

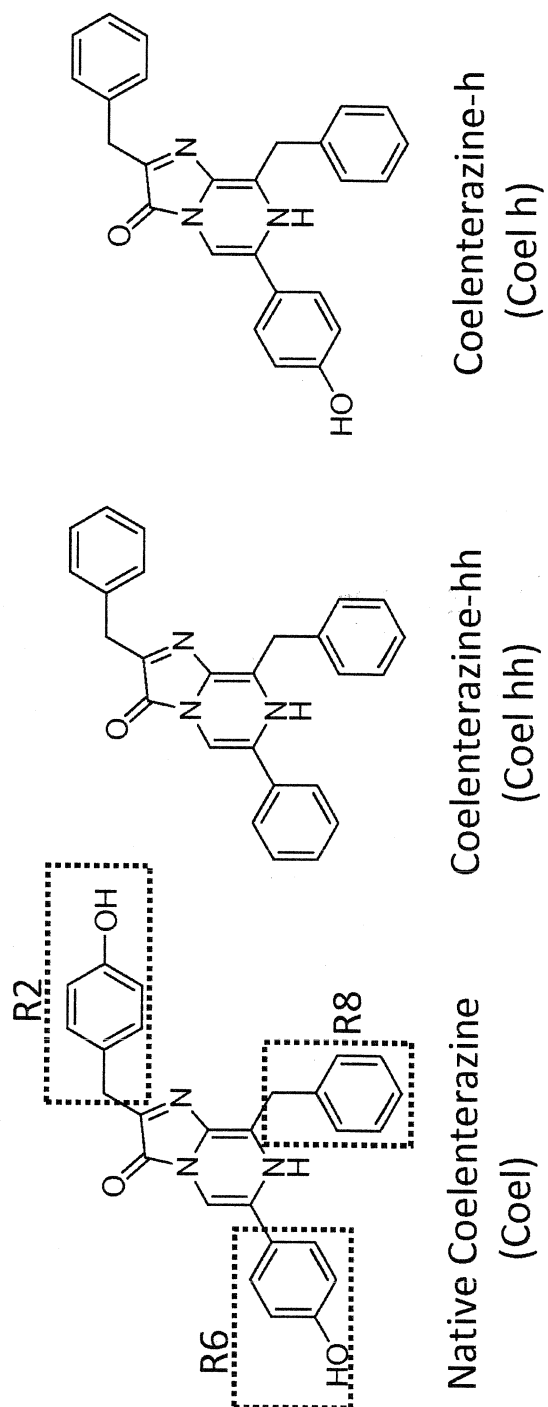


FIG. 1

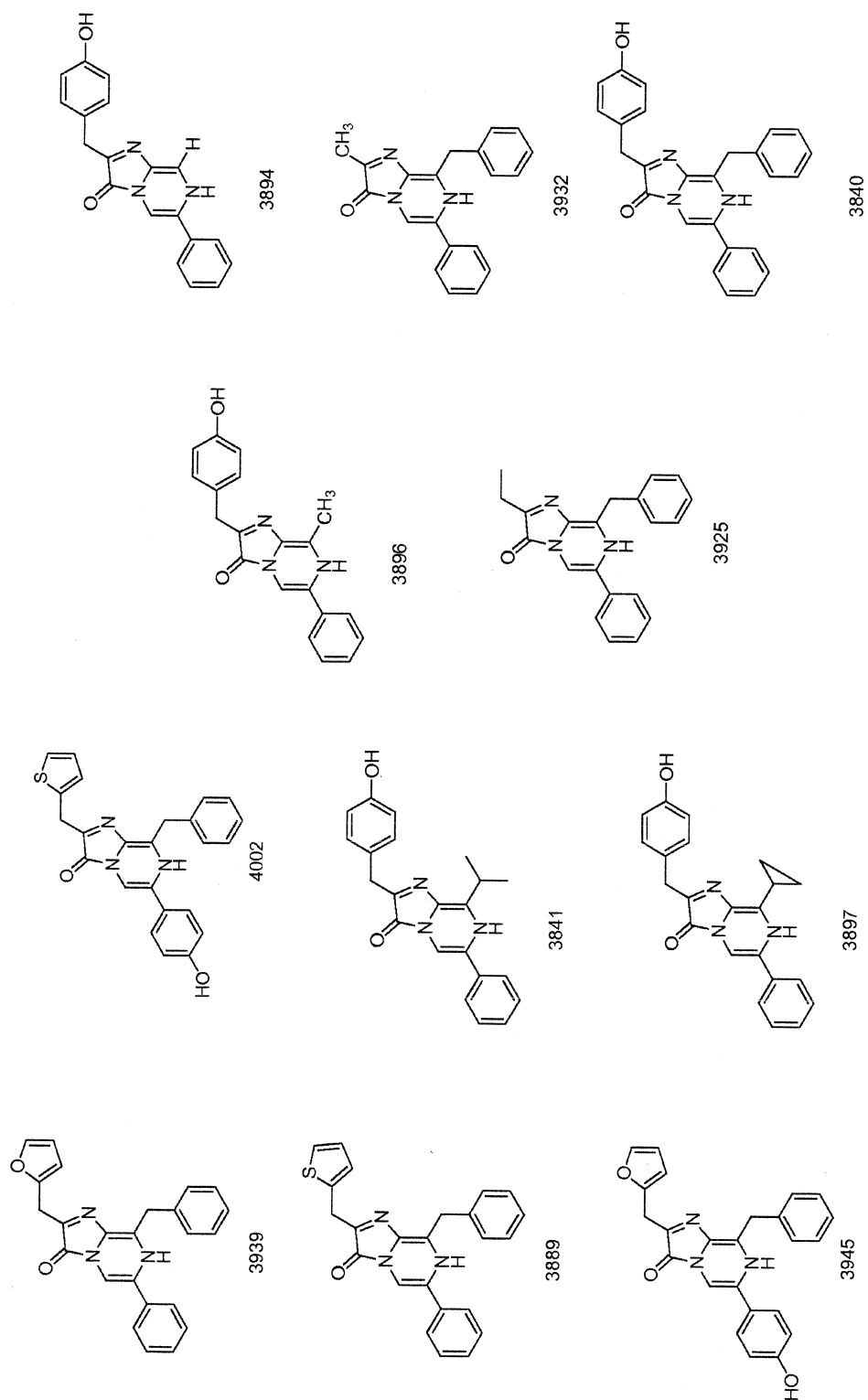


FIG. 2

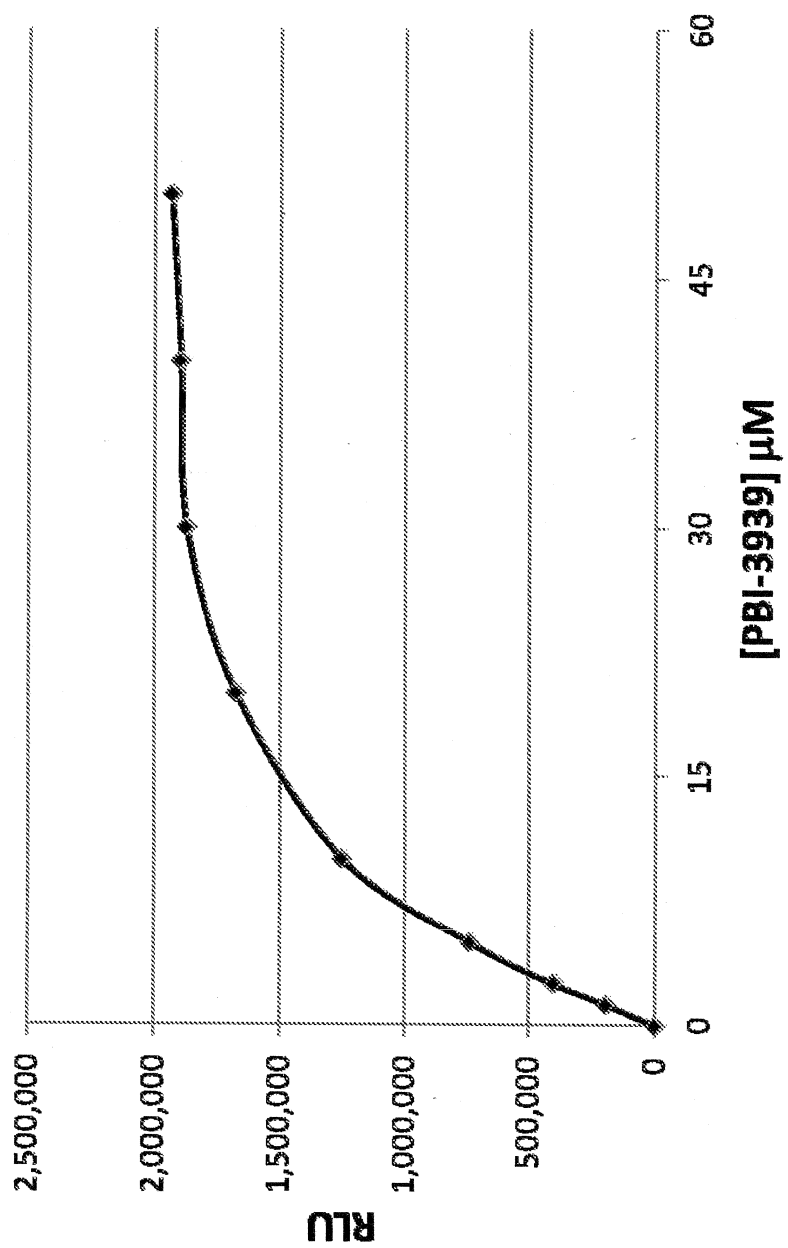


FIG. 3

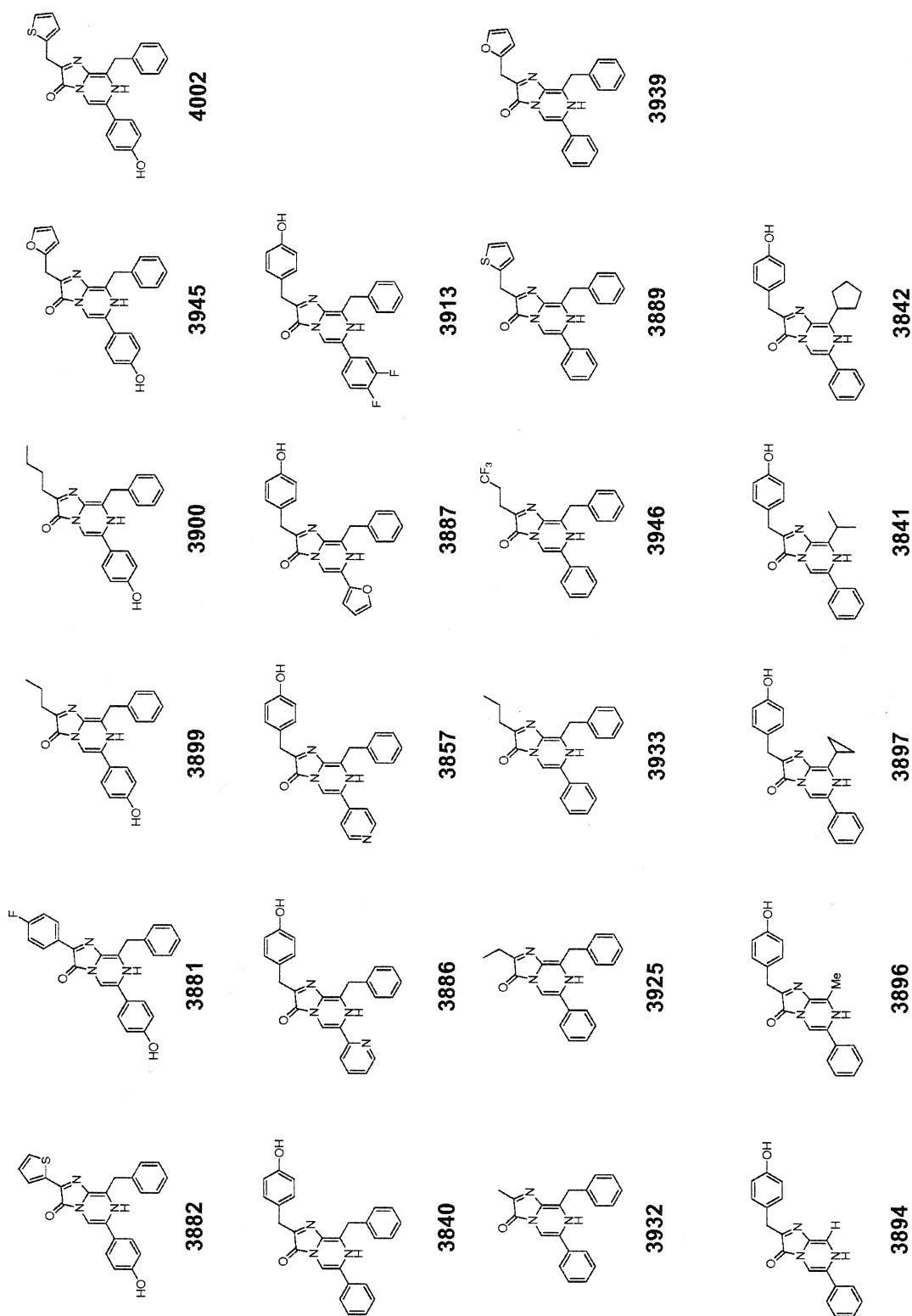


FIG. 4

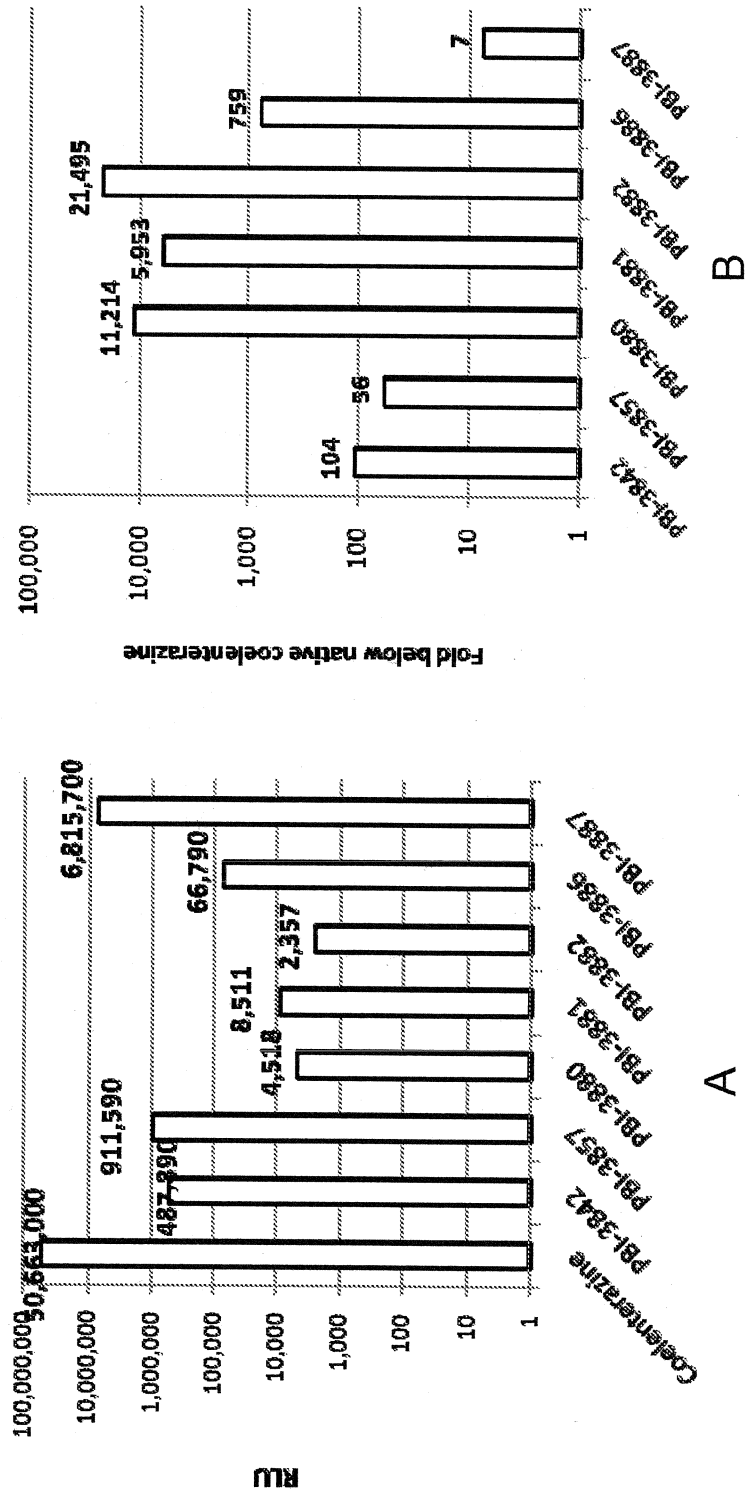


FIG. 5

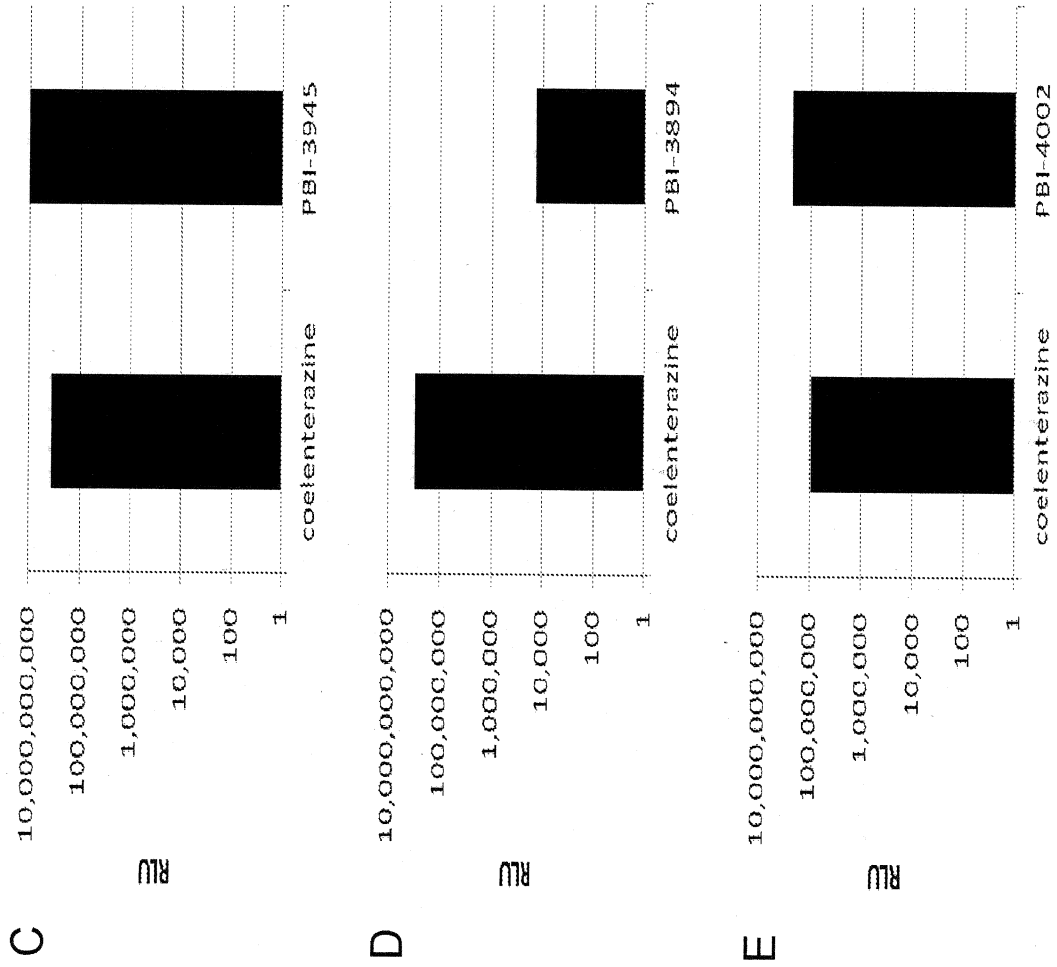


FIG. 5

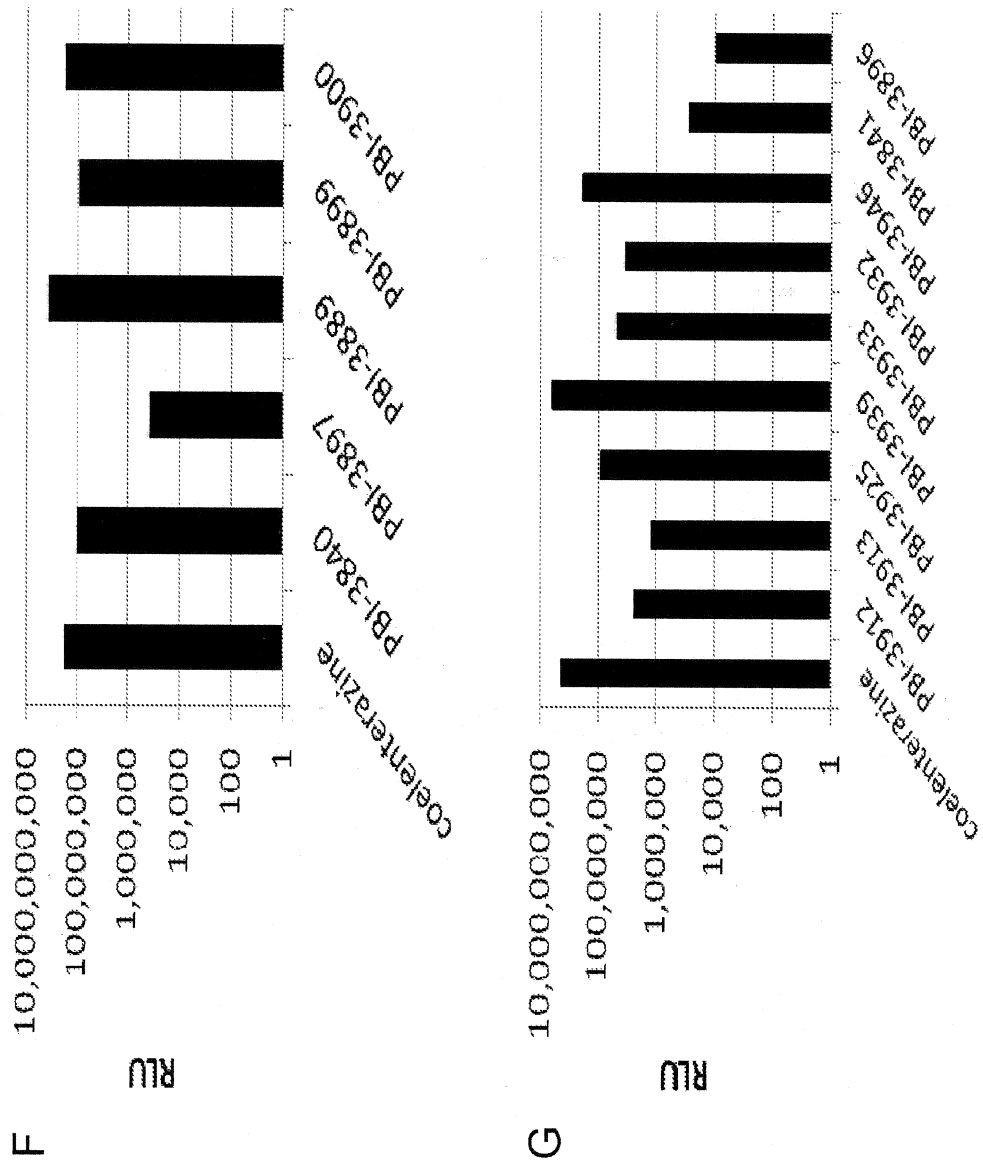


FIG. 5

POSITION	Clone(AA sequence)	Colenteh	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	0.003	0.031	0.003	0.011
18	40H11 (Q18L)	0.38	0.26	0.51	0.20	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
22	14C1 (L22I)	0.17	0.63	0.47	0.41	0.14	0.08	0.43	0.66
27	11E8 (L27V)	0.34	1.74	1.88	0.55	0.35	0.03	2.03	2.22
54	18F8 (F54S)	0.21	0.40	1.78	0.13	0.53	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
58	30E4 (V58I)	0.27	0.89	1.00	0.96	0.43	0.21	1.15	0.65
72	04G7 (L72Q)	4.46	1.25	1.15	9.36	5.65	8.84	1.13	4.04
75	28B2 (M75K)	4.16	1.57	2.30	5.43	4.92	5.67	2.38	4.03
77	18C4 (F77T)	1.62	0.55	1.22	7.82	2.06	2.28	1.33	2.67
77	42E4 (F77C)	0.13	0.46	0.99	3.43	0.20	0.02	1.27	0.56
77	50D12 (F77T)	1.48	0.52	1.15	7.75	1.87	2.20	1.24	2.46
89	35D11 (K89E)	3.02	0.59	1.26	5.27	3.39	5.19	1.41	2.32
90	10F2 (I90T)	2.20	0.48	0.66	0.00	3.90	5.41	0.84	1.36
92	04A12 (L92H)	0.89	0.39	0.61	7.31	3.72	14.25	0.53	0.89
109	43F9 (Y109F)	0.48	0.33	0.97	0.36	1.49	24.83	0.94	0.43
127	30D6 (V127A)	0.23	0.50	0.54	0.26	0.81	1.00	0.57	0.26
139	27B8 (D139G)	0.01	0.03	0.14	0.00	0.03	0.00	0.16	0.00
164	25A1 (C164S)	2.47	1.79	1.53	2.74	3.37	5.10	1.63	2.87
14-89	25F2 (A14V,K89E)	3.05	0.81	1.22	5.43	3.82	6.21	1.58	2.32
27-33	47D8(L27M,K33N)	0.59	1.71	2.05	0.85	0.95	0.27	2.12	1.23
49-72	26F1 (E49K,L72Q)	6.19	1.48	1.35	10.72	6.90	9.99	1.32	4.76
54-66	48G3 (F54I,S66T)	7.33	1.06	0.91	16.39	11.27	8.28	1.42	6.63
54-67	35B8 (F54S,G67S)	0.33	0.75	2.49	0.19	0.78	0.87	2.25	0.27
55-117	05H8 (D55G,I117F)	0.24	0.53	0.62	0.00	0.25	0.22	0.65	0.21
56-93	46A11(I56V,H93R)	0.35	0.61	1.53	0.22	0.42	0.18	1.50	0.36
68-75	04E2 (F68S,M75K)	3.43	1.20	1.53	4.37	3.91	4.12	1.62	3.24
77-126	27B9 (F77T,I126R)	0.87	0.15	0.69	4.87	0.80	1.00	0.79	1.14
77-39	33E2 (F77T,T39I)	0.95	0.34	0.69	6.30	1.32	1.64	0.74	1.47
89-148	50E7 (K89E,S148T)	1.81	0.48	0.85	3.74	2.23	3.20	0.94	1.59
90-77	02D10 (I90V,F77T)	2.05	0.70	1.17	17.39	6.32	10.88	1.05	2.37
106-164	17E6 (M106K,C164S)	4.14	2.46	2.52	3.77	5.06	7.72	2.71	3.80
23-58-169	47G12 (E23K,V58L,A169V)	0.06	0.44	0.49	1.49	0.10	0.01	0.68	0.58
33-76-89	17C5 (K33N,I76N,K89E)	1.27	0.68	2.02	1.33	2.22	1.12	2.24	1.07
68-90-145	04D10 (F68S,I90V,P145L)	4.17	1.40	1.65	0.00	6.09	5.96	1.72	3.82
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

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	11E8 (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	30E4 (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	42E4 (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	04E2 (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	33E2 (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	17E6 (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	0.03	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)	col	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	11E8 (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	30E4 (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	42E4 (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	04E2 (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,I126R)	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	33E2 (F77T,I39I)	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	17E6 (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 (F23K,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	11E8 (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	30E4 (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	42E4 (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	04E2 (F68S,M75K)	4.11	1.78	1.94
77-126	27B9 (F77T,T126R)	0.49	0.47	0.13
77-39	33E2 (F77T,T39I)	0.58	0.44	0.20
89-148	50E7 (K89E,S148T)	2.06	0.85	0.59
90-77	02D10 (I90V,F77T)	1.85	1.01	0.56
106-164	17E6 (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
C144E	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, F77C)	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,I92H,V127A)		x			x	x	x		x
QC#2 2 (Q20R,G71D,F77C,I90V,I92H,V127A)		x		x	x	x	x		x
QC#2 3 (Q20R,G71D,F77C,I92H,V127A)		x		x	x		x		x
QC#2 6 (F54I,G71D,F77C,I92H,V127A)			x	x	x		x		x
QC#2 7 (F54I,G71D,F77C,I90V,I92H)			x	x	x	x	x		
QC#2 8 (F54I,G71D,F77C,I90V,I92H,V127A)			x	x	x	x	x		x
QC#2 11 (Q20R,F54I,G71D,F77C,I90V,I92H,V127A)		x	x	x	x	x	x		x
QC#2 16 (G71D,I90V,I92H,Y109F,V127A)				x		x	x	x	x
QC#2 18 (G71D,I92H,Y109F,V127A)				x			x	x	x
QC#2 21 (Q20R,F54I,G71D,F77C,I90V,I92H,V127A)		x	x	x	x	x	x		x
QC#2 26 (Q18L,G71D,F77Y,I90V,I92H,Y109F,V127A)	x			x	x	x	x	x	x
QC#2 31 (Q18L,F54I,G71D,F77Y,I90V,I92H,Y109F,V127A)	x		x	x	x	x	x	x	x
QC#2 41 (G71D,F77C,I90V,I92H,V127A)				x	x	x	x		x
QC#2 44 (G71D,F77C,I90V,V127A)				x	x	x			x
QC#2 A3 (Q20R,G71D,F77C,I92H)		x		x	x		x		
QC#2 C6 (F54I,G71D,F77C,I92H)			x	x	x		x		
QC#2 A7 (Q20R,F54I,G71D,F77C,I92H,V127A)		x	x	x	x		x		x
QC#2 C10 (I90V,I92H,Y109F,V127A)						x	x	x	x
QC#2 E1 (F54I,I90V,I92H,Y109F,V127A)			x			x	x	x	x
QC#2 E2 (F54I,G71D,I90V,I92H,Y109F)			x	x		x	x	x	
QC#2 E4 (Q18L,G71D,F77Y,I92H,Y109F,V127A)	x			x	x		x	x	x
QC#2 F4 (Q18L,F77Y,I90V,I92H,Y109F,V127A)	x				x	x	x	x	x
QC#2 E7 (Q18L,F54I,F77Y,I90V,I92H,Y109F,V127A)	x		x		x	x	x	x	x
QC#2 E8 (Q18L,F54I,I90V,I92H,Y109F,V127A)	x		x			x	x	x	x
QC#2 F7 (Q18L,F54I,G71D,F77Y,I92H,Y109F,V127A)	x		x	x	x		x	x	x
QC#2 H11 (G71D,F77C)				x	x				
IE1 (I90V/I92H)						x	x		
IE2 (I90V,Y109F)						x		x	
IE3 (I90V/I92H/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,I92H,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,I92H,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,I92H,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,I92H,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,I92H)	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,I92H,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,I92H,V127A)	0.10	0.03	0.05	0.02	0.17	6.66	1.11	0.40	0.24
QC#2 16 (G71D,I90V,I92H,Y109F,I127A)	0.11	0.06	0.04	0.001	0.29	1.49	1.51	7.72	55.11
QC#2 18 (G71D,I92H,Y109F,I127A)	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,I92H,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,I92H,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,I92H,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,I92H,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,I92H)	0.23	0.20	0.22	0.03	0.52	6.75	1.11	0.39	0.24
QC#2 C6 (F54I,G71D,F77C,I92H)	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,I92H,V127A)	0.13	0.05	0.06	0.02	0.28	6.93	1.25	0.32	0.22
QC#2 C10 (I90V,I92H,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,I92H,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,I92H,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,I92H,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,I92H,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,I92H,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,I92H,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,I92H,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 ₁ 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	F11,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,I96A	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 CIA4E 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
I90T, H92R		QC27 #2														X		
F68Y, L72Q, M75K, I90T, H92R, V158I		QC27 #3														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								X			
V21L, I76F, H92R		QC27 #8	X											X			X	
V21L, F68Y, L72Q, M75K, H92R, V158I		QC27 #9A	X	X	X	X	X									X	X	X
V21L, F68Y, L72Q, M75K, I76F, I90T, H92R, V158I		QC27 #10	X	X	X	X	X							X	X	X	X	X
F68Y, L72Q, M75K, H92R		QC27 #12A		X	X	X	X									X		
V21L, H92R		QC27 #13	X														X	
F68Y, L72Q, M75K, H92R, V158I		QC27 #14A		X	X	X	X									X	X	X
V21L, F68Y, L72Q, M75K, I90T, H92R, V158I		QC27 #5	X	X	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				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	L118I,P145Q	42.10	3.20	18.30	2.51	2.37
36C11	18-99	L118V,I99T	125.68	2.64	24.99	2.15	2.17
3E2	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

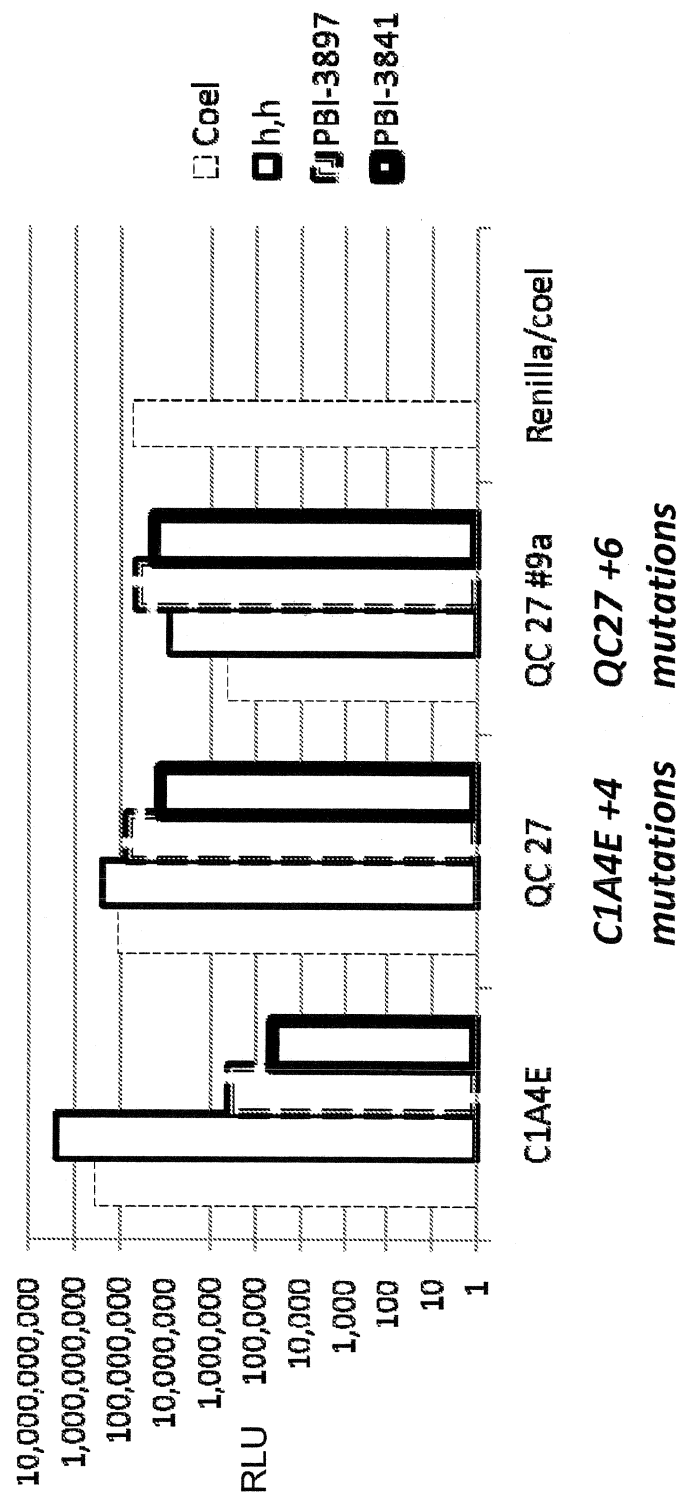


FIG. 17

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,Q82P,E49G,I149I	C1.1		X		X			X					X
Q20P,Q82P,E49G,F77Y,G95D,F110I,I149I	C4.13		X		X			X		X	X	X	X
Q20P,I27N,V38I,I149I	C5.16		X	X		X							X
I27N,V38I,I149I	C5.19			X		X							X
D19N,Q20P,V38I,I149I	C7.26	X	X			X							X
V38I,G95D,F110I,I149I	C8.28					X					X	X	X
D19N,Q20P,V38I,G95D,F110I,I149I	C8.29	X	X			X					X	X	X
I27N,V38F,K43N,I149I	C11.40			X		X	X						X
I27N,V38F,K43N,V58I,I149I	C11.42			X		X	X		X				X
I27N,V38F,K43N,V58I,F110I,I149I	C12.46			X		X	X		X		X	X	X
Q20P,I27N,I149I	C13.50		X	X									X
Q20P,I27N,V38I,F110I	C14.51		X	X		X						X	
I27N,V38I,F110I,I149I	C14.52			X		X						X	X

FIG. 20

Sample	Sequence	Coelenterazine	H	H ₂ H	PBI-3939	PBI-3945	PBI-4002	PBI-3932	PBI-3840
C1.1	Q20P,Q32P,E49G,I149I		0.00006	0.00026	0.00033	0.00069	0.00050	0.00051	0.00008
C4.13	Q20P,Q32P,E49G,F77Y,G95D,F110I,I149I		0.00006	0.00008	0.00009	0.00008	0.00005	0.00006	0.00010
C5.16	Q20P,I27V,V38I,I149I		0.0002	0.0013	0.0003	0.0011	0.0034	0.0036	0.0001
C5.19	L27V,V38I,I149I		0.0010	0.2747	0.0663	0.1595	0.5082	0.5129	0.0003
C7.26	D19N,Q20P,V38I,I149I		0.0001	0.0017	0.0011	0.0020	0.0032	0.0037	0.0000
C8.29	D19N,Q20P,V38I,G95D,F110I,I149I		0.000016	0.000025	0.000036	0.000016	0.000014	0.000031	0.000025
C11.40	L27V,V38F,K43N,I149I		0.00010	0.01172	0.00714	0.01853	0.04124	0.02485	0.00006
C11.42	L27V,V38F,K43N,V58I,I149I		0.0001	0.0016	0.0011	0.0044	0.0093	0.0040	0.0001
C12.46	L27V,V38F,K43N,V58I,F110I,I149I		0.00002	0.00004	0.00005	0.00001	0.00008	0.00006	0.00004
C13.50	Q20P,I27V,I149I		0.0001	0.0124	0.0036	0.0027	0.0109	0.0100	0.0000
C14.51	Q20P,I27V,V38I,F110I		0.00003	0.00010	0.00004	0.00001	0.00008	0.00011	0.00004
C14.52	L27V,V38I,F110I,I149I		0.00007	0.00041	0.00008	0.00006	0.00034	0.00046	0.00008
IVY-H17			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
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		H-H		PBI-3939	PBI-3945	PBI-3889	PBI-4002	PBI-3932	PBI-3840
Cl.1	F6Y,Q18L,K33N,N135D	0.0055128	0.130	0.463	0.444	0.127	0.290	0.159	0.0083435	0.017											
Cl.2	F6Y,Q18L,K33N,N135D,I155T	0.0000068	0.0015688	0.0043713	0.0066058	0.0013479	0.0027420	0.0009303	0.0000844	0.0000782											
Cl.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,L27N,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,L27N,S28Y,P40T	0.0000579	0.0002759	0.0000464	0.0004978	0.0017611	0.0002812	0.0013044	0.0000144	0.0000132											
C6.26	Q18L,L27N,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,L27N,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,L27N,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,I155T	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,L27N,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,L27N,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,L27N,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

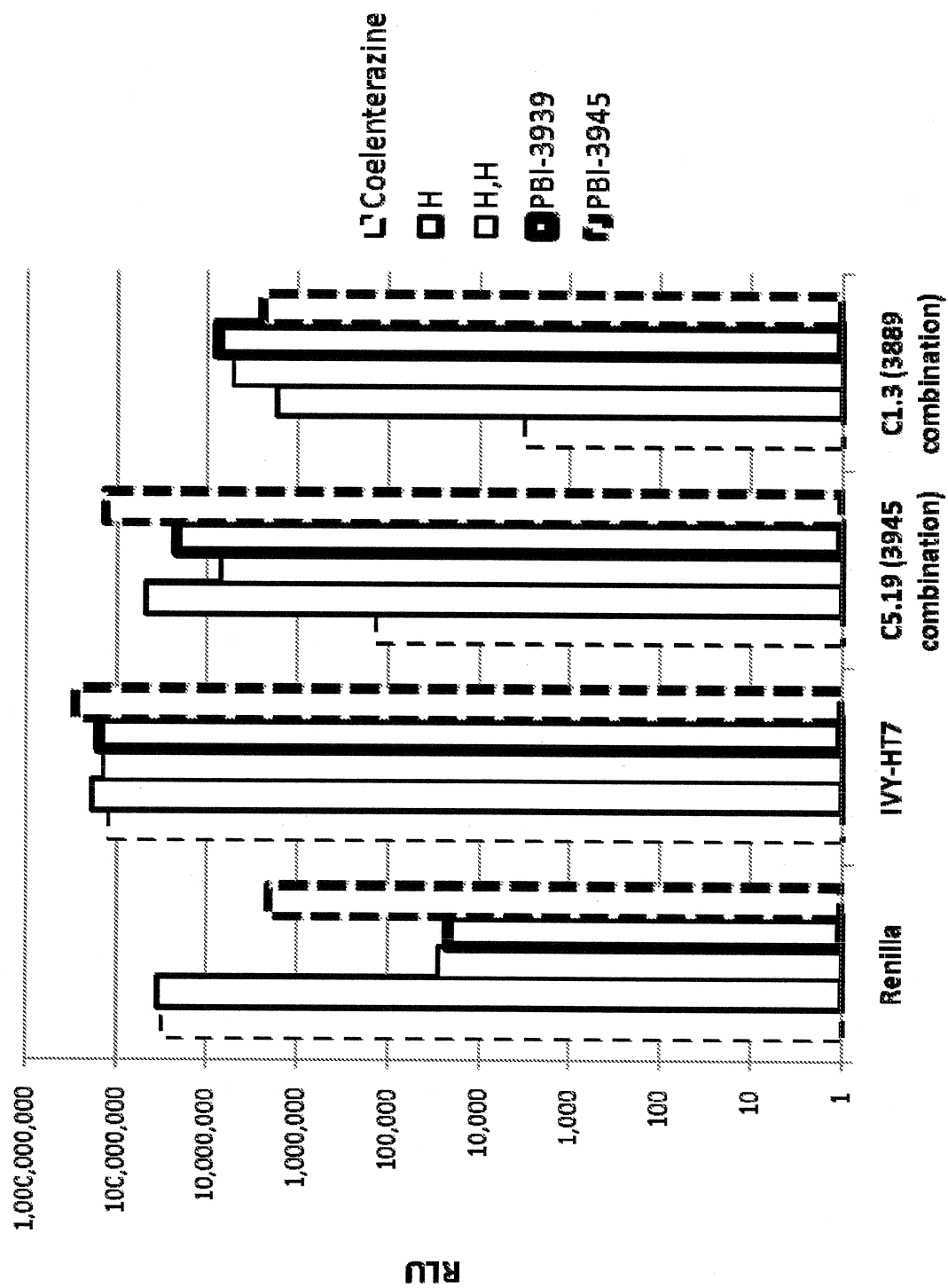


FIG. 24

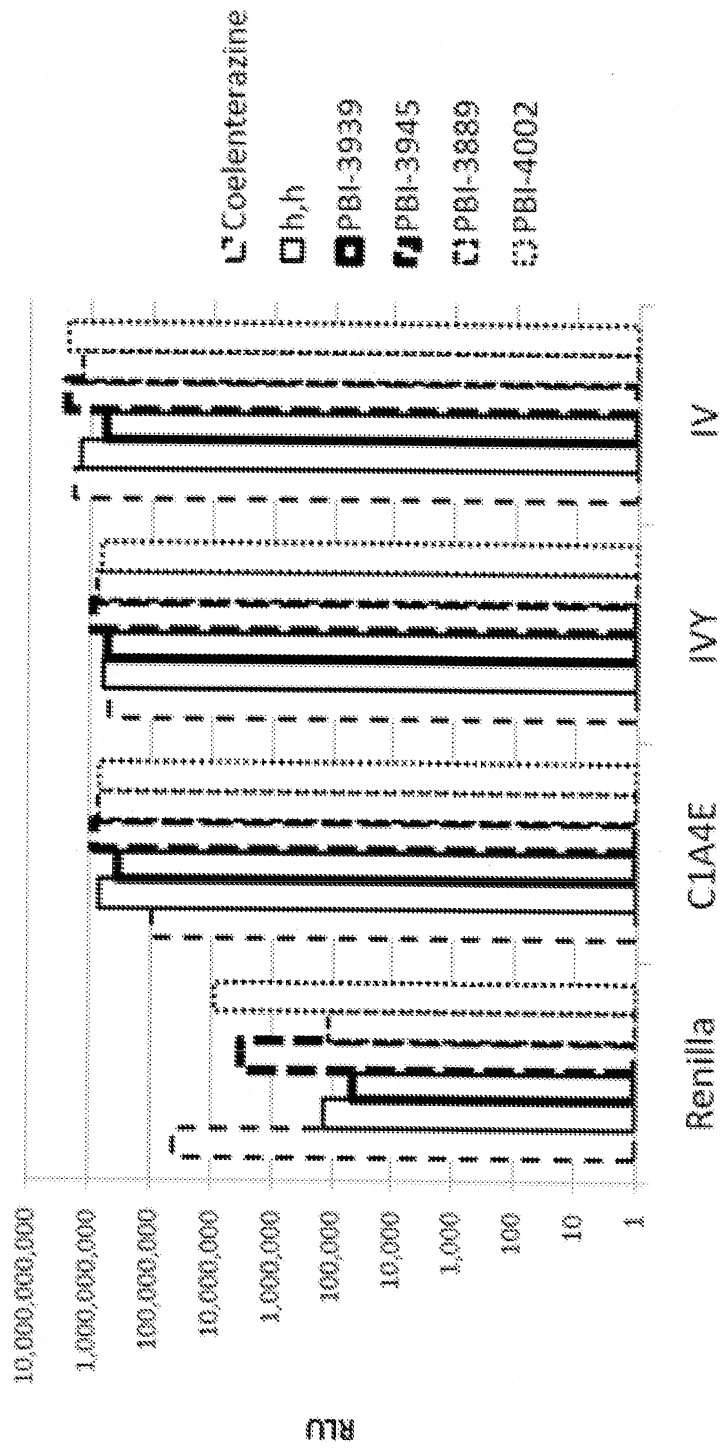


FIG. 25

Sequence	done	1	4	18	27	33	38	68	70	72	75	102
Q18L	1D6			X								
F1J,L27A,V38I	8A3	X			X		X					
F1J,L27V	8A7	X			X							
F1J,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	
F1J,M75K,L72Q,F68Y,L27A,V38I	10A7	X			X		X	X		X	X	
F1J,M75K,L72Q,M70V,Q18L	10G10	X		X					X	X	X	
K33N	14A2					X						
F1J,M75K,L72Q,F68Y,K33N,V102E	14C4	X				X		X		X	X	X
E4IK,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,L27N,V38I	0.01	0.81	0.87	0.43	0.90	0.48	0.77
8A7	F1I,L27N	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	L27N	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,L27N,Q18L,V38I	0.09	2.05	1.67	1.11	1.98	1.04	1.62
10A7	F1I,M75K,L72Q,F68Y,L27N,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

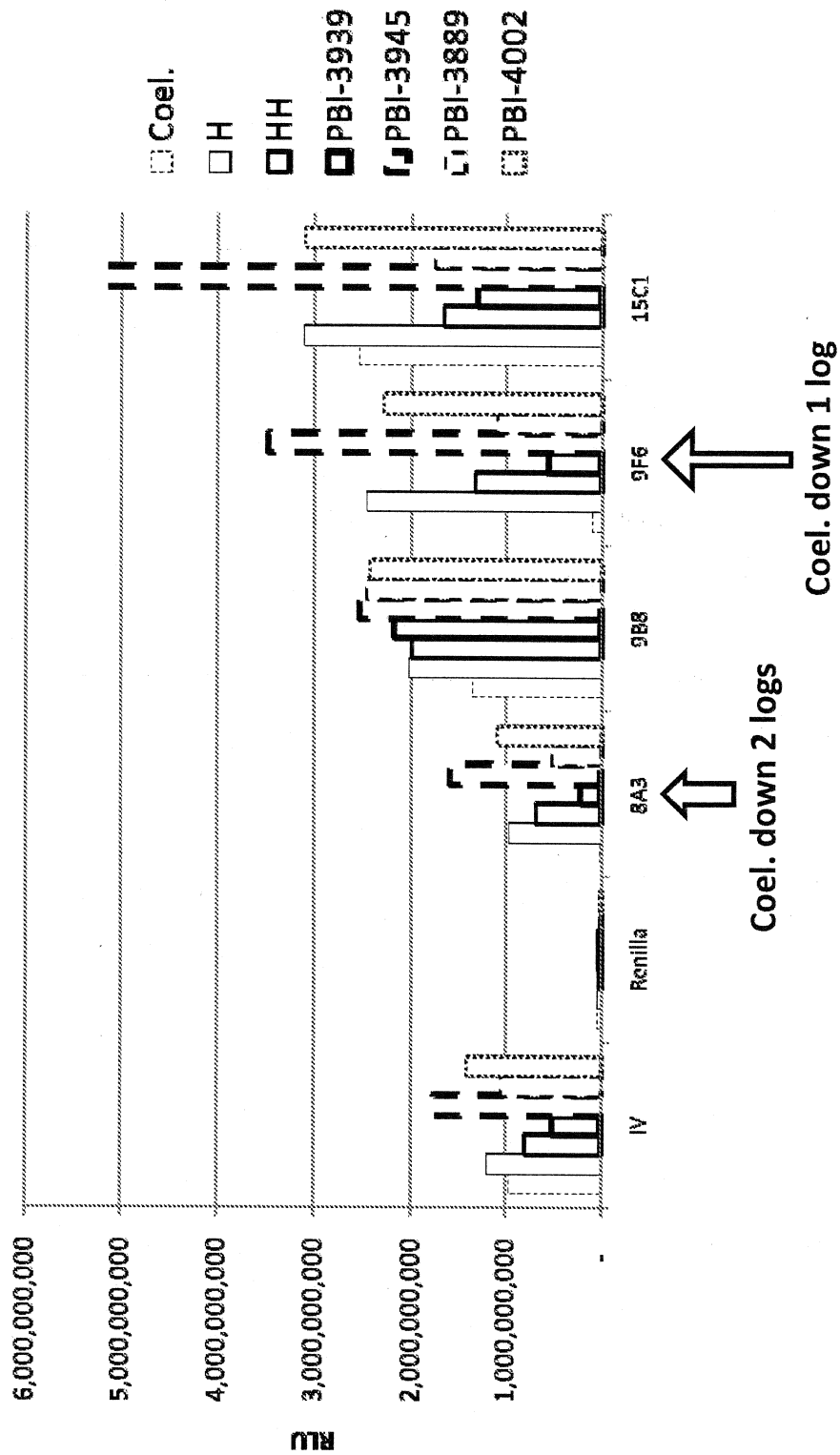


FIG. 28

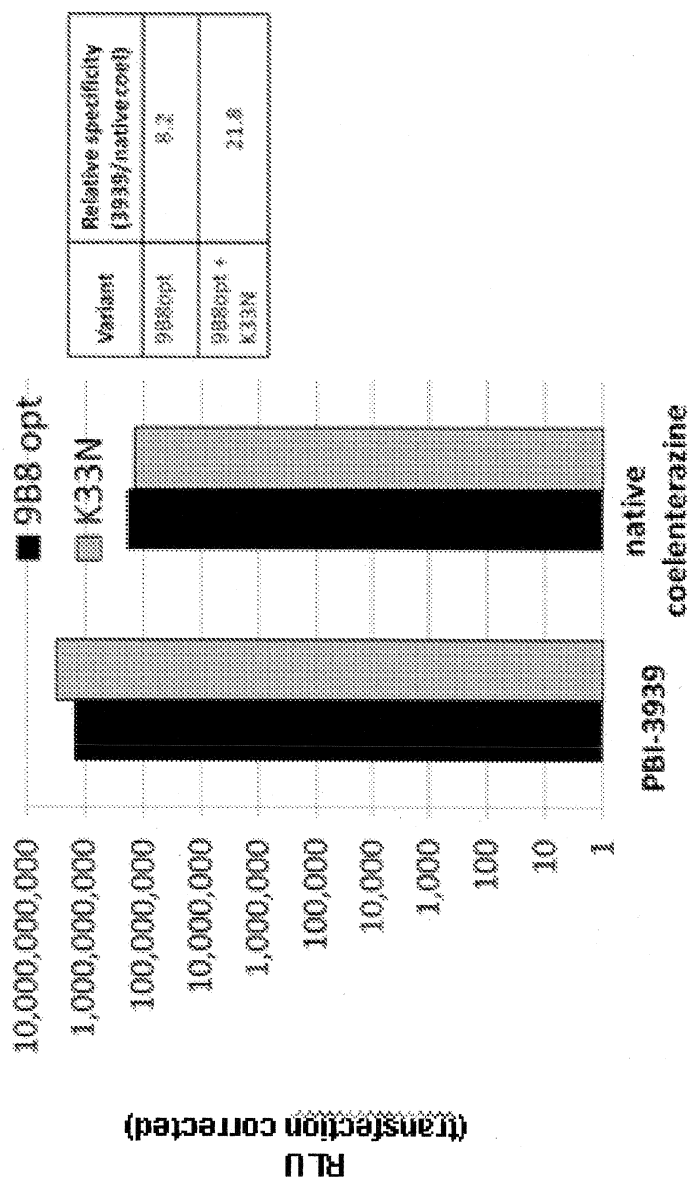


FIG. 29

A	Native coelenterazine		B	Coelenterazine-h	
	Amino acid	Relative activity		Amino acid	Relative activity
	Ala	1.0		Ser	1.1
	Phe	3.2		Ile	1.4
	Lys	19.2		Lys	11.6
	Arg	26.8		Arg	15.9
				Phe	55.9
C	PBI-3939		D	Relative activity (in context of wt OgLuc)	
	Amino acid	Relative activity		Amino acid substitution	Relative activity (in context of N166R)
	Ser	1.8		L27V	62.2
	Glu	4.6		A33N	0.6
	Ile	10.0		K43R	1.1
	Lys	17.5		M75K	65.7
	Arg	26.4		T39T	118.6
	Phe	444.4		L72Q	2.0
				F68D	3.0
					45.4

FIG. 30

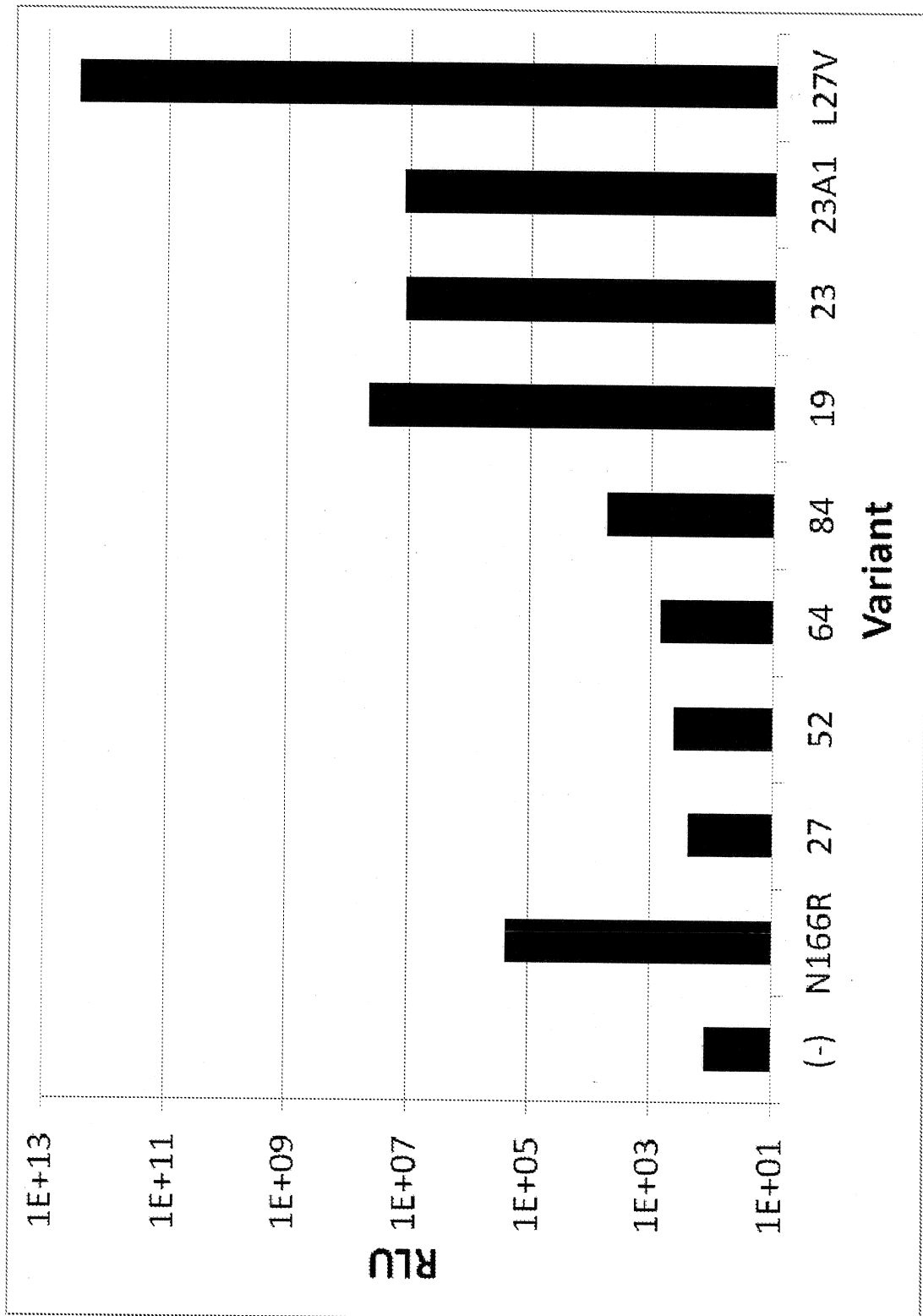


FIG. 31

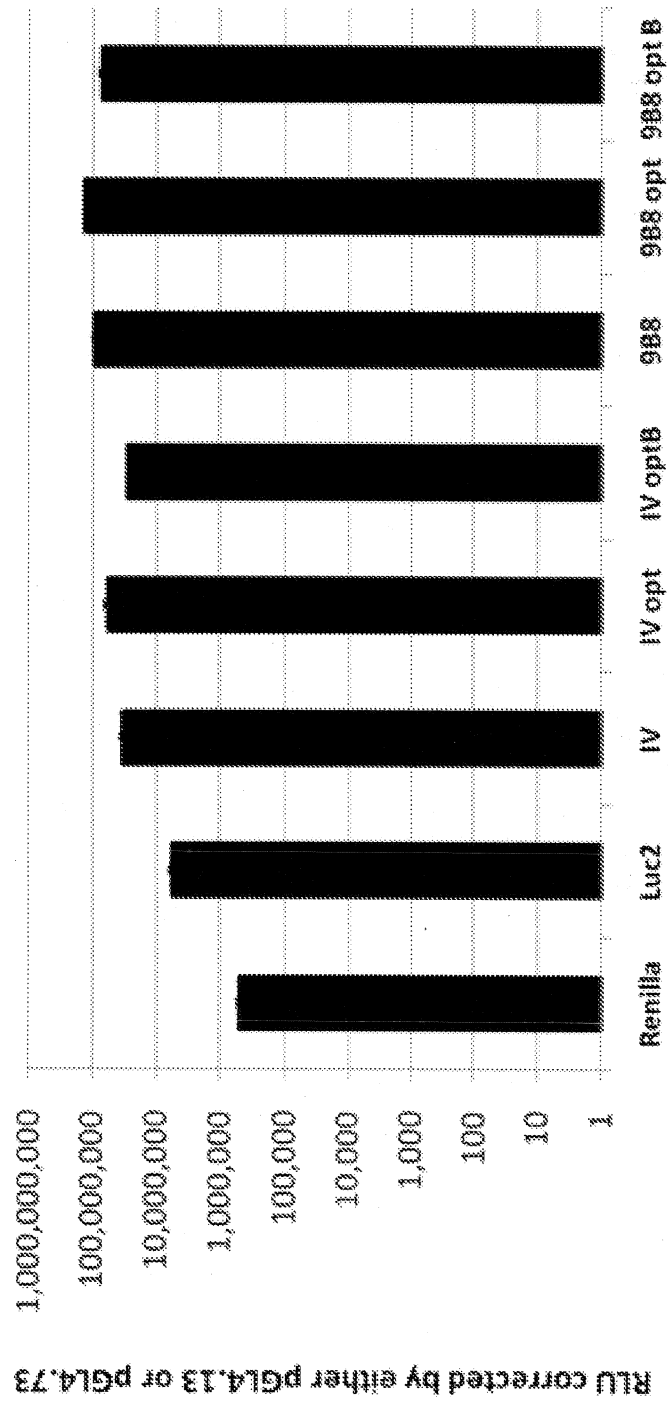


FIG. 32

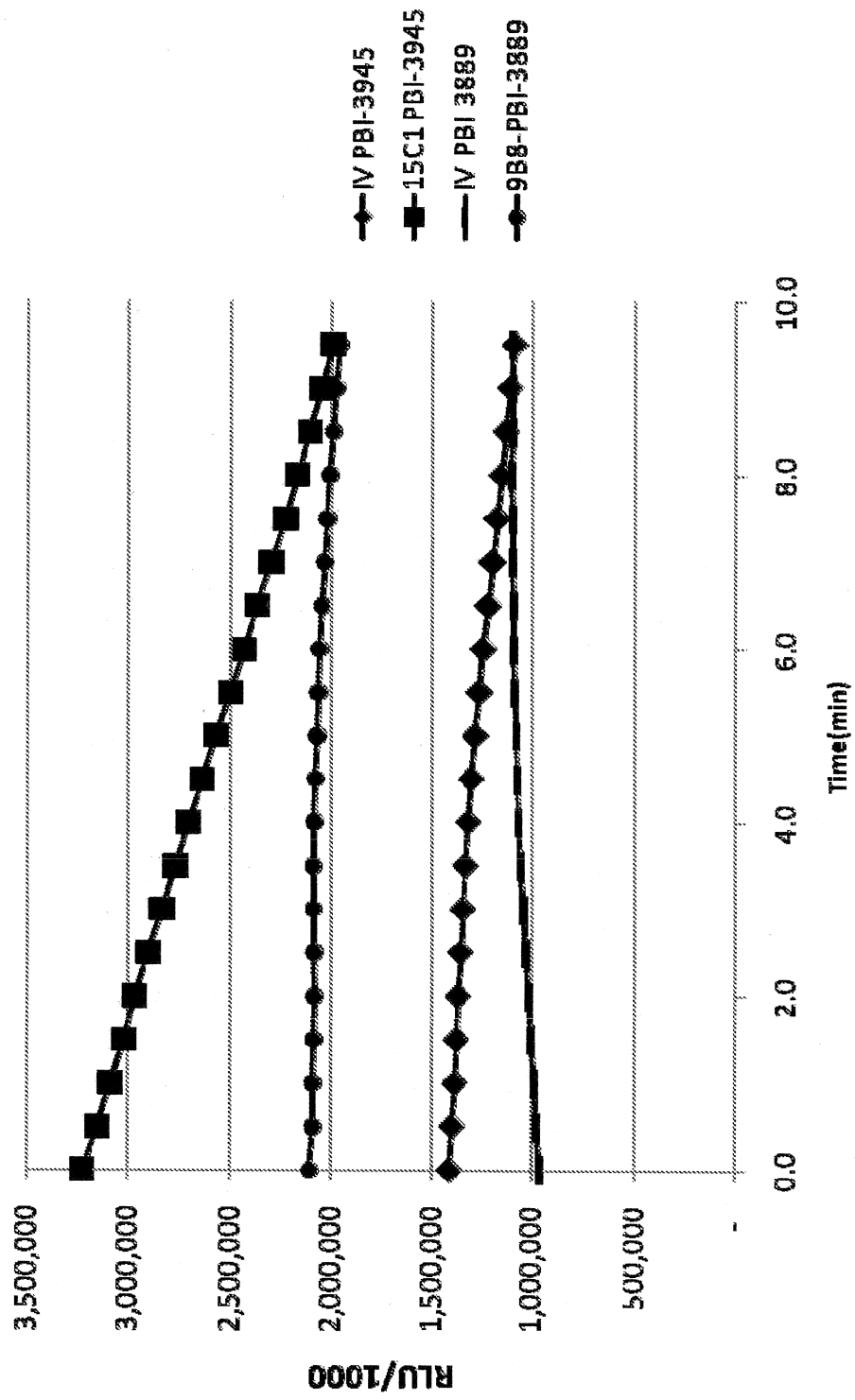


FIG. 33

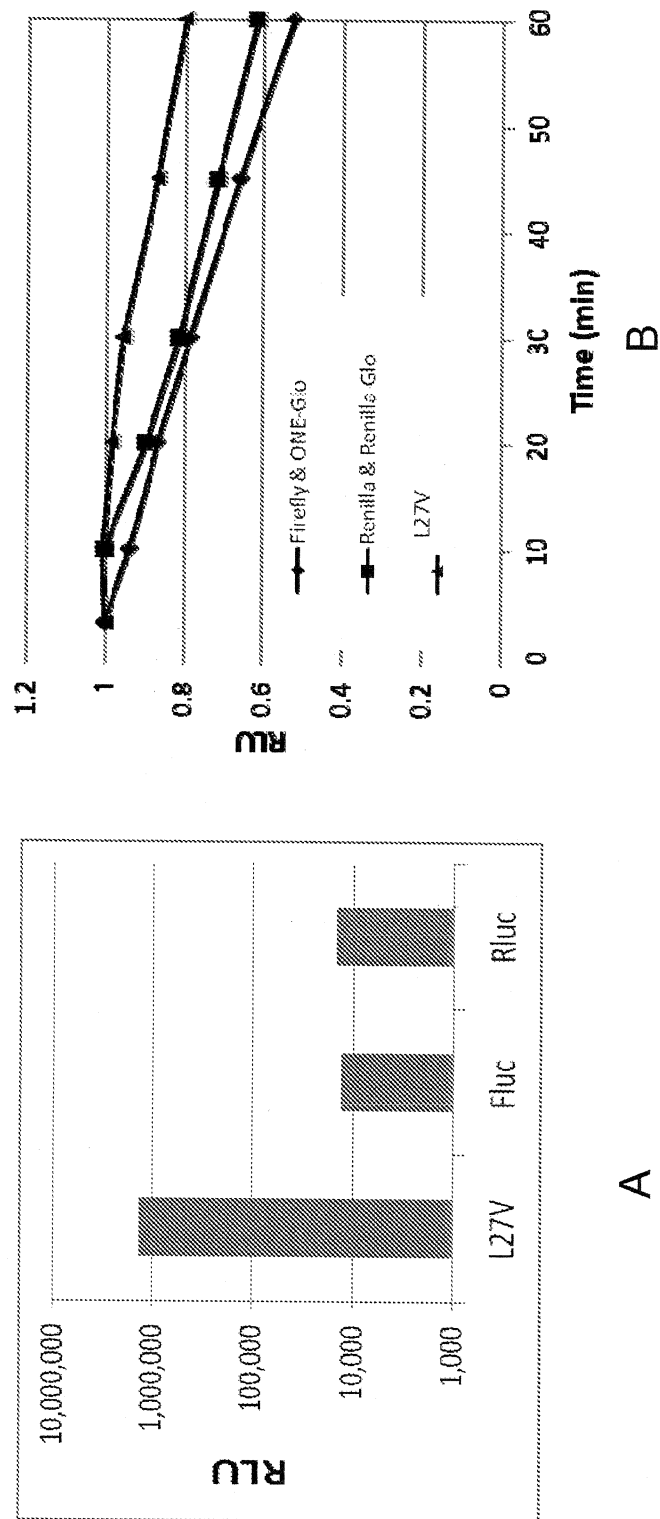


FIG. 34

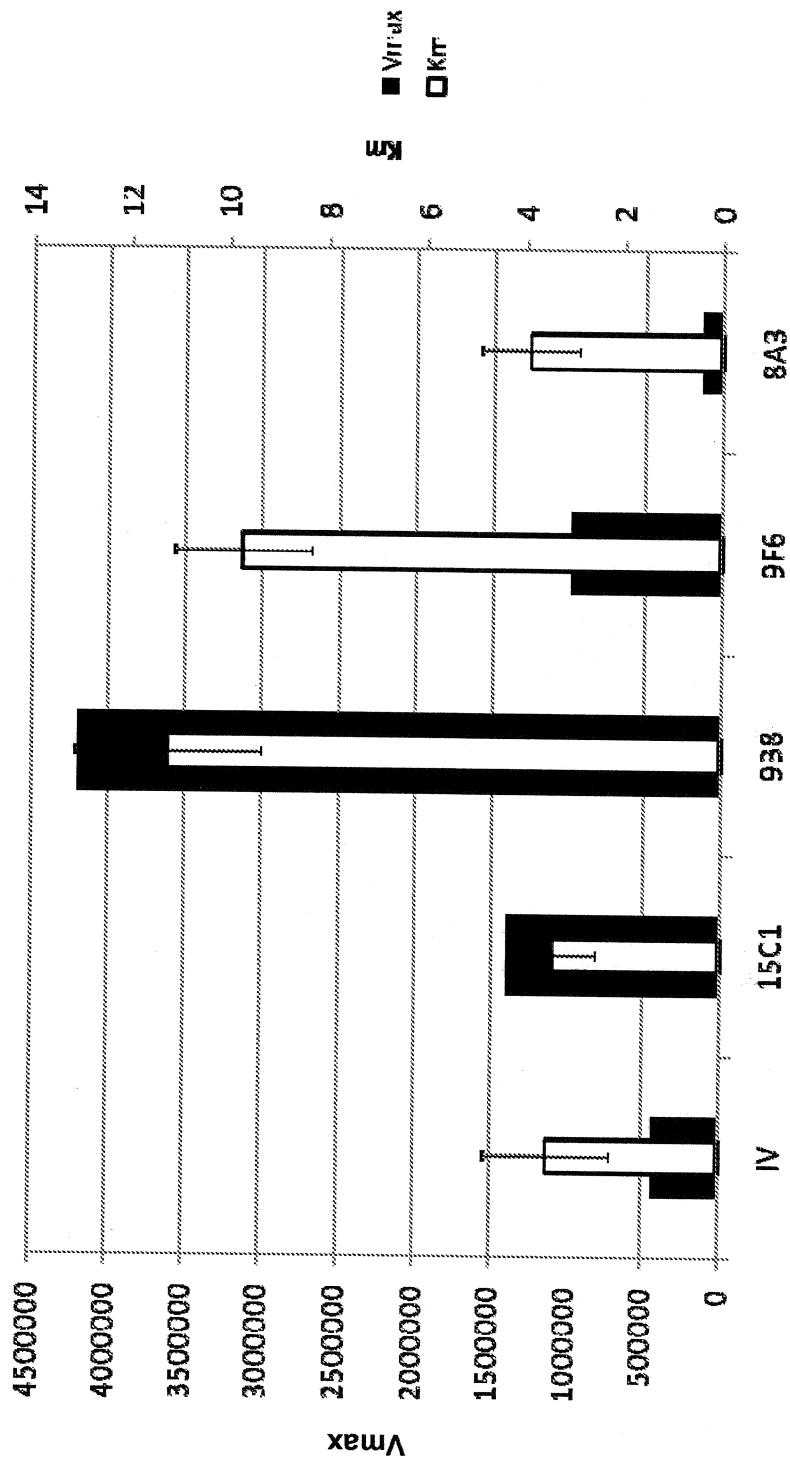
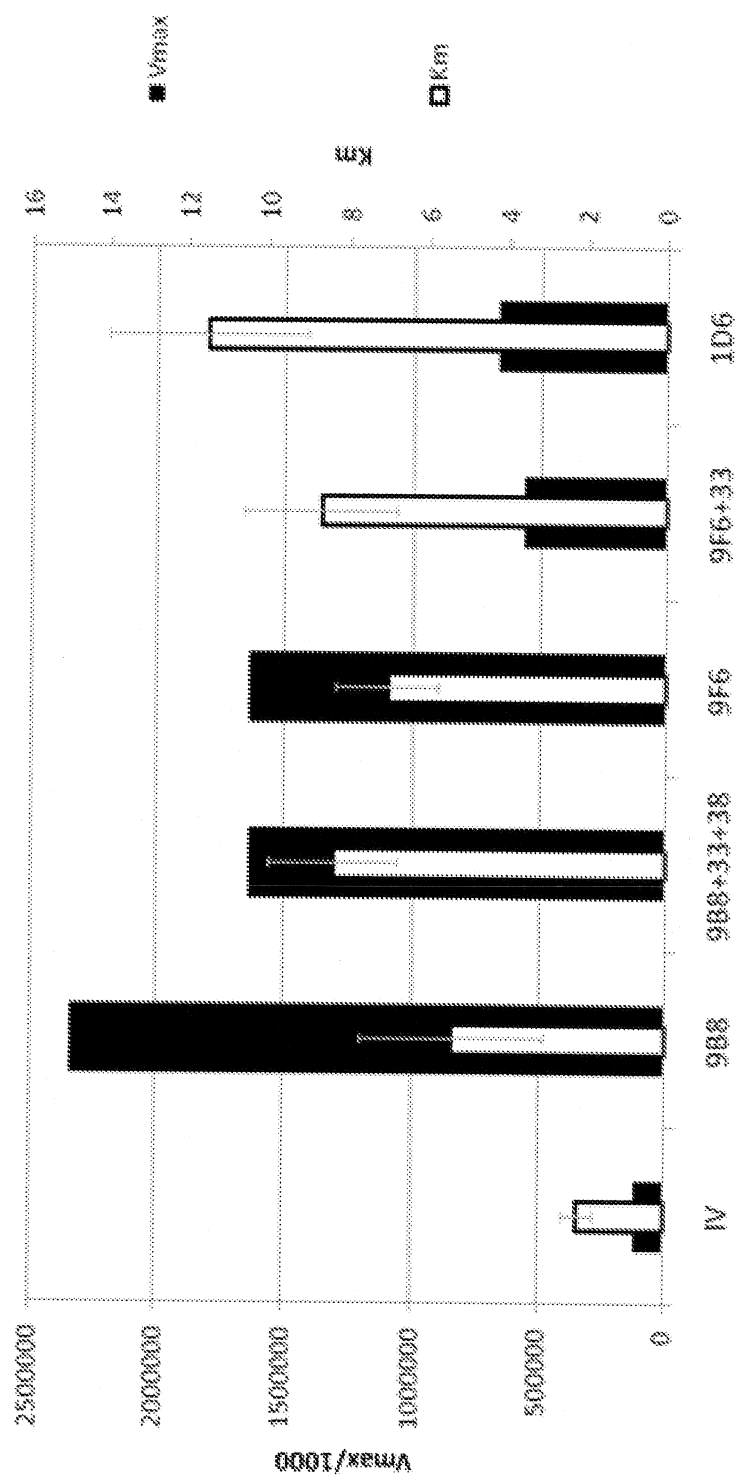


FIG. 35



Position 33 is K33N

Position 38 is V38I

FIG. 36

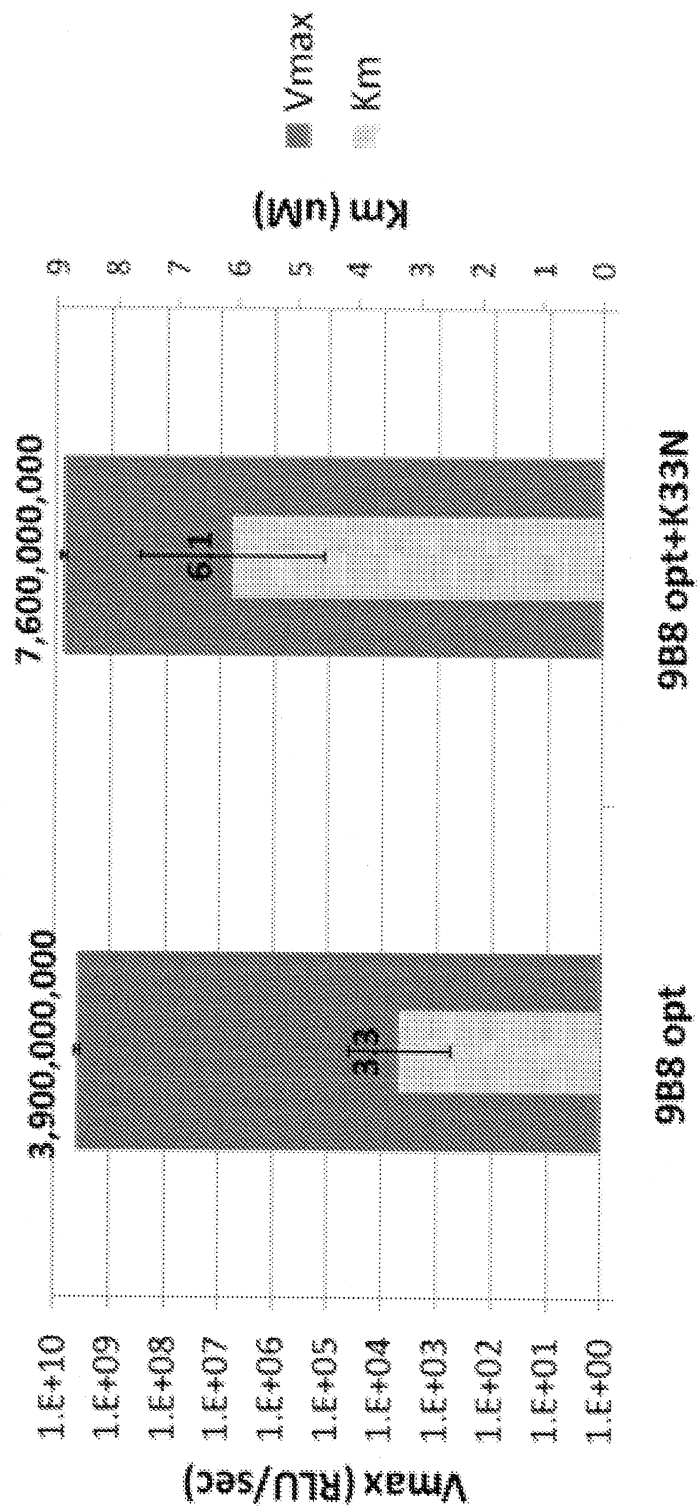


FIG. 37

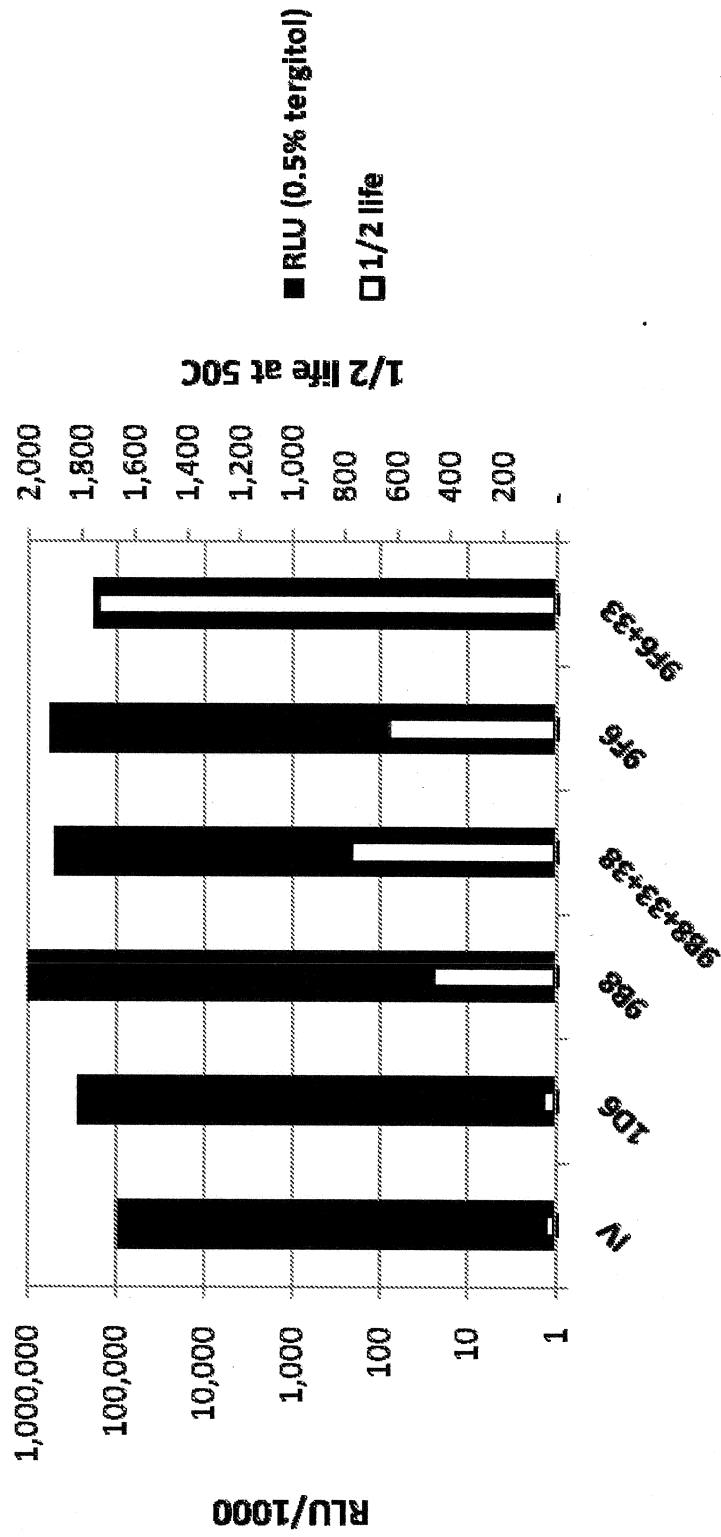


FIG. 38

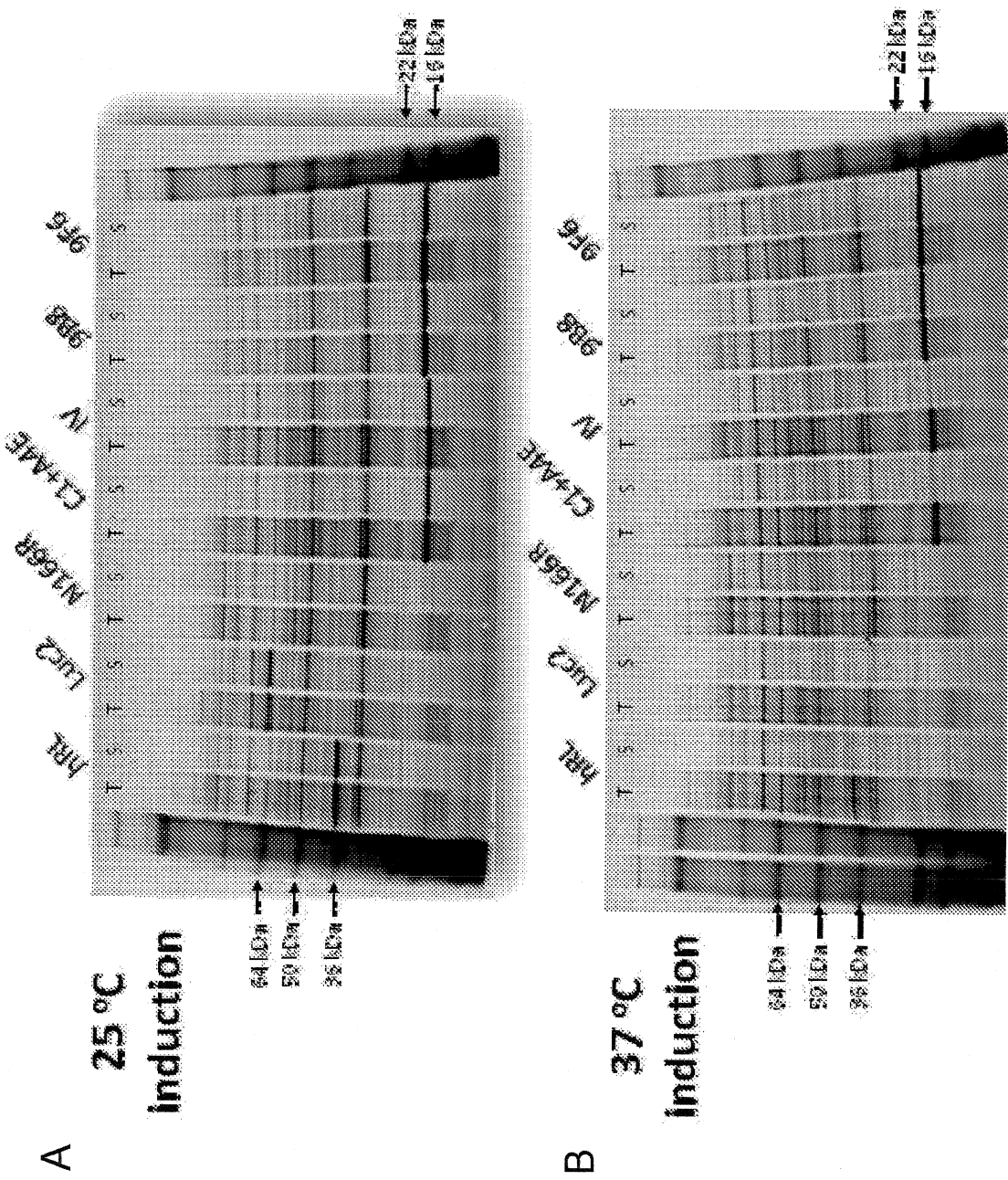
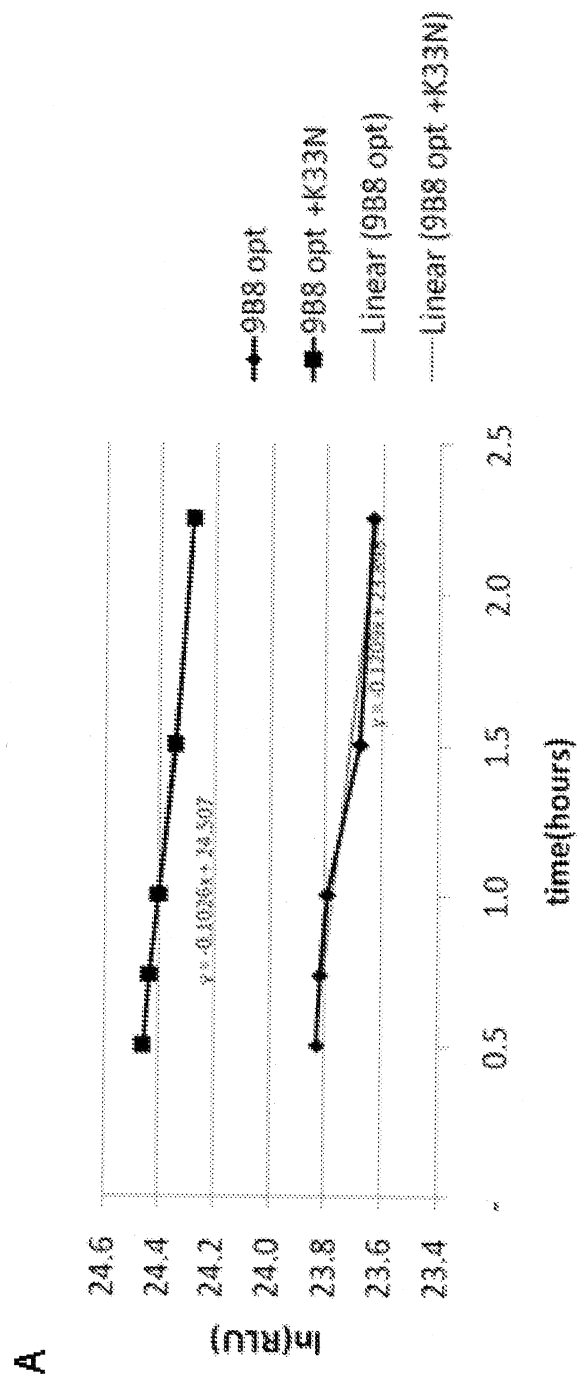


FIG. 39



B

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

FIG. 40

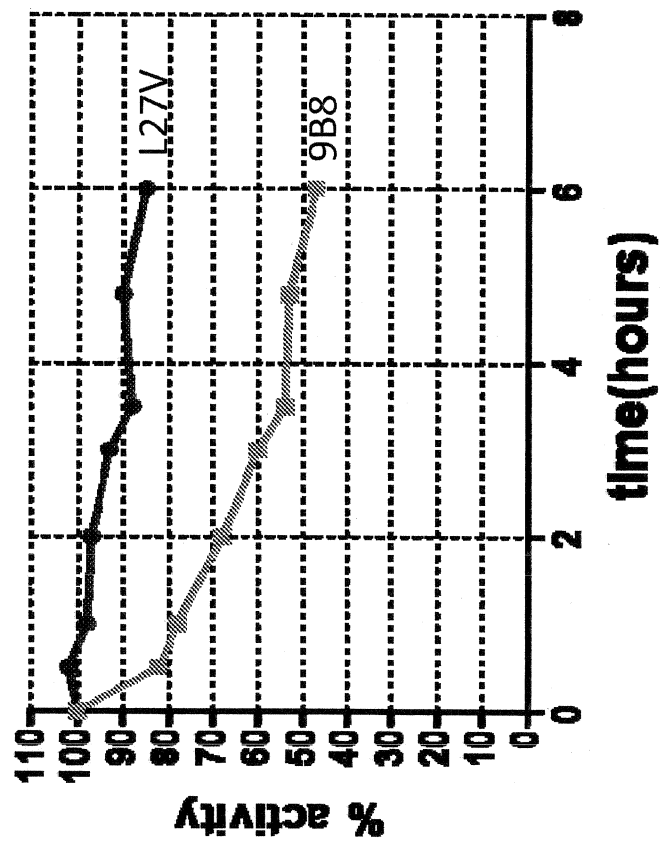


FIG. 41

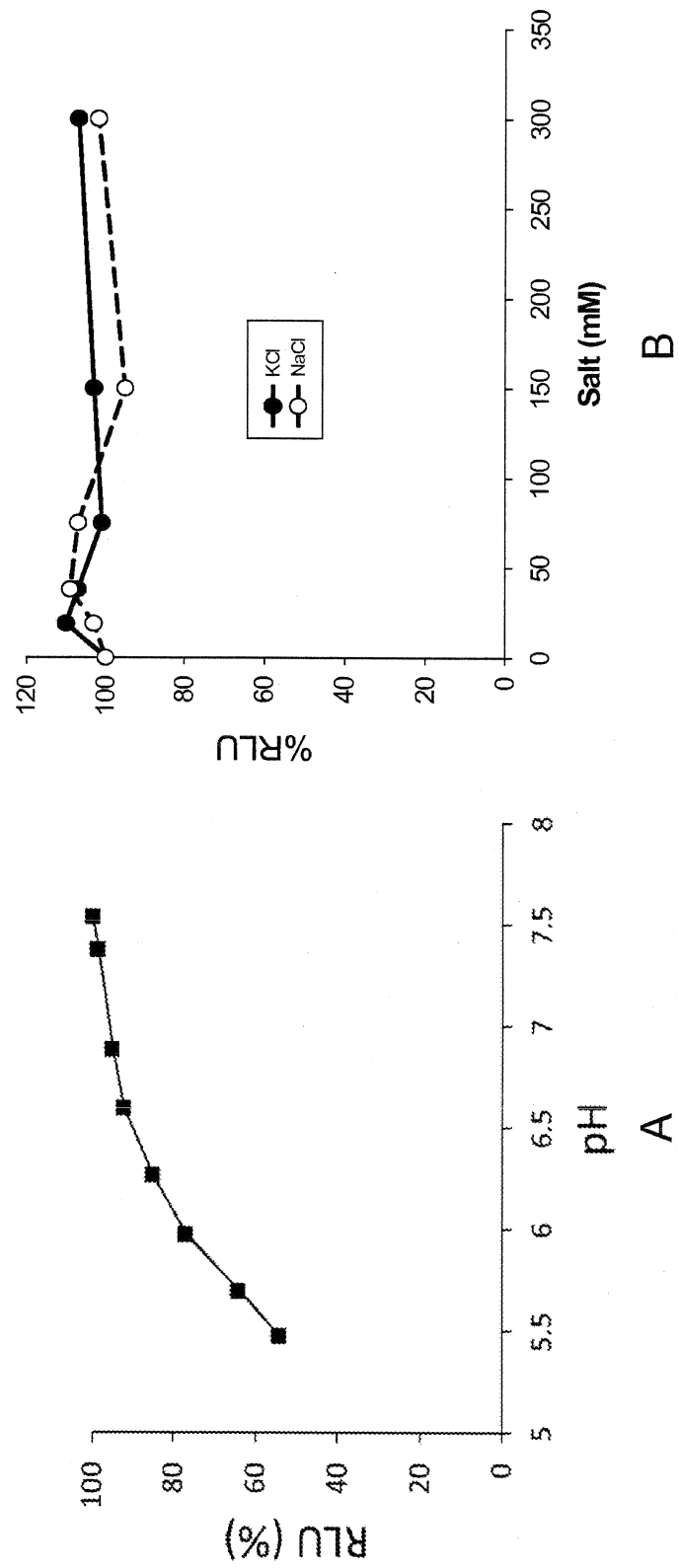


FIG. 42

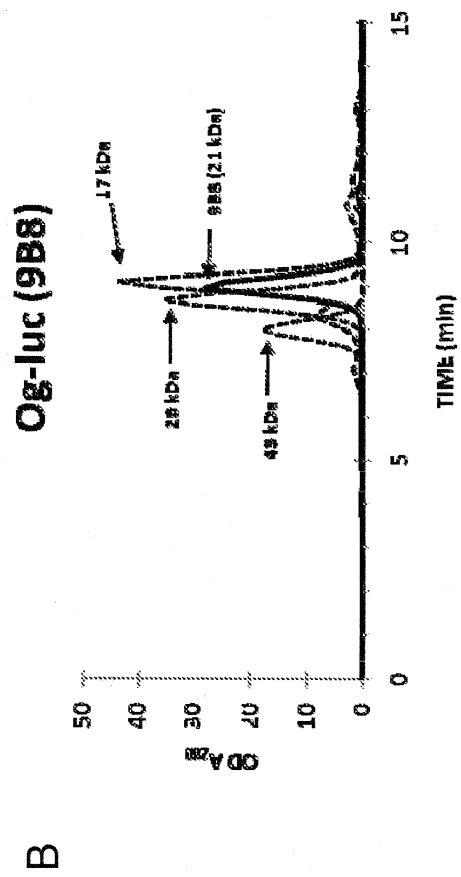
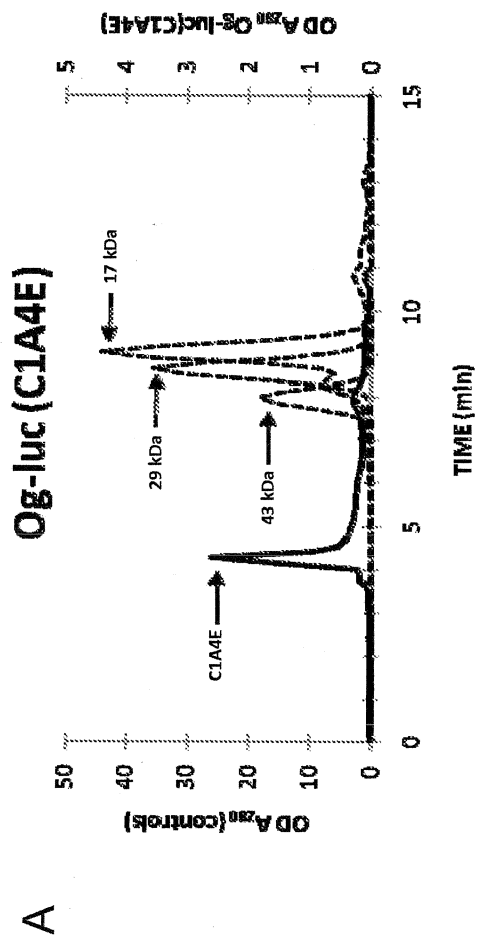


FIG. 43

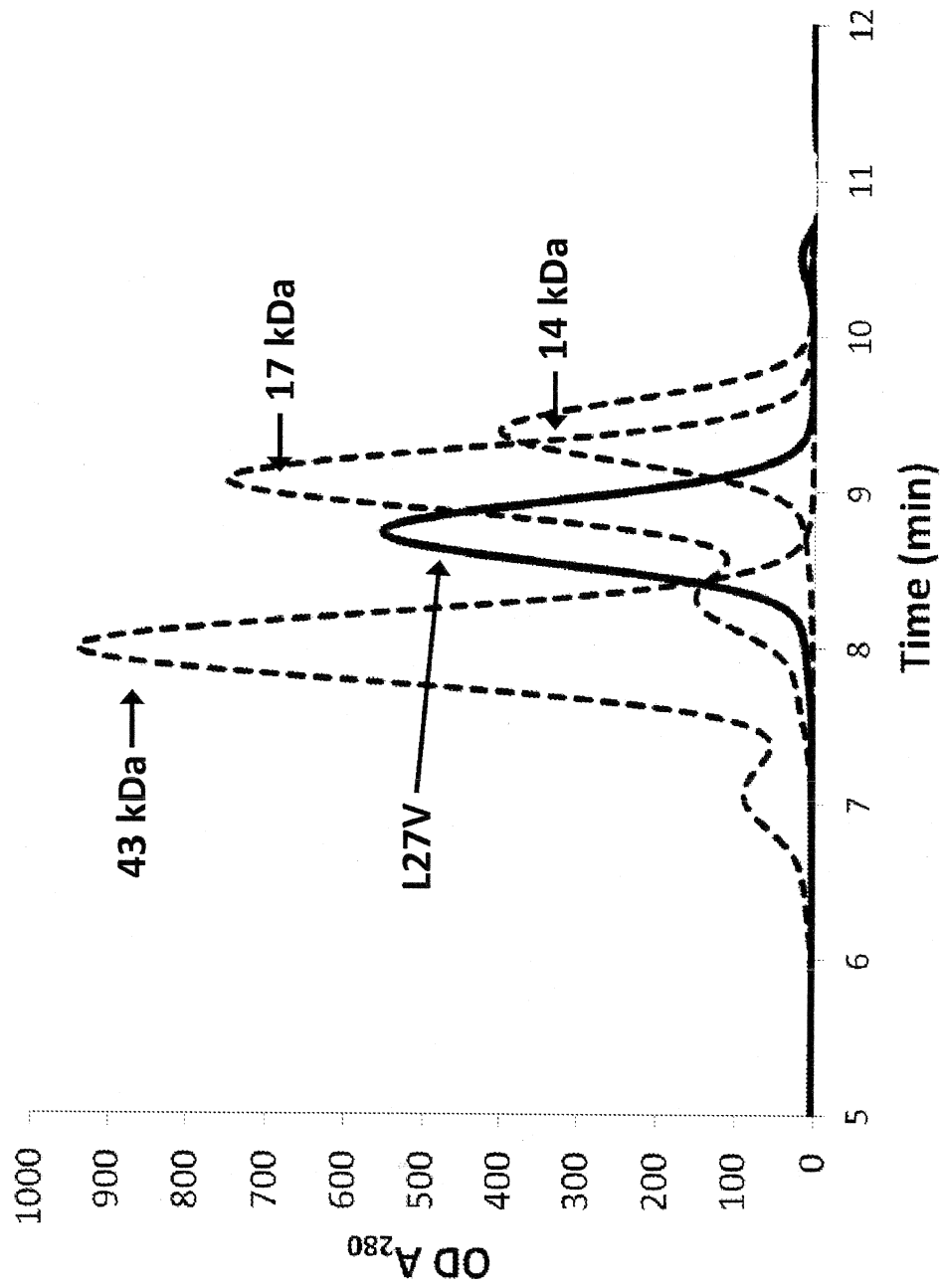


FIG. 44

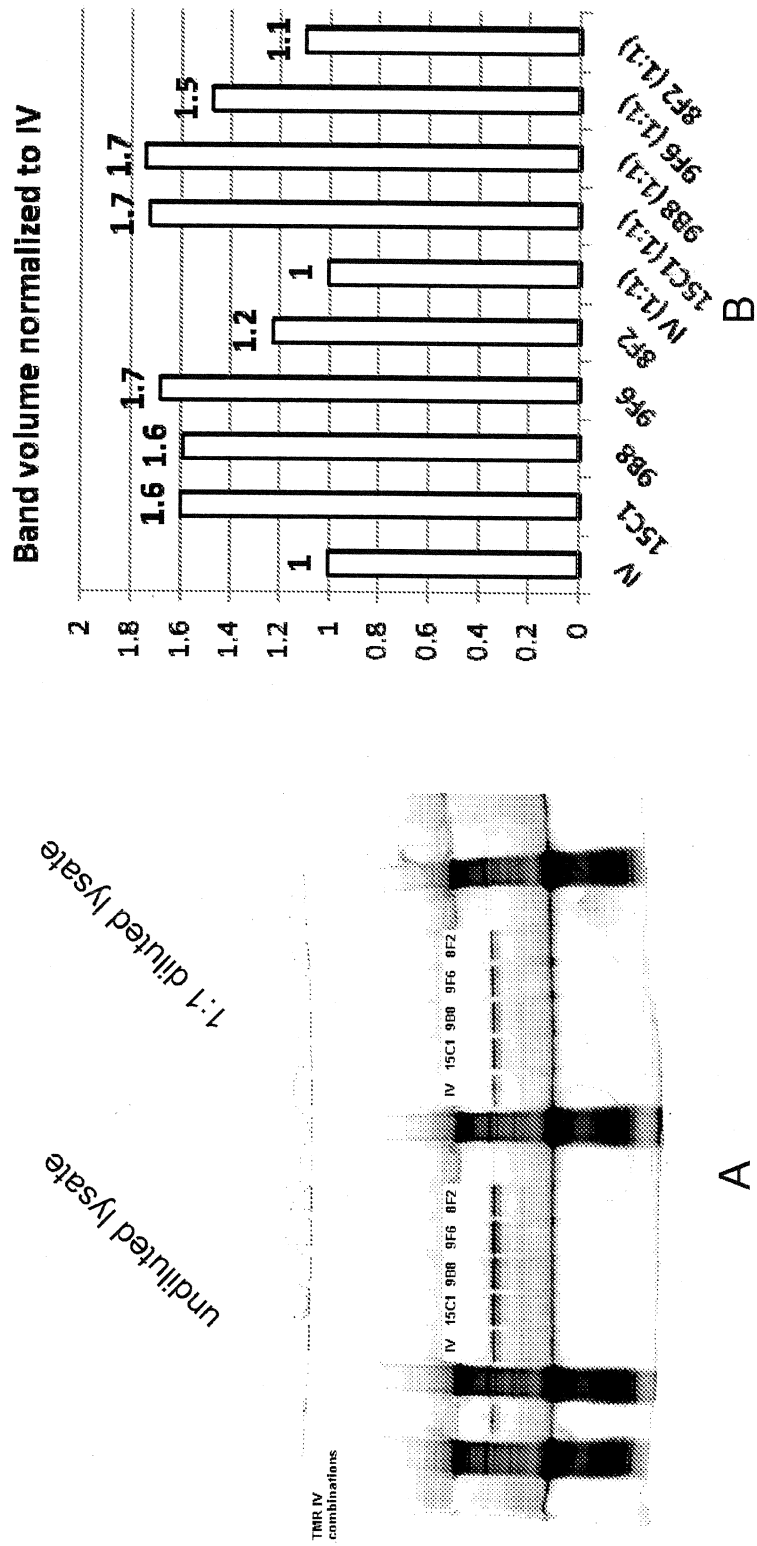


FIG. 45

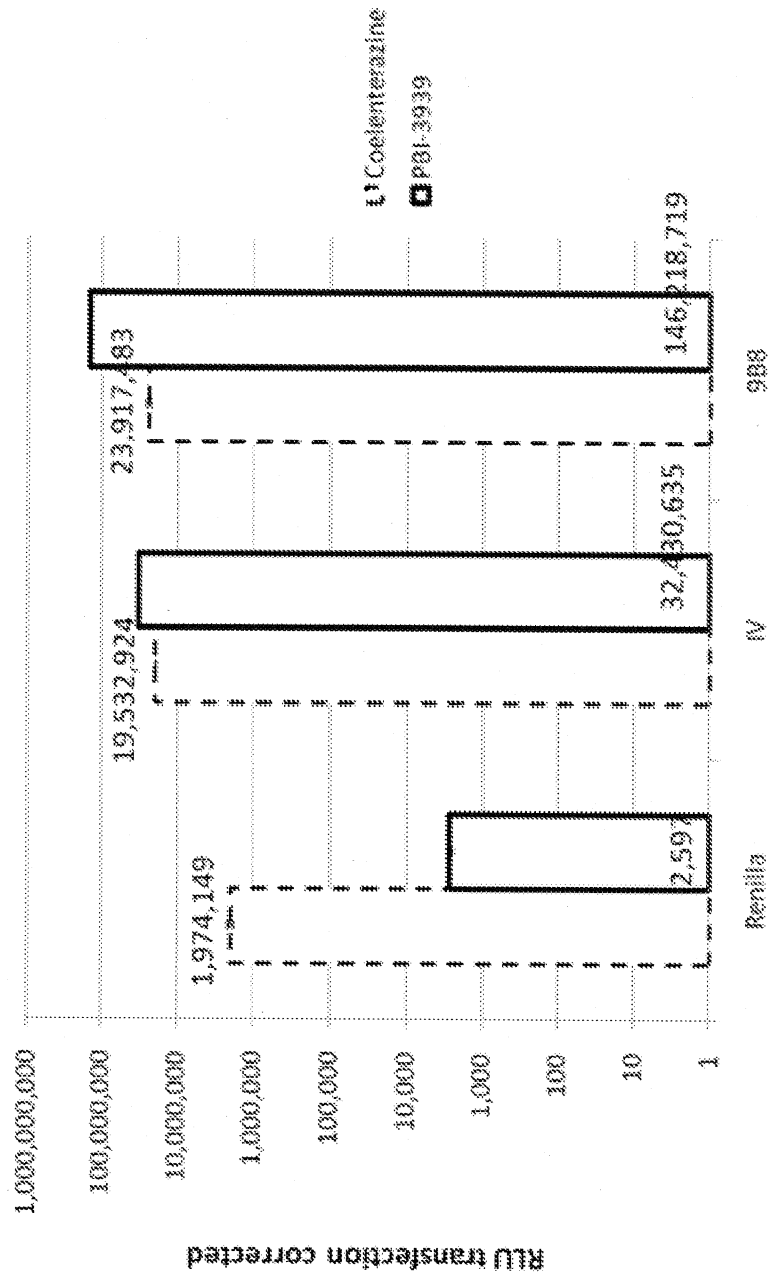


FIG. 47

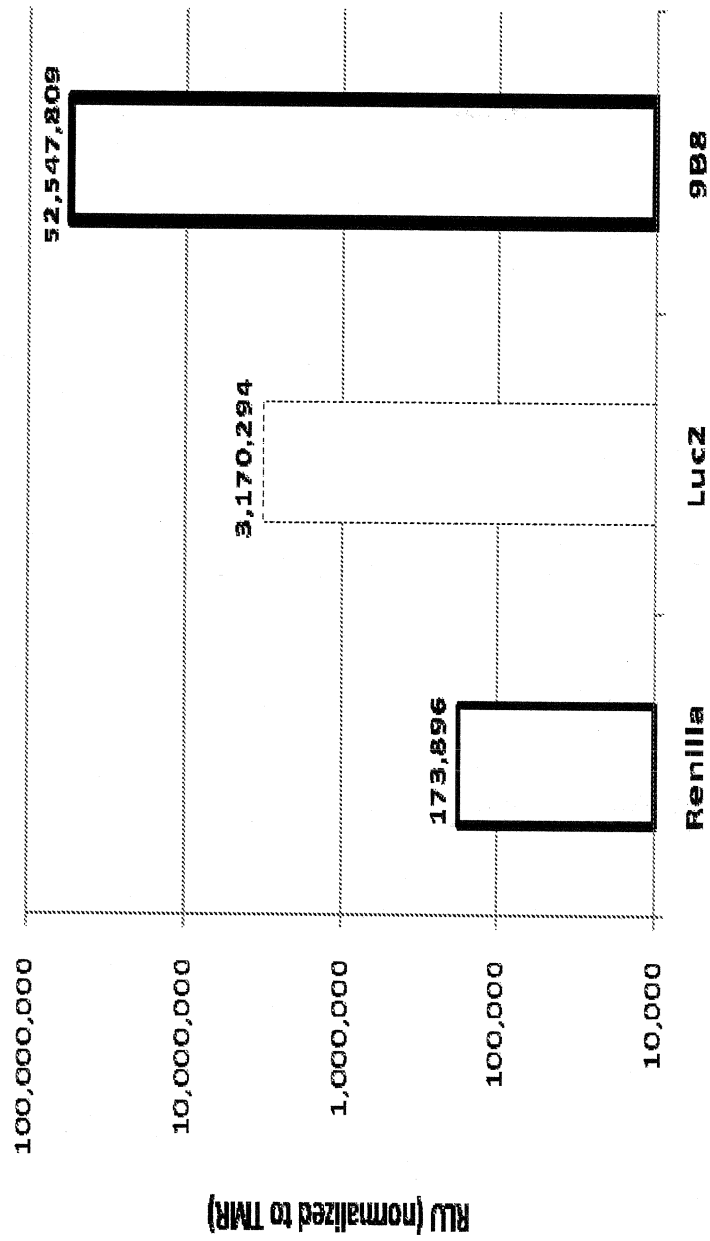


FIG. 48

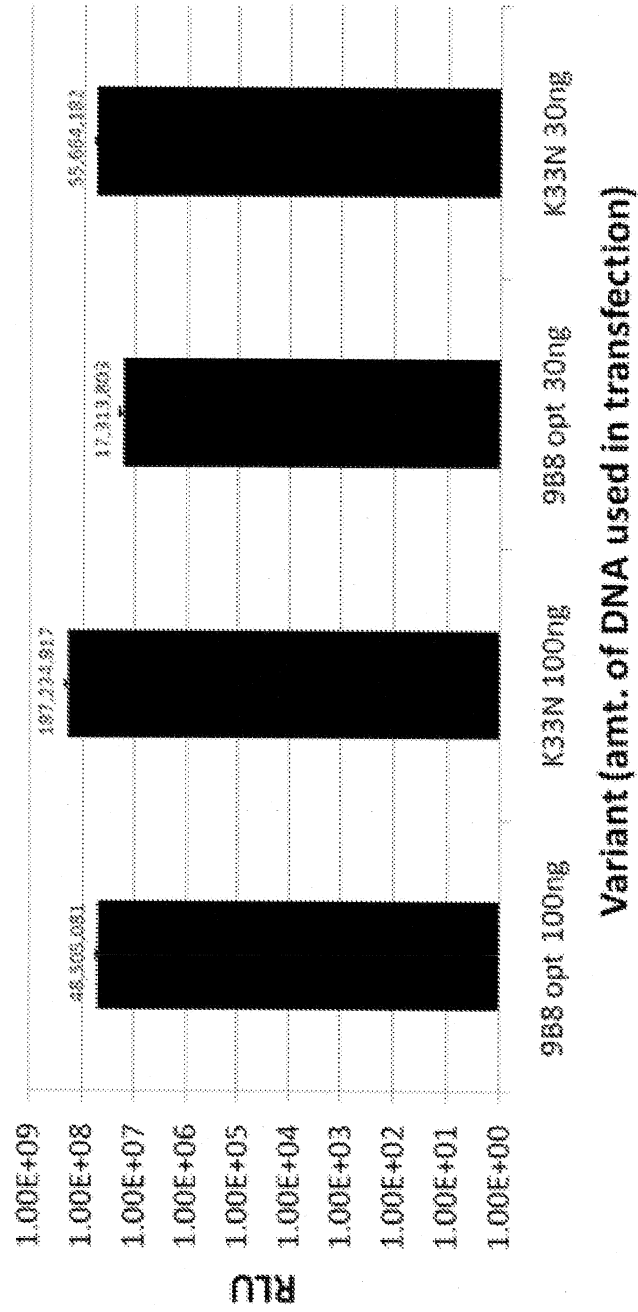


FIG. 49

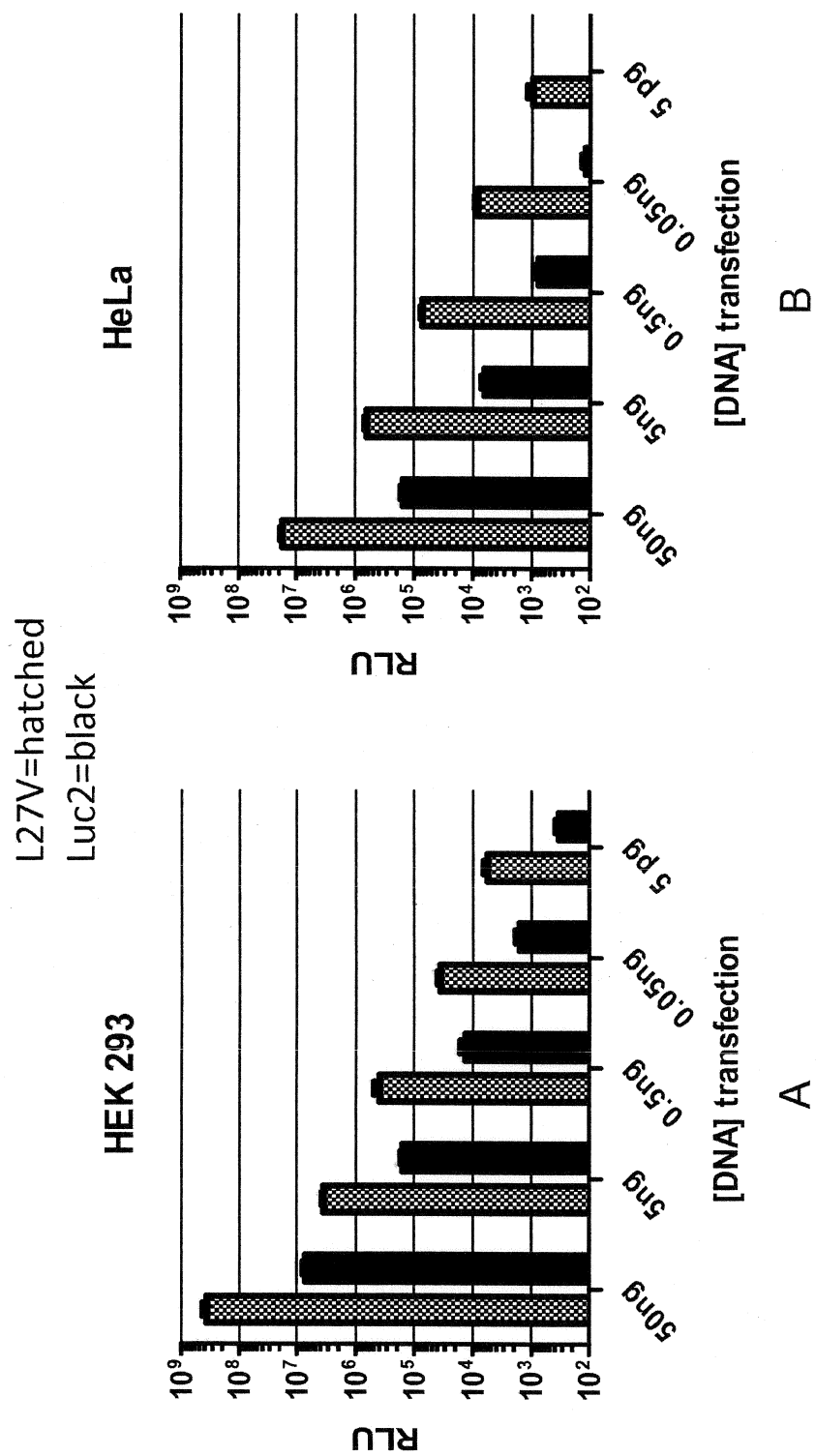


FIG. 50

L27V=hatched,
Luc2=black

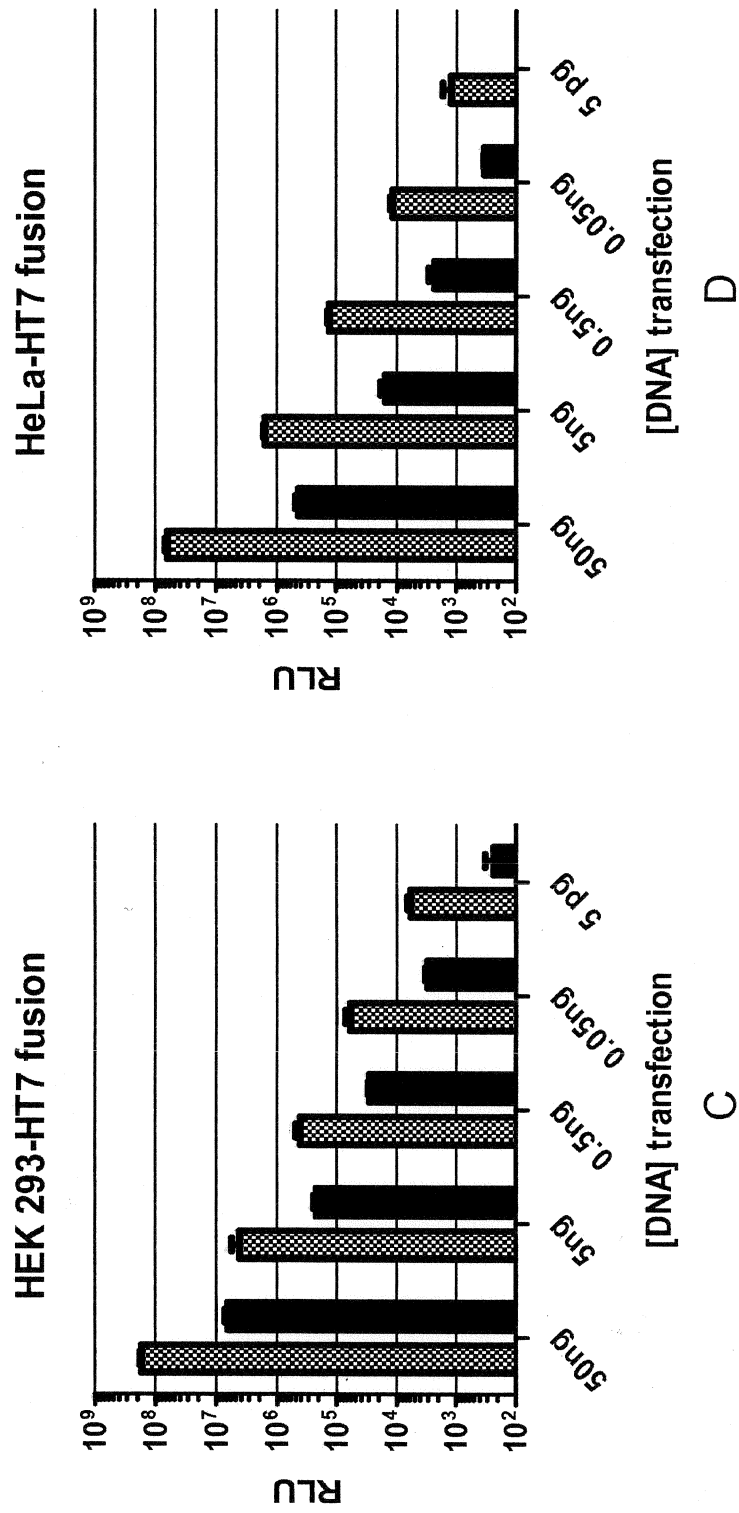
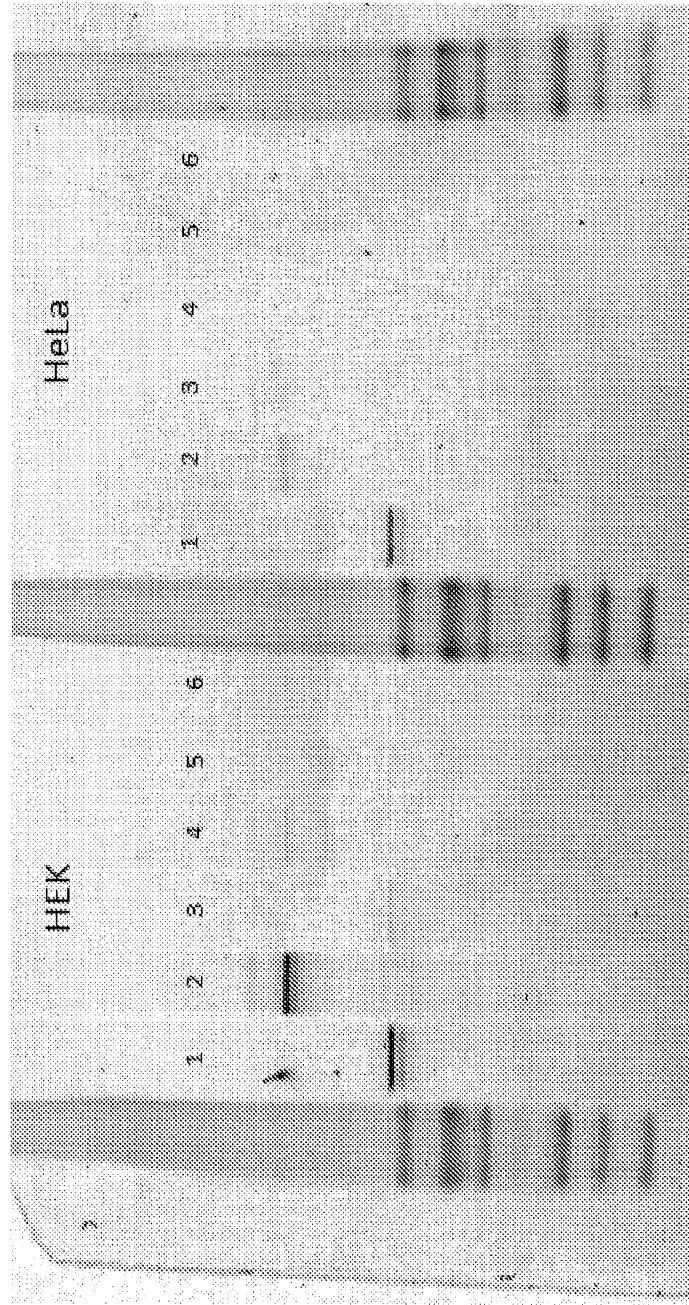


FIG. 50



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

FIG. 50

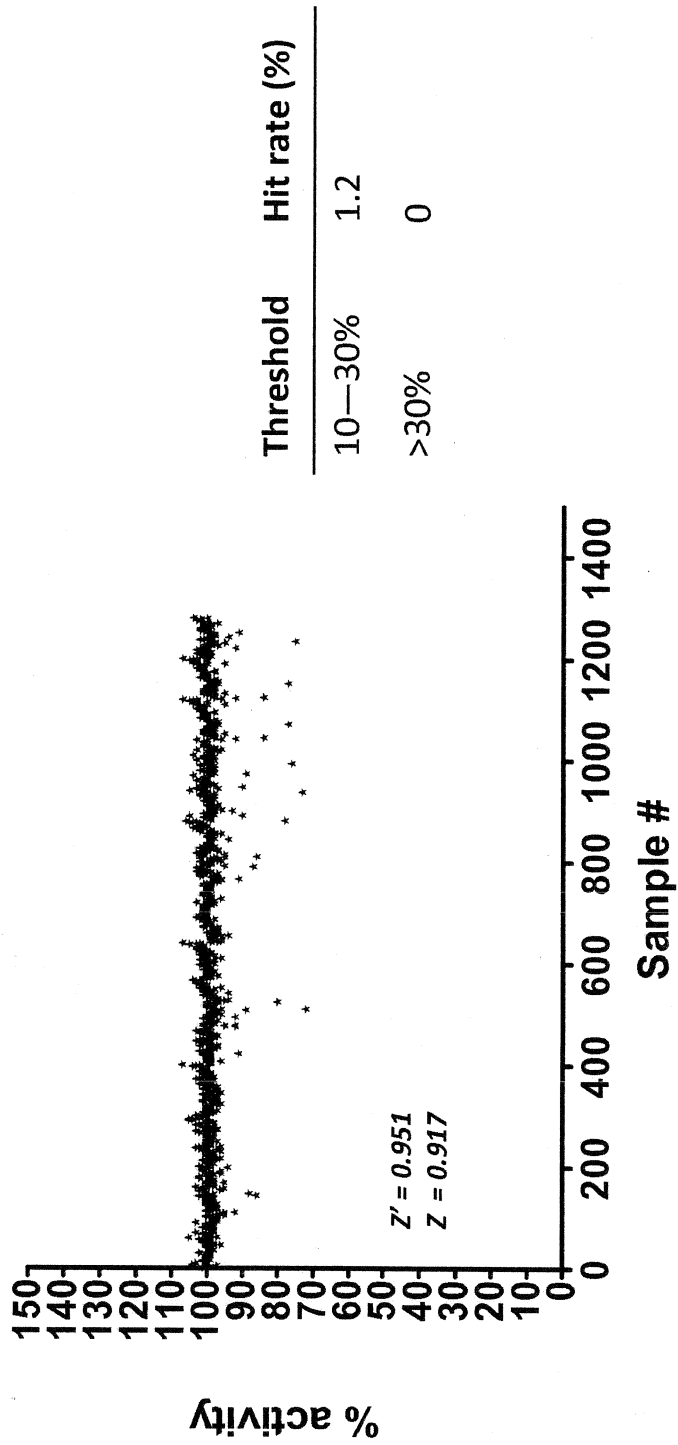
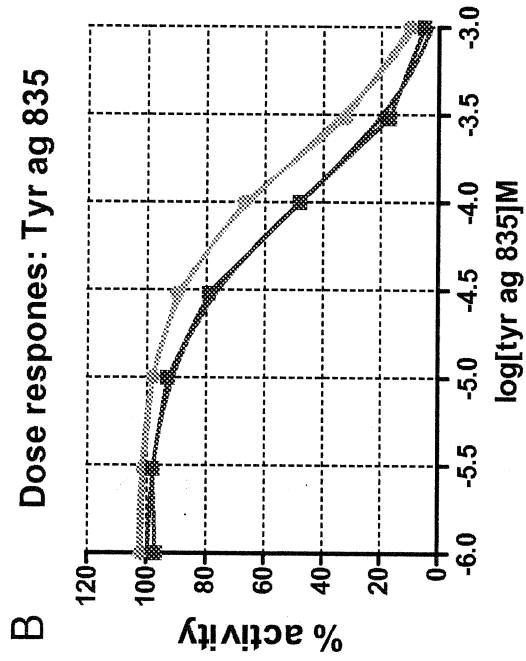
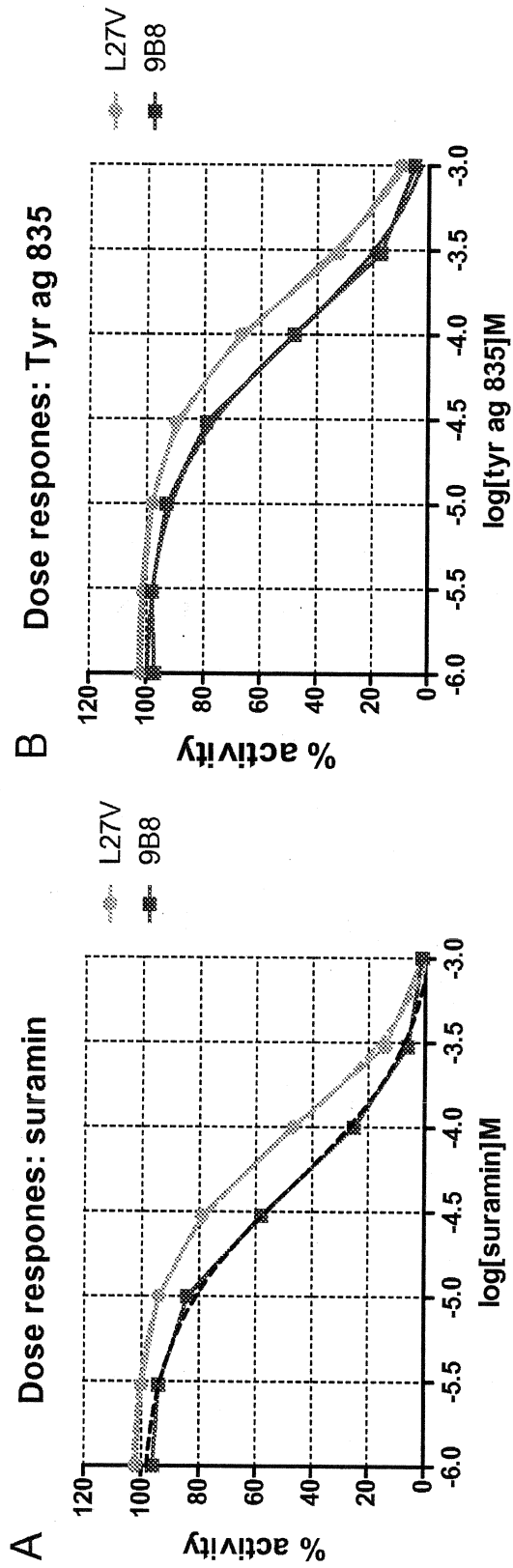
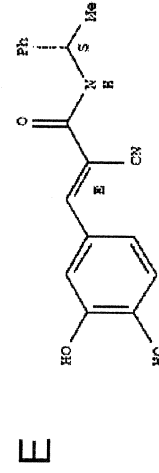
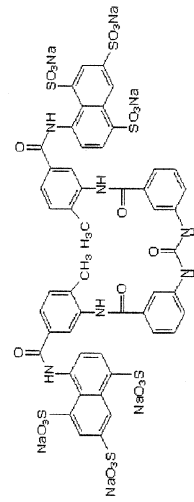


FIG. 51



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



Tyr ag 835

Suramin

FIG. 52

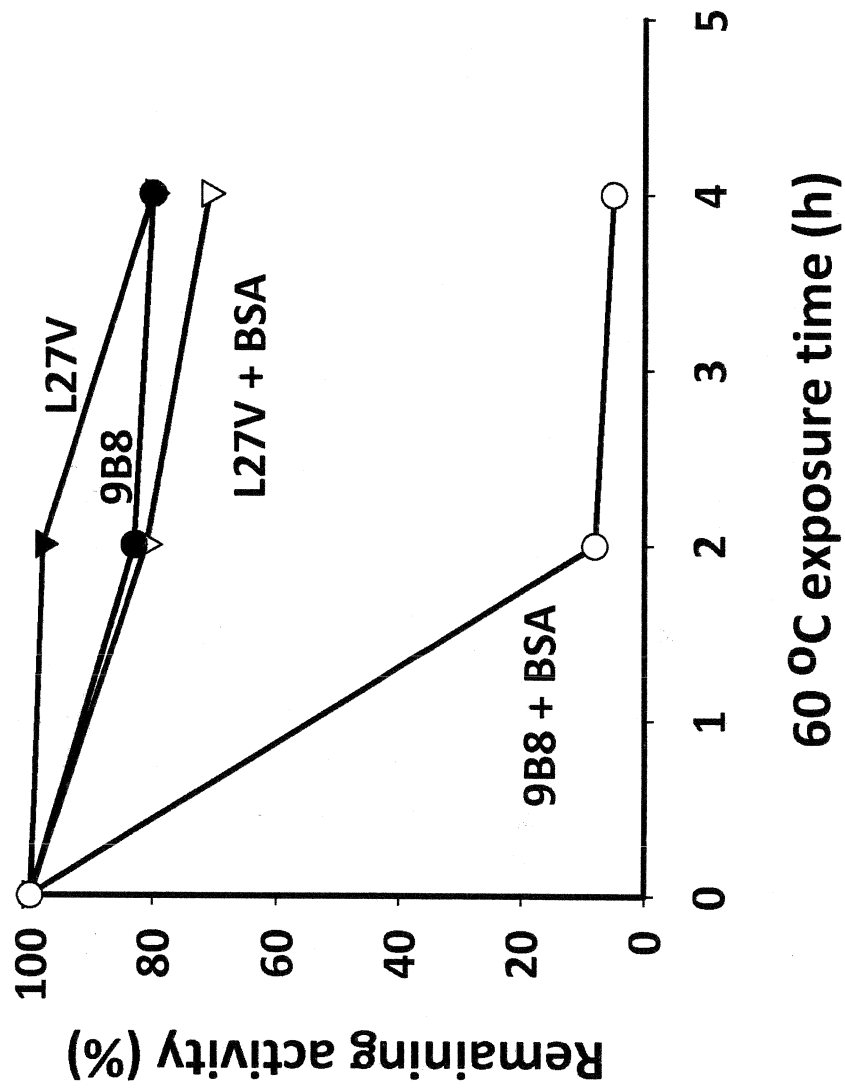


FIG. 53

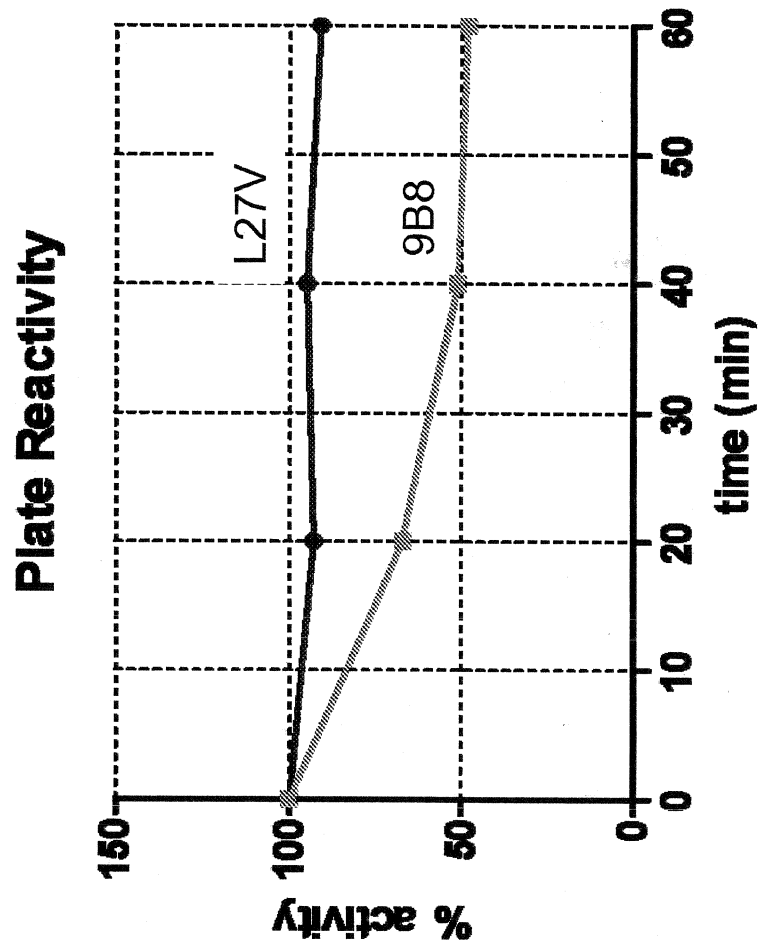


FIG. 54

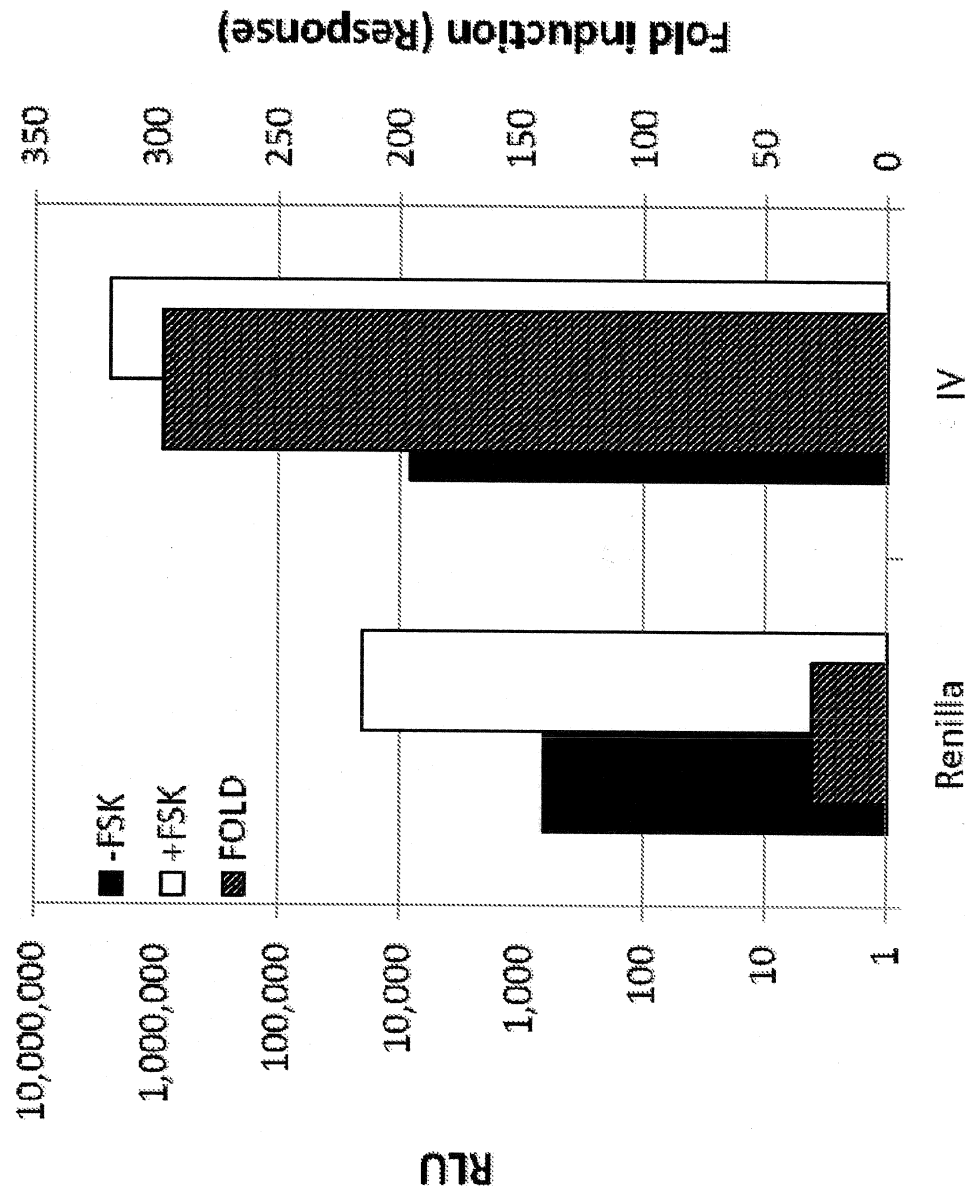


FIG. 55

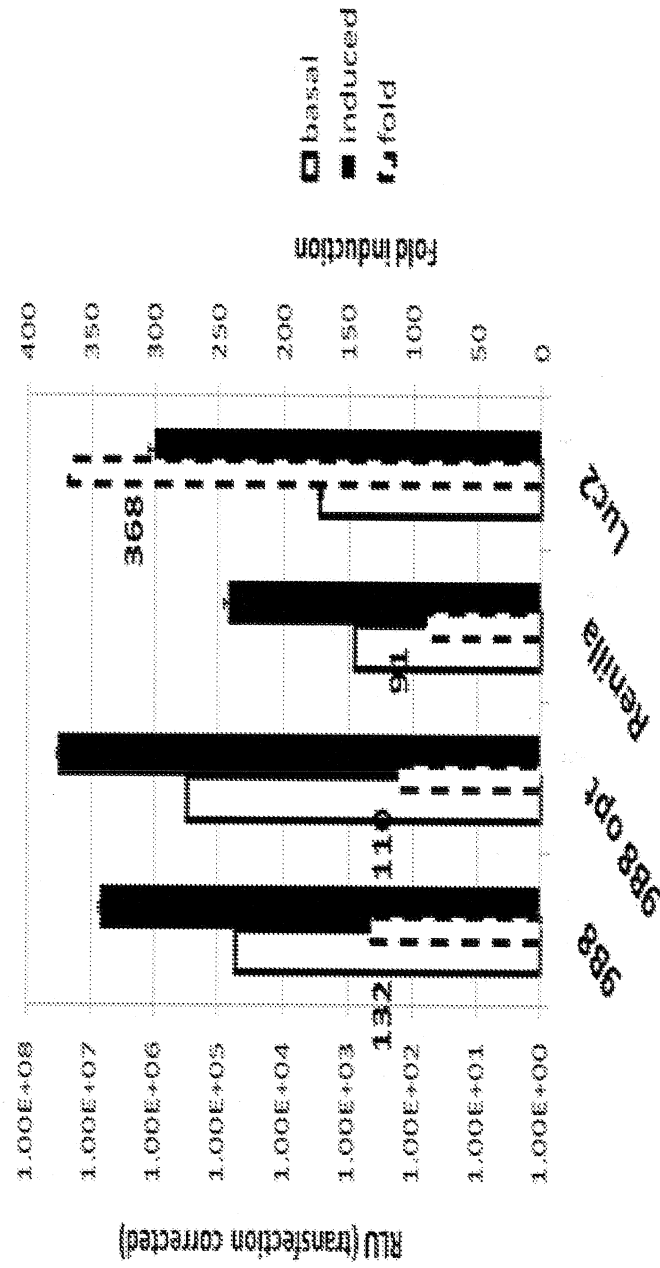


FIG. 56

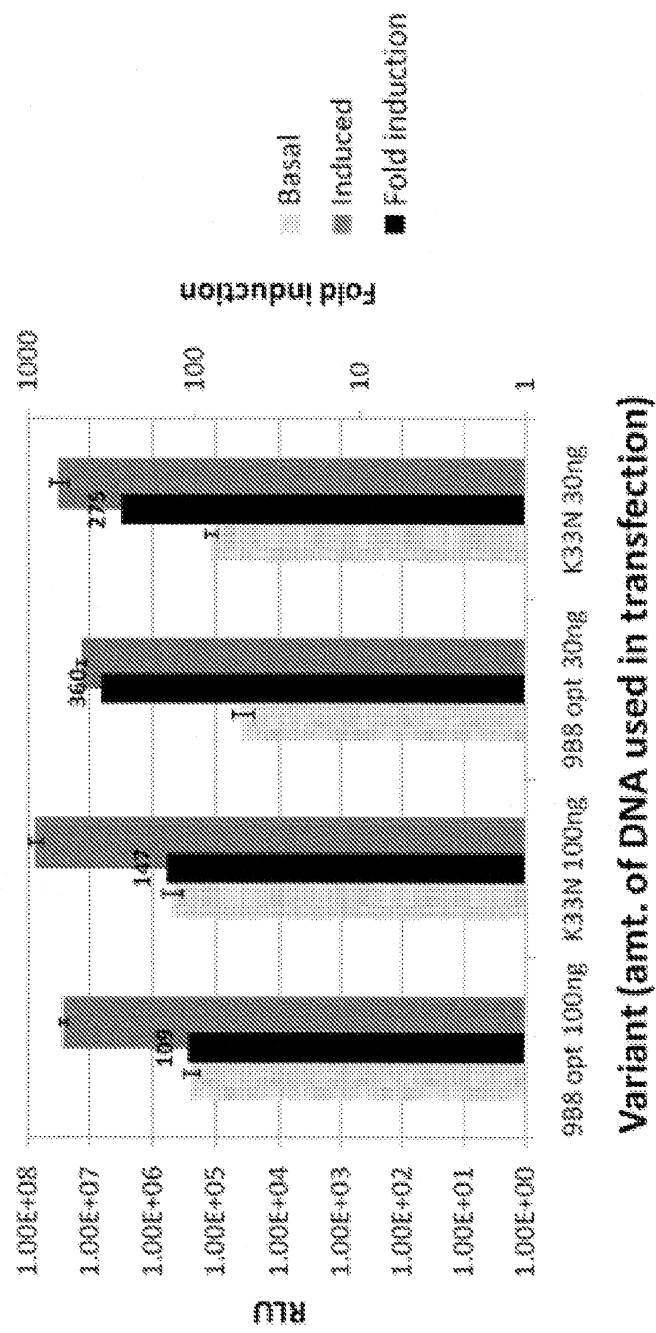


FIG. 57

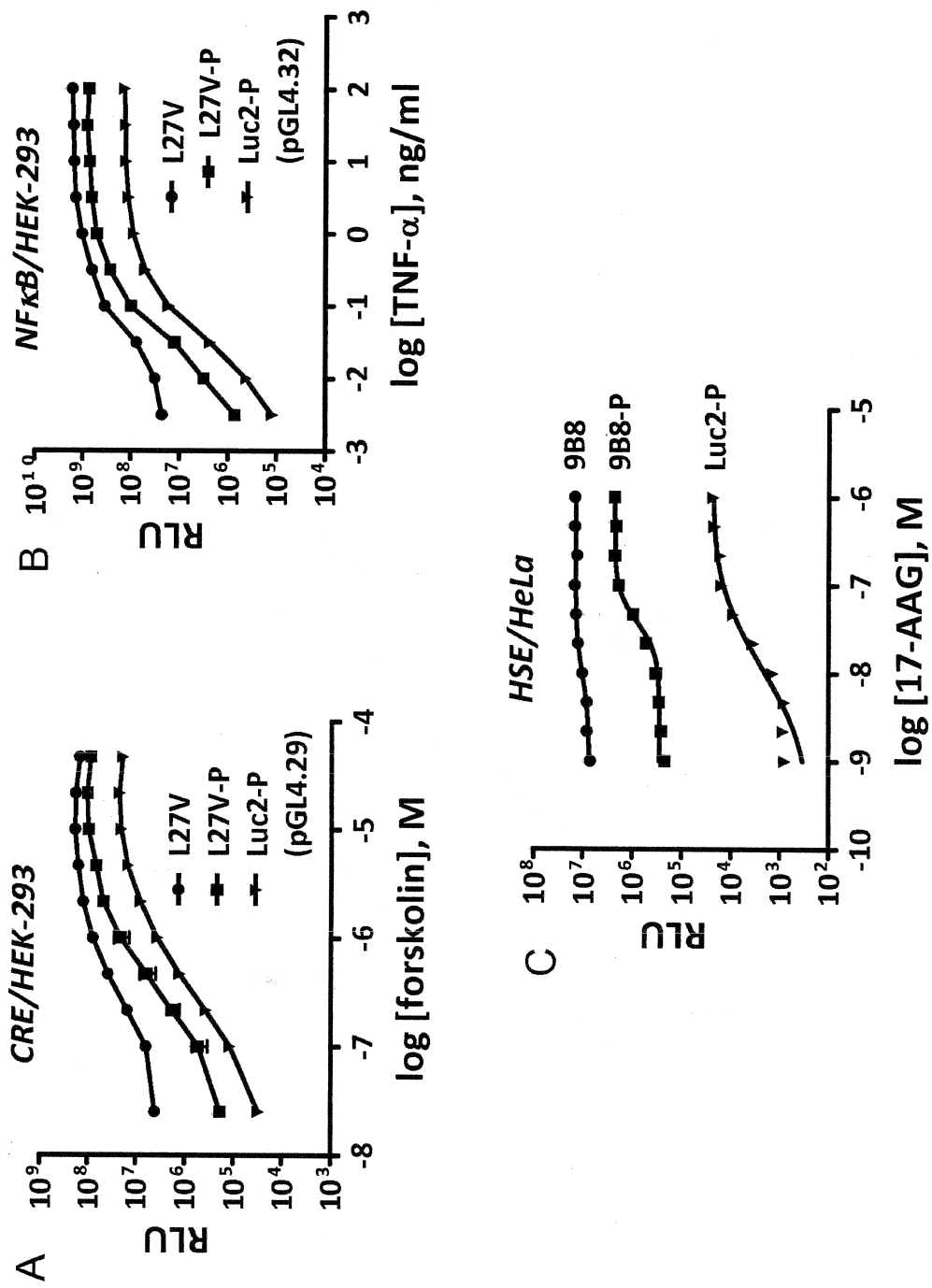
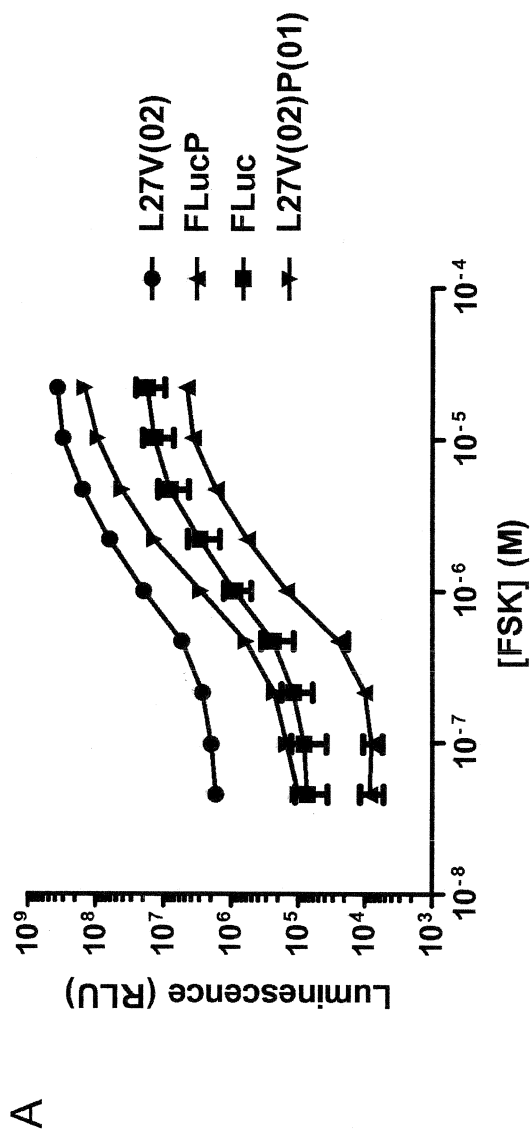


FIG. 58



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

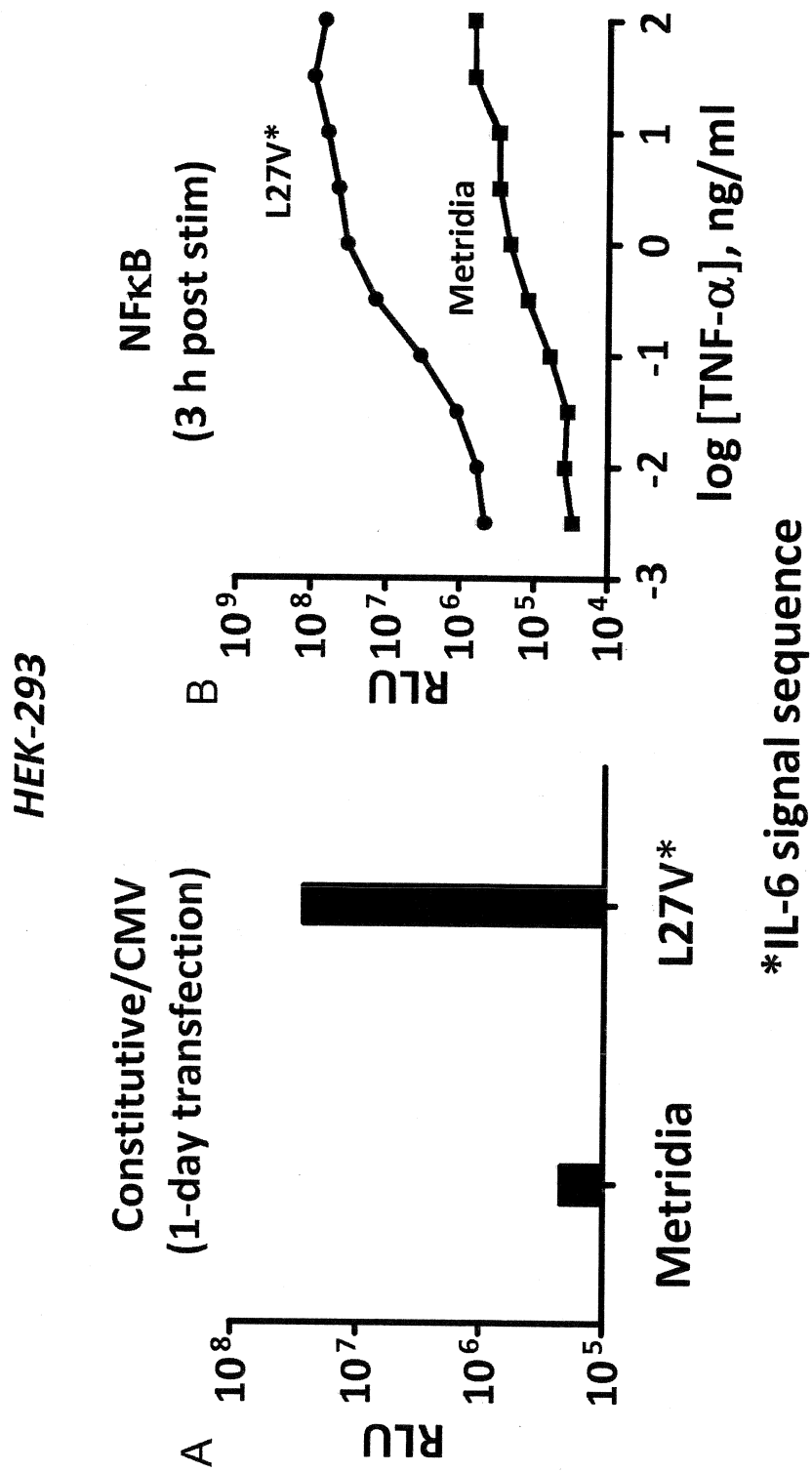


FIG. 60

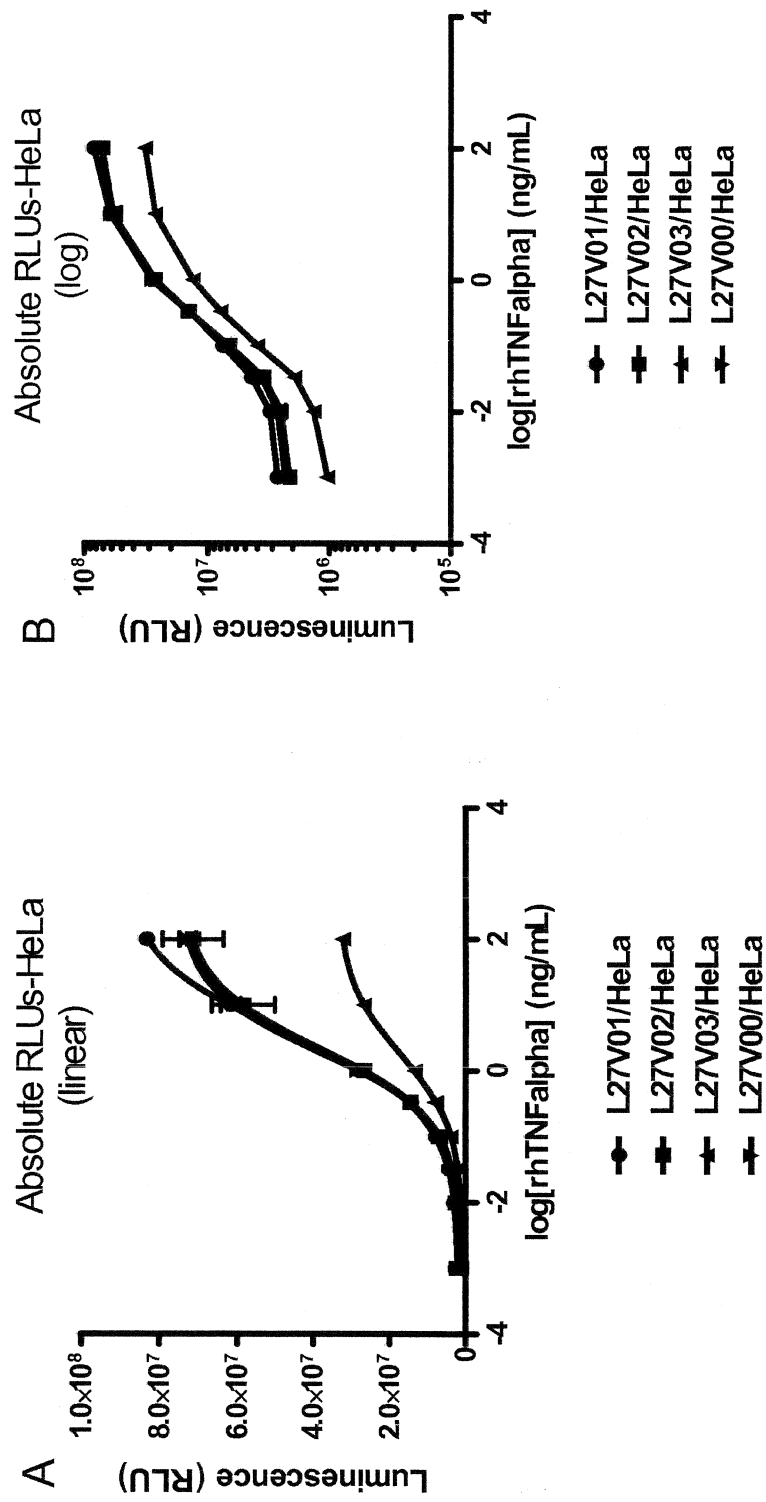


FIG. 61

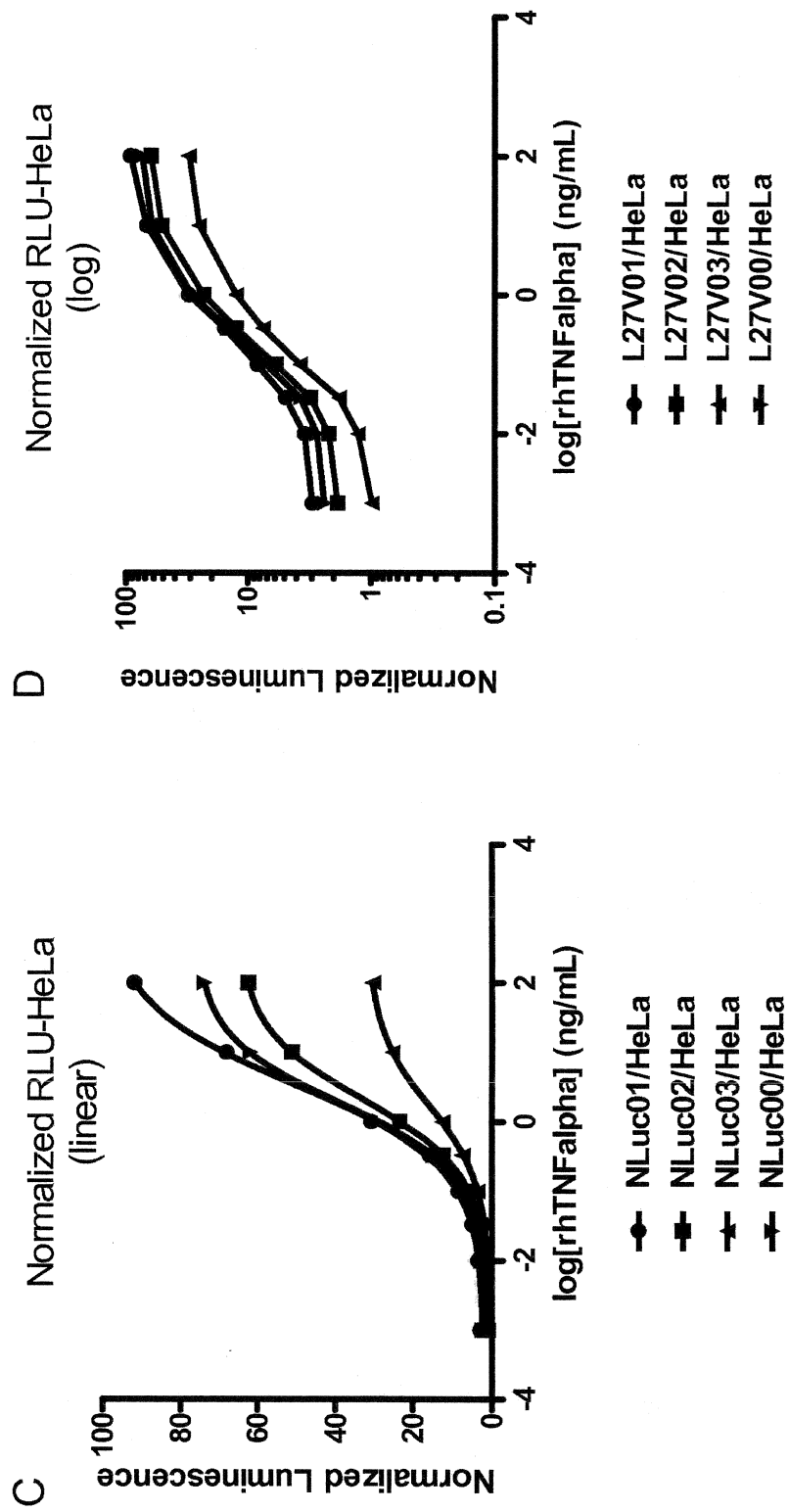


FIG. 61

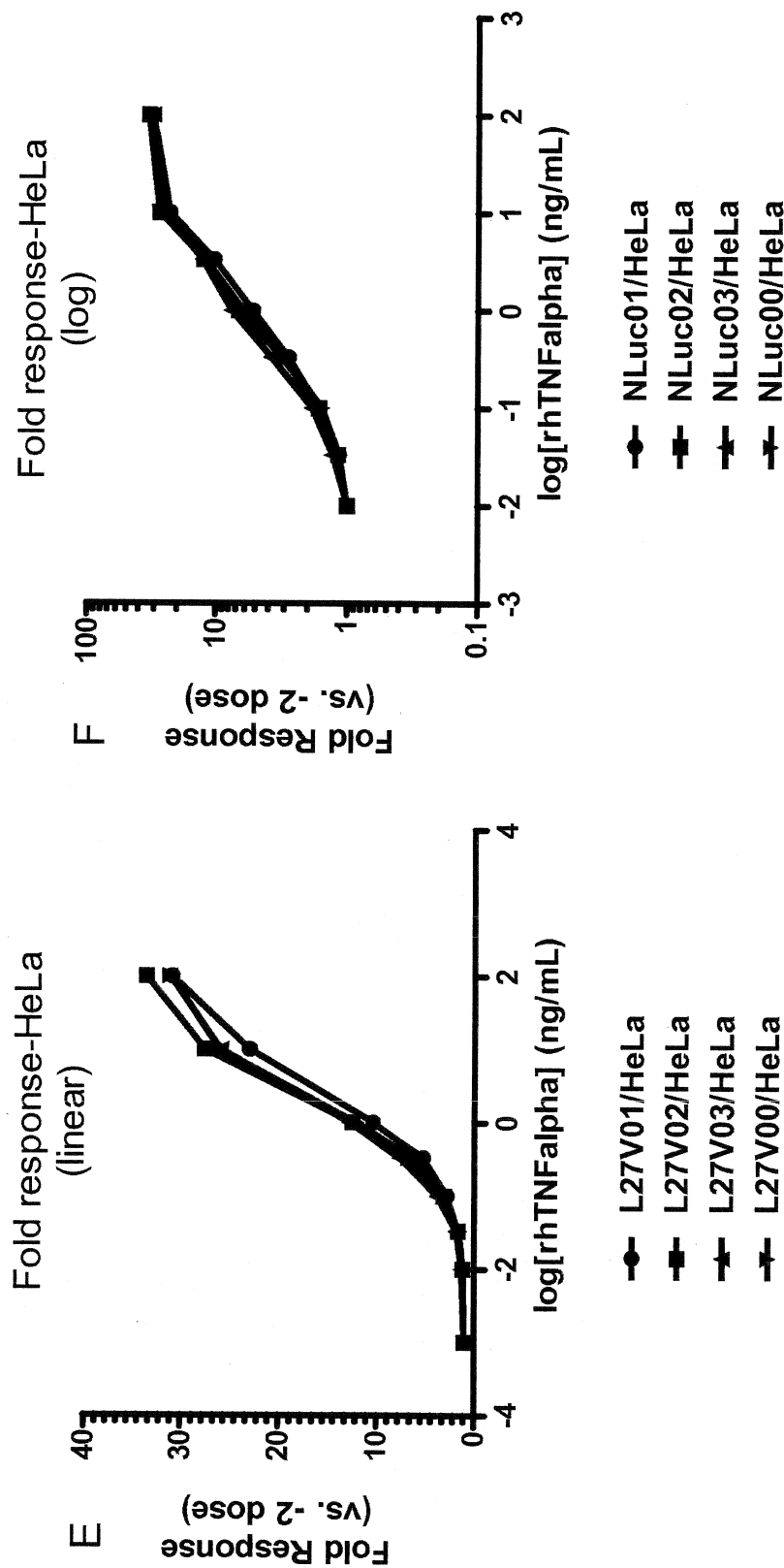


FIG. 61

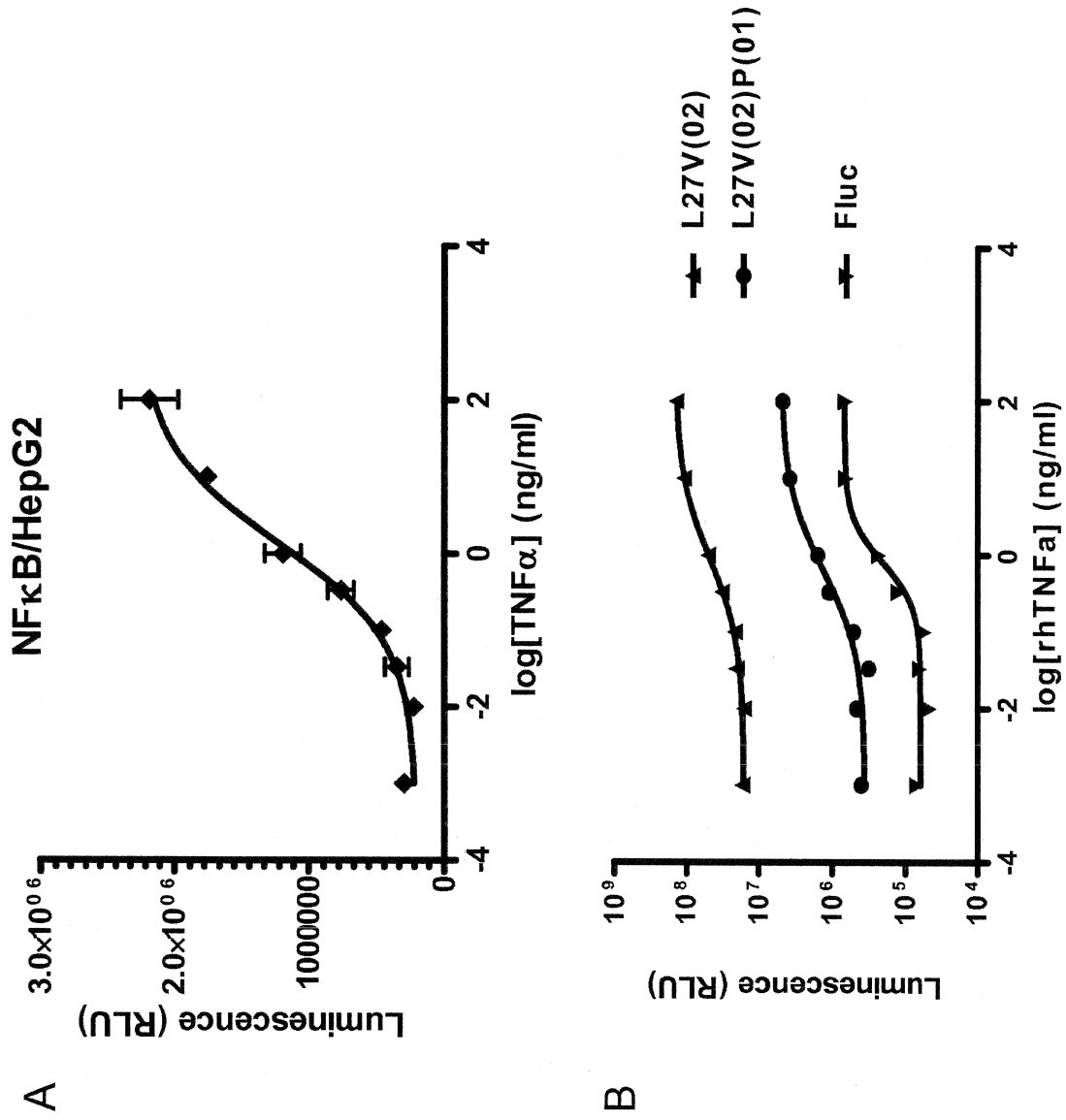


FIG. 62

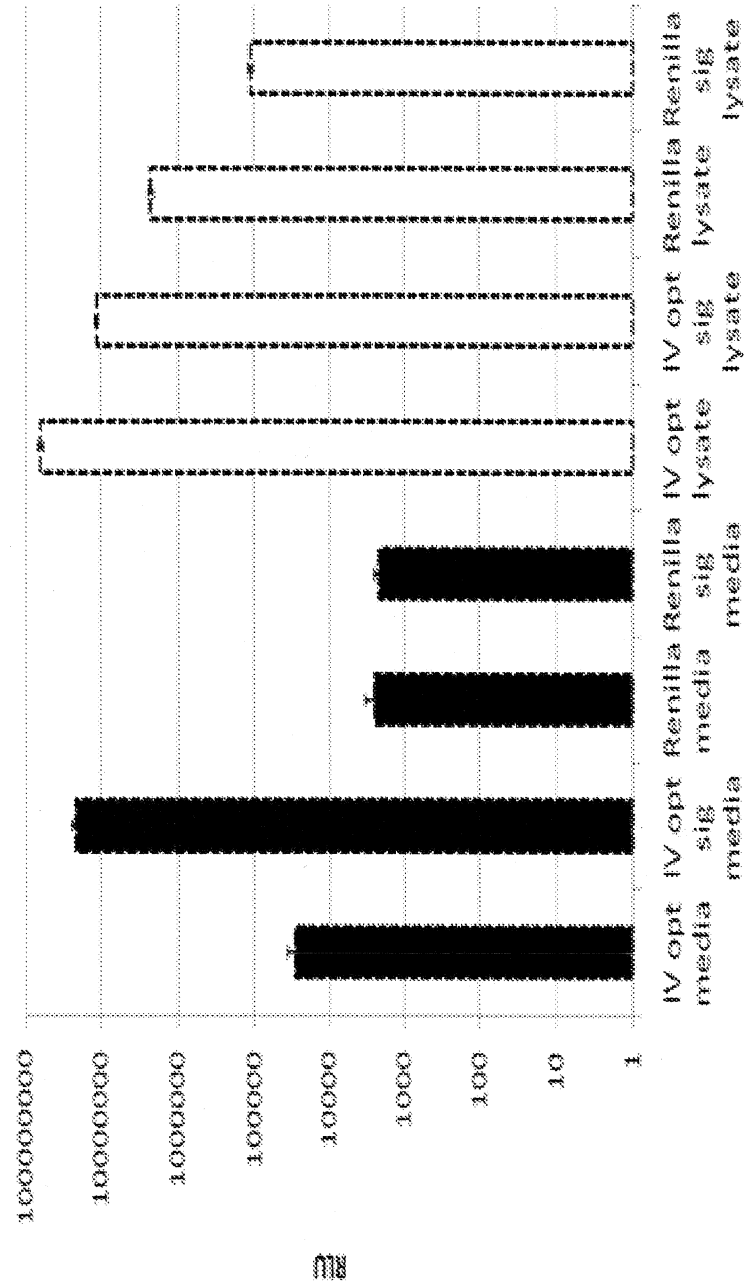
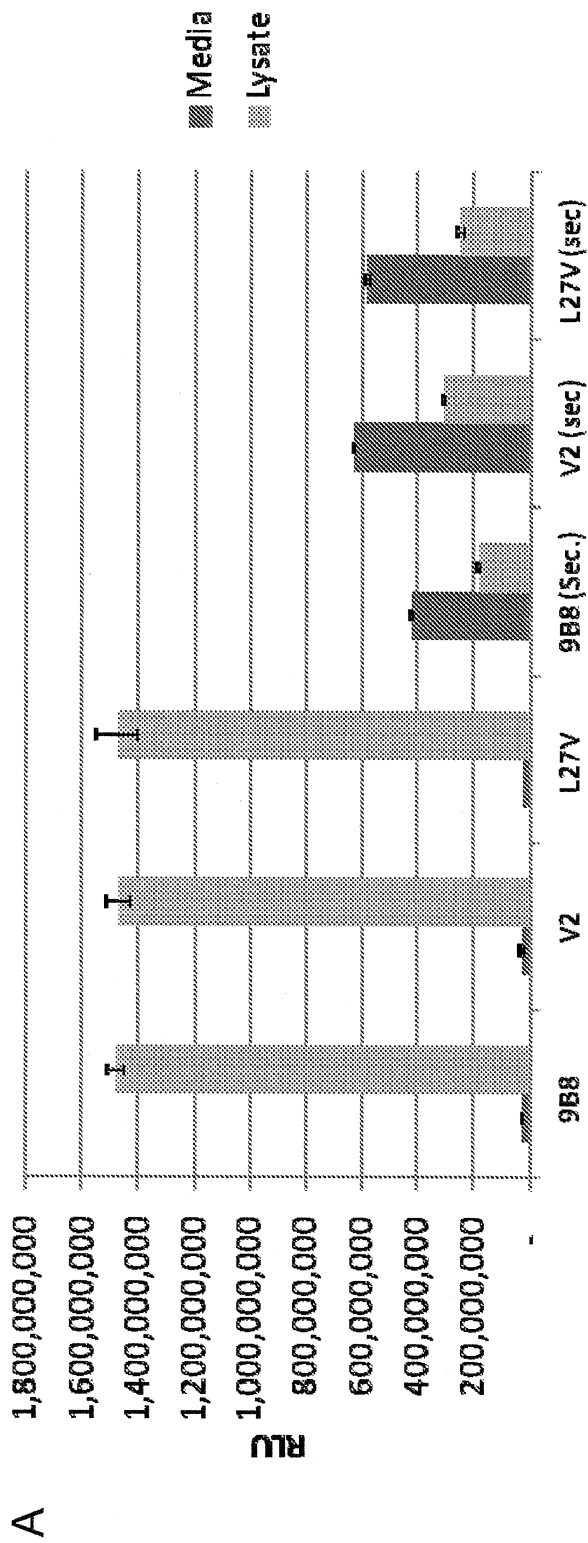


FIG. 63



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

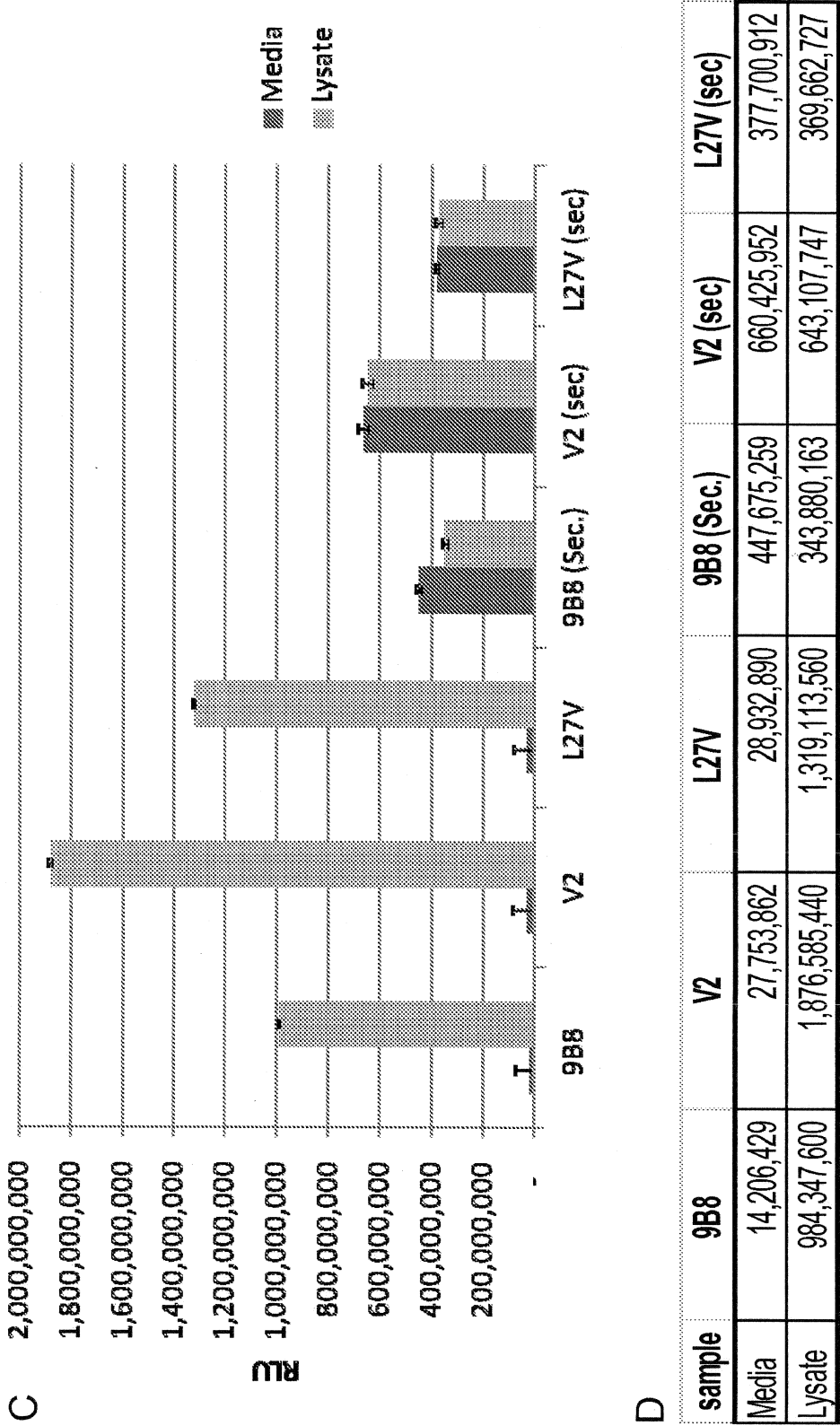


FIG. 64

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

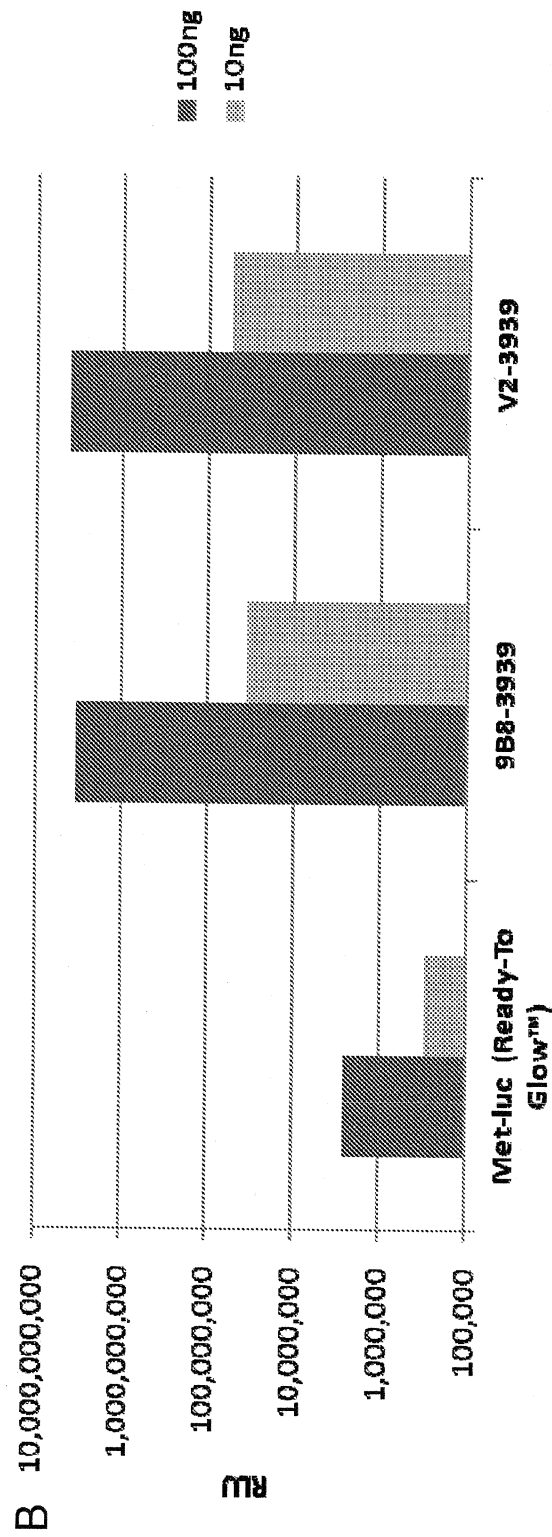


FIG. 65

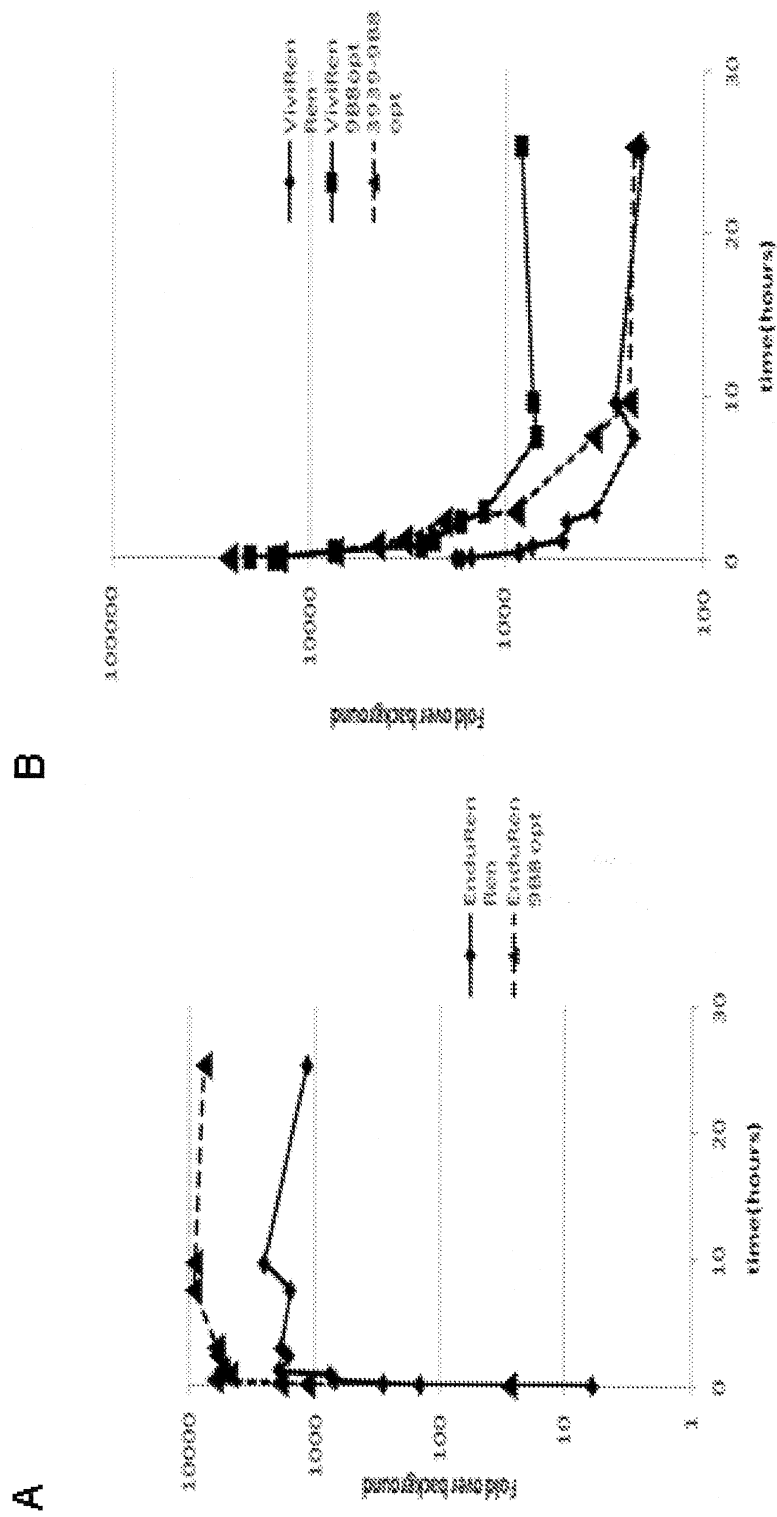
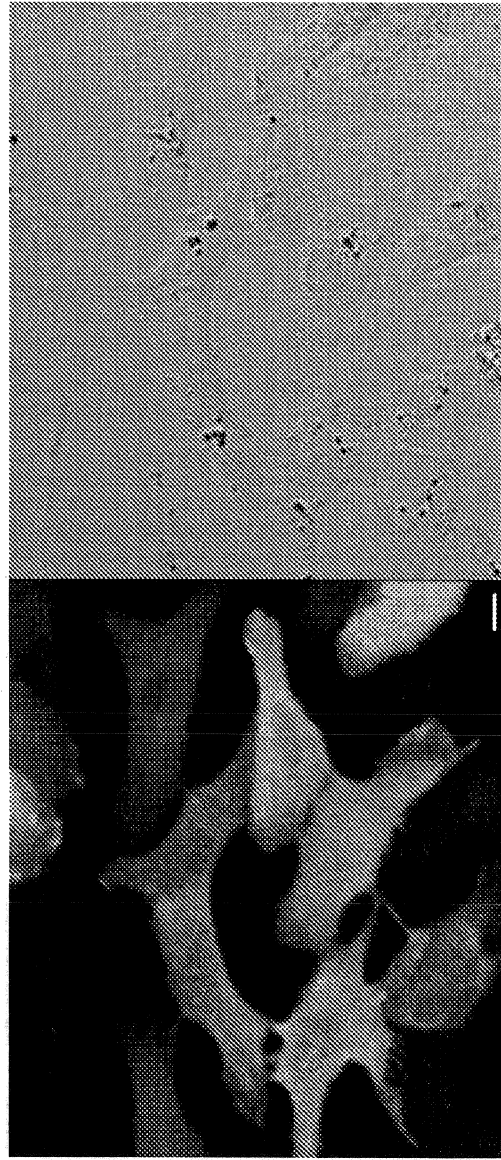


FIG. 66



A

FIG. 67

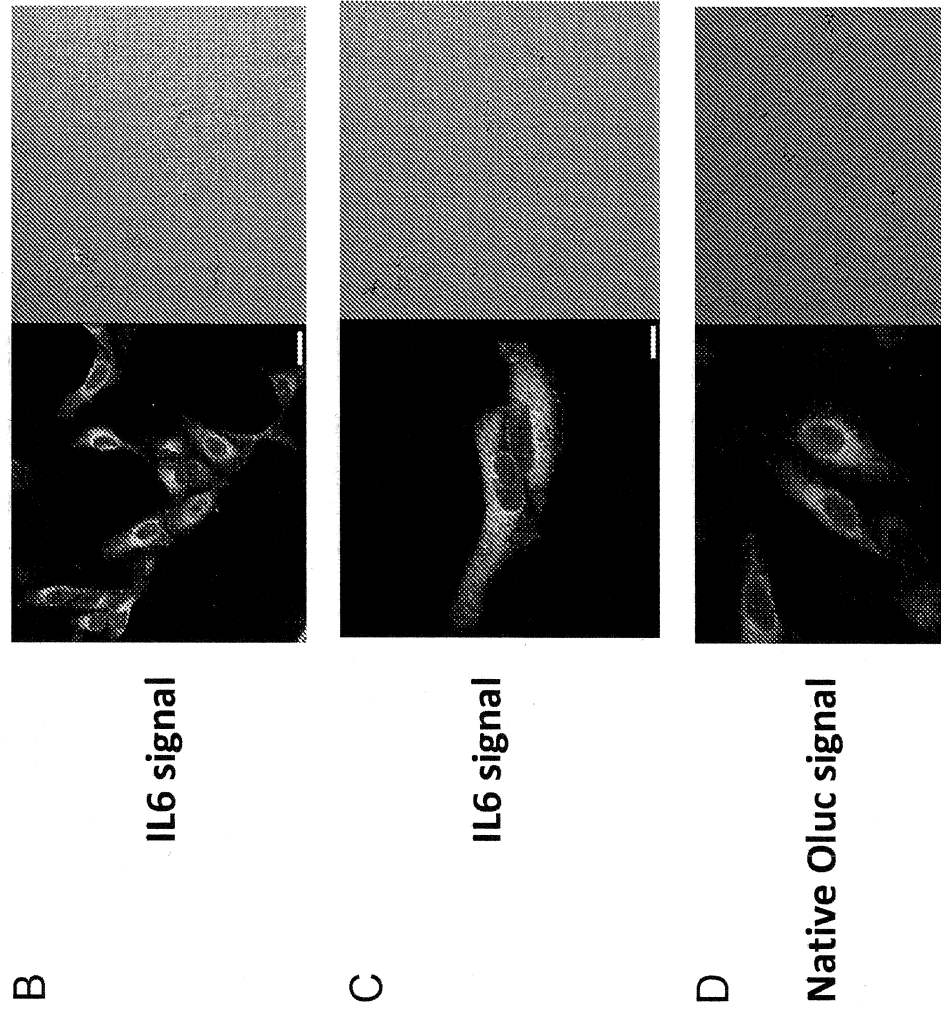


FIG. 67

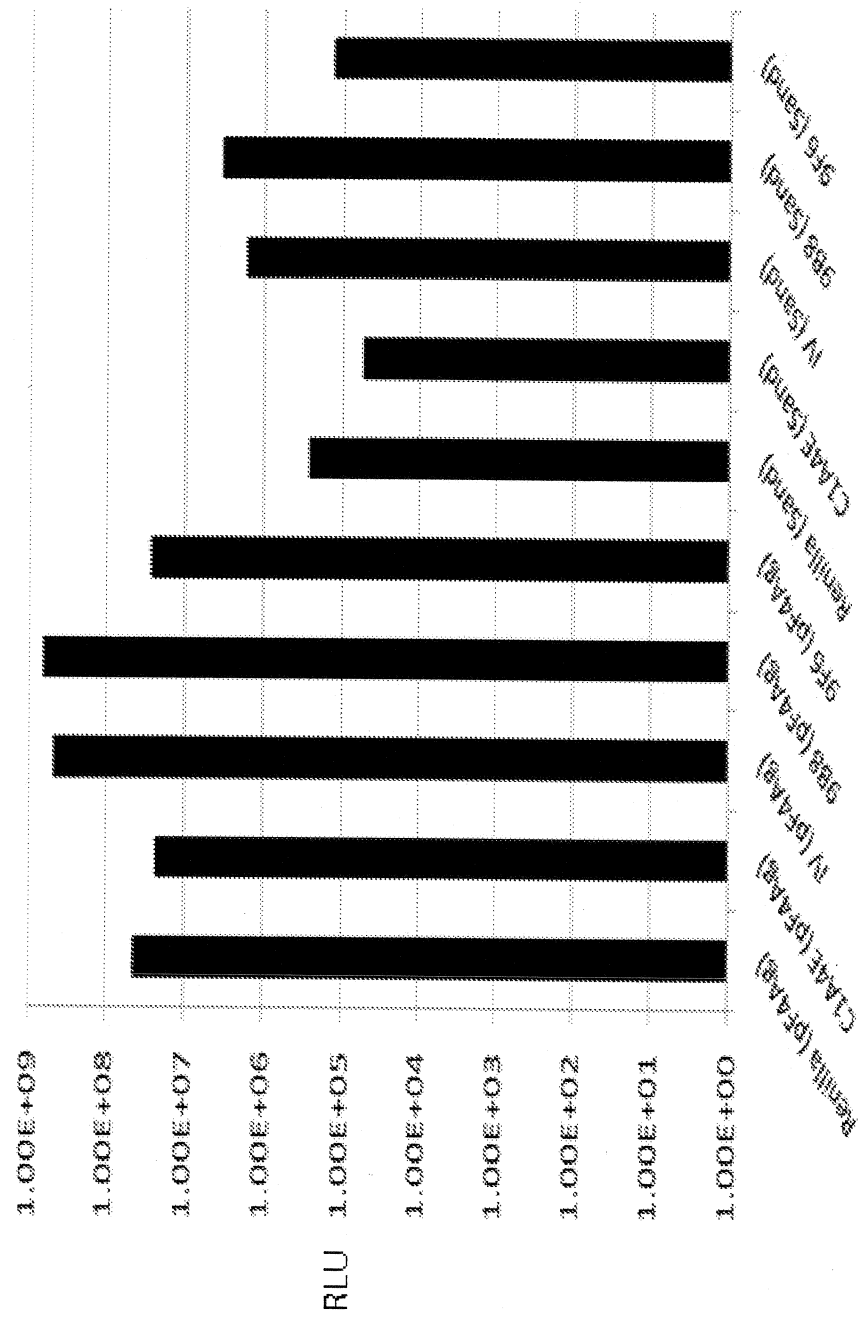


FIG. 68

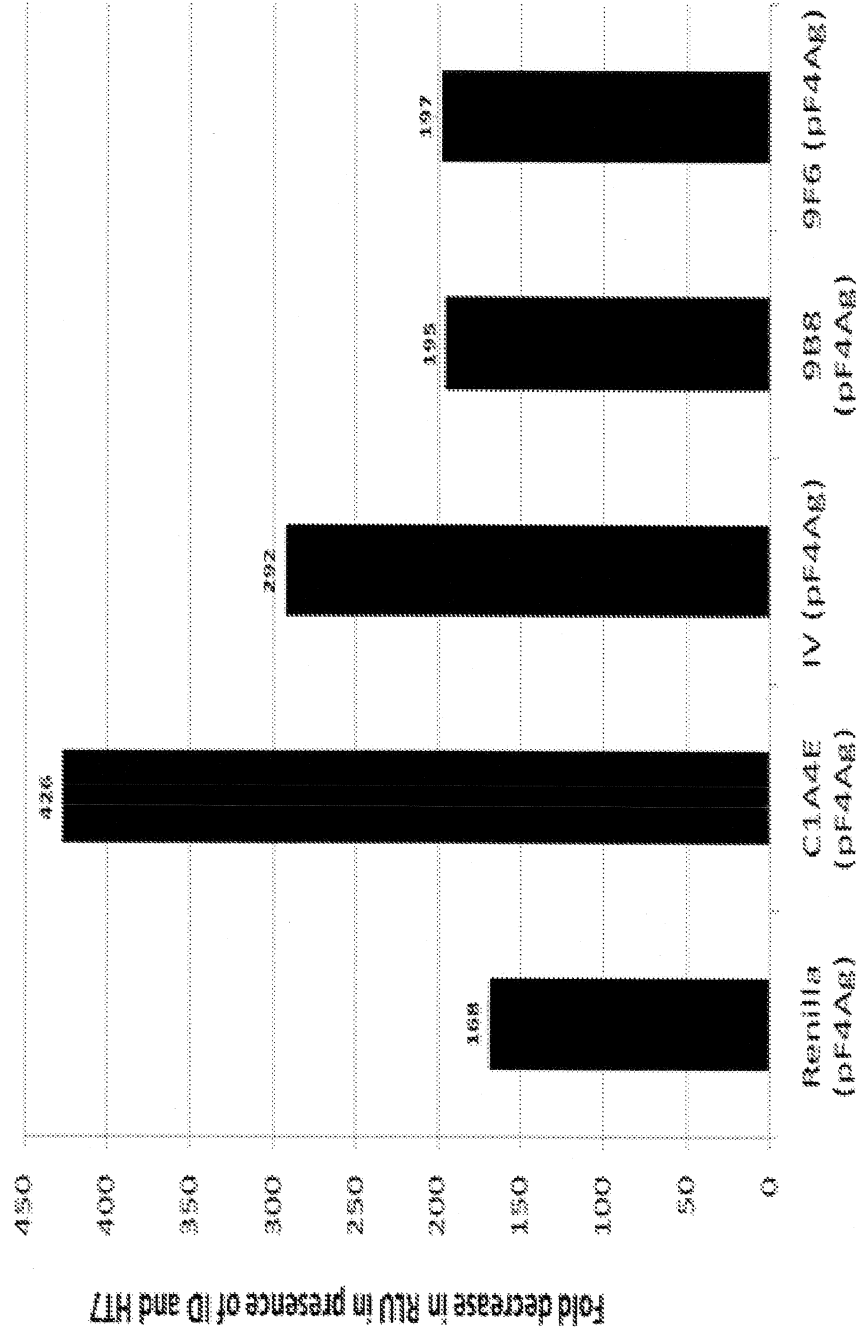


FIG. 69

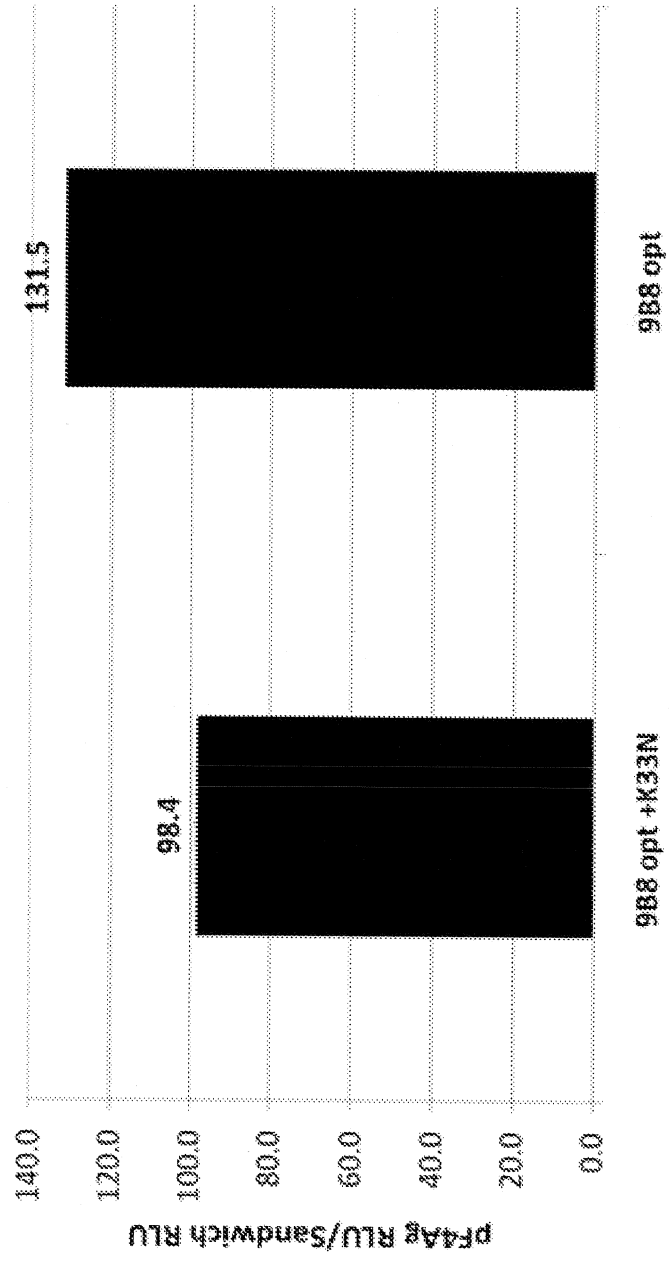


FIG. 70

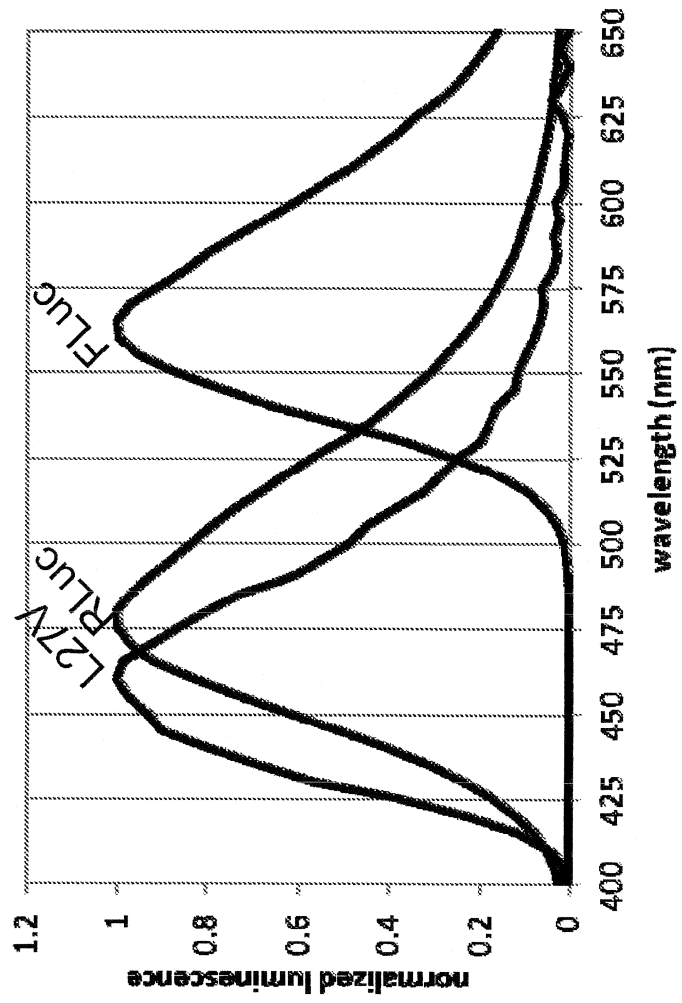


FIG. 71

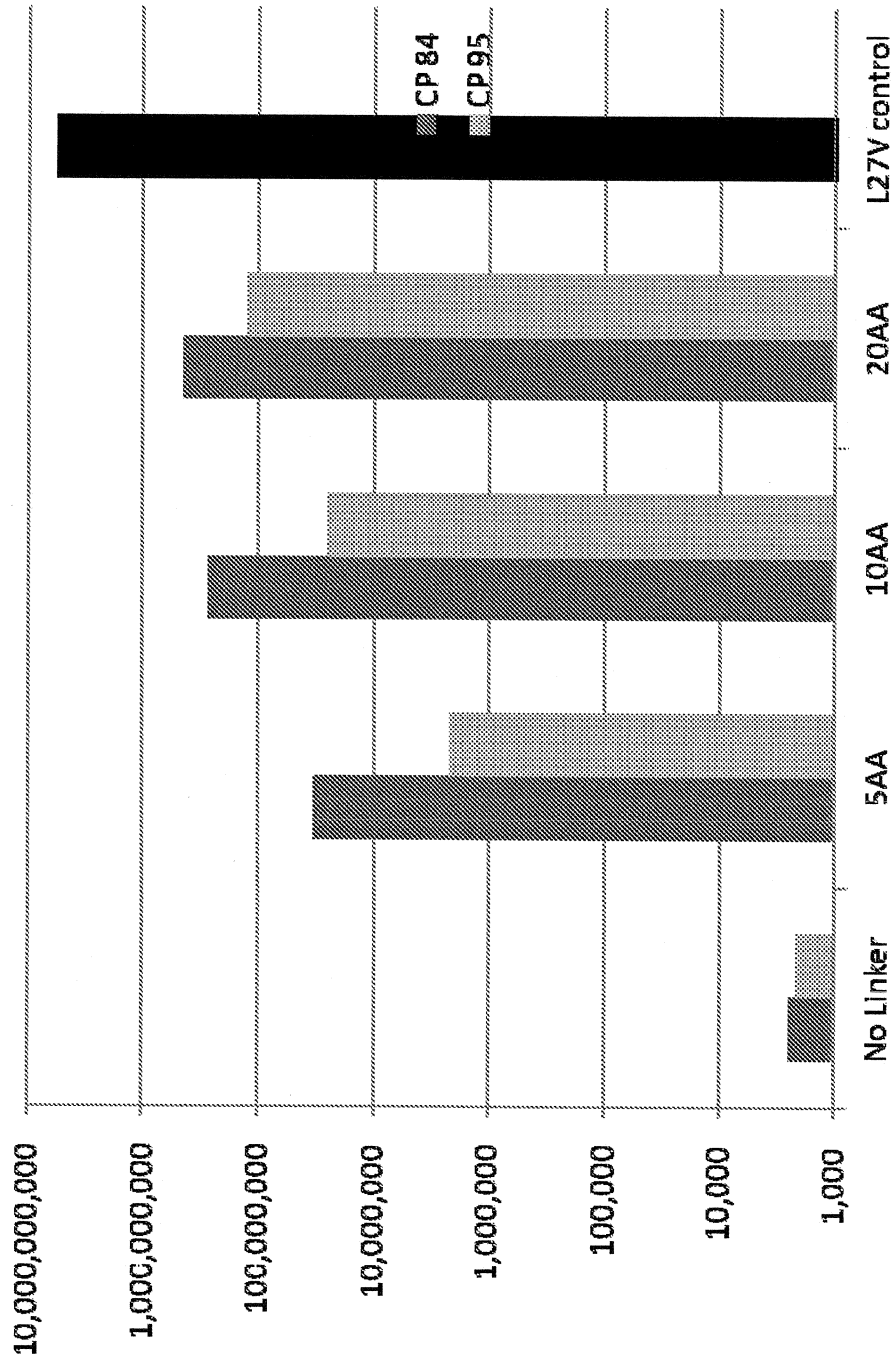


FIG. 72

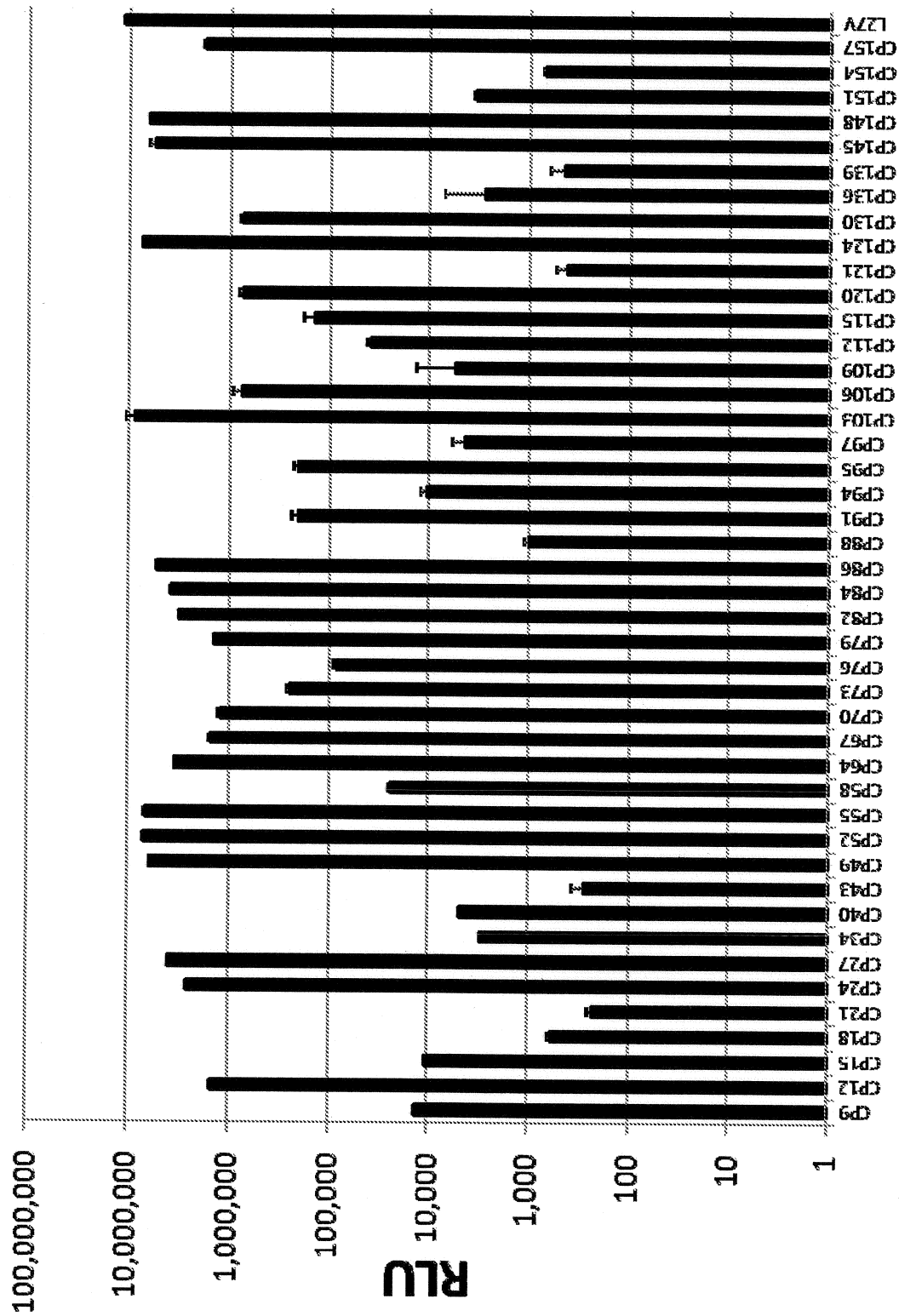


FIG. 73A

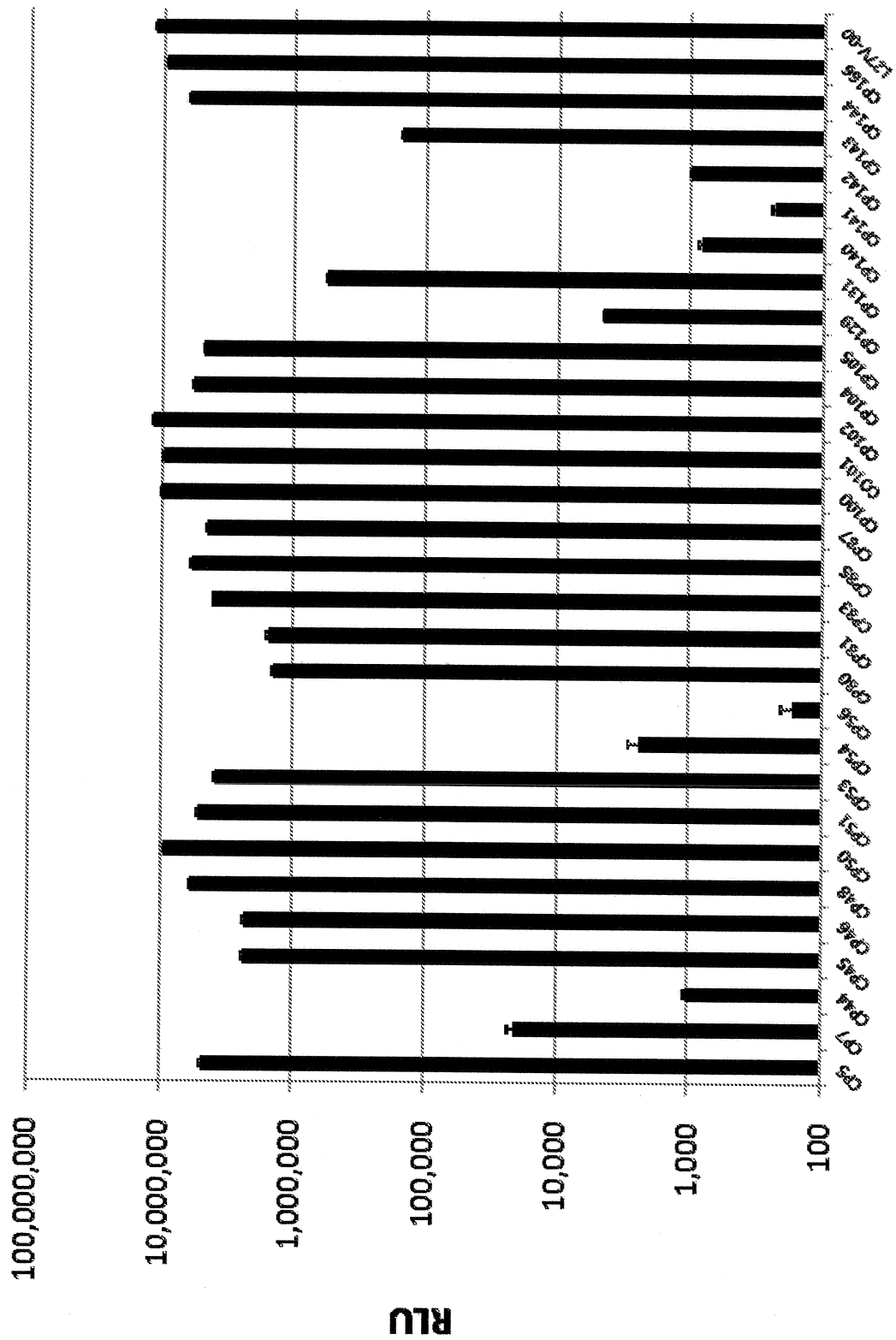


FIG. 73B

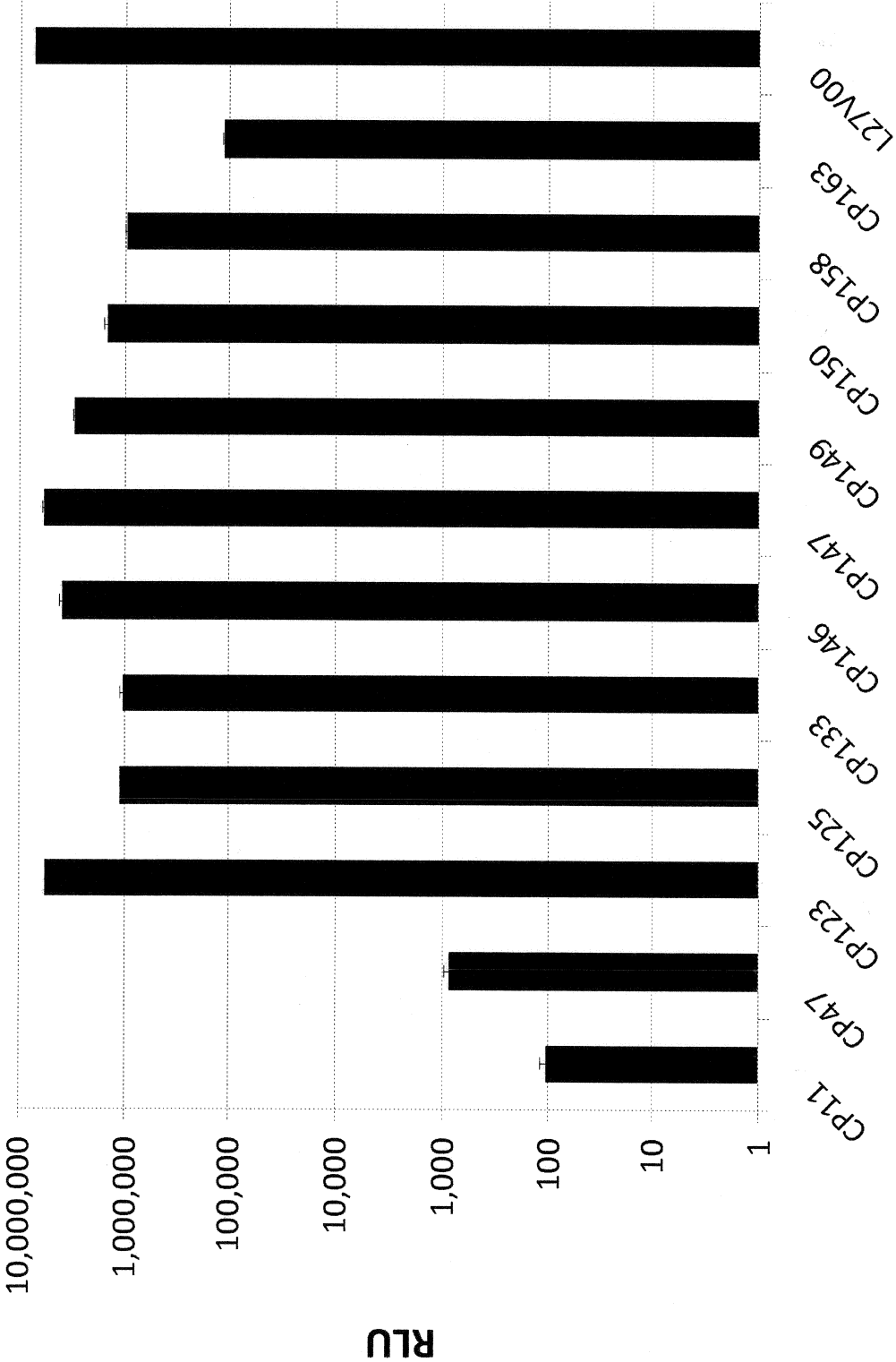


FIG. 73C

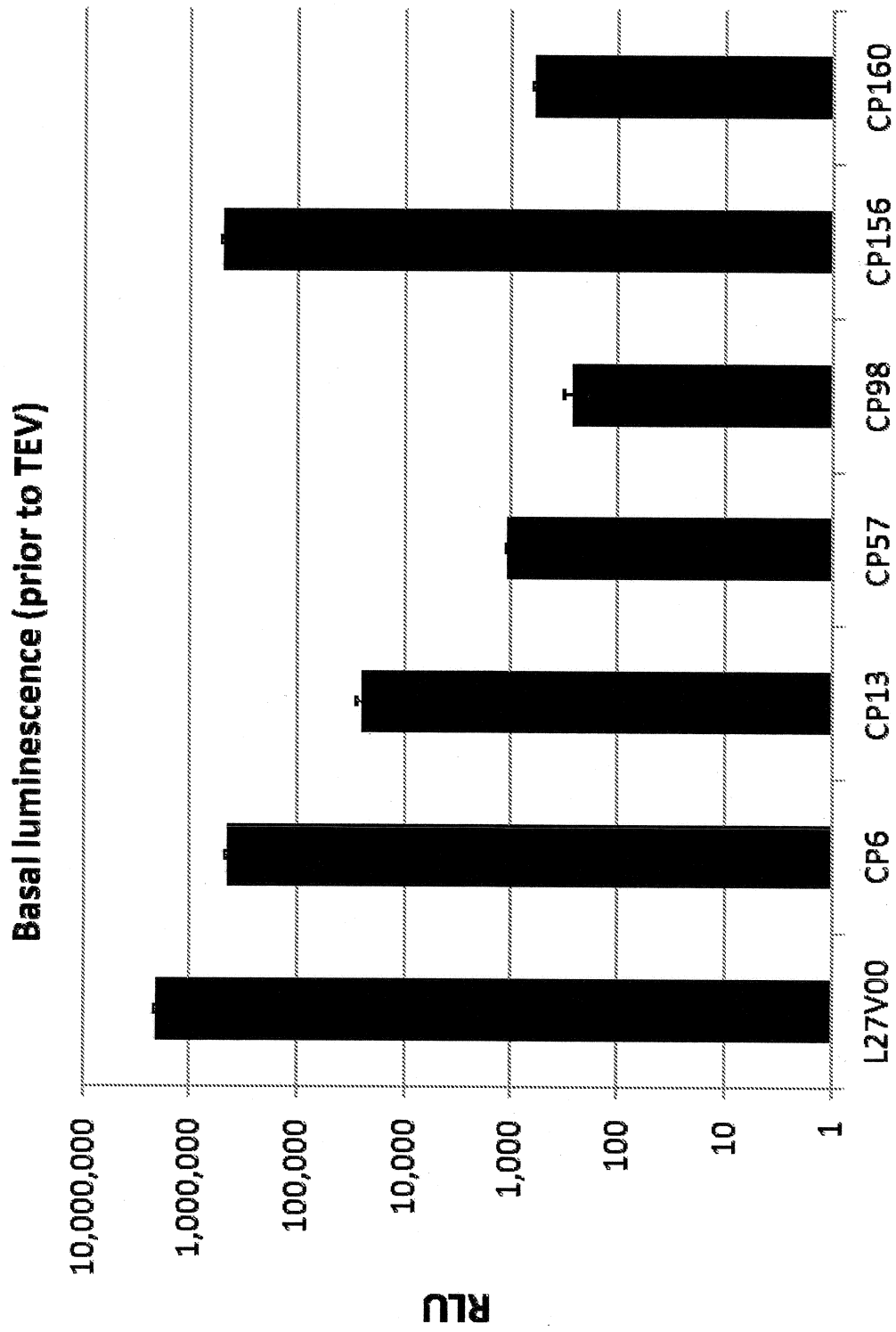


FIG. 73D

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	113	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

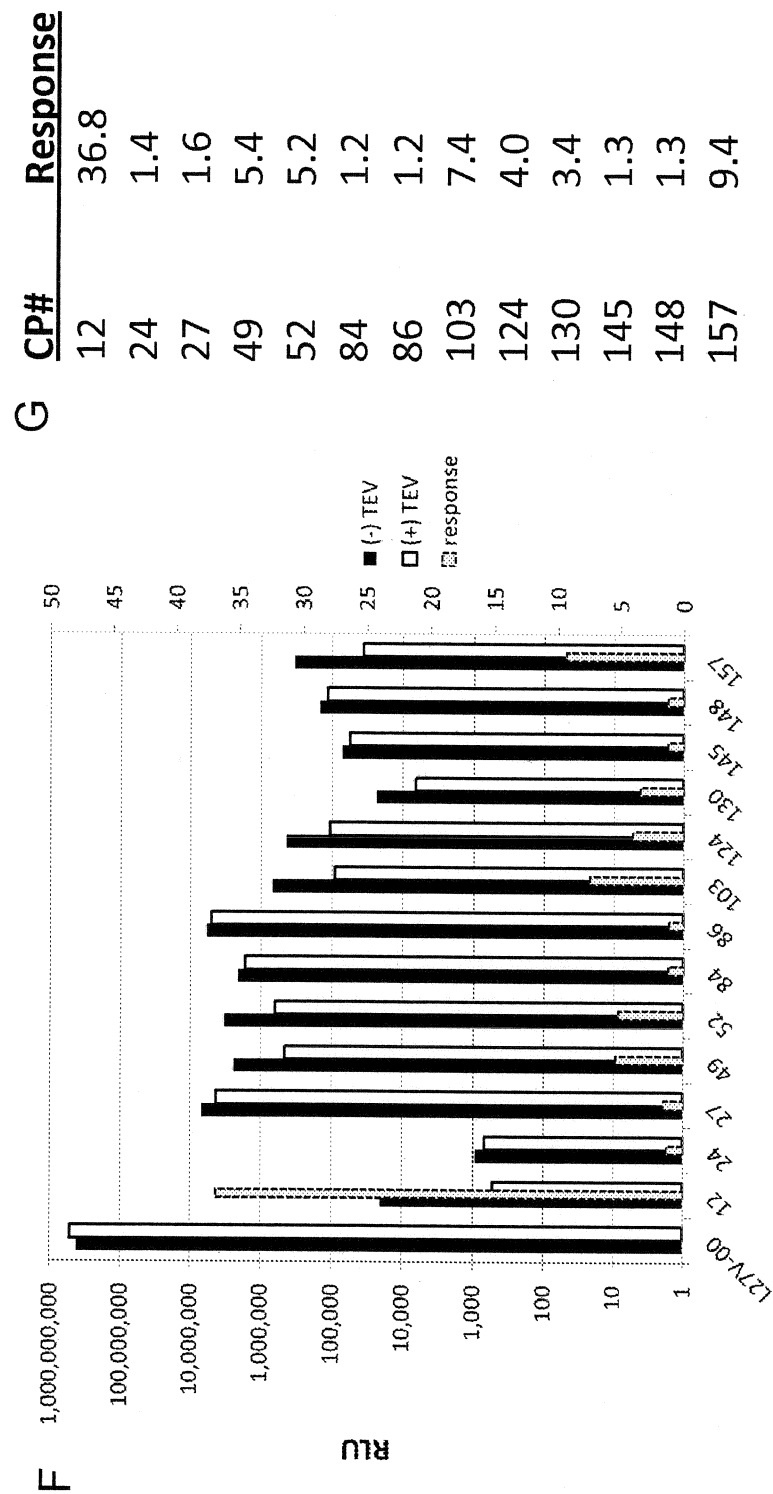


FIG. 73

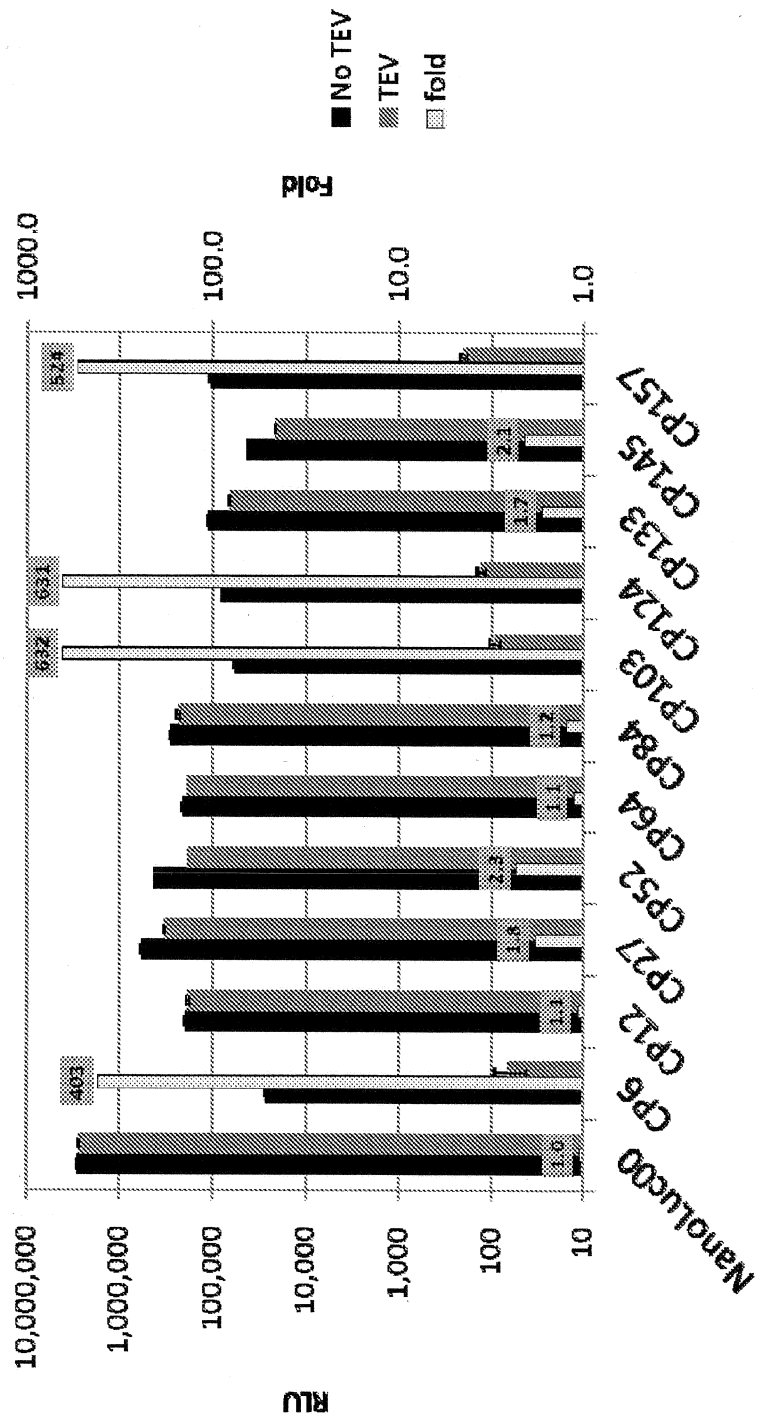


FIG. 73H

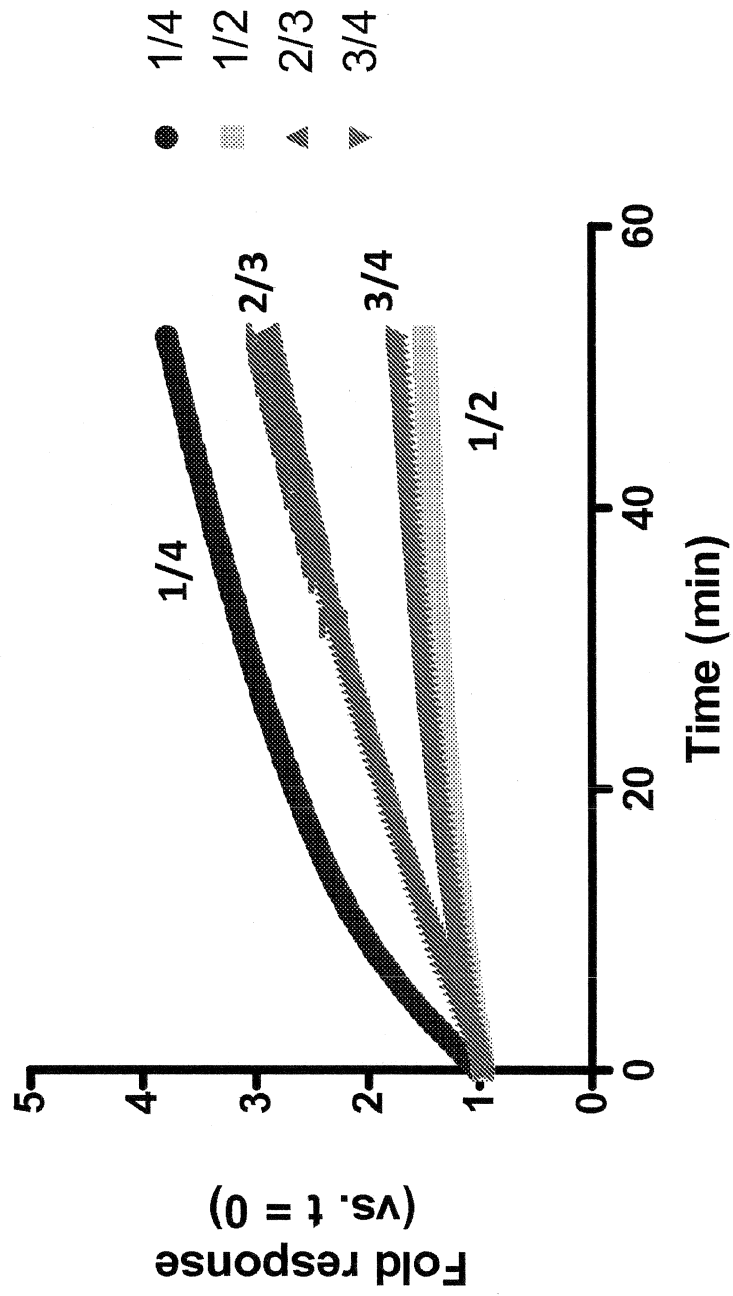


FIG. 74

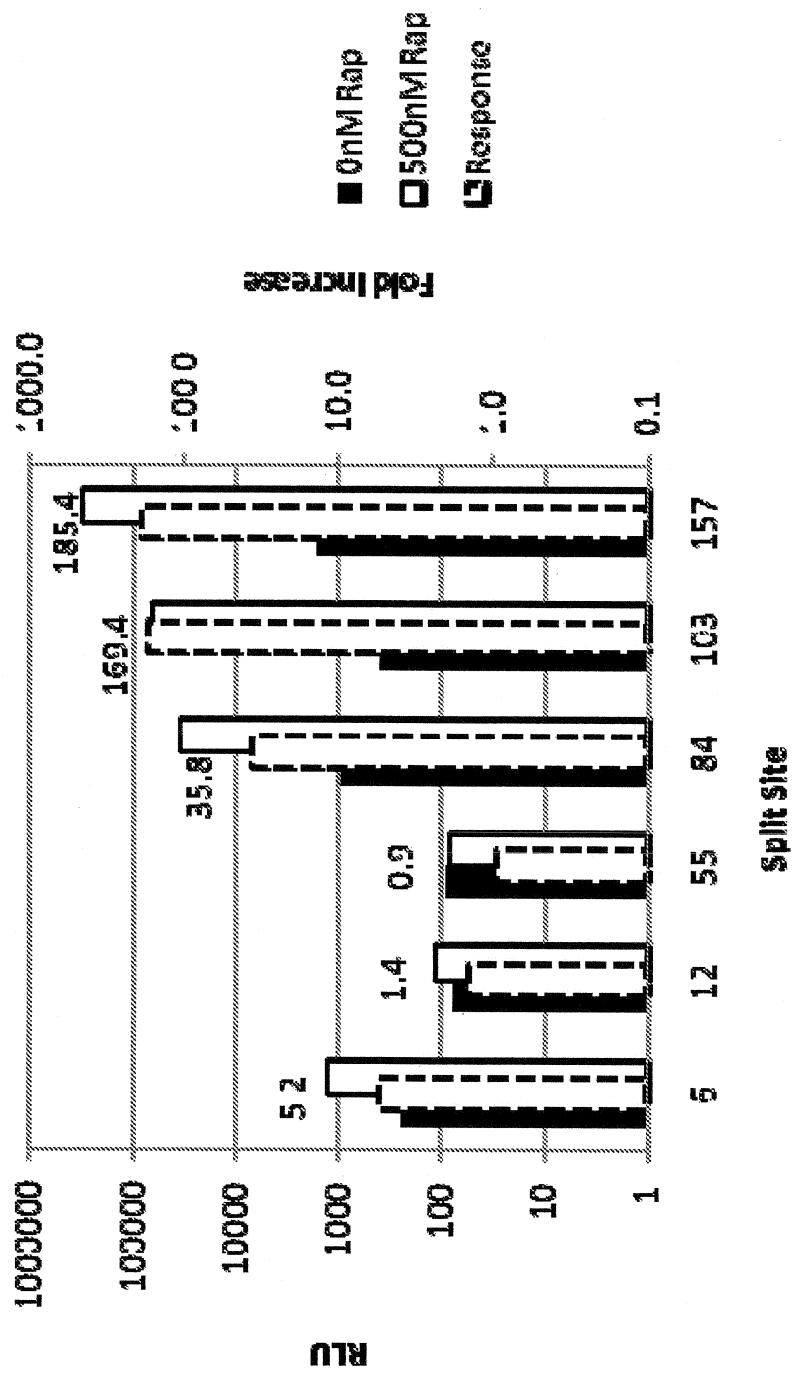


FIG. 75A

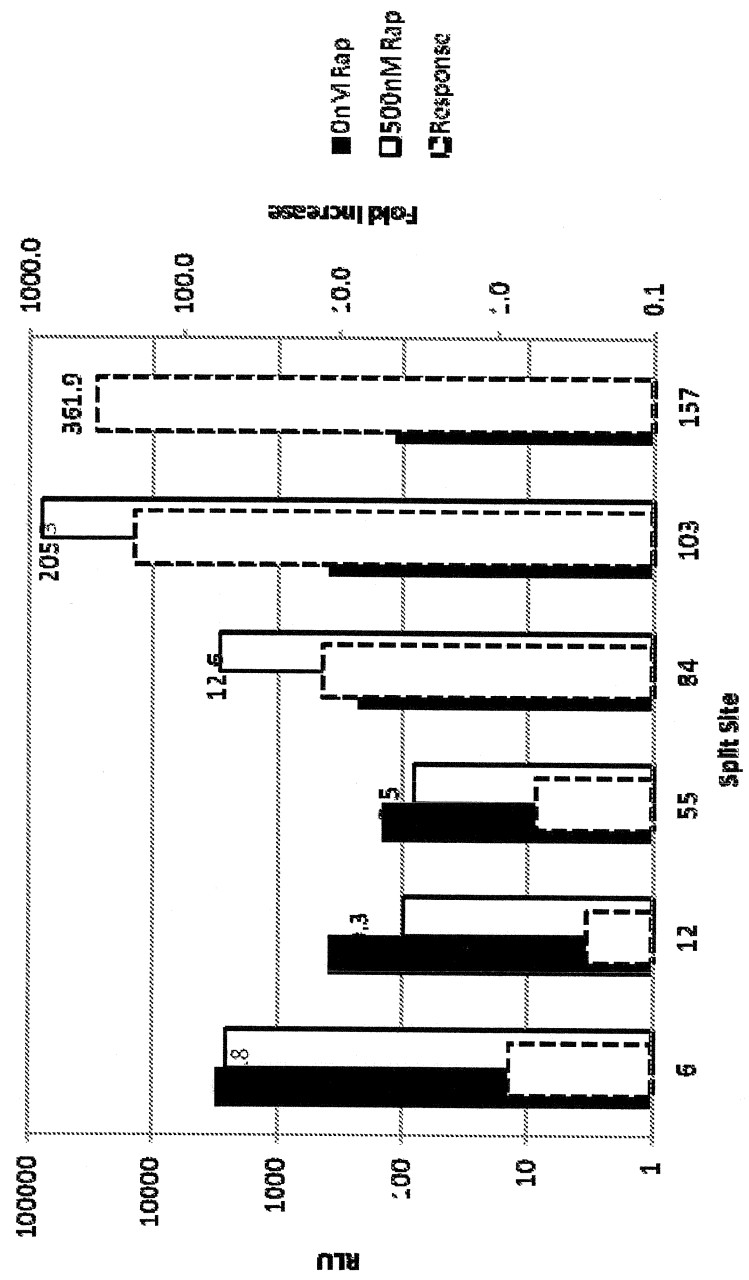


FIG. 75B

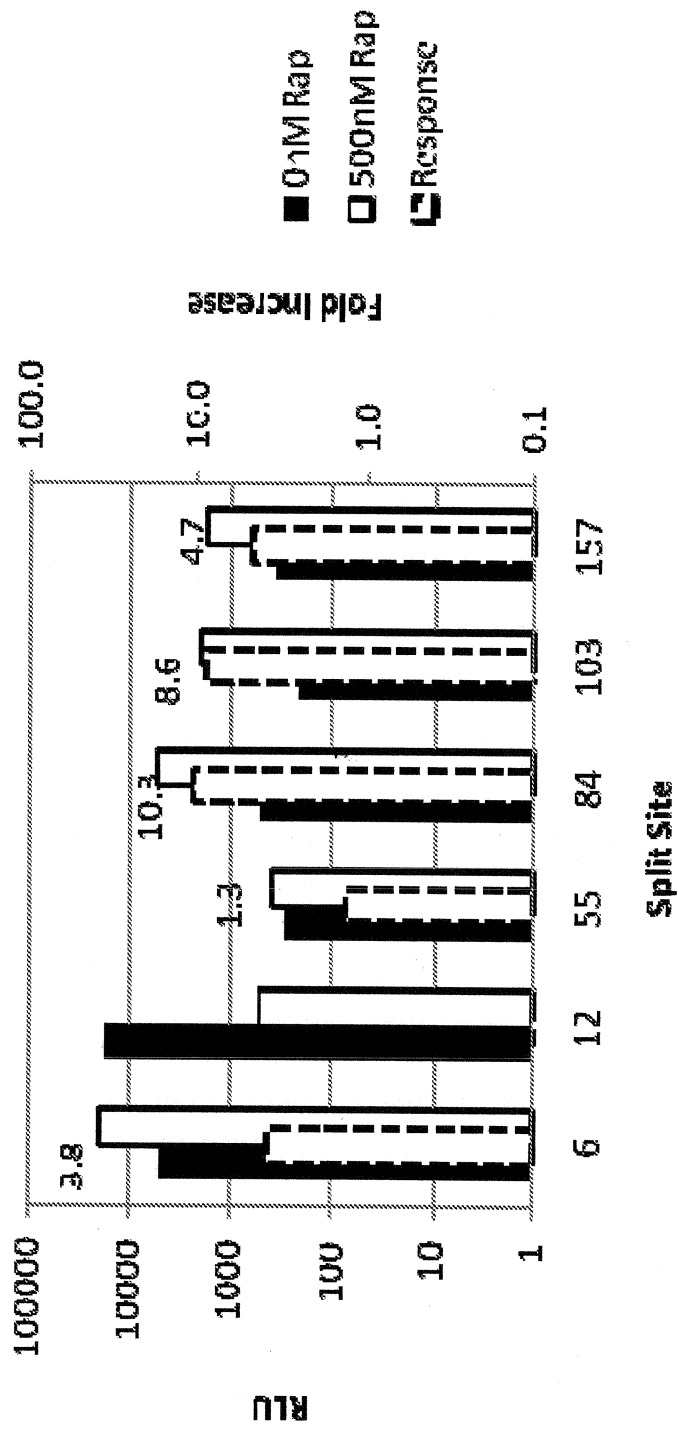


FIG. 75C

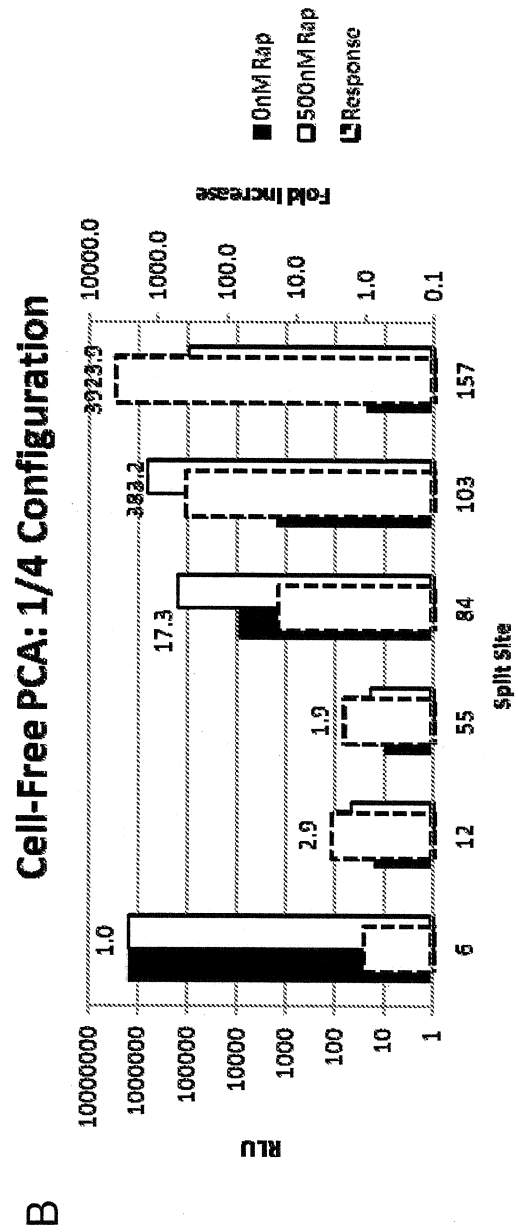
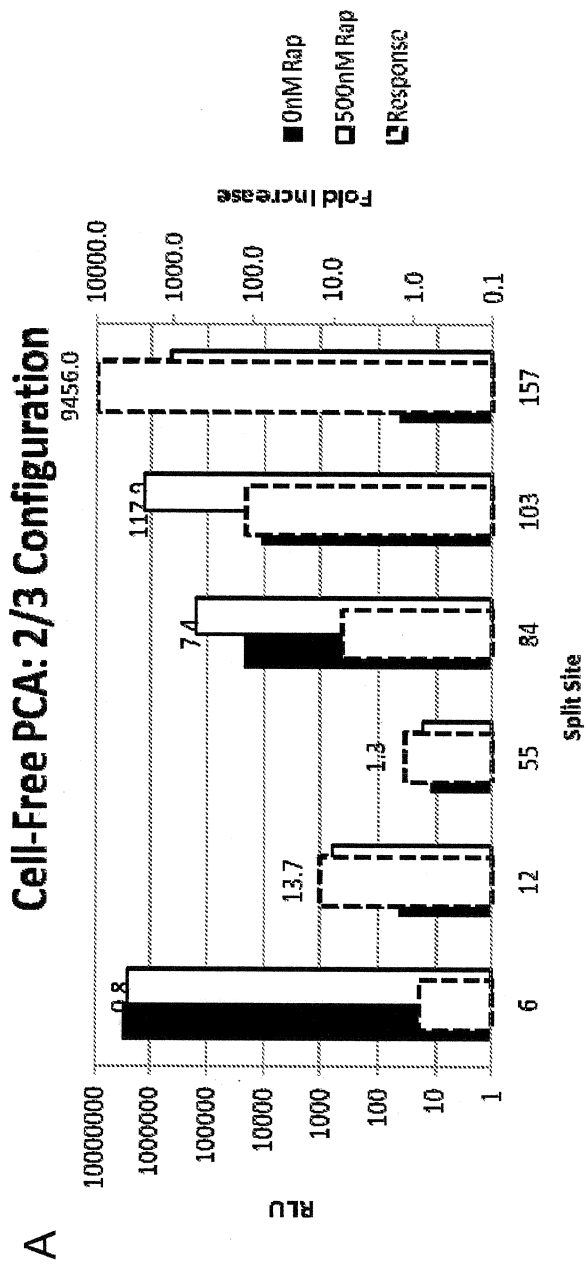


FIG. 76

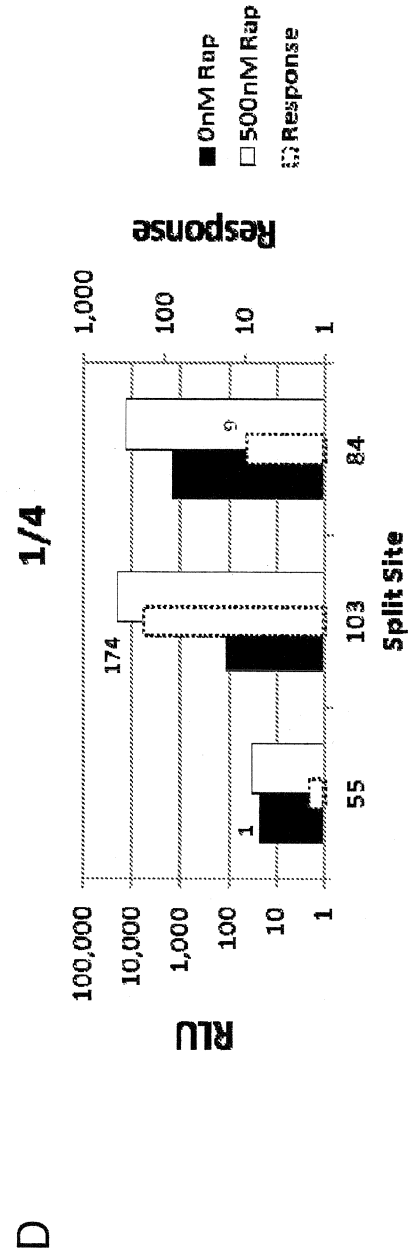
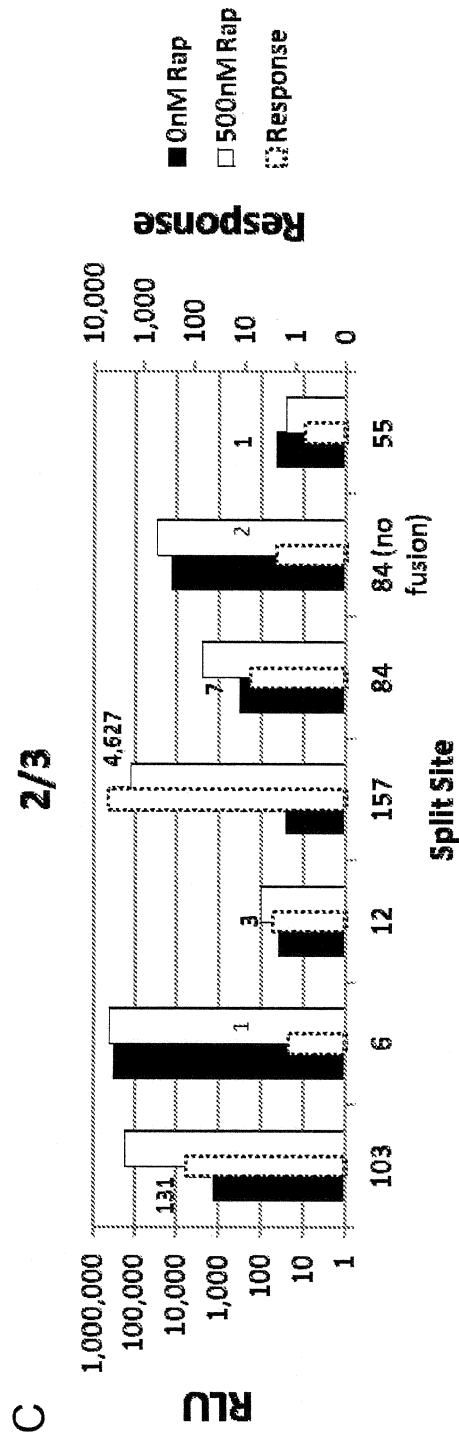


FIG. 76

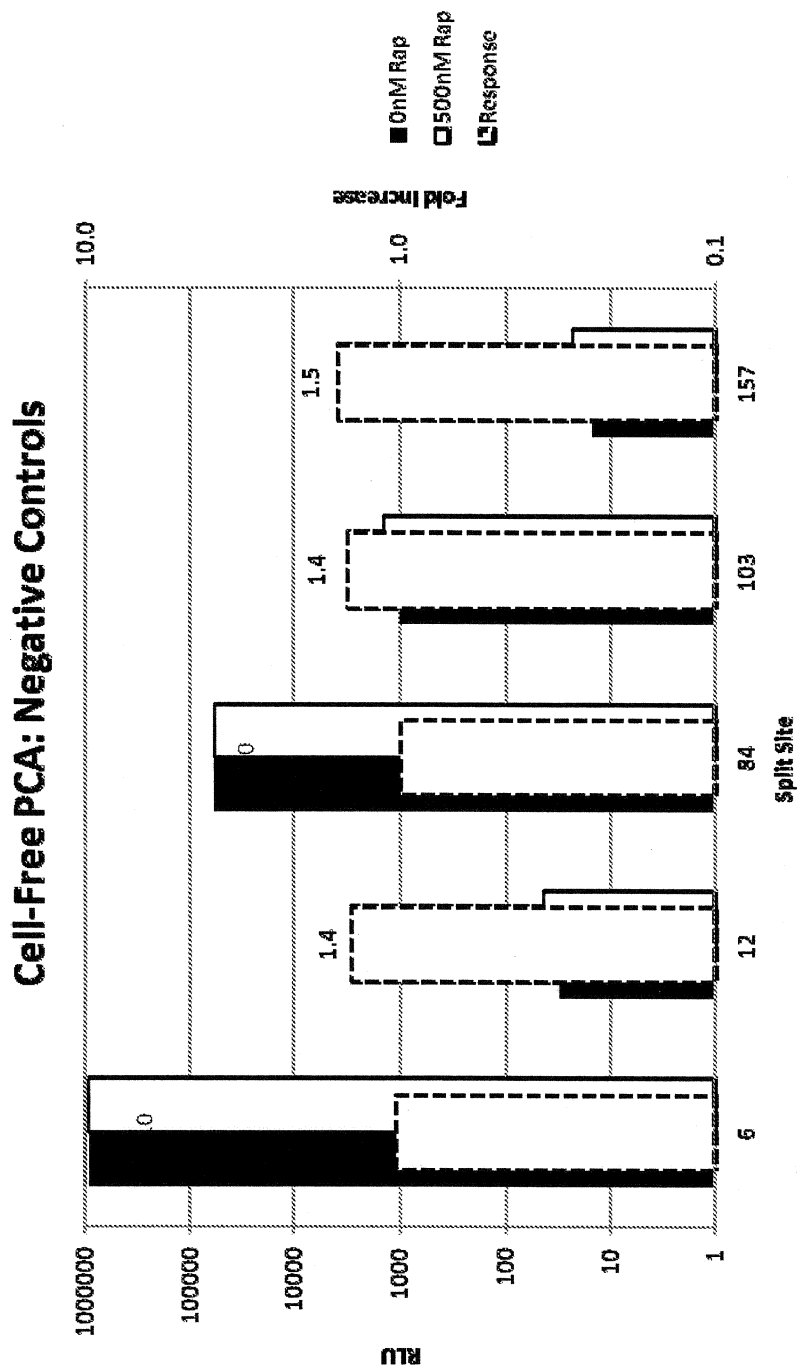


FIG. 76E

PCA (Cell-Free, "1/4")

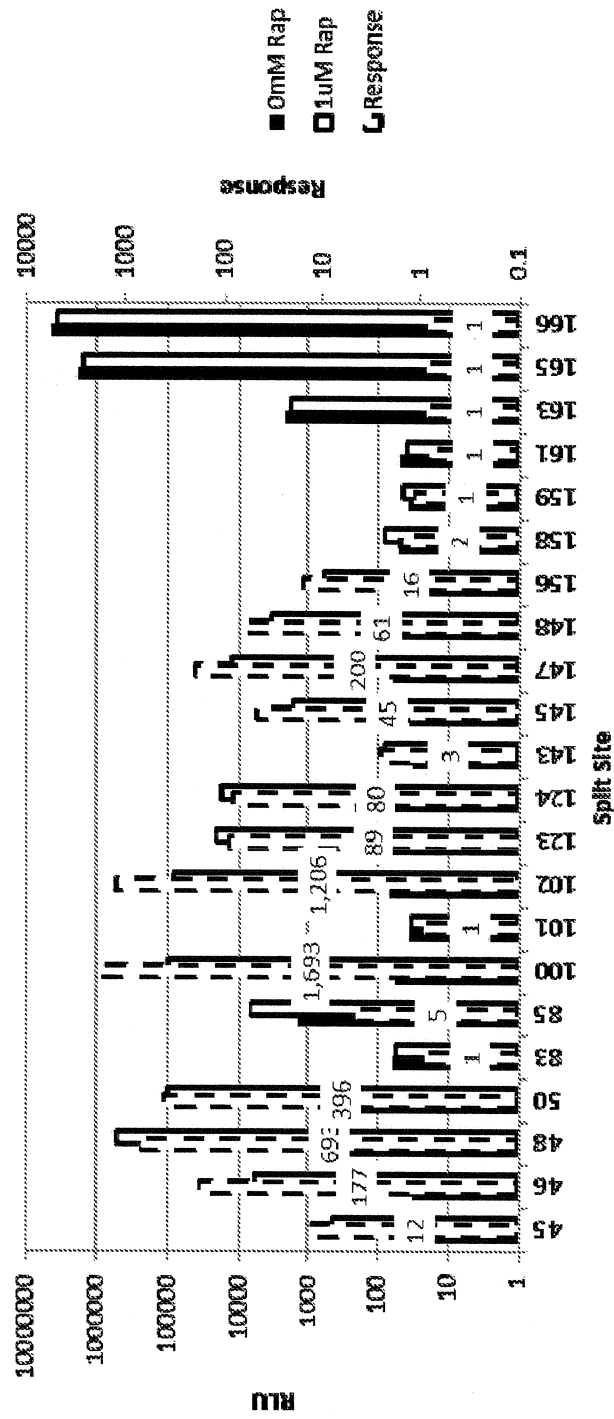


FIG. 76F

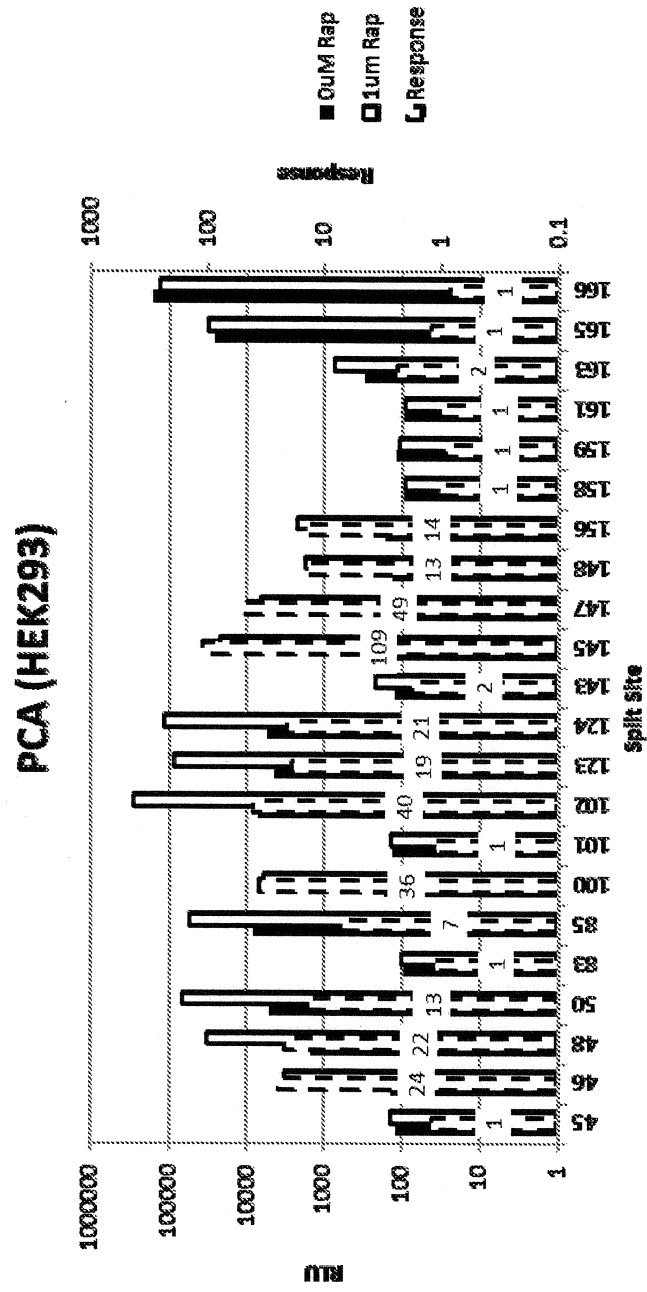


FIG. 76G

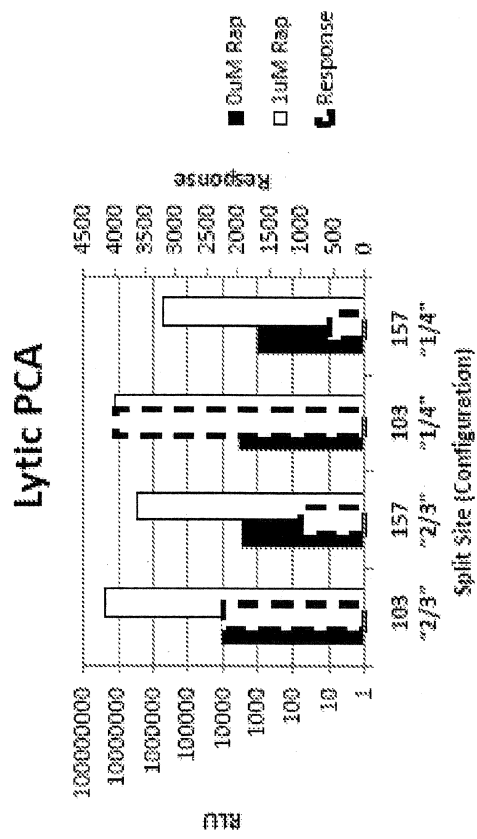


FIG. 76H

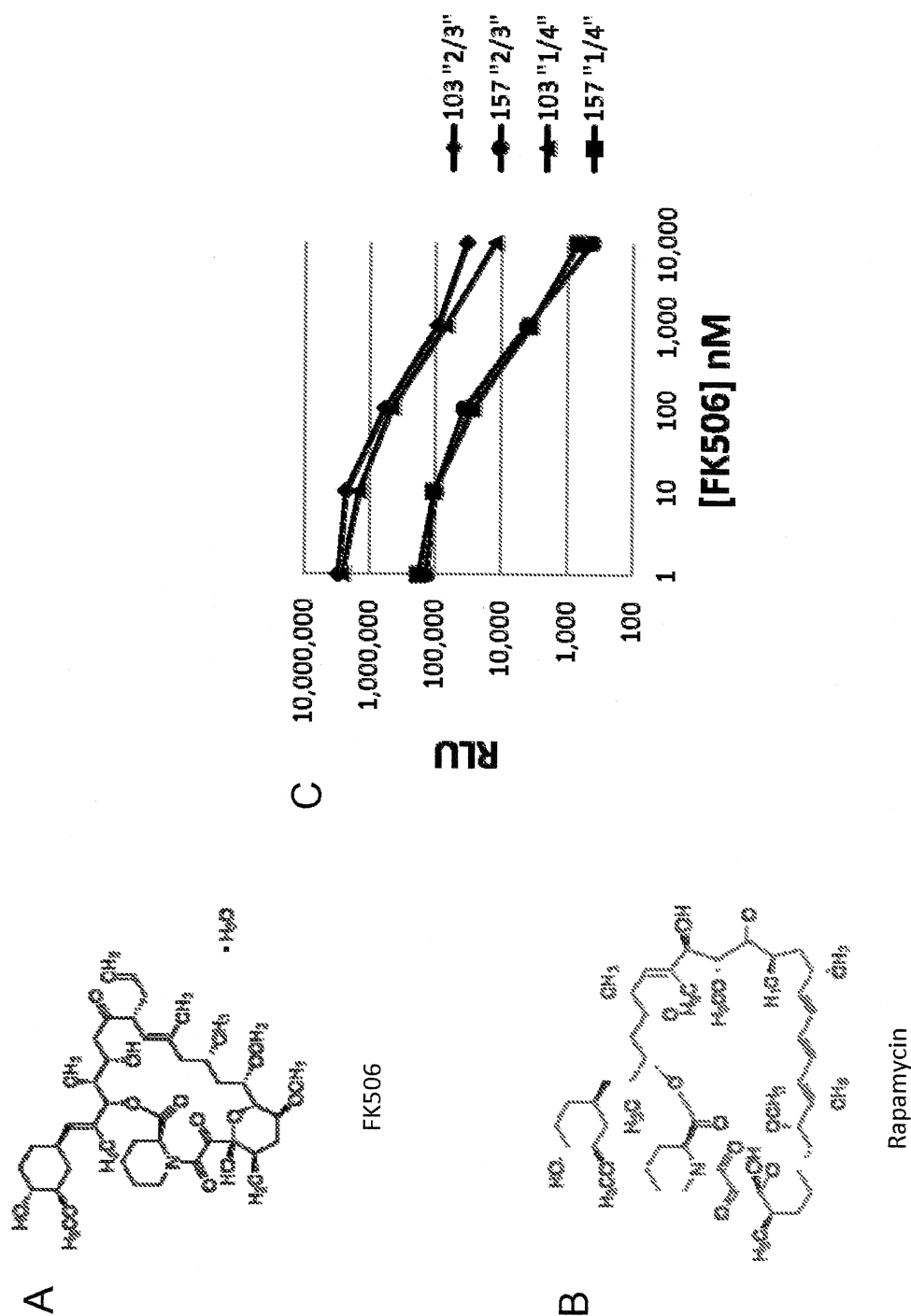


FIG. 77

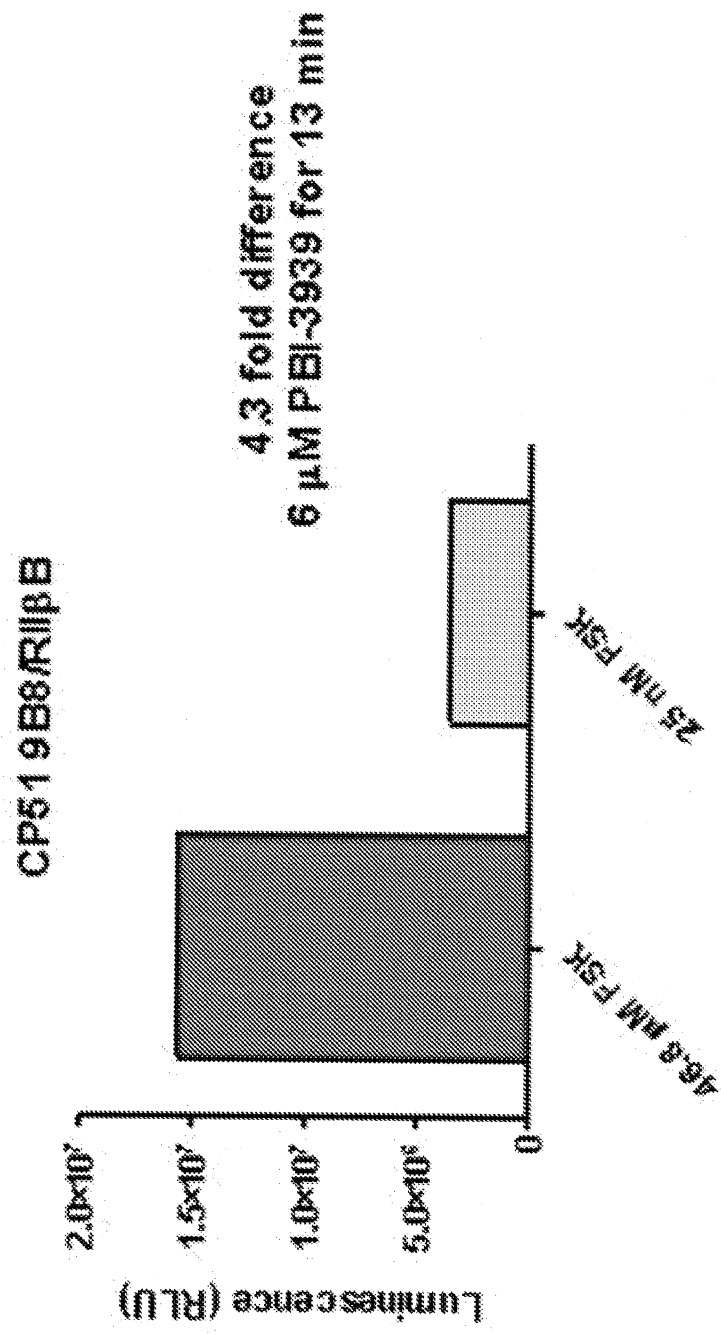


FIG. 78

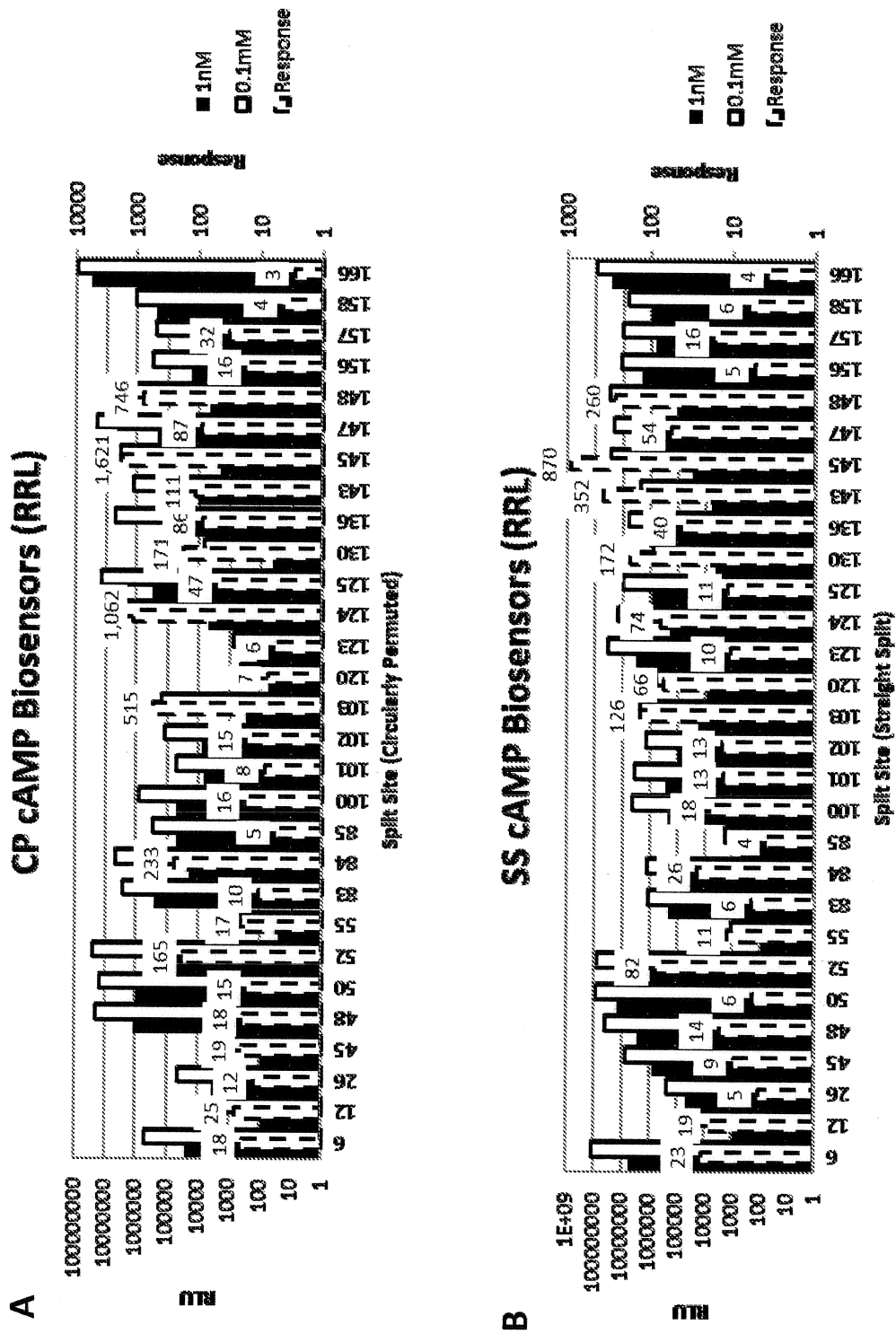


FIG. 79

C CP cAMP Biosensors (HEK293)



D SS cAMP Biosensors (HEK293)

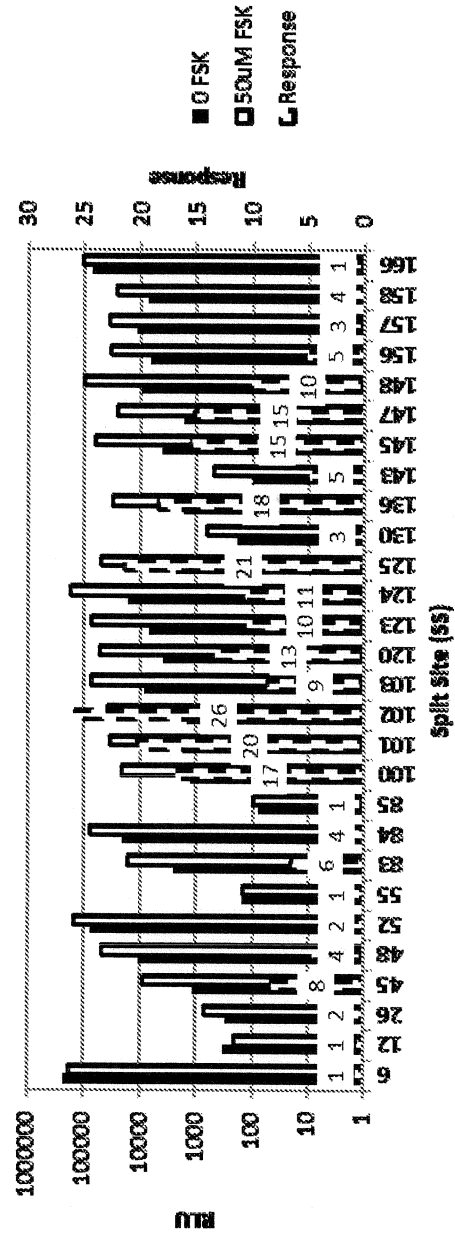


FIG. 79

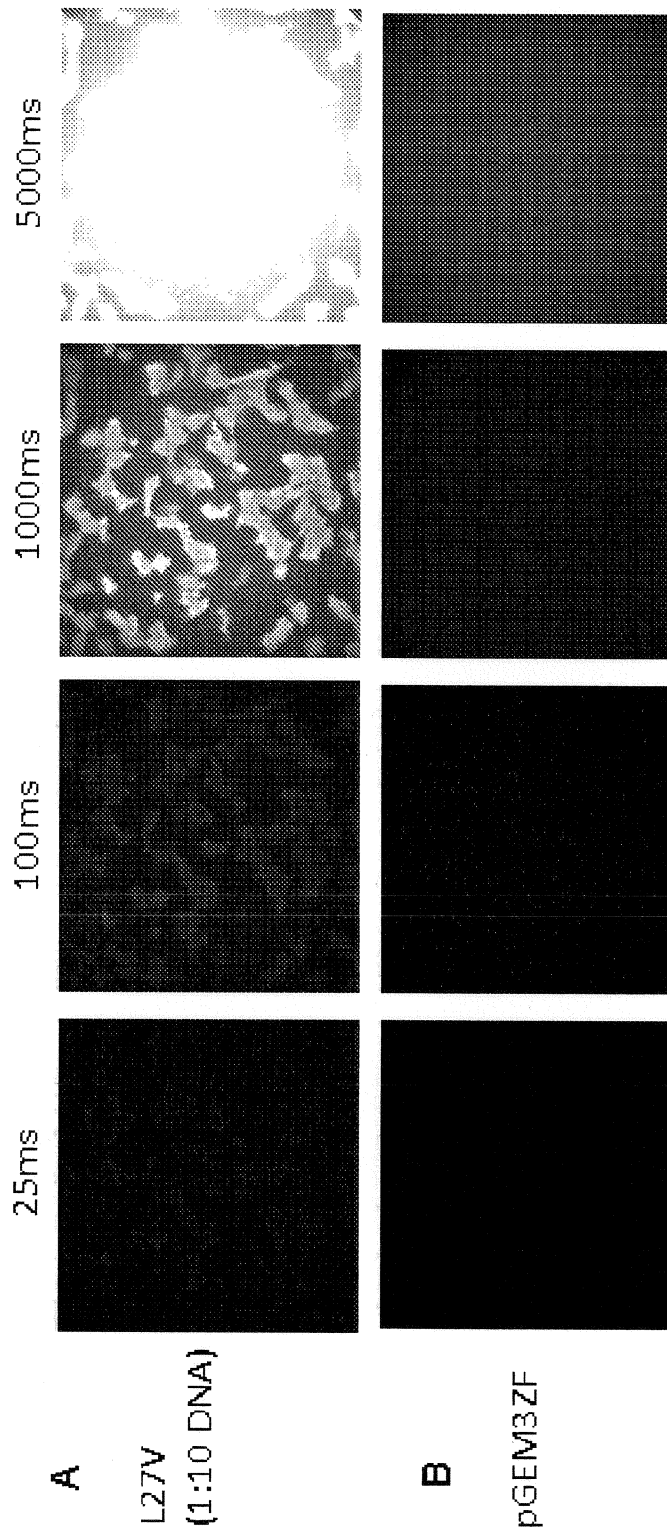


FIG. 80

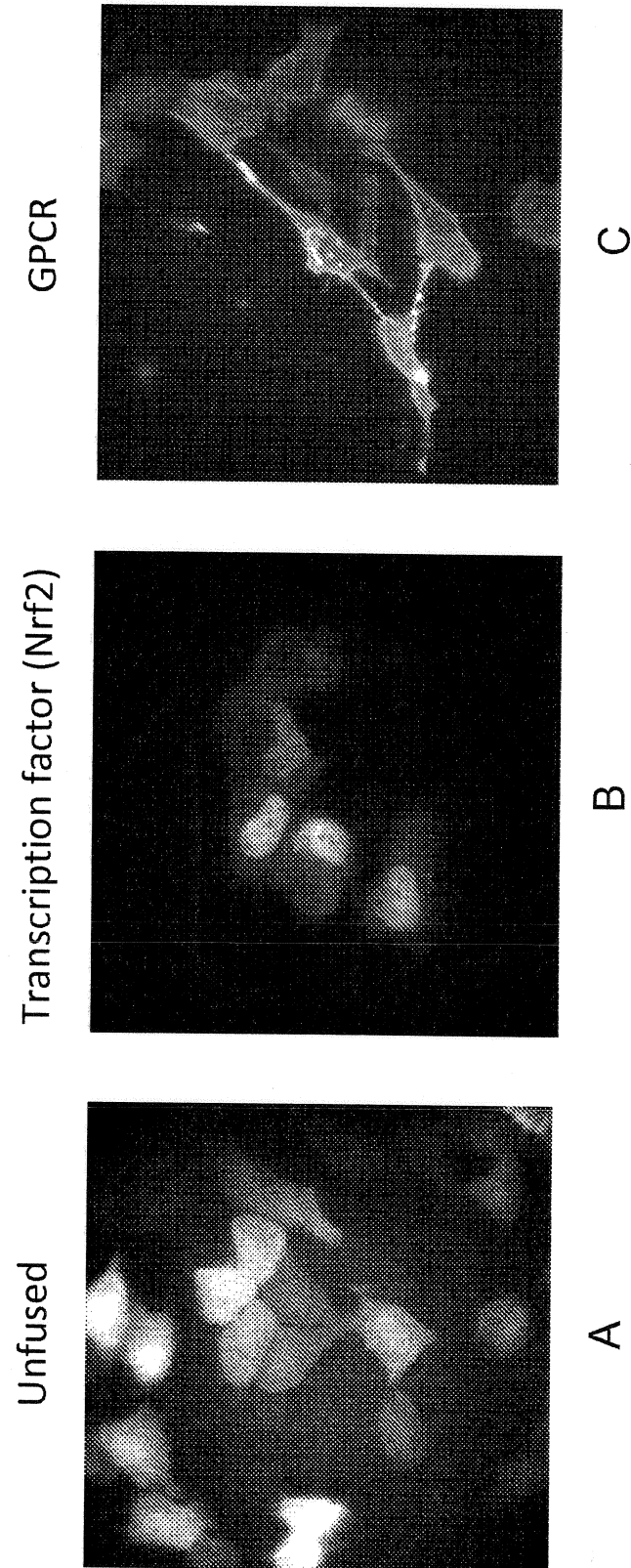


FIG. 81

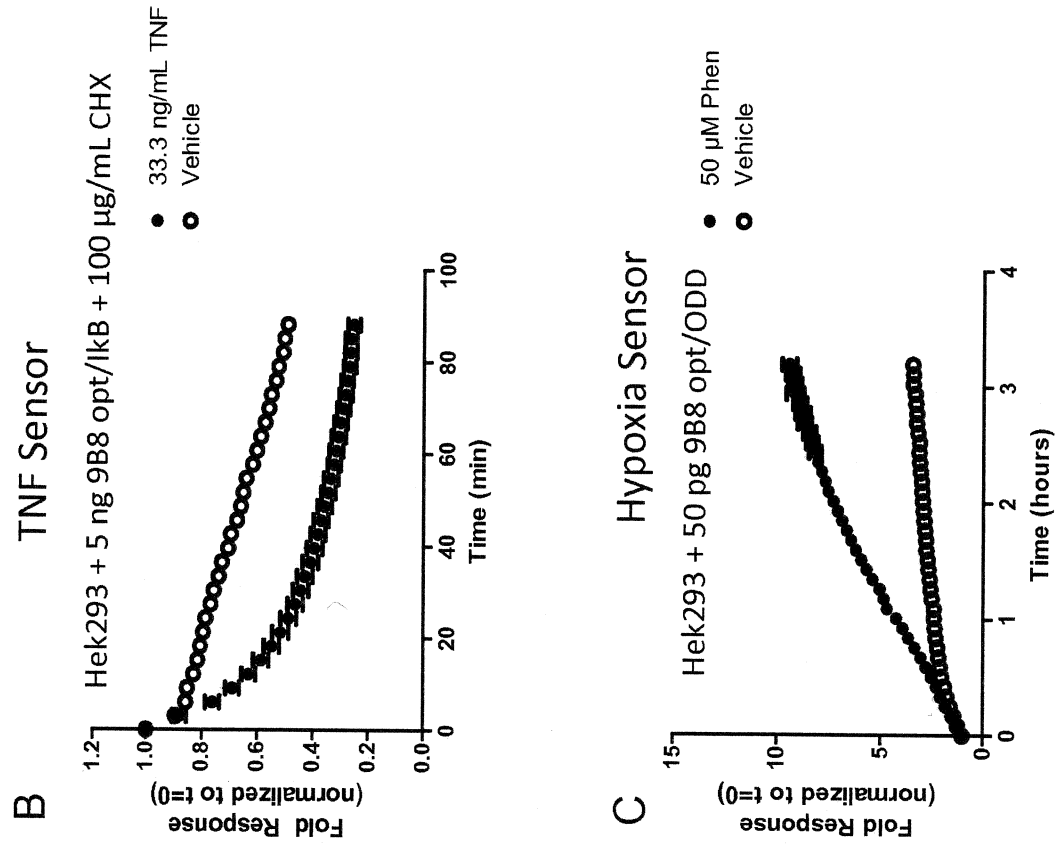
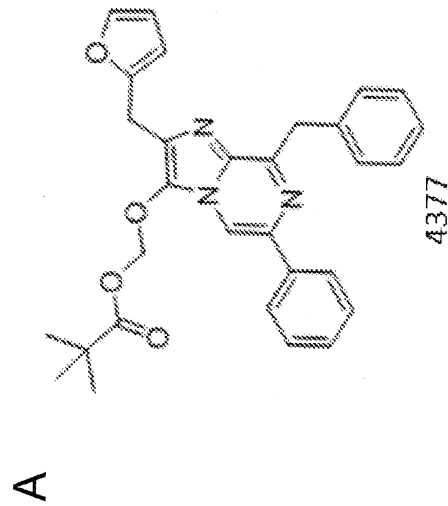


FIG. 82



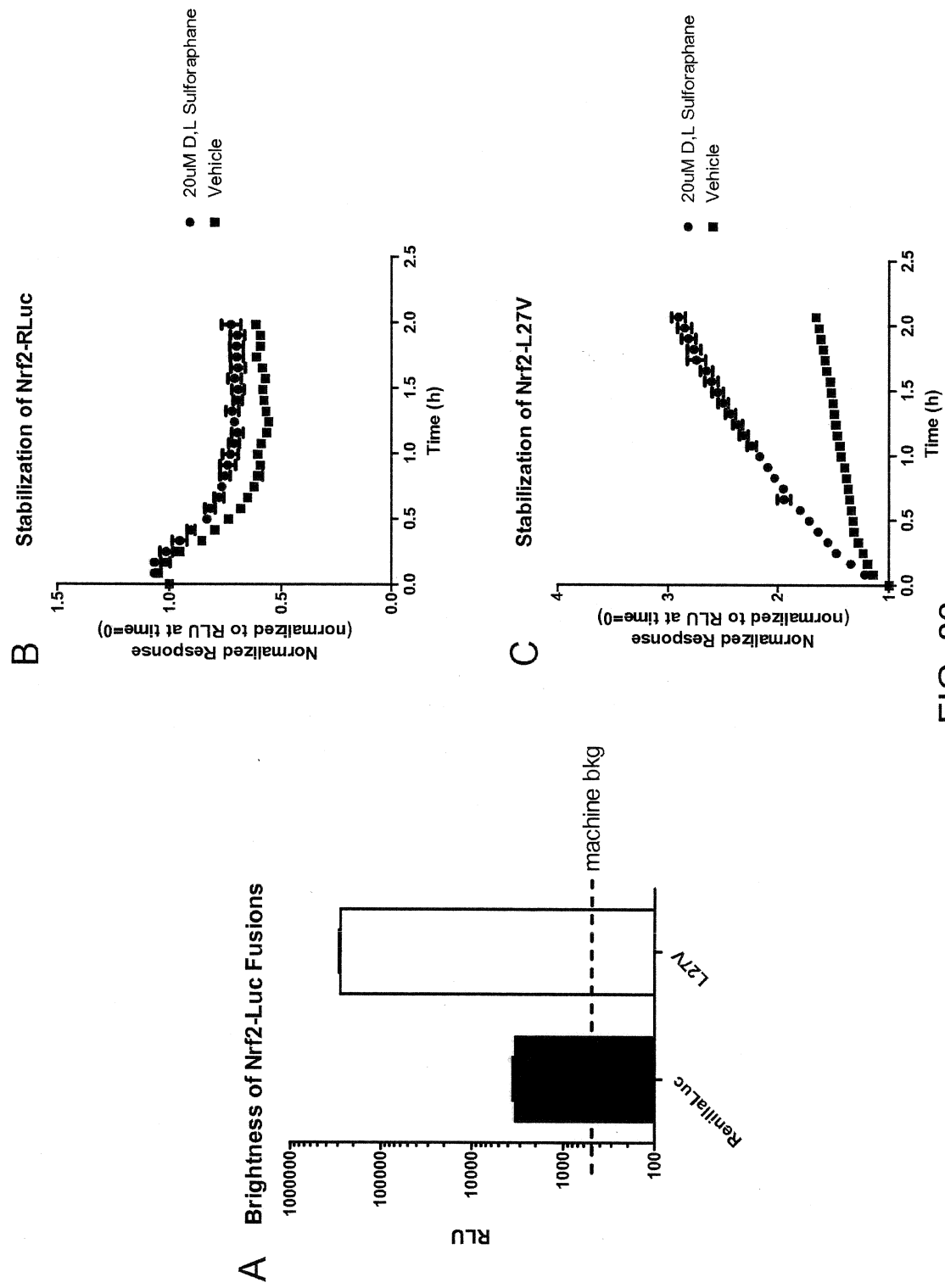


FIG. 83

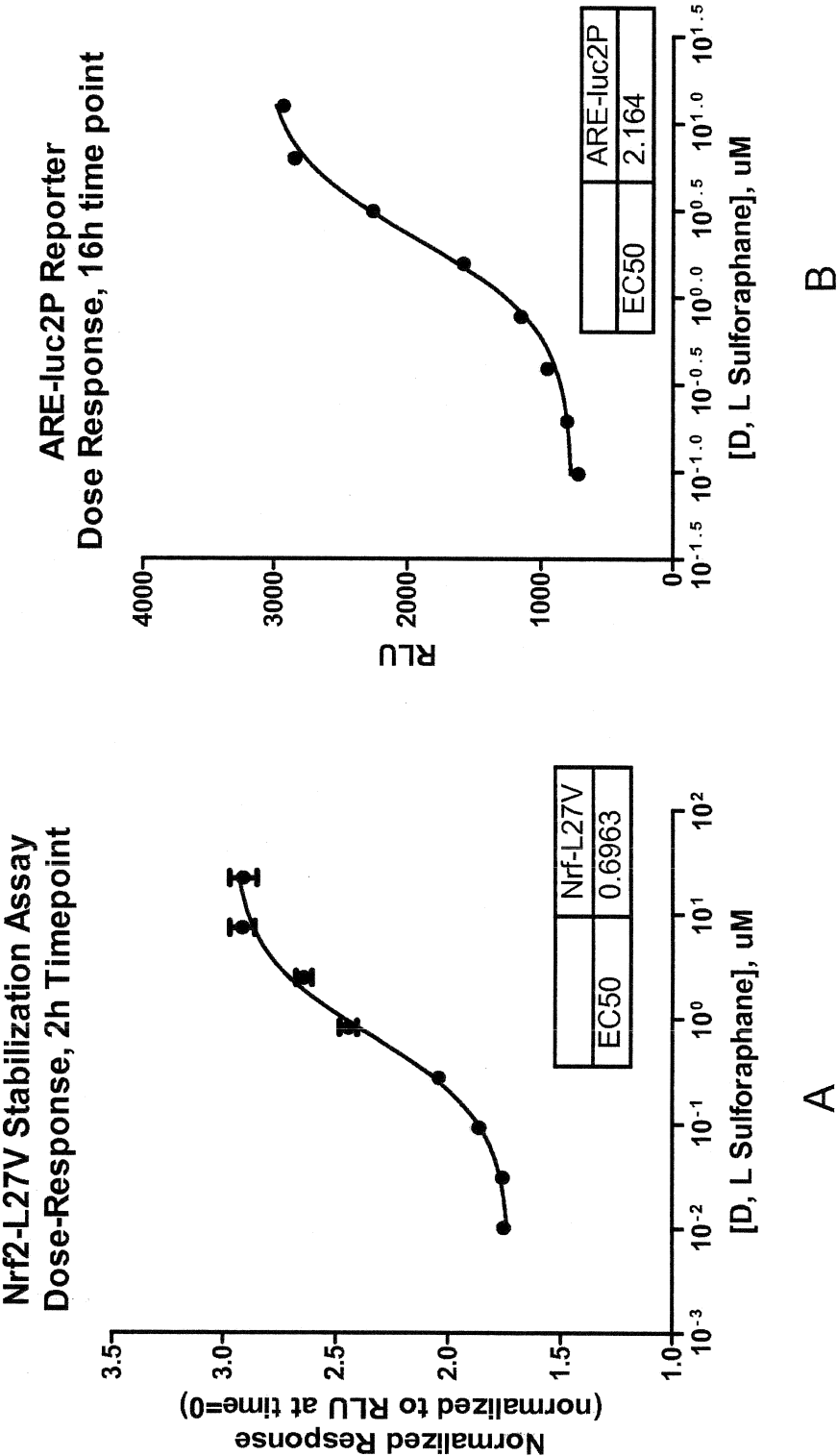
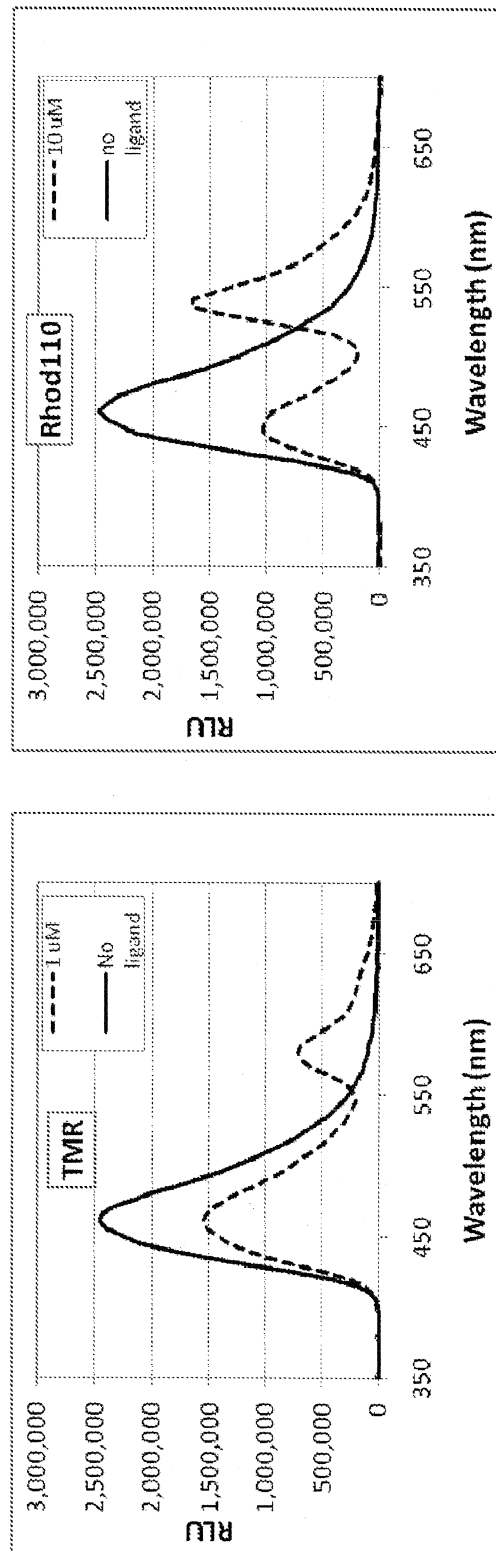


FIG. 84



A

B

FIG. 85

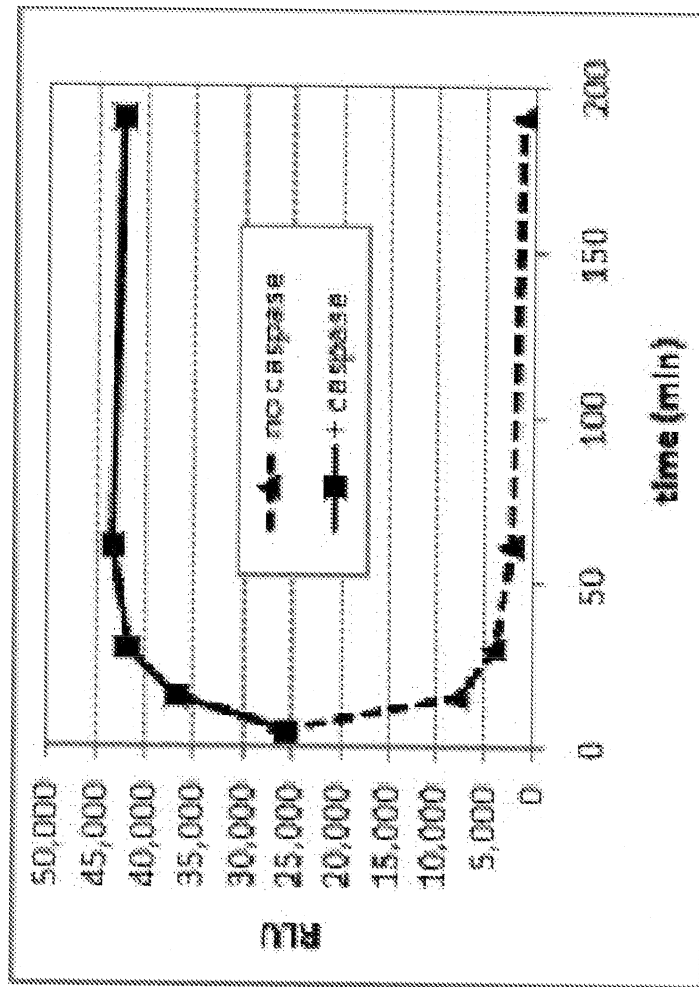


FIG. 86

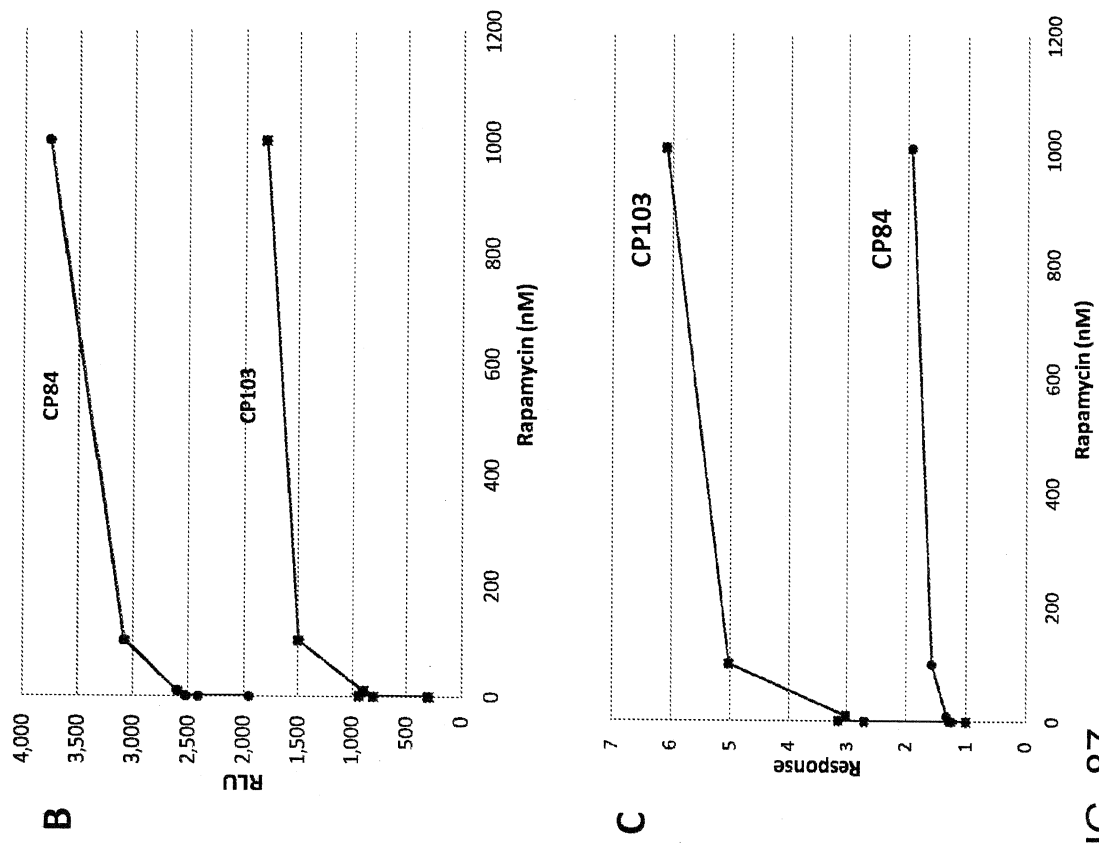


FIG. 87

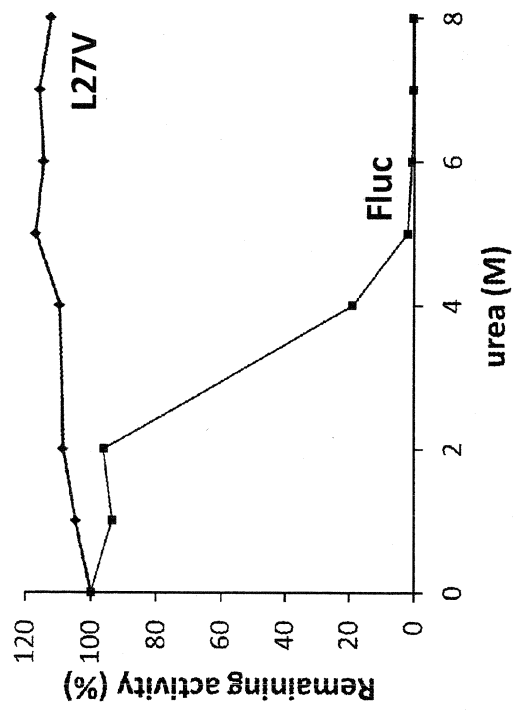


FIG. 88

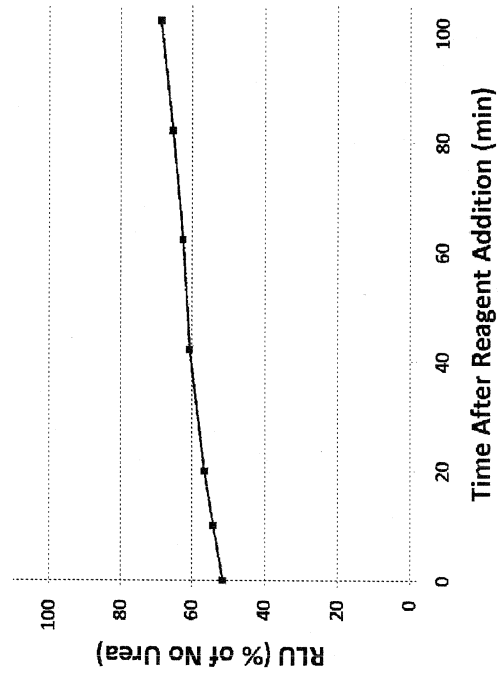


FIG. 89

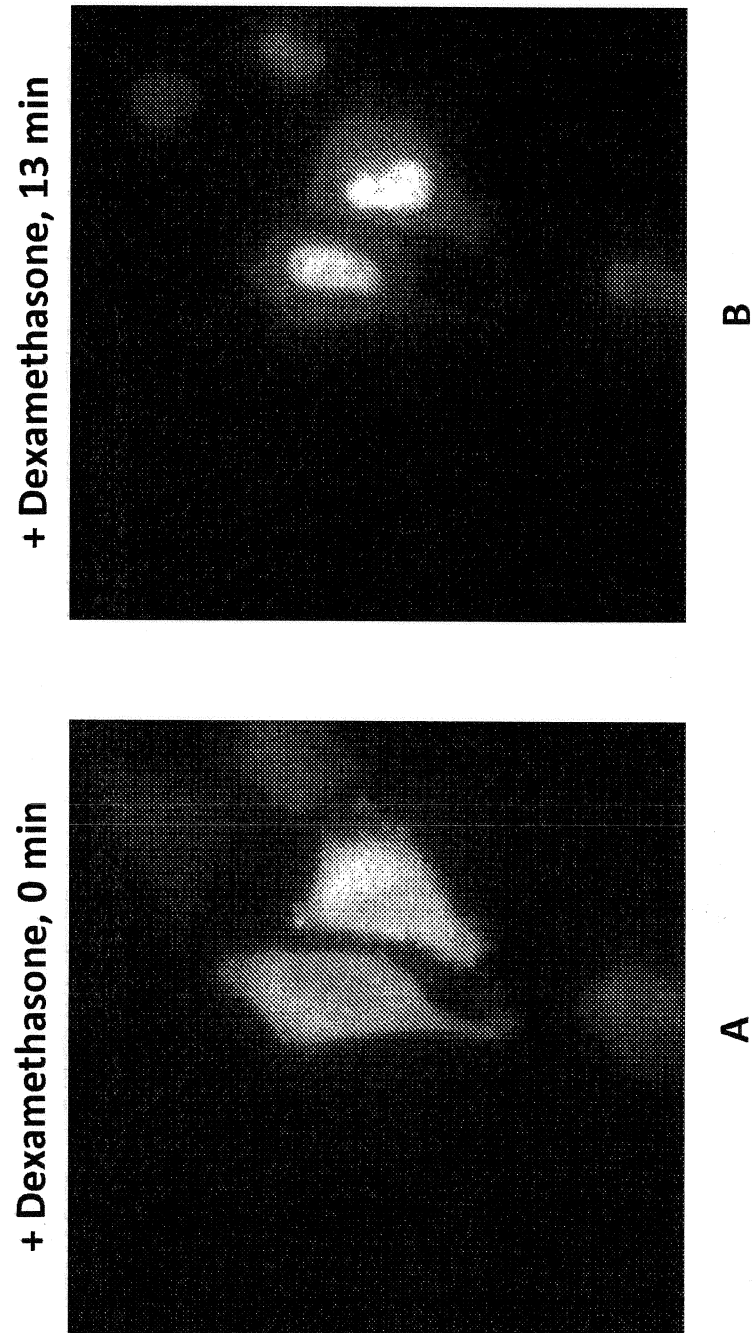


FIG. 90

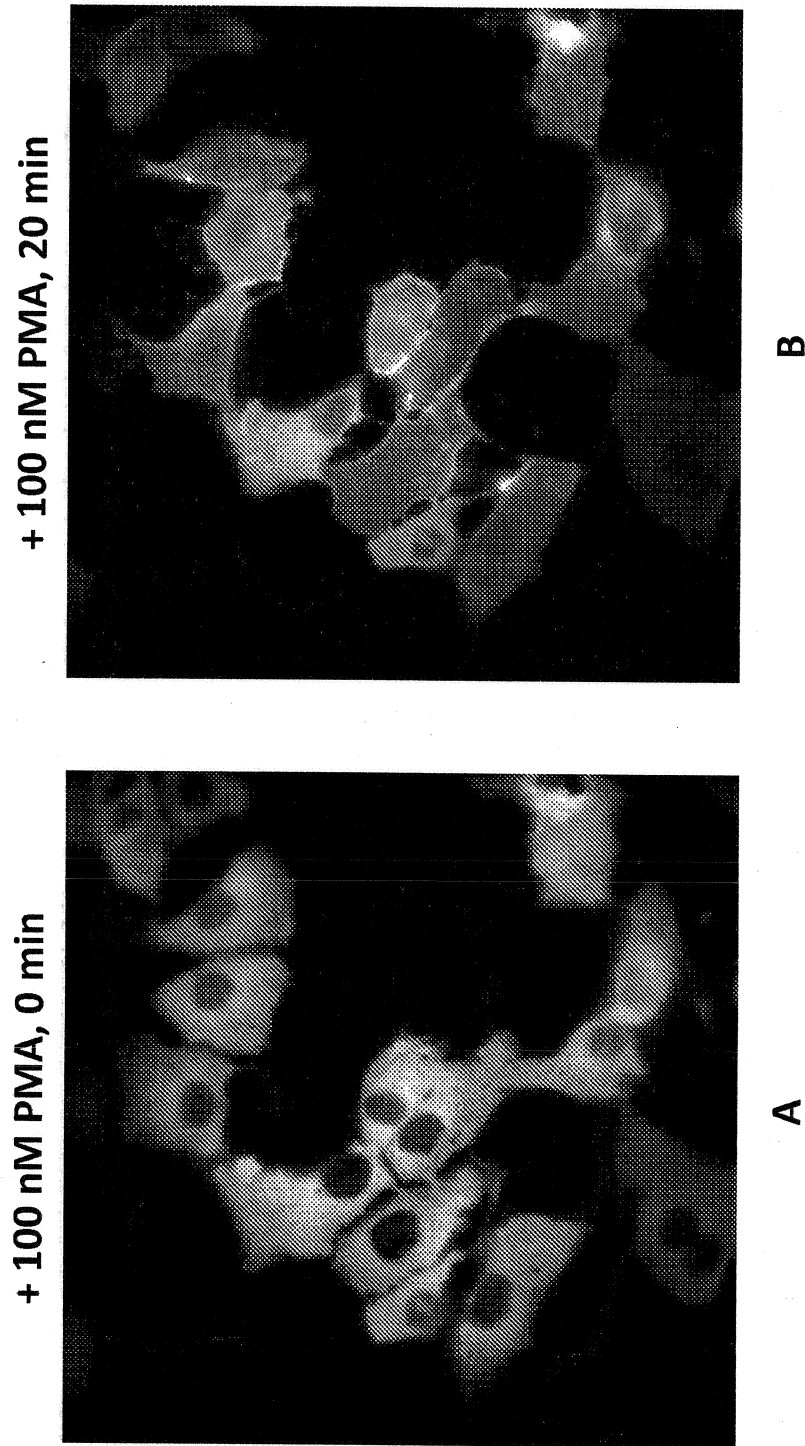


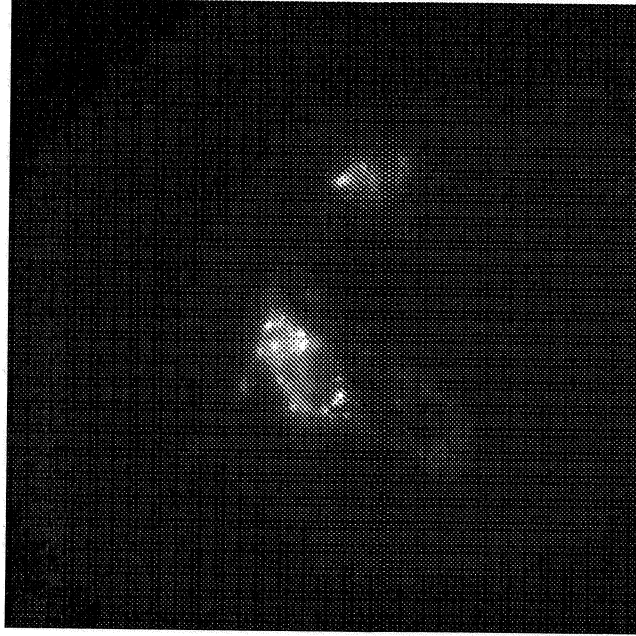
FIG. 91

untreated



A

+ 50 μ M Chloroquine, 16 h



B

FIG. 92