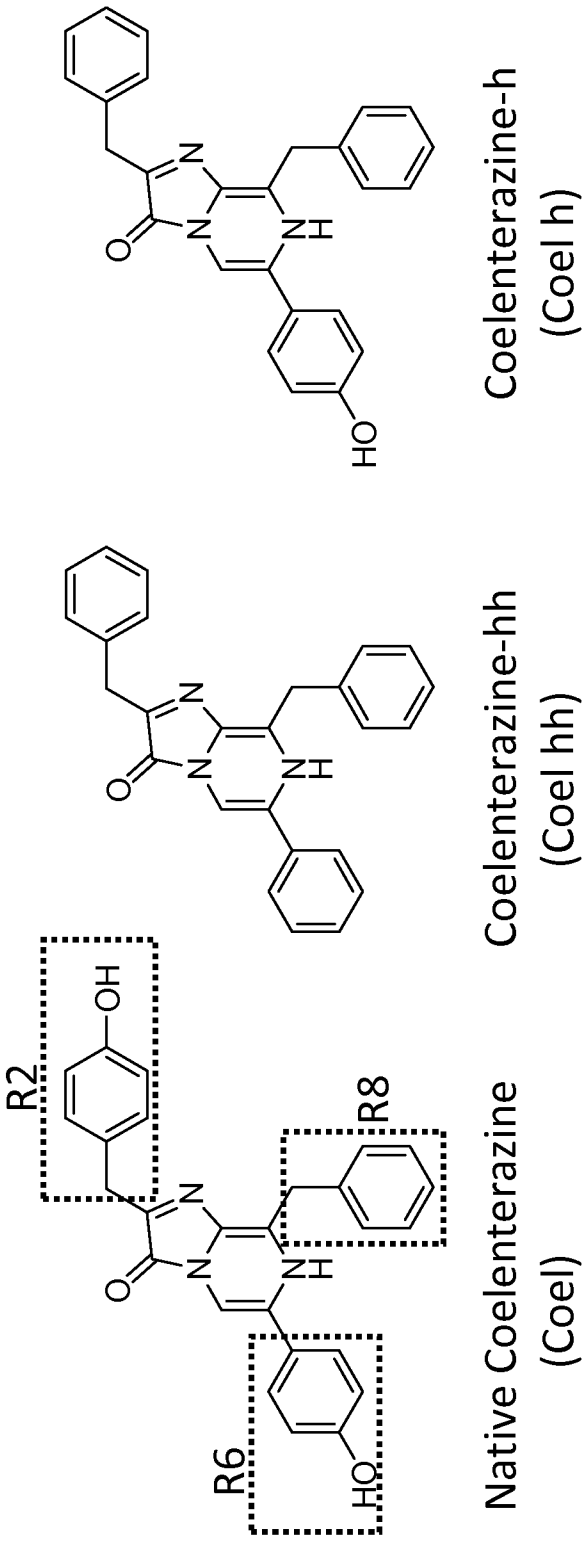

R2
OH
R8
R6
HO
N
N
NH
O
Native Coelenterazine
(Coel)
Coelenterazine-hh
(Coel hh)
Coelenterazine-h
(Coel h)

FIG. 1

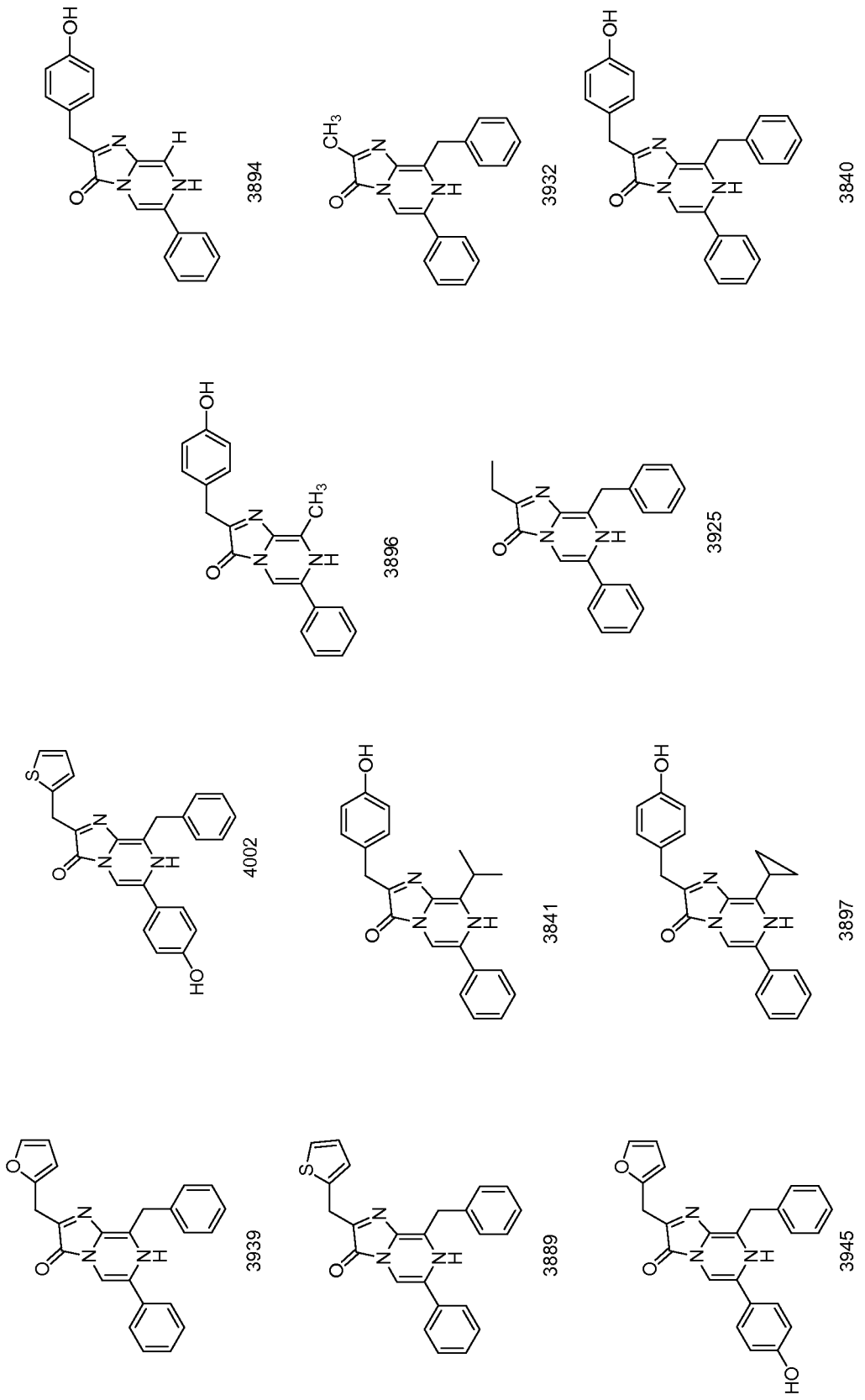


FIG. 2

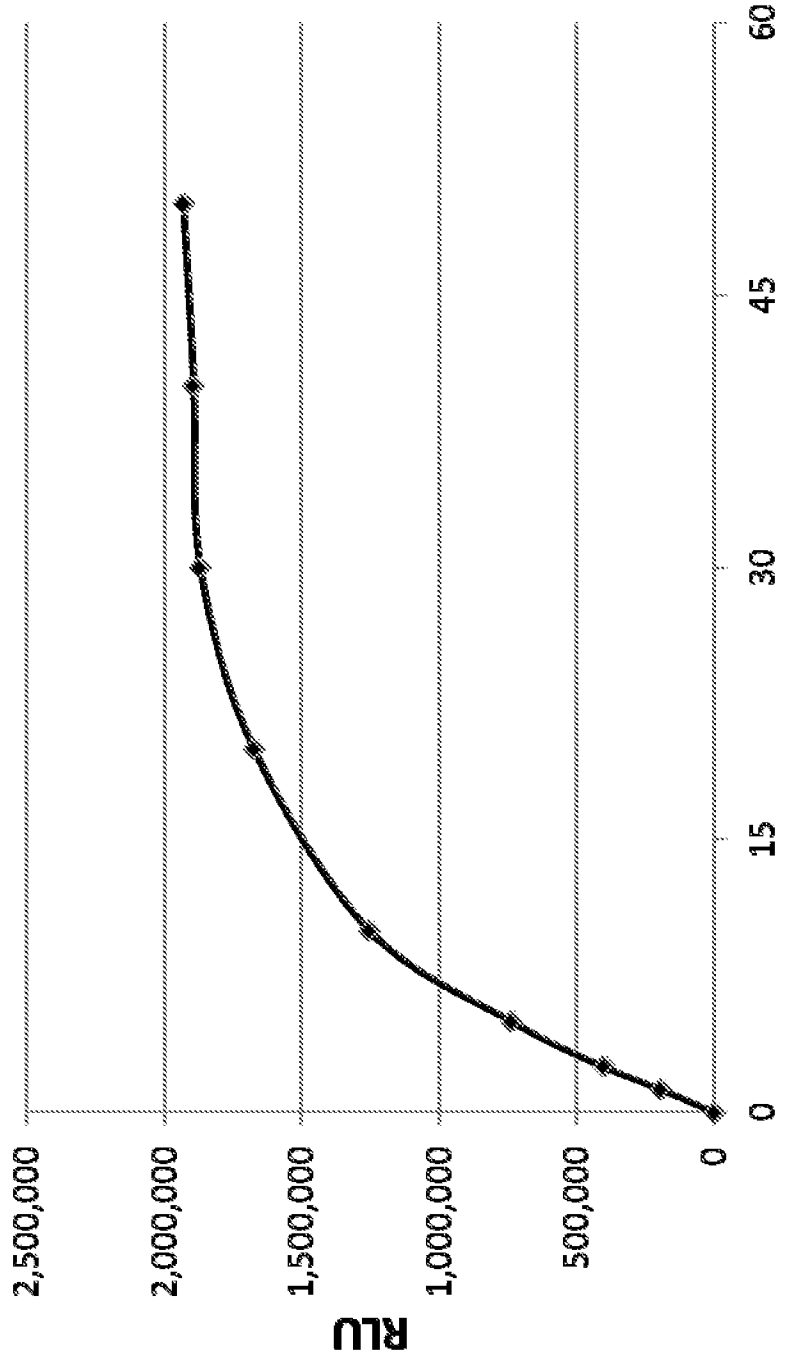

RLU
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
0
15
30
45
60
[PBI-3939] µM

FIG. 3

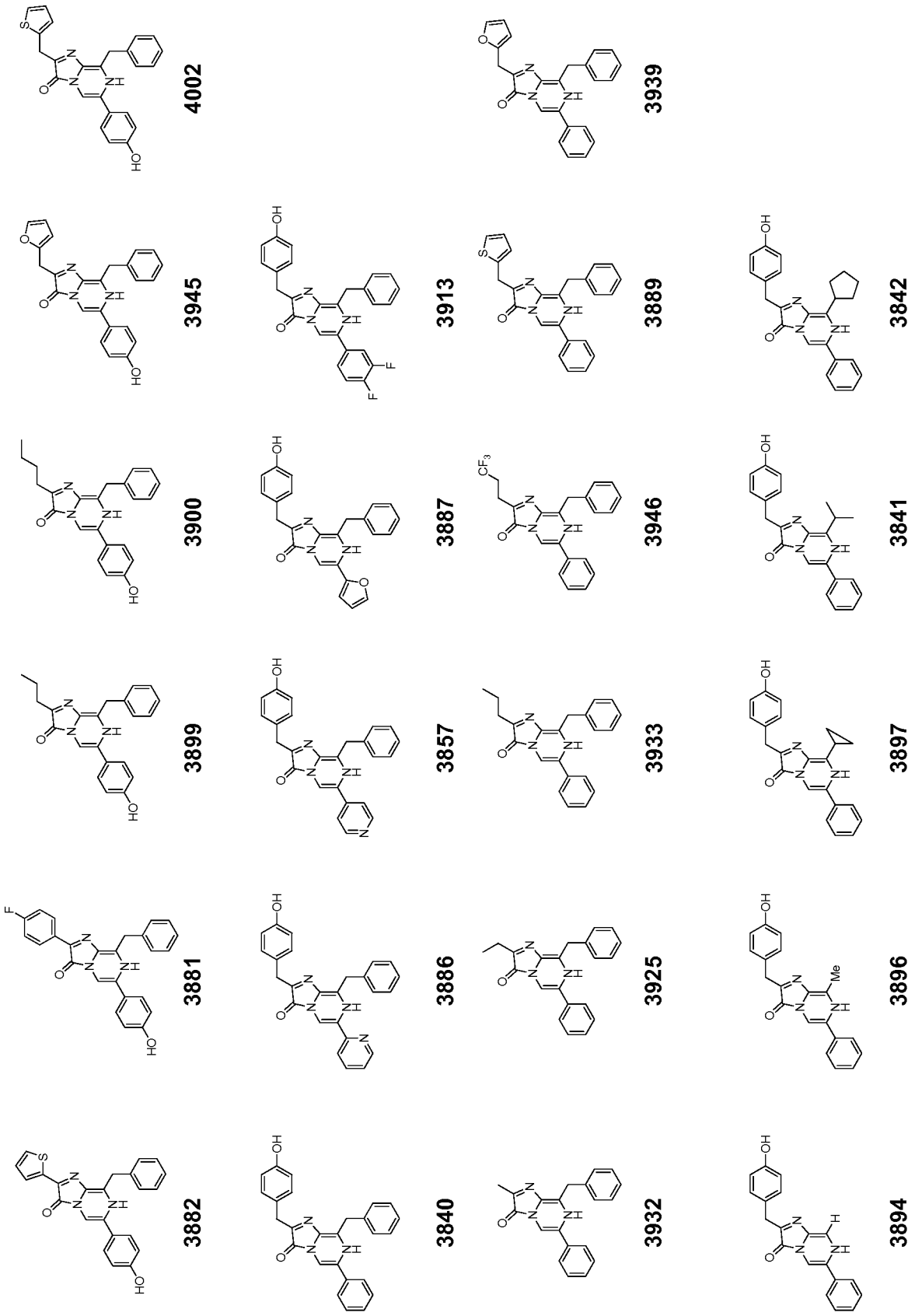

4002
3939
3945
3913
3889
3842
3900
3887
3946
3841
3899
3857
3933
3897
3881
3886
3925
3896
3882
3840
3932
3894

FIG. 4

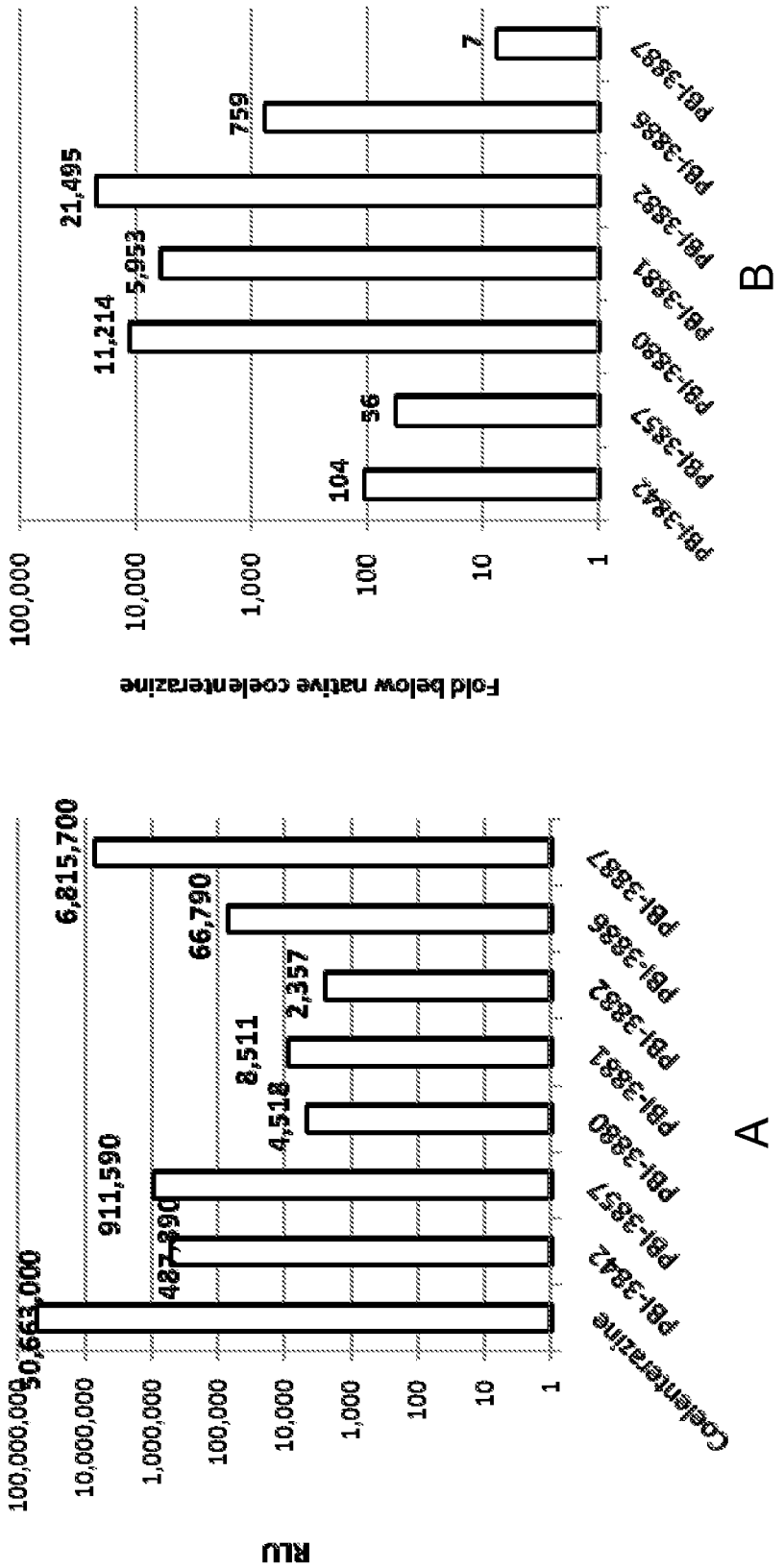

Fold below native coelenterazine
100,000
10,000
1,000
100
10
1
104
56
11,214
5,953
21,495
759
7
PBI-3842
PBI-3857
PBI-3880
PBI-3881
PBI-3882
PBI-3886
PBI-3887
B
RLU
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
30,663,000
487,390
911,590
4,518
8,511
2,357
66,790
6,815,700
Coelenterazine
PBI-3842
PBI-3857
PBI-3880
PBI-3881
PBI-3882
PBI-3886
PBI-3887
A

FIG. 5

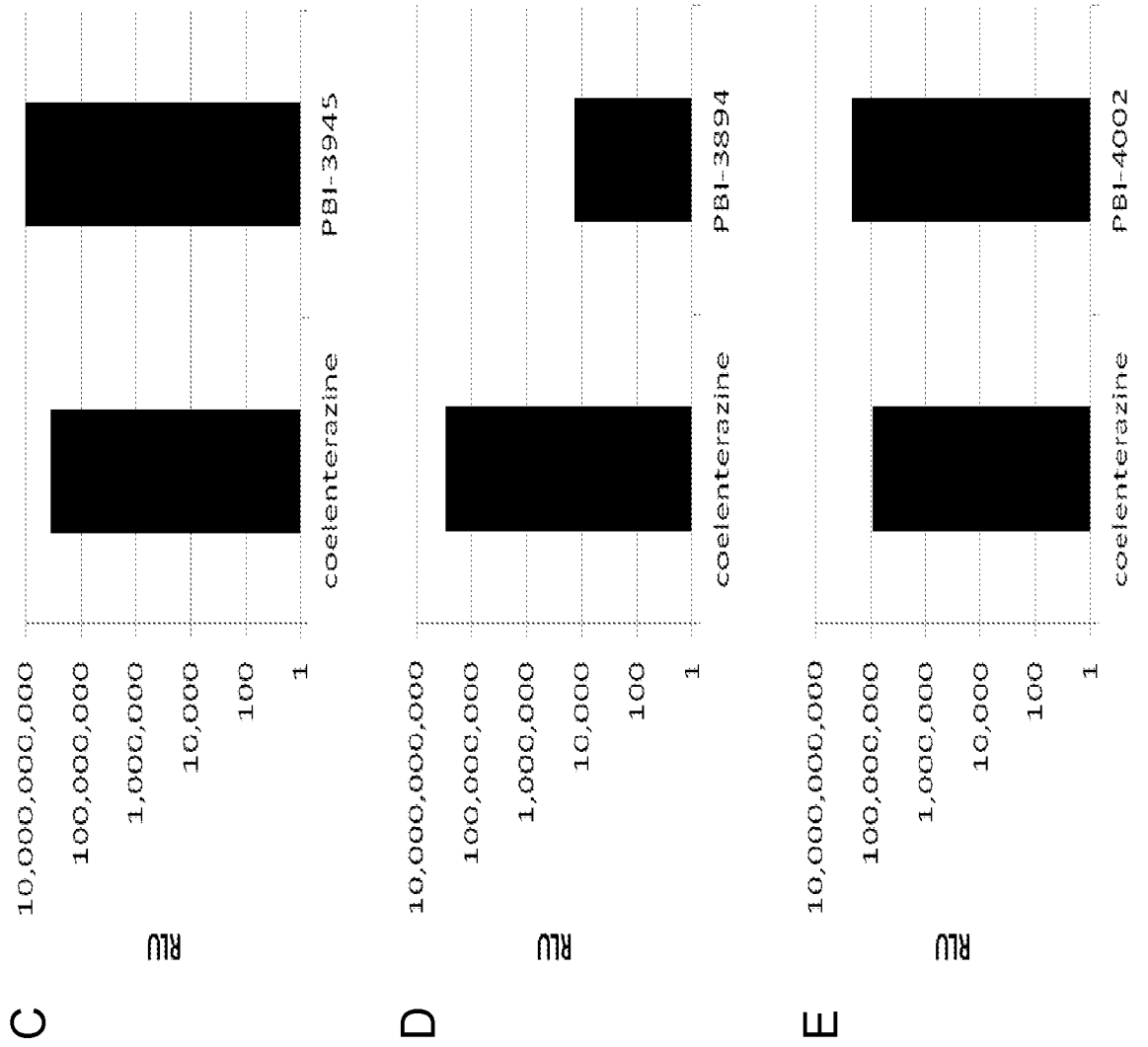

C
RLU
10,000,000,000
100,000,000
1,000,000
10,000
100
1
coelenterazine
PBI-3945
D
RLU
10,000,000,000
100,000,000
1,000,000
10,000
100
1
coelenterazine
PBI-3894
E
RLU
10,000,000,000
100,000,000
1,000,000
10,000
100
1
coelenterazine
PBI-4002

FIG. 5

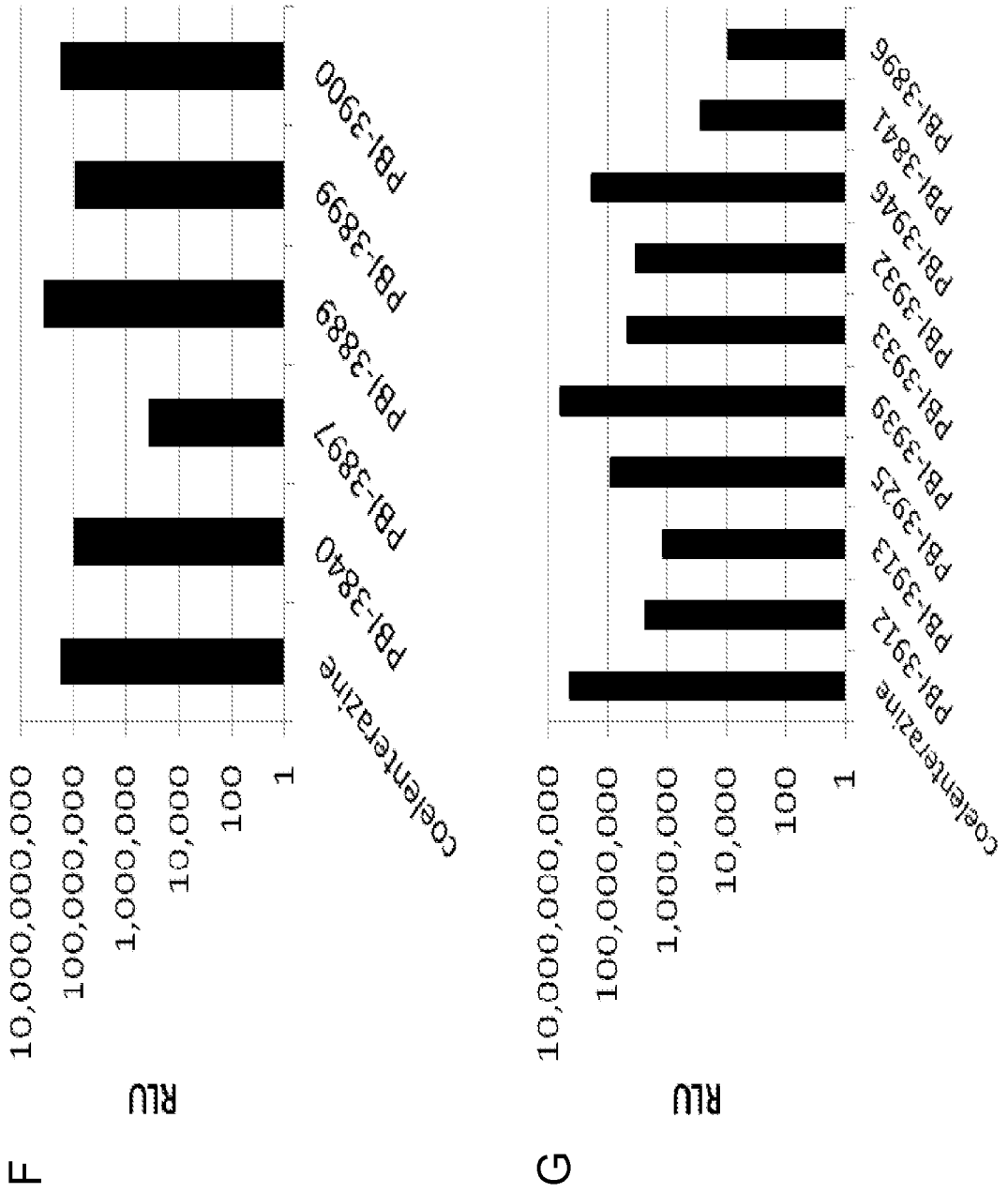

F
RLU
10,000,000,000
100,000,000
1,000,000
10,000
100
1
coelenterazine
PBI-3840
PBI-3897
PBI-3889
PBI-3899
PBI-3900
G
RLU
10,000,000,000
100,000,000
1,000,000
10,000
100
1
coelenterazine
PBI-3912
PBI-3913
PBI-3925
PBI-3939
PBI-3933
PBI-3932
PBI-3946
PBI-3841
PBI-3896

FIG. 5

POSITION	Clone(AA sequence)	Colente	h	h,h	2-me	PBI-3840	PBI-3897	PBI-3889	PBI-3899	PBI-3900
15	03F2 (G15R)	0.004	0.059	0.024	0.004	0.008	0.003	0.031	0.003	0.011
18	40H11 (Q18L)	0.38	0.26	0.51	0.20	0.28	**4.46**	0.53	0.17	0.43
20	16C5 (Q20R)	**5.66**	**1.96**	**1.88**	**9.02**	**4.72**	**6.50**	**1.61**	**4.92**	**2.41**
22	14C1 (L22I)	0.17	0.63	0.47	0.41	0.14	0.08	0.43	0.49	0.66
27	11E 8 (L27V)	0.34	**1.74**	**1.88**	0.55	0.35	0.03	**2.03**	0.88	**2.22**
54	18F8 (F54S)	0.21	0.40	**1.78**	0.13	0.53	0.55	**1.62**	0.18	0.69
54	29H7 (F54I)	**11.30**	**1.71**	**1.48**	**24.59**	**18.11**	**18.09**	**2.32**	**10.57**	**2.99**
58	30E 4 (V58I)	0.27	0.89	1.00	0.96	0.43	0.21	1.15	0.65	1.02
72	04G7 (L72Q)	**4.46**	1.25	1.15	**9.36**	**5.65**	**8.84**	1.13	**4.04**	**1.53**
75	28B2 (M75K)	**4.16**	**1.57**	**2.30**	**5.43**	**4.92**	**5.67**	**2.38**	**4.03**	**2.11**
77	18C4 (F77T)	**1.62**	0.55	1.22	**7.82**	**2.06**	**2.28**	**1.33**	**2.67**	0.86
77	42E 4 (F77C)	0.13	0.46	0.99	**3.43**	0.20	0.02	1.27	0.56	0.58
77	50D12 (F77T)	**1.48**	0.52	1.15	**7.75**	**1.87**	**2.20**	1.24	**2.46**	0.78
89	35D11 (K89E)	**3.02**	0.59	1.26	**5.27**	**3.39**	**5.19**	**1.41**	**2.32**	0.95
90	10F2 (I90T)	**2.20**	0.48	0.66	0.00	**3.90**	**5.41**	0.84	**1.36**	0.55
92	04A12 (L92H)	0.89	0.39	0.61	**7.31**	**3.72**	**14.25**	0.53	0.89	0.45
109	43F9 (Y109F)	0.48	0.33	0.97	0.36	**1.49**	**24.83**	0.94	0.43	0.56
127	30D6 (V127A)	0.23	0.50	0.54	0.26	0.81	1.00	0.57	0.26	0.42
139	27B8 (D139G)	0.01	0.03	0.14	0.00	0.03	0.00	0.16	0.00	0.02
164	25A1 (C164S)	**2.47**	**1.79**	**1.53**	**2.74**	**3.37**	**5.10**	**1.63**	**2.87**	**2.05**
14-89	25F2 (A14V,K89E)	**3.05**	0.81	1.22	**5.43**	**3.82**	**6.21**	**1.58**	**2.32**	0.91
27-33	47D8(**L27M,K33N**)	0.59	**1.71**	**2.05**	0.85	0.95	0.27	**2.12**	1.23	**1.85**
49-72	26F1 (E49K,**L72Q**)	**6.19**	**1.48**	**1.35**	**10.72**	**6.90**	**9.99**	**1.32**	**4.76**	**1.70**
54-66	48G3 (**F54I,** S66T)	**7.33**	1.06	0.91	**16.39**	**11.27**	**8.28**	**1.42**	**6.63**	**1.85**
54-67	35B8 **(F54S**,G67S)	0.33	0.75	**2.49**	0.19	0.78	0.87	**2.25**	0.27	1.05
55-117	05H8 (D55G,I117F)	0.24	0.53	0.62	0.00	0.25	0.22	0.65	0.21	0.45
56-93	46A11(I56V,H93R)	0.35	0.61	**1.53**	0.22	0.42	0.18	**1.50**	0.36	0.88
68-75	04E 2 (**F68S,M75K**)	**3.43**	1.20	**1.53**	**4.37**	**3.91**	**4.12**	**1.62**	**3.24**	**1.57**
77-126	27B9 (**F77T**,T126R)	0.87	0.15	0.69	**4.87**	0.80	1.00	0.79	1.14	0.32
77-39	33E 2 (**F77T**,T39I)	0.95	0.34	0.69	**6.30**	**1.32**	**1.64**	0.74	**1.47**	0.47
89-148	50 E7 (**K89E**, S148T)	**1.81**	0.48	0.85	**3.74**	**2.23**	**3.20**	0.94	**1.59**	0.65
90-77	02D10 (**I90V,F77T**)	**2.05**	0.70	1.17	**17.39**	**6.32**	**10.88**	1.05	**2.37**	0.73
106-164	17E 6 (M106K,**C164S**)	**4.14**	**2.46**	**2.52**	**3.77**	**5.06**	**7.72**	**2.71**	**3.80**	**2.82**
23-58-169	47G12 (E23K,V58L,A169V)	0.06	0.44	0.49	**1.49**	0.10	0.01	0.68	0.58	0.92
33-76-89	17C5 **(K33N**,I76N,**K89E**)	1.27	0.68	**2.02**	**1.33**	**2.22**	1.12	**2.24**	1.07	0.79
68-90-145	04D10 (**F68S,I90V**,P145L)	**4.17**	**1.40**	**1.65**	0.00	**6.09**	**5.96**	**1.72**	**3.82**	**1.49**
59-109-113-136	41H10 (I59T, **Y109F**, P113T, L136M)	0.06	0.06	0.26	0.04	0.46	**8.09**	0.25	0.06	0.10

FIG. 6A

POSITION	Clone(AA sequence)	Colenterazine	H	h,h	v	PBI-3912	PBI-3913	PBI-3925	PBI-3897	PBI-3899
15	03F2 (G15R)	0.003	0.040	0.018	0.022	0.005	0.019	0.005	0.002	0.003
18	40H11 (Q18L)	0.20	0.18	0.30	0.11	0.13	0.14	0.06	**3.24**	0.09
20	16C5 (Q20R)	**4.08**	1.30	1.14	**5.25**	**5.27**	**5.34**	**4.83**	**3.33**	**3.32**
22	14C1 (L22I)	0.13	0.56	0.40	0.13	0.10	0.11	0.21	0.06	0.39
27	11E 8 (L27V)	0.16	1.01	0.95	0.22	0.07	0.08	0.26	0.02	0.45
54	18F8 (F54S)	0.16	0.37	**1.44**	0.55	0.17	0.22	0.26	0.38	0.14
54	29H7 (F54I)	**10.34**	**1.45**	1.06	**8.28**	**18.52**	**15.79**	**18.92**	**11.15**	**9.62**
58	30E 4 (V58I)	0.17	0.63	0.69	0.20	0.19	0.21	0.83	0.13	0.43
72	04G7 (L72Q)	**2.96**	1.02	0.69	1.03	**5.81**	**5.81**	**5.65**	**7.42**	**3.09**
75	28B2 (M75K)	**2.93**	**1.35**	**1.53**	1.06	**3.98**	**3.89**	**3.86**	**4.73**	**2.93**
77	18C4 (F77T)	1.01	0.53	0.85	**1.31**	0.78	0.74	**7.08**	**1.69**	**2.30**
77	42E 4 (F77C)	0.10	0.34	0.70	0.61	0.03	0.04	0.88	0.01	0.41
77	50D12 (F77T)	0.88	0.46	0.72	1.21	0.62	0.60	**5.94**	**1.41**	**1.92**
89	35D11 (K89E)	**2.17**	0.53	0.89	0.76	**3.64**	**3.98**	**3.51**	**3.89**	**1.77**
90	10F2 (I90T)	**1.86**	0.39	0.49	**1.71**	**10.44**	**11.10**	**3.76**	**3.61**	1.20
92	04A12 (L92H)	0.65	0.08	0.27	**27.76**	**4.48**	**2.79**	**5.01**	**6.72**	0.60
109	43F9 (Y109F)	0.36	0.20	0.76	0.84	0.73	0.60	1.04	**18.04**	0.31
127	30D6 (V127A)	0.16	0.31	0.30	0.16	0.32	0.45	0.50	0.43	0.15
139	27B8 (D139G)	0.00	0.02	0.08	0.10	0.02	0.04	0.01	0.00	0.00
164	25A1 (C164S)	**3.10**	**1.71**	**1.47**	**3.91**	**3.04**	**3.29**	**3.43**	**4.33**	**3.14**
14-89	25F2 (A14V,K89E)	**3.31**	0.75	1.16	**1.34**	**5.26**	**5.90**	**5.28**	**4.68**	**2.46**
27-33	47D8(**L27M,K33N**)	0.52	**1.41**	**1.46**	**1.55**	0.41	0.41	1.21	0.17	1.05
49-72	26F1 (E49K,**L72Q**)	**3.61**	1.09	0.76	**2.24**	**6.83**	**6.59**	**6.42**	**6.33**	**3.40**
54-66	48G3 (**F54I,** S66T)	**10.15**	**1.40**	1.01	**7.67**	**17.73**	**14.90**	**18.29**	**10.14**	**9.44**
54-67	35B8 (**F54S**,G67S)	0.23	0.46	**1.73**	1.28	0.23	0.29	0.32	0.42	0.18
55-117	05H8 (D55G,I117F)	0.18	0.38	0.46	0.44	0.12	0.14	0.10	0.14	0.16
56-93	46A11(I56V,H93R)	0.24	0.39	0.96	0.88	0.10	0.11	0.21	0.11	0.24
68-75	04E 2 (**F68S,M75K**)	**2.39**	1.01	1.04	0.74	**3.01**	**3.04**	**3.61**	**3.58**	**2.47**
77-126	27B9 (**F77T**,T126R)	0.34	0.15	0.37	0.51	0.28	0.24	**2.16**	0.58	0.74
77-39	33E 2 (**F77T**,T39I)	0.50	0.24	0.35	0.67	0.42	0.38	**3.27**	0.96	0.96
89-148	50 E7 (**K89E**, S148T)	**2.97**	0.66	1.15	**1.50**	**4.57**	**5.01**	**4.36**	**3.91**	**2.28**
90-77	02D10 (**I90V,F77T**)	**1.44**	0.63	0.80	**3.79**	**2.80**	**2.47**	**24.43**	**7.82**	**2.14**
106-164	17E 6 (M106K,**C164S**)	**2.71**	**1.50**	**1.52**	**4.80**	**2.79**	**2.92**	**2.93**	**3.47**	**2.48**
23-58-169	47G12 (E23K,V58L,A169V)	0.05	0.41	0.39	0.31	0.03	0.03	1.24	0.01	0.48
33-76-89	17C5 (**K33N**,I76N,**K89E**)	**1.53**	0.63	**1.74**	**2.16**	0.86	0.94	**1.38**	0.91	1.03
68-90-145	04D10 (**F68S,I90V**,P145L)	**2.63**	0.88	0.95	**2.74**	**4.40**	**4.42**	**5.96**	**4.82**	**2.48**
59-109-113-136	41H10 (I59T, **Y109F**, P113T, L136M)	0.08	0.07	0.29	0.20	0.23	0.22	0.41	**7.52**	0.07

FIG. 6B

POSITION	Clone(AA sequence)	coel	h	h,h	2-me	PBI-3889	PBI-3939	PBI-3933	PBI-3932	PBI-3946	PBI-3897	PBI-3841	PB-3896	PBI-3925
15	03F2 (G15R)	0.003	0.041	0.021	0.005	0.025	0.019	0.003	0.009	0.003	0.002	0.003	0.013	0.004
18	40H11 (Q18L)	0.31	0.34	0.48	0.19	0.43	0.39	0.09	0.08	0.09	**3.86**	**4.08**	**61.25**	0.09
20	16C5 (Q20R)	**2.84**	1.04	0.92	**3.91**	0.86	1.15	**2.79**	**4.70**	**2.18**	**2.70**	**3.59**	**3.78**	**3.81**
22	14C1 (L22I)	0.12	0.52	0.39	0.30	0.33	0.29	0.20	0.15	0.20	0.06	0.09	0.12	0.20
27	11E 8 (L27V)	0.25	**1.64**	**1.48**	0.40	**1.53**	**1.44**	0.47	0.52	0.61	0.03	0.04	0.09	0.40
54	18F8 (F54S)	0.2	0.6	**1.6**	0.1	**1.4**	1.3	0.4	0.3	0.5	0.4	0.4	0.5	0.3
54	29H7 (F54I)	**6.77**	1.18	0.78	out of range	**1.57**	0.50	**5.38**	**17.81**	**3.69**	**8.04**	**6.14**	**9.69**	**12.97**
58	30E 4 (V58I)	0.17	0.66	0.72	0.62	0.80	0.91	0.63	0.90	0.48	0.12	0.14	0.27	0.83
72	04G7 (L72Q)	**4.07**	1.27	0.93	**7.23**	1.08	1.27	**5.03**	**7.66**	**3.14**	**6.40**	**7.30**	**6.20**	**6.85**
75	28B2 (M75K)	**3.25**	**1.44**	**1.68**	**3.97**	**1.86**	**2.01**	**3.77**	**4.33**	**3.33**	**3.89**	**3.85**	**3.41**	**4.15**
77	18C4 (F77T)	0.86	0.48	0.74	**4.98**	0.96	0.81	**3.37**	**4.84**	**1.81**	**1.32**	0.56	0.91	**5.04**
77	42E 4 (F77C)	0.07	0.30	0.65	**1.89**	0.77	0.46	0.50	**2.18**	0.34	0.02	0.02	0.08	0.63
77	50D12 (F77T)	0.87	0.50	0.76	**4.89**	0.99	0.85	**3.45**	**4.92**	**1.86**	1.30	0.55	0.88	**5.14**
89	35D11 (K89E)	**2.17**	0.60	0.88	**3.39**	0.97	1.02	**2.28**	**3.65**	**2.09**	**3.17**	**2.95**	**2.56**	**3.01**
90	10F2 (I90T)	**1.68**	0.37	0.51	**2.60**	0.72	0.62	**2.08**	**4.64**	**1.35**	**2.99**	**1.93**	**4.51**	**3.57**
92	04A12 (L92H)	0.59	0.03	0.24	**5.44**	0.23	0.11	**1.86**	**3.25**	0.88	**8.81**	**23.30**	**22.13**	**3.53**
109	43F9 (Y109F)	0.25	0.20	0.65	0.11	0.73	0.64	0.64	0.71	0.44	**13.07**	**4.80**	1.20	0.85
127	30D6 (V127A)	0.22	0.40	0.46	0.15	0.48	0.52	0.65	0.75	0.62	0.50	0.55	0.53	0.66
139	27B8 (D139G)	0.00	0.02	0.11	0.01	0.10	0.09	0.02	0.01	0.02	0.01	0.01	0.04	0.01
164	25A1 (C164S)	**2.10**	**1.30**	1.12	**1.71**	1.28	**1.30**	**2.30**	**2.26**	**2.11**	**3.14**	**5.76**	**1.96**	**2.66**
14-89	25F2 (A14V,K89E)	**1.93**	0.53	0.73	**2.80**	0.98	1.00	**2.15**	**3.78**	**1.88**	**3.07**	**2.32**	**2.94**	**3.01**
27-33	47D8(**L27M,K33N**)	0.38	1.26	**1.37**	0.46	**1.73**	**1.79**	1.27	0.92	**1.46**	0.16	0.21	0.25	1.02
49-72	26F1 (E49K,**L72Q**)	**4.26**	1.22	0.91	**7.26**	1.10	1.26	**5.21**	**7.70**	**3.23**	**6.66**	**7.28**	**6.41**	**6.91**
54-66	48G3 (**F54I,** S66T)	**10.93**	**1.88**	1.21	out of range	**2.46**	0.76	**8.62**	**28.26**	**5.88**	**9.90**	**7.53**	**11.93**	**20.94**
54-67	35B8 **(F54S**,G67S)	0.19	0.59	**1.64**	0.10	**1.44**	1.30	0.47	0.30	0.58	0.43	0.51	0.47	0.32
55-117	05H8 (D55G,I117F)	0.17	0.37	0.49	0.08	0.51	0.54	0.15	0.09	0.19	0.12	0.10	0.13	0.10
56-93	46A11(I56V,H93R)	0.21	0.45	0.99	0.12	0.97	0.80	0.26	0.13	0.14	0.09	0.06	0.15	0.18
68-75	04E 2 (**F68S,M75K**)	**2.80**	1.14	1.27	**4.47**	**1.50**	**1.62**	**3.71**	**4.35**	**3.04**	**3.91**	**3.46**	**2.89**	**4.20**
77-126	27B9 (**F77T**,T126R)	0.55	0.26	0.54	**3.68**	0.67	0.59	**1.91**	**2.34**	1.06	0.67	0.32	0.49	**2.58**
77-39	33E 2 (**F77T**,T39I)	0.70	0.35	0.51	**4.35**	0.67	0.53	**2.39**	**4.01**	1.20	1.12	0.50	0.67	**3.74**
89-148	50 E7 (**K89E**, S148T)	**2.62**	0.76	1.04	**3.34**	1.21	1.24	**2.76**	**4.62**	**2.49**	**2.89**	**2.61**	**2.58**	**3.71**
90-77	02D10 (**I90V,F77T**)	**1.55**	0.60	0.82	**13.12**	1.08	0.84	**6.71**	**30.62**	**1.77**	**8.13**	**4.09**	**12.85**	**20.94**
106-164	17E 6 (M106K,**C164S**)	**1.65**	1.06	1.11	**1.32**	1.27	1.29	**1.98**	**1.77**	**1.82**	**2.58**	**4.79**	**1.60**	**2.16**
23-58-169	47G12 (E23K,V58L,A169V)	0.05	0.42	0.46	1.16	0.62	0.79	0.84	**1.54**	0.30	0.02	0.03	0.12	**1.32**
33-76-89	17C5 **(K33N**,I76N,**K89E**)	1.11	0.59	**1.48**	0.93	**1.89**	**1.79**	1.30	1.13	**1.56**	0.74	0.32	0.65	0.99
68-90-145	04D10 (**F68S,I90V**,P145L)	**3.91**	**1.33**	**1.45**	**5.59**	**1.74**	**1.99**	**6.12**	**8.68**	**4.09**	**3.86**	**3.64**	**6.08**	**8.66**
59-109-113-136	41H10 (I59T, **Y109F**, P113T, L136M)	0.06	0.07	0.24	0.03	0.26	0.20	0.25	0.30	0.16	**5.44**	**2.21**	0.43	0.36

FIG. 6C

POSITION	Clone(AA sequence)	coel.	h,h	PBI-3945
15	03F2 (G15R)	0.002	0.010	0.024
18	40H11 (Q18L)	0.38	0.53	0.39
20	16C5 (Q20R)	**2.58**	0.82	0.80
22	14C1 (L22I)	0.12	0.33	0.44
27	11E 8 (L27V)	0.21	1.20	**1.30**
54	18F8 (F54S)	0.59	0.25	0.03
54	29H7 (F54I)	**6.20**	0.65	1.29
58	30E 4 (V58I)	0.18	0.69	0.77
72	04G7 (L72Q)	**4.19**	1.01	**1.49**
75	28B2 (M75K)	**4.11**	**2.21**	**2.06**
77	18C4 (F77T)	0.93	0.74	0.33
77	42E 4 (F77C)	0.08	0.75	0.28
77	50D12 (F77T)	0.81	0.64	0.29
89	35D11 (K89E)	**2.18**	0.88	0.61
90	10F2 (I90T)	**1.45**	0.38	0.32
92	04A12 (L92H)	0.62	0.27	0.03
109	43F9 (Y109F)	0.22	0.54	0.07
127	30D6 (V127A)	0.11	0.22	0.19
139	27B8 (D139G)	0.00	0.08	0.01
164	25A1 (C164S)	0.56	0.61	0.63
14-89	25F2 (A14V,K89E)	**1.91**	0.66	0.49
27-33	47D8(L27M,K33N)	0.40	1.24	1.12
49-72	26F1 (E49K,L72Q)	1.17	1.08	1.14
54-66	48G3 (F54I, S66T)	**6.17**	0.66	**1.31**
54-67	35B8 (F54S,G67S)	0.13	0.99	0.24
55-117	05H8 (D55G,I117F)	0.11	0.30	0.30
56-93	46A11(I56V,H93R)	0.15	0.64	0.26
68-75	04E 2 (F68S,M75K)	**4.11**	**1.78**	**1.94**
77-126	27B9 (F77T,T126R)	0.49	0.47	0.13
77-39	33E 2 (F77T,T39I)	0.58	0.44	0.20
89-148	50 E7 (K89E, S148T)	**2.06**	0.85	0.59
90-77	02D10 (I90V,F77T)	**1.85**	1.01	0.56
106-164	17E 6 (M106K,C164S)	**2.16**	**1.30**	**1.35**
23-58-169	47G12 (E23K,V58L,A169V)	0.04	0.37	0.47
33-76-89	17C5 (K33N,I76N,K89E)	**1.31**	**1.75**	0.60
68-90-145	04D10 (F68S,I90V,P145L)	**3.01**	1.11	1.07
59-109-113-136	41H10 (I59T, Y109F, P113T, L136M)	0.05	0.20	0.02

FIG. 6D

Clone	18	20	54	59	72	77	89	92	109	113	127	136	164
QC#1 (Q20R, L92H, F77C)		X				X		X					
QC#3 (F77C)						X							
QC#5 (L92H)								X					
QC#6 (F54I, F77C)			X			X							
QC#7 (Q20R, F54I, F77C)		X	X			X							
QC#8 (Q20R, F54I)		X	X										
QC#9 (F54I, F77C, L92H)			X			X		X					
QC#10 (Q20R, F54I, F77C, L92H)		X	X			X		X					
QC#13 (Q20R, Y109F)		X							X				
QC#14 (L92H, Y109F)								X	X				
QC#15 (Y109F)									X				
QC#16 (F54I, L92H, Y109F)			X					X	X				
QC#20 (F54I, Y109F)			X						X				
QC#22 (Q18L, L92H, Y109F)	X							X	X				
QC#27 (Q18L, F54I, L92H, Y109F)	X		X					X	X				
QC#32 (Q18L, I59T, L92H, Y109F, P113T, V127A, K136M)	X			X				X	X	X	X	X	
QC#36 (Q20R,F54I, L72Q, K89E, C164S)		X	X		X		X						X
QC#37 (F54I, L72Q, K89E, C164S)			X		X		X						X
QC#38 (F54I, C164S)			X										X
QC #1 C2 (Q18L, Y109F)	X								X				
QC #1 B4 (Q18L, F54I)	X		X										
QC #1 C3 (Q18L, F54I, Y109F)	X		X						X				
QC#1 A7 (F54I, K89E, C164S)			X				X						X
QC#1 A11 (Q20R, F54I, L72Q, K89E)		X	X		X		X						
QC#1 B9 Q20R,F54I, L72Q, C164S)		X	X		X								X
QC#1 D11 (F54I, L72Q,K89E,C164S)			X		X		X						X

FIG. 7

Sample	coelenterazine	h	h,h	PBI-3840	PBI-3925	PBI-3912
QC#1 (Q20R, L92H, F77C)	0.17	0.03	0.19	0.74	1.03	0.56
QC#3 (F77C)	0.09	0.36	0.89	0.16	1.06	0.04
QC#5 (L92H)	0.73	0.04	0.38	**4.49**	**6.92**	**5.64**
QC#6 (F54I, F77C)	0.85	0.59	0.91	**1.89**	**3.80**	0.41
QC#7 (Q20R, F54I, F77C)	0.72	0.22	0.20	**1.85**	**2.83**	**1.50**
QC#8 (Q20R, F54I)	**6.48**	0.89	0.34	**11.73**	**21.51**	**35.04**
QC#9 (F54I, F77C, L92H)	0.39	0.07	0.16	0.81	**1.58**	0.64
QC#10 (Q20R, F54I, F77C, L92H)	0.27	0.05	0.07	0.75	**2.24**	**1.53**
QC#13 (Q20R, Y109F)	0.21	0.07	0.25	1.08	**1.74**	**4.18**
QC#14 (L92H, Y109F)	0.06	0.00	0.08	0.78	**1.84**	**2.63**
QC#15 (Y109F)	0.21	0.15	0.54	0.95	0.68	0.46
QC#16 (F54I, L92H, Y109F)	0.35	0.02	0.05	1.01	**3.73**	**8.75**
QC#20 (F54I, Y109F)	**4.49**	0.82	0.51	**9.41**	**12.43**	**10.39**
QC#22 (Q18L, L92H, Y109F)	0.05	0.01	0.05	0.15	0.06	0.14
QC#27 (Q18L, F54I, L92H, Y109F)	0.08	0.01	0.10	0.68	0.59	1.06
QC#32 (Q18L, I59T, L92H, Y109F, P113T, V127A, K136M)	0.003	0.001	0.007	0.022	0.015	0.020
QC#36 (Q20R,F54I, L72Q, K89E, C164S)	0.99	0.14	0.06	**1.34**	**1.86**	**16.93**
QC#37 (F54I, L72Q, K89E, C164S)	**2.01**	0.30	0.14	**3.16**	**4.63**	**23.45**
QC#38 (F54I, C164S)	**3.39**	0.65	0.48	**5.25**	**4.56**	**9.48**
C1A4E	1.00	1.00	1.00	1.00	1.00	1.00

FIG. 8A

Sample	Coel.	h	h,h	2-me	PBI-3889	PBI-3939	PBI-3933	PBI-3932	PBI-3946	PBI-3841	PBI-3896	PBI-3925
QC#1 (Q20R, L92H, F77C)	0.13	0.02	0.11	**1.62**	0.15	0.12	0.45	**3.87**	0.18	0.25	0.17	0.58
QC#3 (F77C)	0.07	0.25	0.55	1.14	0.63	0.38	0.41	**1.79**	0.28	0.04	0.04	0.55
QC#5 (L92H)	0.51	0.03	0.24	**4.99**	0.23	0.10	**1.68**	**2.88**	0.80	**25.89**	**23.26**	**3.28**
QC#6 (F54I, F77C)	0.69	0.48	0.67	**22.92**	0.31	0.07	0.51	**35.10**	0.44	0.08	0.15	**2.55**
QC#7 (Q20R, F54I, F77C)	0.54	0.18	0.15	**22.57**	0.09	0.03	0.39	**32.08**	0.21	0.39	0.41	**2.24**
QC#8 (Q20R, F54I)	**5.32**	0.79	0.28	**28.12**	0.49	0.11	**2.80**	**40.88**	**1.43**	**26.40**	**43.55**	**17.18**
QC#9 (F54I, F77C, L92H)	0.26	0.05	0.10	**3.69**	0.13	0.09	0.55	**5.63**	0.22	0.21	0.23	1.03
QC#10 (Q20R, F54I, F77C, L92H)	0.20	0.03	0.05	**5.91**	0.07	0.05	0.45	**11.18**	0.19	0.54	0.45	**1.58**
QC#13 (Q20R, Y109F)	0.26	0.10	0.31	1.05	0.37	0.29	0.66	**3.37**	0.25	**19.06**	**74.46**	**1.82**
QC#14 (L92H, Y109F)	0.10	0.003	0.11	1.18	0.11	0.06	0.52	**2.24**	0.07	**13.47**	**65.50**	**1.64**
QC#15 (Y109F)	0.29	0.22	0.74	0.38	0.83	0.69	0.68	0.74	0.47	**6.43**	**1.61**	0.89
QC#16 (F54I, L92H, Y109F)	0.44	0.02	0.06	**6.00**	0.11	0.06	0.46	**9.48**	0.03	**5.53**	**269.14**	**3.50**
QC#20 (F54I, Y109F)	**4.22**	0.90	0.50	**5.86**	1.19	0.61	**3.68**	**14.10**	**1.82**	**44.89**	**15.18**	**12.45**
QC#22 (Q18L, L92H, Y109F)	0.06	0.01	0.06	0.06	0.06	0.05	0.05	0.03	0.01	**14.10**	**242.95**	0.04
QC#27 (Q18L, F54I, L92H, Y109F)	0.13	0.02	0.12	0.33	0.15	0.12	0.30	0.24	0.10	0.60	**561.32**	0.40
QC#32 (Q18L, I59T, L92H, Y109F, P113T, V127A, K136M)	0.004	0.001	0.011	0.01	0.01	0.01	0.01	0.01	0.00	**16.29**	**51.72**	0.02
QC#36 (Q20R,F54I, L72Q, K89E, C164S)	0.78	0.14	0.07	**23.49**	0.02	0.002	0.09	**28.30**	0.06	**64.90**	**49.30**	**2.04**
QC#37 (F54I, L72Q, K89E, C164S)	**1.87**	0.32	0.17	**26.86**	0.08	0.01	0.32	**33.42**	0.22	**71.56**	**37.60**	**5.30**
QC#38 (F54I, C164S)	**4.51**	0.92	0.67	**11.22**	0.49	0.06	1.15	**12.46**	1.13	**22.07**	**11.03**	**6.46**
C1A4E	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

FIG. 8B

Sample	Coel	h,h	PBI-3939	PBI-3945	PBI-3840	PBI-3932	PBI-3925	PBI-9894	PBI-3896
QC C2 (Q18L, Y109F)	0.01	0.06	0.04	0.04	0.03	0.01	0.01	19.87	24.80
QC B4 (Q18L, F54I)	5.71	1.84	1.23	1.19	6.04	8.23	7.02	67.35	719.58
QC C3 (Q18L, F54I, Y109F)	0.18	0.52	0.65	0.22	0.49	0.13	0.46	407.08	454.48
QC E6 (Q18L, I59T, Y109F, P113T, V127A,K136M)	0.0004	0.01	0.01	0.002	0.01	0.01	0.00	3.88	9.03
QC A7 (F54I, K89E, C164S)	2.63	0.21	0.01	0.28	4.05	17.21	4.86	11.82	23.95
QC A11 (Q20R, F54I, L72Q, K89E)	3.76	0.13	0.02	0.31	3.21	94.34	15.76	46.29	127.88
QC B9 Q20R,F54I, L72Q, C164S)	1.67	0.10	0.005	0.10	1.89	25.28	3.37	13.58	42.27
QC D11 (F54I, L72Q,K89E,C164S	1.95	0.13	0.01	0.16	2.45	27.69	4.84	13.38	33.69
QC#6 (F54I,F77C)	0.67	0.76	0.06	0.36	1.24	39.37	3.14	0.52	1.32
QC# 7 (Q20R,F54I,F77C0	0.51	0.15	0.03	0.07	1.01	32.09	2.26	1.67	1.30
QC#9 (F54I,F77C,L92H)	0.22	0.10	0.09	0.06	0.33	4.97	1.01	0.26	0.32
QC #8 (Q20R,F54I)	6.37	0.35	0.14	0.93	8.42	46.87	21.22	13.40	33.57
QC# 13(Q20R,Y109F)	0.18	0.26	0.25	0.02	0.79	2.54	1.58	6.85	49.23
QC#14 (L92H,Y109F)	0.04	0.07	0.04	0.001	0.29	1.35	1.43	4.53	37.03
QC#16 (F54I,L92H,Y109F)	0.30	0.05	0.05	0.01	0.46	7.07	3.42	19.64	157.70
QC# 20 (F54I,Y109F)	4.25	0.56	0.62	1.04	6.93	13.08	11.84	8.73	10.47
QC#22 (Q18L, L92H, Y109F)	0.04	0.05	0.04	0.01	0.09	0.03	0.05	38.41	138.45
QC#27 (Q18L, F54I, L92H, Y109F)	0.07	0.08	0.10	0.01	0.28	0.17	0.42	392.98	283.85
QC#32 (Q18L, I59T, L92H, Y109F, P113T, V127A, K136M)	0.003	0.01	0.01	0.0002	0.02	0.01	0.02	11.79	33.46
QC#36 (Q20R,F54I, L72Q, K89E, C164S)	0.92	0.06	0.002	0.05	1.07	24.56	2.12	10.40	35.04

FIG. 9

Clone	18	20	54	71	77	90	92	109	127
QC#2 1 (Q20R,F77C,I90V,L92H,V127A)		x			x	x	x		x
QC#2 2 (Q20R,G71D,F77C,I90V,L92H,V127A)		x		x	x	x	x		x
QC#2 3 (Q20R,G71D,F77C,L92H,V127A)		x		x	x		x		x
QC#2 6 (F54I,G71D,F77C,L92H,V127A)			x	x	x		x		x
QC#2 7 (F54I,G71D,F77C,I90V,L92H)			x	x	x	x	x		
QC#2 8 (F54I,G71D,F77C,I90V,L92H,V127A)			x	x	x	x	x		x
QC#2 11 (Q20R,F54I,G71D,F77C,I90V,L92H,V127A)		x	x	x	x	x	x		x
QC#2 16 (G71D,I90V,L92H,Y109F,L127A)				x		x	x	x	x
QC#2 18 (G71D,L92H,Y109F,L127A)				x			x	x	x
QC#2 21(Q20R,F54I,G71D,F77C,I90V,L92H,V127A)		x	x	x	x	x	x		x
QC#2 26 (Q18L,G71D,F77Y,I90V,L92H,Y109F,V127A)	x			x	x	x	x	x	x
QC#2 31 (Q18L,F54I,G71D,F77Y,I90V,L92H,Y109F,V127A)	x		x	x	x	x	x	x	x
QC#2 41 (G71D,F77C,I90V,L92H,V127A)				x	x	x	x		x
QC#2 44 (G71D,F77C,I90V,V127A)				x	x	x			x
QC#2 A3 (Q20R,G71D,F77C,L92H)		x		x	x		x		
QC#2 C6 (F54I,G71D,F77C,L92H)			x	x	x		x		
QC#2 A7 (Q20R,F54I,G71D,F77C,L92H,V127A)		x	x	x	x		x		x
QC#2 C10 (I90V,L92H,Y109F,V127A)						x	x	x	x
QC#2 E1 (F54I,I90V,L92H,Y109F,V127A)			x			x	x	x	x
QC#2 E2 (F54I,G71D,I90V,L92H,Y109F)			x	x		x	x	x	
QC#2 E4 (Q18L,G71D,F77Y,L92H,Y109F,V127A)	x			x	x		x	x	x
QC#2 F4(Q18L,F77Y,I90V,L92H,Y109F,V127A)	x				x	x	x	x	x
QC#2 E7 (Q18L,F54I,F77Y,I90V,L92H,Y109F,V127A)	x		x		x	x	x	x	x
QC#2 E8 (Q18L,F54I,I90V,L92H,Y109F,V127A)	x		x			x	x	x	x
QC#2 F7 (Q18L,F54I,G71D,F77Y,L92H,Y109F,V127A)	x		x	x	x		x	x	x
QC#2 H11 (G71D,F77C)				x	x				
LE1 (I90V/L92H)						x	x		
LE2 (I90V,Y109F)						x		x	
LE3 (I90V/L92H/Y109F)						x	x	x	

FIG. 10

Sample	Coel	h,h	PBI-3939	PBI-3945	PBI-3840	PBI-3932	PBI-3925	PBI-3894	PBI-3896
QC#2 1 (Q20R,F77C,I90V,L92H,V127A)	0.04	0.04	0.05	0.004	0.17	***4.04***	0.62	0.19	0.13
QC#2 2 (Q20R,G71D,F77C,I90V,L92H,V127A)	0.07	0.05	0.07	0.01	0.24	***6.19***	0.98	0.26	0.20
QC#2 3 (Q20R,G71D,F77C,L92H,V127A)	0.09	0.07	0.09	0.01	0.30	***5.02***	0.88	0.34	0.21
QC#2 6 (F54I,G71D,F77C,L92H,V127A	0.11	0.07	0.08	0.03	0.19	***2.43***	0.51	0.15	0.11
QC#2 7 (F54I,G71D,F77C,I90V,L92H)	0.40	0.11	0.12	0.09	0.39	***13.65***	***2.15***	0.38	0.41
QC#2 8 (F54I,G71D,F77C,I90V,L92H,V127A)	0.11	0.06	0.08	0.03	0.16	***4.12***	0.68	0.15	0.18
QC#2 11 (Q20R,F54I,G71D,F77C,I90V,L92H,V127A)	0.10	0.03	0.05	0.02	0.17	***6.66***	1.11	0.40	0.24
QC#2 16 (G71D,I90V,L92H,Y109F,L127A,)	0.11	0.06	0.04	0.001	0.29	***1.49***	***1.51***	***7.72***	***55.11***
QC#2 18 (G71D,L92H,Y109F,L127A)	0.08	0.04	0.03	0.0005	0.28	***1.33***	1.29	***7.30***	***48.97***
QC#2 21(Q20R,F54I,G71D,F77C,I90V,L92H,V127A)	0.27	0.02	0.02	0.004	0.31	***7.57***	***2.49***	***30.02***	***160.54***
QC#2 26 (Q18L,G71D,F77Y,I90V,L92H,Y109F,V127A)	0.00	0.01	0.01	0.001	0.00	0.01	0.02	***1.38***	***7.86***
QC#2 31 (Q18L,F54I,G71D,F77Y,I90V,L92H,Y109F,V127A)	0.01	0.03	0.03	0.004	0.01	0.05	0.06	***8.04***	***77.32***
QC#2 41 (G71D,F77C,I90V,L92H,V127A)	0.05	0.07	0.08	0.01	0.12	***2.50***	0.37	0.10	0.07
QC#2 44 (G71D,F77C,I90V,V127A)	0.17	0.15	0.15	0.03	0.15	***2.92***	0.41	0.18	0.10
QC#2 A3 (Q20R,G71D,F77C,L92H)	0.23	0.20	0.22	0.03	0.52	***6.75***	1.11	0.39	0.24
QC#2 C6 (F54I,G71D,F77C,L92H)	0.44	0.18	0.18	0.13	0.60	***12.27***	***2.43***	0.50	0.30
QC#2 A7 (Q20R,F54I,G71D,F77C,L92H,V127A)	0.13	0.05	0.06	0.02	0.28	***6.93***	1.25	0.32	0.22
QC#2 C10 (I90V,L92H,Y109F,V127A)	0.06	0.04	0.02	0.0004	0.22	1.08	1.05	***4.33***	***34.52***
QC#2 E1 (F54I,I90V,L92H,Y109F,V127A)	0.18	0.04	0.03	0.004	0.25	***2.74***	***1.66***	***6.96***	***49.09***
QC#1 E2 (F54I,G71D,I90V,L92H,Y109F)	0.39	0.06	0.04	0.01	0.24	***9.71***	***3.63***	***25.77***	***185.63***
QC#2 E4 (Q18L,G71D,F77Y,L92H,Y109F,V127A)	0.0005	0.009	0.008	0.0005	0.001	0.009	0.004	0.58	***3.48***
QC#2 F4(Q18L,F77Y,I90V,L92H,Y109F,V127A)	0.0002	0.0057	0.0062	0.0003	0.0013	0.0069	0.0048	0.56	***4.18***
QC#2 E7 (Q18L,F54I,F77Y,I90V,L92H,Y109F,V127A)	0.003	0.013	0.015	0.002	0.006	0.020	0.024	***3.17***	***36.43***
QC#2 E8 (Q18L,F54I,I90V,L92H,Y109F,V127A)	0.06	0.04	0.05	0.002	0.13	0.05	0.11	***145.44***	***331.26***
QC#2 F7 (Q18L,F54I,G71D,F77Y,L92H,Y109F,V127A	0.01	0.03	0.03	0.004	0.01	0.03	0.04	***3.22***	***30.50***
QC#2 H11 (G71D,F77C)	0.07	0.69	0.61	0.24	0.13	***3.46***	1.02	0.16	0.10
C1A4E	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

FIG. 11

Sample	Coel.	hh	PBI-3939	PBI-3945	PBI-3889	PBI-3840	PBI-3932	PBI-3925	PBI-3894	PBI-3896	PBI-3897
C1A4E	1	1	1	1	1	1	1	1	1	1	1
I90V	**1.49**	0.77	1.21	0.51	1.12	**2.86**	**22.13**	**14.69**	**8.48**	**2.30**	**6.43**
Y109F	0.13	0.42	0.35	0.15	0.42	0.58	0.37	0.41	1.05	0.35	**12.70**
LE2 (I90V,Y109F)	0.31	0.29	0.26	0.06	0.33	**1.80**	**10.20**	**6.09**	**5.15**	**3.04**	**60.24**
QC #27(Q18L/L92H/Y109F/F54I)	0.15	0.06	0.08	0.01	0.06	0.23	0.12	0.20	**196.86**	**140.68**	**13.83**
QC#2 E7 (Q18L,F54I,F77Y,I90V,L92H,Y109F,V127A)	0.002	0.02	0.02	0.0022	0.0076	0.0054	0.02	0.02	**1.83**	**6.57**	**31.17**
QC#2 F4(Q18L,F77Y,I90V,L92H,Y109F,V127A)	0.0008	0.005	0.0046	0.0004	0.0021	0.0021	0.01	0.05	0.24	0.80	**7.04**
QC#1 A11 (Q20R, F54I, L72Q, K89E)	**3.50**	0.12	0.02	0.16	0.08	**3.51**	**82.84**	**11.19**	**59.12**	**24.00**	**42.51**

FIG. 12

Sample	POSITION	Sequence	COELENTERAZINE	H,H	PBI-3897	PBI-3896	PBI-3894
29H3	18	L18I	1.17	0.32	0.94	**2.37**	**3.25**
25A11	21	V21L	0.06	0.11	0.53	0.55	0.19
4F3	67	G67S	**1.75**	0.63	**1.37**	1.24	1.06
24B12	68	F68Y	**1.88**	0.88	**1.52**	**1.62**	**1.44**
29C4	72	L72Q	**1.69**	0.77	**1.37**	**2.33**	**2.38**
3H11	75	M75K	**2.33**	1.09	**1.90**	**2.19**	**1.90**
3F10	76	I76F	0.50	0.81	1.27	0.17	0.08
27E 4	90	I90T	0.24	0.17	0.76	0.10	0.04
1B6	92	H92R	0.10	0.46	0.98	0.06	0.02
47H12	22-74	L22F-E74K	0.03	0.12	0.29	0.02	0.03
11F10	158-1	F1I,V158I	**2.49**	**2.53**	**2.37**	**1.67**	**1.40**
48D5	11-109	R11Q,F109Y	**6.24**	**2.74**	0.39	1.04	0.17
18A8	18-98	**L18I,** V98F	0.64	0.19	0.54	1.25	**1.56**
43G9	21-106	**V21M,M106I**	0.02	0.04	0.05	0.23	0.49
33A5	31-102	F31I,**V102M**	0.73	0.68	0.67	1.06	1.16
11G9	32-92	Q32H,**H92R**	0.10	0.47	1.02	0.06	0.01
9D7	45-76	V45E,**I76V**	**1.66**	0.71	**1.33**	**1.72**	**1.80**
33H2	92-159	**H92R,**T159S	0.05	0.37	0.58	0.04	0.01
1G5	92-170	**H92R**,G170R	0.58	**N/A**	**1.50**	0.16	0.31
19C10	18-49-86	**L18Q**,E49D,H86R	**3.29**	0.15	0.52	0.05	0.05
44H1	21-47-51	**V21M,** S47P, G51E	0.01	0.02	0.02	0.10	0.22
14C2	46-106-168	L46Q, **M106I**, L168F	**1.77**	0.97	**1.52**	**1.51**	**1.33**
24D7	48-99-76	G48R,**I99V,I76F**	0.38	0.63	1.04	0.10	0.05
18E 8	55-92-96	D55E,**H92Q,**T96A	**1.40**	**3.78**	**2.41**	0.39	0.36
7D9	74-92-99	E74I,**H92R,I99T**	0.14	0.36	0.77	0.04	0.01
8C2	21-68-69-142	**V21M,F68L**,Q69H,L142V	0.01	0.02	0.02	0.11	0.22
QC #27 HT7			1.00	1.00	1.00	1.00	1.00

Positions in common with C1A4E library
18
22
68
72
75
90
92
106
109

FIG. 13

Sequence	Clone	21	68	72	75	76	90	92	158
F68Y, L72Q, M75K, V158I	QC27#1		X	X	X				X
I90T, H92R	QC27#2						X	X	
F68Y, L72Q, M75K, I90T, H92R, V158I	QC27#3		X	X	X		X	X	X
V21L, I76F, I90T, H92R	QC27#4	X				X	X	X	
V21L,I76F	QC27#6A	X				X			
V21L,F68Y,L72Q,M75K,I90T	QC27#7A	X	X	X	X		X		
V21L,I76F,H92R	QC27#8	X				X		X	
V21L,F68Y,L72Q,M75K,H92R,V158I	QC27#9A	X	X	X	X			X	X
V21L,F68Y,L72Q,M75K,I76F,I90T,H92R,V158I	QC27#10	X	X	X	X	X	X	X	X
F68Y,L72Q,M75K,H92R	QC27#12A		X	X	X			X	
V21L,H92R	QC27#13	X						X	
F68Y,L72Q,M75K,H92R,V158I	QC27#14A		X	X	X			X	X
V21L, F68Y, L72Q, M75K, I90T, H92R, V158I	QC27#5	X	X	X	X		X	X	X
V21L, I90T, I76F	QC27#6	X				X	X		
V21L,F68Y,L72Q,M75K, I76F,V158I	QC27#9B	X	X	X	X	X			X

FIG. 14

Sample	AA change	Coelenterazine	h,h	PBI-3897	PBI-3841	PBI-3896	PBI-3894
QC27 #1	F68Y, L72Q, M75K, V158I	**2.59**	**3.95**	**2.90**	**2.90**	**5.19**	**6.72**
QC27 #2	I90T, H92R	0.0189	0.17	0.19	0.19	0.02	0.00
QC27 #3	F68Y, L72Q, M75K, V158I, I90T, H92R	0.1212	0.77	0.88	0.92	0.10	0.02
QC27 #4	V21L, I76F, I90T, H92R	0.0006	0.00	0.06	0.08	0.04	0.00
QC27 #6A	V21L,I76F	0.0060	0.07	0.26	0.20	0.12	0.03
QC27 #7A	V21L,F68Y,L72Q,M75K,I90T	0.0370	0.40	**1.76**	**1.54**	1.09	0.16
QC27 #8	V21L,I76F,H92R	0.0730	0.21	0.50	0.59	0.28	0.11
QC27 #9A	V21L,F68Y,L72Q,M75K,H92R,V158I	0.0041	0.08	0.81	**1.35**	0.32	0.03
QC27 #10	V21L,F68Y,L72Q,M75K,I76F,I90T,H92R,V158I	0.0049	0.01	0.33	0.48	0.20	0.02
QC27 #12A	F68Y,L72Q,M75K,H92R	0.2767	**2.64**	**2.82**	**3.17**	0.31	0.09
QC27 #13	V21L,H92R	0.0007	0.01	0.12	0.20	0.05	0.00
QC27 #14A	F68Y,L72Q,M75K,H92R,V158I	0.2283	**2.38**	**2.51**	**2.71**	0.26	0.07
QC27 #5	V21L, F68Y, L72Q, M75K, I90T, H92R, V158I	0.0013	0.02	0.15	0.25	0.07	0.01
QC27 #6	V21L, I90T, I76F	0.0014	0.00	0.09	0.05	0.03	0.00
QC27 #9B	V21L,F68Y,L72Q,M75K,I76F,V158I	0.0530	0.43	1.14	1.19	0.93	0.32
QC27 ctrl.		1	1	1	1	1	1

FIG. 15

Sample	Position	AA change	Coelenterazine	H	HH	PBI-3841	PBI-3897
30D12	22	L22F	0.08	0.01	0.08	0.28	0.26
26D4	36	V36M	**1.70**	**1.40**	**2.19**	1.21	1.08
40B10	76	I76F	**3.66**	0.54	0.57	**1.65**	**1.97**
5C3	128	T128A	0.19	0.26	0.45	0.43	0.47
22C2	128-154	T128S,T154S	0.24	0.33	0.52	0.43	0.46
22G9	135-152	N135Y,R152H	0.09	0.13	0.16	0.20	0.24
39H11	18-145	L18I,P145Q	**42.10**	**3.20**	**18.30**	**2.51**	**2.37**
36C11	18-99	L18V,I99T	**125.68**	**2.64**	**24.99**	**2.15**	**2.17**
3E 2	67-159	G67D,T159I	0.39	0.51	0.63	0.77	0.80
48C5	46-51-138	L46Q,G51R,I138S	0.43	0.49	0.96	0.87	0.95
3C10	51-112-113	G51V,R112C,P113H	0.24	0.31	0.72	0.93	0.67
QC279a HT			1	1	1	1	1

FIG. 16

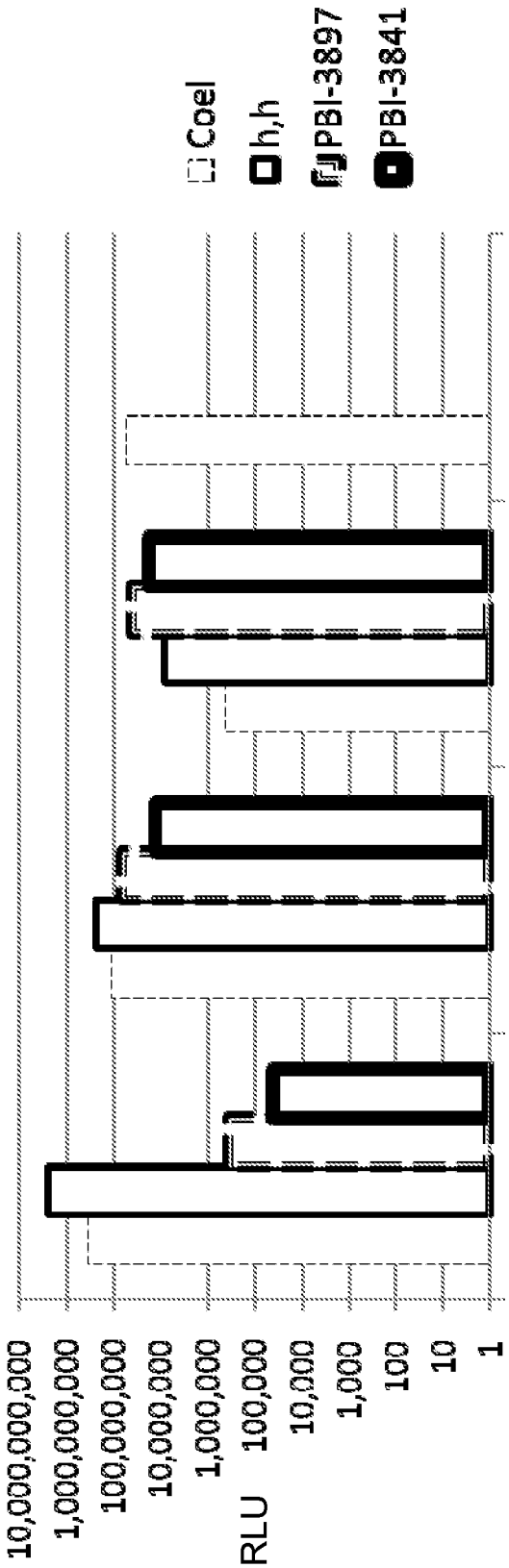

Coel
h,h
PBI-3897
PBI-3841
RLU
10,000,000,000
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
C1A4E
QC 27
QC 27 #9a
Renilla/coel
C1A4E +4 mutations
QC27 +6 mutations

FIG. 17

Sample	Position	AA change	Coelenterazine	h,h	PBI-3939	PBI-3945	PBI-3889	PBI-4002
IVY 29H12	19	D19N	0.002	0.03	0.04	0.15	0.03	0.11
IVY 47E11	20	Q20P	0.054	1.05	**1.36**	1.22	1.22	1.12
IVY 36E11	38	V38I	0.169	0.96	1.26	1.18	1.23	1.09
IVY 21D1	44	I44F	0.128	0.67	0.65	0.39	0.73	0.38
IVY 5B8	76	I76N	0.208	0.32	0.39	0.50	0.36	0.44
IVY 50G8	77	Y77N	0.141	0.86	0.23	0.61	0.62	0.85
IVY 27E6	95	G95S	0.106	0.73	0.70	0.61	0.78	0.62
IVY 2G6	95	G95D	0.004	0.07	0.07	0.33	0.06	0.30
IVY 27B7	110	F110I	0.022	0.13	0.18	0.44	0.17	0.37
IVY 26H7	149	L149I	0.266	0.94	0.82	0.90	0.89	0.94
IVY 21A4	152	R152S	0.242	0.78	0.83	0.63	0.99	0.60
IVY 5C2	38-43	V38F,K43N	0.008	0.45	0.51	0.56	0.46	0.51
IVY 43D3	49-32	E49G,Q32P	0.170	0.93	1.23	1.15	1.20	1.07
IVY 20E12	60-119	I60V,V119M	0.289	0.38	0.44	0.62	0.43	0.57
IVY 26B6	18-69-124	Q18H,Q69H,K124M	0.006	0.29	0.39	0.29	0.40	0.26
IVY 23F3	33-43-94	K33N,K43N,Y94F	0.004	0.24	0.25	0.34	0.21	0.34
IVY+HT7			1.00	1.00	1.00	1.00	1.00	1.00

FIG. 18

Sample	position	AA	Coel.	H	H,H	PBI-3939	PBI-3945	PBI-3889	PBI-4002
34B9	18	Q18L	0.22	0.52	0.74	0.92	0.38	1.12	0.48
8E 3	27	L27V	0.19	1.06	1.12	**1.35**	1.17	**1.33**	1.11
33C4	28	S28Y	0.07	0.83	0.80	0.78	0.88	0.83	0.80
21C5	33	K33N	0.96	**1.41**	**1.68**	**1.68**	**1.36**	**1.74**	**1.34**
25F10	36	V36E	0.14	0.39	0.46	0.40	0.36	0.60	0.41
25H7	40	P40T	0.003	0.13	0.27	0.47	0.28	0.46	0.23
28A8	135	N135D	0.04	0.50	0.69	0.73	0.51	0.86	0.47
44G12	155	I155T	0.08	0.51	0.85	0.63	0.48	0.87	0.48
46A6	32-6	F6Y,Q32L	0.12	0.61	0.76	0.75	0.57	0.80	0.56
26D5	18-51-86	Q18L,G51R,H86L	0.47	0.95	**1.39**	**1.58**	0.62	**1.95**	0.83
3E 12	6-42-50	F6Y,Q42H,N50K	0.12	0.60	0.84	1.03	0.61	1.04	0.61

FIG. 19

Sequence	Sample	19	20	27	32	38	43	49	58	77	95	110	149
Q20P,Q32P,E49G,L149I	C1.1		X		X			X					X
Q20P,Q32P,E49G,F77Y,G95D,F110I,L149I	C4.13		X		X			X		X	X	X	X
Q20P,L27V,V38I,L149I	C5.16		X	X		X							X
L27V,V38I,L149I	C5.19			X		X							X
D19N,Q20P,V38I,L149I	C7.26	X	X			X							X
V38I,G95D,F110I,L149I	C8.28					X					X	X	X
D19N,Q20P,V38I,G95D,F110I,L149I	C8.29	X	X			X					X	X	X
L27V,V38F,K43N,L149I	C11.40			X		X	X						X
L27V,V38F,K43N,V58I,L149I	C11.42			X		X	X		X				X
L27V,V38F,K43N,V58I,F110I,L149I	C12.46			X		X	X		X			X	X
Q20P,L27V,L149I	C13.50		X	X									X
Q20P,L27V,V38I,F110I	C14.51		X	X		X						X	
L27V,V38I,F110I,L149I	C14.52			X		X						X	X

FIG. 20

Sample	Sequence	Coelenterazine	H	H,H	PBI-3939	PBI-3945	PBI-4002	PBI-3932	PBI-3840
C1.1	Q20P,Q32P,E49G,L149I	0.00006	0.00026	0.00033	0.00069	0.00050	0.00051	0.00014	0.00008
C4.13	Q20P,Q32P,E49G,F77Y,G95D,F110I,L149I	0.00006	0.00008	0.00009	0.00008	0.00005	0.00006	0.00013	0.00010
C5.16	Q20P,L27V,V38I,L149I	0.0002	0.0013	0.0003	0.0011	0.0034	0.0036	0.0001	0.0001
C5.19	L27V,V38I,L149I	0.0010	0.2747	0.0663	0.1595	0.5082	0.5129	0.0015	0.0003
C7.26	D19N,Q20P,V38I,L149I	0.0001	0.0017	0.0011	0.0020	0.0032	0.0037	0.0001	0.0000
C8.29	D19N,Q20P,V38I,G95D,F110I,L149I	0.000016	0.000025	0.000036	0.000016	0.000014	0.000031	0.000040	0.000025
C11.40	L27V,V38F,K43N,L149I	0.00010	0.01172	0.00714	0.01853	0.04124	0.02485	0.00036	0.00006
C11.42	L27V,V38F,K43N,V58I,L149I	0.0001	0.0016	0.0011	0.0044	0.0093	0.0040	0.0001	0.0001
C12.46	L27V,V38F,K43N,V58I,F110I,L149I	0.00002	0.00004	0.00005	0.00001	0.00008	0.00006	0.00006	0.00004
C13.50	Q20P,L27V,L149I	0.0001	0.0124	0.0036	0.0027	0.0109	0.0100	0.0001	0.0000
C14.51	Q20P,L27V,V38I,F110I	0.00003	0.00010	0.00004	0.00001	0.00008	0.00011	0.00003	0.00004
C14.52	L27V,V38I,F110I,L149I	0.00007	0.00041	0.00008	0.00006	0.00034	0.00046	0.00012	0.00008
IVY-HT7		1	1	1	1	1	1	1	1

FIG. 21

Sequence	clone	6	18	27	28	33	34	36	40	50	51	135	155
F6Y,Q18L,K33N,N135D	C1.1	X	X			X						X	
F6Y,Q18L,K33N,N135D,I155T	C1.2	X	X			X						X	X
F6Y,K33N,N135D,I155T	C1.3	X				X						X	X
K33N,P40T,N135D,I155T	C2.6					X			X			X	X
F6Y,Q18L,K33N,P40T,N135D,I155T	C2.7	X	X			X			X			X	X
F6Y,K33N,P40T,N135D,I155T	C2.8	X				X			X			X	X
F6Y,Q18L,K33N,V36E,P40T,N50S,G51RN135D,I155T	C3.13	X	X			X		X	X	X	X	X	X
F6Y,Q18L,N135D,I155T	C4.16	X	X									X	X
Q18L,N135D,I155T	C4.17		X									X	X
F6Y,Q18L,L27V,S28Y,N135D,I155T	C4.18	X	X	X	X							X	X
F6Y,Q18L,L27V,S28Y,P40T	C5.23	X	X	X	X				X				
Q18L,L27V,S28Y,K33N,V36E,N135D,I155T	C6.26		X	X	X	X		X				X	X
F6Y,Q18L,L27V,S28Y,K33N,V36E,N135D,I155T	C6.28	X	X	X	X	X		X				X	X
F6Y,Q18L,P40T,N135D,I155T	C7.31	X	X						X			X	X
F6Y,Q18L,L27V,S28Y,K33N,P40T,N135D,I155T	C7.33	X	X	X	X	X			X			X	X
Q18L,P40T,N135D,I155T	C7.34		X						X			X	X
F6Y,Q18L,K33M,L34M,N135D,I155T	C8.36	X	X			X	X					X	X
Q18L,K33M,L34M,N135D,I155T	C8.38		X			X	X					X	X
F6Y,Q18L,K33M,L34M,I55T	C8.40	X	X			X	X						X
F6Y,Q18L,K33M,L34M,P40T,N135D	C9.44	X	X			X	X		X			X	
F6Y,Q18L,L27V,S28Y,N50S,G51R,N135D	C10.46	X	X	X	X					X	X	X	
F6Y,Q18L,N50S,G51R,N135D,I155T	C10.48	X	X							X	X	X	X
Q18L,N50S,G51R,N135D,I155T	C10.49		X							X	X	X	X
Q18L,L27V,S28Y,N50S,G51R,N135D,I155T	C10.50		X	X	X					X	X	X	X
F6Y,Q18L,L27V,S28Y,N50S,G51R,N135D,I155T	C11.54	X	X	X	X					X	X	X	X

FIG. 22

Sample	Sequence	Coelenterazine	H	HH	PBI-3939	PBI-3945	PBI-3889	PBI-4002	PBI-3932	PBI-3840
C1.1	F6Y,Q18L,K33N,N135D	0.0055128	0.130	0.463	0.444	0.127	0.290	0.159	0.0083435	0.017
C1.2	F6Y,Q18L,K33N,N135D,I155T	0.0000068	0.0015688	0.0043713	0.0066058	0.0013479	0.0027420	0.0009303	0.0000844	0.0000782
C1.3	F6Y,K33N,N135D,I155T	0.0000257	0.0088297	0.036	0.048	0.0083438	0.023	0.0057396	0.0002600	0.0004430
C2.6	K33N,P40T,N135D,I155T	0.0000009	0.0000288	0.0001711	0.0017877	0.0001365	0.0002859	0.0000858	0.0000035	0.0000025
C2.7	F6Y,Q18L,K33N,P40T,N135D,I155T	0.0000008	0.0000091	0.0000318	0.0000611	0.0002445	0.0003744	0.0002560	0.0000013	0.0000020
C2.8	F6Y,K33N,P40T,N135D,I155T	0.0000004	0.0000107	0.0000641	0.0007157	0.0000848	0.0001958	0.0000447	0.0000019	0.0000011
C3.13	F6Y,Q18L,K33N,V36E,P40T,N50S,G51R,N135D,I155T	0.0000003	0.0000006	0.0000015	0.0000037	0.0000010	0.0000031	0.0000018	0.0000064	0.0000017
C4.16	F6Y,Q18L,N135D,I155T	0.0000299	0.0046508	0.012	0.017	0.0052284	0.010	0.0037895	0.0000946	0.0002504
C4.17	Q18L,N135D,I155T	0.0000222	0.0032164	0.0081703	0.011	0.0034948	0.0066376	0.0026060	0.0000717	0.0001691
C4.18	F6Y,Q18L,L27V,S28Y,N135D,I155T	0.0000008	0.0000325	0.0001094	0.0003355	0.0001157	0.0001447	0.0000548	0.0000060	0.0000025
C5.23	F6Y,Q18L,L27V,S28Y,P40T	0.0000579	0.0002759	0.0000464	0.0004978	0.0017611	0.0002812	0.0013044	0.0000144	0.0000132
C6.26	Q18L,L27V,S28Y,K33N,V36E,N135D,I155T	0.0000055	0.0000276	0.0001950	0.0004882	0.0000838	0.0003453	0.0000663	0.0003772	0.0000586
C6.28	F6Y,Q18L,L27V,S28Y,K33N,V36E,N135D,I155T	0.0000016	0.0000067	0.0000451	0.0001166	0.0000200	0.0000805	0.0000158	0.0000852	0.0000157
C7.31	F6Y,Q18L,P40T,N135D,I155T	0.0000003	0.0000017	0.0000024	0.0000174	0.0000084	0.0000113	0.0000067	0.0000018	0.0000005
C7.33	F6Y,Q18L,L27V,S28Y,K33N,P40T,N135D,I155T	0.0000004	0.0000007	0.0000013	0.0000017	0.0000008	0.0000011	0.0000006	0.0000016	0.0000009
C7.34	Q18L,P40T,N135D,I155T	0.0000007	0.0000021	0.0000024	0.0000253	0.0000131	0.0000159	0.0000101	0.0000048	0.0000009
C8.36	F6Y,Q18L,K33M,L34M,N135D,I155T	0.0000024	0.0000249	0.0000898	0.0001137	0.0000298	0.0000823	0.0000269	0.0000163	0.0000195
C8.38	Q18L,K33M,L34M,N135D,I155T	0.0000047	0.0000956	0.0003420	0.0004501	0.0001128	0.0003207	0.0001027	0.0000476	0.0000710
C8.40	F6Y,Q18L,K33M,L34M,I55T	0.0002632	0.010	0.015	0.018	0.011	0.015	0.0097703	0.0008447	0.0018540
C9.44	F6Y,Q18L,K33M,L34M,P40T,N135D	0.0000041	0.0000139	0.0000153	0.0000379	0.0000423	0.0000474	0.0000534	0.0000284	0.0000486
C10.46	F6Y,Q18L,L27V,S28Y,N50S,G51R,N135D	0.0002613	0.016	0.015	0.030	0.026	0.014	0.014	0.0004421	0.0002678
C10.48	F6Y,Q18L,N50S,G51R,N135D,I155T	0.0000064	0.0005384	0.0011439	0.0016621	0.0006785	0.0010704	0.0004835	0.0000176	0.0000290
C10.49	Q18L,N50S,G51R,N135D,I155T	0.0000184	0.0023853	0.0052253	0.0072753	0.0028335	0.0045995	0.0021987	0.0000853	0.0001334
C10.50	Q18L,L27V,S28Y,N50S,G51R,N135D,I155T	0.0000095	0.0000547	0.0001427	0.0004411	0.0002044	0.0001929	0.0000965	0.0000697	0.0000099
C11.54	F6Y,Q18L,L27V,S28Y,N50S,G51R,N135D,I155T	0.0000009	0.0000157	0.0000467	0.0001215	0.0000541	0.0000547	0.0000266	0.0000065	0.0000023

FIG. 23

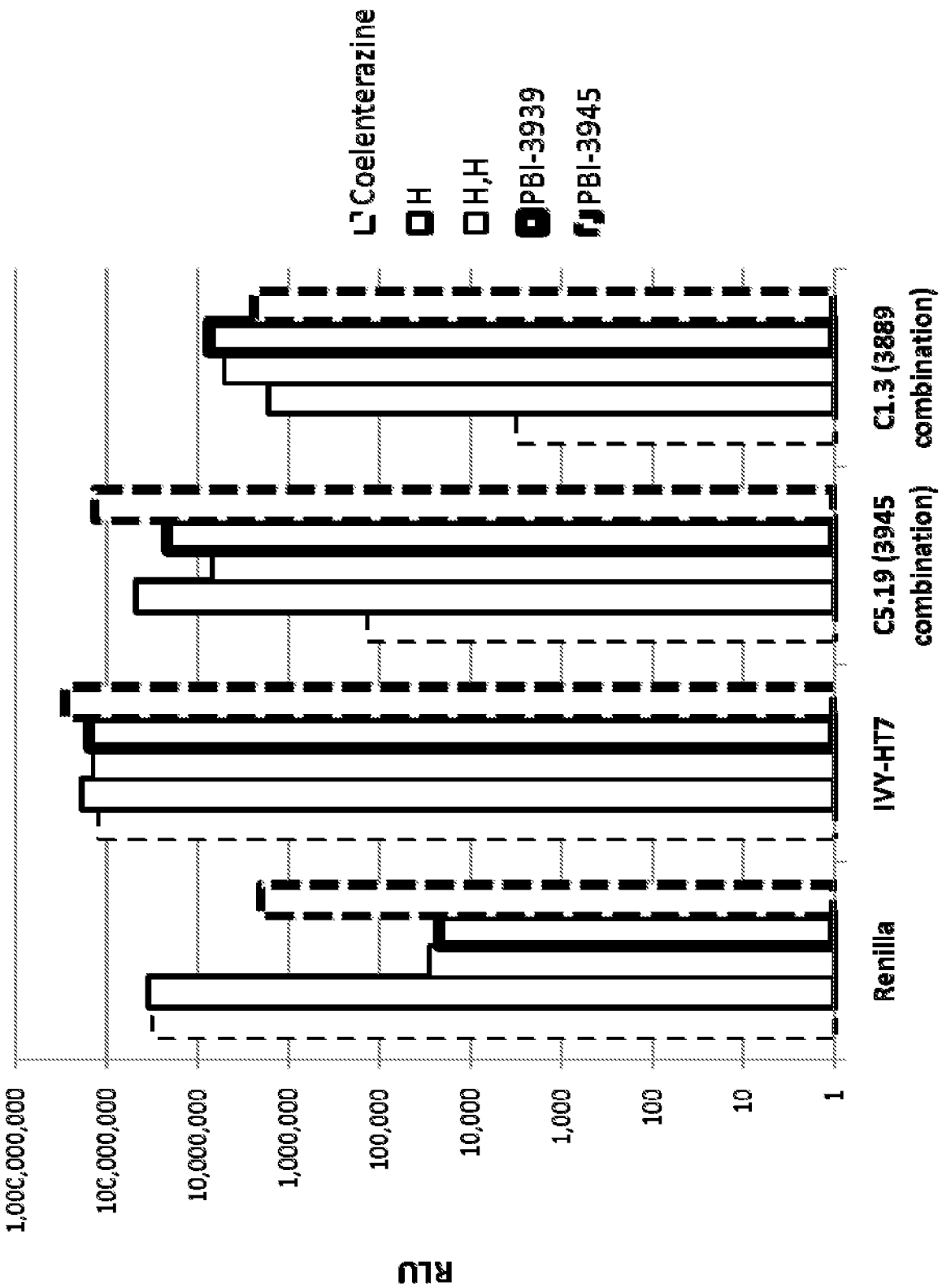

RLU
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
Coelenterazine
H
H,H
PBI-3939
PBI-3945
Renilla
IVY-HT7
C5.19 (3945 combination)
C1.3 (3889 combination)

FIG. 24

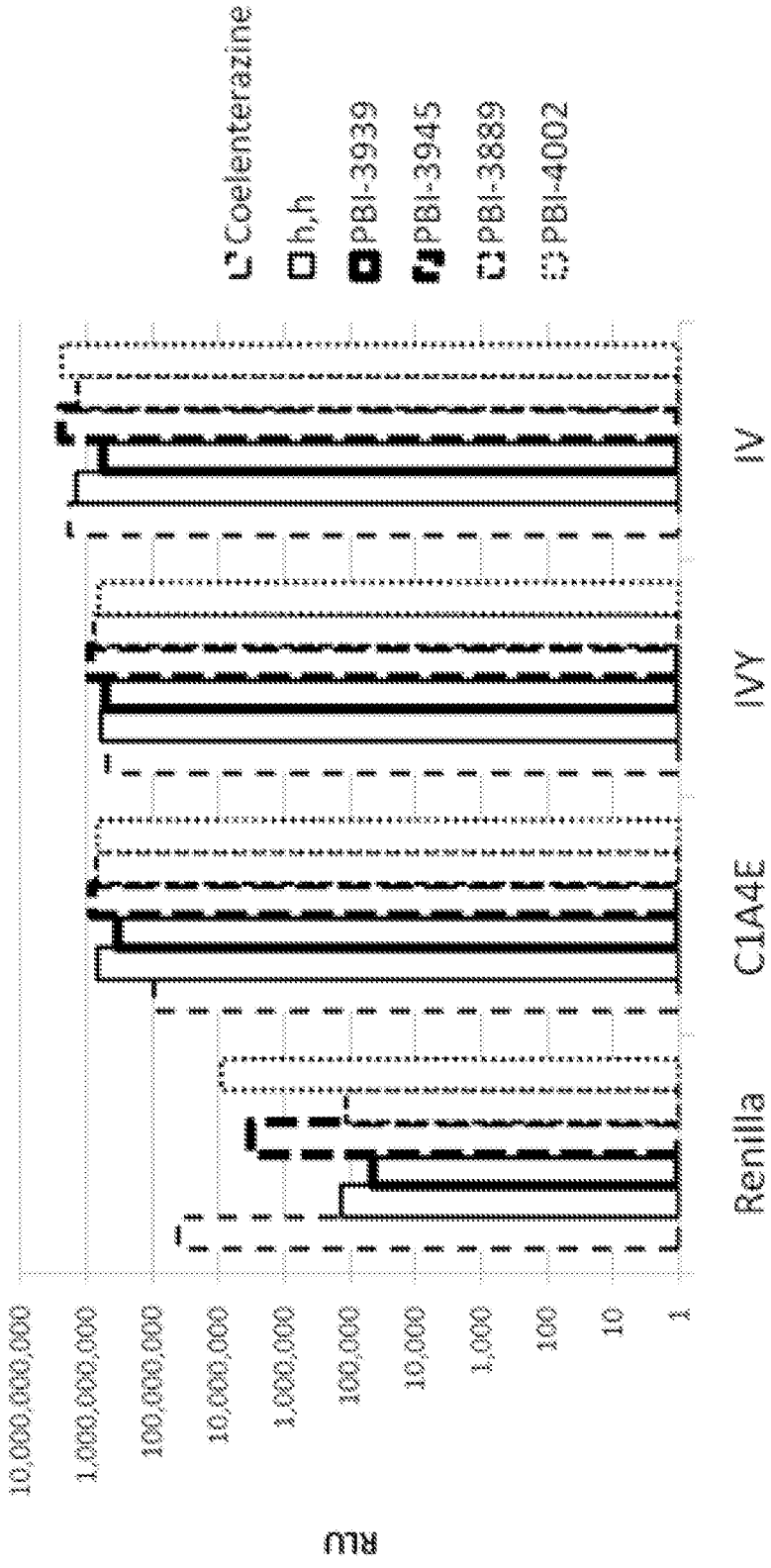

Coelenterazine
h,h
PBI-3939
PBI-3945
PBI-3889
PBI-4002
RLU
10,000,000,000
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
Renilla
C1A4E
IVY
IV

FIG. 25

Sequence	clone	1	4	18	27	33	38	68	70	72	75	102
Q18L	1D6			X								
F1I,L27V,V38I	8A3	X			X		X					
F1I,L27V	8A7	X			X							
F1I,V38I	8E2	X					X					
L27V	8F2				X							
M75K,L72Q,F68Y,Q18L	9B8			X				X		X	X	
M75K,L72Q,F68Y,L27V,Q18L,V38I	9F6			X	X		X	X		X	X	
F1I,M75K,L72Q,F68Y,L27V,V38I	10A7	X			X		X	X		X	X	
F1I,M75K,L72Q,M70V,Q18L	10G10	X		X					X	X	X	
K33N	14A2					X						
F1I,M75K,L72Q,F68Y,K33N,M102E	14C4	X				X		X		X	X	X
E4K,M75K,L72Q,F68Y,K33N	15C1		X			X		X		X	X	

FIG. 26

Sample	Sequence	Coel.	H	HH	PBI-3939	PBI-3945	PBI-3889	PBI-4002
IV		1	1	1	1	1	1	1
1D6	Q18L	0.77	0.92	**2.16**	**4.08**	1.04	**2.27**	**1.32**
8A3	F1I,L27V,V38I	0.01	0.81	0.87	0.43	0.90	0.48	0.77
8A7	F1I,L27V	0.43	**1.55**	**1.95**	1.26	**1.50**	**1.50**	**1.50**
8E 2	F1I,V38I	0.13	0.53	0.61	0.75	0.60	0.64	0.51
8F2	L27V	0.40	**1.54**	**1.87**	1.06	**1.53**	**1.37**	**1.49**
9B8	M75K,L72Q,F68Y,Q18L	**1.40**	**1.69**	**2.52**	**4.37**	**1.44**	**2.35**	**1.72**
9F6	M75K,L72Q,F68Y,L27V,Q18L,V38I	0.09	**2.05**	**1.67**	1.11	**1.98**	1.04	**1.62**
10A7	F1I,M75K,L72Q,F68Y,L27V,V38I	0.08	**1.40**	1.17	0.82	**1.74**	0.89	1.23
10G10	F1I,M75K,L72Q,M70V,Q18L	1.03	**1.51**	**2.30**	**4.25**	**1.35**	**2.33**	**1.60**
14A2	K33N	1.12	**1.62**	**1.93**	**1.80**	**1.80**	**2.23**	**1.66**
14C4	F1I,M75K,L72Q,F68Y,K33N,V102E	1.27	**1.33**	1.08	**1.36**	**1.53**	0.84	1.11
15C1	E4K,M75K,L72Q,F68Y,K33N	**2.63**	**2.61**	**2.10**	**2.60**	**2.94**	**1.67**	**2.20**

FIG. 27

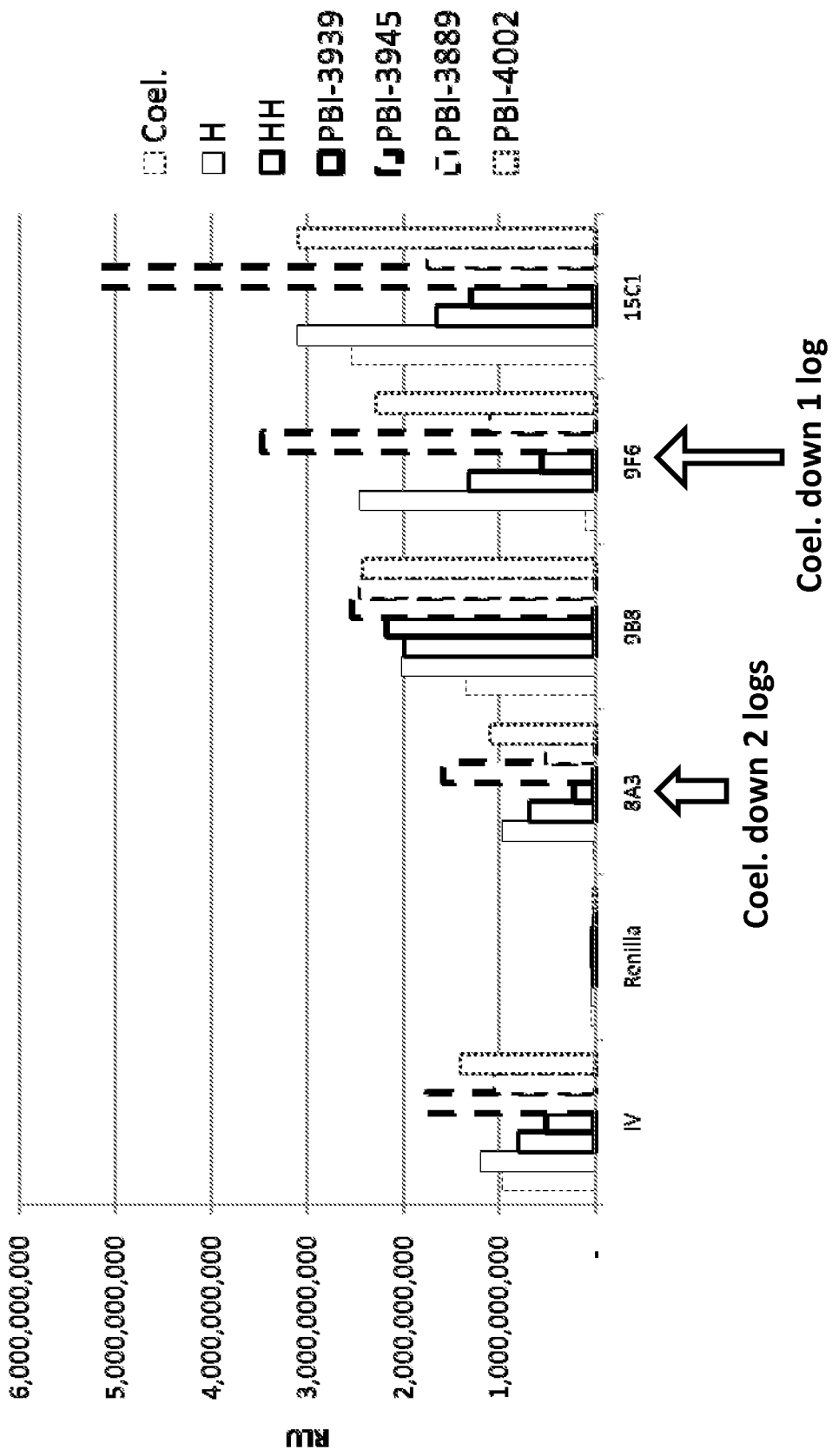

Coel.
H
HH
PBI-3939
PBI-3945
PBI-3889
PBI-4002
RLU
6,000,000,000
5,000,000,000
4,000,000,000
3,000,000,000
2,000,000,000
1,000,000,000
-
IV
Renilla
8A3
9B8
9F6
15C1
Coel. down 2 logs
Coel. down 1 log

FIG. 28

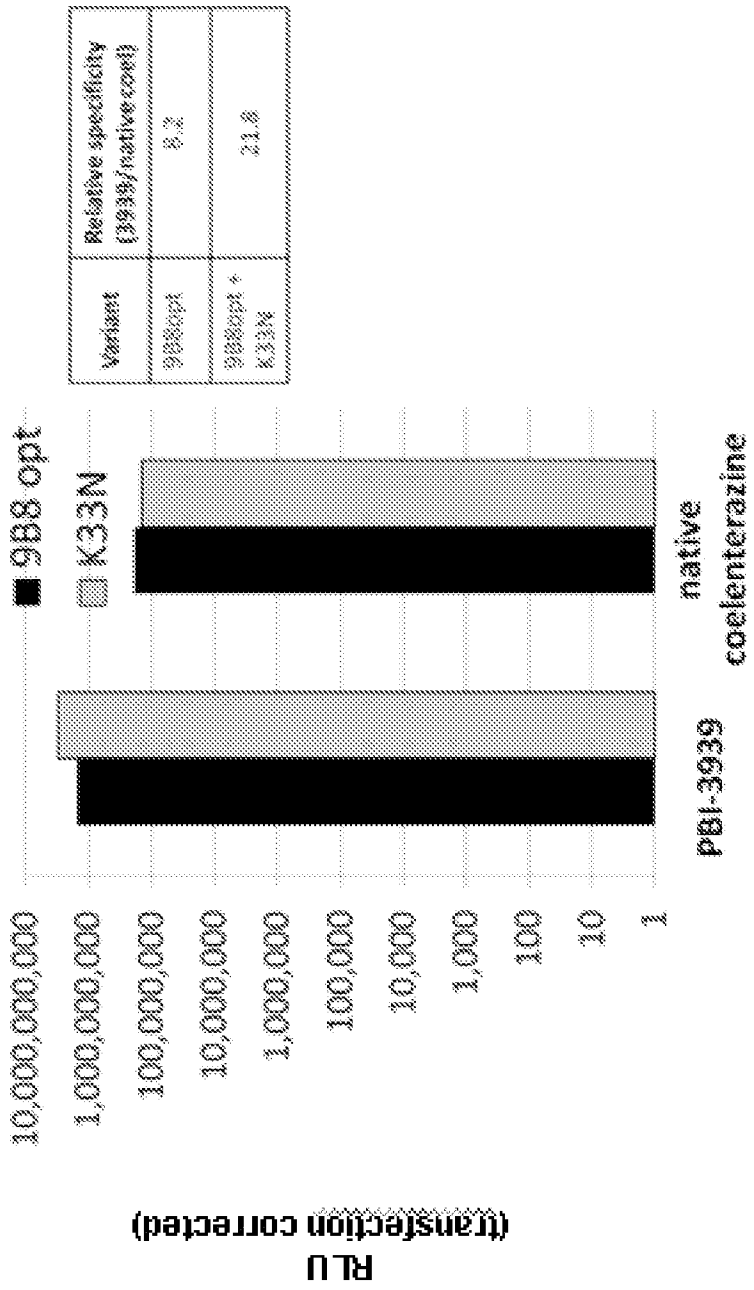

Variant
Relative specificity (3939/native coel)
9B8opt
8.2
9B8opt + K33N
21.6
9B8 opt
K33N
RLU
(transfection corrected)
10,000,000,000
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
PBI-3939
native coelenterazine

FIG. 29

A

Native coelenterazine	
Amino acid	Relative activity
Ala	1.0
Phe	3.2
Lys	19.2
Arg	26.8

B

Coelenterazine-h	
Amino acid	Relative activity
Ser	1.1
Ile	1.4
Lys	11.6
Arg	15.9
Phe	55.9

C

PBI-3939	
Amino acid	Relative activity
Ser	1.8
Glu	4.6
Ile	10.0
Lys	17.5
Arg	26.4
Phe	444.4

D

Amino acid substitution	Relative activity (in context of wt OgLuc)	Relative activity (in context of N166R)
L27V	62.2	45.4
A33N	0.6	1.3
K43R	1.1	17.1
M75K	65.7	467.7
T39T	118.6	1.5
L72Q	2.0	5.0
F68D	3.0	5.4

FIG. 30

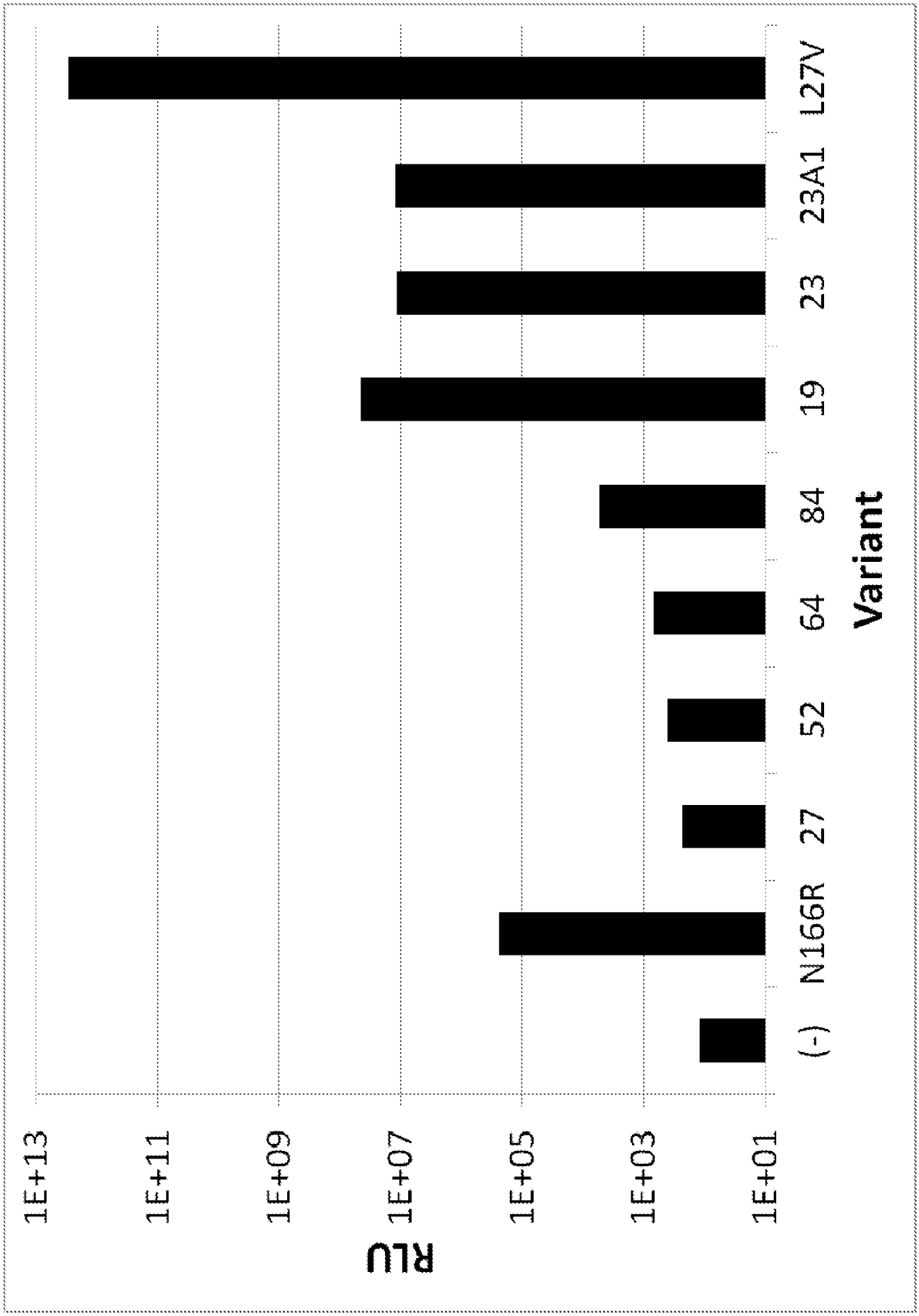

RLU
1E+13
1E+11
1E+09
1E+07
1E+05
1E+03
1E+01
(-)
N166R
27
52
64
84
19
23
23A1
L27V
Variant

FIG. 31

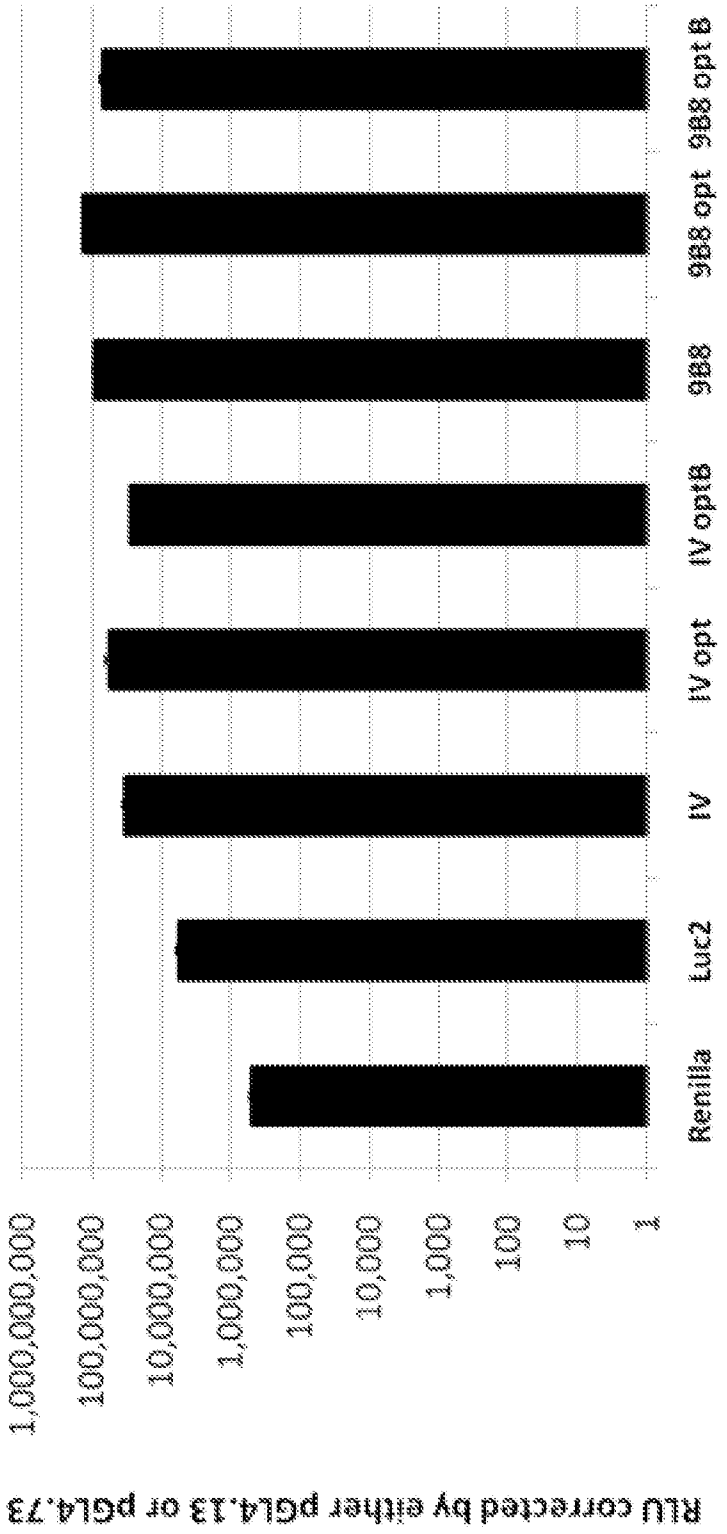

RLU corrected by either pGL4.13 or pGL4.73
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
Renilla
Luc2
IV
IV opt
IV opt8
988
988 opt
988 opt 8

FIG. 32

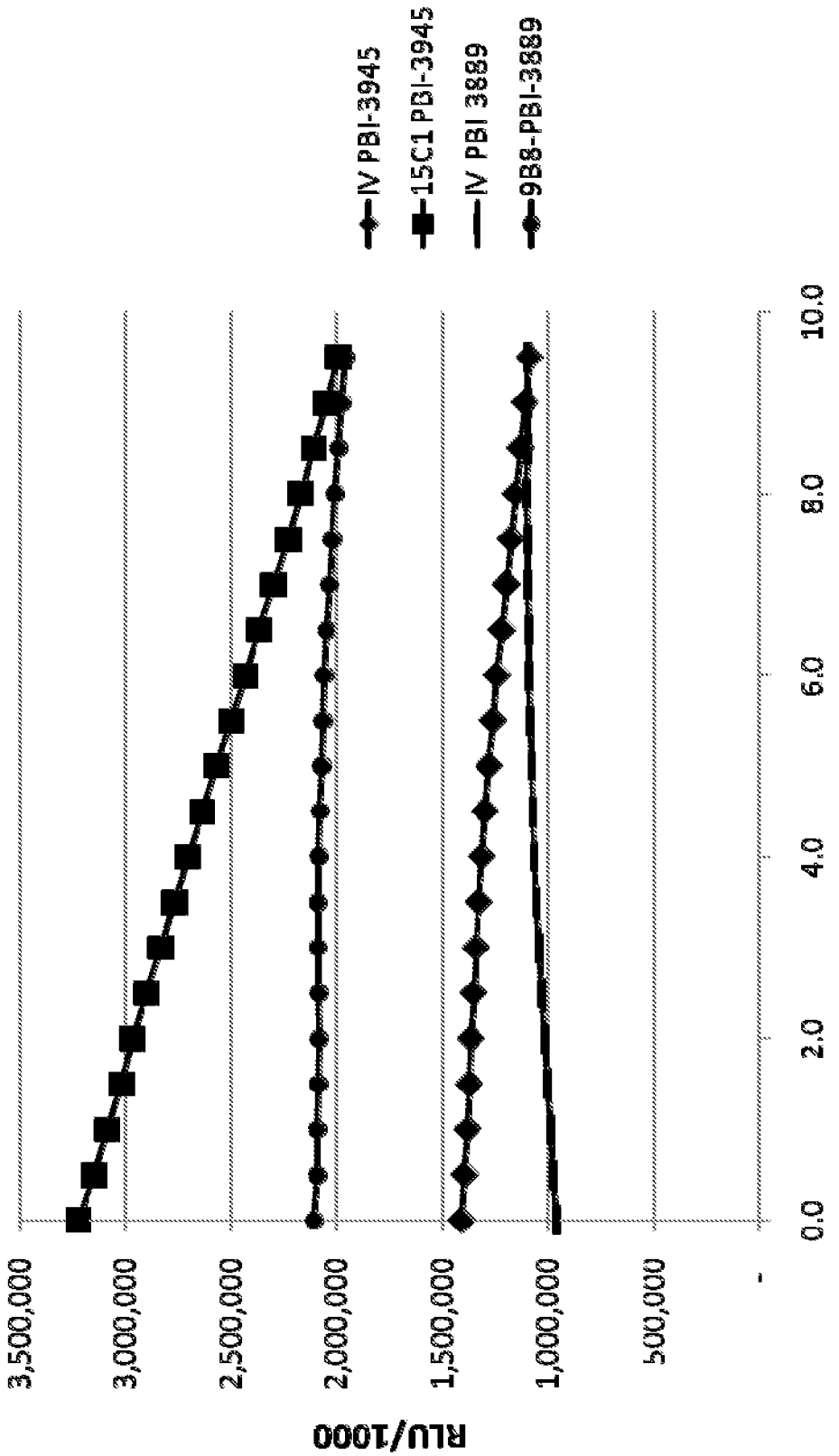
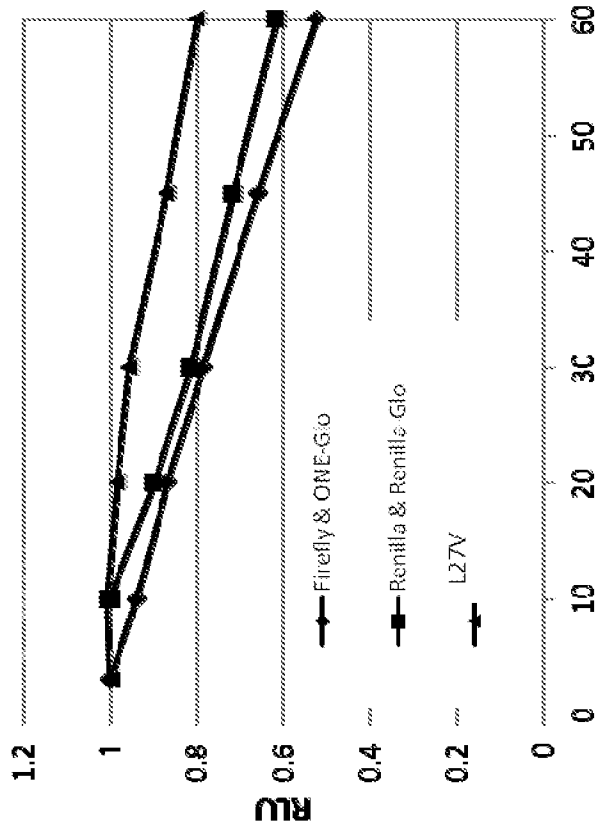
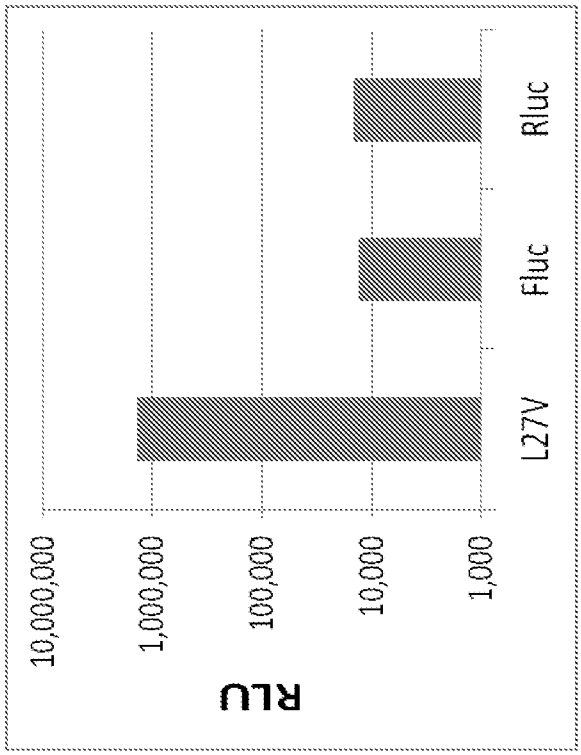

IV PBI-3945
15C1 PBI-3945
IV PBI 3889
9B8-PBI-3889
RLU/1000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
-
0.0
2.0
4.0
6.0
8.0
10.0
Time(min)

FIG. 33


1.2
1
0.8
0.6
0.4
0.2
0
RLU
0
10
20
30
40
50
60
Time (min)
Firefly & ONE-Glo
Renilla & Renilla Glo
L27V

B


10,000,000
1,000,000
100,000
10,000
1,000
RLU
L27V
Fluc
Rluc

A

FIG. 34

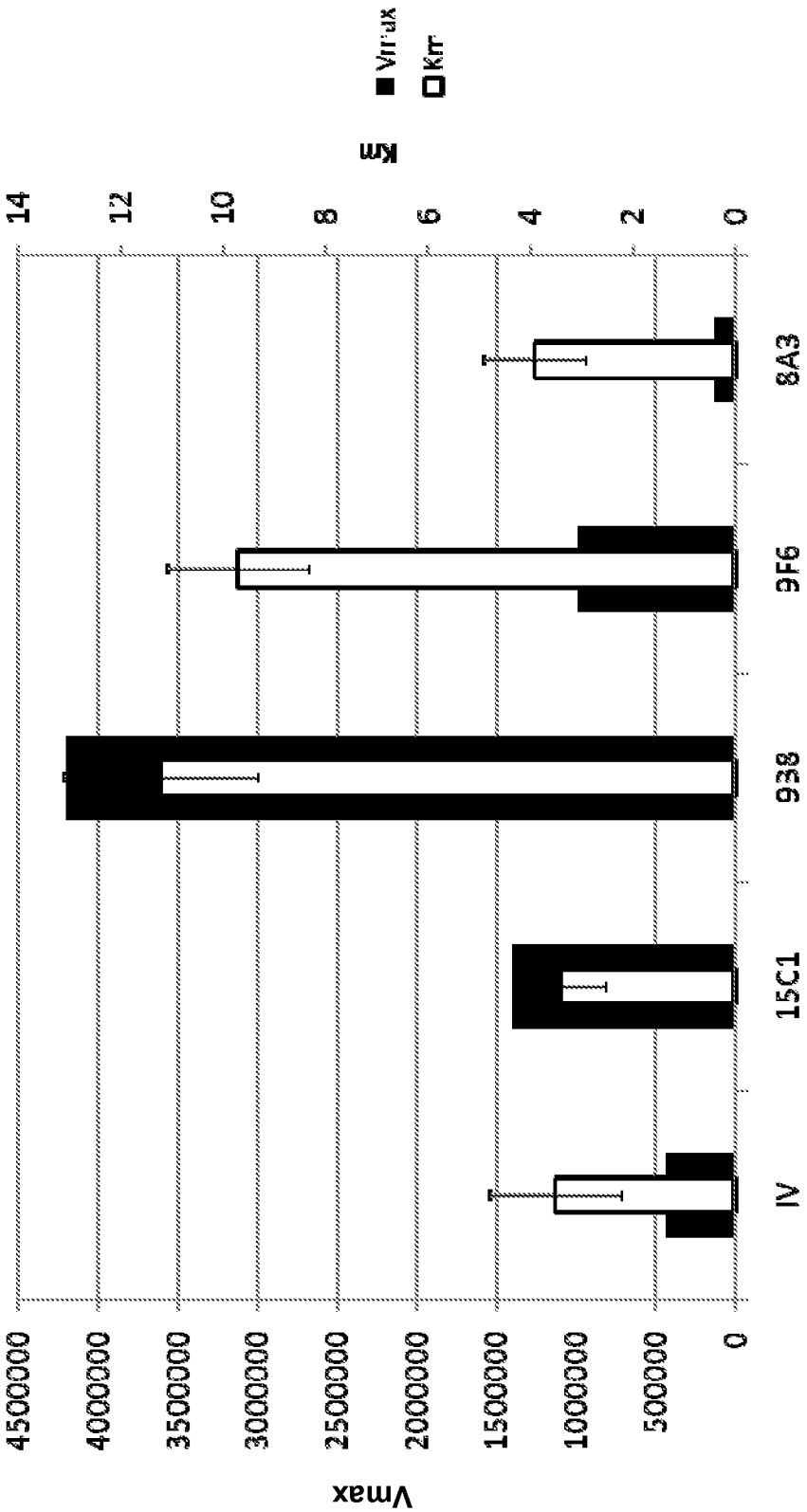
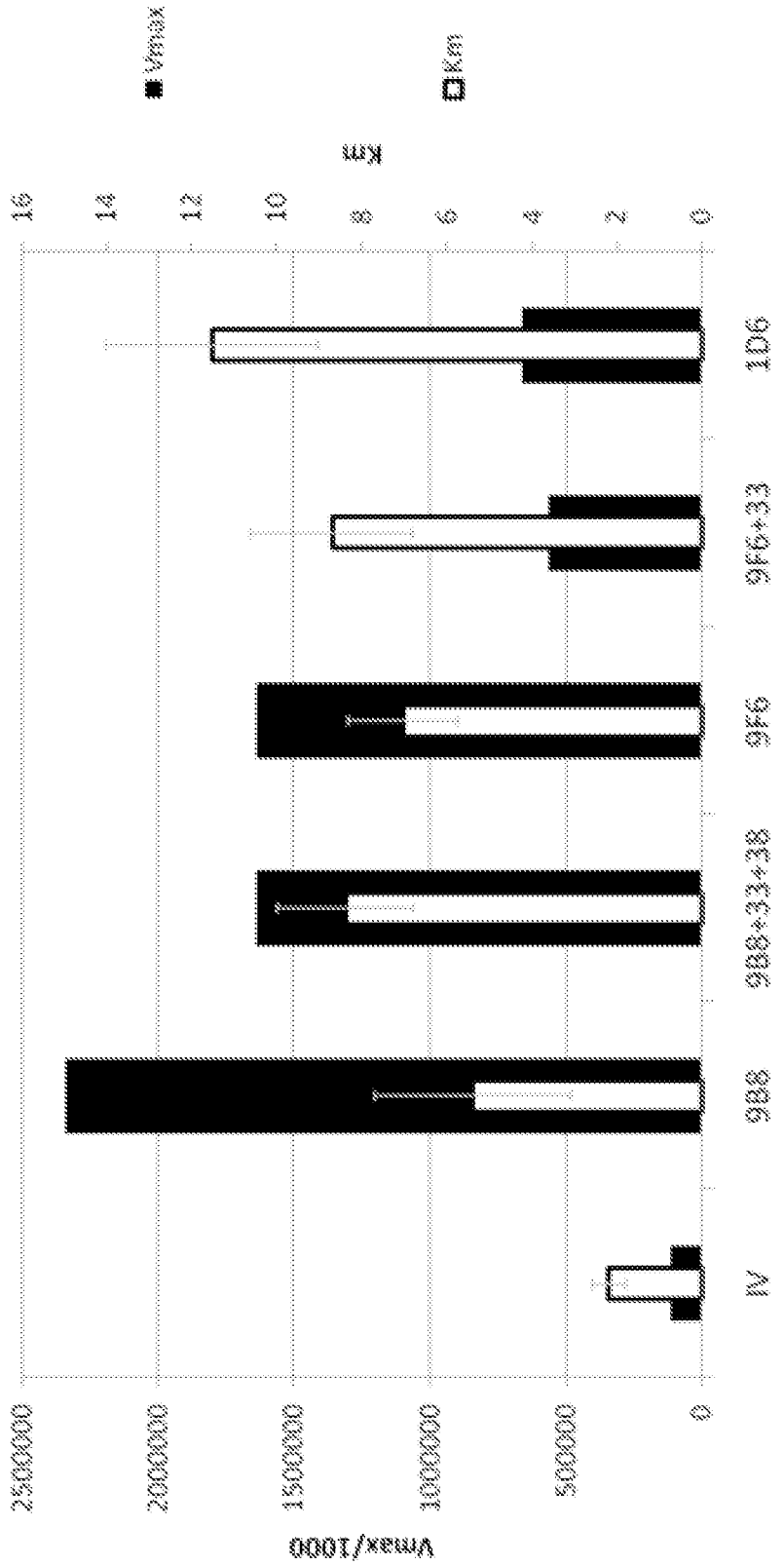

Vmax
Km
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
14
12
10
8
6
4
2
0
IV
15C1
938
9F6
8A3

FIG. 35



Position 33 is K33N
Position 38 is V38I

FIG. 36

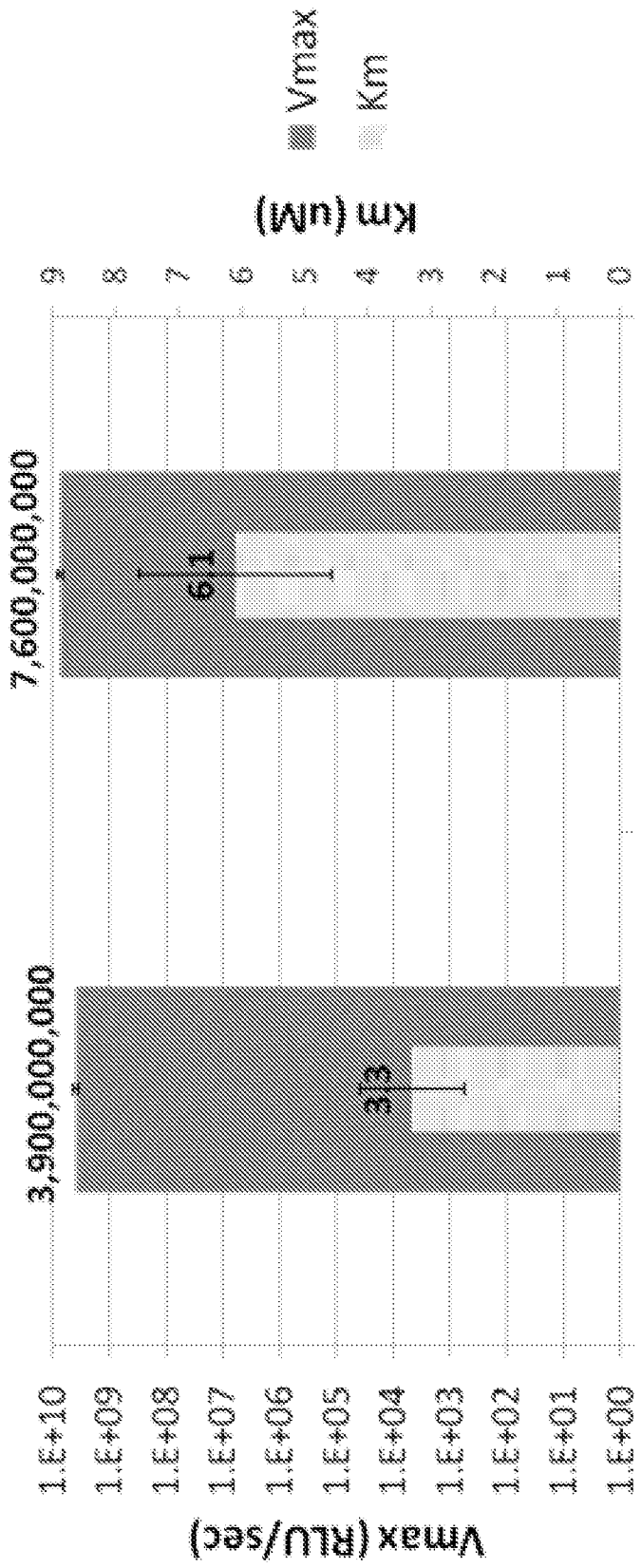

Vmax
Km
Km (uM)
9
8
7
6
5
4
3
2
1
0
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Vmax (RLU/sec)
3,900,000,000
7,600,000,000
313
61
9B8 opt
9B8 opt+K33N

FIG. 37

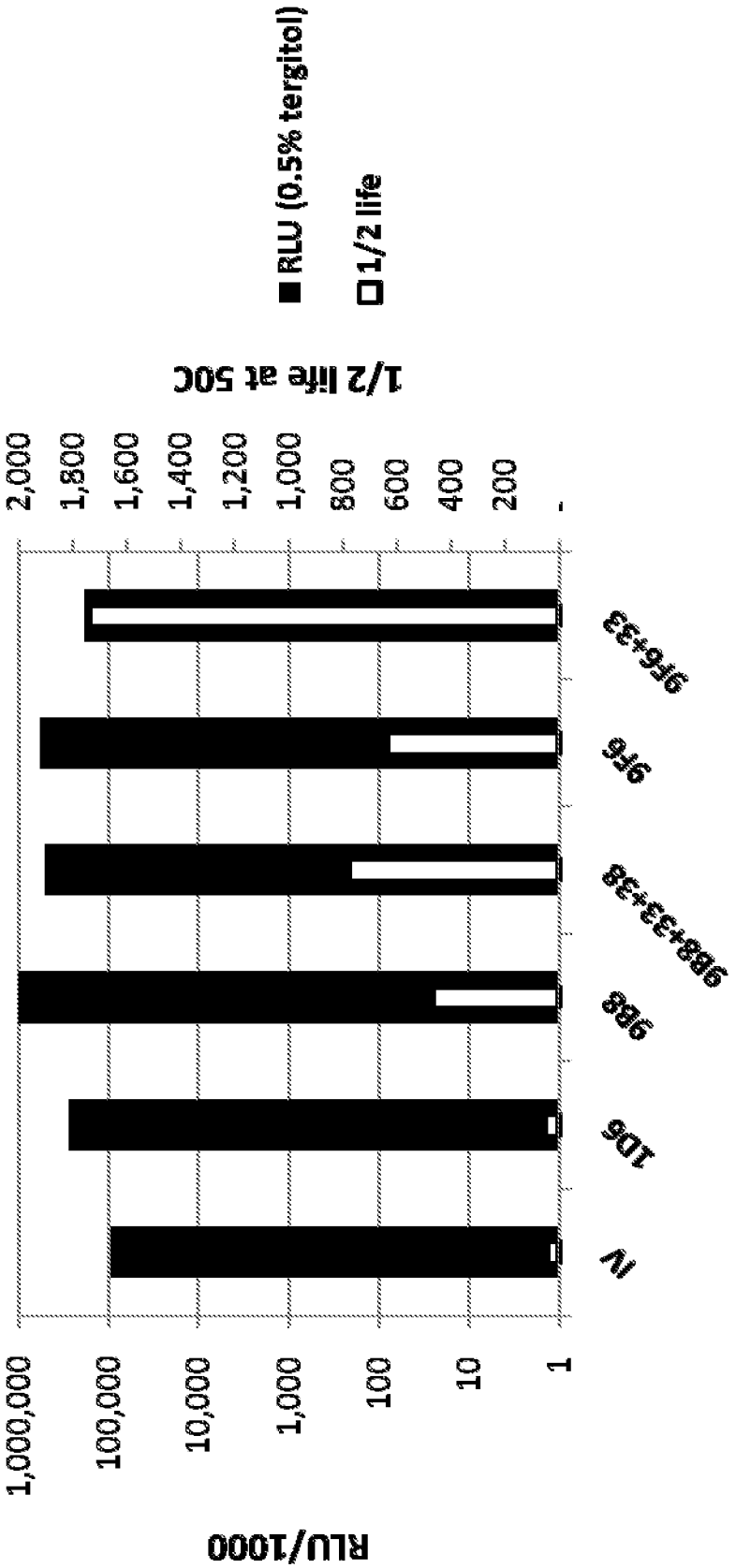

RLU (0.5% tergitol)
1/2 life
1/2 life at 50C
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
-
RLU/1000
1,000,000
100,000
10,000
1,000
100
10
1
IV
1D6
9B6
9B6+33+38
9F6
9F6+33

FIG. 38

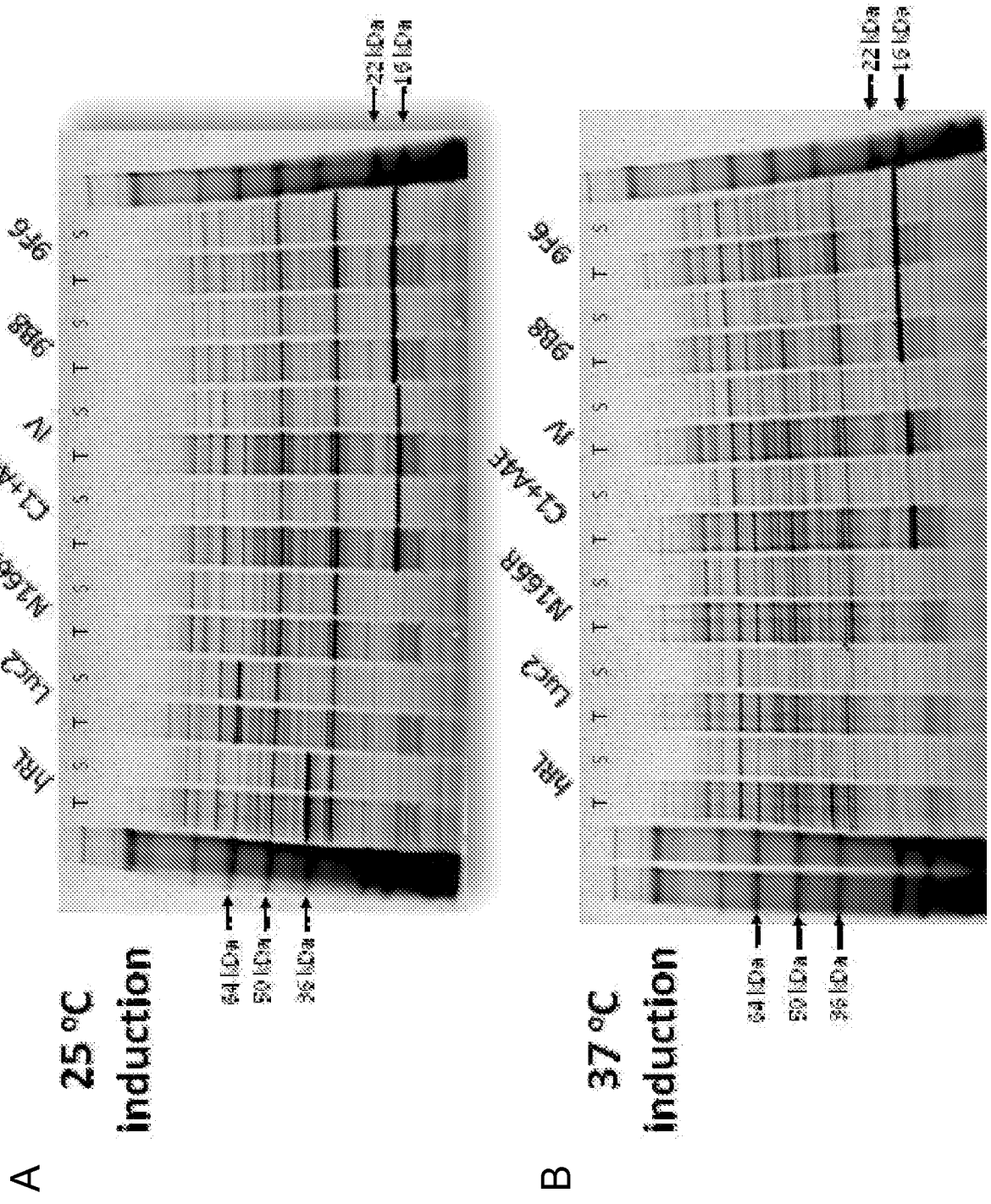
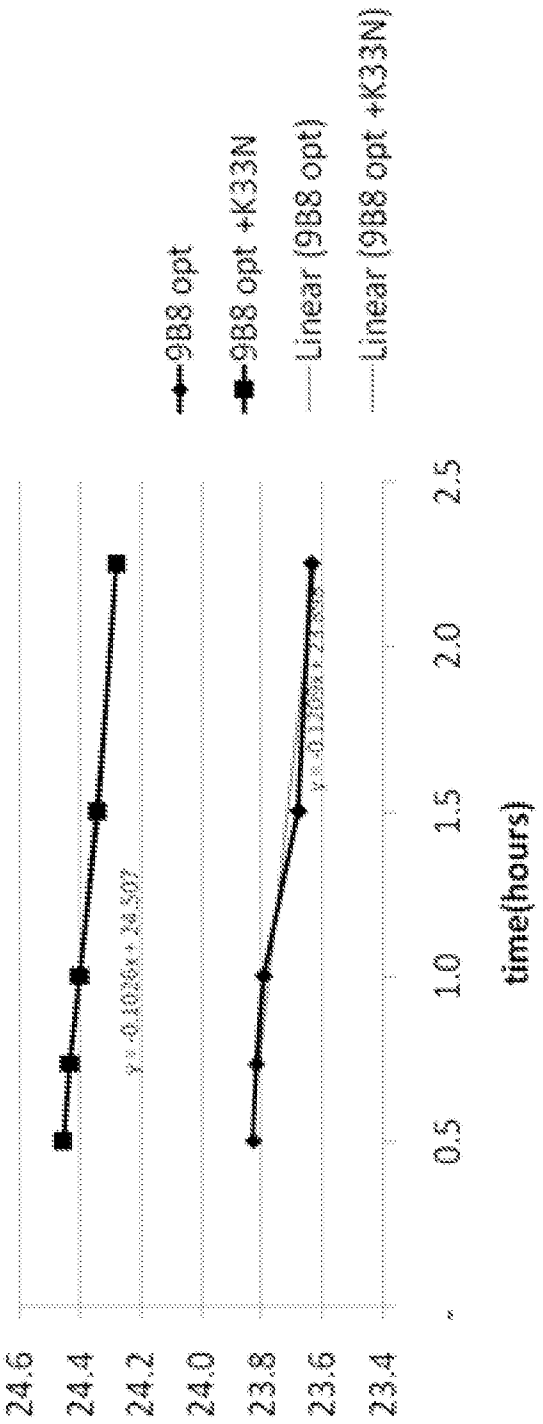

A
25 °C
induction
64 kDa
50 kDa
36 kDa
22 kDa
16 kDa
PBL
uc2
N166R
C1+A4E
N
9B8
9f6
B
37 °C
induction
64 kDa
50 kDa
36 kDa
22 kDa
16 kDa
PBL
uc2
N166R
C1+A4E
N
9B8
9f6

FIG. 39

A



B

Sample	1/2 life (hours) 60C
988 opt	5.7
988 opt +K33N	6.8

FIG. 40

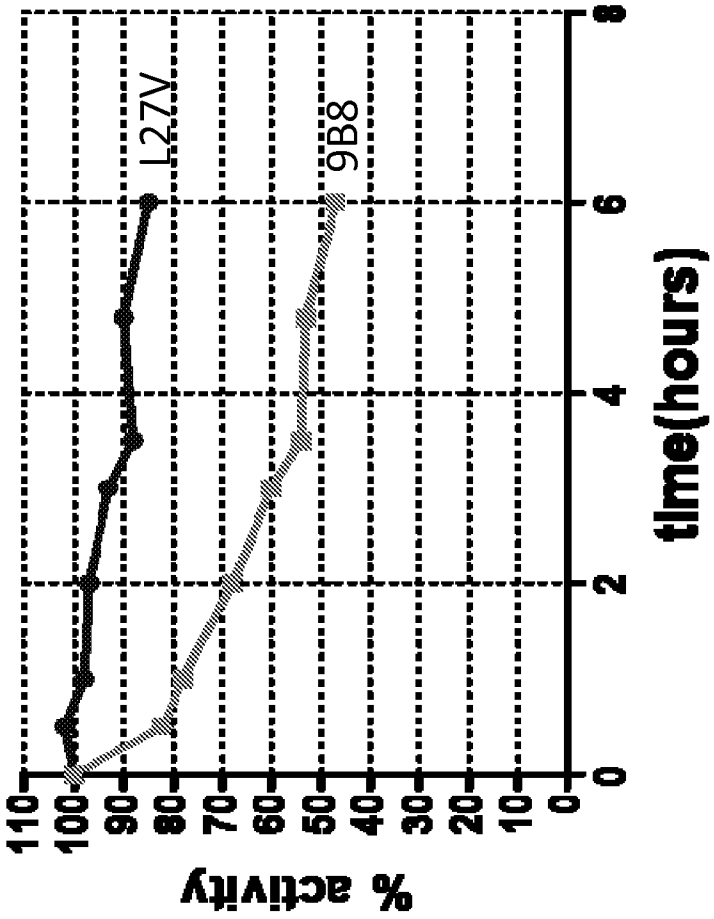
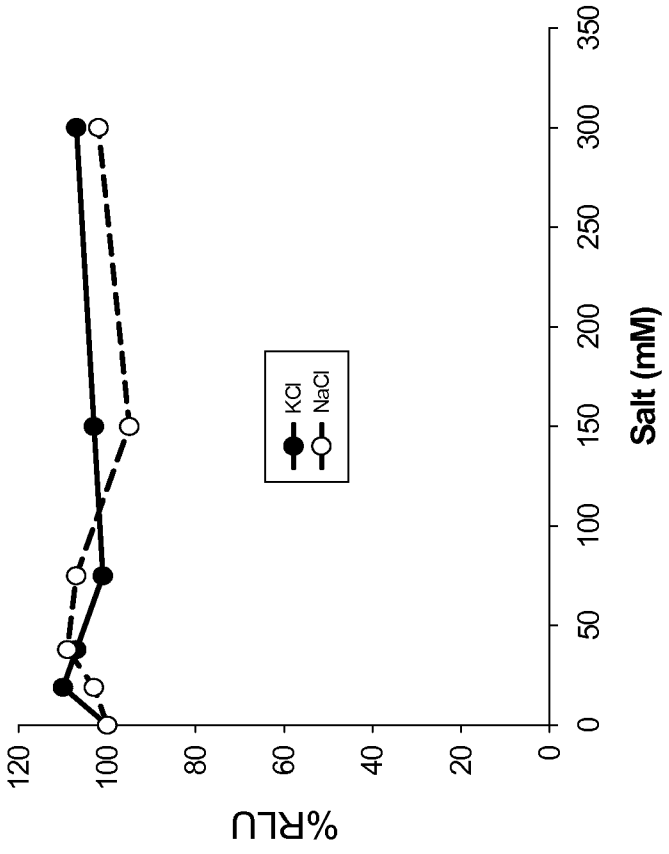

% activity
time(hours)
L27V
9B8
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8

FIG. 41


%RLU
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
Salt (mM)
KCl
NaCl
B

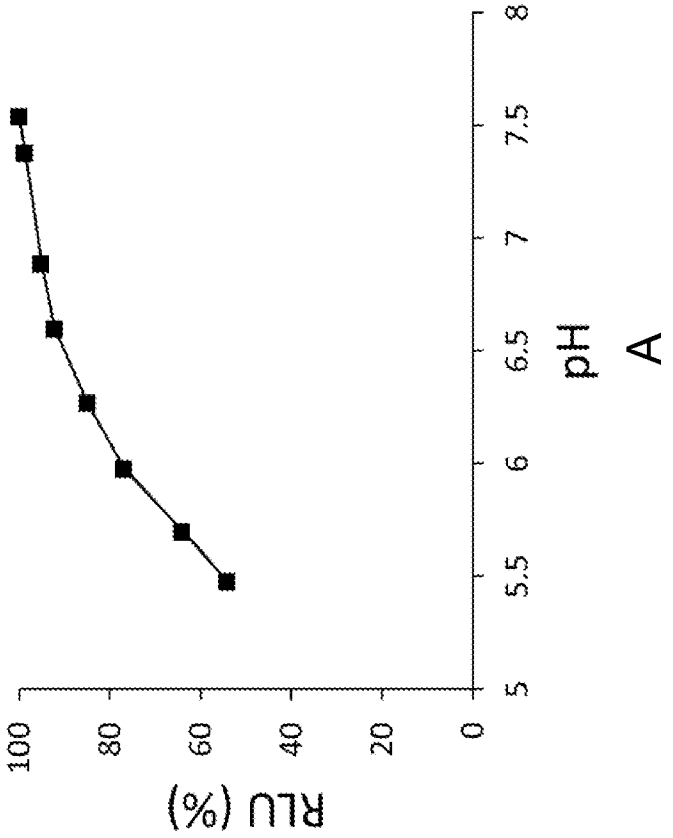

RLU (%)
100
80
60
40
20
0
5
5.5
6
6.5
7
7.5
8
pH
A

FIG. 42

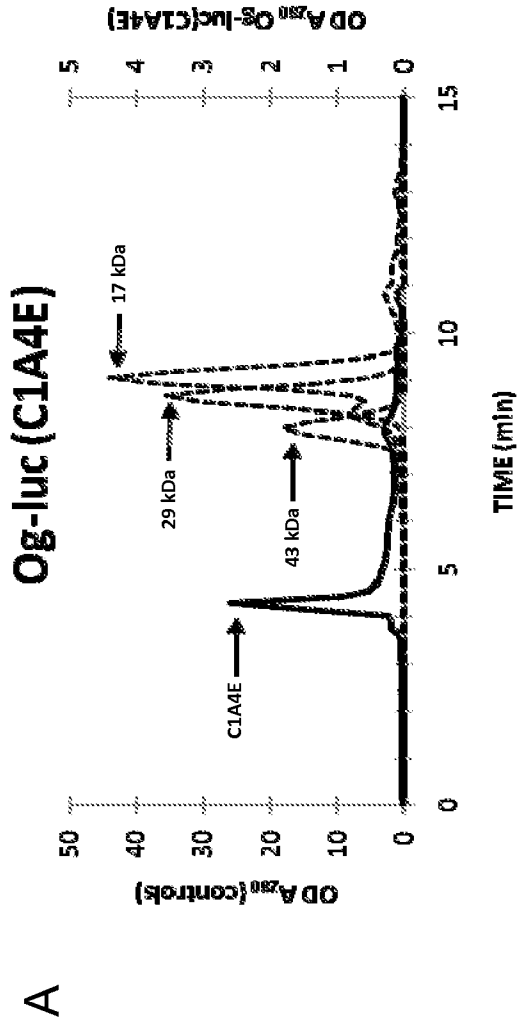

A
Og-luc (C1A4E)
OD A280 (controls)
OD A280 Og-luc(C1A4E)
TIME (min)
C1A4E
29 kDa
43 kDa
17 kDa
B
Og-luc (9B8)
OD A280
TIME (min)
17 kDa
29 kDa
43 kDa

FIG. 43

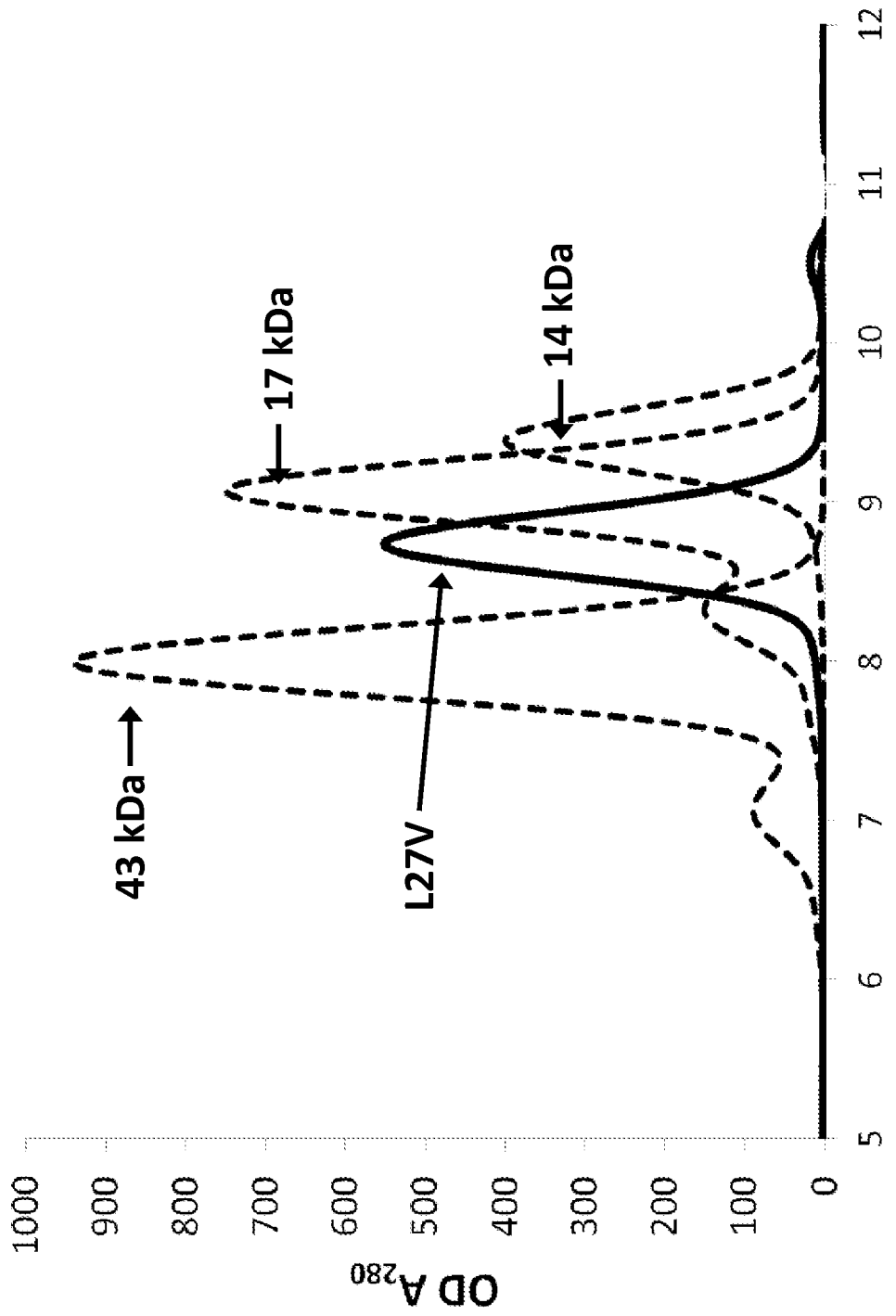

OD A280
1000
900
800
700
600
500
400
300
200
100
0
5
6
7
8
9
10
11
12
Time (min)
43 kDa
L27V
17 kDa
14 kDa

FIG. 44

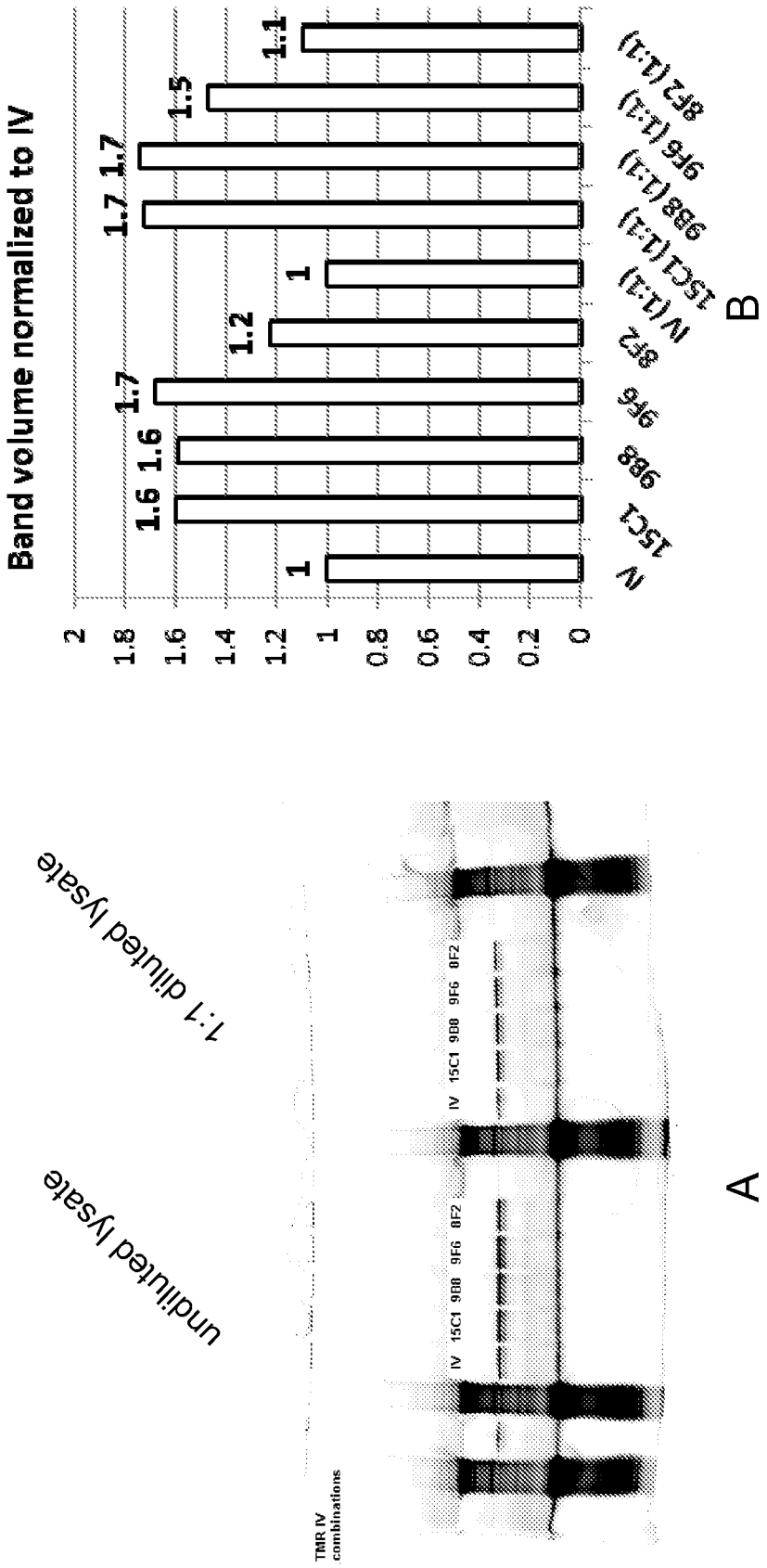

Band volume normalized to IV
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
1.6 1.6
1.7
1.2
1
1.7 1.7
1.5
1.1
IV
15C1
9B8
9F6
8F2
IV (1:1)
15C1 (1:1)
9B8 (1:1)
9F6 (1:1)
8F2 (1:1)
B
undiluted lysate
1:1 diluted lysate
IV 15C1 9B8 9F6 8F2
IV 15C1 9B8 9F6 8F2
TMR IV combinations
A

FIG. 45

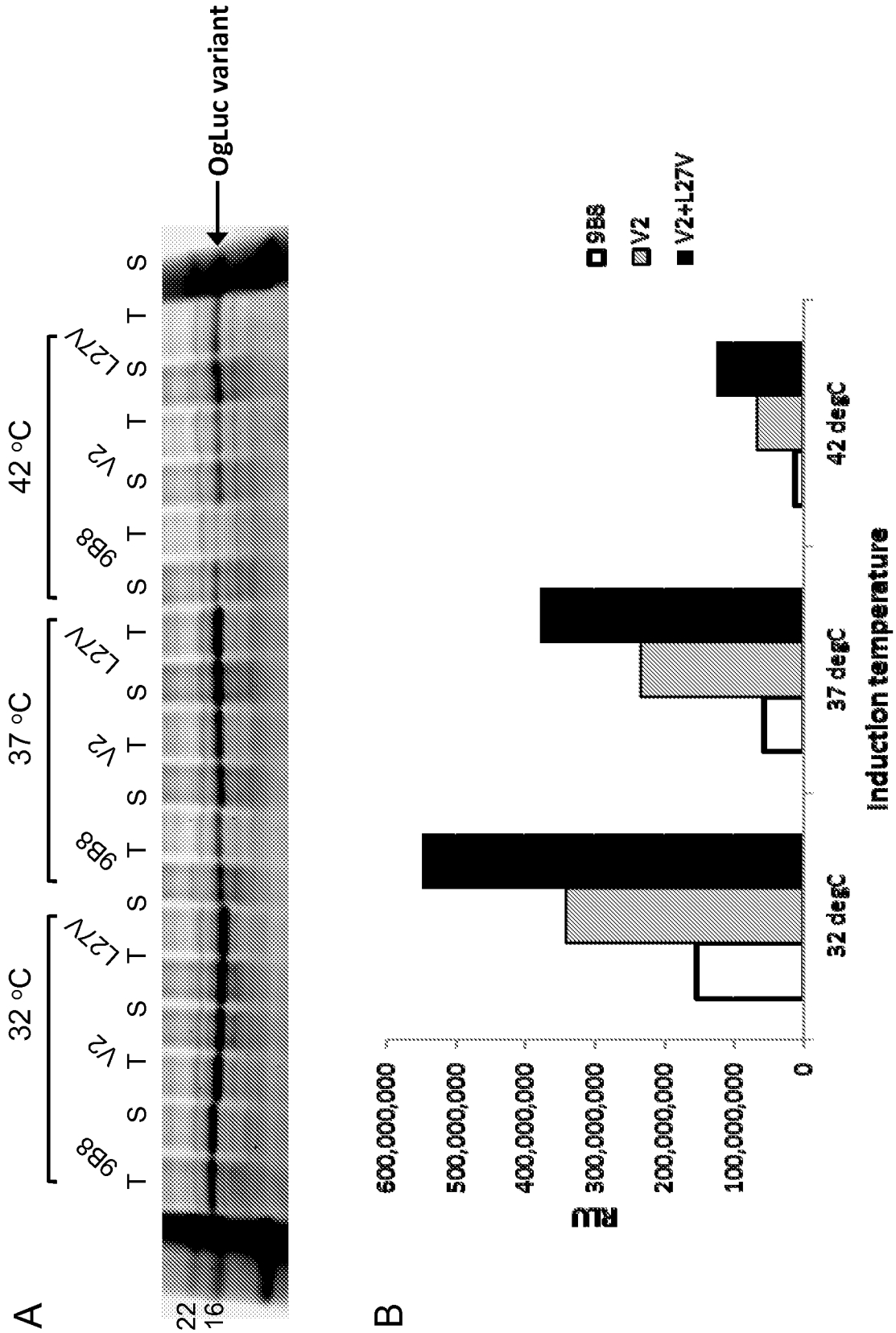

A
B
OgLuc variant
32 °C
37 °C
42 °C
9B8
V2
L27V
T
S
22
16
RLU
600,000,000
500,000,000
400,000,000
300,000,000
200,000,000
100,000,000
0
9B8
V2
V2+L27V
32 degC
37 degC
42 degC
induction temperature

FIG. 46

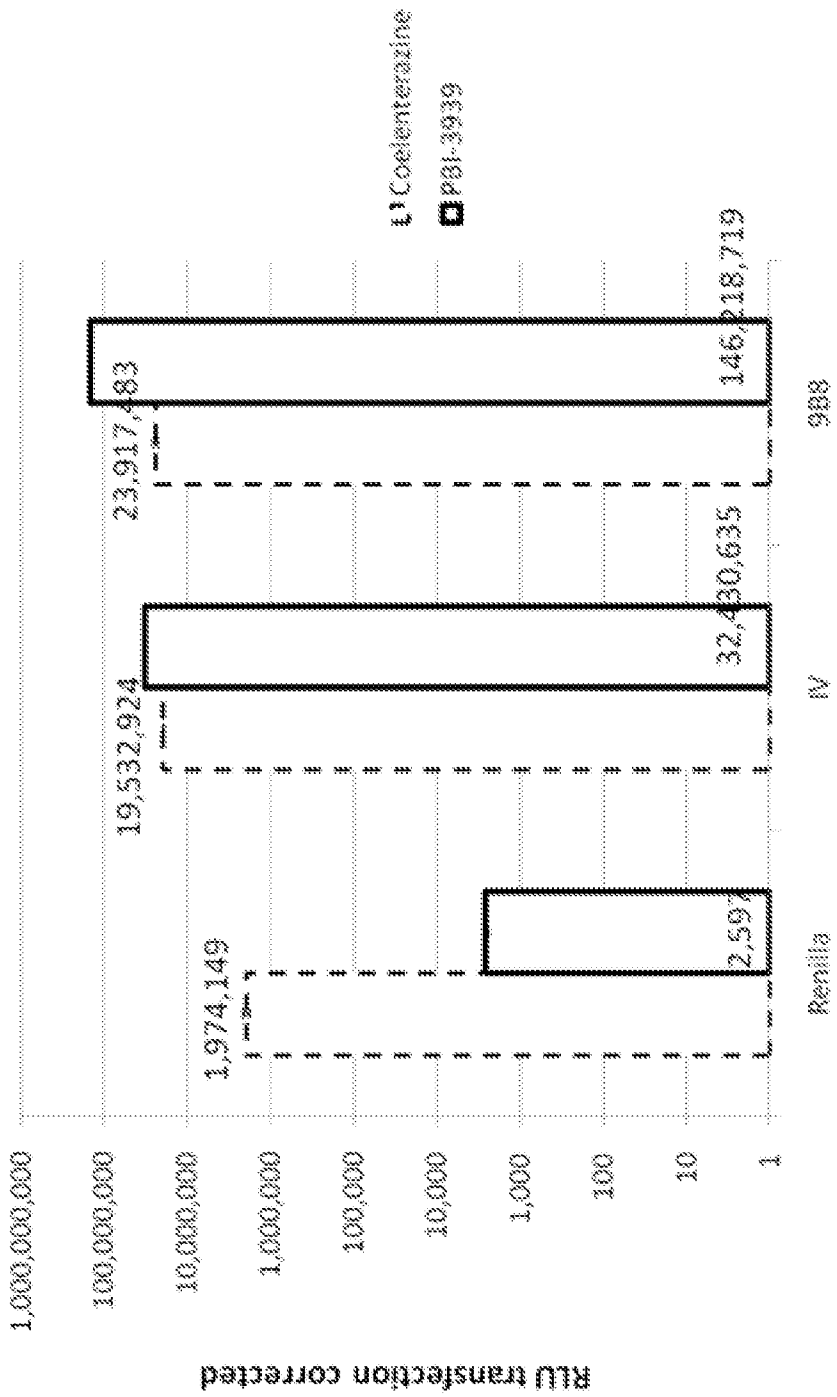

Coelenterazine
PBI-3939
RLU transfection corrected
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
1,974,149
19,532,924
23,917,483
2,597
32,430,635
146,818,719
Renilla
IV
SBB

FIG. 47

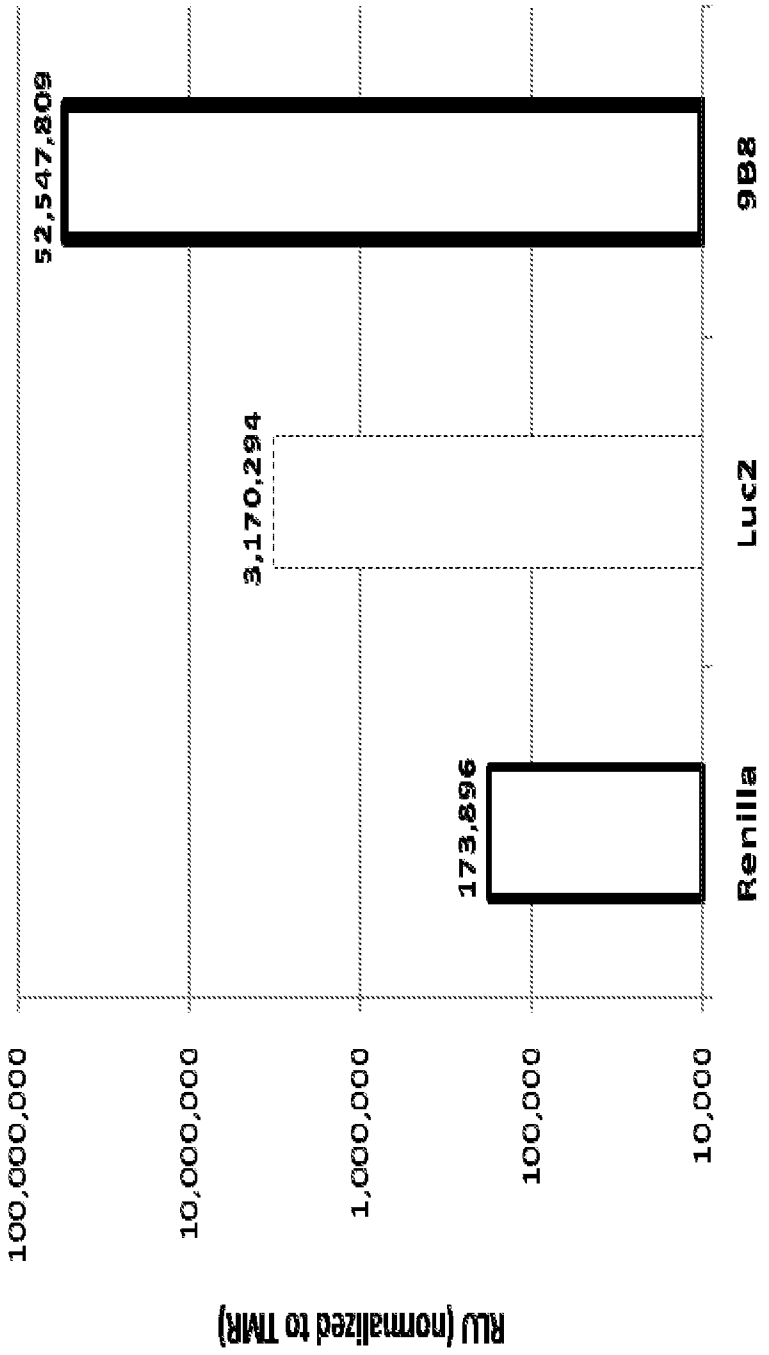

RLU (normalized to TMR)
100,000,000
10,000,000
1,000,000
100,000
10,000
52,547,809
3,170,294
173,896
988
LucZ
Renilla

FIG. 48

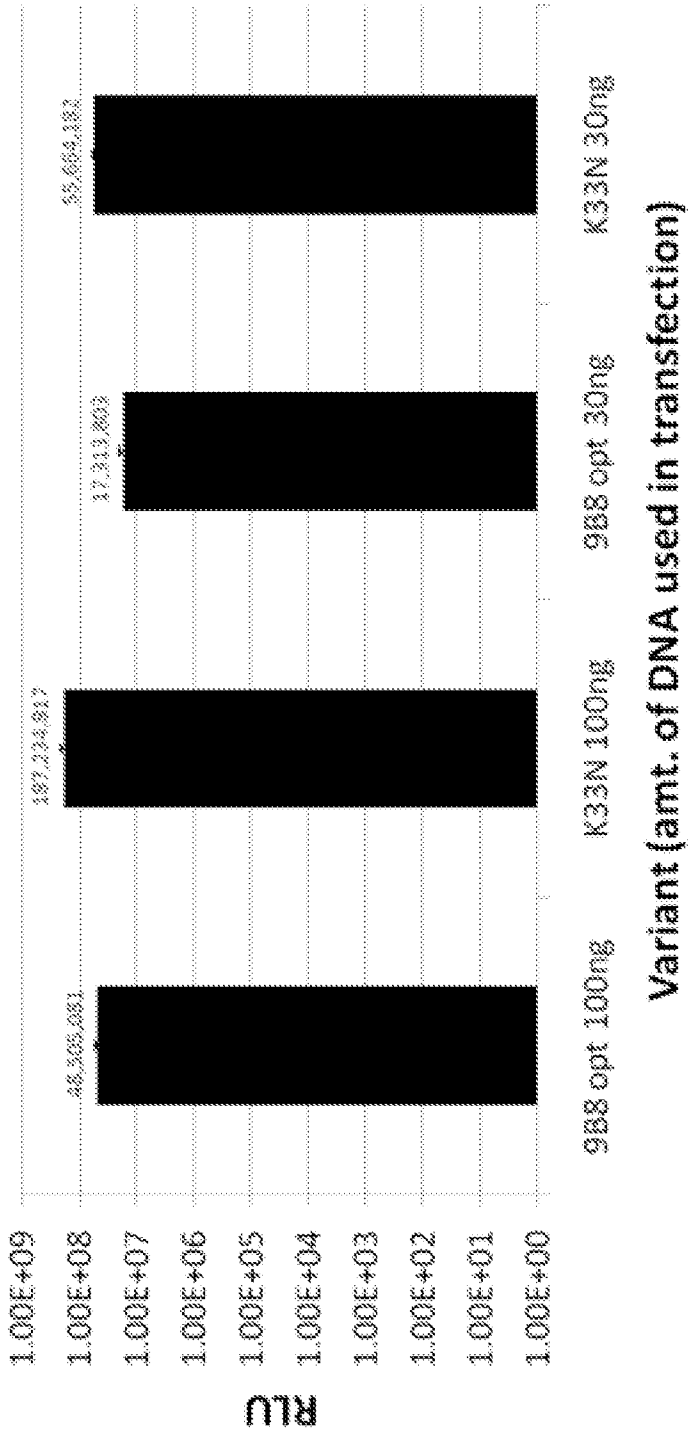

RLU
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
46,305,081
187,214,817
17,313,803
33,664,182
988 opt 100ng
K33N 100ng
988 opt 30ng
K33N 30ng
Variant (amt. of DNA used in transfection)

FIG. 49

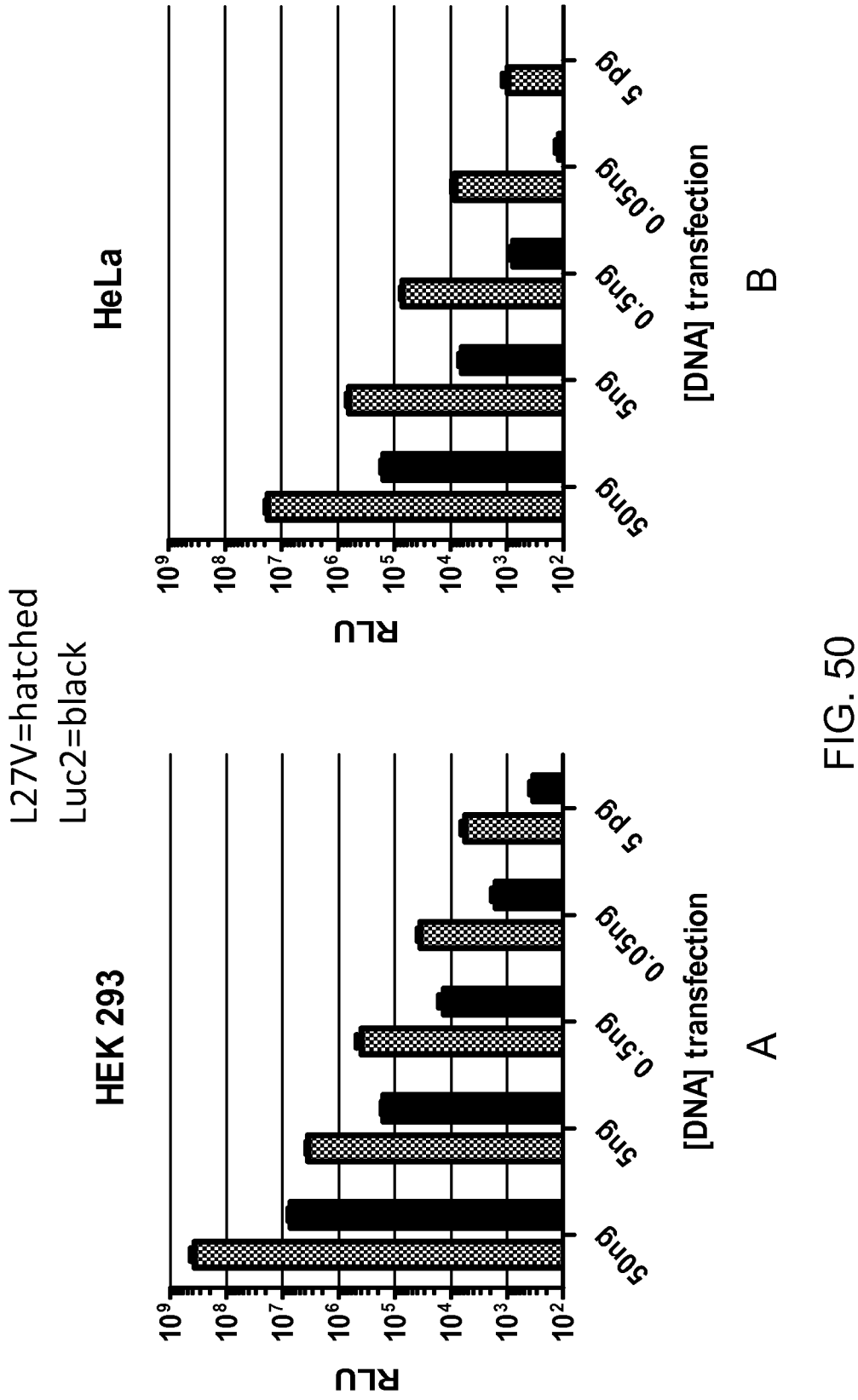

L27V=hatched
Luc2=black
HEK 293
HeLa
RLU
[DNA] transfection
50ng
5ng
0.5ng
0.05ng
5 pg
A
B

FIG. 50

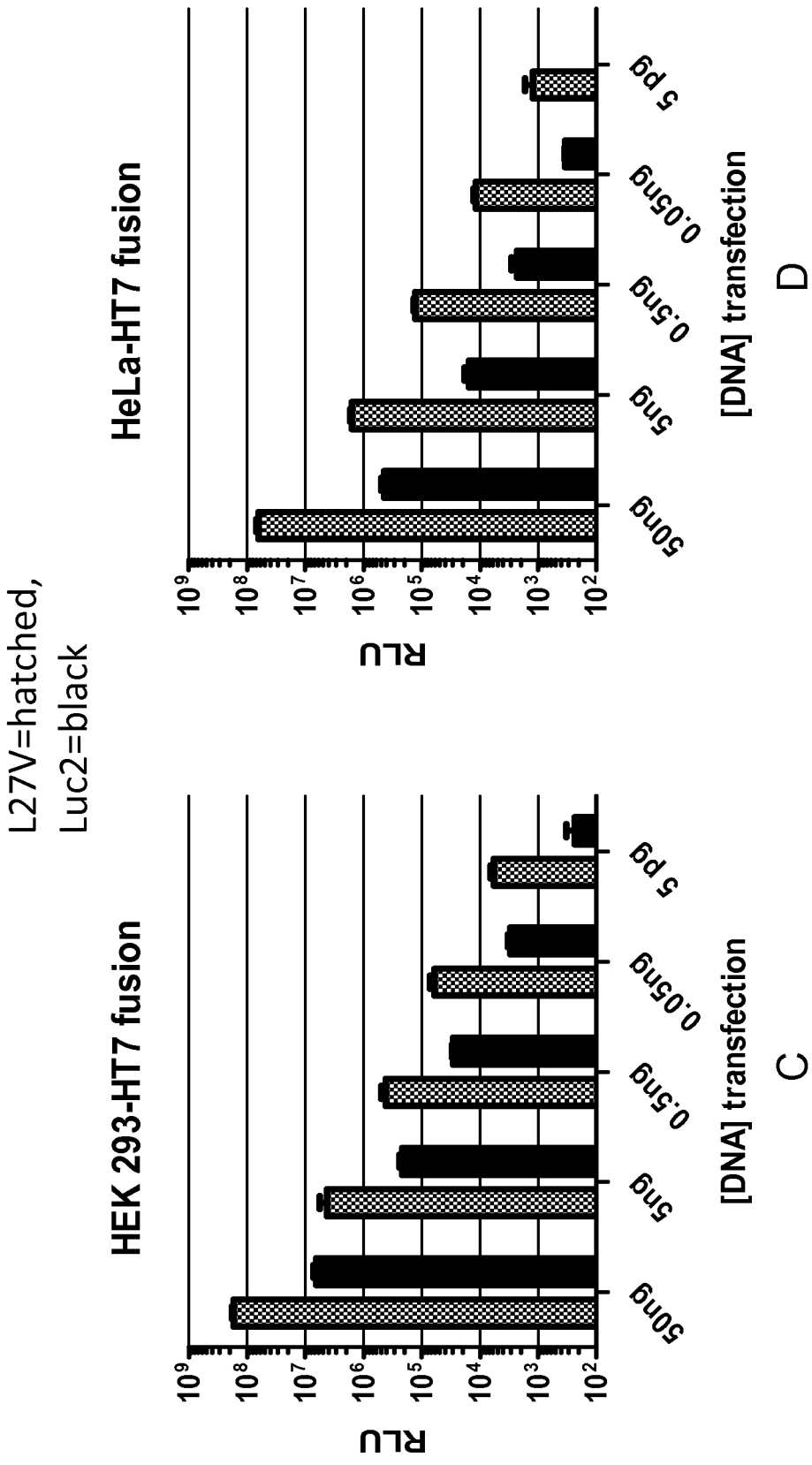
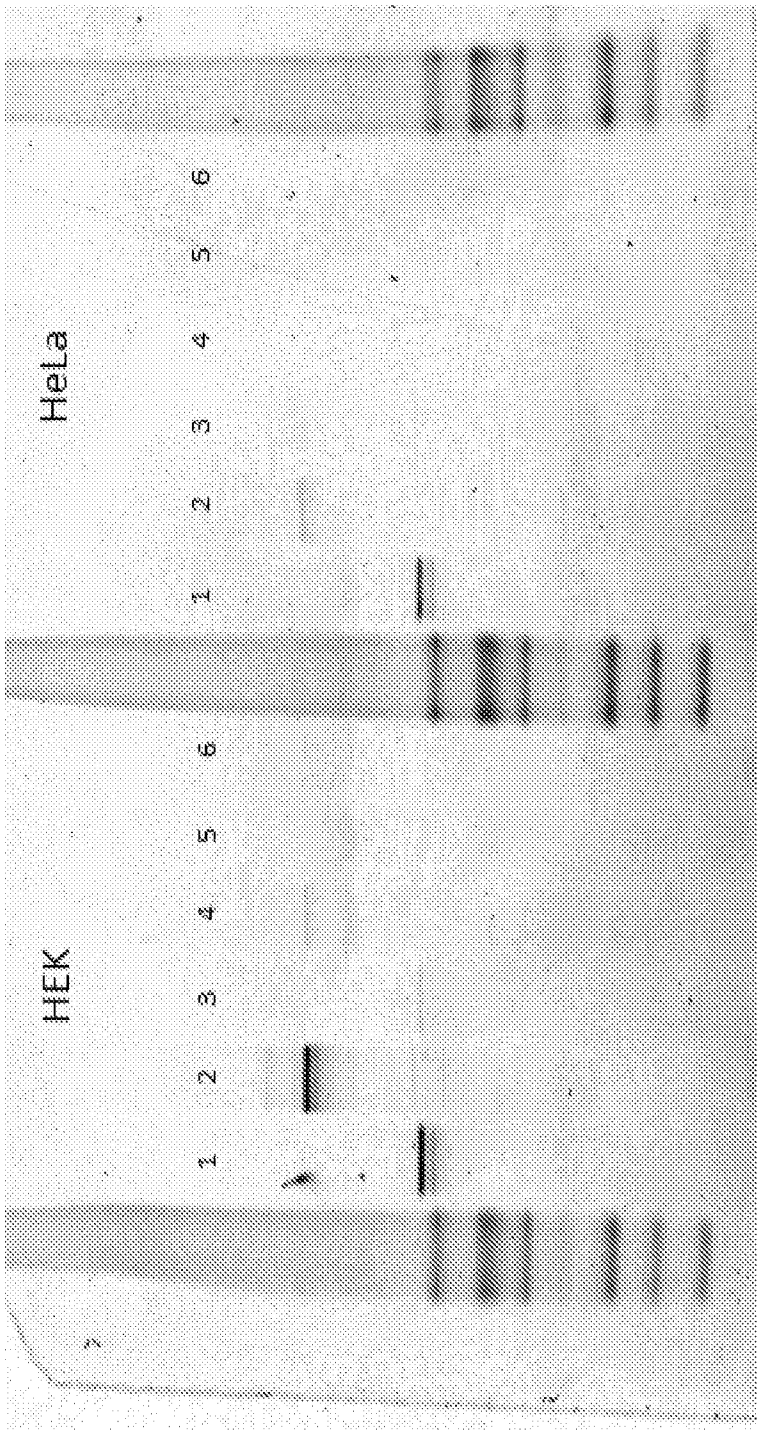

L27V=hatched,
Luc2=black
HEK 293-HT7 fusion
HeLa-HT7 fusion
RLU
[DNA] transfection
50ng
5ng
0.5ng
0.05ng
5 pg
C
D

FIG. 50


HEK
HeLa
1
2
3
4
5
6
1. L27V 50ng
2. Luc2 50ng
3. L27V 5 ng
4. Luc2 5 ng
5. L27V 0.5ng
6. Luc2 0.5ng

E

FIG. 50

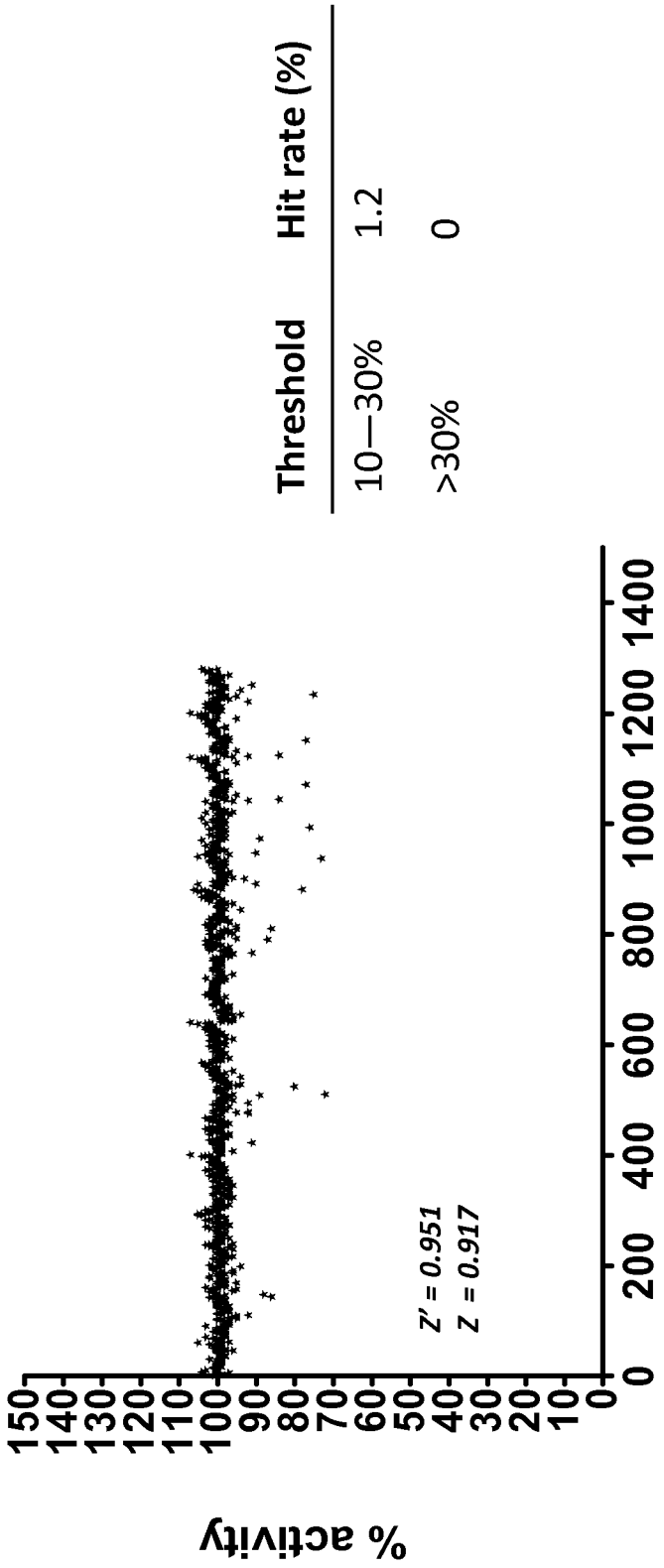
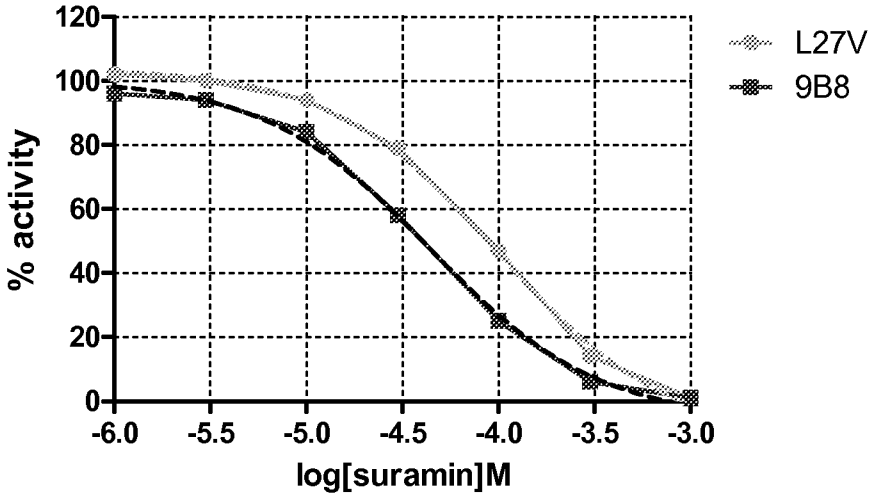
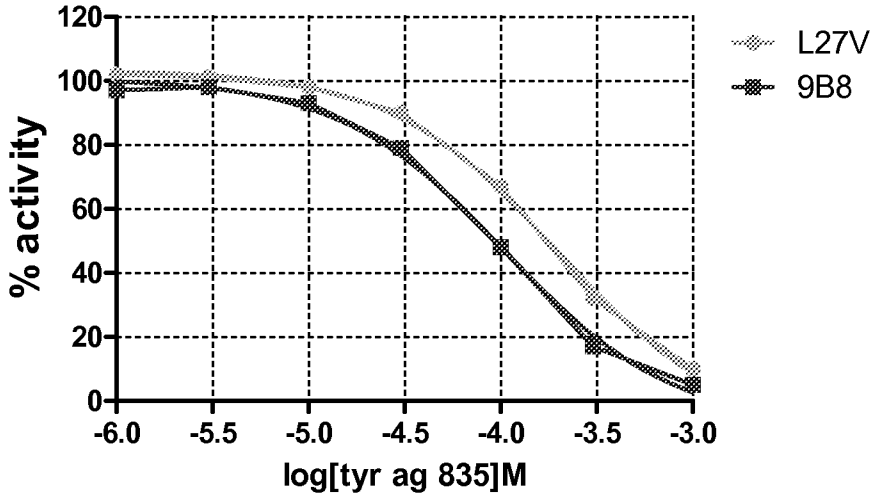


FIG. 51

A



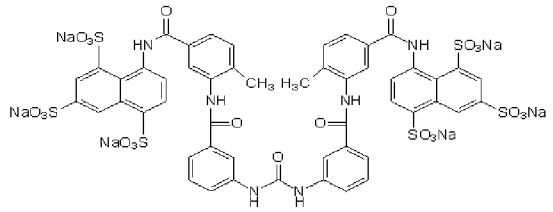
B



C

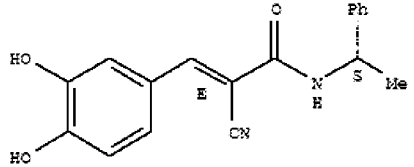
Sample	9B8 IC50 (uM)	L27V IC50(uM)
Suramin	44.4	100.4
Tyr ag 835	105	198

D



Suramin

E



Tyr ag 835

FIG. 52

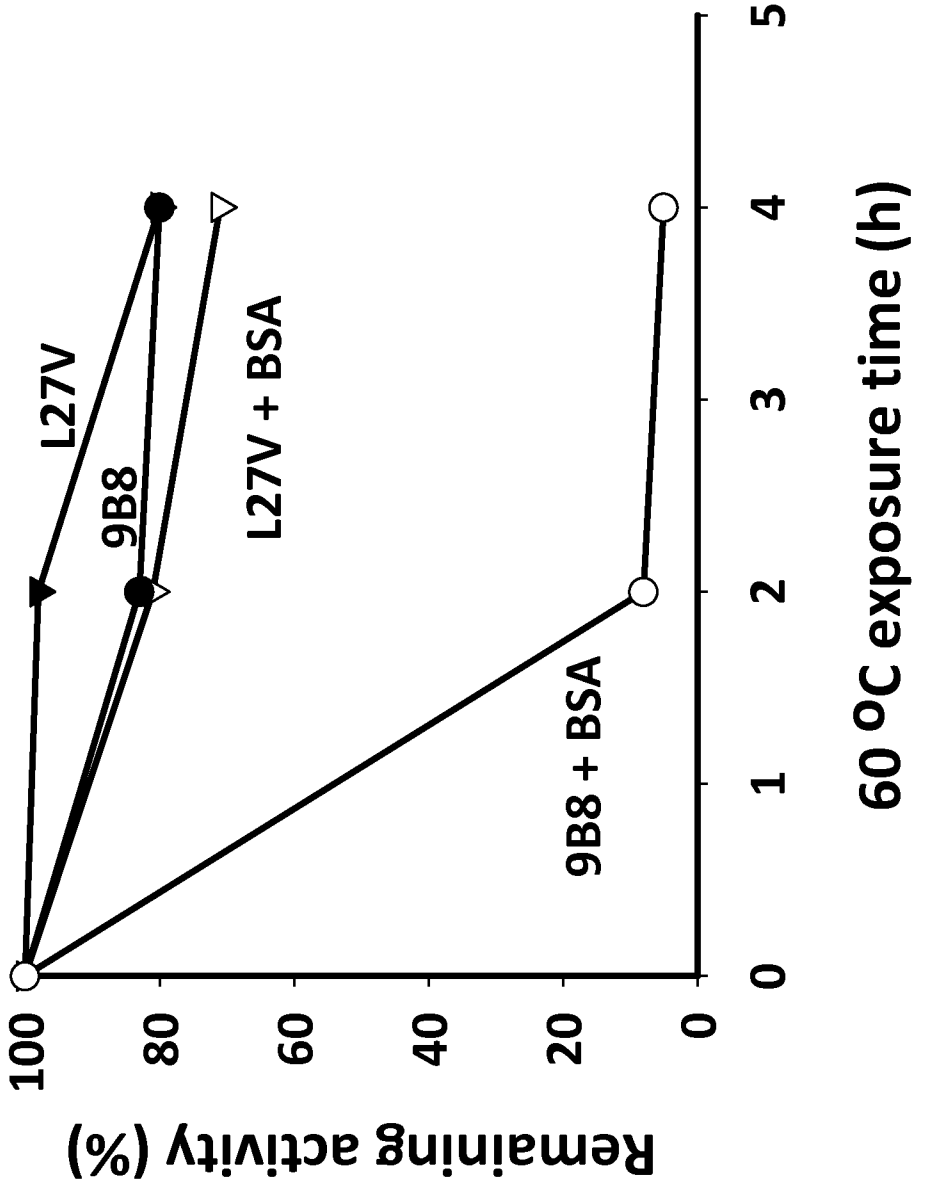

Remaining activity (%)
100
80
60
40
20
0
0
1
2
3
4
5
60 °C exposure time (h)
L27V
9B8
L27V + BSA
9B8 + BSA

FIG. 53

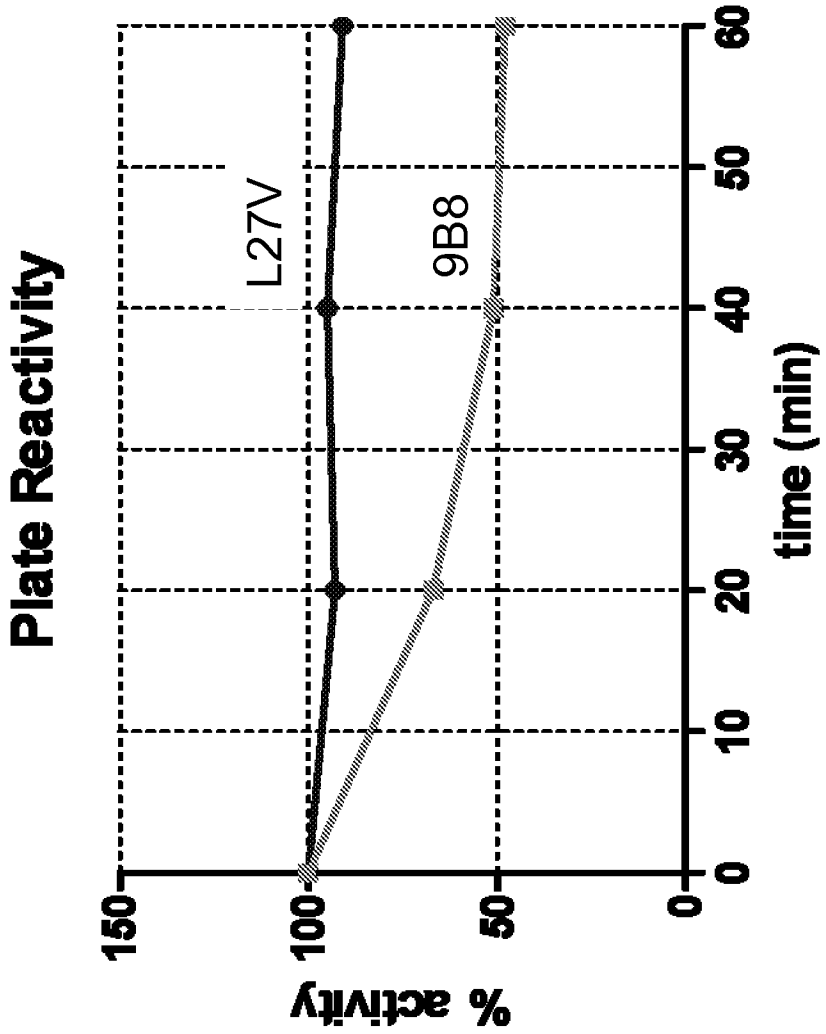

Plate Reactivity
L27V
9B8
150
100
50
0
% activity
0
10
20
30
40
50
60
time (min)

FIG. 54

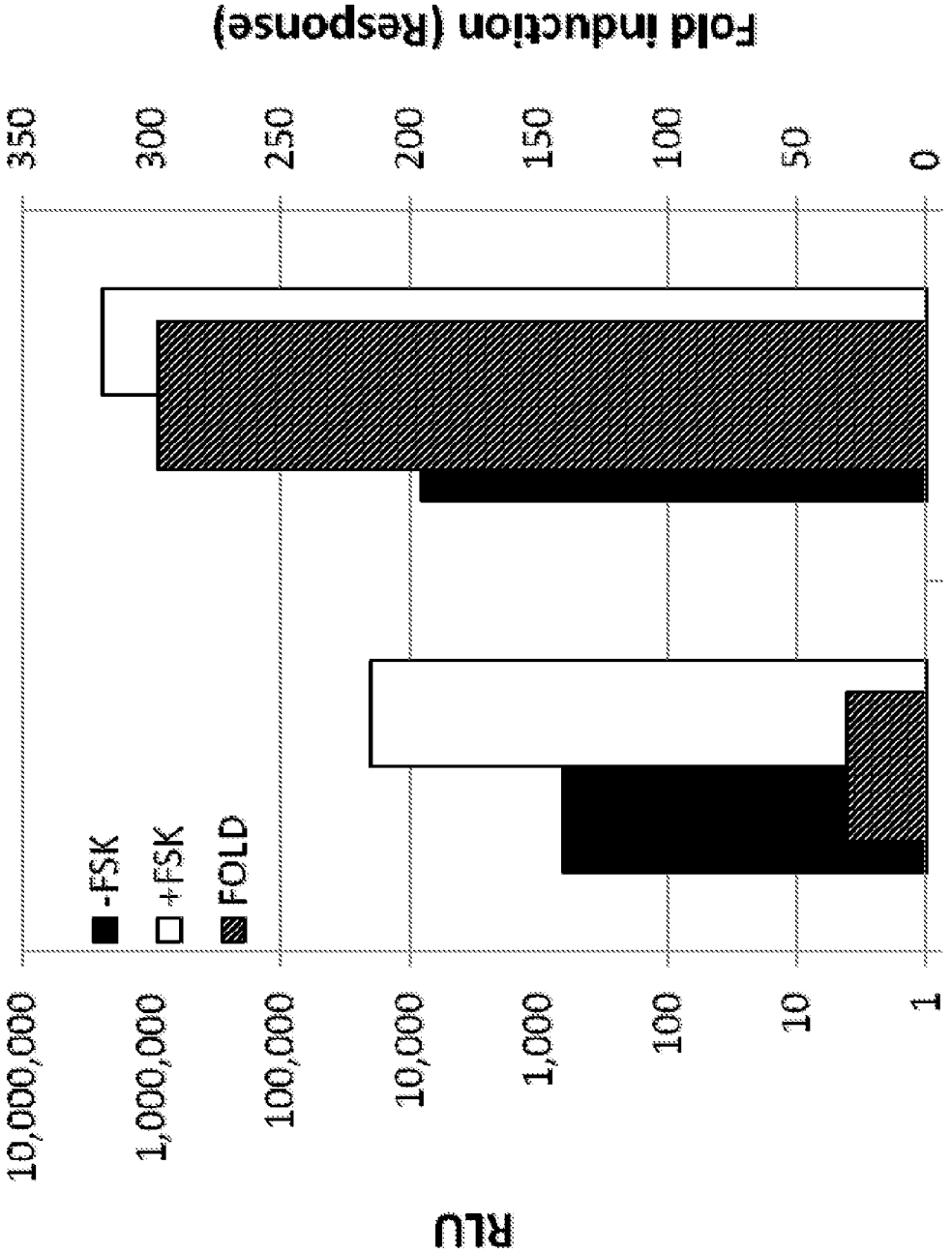

Fold induction (Response)
350
300
250
200
150
100
50
0
RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
-FSK
+FSK
FOLD
Renilla
IV

FIG. 55

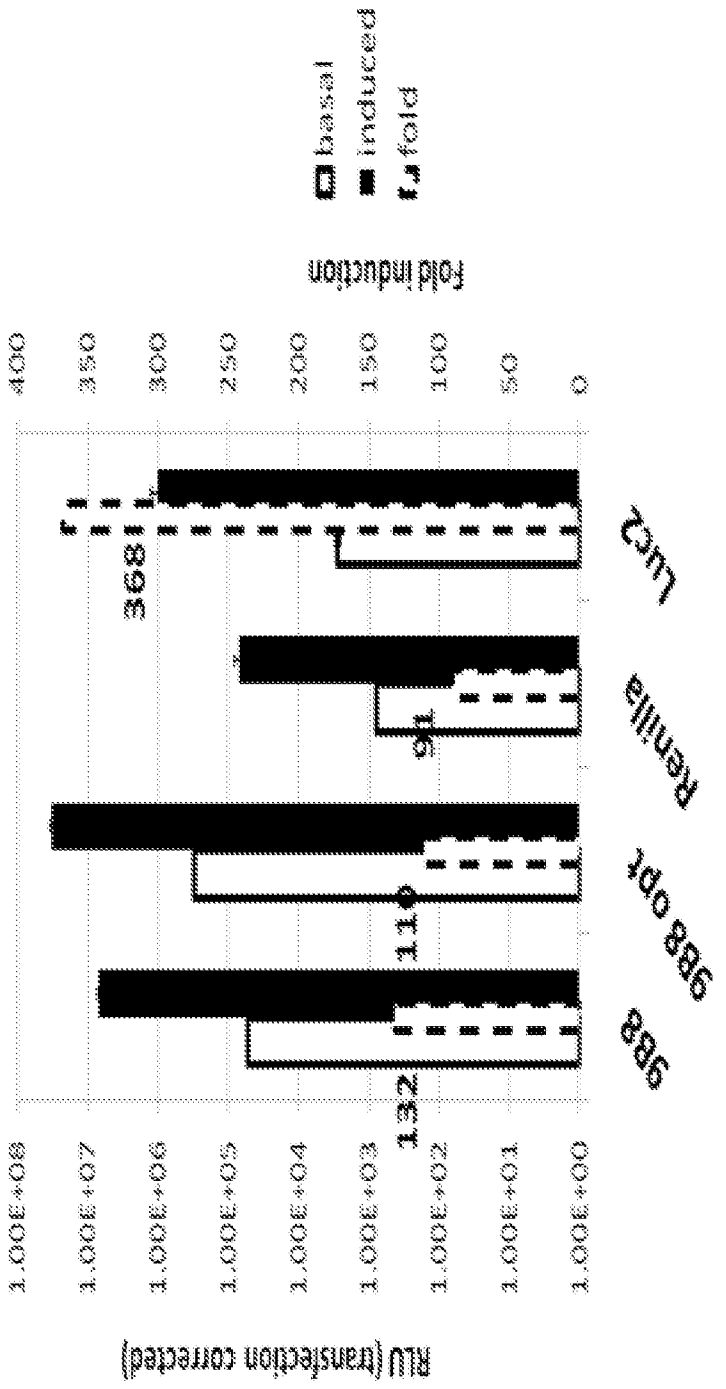

RLU (transfection corrected)
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
fold induction
400
350
300
250
200
150
100
50
0
basal
induced
fold
132
110
16
3981
9a9
9a9 opt
renilla
luc2

FIG. 56

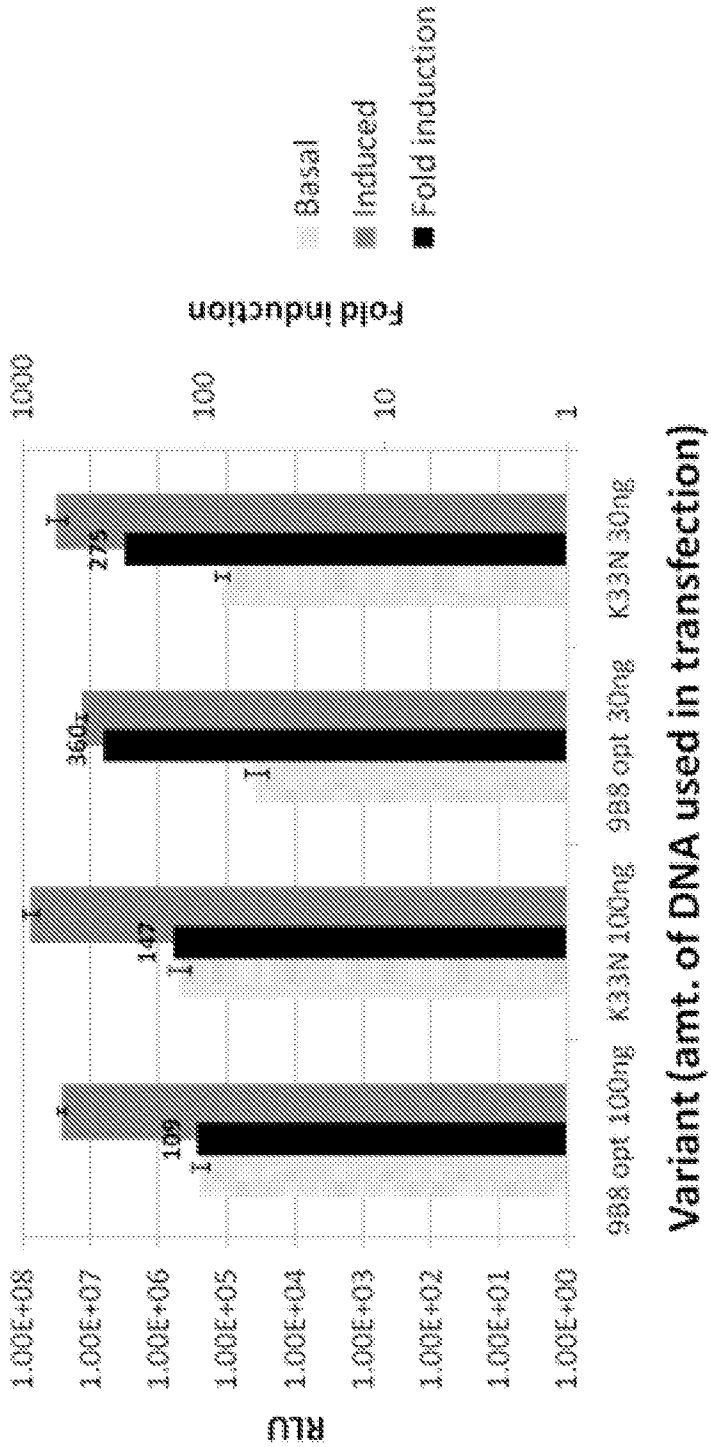

Basal
Induced
Fold induction
Fold induction
1000
100
10
1
RLU
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
1
109
1
1
147
1
1
360
1
1
275
1
988 opt 100ng
K33N 100ng
988 opt 30ng
K33N 30ng
Variant (amt. of DNA used in transfection)

FIG. 57

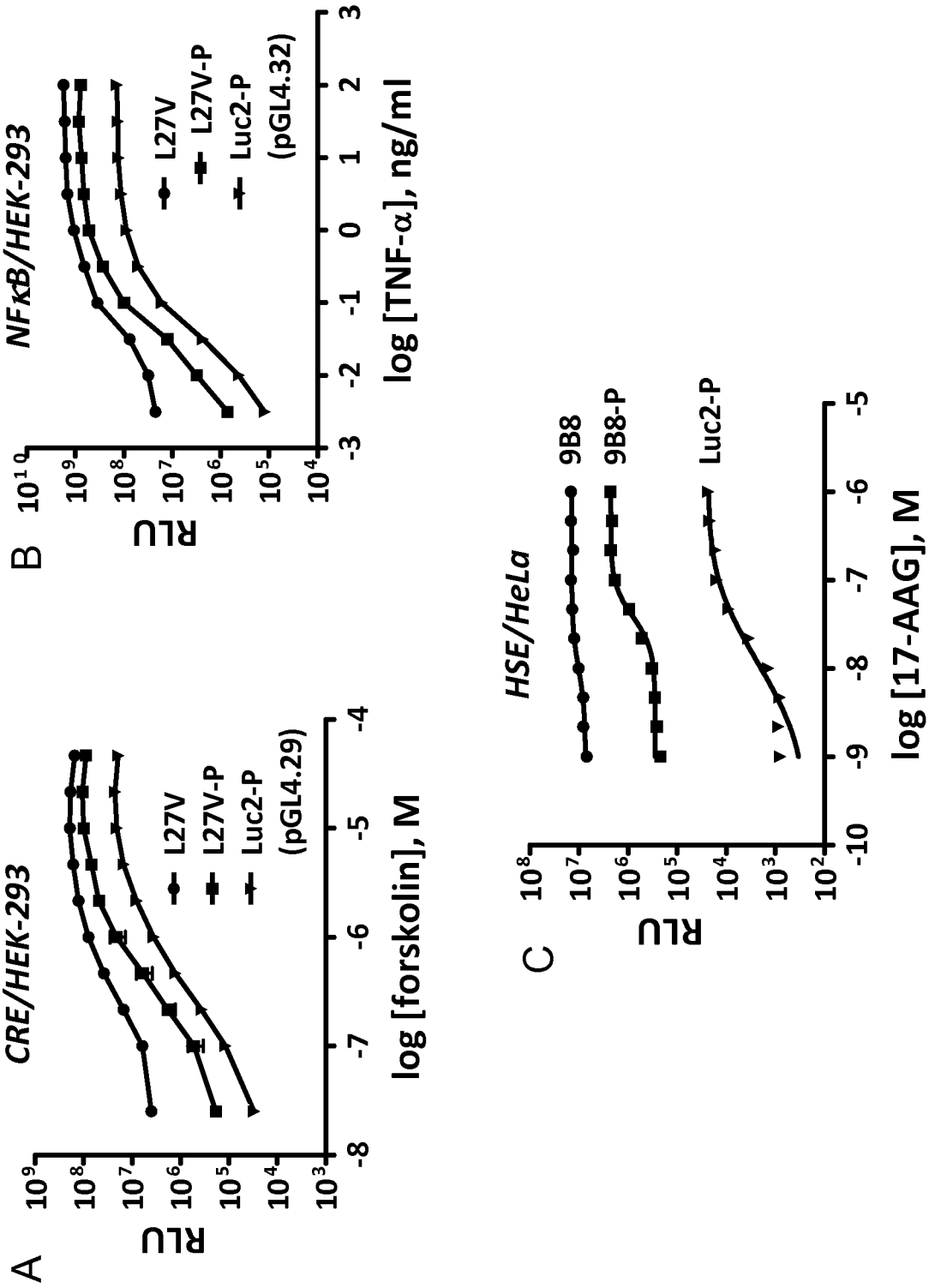
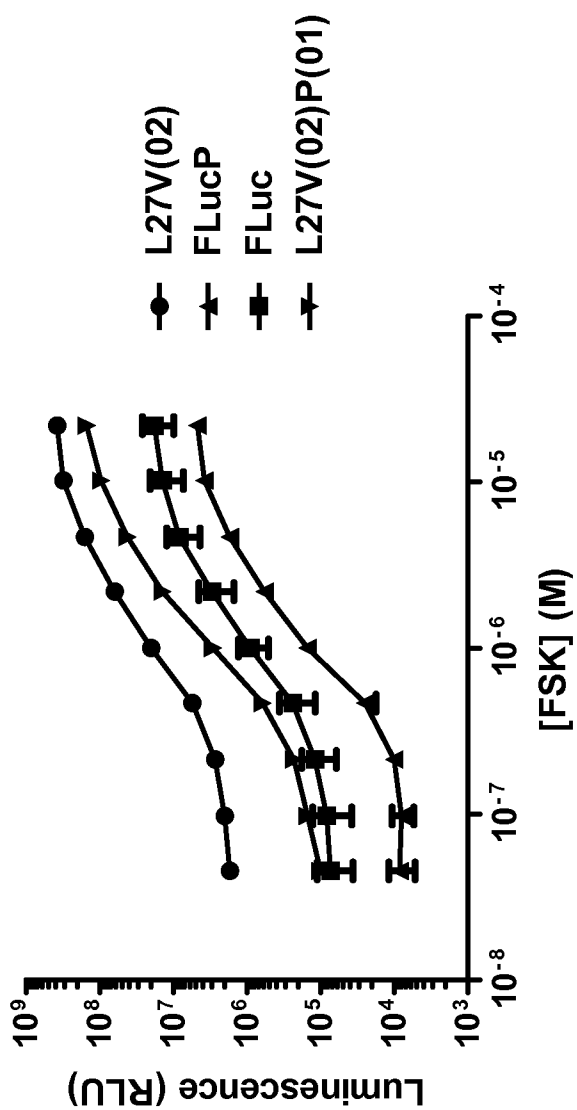

A
CRE/HEK-293
RLU
L27V
L27V-P
Luc2-P
(pGL4.29)
log [forskolin], M
B
NFκB/HEK-293
RLU
L27V
L27V-P
Luc2-P
(pGL4.32)
log [TNF-α], ng/ml
C
HSE/HeLa
RLU
9B8
9B8-P
Luc2-P
log [17-AAG], M

FIG. 58

A



B

	FLucP	FLuc	L27V02P	L27V02
Avg. RLU / luc2P	1.00	7.5	21.6	168.5
S/B	781	268	1768	224
EC50 (μM)	6.3	5.9	10.3	5.9

FIG. 59

C

Exp. system	RLUs Oluc/FLuc	RLUs OLucP/FLucP	S/B, Oluc	S/B, OLucP	S/B, FLuc	S/B, FLucP
293/CRE	22	22	224	1768	268	781
293/NFkB	75	8	22	514	233	347
Hela/NFkB	14	2	40	47	54	44
HepG2/NFkB	267	5	7	11	11	19
Jurkat/NFkB	16	6	18	15	6	11
Jurkat/CRE	19	4	14	123	28	40
ME180/CRE	56	12	2	6	4	5
ME180/NFkB	149	21	7	19	7	25
HCT116/NFkB	22	16	10	605	4	47
U2OS/NFkB	114	16	15	18	11	20

FIG. 59

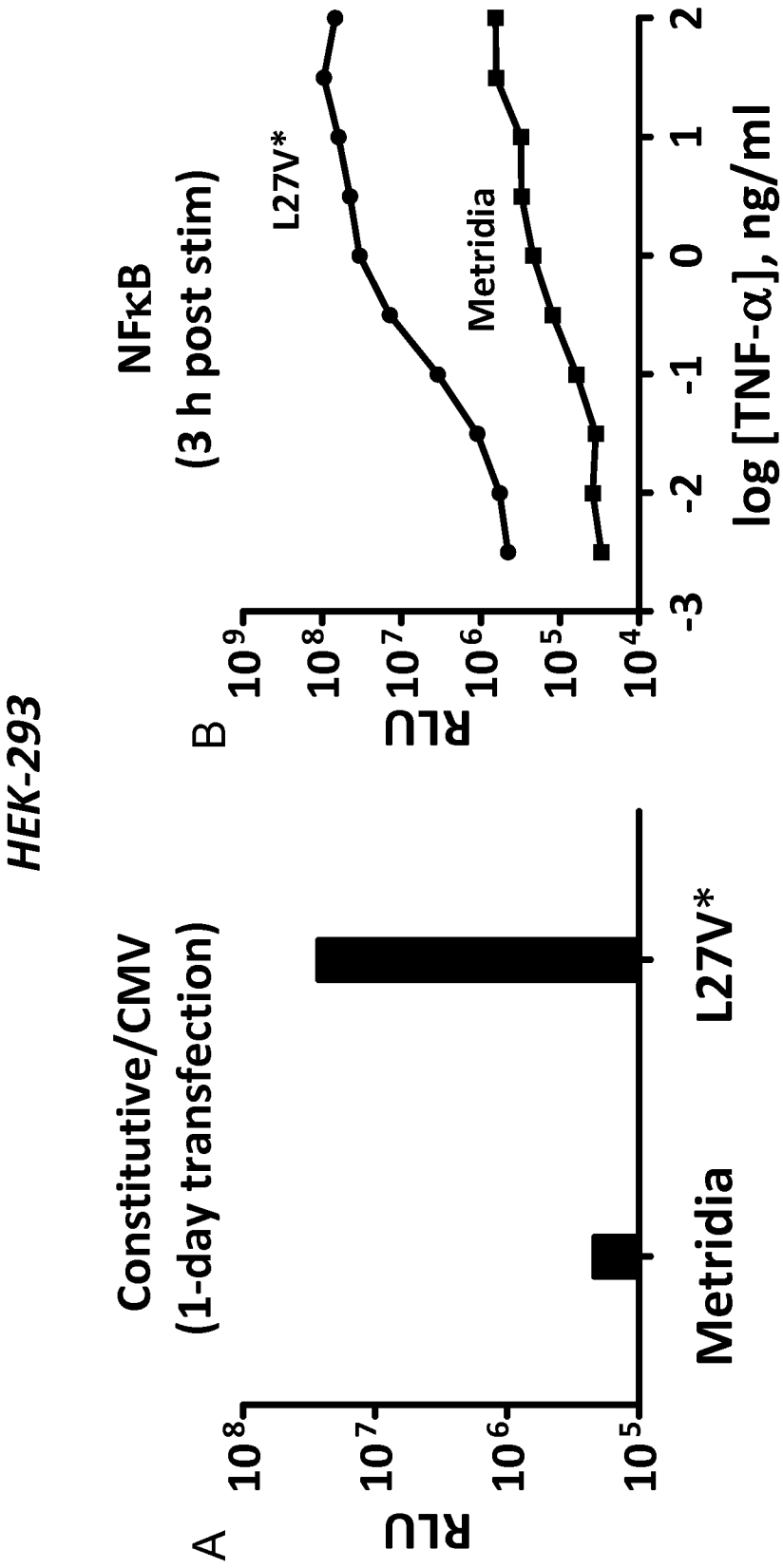

HEK-293
A
Constitutive/CMV
(1-day transfection)
RLU
10^8
10^7
10^6
10^5
Metridia
L27V*
*IL-6 signal sequence
B
NFκB
(3 h post stim)
RLU
10^9
10^8
10^7
10^6
10^5
10^4
L27V*
Metridia
-3
-2
-1
0
1
2
log [TNF-α], ng/ml

FIG. 60

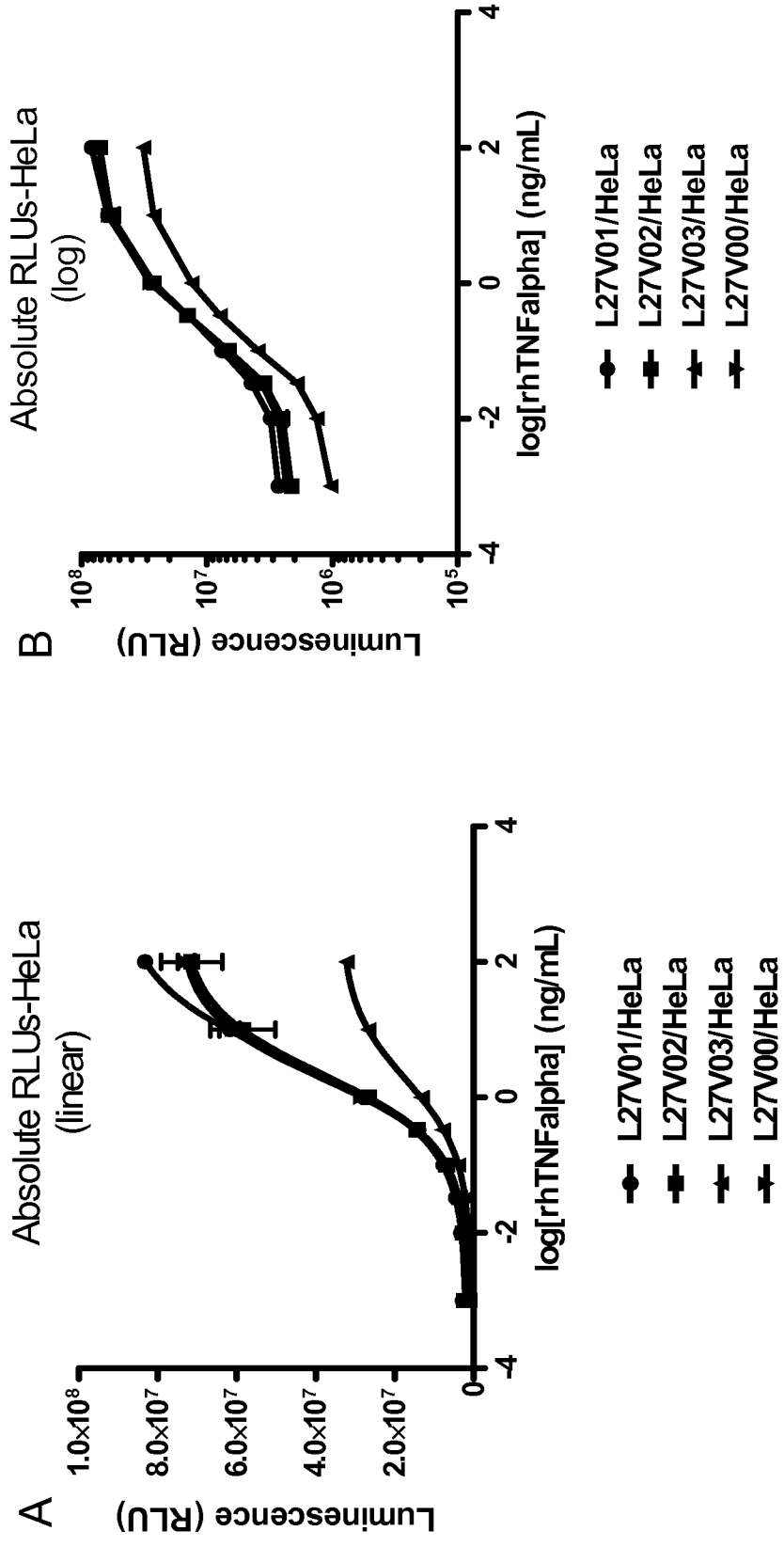

A
Absolute RLUs-HeLa
(linear)
Luminescence (RLU)
1.0x10^8
8.0x10^7
6.0x10^7
4.0x10^7
2.0x10^7
0
-4
-2
0
2
4
log[rhTNFalpha] (ng/mL)
L27V01/HeLa
L27V02/HeLa
L27V03/HeLa
L27V00/HeLa
B
Absolute RLUs-HeLa
(log)
Luminescence (RLU)
10^8
10^7
10^6
10^5
-4
-2
0
2
4
log[rhTNFalpha] (ng/mL)
L27V01/HeLa
L27V02/HeLa
L27V03/HeLa
L27V00/HeLa

FIG. 61

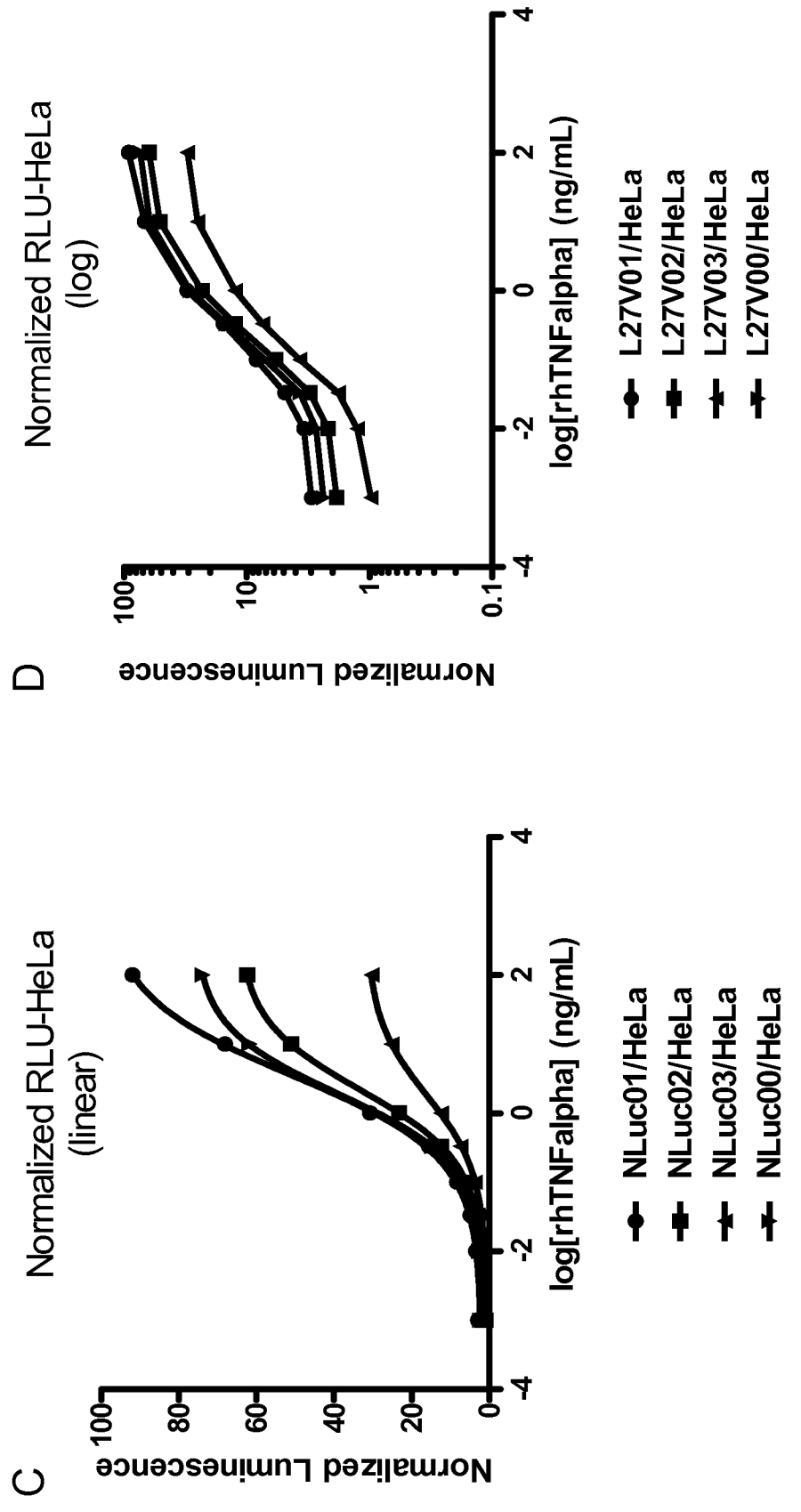

C
Normalized RLU-HeLa
(linear)
Normalized Luminescence
100
80
60
40
20
0
-4
-2
0
2
4
log[rhTNFalpha] (ng/mL)
NLuc01/HeLa
NLuc02/HeLa
NLuc03/HeLa
NLuc00/HeLa
D
Normalized RLU-HeLa
(log)
Normalized Luminescence
100
10
1
0.1
-4
-2
0
2
4
log[rhTNFalpha] (ng/mL)
L27V01/HeLa
L27V02/HeLa
L27V03/HeLa
L27V00/HeLa

FIG. 61

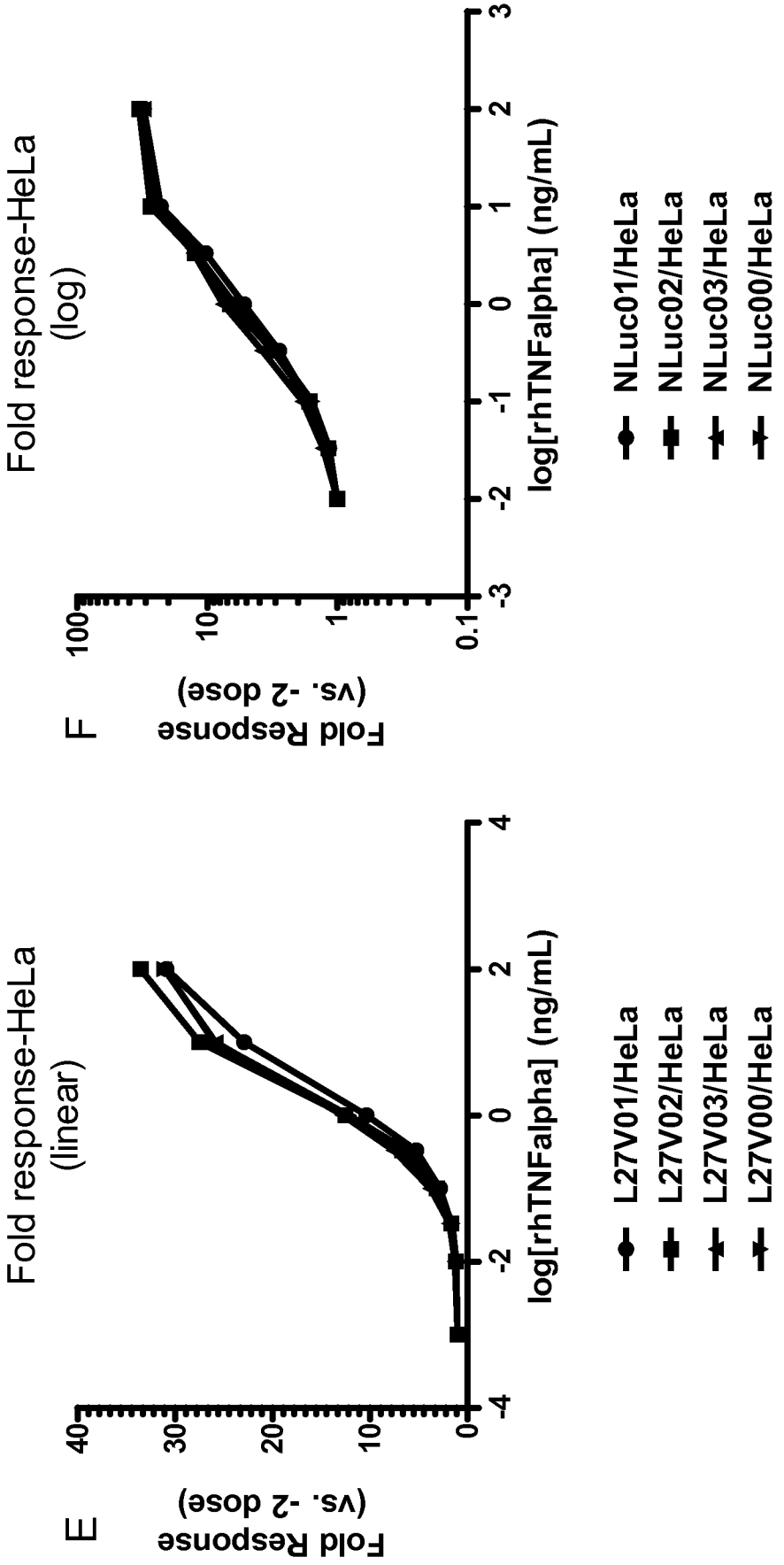

F
Fold Response (vs. -2 dose)
100
10
1
0.1
-3
-2
-1
0
1
2
3
log[rhTNFalpha] (ng/mL)
Fold response-HeLa (log)
NLuc01/HeLa
NLuc02/HeLa
NLuc03/HeLa
NLuc00/HeLa
E
Fold Response (vs. -2 dose)
40
30
20
10
0
-4
-2
0
2
4
log[rhTNFalpha] (ng/mL)
Fold response-HeLa (linear)
L27V01/HeLa
L27V02/HeLa
L27V03/HeLa
L27V00/HeLa

FIG. 61

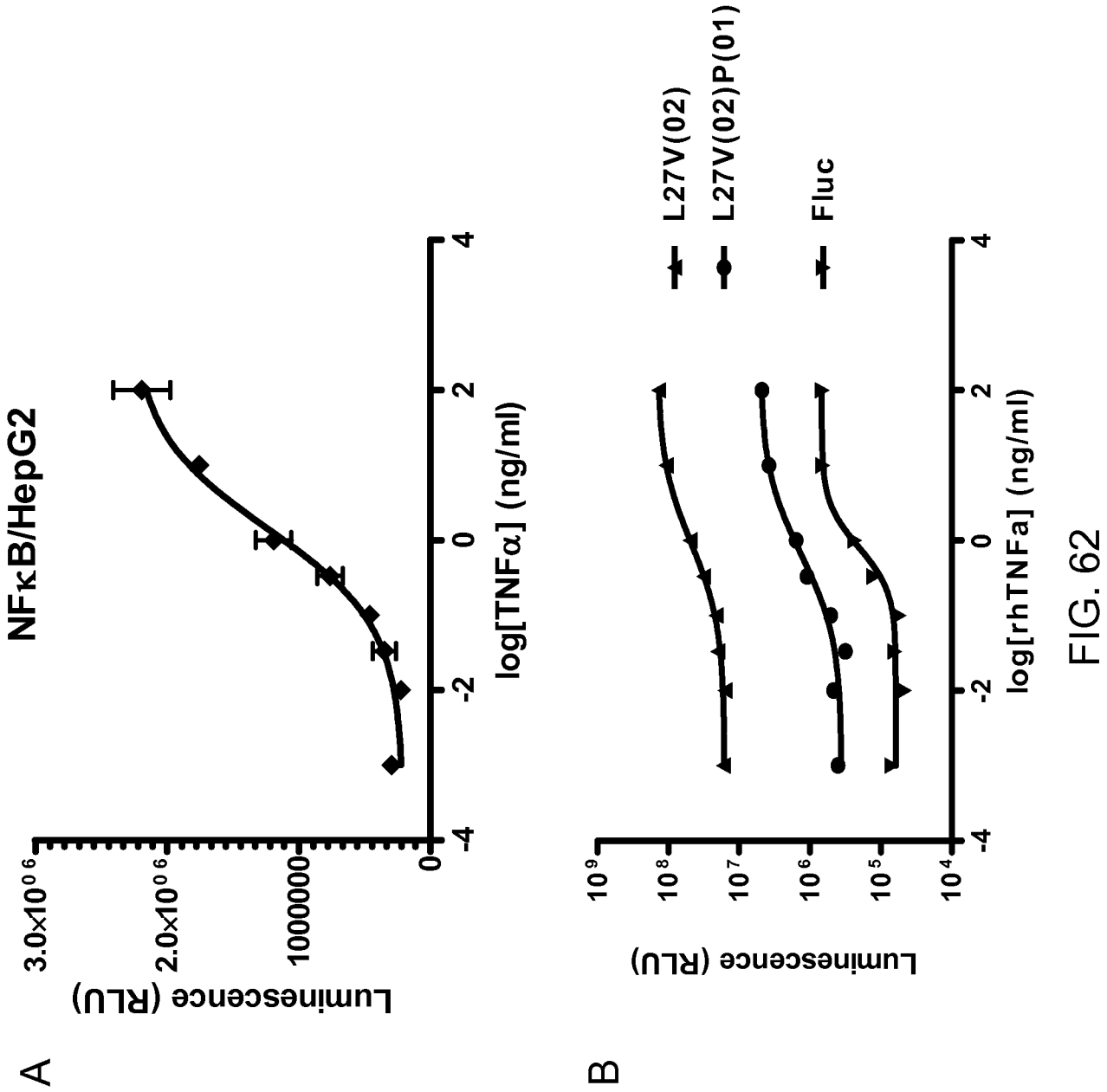

A
NFκB/HepG2
Luminescence (RLU)
3.0×10^0 6
2.0×10^0 6
1000000
0
-4
-2
0
2
4
log[TNFα] (ng/ml)
B
L27V(02)
L27V(02)P(01)
Fluc
Luminescence (RLU)
10^9
10^8
10^7
10^6
10^5
10^4
-4
-2
0
2
4
log[rhTNFa] (ng/ml)

FIG. 62

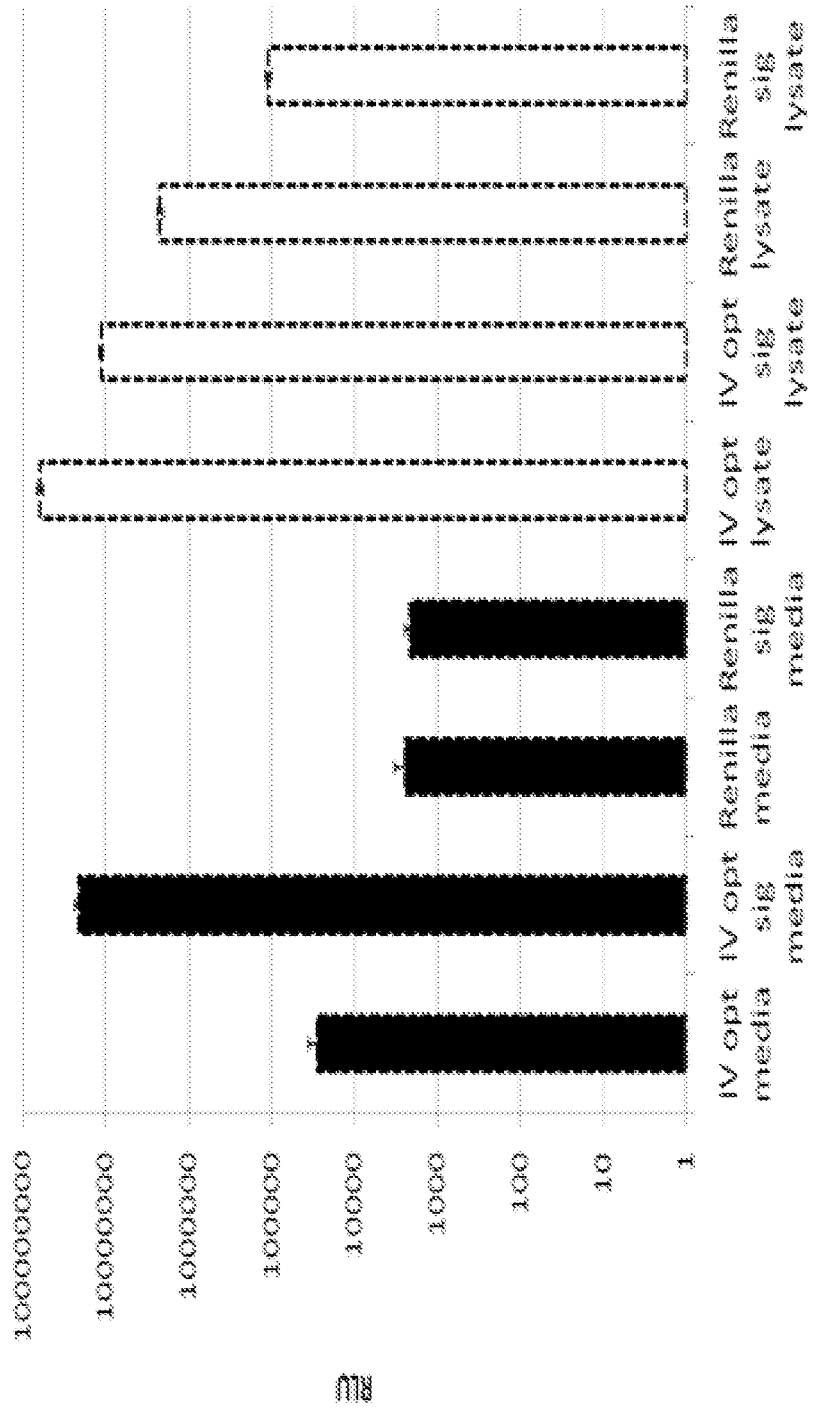
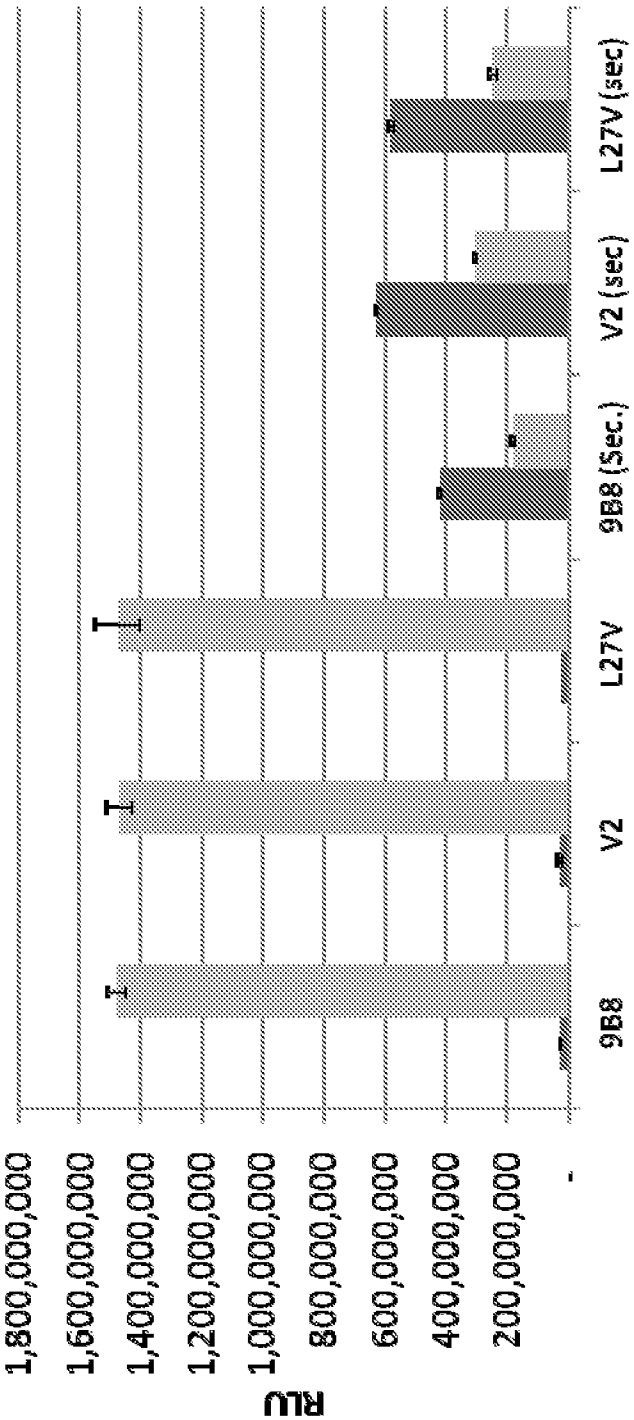

RLU
10000000
1000000
100000
10000
1000
100
10
1
IV opt media
IV opt sig media
Renilla media
Renilla sig media
IV opt lysate
IV opt sig lysate
Renilla lysate
Renilla sig lysate

FIG. 63

A

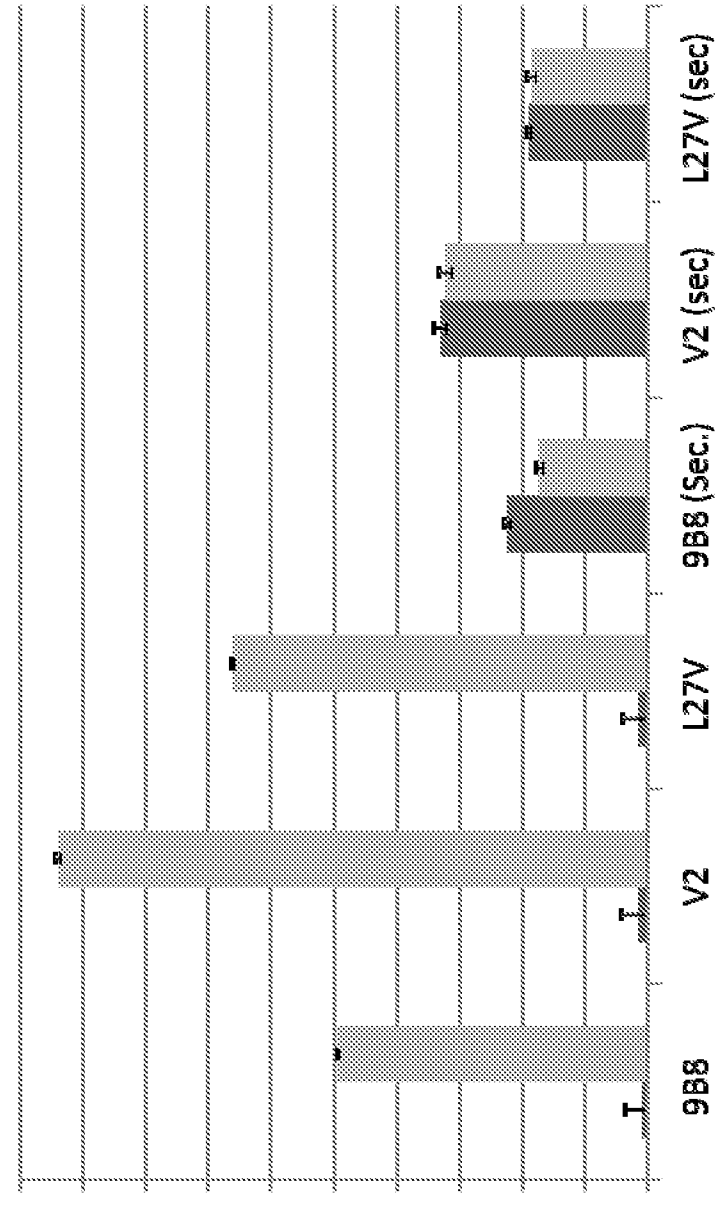


B

sample	9B8	V2	L27V	9B8 (Sec.)	V2 (sec)	L27V (sec)
Media	21,284,716	23,787,770	18,299,776	417,440,788	625,387,280	578,186,357
Lysate	1,478,846,480	1,468,338,592	1,474,480,208	177,409,200	299,355,128	243,323,506

FIG. 64

C



D

sample	9B8	V2	L27V	9B8 (Sec.)	V2 (sec)	L27V (sec)
Media	14,206,429	27,753,862	28,932,890	447,675,259	660,425,952	377,700,912
Lysate	984,347,600	1,876,585,440	1,319,113,560	343,880,163	643,107,747	369,662,727

FIG. 64

A

DNA	Met-luc (Ready-To Glow™)	9B8-3939	V2-3939
100ng	2,502,116	3,307,464,875	3,992,245,760
10ng	297,024	33,947,989	51,683,999

B

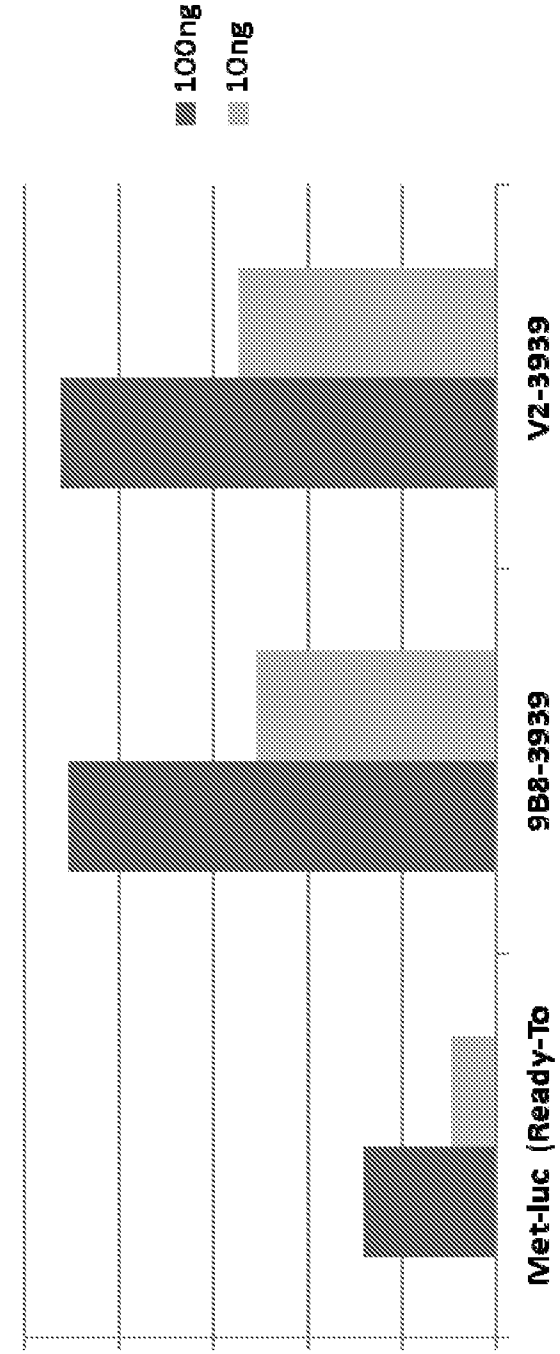


FIG. 65

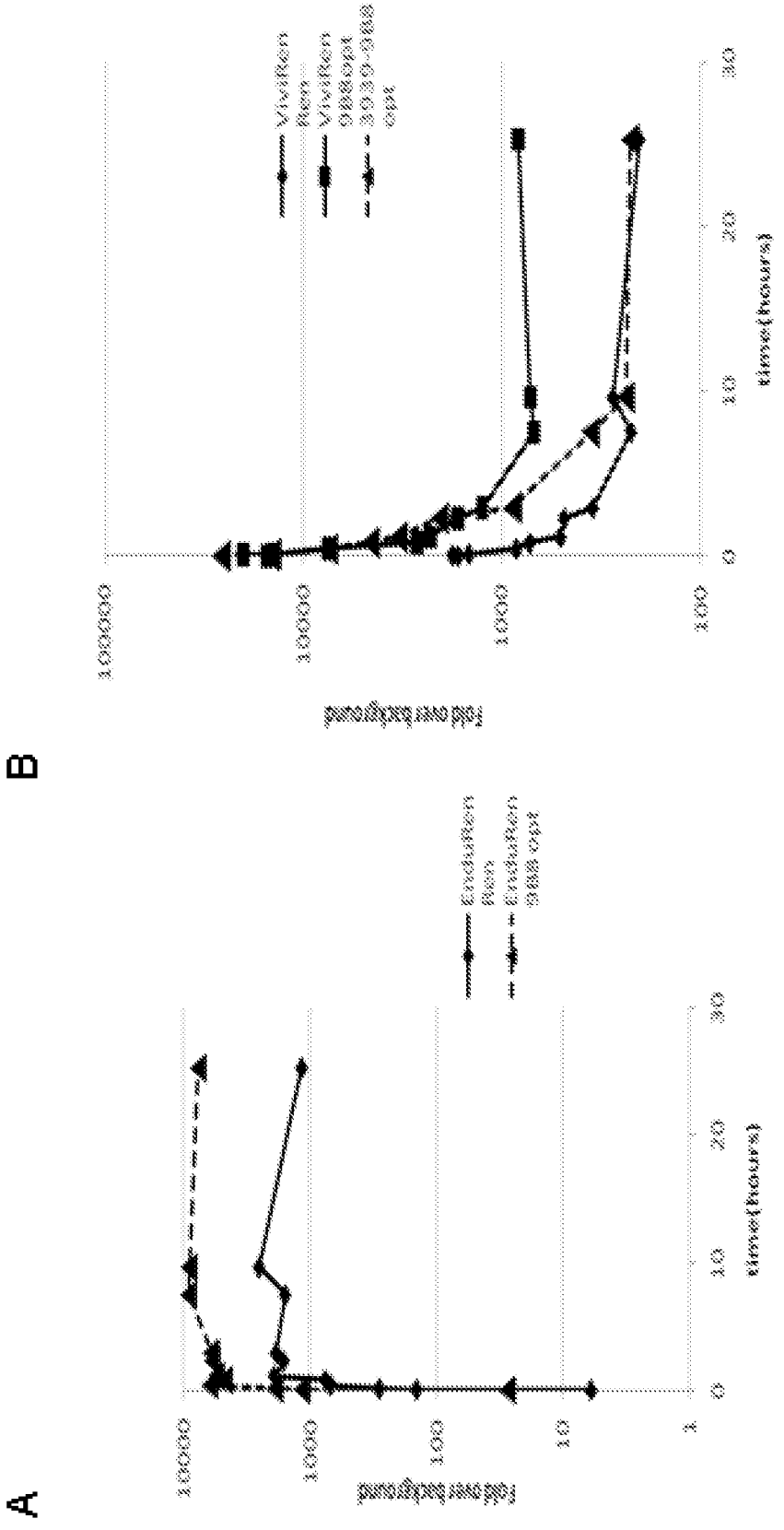
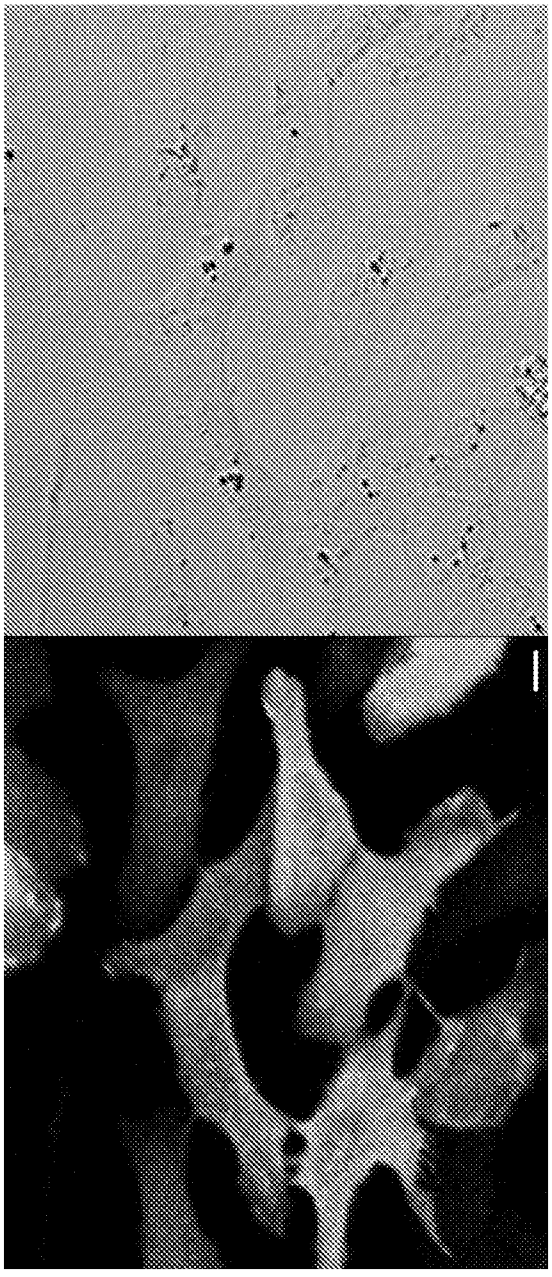

A
B
fold over background
Time (hours)
Endostatin 9888 opt
Vivitrol 9888 opt
0
10
20
30
1
10
100
1000
10000
100000

FIG. 66



A

FIG. 67

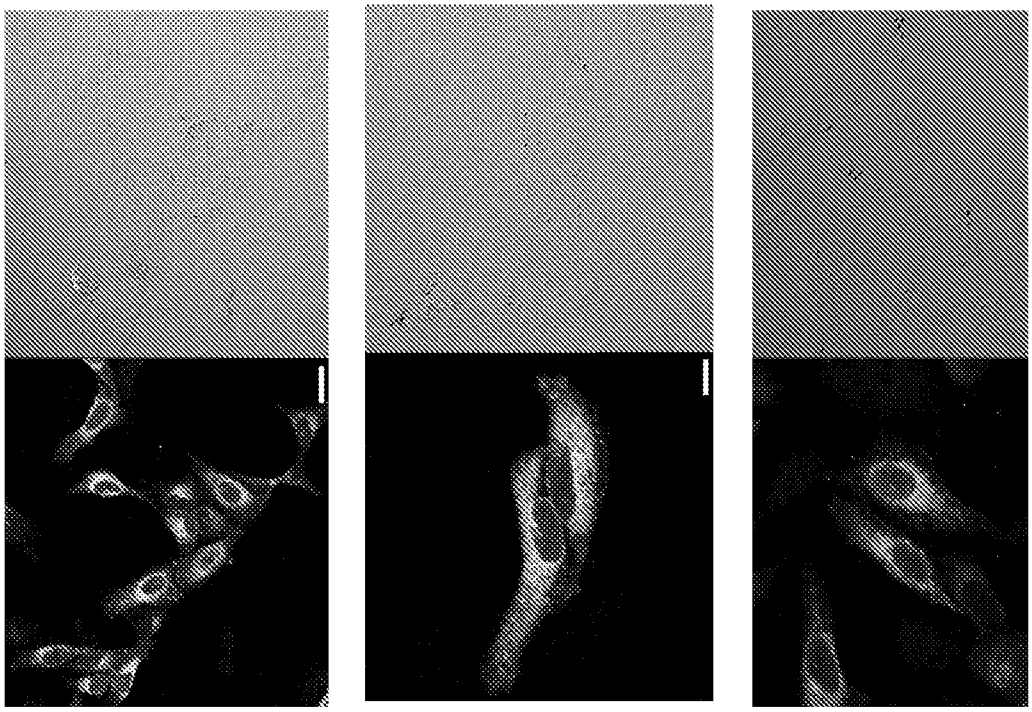


FIG. 67

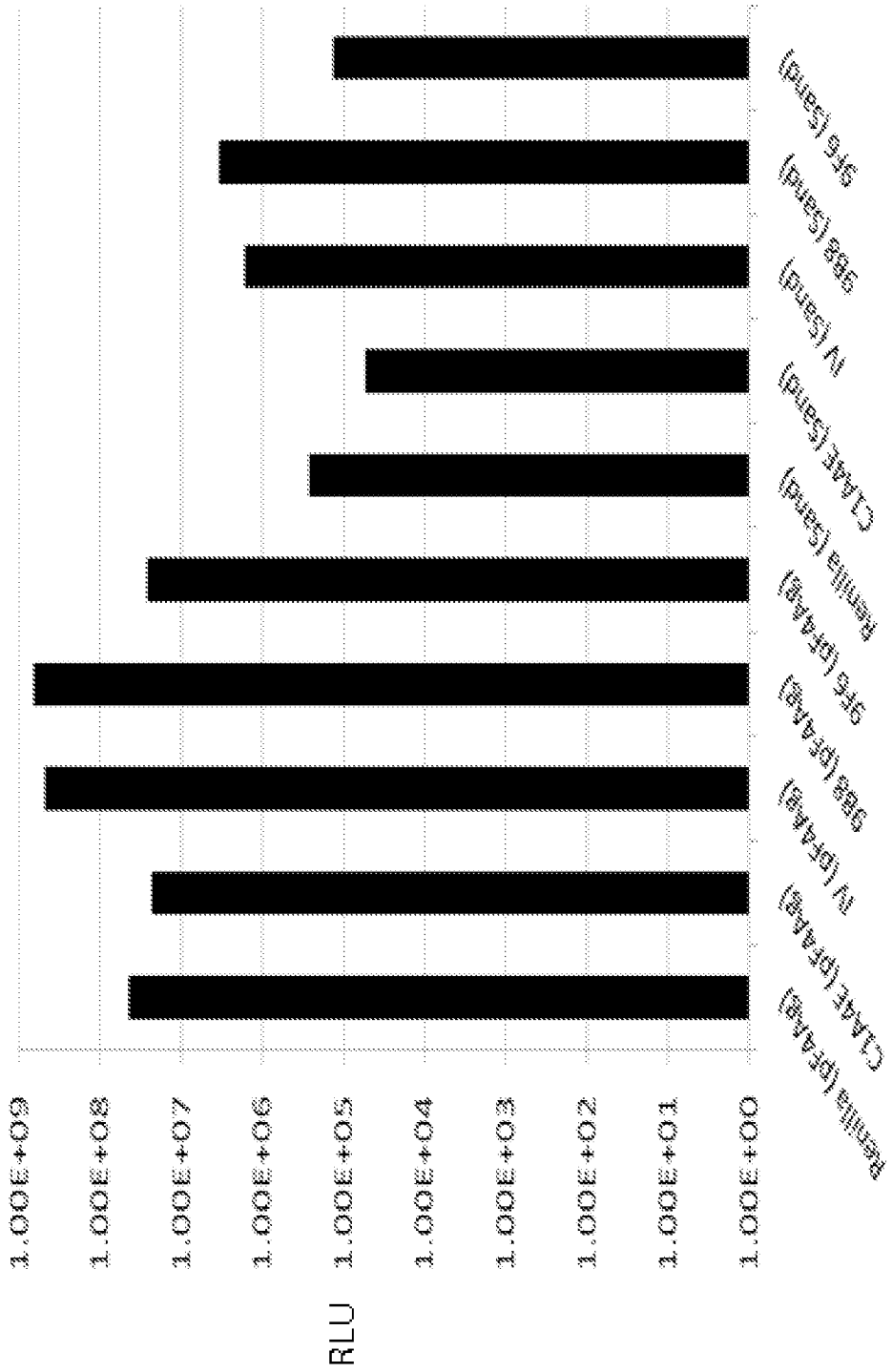

RLU
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Renilla (GFrAAp)
C1A4E (GFrAAp)
IV (GFrAAp)
9B8 (GFrAAp)
9F6 (GFrAAp)
Renilla (Sarnd)
C1A4E (Sarnd)
IV (Sarnd)
9B8 (Sarnd)
9F6 (Sarnd)

FIG. 68

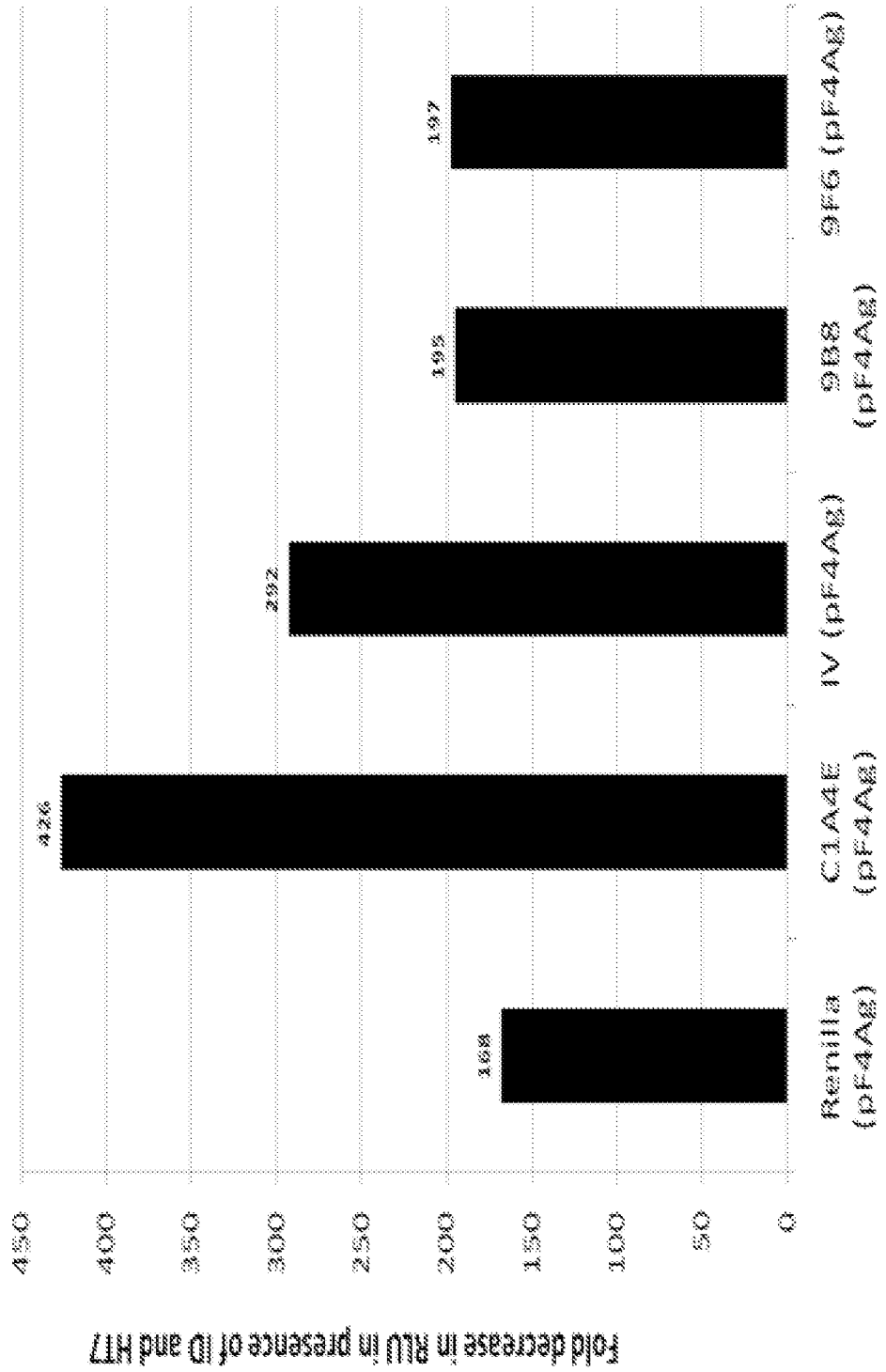

fold decrease in RLU in presence of ID and HT7
450
400
350
300
250
200
150
100
50
0
168
42%
292
19%
197
Renilla (pF4A8)
C1A4E (pF4A8)
IV (pF4A8)
9B8 (pF4A8)
9F6 (pF4A8)

FIG. 69

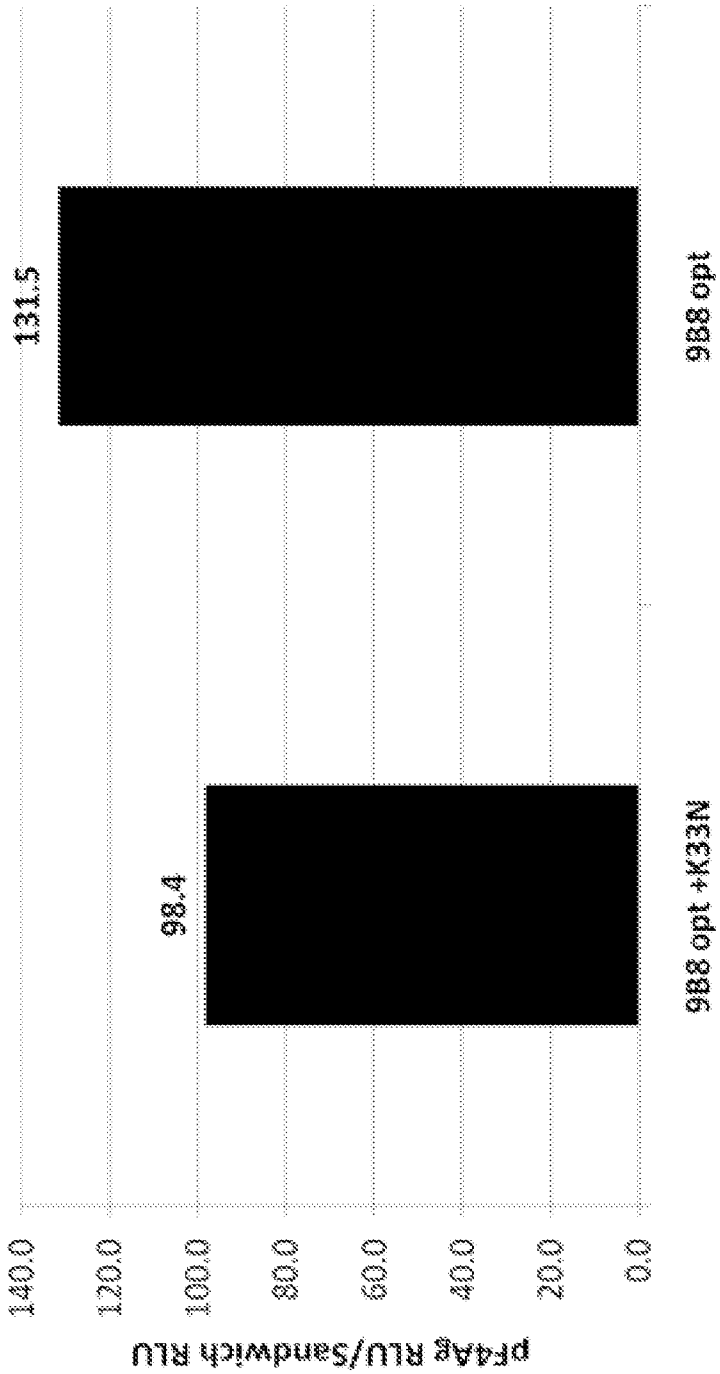

pf4Ag RLU/Sandwich RLU
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
131.5
98.4
988 opt
988 opt +K33N

FIG. 70

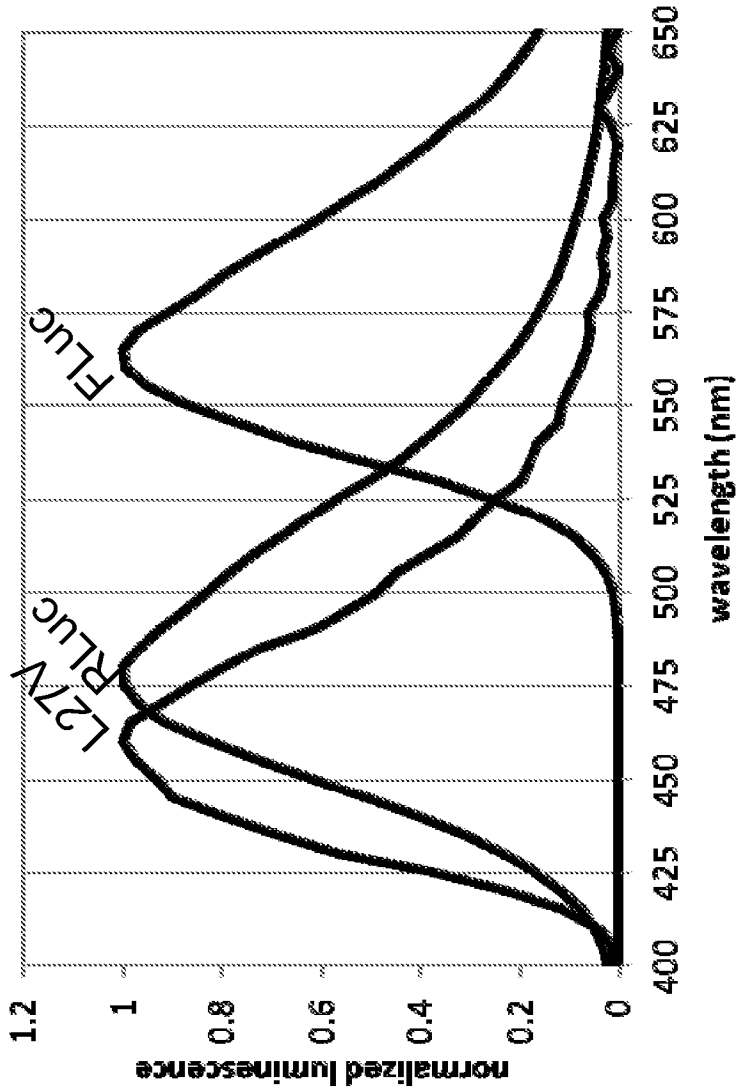

normalized luminescence
1.2
1
0.8
0.6
0.4
0.2
0
400
425
450
475
500
525
550
575
600
625
650
wavelength (nm)
L2ZV-RLuc
FLuc

FIG. 71

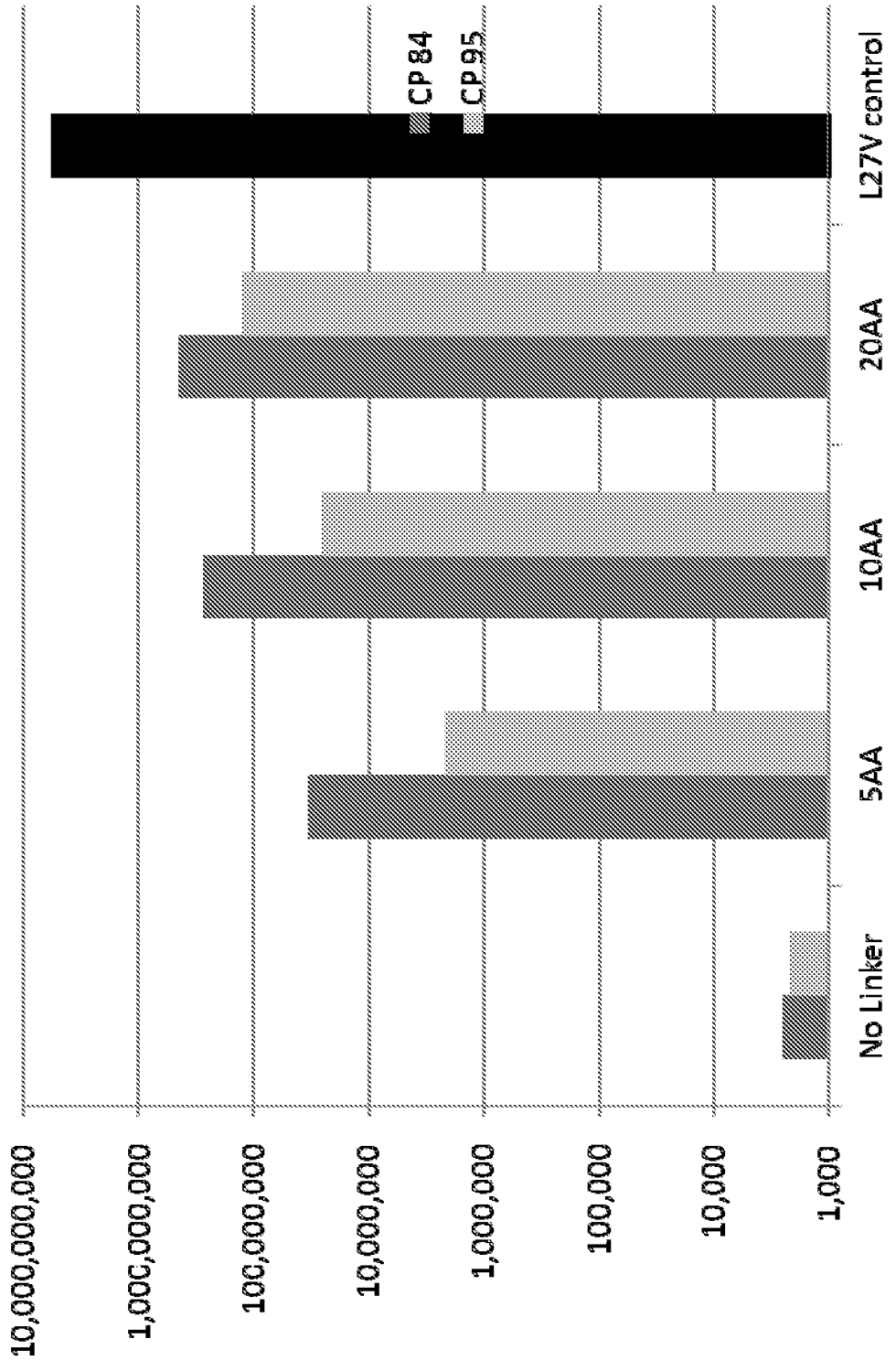

10,000,000,000
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
CP84
CP95
No Linker
5AA
10AA
20AA
L27V control

FIG. 72

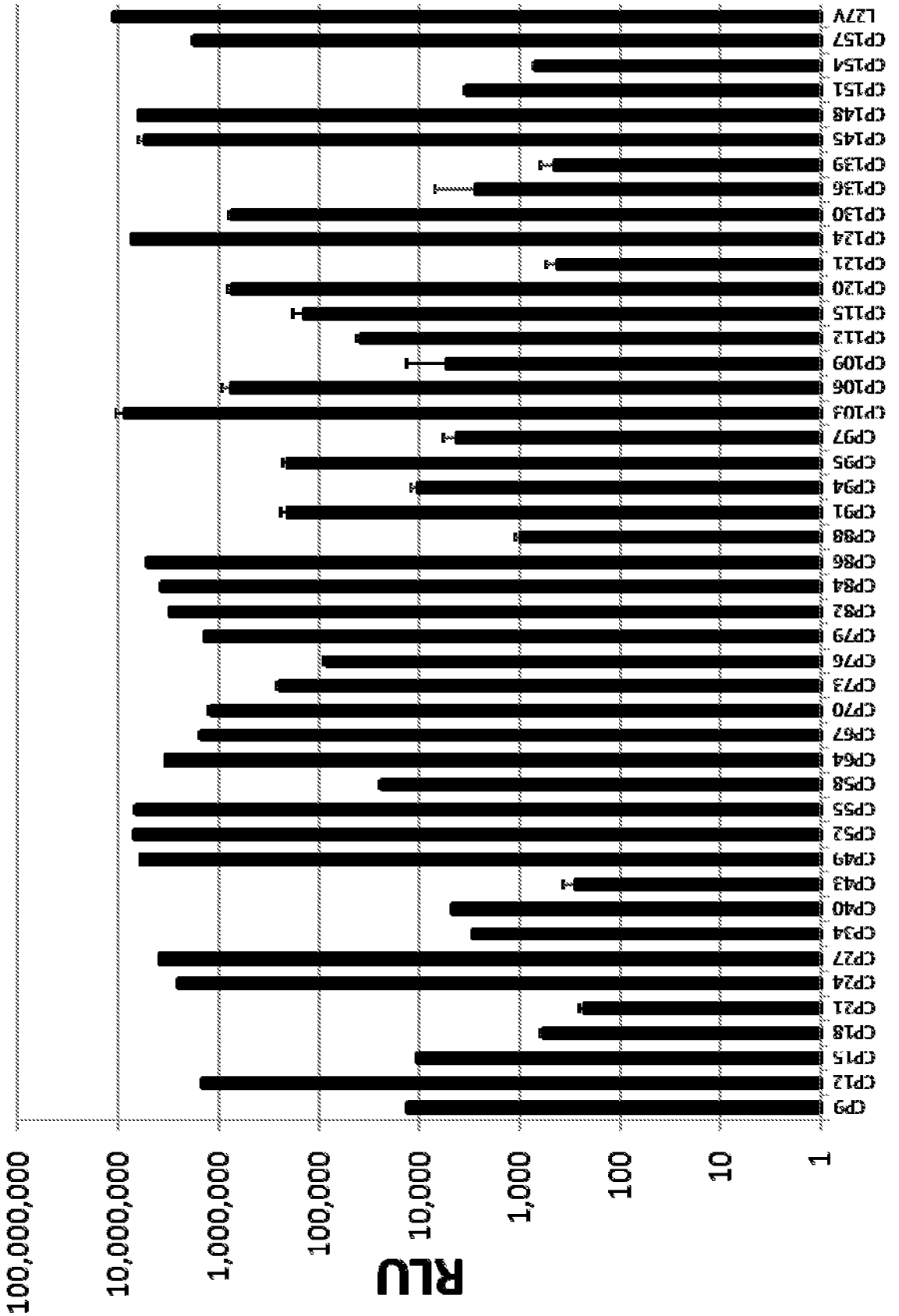

RLU
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
CP9
CP12
CP15
CP18
CP21
CP24
CP27
CP34
CP40
CP43
CP49
CP52
CP55
CP58
CP64
CP67
CP70
CP73
CP76
CP79
CP82
CP84
CP86
CP88
CP91
CP94
CP95
CP97
CP103
CP106
CP109
CP112
CP115
CP120
CP121
CP124
CP130
CP136
CP139
CP145
CP148
CP151
CP154
CP157
LZ7V

FIG. 73A

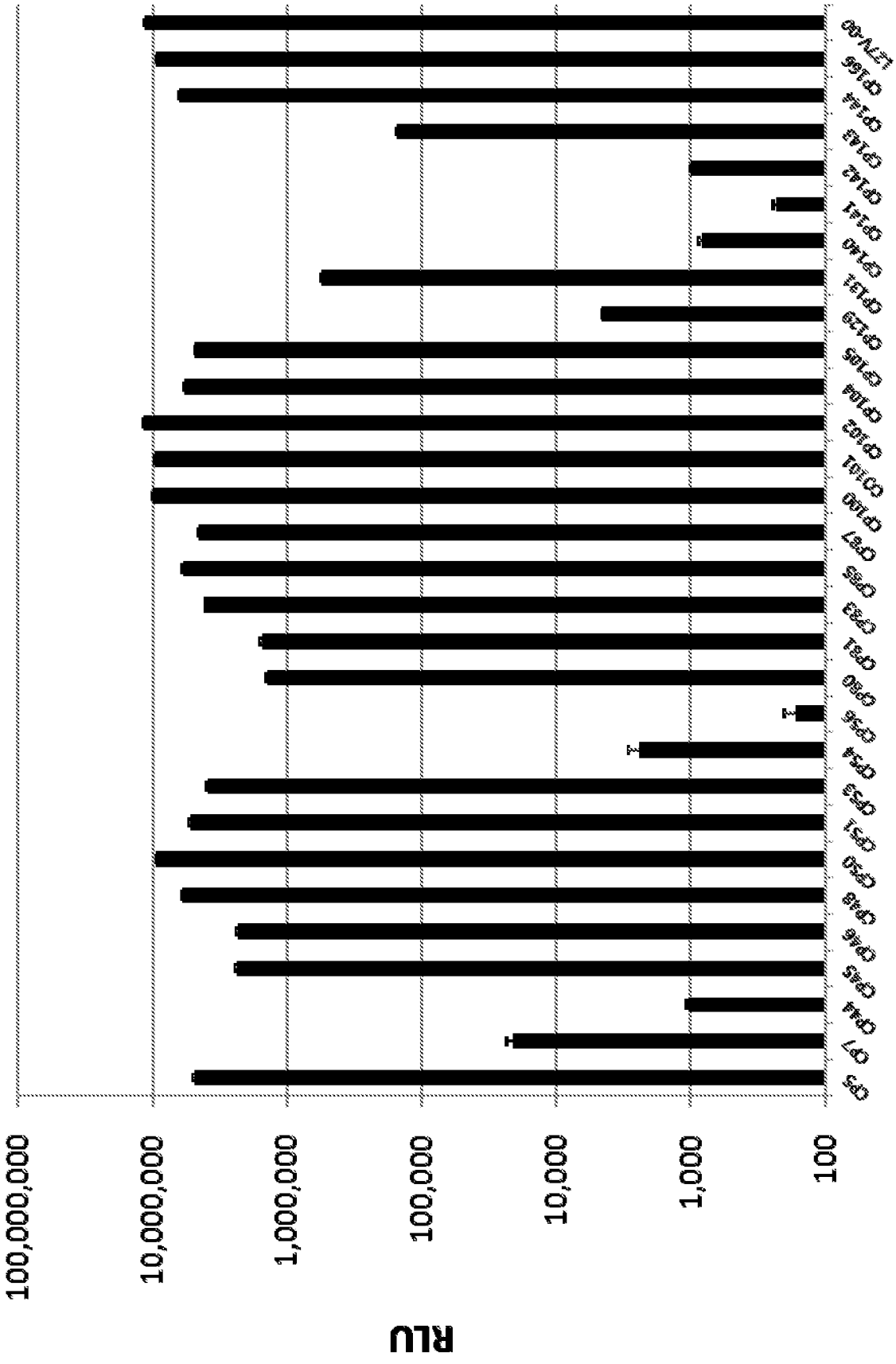

RLU
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
CP5
CP7
CP14
CP15
CP16
CP17
CP18
CP19
CP20
CP21
CP22
CP23
CP24
CP25
CP26
CP27
CP28
CP29
CP30
CP31
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CP366
CP367
CP368
CP369
CP370
CP371
CP372
CP373
CP374
CP375
CP376
CP377
CP378
CP379
CP380
CP381
CP382
CP383
CP384

FIG. 73B

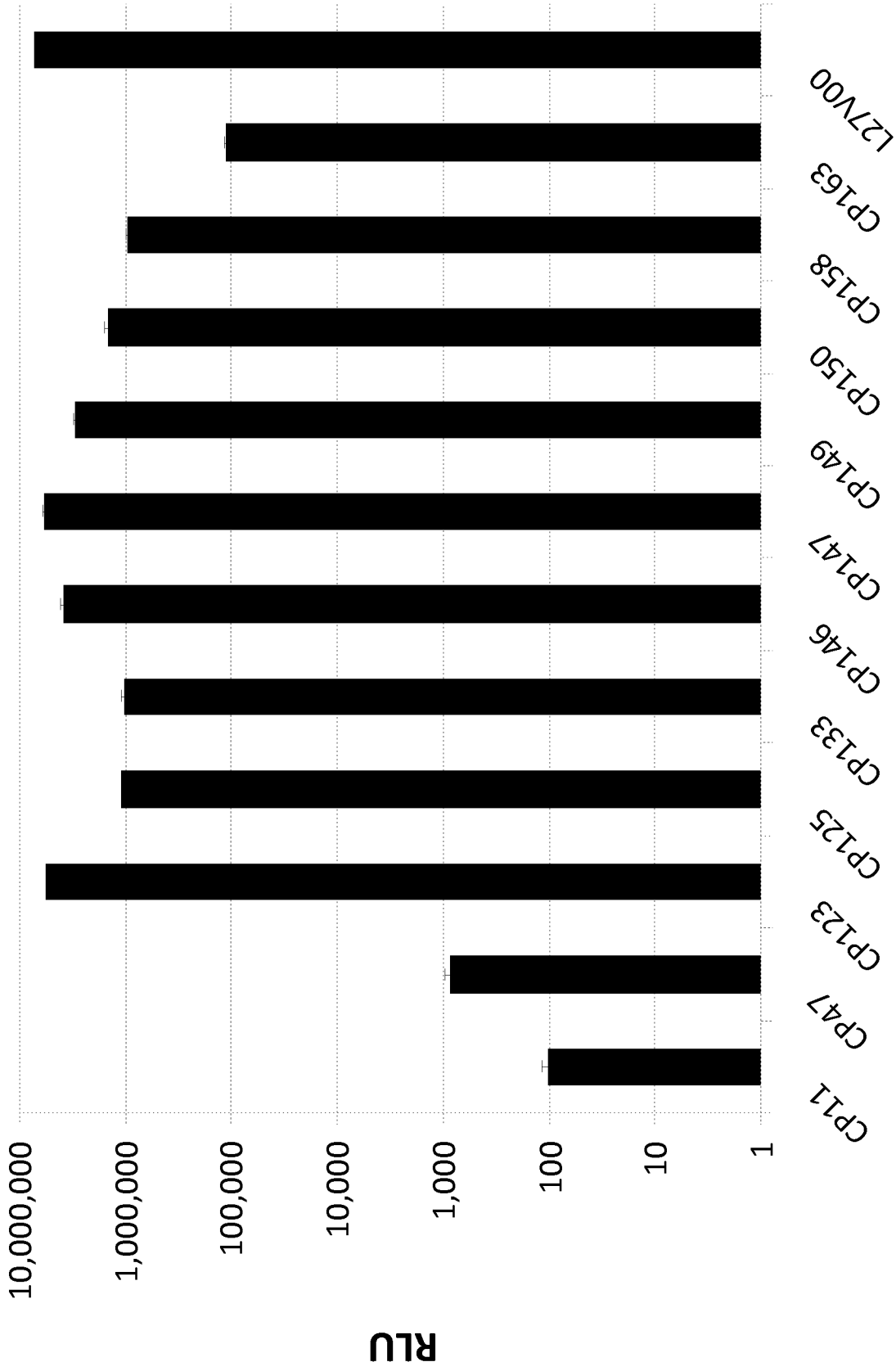

RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
CP11
CP47
CP123
CP125
CP133
CP146
CP147
CP149
CP150
CP158
CP163
L27V00

FIG. 73C

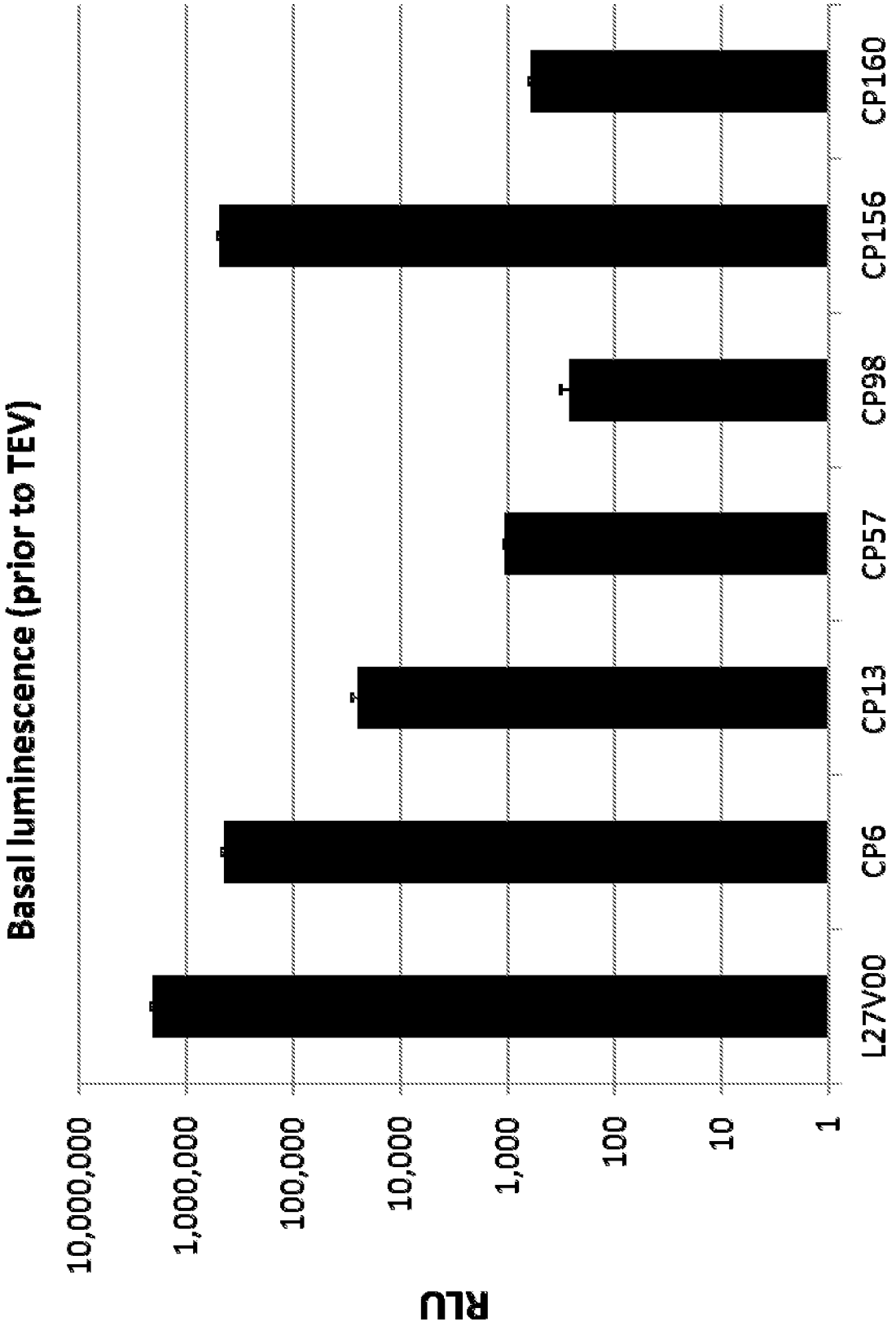

Basal luminescence (prior to TEV)
RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
L27V00
CP6
CP13
CP57
CP98
CP156
CP160

FIG. 73D

CP site ("CP#")	SEQ ID NOs:	response to TEV	CP site ("CP#")	SEQ ID NOs:	response to TEV	CP site ("CP#")	SEQ ID NOs:	response to TEV	CP site ("CP#")	SEQ ID NOs:	response to TEV
5	114 and 115	1.3	53	160 and 161	2100	95	204 and 205	512.9	136	246 and 247	/
6	116 and 117	7.0	54	162 and 163	12.3	97	206 and 207	9.1	139	248 and 249	1.9
7	118 and 119	/	55	164 and 165	7744.3	98	447 and 448	5.4	140	250 and 251	/
9	120 and 121	2.2	56	166 and 167	/	100	208 and 209	7515.2	141	252 and 253	/
11	122 and 123	/	57	445 and 446	17.6	101	210 and 211	6329.9	142	254 and 255	5.3
12	124 and 125	1.6	58	168 and 169	116.7	102	212 and 213	3205.1	143	256 and 257	871.4
15	126 and 127	/	64	170 and 171	1.4	103	214 and 215	3456.8	144	258 and 259	1.6
18	128 and 129	2.1	67	172 and 173	/	104	216 and 217	3036.9	145	260 and 261	3.7
21	130 and 131	/	70	174 and 175	/	105	218 and 219	4931.6	146	262 and 263	6.6
24	132 and 133	3.8	73	176 and 177	/	106	220 and 221	5.3	147	264 and 265	2.0
27	134 and 135	3.1	76	178 and 179	/	109	222 and 223	12.3	148	266 and 267	3.8
34	136 and 137	9.3	79	180 and 181	/	112	224 and 225	82.7	149	268 and 269	4.4
40	138 and 139	1.8	80	182 and 183	1.3	13	443 and 444	160.8	150	270 and 271	867.4
43	140 and 141	/	81	184 and 185	1.3	115	226 and 227	/	151	272 and 273	6.3
44	142 and 143	12.9	82	186 and 187	1.5	120	228 and 229	1661	154	274 and 275	/
45	144 and 145	1057.7	83	188 and 189	1.5	121	230 and 231	1.5	156	276 and 277	942.1
46	146 and 147	1277.9	84	190 and 191	1.2	123	232 and 233	323.6	157	278 and 279	1467.9
47	148 and 149	1.5	85	192 and 193	1.4	124	234 and 235	1548.2	158	280 and 281	1526.5
48	150 and 151	6.7	86	194 and 195	1.2	125	236 and 237	1141.8	160	282 and 283	12.4
49	152 and 153	12.5	87	196 and 197	1.5	129	238 and 239	40.2	163	284 and 285	/
50	154 and 155	2.2	88	198 and 199	1.8	130	240 and 241	2,043.0	166	286 and 287	/
51	156 and 157	2.8	91	200 and 201	324.2	131	242 and 243	612.5			
52	158 and 159	15.9	94	202 and 203	5.5	133	244 and 245	1.5			

FIG. 73E

G

CP#	Response
12	36.8
24	1.4
27	1.6
49	5.4
52	5.2
84	1.2
86	1.2
103	7.4
124	4.0
130	3.4
145	1.3
148	1.3
157	9.4

F

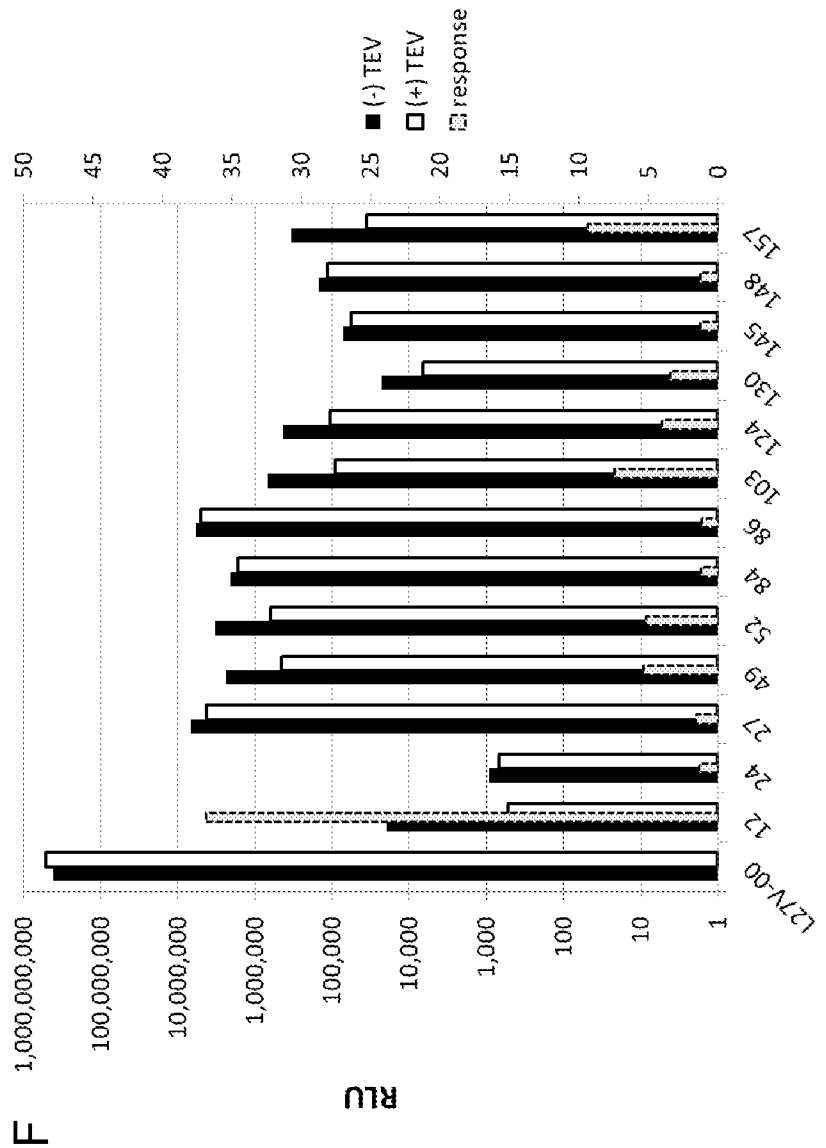


FIG. 73

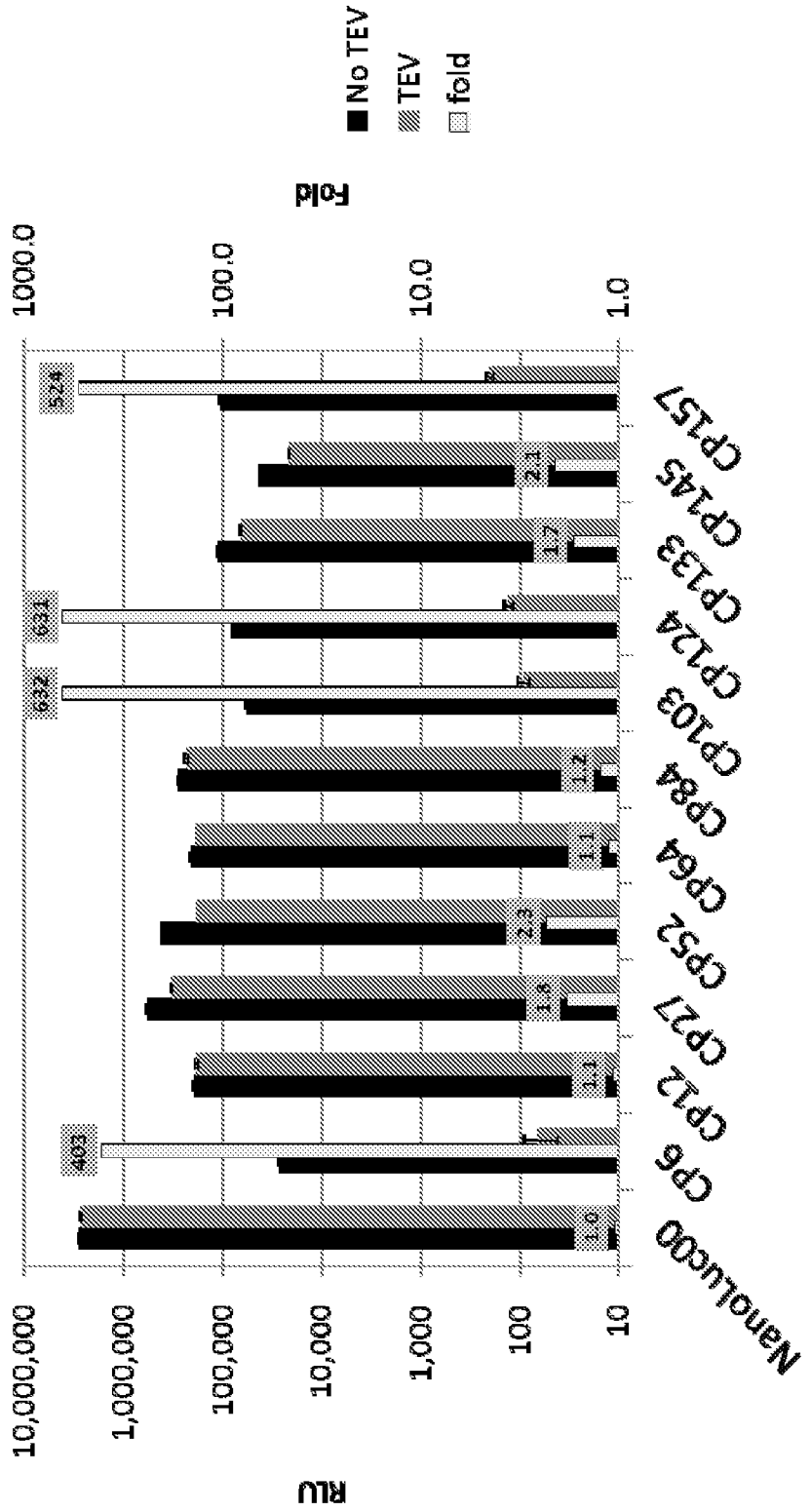

No TEV
TEV
fold
Fold
1000.0
100.0
10.0
1.0
RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
10
NanoLuc00
CP6
CP12
CP27
CP52
CP64
CP84
CP103
CP124
CP133
CP145
CP157
524
403
632
631
1.0
1.1
1.6
2.3
1.1
1.2
2.1
1.7

FIG. 73H

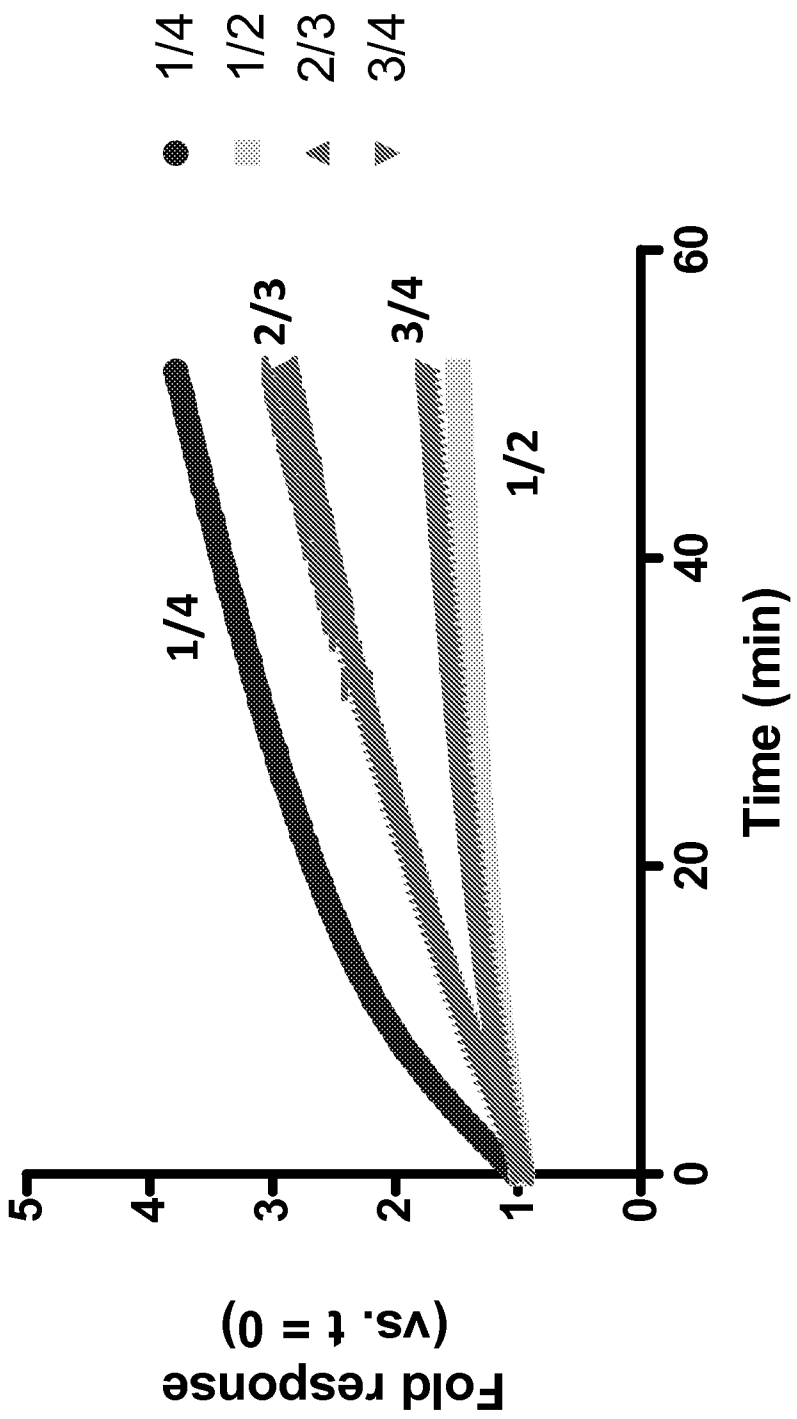

1/4
1/2
2/3
3/4
Fold response
(vs. t = 0)
Time (min)
0
20
40
60
1
2
3
4
5
1/4
2/3
3/4
1/2

FIG. 74

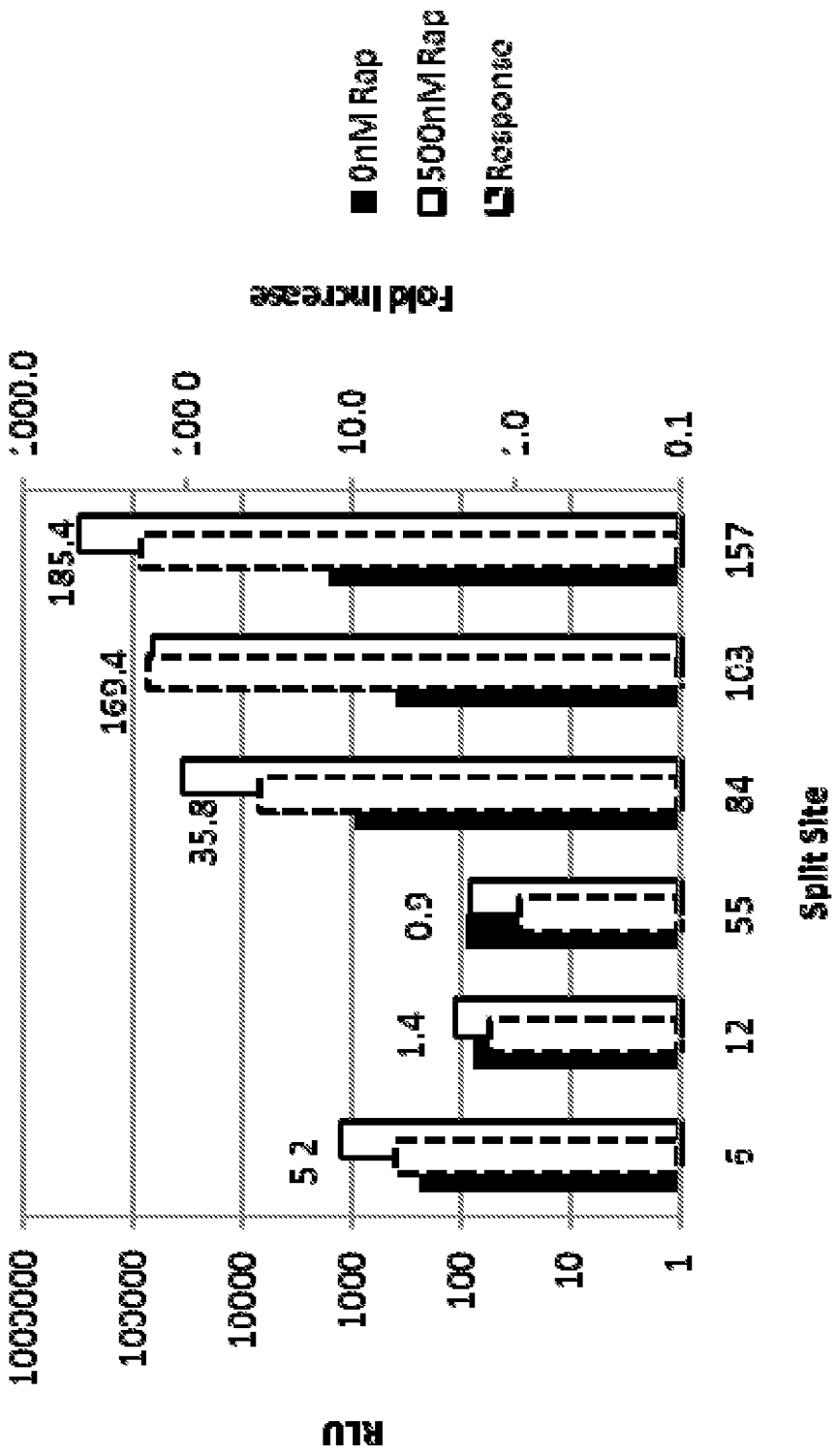

0nM Rap
500nM Rap
Response
Fold Increase
1000.0
100.0
10.0
2.0
0.1
185.4
169.4
35.8
0.9
1.4
5.2
157
103
84
55
12
5
Split Site
RLU
1000000
100000
10000
1000
100
10
1

FIG. 75A

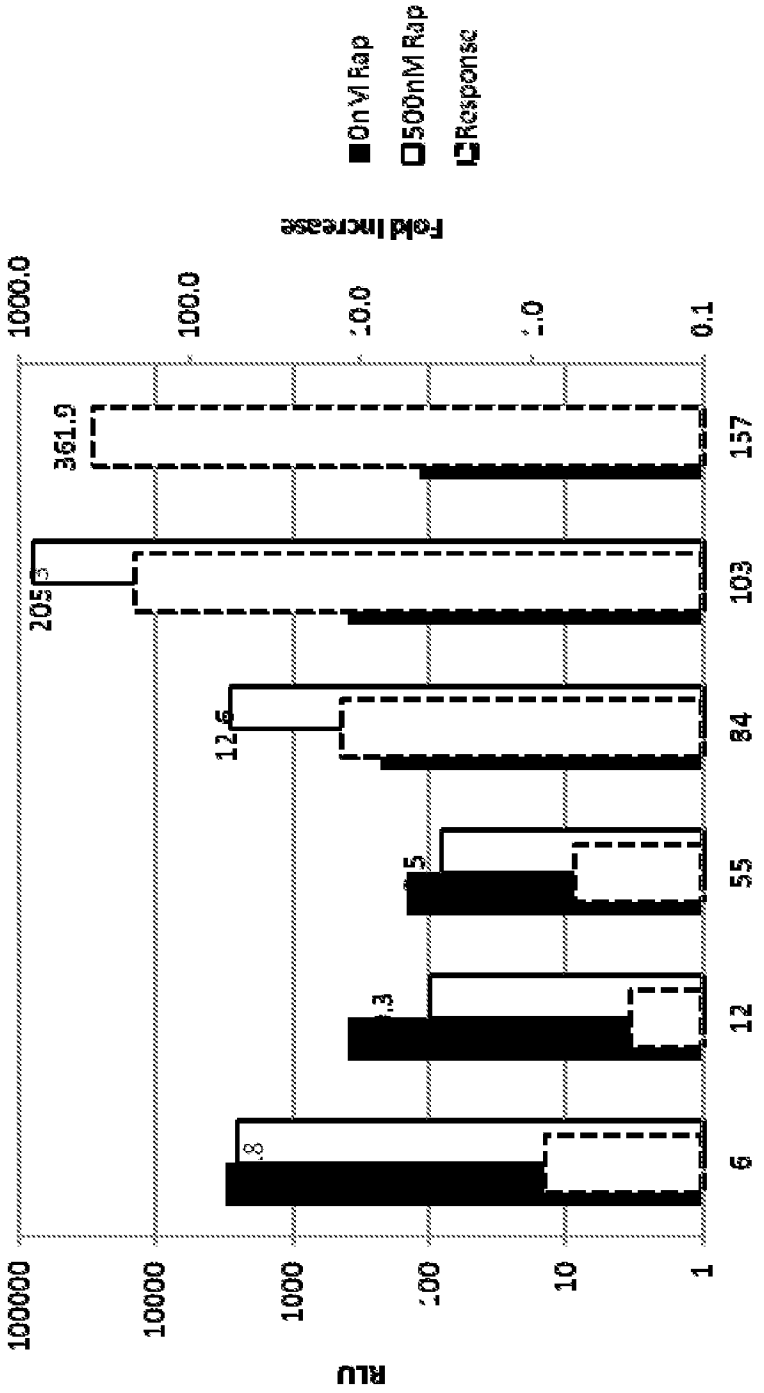

0nM Rap
500nM Rap
Response
Fold Increase
1000.0
100.0
10.0
1.0
0.1
361.9
157
103
84
55
12
6
Split Site
RLU
100000
10000
1000
100
10
1

FIG. 75B

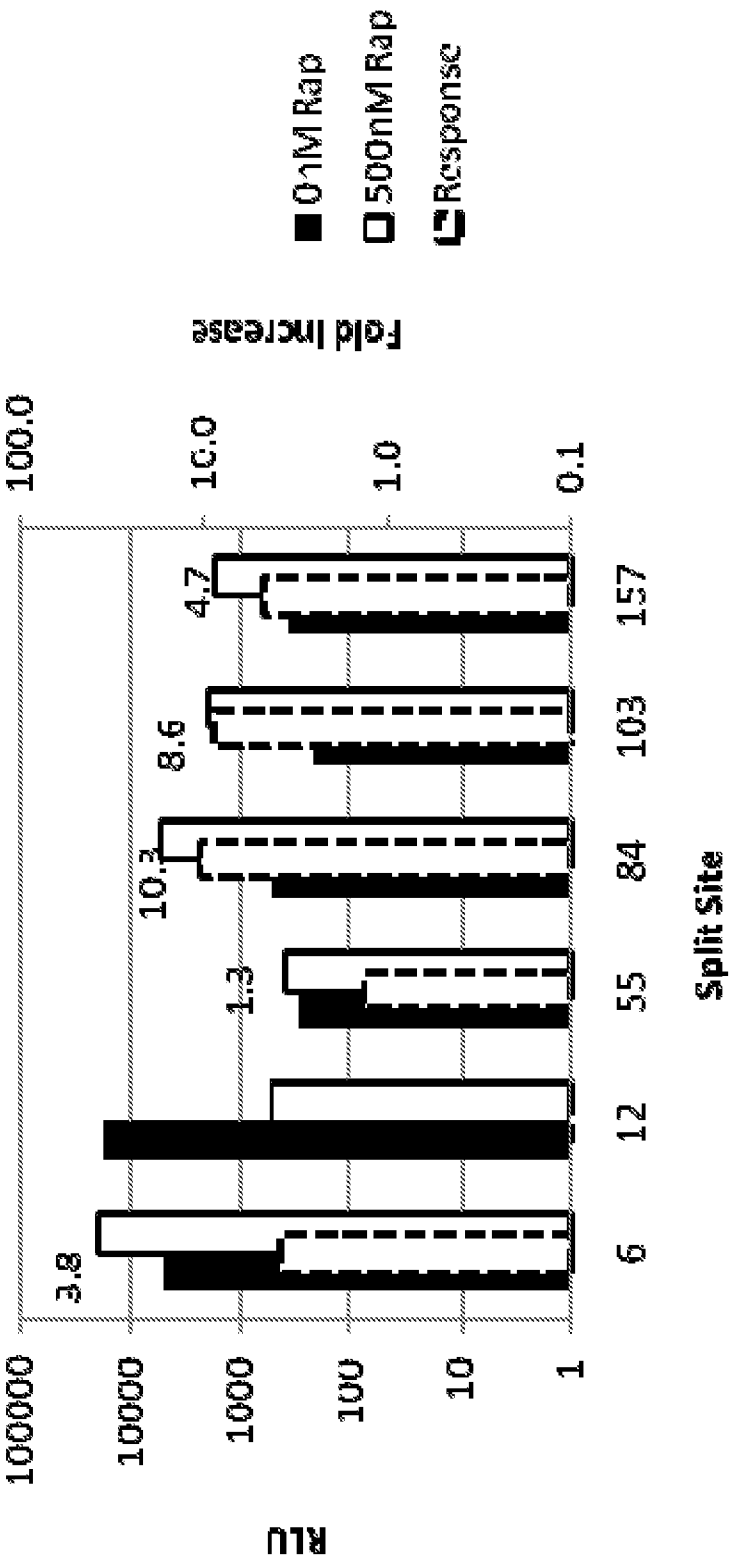

0nM Rap
500nM Rap
Response
Fold Increase
100.0
10.0
1.0
0.1
100000
10000
1000
100
10
1
RLU
3.8
1.3
10.3
8.6
4.7
6
12
55
84
103
157
Split Site

FIG. 75C

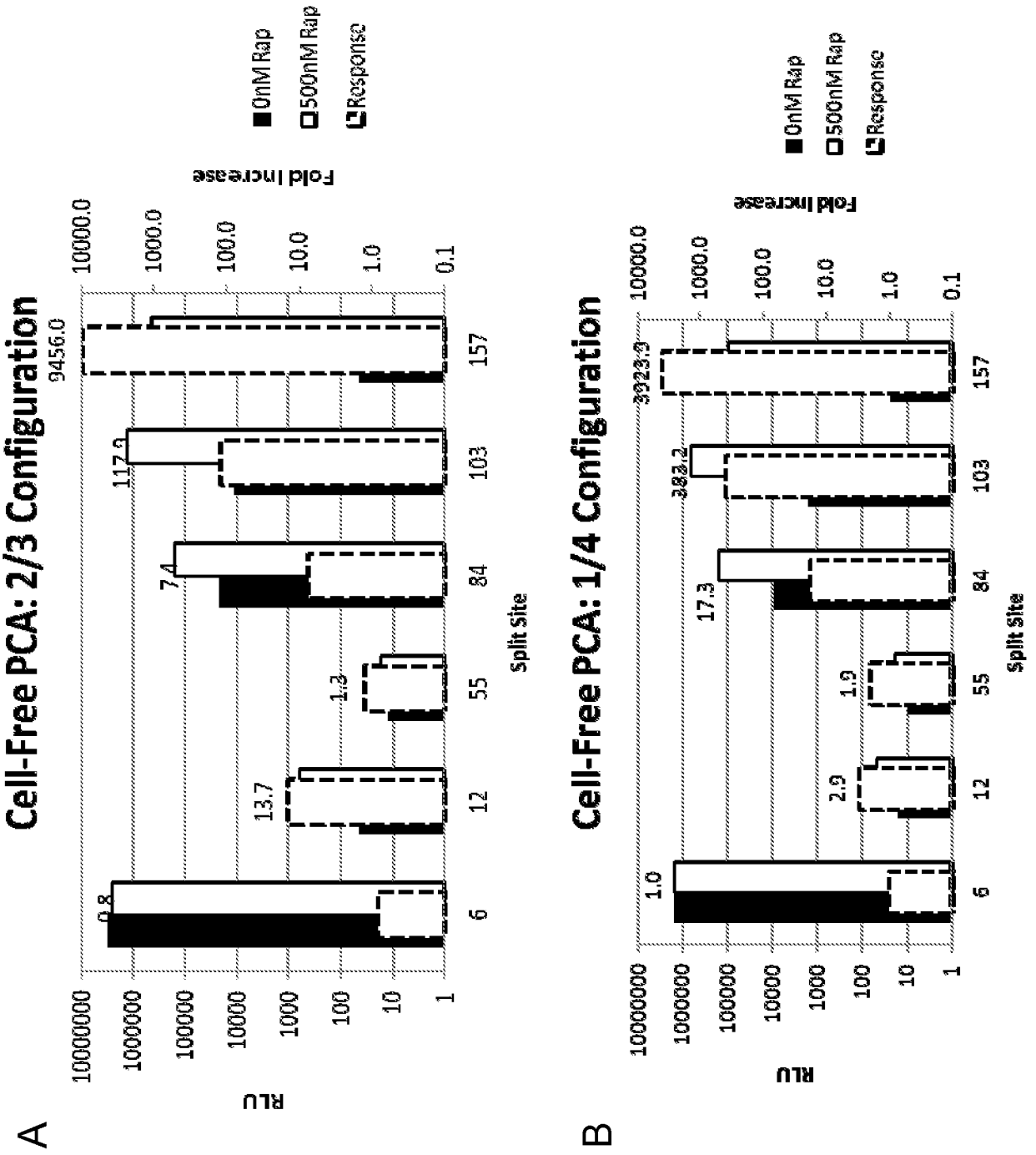

A
Cell-Free PCA: 2/3 Configuration
0nM Rap
500nM Rap
Response
Fold Increase
RLU
Split Site
9456.0
117.9
7.4
13.7
1.3
0.8
B
Cell-Free PCA: 1/4 Configuration
0nM Rap
500nM Rap
Response
Fold Increase
RLU
Split Site
3923.9
383.2
17.3
2.9
1.9
1.0

FIG. 76

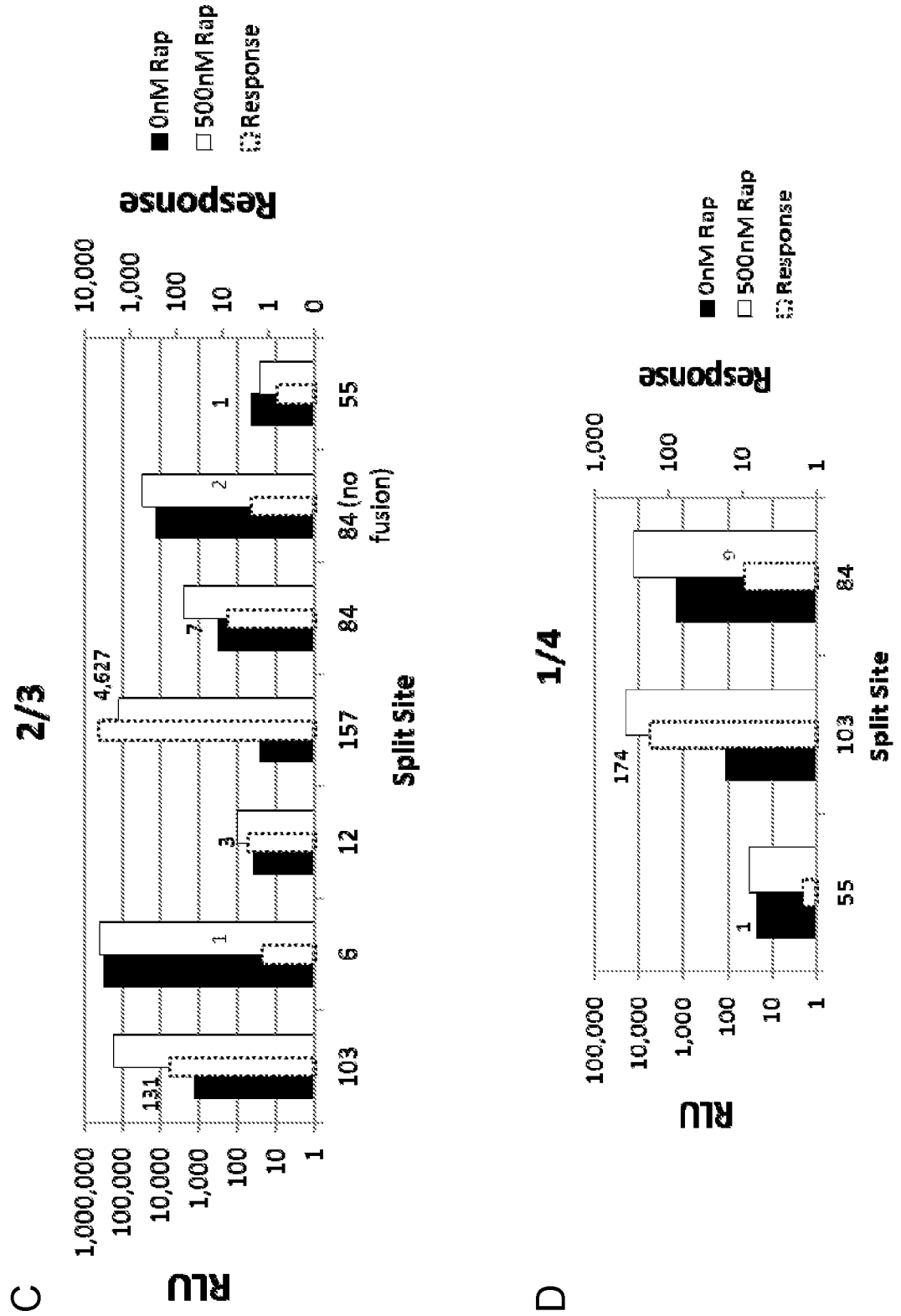

C
2/3
RLU
1,000,000
100,000
10,000
1,000
100
10
1
Response
10,000
1,000
100
10
1
0
0nM Rap
500nM Rap
Response
131
1
3
4,627
1
7
2
1
103
6
12
157
84
55
84 (no fusion)
Split Site
D
1/4
RLU
100,000
10,000
1,000
100
10
1
Response
1,000
100
10
1
0nM Rap
500nM Rap
Response
1
174
9
1
55
103
84
Split Site

FIG. 76

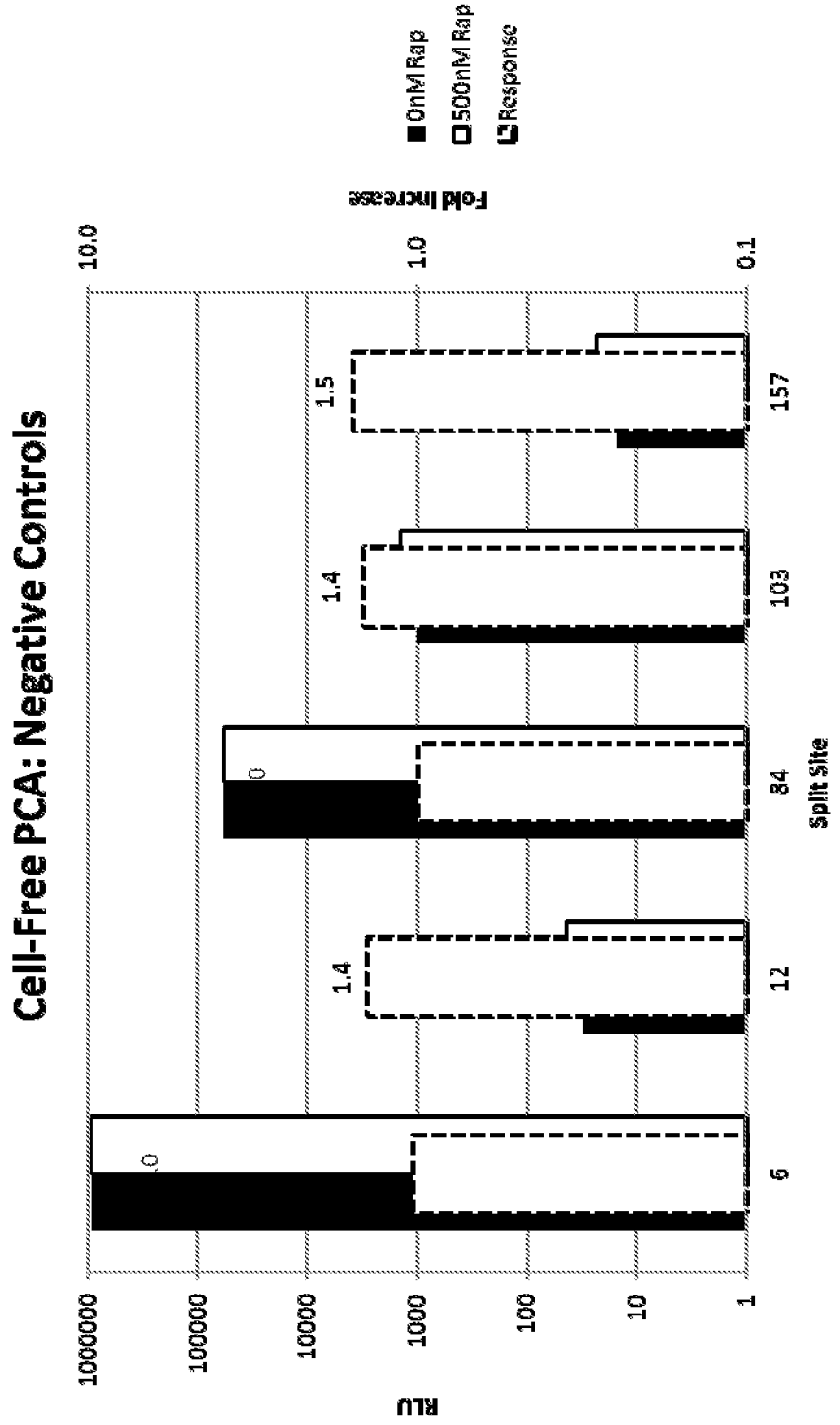

Cell-Free PCA: Negative Controls
0nM Rap
500nM Rap
Response
Fold Increase
10.0
1.0
0.1
RLU
1000000
100000
10000
1000
100
10
1
0
1.4
1.4
1.5
6
12
84
103
157
Split Site

FIG. 76E

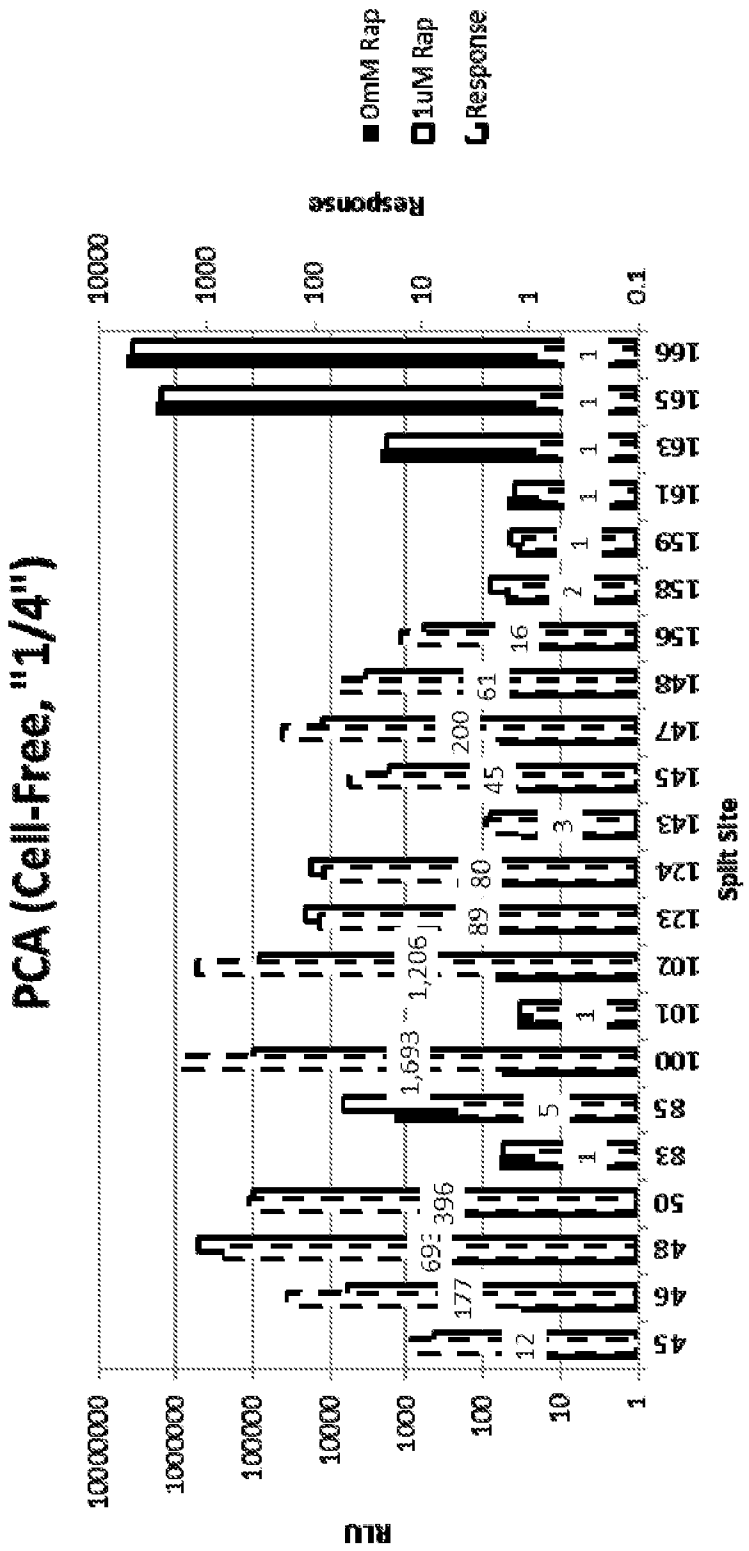

PCA (Cell-Free, "1/4")
0mM Rap
1uM Rap
Response
RLU
Response
Split Site
45
46
48
50
83
85
100
101
102
123
124
143
145
147
148
156
158
159
161
163
165
166

FIG. 76F

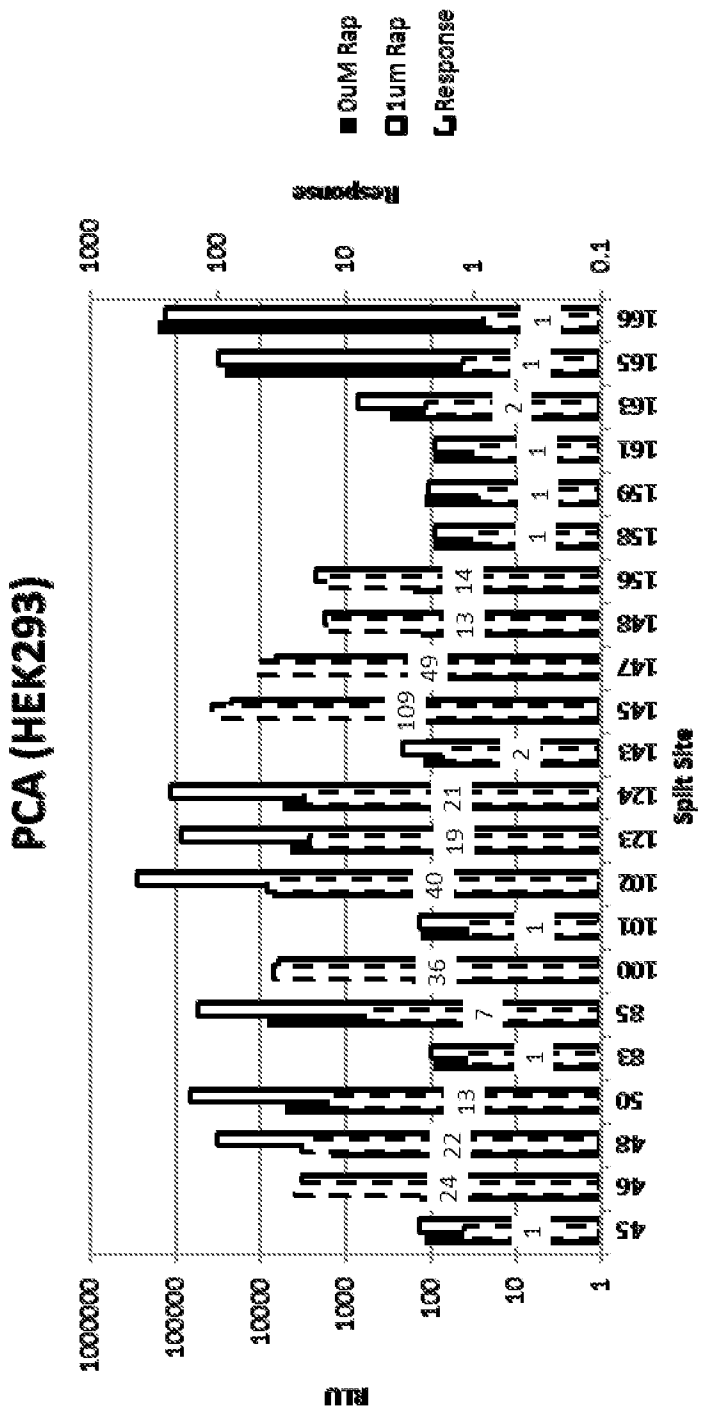


FIG. 76G

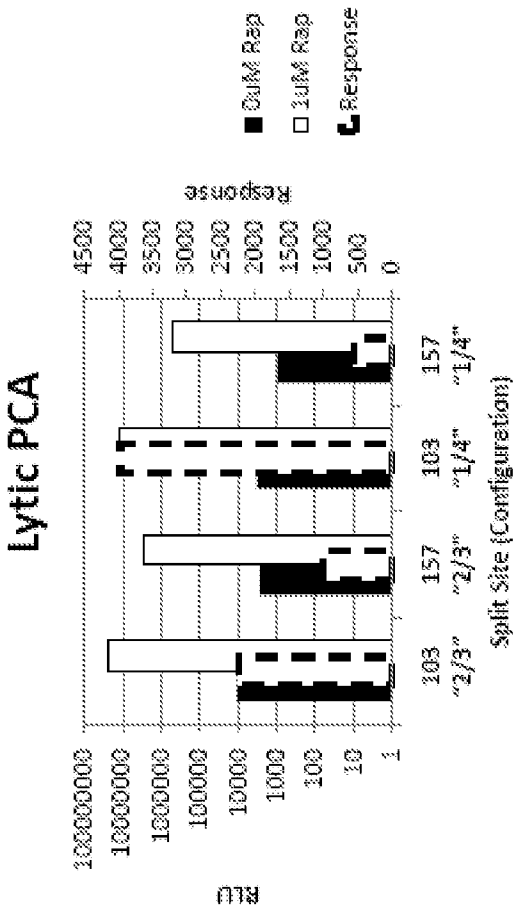

Lytic PCA
RLU
Response
Split Site (Configuration)
0.5uM Rap
1uM Rap
Response
"2/3"
"1/4"
103
157

FIG. 76H

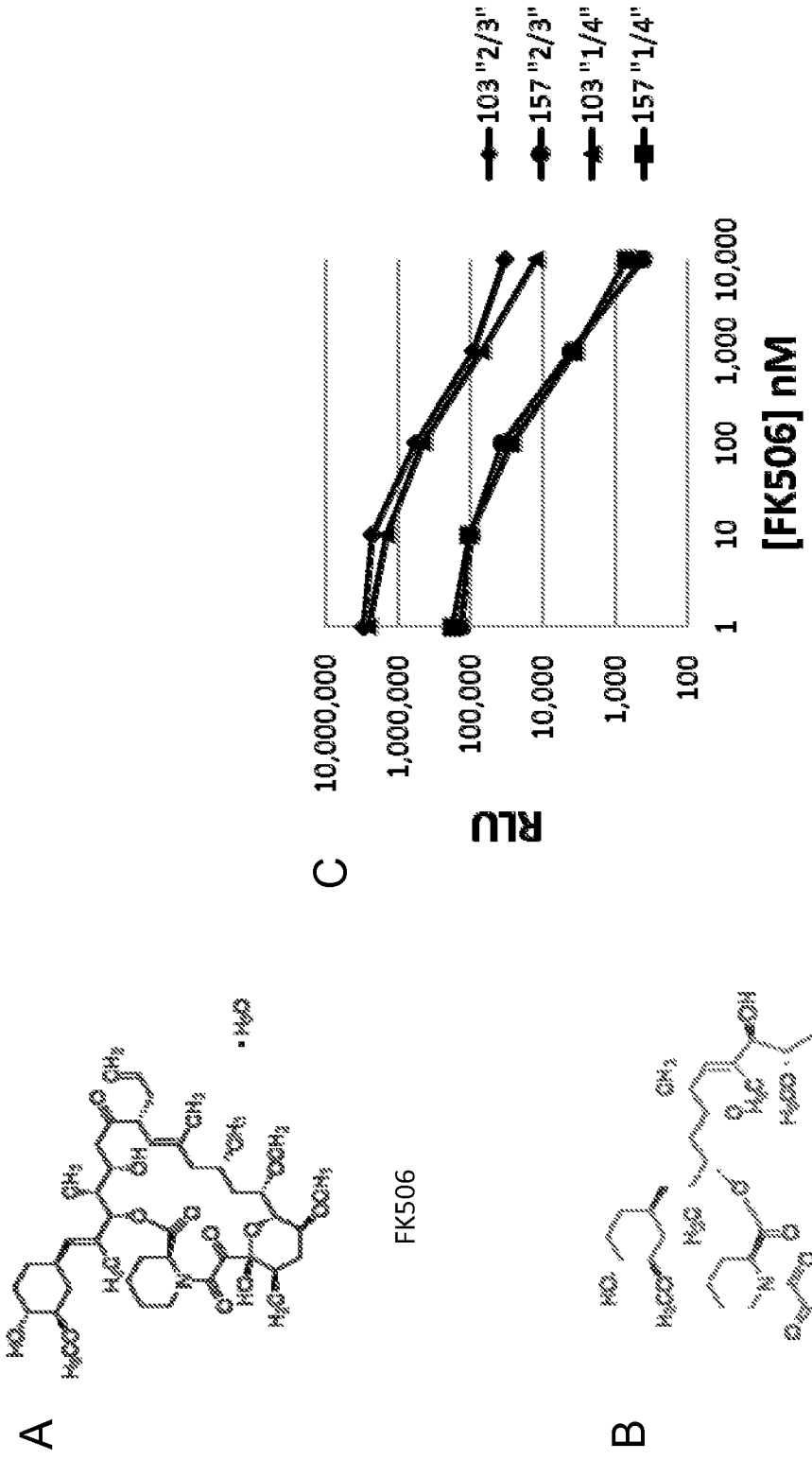

A
FK506
B
Rapamycin
C
RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
1
10
100
1,000
10,000
[FK506] nM
103 "2/3"
157 "2/3"
103 "1/4"
157 "1/4"

FIG. 77

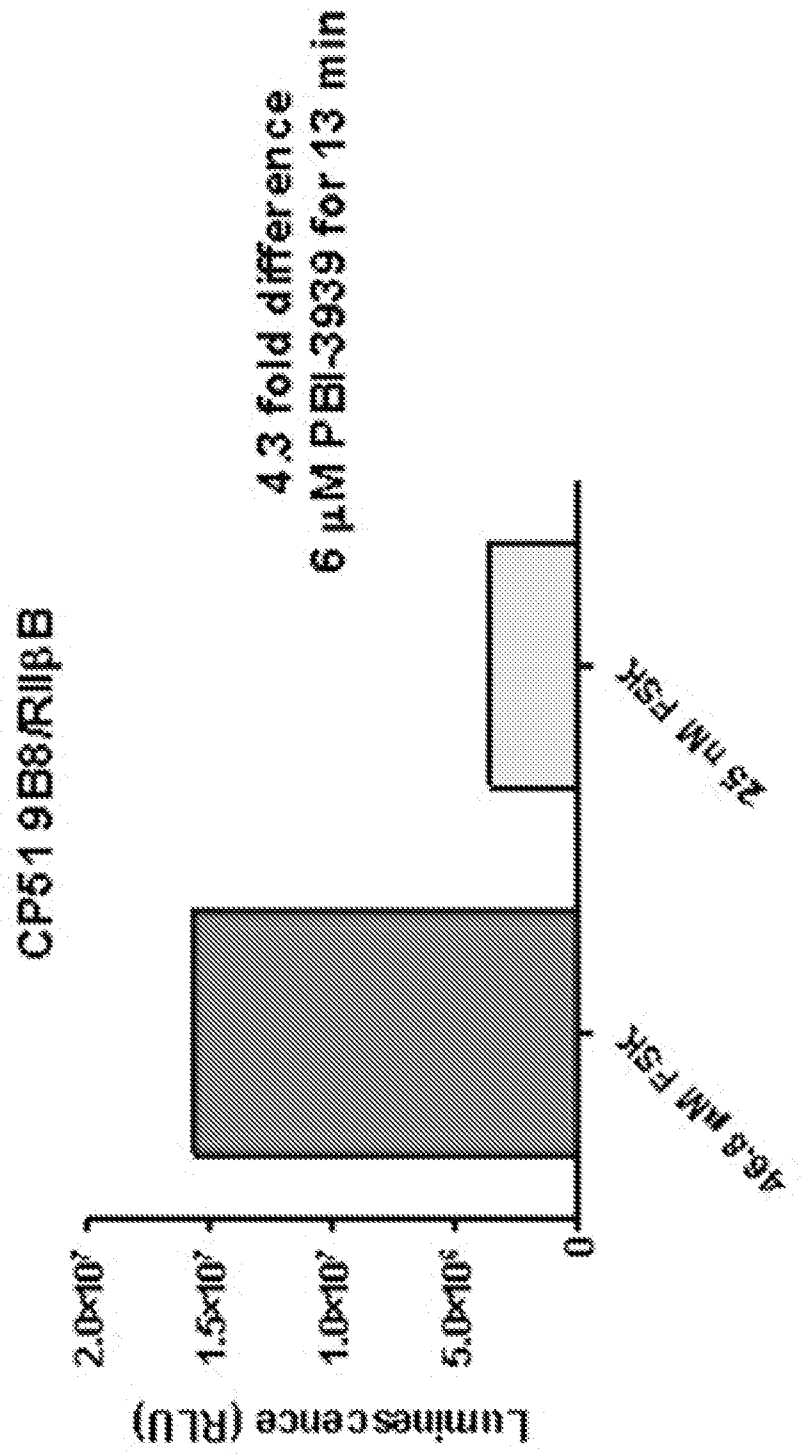

4.3 fold difference
6 µM PBI-3939 for 13 min
CP519B8/RIIβB
Luminescence (RLU)
2.0x10⁷
1.5x10⁷
1.0x10⁷
5.0x10⁶
0
46.8 nM FSK
25 nM FSK

FIG. 78

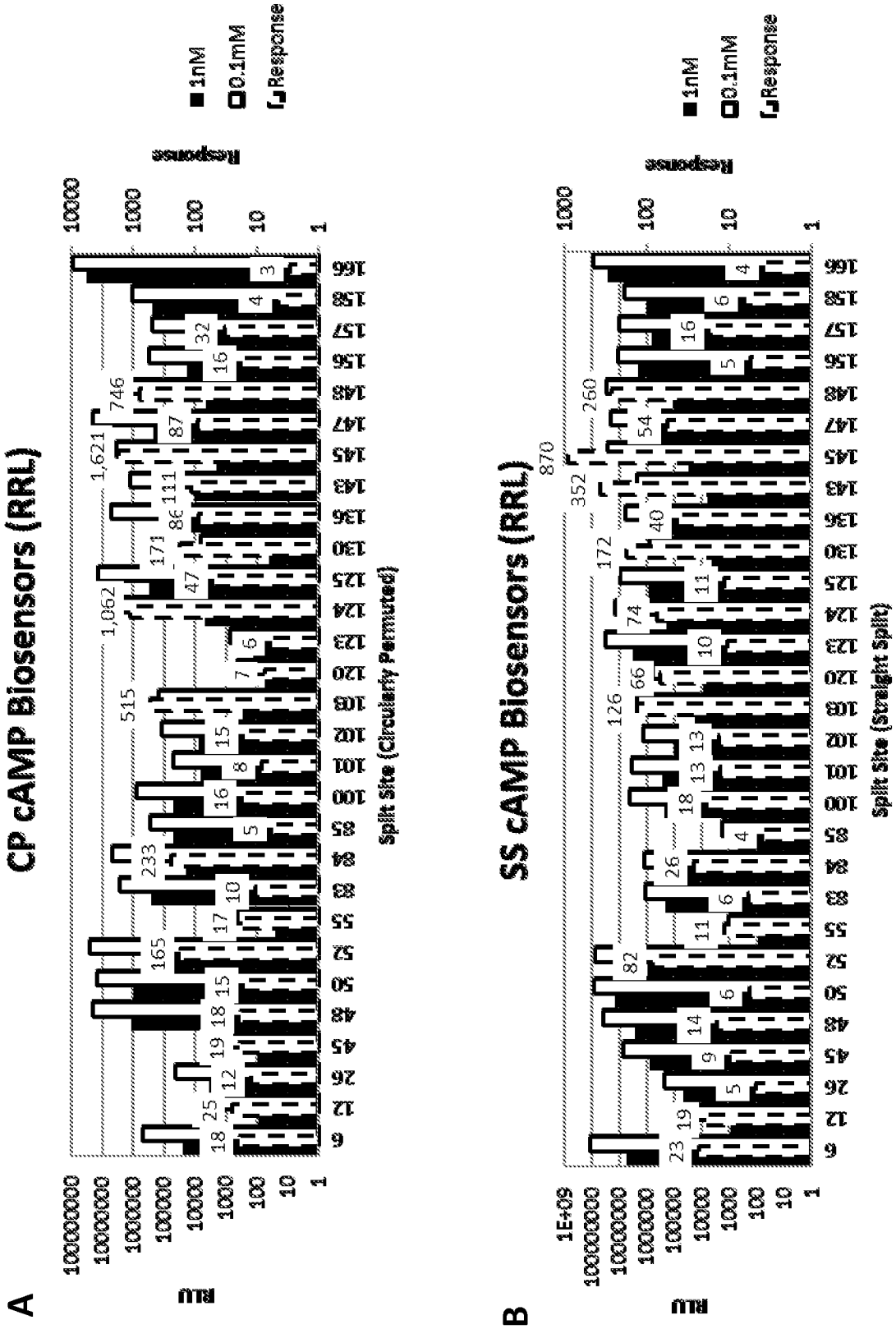

A
CP cAMP Biosensors (RRL)
RLU
Response
1nM
0.1mM
Response
Split Site (Circularly Permuted)
B
SS cAMP Biosensors (RRL)
RLU
Response
1nM
0.1mM
Response
Split Site (Straight Split)

FIG. 79

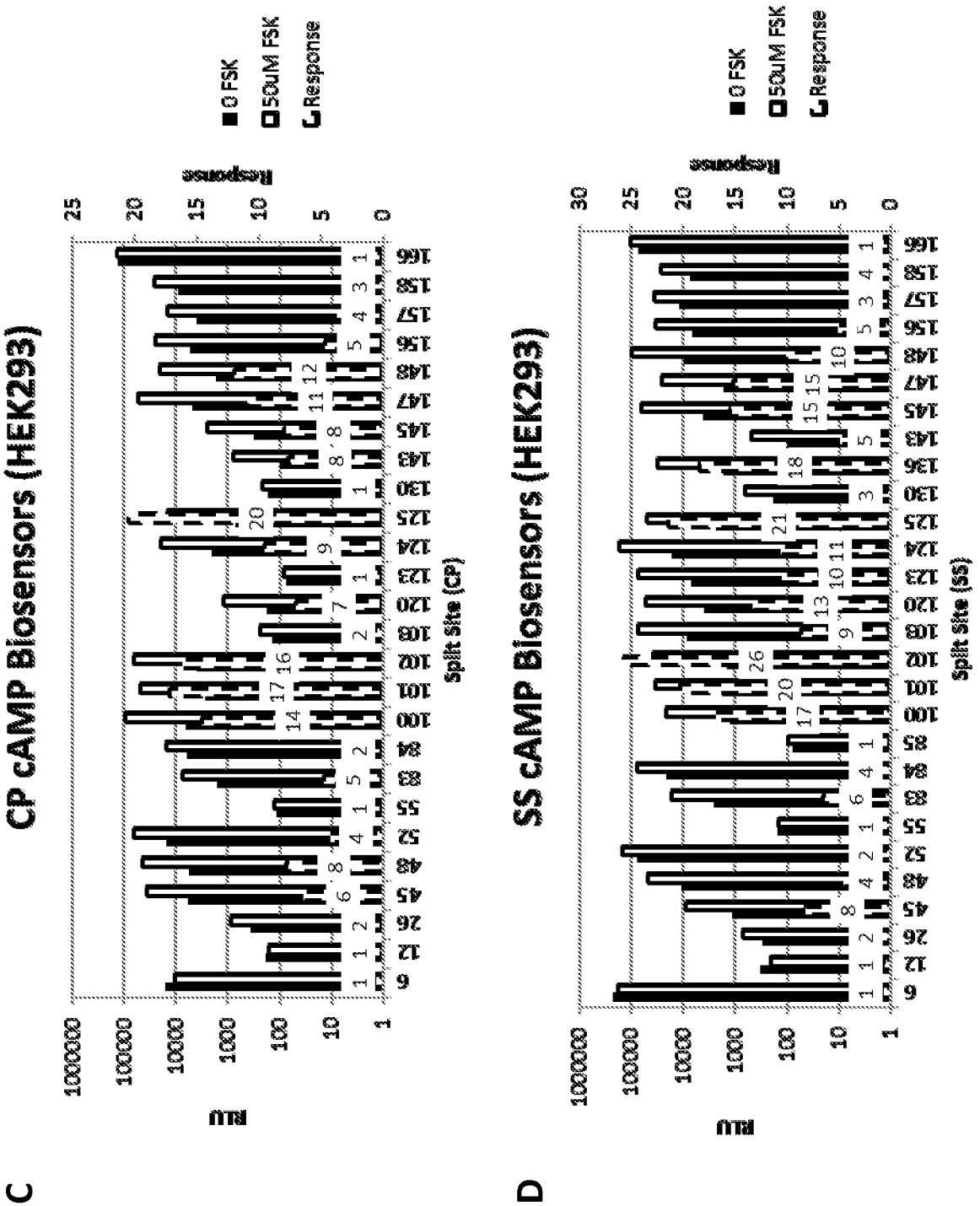

C
CP cAMP Biosensors (HEK293)
RLU
Response
0 FSK
50uM FSK
Response
split site (CP)
D
SS cAMP Biosensors (HEK293)
RLU
Response
0 FSK
50uM FSK
Response
split site (SS)

FIG. 79

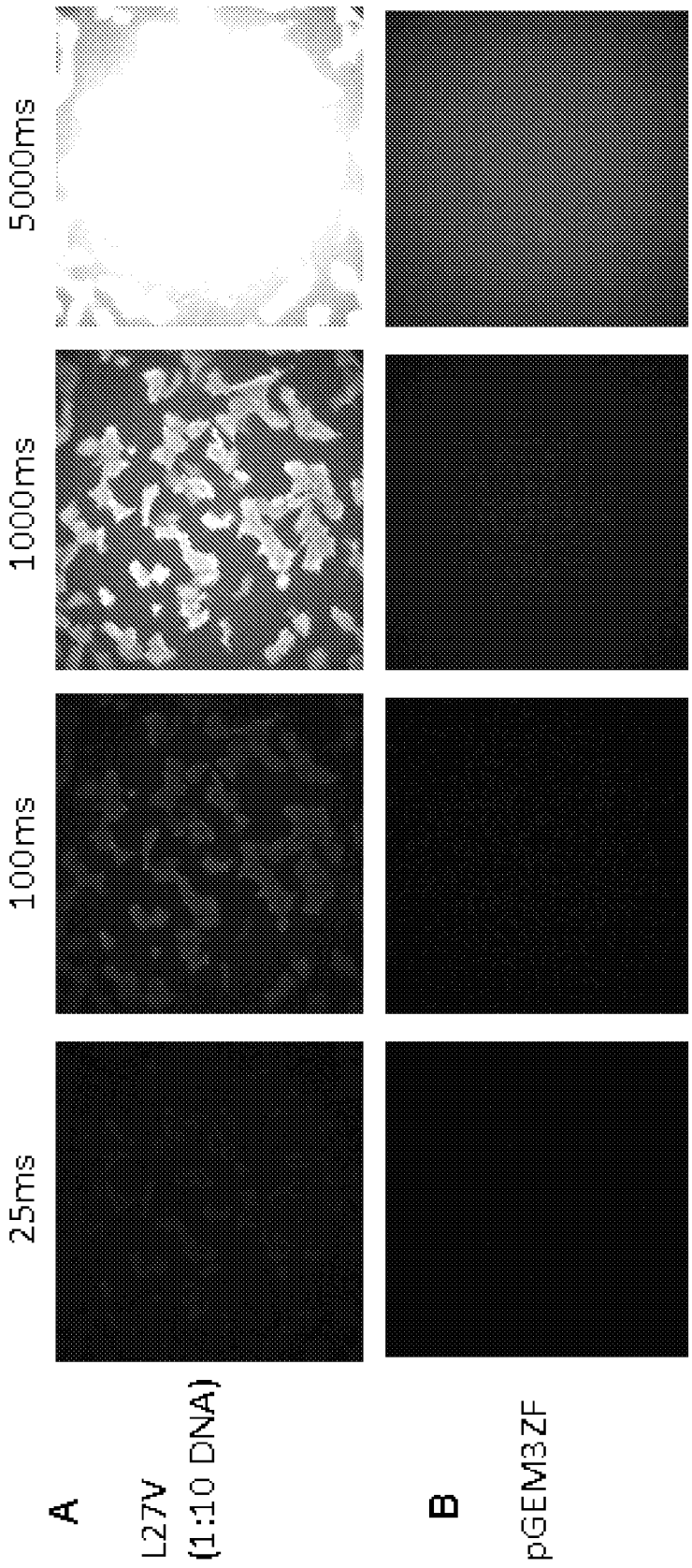


FIG. 80

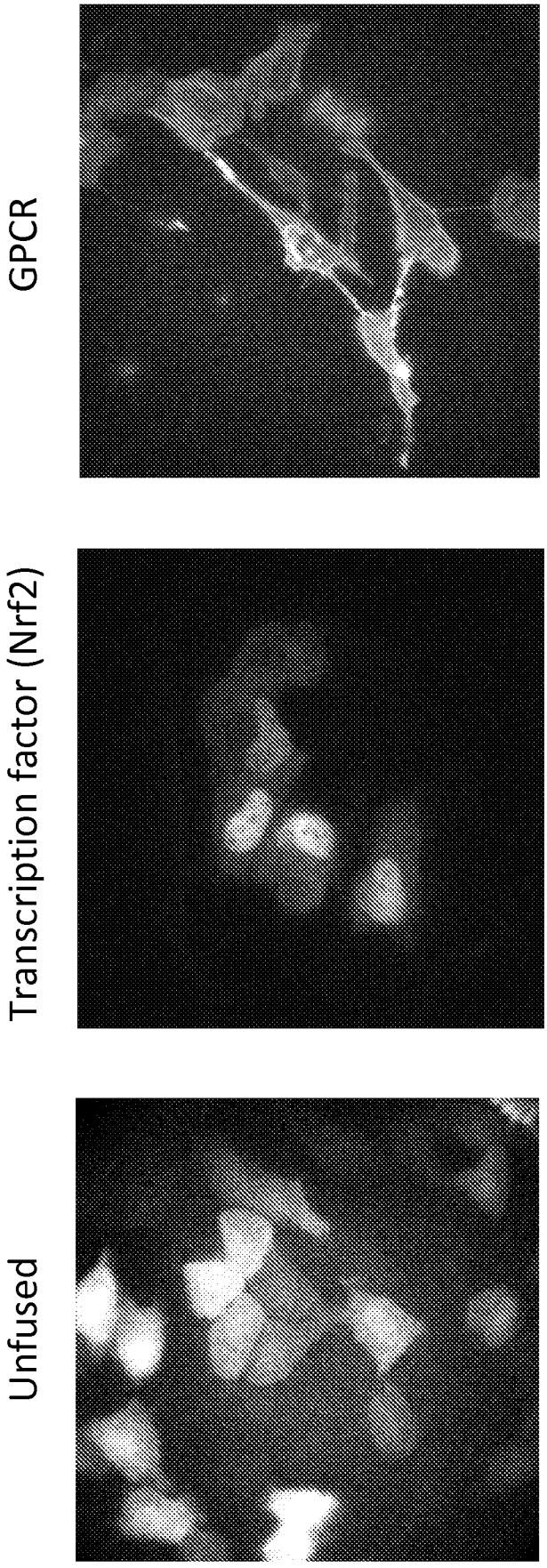


FIG. 81

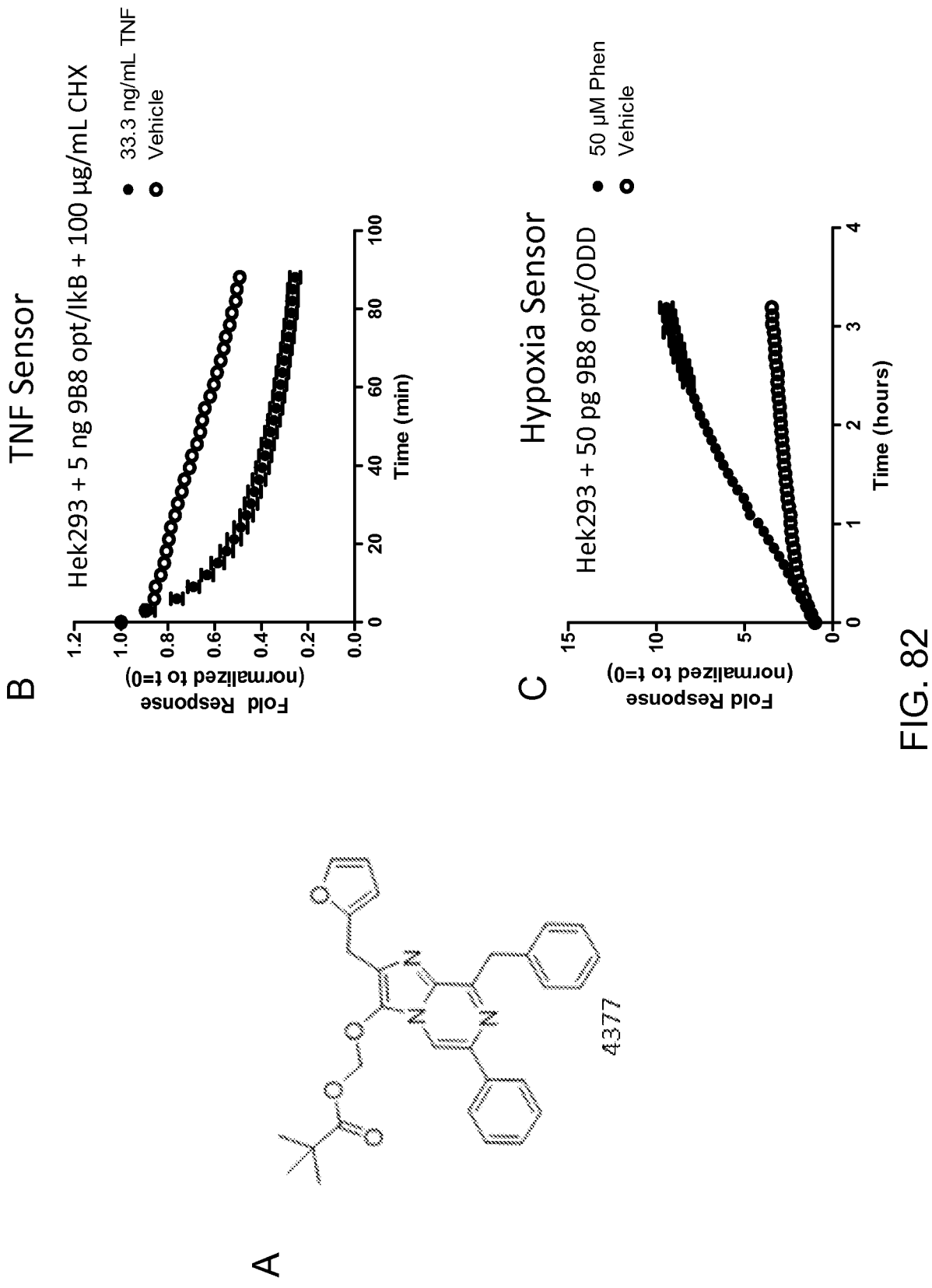

A
4377
B
TNF Sensor
Hek293 + 5 ng 9B8 opt/IkB + 100 µg/mL CHX
33.3 ng/mL TNF
Vehicle
Fold Response (normalized to t=0)
Time (min)
C
Hypoxia Sensor
Hek293 + 50 pg 9B8 opt/ODD
50 µM Phen
Vehicle
Fold Response (normalized to t=0)
Time (hours)

FIG. 82

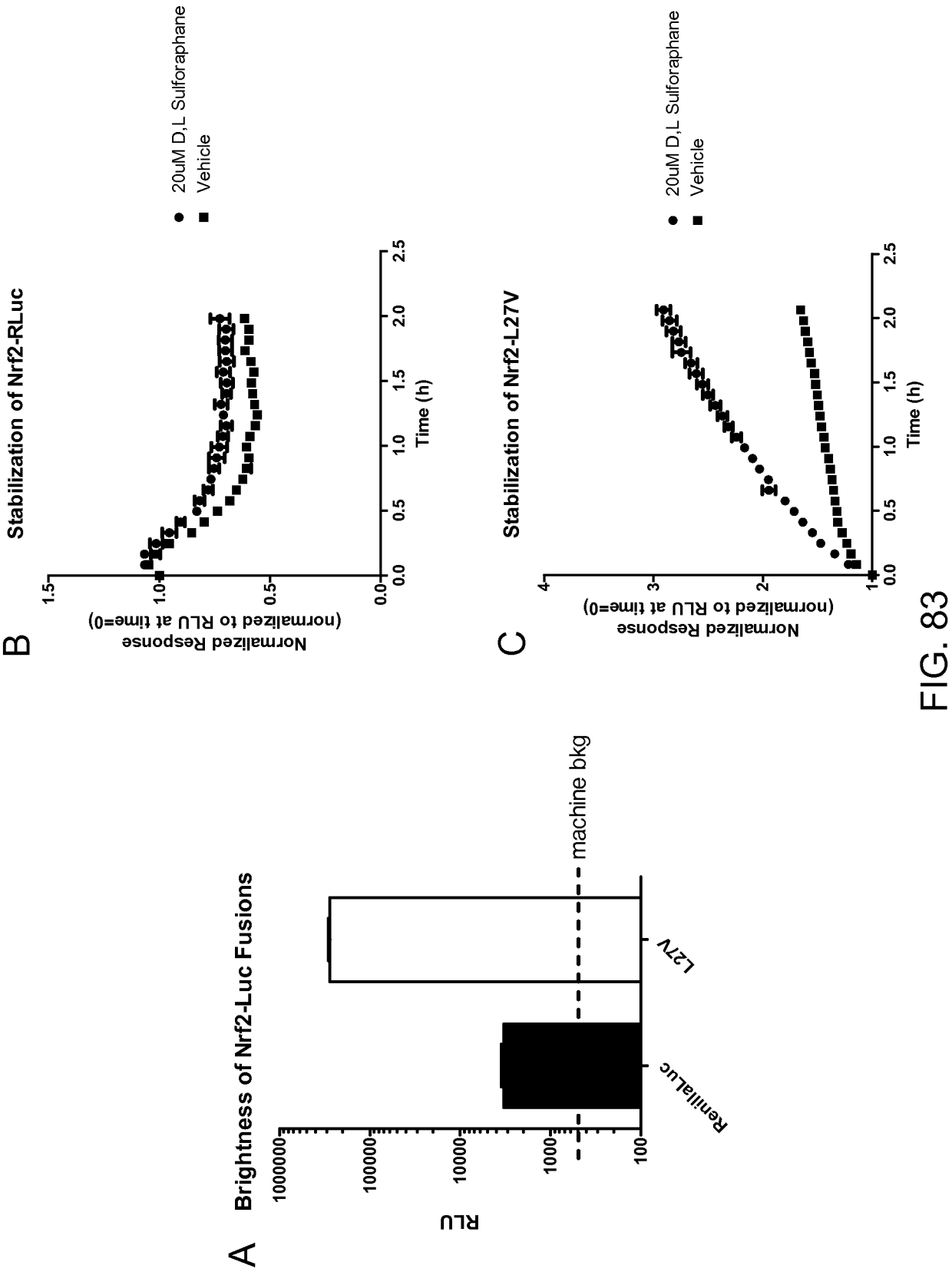

A
Brightness of Nrf2-Luc Fusions
RLU
1000000
100000
10000
1000
100
machine bkg
RenillaLuc
L27V
B
Stabilization of Nrf2-RLuc
20uM D,L Sulforaphane
Vehicle
Normalized Response
(normalized to RLU at time=0)
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5
Time (h)
C
Stabilization of Nrf2-L27V
20uM D,L Sulforaphane
Vehicle
Normalized Response
(normalized to RLU at time=0)
4
3
2
1
0.0
0.5
1.0
1.5
2.0
2.5
Time (h)

FIG. 83

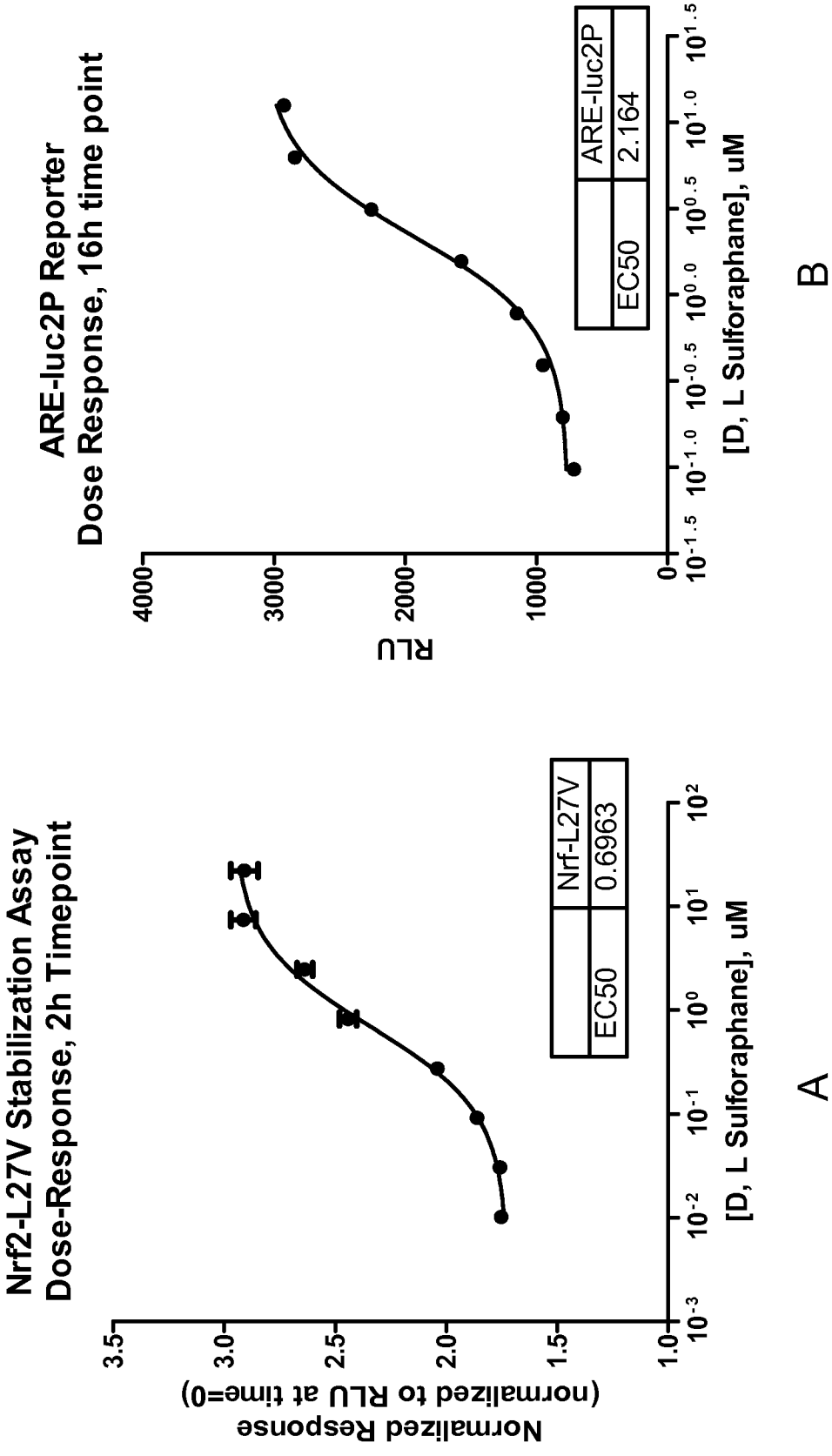
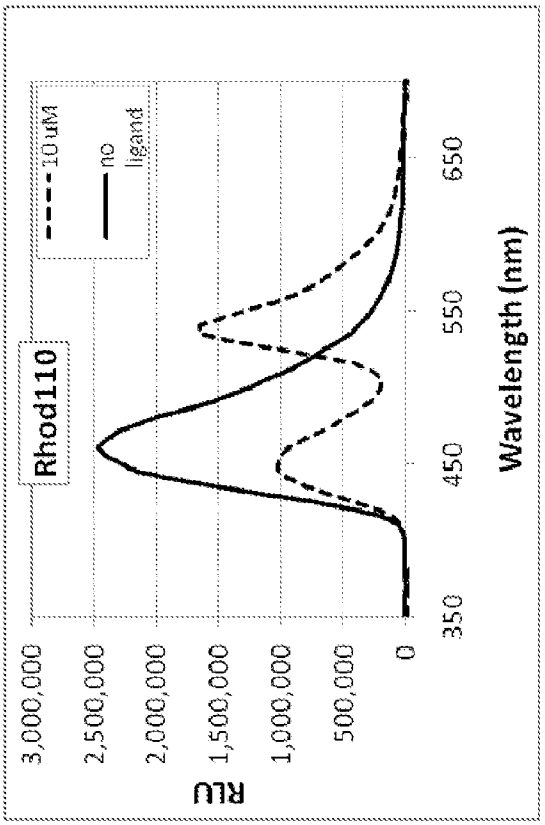
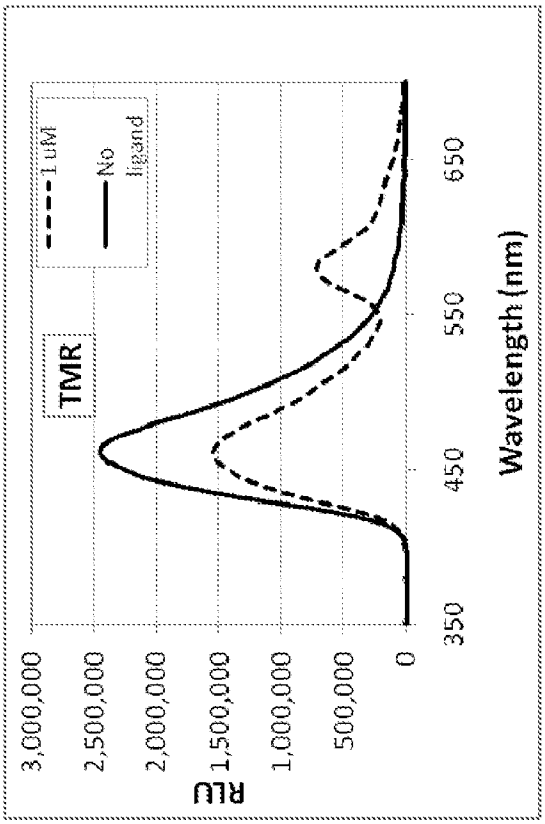

Nrf2-L27V Stabilization Assay
Dose-Response, 2h Timepoint
Normalized Response
(normalized to RLU at time=0)
[D, L Sulforaphane], uM
Nrf-L27V
EC50
0.6963
A
ARE-luc2P Reporter
Dose Response, 16h time point
RLU
[D, L Sulforaphane], uM
ARE-luc2P
EC50
2.164
B

FIG. 84


Rhod110
10 uM
no ligand
RLU
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
350
450
550
650
Wavelength (nm)

B


TMR
1 uM
No ligand
RLU
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
350
450
550
650
Wavelength (nm)

A

FIG. 85

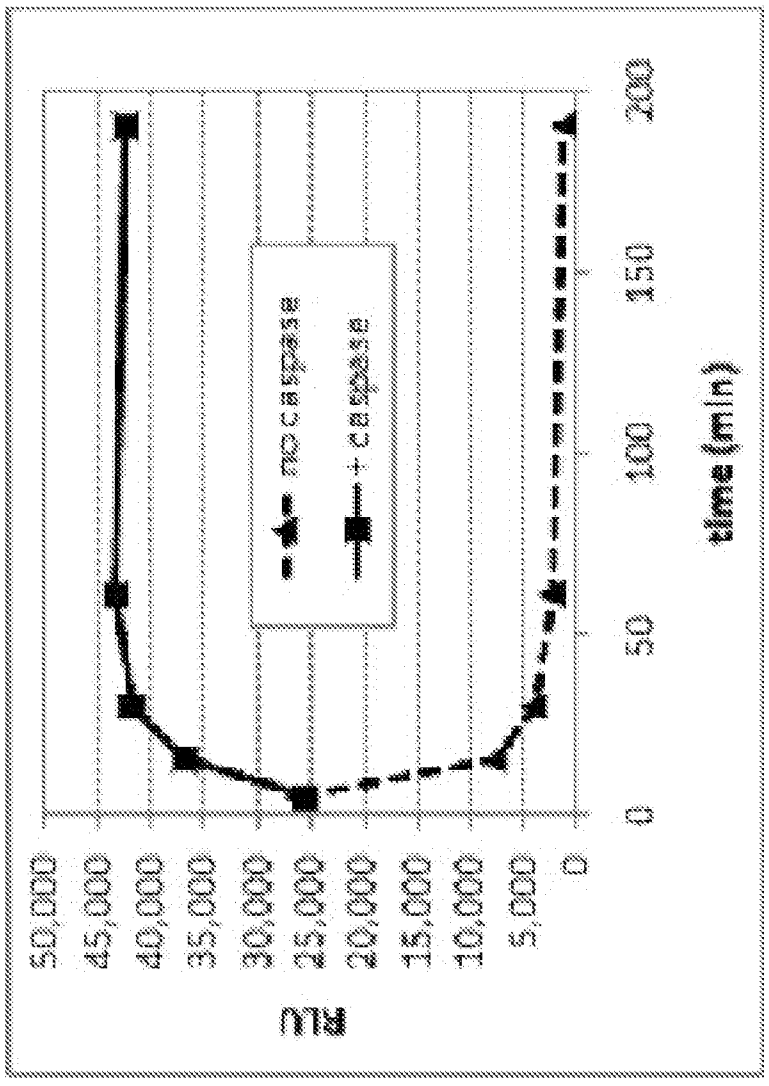

RLU
50,000
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
0
50
100
150
200
time (min)
- caspase
+ caspase

FIG. 86

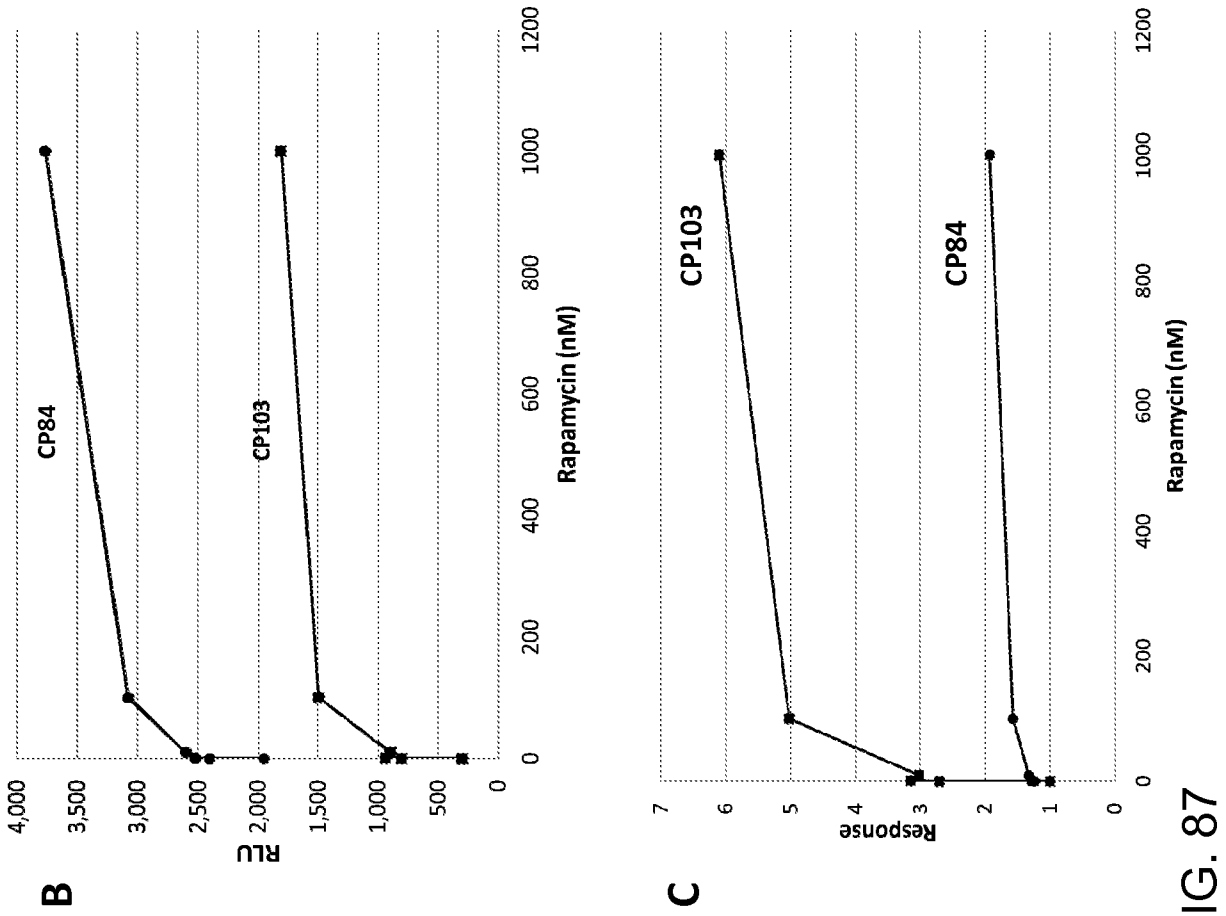
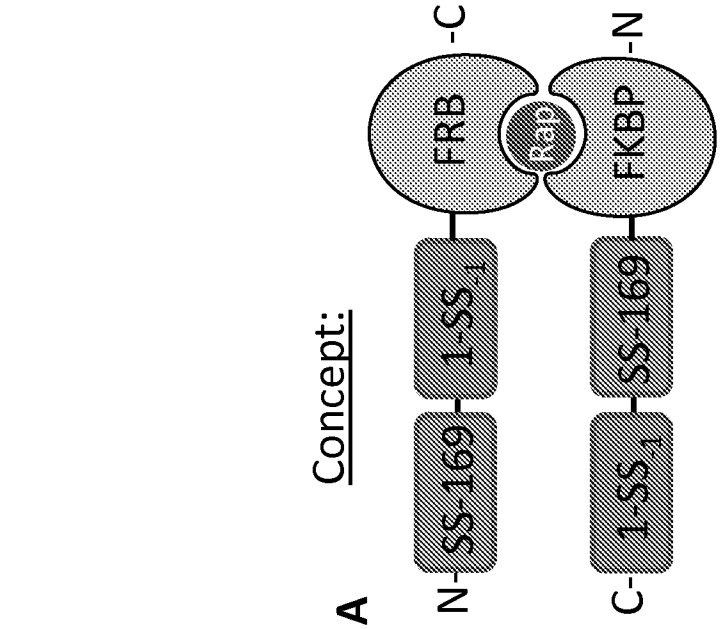

B
RLU
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0
CP84
CP103
0
200
400
600
800
1000
1200
Rapamycin (nM)
C
Response
7
6
5
4
3
2
1
0
CP103
CP84
0
200
400
600
800
1000
1200
Rapamycin (nM)

FIG. 87


A
Concept:
N-
SS-169
1-SS1
FRB
-C
Rap
FKBP
-N
C-
1-SS1
SS-169

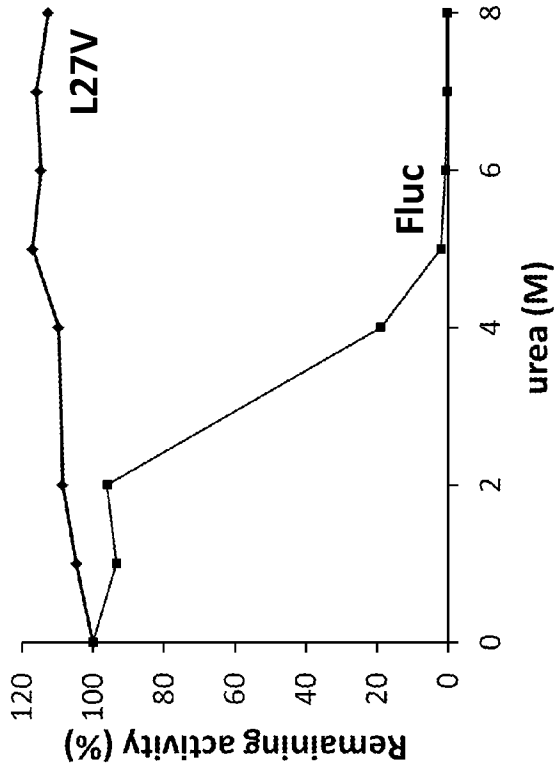

L27V
Fluc
Remaining activity (%)
120
100
80
60
40
20
0
0
2
4
6
8
urea (M)

FIG. 88

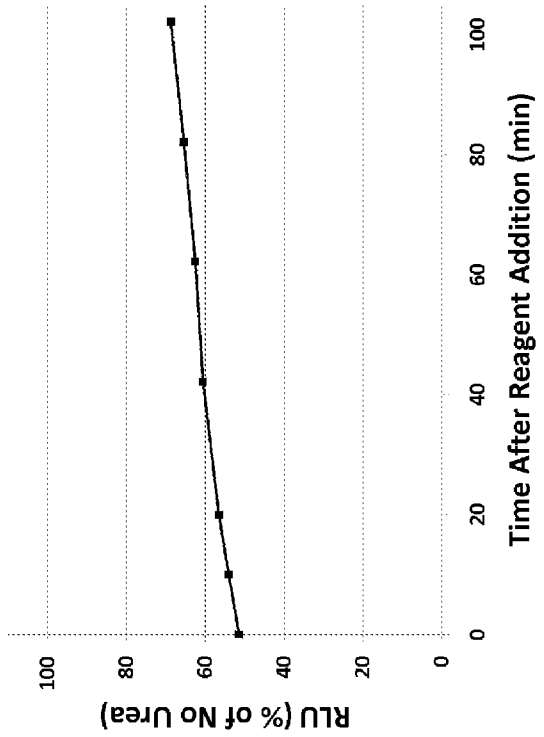

RLU (% of No Urea)
100
80
60
40
20
0
0
20
40
60
80
100
Time After Reagent Addition (min)

FIG. 89

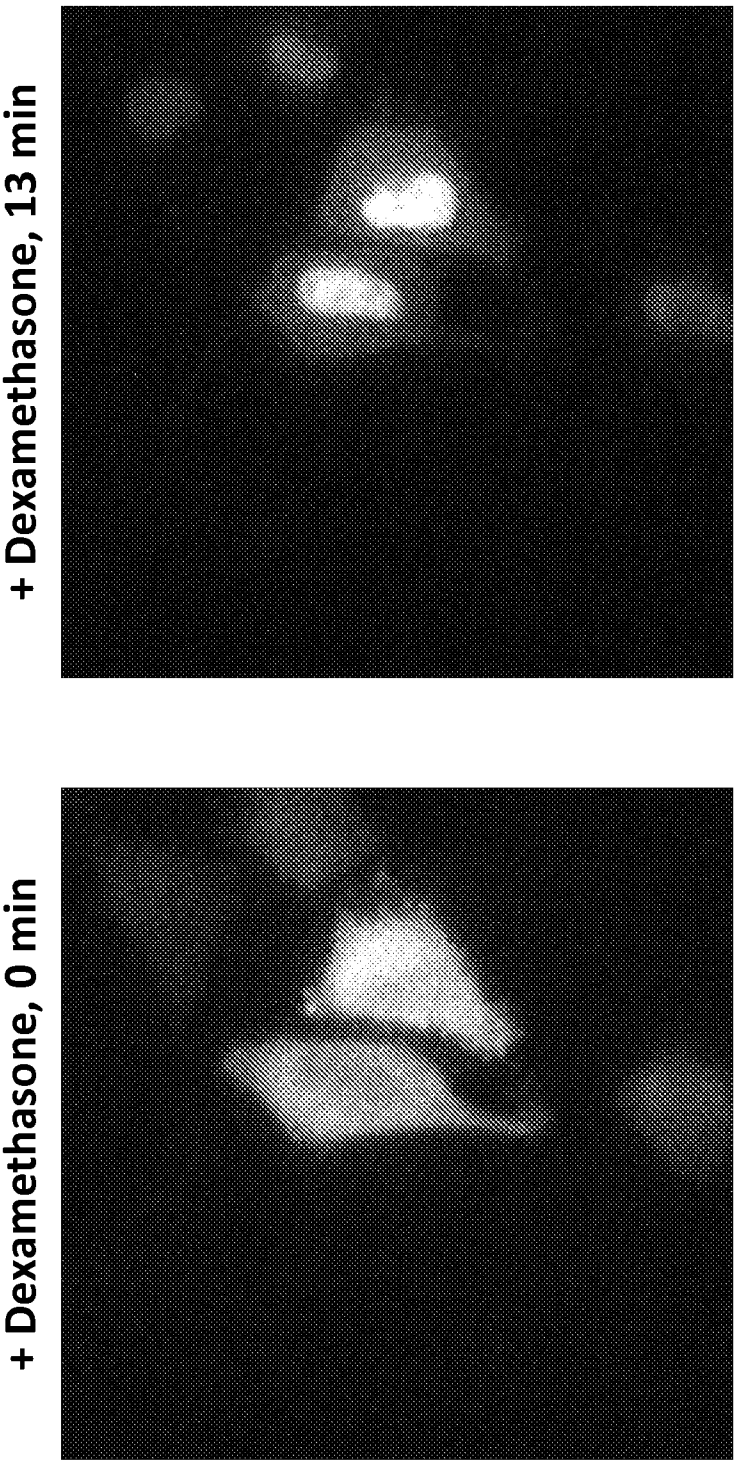


FIG. 90

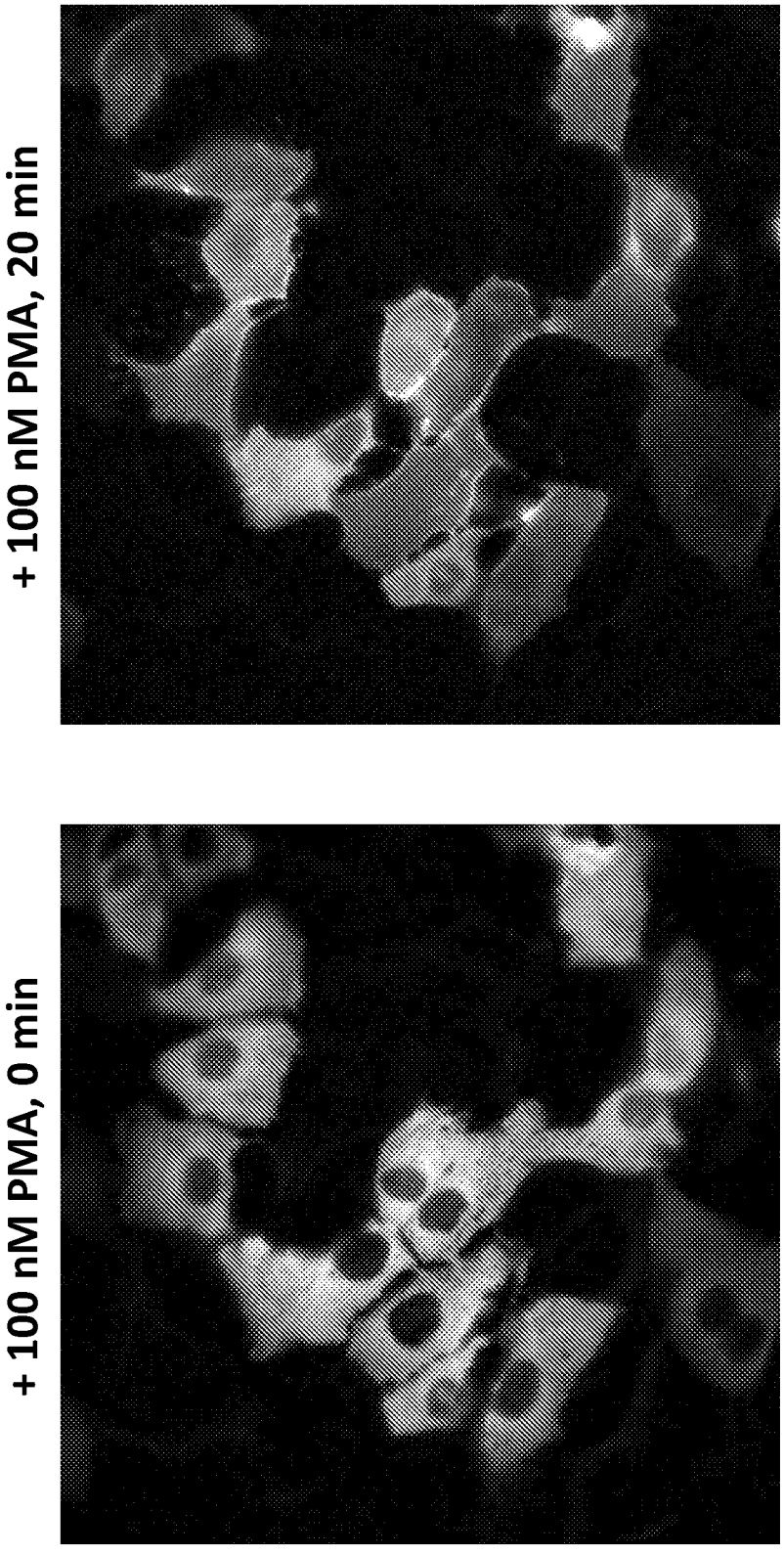


FIG. 91

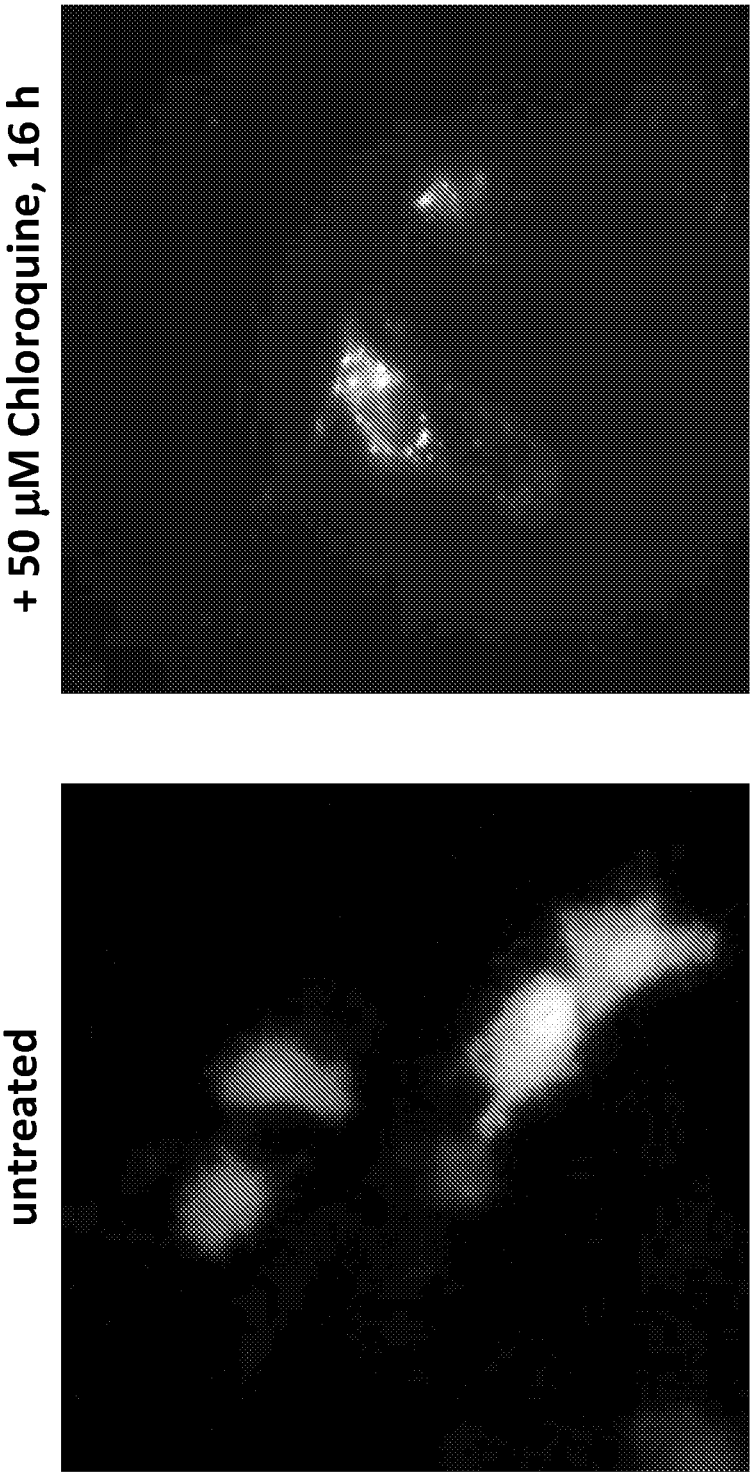


FIG. 92