1 VS 4	1 VS 5	2 VS 3	2 VS 4	2 VS 5	3 VS 4	3 VS 5	4 VS 5
CD3+ICOS+	0.11	0.10	0.48	0.09	0.16	0.82	0.05	0.10	0.65	0.60	0.09	0.14
CD3+KI67+	0.25	0.18	0.70	0.10	0.23	0.94	0.02	0.13	0.78	0.90	0.11	0.22
CD3Tcells	0.09	0.16	0.83	0.07	0.14	0.76	0.10	0.13	0.66	0.54	0.18	0.19
CD4+ICOS+	0.14	0.19	0.18	0.25	0.41	0.41	0.05	0.14	0.48	0.90	0.10	0.24
CD4+KI67+	0.13	0.00	0.01	0.08	0.88	0.05	0.00	0.05	0.31	0.37	0.00	0.15
CD4Tcells	0.10	0.25	0.25	0.25	0.38	0.62	0.07	0.16	0.45	0.81	0.15	0.28
CD8+ICOS+	0.01	0.22	0.33	0.08	0.05	0.54	0.59	0.18	1.00	0.48	0.70	0.24
CD8+KI67+	0.50	0.29	0.27	0.21	0.08	0.44	0.84	0.26	0.87	0.30	0.76	0.27
CD8Tcells	0.01	0.16	0.18	0.08	0.02	0.54	0.82	0.18	0.94	0.48	0.82	0.24
Tregs	0.02	0.00	0.01	0.93	0.54	0.01	0.00	0.06	0.59	0.13	0.00	0.13
Tregs+ICOS+	0.03	0.00	0.00	0.66	0.08	0.00	0.00	0.06	0.59	0.09	0.00	0.13
Tregs+KI67+	0.04	0.01	0.01	0.66	0.93	0.02	0.00	0.09	0.82	0.94	0.00	0.13

FIG. 19F Intratumoral

Key for statistics and bar graph:

	Bartlett	NOVA/Krusk	1 VS 2	1 VS 3	1 VS 4	1 VS 5	2 VS 3	2 VS 4	2 VS 5	3 VS 4	3 VS 5	4 VS 5
CD3+ICOS+	0.00	0.03	0.08	0.82	0.09	0.03	0.13	0.66	0.79	0.06	0.03	0.24
CD3+KI67+	0.00	0.10	0.08	0.59	0.31	0.24	0.03	0.18	0.66	0.24	0.09	0.31
CD3Tcells	0.26	0.08	0.13	0.49	0.32	0.04	0.22	0.38	0.55	0.65	0.08	0.15
CD4+ICOS+	0.02	0.05	0.05	0.59	0.18	0.09	0.05	0.18	0.93	0.18	0.06	0.18
CD4+KI67+	0.00	0.02	0.01	0.82	0.18	0.18	0.01	0.05	0.79	0.06	0.09	0.31
CD4Tcells	0.17	0.19	0.12	0.52	0.35	0.08	0.20	0.43	0.86	0.58	0.13	0.33
CD8+ICOS+	0.14	0.03	0.11	0.47	0.26	0.04	0.22	0.31	0.39	0.67	0.06	0.09
CD8+KI67+	0.00	0.09	0.05	0.70	0.13	0.04	0.13	0.43	0.79	0.39	0.13	0.39
CD8Tcells	0.24	0.05	0.12	0.45	0.31	0.04	0.26	0.32	0.44	0.82	0.08	0.10
Tregs	0.03	0.11	0.93	0.00	0.09	0.82	0.25	0.43	0.54	0.31	0.13	0.59
Tregs+ICOS+	0.03	0.02	0.54	0.00	0.02	0.04	0.13	0.54	0.79	0.09	0.13	0.70
Tregs+KI67+	0.00	0.06	0.01	0.59	0.09	0.18	0.02	0.13	1.00	0.39	0.31	0.48

FIG. 20A


Antibody 2-10 (20 µg)
Antibody 2-10 (7 µg)
RSV-m control (2 µg)
Antibody 2-6 (20 µg)
Antibody 2-6 (7 µg)
Antibody 2-6 (2 µg)
Tumor Volume (mm3)
Day of Study

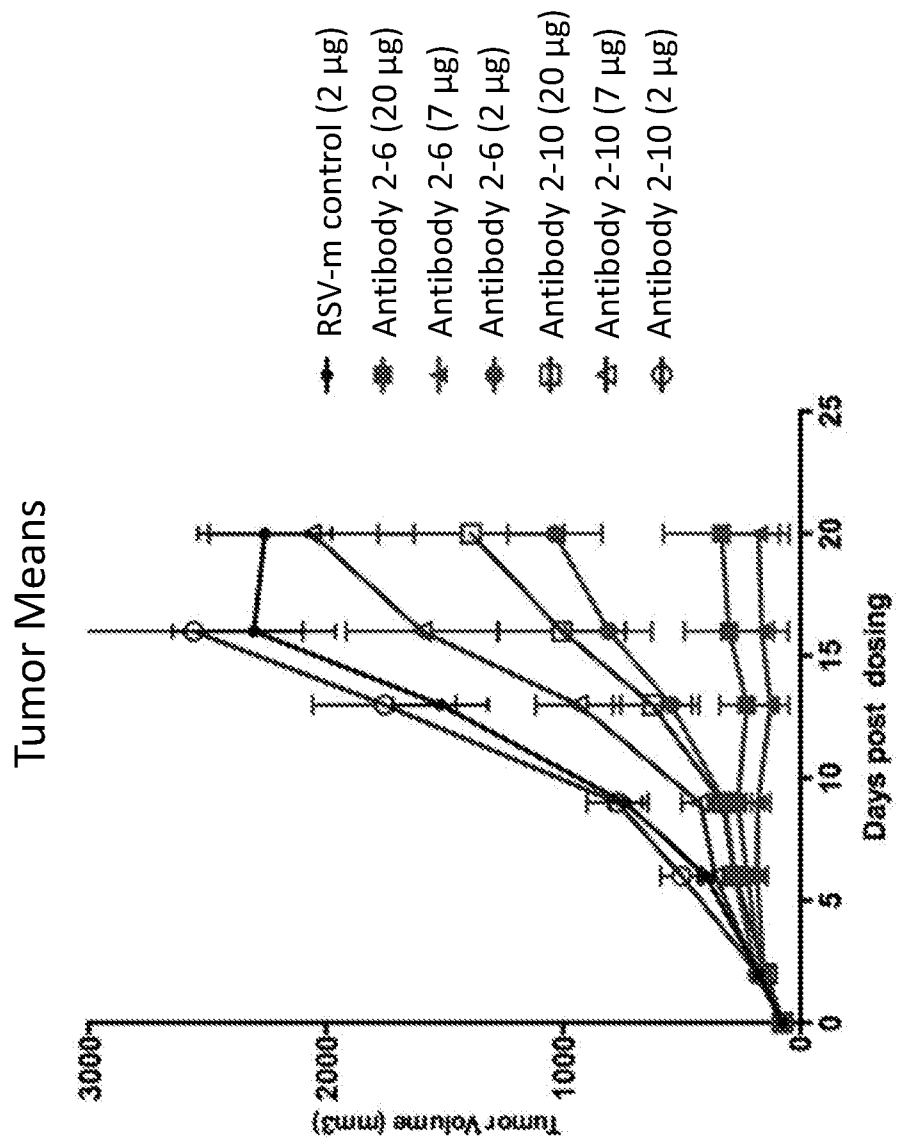
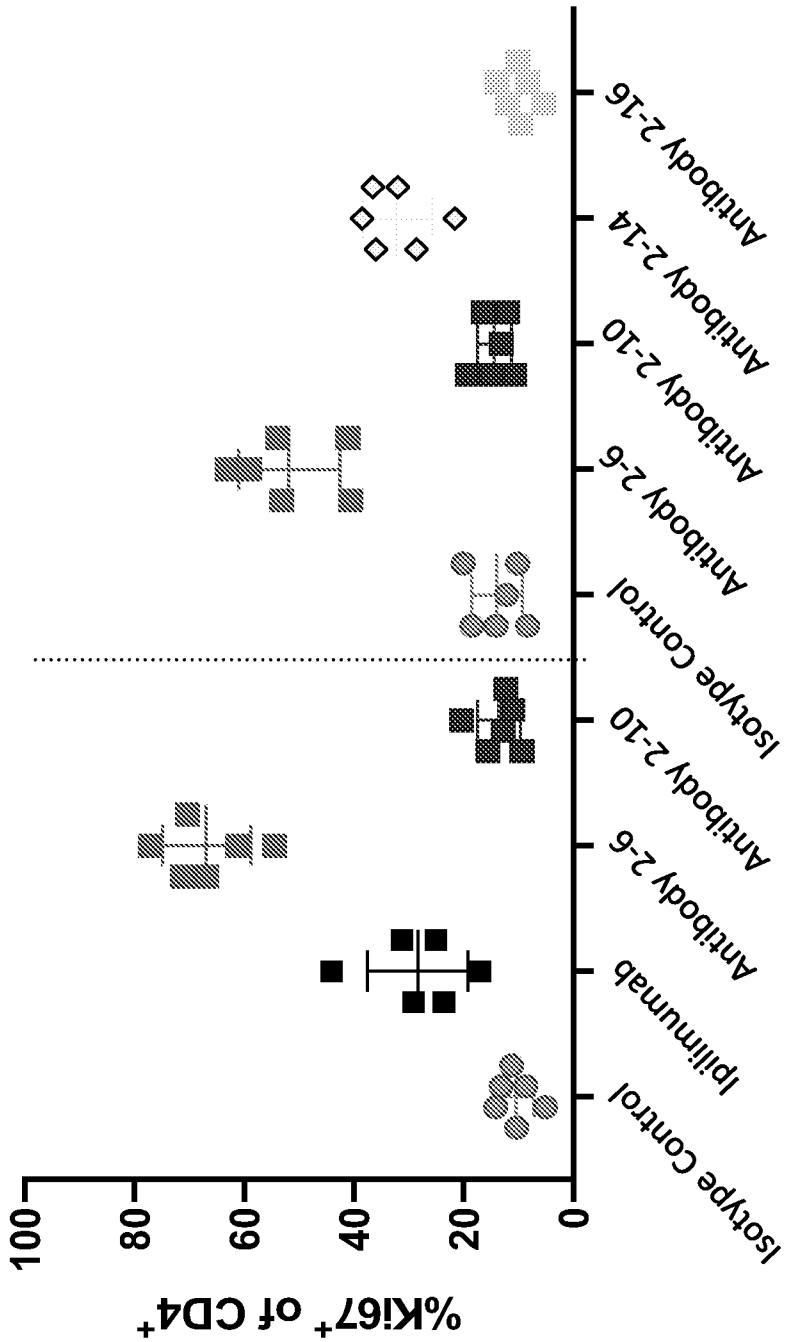

Tumor Means
RSV-m control (2 µg)
Antibody 2-6 (20 µg)
Antibody 2-6 (7 µg)
Antibody 2-6 (2 µg)
Antibody 2-10 (20 µg)
Antibody 2-10 (7 µg)
Antibody 2-10 (2 µg)
Tumor Volume (mm3)
3000
2000
1000
0
0
5
10
15
20
25
Days post dosing

FIG. 20B

FIG. 21


Experiment 1
Experiment 2
%KI67+ of CD4+
100
80
60
40
20
0
Isotype Control
Ipilimumab
Antibody 2-6
Antibody 2-10
Isotype Control
Antibody 2-6
Antibody 2-10
Antibody 2-14
Antibody 2-16

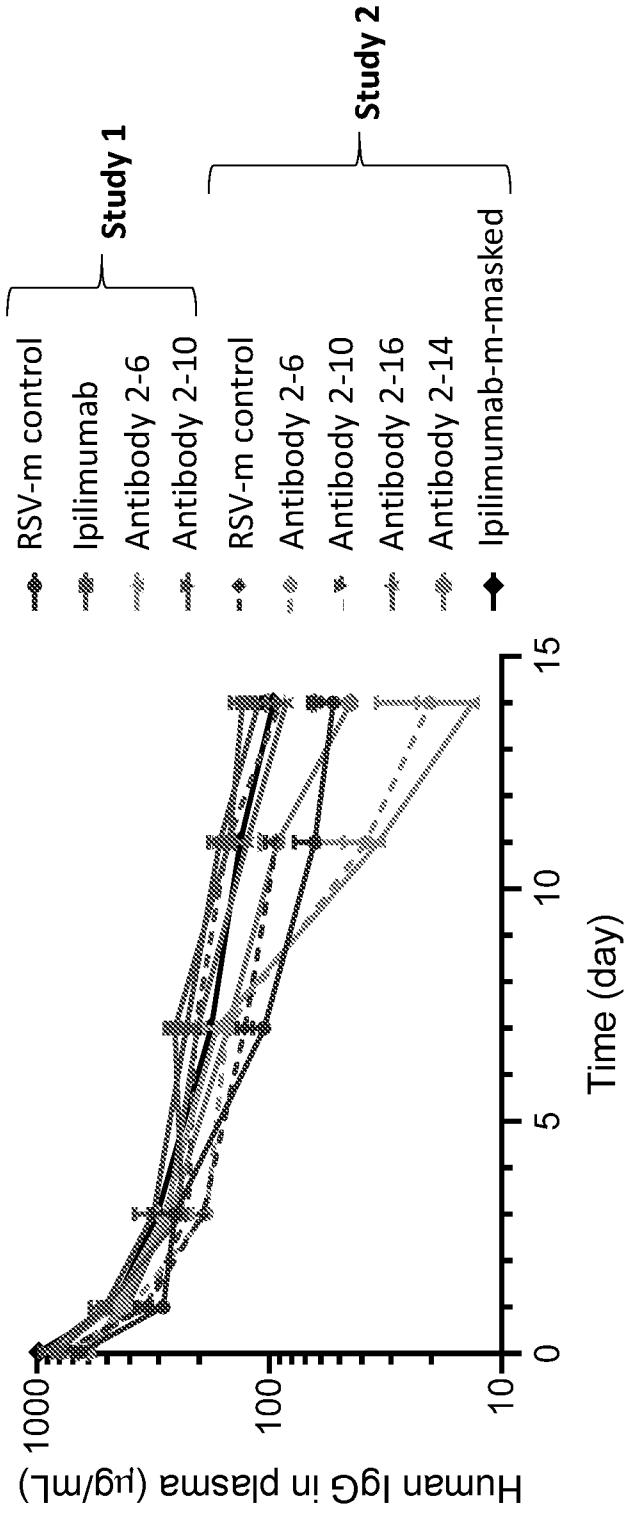

Study 1
RSV-m control
Ipilimumab
Antibody 2-6
Antibody 2-10
Study 2
RSV-m control
Antibody 2-6
Antibody 2-10
Antibody 2-16
Antibody 2-14
Ipilimumab-m-masked
Human IgG in plasma (μg/mL)
1000
100
10
0
5
10
15
Time (day)

FIG. 22

FIG. 23A

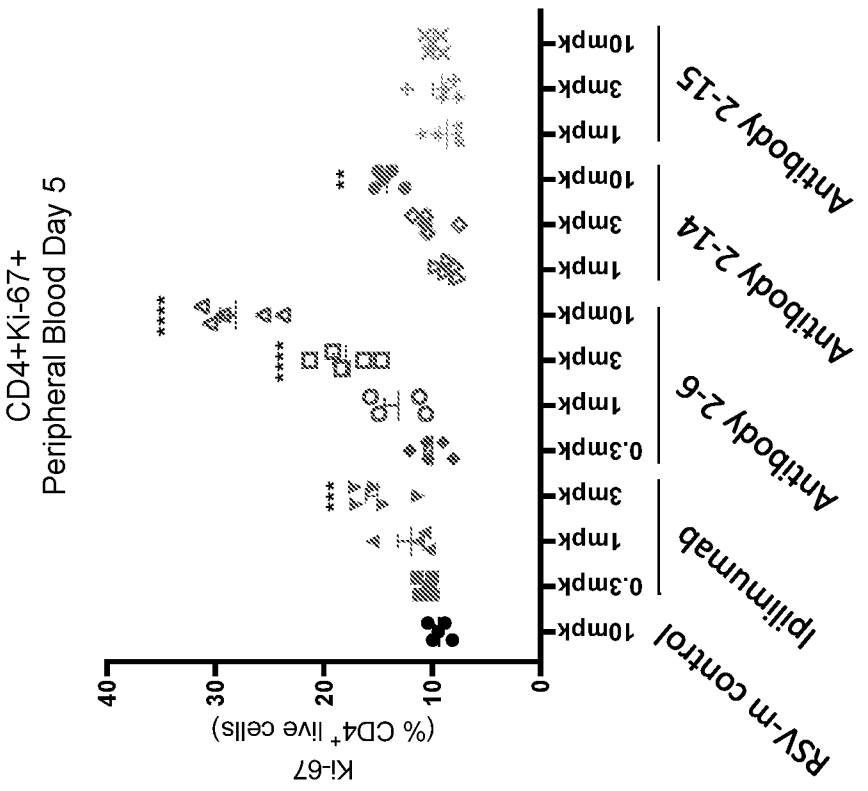

CD4+Ki-67+
Peripheral Blood Day 5
Ki-67
(% CD4+ live cells)
40
30
20
10
0
****
****
***
**
RSV-m control
Ipilimumab
Antibody 2-6
Antibody 2-14
Antibody 2-15
10mpk
0.3mpk
1mpk
3mpk
10mpk
0.3mpk
1mpk
3mpk
10mpk
1mpk
3mpk
10mpk
1mpk
3mpk
10mpk
CD4+ICOS+
Peripheral Blood Day 5
ICOS
(% CD4+ live cells)
25
20
15
10
5
0
****
****
****
RSV-m control
Ipilimumab
Antibody 2-6
Antibody 2-14
Antibody 2-15
10mpk
0.3mpk
1mpk
3mpk
10mpk
0.3mpk
1mpk
3mpk
10mpk
1mpk
3mpk
10mpk
1mpk
3mpk
10mpk

FIG. 23B

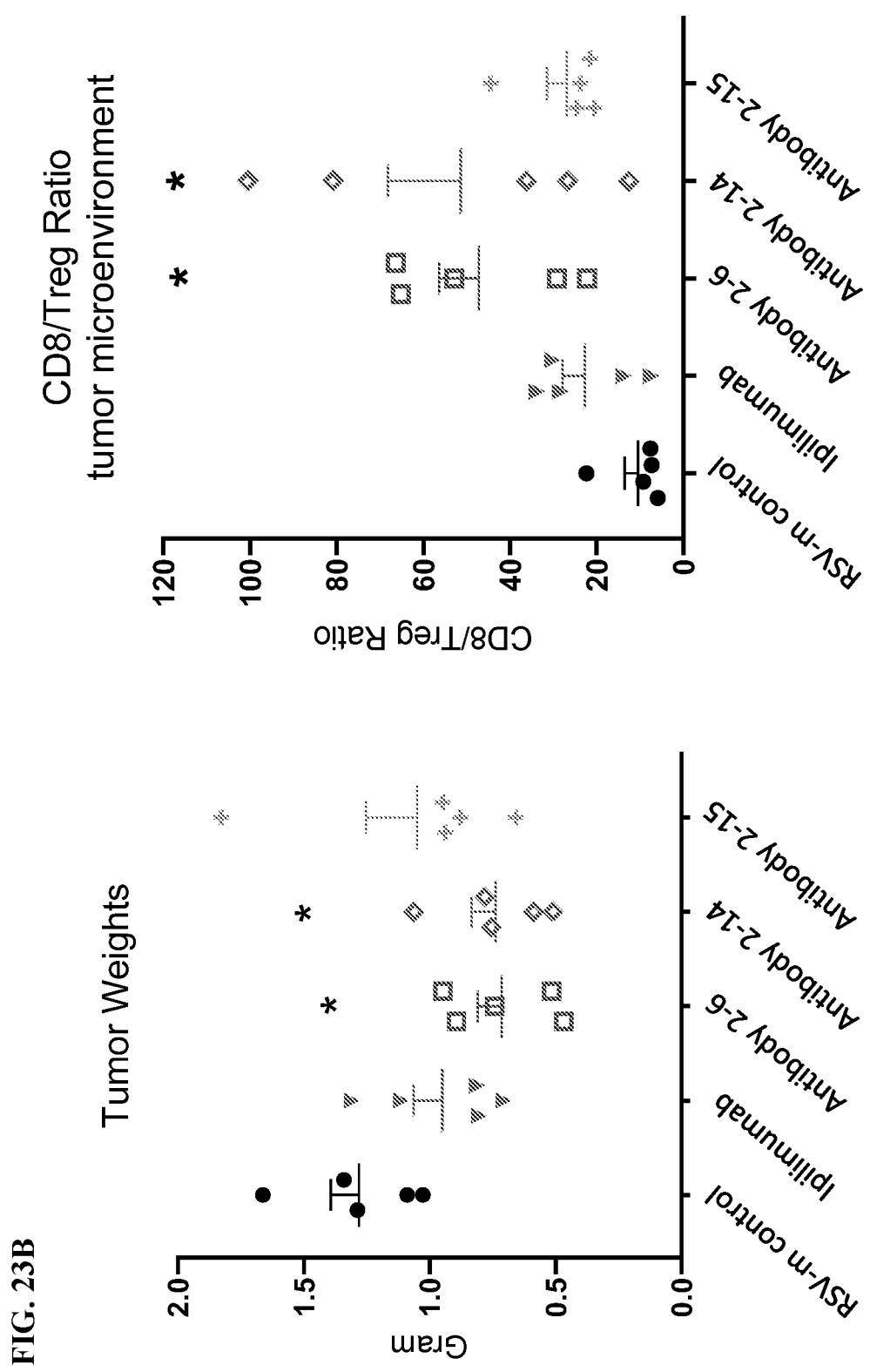

CD8/Treg Ratio
tumor microenvironment
CD8/Treg Ratio
120
100
80
60
40
20
0
RSV-m control
Ipilimumab
Antibody 2-6
Antibody 2-14
Antibody 2-15
Tumor Weights
Gram
2.0
1.5
1.0
0.5
0.0
RSV-m control
Ipilimumab
Antibody 2-6
Antibody 2-14
Antibody 2-15

FIG. 23C

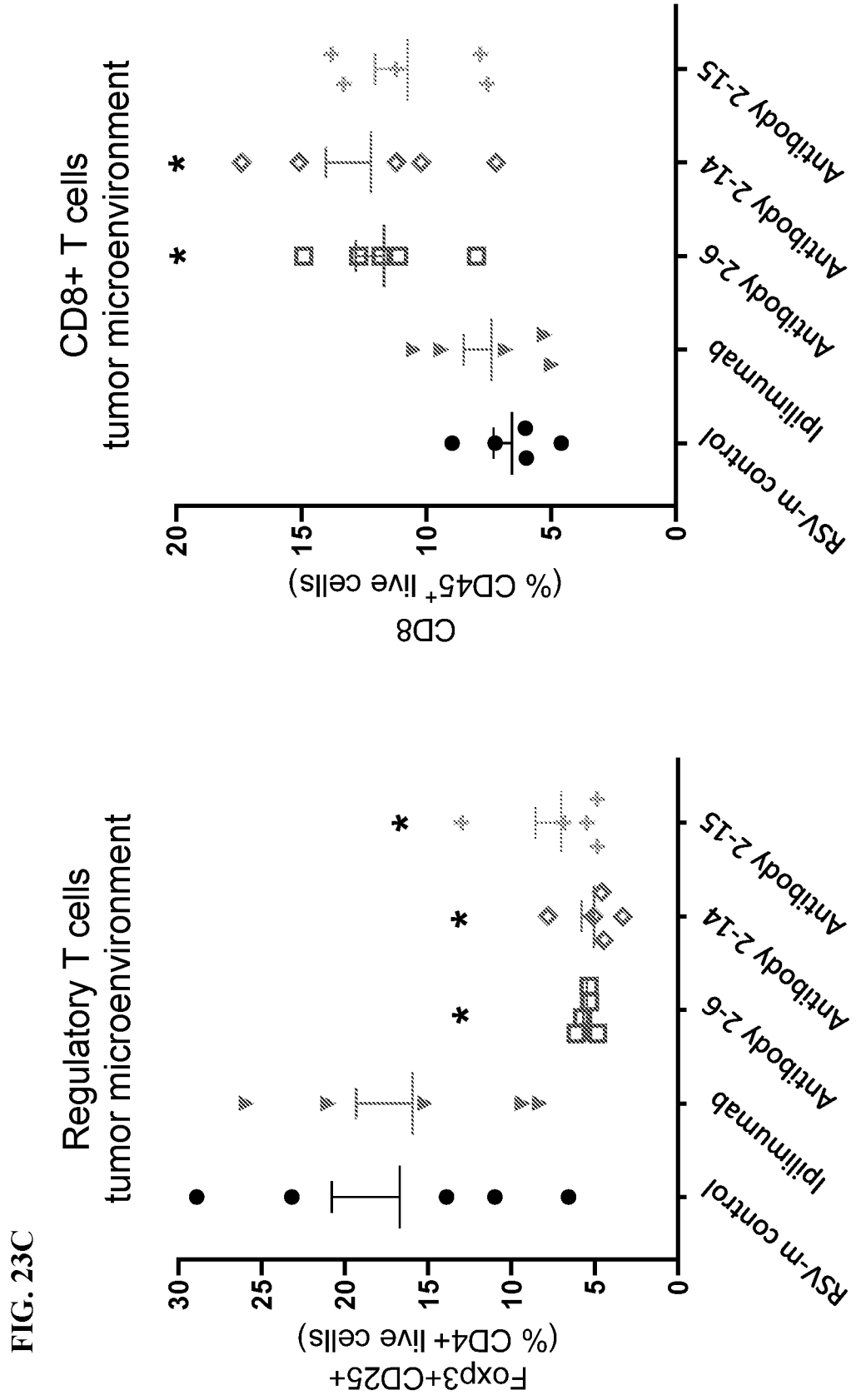

Regulatory T cells
tumor microenvironment
Foxp3+CD25+
(% CD4+ live cells)
30
25
20
15
10
5
0
RSV-m control
Ipilimumab
Antibody 2-6
Antibody 2-14
Antibody 2-15
CD8+ T cells
tumor microenvironment
CD8
(% CD45+ live cells)
20
15
10
5
0
RSV-m control
Ipilimumab
Antibody 2-6
Antibody 2-14
Antibody 2-15

FIG. 23D

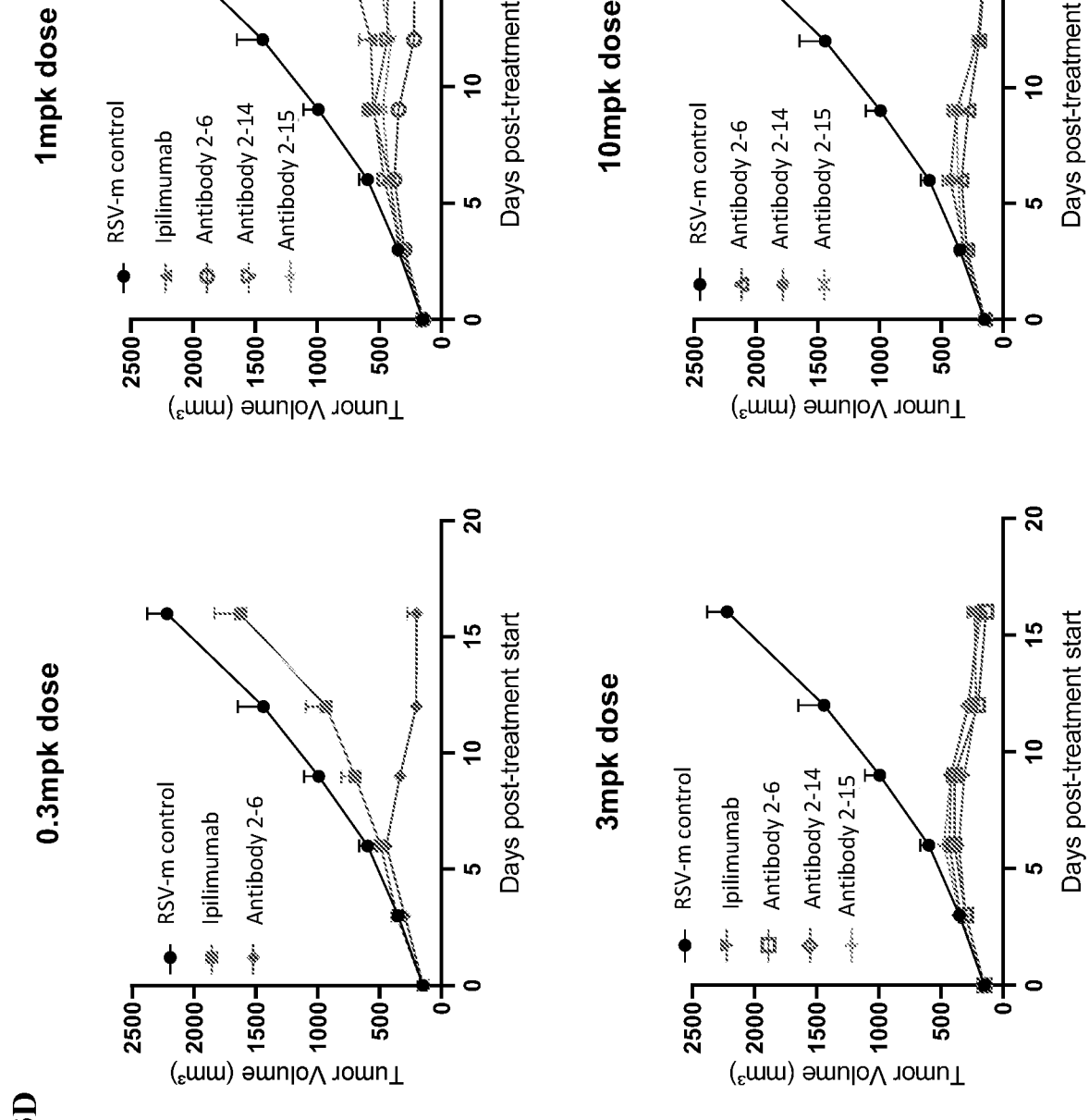

0.3mpk dose
1mpk dose
3mpk dose
10mpk dose
RSV-m control
Ipilimumab
Antibody 2-6
Antibody 2-14
Antibody 2-15
Tumor Volume (mm³)
Days post-treatment start
0
500
1000
1500
2000
2500
0
5
10
15
20

FIG. 24A

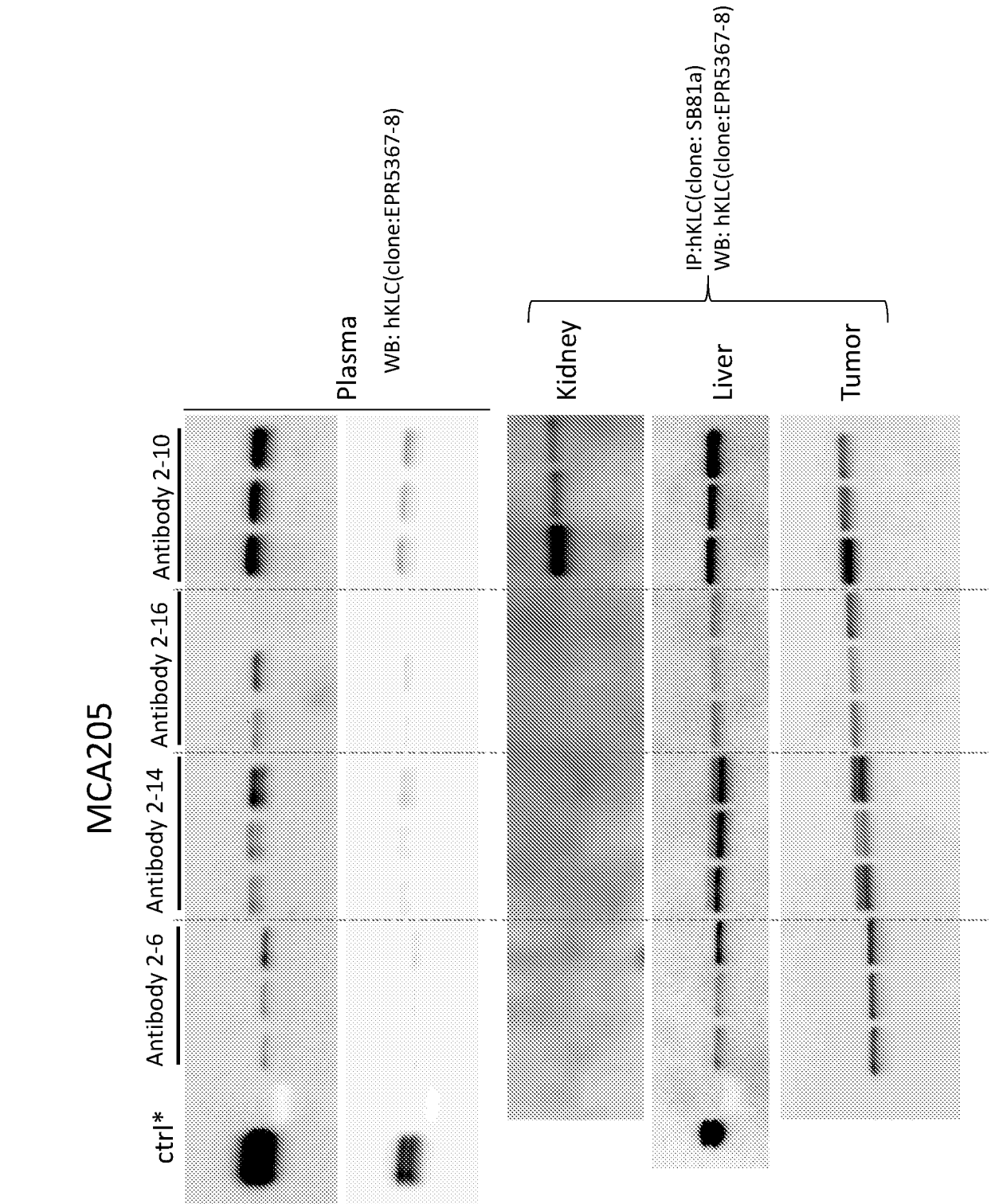

MCA205
ctrl*
Antibody 2-6
Antibody 2-14
Antibody 2-16
Antibody 2-10
Plasma
WB: hKLC(clone:EPR5367-8)
long exposure
short exposure
IP:hKLC(clone: SB81a)
WB: hKLC(clone:EPR5367-8)
Kidney
Liver
Tumor

FIG. 24B

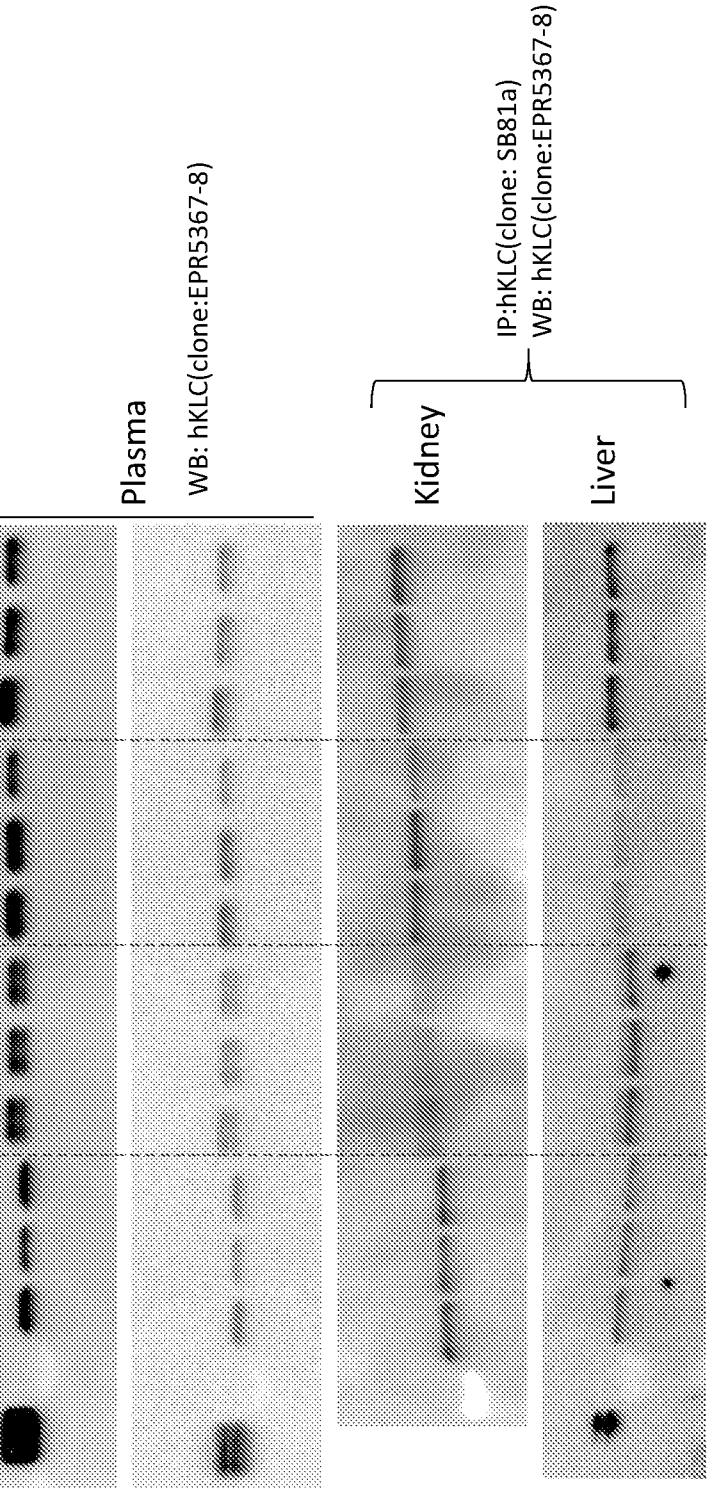

MB49
ctrl*
Antibody 2-6
Antibody 2-14
Antibody 2-16
Antibody 2-10
Plasma
WB: hKLC(clone:EPR5367-8)
long exposure
short exposure
IP:hKLC(clone: SB81a)
WB: hKLC(clone:EPR5367-8)
Kidney
Liver

FIG. 24C

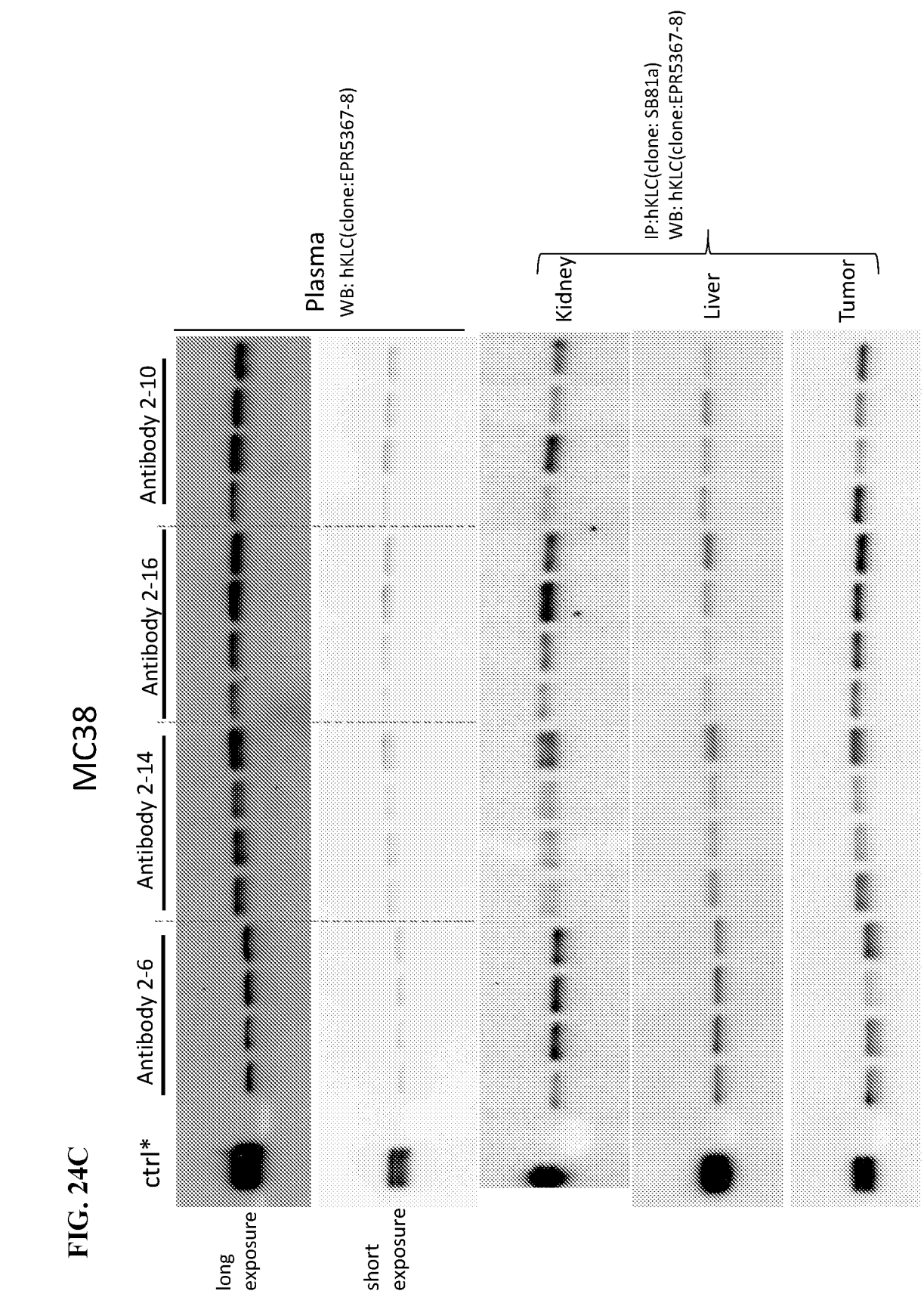

MC38
ctrl*
Antibody 2-6
Antibody 2-14
Antibody 2-16
Antibody 2-10
Plasma
WB: hKLC(clone:EPR5367-8)
long exposure
short exposure
IP:hKLC(clone: SB81a)
WB: hKLC(clone:EPR5367-8)
Kidney
Liver
Tumor

FIG. 24D

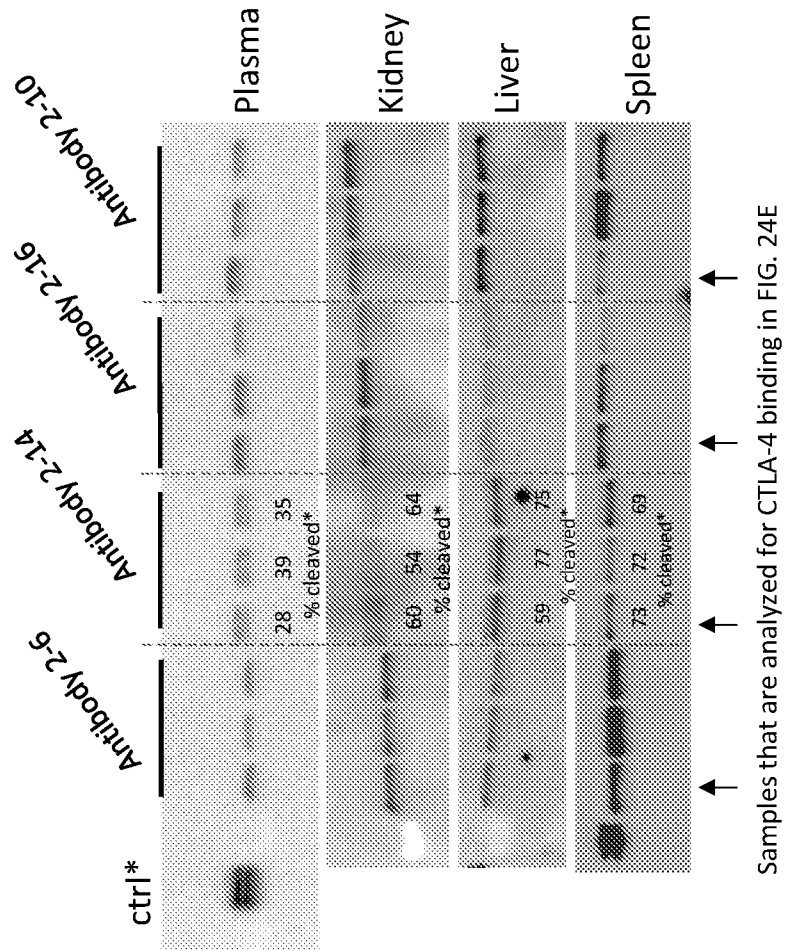

Plasma
Kidney
Liver
Spleen
ctrl*
Antibody 2-6
Antibody 2-14
Antibody 2-16
Antibody 2-10
28 39 35
% cleaved*
60 54 64
% cleaved*
59 77 75
% cleaved*
73 72 69
% cleaved*
Samples that are analyzed for CTLA-4 binding in FIG. 24E

FIG. 24E

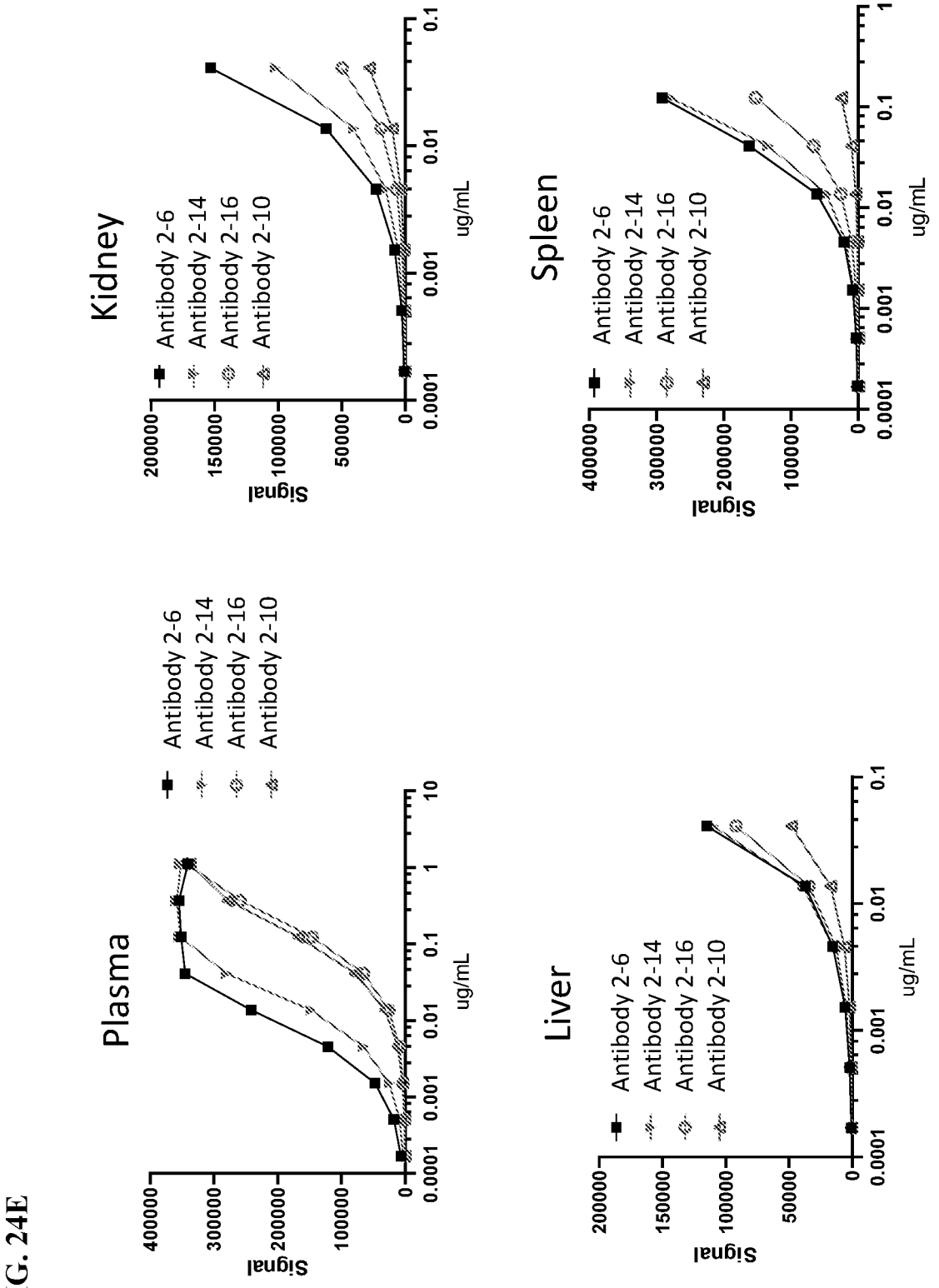

Plasma
Kidney
Liver
Spleen
Antibody 2-6
Antibody 2-14
Antibody 2-16
Antibody 2-10
Signal
ug/mL
0
50000
100000
150000
200000
300000
400000
0.0001
0.001
0.01
0.1
1
10

FIG. 24F

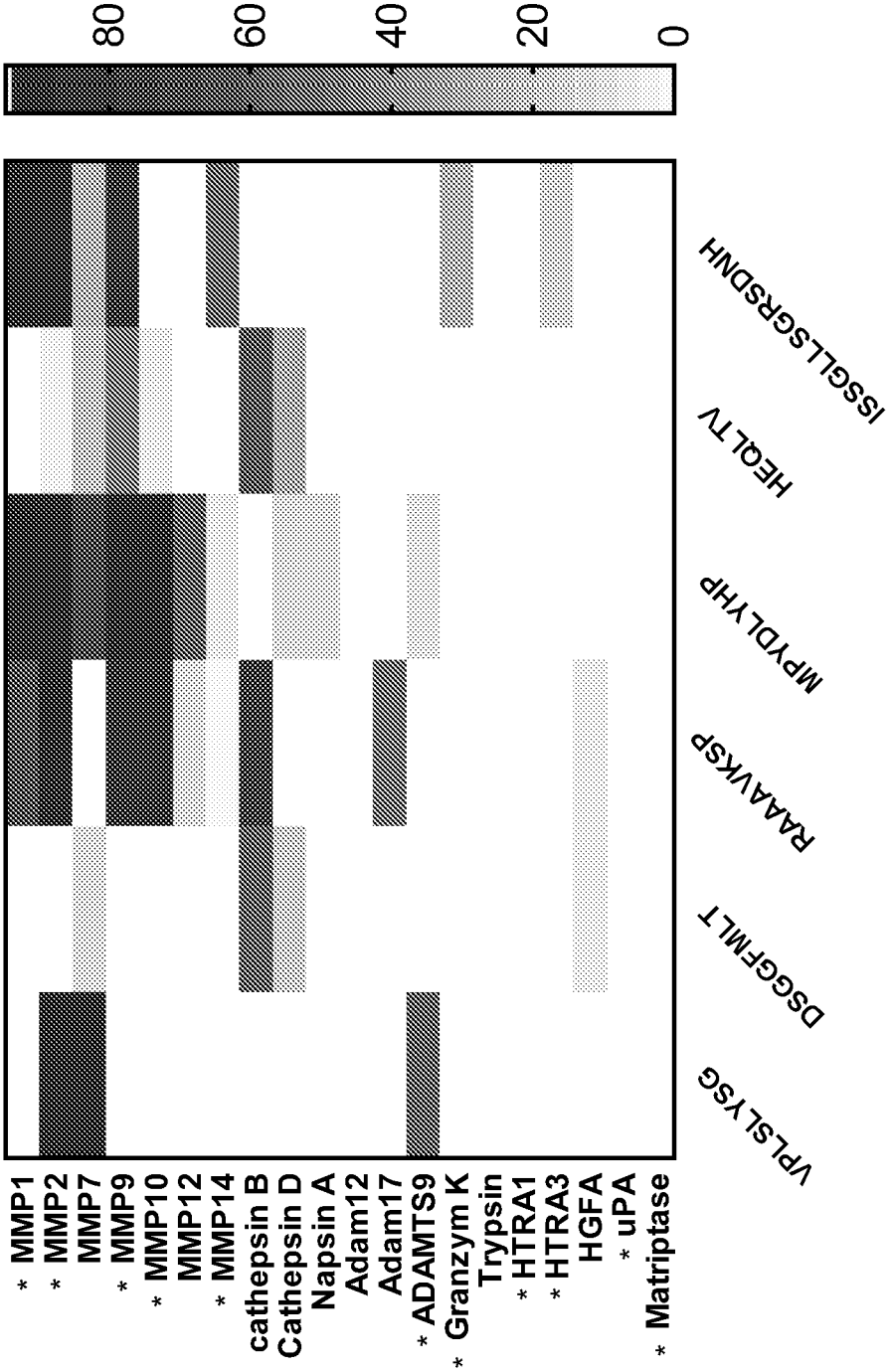
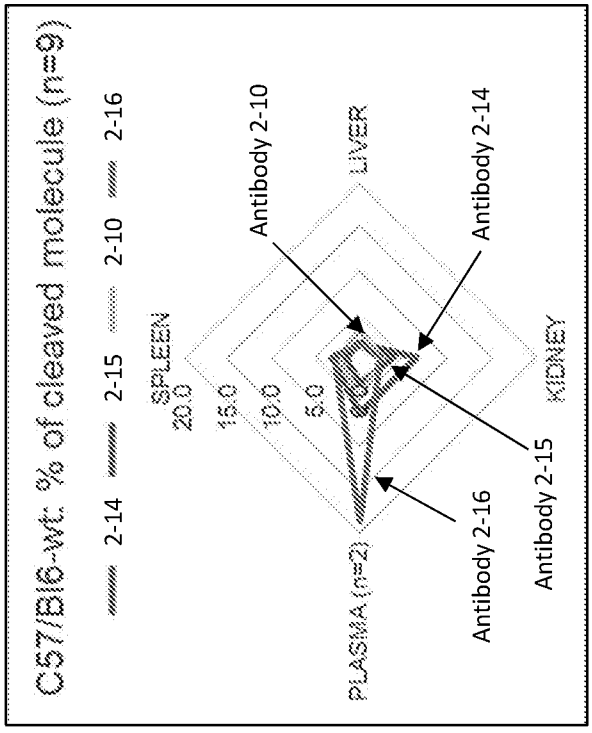

Confluence: in vitro cleavage
0
20
40
60
80
* MMP1
* MMP2
MMP7
* MMP9
* MMP10
MMP12
* MMP14
cathepsin B
Cathepsin D
Napsin A
Adam12
Adam17
* ADAMTS9
* Granzym K
Trypsin
* HTRA1
* HTRA3
HGFA
* uPA
* Matriptase
VPLSLYSG
DSGGFMLT
RAAAVKSP
MPYDLVHP
HEGLTV
ISSGLLSGRSDNH

FIG. 25A


C57/Bl6-wt: % of cleaved molecule (n=9)
2-14
2-15
2-10
2-16
SPLEEN
20.0
15.0
10.0
5.0
LIVER
KIDNEY
PLASMA (n=2)
Antibody 2-10
Antibody 2-14
Antibody 2-16
Antibody 2-15

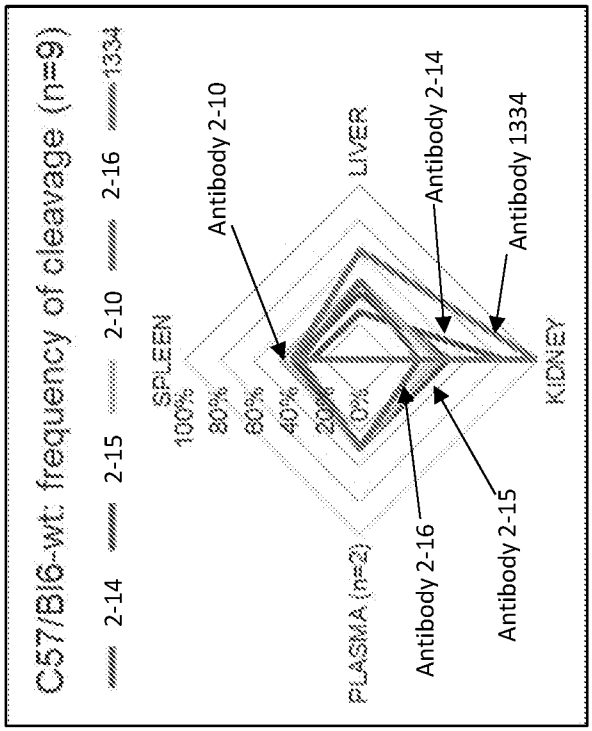

C57/Bl6-wt: frequency of cleavage (n=9)
2-14
2-15
2-10
2-16
1334
SPLEEN
100%
80%
60%
40%
20%
0%
LIVER
KIDNEY
PLASMA (n=2)
Antibody 2-10
Antibody 2-14
Antibody 1334
Antibody 2-16
Antibody 2-15

FIG. 25B

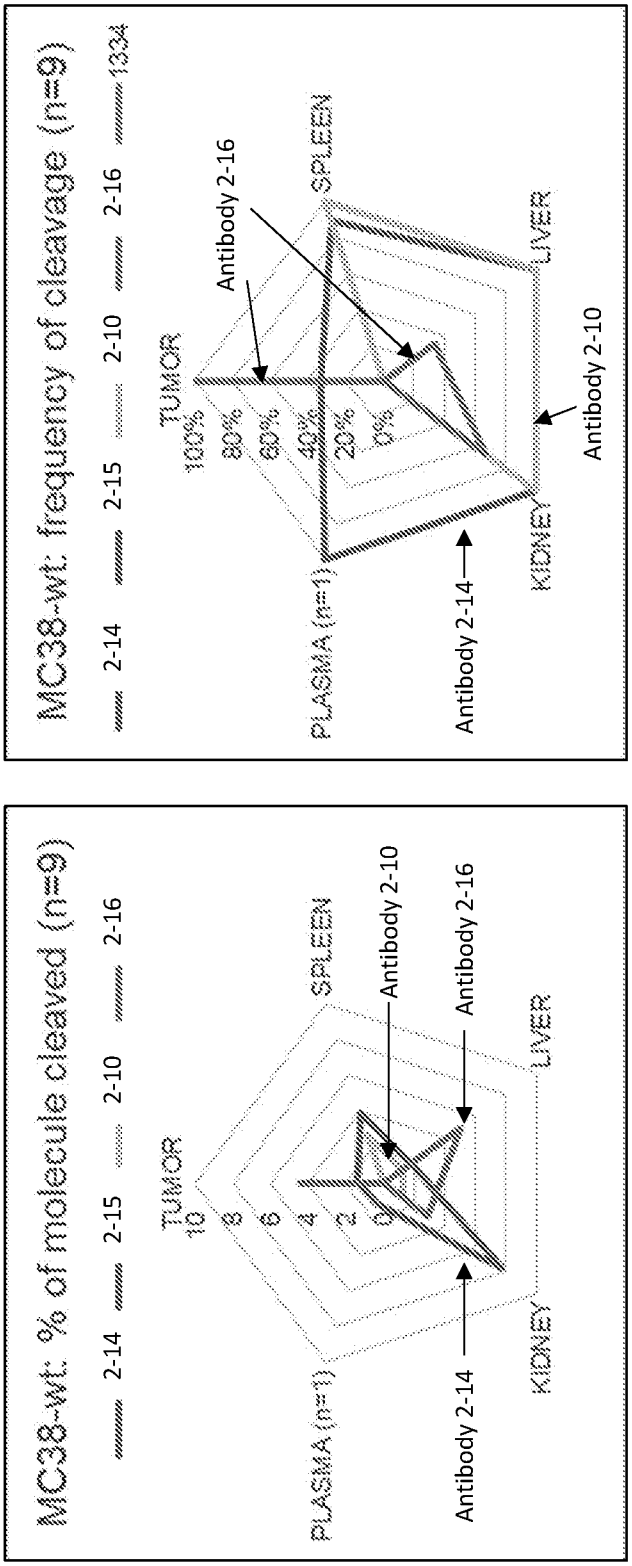
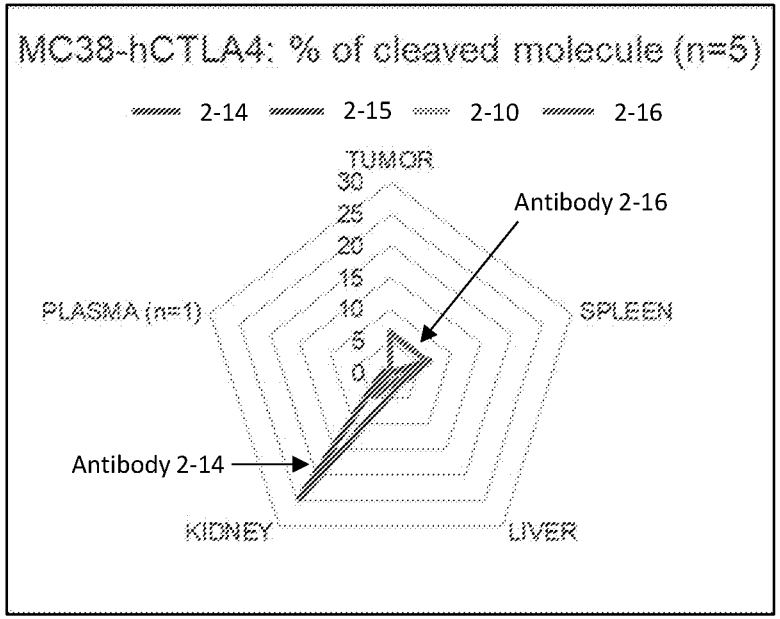

MC38-wt: % of molecule cleaved (n=9)
2-14
2-15
2-10
2-16
TUMOR
10
8
6
4
2
0
SPLEEN
PLASMA (n=1)
LIVER
KIDNEY
Antibody 2-10
Antibody 2-16
Antibody 2-14
MC38-wt: frequency of cleavage (n=9)
2-14
2-15
2-10
2-16
1334
TUMOR
100%
80%
60%
40%
20%
0%
Antibody 2-16
SPLEEN
PLASMA (n=1)
LIVER
Antibody 2-14
KIDNEY
Antibody 2-10

FIG. 25C


MC38-hCTLA4: % of cleaved molecule (n=5)
2-14
2-15
2-10
2-16
TUMOR
30
25
20
15
10
5
0
Antibody 2-16
PLASMA (n=1)
SPLEEN
Antibody 2-14
KIDNEY
LIVER

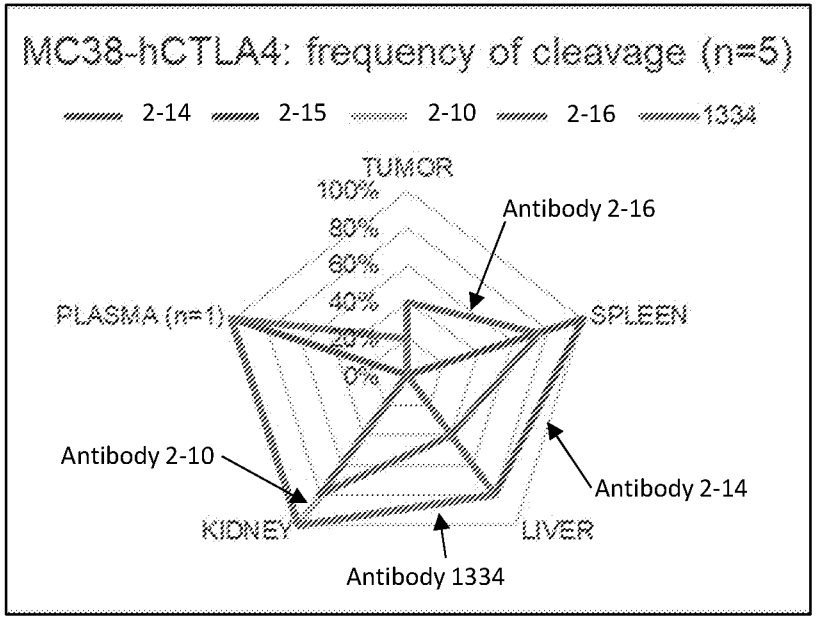

MC38-hCTLA4: frequency of cleavage (n=5)
2-14
2-15
2-10
2-16
1334
TUMOR
100%
80%
60%
40%
20%
0%
Antibody 2-16
PLASMA (n=1)
SPLEEN
Antibody 2-10
Antibody 2-14
KIDNEY
LIVER
Antibody 1334

FIG. 25D

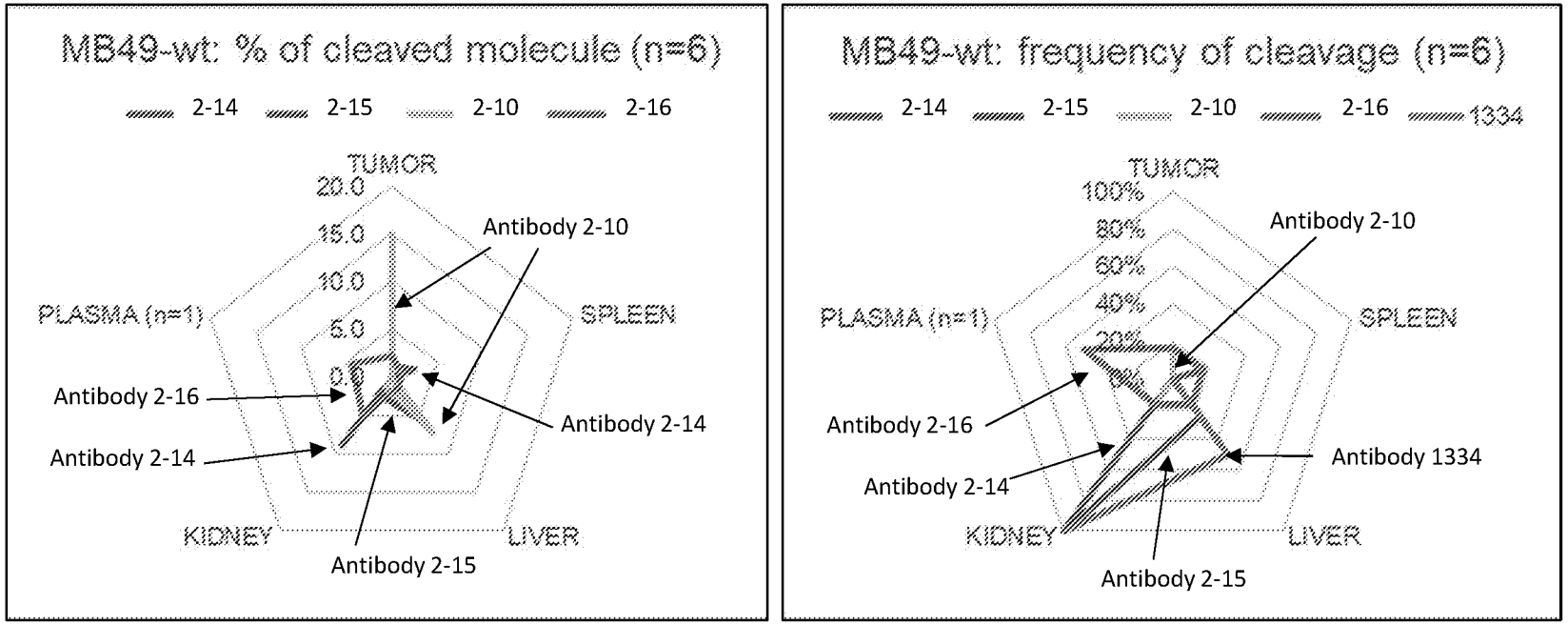

MB49-wt: % of cleaved molecule (n=6)
2-14
2-15
2-10
2-16
TUMOR
20.0
15.0
10.0
5.0
0
Antibody 2-10
PLASMA (n=1)
SPLEEN
Antibody 2-16
Antibody 2-14
Antibody 2-14
KIDNEY
LIVER
Antibody 2-15
MB49-wt: frequency of cleavage (n=6)
2-14
2-15
2-10
2-16
1334
TUMOR
100%
80%
60%
40%
20%
0%
Antibody 2-10
PLASMA (n=1)
SPLEEN
Antibody 2-16
Antibody 1334
Antibody 2-14
KIDNEY
LIVER
Antibody 2-15

FIG. 25E

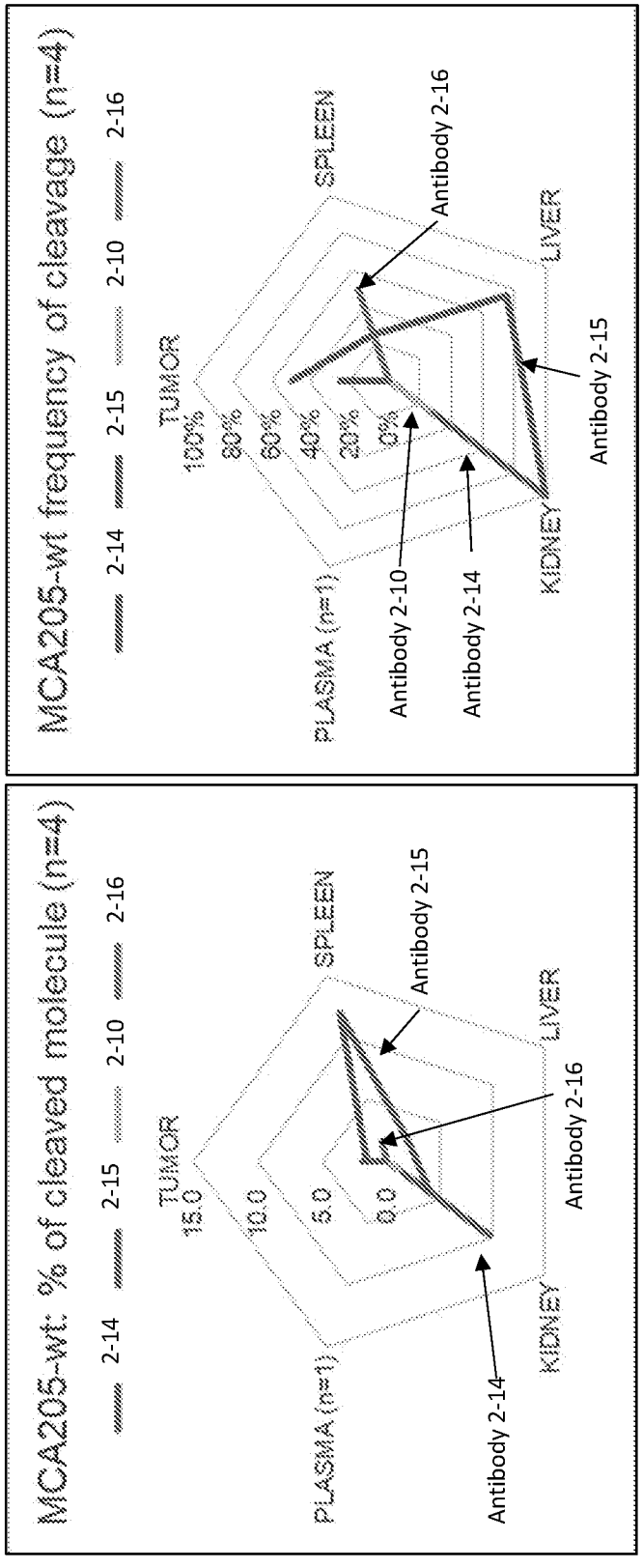

MCA205-wt: % of cleaved molecule (n=4)
2-14
2-15
2-10
2-16
TUMOR
15.0
10.0
5.0
0.0
PLASMA (n=1)
SPLEEN
LIVER
KIDNEY
Antibody 2-15
Antibody 2-16
Antibody 2-14
MCA205-wt frequency of cleavage (n=4)
2-14
2-15
2-10
2-16
TUMOR
100%
80%
60%
40%
20%
0%
PLASMA (n=1)
SPLEEN
LIVER
KIDNEY
Antibody 2-16
Antibody 2-10
Antibody 2-14
Antibody 2-15

FIG. 25F

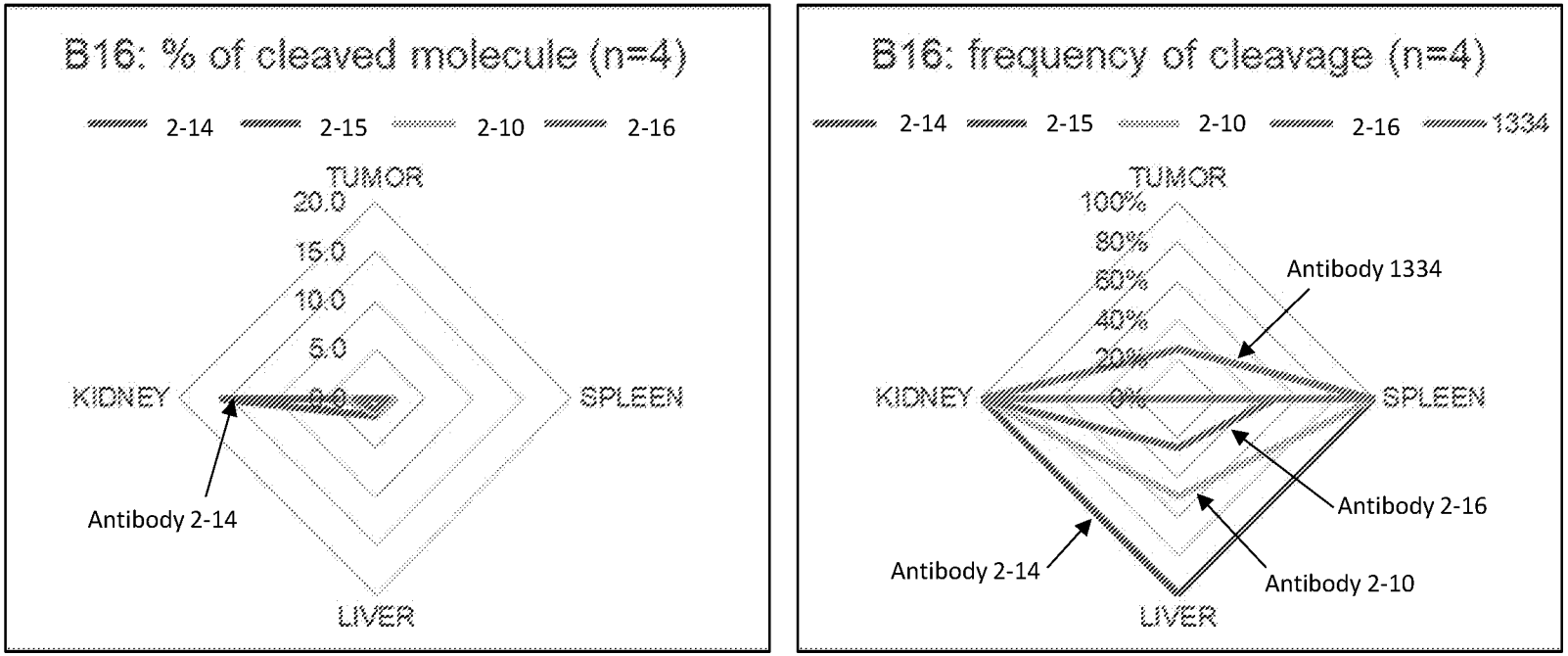

B16: % of cleaved molecule (n=4)
2-14
2-15
2-10
2-16
TUMOR
20.0
15.0
10.0
5.0
KIDNEY
SPLEEN
Antibody 2-14
LIVER
B16: frequency of cleavage (n=4)
2-14
2-15
2-10
2-16
1334
TUMOR
100%
80%
60%
40%
20%
0%
Antibody 1334
KIDNEY
SPLEEN
Antibody 2-16
Antibody 2-14
Antibody 2-10
LIVER

FIG. 26A

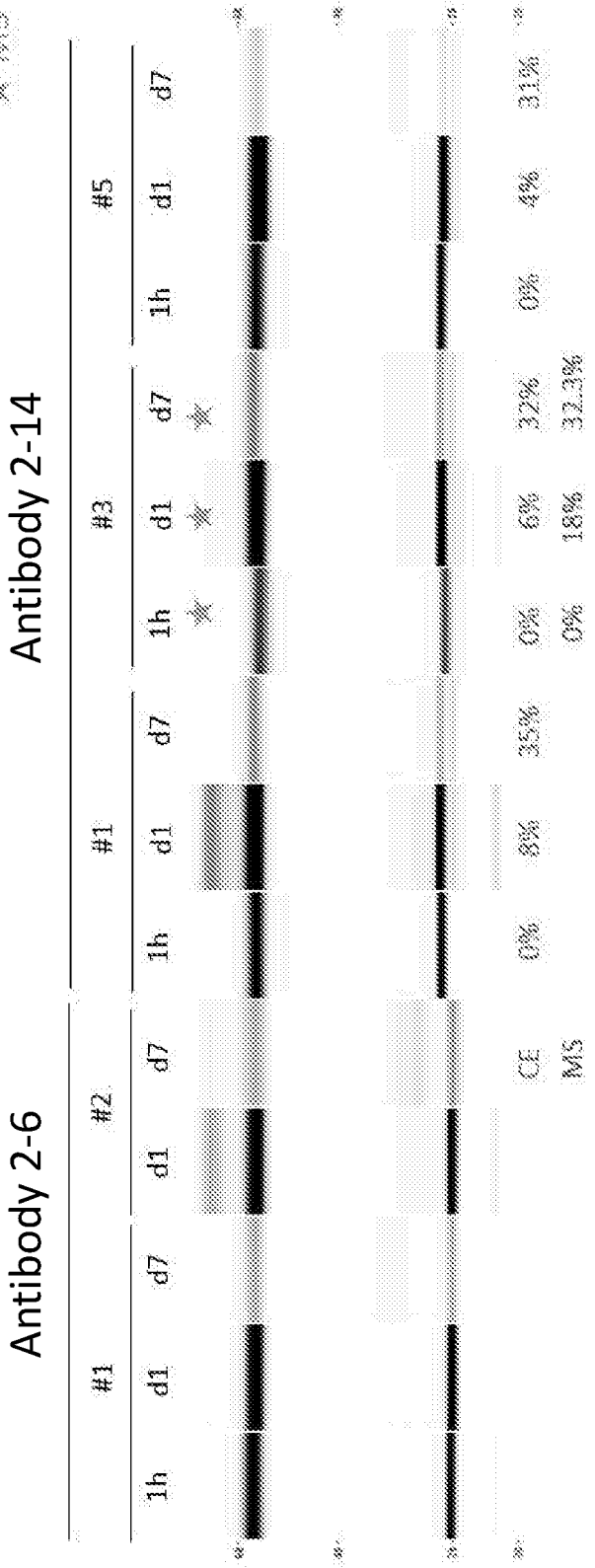

Antibody 2-6
Antibody 2-14
MS
#1
#2
#3
#5
1h
d1
d7
CE
MS
0%
8%
35%
0%
6%
32%
0%
4%
31%
0%
18%
32.3%

FIG. 26B

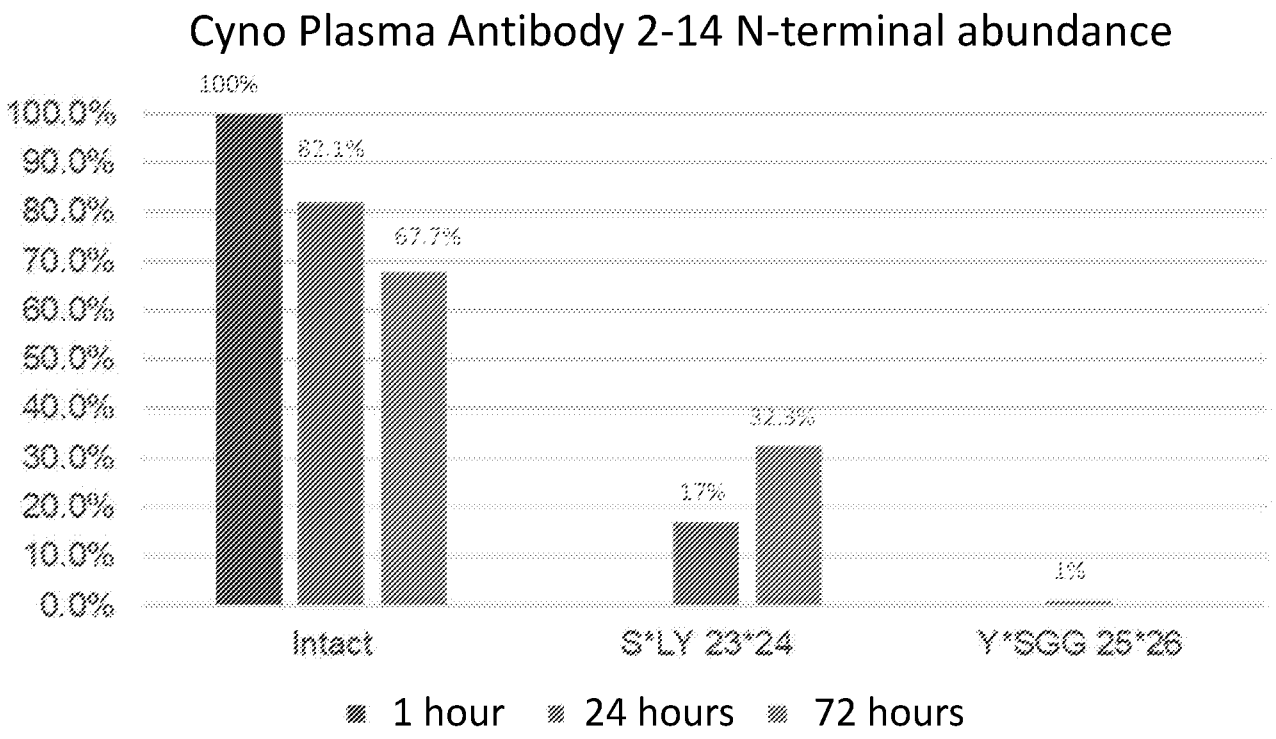

Cyno Plasma Antibody 2-14 N-terminal abundance
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
100%
82.1%
67.7%
1.7%
32.3%
1%
Intact
S*LY 23*24
Y*SGG 25*26
1 hour
24 hours
72 hours

FIG. 26C

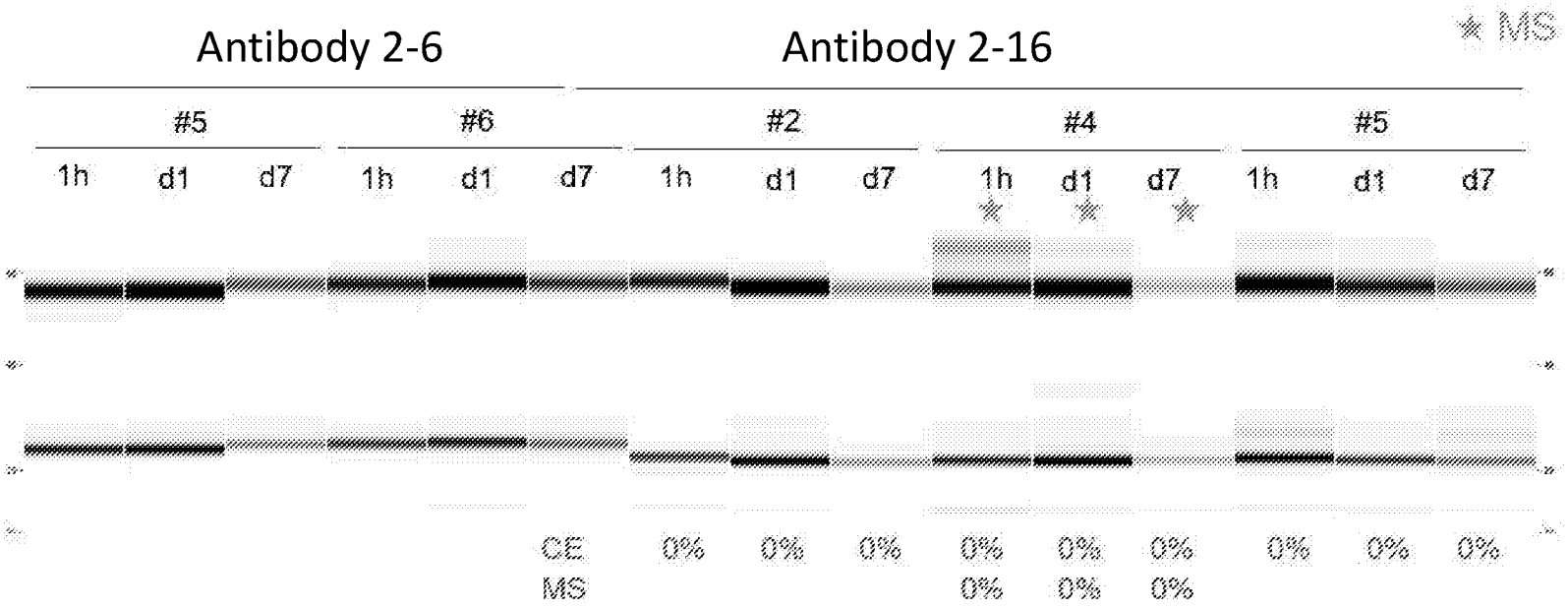


FIG. 27A

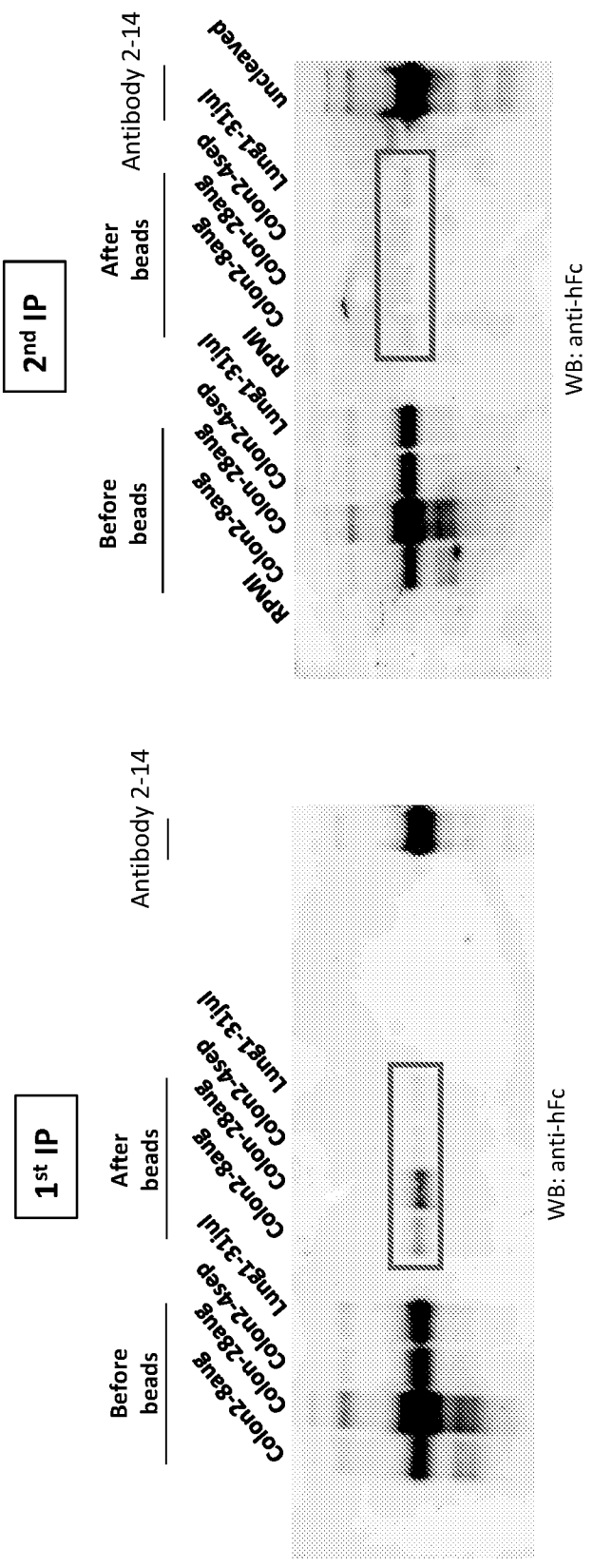

1st IP
Before beads
After beads
Antibody 2-14
Colon2-8aug
Colon-28aug
Lung2-4sep
Lung1-31jul
WB: anti-hFc
2nd IP
Before beads
After beads
Antibody 2-14
RPMI
Colon2-8aug
Colon-28aug
Lung2-4sep
Lung1-31jul
uncleaved
WB: anti-hFc

FIG. 27B

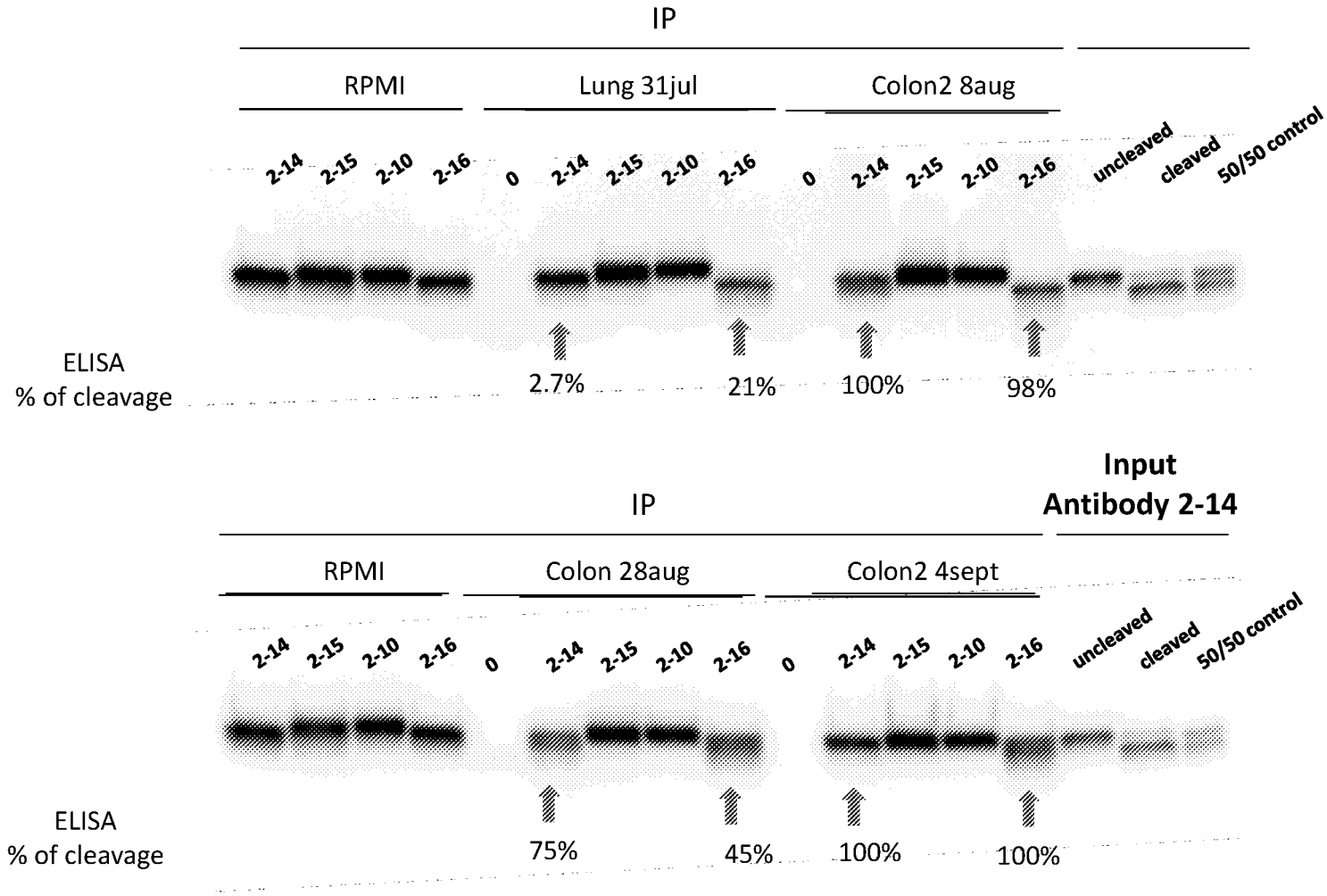

IP
RPMI
Lung 31jul
Colon2 8aug
2-14
2-15
2-10
2-16
0
uncleaved
cleaved
50/50 control
ELISA
% of cleavage
2.7%
21%
100%
98%
IP
Input
Antibody 2-14
RPMI
Colon 28aug
Colon2 4sept
75%
45%
100%
100%

FIG. 28A

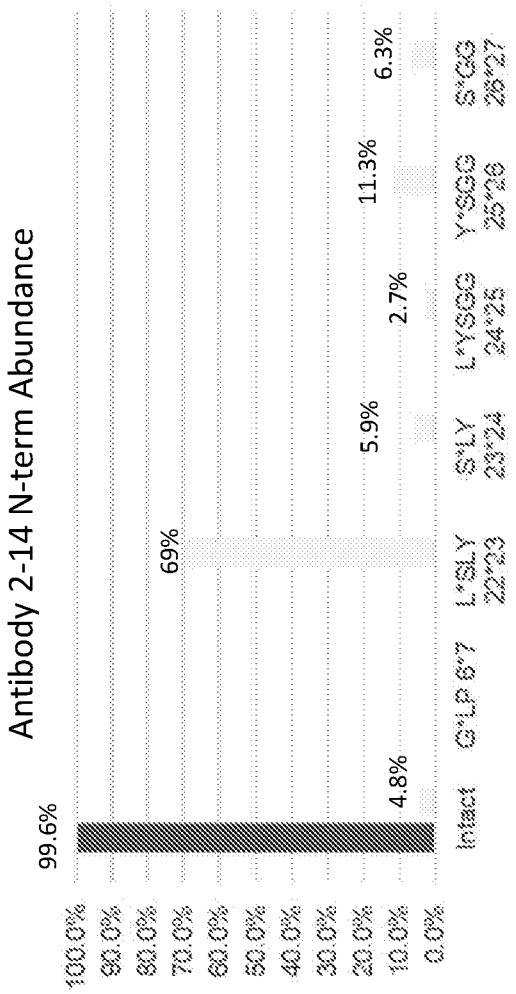


FIG. 28B

FIG. 28C

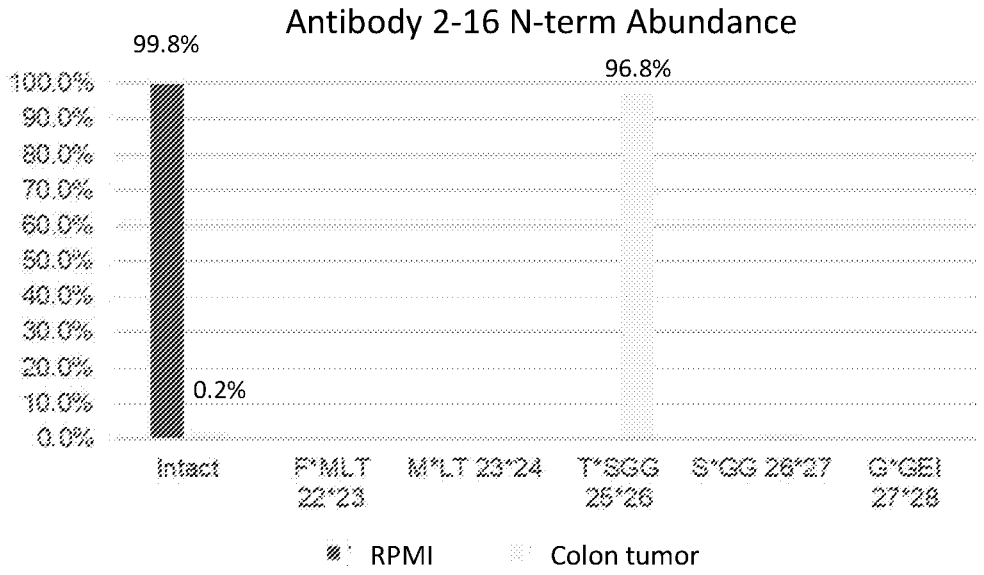


FIG. 28D

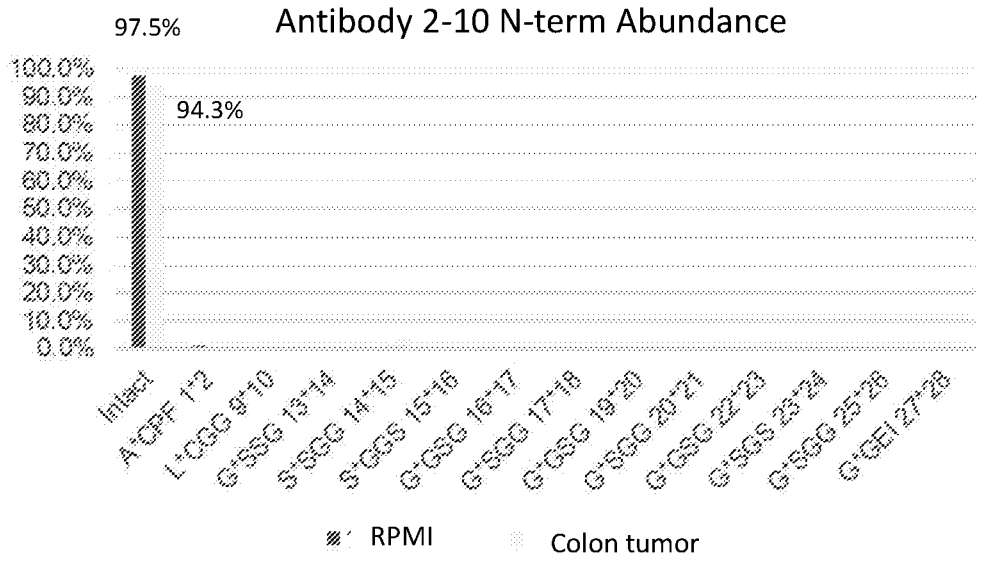
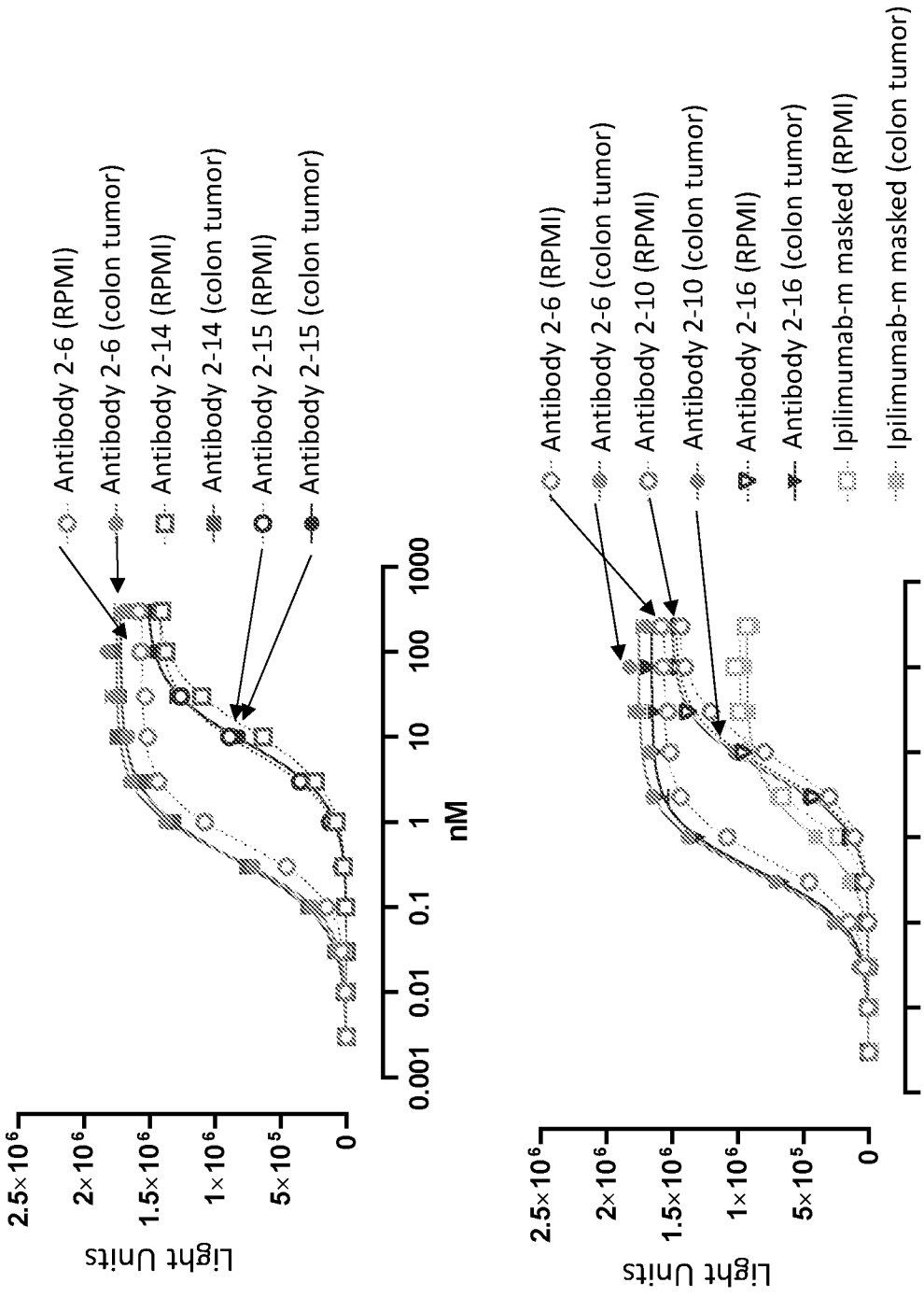


FIG. 28E


Antibody 2-6 (RPMI)
Antibody 2-6 (colon tumor)
Antibody 2-14 (RPMI)
Antibody 2-14 (colon tumor)
Antibody 2-15 (RPMI)
Antibody 2-15 (colon tumor)
Light Units
2.5×10^6
2×10^6
1.5×10^6
1×10^6
5×10^5
0
0.001
0.01
0.1
1
10
100
1000
nM
Antibody 2-6 (RPMI)
Antibody 2-6 (colon tumor)
Antibody 2-10 (RPMI)
Antibody 2-10 (colon tumor)
Antibody 2-16 (RPMI)
Antibody 2-16 (colon tumor)
Ipilimumab-m masked (RPMI)
Ipilimumab-m masked (colon tumor)
Light Units
2.5×10^6
2×10^6
1.5×10^6
1×10^6
5×10^5
0
0.001
0.01
0.1
1
10
100
1000
nM

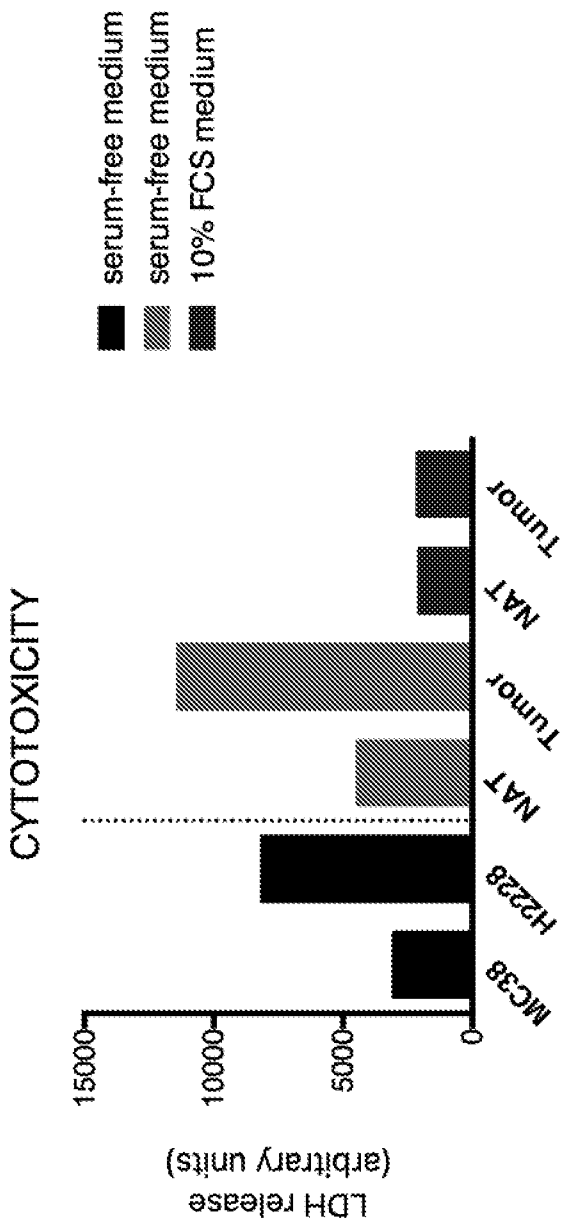
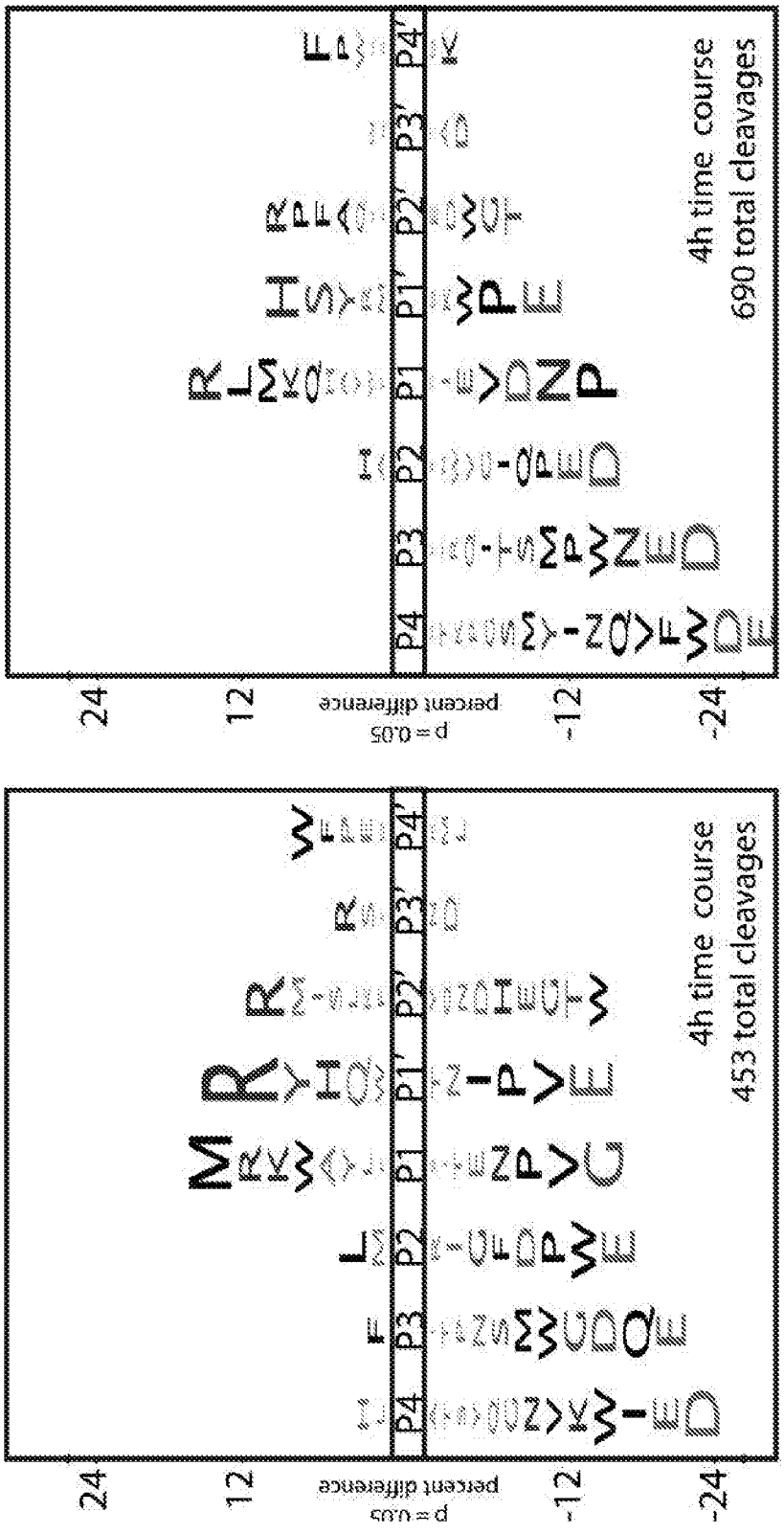

CYTOTOXICITY
serum-free medium
serum-free medium
10% FCS medium
LDH release
(arbitrary units)
15000
10000
5000
0
MC38
H228
NAT
Tumor
NAT
Tumor

FIG. 29

FIG. 30


MC38 conditioned media
H2228 conditioned media
24
12
-12
-24
percent difference
p = 0.05
P4
P3
P2
P1
P1'
P2'
P3'
P4'
4h time course
453 total cleavages
4h time course
690 total cleavages

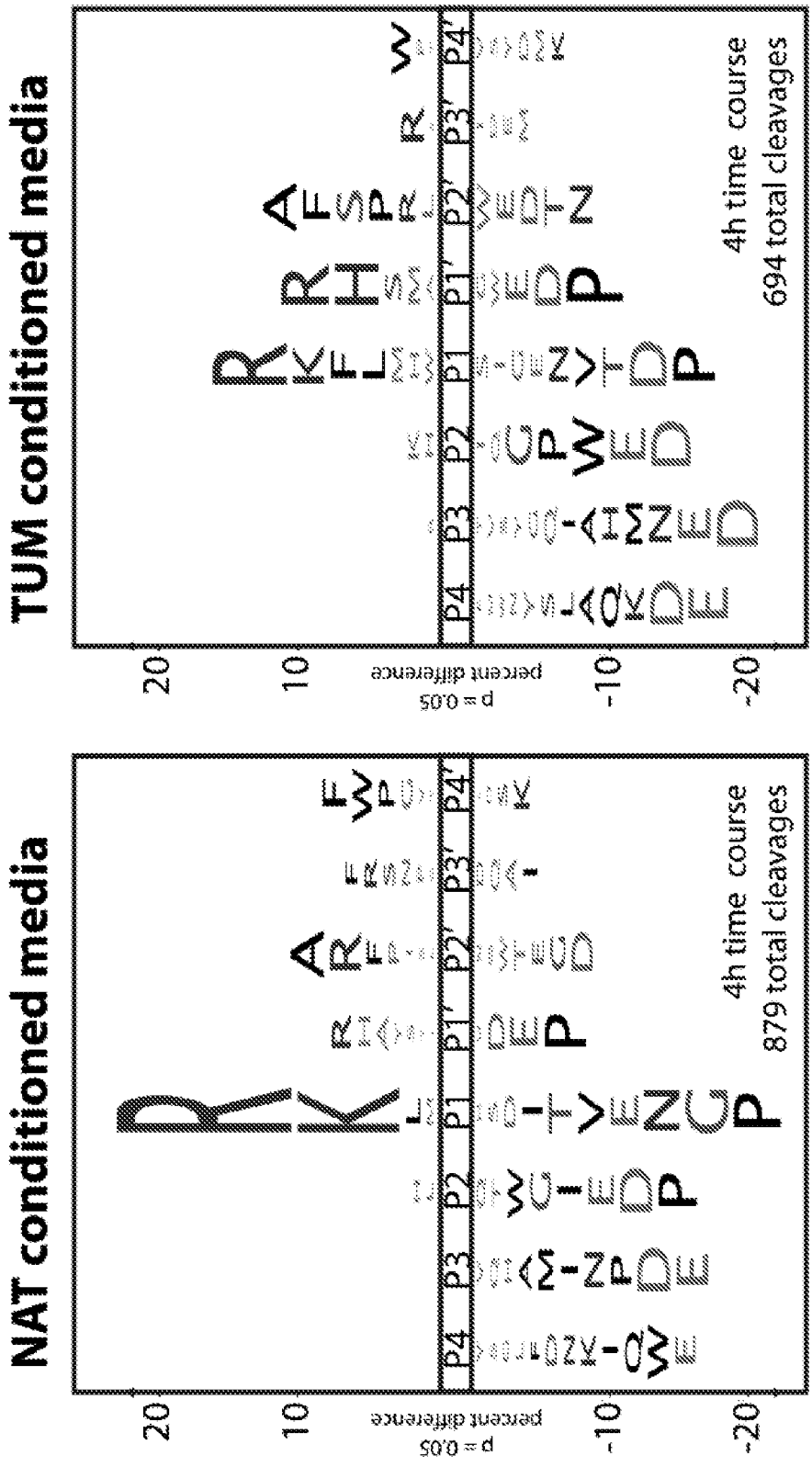

NAT conditioned media
TUM conditioned media
20
10
-10
-20
percent difference
p = 0.05
P4 P3 P2 P1 P1' P2' P3' P4'
4h time course
879 total cleavages
4h time course
694 total cleavages

FIG. 31A

FIG. 31B

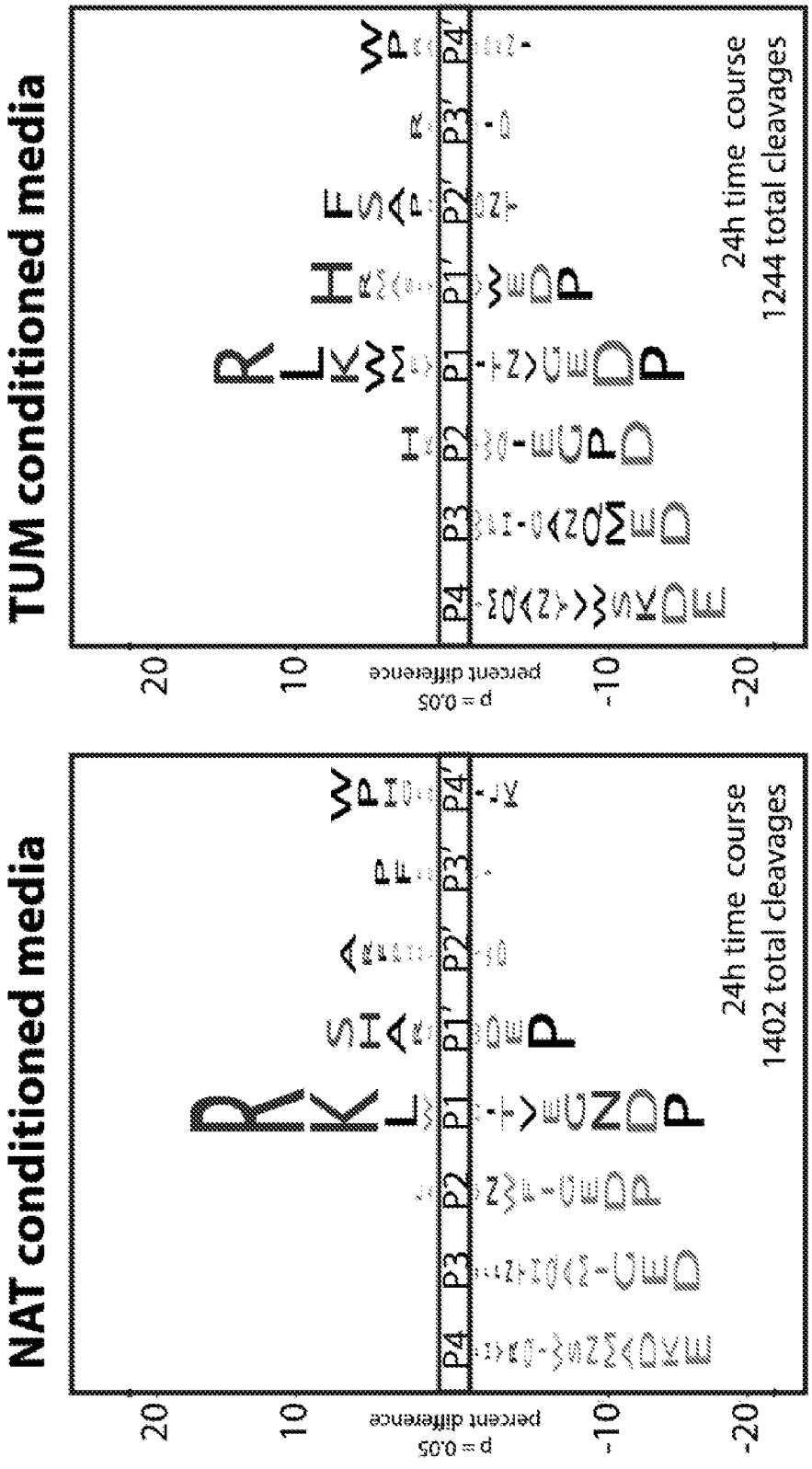

NAT conditioned media
TUM conditioned media
20
10
-10
-20
percent difference
p = 0.05
P4
P3
P2
P1
P1′
P2′
P3′
P4′
24h time course
1402 total cleavages
24h time course
1244 total cleavages

FIG. 31C

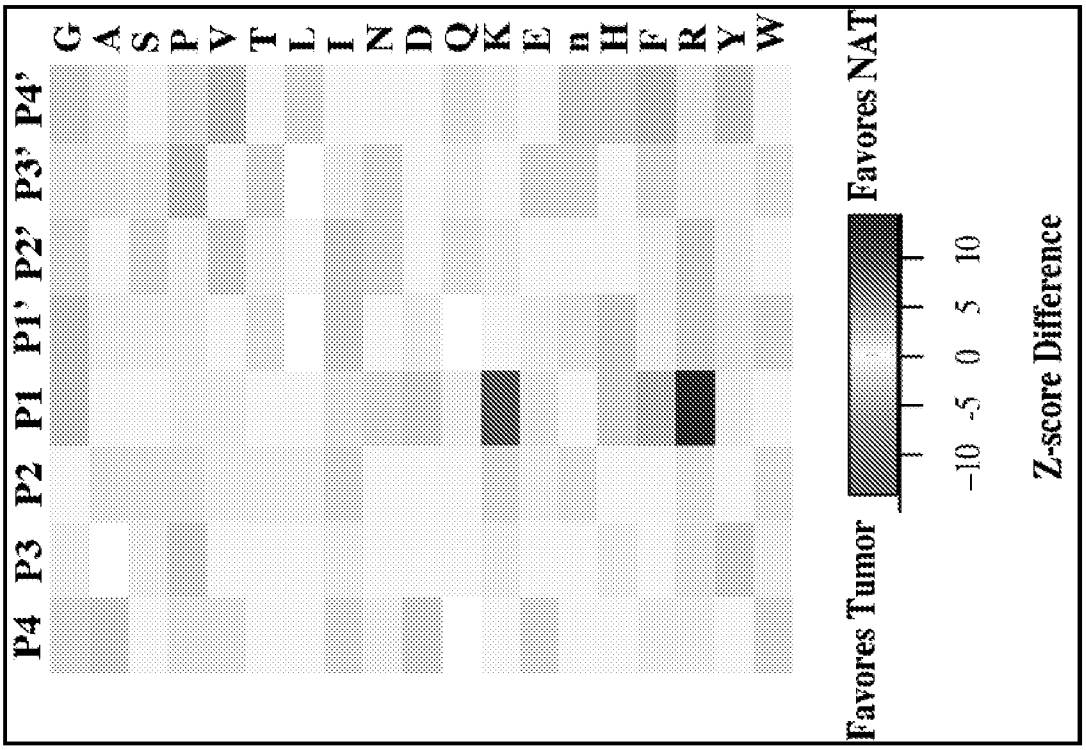
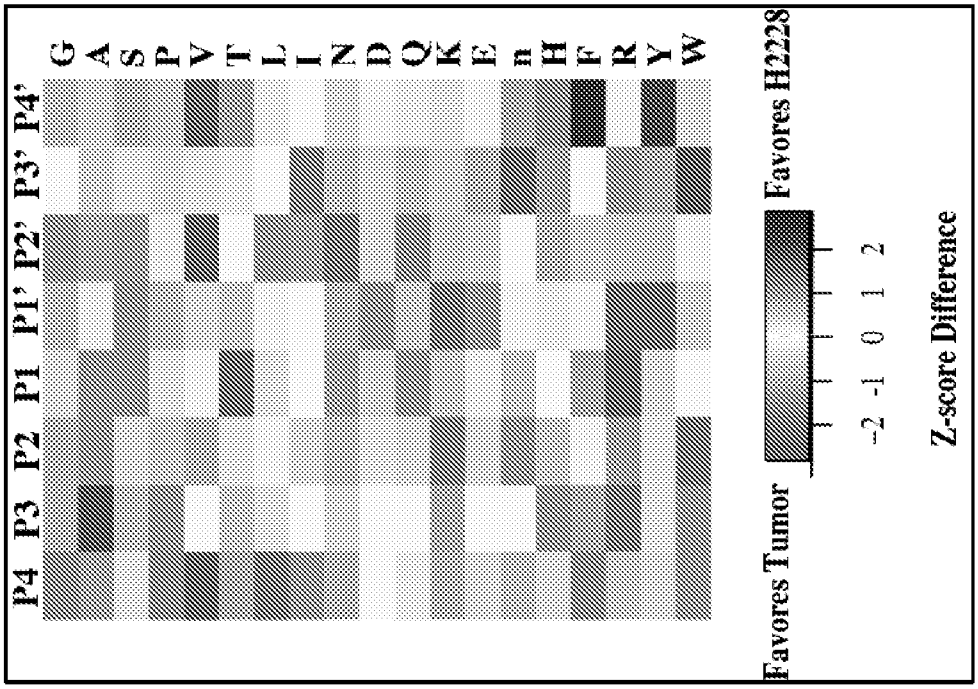

P4
P3
P2
P1
P1'
P2'
P3'
P4'
G
A
S
P
V
T
L
I
N
D
Q
K
E
n
H
F
R
Y
W
Favores Tumor
Favores NAT
−10
-5
0
5
10
Z-score Difference

FIG. 31D


P4 P3 P2 P1 P1' P2' P3' P4'
G A S P V T L I N D Q K E n H F R Y W
Favores Tumor
Favores H2228
−2 -1 0 1 2
Z-score Difference

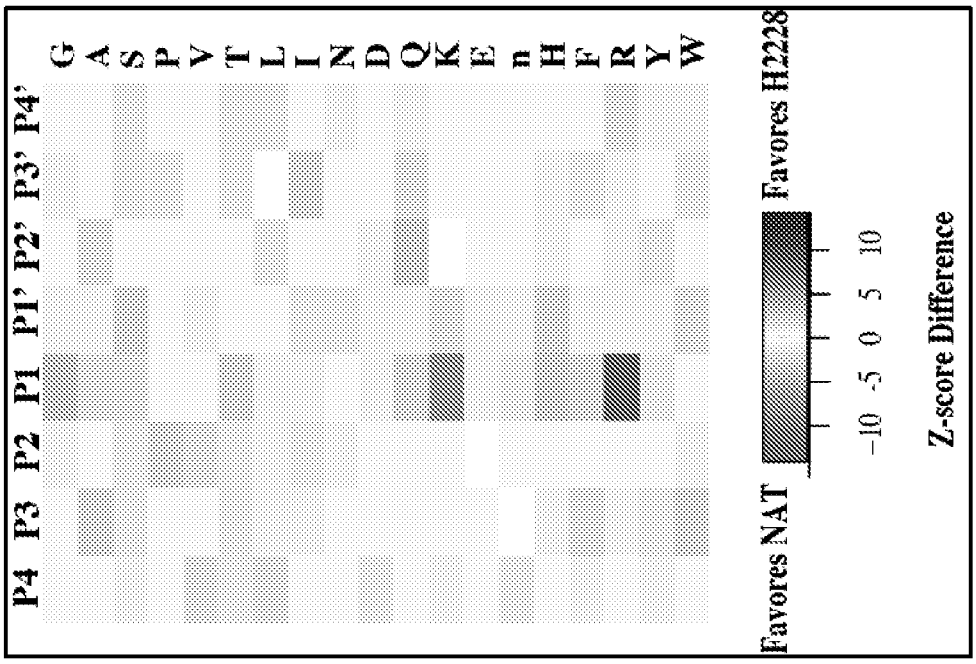

P4 P3 P2 P1 P1' P2' P3' P4'
G A S P V T L I N D Q K E n H F R Y W
Favores NAT
Favores H2228
−10 -5 0 5 10
Z-score Difference

FIG. 32A

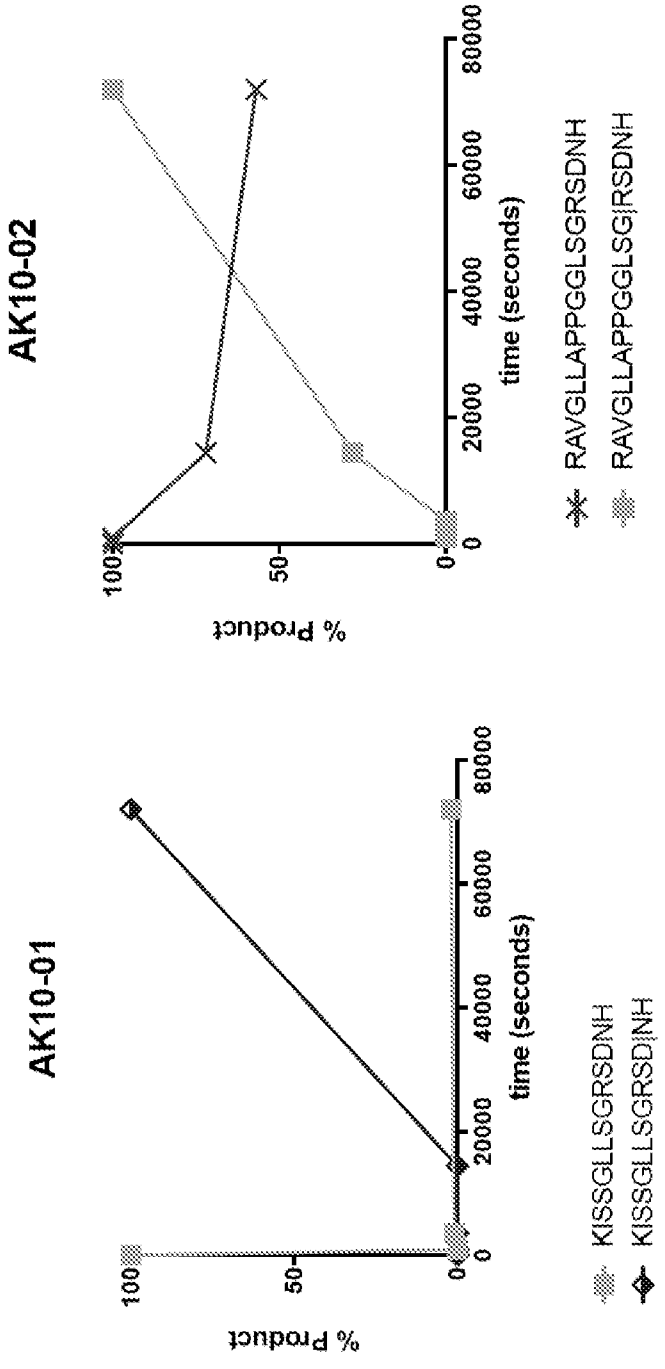

AK10-02
% Product
100
50
0
0
20000
40000
60000
80000
time (seconds)
RAVGLLAPPGGLSGRSDNH
RAVGLLAPPGGLSGRSDNH
AK10-01
% Product
100
50
0
0
20000
40000
60000
80000
time (seconds)
KISSGLLSGRSDNH
KISSGLLSGRSDNH

FIG. 32B

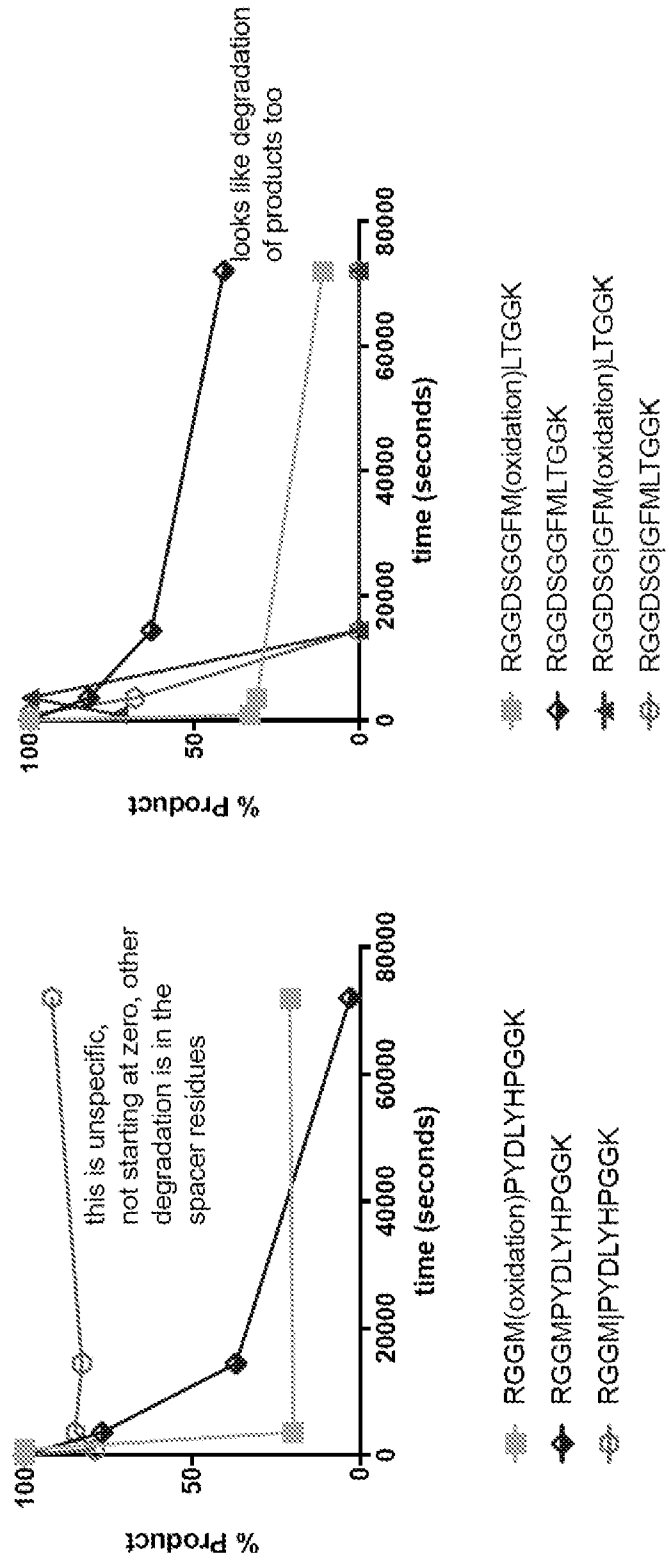
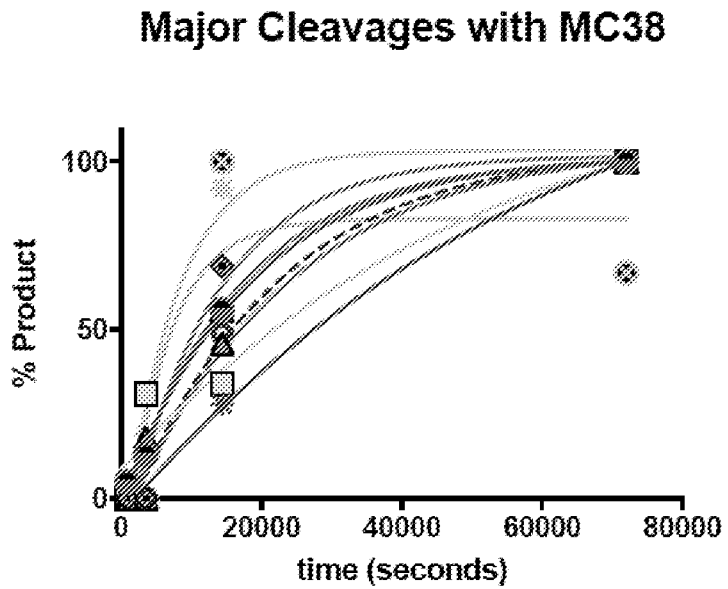

AK10-04
AK10-05
% Product
time (seconds)
0
20000
40000
60000
80000
50
100
this is unspecific, not starting at zero, other degradation is in the spacer residues
looks like degradation of products too
RGGM(oxidation)PYDLYHPGGK
RGGMPYDLYHPGGK
RGGM|PYDLYHPGGK
RGGDSGGFM(oxidation)LTGGK
RGGDSGGFMLTGGK
RGGDSG|GFM(oxidation)LTGGK
RGGDSG|GFMLTGGK

FIG. 33



	Peptide	relative efficiency	r^2
MPYDLYHPRQA|RVVG	AK-10	1.00	0.78
LS|GRSNAMPYDLYHP	AK-09	0.79	0.97
LSG|RSNAM(oxidation)PYDLYHP	AK-09	0.47	0.96
LSG|RSNAMPYDLYHP	AK-09	0.36	0.99
MPYDLYHPRQ|ARVVG	AK-10	0.33	0.99
LSGR|SNAMPYDLYHP	AK-09	0.29	0.98
LS|GRSNAM(oxidation)PYDLYHP	AK-09	0.26	0.98
L|SGRSNAMPYDLYHP	AK-09	0.12	0.93
RAVGLLAPPGGLSG|RSDNH	AK-02	0.10	0.99

FIG. 34A

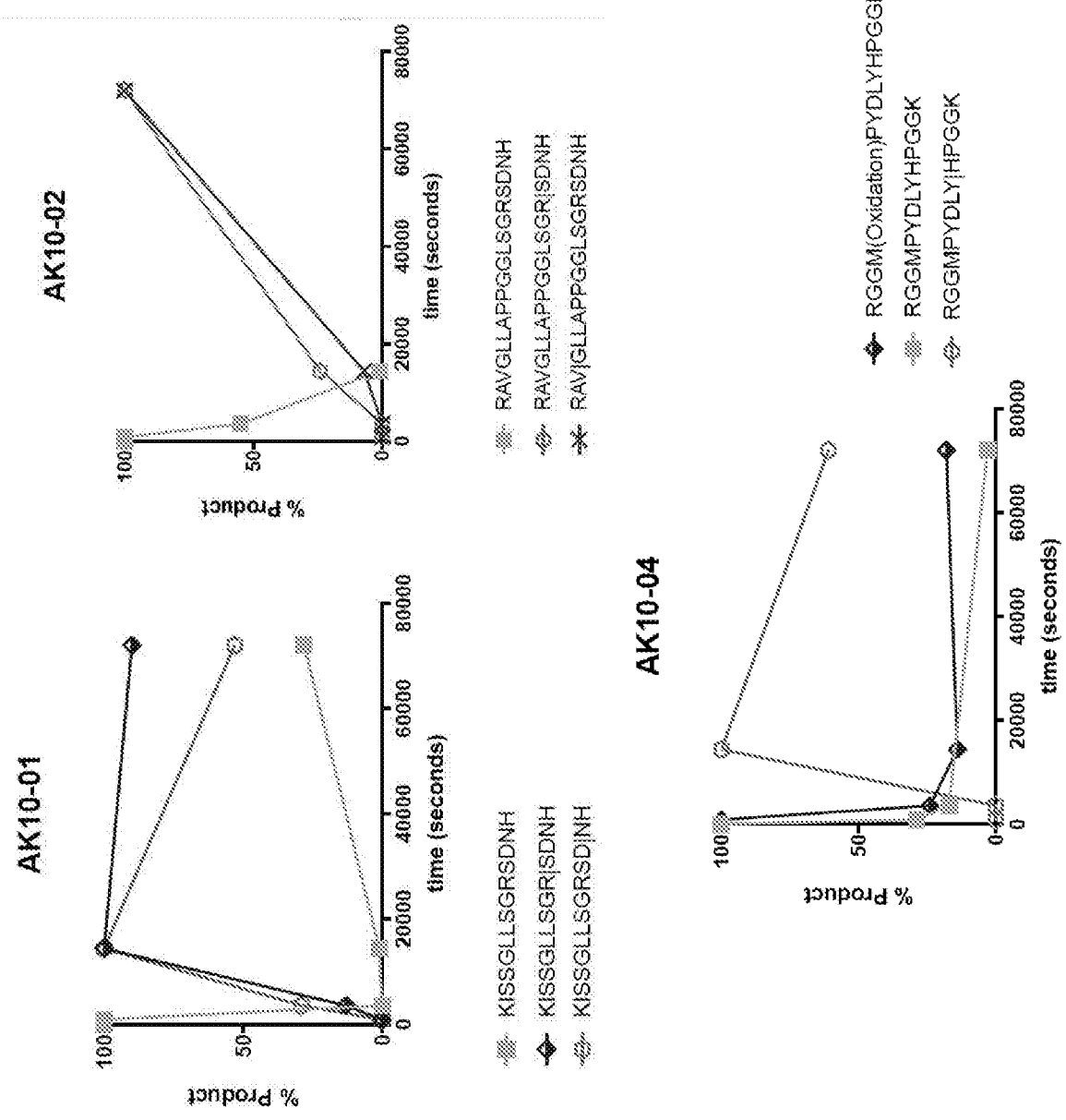

AK10-01
AK10-02
AK10-04
% Product
time (seconds)
0
20000
40000
60000
80000
50
100
KISSGLLSGRSDNH
KISSGLLSGR|SDNH
KISSGLLSGRSD|NH
RAVGLLAPPGGLSGRSDNH
RAVGLLAPPGGLSGR|SDNH
RAV|GLLAPPGGLSGRSDNH
RGGM(Oxidation)PYDLYHPGGK
RGGMPYDLYHPGGK
RGGMPYDLY|HPGGK

FIG. 34B

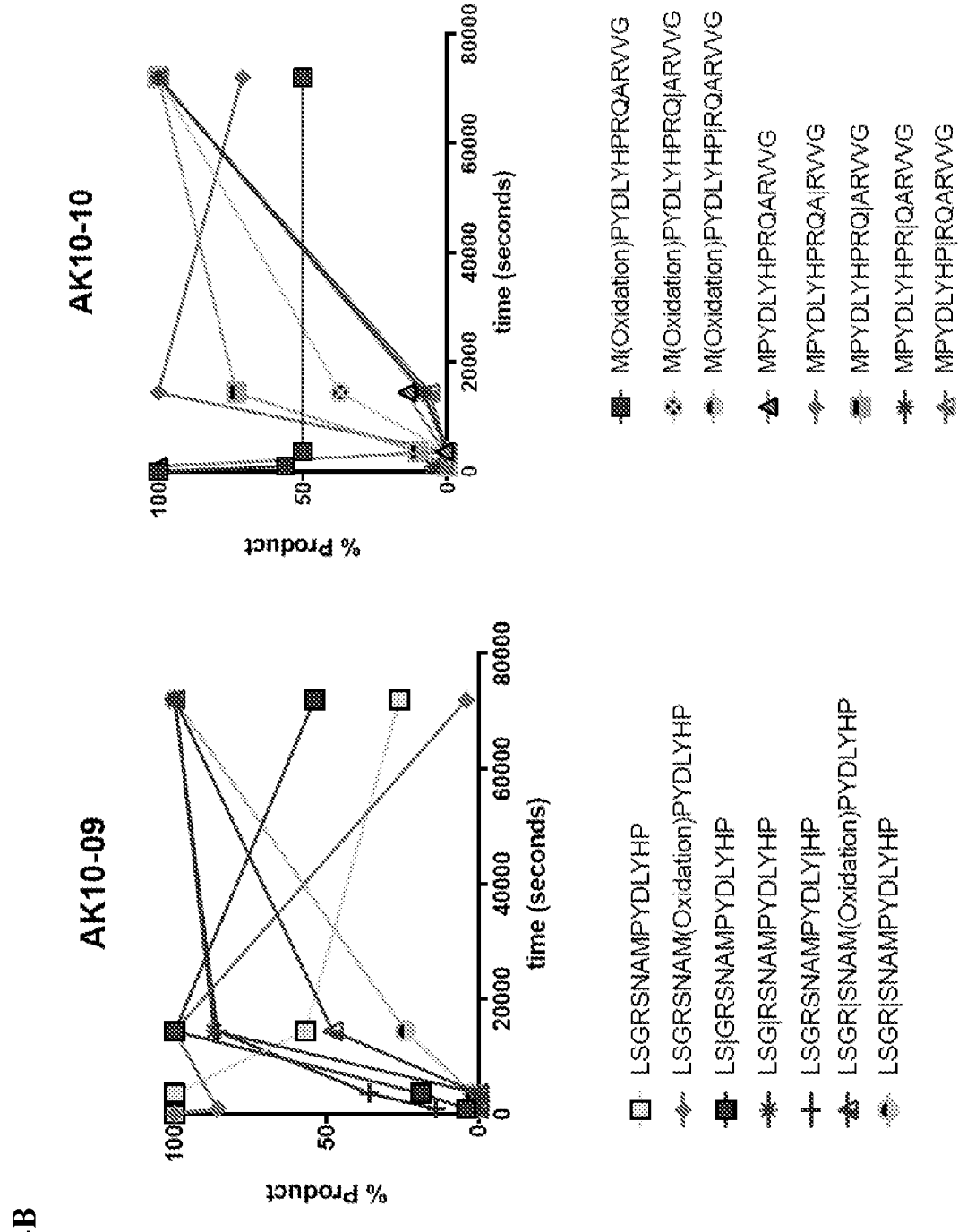
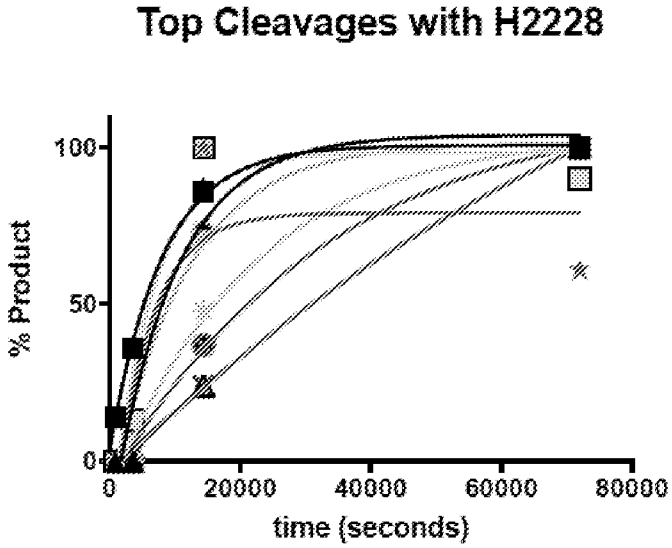

AK10-09
% Product
100
50
0
0
20000
40000
60000
80000
time (seconds)
AK10-10
% Product
100
50
0
0
20000
40000
60000
80000
time (seconds)
LSGRSNAMPYDLYHP
LSGRSNAM(Oxidation)PYDLYHP
LS|GRSNAMPYDLYHP
LSG|RSNAMPYDLYHP
LSGRSNAMPYDL|HP
LSGR|SNAM(Oxidation)PYDLYHP
LSGR|SNAMPYDLYHP
M(Oxidation)PYDLYHPRQARVVG
M(Oxidation)PYDLYHPRQ|ARVVG
M(Oxidation)PYDLYHP|RQARVVG
MPYDLYHPRQARVVG
MPYDLYHPRQA|RVVG
MPYDLYHPRQ|ARVVG
MPYDLYHPR|QARVVG
MPYDLYHP|RQARVVG

FIG. 35



	substrate	relative efficiency	r^2
RGGMPYDLY|HPGGK	AK-04	not fit	
KISSGLLSGR|SDNH	AK-01	1.00	0.92
LSGRSNAMPYDLY|HP	AK-09	0.71	0.99
LSG|RSNAMPYDLYHP	AK-09	0.65	0.93
MPYDLYHPRQ|ARVVG	AK-10	0.58	0.98
LSGR|SNAM(Oxidation)PYDLYHP	AK-09	0.30	0.98
M(Oxidation)PYDLYHPRQ|ARVVG	AK-10	0.20	0.99
RAVGLLAPPGGLSGR|SDNH	AK-02	0.07	0.99
LSGR|SNAMPYDLYHP	AK-09	0.06	0.99

FIG. 36A

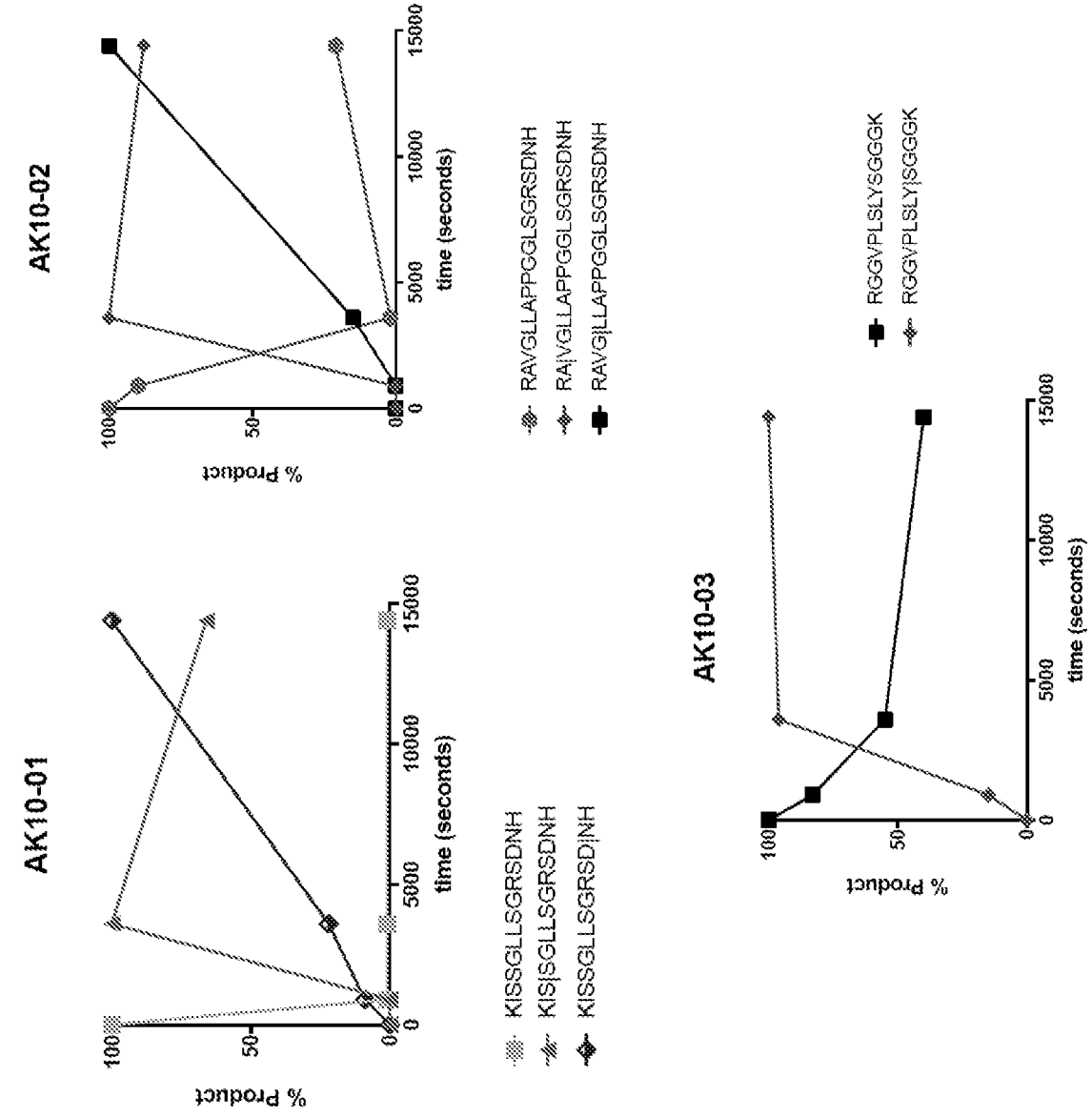

AK10-01
AK10-02
AK10-03
% Product
time (seconds)
0
50
100
5000
10000
15000
KISSGLLSGRSDNH
KIS|SGLLSGRSDNH
KISSGLLSGRSD|NH
RAVGLLAPPGGLSGRSDNH
RA|VGLLAPPGGLSGRSDNH
RAVG|LLAPPGGLSGRSDNH
RGGVPLSLYSGGGK
RGGVPLSLY|SGGGK

FIG. 36B

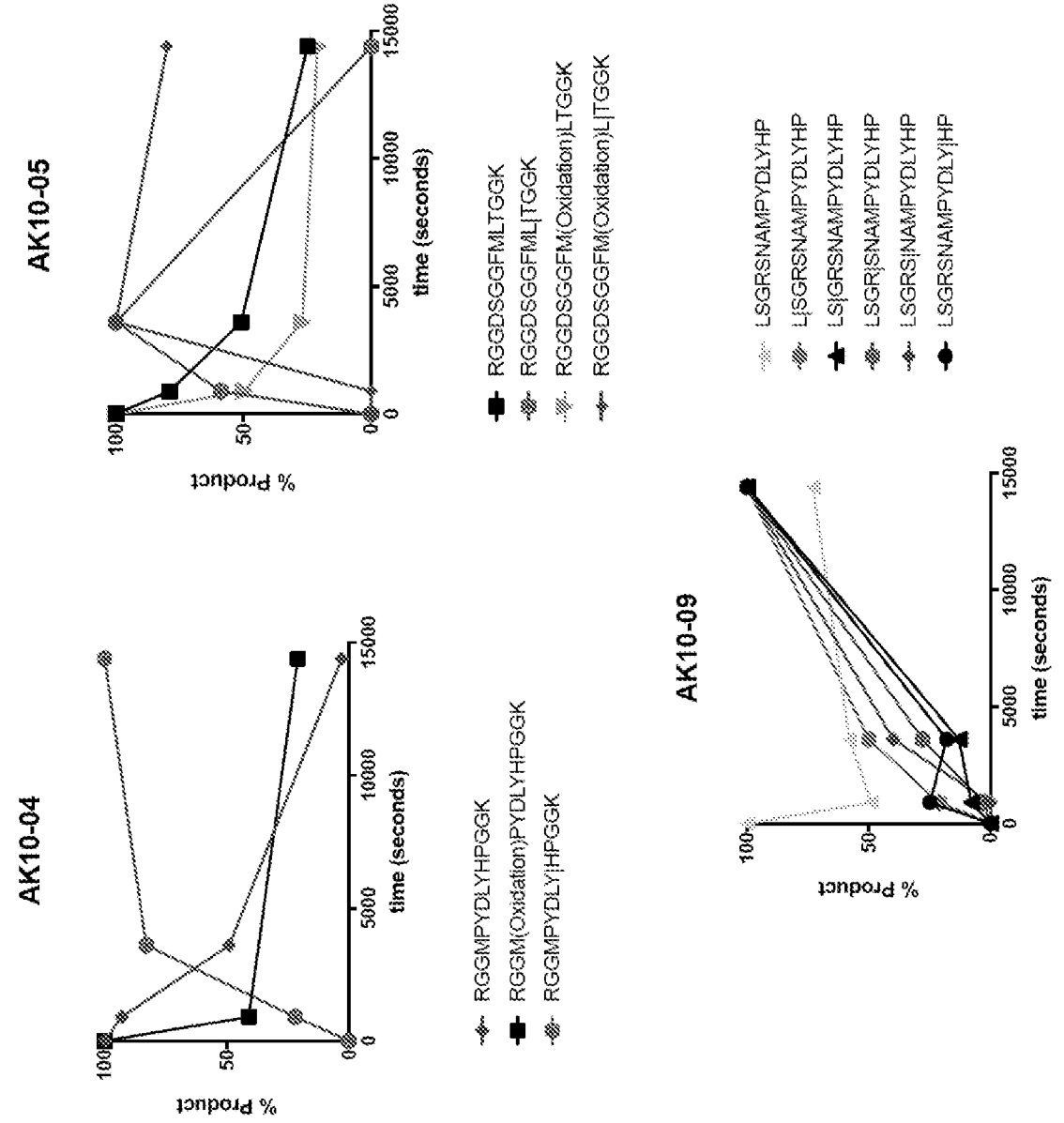

AK10-04
AK10-05
AK10-09
% Product
time (seconds)
0
50
100
5000
10000
15000
RGGMPYDLYHPGGK
RGGM(Oxidation)PYDLYHPGGK
RGGMPYDLY(HP)GGK
RGGDSGGFMLTGGK
RGGDSGGFML(TGGK
RGGDSGGFM(Oxidation)LTGGK
RGGDSGGFM(Oxidation)L(TGGK
LSGRSNAMPYDLYHP
L(SGRSNAMPYDLYHP
LS(GRSNAMPYDLYHP
LSGR(SNAMPYDLYHP
LSGRS(NAMPYDLYHP
LSGRSNAMPYDLY(HP

FIG. 36C

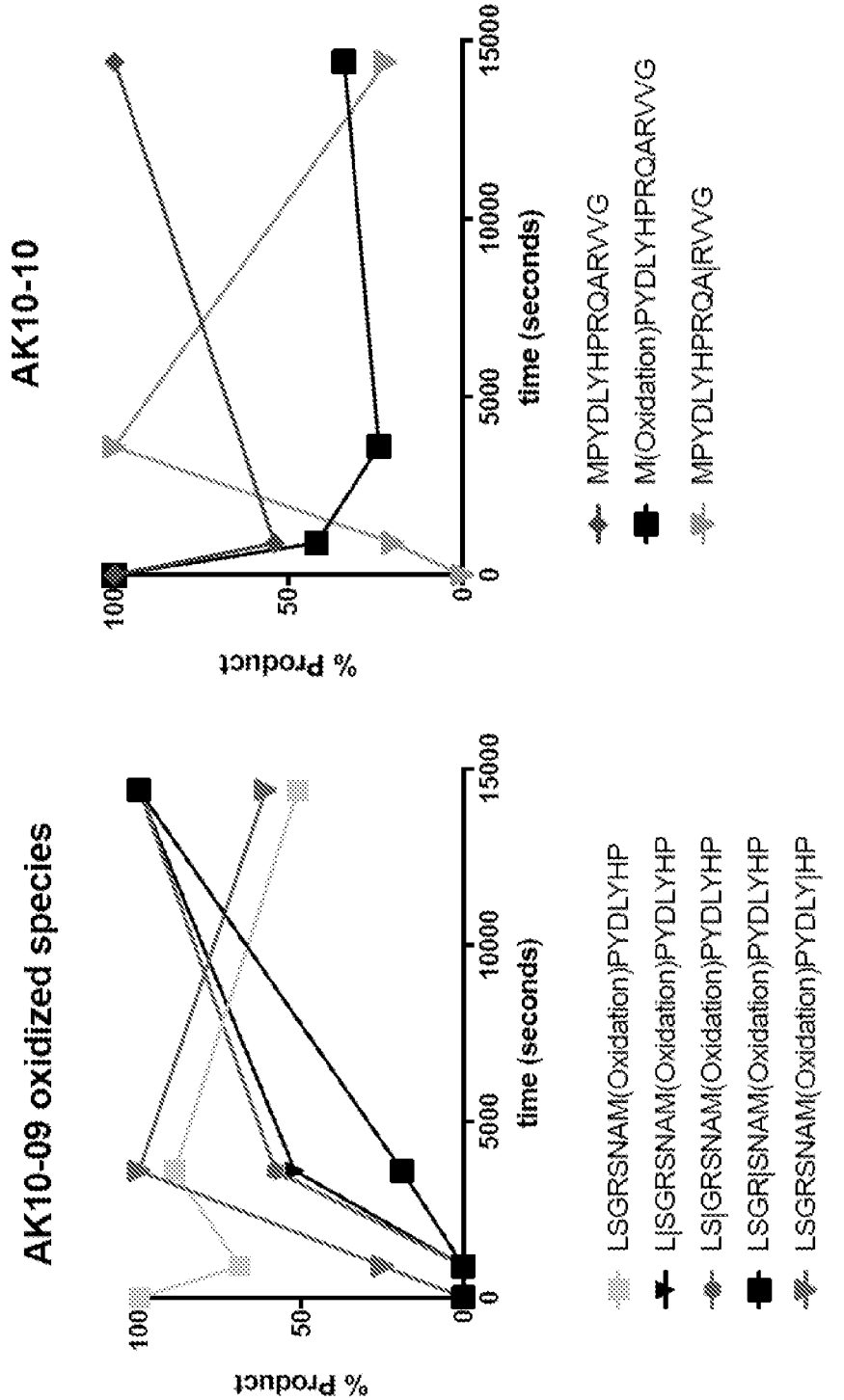
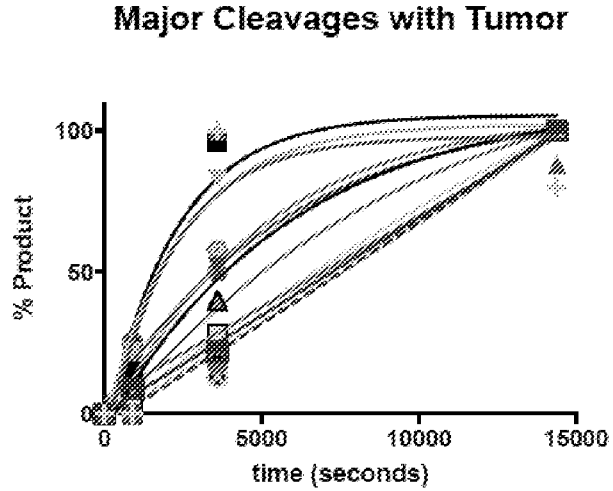

AK10-09 oxidized species
% Product
100
50
0
0
5000
10000
15000
time (seconds)
LSGRSNAM(Oxidation)PYDLYHP
L|SGRSNAM(Oxidation)PYDLYHP
LS|GRSNAM(Oxidation)PYDLYHP
LSGR|SNAM(Oxidation)PYDLYHP
LSGRSNAM(Oxidation)PYDLY|HP
AK10-10
% Product
100
50
0
0
5000
10000
15000
time (seconds)
MPYDLYHPRQARVVG
M(Oxidation)PYDLYHPRQARVVG
MPYDLYHPRQA|RVVG

FIG. 36D



	Peptide	relative efficiency	r^2
RGGDSGGFM(Oxidation)L|TGGK	AK-02	>1.00	no fit
RA|VGLLAPPGGLSGRSDNH	AK-05	>1.00	no fit
RGGVPLSLY|SGGGK	AK-03	1.00	0.78
RGGMPYDLY|HPGGK	AK-04	0.90	0.97
LS|GRSNAM(Oxidation)PYDLYHP	AK-09	0.46	0.96
L|SGRSNAMPYDLYHP	AK-09	0.38	0.99
L|SGRSNAM(Oxidation)PYDLYHP	AK-09	0.38	0.99
LSGRS|NAMPYDLYHP	AK-09	0.23	0.98
LSGR|SNAMPYDLYHP	AK-09	0.05	0.98
KISSGLLSGRSD|NH	AK-01	<0.01	no fit
LSGRSNAMPYDLY|HP	AK-09	<0.01	no fit
LSGR|SNAM(Oxidation)PYDLYHP	AK-09	<0.01	no fit
LS|GRSNAMPYDLYHP	AK-09	<0.01	no fit

FIG. 37A

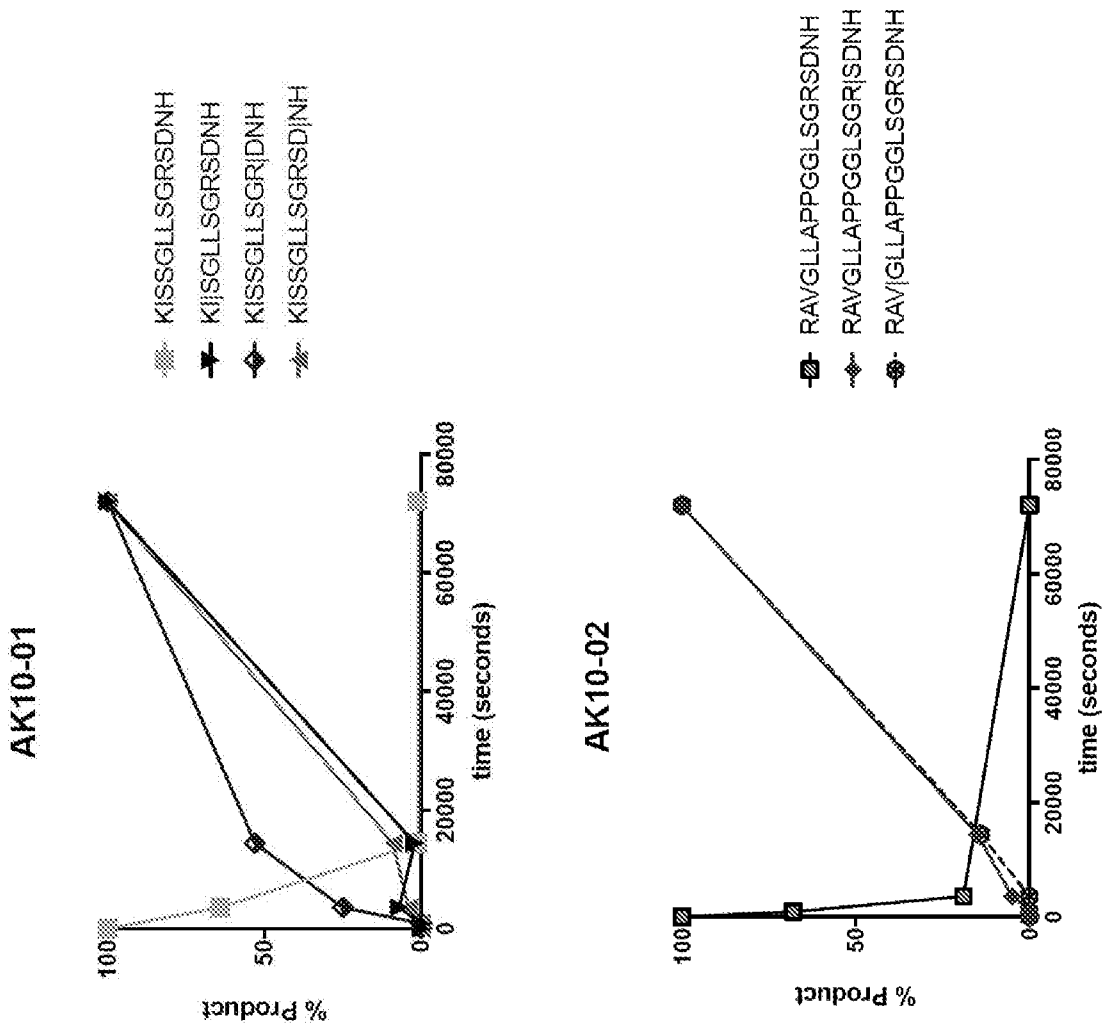

AK10-01
KISSGLLSGRSDNH
KI|SGLLSGRSDNH
KISSGLLSGR|DNH
KISSGLLSGRSD|NH
% Product
time (seconds)
0
50
100
0
20000
40000
60000
80000
AK10-02
RAV\GLLAPPGGLSGRSDNH
RAV\GLLAPPGGLSGR|SDNH
RAV|GLLAPPGGLSGRSDNH
% Product
time (seconds)

FIG. 37B

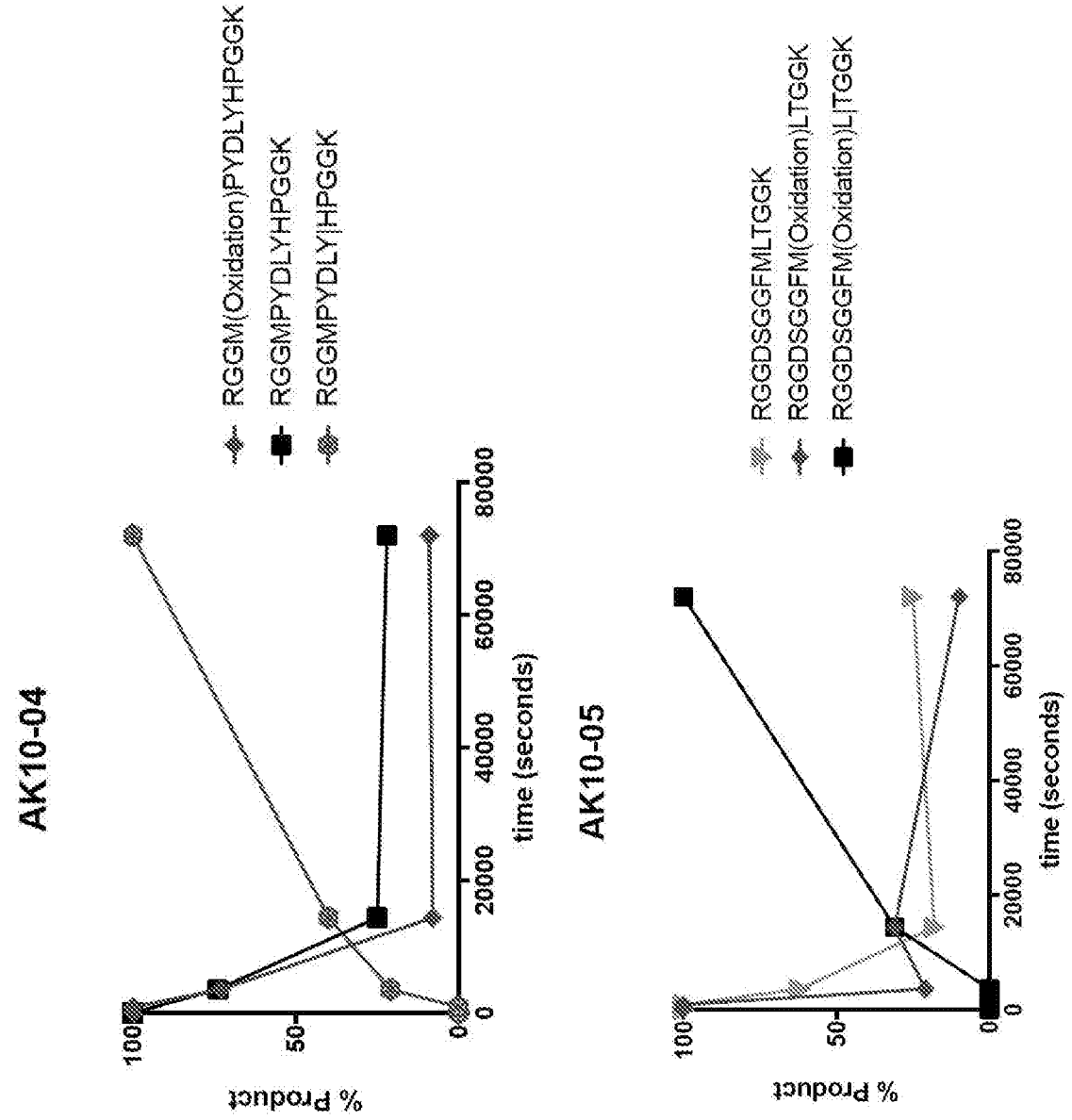

AK10-04
RGGM(Oxidation)PYDLYHPGGK
RGGMPYDLYHPGGK
RGGMPYDLY|HPGGK
% Product
100
50
0
0
20000
40000
60000
80000
time (seconds)
AK10-05
RGGDSGGFMLTGGK
RGGDSGGFM(Oxidation)LTGGK
RGGDSGGFM(Oxidation)L|TGGK
% Product
100
50
0
0
20000
40000
60000
80000
time (seconds)

FIG. 37C

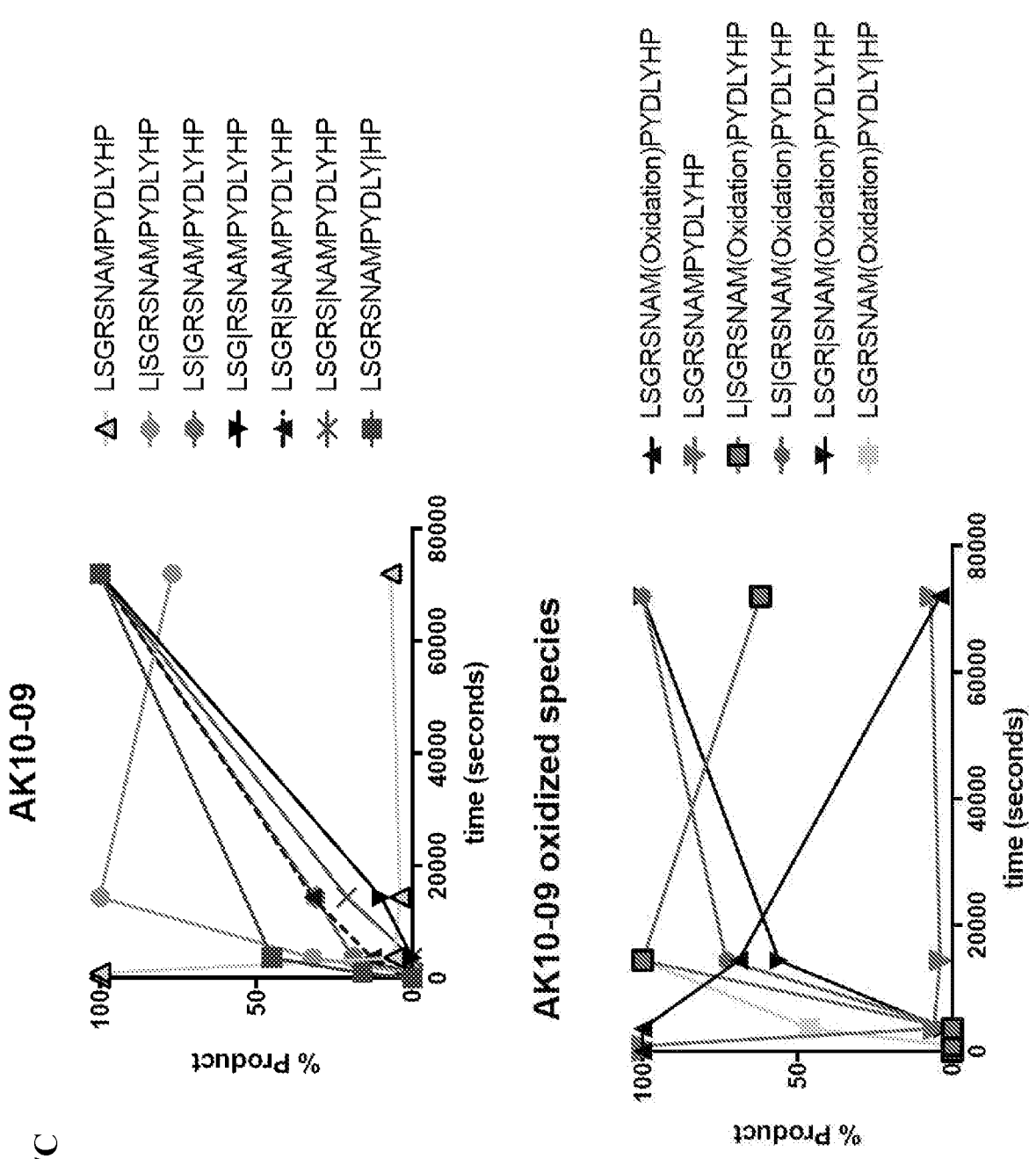

AK10-09
% Product
time (seconds)
0
50
100
0
20000
40000
60000
80000
LSGRSNAMPYDLYHP
L|SGRSNAMPYDLYHP
LS|GRSNAMPYDLYHP
LSG|RSNAMPYDLYHP
LSGR|SNAMPYDLYHP
LSGRS|NAMPYDLYHP
LSGRSNAMPYDLY|HP
AK10-09 oxidized species
% Product
time (seconds)
LSGRSNAM(Oxidation)PYDLYHP
LSGRSNAMPYDLYHP
L|SGRSNAM(Oxidation)PYDLYHP
LS|GRSNAM(Oxidation)PYDLYHP
LSGR|SNAM(Oxidation)PYDLYHP
LSGRSNAM(Oxidation)PYDLY|HP

FIG. 37D

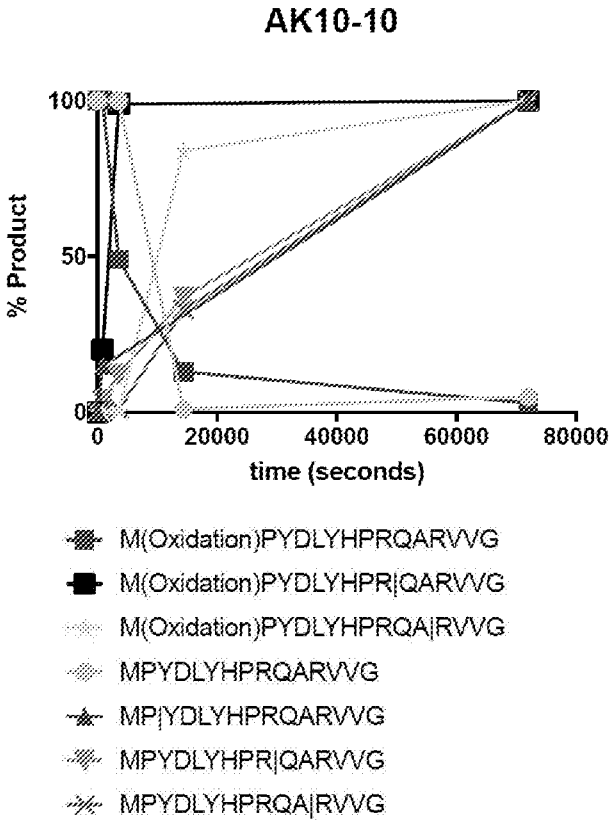
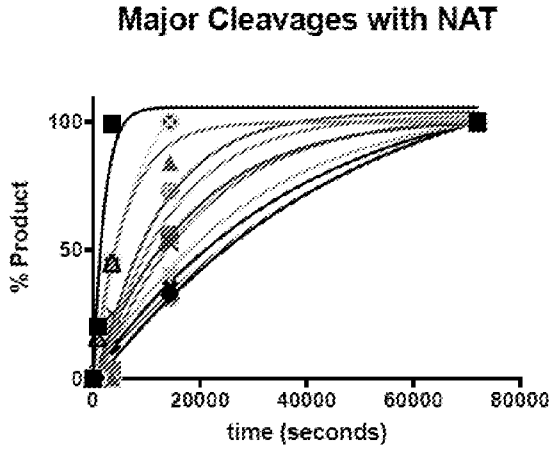


FIG. 37E



	Substrate	relative efficiency	r^2
M(Oxidation)PYDLYHPR|QARVVG	AK-10	1.00	0.94
LSGRSNAMPYDLY|HP	AK-09	0.34	0.99
LSGRSNAM(Oxidation)PYDLY|HP	AK-09	0.28	0.98
M(Oxidation)PYDLYHPRQA|RVVG	AK-10	0.18	0.93
LS|GRSNAM(Oxidation)PYDLYHP	AK-09	0.15	0.95
KISSGLLSGR|DNH	AK-01	0.11	0.98
LSGR|SNAM(Oxidation)PYDLYHP	AK-09	0.11	0.98
RGGMPYDLY|HPGGK	AK-04	0.07	0.98
MPYDLYHPR|QARVVG	AK-10	0.05	0.99
MPYDLYHPRQA|RVVG	AK-10	0.04	0.99
LS|GRSNAMPYDLYHP	AK-09	0.04	0.98
LSGR|SNAMPYDLYHP	AK-09	0.04	0.99

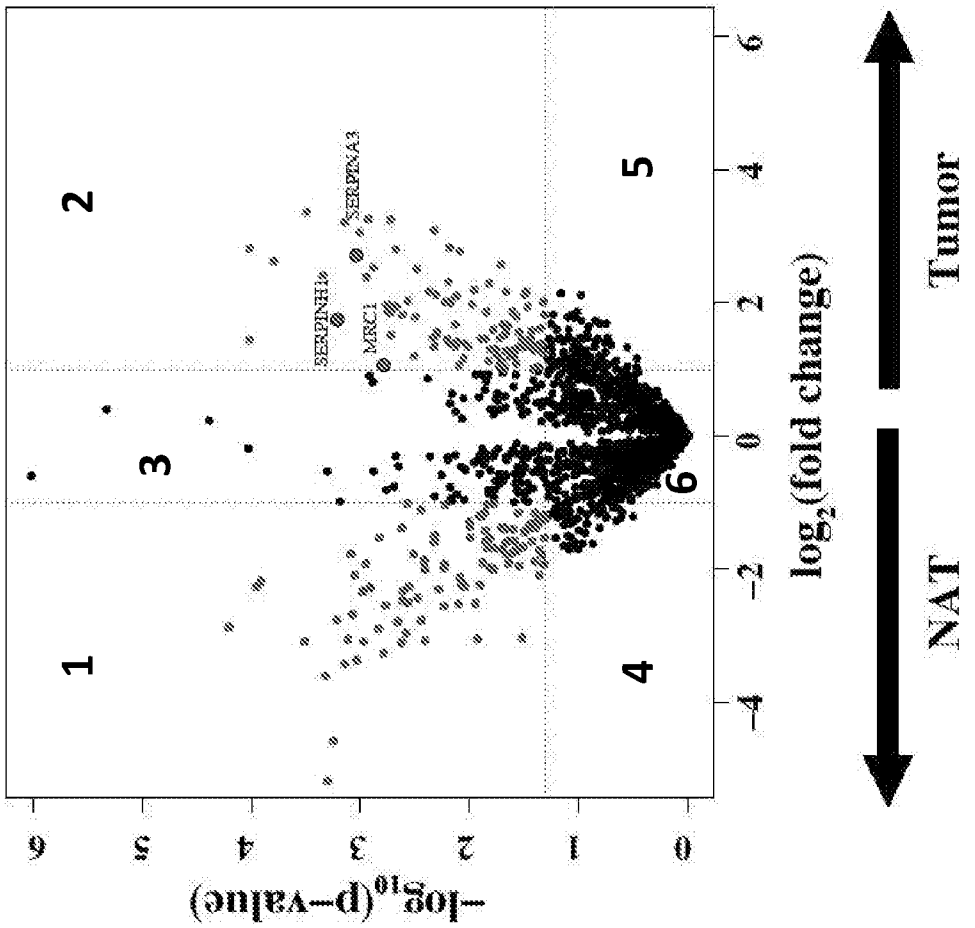

1
2
3
4
5
6
SERPINH1
SERPINA3
MRC1
−log10(p−value)
log2(fold change)
NAT
Tumor

FIG. 38