FIG. 1

Light chain variable region

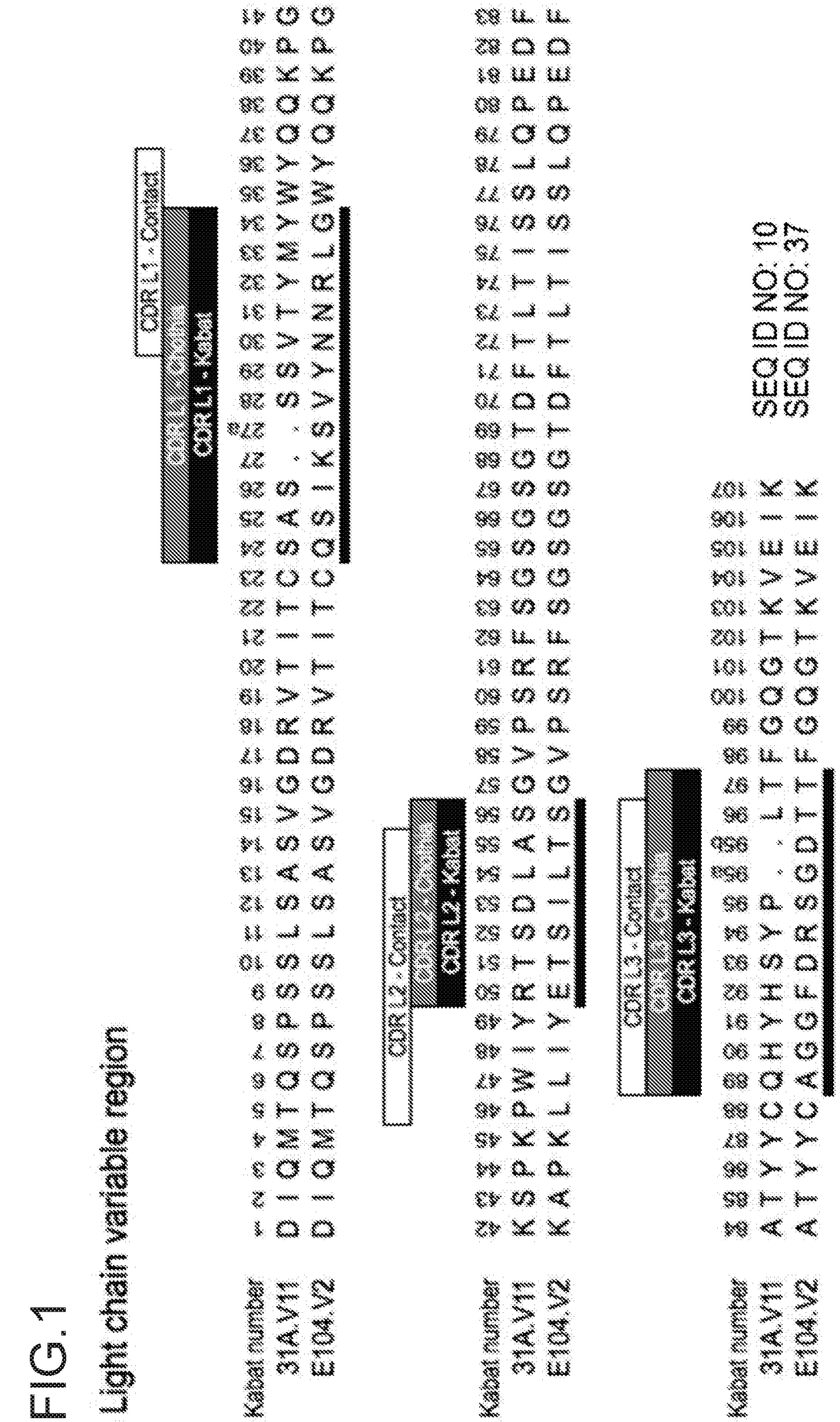


FIG. 1 cont.

Heavy chain variable region

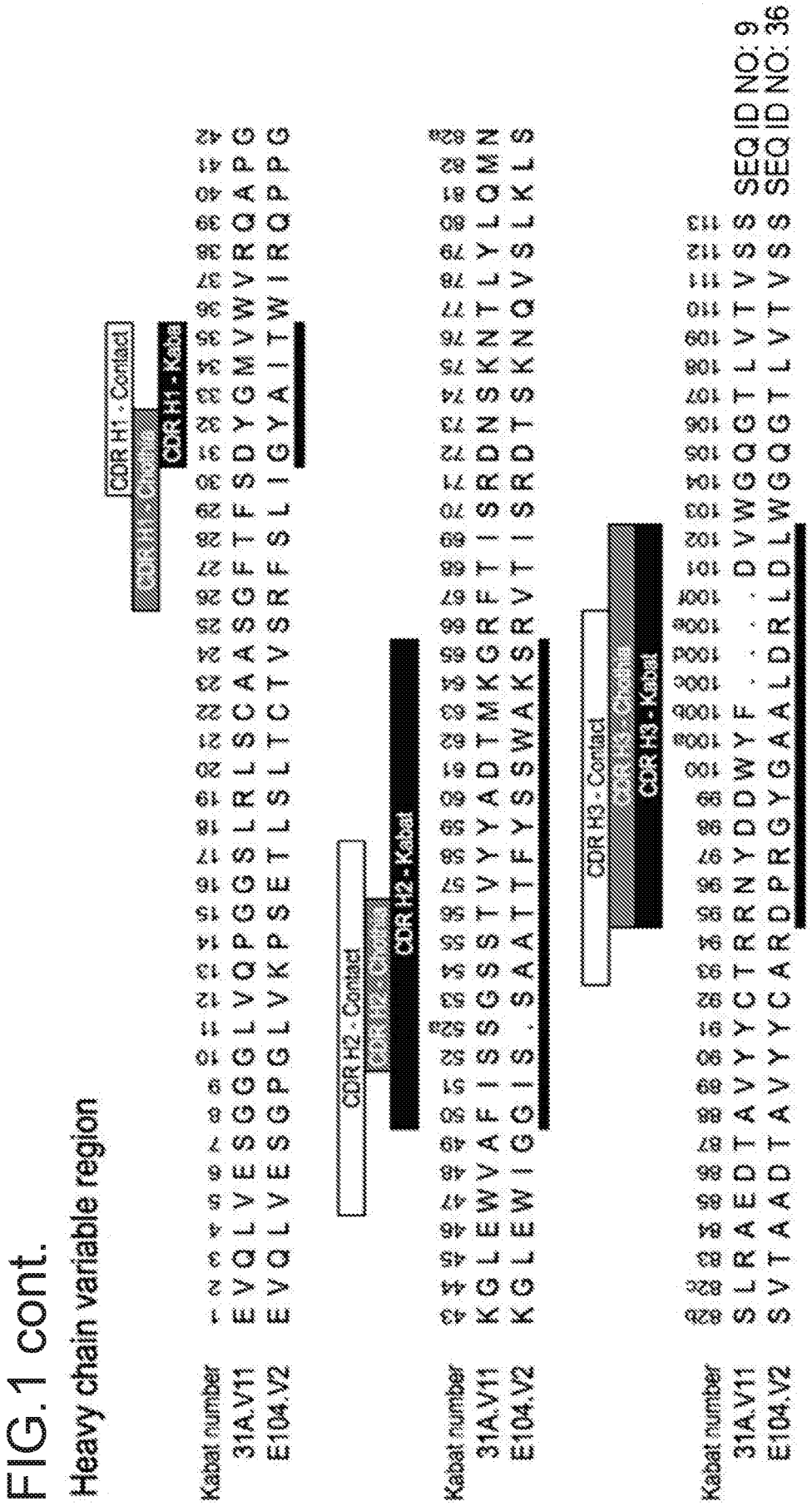


FIG. 2A

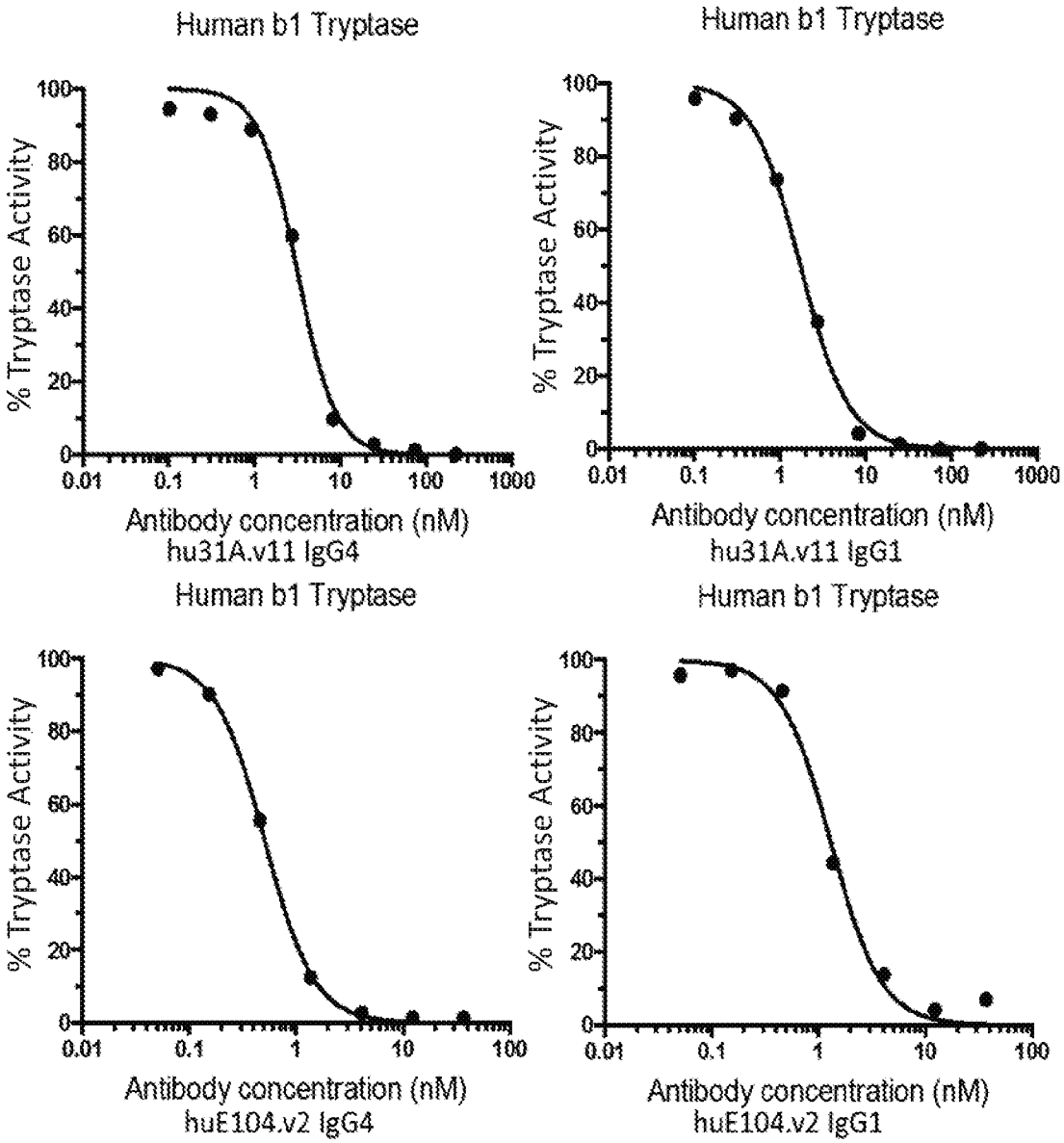

Human b1 Tryptase
% Tryptase Activity
Antibody concentration (nM)
hu31A.v11 IgG4
Human b1 Tryptase
% Tryptase Activity
Antibody concentration (nM)
hu31A.v11 IgG1
Human b1 Tryptase
% Tryptase Activity
Antibody concentration (nM)
huE104.v2 IgG4
Human b1 Tryptase
% Tryptase Activity
Antibody concentration (nM)
huE104.v2 IgG1

FIG. 2B

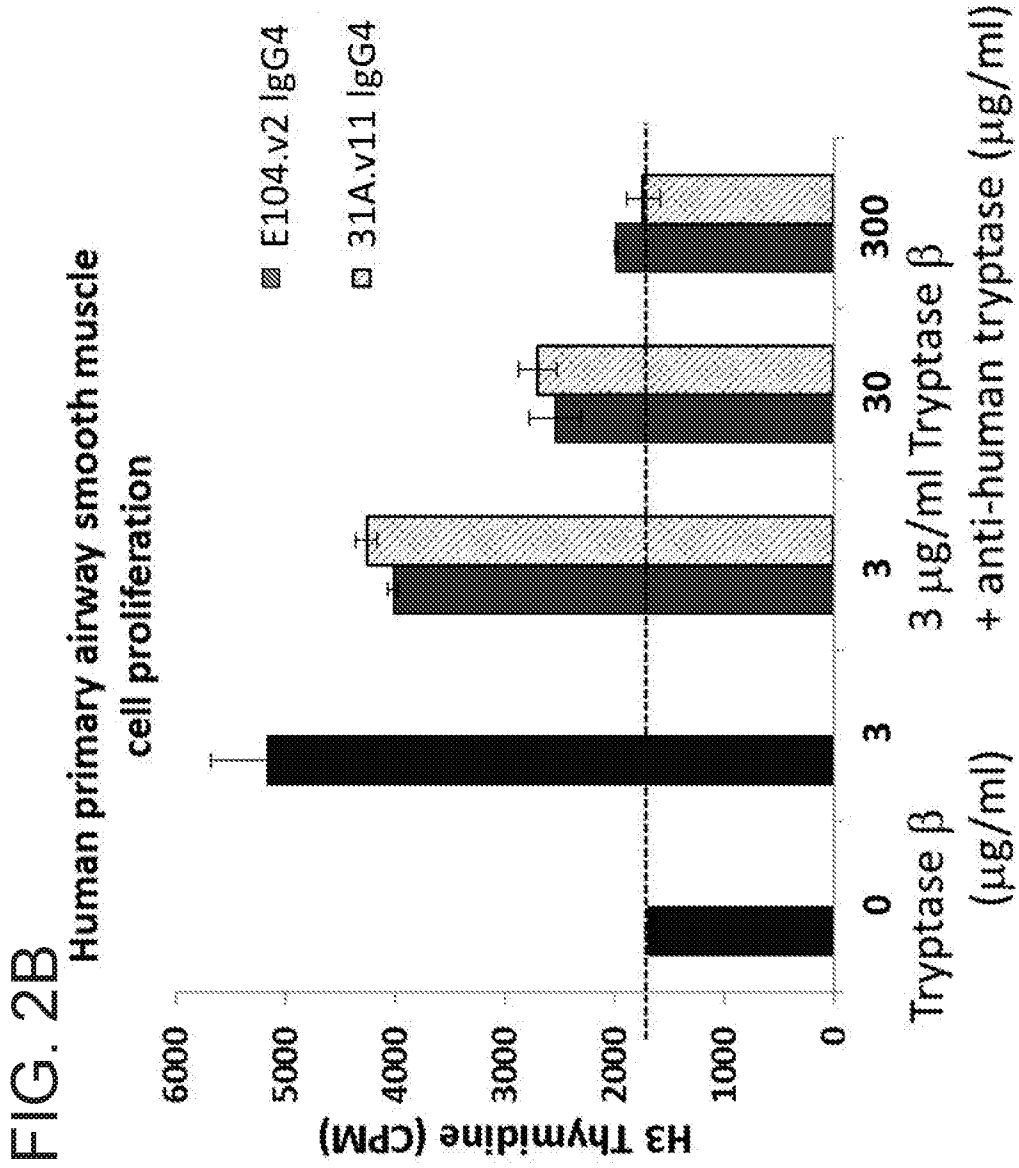
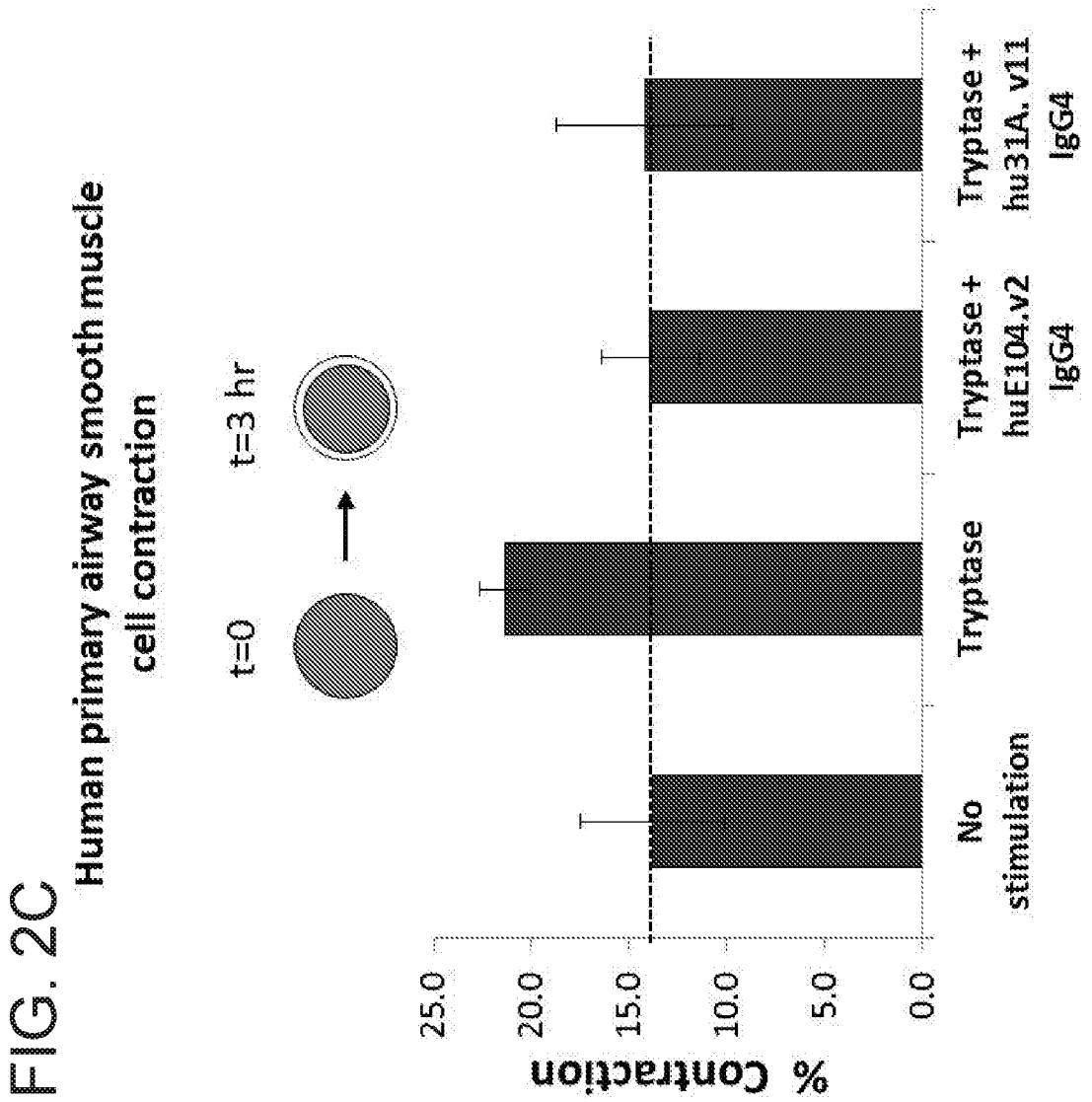

Human primary airway smooth muscle cell proliferation
E104.v2 IgG4
31A.v11 IgG4
H3 Thymidine (CPM)
6000
5000
4000
3000
2000
1000
0
0
3
3
30
300
Tryptase β (µg/ml)
3 µg/ml Tryptase β + anti-human tryptase (µg/ml)

FIG. 2C


Human primary airway smooth muscle cell contraction
t=0
t=3 hr
% Contraction
25.0
20.0
15.0
10.0
5.0
0.0
No stimulation
Tryptase
Tryptase + huE104.v2 IgG4
Tryptase + hu31A.v11 IgG4

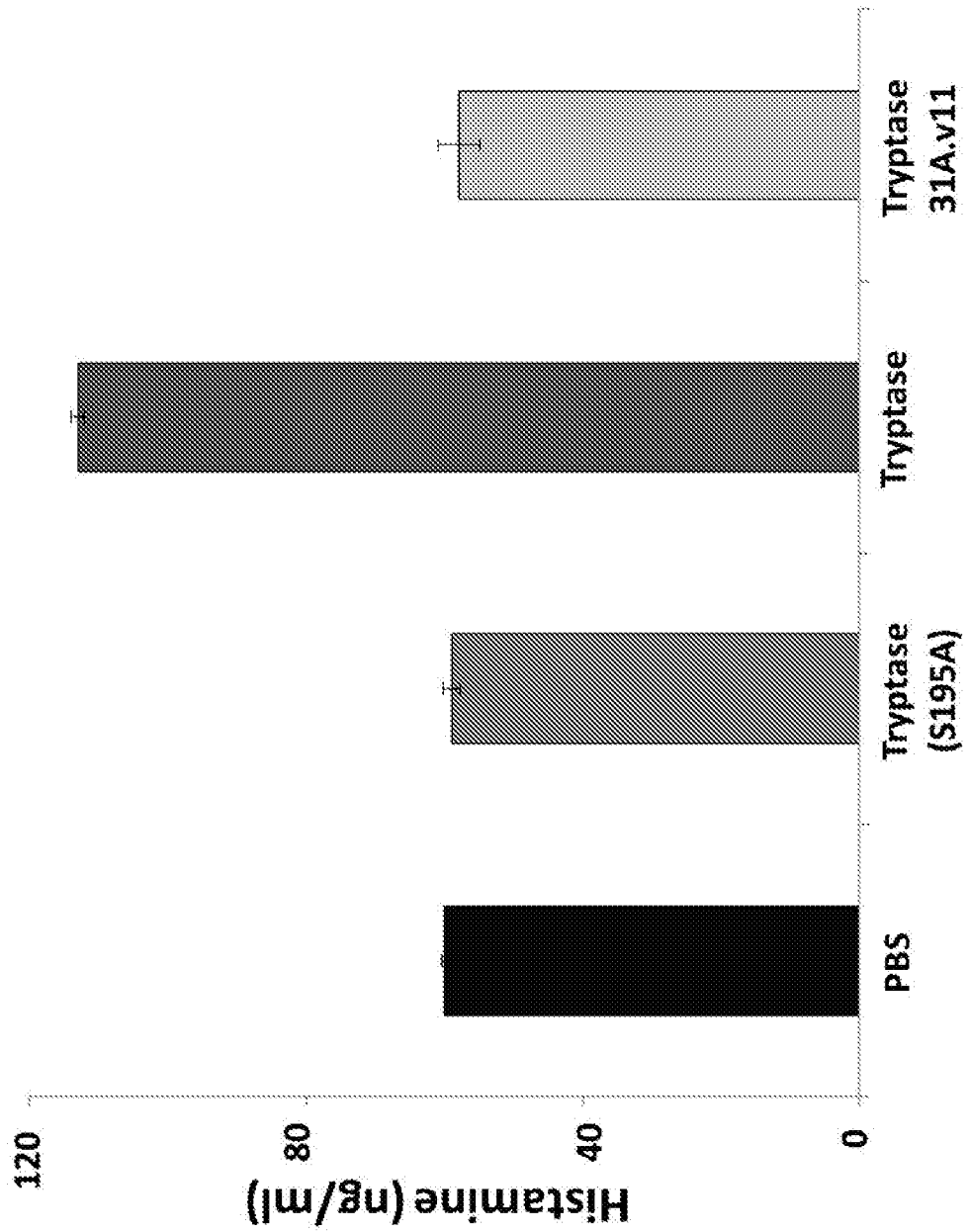

Histamine (ng/ml)
120
80
40
0
PBS
Tryptase (S195A)
Tryptase
Tryptase 31A.v11

FIG. 2D

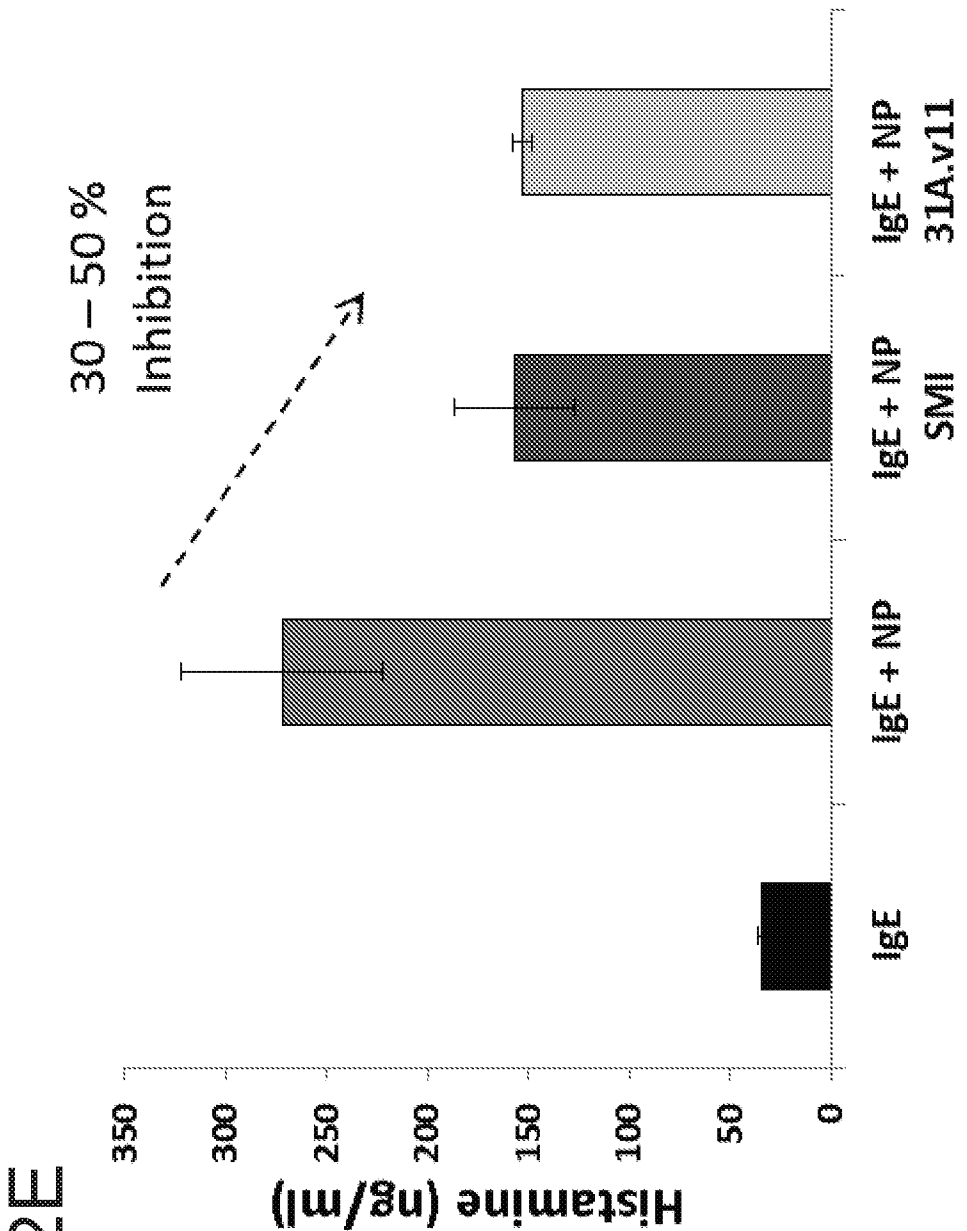

30 – 50 %
Inhibition
Histamine (ng/ml)
350
300
250
200
150
100
50
0
IgE
IgE + NP
IgE + NP
SMI
IgE + NP
31A.v11

FIG. 2E

FIG. 3A

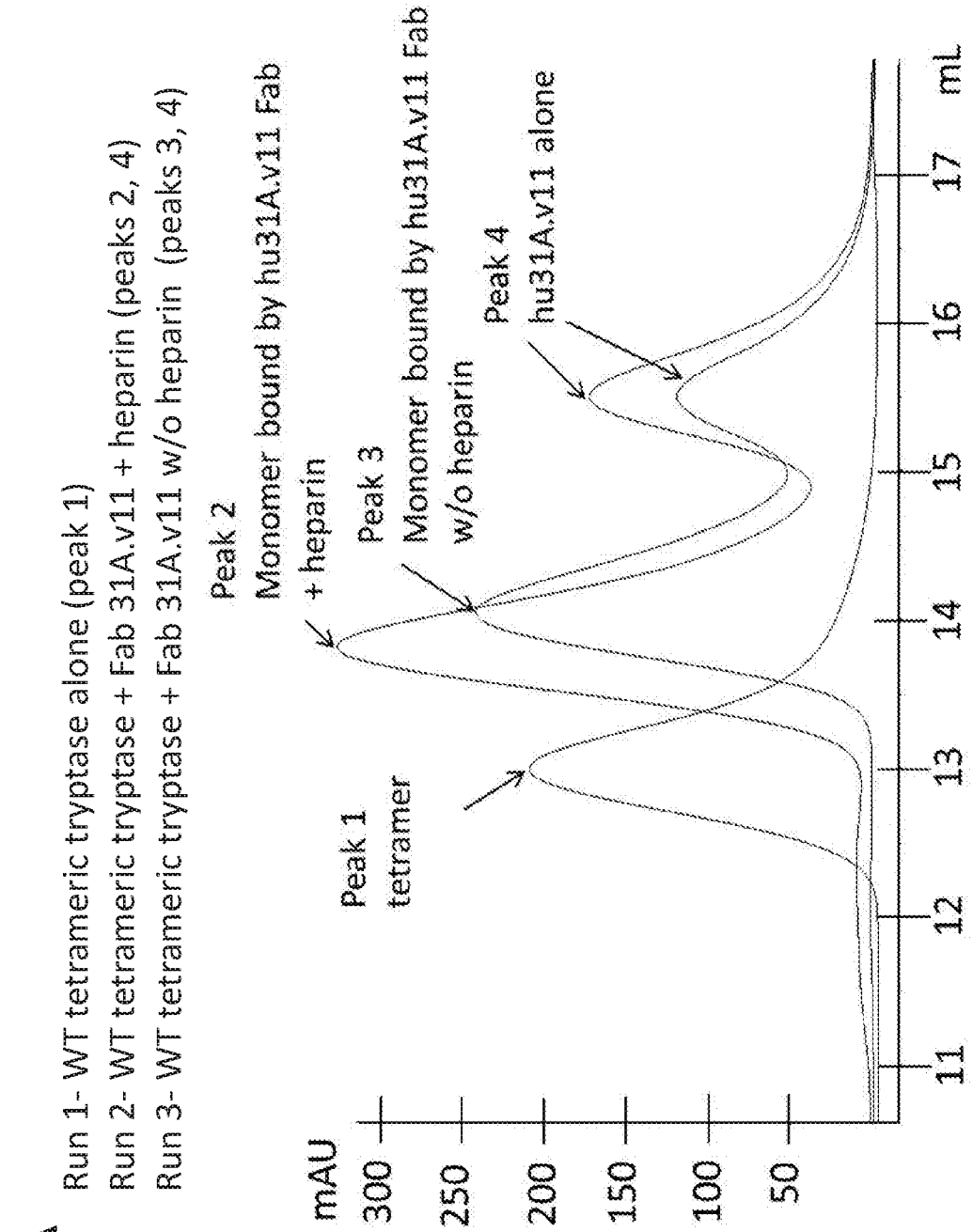

Run 1- WT tetrameric tryptase alone (peak 1)
Run 2- WT tetrameric tryptase + Fab 31A.v11 + heparin (peaks 2, 4)
Run 3- WT tetrameric tryptase + Fab 31A.v11 w/o heparin (peaks 3, 4)
Peak 2
Monomer bound by hu31A.v11 Fab
+ heparin
Peak 3
Monomer bound by hu31A.v11 Fab
w/o heparin
Peak 4
hu31A.v11 alone
Peak 1
tetramer
mAU
300
250
200
150
100
50
11
12
13
14
15
16
17
mL

FIG.3B

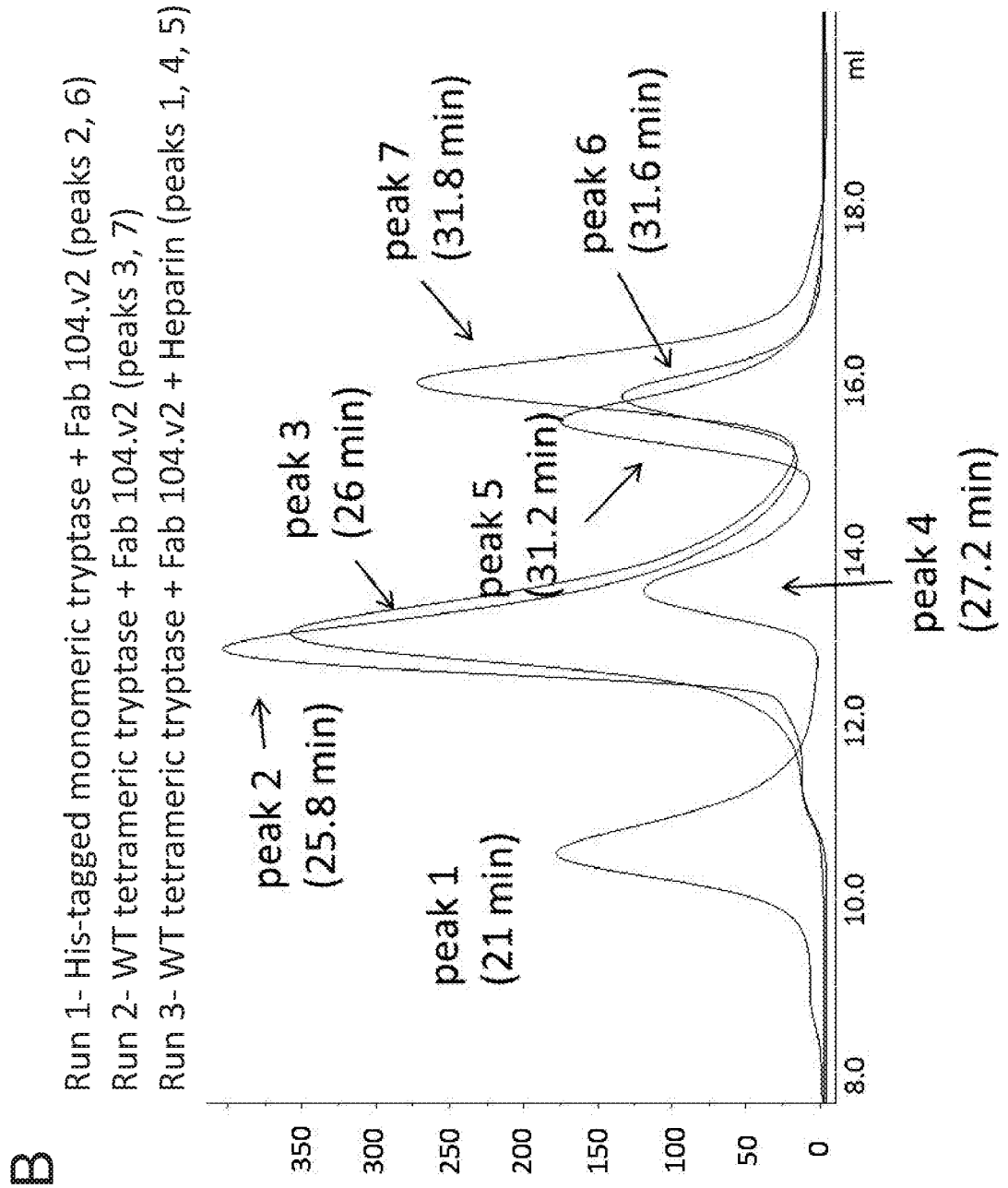

Run 1- His-tagged monomeric tryptase + Fab 104.v2 (peaks 2, 6)
Run 2- WT tetrameric tryptase + Fab 104.v2 (peaks 3, 7)
Run 3- WT tetrameric tryptase + Fab 104.v2 + Heparin (peaks 1, 4, 5)
peak 2 (25.8 min)
peak 3 (26 min)
peak 7 (31.8 min)
peak 6 (31.6 min)
peak 5 (31.2 min)
peak 1 (21 min)
peak 4 (27.2 min)
350
300
250
200
150
100
50
0
8.0
10.0
12.0
14.0
16.0
18.0
ml

FIG. 4A

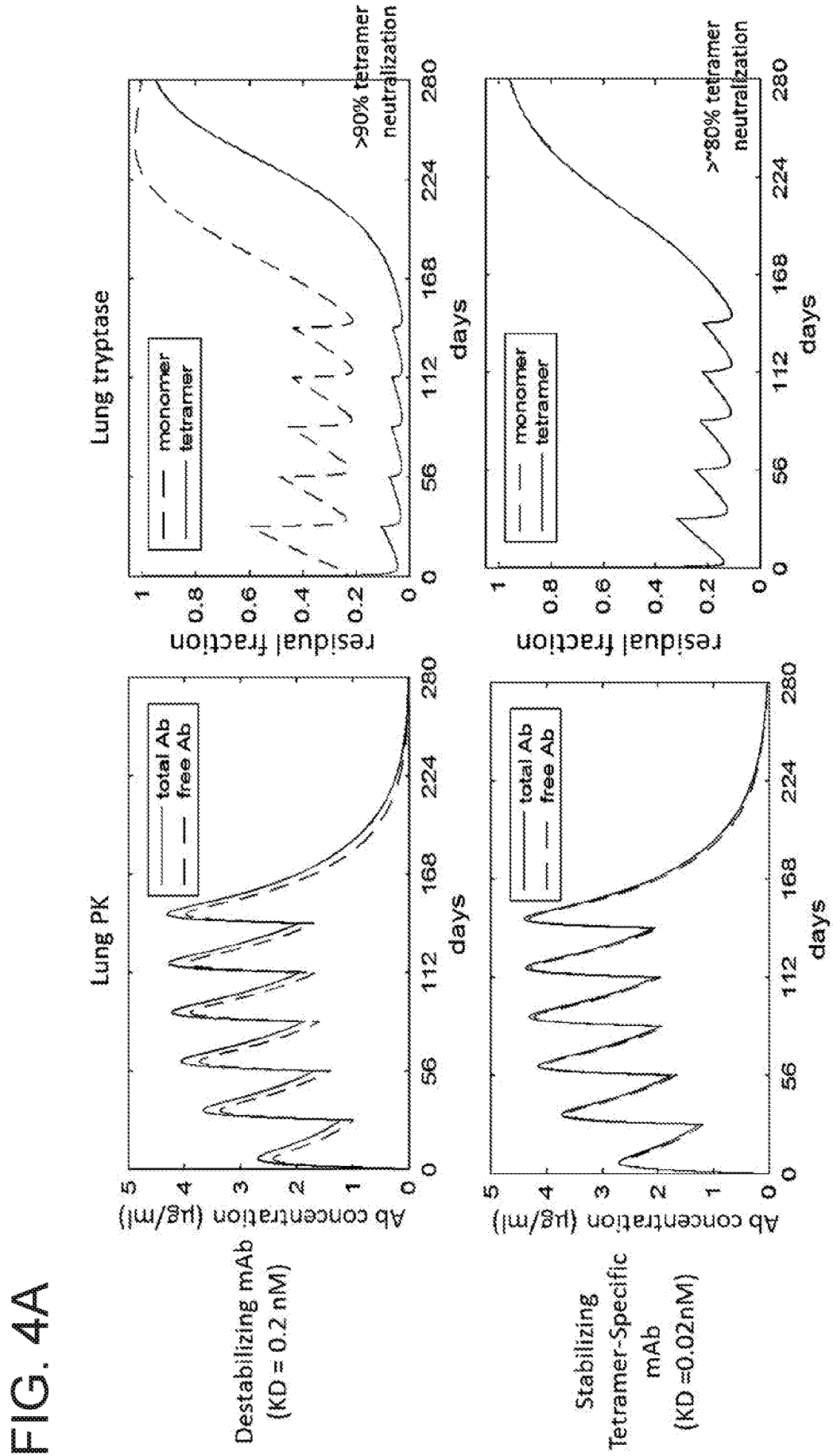

Lung PK
Lung tryptase
Ab concentration (µg/ml)
residual fraction
days
total Ab
free Ab
monomer
tetramer
>90% tetramer neutralization
>~80% tetramer neutralization
Destabilizing mAb (KD = 0.2 nM)
Stabilizing Tetramer-Specific mAb (KD =0.02nM)

FIG. 4B

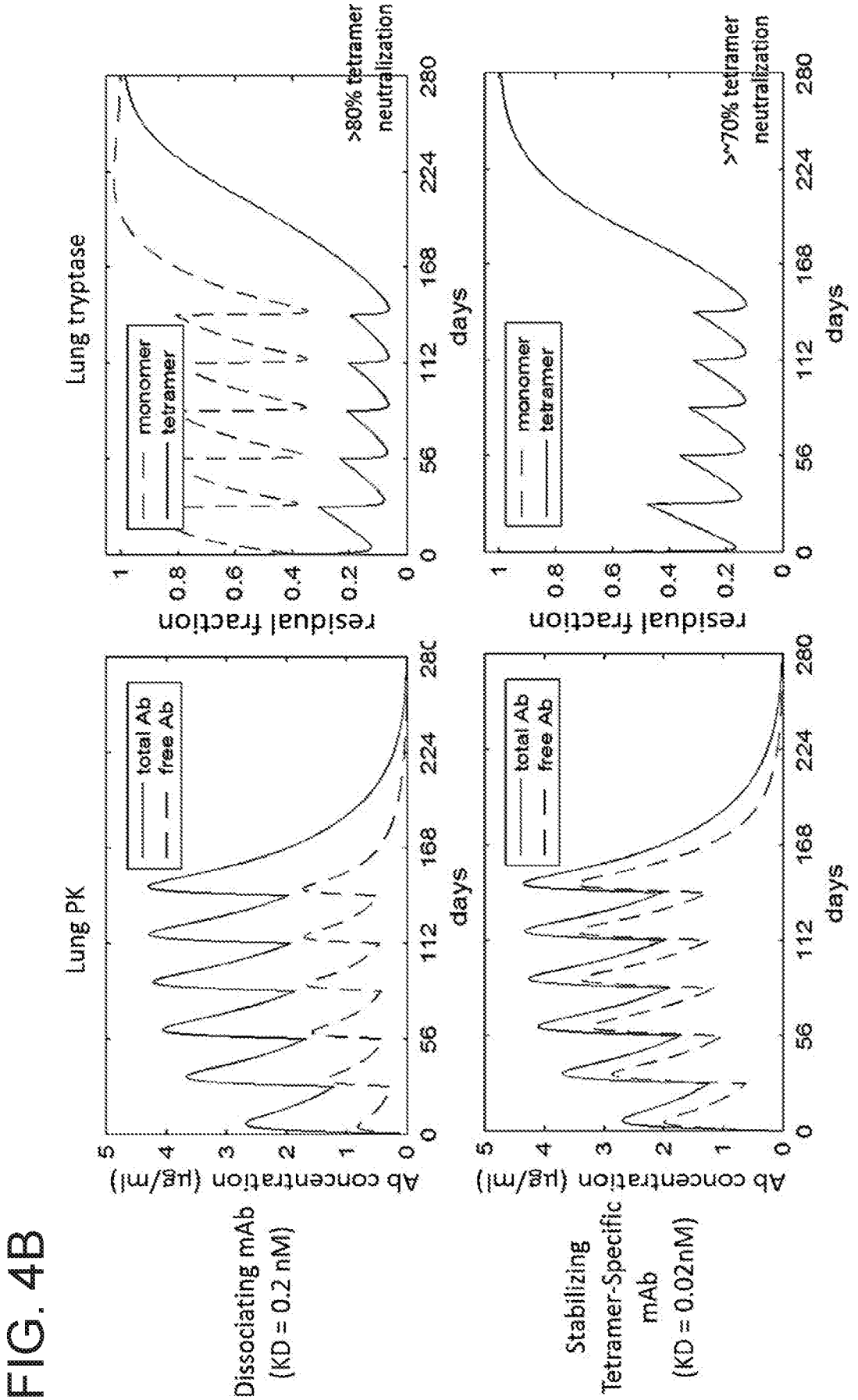

Lung PK
Lung tryptase
Dissociating mAb
(KD = 0.2 nM)
Stabilizing
Tetramer-Specific
mAb
(KD = 0.02nM)
Ab concentration (μg/ml)
residual fraction
days
total Ab
free Ab
monomer
tetramer
>80% tetramer
neutralization
>~70% tetramer
neutralization

FIG. 4C

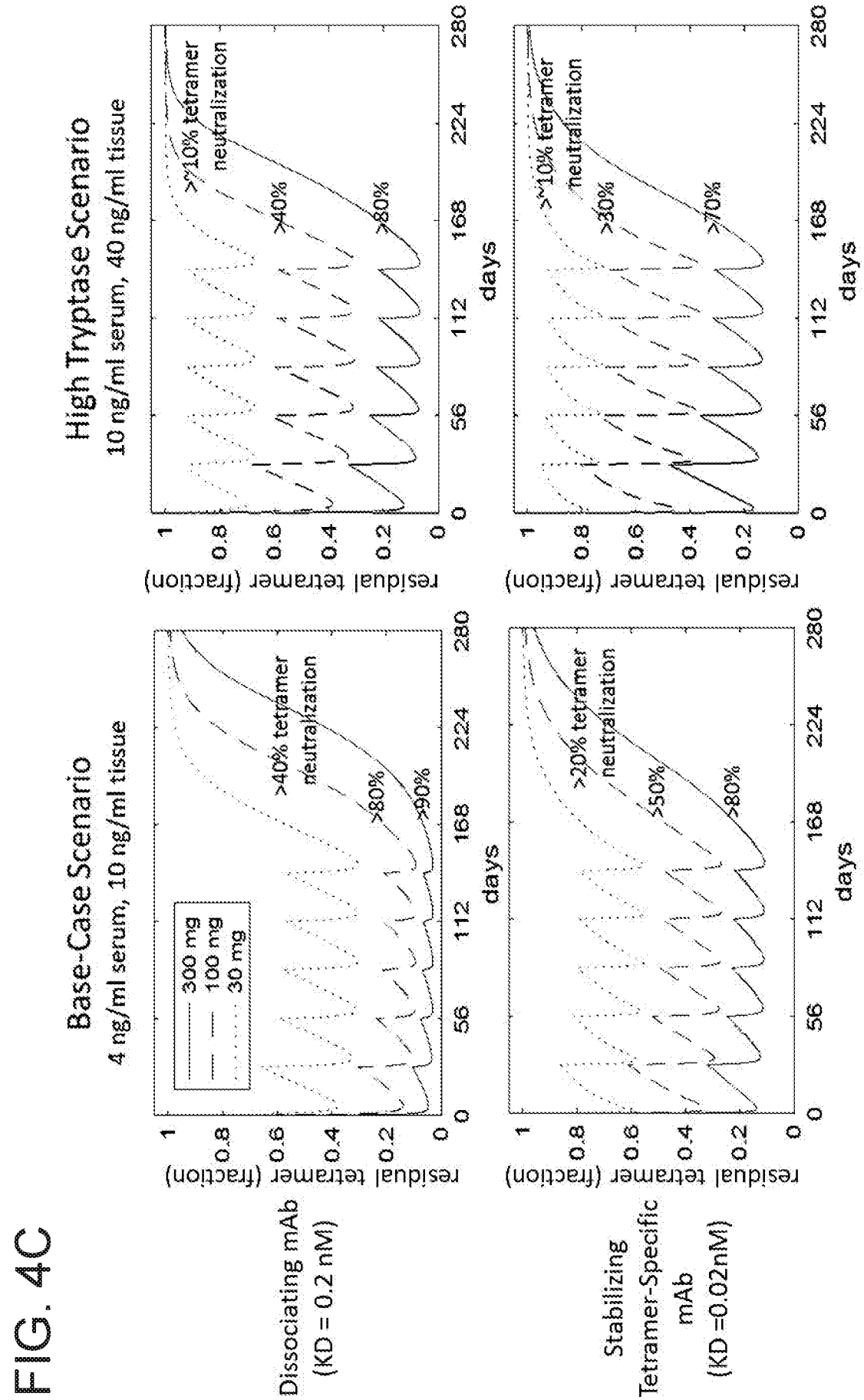
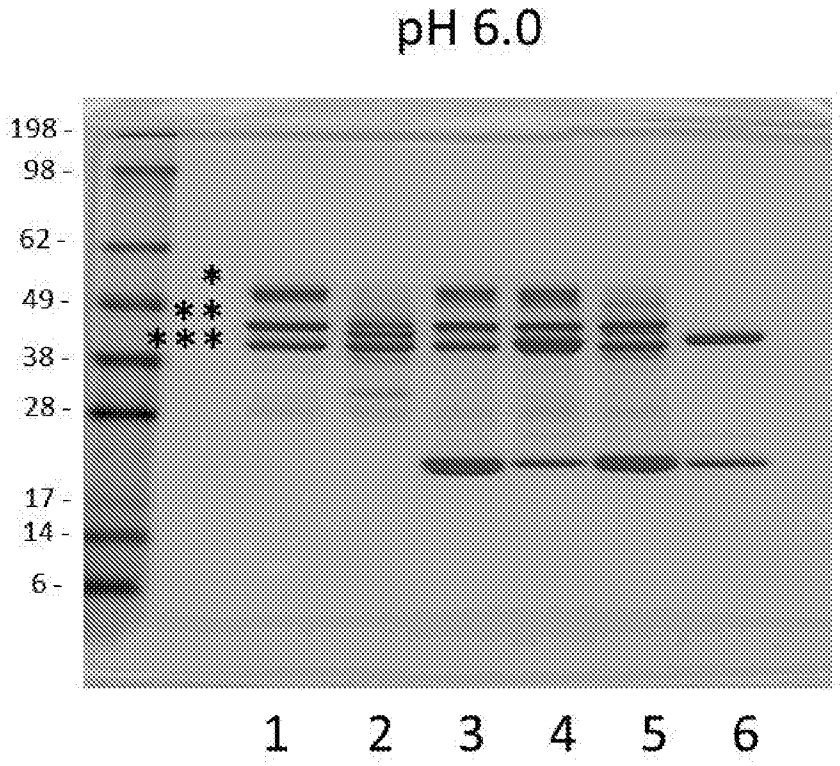

Base-Case Scenario
4 ng/ml serum, 10 ng/ml tissue
High Tryptase Scenario
10 ng/ml serum, 40 ng/ml tissue
Dissociating mAb
(KD = 0.2 nM)
Stabilizing
Tetramer-Specific
mAb
(KD =0.02nM)
residual tetramer (fraction)
days
300 mg
100 mg
30 mg
>40% tetramer neutralization
>80%
>90%
>10% tetramer neutralization
>40%
>80%
>20% tetramer neutralization
>50%
>80%
>10% tetramer neutralization
>30%
>70%

FIG. 5A


pH 6.0
198 -
98 -
62 -
49 -
38 -
28 -
17 -
14 -
6 -
1 2 3 4 5 6

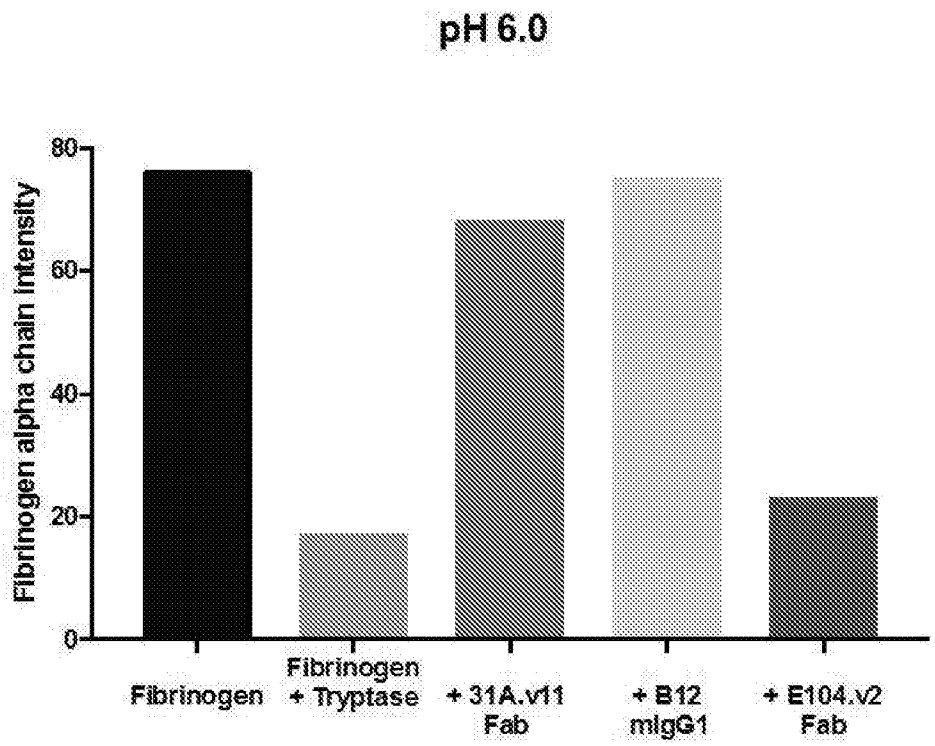
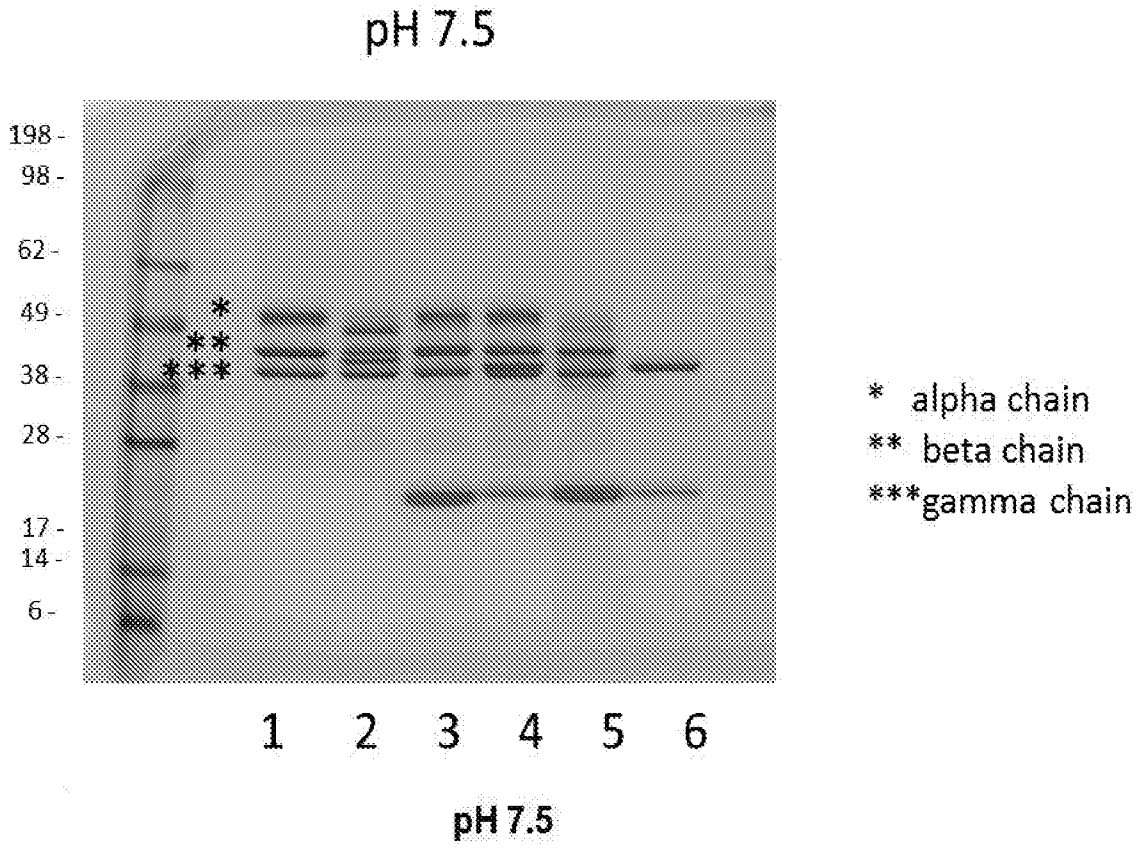

pH 6.0
Fibrinogen alpha chain intensity
80
60
40
20
0
Fibrinogen
Fibrinogen + Tryptase
+ 31A.v11 Fab
+ B12 mIgG1
+ E104.v2 Fab

FIG. 5B


pH 7.5
198 -
98 -
62 -
49 -
38 -
28 -
17 -
14 -
6 -
*
**
***
1 2 3 4 5 6
* alpha chain
** beta chain
***gamma chain

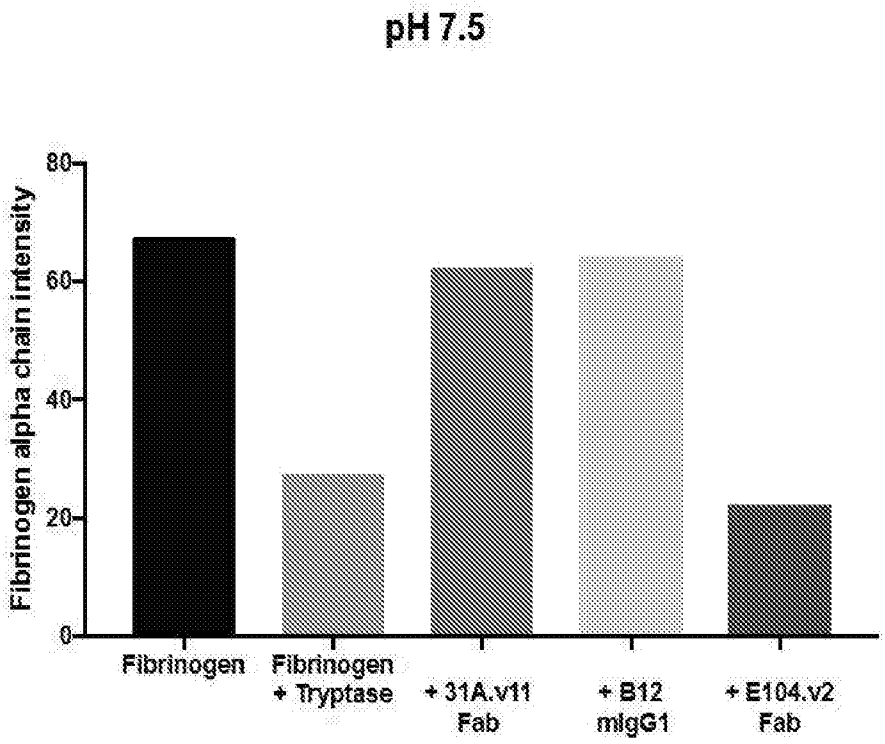

pH 7.5
Fibrinogen alpha chain intensity
80
60
40
20
0
Fibrinogen
Fibrinogen + Tryptase
+ 31A.v11 Fab
+ B12 mIgG1
+ E104.v2 Fab

FIG. 6A

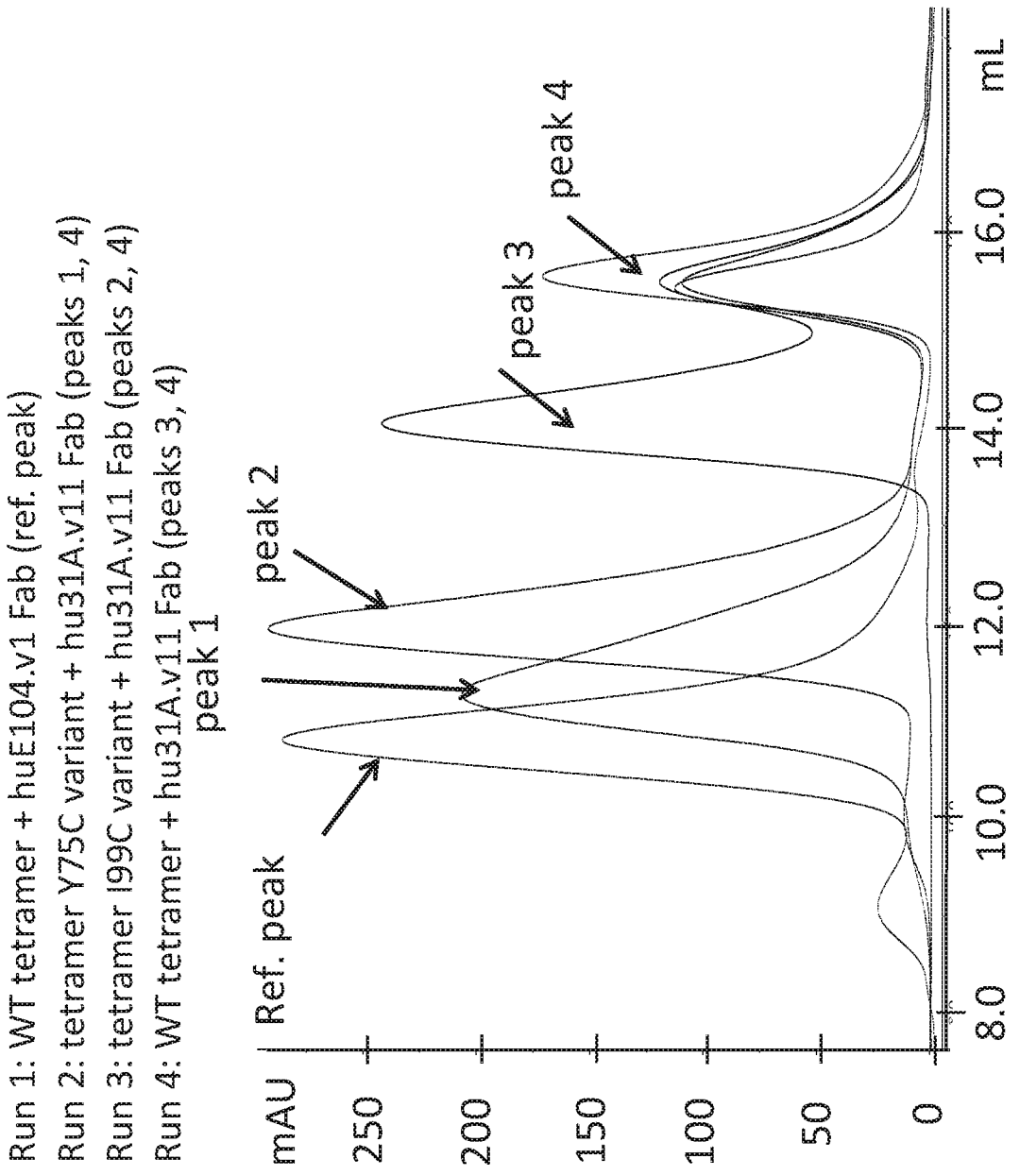

Run 1: WT tetramer + huE104.v1 Fab (ref. peak)
Run 2: tetramer Y75C variant + hu31A.v11 Fab (peaks 1, 4)
Run 3: tetramer I99C variant + hu31A.v11 Fab (peaks 2, 4)
Run 4: WT tetramer + hu31A.v11 Fab (peaks 3, 4)
peak 1
Ref. peak
peak 2
peak 3
peak 4
mAU
250
200
150
100
50
0
8.0
10.0
12.0
14.0
16.0
mL

FIG. 6B

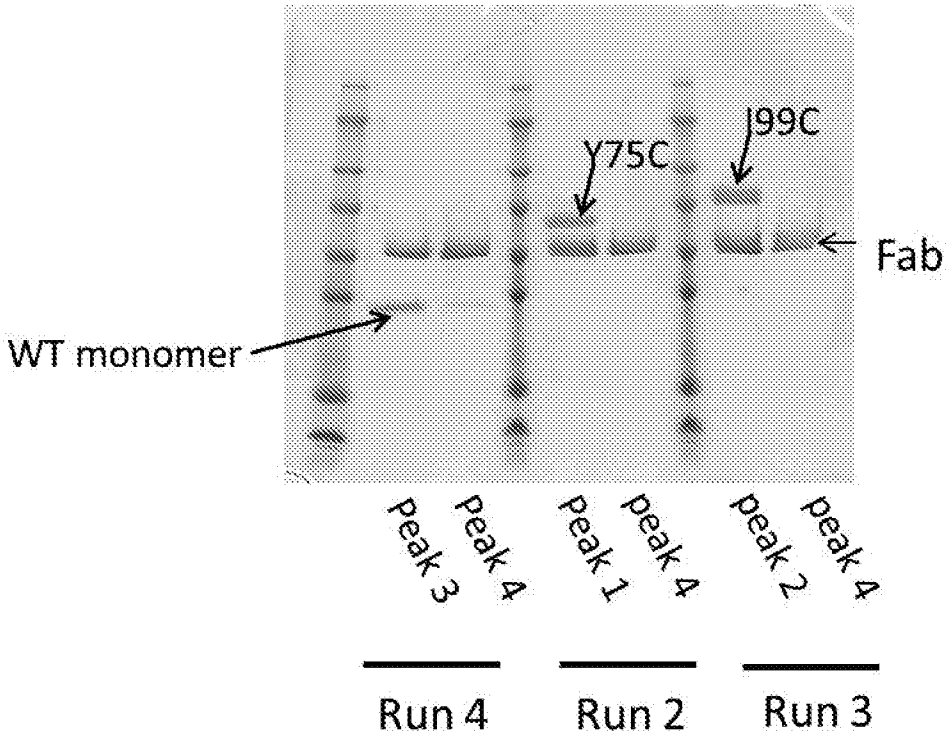

Y75C
I99C
Fab
WT monomer
peak 3
peak 4
peak 1
peak 4
peak 2
peak 4
Run 4
Run 2
Run 3

FIG. 6C

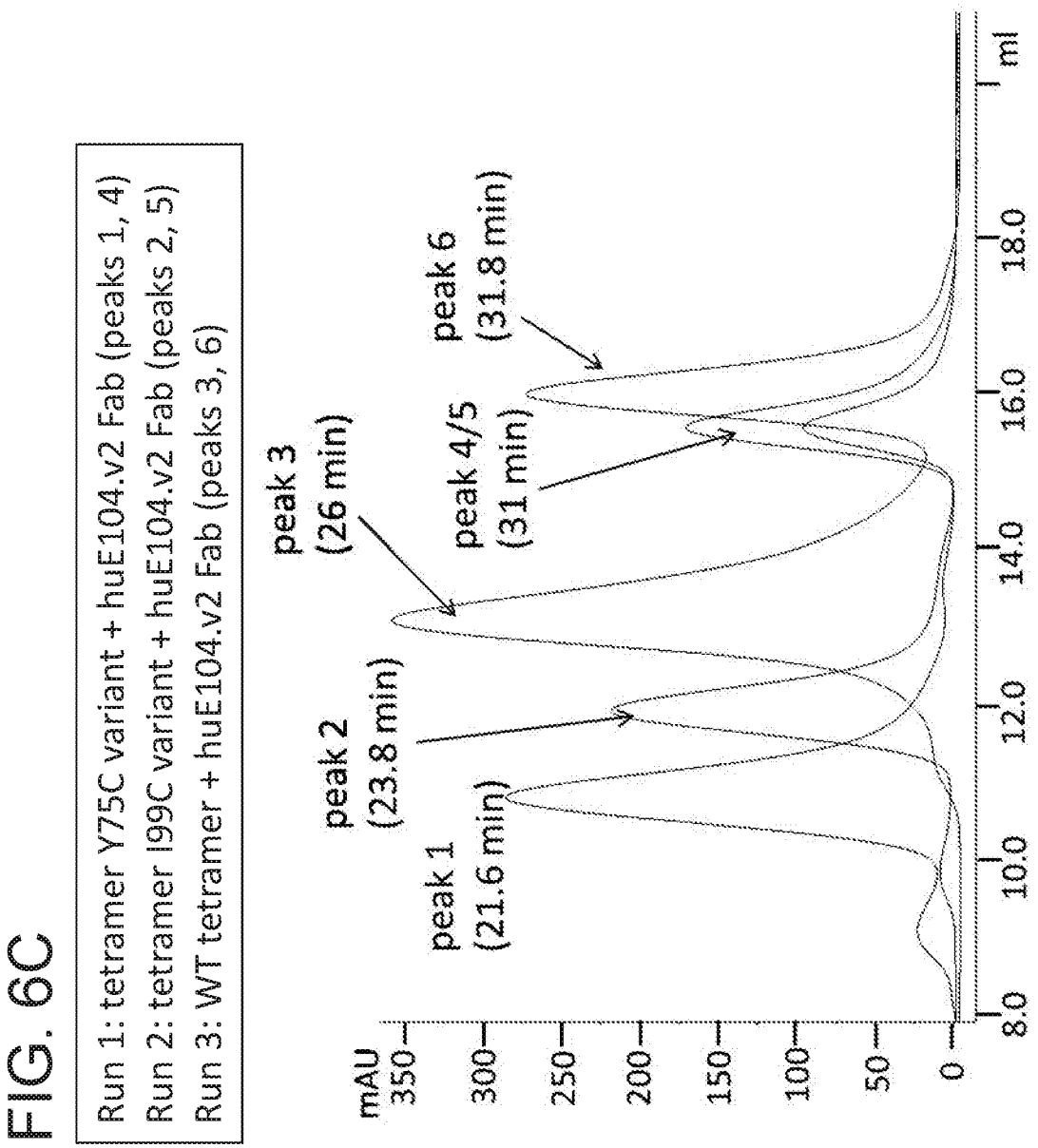

Run 1: tetramer Y75C variant + huE104.v2 Fab (peaks 1, 4)
Run 2: tetramer I99C variant + huE104.v2 Fab (peaks 2, 5)
Run 3: WT tetramer + huE104.v2 Fab (peaks 3, 6)
peak 1 (21.6 min)
peak 2 (23.8 min)
peak 3 (26 min)
peak 4/5 (31 min)
peak 6 (31.8 min)
mAU
350
300
250
200
150
100
50
0
8.0
10.0
12.0
14.0
16.0
18.0
ml

FIG. 7

	structure: chymo-numb	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	a	b	38	39	40
	gene-sequential	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
Mature human tryptase beta 1	amino acid sequence	I	V	G	G	Q	E	A	P	R	S	K	W	P	W	Q	V	S	L	R	V	H	G	P	Y	W	M	H

	structure: chymo-numb	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	a	b	c	d	e
	gene-sequential	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82
Mature human tryptase beta 1	amino acid sequence	F	C	G	G	S	L	I	H	P	Q	W	V	L	T	A	A	H	C	V	G	P	D	V	K	D

	structure: chymo-numb	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	79	80	81	82	83	84	85	86	87	88	89	90
	gene-sequential	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109
Mature human tryptase beta 1	amino acid sequence	L	A	A	L	R	V	Q	L	R	E	Q	H	L	Y	Y	Q	D	Q	L	L	P	V	S	R	I	I	V

	structure: chymo-numb	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114
	gene-sequential	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133
Mature human tryptase beta 1	amino acid sequence	V	H	P	Q	F	Y	T	A	Q	I	G	A	D	I	A	L	L	E	L	E	E	P	V	N	V

	structure: chymo-numb	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	138
	gene-sequential	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158
Mature human tryptase beta 1	amino acid sequence	S	S	H	V	H	T	V	T	L	P	P	A	S	E	T	F	P	P	G	M	P	C	W	V	T

FIG. 7 cont.

structure: chymo-numb	140	141	142	143	144	145	146	147	149	150	151	152	a	b	153	154	155	156	157	158	159	160	161	162	163	164
gene-sequential	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184
Mature human tryptase beta 1 amino acid sequence	G	W	G	D	V	D	N	D	E	R	L	P	P	P	F	P	L	K	Q	V	K	V	P	I	M	E

structure: chymo-numb	165	166	167	168	169	170	171	172	173	a	b	c	d	e	f	g	h	i	174	175	176	177	178	179	180	181
gene-sequential	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
Mature human tryptase beta 1 amino acid sequence	N	H	I	C	D	A	K	Y	H	L	G	A	Y	T	G	D	D	V	R	I	V	R	D	D	M	L

structure: chymo-numb	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206
gene-sequential	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235
Mature human tryptase beta 1 amino acid sequence	C	A	G	N	T	R	R	D	S	C	Q	G	D	S	G	G	P	L	V	C	K	V	N	G	T

structure: chymo-numb	207	208	209	210	211	212	213	214	215	216	217	219	220	221a	221	222	223	224	225	226	227	228	229	230	231
gene-sequential	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260
Mature human tryptase beta 1 amino acid sequence	W	L	Q	A	G	V	V	S	W	G	E	G	C	A	Q	P	N	R	P	G	I	Y	T	R	V

structure: chymo-numb	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246
gene-sequential	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275
Mature human tryptase beta 1 amino acid sequence	T	Y	Y	L	D	W	I	H	H	Y	V	P	K	K	P

SEQ. ID NO.:97

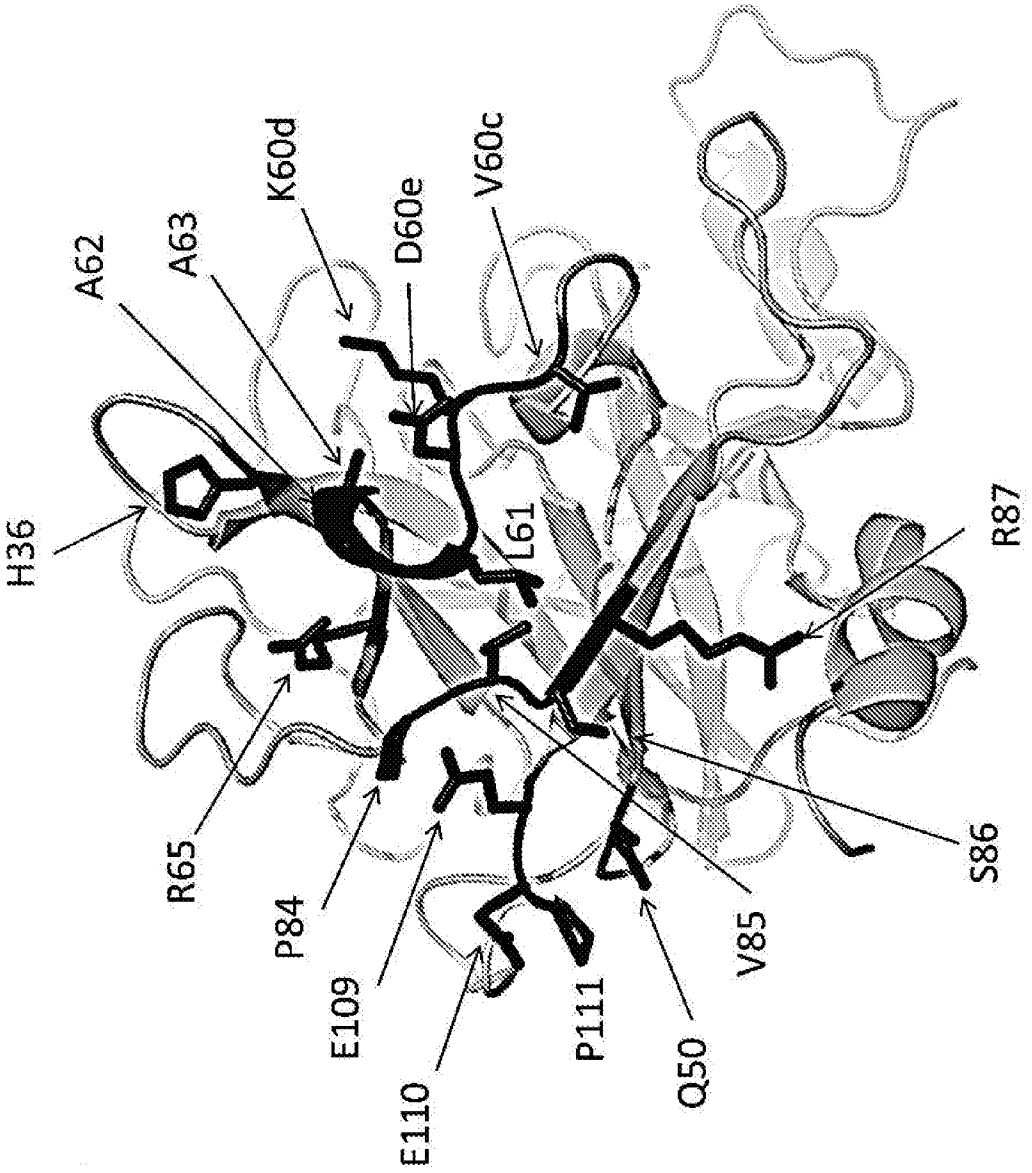

H36
A62
A63
K60d
D60e
V60c
L61
R87
R65
P84
E109
E110
P111
Q50
V85
S86

FIG. 8

FIG. 9

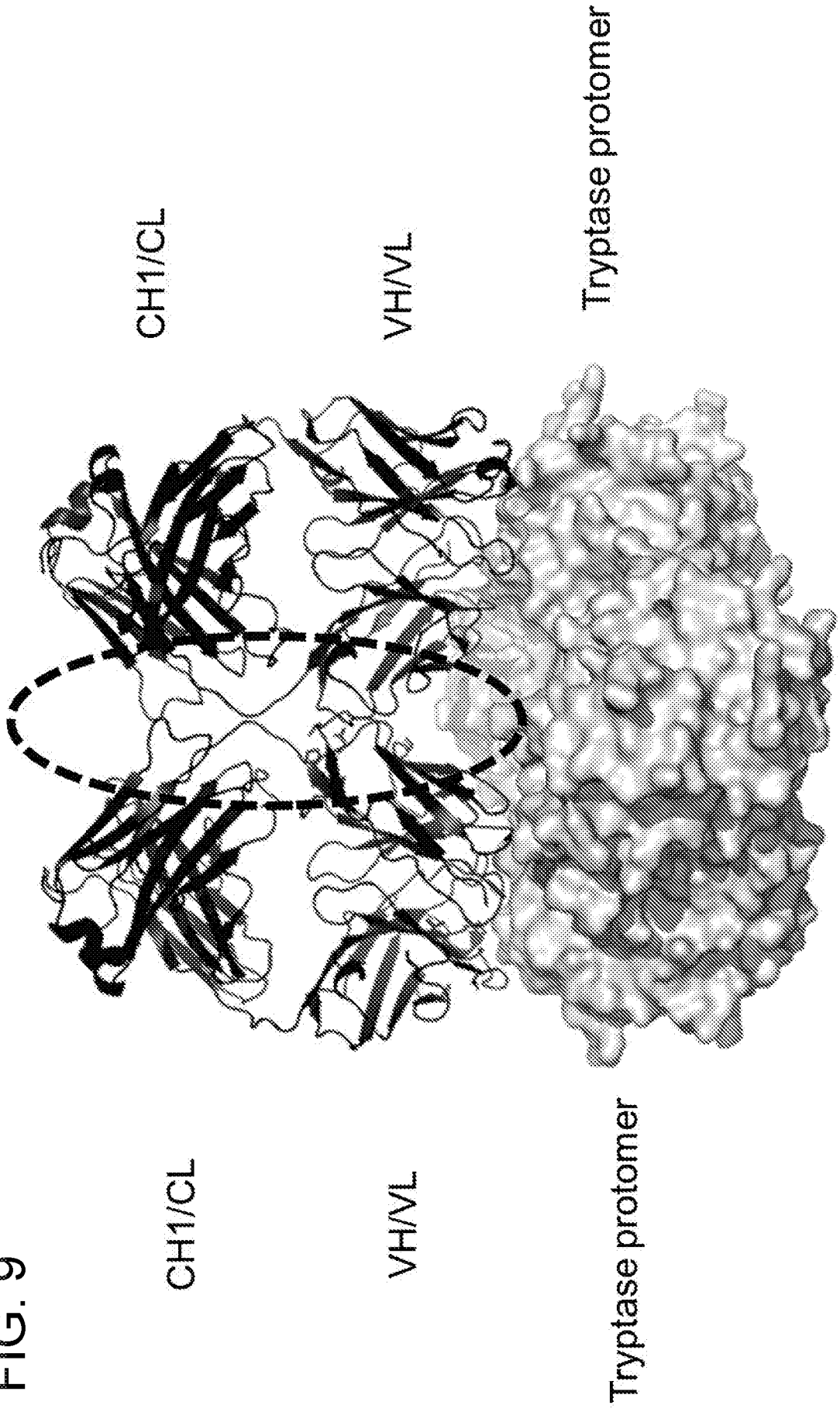

CH1/CL
VH/VL
Tryptase protomer
CH1/CL
VH/VL
Tryptase protomer

FIG. 10

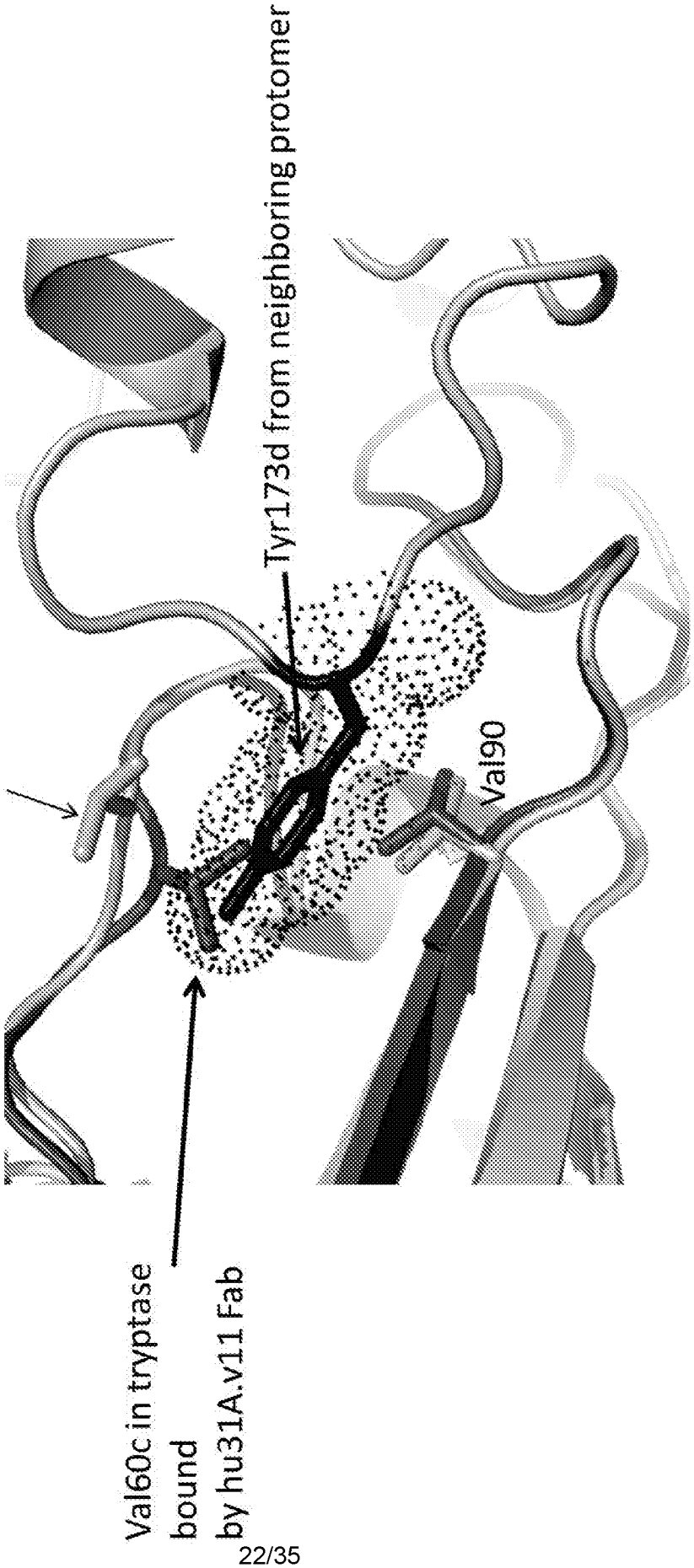
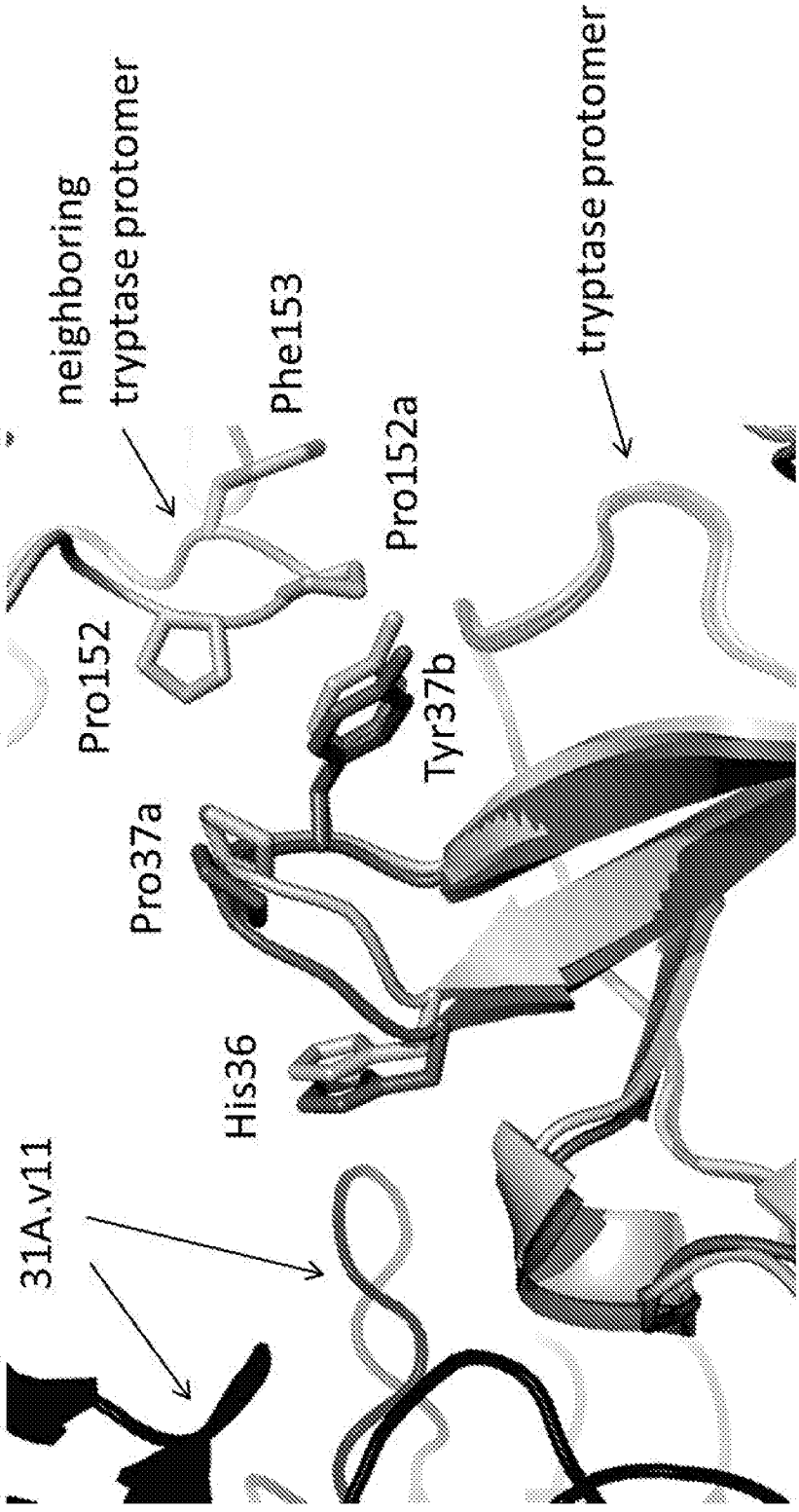

Val60c in unbound tryptase
Tyr173d from neighboring protomer
Val90
Val60c in tryptase bound by hu31A.v11 Fab

FIG. 11


31A.v11
His36
Pro37a
Pro152
neighboring tryptase protomer
Phe153
Pro152a
Tyr37b
tryptase protomer

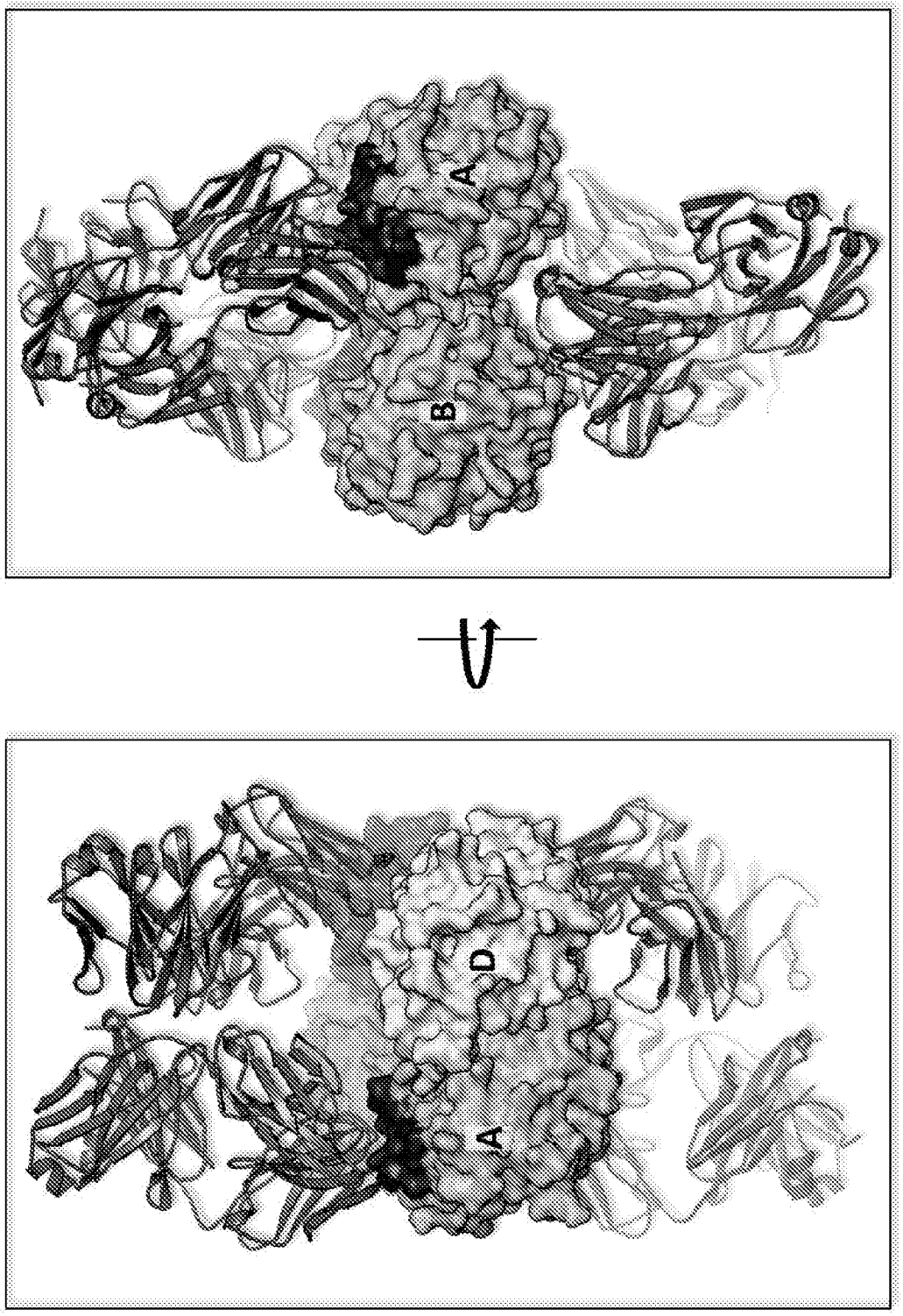


FIG. 12A

FIG. 12B

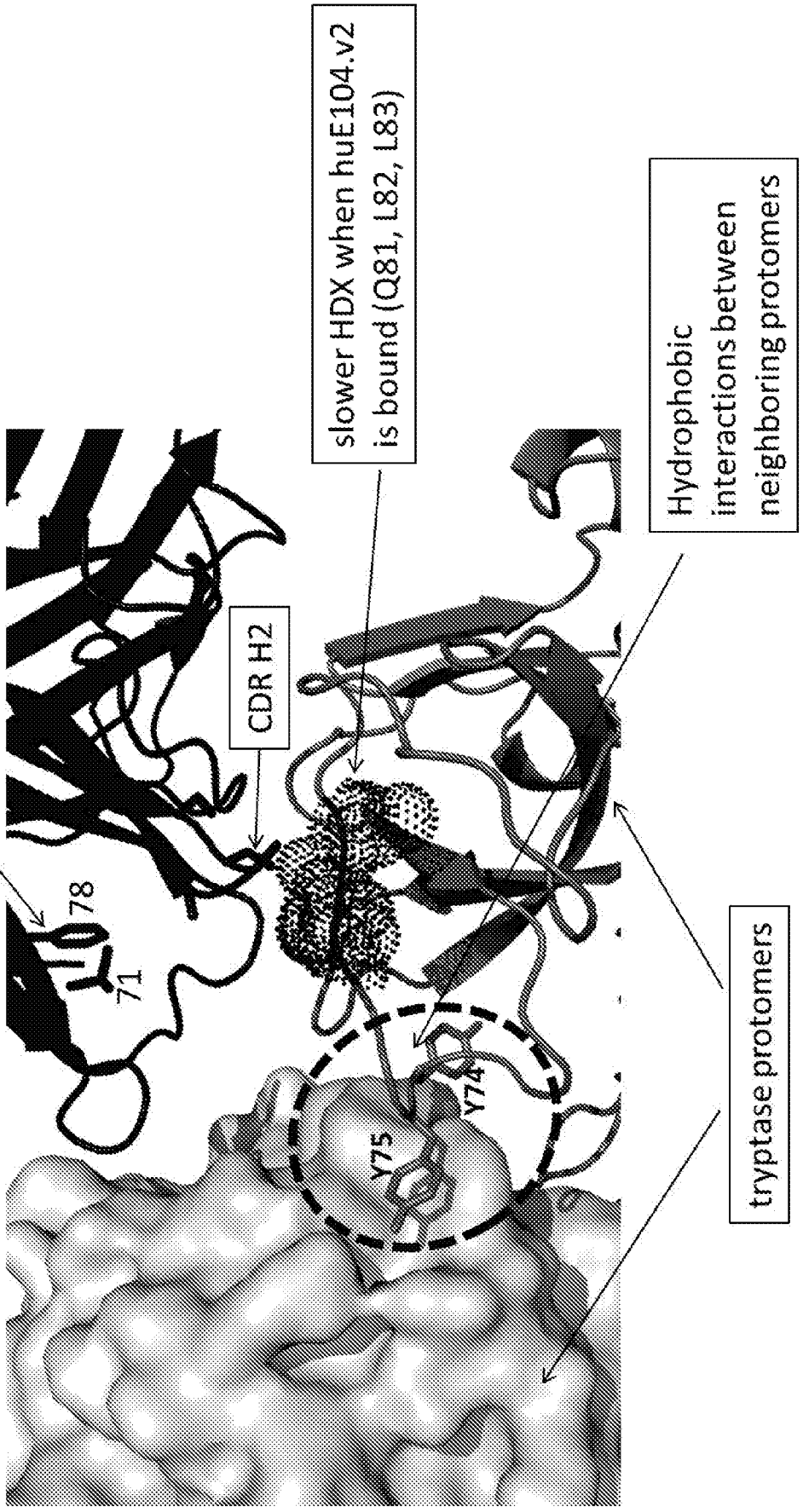
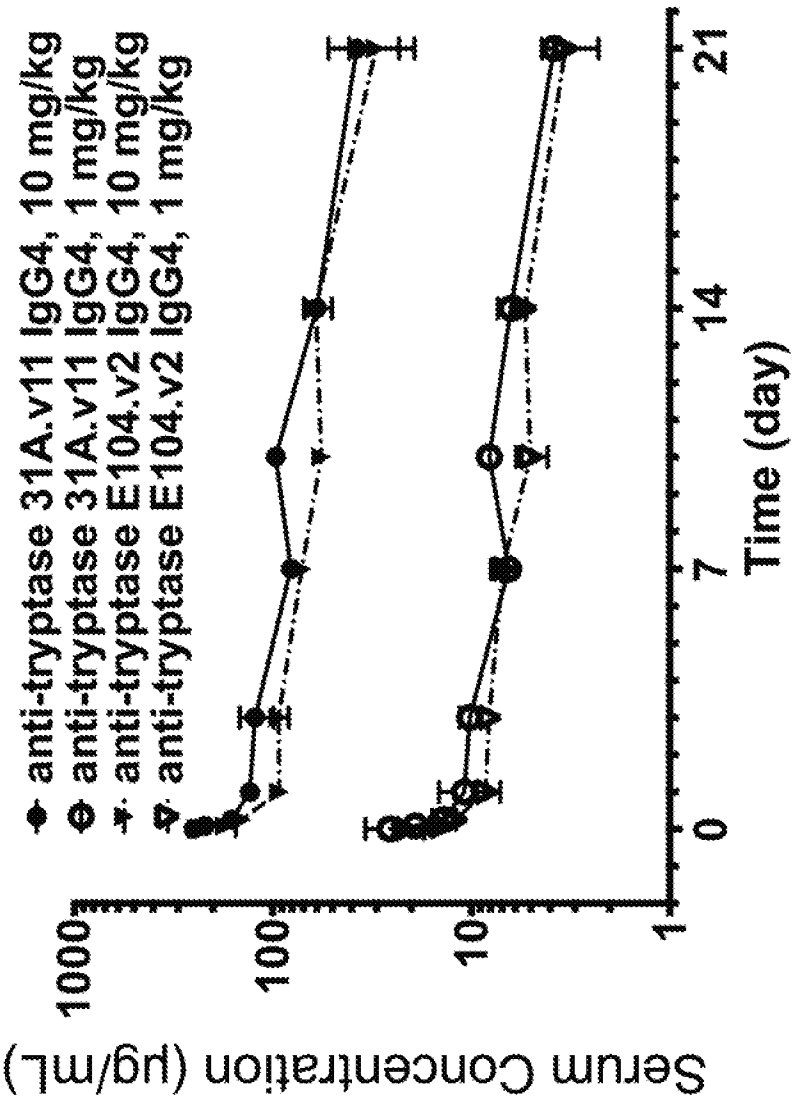

V71 and F78 in the grafted HC FR3 as in huE104.v1
78
71
CDR H2
slower HDX when huE104.v2 is bound (Q81, L82, L83)
Hydrophobic interactions between neighboring protomers
Y74
Y75
tryptase protomers

FIG. 13


anti-tryptase 31A.v11 IgG4, 10 mg/kg
anti-tryptase 31A.v11 IgG4, 1 mg/kg
anti-tryptase E104.v2 IgG4, 10 mg/kg
anti-tryptase E104.v2 IgG4, 1 mg/kg
Serum Concentration (µg/mL)
1000
100
10
1
0
7
14
21
Time (day)

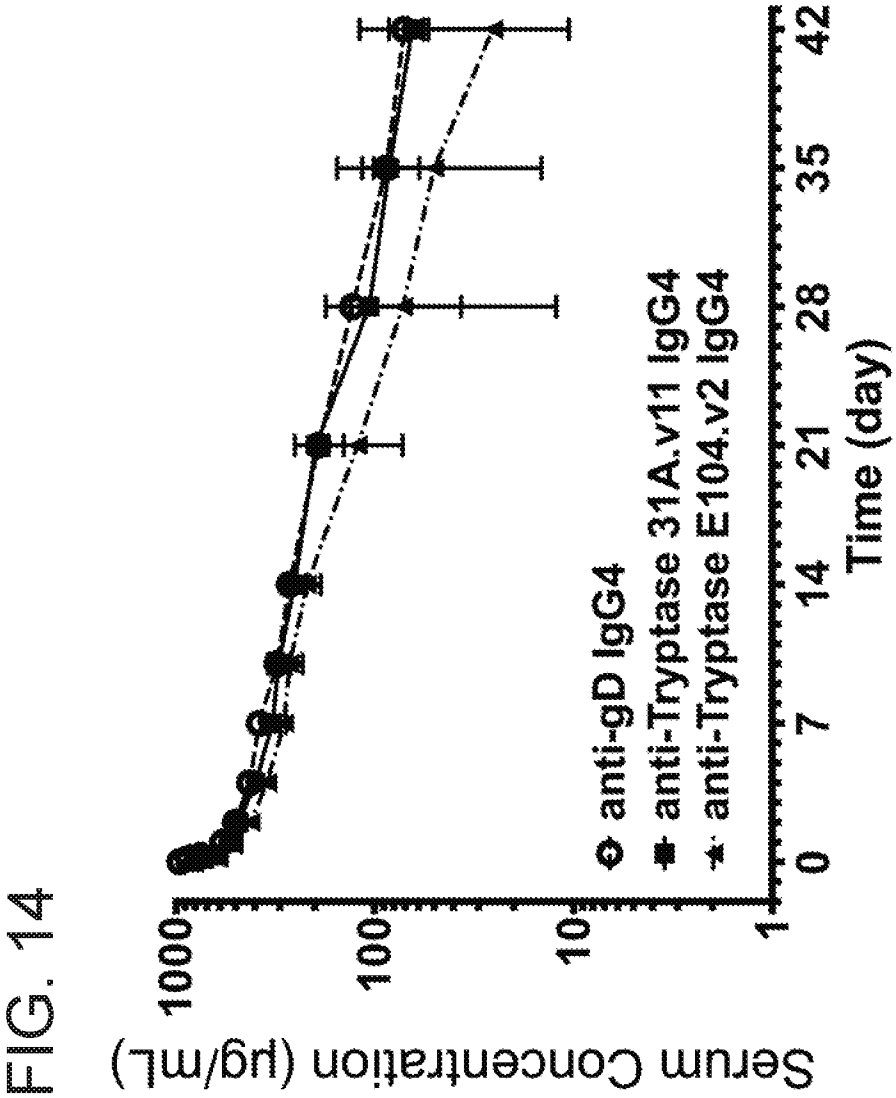

Serum Concentration (μg/mL)
1000
100
10
1
0
7
14
21
28
35
42
Time (day)
anti-gD IgG4
anti-Tryptase 31A.v11 IgG4
anti-Tryptase E104.v2 IgG4

FIG. 14

FIG. 15

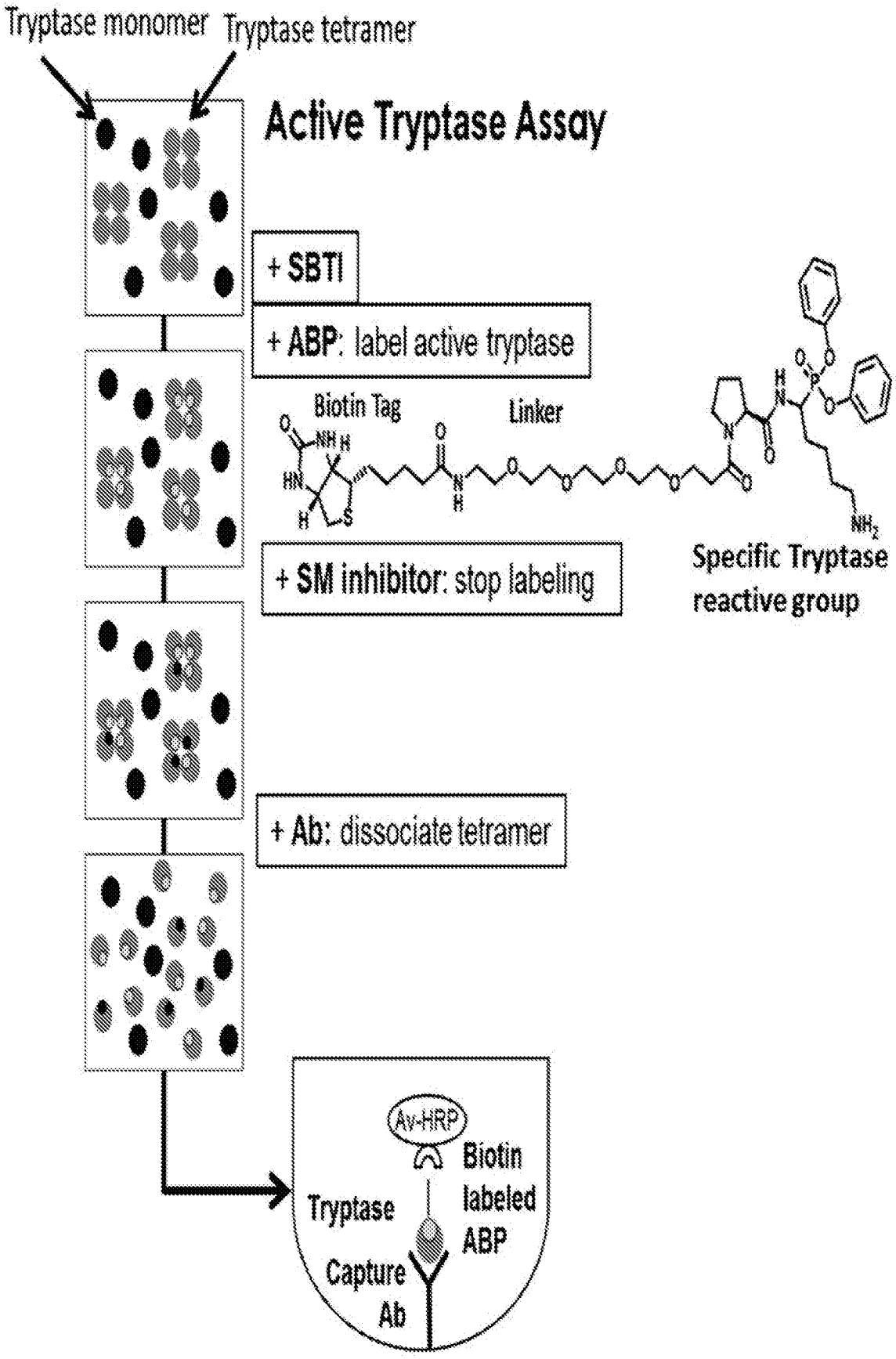

Tryptase monomer
Tryptase tetramer
Active Tryptase Assay
+ SBTI
+ ABP: label active tryptase
Biotin Tag
Linker
+ SM inhibitor: stop labeling
Specific Tryptase reactive group
+ Ab: dissociate tetramer
Av-HRP
Biotin labeled ABP
Tryptase
Capture Ab

FIG. 15 cont.

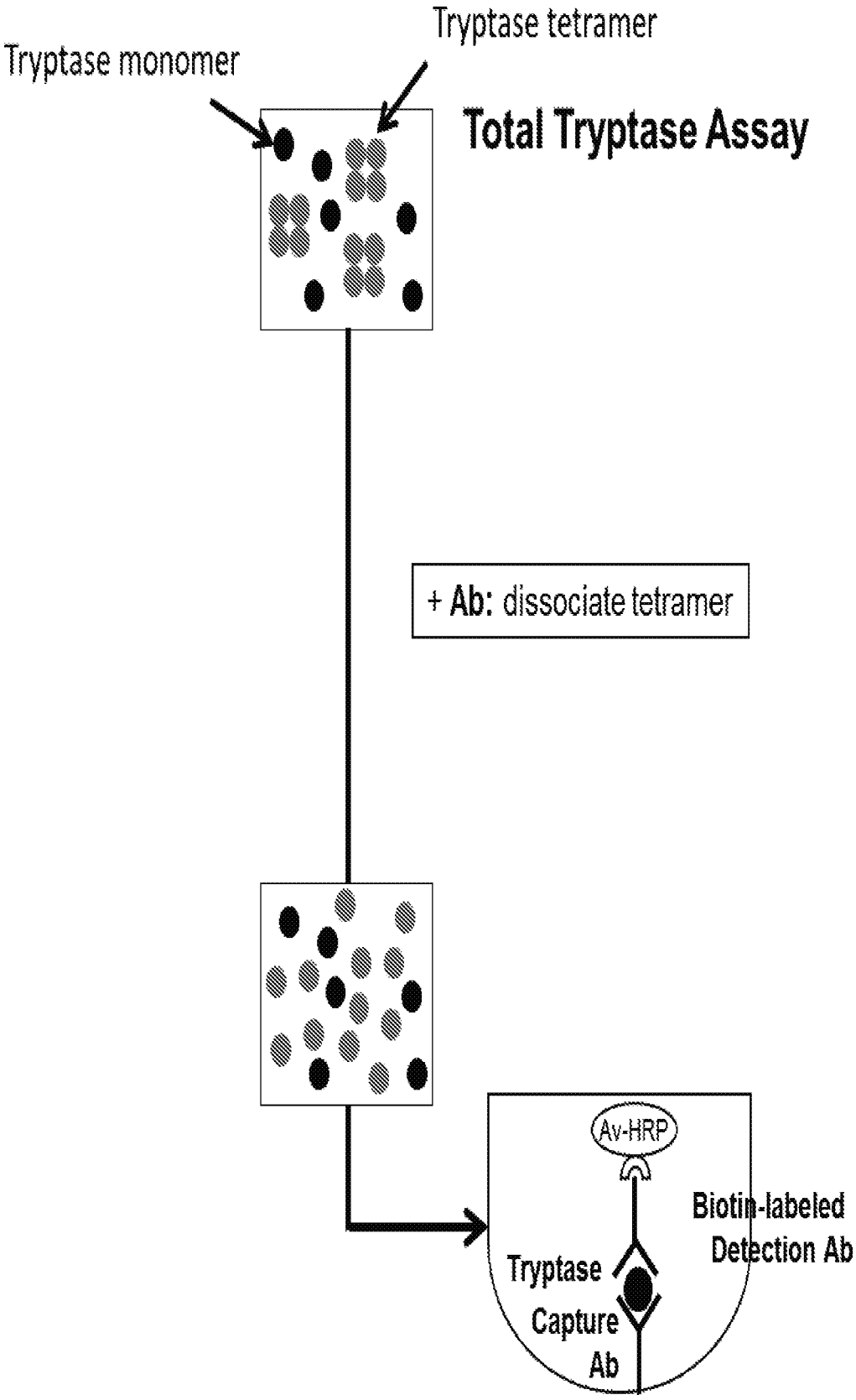

Tryptase monomer
Tryptase tetramer
Total Tryptase Assay
+ Ab: dissociate tetramer
Av-HRP
Biotin-labeled
Detection Ab
Tryptase
Capture
Ab

FIG. 16

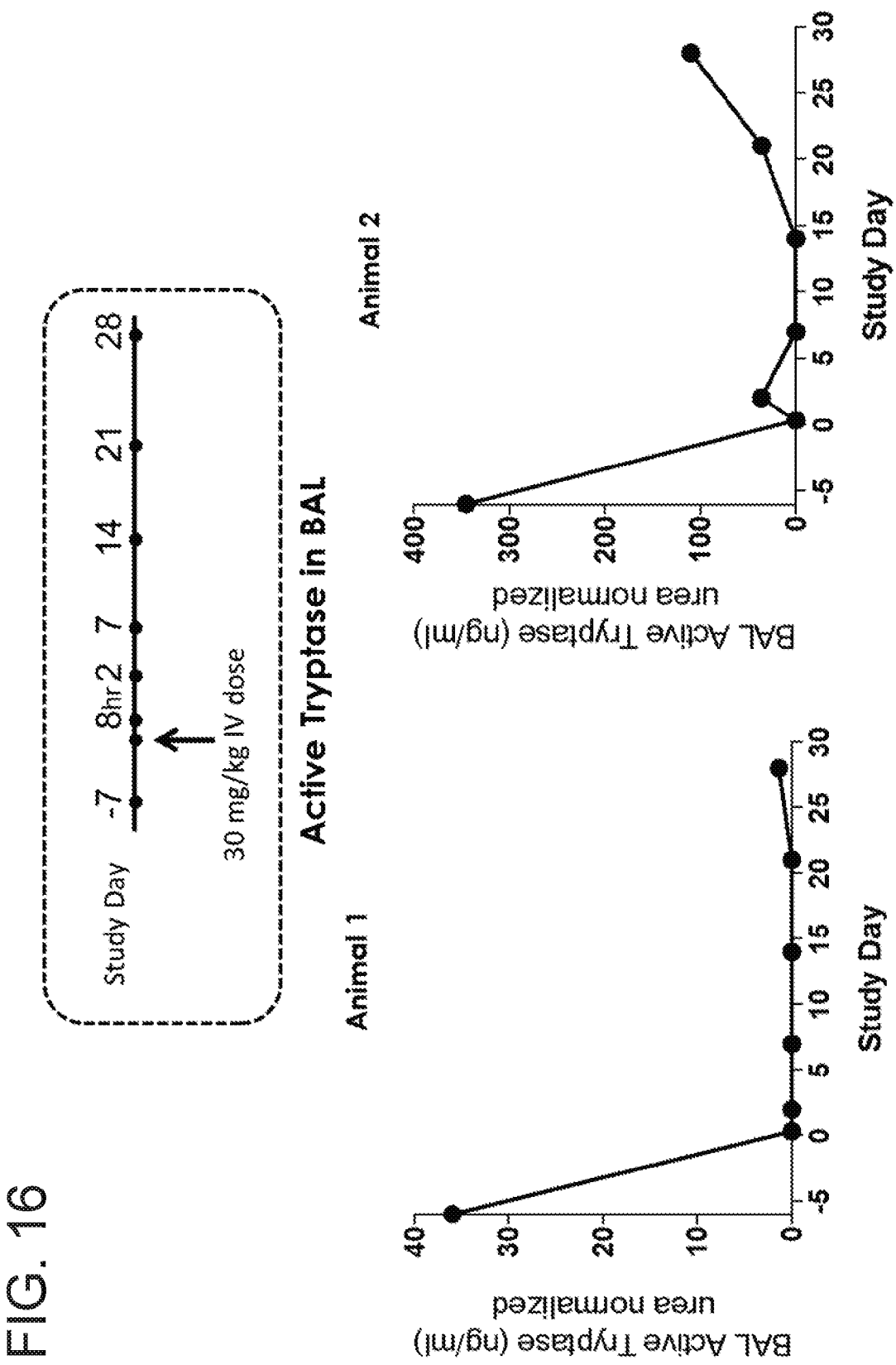

Study Day
-7
8hr
2
7
14
21
28
30 mg/kg IV dose
Active Tryptase in BAL
Animal 1
BAL Active Tryptase (ng/ml)
urea normalized
Study Day
Animal 2
BAL Active Tryptase (ng/ml)
urea normalized
Study Day

FIG. 17

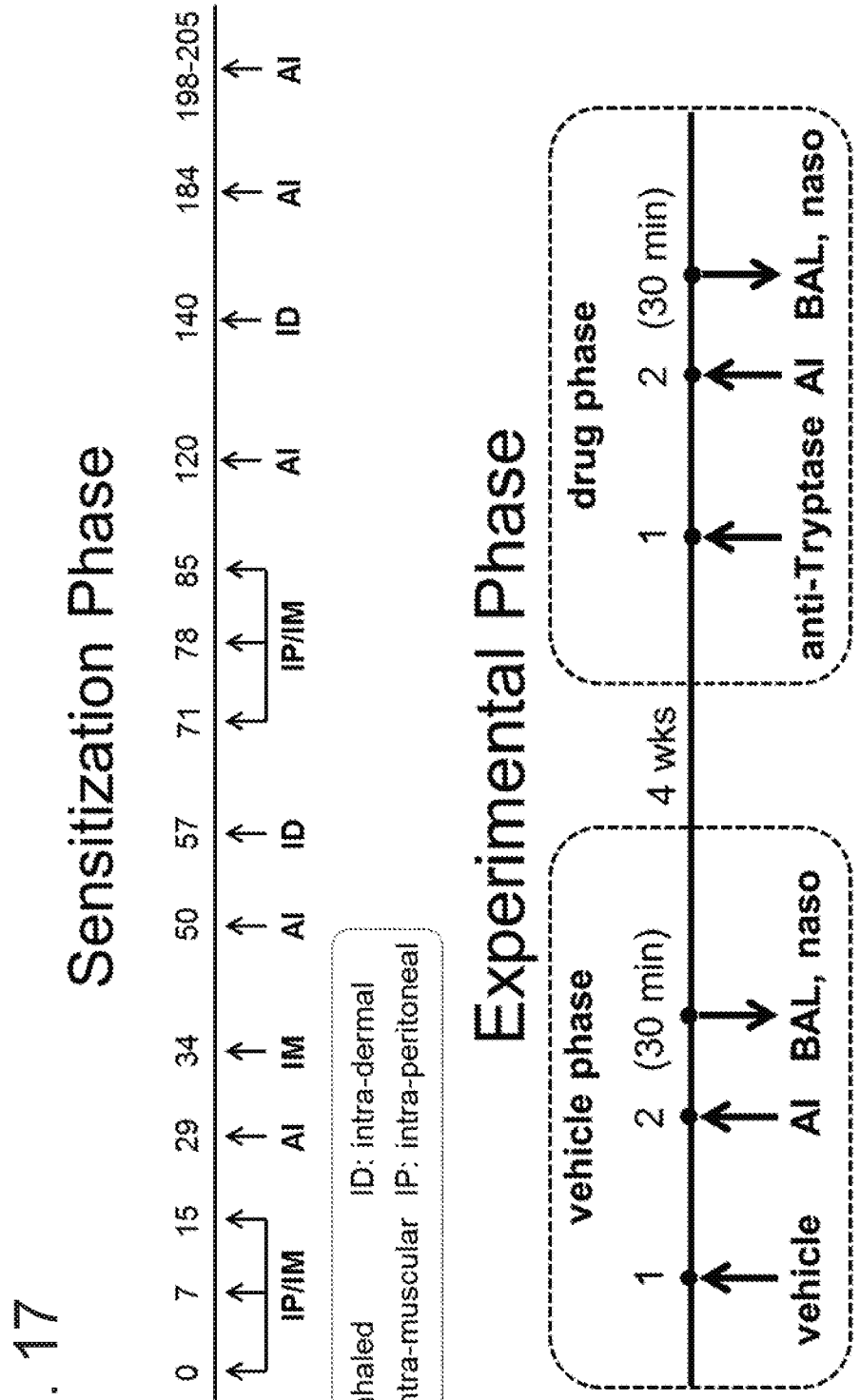
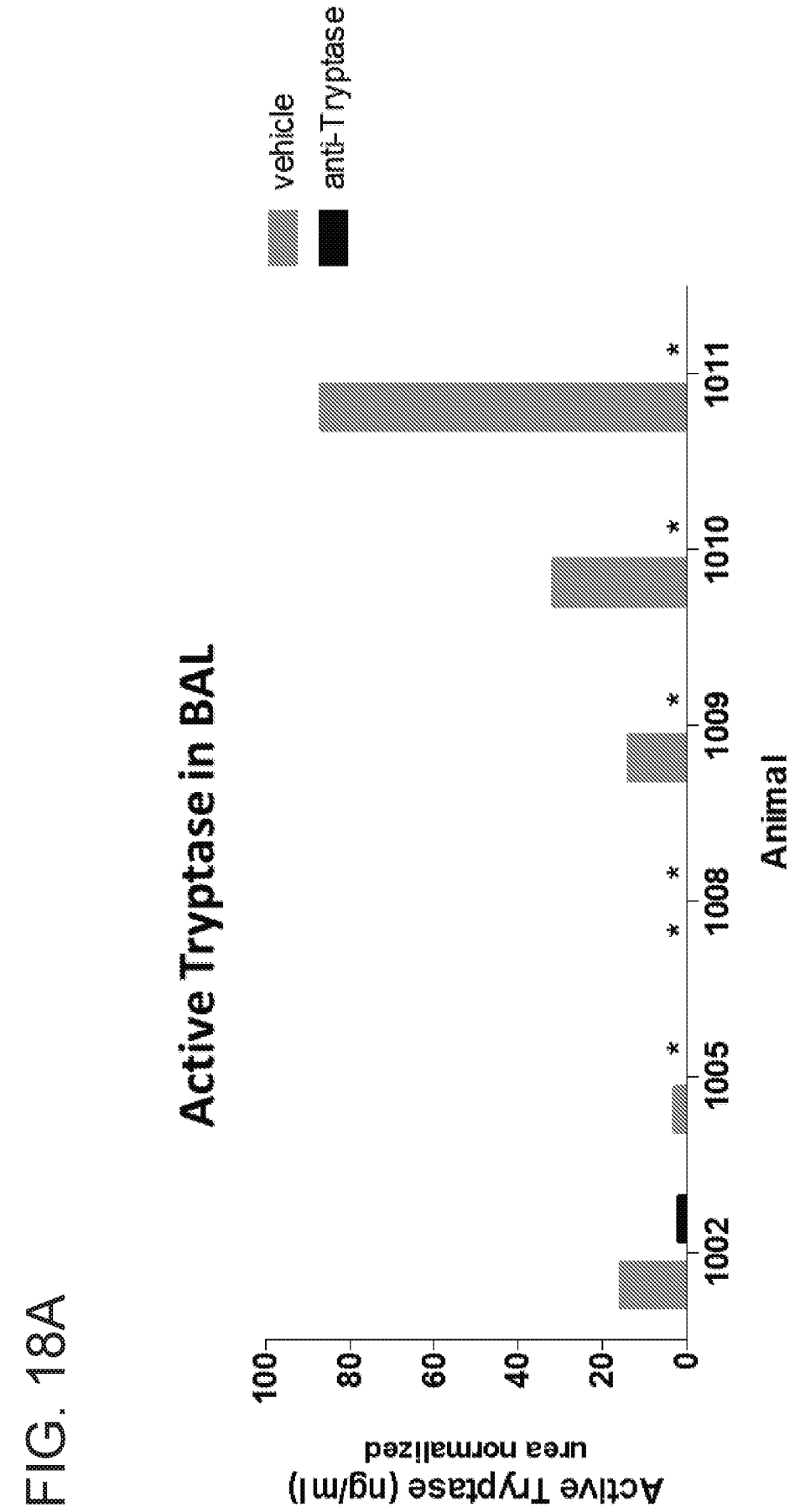

Sensitization Phase
-7 0 7 15 29 34 50 57 71 78 85 120 140 184 198-205
ID IP/IM AI IM AI ID IP/IM AI ID AI AI
AI: inhaled ID: intra-dermal
IM: intra-muscular IP: intra-peritoneal
Experimental Phase
vehicle phase
drug phase
1 2 (30 min) 4 wks 1 2 (30 min)
vehicle AI BAL, naso anti-Tryptase AI BAL, naso

FIG. 18A


Active Tryptase in BAL
vehicle
anti-Tryptase
Active Tryptase (ng/ml)
urea normalized
100
80
60
40
20
0
1002
1005
1008
1009
1010
1011
Animal
*
*
*
*
*
*

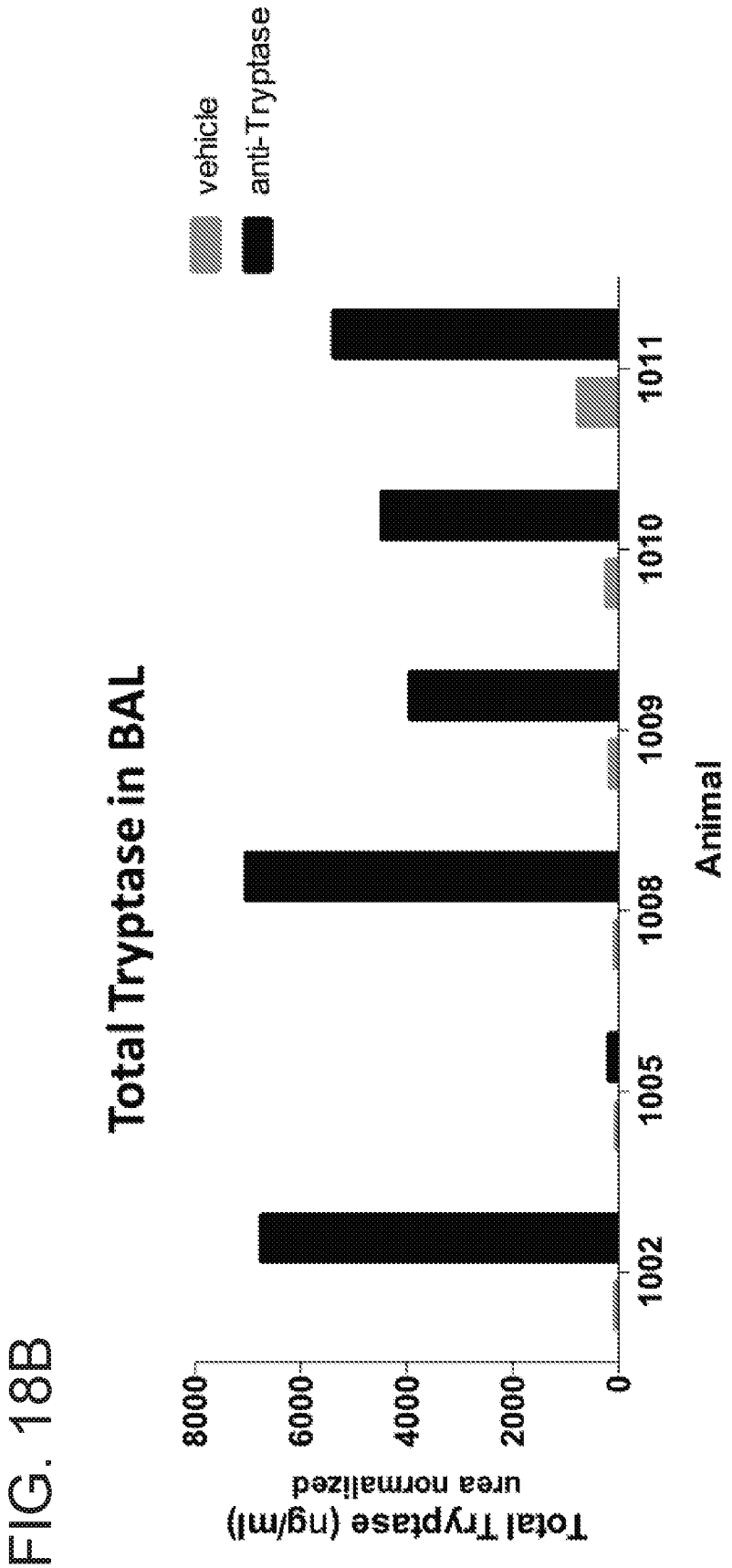

Total Tryptase in BAL
vehicle
anti-Tryptase
Total Tryptase (ng/ml)
urea normalized
8000
6000
4000
2000
0
1002
1005
1008
1009
1010
1011
Animal

FIG. 18B

FIG. 18C

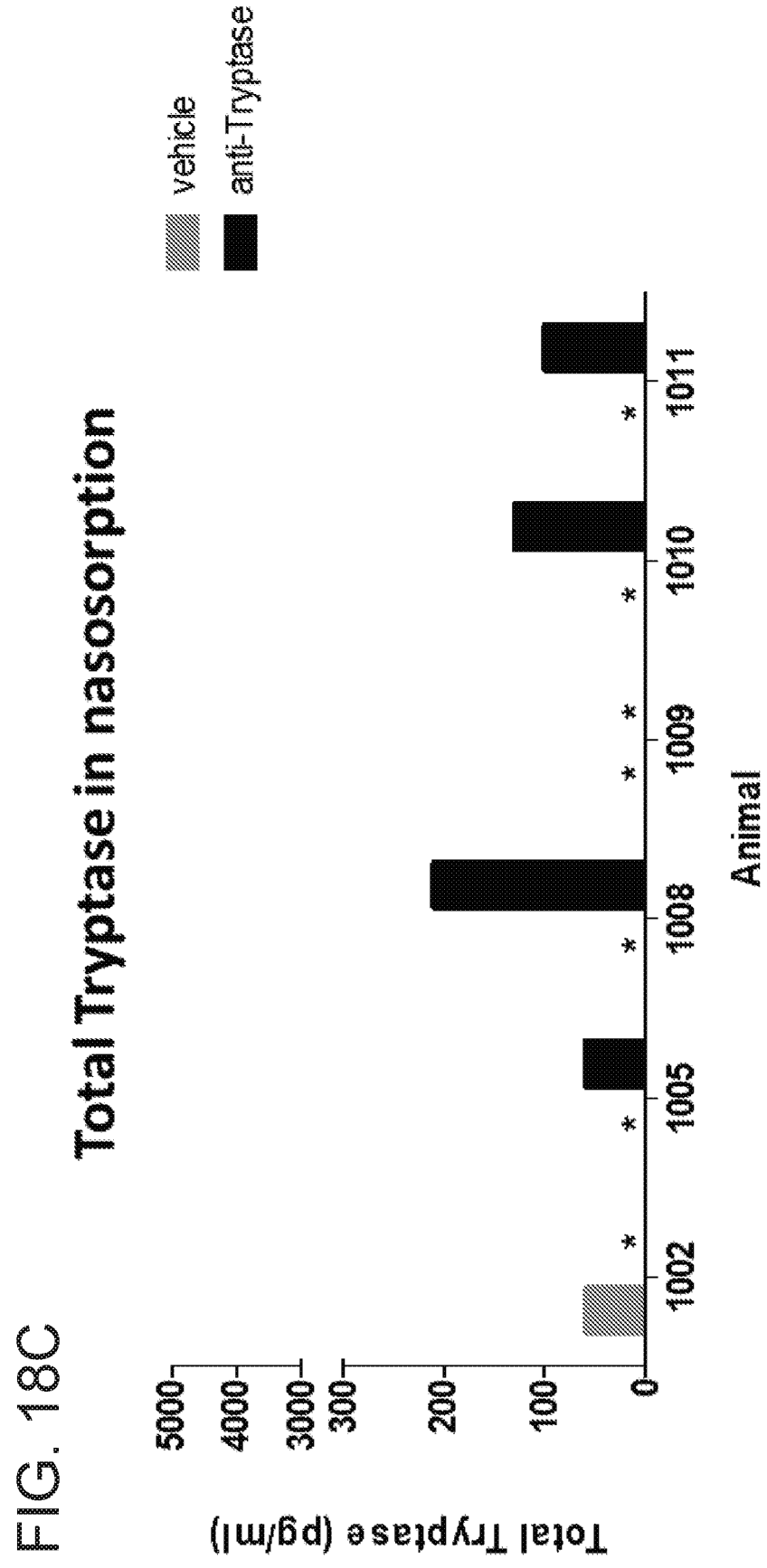
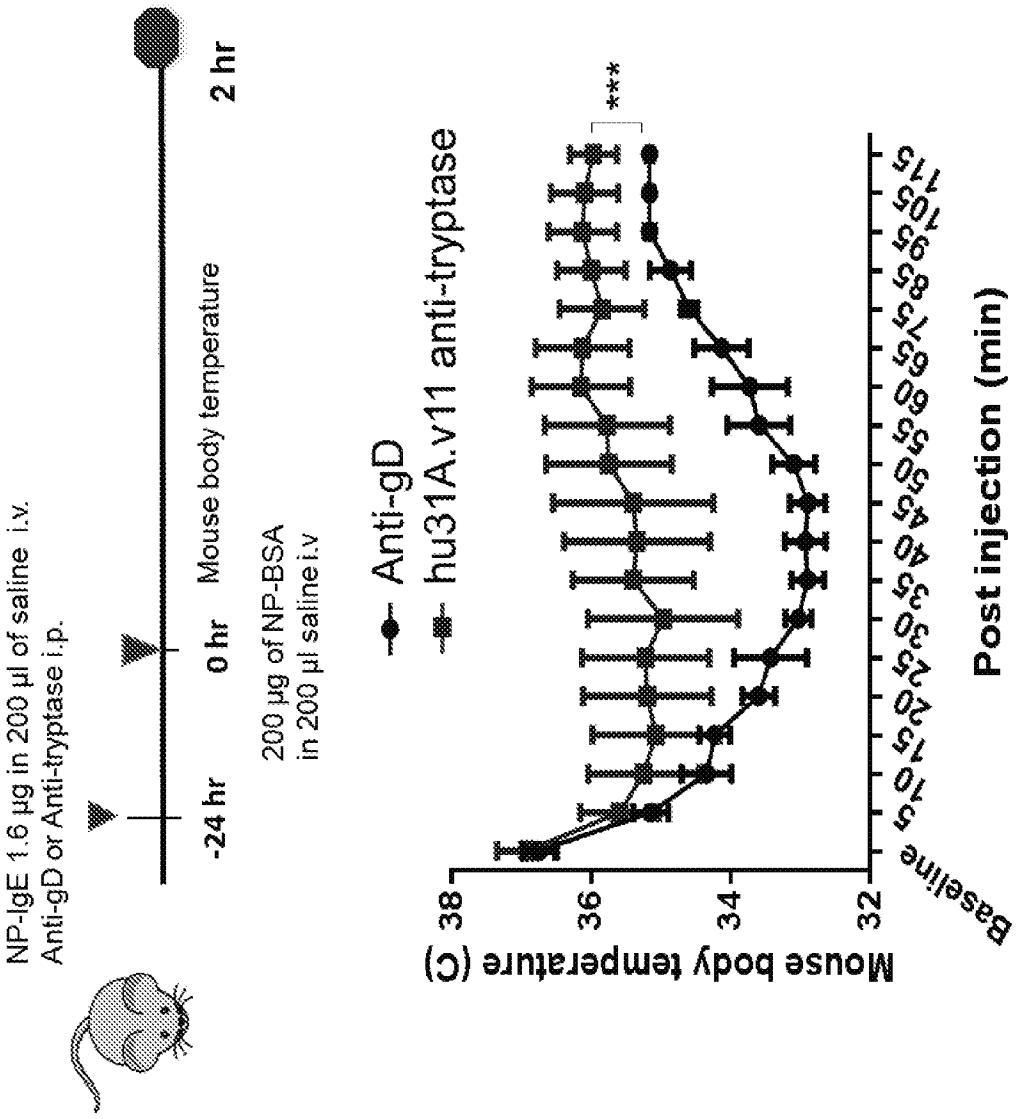

Total Tryptase in nasosorption
vehicle
anti-Tryptase
Total Tryptase (pg/ml)
5000
4000
3000
300
200
100
0
1002
1005
1008
1009
1010
1011
Animal
*

FIG. 19


NP-IgE 1.6 µg in 200 µl of saline i.v.
Anti-gD or Anti-tryptase i.p.
-24 hr
0 hr
Mouse body temperature
2 hr
200 µg of NP-BSA
in 200 µl saline i.v
Anti-gD
hu31A.v11 anti-tryptase
***
Mouse body temperature (C)
38
36
34
32
Baseline
5
10
15
20
25
30
35
40
45
50
55
60
65
75
85
95
105
115
Post injection (min)