FIG. 1A

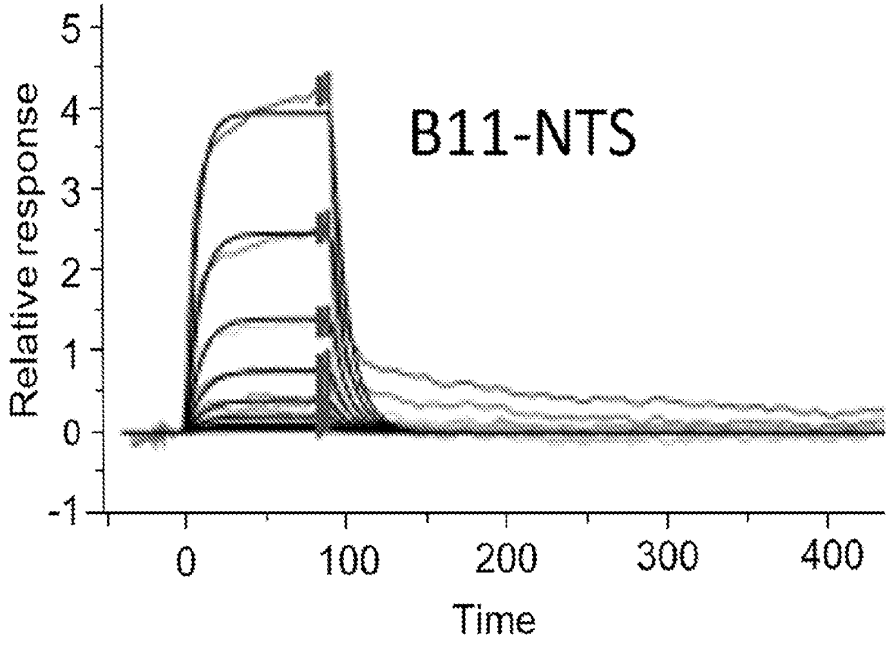

B11-NTS
Relative response
5
4
3
2
1
0
-1
0
100
200
300
400
Time

FIG. 1B

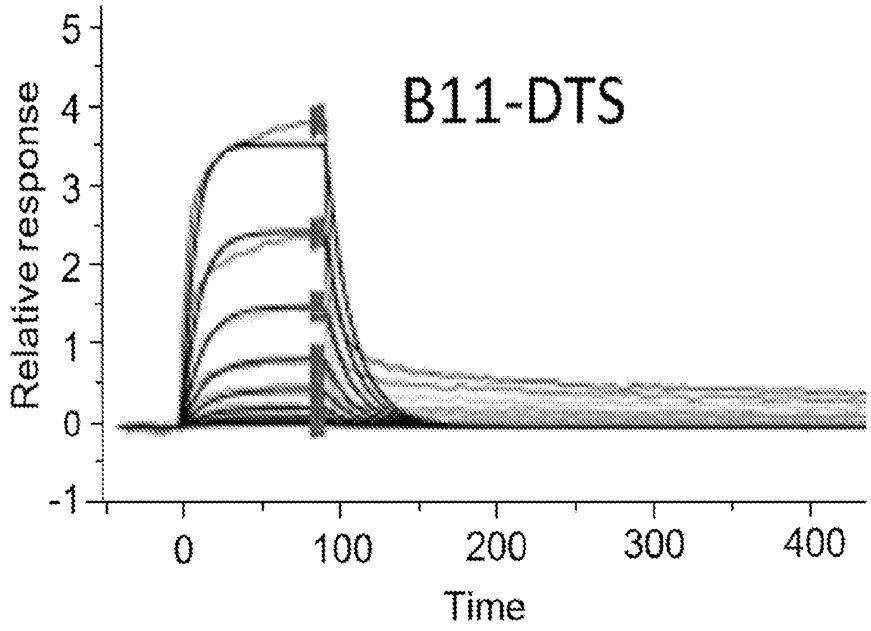

B11-DTS
Relative response
5
4
3
2
1
0
-1
0
100
200
300
400
Time

FIG. 1C

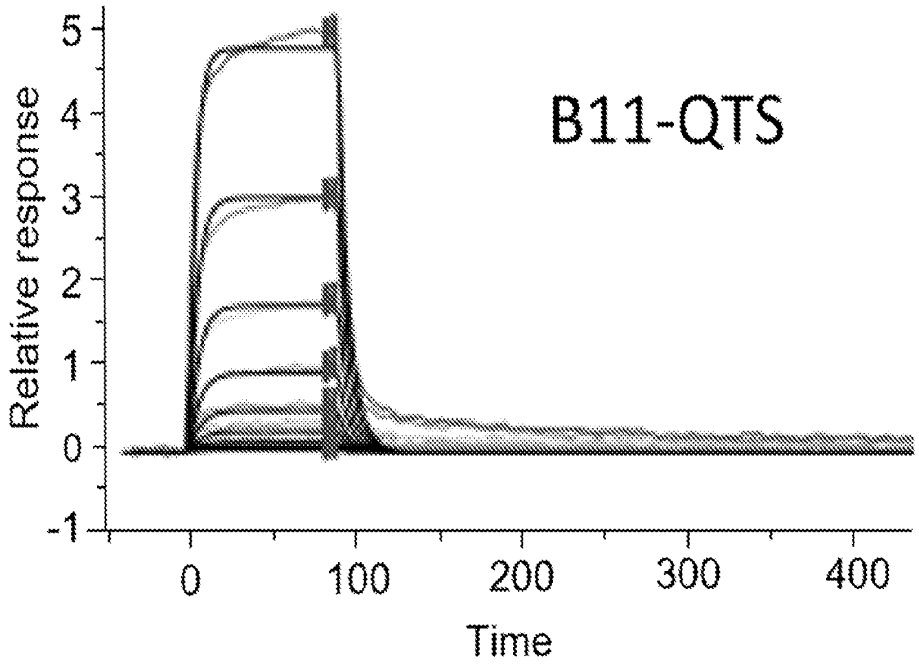


FIG. 1D

Antibody	Kass (1/Ms)	Kdis (1/s)	KD (M)
B11-NTS	6.52E+05	9.89E-02	1.52E-07
B11-DTS	7.52E+05	6.14E-02	8.15E-08
B11-QTS	1.09E+06	1.53E-01	1.41E-07

FIG. 2

Library	CDRs Mutated	Library Size	Theoretical Diversity
Lib-H3	HC CDR3	1.0×10^8	1.4×10^4
Lib-L3	LC CDR3	5.6×10^7	1.4×10^4
Lib-H3&L3	HC CDR3 and LC CDR3	4.2×10^8	2.1×10^8
Lib-H1&H3	HC CDR1 and HC CDR3	1.8×10^8	5.8×10^7
Lib-H3&L1	HC CDR3 and LC CDR1	2.2×10^9	3.2×10^8
Lib-H3&L3	LC CDR3 and LC CDR1	1.2×10^9	3.2×10^8

FIG. 3A

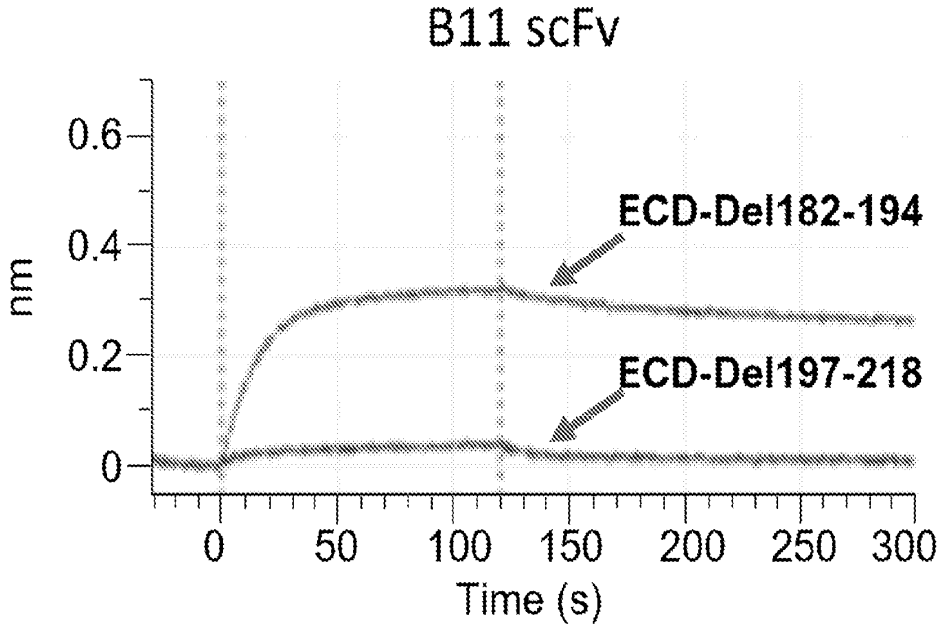

B11 scFv
0.6
0.4
0.2
0
nm
ECD-Del182-194
ECD-Del197-218
0
50
100
150
200
250
300
Time (s)

FIG. 3B

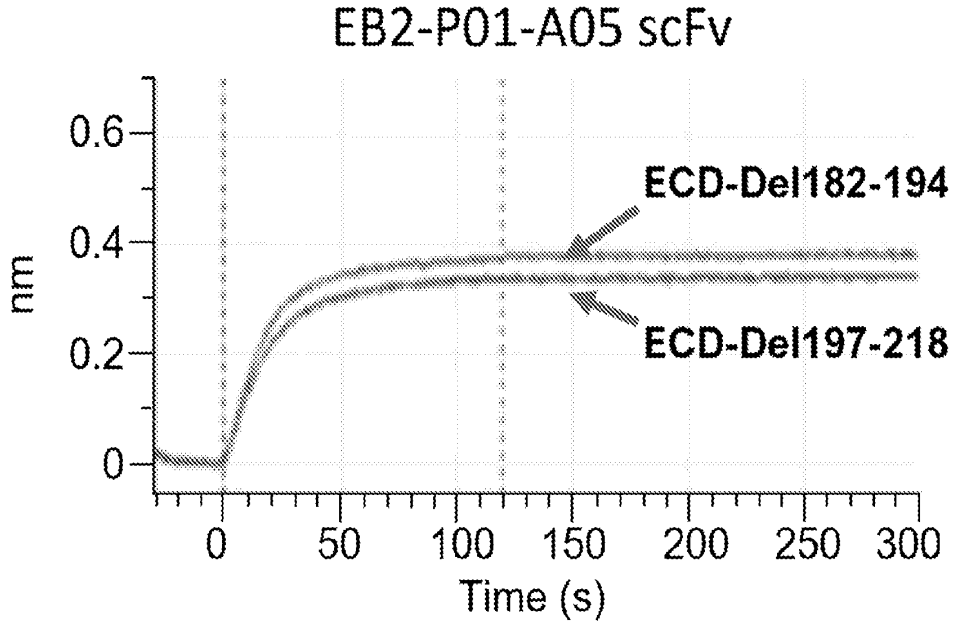
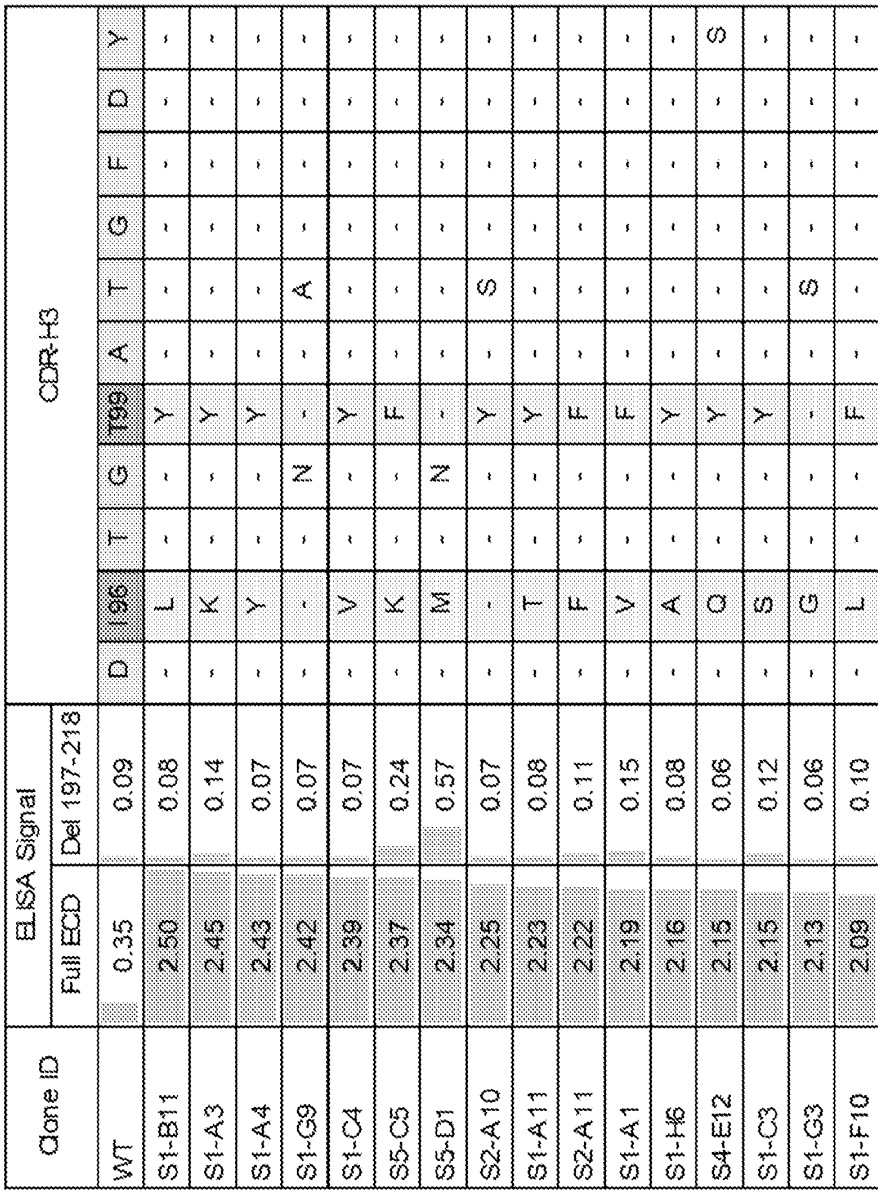

EB2-P01-A05 scFv
0.6
0.4
0.2
0
nm
ECD-Del182-194
ECD-Del197-218
0
50
100
150
200
250
300
Time (s)

FIG. 4A



Clone ID	ELISA Signal		CDR-H3									
	Full ECD	Del 197-218	D	196	T	G	T99	A	T	G	F	D	Y
WT	0.35	0.09	-	L	-	-	Y	-	-	-	-	-	-
S1-B11	2.50	0.08	-	K	-	-	Y	-	-	-	-	-	-
S1-A3	2.45	0.14	-	Y	-	-	Y	-	-	-	-	-	-
S1-A4	2.43	0.07	-	-	-	N	-	-	A	-	-	-	-
S1-G9	2.42	0.07	-	V	-	-	Y	-	-	-	-	-	-
S1-C4	2.39	0.07	-	K	-	-	F	-	-	-	-	-	-
S5-C5	2.37	0.24	-	M	-	N	-	-	-	-	-	-	-
S5-D1	2.34	0.57	-	-	-	-	Y	-	S	-	-	-	-
S2-A10	2.25	0.07	-	T	-	-	Y	-	-	-	-	-	-
S1-A11	2.23	0.08	-	F	-	-	F	-	-	-	-	-	-
S2-A11	2.22	0.11	-	V	-	-	F	-	-	-	-	-	-
S1-A1	2.19	0.15	-	A	-	-	Y	-	-	-	-	-	-
S1-H6	2.16	0.08	-	Q	-	-	Y	-	-	-	-	-	S
S4-E12	2.15	0.06	-	S	-	-	Y	-	-	-	-	-	-
S1-C3	2.15	0.12	-	G	-	-	-	-	S	-	-	-	-
S1-G3	2.13	0.06	-	L	-	-	F	-	-	-	-	-	-
S1-F10	2.09	0.10	-	-	-	-	-	-	-	-	-	-	-

FIG. 4A (Continued)

Clone ID	ELISA Signal		CDR-H3												
	Full ECD	Del 197-218													
S2-H1	2.08	0.31	-	Y	-	-	F	-	-	-	-	-	-	-	-
S1-G2	2.06	0.13	-	-	-	-	F	-	S	-	-	-	-	-	-
S3-C7	2.05	0.09	-	R	-	-	Y	-	-	-	-	-	-	-	-
S2-D4	2.01	0.06	-	T	-	-	Y	-	S	-	-	-	-	-	-
S1-E5	1.99	0.06	-	F	-	-	Y	-	-	-	-	-	-	-	-
S1-G1	1.98	0.09	~	-	~	~	Y	~	~	~	~	L	~	~	~
S1-G10	1.96	0.06	~	W	~	~	Y	~	~	~	~	~	~	~	~
S1-C2	1.94	0.07	-	Q	-	-	Y	-	-	-	-	-	-	-	-
S1-A6	1.90	0.13	-	A	-	-	F	-	-	-	-	-	-	-	-
S2-C6	1.89	0.03	~	H	~	~	Y	~	~	~	~	~	~	~	~
S2-C12	1.82	0.26	~	S	~	N	-	~	~	~	~	~	~	~	~
S1-A2	1.88	0.08	-	-	~	~	Y	~	~	~	~	~	~	~	~
S3-E9	1.79	0.17	-	S	-	-	Y	-	-	K	-	-	-	-	-
S5-H2	1.78	0.15	~	T	~	~	F	~	~	~	~	~	~	~	~
S4-F9	1.71	0.05	~	M	~	~	Y	~	~	~	~	~	~	~	~
S5-E7	1.70	0.16	~	R	~	~	S	~	S	~	~	~	~	~	~
S1-F12	1.70	0.07	~	N	~	~	Y	~	~	~	~	~	~	~	~
S5-H4	1.56	0.06	~	G	~	~	Y	~	~	~	~	~	~	~	~
S3-F2	1.54	0.06	~	-	~	~	Y	~	A	~	~	~	~	~	~
S1-B3	1.52	0.06	~	G	~	~	-	~	A	~	~	~	~	~	~

FIG. 4B

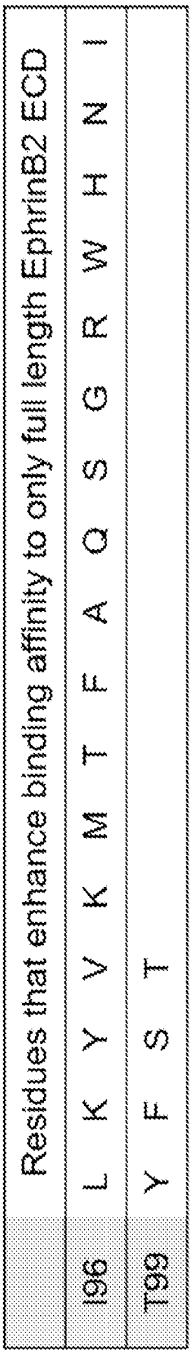
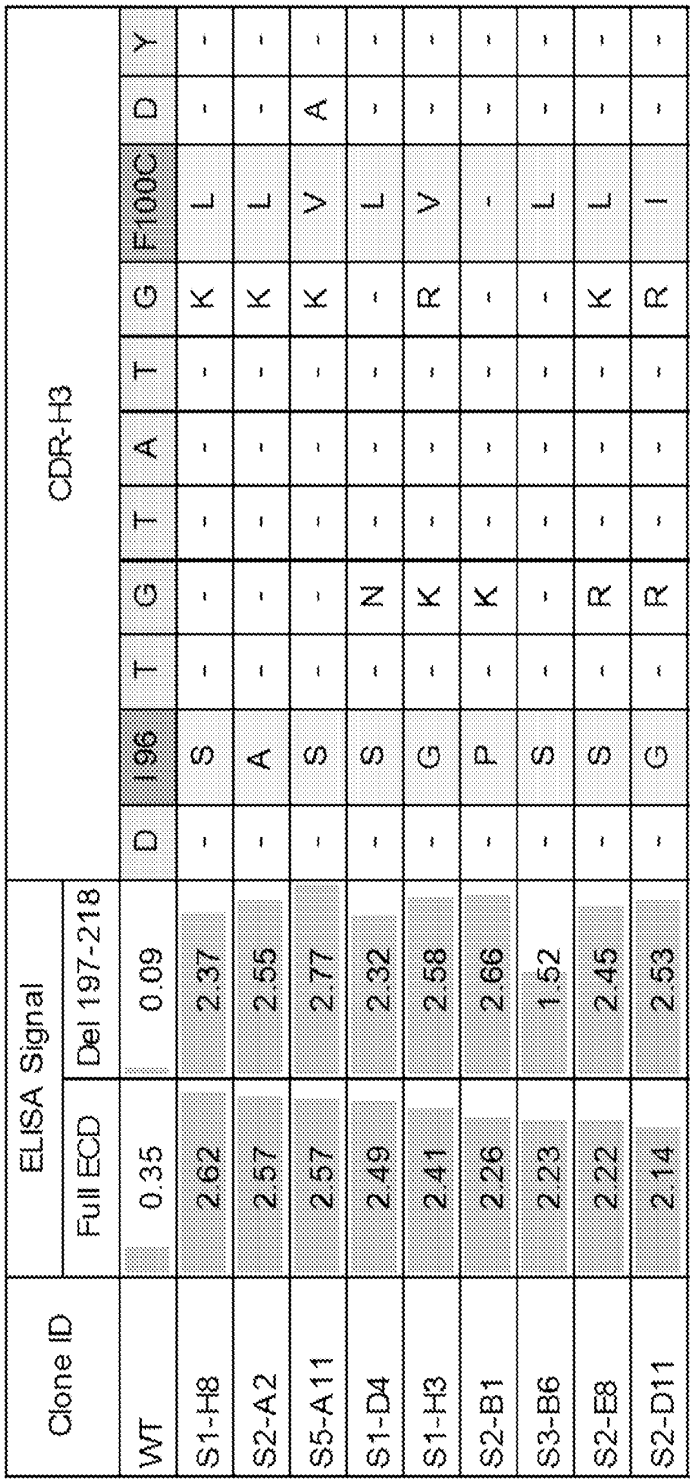

Residues that enhance binding affinity to only full length EphrinB2 ECD
I96 L K Y V K M T F A Q S G R W H N I
T99 Y F S T

FIG. 5A



Clone ID	ELISA Signal		CDR-H3									
	Full ECD	Del 197-218	D	196	T	G	T	A	T	G	F100C	D	Y
WT	0.35	0.09	-	S	-	-	-	-	-	K	L	-	-
S1-H8	2.62	2.37	-	A	-	-	-	-	-	K	L	-	-
S2-A2	2.57	2.55	-	S	-	-	-	-	-	K	V	A	-
S5-A11	2.57	2.77	-	S	-	N	-	-	-	-	L	-	-
S1-D4	2.49	2.32	-	G	-	K	-	-	-	R	V	-	-
S1-H3	2.41	2.58	-	P	-	K	-	-	-	-	-	-	-
S2-B1	2.26	2.66	-	S	-	-	-	-	-	-	L	-	-
S3-B6	2.23	1.52	-	S	-	R	-	-	-	K	L	-	-
S2-E8	2.22	2.45	-	S	-	R	-	-	-	K	I	-	-
S2-D11	2.14	2.53	-	G	-	R	-	-	-	R	L	-	-

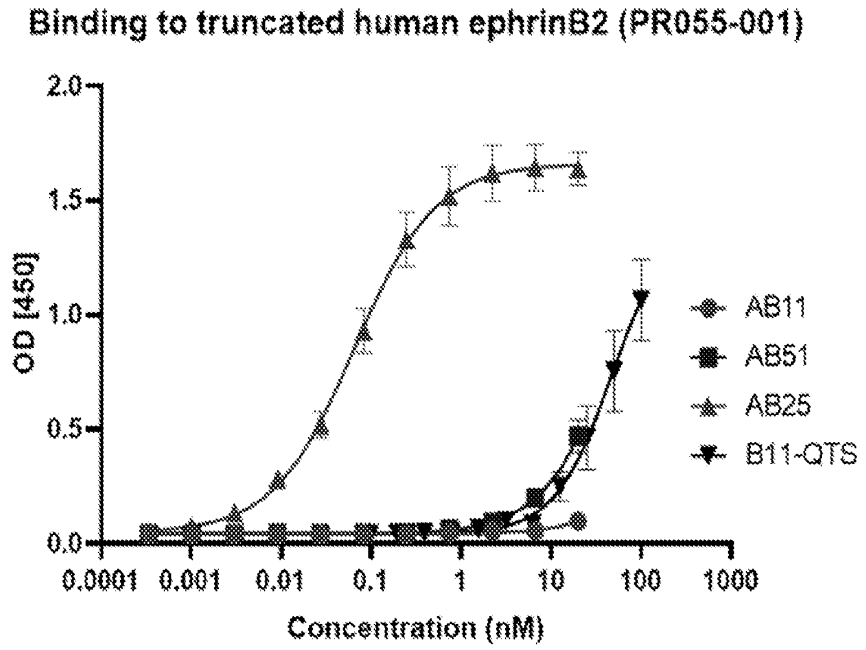
FIG. 5A (Continued)

Clone ID	ELISA Signal		CDR-H3										
	Full ECD	Del 197-218											
S3-C9	2.14	1.76	-	R	-	-	-	-	-	-	L	-	-
S3-B3	2.07	1.20	-	K	-	-	-	-	-	-	L	-	-
S1-A9	1.98	2.20	-	S	-	-	-	-	-	K	I	-	-
S1-F5	1.88	2.13	-	S	-	-	-	-	-	K	V	-	-
S1-B5	1.70	2.01	-	S	-	-	-	-	-	R	V	-	-
PLATE3-F11	2.14	1.16	-	A	-	-	-	-	-	-	L	-	-
PLATE3-F12	2.11	2.44	-	C	-	-	-	-	-	-	V	-	-
PLATE3-A2	1.96	1.98	-	-	-	N	-	-	-	-	I	-	-
S1-B4	1.41	1.73	-	S	-	-	-	-	-	R	I	-	-
S5-B9	1.36	1.80	-	S	-	N	-	-	-	-	V	-	-
S2-B8	1.30	1.56	-	M	-	N	-	-	-	-	V	-	-

FIG. 5B

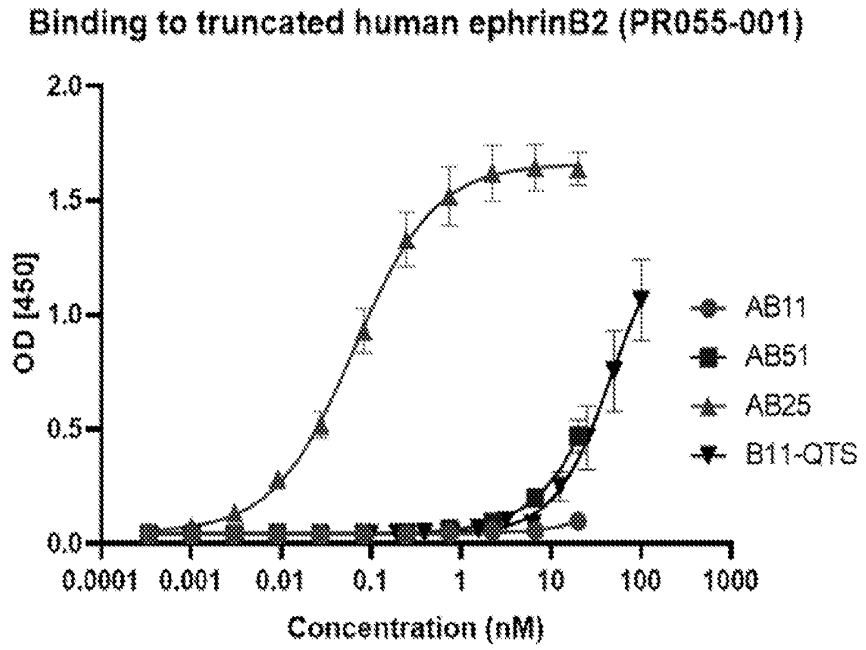
	Residues that enhance binding to both EphrinB2 constructs
I96	S A G P R K C M I
G98	N K R G
G100B	K R G
F100C	L V I F

FIG. 6A



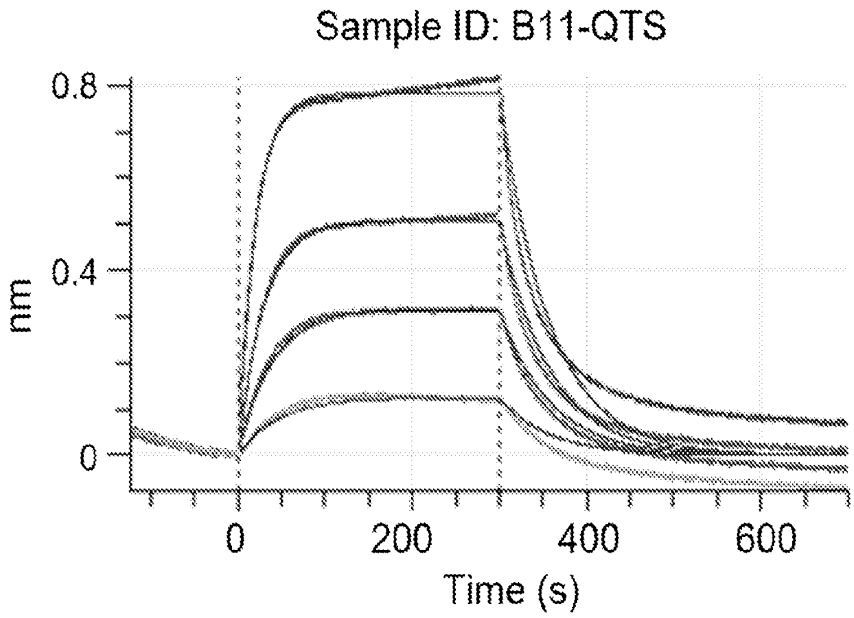
Registry ID	Mutations	EC50 [nM]
B11-QTS	Parental	>100
AB11	B11-huG1-QTS-H3_LY	>20
AB51	B11-huG1-QTS-_H3_MY	>20
AB25	B11-huG1-QTS-H3_GKRV	0.0652

FIG. 6B



Registry ID	Mutations	EC50 [nM]
B11-QTS	Parental	>100
AB11	B11-huG1-QTS-H3_LY	>20
AB51	B11-huG1-QTS-_H3_MY	>20
AB25	B11-huG1-QTS-H3_GKRV	0.0652

FIG. 7A



K_D (nM)= **14.36 nM**

K_{on} ($M^{-1}S^{-1}$)=9.677E+05

K_{dis} (S^{-1})=1.390E-02

FIG. 7B

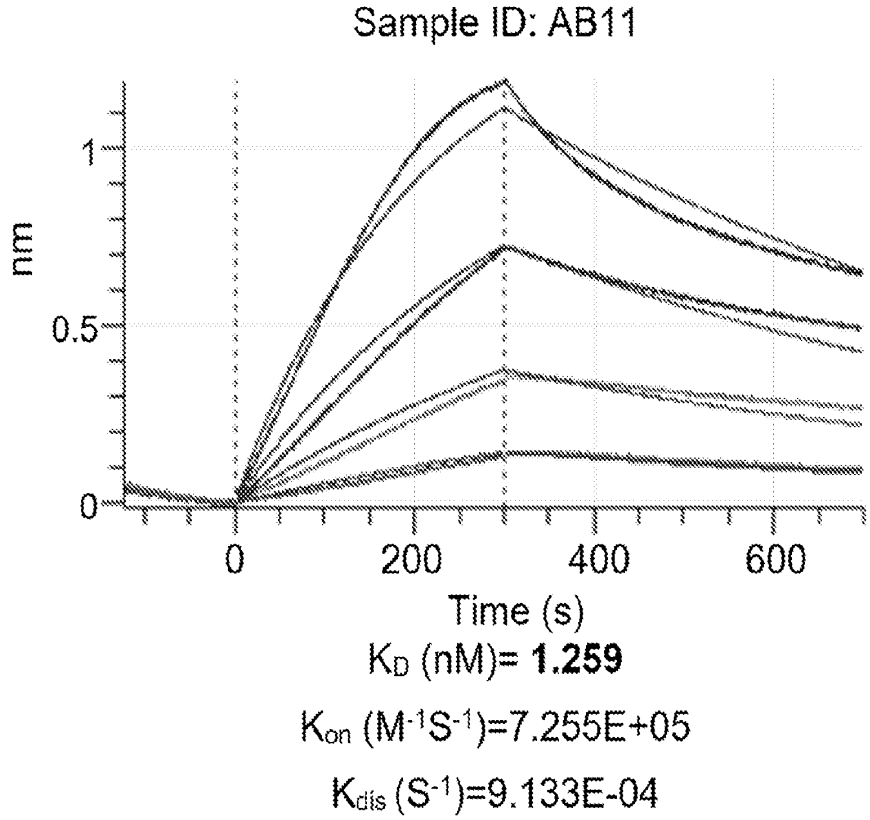
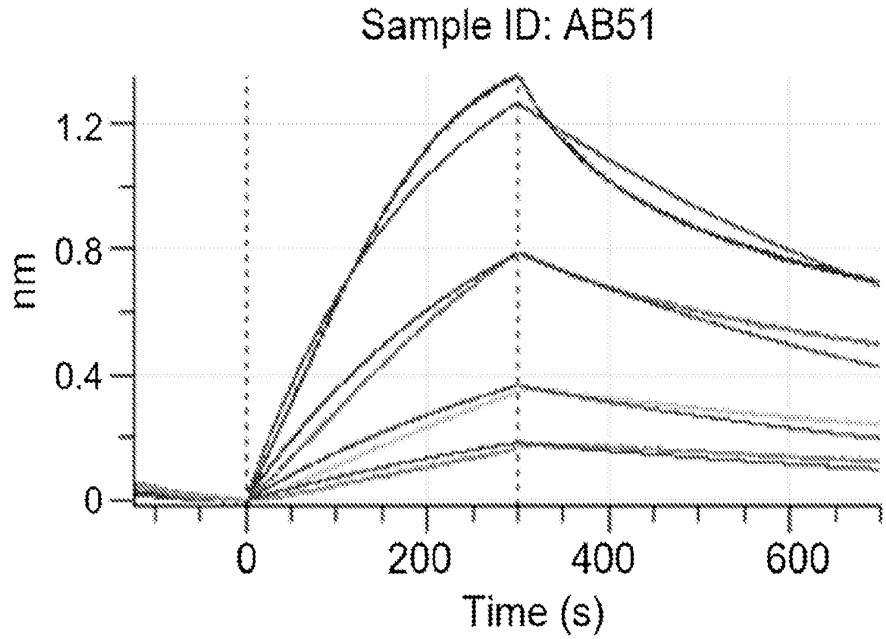

Sample ID: AB11
nm
1
0.5
0
0
200
400
600
Time (s)
K_D (nM)= **1.259**
K_{on} ($M^{-1}S^{-1}$)=7.255E+05
K_{dis} (S^{-1})=9.133E-04

FIG. 7C



K_D (nM)= **1.496**

K_{on} ($M^{-1}S^{-1}$)=7.099E+05

K_{dis} (S^{-1})=1.062E-03

FIG. 7D

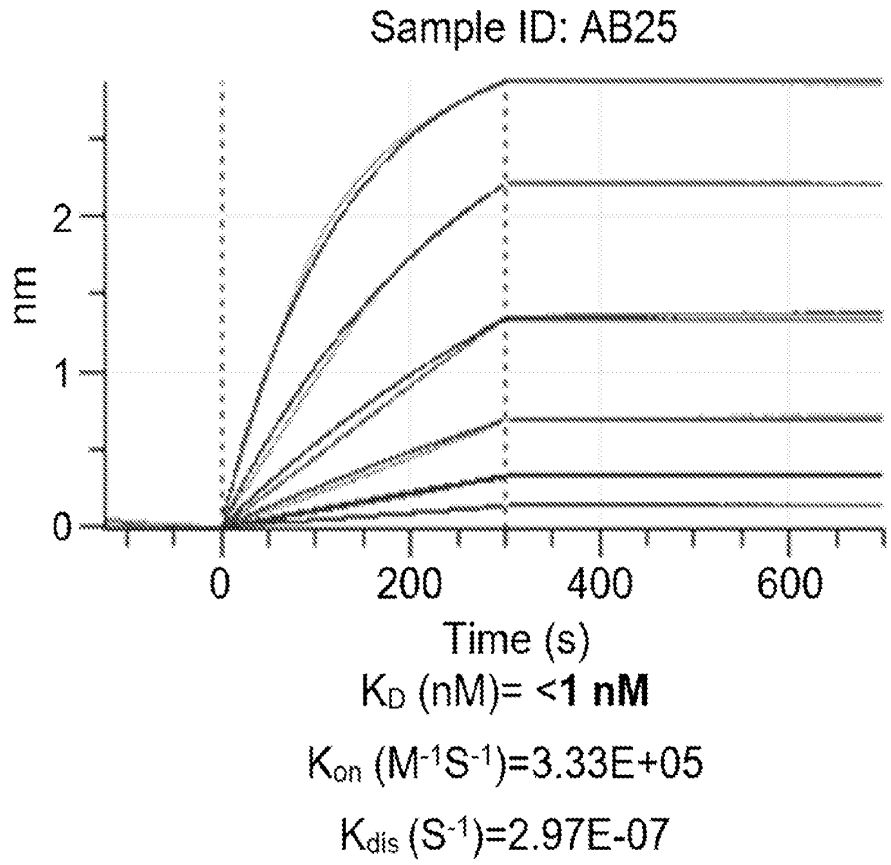
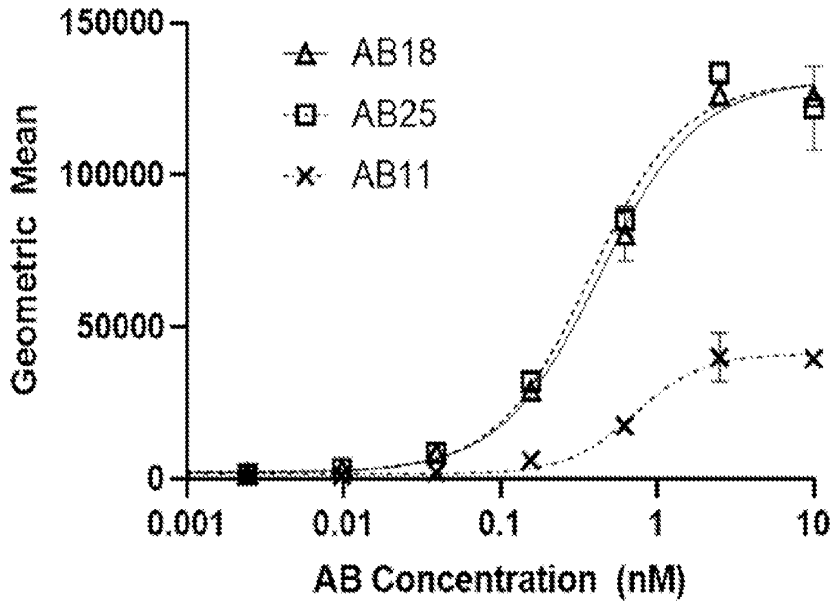

Sample ID: AB25
nm
2
1
0
0
200
400
600
Time (s)
K_D (nM)= <1 nM
K_{on} ($M^{-1}S^{-1}$)=3.33E+05
K_{dis} (S^{-1})=2.97E-07

FIG. 8



AB	EC50 (nM)
AB18	0.4275
AB25	0.3738
AB11	0.7196

FIG. 9A

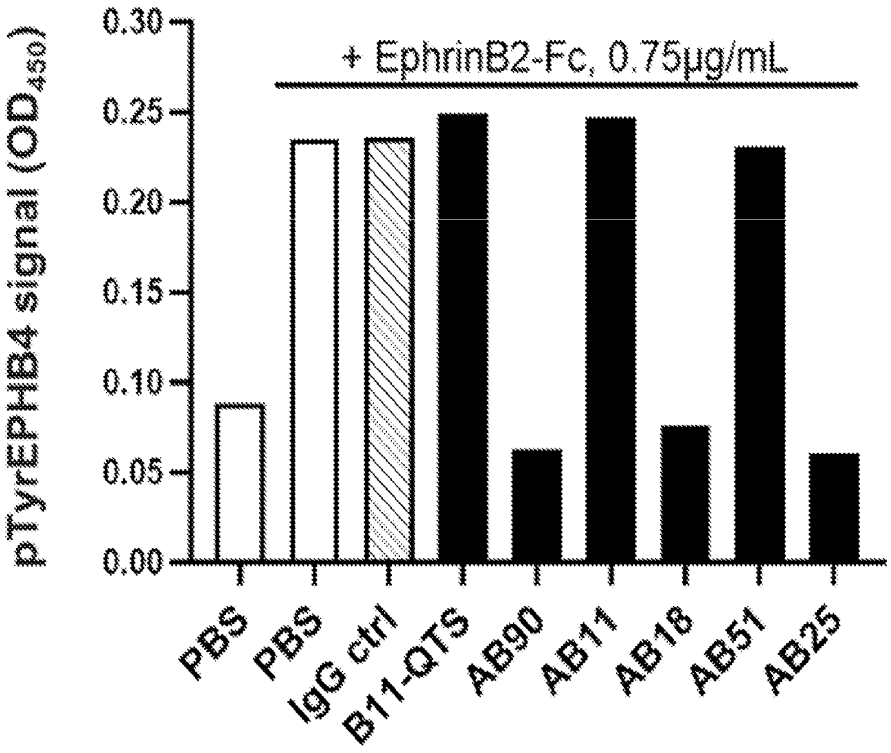

+ EphrinB2-Fc, 0.75µg/mL
pTyrEPHB4 signal (OD450)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
PBS
PBS
IgG ctrl
B11-QTS
AB90
AB11
AB18
AB51
AB25

FIG. 9B

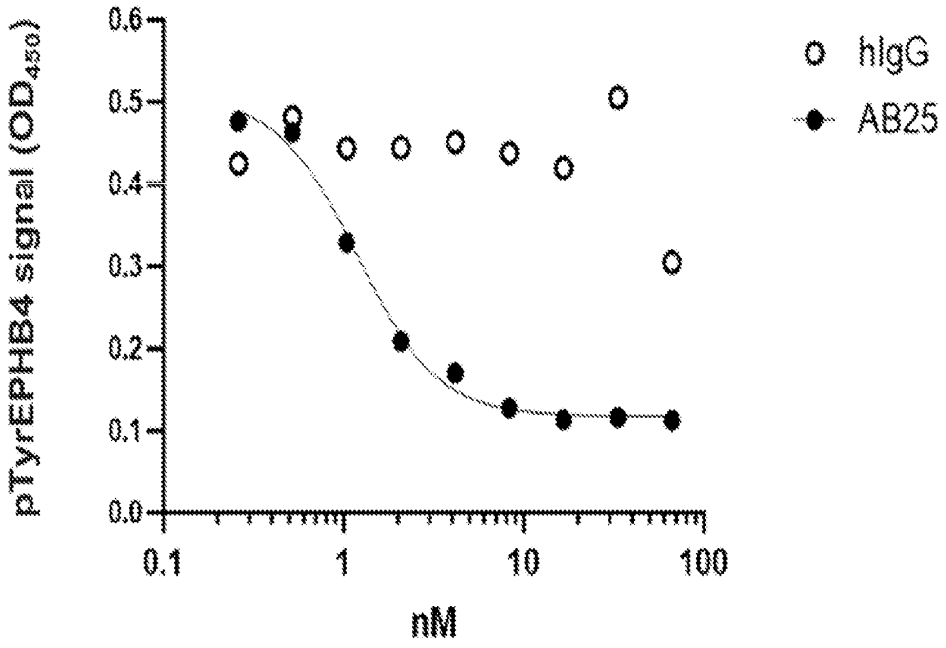

pTyrEPHB4 signal (OD450)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.1
1
10
100
nM
hIgG
AB25

FIG. 10A

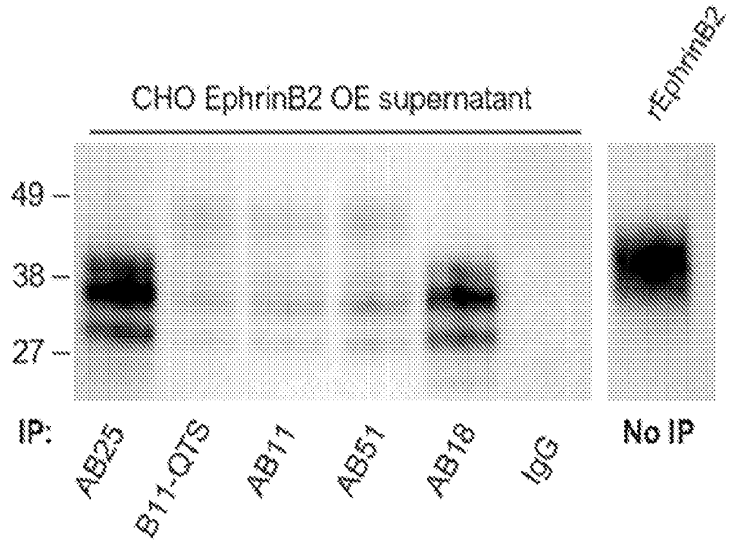


FIG. 10B

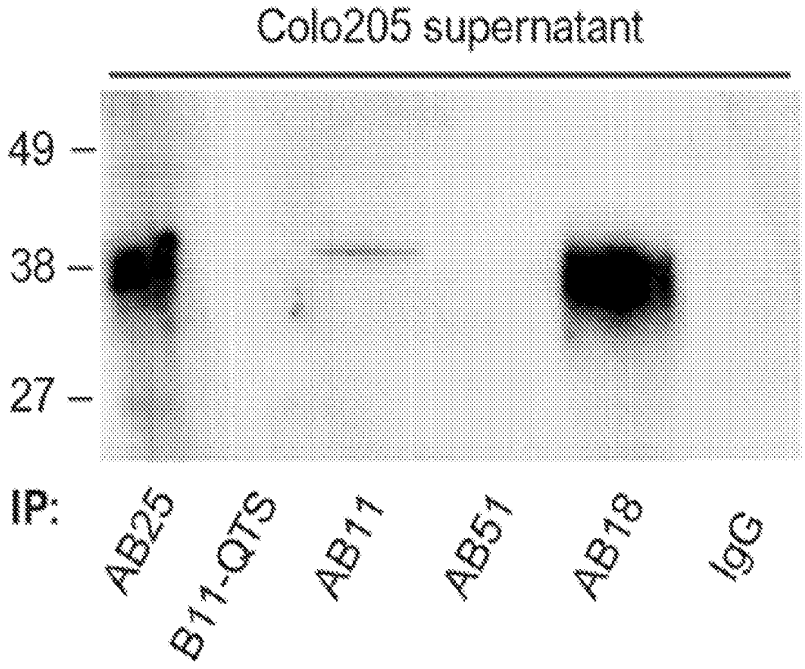


FIG. 10C

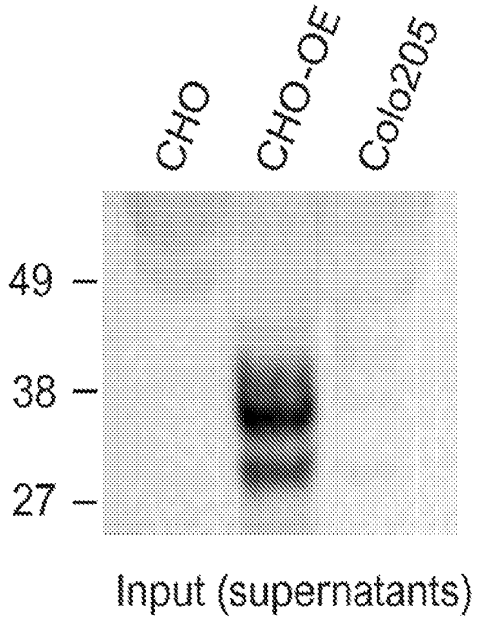


FIG. 10D

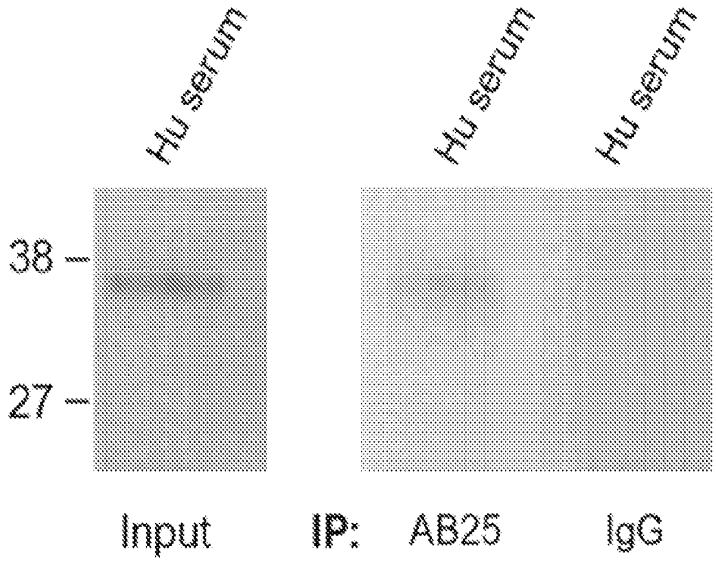


FIG. 11A

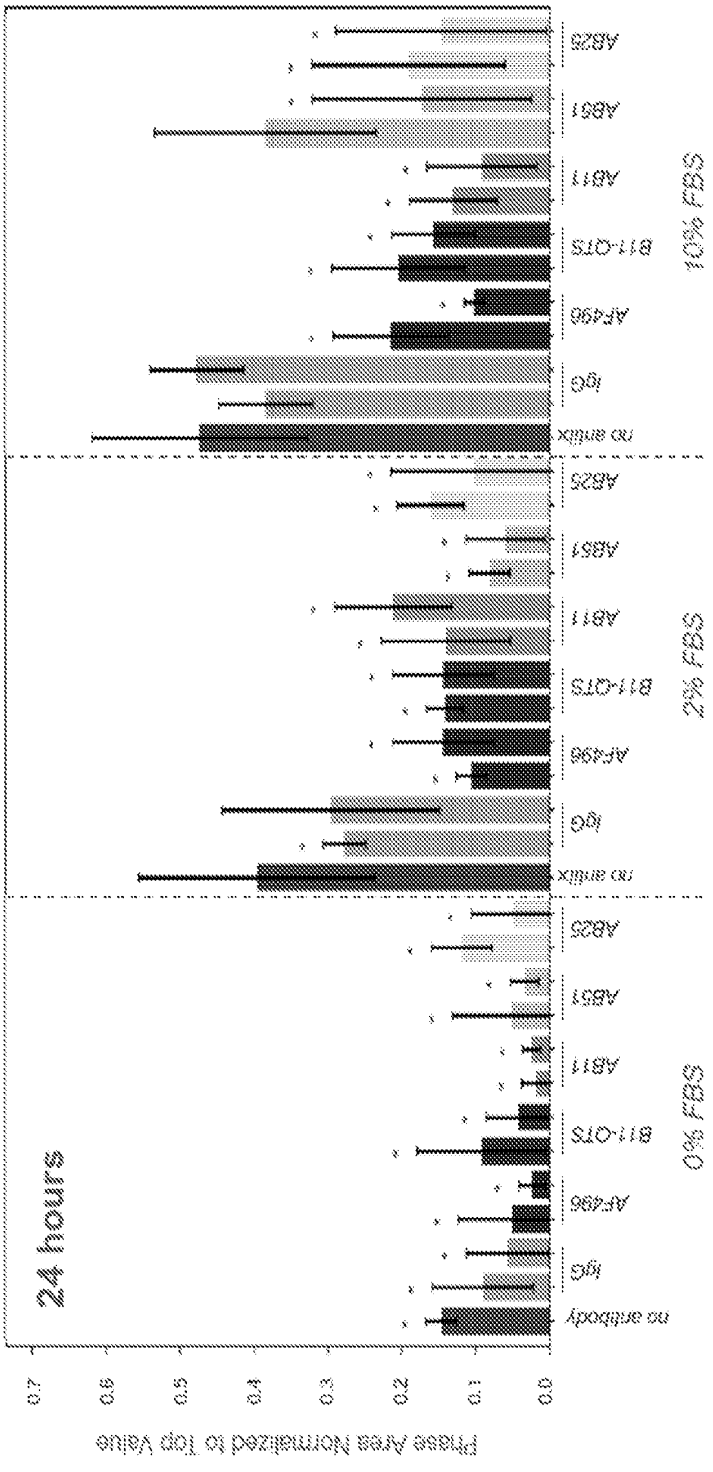

24 hours
Phase Area Normalized to Top Value
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0% FBS
2% FBS
10% FBS
no antibody
IgG
AF496
811-OTS
AB11
AB51
AB25
no antibody
IgG
AF496
811-OTS
AB11
AB51
AB25
no antibody
IgG
AF496
811-OTS
AB11
AB51
AB25

FIG. 11B

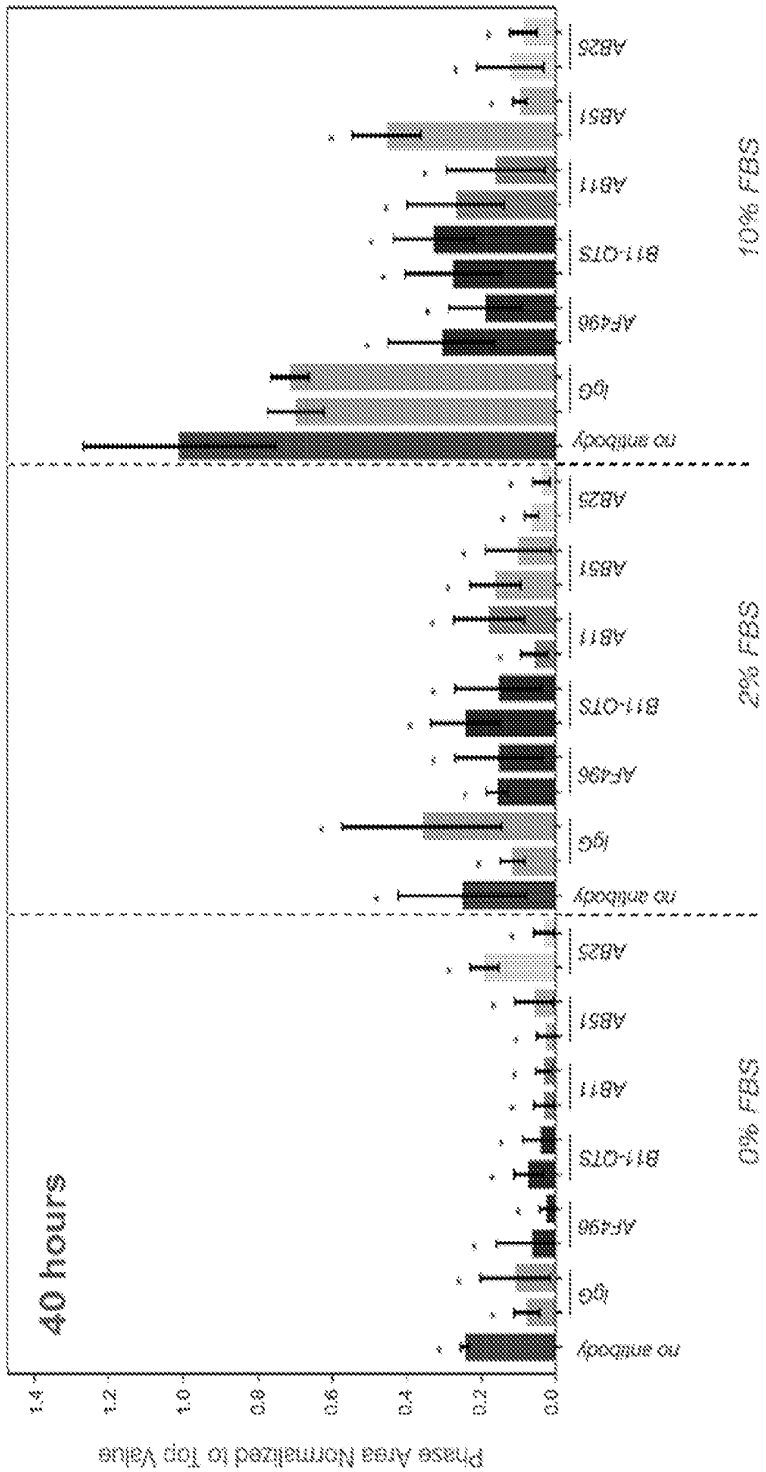

40 hours
Pmase Area Normalized to Top Value
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
no antibody
IgG
AF498
B11-OTS
AB11
AB51
AB25
0% FBS
2% FBS
10% FBS

FIG. 12A

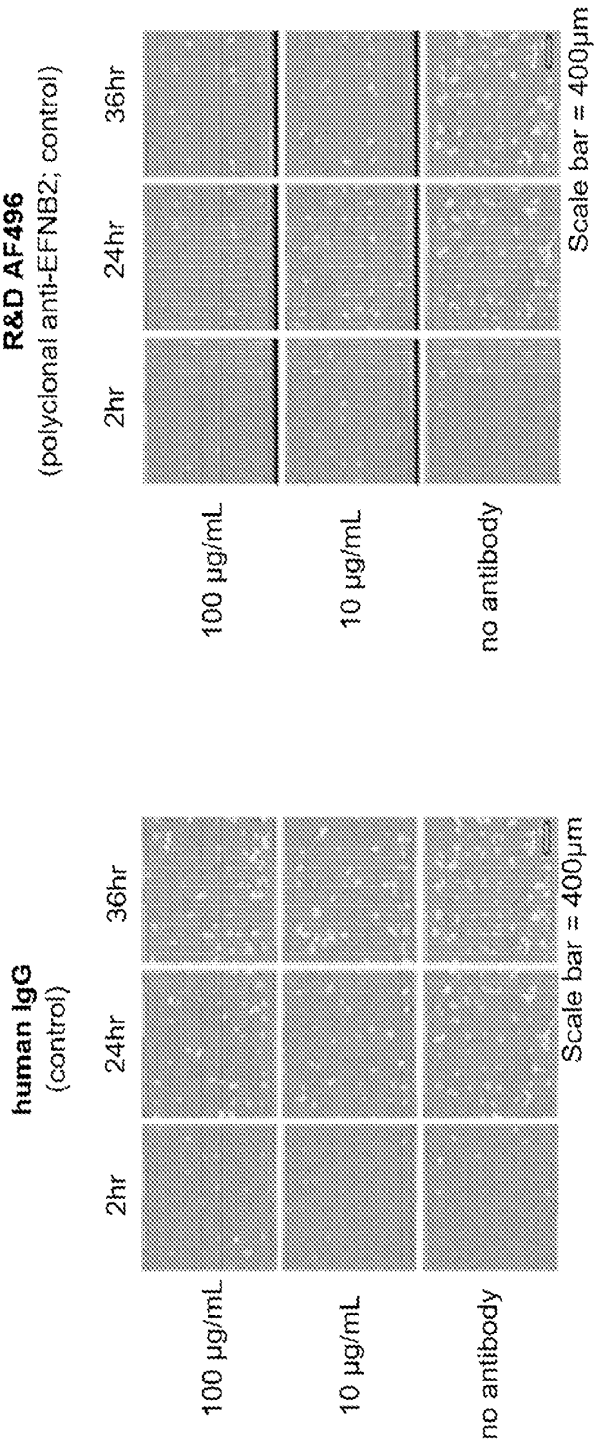

human IgG
(control)
R&D AF496
(polyclonal anti-EFNB2; control)
2hr
24hr
36hr
100 µg/mL
10 µg/mL
no antibody
Scale bar = 400µm

FIG. 12B

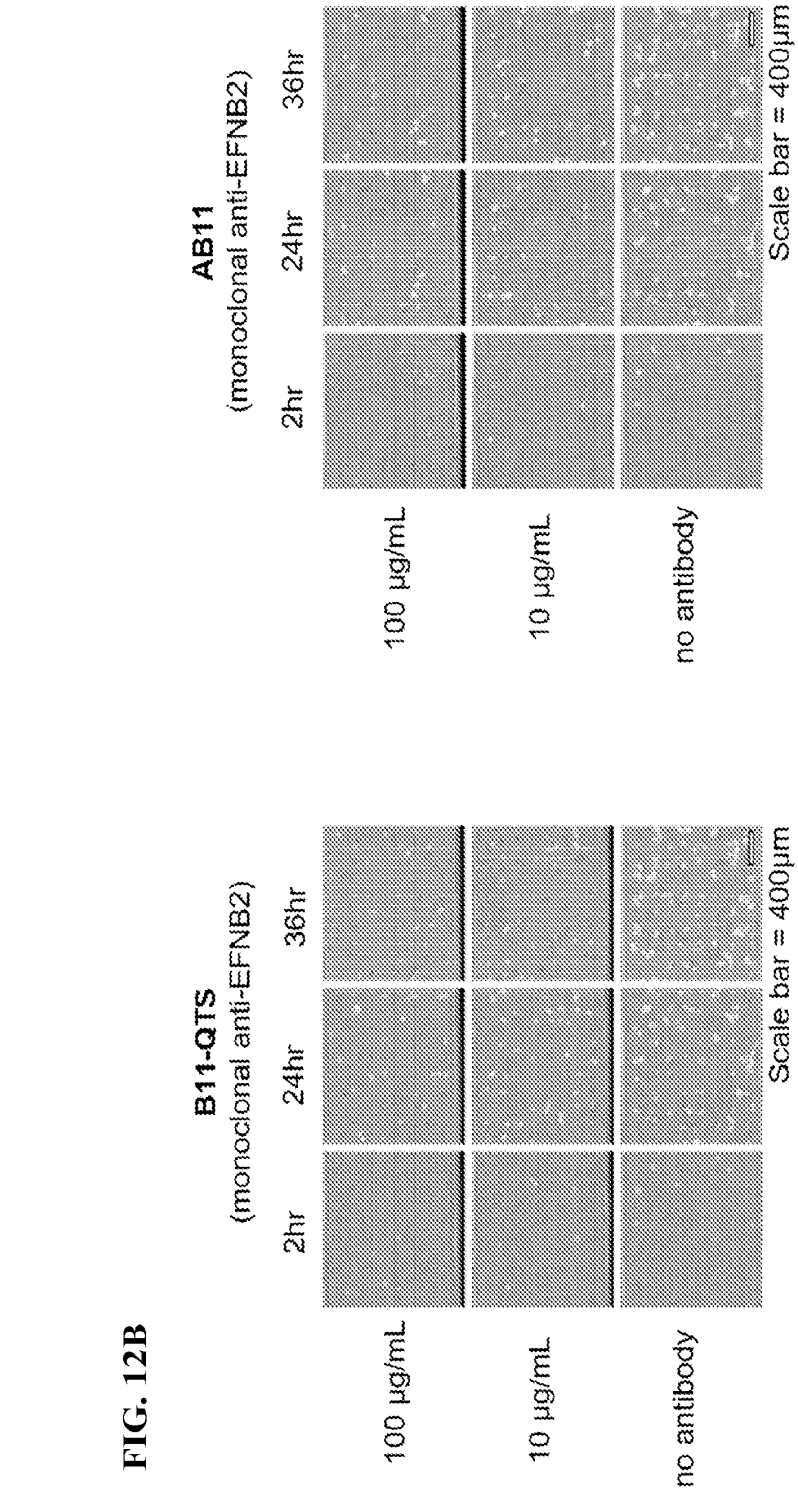

B11-QTS
(monoclonal anti-EFNB2)
2hr
24hr
36hr
100 µg/mL
10 µg/mL
no antibody
Scale bar = 400µm
AB11
(monoclonal anti-EFNB2)
2hr
24hr
36hr
100 µg/mL
10 µg/mL
no antibody
Scale bar = 400µm

FIG. 12C

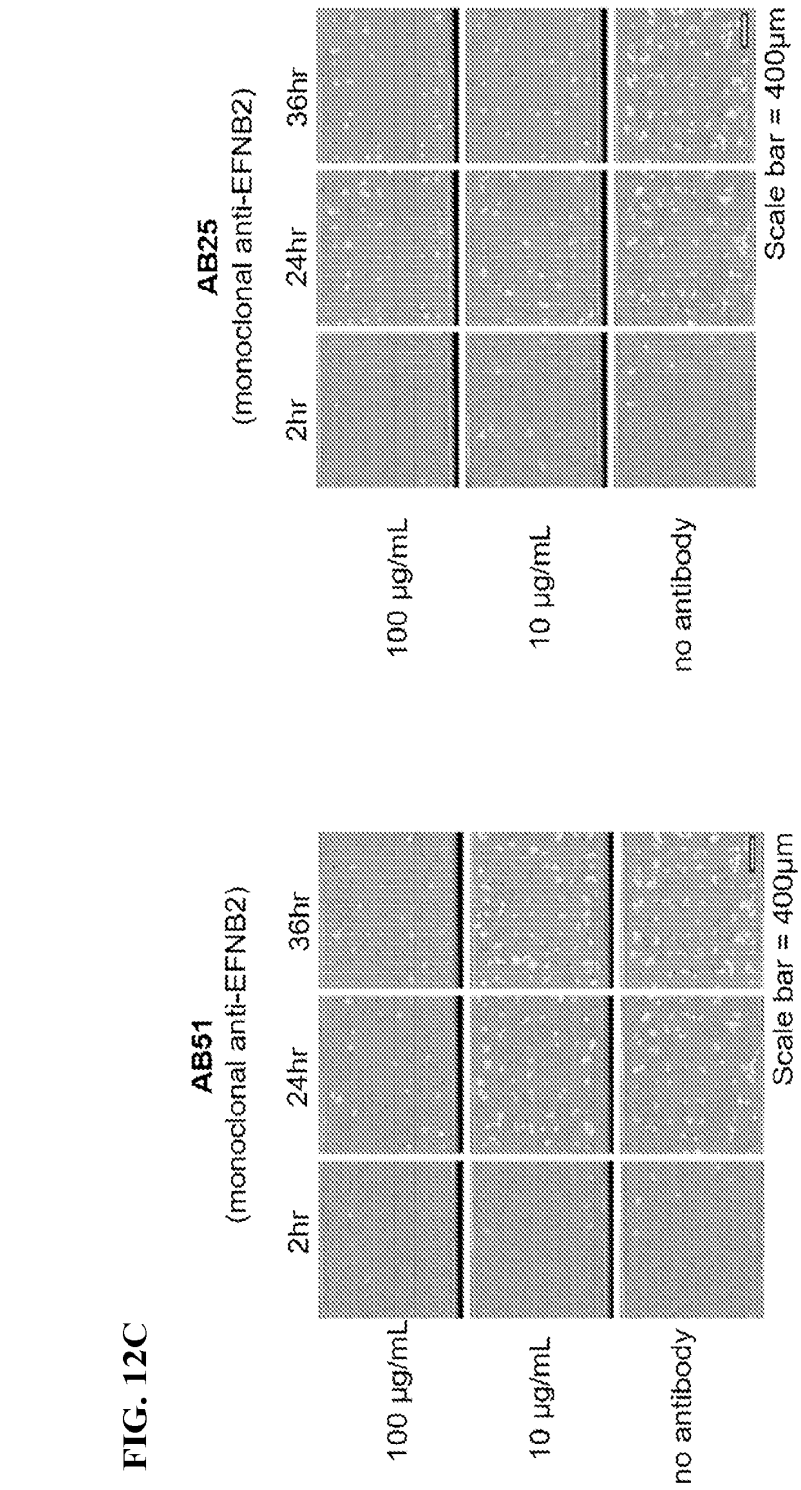

AB51
(monoclonal anti-EFNB2)
2hr
24hr
36hr
100 µg/mL
10 µg/mL
no antibody
Scale bar = 400µm
AB25
(monoclonal anti-EFNB2)
2hr
24hr
36hr
100 µg/mL
10 µg/mL
no antibody
Scale bar = 400µm

FIG. 13A

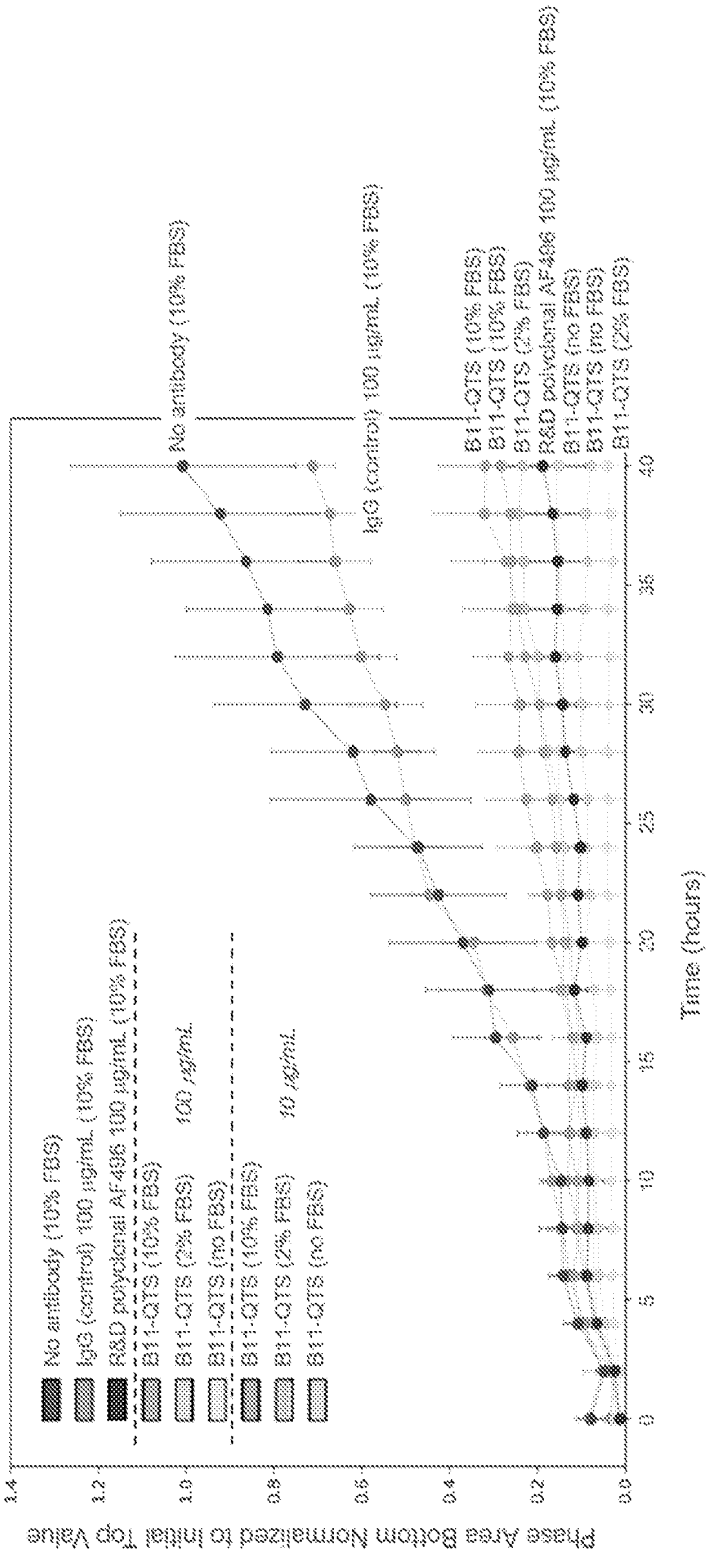

Phase Area Bottom Normalized to Initial Top Value
Time (hours)
No antibody (10% FBS)
IgG (control) 100 ng/mL (10% FBS)
R&D polyclonal AF406 100 µg/mL (10% FBS)
B11-QTS (10% FBS)
B11-QTS (2% FBS)
100 µg/mL
B11-QTS (no FBS)
B11-QTS (10% FBS)
B11-QTS (2% FBS)
10 µg/mL
B11-QTS (no FBS)
No antibody (10% FBS)
IgG (control) 100 ng/mL (10% FBS)
B11-QTS (10% FBS)
B11-QTS (10% FBS)
B11-QTS (2% FBS)
R&D polyclonal AF406 100 ng/mL (10% FBS)
B11-QTS (no FBS)
B11-QTS (no FBS)
B11-QTS (2% FBS)

FIG. 13B

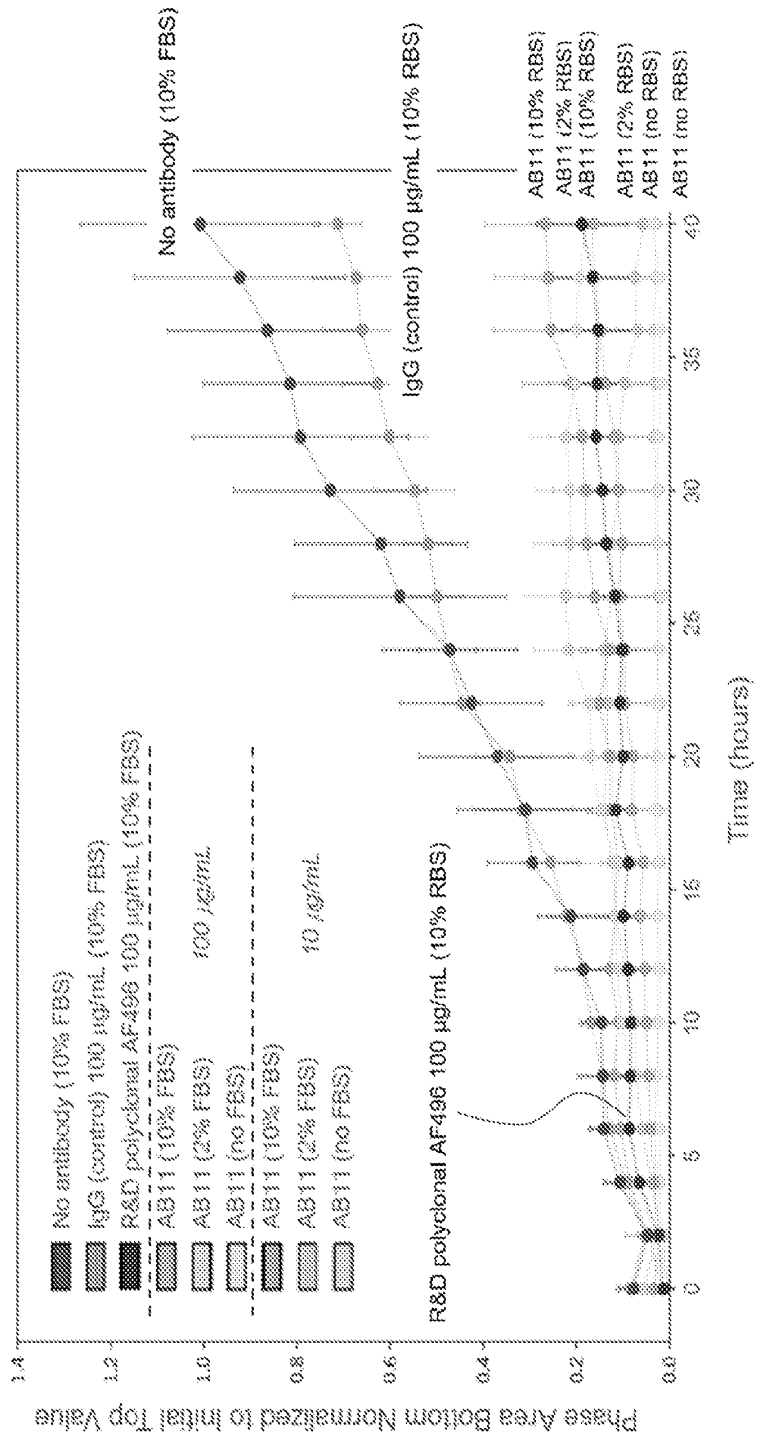

Phase Area Bottom Normalized to Initial Top Value
Time (hours)
No antibody (10% FBS)
IgG (control) 100 µg/mL (10% FBS)
R&D polyclonal AF496 100 µg/mL (10% FBS)
AB11 (10% FBS)
AB11 (2% FBS)
AB11 (no FBS)
100 µg/mL
10 µg/mL
R&D polyclonal AF496 100 µg/mL (10% RBS)
IgG (control) 100 µg/mL (10% RBS)
No antibody (10% FBS)
AB11 (10% RBS)
AB11 (2% RBS)
AB11 (10% RBS)
AB11 (2% RBS)
AB11 (no RBS)
AB11 (no RBS)

FIG. 13C

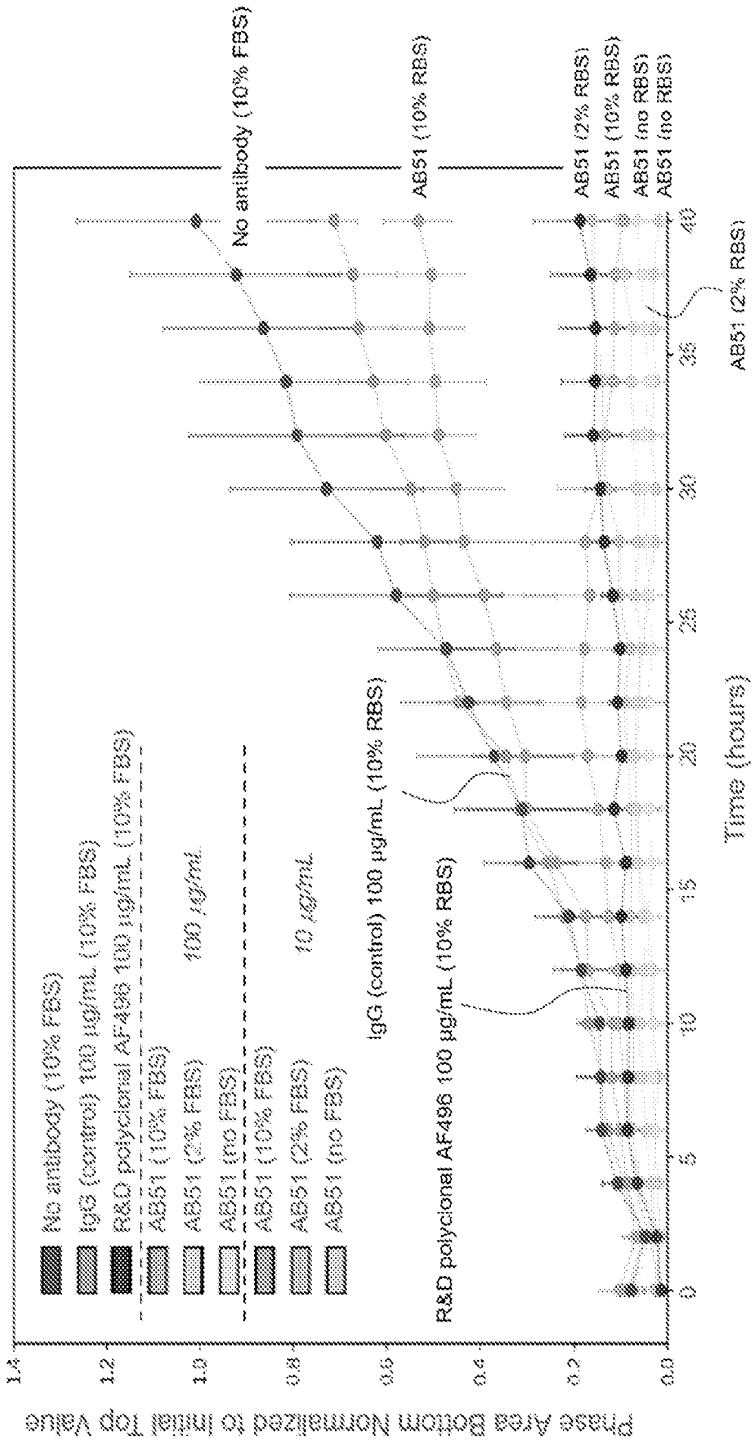

No antibody (10% FBS)
IgG (control) 100 µg/mL (10% FBS)
R&D polyclonal AF496 100 µg/mL (10% FBS)
AB51 (10% FBS)
AB51 (2% FBS)
100 µg/mL
AB51 (no FBS)
AB51 (10% FBS)
AB51 (2% FBS)
10 µg/mL
AB51 (no FBS)
IgG (control) 100 µg/mL (10% RBS)
R&D polyclonal AF496 100 µg/mL (10% RBS)
No antibody (10% FBS)
AB51 (10% RBS)
AB51 (2% RBS)
AB51 (10% RBS)
AB51 (no RBS)
AB51 (no RBS)
AB51 (2% RBS)
Phase Area Bottom Normalized to Initial Top Value
Time (hours)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
5
10
15
20
25
30
35
40

FIG. 13D

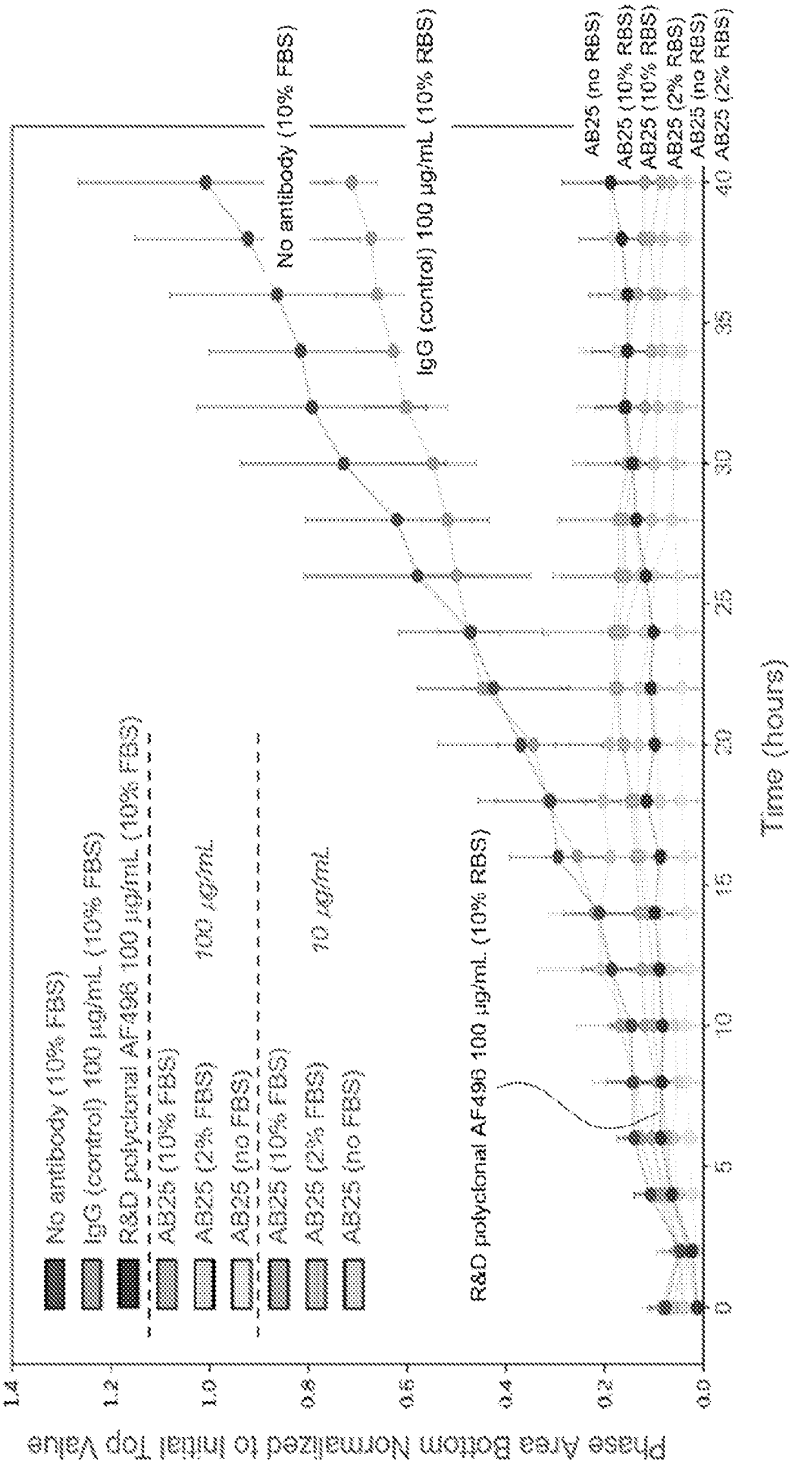

Phase Area Bottom Normalized to Initial Top Value
Time (hours)
No antibody (10% FBS)
IgG (control) 100 µg/mL (10% FBS)
R&D polyclonal AF496 100 µg/mL (10% FBS)
AB25 (10% FBS)
AB25 (2% FBS)
AB25 (no FBS)
100 µg/mL
AB25 (10% FBS)
AB25 (2% FBS)
AB25 (no FBS)
10 µg/mL
R&D polyclonal AF496 100 µg/mL (10% RBS)
No antibody (10% FBS)
IgG (control) 100 µg/mL (10% RBS)
AB25 (no RBS)
AB25 (10% RBS)
AB25 (10% RBS)
AB25 (2% RBS)
AB25 (no RBS)
AB25 (2% RBS)

FIG. 14A

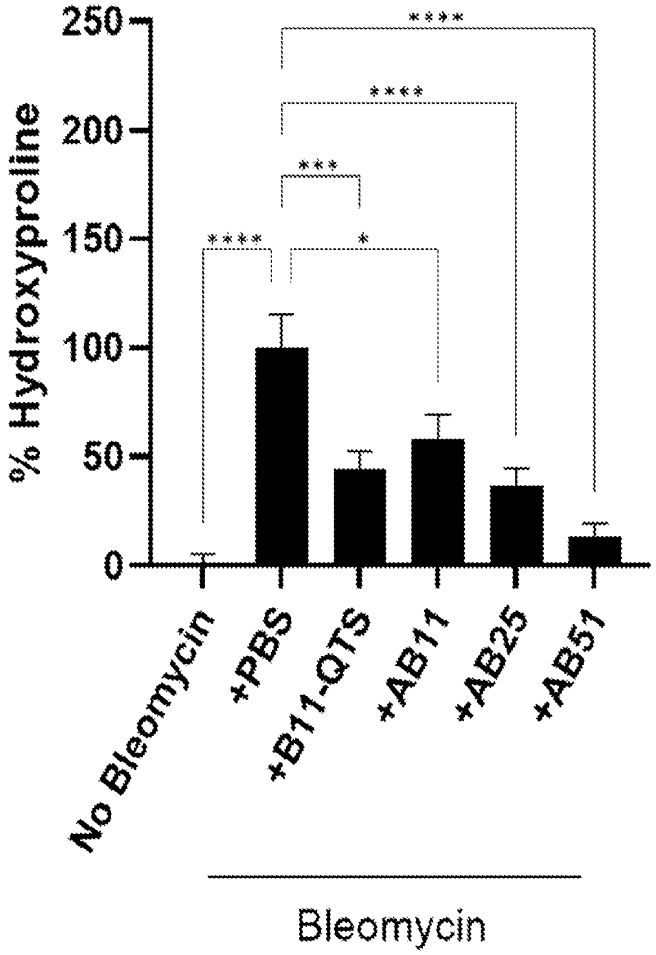

250
200
150
100
50
0
% Hydroxyproline
****
****
***
****
*
No Bleomycin
+PBS
+B11-QTS
+AB11
+AB25
+AB51
Bleomycin

FIG. 14B

	+B11-QTS	+AB11	+AB25	+AB51
% Reduction in Hydroxyproline	55	42	63	87

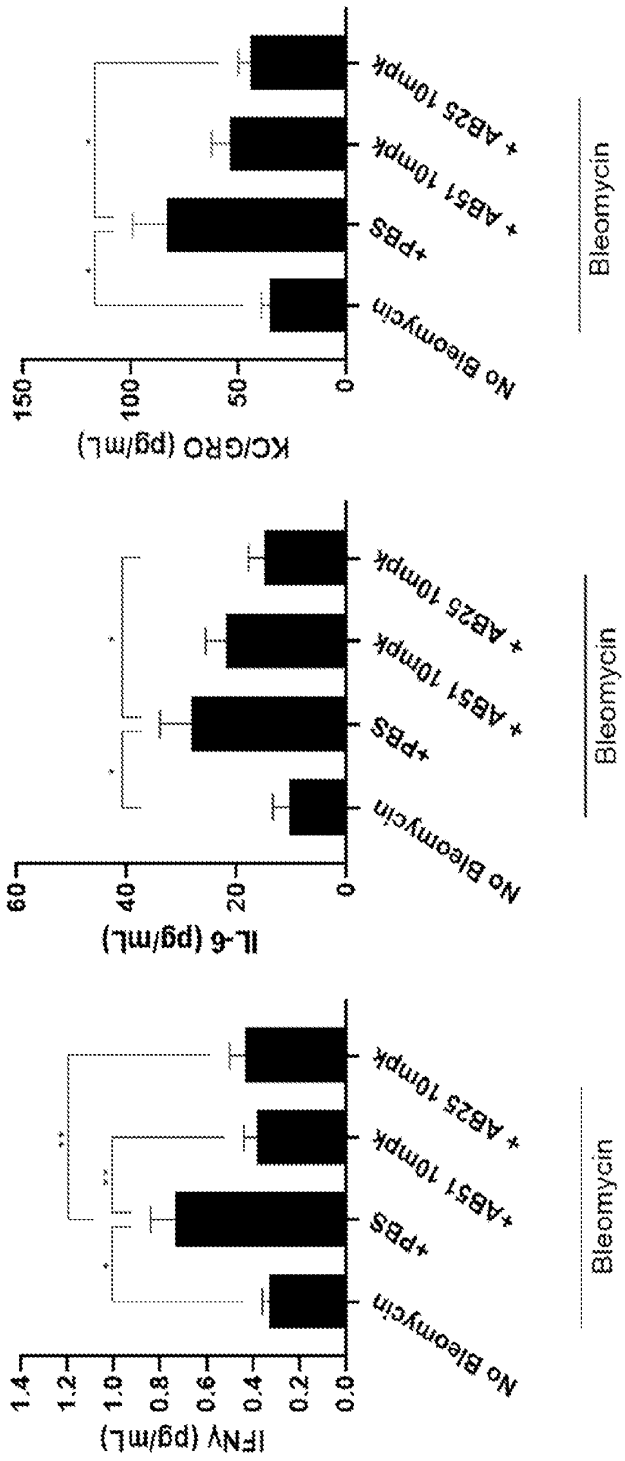

IFNγ (pg/mL)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
IL-6 (pg/mL)
0
20
40
60
KC/GRO (pg/mL)
0
50
100
150
No Bleomycin
+PBS
+ ABS1 10mpk
+ ABS25 10mpk
Bleomycin

FIG. 15A

FIG. 15B

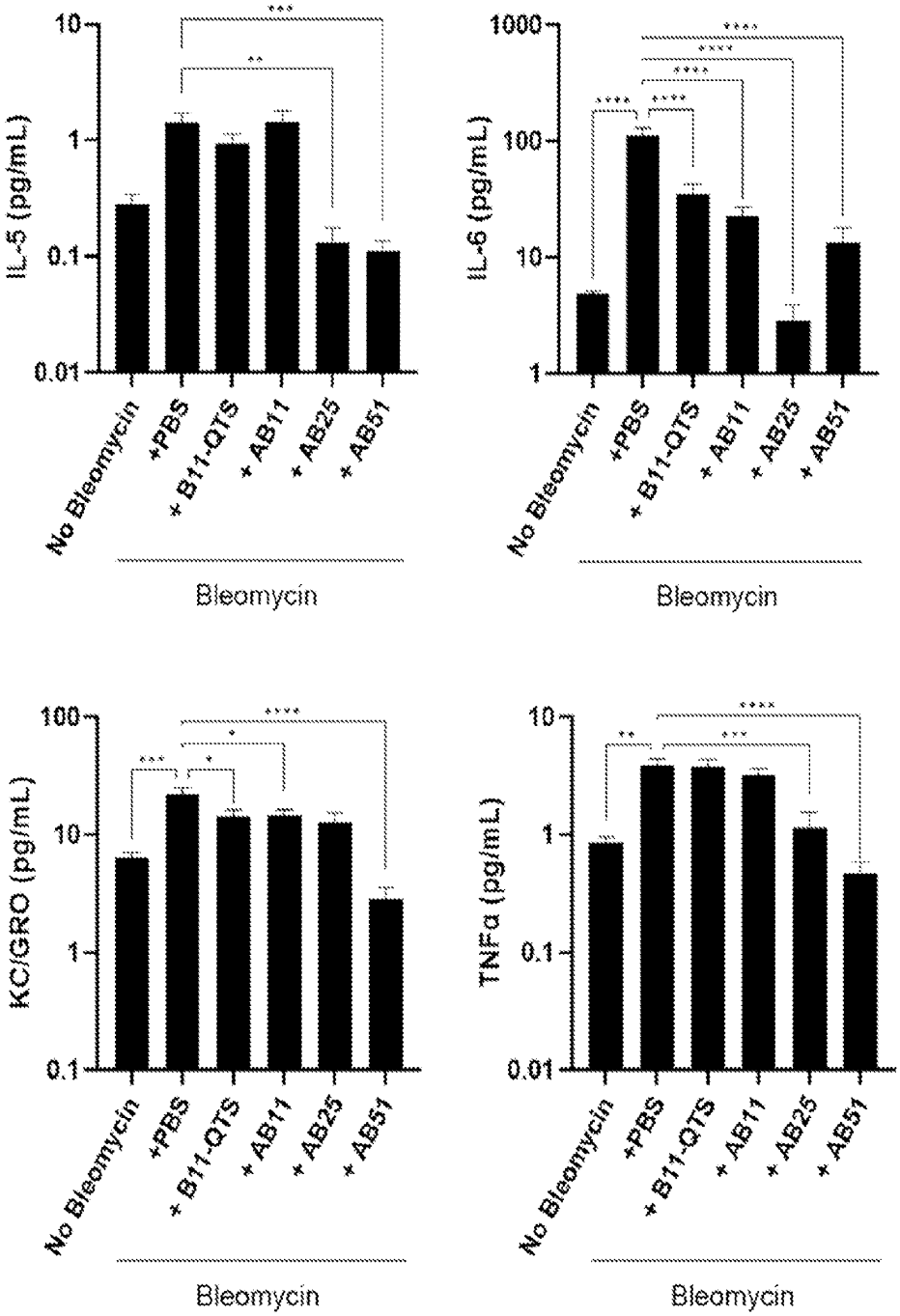

IL-5 (pg/mL)
10
1
0.1
0.01
No Bleomycin
+PBS
+ B11-QTS
+ AB11
+ AB25
+ AB51
Bleomycin
IL-6 (pg/mL)
1000
100
10
1
Bleomycin
KC/GRO (pg/mL)
100
10
1
0.1
Bleomycin
TNFα (pg/mL)
10
1
0.1
0.01
Bleomycin

FIG. 16A

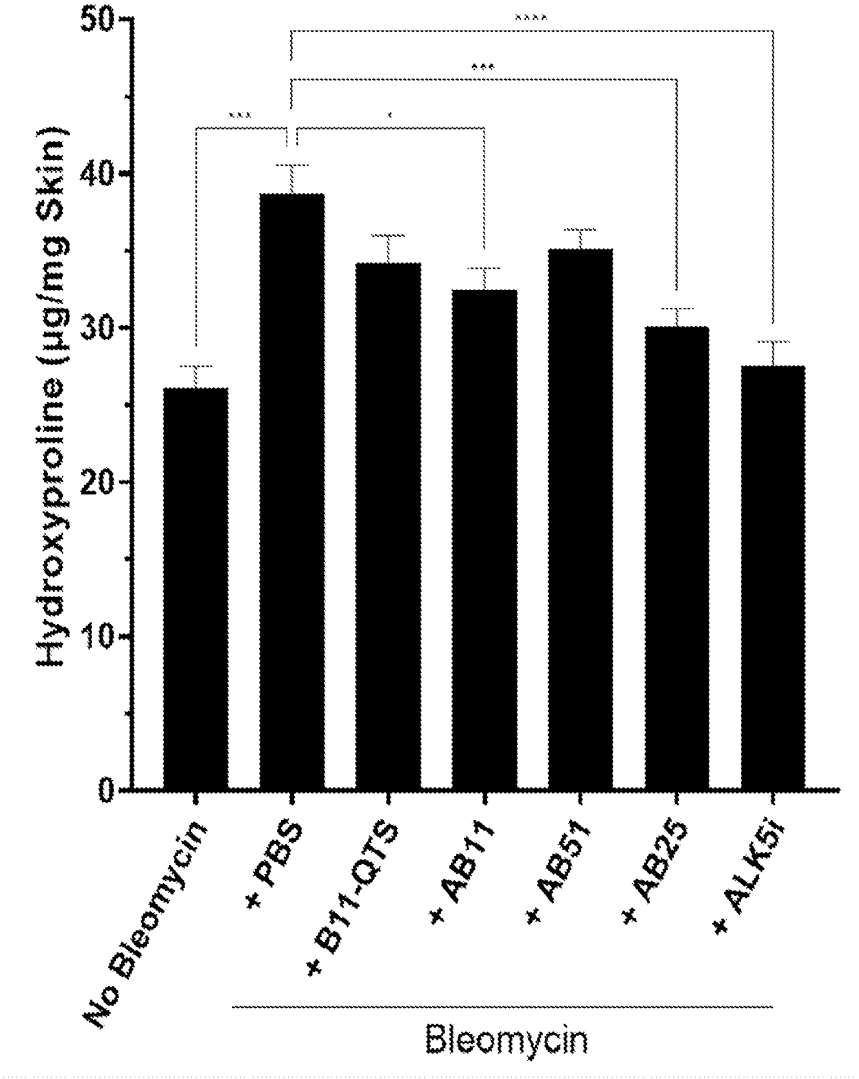

Hydroxyproline (µg/mg Skin)
50
40
30
20
10
0
No Bleomycin
+ PBS
+ B11-QTS
+ AB11
+ AB51
+ AB25
+ ALK5i
Bleomycin

FIG. 16B

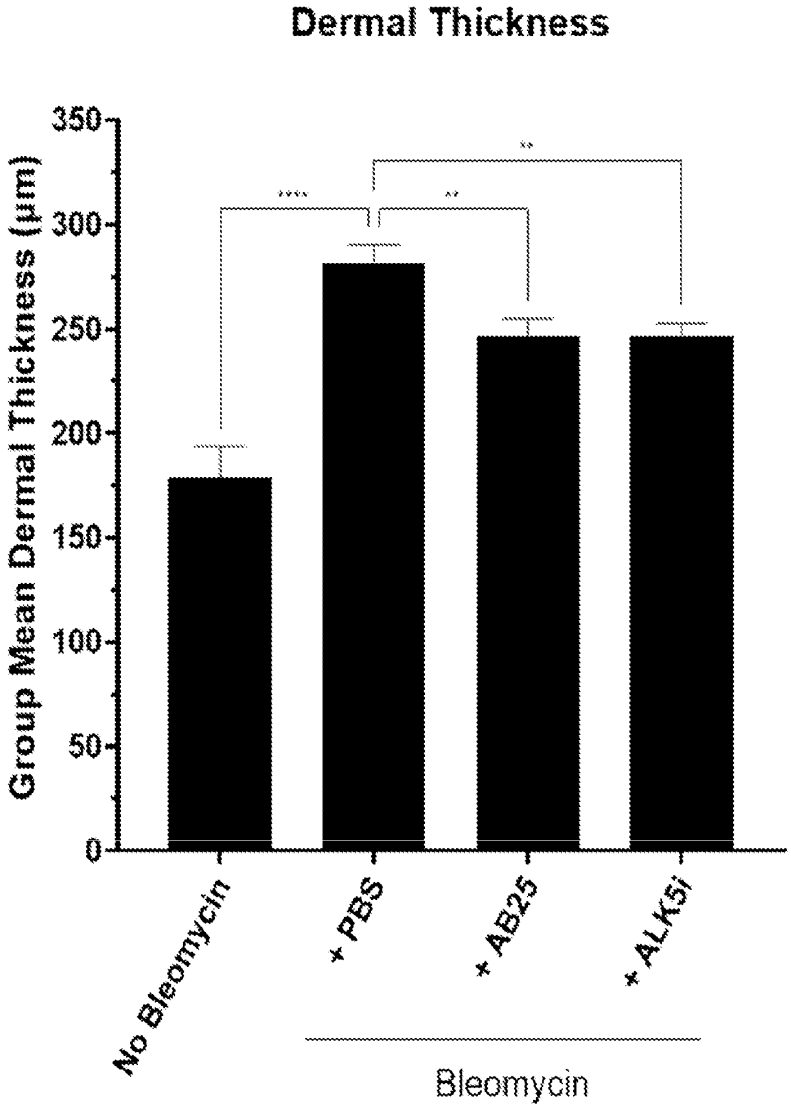

Dermal Thickness
Group Mean Dermal Thickness (µm)
350
300
250
200
150
100
50
0
No Bleomycin
+ PBS
+ AB25
+ ALK5i
Bleomycin

FIG. 16C

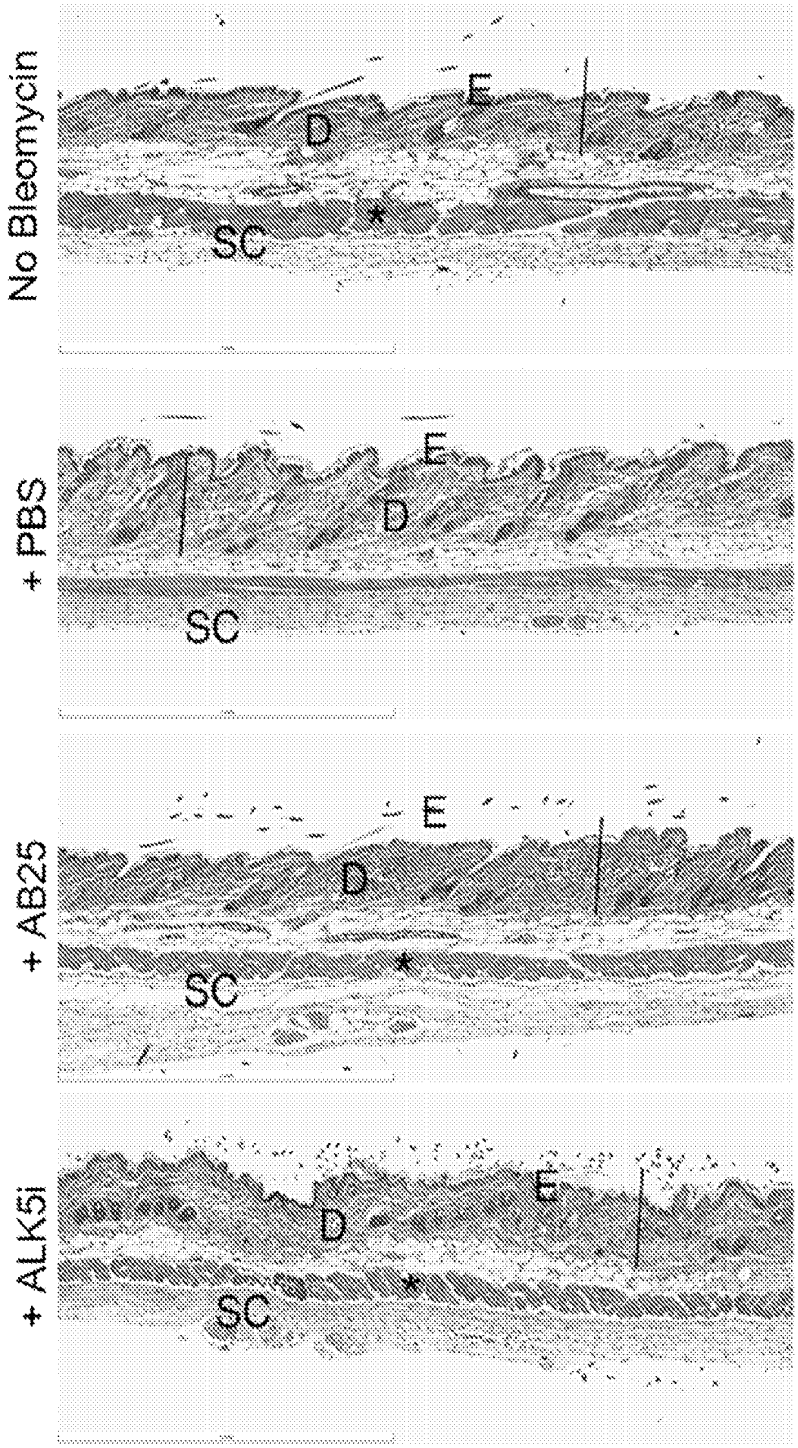


FIG. 17A

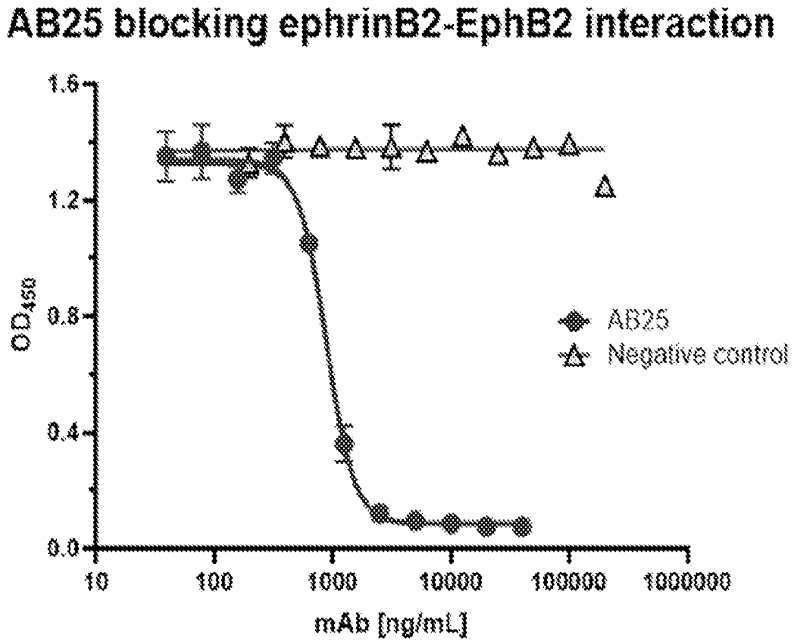


FIG. 17B

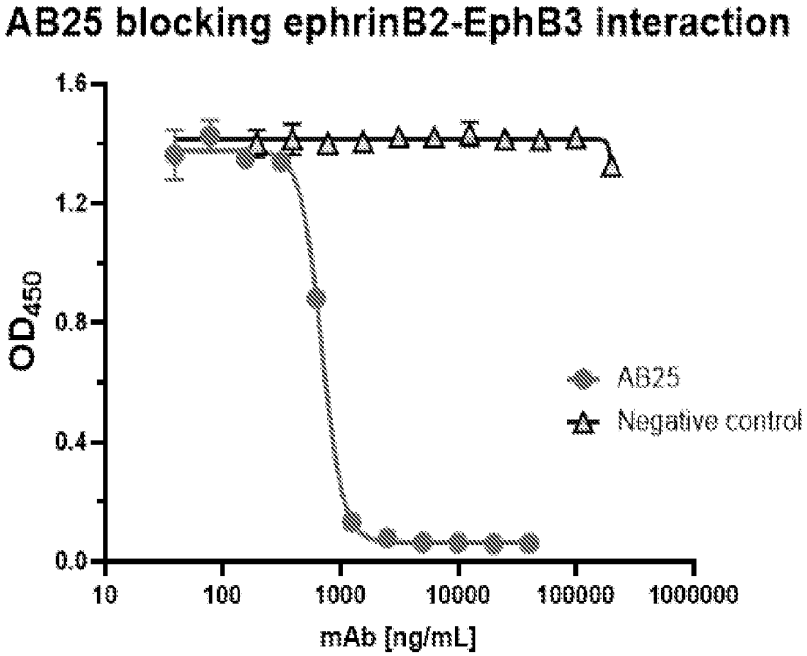


FIG. 17C

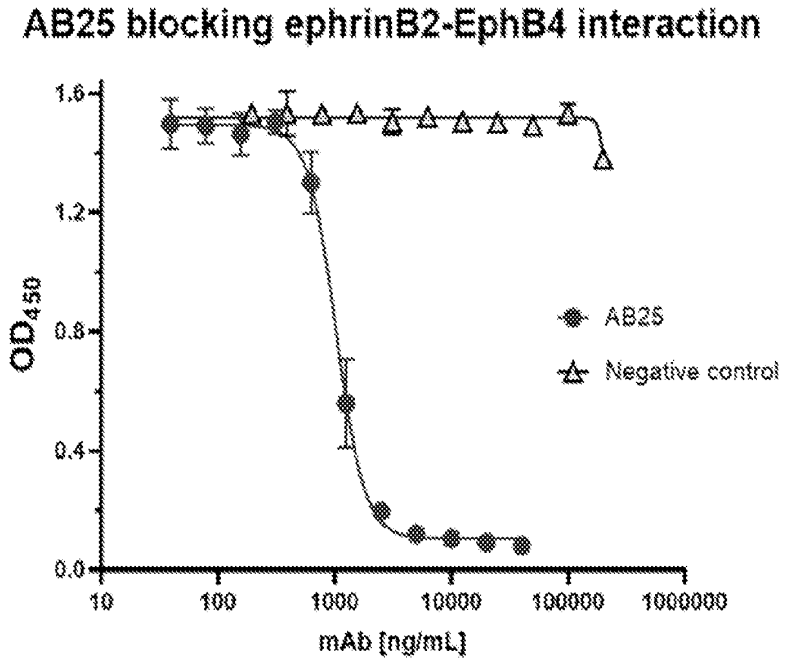


FIG. 17D

Antibody ID	IC_{50} (ng/mL)		
	EphB2	EphB3	EphB4
AB25	879	693.4	930.5
Negative control	Unstable	Unstable	Unstable

FIG. 18A

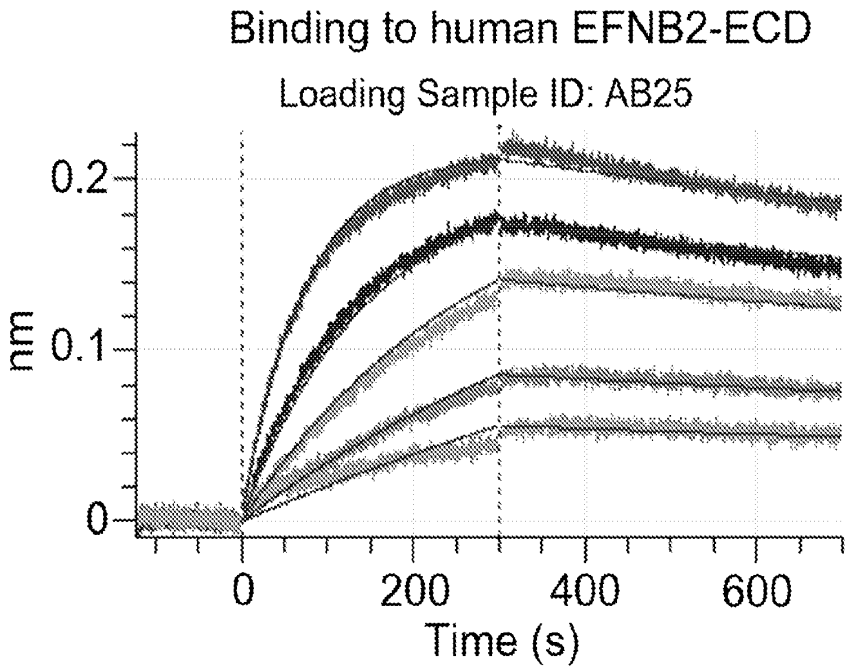


FIG. 18B

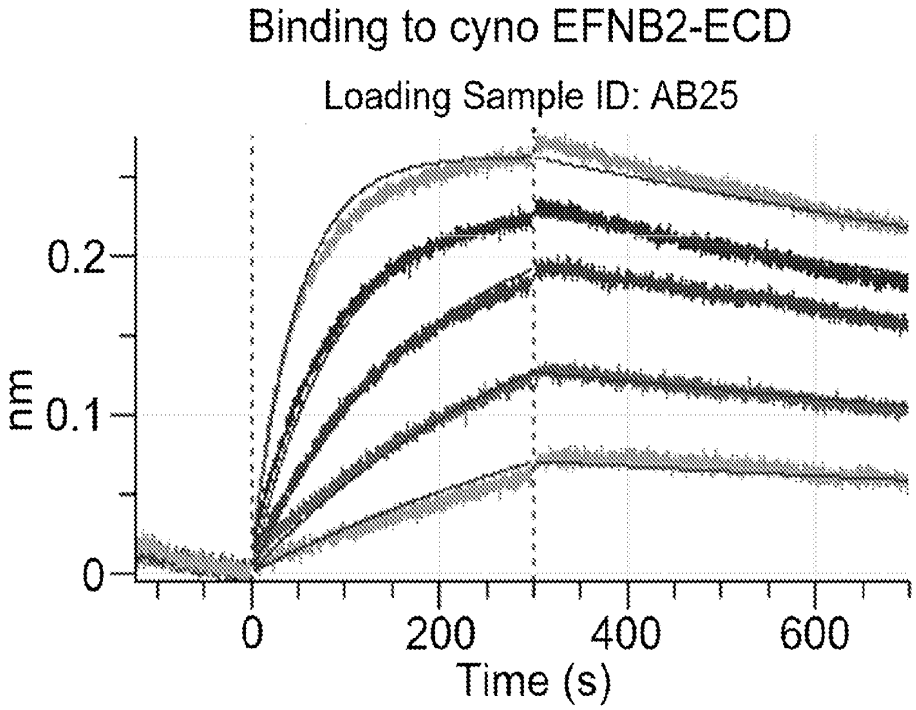


FIG. 18C

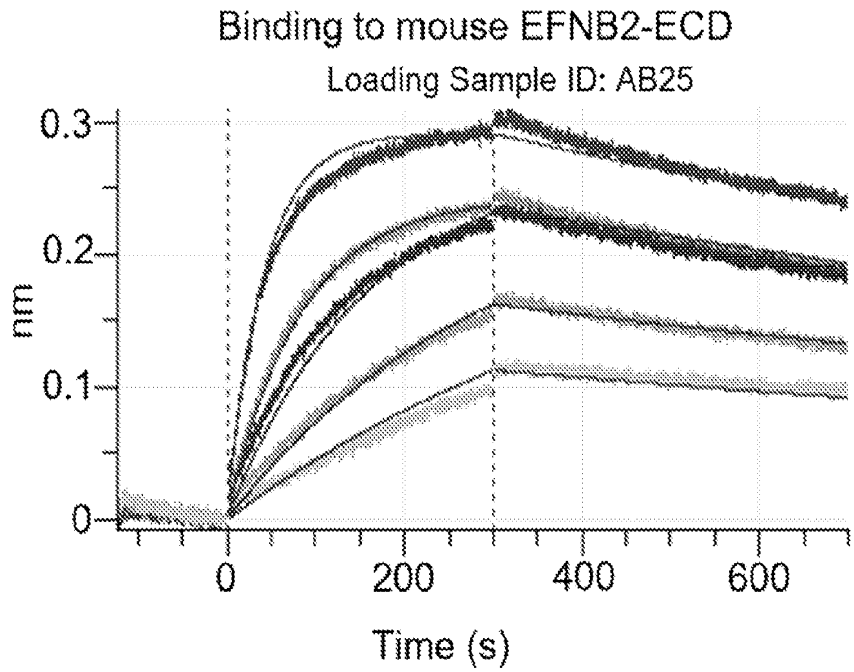


FIG. 18D

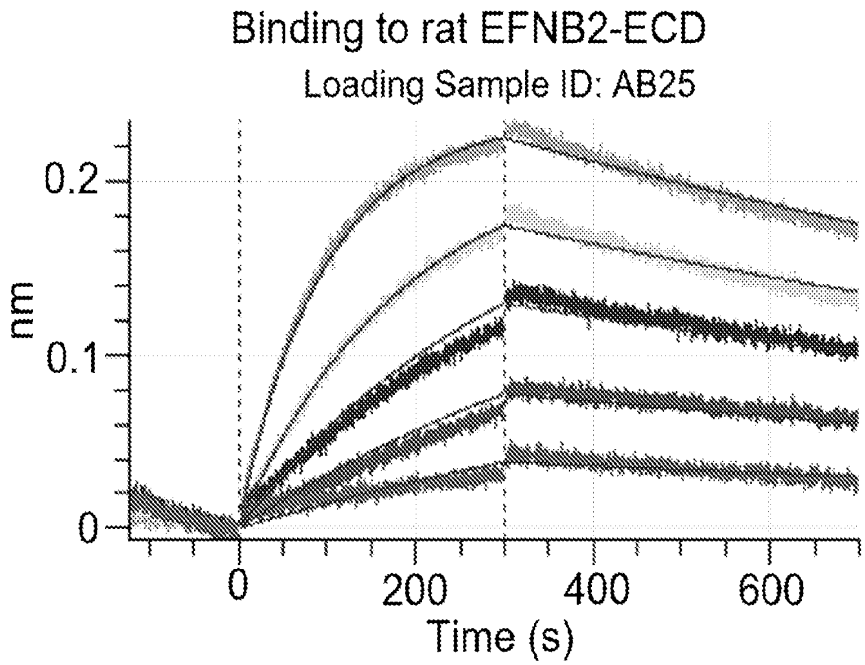


FIG. 19

Ligand	Analyte	KD (M)	KD Error	ka (1/Ms)	ka Error	kdis (1/s)	kdis Error
AB5	Hu EphrinB2-ECD	4.68E-10	4.01E-12	6.70E+05	2.11E+03	3.14E-04	2.49E-06
AB5	Cyno EFNB2-ECD	4.54E-10	2.35E-12	1.03E+06	2.58E+03	4.69E-04	2.13E-06
AB5	mu EFNB2-ECD	4.31E-10	2.23E-12	1.18E+06	3.16E+03	5.07E-04	2.24E-06
AB5	Rat ephrinB2-ECD	1.26E-09	7.21E-12	4.91E+05	1.77E+03	6.21E-04	2.74E-06

FIG. 20

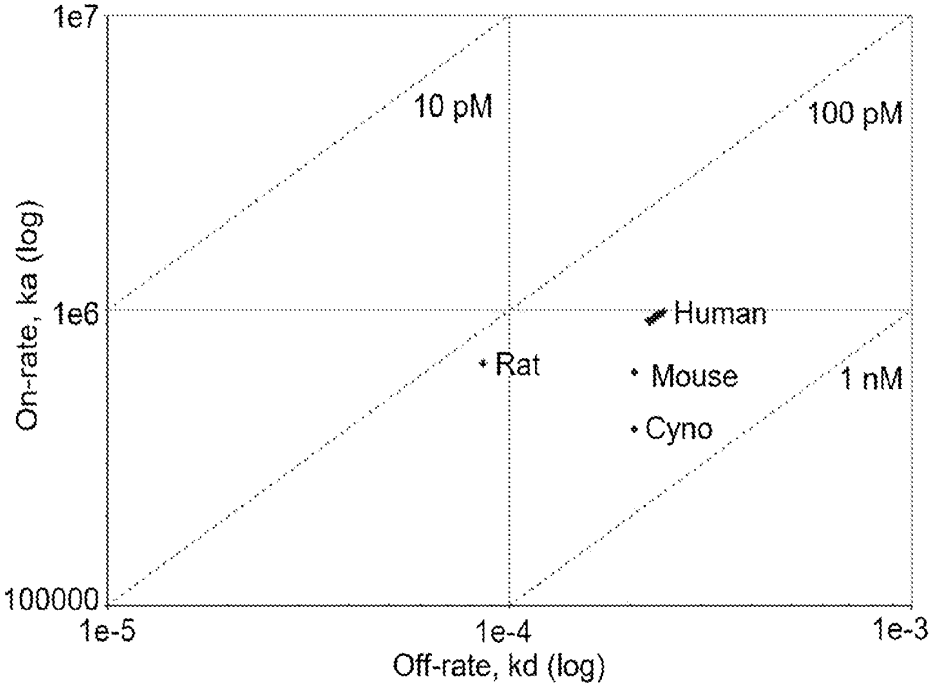
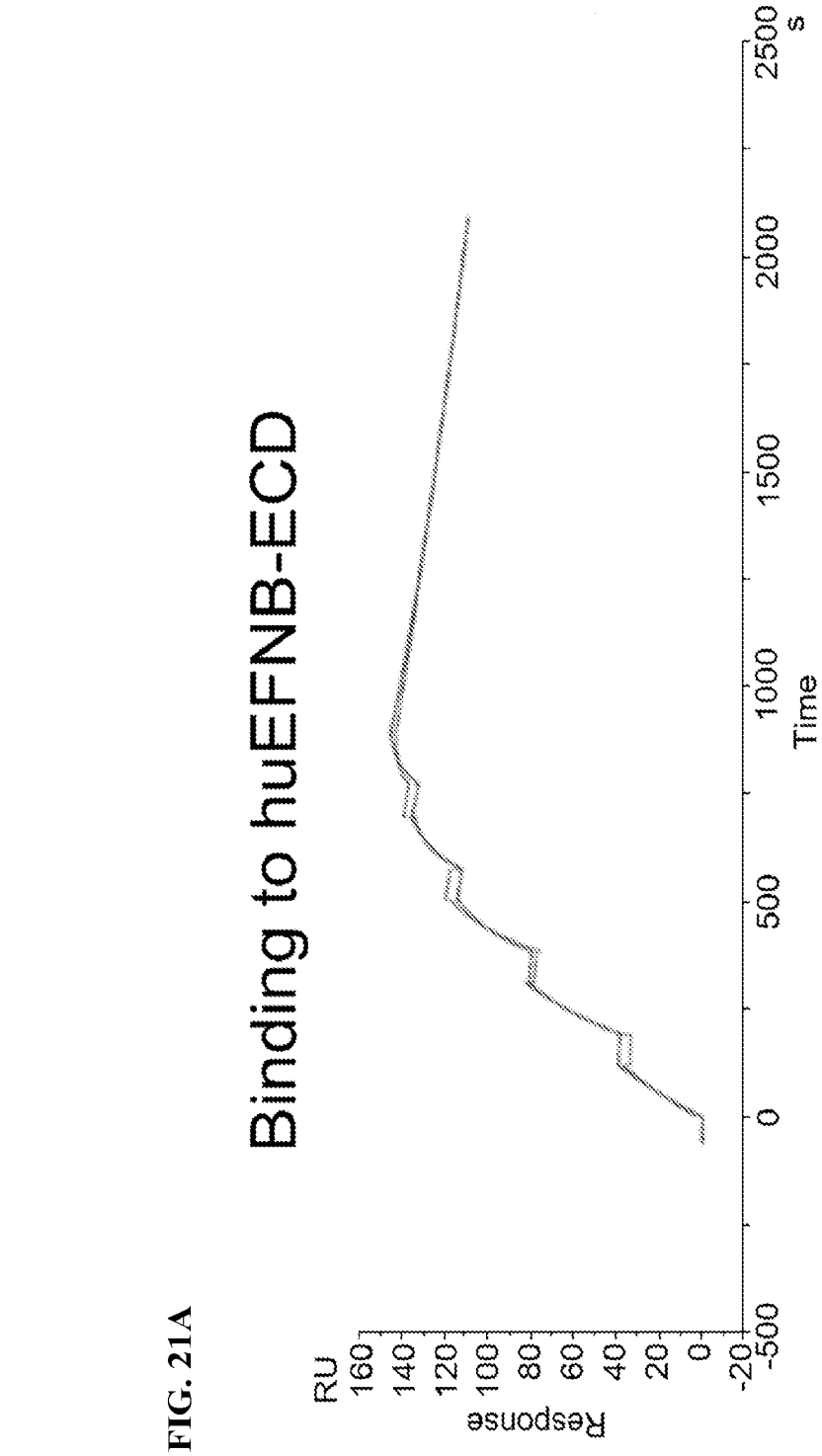

1e7
1e6
100000
On-rate, ka (log)
10 pM
100 pM
1 nM
Human
Rat
Mouse
Cyno
1e-5
1e-4
1e-3
Off-rate, kd (log)

FIG. 21A


Binding to huEFNB-ECD
Response
RU
160
140
120
100
80
60
40
20
0
-20
-500
0
500
1000
1500
2000
2500
Time
s

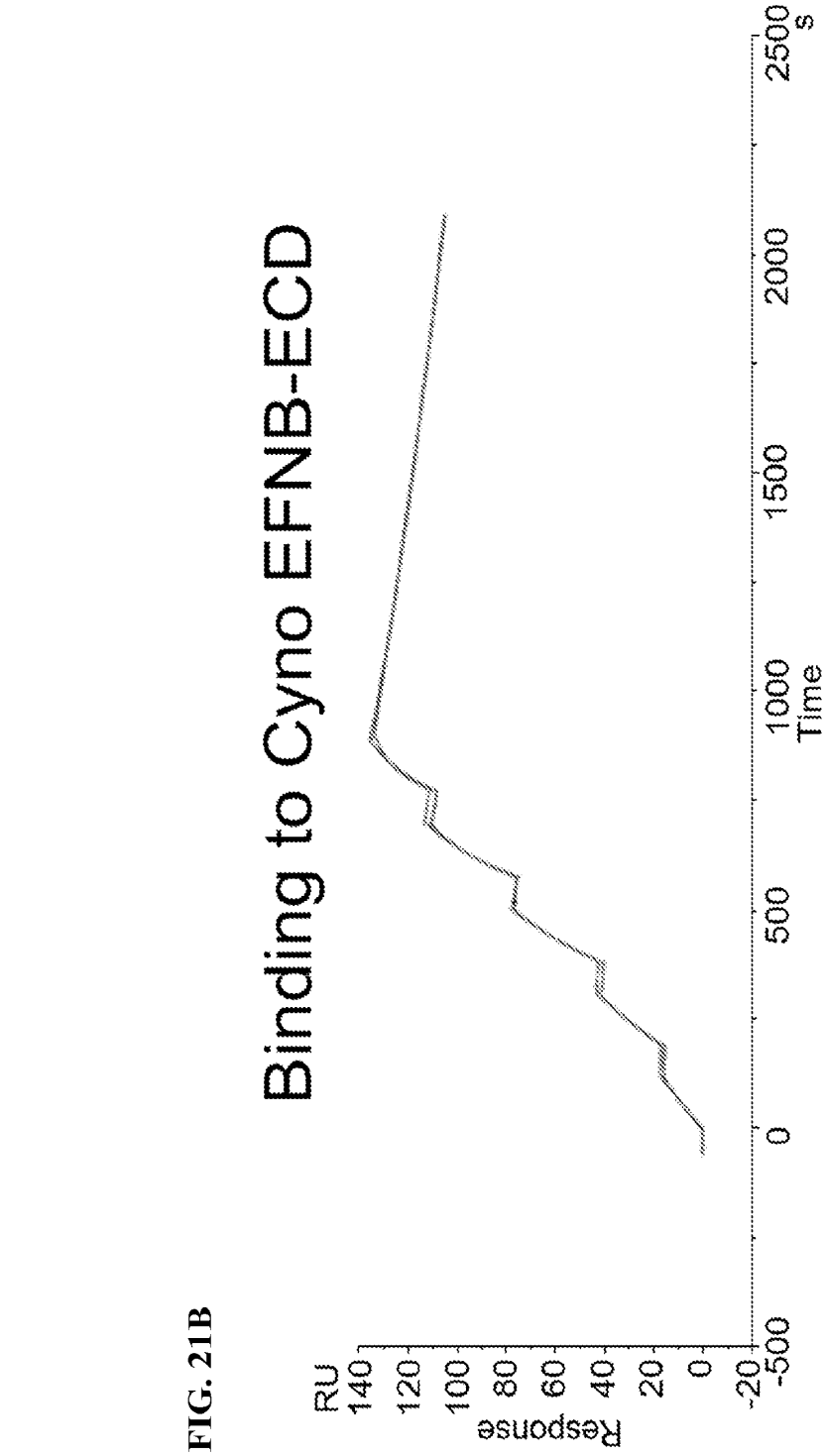

Binding to Cyno EFNB-ECD
RU
140
120
100
80
60
40
20
0
-20
Response
-500
0
500
1000
1500
2000
2500
Time
s

FIG. 21B

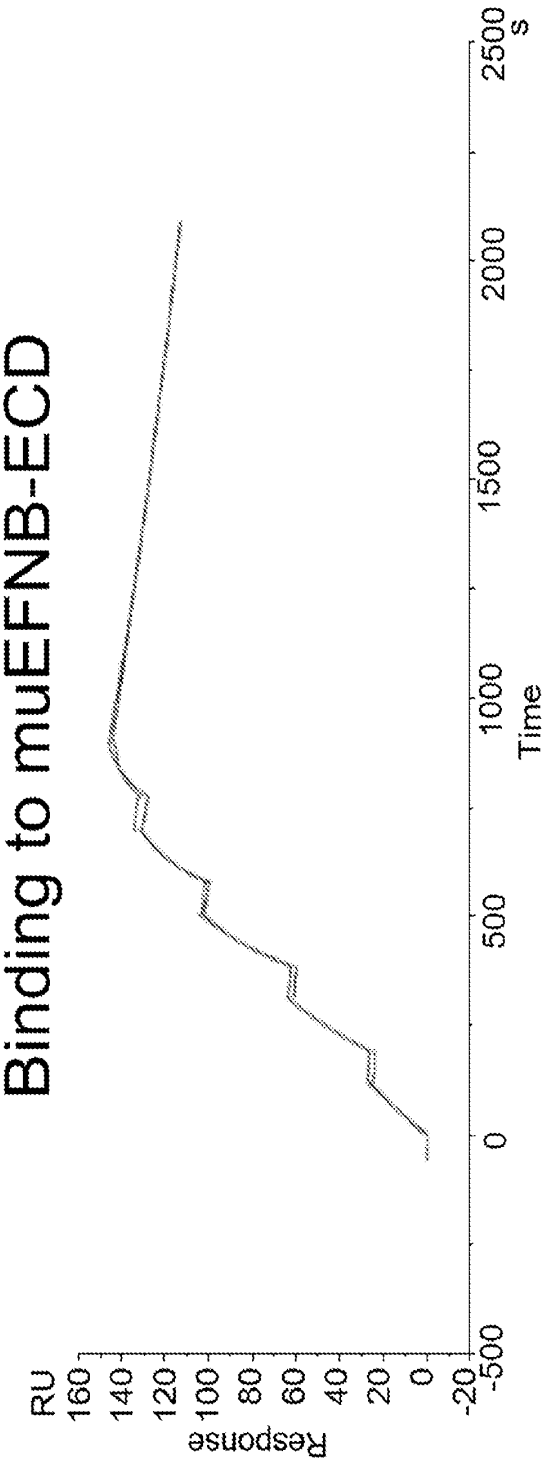

Binding to muEFNB-ECD
RU
160
140
120
100
80
60
40
20
0
-20
Response
-500
0
500
1000
1500
2000
2500
s
Time

FIG. 21C

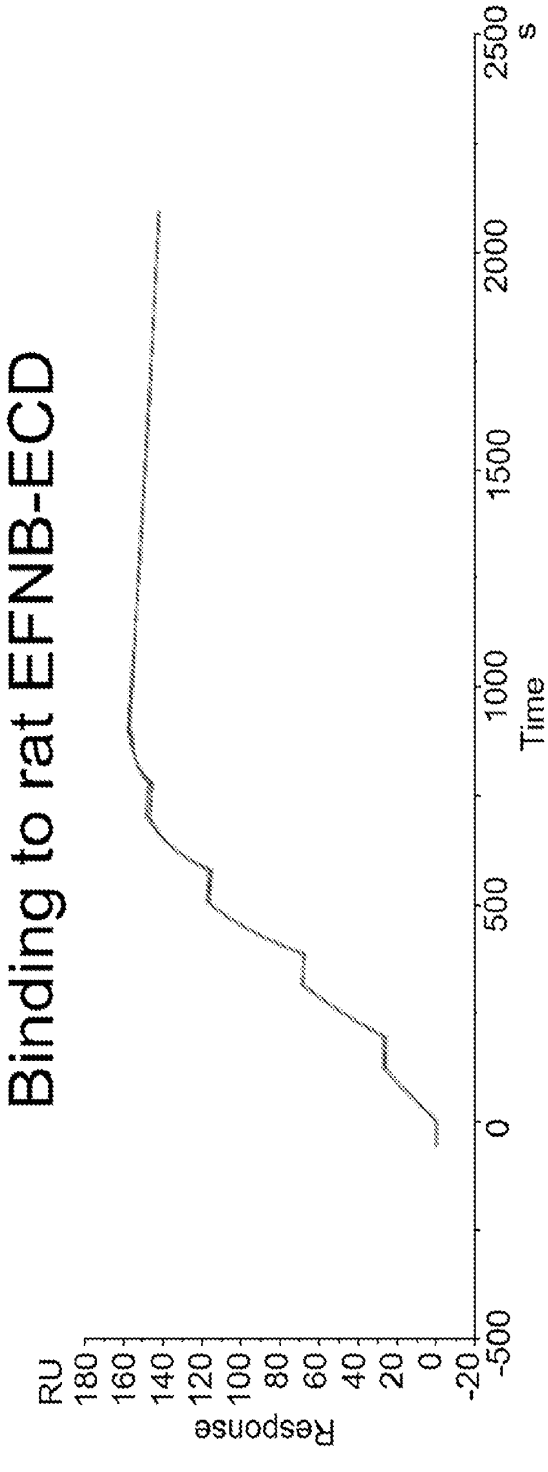

Binding to rat EFNB-ECD
RU
Response
180
160
140
120
100
80
60
40
20
0
-20
-500
0
500
1000
1500
2000
2500
Time
s

FIG. 21D

FIG. 22

Type of bond	CDR region	Residue in AB25 VH/VL	Residue in hEFNB2	Distance (Å)	Energy
H-bond	HCDR 1	Tyr32	Glu94	2.63	-3.40
Pi H-bond		Tyr 33	Gly123	2.71	-3.30
H-bond			Leu121	4.27	-0.60
H-bond	HCDR 2	Asn52	Glu125	2.80	-6.80
H-bond		Ser55	Glu125	2.65	-3.30
H-bond		Asn57	Ser118	2.74	-2.90
H-bond			Glu125	2.75	-6.70
H-bond		Trp50	Leu121	2.87	-2.50
H-bond		Thr58	Leu121	3.05	-1.90
H-bond		Ser54	Gln127	2.97	-1.10
Ionic	HCDR 3	Arg98	Glu94	3.16	-8.89
H-bond		Thr101	Thr96	2.65	-1.30
Ionic bond		Lys102	Glu94		
H-bond		Arg106	Asn95	2.76	-11.10
H-bond	LCDR 1	Ser32	Lys109	3.25	-1.30
H-bond		Asn33	Leu98	2.91	-1.60
H-bond			Asn100	2.79	-6.10
H-bond		Gly34	Asn100	2.88	-2.20
H-bond	LCDR 3	Leu97	Trp122	3.22	-1.20
Arene		Ser99	Trp122	3.86	-0.60

FIG. 23

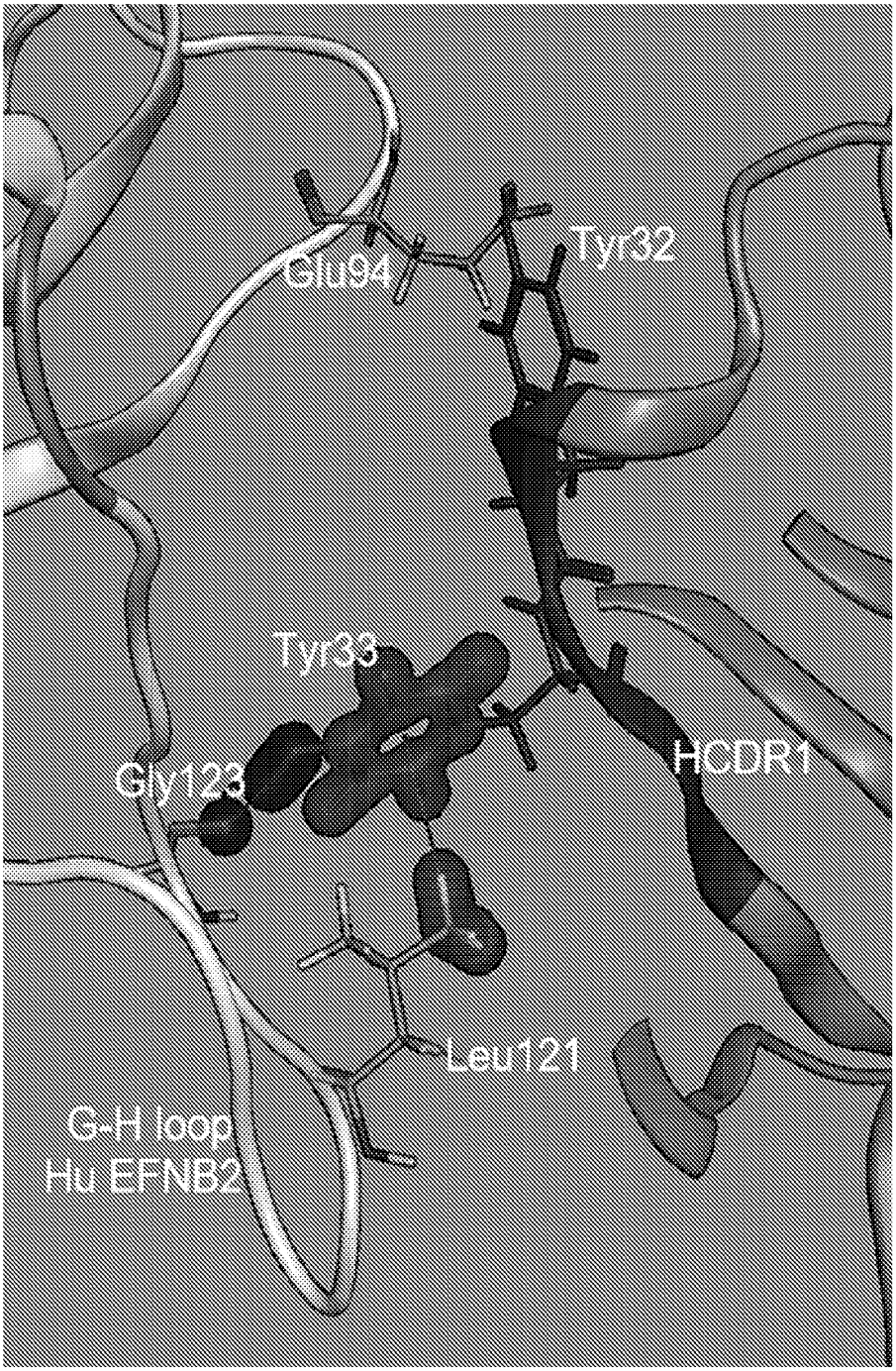


FIG. 24

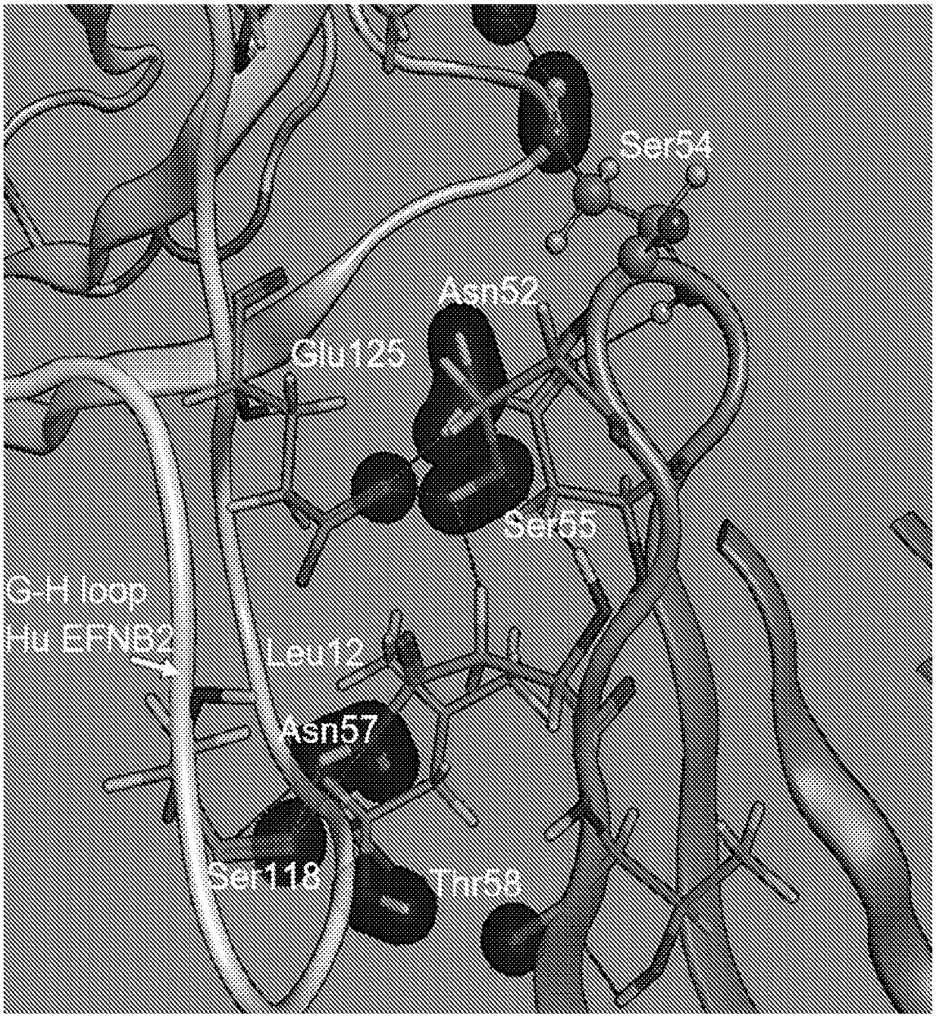

Ser54
Asn52
Glu125
Ser55
G-H loop
Hu EFNB2
Leu12
Asn57
Ser118
Thr58

FIG. 25

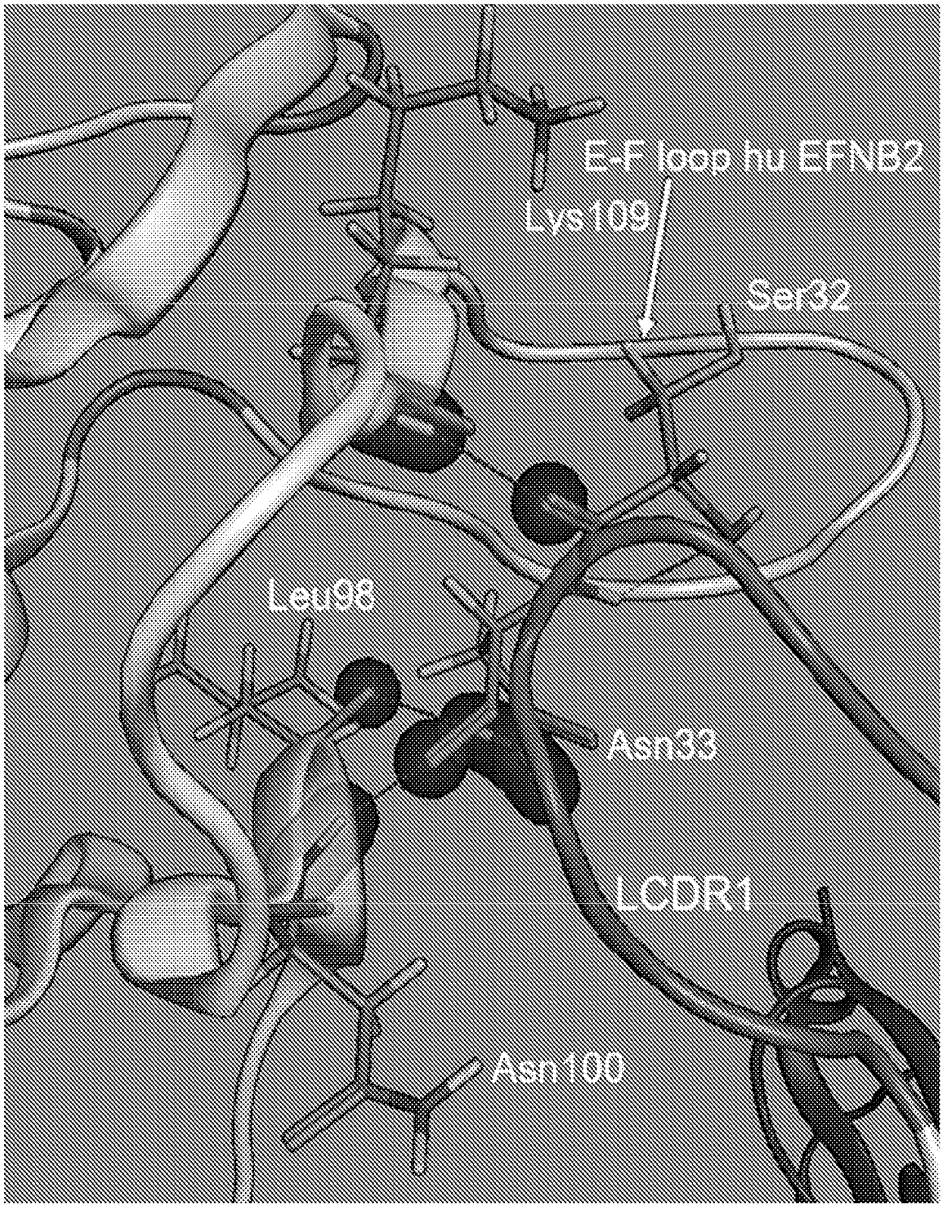


FIG. 26

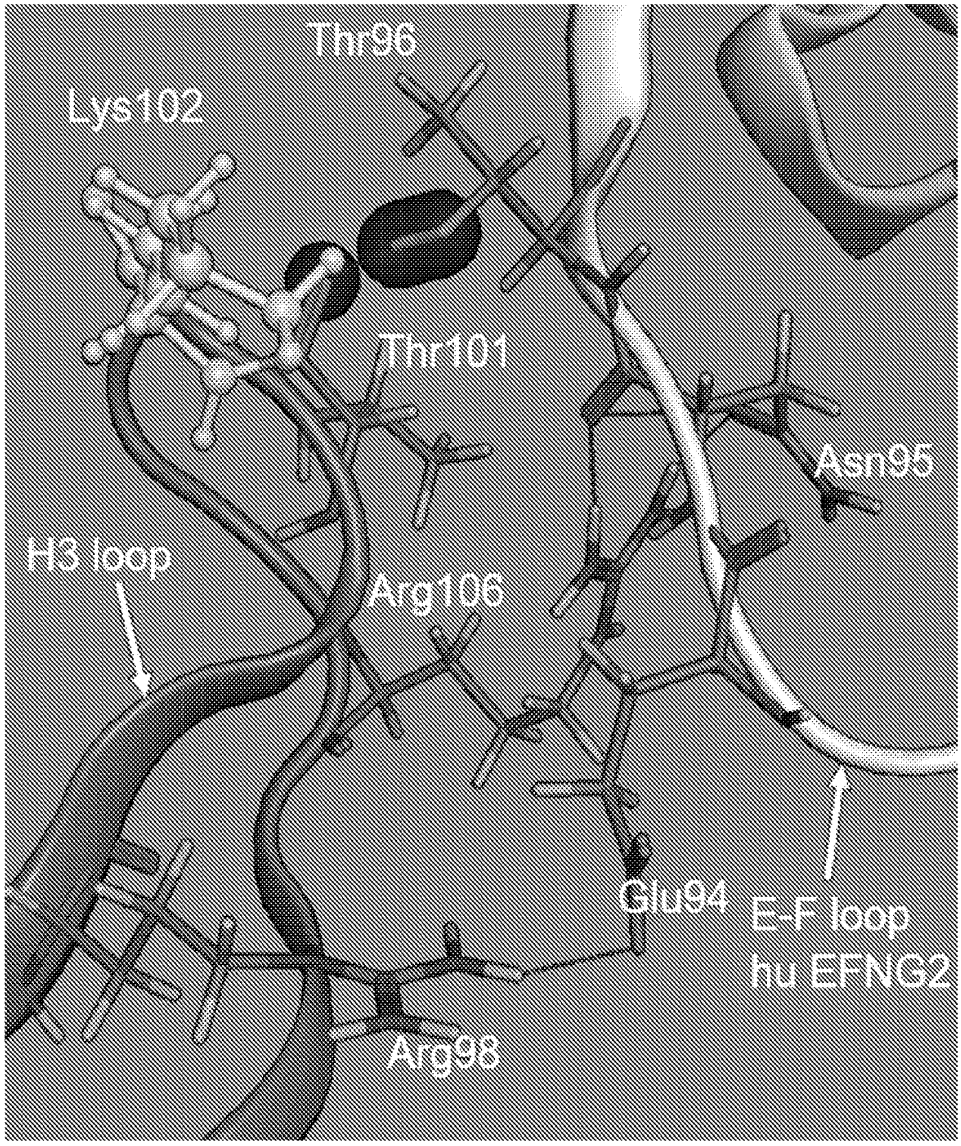

Thr96
Lys102
Thr101
Asn95
H3 loop
Arg106
Glu94
E-F loop
hu EFNG2
Arg98

FIG. 27

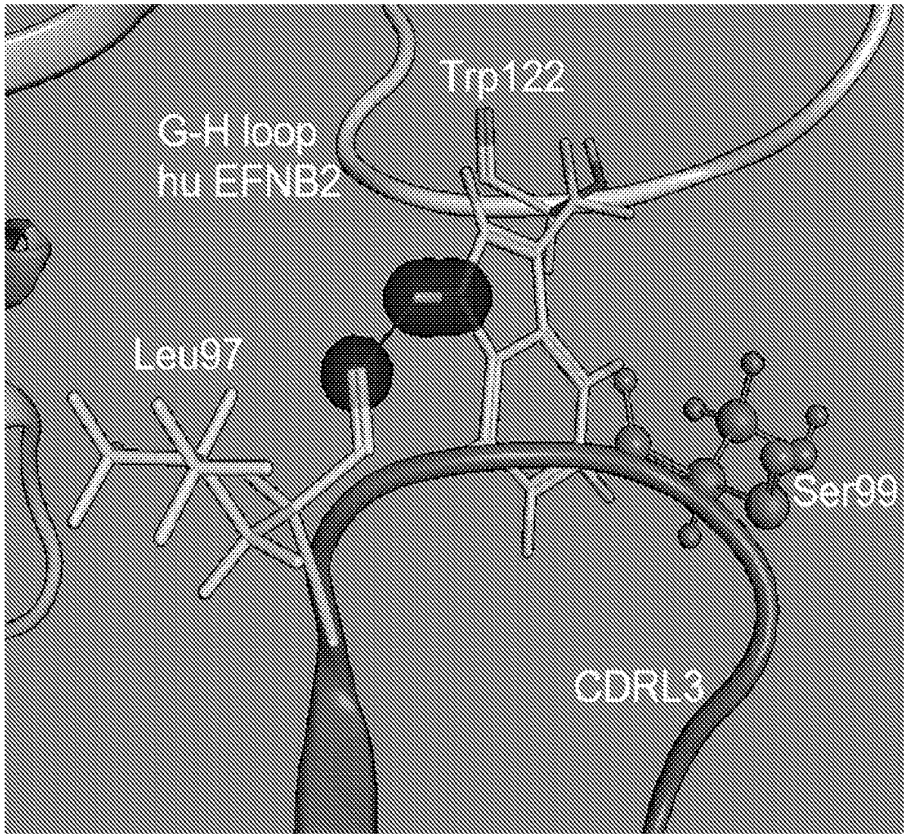
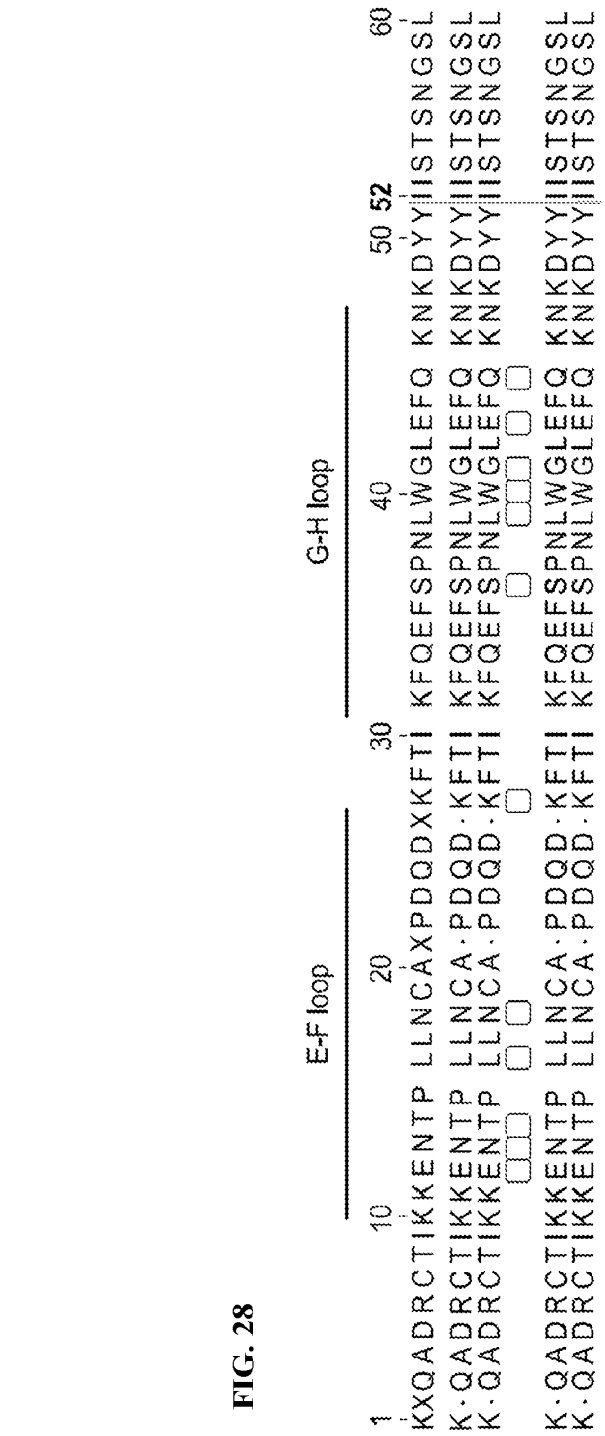


FIG. 28


E-F loop
G-H loop
1
10
20
30
40
50
52
60
KXQADRCTIKKENTP LLNCAXPDQDXKFTI KFQEFSPNLWGLEFQ KNKDYYIISTSNGSL
K·QADRCTIKKENTP LLNCA·PDQD·KFTI KFQEFSPNLWGLEFQ KNKDYYIISTSNGSL
K·QADRCTIKKENTP LLNCA·PDQD·KFTI KFQEFSPNLWGLEFQ KNKDYYIISTSNGSL
K·QADRCTIKKENTP LLNCA·PDQD·KFTI KFQEFSPNLWGLEFQ KNKDYYIISTSNGSL
K·QADRCTIKKENTP LLNCA·PDQD·KFTI KFQEFSPNLWGLEFQ KNKDYYIISTSNGSL