FIG. 1A

Antibody	Back-mutations VH / VL	SEQ ID NO H / L chains	Transient expression (mg/L)	FAB Tm (°C)	HPB-ALL staining relative to OKT3 chimera
Chimeric OKT3	N.A.	25/26	20	80.7	+++
VH/VL	- / -	27/39	50	88.1	-
VH/VL2	- / M4L	27/41	47	90.6	-
VH/VL3	- / M4L- F71Y	27/42	43	89.8	+
VH1/VL1	A49G / F71Y	28/40	22	90.1	-
VH1/VL2	A49G / M4L	28/41	42	90.5	-
VH1/VL3	A49G / M4L-F71Y	28/42	40	90.7	+
VH2/VL1	I34M-A49G / F71Y	29/40	51	89.5	-
VH2/VL2	I34M-A49G / M4L	29/41	43	90.1	-
VH2/VL3	I34M-A49G / M4L- F71Y	29/42	42.5	89.5	+
VH3/VL1	A49G-A71T / F71Y	30/40	33.5	89.7	-
VH3/VL2	A49G-A71T / M4L	30/41	42	90.4	-
VH3/VL3	A49G-A71T / M4L- F71Y	30/42	56.5	89.8	+

FIG. 1B

Antibody	Back-mutations	SEQ ID NO H / L chains	Transient expression (mg/l)	FAB Tm (°C)	HPB-ALL staining relative to OKT3 chimera
VH4/VL2	I34M-A49G-A71T / M4L	31/41	20	88.4	+ +
VH4/VL3	I34M-A49G-A71T / M4L-F71Y	31/42	34	88.7	+ +
VH5/VL2	I34M-A49G-I69L-A71T-T73K / M4L	32/41	40	87	+ +
VH5/VL3	I34M-A49G-I69L-A71T-T73K / M4L-F71Y	32/42	36	87.2	+ +
VH5/VL4	I34M-A49G-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	32/43	12	80.3	+++
VH5/VL6	I34M-A49G-I69L-A71T-T73K / M4L-L46R-L47W-F71Y-P96F	32/45	15.6	78.6	+++
VH5/VL7	I34M-A49G-I69L-A71T-T73K / M4L-V33M-A34N-F71Y-P96F	32/46	35.4	84.1	++
VH6/VL3	I34M-V48I-A49G-I69L-A71T-T73K / M4L-F71Y	33/42	23	88.3	++
VH6/VL4	I34M-V48I-A49G-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	33/43	14	80.8	+++
VH6/VL5	I34M-V48I-A49G-I69L-A71T-T73K / M4L-V33M-A34N-F71Y	33/44	26	86.1	++
VH6/VL6	I34M-V48I-A49G-I69L-A71T-T73K / M4L-L46R-L47W-F71Y-P96F	33/45	14.8	79.1	+++
VH6/VL7	I34M-V48I-A49G-I69L-A71T-T73K / M4L-V33M-A34N-F71Y-P96F	33/46	32	85.3	++

FIG. 1C

Antibody	Back-mutations	SEQ ID NO H / L chains	Transient expression (mg/l)	FAB Tm (°C)	HPB-ALL staining relative to OKT3 chimera
VH6/VL8	I34M-V48I-A49G-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	33/47	7	80.6	+++
VH7/VL3	I34M-A49G-R58N-I69L-A71T-T73K / M4L-F71Y	34/42	21	86.1	++
VH7/VL4	I34M-A49G-R58N-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	34/43	25	80.5	+++
VH7/VL5	I34M-A49G-R58N-I69L-A71T-T73K / M4L-V33M-A34N-F71Y	34/44	26	84.12	++
VH8/VL4	I34M-V48I-A49G-R58Y-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	35/43	7	80.9	+++
VH8/VL8	I34M-V48I-A49G-R58Y-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	35/47	23	83.5	+++
VH9/VL8	I34M-V48I-A49G-R58Y-G65S-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	36/47	13	82	+++
VH10/VL4	I34M-V48I-A49G-R58Y-G65S-F67A-I69L-A71T-T73K / M4L-L46R-L47W-F71Y	37/43	7	78.6	+++
VH10/VL8	I34M-V48I-A49G-R58Y-G65S-F67A-I69L-A71T-T73K / M4L-L46R-L47W-R66G-F71Y	37/47	10	80.4	+++
VH11/VL4	I34M-V48I-A49G-R58Y-I69L-A71T-T73K-N82aS / M4L-L46R-L47W-F71Y	38/43	8	80.3	+++
VH11/VL8	I34M-V48I-A49G-R58Y-I69L-A71T-T73K-N82aS / M4L-L46R-L47W-R66G-F71Y	38/47	15	82.3	+++

FIG. 1D

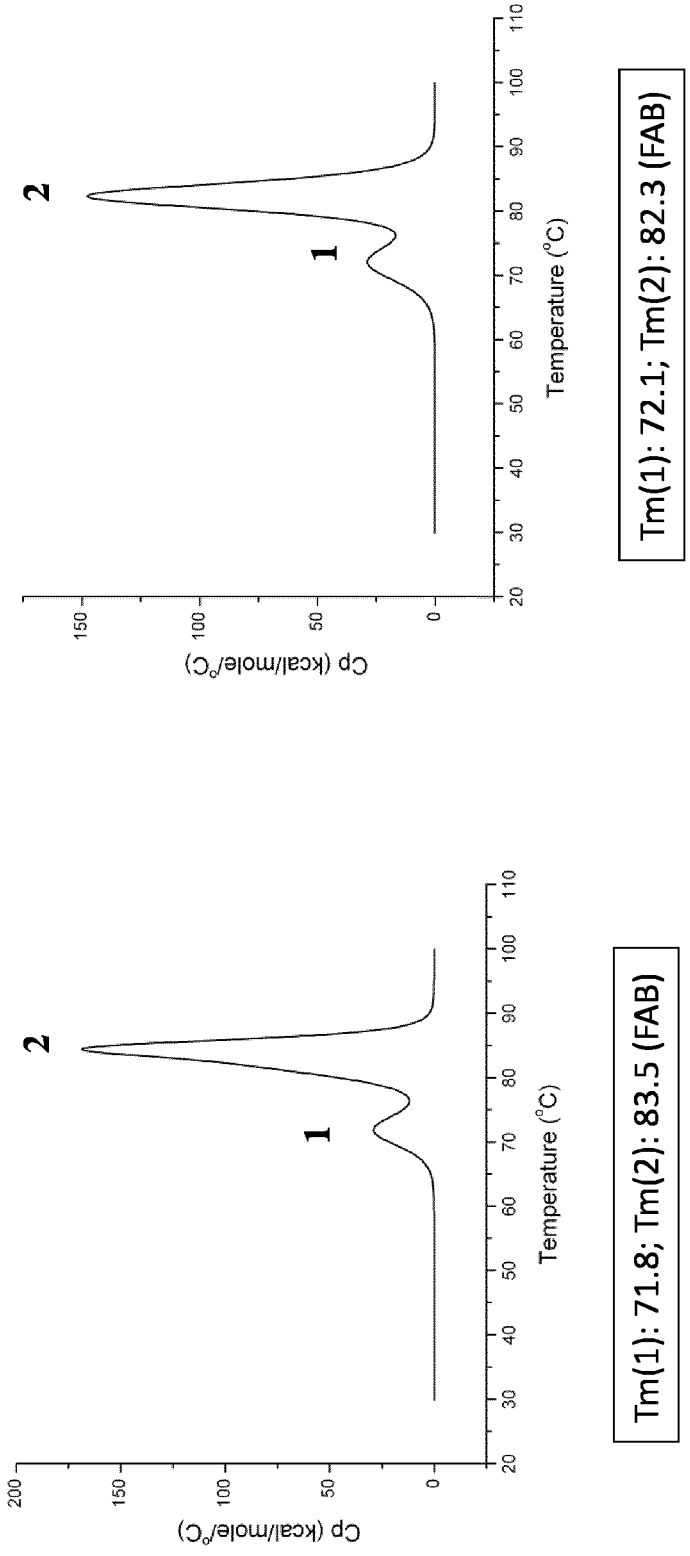

hOKT3 VH11/VL8 IgG1
2
1
Cp (kcal/mole/°C)
Temperature (°C)
Tm(1): 72.1; Tm(2): 82.3 (FAB)
hOKT3 VH8/VL8 IgG1
2
1
Cp (kcal/mole/°C)
Temperature (°C)
Tm(1): 71.8; Tm(2): 83.5 (FAB)

FIG. 1E

scFv-Fc fusion	SEQ ID NO	Transient expression (mg/L)	scFv Tm (°C)	HPB-ALL staining relative to OKT3 chimera
Mouse OKT3	52	0.35	-	++
VH5-VL3	53	21	71.2	+
VH6-VL4	54	29	65.3	++
VH6-VL5	55	27	71.2	++
VH8-VL4	56	28	66.4	+++
VH8-VL8	57	28	69.2	+++

FIG. 1F

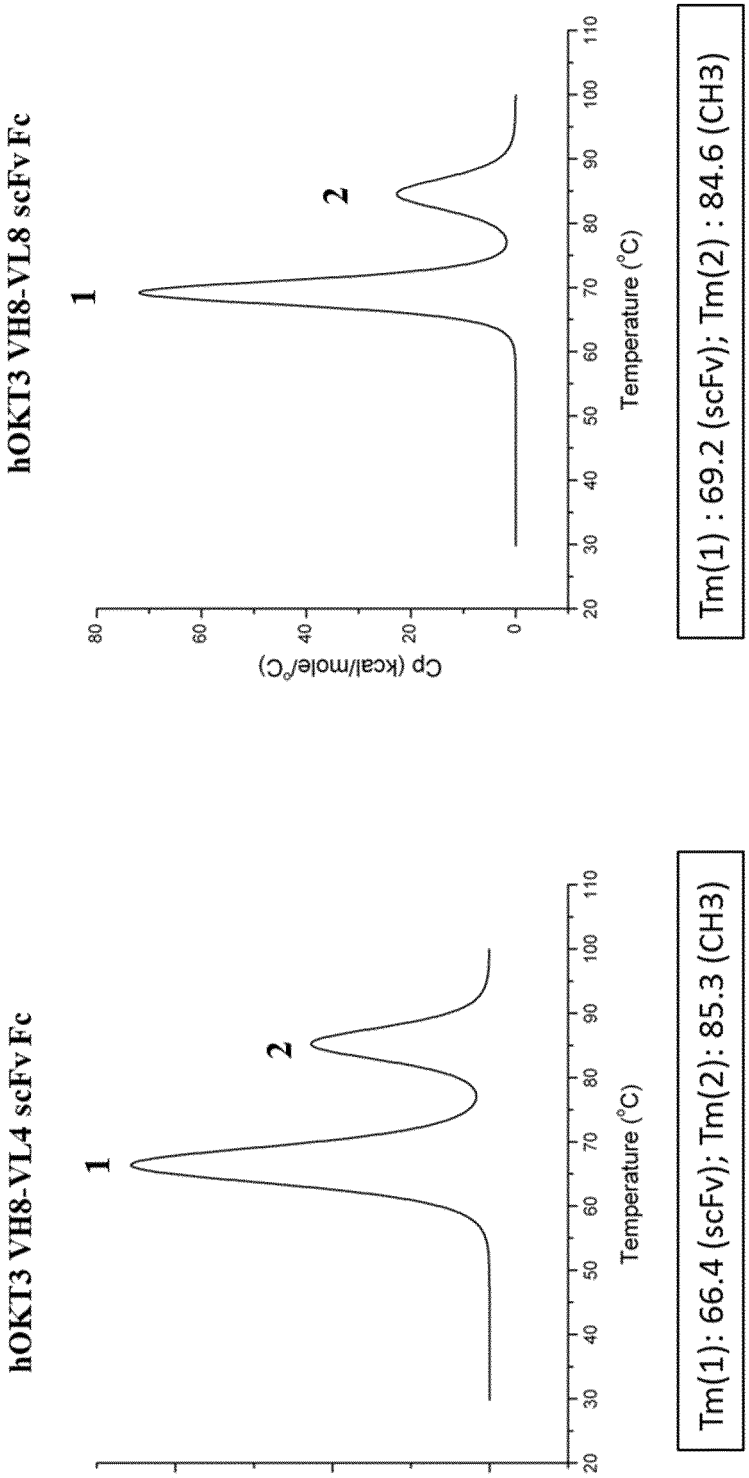

hOKT3 VH8-VL4 scFv Fc
1
2
Cp (kcal/mole/°C)
Temperature (°C)
Tm(1): 66.4 (scFv); Tm(2): 85.3 (CH3)
hOKT3 VH8-VL8 scFv Fc
1
2
Cp (kcal/mole/°C)
Temperature (°C)
Tm(1) : 69.2 (scFv); Tm(2) : 84.6 (CH3)

FIG. 2A

Antibody	Back-mutations VH/VL	SEQ ID NO H/L chains	Format	Transient expression (mg/L)	SPR-Binding to human/cynomolgus monkey CD3 epsilon 1-26_Fc proteins
Chimeric SP34	N.A.	62/63	IgG1	7	++++
VH1/VL1	-/-	64/69	IgG1	8	+
VH1/VL2	-/Q89A	64/70	IgG1	1	++++
VH1/VL3	-/deleted 8P	64/71	IgG1	0.5	-
VH1/VL4	-/deleted 8P-Q89A	64/72	IgG1	0.5	++++
VH1/VL5	-/A2I-Q89A	64/73	IgG1	2.6	++++
VH1/VL6	-/F44P-Q89A	64/74	IgG1	0.9	++++
VH1/VL7	-/A2I-F44P-Q89A	64/75	IgG1	No expression	Not tested
VH1/VL8	-/L66G-Q89A	64/76	IgG1	0.8	+
VH1/VL9	-/A2I-L66G-Q89A	64/77	IgG1	No expression	Not tested
VH1/VL10	-/F87Y-Q89A	64/78	IgG1	6	++++
VH1/VL11	-/L66G-D69T-Q89A	64/79	IgG1	0.8	+
VH1/VL12	-/D69T-Q89A	64/80	IgG1	1.5	++
VH1/VL13	-/S25A/Q89A	64/81	IgG1	1	+++
VH1/VL14	-/G46L-Q89A	64/82	IgG1	3	-
VH1/VL15	-/E38Q-Q89A	64/83	IgG1	3	++++
VH1/VL16	-/A2I-D69T-Q89A	64/84	IgG1	0.5	+
VH1/VL17	-/A2I-S25A-Q89A	64/85	IgG1	1	++
VH1/VL18	-/A2I-Q89A-Q100G	64/86	IgG1	1	++
VH1/VL19	-/A2I-D69T-F87Y-Q89A	64/87	IgG1	0.2	+
VH1/VL20	-/A2I-E38Q-D69T-F87Y-Q89A	64/88	IgG1	No expression	Not tested
VH1/VL21	-/A2I-F87Y-Q89A	64/89	IgG1	8	++++
VH1/VL22	-/A2I-E38Q-F87Y-Q89A	64/90	IgG1	2	+++

FIG. 2B

ScFv-Fc fusion	Back-mutations VH/VL	SEQ ID NO H/L chains	Format	Transient expression (mg/L)	SPR-Binding to human/cynomolgus monkey CD3 epsilon 1-26_Fc proteins
VH2/VL21	G65S /A2I -F87Y-Q89A	91	scFv-Fc	5	+++
VH3/VL23	G65S-W100eY /A2I-F87Y-Q89A-W91F	92	scFv-Fc	10	++
VH4/VL23	G65S-W100eF /A2I-F87Y-Q89A-W91F	93	scFv-Fc	5.5	++++
VH5/VL23	W100eY/A2I-F87Y-Q89A-W91F	94	scFv-Fc	15	++++
VH1/VL24	-/A2I-T27A-G27aA- F87Y-Q89A	349	scFvFc	No expression	Not tested
VH1/VL25	-/A2I-V27cA-T28A- F87Y-Q89A	350	scFvFc	4	-
VH1/VL26	-/A2I-T29A-S30A- F87Y-Q89A	351	scFvFc	12	++++
VH1/VL27	-/A2I-N31A-Y32A- F87Y-Q89A	95	scFv-Fc	2	-
VH1/VL28	-/A2I-N52A-K53A- F87Y-Q89A	96	scFv-Fc	No expression	Not tested
VH1/VL29	-/A2I-R54A-P56A- F87Y-Q89A	97	scFv-Fc	4	-
VH1/VL30	-/A2I-Y92A-S93A- F87Y-Q89A	98	scFv-Fc	2	+
VH1/VL31	-/A2I-N94A -F87Y-Q89A	99	scFv-Fc	2	++
VH5/VL32	W100eY/A2I-T29A-S30A-T51A-F87Y-Q89A-W91F	100	scFv-Fc	25	++++

FIG. 3

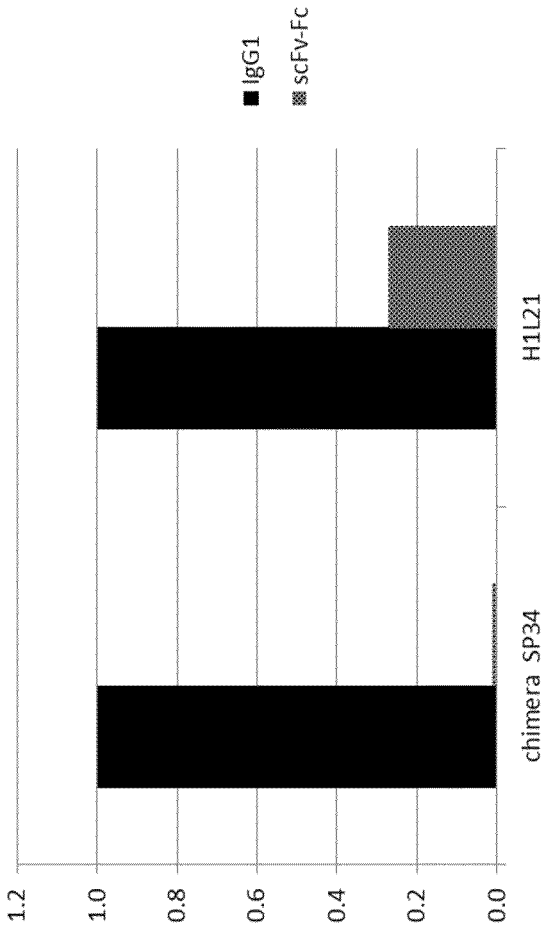


FIG. 4

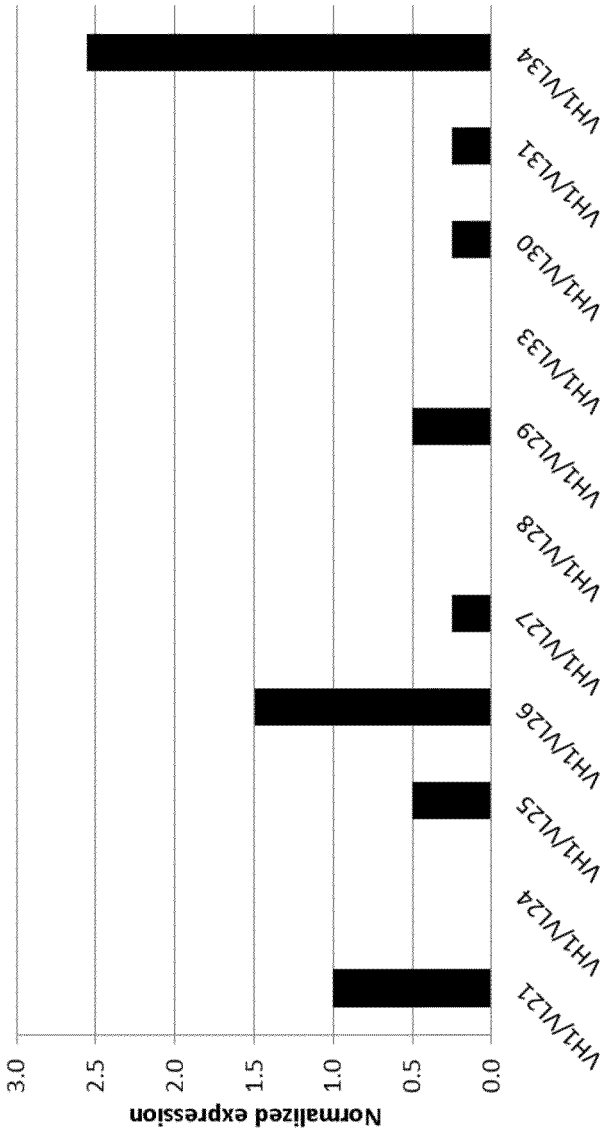

Normalized expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
VH1/VL34
VH1/VL31
VH1/VL30
VH1/VL33
VH1/VL29
VH1/VL28
VH1/VL27
VH1/VL26
VH1/VL25
VH1/VL24
VH1/VL23

FIG. 5

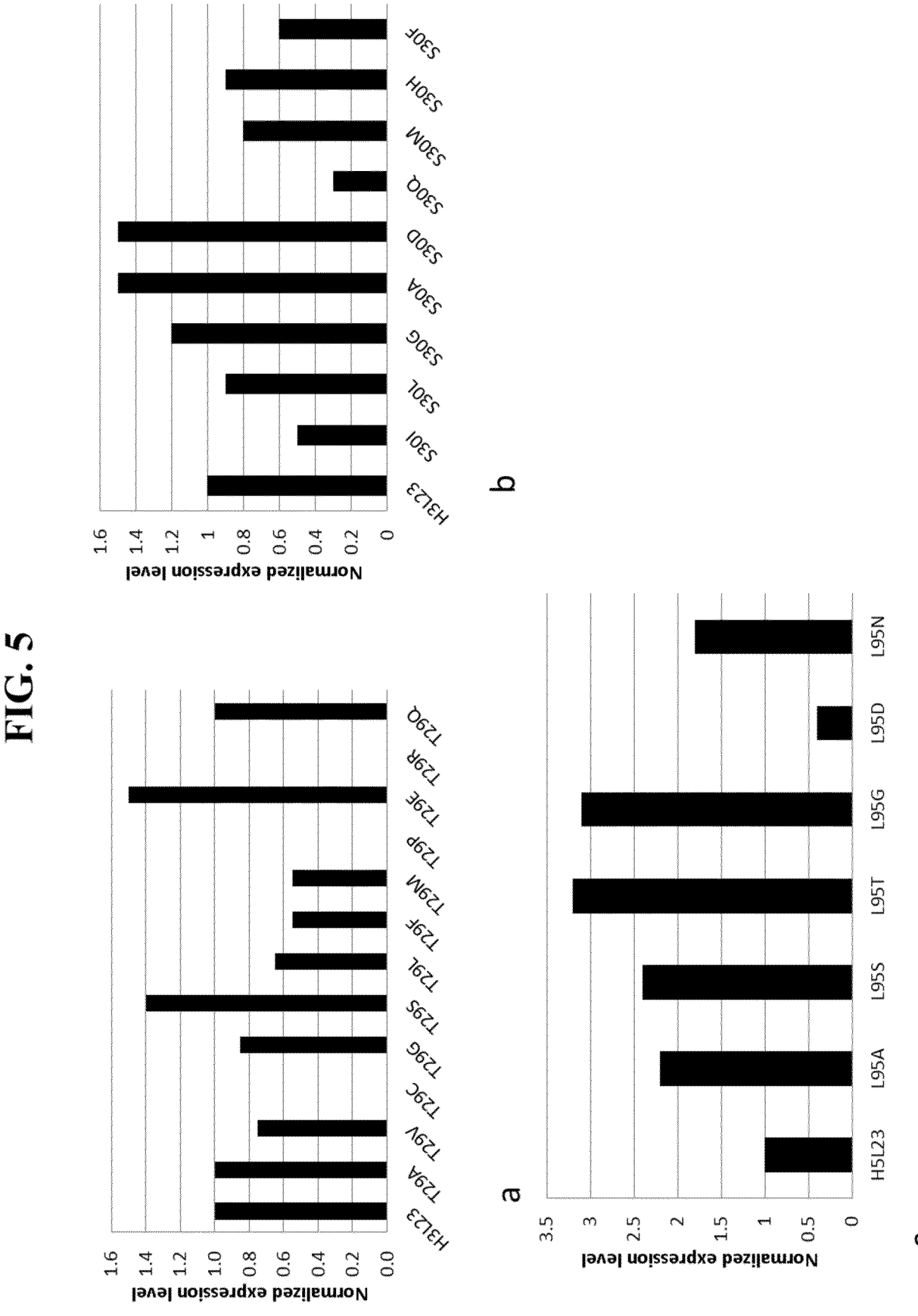

a
Normalized expression level
3.5
3
2.5
2
1.5
1
0.5
0
H5L23
L95A
L95S
L95T
L95G
L95D
L95N
b
Normalized expression level
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
H3L23
S30I
S30L
S30G
S30A
S30D
S30Q
S30M
S30H
S30F
c
Normalized expression level
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
H3L23
T29A
T29V
T29C
T29G
T29S
T29L
T29F
T29M
T29P
T29E
T29R
T29Q

FIG. 6

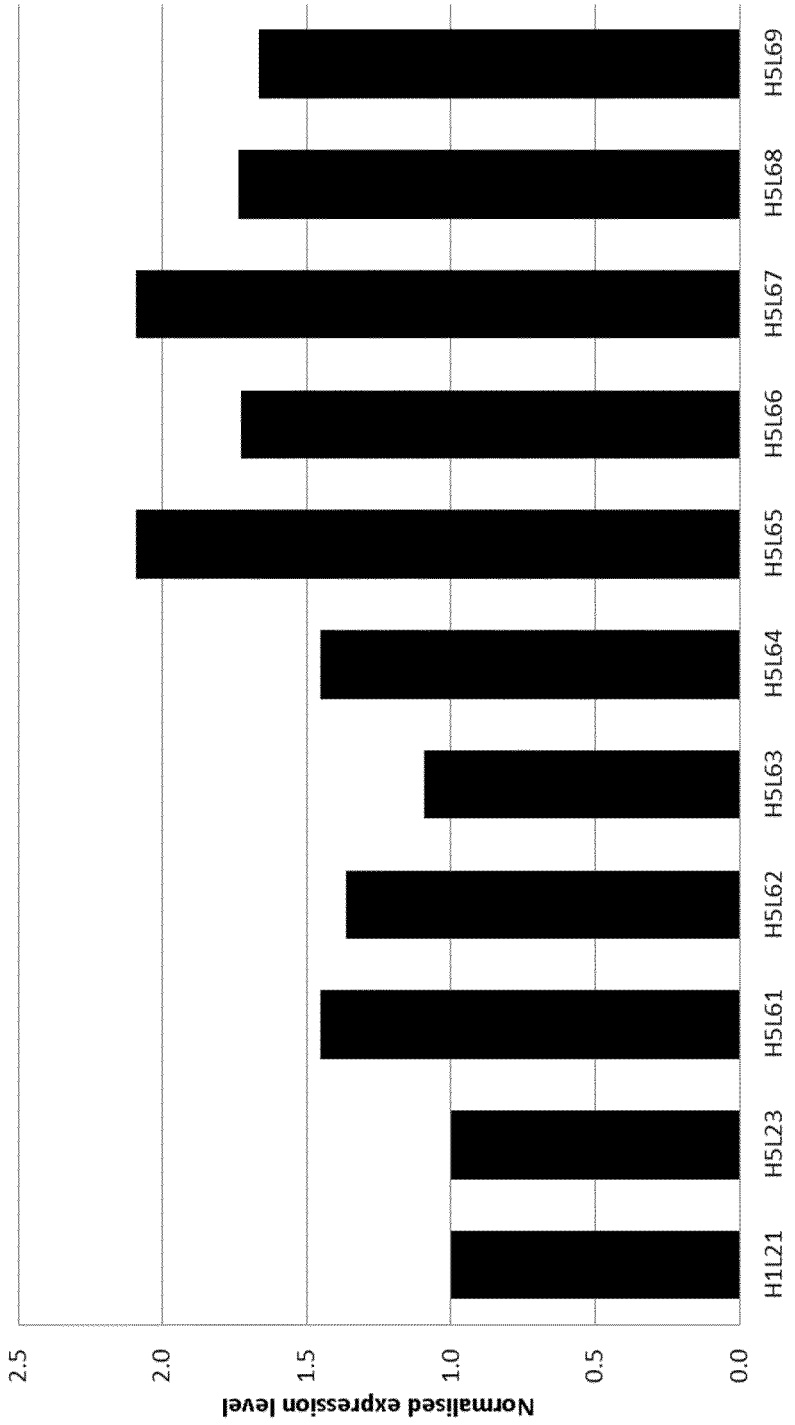

Normalised expression level
0.0
0.5
1.0
1.5
2.0
2.5
H1L21
H5L23
H5L61
H5L62
H5L63
H5L64
H5L65
H5L66
H5L67
H5L68
H5L69

FIG. 7A

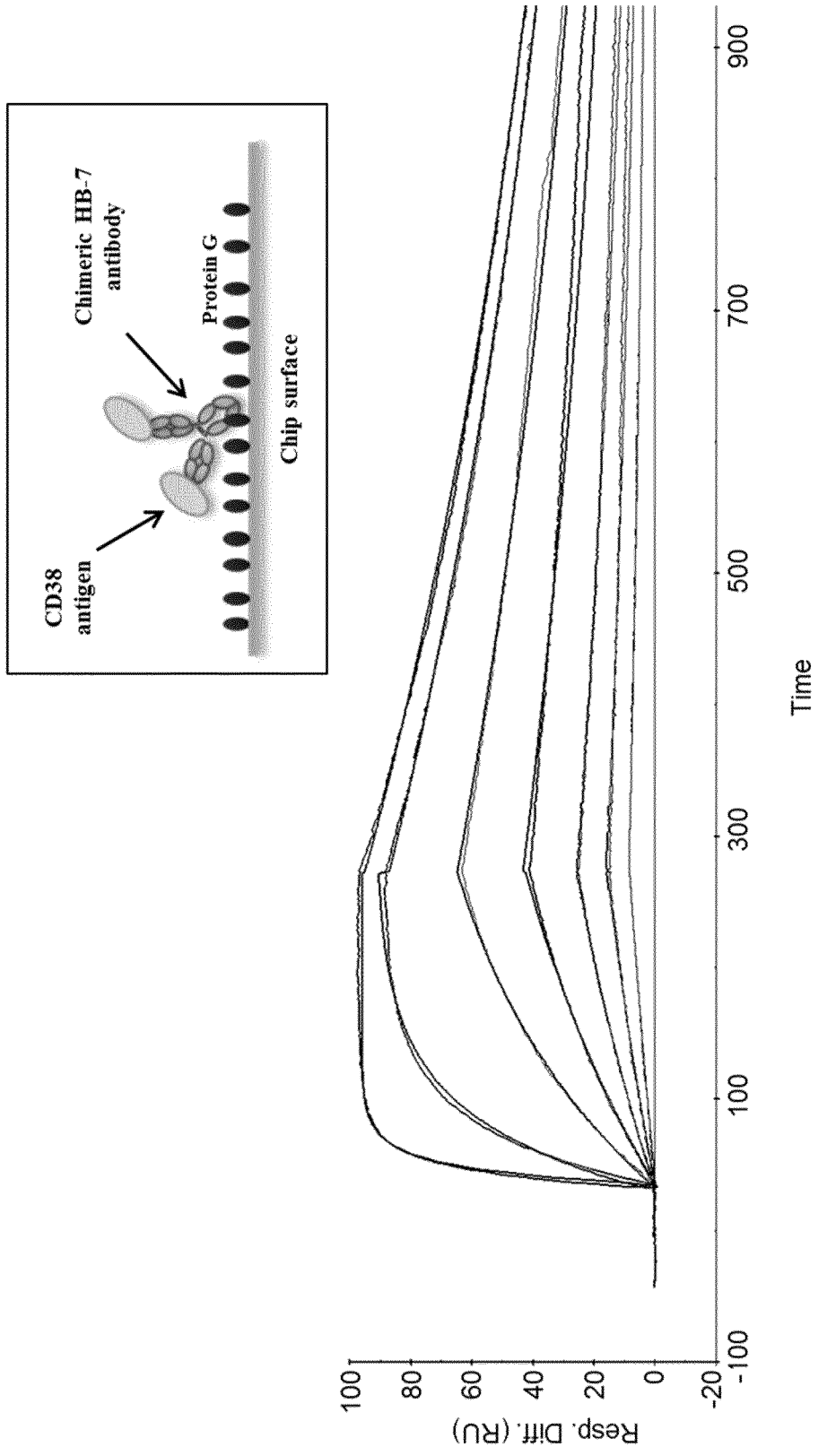

CD38 antigen
Chimeric HB-7 antibody
Protein G
Chip surface
Resp. Diff. (RU)
100
80
60
40
20
0
-20
-100
100
300
500
700
900
Time

FIG. 7B

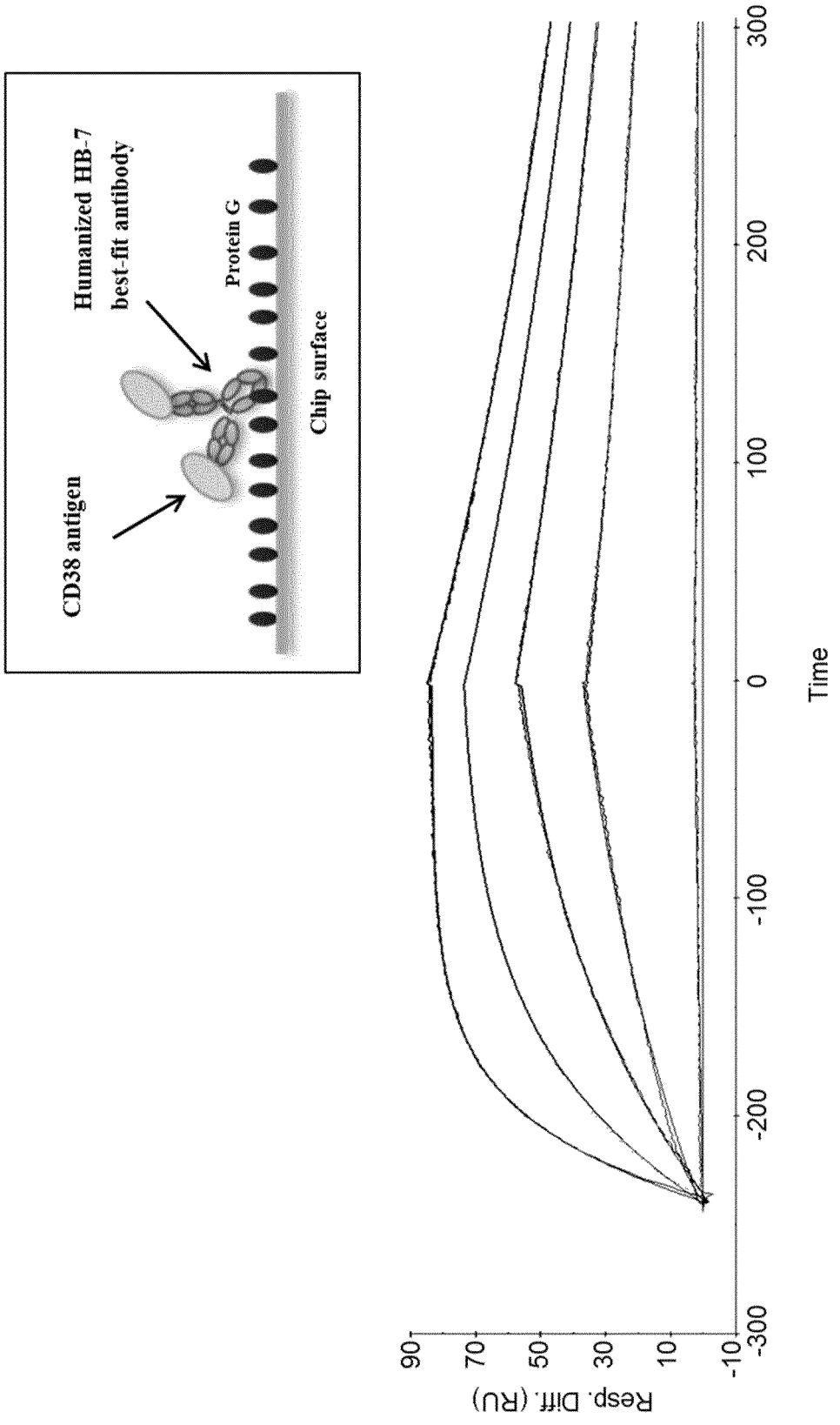

CD38 antigen
Humanized HB-7
best-fit antibody
Protein G
Chip surface
Resp. Diff. (RU)
90
70
50
30
10
-10
-300
-200
-100
0
100
200
300
Time

FIG. 7C

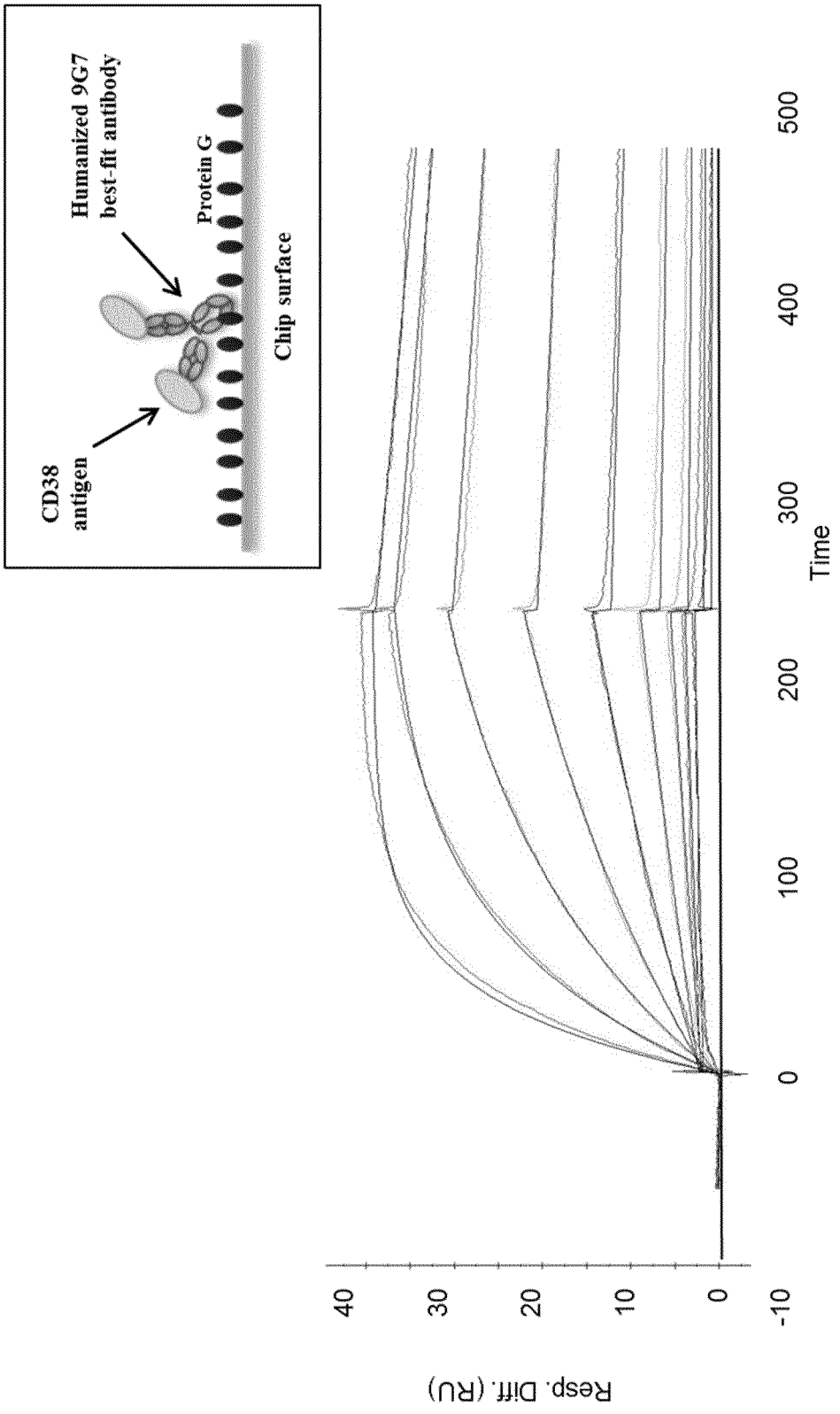

CD38 antigen
Humanized 9G7 best-fit antibody
Protein G
Chip surface
Resp. Diff. (RU)
40
30
20
10
0
-10
0
100
200
300
400
500
Time

FIG. 7D

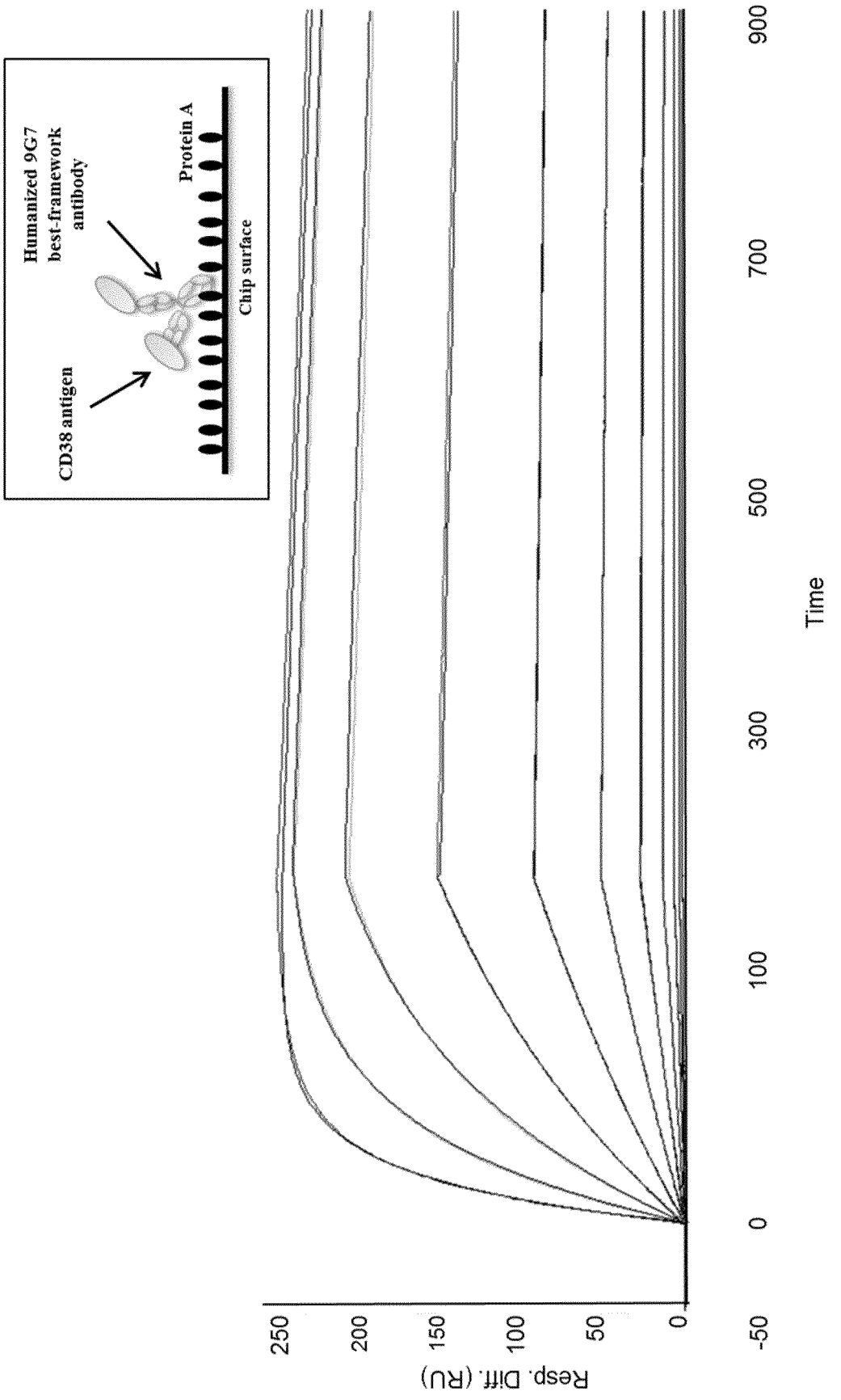
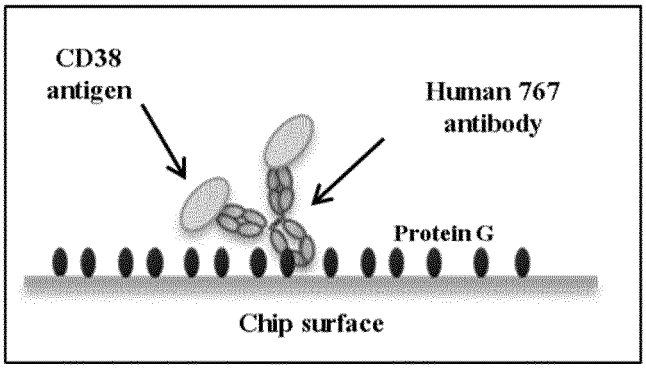

Humanized 9G7
best-framework
antibody
CD38 antigen
Protein A
Chip surface
Resp. Diff. (RU)
250
200
150
100
50
0
-50
0
100
300
500
700
900
Time

FIG. 7E


CD38
antigen
Human 767
antibody
Protein G
Chip surface

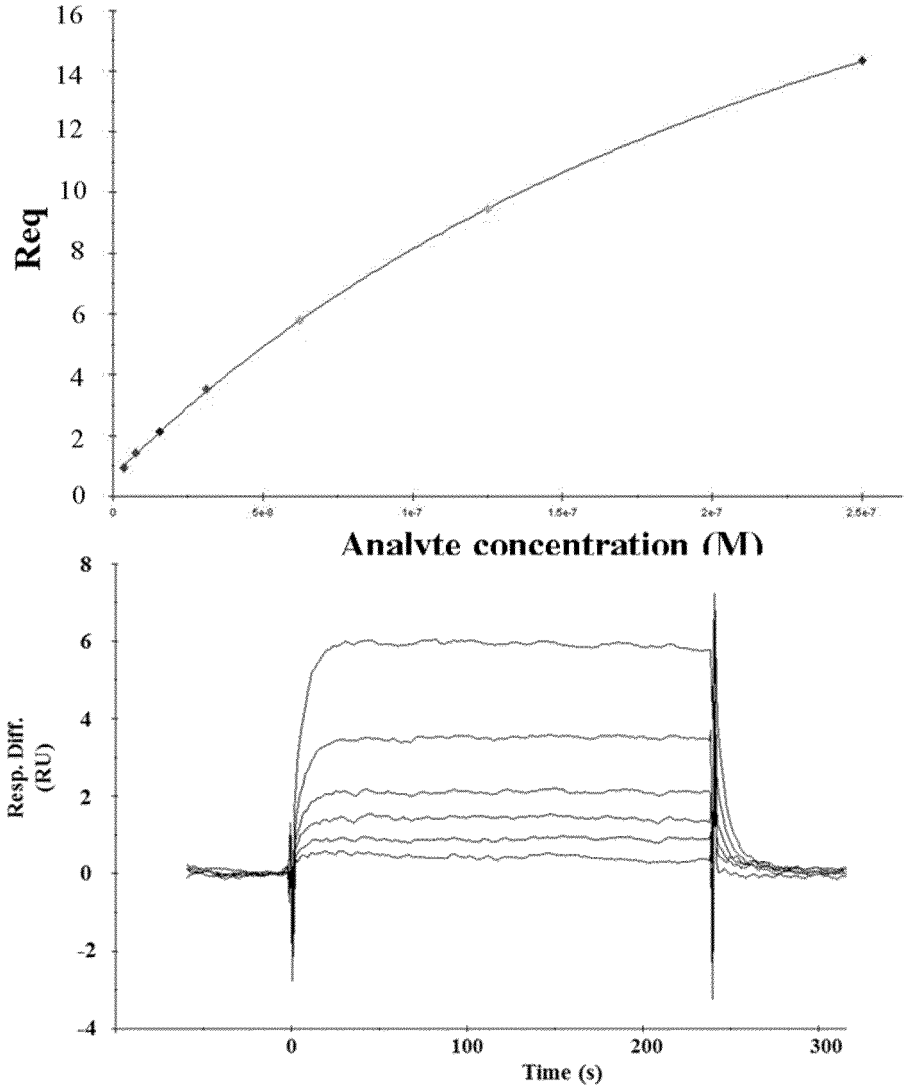

Req
16
14
12
10
8
6
4
2
0
0
5e-8
1e-7
1.5e-7
2e-7
2.5e-7
Analyte concentration (M)
Resp. Diff.
(RU)
8
6
4
2
0
-2
-4
0
100
200
300
Time (s)

FIG. 7F

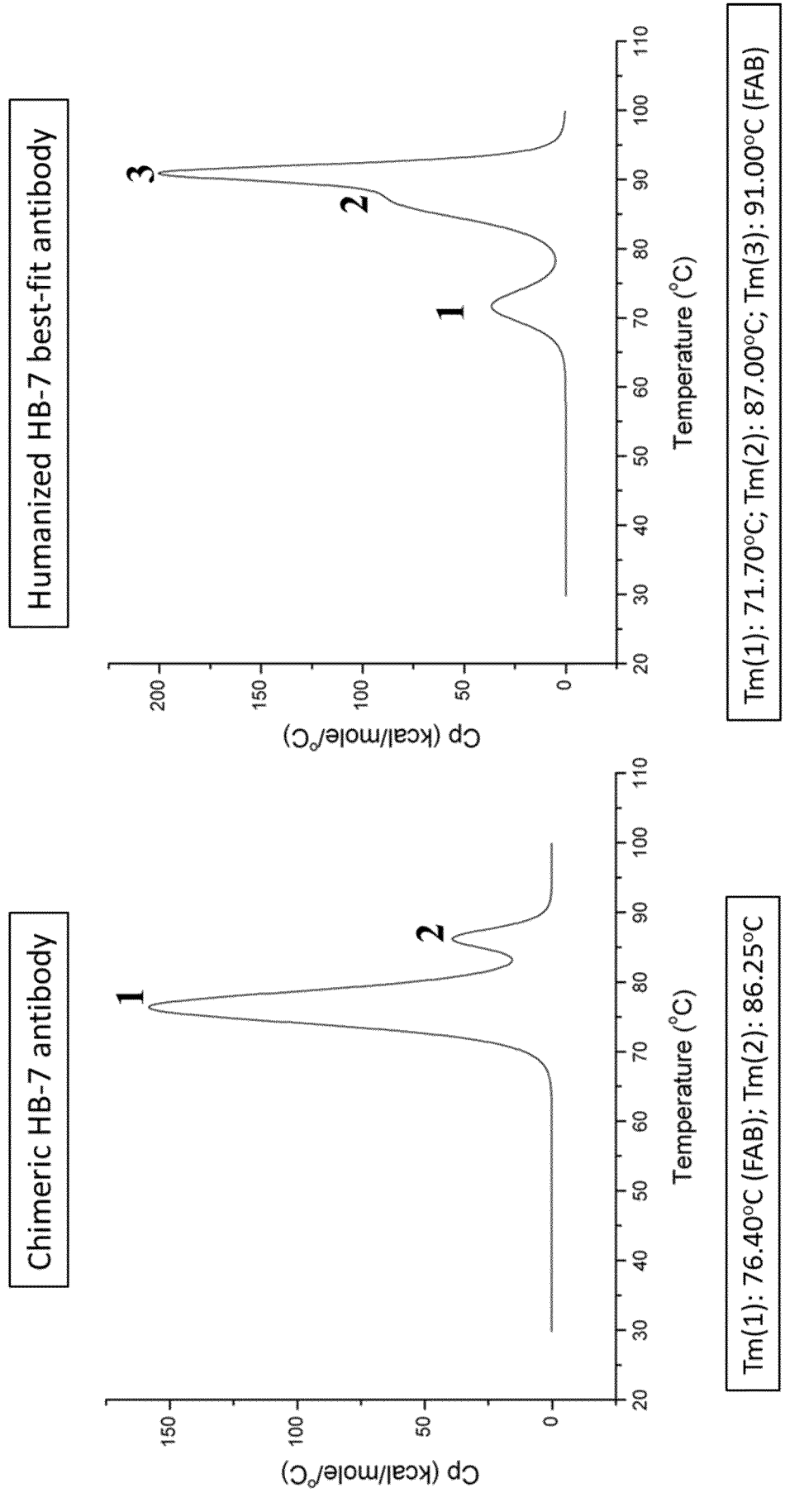

Chimeric HB-7 antibody
Humanized HB-7 best-fit antibody
Cp (kcal/mole/°C)
Temperature (°C)
1
2
3
Tm(1): 76.40°C (FAB); Tm(2): 86.25°C
Tm(1): 71.70°C; Tm(2): 87.00°C; Tm(3): 91.00°C (FAB)

FIG. 7G

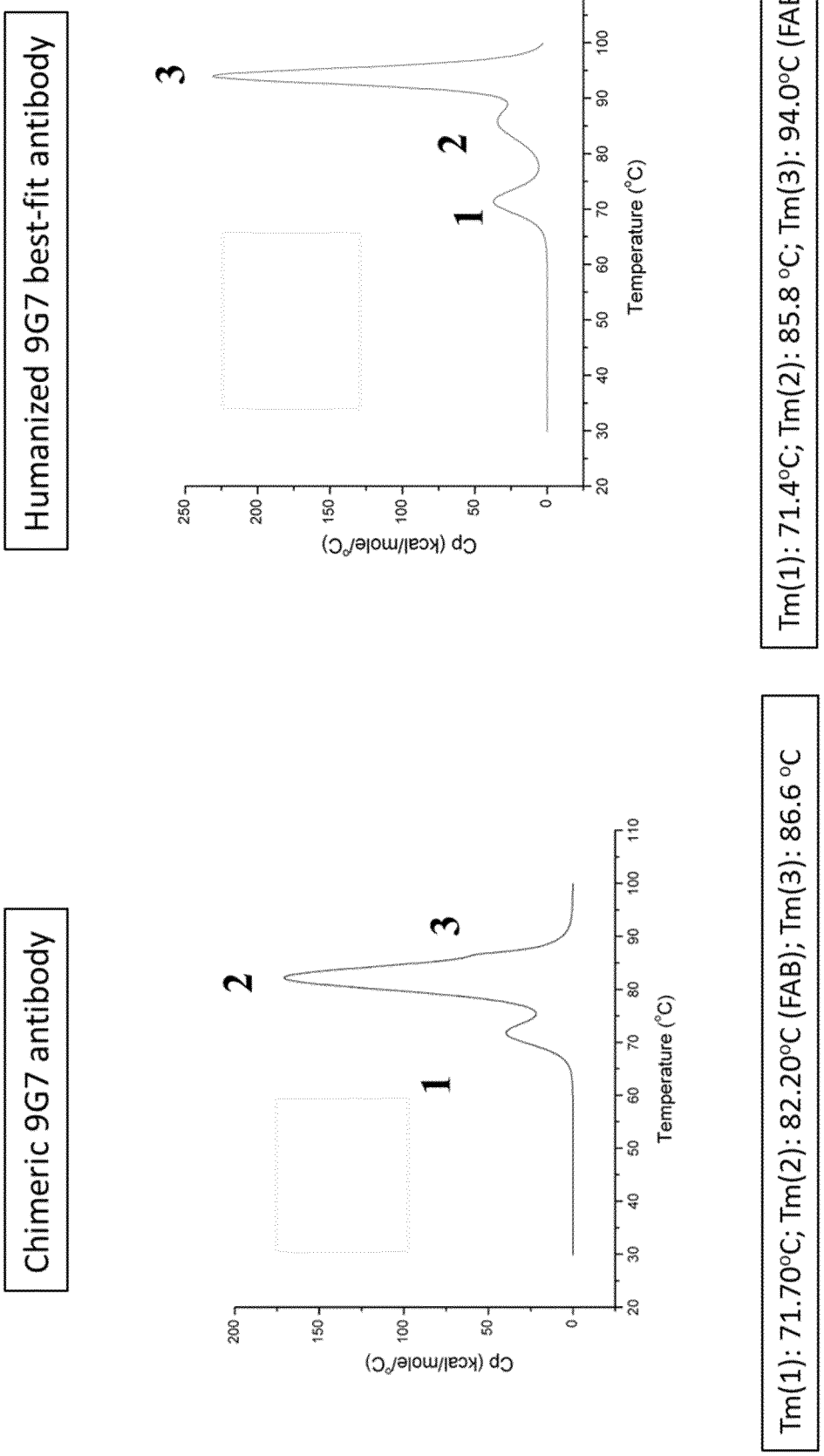

Humanized 9G7 best-fit antibody
Chimeric 9G7 antibody
Cp (kcal/mole/°C)
Temperature (°C)
1
2
3
Tm(1): 71.4°C; Tm(2): 85.8 °C; Tm(3): 94.0°C (FAB)
Tm(1): 71.70°C; Tm(2): 82.20°C (FAB); Tm(3): 86.6 °C

FIG. 7H

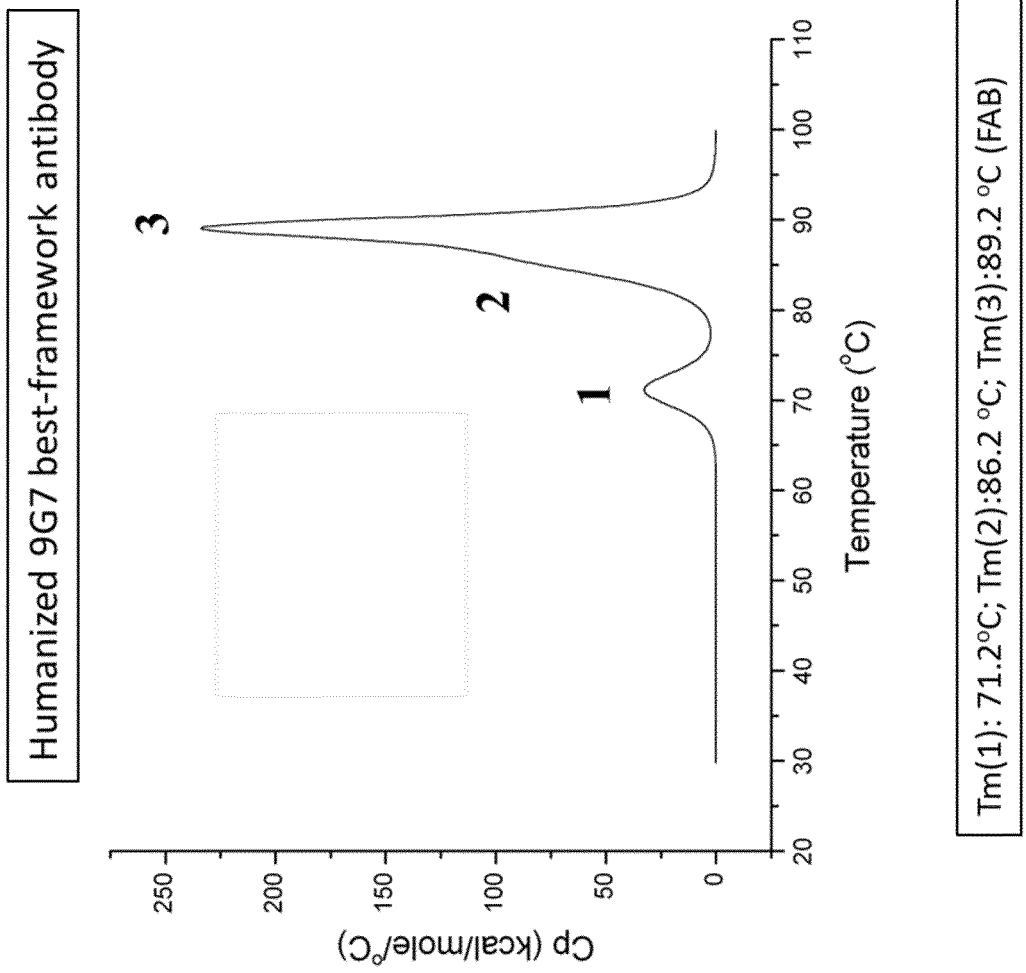

Humanized 9G7 best-framework antibody
Cp (kcal/mole/°C)
Temperature (°C)
1
2
3
Tm(1): 71.2°C; Tm(2):86.2 °C; Tm(3):89.2 °C (FAB)

FIG. 7I

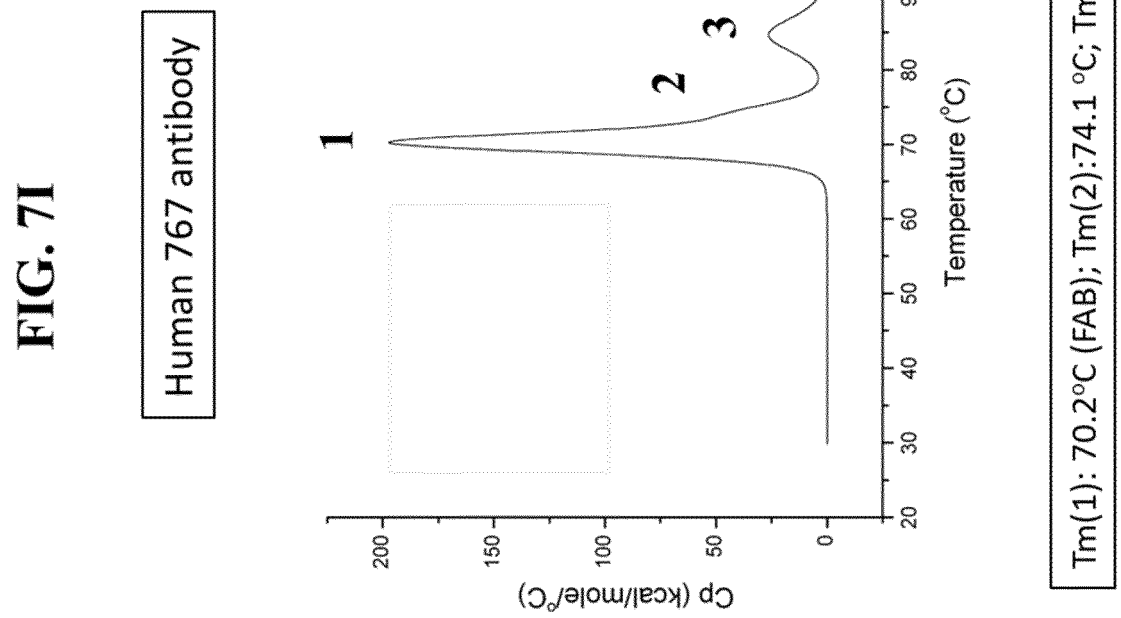

Human 767 antibody
1
2
3
Cp (kcal/mole/°C)
Temperature (°C)
0
50
100
150
200
20
30
40
50
60
70
80
90
100
110
Tm(1): 70.2°C (FAB); Tm(2):74.1 °C; Tm(3):84.7 °C

FIG. 7J

	Chimeric 9G7 antibody	Humanized 9G7 best-fit antibody	Humanized 9G7 best-framework antibody
HC/LC SEQ ID NO	**126/127**	**124/128**	**131/132**
Transient expression levels (mg/l)	**20**	**11**	**17**
KD human CD38 (nM)	**0.4**	**0.5**	**0.4**
KD cynomolgus monkey CD38 (nM)	**1**	**3.2**	**1**
FAB Tm (^{o}C)	**82.2**	**94**	**89.2**

FIG. 8

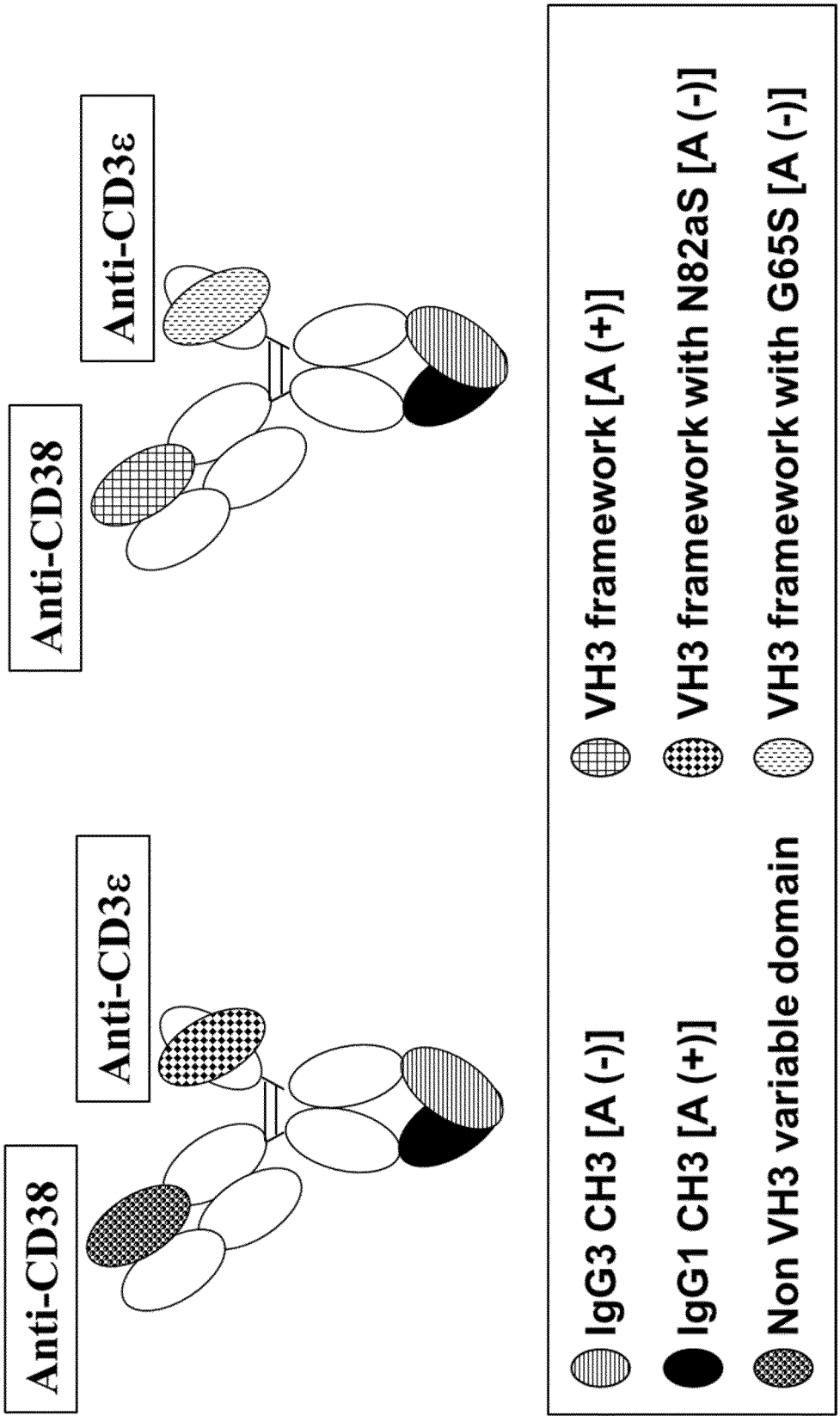

A
Anti-CD38
Anti-CD3ε
B
Anti-CD38
Anti-CD3ε
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
Non VH3 variable domain
VH3 framework [A (+)]
VH3 framework with N82aS [A (-)]
VH3 framework with G65S [A (-)]

FIG. 9A

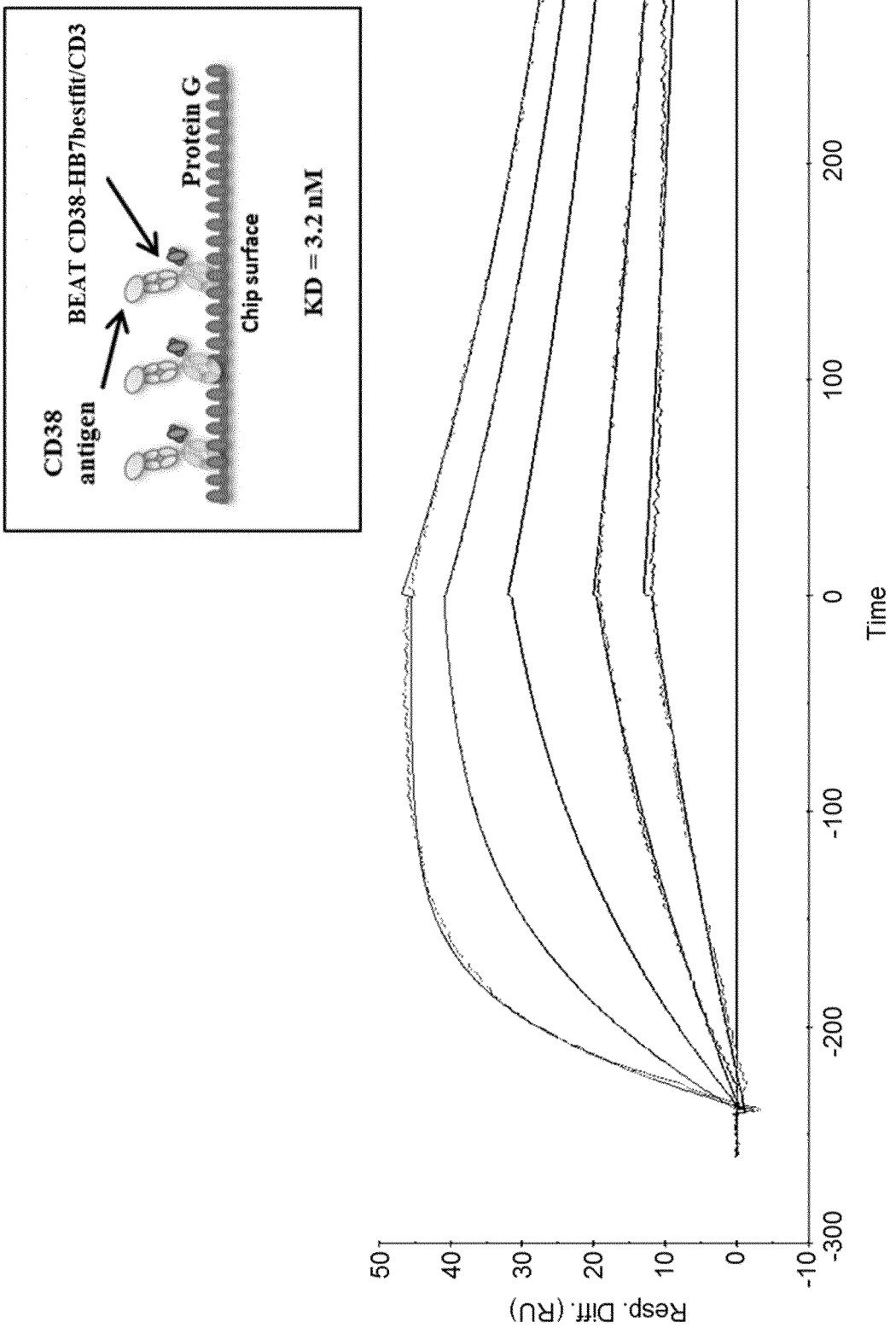

CD38 antigen
BEAT CD38-HB7bestfit/CD3
Protein G
Chip surface
KD = 3.2 nM
Resp. Diff. (RU)
50
40
30
20
10
0
-10
-300
-200
-100
0
100
200
Time

FIG. 9B

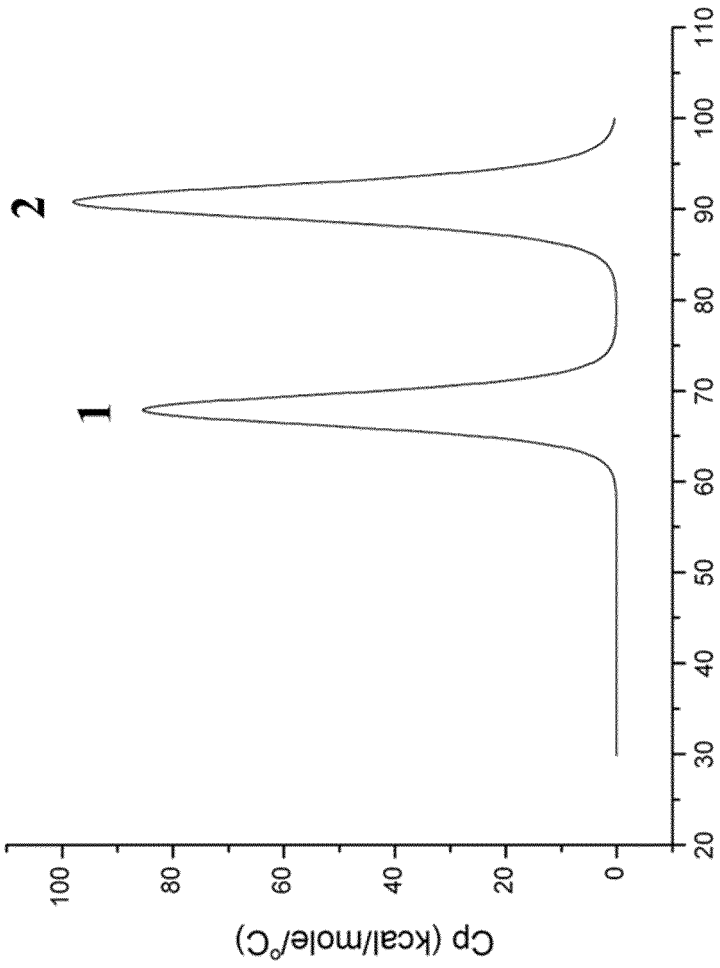

1
2
Cp (kcal/mole/°C)
Temperature (°C)
0
20
40
60
80
100
20
30
40
50
60
70
80
90
100
110
Tm(1): 67.9°C (scFv); Tm(2): 90.8°C (FAB)

FIG. 10

RPMI 8226

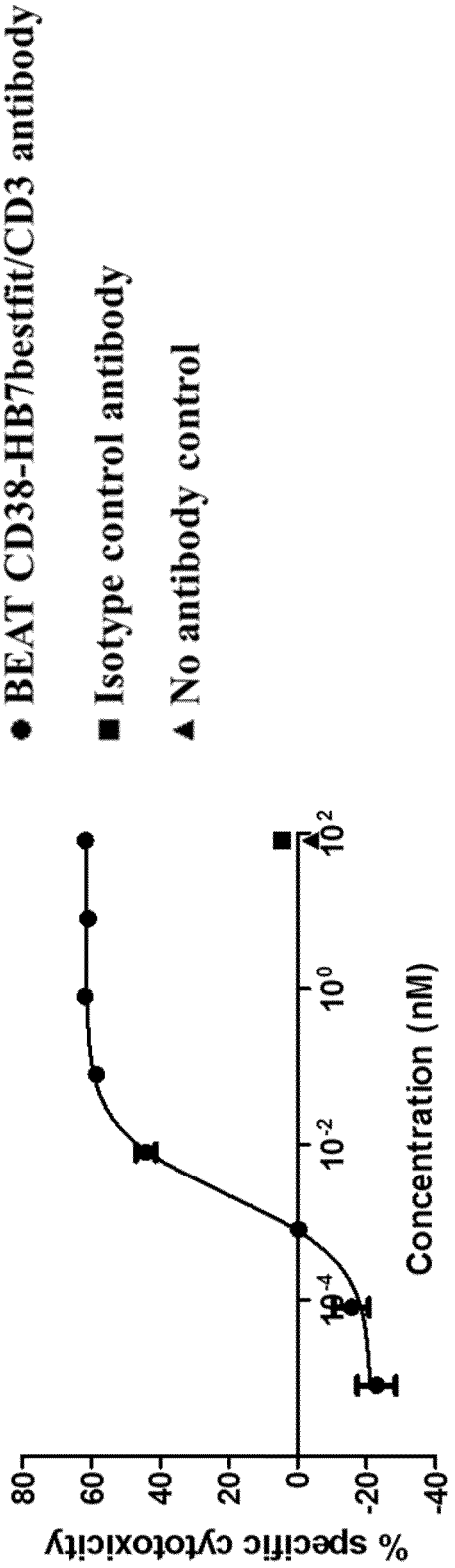


FIG. 11

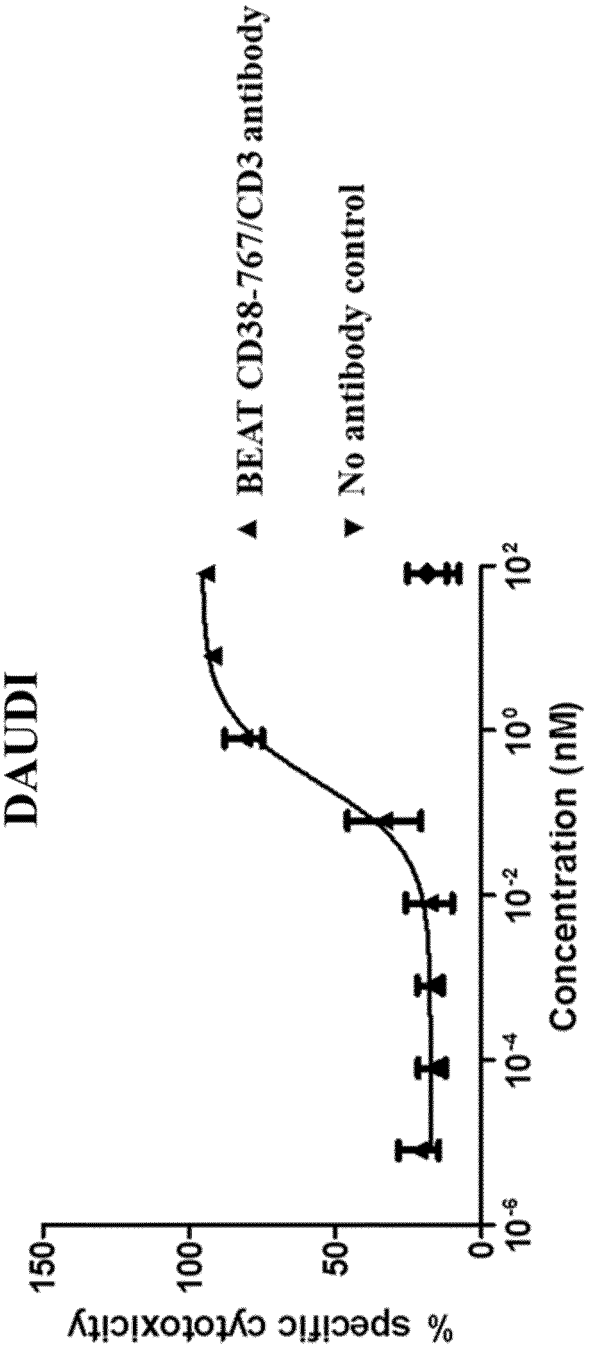

DAUDI
BEAT CD38-767/CD3 antibody
No antibody control
% specific cytotoxicity
150
100
50
0
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
Concentration (nM)

FIG. 12

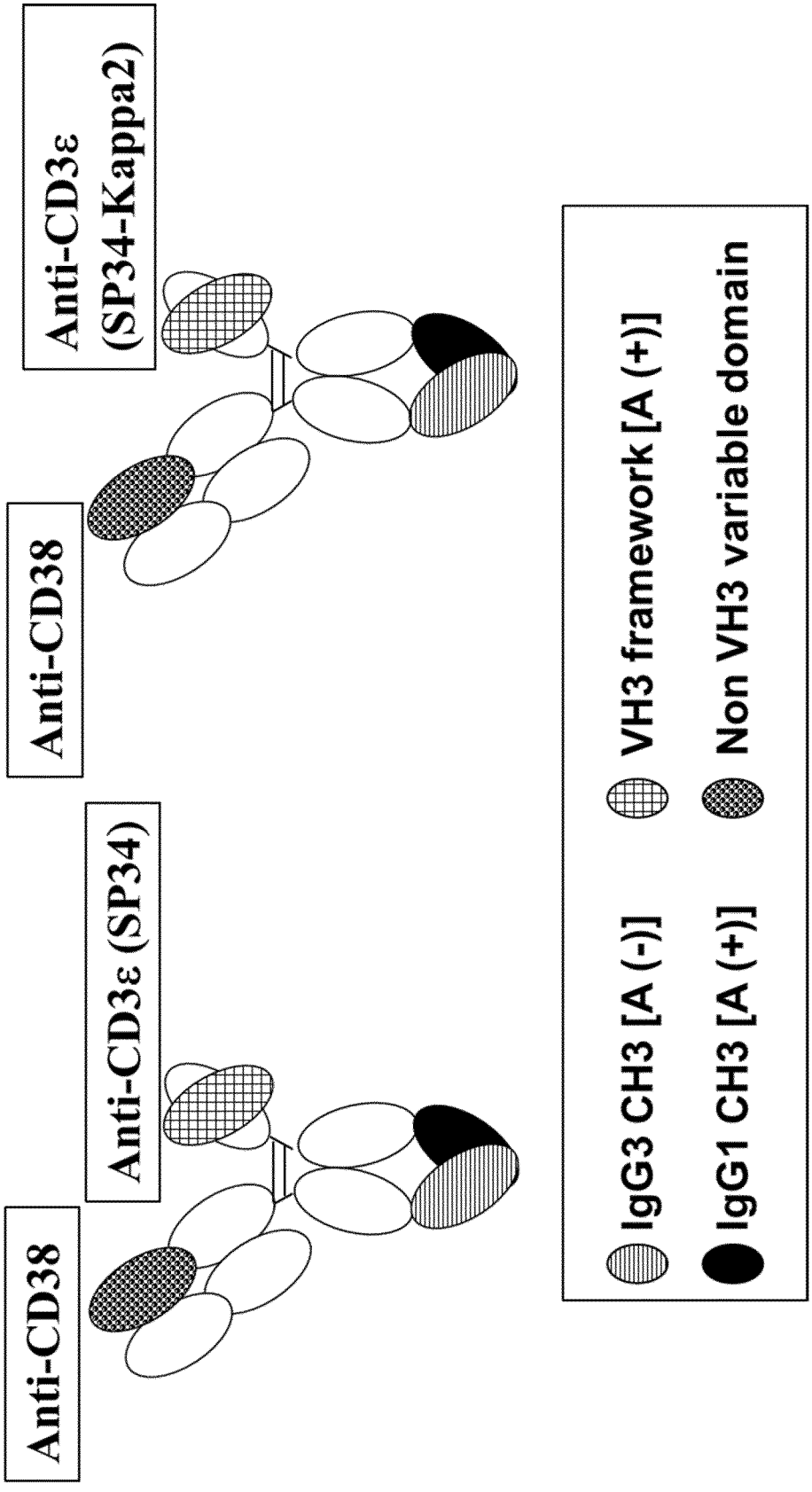

A
Anti-CD38
Anti-CD3ε (SP34)
B
Anti-CD38
Anti-CD3ε (SP34-Kappa2)
IgG3 CH3 [A (-)]
IgG1 CH3 [A (+)]
VH3 framework [A (+)]
Non VH3 variable domain

FIG. 13

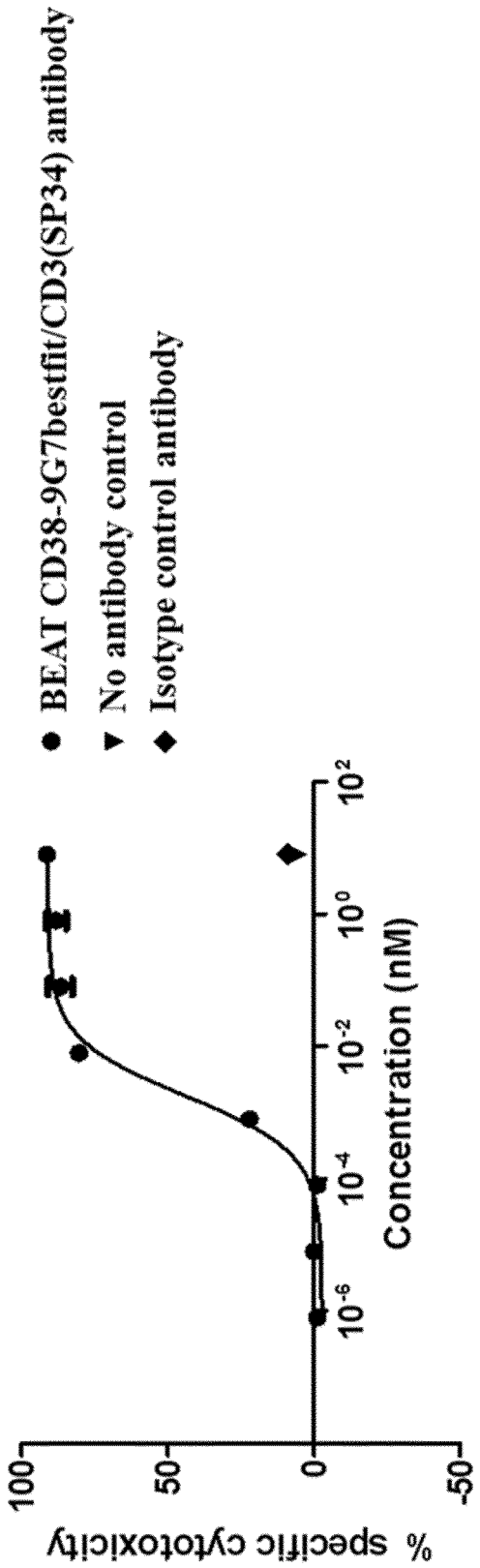

DAUDI
BEAT CD38-9G7bestfit/CD3(SP34) antibody
No antibody control
Isotype control antibody
% specific cytotoxicity
100
50
0
-50
10-6
10-4
10-2
100
102
Concentration (nM)

FIG. 13

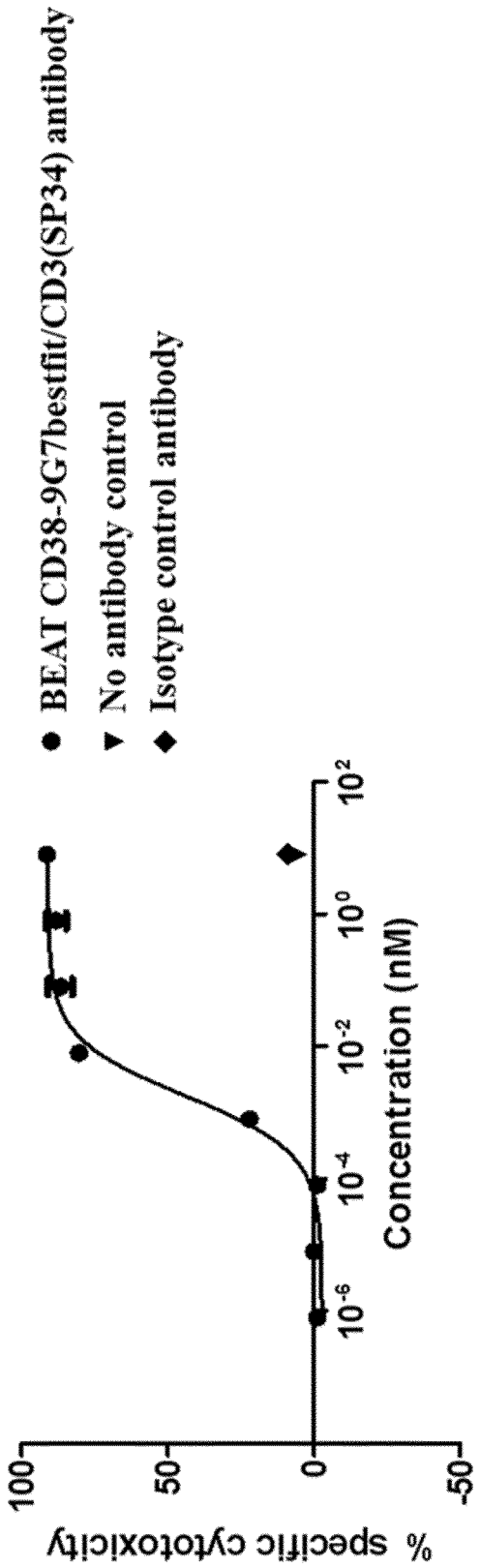

DAUDI
BEAT CD38-9G7bestfit/CD3(SP34) antibody
No antibody control
Isotype control antibody
% specific cytotoxicity
100
50
0
-50
10-6
10-4
10-2
100
102
Concentration (nM)

FIG. 14

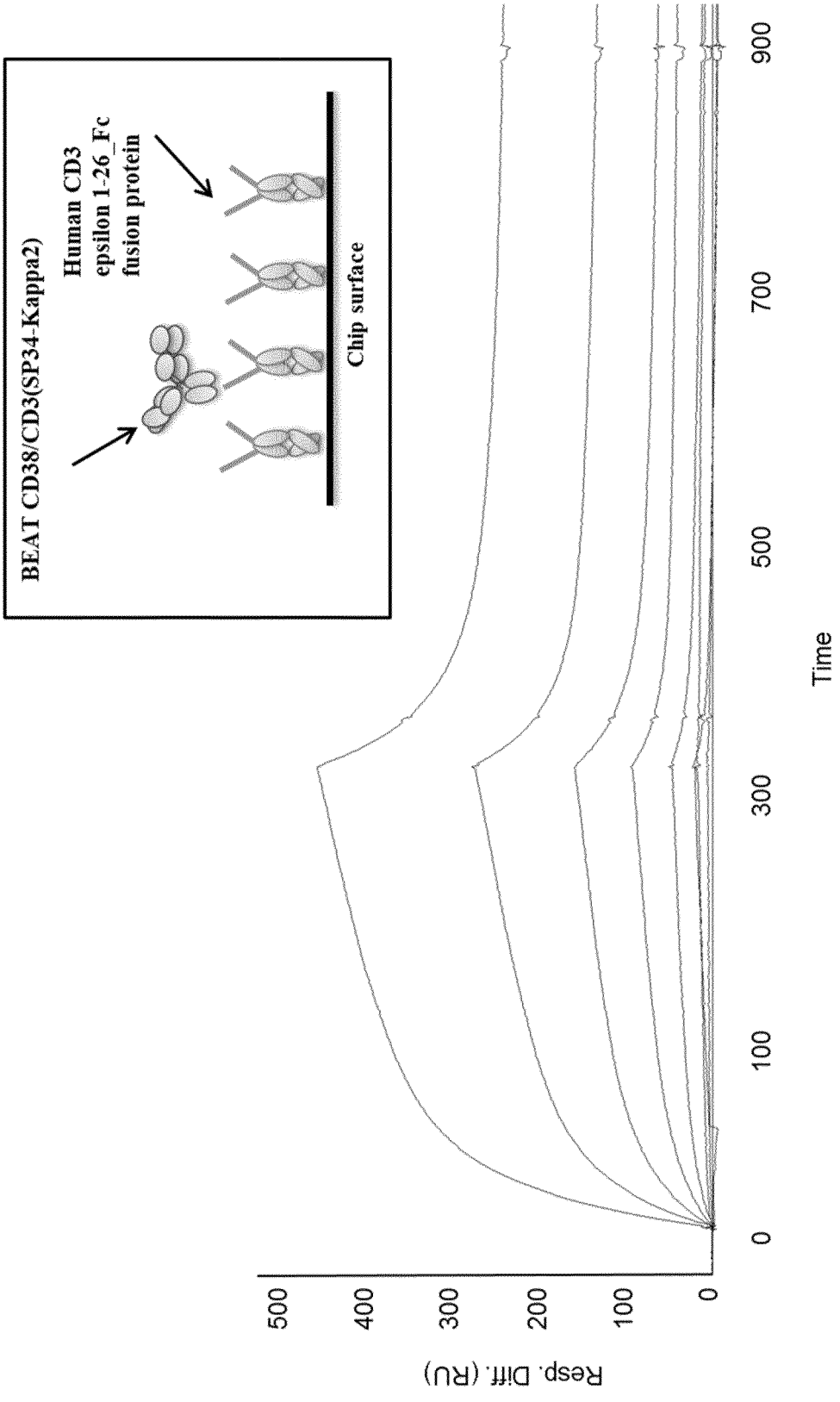

BEAT CD38/CD3(SP34-Kappa2)
Human CD3 epsilon 1-26_Fc fusion protein
Chip surface
Resp. Diff. (RU)
0
100
200
300
400
500
Time
0
100
300
500
700
900

FIG. 15

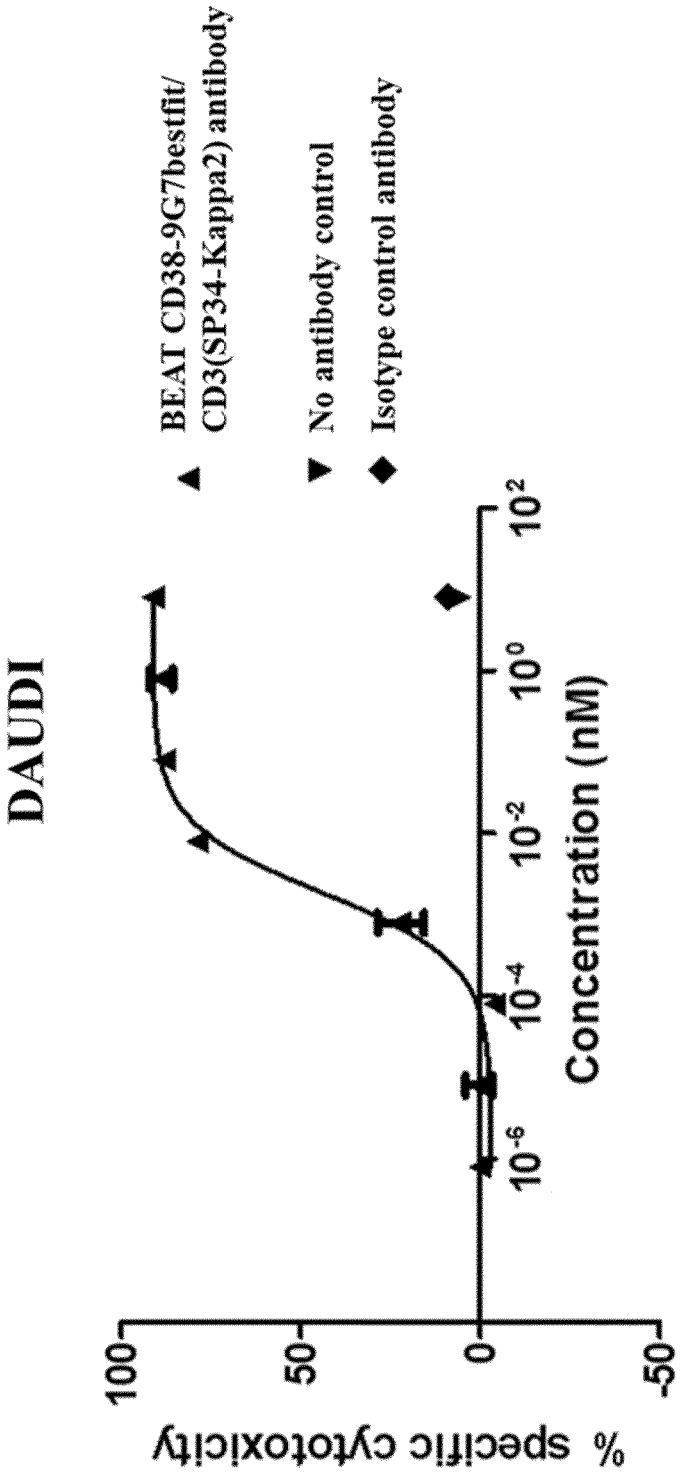
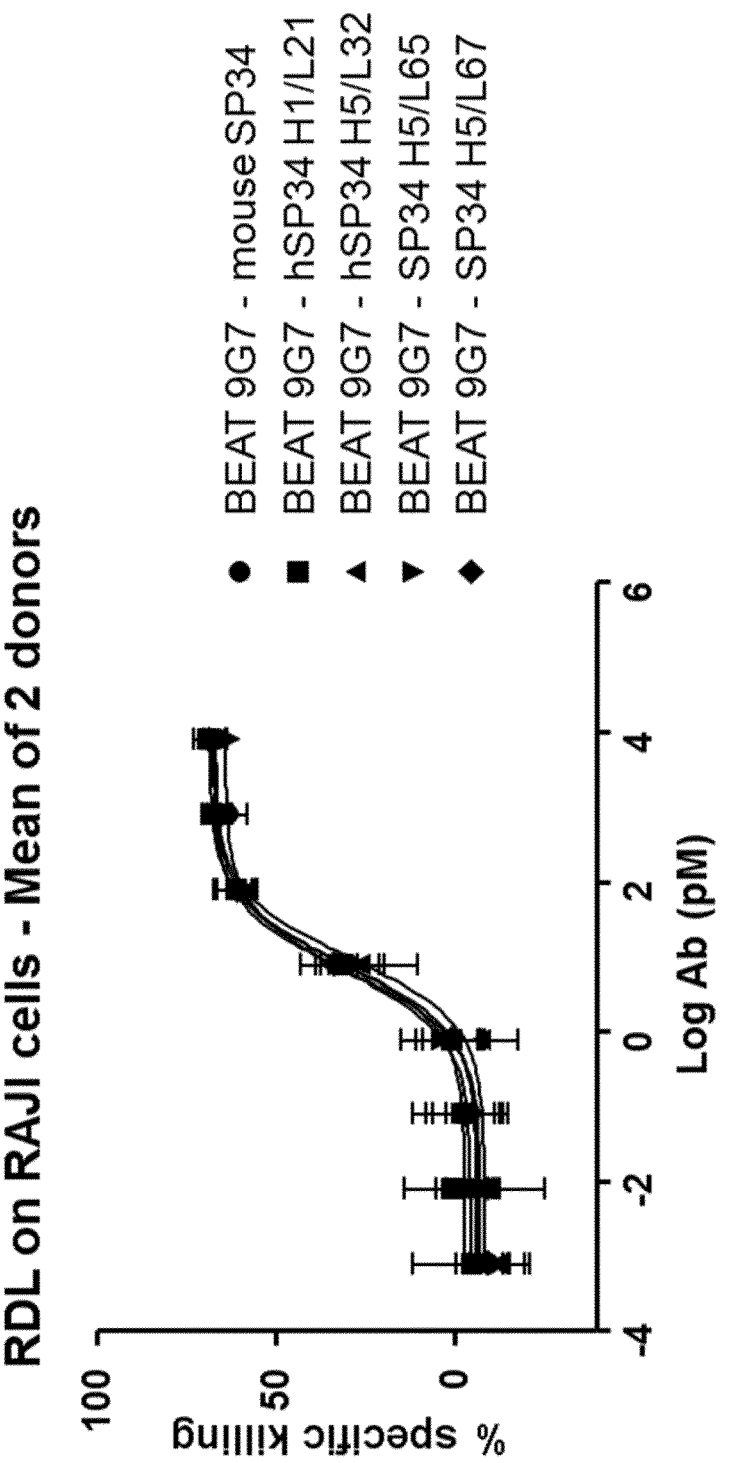

DAUDI
BEAT CD38-9G7bestfit/ CD3(SP34-Kappa2) antibody
No antibody control
Isotype control antibody
% specific cytotoxicity
100
50
0
-50
Concentration (nM)
10^-6
10^-4
10^-2
10^0
10^2

FIG. 16



	EC50 BEAT 9G7 - mouse SP34	EC50 BEAT 9G7 - hSP34 H1L21	EC50 BEAT 9G7 - hSP34 H5L32	EC50 BEAT 9G7 - hSP34 H5L65	EC50 BEAT 9G7 - hSP34 H5L67
Mean	7.883	8.807	10.09	6.170	6.493
Std. Deviation	2.879	2.070	5.098	1.185	1.526

FIG. 17

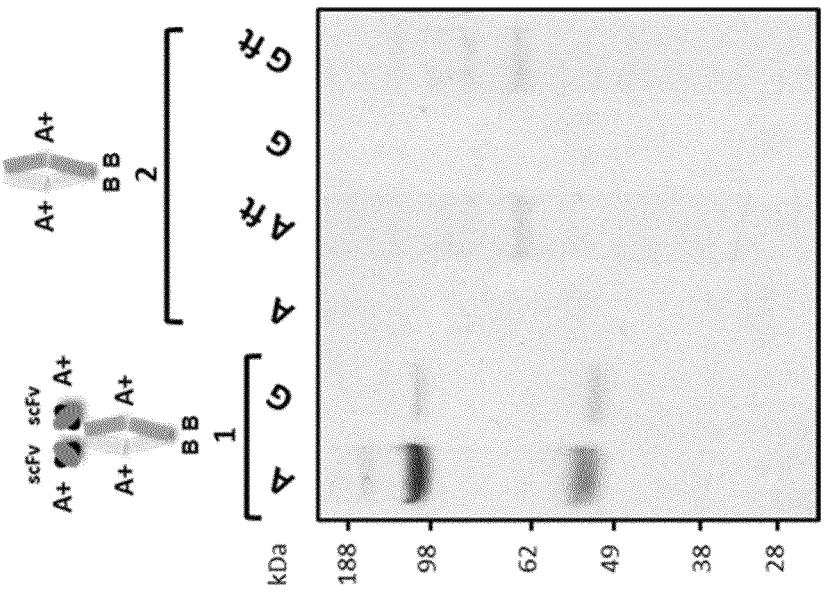


FIG. 18

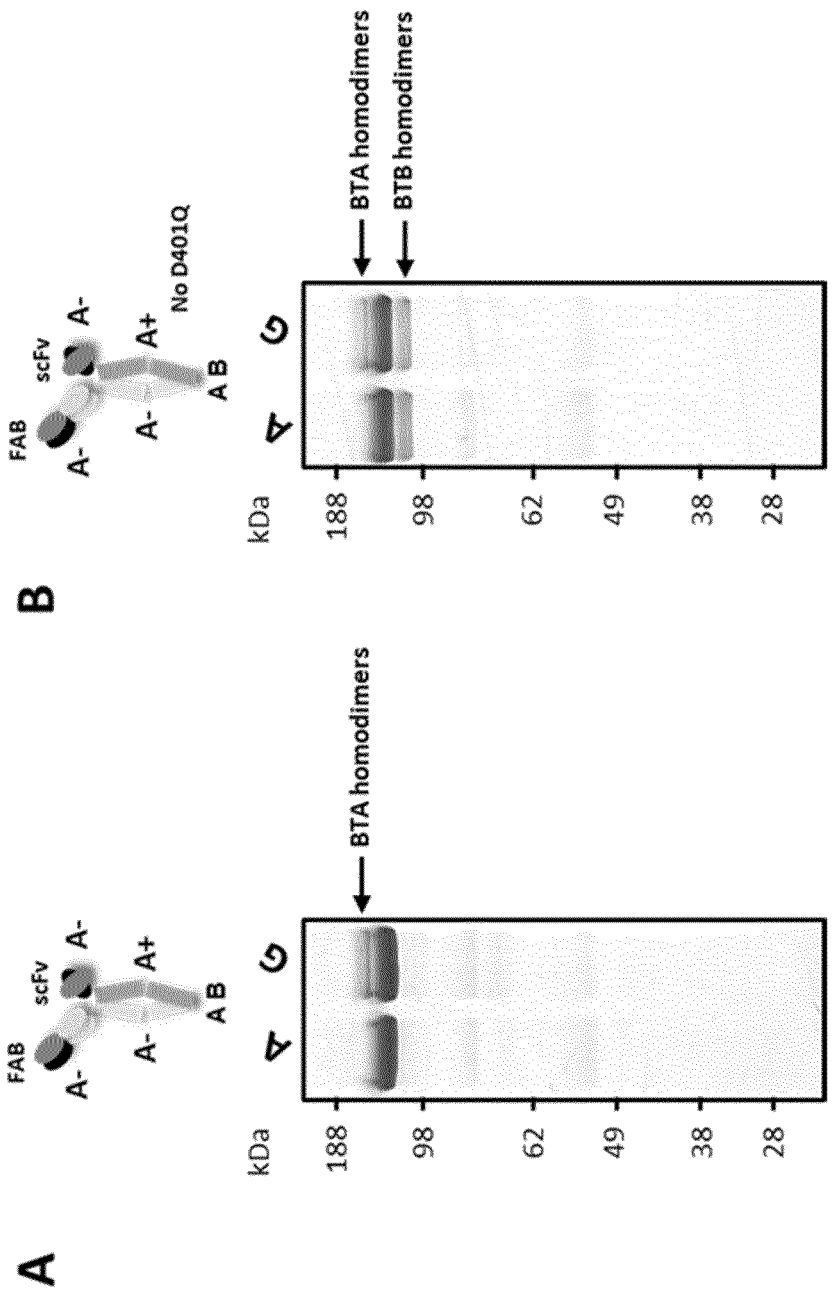

A
FAB
scFv
A-
A-
A-
A+
A B
kDa
188
98
62
49
38
28
BTA homodimers
B
FAB
scFv
A-
A-
A-
A+
No D401Q
A B
kDa
188
98
62
49
38
28
BTA homodimers
BTB homodimers

FIG. 19

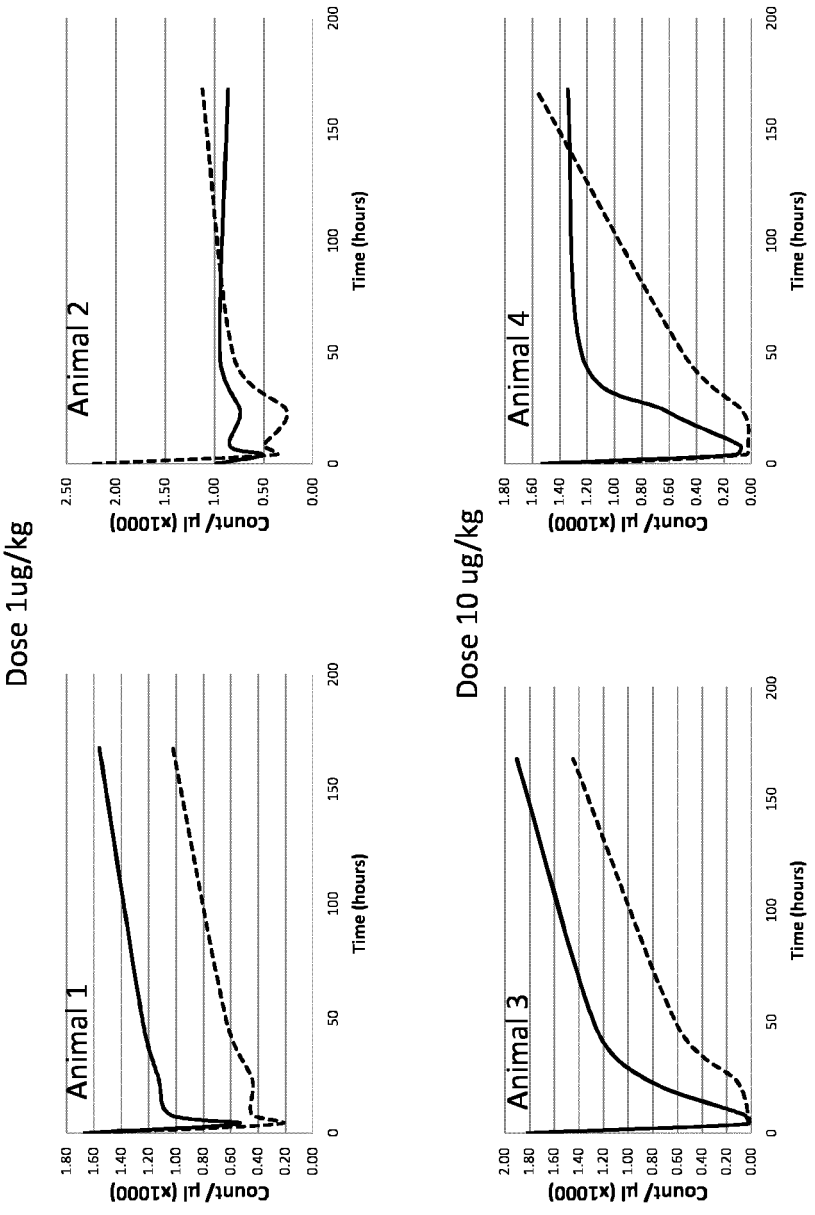

Dose 1ug/kg
Dose 10 ug/kg
Animal 1
Animal 2
Animal 3
Animal 4
Count/ µl (x1000)
Time (hours)

FIG. 20

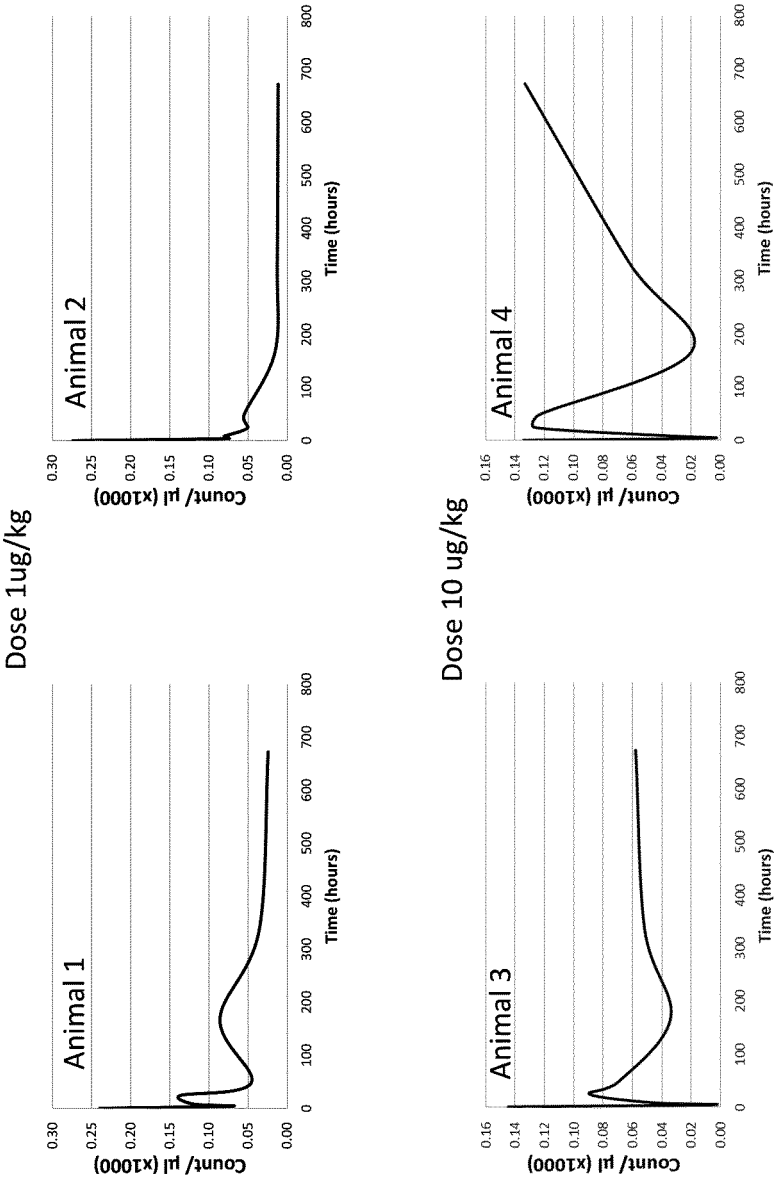

Dose 1ug/kg
Dose 10 ug/kg
Animal 1
Animal 2
Animal 3
Animal 4
Count/ µl (x1000)
Time (hours)

FIG. 21

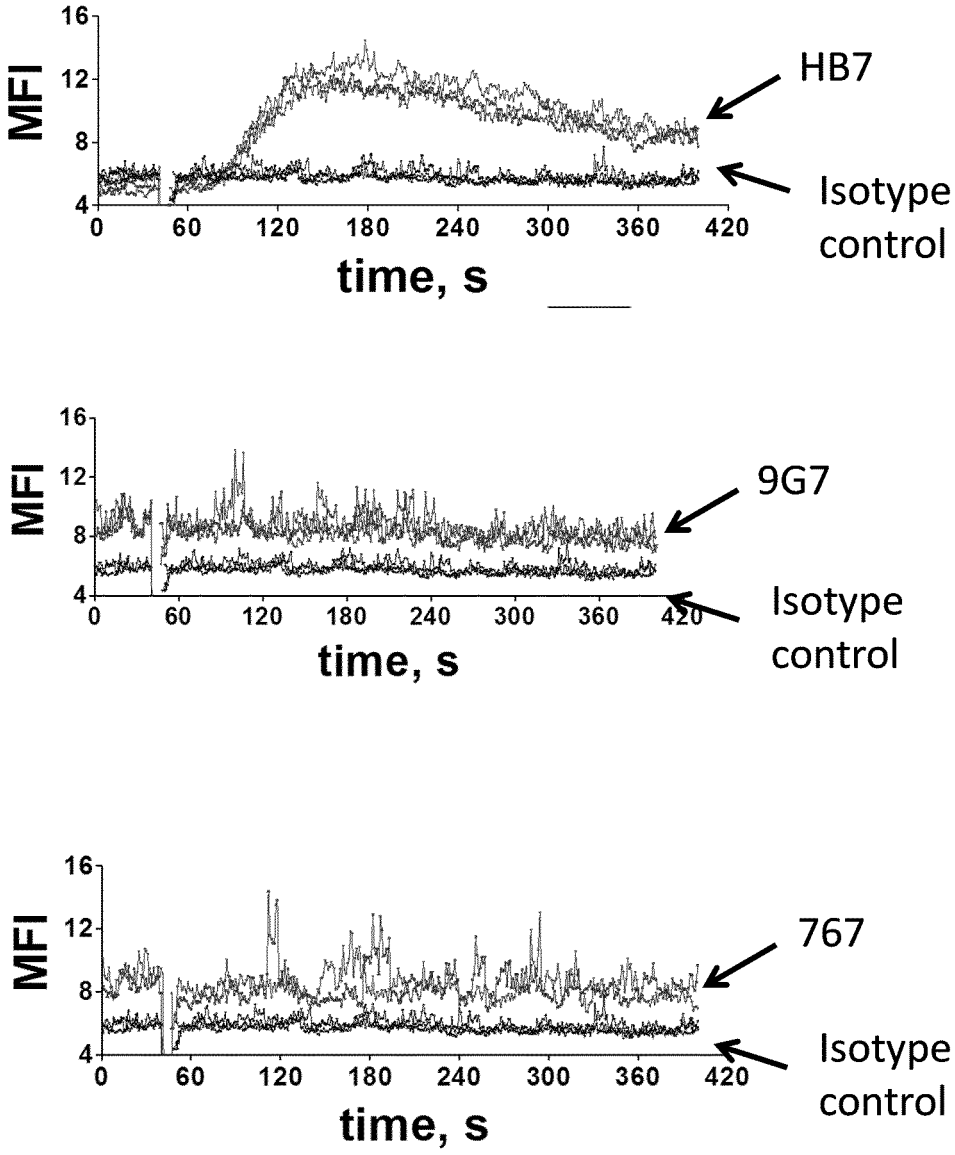

MFI
16
12
8
4
0
60
120
180
240
300
360
420
time, s
HB7
Isotype control
9G7
Isotype control
767
Isotype control

FIG. 22

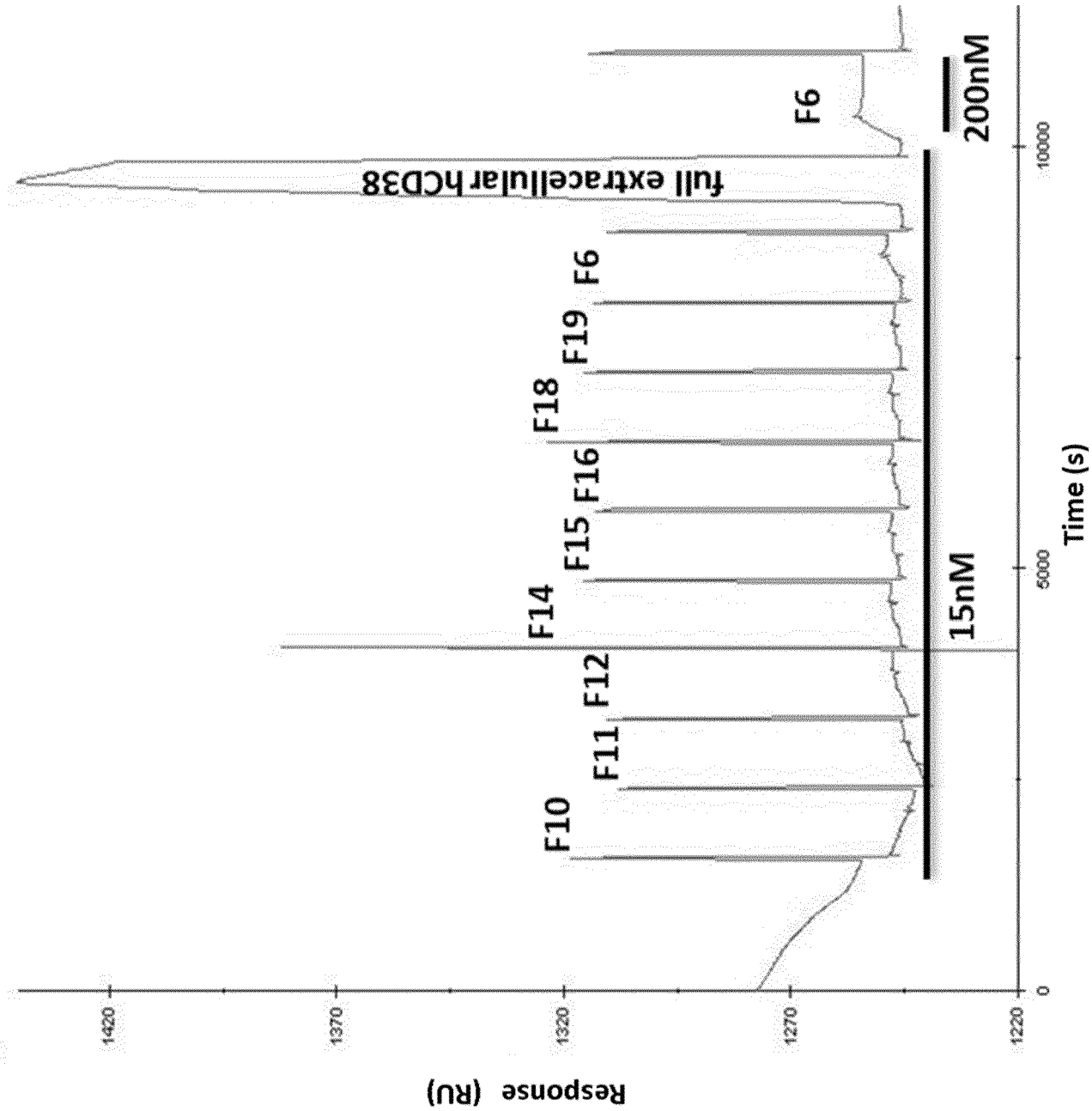

Response (RU)
1420
1370
1320
1270
1220
0
5000
10000
Time (s)
200nM
15nM
F6
full extracellular hCD38
F6
F19
F18
F16
F15
F14
F12
F11
F10

FIG. 23

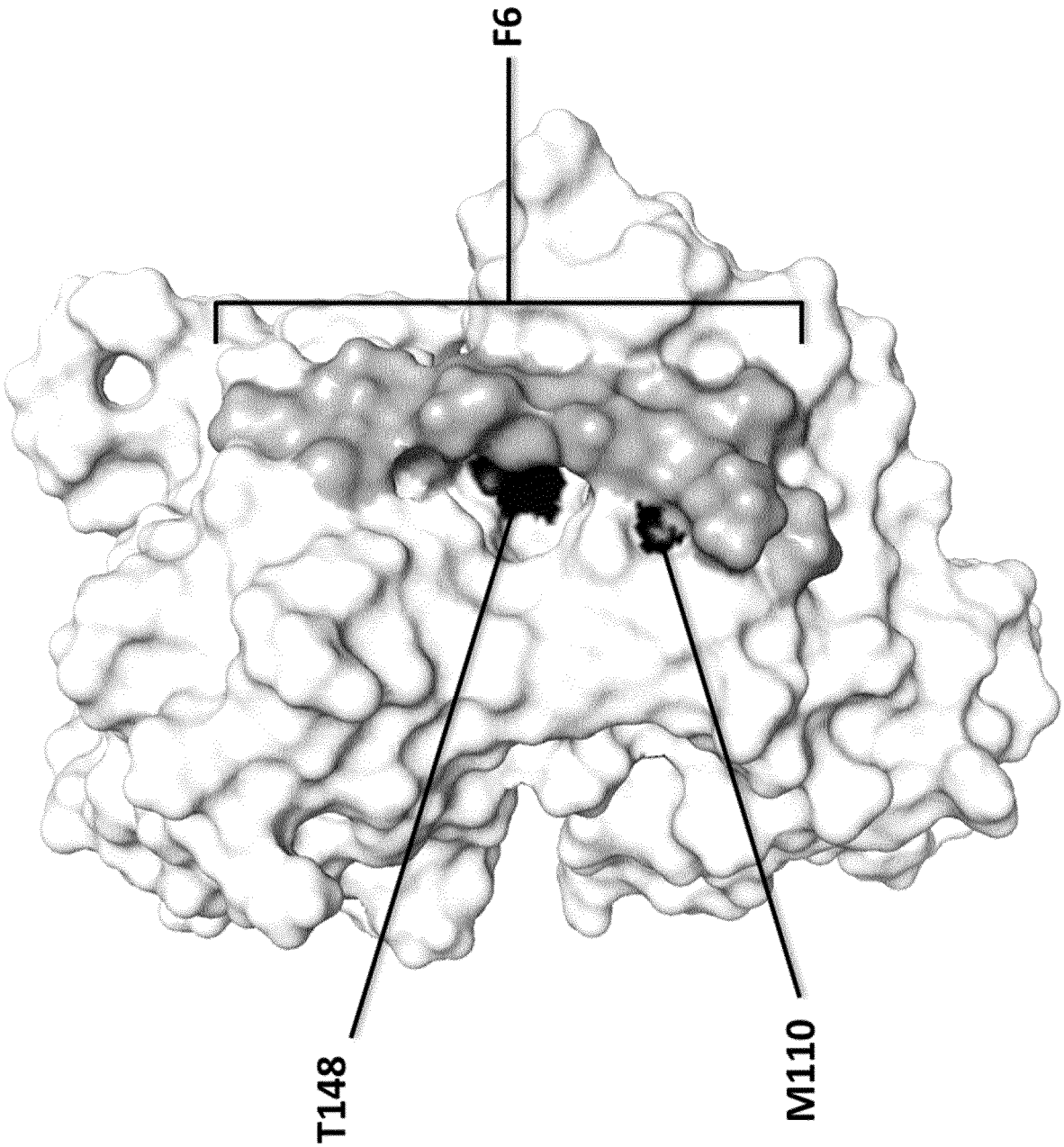


FIG. 24

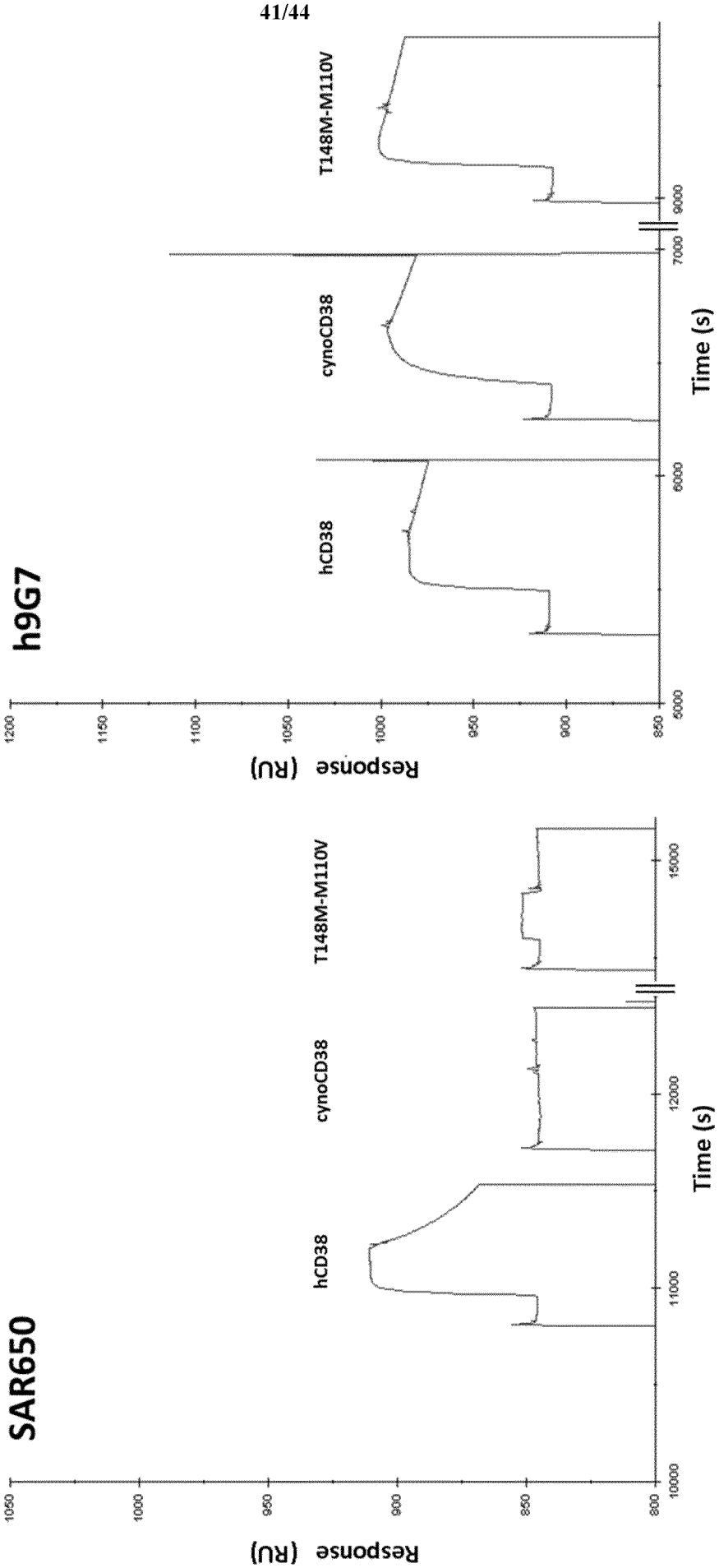

h9G7
Response (RU)
1200
1150
1100
1050
1000
950
900
850
5000
6000
7000
9000
Time (s)
hCD38
cynoCD38
T148M-M110V
SAR650
Response (RU)
1050
1000
950
900
850
800
10000
11000
12000
15000
Time (s)
hCD38
cynoCD38
T148M-M110V

FIG. 25

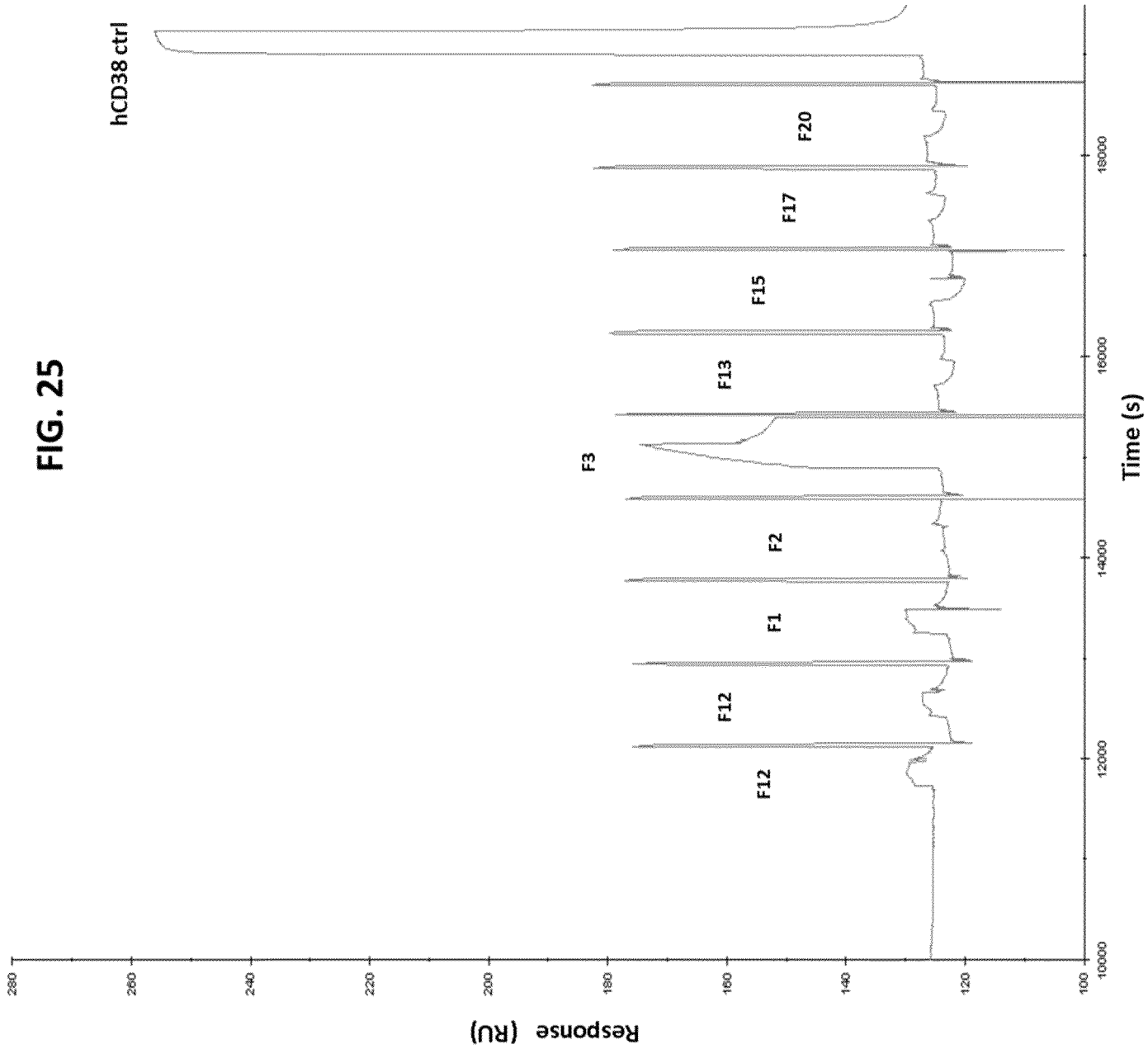

hCD38 ctrl
F20
F17
F15
F13
F3
F2
F1
F12
F12
Response (RU)
Time (s)
280
260
240
220
200
180
160
140
120
100
10000
12000
14000
16000
18000

FIG. 26

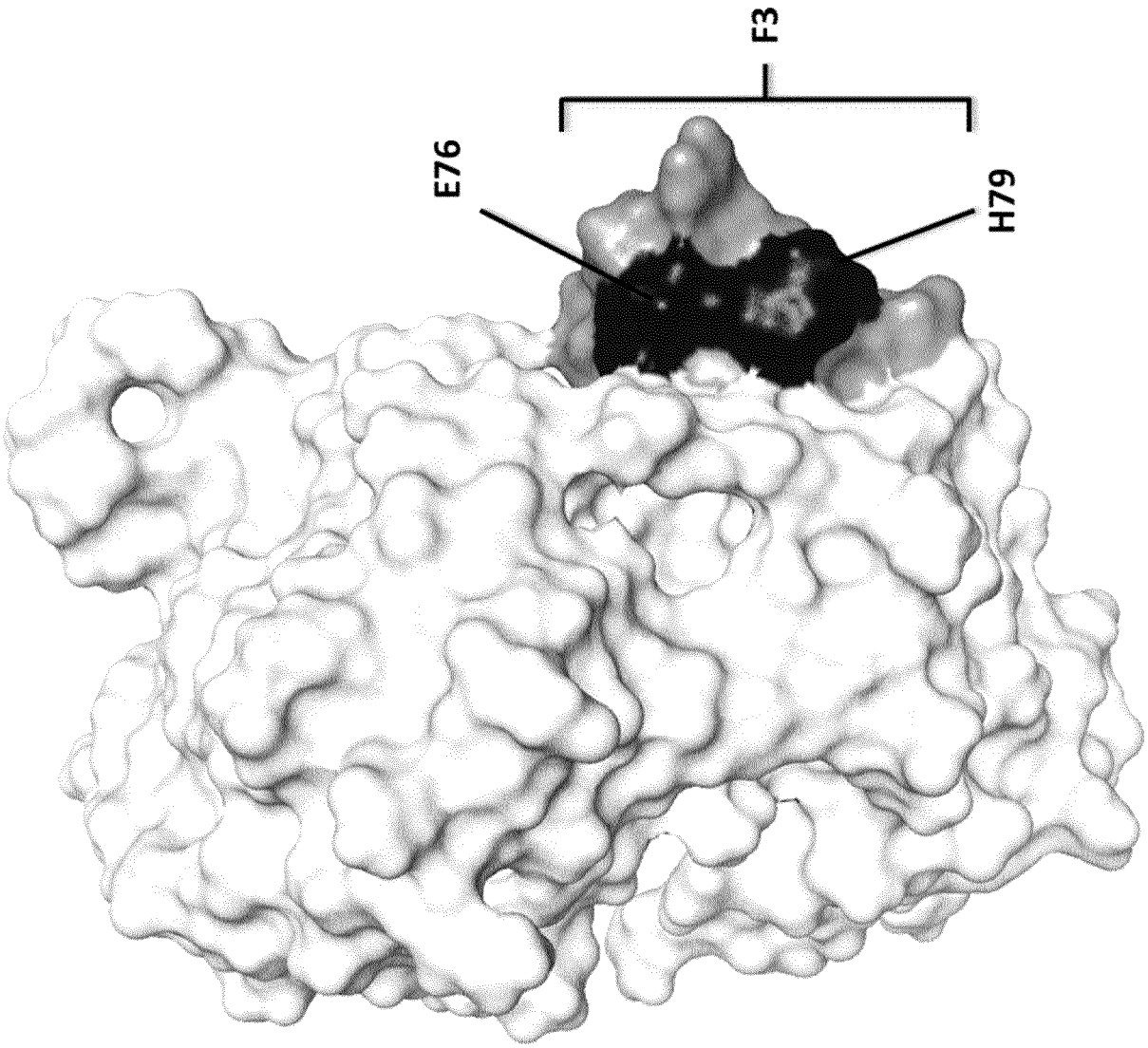
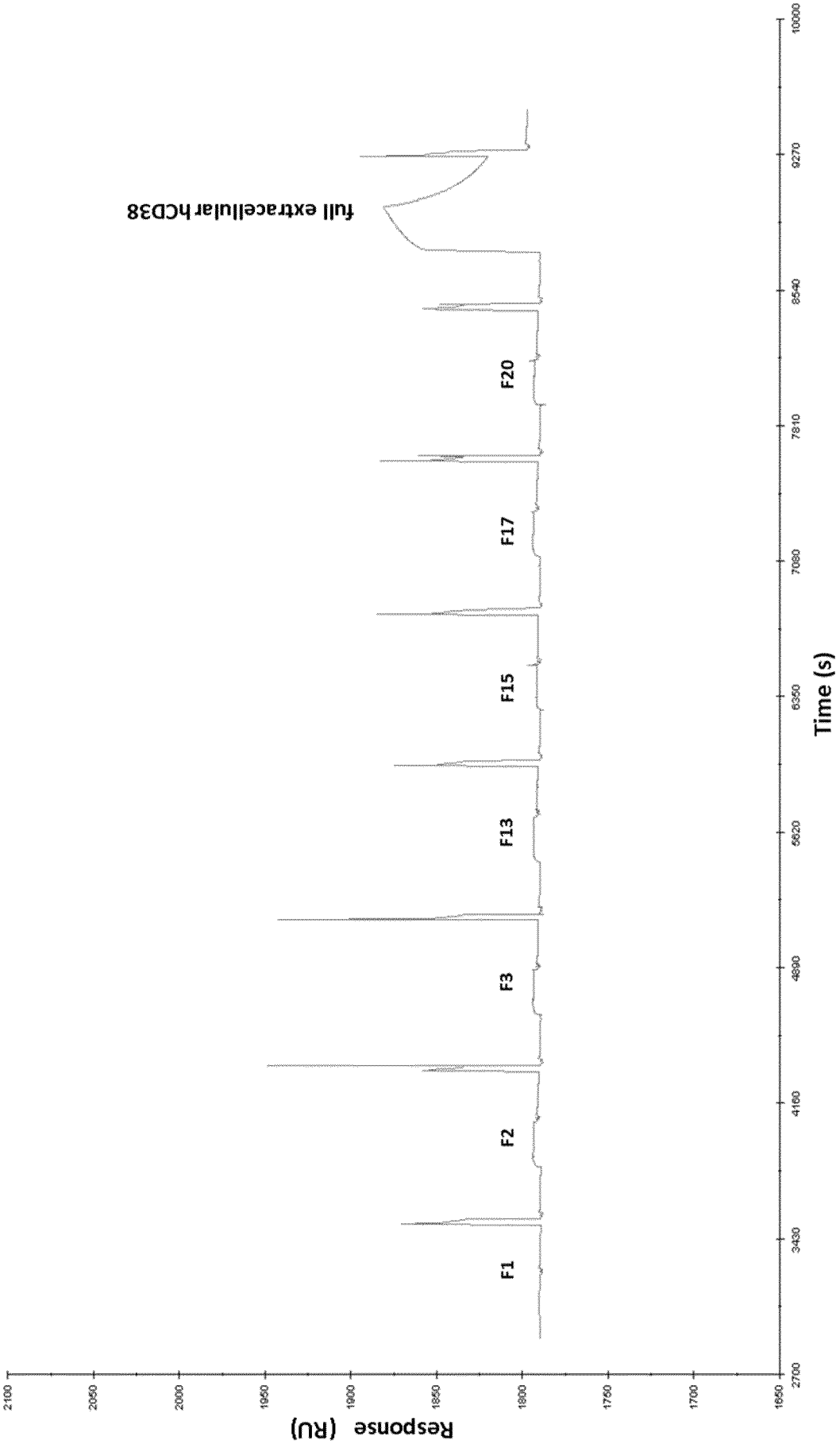


FIG. 27


full extracellular hCD38
F1
F2
F3
F13
F15
F17
F20
Response (RU)
Time (s)
2100
2050
2000
1950
1900
1850
1800
1750
1700
1650
2700
3430
4160
4890
5620
6350
7080
7810
8540
9270
10000