Figure 1

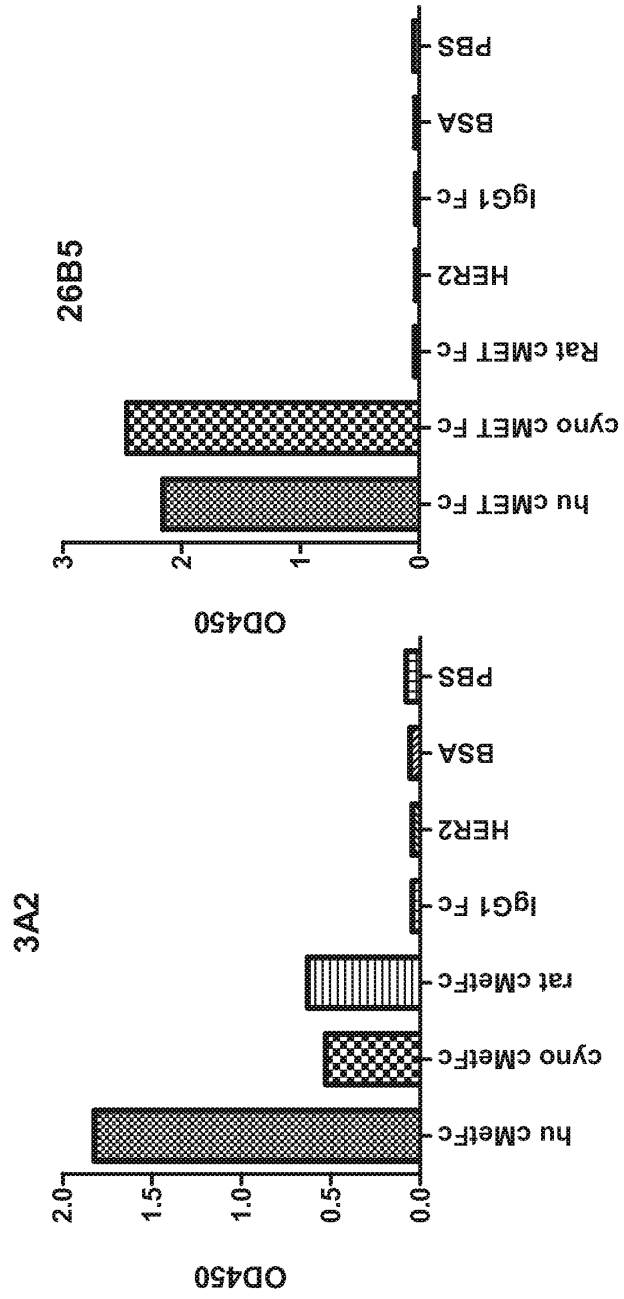

26B5
hu cMET Fc
cyno cMET Fc
Rat cMET Fc
HER2
IgG1 Fc
BSA
PBS
0
1
2
3
OD450
3A2
hu cMetFc
cyno cMetFc
rat cMetFc
IgG1 Fc
HER2
BSA
PBS
0.0
0.5
1.0
1.5
2.0
OD450

Figure 2a

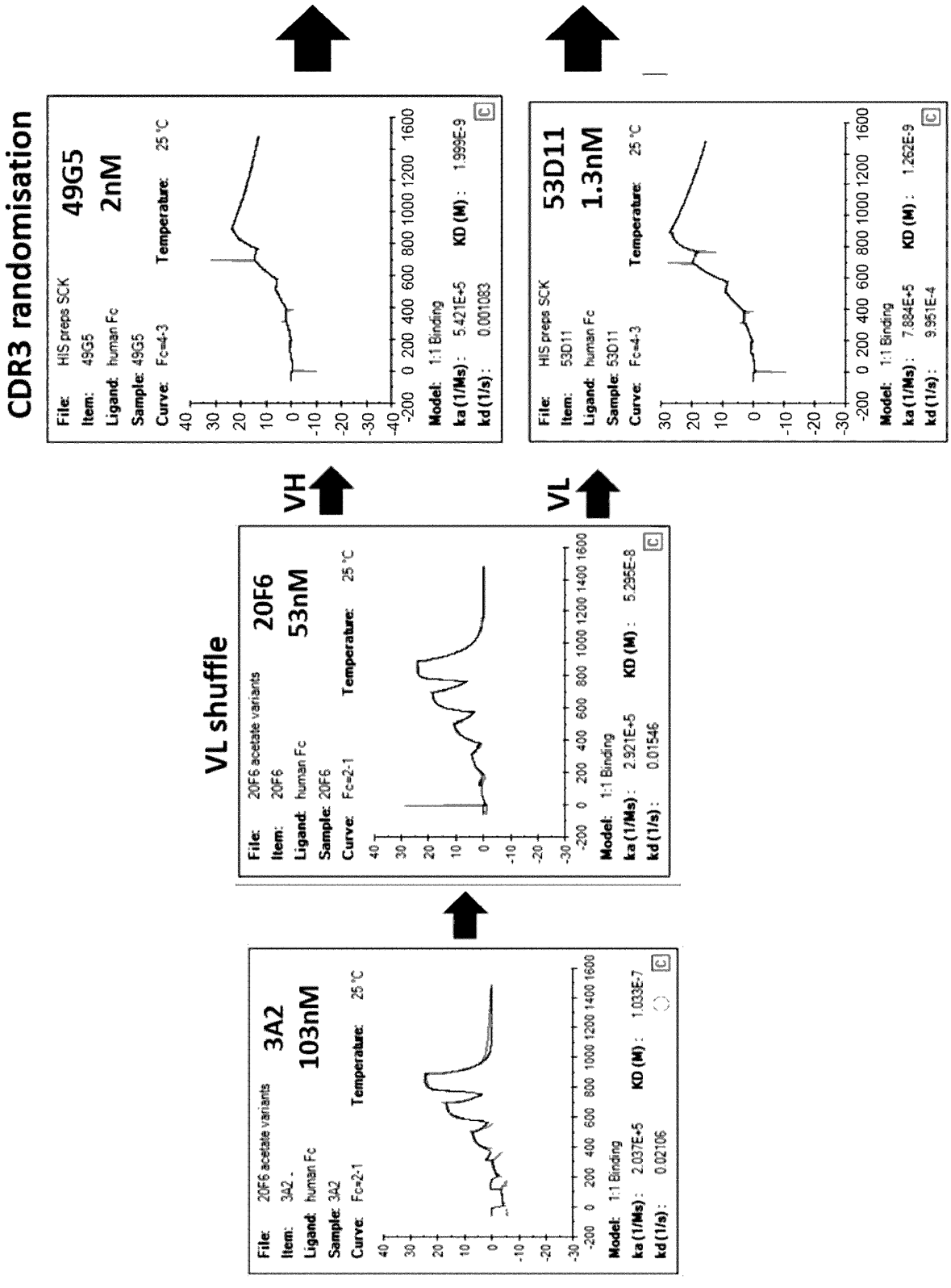

CDR3 randomisation
VH
VL
VL shuffle
49G5
2nM
53D11
1.3nM
20F6
53nM
3A2
103nM

Figure 2a (continued)

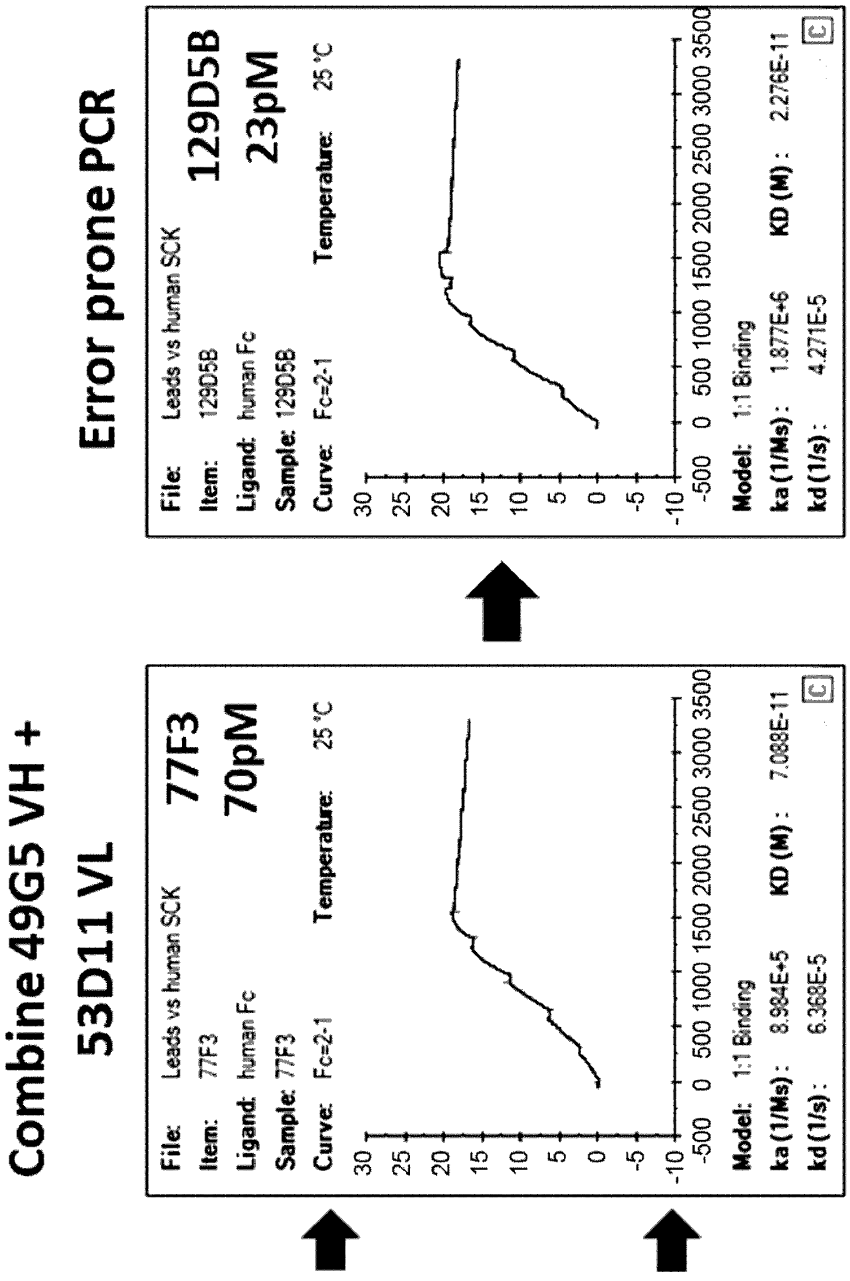


Figure 2b

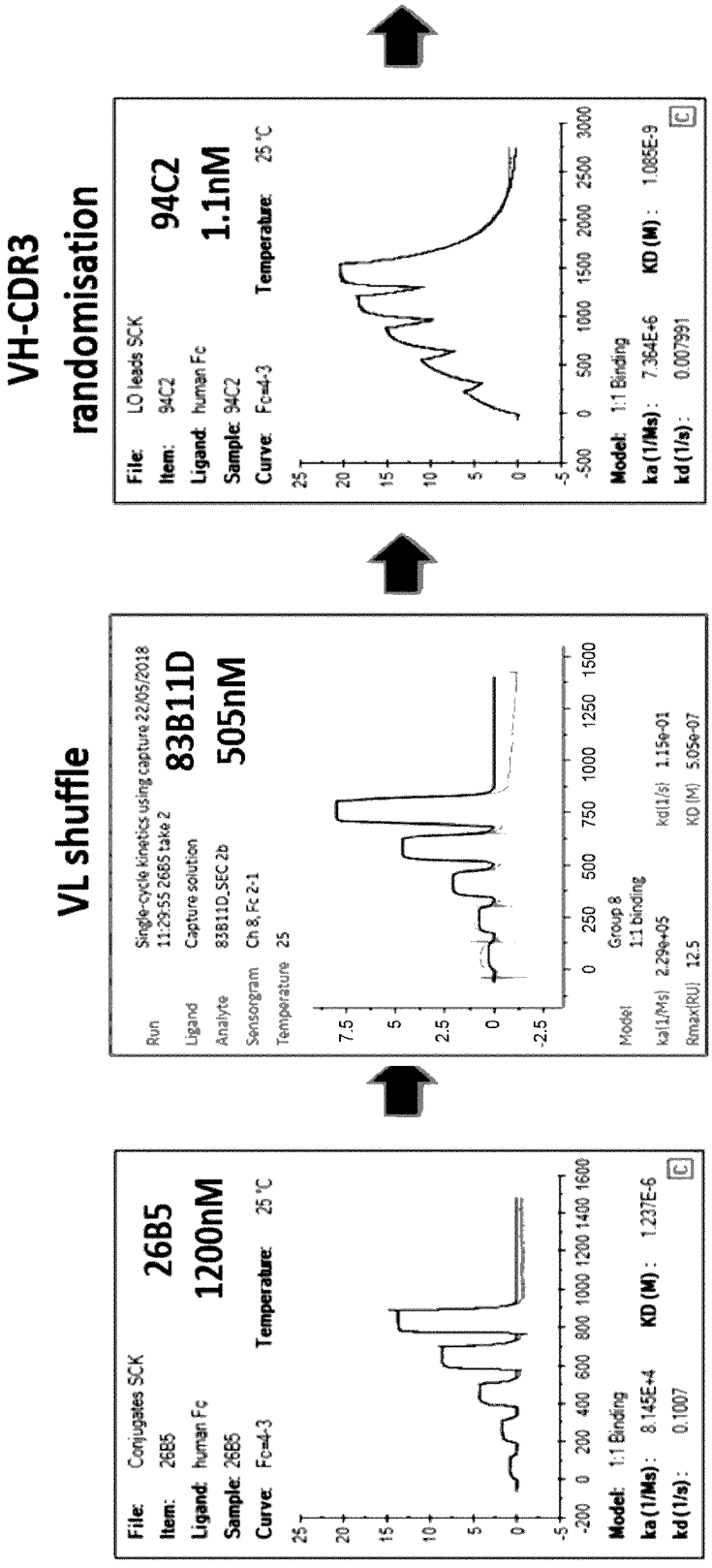

VH-CDR3
randomisation
VL shuffle
File: LO leads SCK
Item: 94C2
Ligand: human Fc
Sample: 94C2
Curve: Fc=4-3
Temperature: 25 °C
94C2
1.1nM
Model: 1:1 Binding
ka (1/Ms): 7.364E+6
KD (M): 1.085E-9
kd (1/s): 0.007991
Run
Ligand
Analyte
Sensorgram
Temperature
Single-cycle kinetics using capture 22/05/2018 11:29:55 26B5 take 2
Capture solution
83B11D_SEC 2b
Ch 8, Fc 2-1
25
83B11D
505nM
Model
Group 8
1:1 binding
ka(1/Ms) 2.29e+05
kd(1/s) 1.15e-01
Rmax(RU) 12.5
KD (M) 5.05e-07
File: Conjugates SCK
Item: 26B5
Ligand: human Fc
Sample: 26B5
Curve: Fc=4-3
Temperature: 25 °C
26B5
1200nM
Model: 1:1 Binding
ka (1/Ms): 8.145E+4
KD (M): 1.237E-6
kd (1/s): 0.1007

Figure 2b (continued)

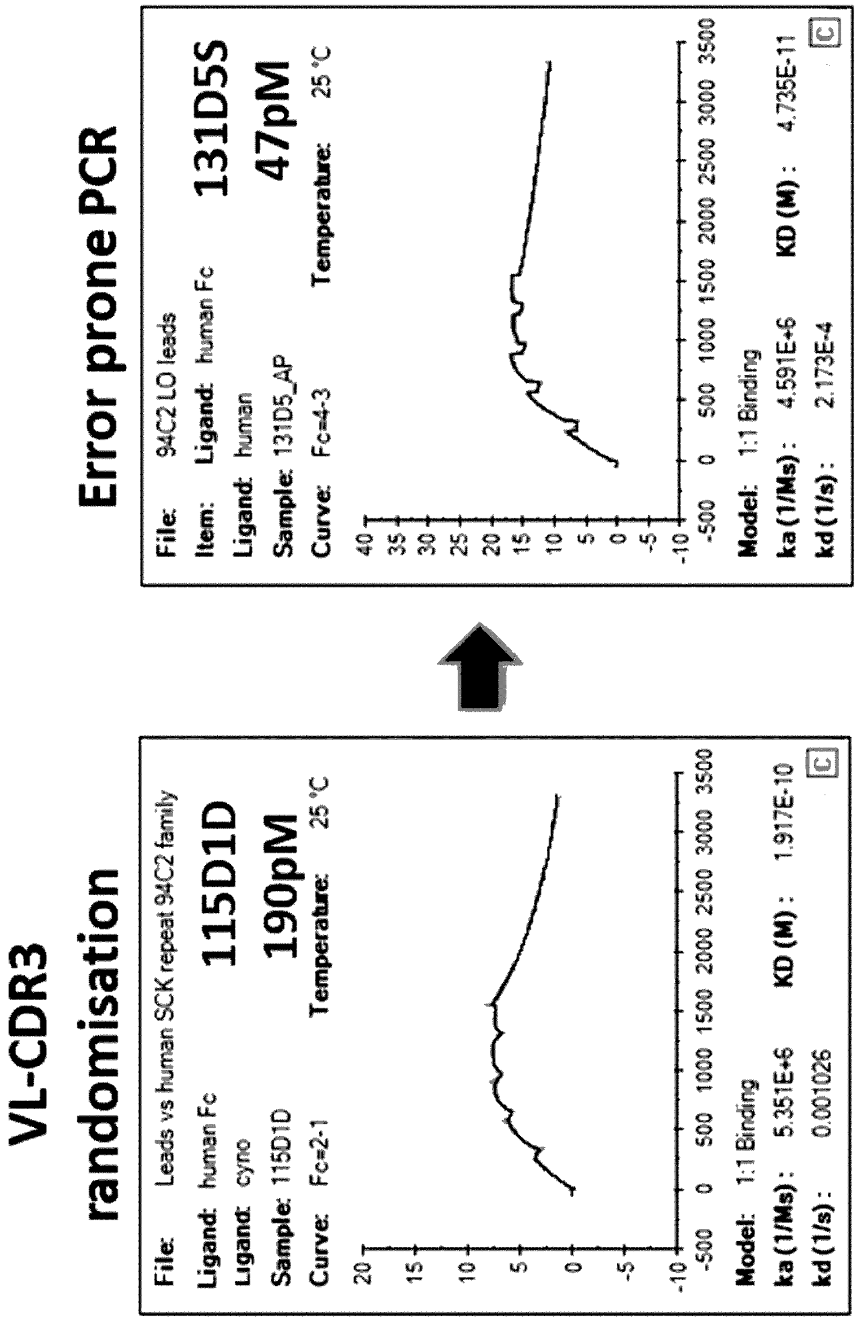


Figure 2c

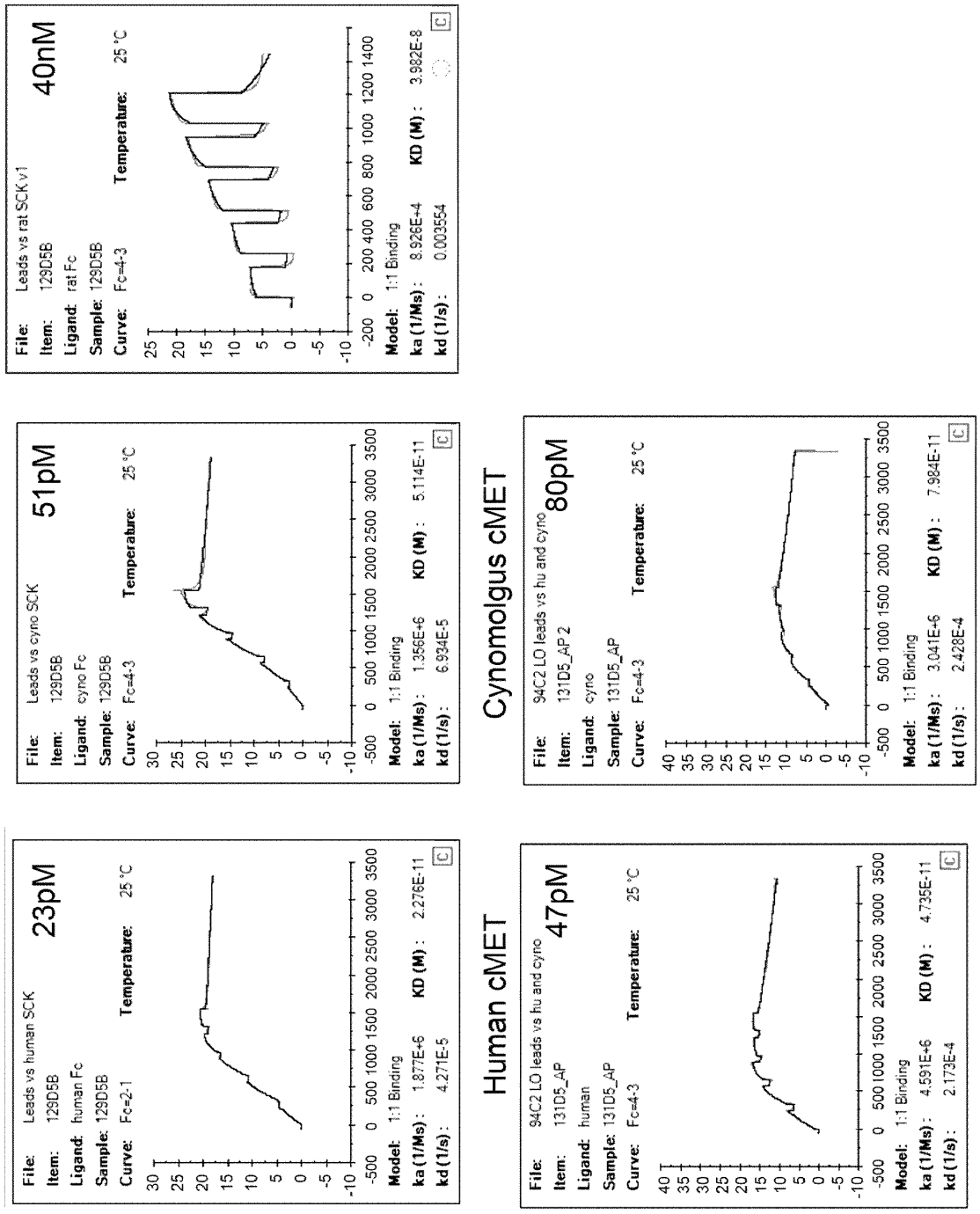
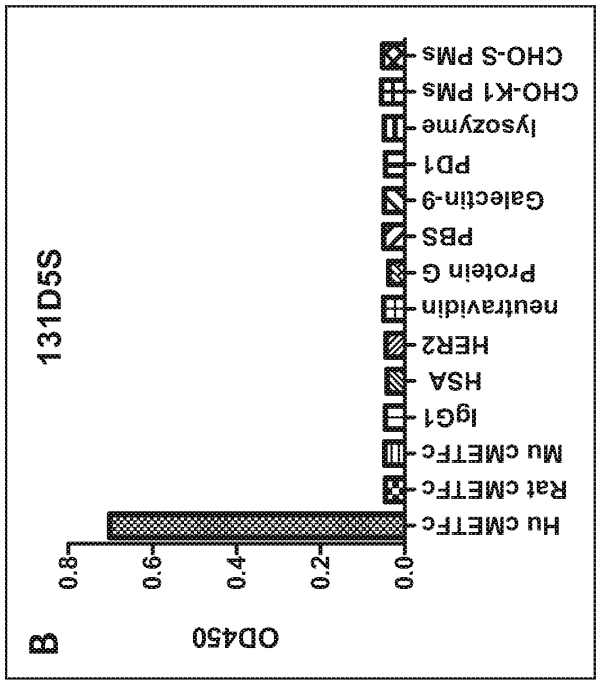

Human cMET
Cynomolgus cMET
Rat cMET
129D5B scFv
A
131D5S scFv
B
File: Leads vs human SCK
Item: 129D5B
Ligand: human Fc
Sample: 129D5B
Curve: Fc=2-1
Temperature: 25 °C
23pM
Model: 1:1 Binding
ka (1/Ms): 1.877E+6
KD (M): 2.276E-11
kd (1/s): 4.271E-5
File: Leads vs cyno SCK
Item: 129D5B
Ligand: cyno Fc
Sample: 129D5B
Curve: Fc=4-3
Temperature: 25 °C
51pM
Model: 1:1 Binding
ka (1/Ms): 1.356E+6
KD (M): 5.114E-11
kd (1/s): 6.934E-5
File: Leads vs rat SCK v1
Item: 129D5B
Ligand: rat Fc
Sample: 129D5B
Curve: Fc=4-3
Temperature: 25 °C
40nM
Model: 1:1 Binding
ka (1/Ms): 8.926E+4
KD (M): 3.982E-8
kd (1/s): 0.003554
File: 94C2 LO leads vs hu and cyno
Item: 131D5_AP
Ligand: human
Sample: 131D5_AP
Curve: Fc=4-3
Temperature: 25 °C
47pM
Model: 1:1 Binding
ka (1/Ms): 4.591E+6
KD (M): 4.735E-11
kd (1/s): 2.173E-4
File: 94C2 LO leads vs hu and cyno
Item: 131D5_AP 2
Ligand: cyno
Sample: 131D5_AP
Curve: Fc=4-3
Temperature: 25 °C
80pM
Model: 1:1 Binding
ka (1/Ms): 3.041E+6
KD (M): 7.984E-11
kd (1/s): 2.428E-4

Figure 3


B
131D5S
OD450
0.0
0.2
0.4
0.6
0.8
Hu cMETFc
Rat cMETFc
Mu cMETFc
IgG1
HSA
HER2
neutravidin
Protein G
PBS
Galectin-9
PD1
lysozyme
CHO-K1 PMs
CHO-S PMs

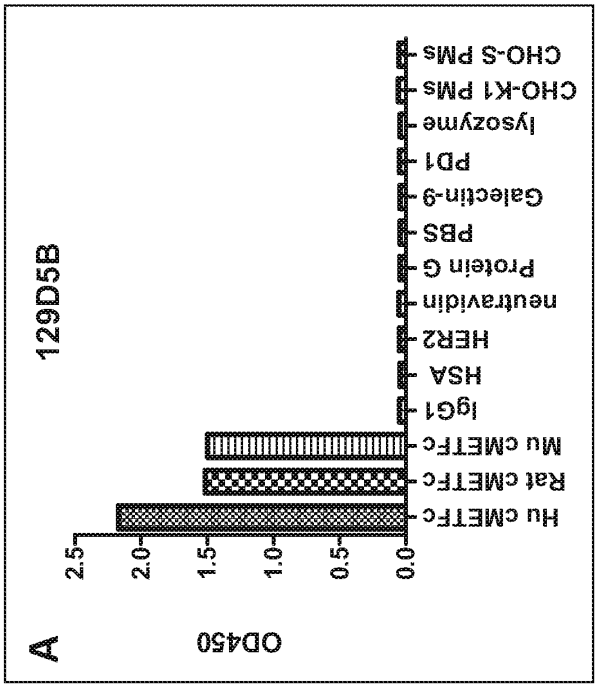

A
129D5B
OD450
0.0
0.5
1.0
1.5
2.0
2.5
Hu cMETFc
Rat cMETFc
Mu cMETFc
IgG1
HSA
HER2
neutravidin
Protein G
PBS
Galectin-9
PD1
lysozyme
CHO-K1 PMs
CHO-S PMs

Figure 4

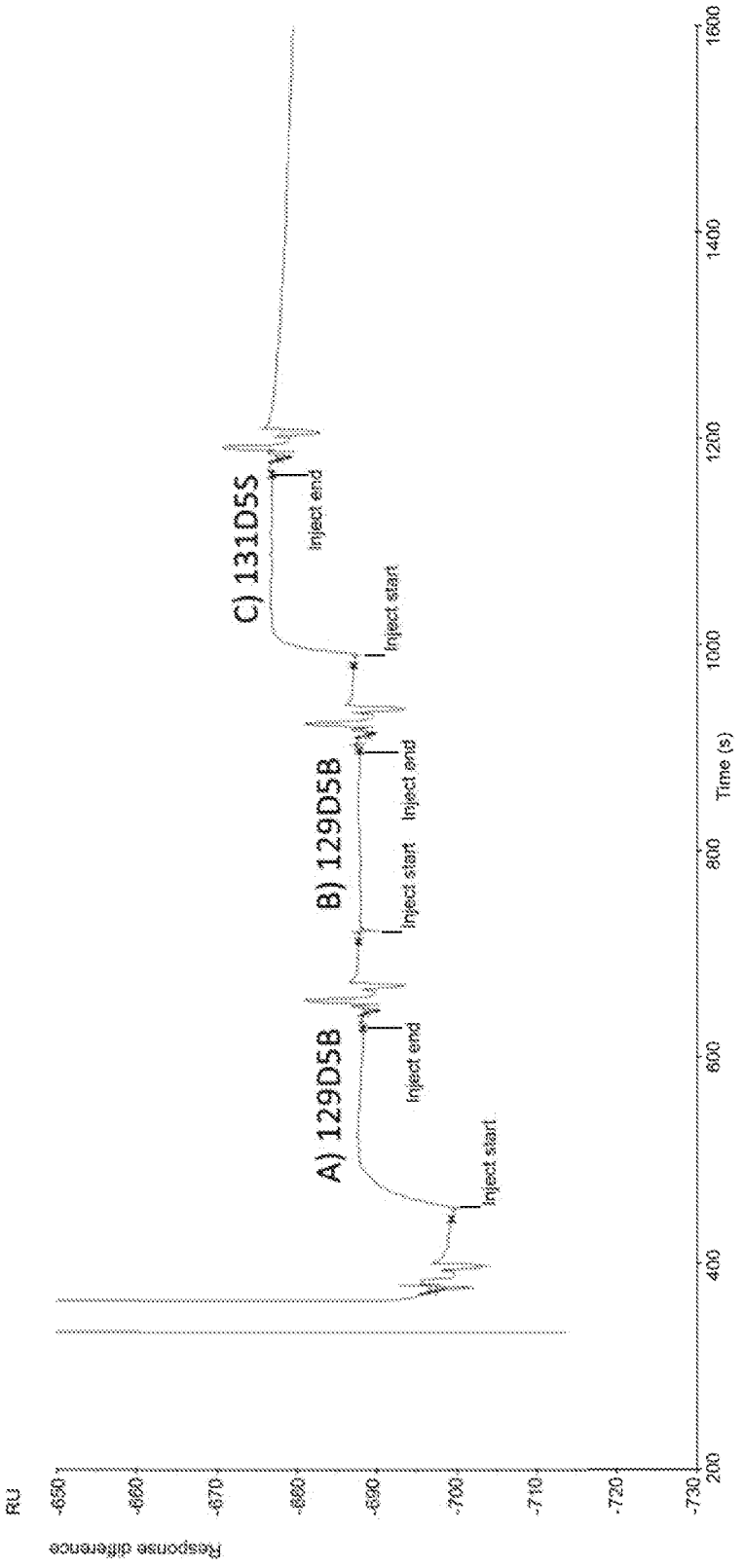

Response difference
RU
-650
-660
-670
-680
-690
-700
-710
-720
-730
200
400
600
800
1000
1200
1400
1600
Time (s)
A) 129D5B
Inject start
Inject end
B) 129D5B
Inject start
Inject end
C) 131D5S
Inject start
Inject end

Figure 5

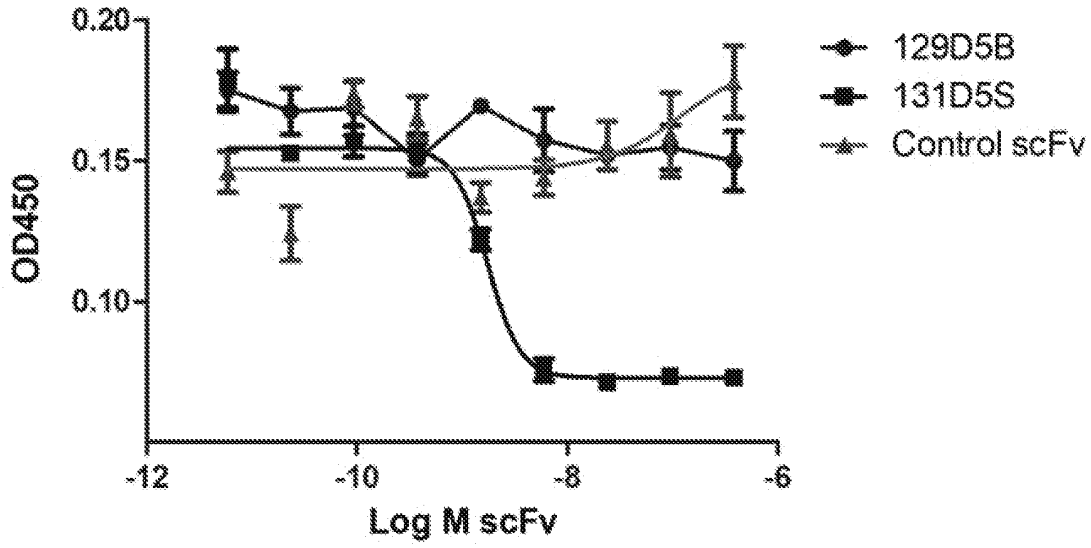

0.20
0.15
0.10
OD450
-12
-10
-8
-6
Log M scFv
129D5B
131D5S
Control scFv

Figure 6

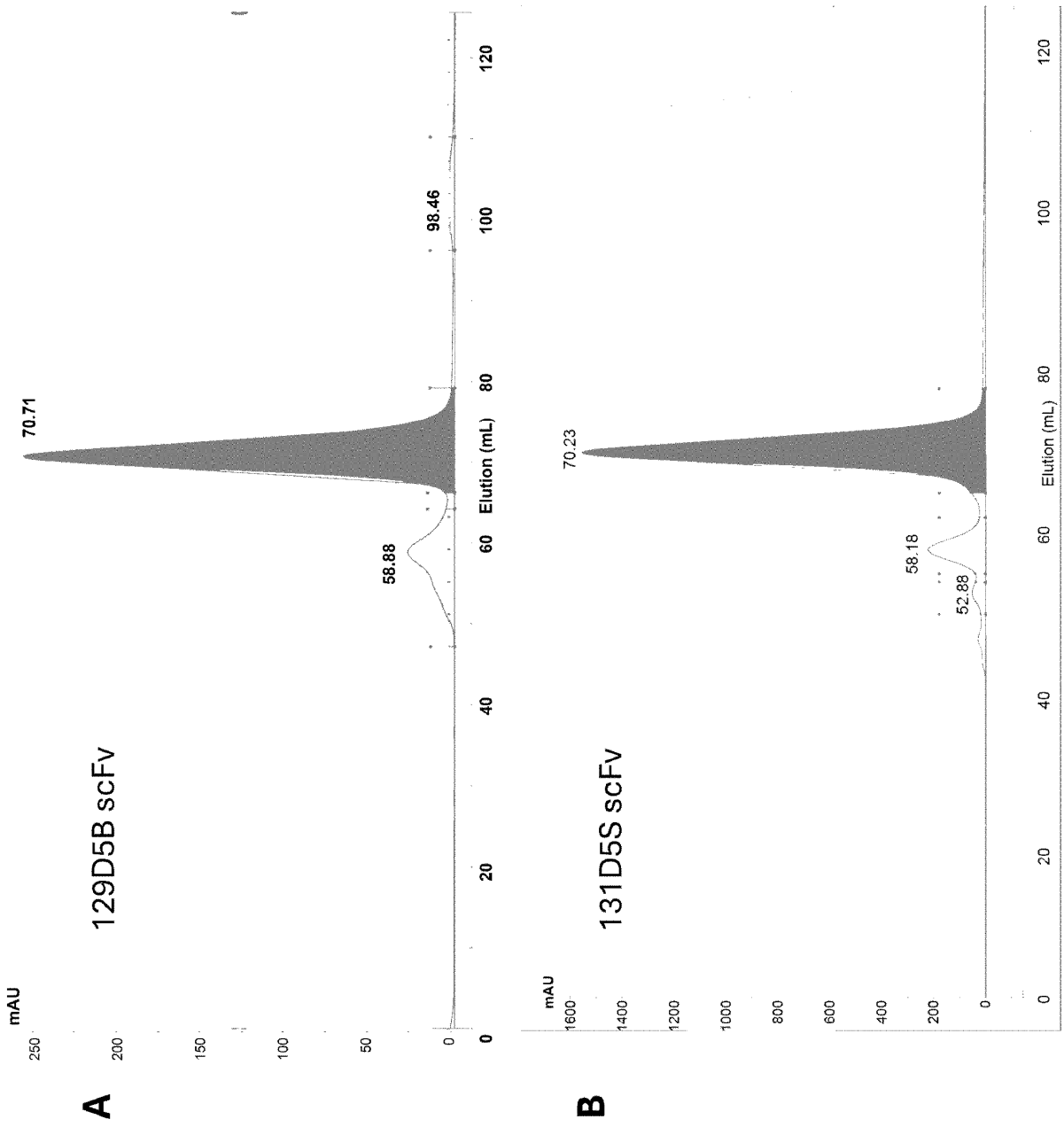
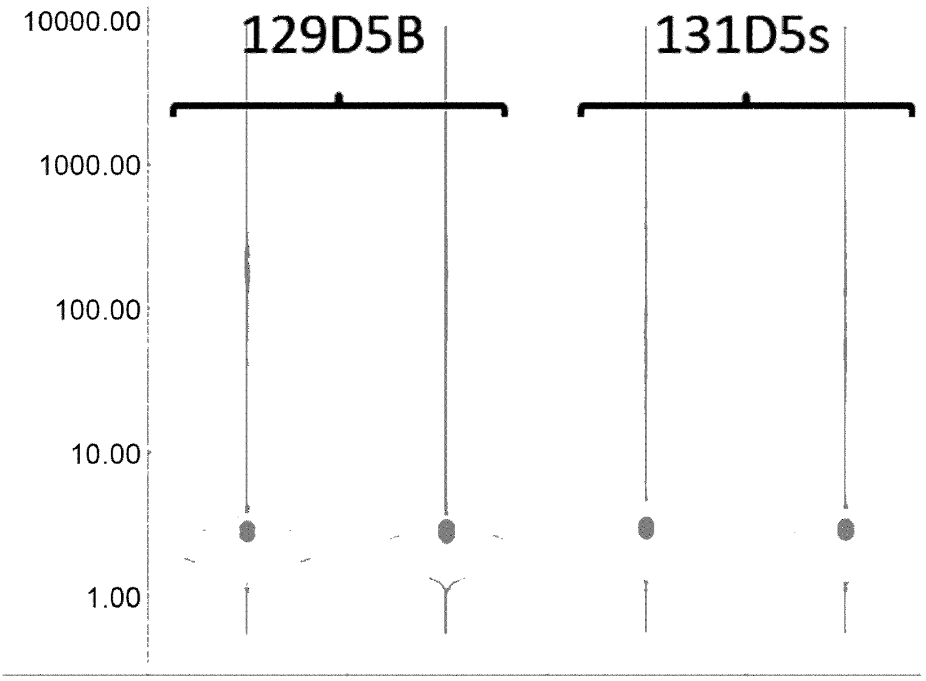

A
129D5B scFv
mAU
250
200
150
100
50
0
0
20
40
60
80
100
120
Elution (mL)
70.71
58.88
98.46
B
131D5S scFv
mAU
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
Elution (mL)
70.23
58.18
52.88

Figure 6 (continued)

C



Sample name	cMET-129D5B	cMET-129D5B	cMET-131D5s	cMET-131D5s
Sample conc.	4mg/mL	4mg/mL	4mg/mL	4mg/mL
Buffer	PBS	PBS	PBS	PBS
Cumm. Radius	2.83 ±0.09nm	2.80 ±0.09nm	2.96 ±0.09nm	2.91 ±0.09nm
Polydispersity	0.08 ±0.02	0.04 ±0.04	0.13 ±0.04	0.07 ±0.03
SLS Intensity	733.55 ±24.9k	821.55 ±31.2k	496.46 ±12.7k	581.16 ±10.6k
Signal/Noise	177.16	146.87	162.10	136.77

12/120

Figure 7

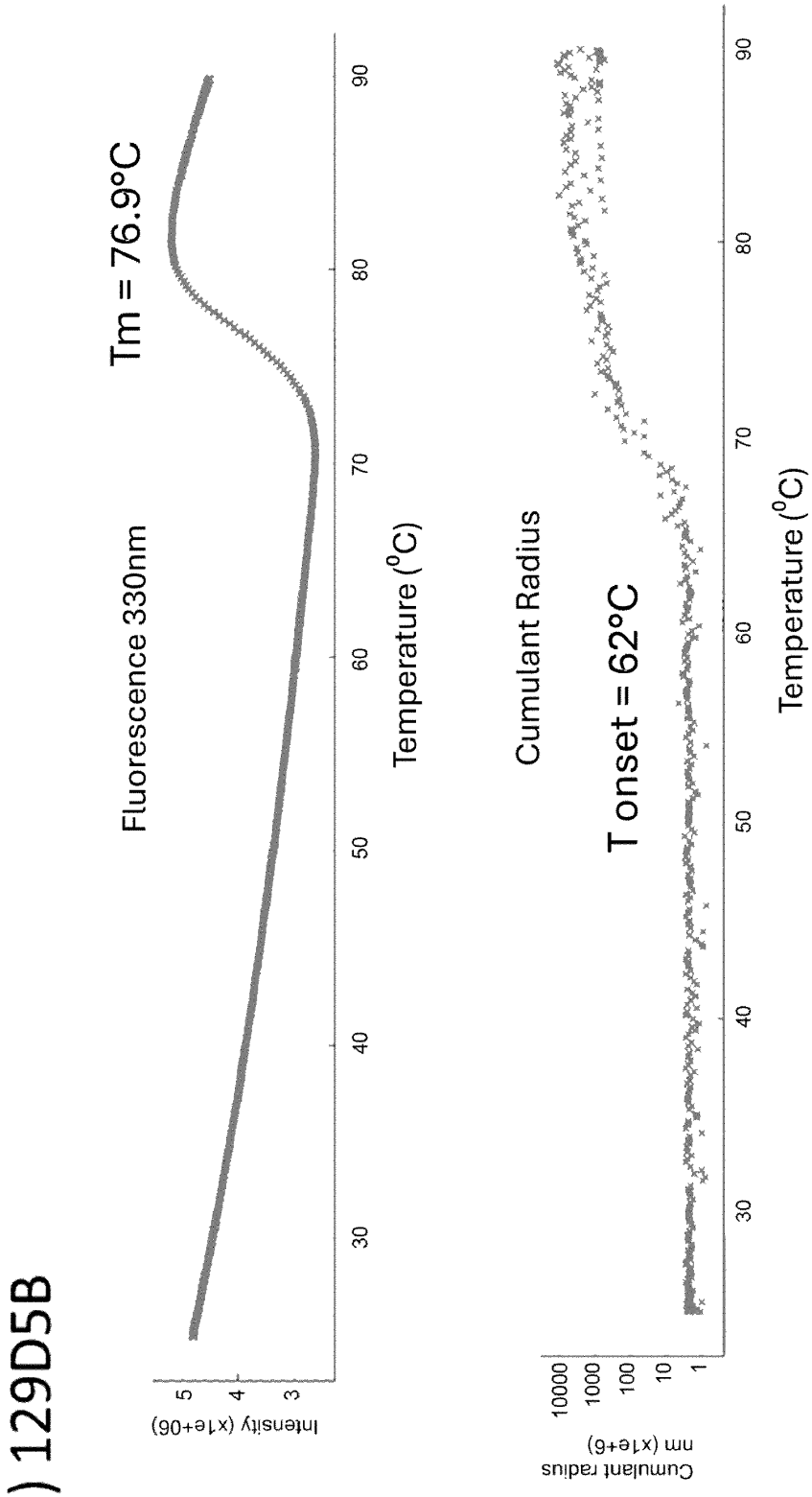


Figure 7 continued

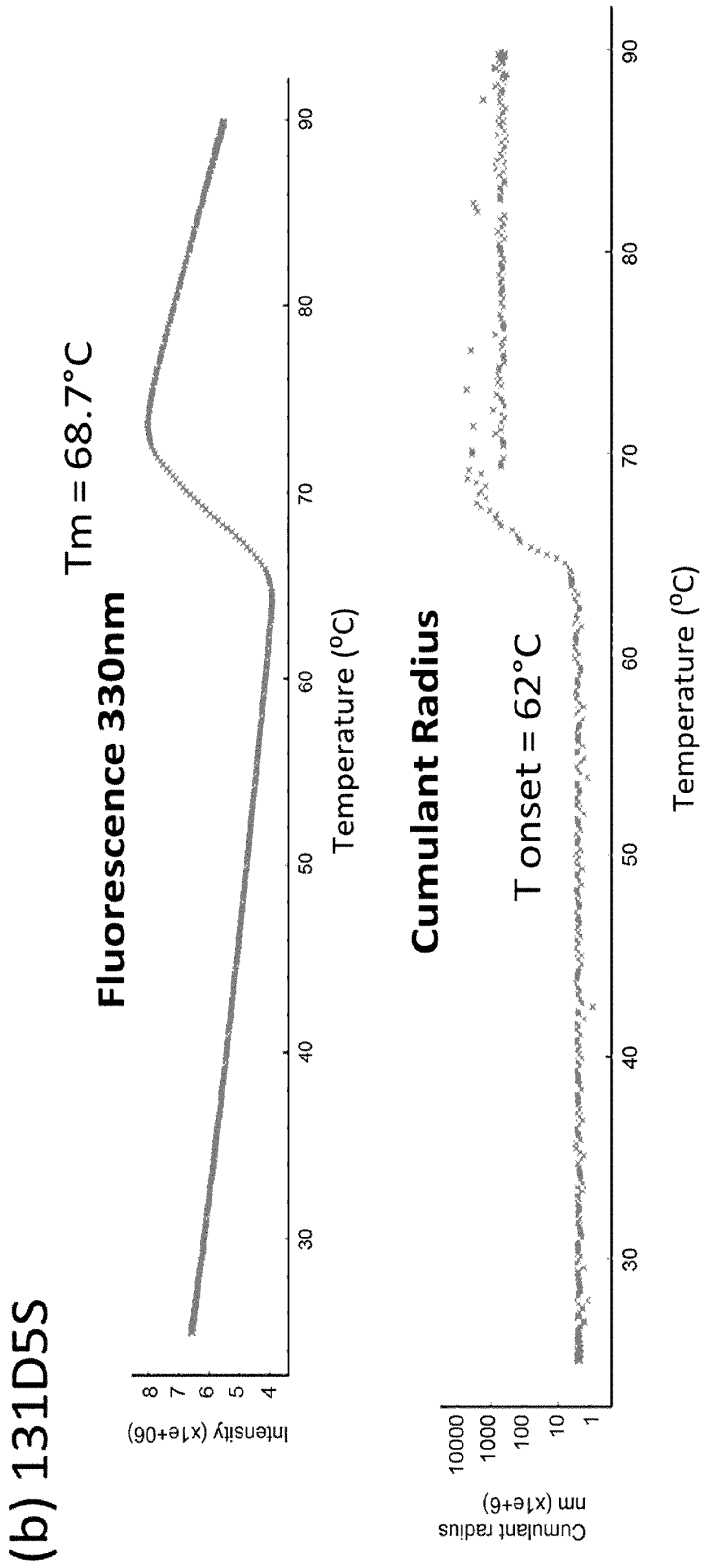
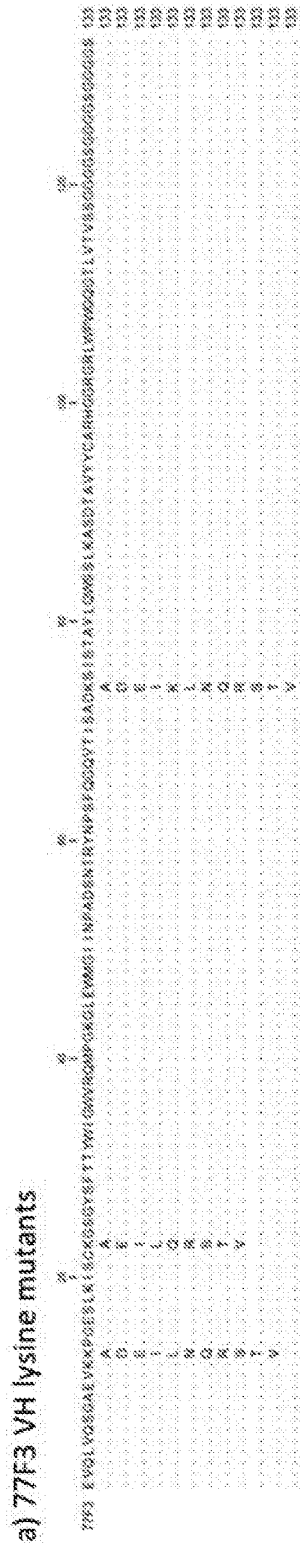


Figure 8



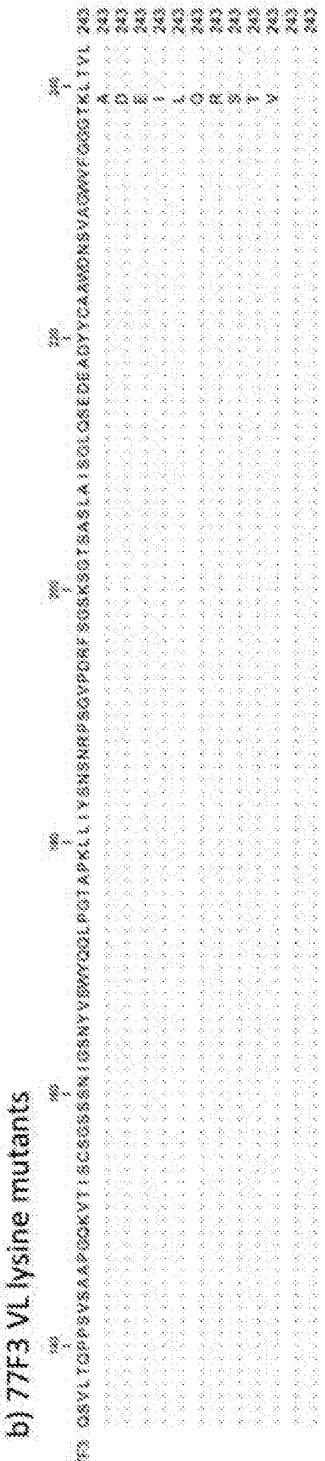
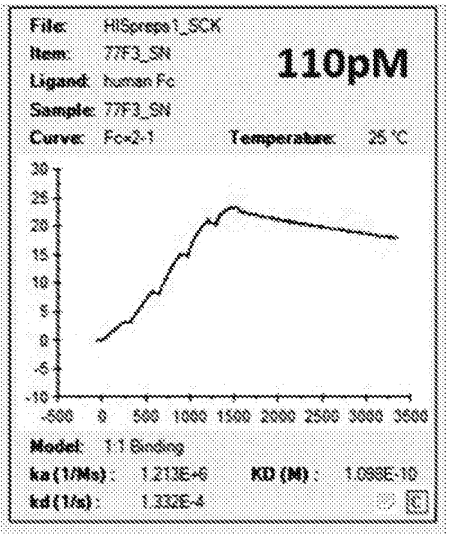
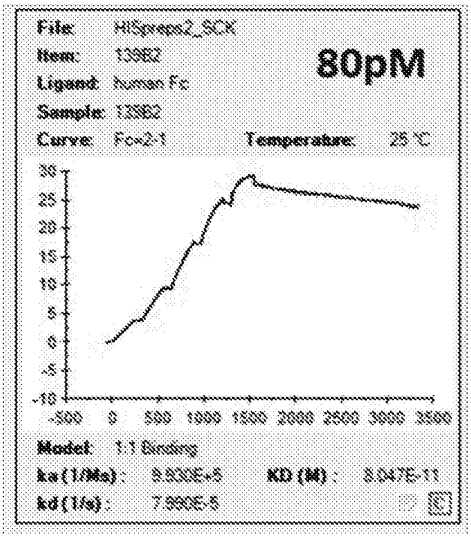


Figure 9

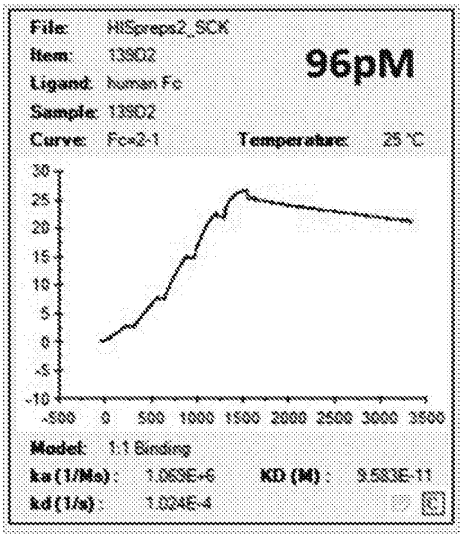
77F3



139B2



139D2



139D7

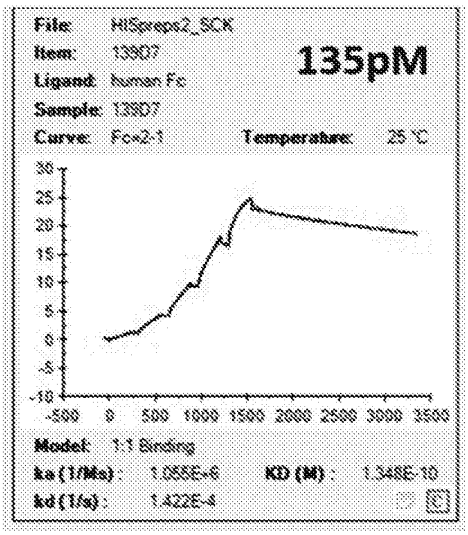


Figure 10

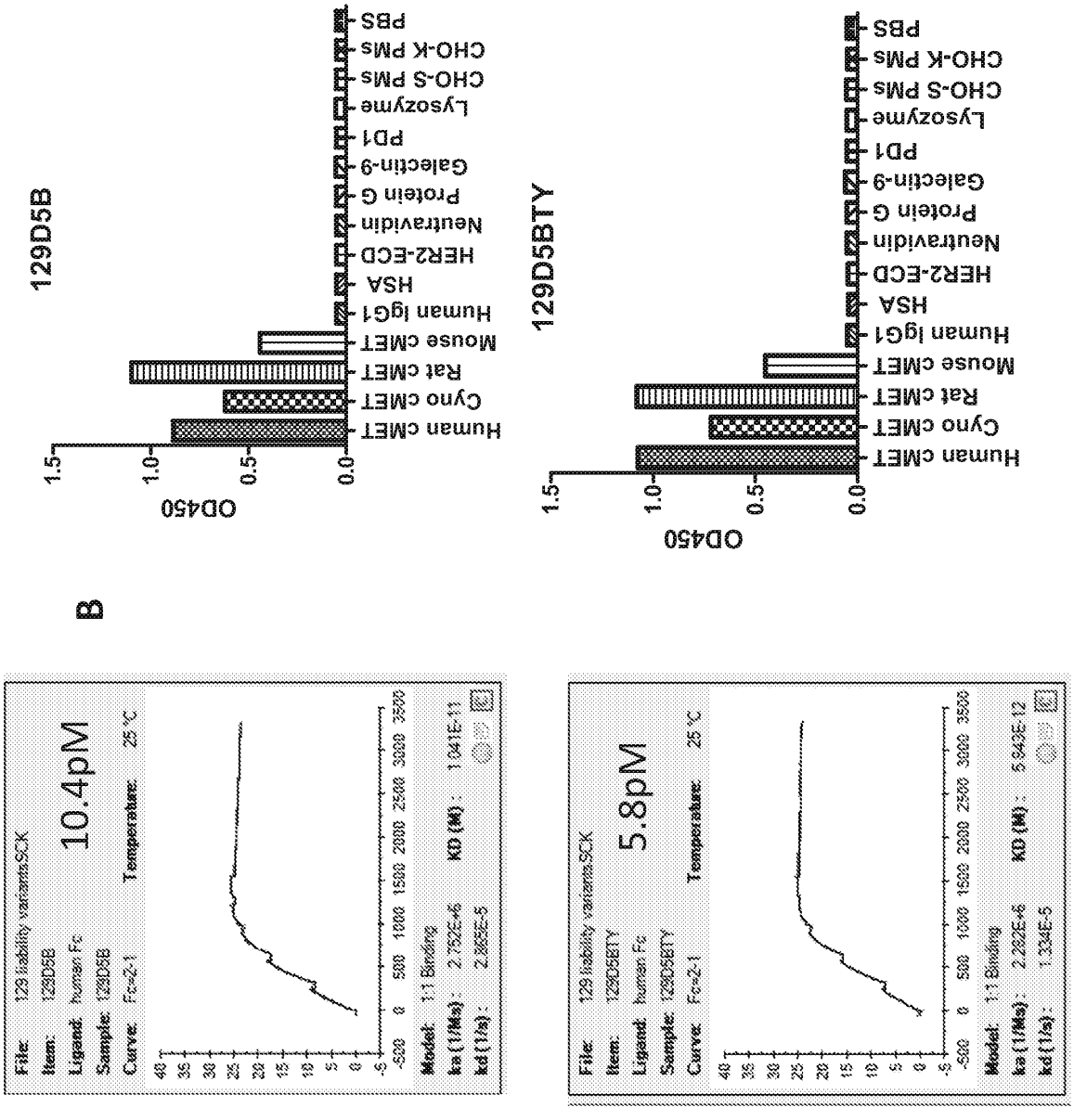

A
B
129D5B
129D5BTY
OD450
1.5
1.0
0.5
0.0
Human cMET
Cyno cMET
Rat cMET
Mouse cMET
Human IgG1
HSA
HER2-ECD
Neutravidin
Protein G
Galectin-9
PD1
Lysozyme
CHO-S PMs
CHO-K PMs
PBS
10.4pM
5.8pM

Figure 11

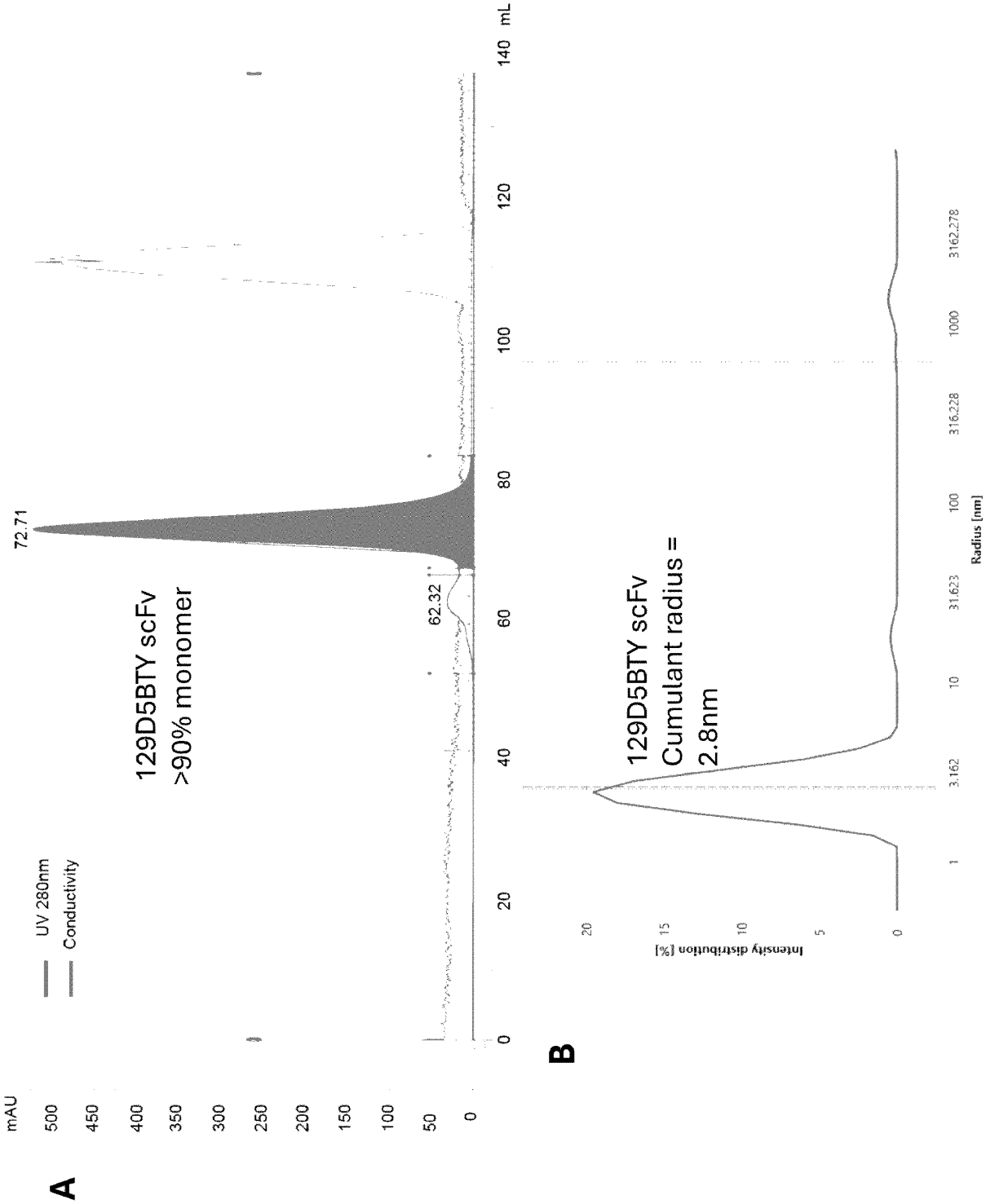
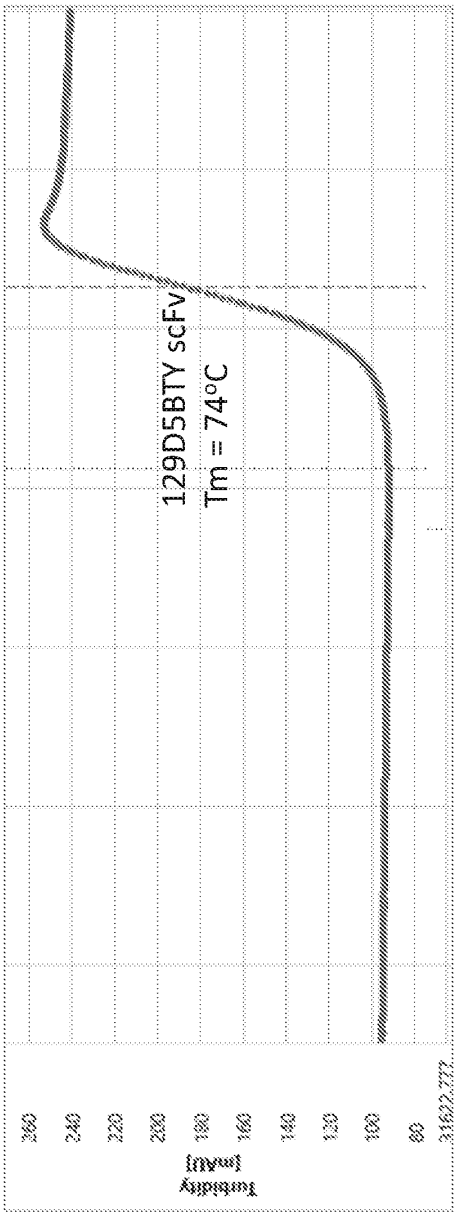

A
mAU
500
450
400
350
300
250
200
150
100
50
0
UV 280nm
Conductivity
72.71
62.32
129D5BTY scFv
>90% monomer
0
20
40
60
80
100
120
140 mL
B
129D5BTY scFv
Cumulant radius =
2.8nm
Intensity distribution [%]
20
15
10
5
0
1
3.162
10
31.623
100
316.228
1000
3162.278
Radius [nm]

Figure 11 (continued)



C

Figure 12

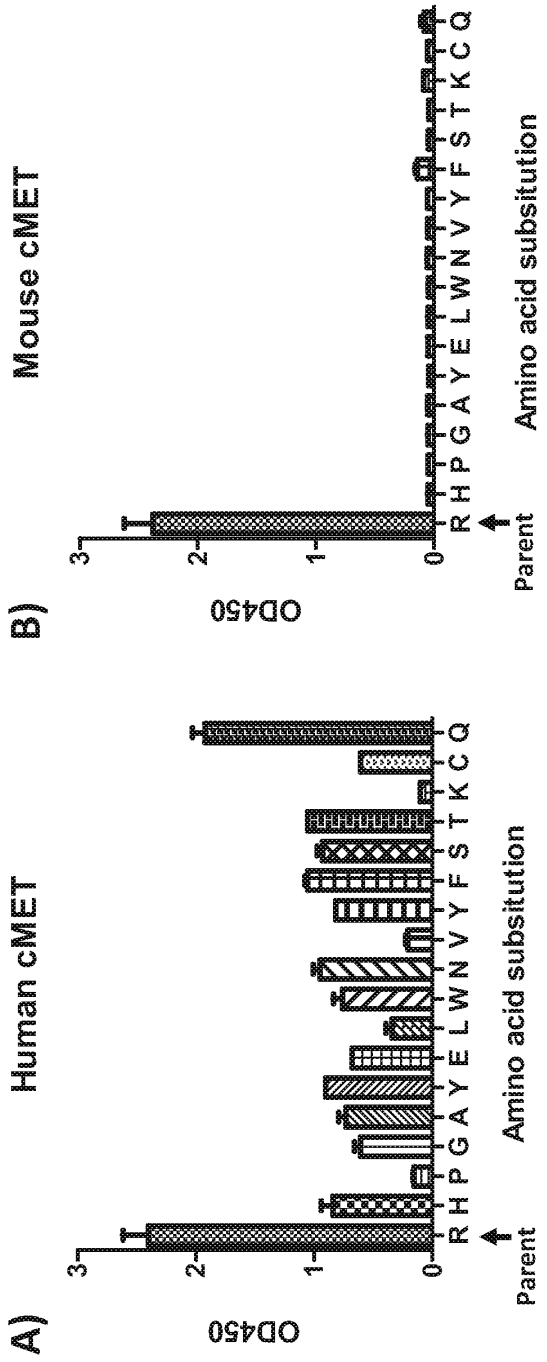
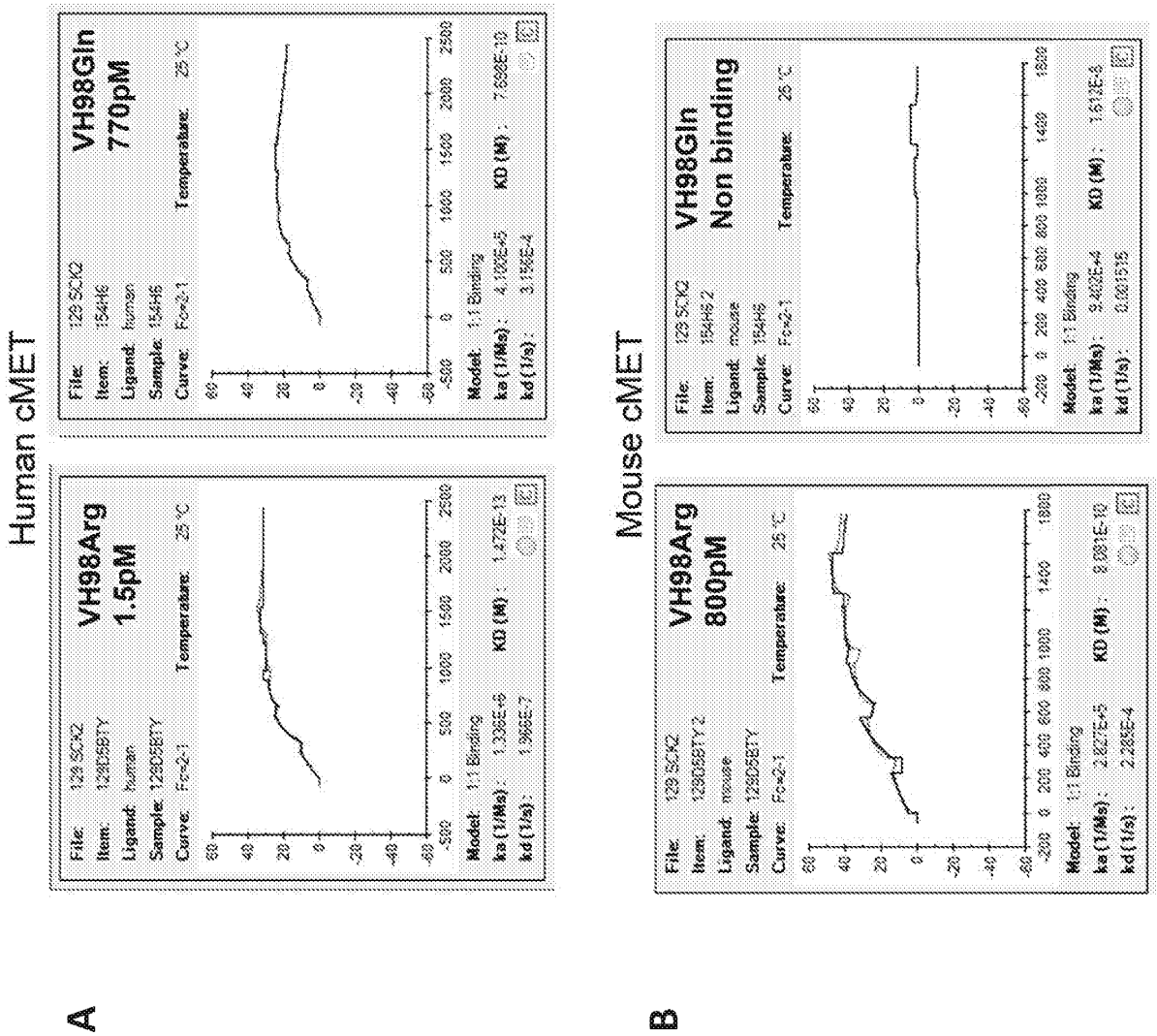

A)
Human cMET
OD450
0
1
2
3
R H P G A Y E L W N V Y F S T K C Q
Parent
Amino acid subsitution
B)
Mouse cMET
OD450
0
1
2
3
R H P G A Y E L W N V Y F S T K C Q
Parent
Amino acid subsitution

Figure 13


A
Human cMET
VH98Arg
1.5pM
VH98Gln
770pM
B
Mouse cMET
VH98Arg
800pM
VH98Gln
Non binding

21/120

Figure 14

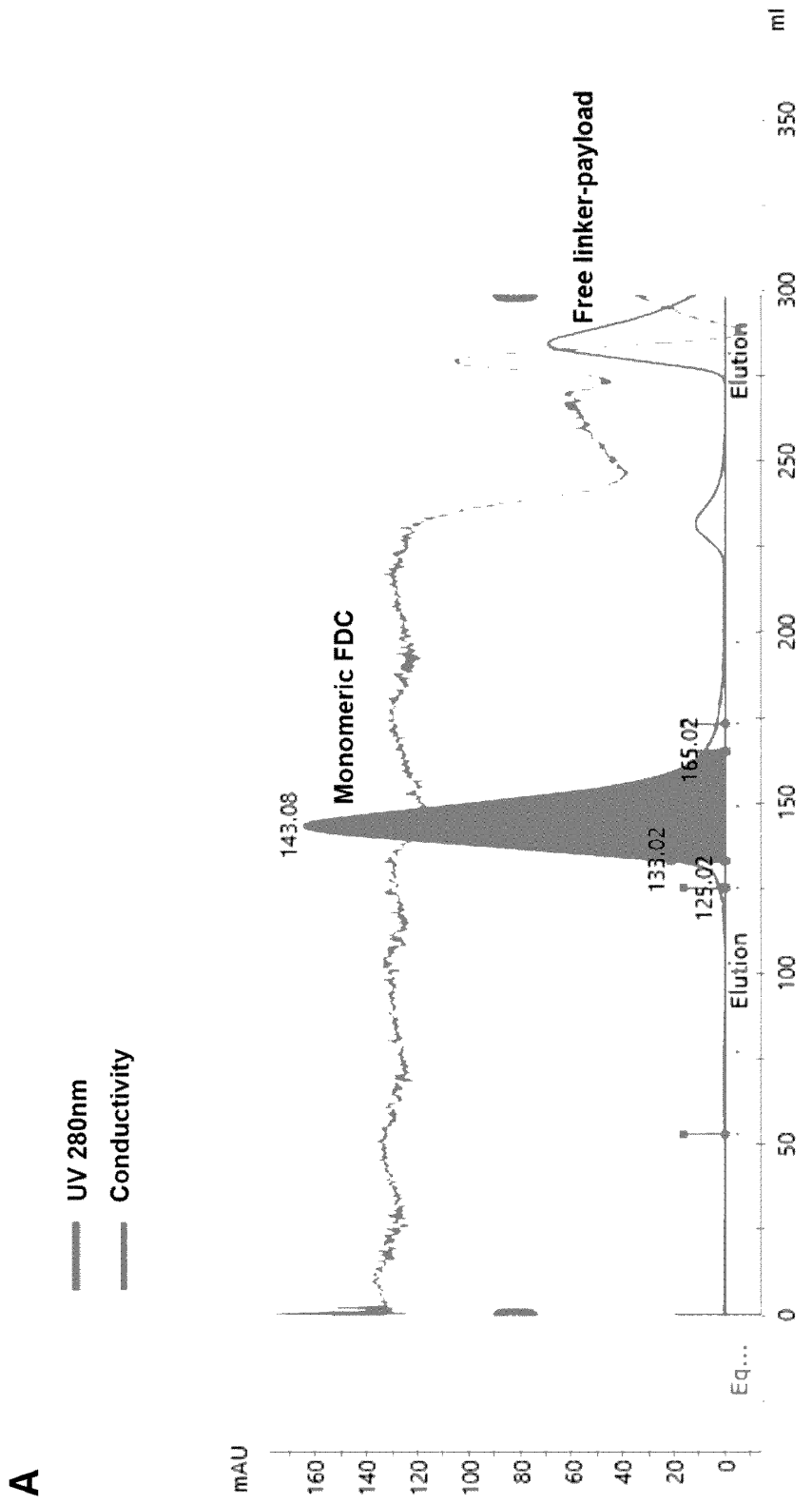
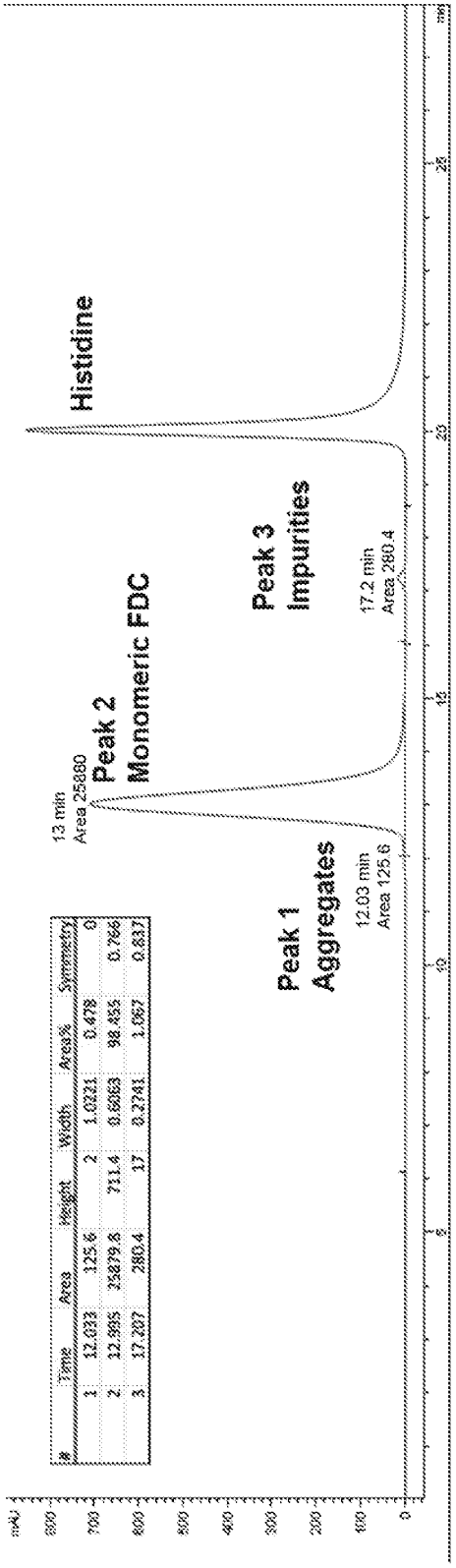


Figure 14 continued

B



#	Time	Area	Height	Width	Area%	Symmetry
1	12.033	125.6	2	1.0231	0.478	0
2	12.995	25879.8	711.4	0.6063	98.455	0.766
3	17.207	280.4	17	0.2741	1.067	0.837

Figure 14 continued

C

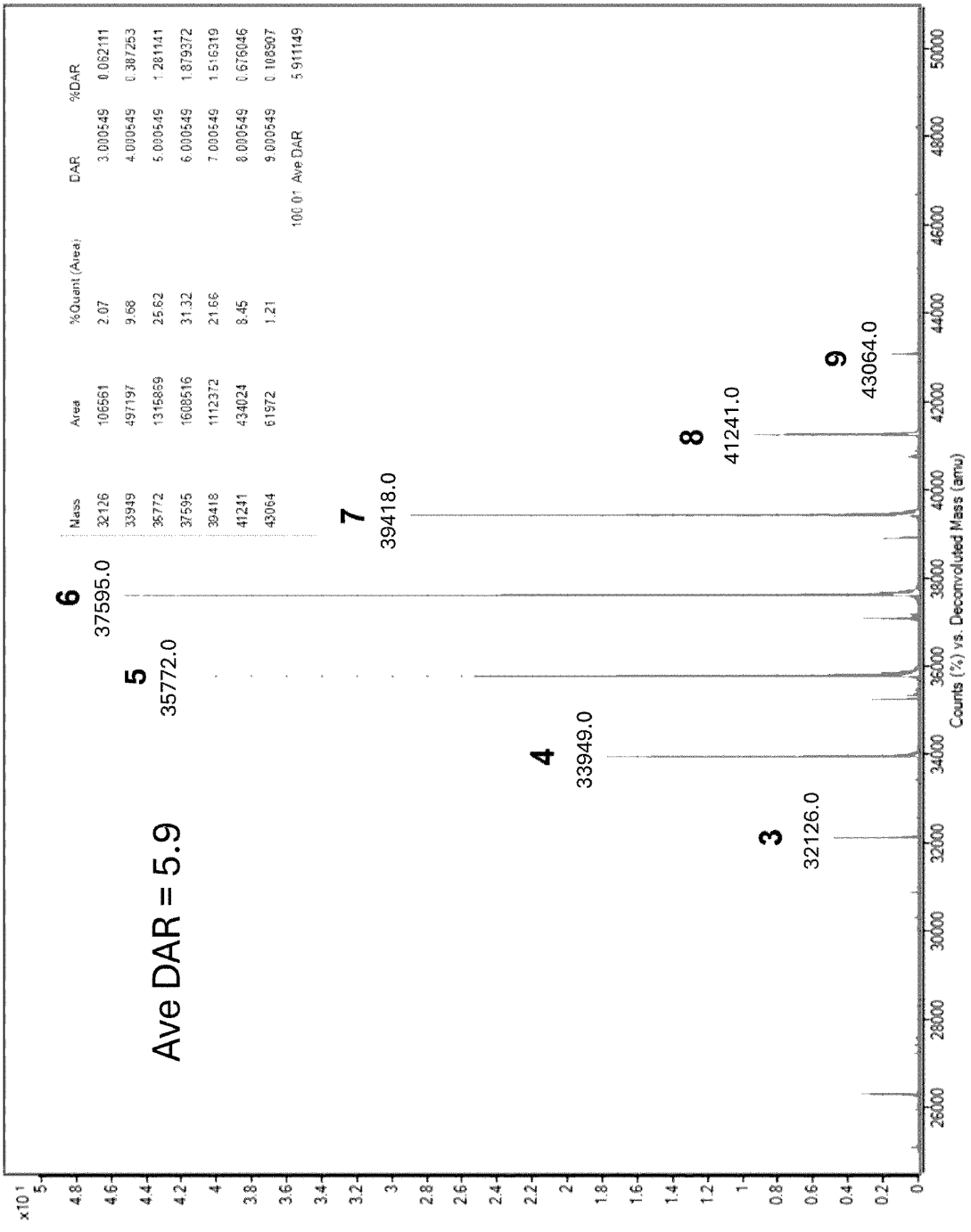


Figure 14 continued

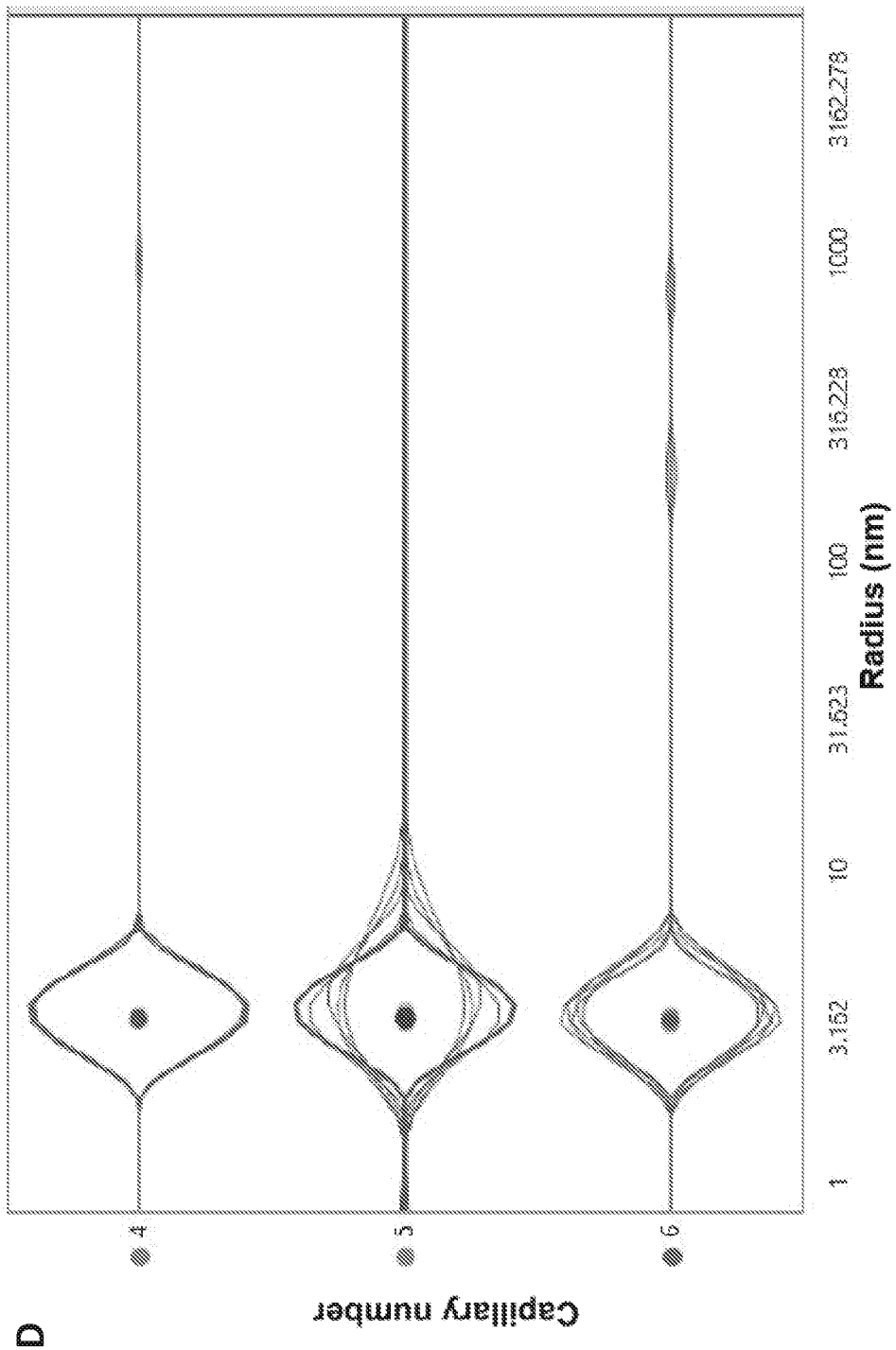
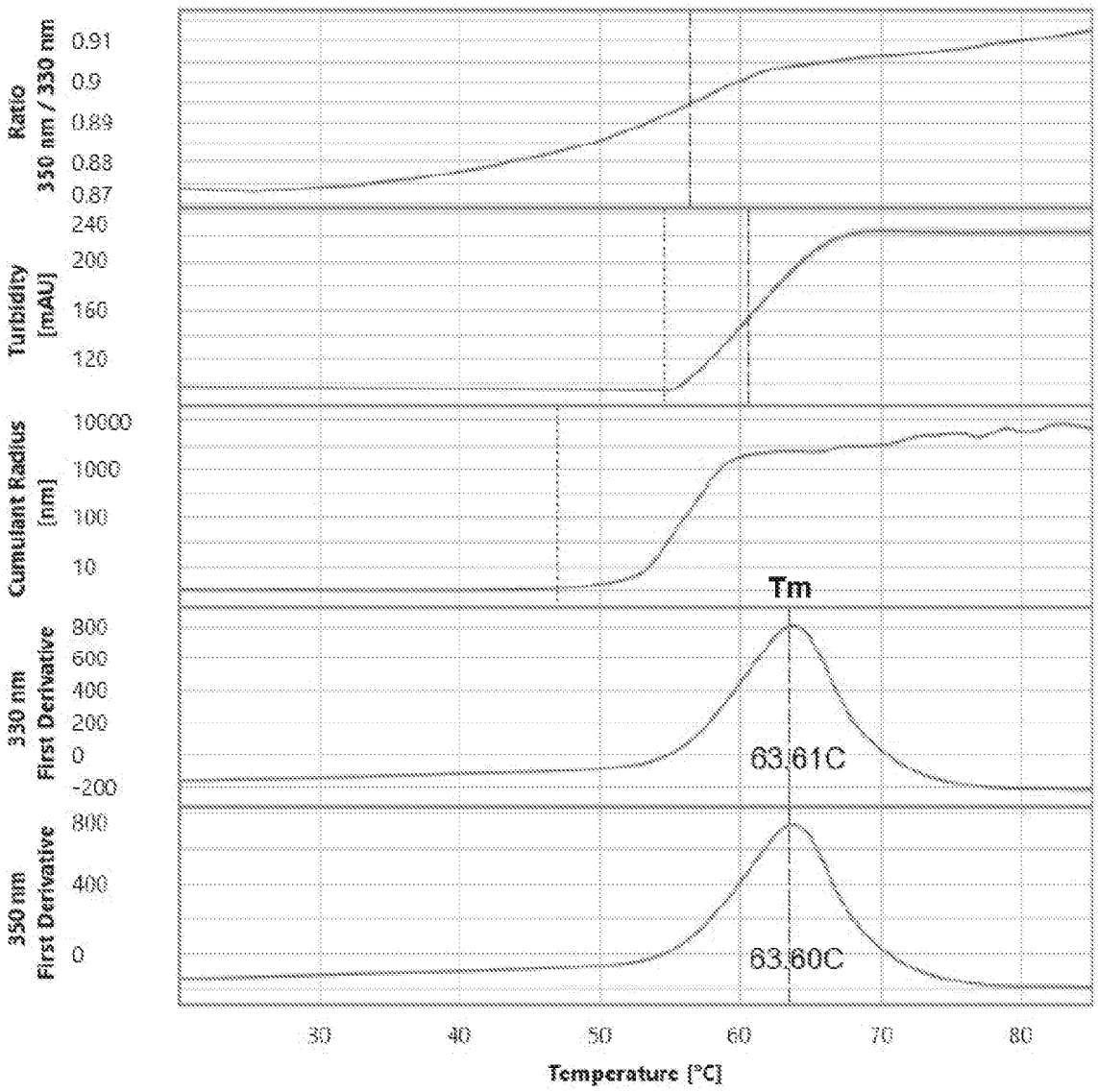


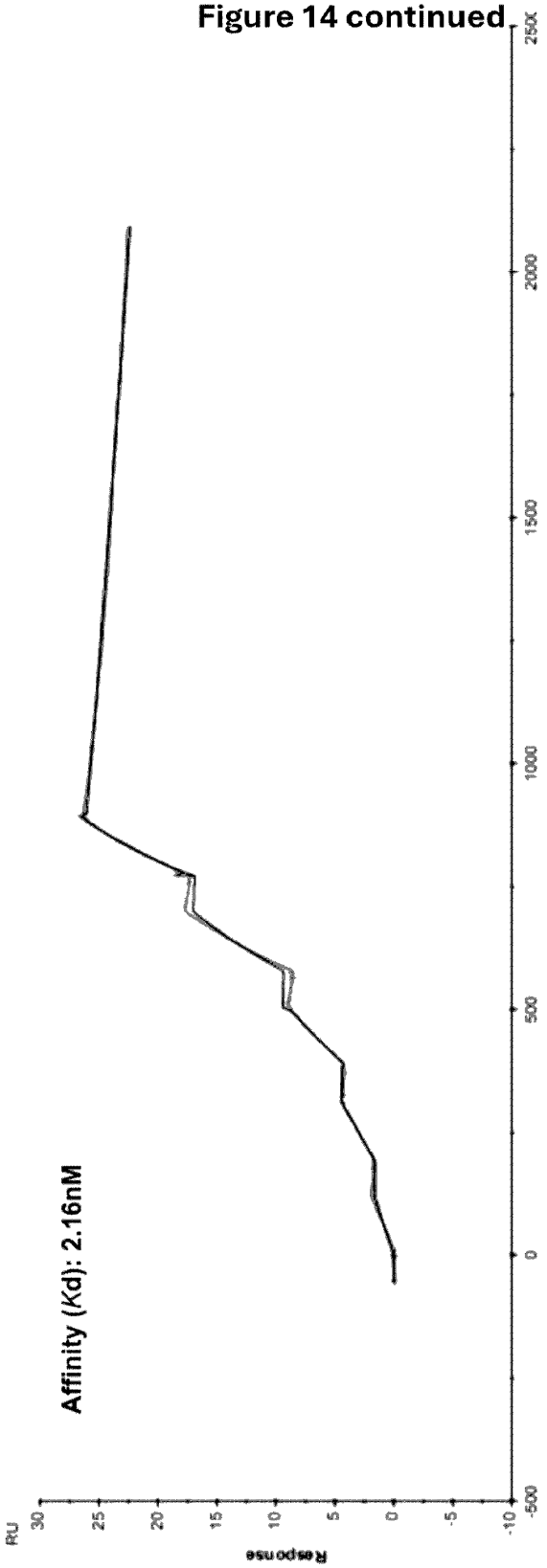
Figure 14 continued



E

Figure 14 continued.

F



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
	5.887E+4	1.269E-4	2.156E-9			3.760E+9				0.0693	1
Cycle: 10				34.94	6.490E-9		30.00	1.168E+10	0.06334		
					1.298E-8				0.01330		
					2.597E-8				-0.4294		
					5.194E-8				-0.1615		
					1.039E-7				0.4649		

Figure 15

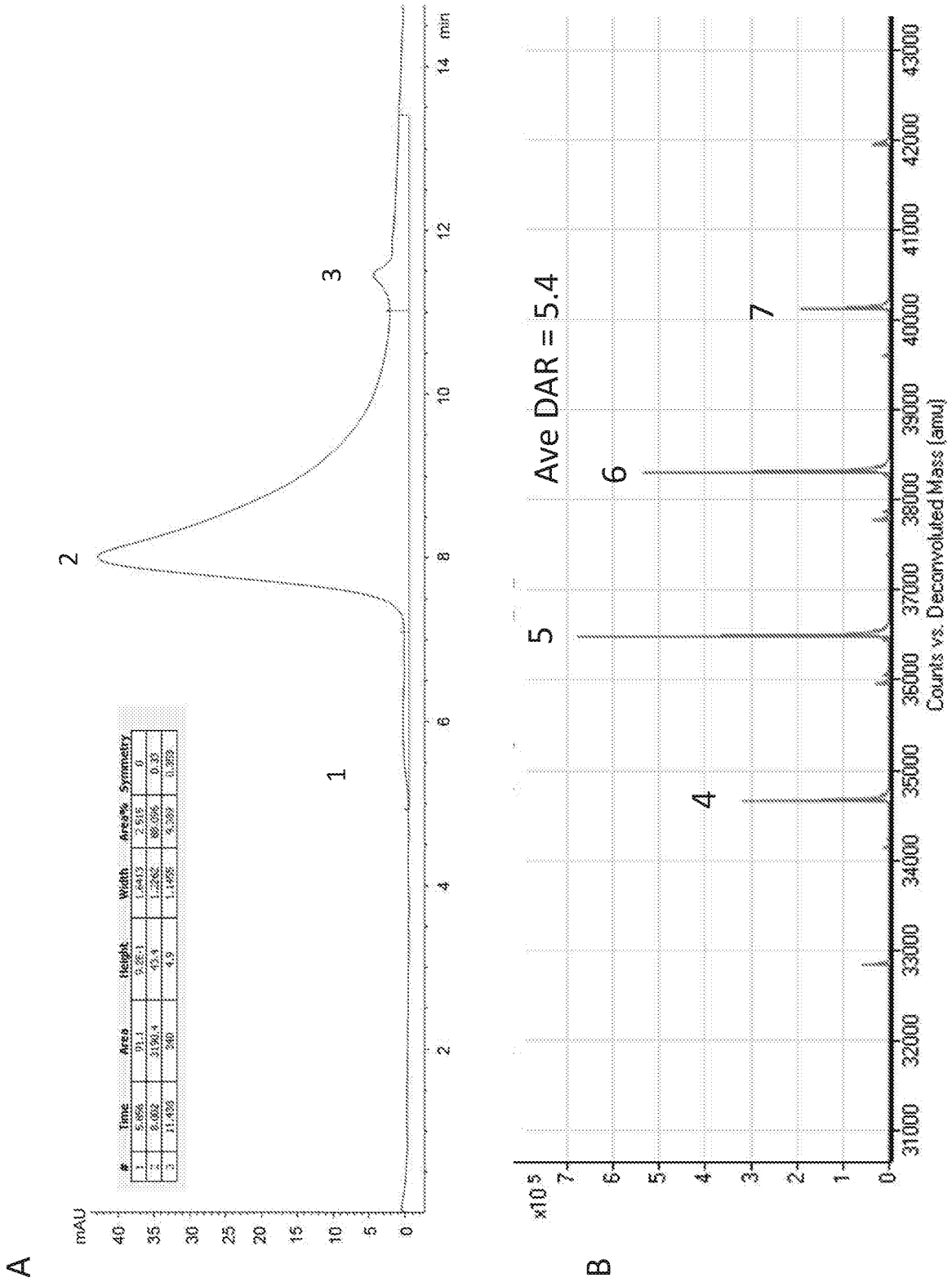
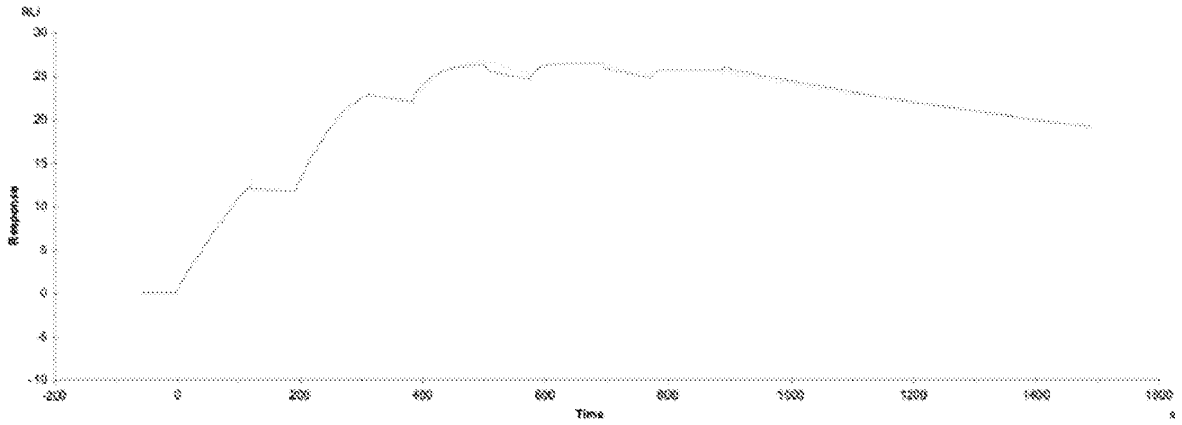

A
mAU
40
35
30
25
20
15
10
5
0
2
4
6
8
10
12
14
min
1
2
3
B
x10 5
7
6
5
4
3
2
1
0
31000
32000
33000
34000
35000
36000
37000
38000
39000
40000
41000
42000
43000
Counts vs. Deconvoluted Mass (amu)
Ave DAR = 5.4
4
5
6
7

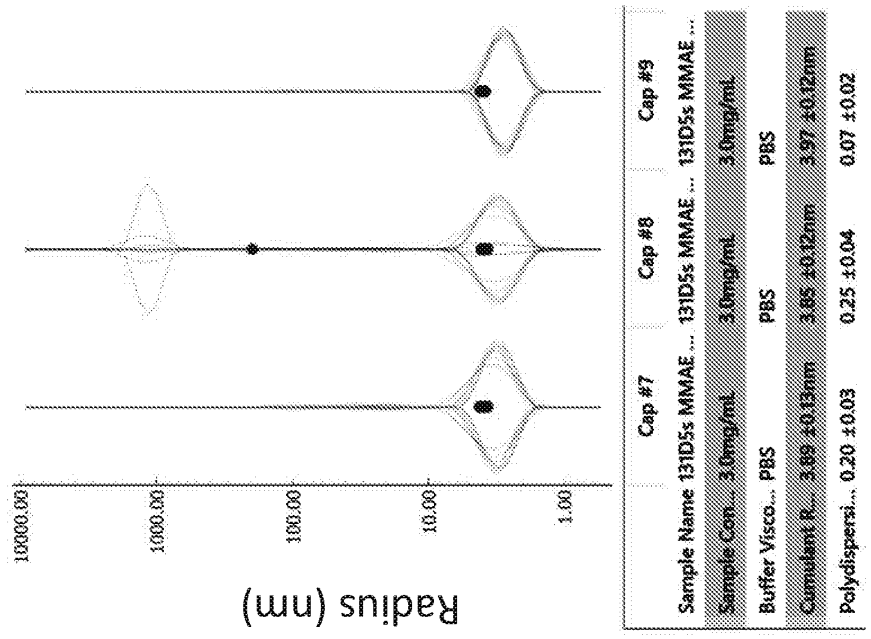
Figure 15 continued

KD = 112pM



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
	5.144E+6	5.762E-4	1.120E-10			5.036E+7				0.105	1
Cycle: 8				26.01	1.676E-9		30.00	1.565E+8	0.4423		
					3.353E-9				0.1193		
					6.705E-9				0.6144		
					1.341E-8				0.5142		
					2.682E-8				-0.2512		

D



C

Figure 16

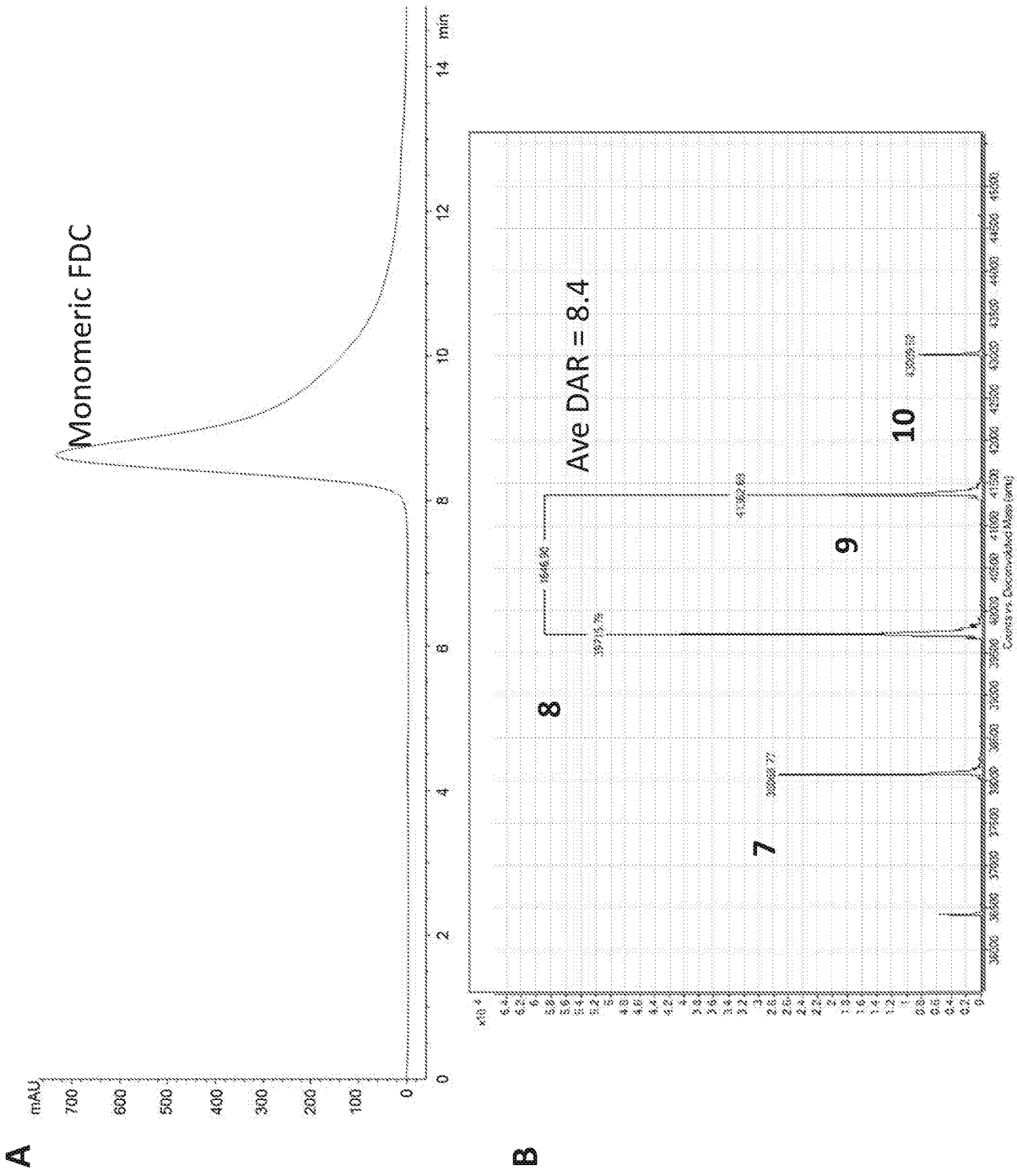

A
mAU
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
min
Monomeric FDC
B
Ave DAR = 8.4
7
8
9
10

Figure 17

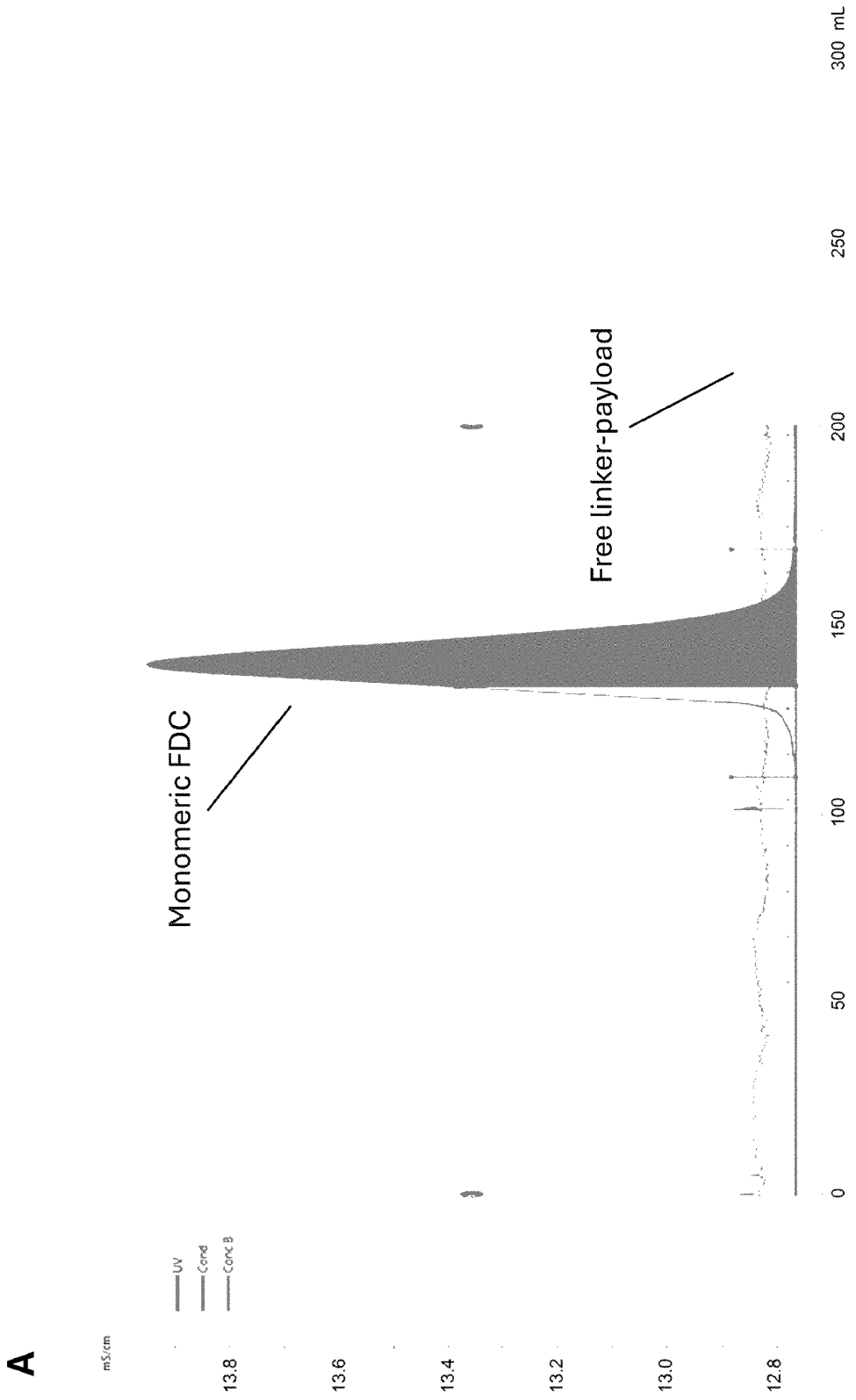

A
mS/cm
UV
Cond
Conc B
13.8
13.6
13.4
13.2
13.0
12.8
Monomeric FDC
Free linker-payload
0
50
100
150
200
250
300 mL

Figure 17 continued

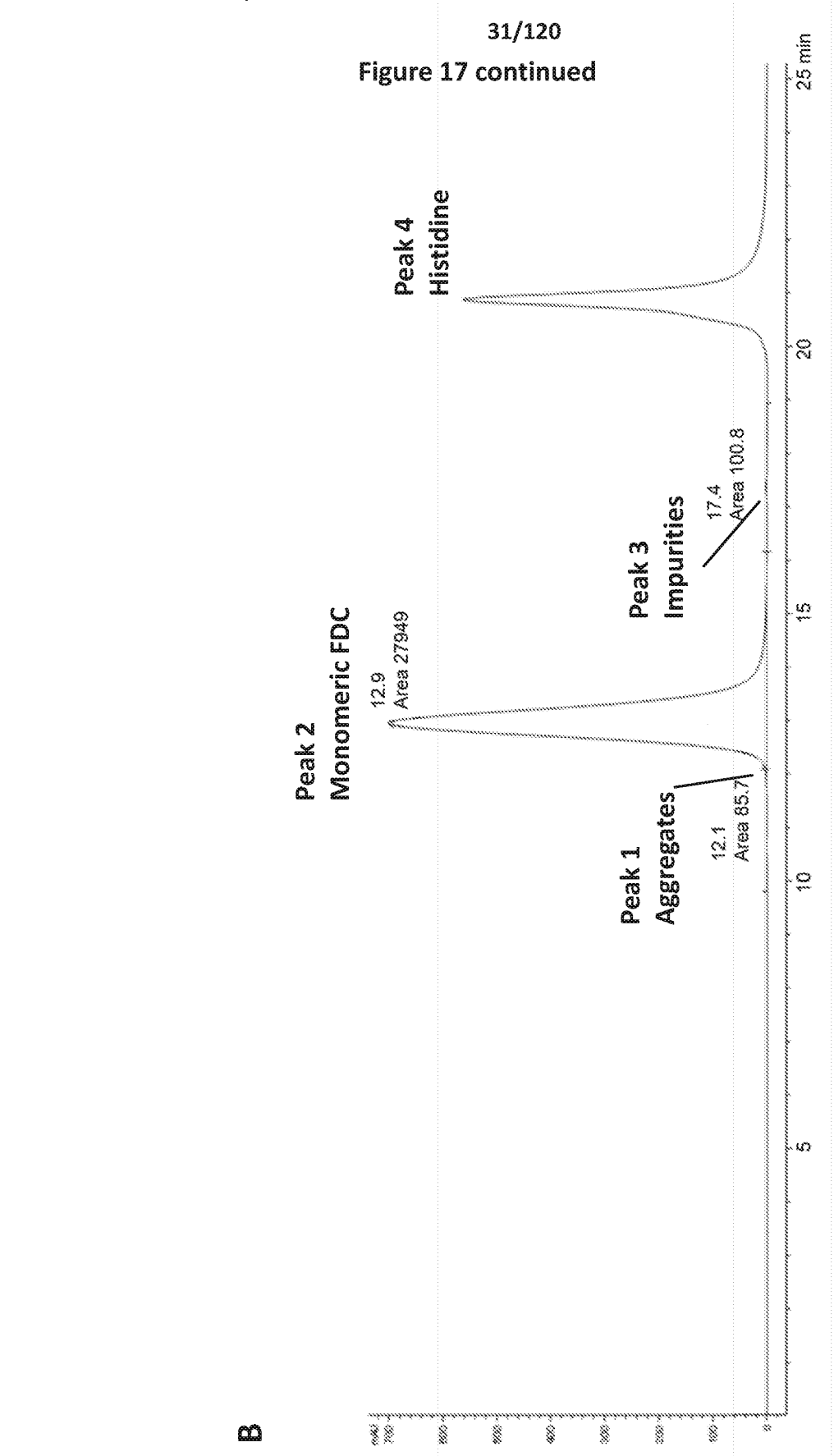


Figure 17 continued

C

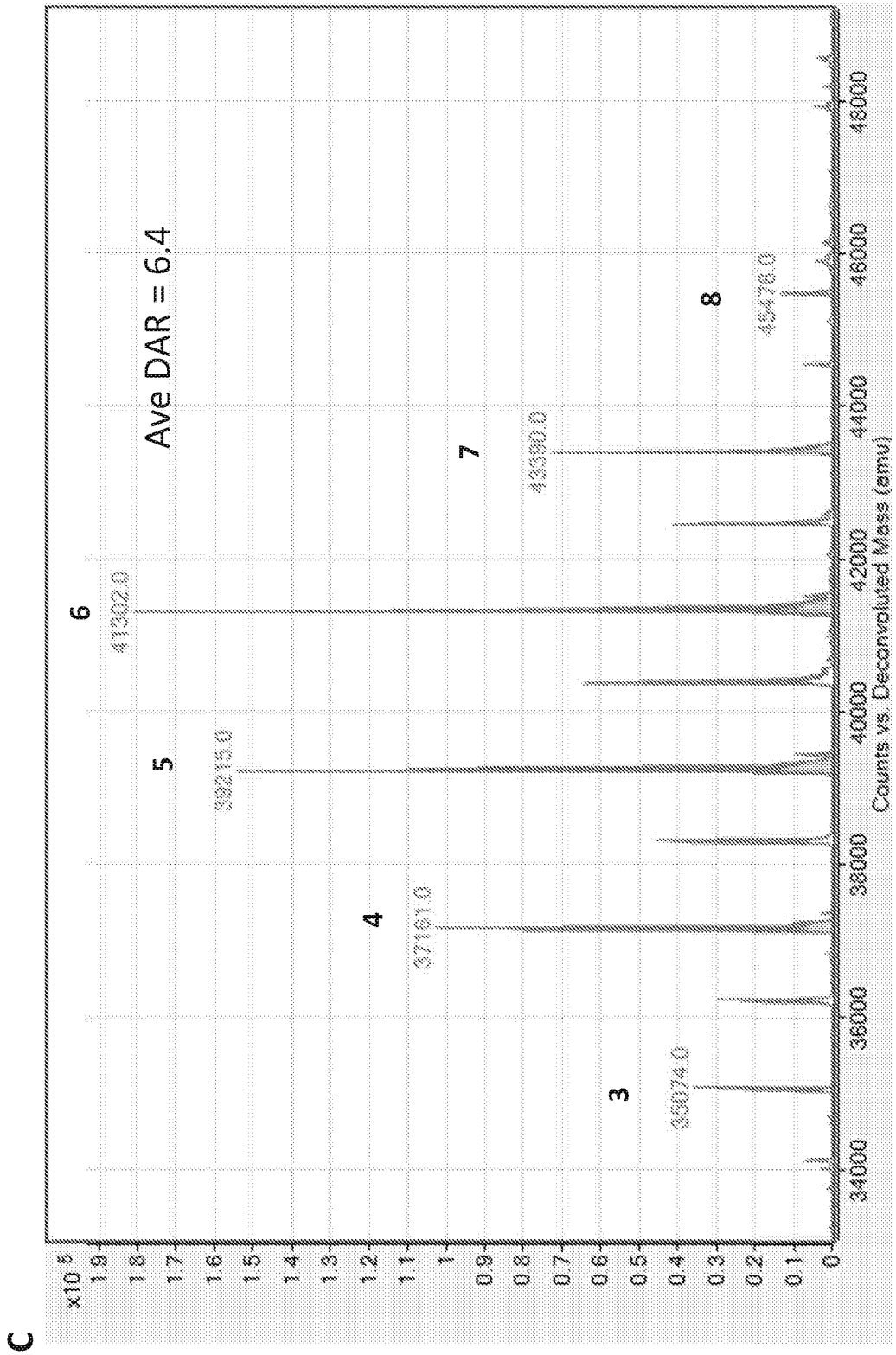
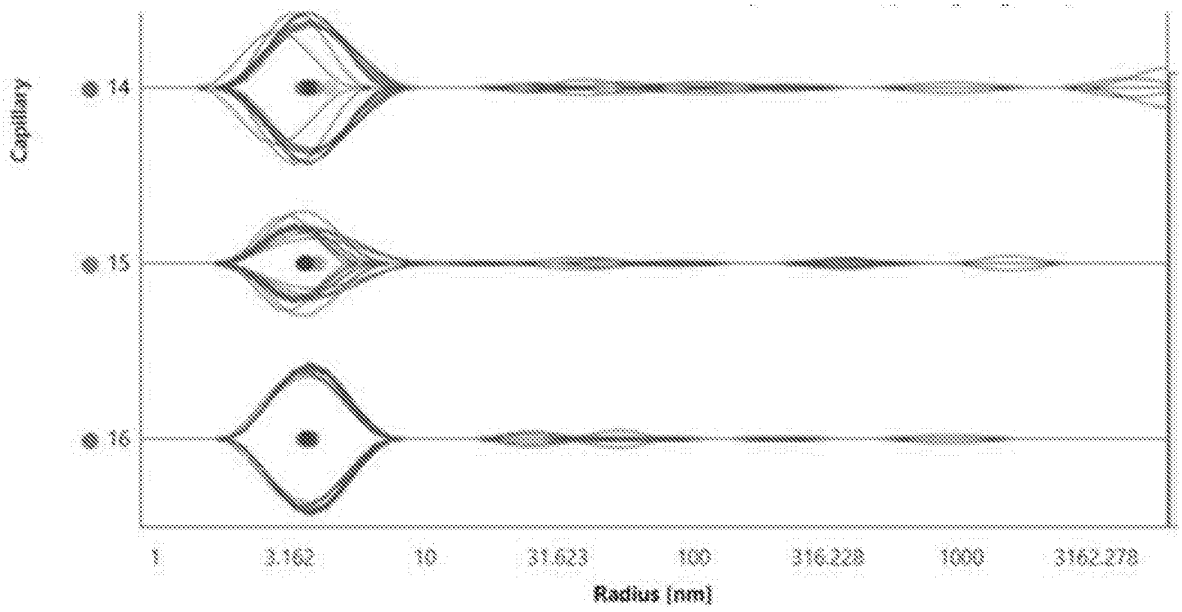
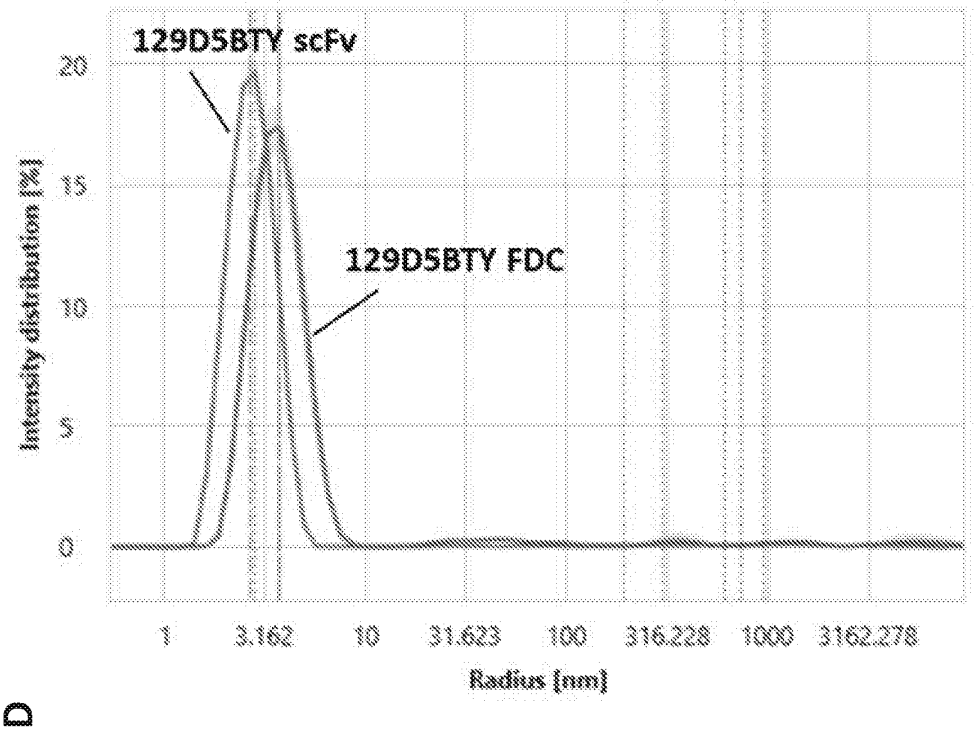


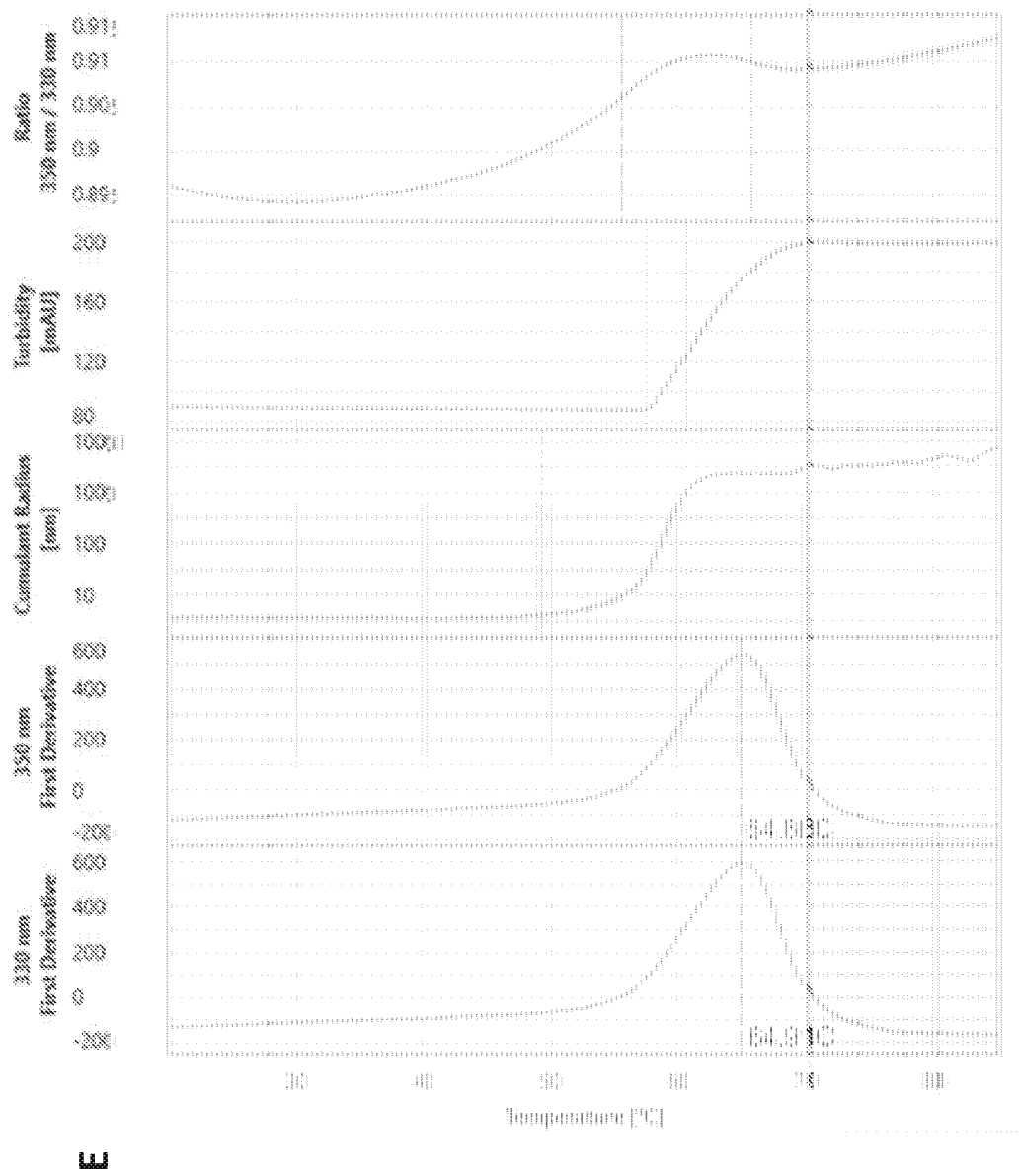
Figure 17 continued





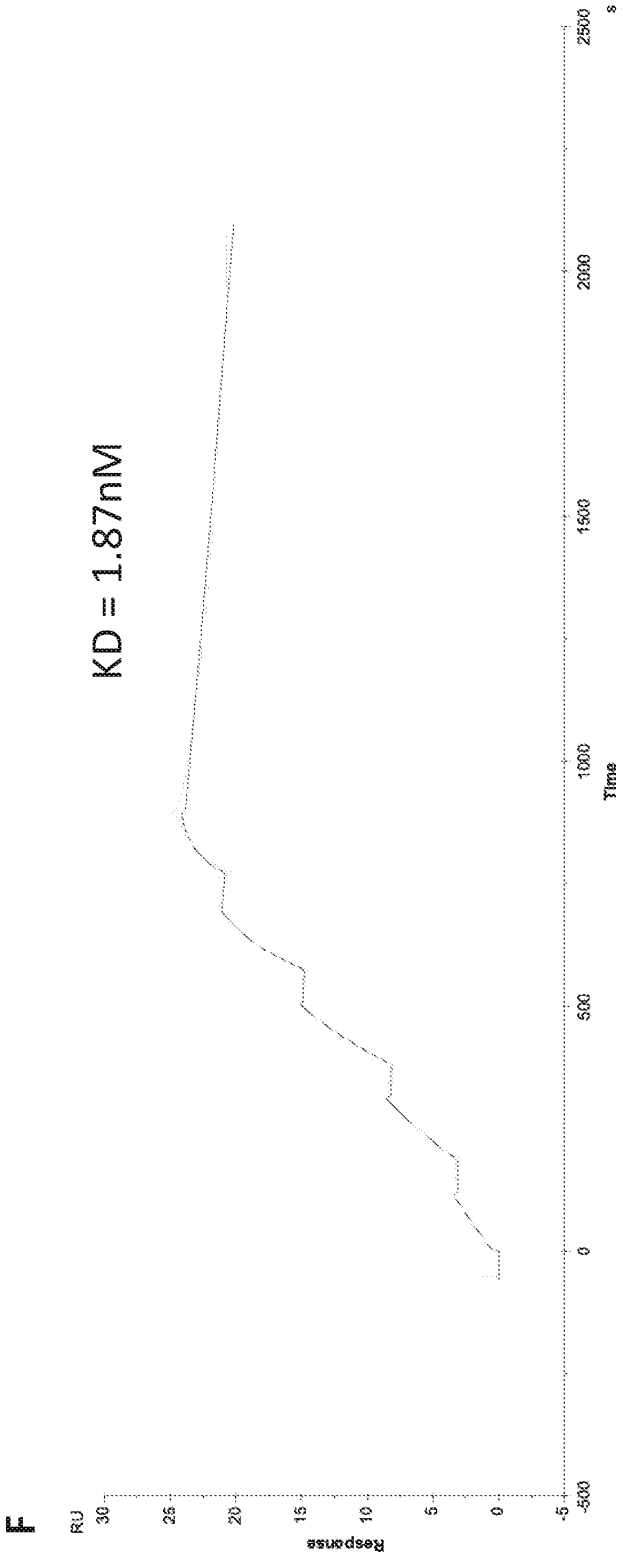
D

Figure 17 continued



FIG

Figure 17 continued



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	Rt (RU)	Chi² (RU²)	U-value
Cycle: 8	7.428E+4	1.386E-4	1.866E-9	24.38	1.564E-8	1.486E+17	30.00	4.649E+17	0.4415	0.0410	1
					3.128E-8				0.3881		
					6.257E-8				0.2309		
					1.252E-7				0.1415		
					2.503E-7				0.2867		

Figure 18

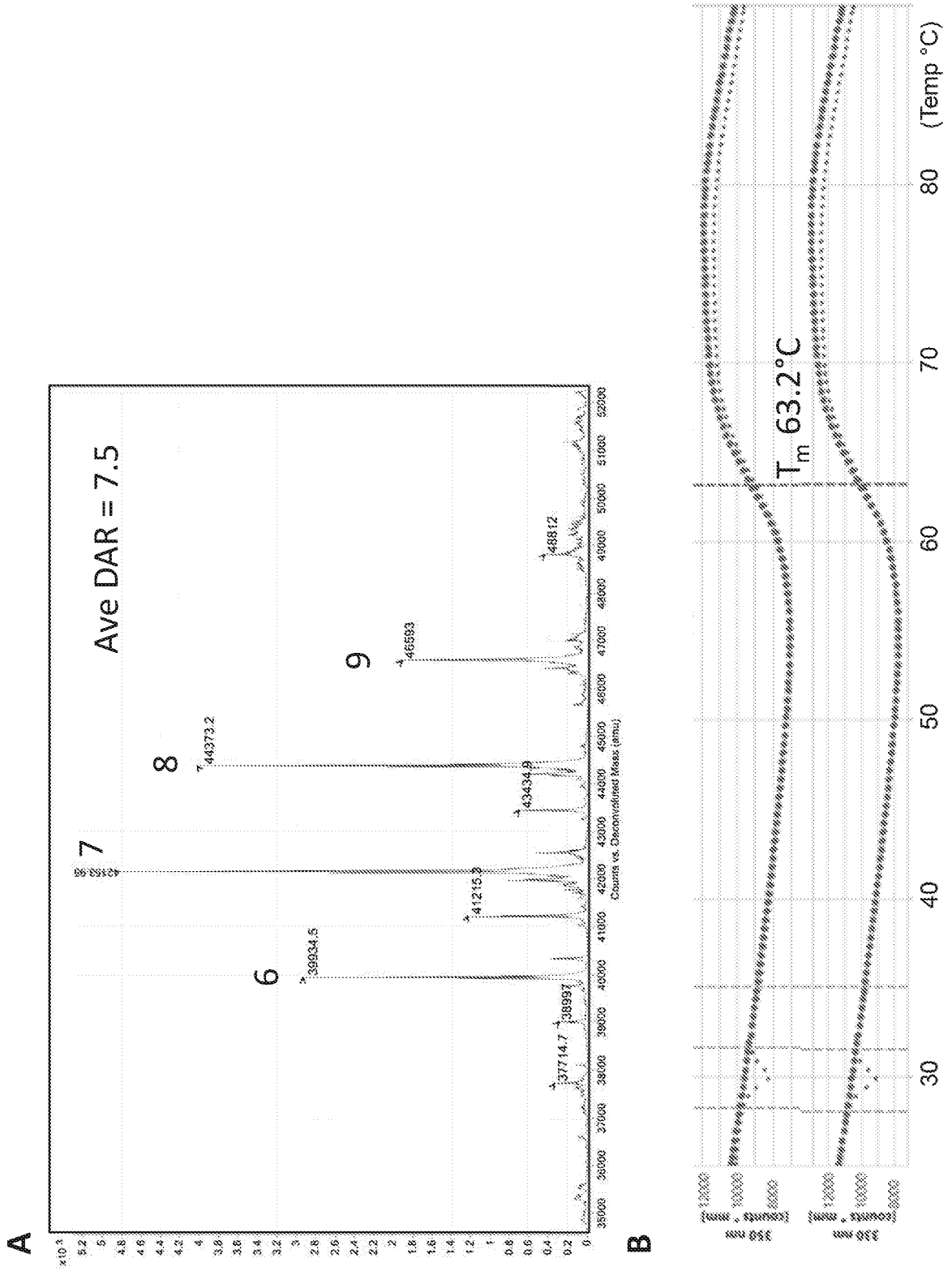
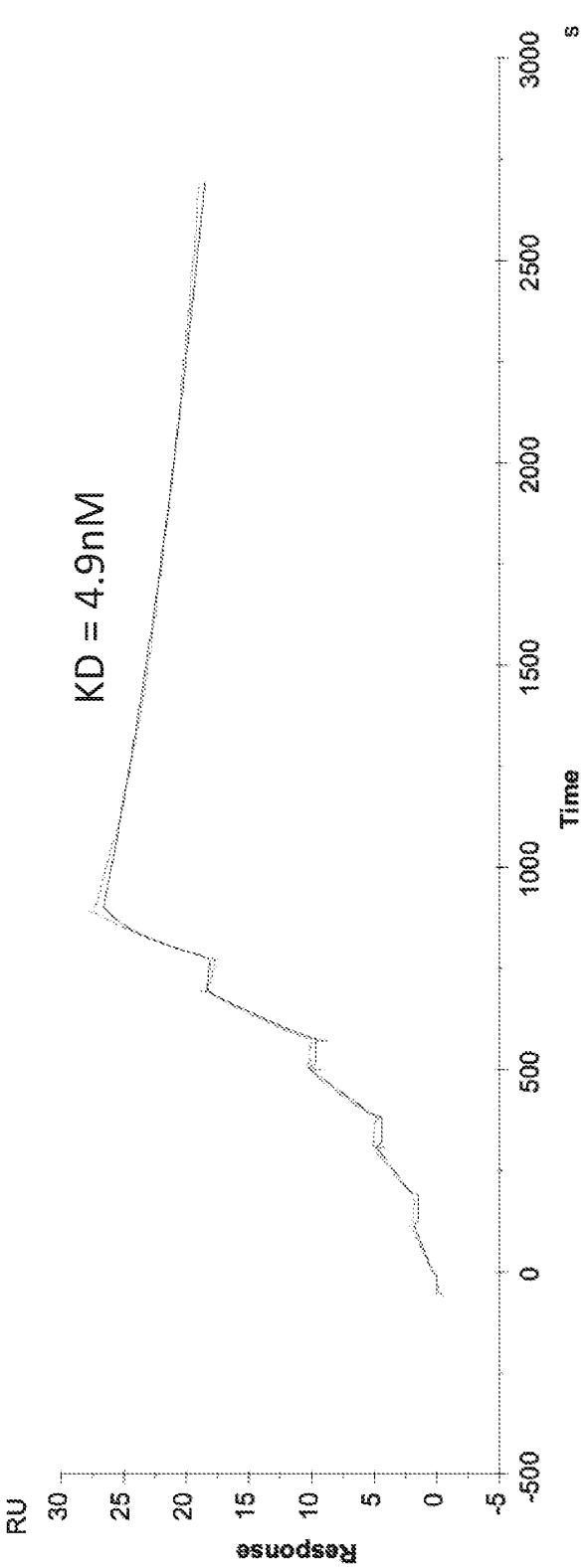

A
Ave DAR = 7.5
6
7
8
9
39934.5
41215.3
43434.9
44373.2
46593
48812
(Counts vs. Deconvoluted Mass (amu))
B
T_m 63.2°C
(Temp °C)

Figure 18 continued

C



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
	5.781E+4	2.845E-4	4.921E-9			2.929E+5				0.0587	1
Cycle: 5				28.70	2.169E-8		30.00	9.102E+5	0.3450		
					4.338E-8				0.4898		
					8.676E-8				0.5141		
					1.735E-7				0.08424		
					3.470E-7				-0.3162		

Figure 19

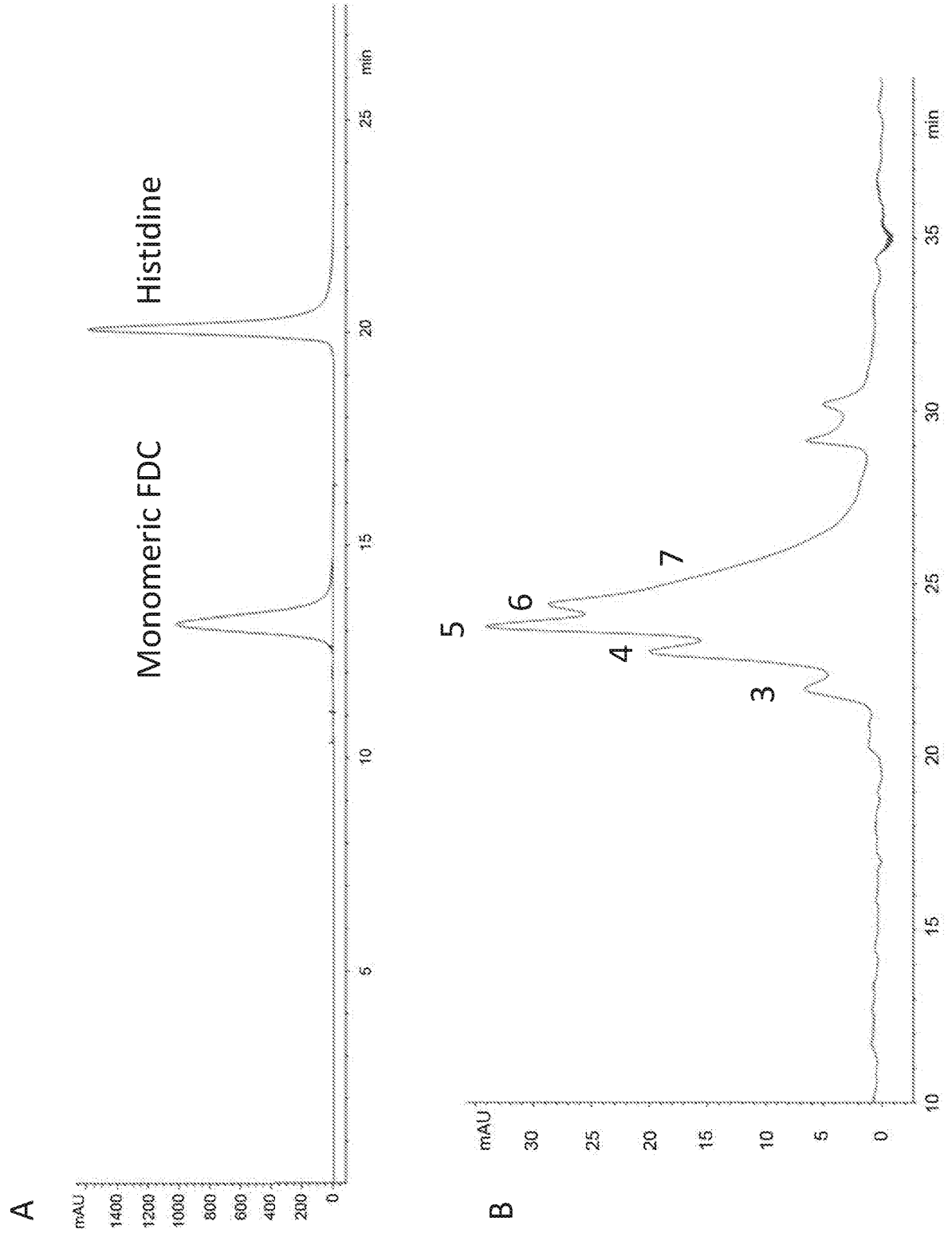

A
mAU
1400
1200
1000
800
600
400
200
0
5
10
15
20
25
min
Histidine
Monomeric FDC
B
mAU
30
25
20
15
10
5
0
10
15
20
25
30
35
min
3
4
5
6
7

Figure 19 continued

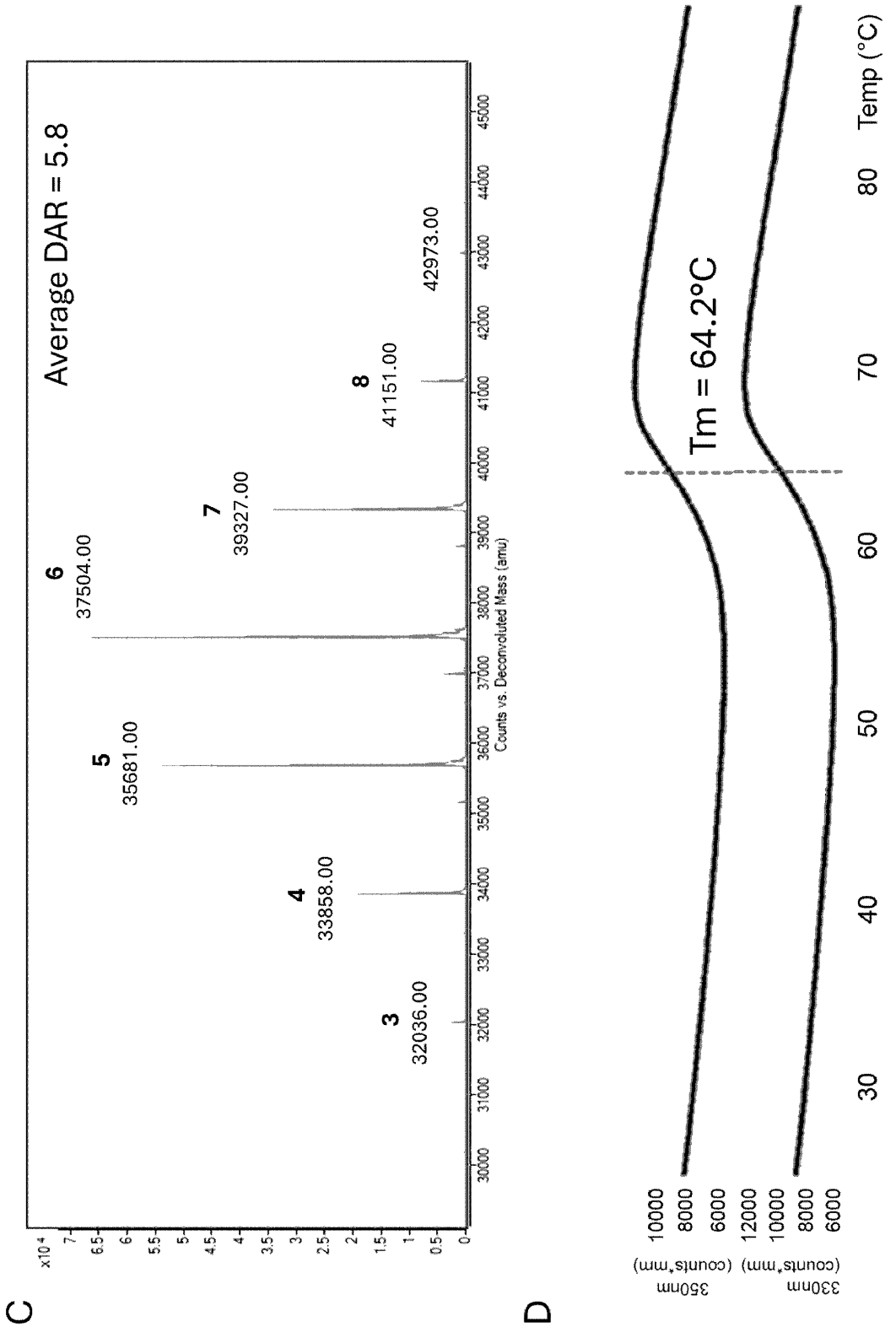
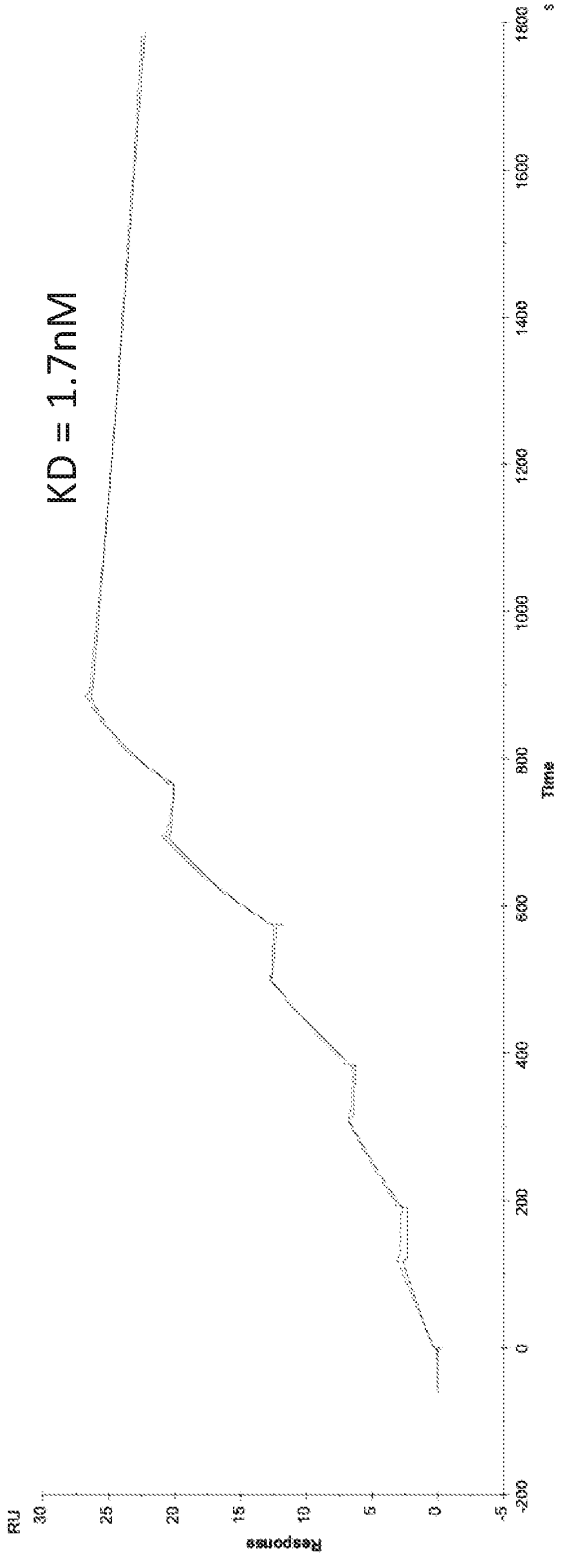


Figure 19 continued

E



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
						1.757E+17				0.8281	1
Cycle: 6	1.058E+5	1.839E-4	1.738E-9	28.81	6.731E-9		30.00	5.461E+17	0.3918		
					1.34E-8				0.5180		
					2.692E-8				0.2343		
					5.385E-8				0.2683		
					1.077E-7				0.1822		

Figure 20

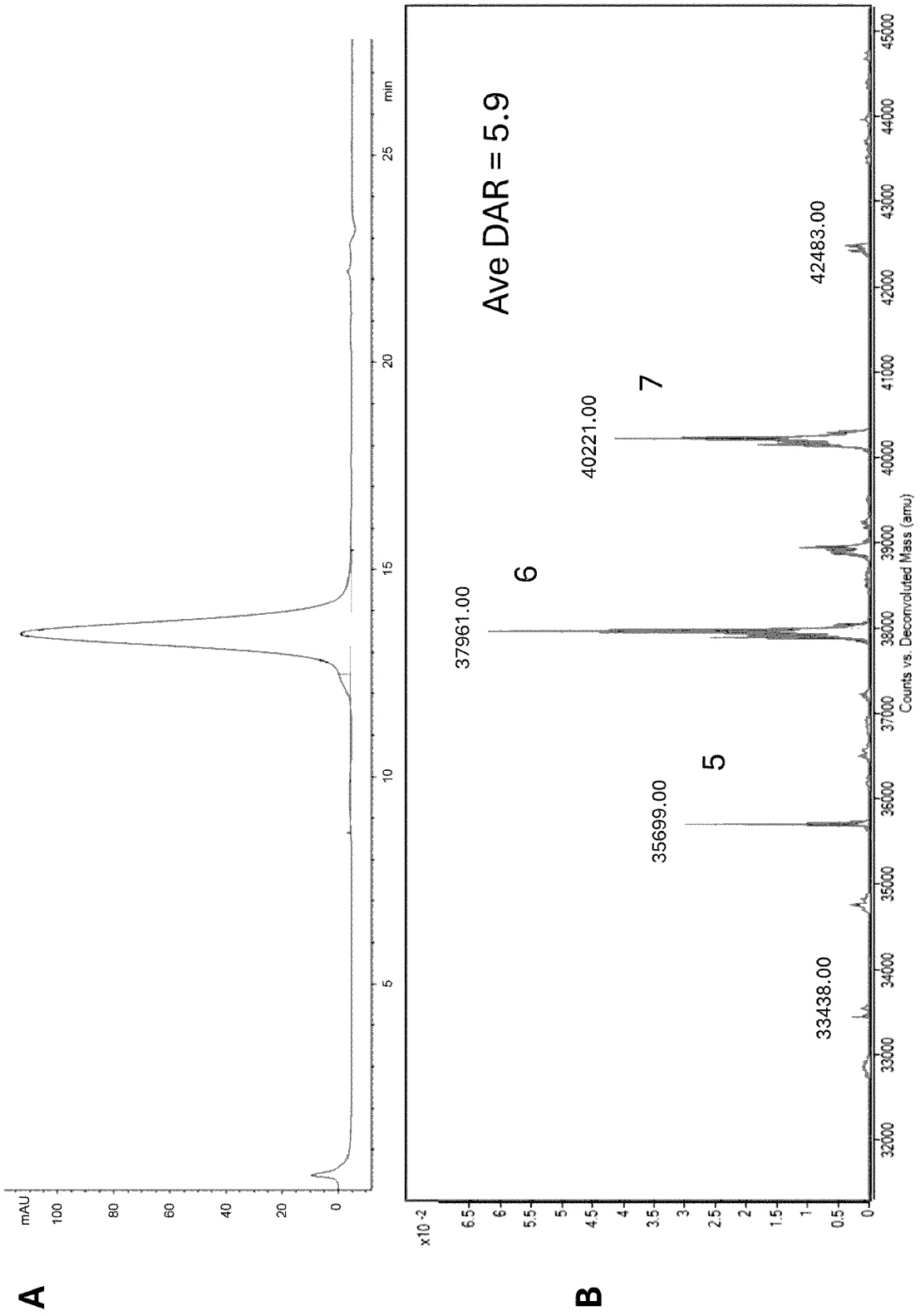

A
mAU
100
80
60
40
20
0
5
10
15
20
25
min
B
x10-2
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
32000
33000
34000
35000
36000
37000
38000
39000
40000
41000
42000
43000
44000
45000
Counts vs. Deconvoluted Mass (amu)
Ave DAR = 5.9
33438.00
35699.00
37961.00
40221.00
42483.00
5
6
7

Figure 20 continued

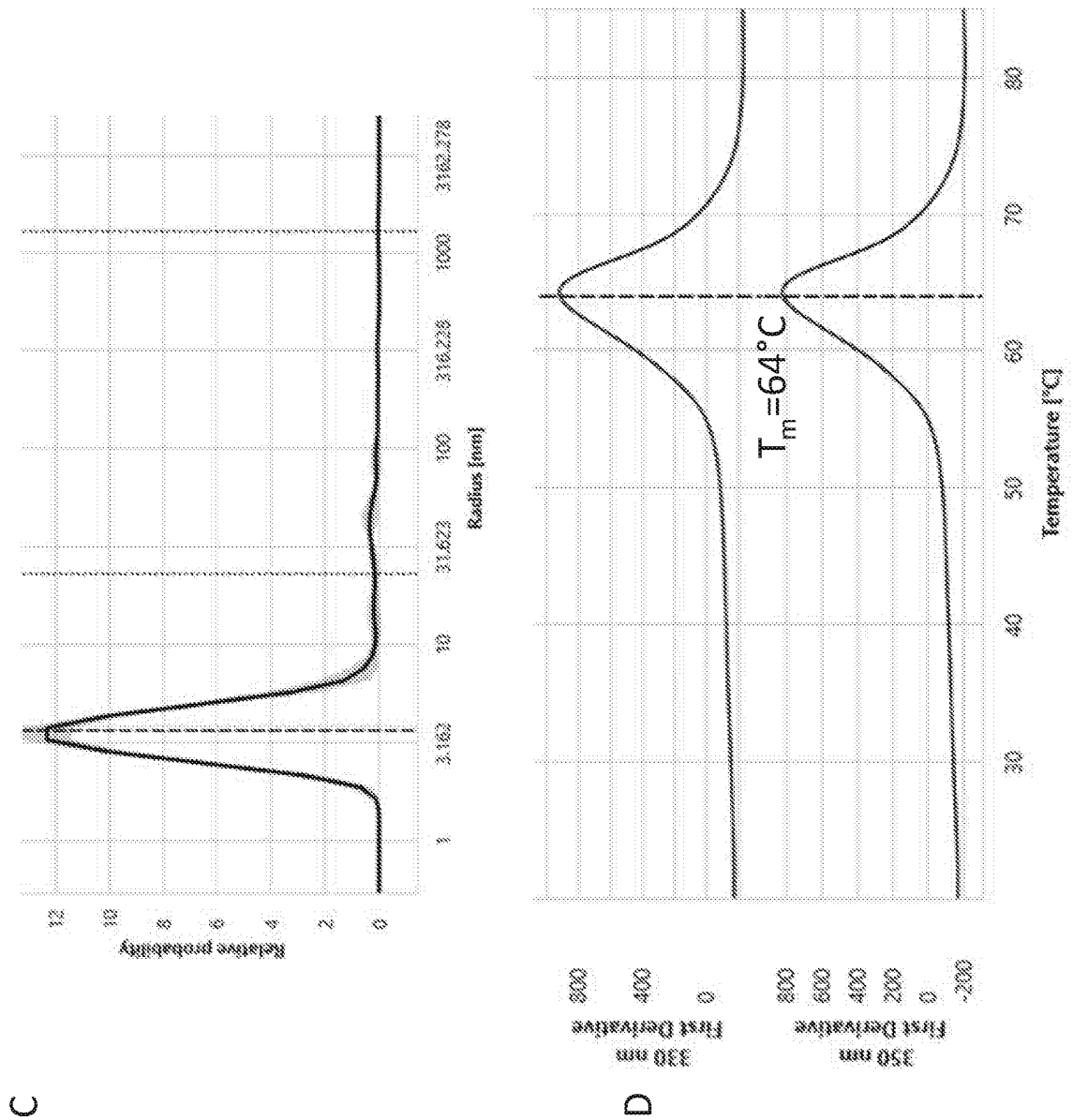
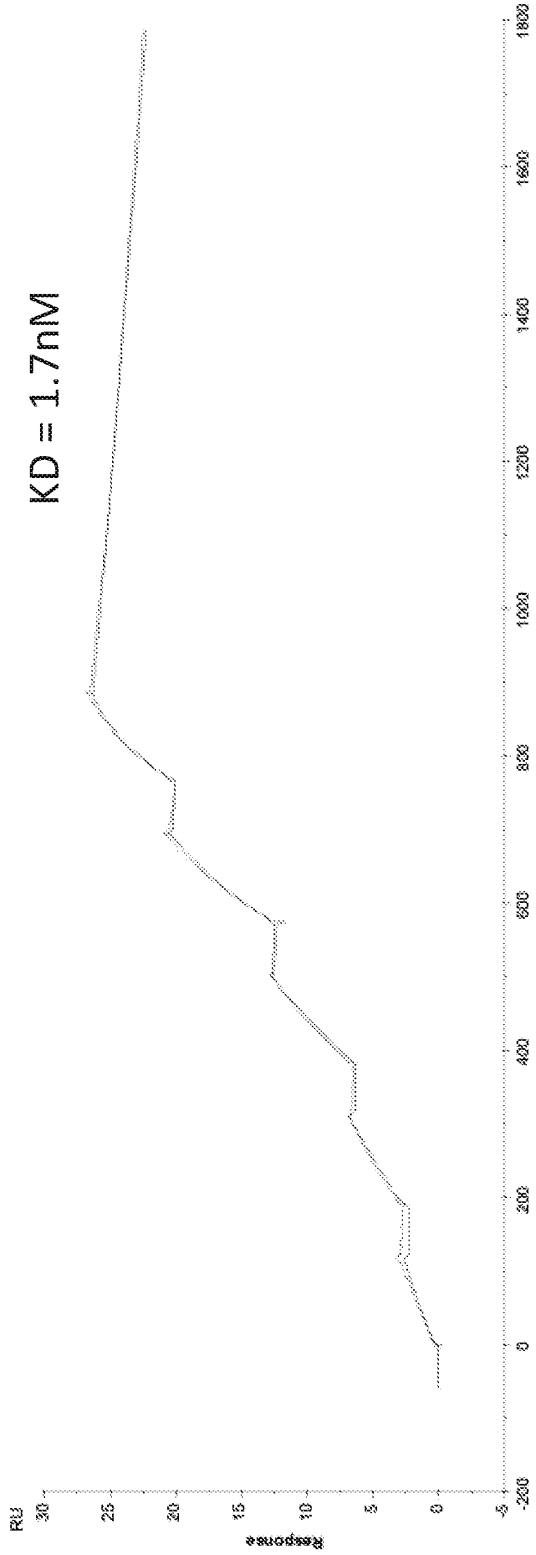


Figure 20 continued

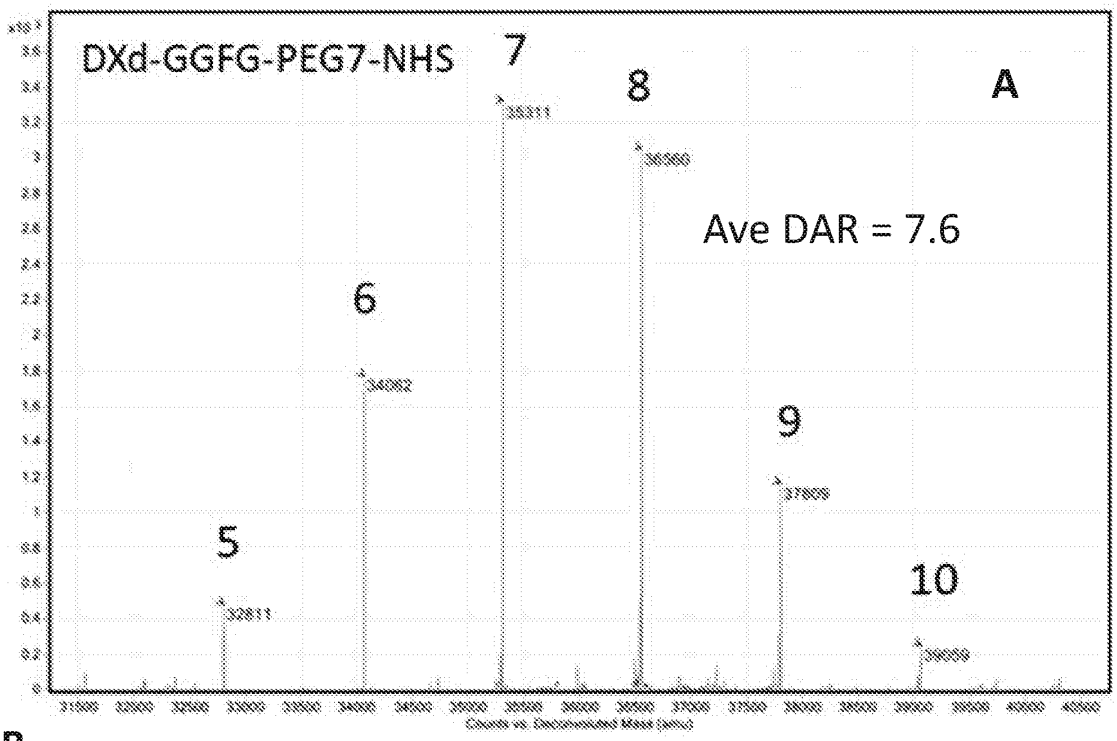
E



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
Cycle: 6	1.058E+5	1.839E-4	1.738E-9	28.81	6.731E-9	1.757E+17	30.00	5.461E+17	0.3918	0.0281	1
					1.346E-8				0.5180		
					2.692E-8				0.2343		
					5.385E-8				0.2683		
					1.077E-7				0.1822		

Figure 21

A


DXd-GGFG-PEG7-NHS
A
5
6
7
8
9
10
Ave DAR = 7.6

B

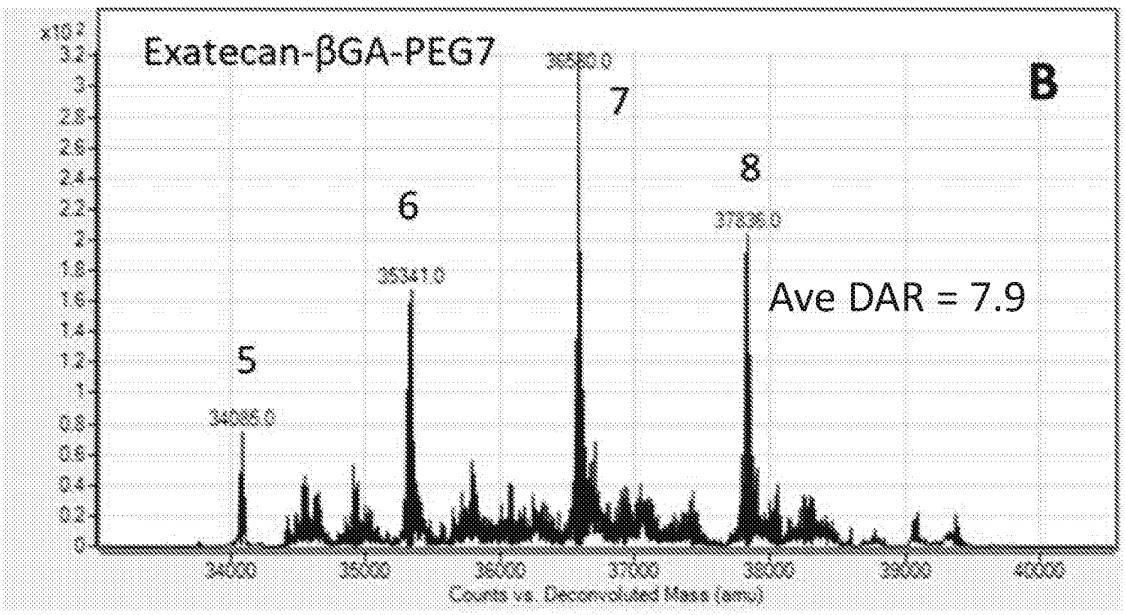
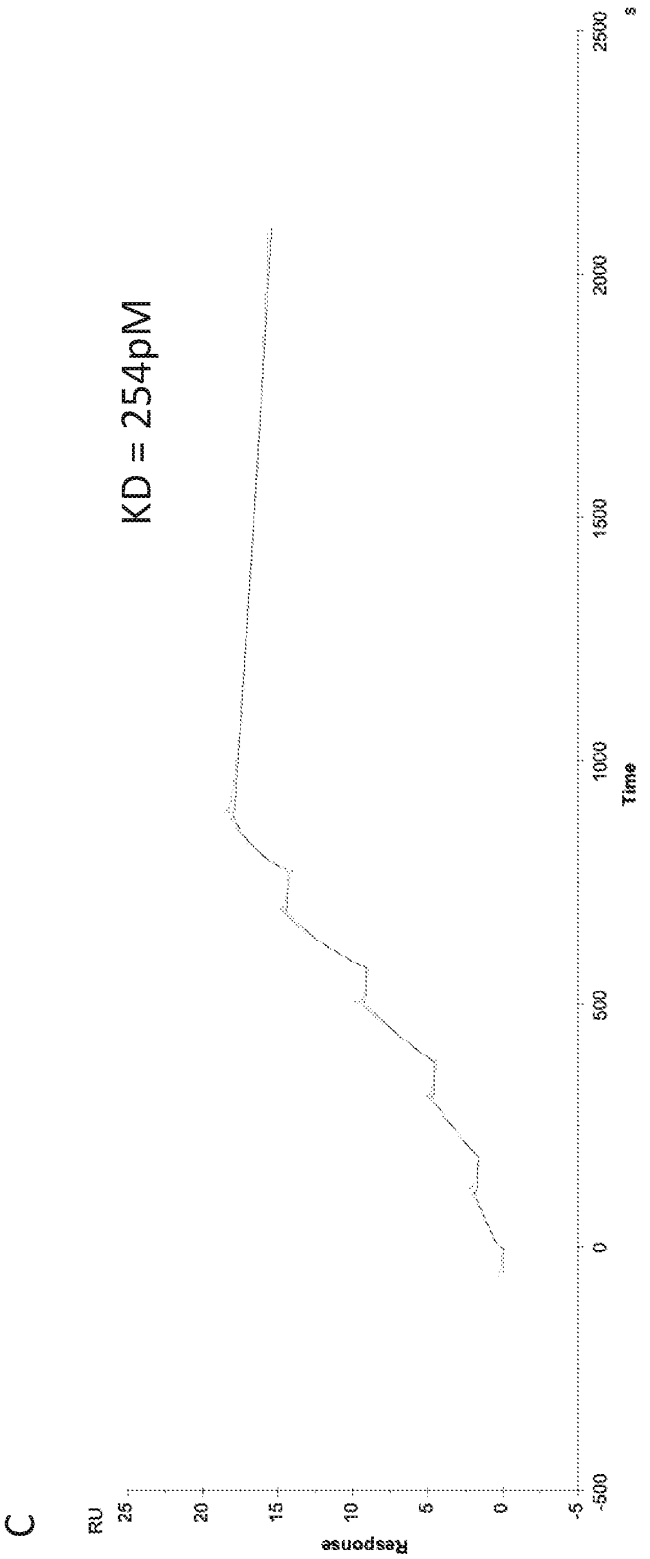
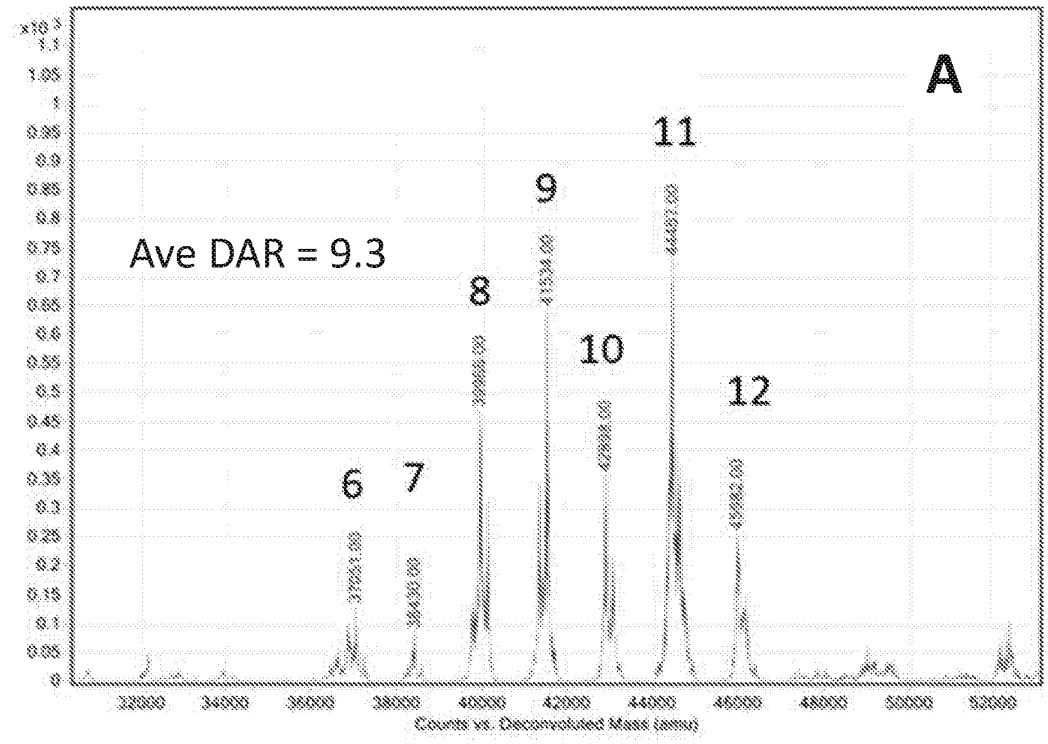

Exatecan-βGA-PEG7
B
5
6
7
8
Ave DAR = 7.9
Counts vs. Deconvoluted Mass (amu)

Figure 21 continued



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	Lc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
Cycle: 9	4.932E+5	1.255E-4	2.541E-10	18.90	1.750E-9	2.838E+7	30.00	8.818E+7	0.3542	0.0212	1
					3.500E-9				0.2426		
					7.094E-9				0.1700		
					1.419E-8				0.2387		
					2.838E-8				0.1682		

Figure 22


A
Ave DAR = 9.3
6
7
8
9
10
11
12
Counts vs. Deconvoluted Mass (amu)

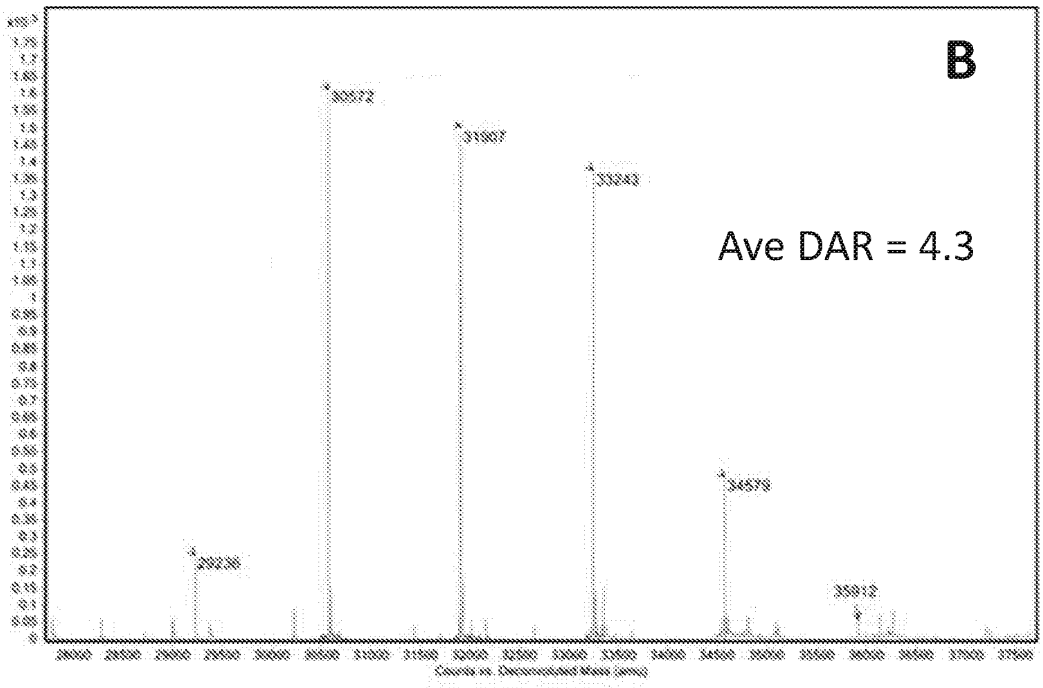
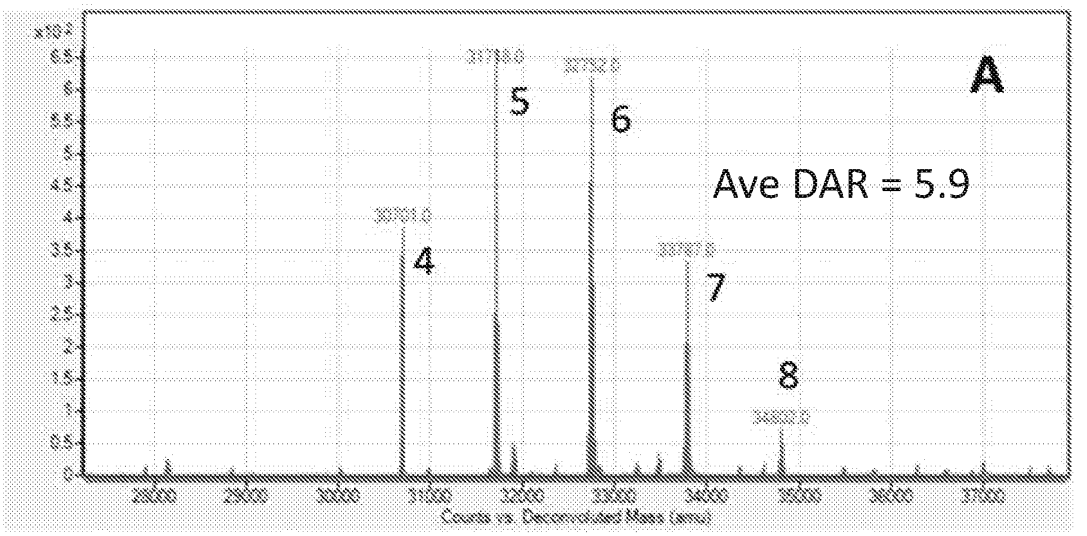

B
Ave DAR = 4.3

Figure 23



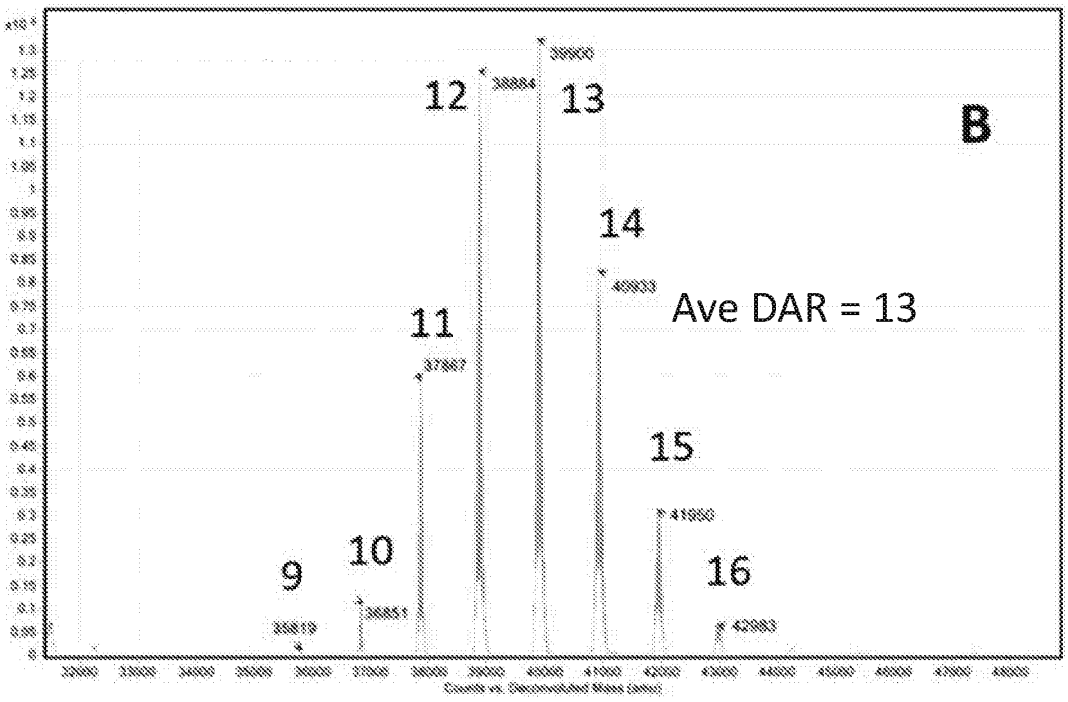
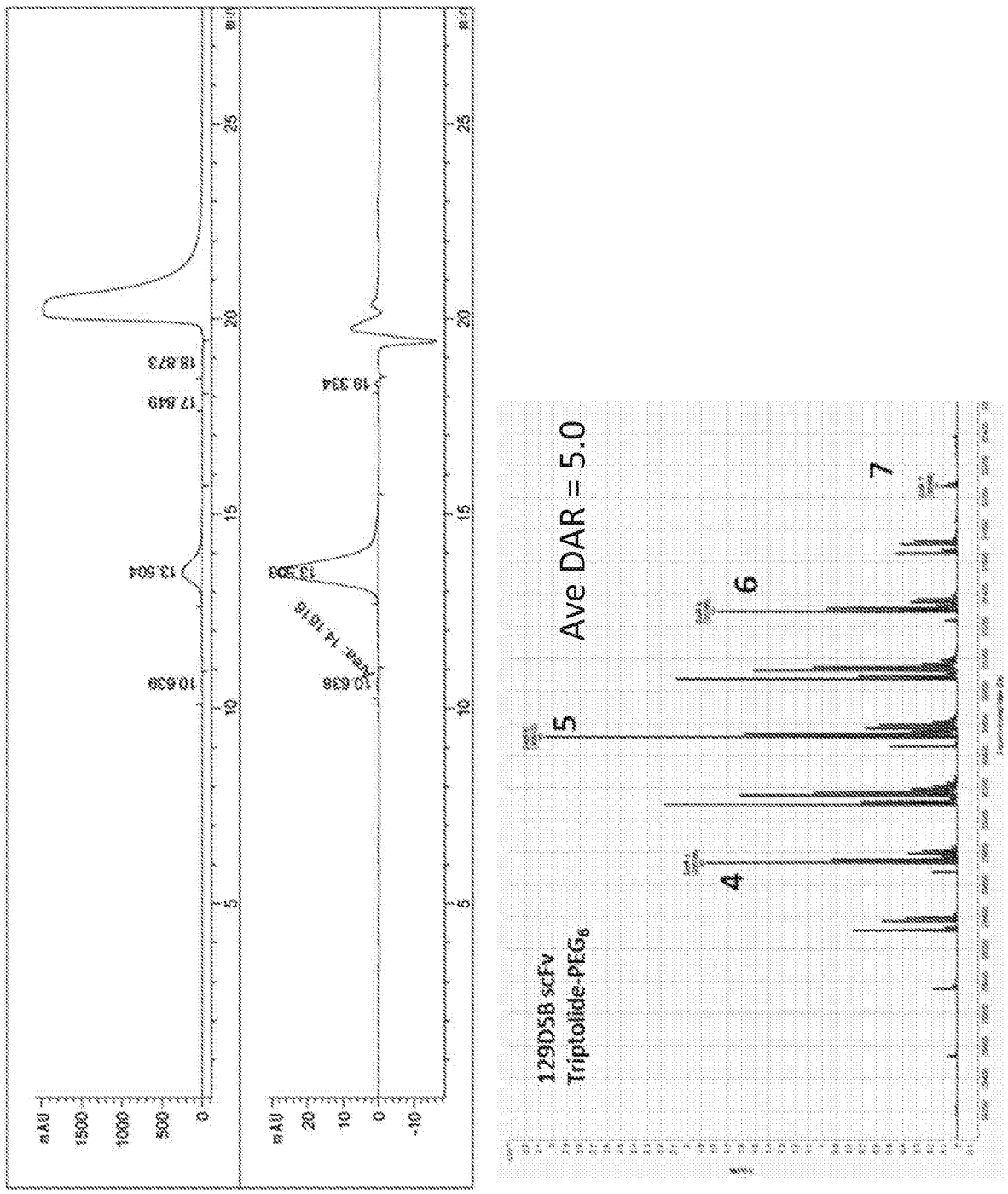


Figure 23


mAU
1500
1000
500
0
10.639
13.504
17.849
18.873
5
10
15
20
25
mAU
20
10
0
-10
10.636
Area: 14.1616
13.503
16.334
5
10
15
20
25
129D5B scFv
Triptolide-PEG6
Ave DAR = 5.0
4
5
6
7

C

D

Figure 24

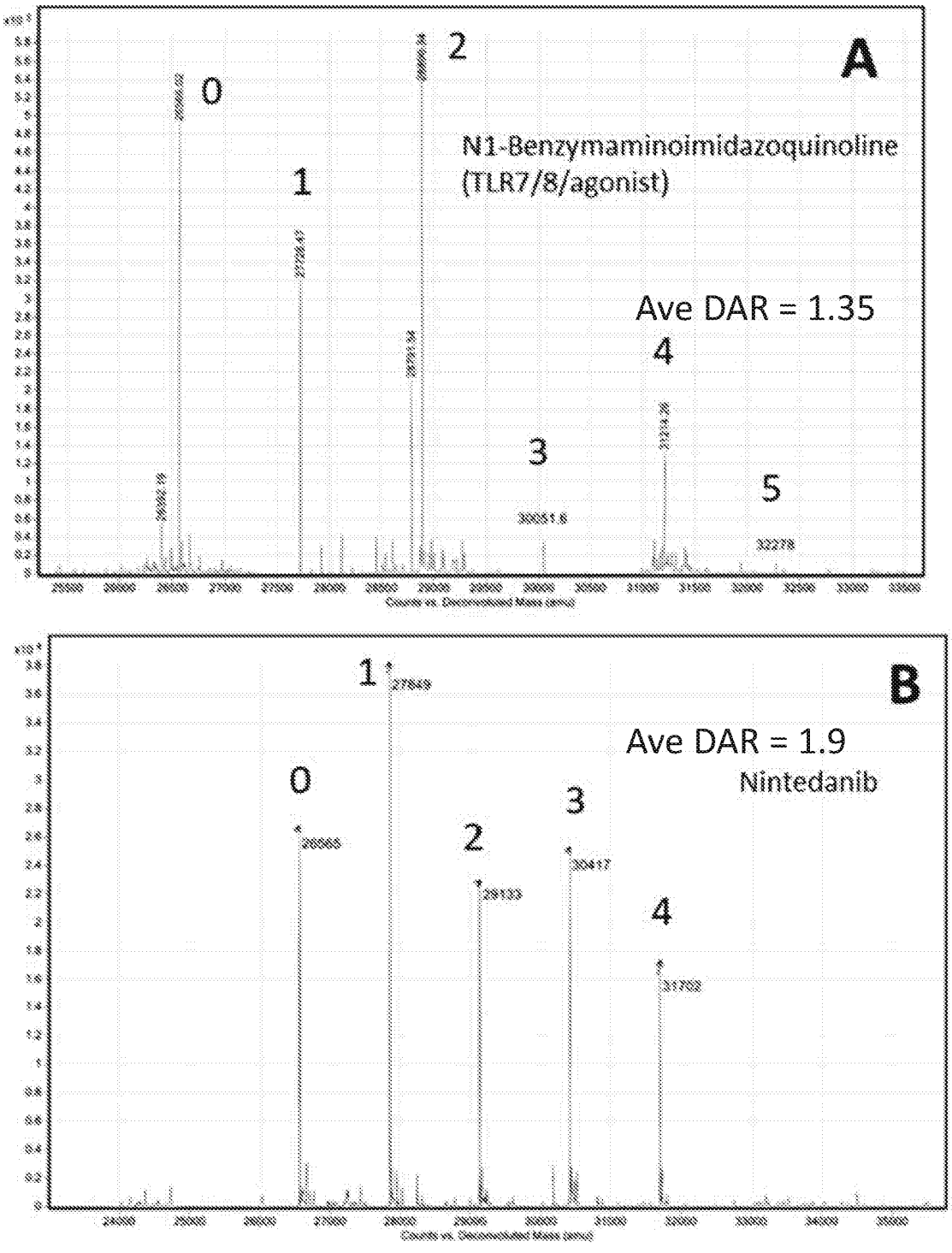
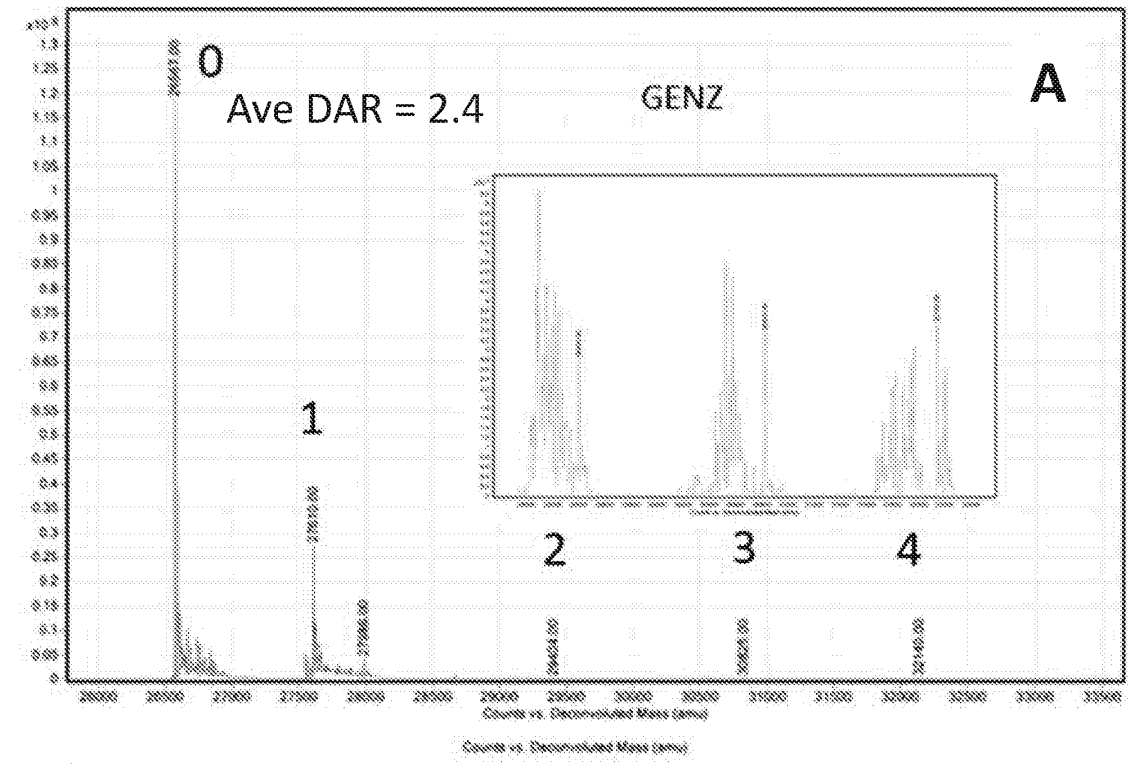

A
N1-Benzymaminoimidazoquinoline
(TLR7/8/agonist)
Ave DAR = 1.35
0
1
2
3
4
5
B
Ave DAR = 1.9
Nintedanib
0
1
2
3
4

Figure 25


0
Ave DAR = 2.4
GENZ
A
1
2
3
4
Counts vs. Deconvoluted Mass (amu)

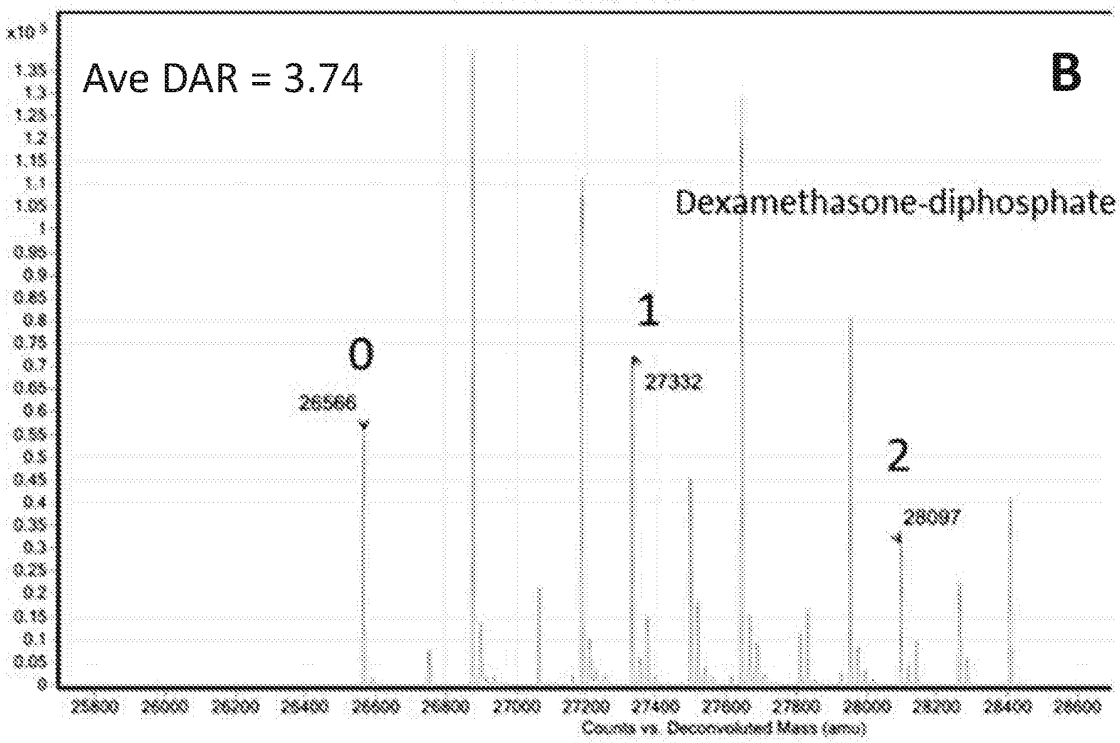
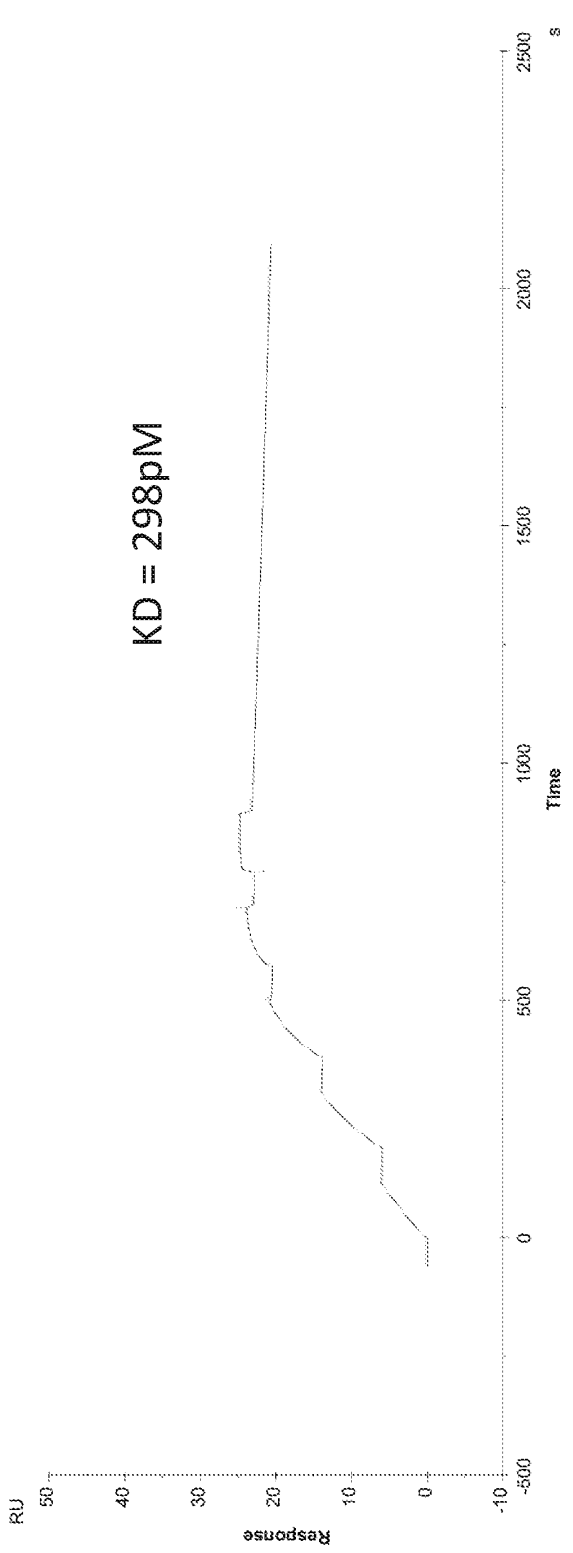

Ave DAR = 3.74
B
Dexamethasone-diphosphate
0
26566
1
27332
2
28097
Counts vs. Deconvoluted Mass (amu)

Figure 25 continued

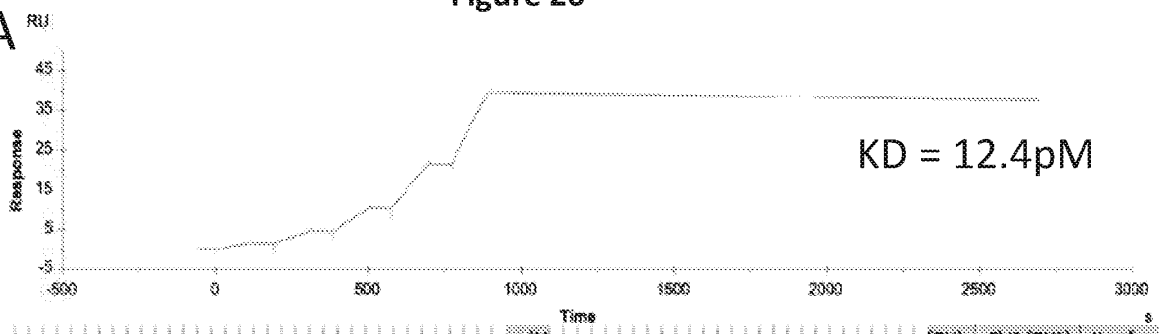
C



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
	3.240E+5	9.680E-5	2.988E-10			1.367E+7				0.0879	2
Cycle: 7				23.17	8.851E-9		30.00	4.248E+7	0.2623		
					1.770E-8				0.3827		
					3.541E-8				0.4678		
					7.081E-8				0.9168		
					1.416E-7				1.619		

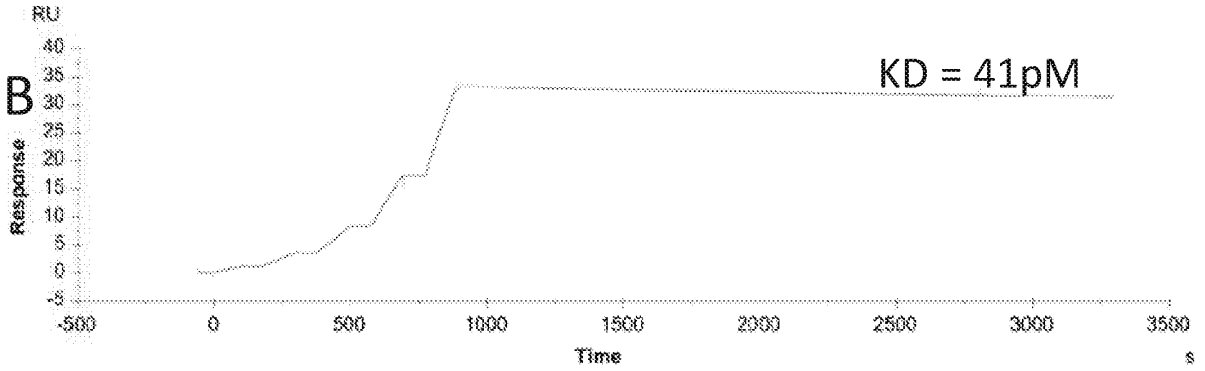
Figure 26

A



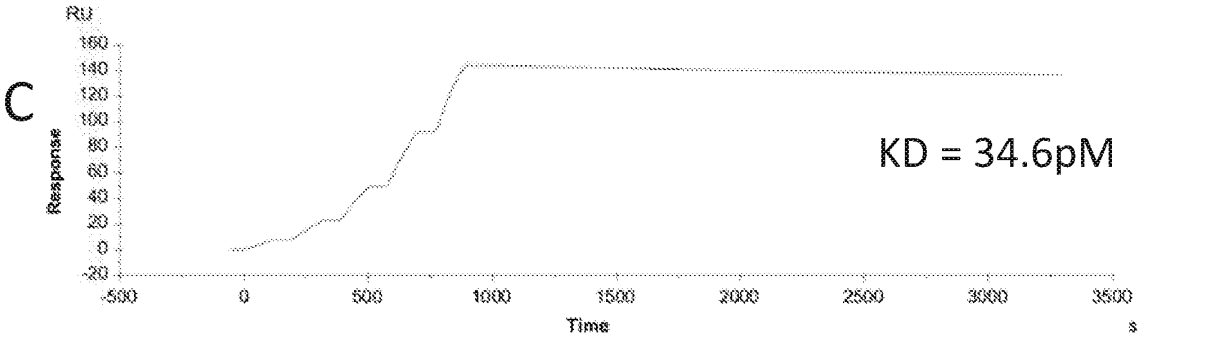
	nc (M		(RU)	Chi² (RU²)	U-value
				0.0791	2
	329E-1		08145		
	159E-1		05929		
	319E-1		03147		
	.063E-		04164		
	.127E-		1.1973		

B



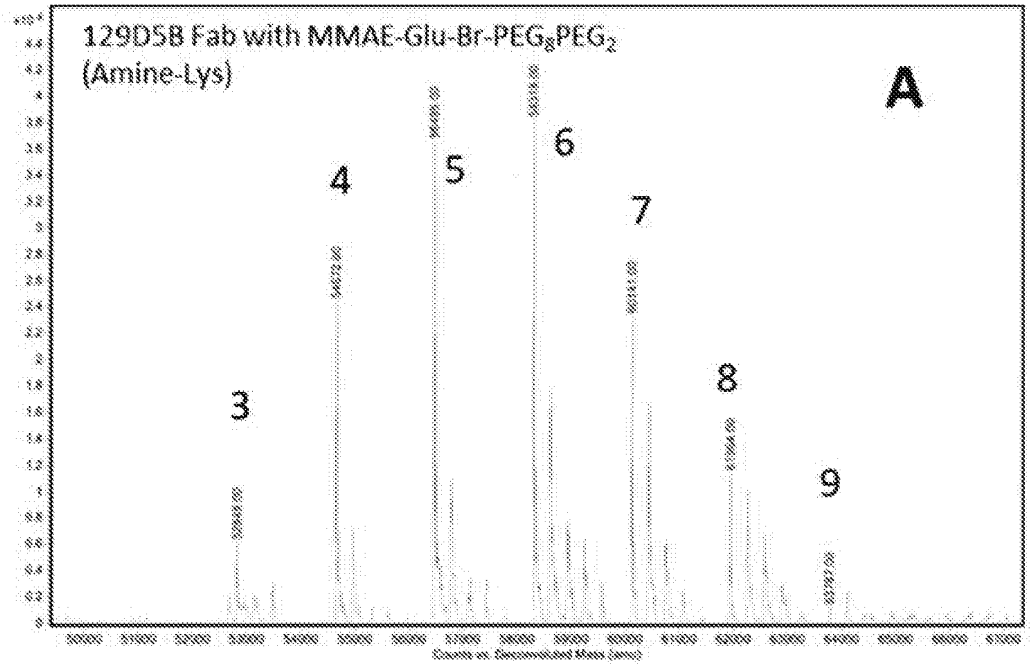
Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
	1.237E+6	5.062E-5	4.092E-11			8.351E+6				0.0192	1
Cycle: 13				56.38	5.377E-10		30.00	2.595E+7	0.1895		
					1.076E-9				0.3073		
					2.151E-9				0.2331		
					4.302E-9				0.3166		
					8.604E-9				0.4874		

C



Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
	6.927E+5	2.394E-5	3.456E-11			1.801E+8				0.266	2
Cycle: 9				189.8	6.250E-10		30.00	5.597E+8	0.08455		
					1.249E-9				0.2616		
					2.499E-9				0.3645		
					4.999E-9				0.4596		
					9.999E-9				1.161		

Figure 27


129D5B Fab with MMAE-Glu-Br-PEG8PEG2
(Amine-Lys)
A
3
4
5
6
7
8
9

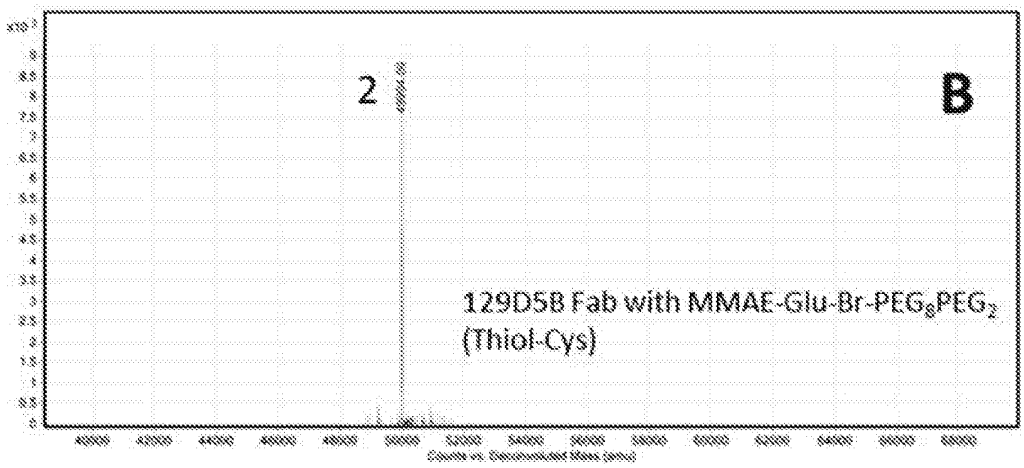

2
B
129D5B Fab with MMAE-Glu-Br-PEG8PEG2
(Thiol-Cys)

Figure 27 continued

C

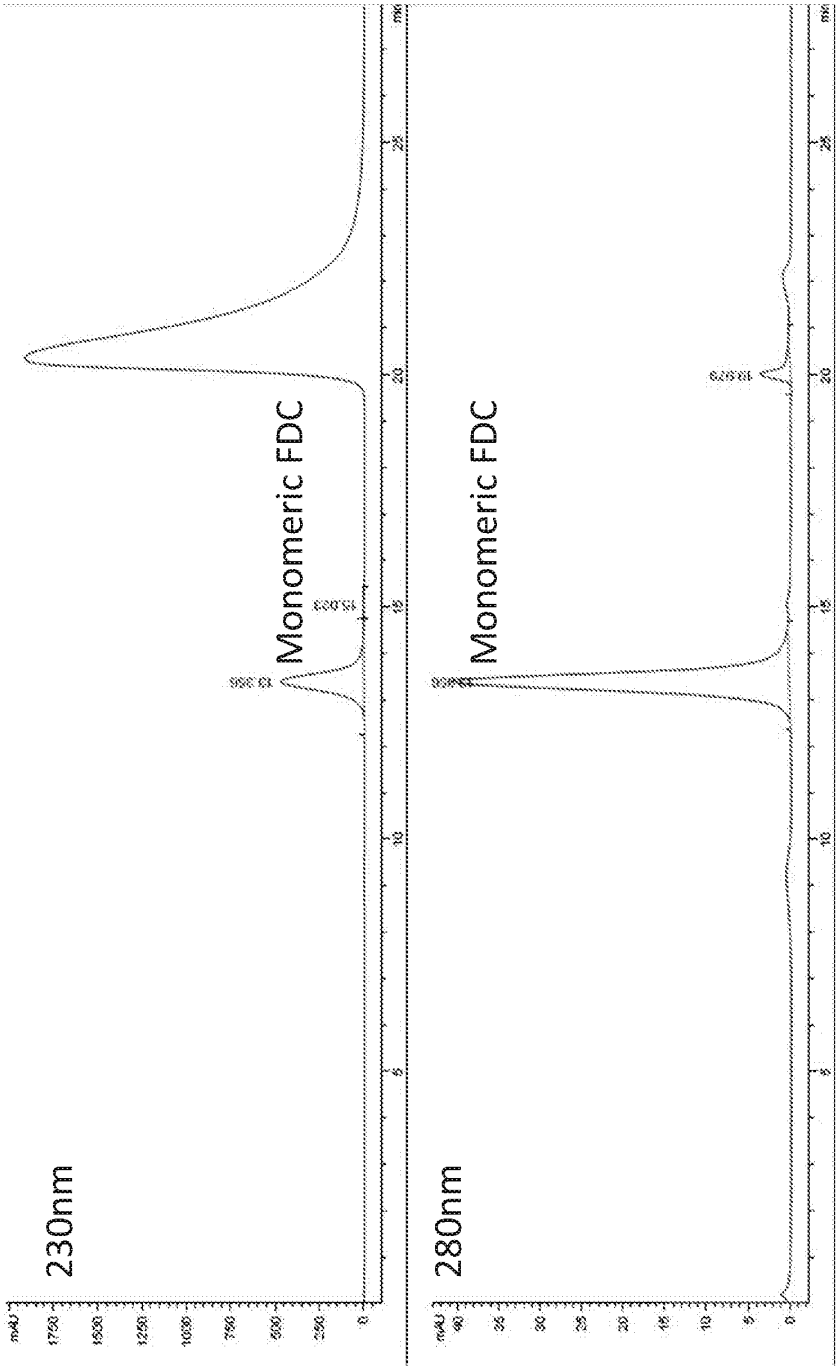


Figure 28

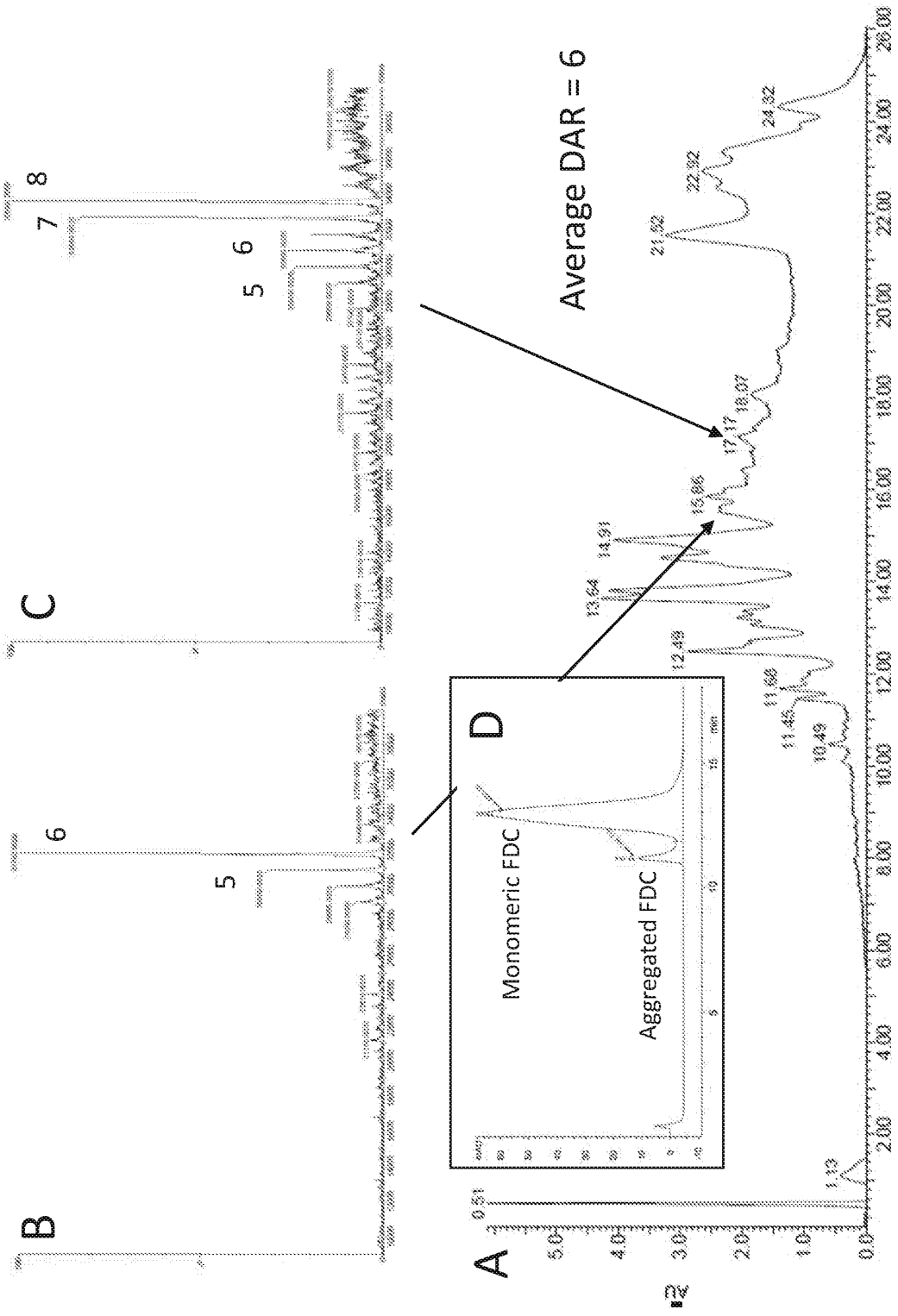

A
B
C
D
Average DAR = 6
Monomeric FDC
Aggregated FDC
AU
0.51
1.13
10.49
11.45
11.68
12.49
13.64
14.91
15.86
17.17
18.07
21.52
22.92
24.32
5
6
7
8

Figure 28 continued

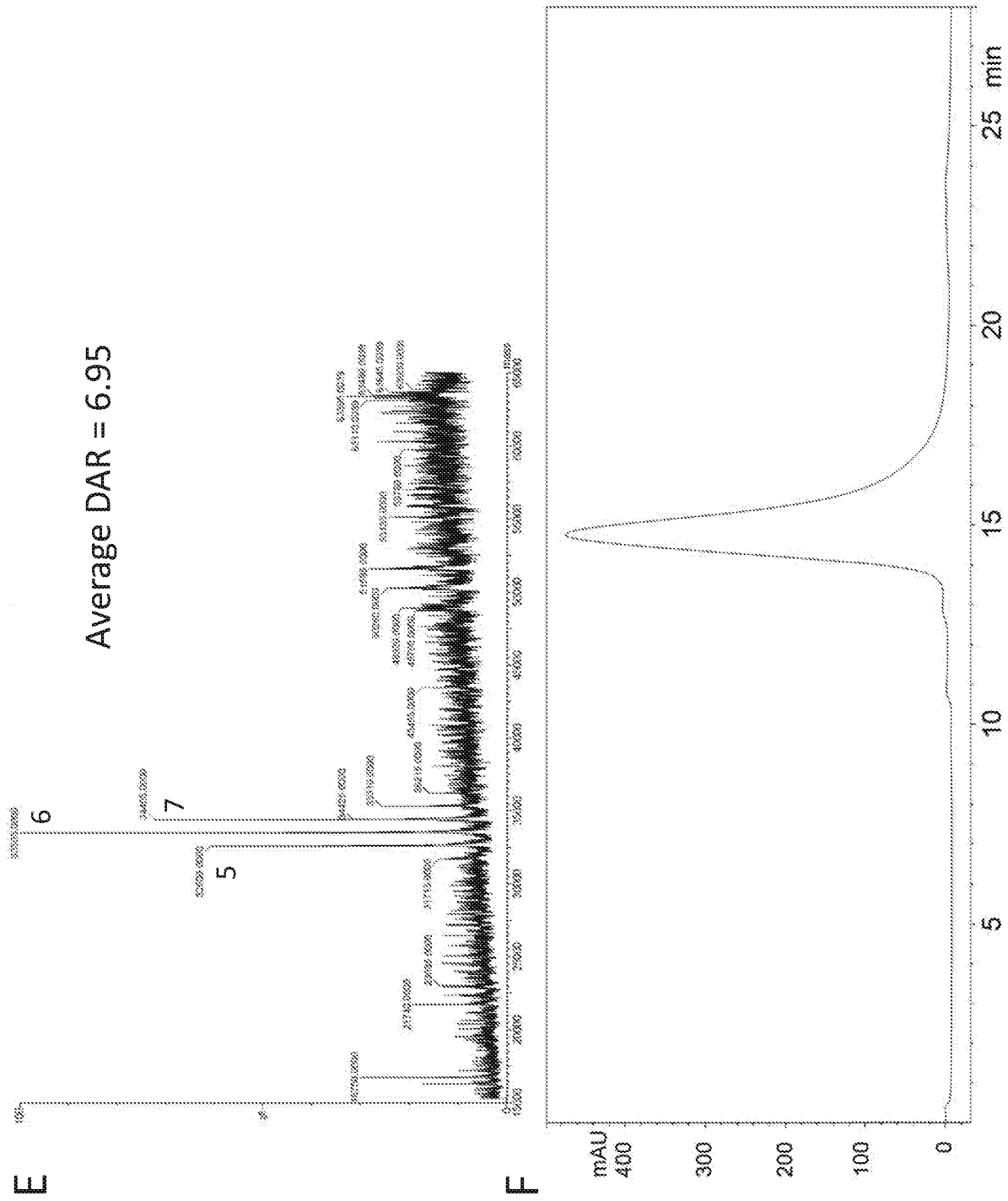


Figure 29

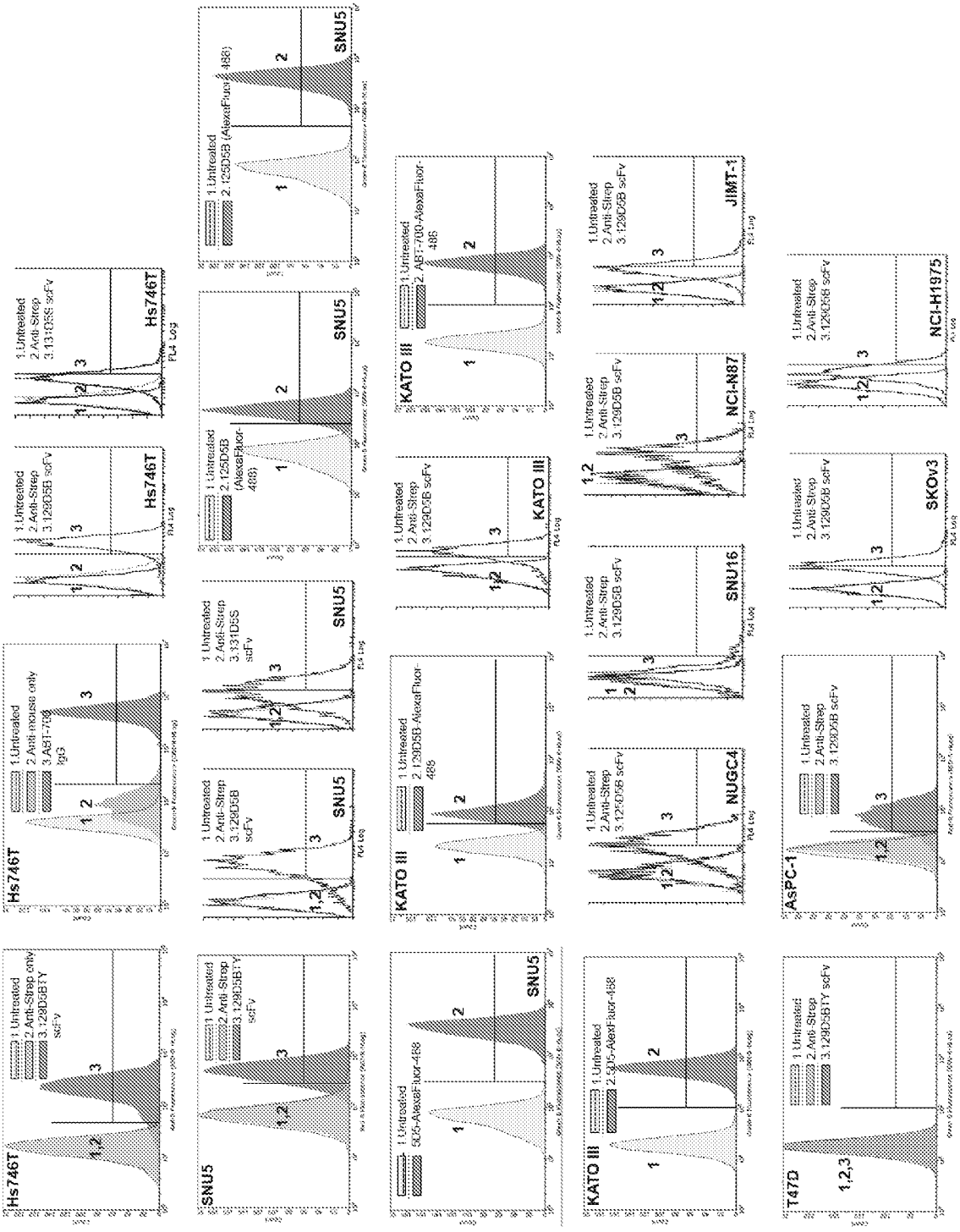

Hs746T
1.Untreated
2.Anti-Strep only
3.129D5BTY scFv
Hs746T
1.Untreated
2.Anti-mouse only
3.ABT-700 IgG
Hs746T
1.Untreated
2.Anti-Strep
3.129D5B scFv
Hs746T
1.Untreated
2.Anti-Strep
3.131D5S scFv
SNU5
1.Untreated
2.Anti-Strep
3.129D5BTY scFv
SNU5
1.Untreated
2.Anti-Strep
3.129D5B scFv
SNU5
1.Untreated
2.Anti-Strep
3.131D5S scFv
SNU5
1.Untreated
2.125D5B (AlexaFluor-488)
SNU5
1.Untreated
2.125D5B (AlexaFluor-488)
SNU5
1.Untreated
5D5-AlexaFluor-488
KATO III
1.Untreated
2.129D5B-AlexaFluor-488
KATO III
1.Untreated
2.Anti-Strep
3.129D5B scFv
KATO III
1.Untreated
2.ABT-700-AlexaFluor-488
KATO III
1.Untreated
2.5D5-AlexFluor-488
NUGC4
1.Untreated
2.Anti-Strep
3.129D5B scFv
SNU16
1.Untreated
2.Anti-Strep
3.129D5B scFv
NCI-N87
1.Untreated
2.Anti-Strep
3.129D5B scFv
JIMT-1
1.Untreated
2.Anti-Strep
3.129D5B scFv
T47D
1.Untreated
2.Anti-Strep
3.129D5BTY scFv
AsPC-1
1.Untreated
2.Anti-Strep
3.129D5B scFv
SKOv3
1.Untreated
2.Anti-Strep
3.129D5B scFv
NCI-H1975
1.Untreated
2.Anti-Strep
3.129D5B scFv

Figure 30

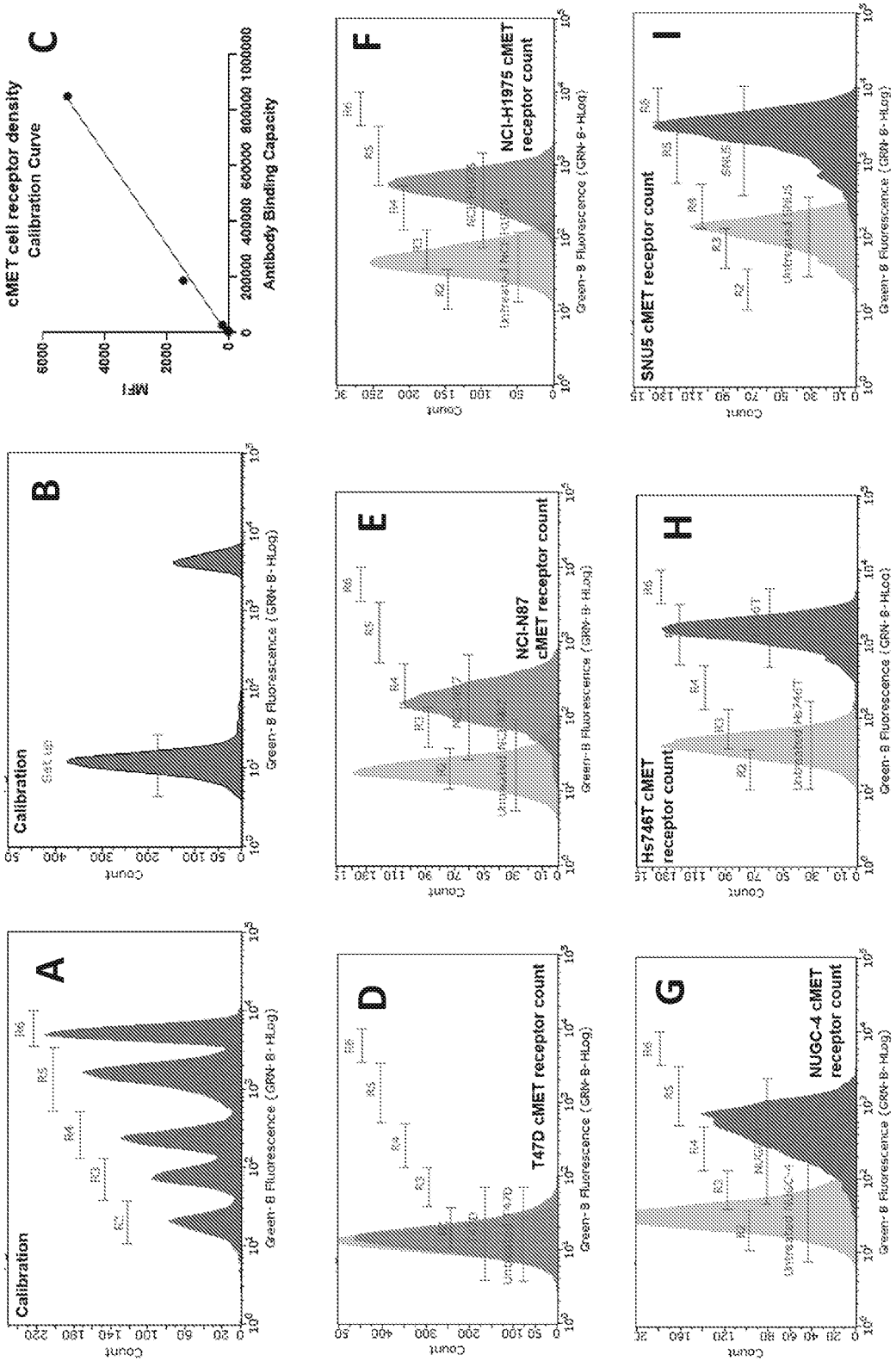

A
Calibration
B
Calibration
C
cMET cell receptor density
Calibration Curve
MFI
Antibody Binding Capacity
D
T47D cMET receptor count
E
NCI-N87
cMET receptor count
F
NCI-H1975 cMET
receptor count
G
NUGC-4 cMET
receptor count
H
Hs746T cMET
receptor count
I
SNU5 cMET receptor count
Count
Green-B Fluorescence (GRN-B-HLog)

Figure 31

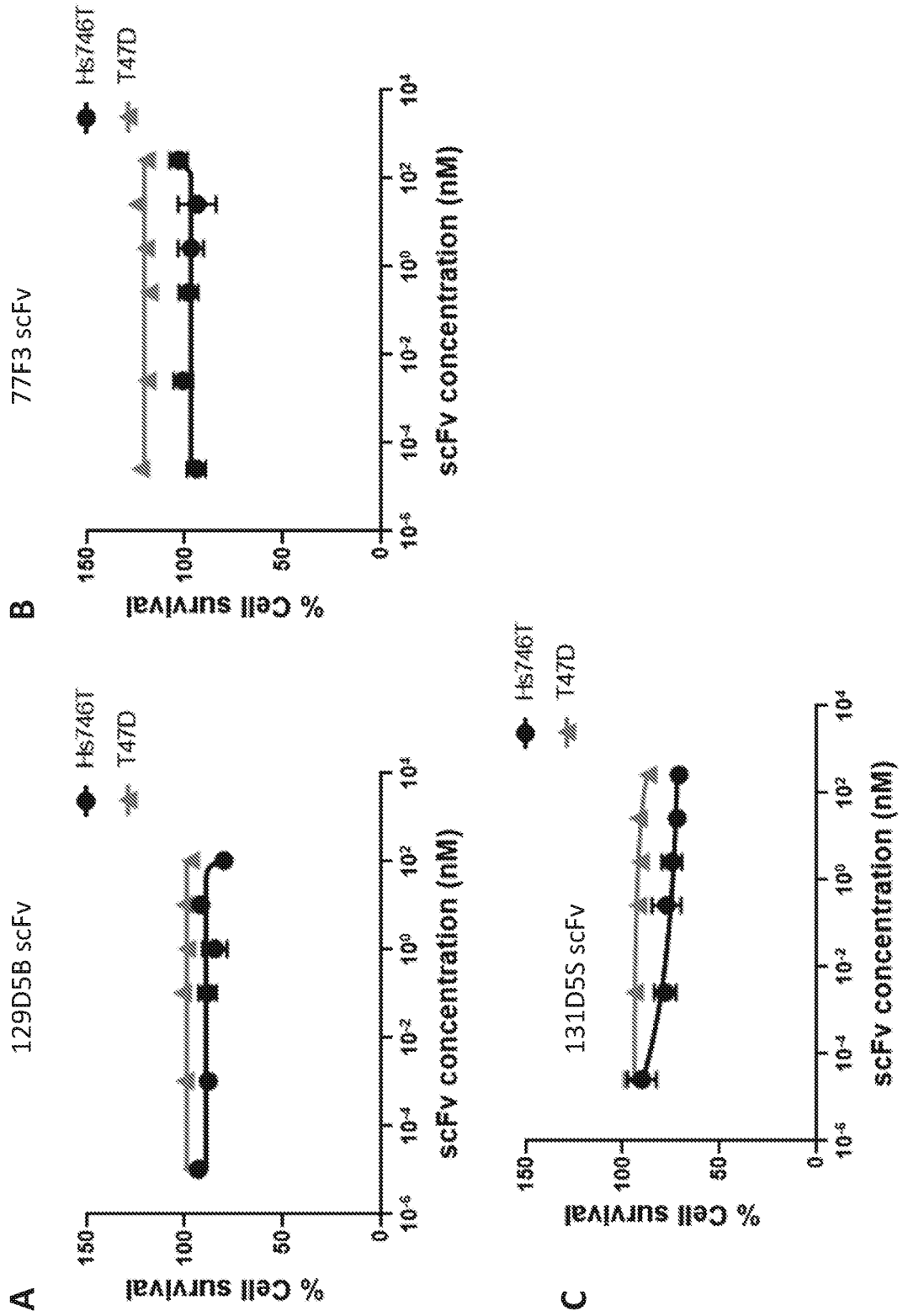

A
129D5B scFv
Hs746T
T47D
% Cell survival
scFv concentration (nM)
B
77F3 scFv
Hs746T
T47D
% Cell survival
scFv concentration (nM)
C
131D5S scFv
Hs746T
T47D
% Cell survival
scFv concentration (nM)

Figure 32

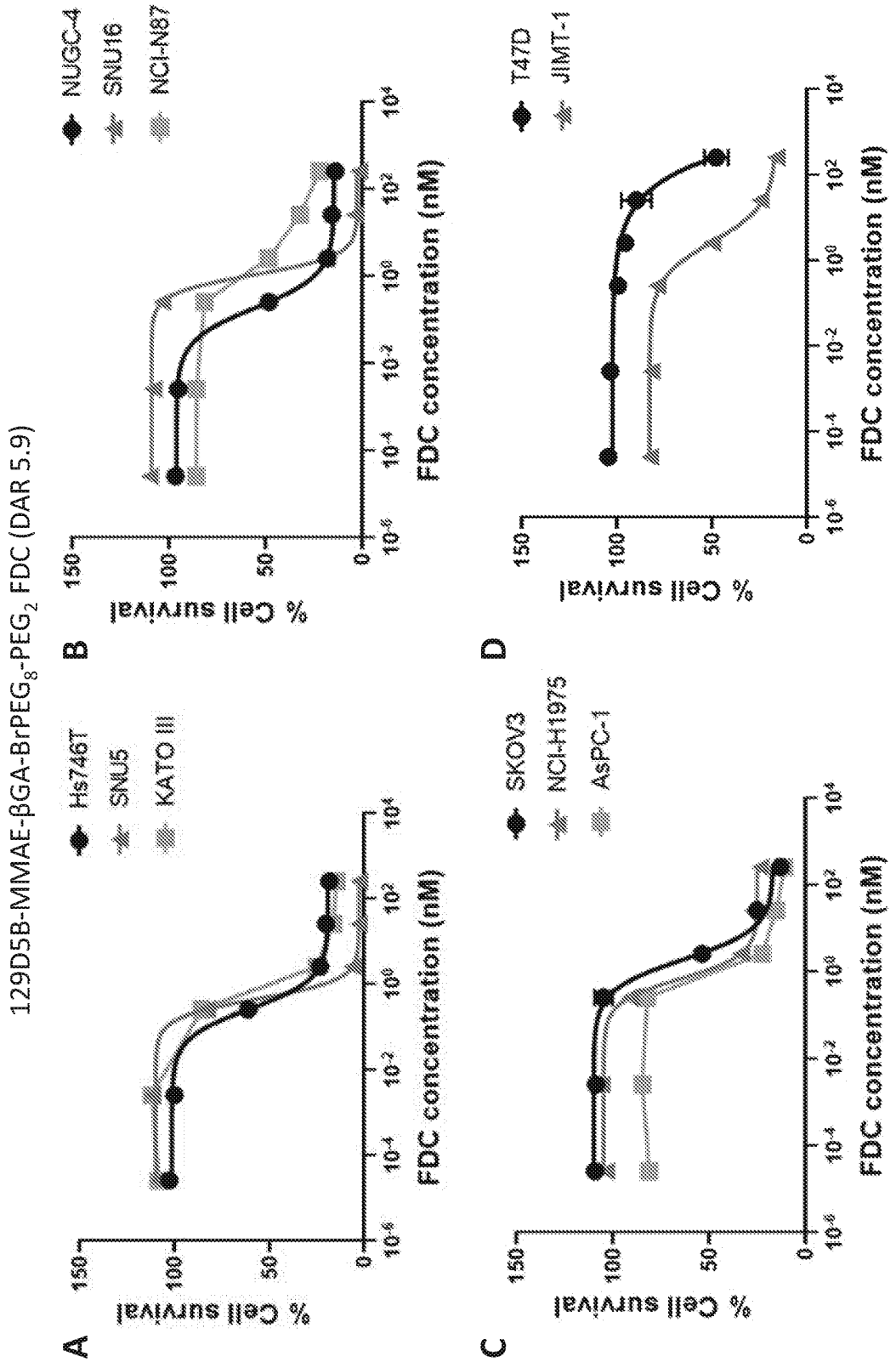

129D5B-MMAE-βGA-BrPEG8-PEG2 FDC (DAR 5.9)
A
Hs746T
SNU5
KATO III
% Cell survival
FDC concentration (nM)
B
NUGC-4
SNU16
NCI-N87
% Cell survival
FDC concentration (nM)
C
SKOV3
NCI-H1975
AsPC-1
% Cell survival
FDC concentration (nM)
D
T47D
JIMT-1
% Cell survival
FDC concentration (nM)

Figure 33

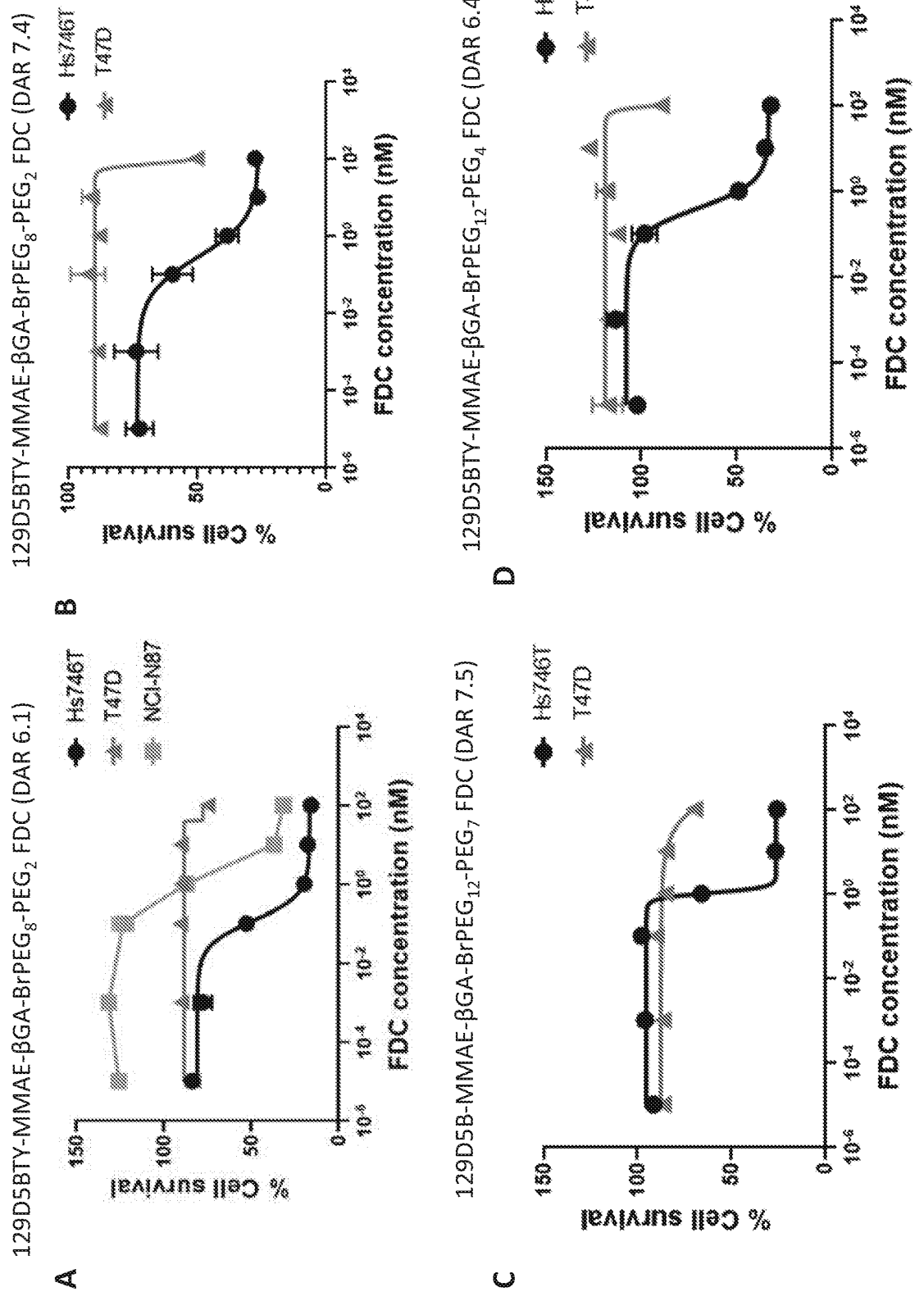

A
129D5BTY-MMAE-βGA-BrPEG8-PEG2 FDC (DAR 6.1)
Hs746T
T47D
NCI-N87
% Cell survival
FDC concentration (nM)
B
129D5BTY-MMAE-βGA-BrPEG8-PEG2 FDC (DAR 7.4)
Hs746T
T47D
% Cell survival
FDC concentration (nM)
C
129D5B-MMAE-βGA-BrPEG12-PEG7 FDC (DAR 7.5)
Hs746T
T47D
% Cell survival
FDC concentration (nM)
D
129D5BTY-MMAE-βGA-BrPEG12-PEG4 FDC (DAR 6.4)
Hs746T
T47D
% Cell survival
FDC concentration (nM)

Figure 34

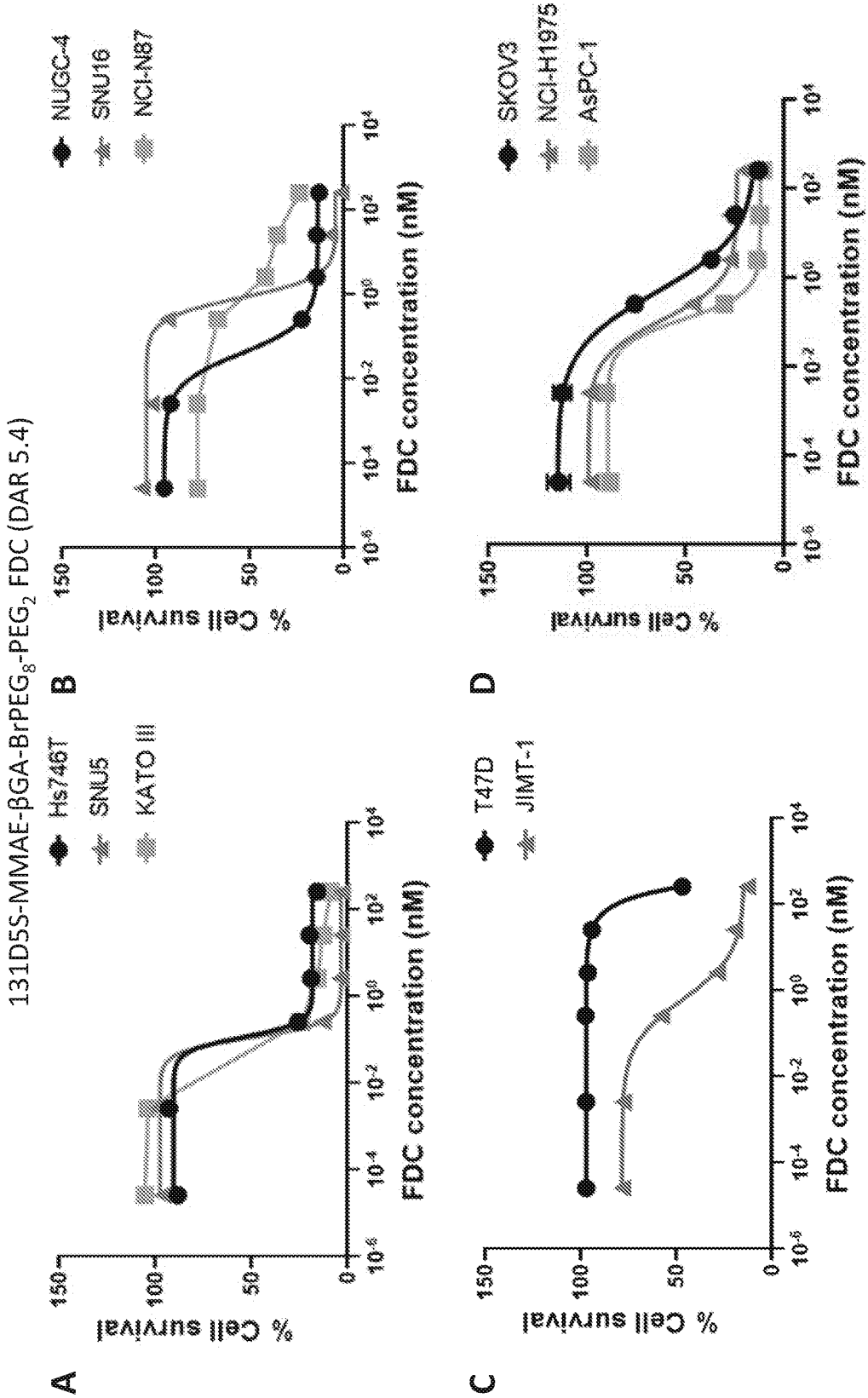

131D5S-MMAE-βGA-BrPEG8-PEG2 FDC (DAR 5.4)
A
B
C
D
% Cell survival
FDC concentration (nM)
Hs746T
SNU5
KATO III
NUGC-4
SNU16
NCI-N87
T47D
JIMT-1
SKOV3
NCI-H1975
AsPC-1

Figure 35

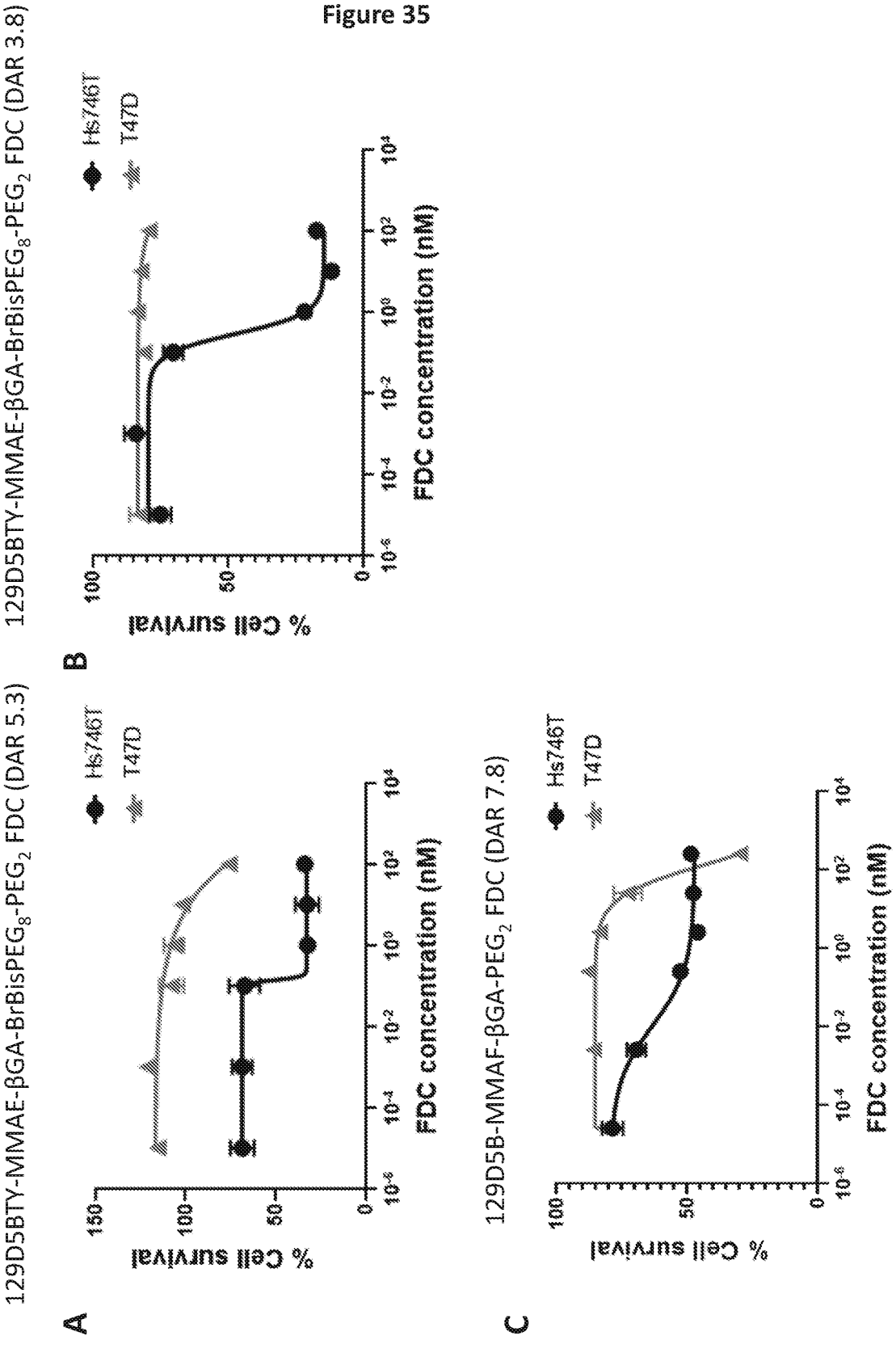

A
129D5BTY-MMAE-βGA-BrBisPEG8-PEG2 FDC (DAR 5.3)
Hs746T
T47D
% Cell survival
FDC concentration (nM)
B
129D5BTY-MMAE-βGA-BrBisPEG8-PEG2 FDC (DAR 3.8)
Hs746T
T47D
% Cell survival
FDC concentration (nM)
C
129D5B-MMAF-βGA-PEG2 FDC (DAR 7.8)
Hs746T
T47D
% Cell survival
FDC concentration (nM)

Figure 36

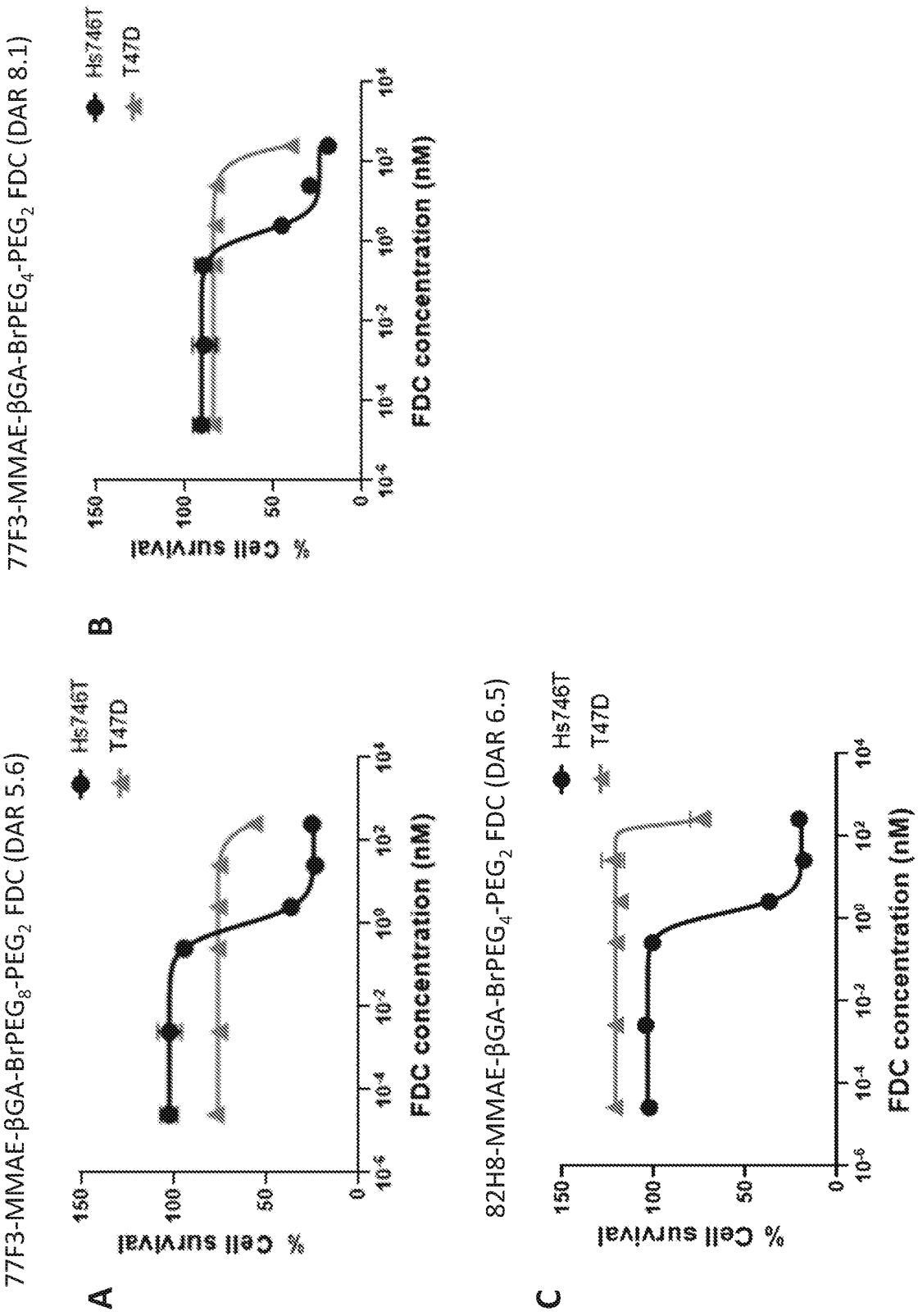

77F3-MMAE-βGA-BrPEG8-PEG2 FDC (DAR 5.6)
A
Hs746T
T47D
% Cell survival
FDC concentration (nM)
77F3-MMAE-βGA-BrPEG4-PEG2 FDC (DAR 8.1)
B
Hs746T
T47D
% Cell survival
FDC concentration (nM)
82H8-MMAE-βGA-BrPEG4-PEG2 FDC (DAR 6.5)
C
Hs746T
T47D
% Cell survival
FDC concentration (nM)

Figure 37

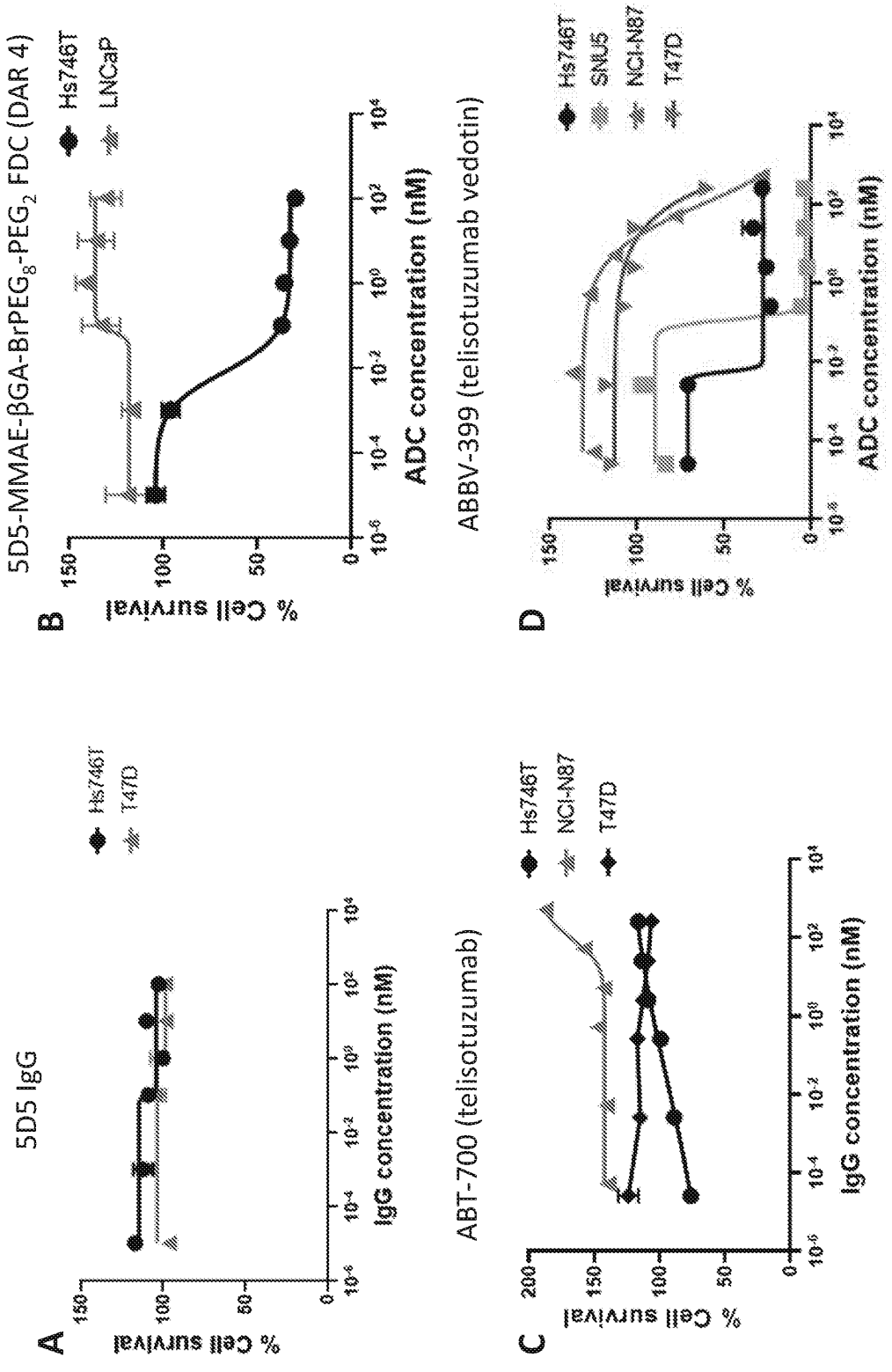

A
5D5 IgG
Hs746T
T47D
% cell survival
IgG concentration (nM)
B
5D5-MMAE-βGA-BrPEG8-PEG2 FDC (DAR 4)
Hs746T
LNCaP
% Cell survival
ADC concentration (nM)
C
ABT-700 (telisotuzumab)
Hs746T
NCI-N87
T47D
% Cell survival
IgG concentration (nM)
D
ABBV-399 (telisotuzumab vedotin)
Hs746T
SNU5
NCI-N87
T47D
% Cell survival
ADC concentration (nM)

Figure 38

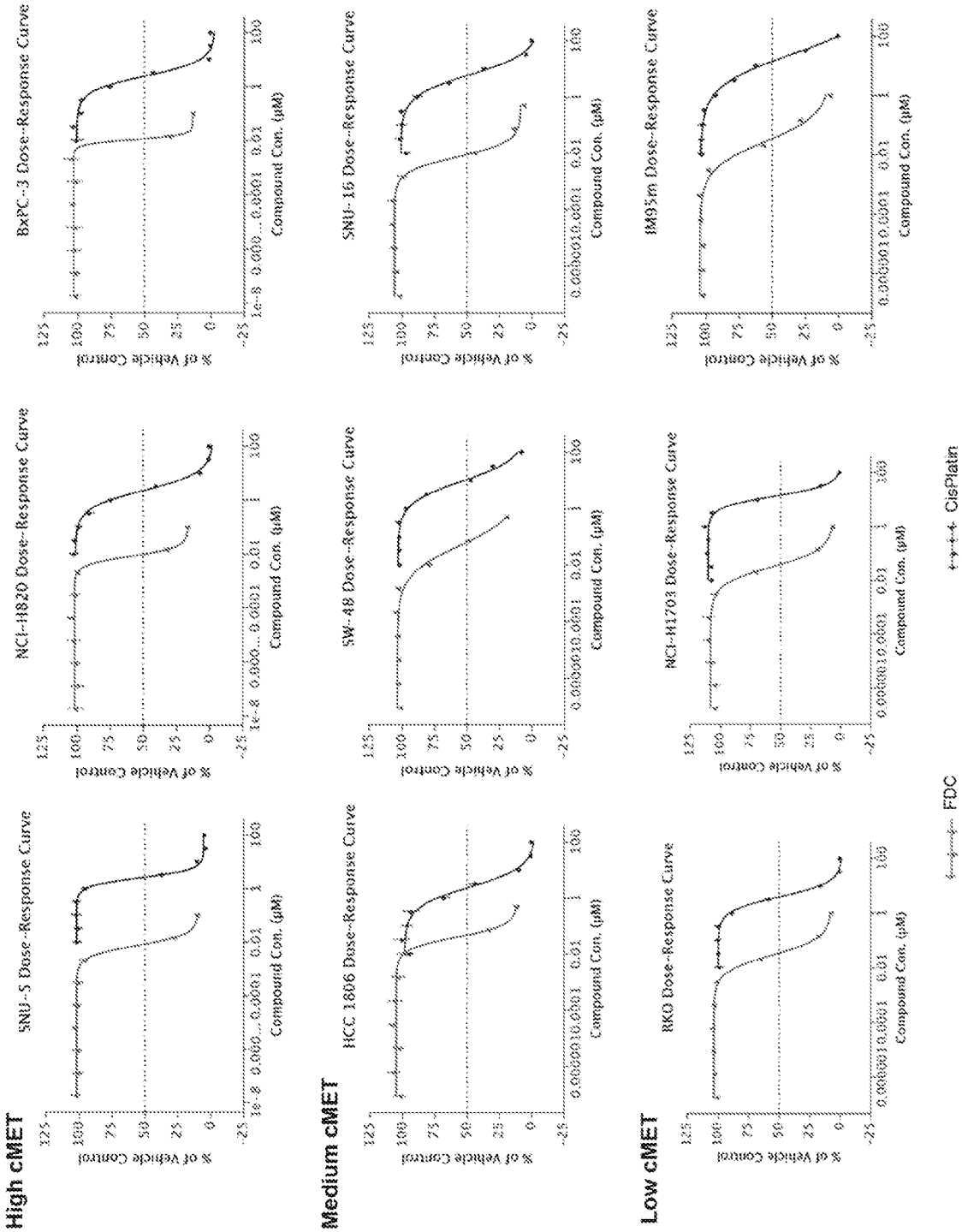

High cMET
Medium cMET
Low cMET
SNU-5 Dose-Response Curve
NCI-H820 Dose-Response Curve
BxPC-3 Dose-Response Curve
HCC 1806 Dose-Response Curve
SW-48 Dose-Response Curve
SNU-16 Dose-Response Curve
RKO Dose-Response Curve
NCI-H1703 Dose-Response Curve
IM95m Dose-Response Curve
% of Vehicle Control
Compound Con. (μM)
FDC
CisPlatin

Figure 39

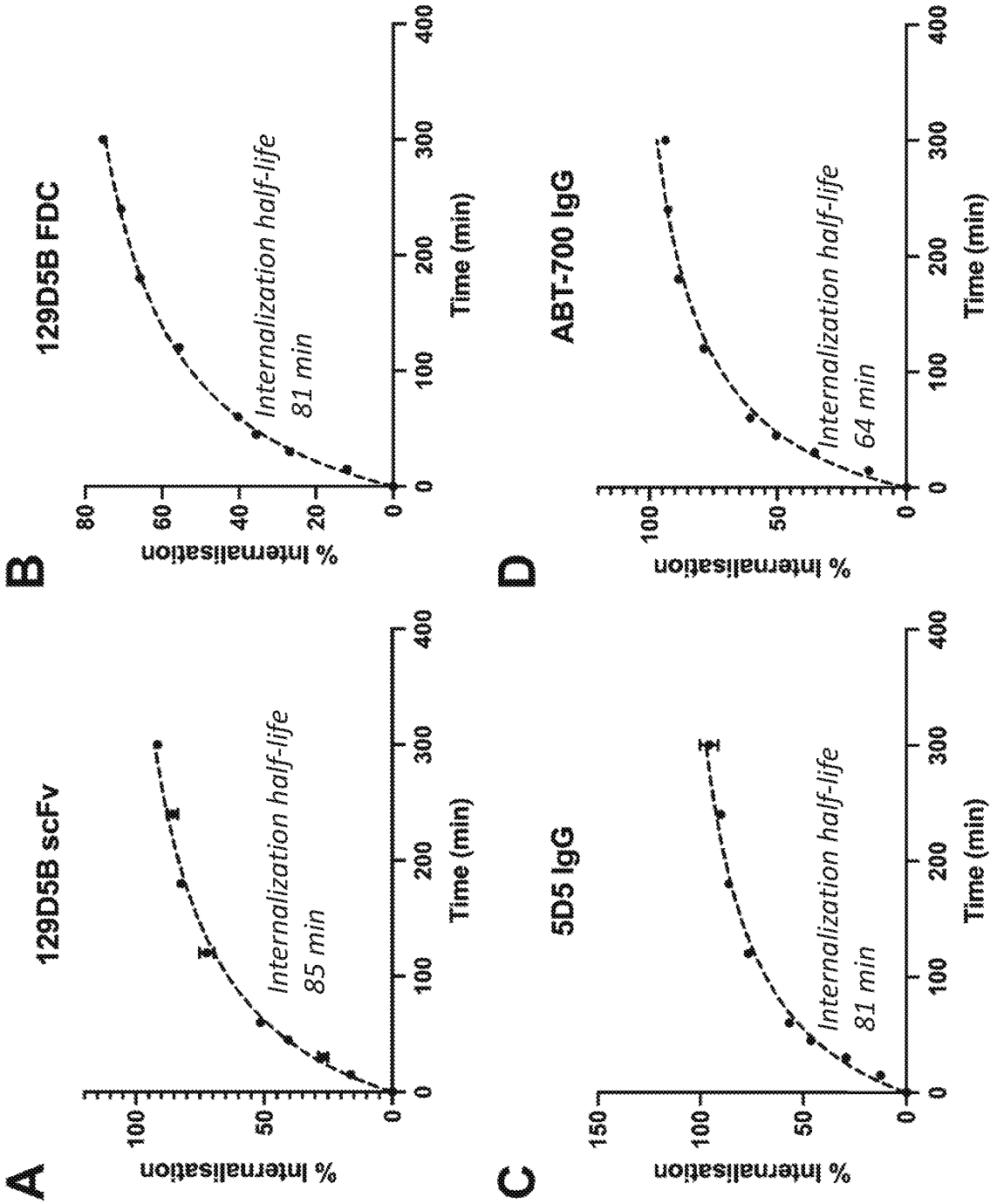

A
129D5B scFv
% internalisation
Time (min)
Internalization half-life
85 min
B
129D5B FDC
% internalisation
Time (min)
Internalization half-life
81 min
C
5D5 IgG
% internalisation
Time (min)
Internalization half-life
81 min
D
ABT-700 IgG
% internalisation
Time (min)
Internalization half-life
64 min

Figure 40

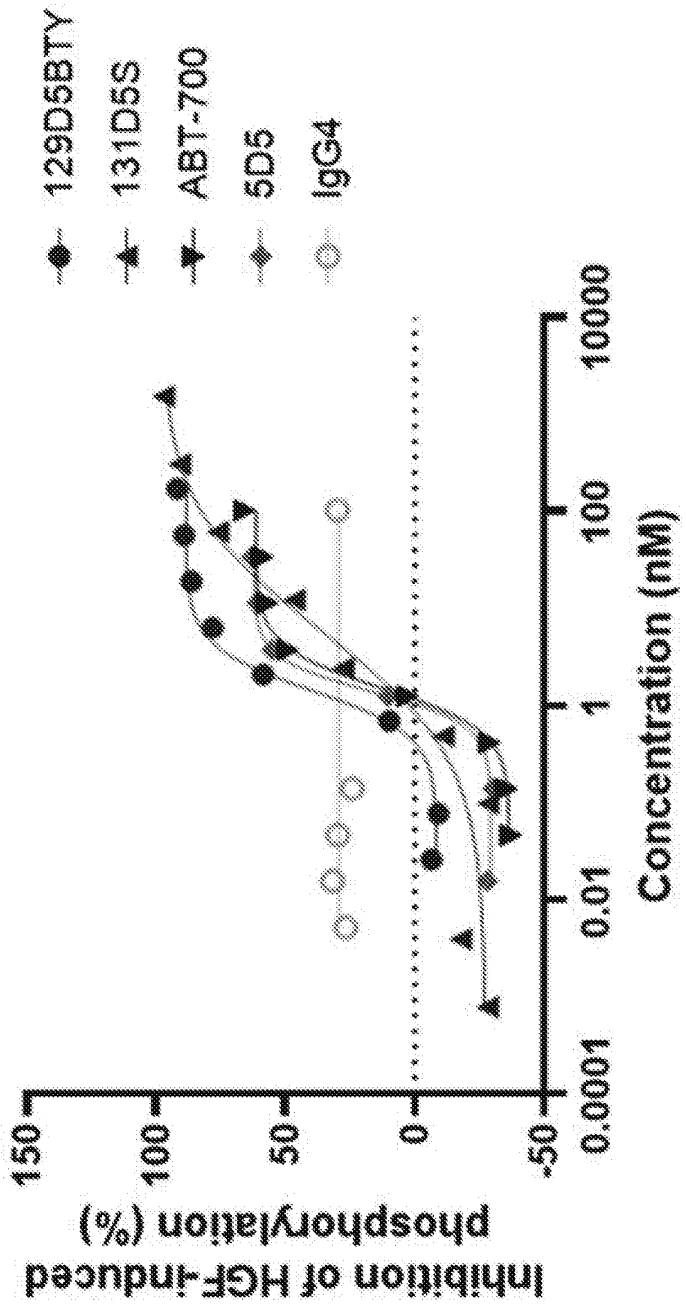

129D5BTY
131D5S
ABT-700
5D5
IgG4
Inhibition of HGF-induced phosphorylation (%)
150
100
50
0
-50
0.0001
0.01
1
100
10000
Concentration (nM)

Figure 41

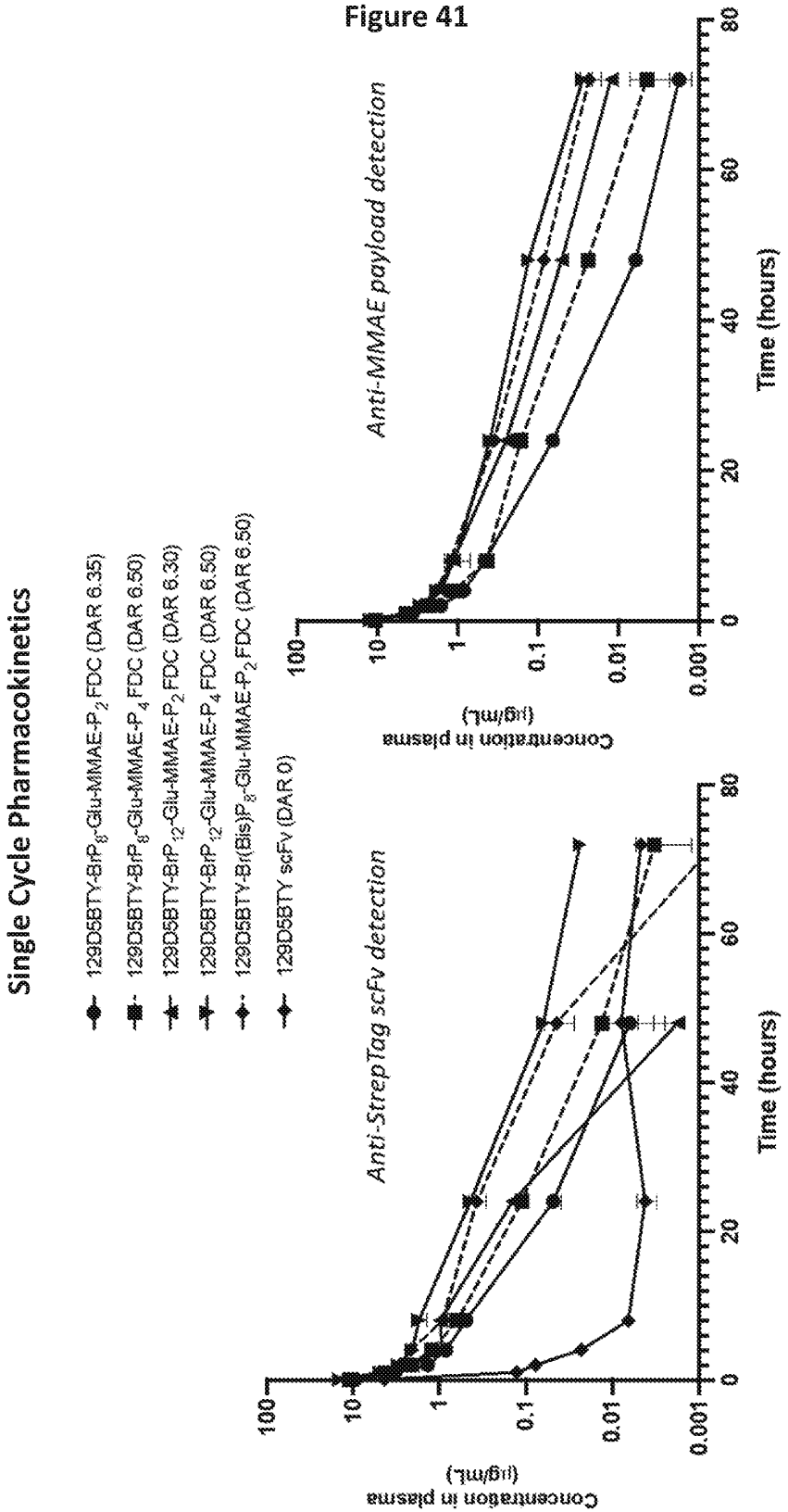

Single Cycle Pharmacokinetics
129D5BTY-BrP8-Glu-MMAE-P2 FDC (DAR 6.35)
129D5BTY-BrP8-Glu-MMAE-P4 FDC (DAR 6.50)
129D5BTY-BrP12-Glu-MMAE-P2 FDC (DAR 6.30)
129D5BTY-BrP12-Glu-MMAE-P4 FDC (DAR 6.50)
129D5BTY-Br(Bis)P8-Glu-MMAE-P2 FDC (DAR 6.50)
129D5BTY scFv (DAR 0)
Anti-MMAE payload detection
Anti-StrepTag scFv detection
Concentration in plasma (µg/mL)
Time (hours)
100
10
1
0.1
0.01
0.001
0
20
40
60
80

Figure 42

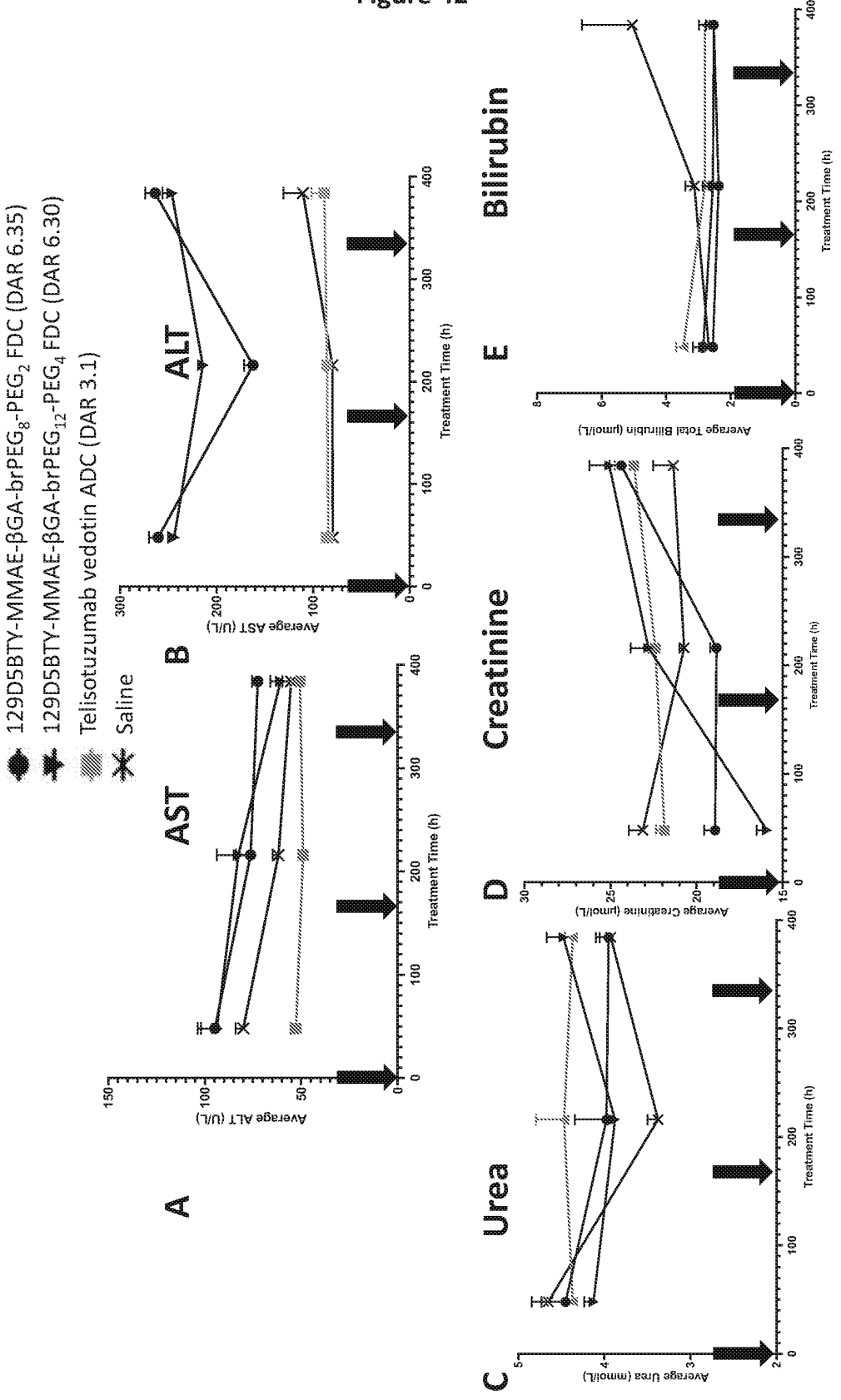
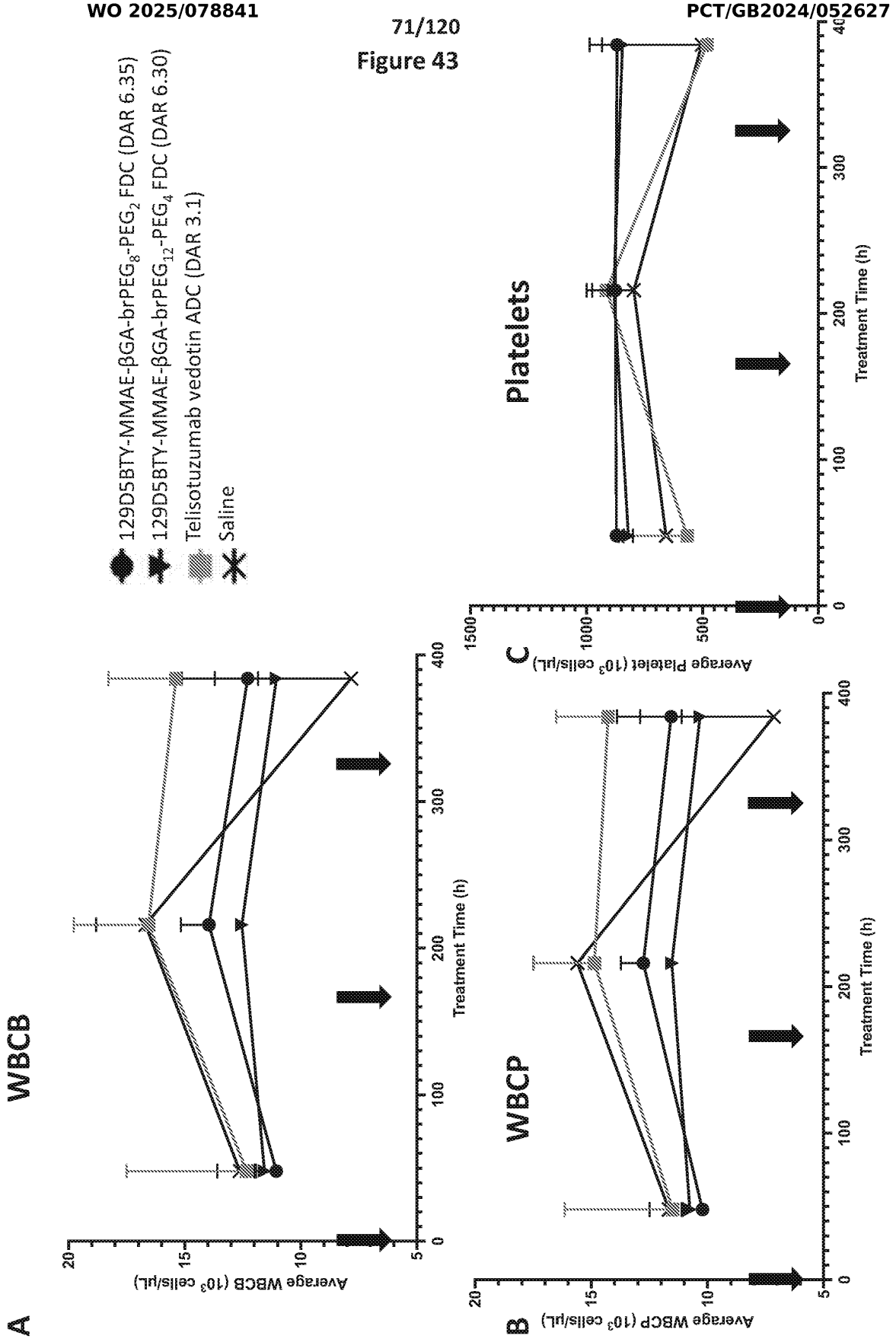

129D5BTY-MMAE-βGA-brPEG8-PEG2 FDC (DAR 6.35)
129D5BTY-MMAE-βGA-brPEG12-PEG4 FDC (DAR 6.30)
Telisotuzumab vedotin ADC (DAR 3.1)
Saline
A
AST
Average ALT (U/L)
B
ALT
Average AST (U/L)
C
Urea
Average Urea (mmol/L)
D
Creatinine
Average Creatinine (µmol/L)
E
Bilirubin
Average Total Bilirubin (µmol/L)
Treatment Time (h)

Figure 43


A
WBCB
Average WBCB (10^3 cells/µL)
Treatment Time (h)
B
WBCP
Average WBCP (10^3 cells/µL)
Treatment Time (h)
C
Platelets
Average Platelet (10^3 cells/µL)
Treatment Time (h)
129D5BTY-MMAE-βGA-brPEG$_8$-PEG$_2$ FDC (DAR 6.35)
129D5BTY-MMAE-βGA-brPEG$_{12}$-PEG$_4$ FDC (DAR 6.30)
Telisotuzumab vedotin ADC (DAR 3.1)
Saline

72/120

Figure 44

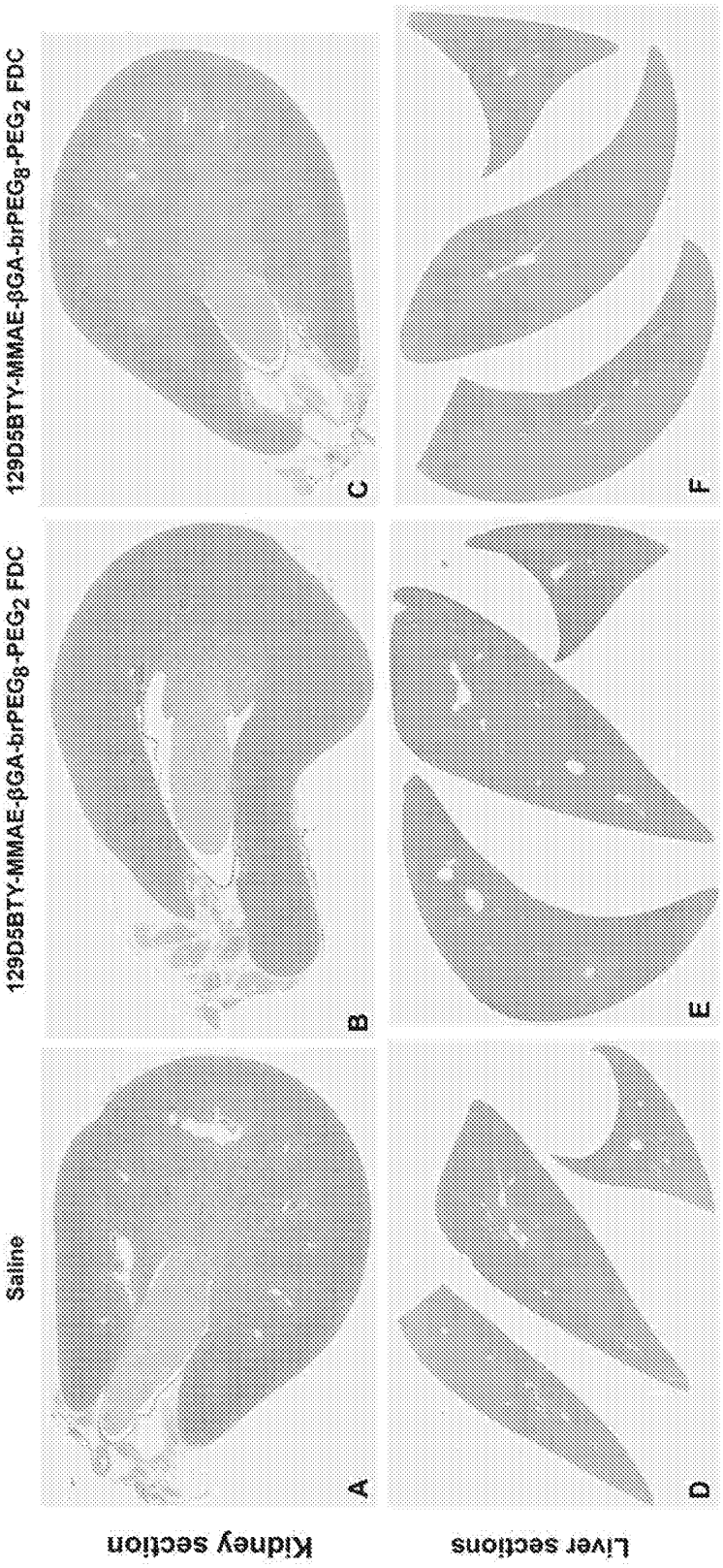


Figure 45

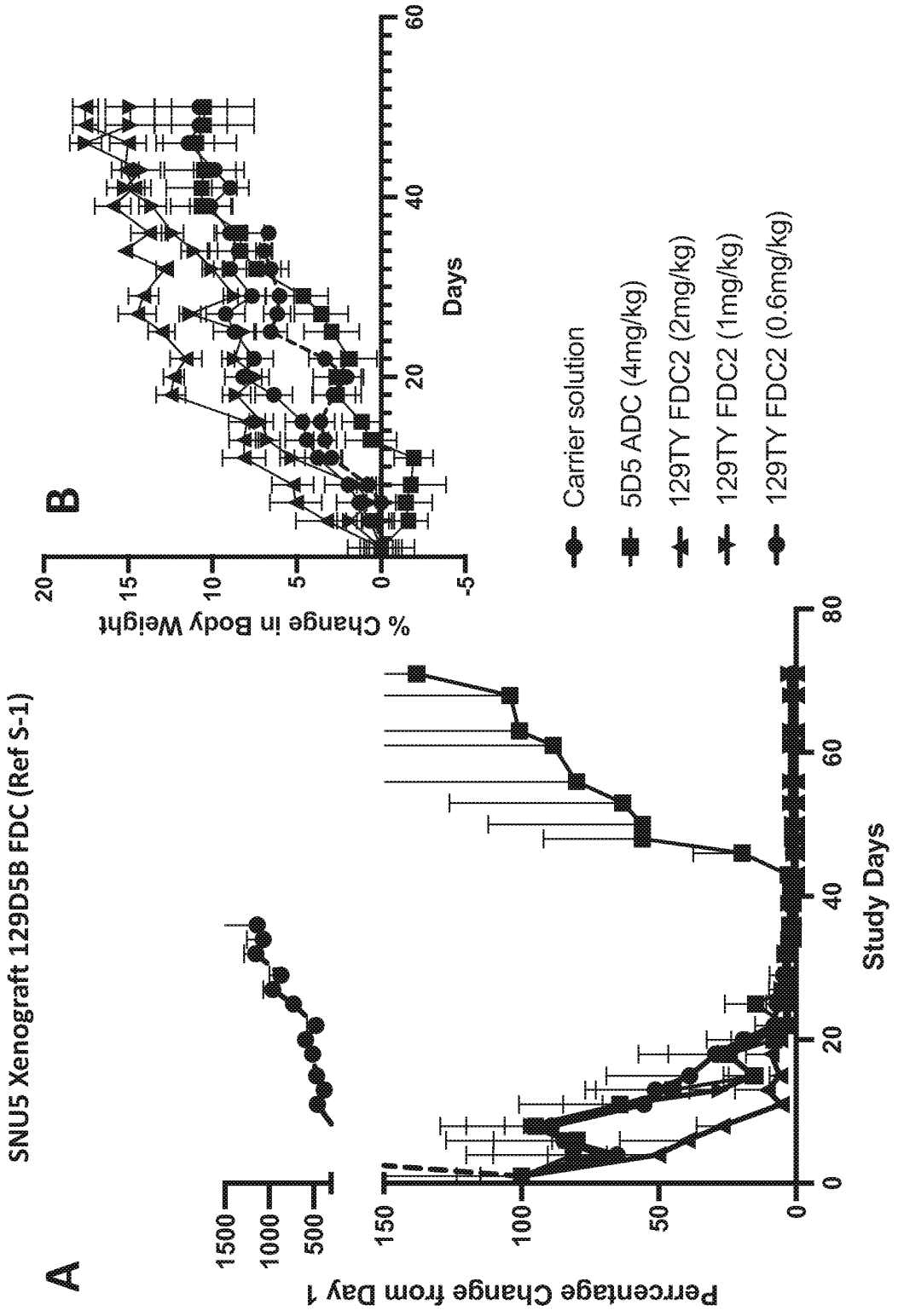

A
SNU5 Xenograft 129D5B FDC (Ref S-1)
Percentage Change from Day 1
Study Days
B
% Change in Body Weight
Days
Carrier solution
5D5 ADC (4mg/kg)
129TY FDC2 (2mg/kg)
129TY FDC2 (1mg/kg)
129TY FDC2 (0.6mg/kg)

Figure 46

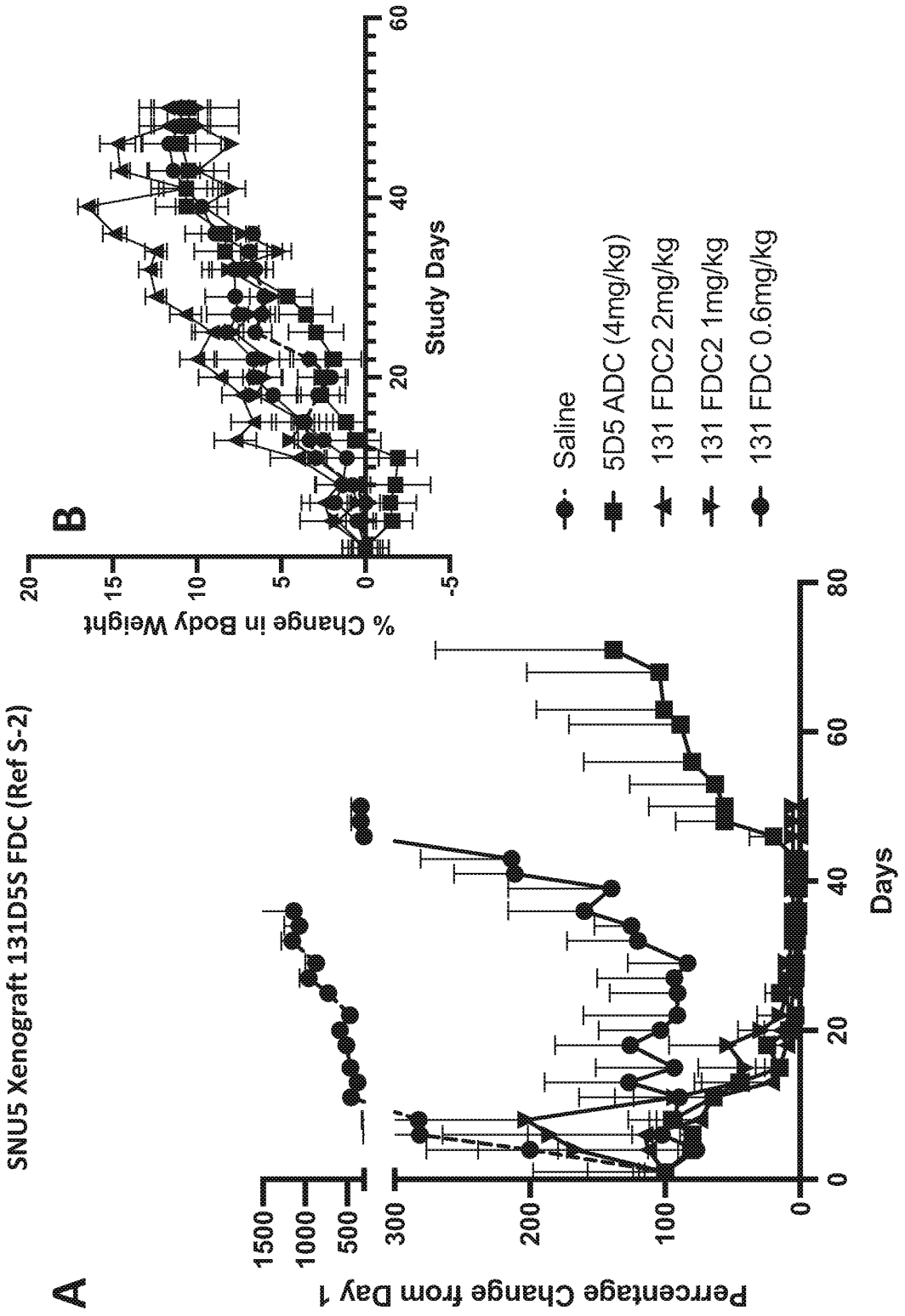

A
SNU5 Xenograft 131D5S FDC (Ref S-2)
Percentage Change from Day 1
1500
1000
500
300
200
100
0
0
20
40
60
80
Days
Saline
5D5 ADC (4mg/kg)
131 FDC2 2mg/kg
131 FDC2 1mg/kg
131 FDC 0.6mg/kg
B
% Change in Body Weight
20
15
10
5
0
-5
0
20
40
60
Study Days

Figure 47

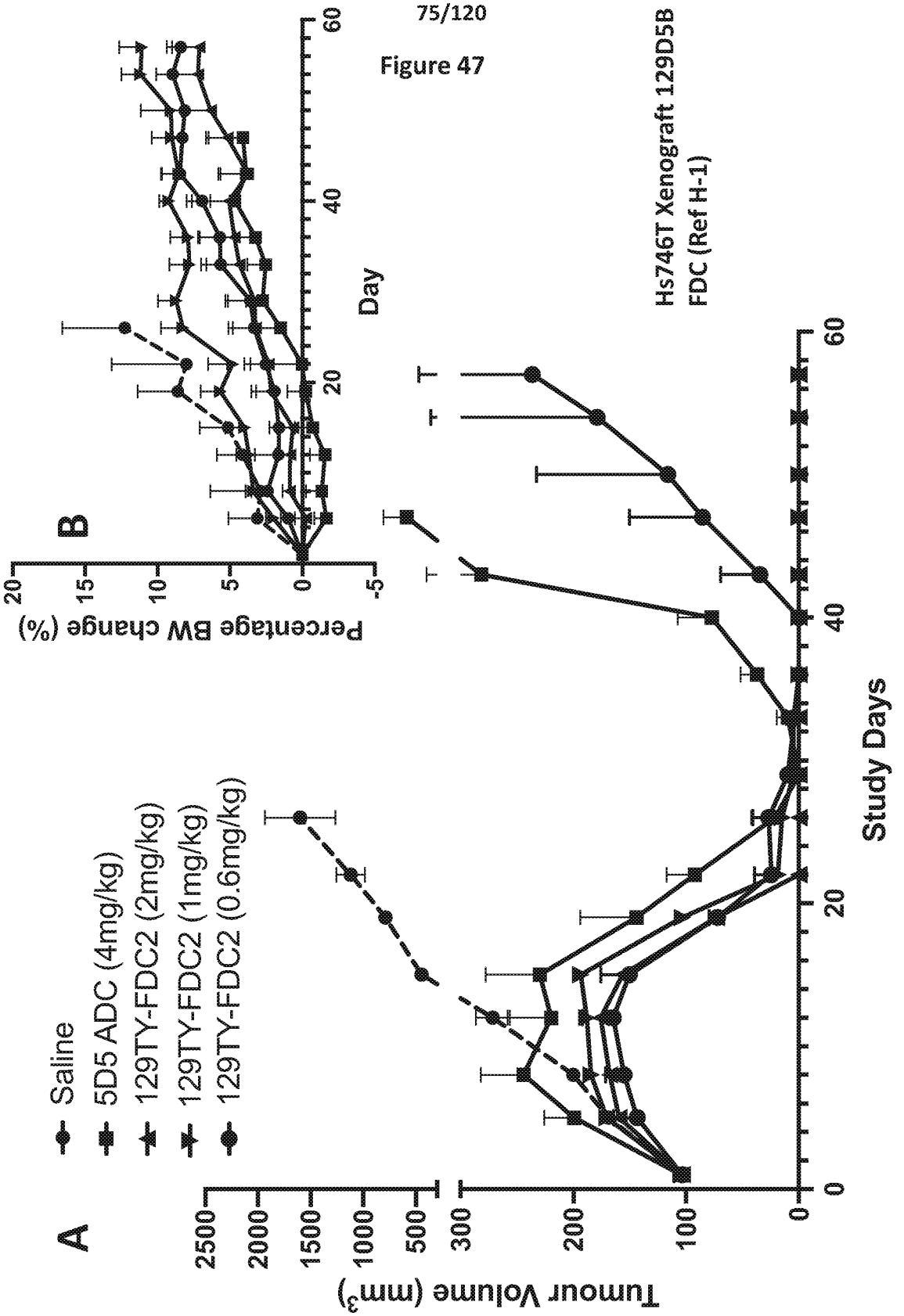
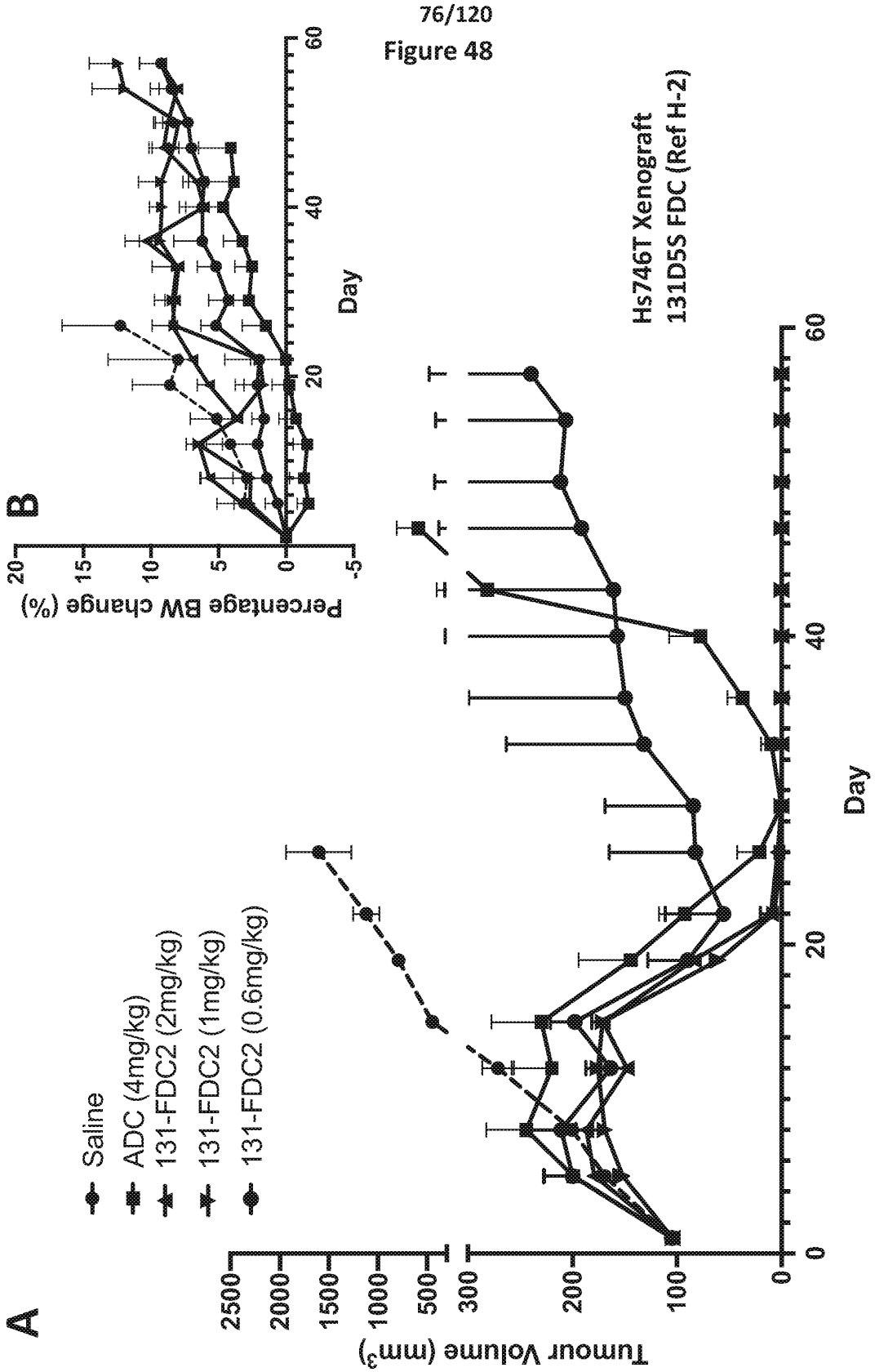

A
B
Saline
5D5 ADC (4mg/kg)
129TY-FDC2 (2mg/kg)
129TY-FDC2 (1mg/kg)
129TY-FDC2 (0.6mg/kg)
Tumour Volume (mm³)
Study Days
Hs746T Xenograft 129D5B
FDC (Ref H-1)
Percentage BW change (%)
Day

Figure 48


A
Hs746T Xenograft
131D5S FDC (Ref H-2)
Tumour Volume (mm3)
Day
Saline
ADC (4mg/kg)
131-FDC2 (2mg/kg)
131-FDC2 (1mg/kg)
131-FDC2 (0.6mg/kg)
B
Percentage BW change (%)
Day

77/120

Figure 49

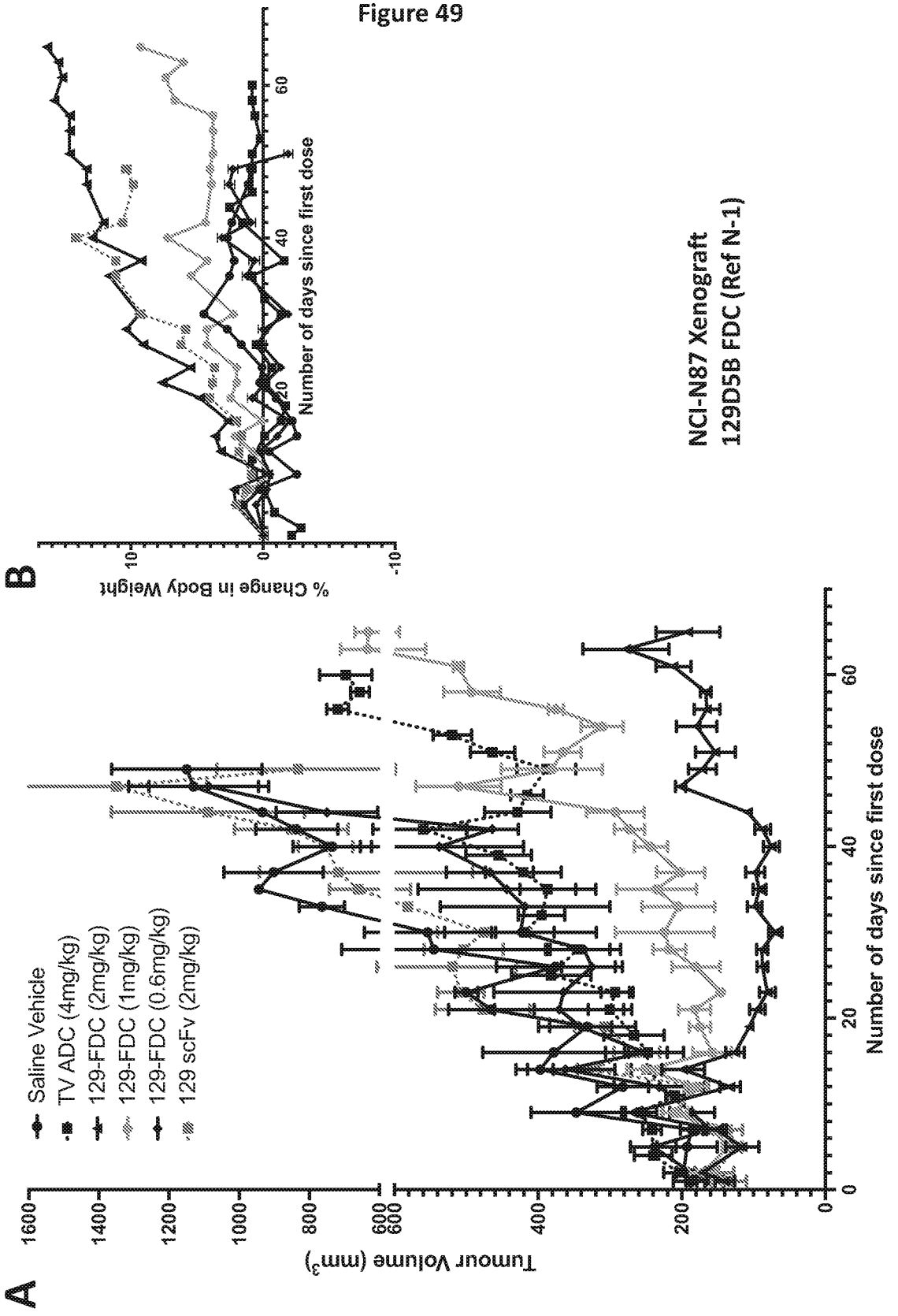


Figure 50

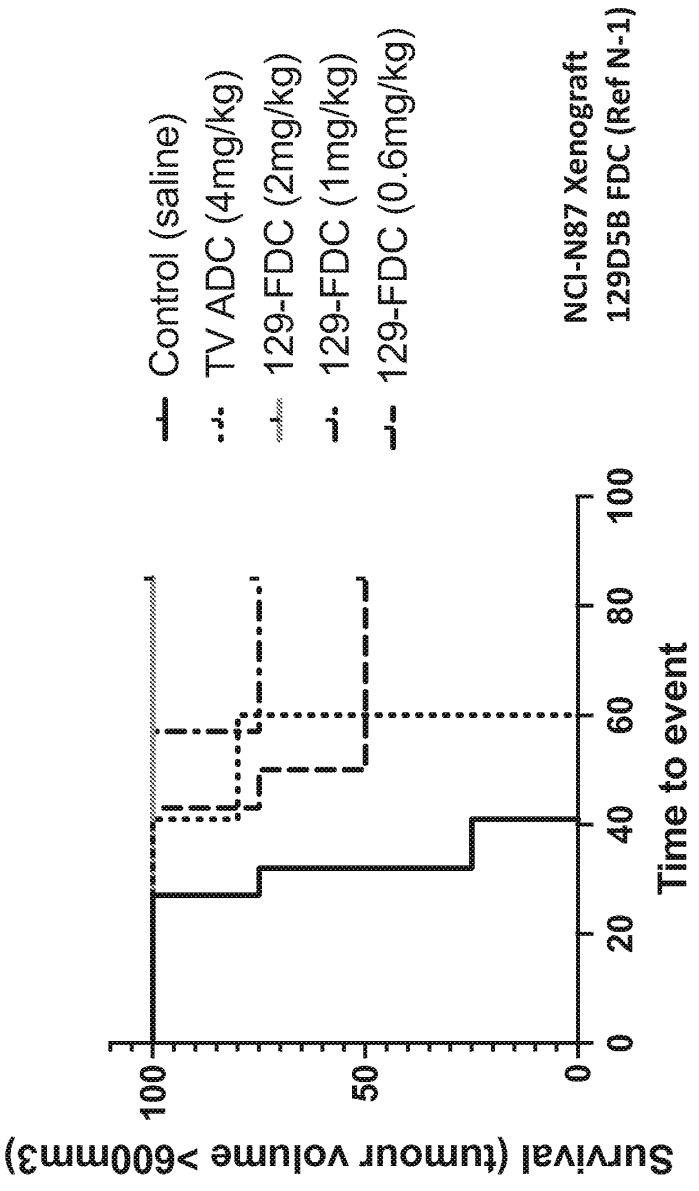

Survival (tumour volume >600mm3)
100
50
0
0
20
40
60
80
100
Time to event
Control (saline)
TV ADC (4mg/kg)
129-FDC (2mg/kg)
129-FDC (1mg/kg)
129-FDC (0.6mg/kg)
NCI-N87 Xenograft
129D5B FDC (Ref N-1)

Figure 51

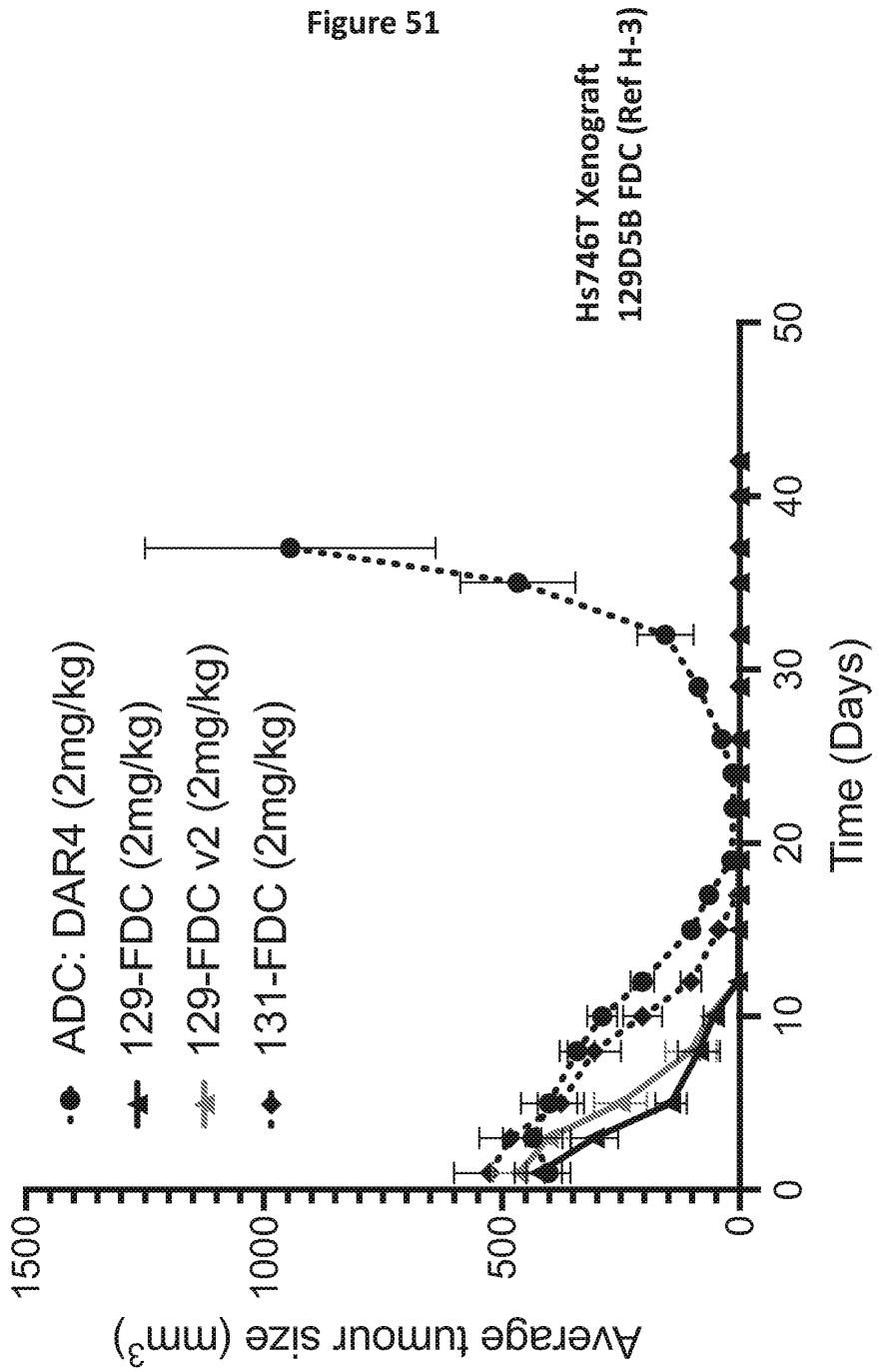

Hs746T Xenograft
129D5B FDC (Ref H-3)
ADC: DAR4 (2mg/kg)
129-FDC (2mg/kg)
129-FDC v2 (2mg/kg)
131-FDC (2mg/kg)
Average tumour size (mm3)
Time (Days)
0
500
1000
1500
0
10
20
30
40
50

Figure 52

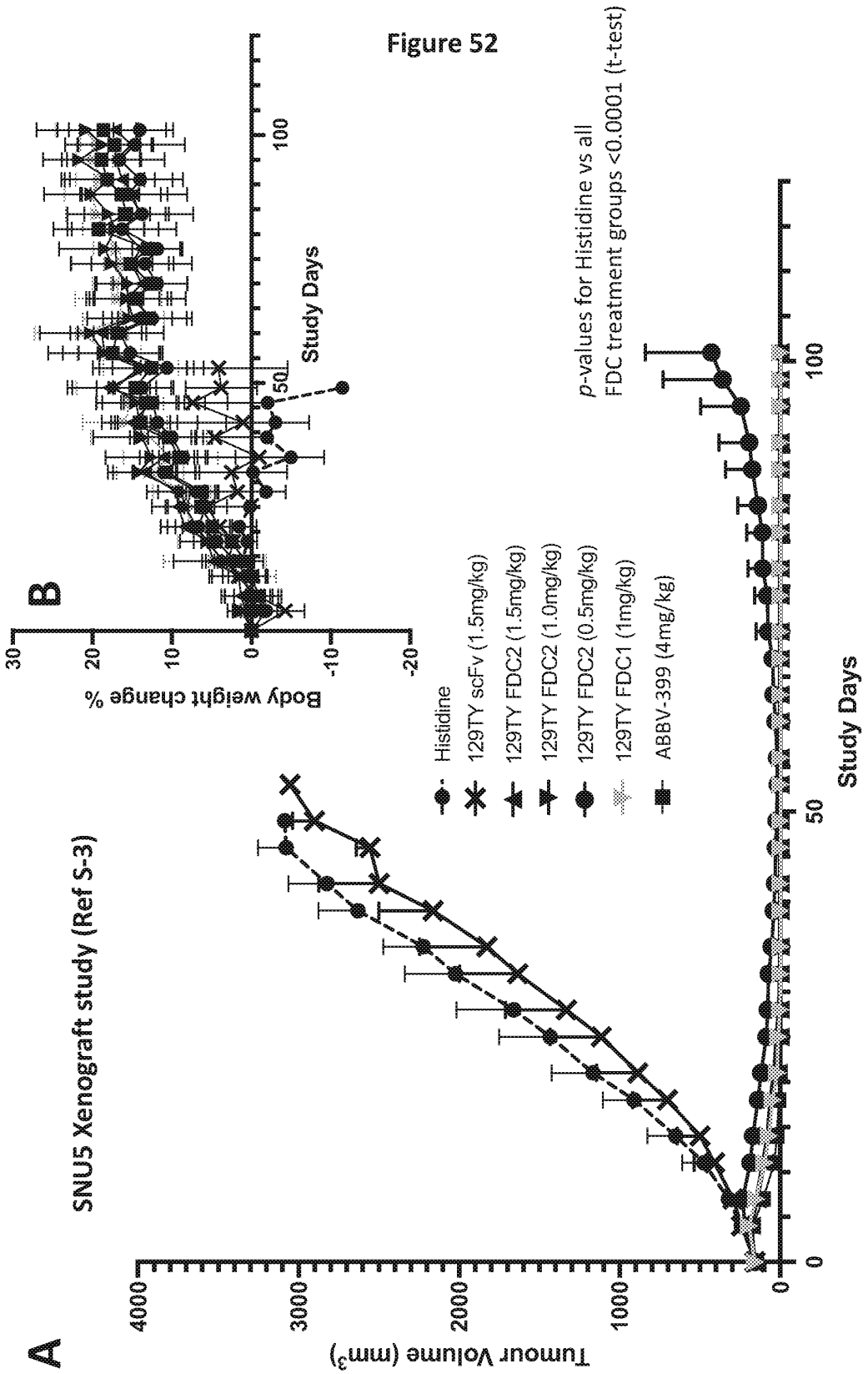

A
SNU5 Xenograft study (Ref S-3)
Tumour Volume (mm³)
4000
3000
2000
1000
0
0
50
100
Study Days
Histidine
129TY scFv (1.5mg/kg)
129TY FDC2 (1.5mg/kg)
129TY FDC2 (1.0mg/kg)
129TY FDC2 (0.5mg/kg)
129TY FDC1 (1mg/kg)
ABBV-399 (4mg/kg)
p-values for Histidine vs all
FDC treatment groups <0.0001 (t-test)
B
Body weight change %
30
20
10
0
-10
-20
0
50
100
Study Days

Figure 53

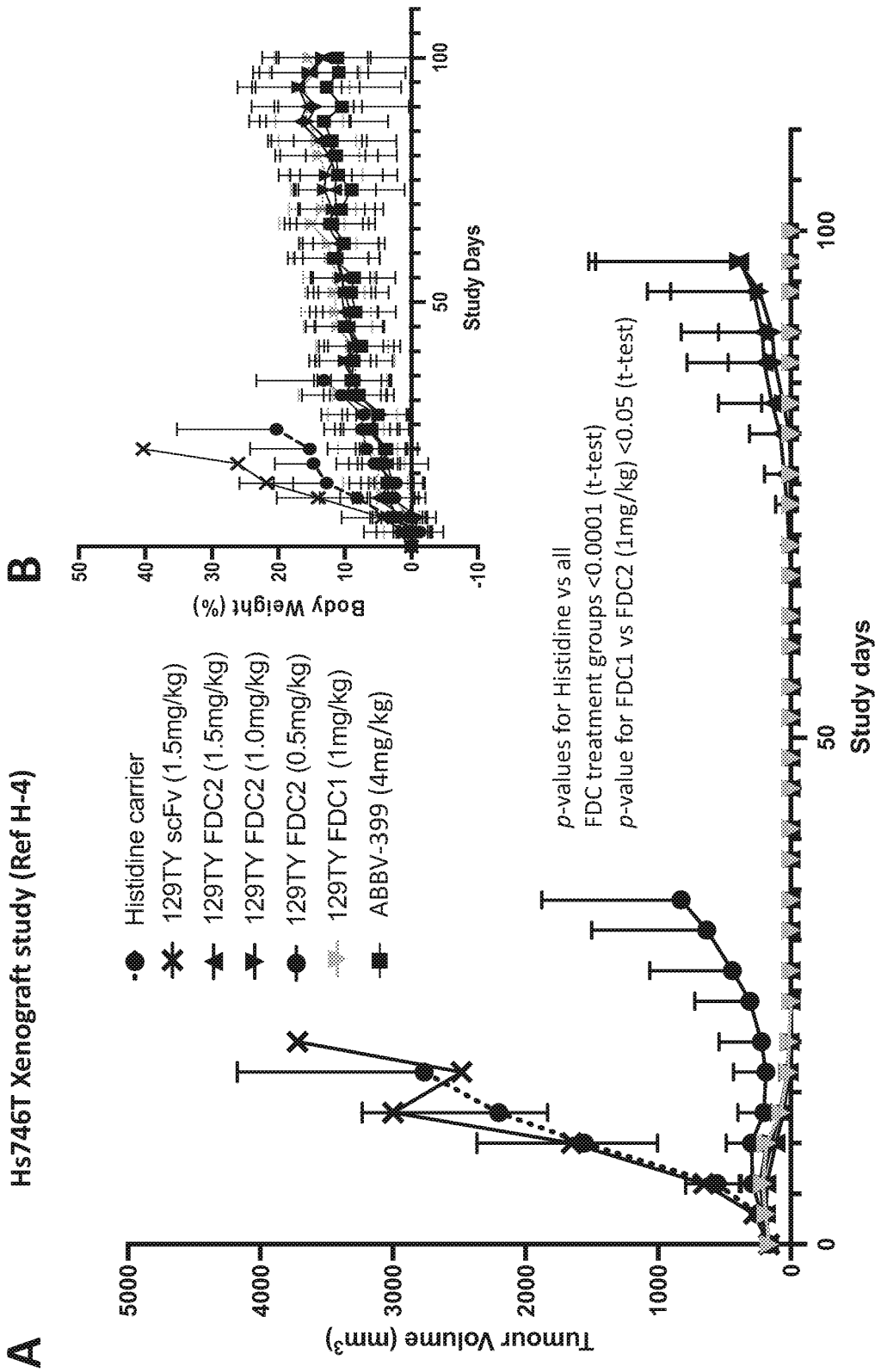

A
Hs746T Xenograft study (Ref H-4)
Tumour Volume (mm3)
5000
4000
3000
2000
1000
0
0
50
100
Study days
Histidine carrier
129TY scFv (1.5mg/kg)
129TY FDC2 (1.5mg/kg)
129TY FDC2 (1.0mg/kg)
129TY FDC2 (0.5mg/kg)
129TY FDC1 (1mg/kg)
ABBV-399 (4mg/kg)
p-values for Histidine vs all FDC treatment groups <0.0001 (t-test)
p-value for FDC1 vs FDC2 (1mg/kg) <0.05 (t-test)
B
Body Weight (%)
50
40
30
20
10
0
-10
0
50
100
Study Days

Figure 54

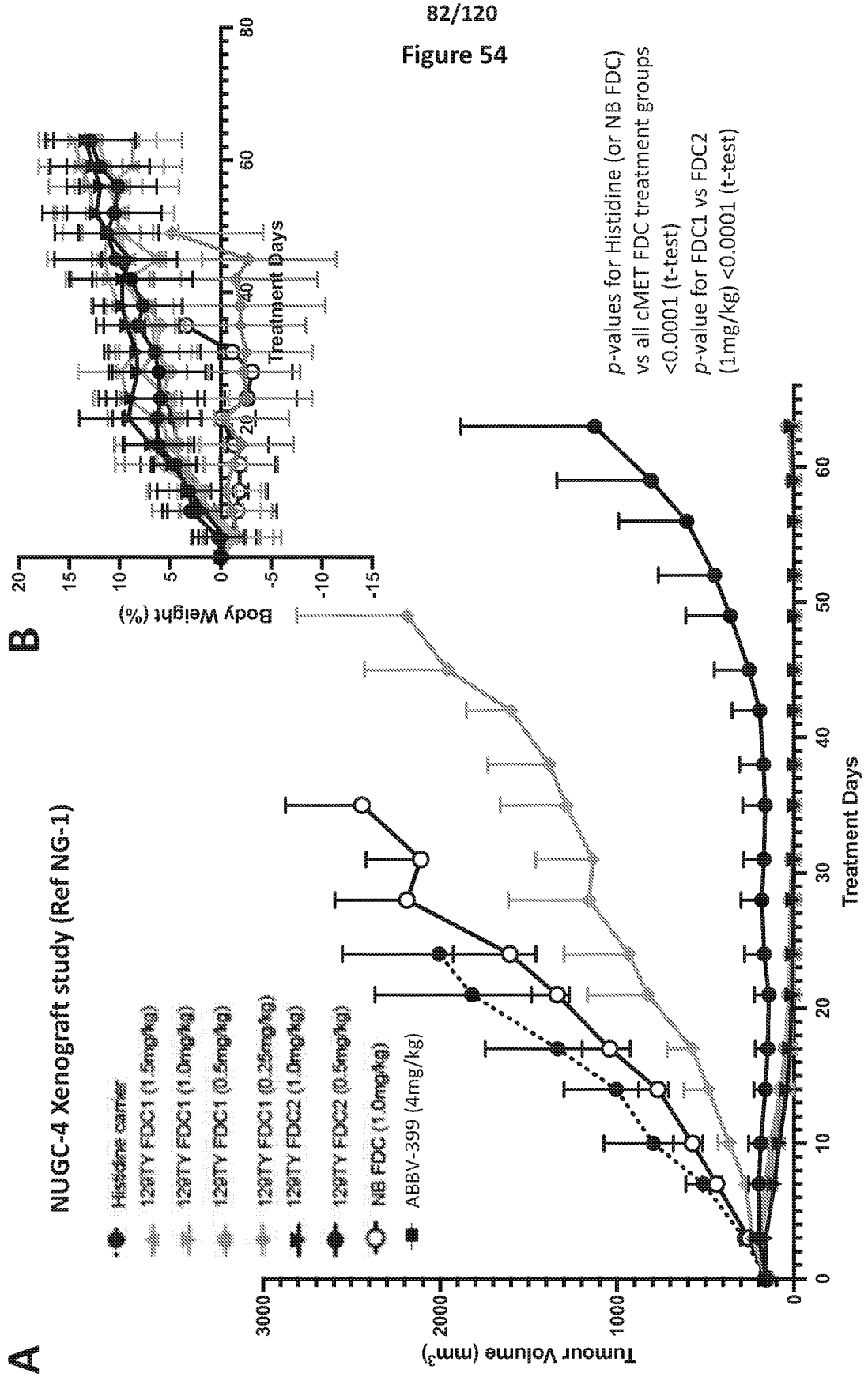

A
NUGC-4 Xenograft study (Ref NG-1)
Histidine carrier
123TY FDC1 (1.5mg/kg)
123TY FDC1 (1.0mg/kg)
123TY FDC1 (0.5mg/kg)
123TY FDC1 (0.25mg/kg)
123TY FDC2 (1.0mg/kg)
123TY FDC2 (0.5mg/kg)
NB FDC (1.0mg/kg)
ABBV-399 (4mg/kg)
Tumour Volume (mm³)
Treatment Days
p-values for Histidine (or NB FDC) vs all cMET FDC treatment groups <0.0001 (t-test)
p-value for FDC1 vs FDC2 (1mg/kg) <0.0001 (t-test)
B
Body Weight (%)
Treatment Days

Figure 55

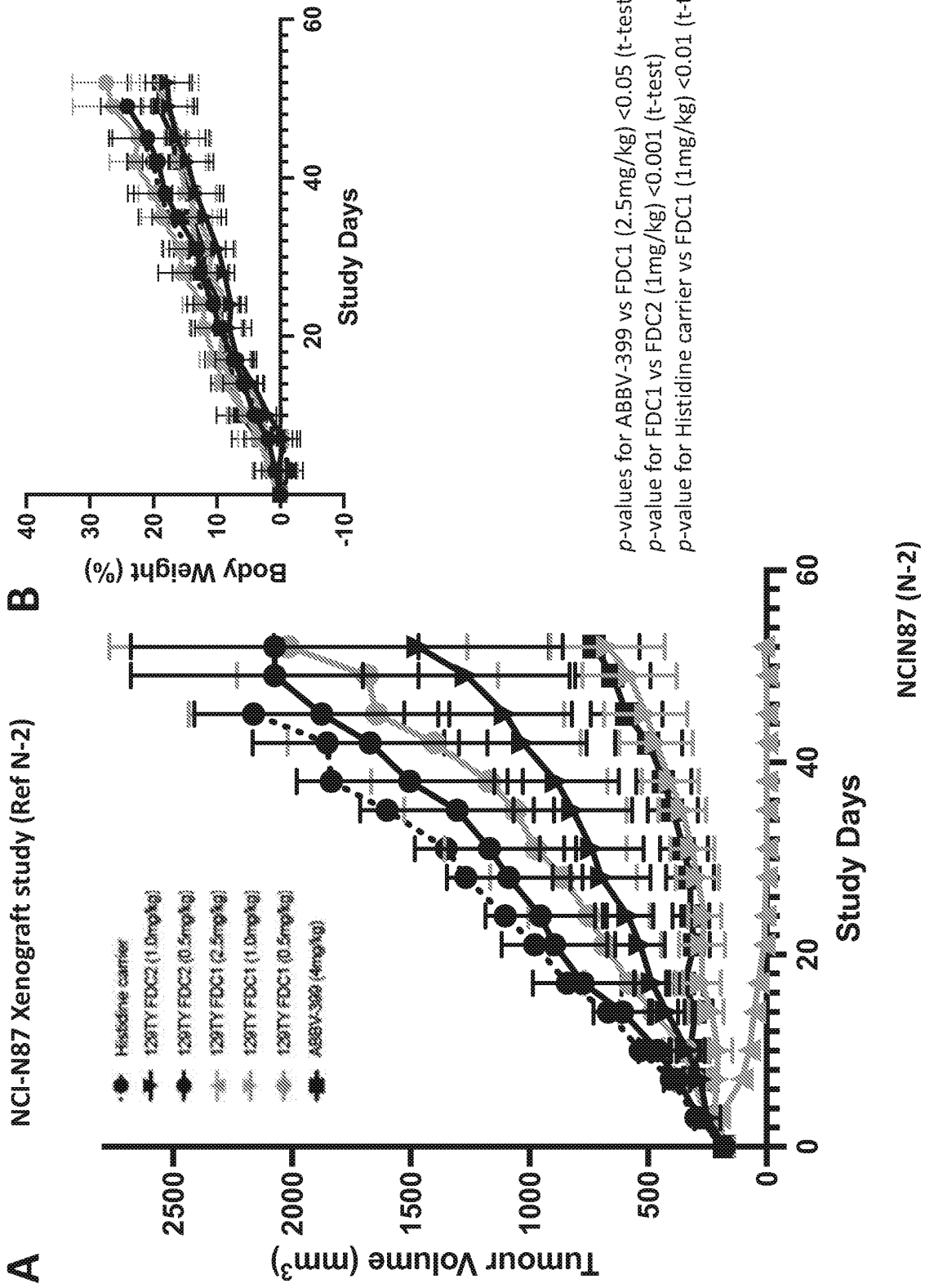

A
NCI-N87 Xenograft study (Ref N-2)
Histidine carrier
129TY FDC2 (1.0mg/kg)
129TY FDC2 (0.5mg/kg)
129TY FDC1 (2.5mg/kg)
129TY FDC1 (1.0mg/kg)
129TY FDC1 (0.5mg/kg)
ABBV-399 (4mg/kg)
Tumour Volume (mm3)
2500
2000
1500
1000
500
0
0
20
40
60
Study Days
B
Body Weight (%)
40
30
20
10
0
-10
0
20
40
60
Study Days
p-values for ABBV-399 vs FDC1 (2.5mg/kg) <0.05 (t-test)
p-value for FDC1 vs FDC2 (1mg/kg) <0.001 (t-test)
p-value for Histidine carrier vs FDC1 (1mg/kg) <0.01 (t-test)
NCIN87 (N-2)

Figure 56

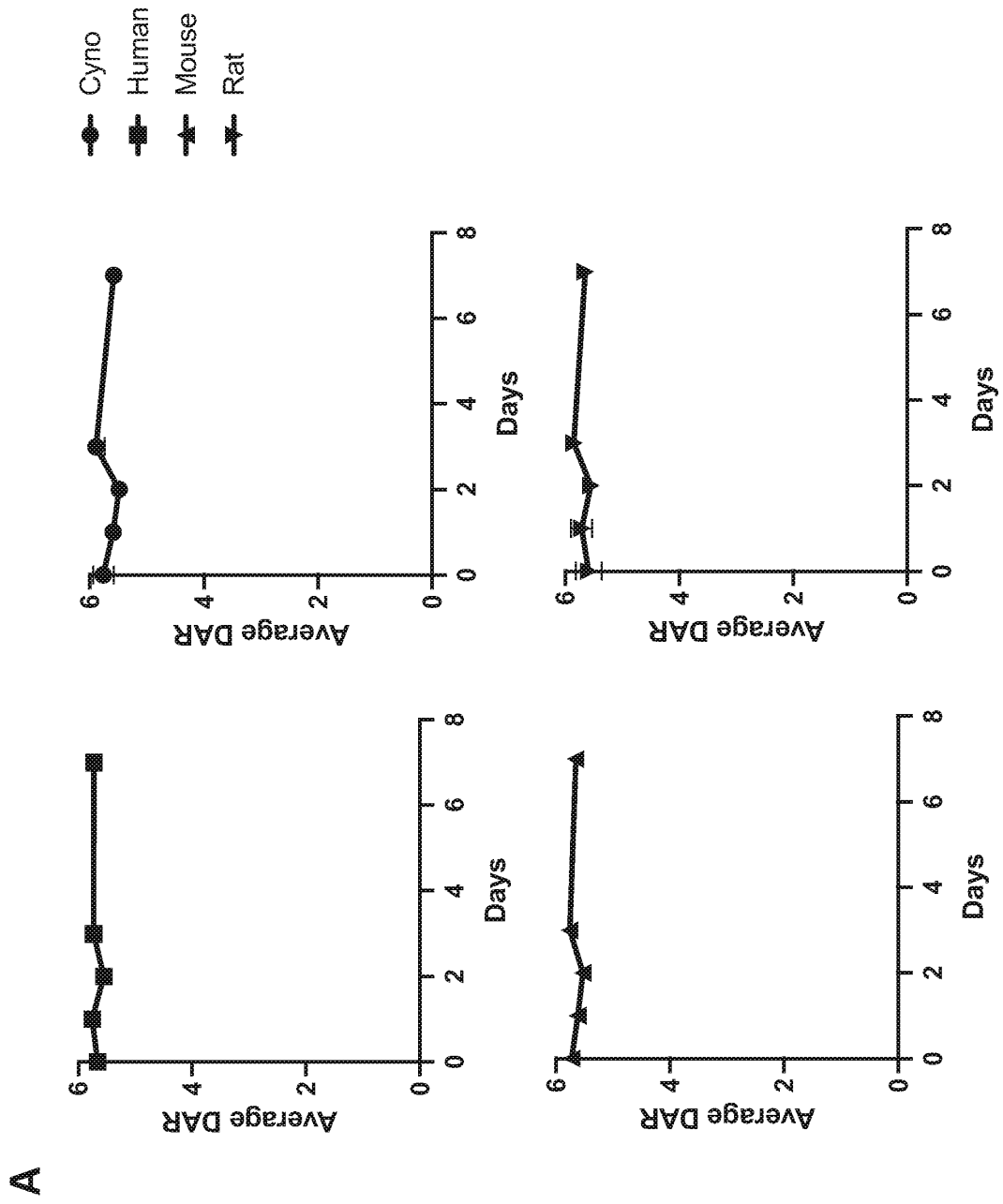


Figure 56 continued

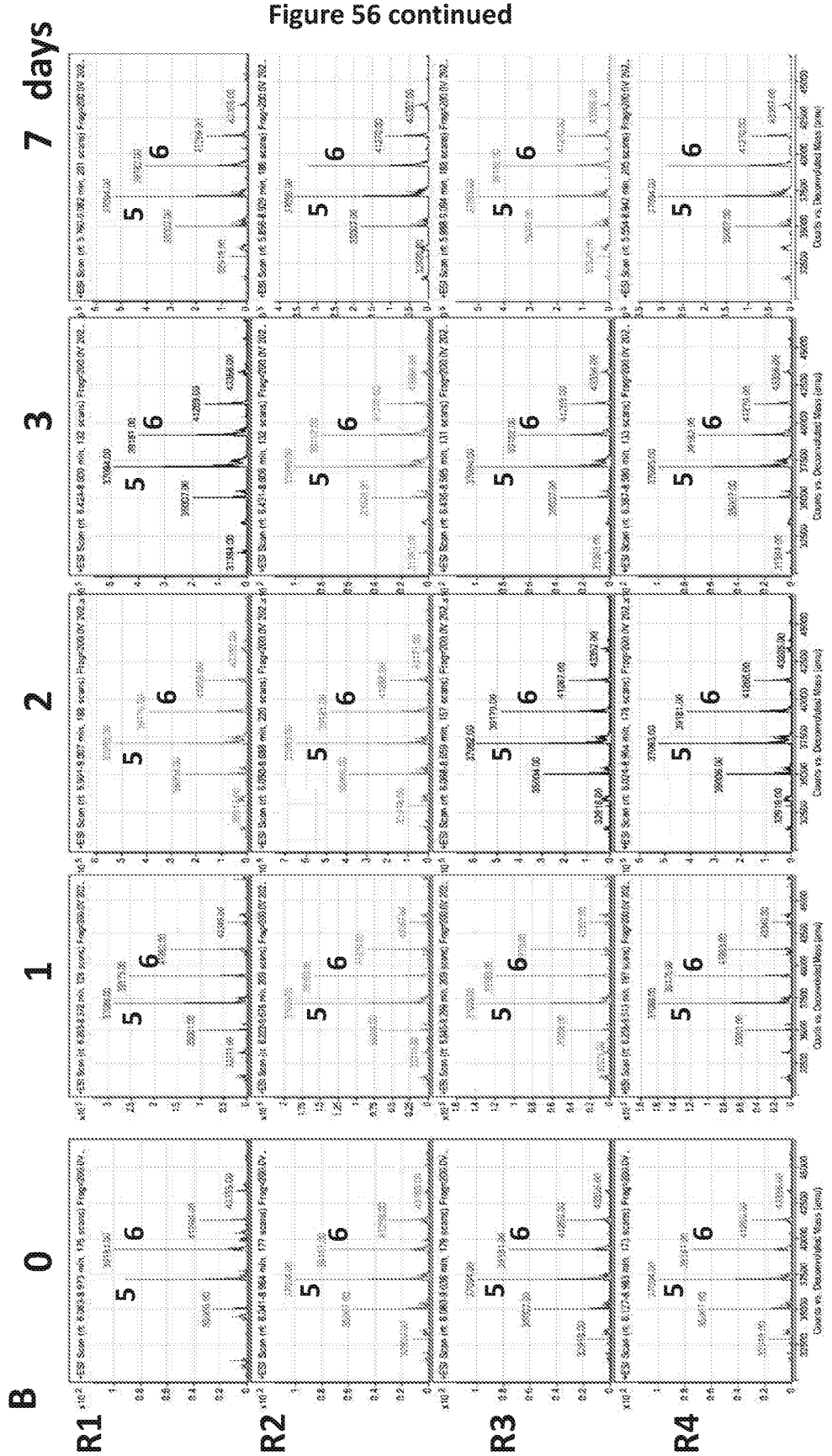


Figure 57

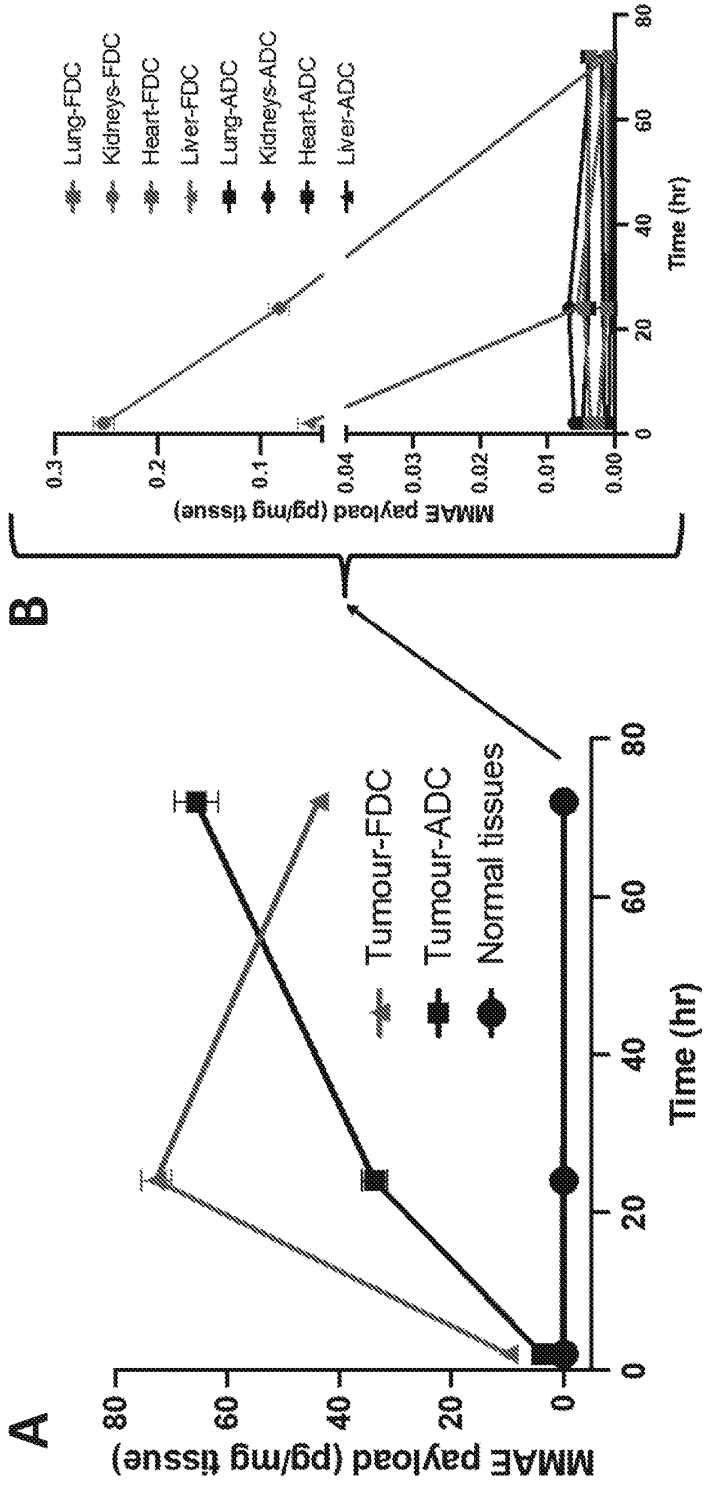

A
MMAE payload (pg/mg tissue)
0
20
40
60
80
0
20
40
60
80
Time (hr)
Tumour-FDC
Tumour-ADC
Normal tissues
B
MMAE payload (pg/mg tissue)
0.00
0.01
0.02
0.03
0.04
0.1
0.2
0.3
0
20
40
60
80
Time (hr)
Lung-FDC
Kidneys-FDC
Heart-FDC
Liver-FDC
Lung-ADC
Kidneys-ADC
Heart-ADC
Liver-ADC

Figure 58

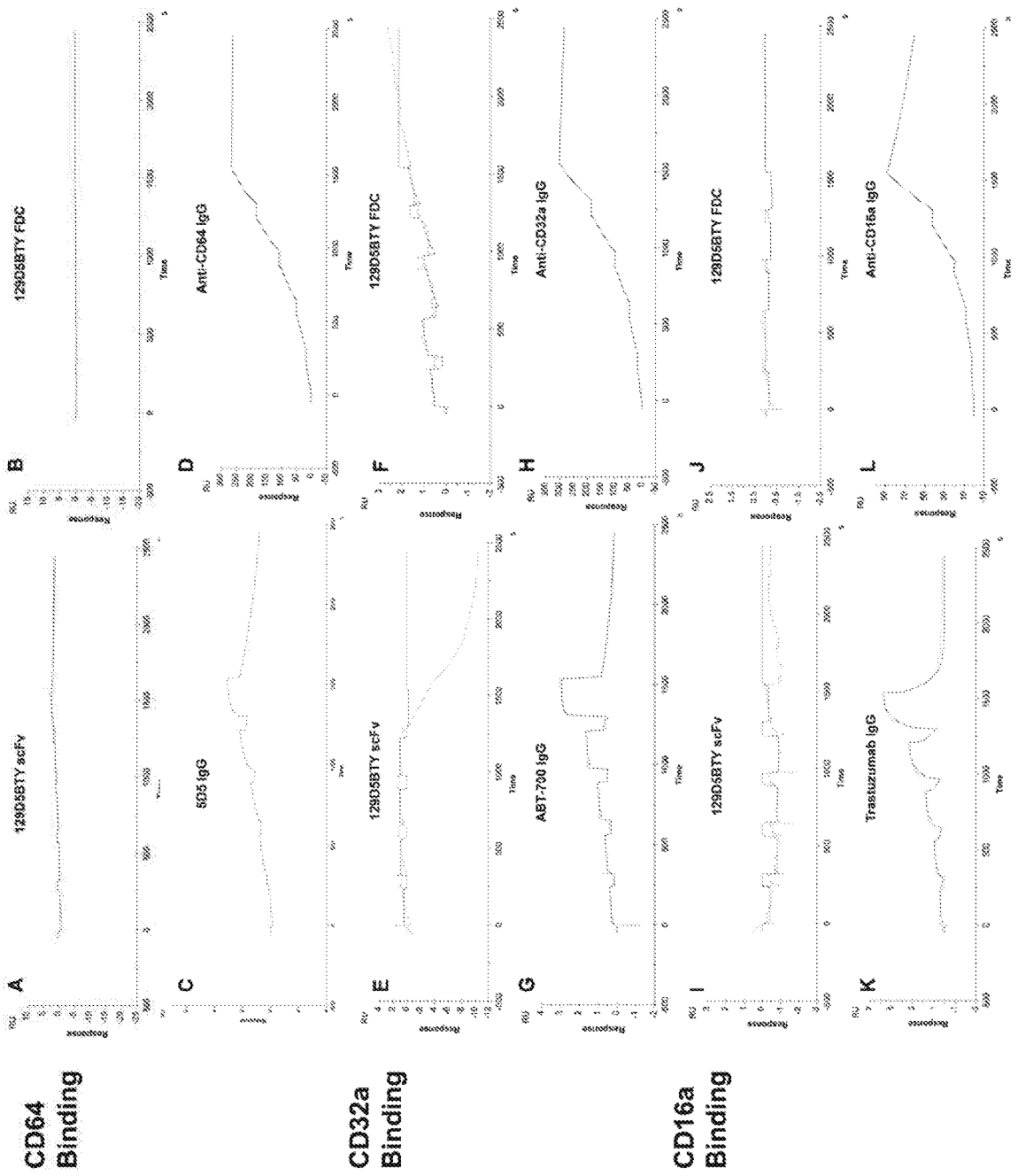

CD64 Binding
CD32a Binding
CD16a Binding
A
129D5BTY scFv
B
129D5BTY FDC
C
5G5 IgG
D
Anti-CD64 IgG
E
129D5BTY scFv
F
129D5BTY FDC
G
ABT-700 IgG
H
Anti-CD32a IgG
I
129D5BTY scFv
J
129D5BTY FDC
K
Trastuzumab IgG
L
Anti-CD16a IgG
Response
Time

Figure 59

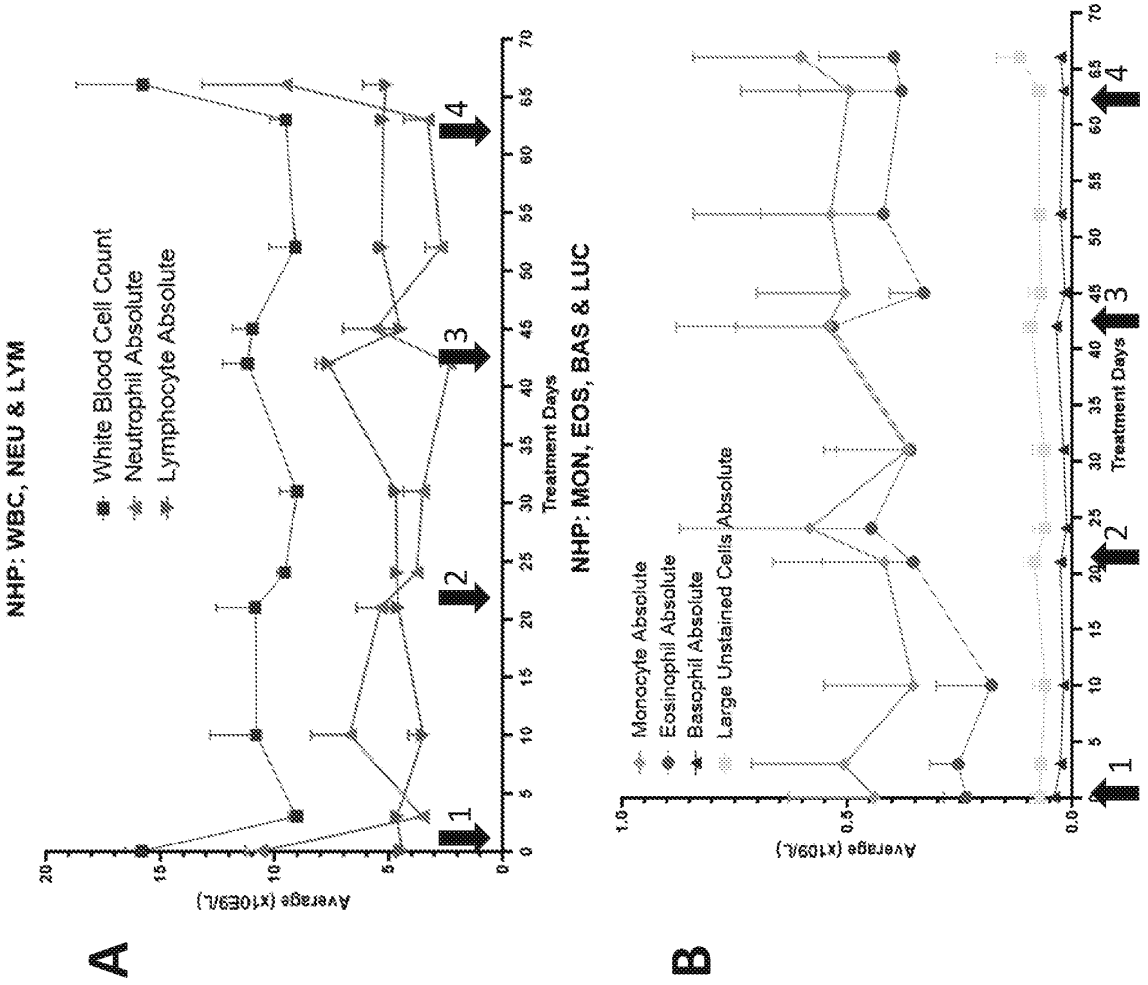

A
NHP: WBC, NEU & LYM
White Blood Cell Count
Neutrophil Absolute
Lymphocyte Absolute
Average (x10E3/L)
Treatment Days
1
2
3
4
B
NHP: MON, EOS, BAS & LUC
Monocyte Absolute
Eosinophil Absolute
Basophil Absolute
Large Unstained Cells Absolute
Average (x10E3/L)
Treatment Days
1
2
3
4

Figure 60

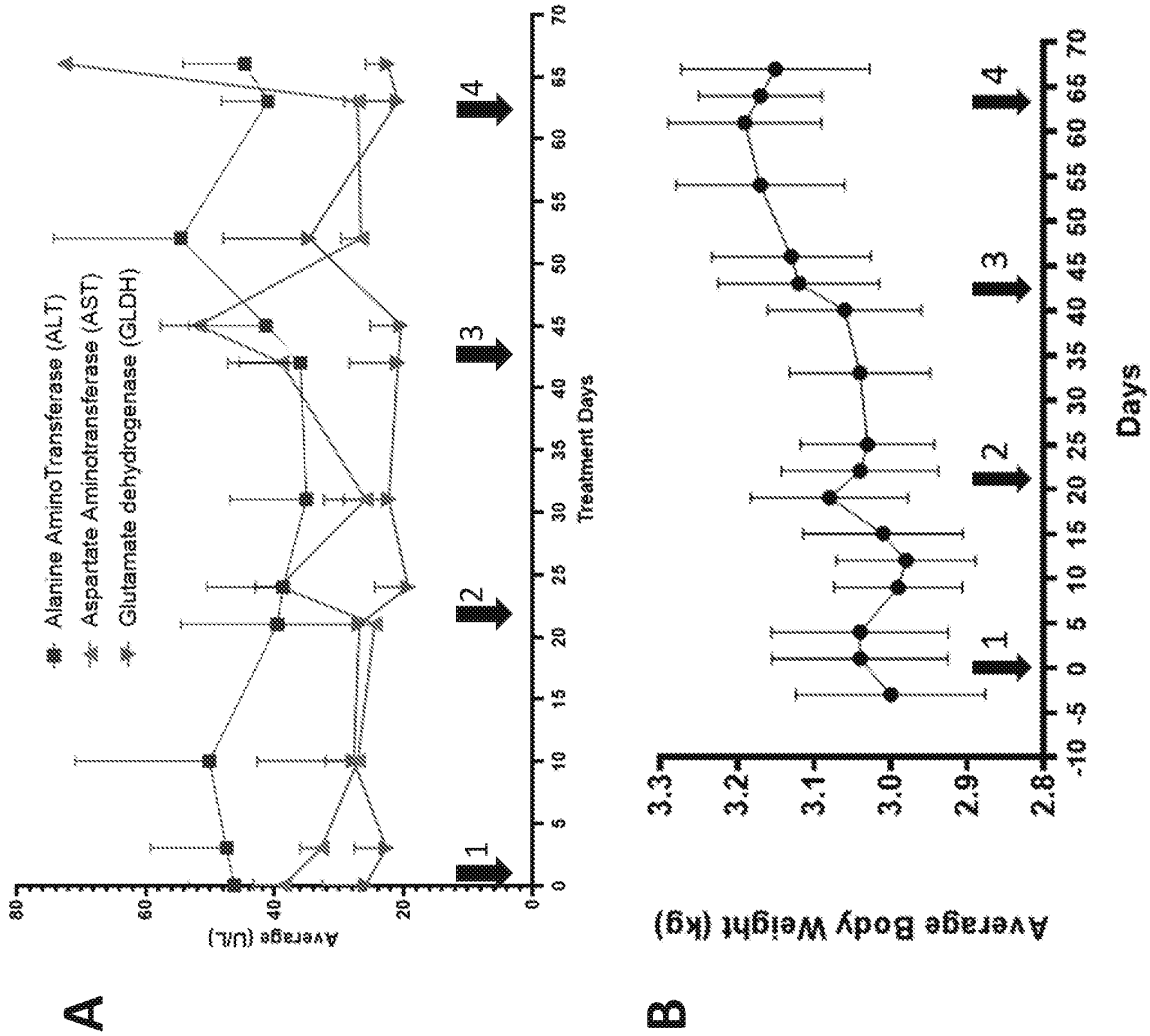

A
NHP: ALT, AST & GLDH
Alanine AminoTransferase (ALT)
Aspartate Aminotransferase (AST)
Glutamate dehydrogenase (GLDH)
Average (U/L)
Treatment Days
1
2
3
4
B
Average Body Weight (kg)
Days

Figure 61

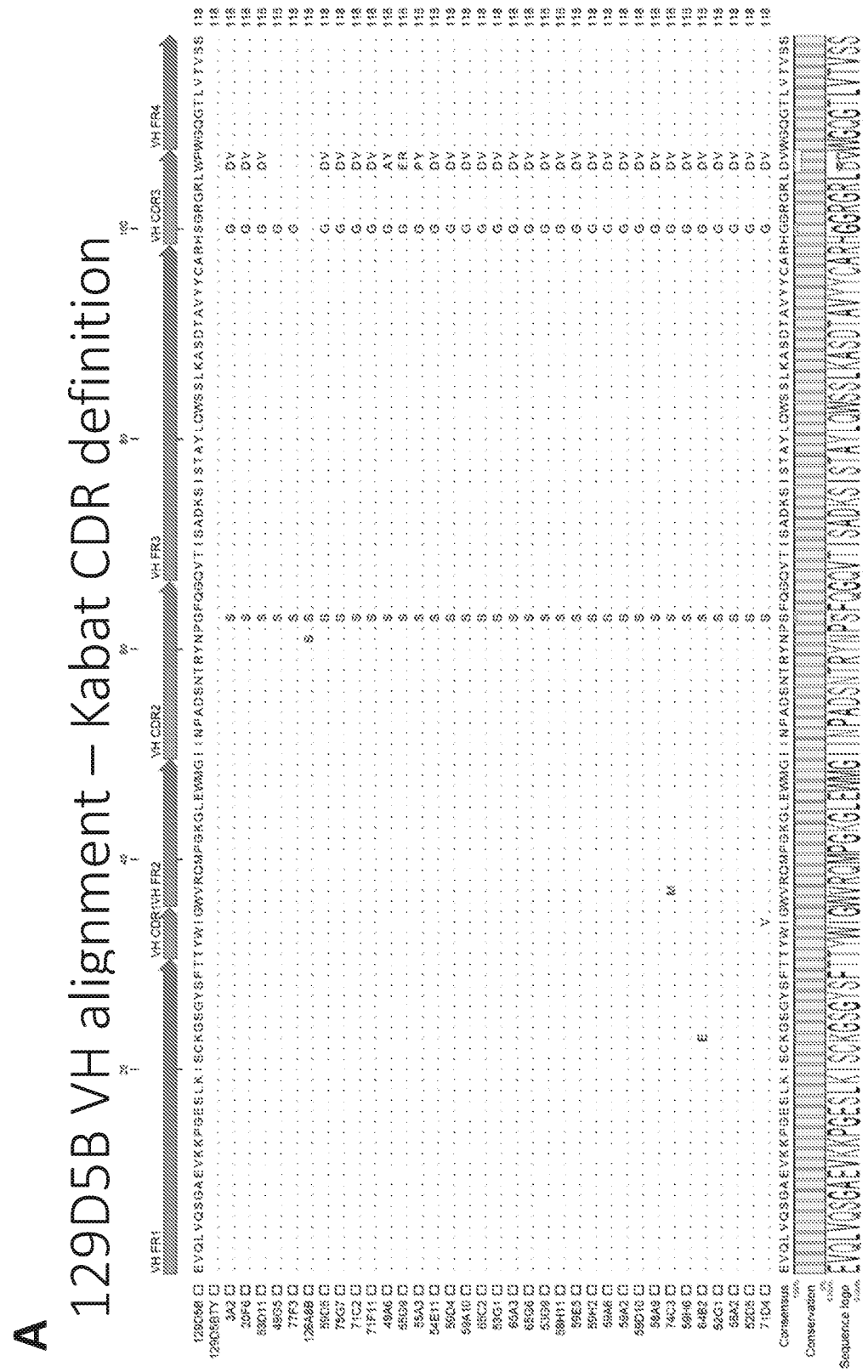

A
129D5B VH alignment – Kabat CDR definition
VH FR1
VH CDR1
VH FR2
VH CDR2
VH FR3
VH CDR3
VH FR4
Consensus
Conservation
Sequence logo

Figure 61 continued

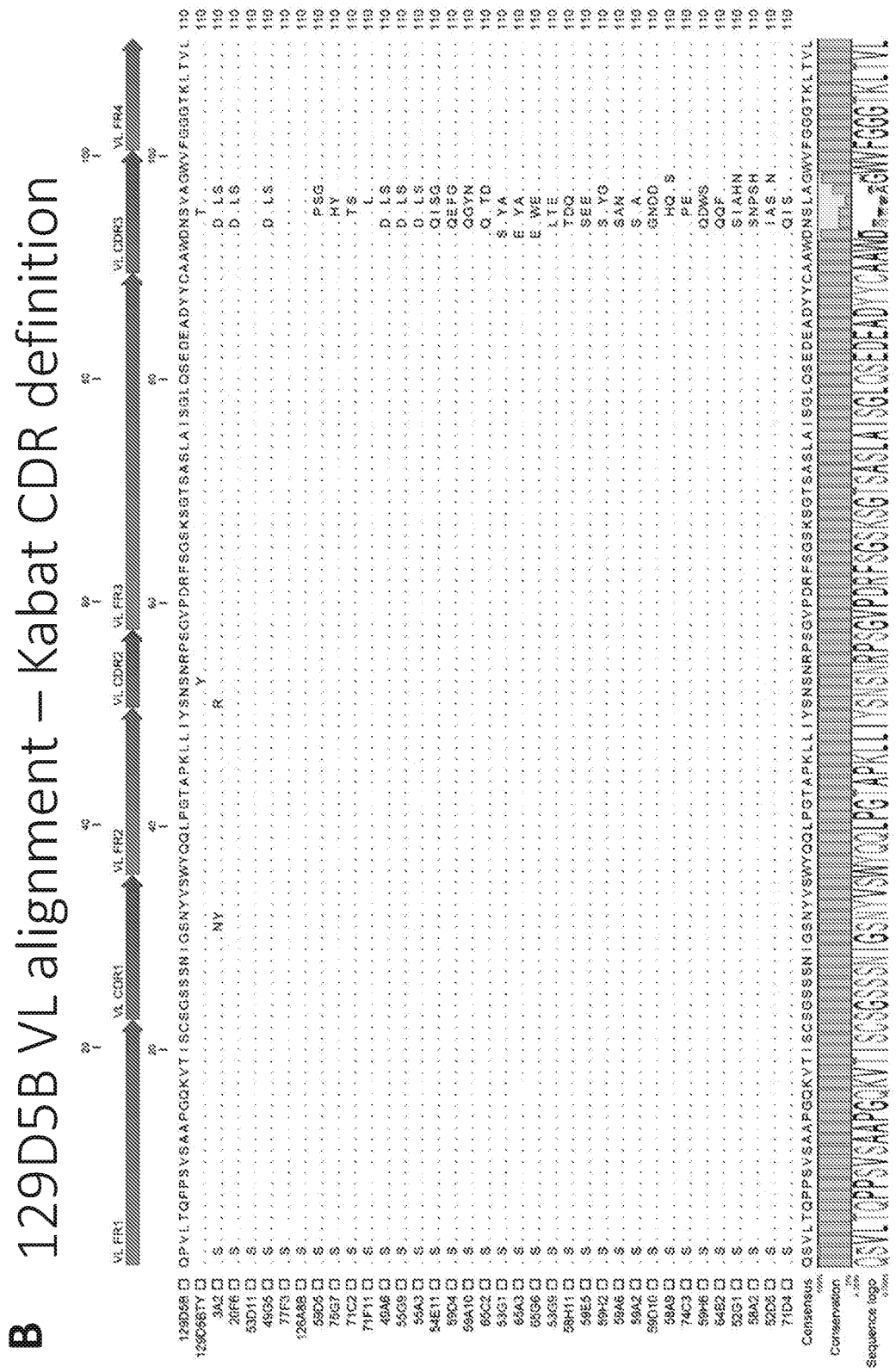


Figure 61 continued

c

Summary of diversity in 129D5B family

129D5BTY whole scFv (18/228 amino acids variance = 7.9%)														
VH (8/118; 7%)							VL (10/110; 9%)							
FR1 (1/30; 3%)	CDR1 (1/5; 20%)	FR2 (1/14; 7%)	CDR2 (2/17; 12%)	FR3 (0/32; 0%)	CDR3 (3/9; 33%)	FR4 (0/11; 0%)	FR1 (1/22; 4%)	CDR1 (2/13; 15%)	FR2 (0/15; 0%)	CDR2 (2/7; 29%)	FR3 (0/32; 0%)	CDR3 (5/11; 45%)	FR4 (0/10; 0%)

Figure 62

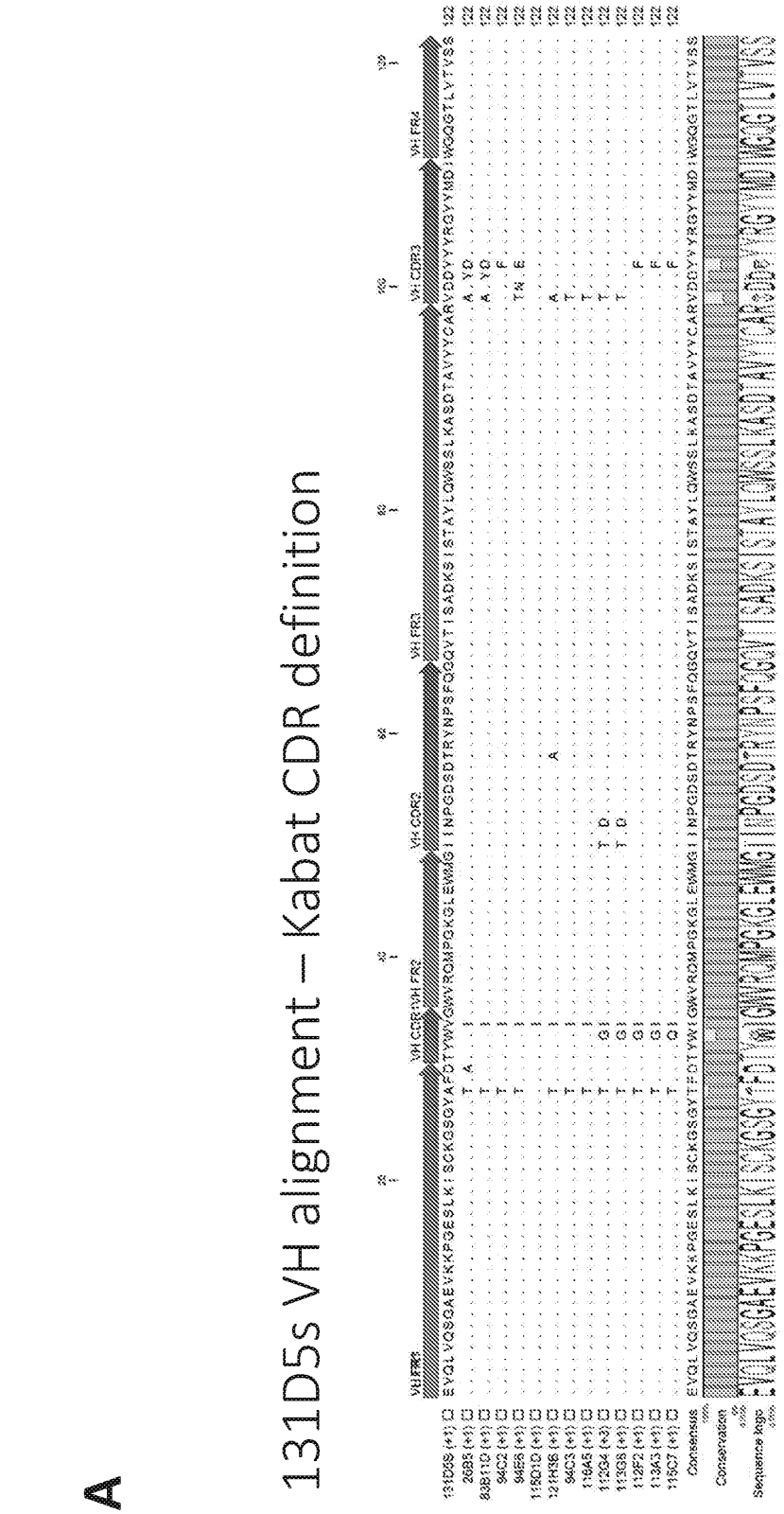

A
131D5s VH alignment – Kabat CDR definition
VH FR1
VH CDR1
VH FR2
VH CDR2
VH FR3
VH CDR3
VH FR4
Consensus
Conservation
Sequence logo

Figure 62 continued

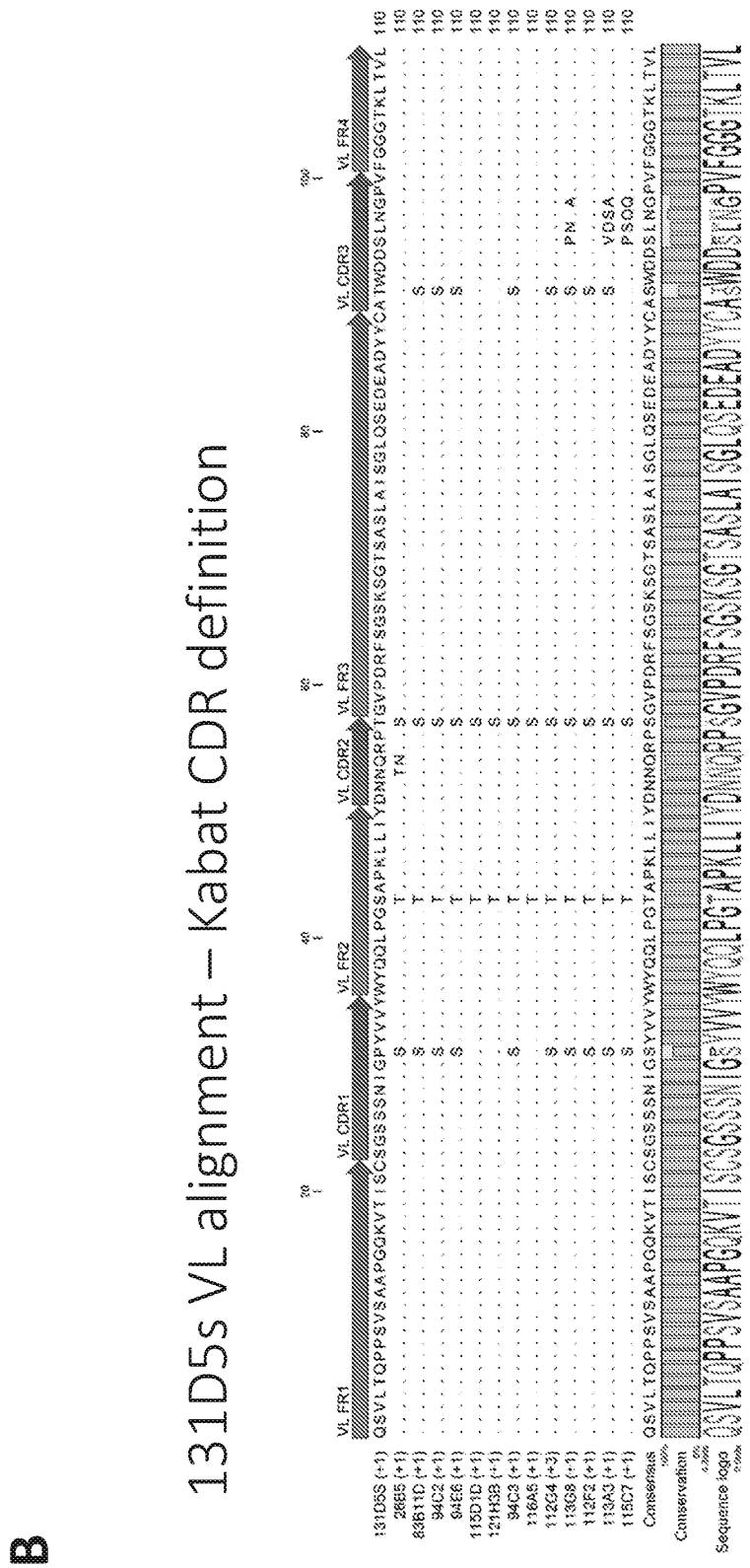
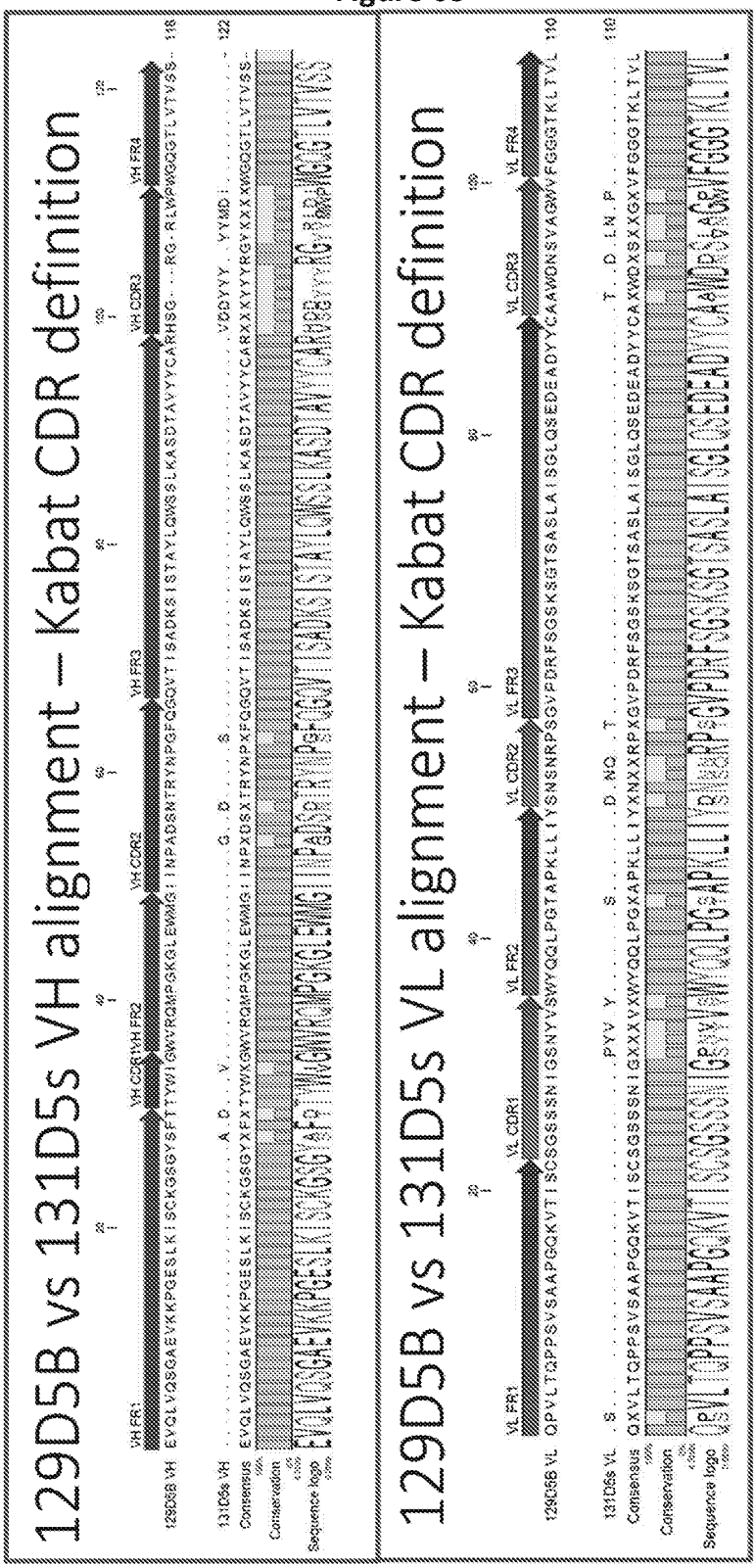


Figure 62 continued

C Summary of diversity in 131D5s family

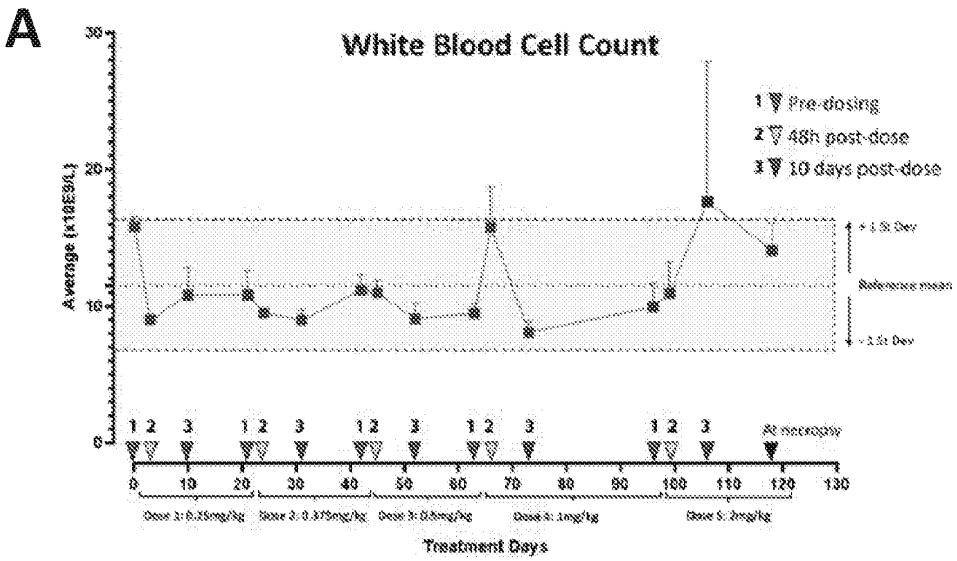
131D5s whole scFv (21/232 amino acids variance = 9%)														
VH (11/122; 9%)							VL (10/110; 9%)							
FR1 (2/30; 7%)	CDR1 (2/5; 40%)	FR2 (0/14; 0%)	CDR2 (3/17; 18%)	FR3 (0/32; 0%)	CDR3 (4/13; 31%)	FR4 (0/11; 0%)	FR1 (0/22; 4%)	CDR1 (1/13; 8%)	FR2 (1/15; 7%)	CDR2 (3/7; 43%)	FR3 (0/32; 0%)	CDR3 (5/11; 45%)	FR4 (0/10; 0%)

Figure 63



129D5BTY whole scFv (28/228 amino acids variance = 12%)															
VH (13/118; 11%)							VL (15/110; 14%)								
FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
(2/30; 7%)	(1/5; 20%)	(0/14; 7%)	(3/17; 18%)	(0/32; 0%)	(7/9; 78%)	(0/11; 0%)	(1/22; 4%)	(4/13; 31%)	(1/15; 7%)	(4/7; 57%)	(0/32; 0%)	(5/11; 45%)	(0/10; 0%)

Figure 64


A
White Blood Cell Count
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (x10E9/L)
Reference mean
At necropsy
Treatment Days

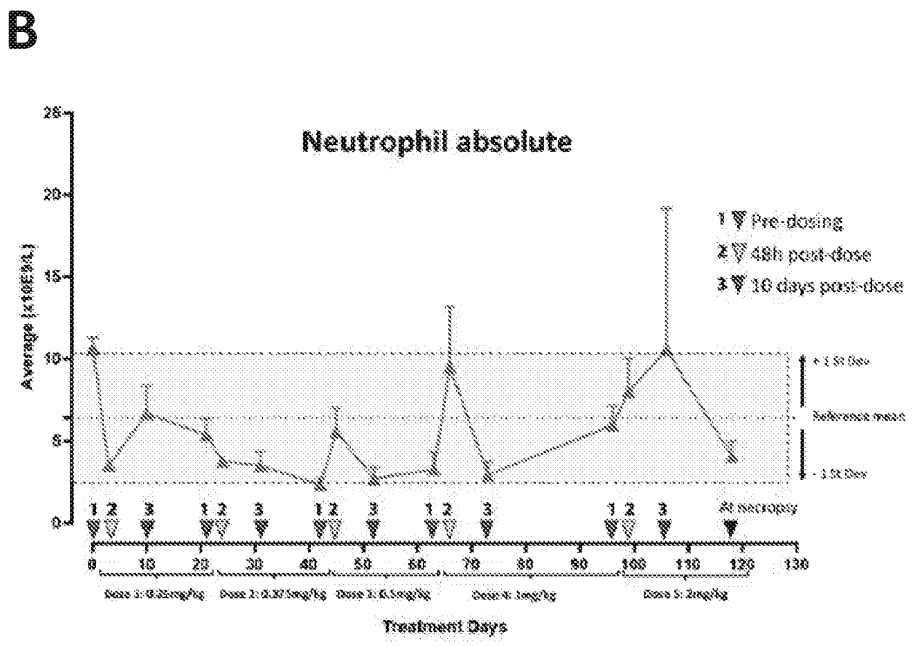
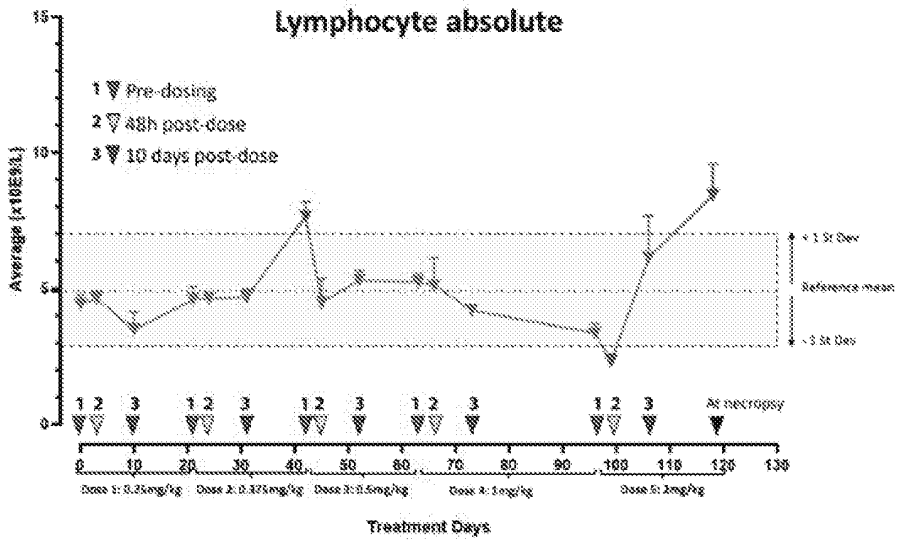

B
Neutrophil absolute
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (x10E9/L)
Reference mean
At necropsy
Treatment Days

Figure 64

C


Lymphocyte absolute
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (x10E9/L)
Reference mean
At necropsy
Treatment Days

D

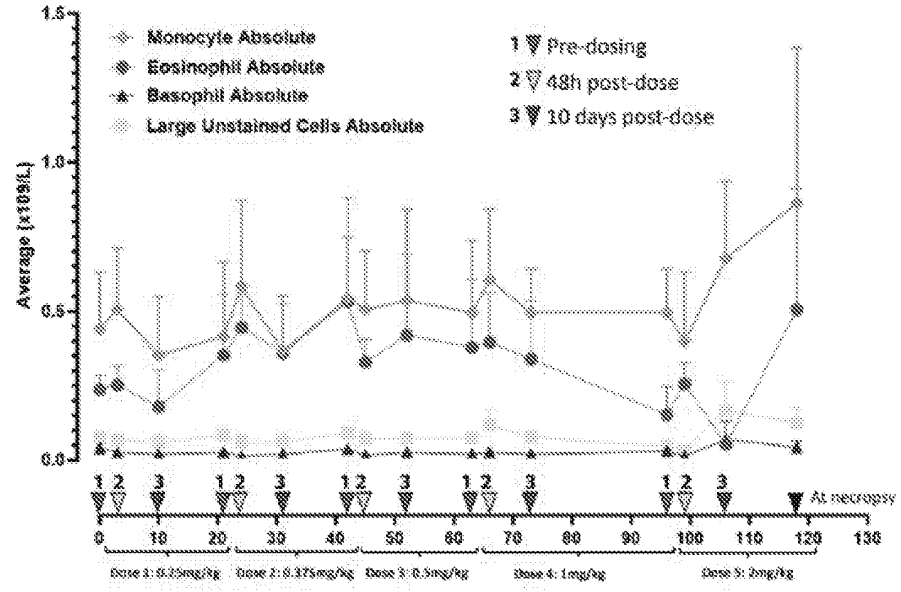
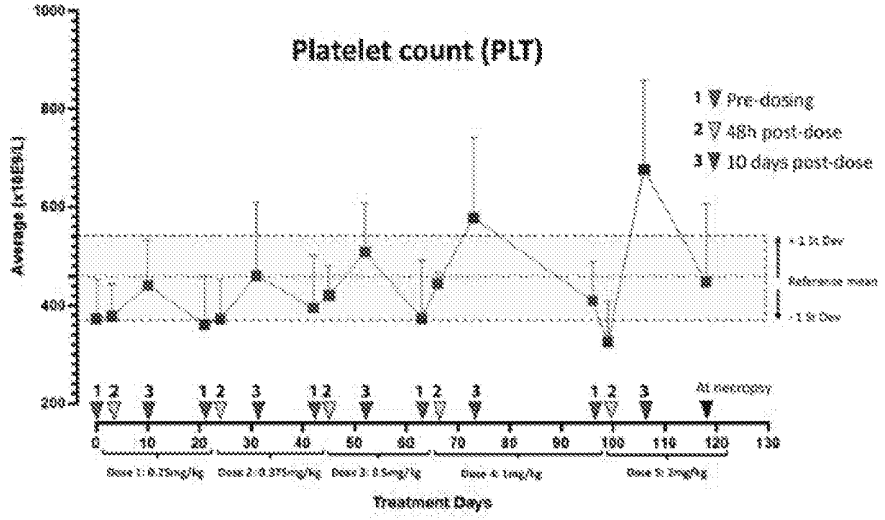

Monocyte Absolute
Eosinophil Absolute
Basophil Absolute
Large Unstained Cells Absolute
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (x10E9/L)
At necropsy

Figure 64

E


Platelet count (PLT)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (x10E9/L)
Reference mean
At necropsy
Treatment Days

F

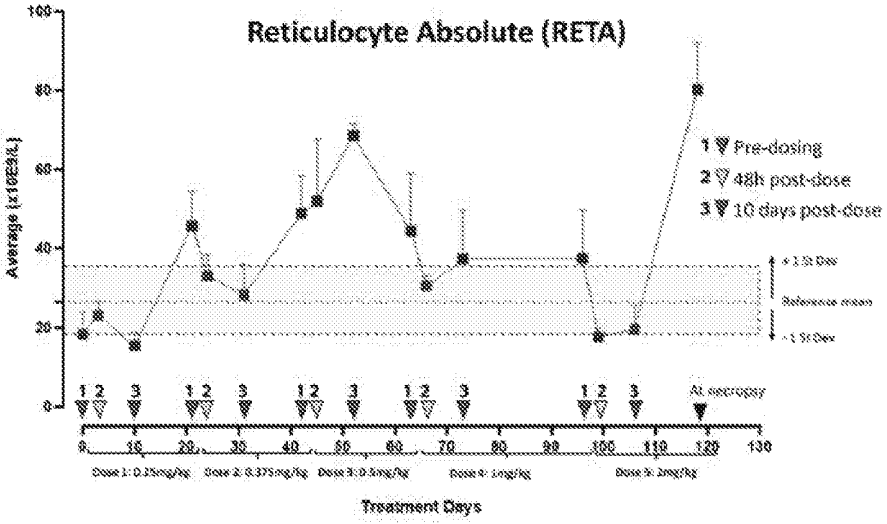
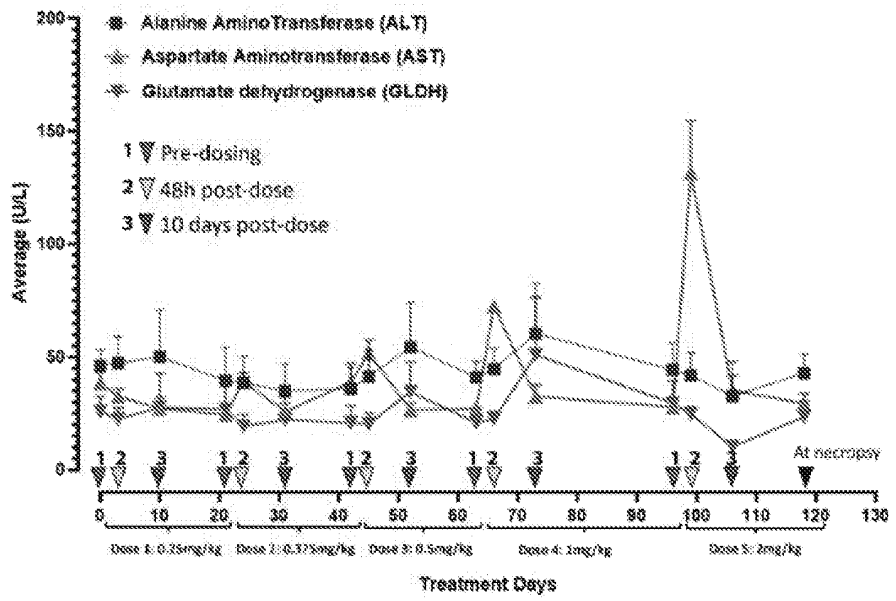

Reticulocyte Absolute (RETA)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (x10E9/L)
Reference mean
At necropsy
Treatment Days

Figure 65

A


Alanine AminoTransferase (ALT)
Aspartate Aminotransferase (AST)
Glutamate dehydrogenase (GLDH)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (U/L)
200
150
100
50
0
At necropsy
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Dose 1: 0.25mg/kg
Dose 2: 0.375mg/kg
Dose 3: 0.5mg/kg
Dose 4: 1mg/kg
Dose 5: 2mg/kg
Treatment Days

B

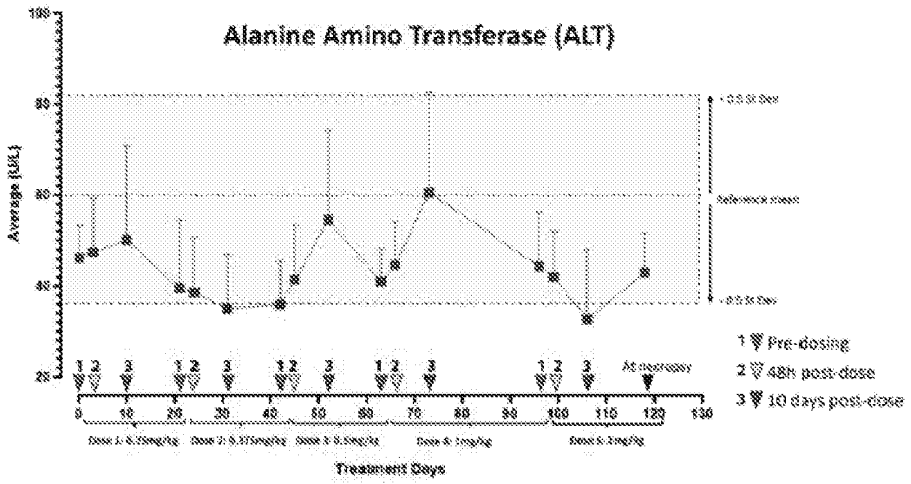
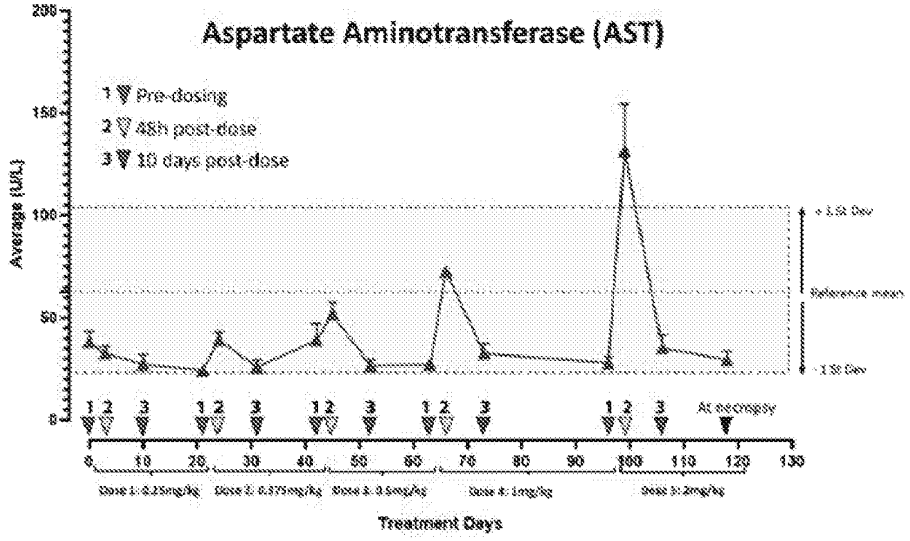

Alanine Amino Transferase (ALT)
Average (U/L)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
At necropsy
Treatment Days

Figure 65

C


Aspartate Aminotransferase (AST)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (U/L)
Reference mean
At necropsy
Treatment Days

D

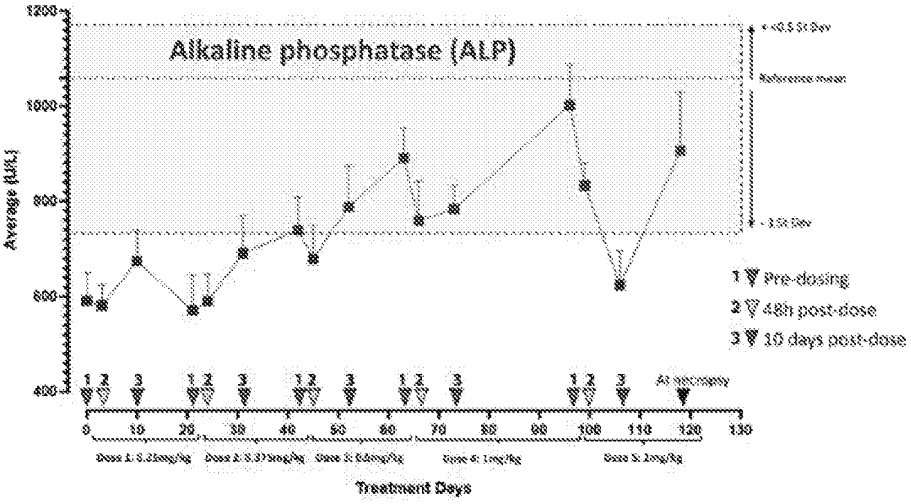

Alkaline phosphatase (ALP)
Average (U/L)
Reference mean
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
At necropsy
Treatment Days

Figure 65

E

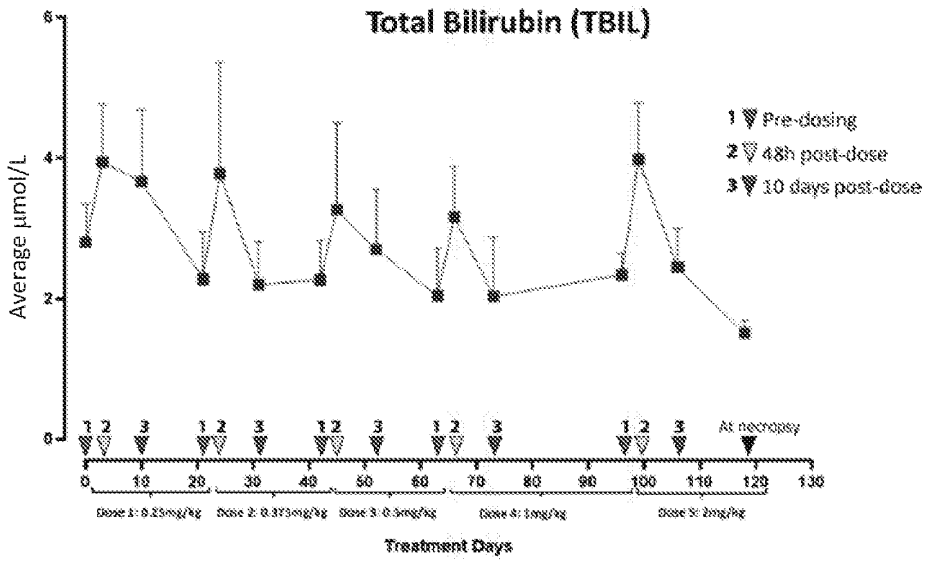
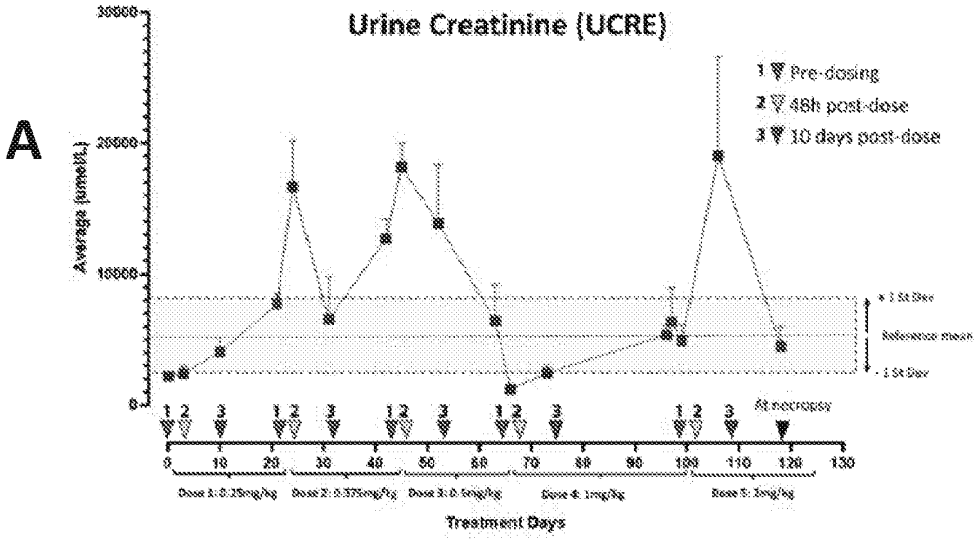

Total Bilirubin (TBIL)
Average µmol/L
0
2
4
6
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 At necropsy
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Dose 1: 0.25mg/kg
Dose 2: 0.375mg/kg
Dose 3: 0.5mg/kg
Dose 4: 1mg/kg
Dose 5: 2mg/kg
Treatment Days

Figure 66


A
Urine Creatinine (UCRE)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (umol/L)
30000
20000
10000
0
At necropsy
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Dose 1: 0.25mg/kg
Dose 2: 0.375mg/kg
Dose 3: 0.5mg/kg
Dose 4: 1mg/kg
Dose 5: 2mg/kg
Treatment Days

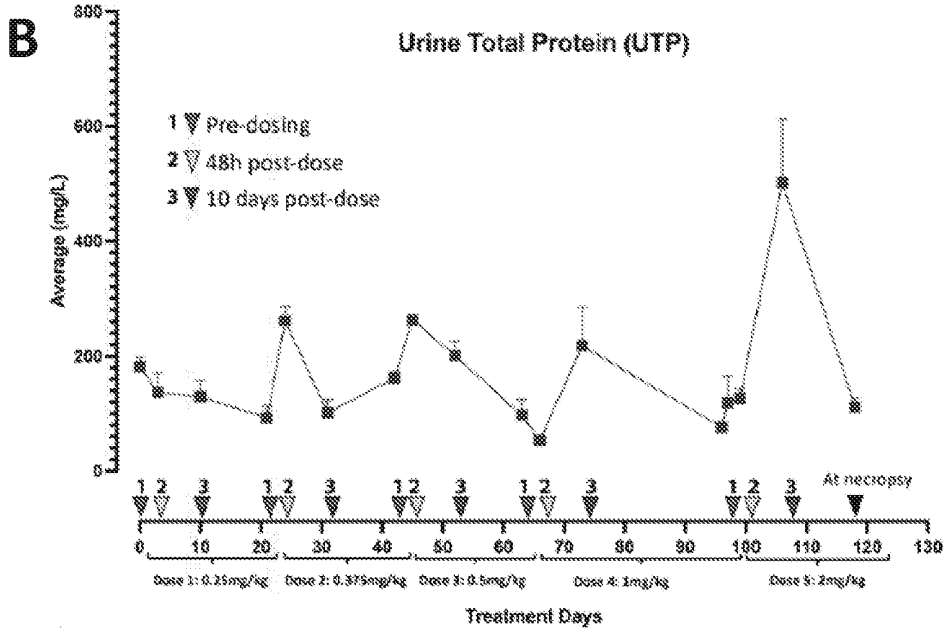
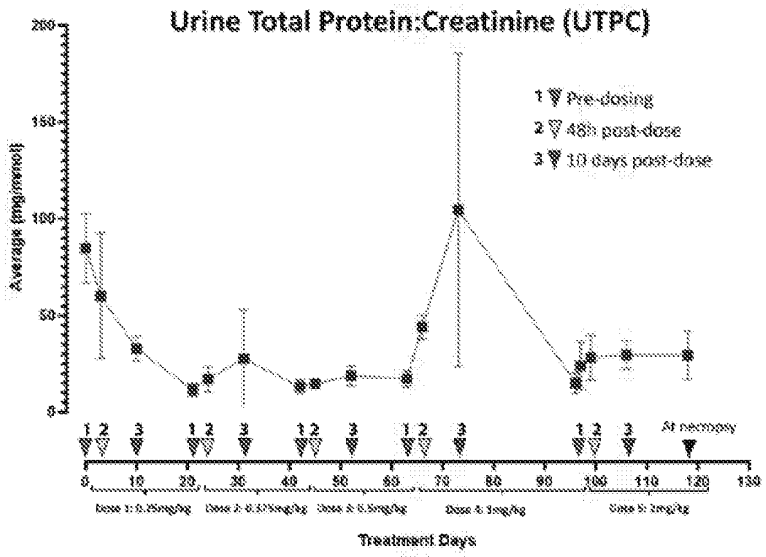

B
Urine Total Protein (UTP)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (mg/L)
800
600
400
200
0
At necropsy
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Dose 1: 0.25mg/kg
Dose 2: 0.375mg/kg
Dose 3: 0.5mg/kg
Dose 4: 1mg/kg
Dose 5: 2mg/kg
Treatment Days

Figure 66

C


Urine Total Protein:Creatinine (UTPC)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (mg/mmol)
At necropsy
Treatment Days

D

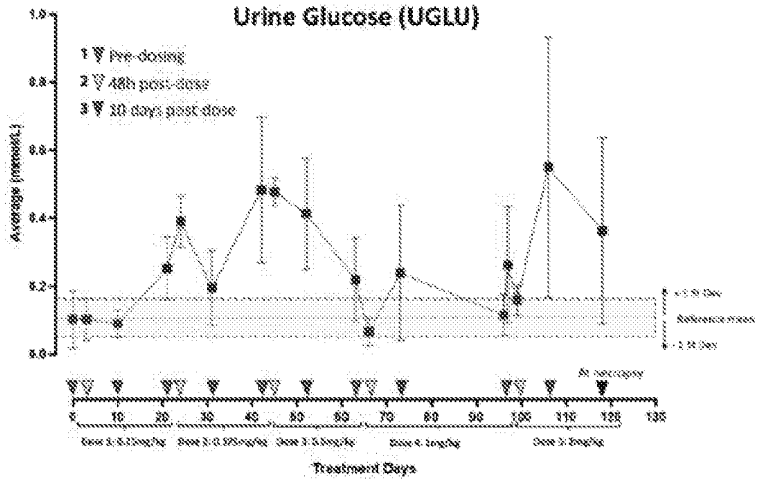

Urine Glucose (UGLU)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (mmol/L)
At necropsy
Treatment Days

Figure 66

E

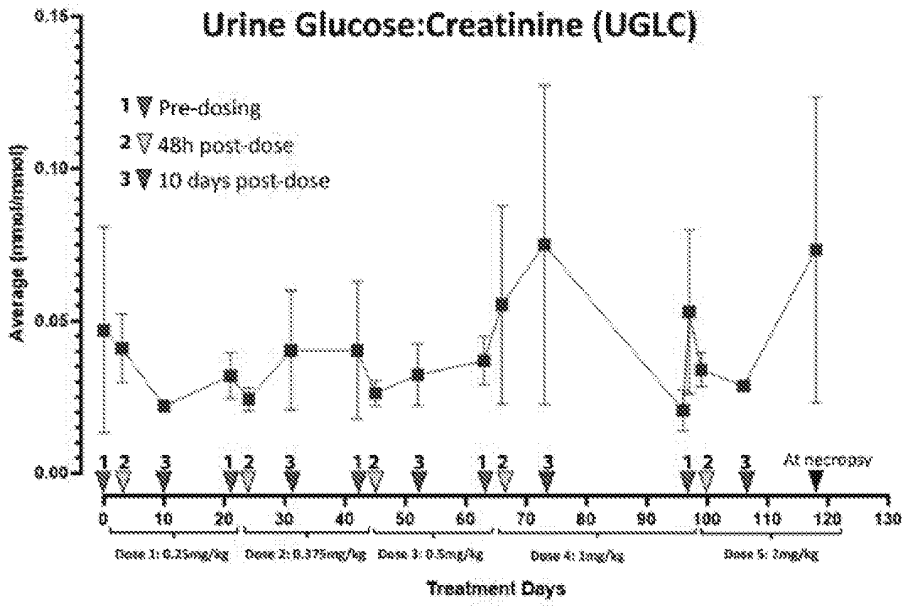

Urine Glucose:Creatinine (UGLC)
1 Pre-dosing
2 48h post-dose
3 10 days post-dose
Average (mmol/mmol)
0.15
0.10
0.05
0.00
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 At necropsy
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Dose 1: 0.25mg/kg
Dose 2: 0.375mg/kg
Dose 3: 0.5mg/kg
Dose 4: 1mg/kg
Dose 5: 2mg/kg
Treatment Days

Figure 67

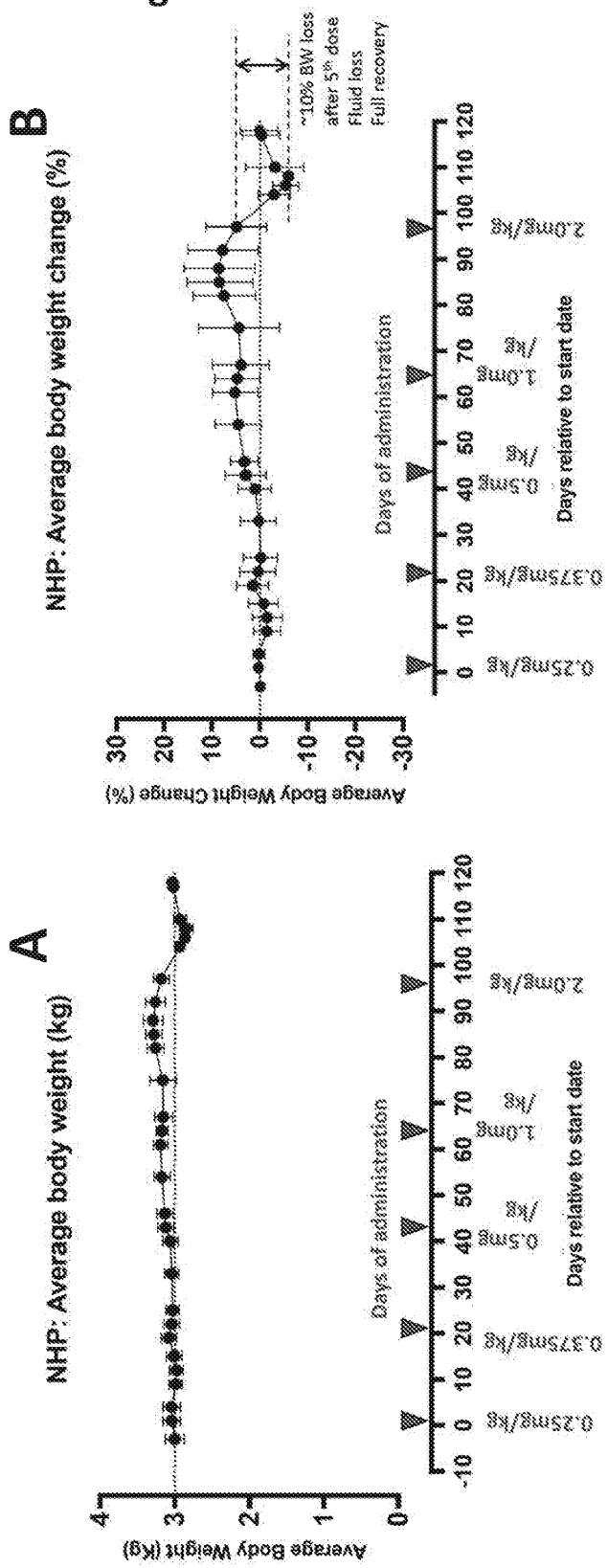

B
NHP: Average body weight change (%)
Average Body Weight Change (%)
30
20
10
0
-10
-20
-30
~10% BW loss
after 5th dose
Fluid loss
Full recovery
Days of administration
0.25mg/kg
0.375mg/kg
0.5mg/kg
1.0mg/kg
2.0mg/kg
0
10
20
30
40
50
60
70
80
90
100
110
120
Days relative to start date
A
NHP: Average body weight (kg)
Average Body Weight (kg)
4
3
2
1
0
-10
Days of administration
0.25mg/kg
0.375mg/kg
0.5mg/kg
1.0mg/kg
2.0mg/kg
Days relative to start date

Figure 68

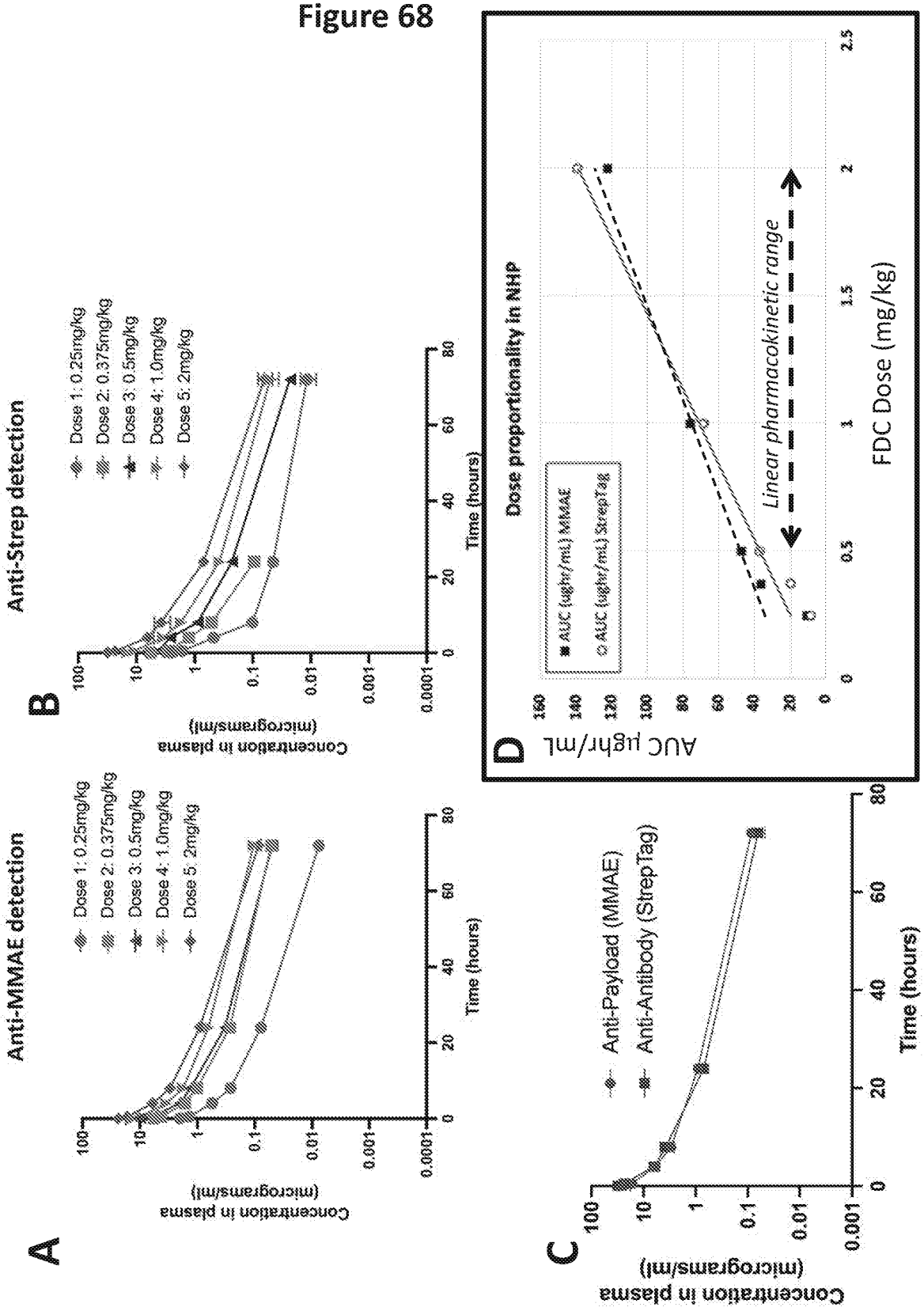

A
Anti-MMAE detection
Dose 1: 0.25mg/kg
Dose 2: 0.375mg/kg
Dose 3: 0.5mg/kg
Dose 4: 1.0mg/kg
Dose 5: 2mg/kg
Concentration in plasma (micrograms/ml)
Time (hours)
B
Anti-Strep detection
Dose 1: 0.25mg/kg
Dose 2: 0.375mg/kg
Dose 3: 0.5mg/kg
Dose 4: 1.0mg/kg
Dose 5: 2mg/kg
Concentration in plasma (micrograms/ml)
Time (hours)
C
Anti-Payload (MMAE)
Anti-Antibody (StrepTag)
Concentration in plasma (micrograms/ml)
Time (hours)
D
Dose proportionality in NHP
AUC (ughr/mL) MMAE
AUC (ughr/mL) StrepTag
Linear pharmacokinetic range
AUC µghr/mL
FDC Dose (mg/kg)

Figure 69

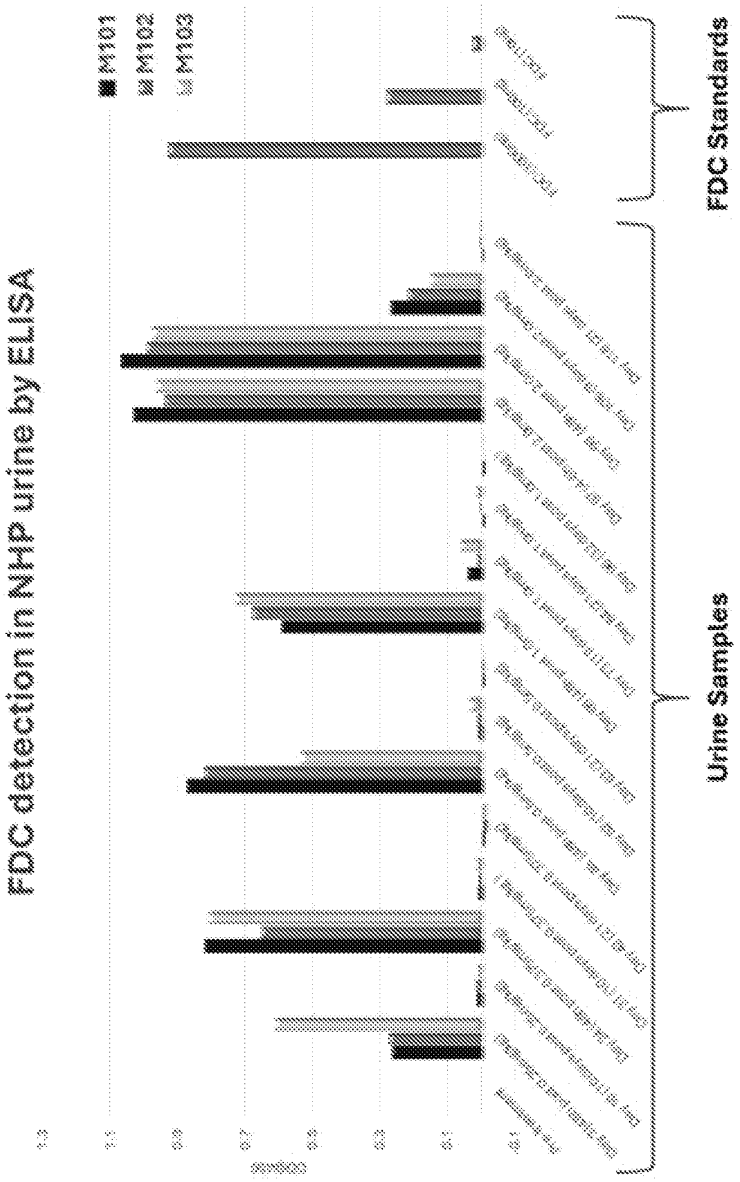

FDC detection in NHP urine by ELISA
M101
M102
M103
FDC Standards
Urine Samples

Figure 70

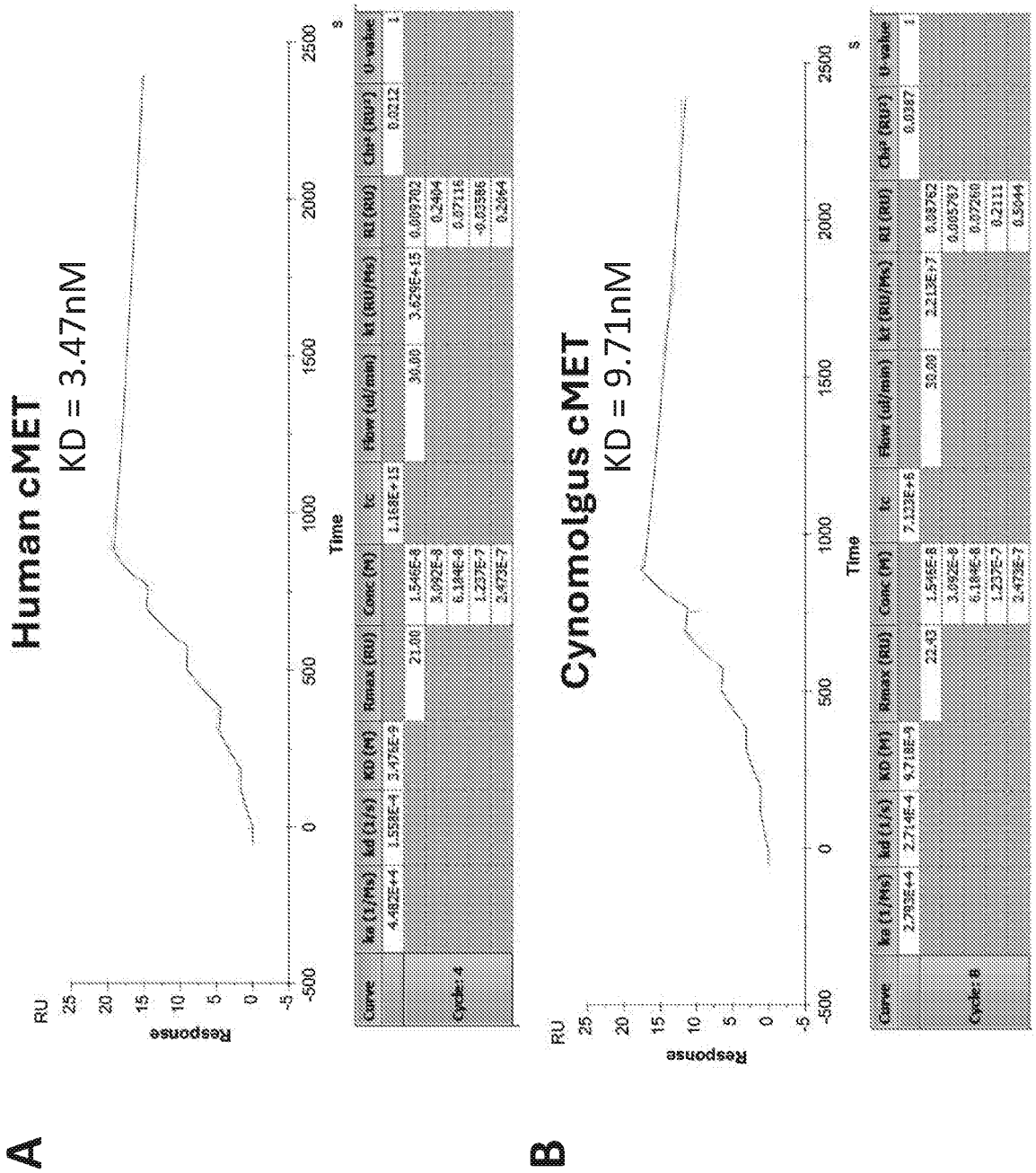
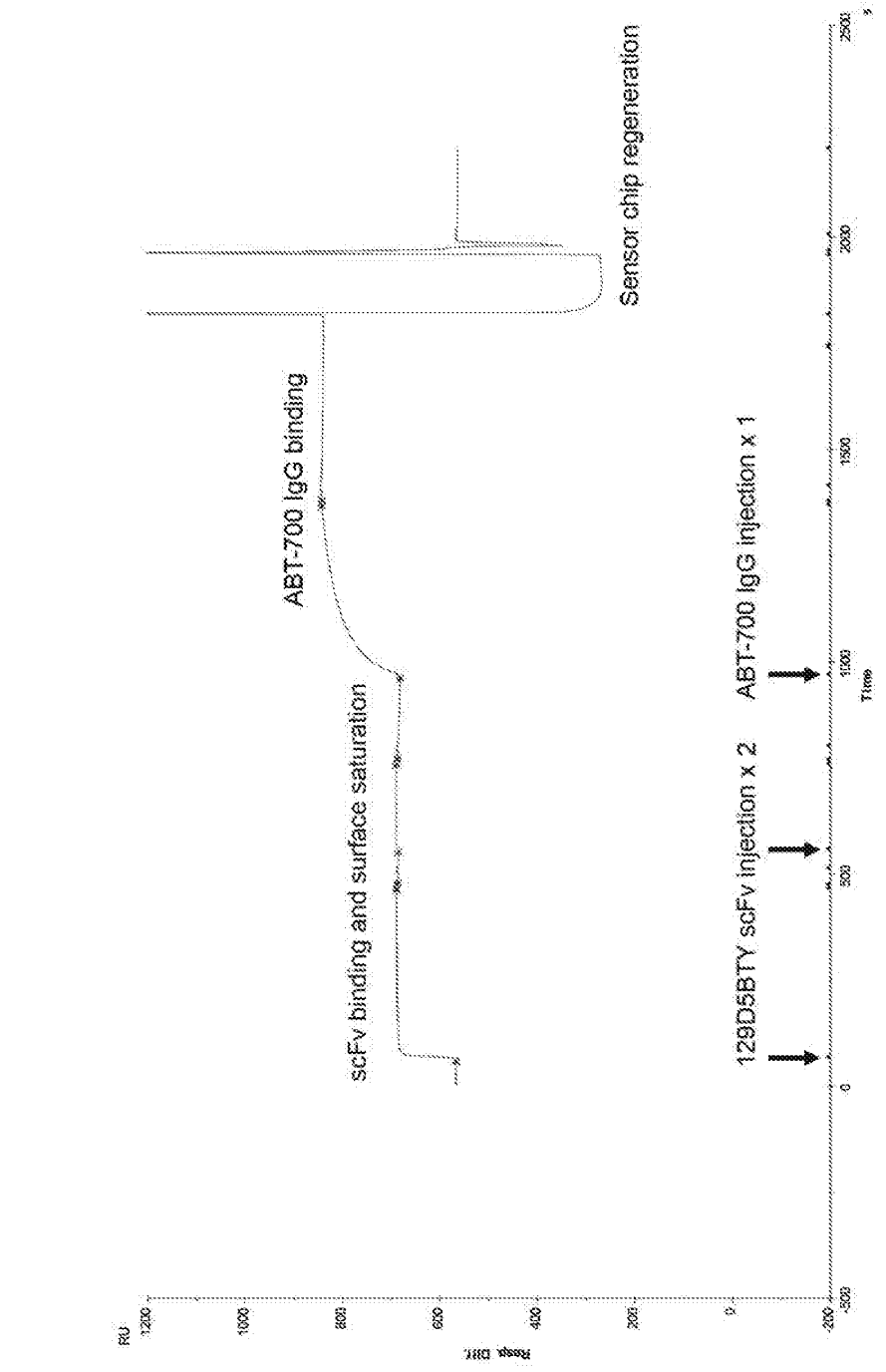

A
Human cMET
KD = 3.47nM
RU
Response
Time
s
B
Cynomolgus cMET
KD = 9.71nM
RU
Response
Time
s

Figure 71


Sensor chip regeneration
ABT-700 IgG binding
scFv binding and surface saturation
129D5BTY scFv injection x 2
ABT-700 IgG injection x 1
RU
Time

A

Figure 71

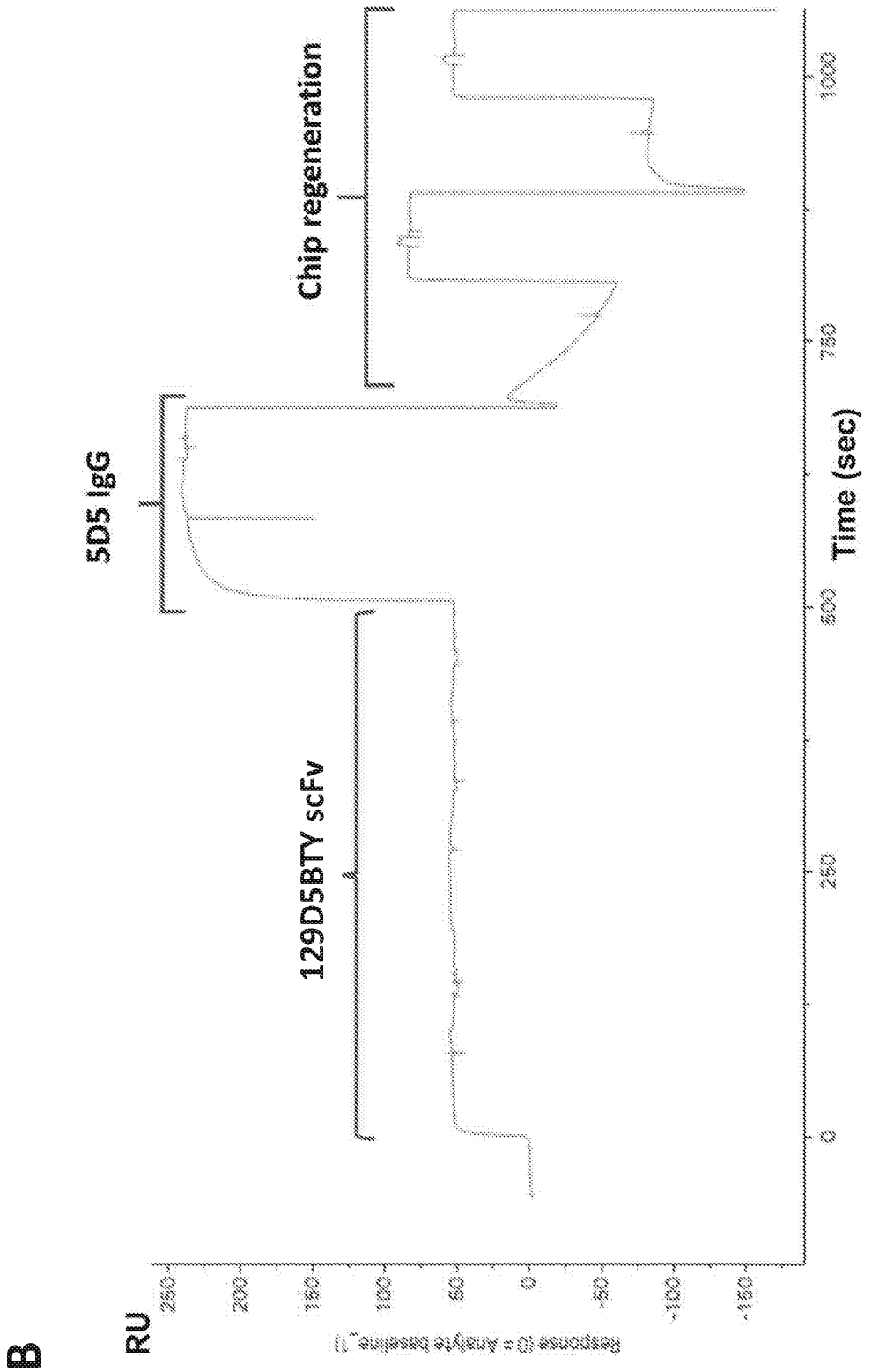

B
RU
250
200
150
100
50
0
-50
-100
-150
Response (0 = Analyte baseline_1)
0
250
500
750
1000
Time (sec)
5D5 IgG
129D5BTY scFv
Chip regeneration

Figure 72

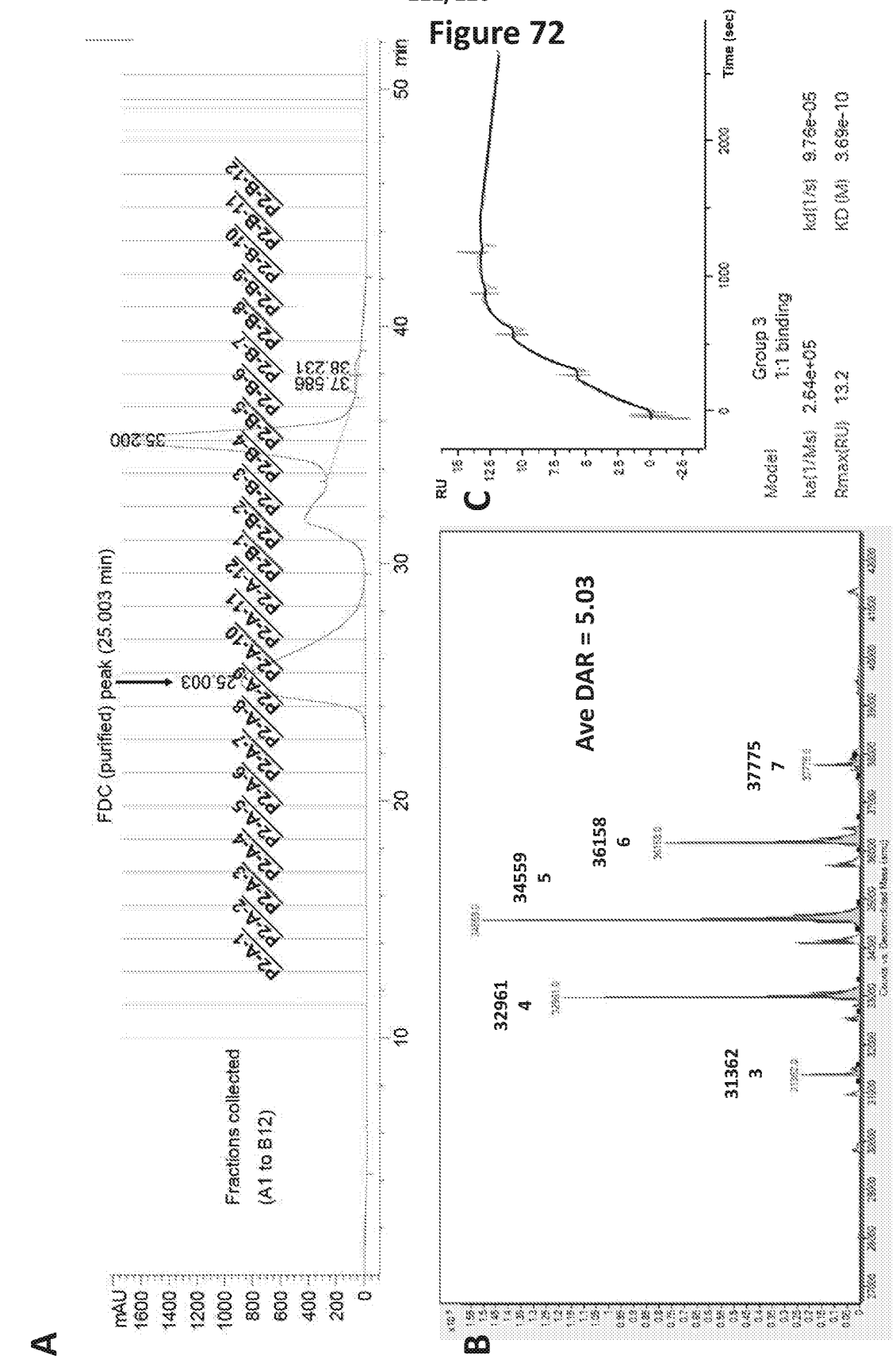

A
mAU
FDC (purified) peak (25.003 min)
25.003
35.200
37.585
38.231
Fractions collected
(A1 to B12)
min
B
Ave DAR = 5.03
32961
4
34559
5
36158
6
37775
7
31362
3
C
RU
Time (sec)
Group 3
1:1 binding
Model
ka(1/Ms) 2.64e+05
kd(1/s) 9.76e-05
Rmax(RU) 13.2
KD (M) 3.69e-10

Figure 72

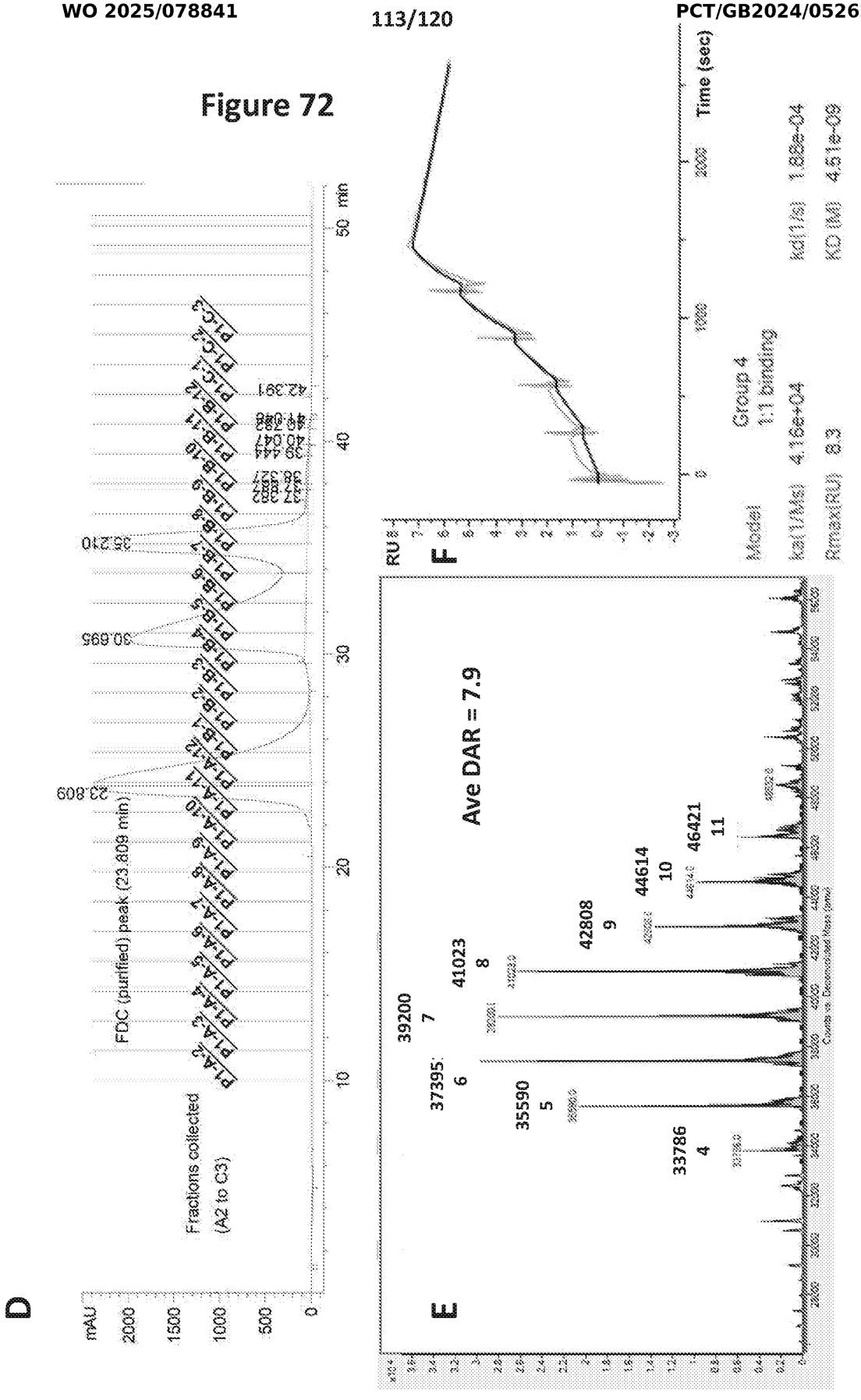

D
mAU
2000
1500
1000
500
0
FDC (purified) peak (23.809 min)
Fractions collected
(A2 to C3)
23.809
30.695
35.210
42.391
10
20
30
40
50 min
E
Ave DAR = 7.9
37395
6
39200
7
41023
8
42808
9
44614
10
46421
11
35590
5
33786
4
F
RU
Time (sec)
Model
Group 4
1:1 binding
kd (1/s) 1.82e-04
ka (1/Ms) 4.16e+04
KD (M) 4.51e-09
Rmax(RU) 8.3

Figure 72

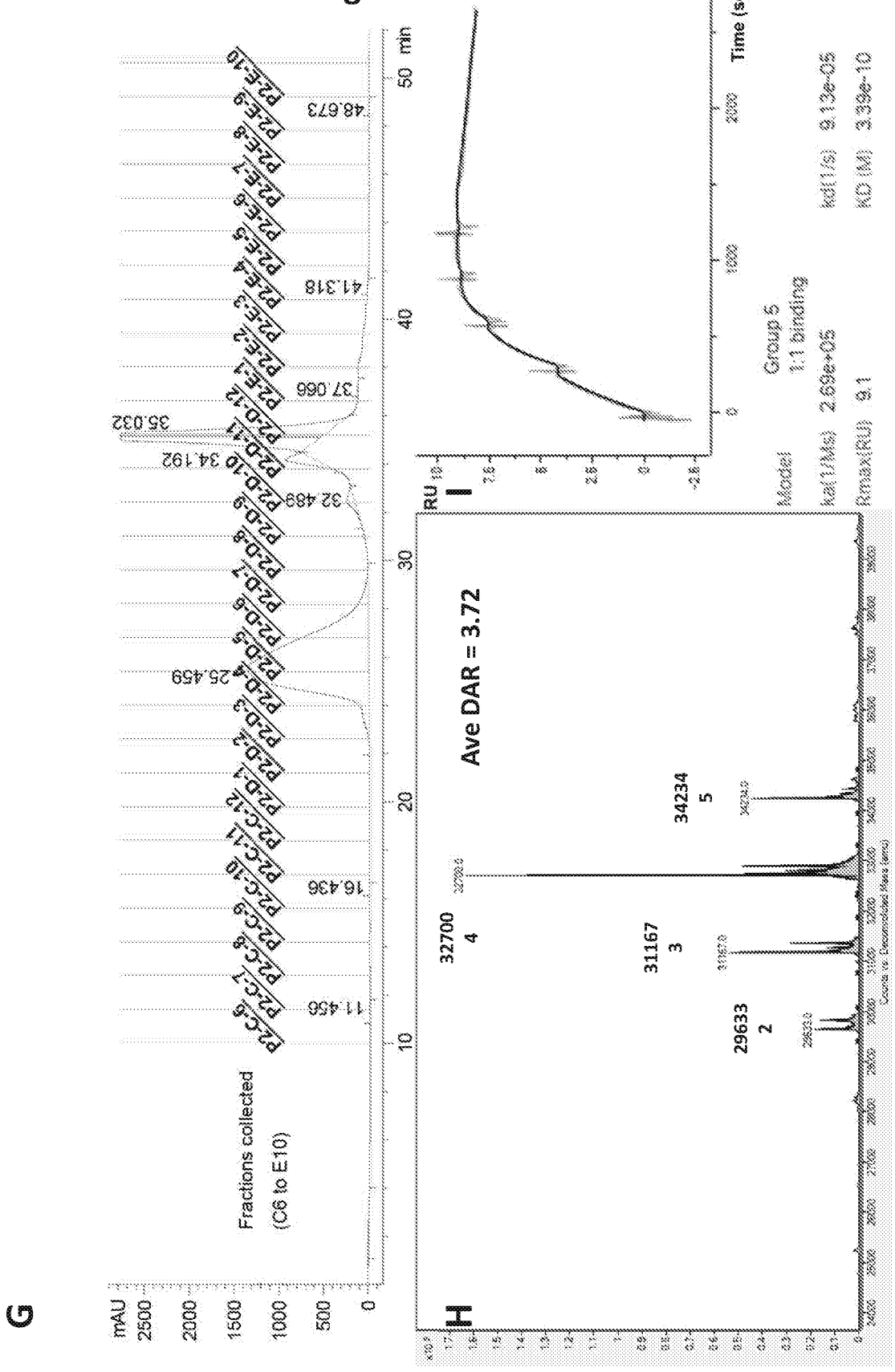

G
mAU
2500
2000
1500
1000
500
0
Fractions collected
(C6 to E10)
11.456
16.436
25.459
32.489
34.192
35.032
37.066
41.318
48.673
min
10
20
30
40
50
H
Ave DAR = 3.72
29633
2
31167
3
32700
4
34234
5
I
RU
Time (sec)
Model
Group 5
1:1 binding
kat (1/Ms) 2.69e+05
Rmax(RU) 9.1
kdl (1/s) 9.13e-05
KD (M) 3.39e-10

Figure 73

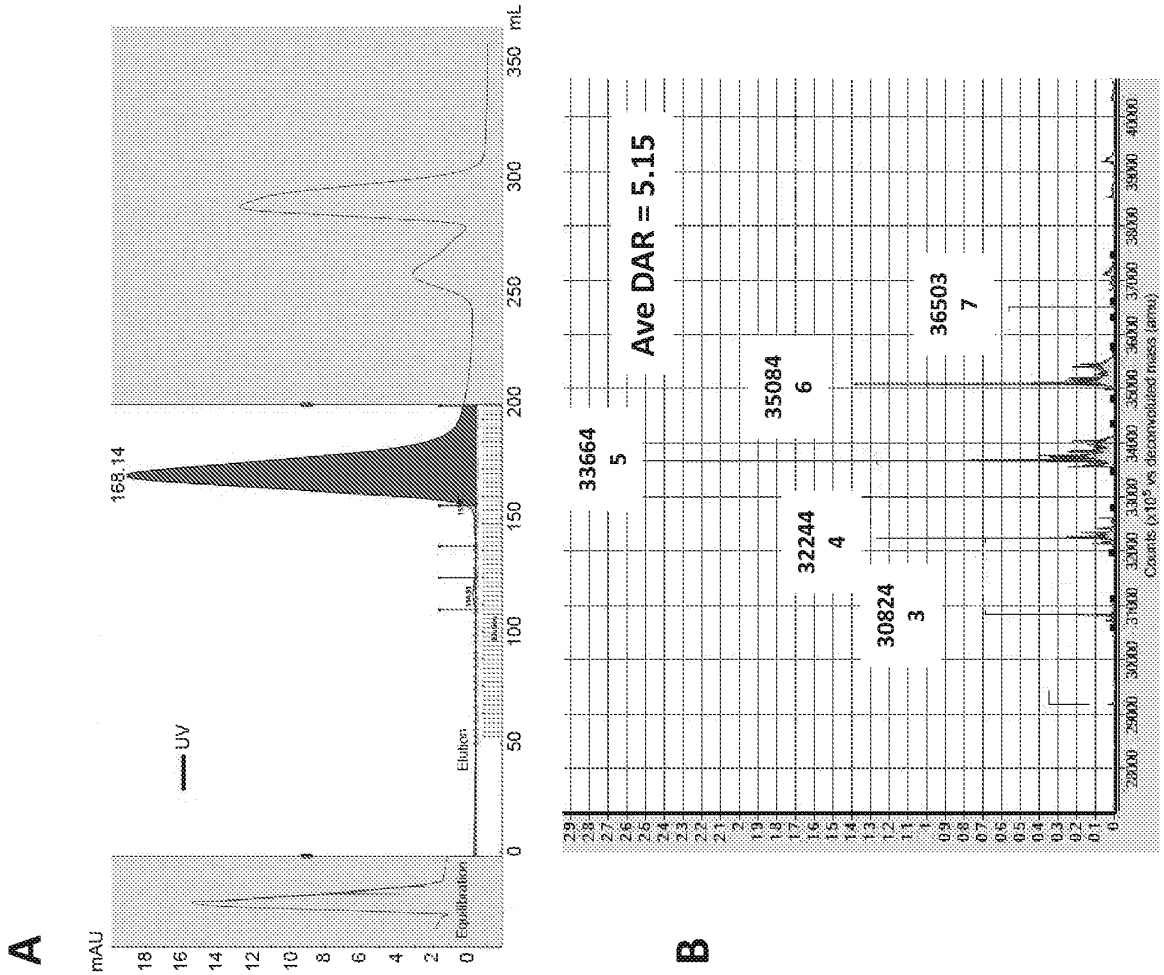

A
mAU
UV
Equilibration
Elution
168.14
mL
B
Ave DAR = 5.15
33664
5
35084
6
32244
4
30824
3
36503
7

Figure 73

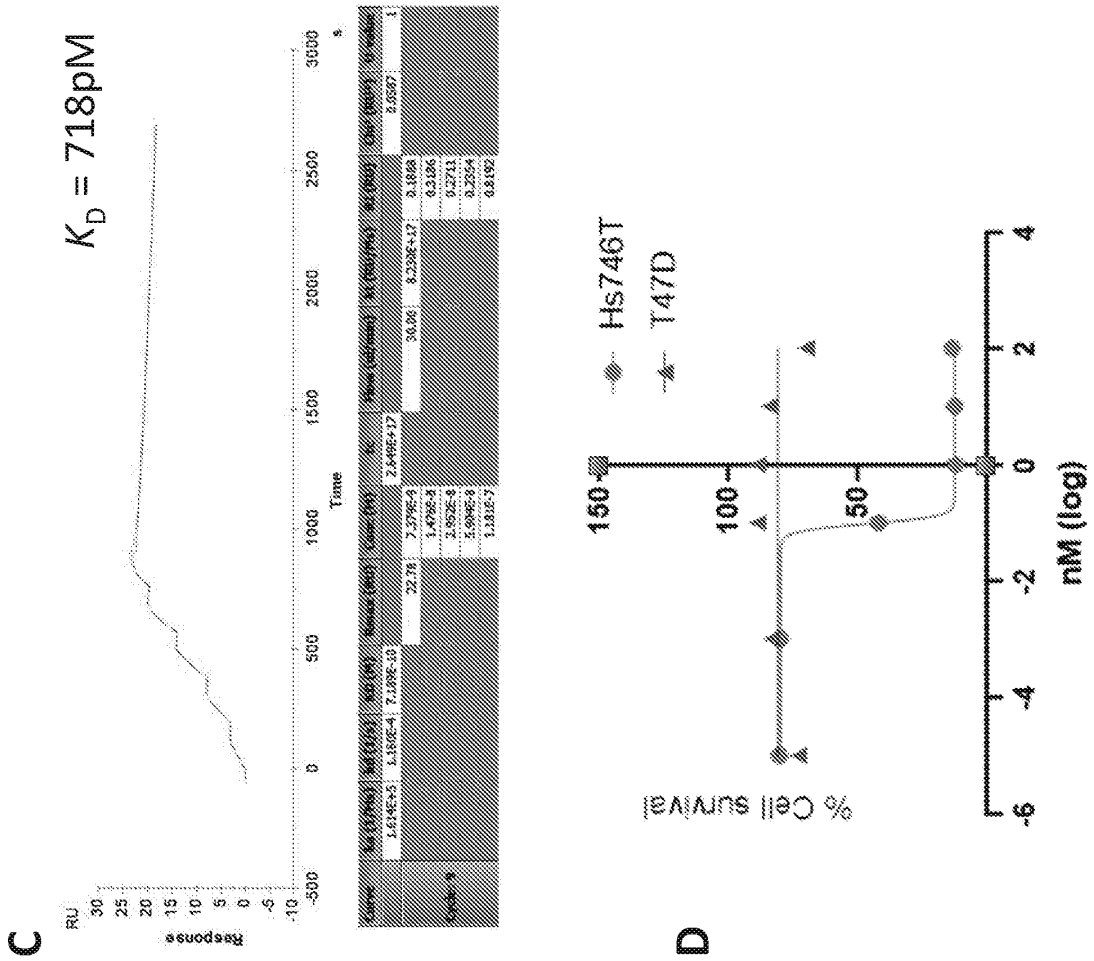

C
K_D = 718pM
RU
Response
Time
s
D
Hs746T
T47D
% Cell survival
nM (log)

Figure 74

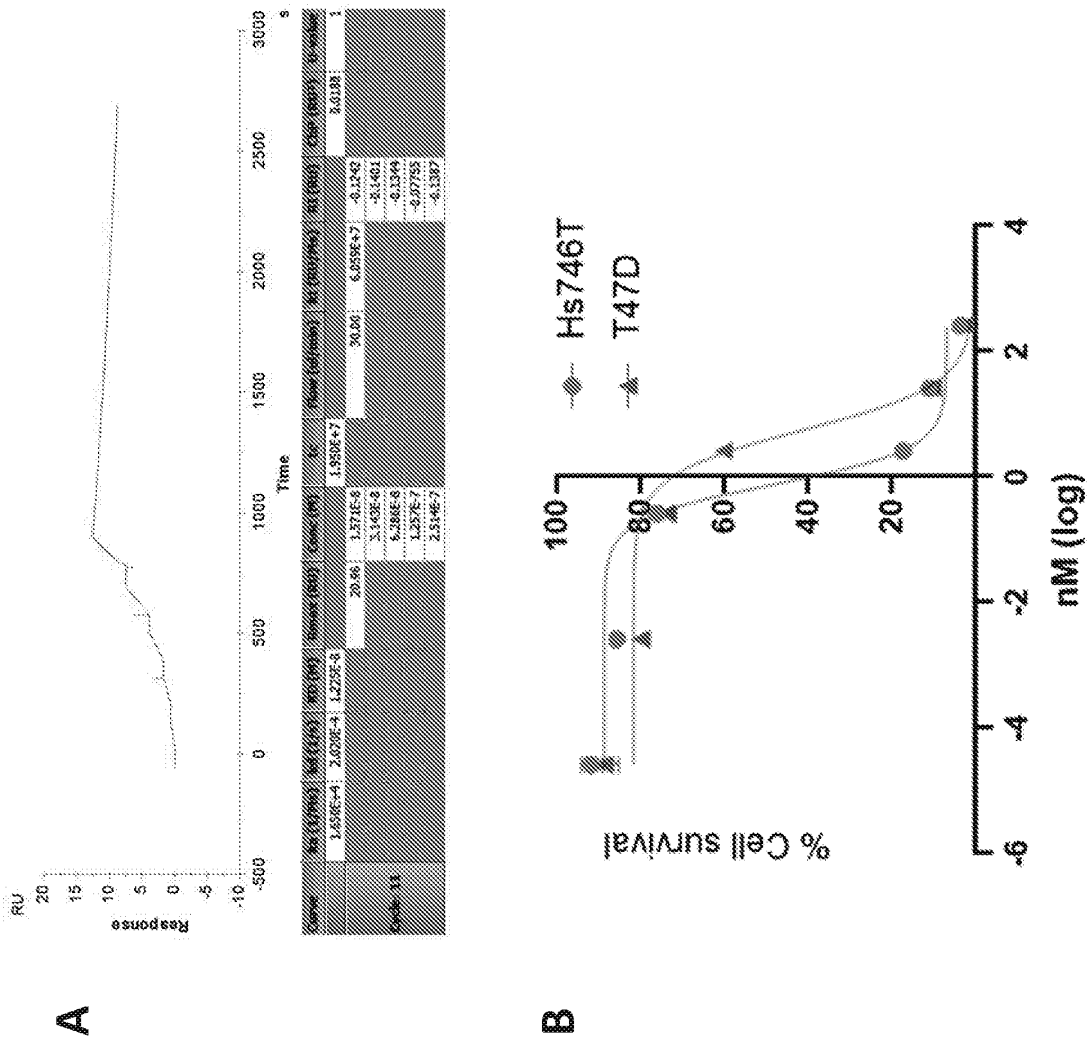


Figure 75

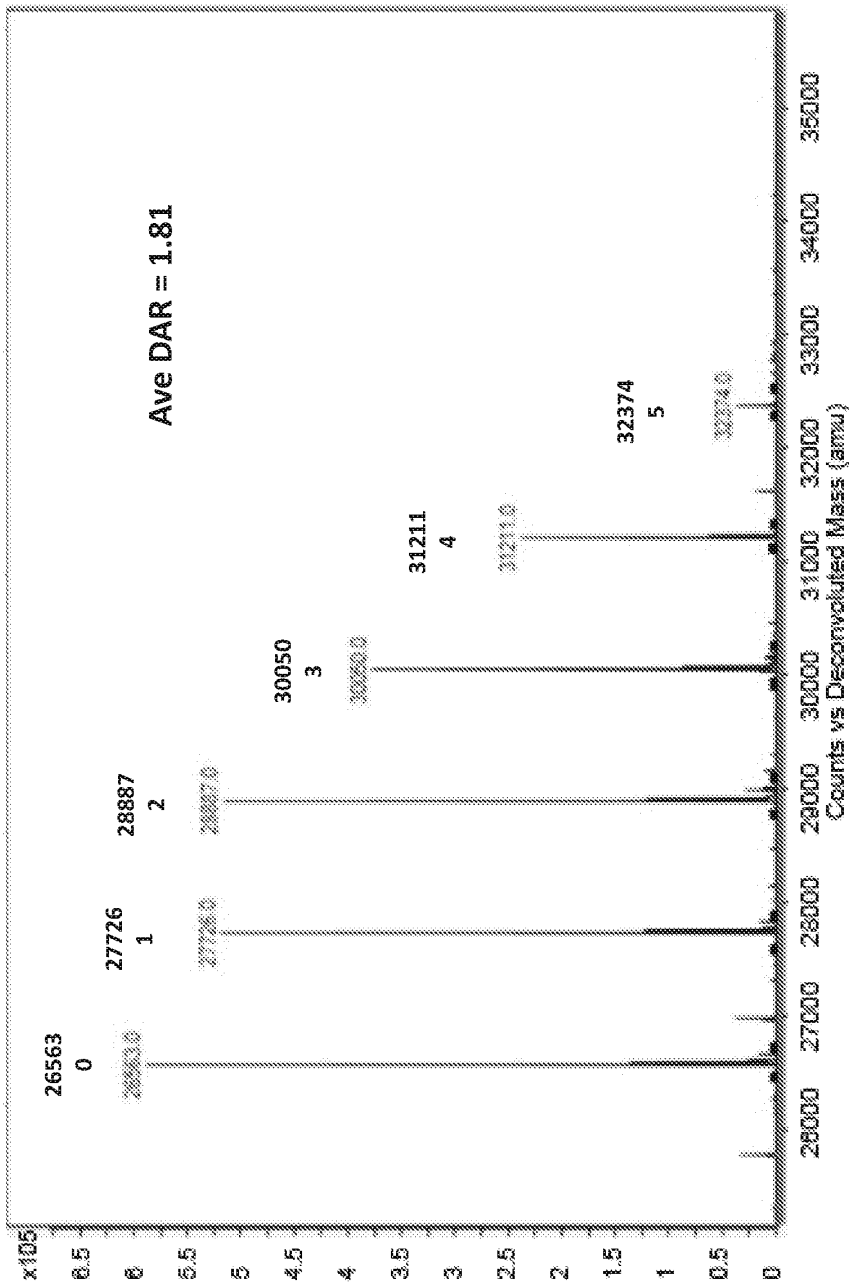
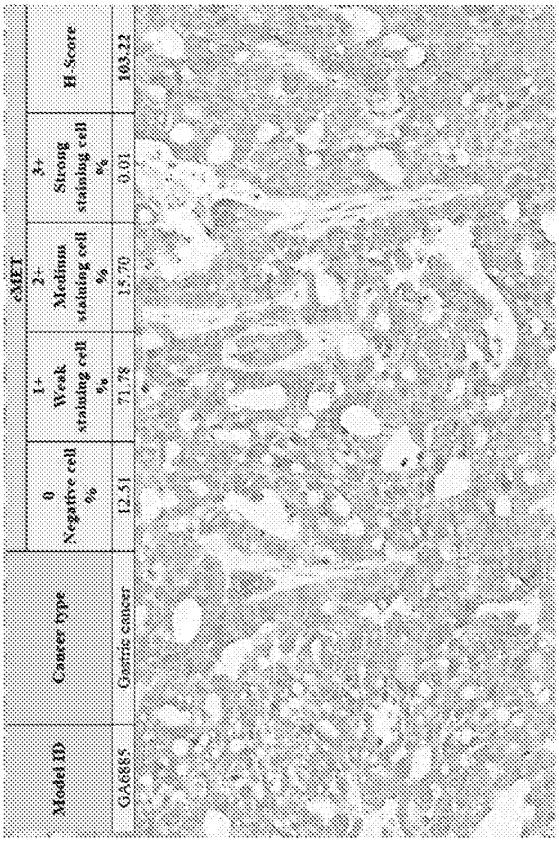

Ave DAR = 1.81
26563
0
27726
1
28887
2
30050
3
31211
4
32374
5
x10^5
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
26000
27000
28000
29000
30000
31000
32000
33000
34000
35000
Counts vs Deconvoluted Mass (amu)

Figure 76

A

Model ID	Cancer type	cMET				
		0 Negative cell %	1+ Weak staining cell %	2+ Medium staining cell %	3+ Strong staining cell %	H Score
GA0885	Gastric cancer	12.51	71.78	15.70	0.01	103.22



B

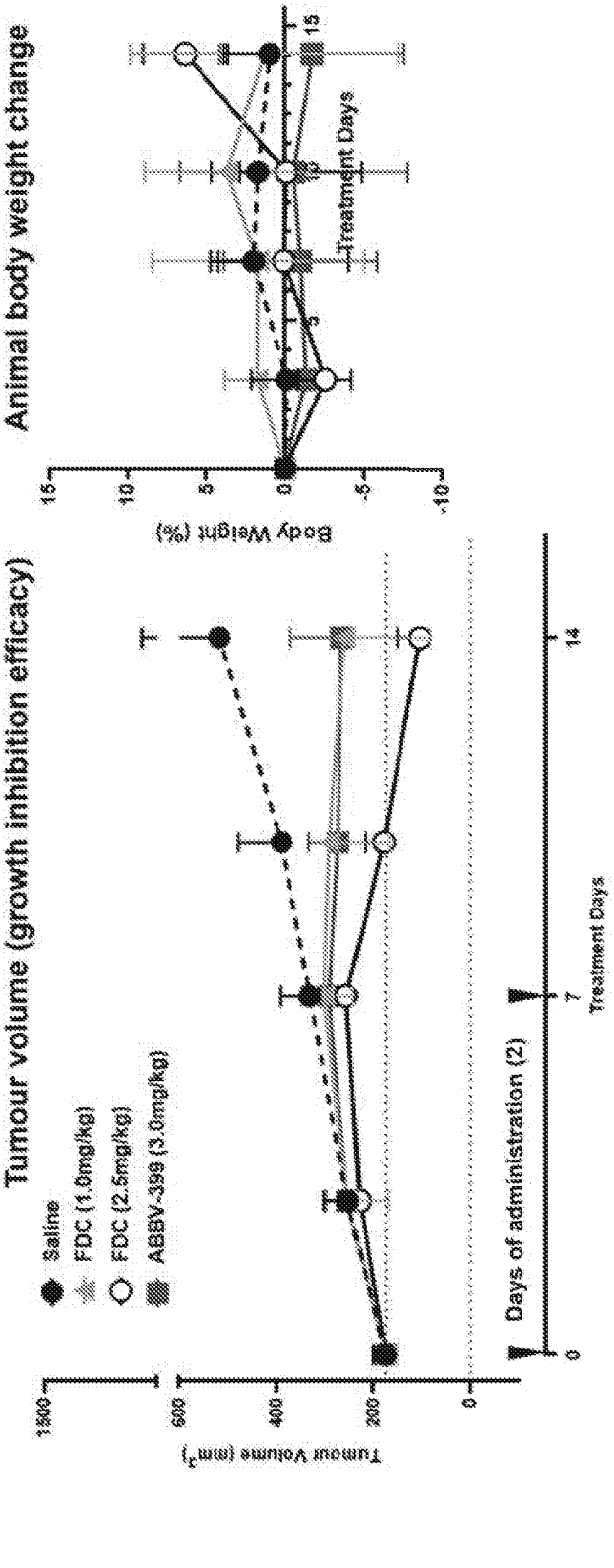
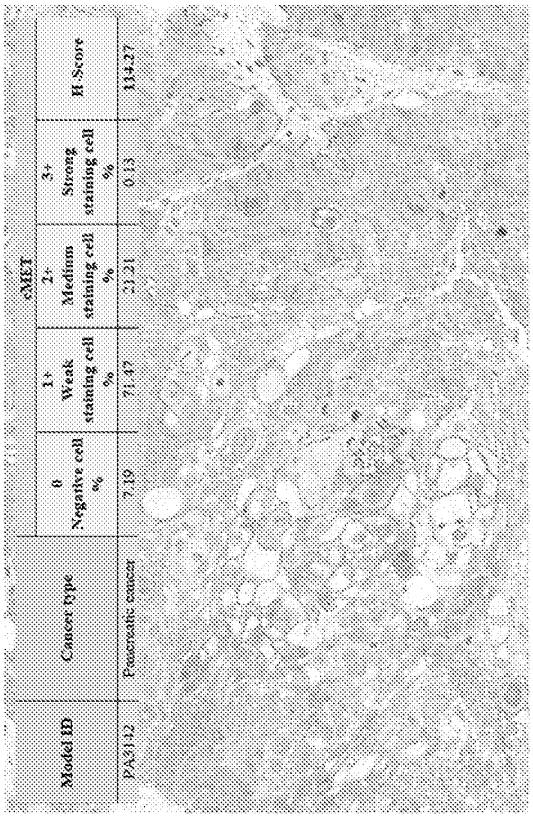


Figure 77

A

Model ID	Cancer type	cMET				
		0 Negative cell %	1+ Weak staining cell %	2+ Medium staining cell %	3+ Strong staining cell %	H-Score
PA342	Pancreatic cancer	7.19	71.47	21.21	0.13	114.27



B

