Figure 1

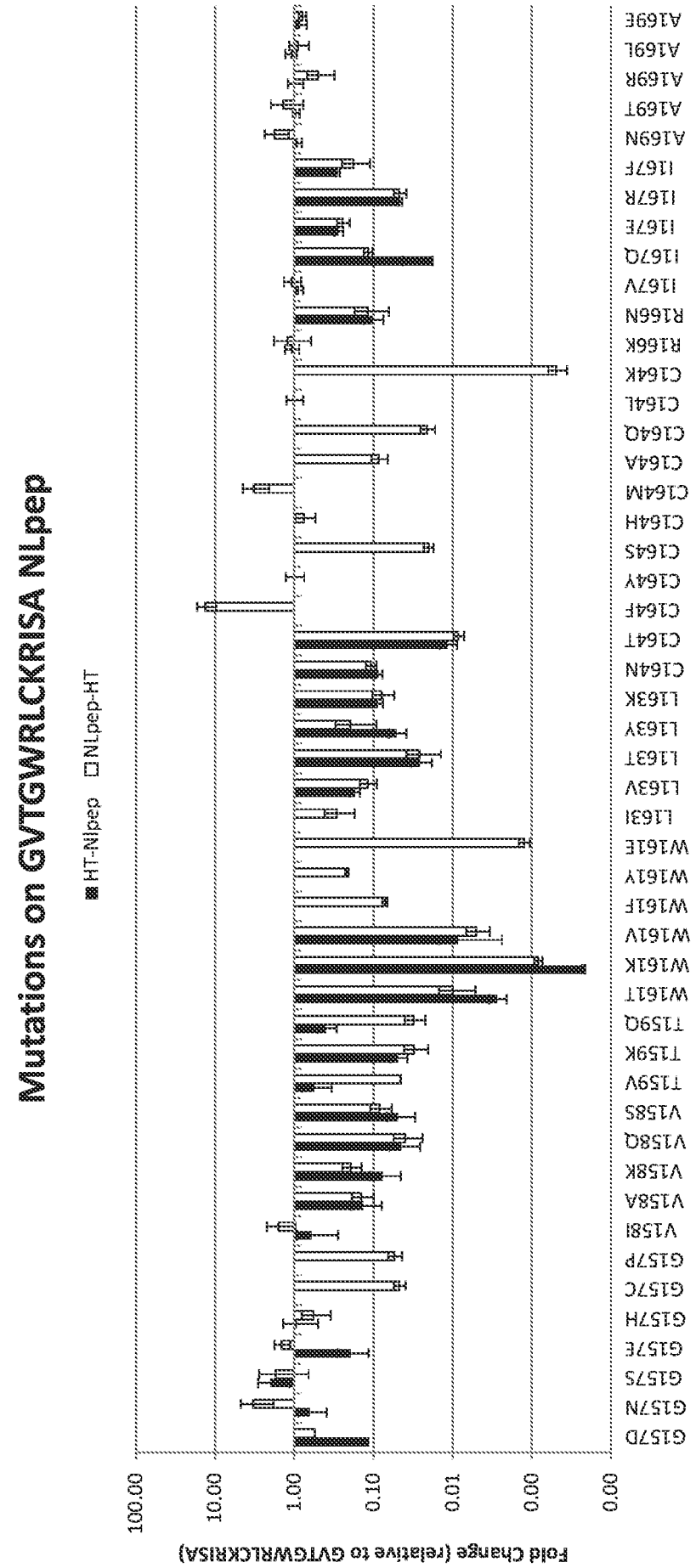

Mutations on GVTGWRLCKRISA NLpep
HT-Nlpep
NLpep-HT
Fold Change (relative to GVTGWRLCKRISA)
100.00
10.00
1.00
0.10
0.01
0.00
0.00
G157D
G157N
G157S
G157E
G157H
G157C
G157P
V158I
V158A
V158K
V158Q
V158S
T159V
T159K
T159Q
W161T
W161K
W161V
W161F
W161Y
W161E
L163I
L163V
L163T
L163Y
L163K
C164N
C164T
C164F
C164V
C164S
C164H
C164M
C164A
C164Q
C164L
C164K
R166K
R166N
I167V
I167Q
I167E
I167R
I167F
A169N
A169T
A169R
A169L
A169E

Figure 2

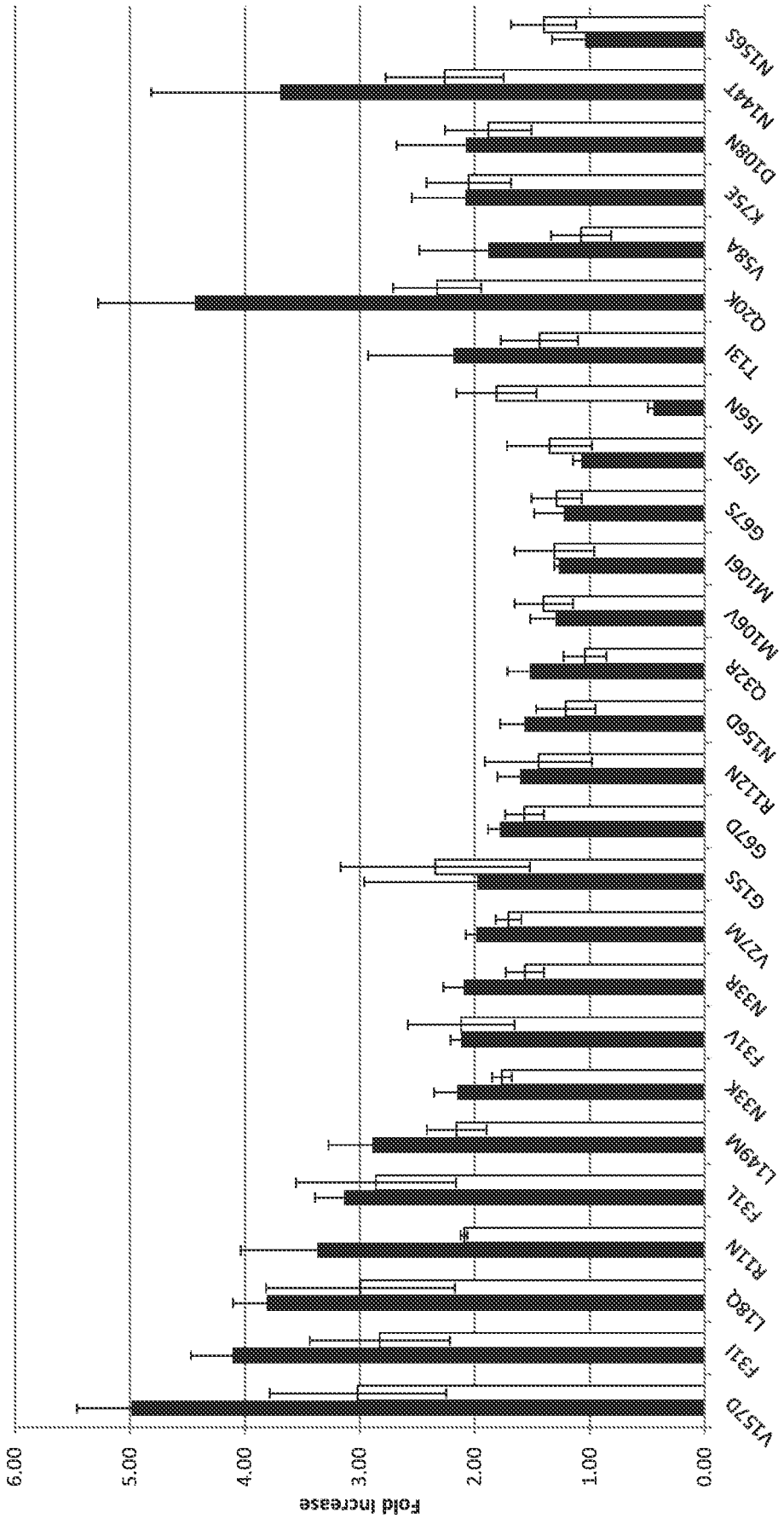
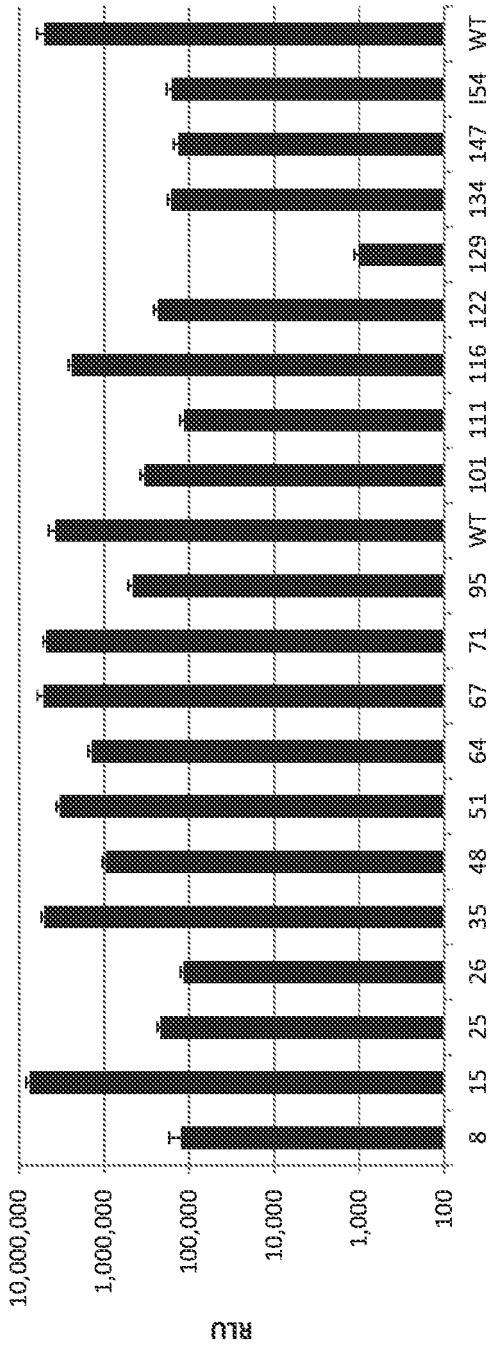
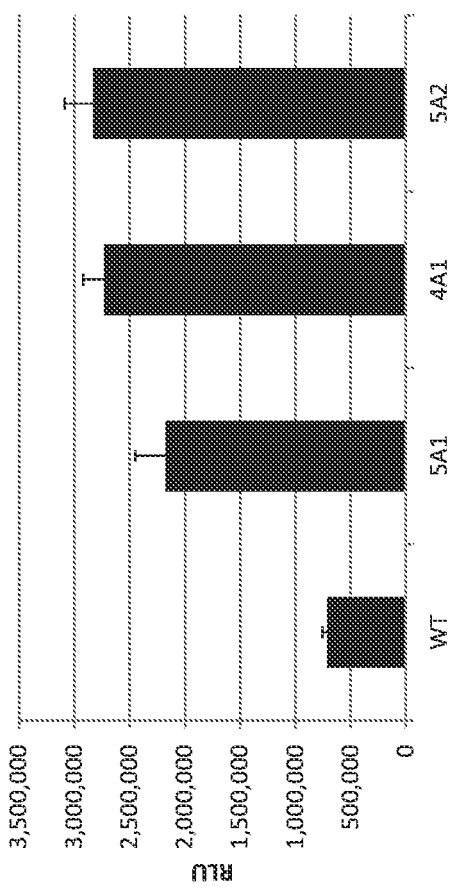

NLpoly Mutations
NLpep: GVTGWRLCKRISA GVTGWRLFKRISA
Fold increase
6.00
5.00
4.00
3.00
2.00
1.00
0.00
V157D
F31I
L18Q
R11N
F31L
L149M
N33K
F31V
N33R
V27M
G15S
G67D
R11N
N156D
Q32R
M106V
M106I
G67S
I59T
I56N
T13I
Q20K
V58A
K75E
D108N
N144T
N156S

Figure 3



Position	8	15	25	26	35	48	51	64	67	71	95	WT	101	111	116	122	129	134	147	I54A	WT
RLU	1.2E+05	7.5E+06	2.2E+05	1.2E+05	5.0E+06	9.5E+05	3.4E+06	1.4E+06	5.2E+06	4.8E+06	4.6E+05	3.7E+06	3.4E+05	1.1E+05	2.4E+06	2.3E+05	1.0E+03	1.6E+05	1.3E+05	1.6E+05	5.1E+06
Fold Increase	0.03	2.01	0.06	0.03	1.35	0.26	0.90	0.37	1.39	1.28	0.12	1.00	0.07	0.02	0.47	0.05	0.00	0.03	0.03	0.03	1.00

Figure 4



Name	G15A	D19A	G35A	G51A	G67A	G71A	Fold Incr.
5A1	Yes	Yes	Yes	Yes	Yes		3.07
4A1	Yes		Yes		Yes	Yes	3.85
5A2	Yes		Yes	Yes	Yes	Yes	3.99

Figure 5

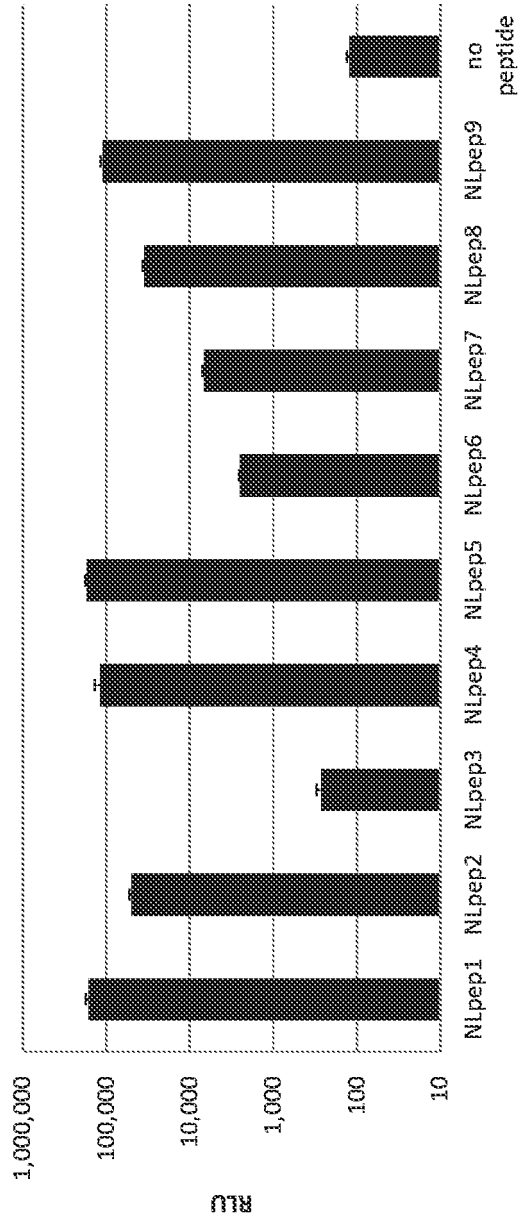

HT-NLpep1-9
RLU
1,000,000
100,000
10,000
1,000
100
10
NLpep1
NLpep2
NLpep3
NLpep4
NLpep5
NLpep6
NLpep7
NLpep8
NLpep9
no peptide

Figure 6

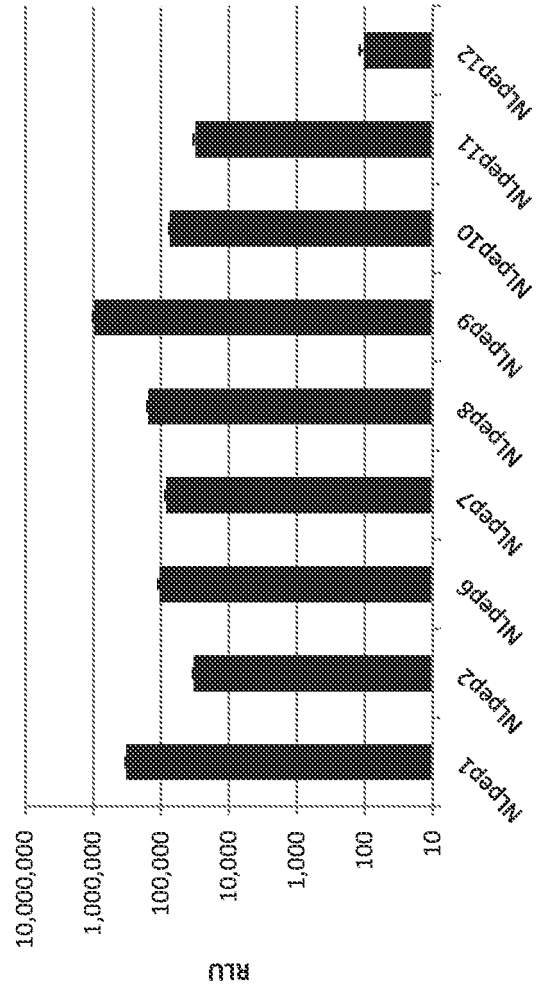

HT-NLpep
RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
10
NLpep1
NLpep2
NLpep6
NLpep7
NLpep8
NLpep9
NLpep10
NLpep11
NLpep12

Figure 7

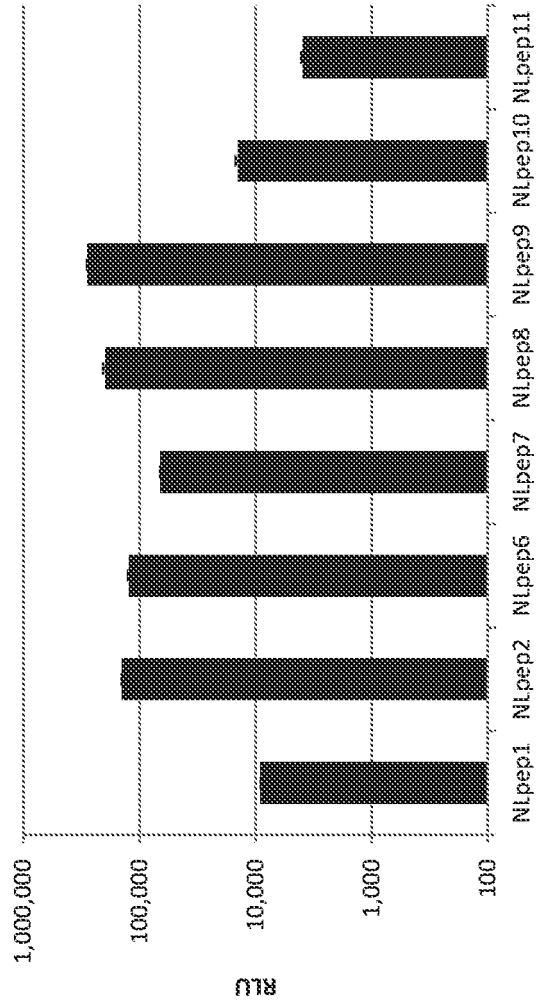

NLpep-HT
RLU
1,000,000
100,000
10,000
1,000
100
NLpep1
NLpep2
NLpep6
NLpep7
NLpep8
NLpep9
NLpep10
NLpep11

Figure 8

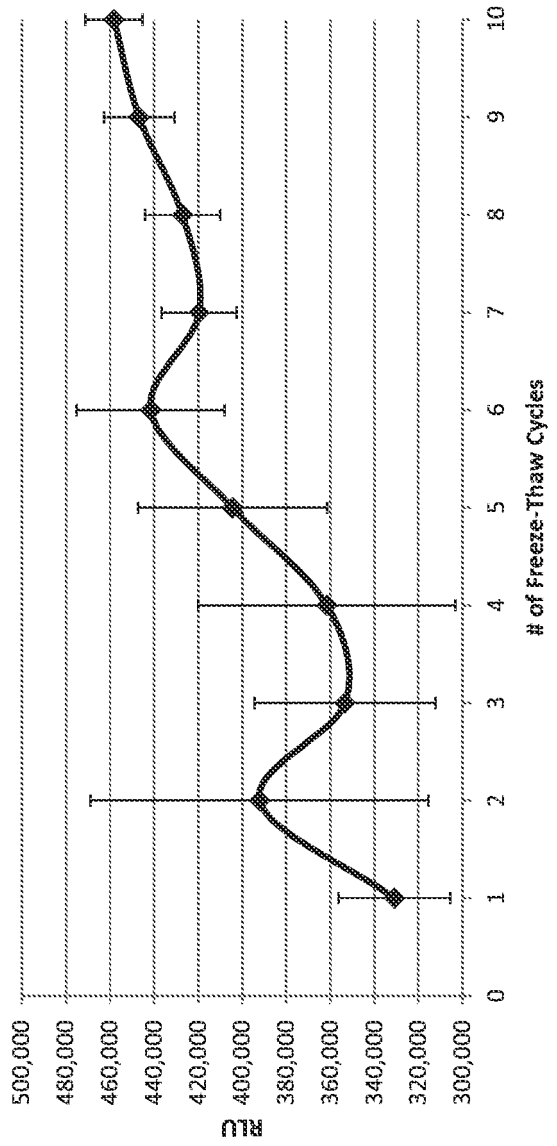

NLpep9-HT Stability
RLU
500,000
480,000
460,000
440,000
420,000
400,000
380,000
360,000
340,000
320,000
300,000
0
1
2
3
4
5
6
7
8
9
10
# of Freeze-Thaw Cycles

Figure 9

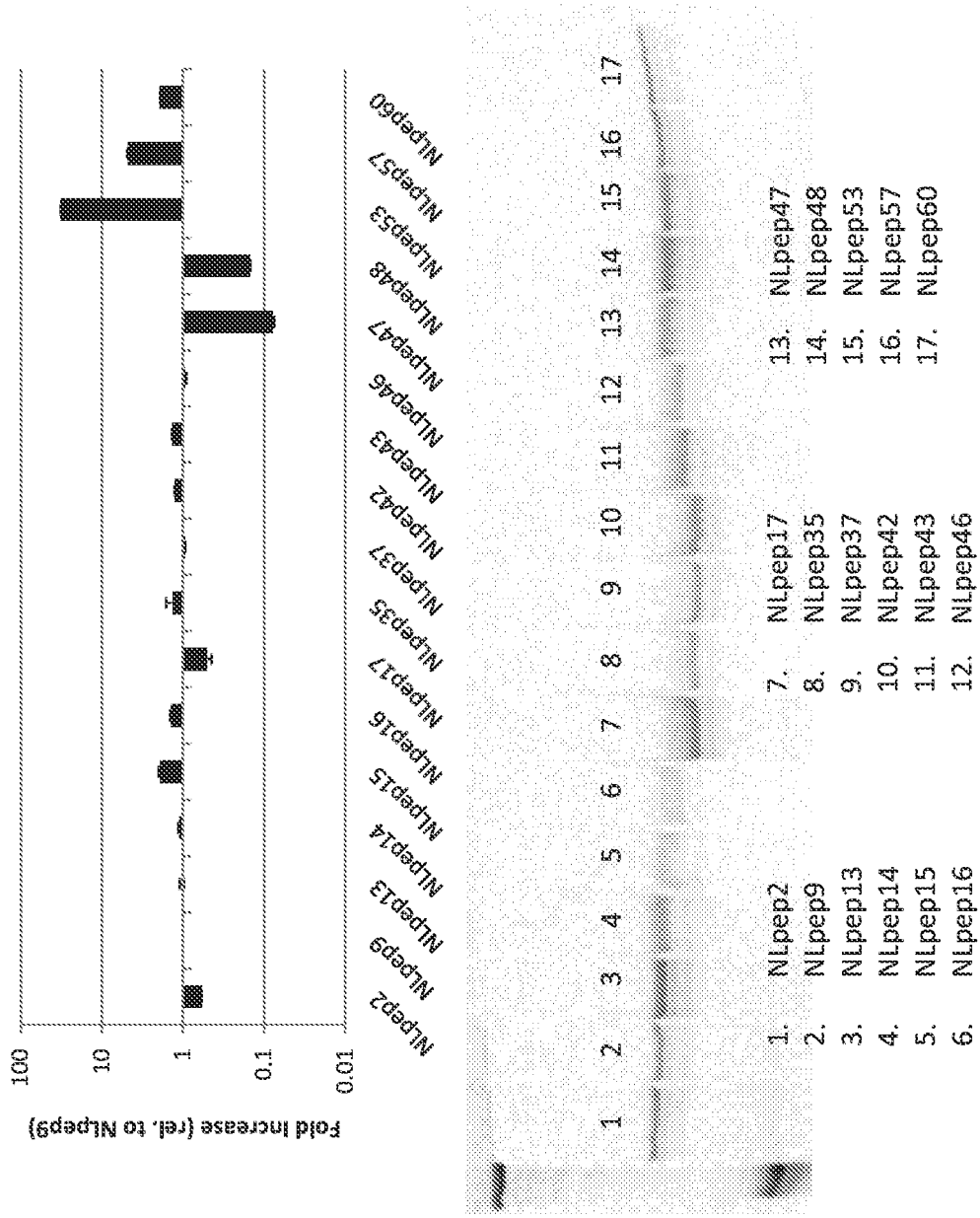

Normalized NLpep Concentration
Fold increase (rel. to NLpep9)
100
10
1
0.1
0.01
NLpep2
NLpep9
NLpep13
NLpep14
NLpep15
NLpep16
NLpep17
NLpep35
NLpep37
NLpep42
NLpep43
NLpep46
NLpep47
NLpep48
NLpep53
NLpep57
NLpep60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
1. NLpep2
2. NLpep9
3. NLpep13
4. NLpep14
5. NLpep15
6. NLpep16
7. NLpep17
8. NLpep35
9. NLpep37
10. NLpep42
11. NLpep43
12. NLpep46
13. NLpep47
14. NLpep48
15. NLpep53
16. NLpep57
17. NLpep60

Figure 10

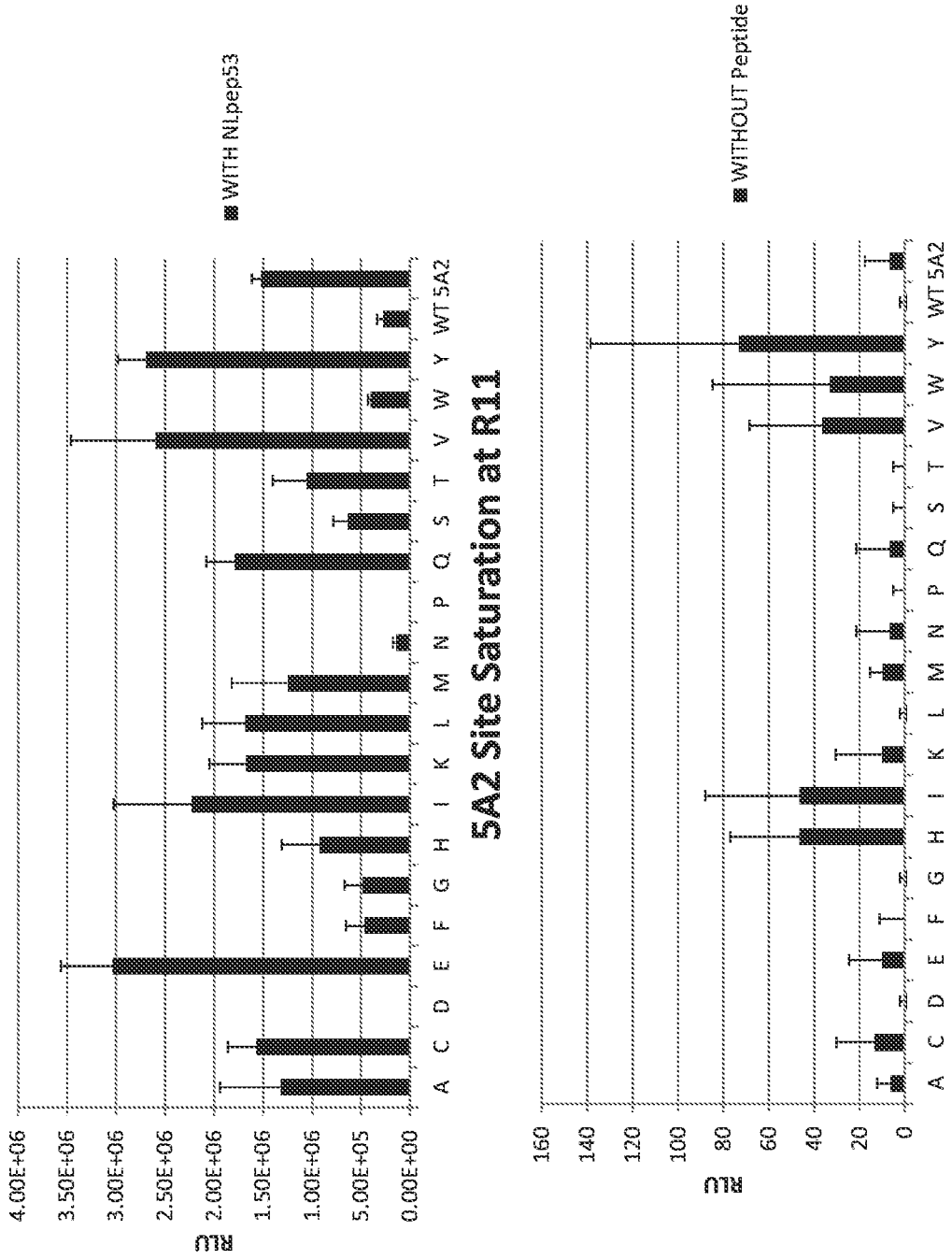
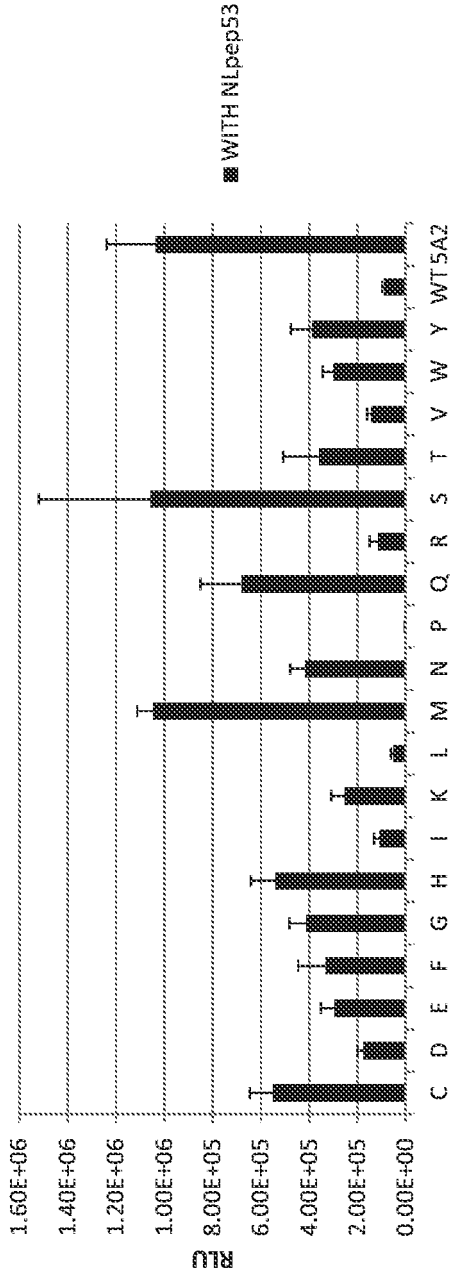

5A2 Site Saturation at R11
WITH Nlpep53
RLU
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
A C D E F G H I K L M N P Q S T V W Y WT 5A2
5A2 Site Saturation at R11
WITHOUT Peptide
RLU
160
140
120
100
80
60
40
20
0
A C D E F G H I K L M N P Q S T V W Y WT 5A2

Figure 11


5A2 Saturation of A15
WITH NLpep53
RLU
1.60E+06
1.40E+06
1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
C D E F G H I K L M N P Q R S T V W Y WT 5A2

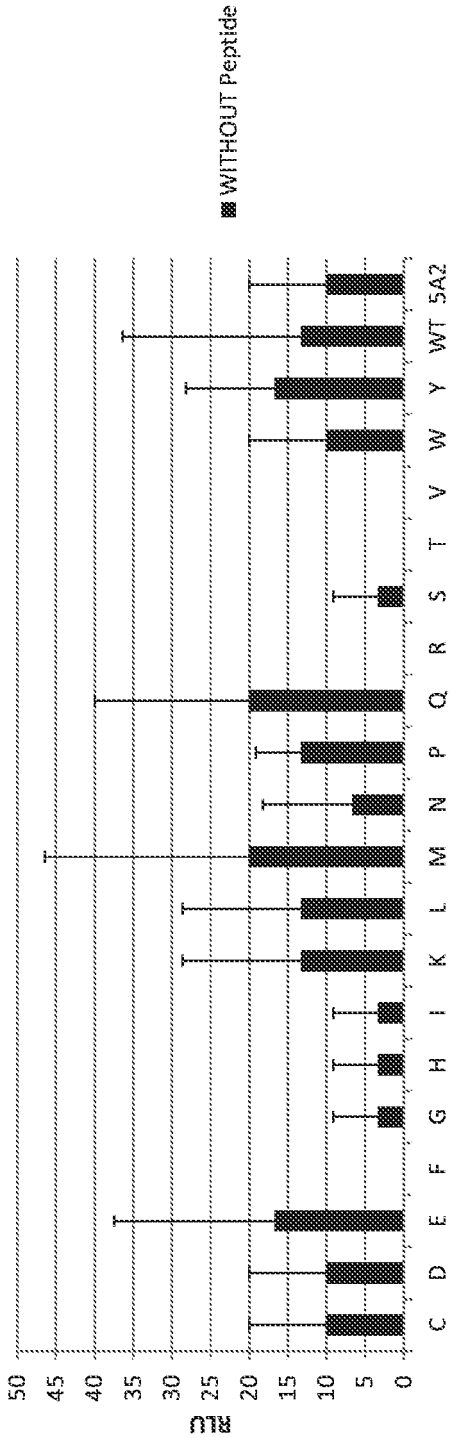

5A2 Site Saturation at A15
WITHOUT Peptide
RLU
50
45
40
35
30
25
20
15
10
5
0
C D E F G H I K L M N P Q R S T V W Y WT 5A2

Figure 12

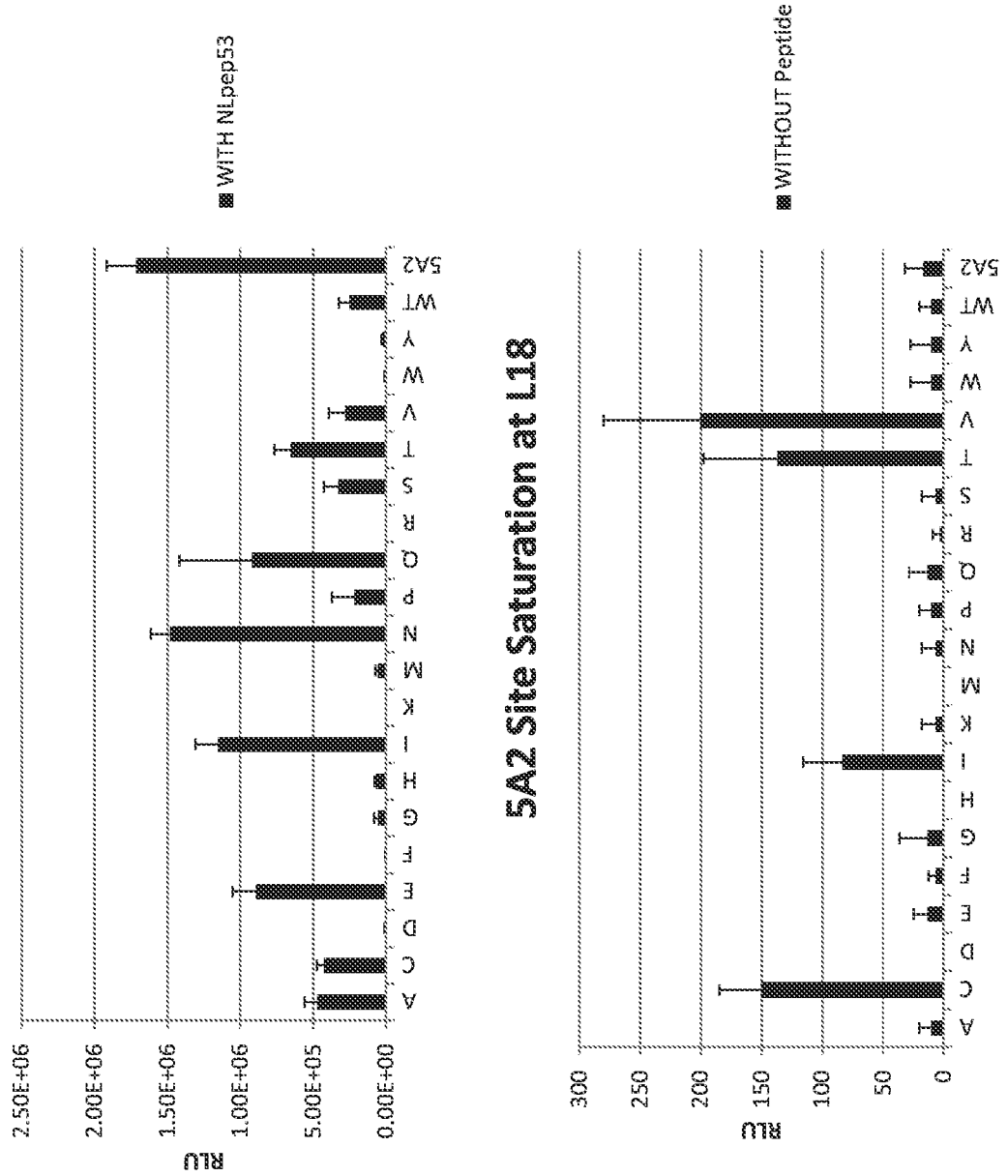

5A2 Site Saturation at L18
WITH NLpep53
RLU
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
A C D E F G H I K M N P Q R S T V W Y WT 5A2
5A2 Site Saturation at L18
WITHOUT Peptide
RLU
300
250
200
150
100
50
0
A C D E F G H I K M N P Q R S T V W Y WT 5A2

Figure 13

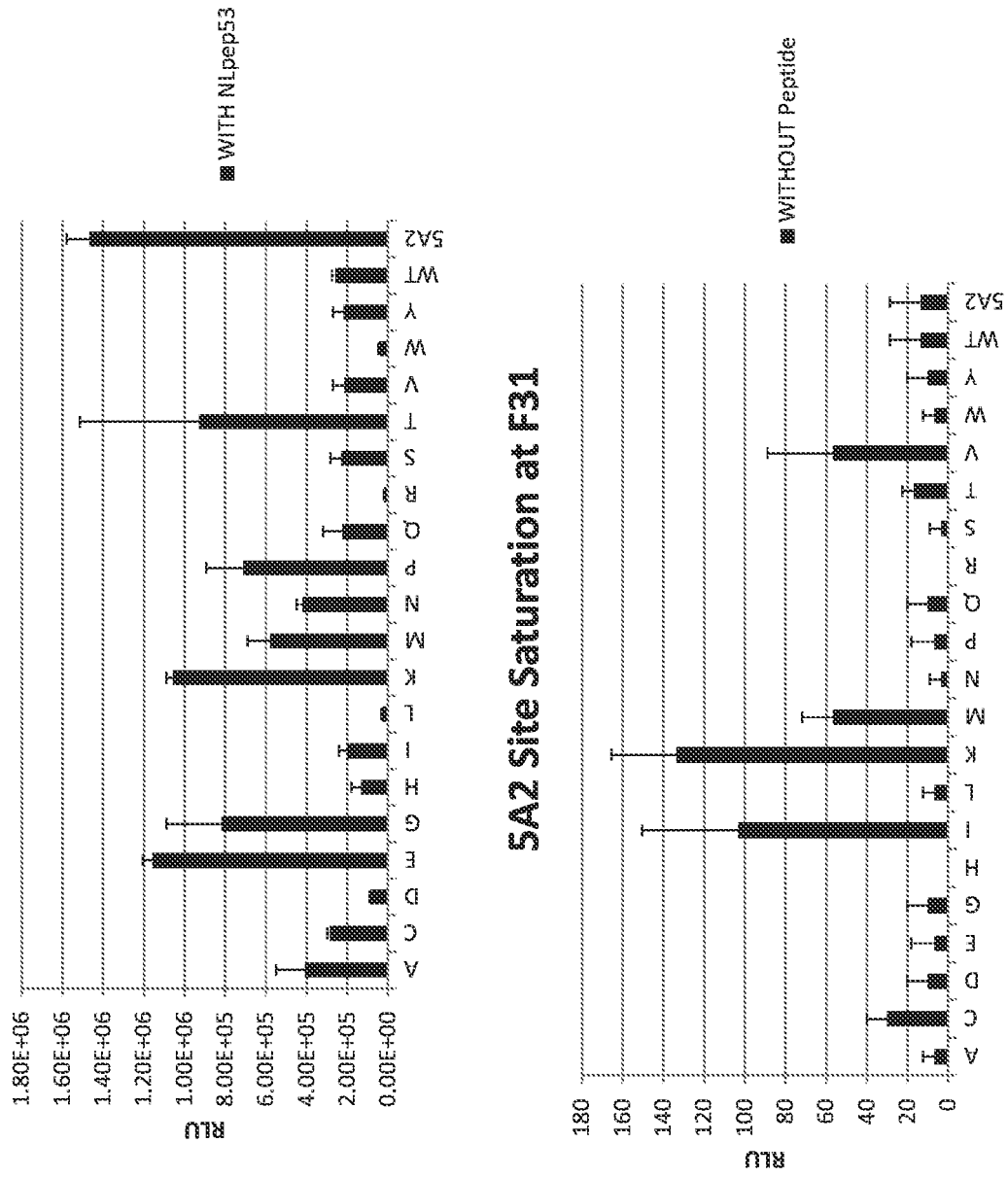

5A2 Site Saturation at F31
WITH NLpep53
RLU
1.80E+06
1.60E+06
1.40E+06
1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
A C D E G H I L K M N P Q R S T V W Y WT 5A2
5A2 Site Saturation at F31
WITHOUT Peptide
RLU
180
160
140
120
100
80
60
40
20
0
A C D E G H I L K M N P Q R S T V W Y WT 5A2

Figure 14

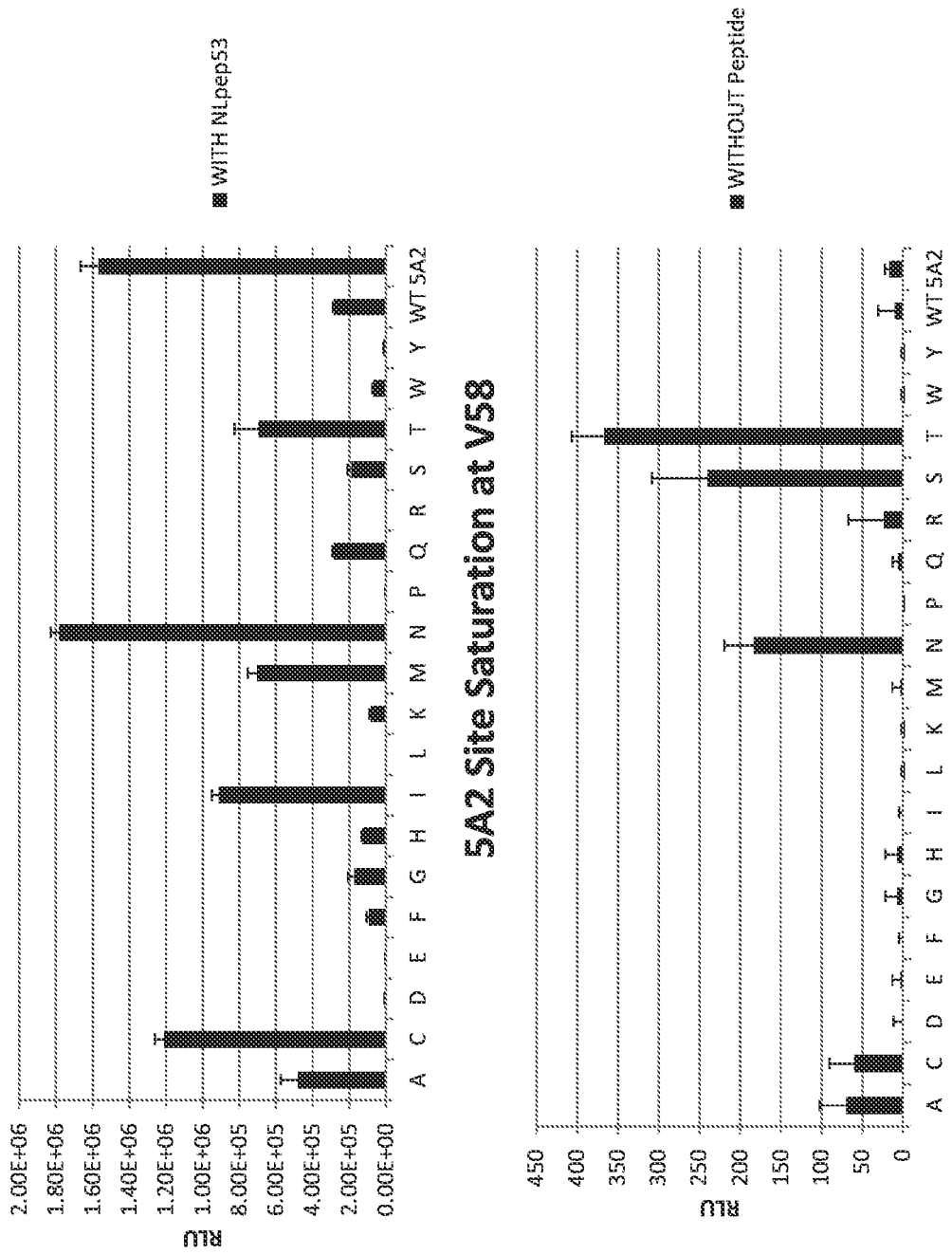

5A2 Site Saturation at V58
WITH NLpep53
RLU
2.00E+06
1.80E+06
1.60E+06
1.40E+06
1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
A C D E F G H I L K M N P Q R S T W Y WT5A2
5A2 Site Saturation at V58
WITHOUT Peptide
RLU
450
400
350
300
250
200
150
100
50
0
A C D E F G H I L K M N P Q R S T W Y WT5A2

Figure 15

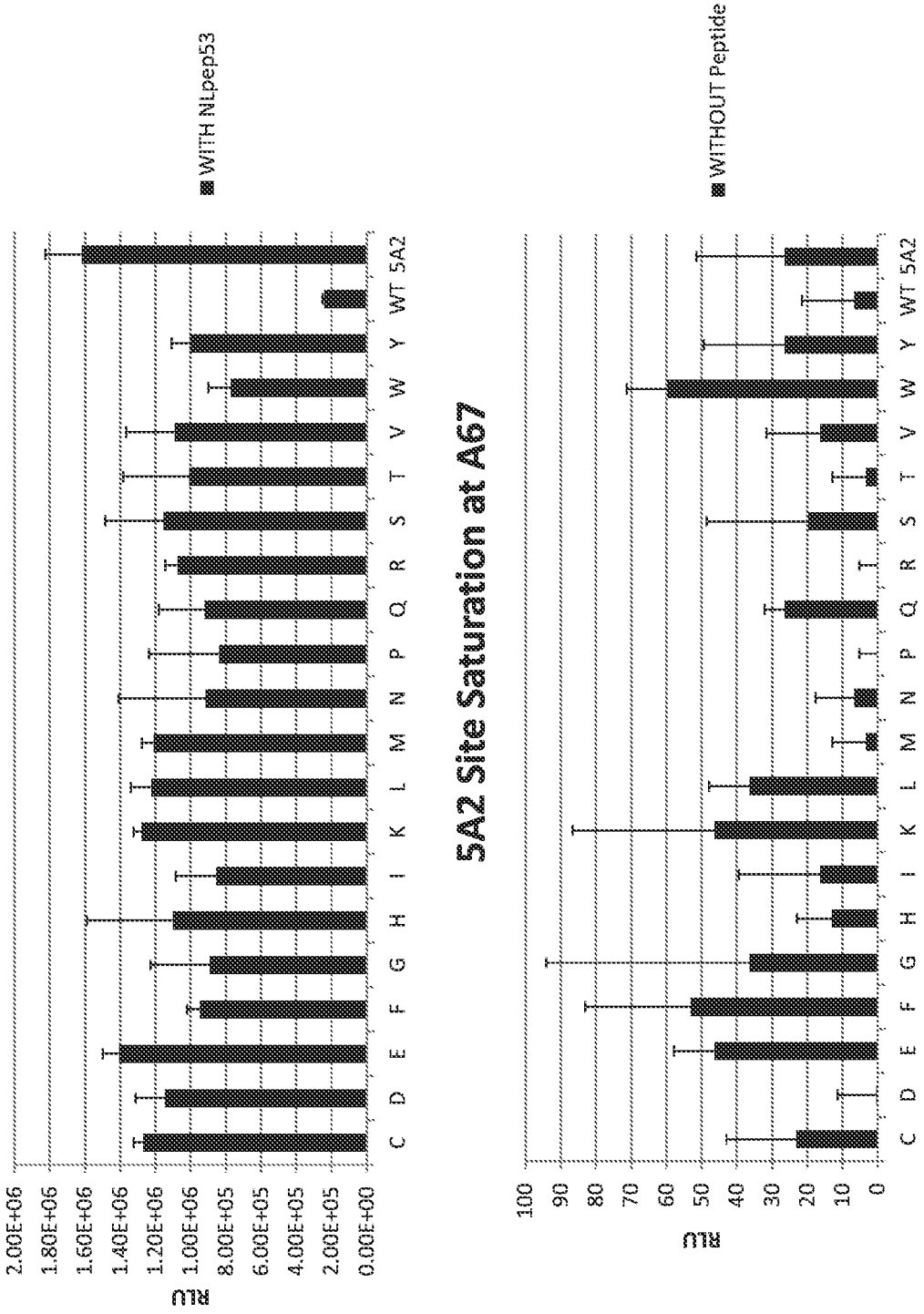

5A2 Site Saturation at A67
WITH Ntpep53
RLU
2.00E+06
1.80E+06
1.60E+06
1.40E+06
1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
C D E F G H I K L M N P Q R S T V W Y WT 5A2
5A2 Site Saturation at A67
WITHOUT Peptide
RLU
100
90
80
70
60
50
40
30
20
10
0
C D E F G H I K L M N P Q R S T V W Y WT 5A2

Figure 16

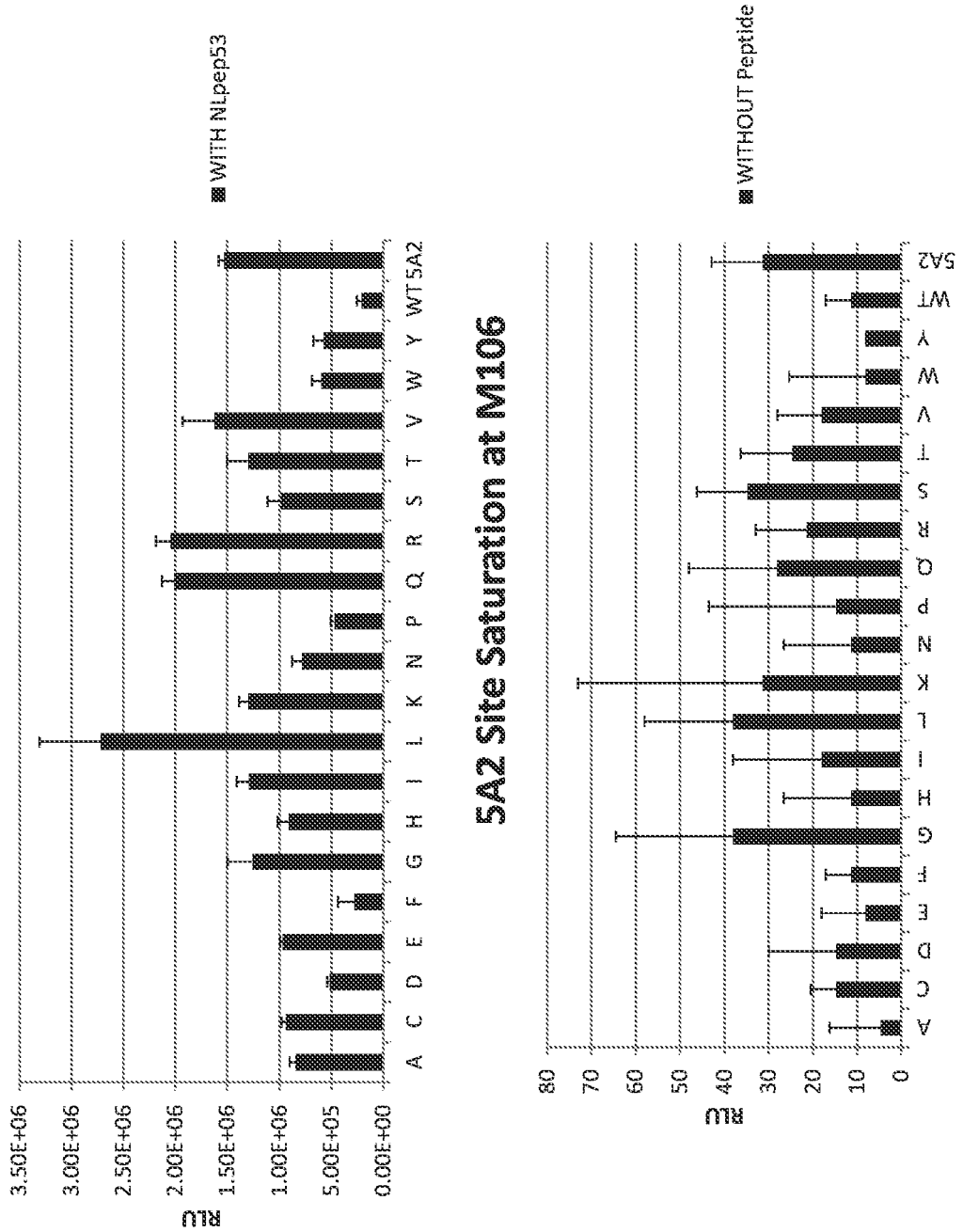

5A2 Site Saturation at M106
WITH NLpep53
RLU
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
A C D E F G H I L K N P Q R S T V W Y WT 5A2
5A2 Site Saturation at M106
WITHOUT Peptide
RLU
80
70
60
50
40
30
20
10
0
A C D E F G H I L K N P Q R S T V W Y WT 5A2

Figure 17

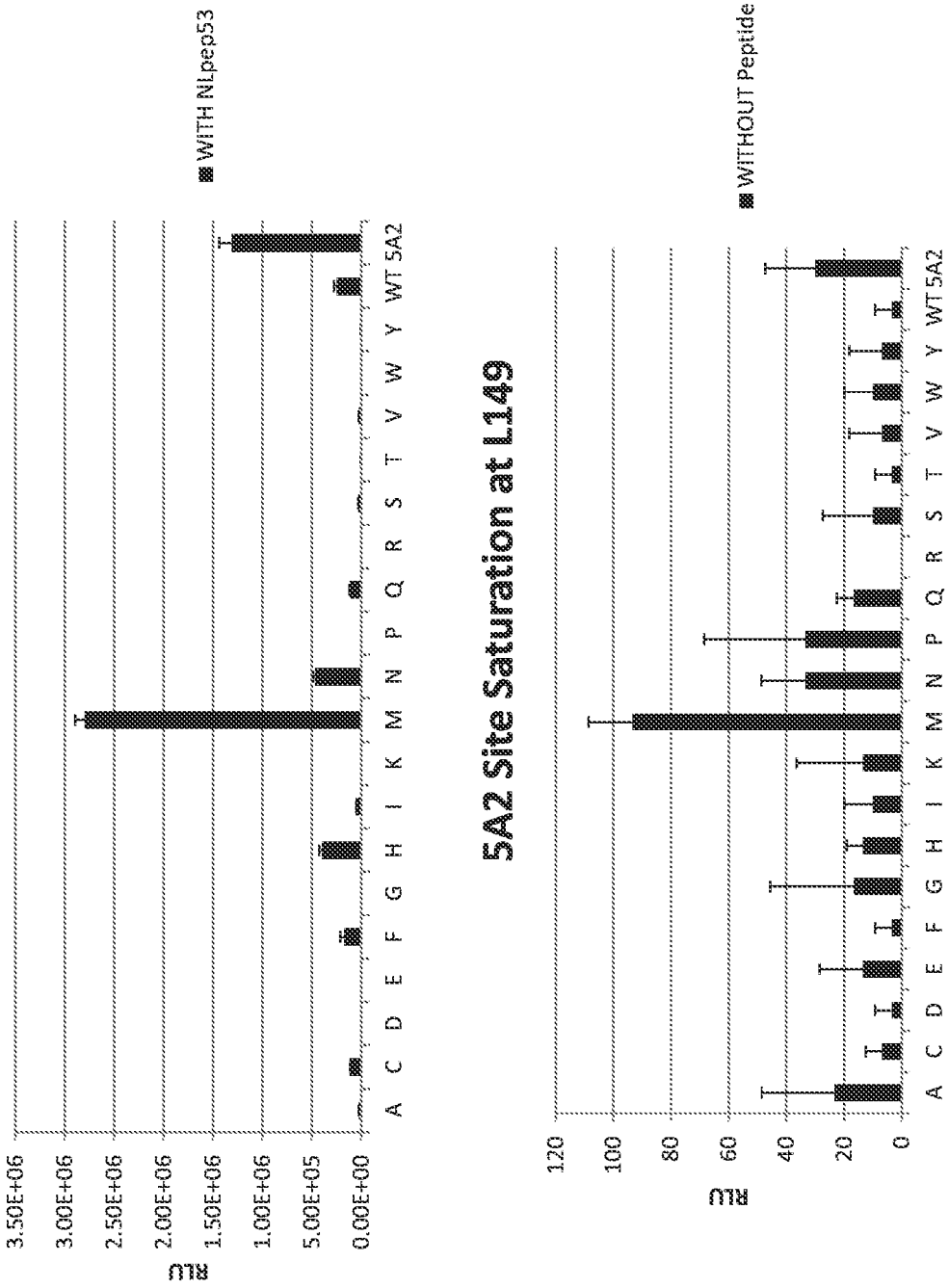

5A2 Site Saturation at L149
WITH NLpep53
RLU
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
A C D E F G H I K M N P Q R S T V W Y WT 5A2
5A2 Site Saturation at L149
WITHOUT Peptide
RLU
120
100
80
60
40
20
0
A C D E F G H I K M N P Q R S T V W Y WT 5A2

Figure 18

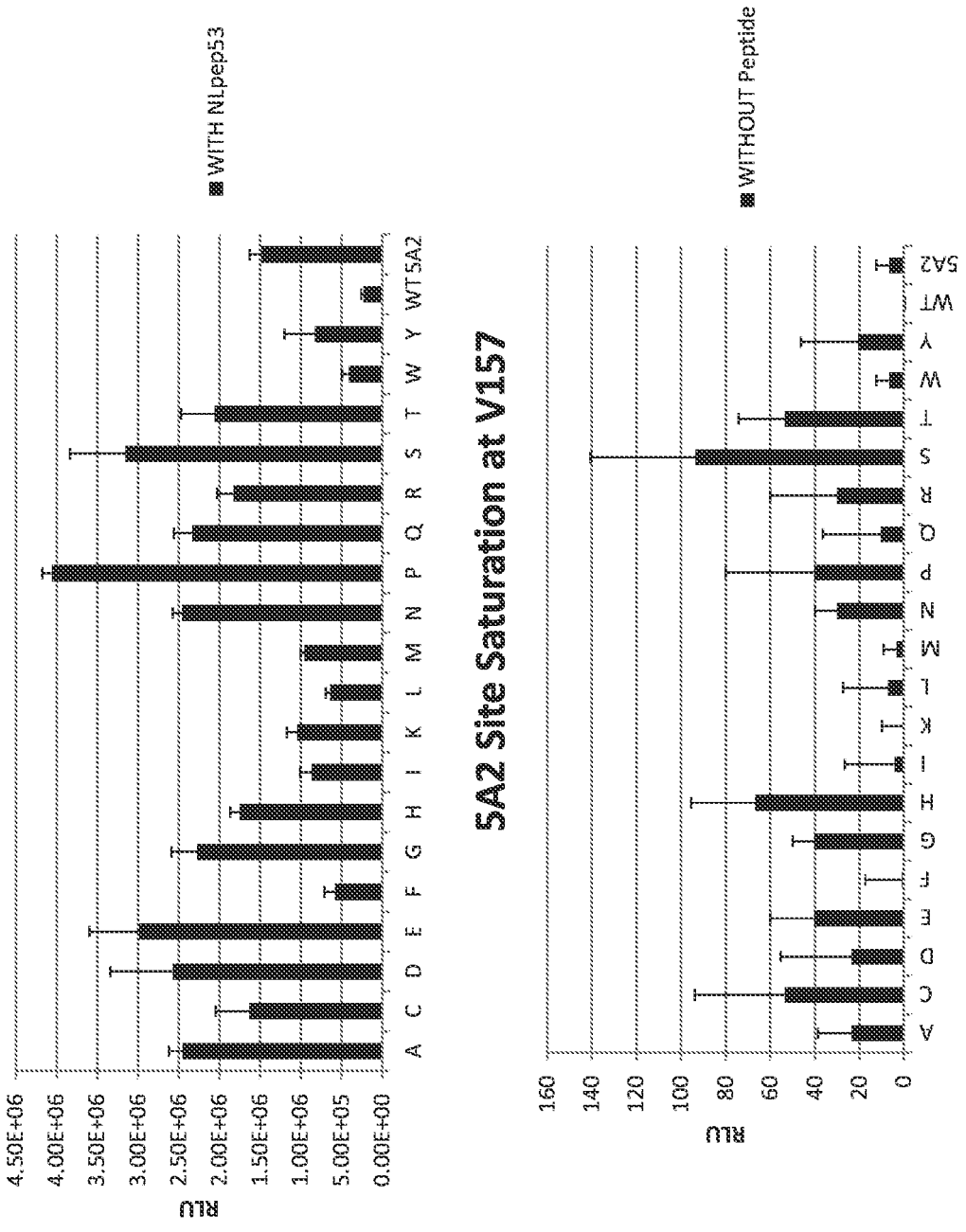

5A2 Site Saturation at V157
WITH NLpep53
RLU
4.50E+06
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
A C D E F G H I K L M N P Q R S T W Y WT5A2
5A2 Site Saturation at V157
WITHOUT Peptide
RLU
160
140
120
100
80
60
40
20
0
A C D E F G H I K L M N P Q R S T W Y WT 5A2

Figure 19

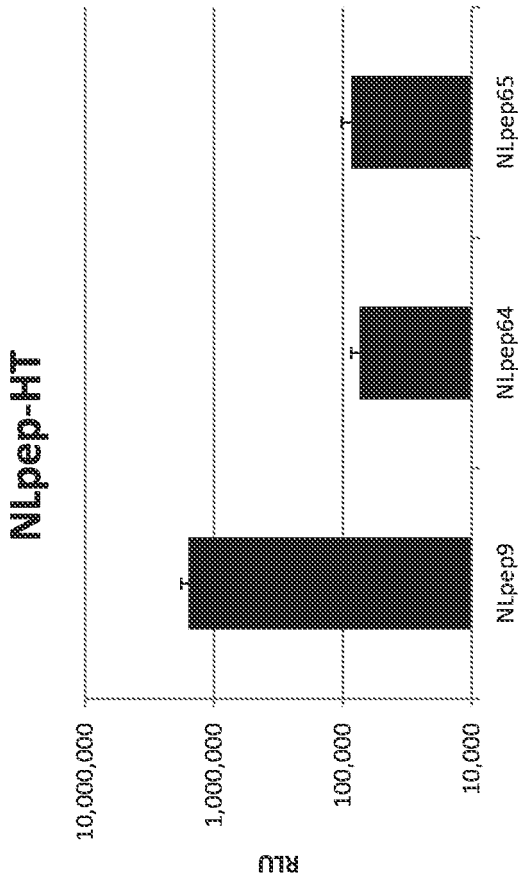

NLpep-HT
RLU
10,000,000
1,000,000
100,000
10,000
NLpep9
NLpep64
NLpep65

Figure 20

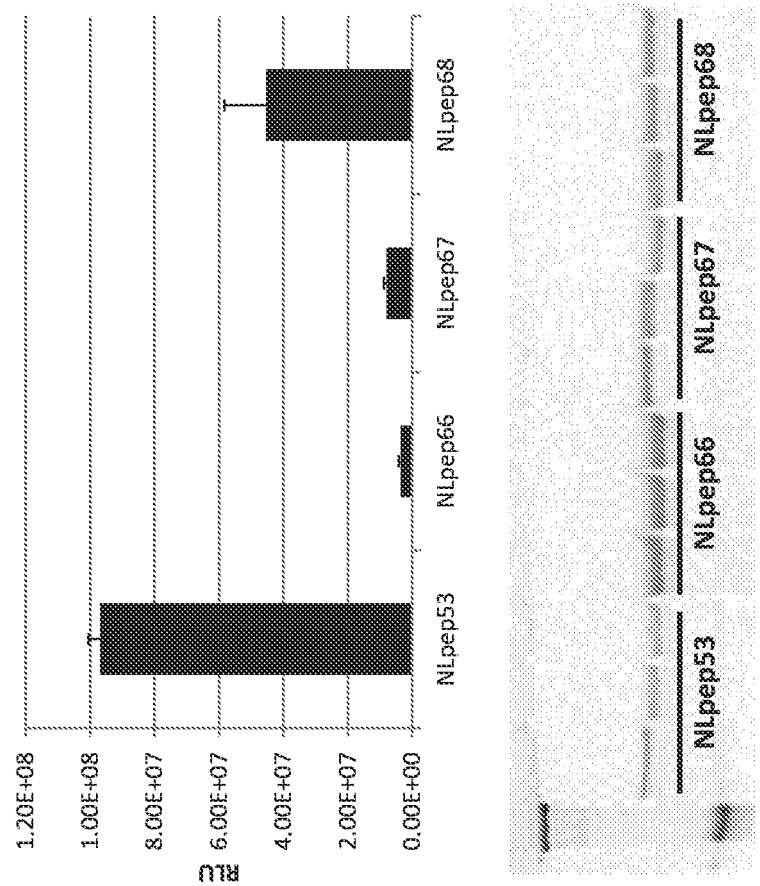

NLpep-HT
RLU
1.20E+08
1.00E+08
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
NLpep53
NLpep66
NLpep67
NLpep68
NLpep53
NLpep66
NLpep67
NLpep68

Figure 21

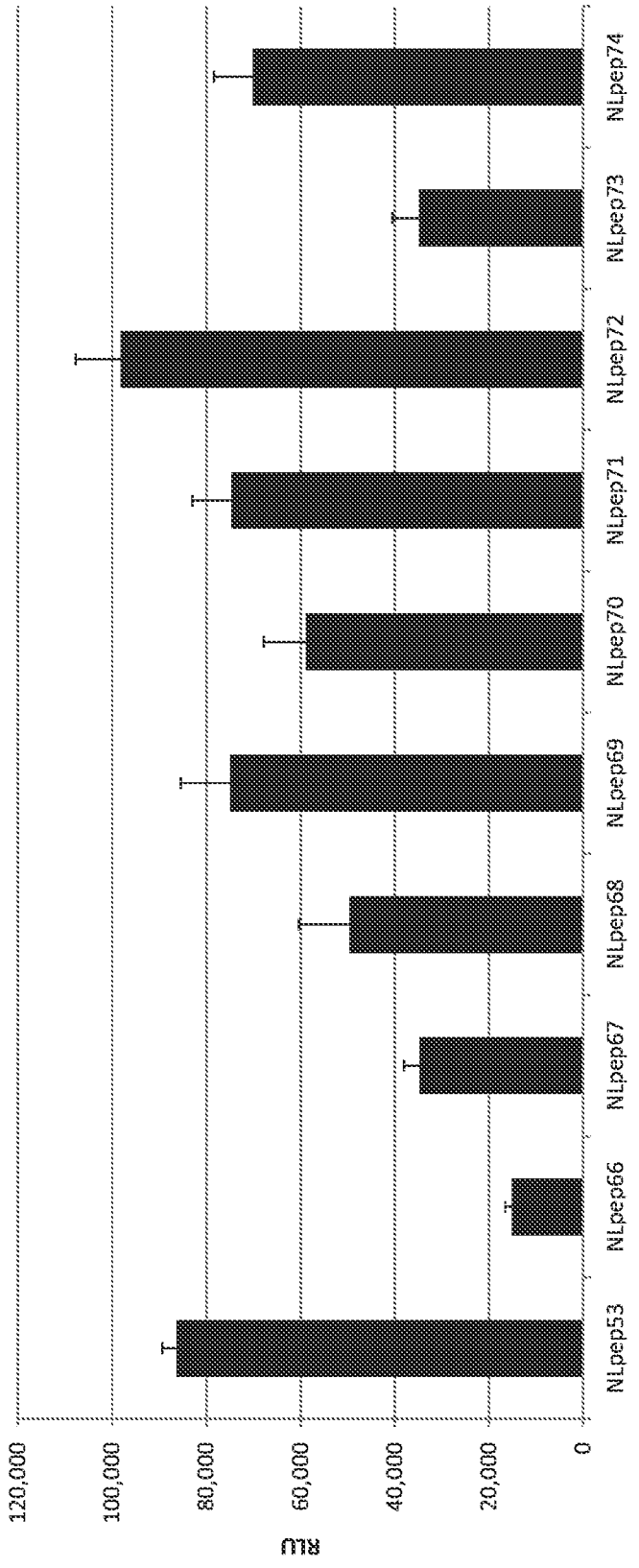

NLpep-HT
RLU
120,000
100,000
80,000
60,000
40,000
20,000
0
NLpep53
NLpep66
NLpep67
NLpep68
NLpep69
NLpep70
NLpep71
NLpep72
NLpep73
NLpep74

Figure 22

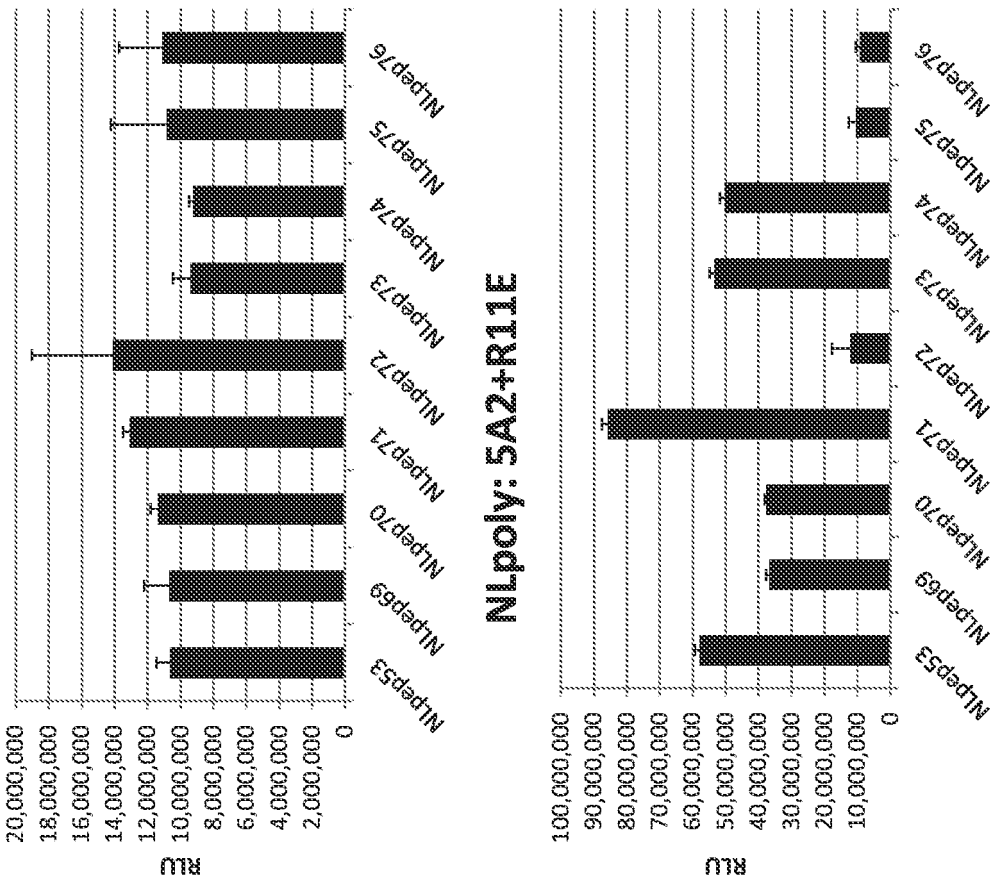

NLpoly: 5A2
RLU
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
NLpep53
NLpep69
NLpep70
NLpep71
NLpep72
NLpep73
NLpep74
NLpep75
NLpep76
NLpoly: 5A2+R11E
RLU
100,000,000
90,000,000
80,000,000
70,000,000
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
0
NLpep53
NLpep69
NLpep70
NLpep71
NLpep72
NLpep73
NLpep74
NLpep75
NLpep76

Figure 23

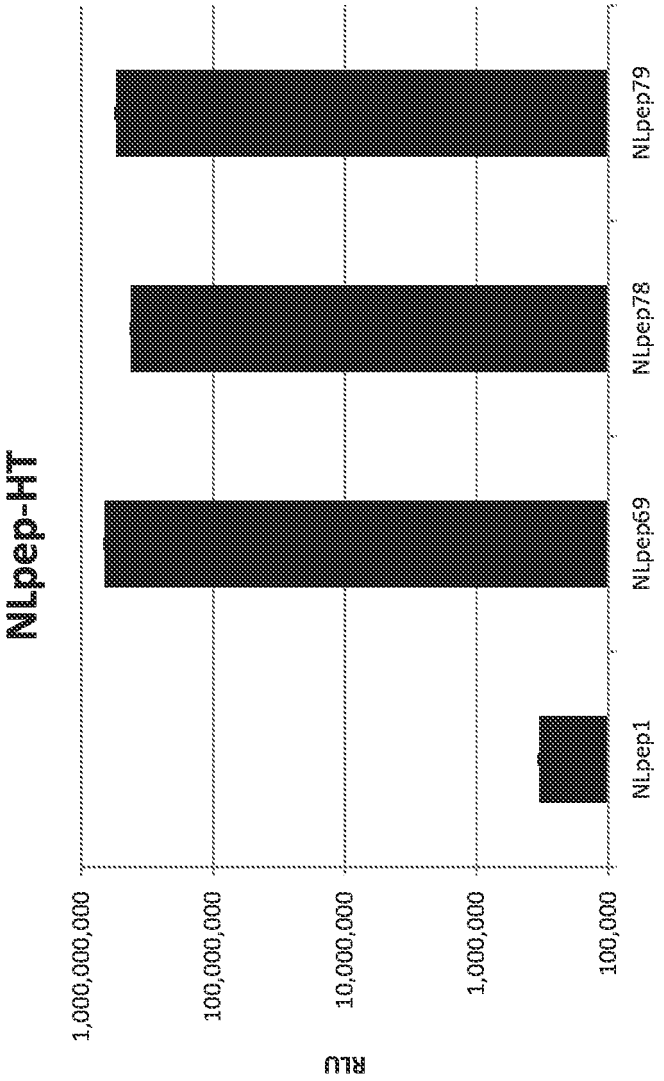

NLpep-HT
RLU
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
NLpep1
NLpep69
NLpep78
NLpep79

Figure 24

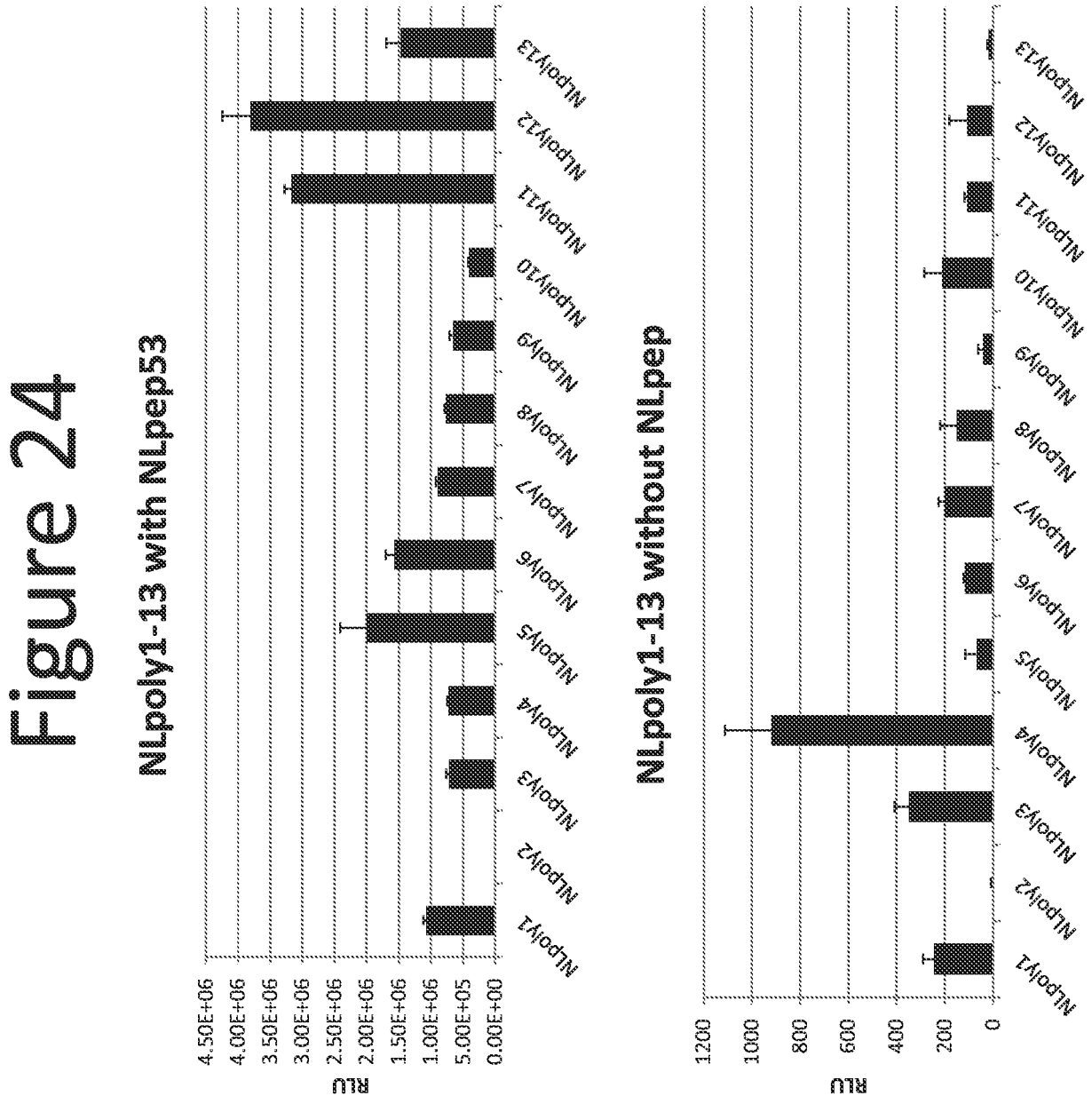

NLpoly1-13 with NLpep53
RLU
4.50E+06
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
NLpoly2
NLpoly3
NLpoly4
NLpoly5
NLpoly6
NLpoly7
NLpoly8
NLpoly9
NLpoly10
NLpoly11
NLpoly12
NLpoly13
NLpoly1-13 without NLpep
RLU
1200
1000
800
600
400
200
0
NLpoly2
NLpoly3
NLpoly4
NLpoly5
NLpoly6
NLpoly7
NLpoly8
NLpoly9
NLpoly10
NLpoly11
NLpoly12
NLpoly13

Figure 25

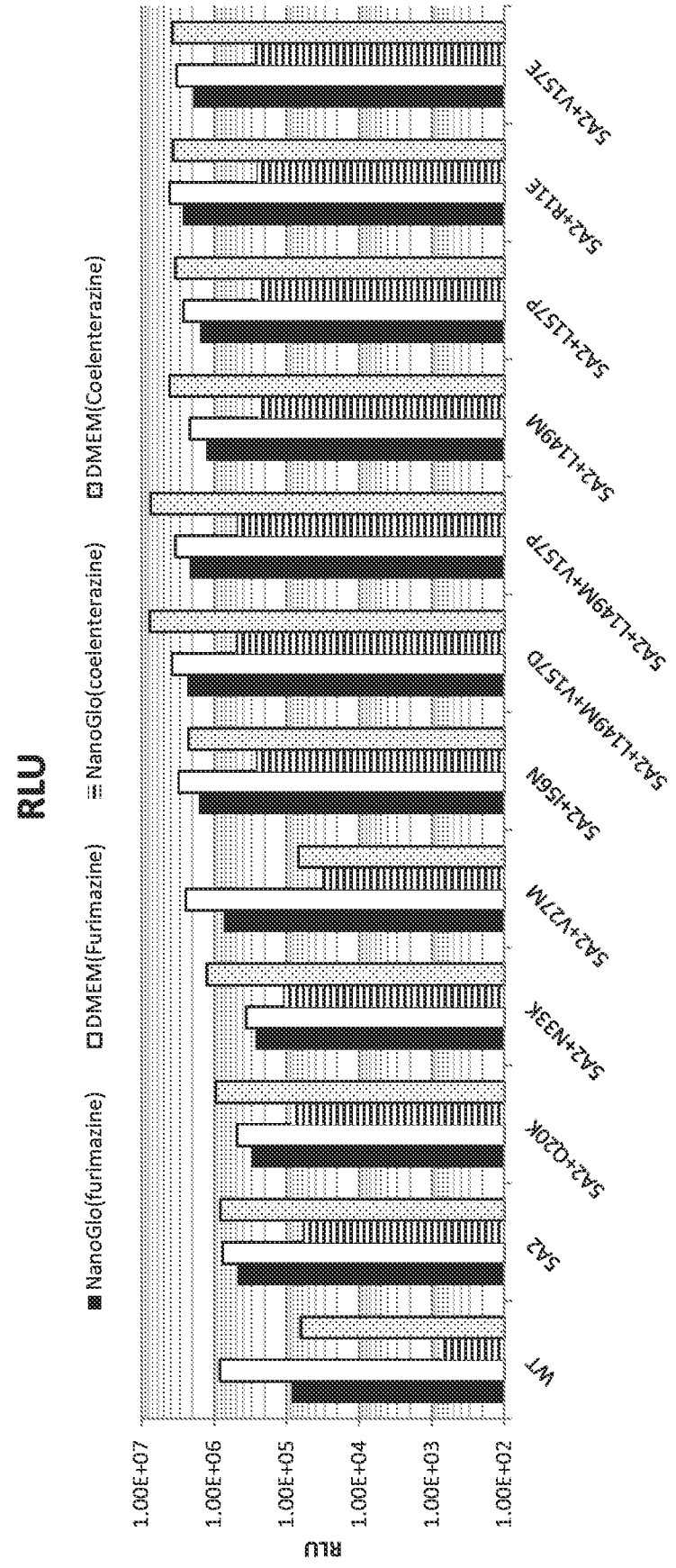

RLU
NanoGlo(furimazine)
DMEM(Furimazine)
NanoGlo(coelenterazine)
DMEM(Coelenterazine)
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
RLU
WT
SA2
SA2+Q20K
SA2+N33K
SA2+V27M
SA2+I56N
SA2+L149M+V157D
SA2+L149M+V157P
SA2+L149M
SA2+L157P
SA2+R11E
SA2+V157E

Figure 26

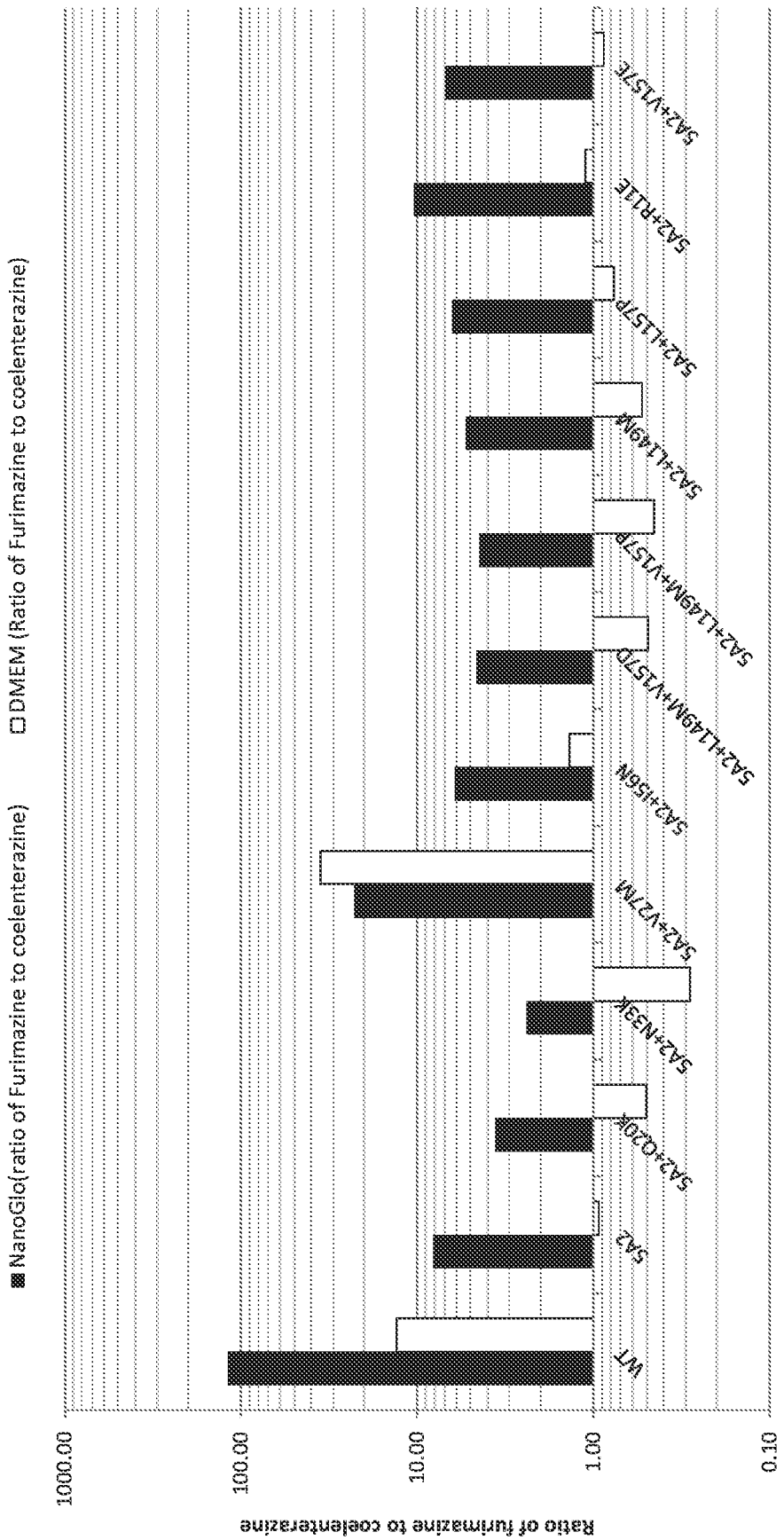

Ratio of Furimazine to Coelenterazine RLU
NanoGlo(ratio of Furimazine to coelenterazine)
DMEM (Ratio of Furimazine to coelenterazine)
Ratio of furimazine to coelenterazine
1000.00
100.00
10.00
1.00
0.10
WT
SA2
SA2+Q20K
SA2+N33K
SA2+V27M
SA2+I55N
SA2+L149M+V157D
SA2+L149M+V157P
SA2+L149N
SA2+L157P
SA2+R1LE
SA2+V157E

Figure 27

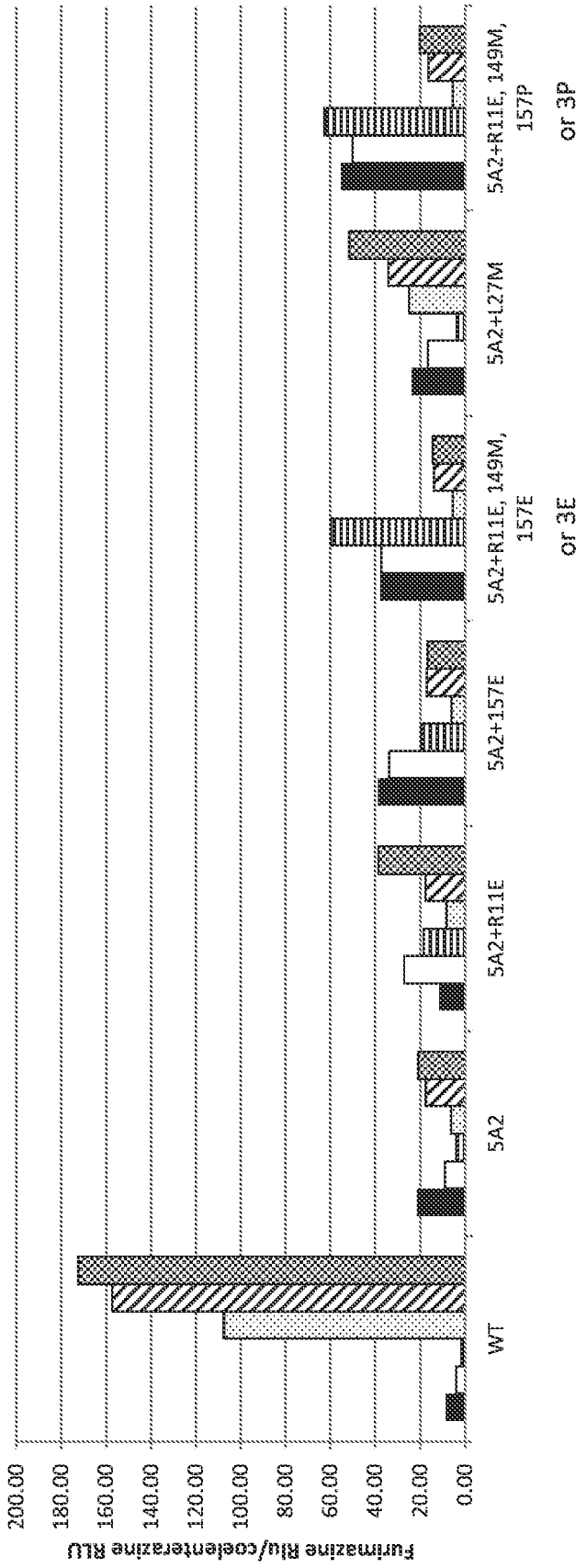

Furimazine RLU divided by coelenterazine RLU
Nl.pep1
Nl.pep9
Nl.pep48
Nl.pep53
Nl.pep69
Nl.pep76
Furimazine Rlu/coelenterazine RLU
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
WT
5A2
5A2+R11E
5A2+157E
5A2+R11E, 149M, 157E or 3E
5A2+L27M
5A2+R11E, 149M, 157P or 3P

Figure 28

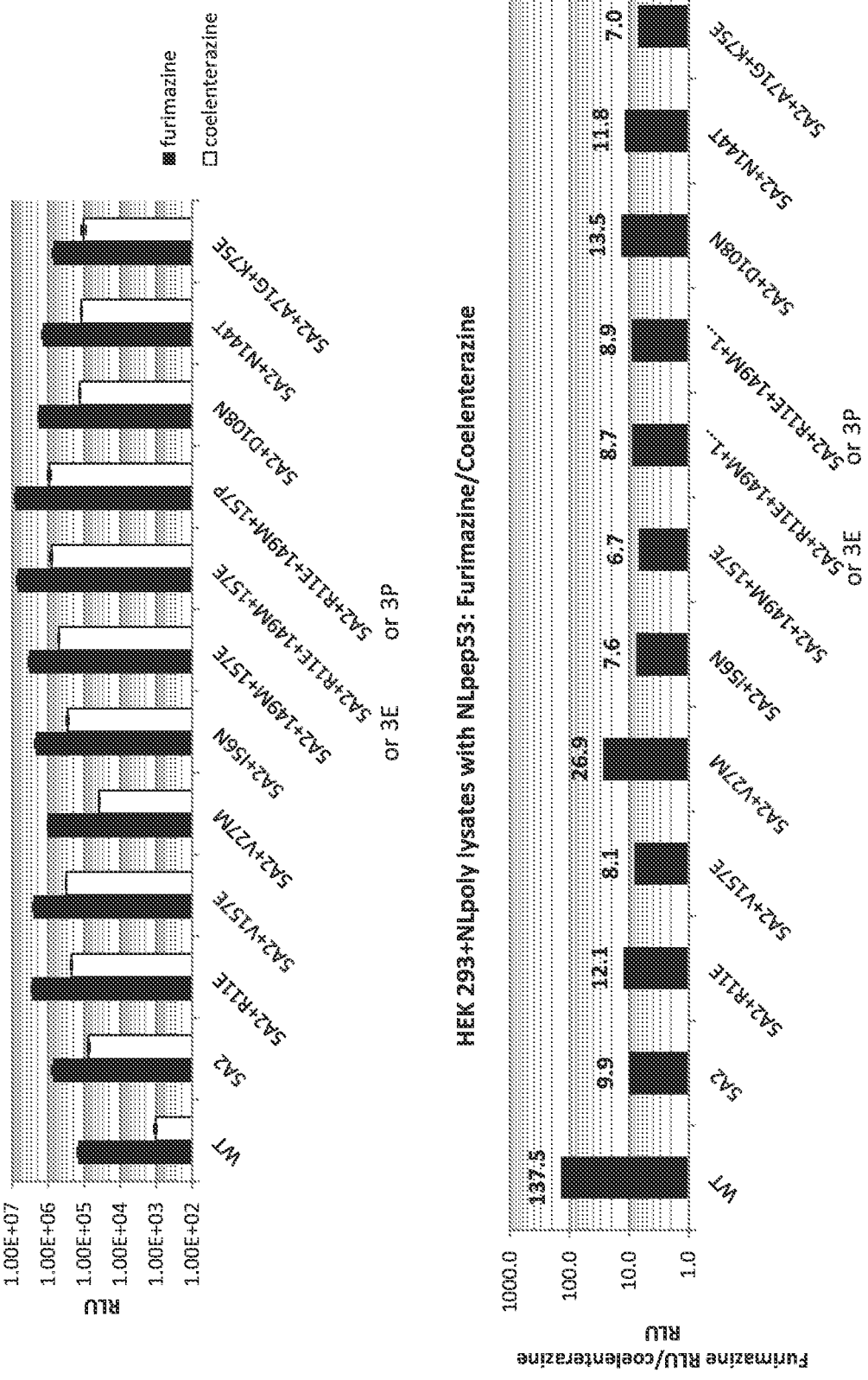

HEK293+NLpoly lysates with NLpep53 in NanoGlo
RLU
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
furimazine
coelenterazine
WT
SA2
SA2+R11E
SA2+V15TE
SA2+V27M
SA2+I56N
SA2+L89M+I57E
SA2+R11E+L89M+I57P
SA2+D108N
SA2+N144T
SA2+A71G+K75E
or 3E
or 3P
HEK 293+NLpoly lysates with NLpep53: Furimazine/Coelenterazine
Furimazine RLU/coelenterazine RLU
1000.0
100.0
10.0
1.0
137.5
9.9
12.1
26.9
8.1
7.6
6.7
8.7
8.9
13.5
11.8
7.0
WT
SA2
SA2+R11E
SA2+V15TE
SA2+V27M
SA2+I56N
SA2+L89M+I57E
SA2+R11E+L89M+I...
SA2+R11E+L89M+I...
SA2+D108N
SA2+N144T
SA2+A71G+K75E
or 3E
or 3P

Figure 29

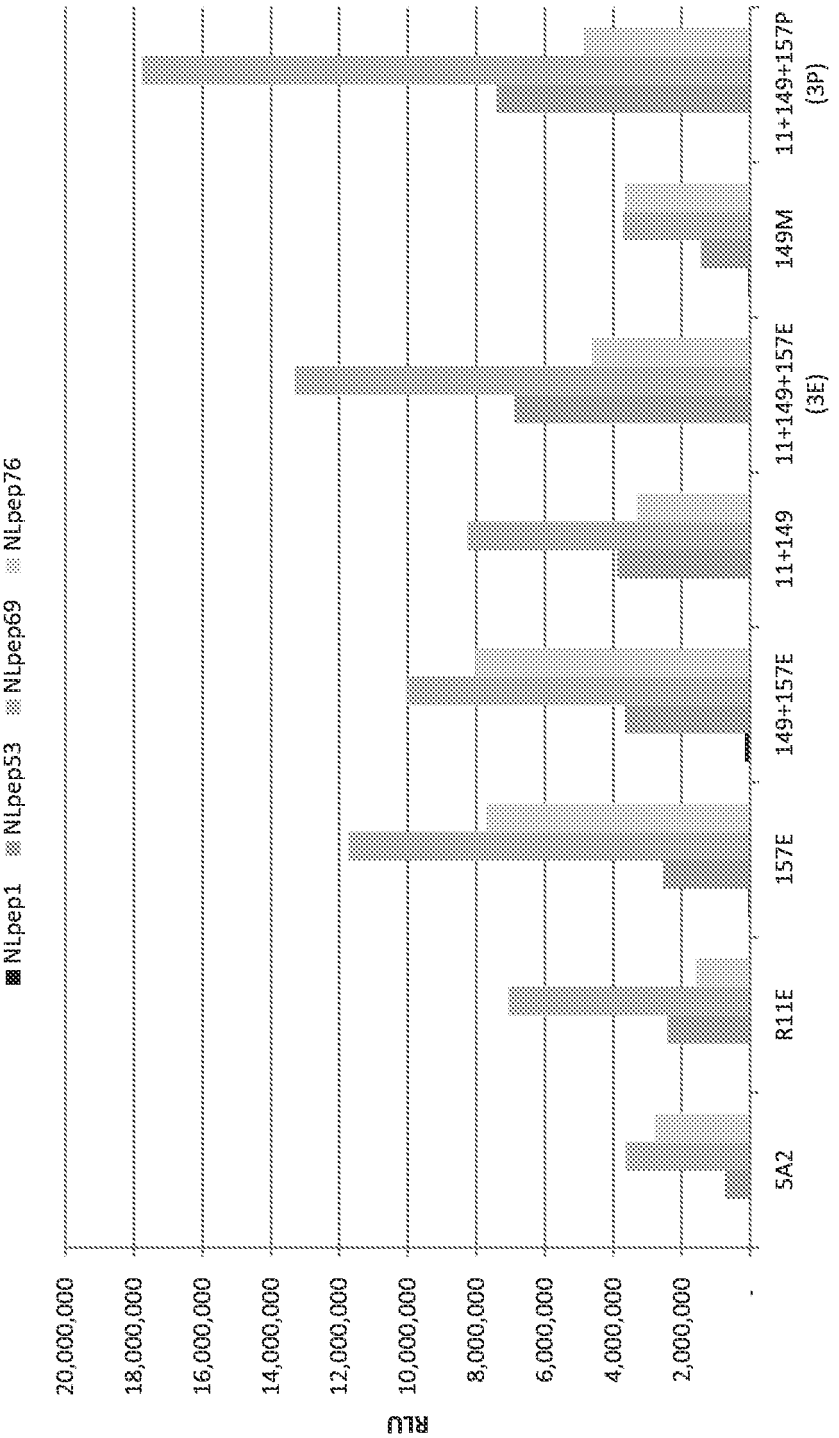

DMEM furimazine
NLpep1
NLpep53
NLpep69
NLpep76
RLU
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
-
5A2
R11E
157E
149+157E
11+149
11+149+157E (3E)
149M
11+149+157P (3P)

Figure 30

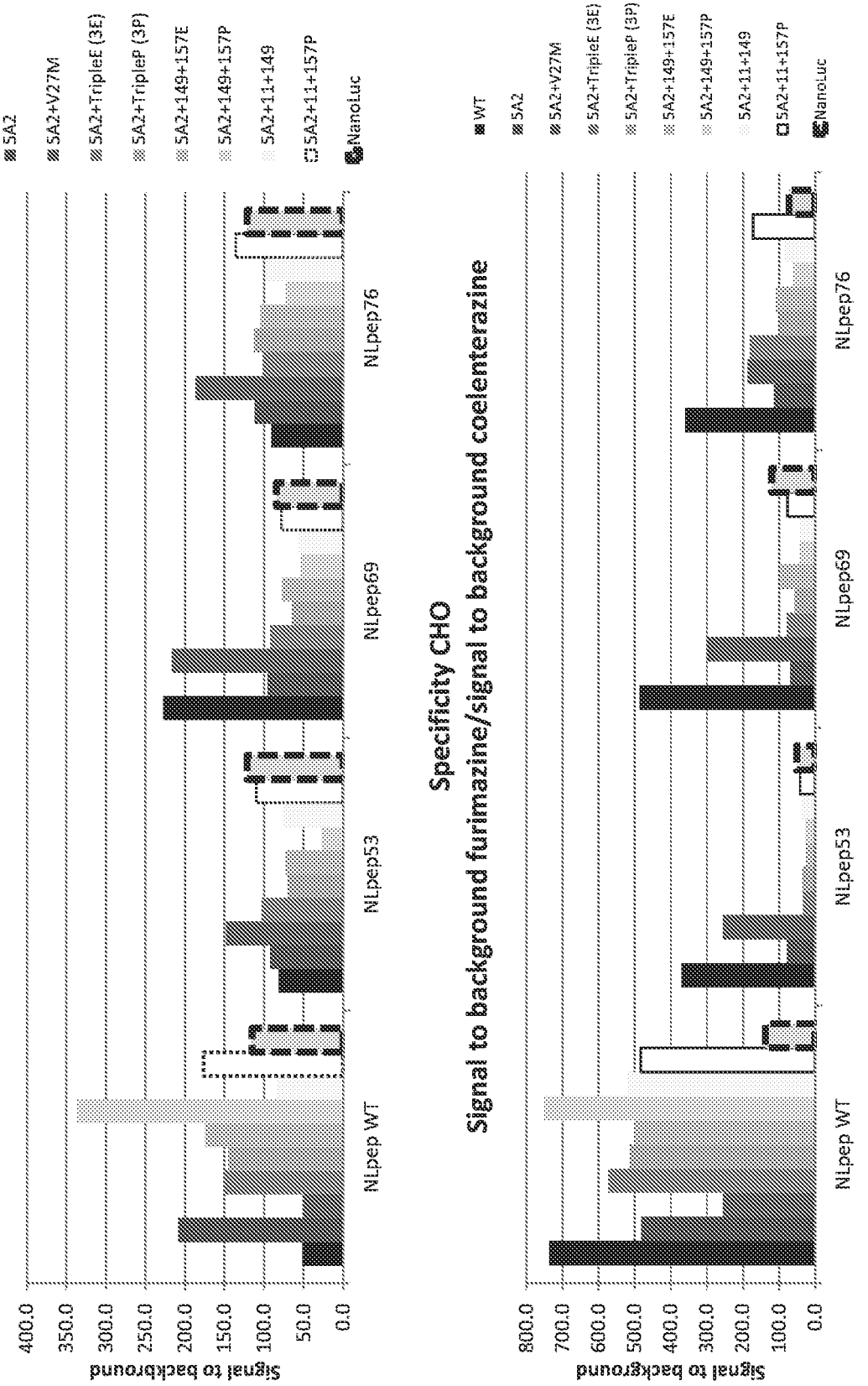

Specificity HEK
Signal to background furimazine/signal to background coelenterazine
Signal to background
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
NLpep WT
NLpep53
NLpep69
NLpep76
WT
5A2
5A2+V27M
5A2+TripleE (3E)
5A2+TripleP (3P)
5A2+149+157E
5A2+149+157P
5A2+11+149
5A2+11+157P
NanoLuc
Specificity CHO
Signal to background furimazine/signal to background coelenterazine
Signal to background
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
NLpep WT
NLpep53
NLpep69
NLpep76
WT
5A2
5A2+V27M
5A2+TripleE (3E)
5A2+TripleP (3P)
5A2+149+157E
5A2+149+157P
5A2+11+149
5A2+11+157P
NanoLuc

Figure 31

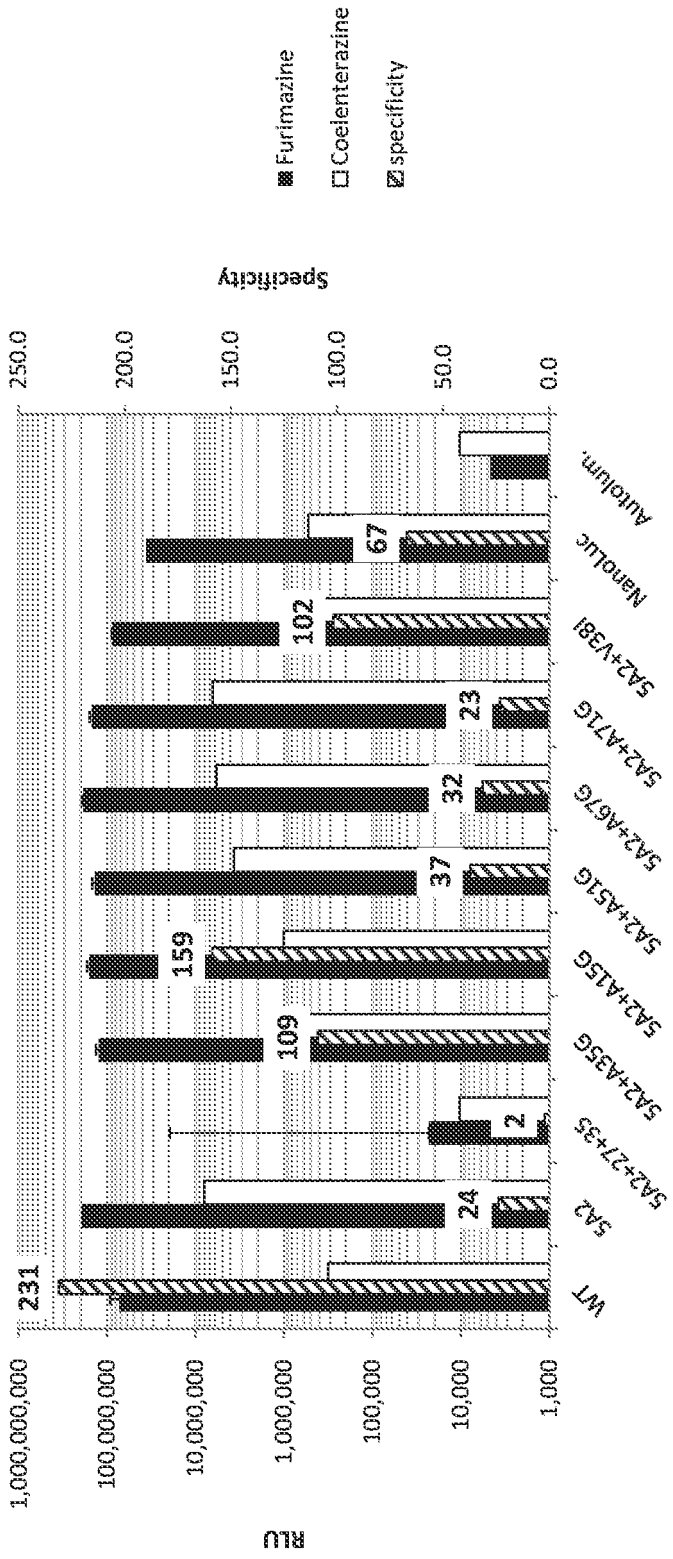

Co-express with NLpep69
RLU
Specificity
Furimazine
Coelenterazine
specificity
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
250.0
200.0
150.0
100.0
50.0
0.0
231
24
2
109
159
37
32
23
102
67
WT
SA2
SA2+27+35
SA2+A35G
SA2+A45G
SA2+A51G
SA2+A67G
SA2+A71G
SA2+V38I
NanoLuc
Autolum.

Figure 32

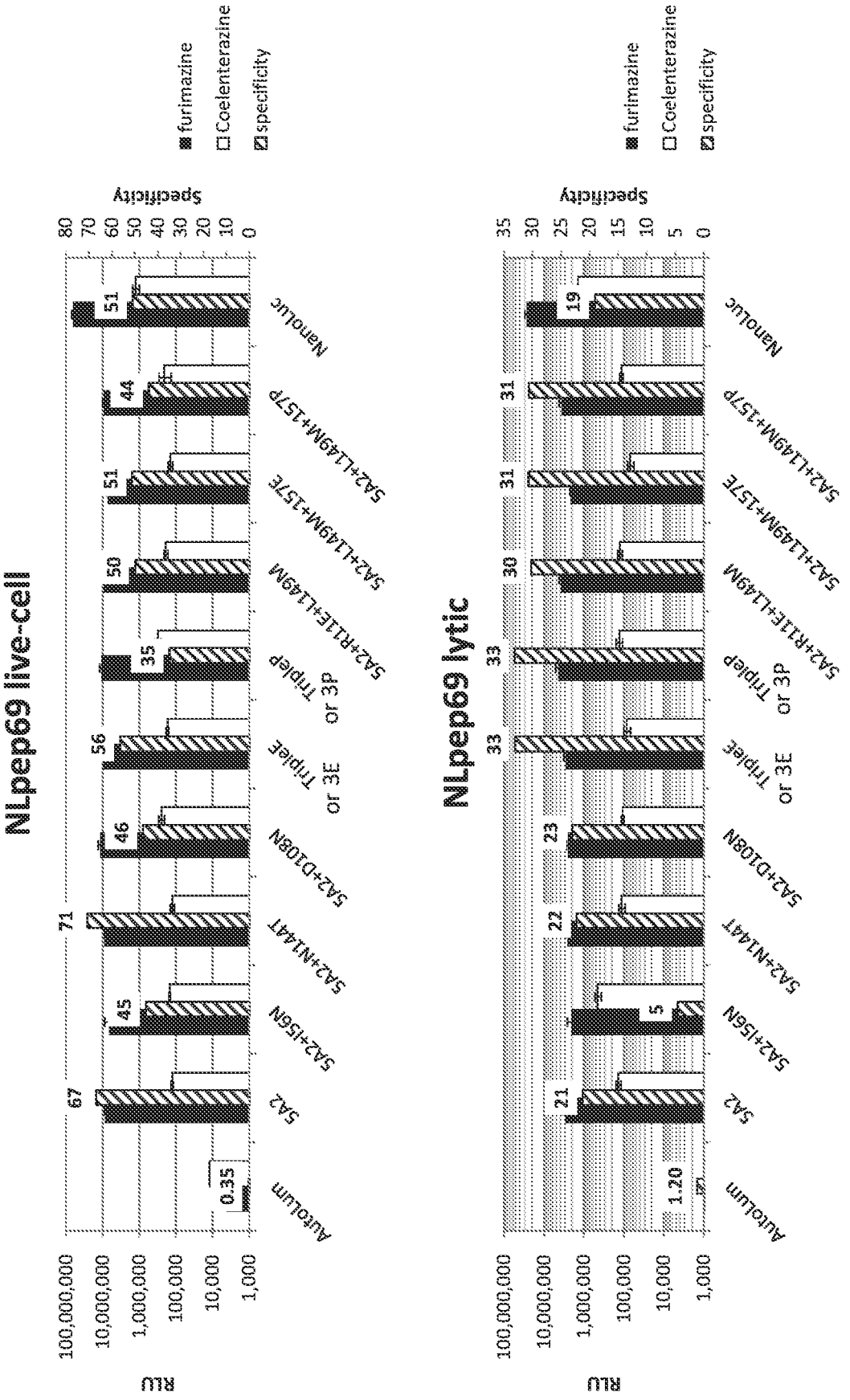

NLpep69 live-cell
furimazine
Coelenterazine
specificity
RLU
Specificity
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
80
70
60
50
40
30
20
10
0
0.35
67
45
71
46
56
35
50
51
44
51
NanoLuc
SA2+L149M+157P
SA2+L149M+157E
SA2+R11E+L149M
Triple or 3P
Triple or 3E
SA2+D108N
SA2+N144T
SA2+I56N
SA2
Autolum
NLpep69 lytic
furimazine
Coelenterazine
specificity
RLU
Specificity
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
35
30
25
20
15
10
5
0
1.20
21
5
22
23
33
33
30
31
31
19
NanoLuc
SA2+L149M+157P
SA2+L149M+157E
SA2+R11E+L149M
Triple or 3P
Triple or 3E
SA2+D108N
SA2+N144T
SA2+I56N
SA2
Autolum

Figure 33

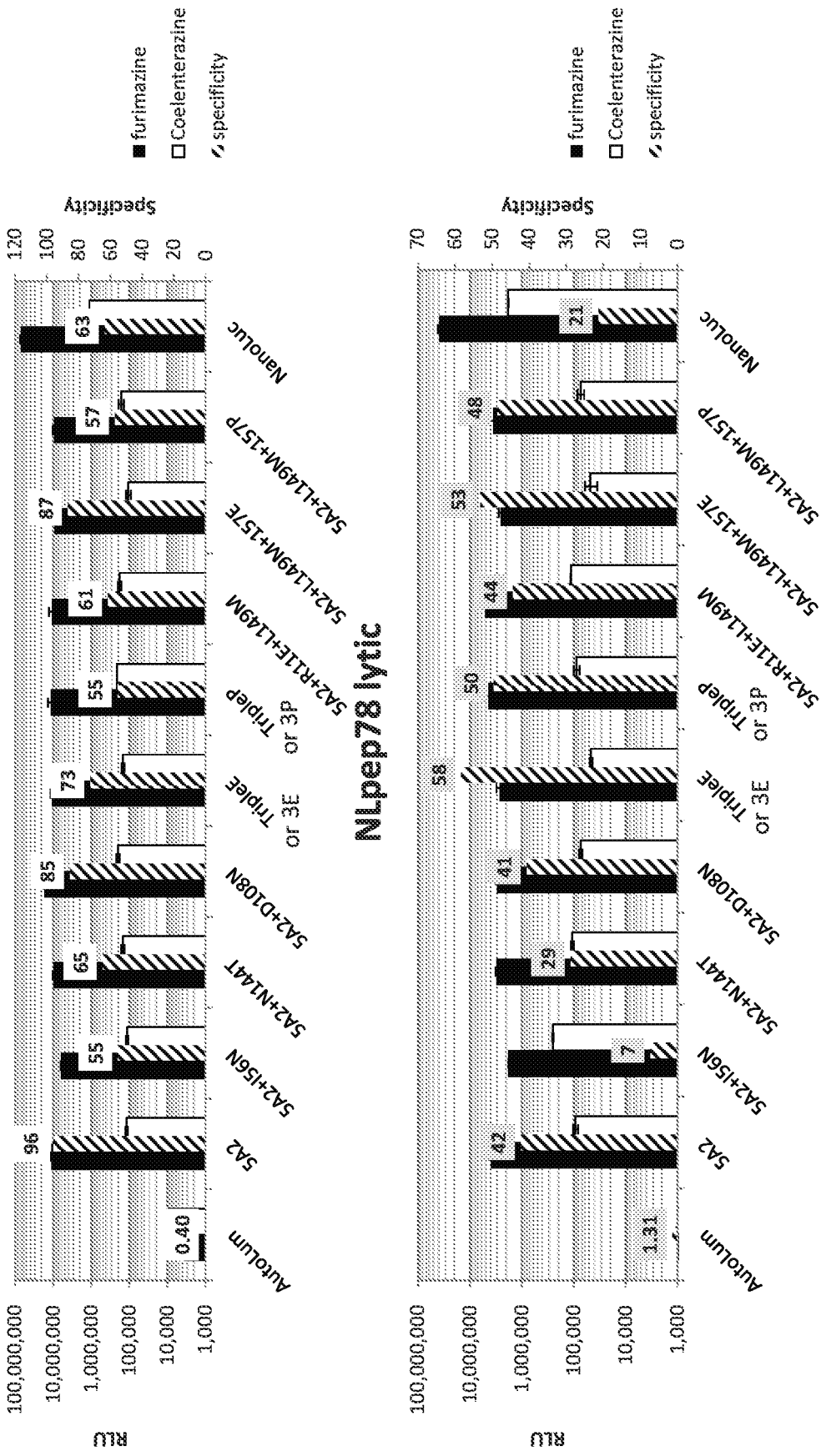

NLpep78 live
NLpep78 lytic
RLU
Specificity
furimazine
Coelenterazine
specificity
Autolum
SA2
SA2+I56N
SA2+N144T
SA2+D108N
SA2+R11E+L149M
SA2+L149M+I57E
SA2+L149M+I57P
NanoLuc
Triple or 3E
Triple or 3P
96
0.40
55
65
85
73
61
55
87
63
57
1.31
42
7
29
41
58
50
44
53
48
21

Figure 34

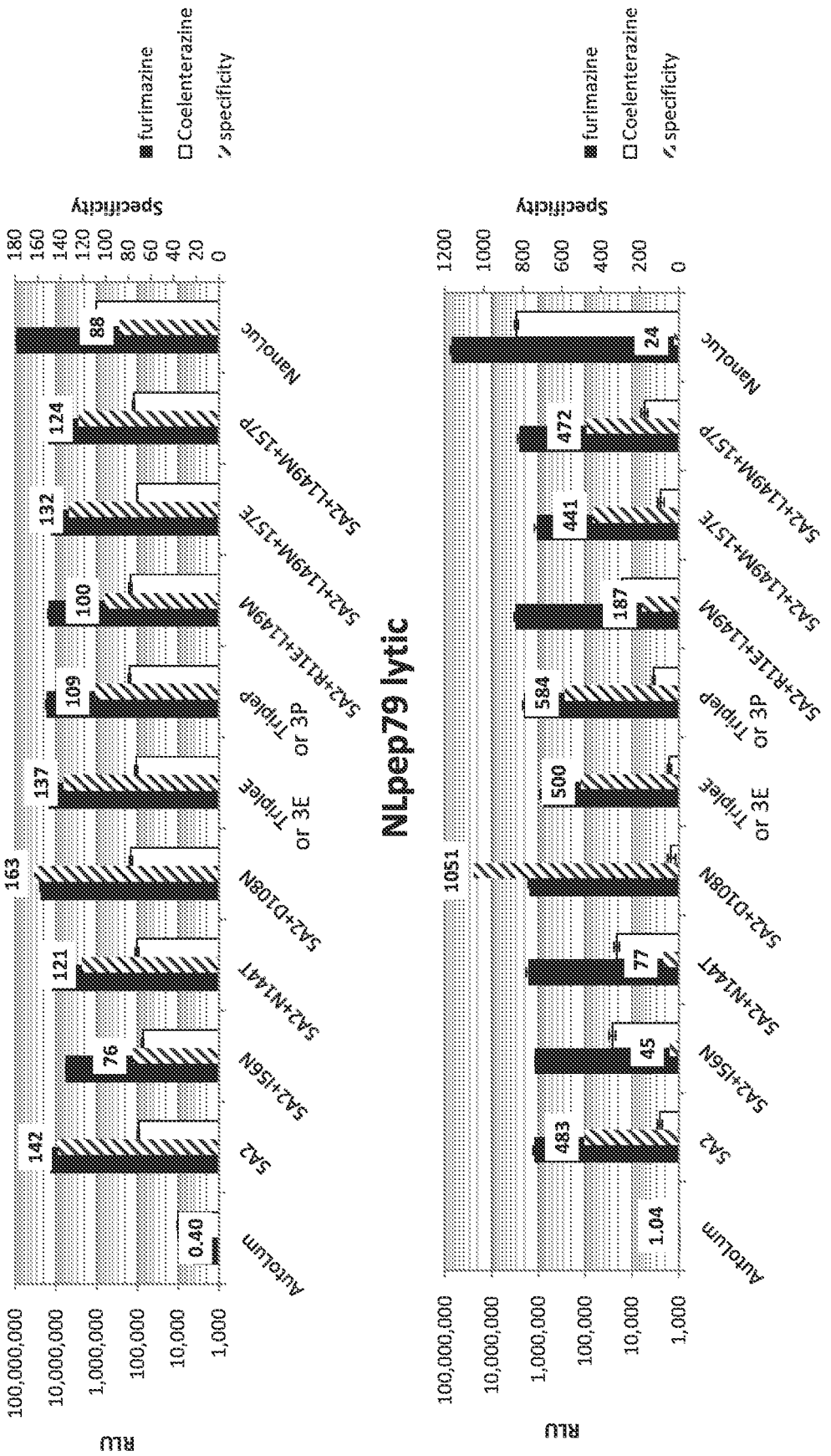

NLpep79 live
NLpep79 lytic
RLU
Specificity
furimazine
Coelenterazine
specificity
Autolum
SA2
SA2+I56N
SA2+N144T
SA2+D108N
Triplex or 3E
Triplep or 3P
SA2+R11E+L149M
SA2+L149M+I57E
SA2+L149M+I57P
NanoLuc
163
142
0.40
76
121
137
109
100
132
124
88
1051
1.04
483
45
77
500
584
187
441
472
24

Figure35

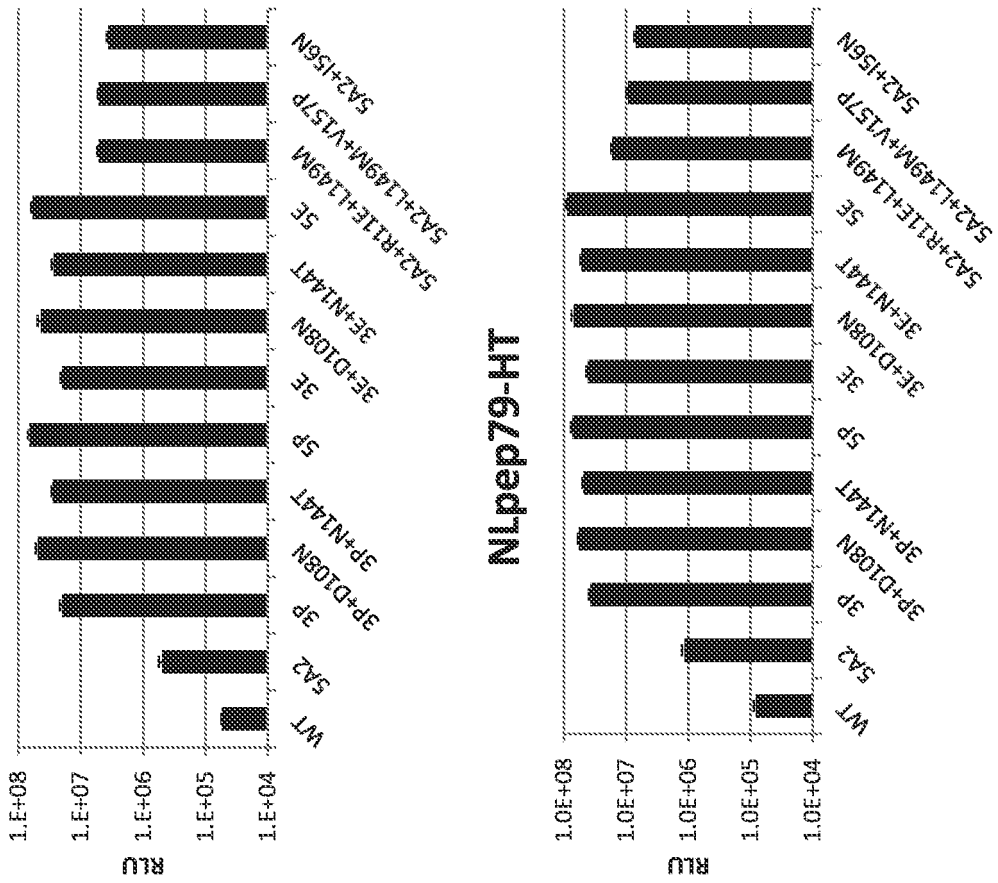

NLpep78-HT
RLU
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
WT
SA2
3P
3P+D108N
3P+N144T
5P
3E
3E+D108N
3E+N144T
5E
SA2+R11E+L149M
SA2+L149M+V157P
SA2+I56N
NLpep79-HT
RLU
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
WT
SA2
3P
3P+D108N
3P+N144T
5P
3E
3E+D108N
3E+N144T
5E
SA2+R11E+L149M
SA2+L149M+V157P
SA2+I56N

Figure 36

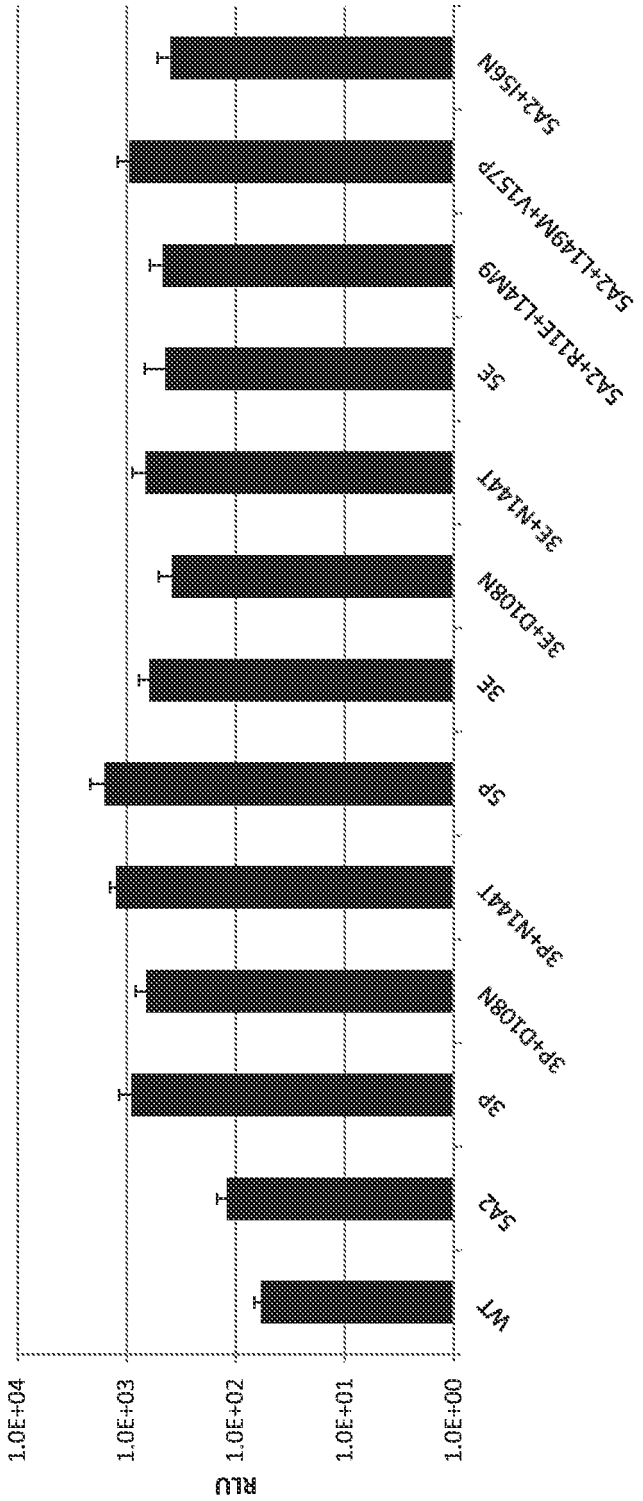

NLpoly without NLpep
RLU
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
WT
5A2
3P
3P+D108N
3P+N144T
5P
3E
3E+D108N
3E+N144T
5E
5A2+R11E+L14N9
5A2+L149M+V157P
5A2+I56N

Figure 37

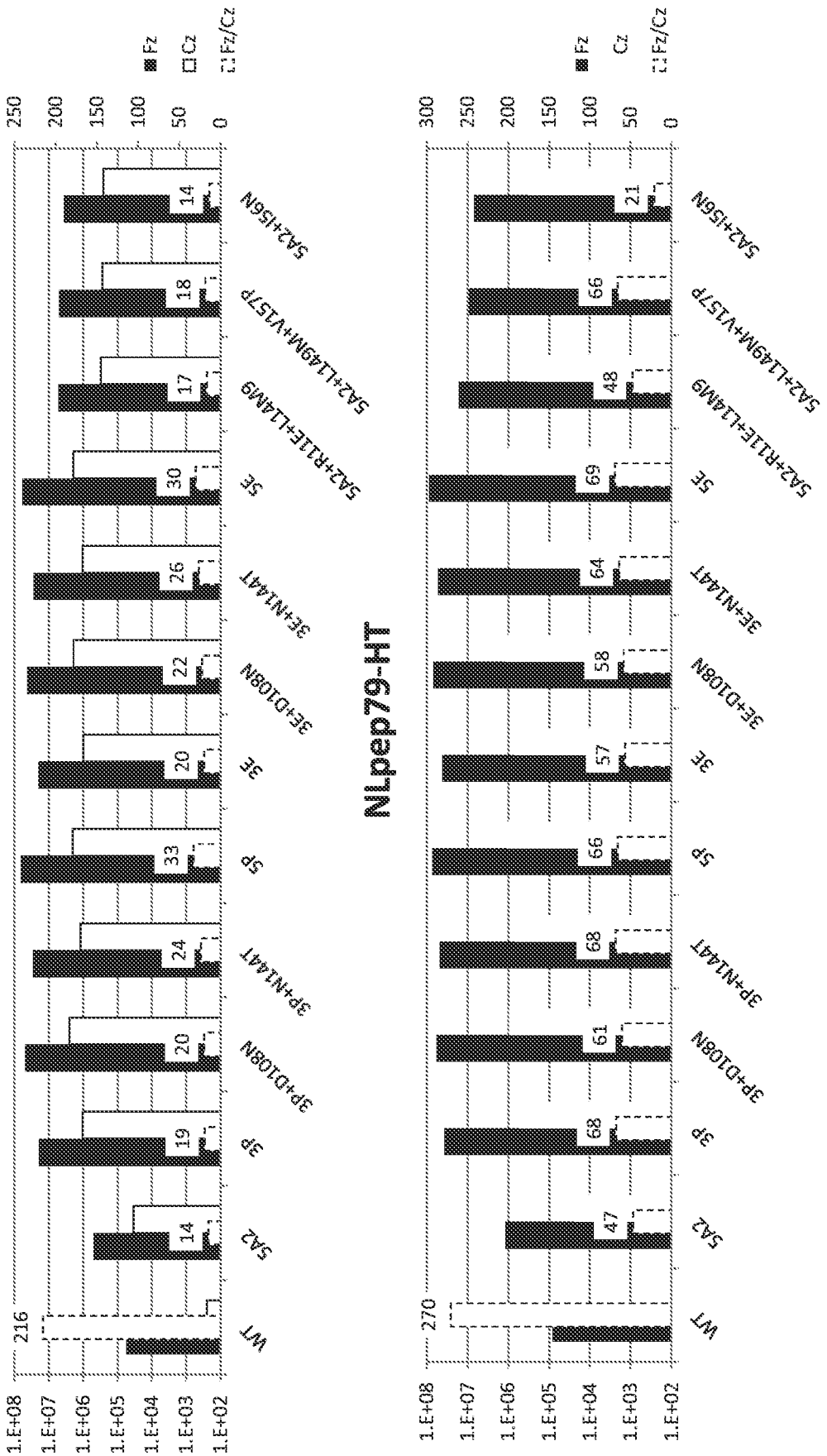

NLpep78-HT
NLpep79-HT
Fz
Cz
Fz/Cz
WT
5A2
3P
3P+D10N
3P+N14T
5P
3E
3E+D10N
3E+N14T
5E
5A2+R11E+L14M9
5A2+L14M9+V15TP
5A2+I5N
216
270

Figure 38

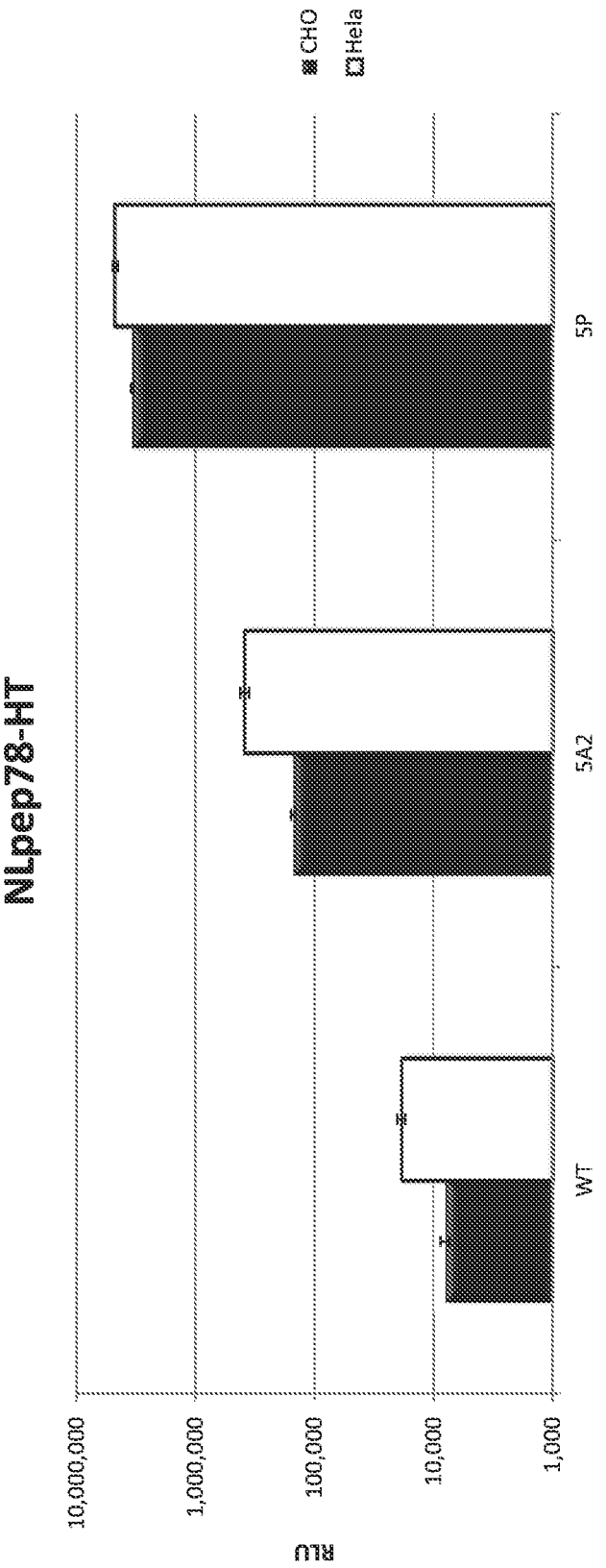

NLpep78-HT
CHO
Hela
RLU
10,000,000
1,000,000
100,000
10,000
1,000
5P
5A2
WT

Figure 39

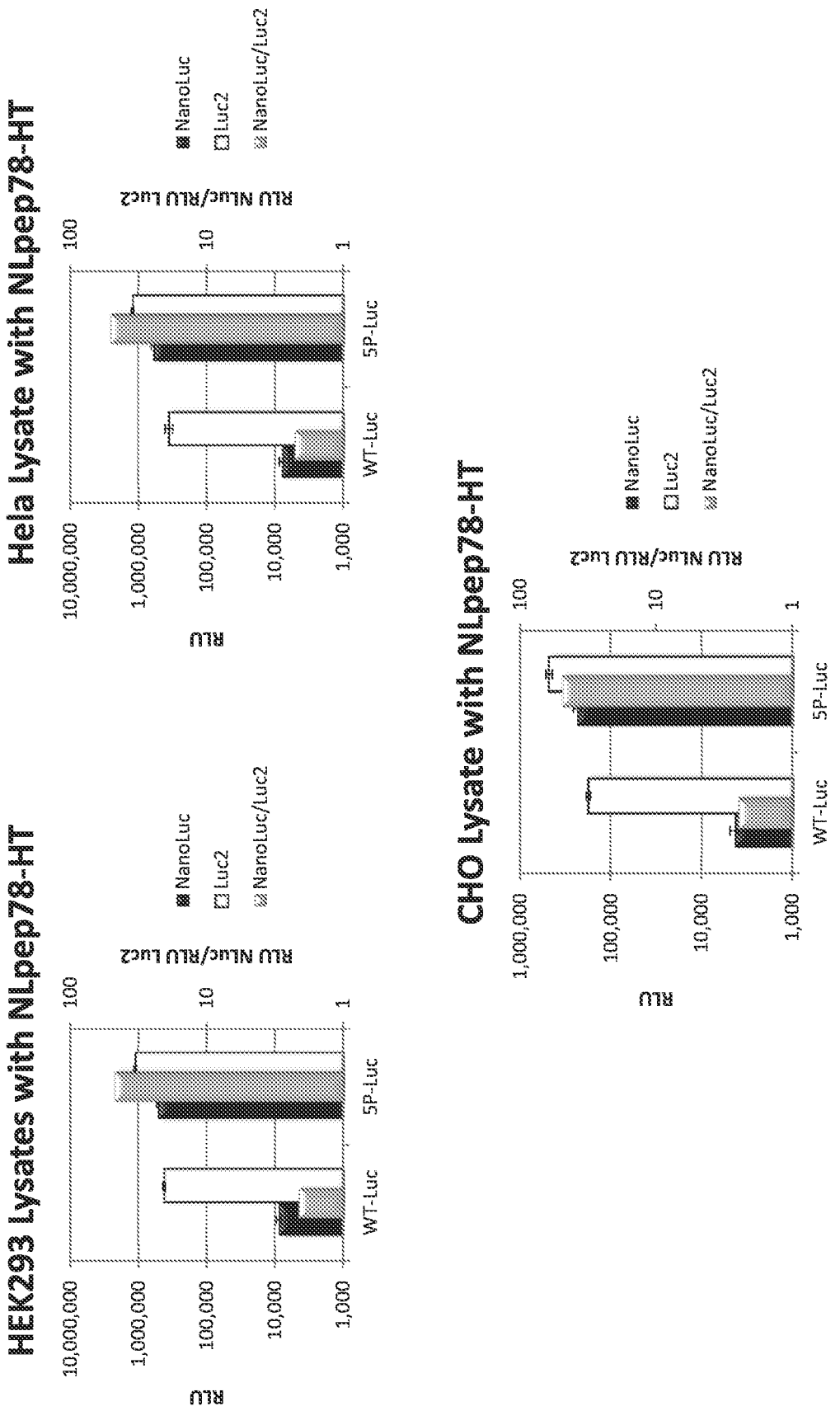

HEK293 Lysates with NLpep78-HT
Hela Lysate with NLpep78-HT
CHO Lysate with NLpep78-HT
RLU
RLU Nluc/RLU luc2
NanoLuc
Luc2
NanoLuc/Luc2
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
WT-Luc
5P-Luc

Figure 40

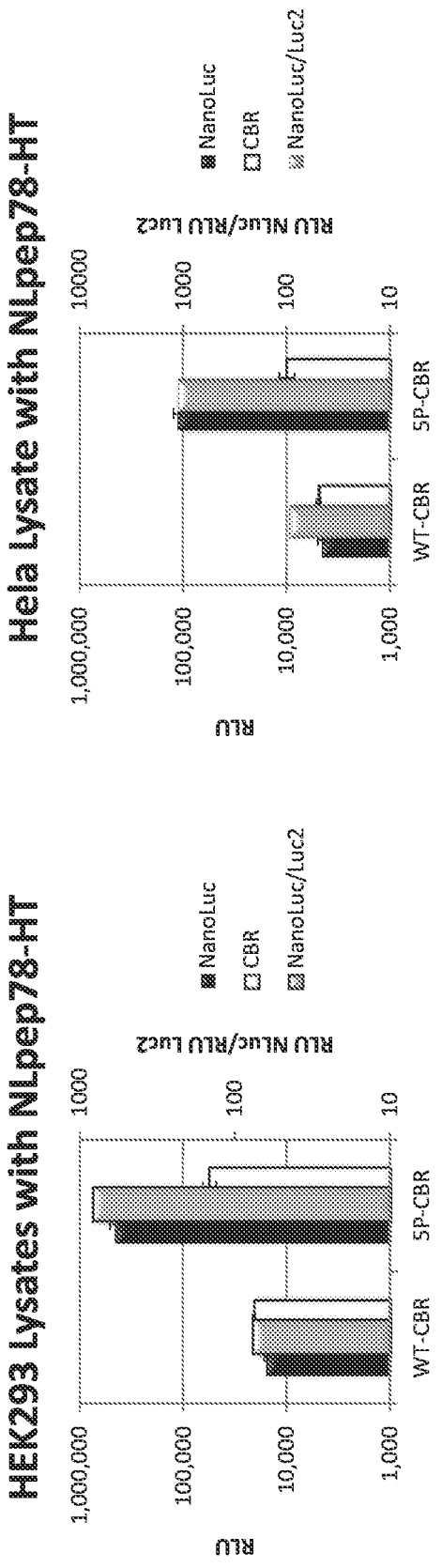

HEK293 Lysates with NLpep78-HT
RLU
1,000,000
100,000
10,000
1,000
WT-CBR
5P-CBR
RLU NLuc/RLU Luc2
1000
100
10
NanoLuc
CBR
NanoLuc/Luc2
Hela Lysate with NLpep78-HT
RLU
1,000,000
100,000
10,000
1,000
WT-CBR
5P-CBR
RLU NLuc/RLU Luc2
10000
1000
100
10
NanoLuc
CBR
NanoLuc/Luc2
CHO Lysate with NLpep78-HT
RLU
100,000
10,000
1,000
WT-CBR
5P-CBR
RLU NLuc/RLU Luc2
1000
100
10
NanoLuc
CBR
NanoLuc/Luc2

Figure 41

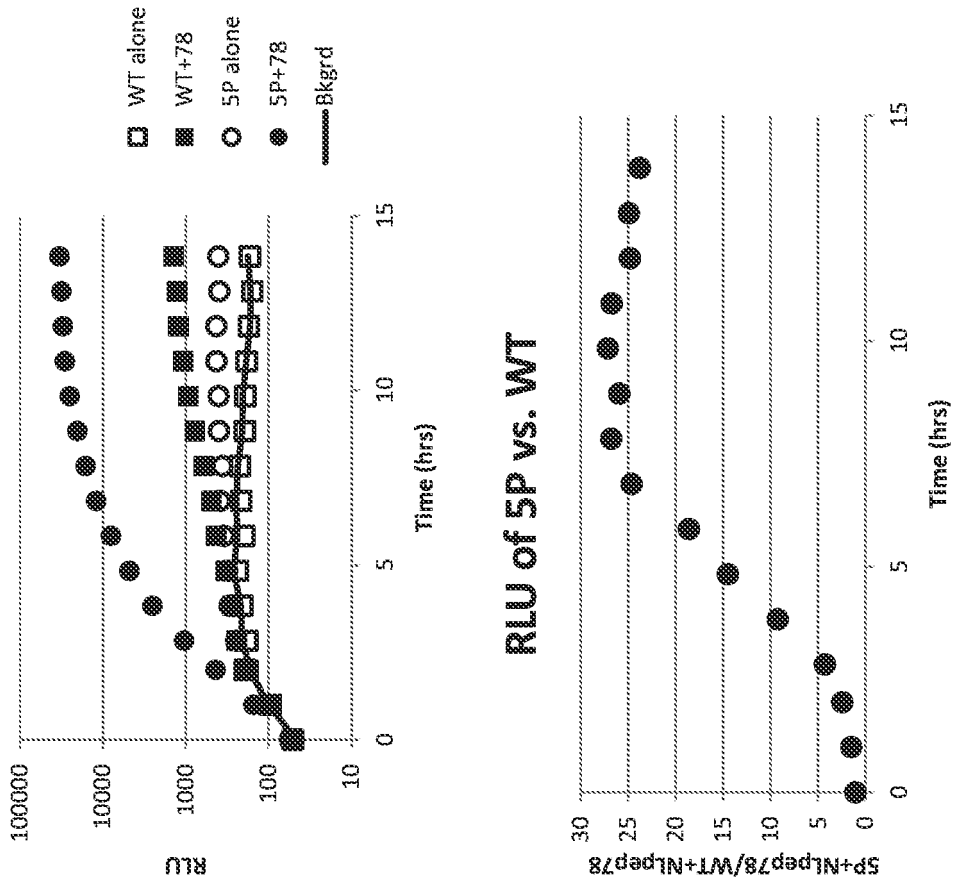

Hela Sequential Transfection, 4377
RLU
100000
10000
1000
100
10
0
5
10
15
Time (hrs)
WT alone
WT+78
5P alone
5P+78
Bkgrd
RLU of 5P vs. WT
5P+NLpep78/WT+NLpep78
30
25
20
15
10
5
0
0
5
10
15
Time (hrs)

Figure 42

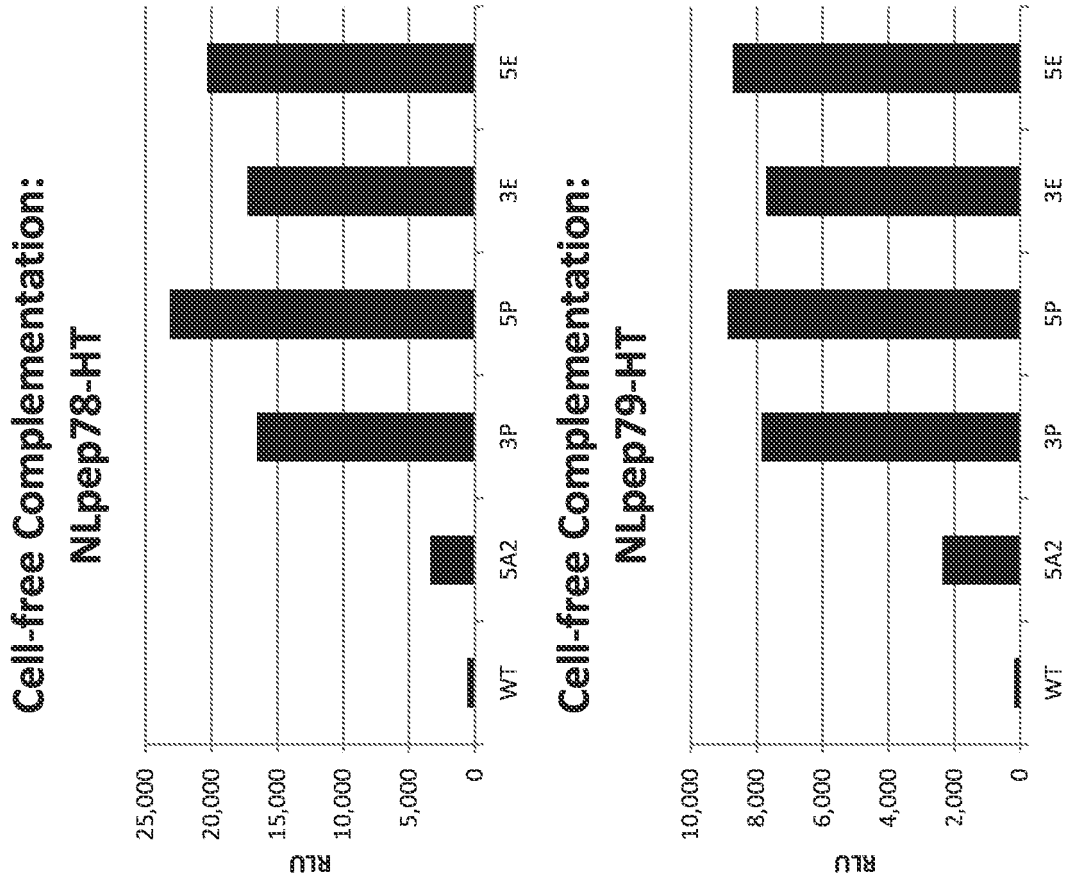

Cell-free Complementation:
NLpep78-HT
RLU
25,000
20,000
15,000
10,000
5,000
0
WT
5A2
3P
5P
3E
5E
Cell-free Complementation:
NLpep79-HT
RLU
10,000
8,000
6,000
4,000
2,000
0
WT
5A2
3P
5P
3E
5E

Figure 43

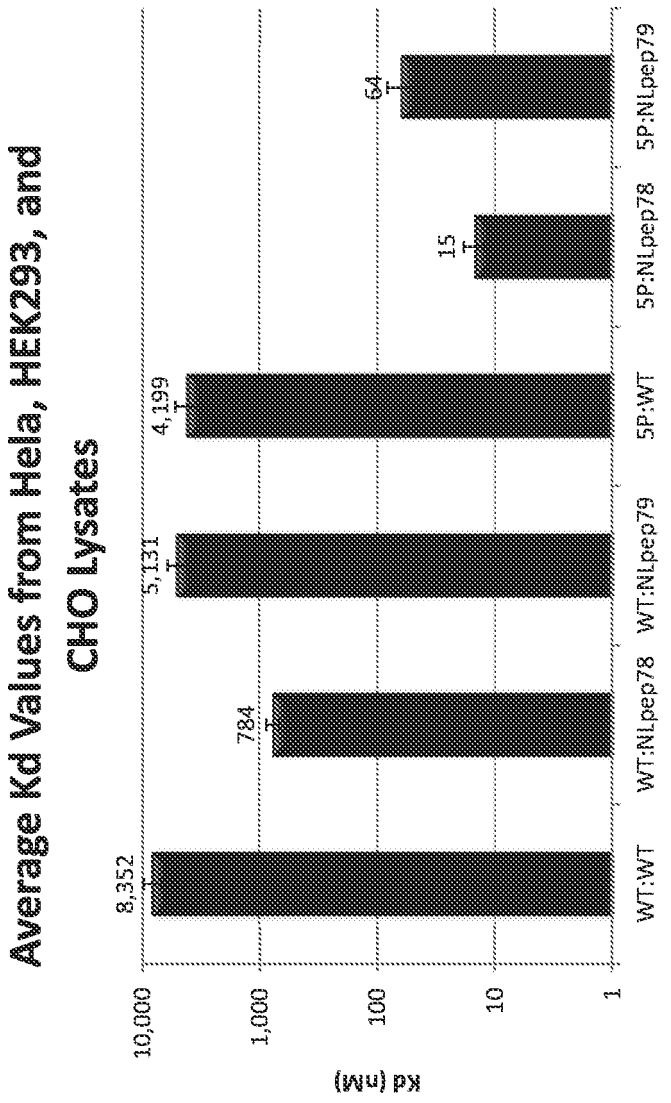

Average Kd Values from Hela, HEK293, and CHO Lysates
Kd (nM)
10,000
1,000
100
10
1
8,352
784
5,131
4,199
15
64
WT:WT
WT:NLpep78
WT:NLpep79
5P:WT
5P:NLpep78
5P:NLpep79

Figure 44

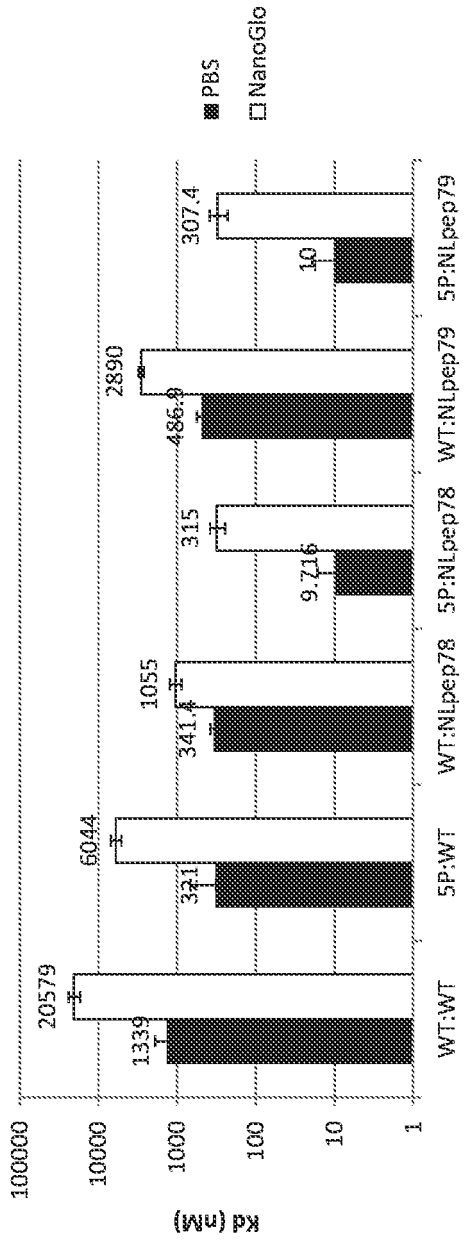
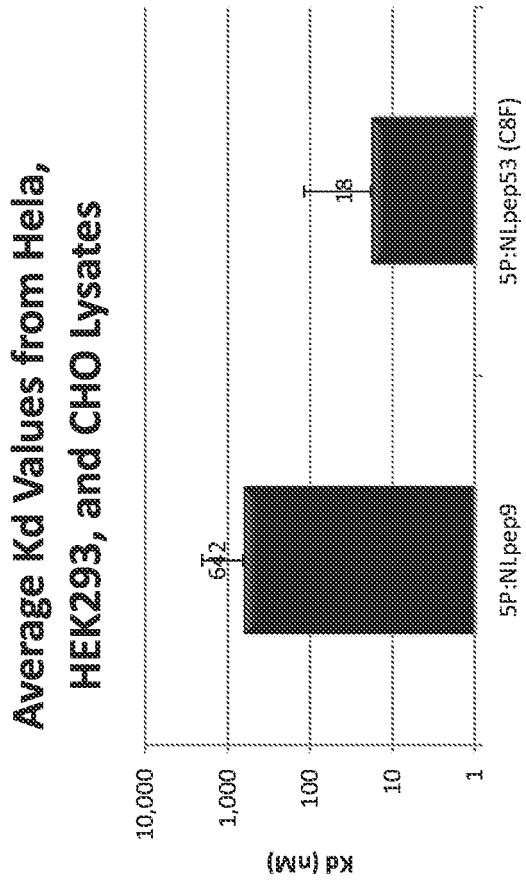
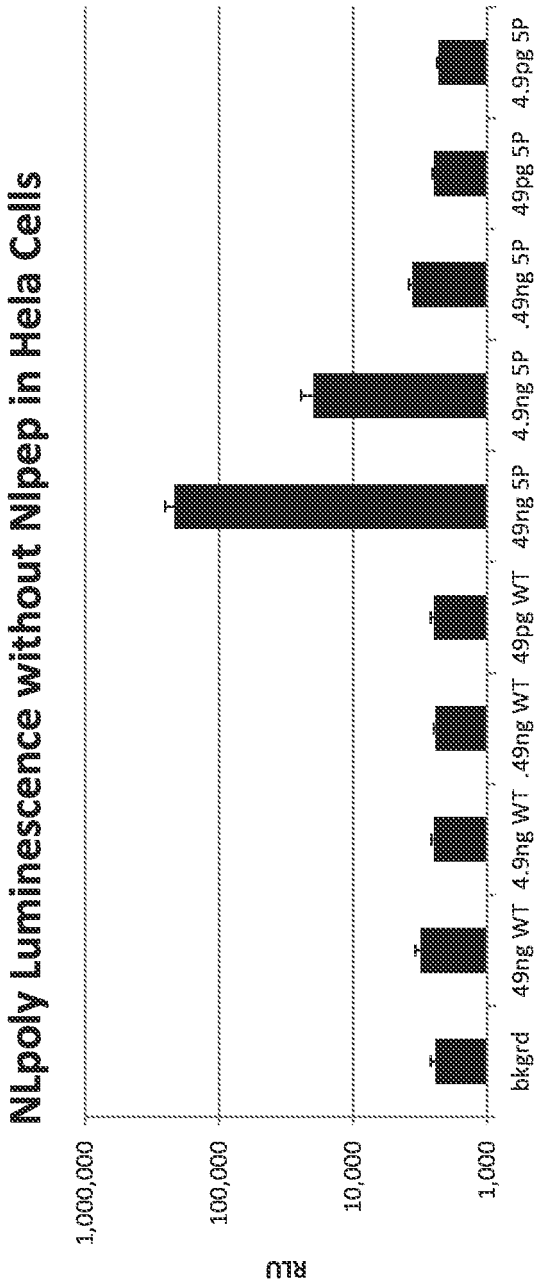

Kd Estimates in PBS and NanoGlo
Kd (nM)
100000
10000
1000
100
10
1
PBS
NanoGlo
20579
1339
6044
321
1055
341.4
315
9.716
2890
486.9
307.4
10
WT:WT
5P:WT
WT:NLpep78
5P:NLpep78
WT:NLpep79
5P:NLpep79

Figure 45



NLpep9	G	V	T	G	W	R	L	C	K		R		I		S		A		
NLpep53	G	V	T	G	W	R	L	F	K		R		I		S		A		

Figure 46


NLpoly Luminescence without Nlpep in Hela Cells
RLU
1,000,000
100,000
10,000
1,000
bkgrd
49ng WT
4.9ng WT
.49ng WT
49pg WT
49ng 5P
4.9ng 5P
.49ng 5P
49pg 5P
4.9pg 5P

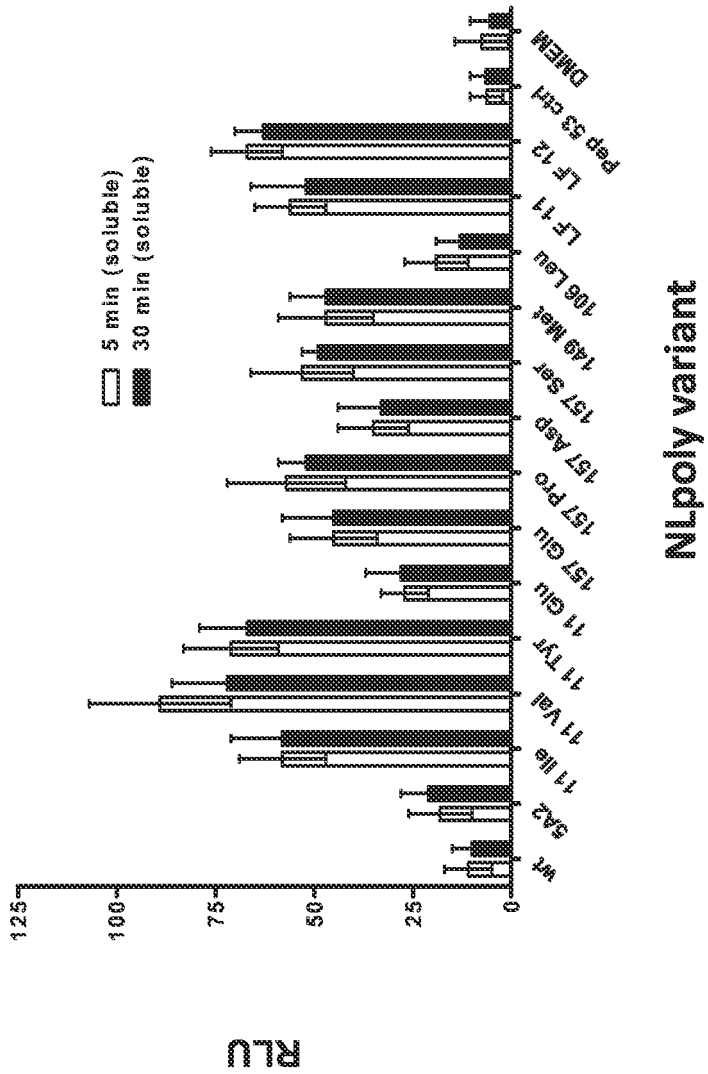

RLU
125
100
75
50
25
0
5 min (soluble)
30 min (soluble)
wt
Δ42
I7 Ile
I1 Val
I1 Tyr
I1 Glu
I57 Glu
I57 Pro
I57 Asp
I57 Ser
I49 Met
I06 Leu
LF I1
LF I2
Pep 53 ctrl
DMEM
NLpoly variant

Figure 47

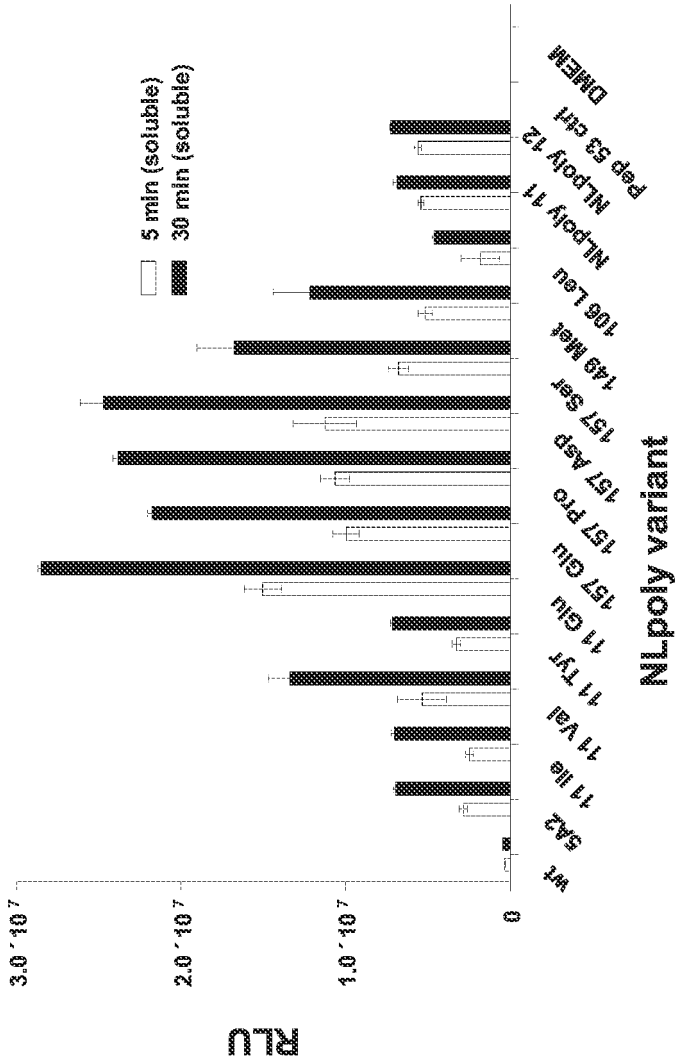

5 min (soluble)
30 min (soluble)
RLU
3.0 ´ 10 7
2.0 ´ 10 7
1.0 ´ 10 7
0
wt
5A2
14 Ile
14 Val
14 Tyr
14 Glu
157 Glu
157 Pro
157 Asp
157 Ser
149 Met
106 Leu
NLpoly 11
NLpoly 12
Pep 53 cut
DMEM
NLpoly variant

Figure 48

Figure 49

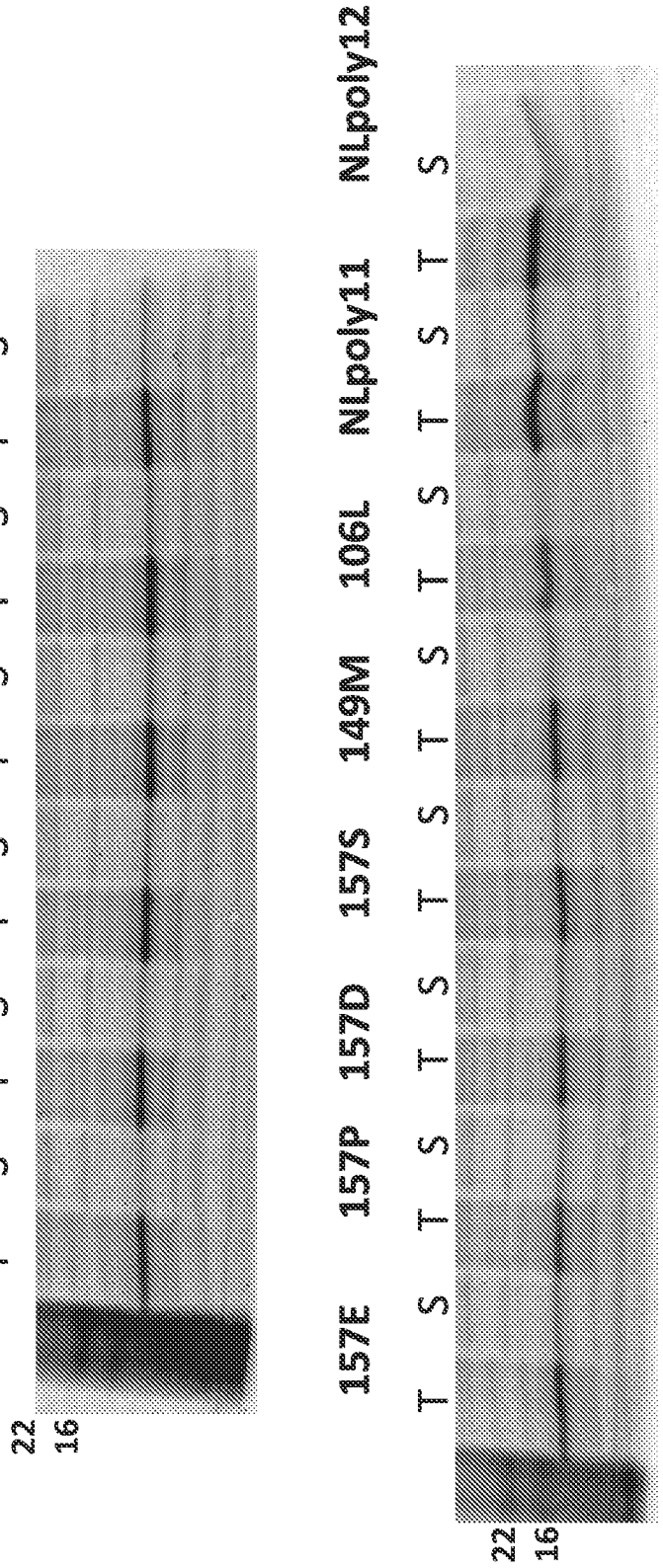

wt 5A2 11I 11V 11Y 11E
T S T S T S T S T S
22
16
157E 157P 157D 157S 149M 106L NLpoly11 NLpoly12
T S T S T S T S T S T S T S
22
16
T=total crude; S=soluble

Figure 50

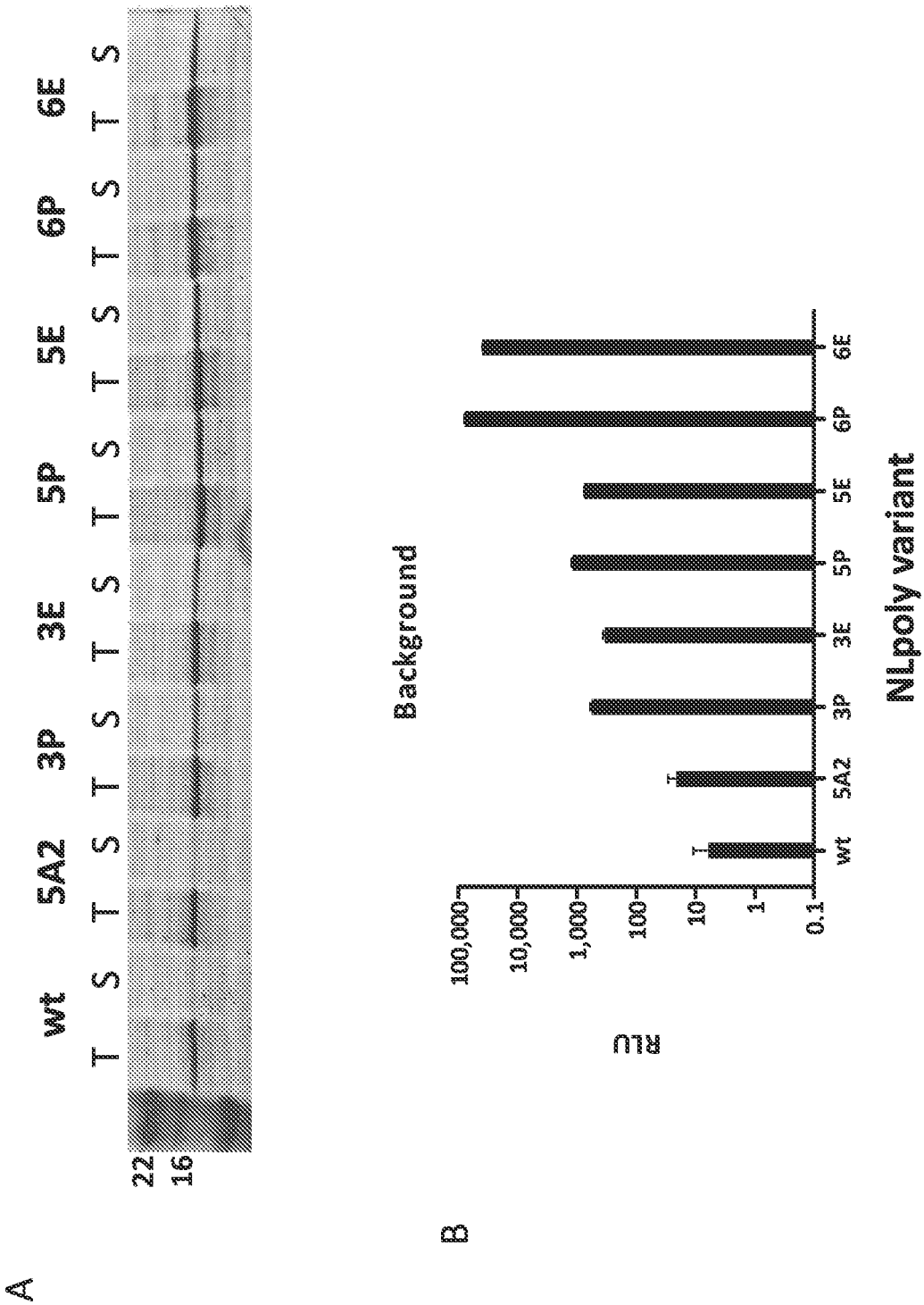

A
wt 5A2 3P 3E 5P 5E 6P 6E
T S T S T S T S T S T S T S T S
22
16
B
Background
RLU
100,000
10,000
1,000
100
10
1
0.1
wt 5A2 3P 3E 5P 5E 6P 6E
NLpoly variant

Figure 51

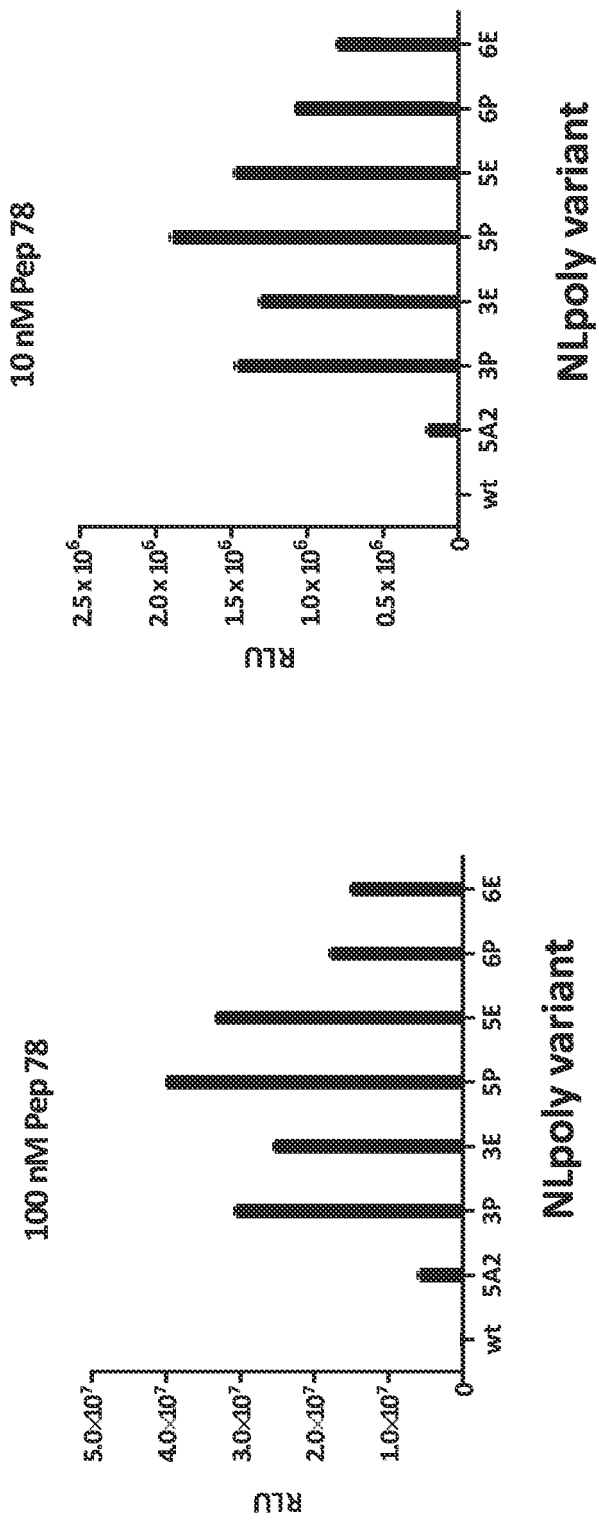

10 nM Pep 78
RLU
2.5 x 10^6
2.0 x 10^6
1.5 x 10^6
1.0 x 10^6
0.5 x 10^6
0
wt
5A2
3P
3E
5P
5E
6P
6E
NLpoly variant
100 nM Pep 78
RLU
5.0x10^7
4.0x10^7
3.0x10^7
2.0x10^7
1.0x10^7
0
wt
5A2
3P
3E
5P
5E
6P
6E
NLpoly variant

Figure 52

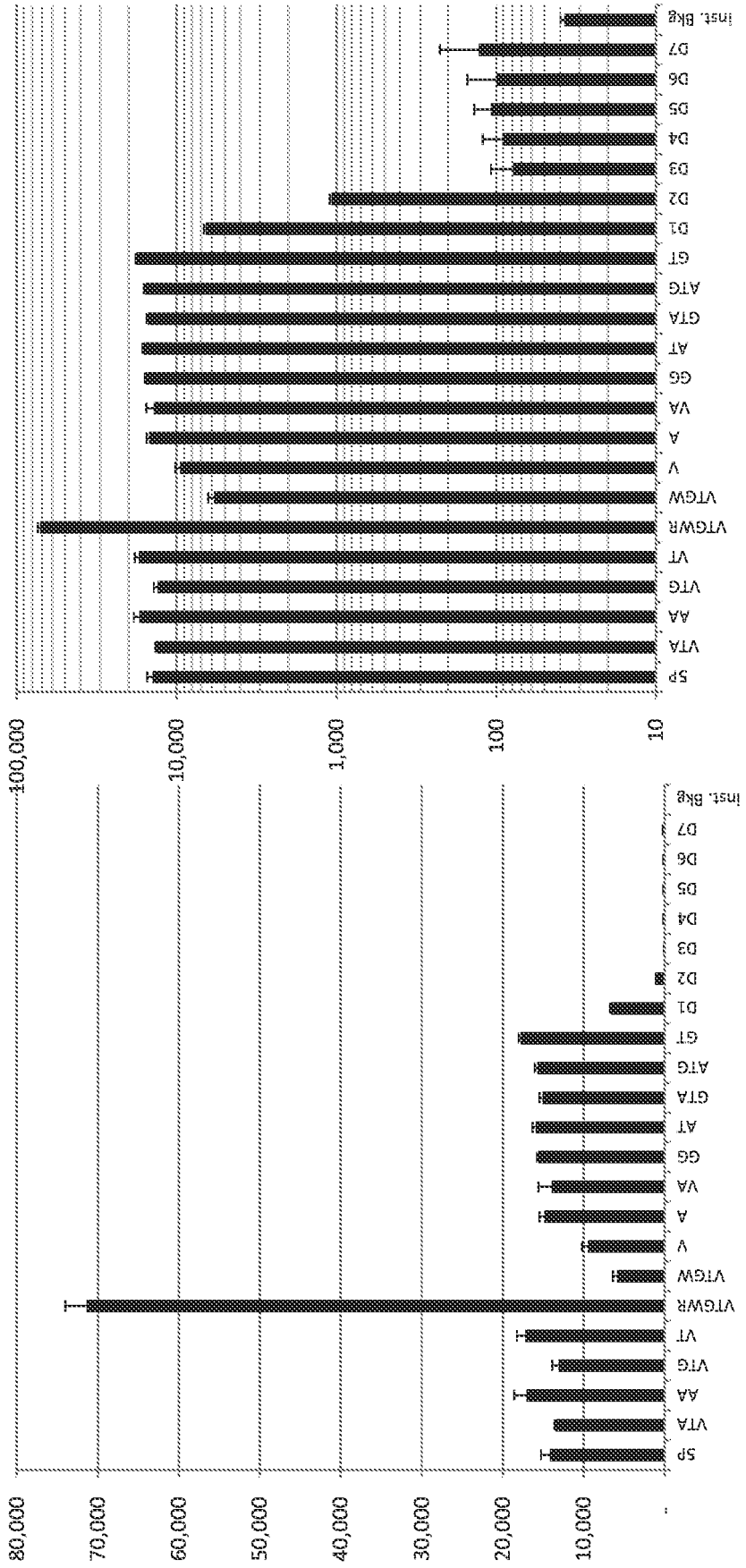

Background
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
-
SP
VTA
AA
VTG
VT
VTGWR
VTGW
V
A
VA
GG
AT
GTA
ATG
GT
D1
D2
D3
D4
D5
D6
D7
Inst. Bkg
Background
100,000
10,000
1,000
100
10
SP
VTA
AA
VTG
VT
VTGWR
VTGW
V
A
VA
GG
AT
GTA
ATG
GT
D1
D2
D3
D4
D5
D6
D7
Inst. Bkg

Figure 53

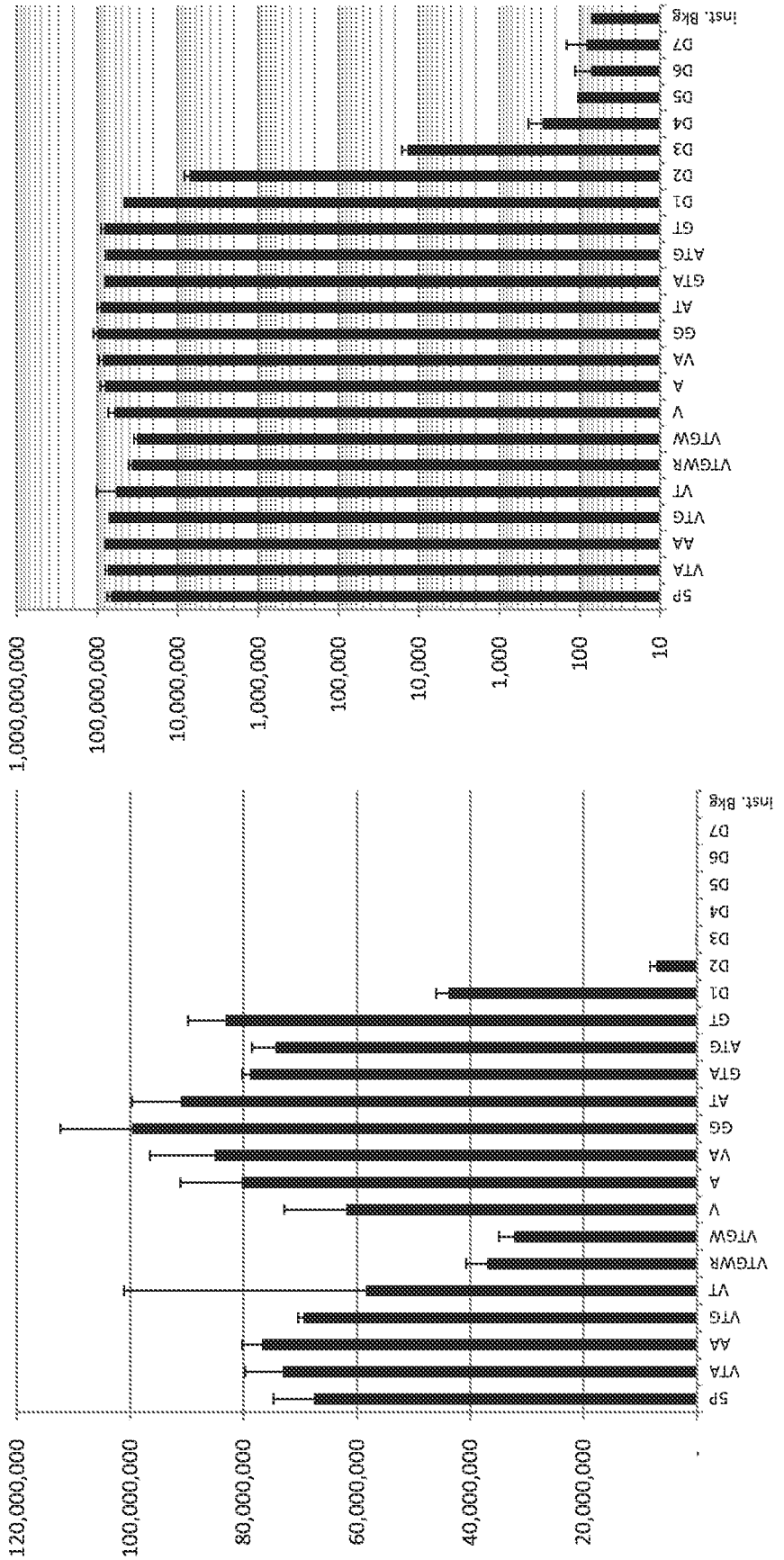

NLpep 78
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
5P
VTA
AA
VTG
VT
VTGWR
VTGW
V
A
VA
GG
AT
GTA
ATG
GT
D1
D2
D3
D4
D5
D6
D7
Inst. Bkg
NLpep 78
120,000,000
100,000,000
80,000,000
60,000,000
40,000,000
20,000,000
5P
VTA
AA
VTG
VT
VTGWR
VTGW
V
A
VA
GG
AT
GTA
ATG
GT
D1
D2
D3
D4
D5
D6
D7
Inst. Bkg

Figure 54

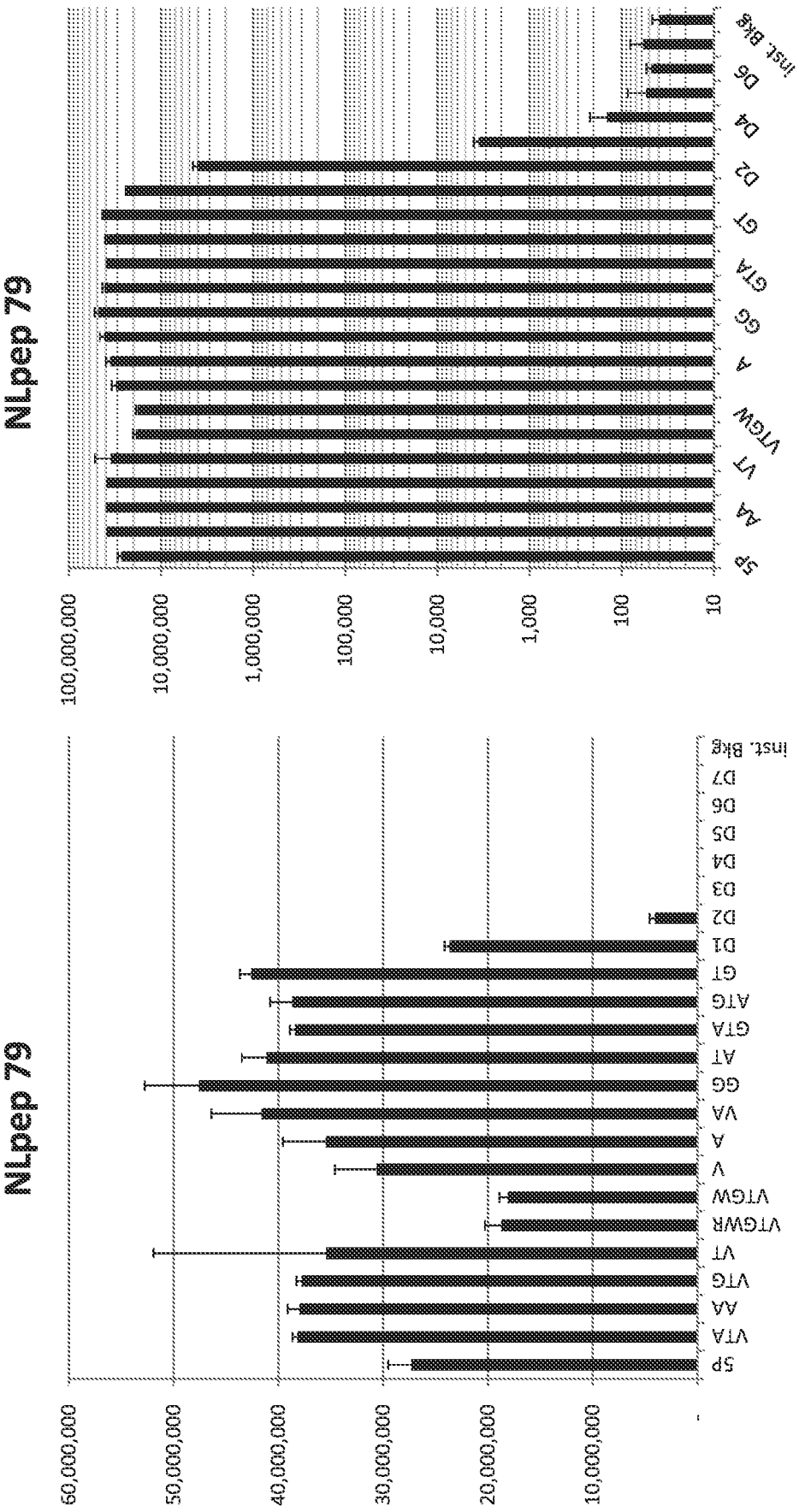

NLpep 79
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
5P
AA
VT
VTGW
A
GG
GTA
GT
D2
D4
D6
Inst. Bkg
NLpep 79
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
-
5P
VTA
AA
VTG
VT
VTGWR
VTGW
V
A
VA
GG
AT
GTA
ATG
GT
D1
D2
D3
D4
D5
D6
D7
Inst. Bkg

Figure 55

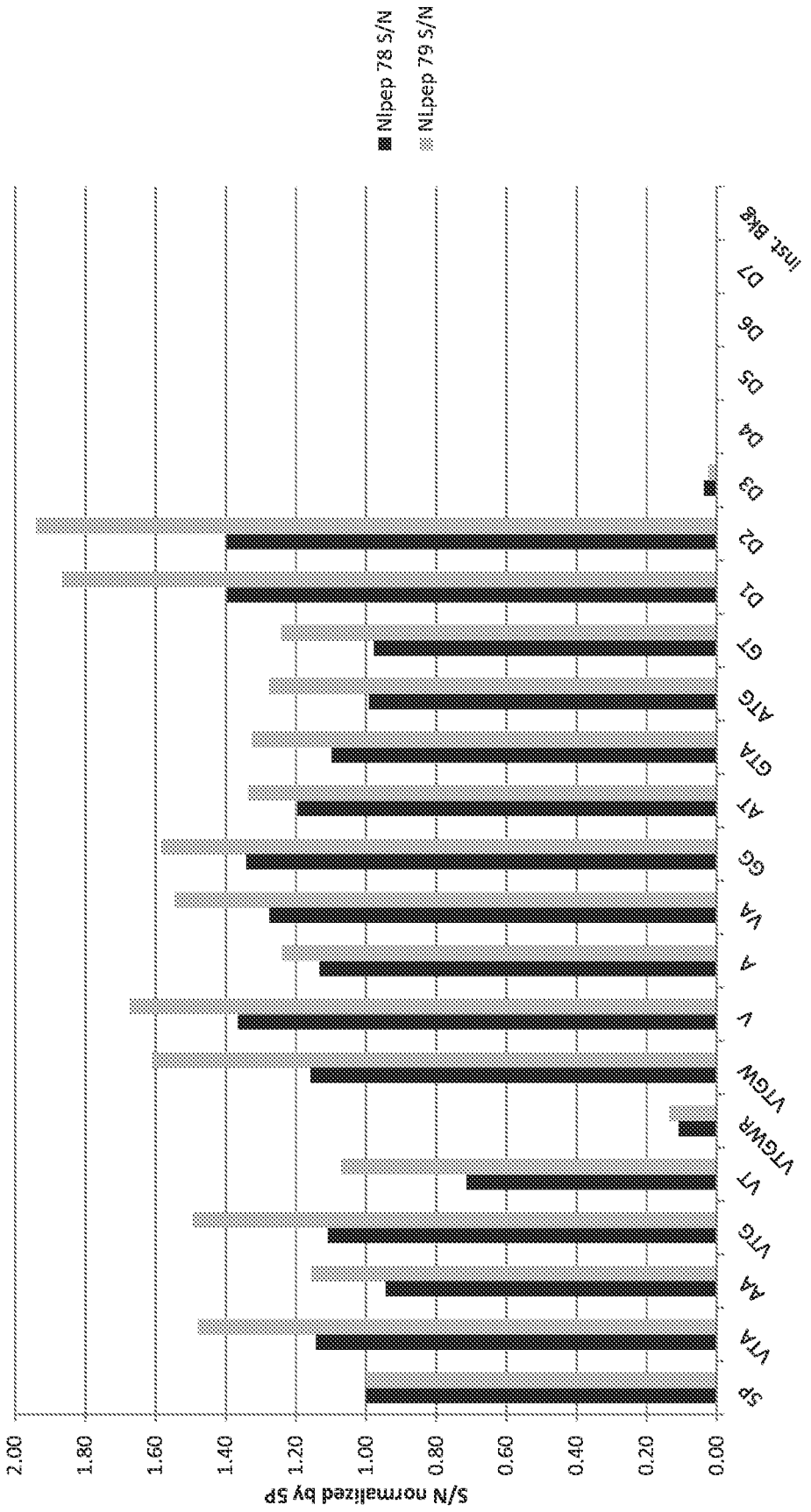

Signal to Background normalized by SP
Nlpep 78 S/N
Nlpep 79 S/N
S/N normalized by SP
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
SP
VTA
AA
VTG
VT
VTGWR
VTGW
V
A
VA
GG
AT
GTA
ATG
GT
D1
D2
D3
D4
D5
D6
D7
Insl. Bkg

Figure 56

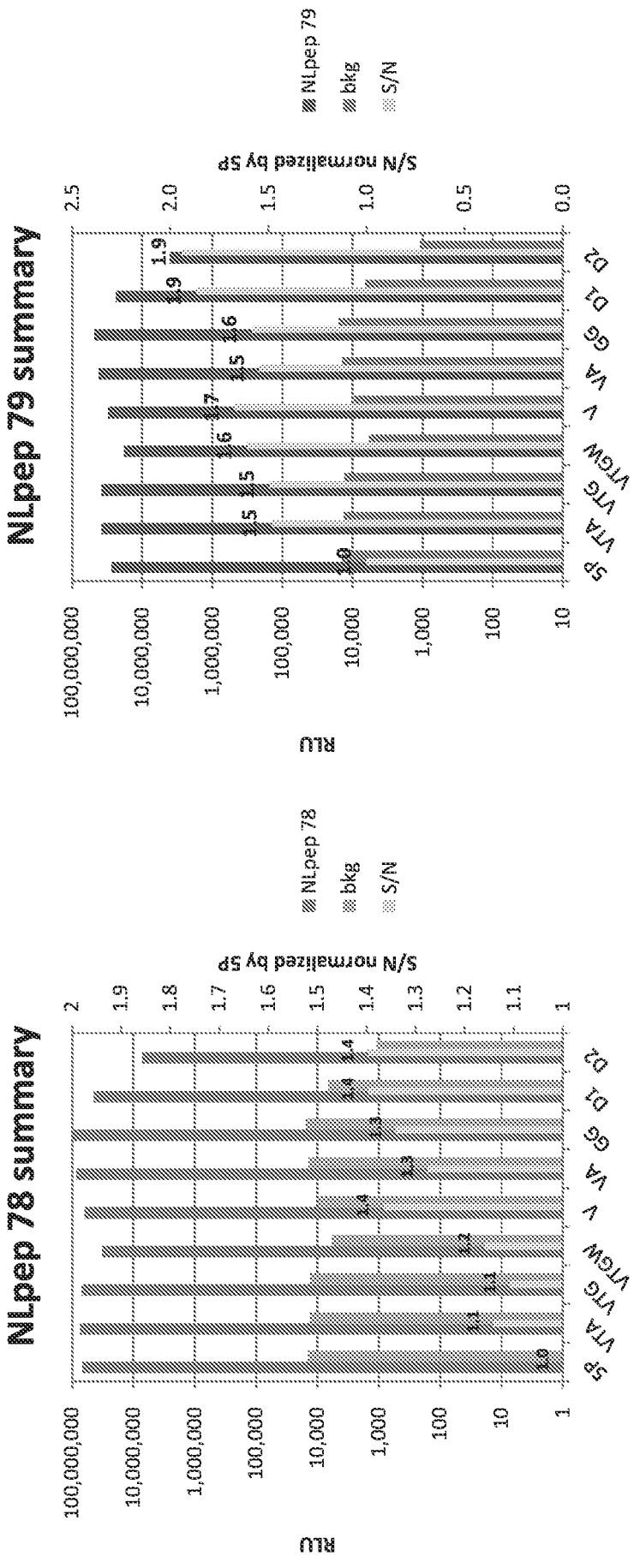

NLpep 78 summary
RLU
S/N normalized by SP
NLpep 78
bkg
S/N
SP
VTA
VTG
VTGW
V
VA
GG
D1
D2
NLpep 79 summary
RLU
S/N normalized by SP
NLpep 79
bkg
S/N
SP
VTA
VTG
VTGW
V
VA
GG
D1
D2

Figure 57

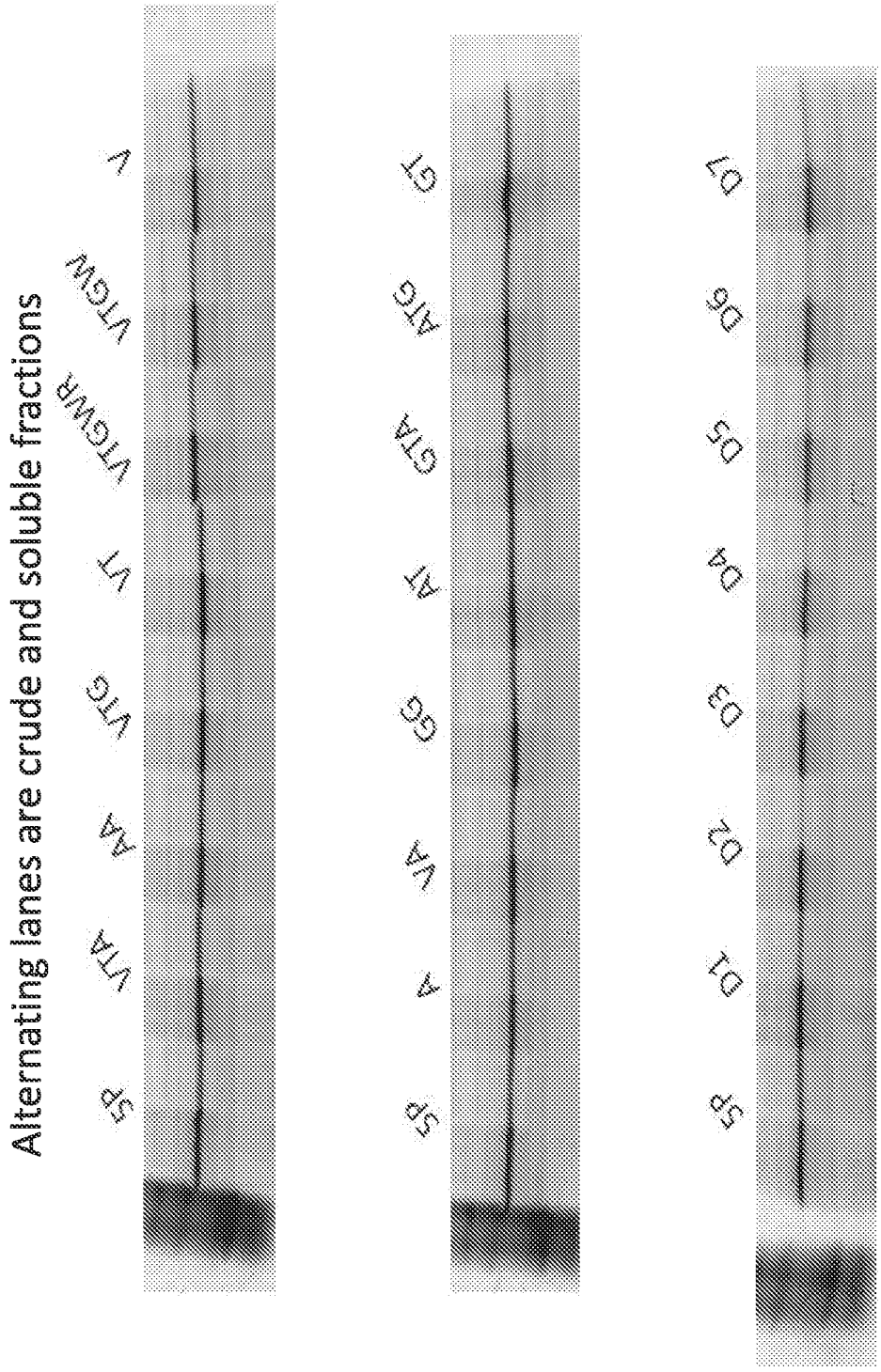
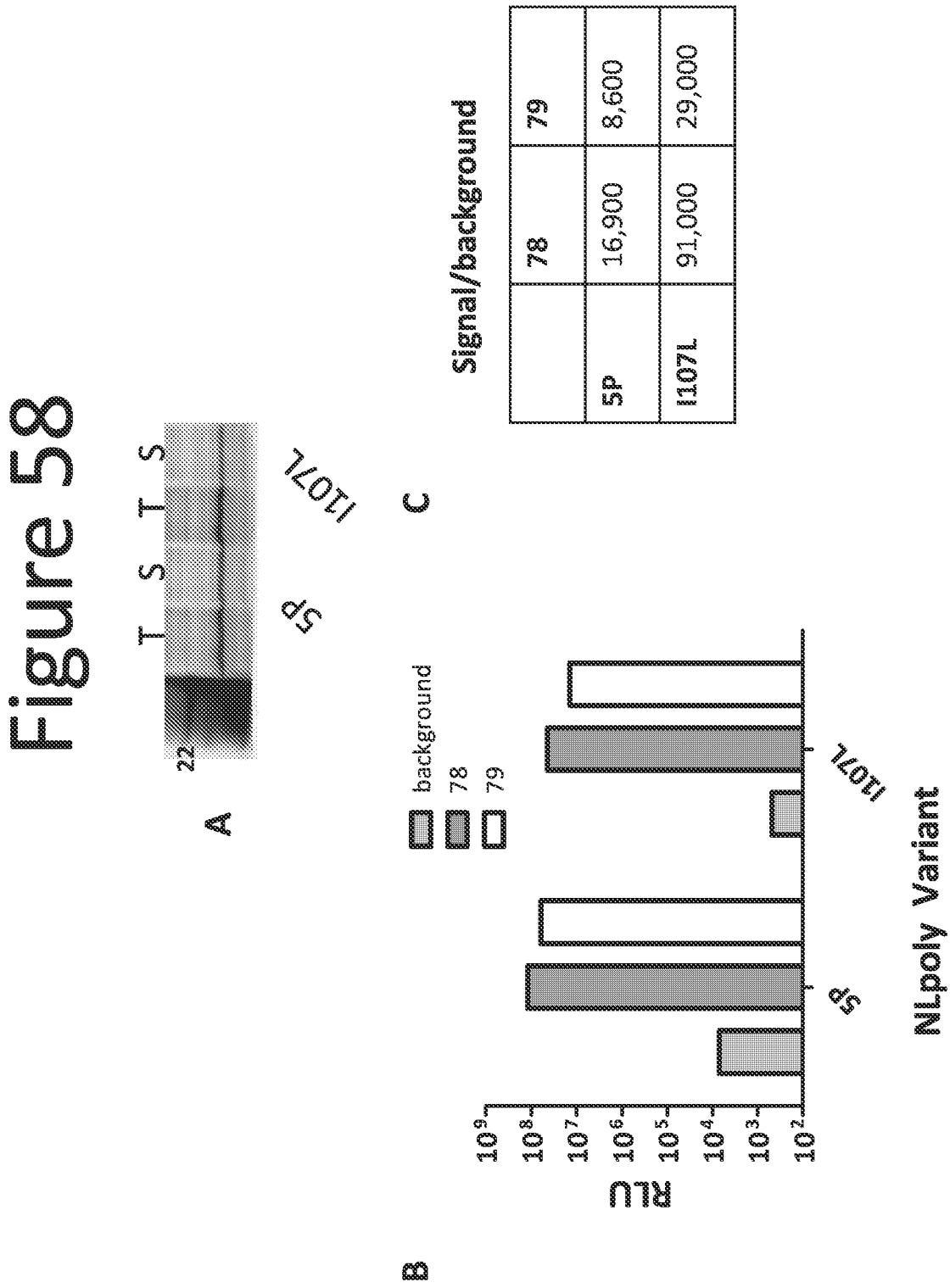

Alternating lanes are crude and soluble fractions
SP
VTA
AA
VTG
VT
VTGWR
VTGW
V
SP
A
VA
GG
AT
GTA
ATG
GT
SP
D1
D2
D3
D4
D5
D6
D7

Figure 58



	78	79
5P	16,900	8,600
I107L	91,000	29,000

Figure 59

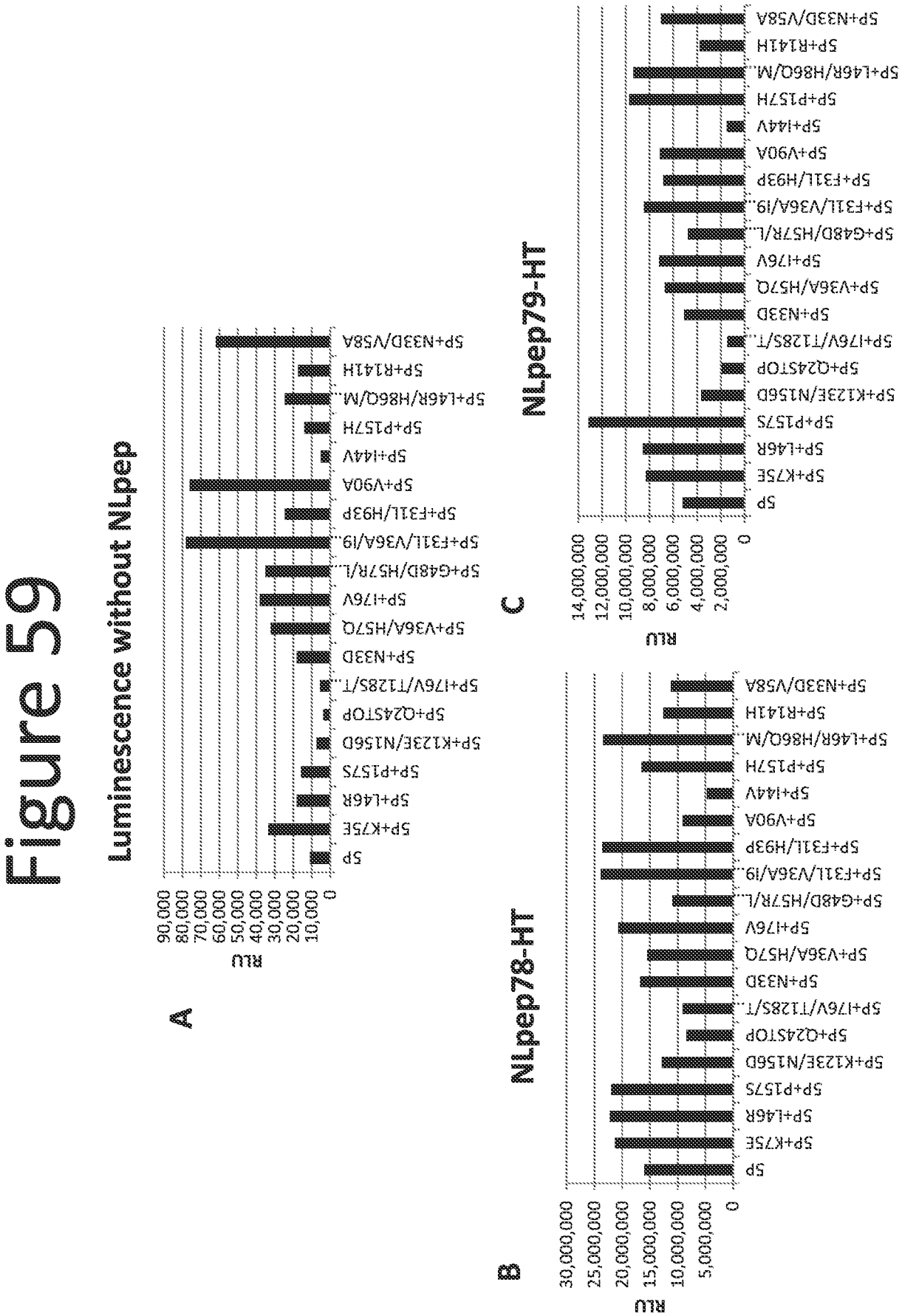

A
Luminescence without NLpep
RLU
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
SP
SP+K75E
SP+L46R
SP+P157S
SP+K123E/N156D
SP+Q245TOP
SP+I76V/T128S/T...
SP+N33D
SP+V36A/H57Q
SP+I76V
SP+G48D/H57R/L...
SP+F31L/V36A/I9...
SP+F31L/H93P
SP+V90A
SP+I44V
SP+P157H
SP+L46R/H86Q/M...
SP+R141H
SP+N33D/V58A
B
NLpep78-HT
RLU
30,000,000
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
SP
SP+K75E
SP+L46R
SP+P157S
SP+K123E/N156D
SP+Q245TOP
SP+I76V/T128S/T...
SP+N33D
SP+V36A/H57Q
SP+I76V
SP+G48D/H57R/L...
SP+F31L/V36A/I9...
SP+F31L/H93P
SP+V90A
SP+I44V
SP+P157H
SP+L46R/H86Q/M...
SP+R141H
SP+N33D/V58A
C
NLpep79-HT
RLU
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
SP
SP+K75E
SP+L46R
SP+P157S
SP+K123E/N156D
SP+Q245TOP
SP+I76V/T128S/T...
SP+N33D
SP+V36A/H57Q
SP+I76V
SP+G48D/H57R/L...
SP+F31L/V36A/I9...
SP+F31L/H93P
SP+V90A
SP+I44V
SP+P157H
SP+L46R/H86Q/M...
SP+R141H
SP+N33D/V58A

Figure 60

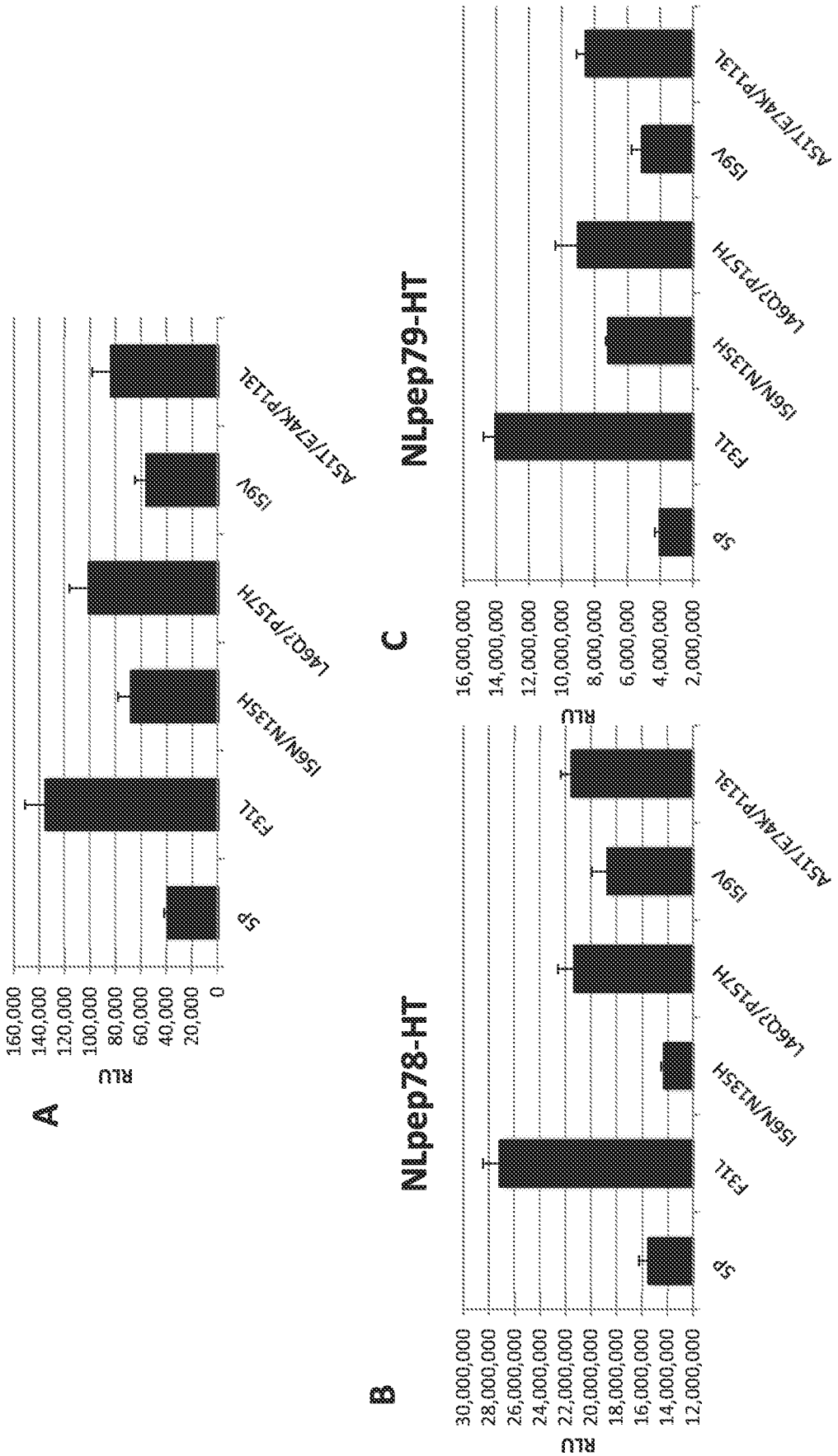

Luminescence without NLpep
A
RLU
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
SP
F31L
I56N/N135H
L46Q?/P157H
I59V
A51T/E74K/P113L
C
NLpep79-HT
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
B
NLpep78-HT
30,000,000
28,000,000
26,000,000
24,000,000
22,000,000
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000

Figure 61

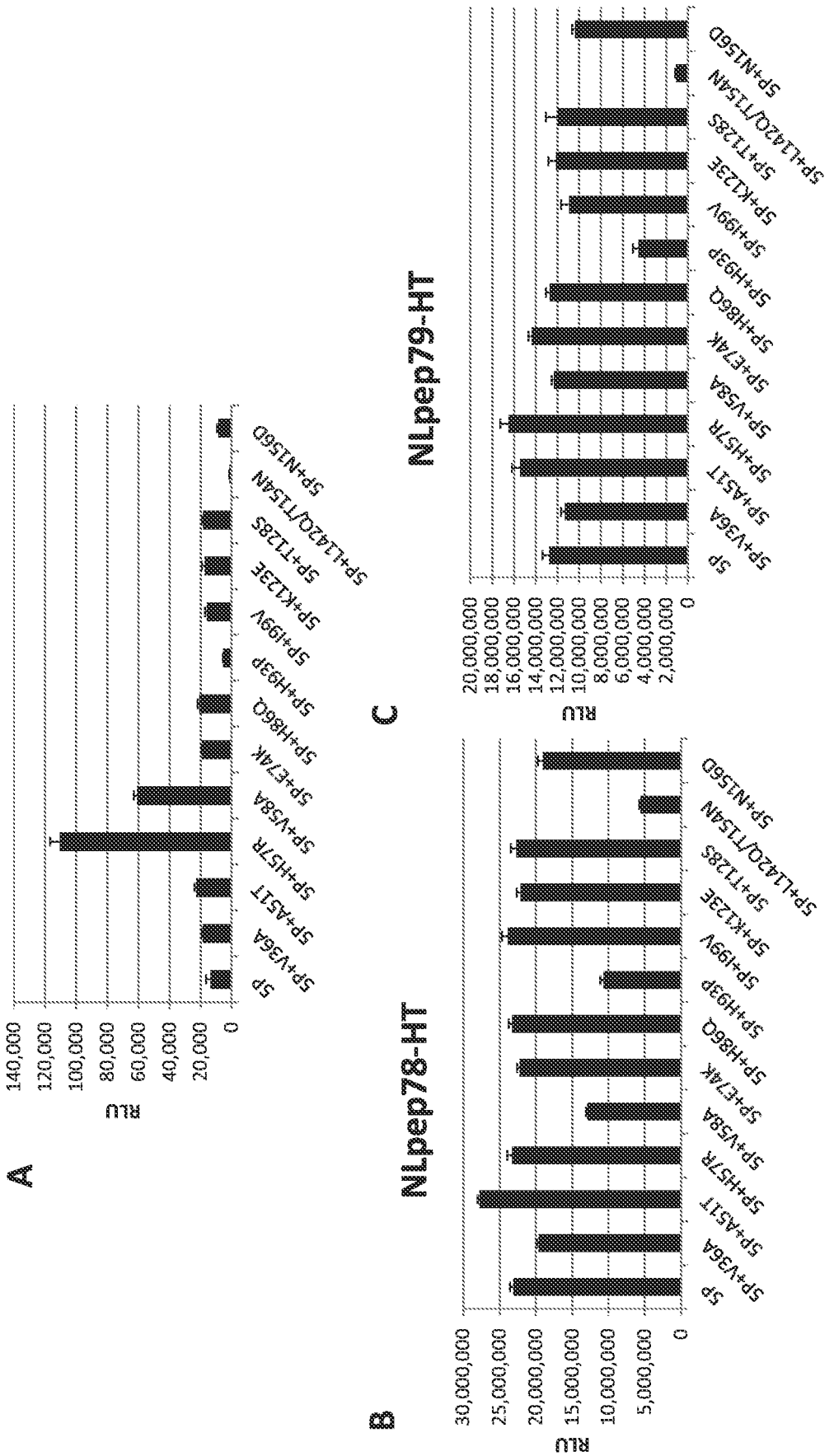

A
Luminescence without NLpep
RLU
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
SP
SP+V36A
SP+A51T
SP+H57R
SP+V58A
SP+E74K
SP+H86Q
SP+H93P
SP+I99V
SP+K123E
SP+T128S
SP+L142Q/T154N
SP+N156D
B
NLpep78-HT
RLU
30,000,000
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
C
NLpep79-HT
RLU
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0

Figure 62

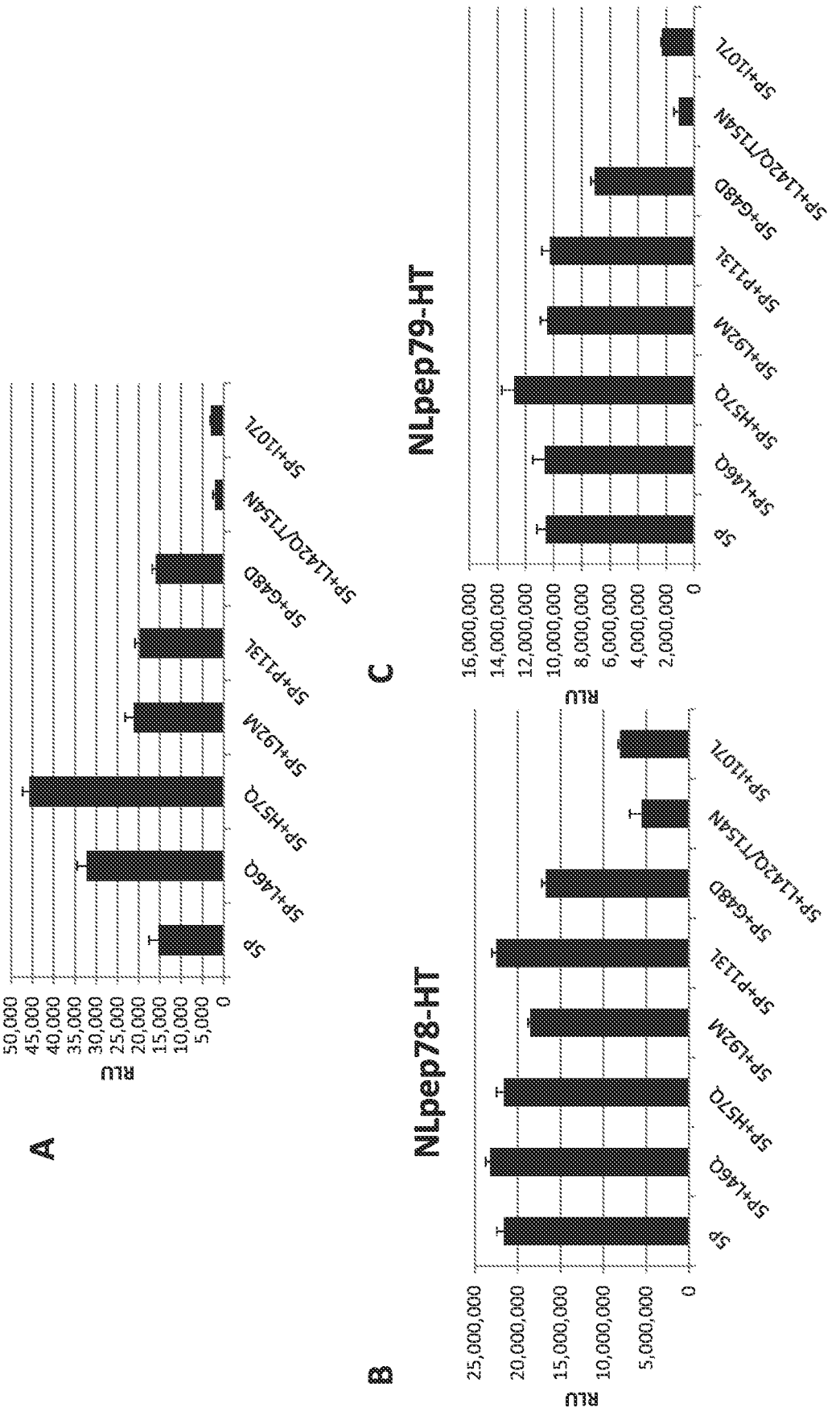
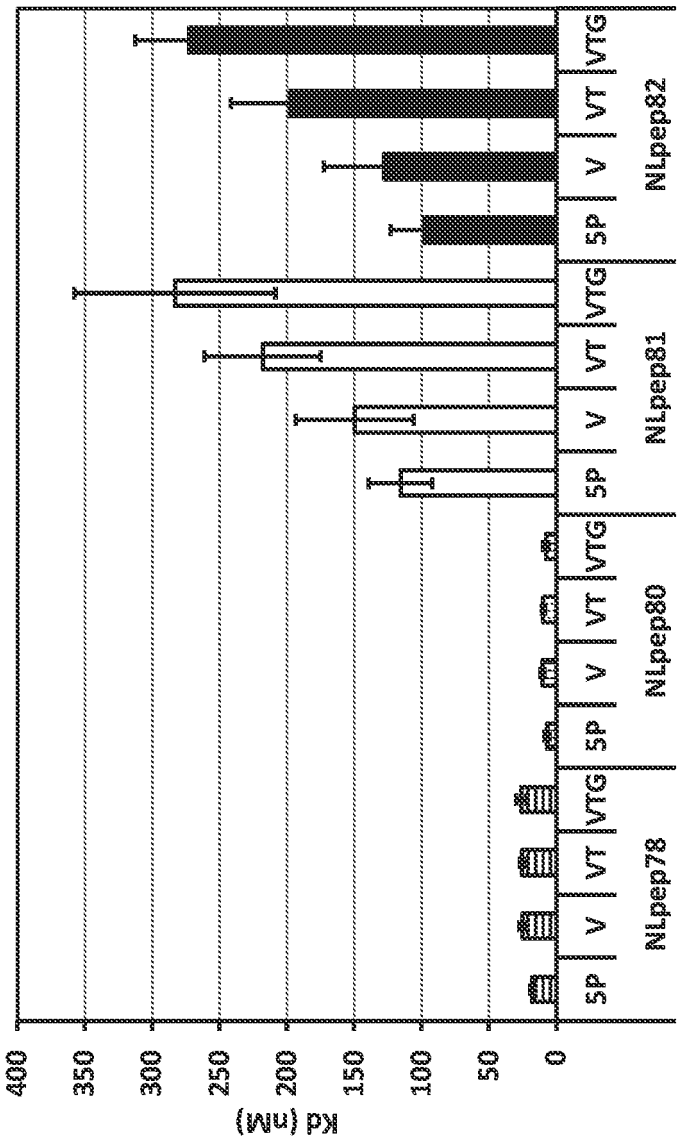

A
Luminescence without NLpep
RLU
50,000
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
SP
SP+L46Q
SP+H57Q
SP+I92M
SP+P113L
SP+G48D
SP+L142Q/T154N
SP+I107L
B
NLpep78-HT
RLU
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
C
NLpep79-HT
RLU
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0

Figure 63



	SP	SP+V	SP+VT	SP+VTG
NLpep78	17.8 ± 1.43	24.9 ± 2.23	25.4 ± 1.41	26.0 ± 3.85
NLpep80	6.9 ± 1.8	10.3 ± 1.57	9.6 ± 1.2	7.5 ± 2.1
NLpep81	115.6 ± 23.7	149.8 ± 43.9	218.0 ± 43.20	283.2 ± 74.7
NLpep82	97.8 ± 25.17	127.9 ± 44.80	197.9 ± 43.70	272.5 ± 40.20

Figure 64

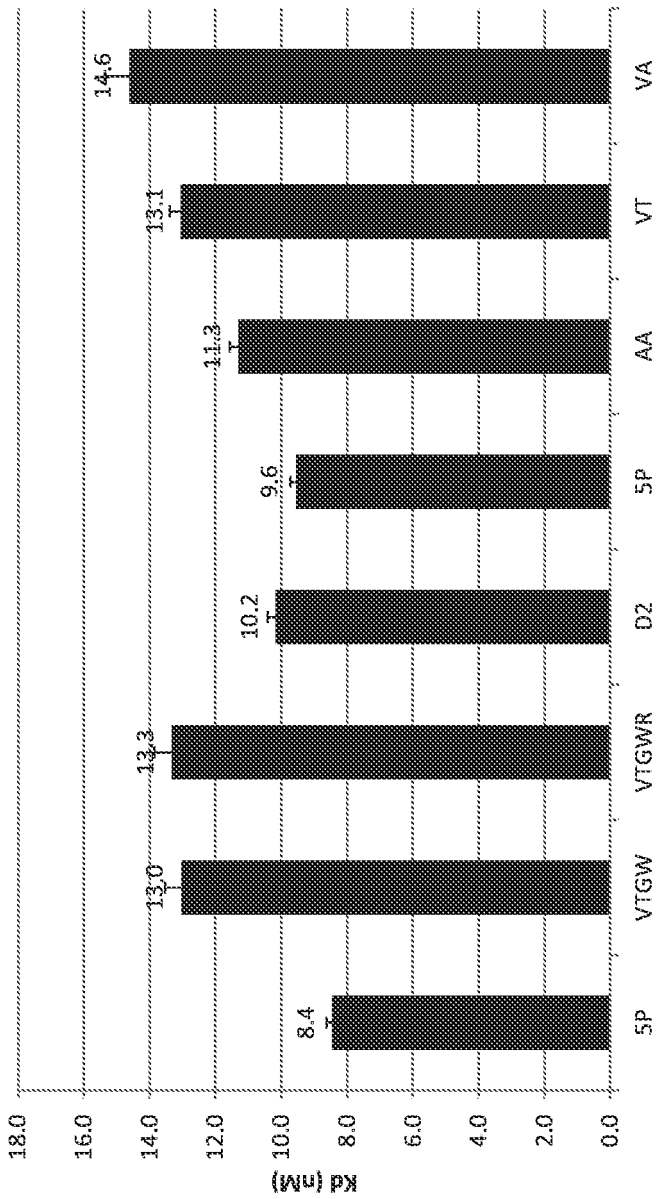
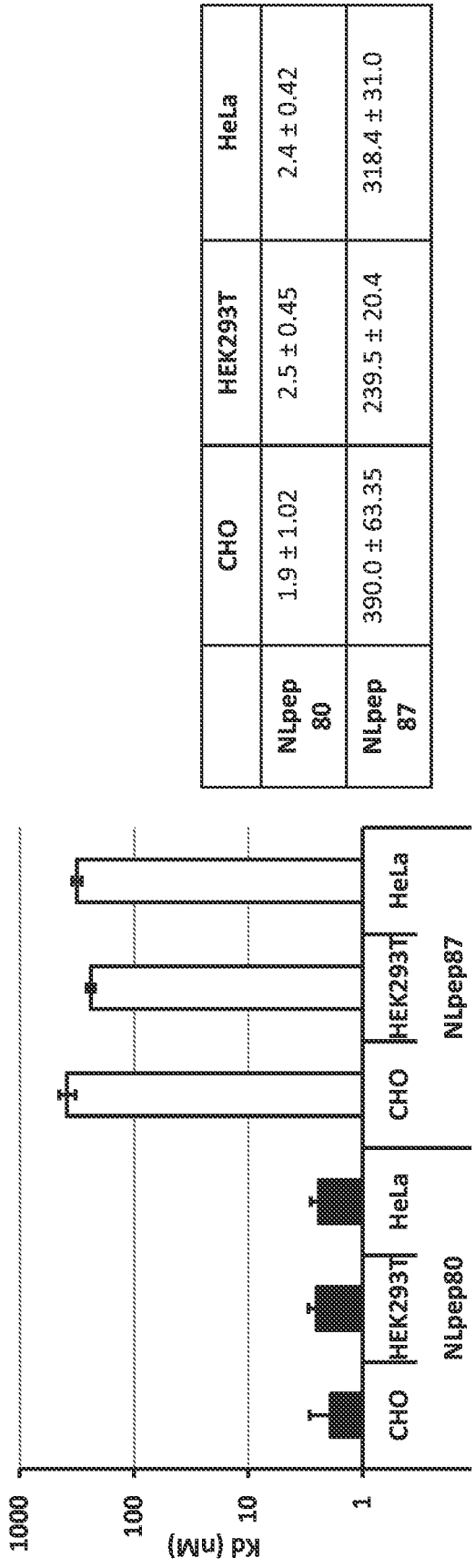
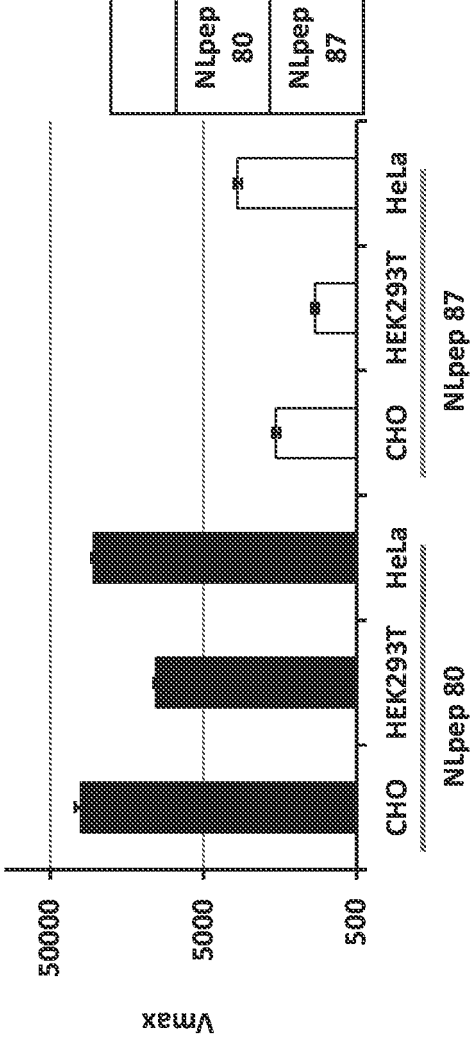

NLpep78
Kd (nM)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
8.4
13.0
13.3
10.2
9.6
11.3
13.1
14.6
5P
VTGW
VTGWR
D2
5P
AA
VT
VA

Figure 65



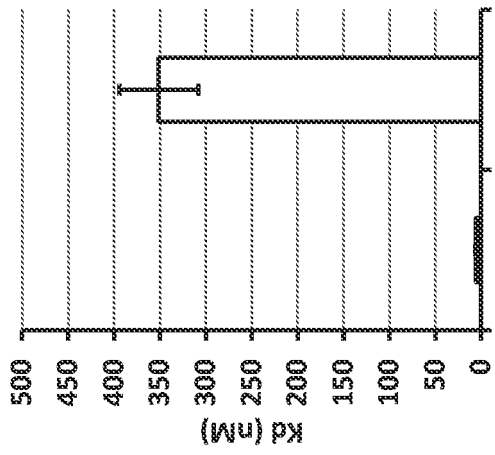
	CHO	HEK293T	HeLa
NLpep 80	1.9 ± 1.02	2.5 ± 0.45	2.4 ± 0.42
NLpep 87	390.0 ± 63.35	239.5 ± 20.4	318.4 ± 31.0



	CHO	HEK293T	HeLa
NLpep 80	31632 ± 2830	10245 ± 322	26027 ± 783
NLpep 87	1677 ± 83	935 ± 43	2993 ± 171

Figure 66

	E.coli
NLpep 80	5.4 ± 1.18
NLpep 87	351.5 ± 43.05



	E.coli
NLpep 80	4.3E+07 ± 2.1E+06
NLpep 87	7.0E+06 ± 5.4E+05

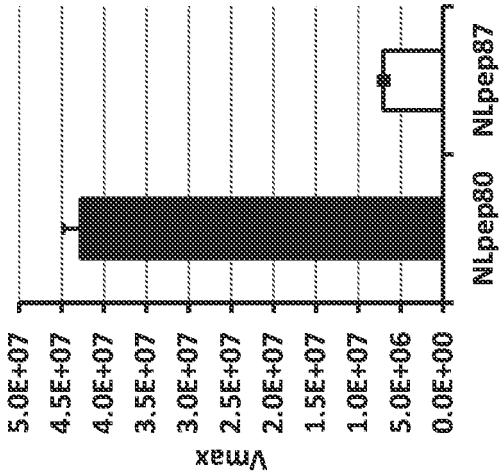
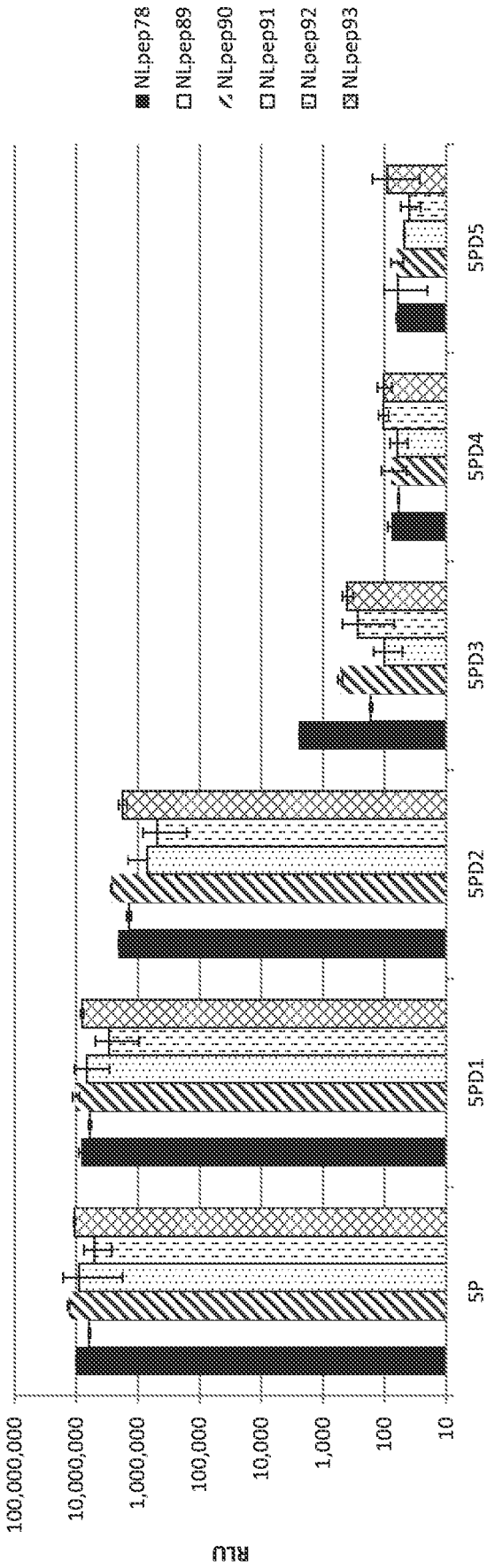


Figure 67



5PD1 has 1 amino acid removed from C-terminus, 5PD2 has 2 amino acids removed from C-terminus, ..., 5PD5 has 5 amino acids removed from the C-terminus.

Figure 68

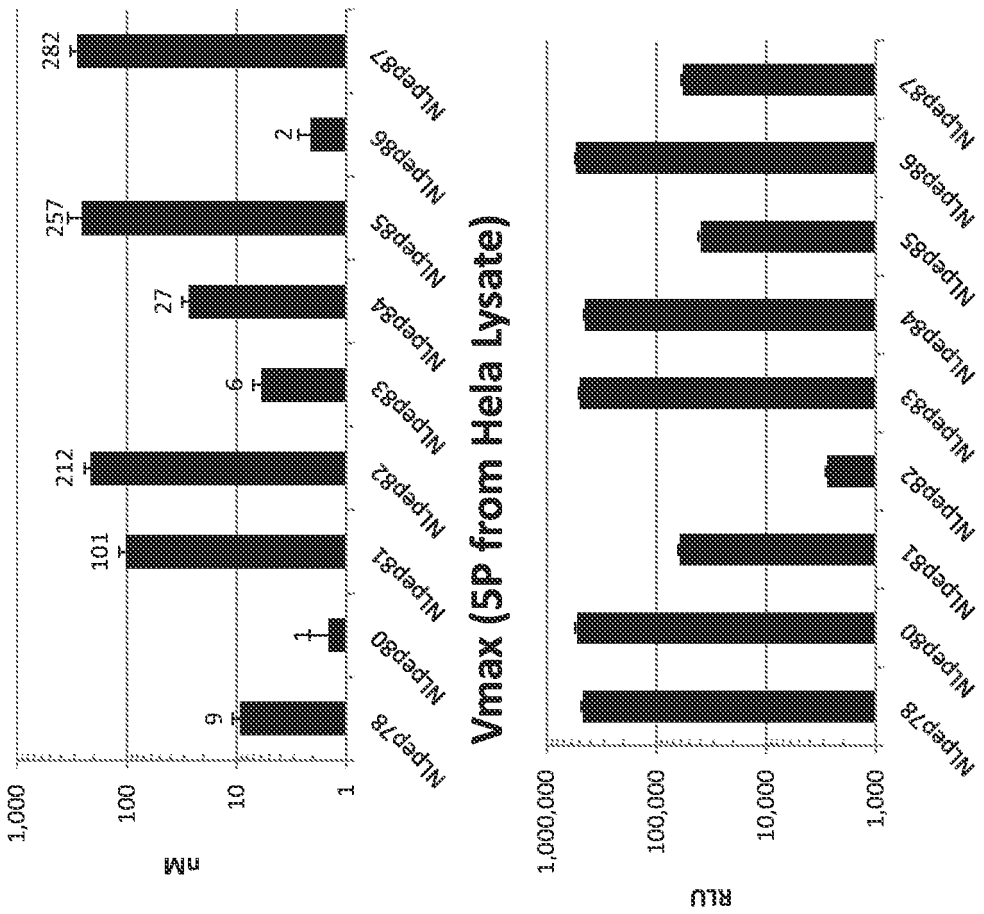

Kd (SP from Hela Lysate)
nM
1,000
100
10
1
9
1
101
212
6
27
257
2
282
NLpep78
NLpep80
NLpep81
NLpep82
NLpep83
NLpep84
NLpep85
NLpep86
NLpep87
Vmax (SP from Hela Lysate)
RLU
1,000,000
100,000
10,000
1,000
NLpep78
NLpep80
NLpep81
NLpep82
NLpep83
NLpep84
NLpep85
NLpep86
NLpep87

Figure 69

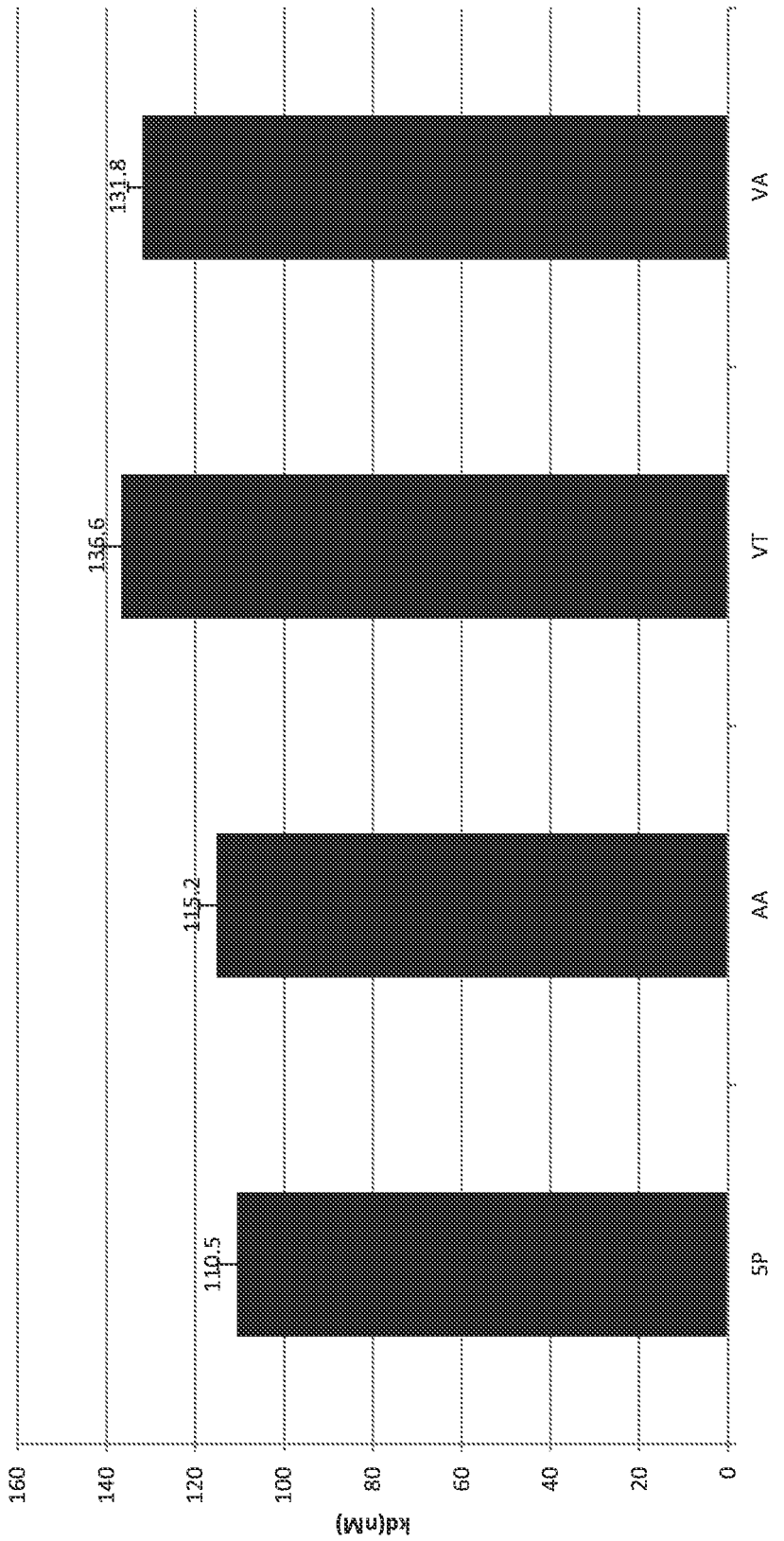

NLpep81
kd(nM)
160
140
120
100
80
60
40
20
0
131.8
136.6
115.2
110.5
VA
VT
AA
SP

Figure 70

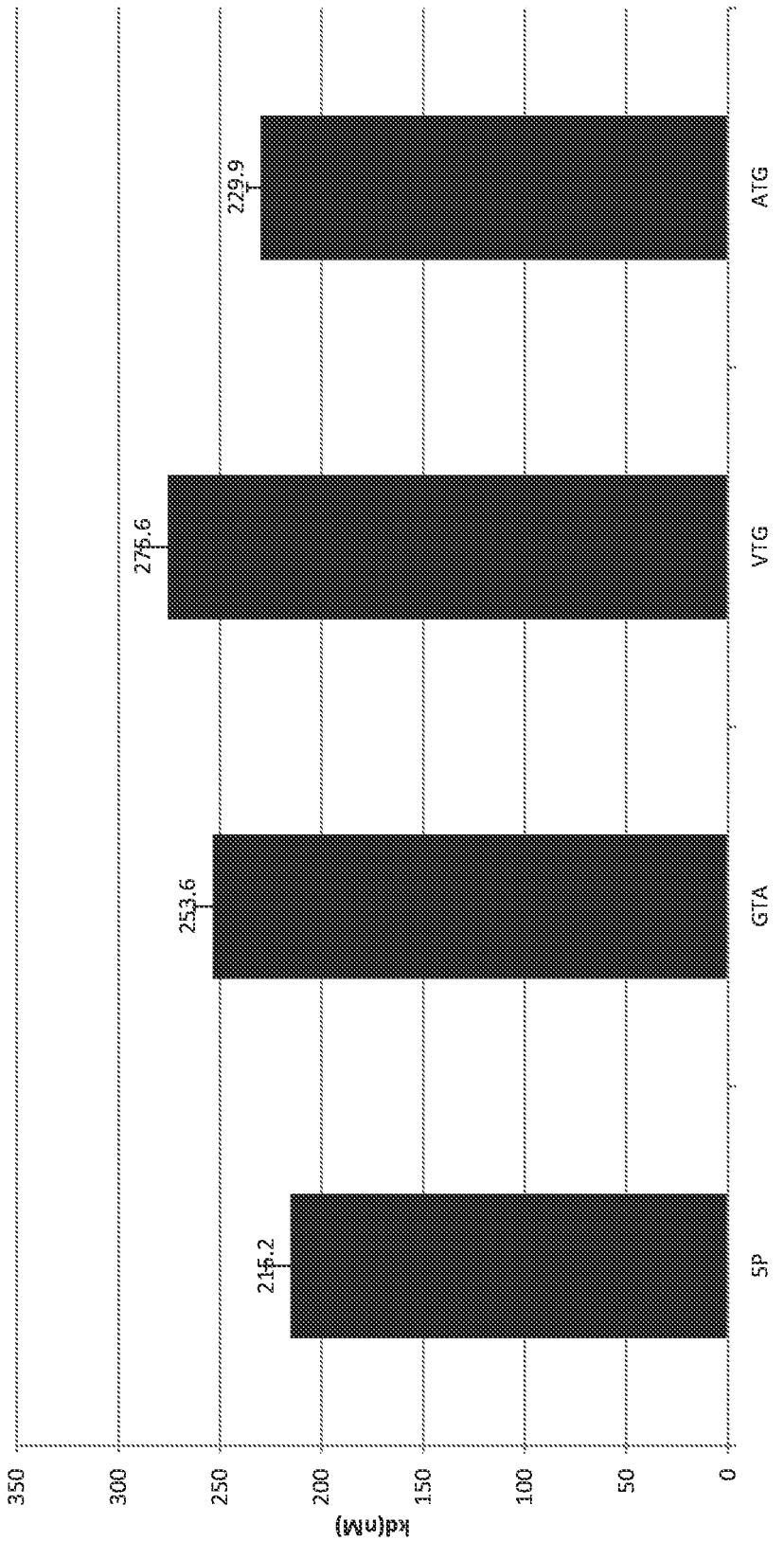
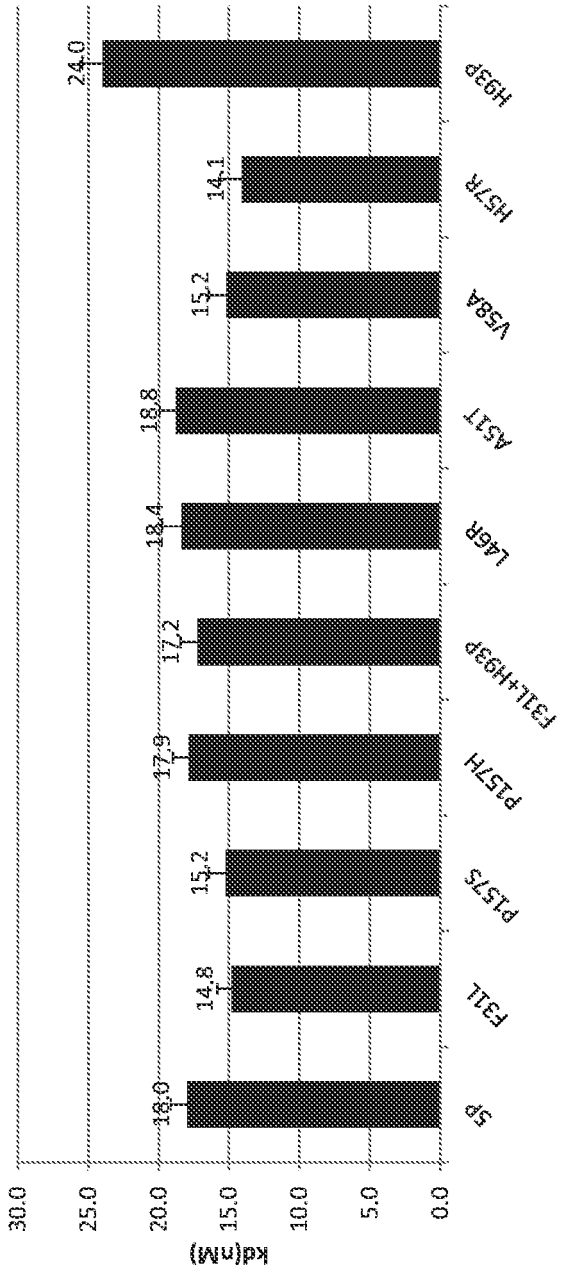

NLpep82
kd(nM)
350
300
250
200
150
100
50
0
215.2
253.6
275.6
229.9
SP
GTA
VTG
ATG

Figure 71



*Indicated mutations are mutations onto 5P

Figure 72

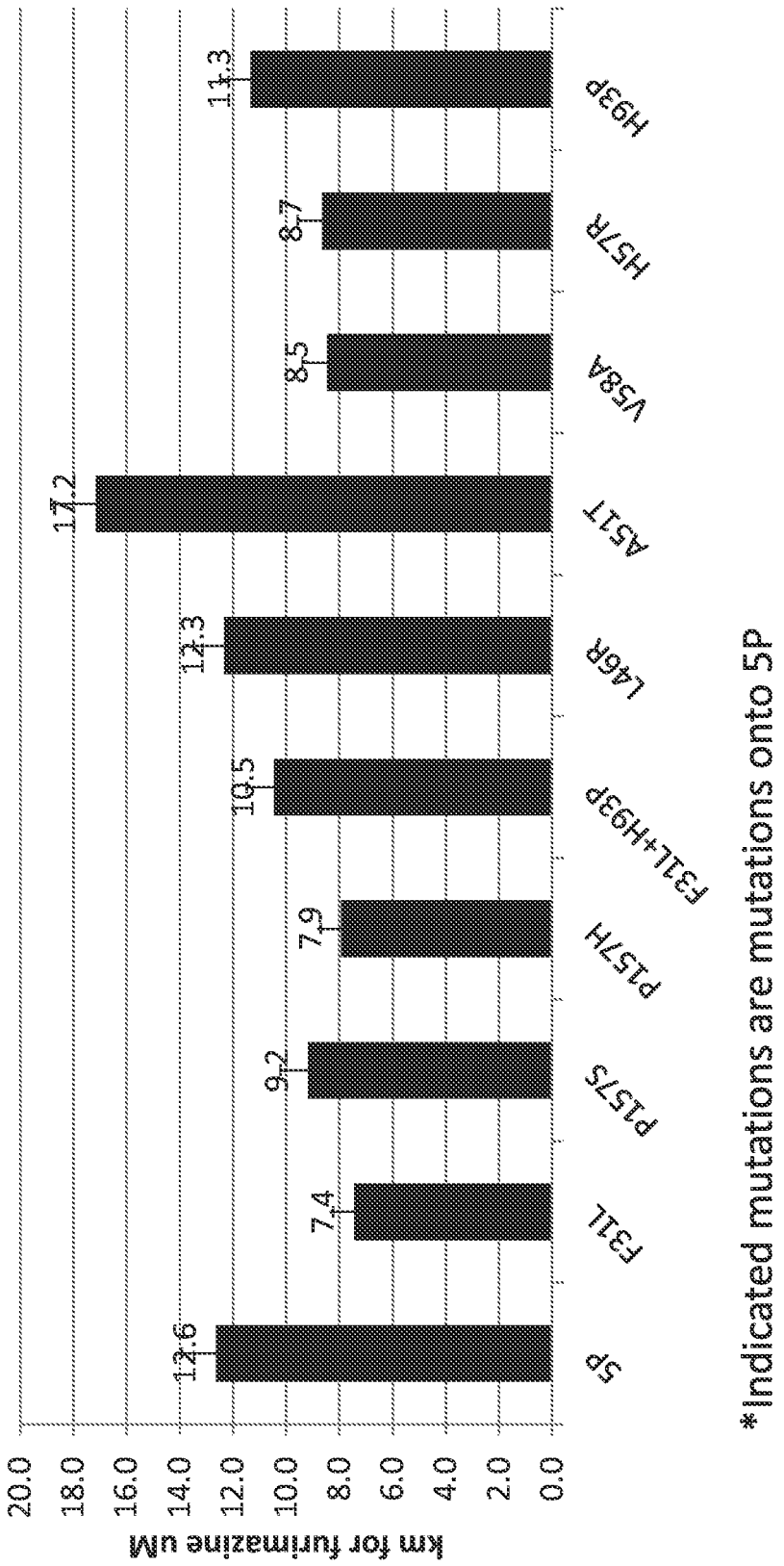

kM calculation (with NLpep78)
km for furimazine uM
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
12.6
7.4
9.2
7.9
10.5
14.3
17.2
8.5
8.7
11.3
5P
F31L
P157S
P157H
F31L+H93P
L46R
A51T
V58A
H57R
H93P
*Indicated mutations are mutations onto 5P

Figure 73

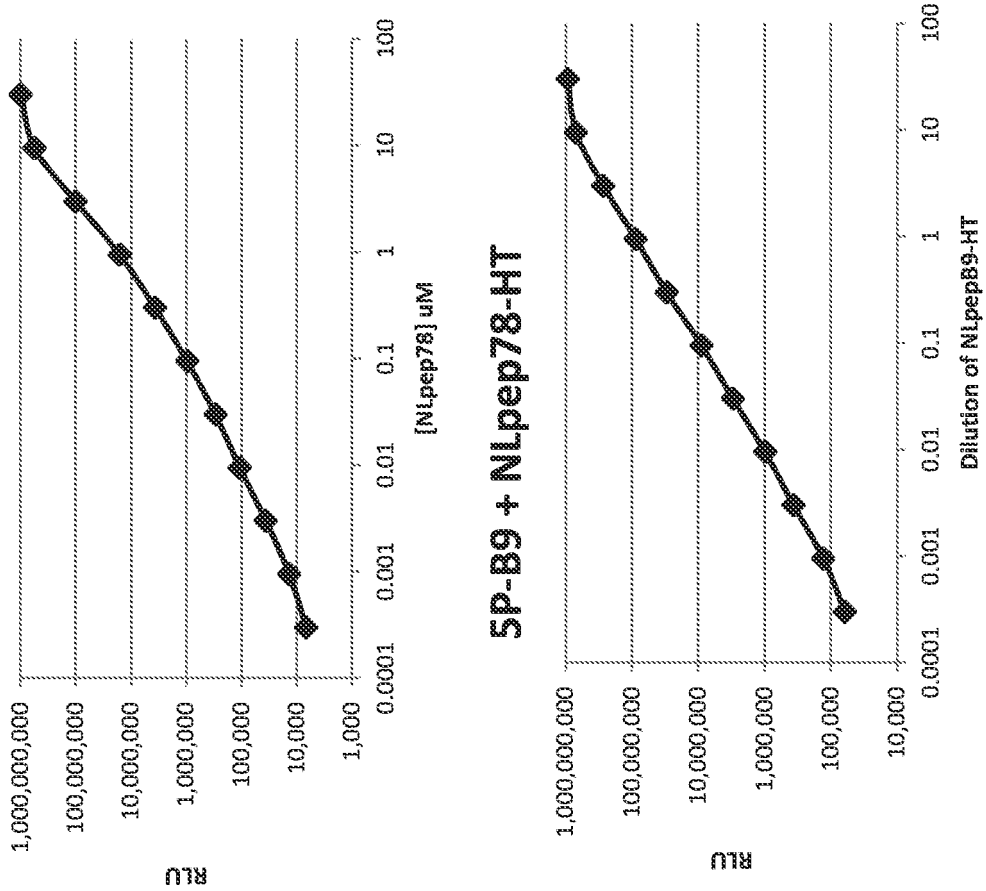
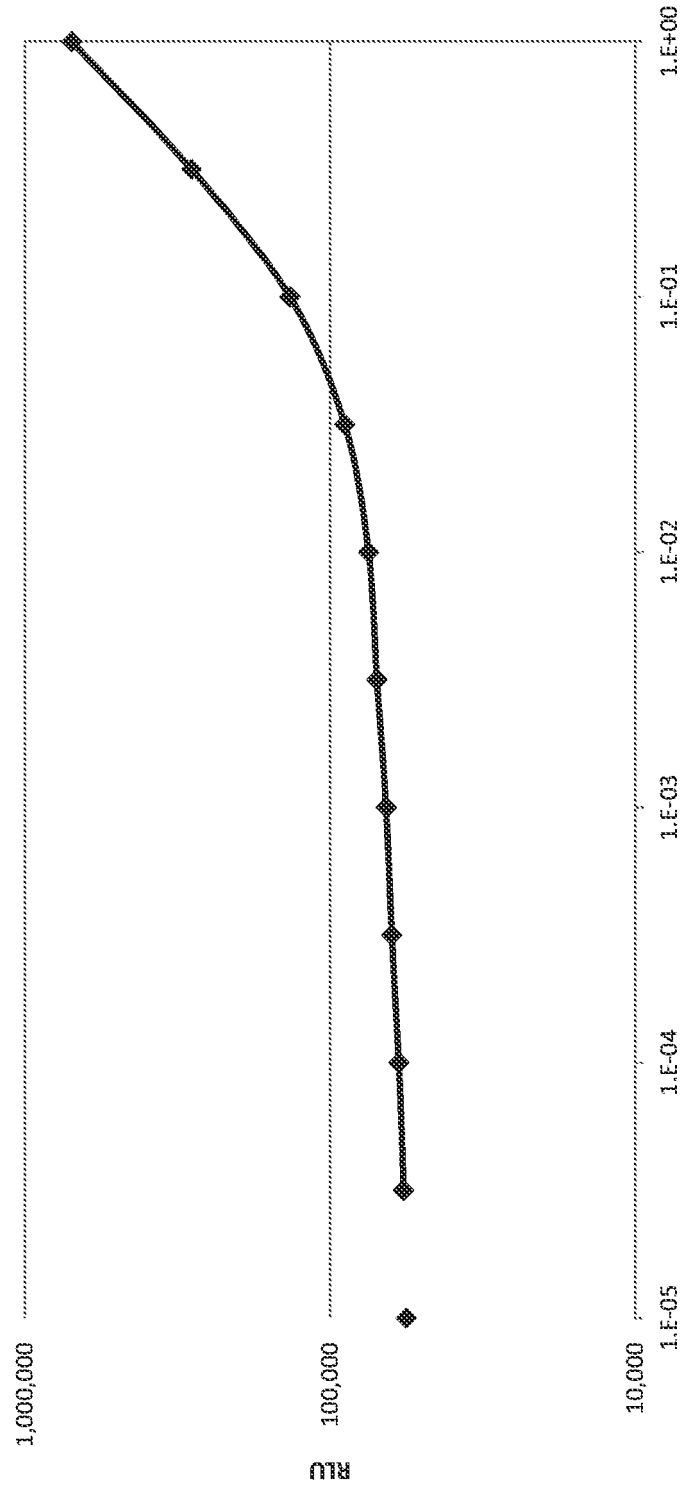

SP-B9 +NLpepB9-HT
RLU
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
0.0001
0.001
0.01
0.1
1
10
100
[NLpep78] uM
SP-B9 + NLpep78-HT
RLU
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
0.0001
0.001
0.01
0.1
1
10
100
Dilution of NLpepB9-HT

Figure 74


SP Titration with NLpep88-HT
RLU
1,000,000
100,000
10,000
1.E-05
1.E-04
1.E-03
1.E-02
1.E-01
1.E+00
Dilution of NLpep88

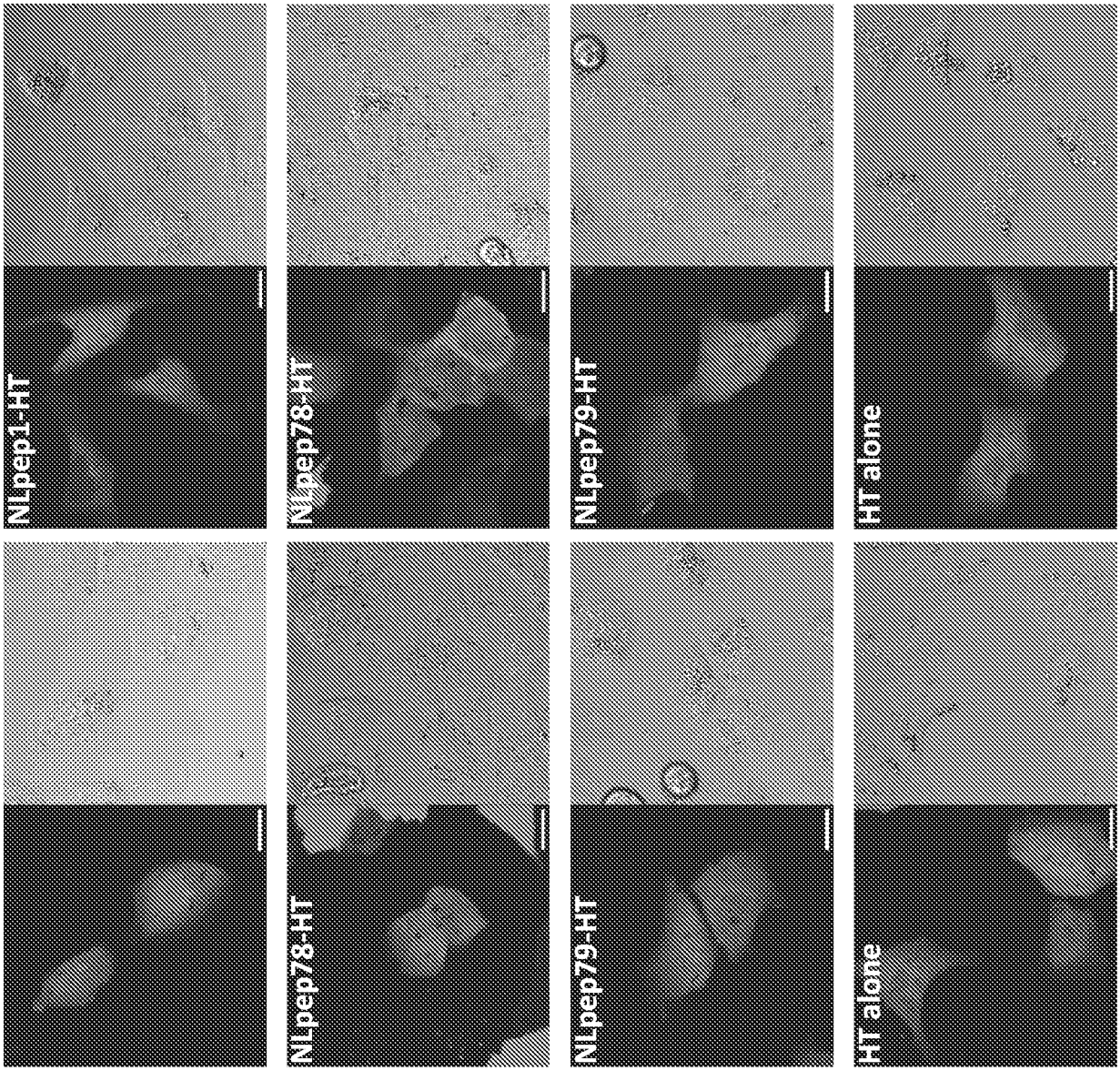
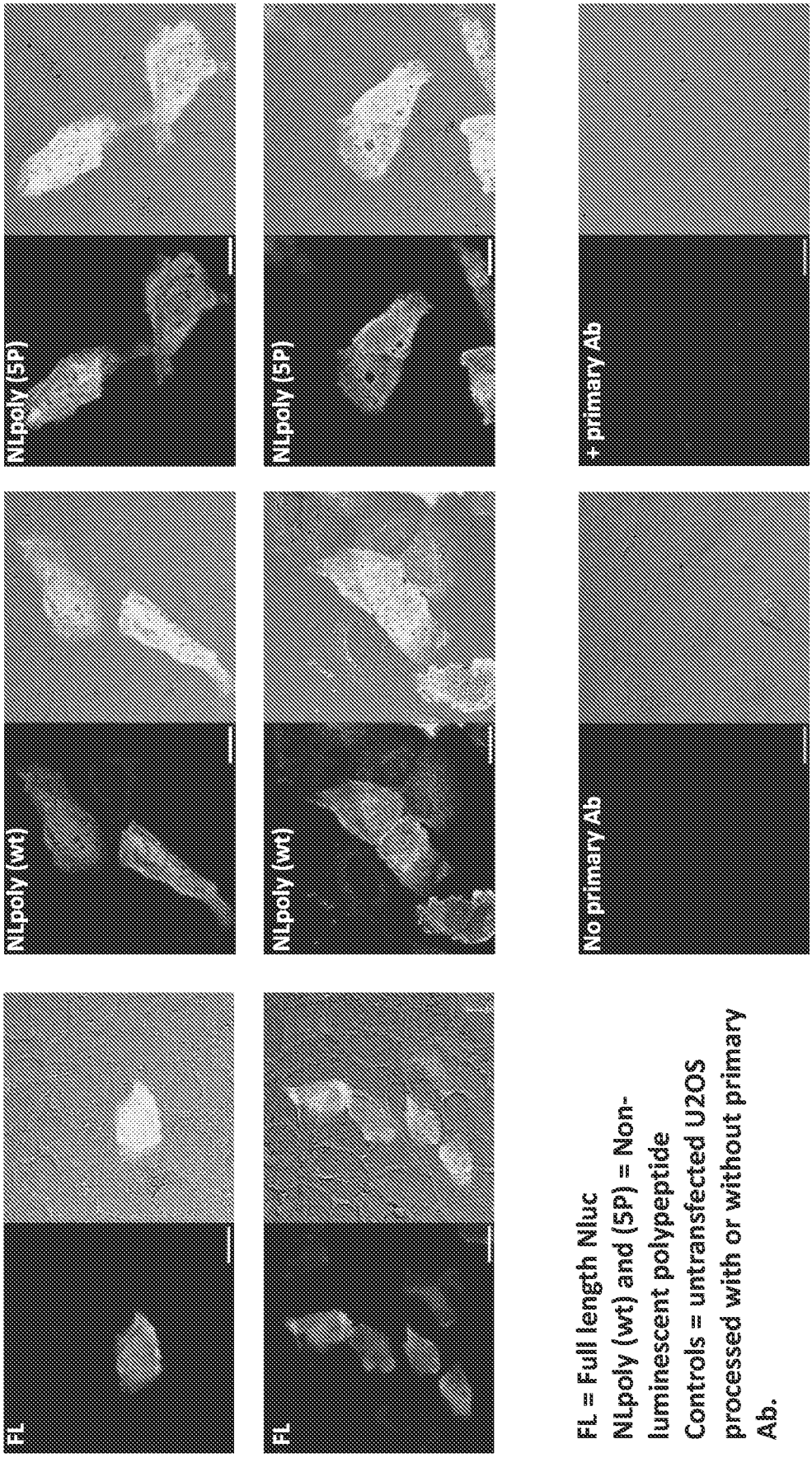

NLpep1-HT
NLpep78-HT
NLpep79-HT
HT alone
NLpep78-HT
NLpep79-HT
HT alone

Figure 75

Figure 76



FL = Full length Nluc
NLpoly (wt) and (SP) = Non-luminescent polypeptide
Controls = untransfected U2OS processed with or without primary Ab.

Scale bars = 20μm

Figure 77

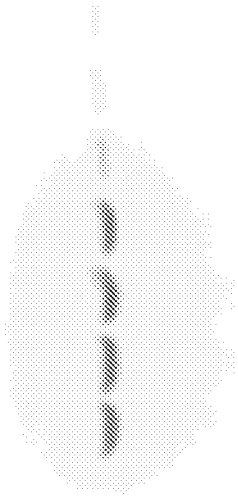


Figure 78

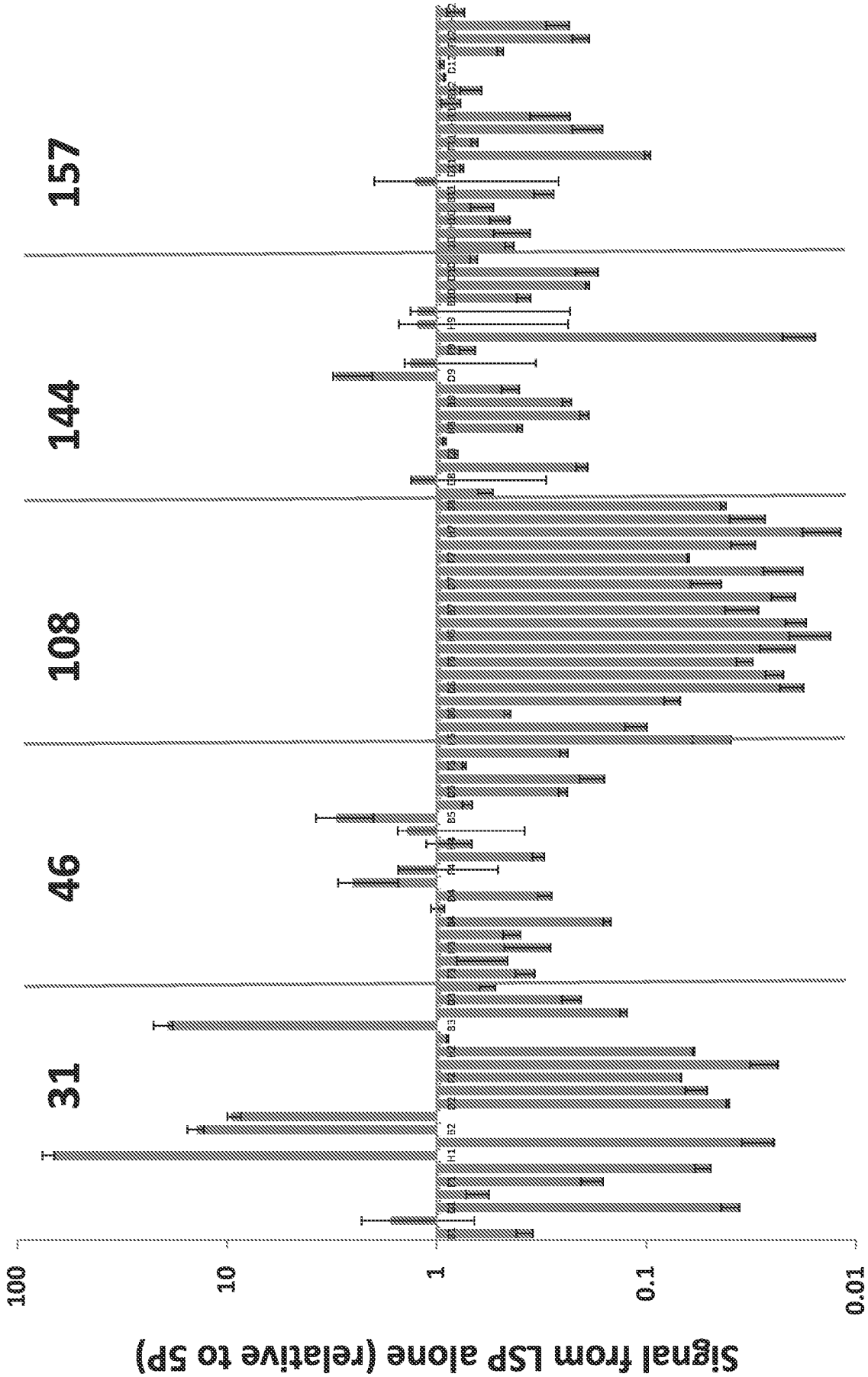

157
144
108
46
31
100
10
1
0.1
0.01
Signal from LSP alone (relative to SP)

Figure 79

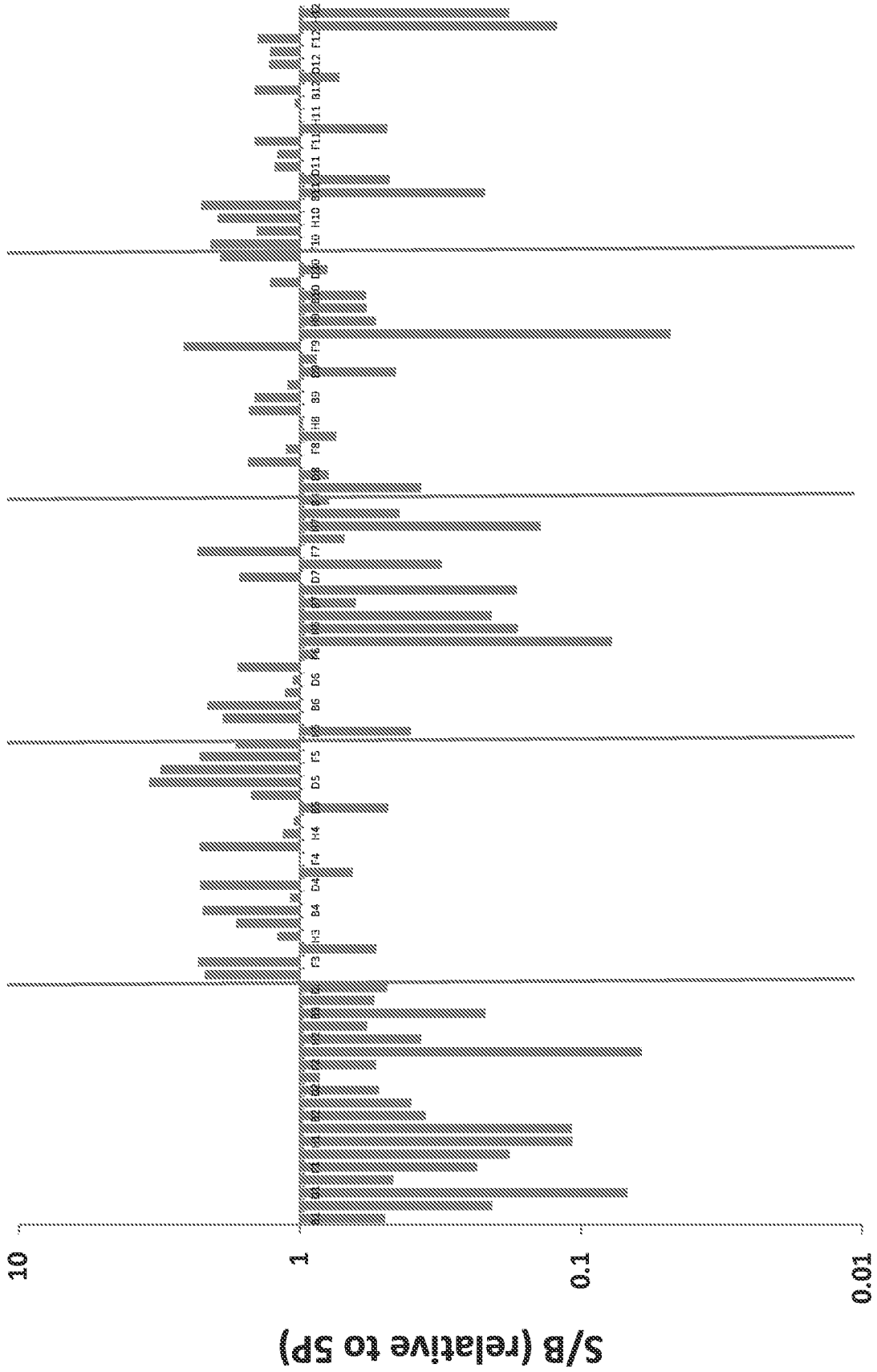
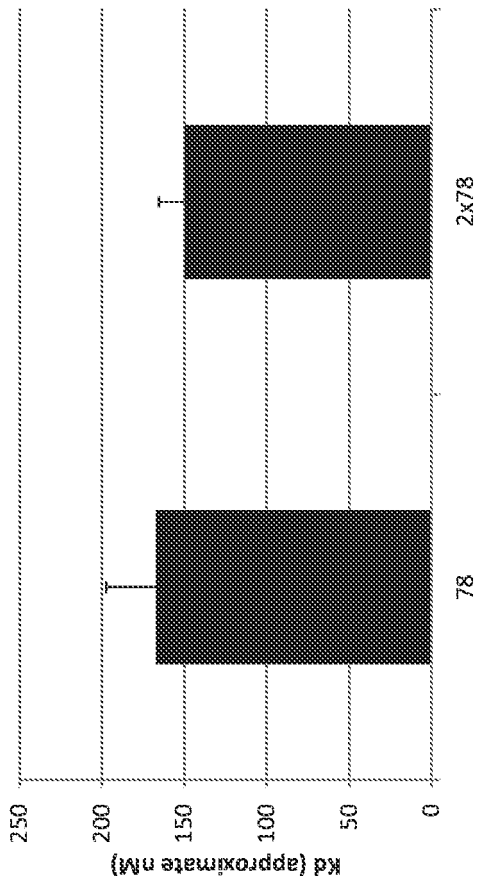
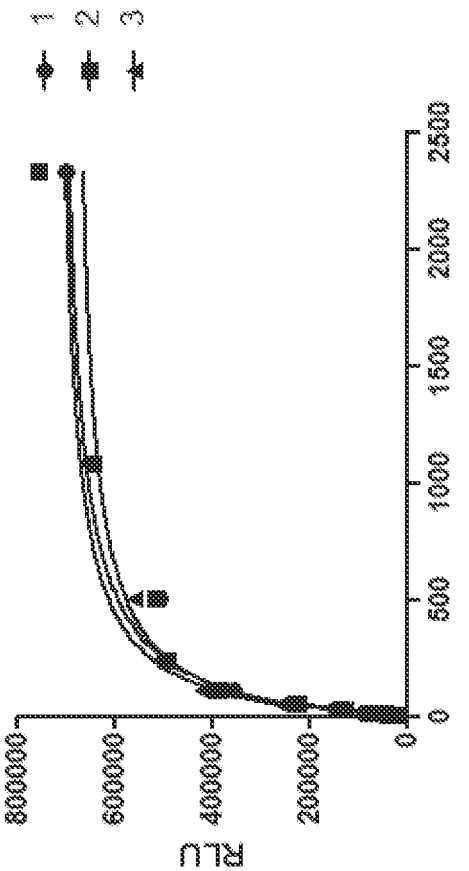


Figure 80



78 = MNVSGWRLFKKISN-HT
2x78 = M(NVSGWRLFKKIS)$_2$N-HT

Figure 81



	1	2	3
One site -- Specific binding			
Best-fit values			
Bmax	692830	725926	728434
Kd	99.14	109.7	93.28
Std. Error			
Bmax	23527	29554	23378
Kd	13.29	17.31	11.95
95% Confidence Intervals			
Bmax	640412 to 745248	660080 to 791773	676347 to 780521
Kd	69.53 to 128.8	71.15 to 148.3	68.65 to 119.9

Figure 82

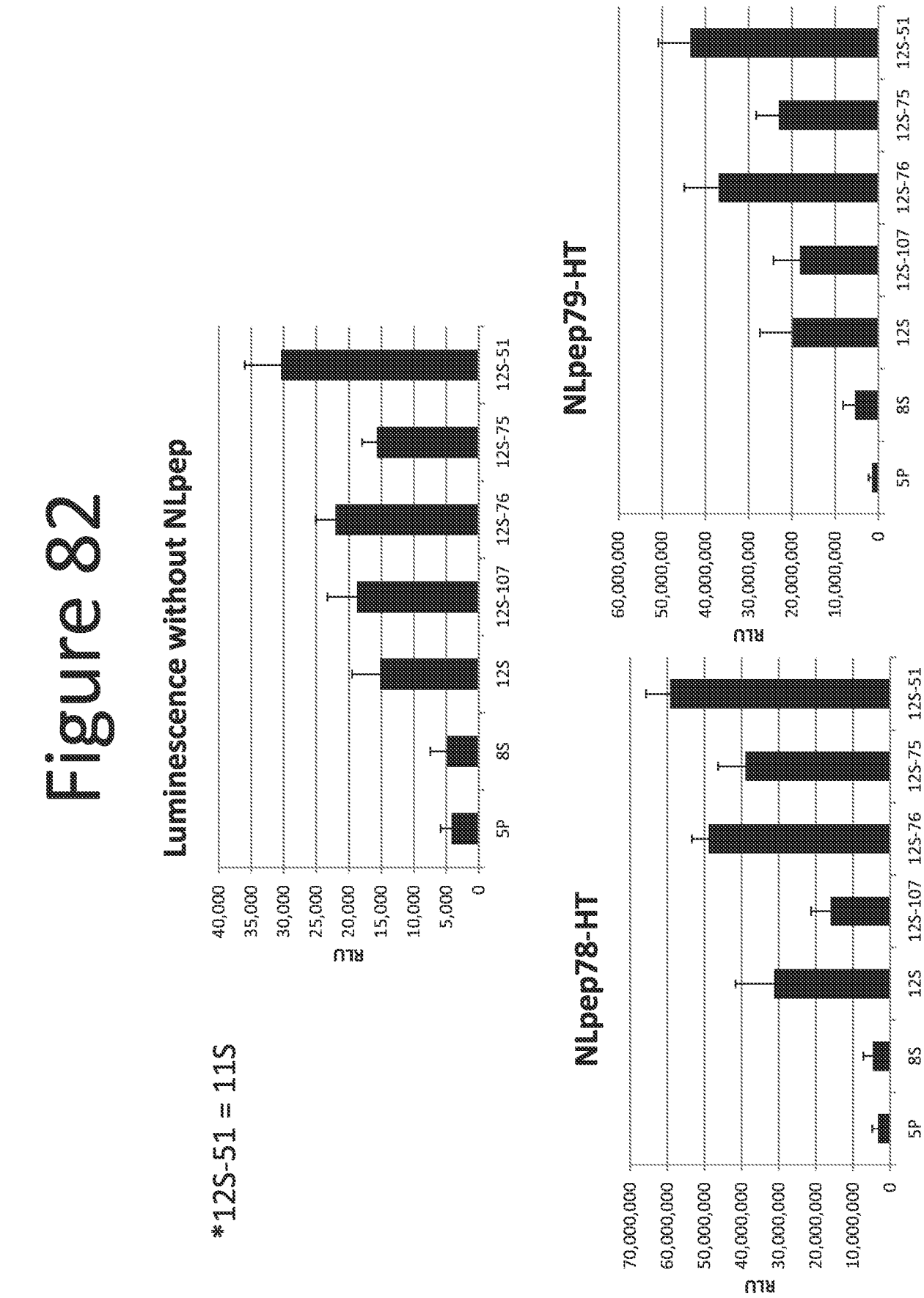

Luminescence without NLpep
RLU
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
5P
8S
12S
12S-107
12S-76
12S-75
12S-51
*12S-51 = 11S
NLpep79-HT
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
NLpep78-HT
70,000,000
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000

Figure 83

Peptide Array 1 Screens

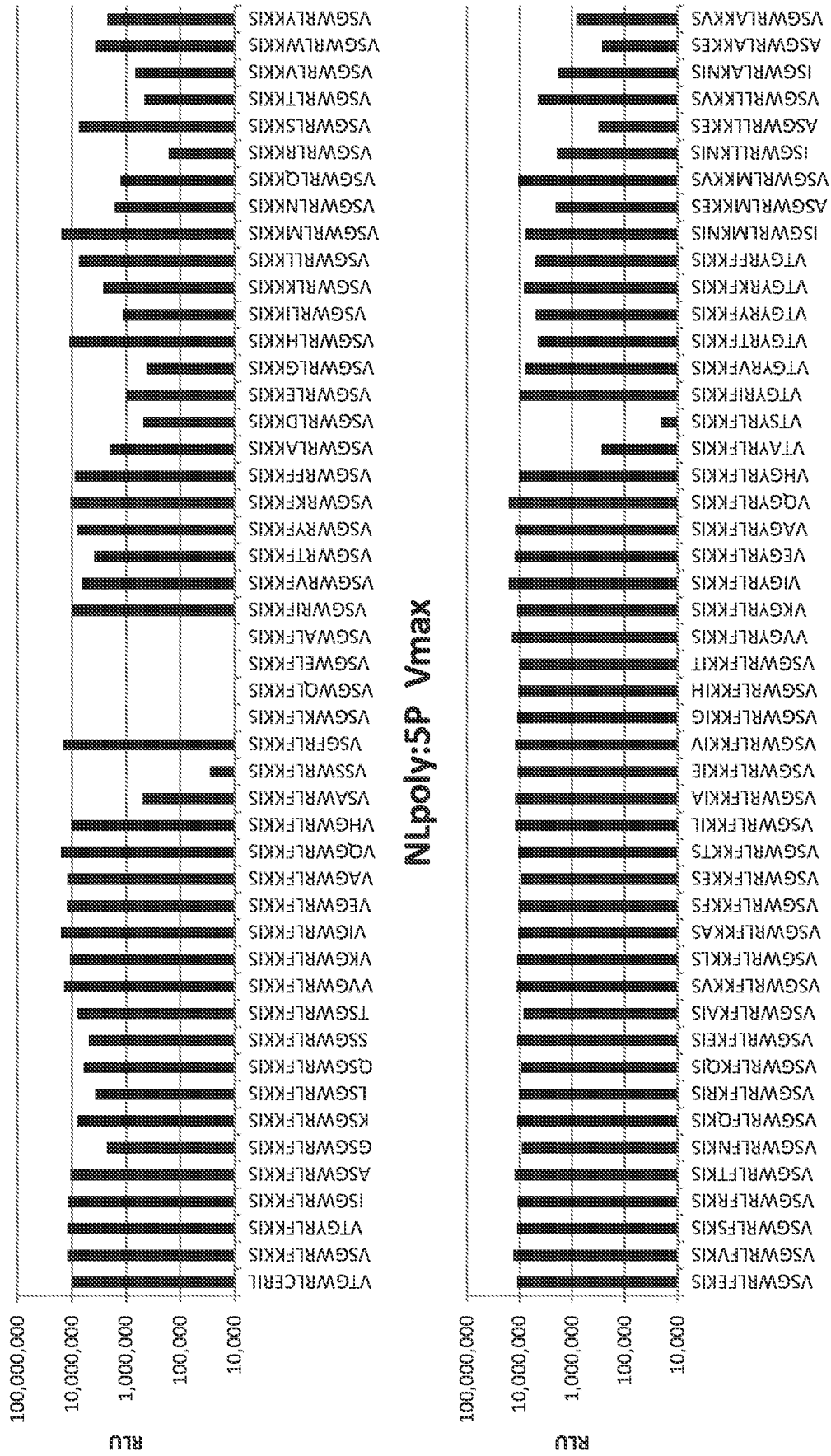


Figure 84

Peptide Array 1 Screens

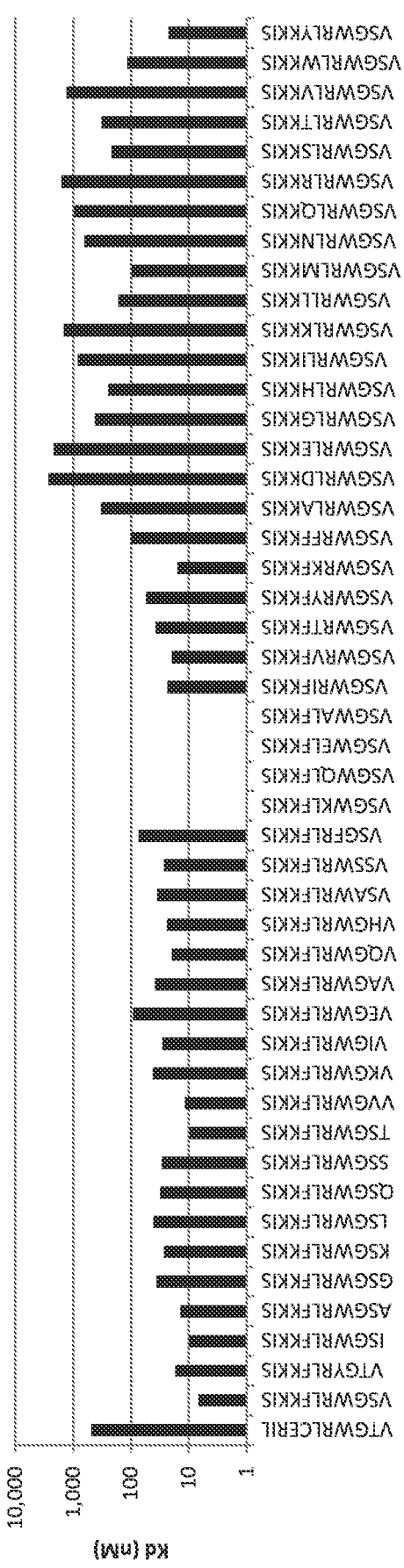


Figure 85

Peptide Array 1 Screens

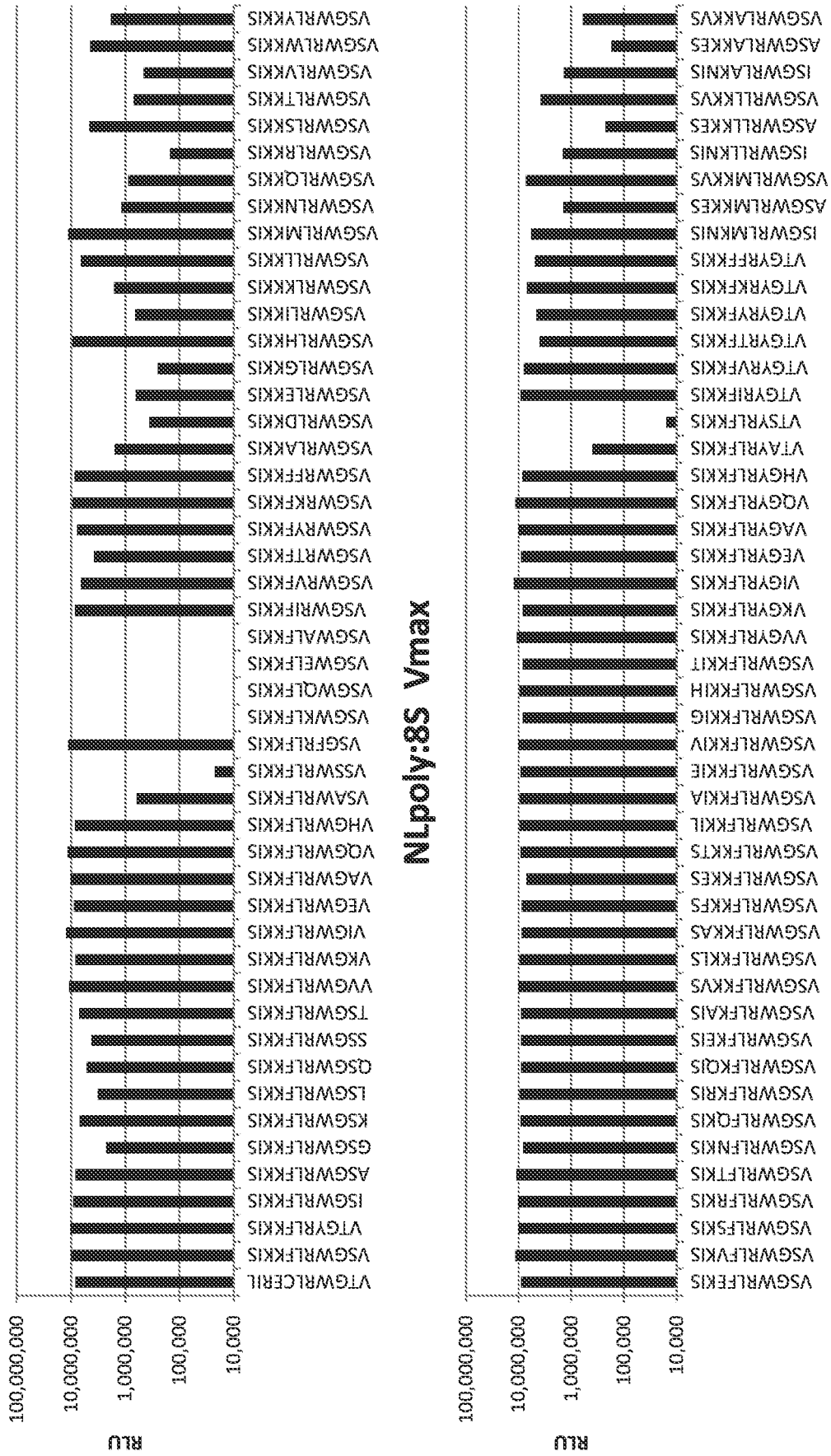
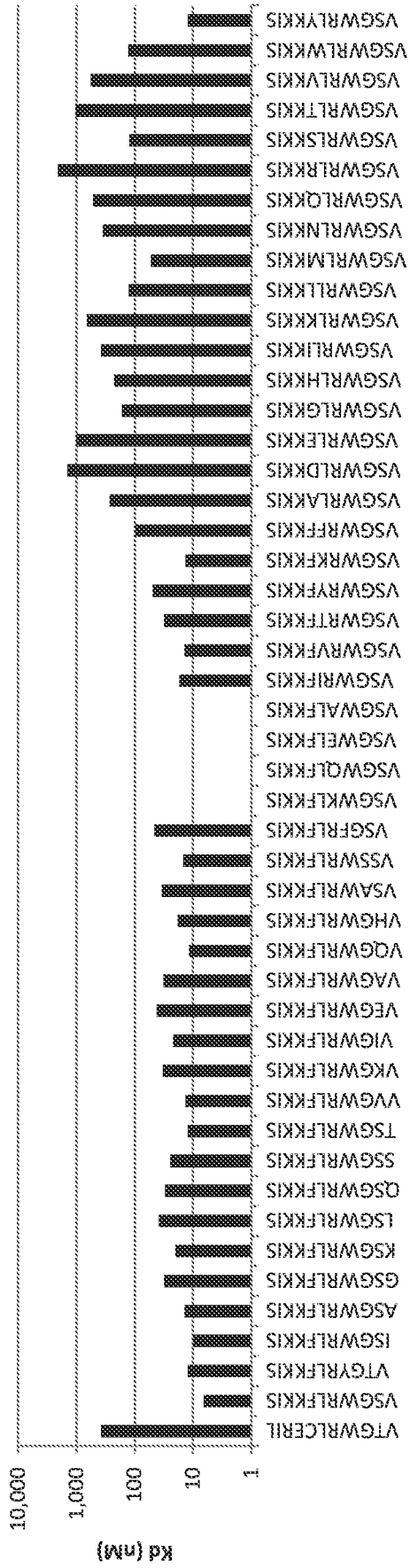


Figure 86

Peptide Array 1 Screens



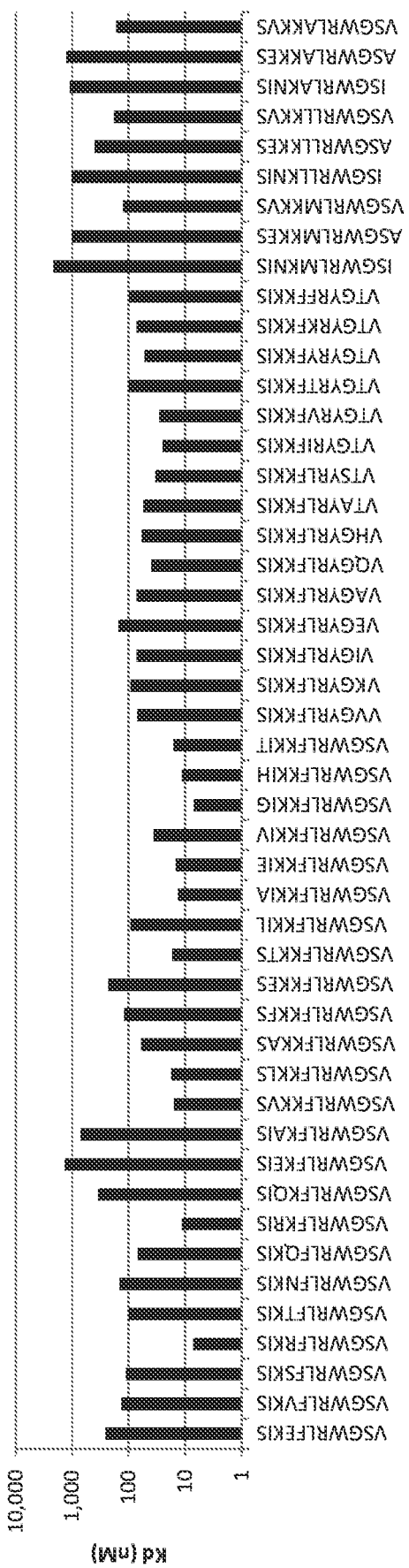


Figure 87

Peptide Array 1 Screens

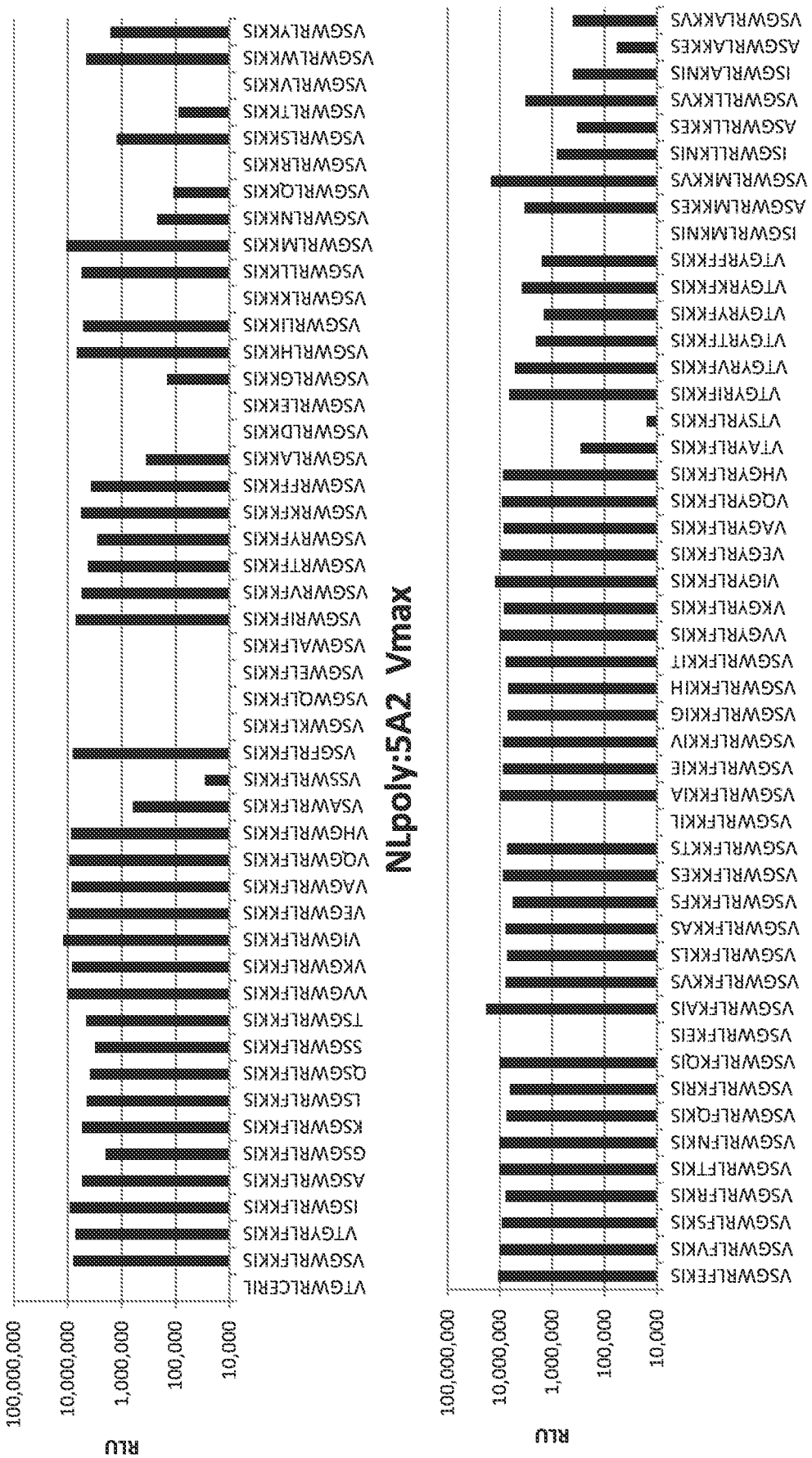
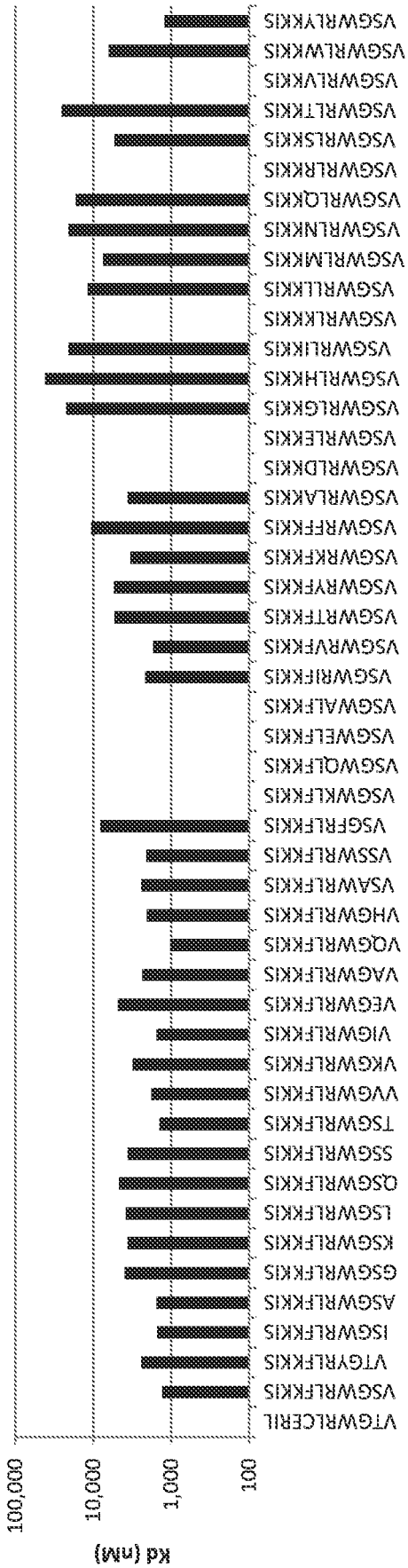


Figure 88

Peptide Array 1 Screens

NLpoly:5A2 Kd



NLpoly:5A2 Kd

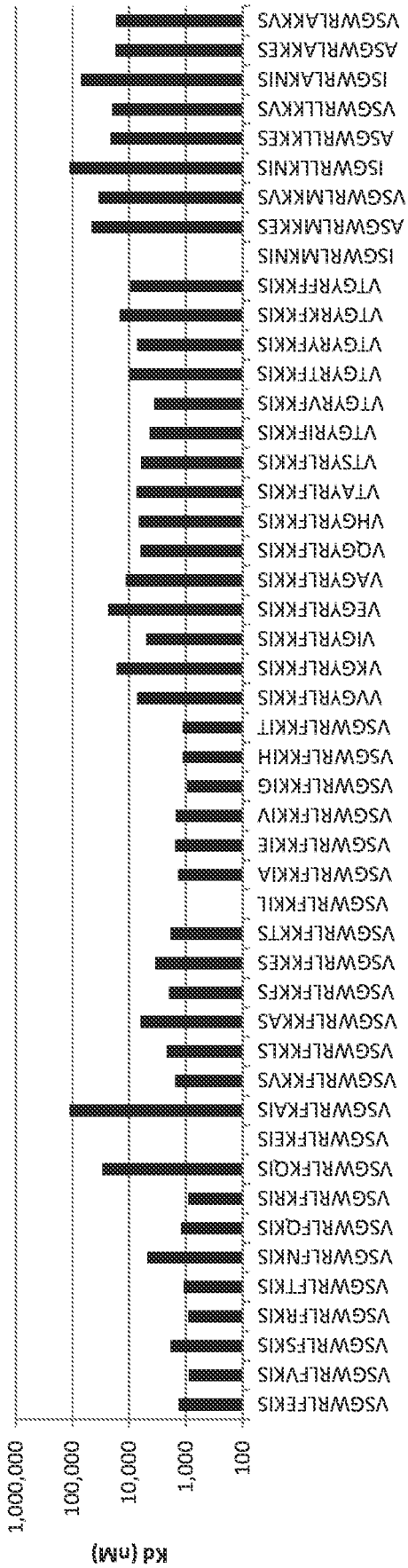


Figure 89

Peptide Array 1 Screens

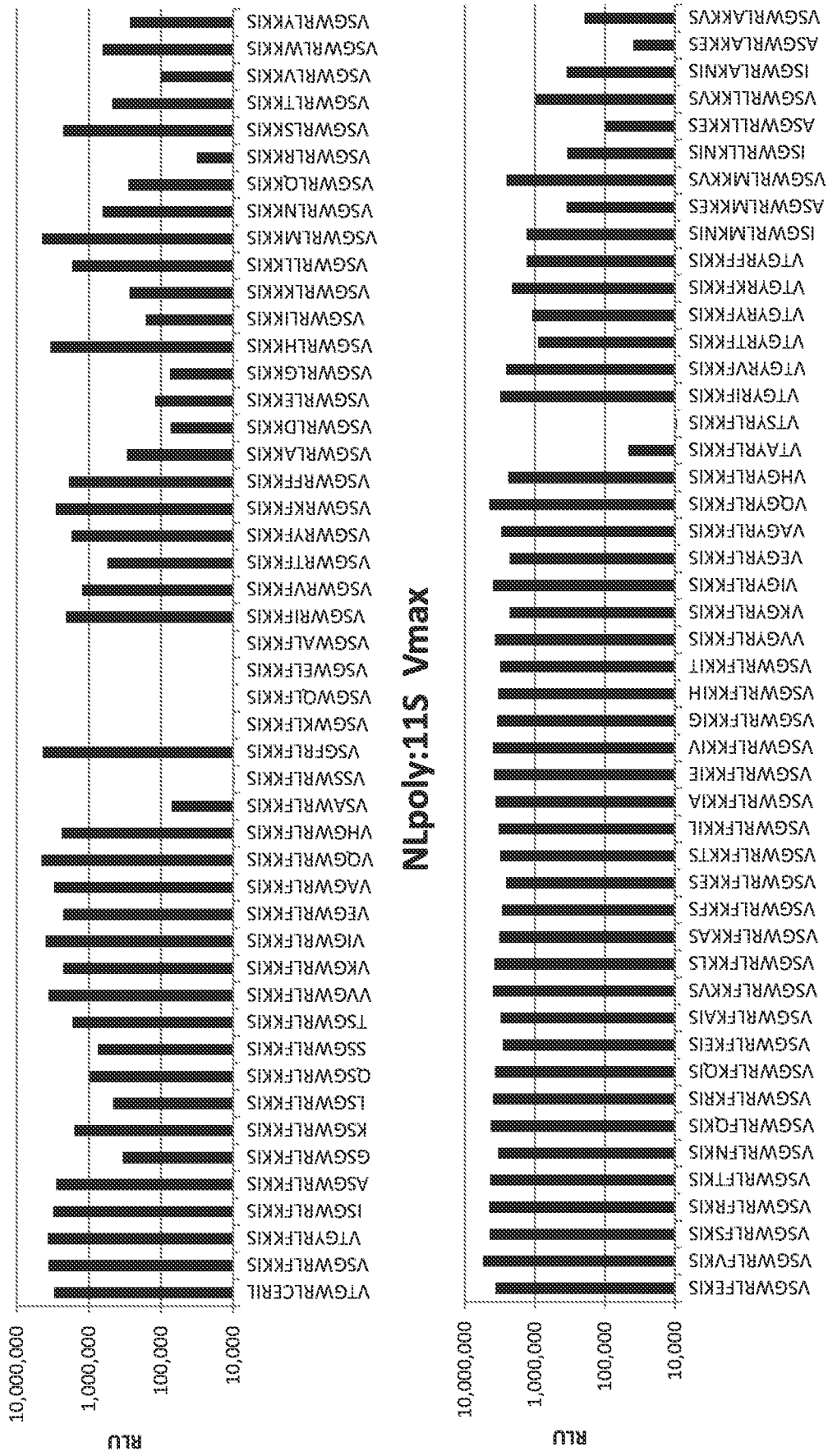
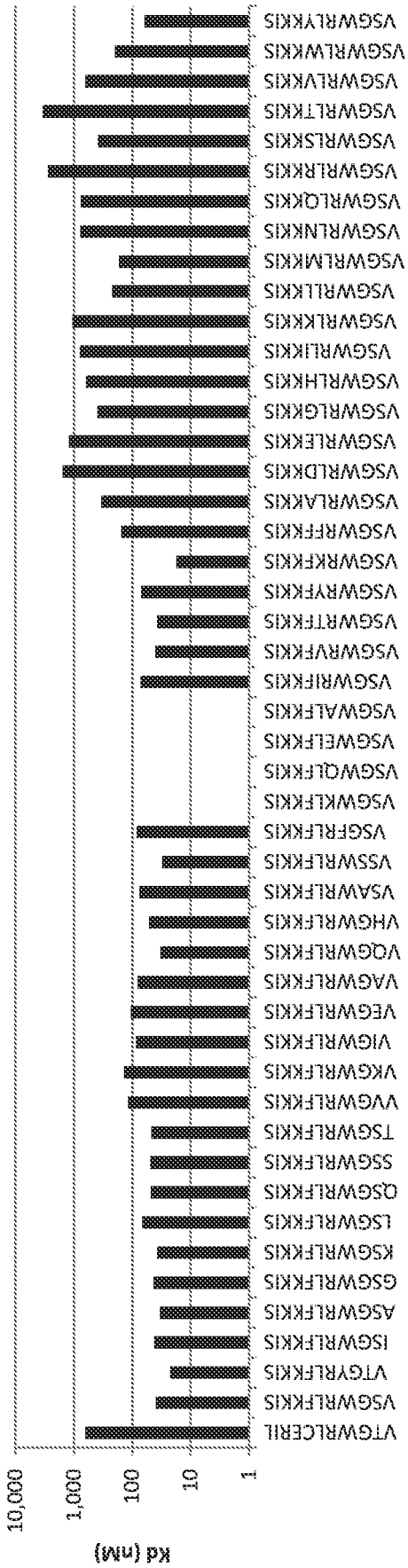


Figure 9O

Peptide Array 1 Screens

NLpoly:11S Kd



NLpoly:11S Kd

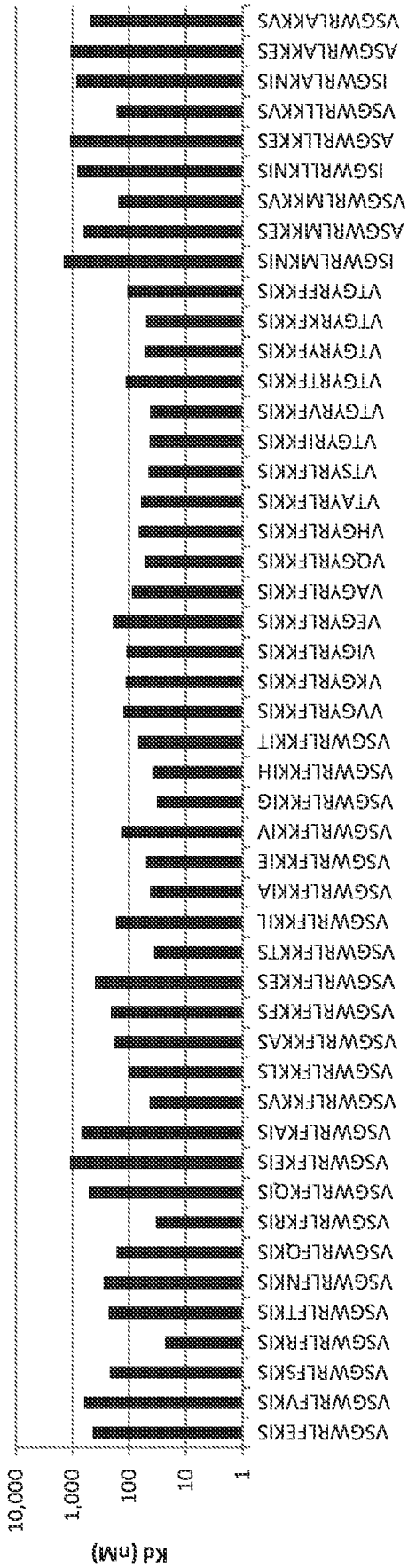


Figure 91

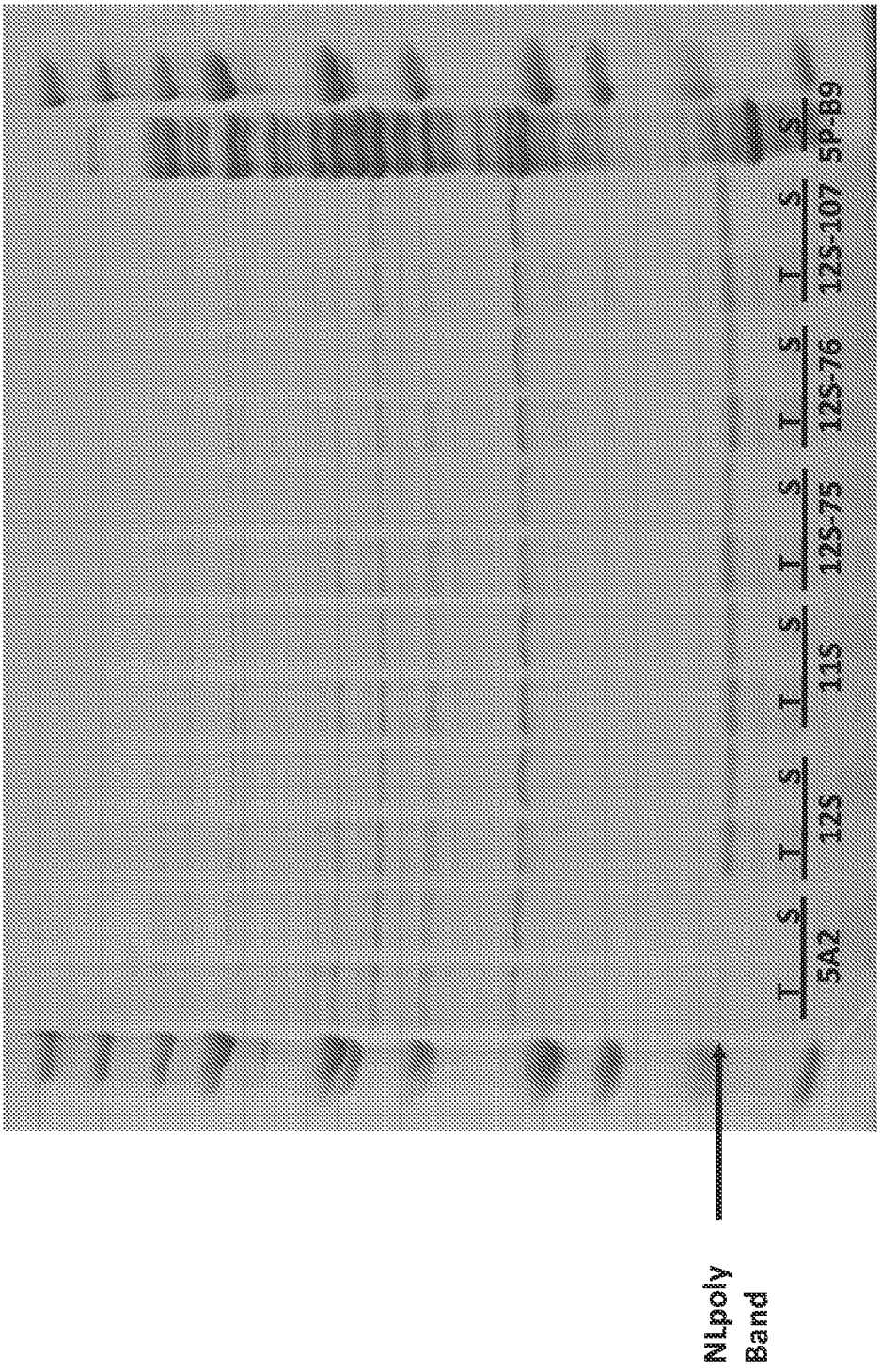
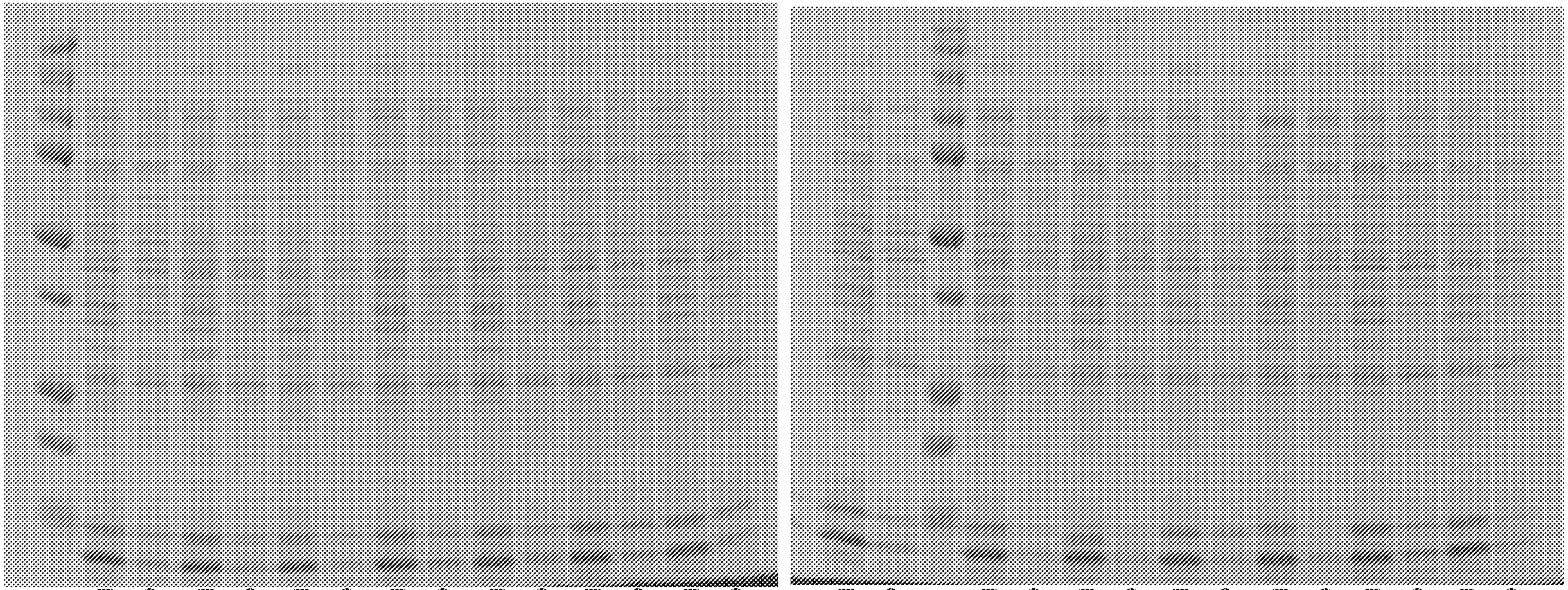

NLpoly Band
I S
5A2
I S
12S
I S
11S
I S
12S-75
I S
12S-76
I S
12S-107
S
SP-89

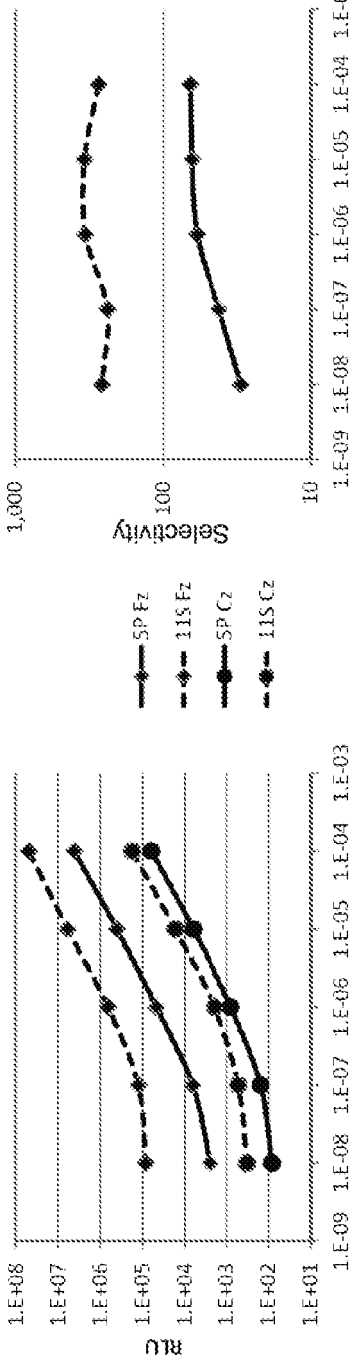
Figure 92



All mutations listed are mutations to 5P

	5P	F31I	F31L	F31M	F31V	L46K	L46R	L46D	N108I	T144L	T144P	P157S	P157Y
Kd	25.42	2.147	9.621	2.279	3.478	30	16.99	14666	140.5	25.11	4240	5.239	7.485
Std. Error													
Kd	3.683	0.5597	1.764	0.8401	0.7314	4.341	2.271	6251	50.81	4.725	938.2	0.9543	1.532
95% Confidence Intervals													
Kd	17.21 to 33.62	0.8565 to 3.438	5.691 to 13.55	0.2237 to 4.335	1.849 to 5.108	20.33 to 39.67	11.93 to 22.05	737.4 to 28594	27.26 to 253.7	14.42 to 35.80	2150 to 6331	3.081 to 7.398	3.953 to 11.02

Figure 93



Dilution	5P Fz	11S Fz	5P Cz	11S Cz	5P Fz/Cz	11S Fz/Cz
1.00E-04	4.11E+06	4.87E+07	6.17E+04	1.77E+05	6.66E+01	2.74E+02
1.00E-05	4.05E+05	5.87E+06	6.30E+03	1.71E+04	6.44E+01	3.43E+02
1.00E-06	4.75E+04	6.62E+05	7.98E+02	1.96E+03	5.95E+01	3.38E+02
1.00E-07	6.57E+03	1.26E+05	1.56E+02	5.26E+02	4.21E+01	2.40E+02
1.00E-08	2.50E+03	8.49E+04	8.40E+01	3.24E+02	2.98E+01	2.62E+02

Figure 94

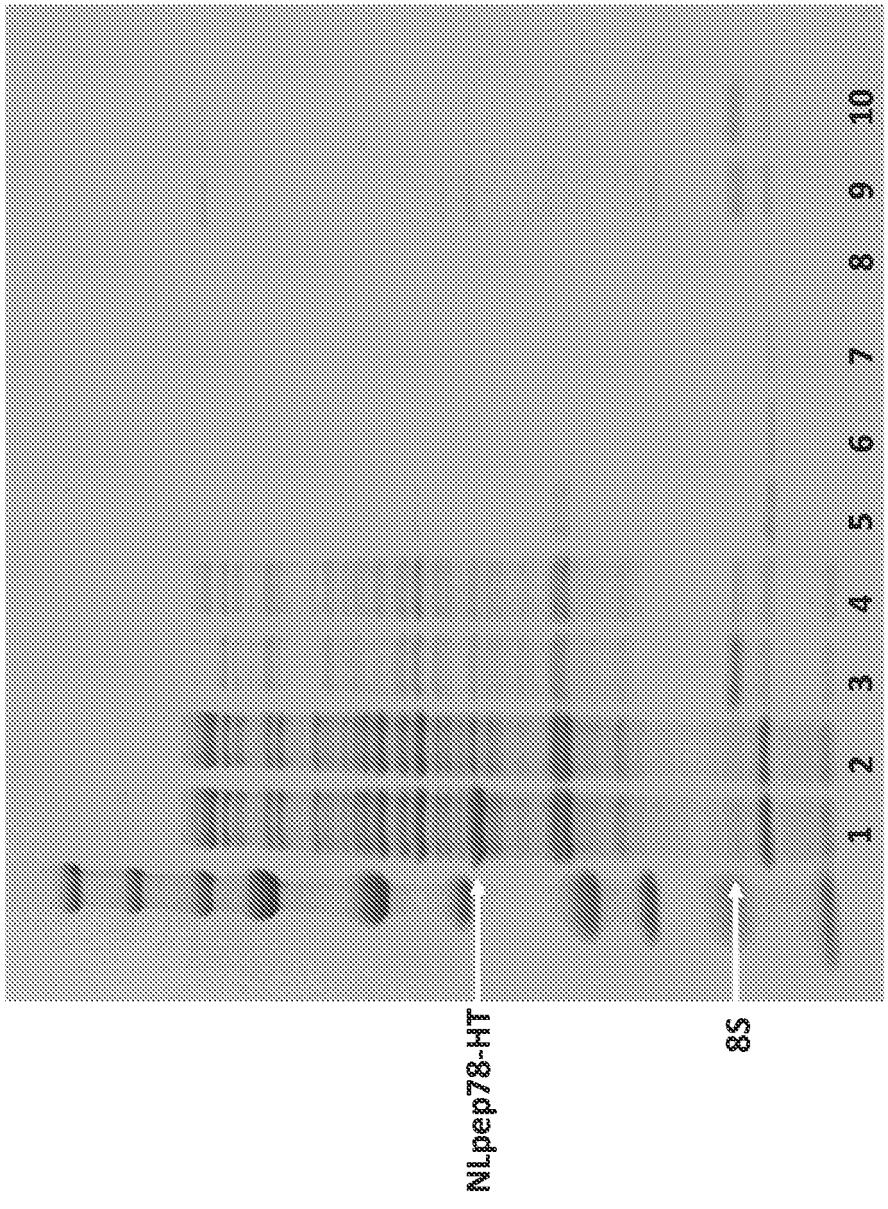
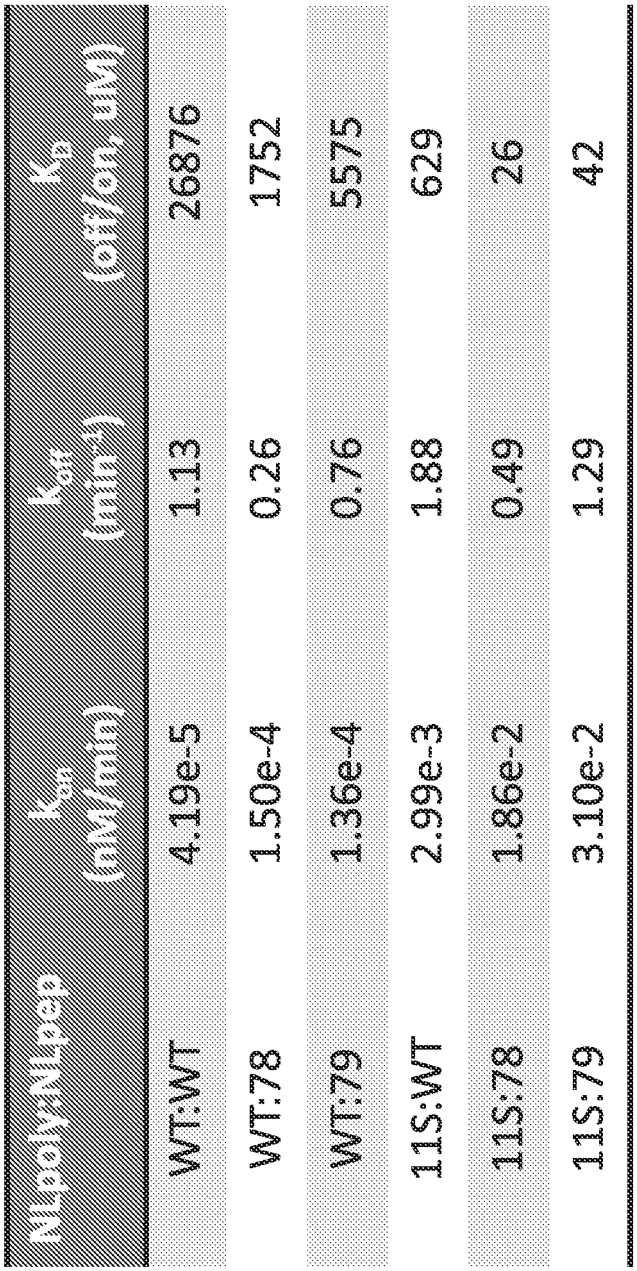

1. NLpep78-HT lysate
2. Flow through of NLpep78-HT lysate
3. 8S lysate
4. Flow through of 8S lysate
5. Wash 1
6. Wash 2
7. Wash 3
8. Wash 4
9. Elution 1
10. Elution 2
1 2 3 4 5 6 7 8 9 10
NLpep78-HT
8S

Figure 95



NLpolyA:NLpep	k_{on} (nM/min)	k_{off} (min^{-1})	K_D (off/on, uM)
WT:WT	4.19e-5	1.13	26876
WT:78	1.50e-4	0.26	1752
WT:79	1.36e-4	0.76	5575
11S:WT	2.99e-3	1.88	629
11S:78	1.86e-2	0.49	26
11S:79	3.10e-2	1.29	42

Figure 96

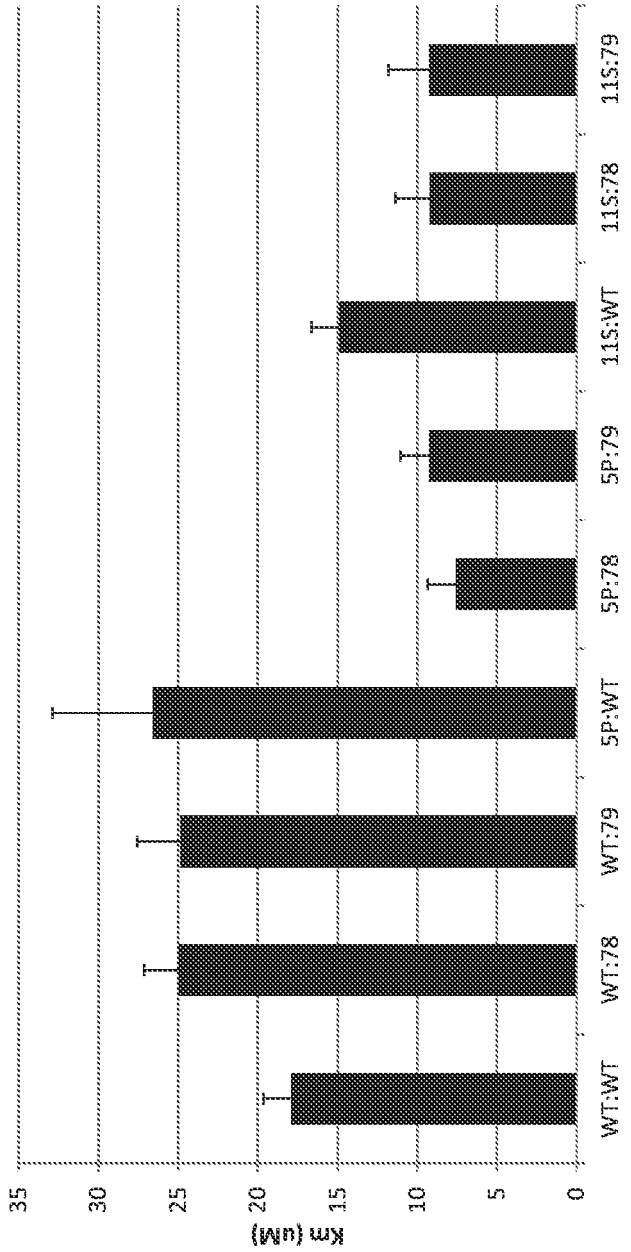

Km (Furimazine)
Km (uM)
35
30
25
20
15
10
5
0
WT:WT
WT:78
WT:79
5P:WT
5P:78
5P:79
11S:WT
11S:78
11S:79

Figure 97

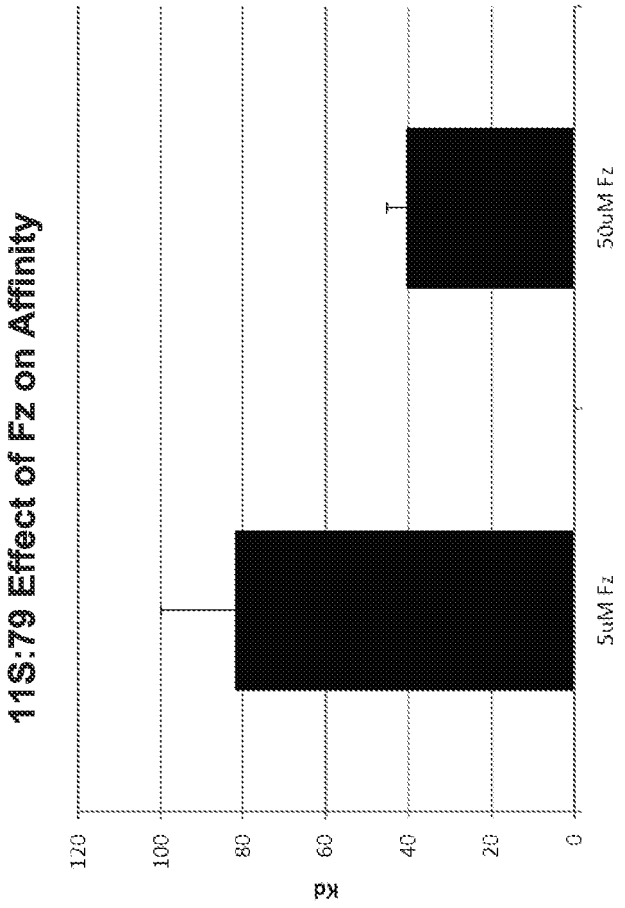

11S:79 Effect of Fz on Affinity
kd
120
100
80
60
40
20
0
5uM Fz
50uM Fz

Figure 98

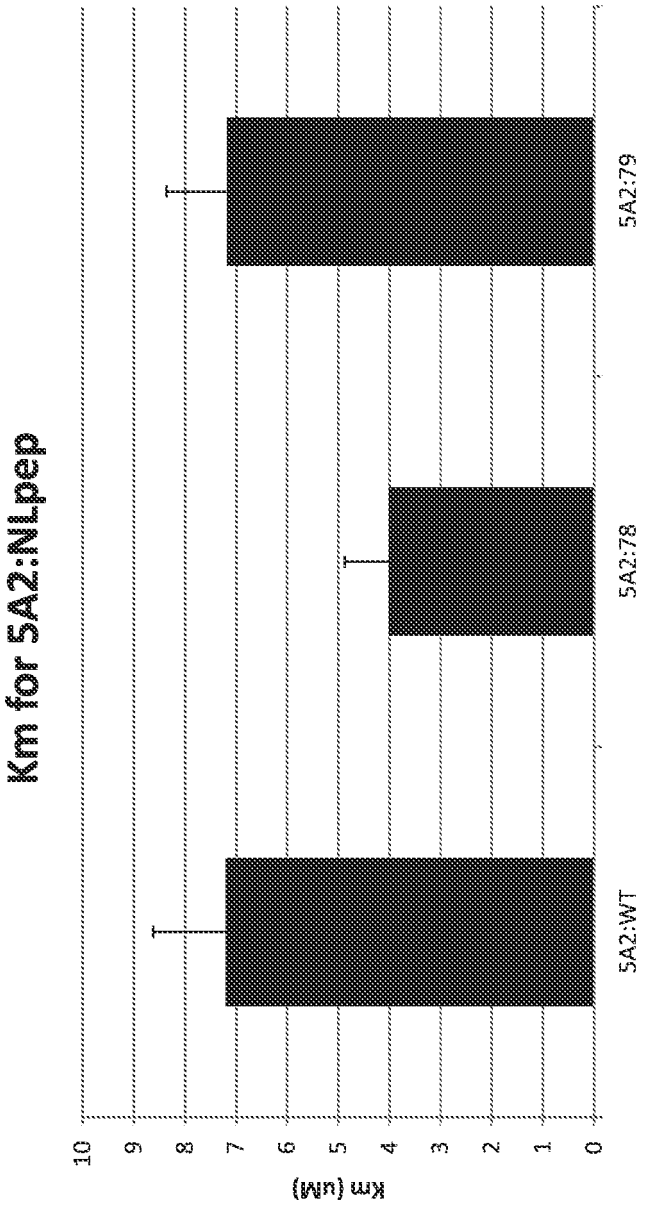


Figure 99

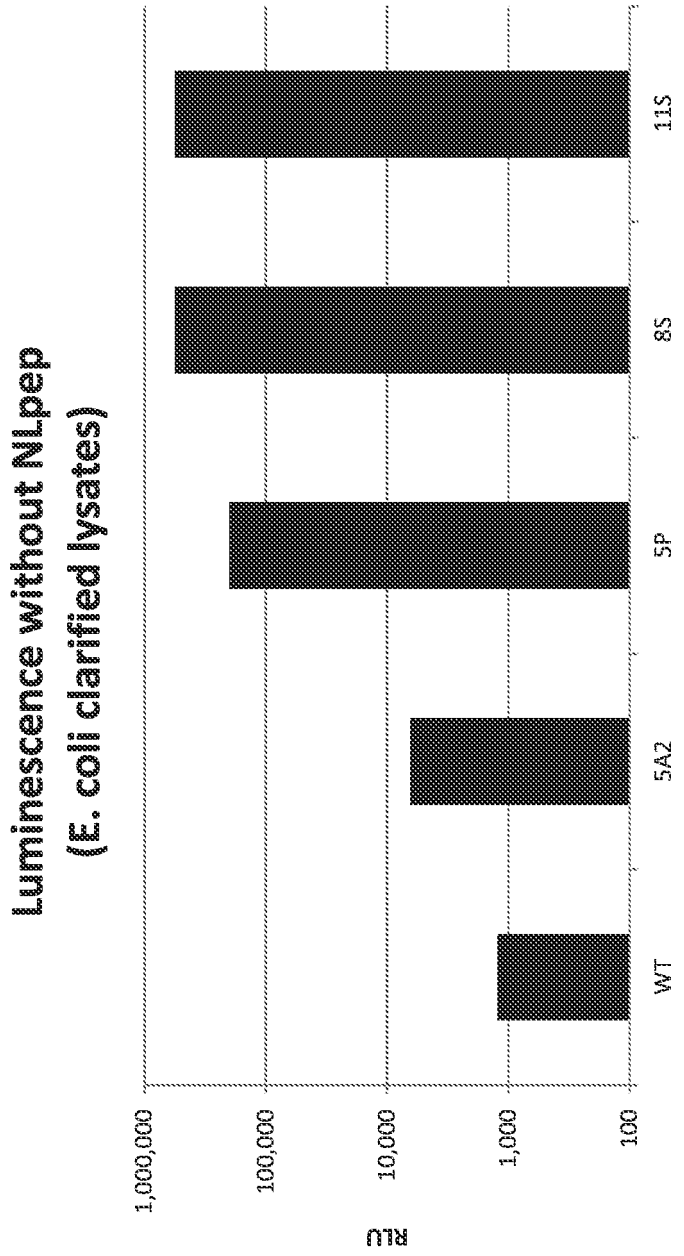

Luminescence without NLpep
(E. coli clarified lysates)
RLU
1,000,000
100,000
10,000
1,000
100
WT
5A2
5P
8S
11S

Figure 100

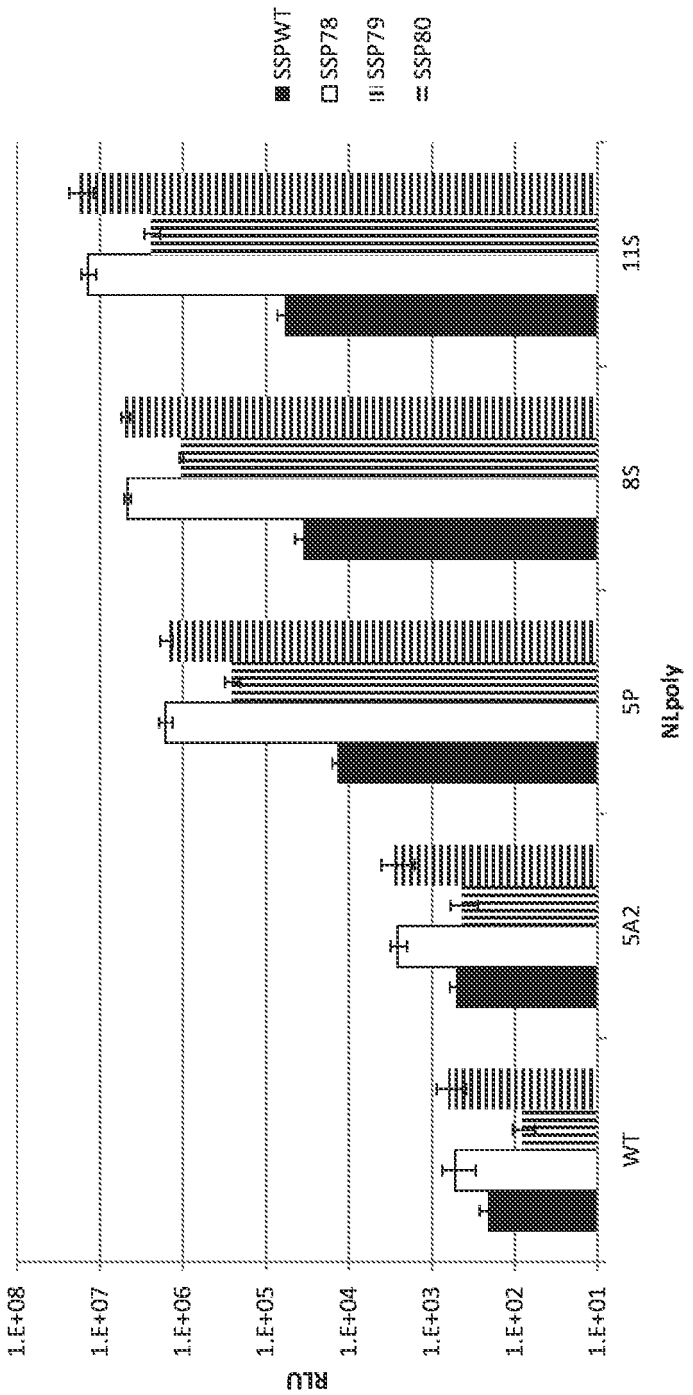

NLpoly (E. coli) with 0.4nM NLpep
SSPWT
SSP78
SSP79
SSP80
RLU
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
WT
5A2
5P
8S
11S
NLpoly

Figure 101

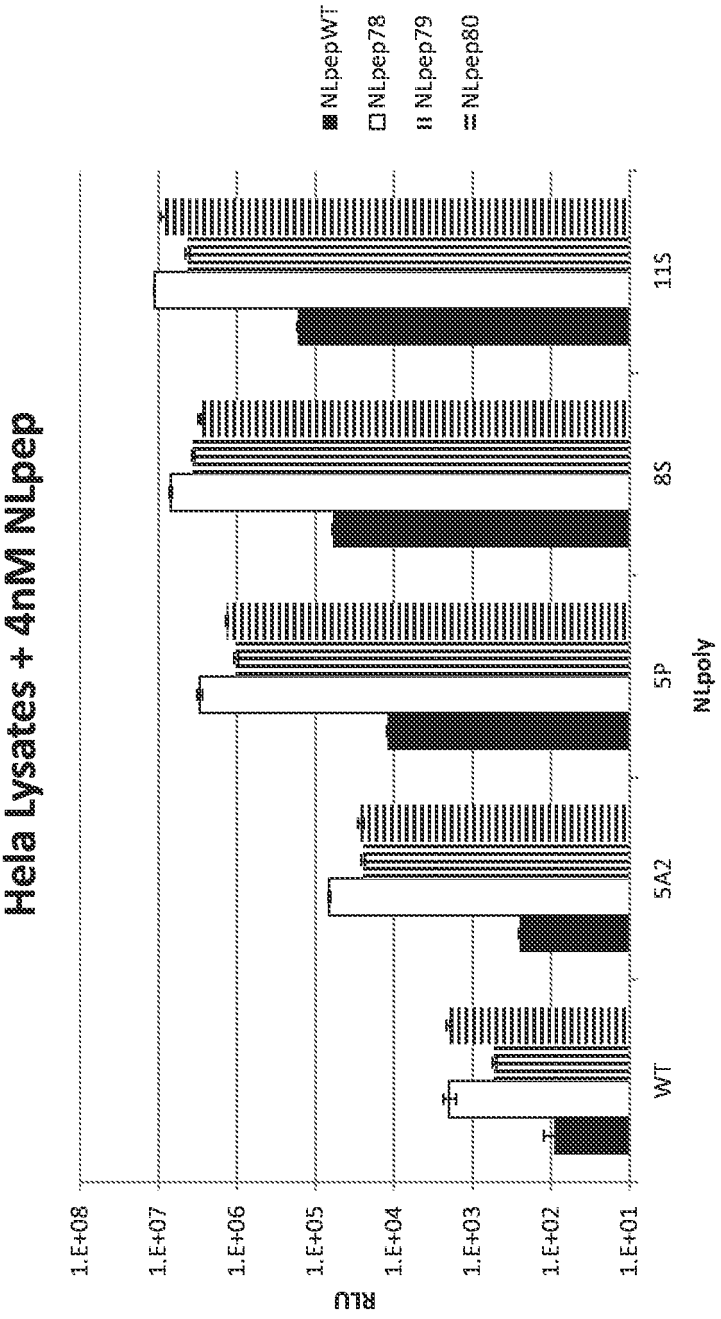

Hela Lysates + 4nM NLpep
NLpepWT
NLpep78
NLpep79
NLpep80
RLU
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
WT
5A2
5P
8S
11S
NLpoly

Figure 102

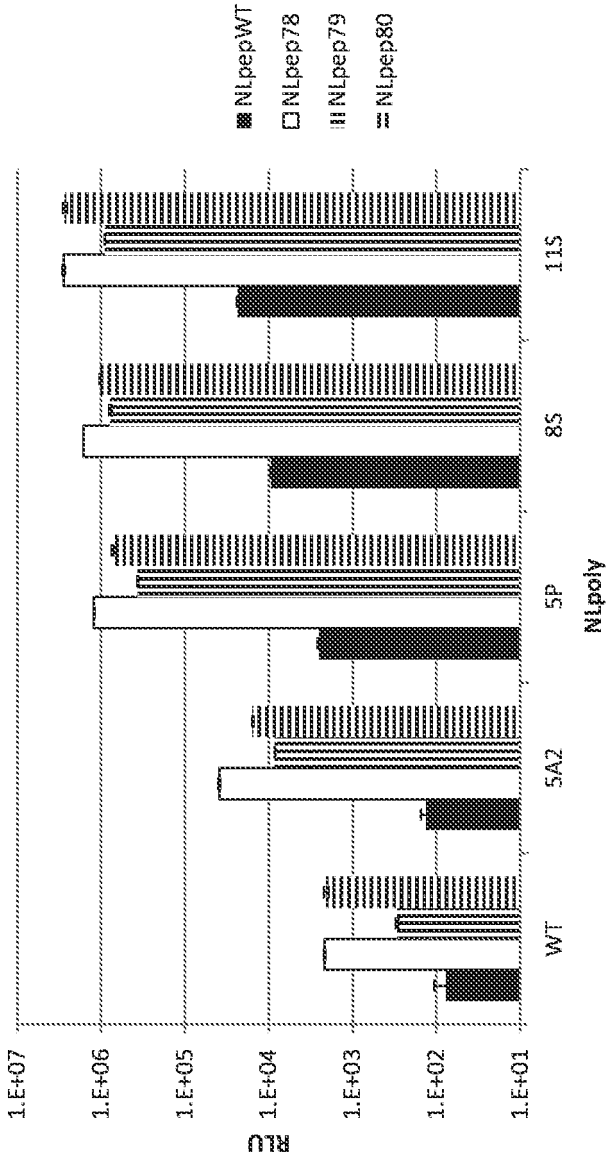

HEK293 Lysates + 4nM NLpep
NLpepWT
NLpep78
NLpep79
NLpep80
RLU
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
WT
5A2
5P
8S
11S
NLpoly

Figure 103

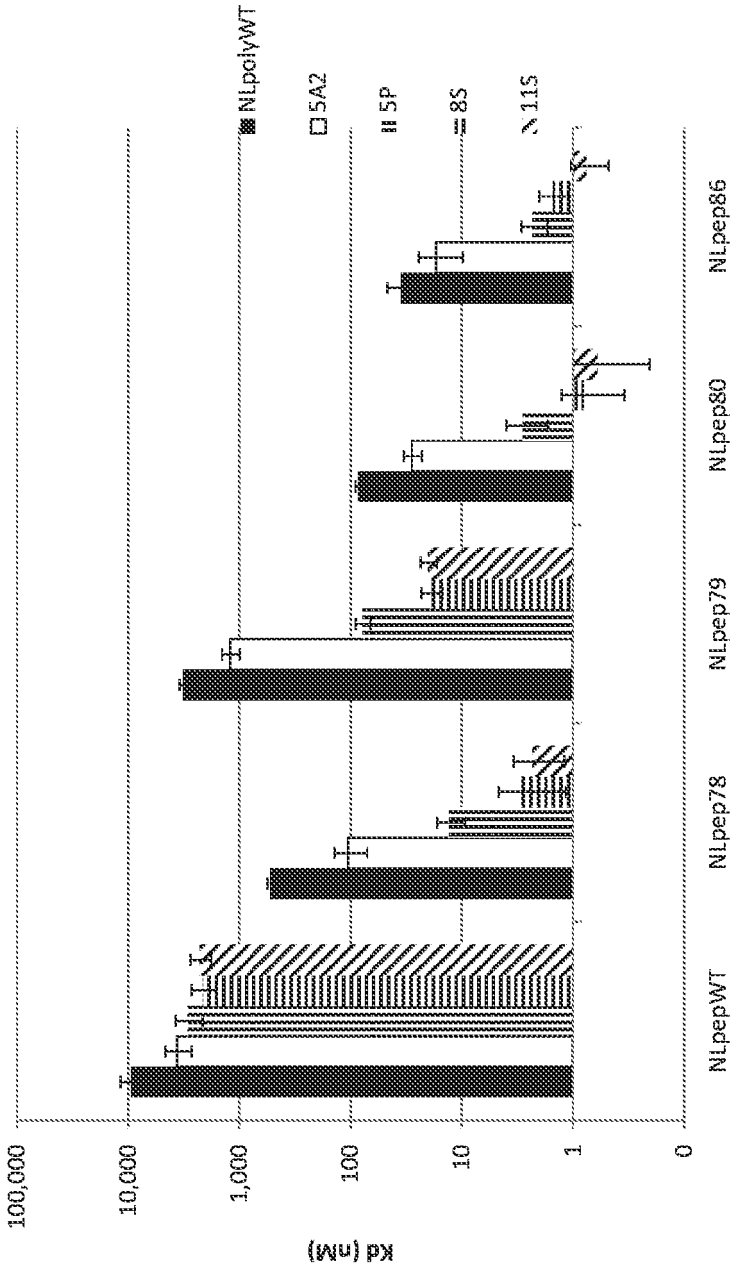

Binding Affinity
NLpolyWT
5A2
5P
8S
11S
Kd (nM)
100,000
10,000
1,000
100
10
1
0
NLpepWT
NLpep78
NLpep79
NLpep80
NLpep86

Figure 104

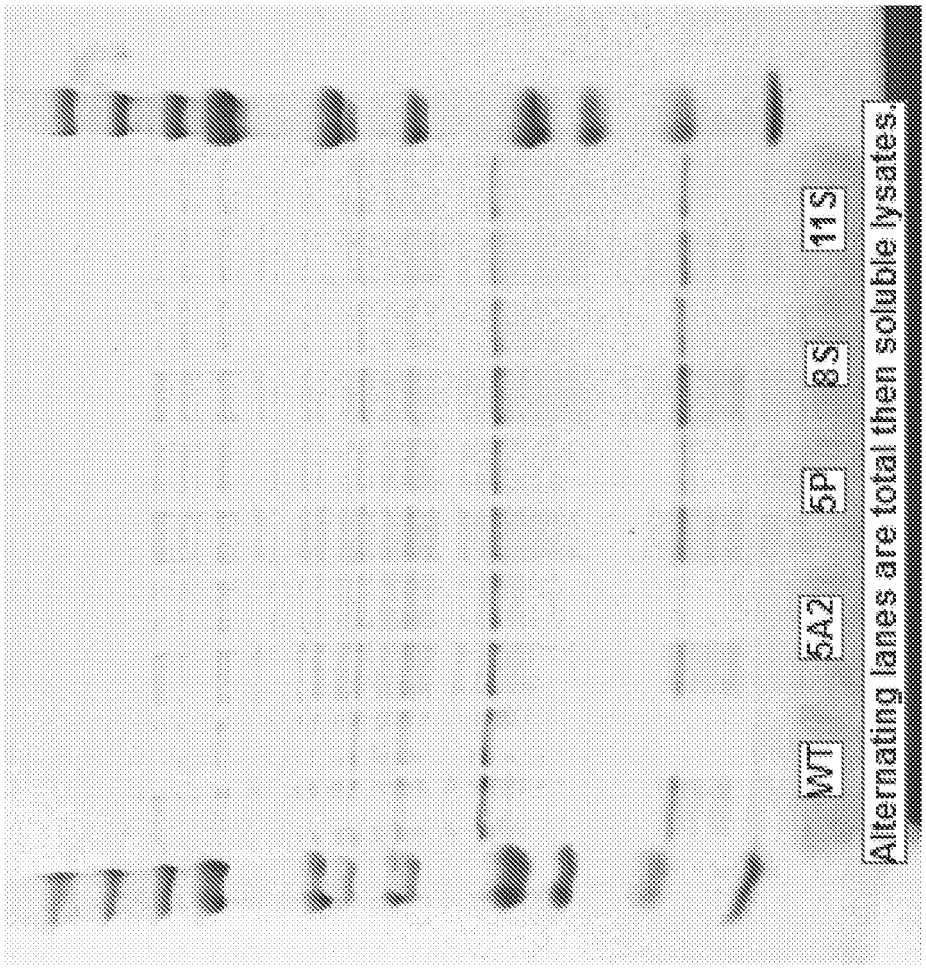

WT
5A2
5P
8S
11S
Alternating lanes are total then soluble lysates.

Figure 105

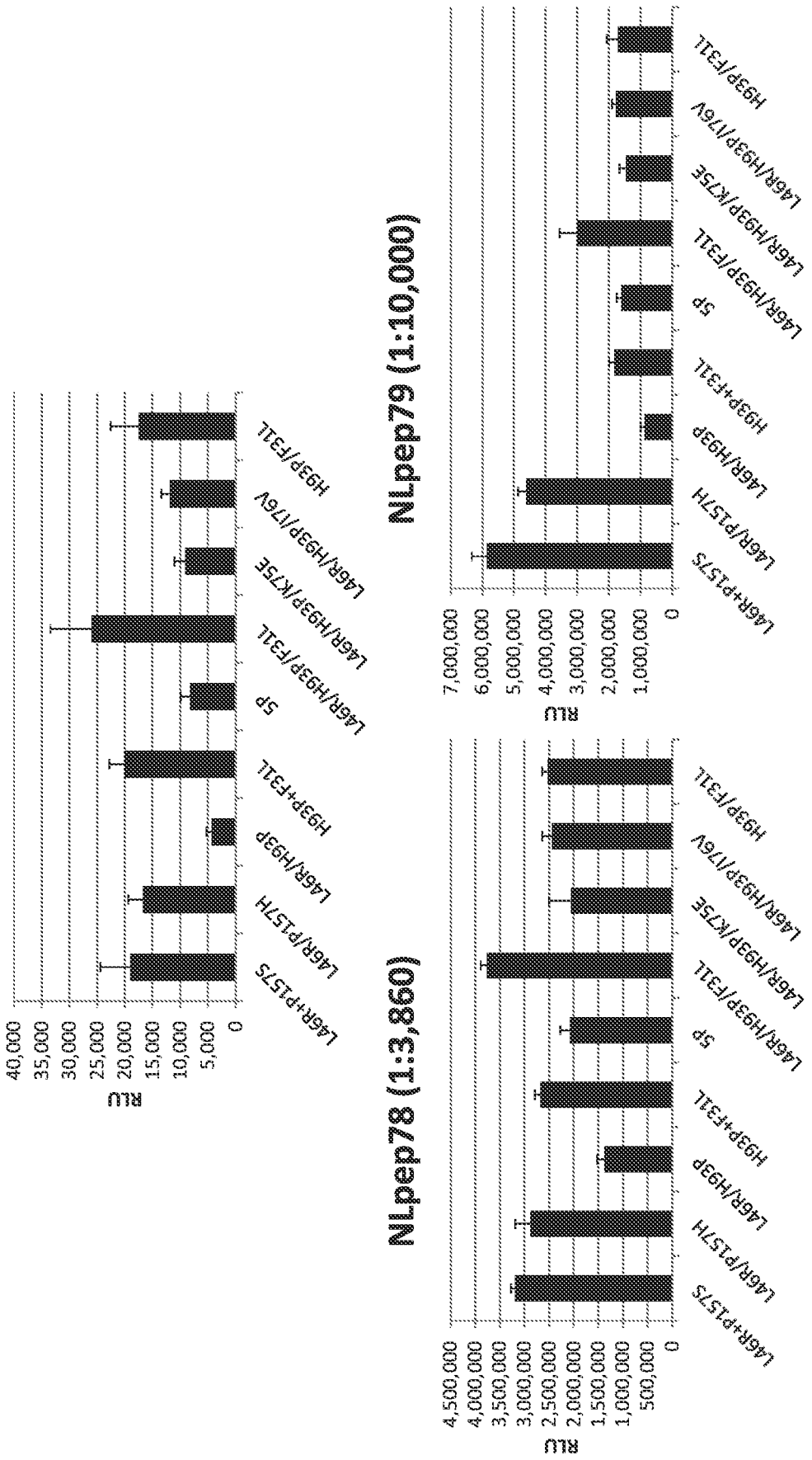

Luminescence without NLpep
RLU
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
L46R+P157S
L46R/P157H
L46R/H93P
H93P+F31L
SP
L46R/H93P/F31L
L46R/H93P/K75E
L46R/H93P/I76V
H93P/F31L
NLpep79 (1:10,000)
RLU
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
L46R+P157S
L46R/P157H
L46R/H93P
H93P+F31L
SP
L46R/H93P/F31L
L46R/H93P/K75E
L46R/H93P/I76V
H93P/F31L
NLpep78 (1:3,860)
RLU
4,500,000
4,000,000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
L46R+P157S
L46R/P157H
L46R/H93P
H93P+F31L
SP
L46R/H93P/F31L
L46R/H93P/K75E
L46R/H93P/I76V
H93P/F31L

Figure 106

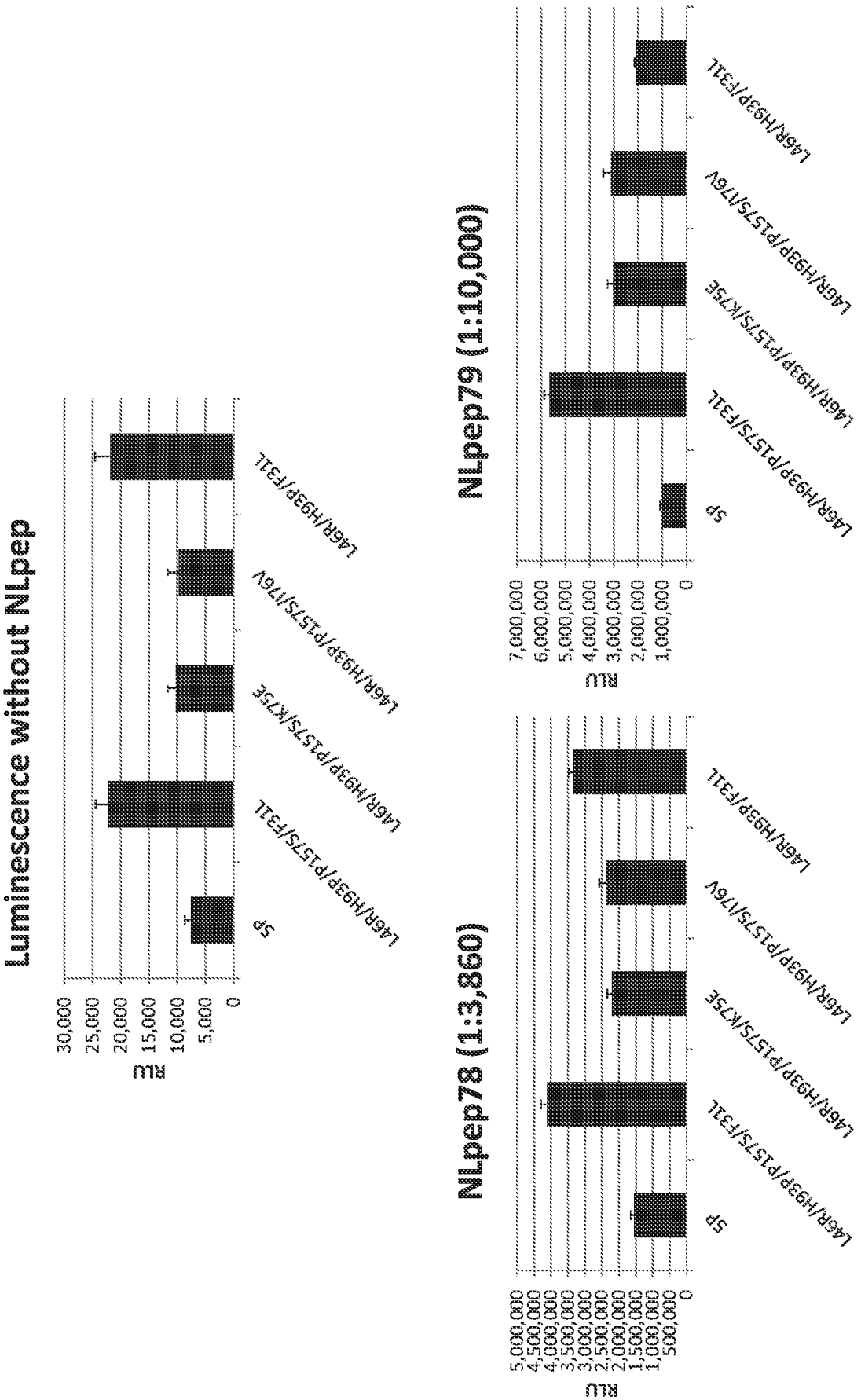

Luminescence without NLpep
RLU
30,000
25,000
20,000
15,000
10,000
5,000
0
SP
L46R/H93P/P157S/F31L
L46R/H93P/P157S/K75E
L46R/H93P/P157S/I76V
L46R/H93P/F31L
NLpep79 (1:10,000)
RLU
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
SP
L46R/H93P/P157S/F31L
L46R/H93P/P157S/K75E
L46R/H93P/P157S/I76V
L46R/H93P/F31L
NLpep78 (1:3,860)
RLU
5,000,000
4,500,000
4,000,000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
SP
L46R/H93P/P157S/F31L
L46R/H93P/P157S/K75E
L46R/H93P/P157S/I76V
L46R/H93P/F31L

Figure 107

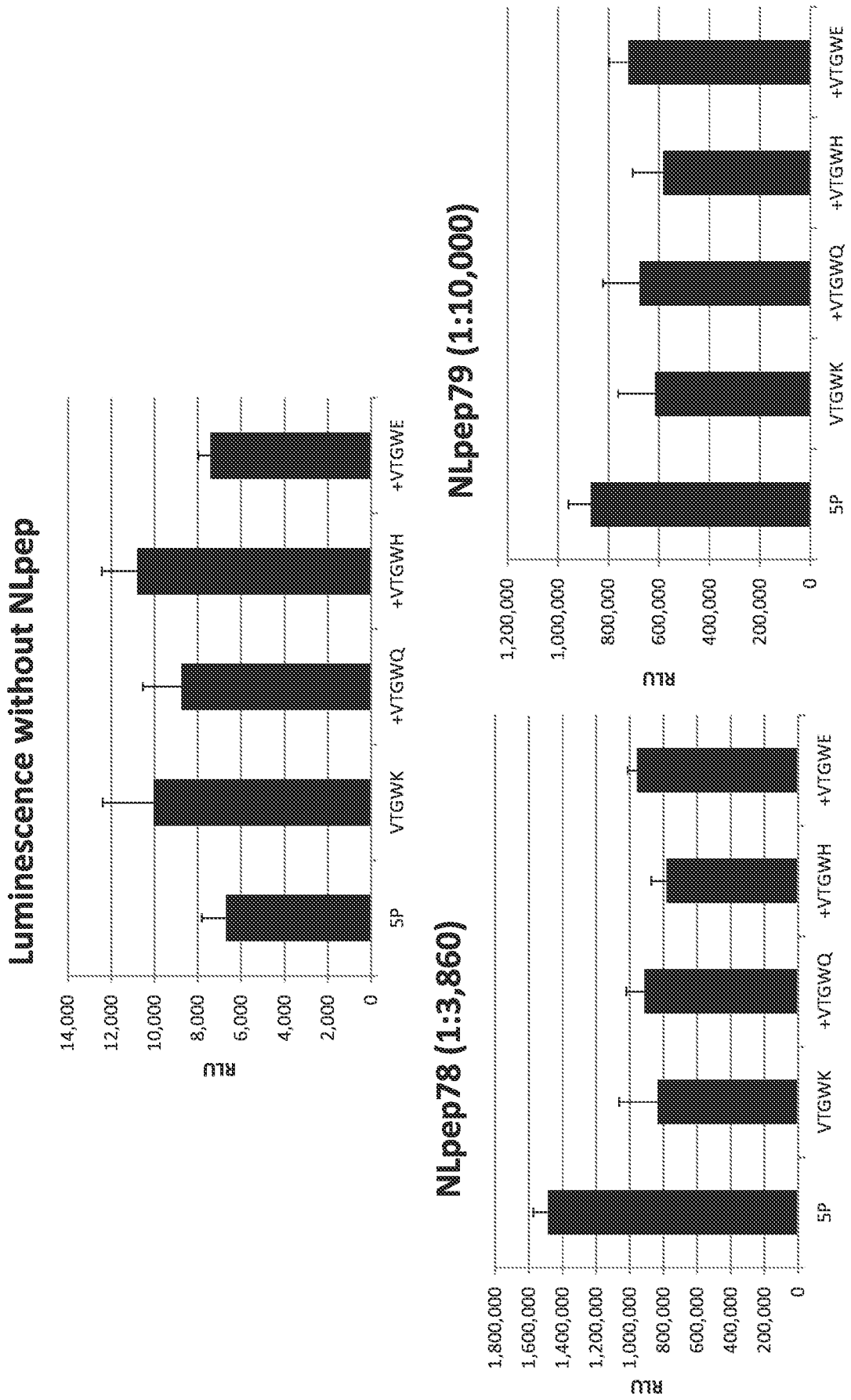
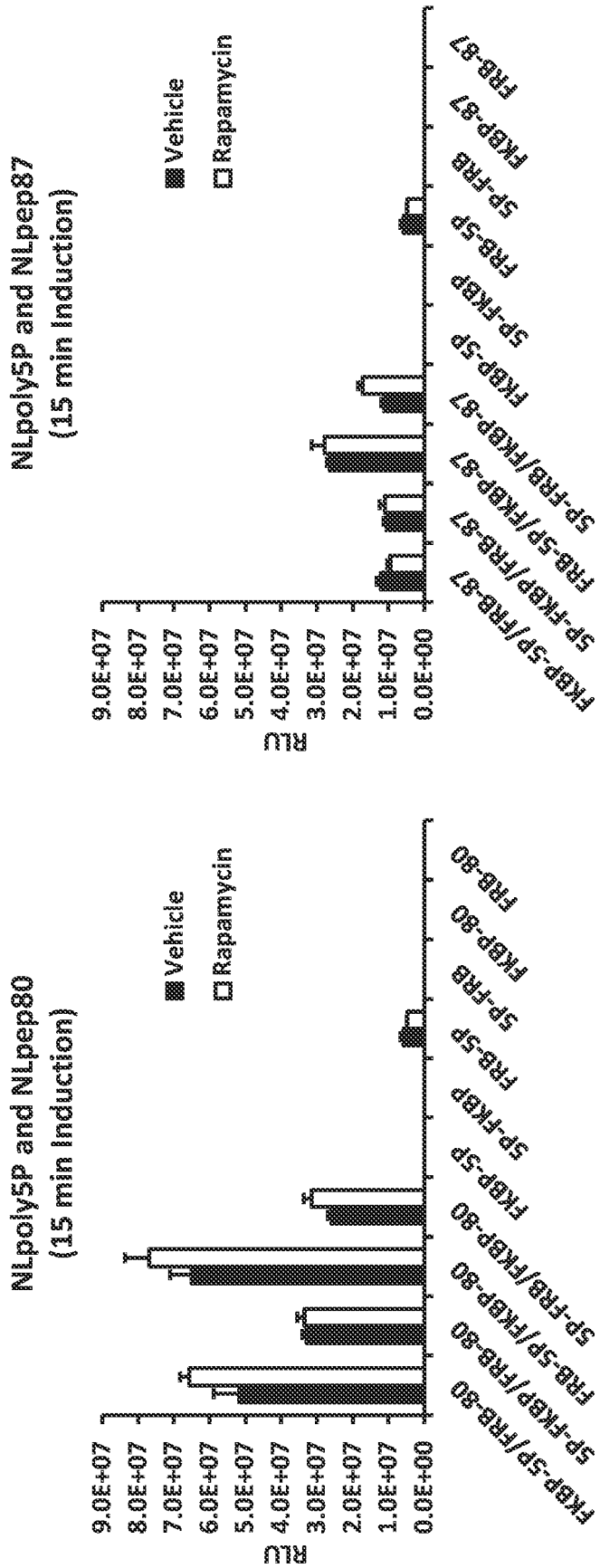

Luminescence without NLpep
RLU
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
5P
VTGWK
+VTGWQ
+VTGWH
+VTGWE
NLpep79 (1:10,000)
1,200,000
1,000,000
800,000
600,000
400,000
200,000
NLpep78 (1:3,860)
1,800,000
1,600,000
1,400,000
1,200,000
1,000,000

Figure 108



Combination	Fold Induction
FKBP-5P/FRB-87	0.8
5P-FKBP/FRB-87	1.0
FRB-5P/FKBP-87	1.1
5P-FRB/FKBP-87	1.5

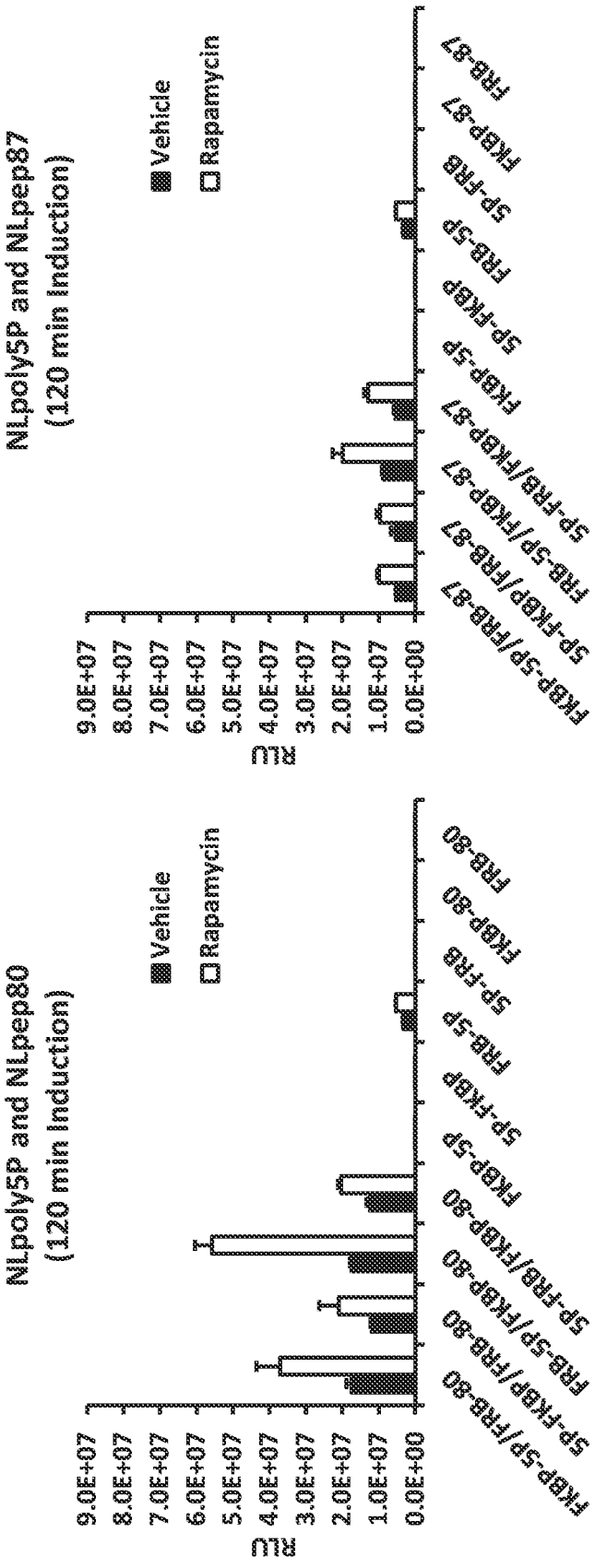
Combination	Fold Induction
FKBP-5P/FRB-80	1.2
5P-FKBP/FRB-80	1.0
FRB-5P/FKBP-80	1.2
5P-FRB/FKBP-80	1.2

Figure 109

Combination	Fold Induction
FKBP-5P/FRB-80	3.3
5P-FKBP/FRB-80	2.1
FRB-5P/FKBP-80	2.9
5P-FRB/FKBP-80	2.2

Combination	Fold Induction
FKBP-5P/FRB-87	1.3
5P-FKBP/FRB-87	1.3
FRB-5P/FKBP-87	2.6
5P-FRB/FKBP-87	2.1

Figure 110



Combination	Fold Induction
FKBP-5P/FRB-80	2.1
5P-FKBP/FRB-80	1.8
FRB-5P/FKBP-80	3.2
5P-FRB/FKBP-80	1.6

Combination	Fold Induction
FKBP-5P/FRB-87	1.9
5P-FKBP/FRB-87	1.8
FRB-5P/FKBP-87	2.3
5P-FRB/FKBP-87	2.4

Figure 111

Configuration	Fold Induction
5P-FRB/87-FKBP	4.1
5P-FRB/FKBP-87	3.6
FRB-5P/87-FKBP	5.1
FRB-5P/FKBP-87	3.1
5P-FKBP/87-FRB	3.8
5P-FKBP/FRB-87	4.8
FKBP-5P/87-FRB	7.0
FKBP-5P/FRB-87	3.7

Configuration	Fold Induction
5P-FRB/80-FKBP	5.1
5P-FRB/FKBP-80	3.3
FRB-5P/80-FKBP	6.1
FRB-5P/FKBP-80	2.4
5P-FKBP/80-FRB	4.6
5P-FKBP/FRB-80	2.0
FKBP-5P/80-FRB	3.5
FKBP-5P/FRB-80	2.3

Configuration	Fold Induction
5P-FRB	0.7
FRB-5P	0.7
5P-FKBP	1.6
FKBP-5P	1.1
80-FRB	0.8
FRB-80	1.0
80-FKBP	0.8
FKBP-80	0.8
87-FRB	0.7
FRB-87	2.0
87-FKBP	1.8
FKBP-87	1.8

Figure 112

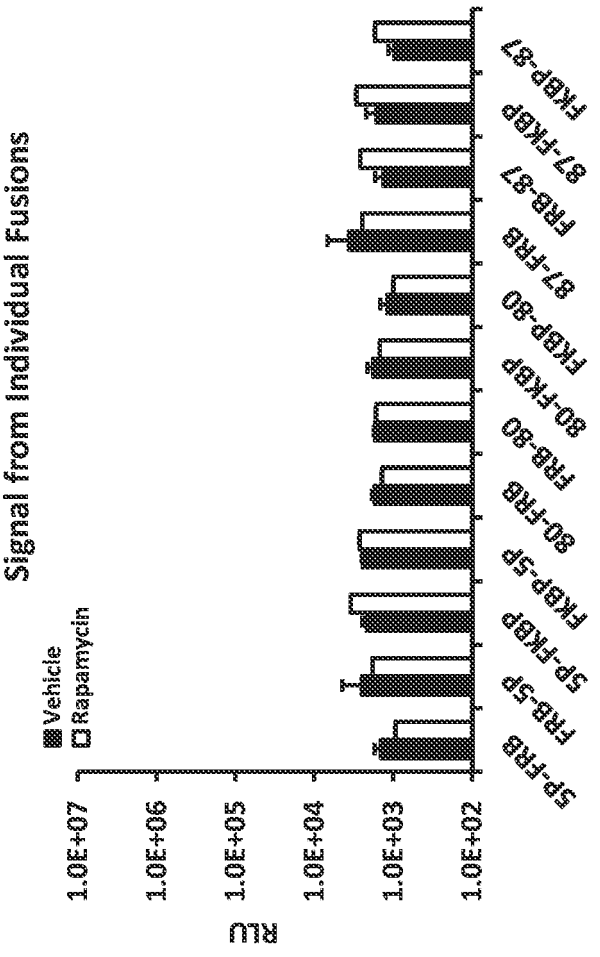
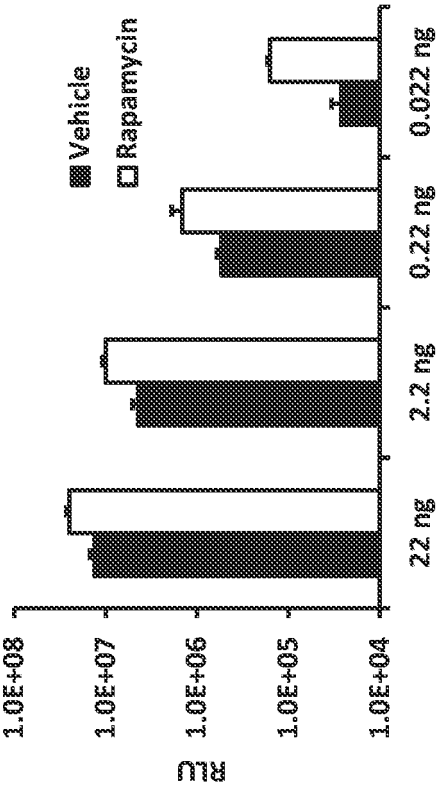
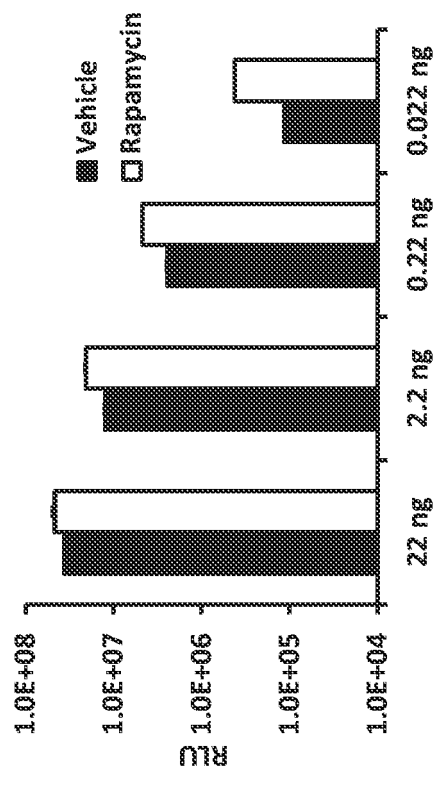


Figure 113



DNA per Well	Fold Induction
22 ng	1.9
2.2 ng	2.2
0.22 ng	2.7
0.022 ng	6.0



DNA per Well	Fold Induction
22 ng	1.3
2.2 ng	1.7
0.22 ng	1.9
0.022 ng	3.7

Figure 114

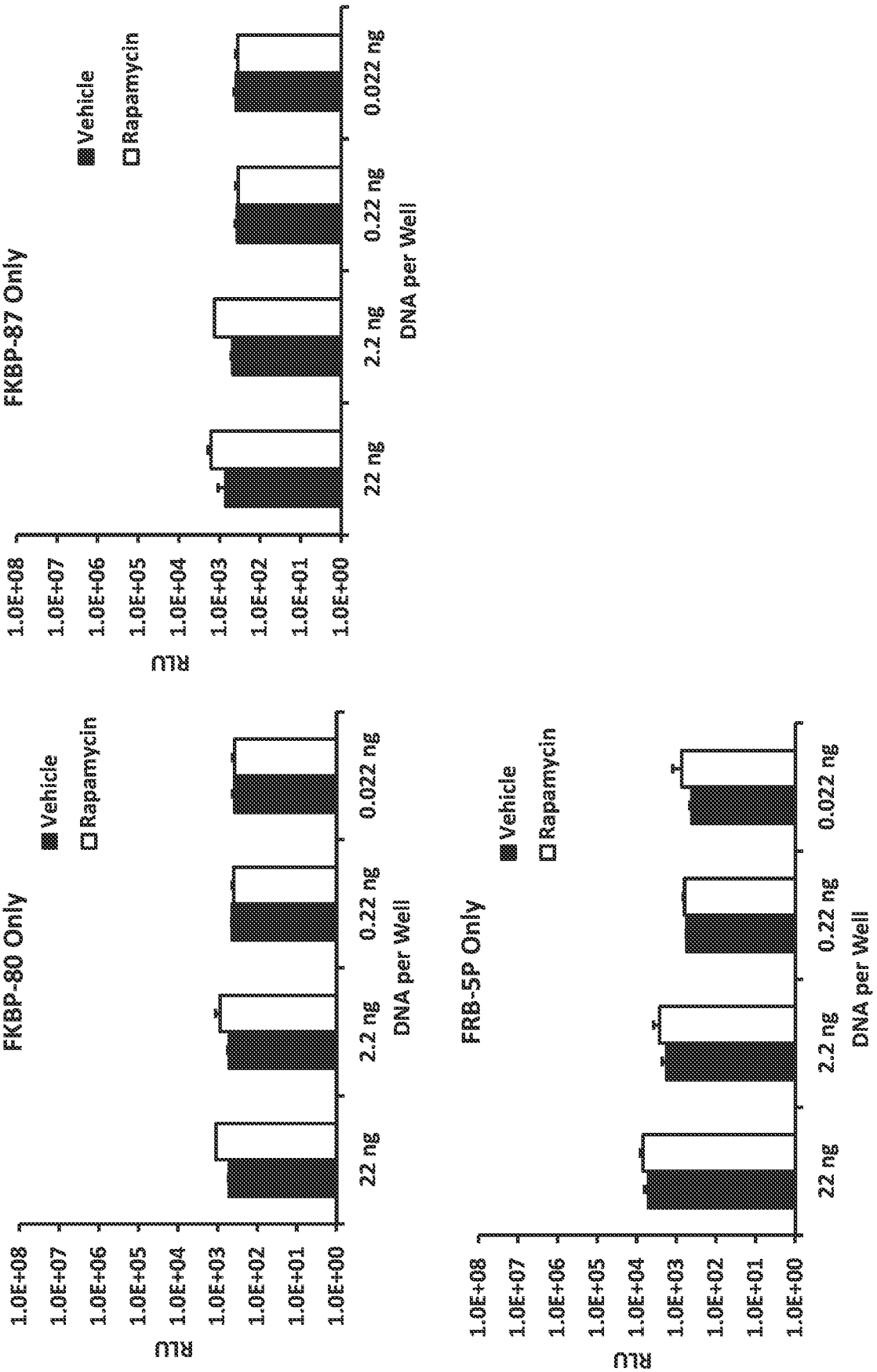
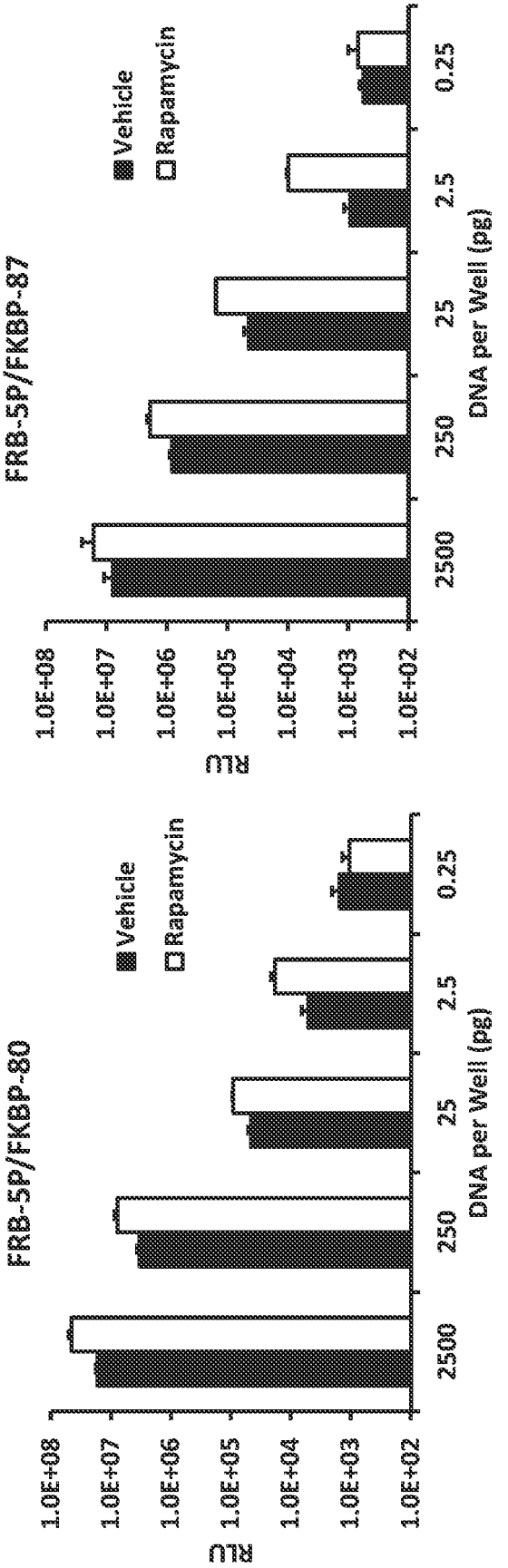

FKBP-87 Only
Vehicle
Rapamycin
RLU
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
22 ng
2.2 ng
0.22 ng
0.022 ng
DNA per Well
FKBP-80 Only
Vehicle
Rapamycin
RLU
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
22 ng
2.2 ng
0.22 ng
0.022 ng
DNA per Well
FRB-5P Only
Vehicle
Rapamycin
RLU
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
22 ng
2.2 ng
0.22 ng
0.022 ng
DNA per Well

Figure 15



DNA per Well (pg)	Fold Induction
2500	2.1
250	2.4
25	3.5
2.5	10.6
0.25	1.2

DNA per Well (pg)	Fold Induction
2500	2.7
250	2.3
25	2.0
2.5	3.6
0.25	0.7

Figure 116

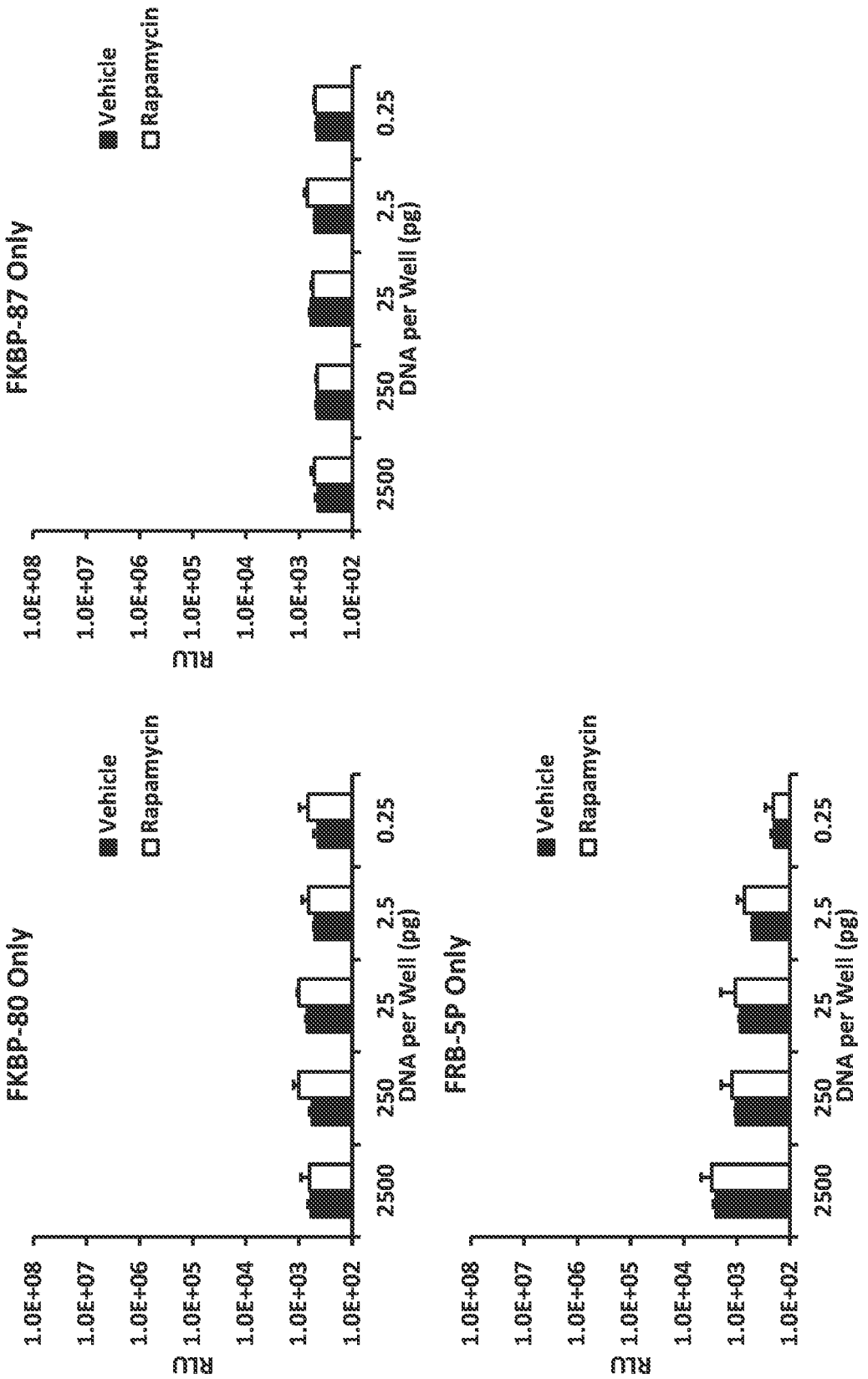

FKBP-87 Only
FKBP-80 Only
FRB-5P Only
Vehicle
Rapamycin
RLU
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
2500
250
25
2.5
0.25
DNA per Well (pg)

Figure 117

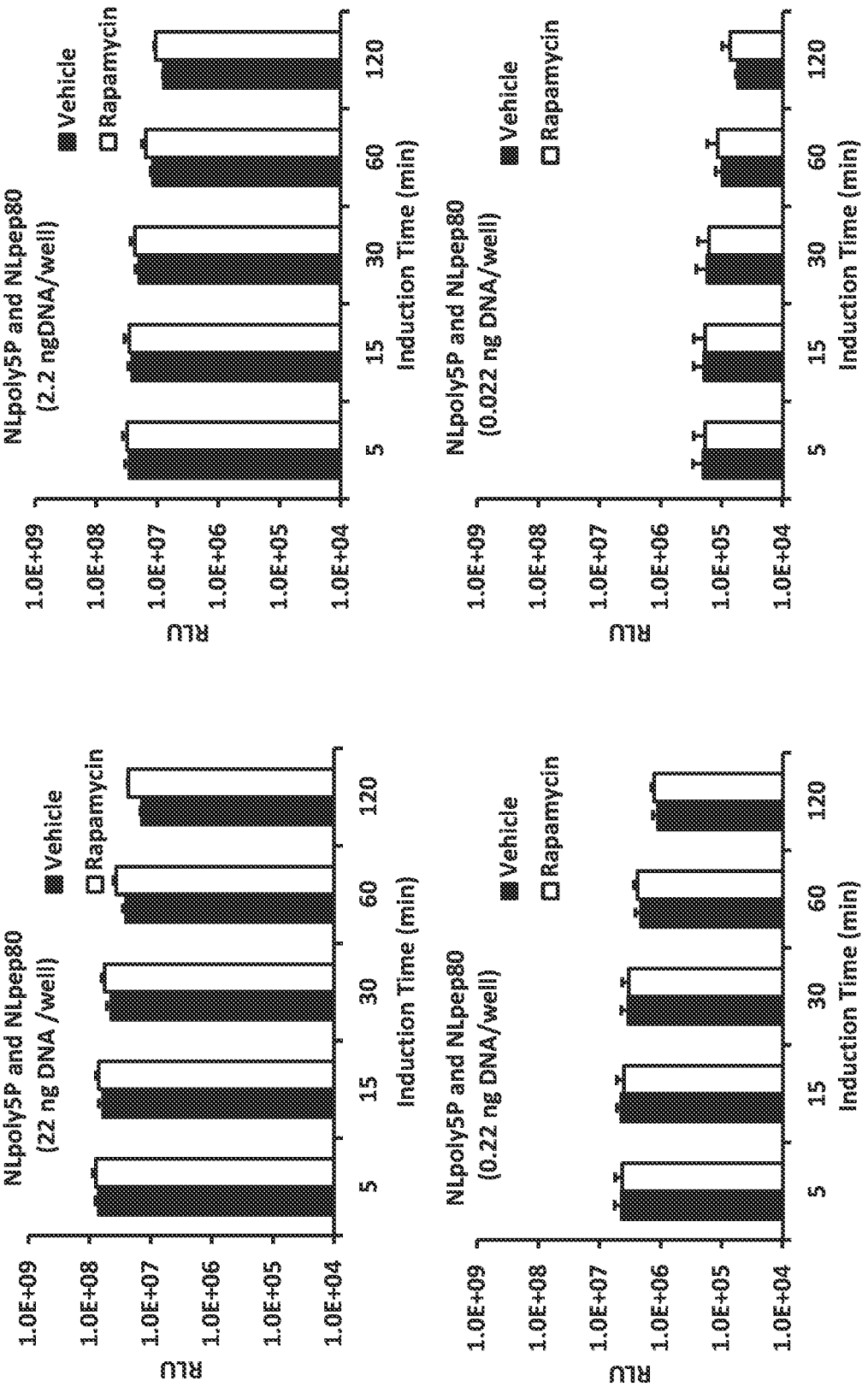

NLpoly5P and NLpep80
(22 ng DNA /well)
Vehicle
Rapamycin
RLU
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
5
15
30
60
120
Induction Time (min)
NLpoly5P and NLpep80
(2.2 ngDNA/well)
NLpoly5P and NLpep80
(0.22 ng DNA/well)
NLpoly5P and NLpep80
(0.022 ng DNA/well)

Figure 118

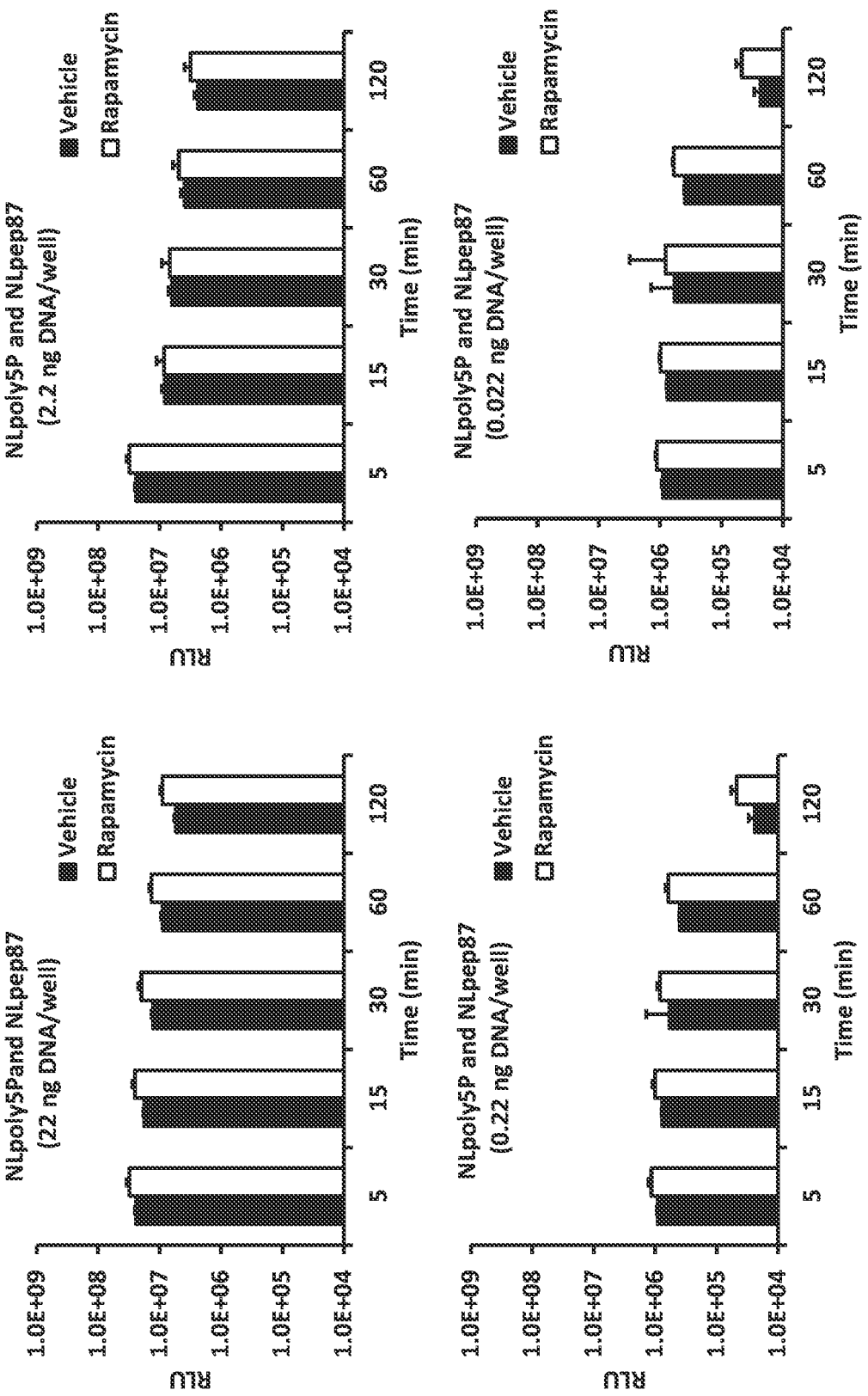
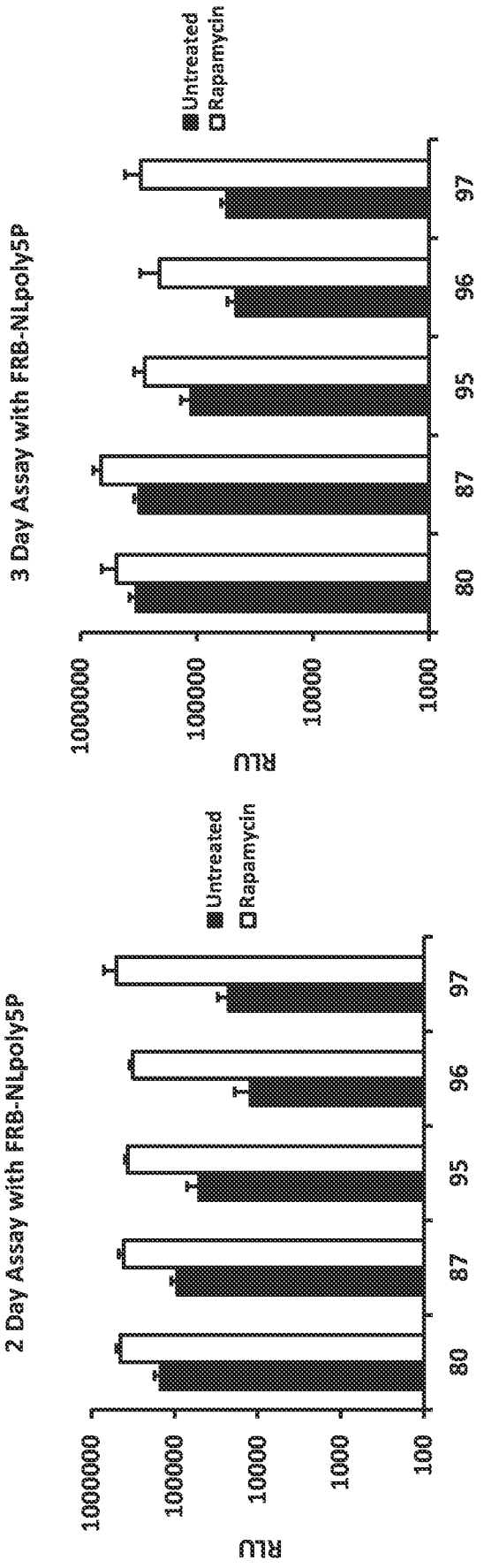

NLpoly5Pand NLpep87
(22 ng DNA/well)
Vehicle
Rapamycin
RLU
Time (min)
NLpoly5P and NLpep87
(2.2 ng DNA/well)
NLpoly5P and NLpep87
(0.22 ng DNA/well)
NLpoly5P and NLpep87
(0.022 ng DNA/well)

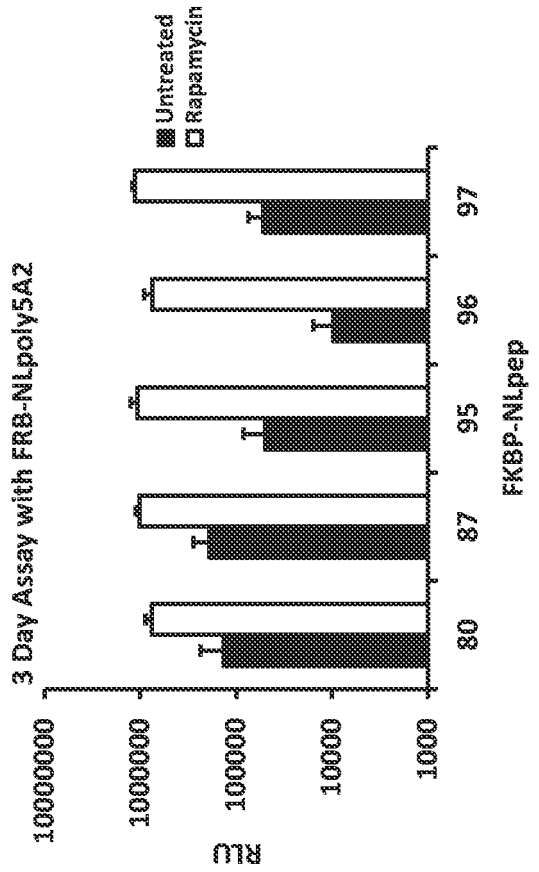
Figure 119



NLpep	Fold Induction
80	3.1
87	4.4
95	7.2
96	26.1
97	22.5

NLpep	Fold Induction
80	1.5
87	2.1
95	2.5
96	4.5
97	5.5

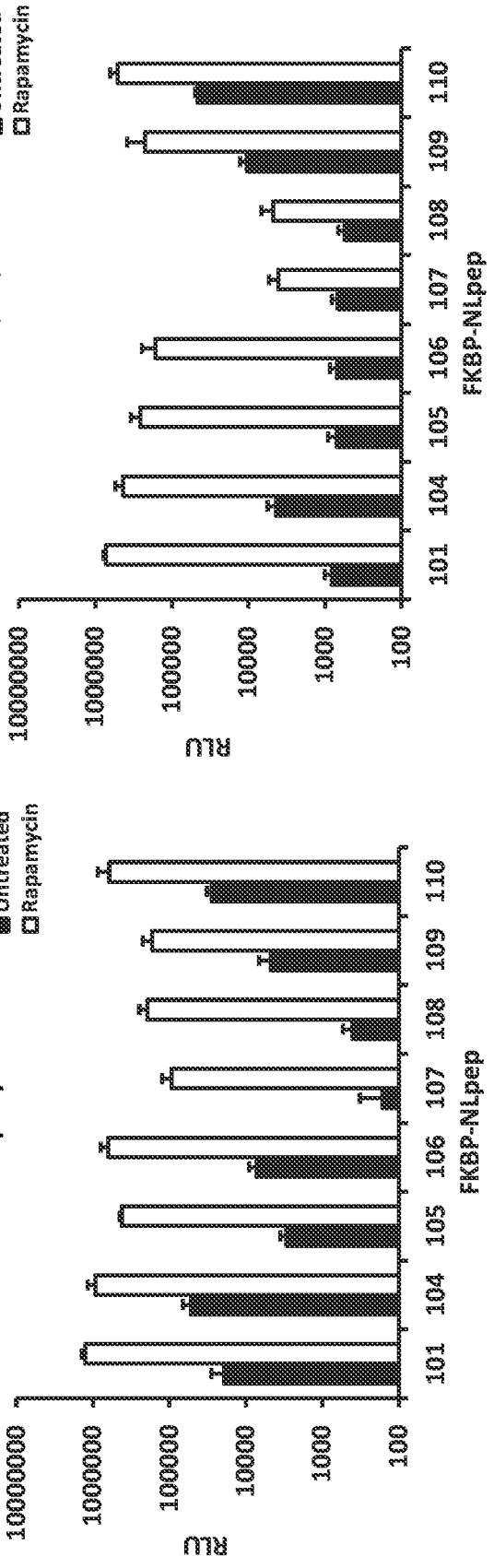
Figure 120



NLpep	Fold Induction
80	5.5
87	5.7
95	22.1
96	60.0
97	12.7

NLpep	Fold Induction
80	5.6
87	5.4
95	21.3
96	77.9
97	21.8

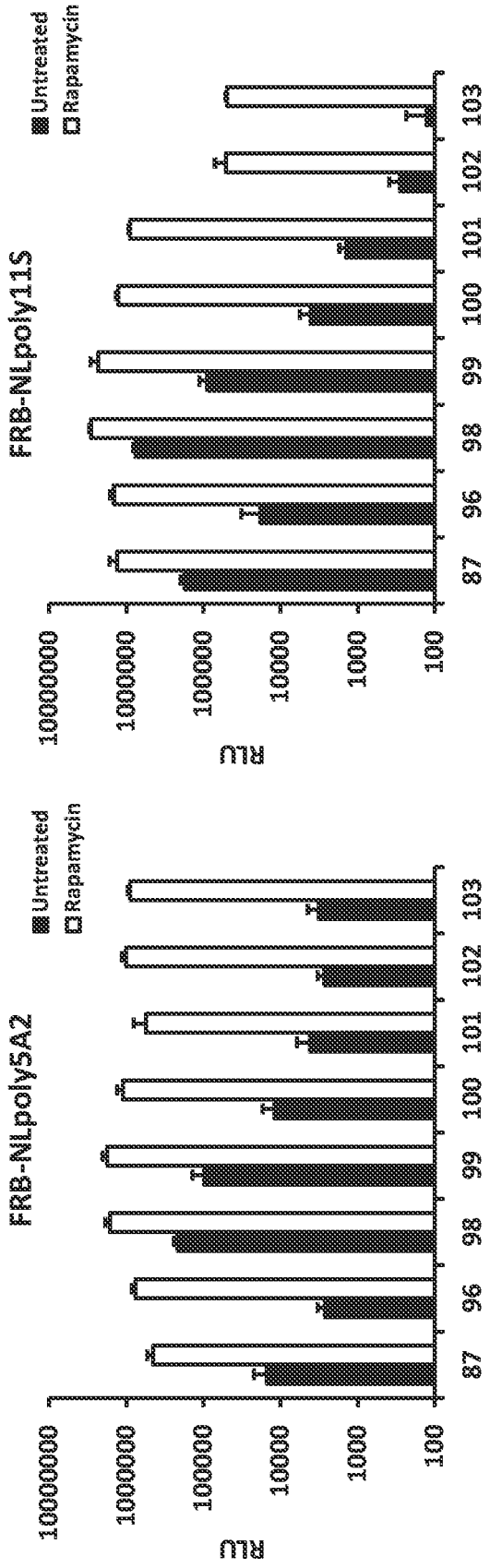
Figure 21



NLpep	Fold Induction
101	65
104	18
105	140
106	86
107	578
108	483
109	36
110	22

NLpep	Fold Induction
101	882
104	101
105	372
106	234
107	6
108	9
109	22
110	11

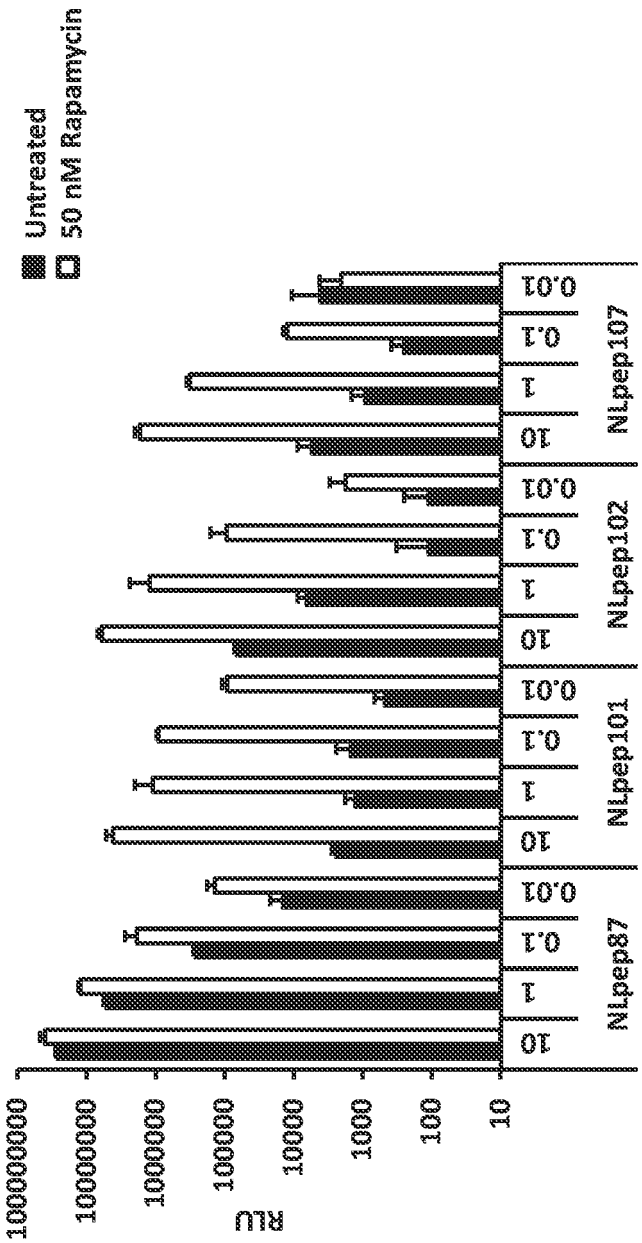
Figure 122



NLpep	Fold Induction
87	30
96	287
98	8
99	18
100	93
101	134
102	375
103	279

NL pep	Fold Induction
87	8
96	79
98	4
99	26
100	310
101	636
102	181
103	388

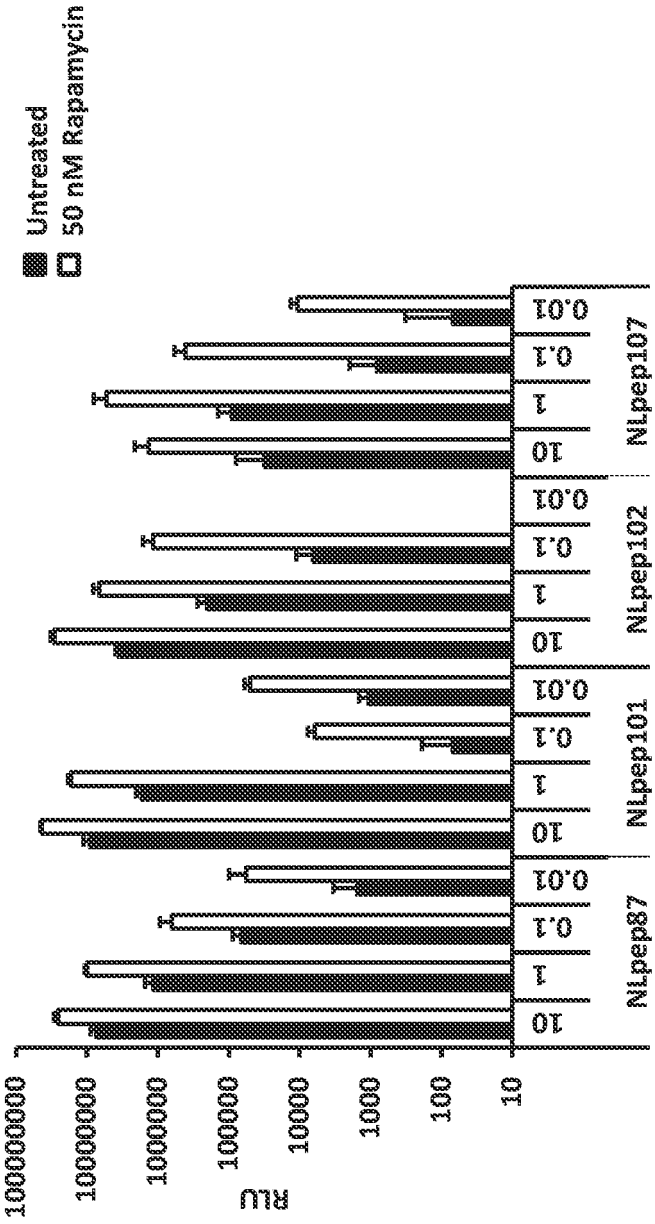
Figure 123



Fold Induction

NLpep	10 ng DNA/well	1 ng DNA/well	0.1 ng DNA/well	0.01 ng DNA/well
87	1	2	7	10
101	1714	862	605	196
102	89	187	865	16
107	314	347	49	0

Figure 124



Fold Induction

NLpep	10 ng DNA/well	1 ng DNA/well	0.1 ng DNA/well	0.01 ng DNA/well
87	3	9	10	37
101	5	10	89	47
102	8	33	179	NA
107	42	58	508	156

Figure 12S

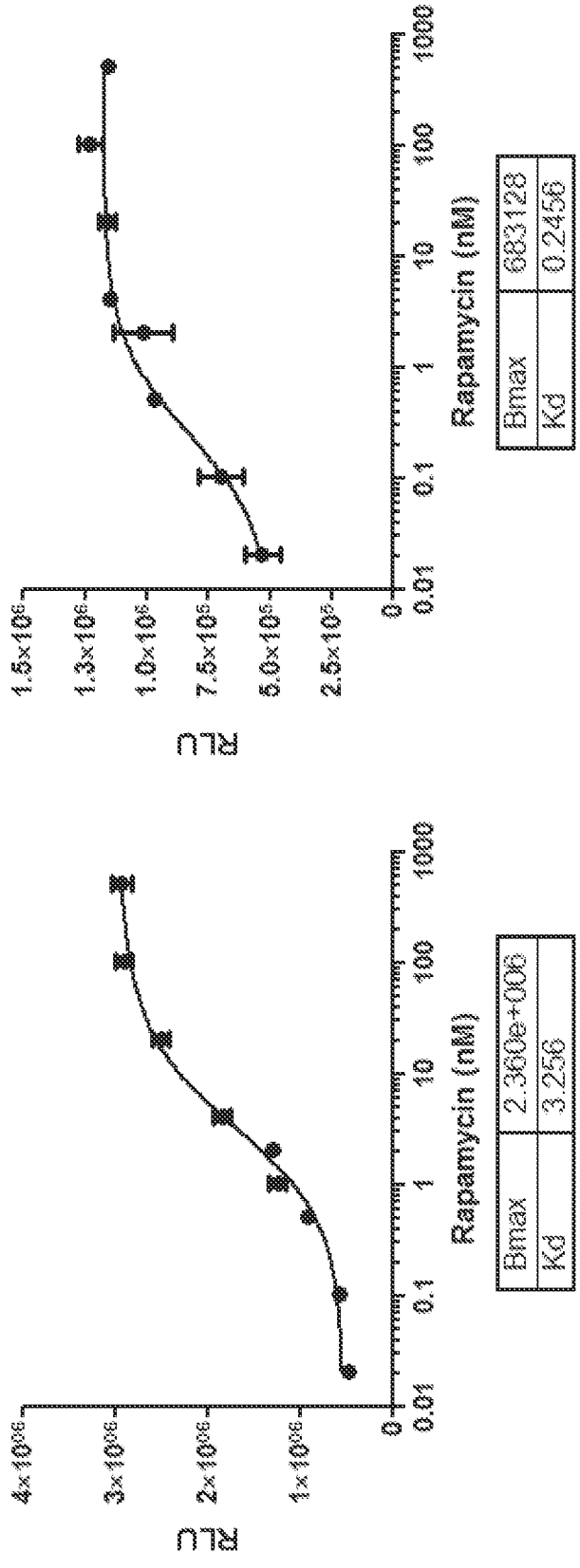
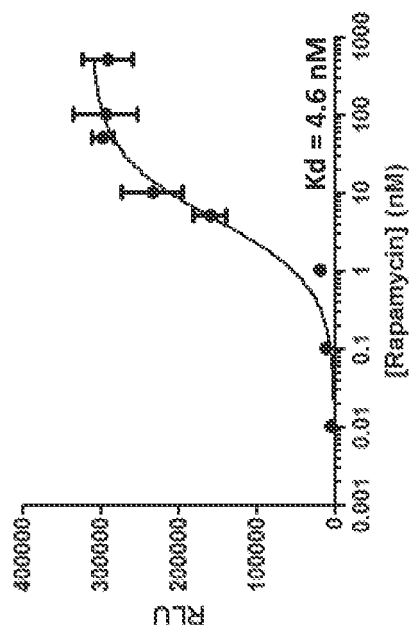
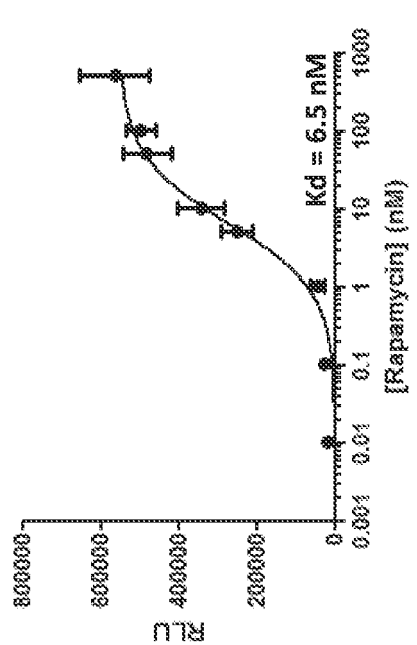
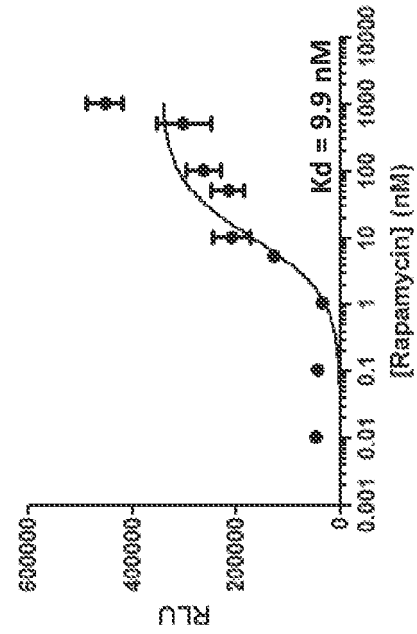

FRB-5P/FKBP-87
RLU
1.5x10^6
1.3x10^6
1.0x10^6
7.5x10^5
5.0x10^5
2.5x10^5
0
0.01
0.1
1
10
100
1000
Rapamycin (nM)
Bmax 683128
Kd 0.2456
FRB-5P/FKBP-80
RLU
4x10^8
3x10^8
2x10^8
1x10^8
0
0.01
0.1
1
10
100
1000
Rapamycin (nM)
Bmax 2.360e+006
Kd 3.256

Figure 126


FRB-11S/FKBP-101
RLU
[Rapamycin] (nM)
Kd = 4.6 nM


FRB-5A2/FKBP-101
RLU
[Rapamycin] (nM)
Kd = 6.5 nM


FRB-11S/FKBP-87
RLU
[Rapamycin] (nM)
Kd = 9.9 nM

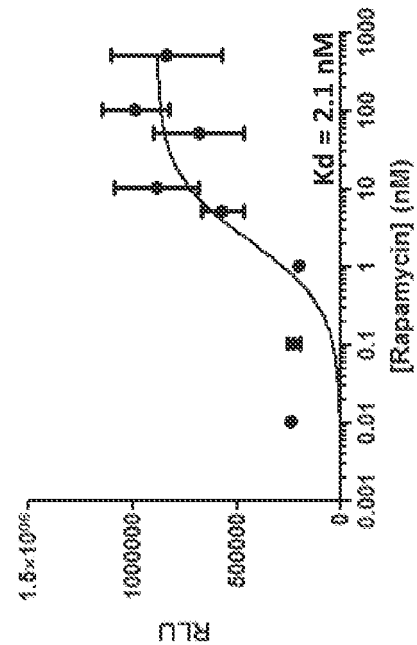
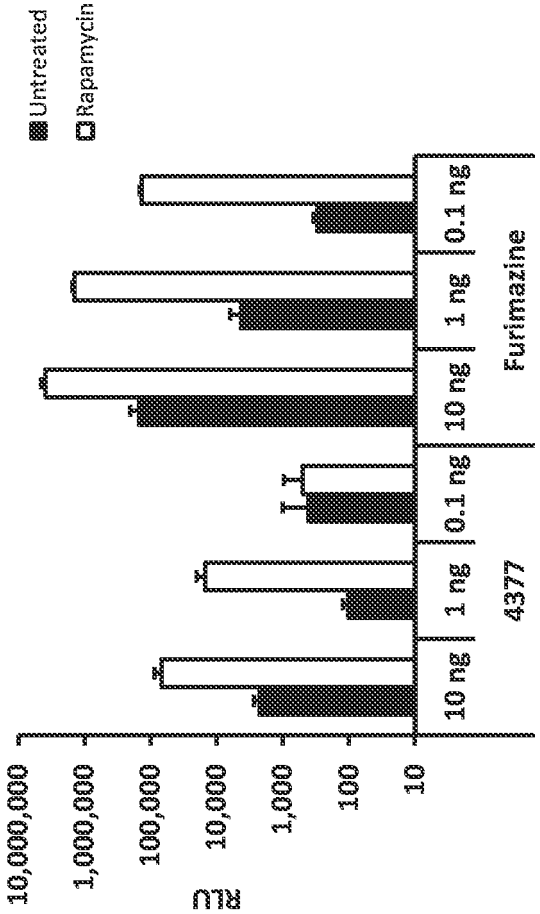

FRB-5A2/FKBP-87
RLU
[Rapamycin] (nM)
Kd = 2.1 nM

Figure 127



Total DNA	Fold induction 4377	Fold induction Furimazine
10 ng	31	26
1 ng	146	335
0.1 ng	1	450

Figure 28

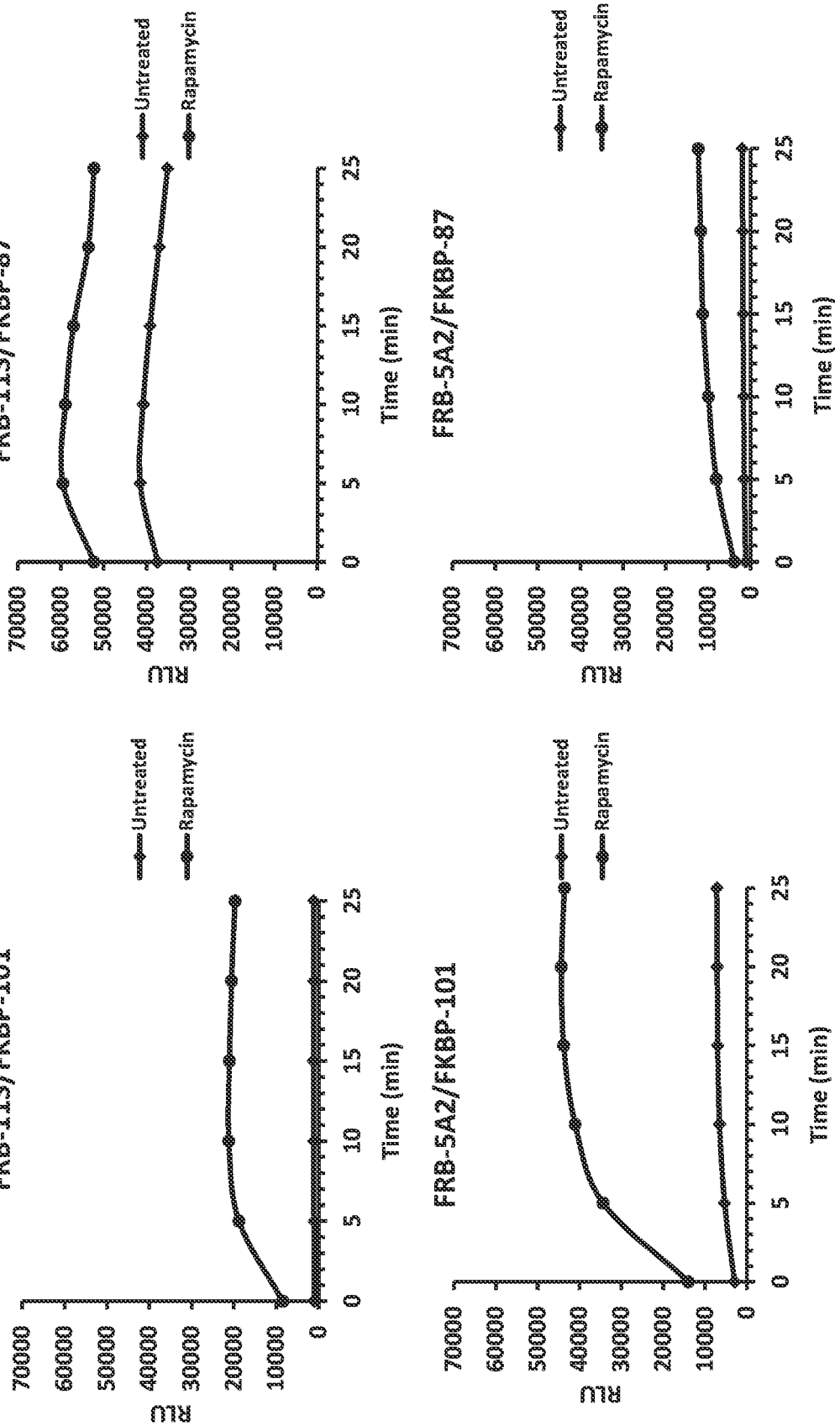

FRB-11S/FKBP-87
FRB-5A2/FKBP-87
FRB-11S/FKBP-101
FRB-5A2/FKBP-101
Untreated
Rapamycin
RLU
Time (min)
70000
60000
50000
40000
30000
20000
10000
0
0
5
10
15
20
25

Figure 129

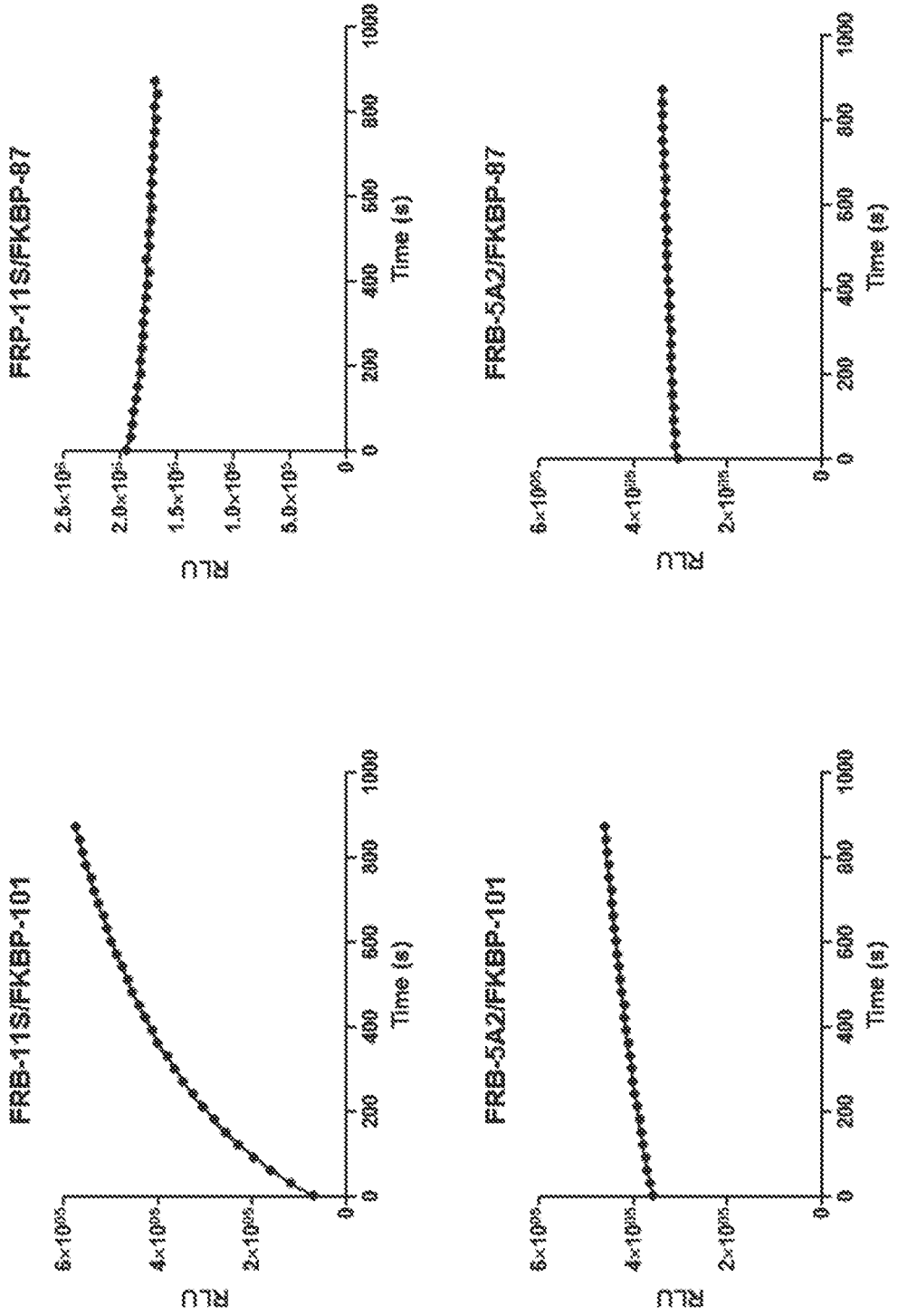

FRP-11S/FKBP-87
FRB-11S/FKBP-101
FRB-5A2/FKBP-87
FRB-5A2/FKBP-101
RLU
Time (s)

Figure 130

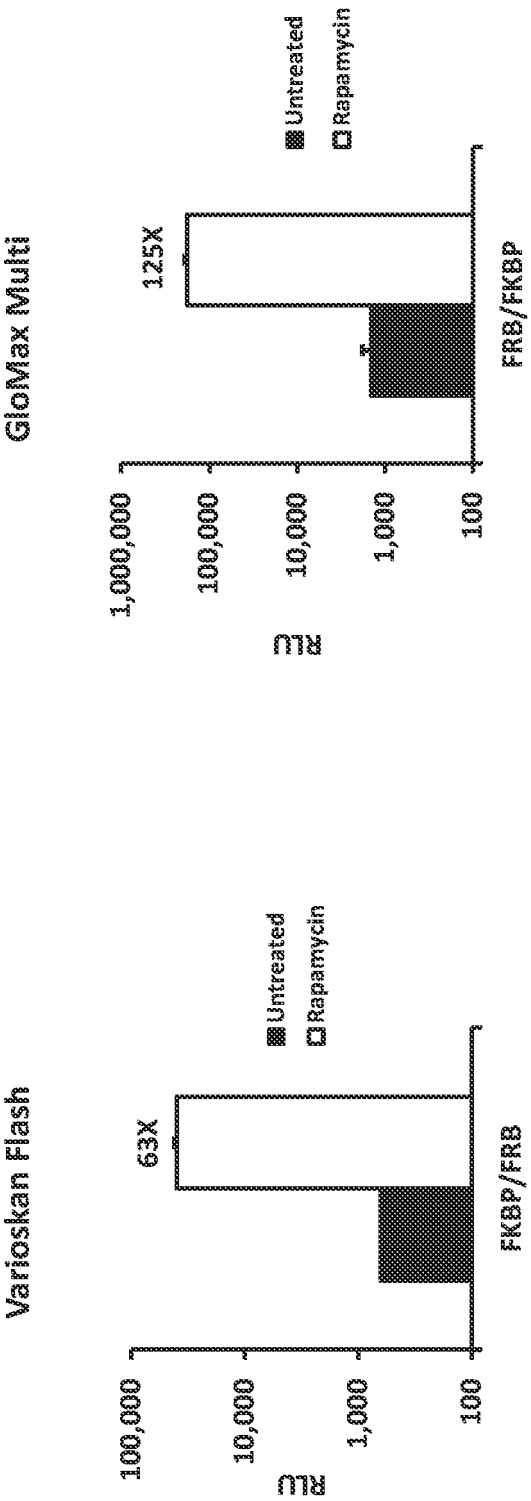

GloMax Multi
RLU
1,000,000
100,000
10,000
1,000
100
125X
Untreated
Rapamycin
FRB/FKBP
Varioskan Flash
RLU
100,000
10,000
1,000
100
63X
Untreated
Rapamycin
FKBP/FRB

Figure 131

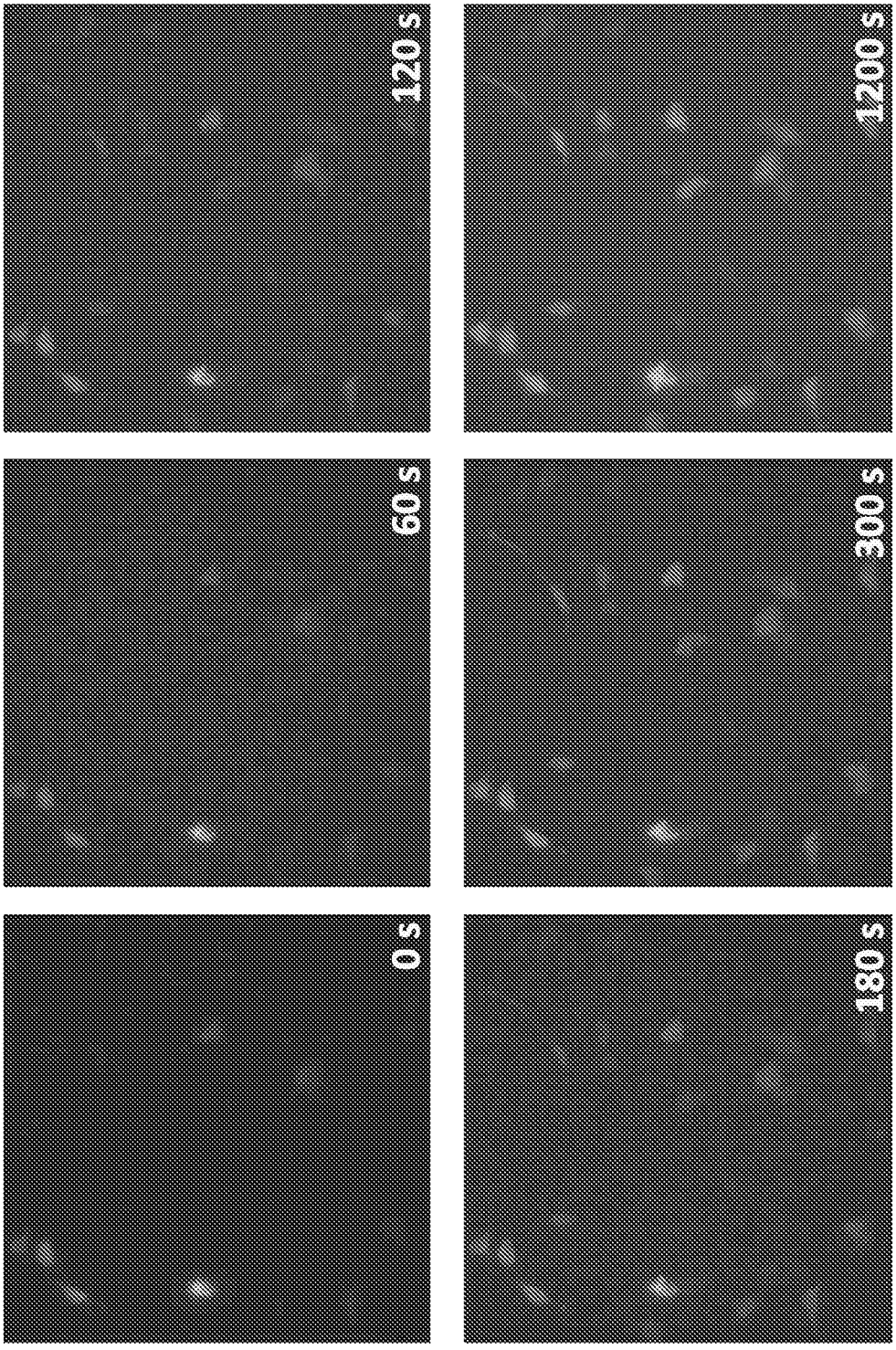


Figure 132

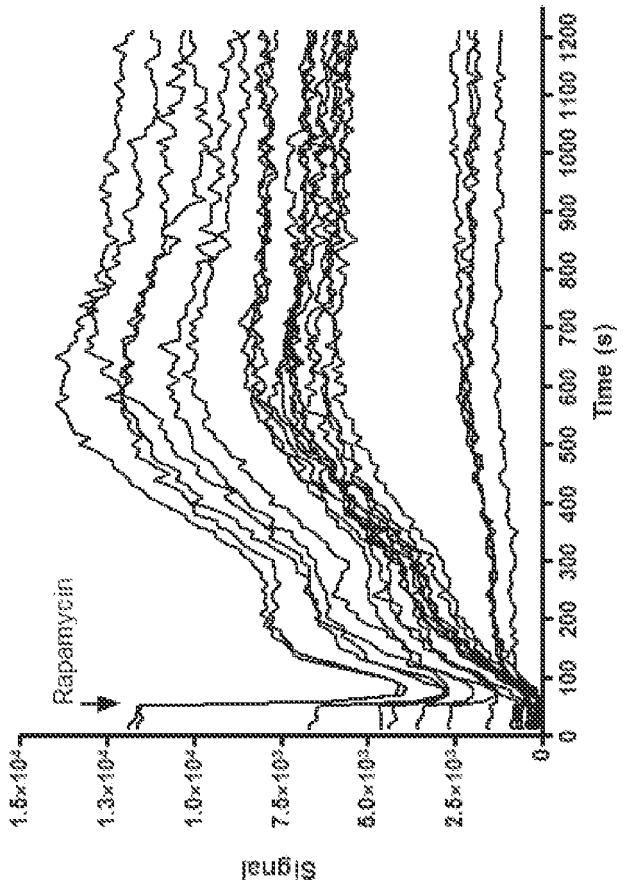
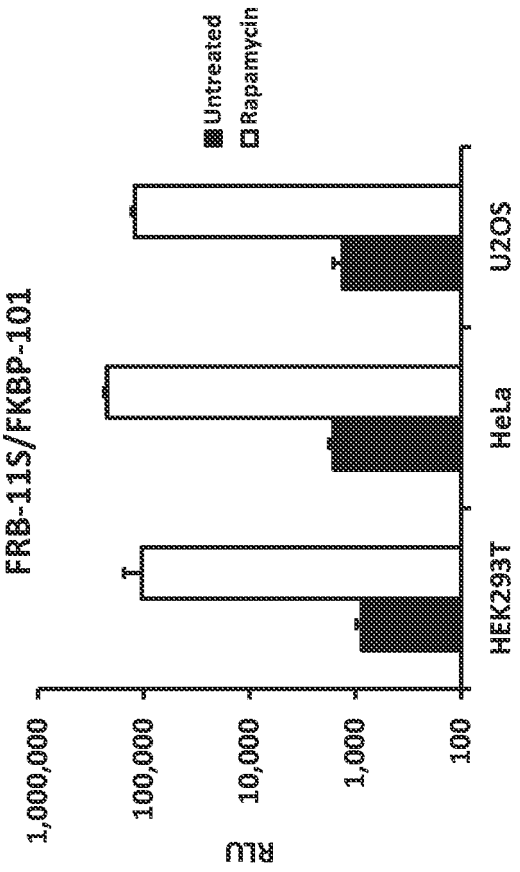

FRB-11S/FKBP-101
Rapamycin
Signal
Time (s)
1.6x10⁴
1.3x10⁴
1.0x10⁴
7.8x10³
5.0x10³
2.5x10³
0
0
100
200
300
400
500
600
700
800
900
1000
1100
1200

Figure 13



Cell Line	Fold Induction
HEK293T	121
HeLa	140
U2OS	92

Figure 134

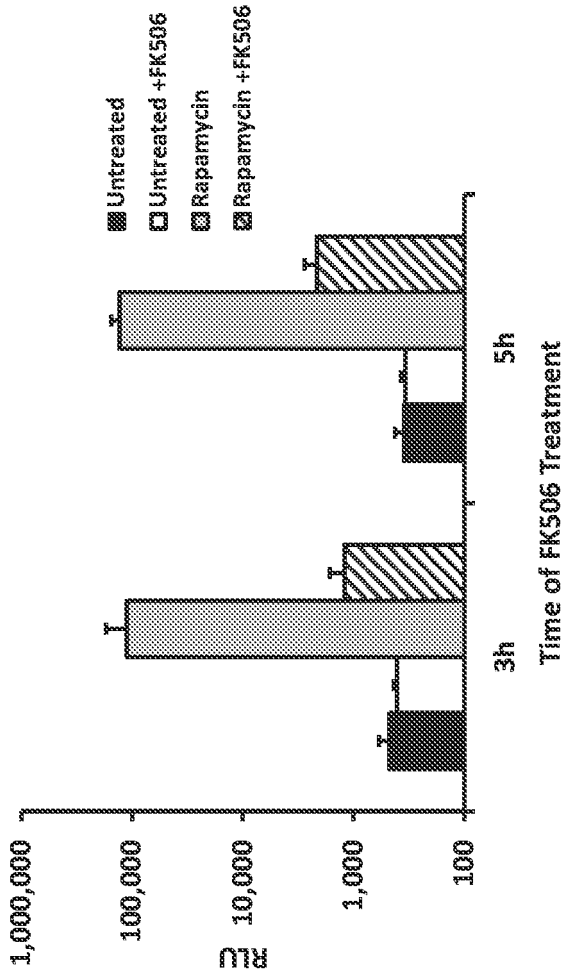

Untreated
Untreated +FK506
Rapamycin
Rapamycin +FK506
1,000,000
100,000
10,000
1,000
100
RLU
3h
5h
Time of FK506 Treatment

Figure 13

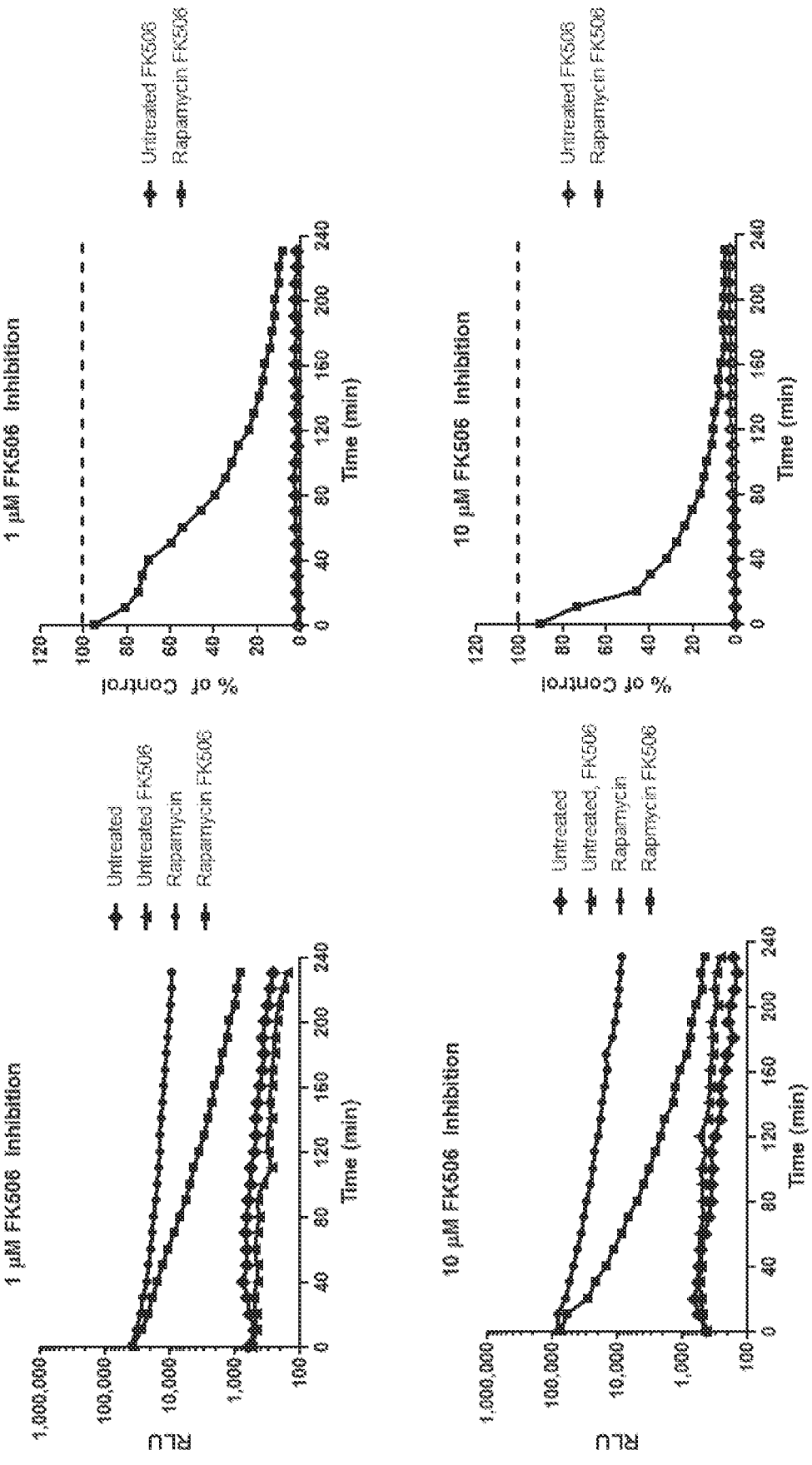

1 μM FK506 Inhibition
% of Control
Time (min)
Untreated FK506
Rapamycin FK506
10 μM FK506 Inhibition
% of Control
Time (min)
Untreated FK506
Rapamycin FK506
1 μM FK506 Inhibition
RLU
Time (min)
Untreated
Untreated FK506
Rapamycin
Rapamycin FK506
10 μM FK506 Inhibition
RLU
Time (min)
Untreated
Untreated, FK506
Rapamycin
Rapamycin FK506

Figure 136

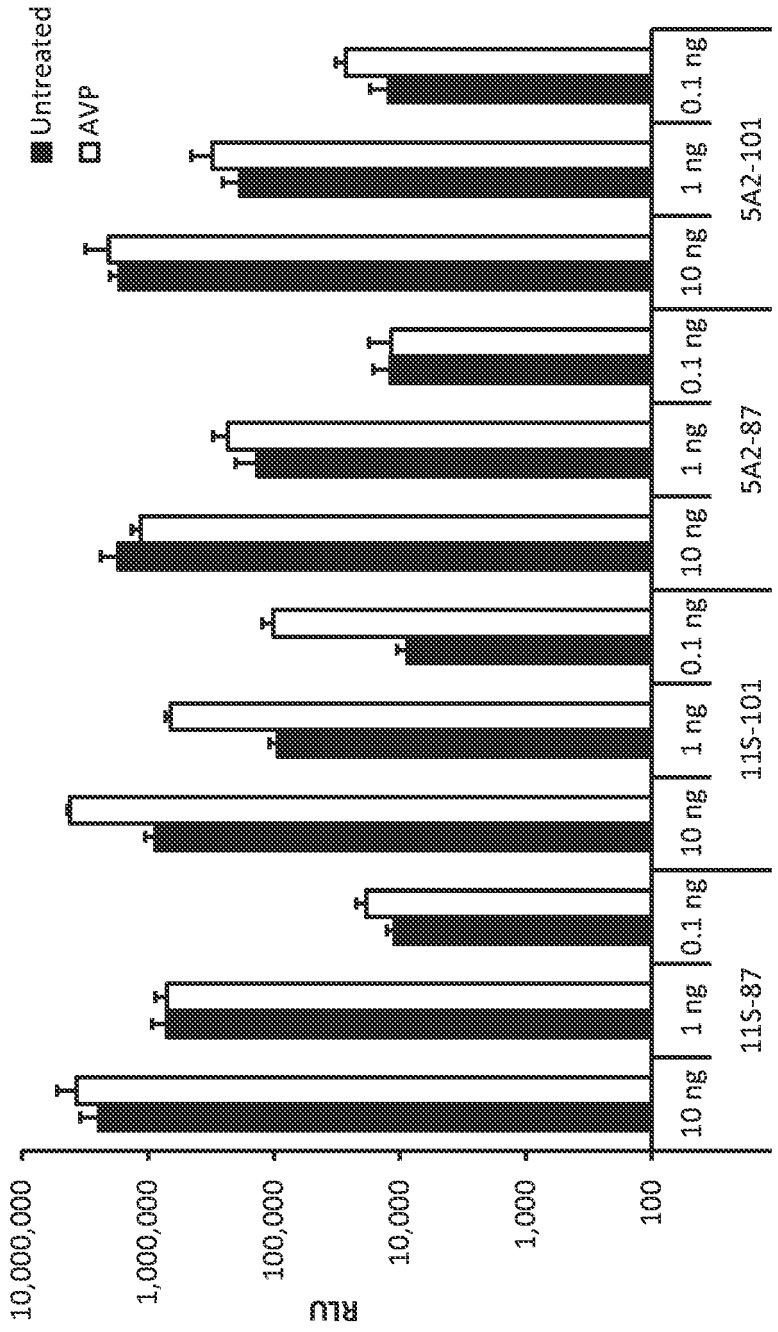

V2R-NLpoly11S and NLpep87/101-ARRB2
Untreated
AVP
RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
10 ng
1 ng
0.1 ng
11S-87
11S-101
5A2-87
5A2-101

Figure 137

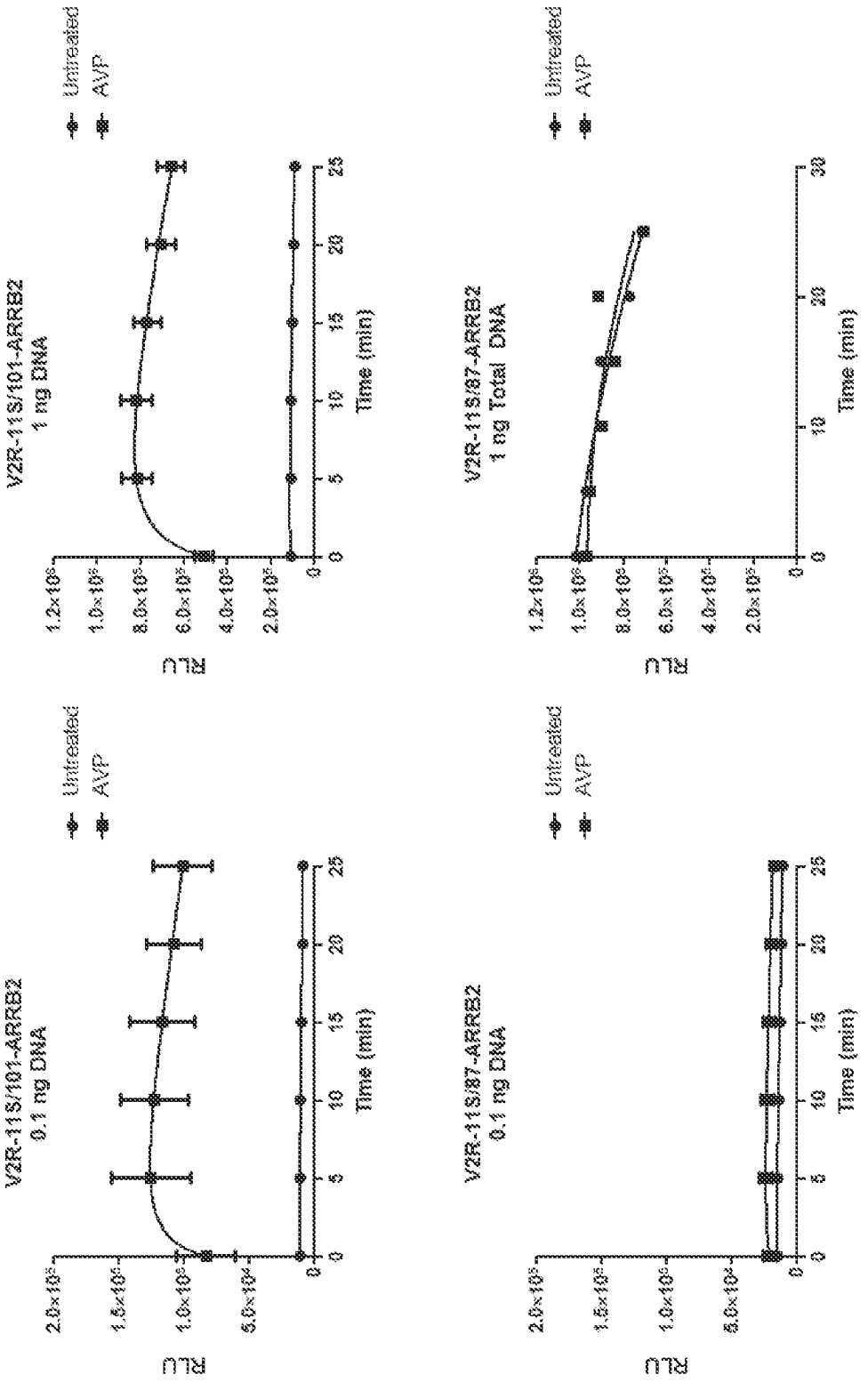

V2R-11S/101-ARRB2
1 ng DNA
Untreated
AVP
RLU
Time (min)
V2R-11S/87-ARRB2
1 ng Total DNA
V2R-11S/101-ARRB2
0.1 ng DNA
V2R-11S/87-ARRB2
0.1 ng DNA

Figure 138

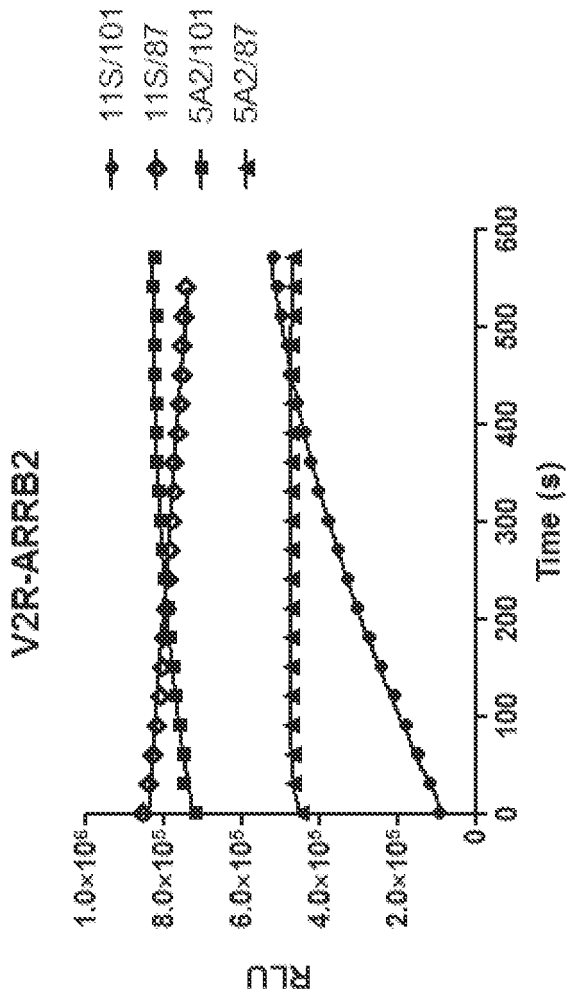

V2R-ARRB2
11S/101
11S/87
5A2/101
5A2/87
RLU
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0
0
100
200
300
400
500
600
Time (s)

Figure 139

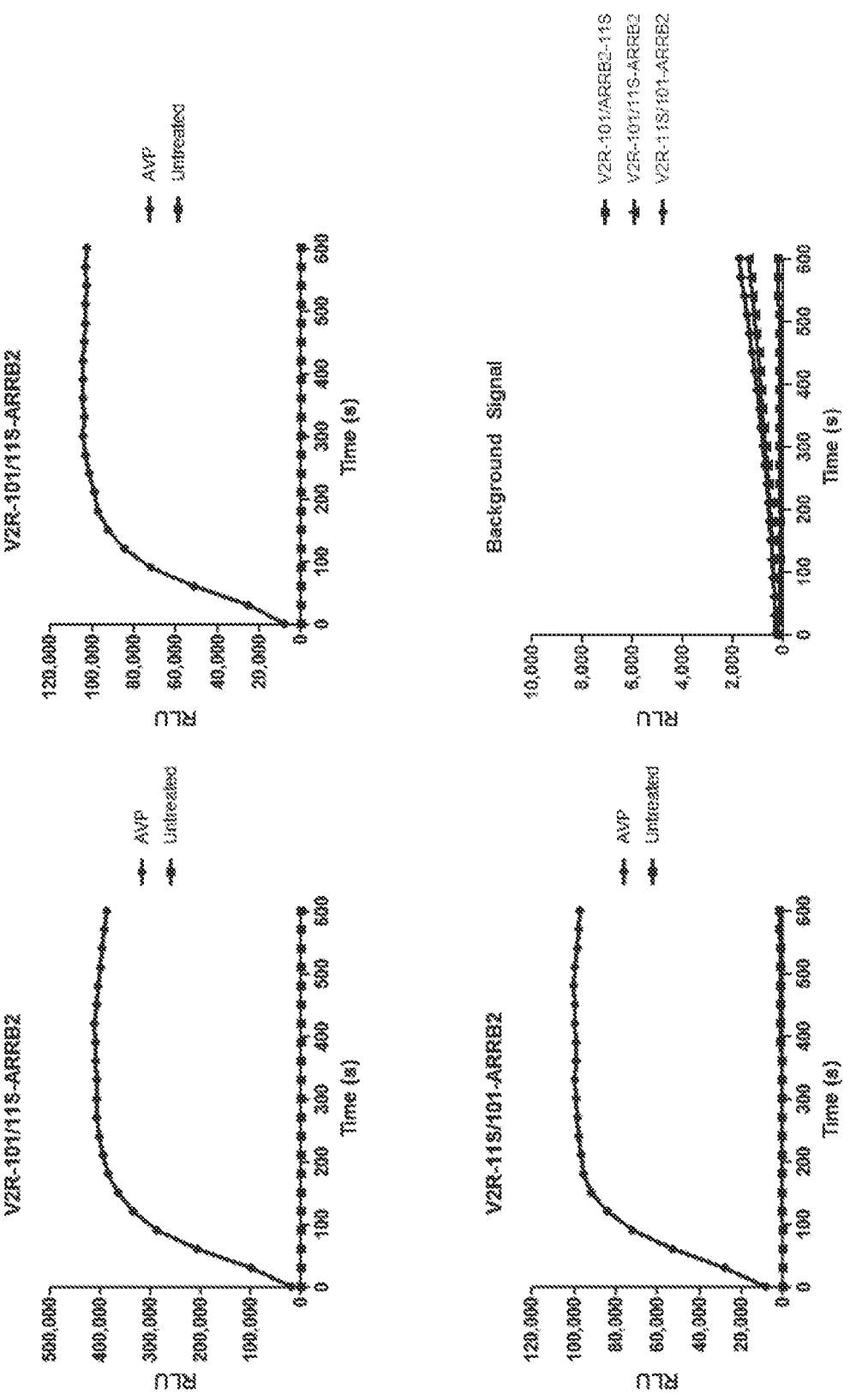
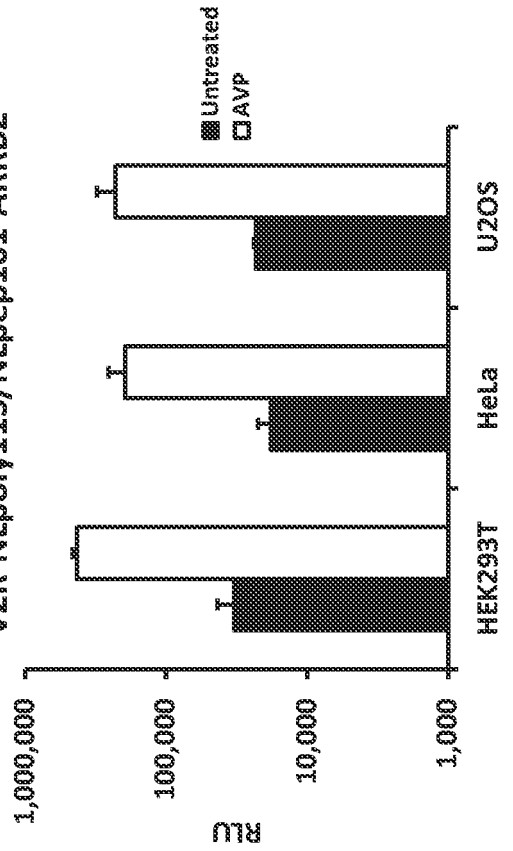

V2R-101/118-ARRB2
AVP
Untreated
RLU
Time (s)
V2R-101/118-ARRB2
AVP
Untreated
RLU
Time (s)
Background Signal
V2R-101/ARRB2-118
V2R-101/118-ARRB2
V2R-118/101-ARRB2
RLU
Time (s)
V2R-118/101-ARRB2
AVP
Untreated
RLU
Time (s)

Figure 140



Cell line	Fold Induction
HEK293T	13
HeLa	11
U2OS	10

Figure 141

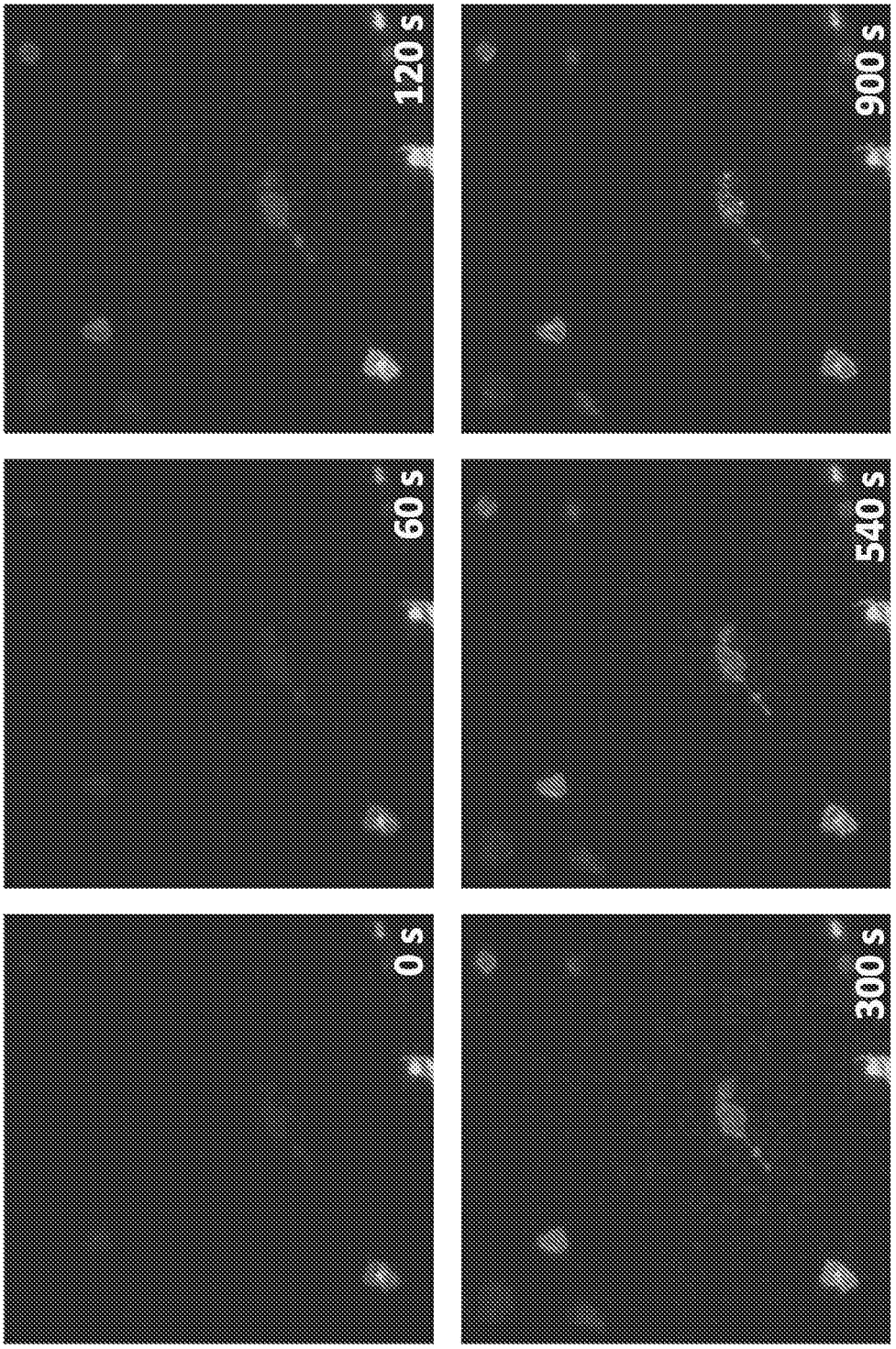


Figure 142

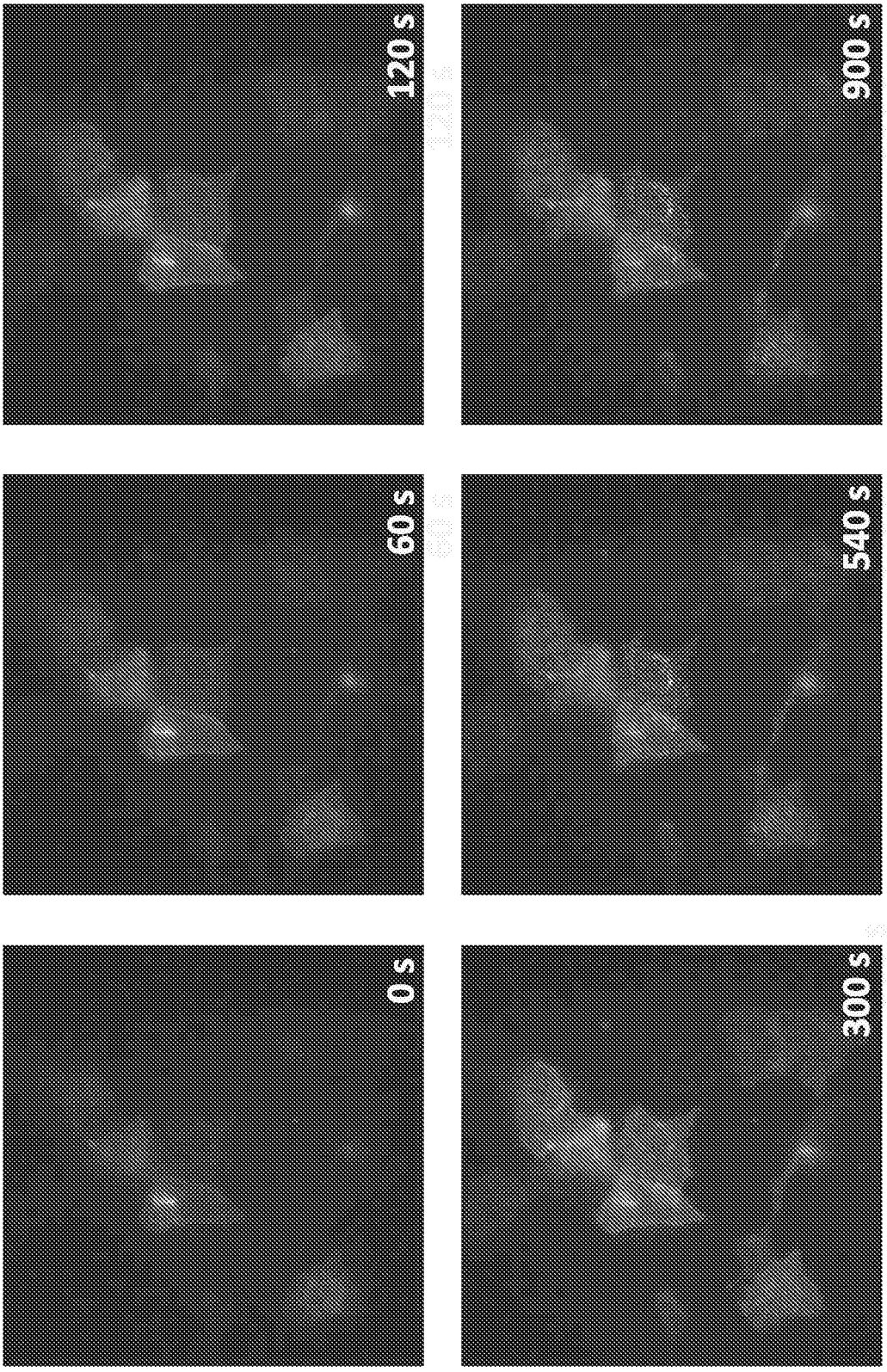
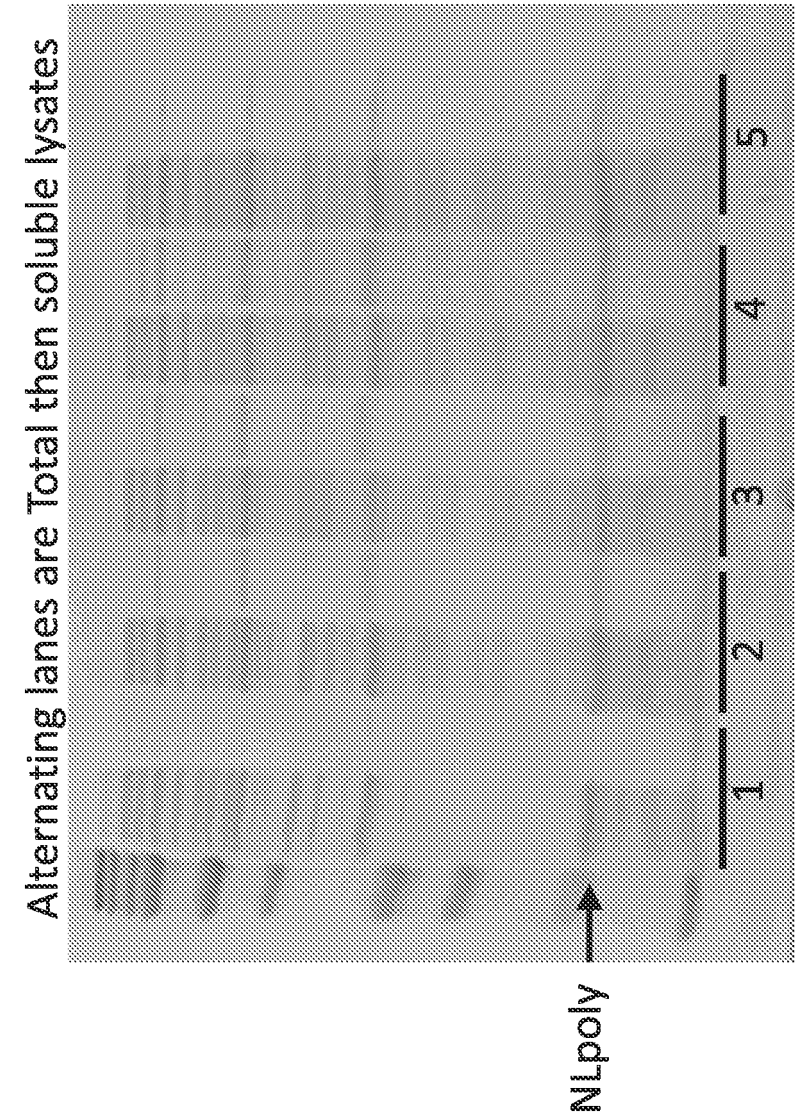


Figure 143



1. 5P
2. 5P+L46R+H93P+P157S
3. 8S
4. 5P+L46R+H93P+P157S+K75E
5. 5P+L46R+H93P+P157S+I76V

Figure 144

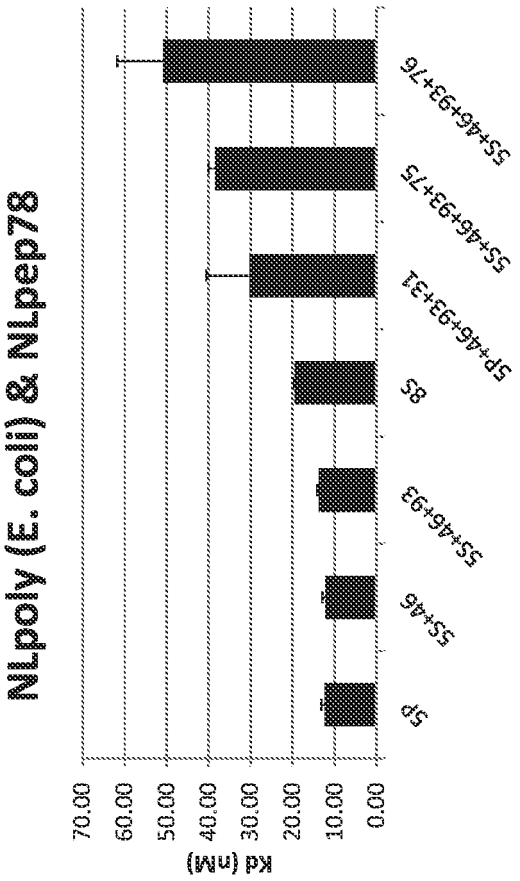

NLpoly (E. coli) & NLpep78
Kd (nM)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
SP
S5+46
S5+46+93
8S
SP+46+93+31
S5+46+93+75
S5+46+93+76

Figure 145

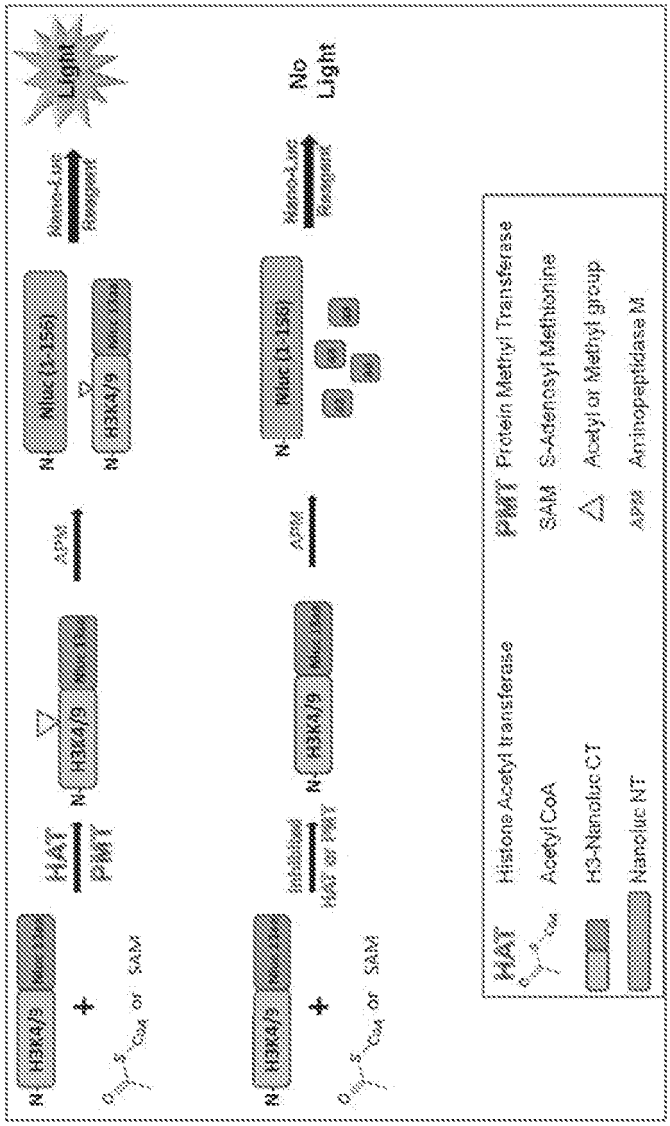

HAT
PMT
APPM
Light
No Light
HAT Histone Acetyl transferase
PMT Protein Methyl Transferase
Acetyl CoA
SAM S-Adenosyl Methionine
H3-Nanoluc CT
Acetyl or Methyl group
Nanoluc NT
APPM Aminopeptidase M

Figure 146

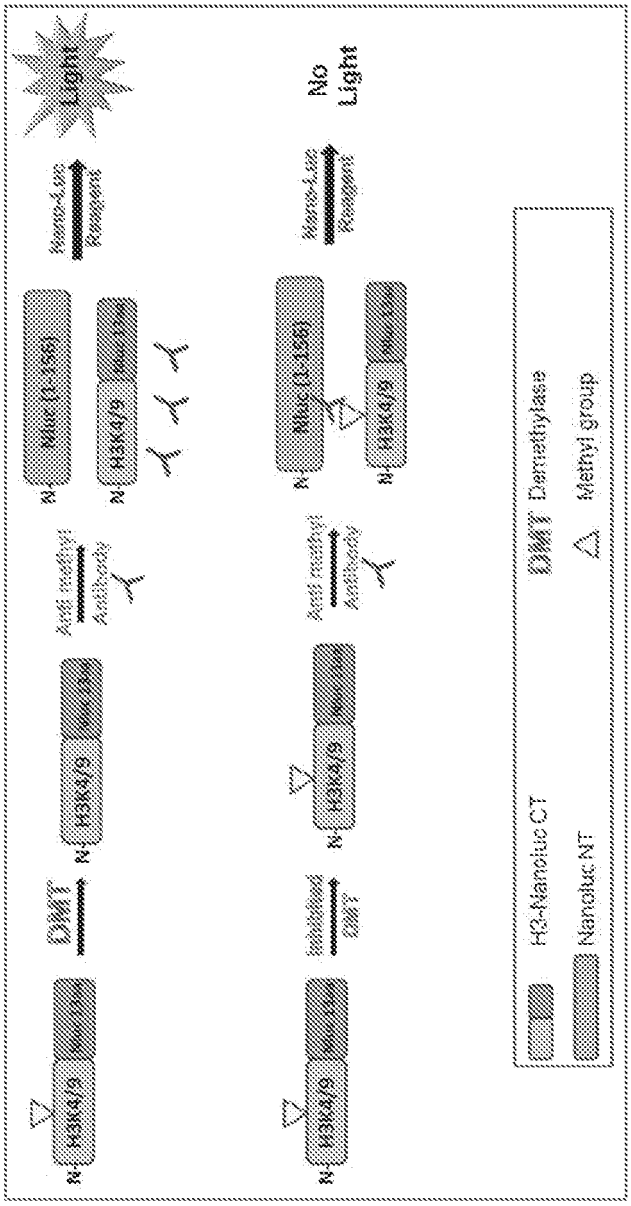
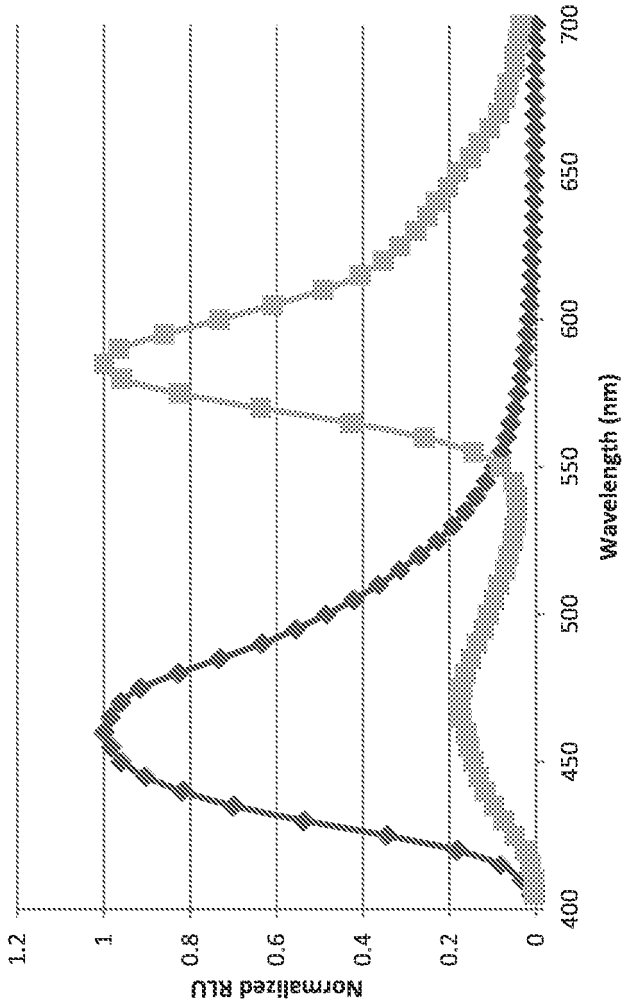

Light
No Light
Nano-Lux Reagent
Anti methyl Antibody
DMT
H3K4/9
H3-Nanoluc CT
Nanoluc NT
Demethylase
Methyl group

Figure 147


Energy Transfer
NLpep1
NLpep1-TMR
Normalized RLU
Wavelength (nm)
1.2
1
0.8
0.6
0.4
0.2
0
400
450
500
550
600
650
700

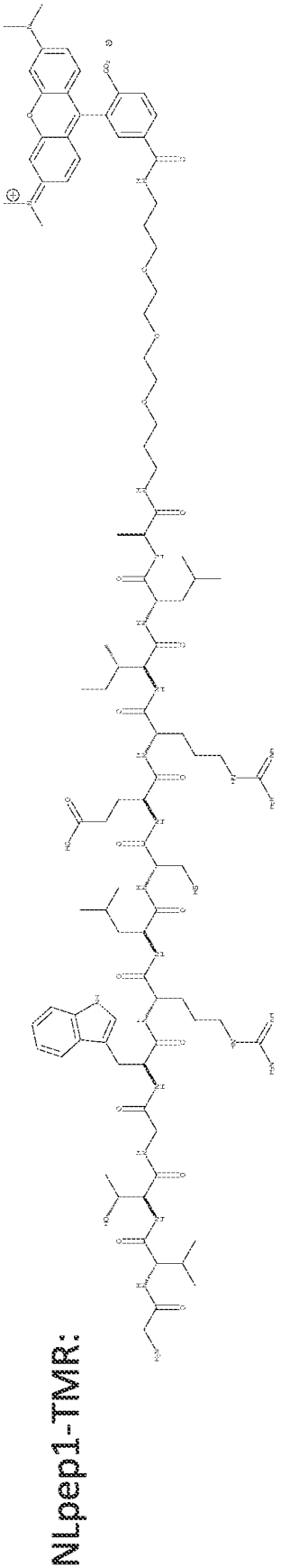
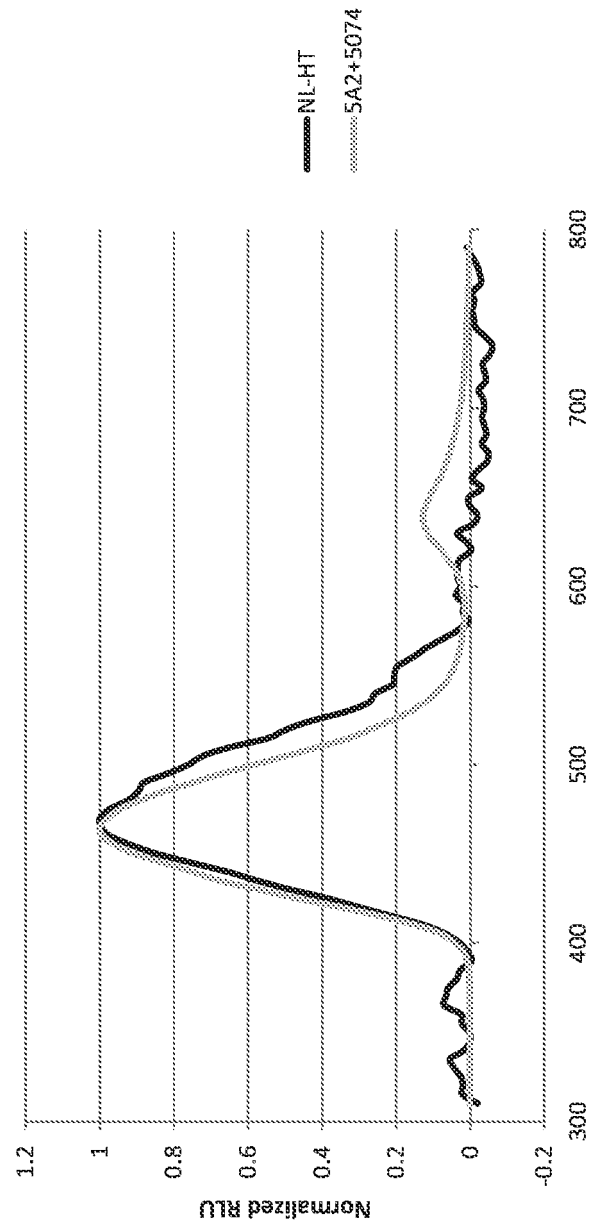

NLpep1-TMR:

Figure 148



5074 = NCT-DEVDGVTGWRLCERILA

Figure 149

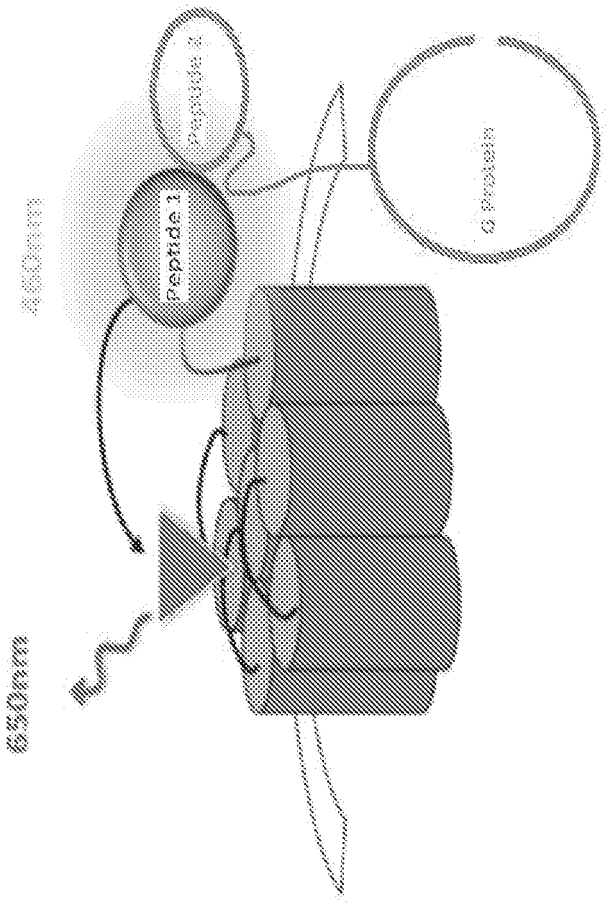
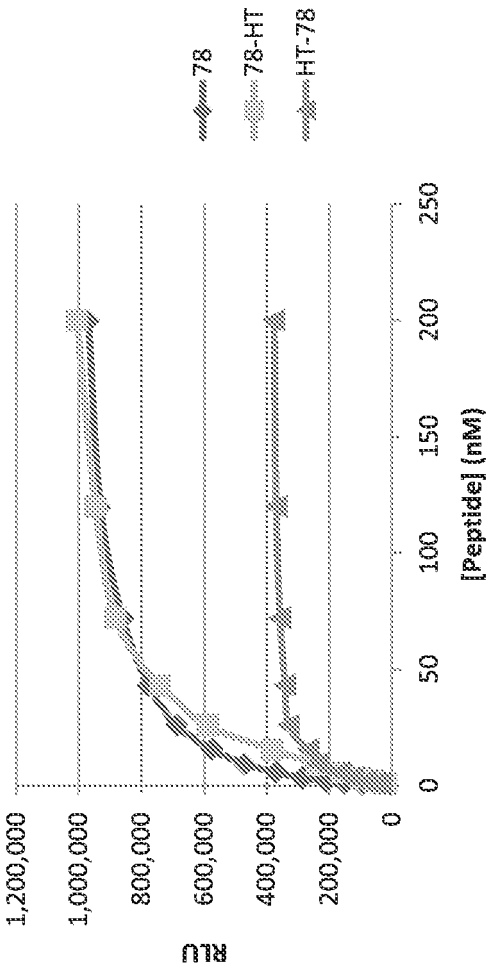
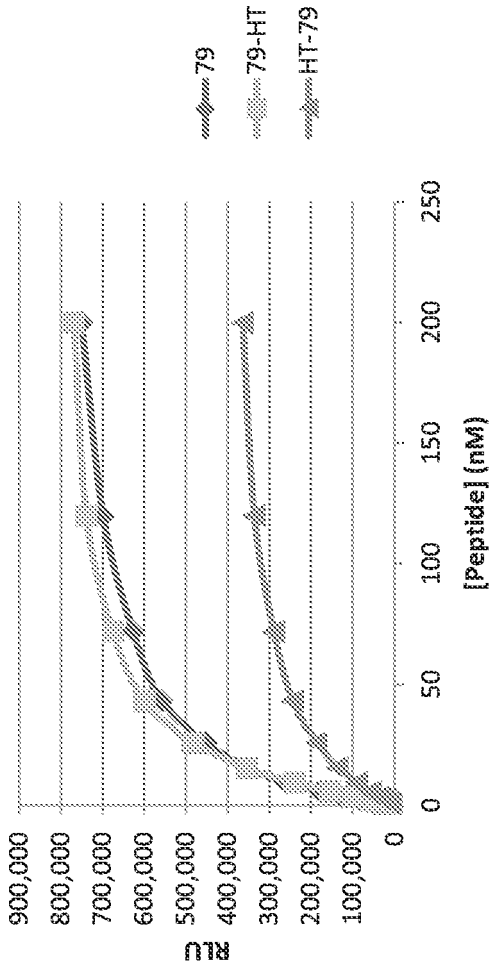


Figure 150



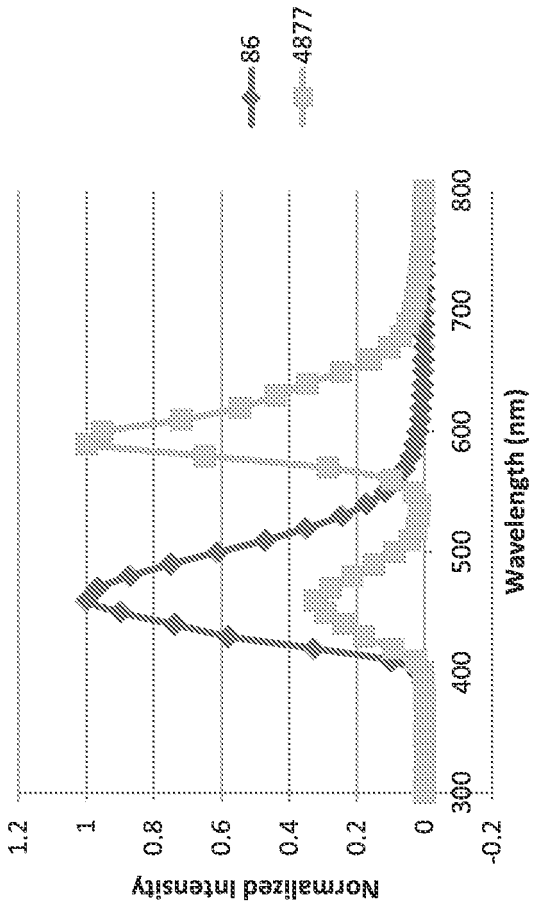
One site -- Specific binding			
	78	78-HT	HT-78
Best-fit values			
Bmax	981727	1200000	396501
Kd	9.558	30.25	7.21
Std. Error			
Bmax	20830	36475	5393
Kd	0.7688	2.667	0.3928

Figure 151



One site -- Specific binding				
	79	79-HT	HT-79	
Best-fit values				
Bmax	816453	879828	427978	
Kd	20.26	22.9	33.87	
Std. Error				
Bmax	5774	15250	3600	
Kd	0.4603	1.237	0.8009	

Figure 152



PBI-4877:

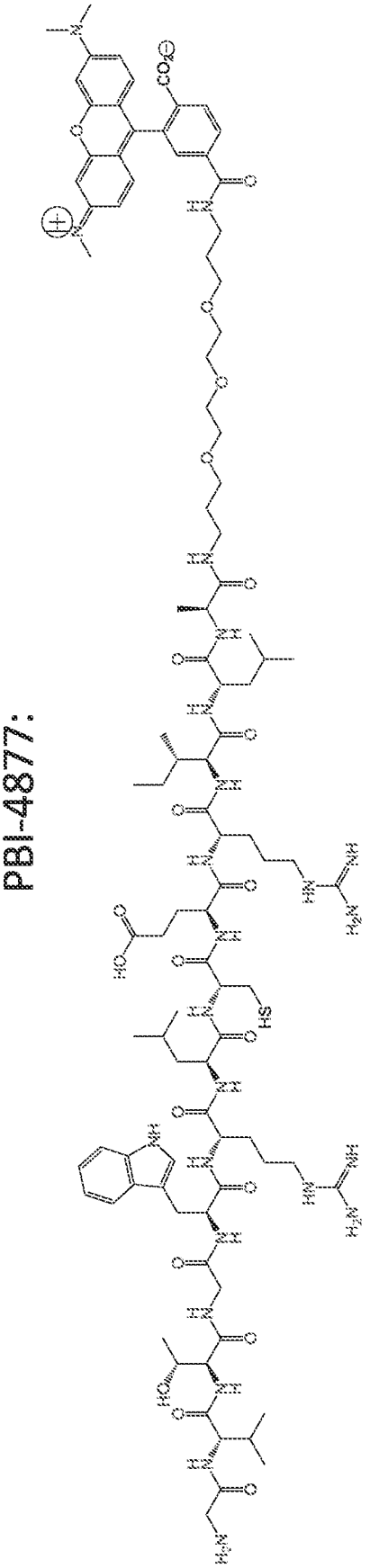
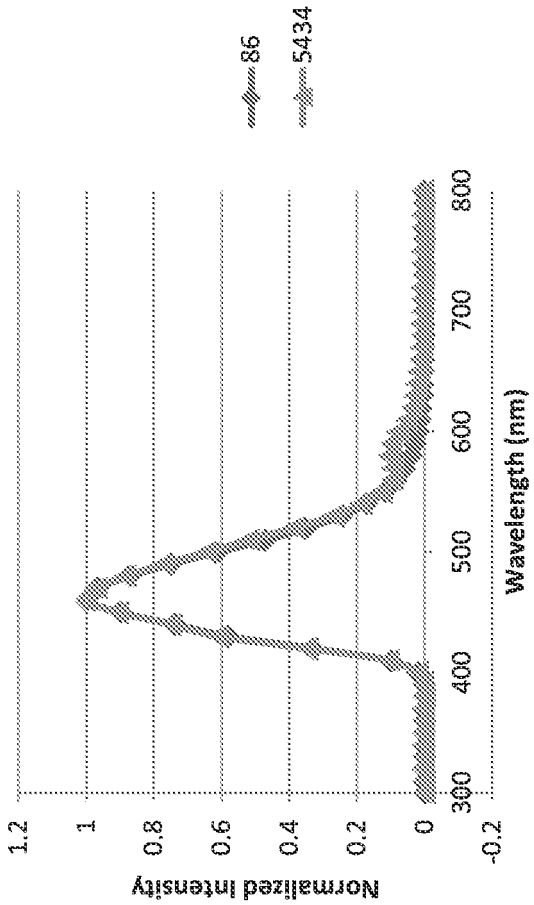


Figure 153



PBI-5434:

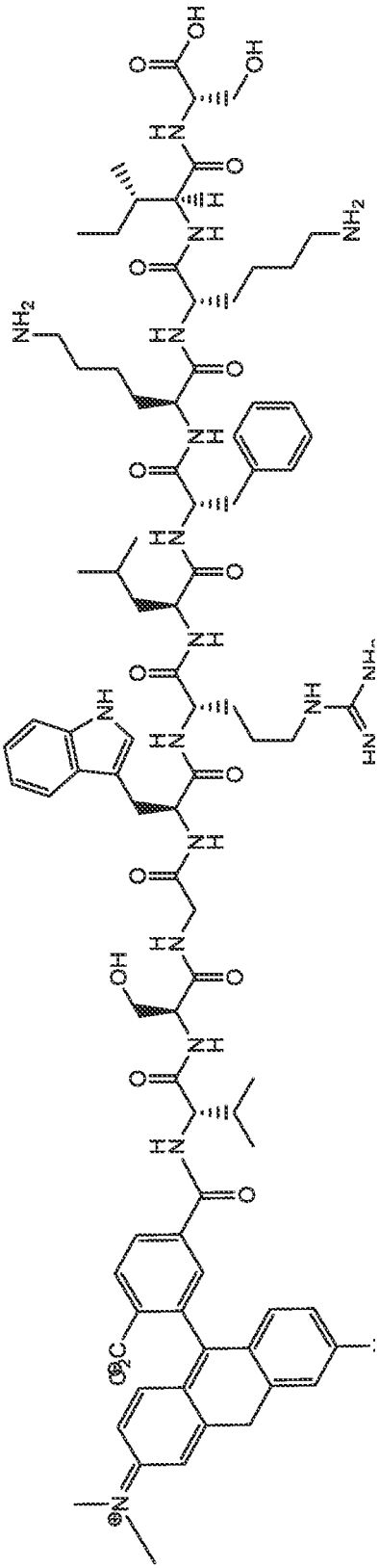
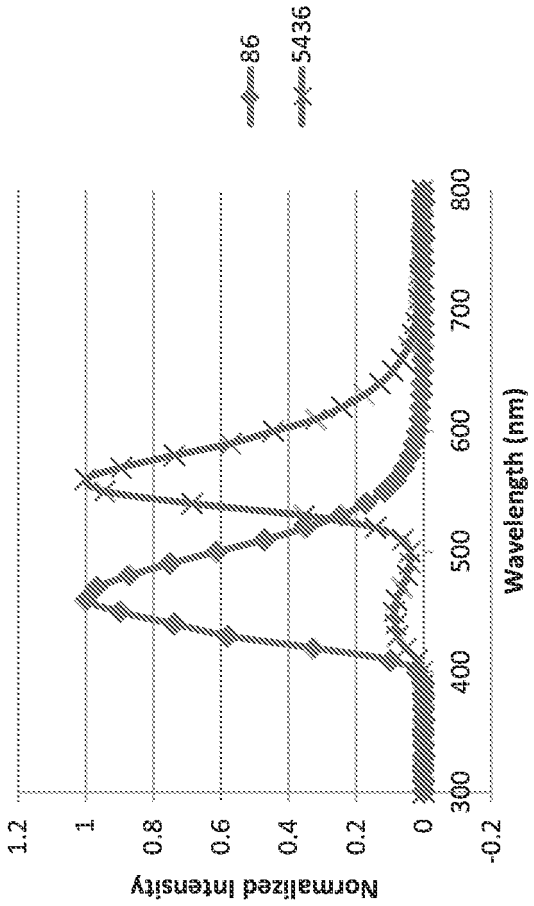


Figure 154



PBI 5436:

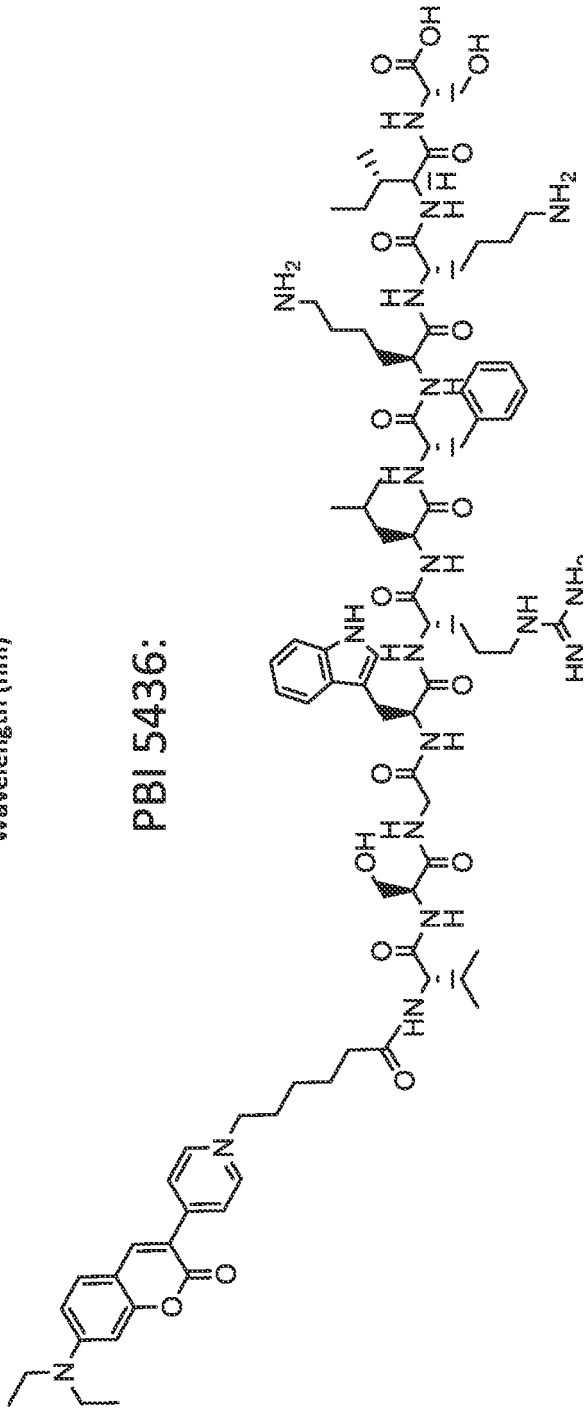


Figure 155

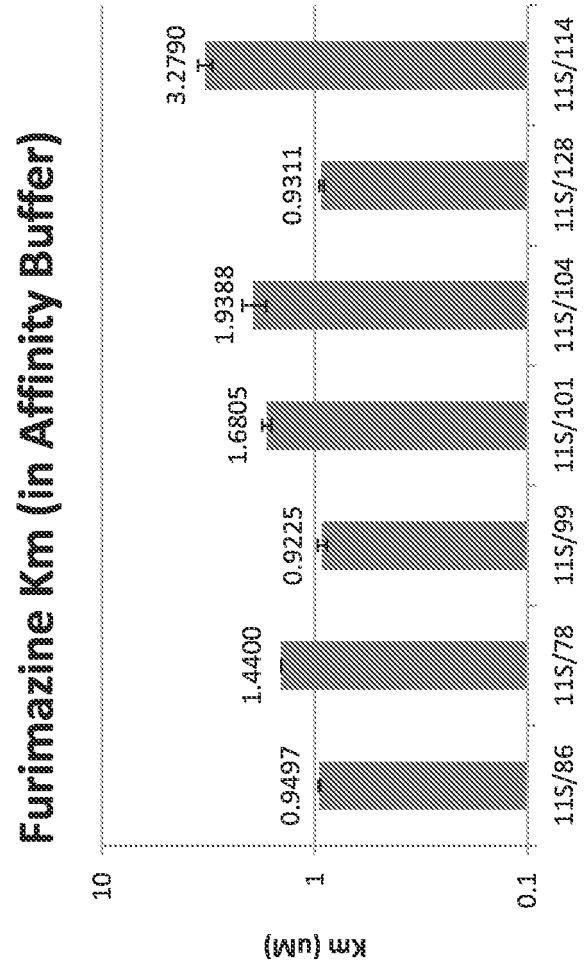

Furimazine Km (in Affinity Buffer)
Km (uM)
10
1
0.1
0.9497
1.4400
0.9225
1.6805
1.9388
0.9311
3.2790
115/86
115/78
115/99
115/101
115/104
115/128
115/114

Figure 156

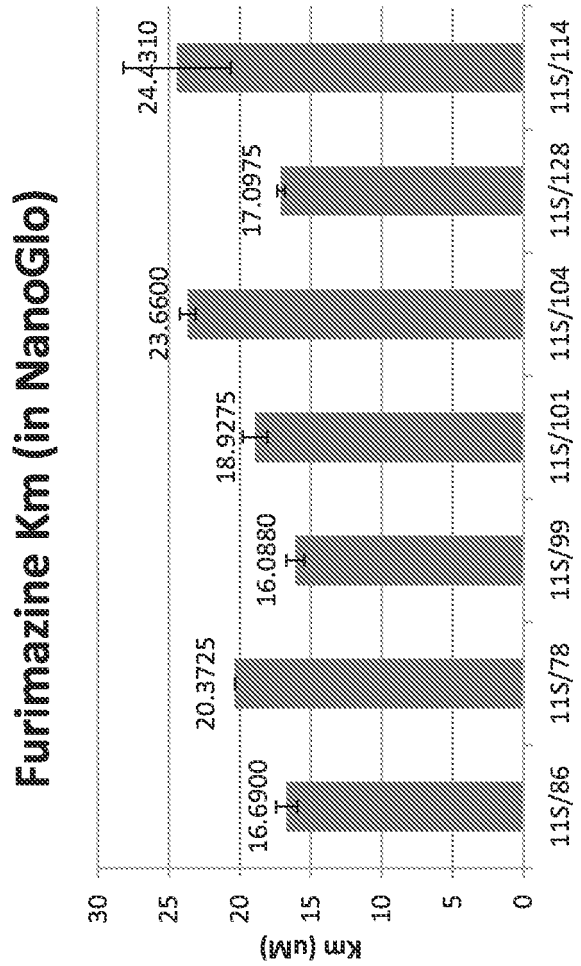
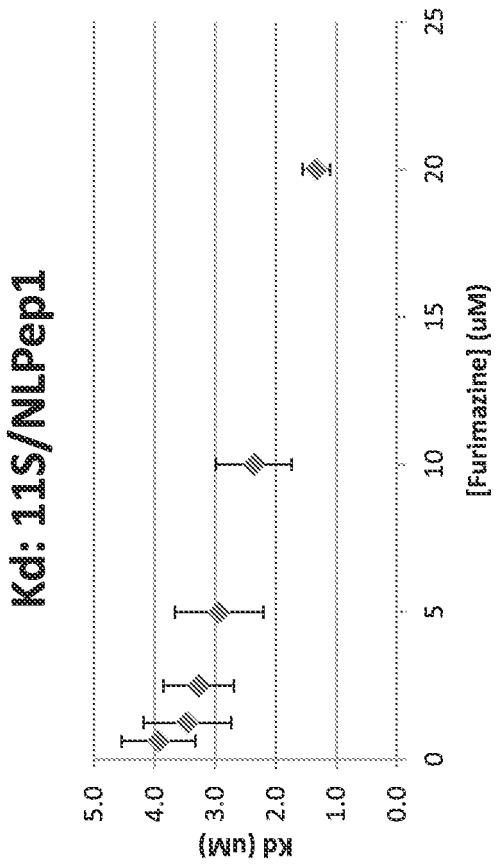

Furimazine Km (in NanoGlo)
Km (uM)
30
25
20
15
10
5
0
16.6900
20.3725
16.0880
18.9275
23.6600
17.0975
24.4310
11S/86
11S/78
11S/99
11S/101
11S/104
11S/128
11S/114

Figure 157


Kd: 11S/NLPep1
Kd (uM)
5.0
4.0
3.0
2.0
1.0
0.0
0
5
10
15
20
25
[Furimazine] (uM)

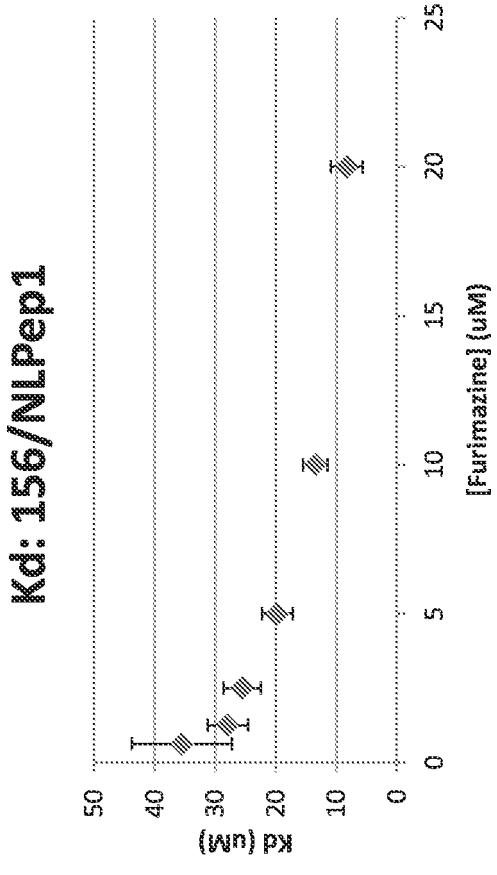
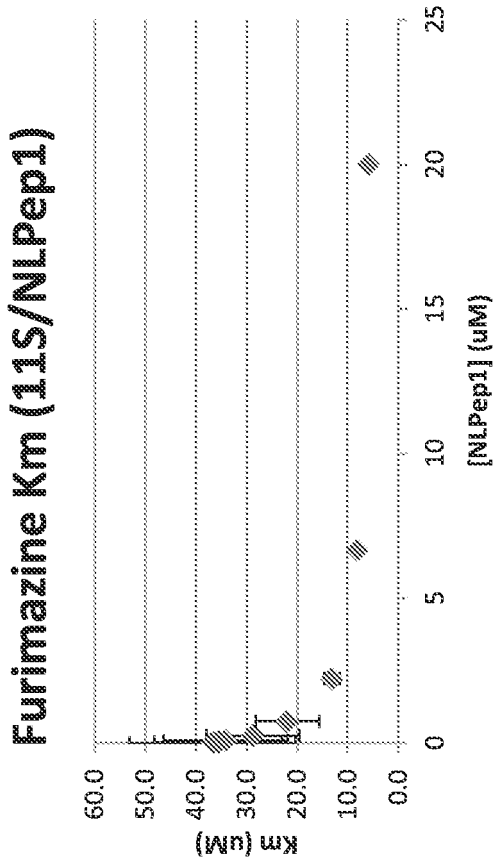

Kd: 156/NLPep1
Kd (uM)
50
40
30
20
10
0
0
5
10
15
20
25
[Furimazine] (uM)

Figure 158


Furimazine Km (11S/NLPep1)
Km (uM)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
5
10
15
20
25
[NLPep1] (uM)

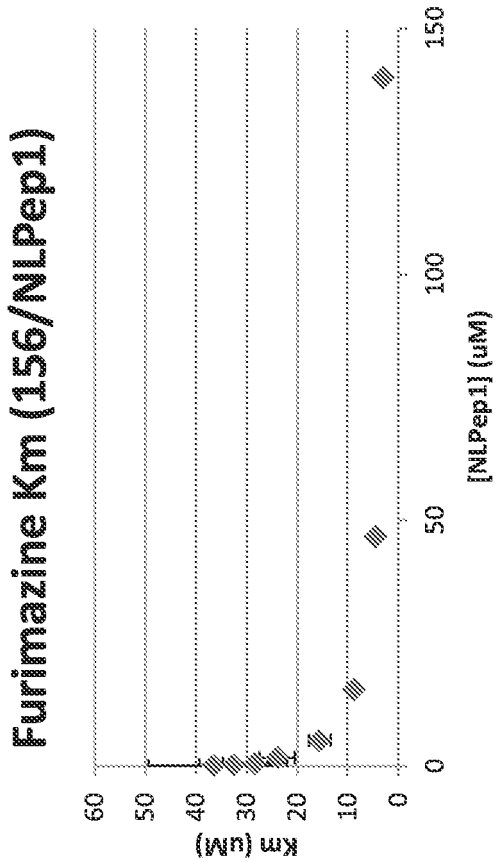

Furimazine Km (156/NLPep1)
Km (uM)
60
50
40
30
20
10
0
0
50
100
150
[NLPep1] (uM)

Figure 159

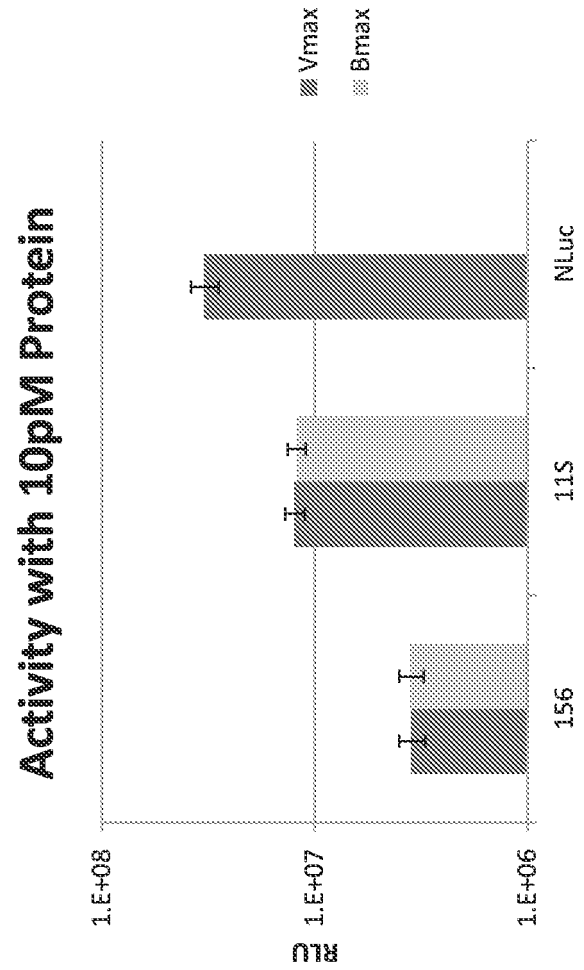
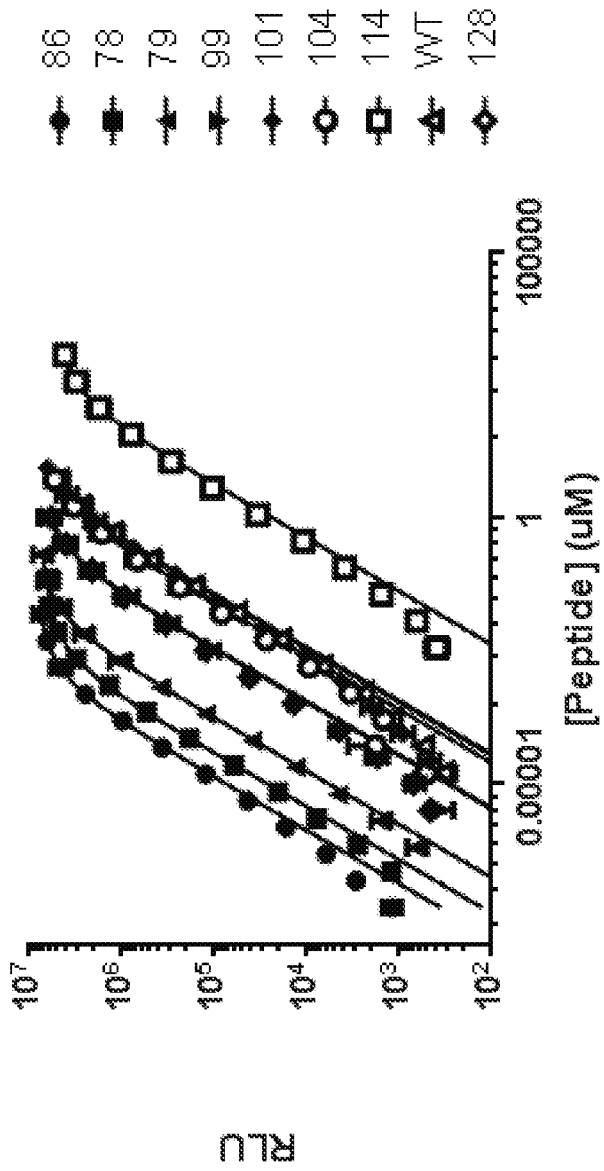

Activity with 10pM Protein
RLU
1.E+08
1.E+07
1.E+06
Vmax
Bmax
NLuc
11S
156

Figure 160



One site -- Specific binding

Best-fit values	86	78	79	99	101	104	114	WT	128
Bmax	7.6e+006	6.3e+006	7.5e+006	7.6e+006	8.5e+006	7.0e+006	4.8e+006	7.2e+006	7.0e+006
Kd (uM)	0.001021	0.002392	0.01306	0.2665	3.124	1.745	199.0	2.461	0.2372
Std. Error									
Bmax	416495	333627	449697	730715	820341	678552	378115	977108	78079
Kd	0.0002090	0.0005326	0.002957	0.06923	0.7336	0.4185	47.23	0.7378	0.007362

Figure 161

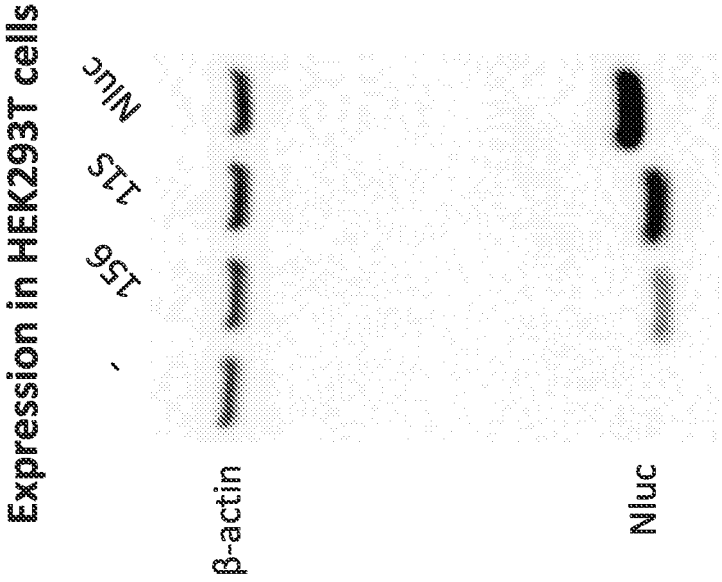
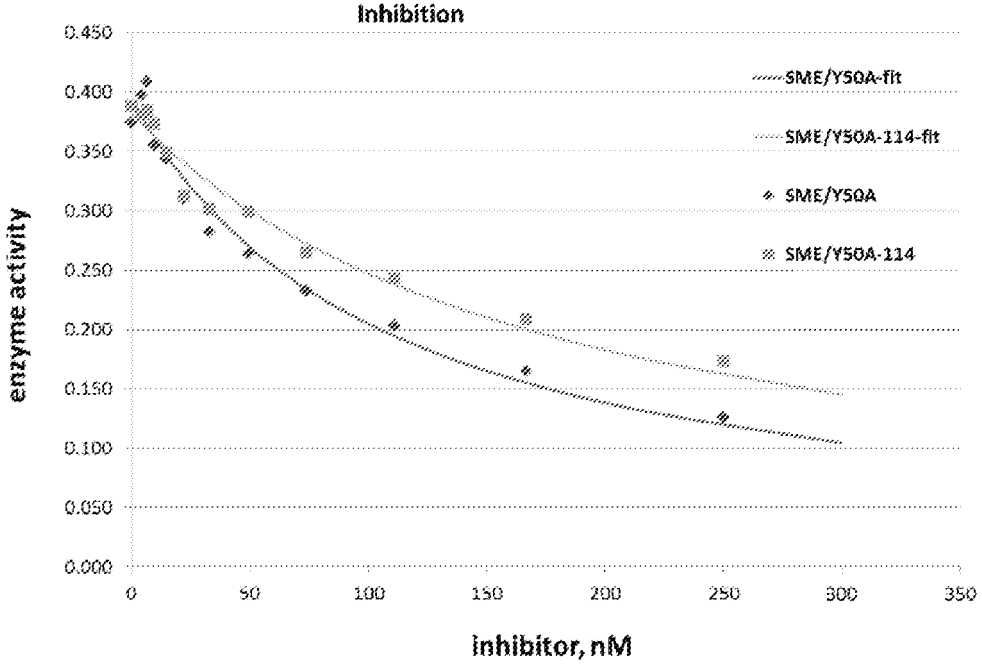
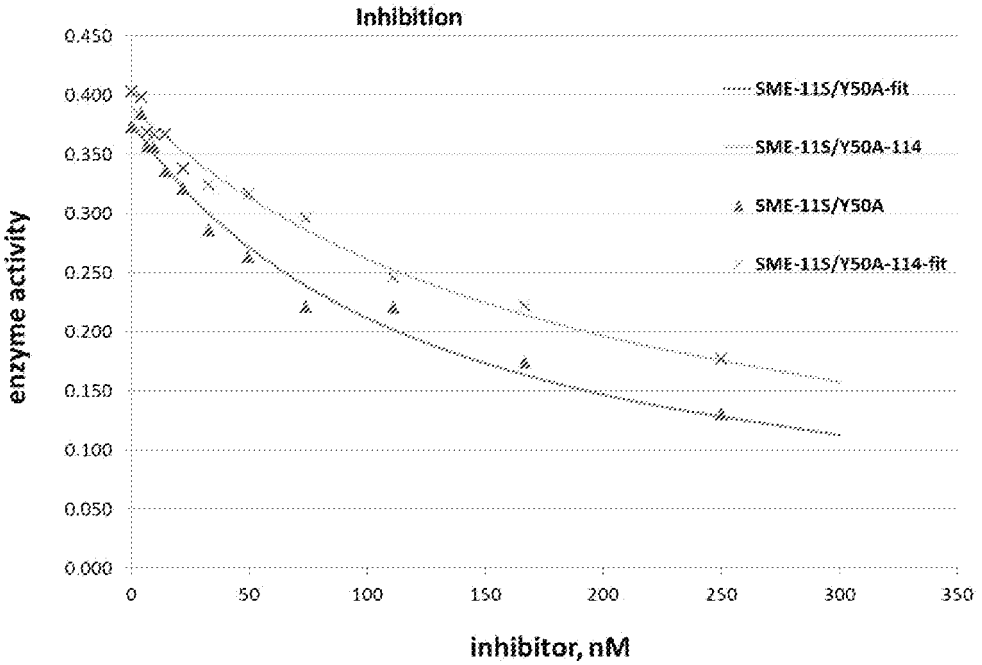
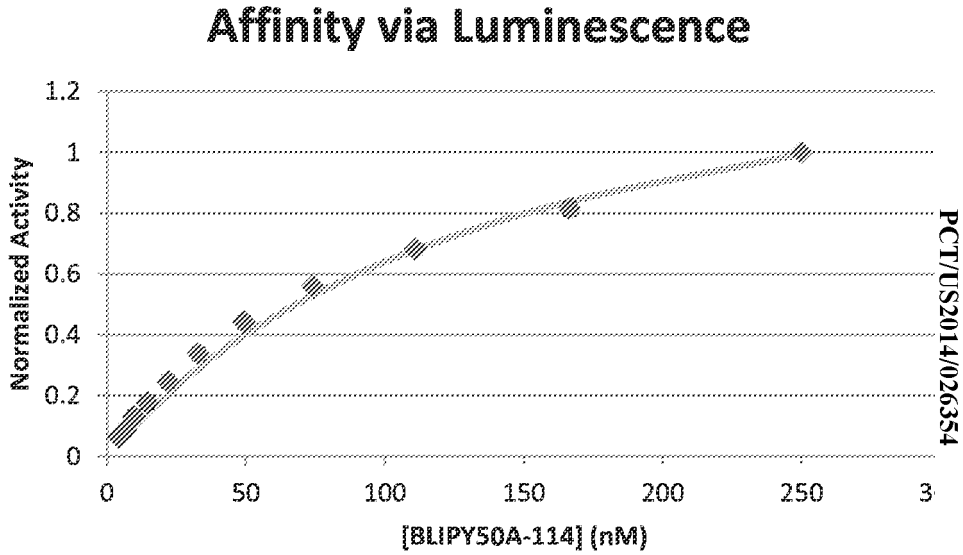


Figure 162





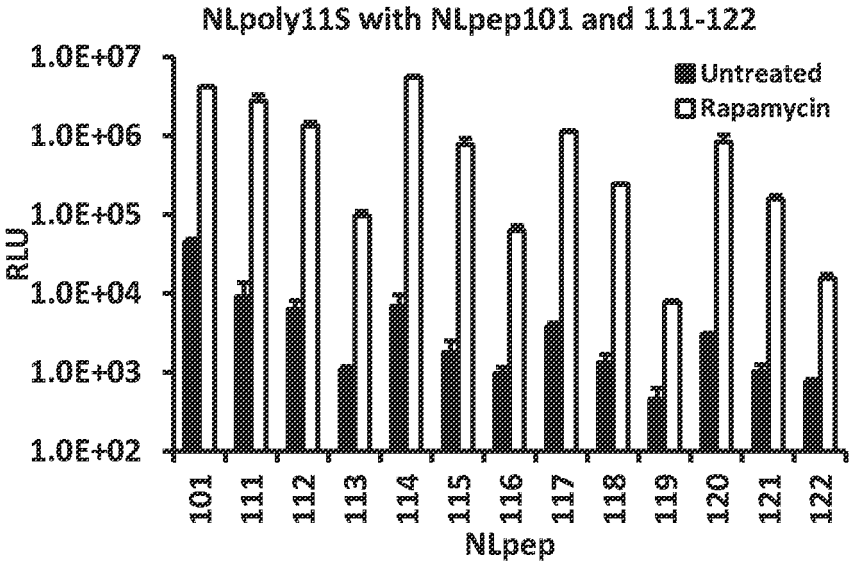
	Inhibition (β-lactamase activity)				Luminescence (NLPep/NLPoly)
SME:	Unfused	+11S	Unfused	+11S	+11S
BLIPY50A:	Unfused	Unfused	+114	+114	+114
Ki/Kd	Ki	Ki	Ki	Ki	Kd
nM	84.1	99.0	142.9	157.3	147.5

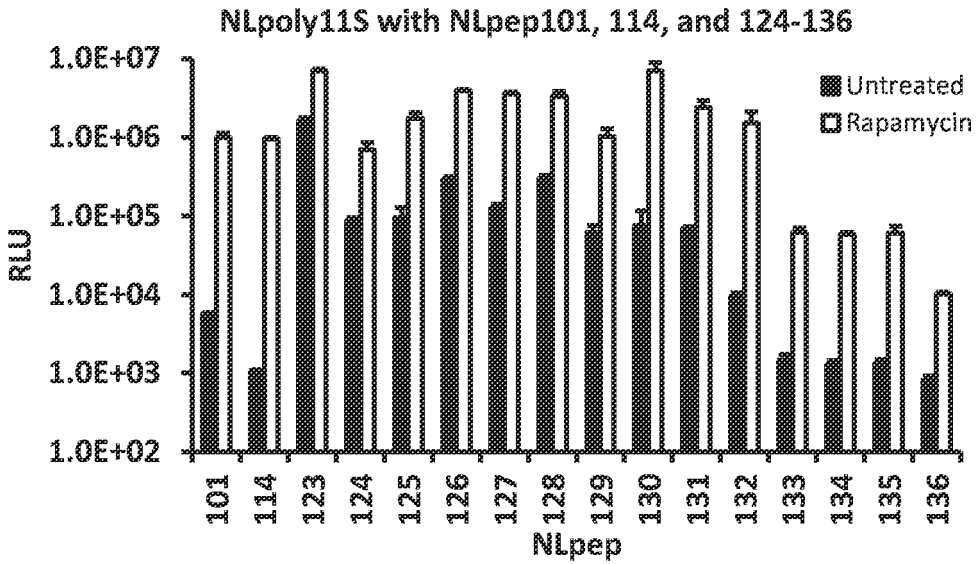


WO 2014/151736
PCT/US2014/026354

SUBSTITUTE SHEET (RULE 26)

Figure 163





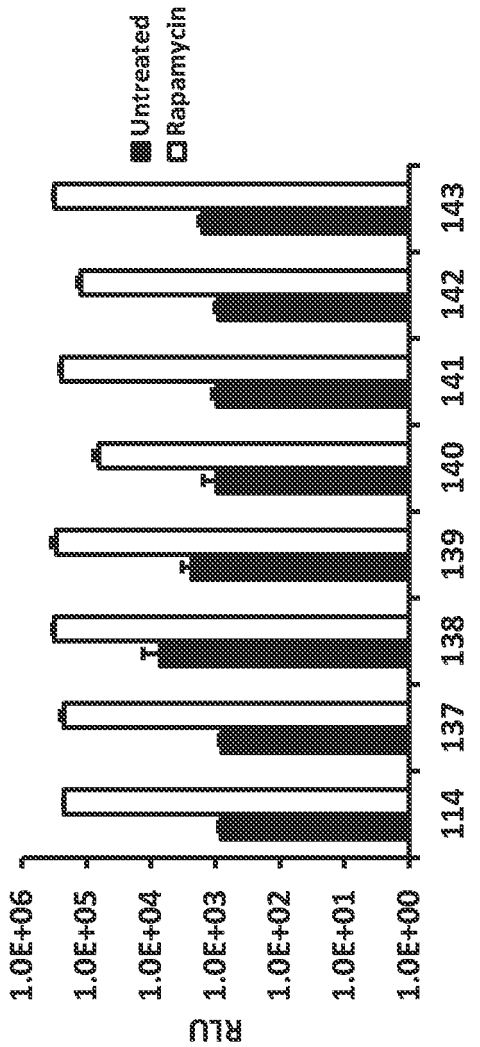
NLpep	Induction
101	92
111	304
112	213
113	90
114	800
115	430
116	64
117	302

NLpep	Induction
118	183
119	17
120	273
121	152
122	21

NLpep	Induction
101	177
114	887
123	4
124	8
125	18
126	14
127	28
128	11

NLpep	Induction
129	16
130	93
131	35
132	161
133	42
134	46
135	46
136	12

Figure 164



NLpep	Induction
114	280
137	281
138	45
139	128
140	67
141	258
142	138
143	201

FIGURE 1

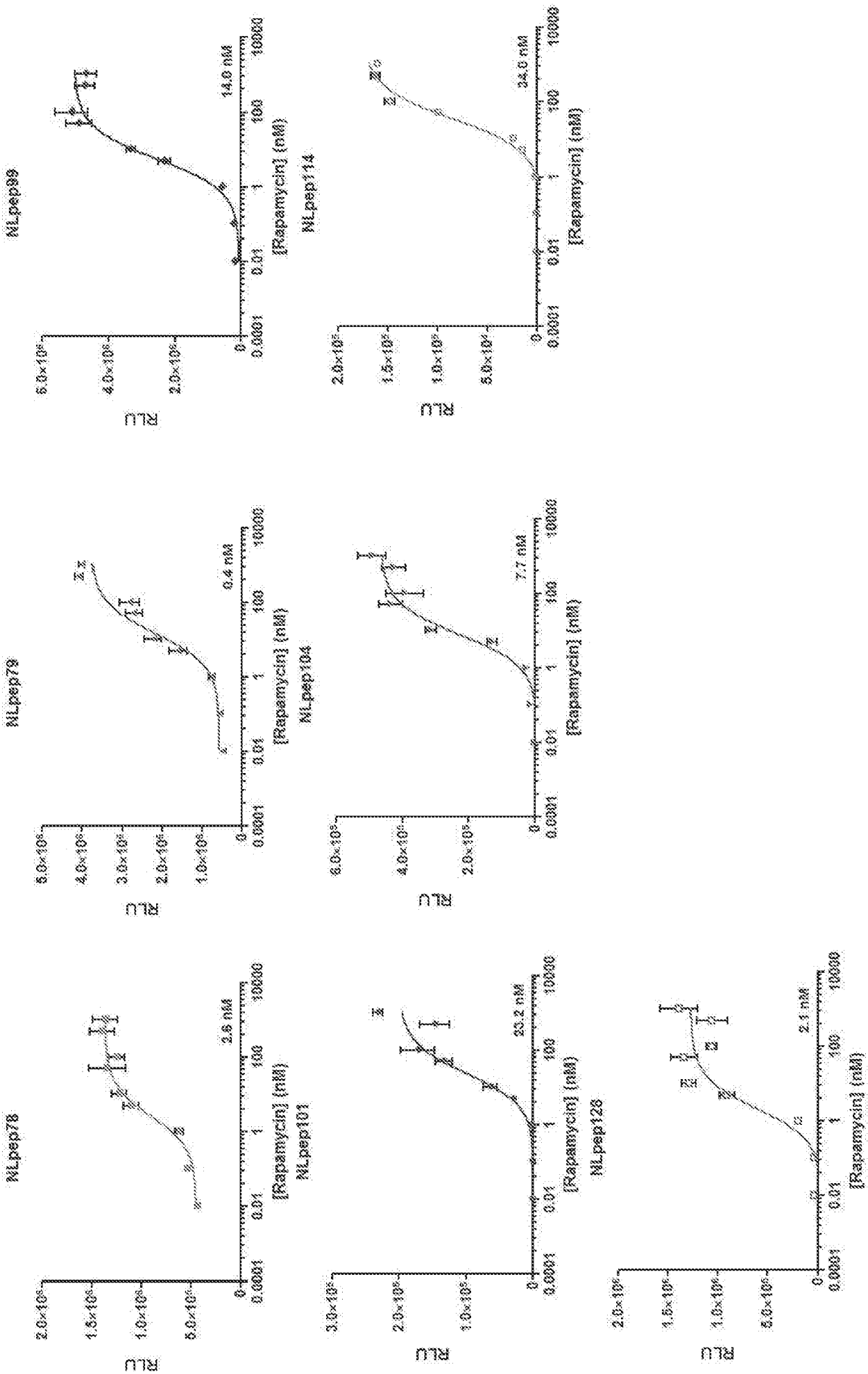

NLpep99
14.0 nM
NLpep114
34.8 nM
NLpep79
0.4 nM
NLpep104
7.7 nM
NLpep78
2.6 nM
NLpep101
23.2 nM
NLpep128
2.1 nM
RLU
[Rapamycin] (nM)

Figure 1nn

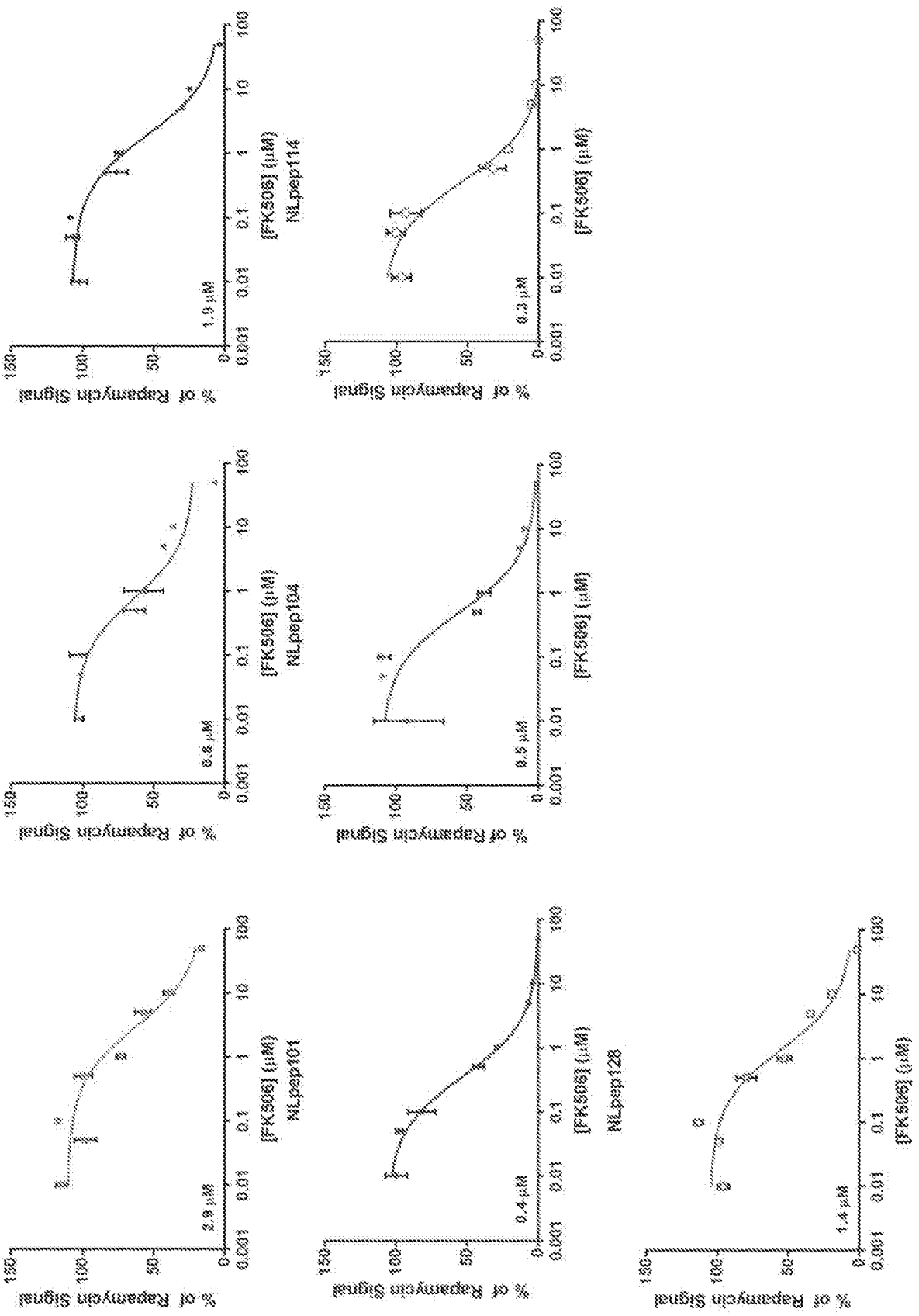

NLpep78
NLpep79
NLpep89
NLpep101
NLpep104
NLpep114
NLpep128
% of Rapamycin Signal
[FK506] (μM)
2.9 μM
0.4 μM
1.9 μM
0.5 μM
0.3 μM
0.4 μM
1.4 μM

Figure 107

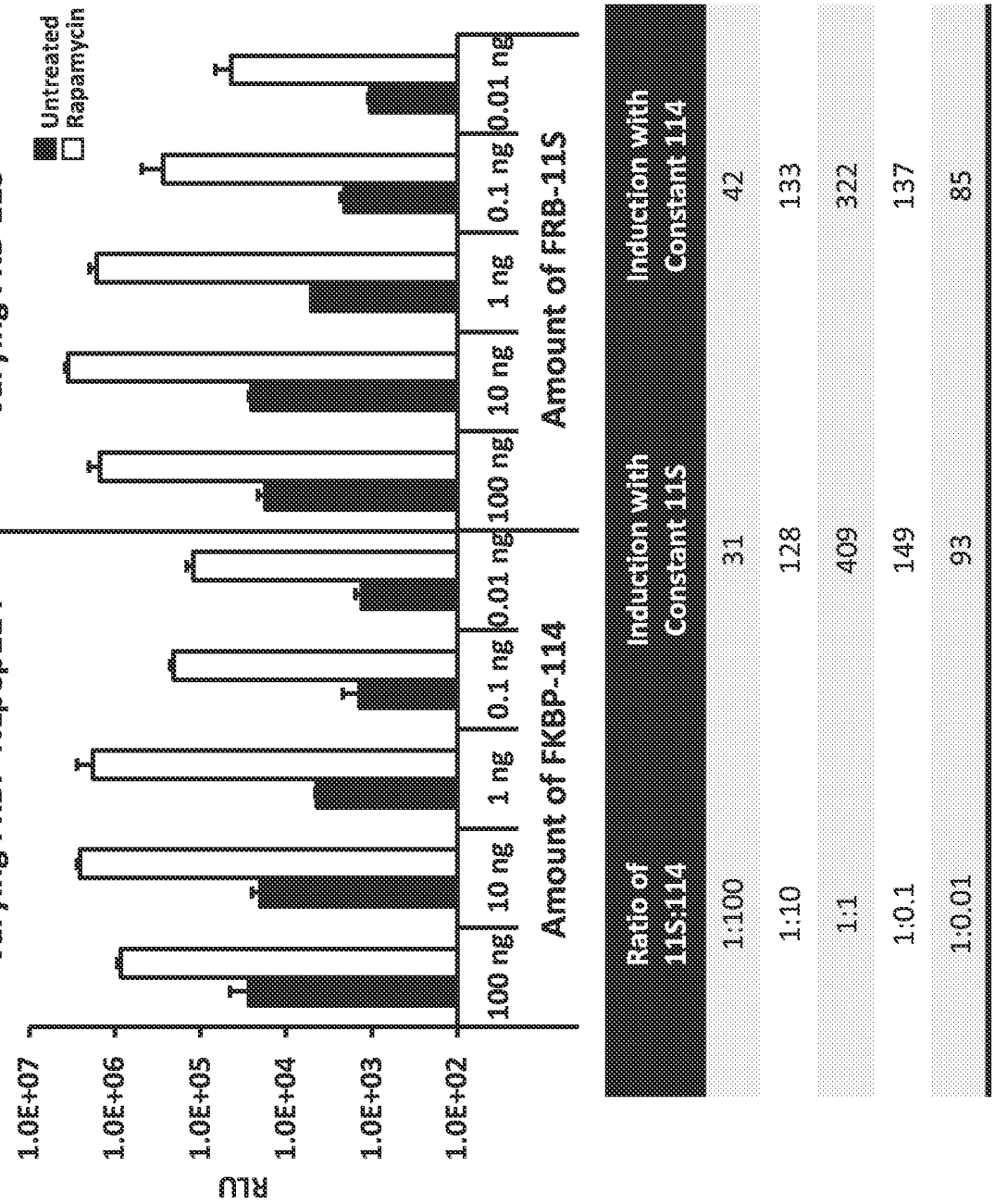

1 ng FRB-NLpoly11S with
Varying FKBP-NLpep114
1 ng FKBP-NLpep114 with
Varying FRB-11S
Untreated
Rapamycin
RLU
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
100 ng
10 ng
1 ng
0.1 ng
0.01 ng
Amount of FKBP-114
Amount of FRB-11S
Ratio of 11S:114
Induction with Constant 11S
Induction with Constant 114
1:100
31
42
1:10
128
133
1:1
409
322
1:0.1
149
137
1:0.01
93
85

Figure 10a

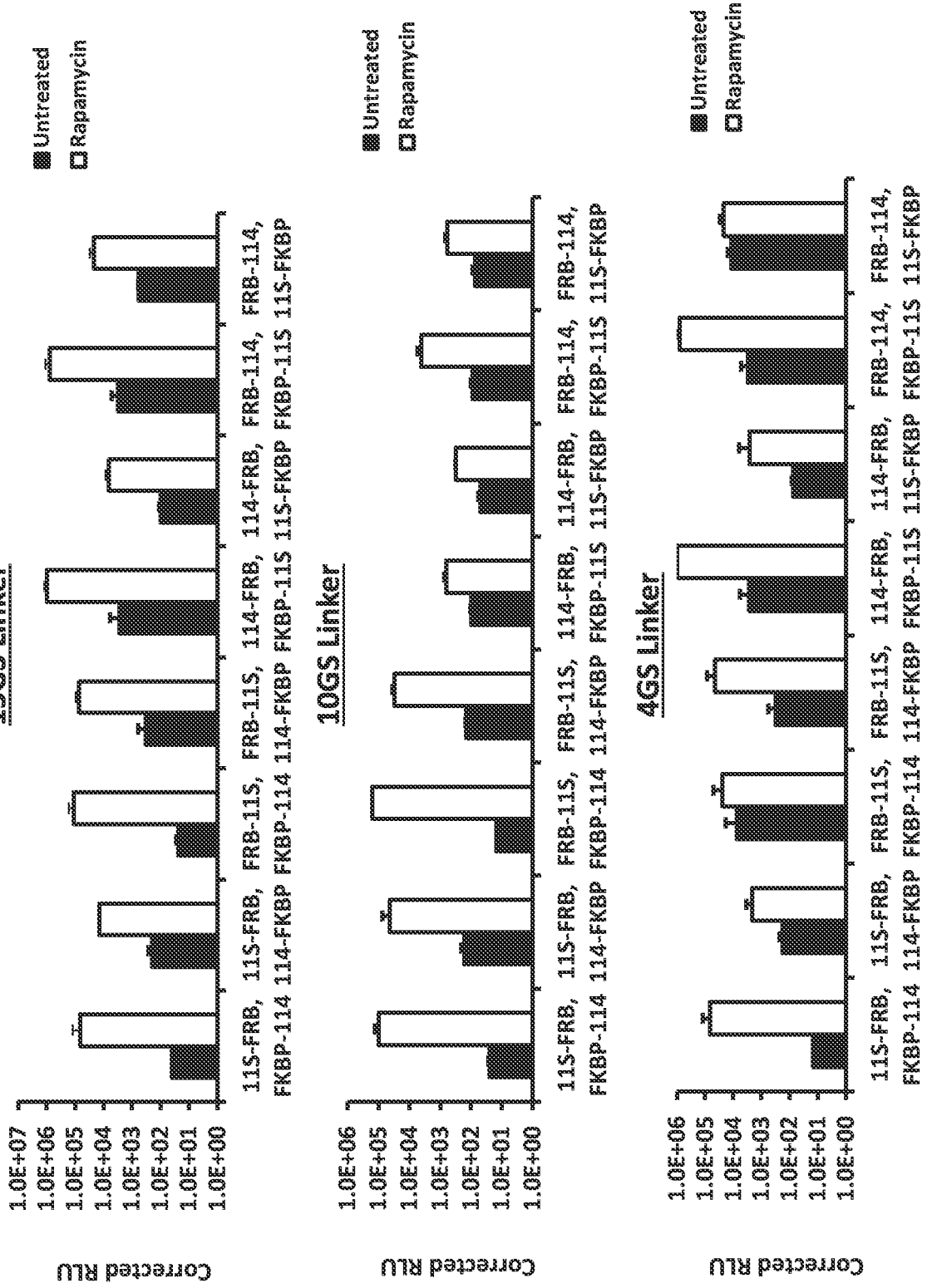
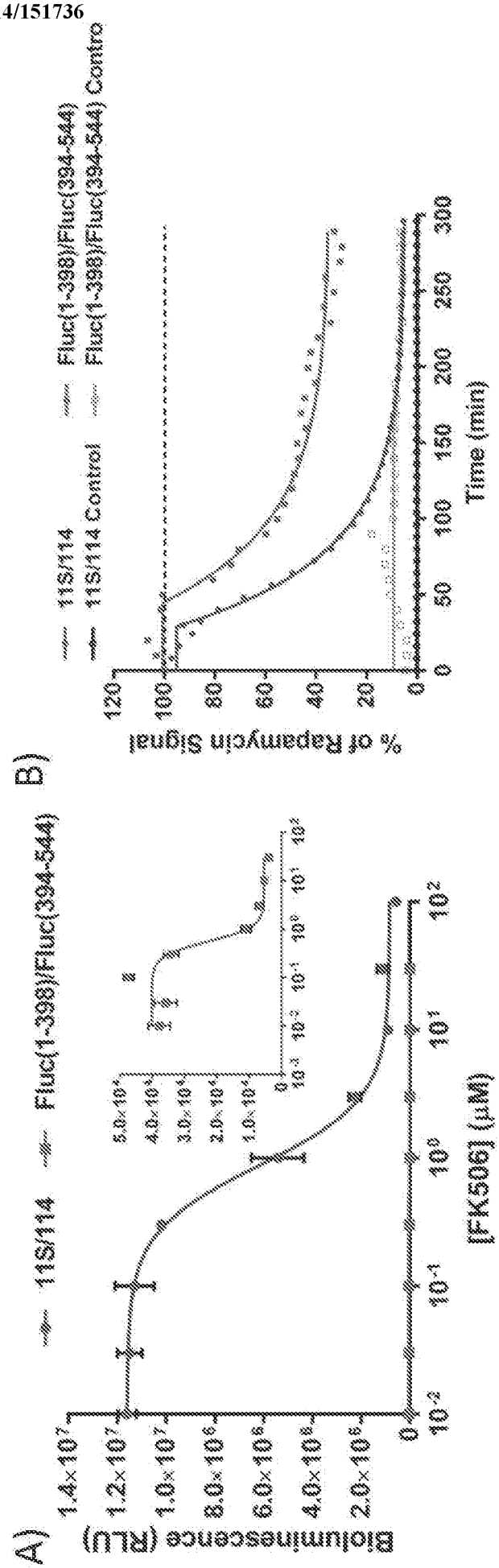

15GS Linker
10GS Linker
4GS Linker
Untreated
Rapamycin
Corrected RLU
1.0E+00
1.0E+01
1.0E+02
1.0E+03
1.0E+04
1.0E+05
1.0E+06
1.0E+07
11S-FRB, 11S-FRB, FRB-11S, FRB-11S, 114-FRB, 114-FRB, FRB-114, FRB-114,
FKBP-114 114-FKBP FKBP-114 114-FKBP FKBP-11S 11S-FKBP FKBP-11S 11S-FKBP

FIGURE 10

	EC50
11S/ 114	1.3 nM
Fluc(1-398)/ Fluc(394-544)	3.4 nM

	Bmax	$t_{1/2}$
11S/ 114	3.7×10^5 RLU	3.8 s
Fluc(1-398)/ Fluc(394-544)	3.1×10^3 RLU	15.5 s

FIGURE 17



	IC50
11S/114	868 nM
Fluc(1-398)/Fluc(394-544)	550 nM

	Lag Time	$t_{1/2}$	Max Inhibition
11S/114	5 min	33 min	95%
Fluc(1-398)/Fluc(394-544)	34 min	54 min	74%

Figure 11

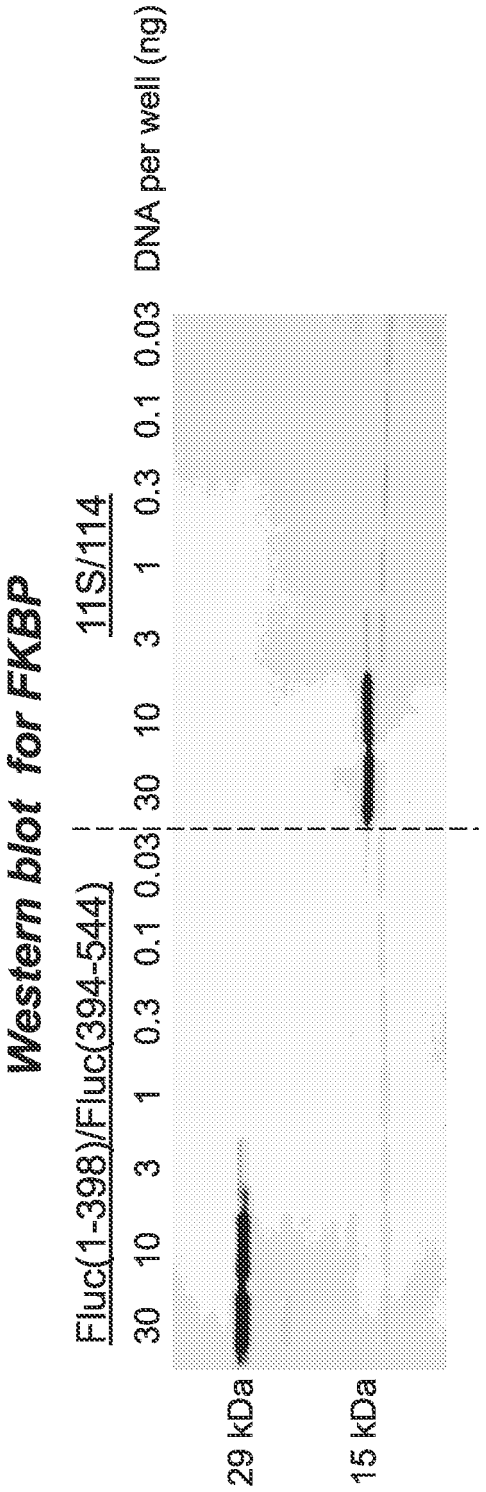

Western blot for FKBP
Fluc(1-398)/Fluc(394-544)
11S/114
30 10 3 1 0.3 0.1 0.03 30 10 3 1 0.3 0.1 0.03
DNA per well (ng)
29 kDa
15 kDa

FIGURE 17

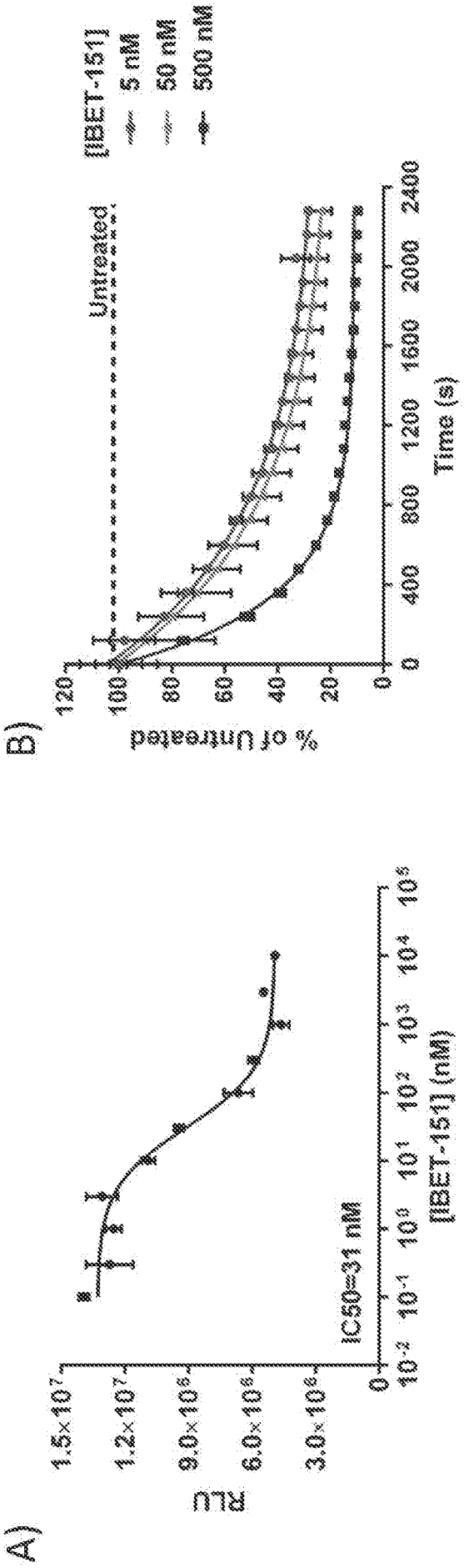

A)
RLU
1.5×10^7
1.2×10^7
9.0×10^6
6.0×10^6
3.0×10^6
0
IC50=31 nM
10^-2 10^-1 10^0 10^1 10^2 10^3 10^4 10^5
[IBET-151] (nM)
B)
% of Untreated
120
100
80
60
40
20
0
Untreated
[IBET-151]
5 nM
50 nM
500 nM
0 400 800 1200 1600 2000 2400
Time (s)

Figure 173

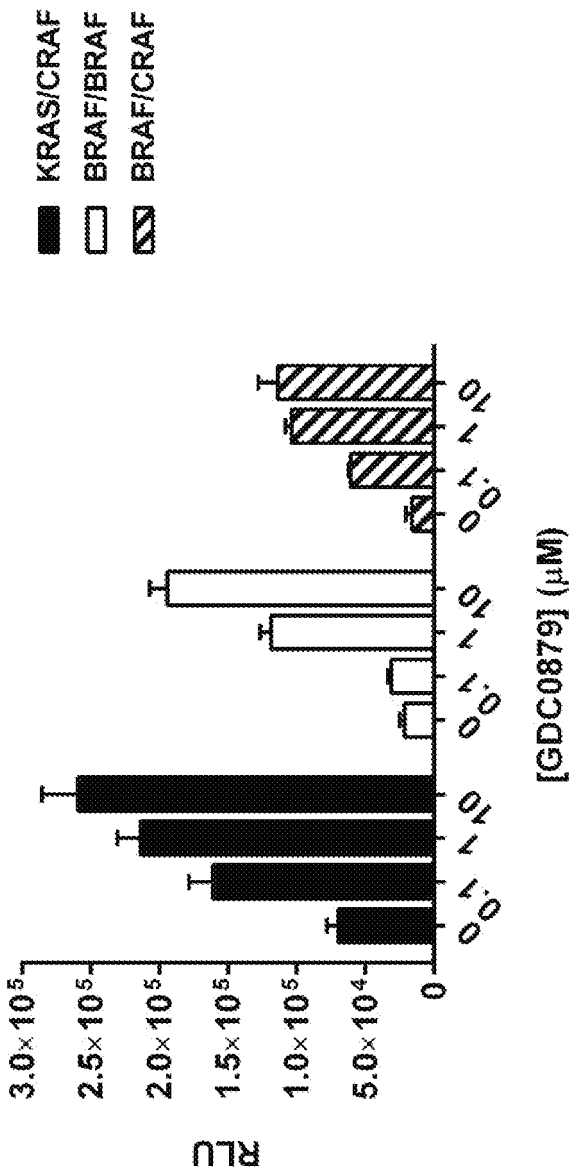

KRAS/CRAF
BRAF/BRAF
BRAF/CRAF
RLU
3.0×10^5
2.5×10^5
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0
0
0.1
1
10
[GDC0879] (μM)

Figure 174

	1	2	3	4	5	6	7	8	9	10	11	12	13
WT	G	V	T	G	W	R	L	C	E	R	I	L	A
86	-	V	S	G	W	R	L	F	K	K	I	S	-
114	-	V	T	G	Y	R	L	F	E	E	I	L	-

High
Low

Need protocol, how much 11S

Figure 175

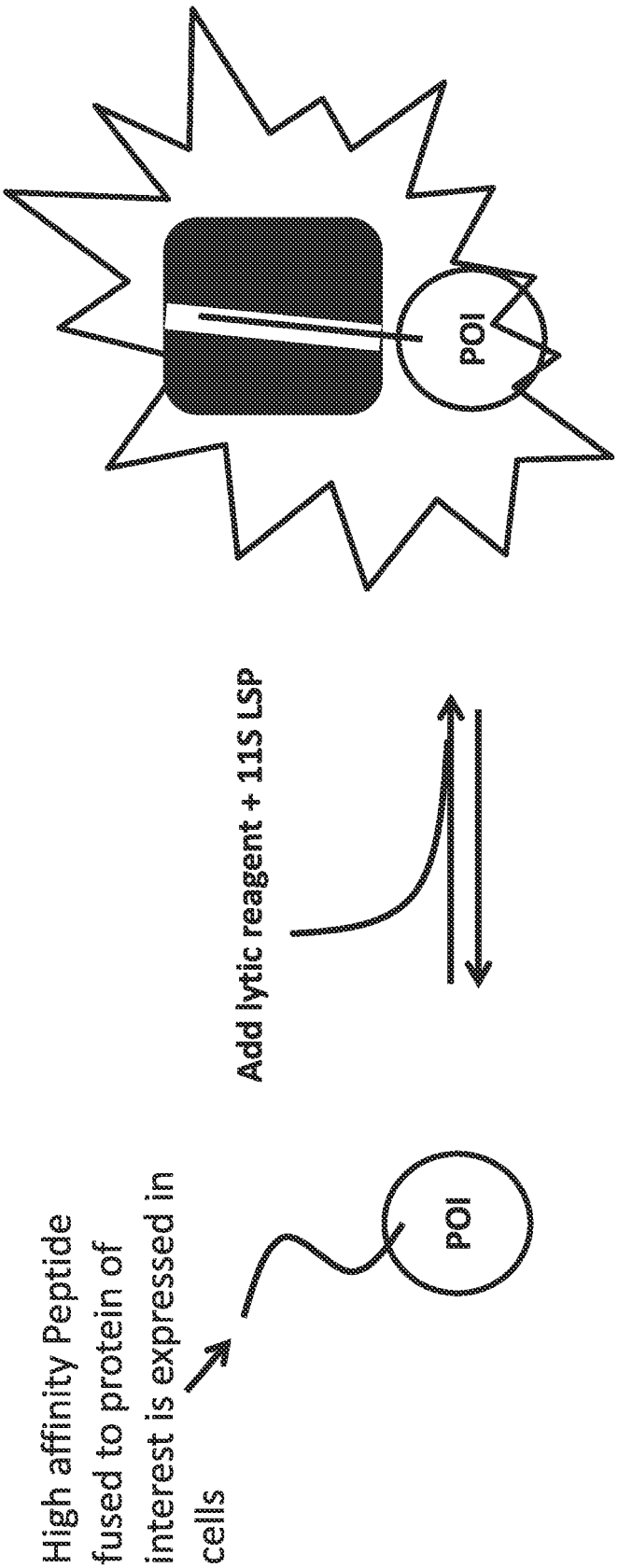

High affinity Peptide fused to protein of interest is expressed in cells
POI
Add lytic reagent + 11S LSP
POI

Figure 176

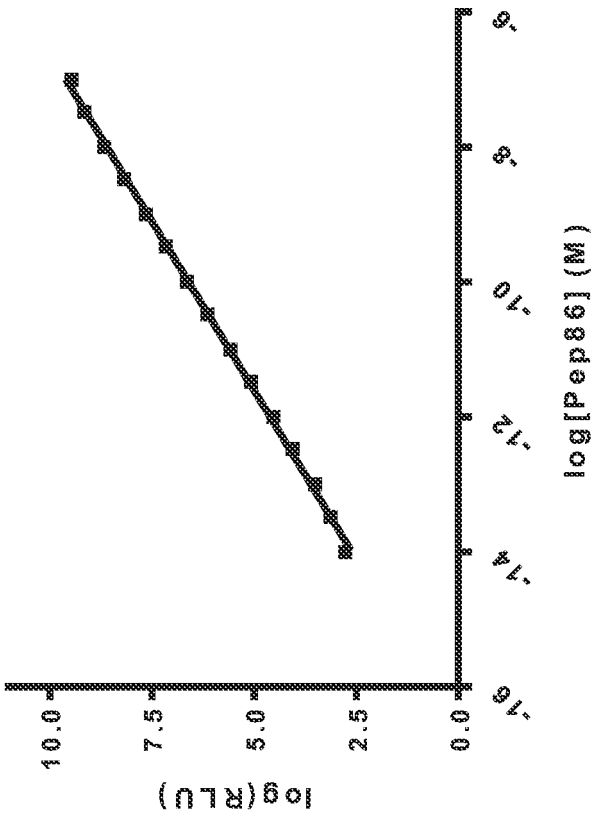


Figure 11

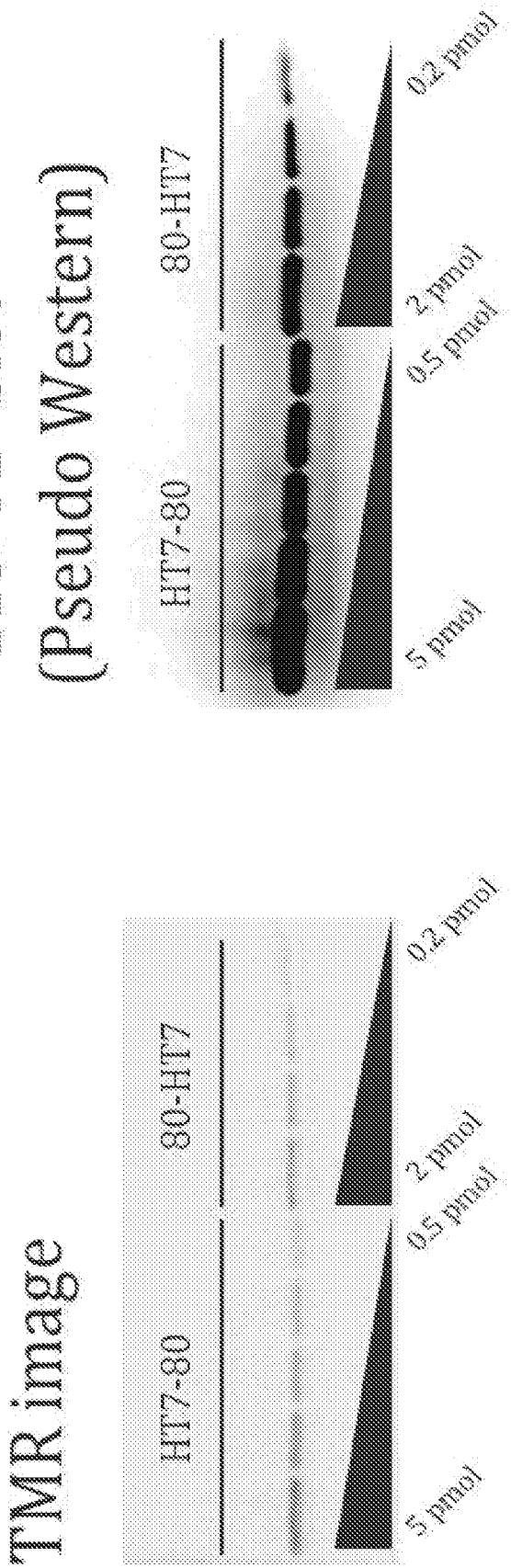

"11S+Fz" blot
(Pseudo Western)
HT7-80
80-HT7
0.2 pmol
2 pmol
0.5 pmol
5 pmol
TMR image
HT7-80
80-HT7
0.2 pmol
2 pmol
0.5 pmol
5 pmol

Figure 1/8

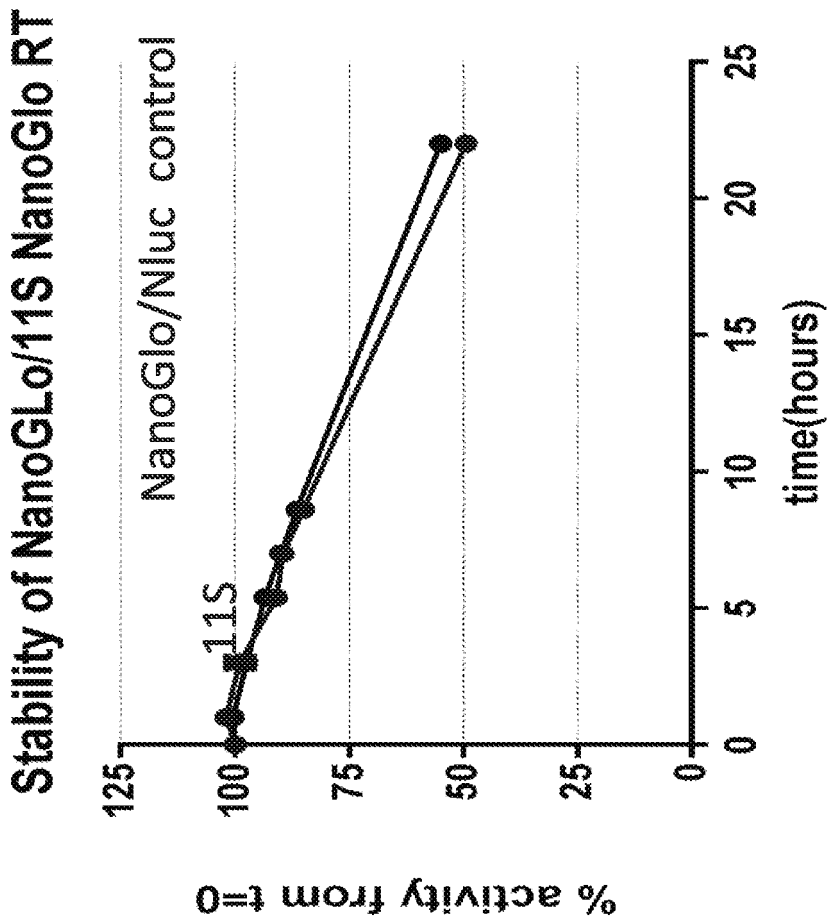
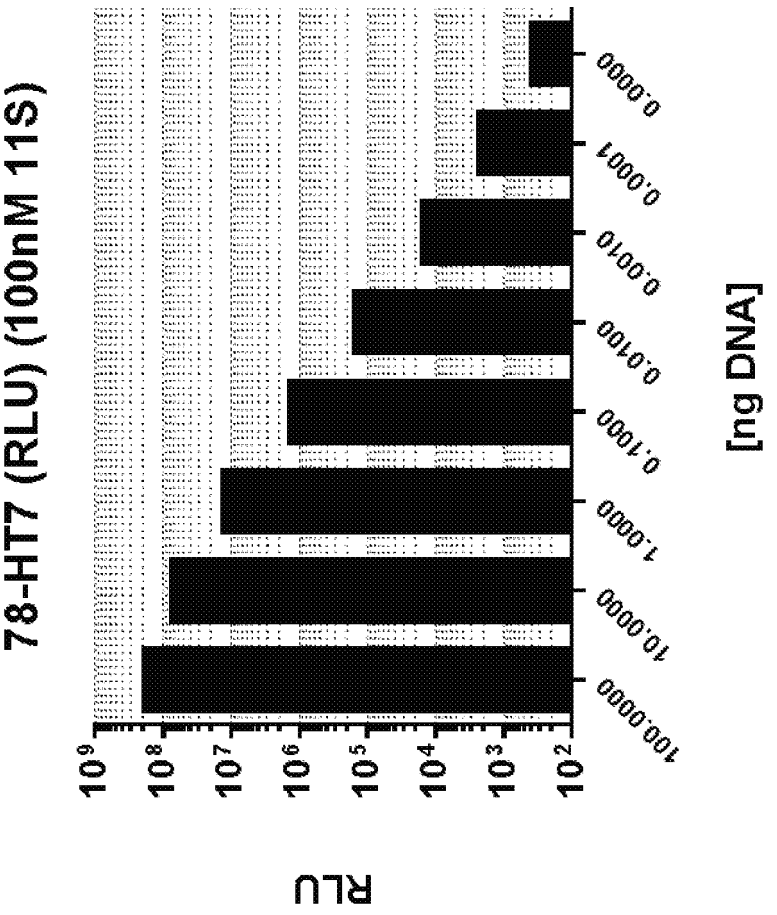

Stability of NanoGLo/11S NanoGlo RT
NanoGlo/Nluc control
11S
% activity from t=0
time(hours)
125
100
75
50
25
0
0
5
10
15
20
25

Figure 179


78-HT7 (RLU) (100nM 11S)
RLU
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
100.0000
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
[ng DNA]

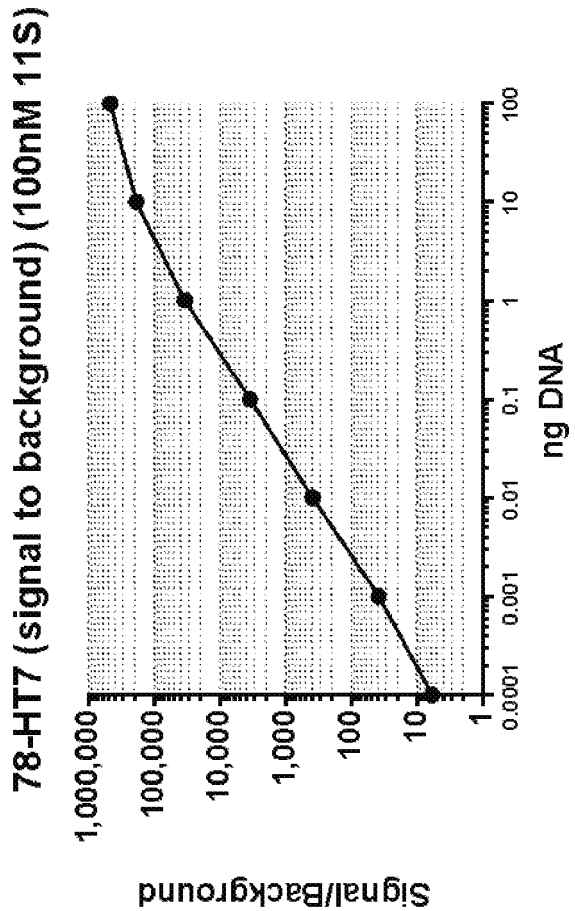

78-HT7 (signal to background) (100nM 11S)
Signal/Background
1,000,000
100,000
10,000
1,000
100
10
1
0.0001
0.001
0.01
0.1
1
10
100
ng DNA

Figure 180

High affinity (spontaneous) peptides

	157													169
Wt	T	I	N	G	V	T	G	W	R	L	C	E	R	I	L	A
78			N		V	S	G	W	R	L	F	K	K	I	S	N
80			-		V	S	G	W	R	L	F	K	K	I	S	N
80*			-		V	S	G	W	R	L	F	K	K	I	S	A
83			N		V	S	G	W	R	L	F	K	K	I	S	-
83*			G		V	S	G	W	R	L	F	K	K	I	S	-
86			-		V	S	G	W	R	L	F	K	K	I	S	-
80+S*			S		V	S	G	W	R	L	F	K	K	I	S	A
80+S			S		V	S	G	W	R	L	F	K	K	I	S	N
80+NS*			N	S	V	S	G	W	R	L	F	K	K	I	S	A
80+NS			N	S	V	S	G	W	R	L	F	K	K	I	S	N
83+S			S	N	V	S	G	W	R	L	F	K	K	I	S	-
83+S*			S	G	V	S	G	W	R	L	F	K	K	I	S	-
83+NS	N	S	N		V	S	G	W	R	L	F	K	K	I	S	-
83+NS*	N	S	G		V	S	G	W	R	L	F	K	K	I	S	-
86+S			S		V	S	G	W	R	L	F	K	K	I	S	-
86+NS	N	S			V	S	G	W	R	L	F	K	K	I	S	-
78+S			S	N	V	S	G	W	R	L	F	K	K	I	S	N
78+NS	N	S	N		V	S	G	W	R	L	F	K	K	I	S	N

Synthetic Dark/Quencher peptides

	157													169
Wt	T	I	N	G	V	T	G	W	R	L	C	E	R	I	L	A
Dark peptides:																
7mer dark							G	W	A	L	F	K	K	K		
Trp 11mer					V	T	G	W	A	L	F	E	E	I	L	
Tyr 11mer					V	T	G	Y	A	L	F	E	E	I	L	
Quencher peptides:																
DAB-G W A L F K K																
DAB-V T G W A L F E E I L																
DAB-V T G Y A L F E E I L																

DAB=Dabcyl(475 nm quencher)+dPEG4 spacer

These dark/quencher peptides can potentially be added to 11S for the purpose of reducing background luminescence produced with Fz substrate in the absence of any peptide. The substitution of Ala for Arg (catalytic) at position 162 should make the peptides essentially dark.

Figure 181

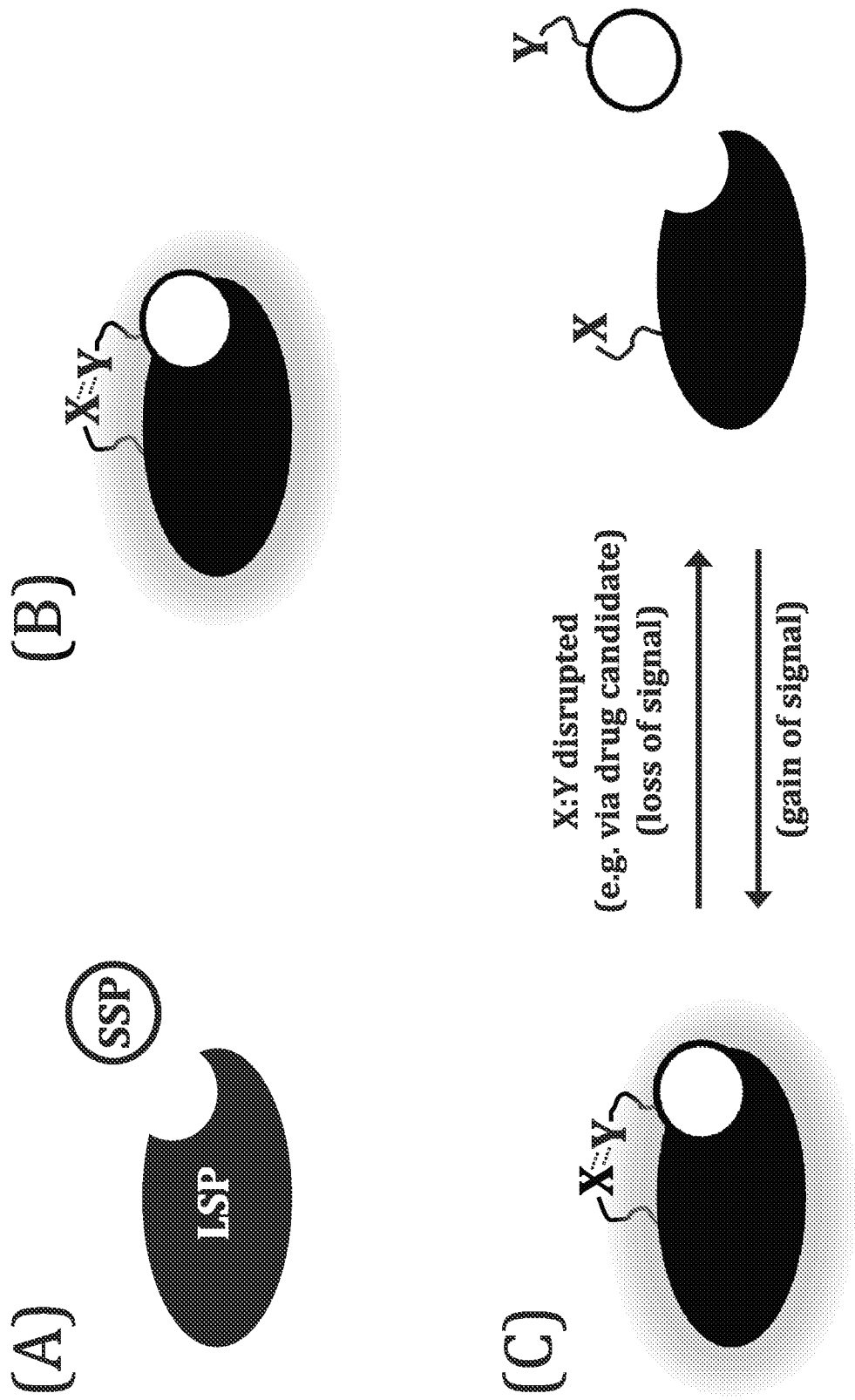

(A)
SSP
LSP
(B)
X:Y
(C)
X:Y disrupted
(e.g. via drug candidate)
(loss of signal)
(gain of signal)
X
Y

Figure 182A

DP = "dark peptide" = a peptide that binds to LSP with relatively high affinity but produces minimal or no luminescence

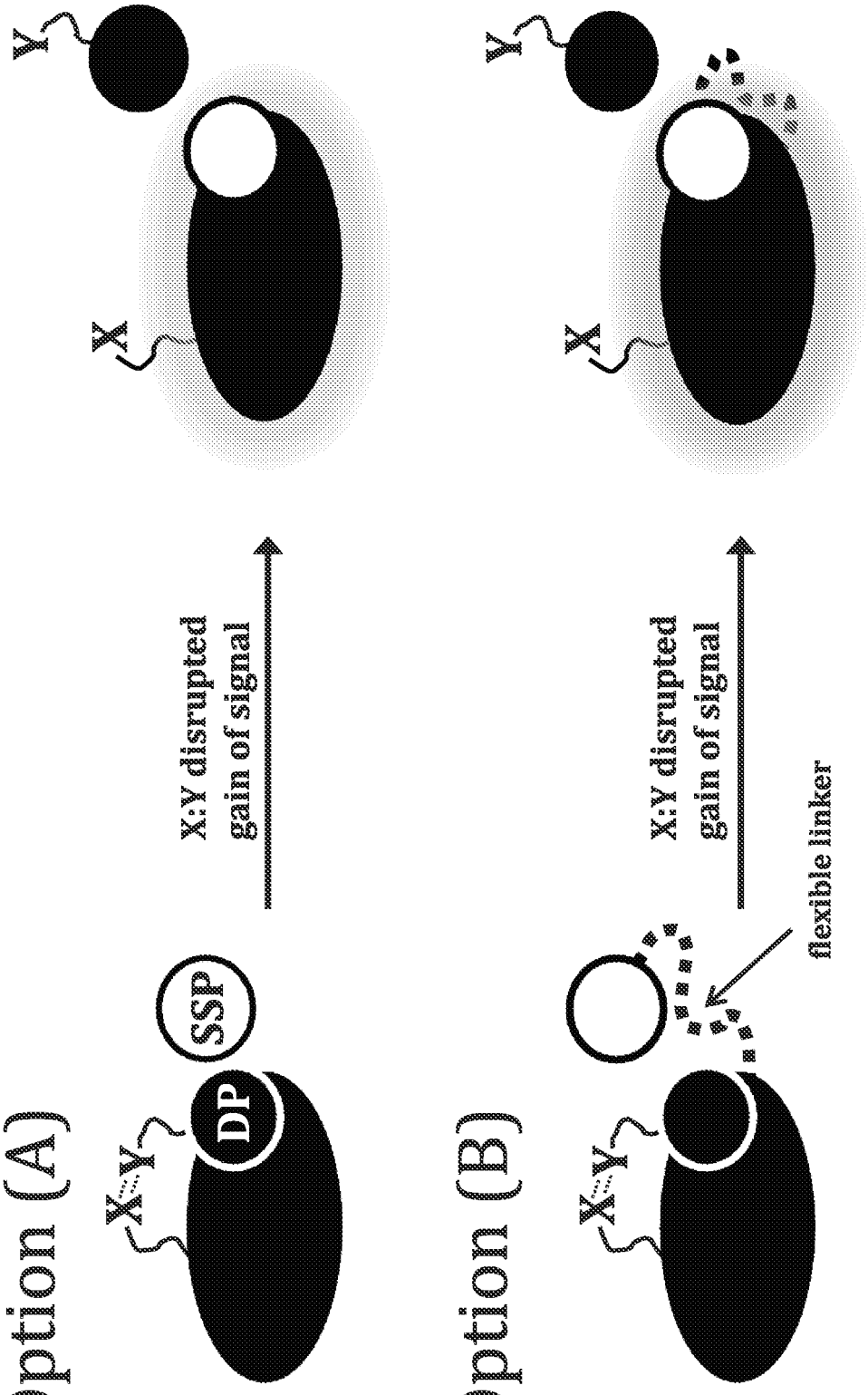


Figure 182B

The ability for this to work is likely dependent on the proper combination of LSP sequence, DP sequence, covalently-attached SSP sequence, and linker sequence. Possible variations and combinations to examine include but are not limited to the following:

X-NLuc(1-156) -- 0aa linker -- NLuc(157-169)
X-NLuc(1-156) -- 0aa linker -- NLuc(158-168)
X-NLuc(1-156) -- 0aa linker -- NLuc(160-168)
X-NLuc(1-156) -- 0aa linker -- NLuc(158-166)
X-NLuc(1-156) -- 0aa linker -- NLuc(160-166)

X-NLuc(1-156) -- 15aa Gly-Ser linker -- NLuc(157-169)
X-NLuc(1-156) -- 15aa Gly-Ser linker -- NLuc(158-168)
X-NLuc(1-156) -- 15aa Gly-Ser linker -- NLuc(160-168)
X-NLuc(1-156) -- 15aa Gly-Ser linker -- NLuc(158-166)
X-NLuc(1-156) -- 15aa Gly-Ser linker -- NLuc(160-166)

X-NLuc(1-156) -- 30aa Gly-Ser linker -- NLuc(157-169)
X-NLuc(1-156) -- 30aa Gly-Ser linker -- NLuc(158-168)
X-NLuc(1-156) -- 30aa Gly-Ser linker -- NLuc(160-168)
X-NLuc(1-156) -- 30aa Gly-Ser linker -- NLuc(158-166)
X-NLuc(1-156) -- 30aa Gly-Ser linker -- NLuc(160-166)

X-NLuc(1-156) -- 45aa Gly-Ser linker -- NLuc(157-169)
X-NLuc(1-156) -- 45aa Gly-Ser linker -- NLuc(158-168)
X-NLuc(1-156) -- 45aa Gly-Ser linker -- NLuc(160-168)
X-NLuc(1-156) -- 45aa Gly-Ser linker -- NLuc(158-166)
X-NLuc(1-156) -- 45aa Gly-Ser linker -- NLuc(160-166)

Figure 183

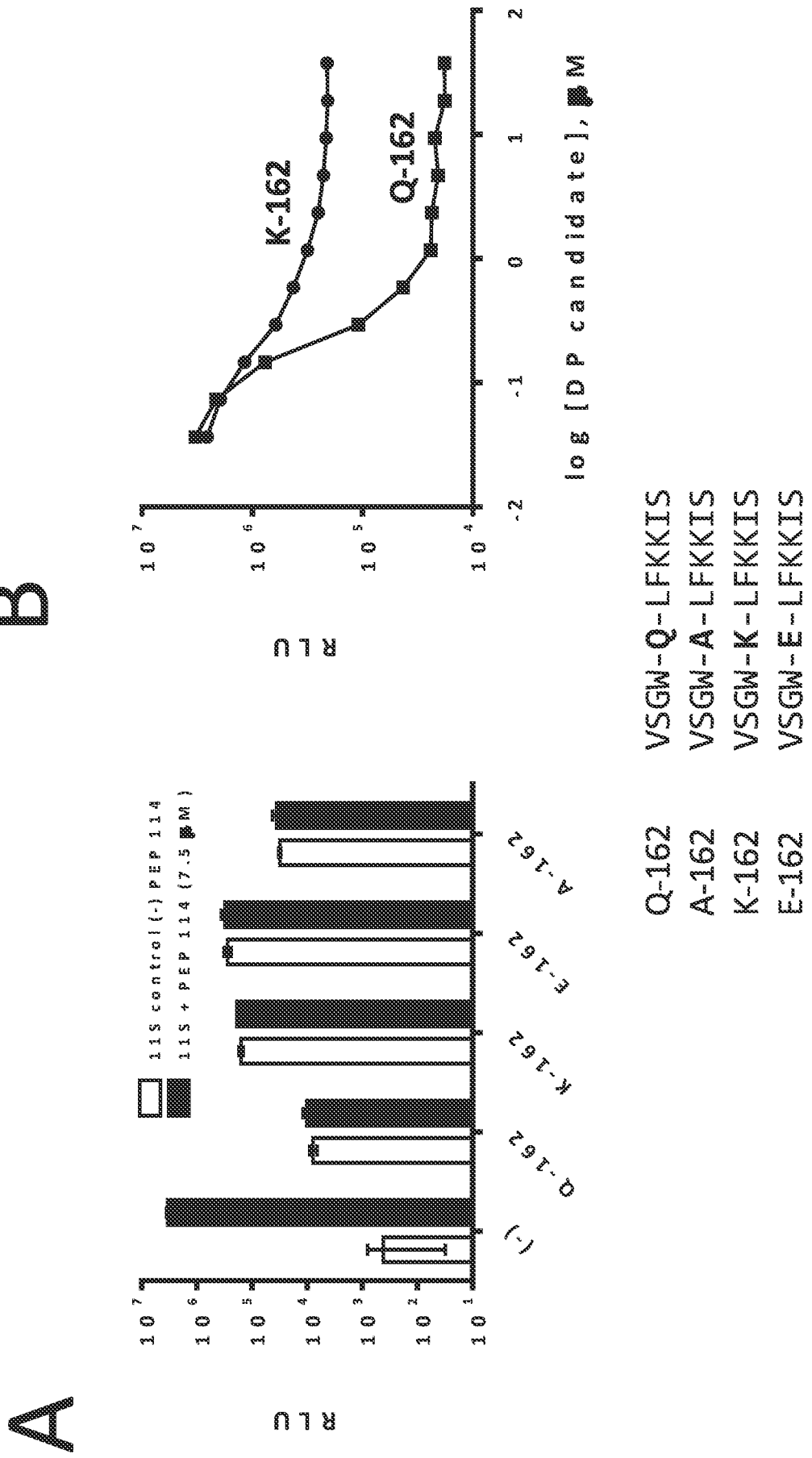

A
RLU
10^7
10^6
10^5
10^4
10^3
10^2
10^1
11S control (-) PEP 114
11S + PEP 114 (7.5 μM)
(-)
Q-162
K-162
E-162
A-162
B
RLU
10^7
10^6
10^5
10^4
K-162
Q-162
-2
-1
0
1
2
log [DP candidate], μM
Q-162 VSGW-Q-LFKKIS
A-162 VSGW-A-LFKKIS
K-162 VSGW-K-LFKKIS
E-162 VSGW-E-LFKKIS

Figure 184

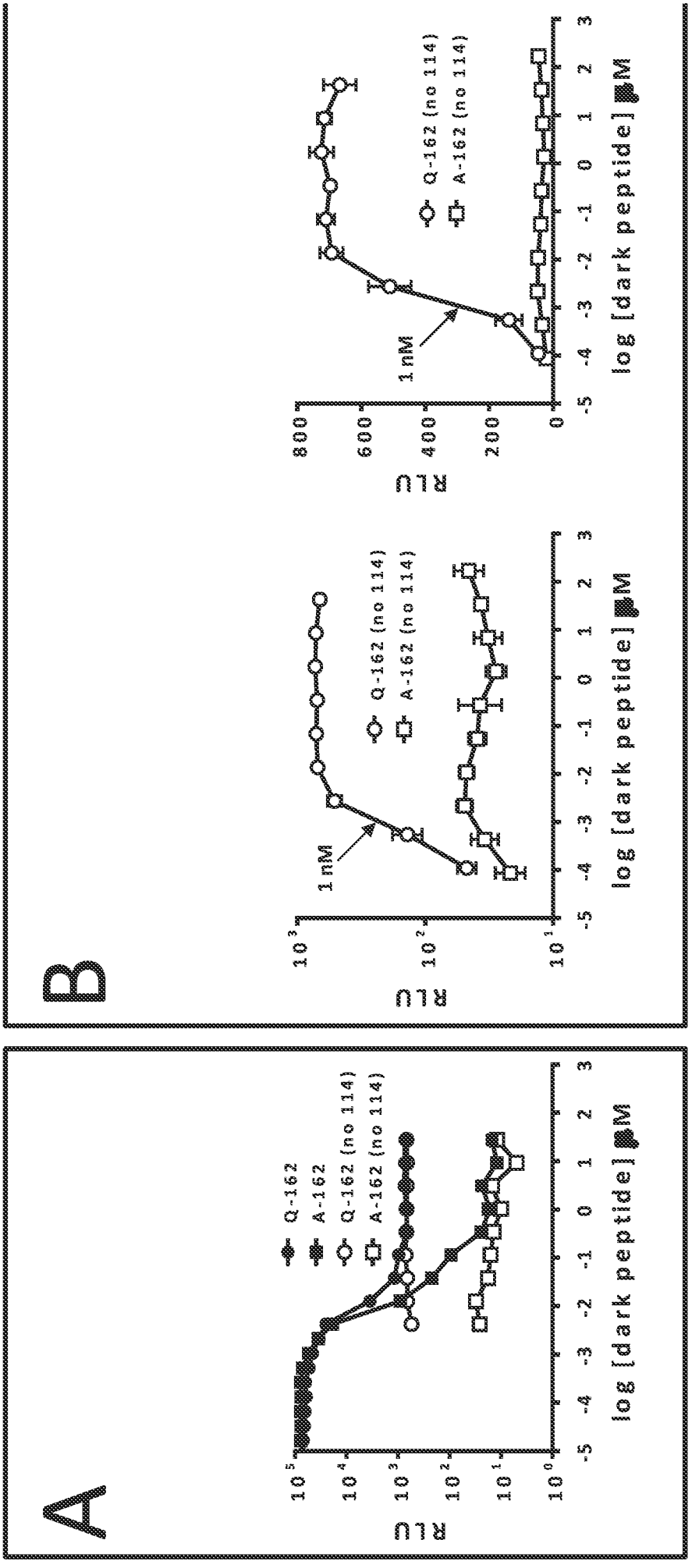

A
RLU
log [dark peptide] M
Q-162
A-162
Q-162 (no 114)
A-162 (no 114)
B
RLU
log [dark peptide] M
1nM
Q-162 (no 114)
A-162 (no 114)

Figure 18b

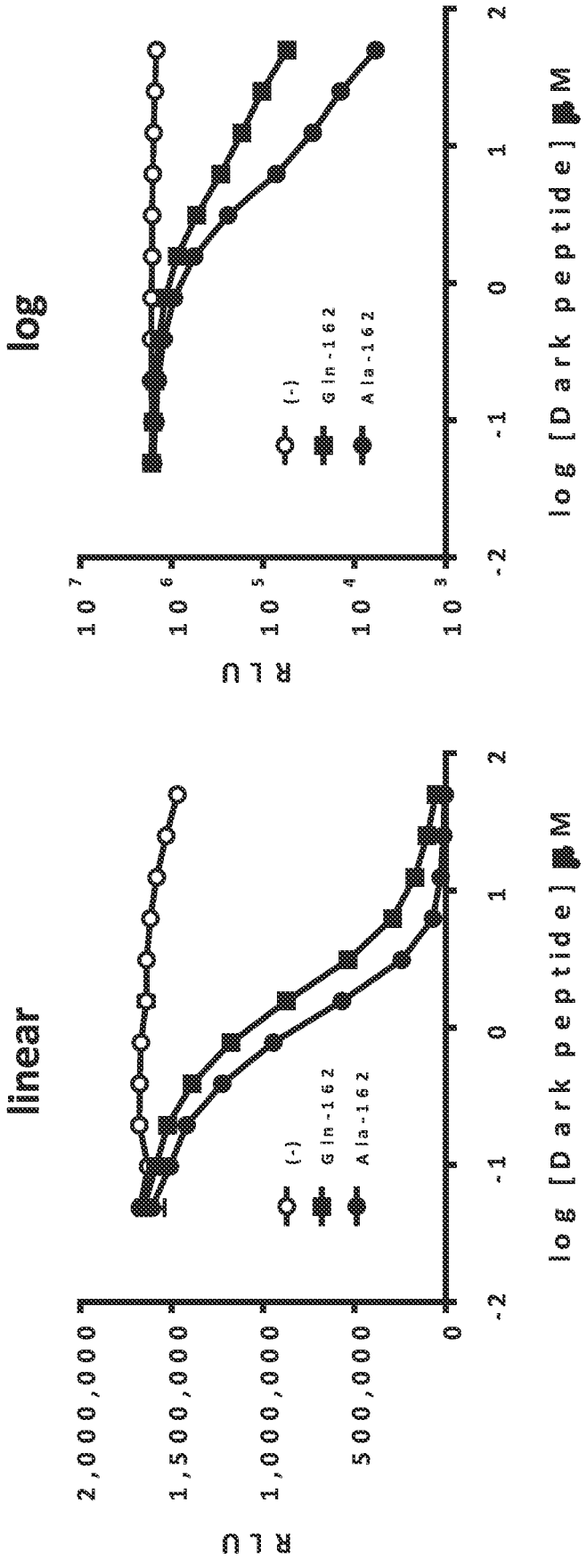

linear
log
RLU
log [Dark peptide] M
(-)
Gln-162
Ala-162
2,000,000
1,500,000
1,000,000
500,000
0
10^7
10^6
10^5
10^4
10^3
-2
-1
0
1
2

Figure 186

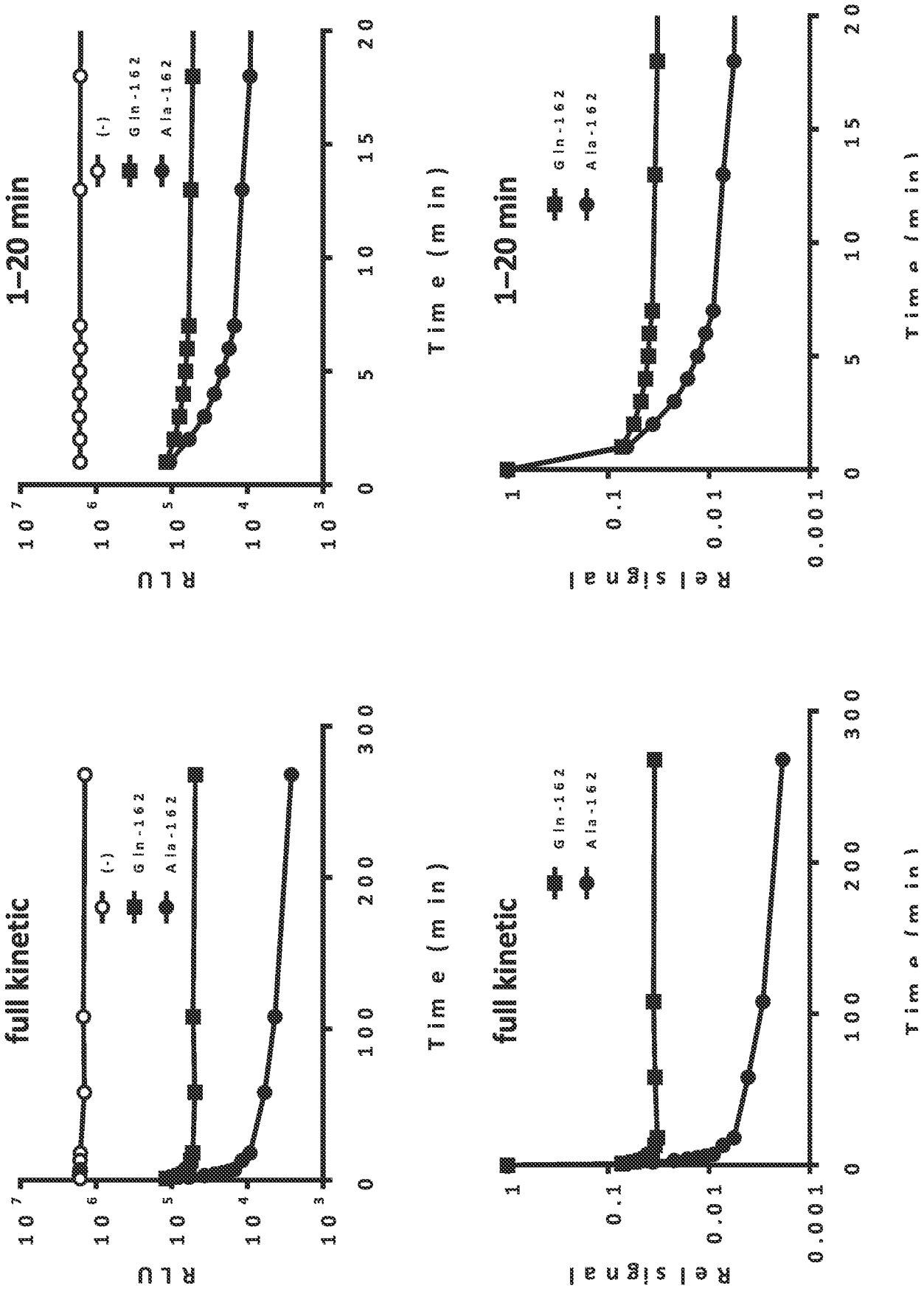


Figure 187

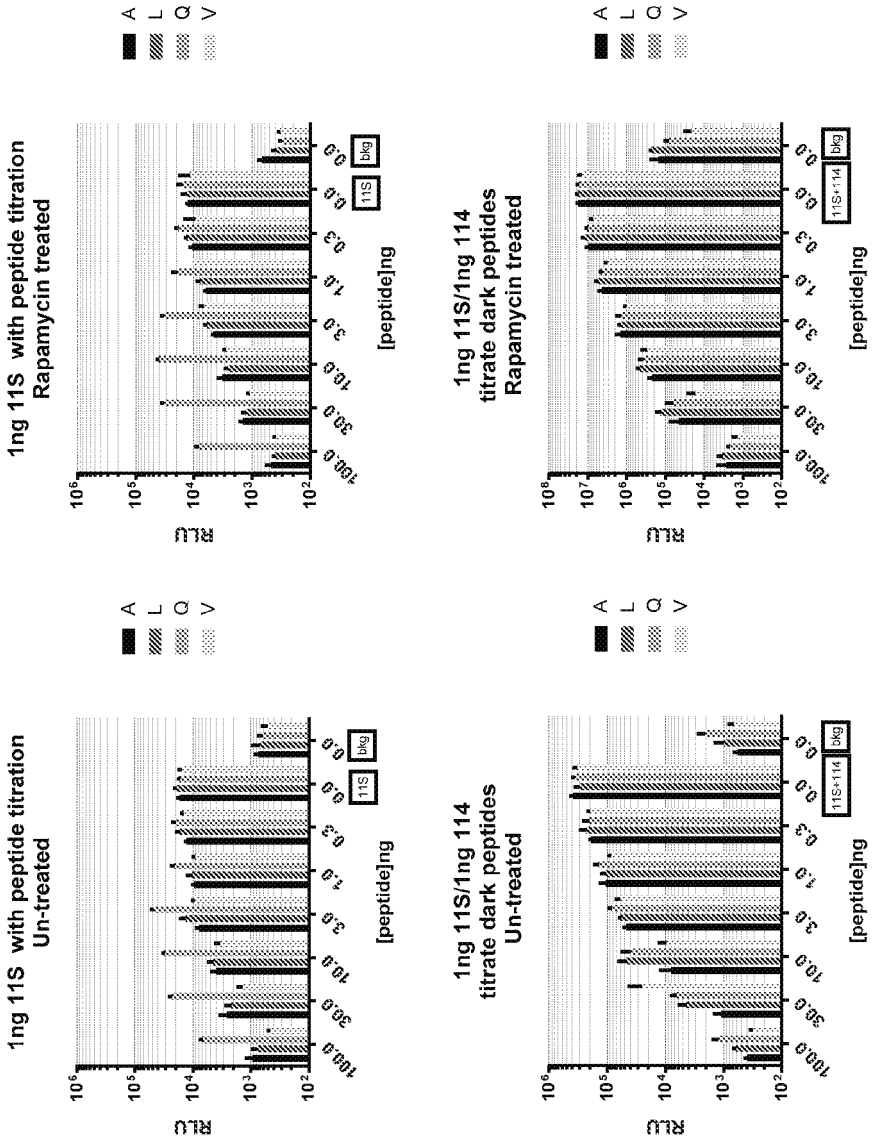

1ng 11S with peptide titration
Un-treated
1ng 11S with peptide titration
Rapamycin treated
1ng 11S/1ng 114
titrate dark peptides
Un-treated
1ng 11S/1ng 114
titrate dark peptides
Rapamycin treated
RLU
[peptide]ng
A
L
Q
V
11S
11S+114
bkg

Figure 18t

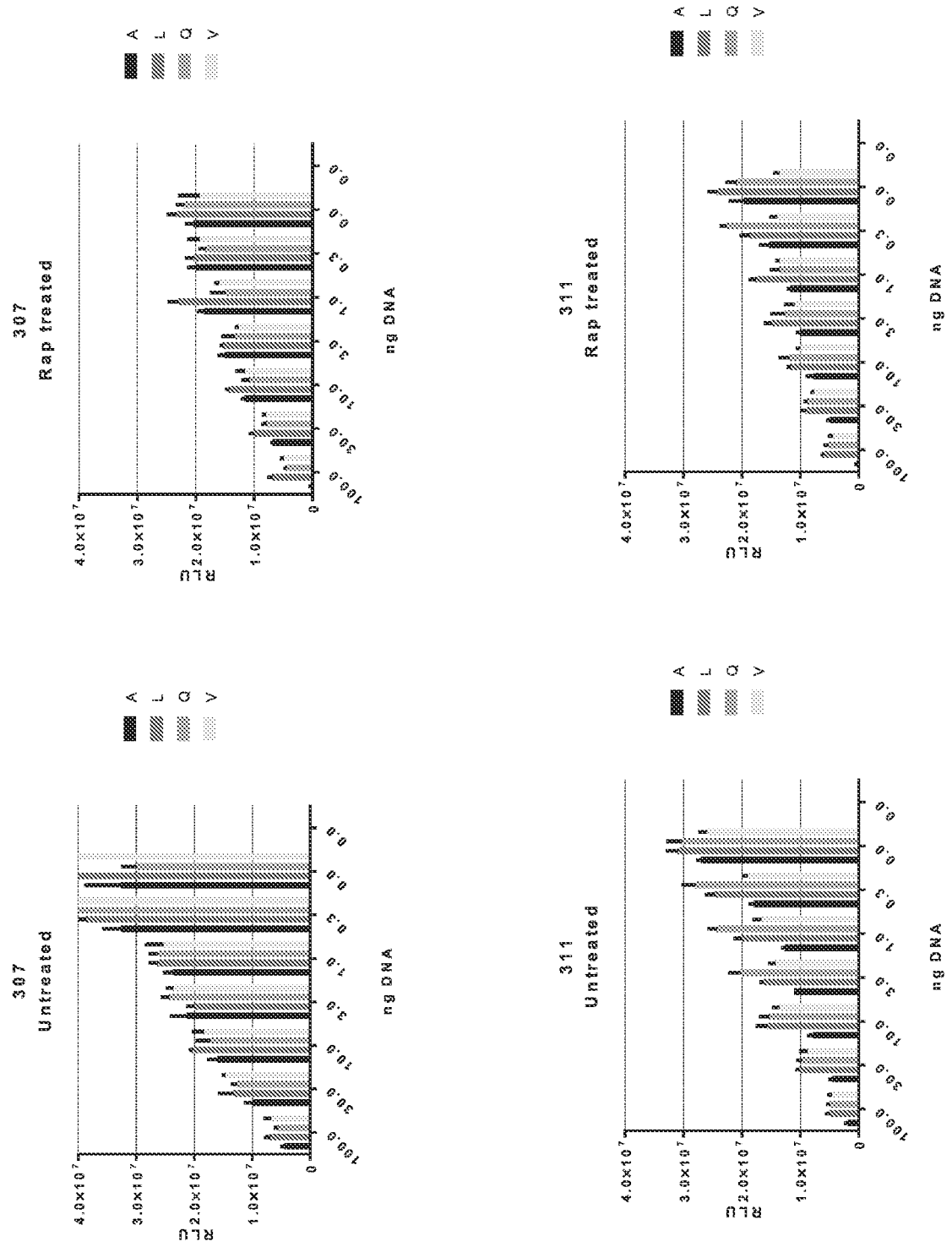

307
Rap treated
307
Untreated
311
Rap treated
311
Untreated
A
L
Q
V
RLU
ng DNA

Figure 189

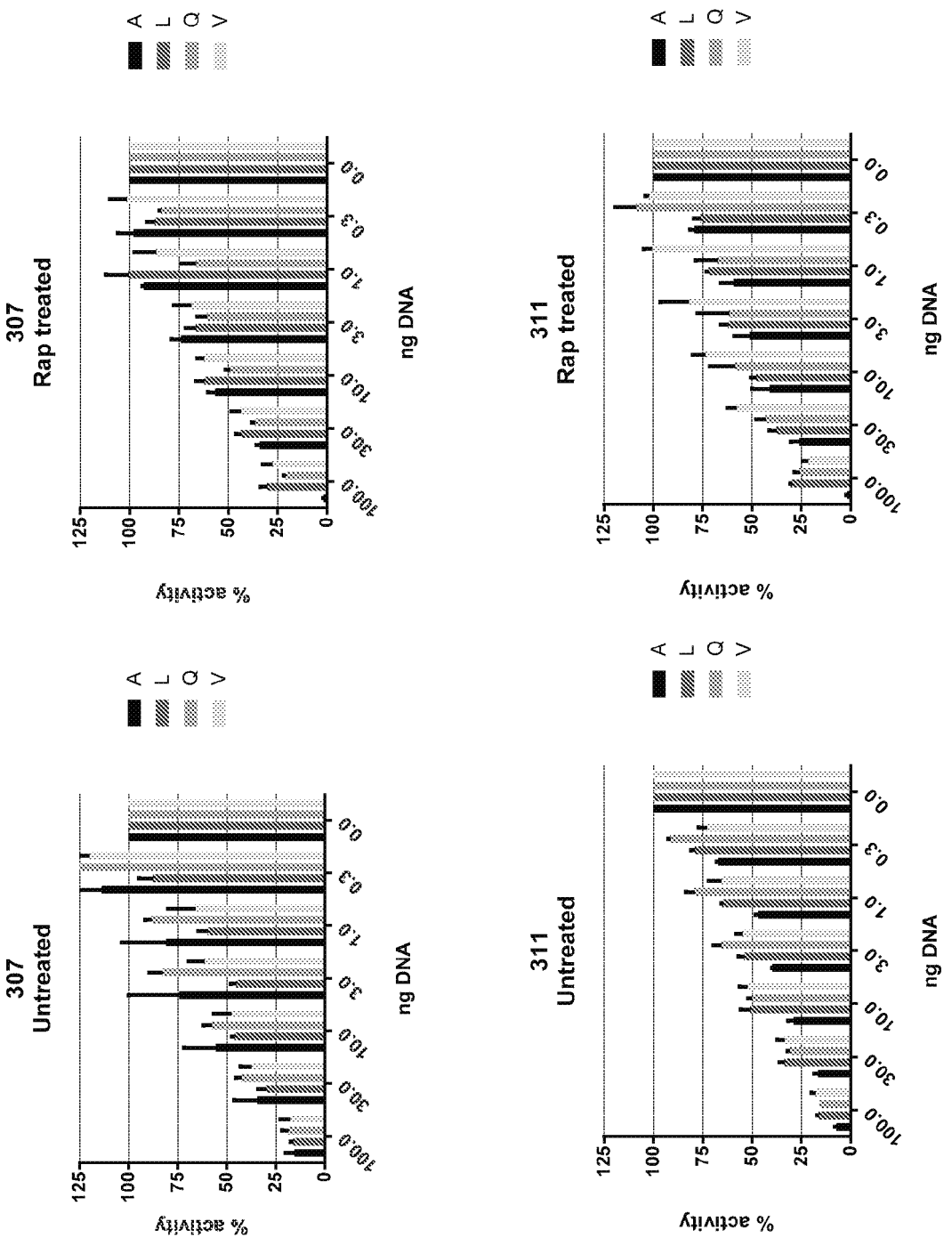

307
Rap treated
311
Rap treated
307
Untreated
311
Untreated
A
L
Q
V
% activity
ng DNA
125
100
75
50
25
0
100.0
30.0
10.0
3.0
1.0
0.3
0.0

Figure 19u

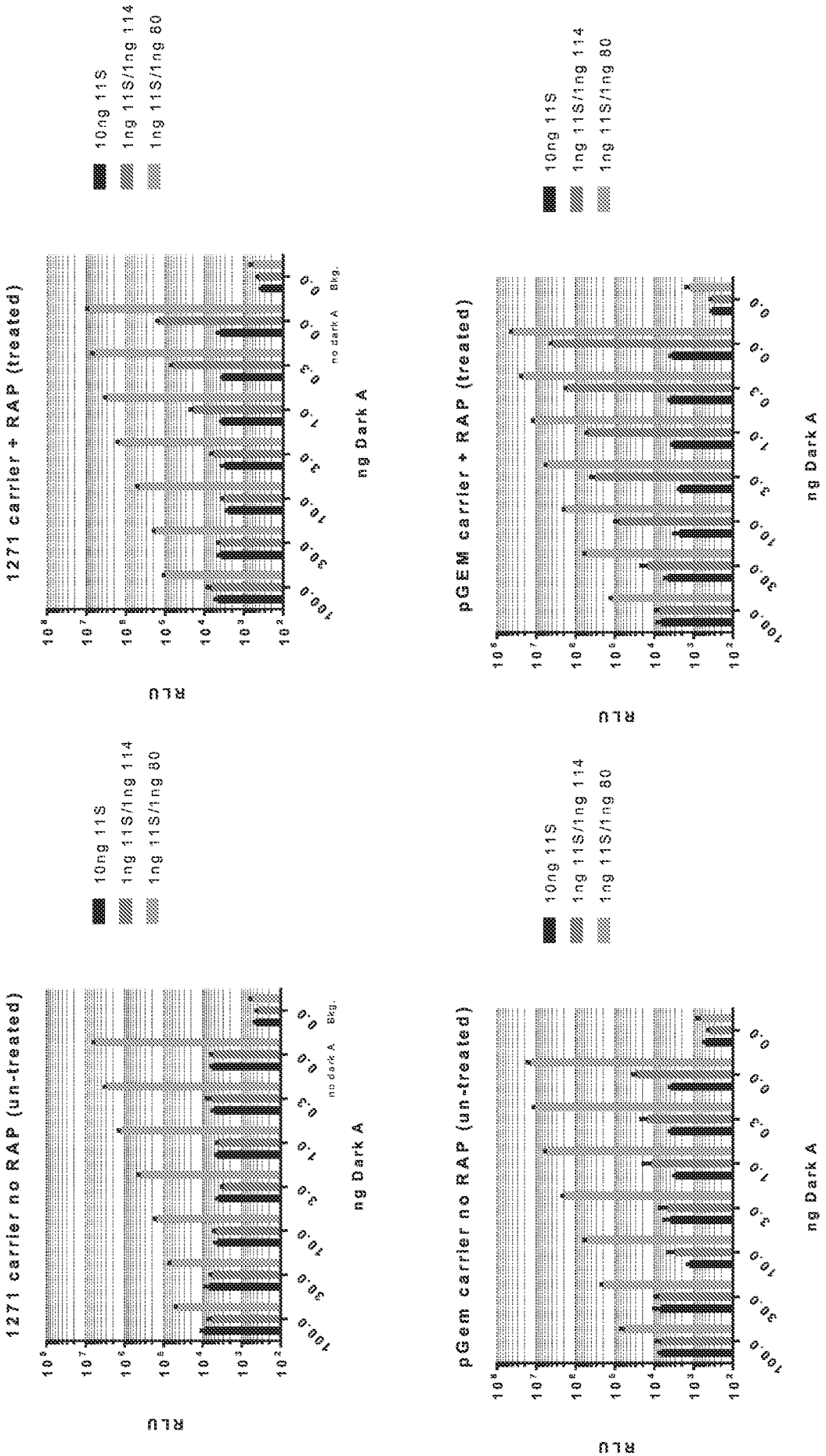

1271 carrier + RAP (treated)
10ng 11S
1ng 11S/1ng 114
1ng 11S/1ng 80
RLU
ng Dark A
no dark A
Bkg.
pGEM carrier + RAP (treated)
1271 carrier no RAP (un-treated)
pGem carrier no RAP (un-treated)

Figure 191

pBC21	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-Fluc (high affinity)
pBC22	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-FlucP (high affinity)
pBC24	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-Fluc-CL1 (high affinity)
pBC25	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-Fluc-PEST12 opt (high affinity)
pBC26	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-Fluc-CP (high affinity)
pBC27	pFC15A/UBQ G76V-VGKLGRQDP-Fluc (EDAKNIKK..)-GGSGGGGSGG-VSGWRLFKKIS (high affinity)
pBC28	pFC15A/UBQ-RGKLGRQDP-Fluc (EDAKNIKK..)-GGSGGGGSGG-VSGWRLFKKIS (high affinity)
pBC29	pFC15A/UBQ-LGKLGRQDP-Fluc (EDAKNIKK..)-GGSGGGGSGG-VSGWRLFKKIS (high affinity)

Figure 192

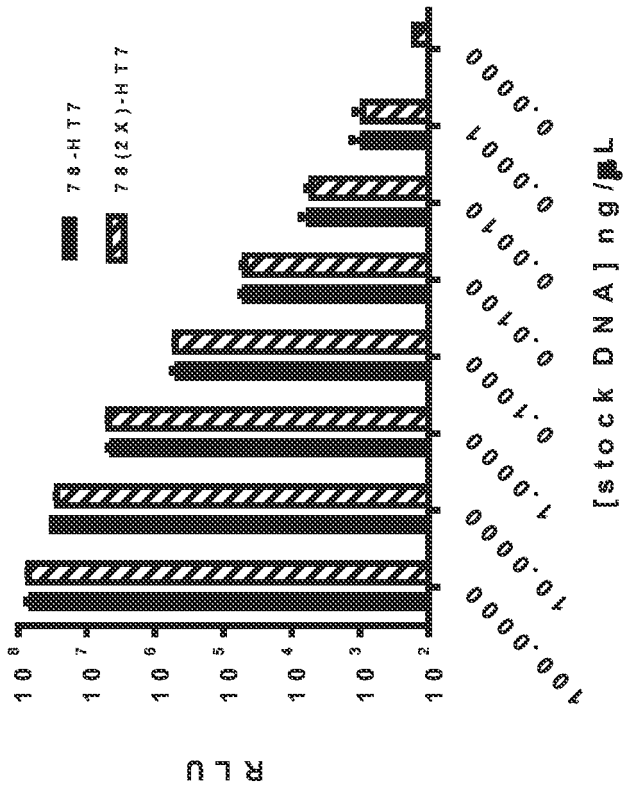

78-HT7
78(2X)-HT7
RLU
10^8
10^7
10^6
10^5
10^4
10^3
10^2
100.0000
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
[stock DNA] ng/µL

Figure 193

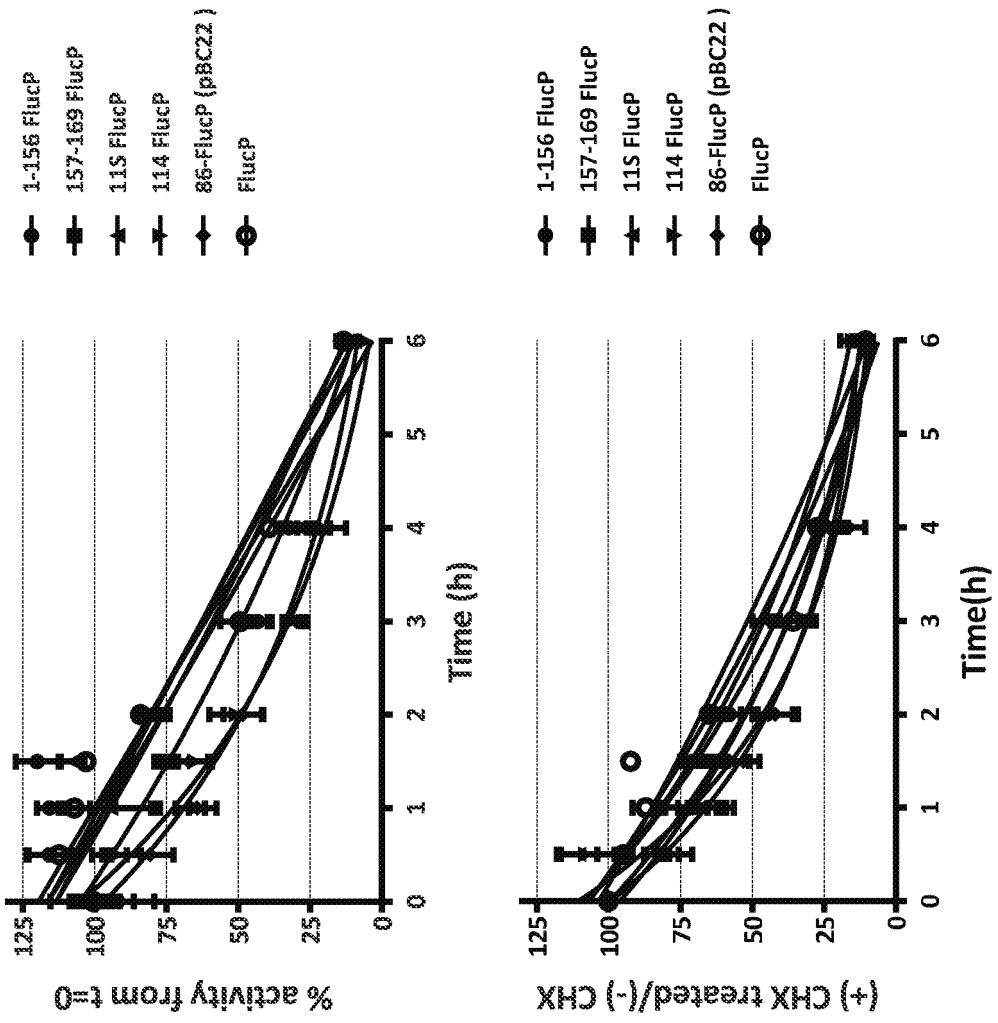

1-156 FlucP
157-169 FlucP
11S FlucP
114 FlucP
86-FlucP (pBC22)
FlucP
% activity from t=0
Time (h)
(+) CHX treated/(-) CHX
Time(h)

Figure 194

Extracellular protease activity (Caspase-1)
Utilize N-terminal cysteine to create peptide-quencher conjugates
Q
CX.WEHD
MLPro
Caspase-1
Quenched luminescence
Q
CX.WEHD
MLPro
Luminescent Bit
Detection with 11S & furimazine
Light

Figure 195

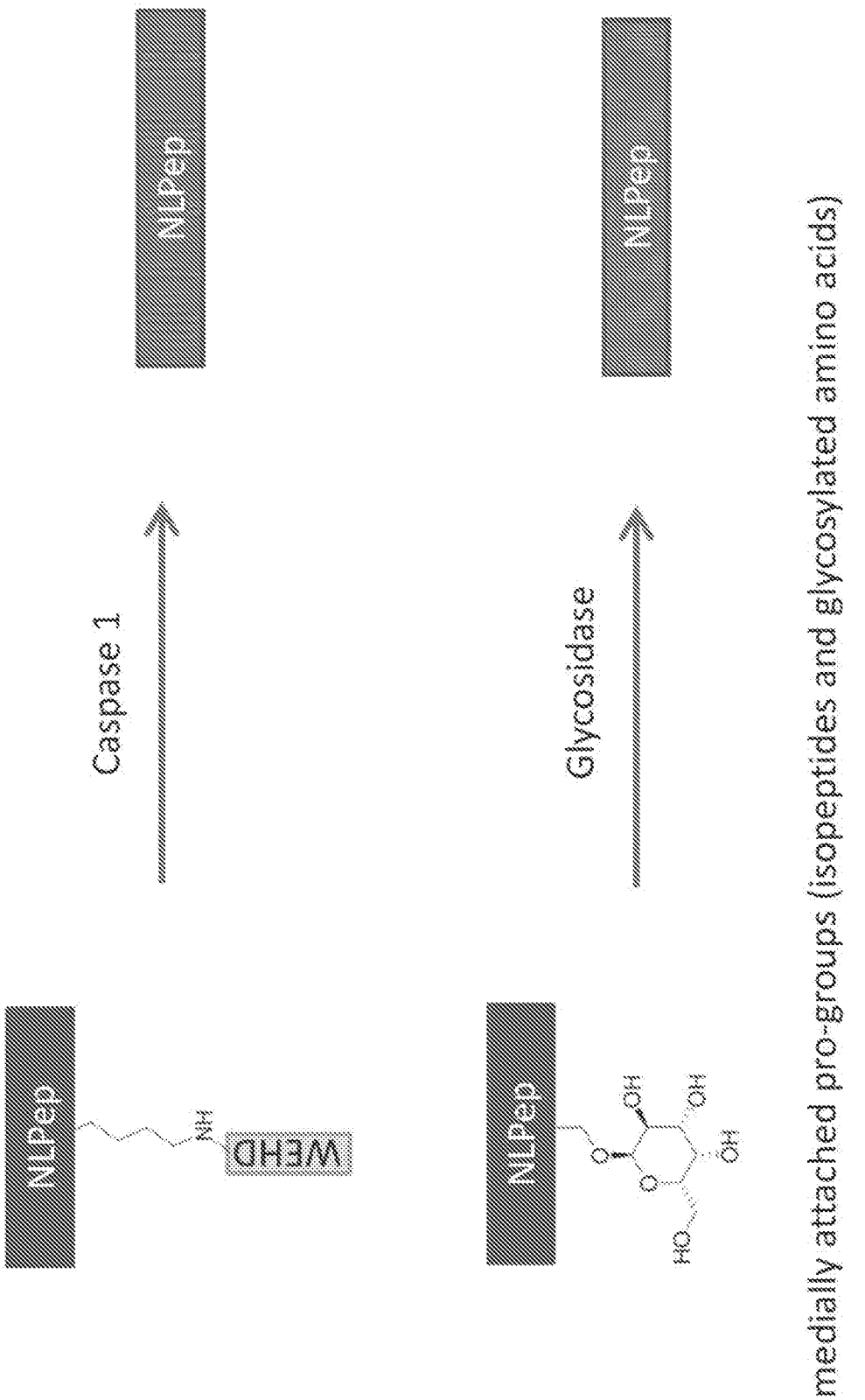

NLPep
Caspase 1
NLPep
WEHD
NH
NLPep
Glycosidase
NLPep
OH
OH
OH
HO
O
O
medially attached pro-groups (isopeptides and glycosylated amino acids)

Figure 196

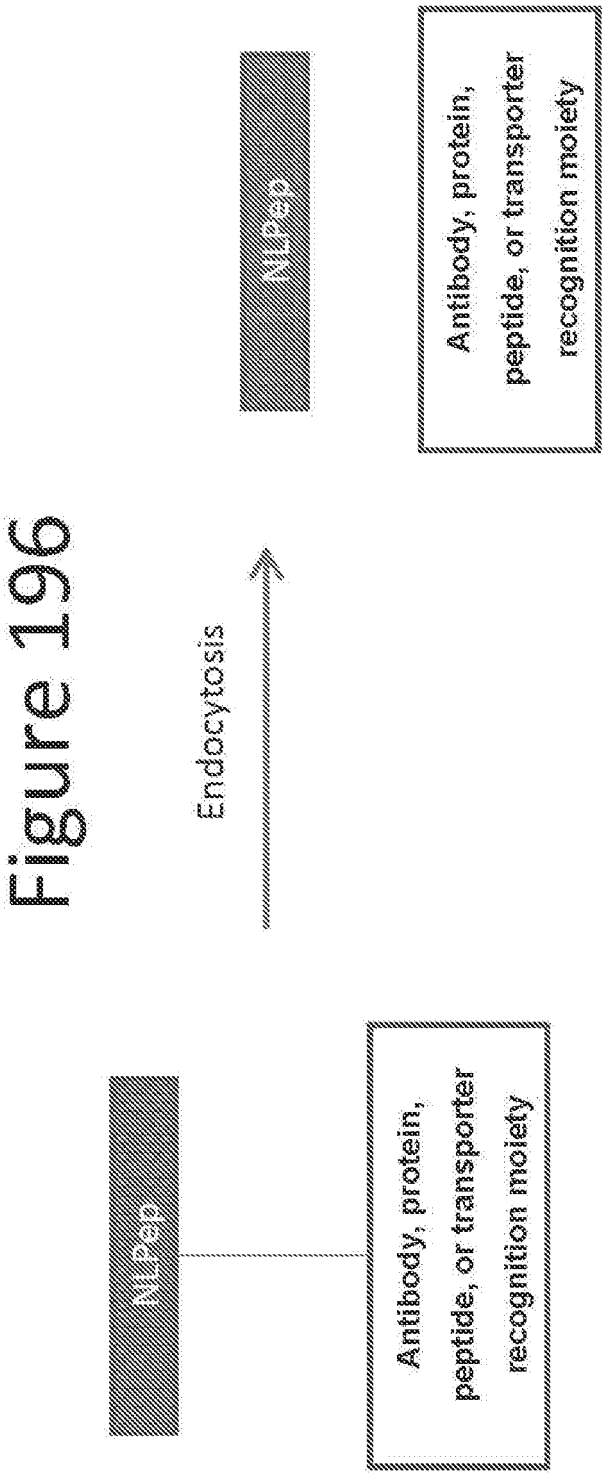

NLPep
Antibody, protein, peptide, or transporter recognition moiety
Endocytosis
NLPep
Antibody, protein, peptide, or transporter recognition moiety

Figure 197

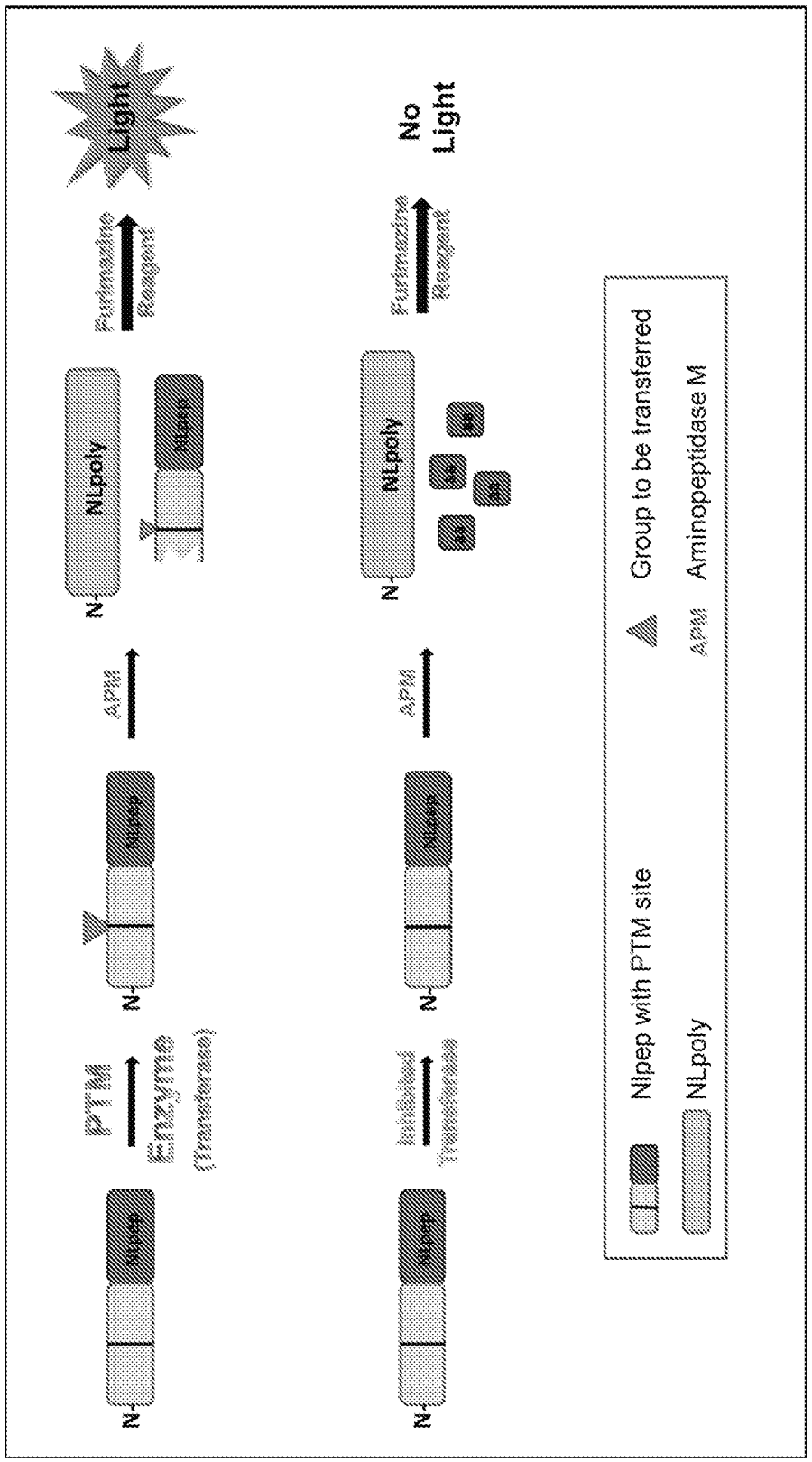

Light
Furimazine Reagent
NLpoly
APM
PTM Enzyme (Transferase)
No Light
Furimazine Reagent
NLpoly
APM
Inhibited Transferase
N
Nlpep with PTM site
Group to be transferred
NLpoly
APM Aminopeptidase M

Figure 198

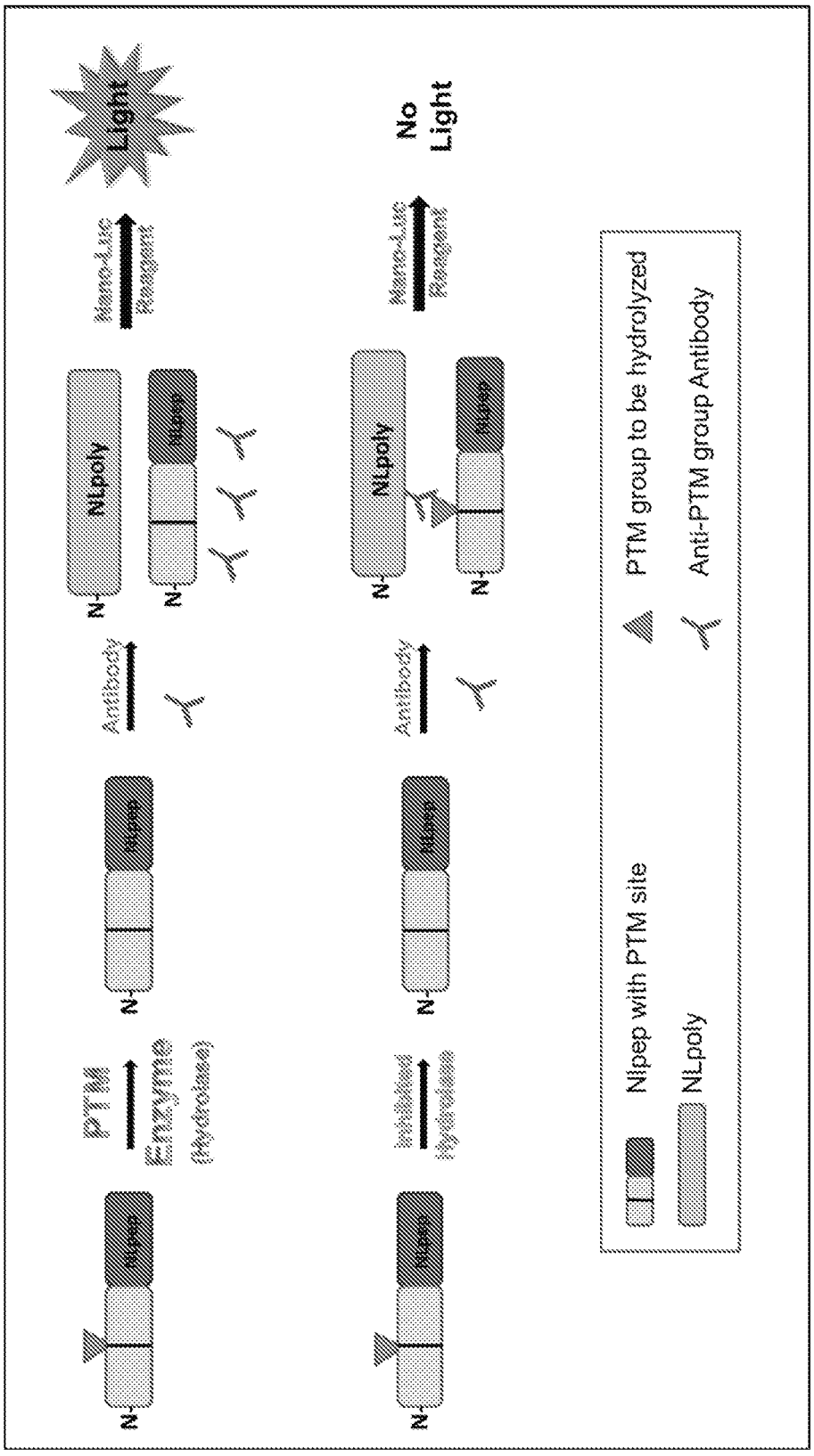
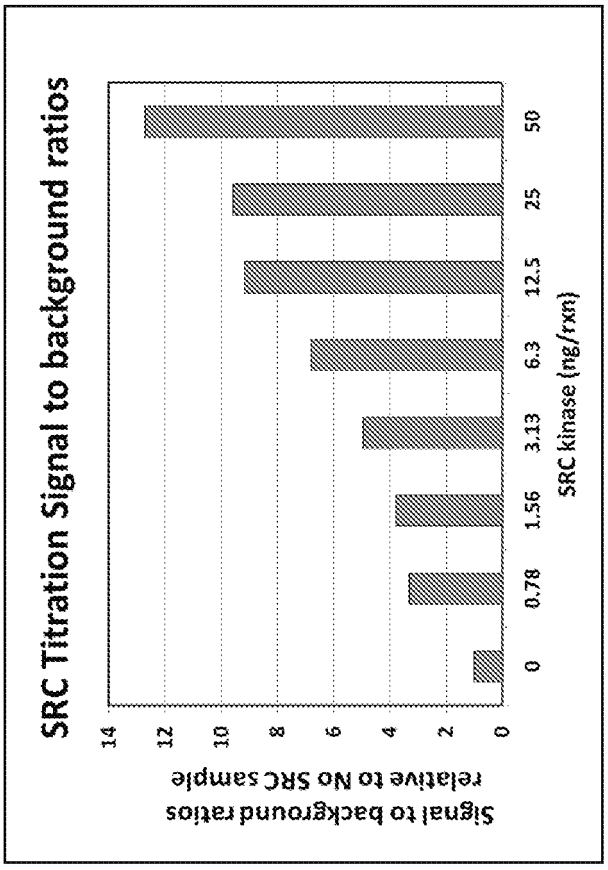

Nipep
N
PTM
Enzyme
(Hydrolyzes)
Antibody
NLpoly
Nano-Luc
Reagent
Light
Initiated
Hydrolysis
No
Light
Nipep with PTM site
NLpoly
PTM group to be hydrolyzed
Anti-PTM group Antibody

Figure 199


SRC Titration Signal to background ratios
Signal to background ratios relative to No SRC sample
0
2
4
6
8
10
12
14
0
0.78
1.56
3.13
6.3
12.5
25
50
SRC kinase (ng/rxn)

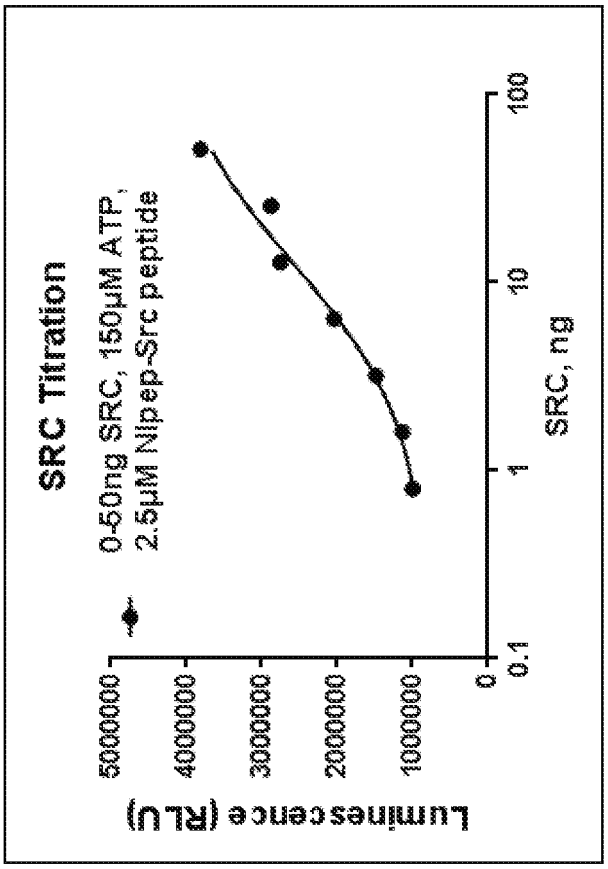
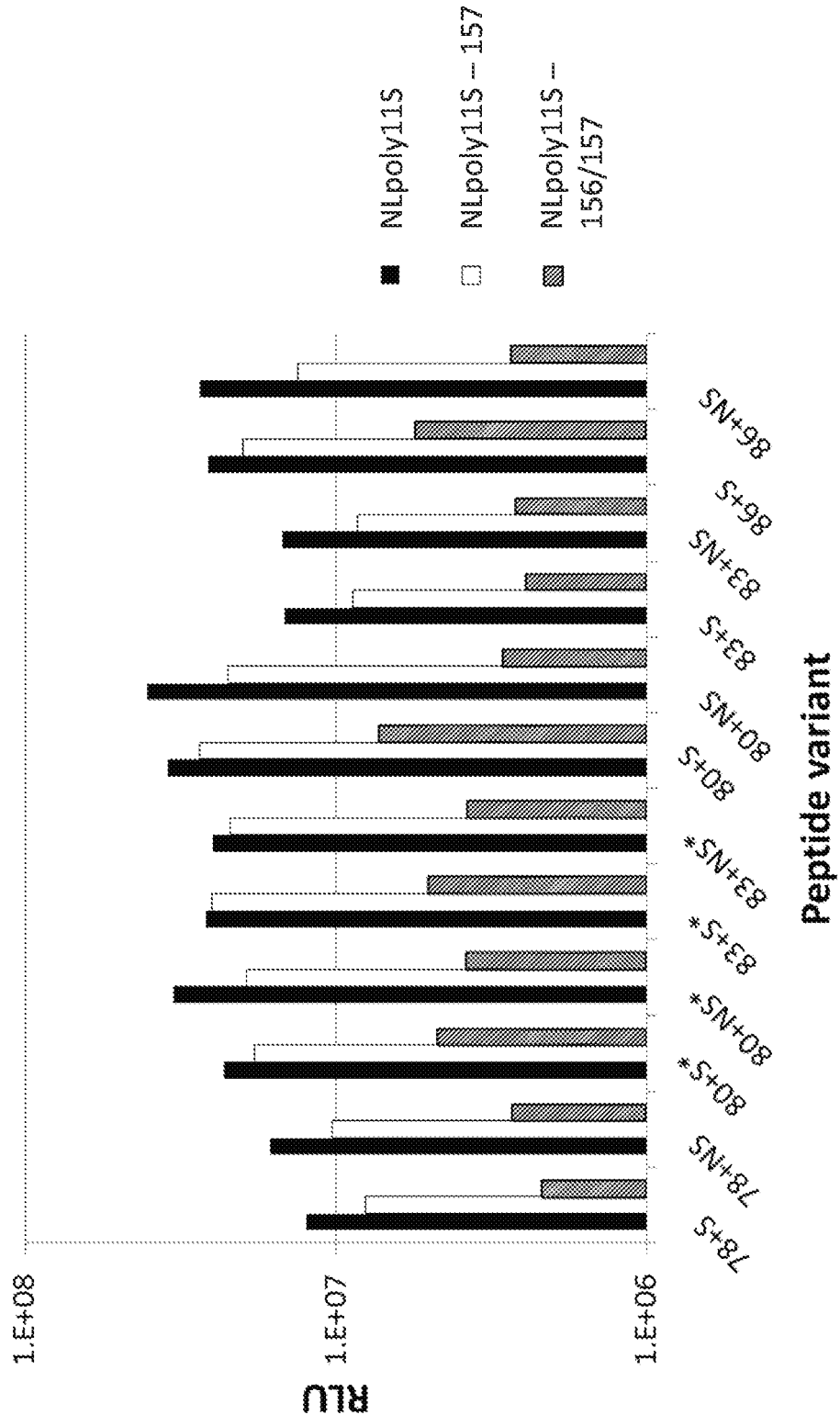

SRC Titration
0-50ng SRC, 150µM ATP,
2.5µM Nipep-Src peptide
Luminescence (RLU)
0
1000000
2000000
3000000
4000000
5000000
0.1
1
10
100
SRC, ng

Figure 200



This data shows that all twelve of the peptide variants can spontaneously interact with NLpoly11S, NLpoly11S (- 157), or NLpoly11S (- 156/ - 157).

Figure 201

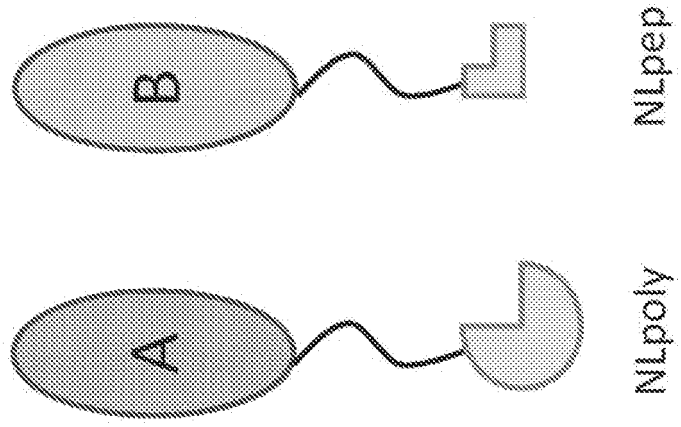


Figure 202

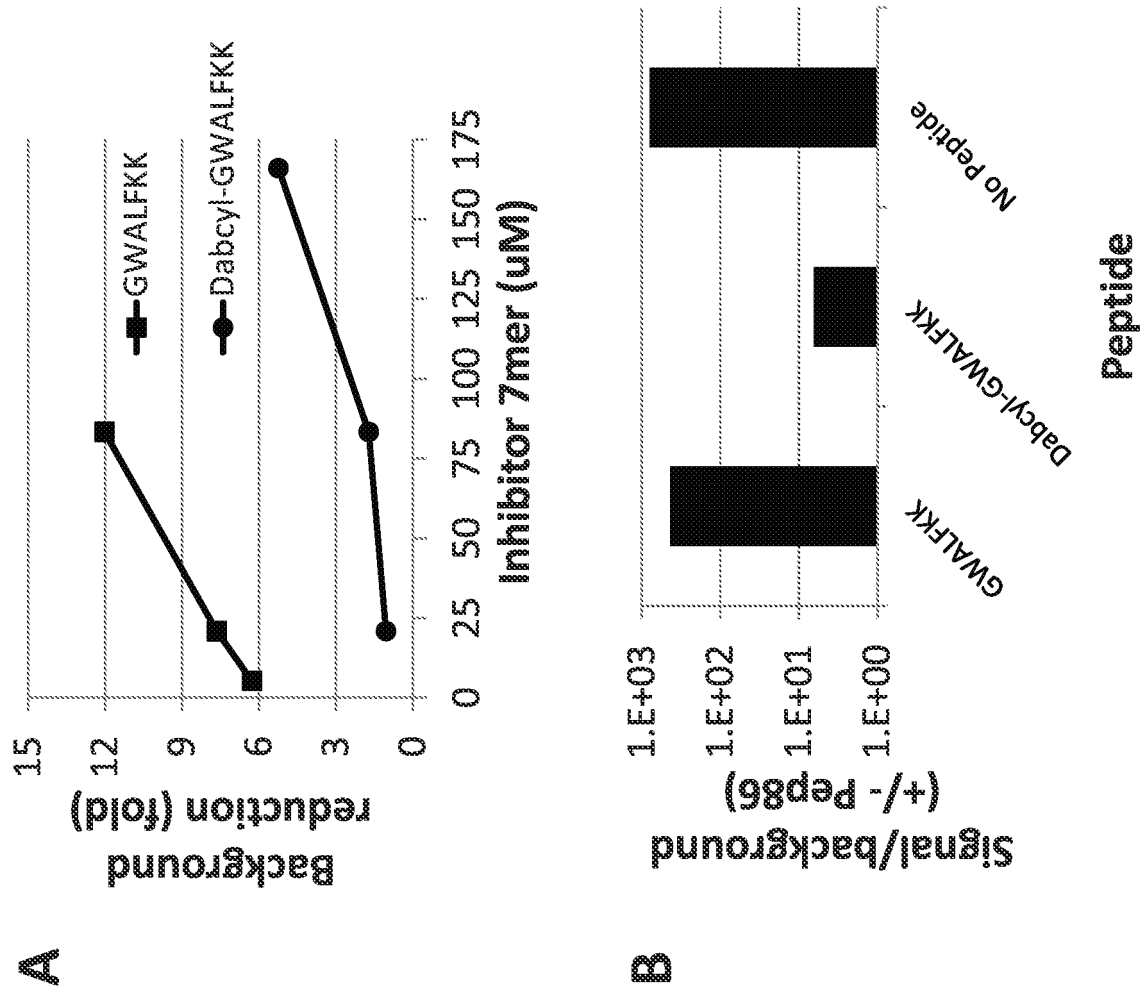
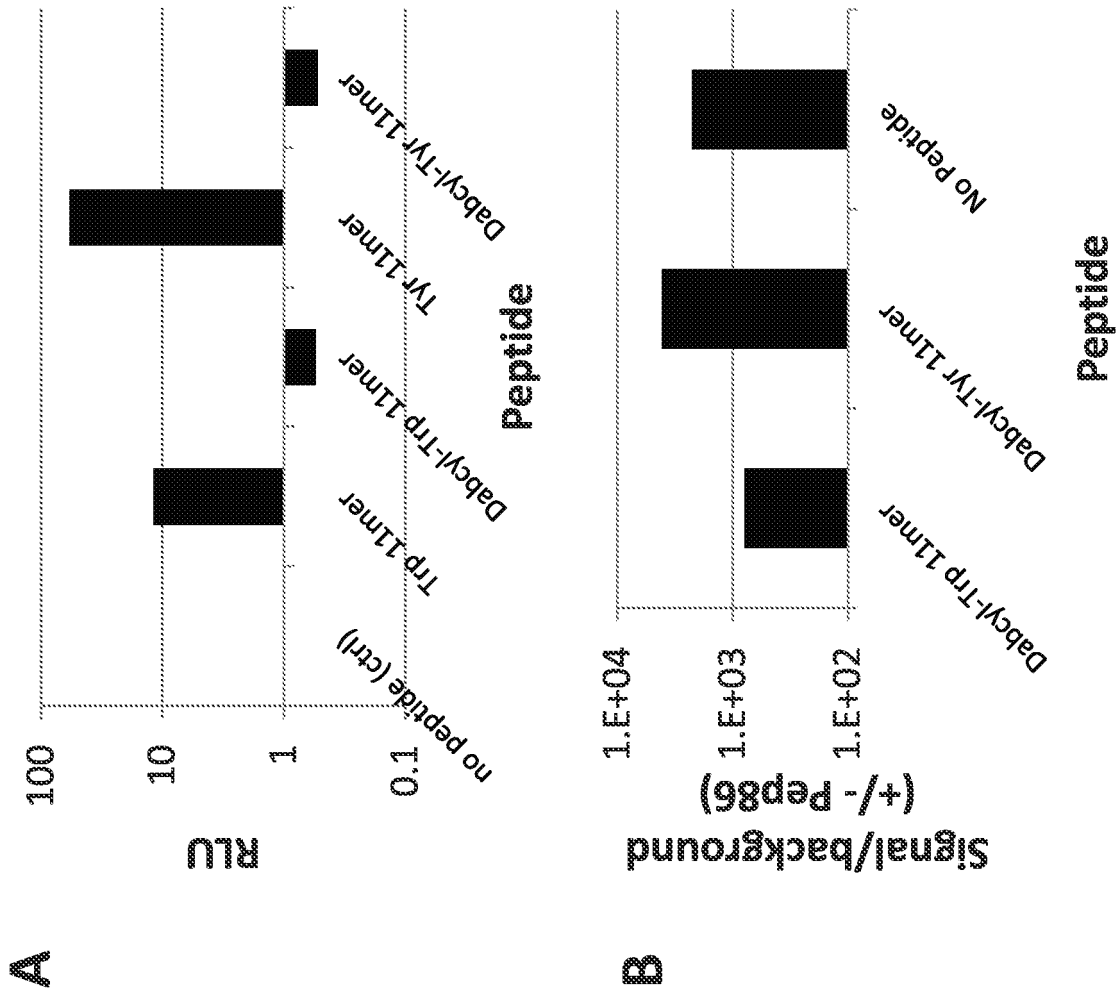

A
Background reduction (fold)
15
12
9
6
3
0
GWALFKK
Dabcyl-GWALFKK
0 25 50 75 100 125 150 175
Inhibitor 7mer (uM)
B
Signal/background (+/- Pep86)
1.E+03
1.E+02
1.E+01
1.E+00
GWALFKK
Dabcyl-GWALFKK
No Peptide
Peptide

Figure 203


A
RLU
100
10
1
0.1
no peptide (ctrl)
Trp-11mer
Dabcyl-Trp-11mer
Tyr-11mer
Dabcyl-Tyr-11mer
Peptide
B
Signal/background
(+/- Pep86)
1.E+04
1.E+03
1.E+02
Dabcyl-Trp-11mer
Dabcyl-Tyr-11mer
No Peptide
Peptide