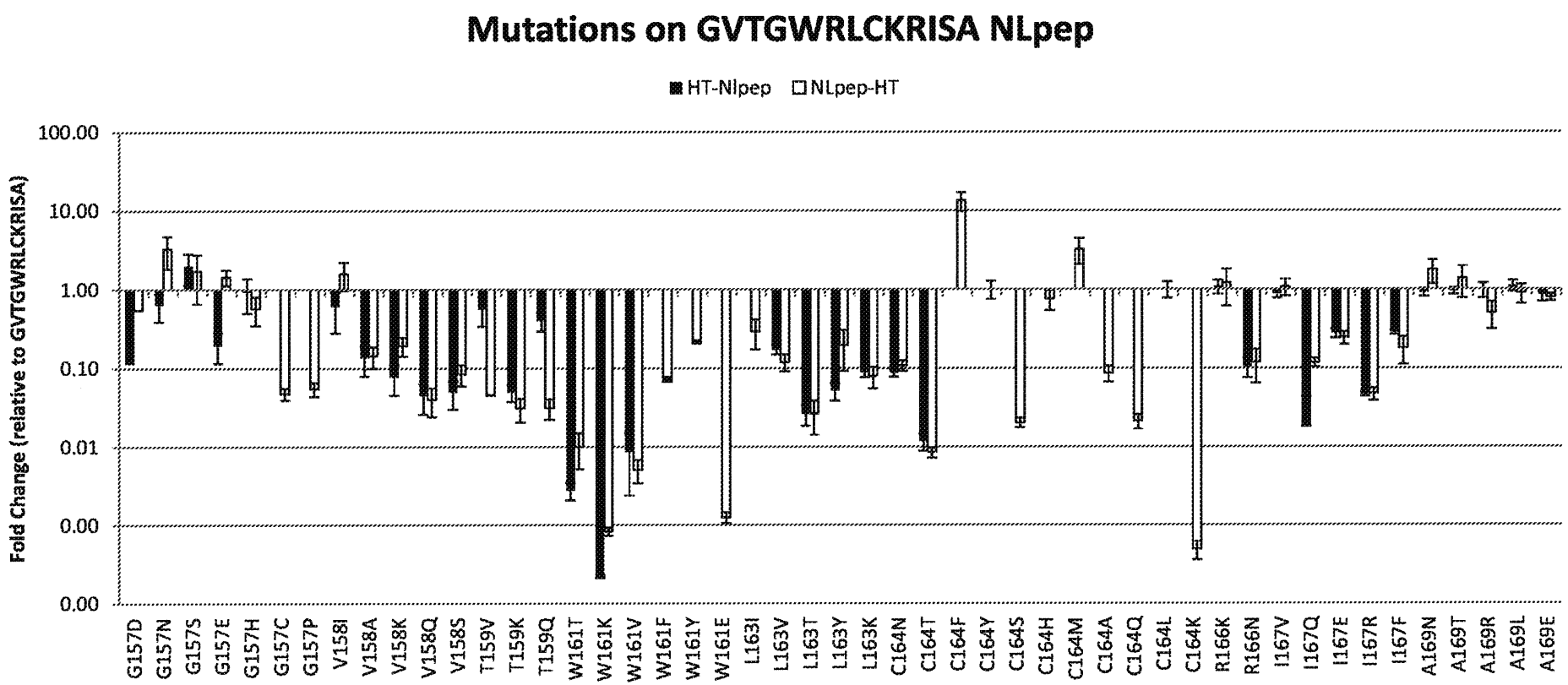
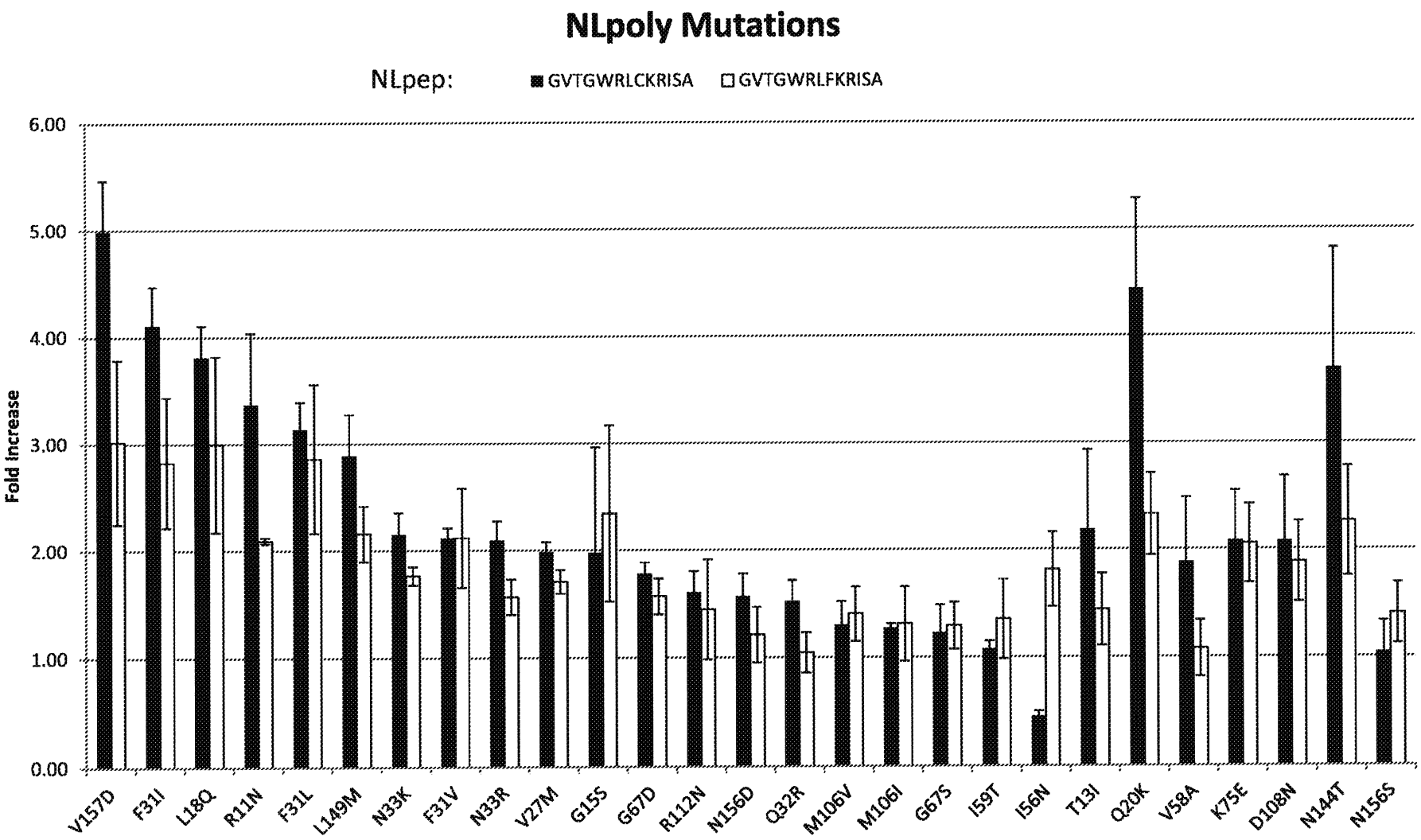
Figure 1



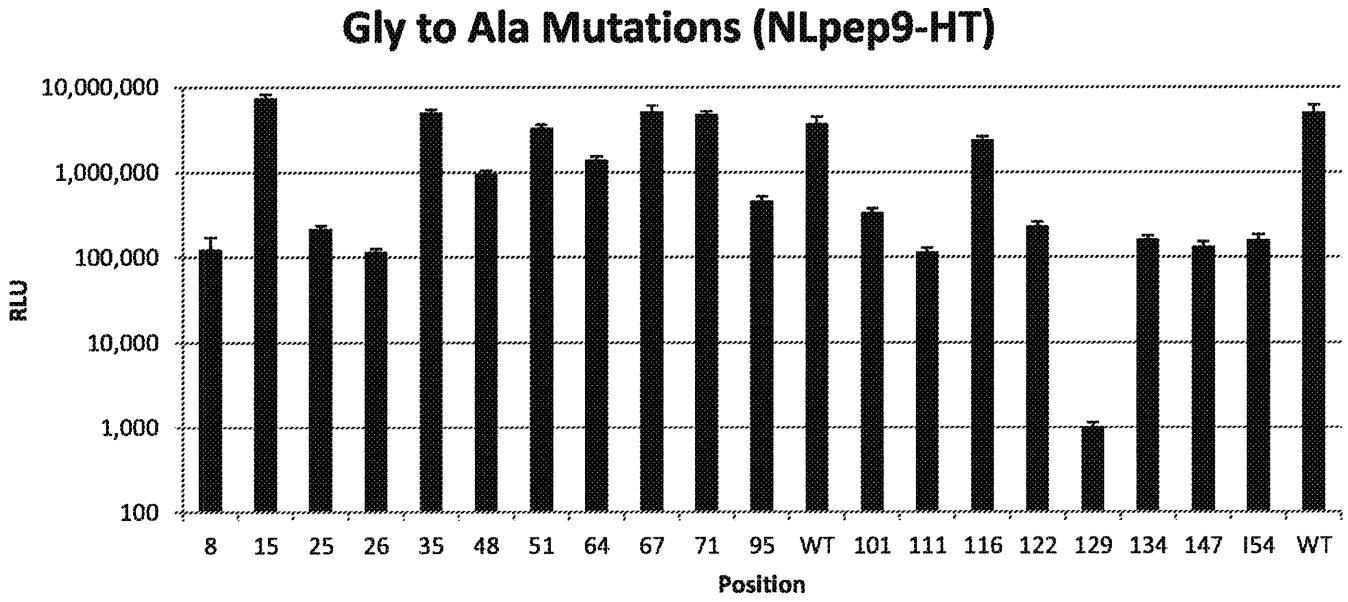
SUBSTITUTE SHEET (RULE 26)
1/204

Figure 2



SUBSTITUTE SHEET (RULE 26)

Figure 3

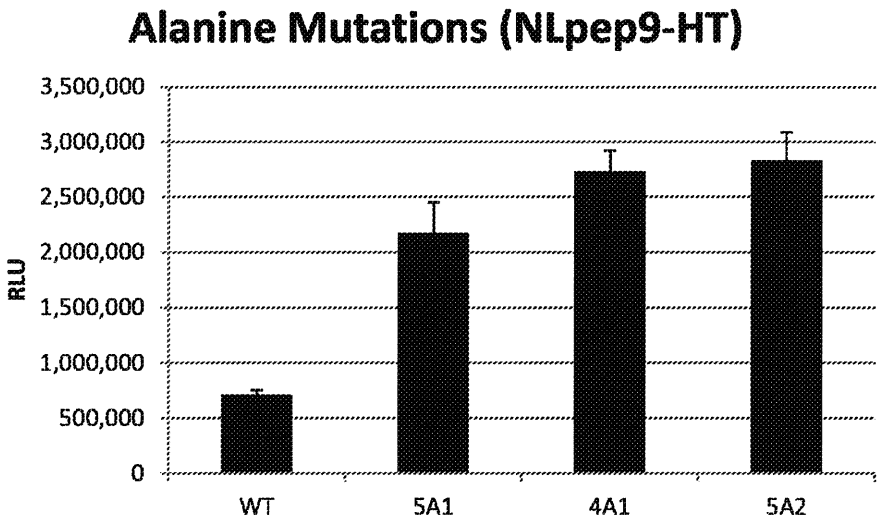


Position	8	15	25	26	35	48	51	64	67	71	95	WT	101	111	116	122	129	134	147	I54A	WT
RLU	1.2E+05	7.5E+06	2.2E+05	1.2E+05	5.0E+06	9.5E+05	3.4E+06	1.4E+06	5.2E+06	4.8E+06	4.6E+05	3.7E+06	3.4E+05	1.1E+05	2.4E+06	2.3E+05	1.0E+03	1.6E+05	1.3E+05	1.6E+05	5.1E+06
Fold Increase	0.03	2.01	0.06	0.03	1.35	0.26	0.90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

SUBSTITUTE SHEET (RULE 26)

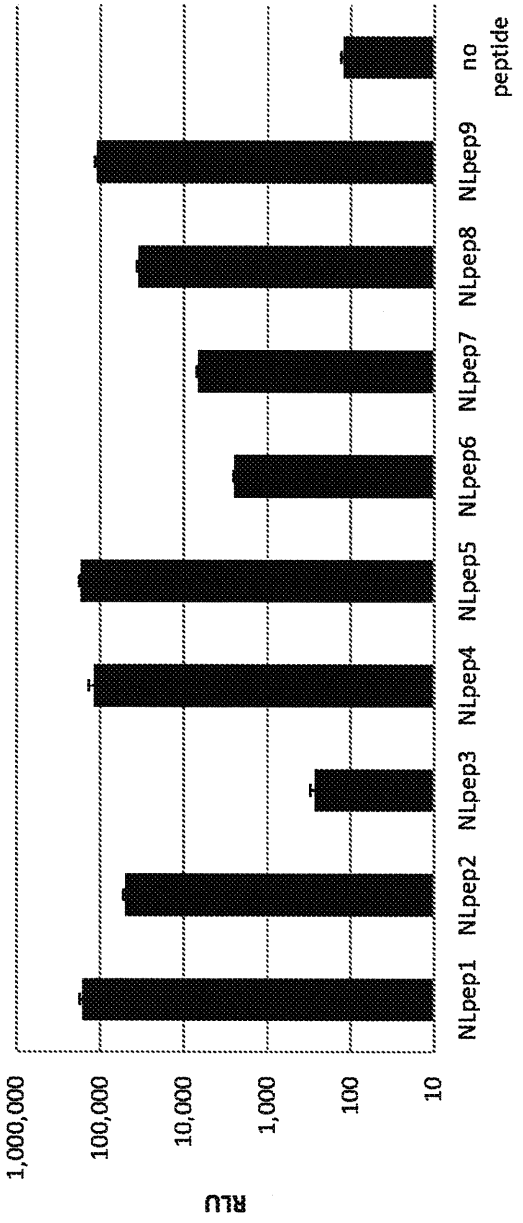
SUBSTITUTE SHEET (RULE 26)

Figure 4



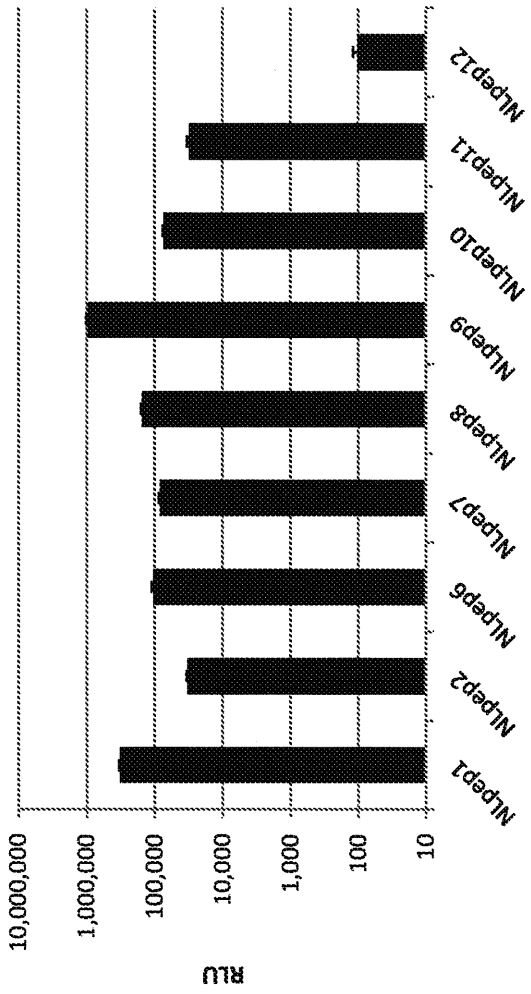
Name	G15A	D19A	G35A	G51A	G67A	G71A	Fold Incr.
5A1	Yes	Yes	Yes	Yes	Yes		3.07
4A1	Yes		Yes		Yes	Yes	3.85
5A2	Yes		Yes	Yes	Yes	Yes	3.99

Figure 5



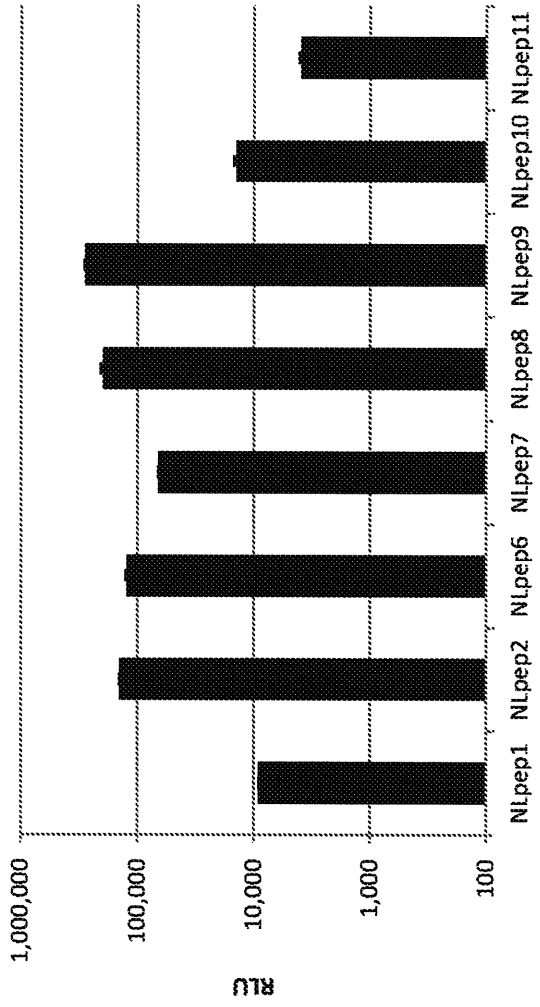
SUBSTITUTE SHEET (RULE 26)

Figure 6



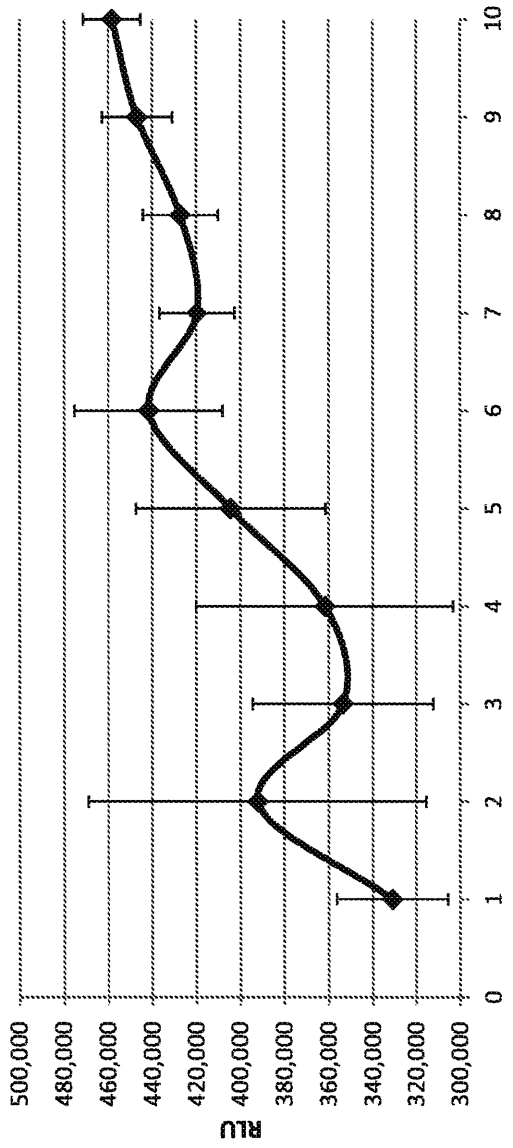
SUBSTITUTE SHEET (RULE 26)

Figure 7



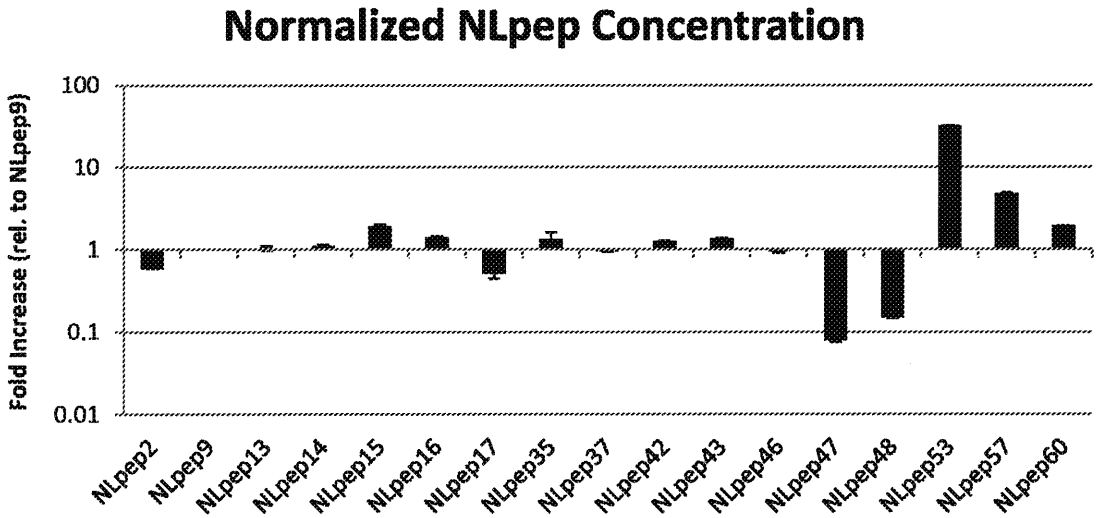
SUBSTITUTE SHEET (RULE 26)

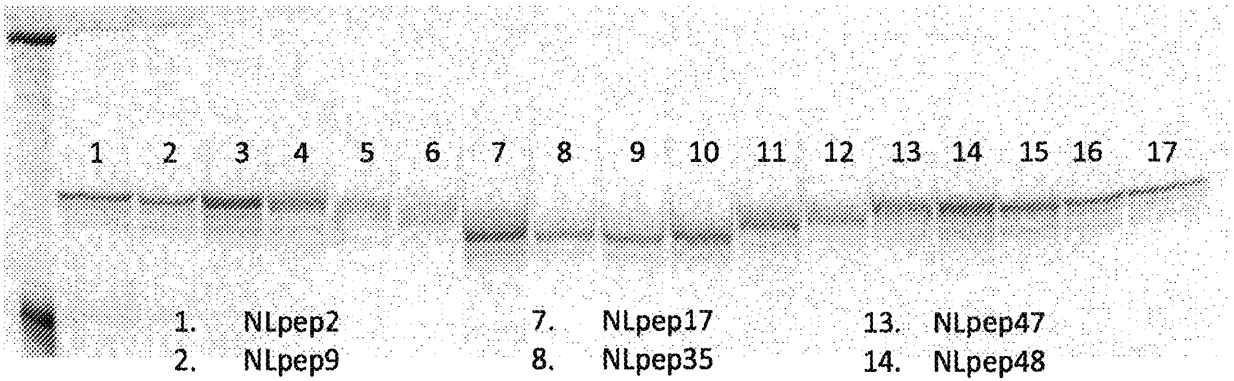
Figure 8



SUBSTITUTE SHEET (RULE 26)

Figure 9





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

SUBSTITUTE SHEET (RULE 26)

Figure 10

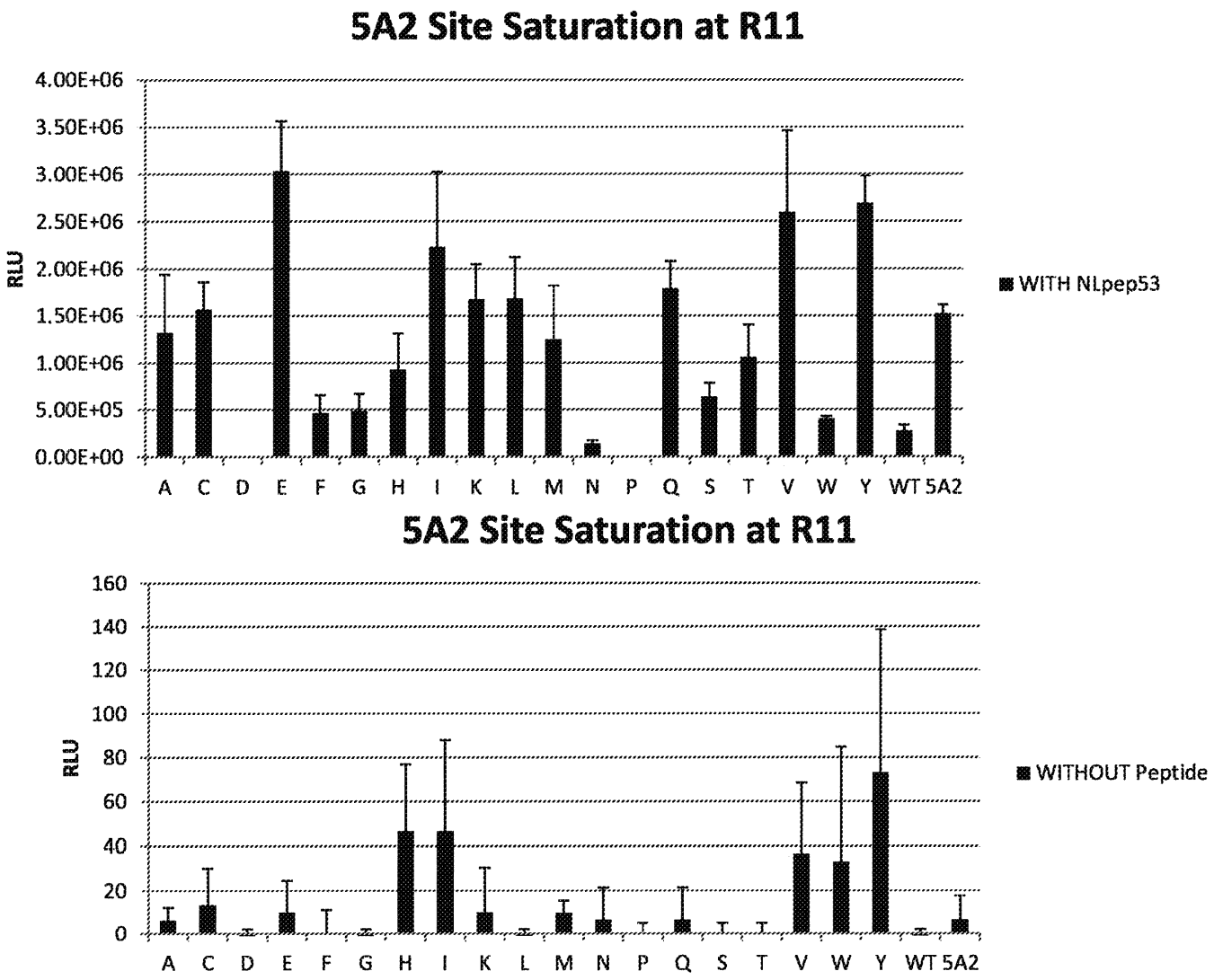
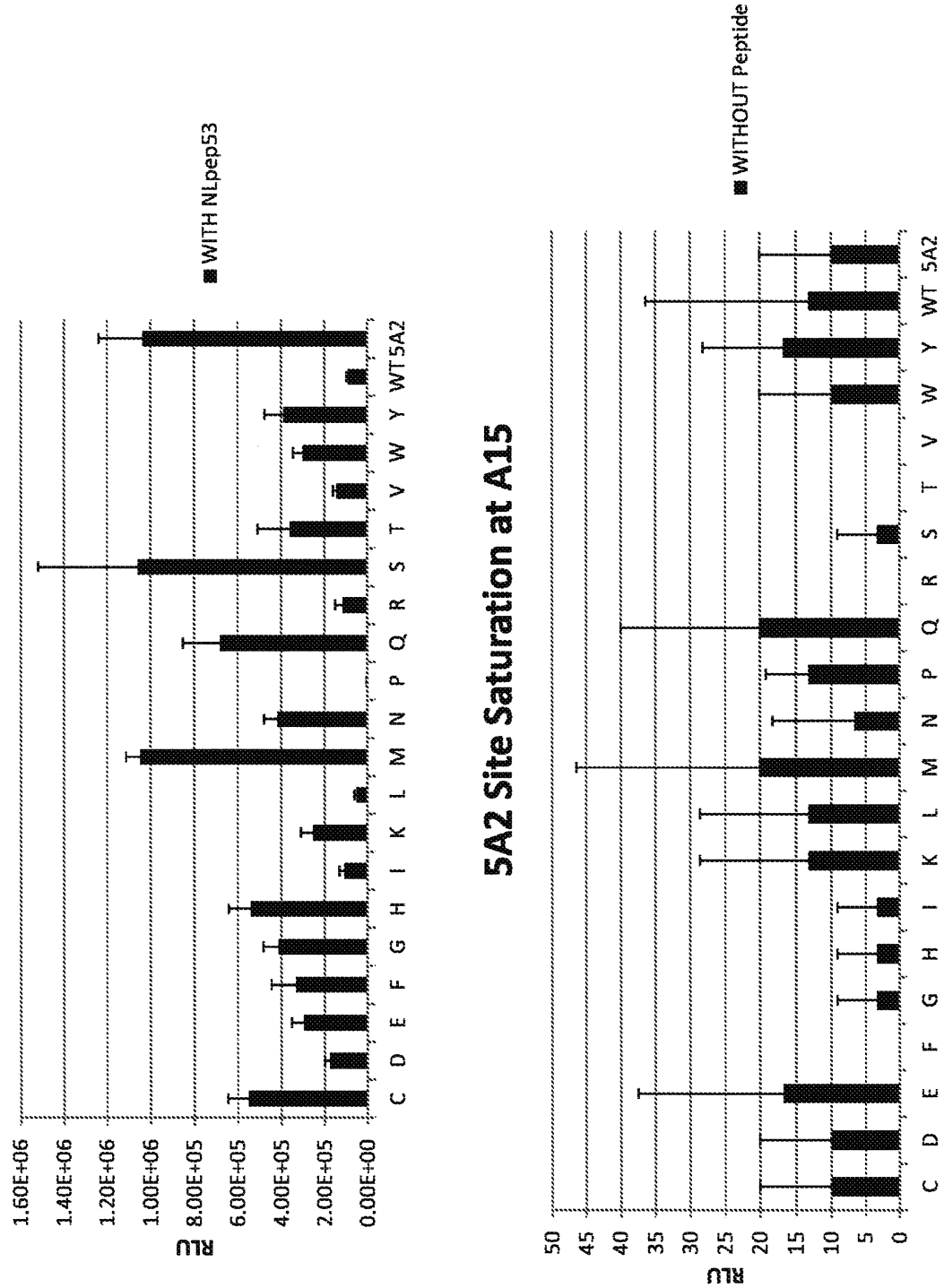
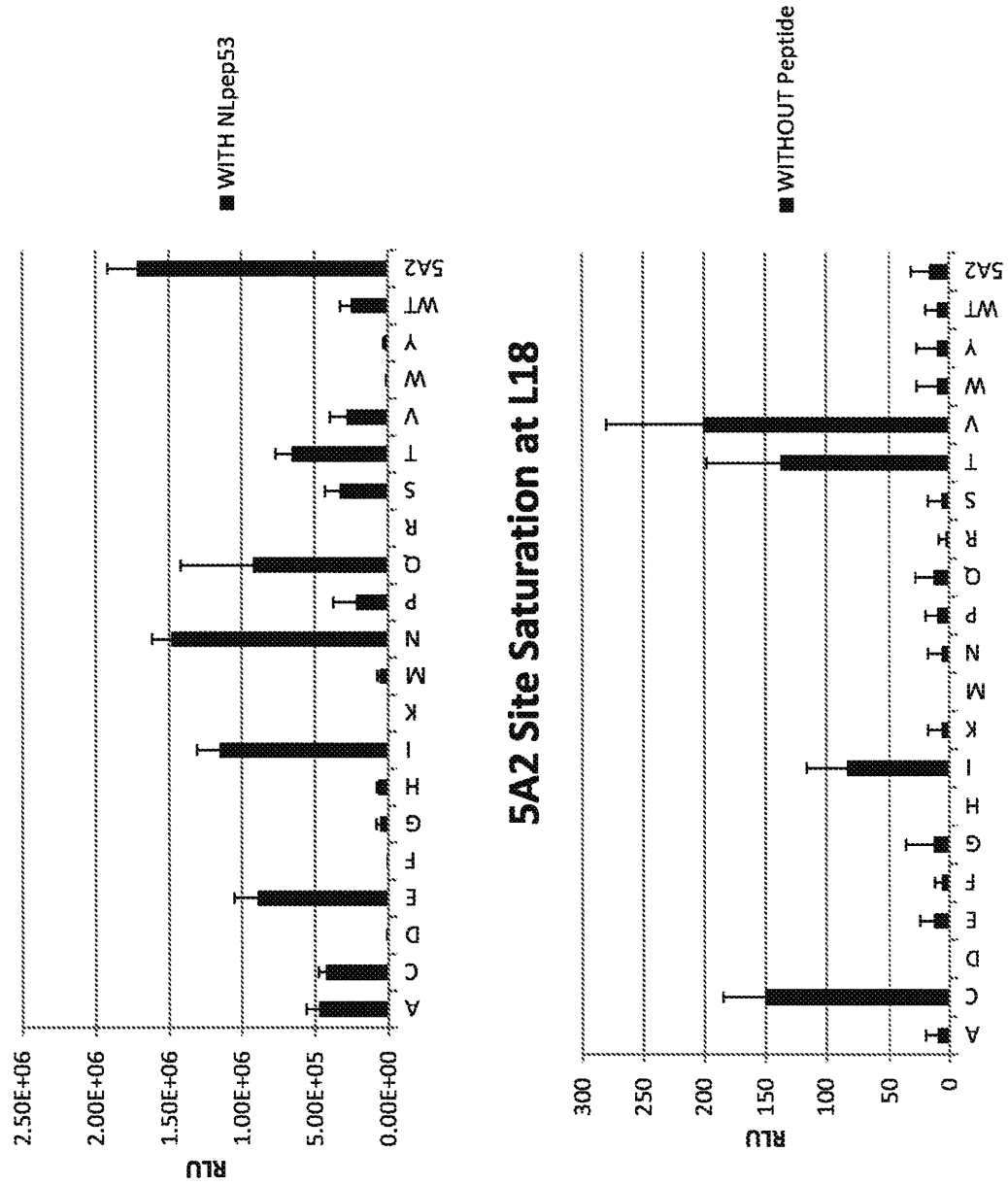

5A2 Site Saturation at R11
RLU
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
A C D E F G H I K L M N P Q S T V W Y WT 5A2
WITH NLpep53
5A2 Site Saturation at R11
RLU
160
140
120
100
80
60
40
20
0
A C D E F G H I K L M N P Q S T V W Y WT 5A2
WITHOUT Peptide

Figure 11



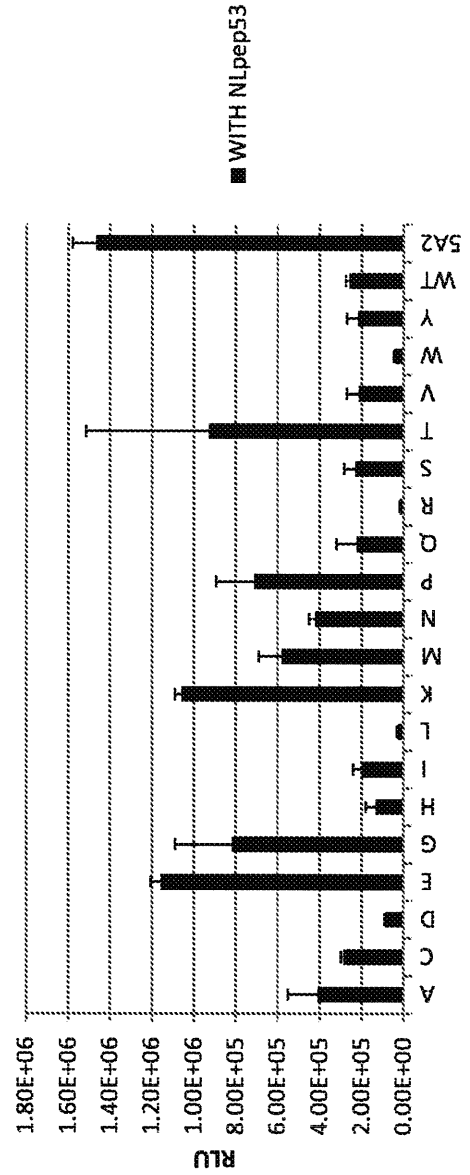
SUBSTITUTE SHEET (RULE 26)

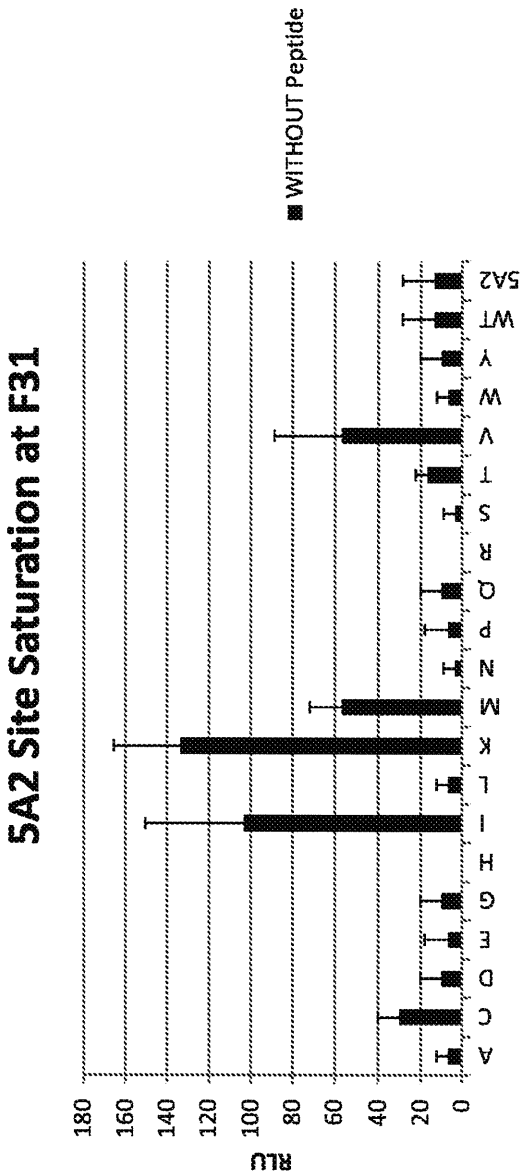
Figure 12



SUBSTITUTE SHEET (RULE 26)

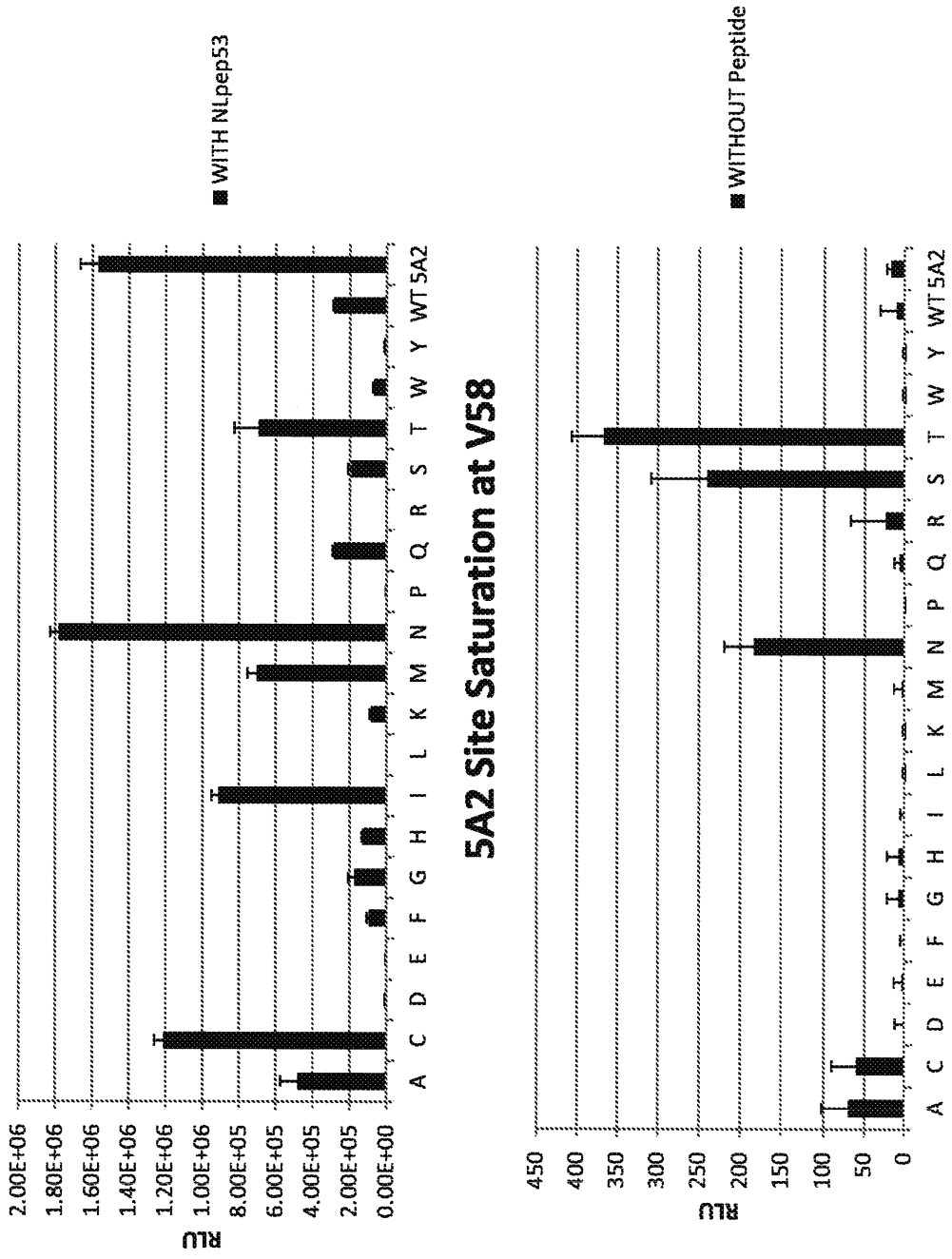
Figure 13





SUBSTITUTE SHEET (RULE 26)

Figure 14



SUBSTITUTE SHEET (RULE 26)

SUBSTITUTE SHEET (RULE 26)

Figure 15

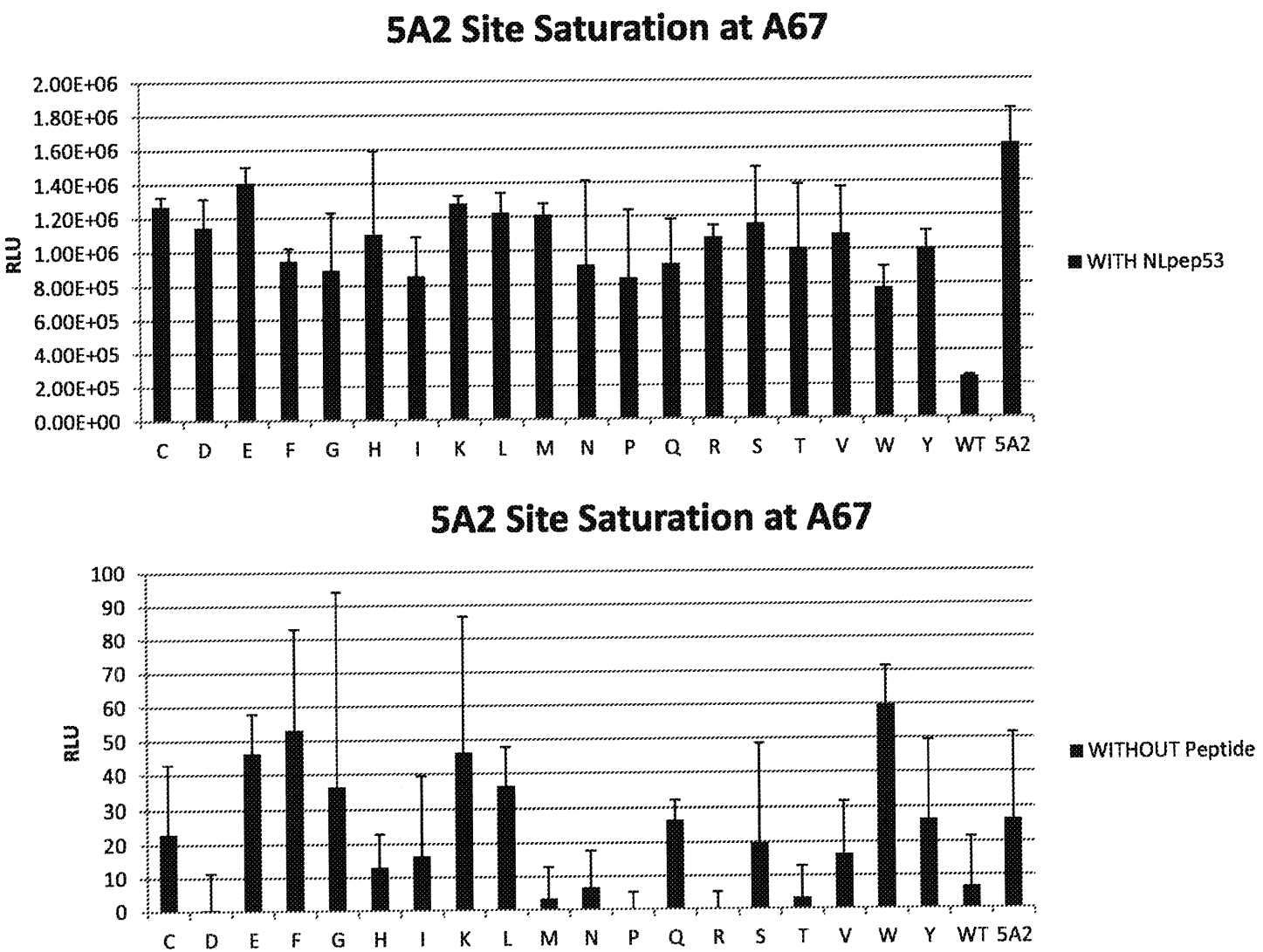
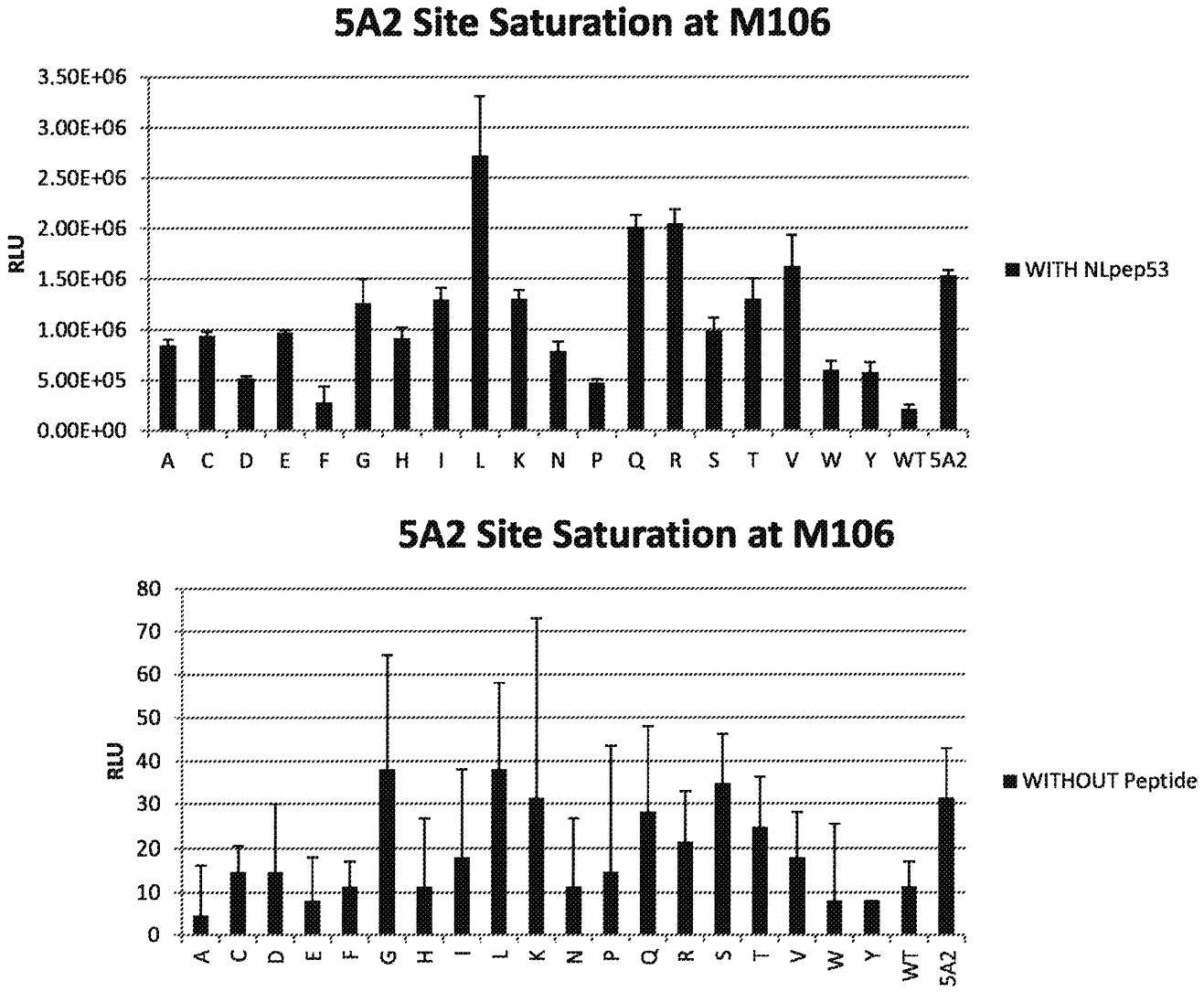


Figure 16


5A2 Site Saturation at M106
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
WITH NLpep53
5A2 Site Saturation at M106
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
WITHOUT Peptide

SUBSTITUTE SHEET (RULE 26)

Figure 17

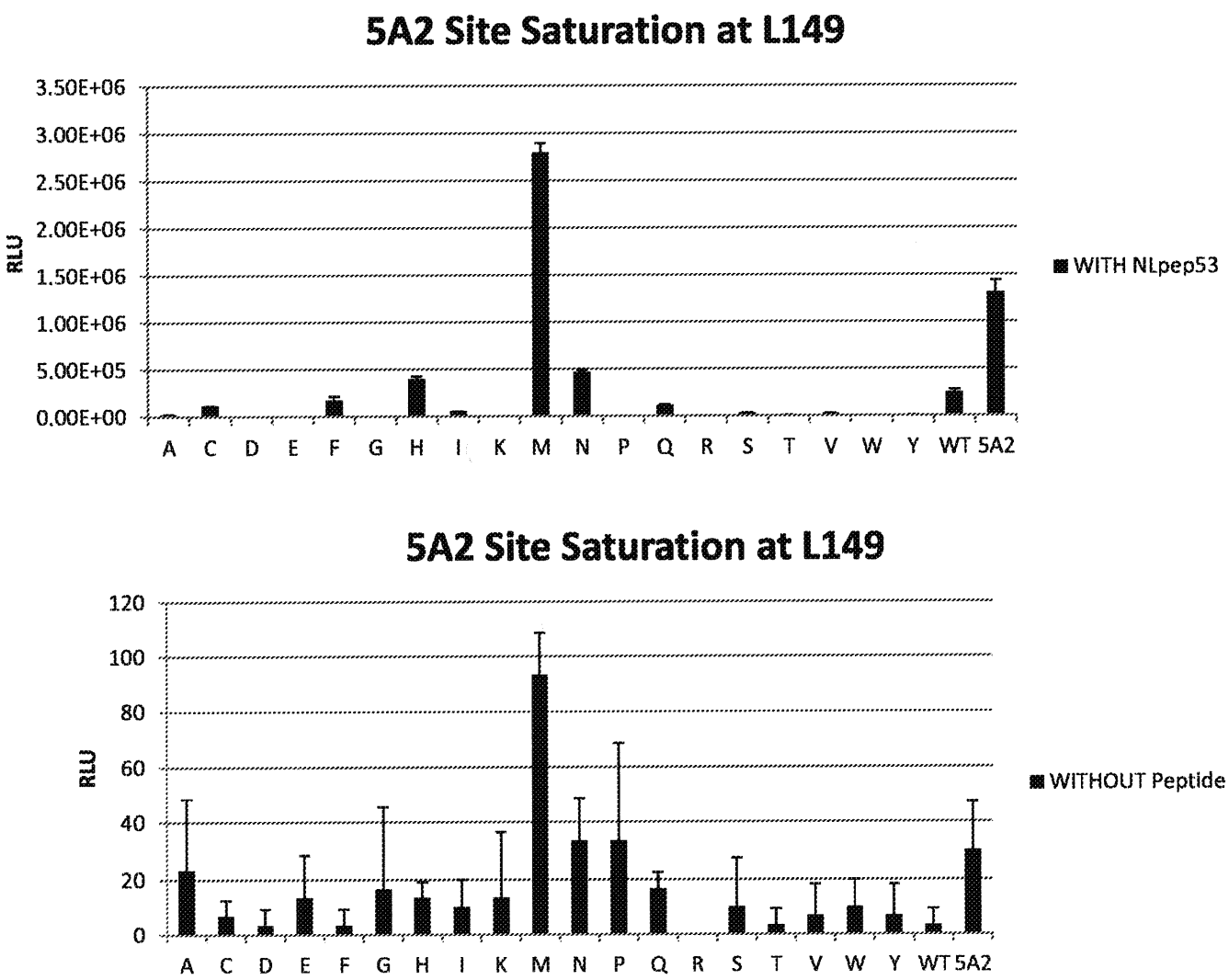
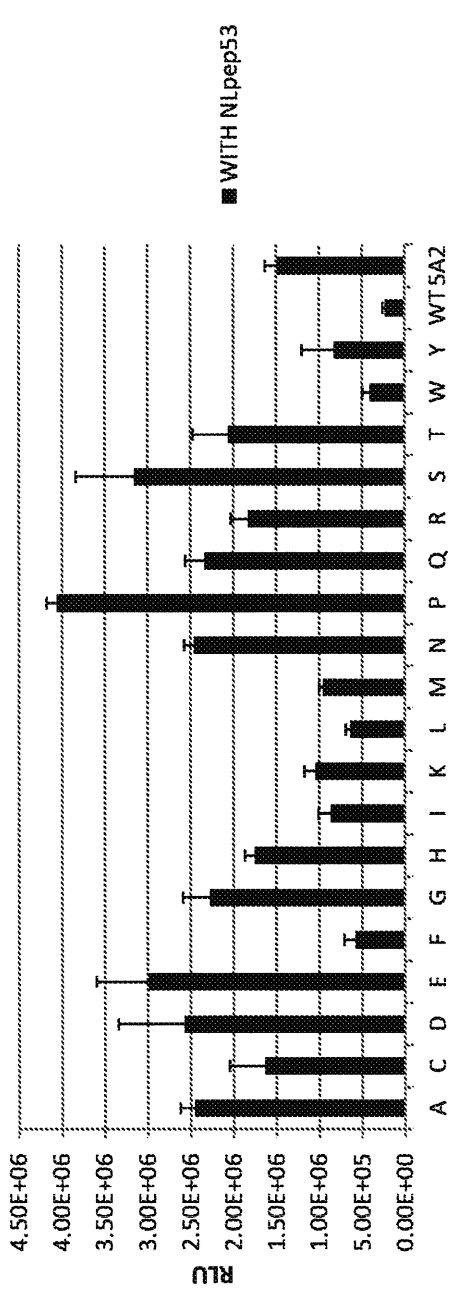
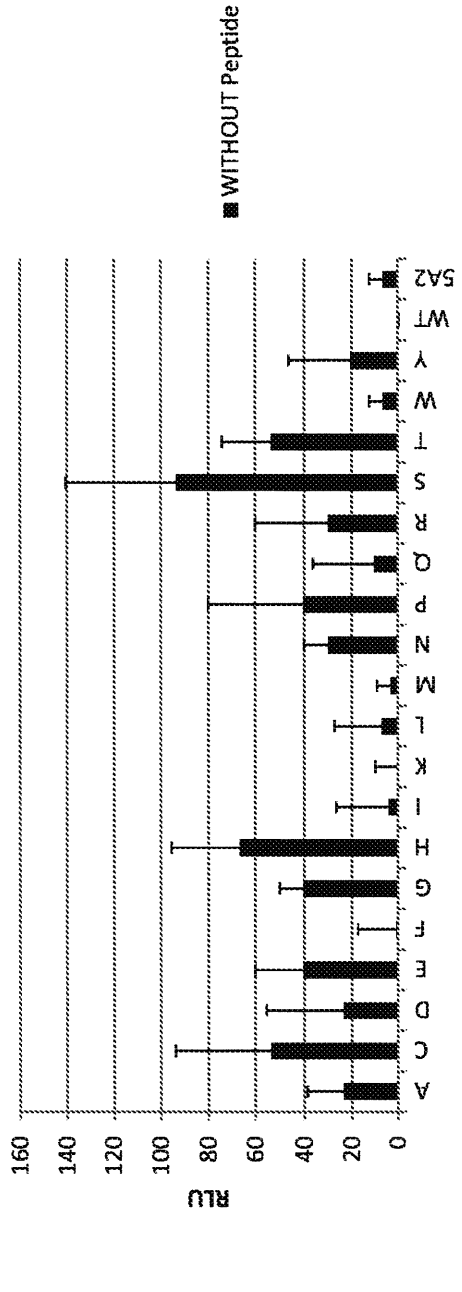


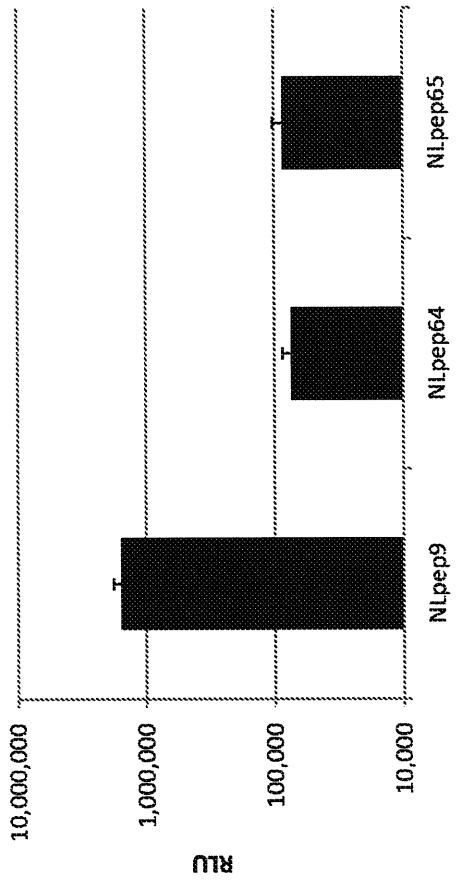
Figure 18





SUBSTITUTE SHEET (RULE 26)

Figure 19



SUBSTITUTE SHEET (RULE 26)

Figure 20

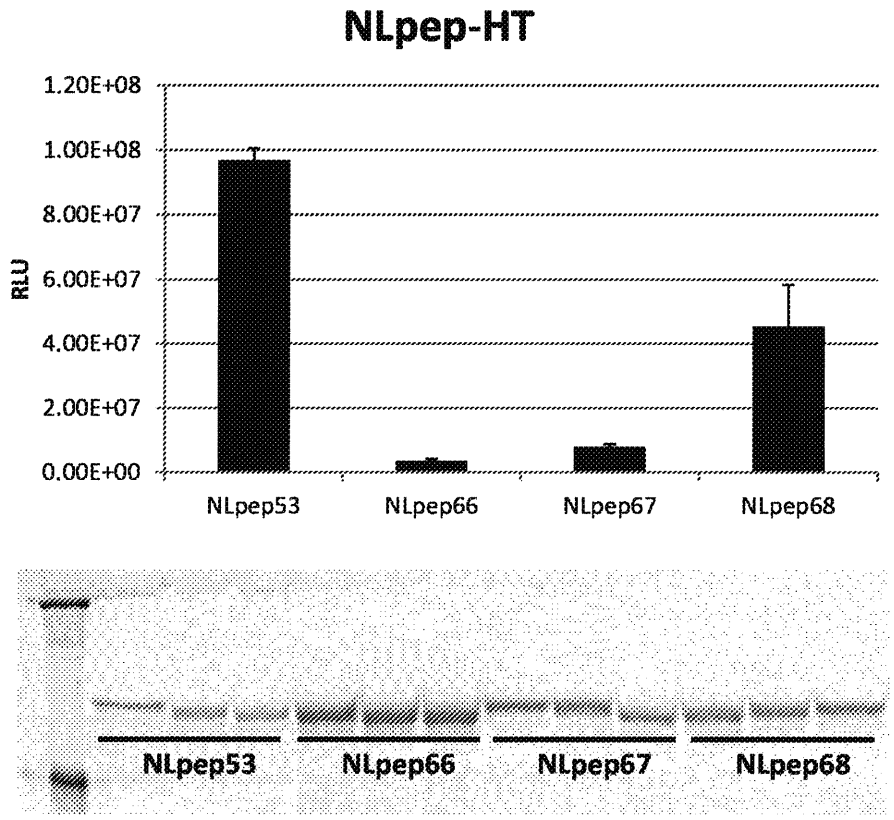
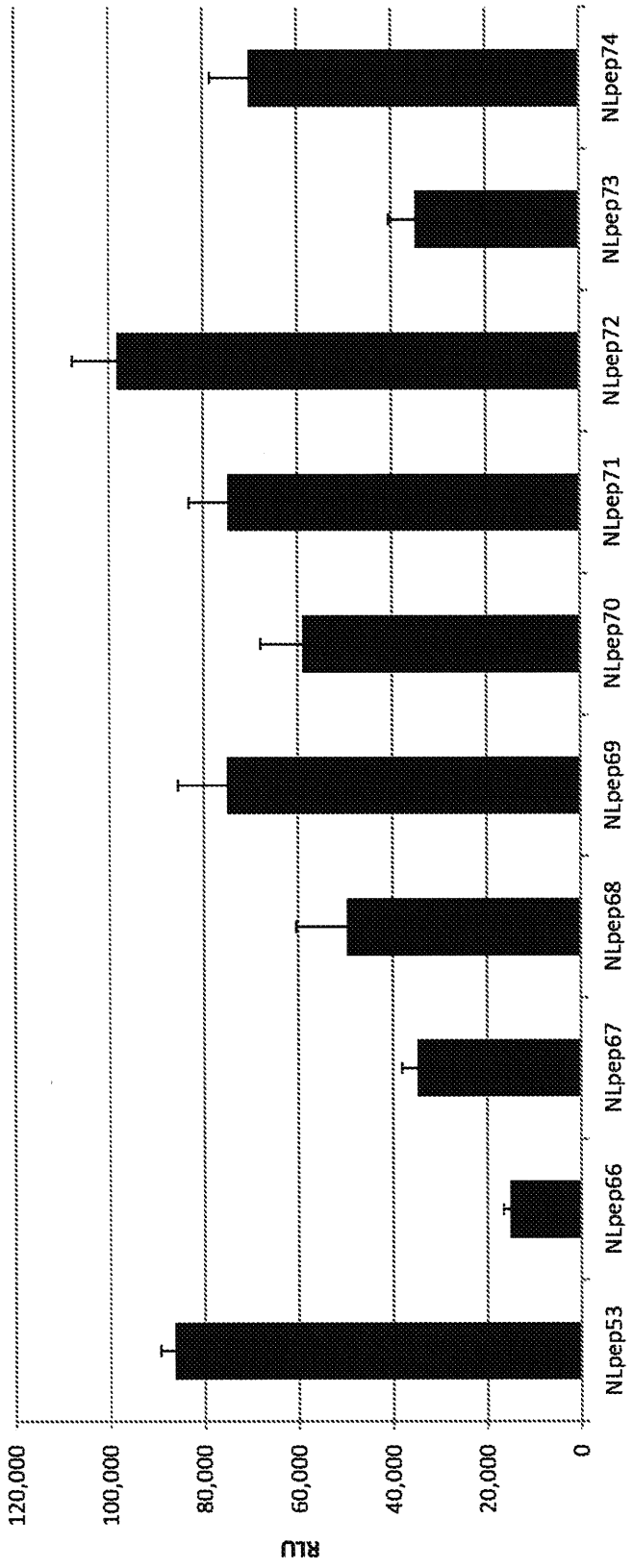

NLpep-HT
RLU
1.20E+08
1.00E+08
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
NLpep53
NLpep66
NLpep67
NLpep68
NLpep53
NLpep66
NLpep67
NLpep68

Figure 21



SUBSTITUTE SHEET (RULE 26)

Figure 22

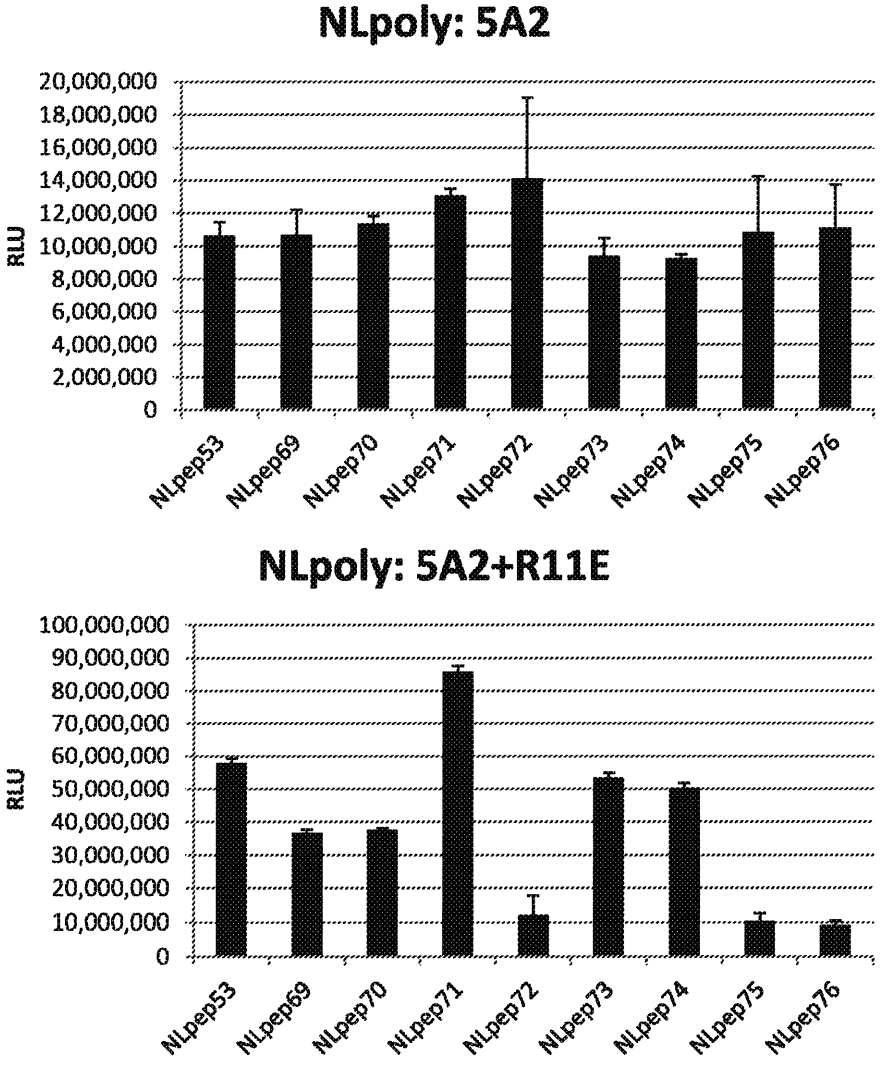
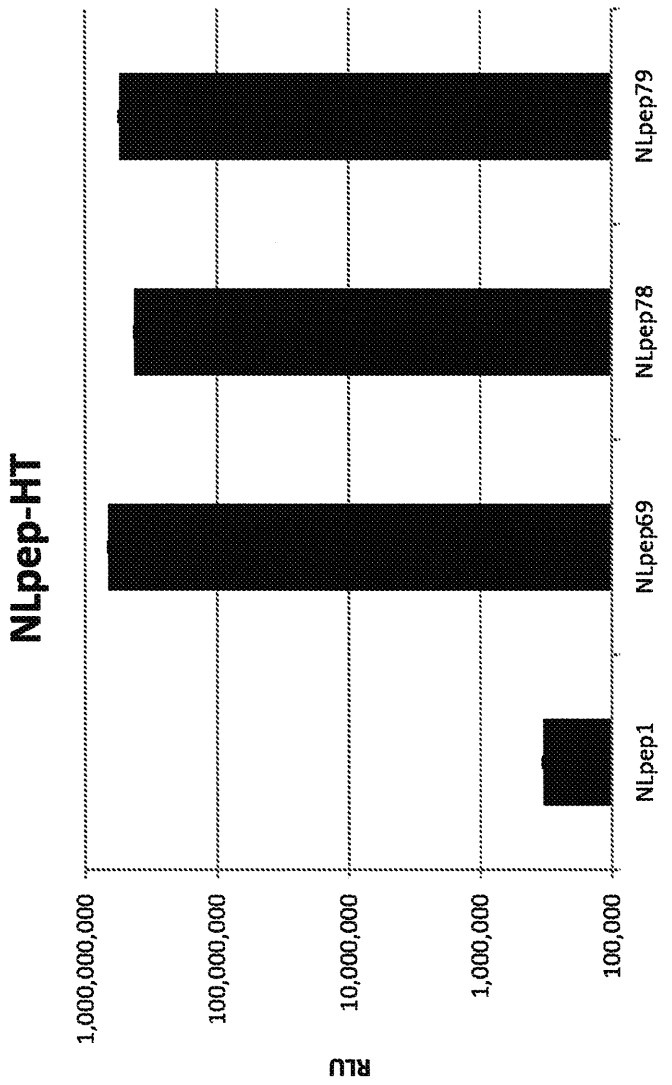

NLpoly: 5A2
RLU
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
NLpep53
NLpep69
NLpep70
NLpep71
NLpep72
NLpep73
NLpep74
NLpep75
NLpep76
NLpoly: 5A2+R11E
RLU
100,000,000
90,000,000
80,000,000
70,000,000
60,000,000
50,000,000
40,000,000
30,000,000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

Figure 23



SUBSTITUTE SHEET (RULE 26)

Figure 24

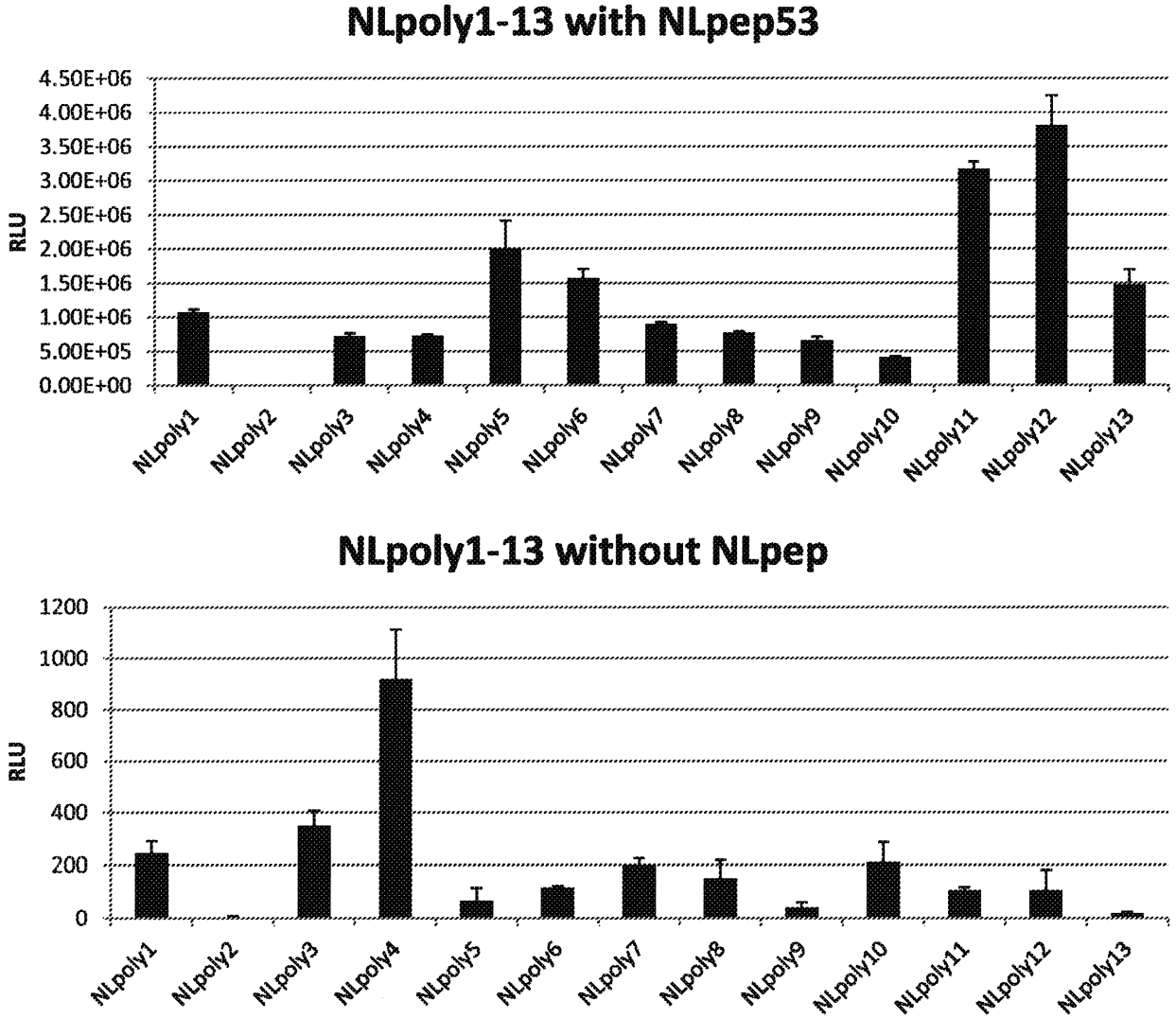
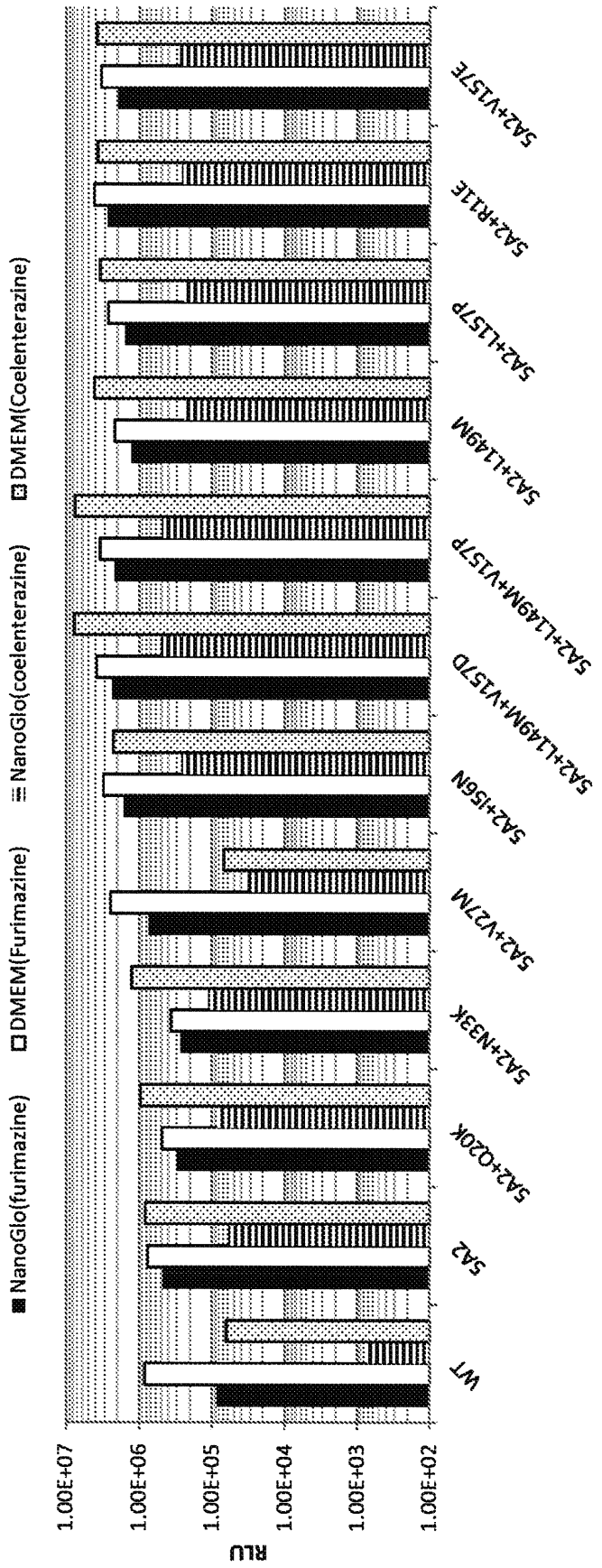
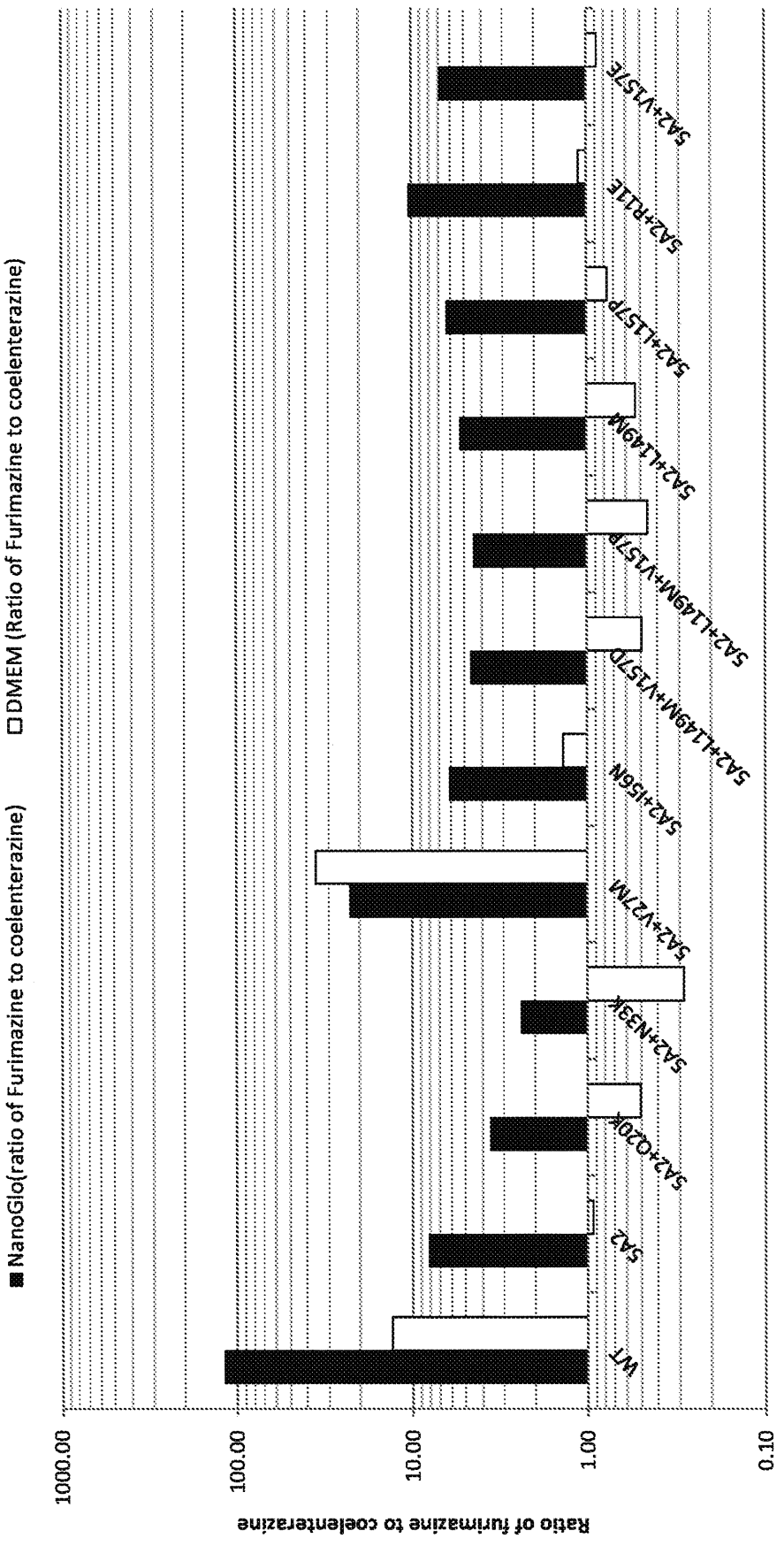

NLpoly1-13 with NLpep53
RLU
4.50E+06
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
NLpoly1
NLpoly2
NLpoly3
NLpoly4
NLpoly5
NLpoly6
NLpoly7
NLpoly8
NLpoly9
NLpoly10
NLpoly11
NLpoly12
NLpoly13
NLpoly1-13 without NLpep
RLU
1200
1000
800
600
400
200
0
NLpoly1
NLpoly2
NLpoly3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

Figure 25



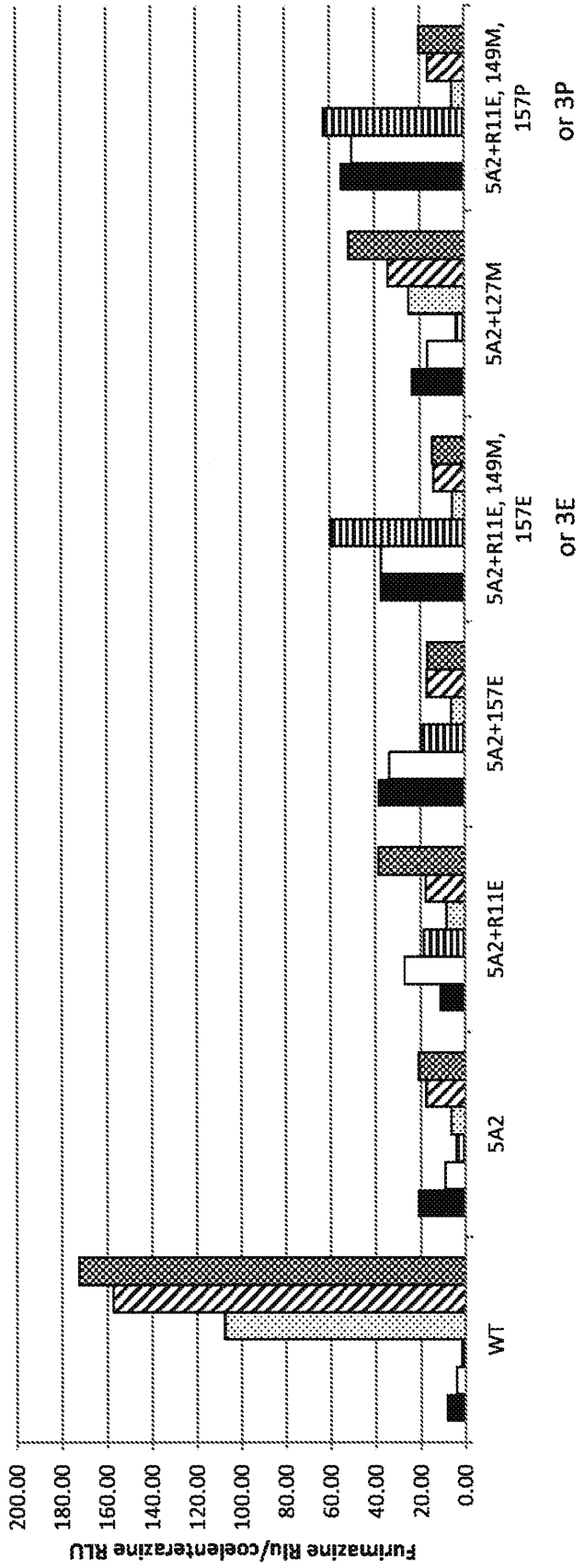
SUBSTITUTE SHEET (RULE 26)

Figure 26



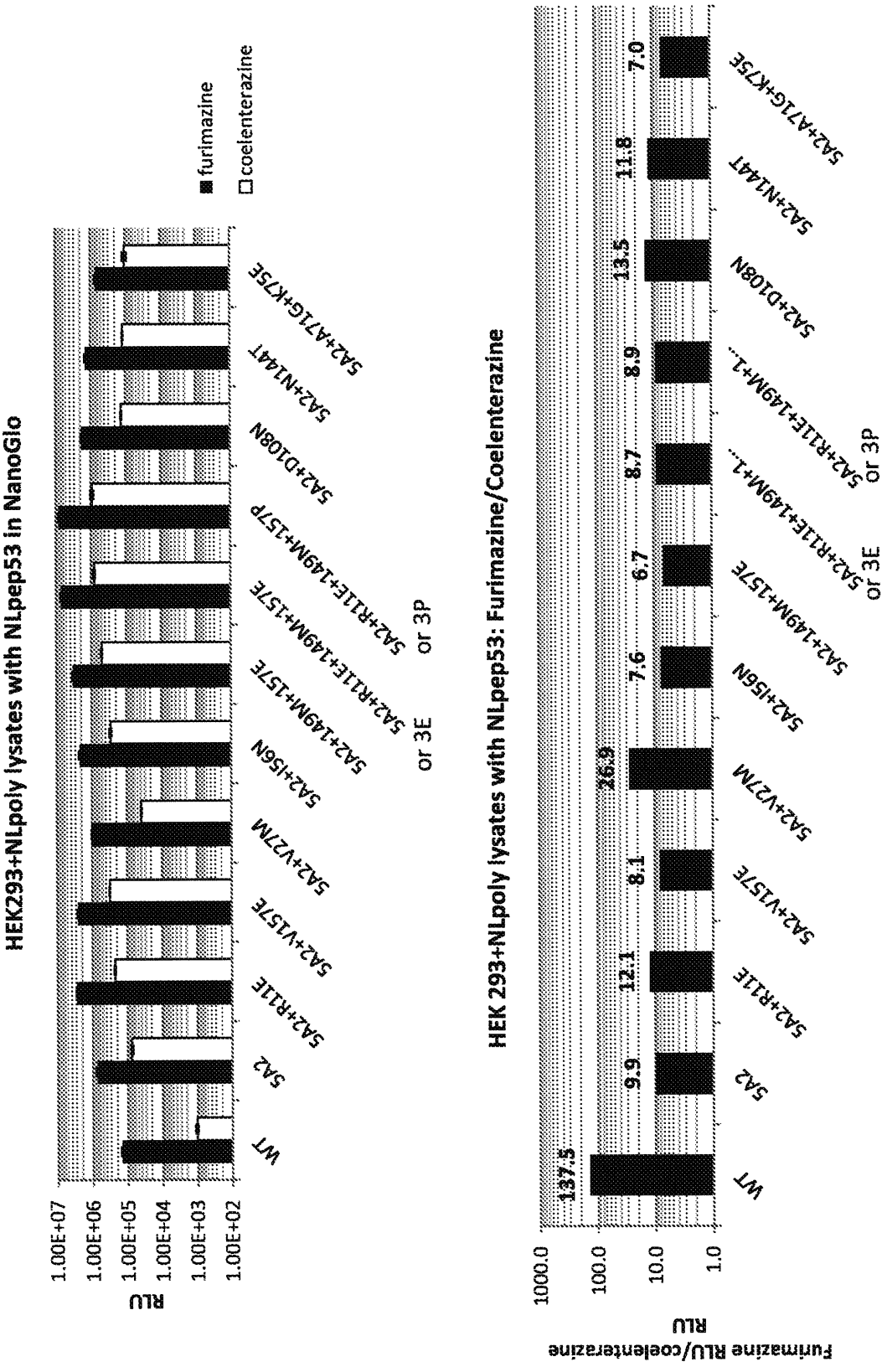
SUBSTITUTE SHEET (RULE 26)

Figure 27



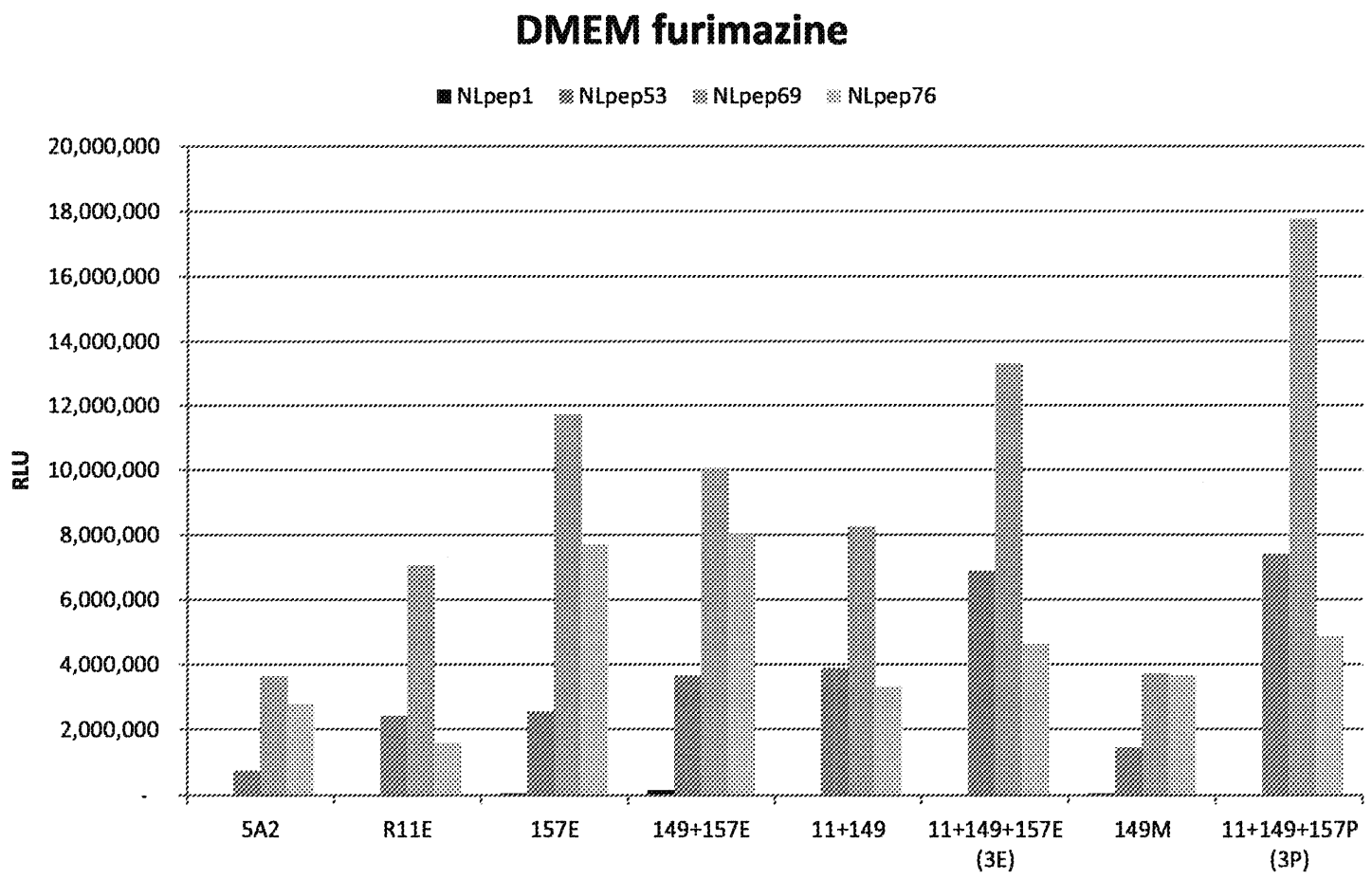
SUBSTITUTE SHEET (RULE 26)

Figure 28



SUBSTITUTE SHEET (RULE 26)

Figure 29



SUBSTITUTE SHEET (RULE 26)

Figure 30

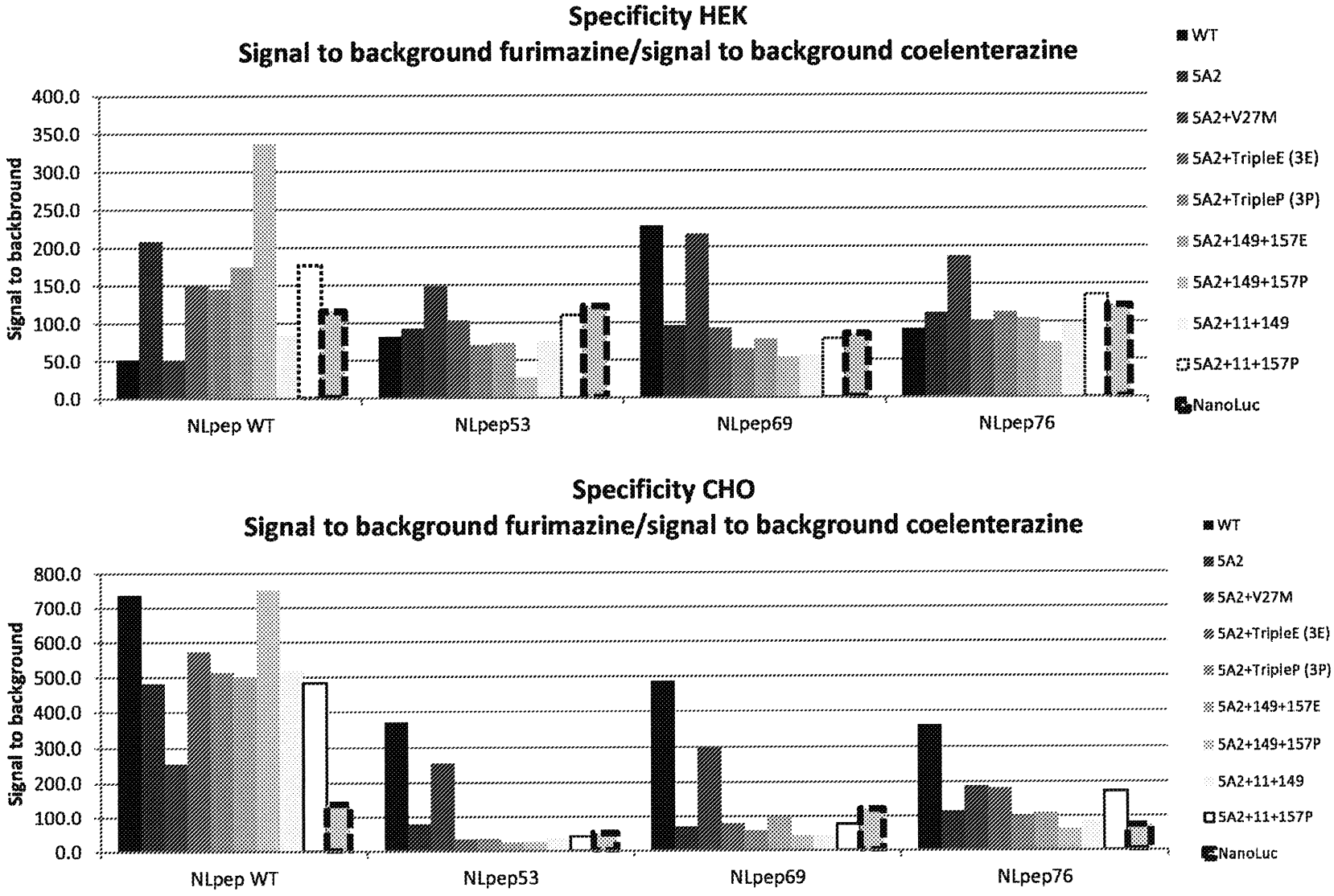
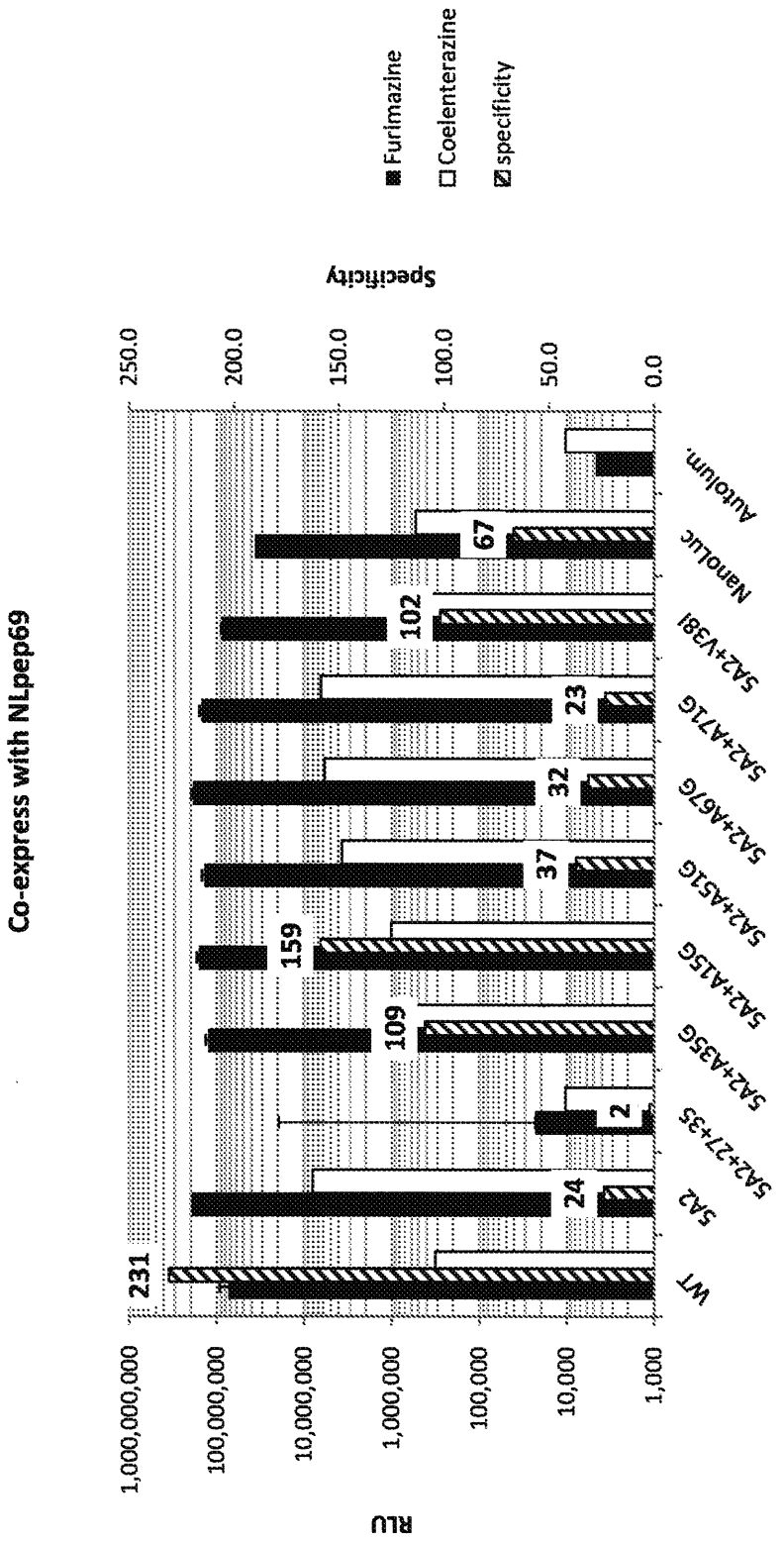

Specificity HEK
Signal to background furimazine/signal to background coelenterazine
Signal to backbround
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
NLpep WT
NLpep53
NLpep69
NLpep76
WT
5A2
5A2+V27M
5A2+TripleE (3E)
5A2+TripleP (3P)
5A2+149+157E
5A2+149+157P
5A2+11+149
5A2+11+157P
NanoLuc
Specificity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



SUBSTITUTE SHEET (RULE 26)

Figure 32

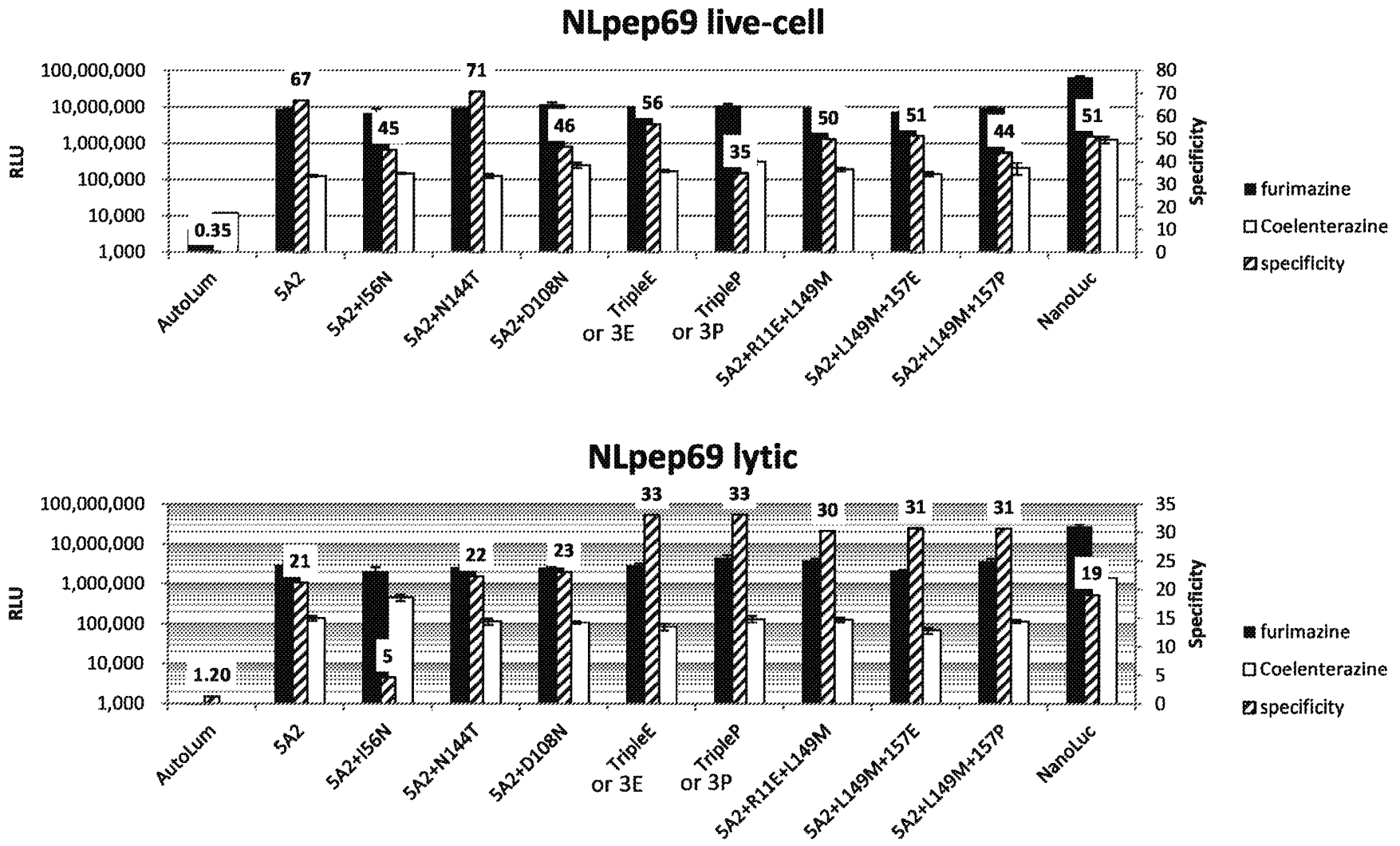
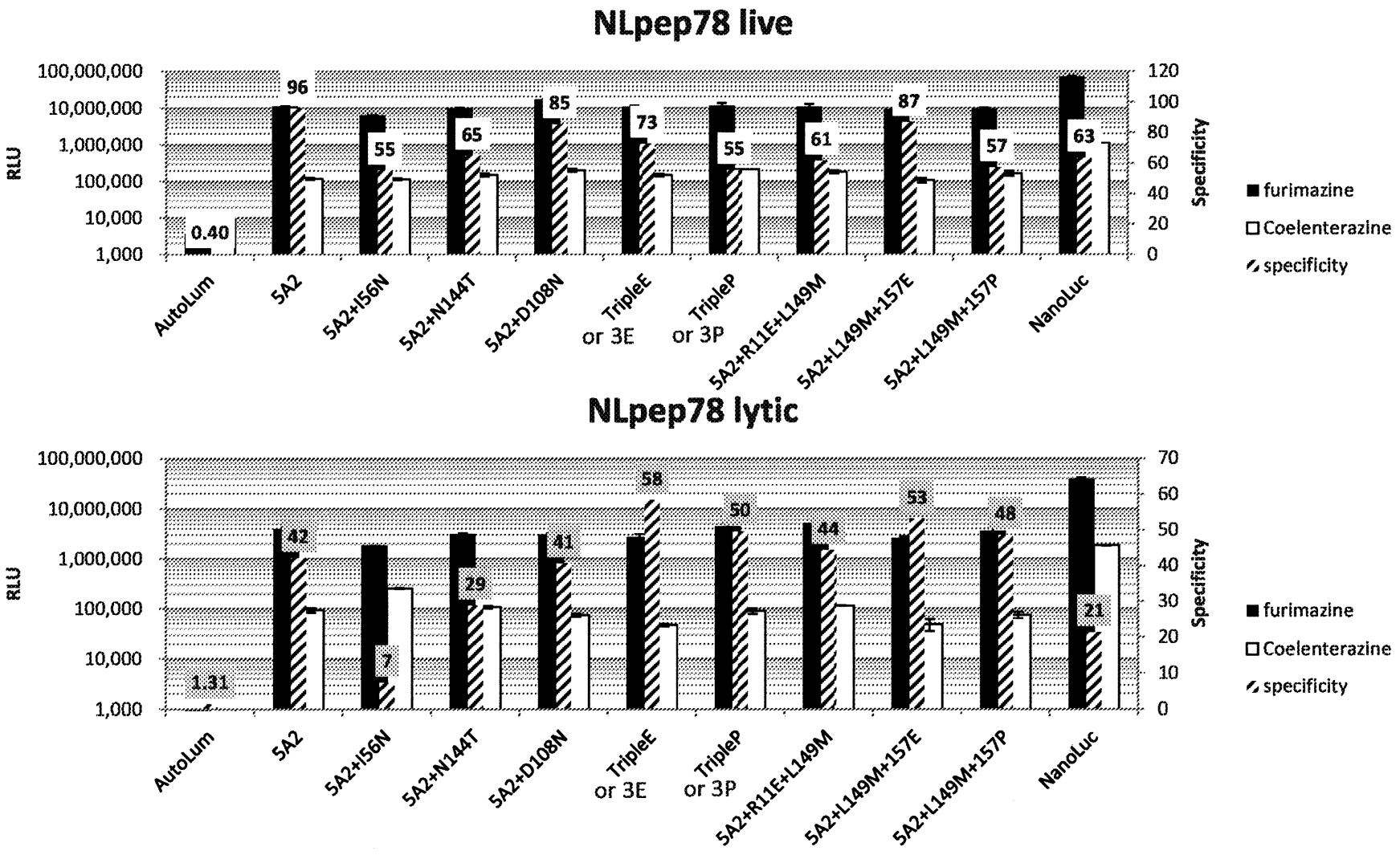

NLpep69 live-cell
RLU
Specificity
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
80
70
60
50
40
30
20
10
0
0.35
67
45
71
46
56
35
50
51
44
51
AutoLum
5A2
5A2+I56N
5A2+N144T
5A2+D108N
TripleE or 3E
TripleP or 3P
5A2+R11E+L149M
5A2+L149M+157E
5A2+L149M+157P
NanoLuc
furimazine
Coelenterazine
specificity
NLpep69 lytic
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
AutoLum
5A2
5A2+I56N
5A2+N144T
5A2+D108N
TripleE or 3E
TripleP or 3P
5A2+R11E+L149M
5A2+L149M+157E
5A2+L149M+157P
NanoLuc
furimazine
Coelenterazine
specificity

Figure 33



SUBSTITUTE SHEET (RULE 26)

Figure 34

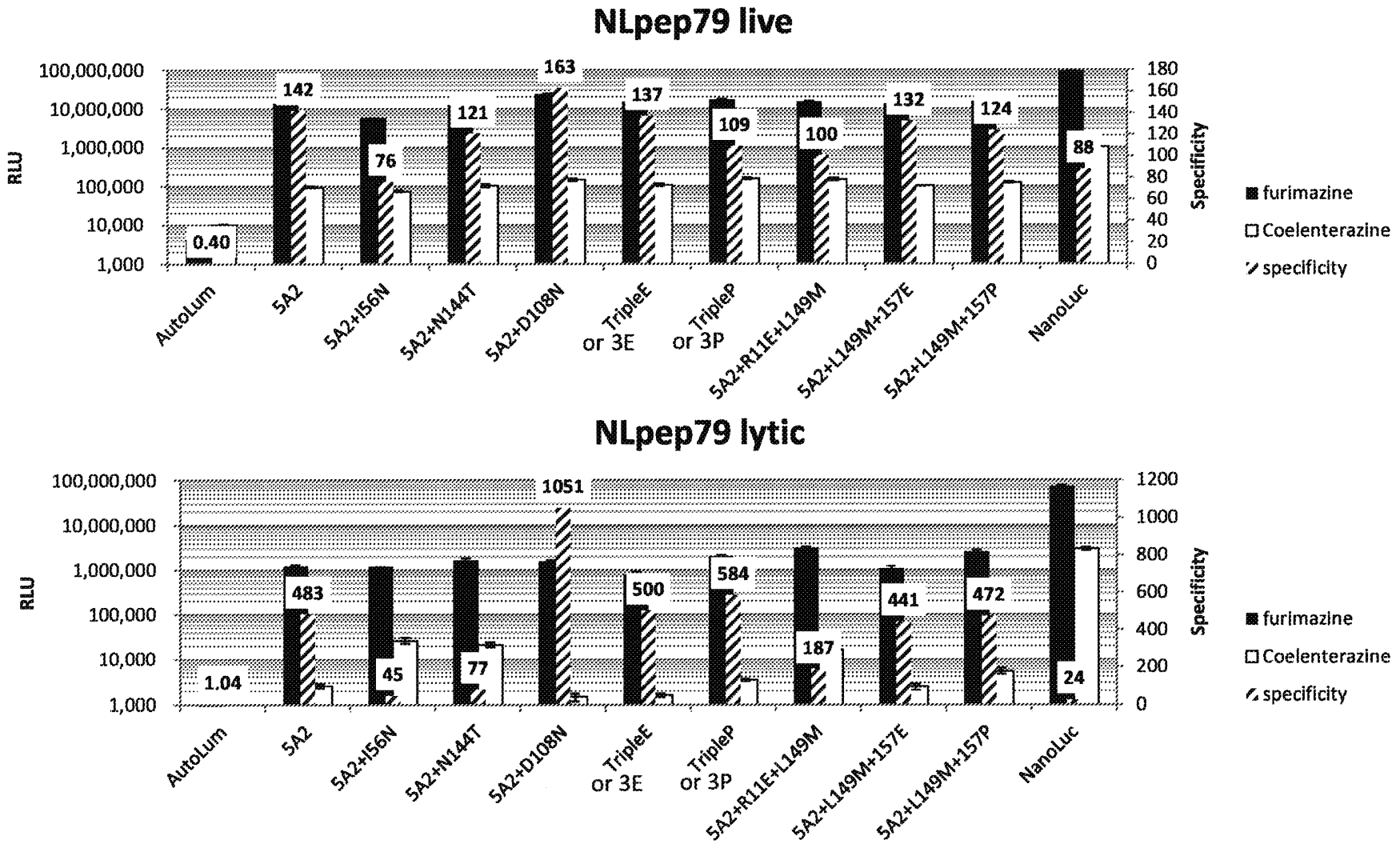

NLpep79 live
RLU
Specificity
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
180
160
140
120
100
80
60
40
20
0
0.40
142
76
121
163
137
109
100
132
124
88
furimazine
Coelenterazine
specificity
AutoLum
5A2
5A2+I56N
5A2+N144T
5A2+D108N
TripleE or 3E
TripleP or 3P
5A2+R11E+L149M
5A2+L149M+157E
5A2+L149M+157P
NanoLuc
NLpep79 lytic
RLU
Specificity
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
1200
1000
800
600
400
200
0
1.04
483
45
77
1051
500
584
187
441
472
24
furimazine
Coelenterazine
specificity
AutoLum
5A2
5A2+I56N
5A2+N144T
5A2+D108N
TripleE or 3E
TripleP or 3P
5A2+R11E+L149M
5A2+L149M+157E
5A2+L149M+157P
NanoLuc

Figure35

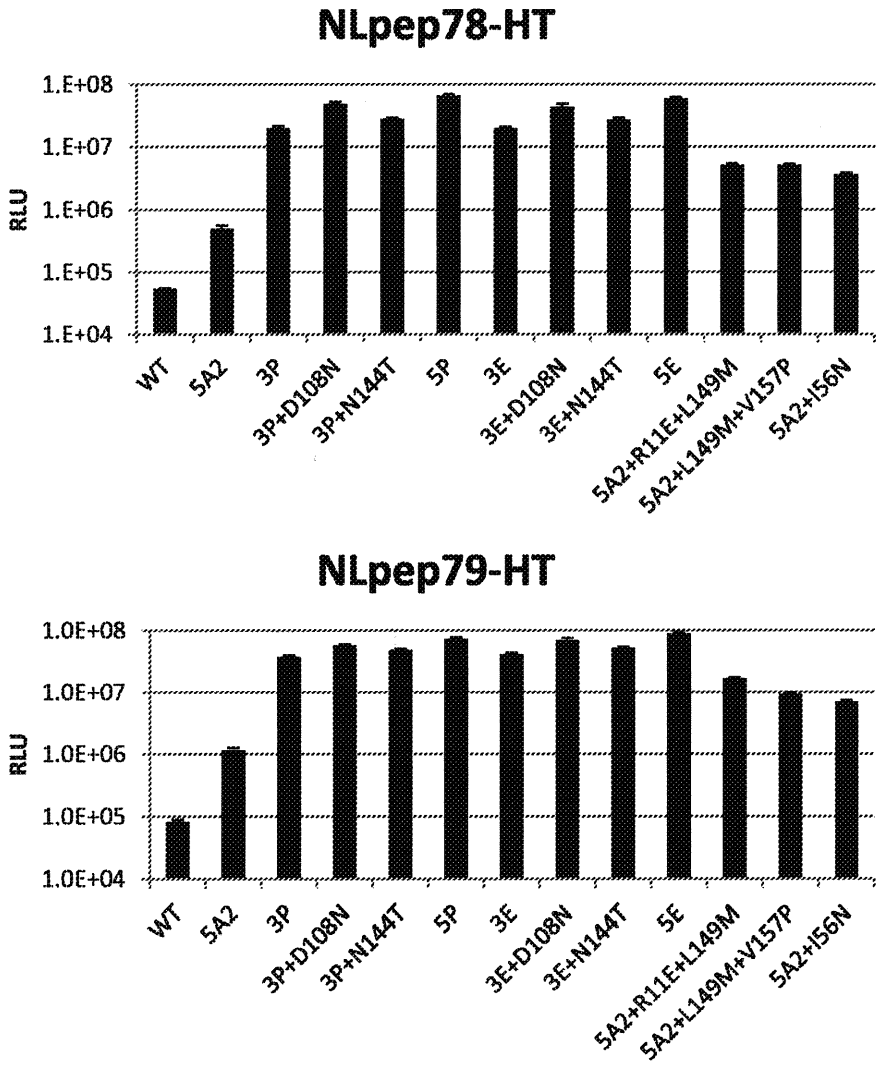
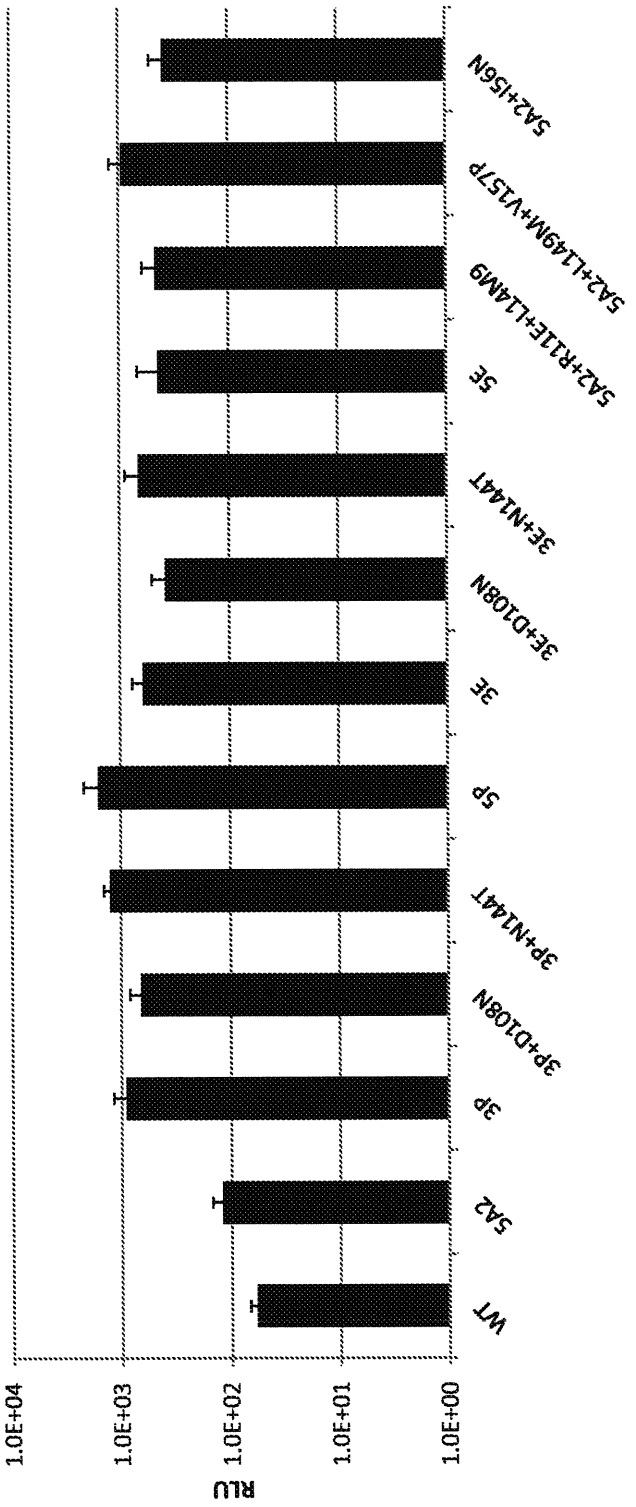
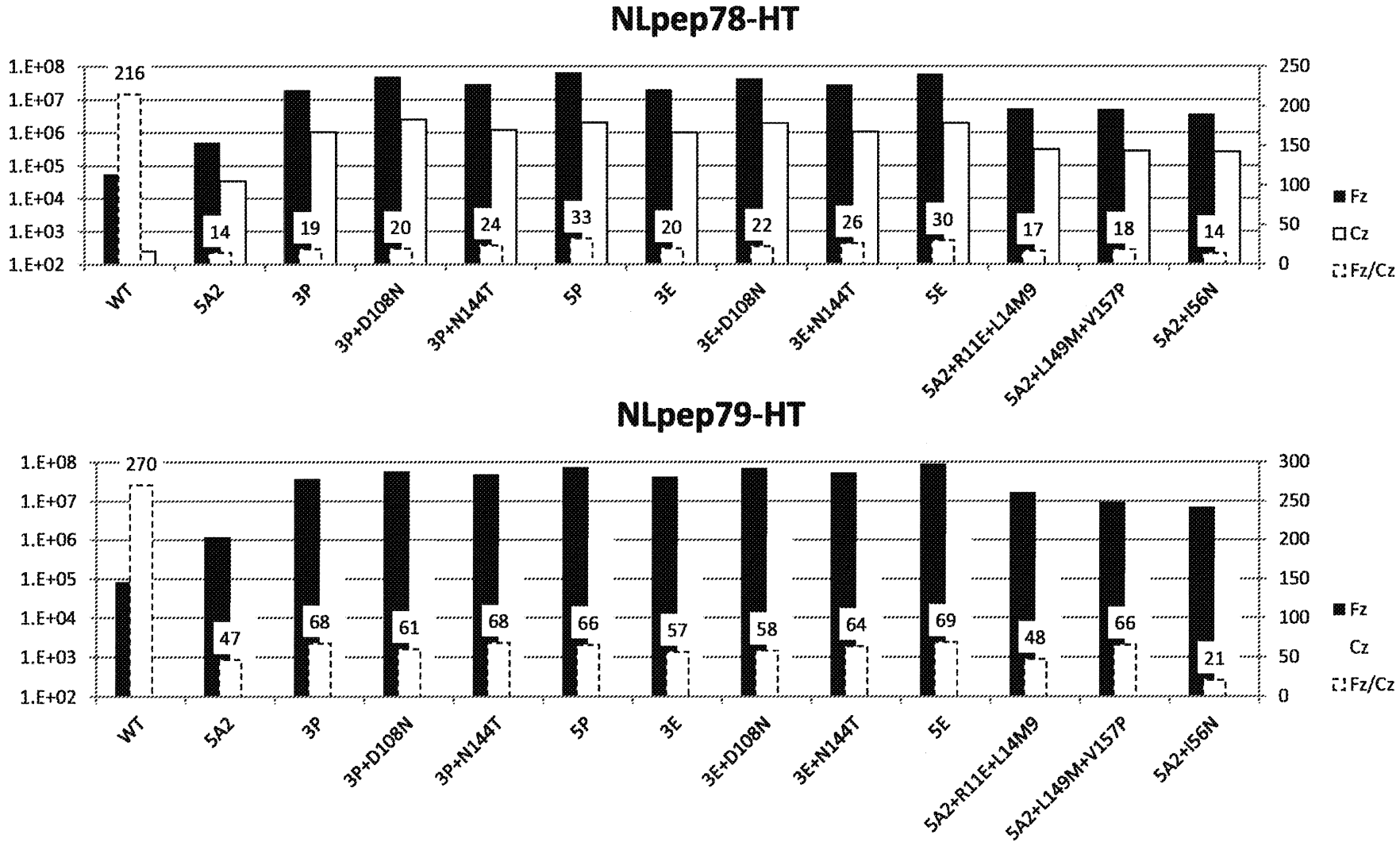

NLpep78-HT
RLU
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
WT
5A2
3P
3P+D108N
3P+N144T
5P
3E
3E+D108N
3E+N144T
5E
5A2+R11E+L149M
5A2+L149M+V157P
5A2+I56N
NLpep79-HT
RLU
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
WT
5A2
3P
3P+D108N
3P+N144T
5P
3E
3E+D108N
3E+N144T
5E
5A2+R11E+L149M
5A2+L149M+V157P
5A2+I56N

Figure 36



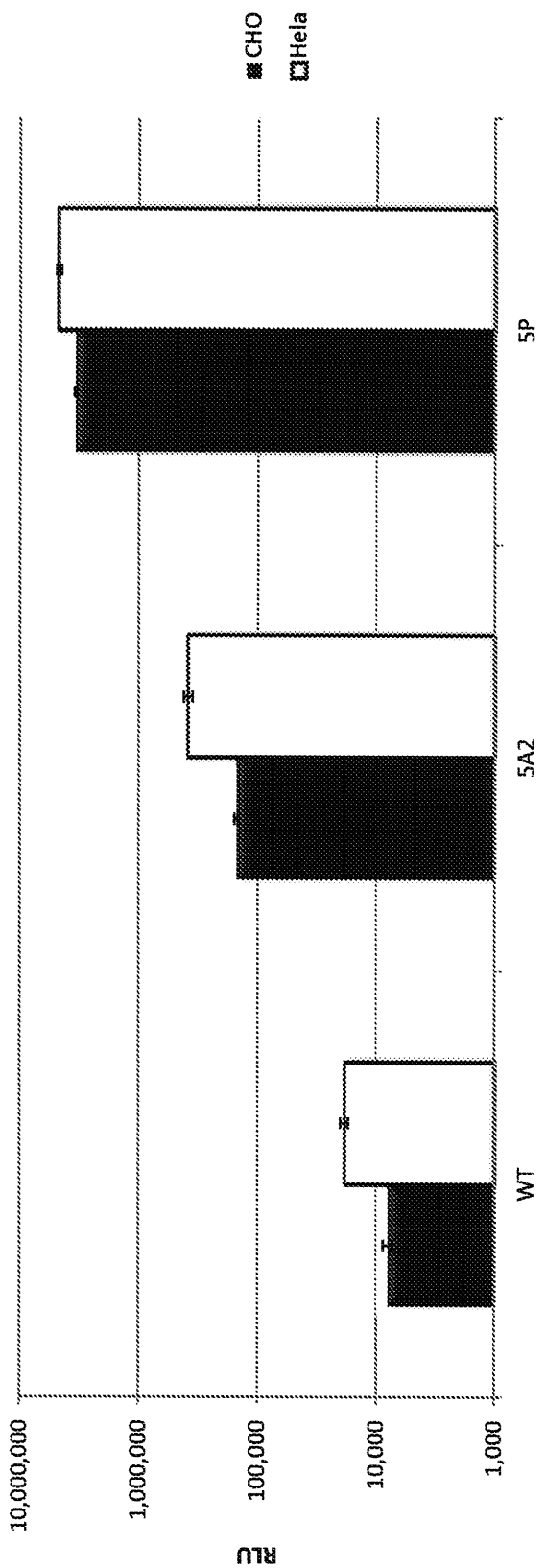
SUBSTITUTE SHEET (RULE 26)

Figure 37



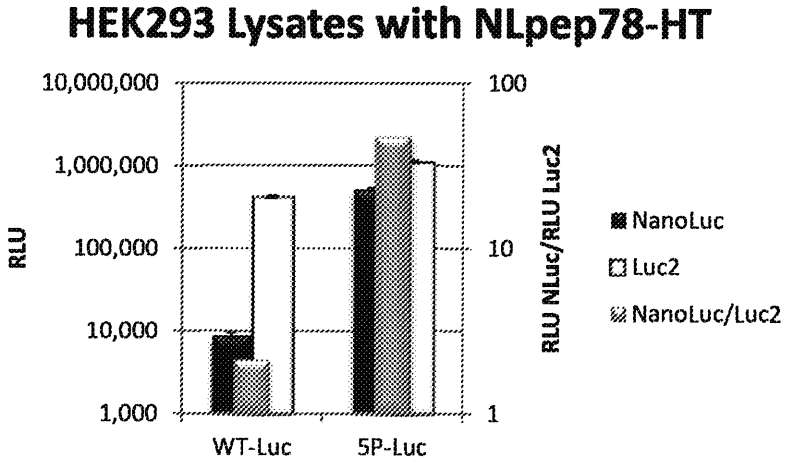
SUBSTITUTE SHEET (RULE 26)

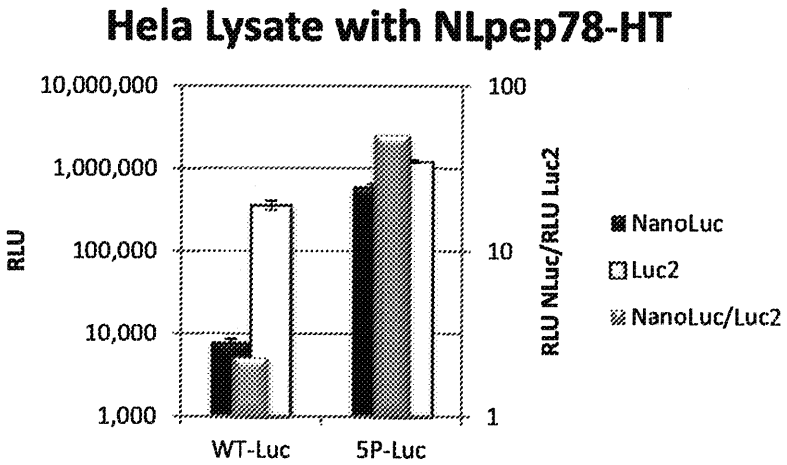
Figure 38



SUBSTITUTE SHEET (RULE 26)

Figure 39


HEK293 Lysates with NLpep78-HT
10,000,000
1,000,000
100,000
10,000
1,000
RLU
100
10
1
RLU NLuc/RLU Luc2
WT-Luc
5P-Luc
NanoLuc
Luc2
NanoLuc/Luc2


Hela Lysate with NLpep78-HT
10,000,000
1,000,000
100,000
10,000
1,000
RLU
100
10
1
RLU NLuc/RLU Luc2
WT-Luc
5P-Luc
NanoLuc
Luc2
NanoLuc/Luc2

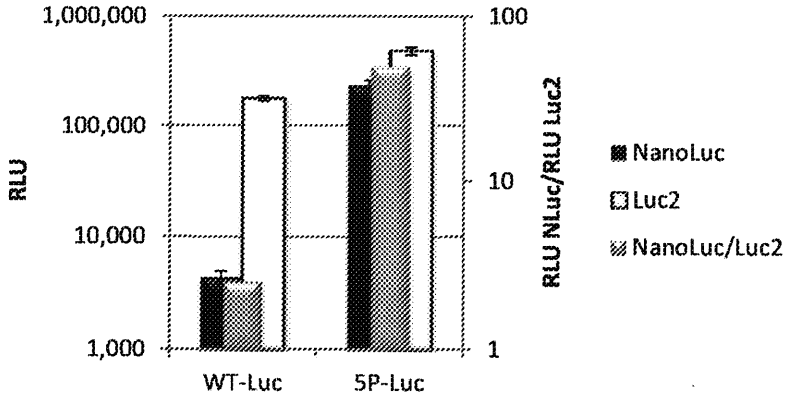

CHO Lysate with NLpep78-HT
1,000,000
100,000
10,000
1,000
RLU
100
10
1
RLU NLuc/RLU Luc2
WT-Luc
5P-Luc
NanoLuc
Luc2
NanoLuc/Luc2

Figure 40

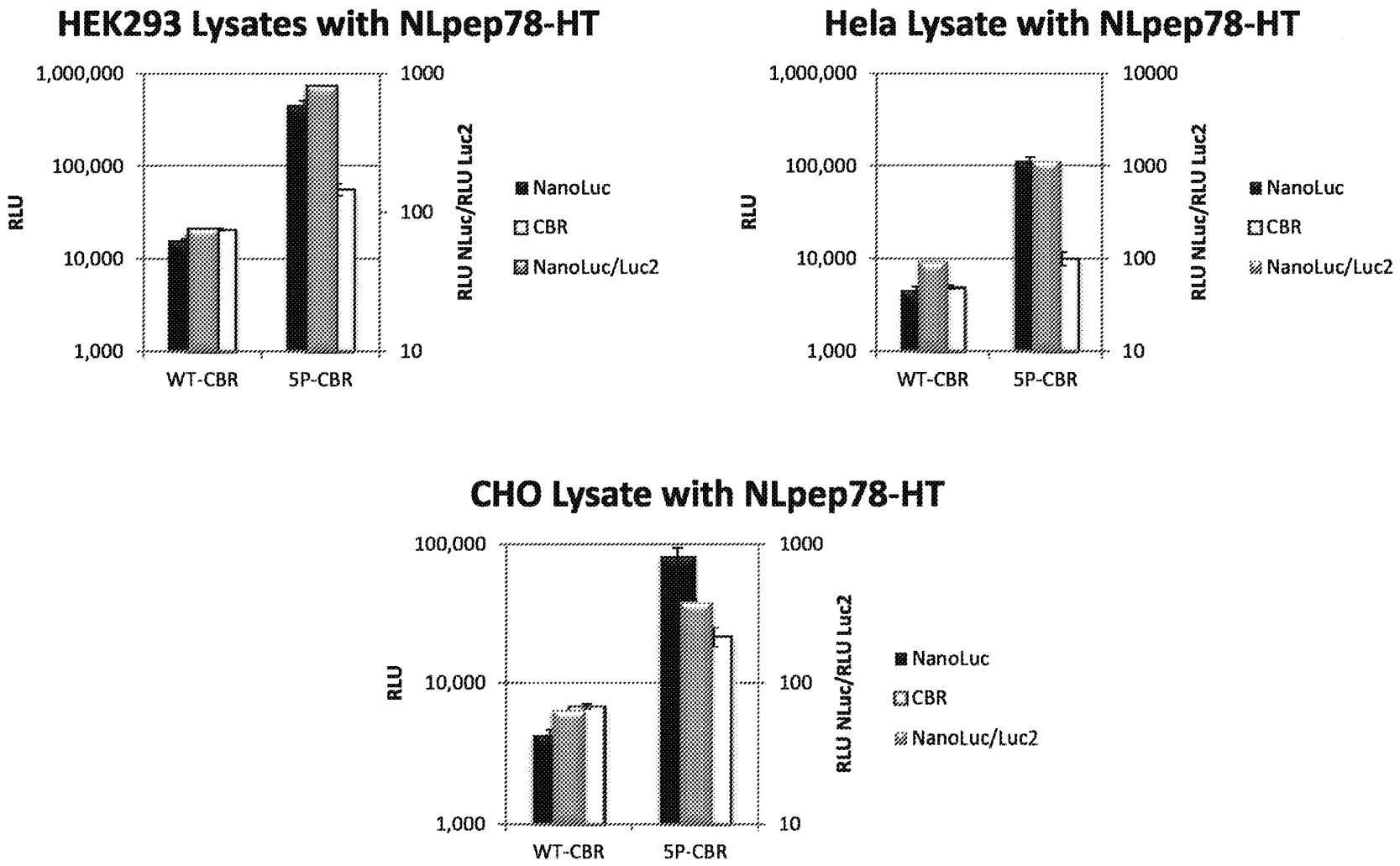
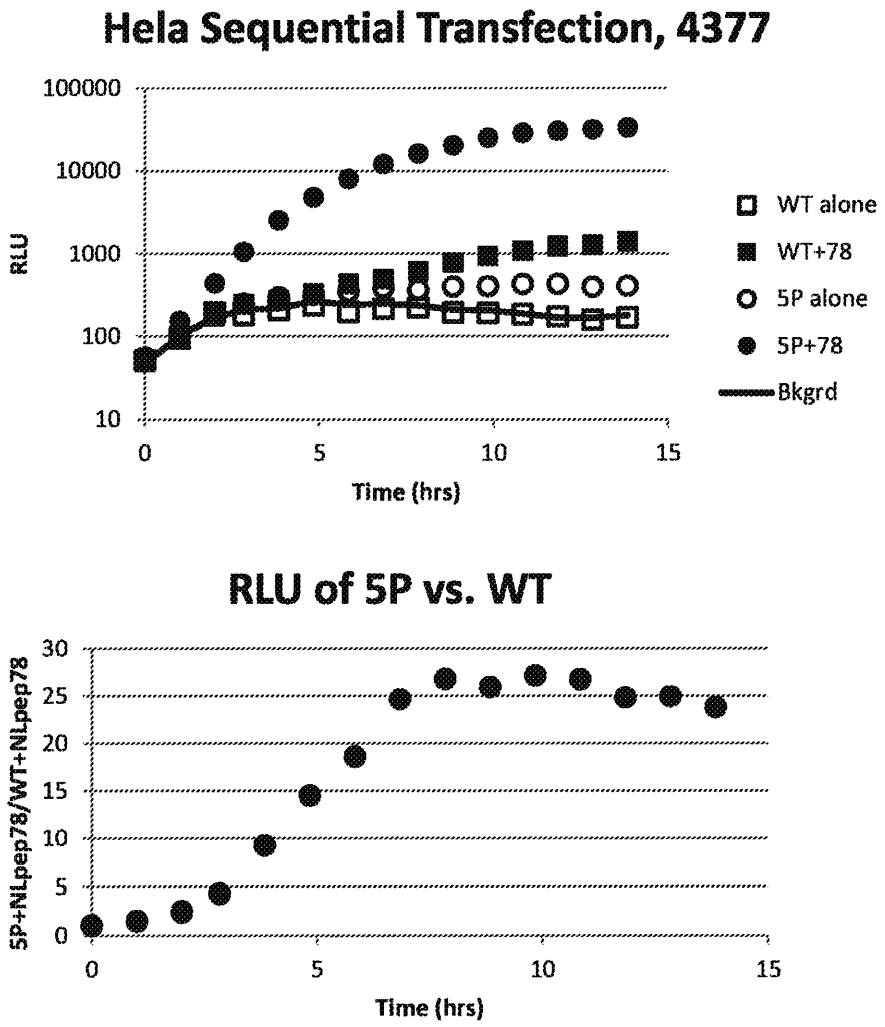

HEK293 Lysates with NLpep78-HT
1,000,000
100,000
10,000
1,000
RLU
1000
100
10
RLU NLuc/RLU Luc2
WT-CBR
5P-CBR
NanoLuc
CBR
NanoLuc/Luc2
Hela Lysate with NLpep78-HT
1,000,000
100,000
10,000
1,000
RLU
10000
1000
100
10
RLU NLuc/RLU Luc2
WT-CBR
5P-CBR
NanoLuc
CBR
NanoLuc/Luc2
CHO Lysate with NLpep78-HT
100,000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

SUBSTITUTE SHEET (RULE 26)

Figure 42

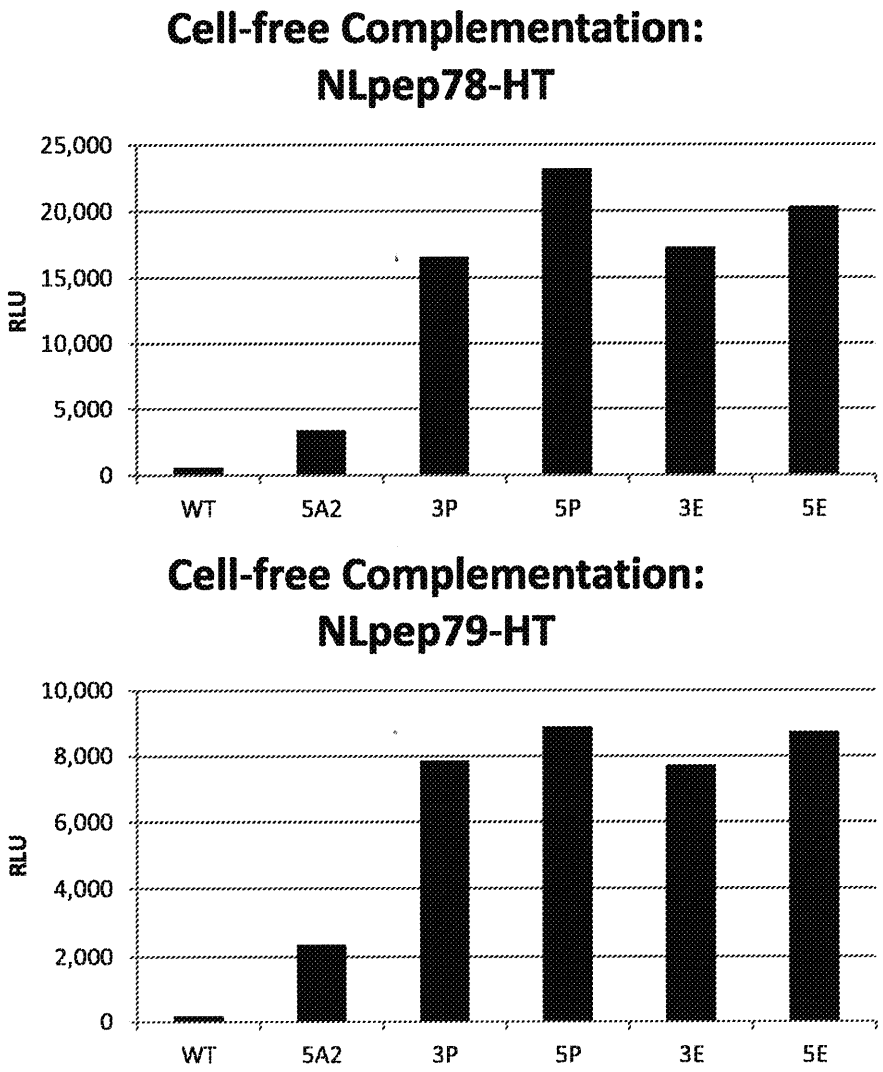
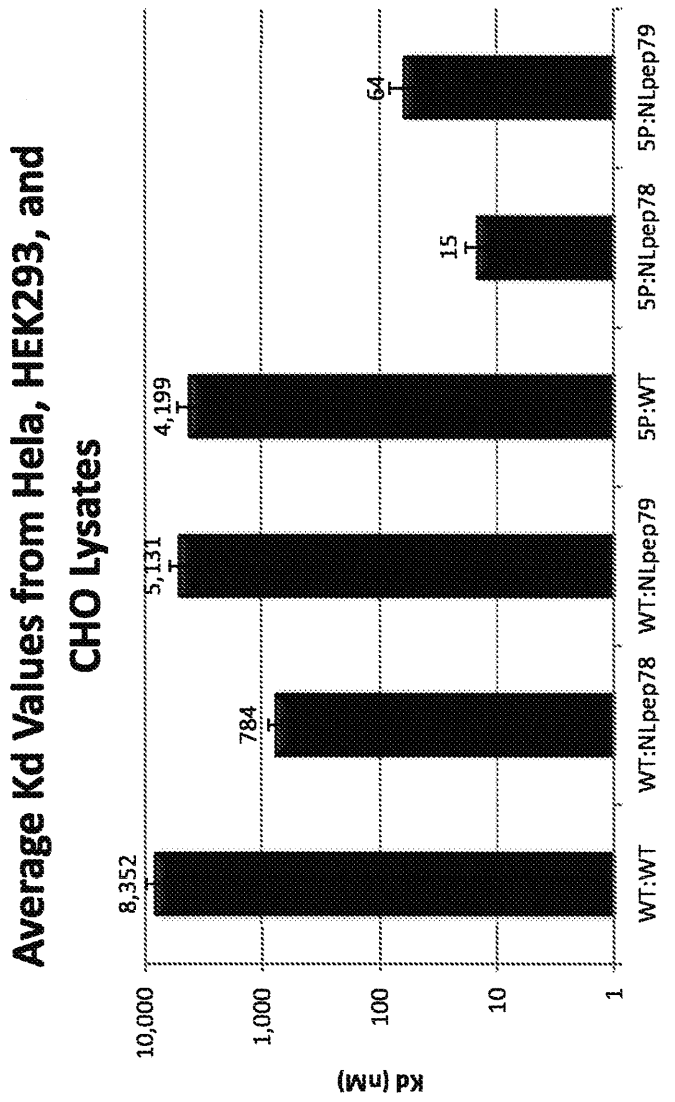
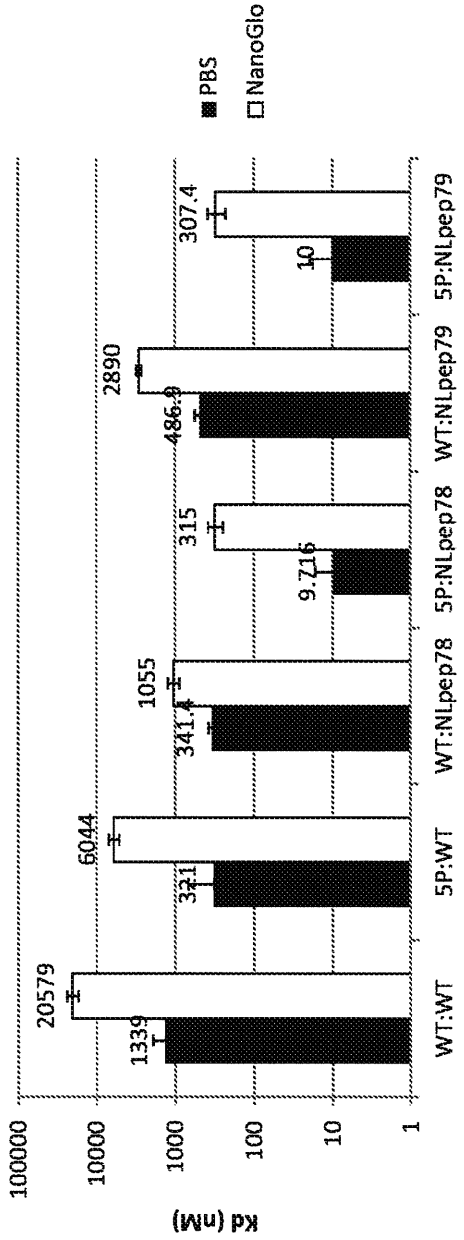


Figure 43



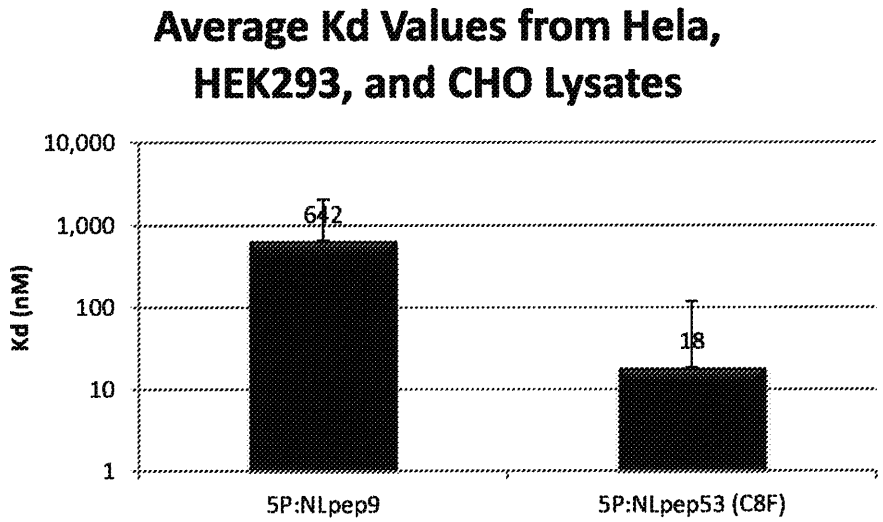
SUBSTITUTE SHEET (RULE 26)

Figure 44



SUBSTITUTE SHEET (RULE 26)

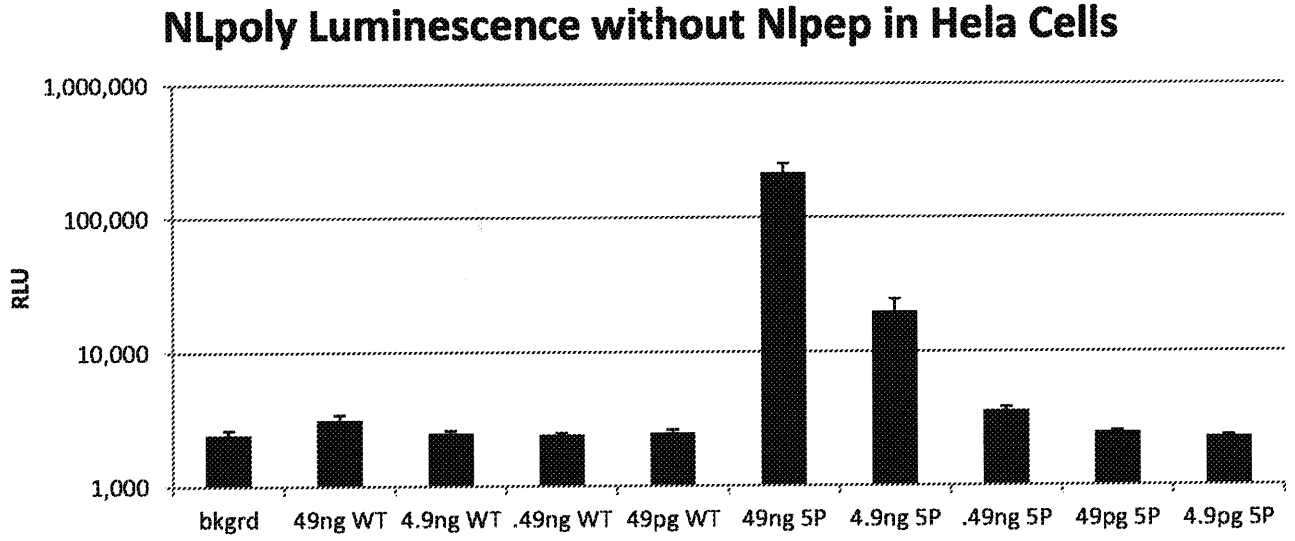
Figure 45



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

SUBSTITUTE SHEET (RULE 26)

Figure 46



SUBSTITUTE SHEET (RULE 26)

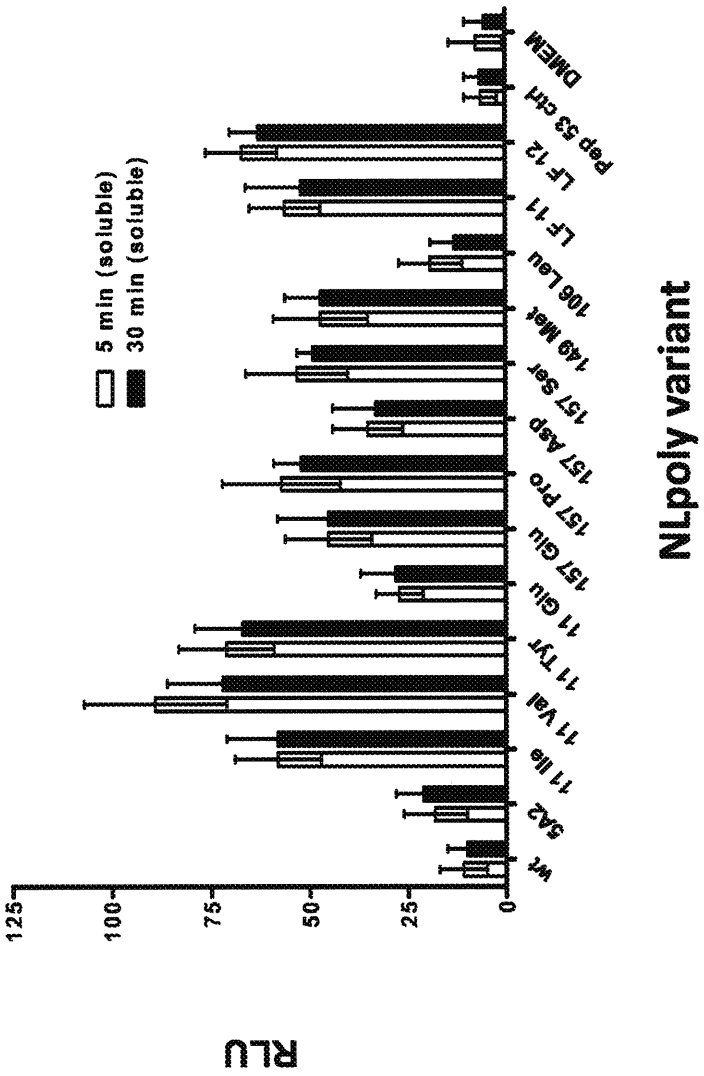
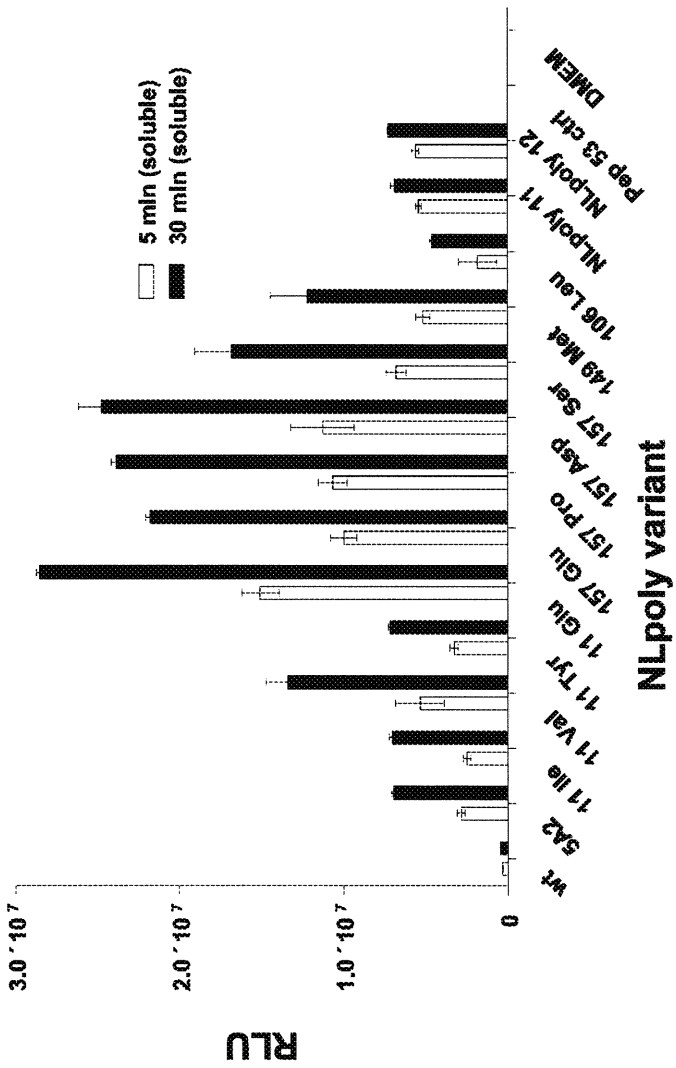


Figure 47

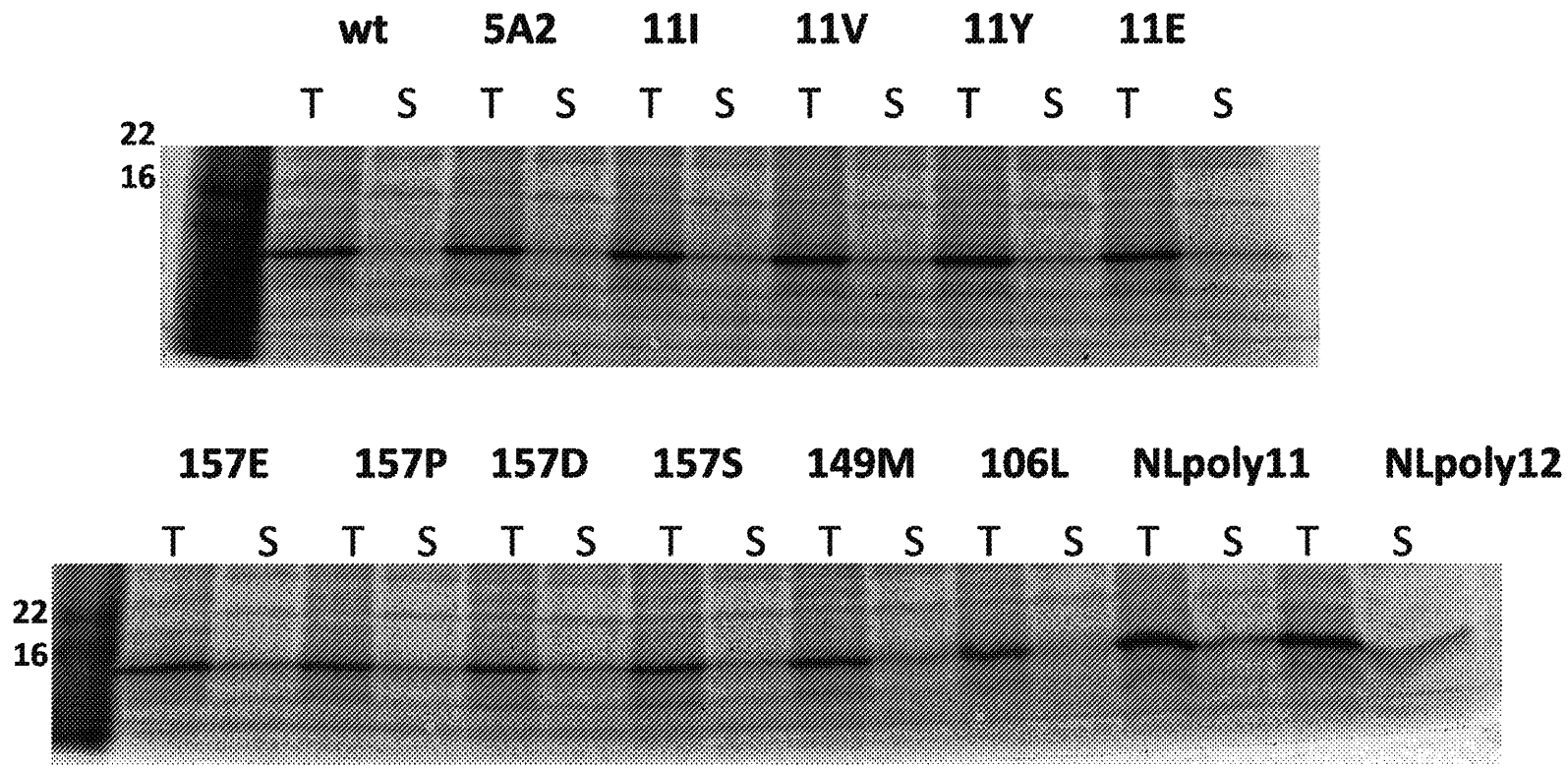
SUBSTITUTE SHEET (RULE 26)

Figure 48



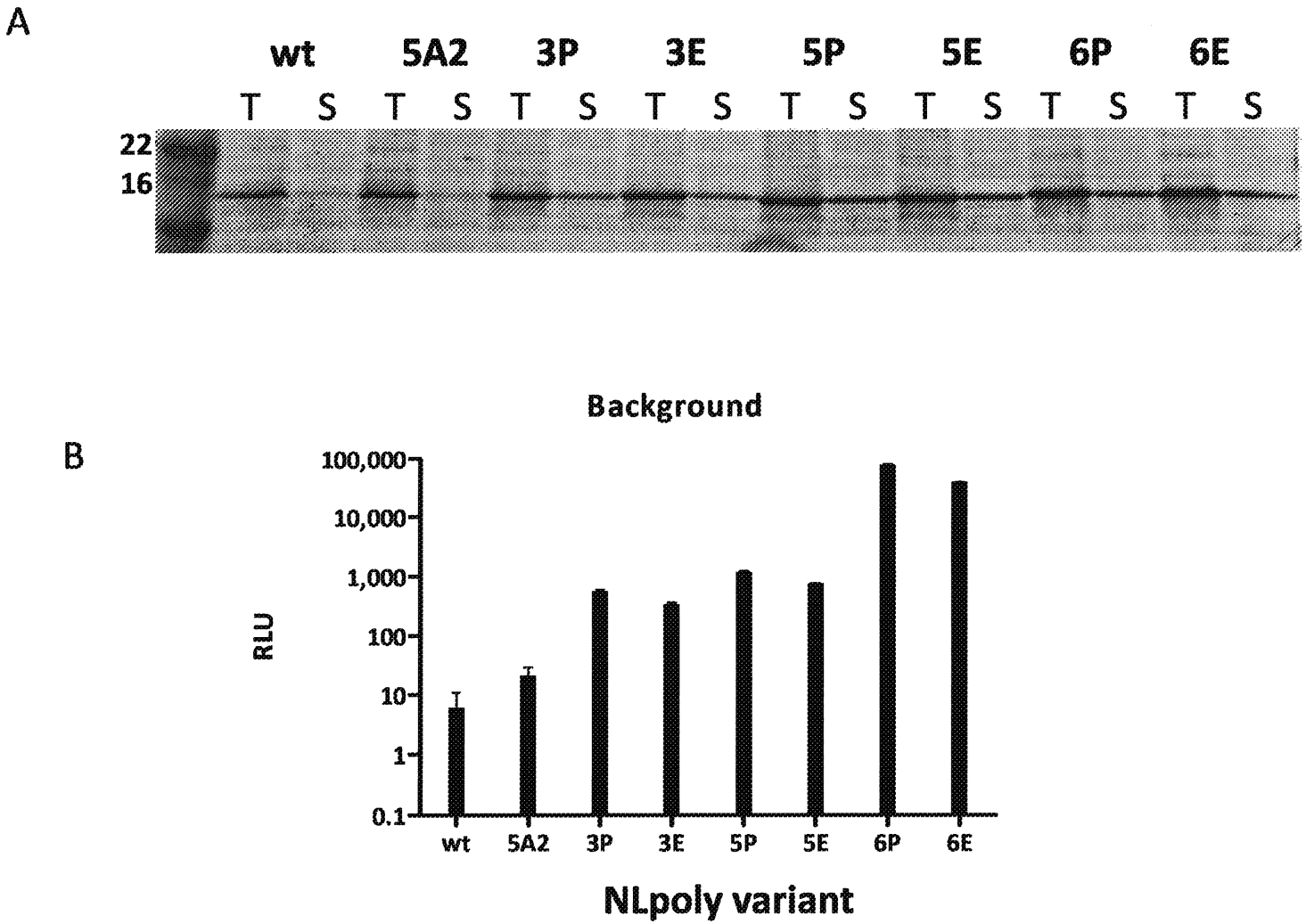
SUBSTITUTE SHEET (RULE 26)

Figure 49



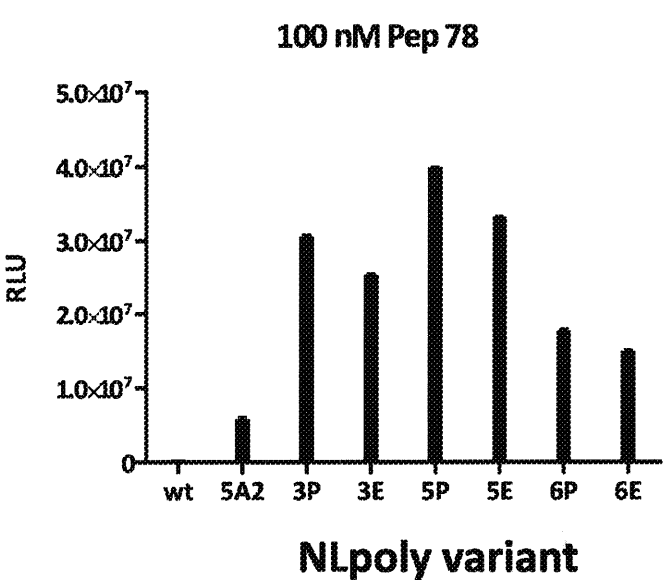
T=total crude; S=soluble

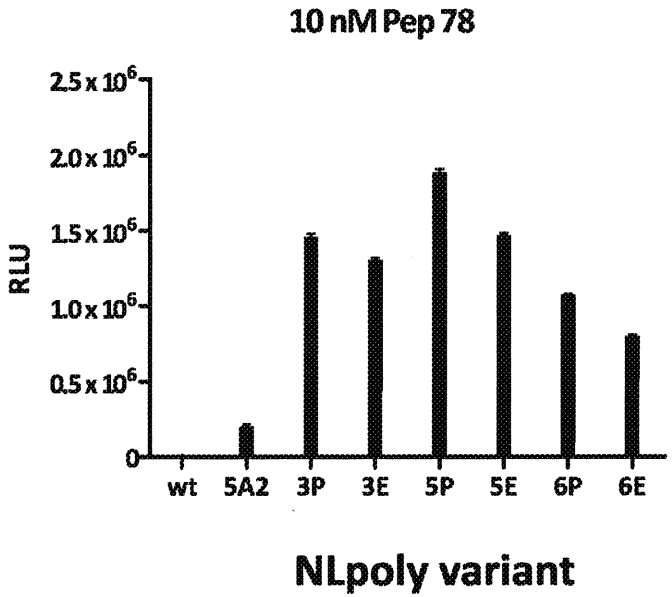
Figure 50



SUBSTITUTE SHEET (RULE 26)

Figure 51





SUBSTITUTE SHEET (RULE 26)

Figure 52

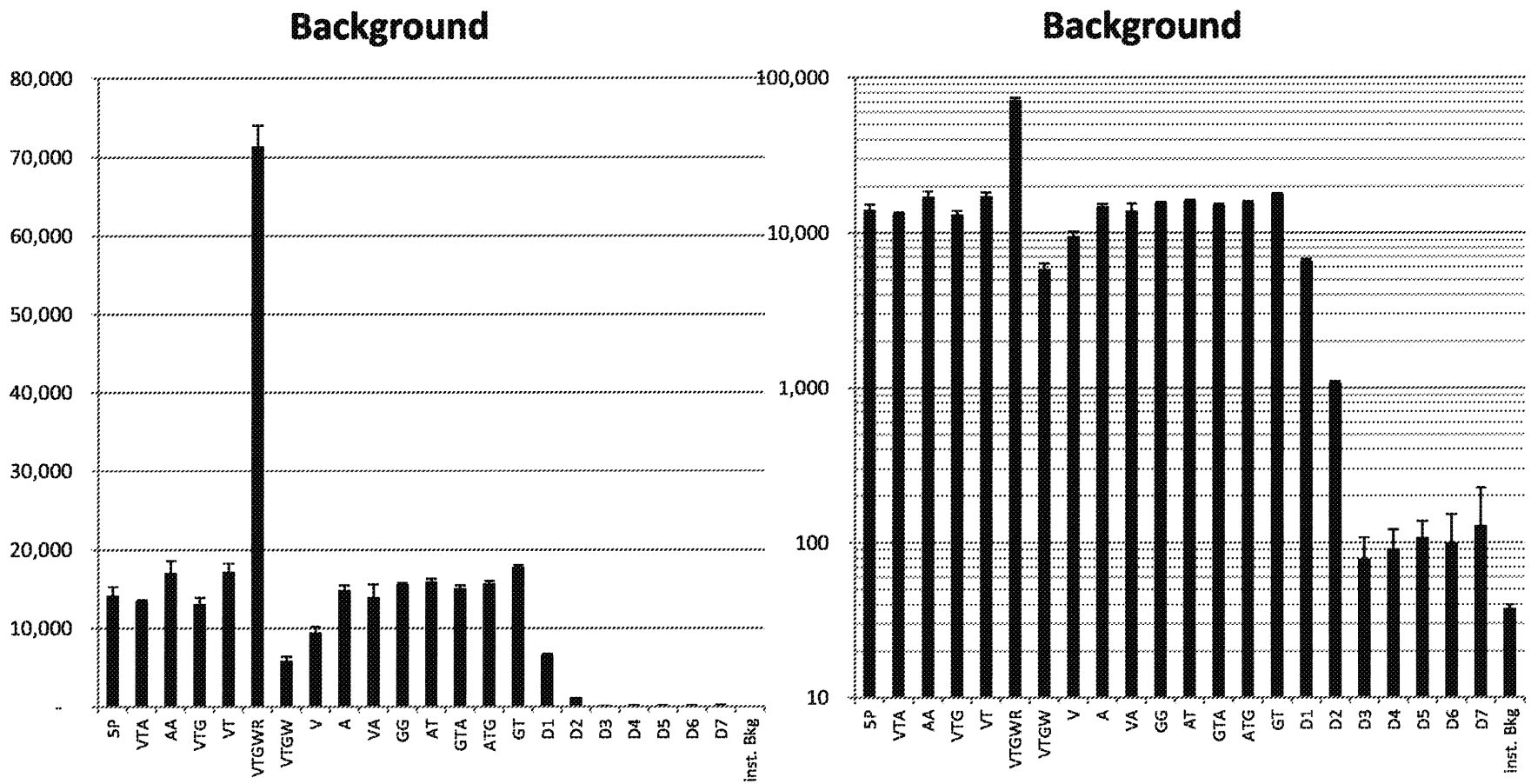
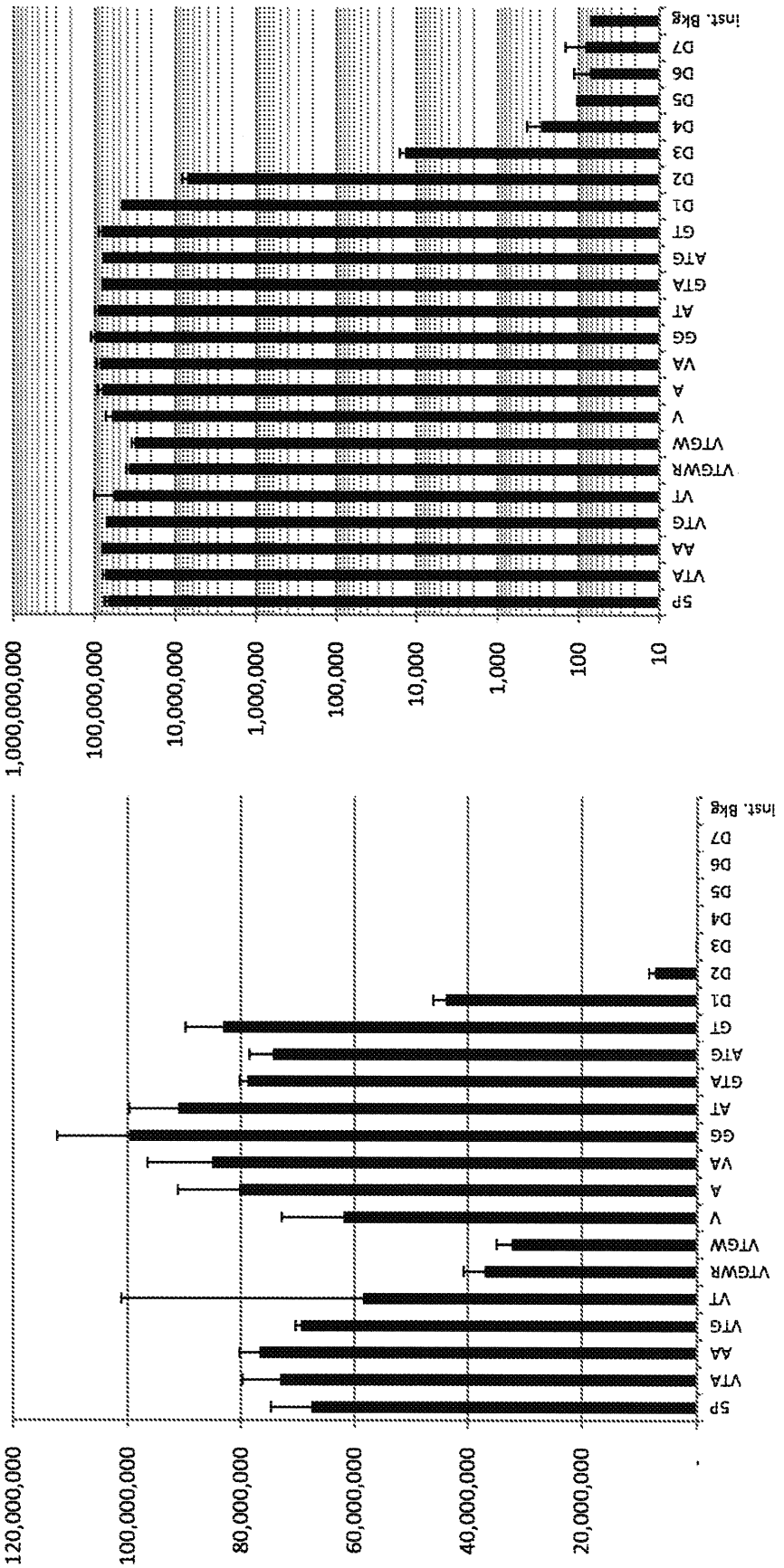
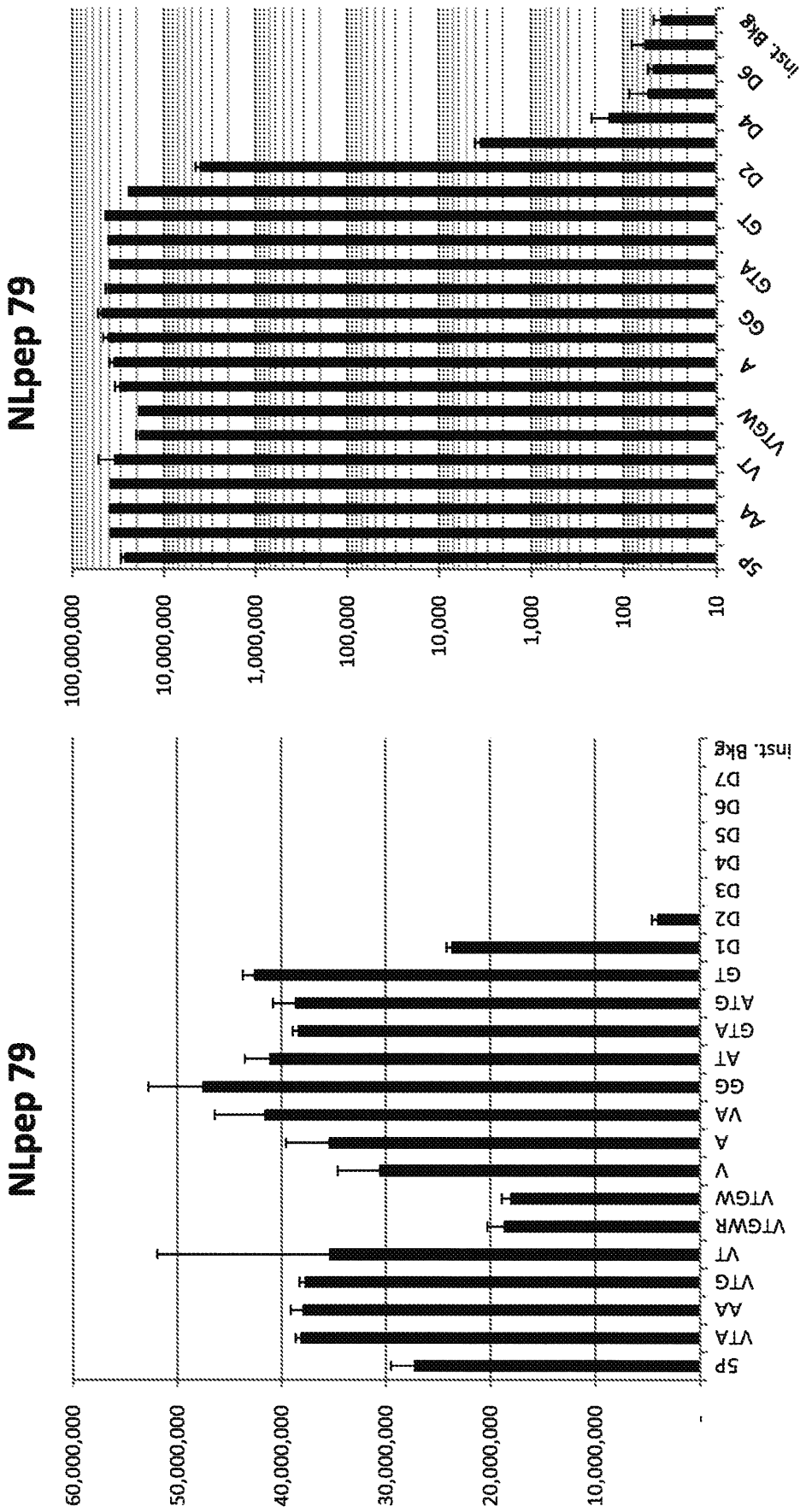

Background
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
-
SP
VTA
AA
VTG
VT
VTGWR
VTGW
V
A
VA
GG
AT
GTA
ATG
GT
D1
D2
D3
D4
D5
D6
D7
inst. Bkg
Background
100,000
10,000
1,000
100
10
SP
VTA
AA
VTG
VT
VTGWR
VTGW
V
A
VA
GG
AT
GTA
ATG
GT
D1
D2
D3
D4
D5
D6
D7
inst. Bkg

Figure 53



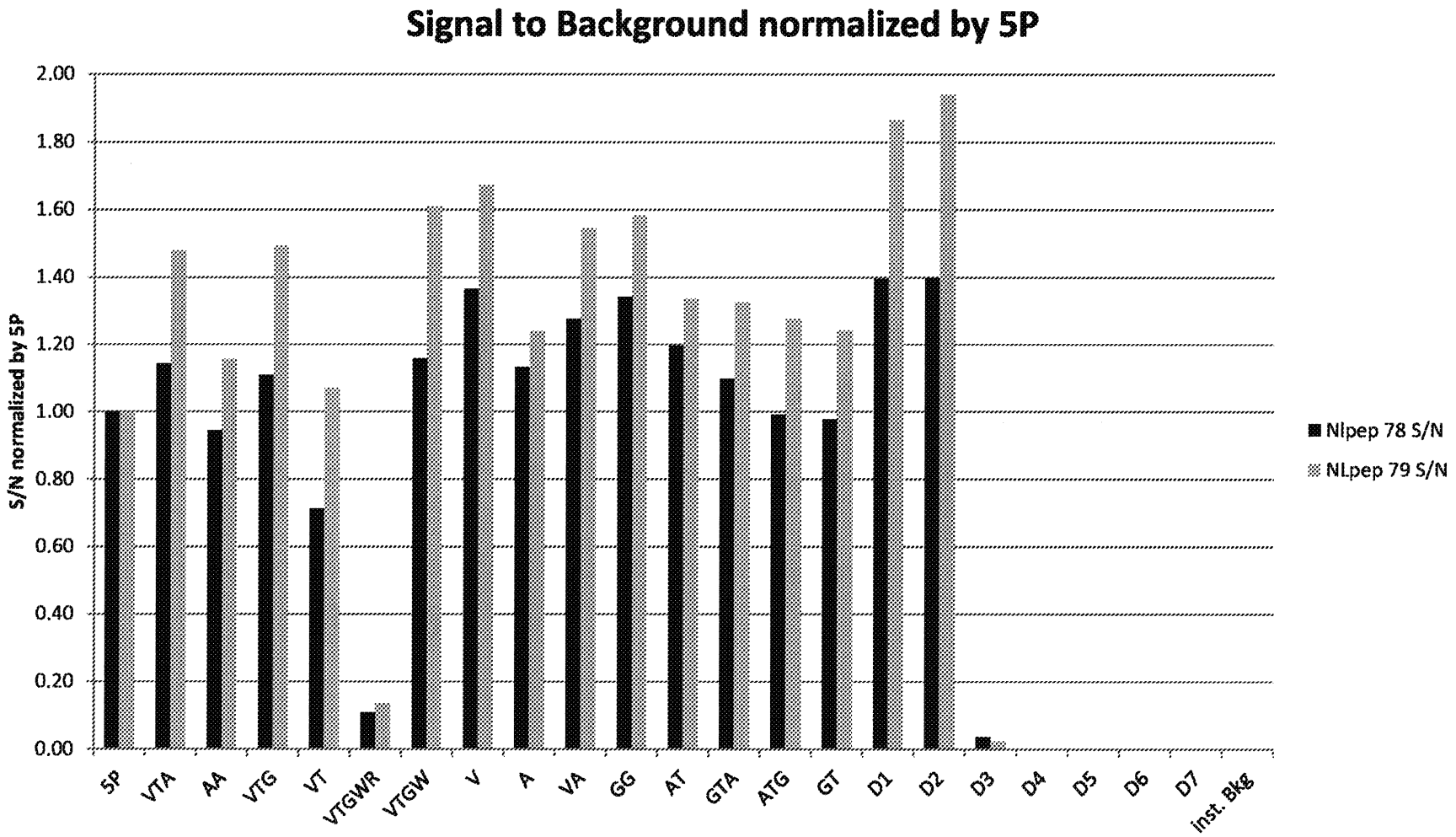
SUBSTITUTE SHEET (RULE 26)

Figure 54



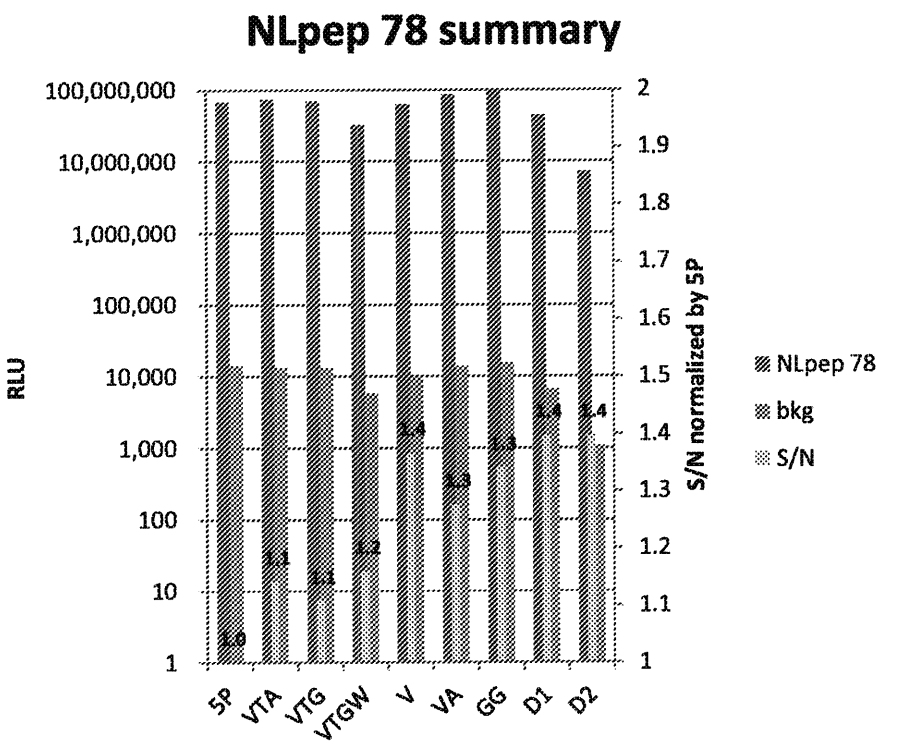
SUBSTITUTE SHEET (RULE 26)

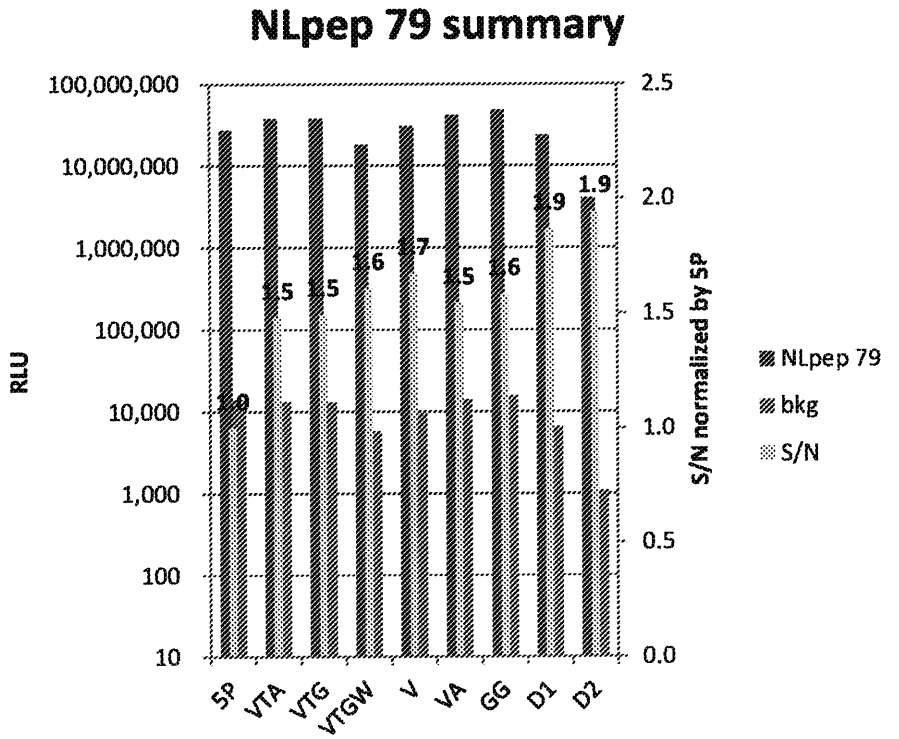
Figure 55



SUBSTITUTE SHEET (RULE 26)

Figure 56

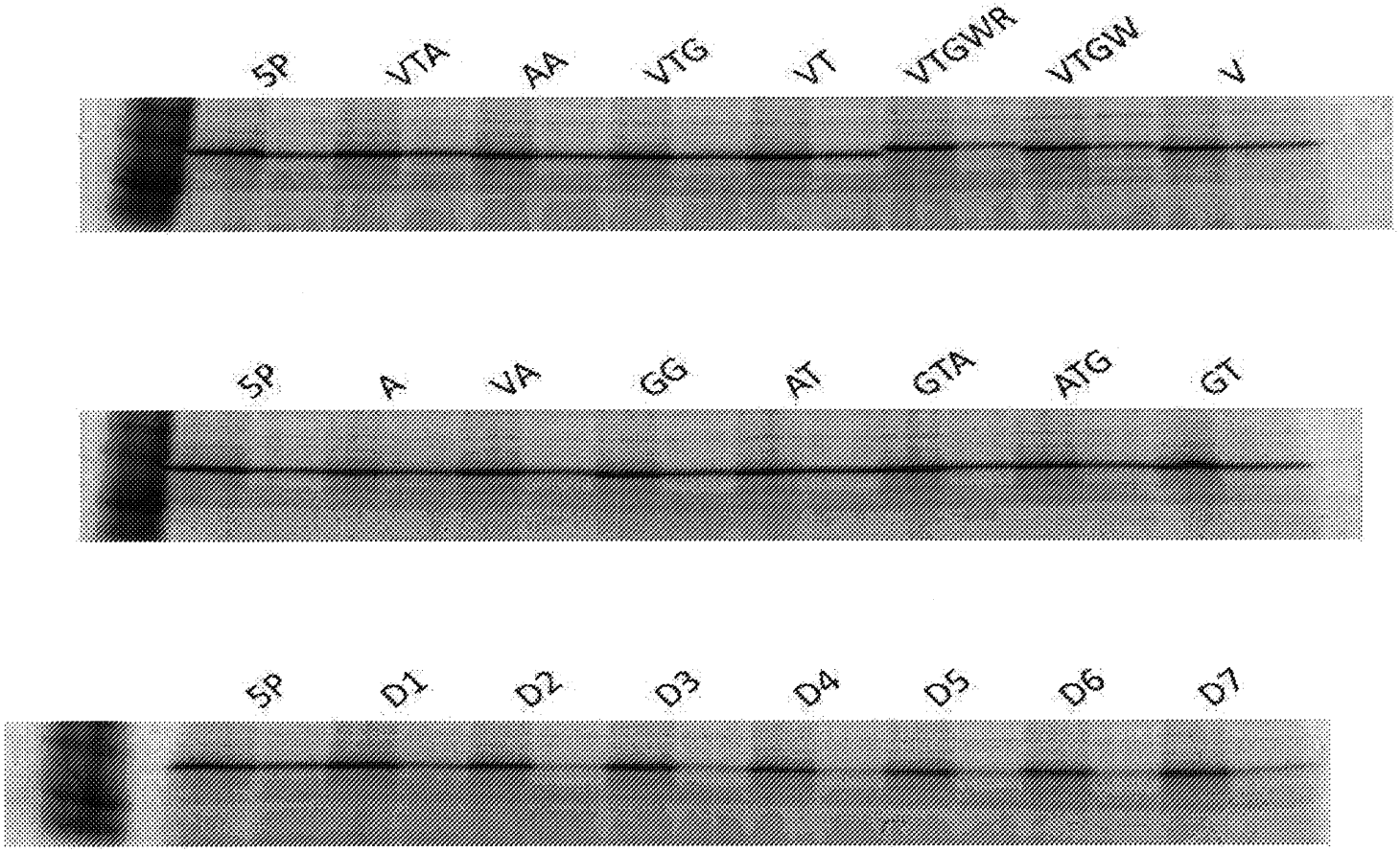




SUBSTITUTE SHEET (RULE 26)

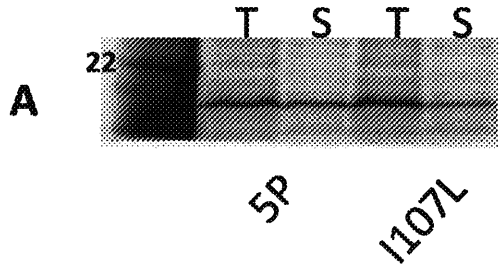
Figure 57

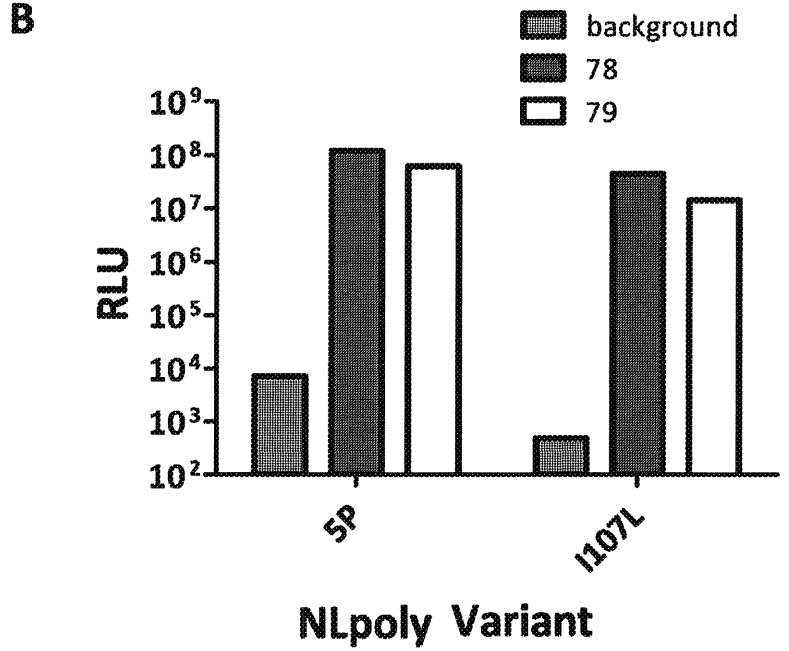
Alternating lanes are crude and soluble fractions



SUBSTITUTE SHEET (RULE 26)

Figure 58





C

Signal/background

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

SUBSTITUTE SHEET (RULE 26)

Figure 59

SUBSTITUTE SHEET (RULE 26)

Figure 60

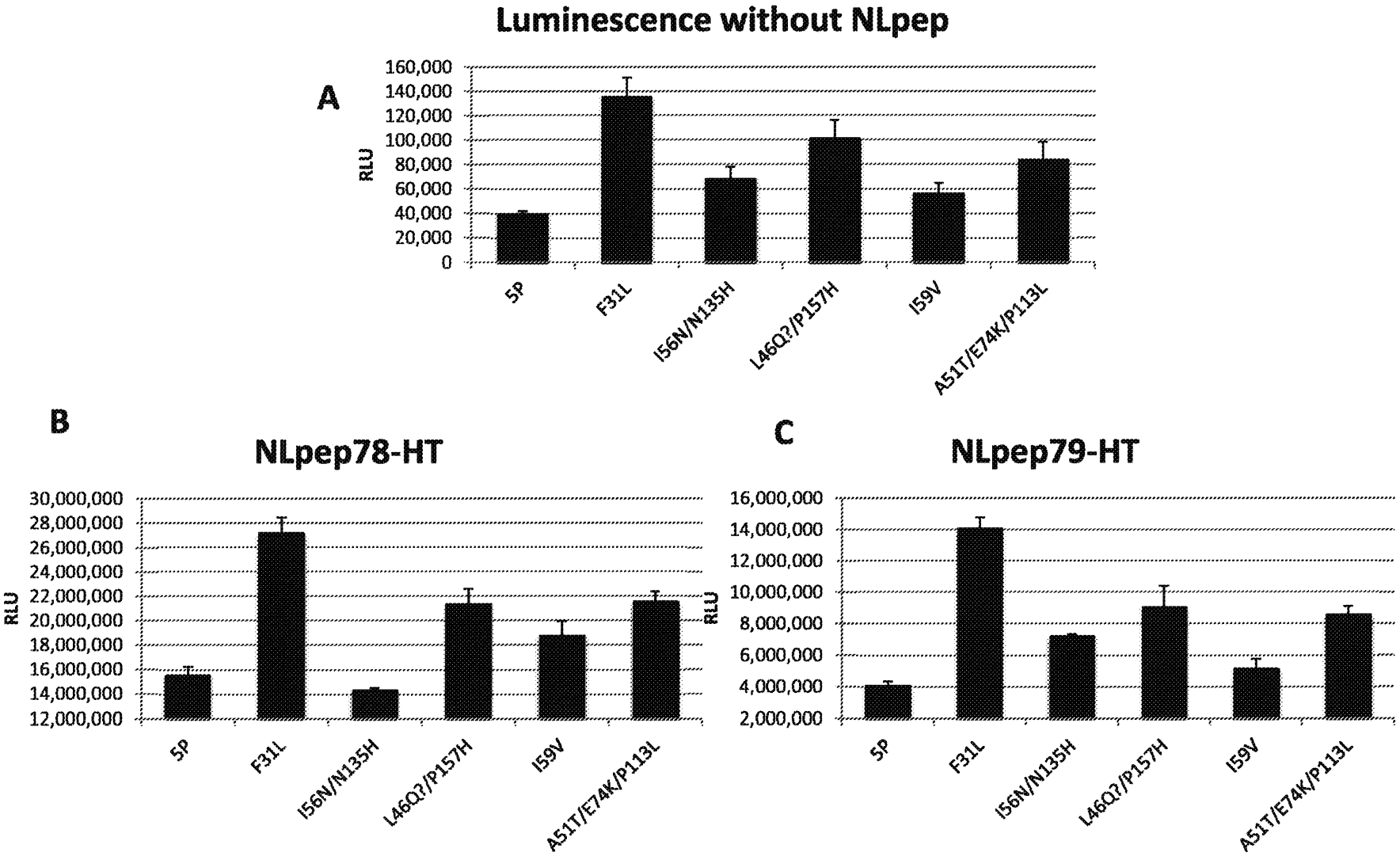

Luminescence without NLpep
A
RLU
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
SP
F31L
I56N/N135H
L46Q?/P157H
I59V
A51T/E74K/P113L
B
NLpep78-HT
RLU
30,000,000
28,000,000
26,000,000
24,000,000
22,000,000
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
SP
F31L
I56N/N135H
L46Q?/P157H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

Figure 61

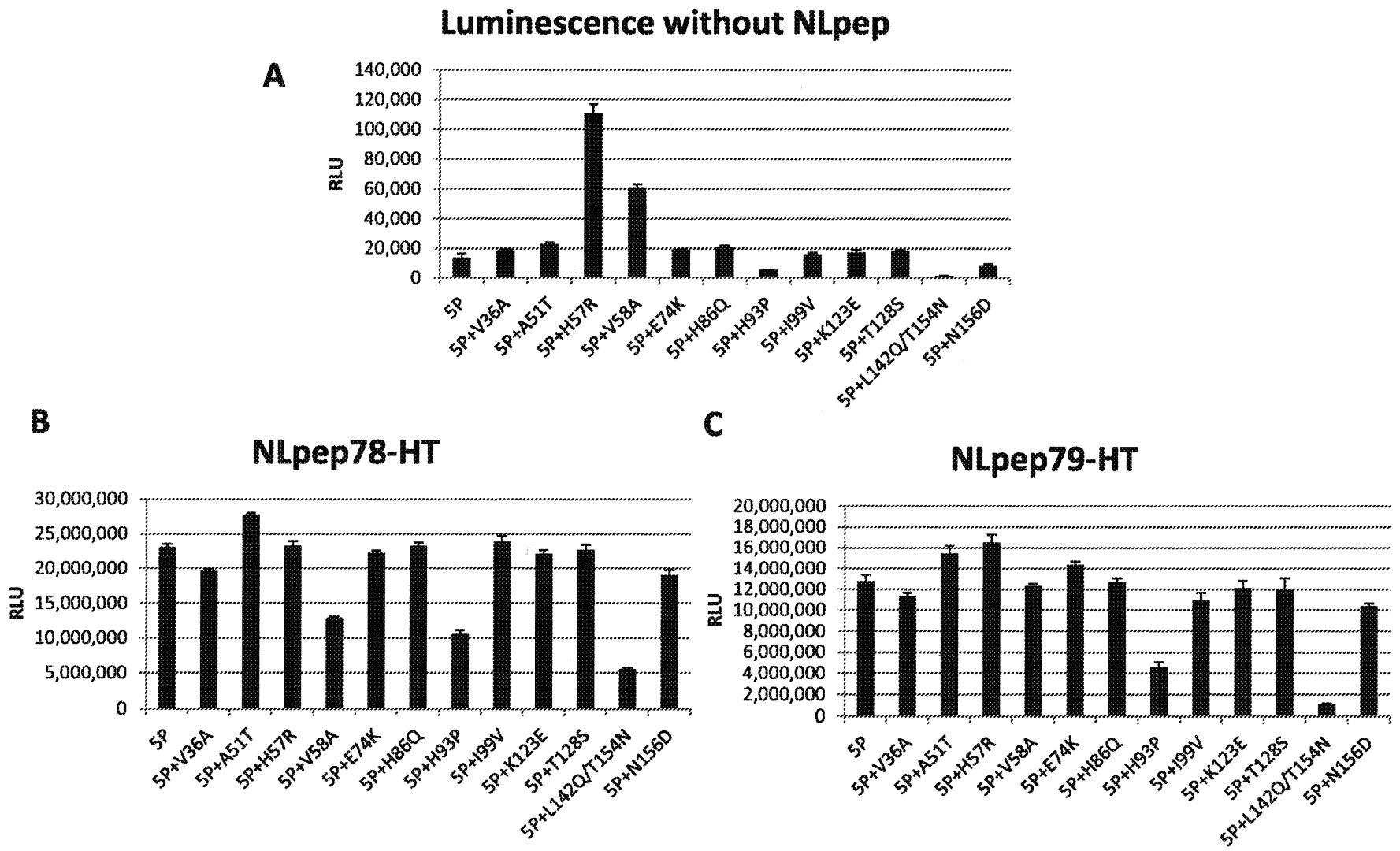

Luminescence without NLpep
A
RLU
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
5P
5P+V36A
5P+A51T
5P+H57R
5P+V58A
5P+E74K
5P+H86Q
5P+H93P
5P+I99V
5P+K123E
5P+T128S
5P+L142Q/T154N
5P+N156D
B
NLpep78-HT
RLU
30,000,000
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
5P
5P+V36A
5P+A51T
5P+H57R
5P+V58A
5P+E74K
5P+H86Q
5P+H93P
5P+I99V
5P+K123E
5P+T128S
5P+L142Q/T154N
5P+N156D
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
5P
5P+V36A
5P+A51T
5P+H57R
5P+V58A
5P+E74K
5P+H86Q
5P+H93P
5P+I99V
5P+K123E
5P+T128S
5P+L142Q/T154N
5P+N156D

Figure 62

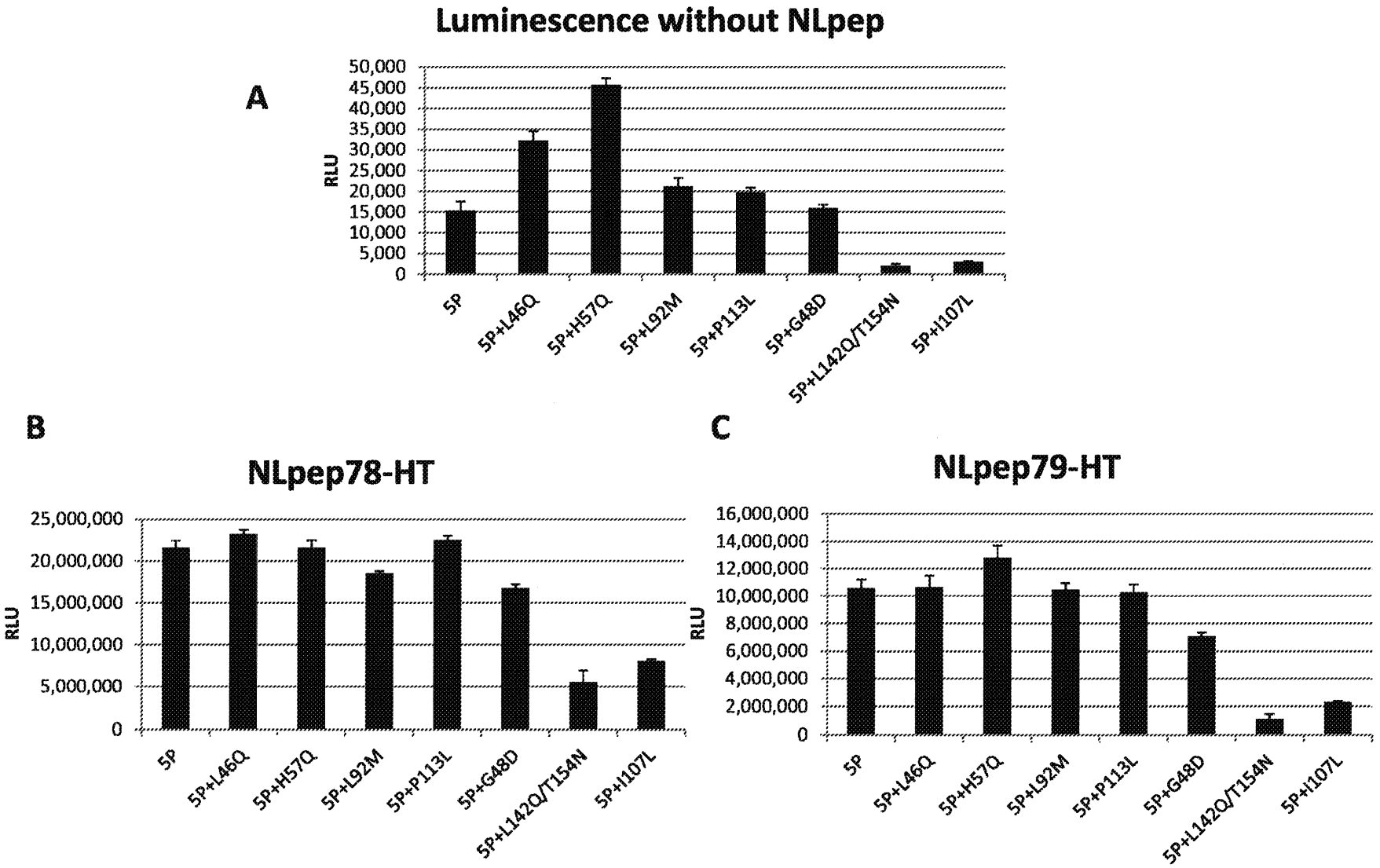
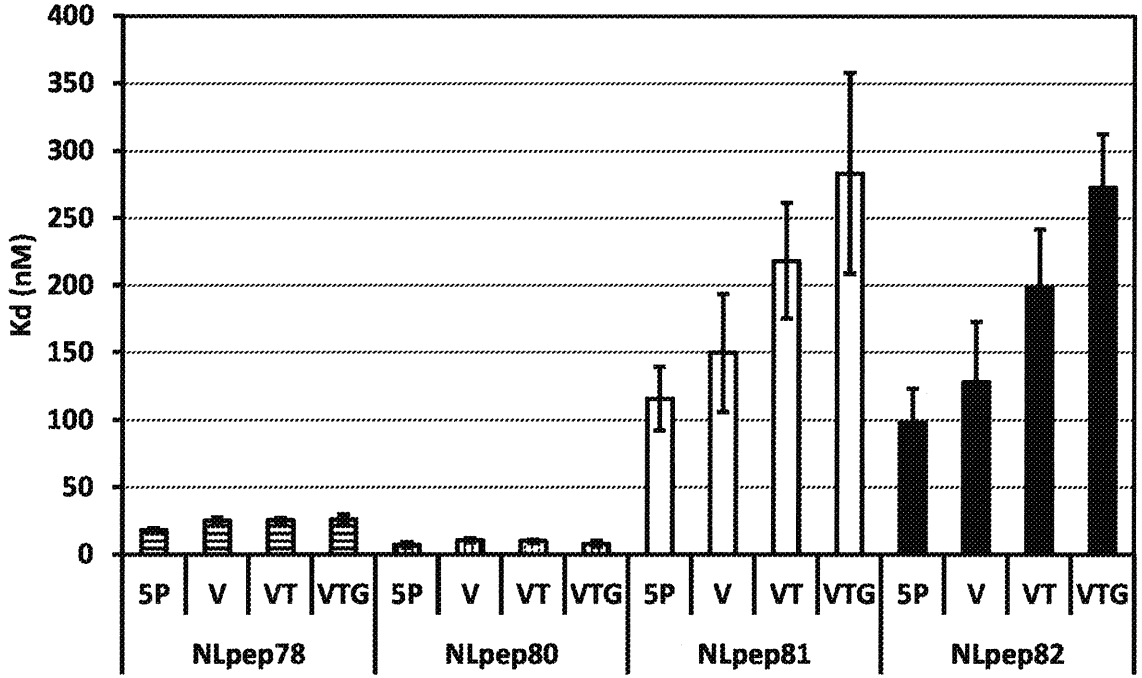
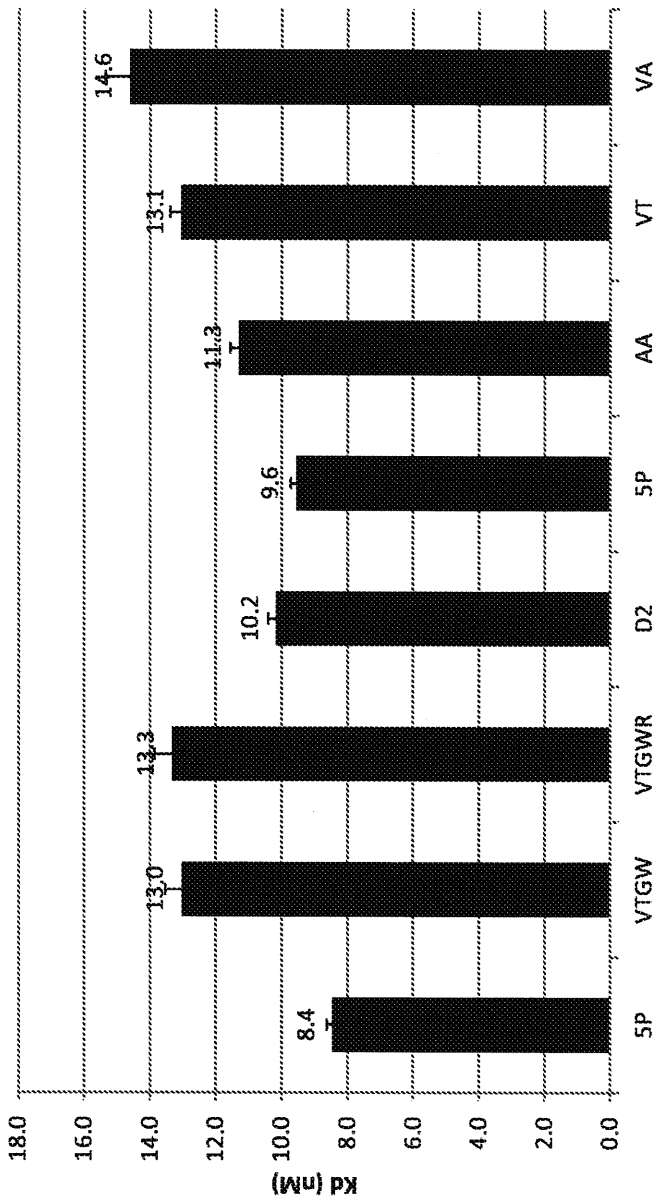

A
Luminescence without NLpep
RLU
50,000
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
5P
5P+L46Q
5P+H57Q
5P+L92M
5P+P113L
5P+G48D
5P+L142Q/T154N
5P+I107L
B
NLpep78-HT
RLU
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
5P
5P+L46Q
5P+H57Q
5P+L92M
5P+P113L
5P+G48D
5P+L142Q/T154N
5P+I107L
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
5P
5P+L46Q
5P+H57Q
5P+L92M
5P+P113L
5P+G48D
5P+L142Q/T154N
5P+I107L

Figure 63



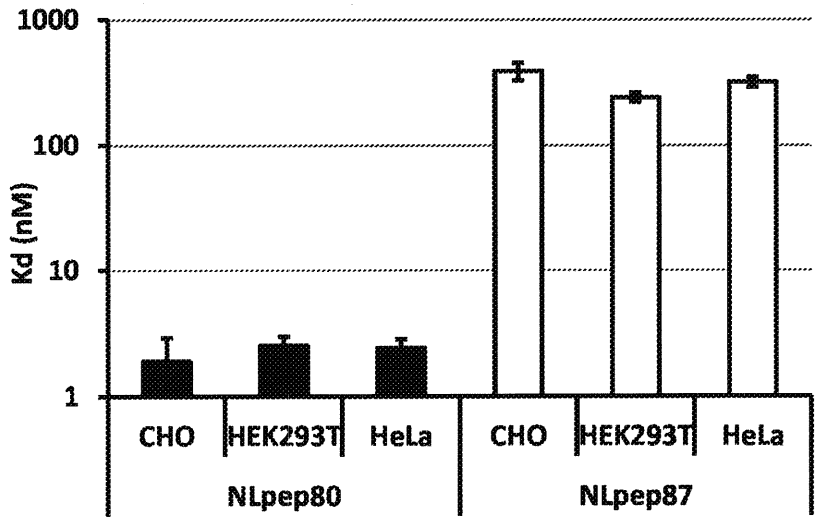
	5P	5P+V	5P+VT	5P+VTG
NLpep78	17.8 ± 1.43	24.9 ± 2.23	25.4 ± 1.41	26.0 ± 3.85
NLpep80	6.9 ± 1.8	10.3 ± 1.57	9.6 ± 1.2	7.5 ± 2.1
NLpep81	115.6 ± 23.7	149.8 ± 43.9	218.0 ± 43.20	283.2 ± 74.7
NLpep82	97.8 ± 25.17	127.9 ± 44.80	197.9 ± 43.70	272.5 ± 40.20

Figure 64

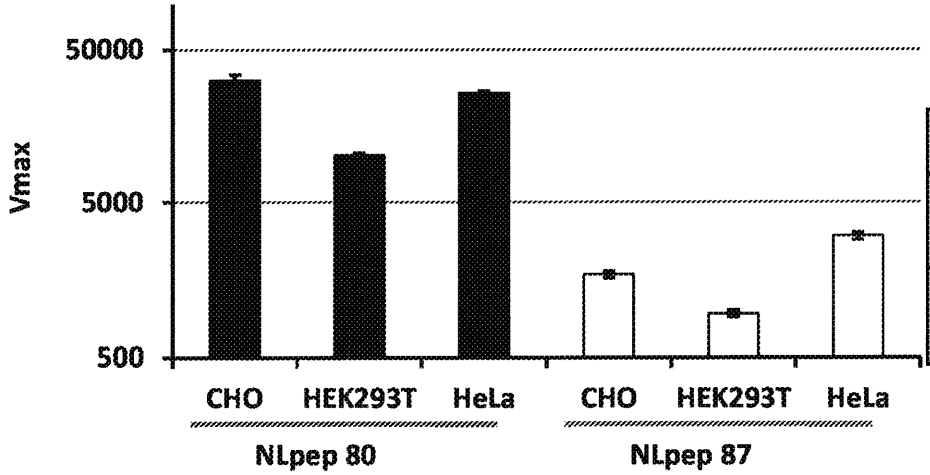


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Figure 65



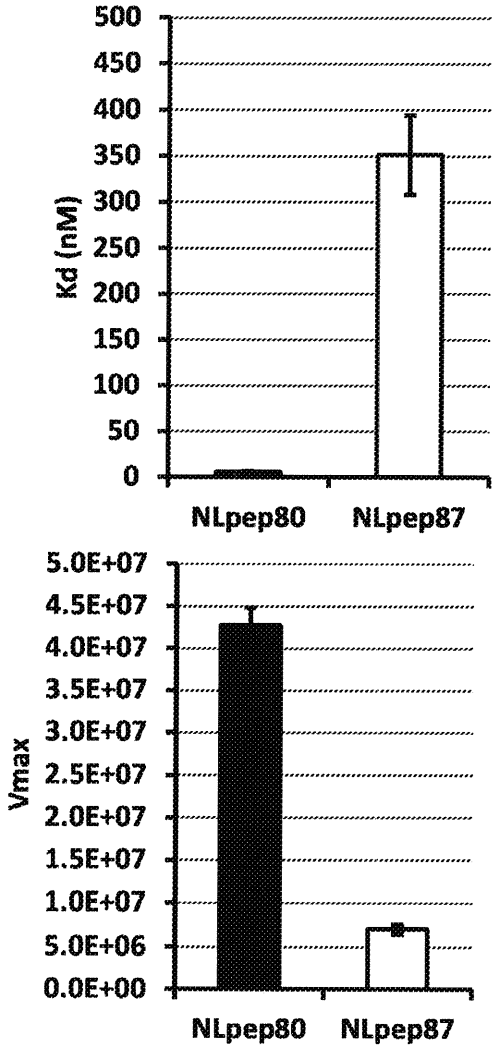
	CHO	HEK293T	HeLa
NLpep 80	1.9 ± 1.02	2.5 ± 0.45	2.4 ± 0.42
NLpep 87	390.0 ± 63.35	239.5 ± 20.4	318.4 ± 31.0



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

SUBSTITUTE SHEET (RULE 26)

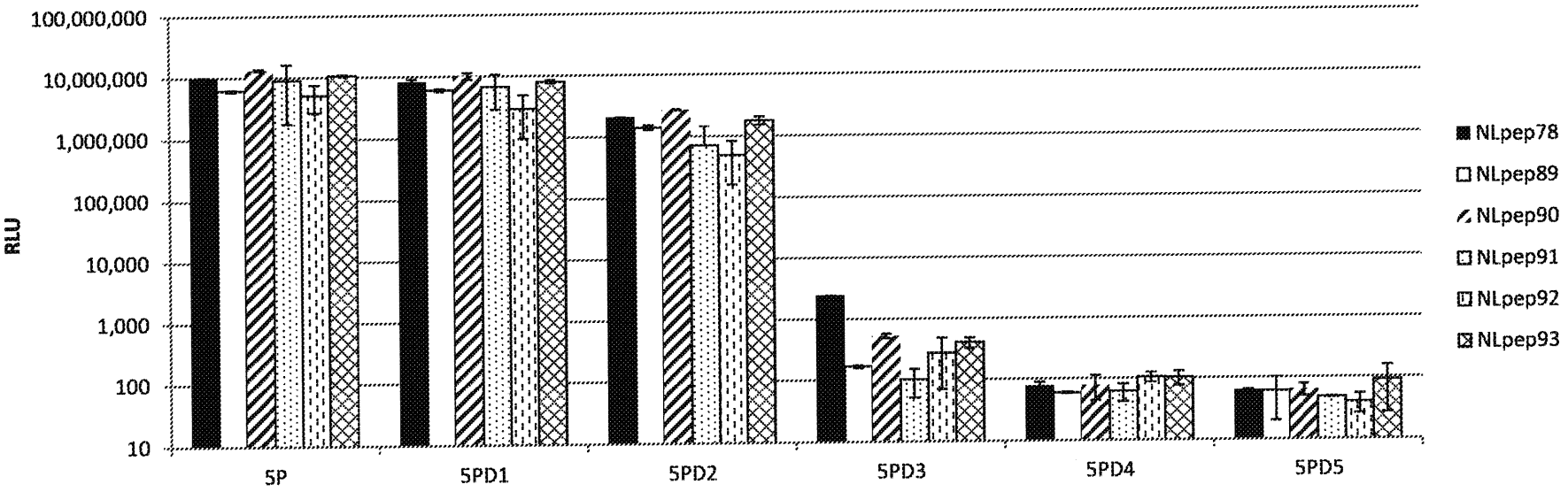
Figure 66



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

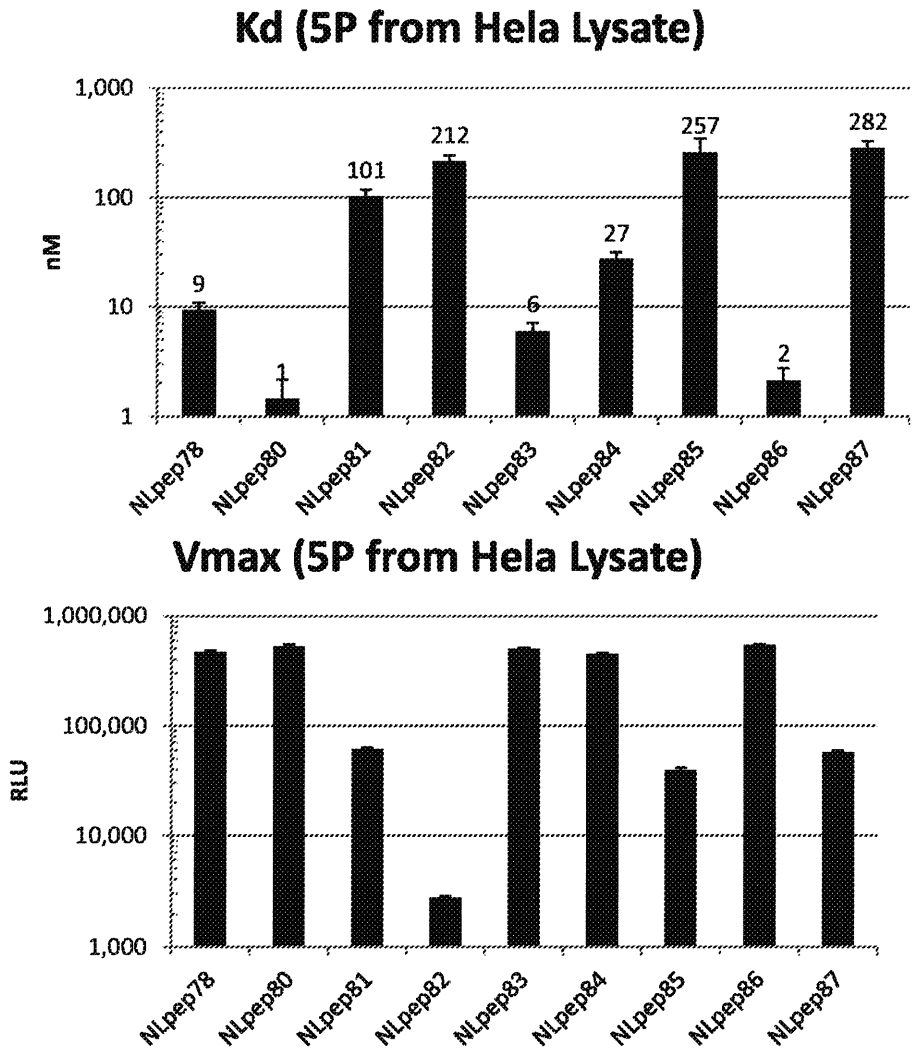
	E.coli
NLpep 80	4.3E+07 ± 2.1E+06
NLpep 87	7.0E+06 ± 5.4E+05

Figure 67



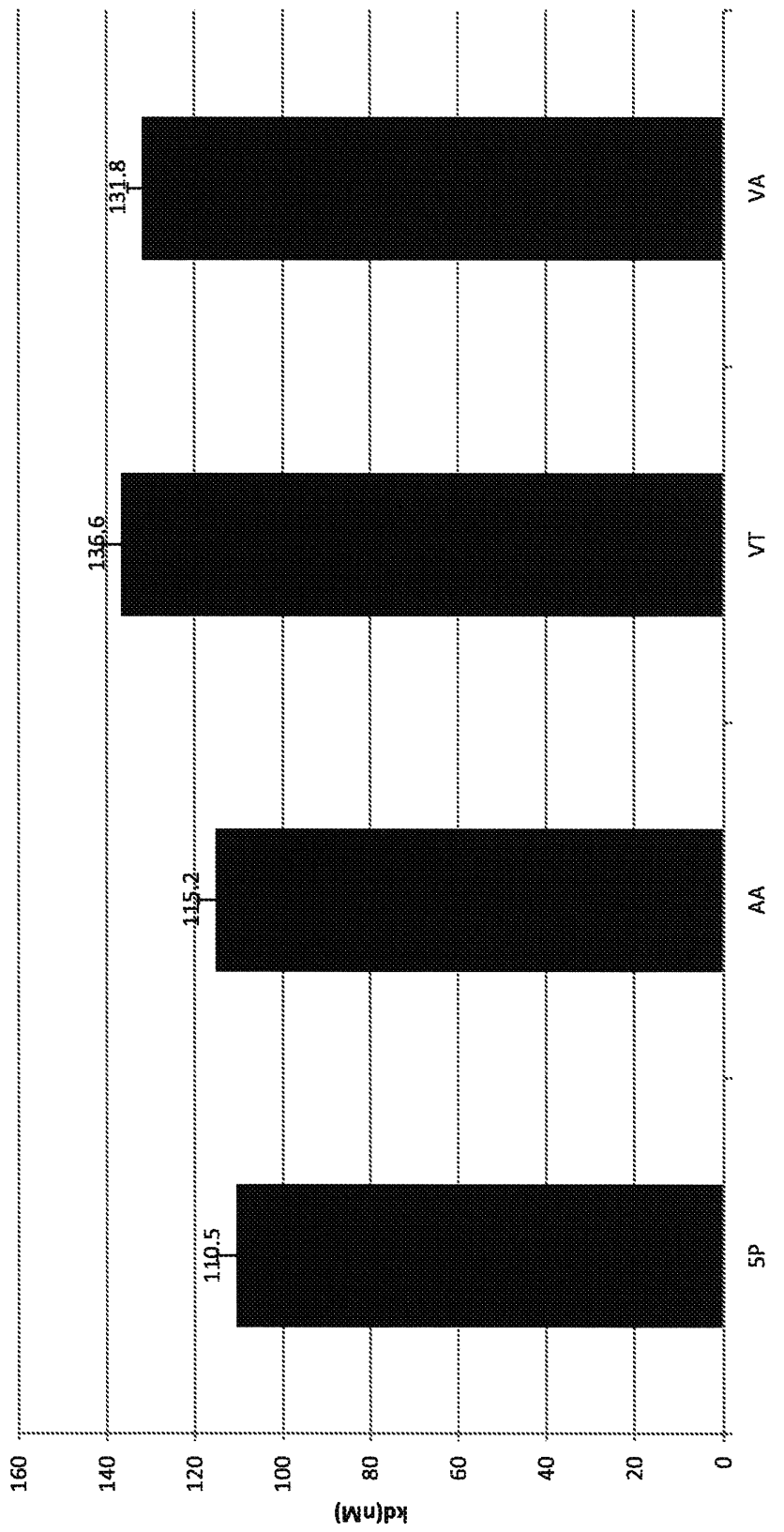
5PD1 has 1 amino acid removed from C-terminus, 5PD2 has 2 amino acids removed from C-terminus, …., 5PD5 has 5 amino acids removed from the C-terminus.

Figure 68



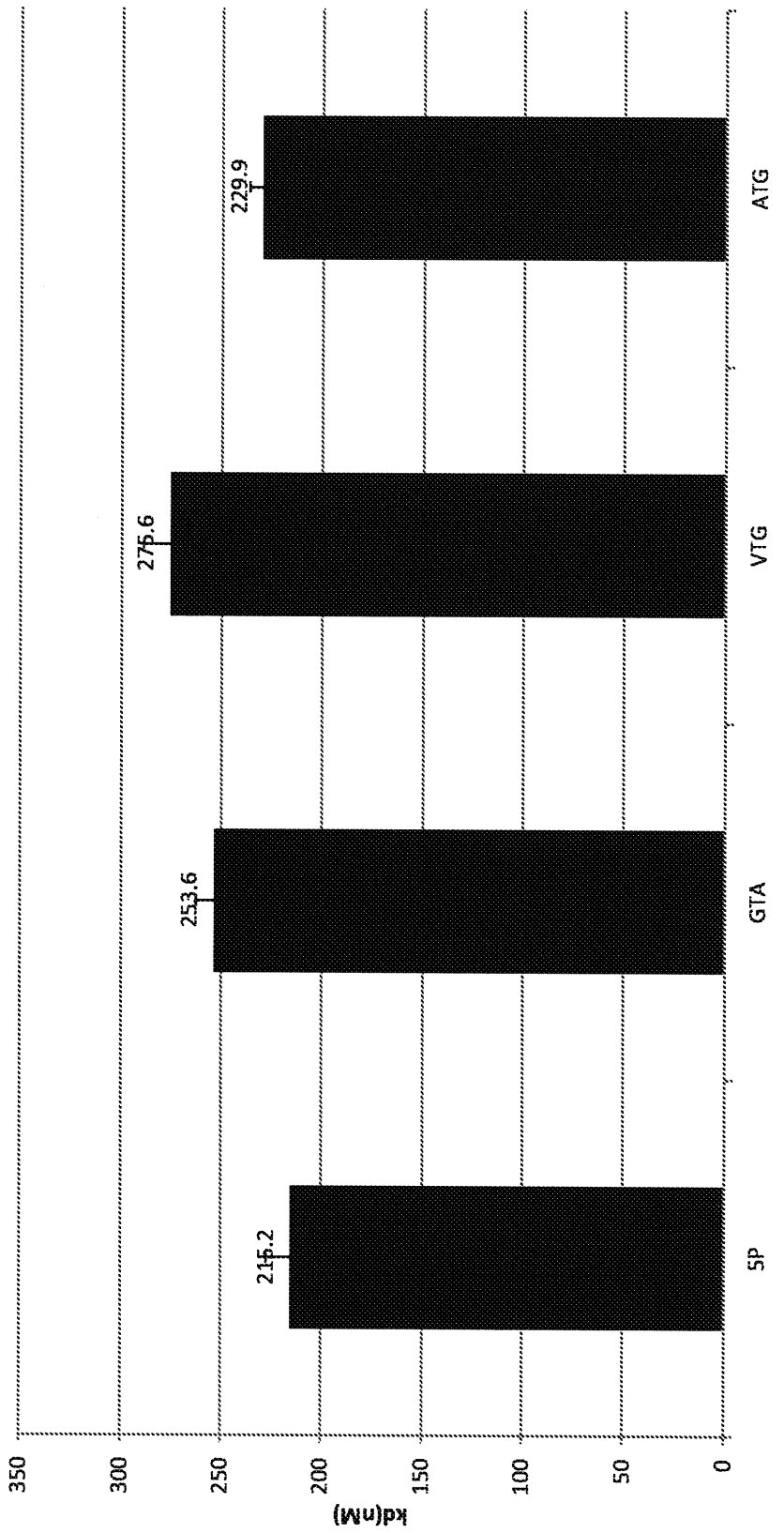
SUBSTITUTE SHEET (RULE 26)

Figure 69



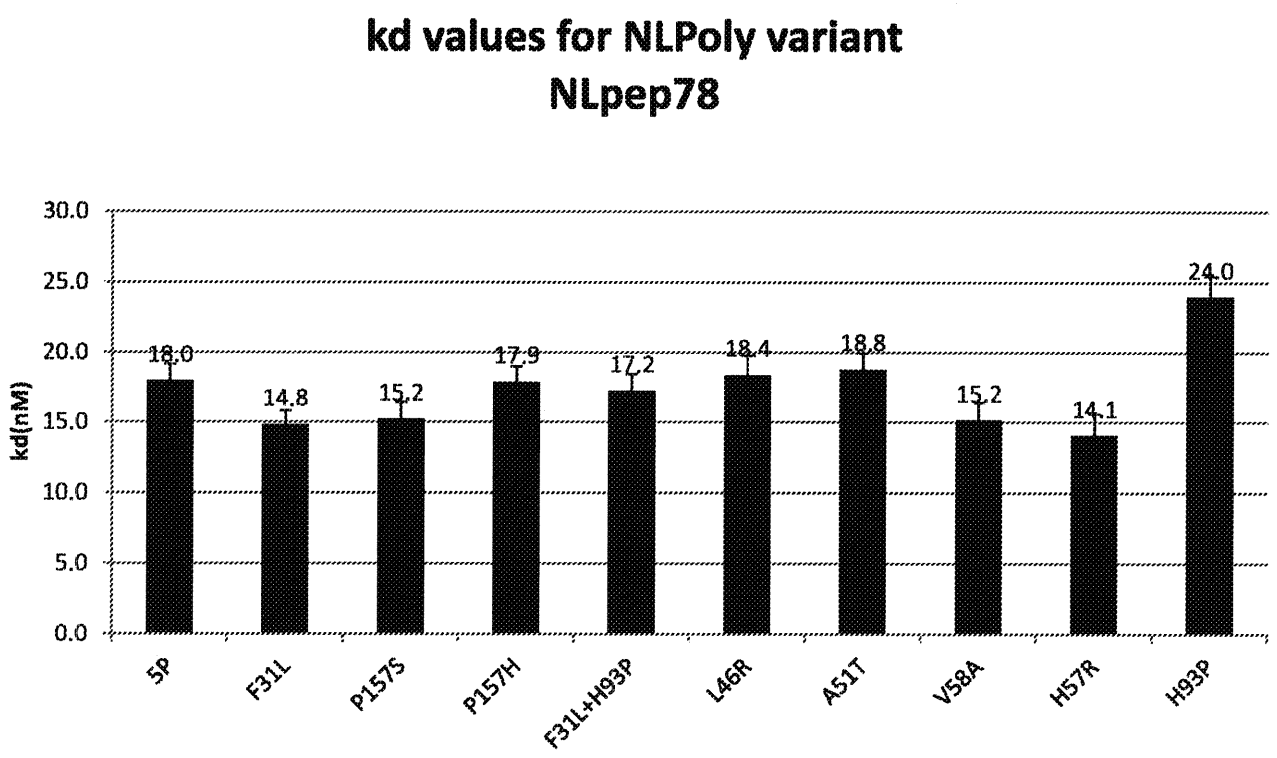
SUBSTITUTE SHEET (RULE 26)

Figure 70



SUBSTITUTE SHEET (RULE 26)

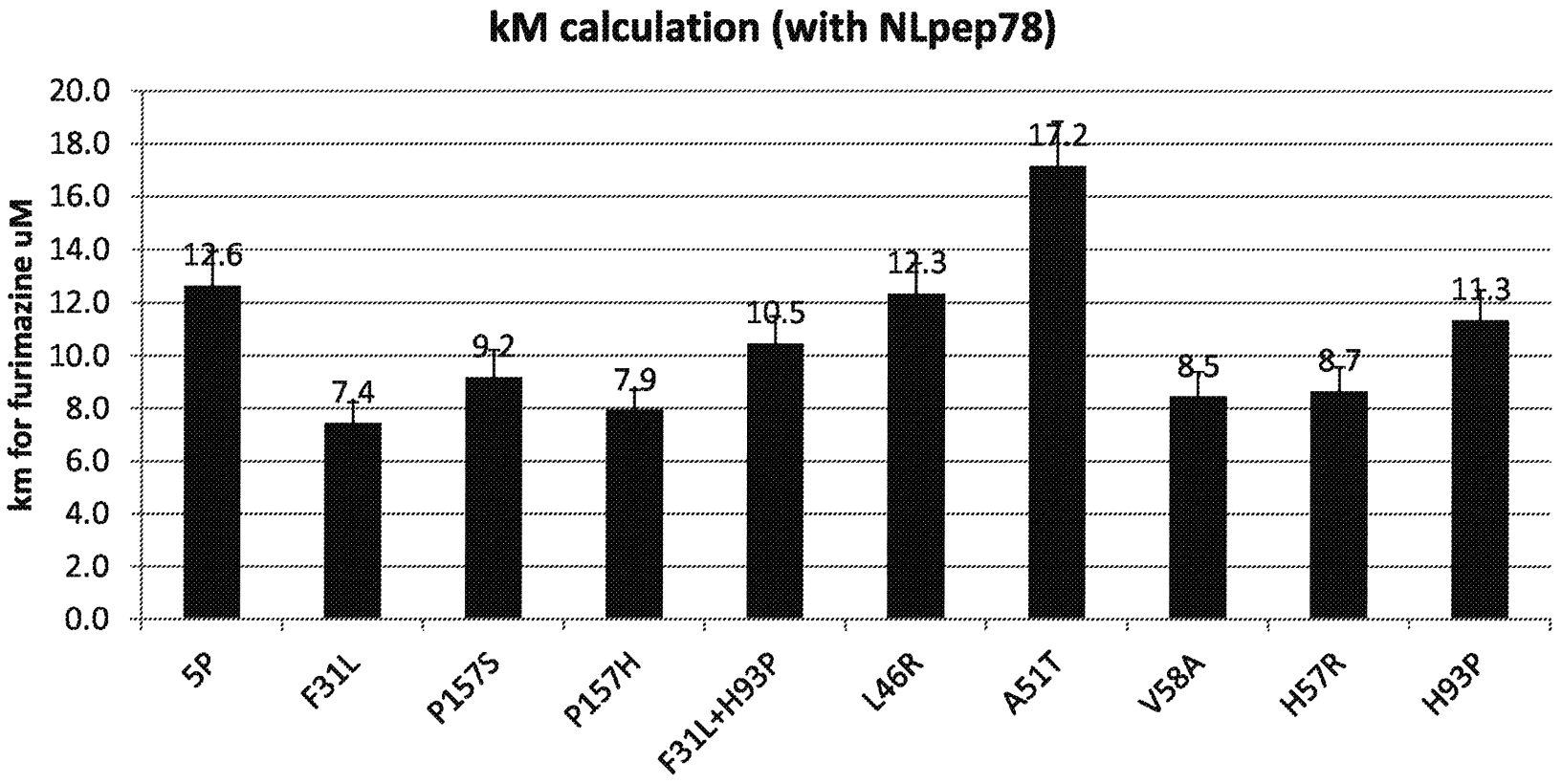
Figure 71



*Indicated mutations are mutations onto 5P

SUBSTITUTE SHEET (RULE 26)

Figure 72



*Indicated mutations are mutations onto 5P

SUBSTITUTE SHEET (RULE 26)

Figure 73

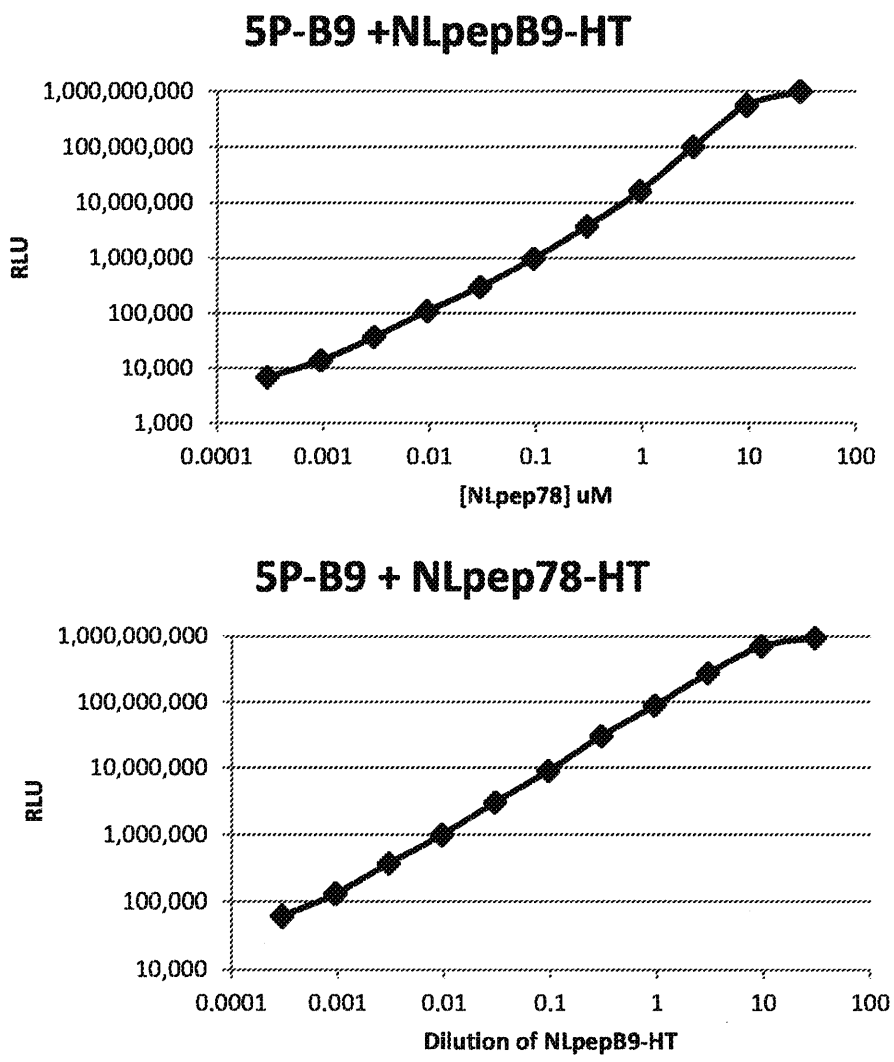
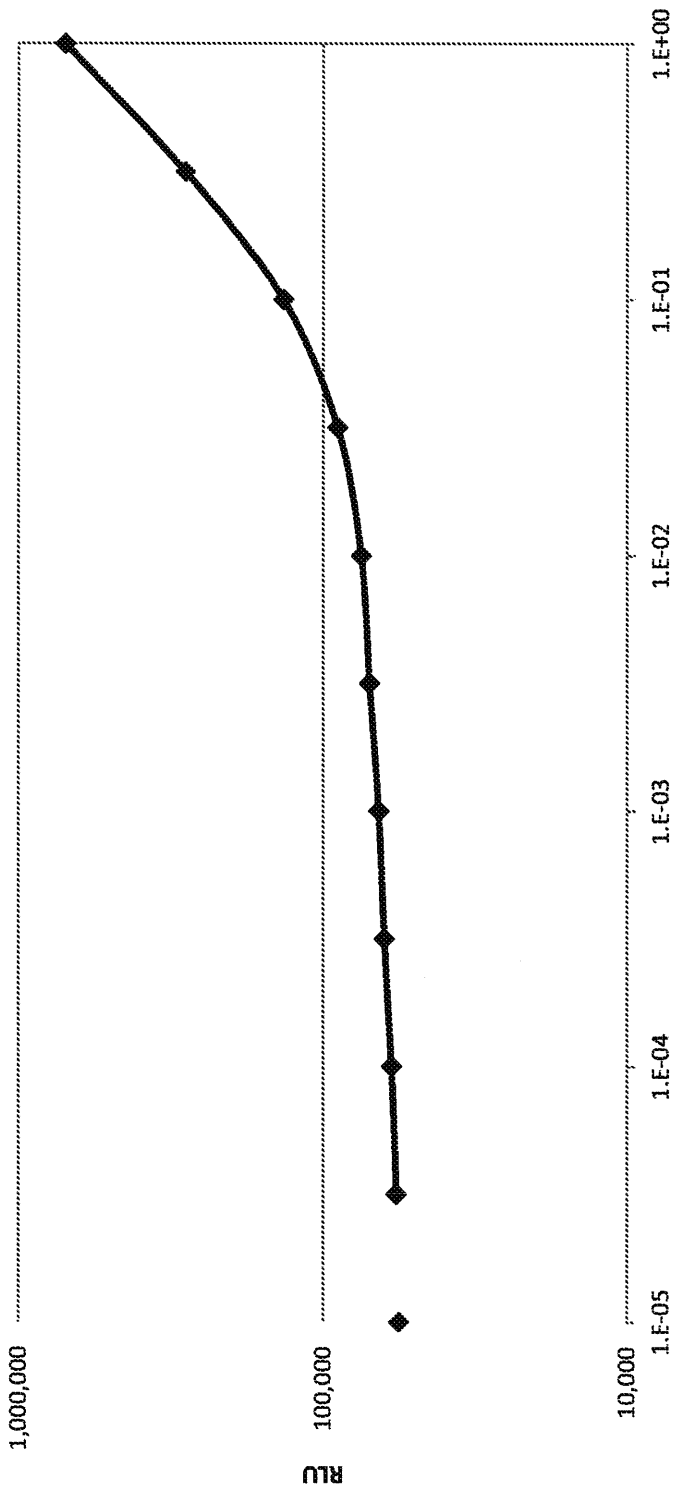
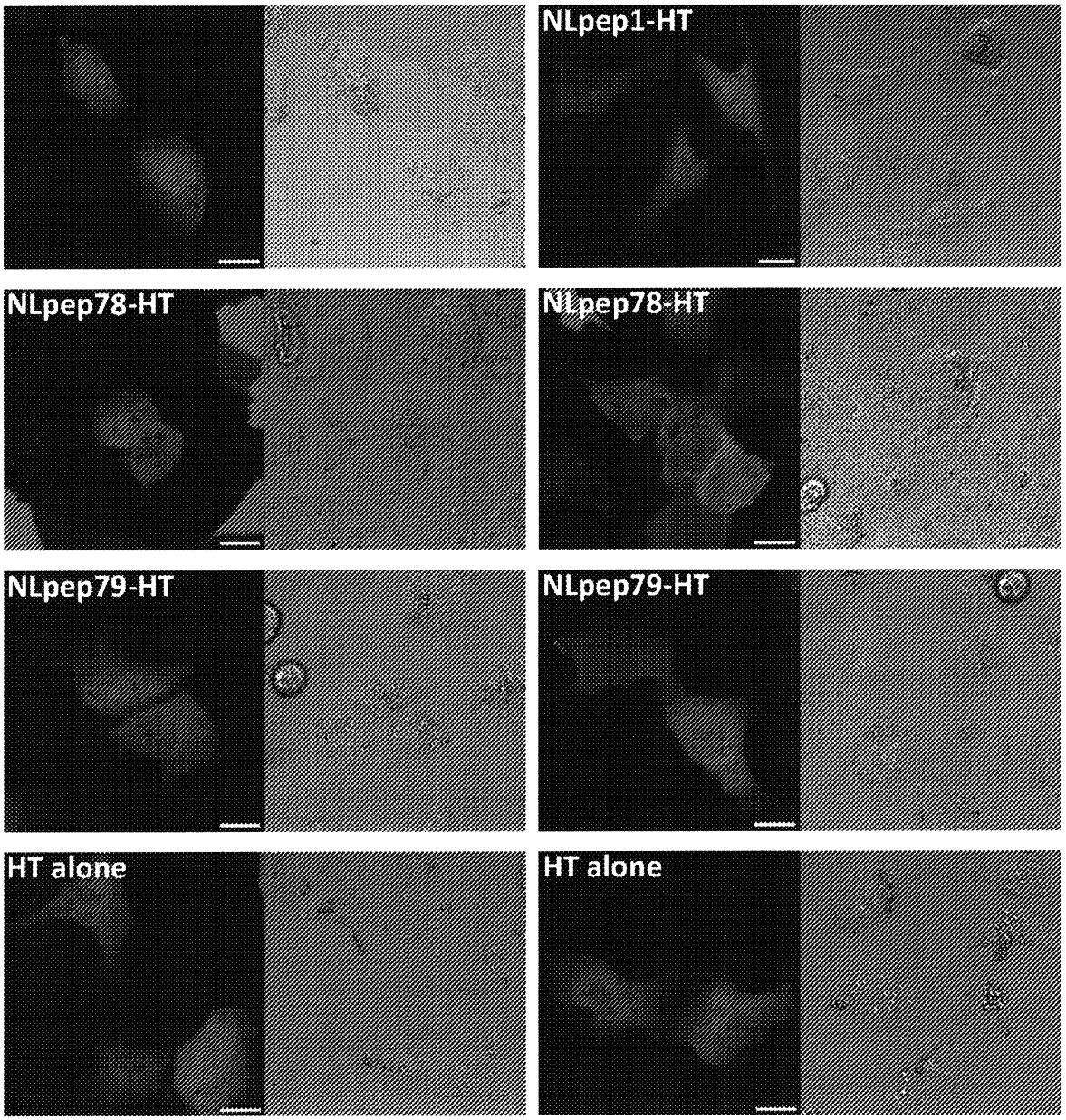

5P-B9 +NLpepB9-HT
RLU
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
0.0001
0.001
0.01
0.1
1
10
100
[NLpep78] uM
5P-B9 + NLpep78-HT
RLU
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
0.0001
0.001
0.01
0.1
1
10
100
Dilution of NLpepB9-HT

Figure 74



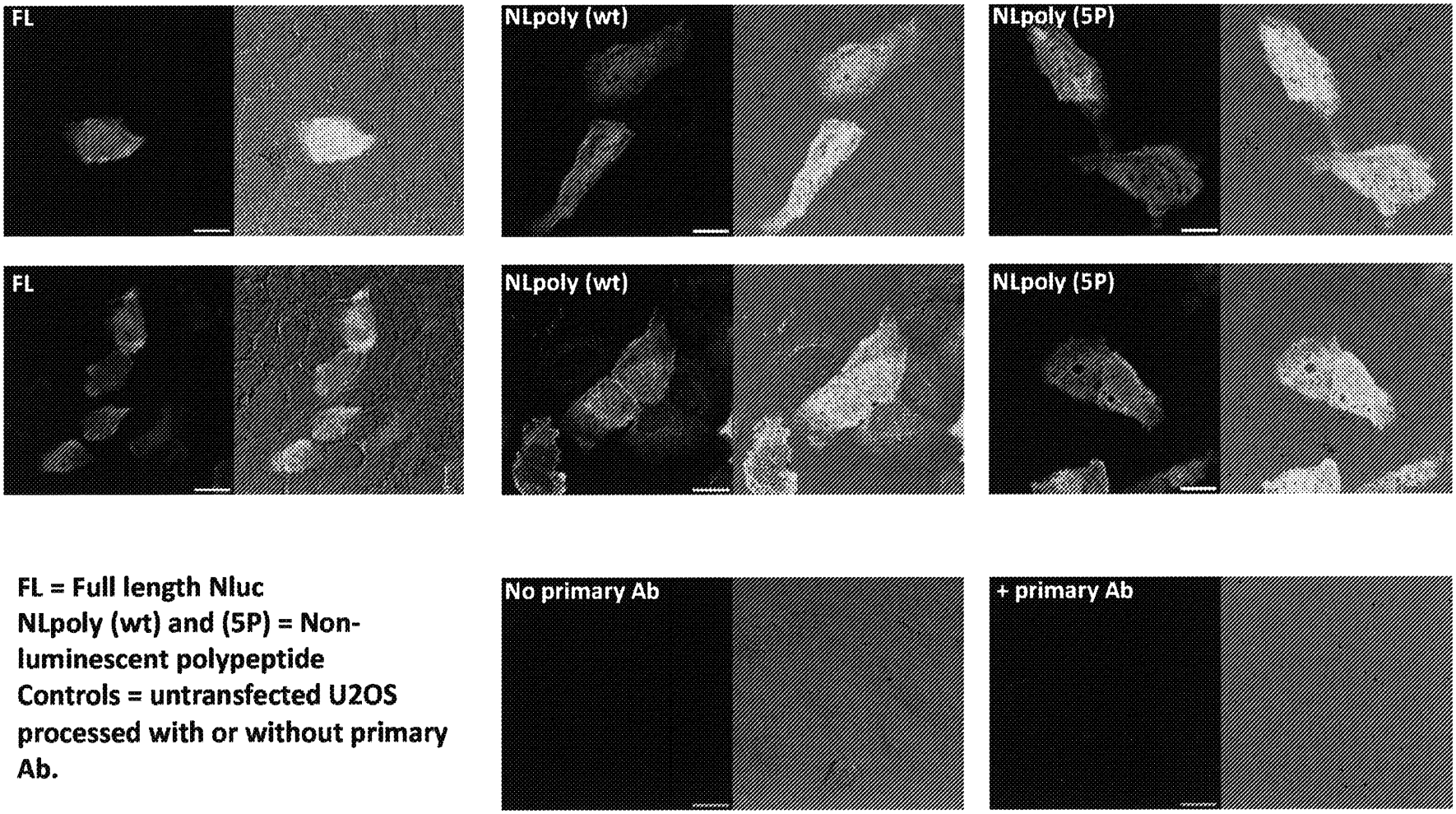
SUBSTITUTE SHEET (RULE 26)

Figure 75



SUBSTITUTE SHEET (RULE 26)

Figure 76



FL = Full length Nluc
NLpoly (wt) and (5P) = Non-luminescent polypeptide
Controls = untransfected U2OS processed with or without primary Ab.

Scale bars = 20µm

Figure 77

SUBSTITUTE SHEET (RULE 26)

Figure 78

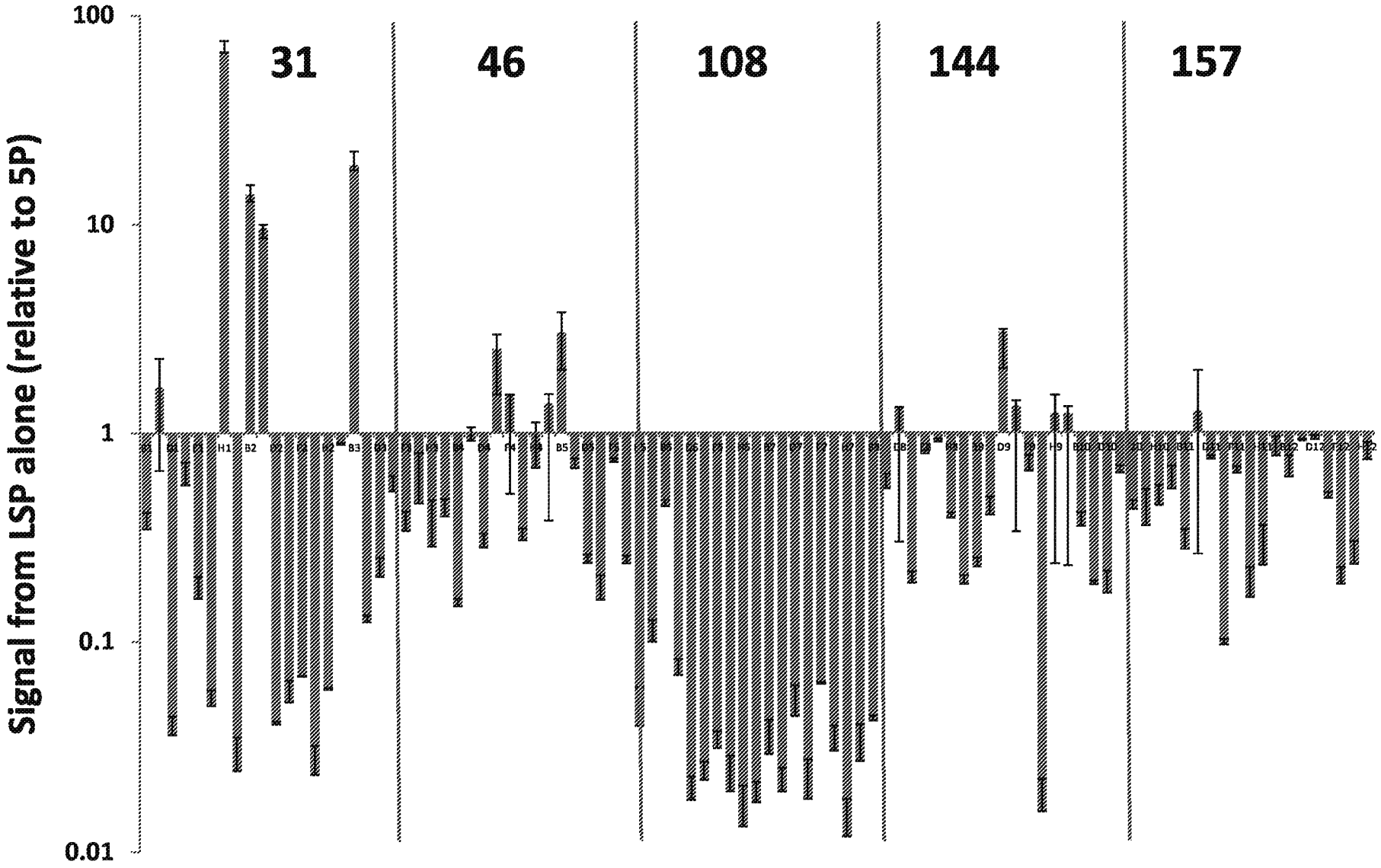
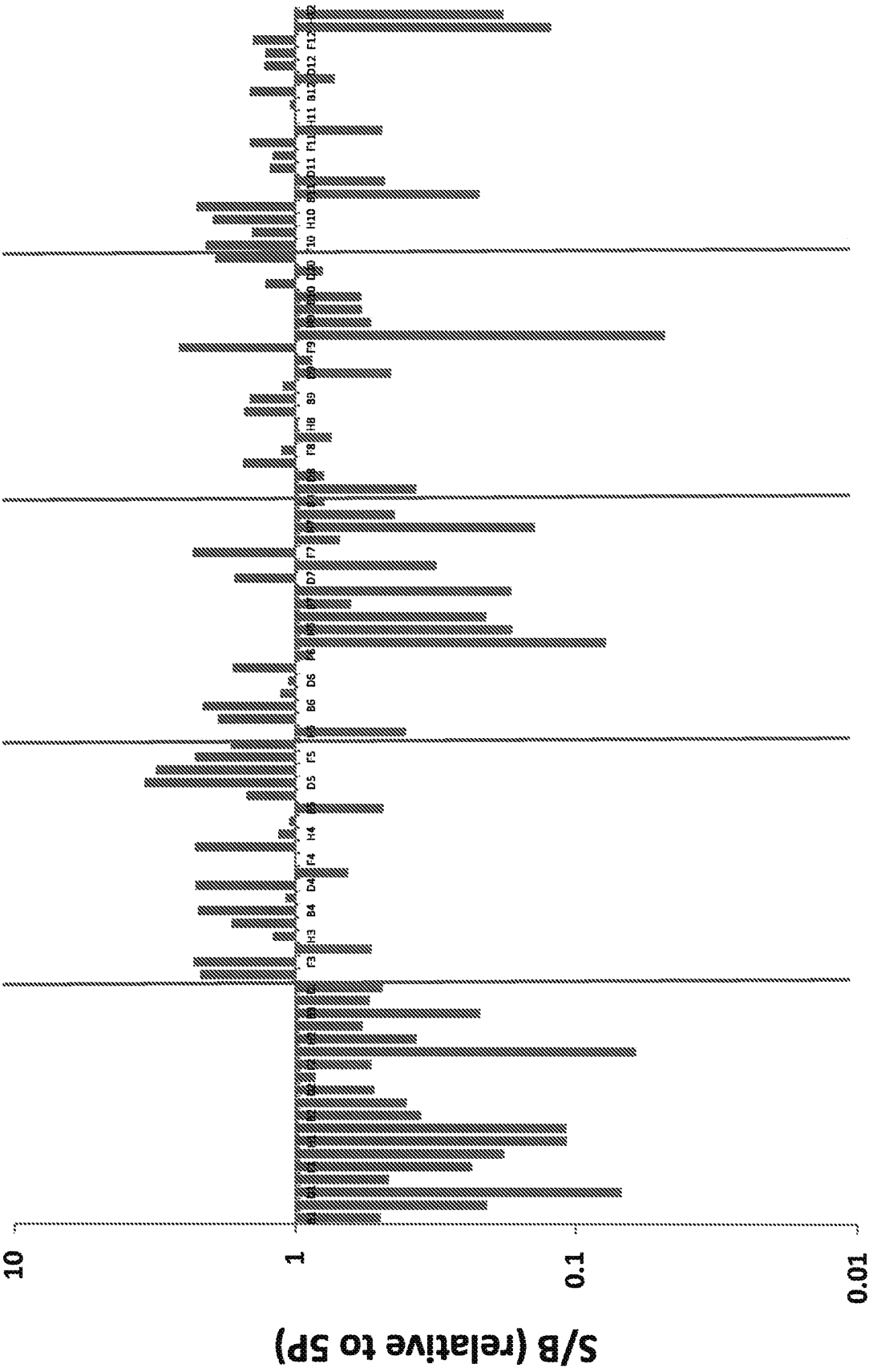
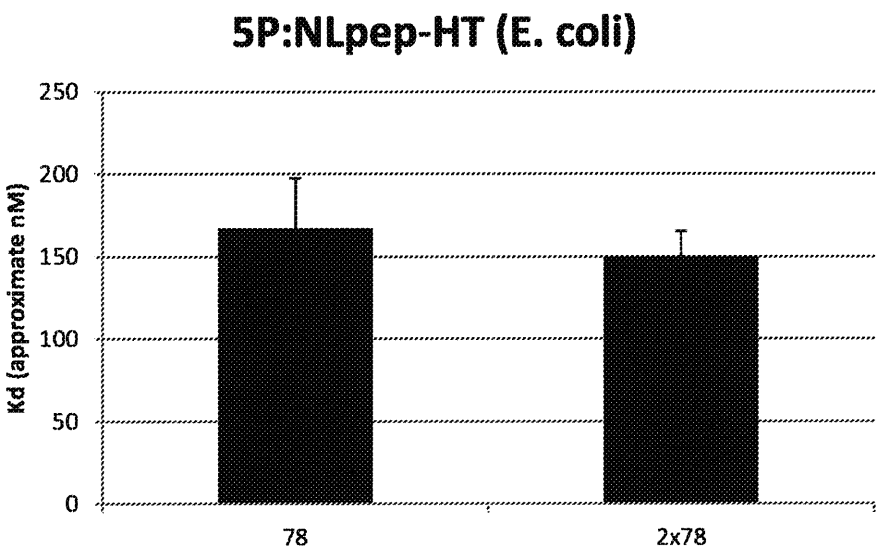

100
10
1
0.1
0.01
Signal from LSP alone (relative to 5P)
31
46
108
144
157

Figure 79



SUBSTITUTE SHEET (RULE 26)

Figure 80

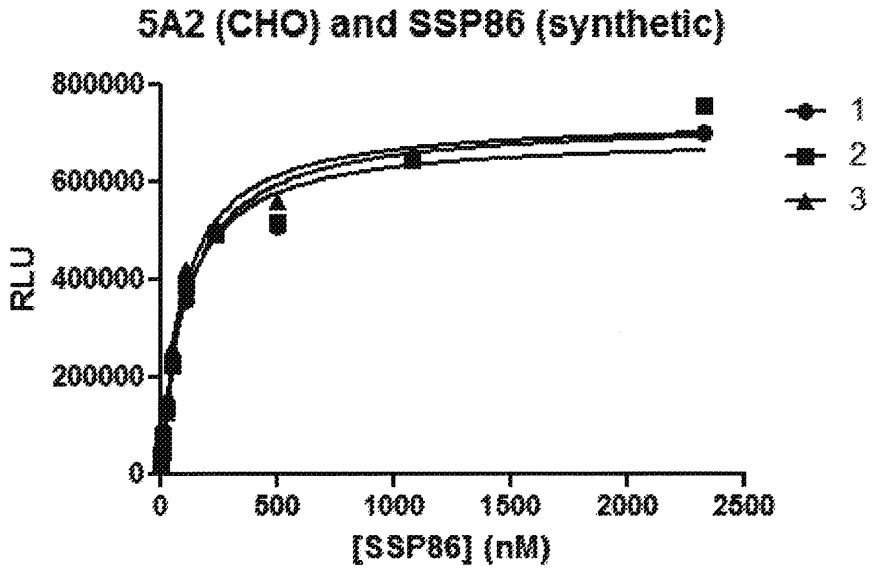


78 = MNVSGWRLFKKISN-HT

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

SUBSTITUTE SHEET (RULE 26)

Figure 81



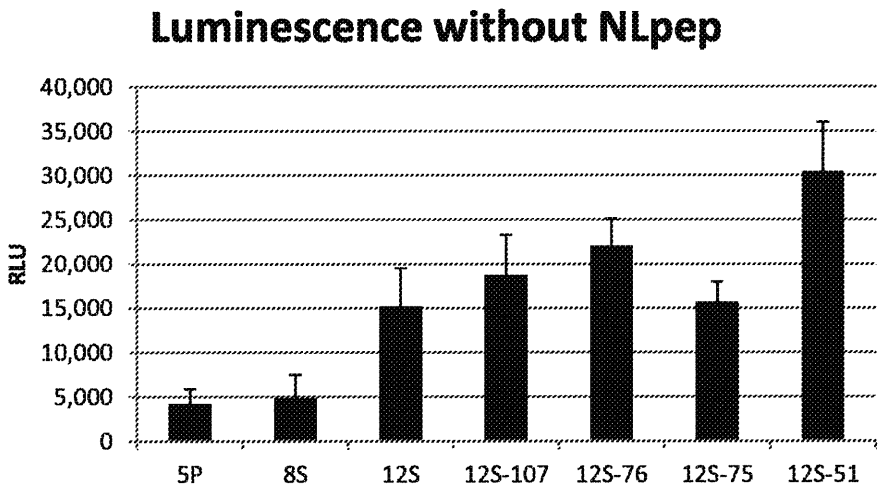
	1	2	3
One site -- Specific binding			
Best-fit values			
Bmax	692830	725926	728434
Kd	99.14	109.7	93.28
Std. Error			
Bmax	23527	29554	23378
Kd	13.29	17.31	11.95
95% Confidence Intervals			
Bmax	640412 to 745248	660080 to 791773	676347 to 780521
Kd	69.53 to 128.8	71.15 to 148.3	66.65 to 119.9

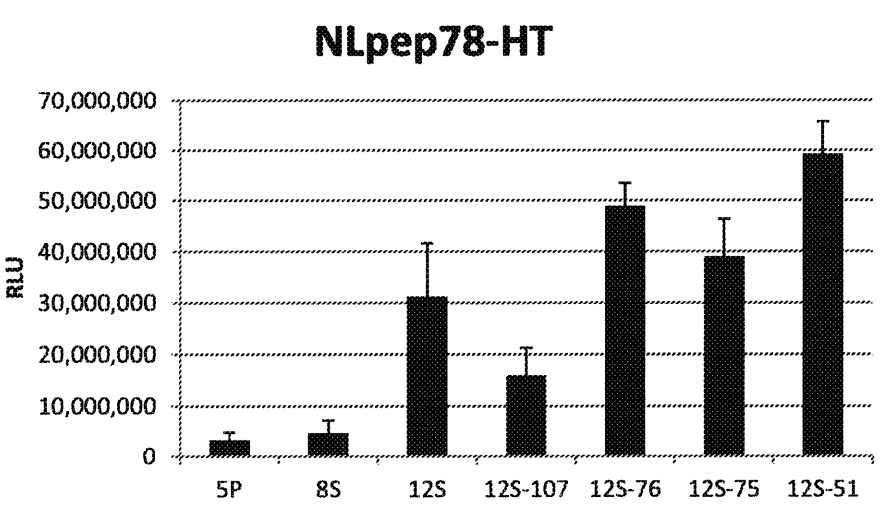
SUBSTITUTE SHEET (RULE 26)

SUBSTITUTE SHEET (RULE 26)

Figure 82

*12S-51 = 11S





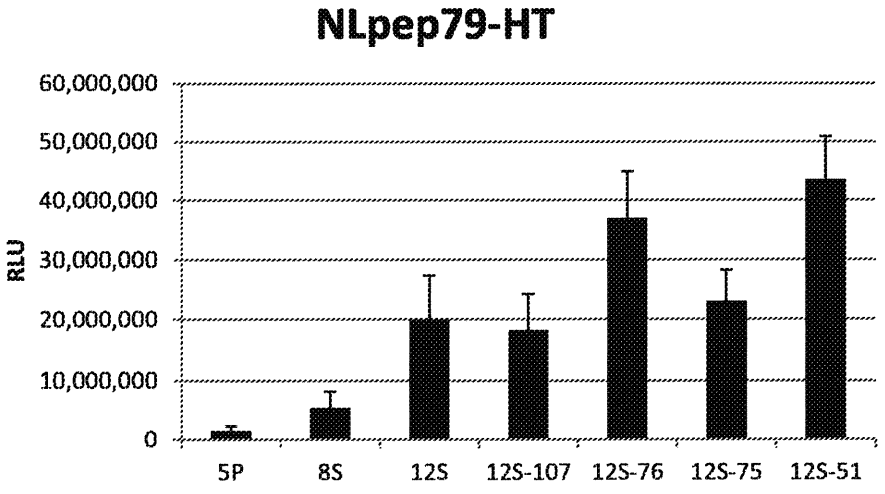


Figure 83

Peptide Array 1 Screens

SUBSTITUTE SHEET (RULE 26)

Figure 84

Peptide Array 1 Screens

NLpoly:5P Kd

NLpoly:5P Kd

SUBSTITUTE SHEET (RULE 26)

Figure 85

Peptide Array 1 Screens

NLpoly:8S Vmax

NLpoly:8S Vmax

SUBSTITUTE SHEET (RULE 26)

Figure 86

Peptide Array 1 Screens

NLpoly:8S Kd

NLpoly:8S Kd

SUBSTITUTE SHEET (RULE 26)

Figure 87

Peptide Array 1 Screens

SUBSTITUTE SHEET (RULE 26)

Figure 88

Peptide Array 1 Screens

NLpoly:5A2 Kd

SUBSTITUTE SHEET (RULE 26)

Figure 89

Peptide Array 1 Screens

NLpoly:11S Vmax

NLpoly:11S Vmax

SUBSTITUTE SHEET (RULE 26)

Figure 90

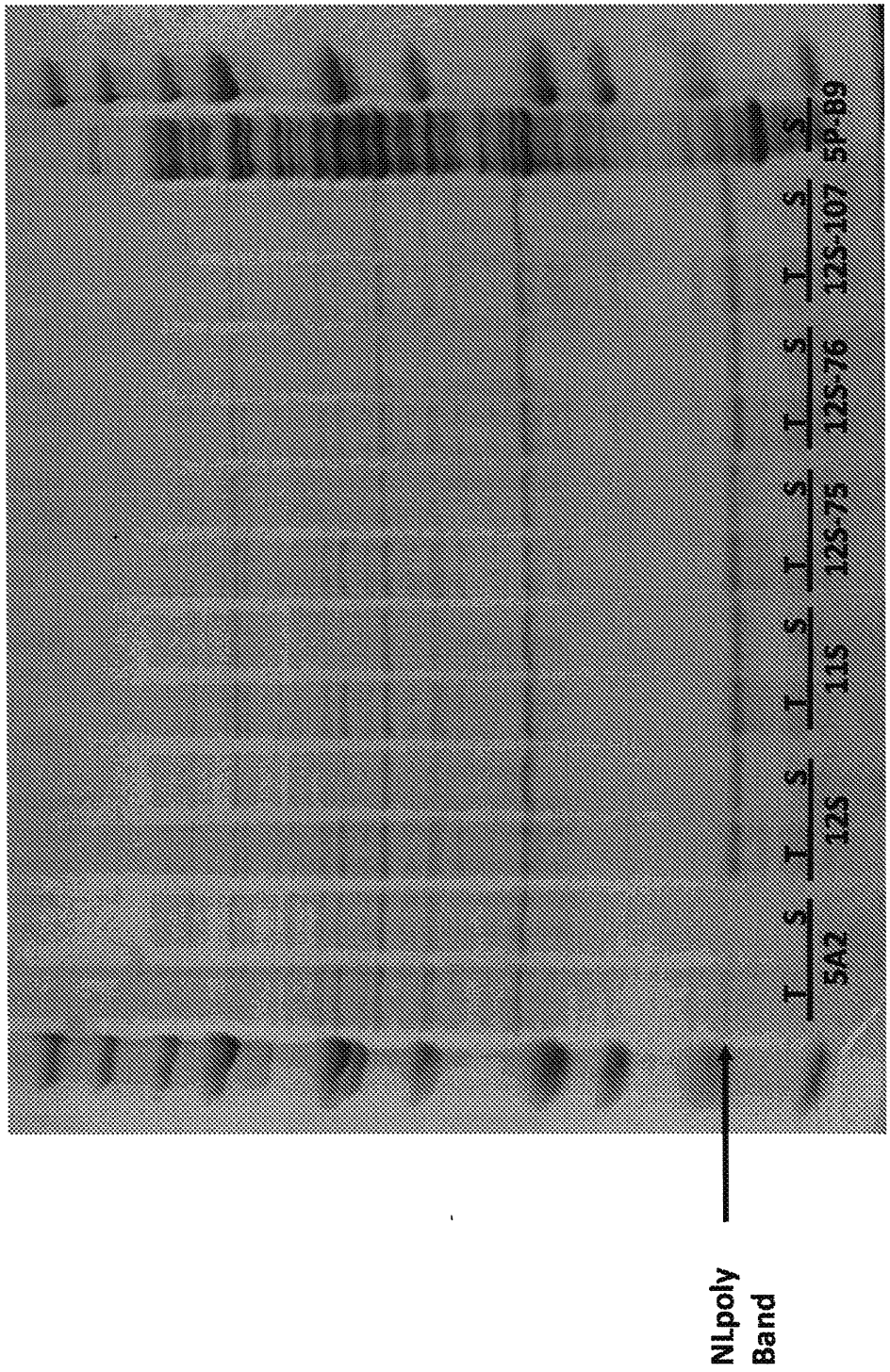
Peptide Array 1 Screens

NLpoly:11S Kd

NLpoly:11S Kd

SUBSTITUTE SHEET (RULE 26)

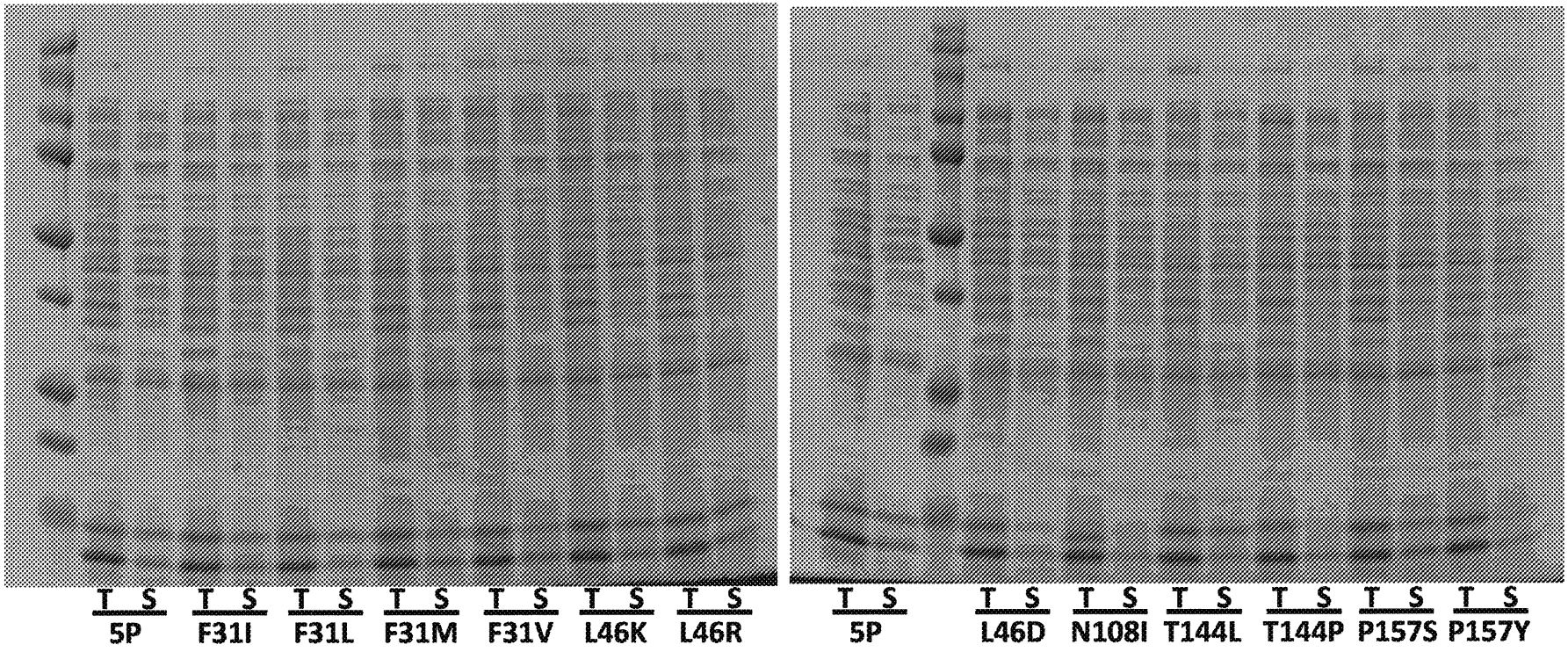
Figure 91



SUBSTITUTE SHEET (RULE 26)

SUBSTITUTE SHEET (RULE 26)

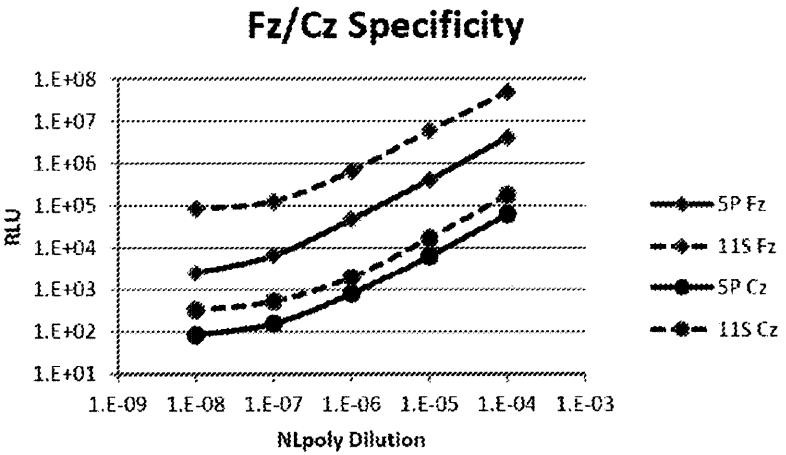
Figure 92

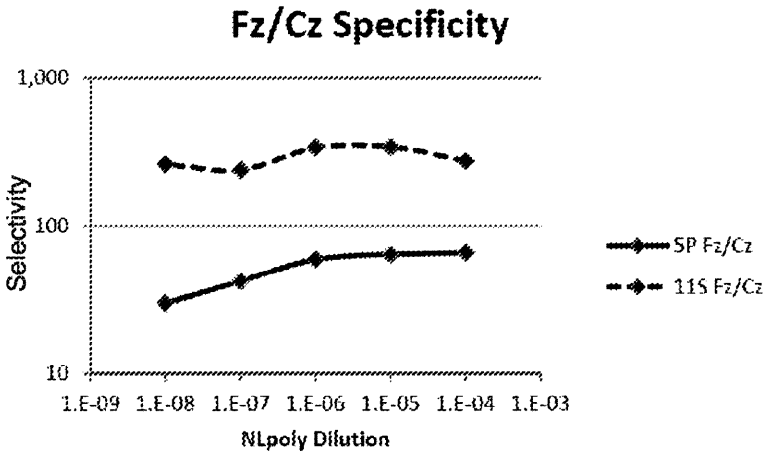


All mutations listed are mutations to 5P

	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

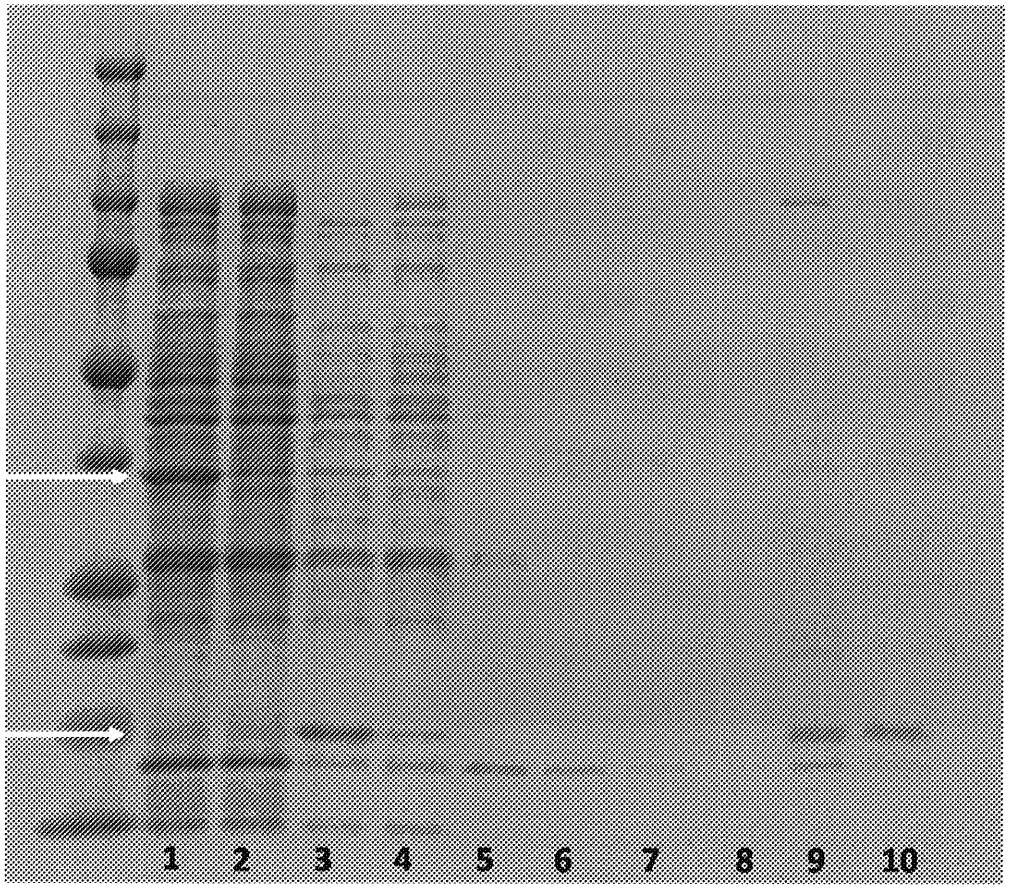




Dilution	5P Fz	11S Fz	5P Cz	11S Cz	5P Fz/Cz	11S Fz/Cz
1.00E-04	4.11E+06	4.87E+07	6.17E+04	1.77E+05	6.66E+01	2.74E+02
1.00E-05	4.05E+05	5.87E+06	6.30E+03	1.71E+04	6.44E+01	3.43E+02
1.00E-06	4.75E+04	6.62E+05	7.98E+02	1.96E+03	5.95E+01	3.38E+02
1.00E-07	6.57E+03	1.26E+05	1.56E+02	5.26E+02	4.21E+01	2.40E+02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

SUBSTITUTE SHEET (RULE 26)

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

SUBSTITUTE SHEET (RULE 26)

Figure 95

NLpoly: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

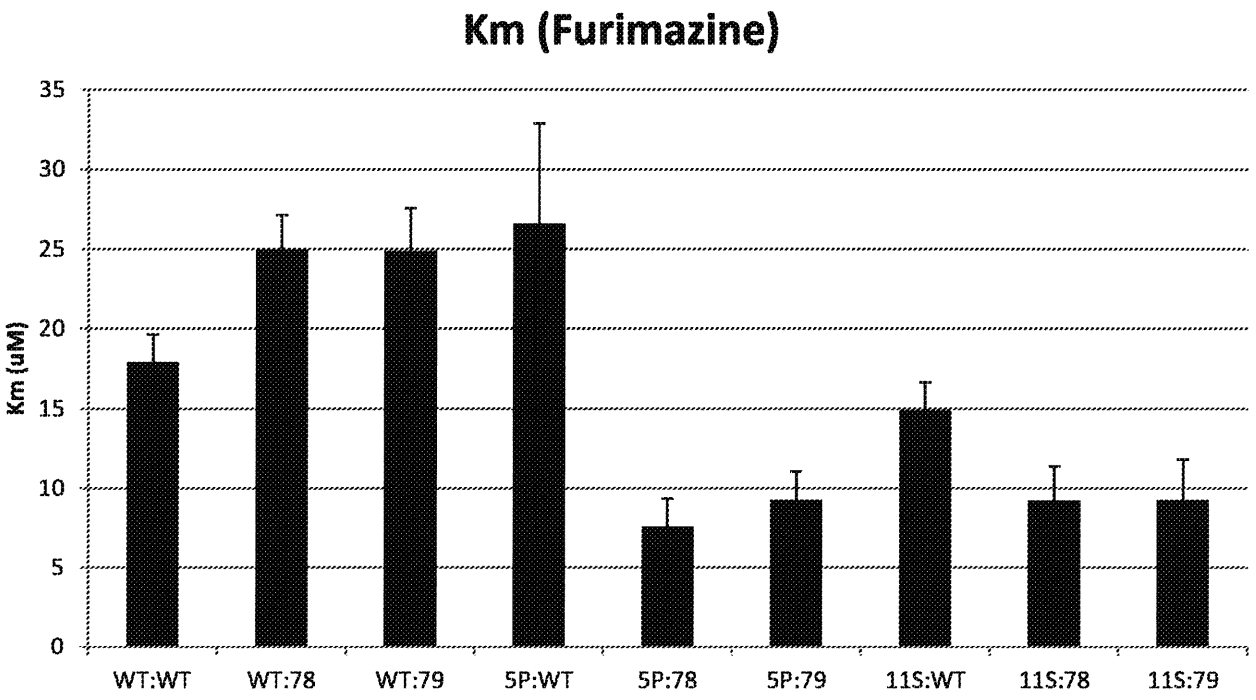
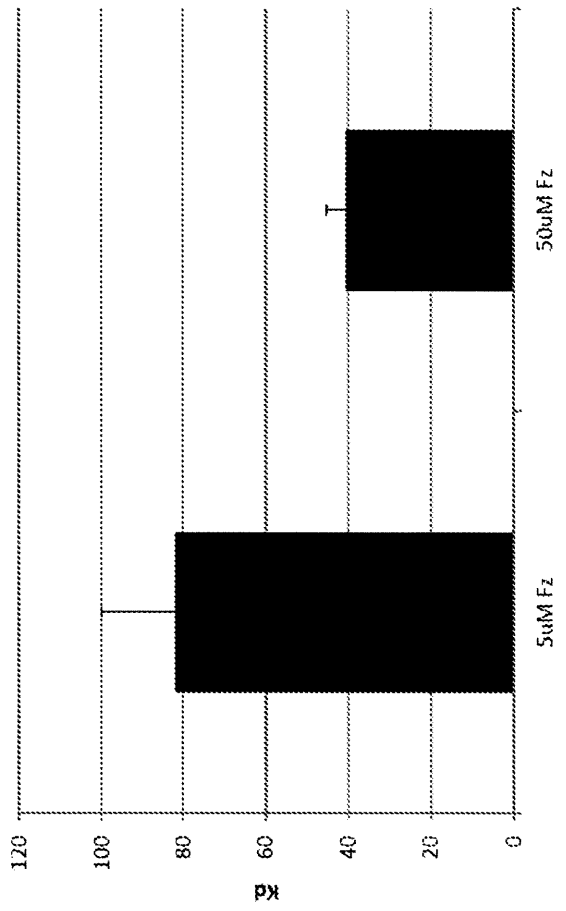
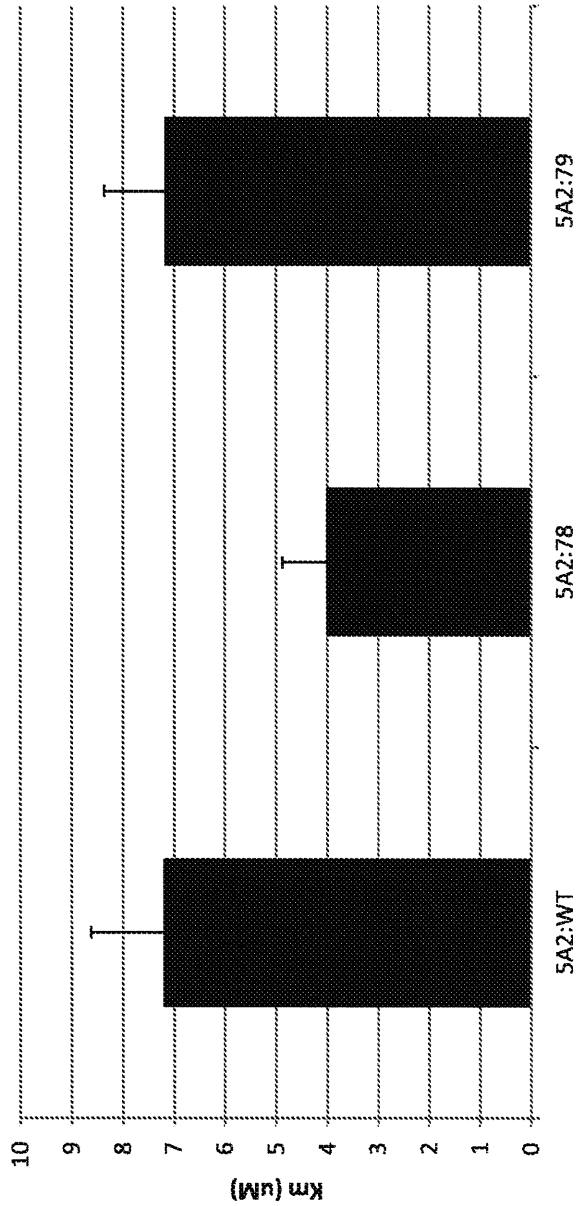

Km (Furimazine)
Km (uM)
35
30
25
20
15
10
5
0
WT:WT
WT:78
WT:79
5P:WT
5P:78
5P:79
11S:WT
11S:78
11S:79

Figure 97



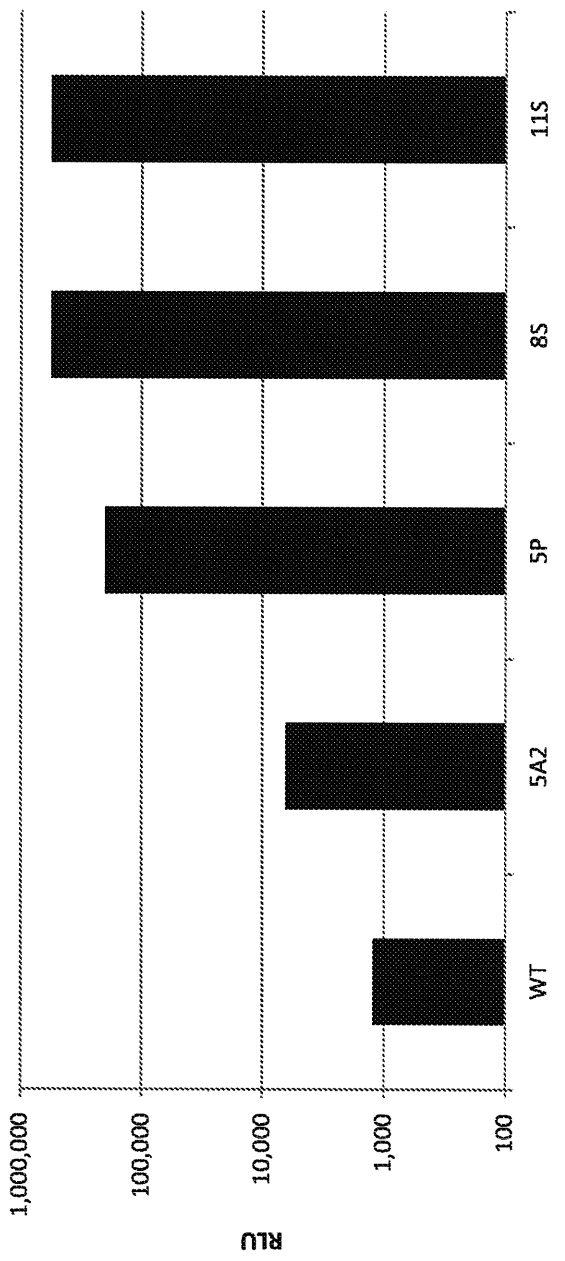
SUBSTITUTE SHEET (RULE 26)

Figure 98



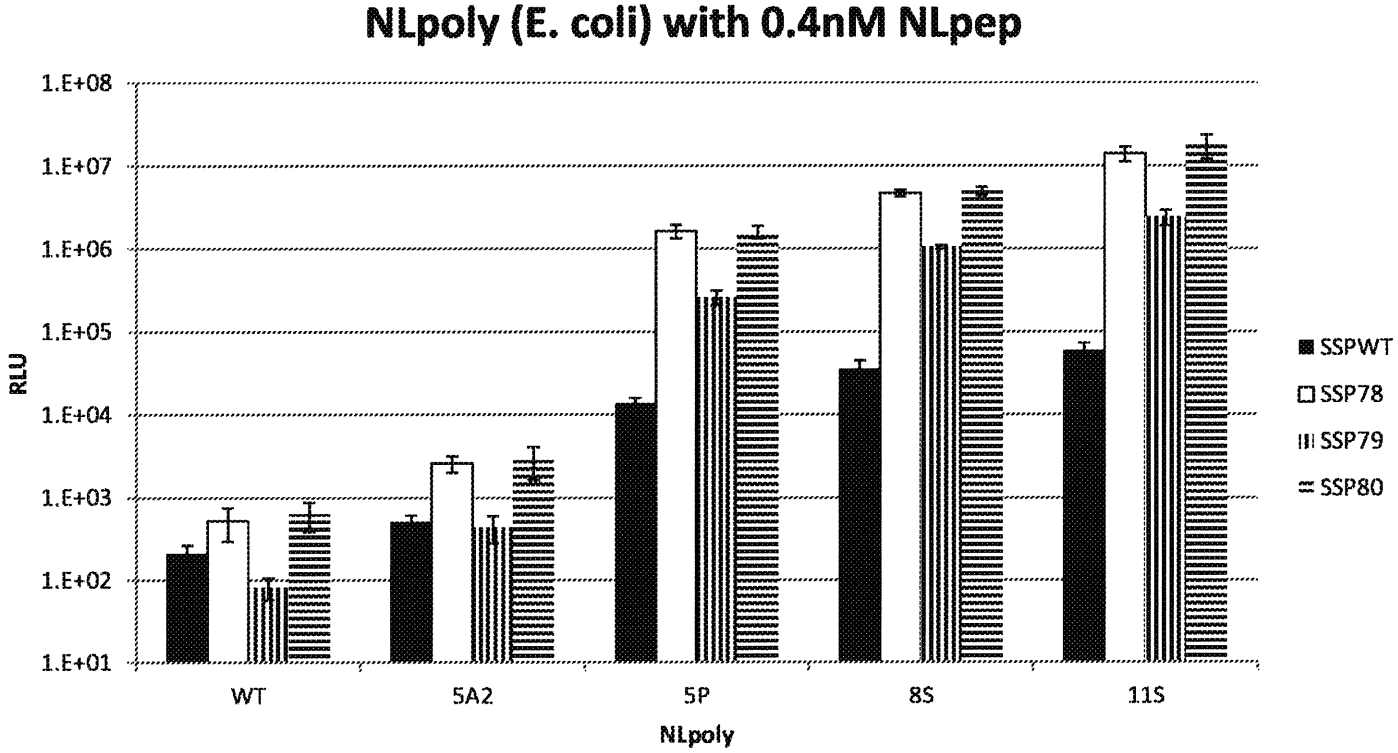
SUBSTITUTE SHEET (RULE 26)

Figure 99



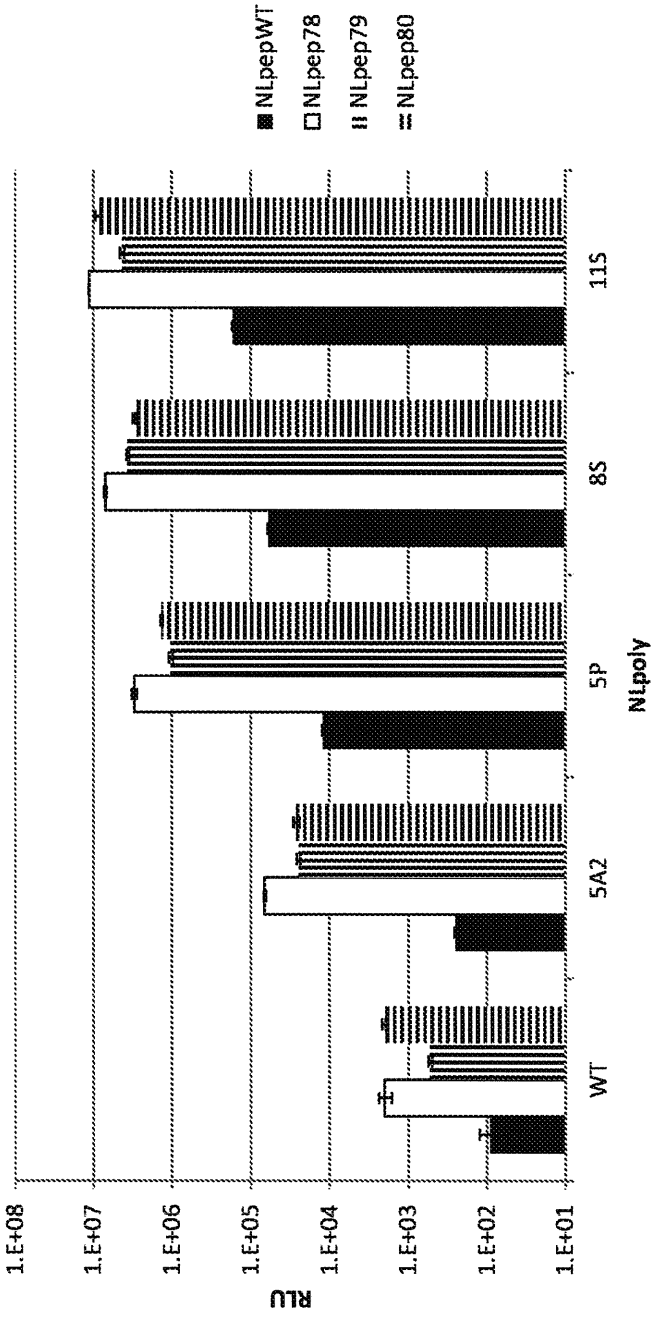
SUBSTITUTE SHEET (RULE 26)

Figure 100



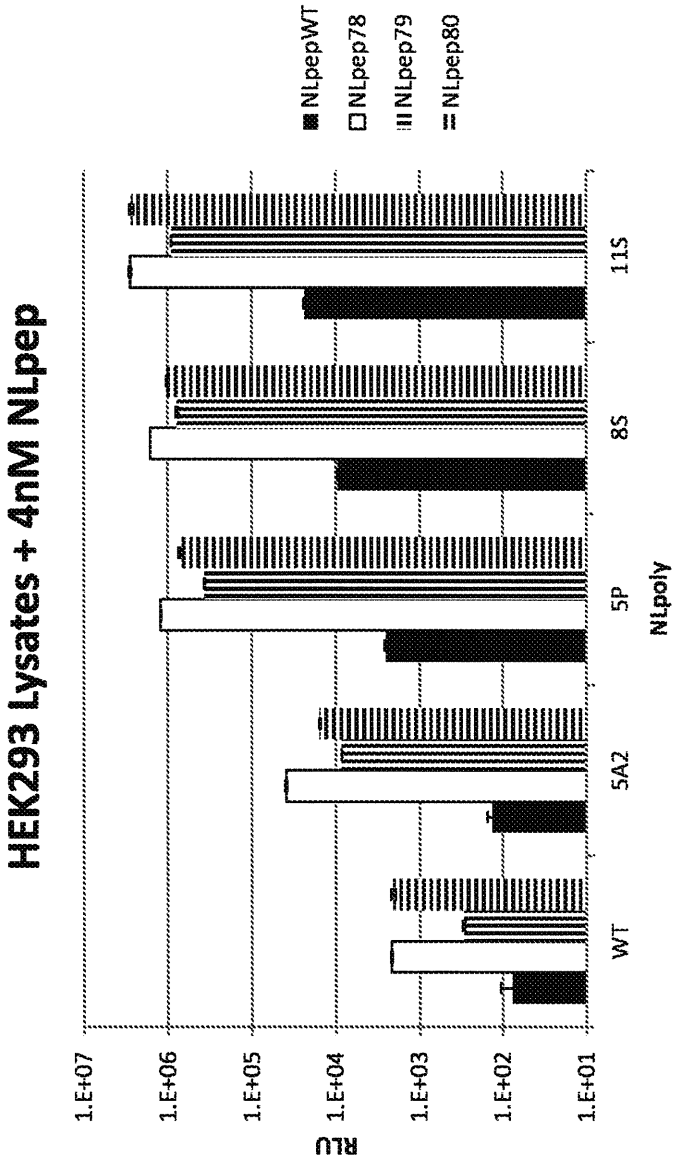
SUBSTITUTE SHEET (RULE 26)

Figure 101



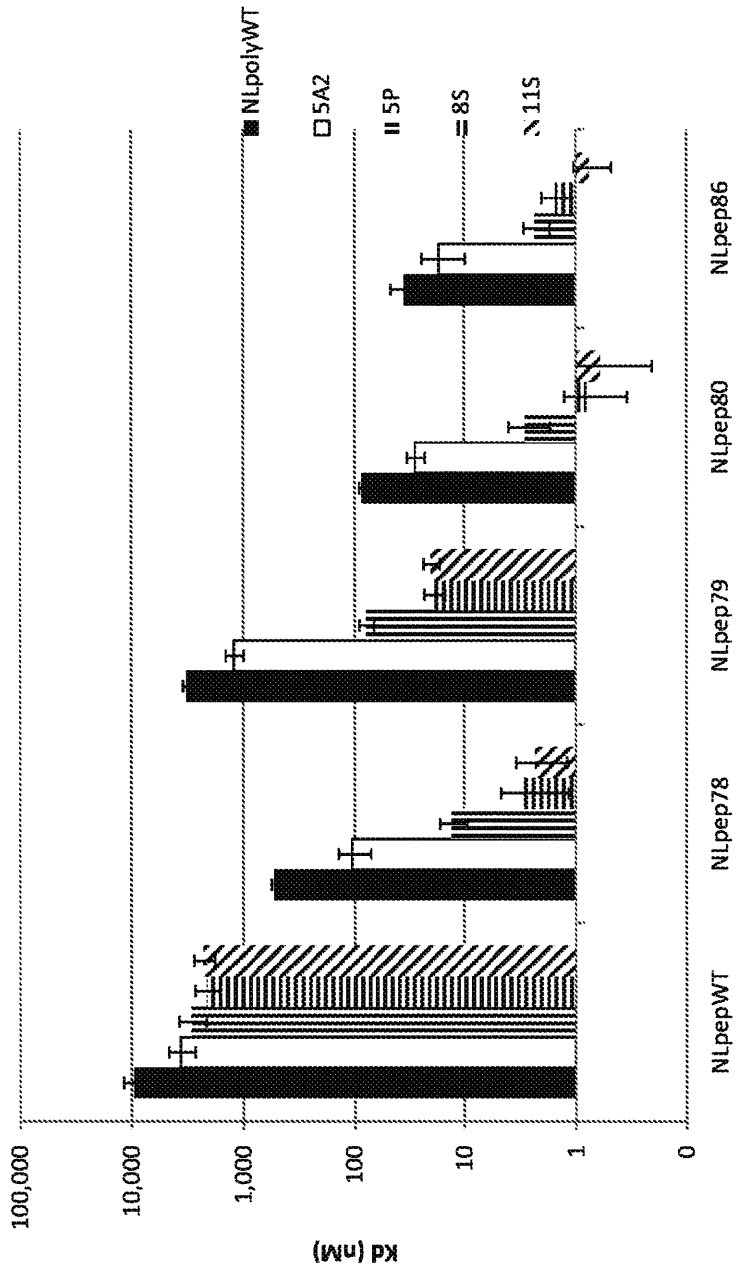
SUBSTITUTE SHEET (RULE 26)

Figure 102



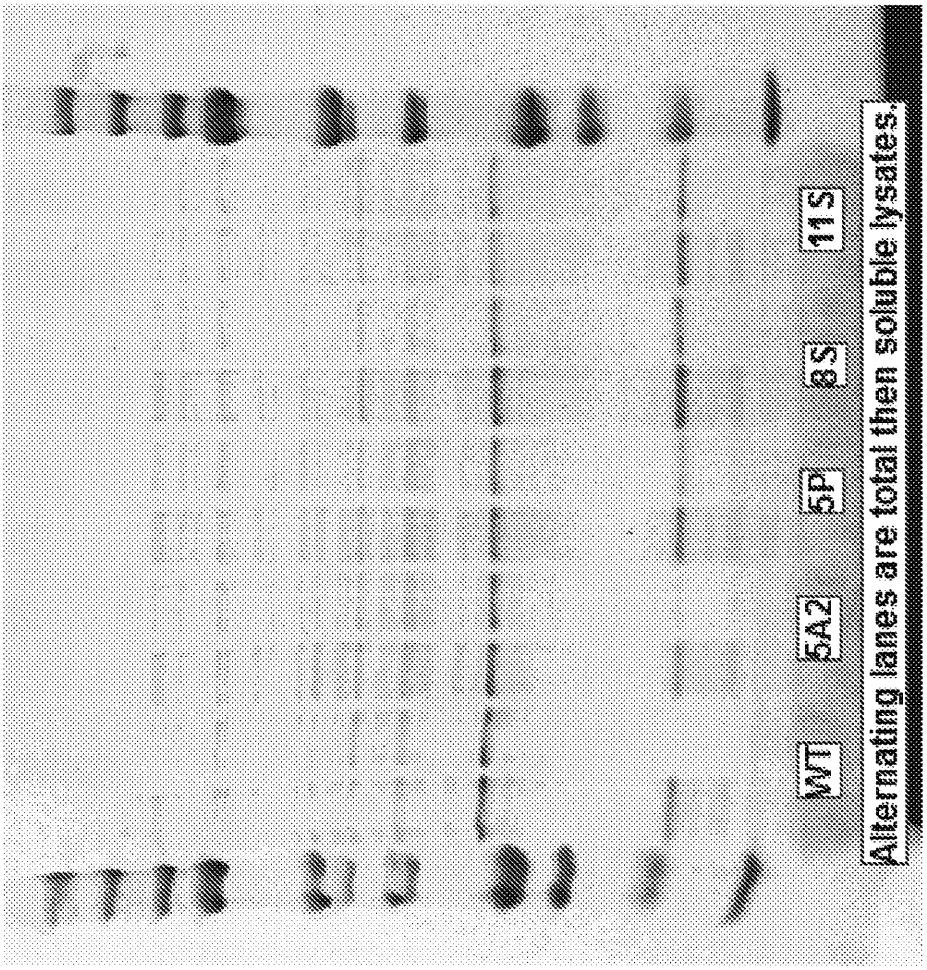
SUBSTITUTE SHEET (RULE 26)

Figure 103



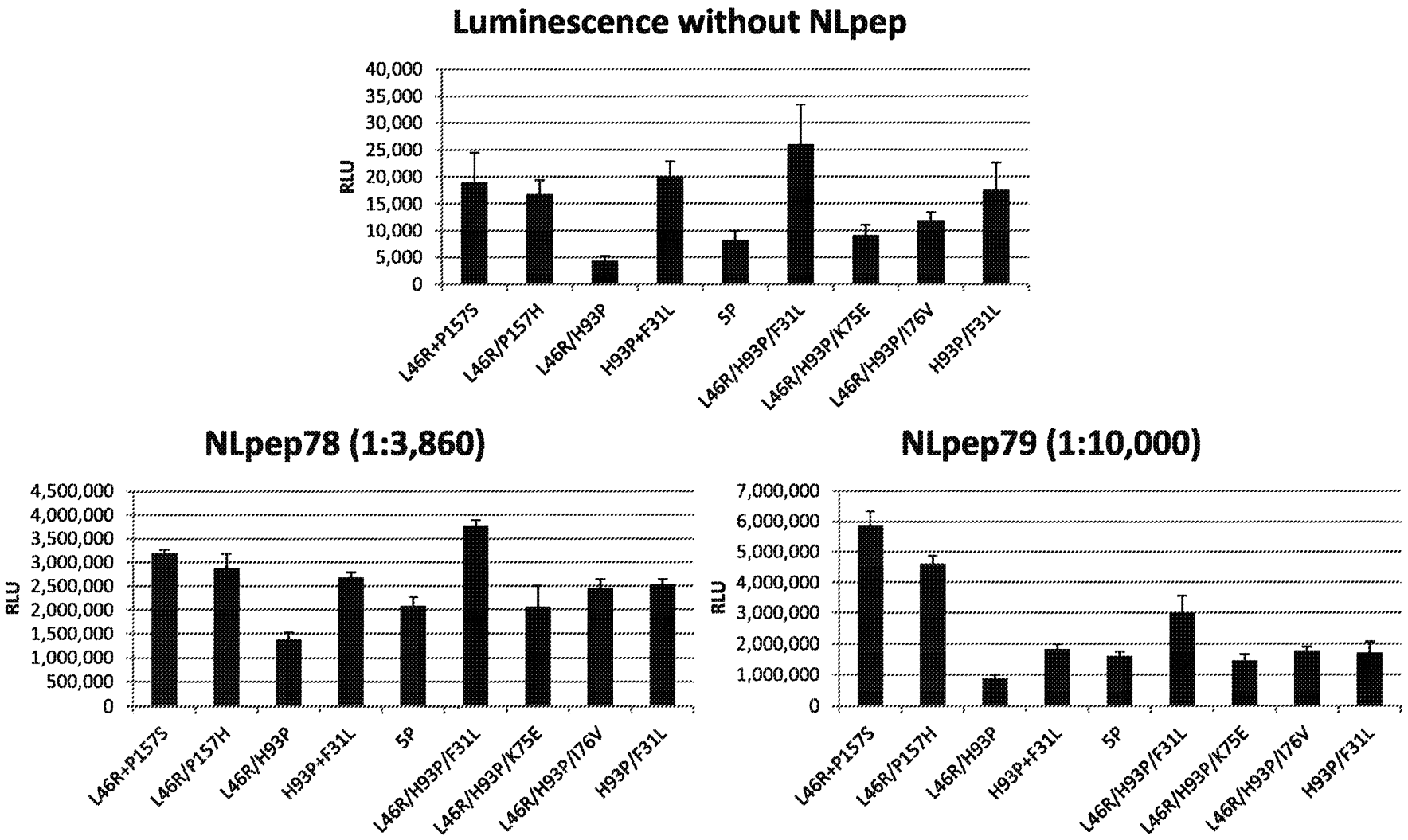
SUBSTITUTE SHEET (RULE 26)

Figure 104



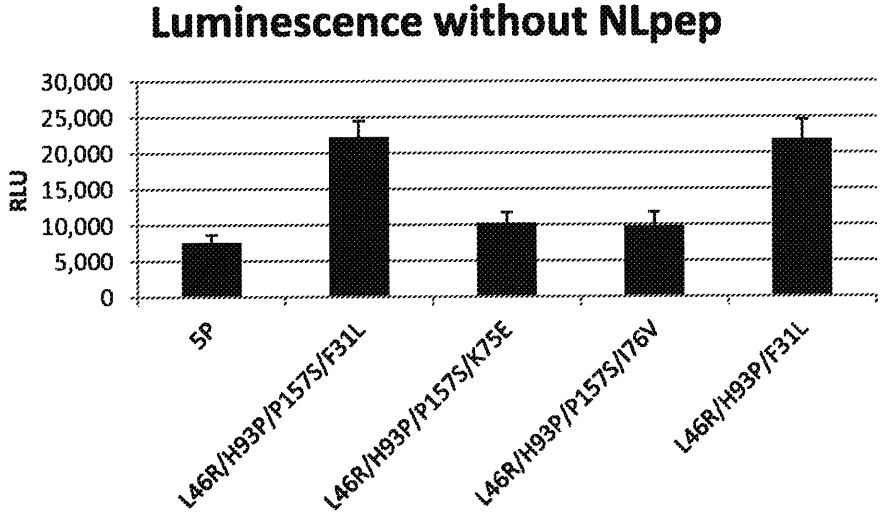
SUBSTITUTE SHEET (RULE 26)

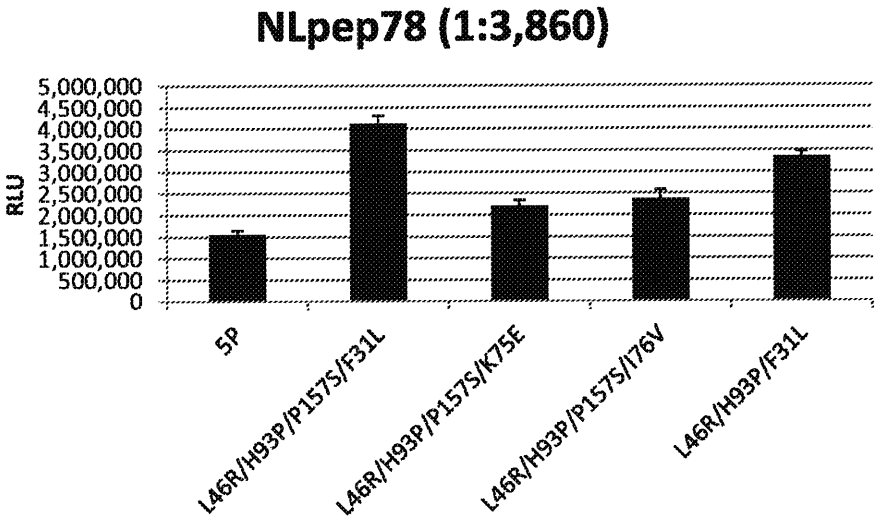
Figure 105

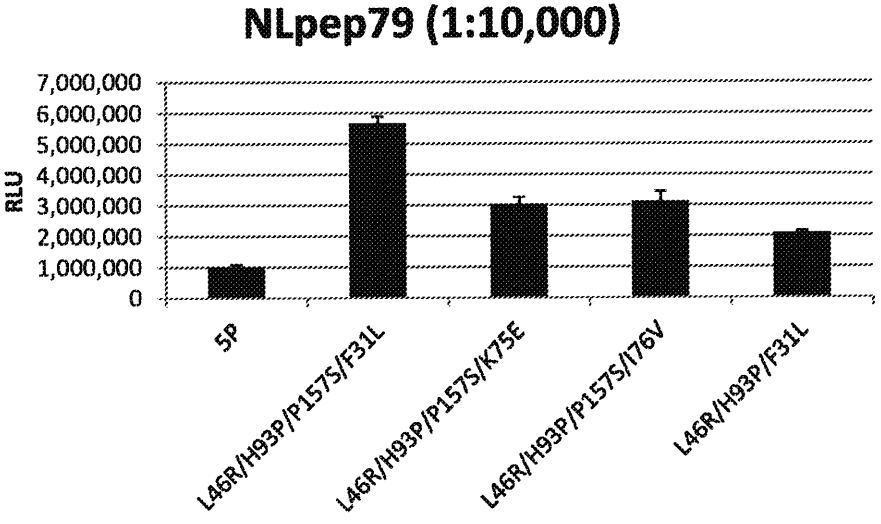

Luminescence without NLpep
RLU
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
L46R+P157S
L46R/P157H
L46R/H93P
H93P+F31L
5P
L46R/H93P/F31L
L46R/H93P/K75E
L46R/H93P/I76V
H93P/F31L
NLpep78 (1:3,860)
RLU
4,500,000
4,000,000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
L46R+P157S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

SUBSTITUTE SHEET (RULE 26)

Figure 106







SUBSTITUTE SHEET (RULE 26)

Figure 107

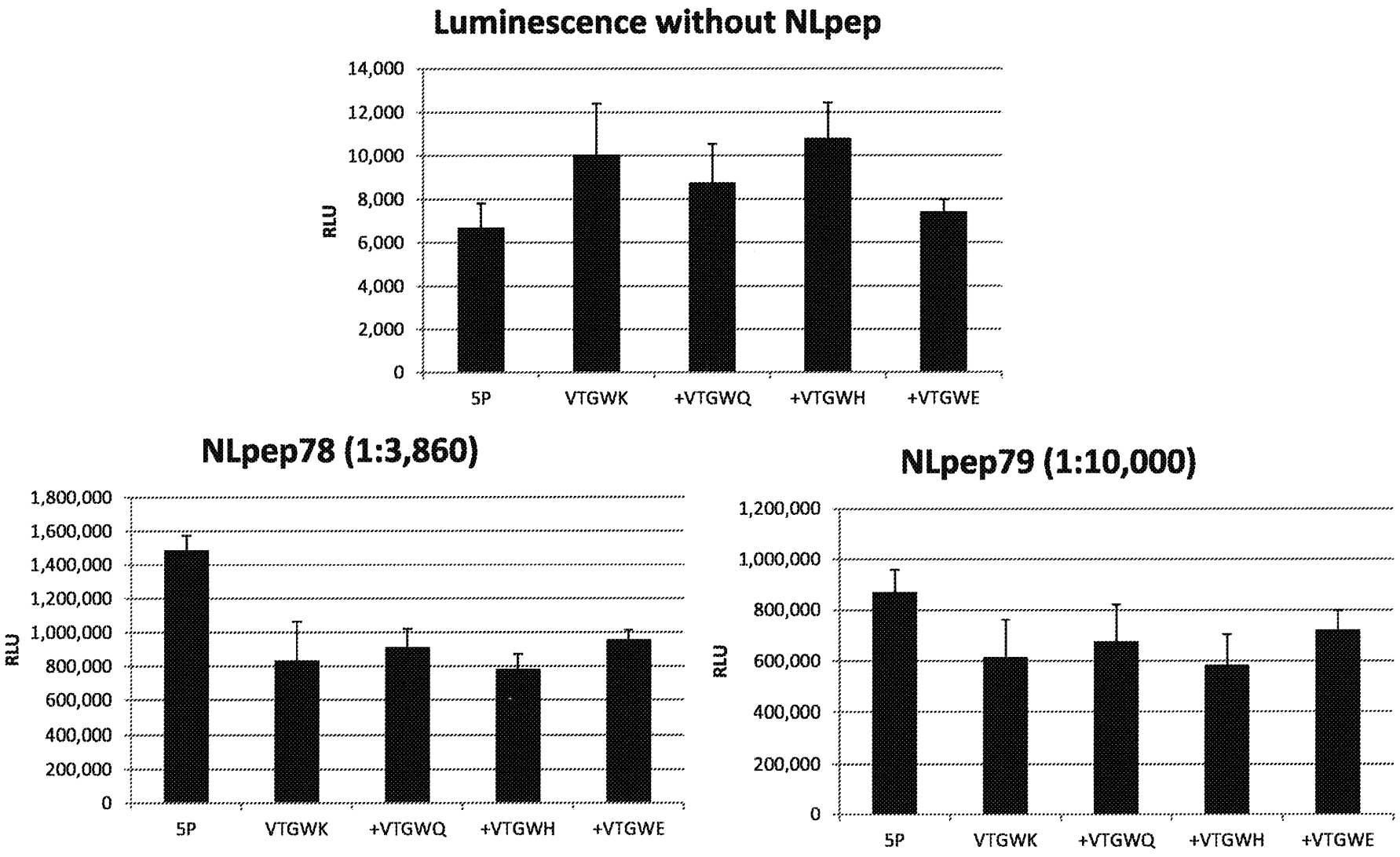
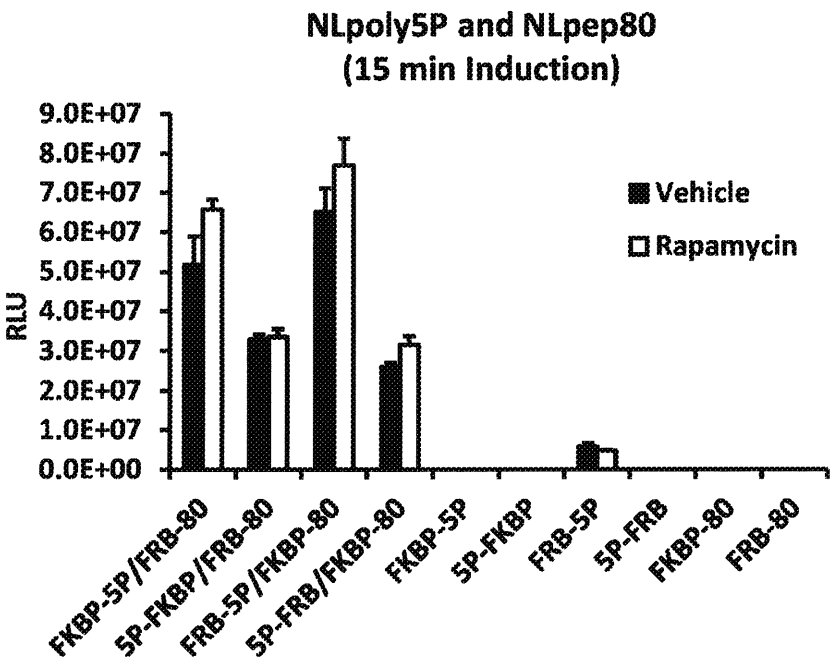
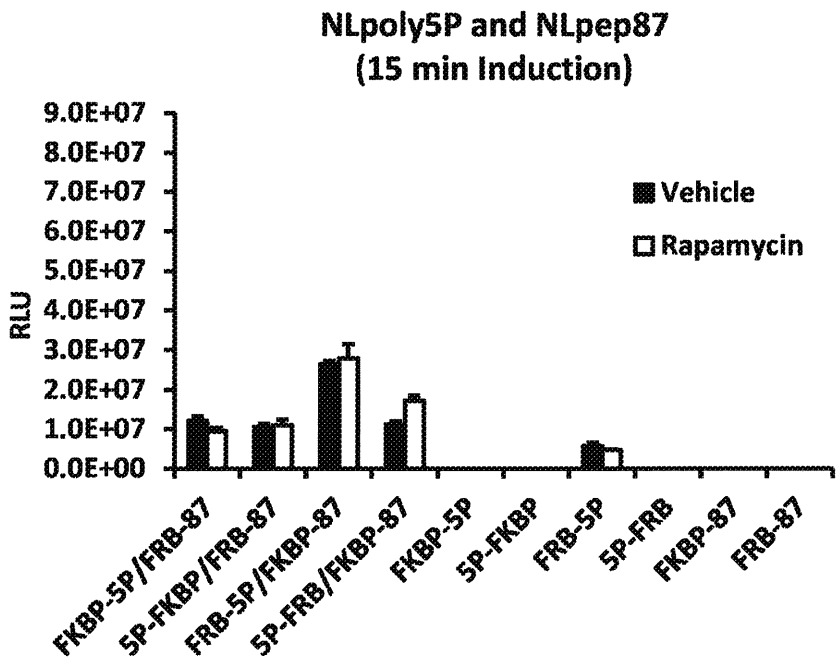


Figure 108



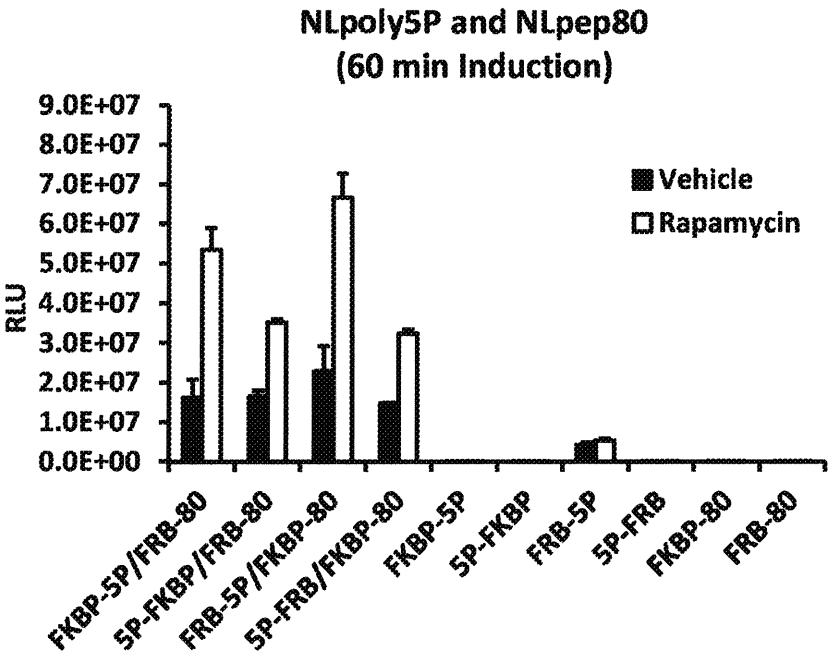


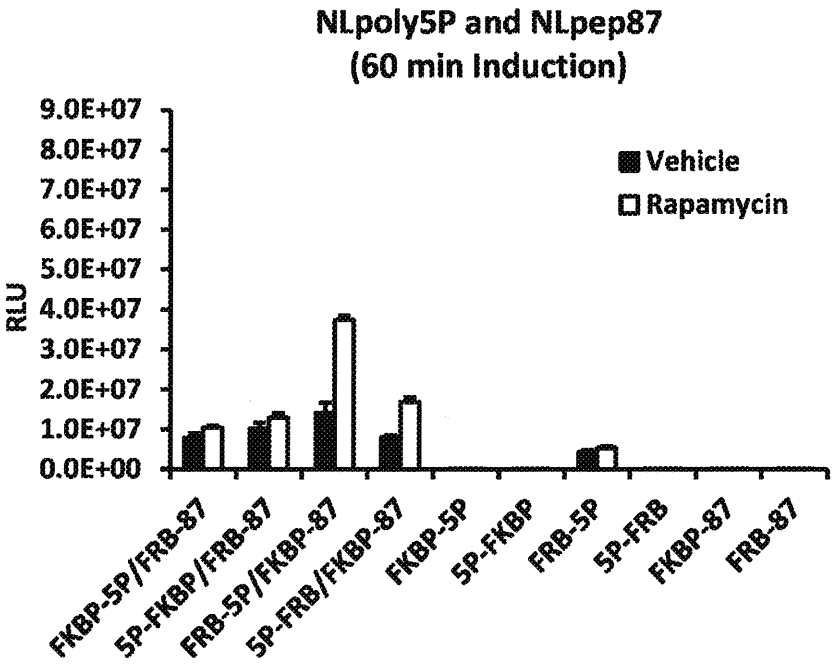
Combination	Fold Induction
FKBP-5P/FRB-80	1.2
5P-FKBP/FRB-80	1.0
FRB-5P/FKBP-80	1.2
5P-FRB/FKBP-80	1.2

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

SUBSTITUTE SHEET (RULE 26)

Figure 109



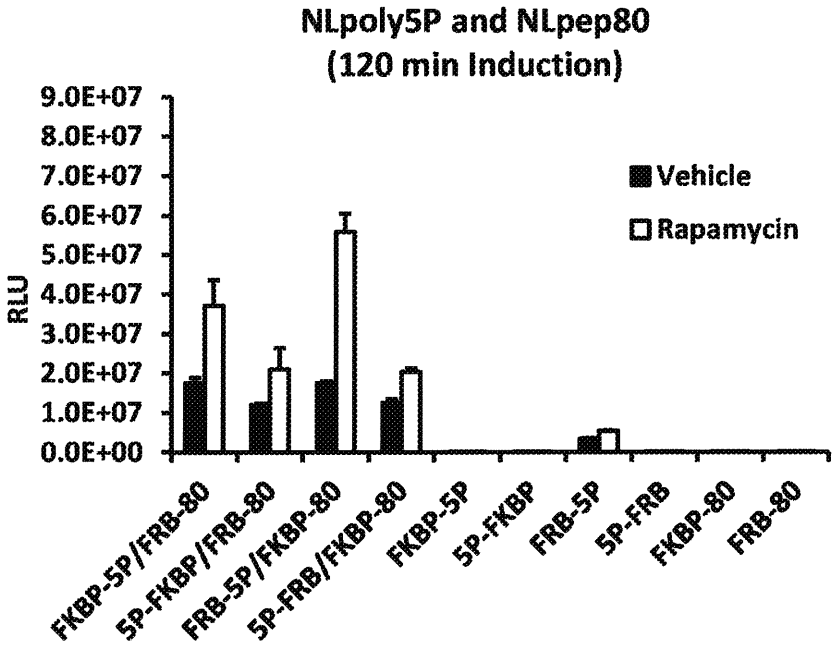


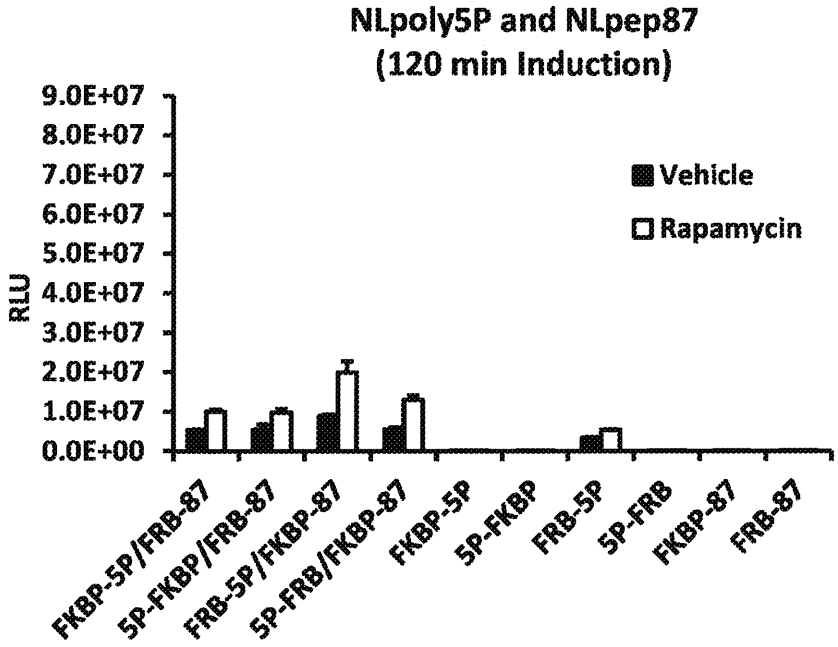
Combination	Fold Induction
FKBP-5P/FRB-80	3.3
5P-FKBP/FRB-80	2.1
FRB-5P/FKBP-80	2.9
5P-FRB/FKBP-80	2.2

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

SUBSTITUTE SHEET (RULE 26)

Figure 110



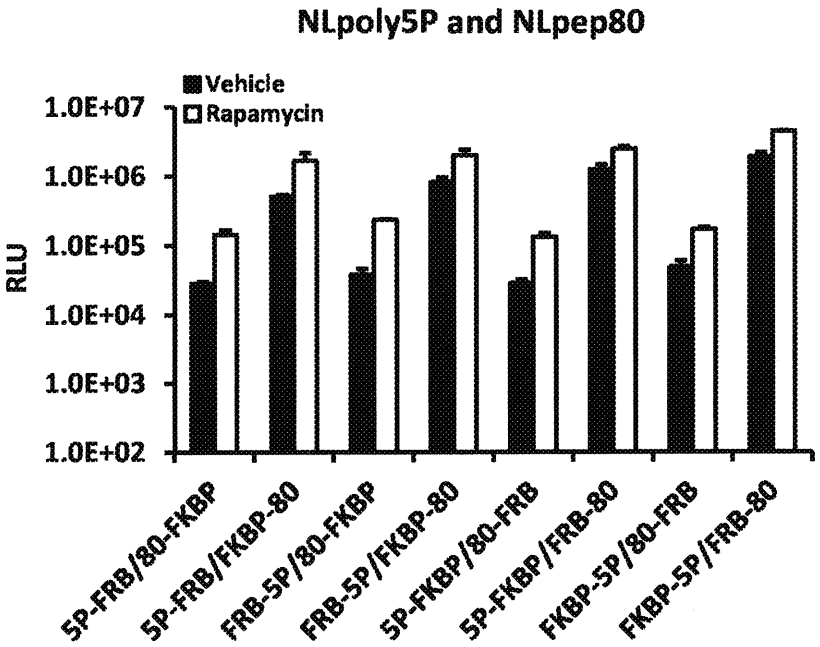


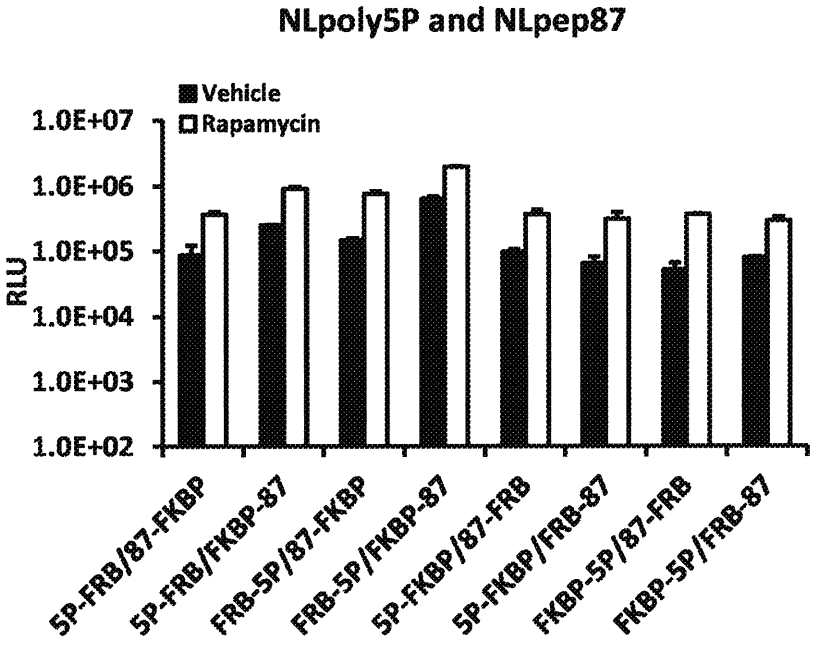
Combination	Fold Induction
FKBP-5P/FRB-80	2.1
5P-FKBP/FRB-80	1.8
FRB-5P/FKBP-80	3.2
5P-FRB/FKBP-80	1.6

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

SUBSTITUTE SHEET (RULE 26)

Figure 111



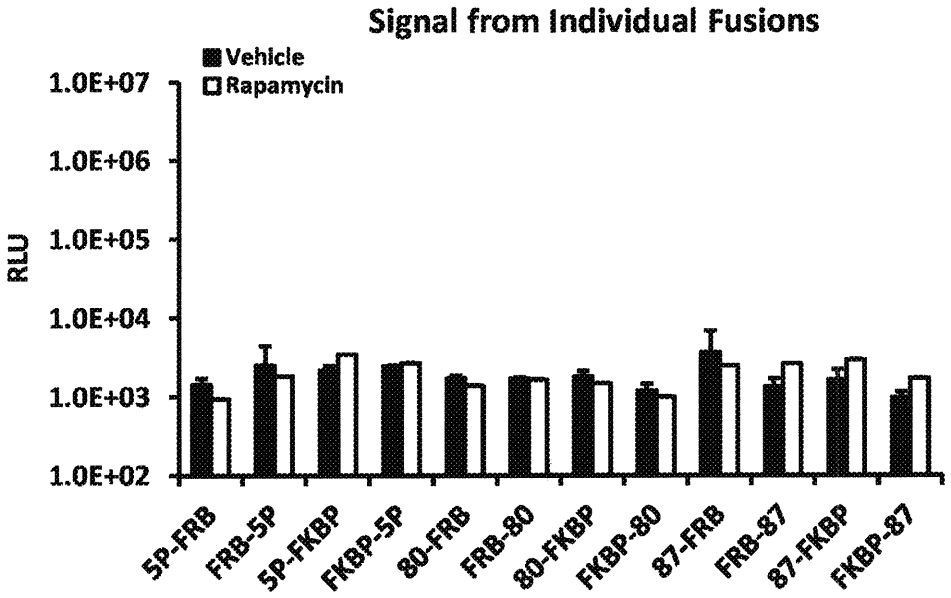


Configuration	Fold Induction
5P-FRB/80-FKBP	5.1
5P-FRB/FKBP-80	3.3
FRB-5P/80-FKBP	6.1
FRB-5P/FKBP-80	2.4
5P-FKBP/80-FRB	4.6
5P-FKBP/FRB-80	2.0
FKBP-5P/80-FRB	3.5
FKBP-5P/FRB-80	2.3

Configuration	Fold Induction
5P-FRB/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

SUBSTITUTE SHEET (RULE 26)

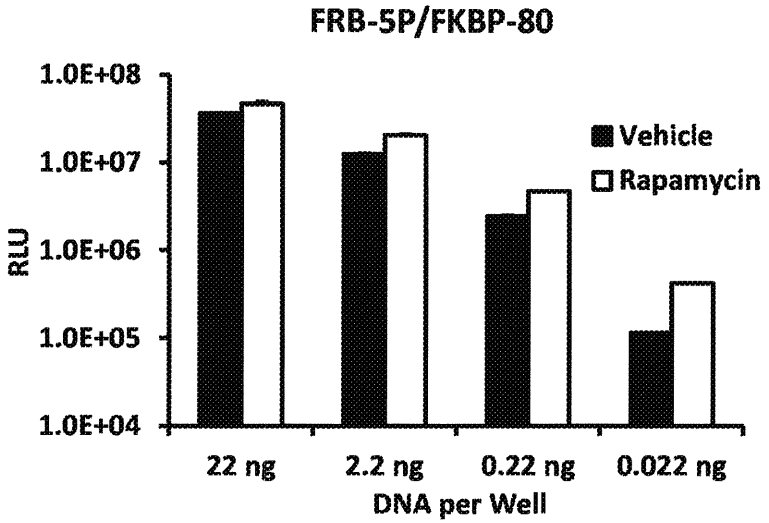
Figure 112



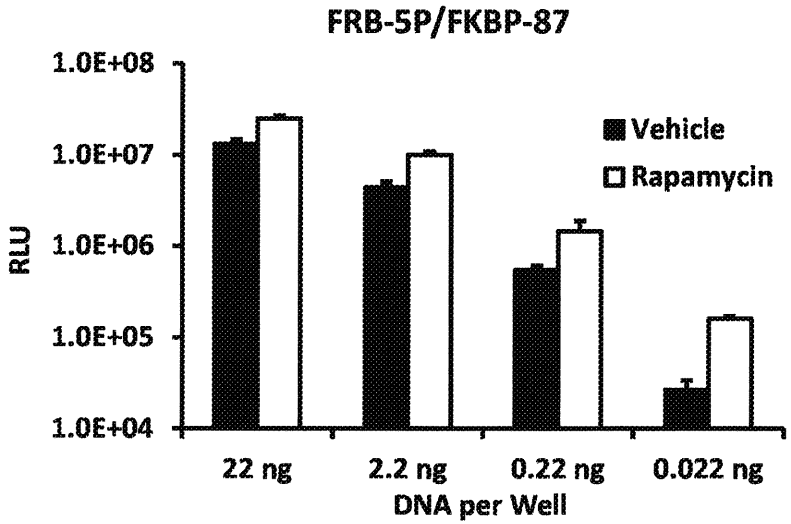
Configuration	Fold Induction
5P-FRB	0.7
FRB-5P	0.7
5P-FKBP	1.6
FKBP-5P	1.1
80-FRB	0.8
FRB-80	1.0
80-FKBP	0.8
FKBP-80	0.8
87-FRB	0.7
FRB-87	2.0
87-FKBP	1.8
FKBP-87	1.8

SUBSTITUTE SHEET (RULE 26)

Figure 113



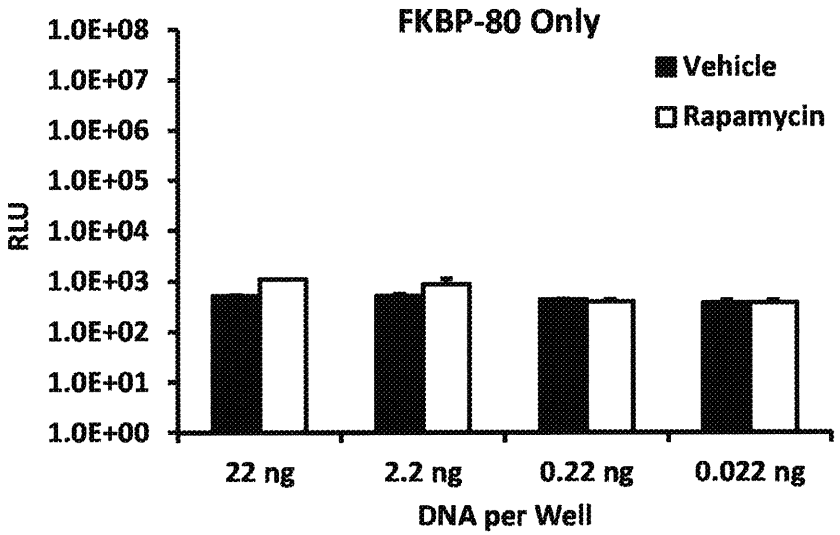
DNA per Well	Fold Induction
22 ng	1.3
2.2 ng	1.7
0.22 ng	1.9
0.022 ng	3.7

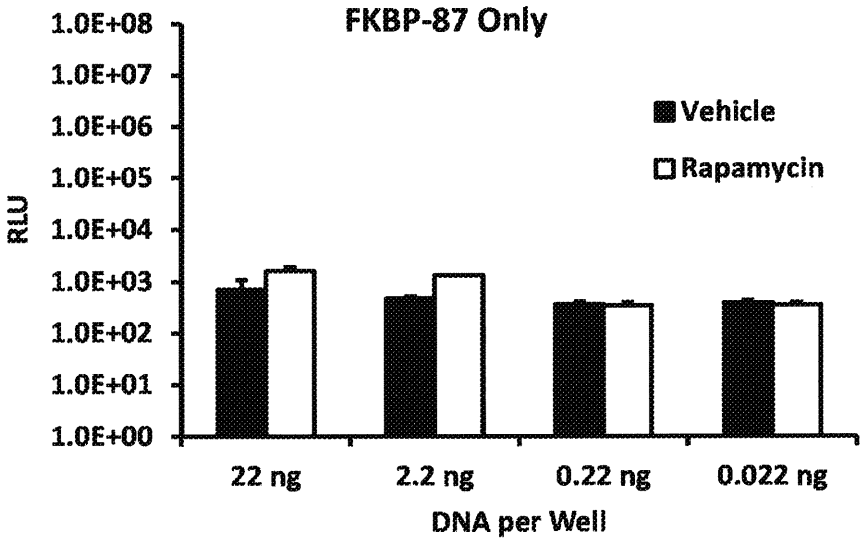


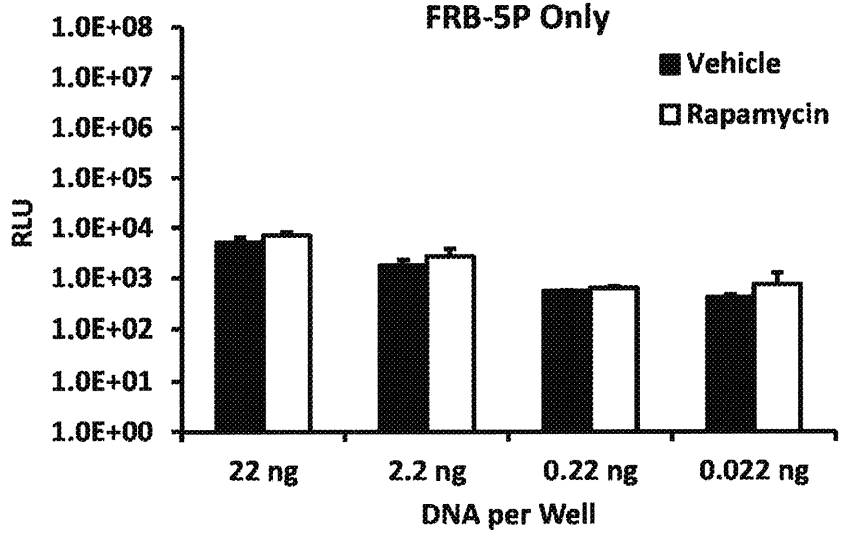
DNA per Well	Fold Induction
22 ng	1.9
2.2 ng	2.2
0.22 ng	2.7
0.022 ng	6.0

SUBSTITUTE SHEET (RULE 26)

Figure 114

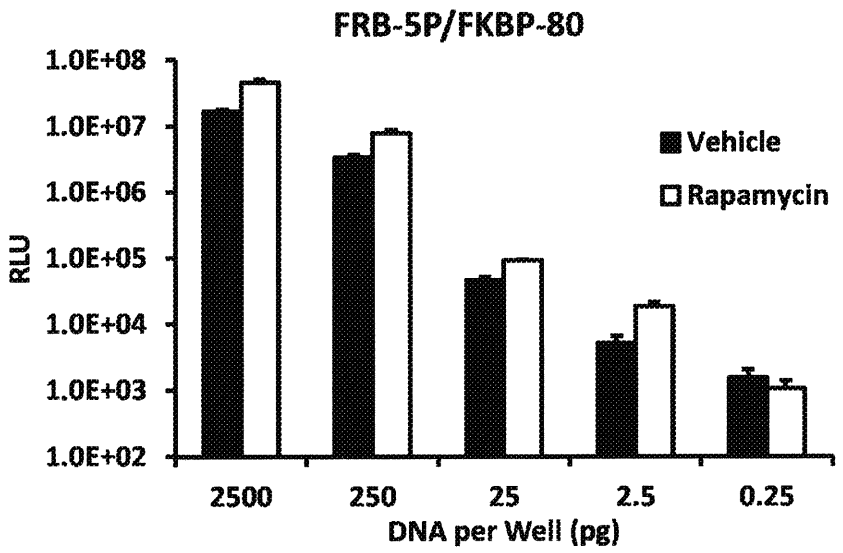


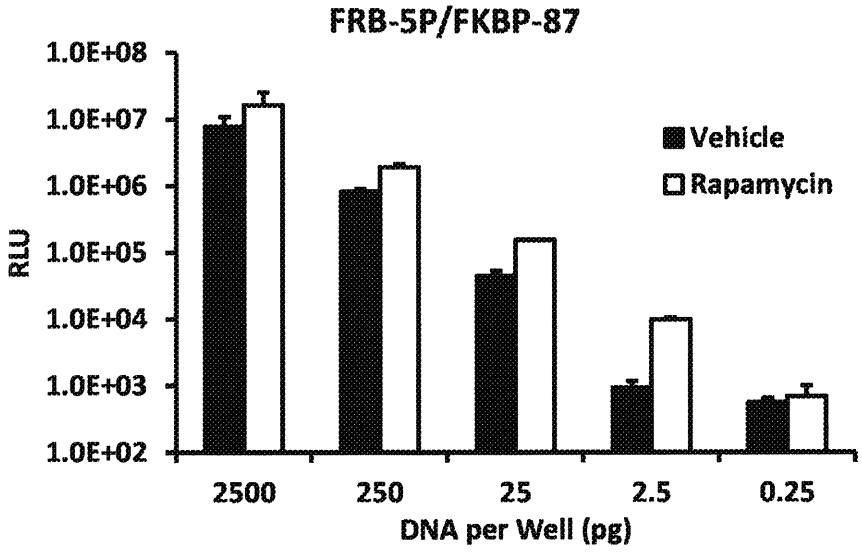




SUBSTITUTE SHEET (RULE 26)

Figure 115



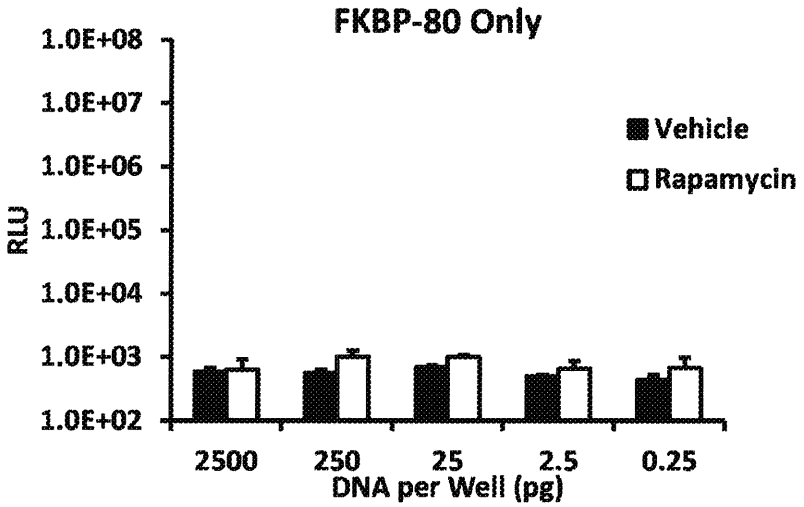


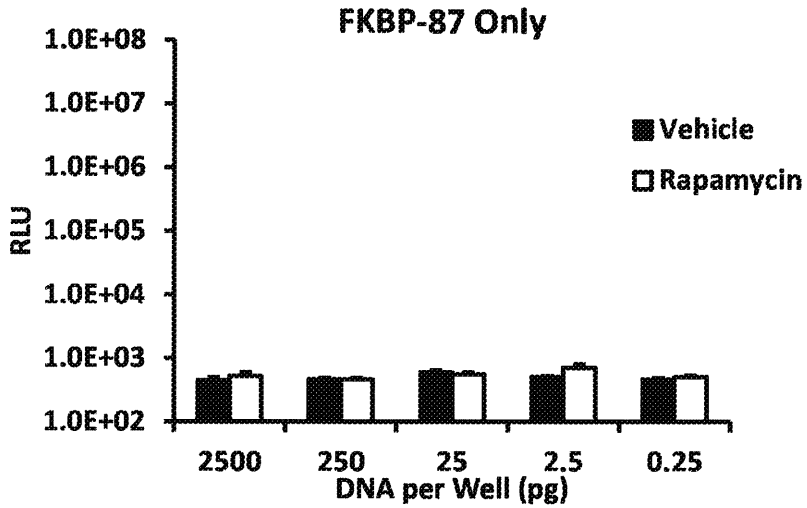
DNA per Well (pg)	Fold Induction
2500	2.7
250	2.3
25	2.0
2.5	3.6
0.25	0.7

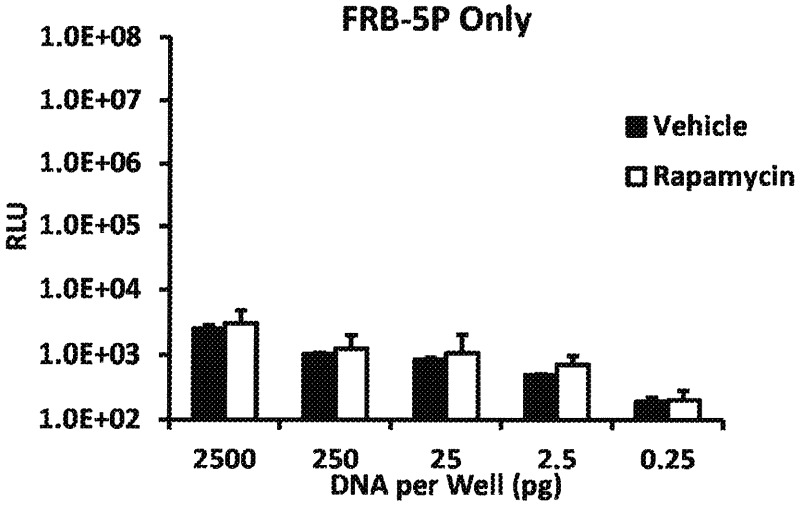
DNA per Well (pg)	Fold Induction
2500	2.1
250	2.4
25	3.5
2.5	10.6
0.25	1.2

SUBSTITUTE SHEET (RULE 26)

Figure 116

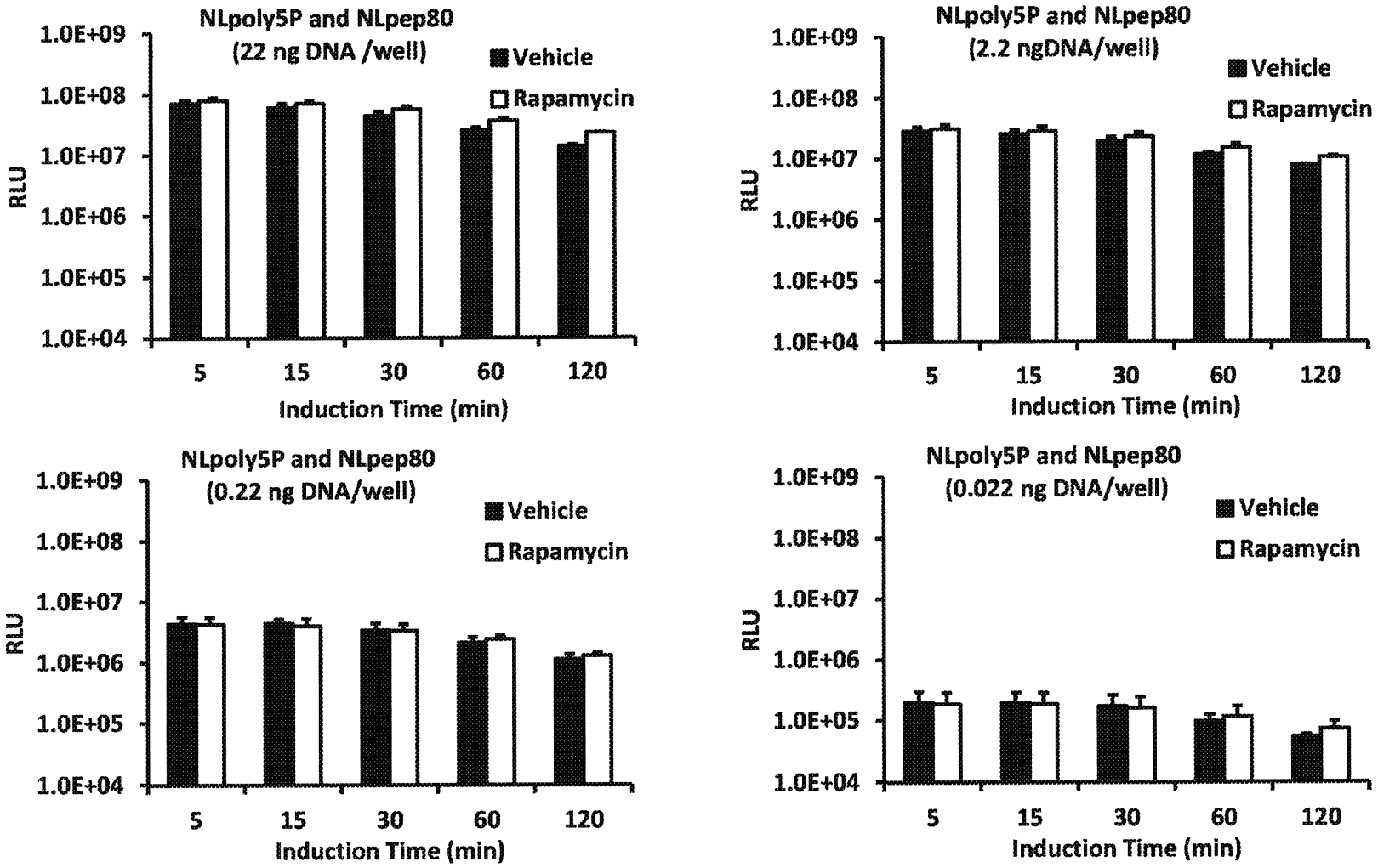






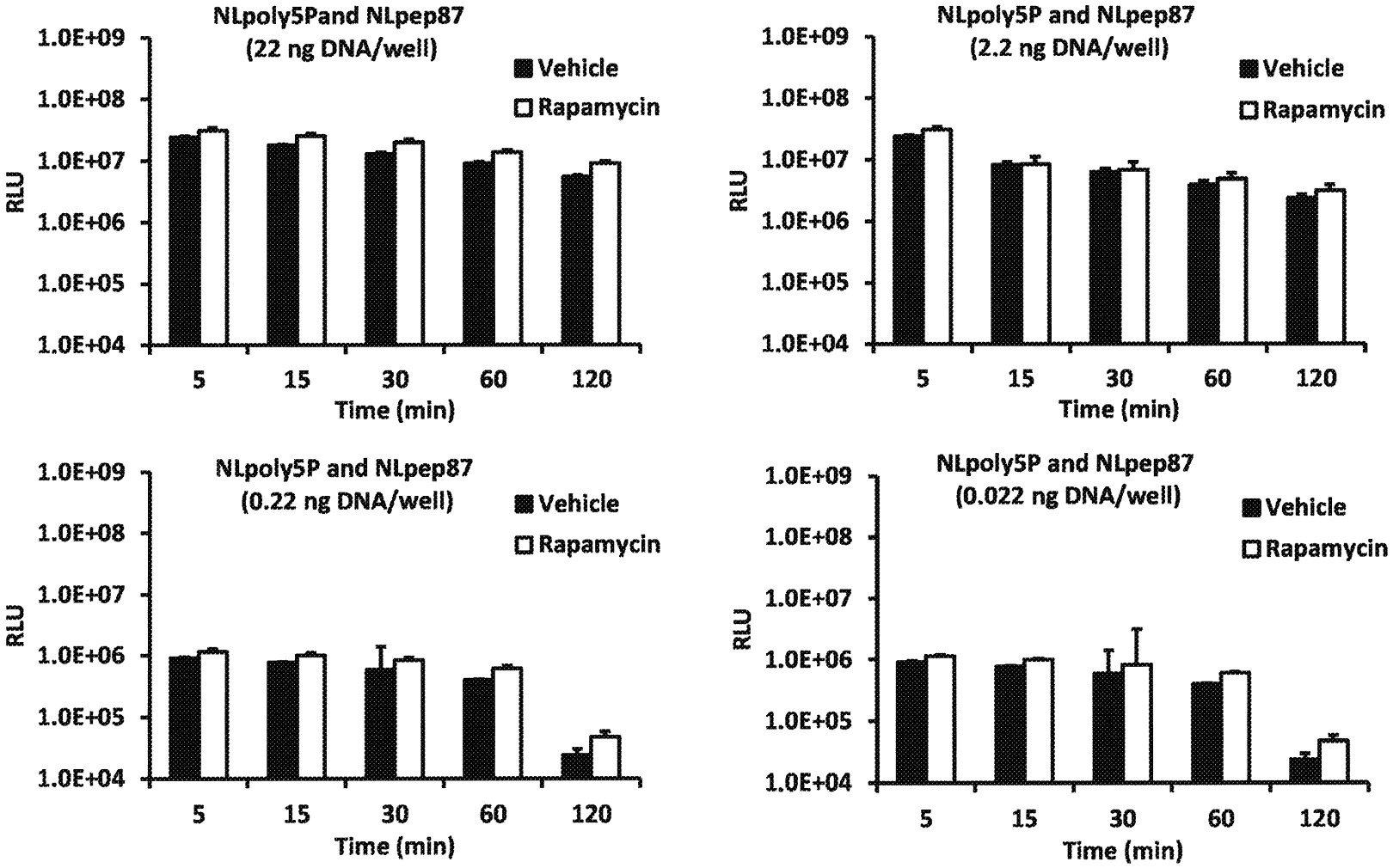
SUBSTITUTE SHEET (RULE 26)

Figure 117



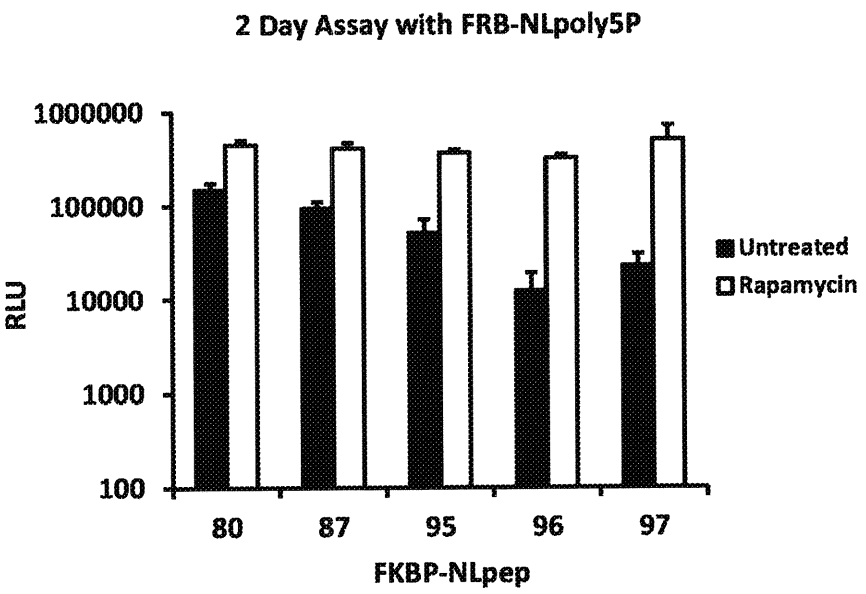
SUBSTITUTE SHEET (RULE 26)

Figure 118

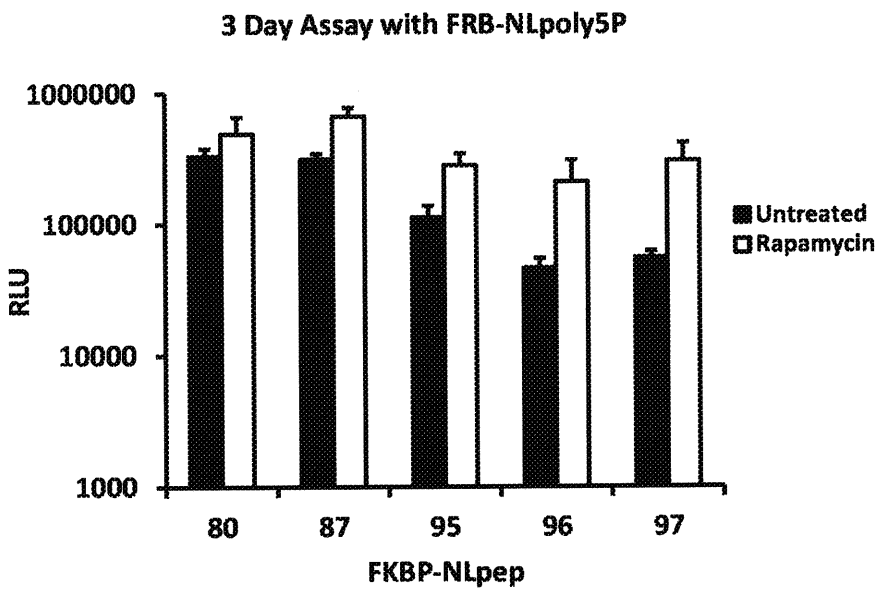


SUBSTITUTE SHEET (RULE 26)

Figure 119



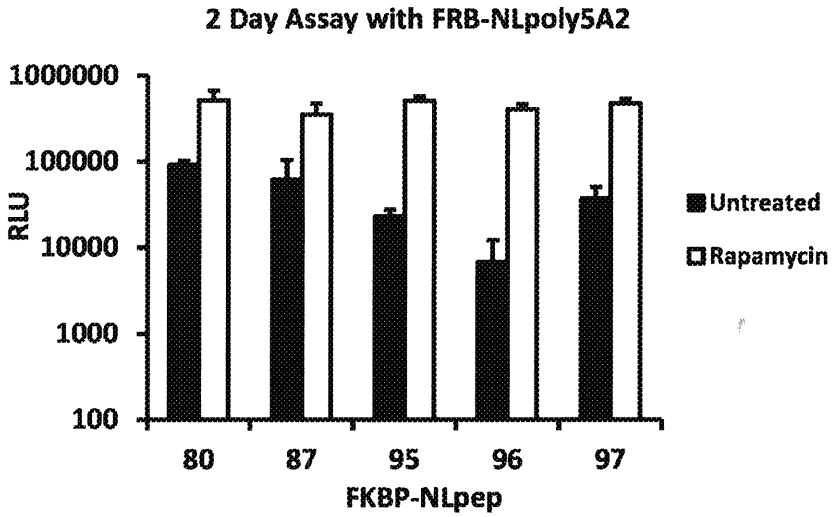
NLpep	Fold Induction
80	3.1
87	4.4
95	7.2
96	26.1
97	22.5



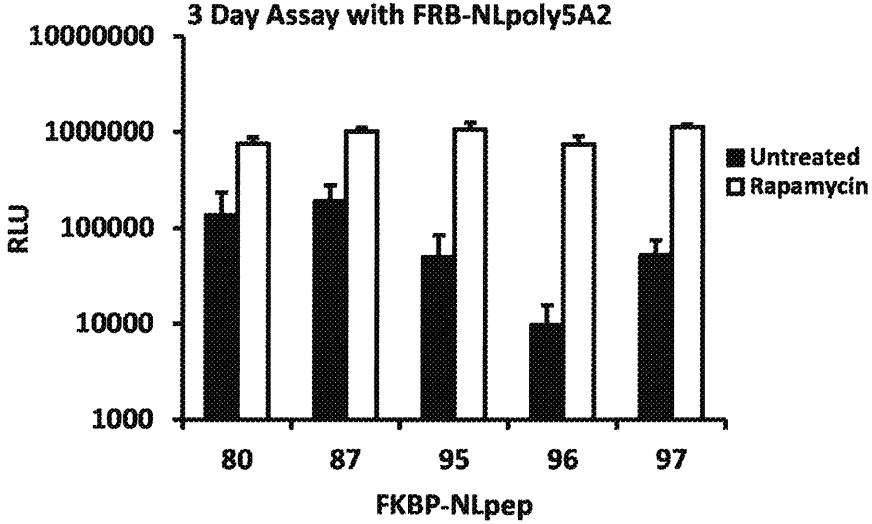
NLpep	Fold Induction
80	1.5
87	2.1
95	2.5
96	4.5
97	5.5

SUBSTITUTE SHEET (RULE 26)

Figure 120

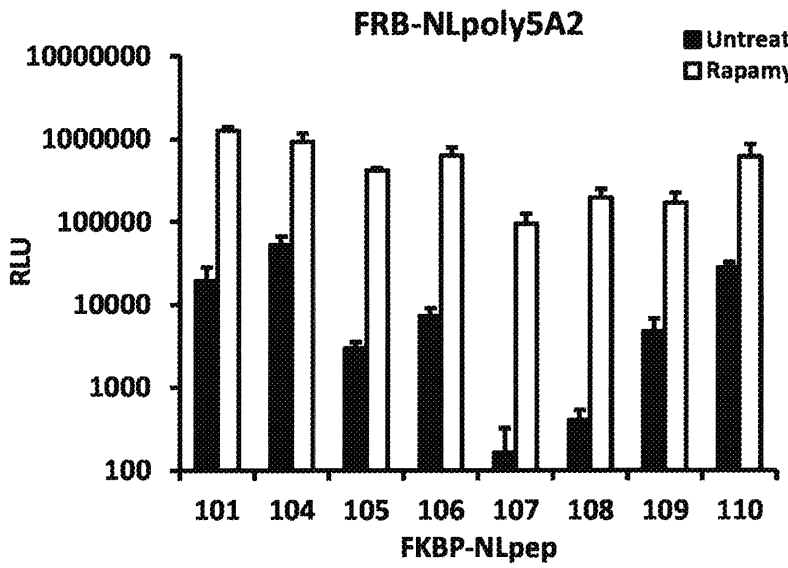


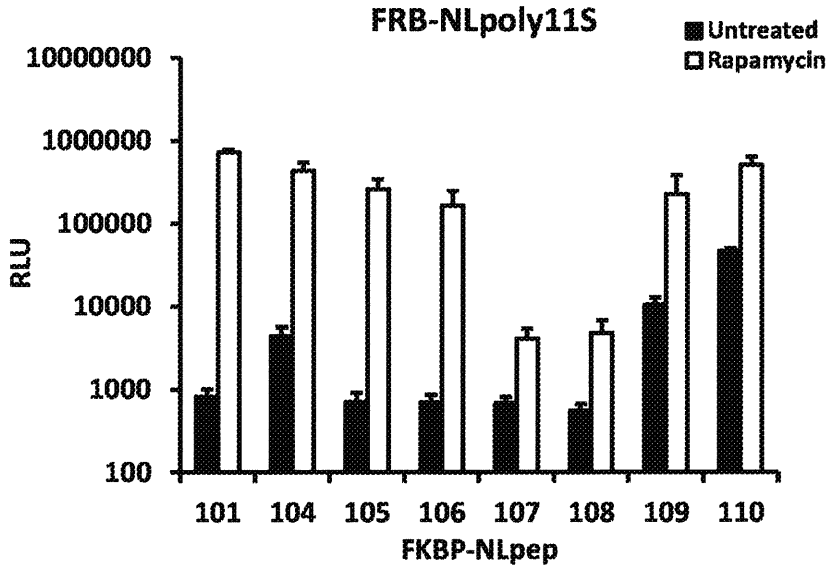
NLpep	Fold Induction
80	5.5
87	5.7
95	22.1
96	60.0
97	12.7



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

Figure 121



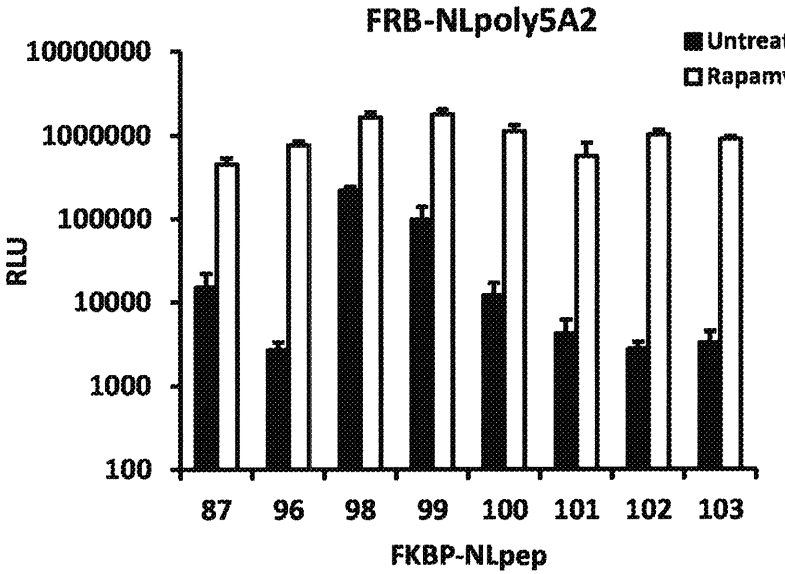


NLpep	Fold Induction
101	65
104	18
105	140
106	86
107	578
108	483
109	36
110	22

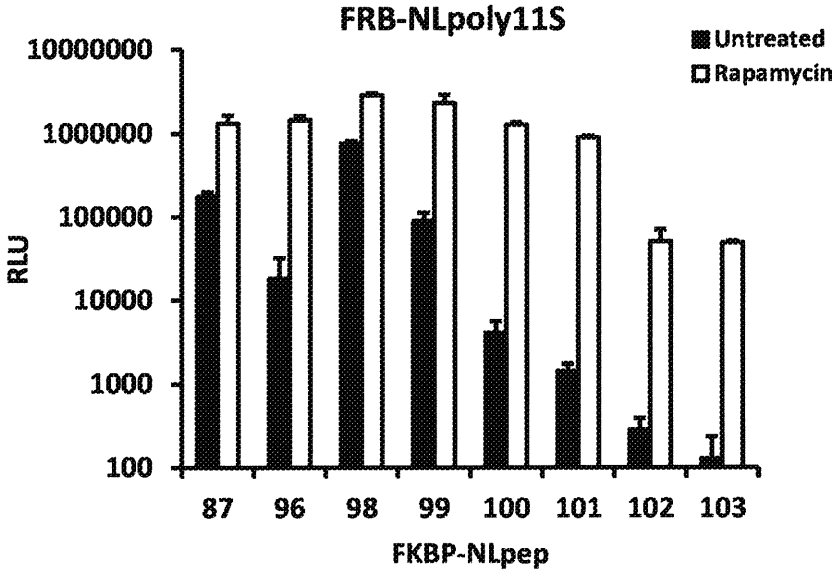
NLpep	Fold Induction
101	882
104	101
105	372
106	234
107	6
108	9
109	22
110	11

SUBSTITUTE SHEET (RULE 26)

Figure 122



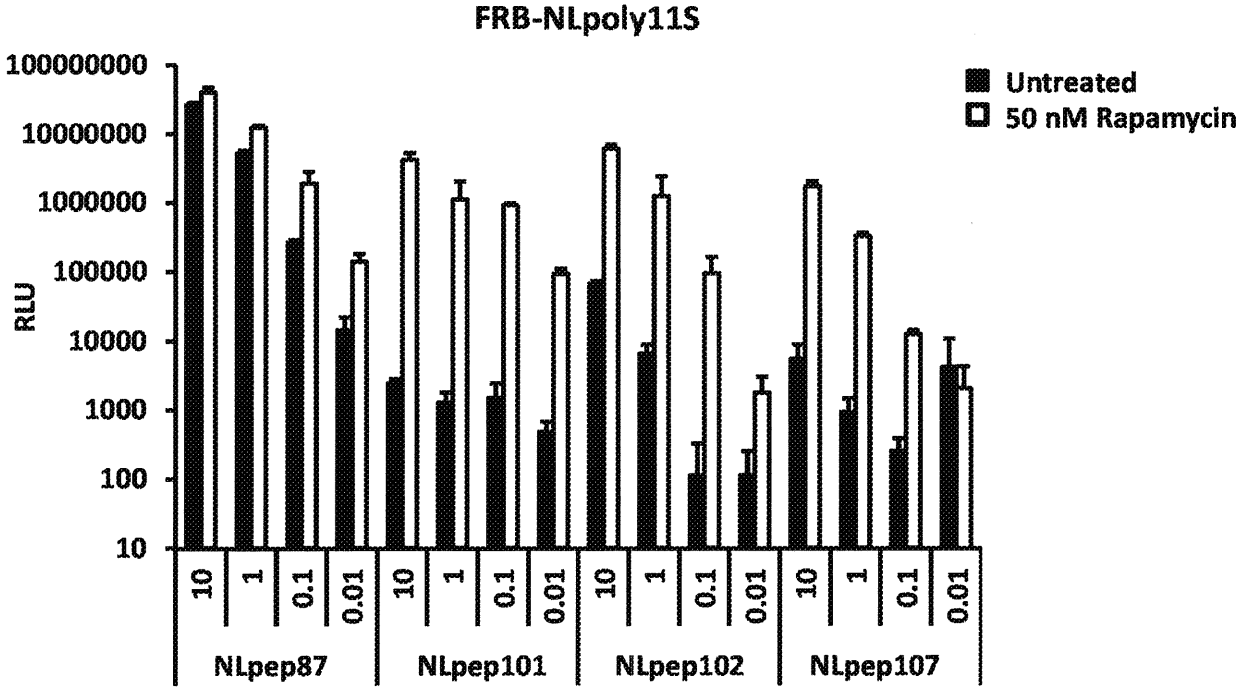
NLpep	Fold Induction
87	30
96	287
98	8
99	18
100	93
101	134
102	375
103	279



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

SUBSTITUTE SHEET (RULE 26)

Figure 123

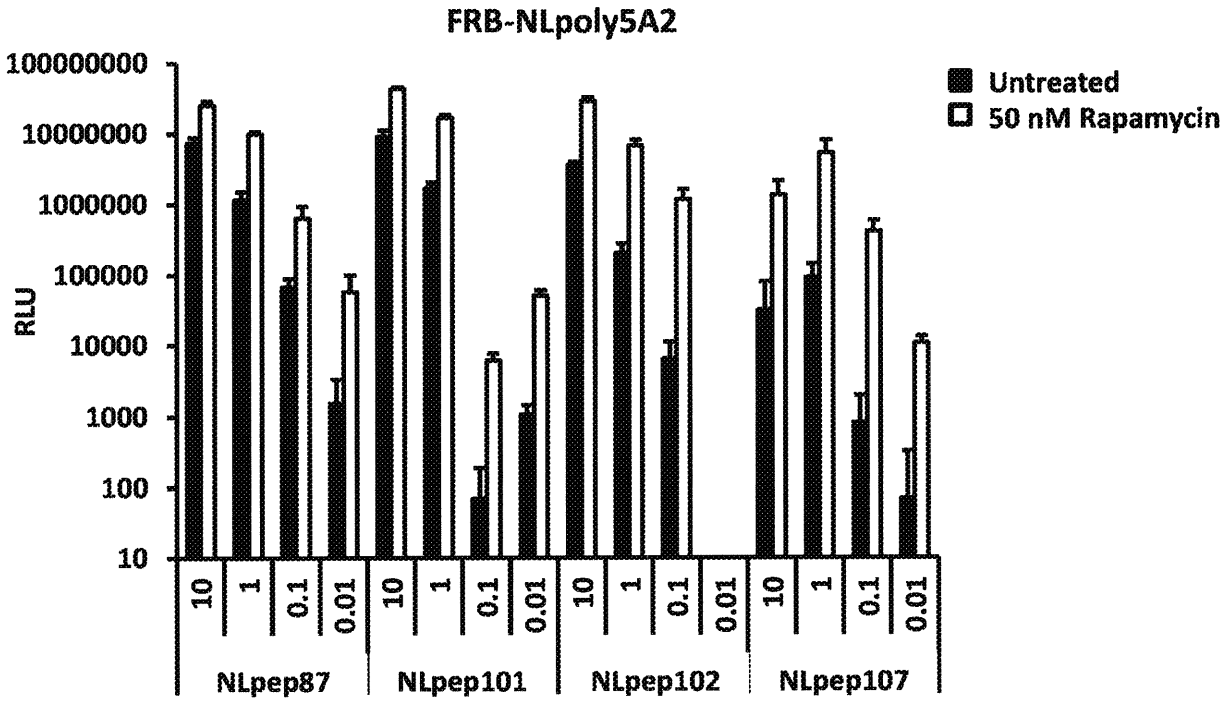


Fold Induction

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

SUBSTITUTE SHEET (RULE 26)

Figure 124

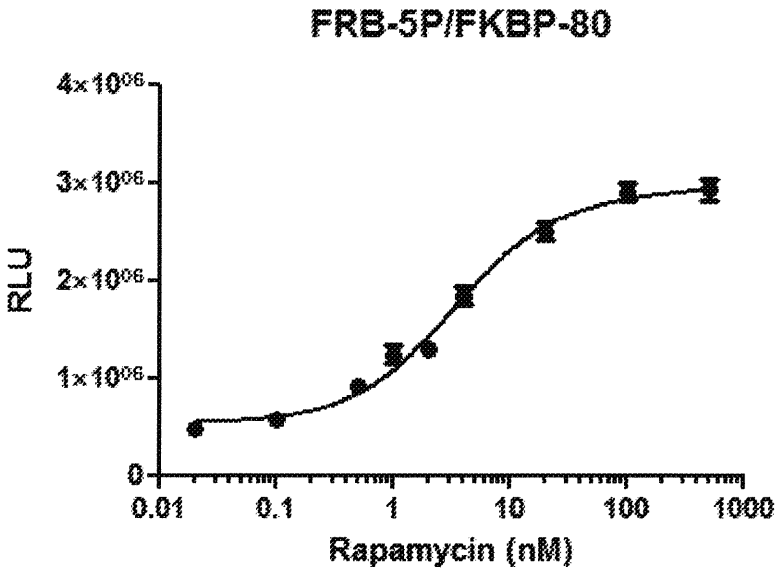


Fold Induction

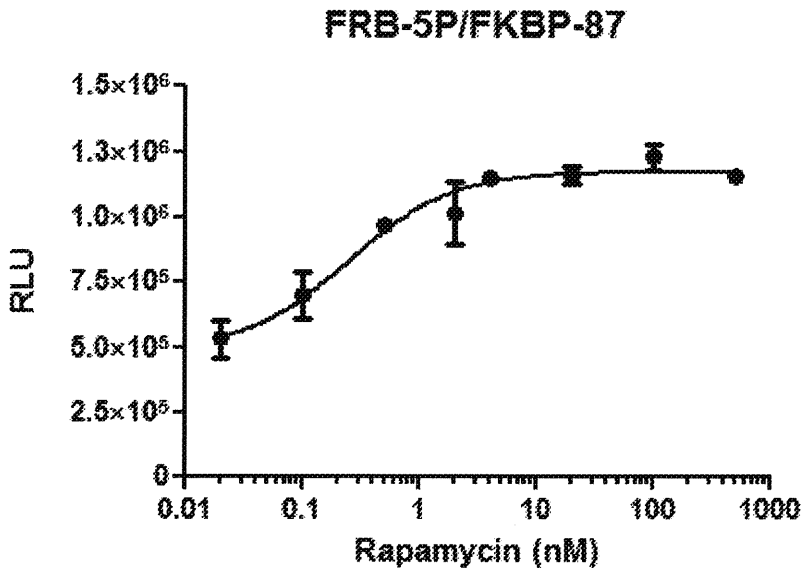
NLpep	10 ng DNA/well	1 ng DNA/well	0.1 ng DNA/well	0.01 ng DNA/well
87	3	9	10	37
101	5	10	89	47
102	8	33	179	NA
107	42	58	508	156

SUBSTITUTE SHEET (RULE 26)

Figure 125



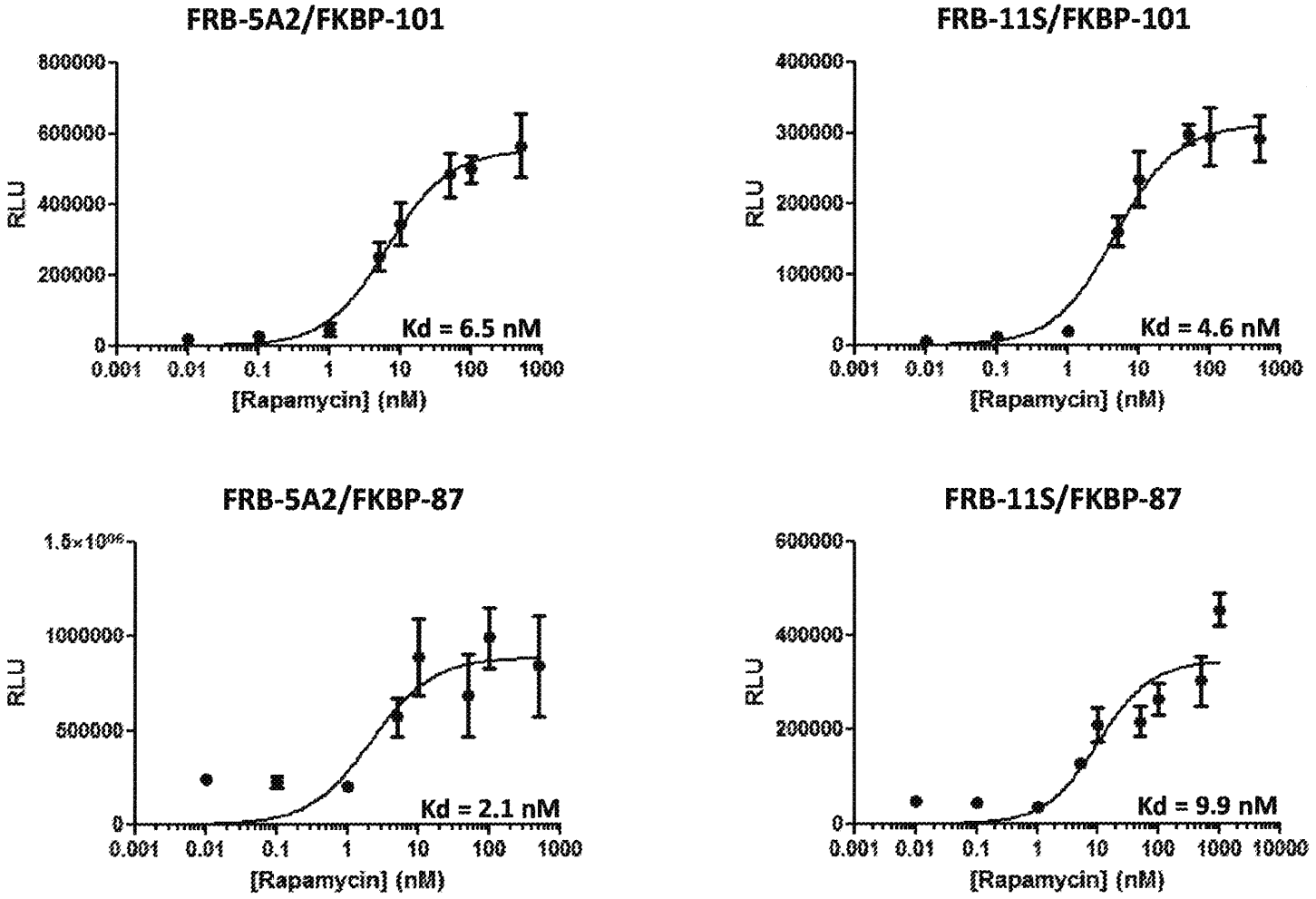
Bmax	2.360e+006
Kd	3.256



Bmax	683128
Kd	0.2456

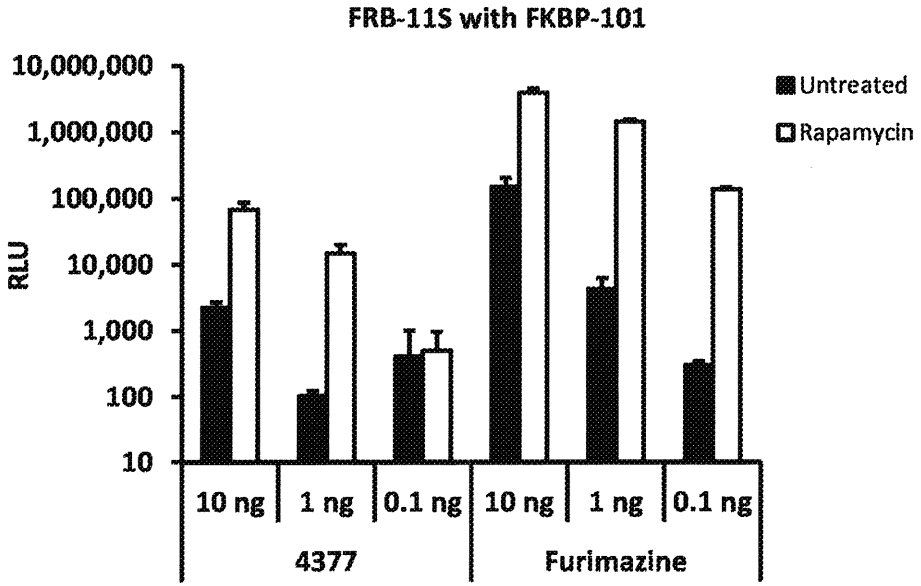
SUBSTITUTE SHEET (RULE 26)

Figure 126



SUBSTITUTE SHEET (RULE 26)

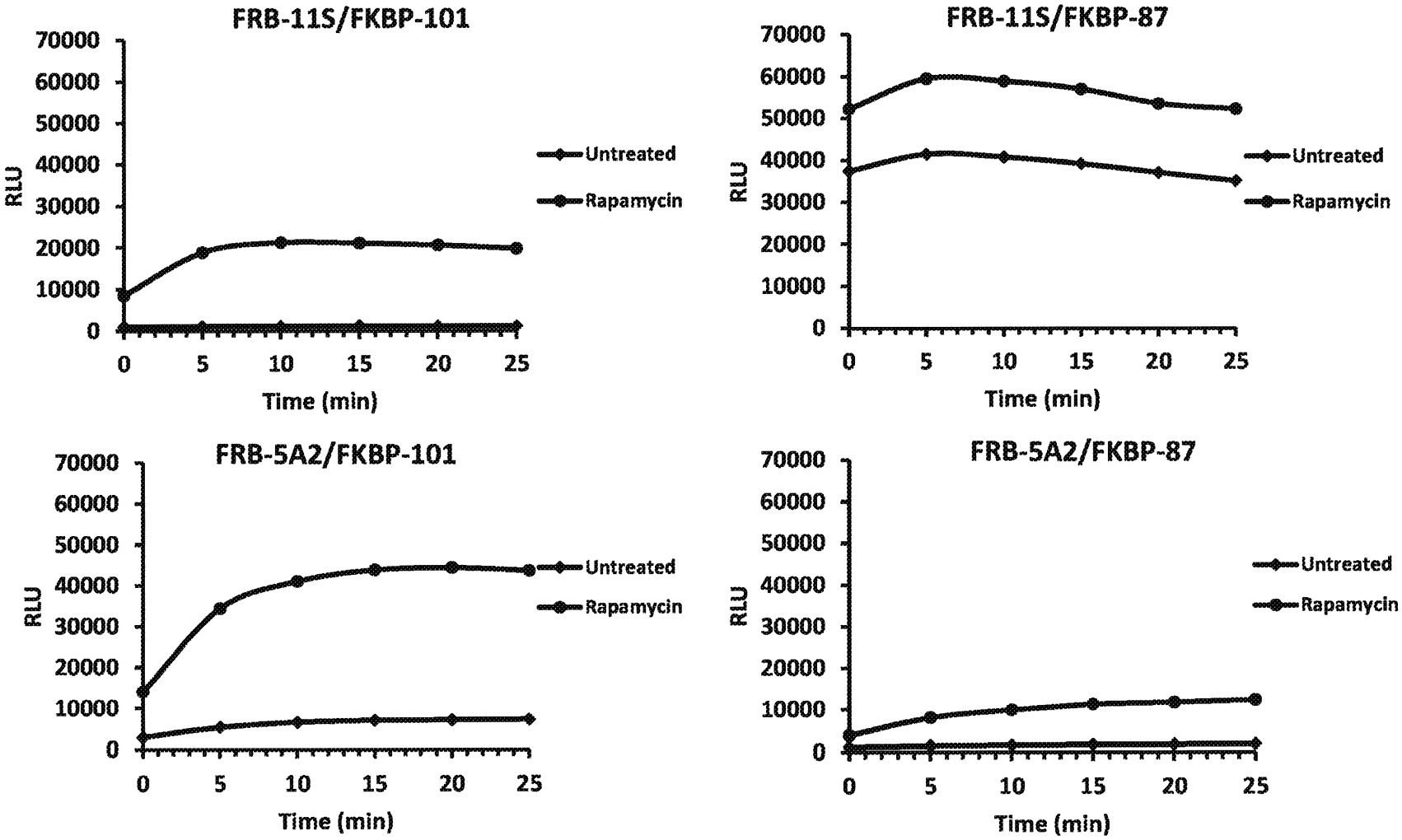
Figure 127



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

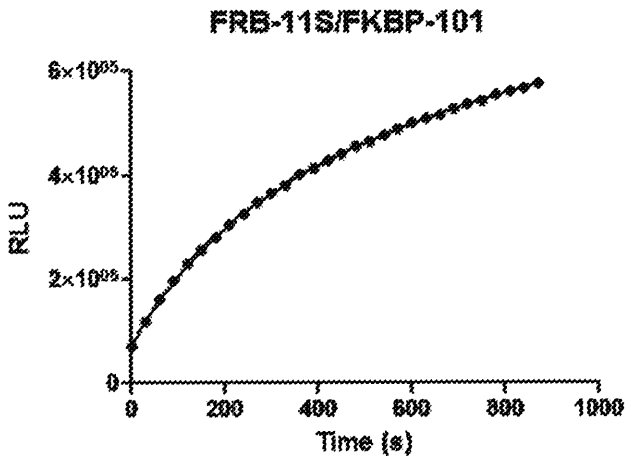
SUBSTITUTE SHEET (RULE 26)

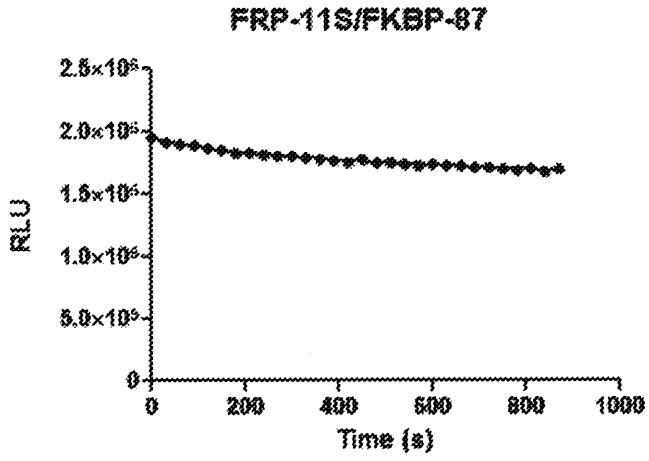
Figure 128

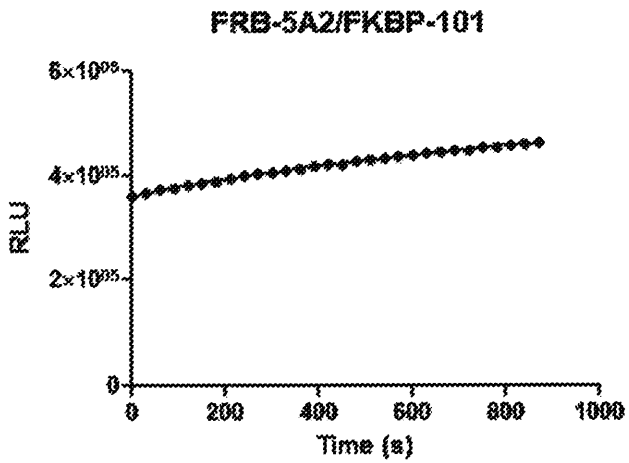


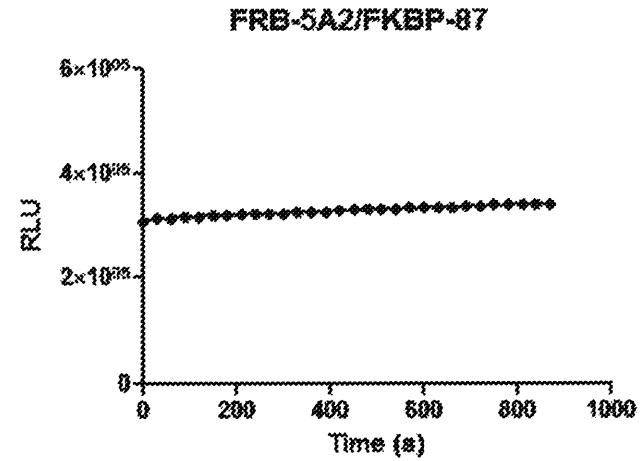
SUBSTITUTE SHEET (RULE 26)

Figure 129



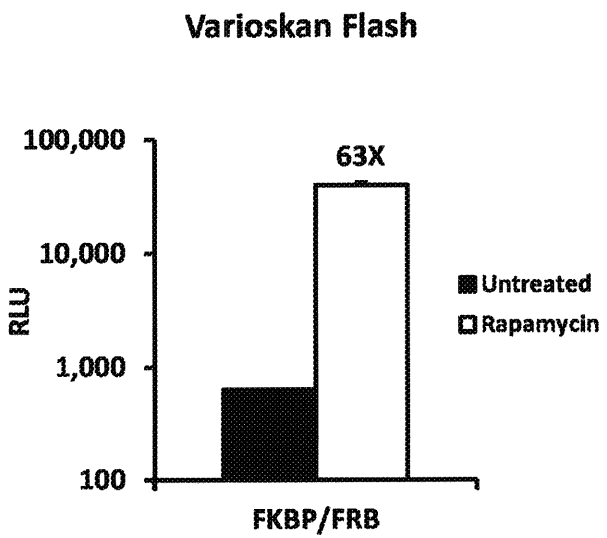


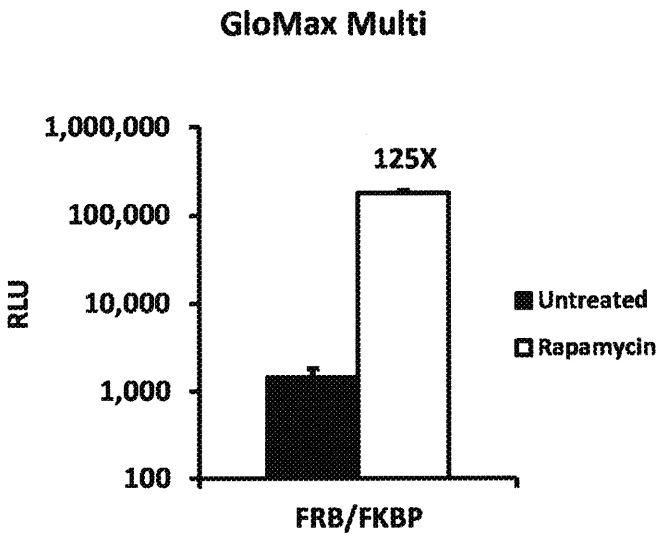




SUBSTITUTE SHEET (RULE 26)

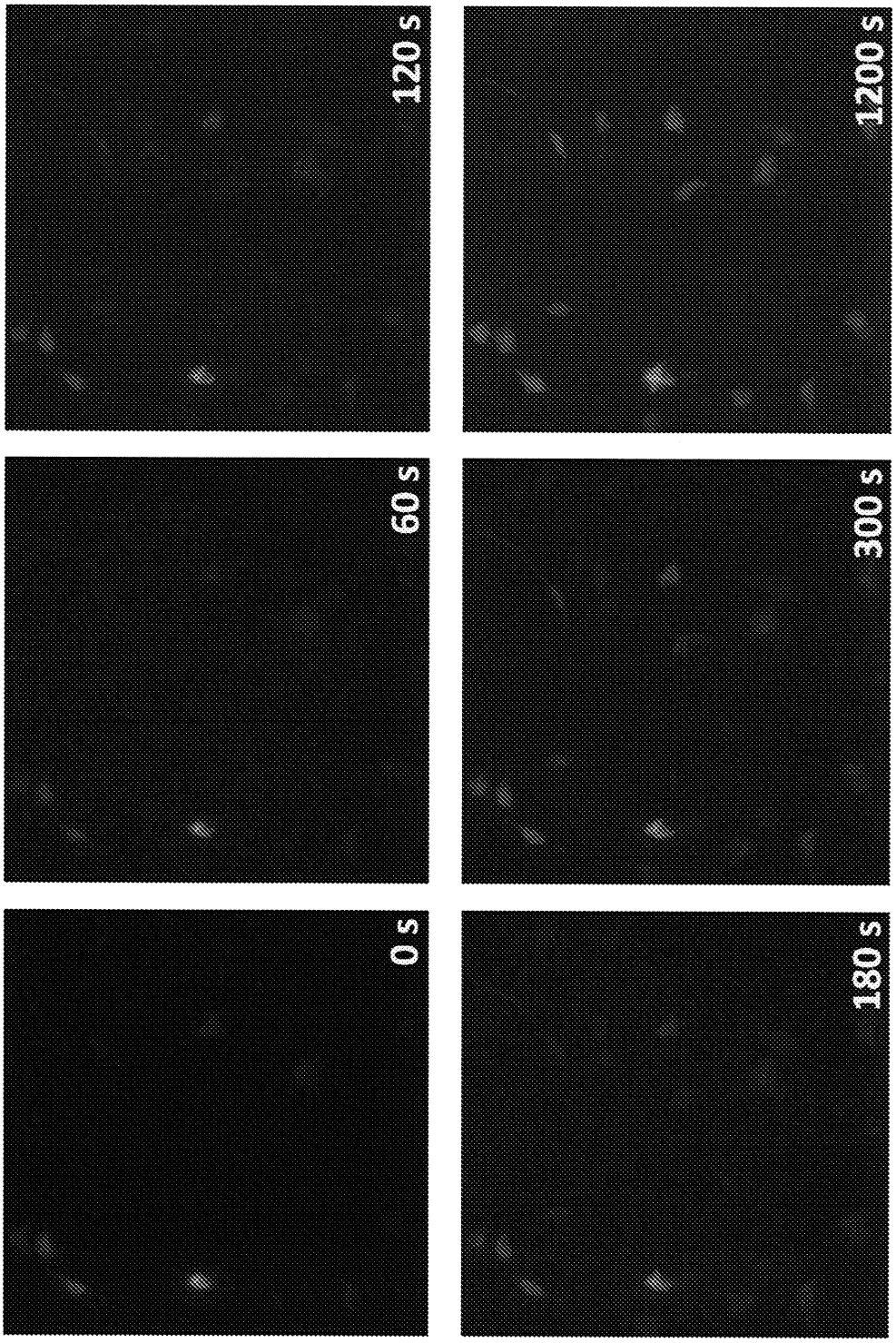
Figure 130





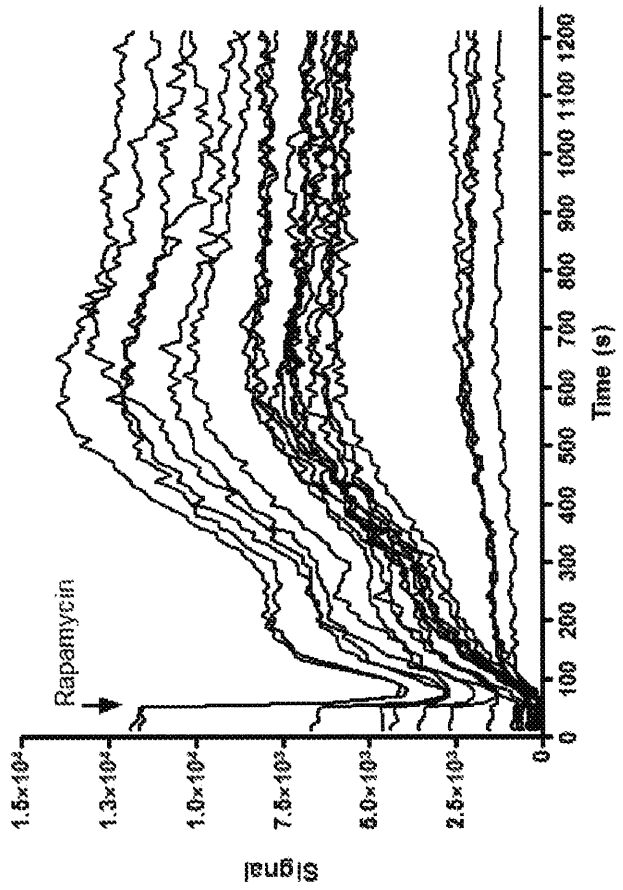
SUBSTITUTE SHEET (RULE 26)

Figure 131



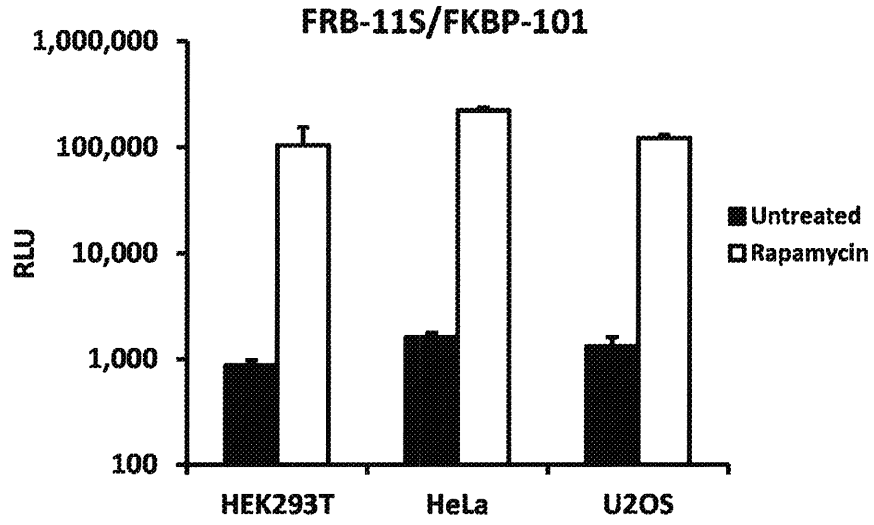
SUBSTITUTE SHEET (RULE 26)

Figure 132



SUBSTITUTE SHEET (RULE 26)

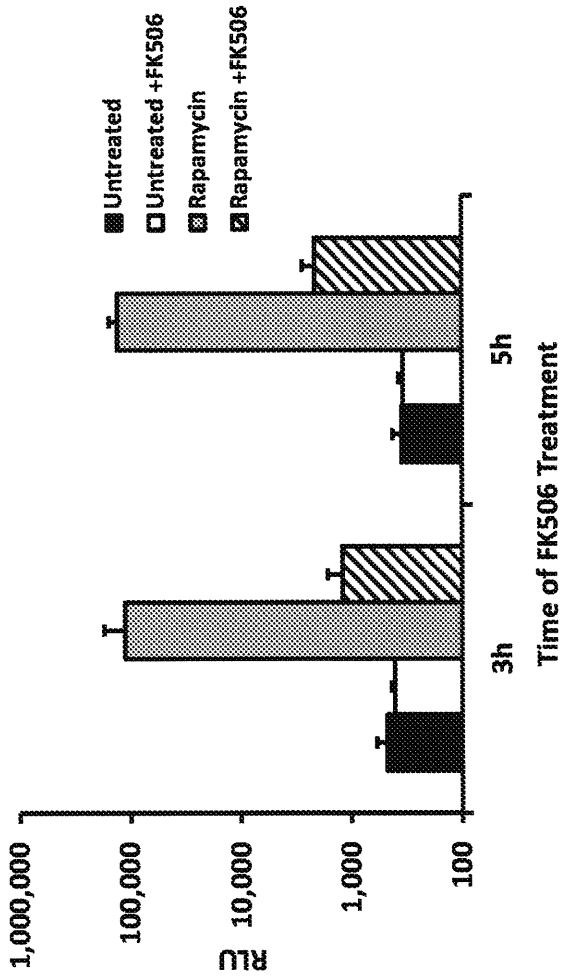
Figure 133



Cell Line	Fold Induction
HEK293T	121
HeLa	140
U2OS	92

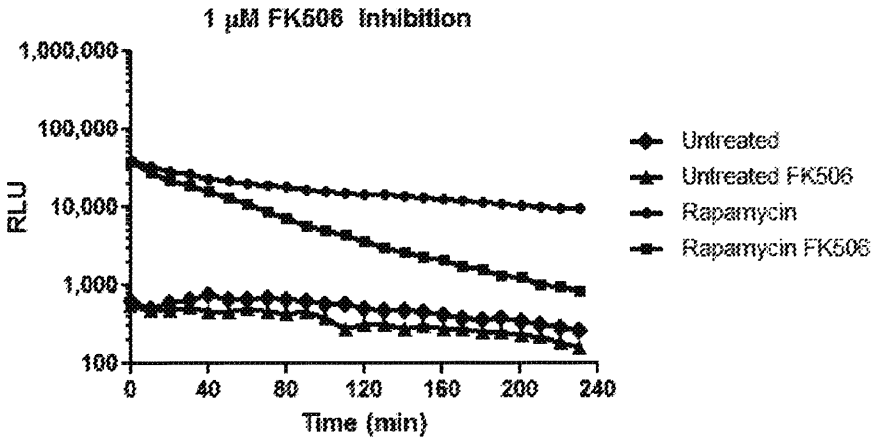
SUBSTITUTE SHEET (RULE 26)

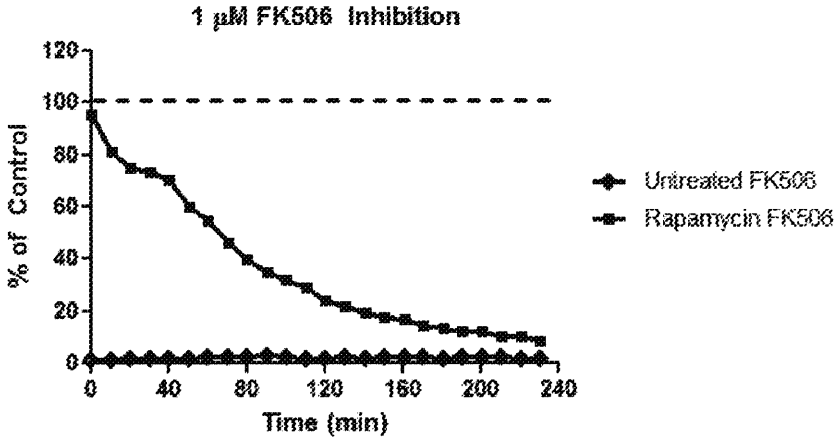
Figure 134

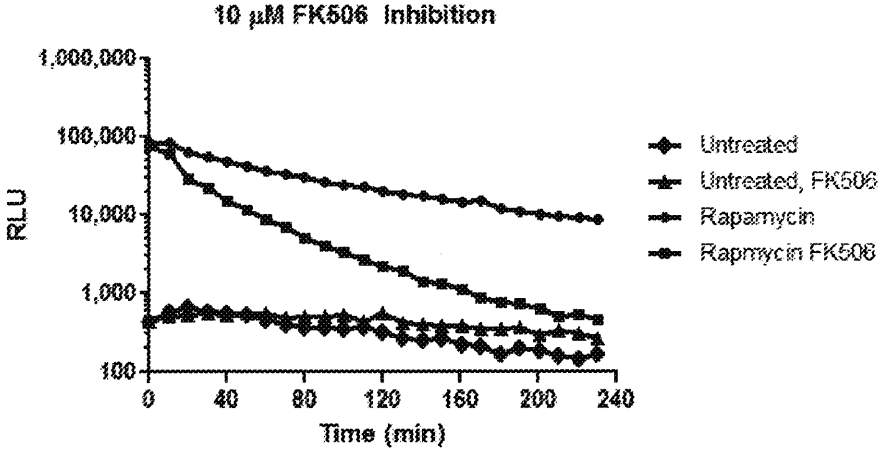


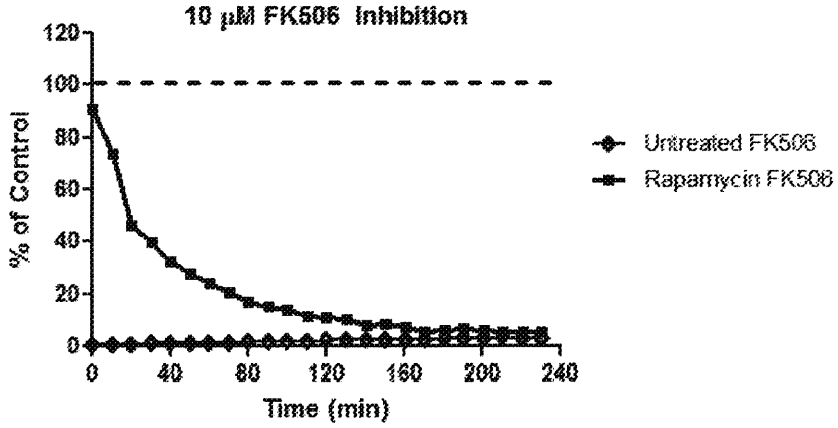
SUBSTITUTE SHEET (RULE 26)

Figure 135









SUBSTITUTE SHEET (RULE 26)

Figure 136

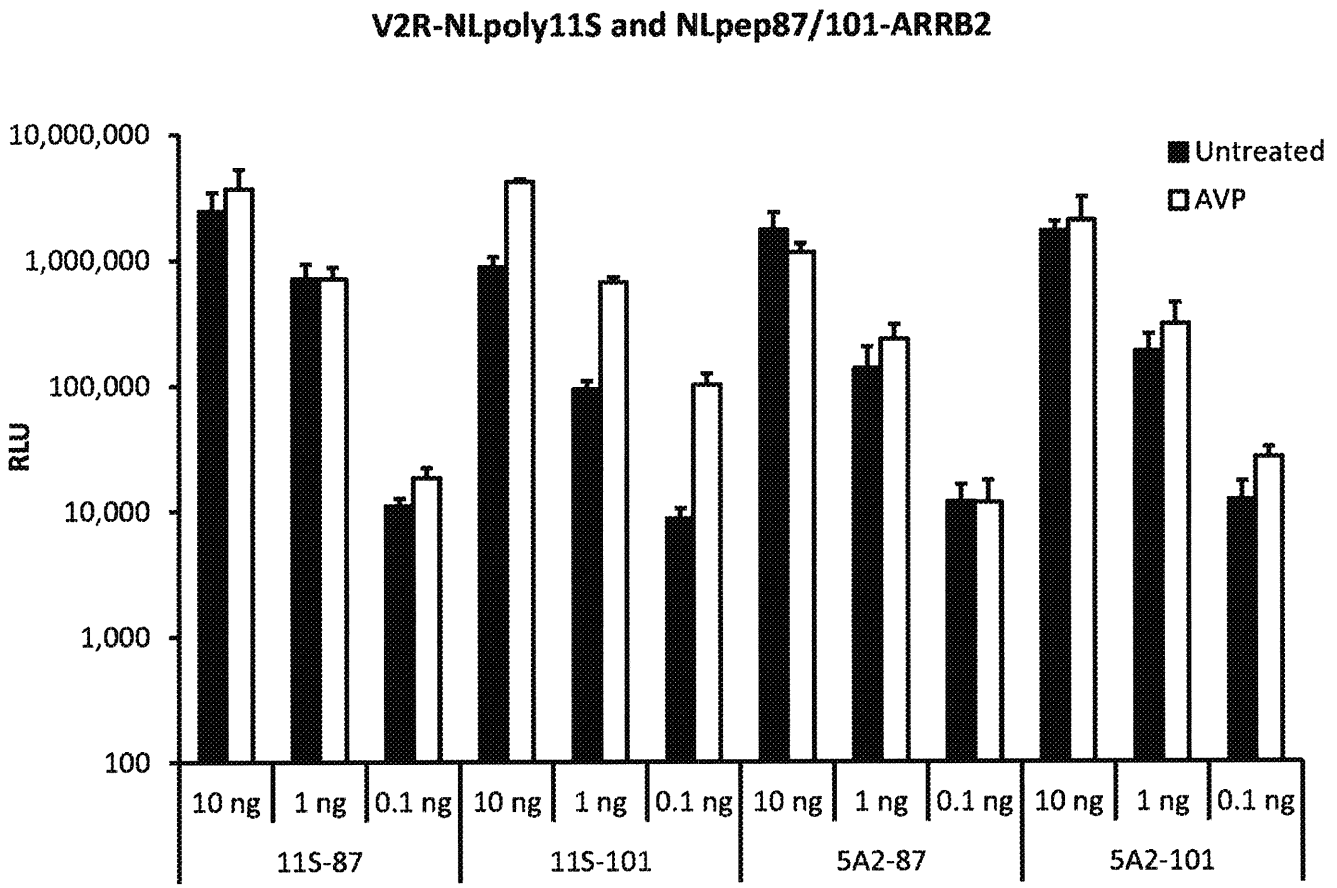

V2R-NLpoly11S and NLpep87/101-ARRB2
RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
Untreated
AVP
10 ng
1 ng
0.1 ng
11S-87
11S-101
5A2-87
5A2-101

Figure 137

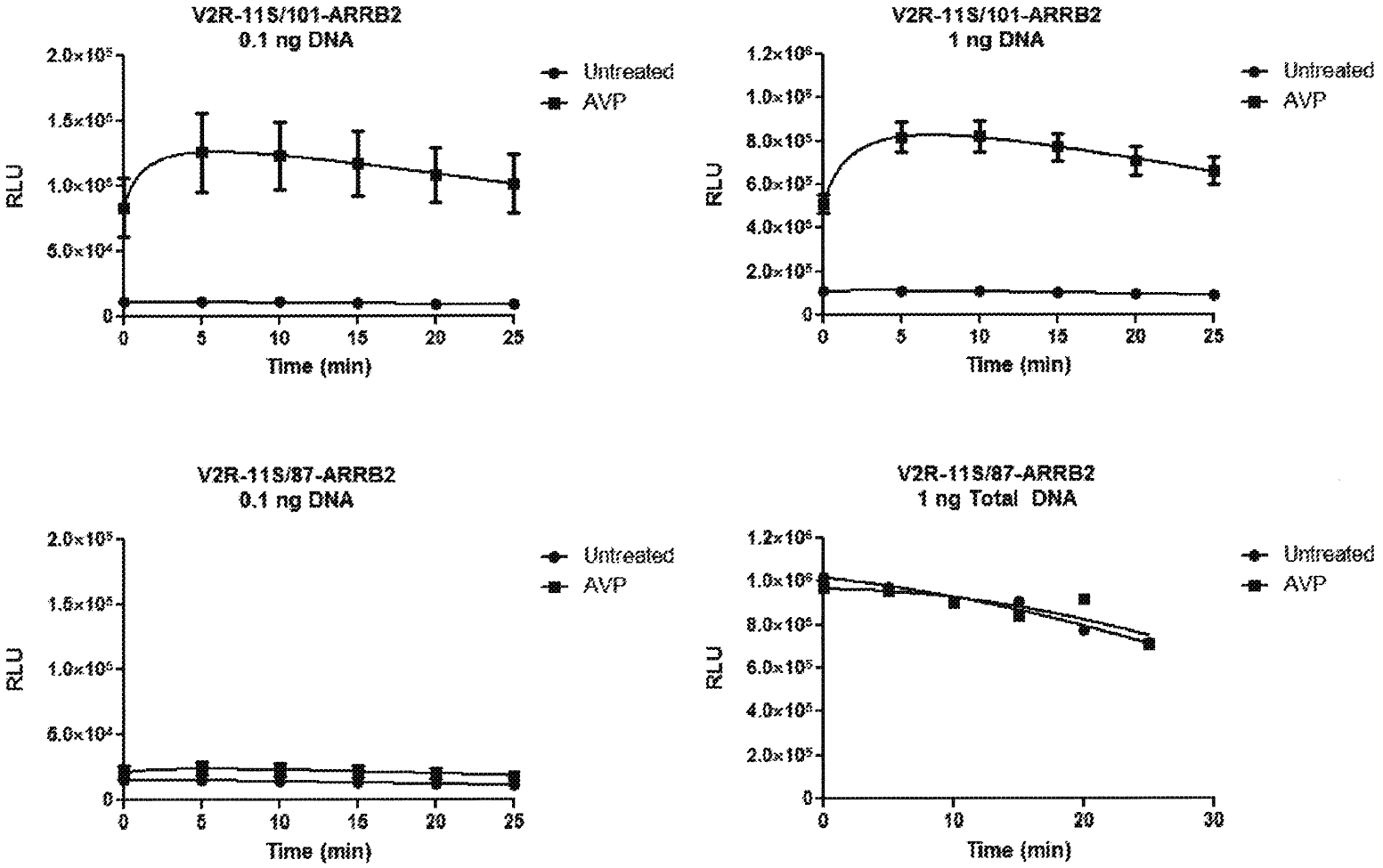
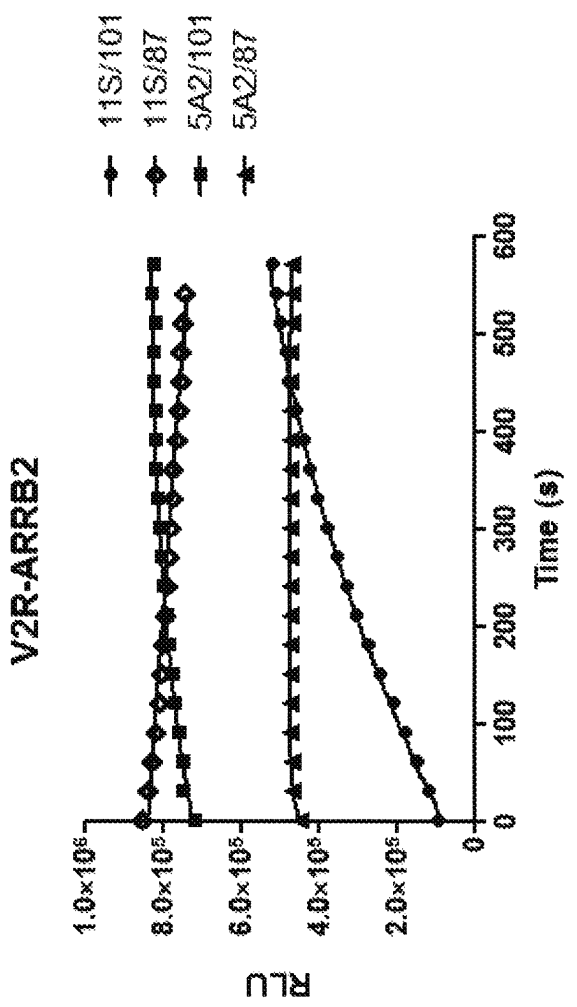
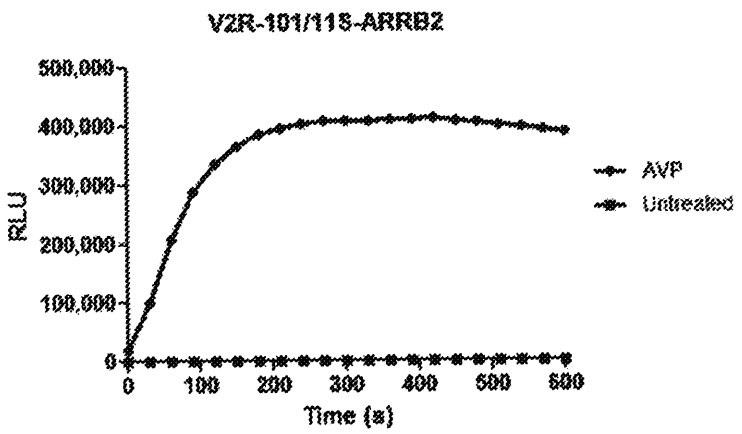

V2R-11S/101-ARRB2
0.1 ng DNA
RLU
Time (min)
Untreated
AVP
V2R-11S/101-ARRB2
1 ng DNA
RLU
Time (min)
Untreated
AVP
V2R-11S/87-ARRB2
0.1 ng DNA
RLU
Time (min)
Untreated
AVP
V2R-11S/87-ARRB2
1 ng Total DNA
RLU
Time (min)
Untreated
AVP

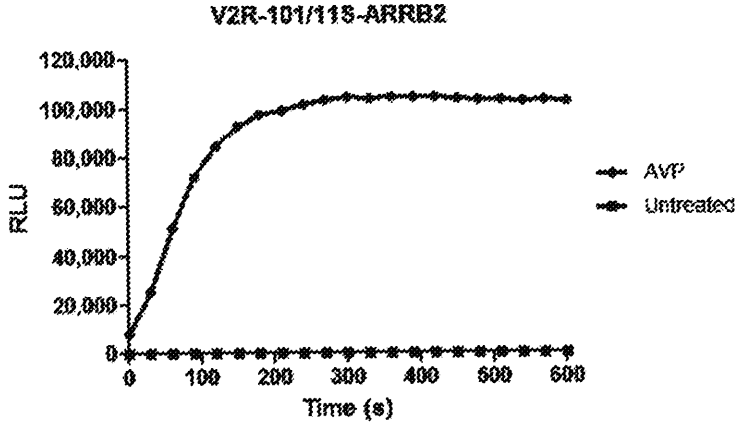
Figure 138

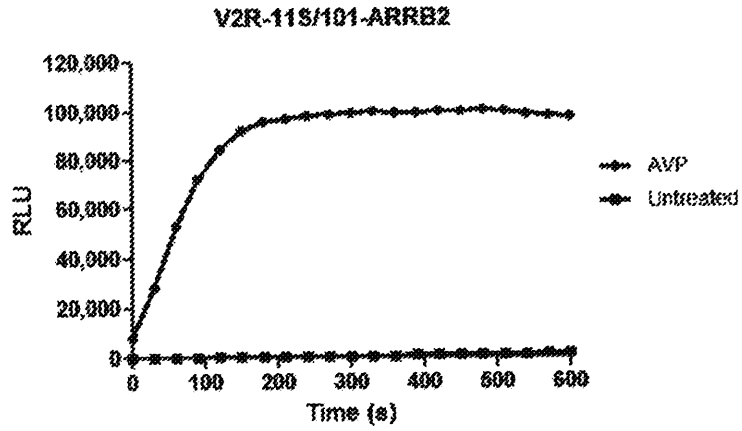


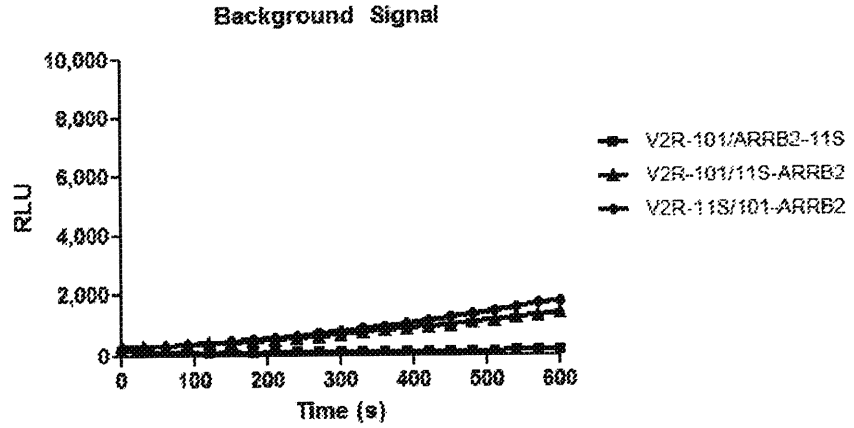
SUBSTITUTE SHEET (RULE 26)

Figure 139



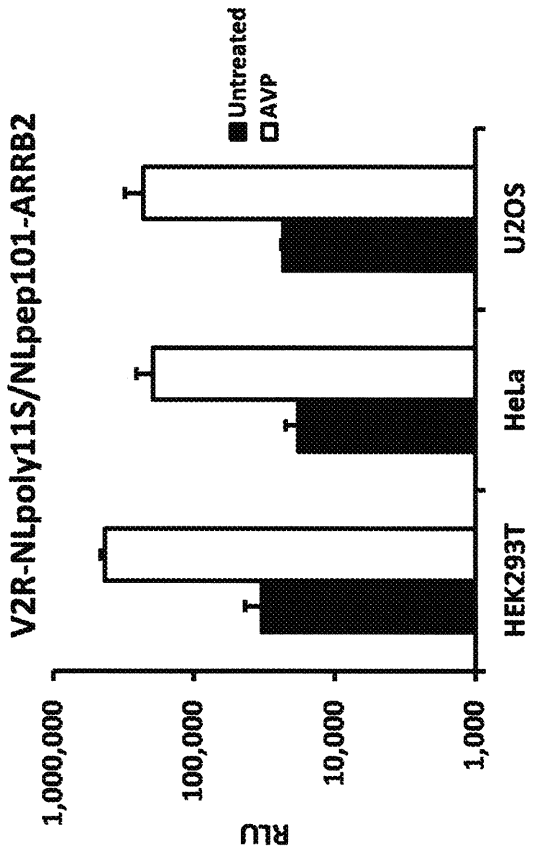






SUBSTITUTE SHEET (RULE 26)

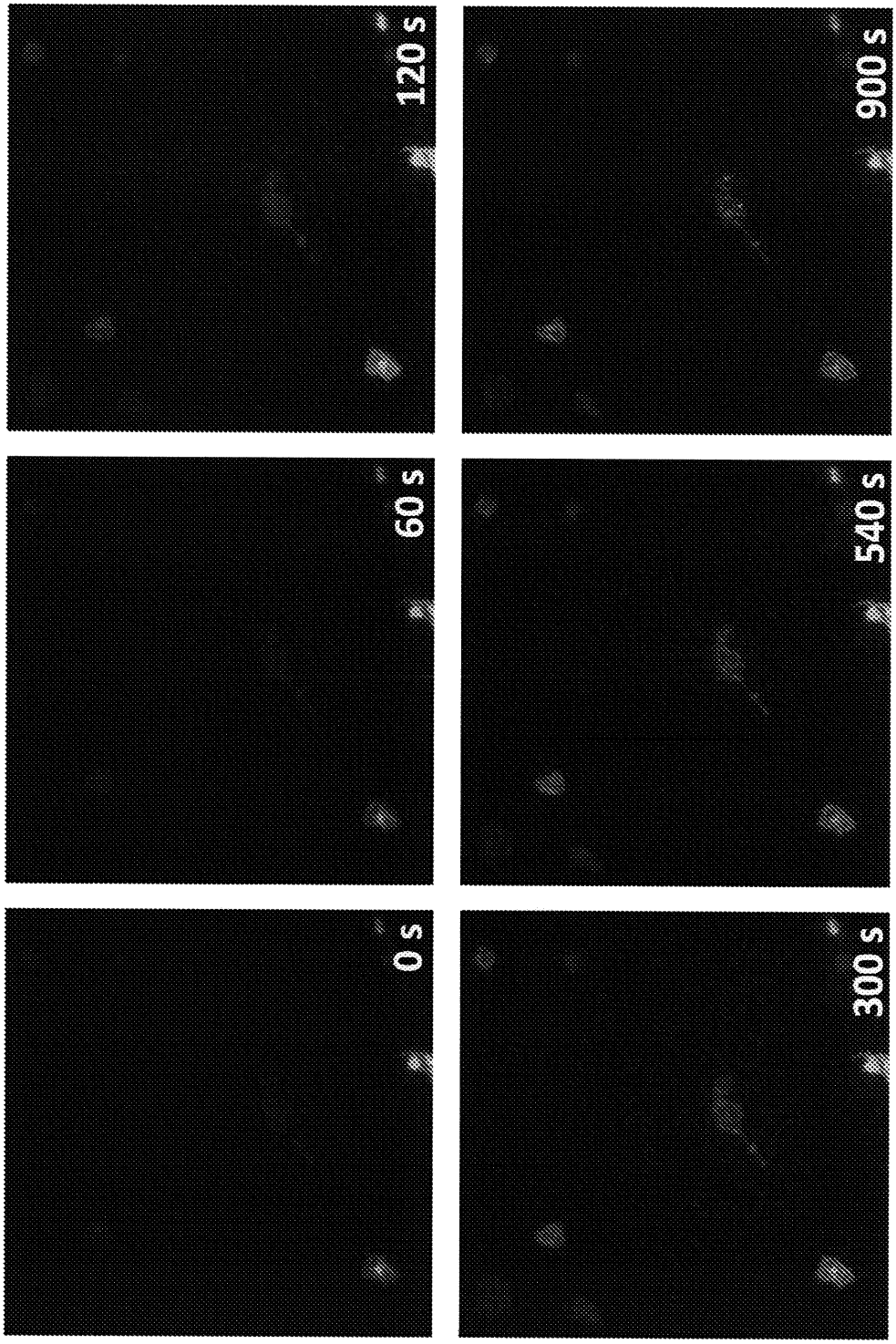
Figure 140



Cell line	Fold Induction
HEK293T	13
HeLa	11
U2OS	10

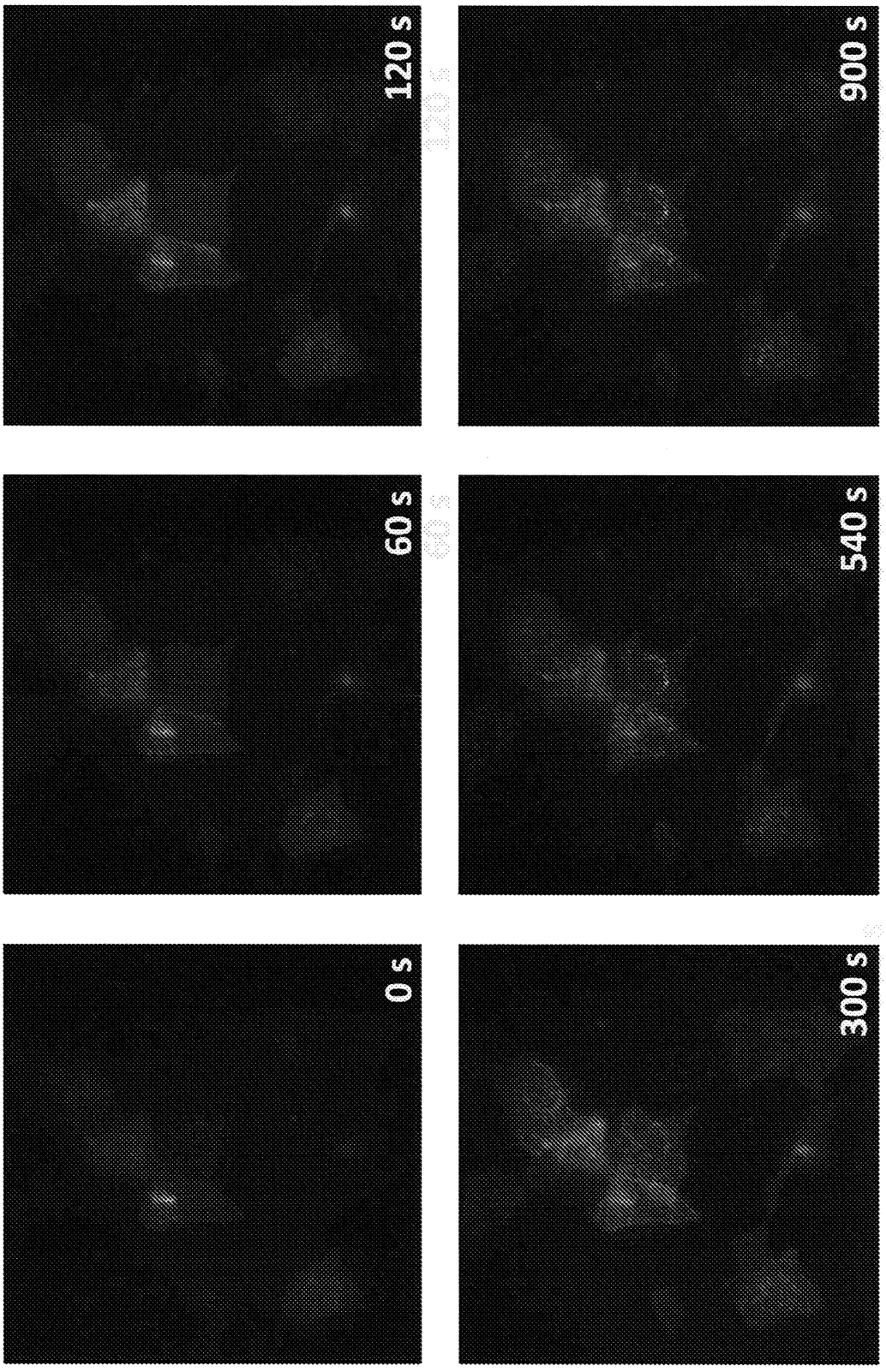
SUBSTITUTE SHEET (RULE 26)

Figure 141



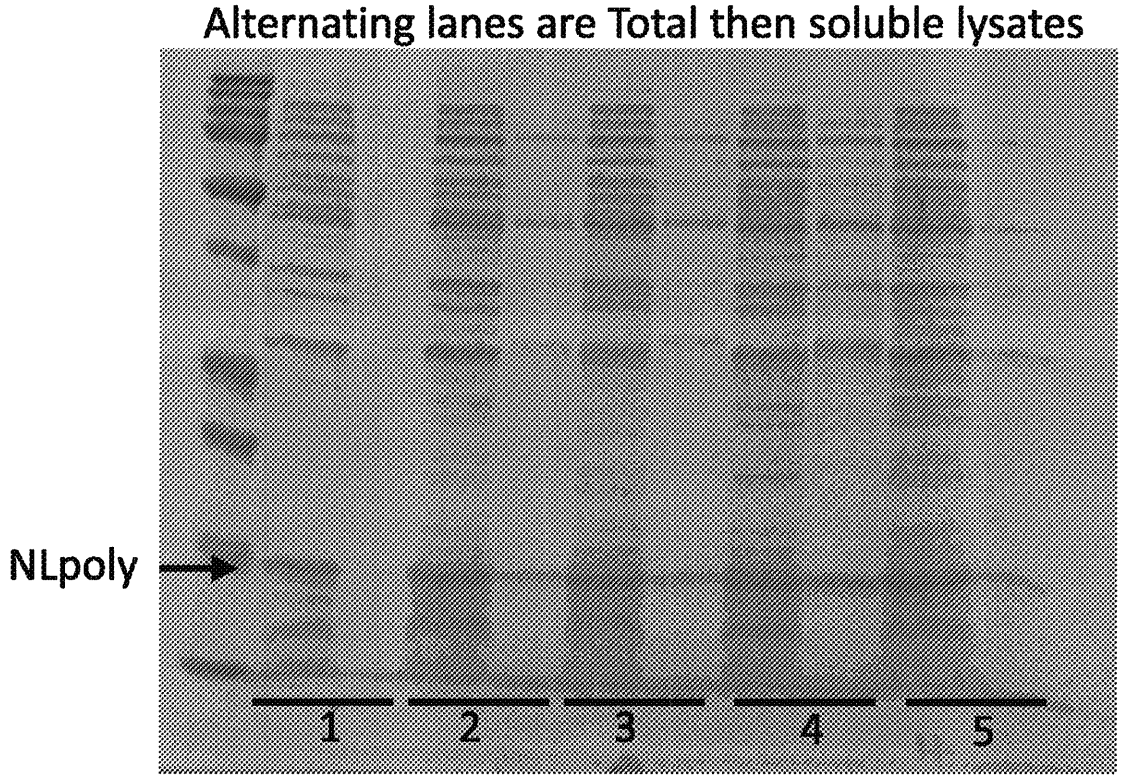
SUBSTITUTE SHEET (RULE 26)

Figure 142



SUBSTITUTE SHEET (RULE 26)

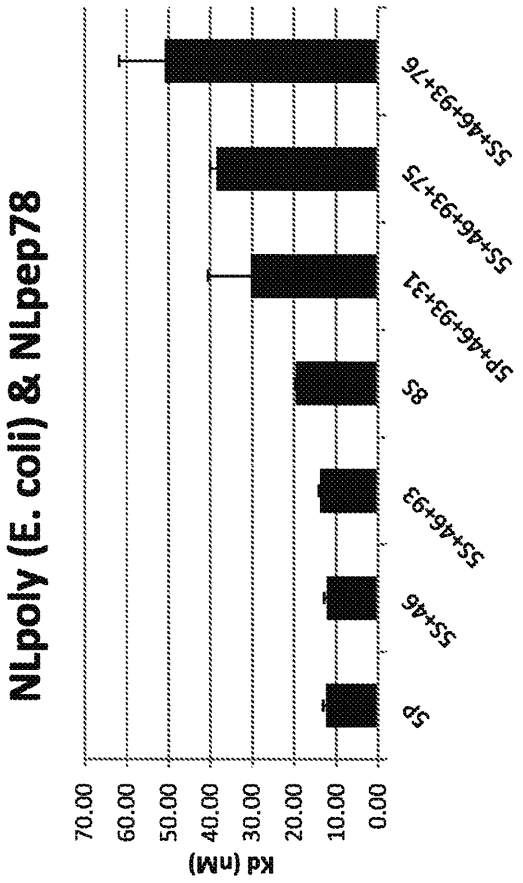
Figure 143



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

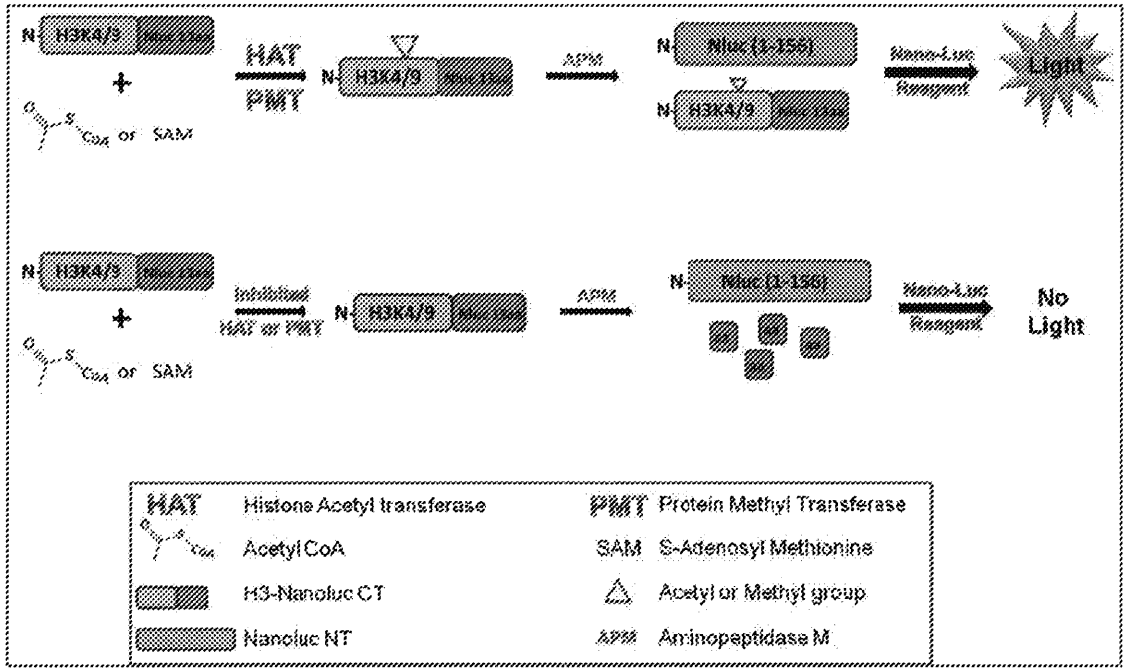
SUBSTITUTE SHEET (RULE 26)

Figure 144



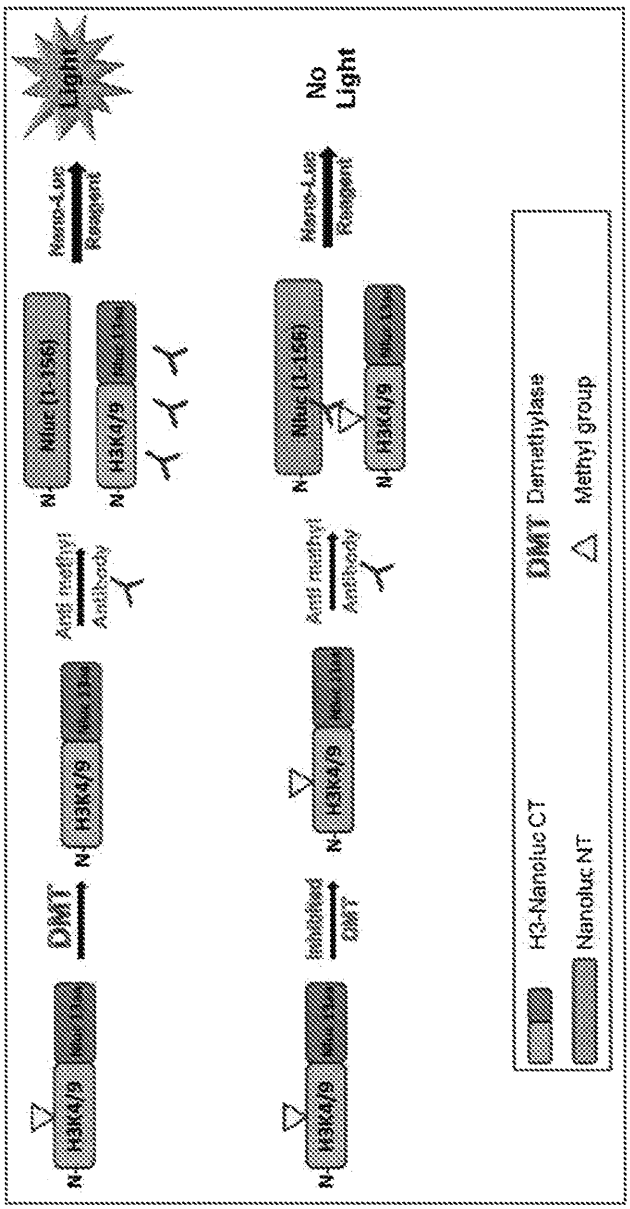
SUBSTITUTE SHEET (RULE 26)

Figure 145



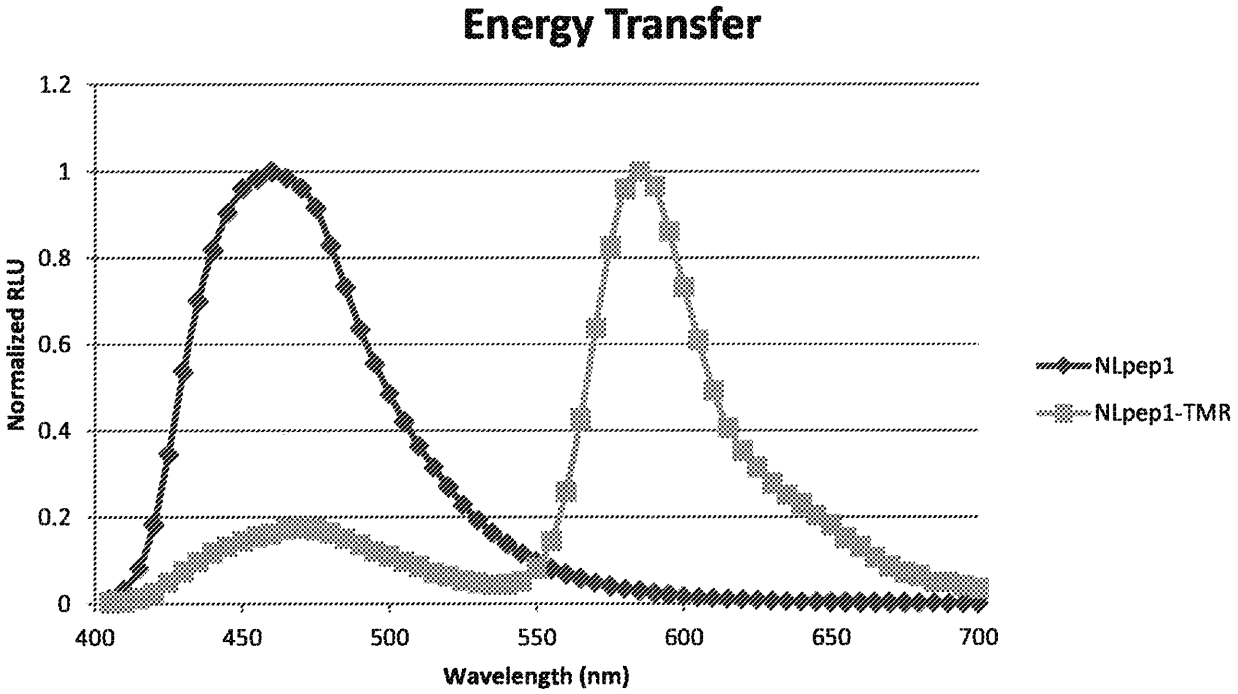
SUBSTITUTE SHEET (RULE 26)

Figure 146

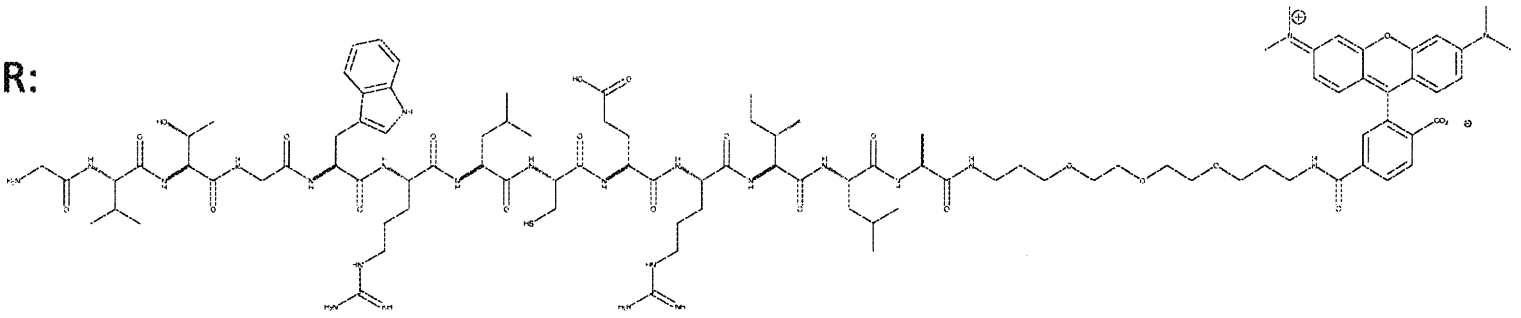


SUBSTITUTE SHEET (RULE 26)

Figure 147

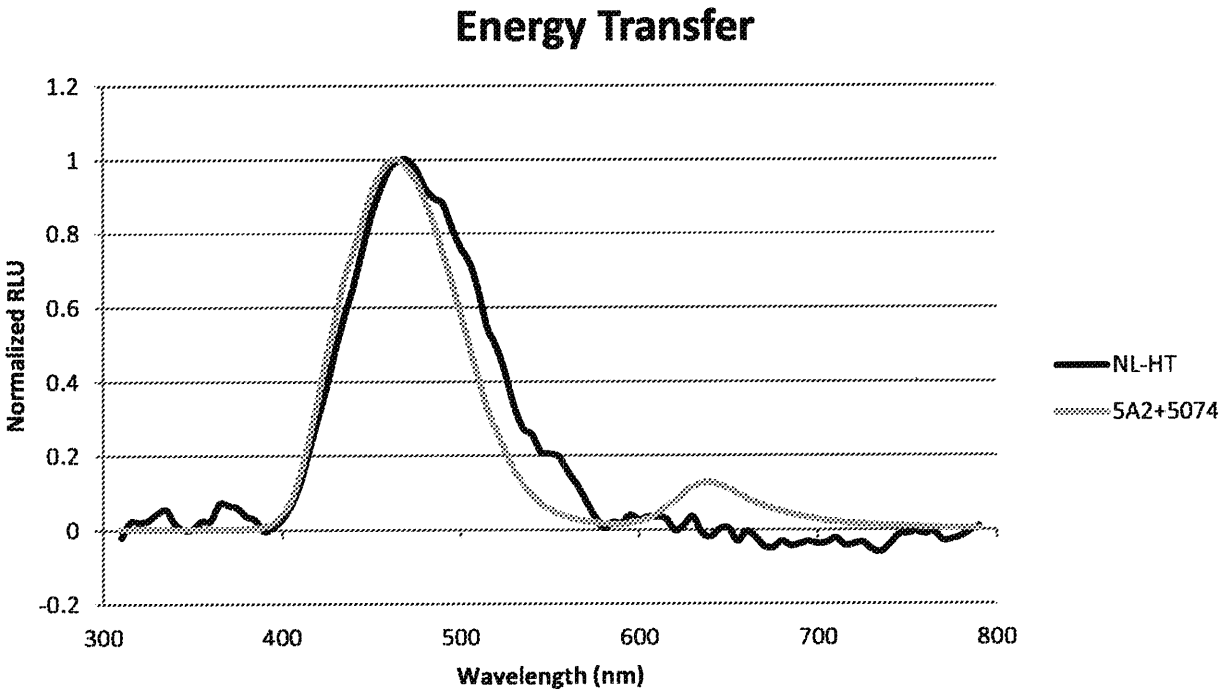


NLpep1-TMR:



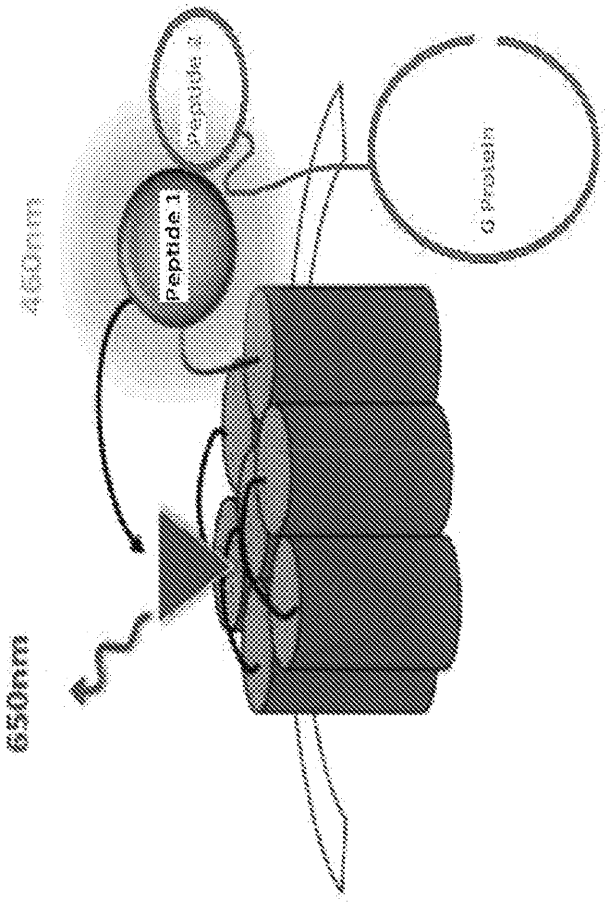
SUBSTITUTE SHEET (RULE 26)

Figure 148



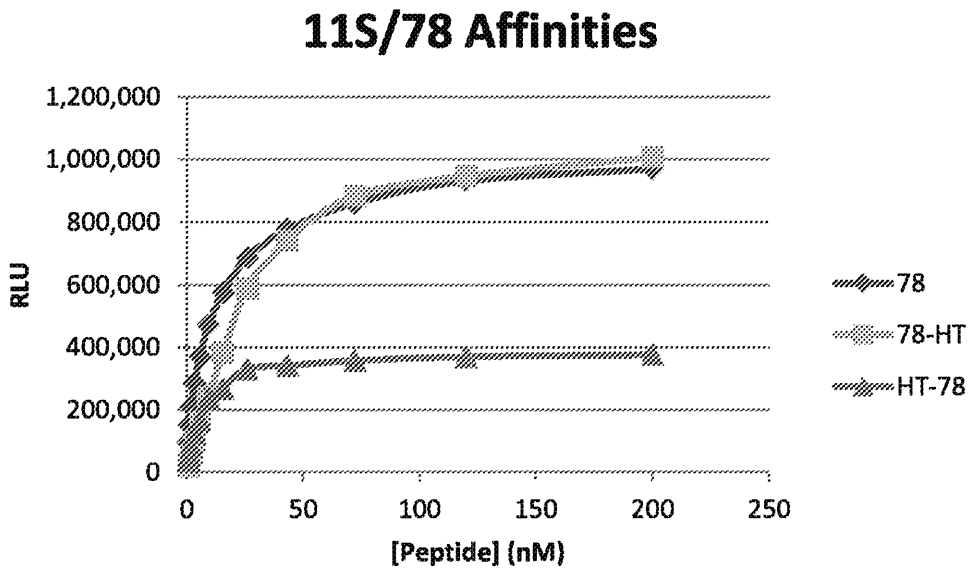
5074 = NCT-DEVDGVTGWRLCERILA

Figure 149



SUBSTITUTE SHEET (RULE 26)

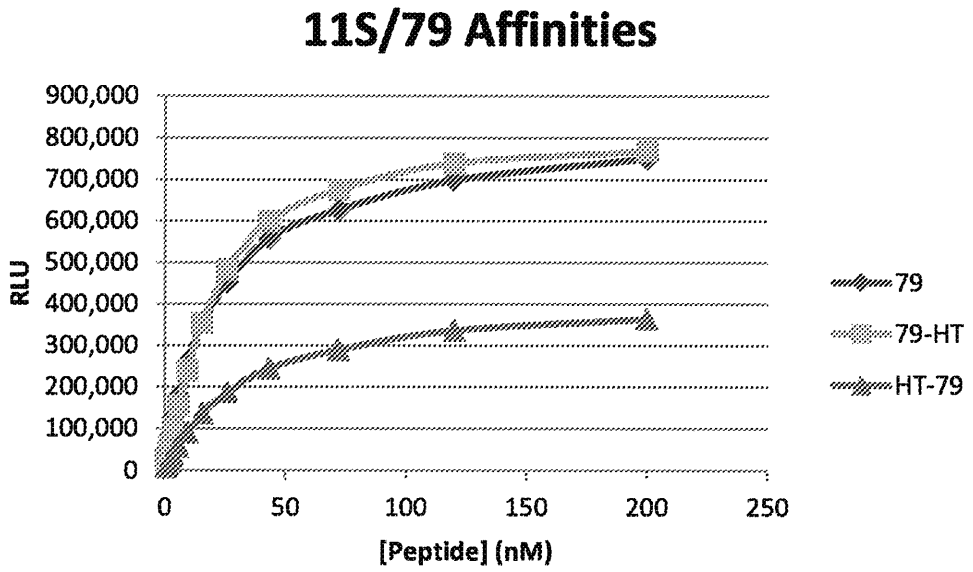
Figure 150



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

SUBSTITUTE SHEET (RULE 26)

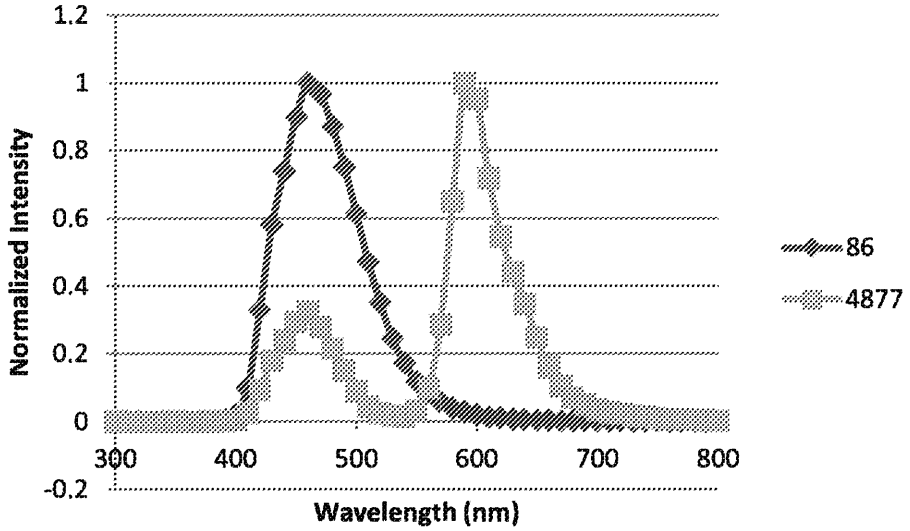
Figure 151



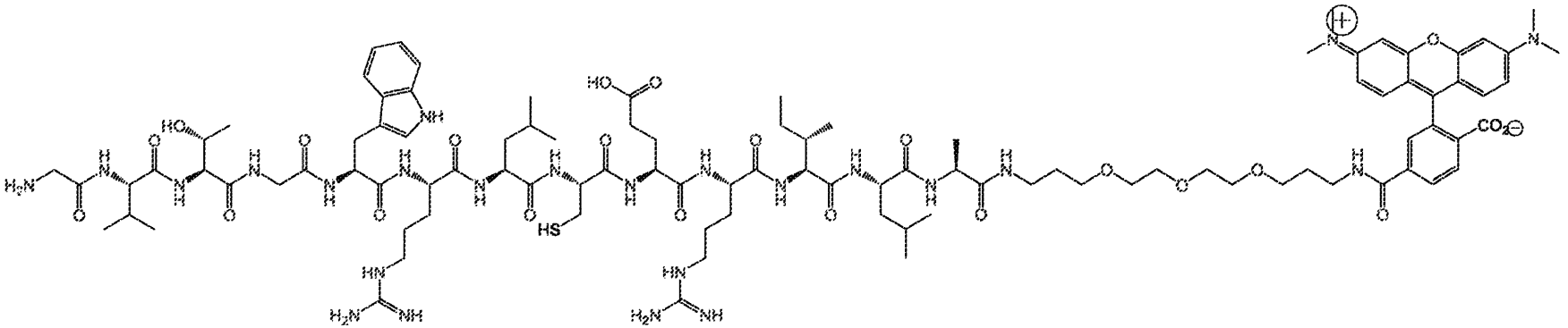
One site -- Specific binding				
	79	79-HT	HT-79	
Best-fit values				
Bmax	816453	879828	427978	
Kd	20.26	22.9	33.87	
Std. Error				
Bmax	5774	15250	3600	
Kd	0.4603	1.237	0.8009	

SUBSTITUTE SHEET (RULE 26)

Figure 152

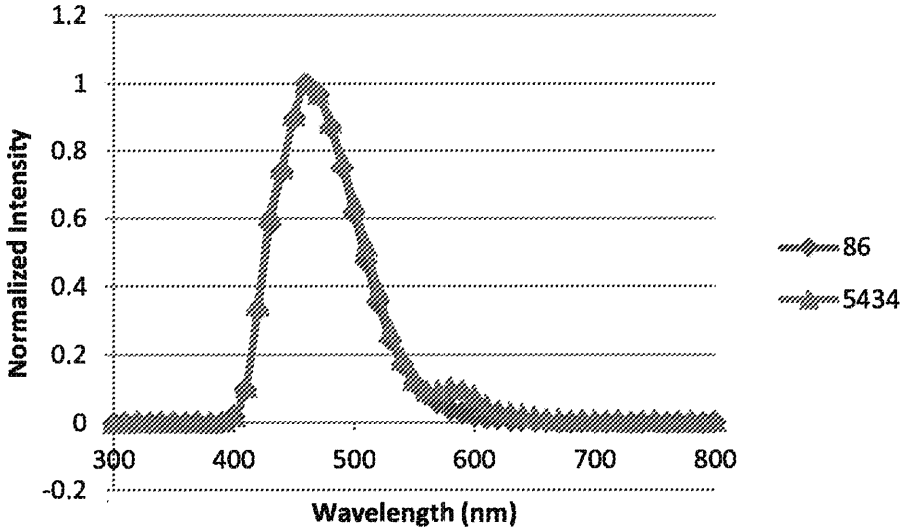


PBI-4877:

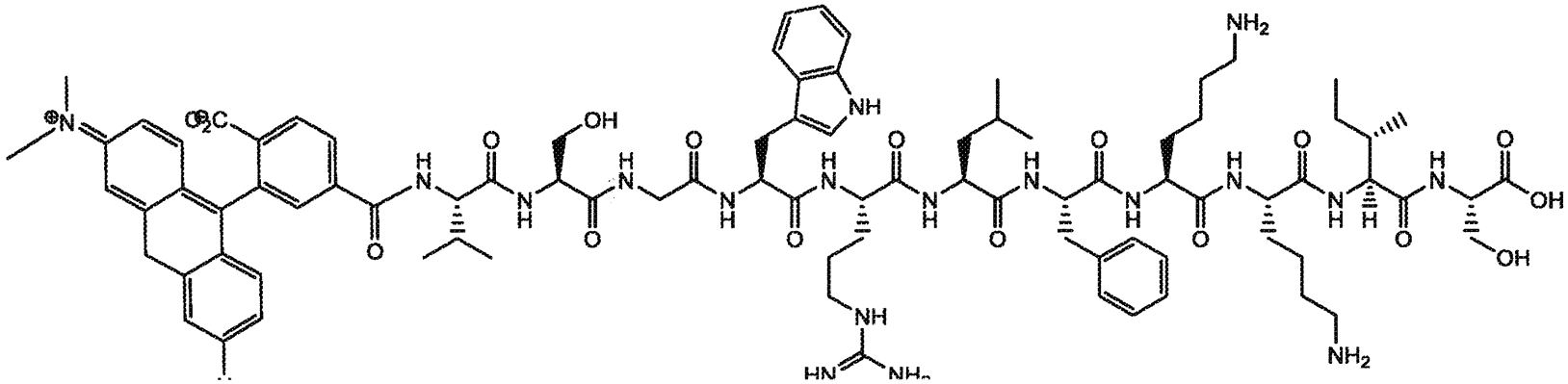


SUBSTITUTE SHEET (RULE 26)

Figure 153

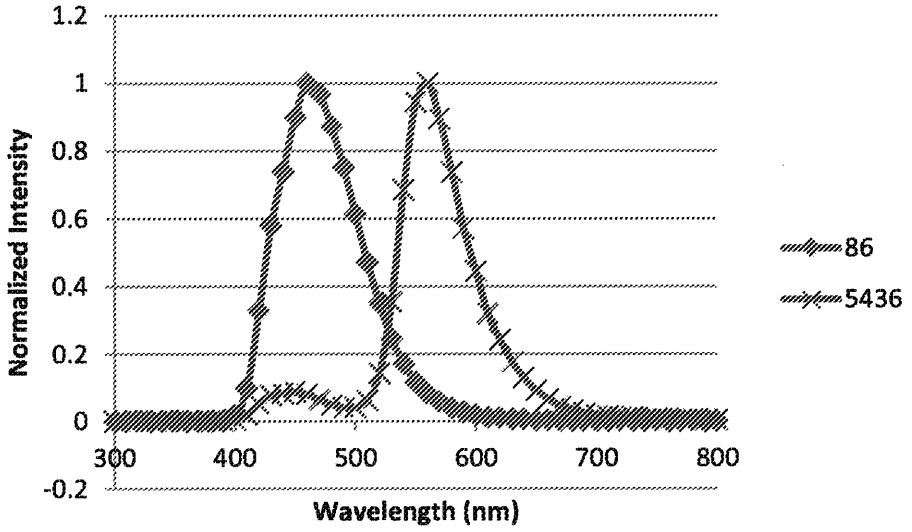


PBI-5434:



SUBSTITUTE SHEET (RULE 26)

Figure 154



PBI 5436:

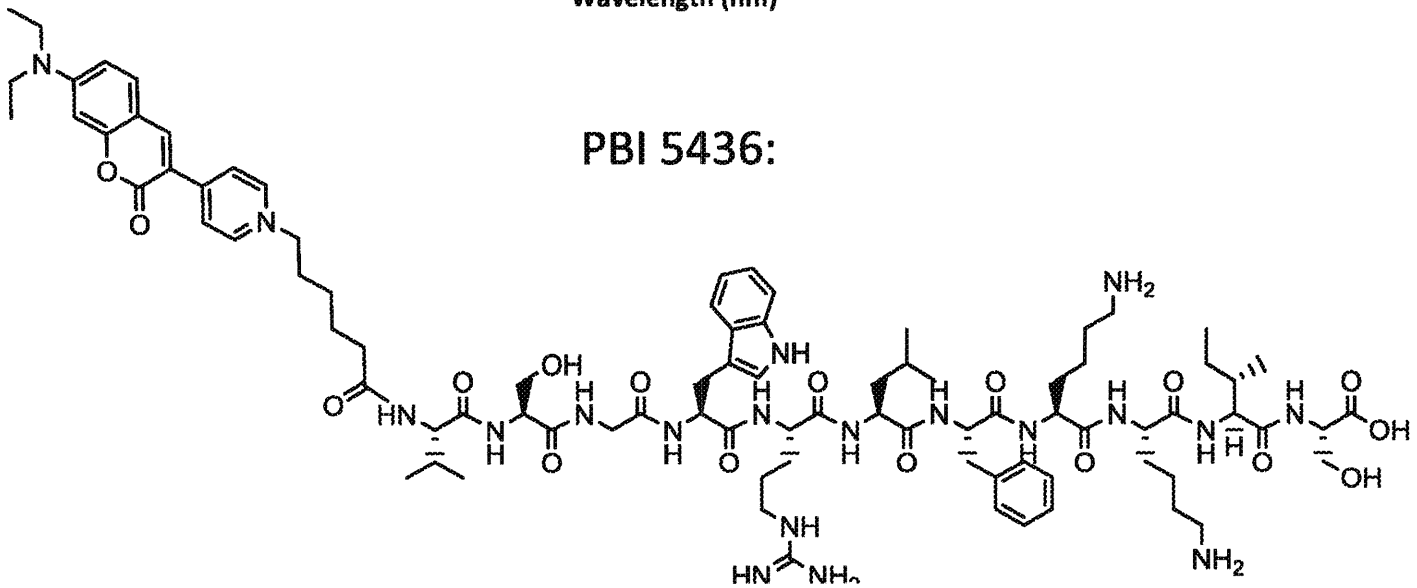
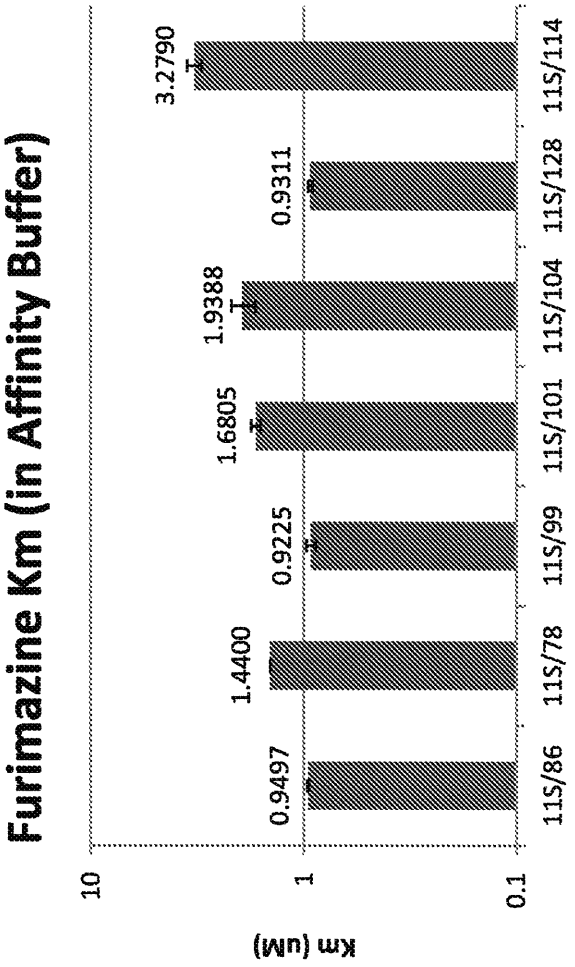
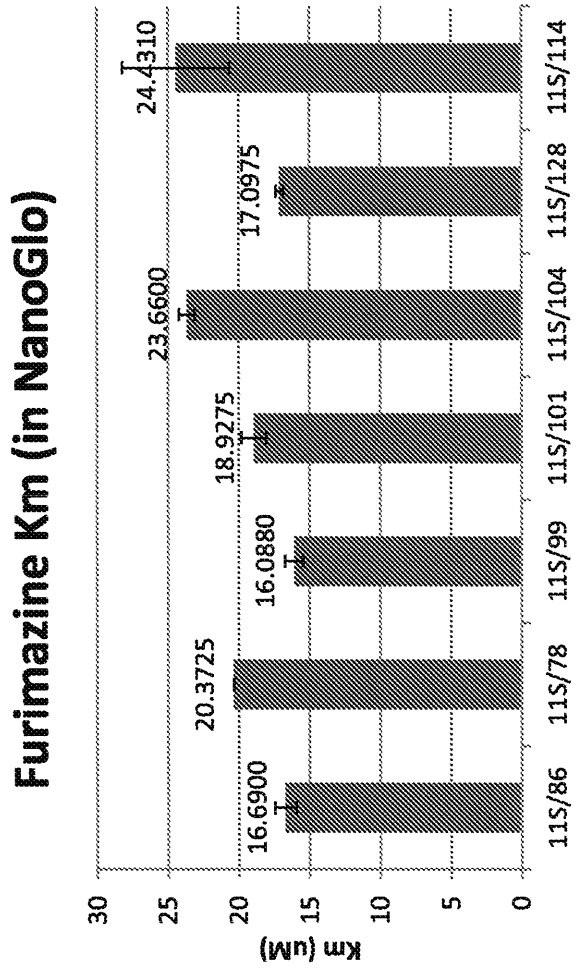


Figure 155



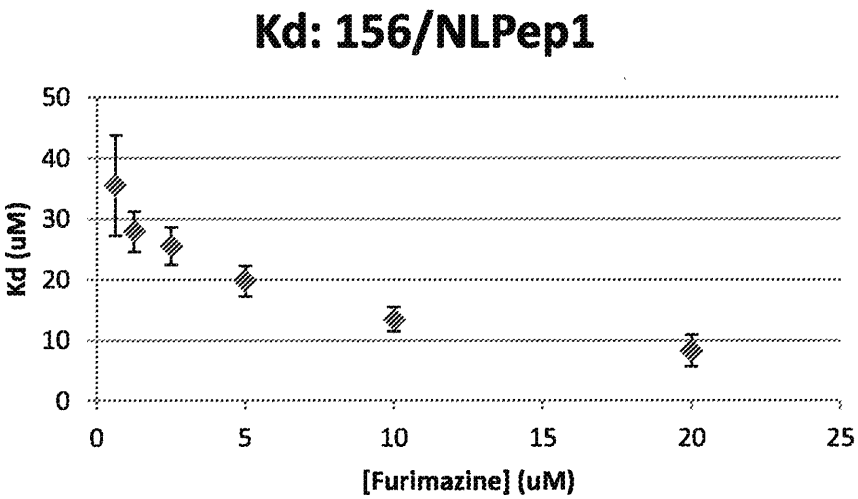
SUBSTITUTE SHEET (RULE 26)

Figure 156



SUBSTITUTE SHEET (RULE 26)

Figure 157


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

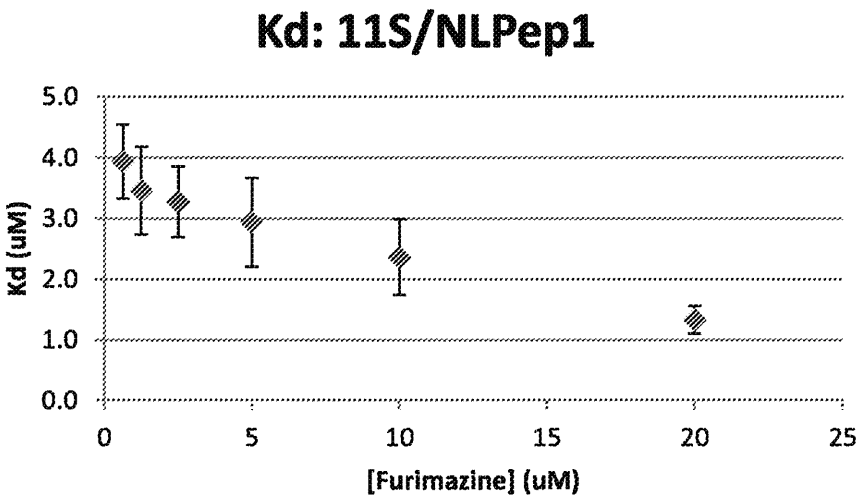
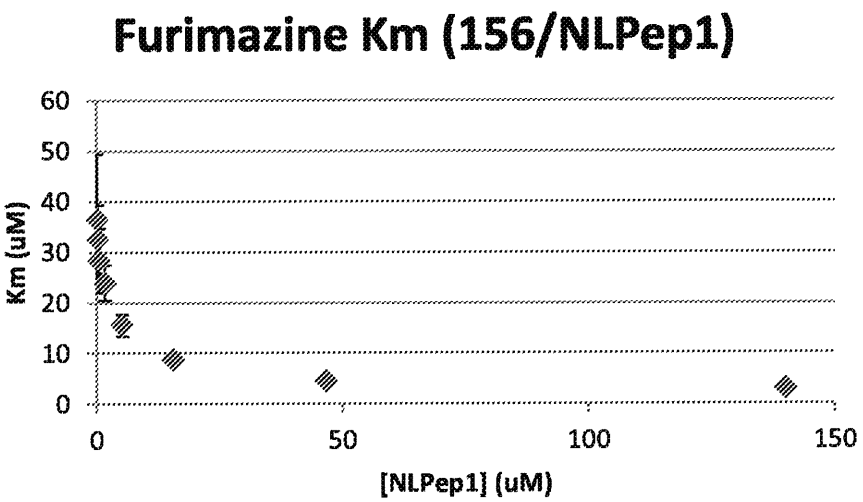

Kd: 11S/NLPep1
Kd (uM)
5.0
4.0
3.0
2.0
1.0
0.0
0
5
10
15
20
25
[Furimazine] (uM)

Figure 158


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

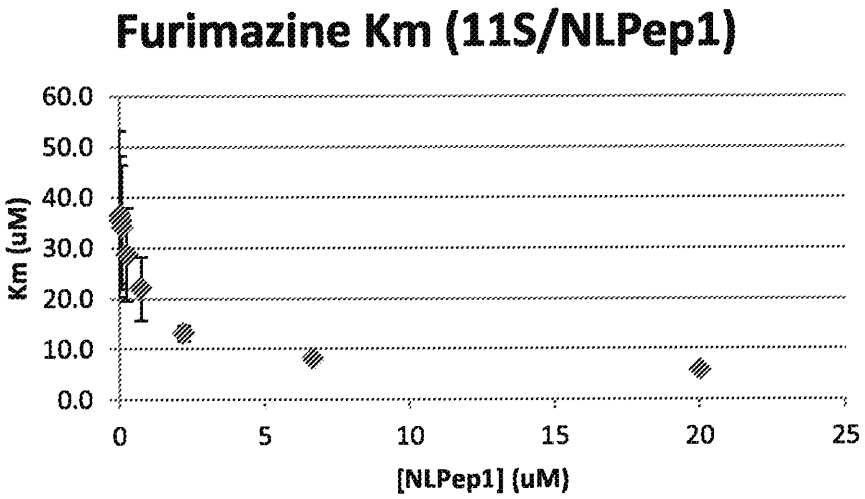
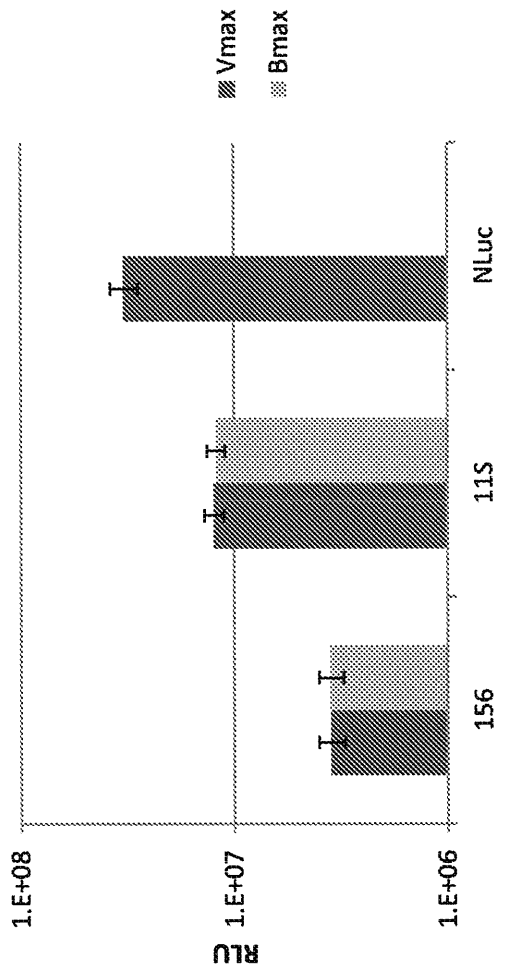
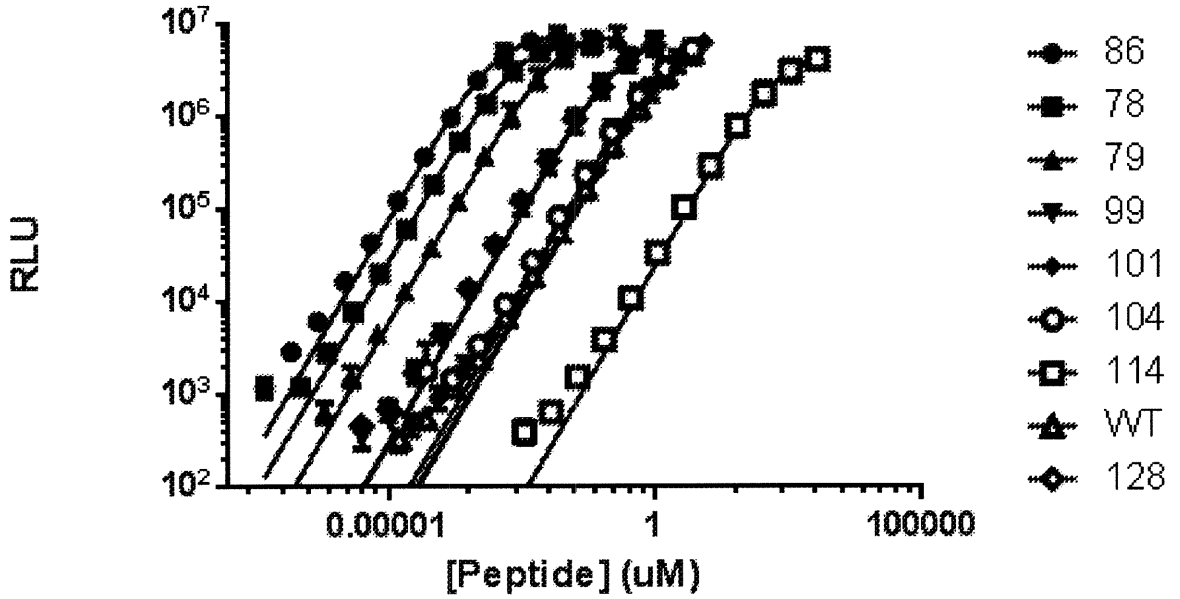

Furimazine Km (11S/NLPep1)
Km (uM)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
5
10
15
20
25
[NLPep1] (uM)

Figure 159



SUBSTITUTE SHEET (RULE 26)

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

SUBSTITUTE SHEET (RULE 26)

Figure 161

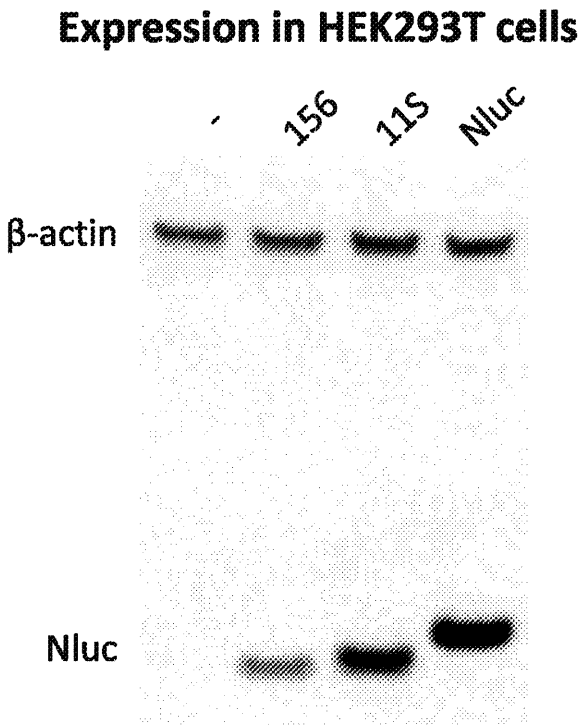
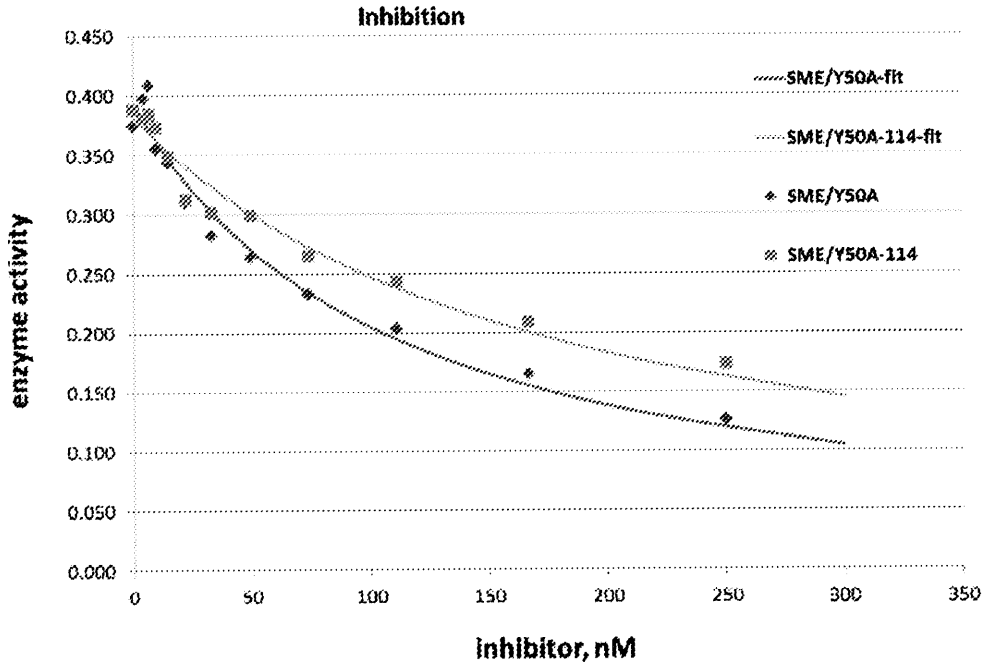
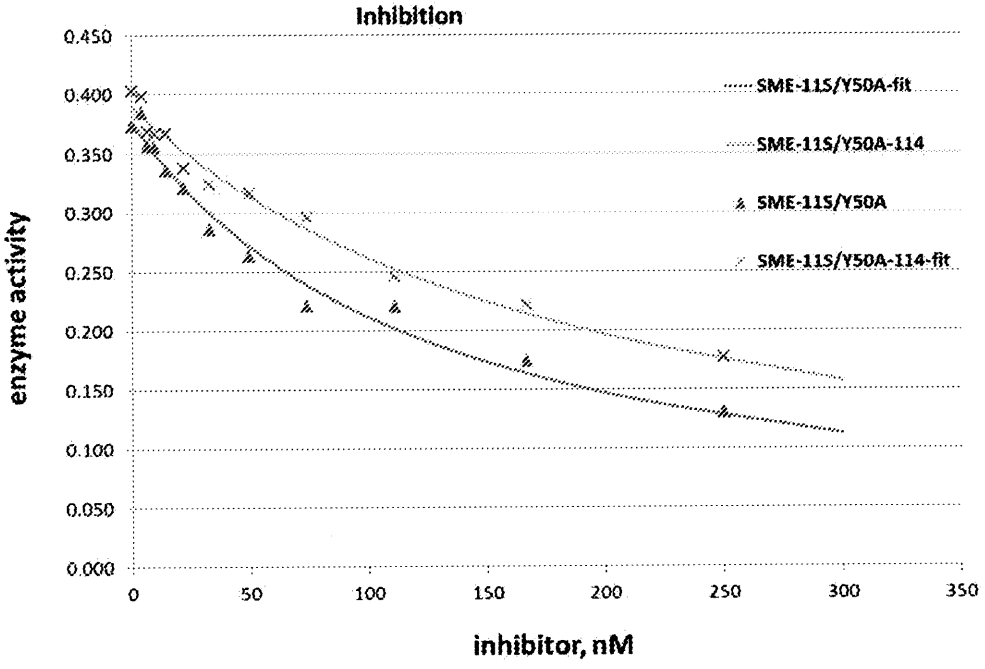
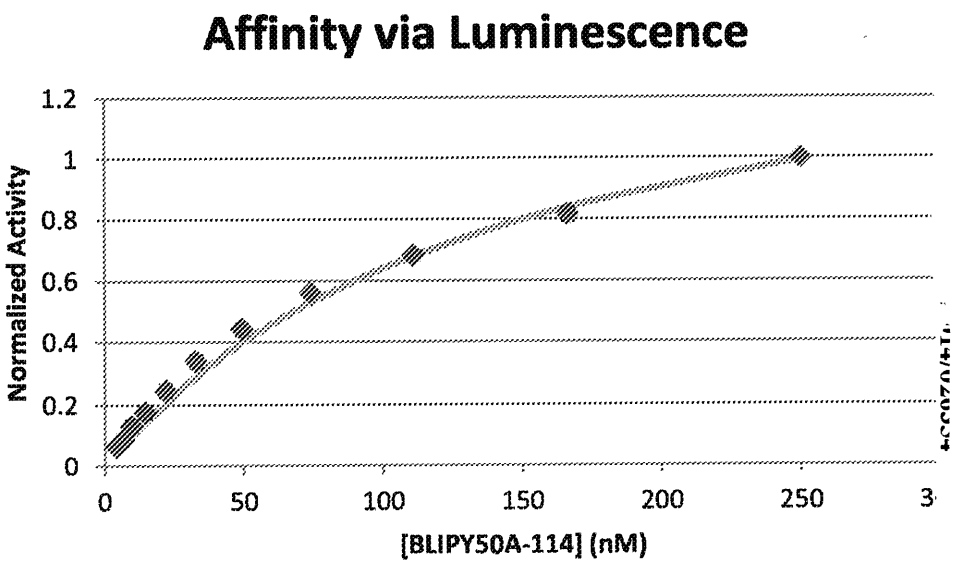


Figure 162



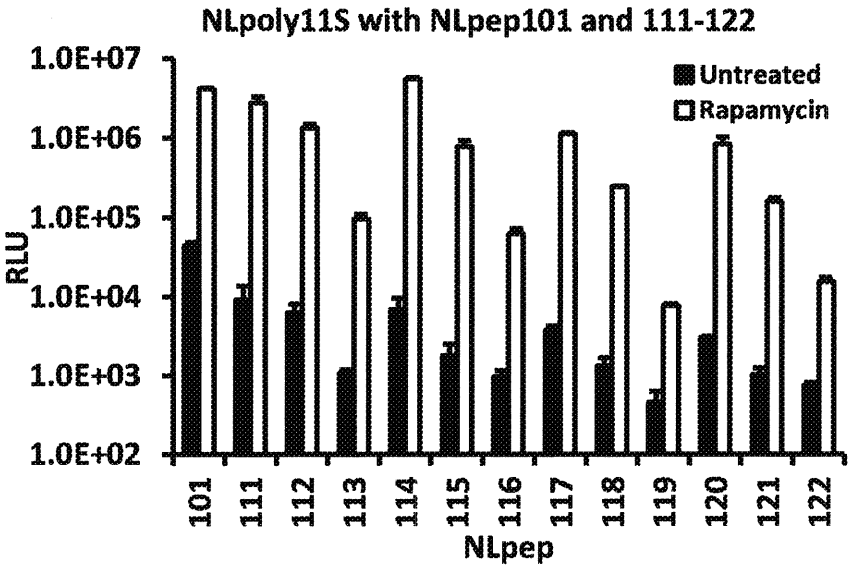


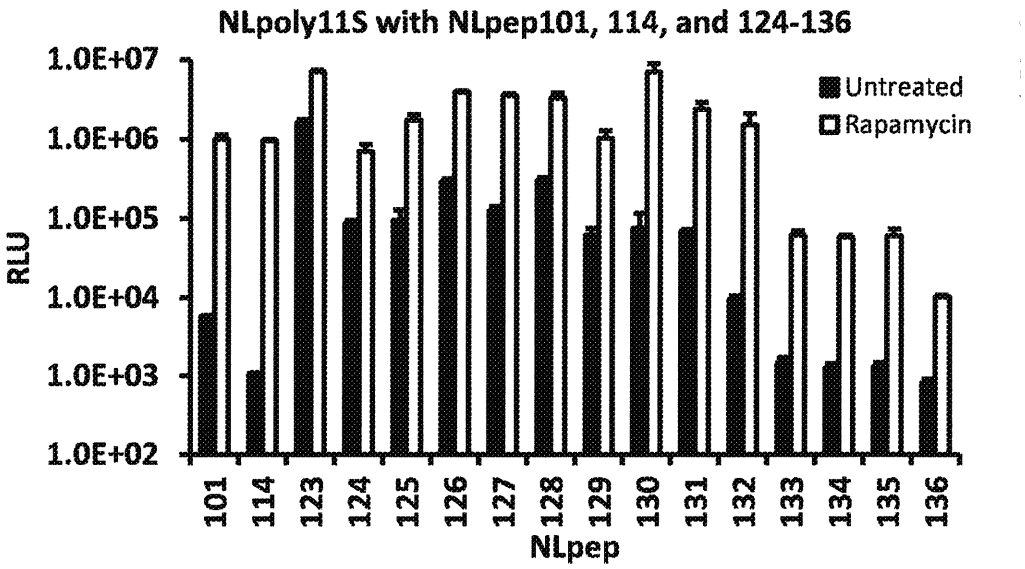
	Inhibition (β-lactamase activity)				Luminescence (NLPep/NLPoly)
SME:	Unfused	+11S	Unfused	+11S	+11S
BLIPY50A:	Unfused	Unfused	+114	+114	+114
Ki/Kd	Ki	Ki	Ki	Ki	Kd
nM	84.1	99.0	142.9	157.3	147.5



SUBSTITUTE SHEET (RULE 26)

Figure 163

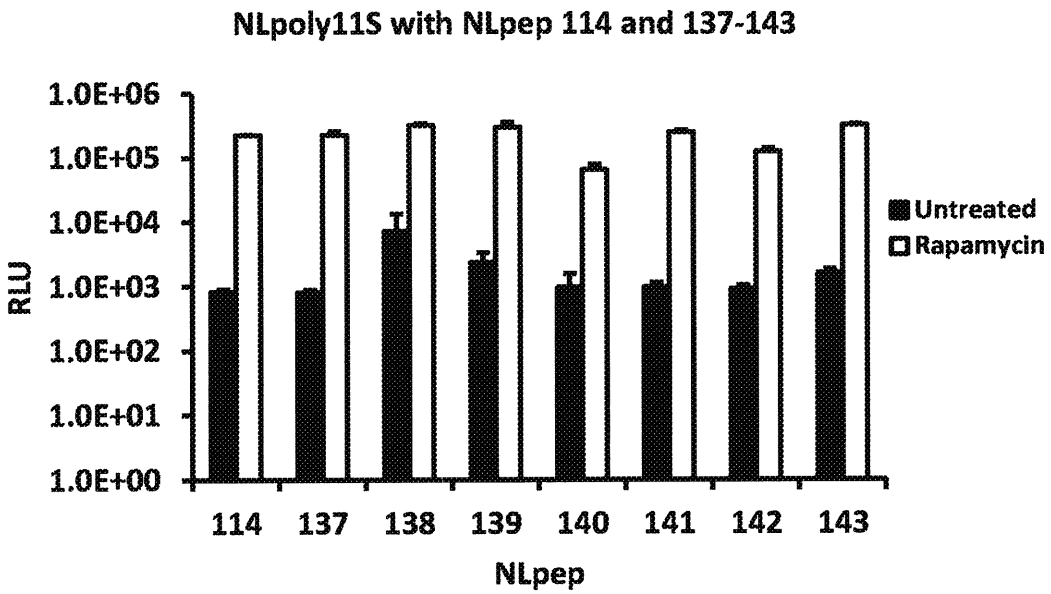




NLpep	Induction	NLpep	Induction
101	92	118	183
111	304	119	17
112	213	120	273
113	90	121	152
114	800	122	21
115	430		
116	64		
117	302		

NLpep	Induction	NLpep	Induction
101	177	129	16
114	887	130	93
123	4	131	35
124	8	132	161
125	18	133	42
126	14	134	46
127	28	135	46
128	11	136	12

Figure 164



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

SUBSTITUTE SHEET (RULE 26)

SUBSTITUTE SHEET (RULE 26)

Figure 10J

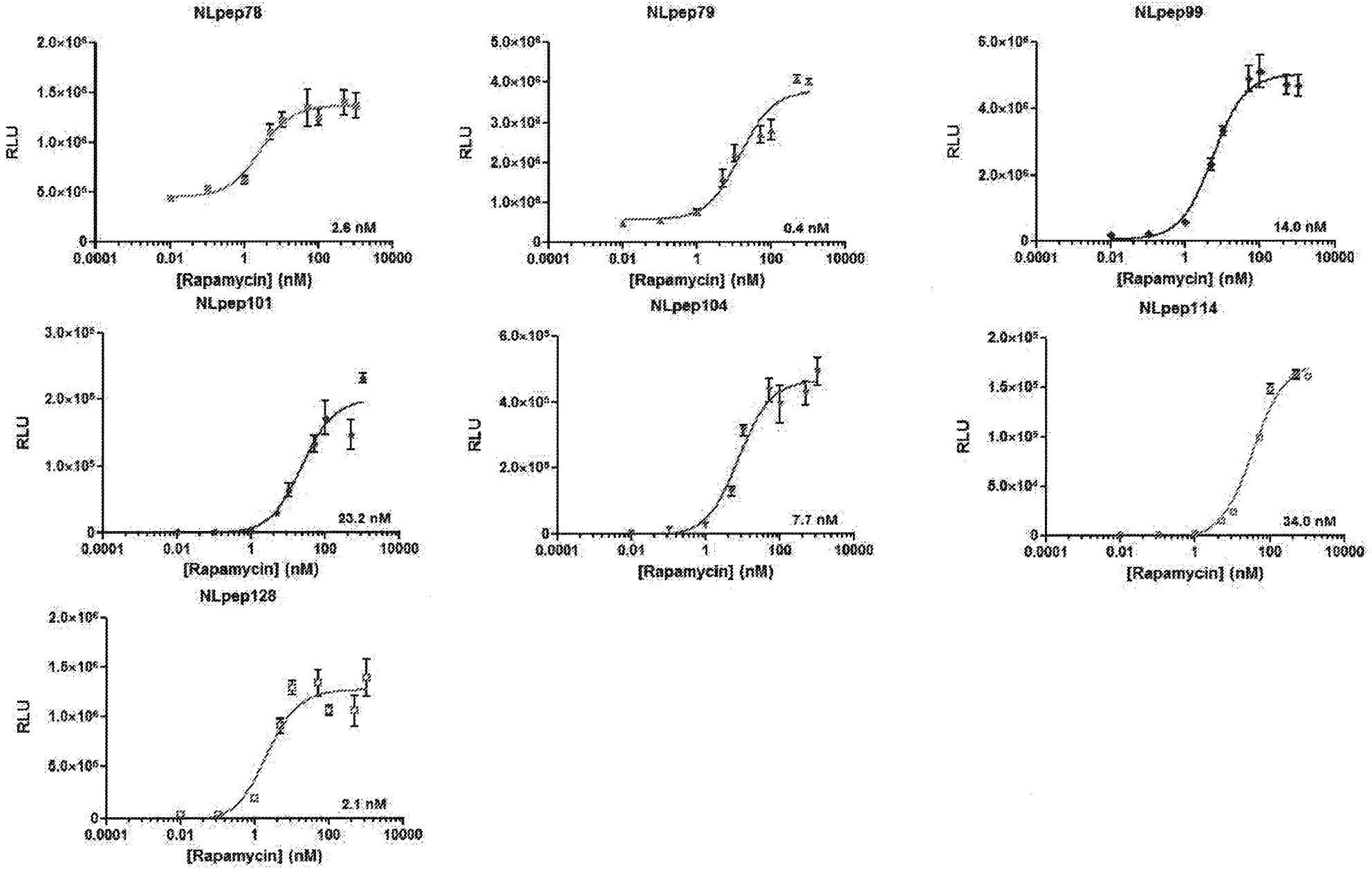
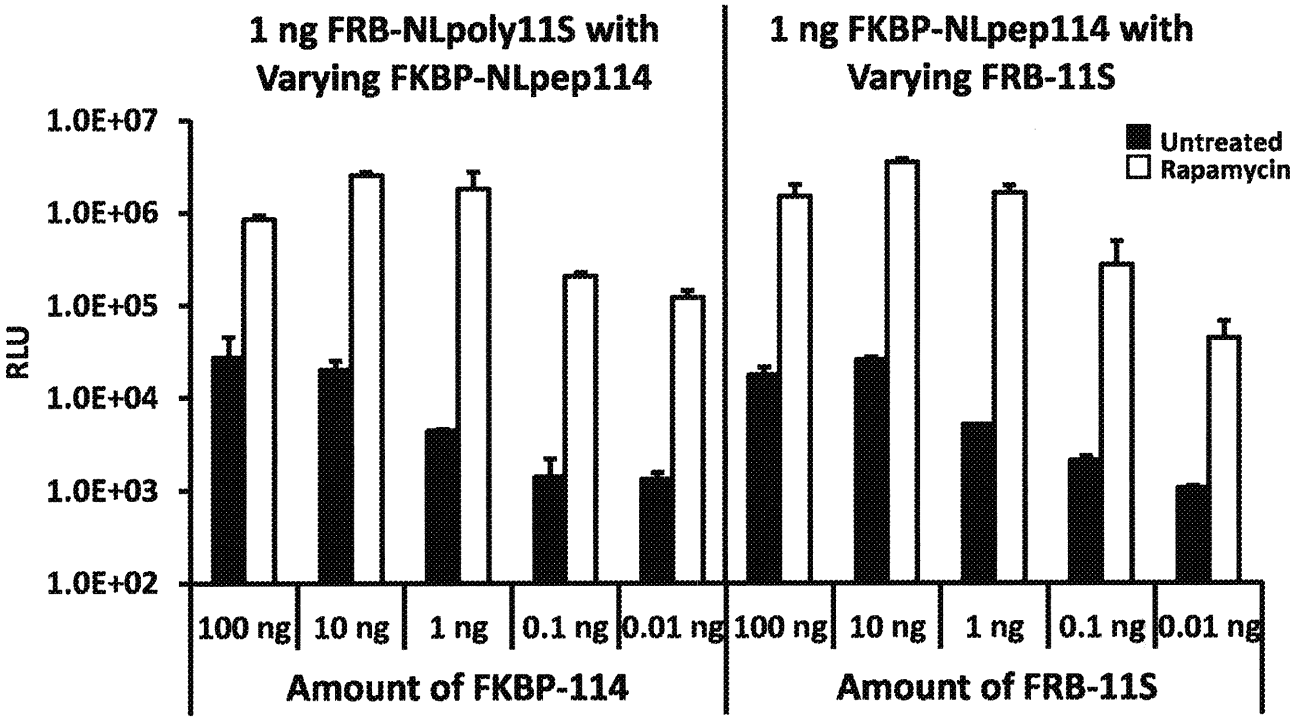


FIGURE 10U

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
1.4 μM

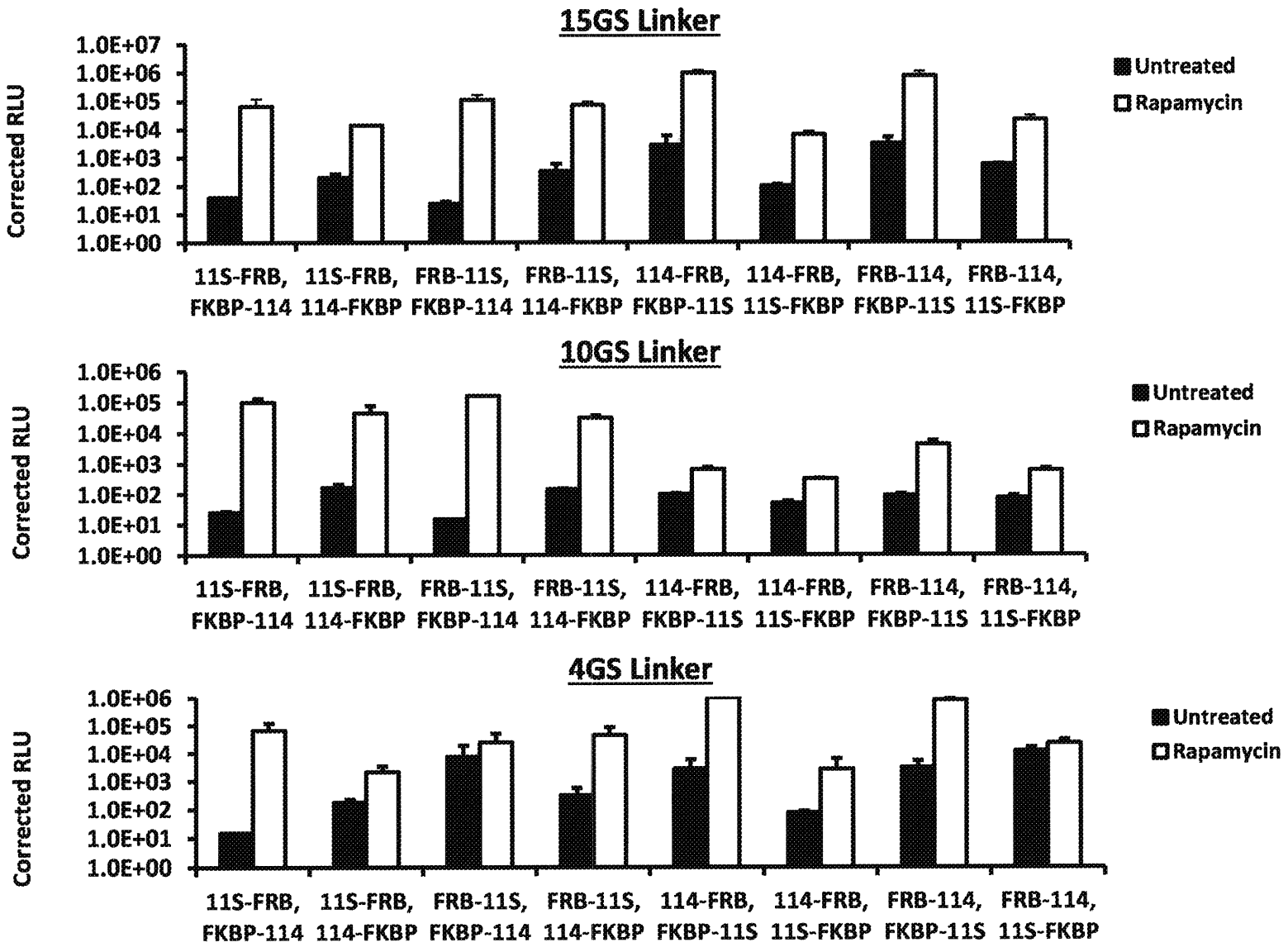
Figure 16/



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

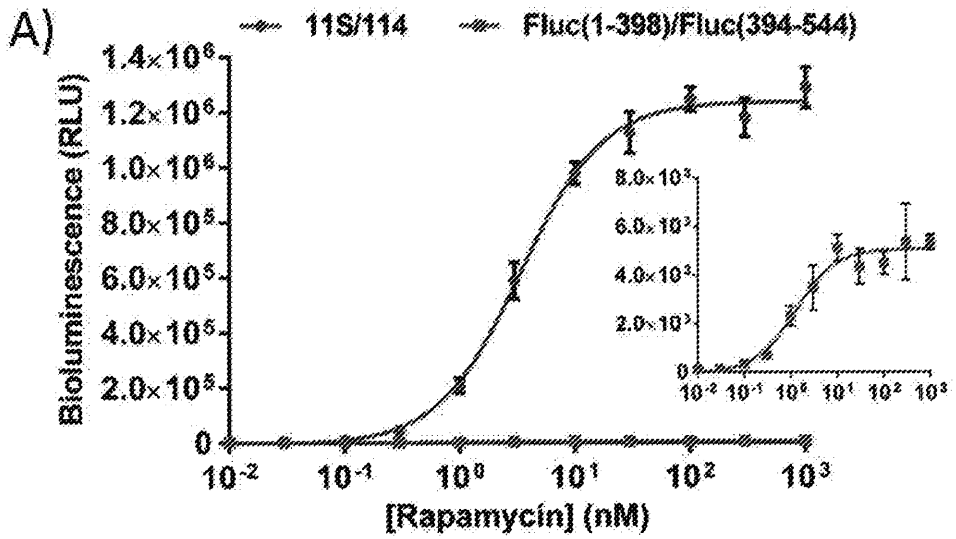
SUBSTITUTE SHEET (RULE 26)

Figure 10B

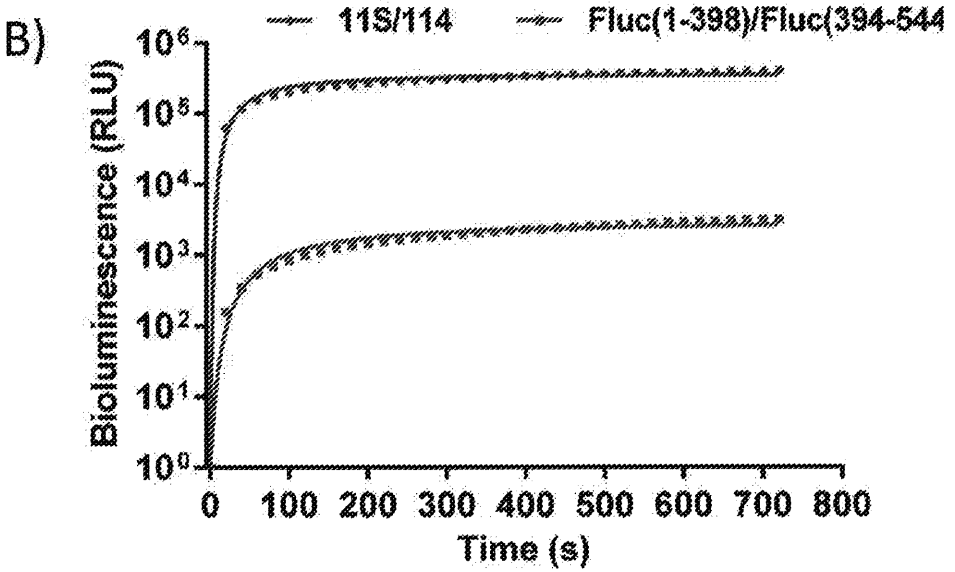


SUBSTITUTE SHEET (RULE 26)

Figure 10B



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

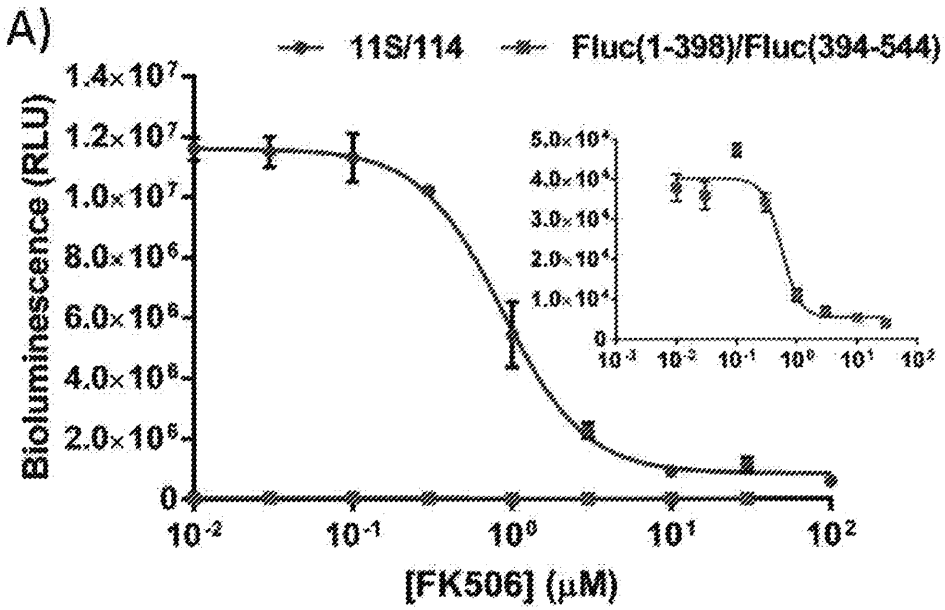


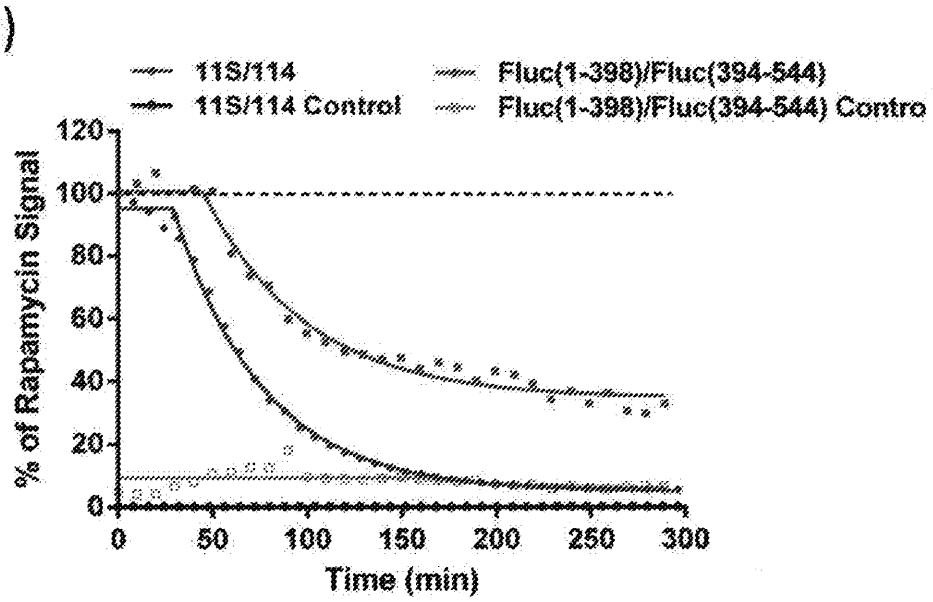
	Bmax	$t_{1/2}$
11S/ 114	3.7×10^5 RLU	3.8 s
Fluc(1-398)/ Fluc(394-544)	3.1×10^3 RLU	15.5 s

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Figure 17C

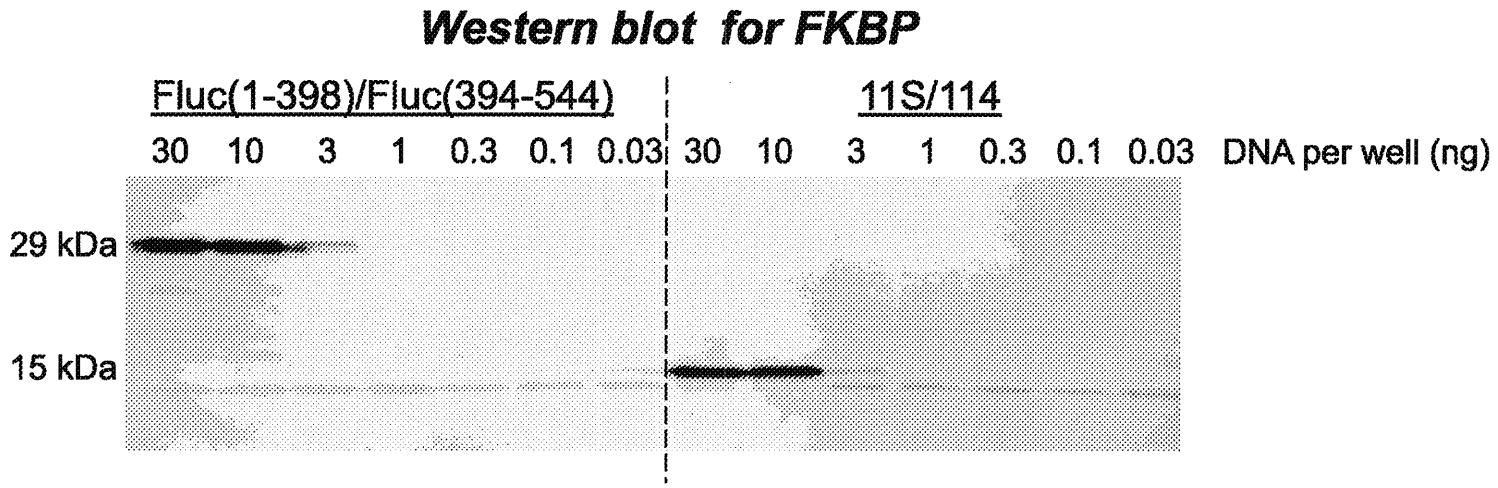




	IC50
11S/ 114	868 nM
Fluc(1-398)/ Fluc(394-544)	550 nM

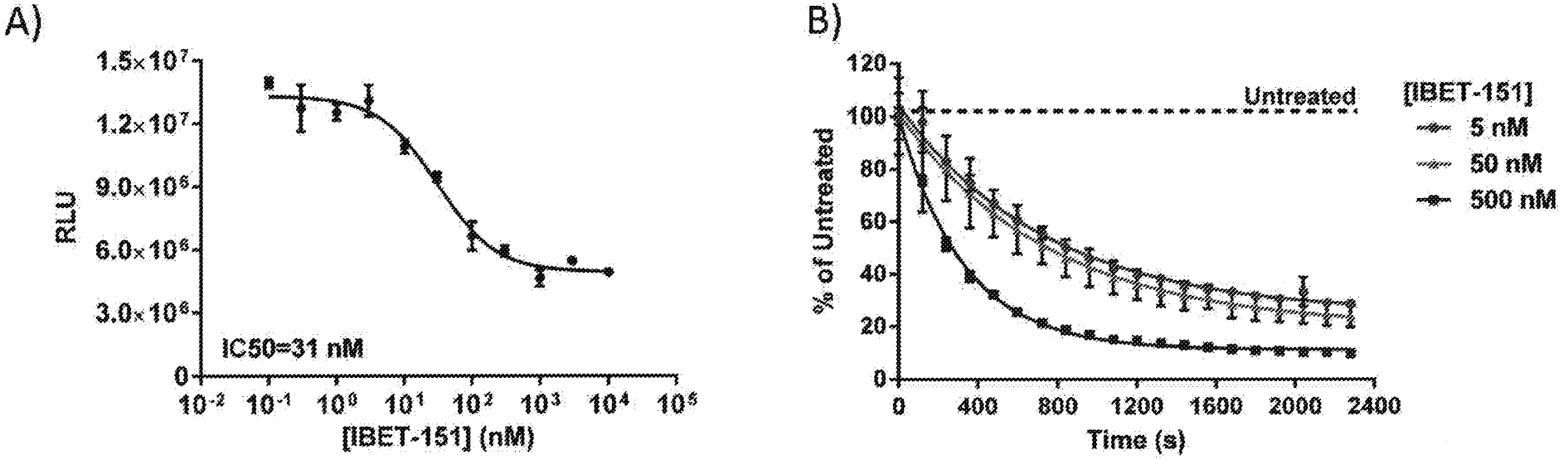
	Lag Time	$t_{1/2}$	Max Inhibition
11S/ 114	5 min	33 min	95%
Fluc(1-398)/ Fluc(394-544)	34 min	54 min	74%

Figure 171



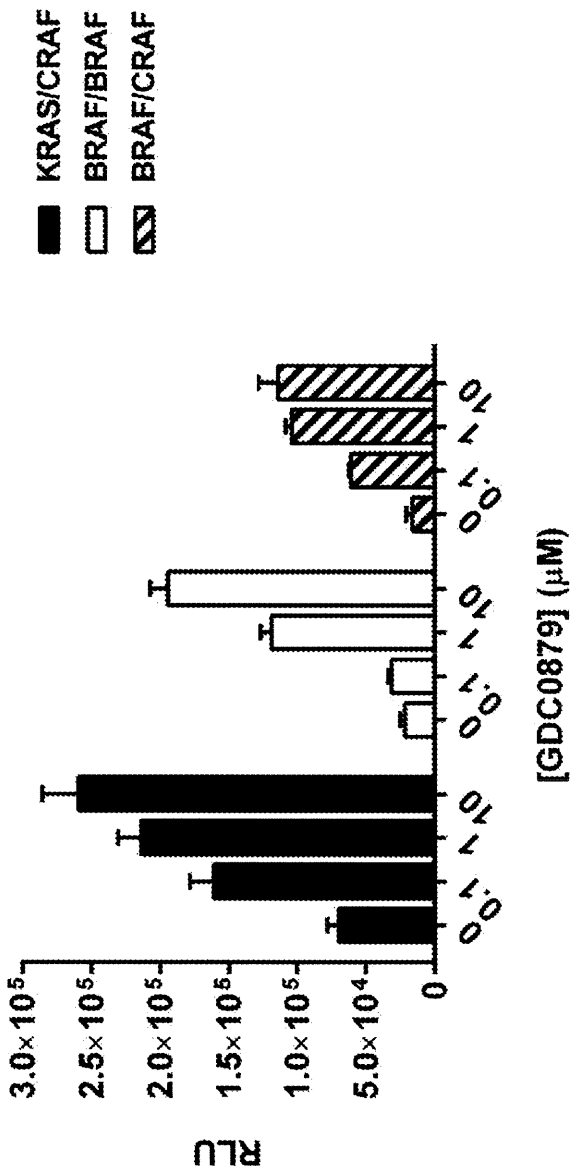
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Figure 17



SUBSTITUTE SHEET (RULE 26)

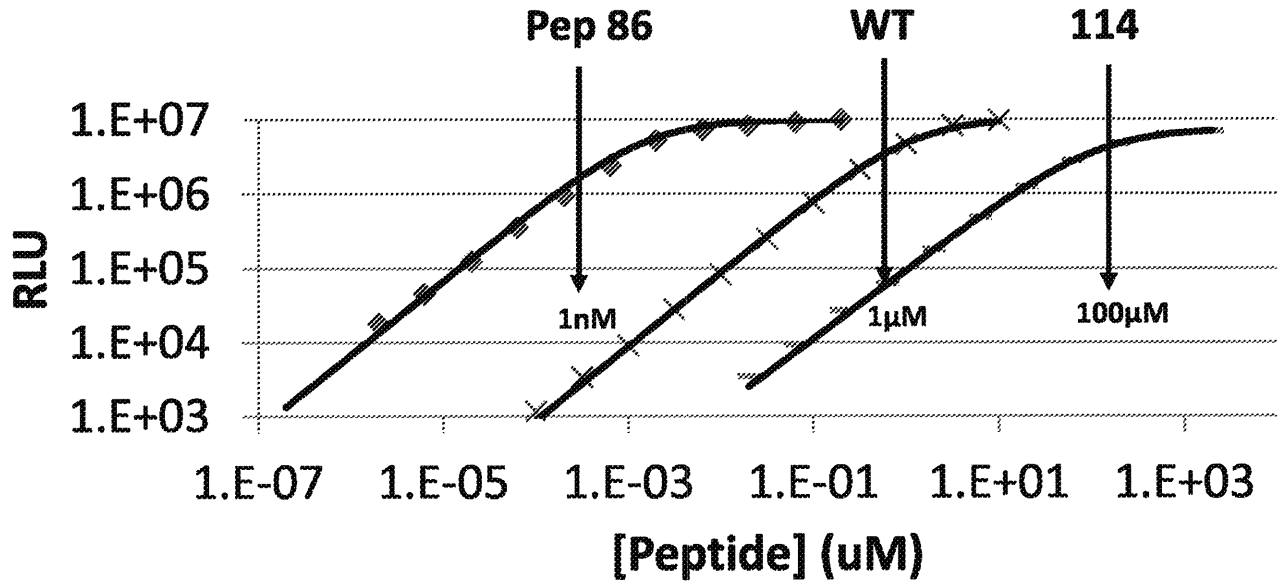
Figure 173



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Figure 174

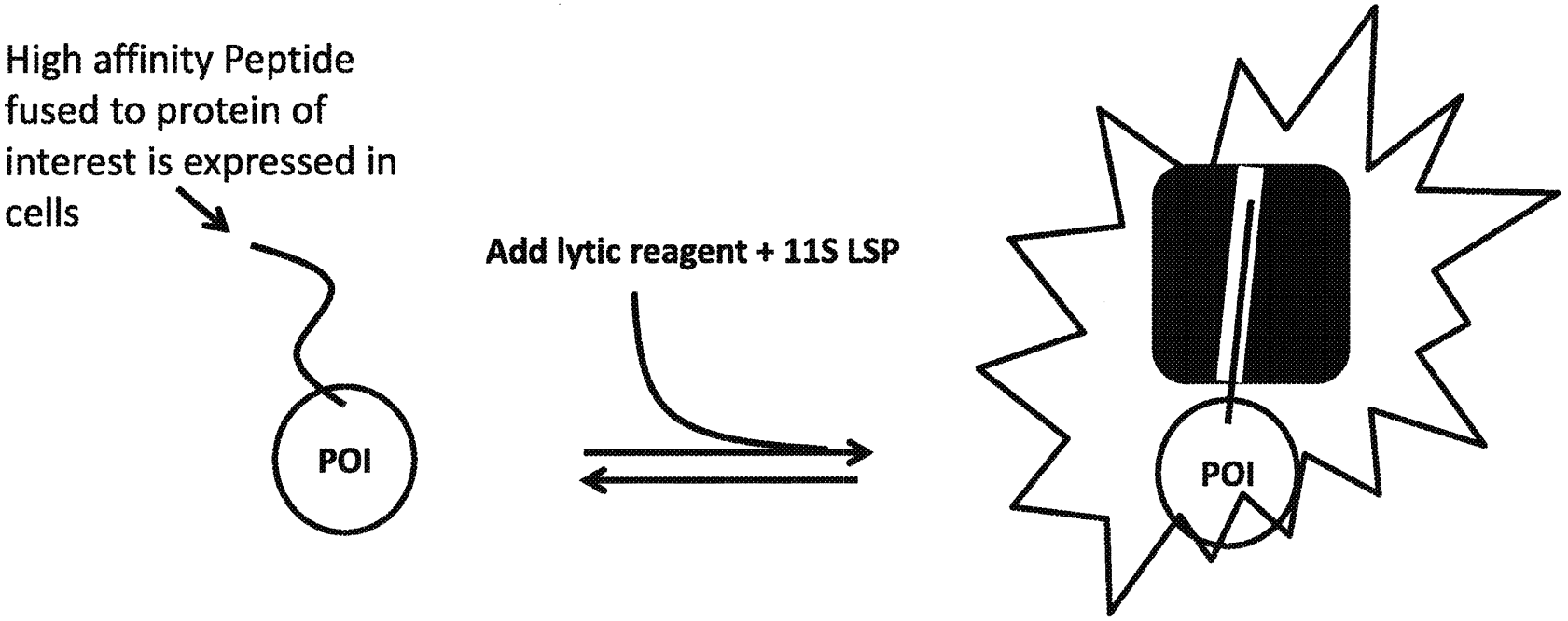
		1	2	3	4	5	6	7	8	9	10	11	12	13
	WT	G	V	T	G	W	R	L	C	E	R	I	L	A
High	86	-	V	S	G	W	R	L	F	K	K	I	S	-
Low	114	-	V	T	G	Y	R	L	F	E	E	I	L	-



Need protocol, how much 11S

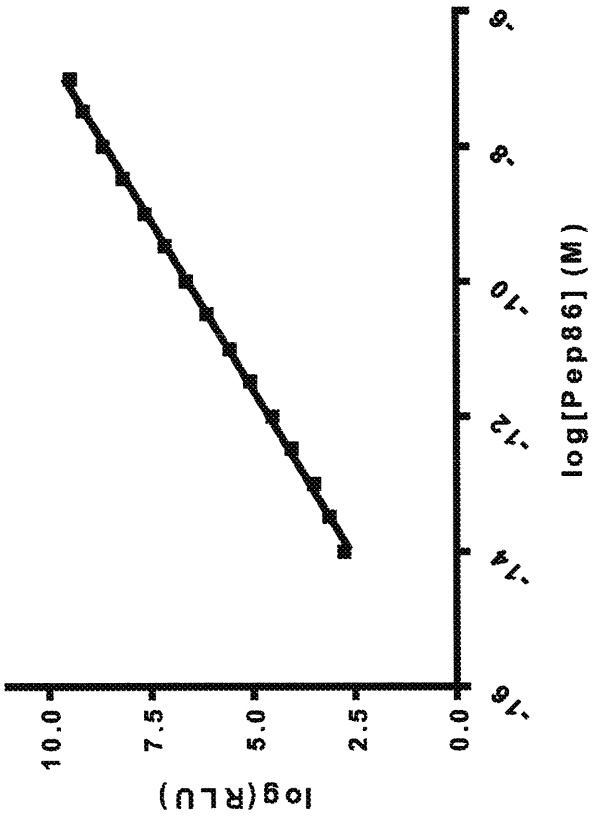
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Figure 175



SUBSTITUTE SHEET (RULE 26)

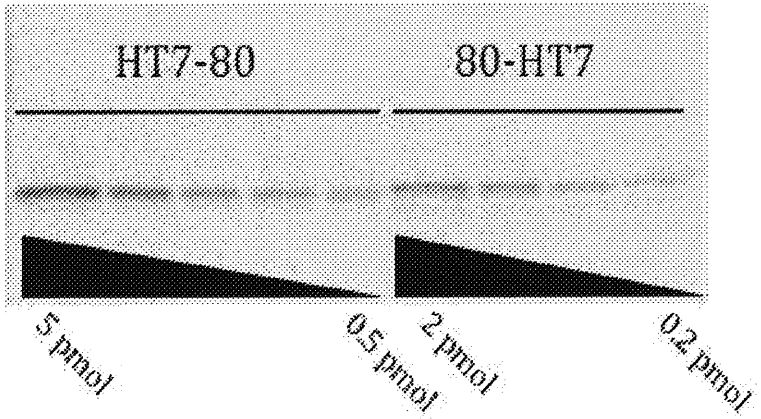
Figure 176



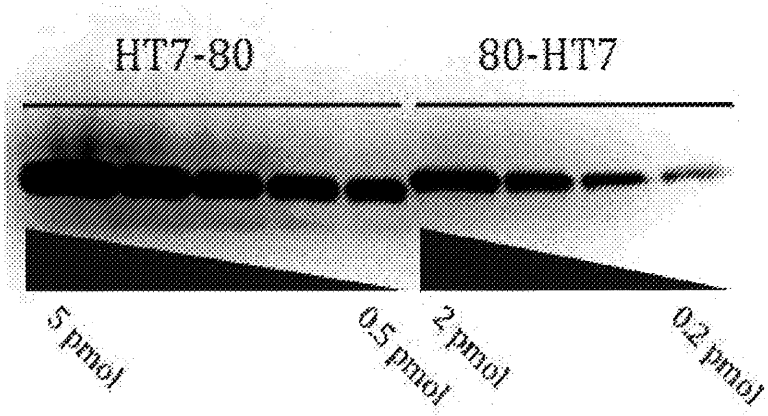
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Figure 11

TMR image

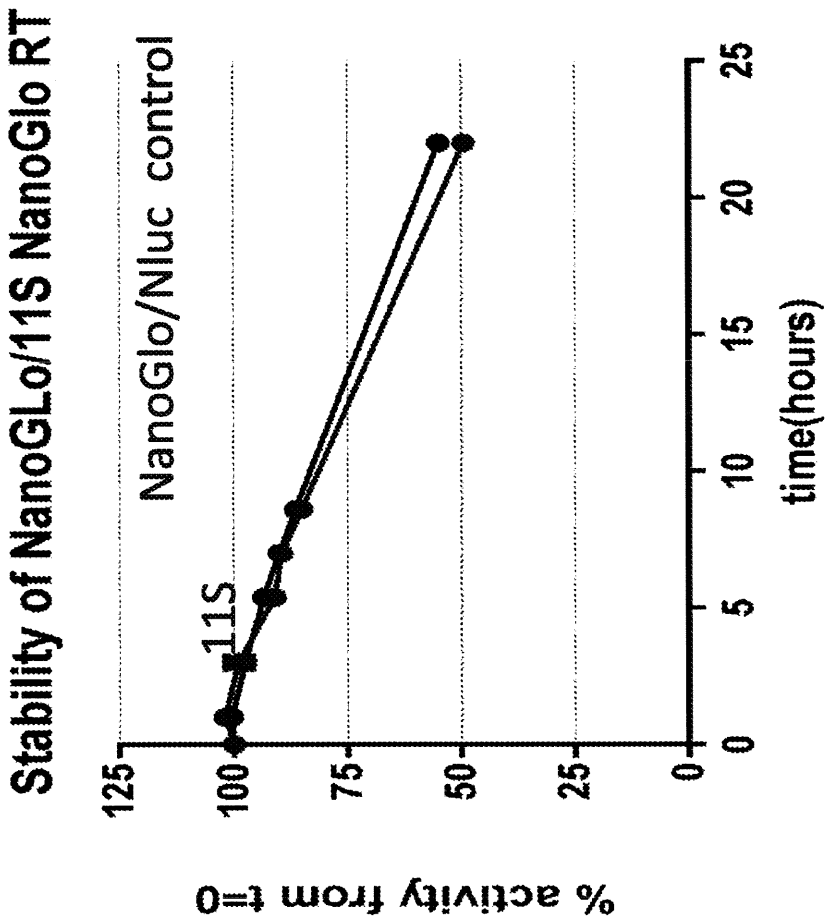


"11S+Fz" blot
(Pseudo Western)



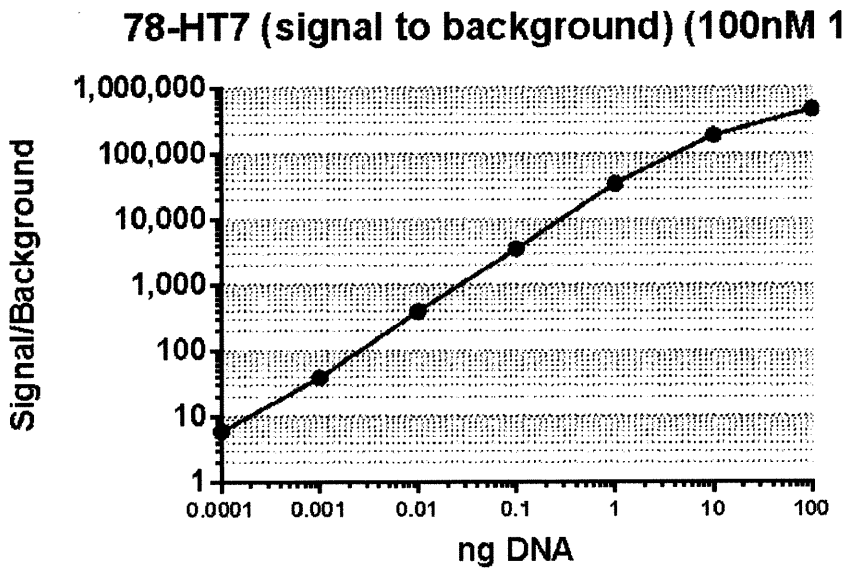
SUBSTITUTE SHEET (RULE 26)

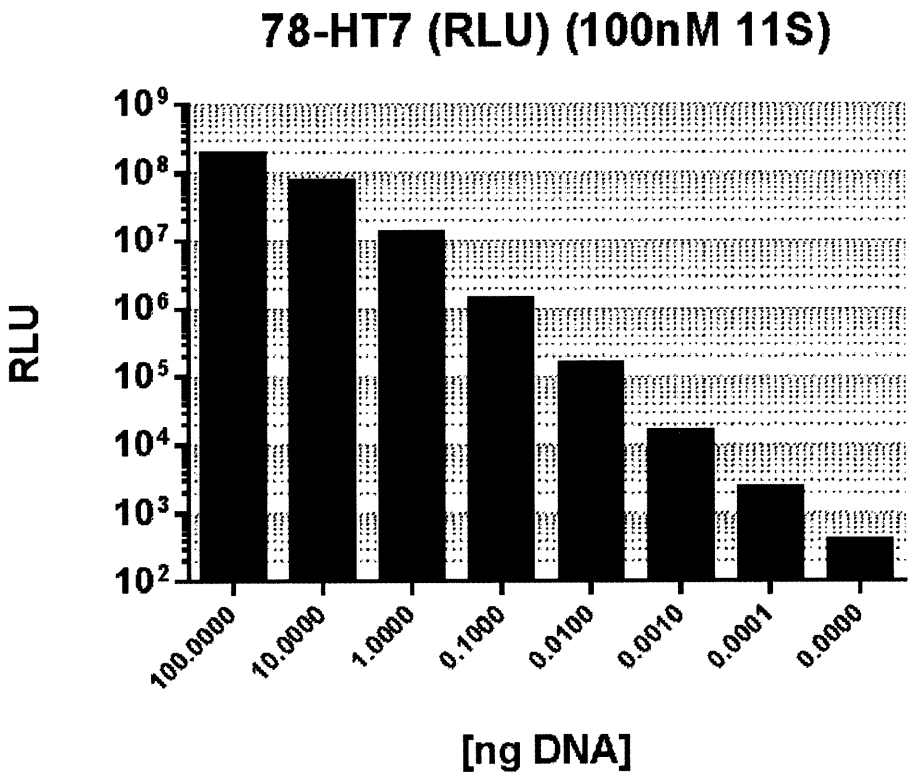
Figure 1/8



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Figure 179





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Figure 180

High affinity (spontaneous) peptides

				157		159		161		163		165		167		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		159		161		163		165		167		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			
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

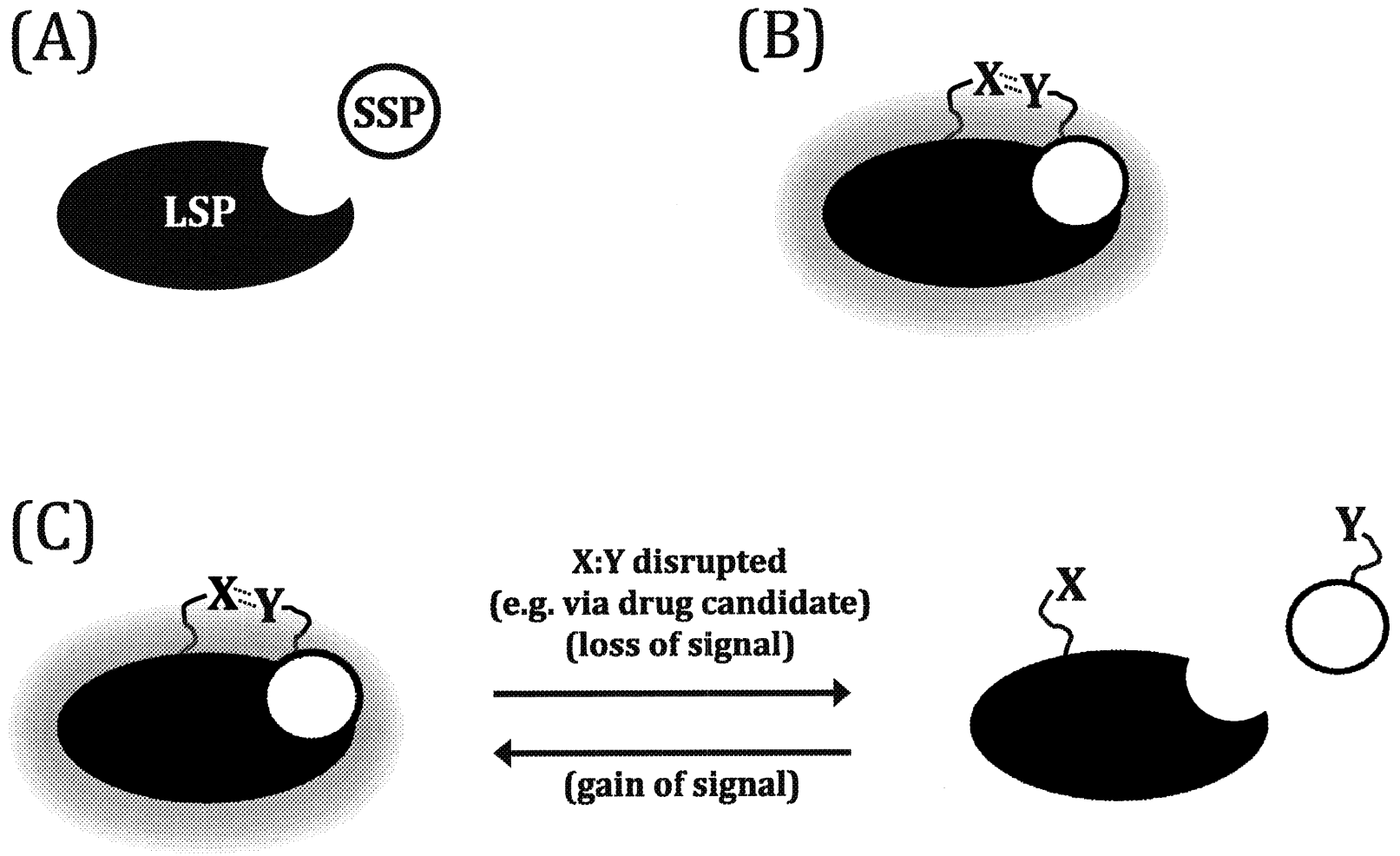
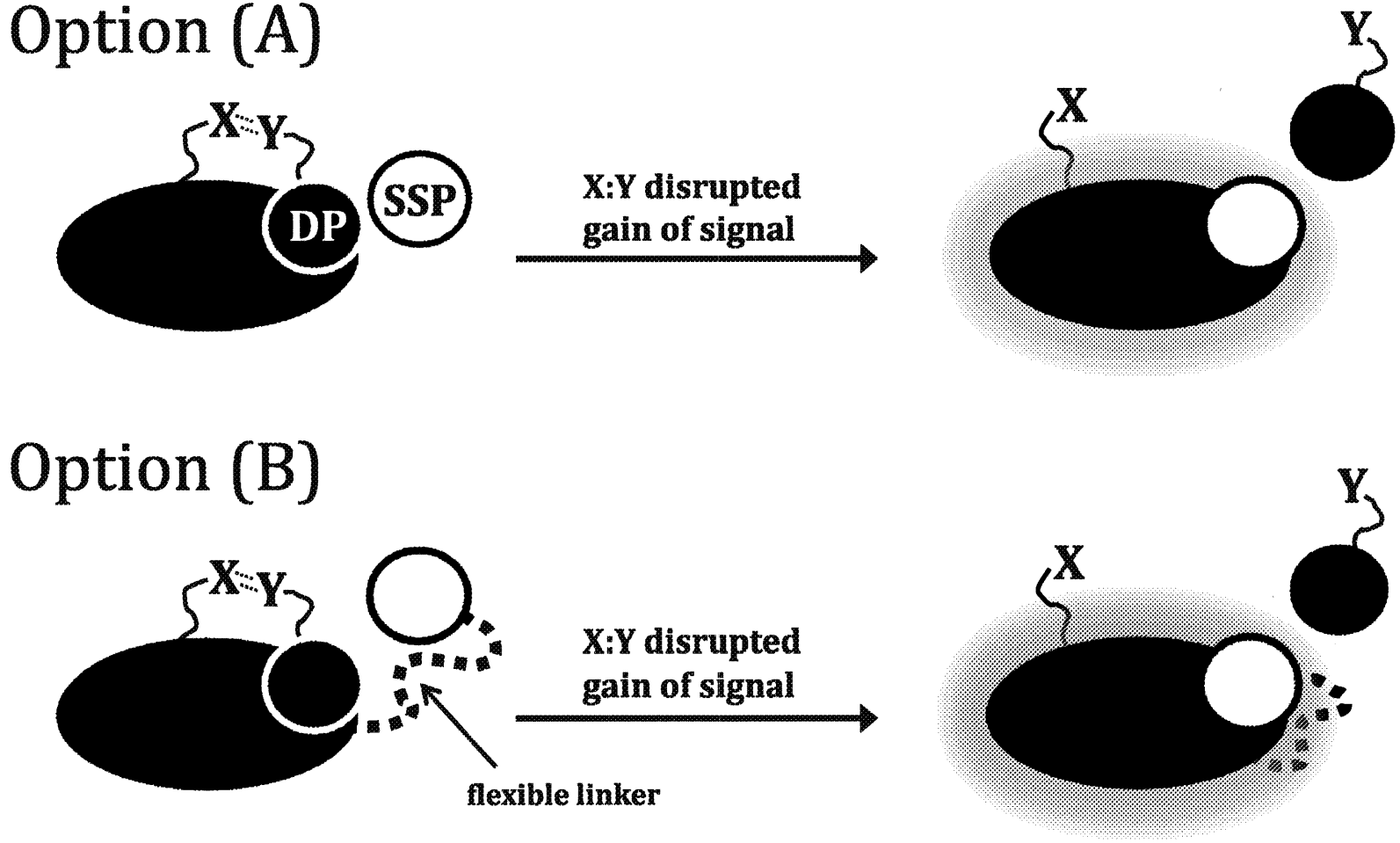

(A)
SSP
LSP
(B)
X
Y
(C)
X
Y
X:Y disrupted
(e.g. via drug candidate)
(loss of signal)
(gain of signal)
X
Y

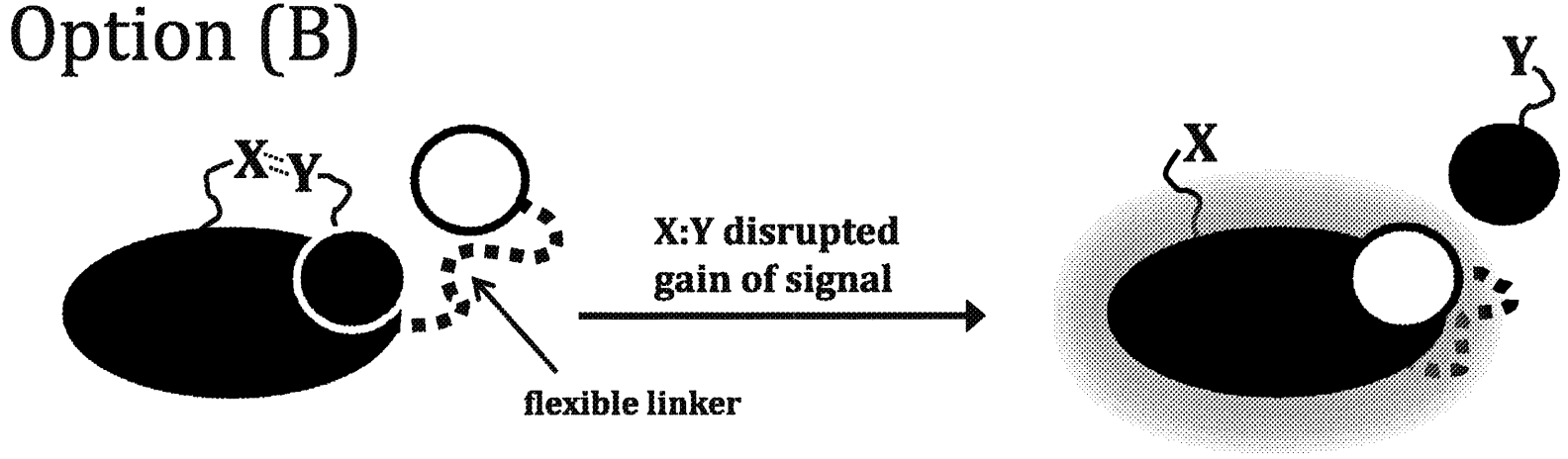
Figure 182A

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



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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)

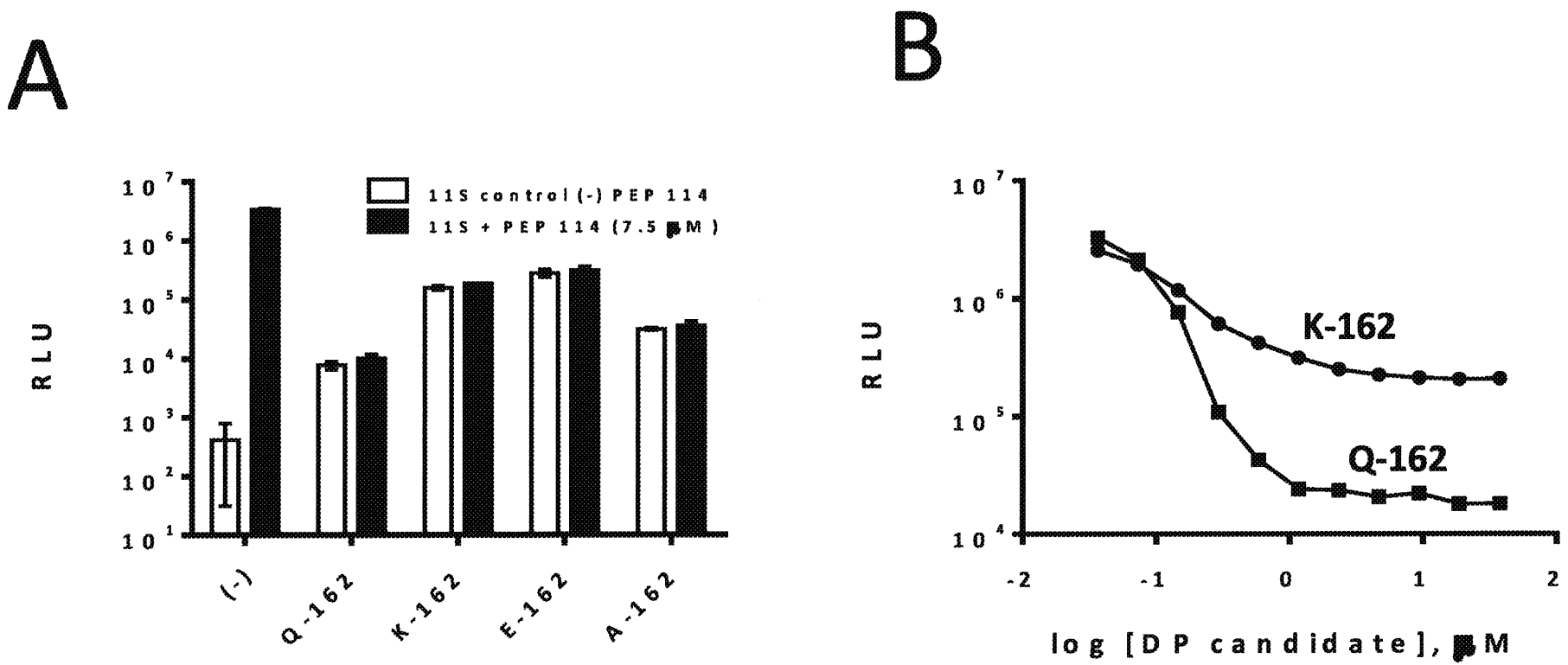
X-NLuc(1-156) – 15aa Gly-Ser linker – NLuc(157-169)
X-NLuc(1-156) – 15aa Gly-Ser linker – NLuc(158-168)
X-NLuc(1-156) – 15aa Gly-Ser linker – NLuc(160-168)
X-NLuc(1-156) – 15aa Gly-Ser linker – NLuc(158-166)
X-NLuc(1-156) – 15aa Gly-Ser linker – NLuc(160-166)

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)

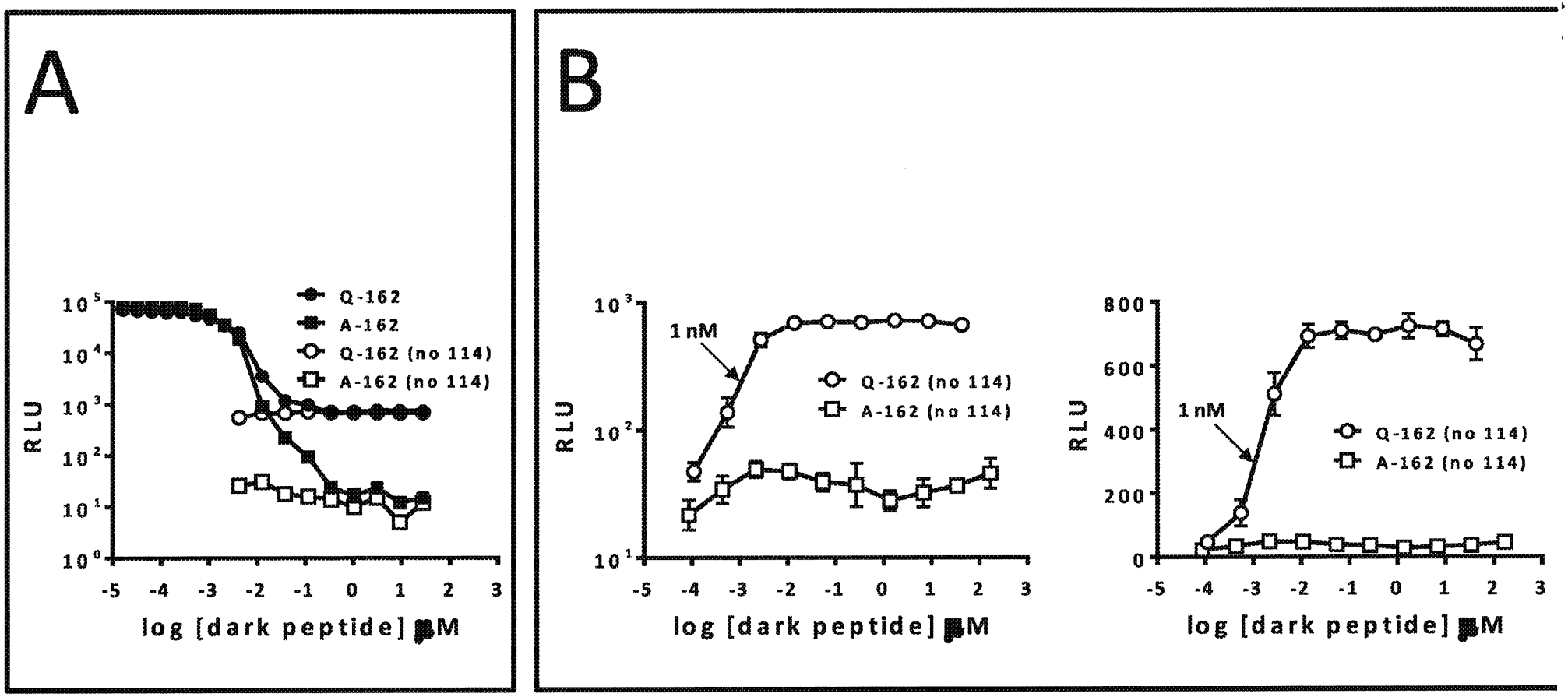
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Figure 183



Q-162	VSGW-**Q**-LFKKIS
A-162	VSGW-**A**-LFKKIS
K-162	VSGW-**K**-LFKKIS
E-162	VSGW-**E**-LFKKIS

Figure 184



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Figure 185

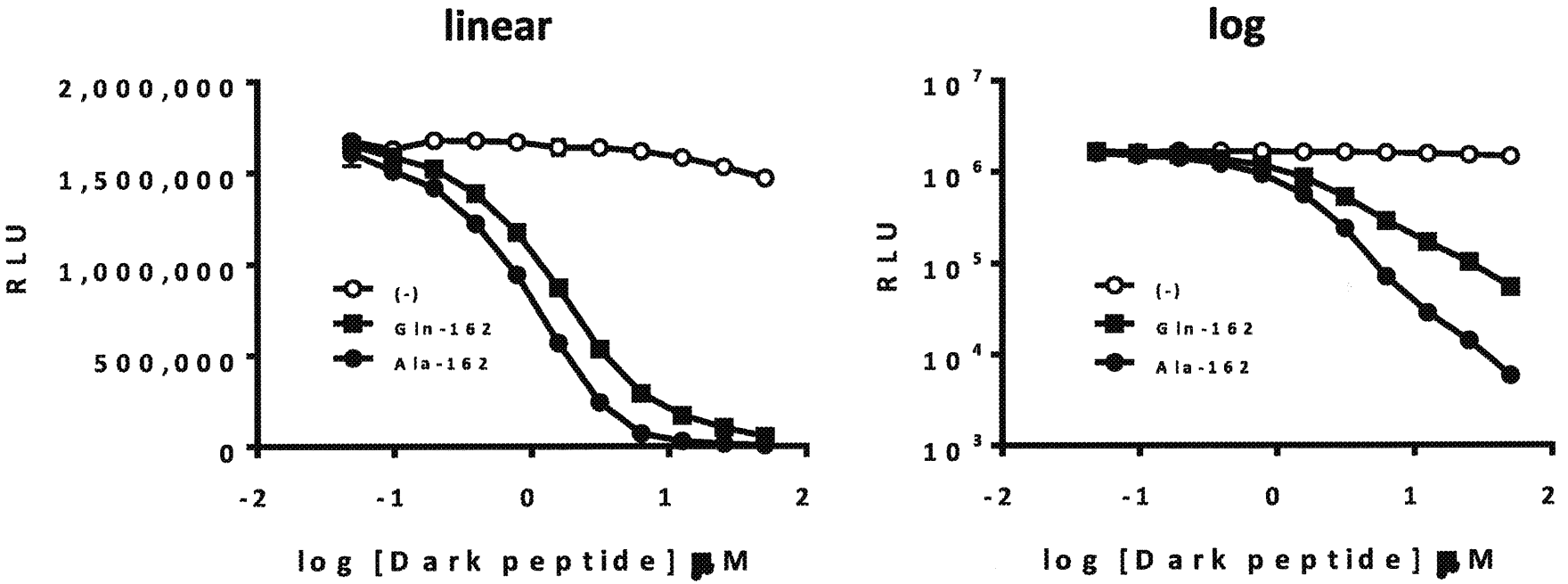
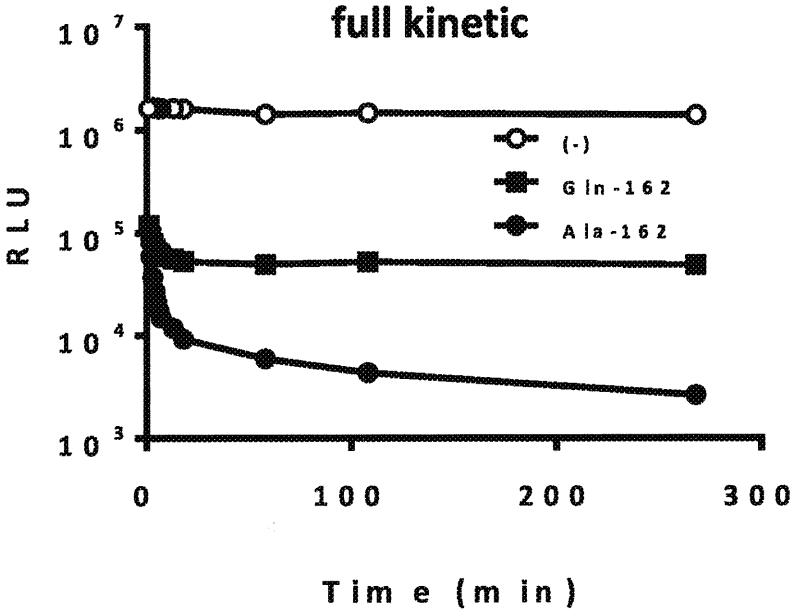
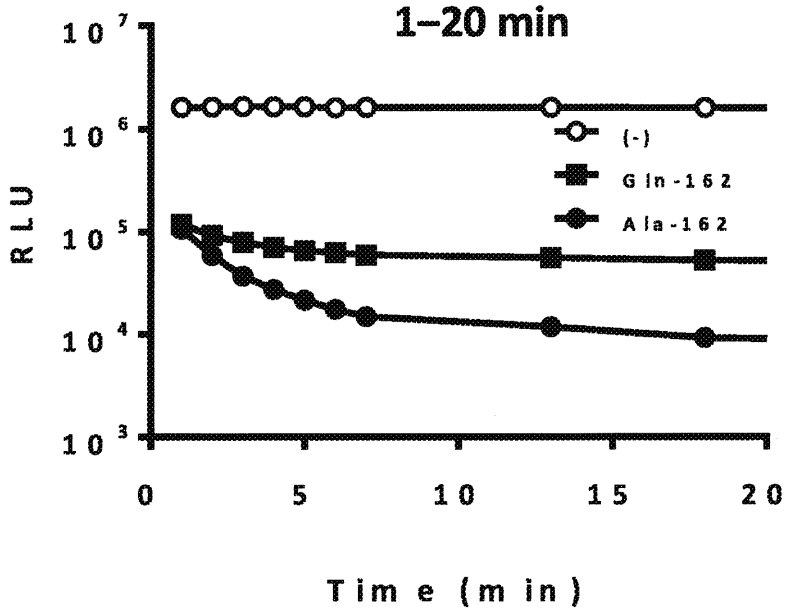
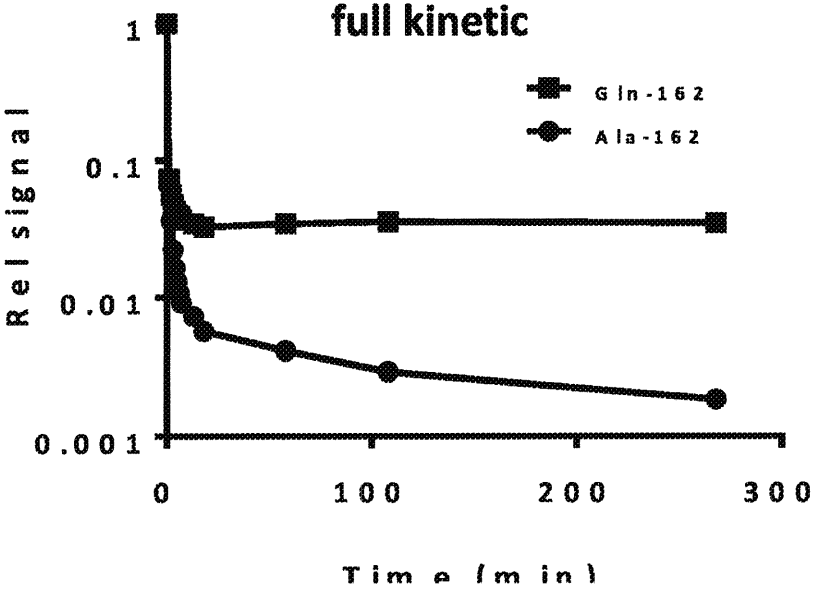

linear
2,000,000
1,500,000
1,000,000
500,000
0
RLU
(-)
Gln-162
Ala-162
-2
-1
0
1
2
log [Dark peptide] M
log
10 7
10 6
10 5
10 4
10 3
RLU
(-)
Gln-162
Ala-162
-2
-1
0
1
2
log [Dark peptide] M

Figure 186


full kinetic
RLU
(-)
Gln-162
Ala-162
Time (min)


1–20 min
RLU
(-)
Gln-162
Ala-162
Time (min)


full kinetic
Rel signal
Gln-162
Ala-162
Time (min)

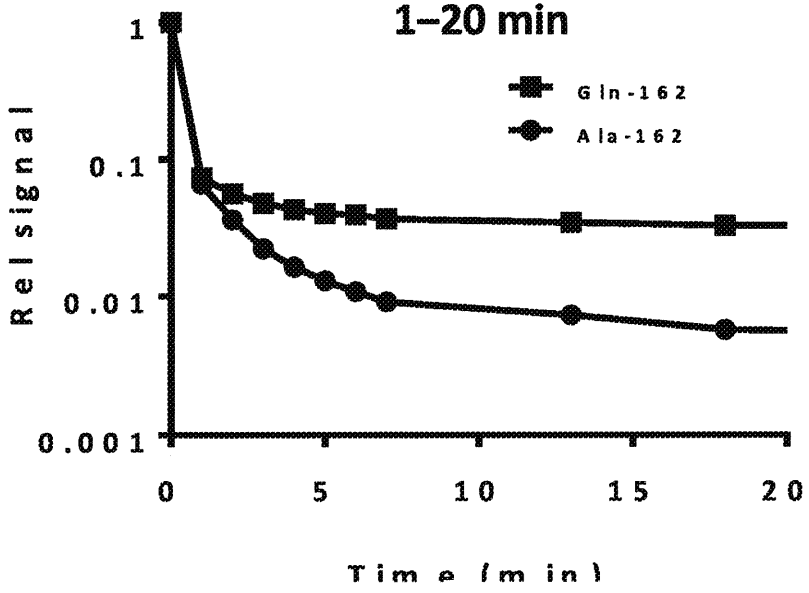
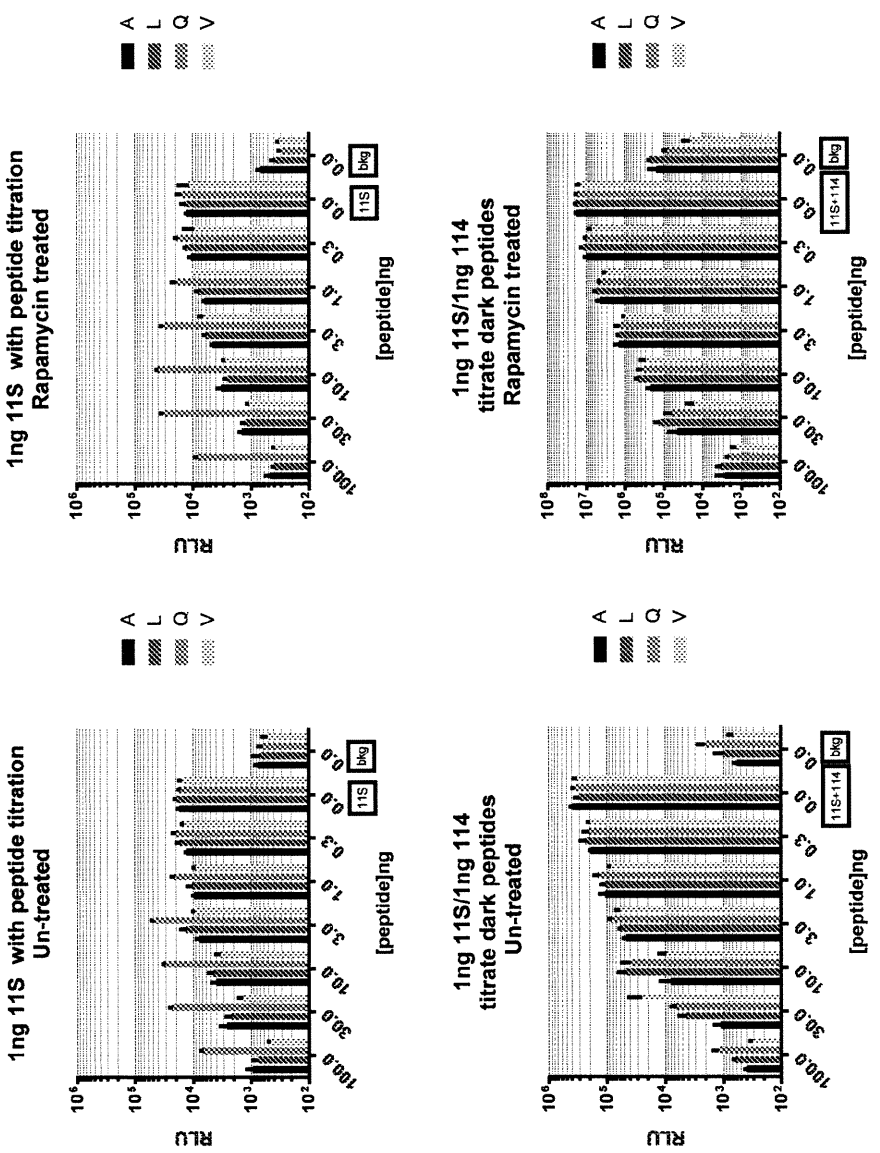

1–20 min
Rel signal
Gln-162
Ala-162
Time (min)

Figure 187



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Figure 188

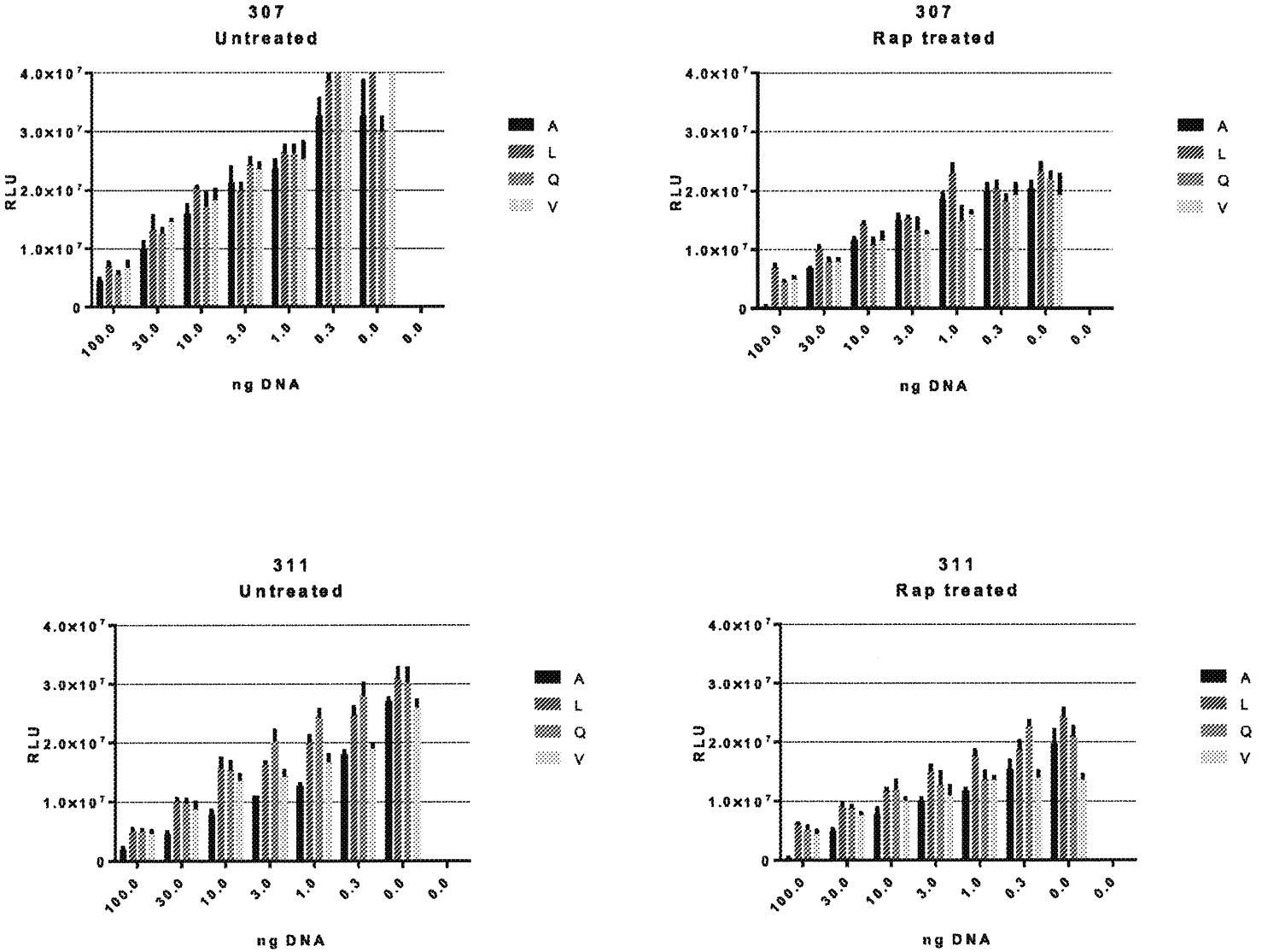
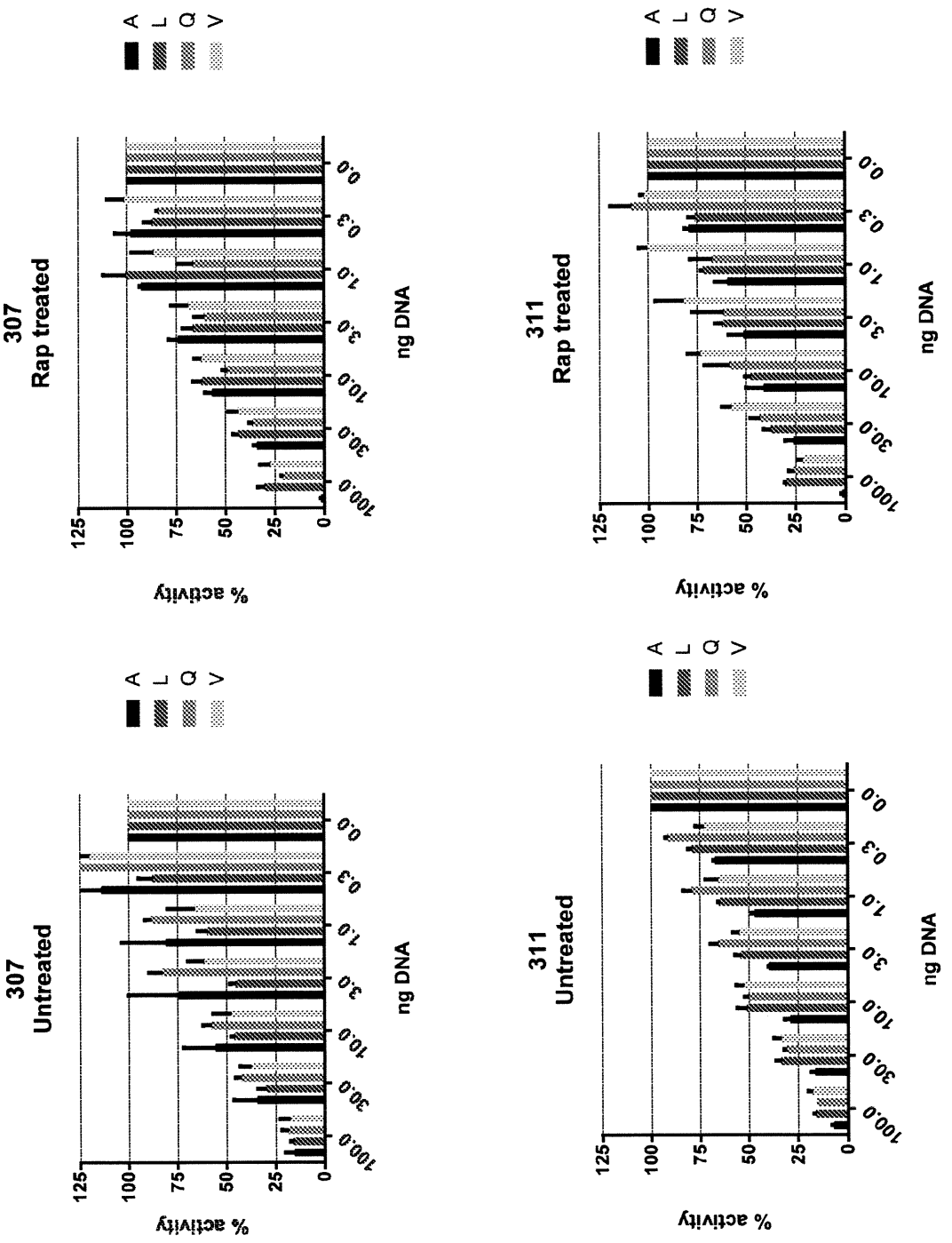
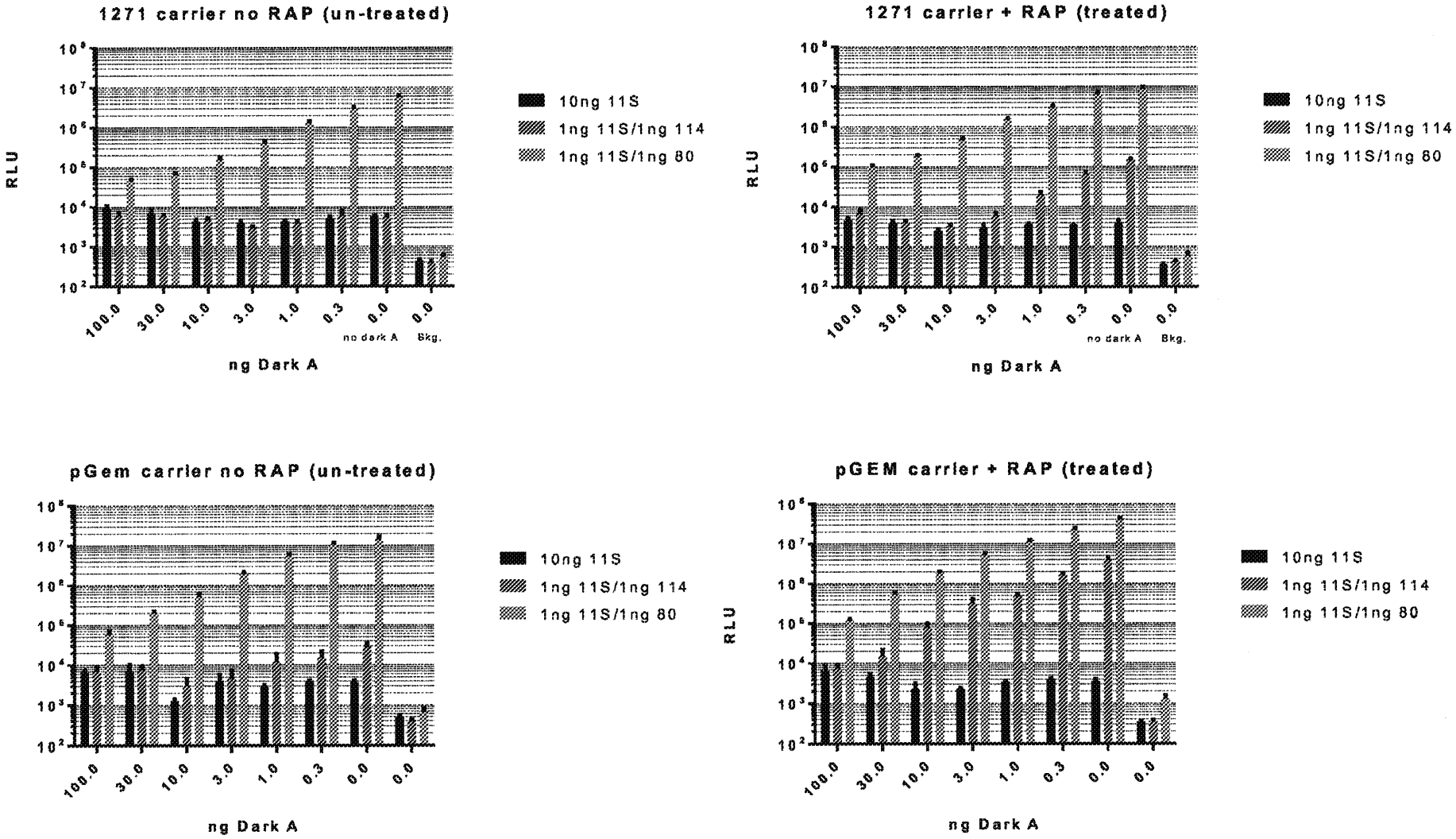


Figure 189



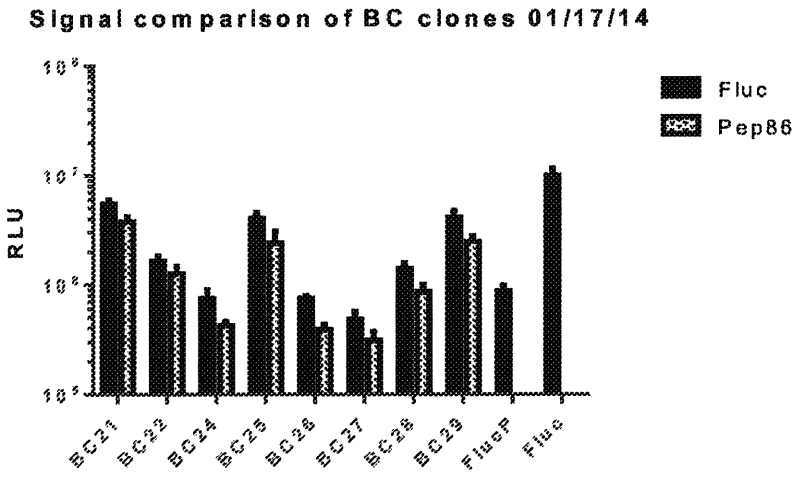
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Figure 190

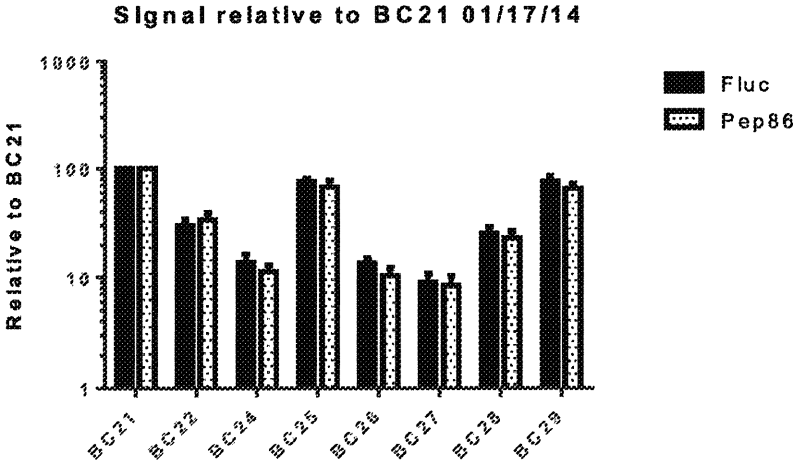


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Figure 191

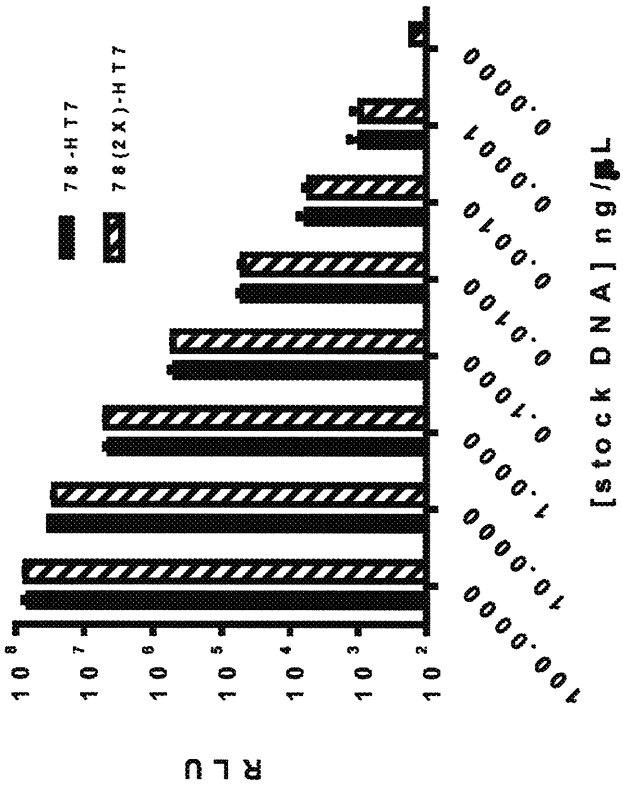


pBC21	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-Fluc (high affinity)
pBC22	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-FlucP (high affinity)
pBC24	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-Fluc-CL1 (high affinity)
pBC25	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-Fluc-PEST12 opt (high affinity)
pBC26	pFC15A/MVSGWRLFKKIS-GGSGGGGSGG-Fluc-CP (high affinity)
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)



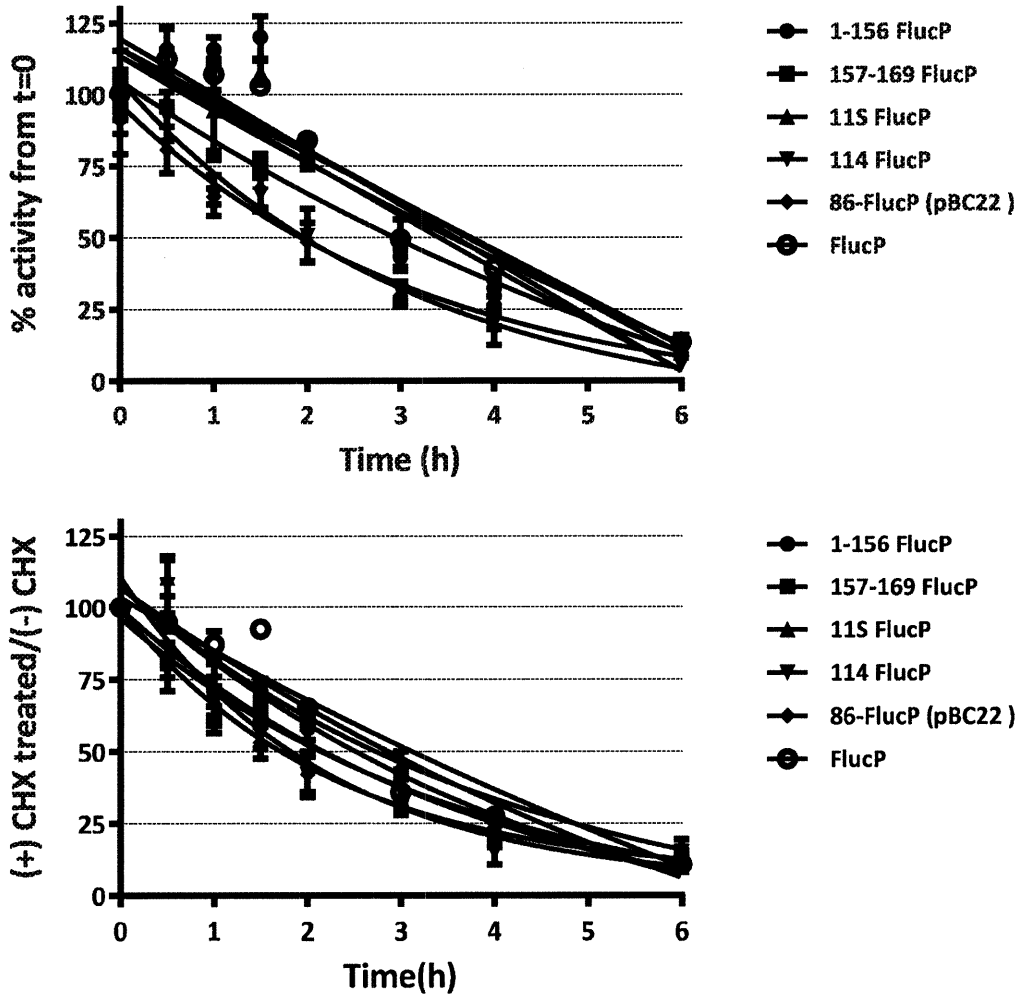
SUBSTITUTE SHEET (RULE 26)

Figure 192



SUBSTITUTE SHEET (RULE 26)

Figure 193



SUBSTITUTE SHEET (RULE 26)

Figure 194

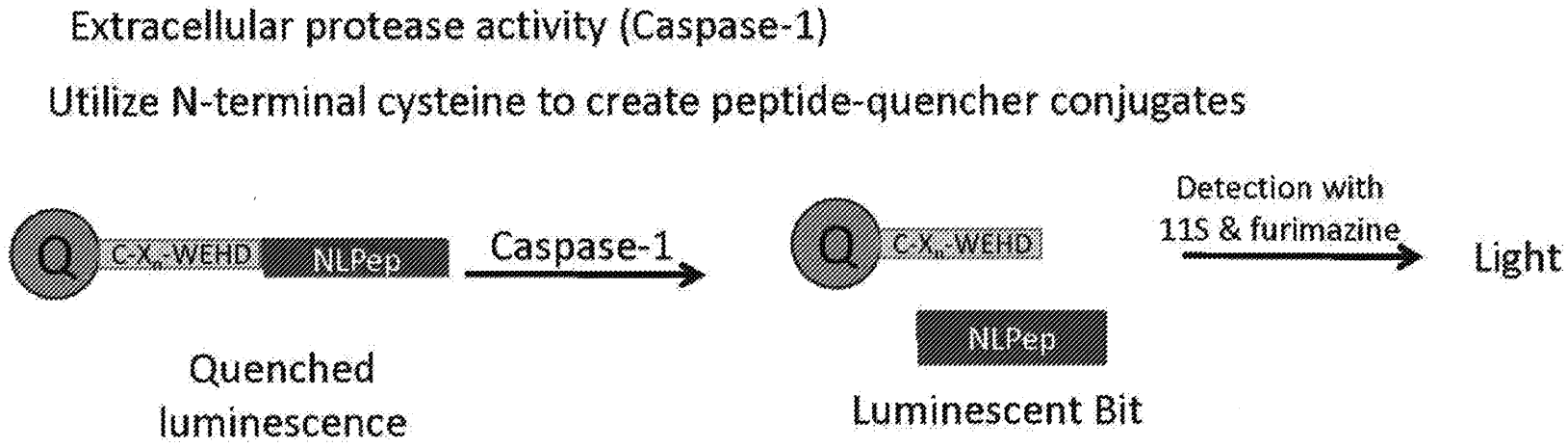
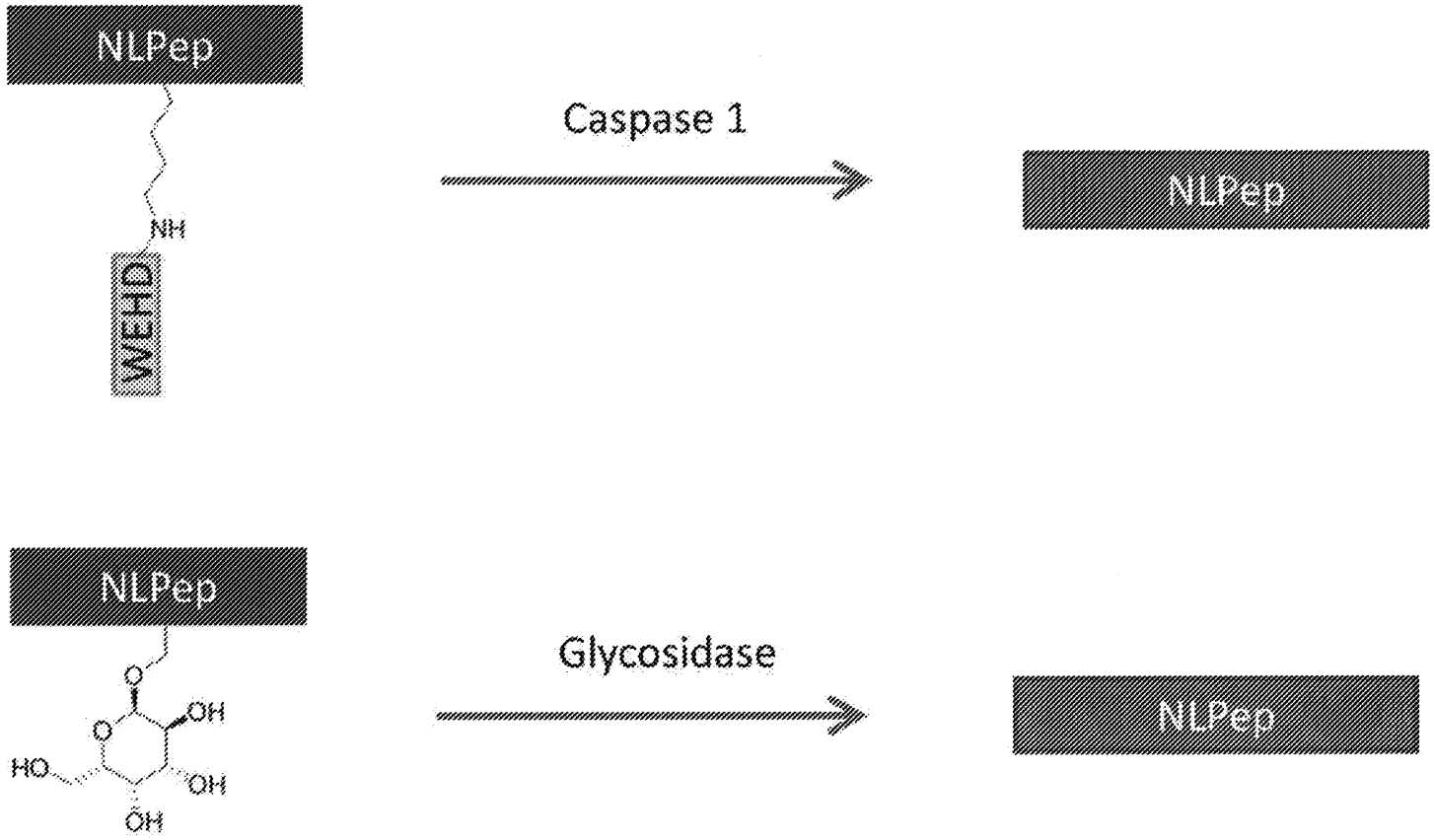

Extracellular protease activity (Caspase-1)
Utilize N-terminal cysteine to create peptide-quencher conjugates
Q
C-X-WEHD
NLPep
Caspase-1
Quenched luminescence
Q
C-X-WEHD
NLPep
Luminescent Bit
Detection with 11S & furimazine
Light

Figure 195



medially attached pro-groups (isopeptides and glycosylated amino acids)

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Figure 196

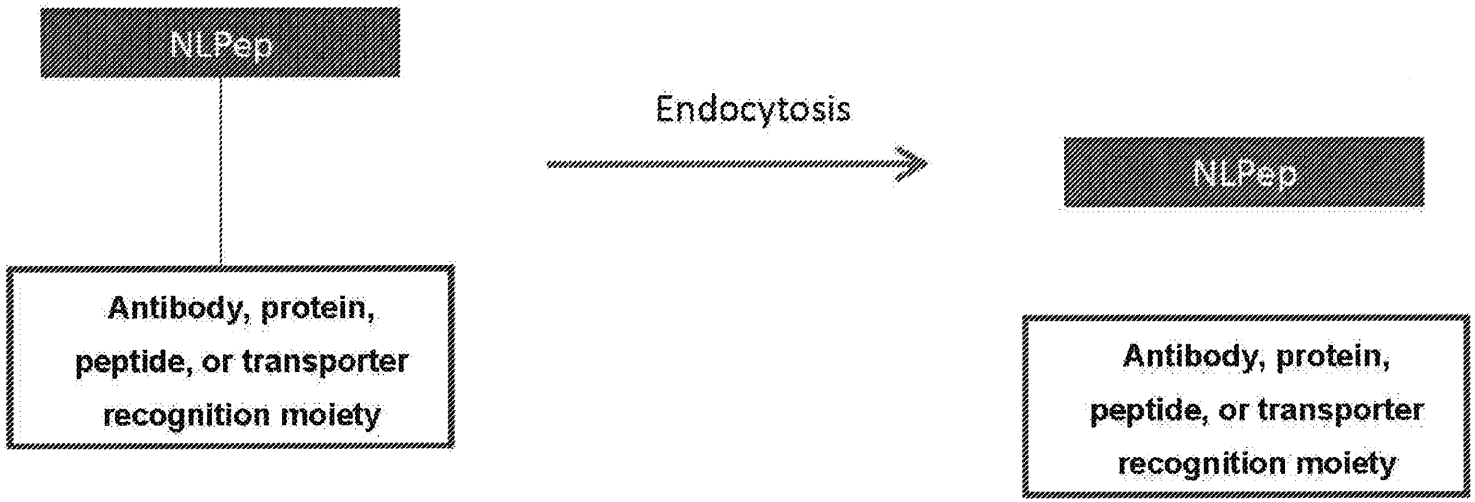
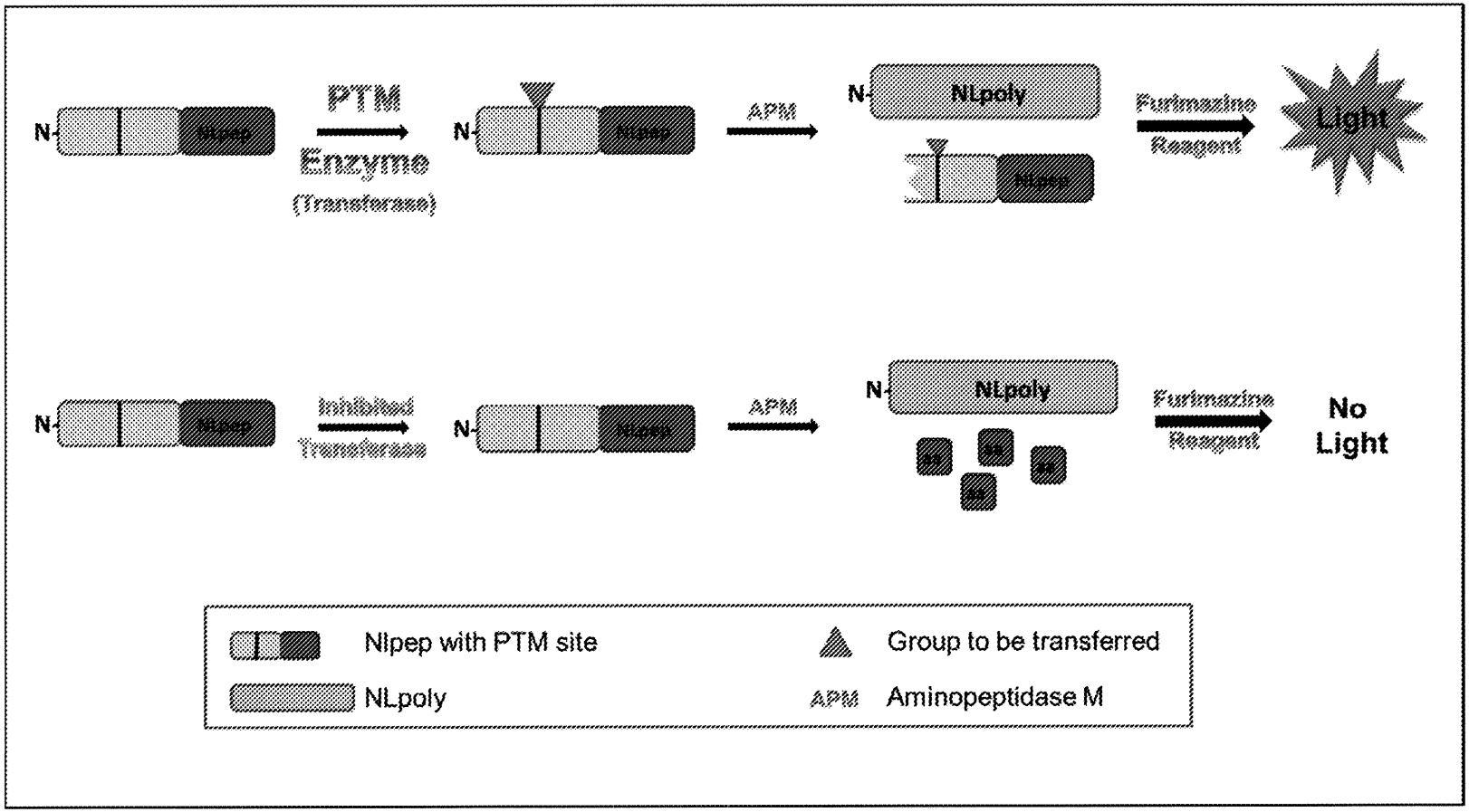


Figure 197



SUBSTITUTE SHEET (RULE 26)

Figure 198

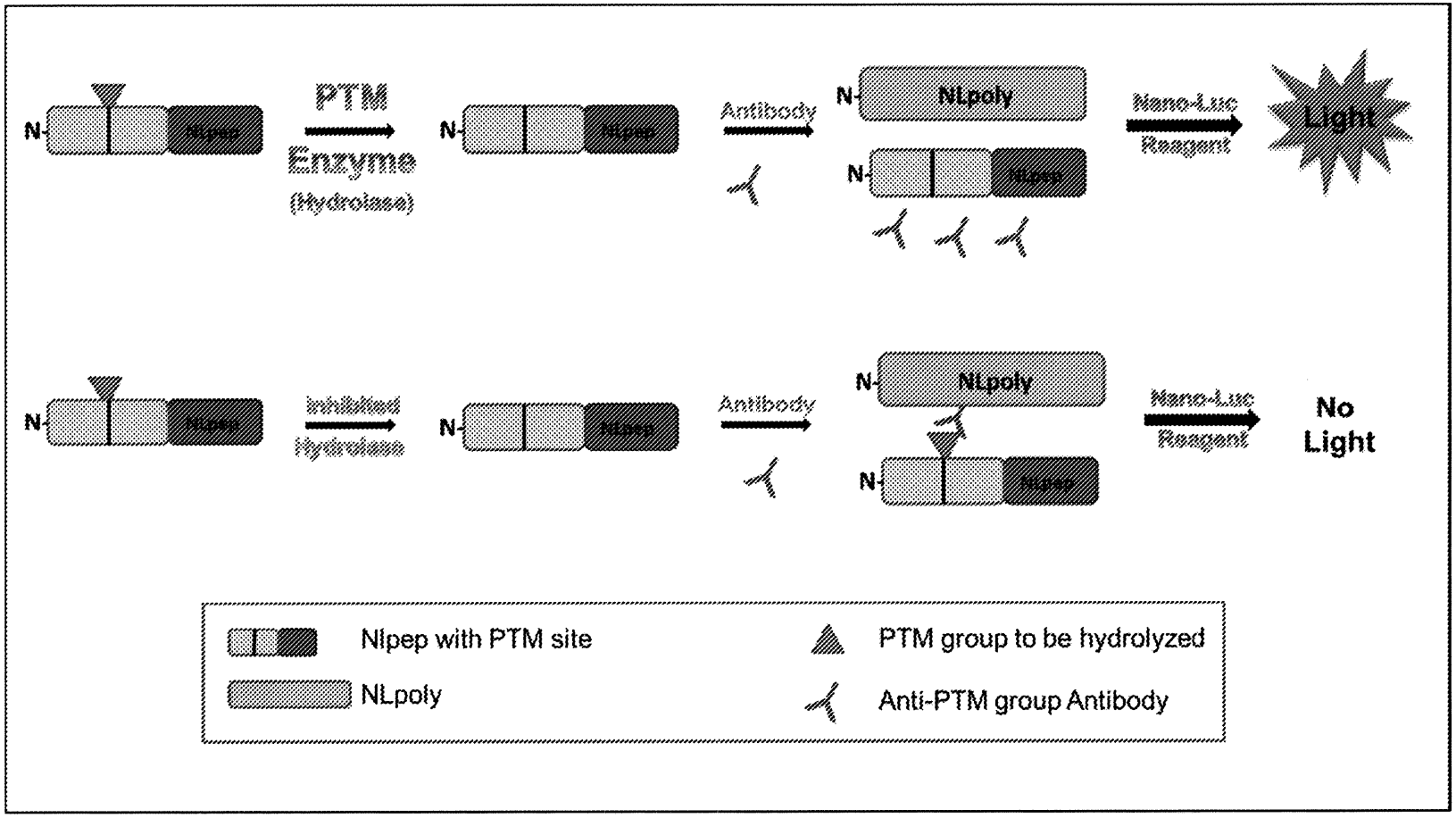
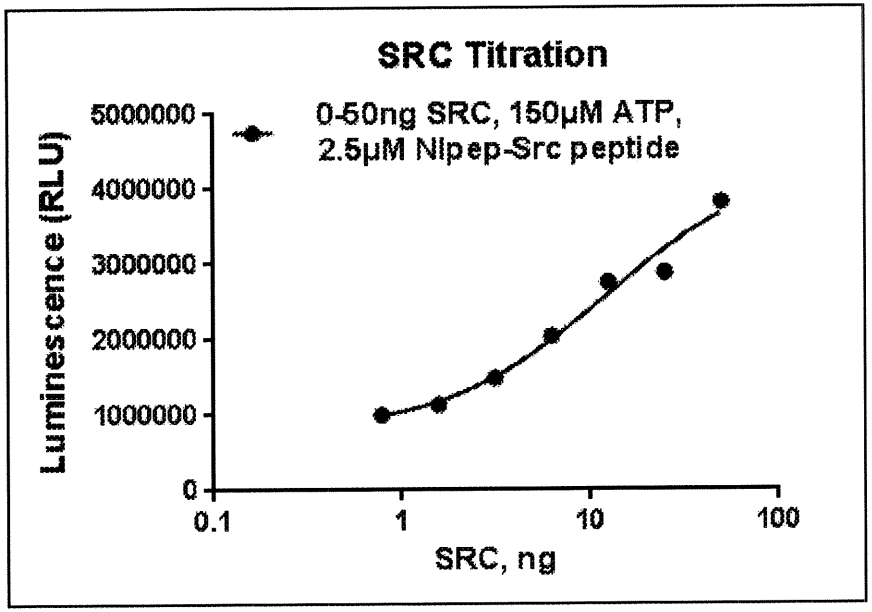

N-
Nlpep
PTM
Enzyme
(Hydrolase)
Antibody
NLpoly
Nano-Luc
Reagent
Light
Inhibited
Hydrolase
No
Light
Nlpep with PTM site
NLpoly
PTM group to be hydrolyzed
Anti-PTM group Antibody

Figure 199


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

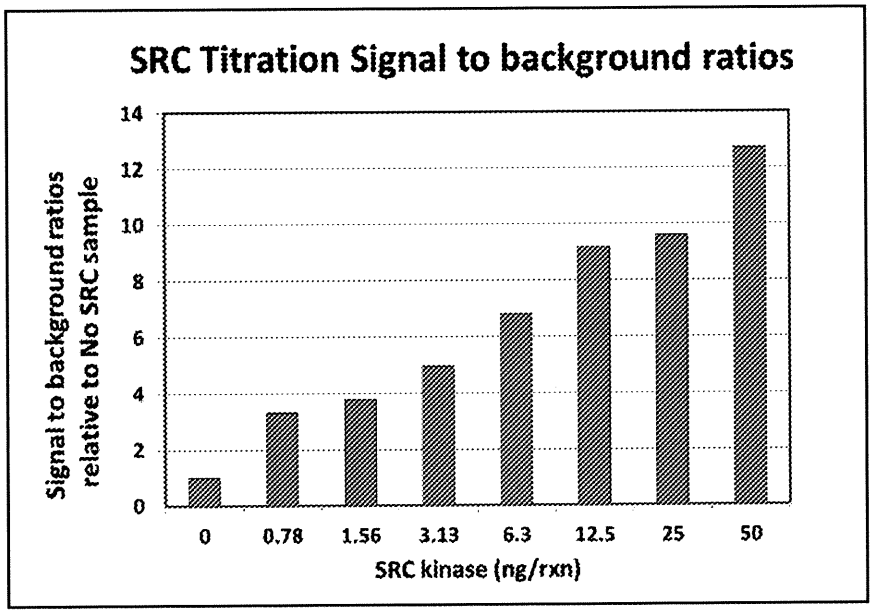
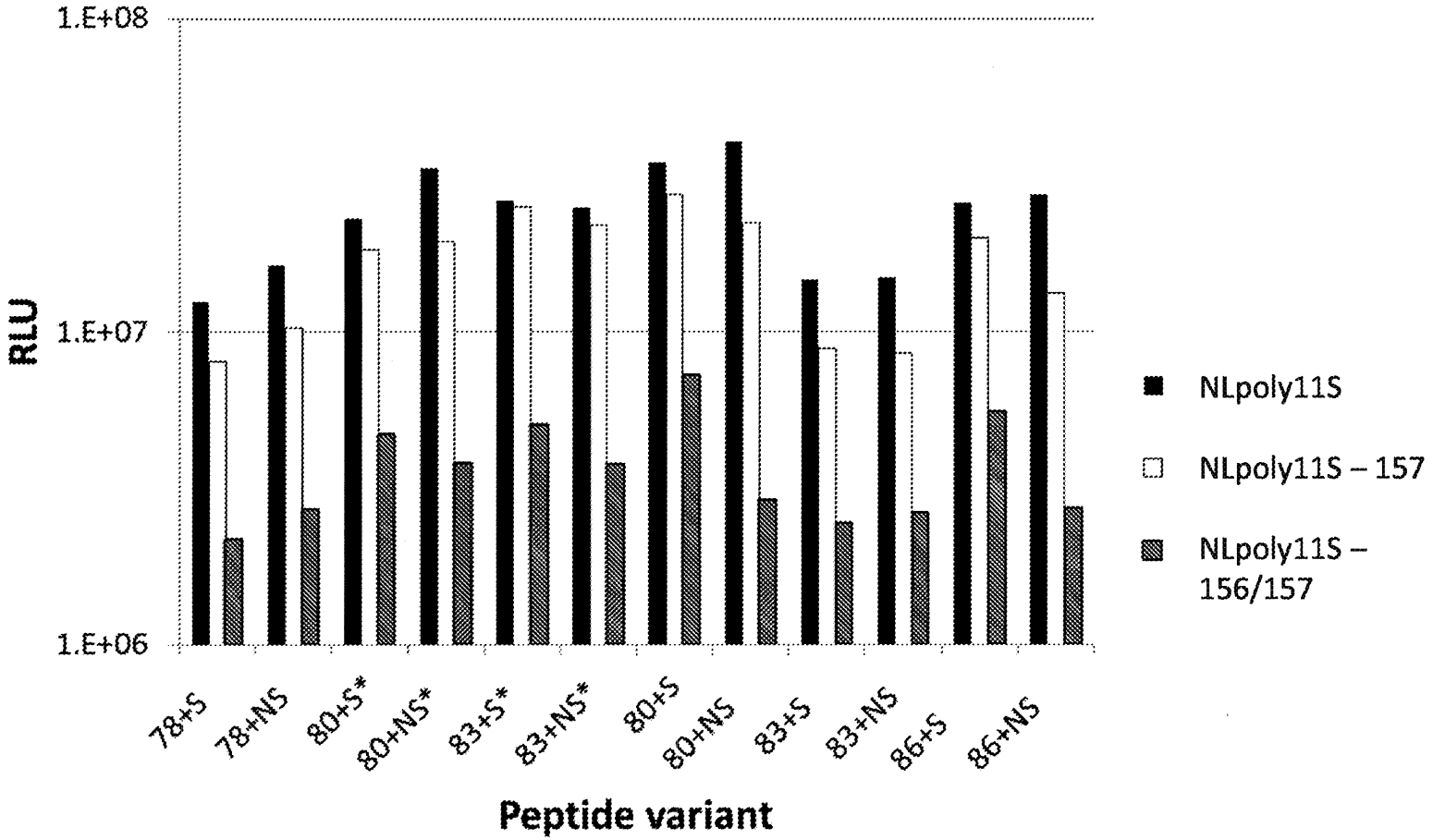

SRC Titration Signal to background ratios
Signal to background ratios relative to No SRC sample
14
12
10
8
6
4
2
0
0
0.78
1.56
3.13
6.3
12.5
25
50
SRC kinase (ng/rxn)

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

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Figure 202

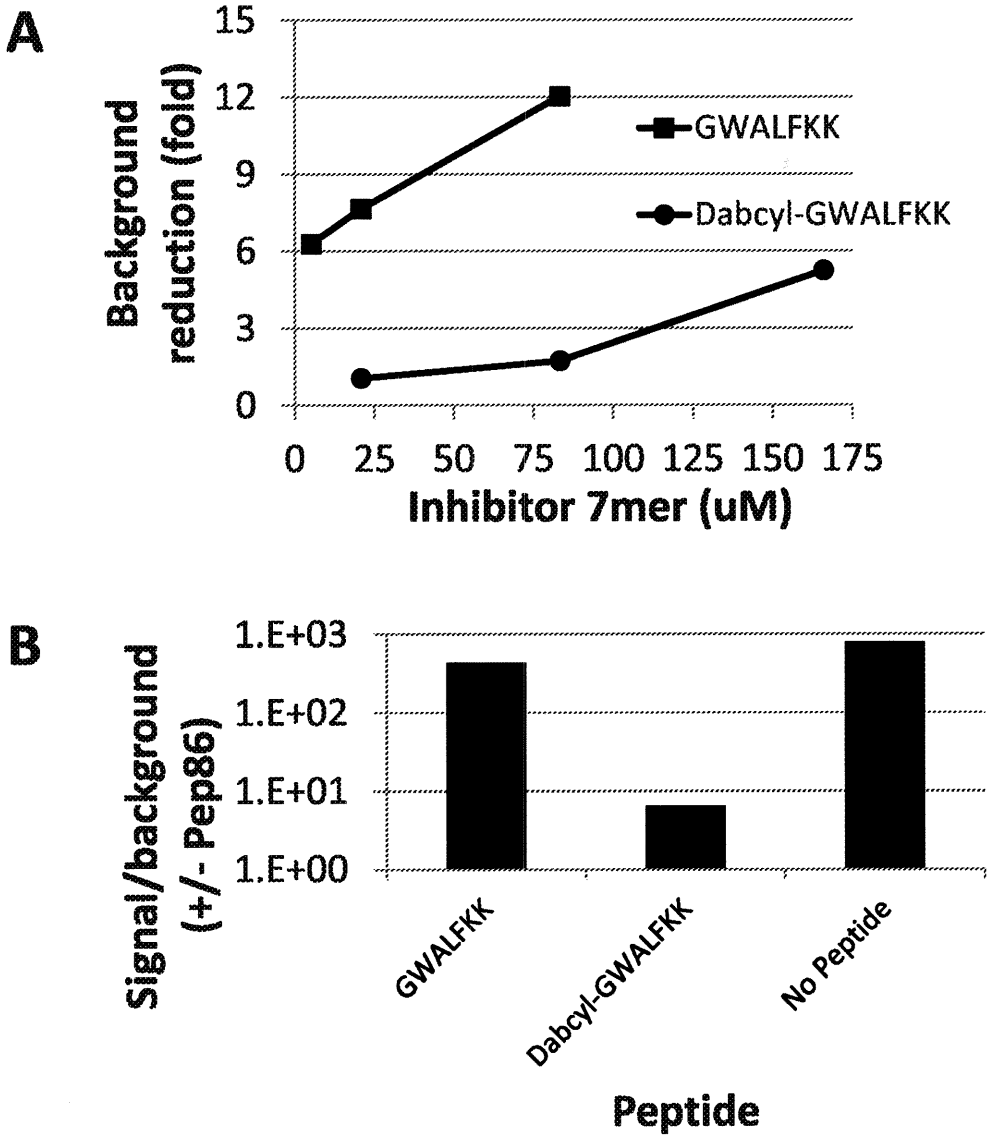
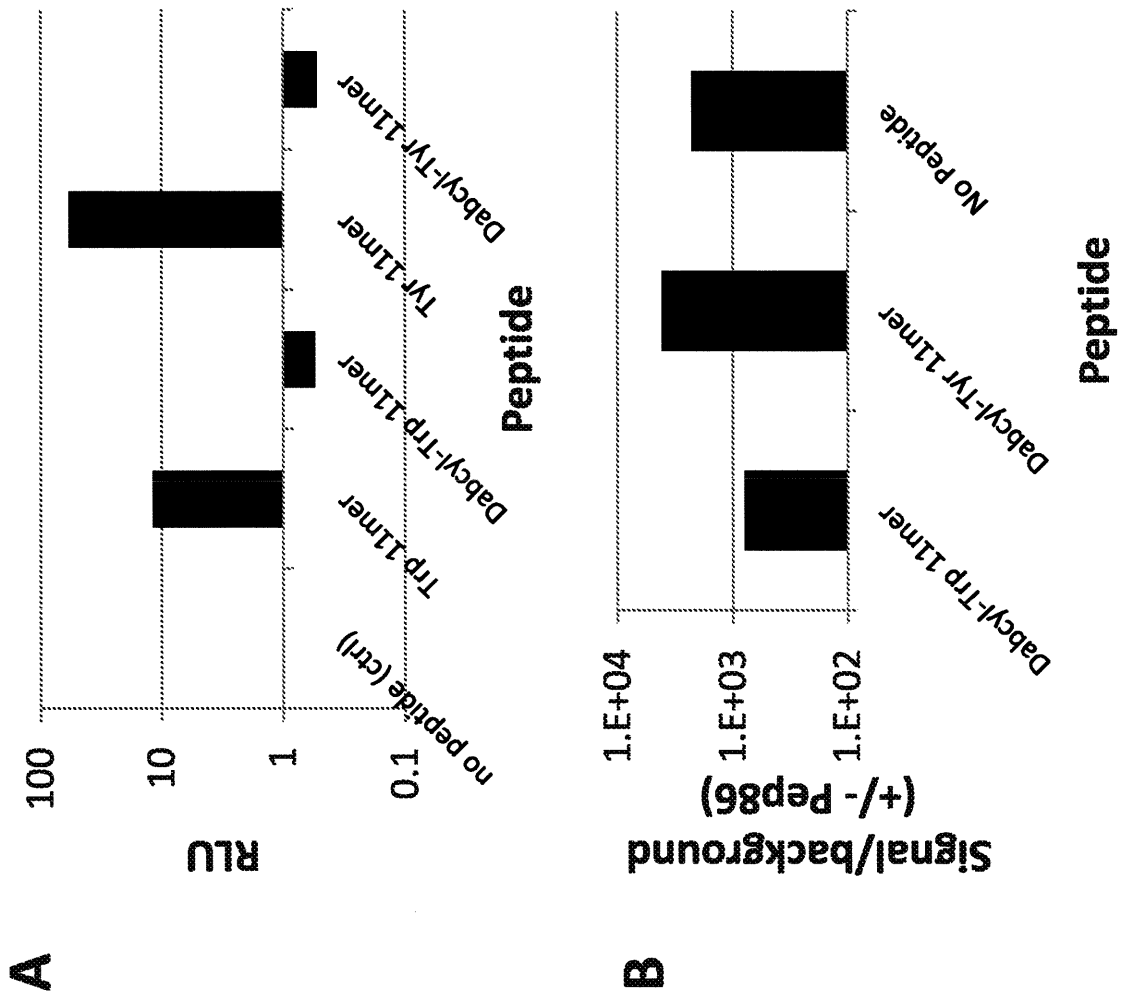

A
Background reduction (fold)
15
12
9
6
3
0
0 25 50 75 100 125 150 175
Inhibitor 7mer (uM)
GWALFKK
Dabcyl-GWALFKK
B
Signal/background (+/- Pep86)
1.E+03
1.E+02
1.E+01
1.E+00
GWALFKK
Dabcyl-GWALFKK
No Peptide
Peptide

Figure 203



SUBSTITUTE SHEET (RULE 26)