Figure 1

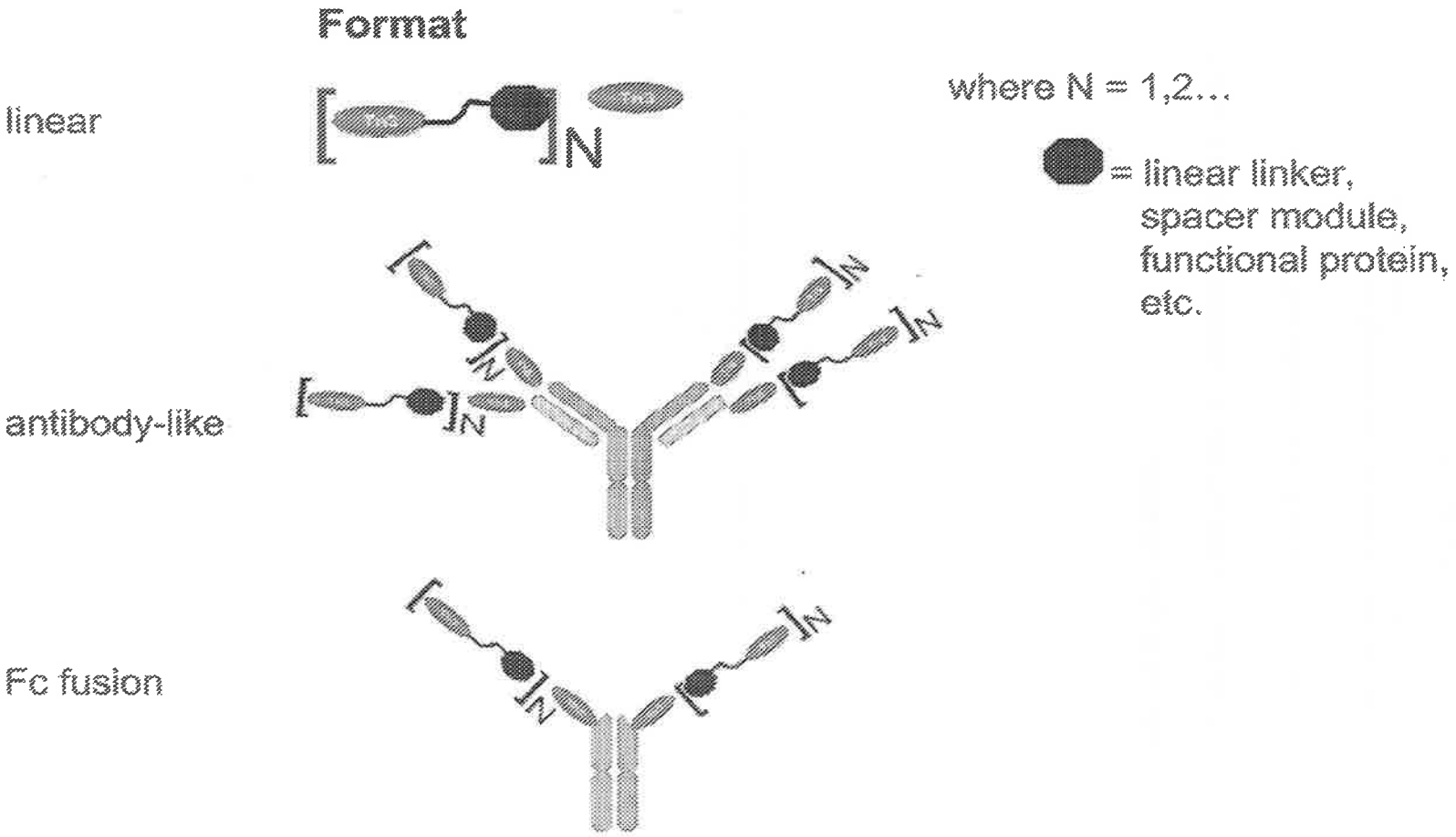

Format
linear
antibody-like
Fc fusion
where N = 1,2…
= linear linker, spacer module, functional protein, etc.

Figure 2

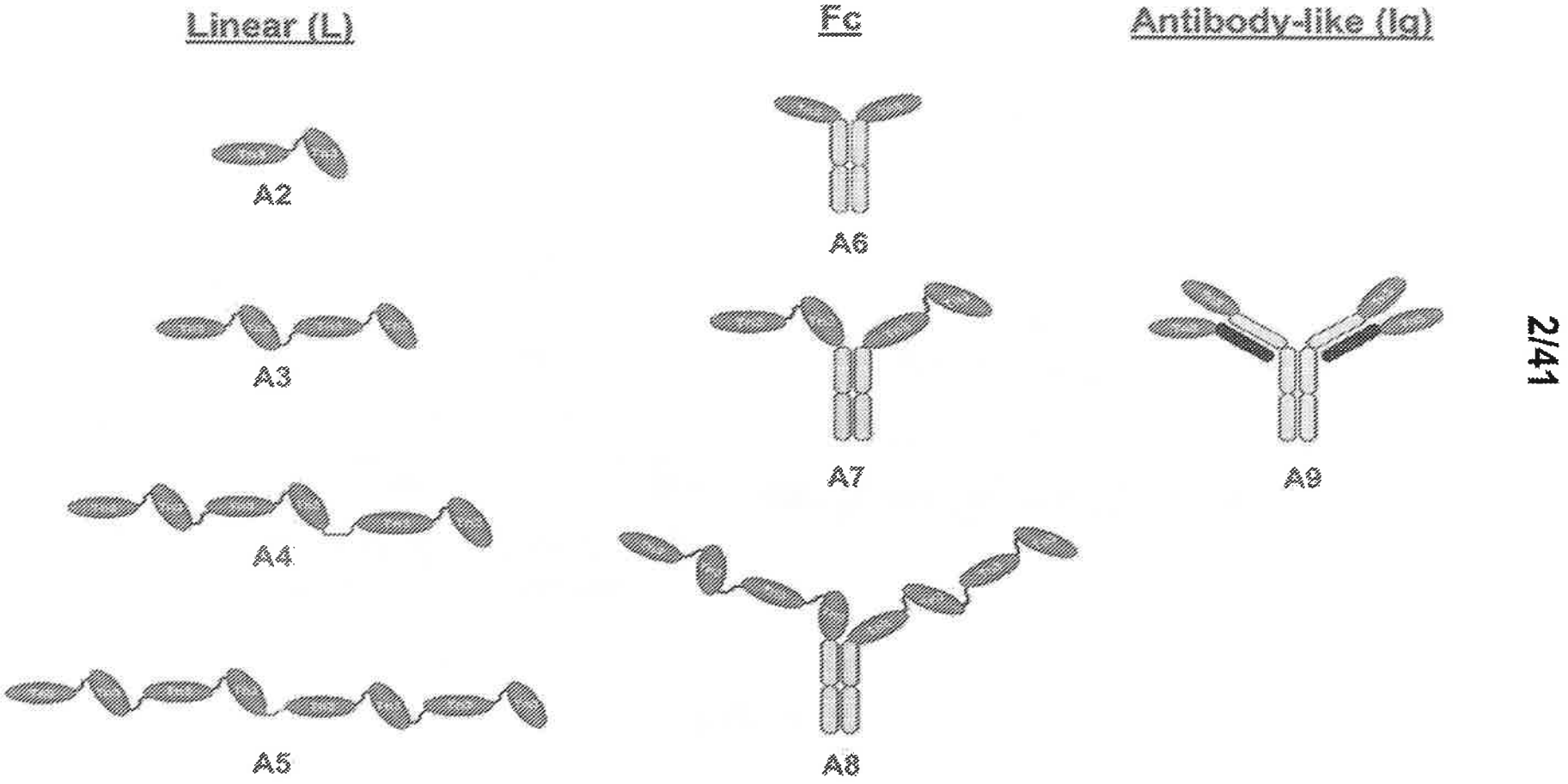
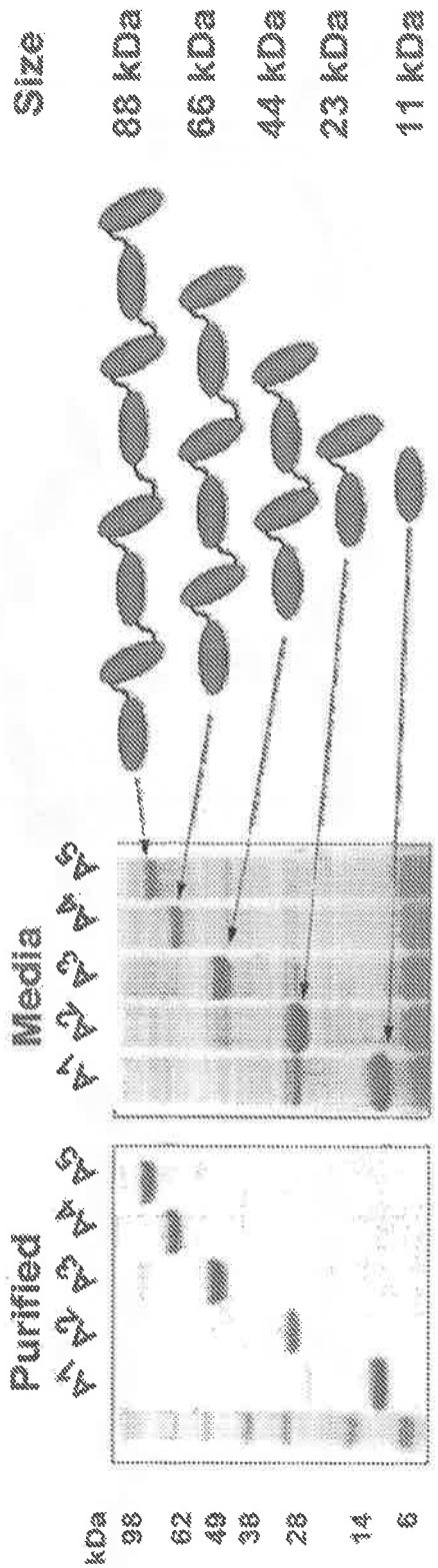


Figure 3


Size
88 kDa
66 kDa
44 kDa
23 kDa
11 kDa
Media
A1 A2 A3 A4 A5
Purified
A1 A2 A3 A4 A5
kDa
98
62
49
38
28
14
6

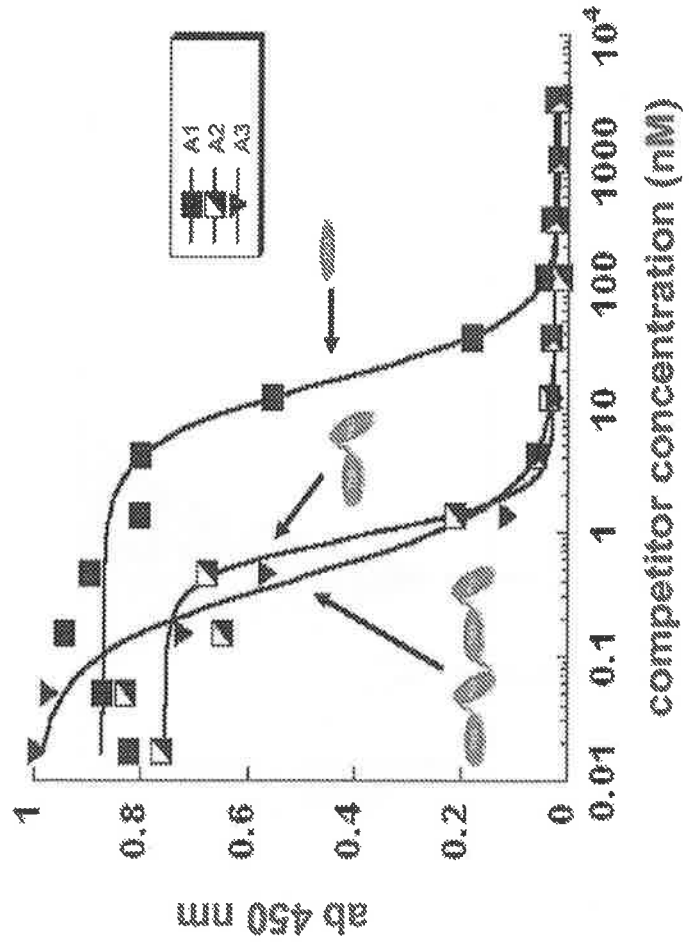

A1
A2
A3
ab 450 nm
competitor concentration (nM)
1
0.8
0.6
0.4
0.2
0
0.01
0.1
1
10
100
1000
10⁴

Figure 4

Figure 5

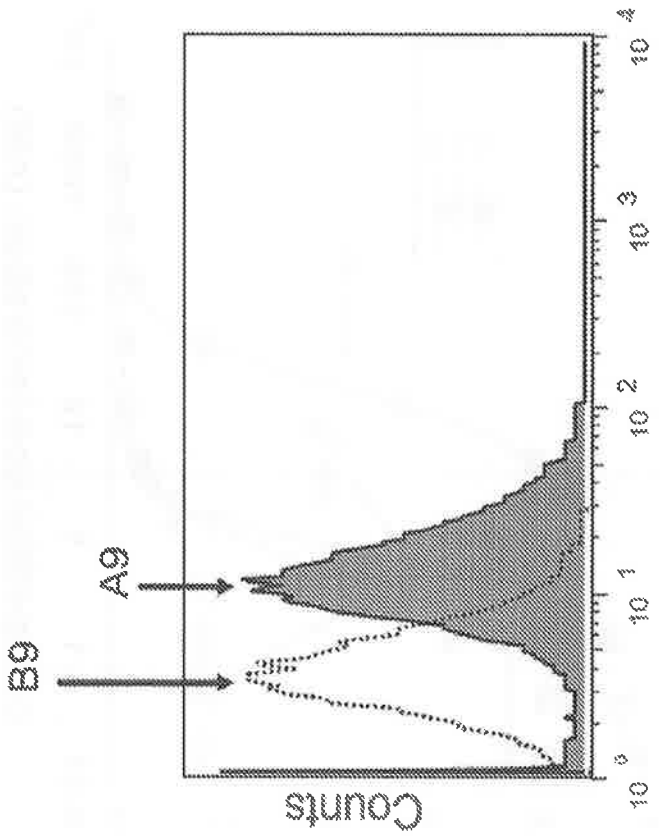


Figure 6A

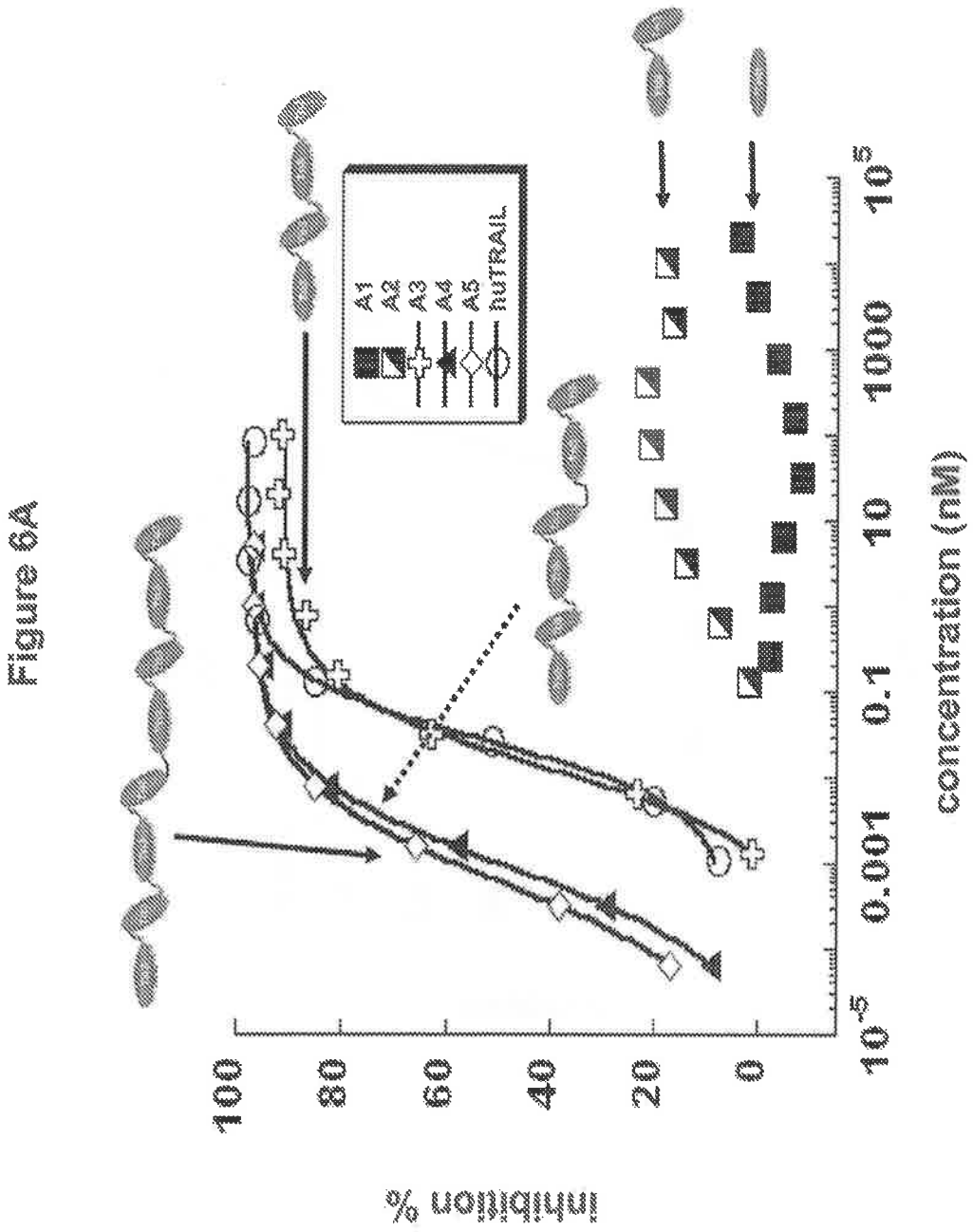

A1
A2
A3
A4
A5
hTRAIL
100
80
60
40
20
0
inhibition %
10^{-5}
0.001
0.1
10
1000
10^{5}
concentration (nM)

Figure 6B

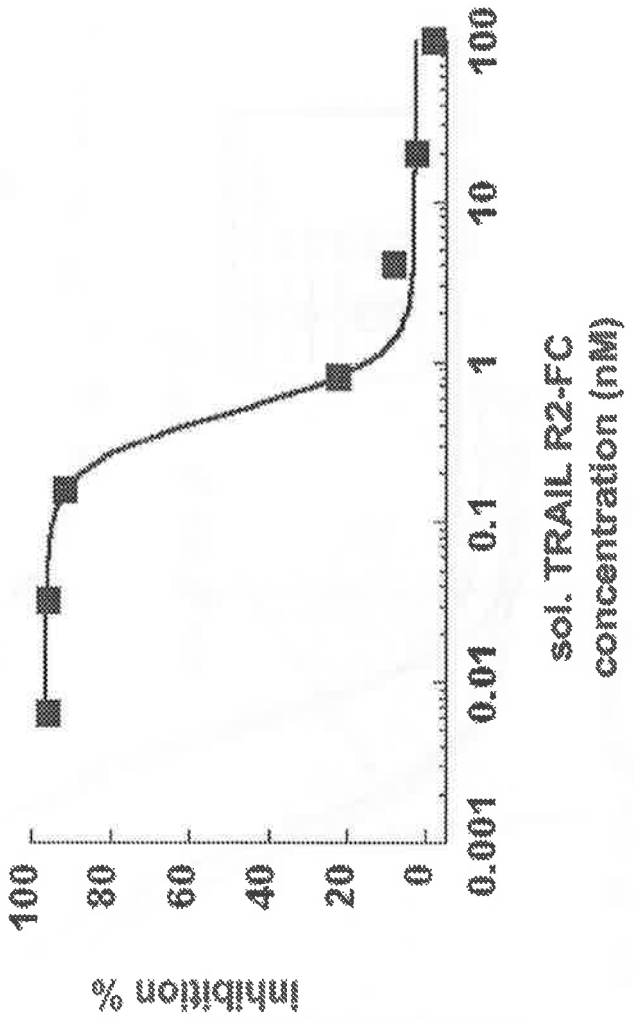

inhibition %
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
sol. TRAIL R2-FC
concentration (nM)

Figure 7A

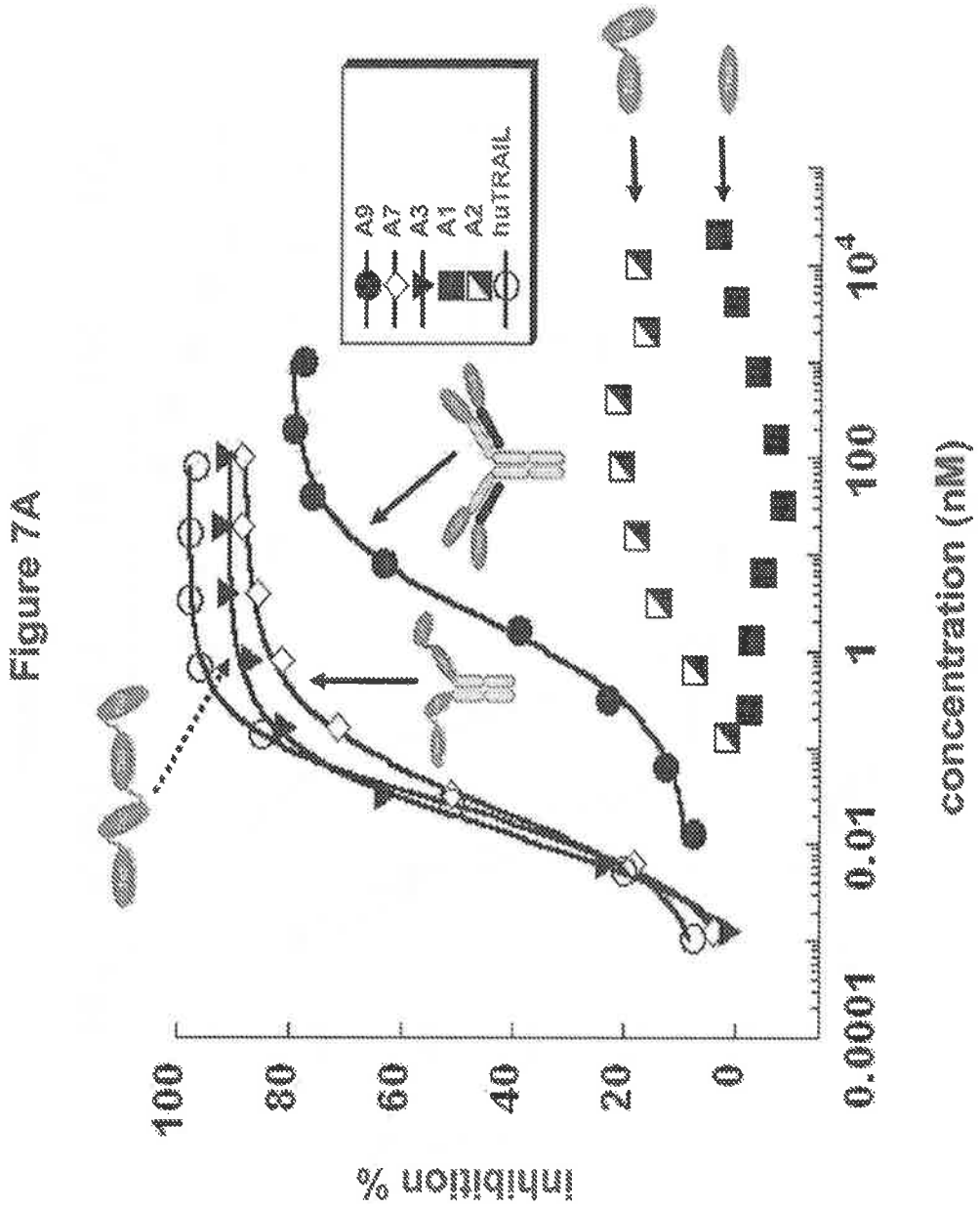

A9
A7
A3
A1
A2
huTRAIL
inhibition %
100
80
60
40
20
0
0.0001
0.01
1
100
10^4
concentration (nM)

Figure 7B

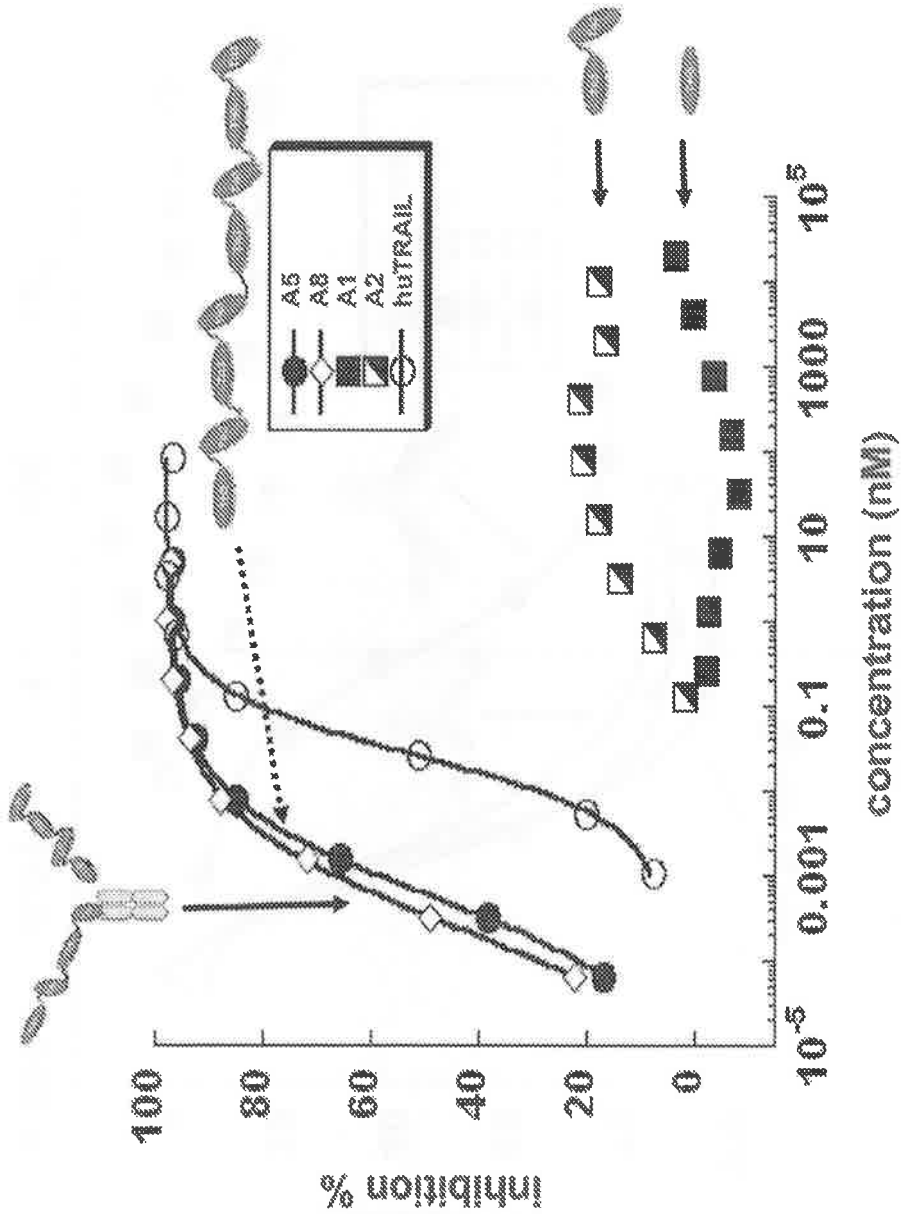
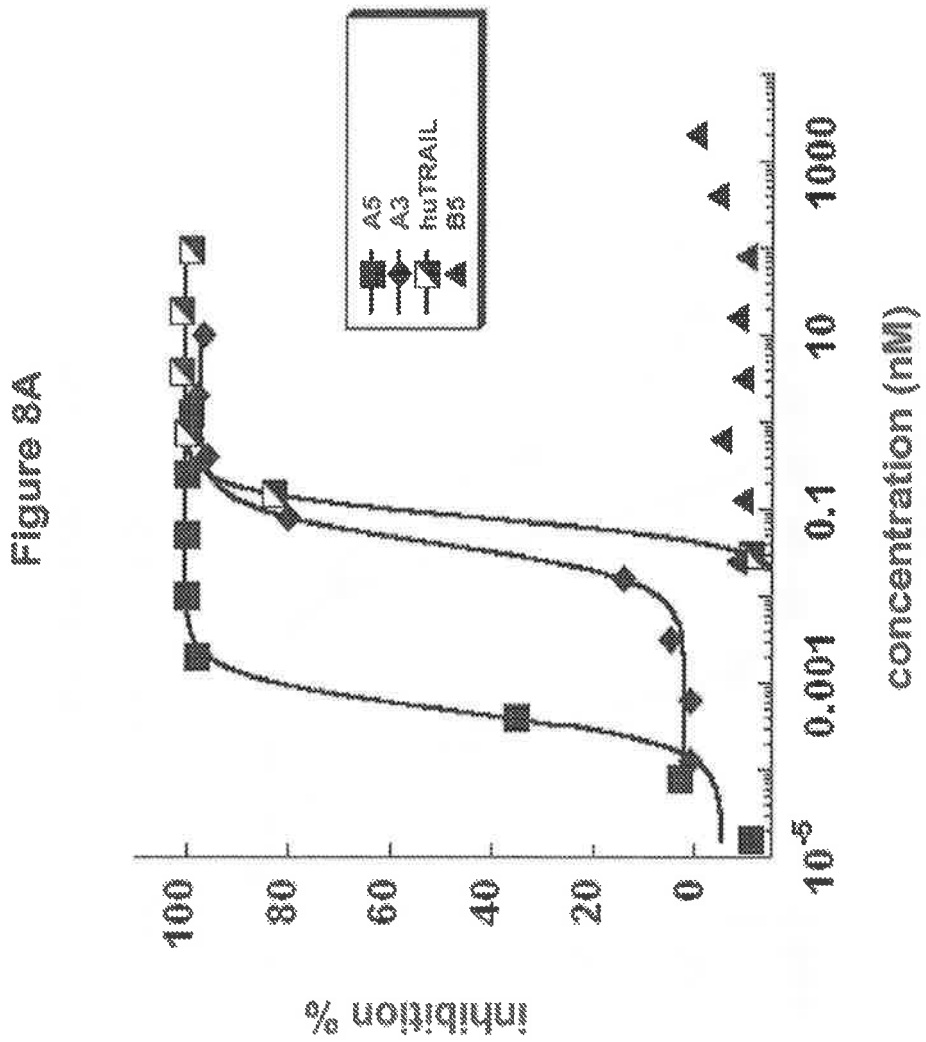

A5
A8
A1
A2
huTRAIL
inhibition %
100
80
60
40
20
0
10^{-5}
0.001
0.1
10
1000
10^{5}
concentration (nM)

Figure 8A


A5
A3
huTRAIL
B5
inhibition %
100
80
60
40
20
0
10^{-5}
0.001
0.1
10
1000
concentration (nM)

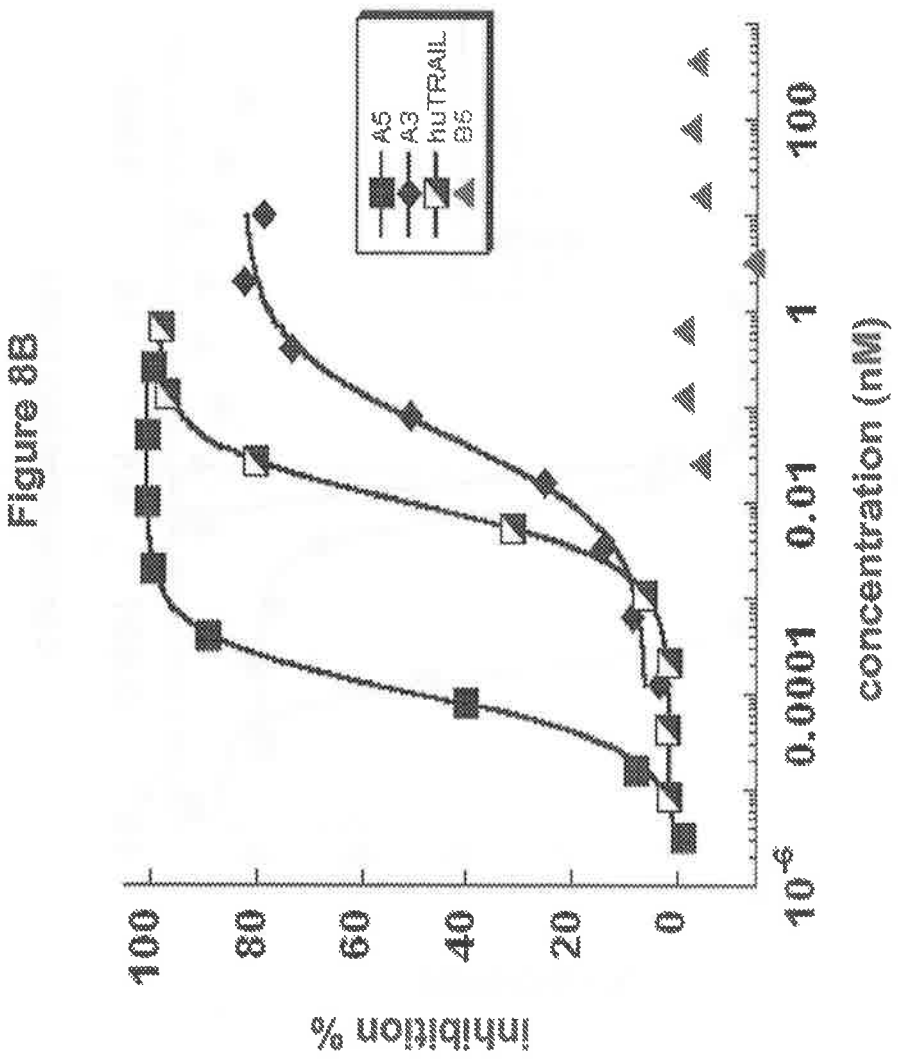
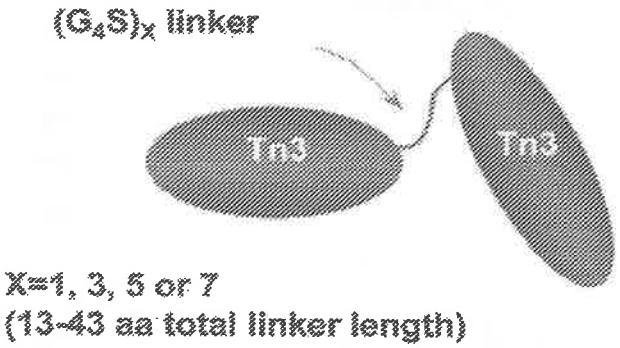

A5
A3
huTRAIL
B5
100
80
60
40
20
0
inhibition %
10⁻⁴
0.0001
0.01
1
100
concentration (nM)

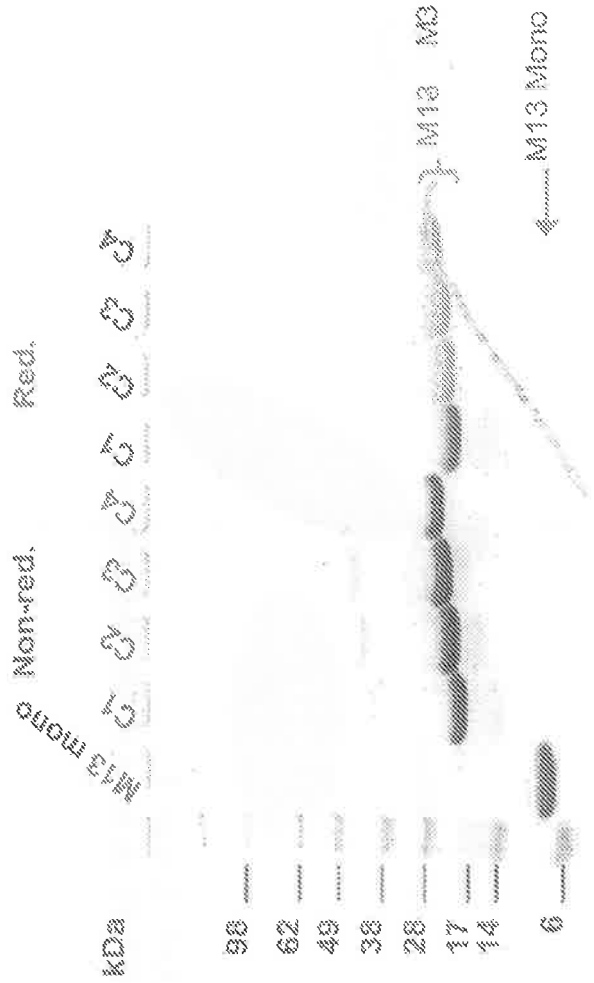
Figure 8B

Figure 9A



Bivalent M13-M13
(MuCD40L-specific)

Figure 9B


kDa
M13 mono
Non-red.
Red.
1
2
3
4
5
6
7
8
9
10
98
62
49
38
28
17
14
6
M13
M3
M13 Mono

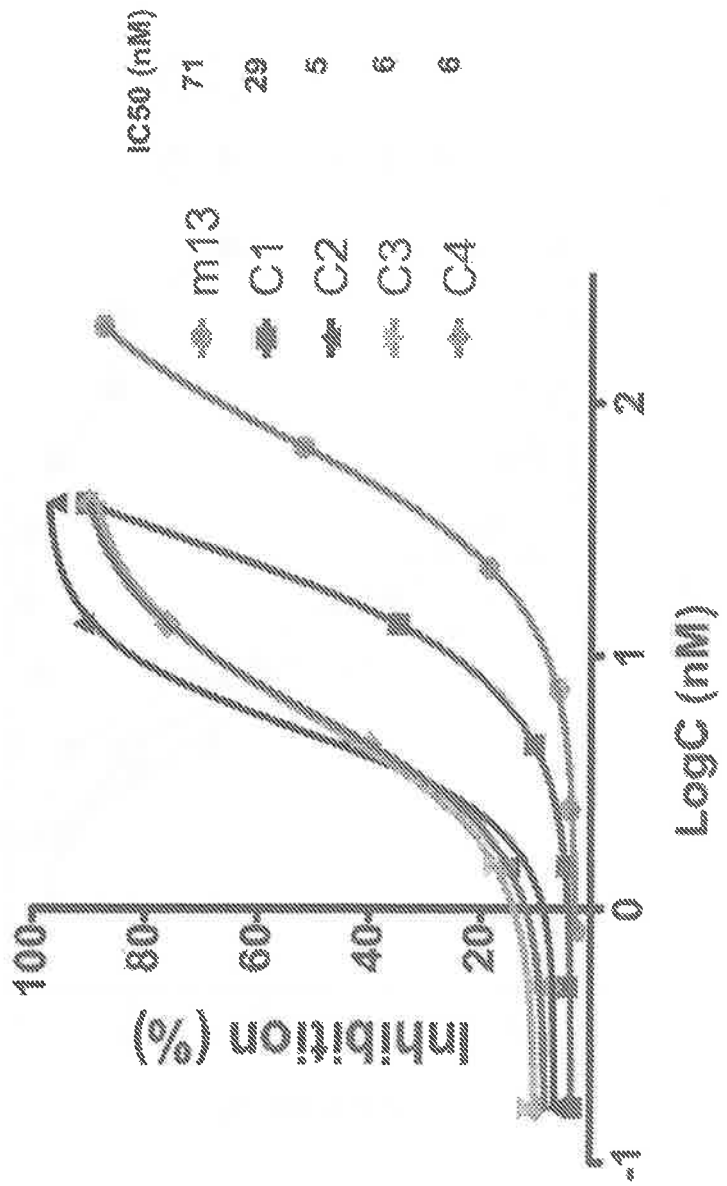

IC50 (nM)
71
29
5
6
6
m13
C1
C2
C3
C4
Inhibition (%)
100
80
60
40
20
0
1
2
-1
LogC (nM)

Figure 9C

Figure 9D

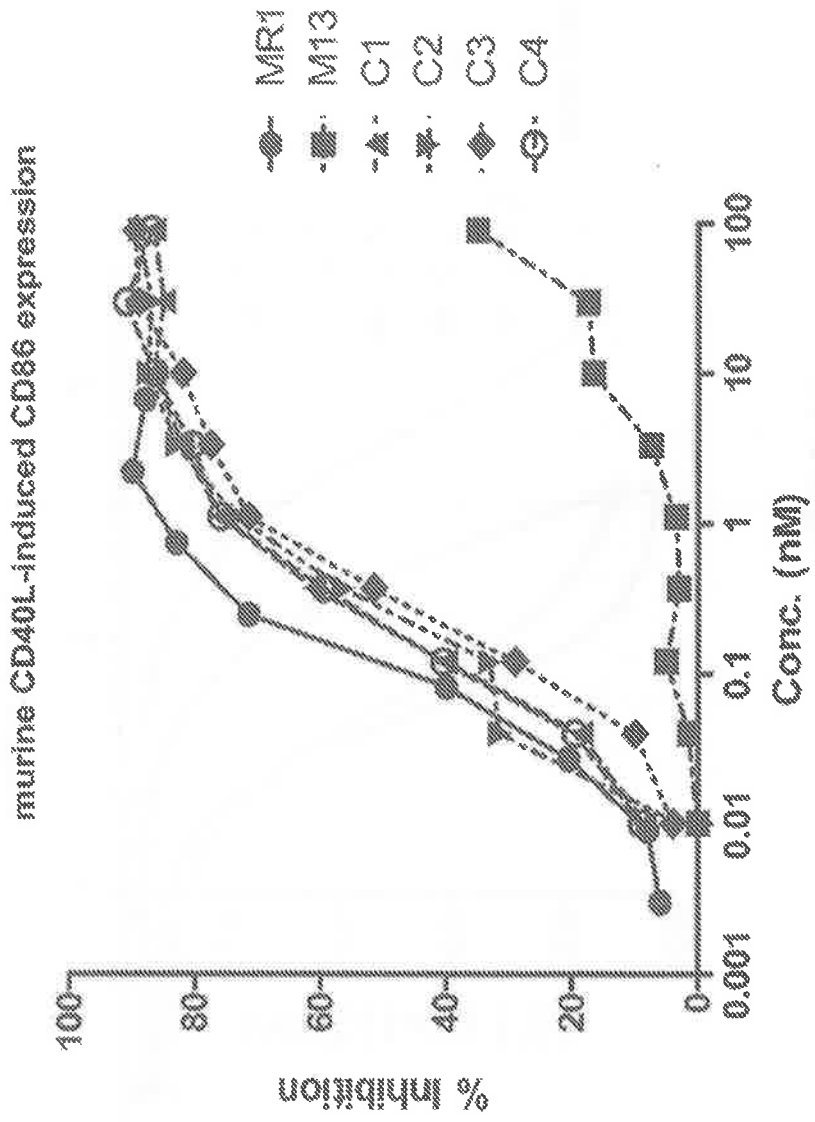

murine CD40L-induced CD86 expression
% Inhibition
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Conc. (nM)
MR1
M13
C1
C2
C3
C4

Figure 10

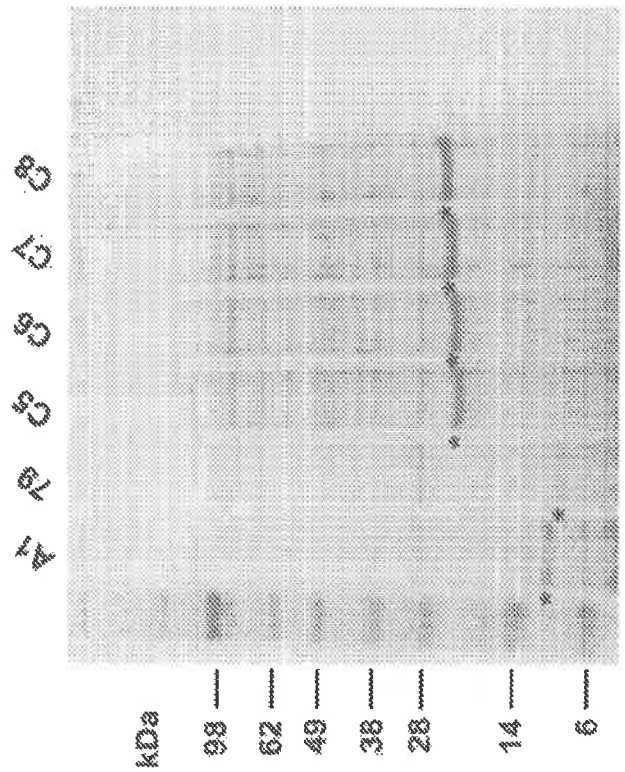


Figure 11A

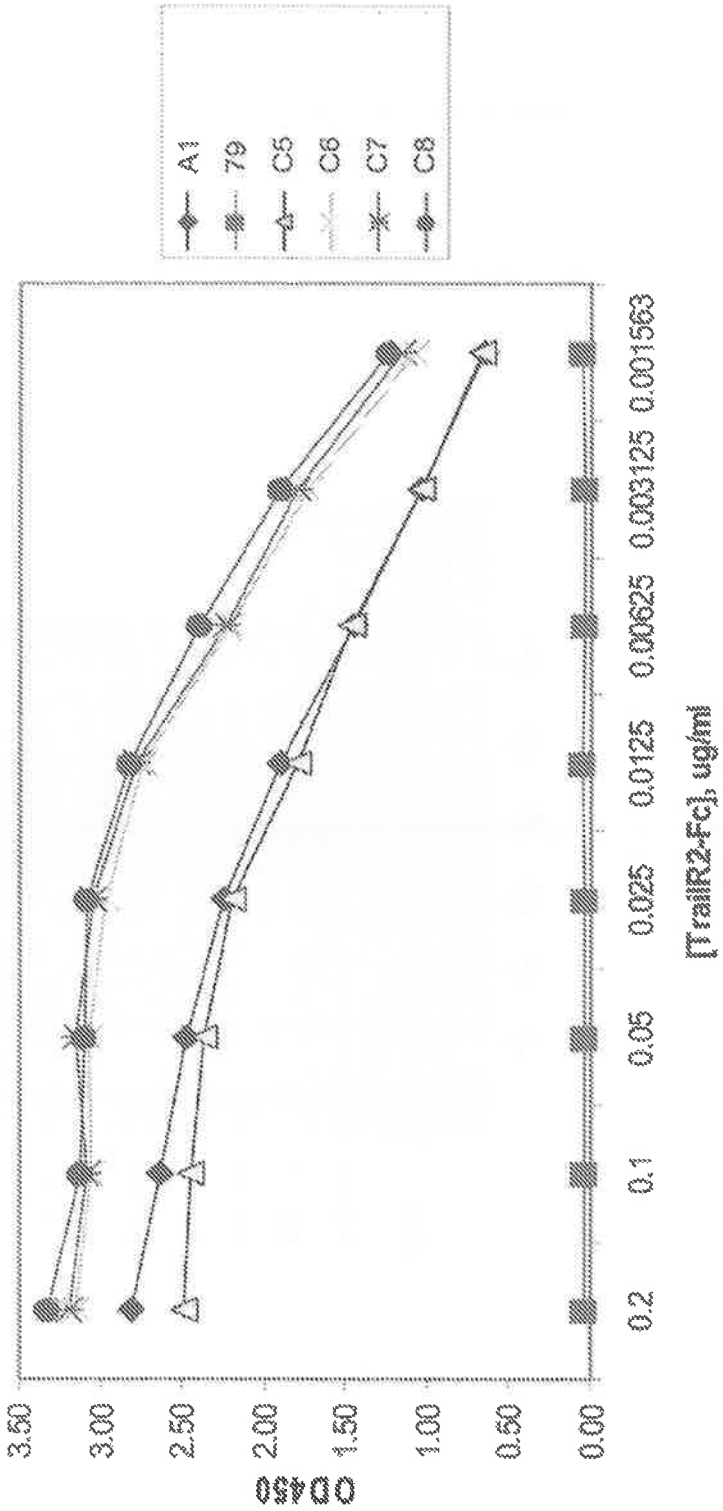

TrailR2 ELISA
A1
79
C5
C6
C7
C8
OD450
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.2
0.1
0.05
0.025
0.0125
0.00625
0.003125
0.001563
[TrailR2-Fc], ug/ml

Figure 11B

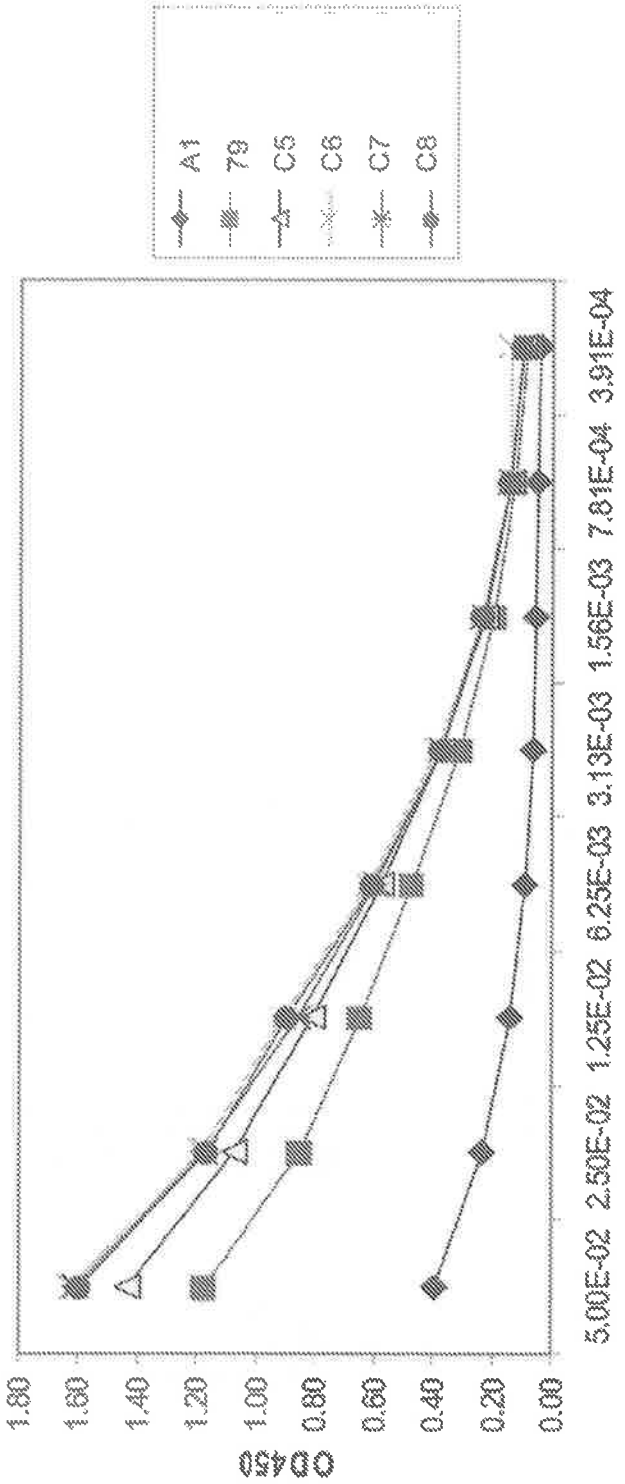

CD40L ELISA
A1
79
C5
C6
C7
C8
OD450
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
5.00E-02
2.50E-02
1.25E-02
6.25E-03
3.13E-03
1.56E-03
7.81E-04
3.91E-04
[CD40L], ug/ml

Figure 12


Simultaneous binding of bispecifics to Trail-R2 and CD40L
AlphaScreen signal
700000
600000
500000
400000
300000
200000
100000
0
165
500
1500
4500
13500
No media
E. coli media dilution factor
A1
79
C5
C6
C7
C8

Figure 13

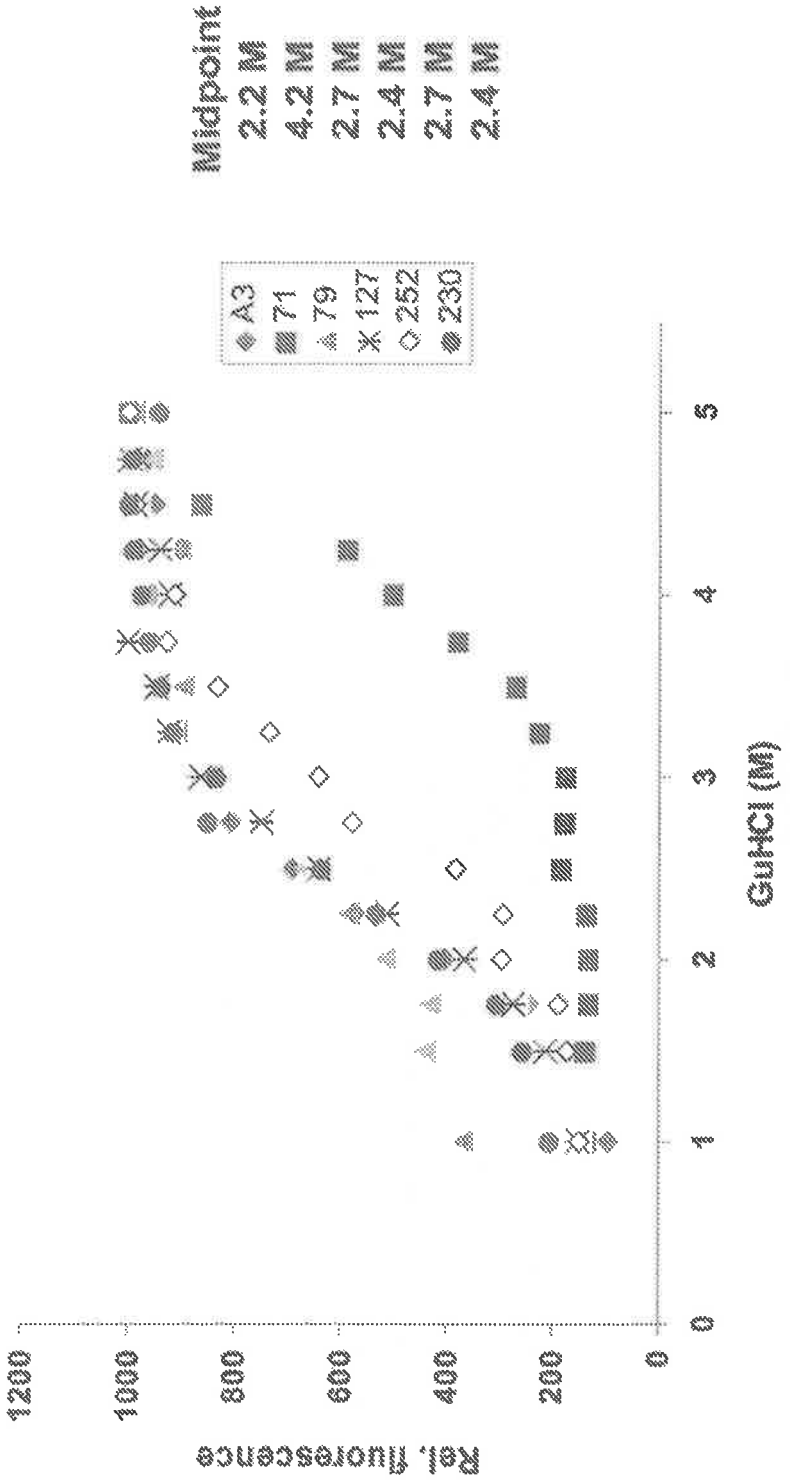

Midpoint
2.2 M
4.2 M
2.7 M
2.4 M
2.7 M
2.4 M
A3
71
79
127
252
230
Rel. fluorescence
1200
1000
800
600
400
200
0
GuHCl (M)
0
1
2
3
4
5

Figure 14

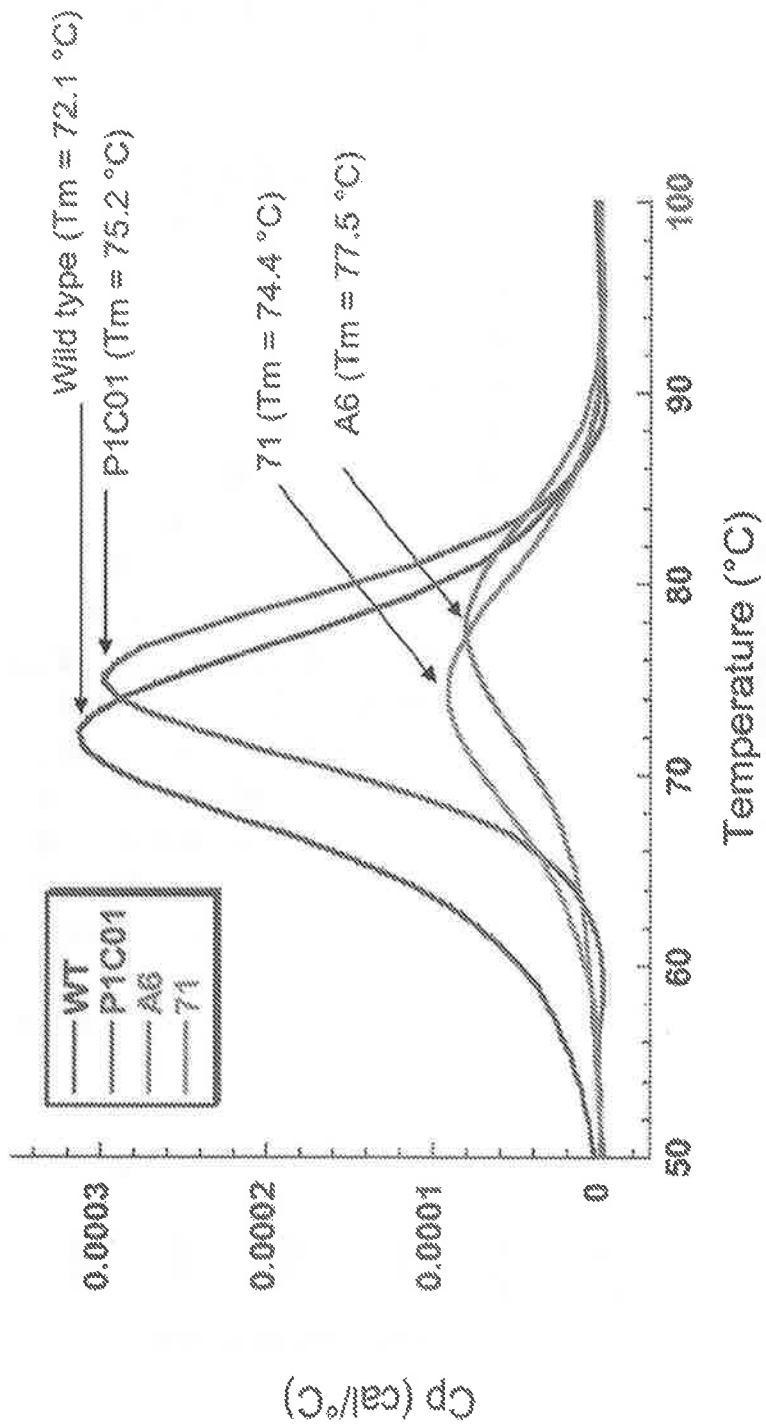

Wild type (Tm = 72.1 °C)
P1C01 (Tm = 75.2 °C)
71 (Tm = 74.4 °C)
A6 (Tm = 77.5 °C)
WT
P1C01
A6
71
0.0003
0.0002
0.0001
0
50
60
70
80
90
100
Temperature (°C)
Cp (cal/°C)

Figure 15

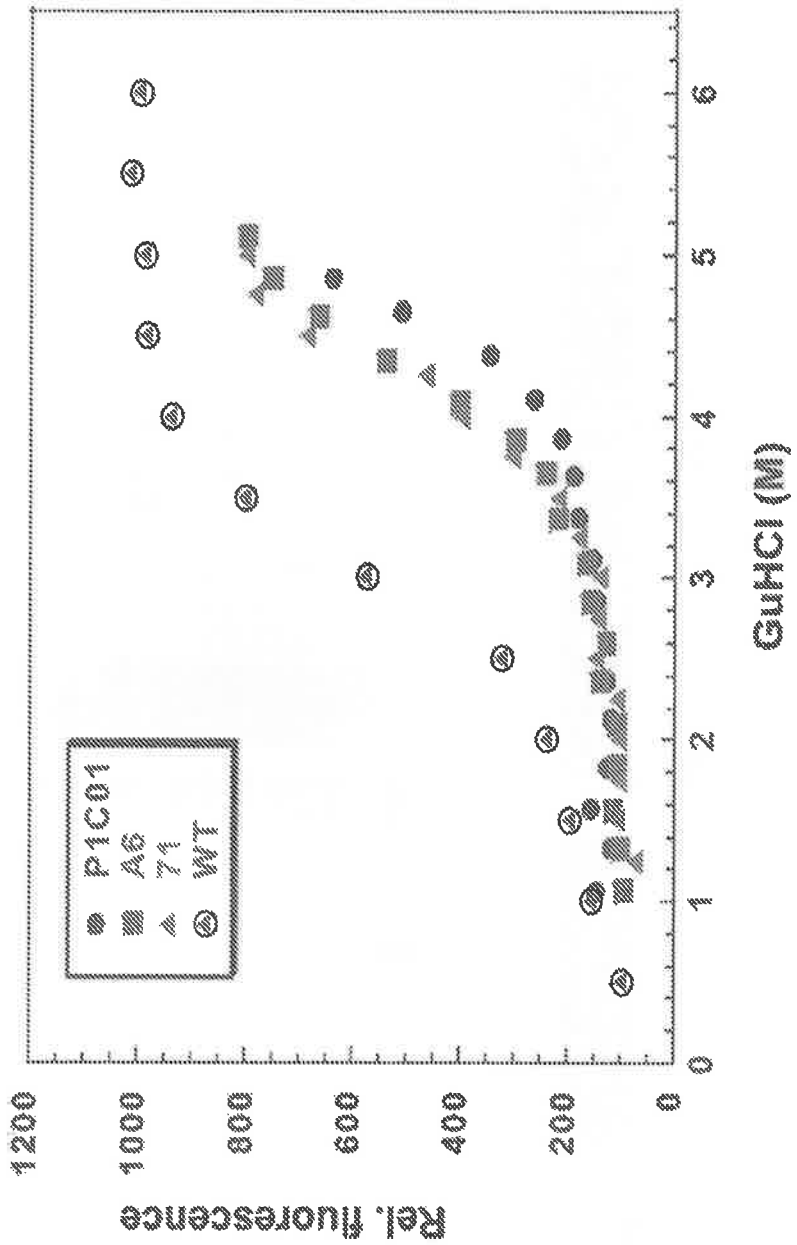

Rel. fluorescence
1200
1000
800
600
400
200
0
0
1
2
3
4
5
6
GuHCl (M)
P1C01
A6
71
WT

Figure 16

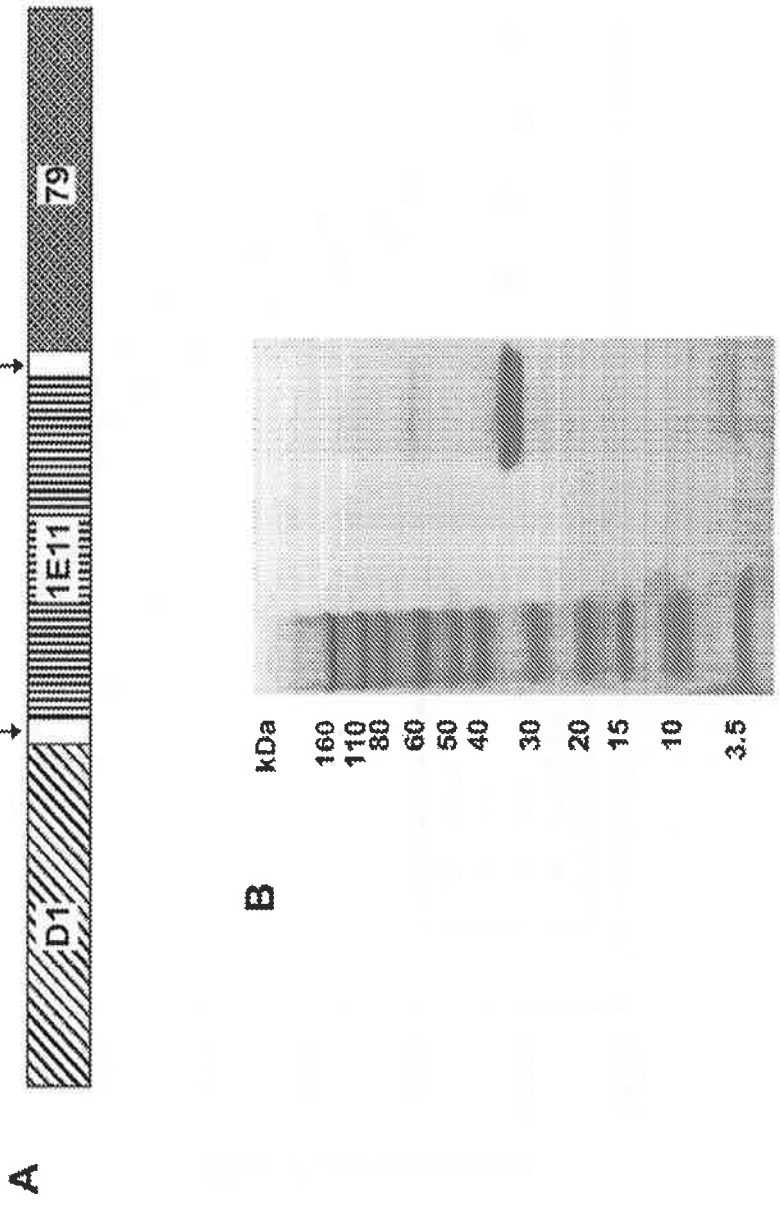


Figure 17A

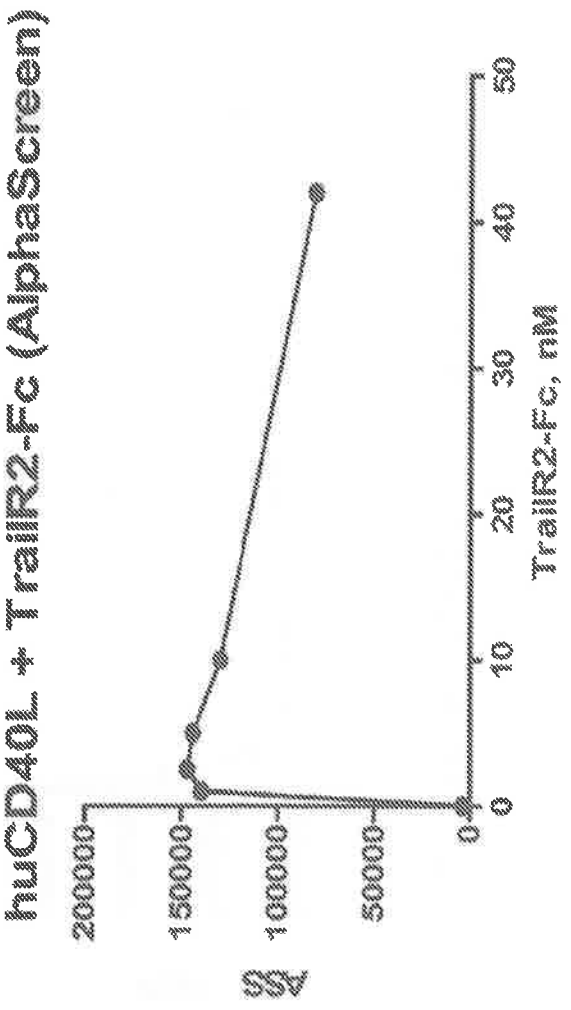

huCD40L + TrailR2-Fc (AlphaScreen)
200000
150000
100000
50000
0
AS
0
10
20
30
40
50
TrailR2-Fc, nM

Figure 17B

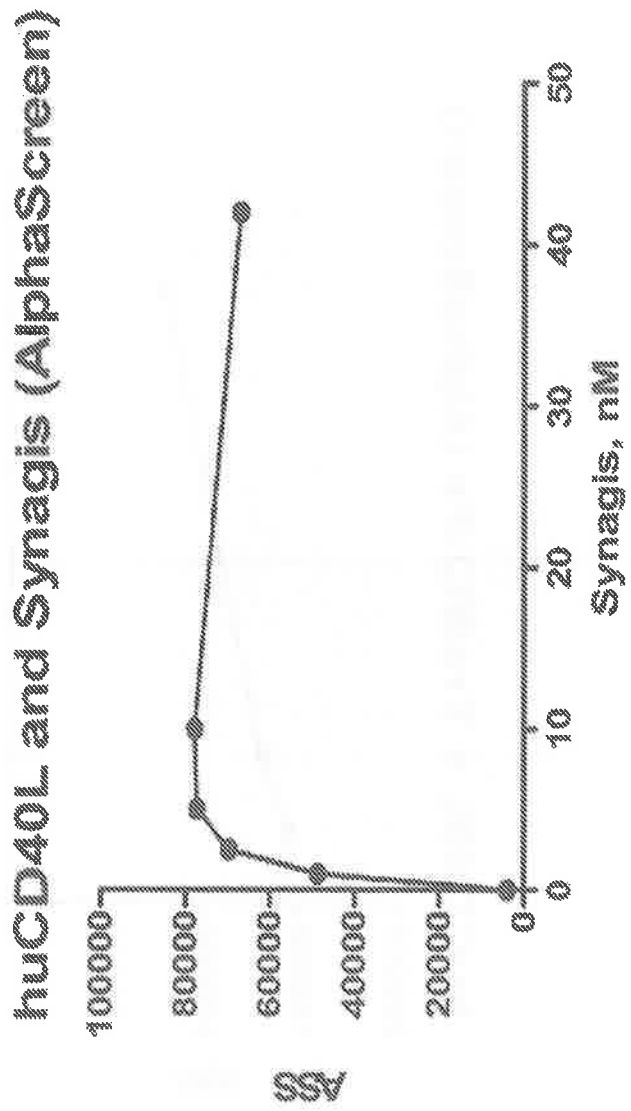

huCD40L and Synagis (AlphaScreen)
ASV
100000
80000
60000
40000
20000
0
0
10
20
30
40
50
Synagis, nM

Figure 18

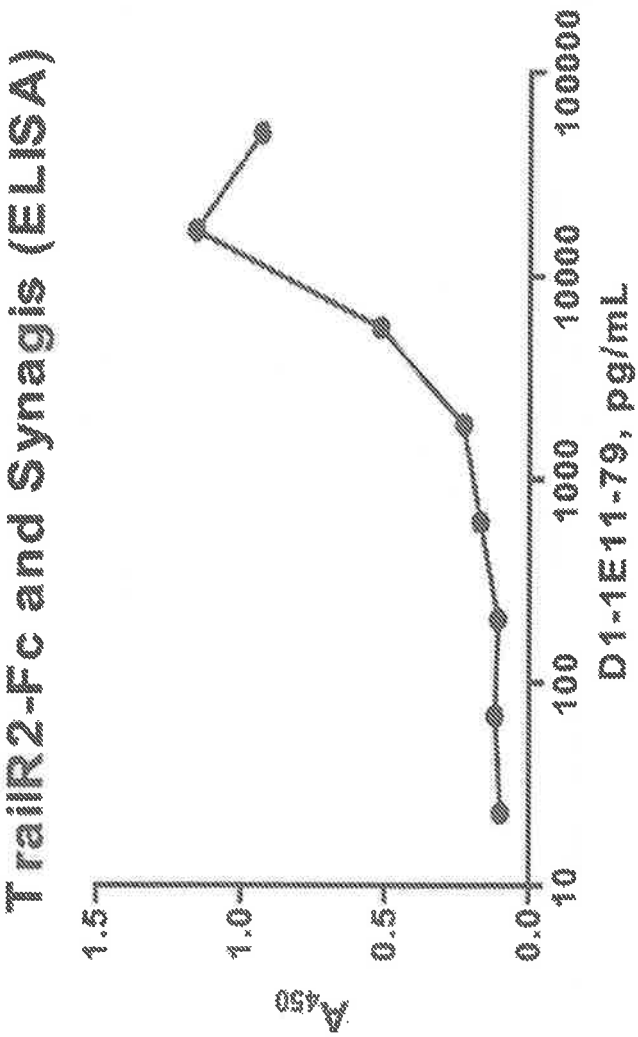

TrailR2-Fc and Synagis (ELISA)
A450
1.5
1.0
0.5
0.0
10
100
1000
10000
100000
D1-1E11-79, pg/mL

Figure 19

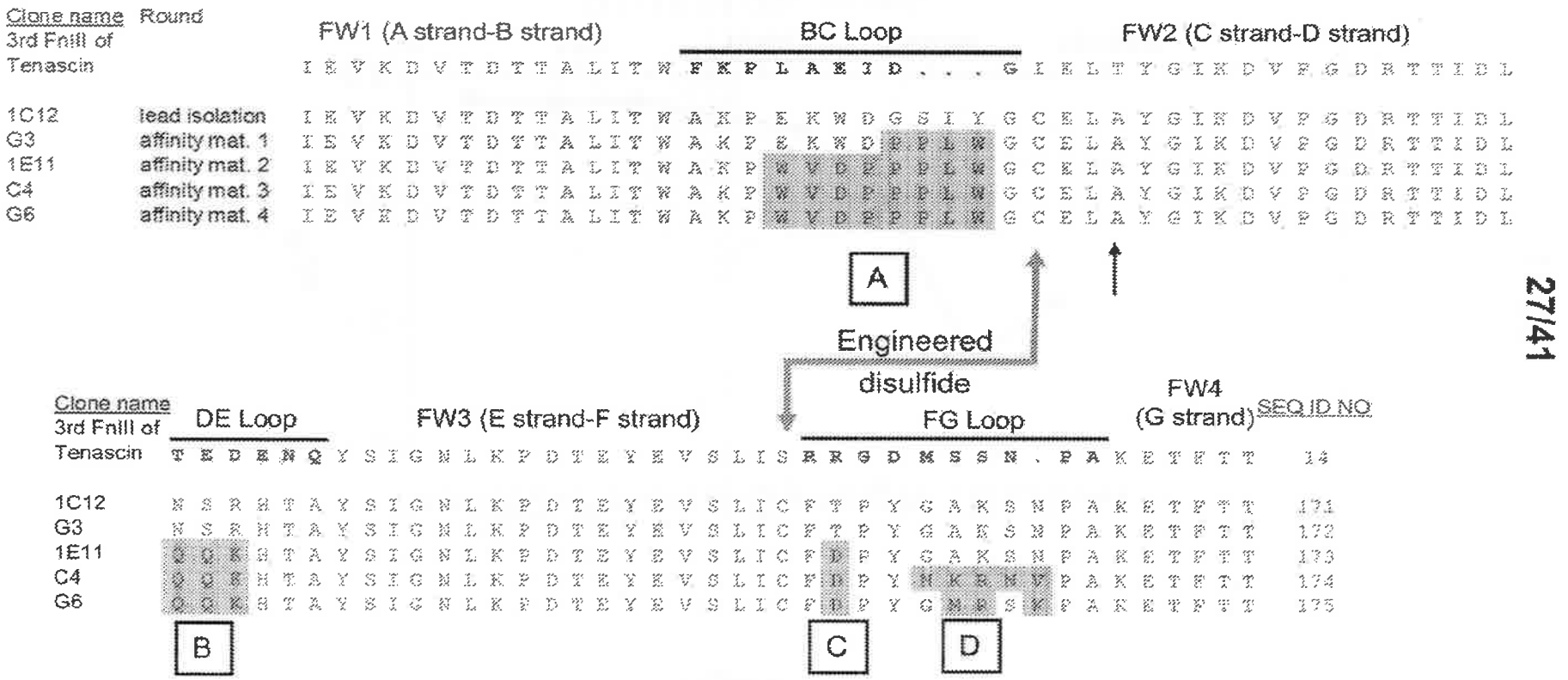
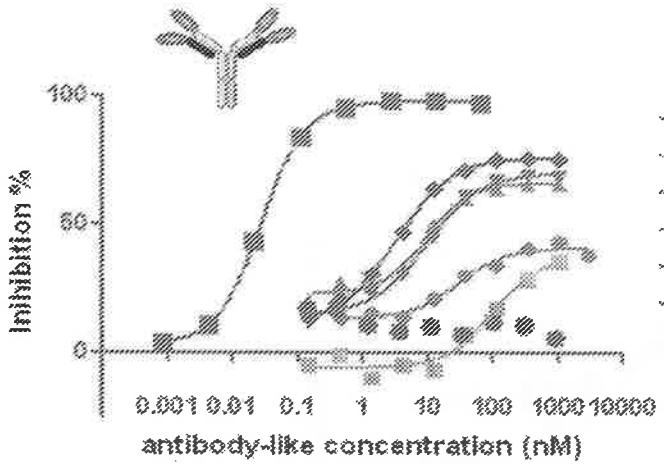

Clone name
3rd FnIII of
Tenascin
Round
FW1 (A strand-B strand)
BC Loop
FW2 (C strand-D strand)
I E V K D V T D T T A L I T W F K P L A E I D . . . G I E L T Y G I K D V P G D R T T I D L
1C12 lead isolation I E V K D V T D T T A L I T W A K P E K W D G S I Y G C E L A Y G I K D V P G D R T T I D L
G3 affinity mat. 1 I E V K D V T D T T A L I T W A K P E K W D P P L W G C E L A Y G I K D V P G D R T T I D L
1E11 affinity mat. 2 I E V K D V T D T T A L I T W A K P W V D P P P L W G C E L A Y G I K D V P G D R T T I D L
C4 affinity mat. 3 I E V K D V T D T T A L I T W A K P W V D P P P L W G C E L A Y G I K D V P G D R T T I D L
G6 affinity mat. 4 I E V K D V T D T T A L I T W A K P W V D P P P L W G C E L A Y G I K D V P G D R T T I D L
A
Engineered
disulfide
Clone name
3rd FnIII of
Tenascin
DE Loop
FW3 (E strand-F strand)
FG Loop
FW4
(G strand)
SEQ ID NO
T E D E N Q Y S I G N L K P D T E Y E V S L I S R R G D M S S N . P A K E T F T T 14
1C12 N S R H T A Y S I G N L K P D T E Y E V S L I C F T P Y G A K S N P A K E T F T T 171
G3 N S R H T A Y S I G N L K P D T E Y E V S L I C F T P Y G A K S N P A K E T F T T 172
1E11 Q Q K H T A Y S I G N L K P D T E Y E V S L I C F D P Y G A K S N P A K E T F T T 173
C4 Q Q R H T A Y S I G N L K P D T E Y E V S L I C F D P Y H K R M V P A K E T F T T 174
G6 Q Q K H T A Y S I G N L K P D T E Y E V S L I C F D P Y G M R S K P A K E T F T T 175
B
C
D

Figure 20



Clone	EC_{50} (nM)	Max Inhibition %
1C12	28 ± 8	41 ± 2
G3	99 ± 39	38 ± 6
1E11	9 ± 1	66 ± 1
C4	9 ± 2	70 ± 2
G6	3 ± 0.2	76 ± 1
TRAIL	0.027 ± 0.0006	98 ± 0.3
nonbinding control		

Figure 21

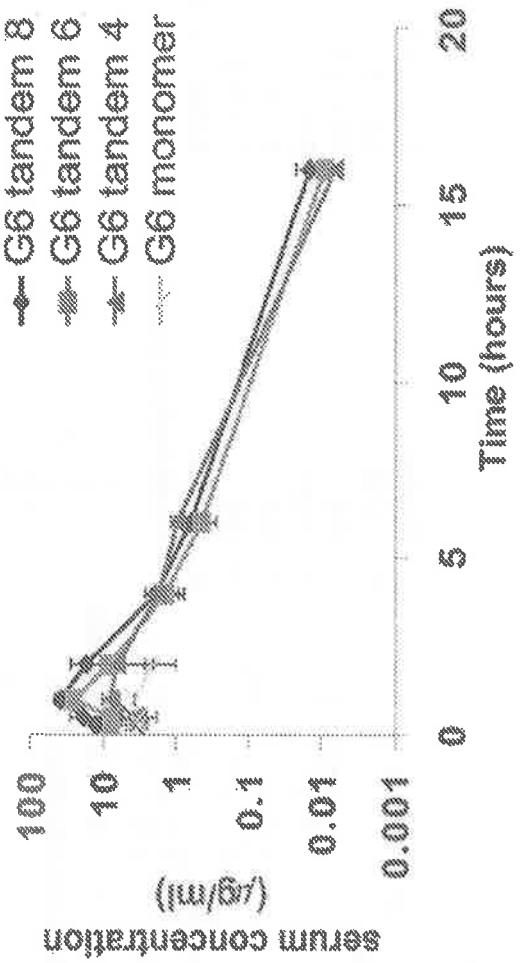

G6 tandem 8
G6 tandem 6
G6 tandem 4
G6 monomer
100
10
1
0.1
0.01
0.001
serum concentration
(µg/ml)
0
5
10
15
20
Time (hours)

Figure 22A

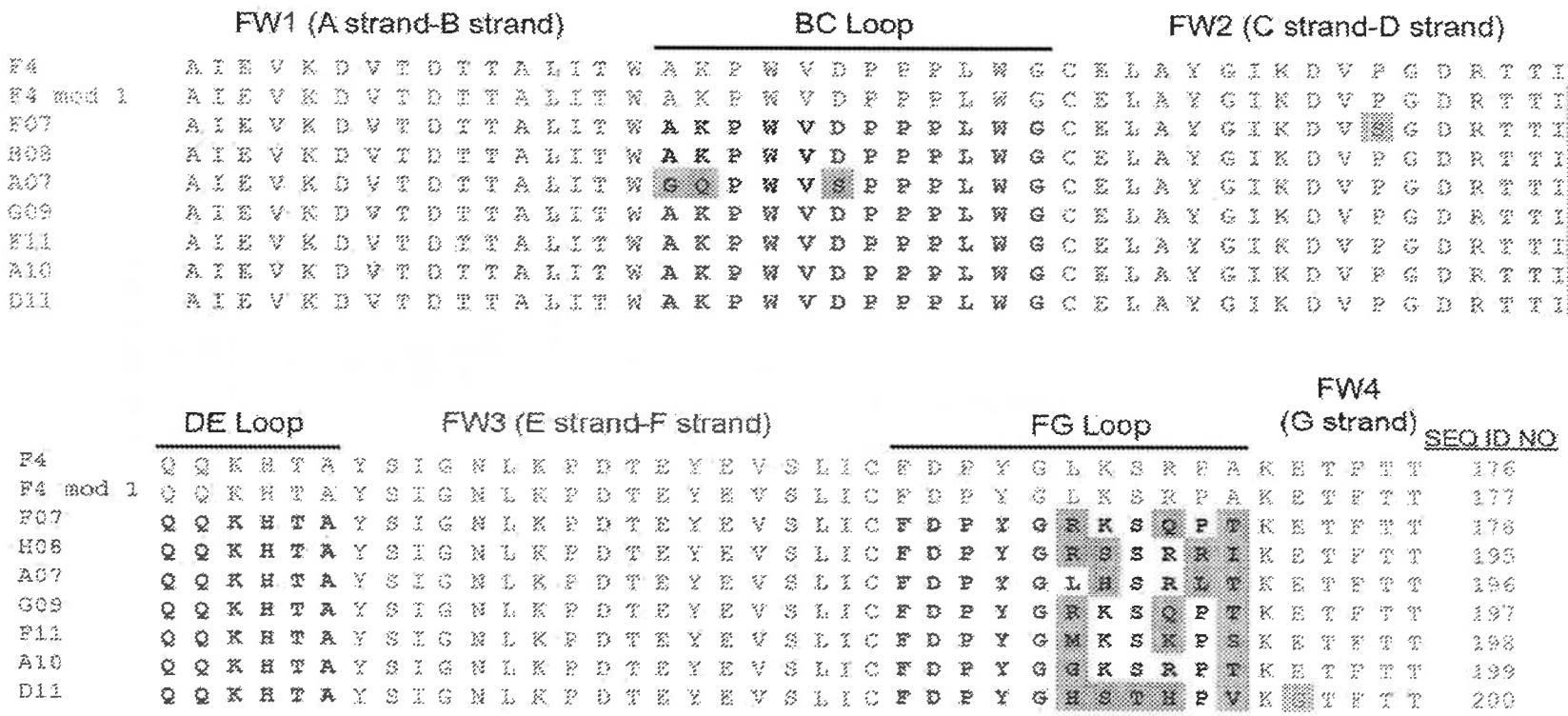

FW1 (A strand-B strand)
BC Loop
FW2 (C strand-D strand)
DE Loop
FW3 (E strand-F strand)
FG Loop
FW4 (G strand)
SEQ ID NO
F4
F4 mod 1
F07
H08
A07
G09
F11
A10
D11
176
177
178
195
196
197
198
199
200

Figure 22B

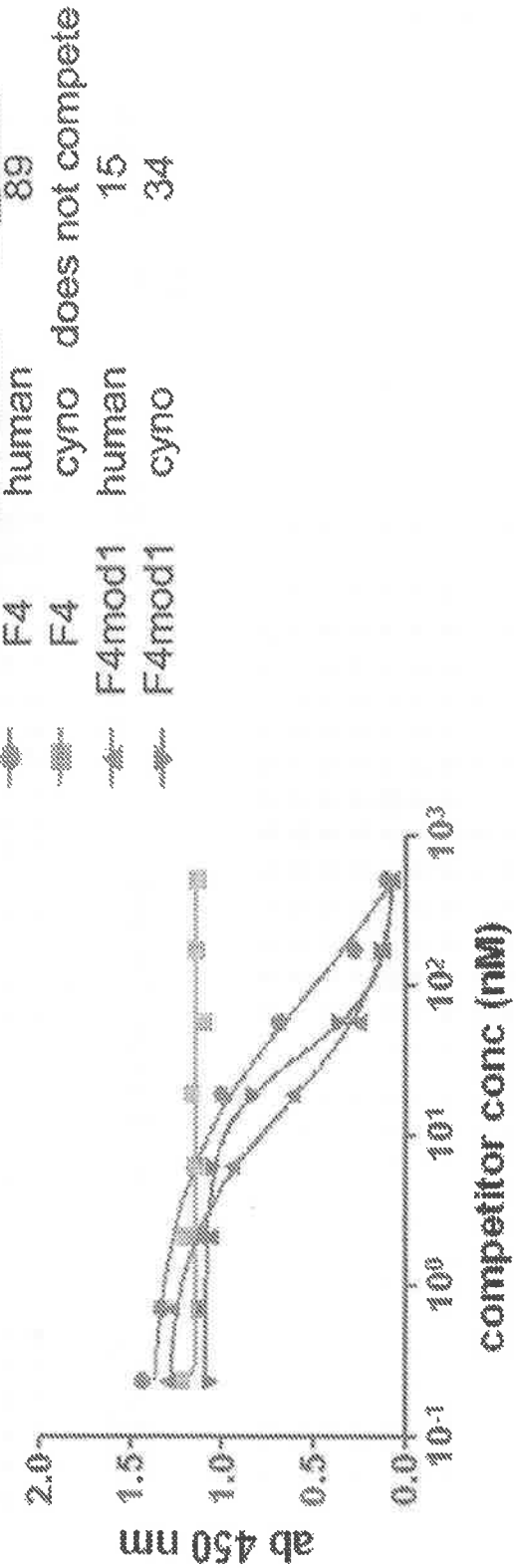

Clone
TRAIL R2
IC50 (nM)
F4
human
89
F4
cyno
does not compete
F4mod1
human
15
F4mod1
cyno
34
ab 450 nm
2.0
1.5
1.0
0.5
0.0
10-1
100
101
102
103
competitor conc (nM)

Figure 23A

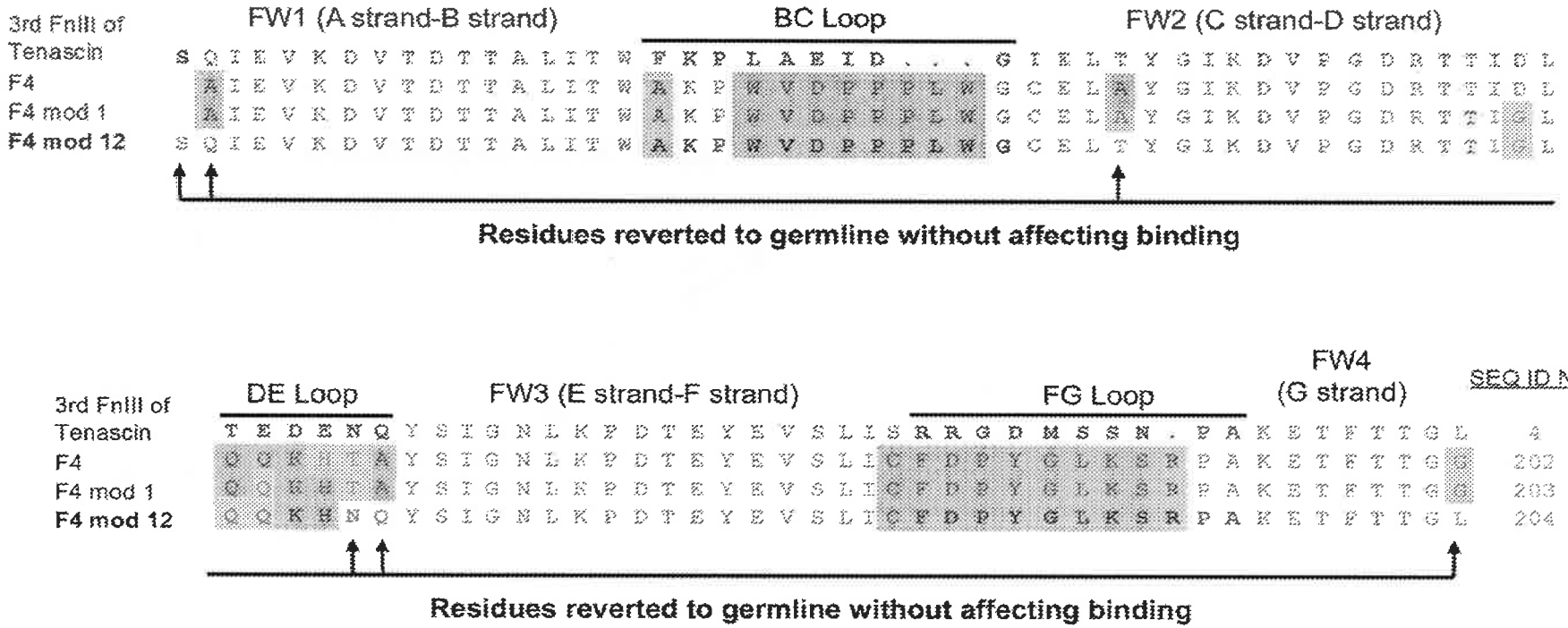

3rd FnIII of Tenascin
F4
F4 mod 1
F4 mod 12
FW1 (A strand-B strand)
BC Loop
FW2 (C strand-D strand)
Residues reverted to germline without affecting binding
DE Loop
FW3 (E strand-F strand)
FG Loop
FW4 (G strand)
SEQ ID NO
4
202
203
204
Residues reverted to germline without affecting binding

Figure 23B

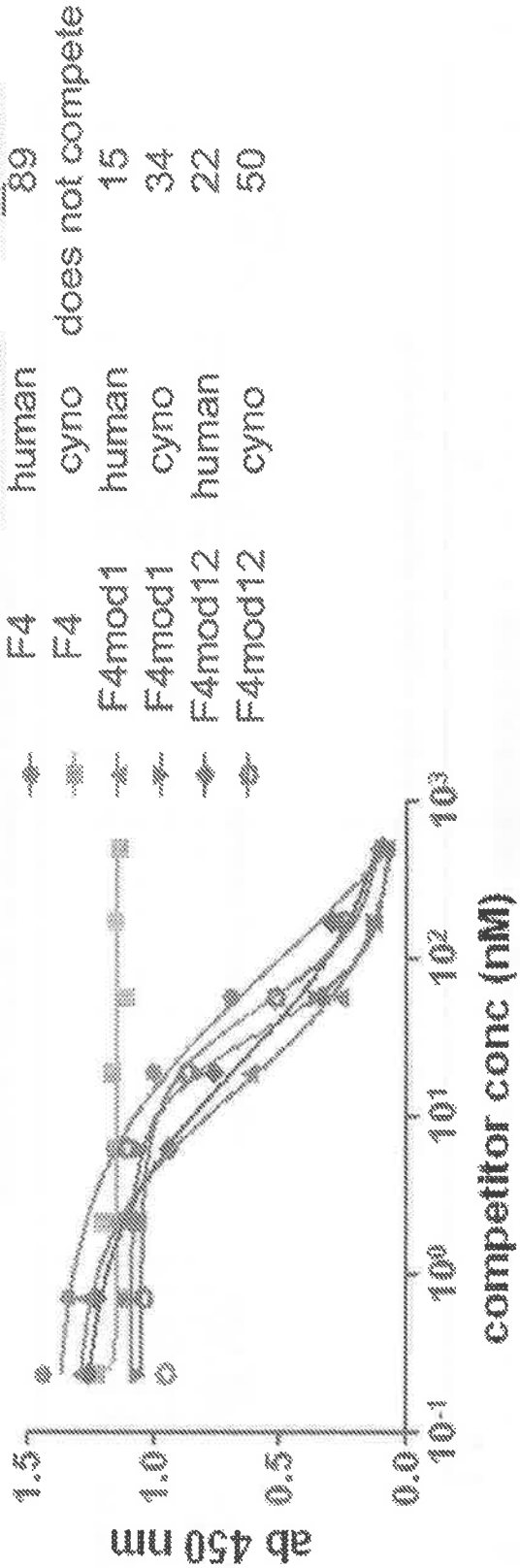

Clone
TRAIL R2
IC50 (nM)
F4
human
89
F4
cyno
does not compete
F4mod1
human
15
F4mod1
cyno
34
F4mod12
human
22
F4mod12
cyno
50
ab 450 nm
1.5
1.0
0.5
0.0
10-1
100
101
102
103
competitor conc (nM)

Figure 23C

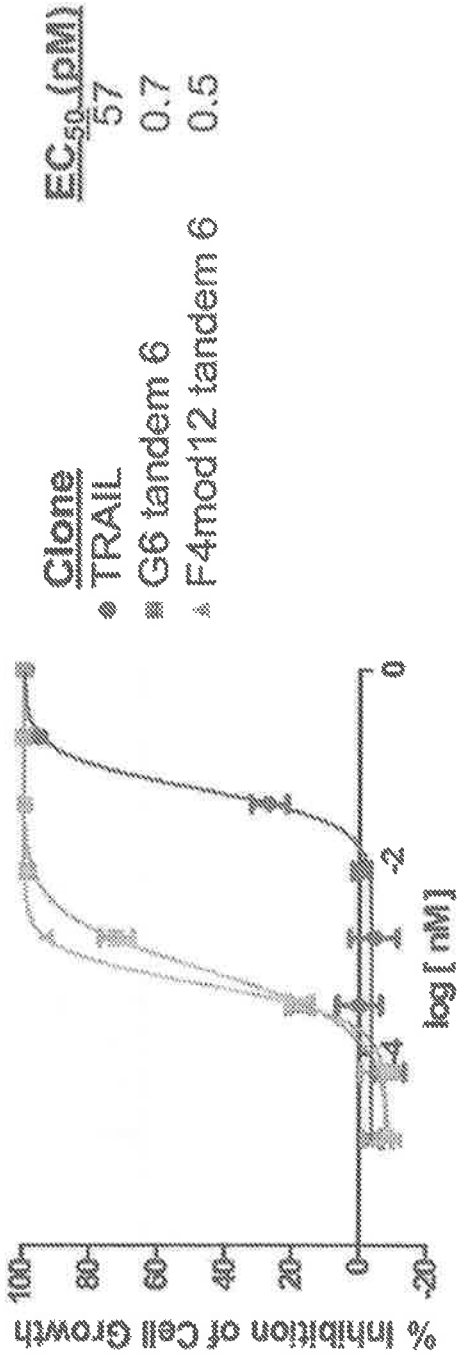
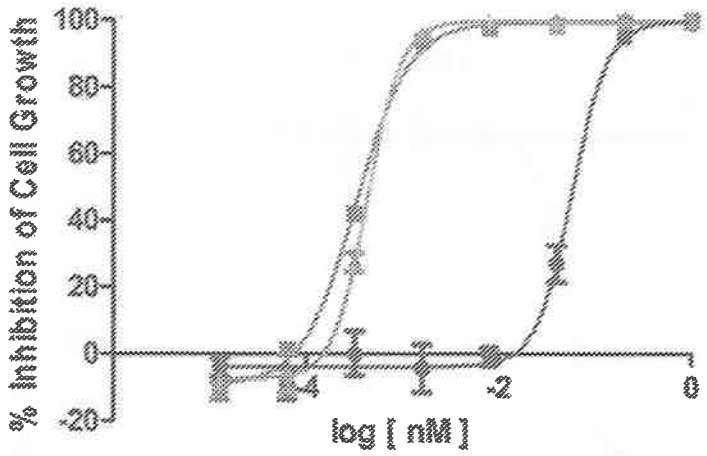
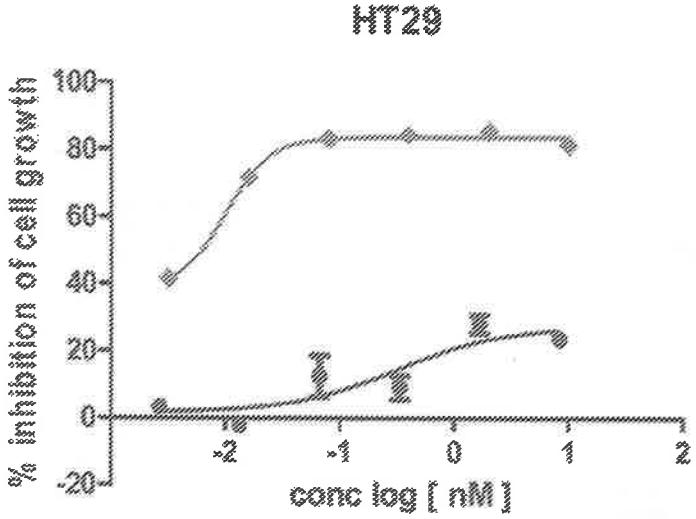

Clone
EC50 (pM)
TRAIL 57
G6 tandem 6 0.7
F4mod12 tandem 6 0.5
% Inhibition of cell Growth
100
80
60
40
20
0
-20
0
-2
-1
-4
log [nM]

Figure 23D



Clone	EC_{50} (pM)
TRAIL	57
G6 tandem 8	0.3
F4mod12 tandem 8	0.4

Figure 24



Clone	EC_{50}(nM)	Max Inhibition %
TRAIL	>8.3	28.0
G6 tandem	80.01	85.4

Figure 25

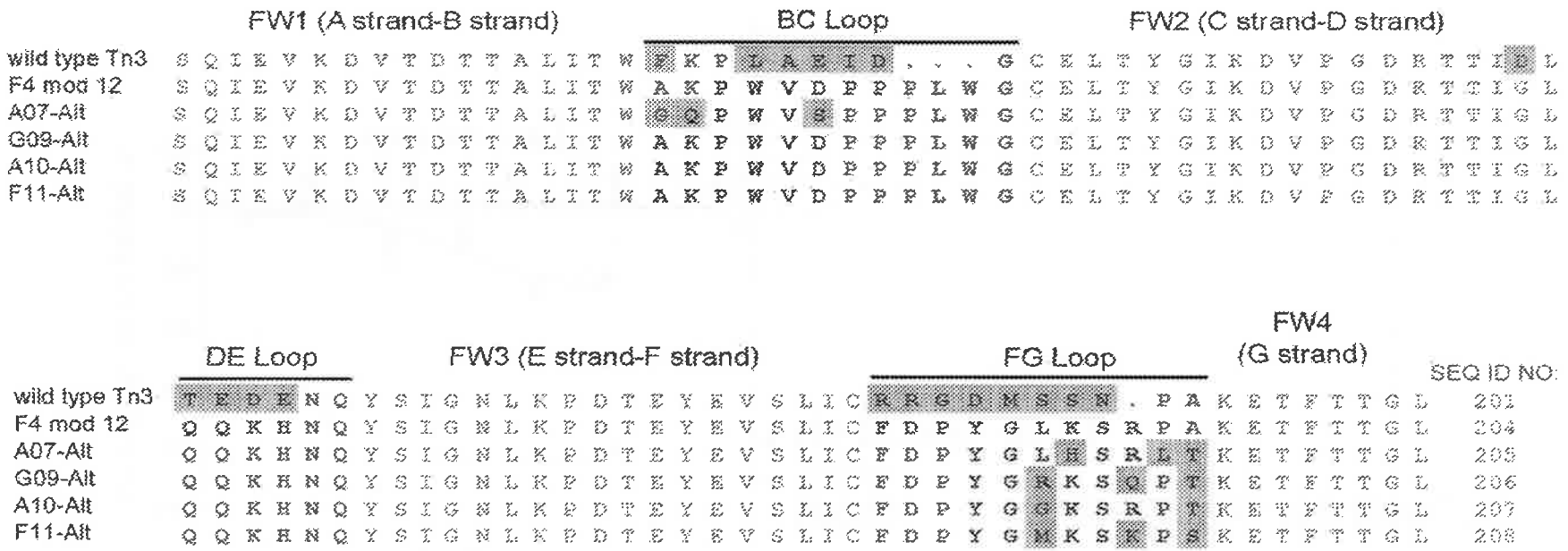

FW1 (A strand-B strand)
BC Loop
FW2 (C strand-D strand)
wild type Tn3 S Q I E V K D V T D T T A L I T W F K P L A E I D . . . G C E L T Y G I K D V P G D R T T I I L
F4 mod 12 S Q I E V K D V T D T T A L I T W A K P W V D P P P L W G C E L T Y G I K D V P G D R T T I G L
A07-Alt S Q I E V K D V T D T T A L I T W G Q P W V S P P P L W G C E L T Y G I K D V P G D R T T I G L
G09-Alt S Q I E V K D V T D T T A L I T W A K P W V D P P P L W G C E L T Y G I K D V P G D R T T I G L
A10-Alt S Q I E V K D V T D T T A L I T W A K P W V D P P P L W G C E L T Y G I K D V P G D R T T I G L
F11-Alt S Q I E V K D V T D T T A L I T W A K P W V D P P P L W G C E L T Y G I K D V P G D R T T I G L
DE Loop
FW3 (E strand-F strand)
FG Loop
FW4
(G strand)
SEQ ID NO:
wild type Tn3 T E D E N Q Y S I G N L K P D T E Y E V S L I C R R G D M S S N . P A K E T F T T G L 201
F4 mod 12 Q Q K H N Q Y S I G N L K P D T E Y E V S L I C F D P Y G L K S R P A K E T F T T G L 204
A07-Alt Q Q K H N Q Y S I G N L K P D T E Y E V S L I C F D P Y G L H S R L T K E T F T T G L 203
G09-Alt Q Q K H N Q Y S I G N L K P D T E Y E V S L I C F D P Y G R K S Q P T K E T F T T G L 206
A10-Alt Q Q K H N Q Y S I G N L K P D T E Y E V S L I C F D P Y G G K S R P T K E T F T T G L 207
F11-Alt Q Q K H N Q Y S I G N L K P D T E Y E V S L I C F D P Y G M K S K P S K E T F T T G L 208

Figure 26A

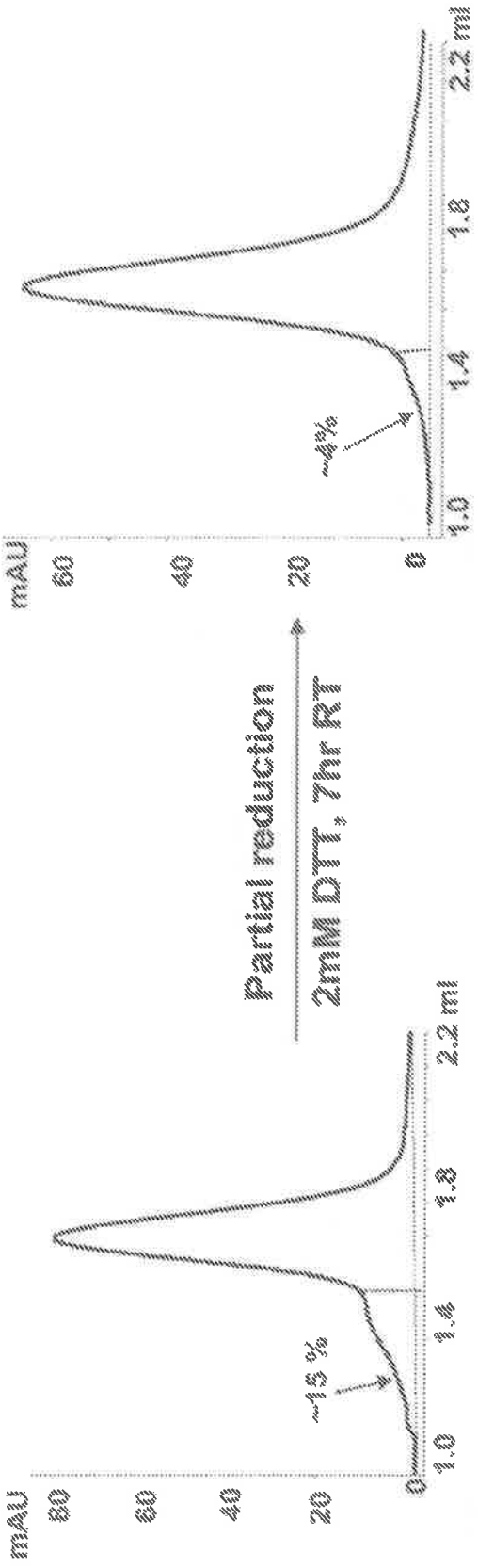

mAU
80
60
40
20
0
1.0
1.4
1.8
2.2 ml
~15 %
Partial reduction
2mM DTT, 7hr RT
mAU
60
40
20
0
1.0
1.4
1.8
2.2 ml
~4%

Figure 26B

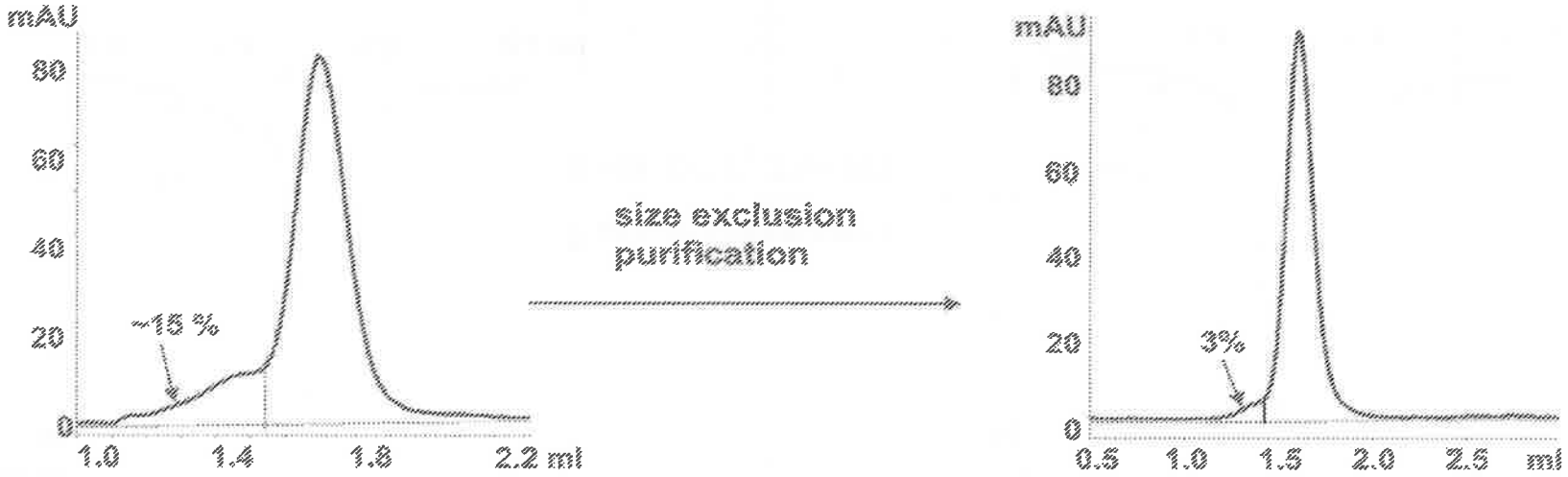

mAU
80
60
40
20
0
~15 %
1.0
1.4
1.8
2.2 ml
size exclusion
purification
mAU
80
60
40
20
0
3%
0.5
1.0
1.5
2.0
2.5
ml

Figure 27

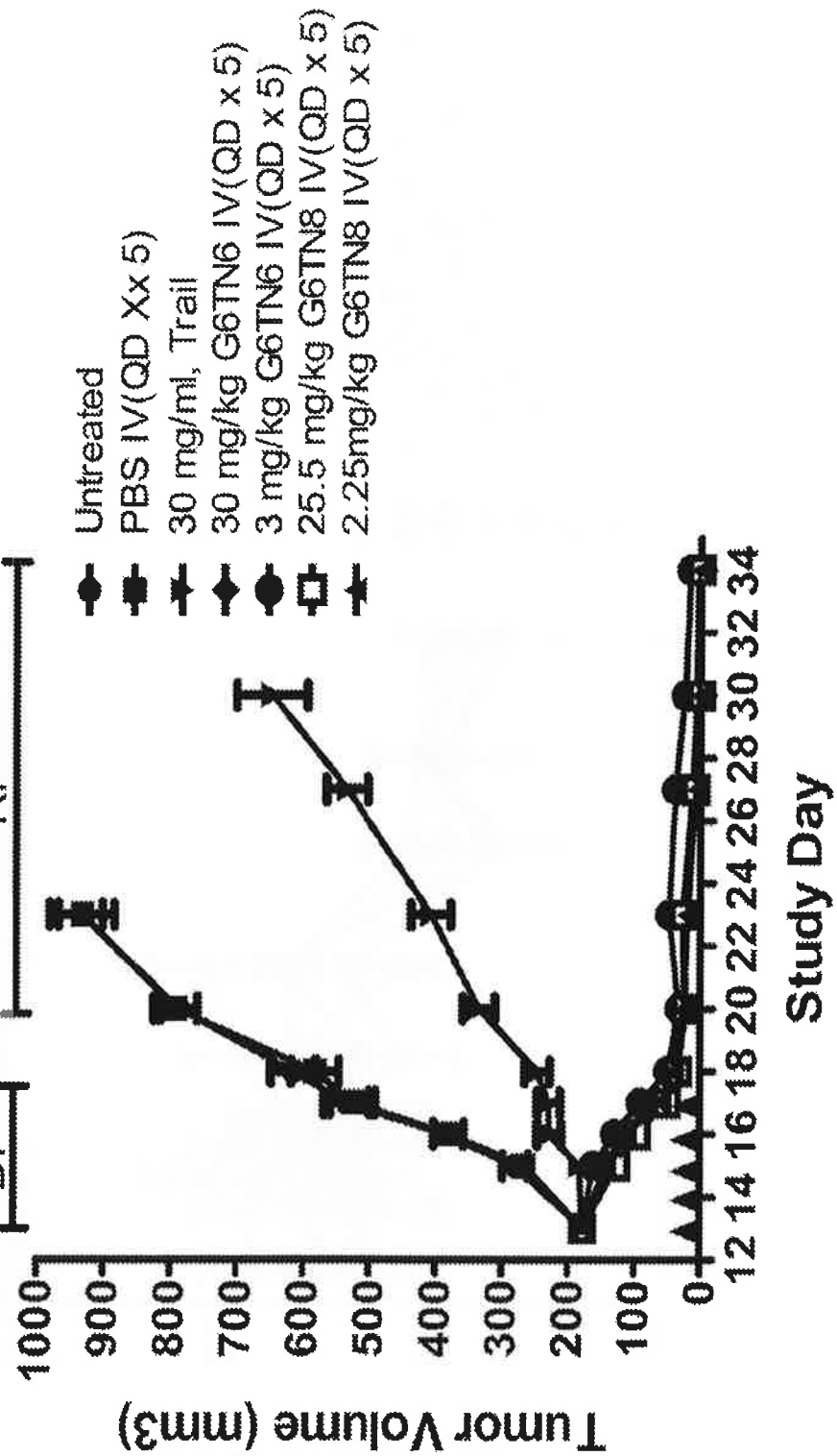
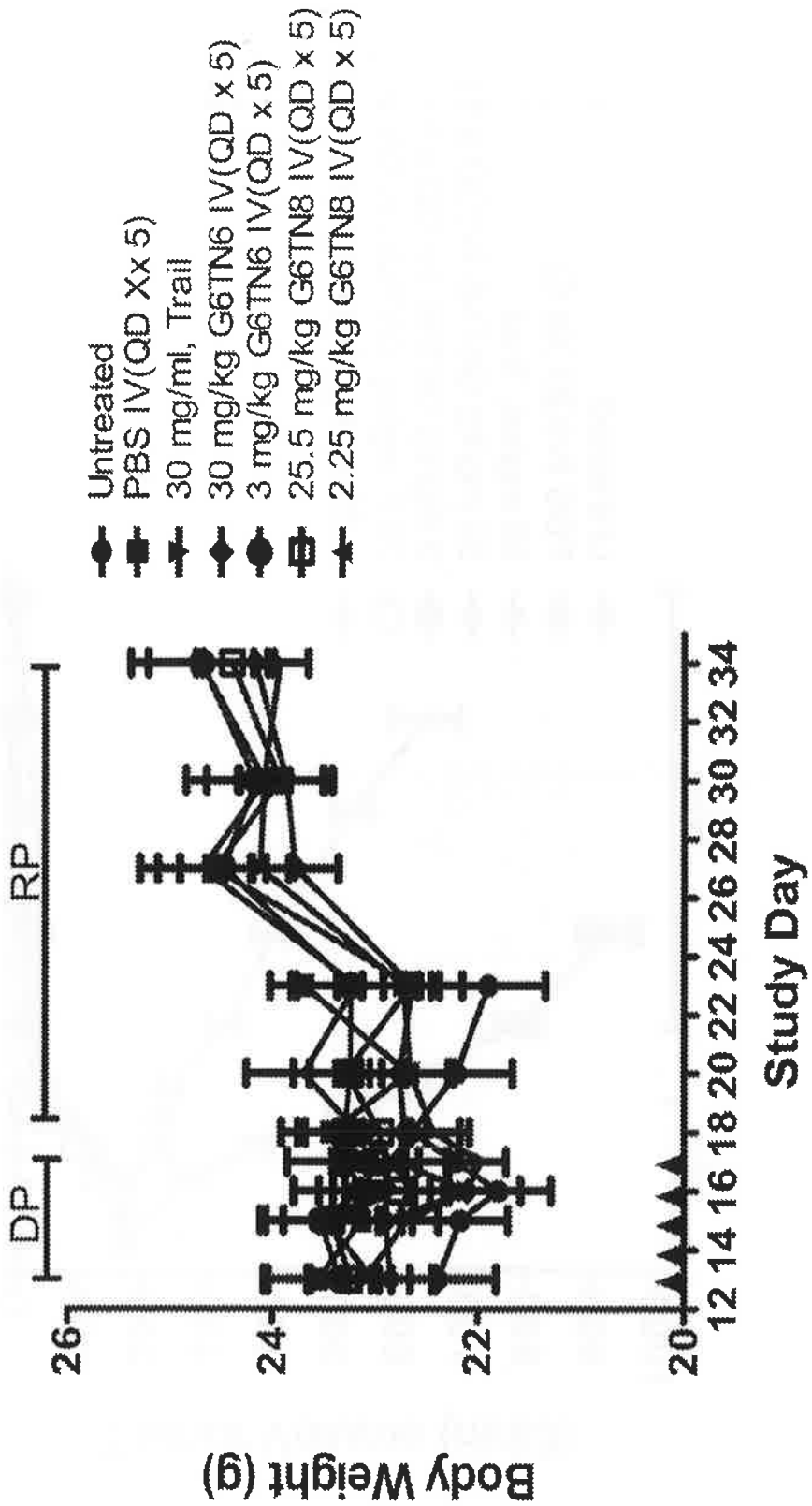

Tumor Volume (mm3)
1000
900
800
700
600
500
400
300
200
100
0
12 14 16 18 20 22 24 26 28 30 32 34
Study Day
DP
RP
Untreated
PBS IV(QD Xx 5)
30 mg/ml, Trail
30 mg/kg G6TN6 IV(QD x 5)
3 mg/kg G6TN6 IV(QD x 5)
25.5 mg/kg G6TN8 IV(QD x 5)
2.25mg/kg G6TN8 IV(QD x 5)

Figure 28


Untreated
PBS IV(QD Xx 5)
30 mg/ml, Trail
30 mg/kg G6TN6 IV(QD x 5)
3 mg/kg G6TN6 IV(QD x 5)
25.5 mg/kg G6TN8 IV(QD x 5)
2.25 mg/kg G6TN8 IV(QD x 5)
DP
RP
26
24
22
20
12 14 16 18 20 22 24 26 28 30 32 34
Study Day
Body Weight (g)