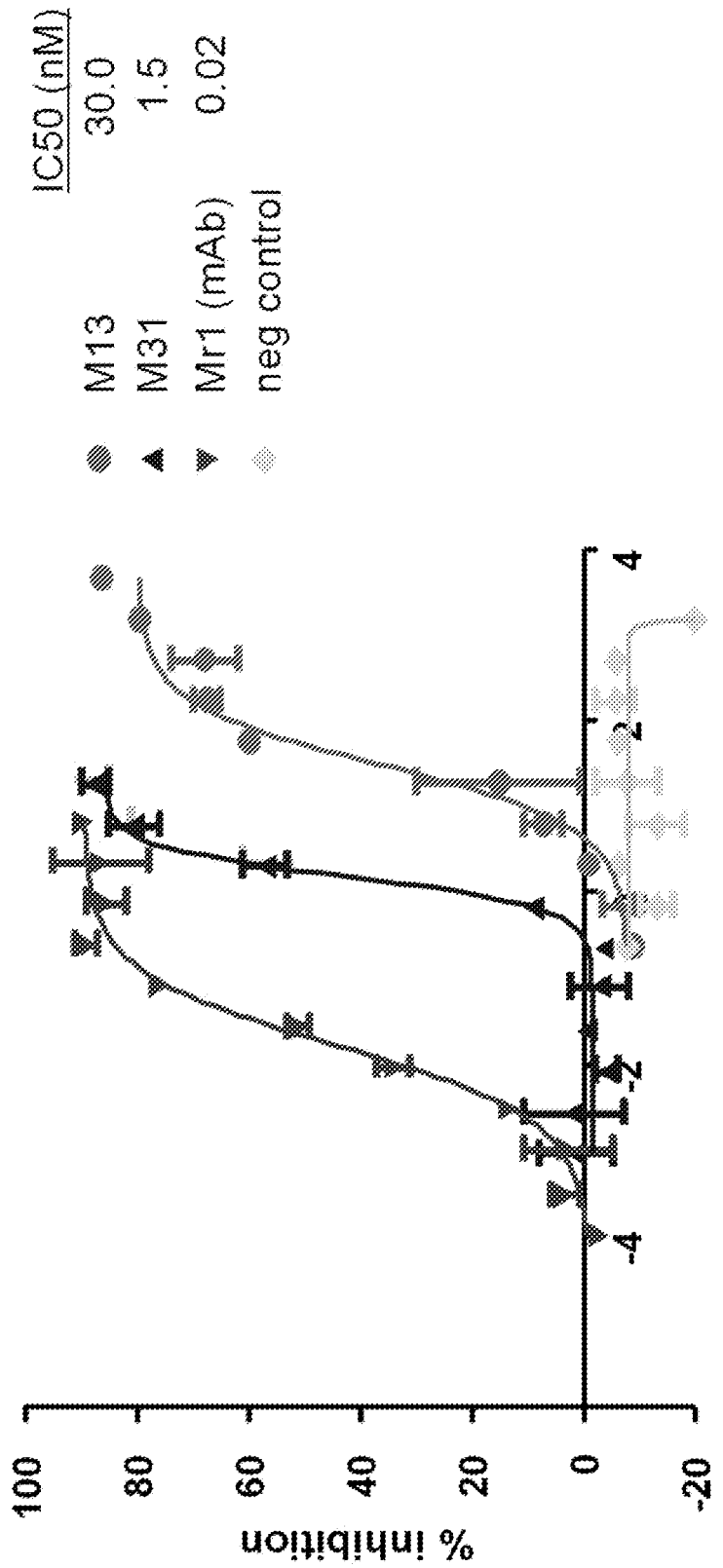

IC50 (nM)
M13 30.0
M31 1.5
Mr1 (mAb) 0.02
neg control
% inhibition
100
80
60
40
20
0
-20
-4
-2
2
4
Log conc. (nM)

Fig. 1A

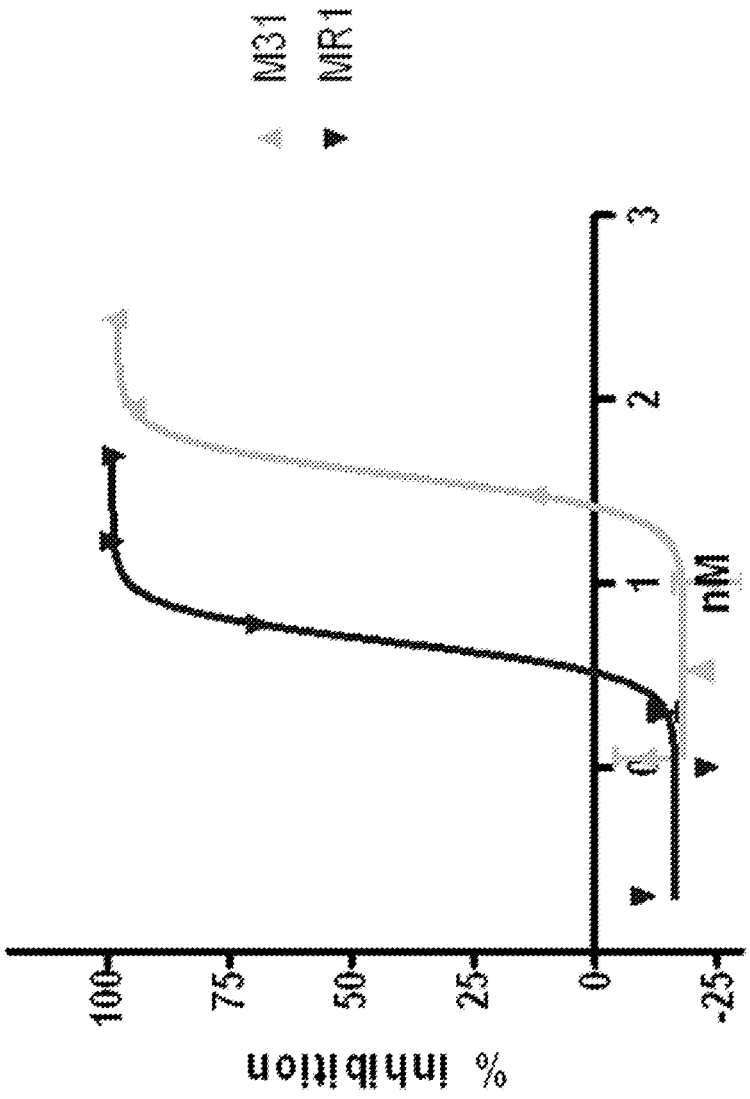
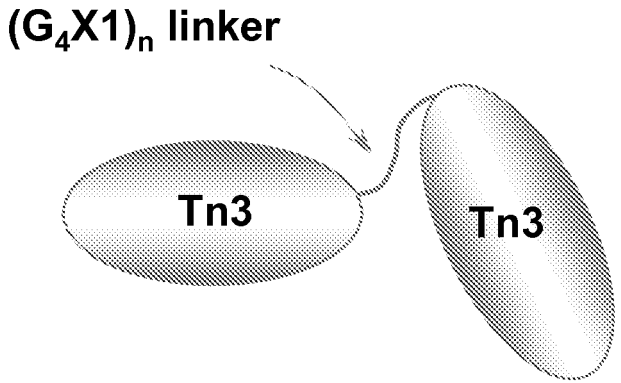
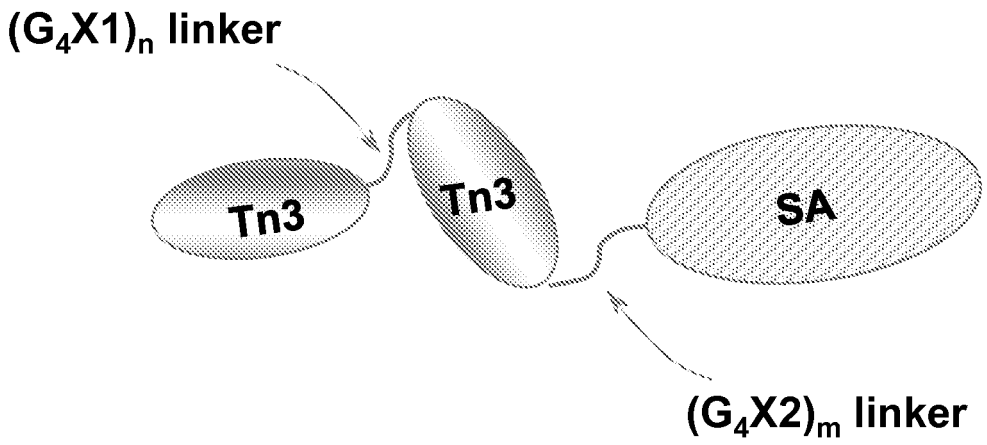



Fig. 1B

**Bivalent CD40L Scaffolds**




**Bivalent CD40L–SerumAlbumin (SA) fusion construct**




X1=S or G or A
X2=S or G or A
n=1-7 (linker may include additional residues)
m=1-7 (linker may include additional residues)

**Fig. 2A**

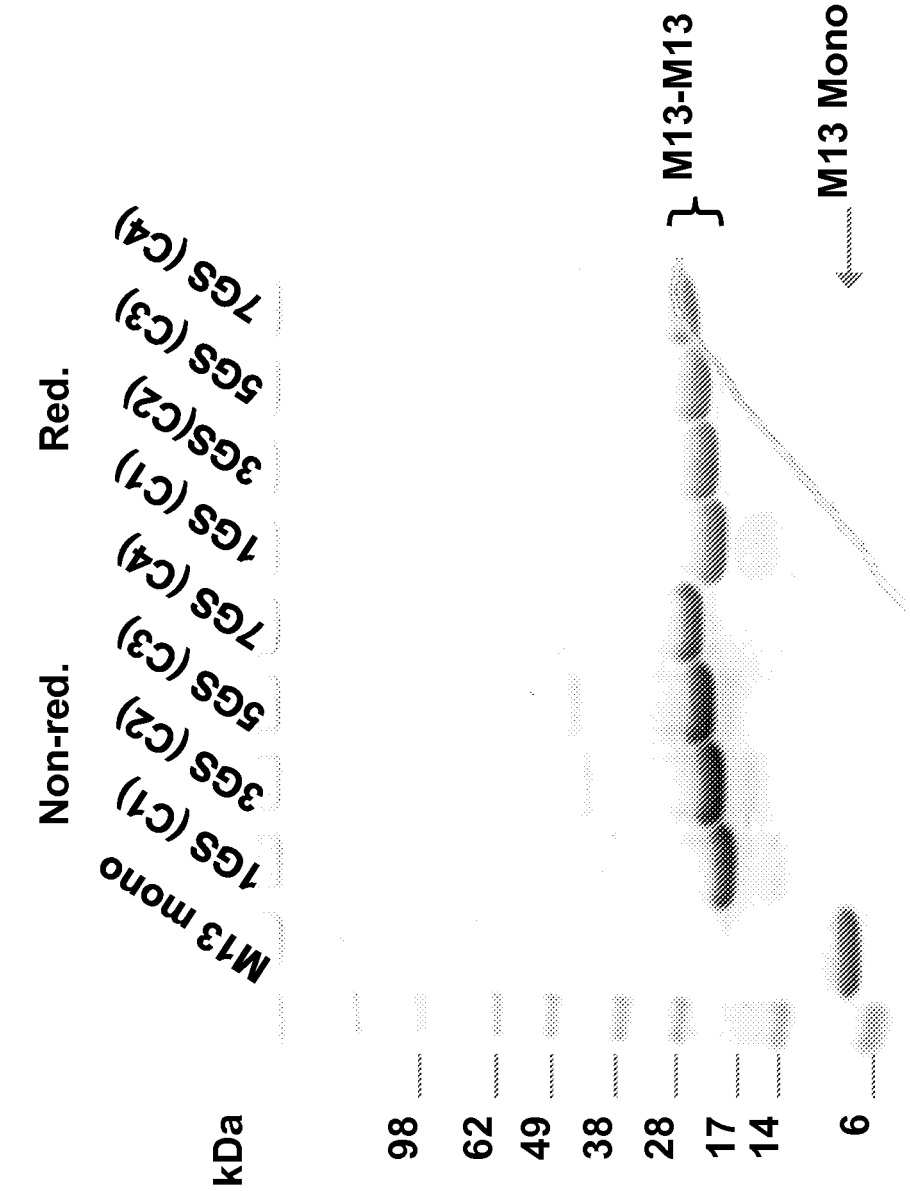

Non-red.
Red.
M13 mono
1GS (C1)
3GS (C2)
5GS (C3)
7GS (C4)
1GS (C1)
3GS(C2)
5GS (C3)
7GS (C4)
kDa
98
62
49
38
28
17
14
6
M13-M13
M13 Mono

Fig. 2B

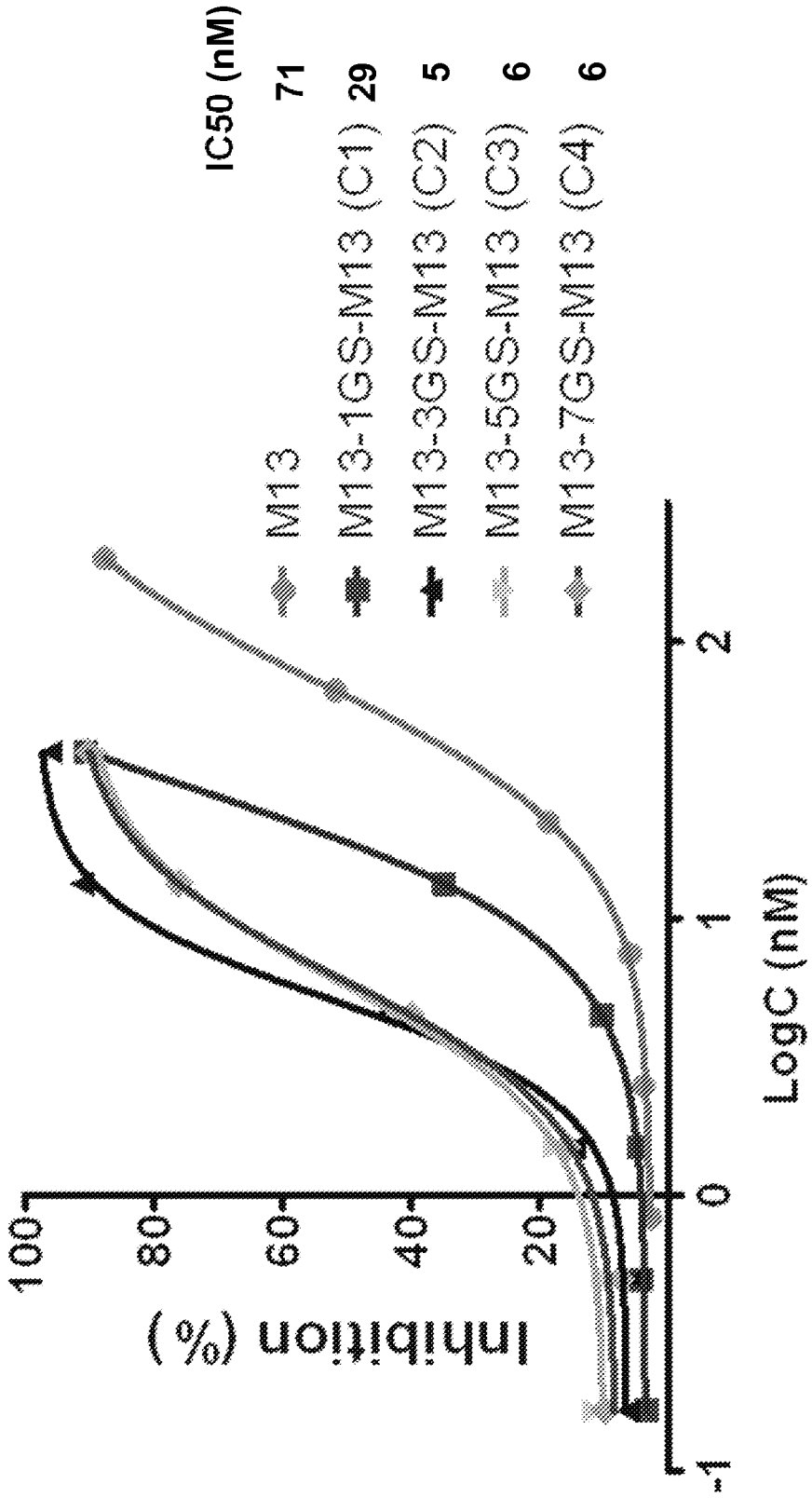

IC50 (nM)
71
M13
29
M13-1GS-M13 (C1)
5
M13-3GS-M13 (C2)
6
M13-5GS-M13 (C3)
6
M13-7GS-M13 (C4)
Inhibition (%)
100
80
60
40
20
0
-1
1
2
LogC (nM)

Fig. 2C

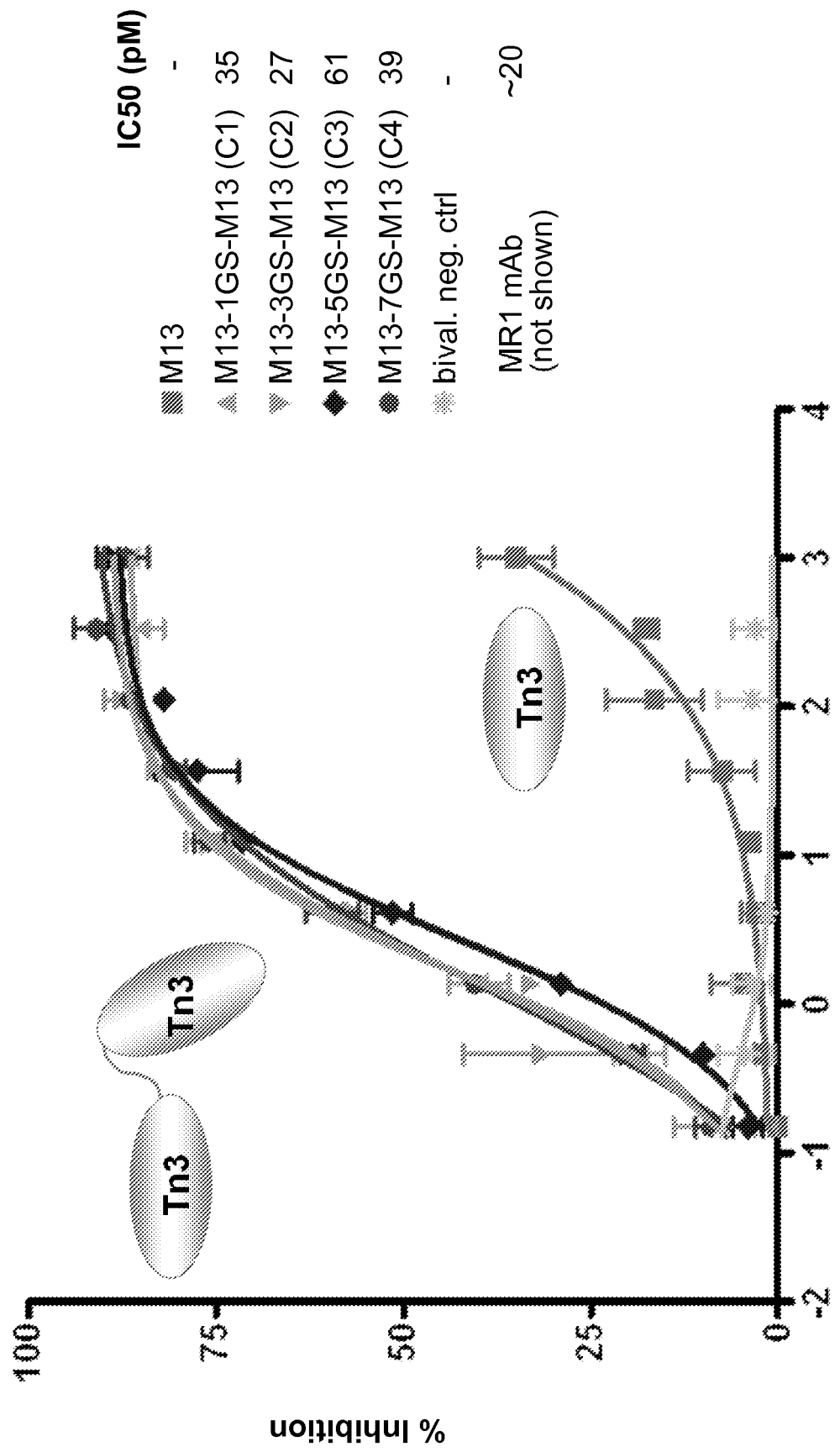

IC50 (pM)
M13 -
M13-1GS-M13 (C1) 35
M13-3GS-M13 (C2) 27
M13-5GS-M13 (C3) 61
M13-7GS-M13 (C4) 39
bival. neg. ctrl -
MR1 mAb (not shown) ~20
Tn3
Tn3
Tn3
% Inhibition
Log Tn3 (ng/mL)
100
75
50
25
0
-2
-1
0
1
2
3
4

Fig. 2D

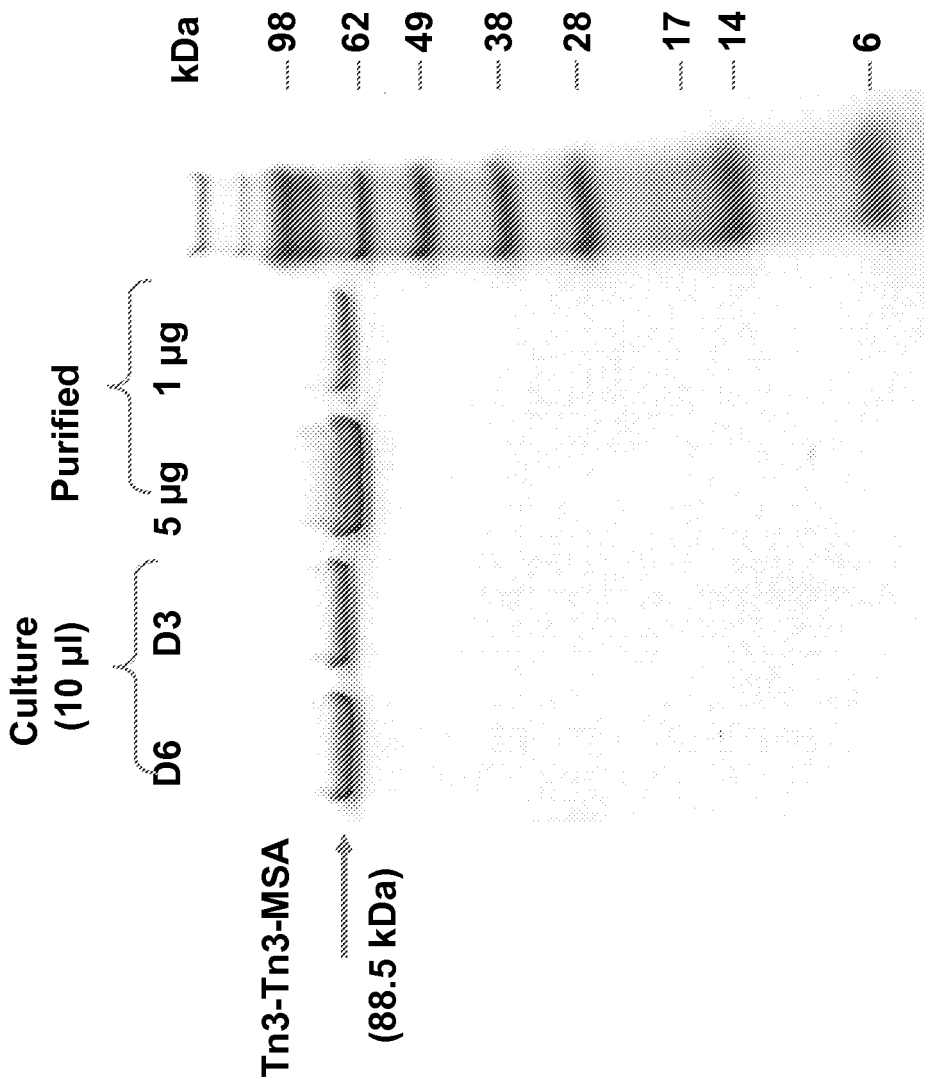

Culture
(10 µl)
D6
D3
Purified
5 µg
1 µg
kDa
98
62
49
38
28
17
14
6
Tn3-Tn3-MSA
(88.5 kDa)

Fig. 3A

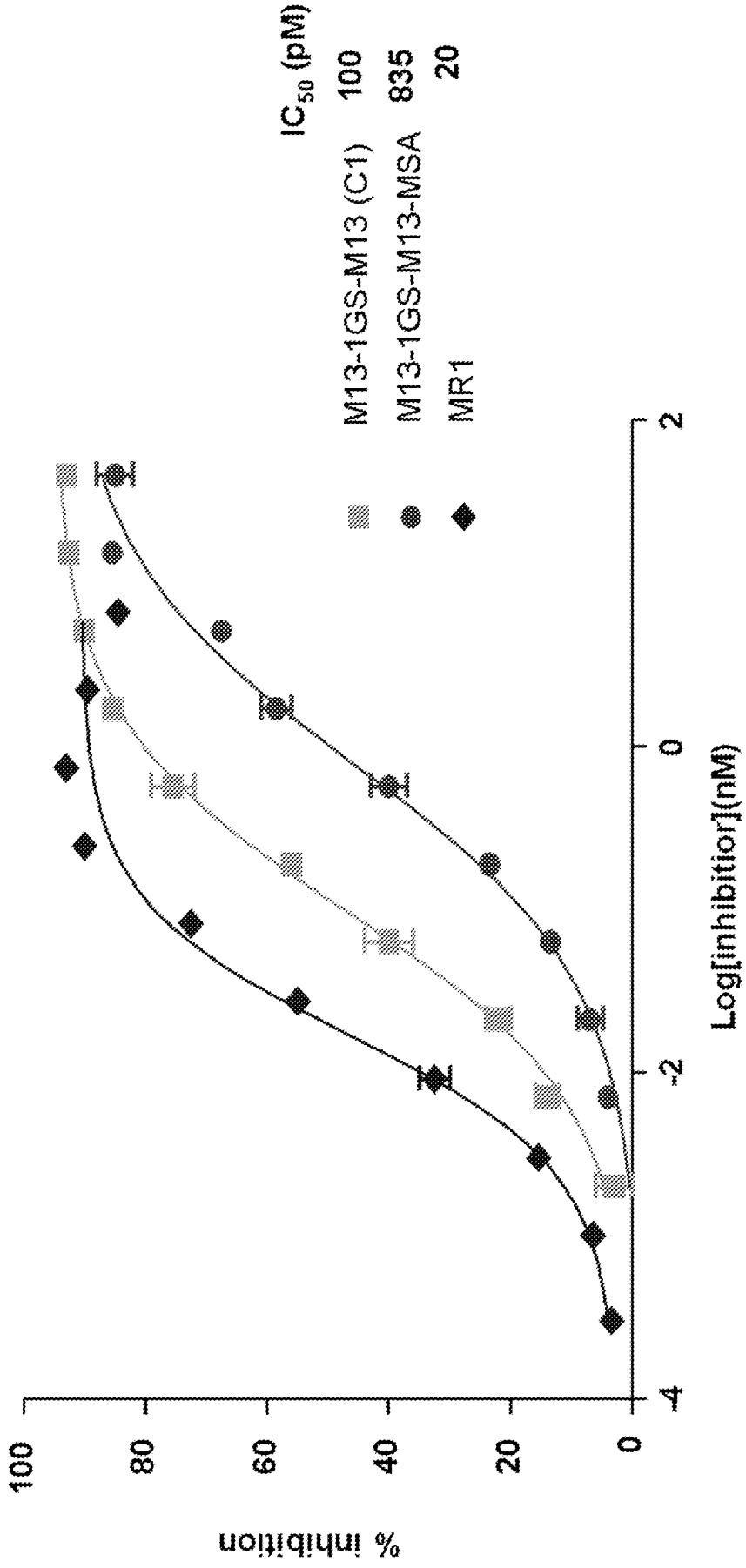

IC50 (pM)
M13-1GS-M13 (C1) 100
M13-1GS-M13-MSA 835
MR1 20
% inhibition
100
80
60
40
20
0
-4
-2
0
2
Log[inhibitior](nM)

Fig. 3B

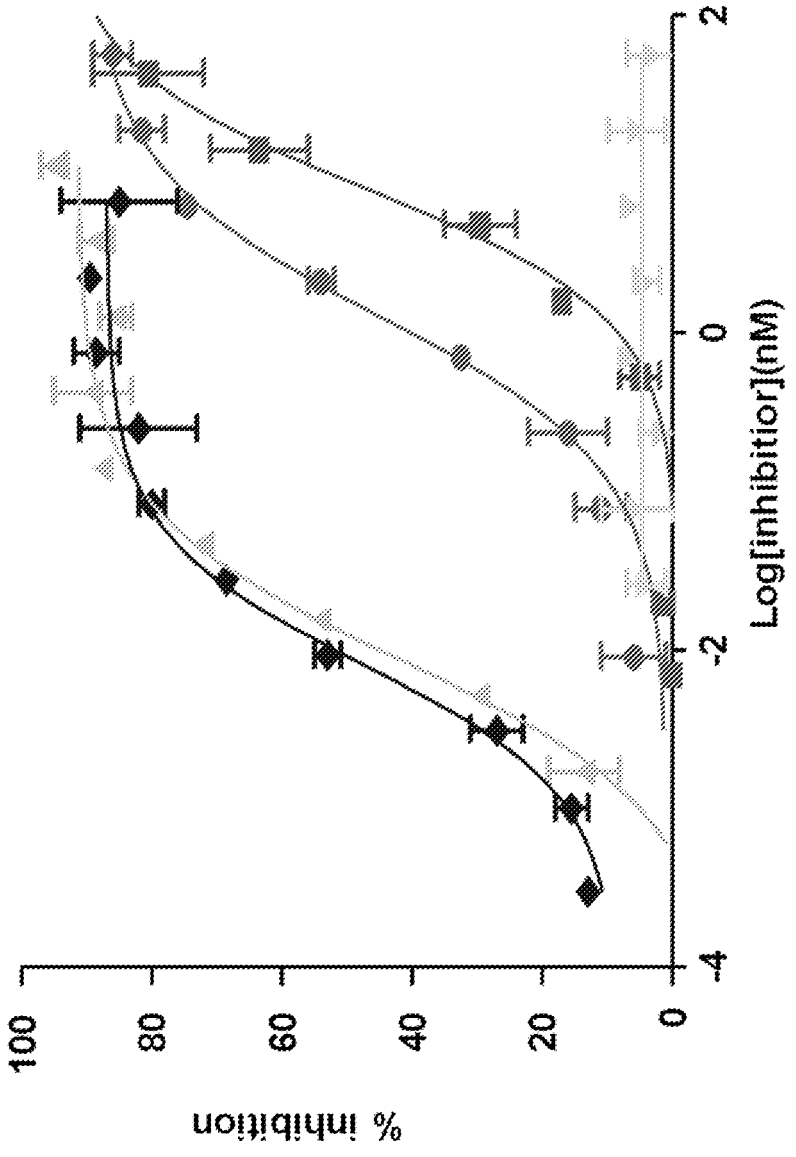

IC50 (pM)
M13-1GS-M13-MSA 1270
M31Mono-MSA 8000
M31-1GS-M31-MSA 9
D1-1GS-D1-MSA -
MR1 8
% inhibition
100
80
60
40
20
0
-4
-2
0
2
Log[inhibitior](nM)

Fig. 3C

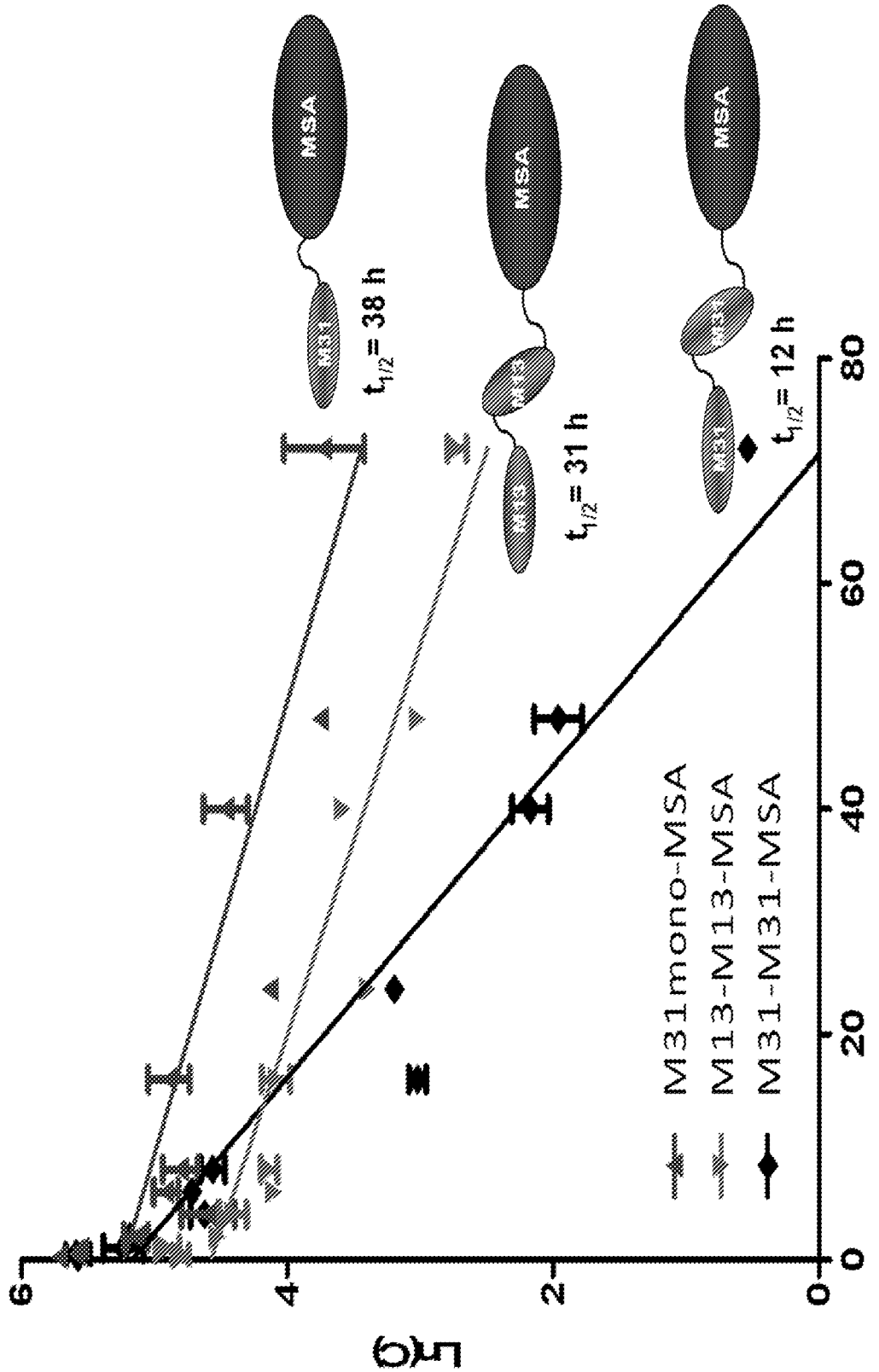

MSA
M31
$t_{1/2}$= 38 h
MSA
M13
M13
$t_{1/2}$= 31 h
MSA
M31
M31
$t_{1/2}$= 12 h
M31mono-MSA
M13-M13-MSA
M31-M31-MSA
Time (h)
0
20
40
60
80
0
2
4
6
Ln(C)

Fig. 4A

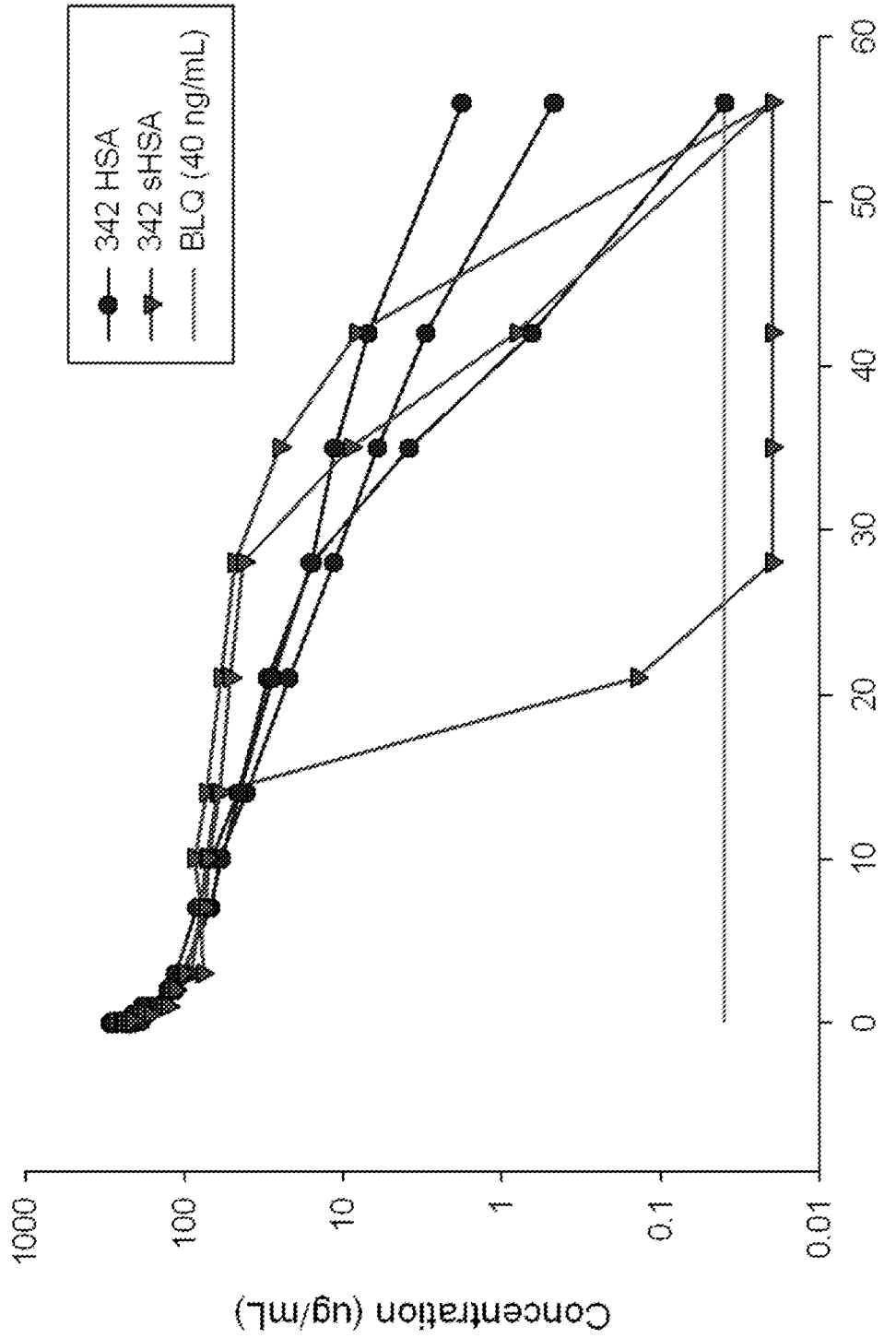

342 HSA
342 sHSA
BLQ (40 ng/mL)
Concentration (ug/mL)
1000
100
10
1
0.1
0.01
0
10
20
30
40
50
60
Time (Days)

Fig. 4B

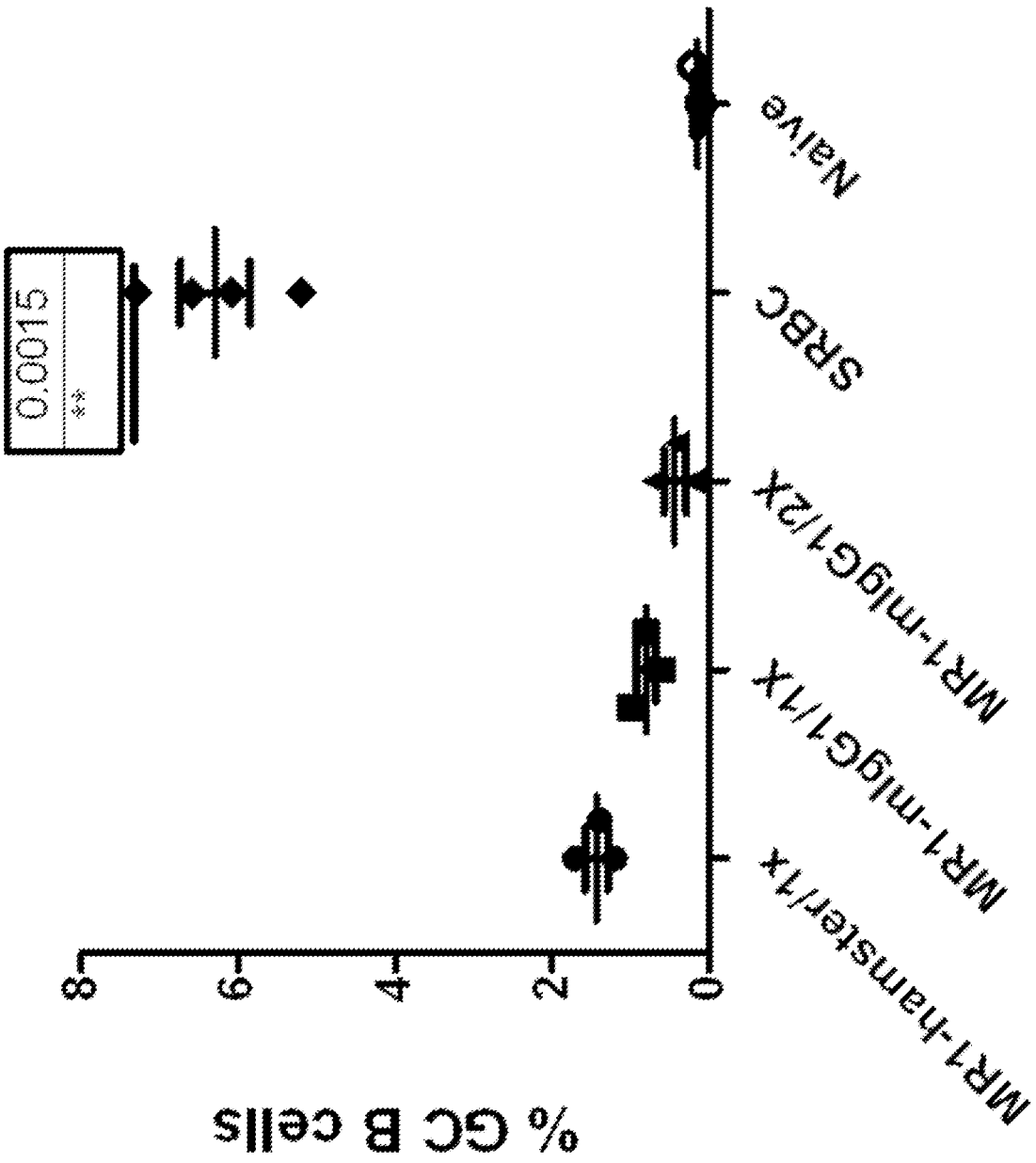

% GC B cells
8
6
4
2
0
MR1-hamster/1x
MR1-mIgG1/1x
MR1-mIgG1/2x
SRBC
Naïve
0.0015
**

Fig. 5A

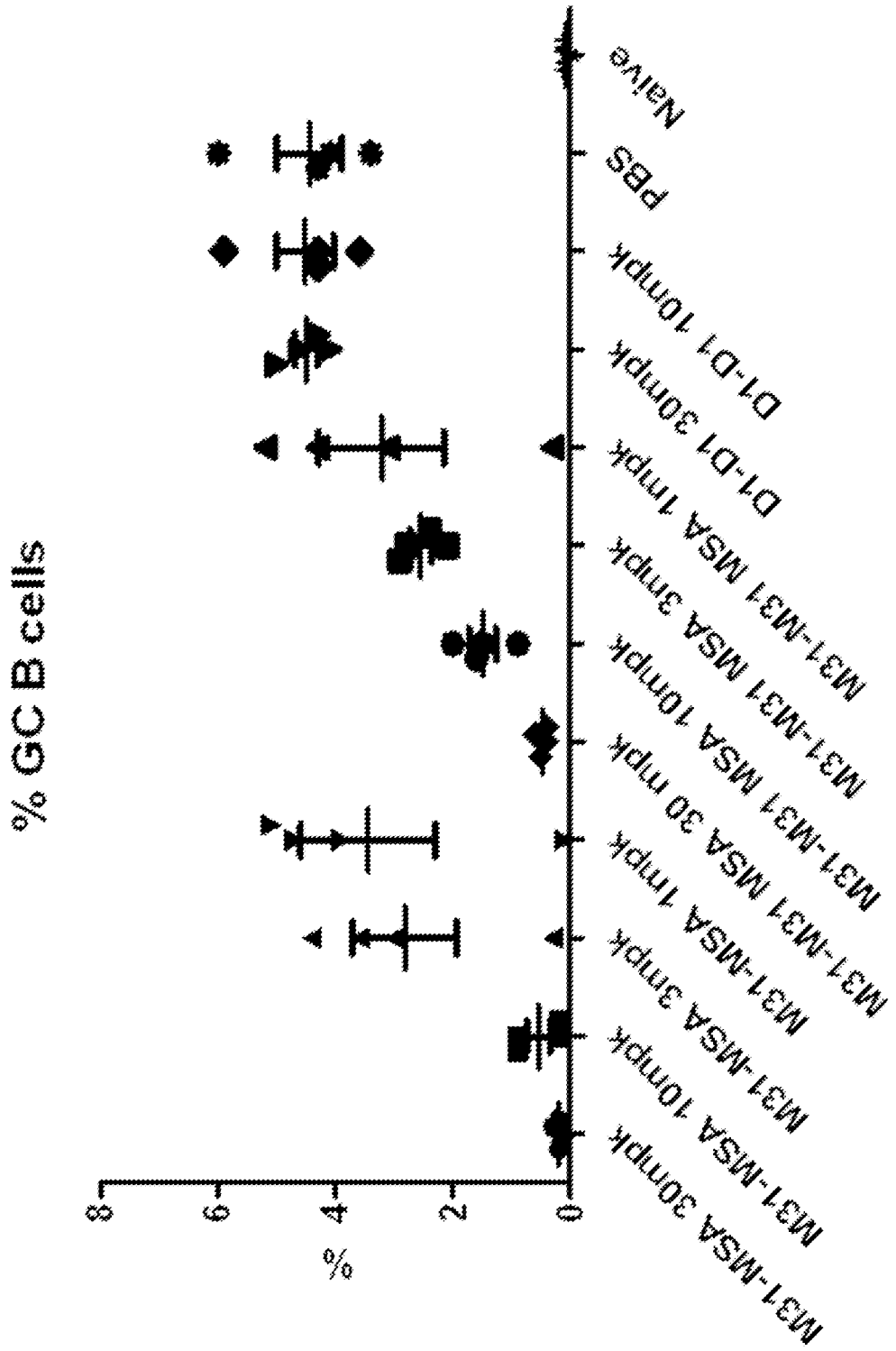

% GC B cells
%
0
2
4
6
8
Naïve
PBS
D1-D1 10mpk
D1-D1 30mpk
M31-M31 MSA 1mpk
M31-M31 MSA 3mpk
M31-M31 MSA 10mpk
M31-M31 MSA 30 mpk
M31-MSA 1mpk
M31-MSA 3mpk
M31-MSA 10mpk
M31-MSA 30mpk

Fig. 5B

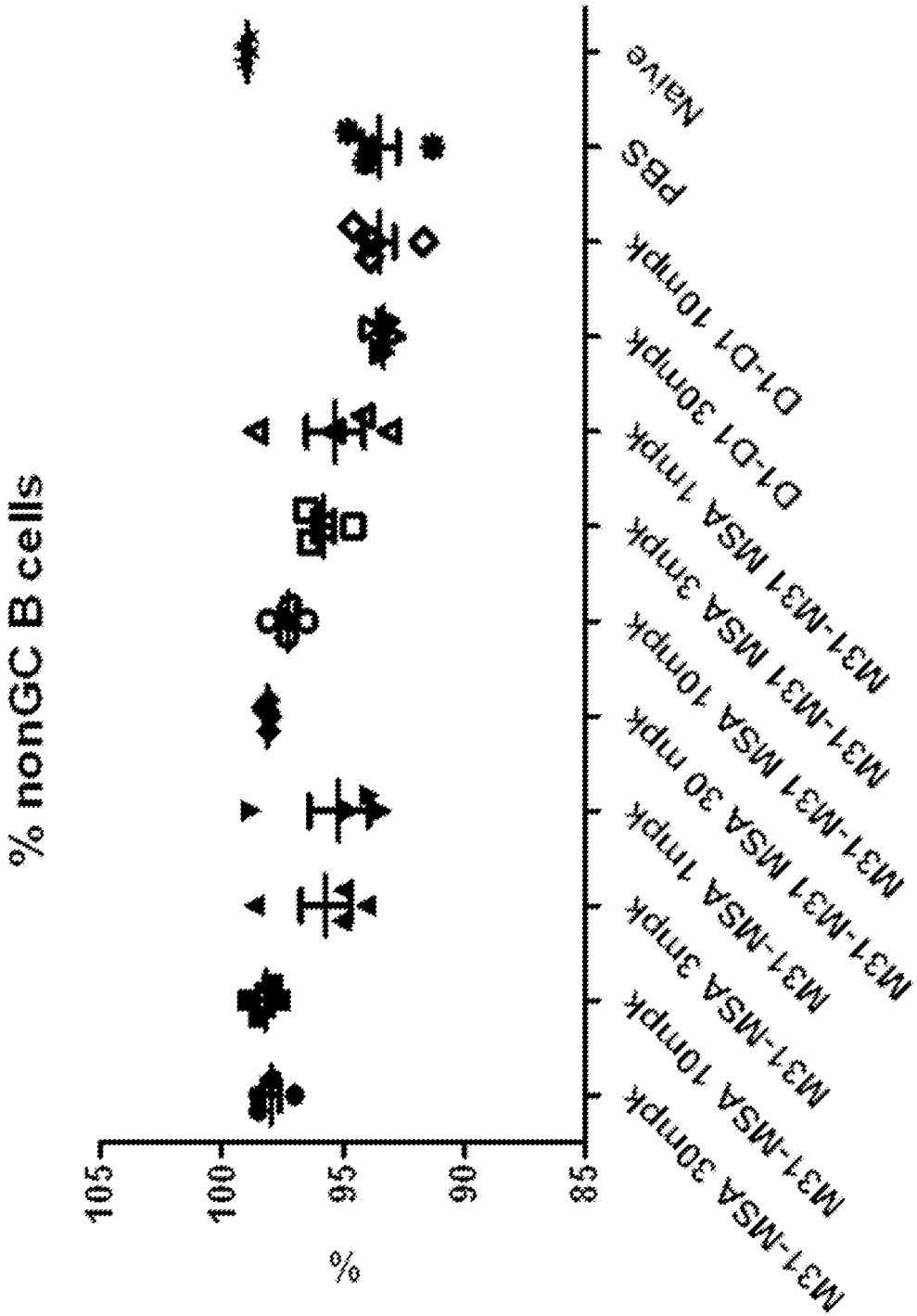

% nonGC B cells
105
100
95
90
85
%
Naive
PBS
D1-D1 10mpk
D1-D1 30mpk
M31-M31 MSA 1mpk
M31-M31 MSA 3mpk
M31-M31 MSA 10mpk
M31-M31 MSA 30 mpk
M31-MSA 1mpk
M31-MSA 3mpk
M31-MSA 10mpk
M31-MSA 30mpk

Fig. 5C

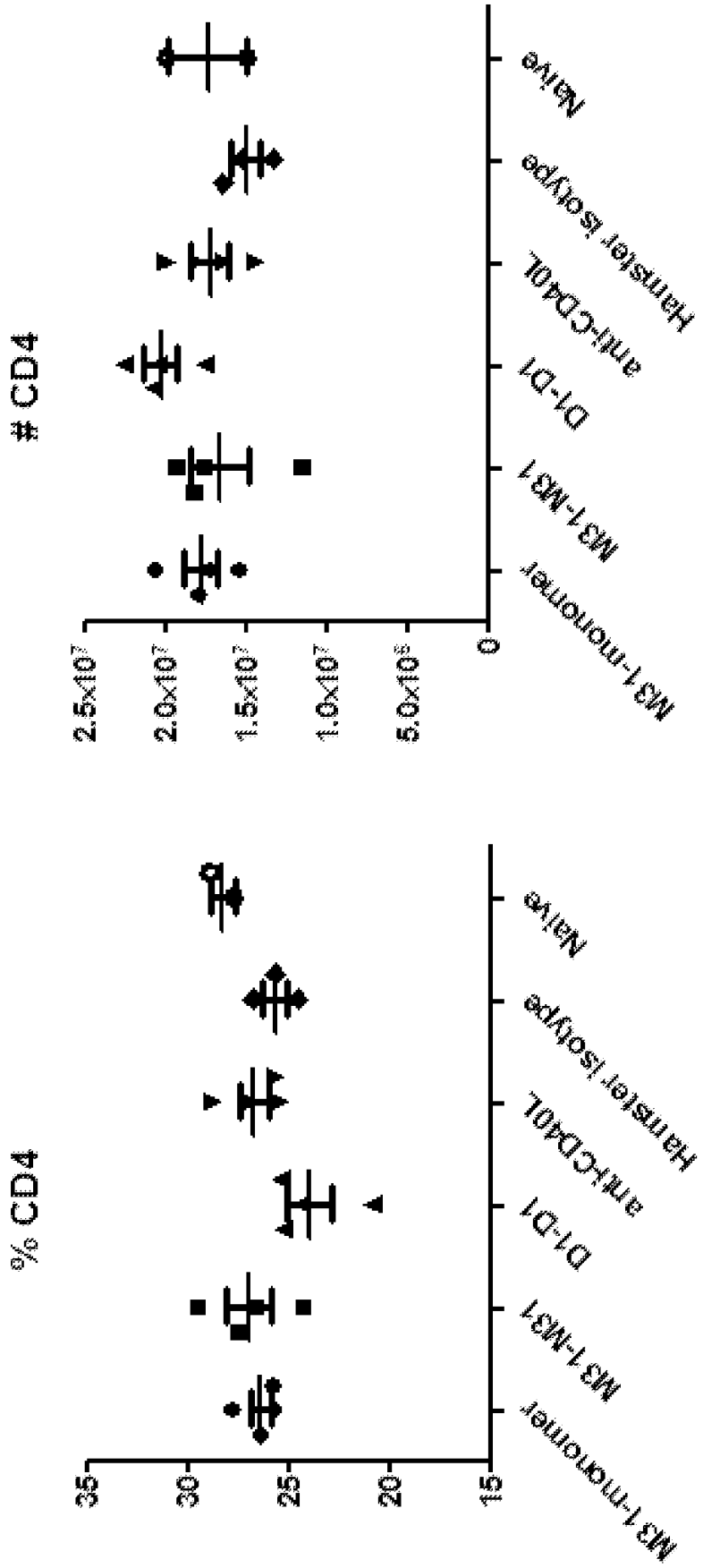

# CD4
2.5x10^7
2.0x10^7
1.5x10^7
1.0x10^7
5.0x10^6
0
M3.1-monomer
M3.1-M3.1
D1-D1
anti-CD40L
Hamster isotype
Naïve
% CD4
35
30
25
20
15
M3.1-monomer
M3.1-M3.1
D1-D1
anti-CD40L
Hamster isotype
Naïve

Fig. 5D

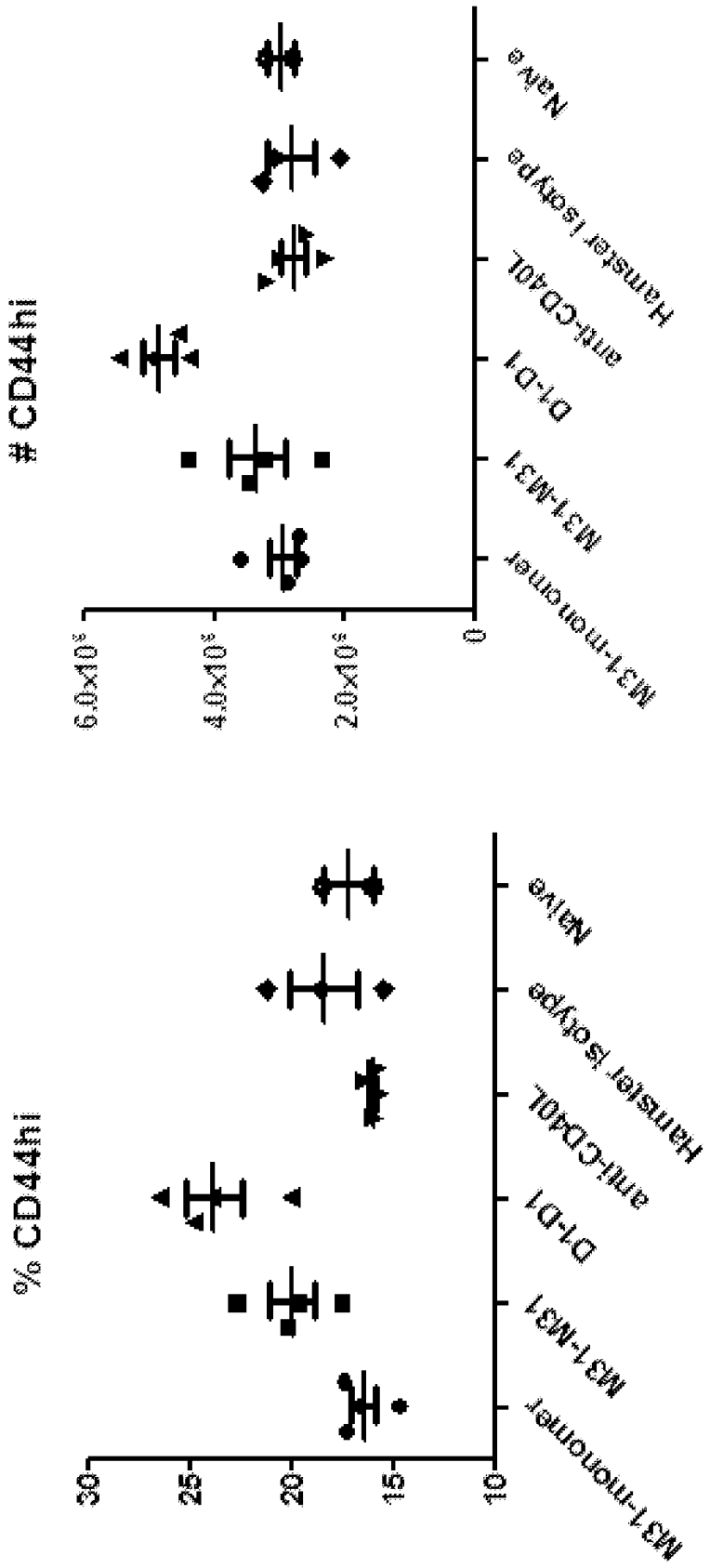

% CD44hi
30
25
20
15
10
M3.1-mononmer
M3.1-M3.1
D1-D1
anti-CD40L
Hamster Isotype
Naïve
# CD44hi
6.0x10^5
4.0x10^5
2.0x10^5
0
M3.1-mononmer
M3.1-M3.1
D1-D1
anti-CD40L
Hamster Isotype
Naïve

Fig. 5E

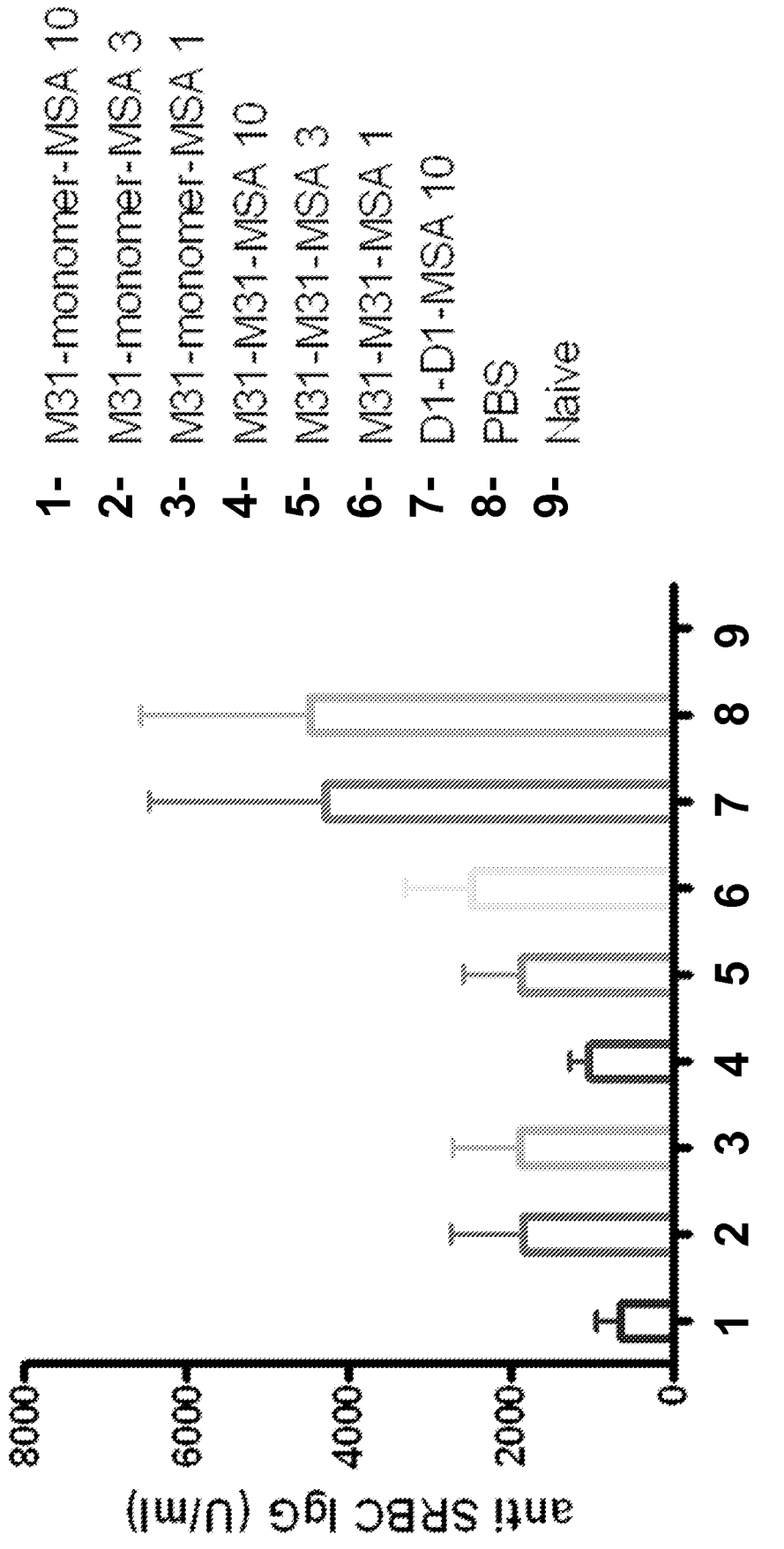

1- M31-monomer-MSA 10
2- M31-monomer-MSA 3
3- M31-monomer-MSA 1
4- M31-M31-MSA 10
5- M31-M31-MSA 3
6- M31-M31-MSA 1
7- D1-D1-MSA 10
8- PBS
9- Naive
anti SRBC IgG (U/ml)
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
9

Fig. 5F

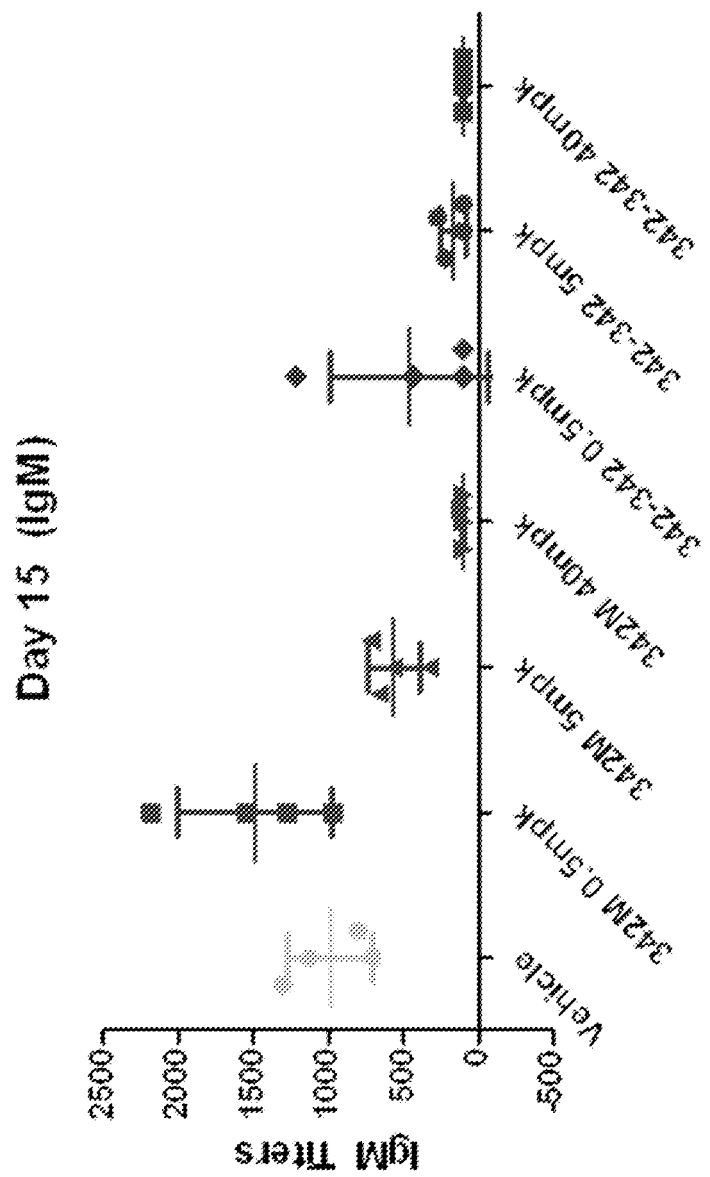

Day 15 (IgM)
IgM Titers
2500
2000
1500
1000
500
0
500
Vehicle
342M 0.5mpk
342M 5mpk
342M 40mpk
342-342 0.5mpk
342-342 5mpk
342-342 40mpk

Fig. 5G

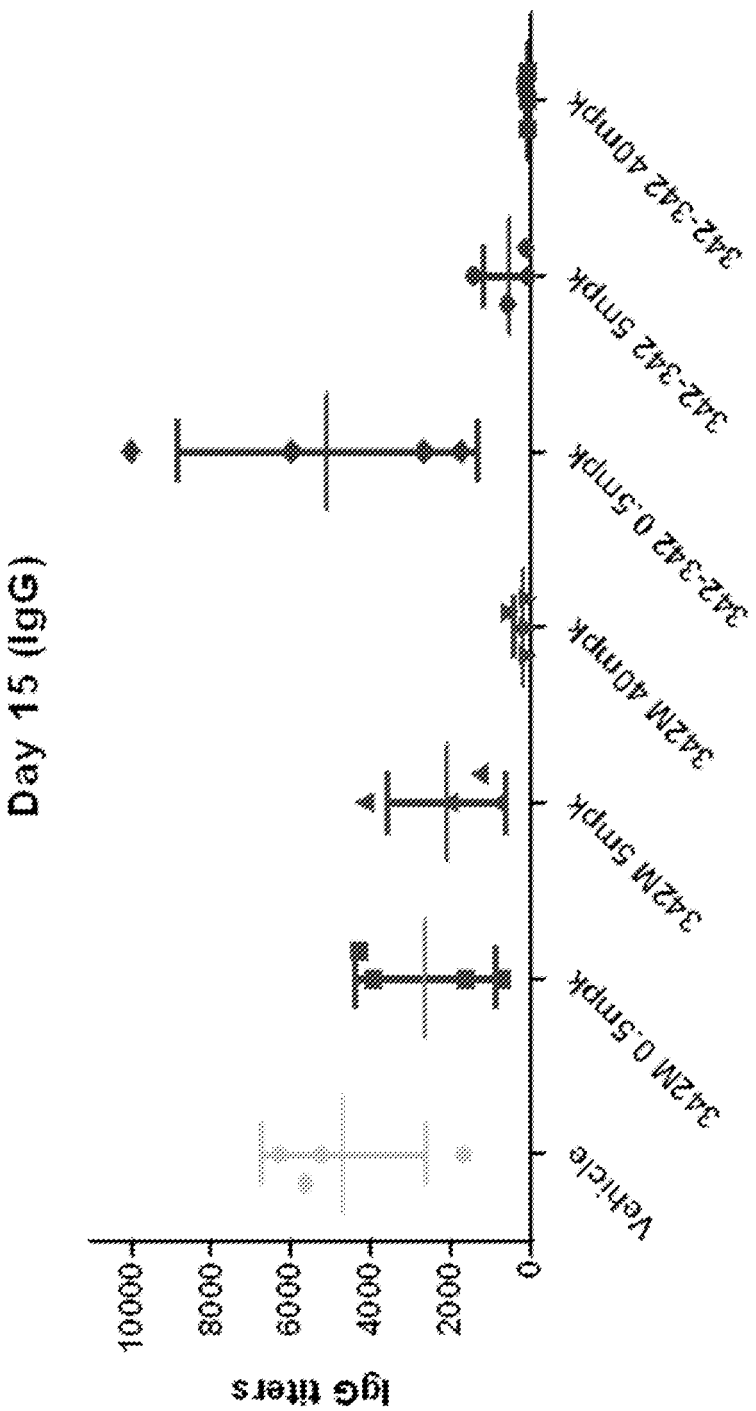

Day 15 (IgG)
IgG titers
10000
8000
6000
4000
2000
0
Vehicle
342M 0.5mpk
342M 5mpk
342M 40mpk
342-342 0.5mpk
342-342 5mpk
342-342 40mpk

Fig. 5H

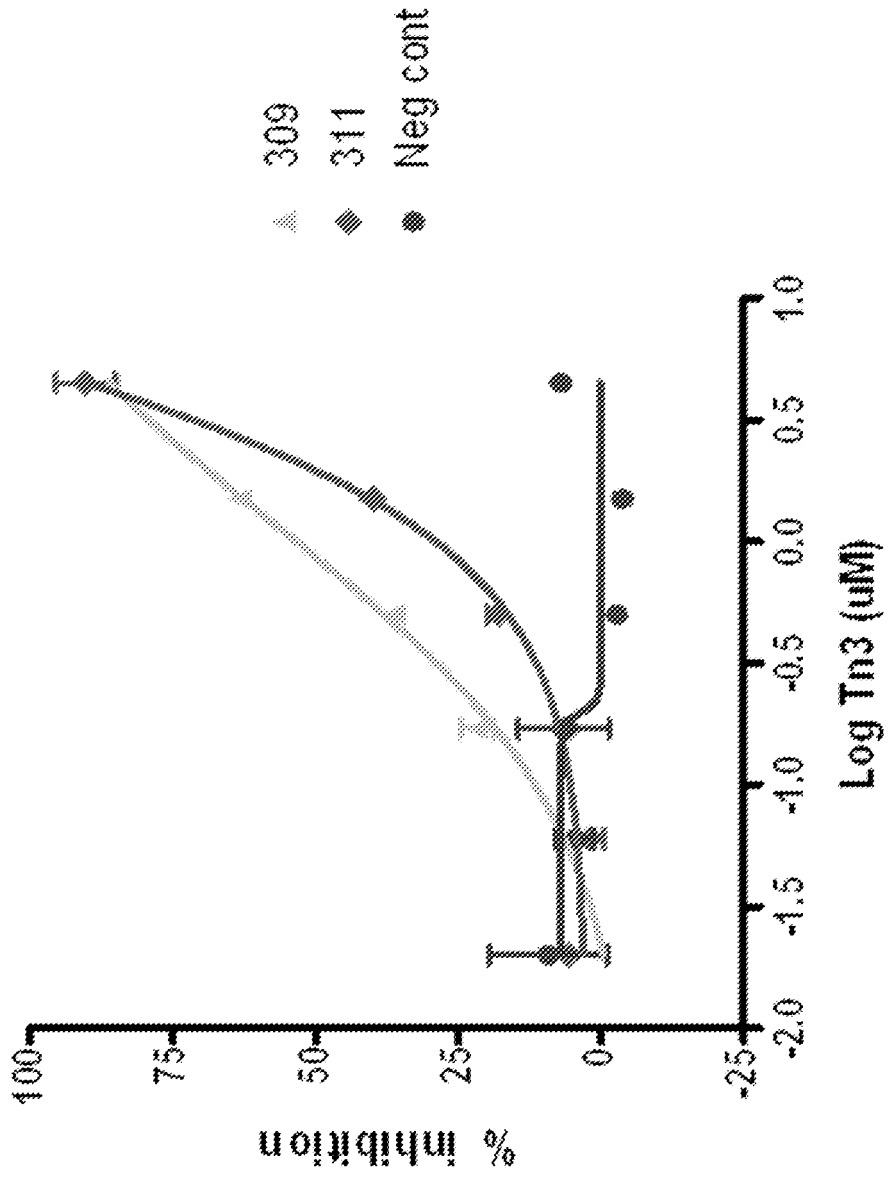

309
311
Neg cont
% inhibition
100
75
50
25
0
-25
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
Log Tn3 (uM)

Fig. 6A

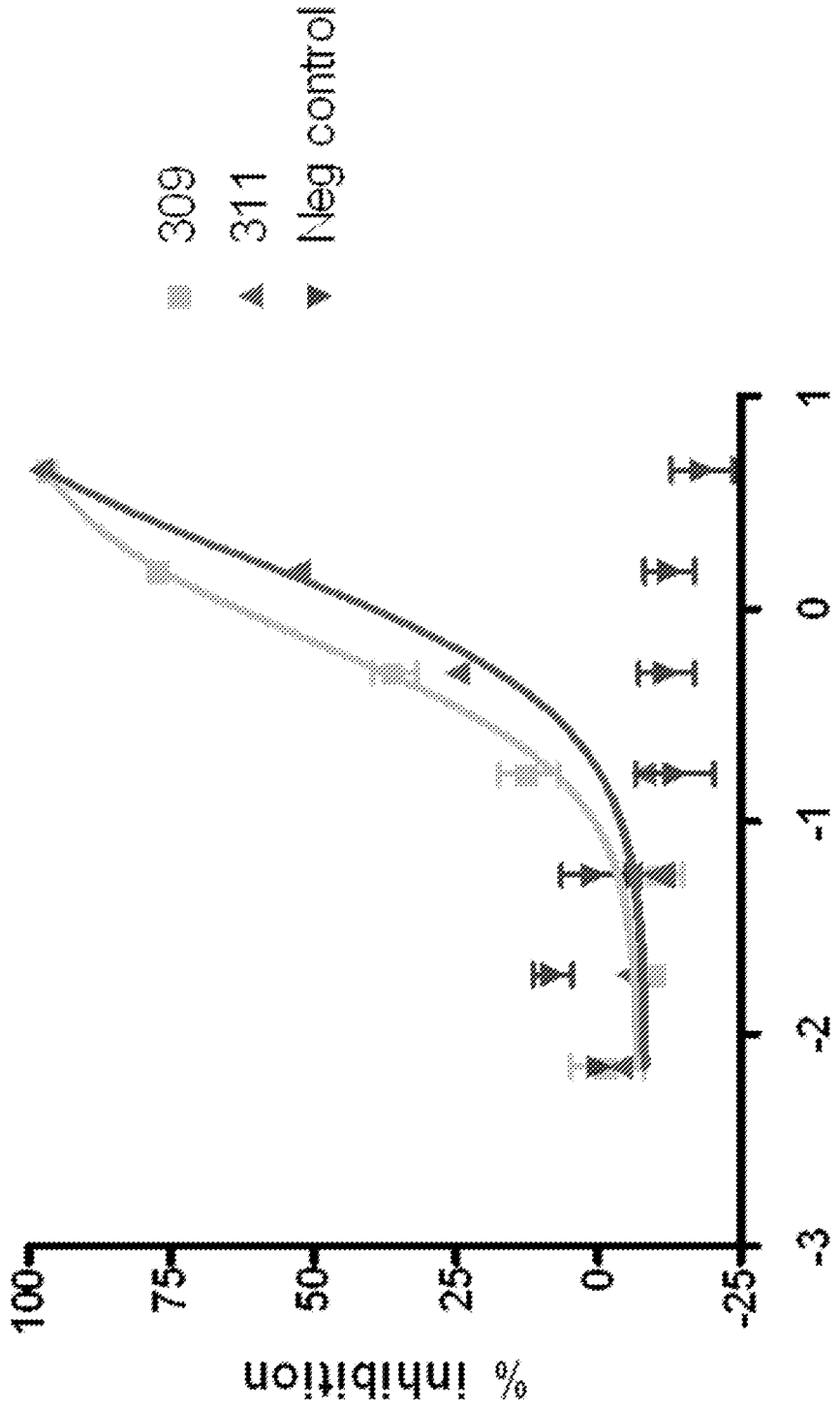
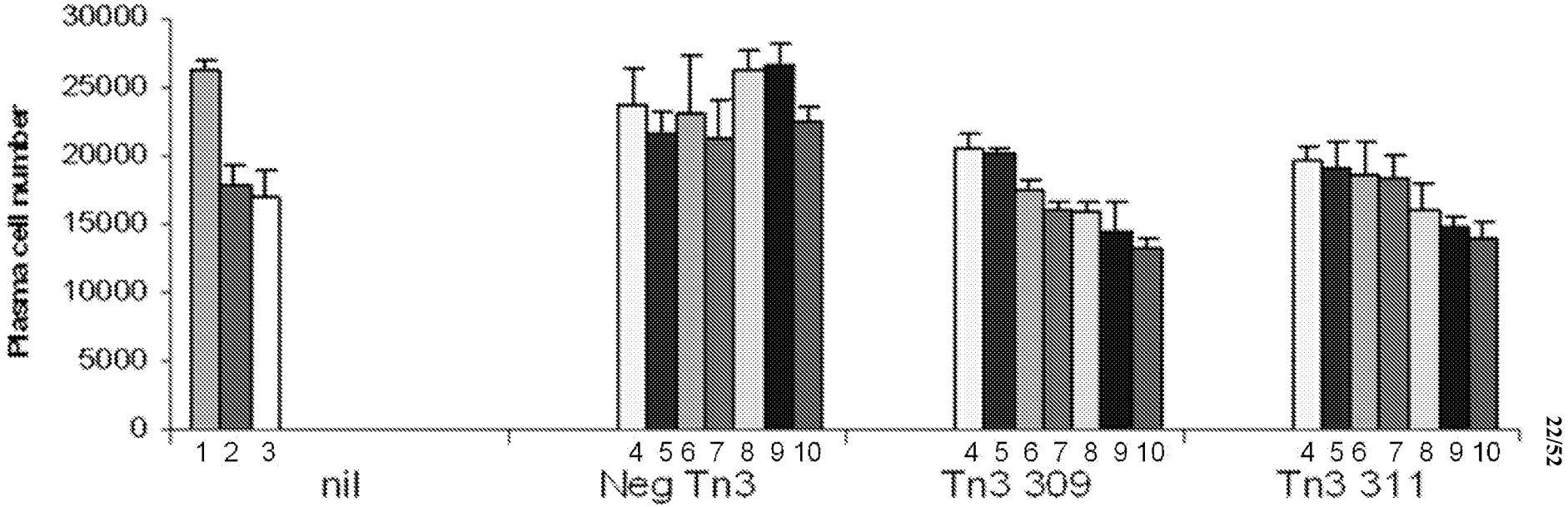

309
311
Neg control
% inhibition
100
75
50
25
0
-25
-3
-2
-1
0
1
Log Tn3 (uM)

Fig. 6B






1 – nil

2 – aCD40L (RE)

3 – aCD40L (Bio)

4 – .008 uM

5 – .025 uM

6 – 0.074 uM

7 – .22 uM

8 – .67 uM

9 – 2 uM

10 – 6 uM

**Fig. 6C**

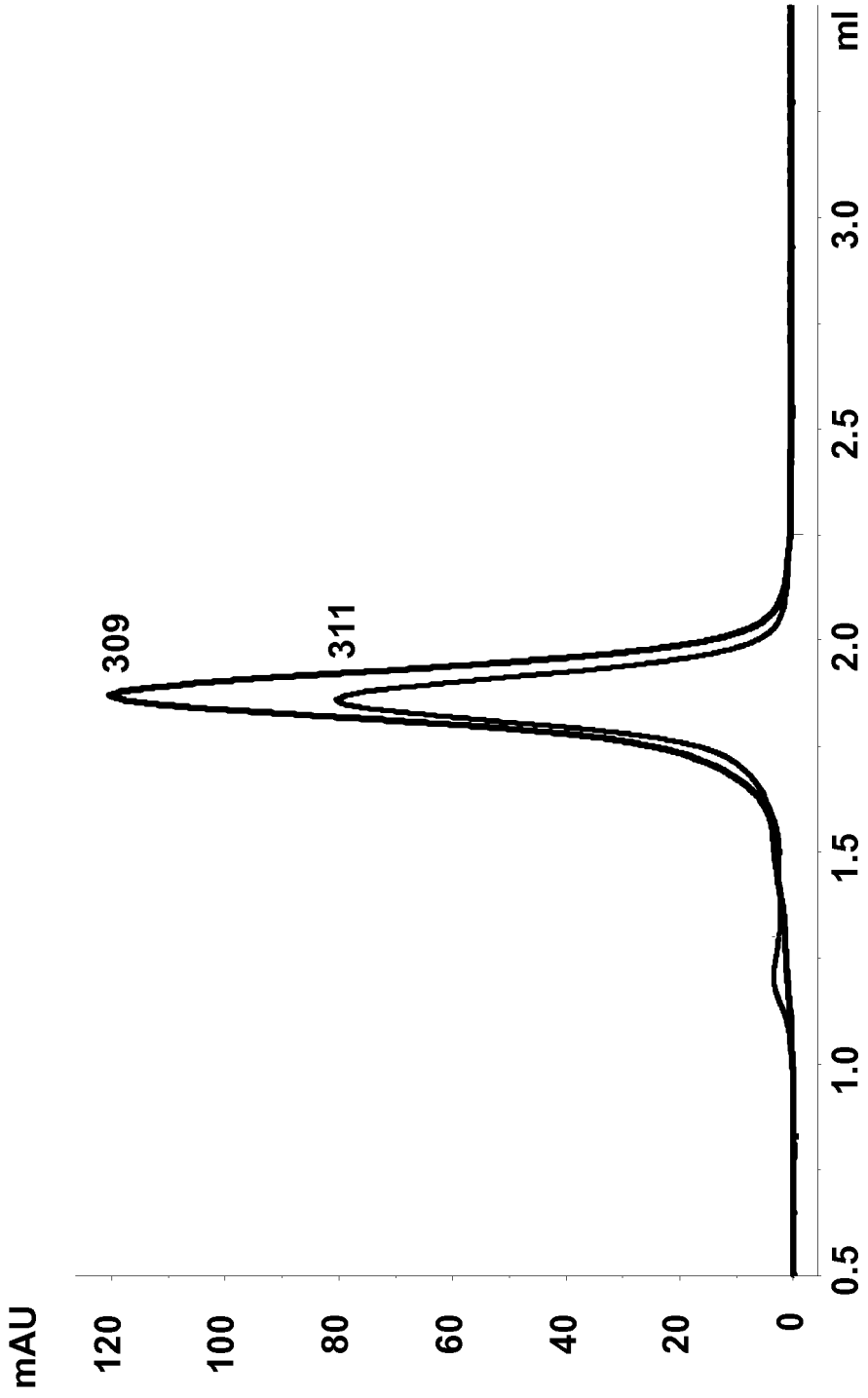



Fig. 7A

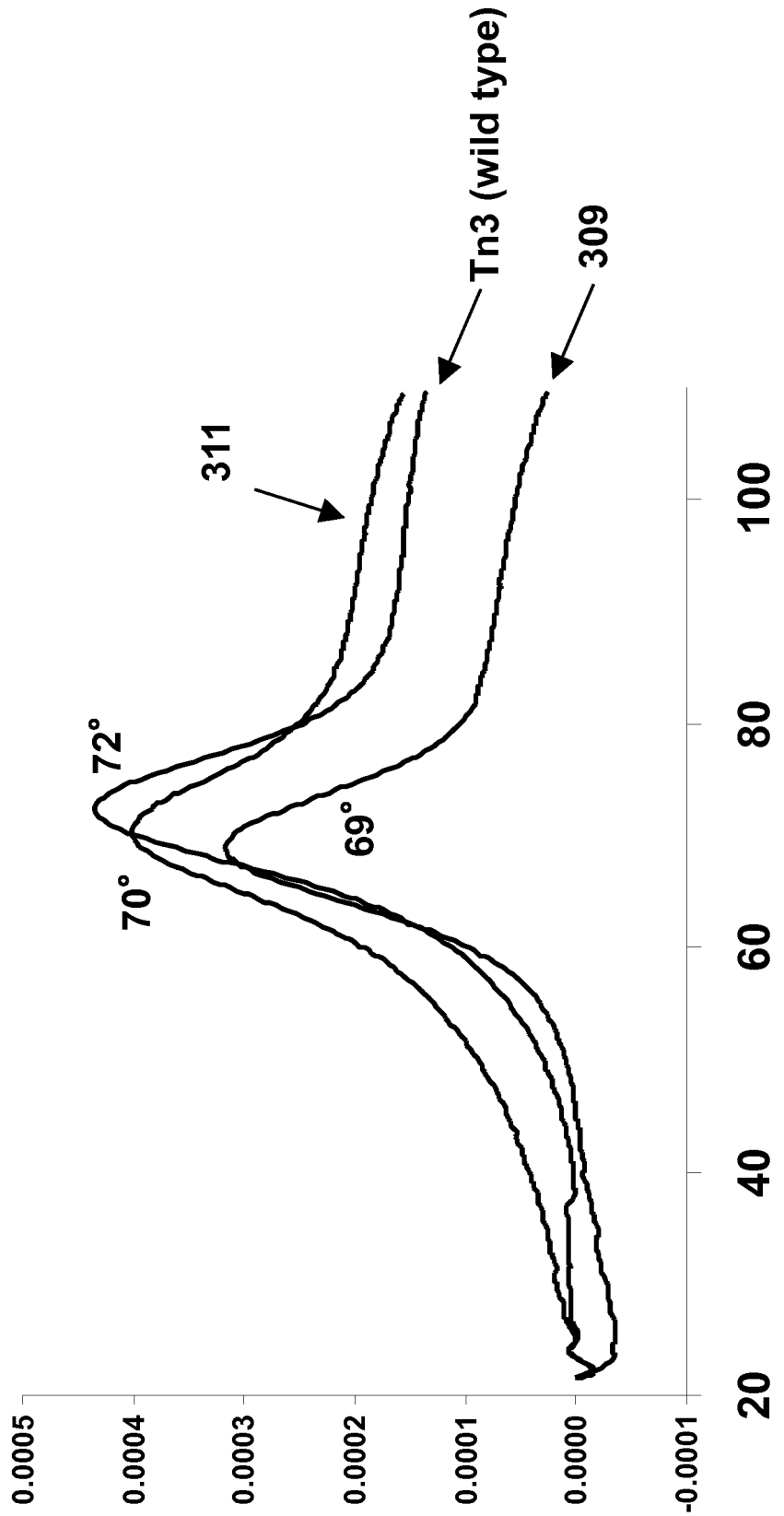

Tn3 (wild type)
309
311
72°
70°
69°
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
-0.0001
20
40
60
80
100

Fig. 7B

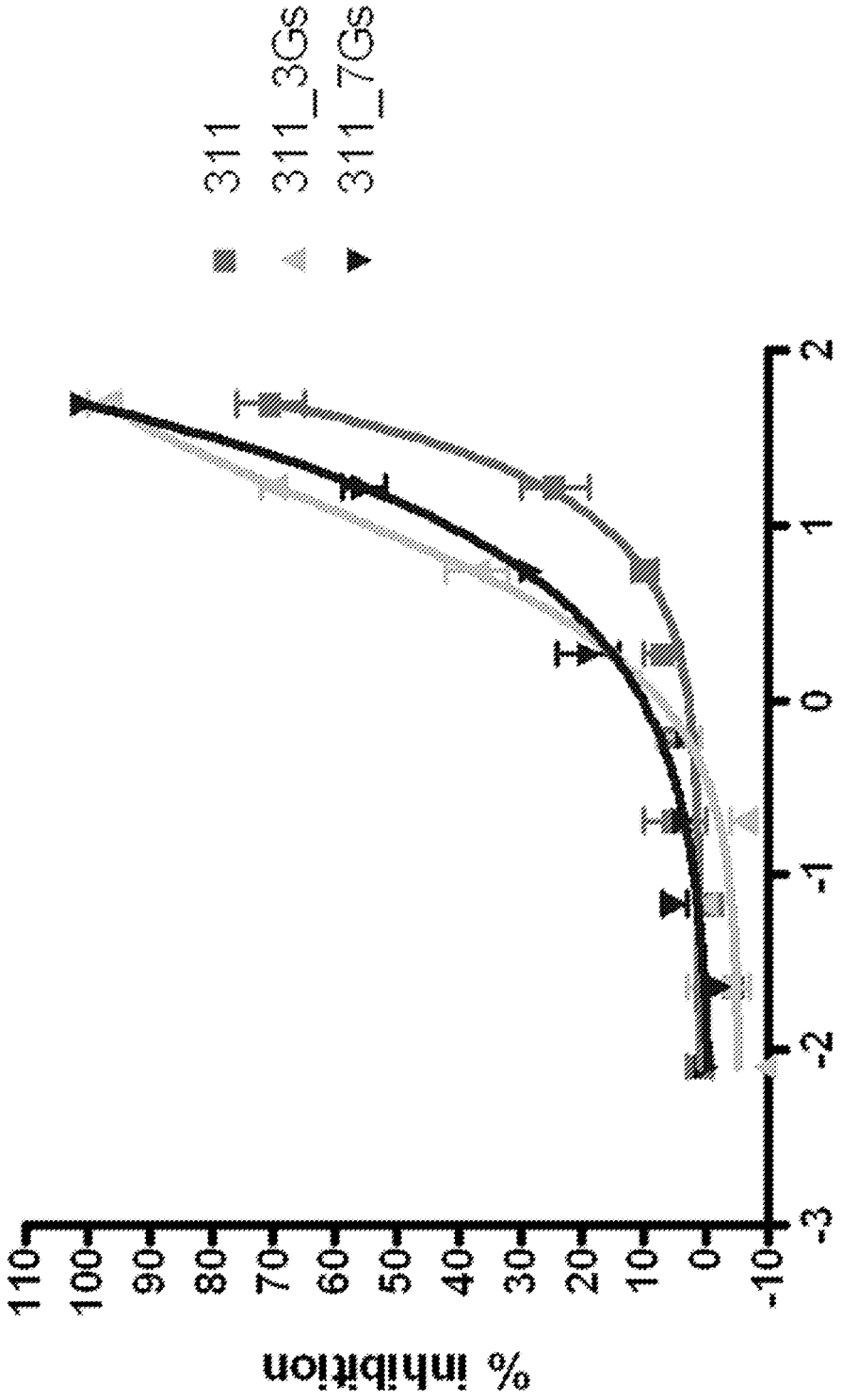

311
311_3Gs
311_7Gs
% inhibition
110
100
90
80
70
60
50
40
30
20
10
0
-10
-3
-2
-1
0
1
2
Log[Tn3](ug/ml)

Fig. 8A

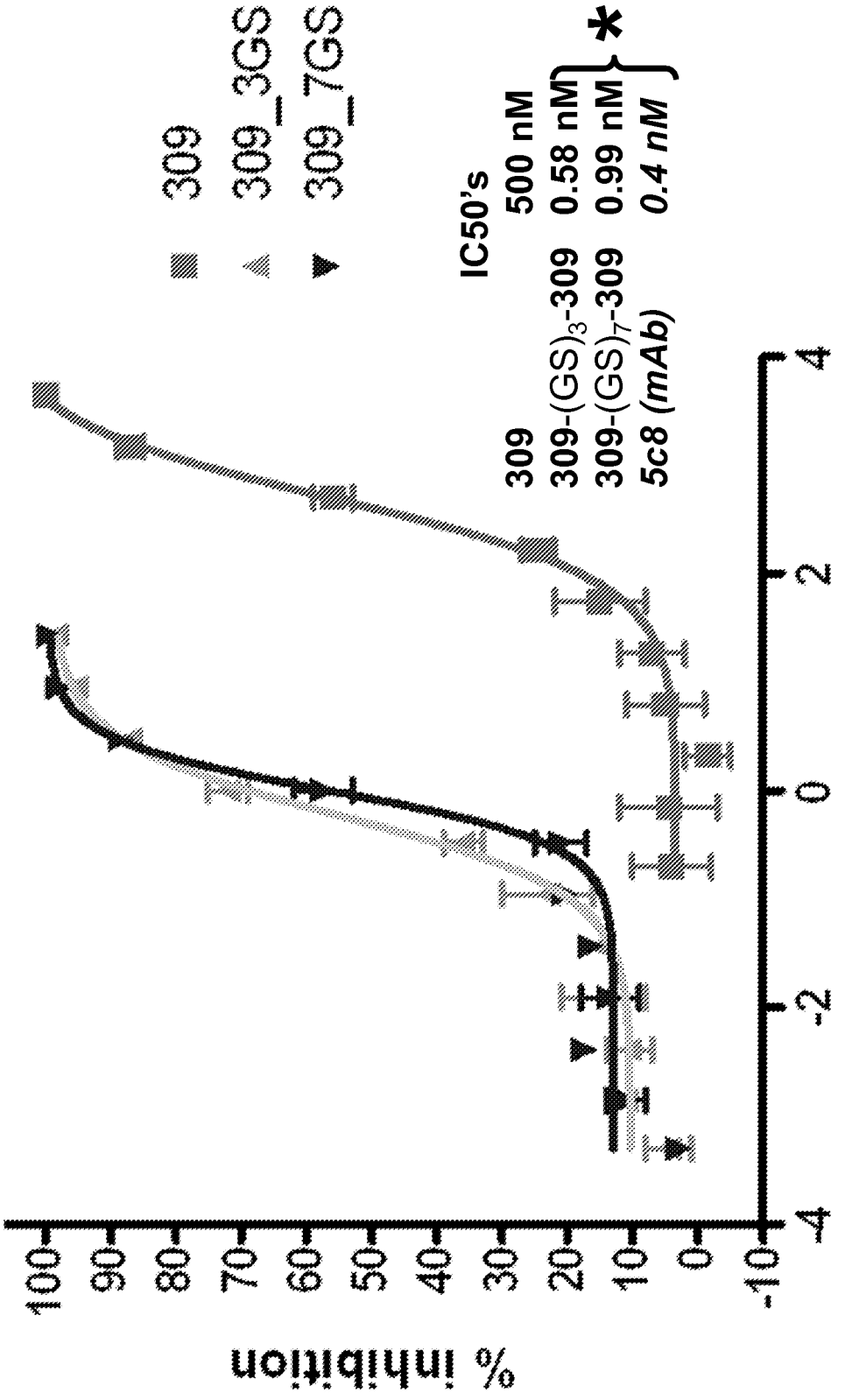

% inhibition
100
90
80
70
60
50
40
30
20
10
0
-10
-4
-2
0
2
4
Log[Tn3](nM)
309
309_3GS
309_7GS
IC50's
309 500 nM
309-(GS)3-309 0.58 nM
309-(GS)7-309 0.99 nM
5c8 (mAb) 0.4 nM
*

Fig. 8B

SQIEVKDVTDTTALITW-**BCloop-C**ELTYGIKDVPGDRTTIDL-**DEloop-**

YSIGNLKPDTEYEVSLI**C**R**X2**GDMSSNPAKETFTT**(GGGGX1)$_N$RLDAP**

SQIEVKDVTDTTALITW-**BCloop-C**ELTYGIKDVPGDRTTIDL-**DEloop-**

YSIGNLKPDTEYEVSLI**C**R**X2**GDMSSNPAKETFTT**(GGGGX1)$_M$** 




X1=S or G or A
X2=R or S or A
N=1-7
M=1-7

**Fig. 9A**

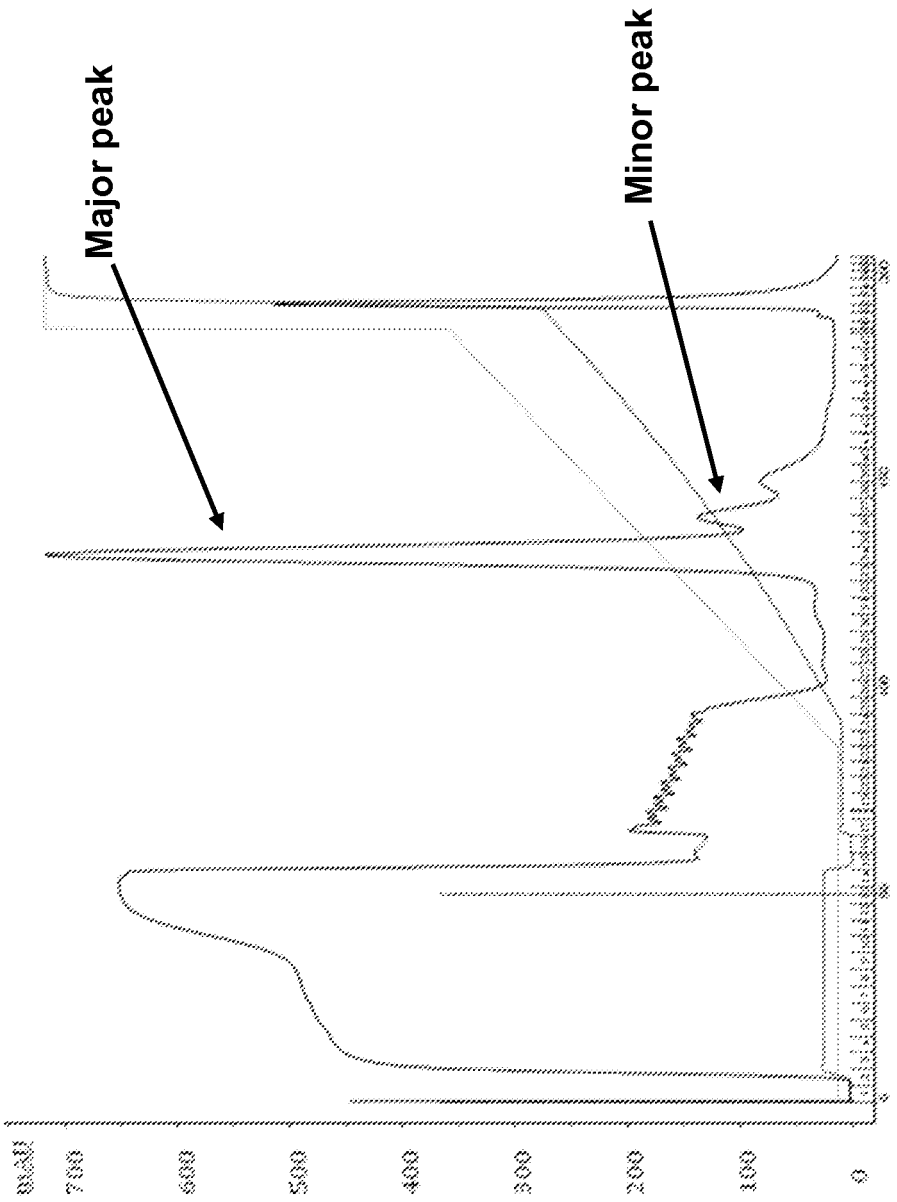



Fig. 9B

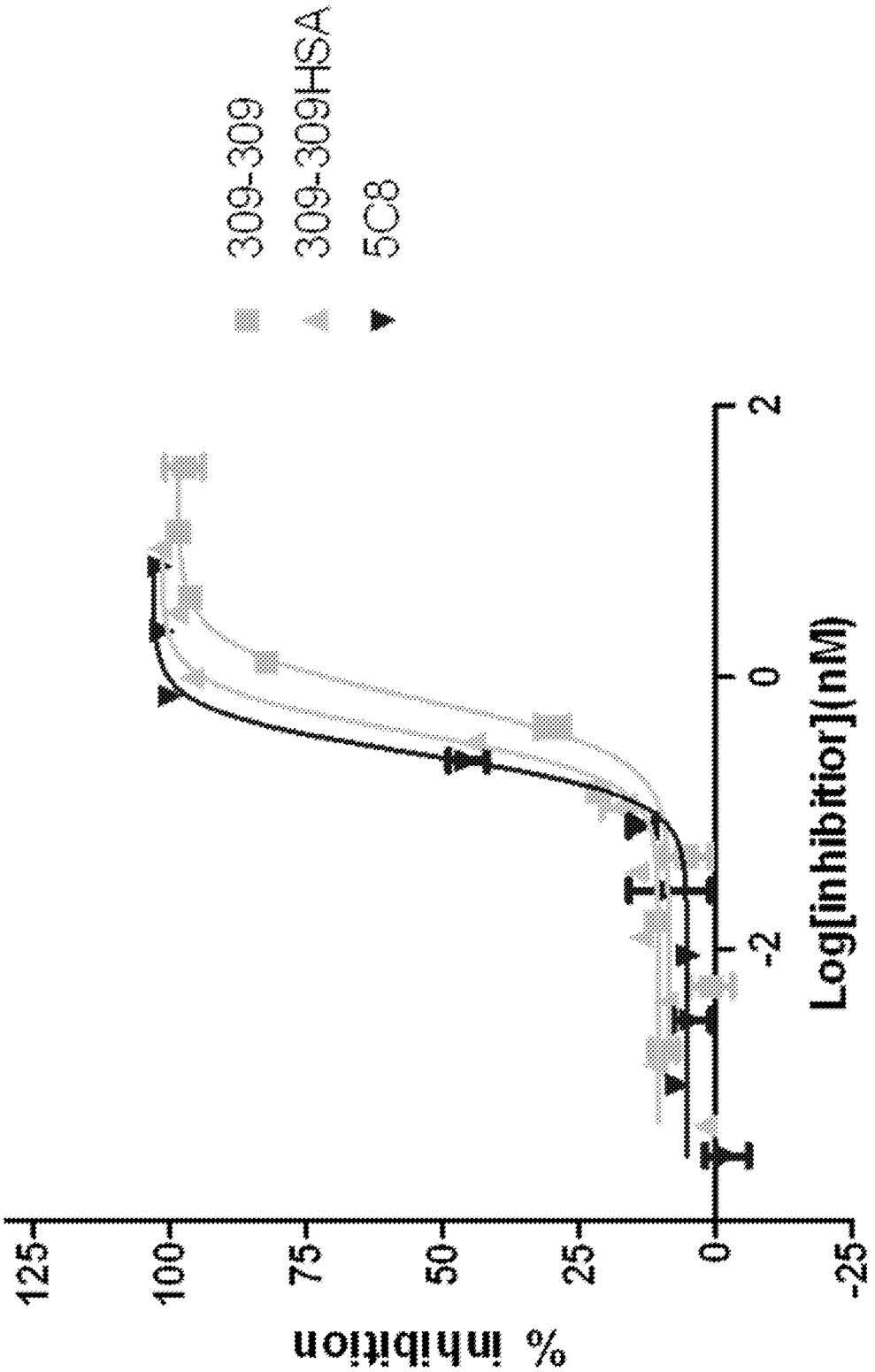

309-309
309-309HSA
5C8
% inhibition
125
100
75
50
25
0
-25
-2
0
2
Log[inhibitior](nM)

Fig. 9C

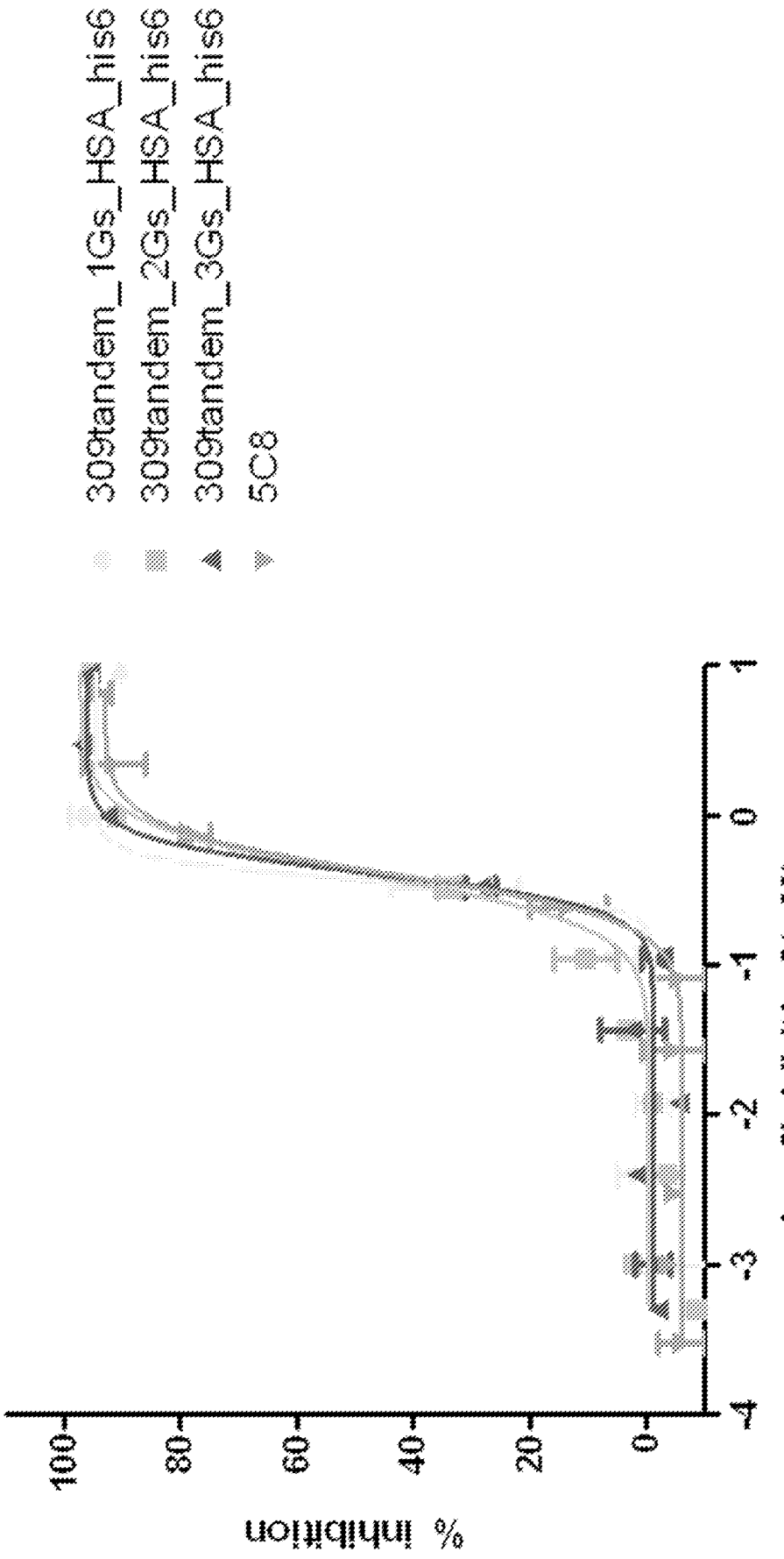

309tandem_1Gs_HSA_his6
309tandem_2Gs_HSA_his6
309tandem_3Gs_HSA_his6
5C8
% inhibition
100
80
60
40
20
0
-4
-3
-2
-1
0
1
Log[inhibition](nM)

Fig. 9D

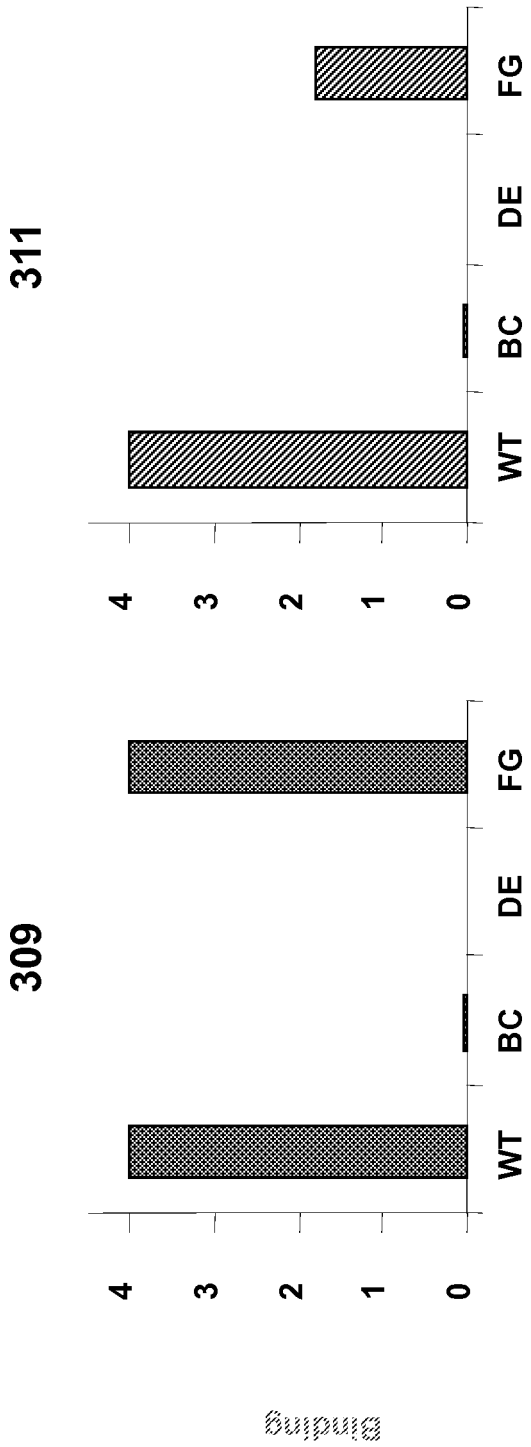



Fig. 10A

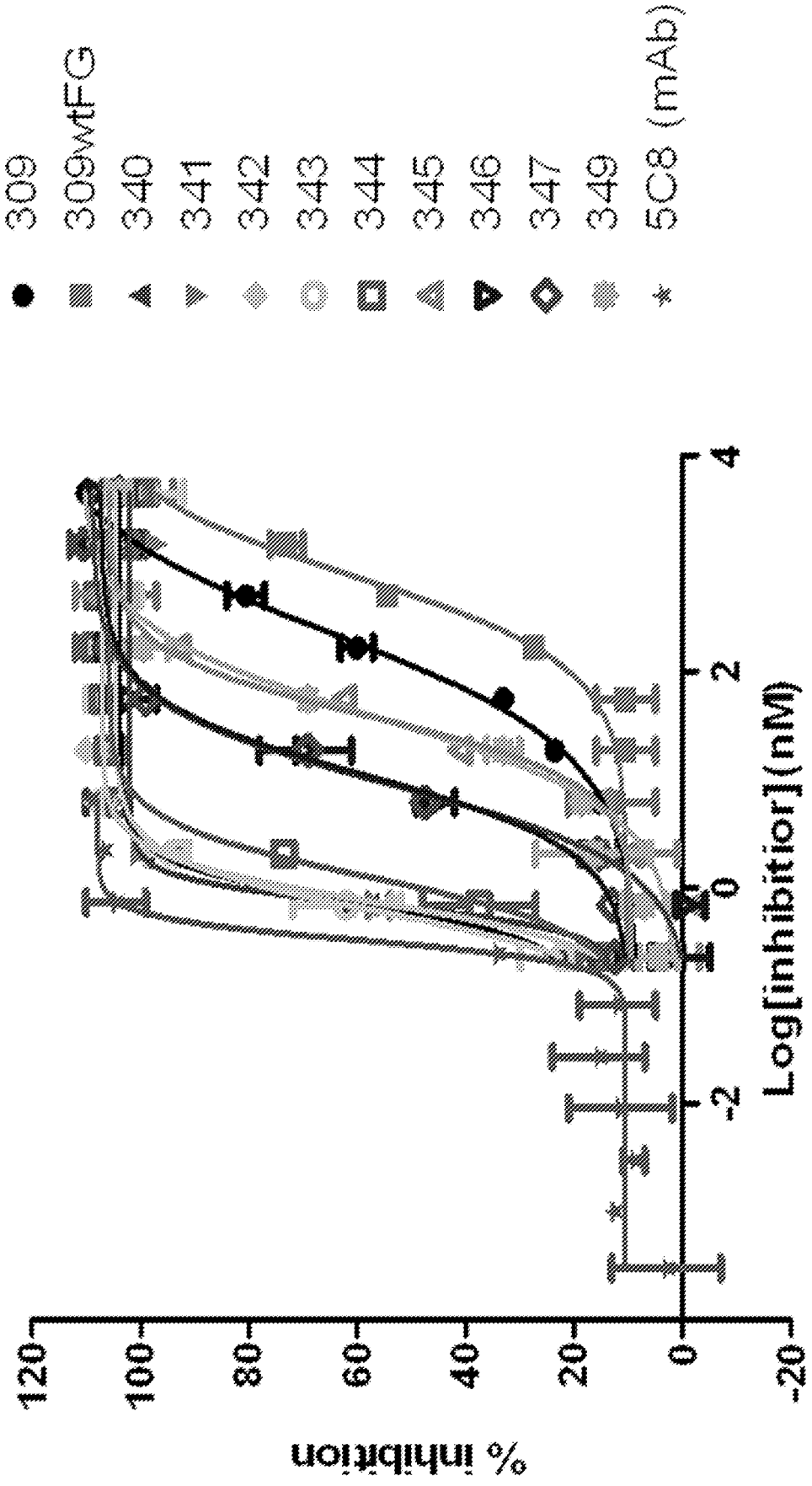

309
309wtFG
340
341
342
343
344
345
346
347
349
5C8 (mAb)
% inhibition
120
100
80
60
40
20
0
-20
-2
0
2
4
Log[inhibitor](nM)

Fig. 10B

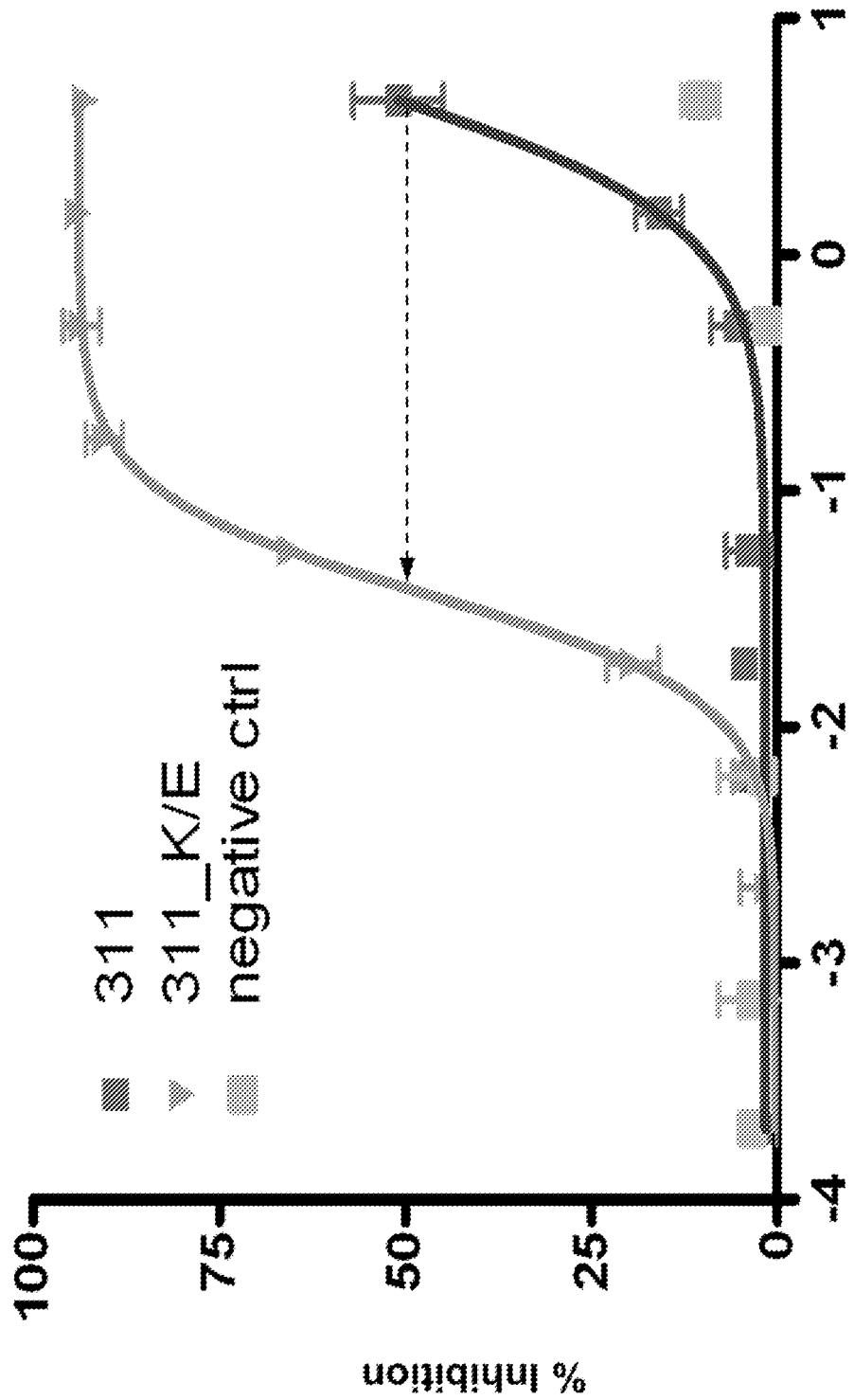

% Inhibition
100
75
50
25
0
-4
-3
-2
-1
0
1
Log Tn3 (μM)
311
311_K/E
negative ctrl

Fig. 10C

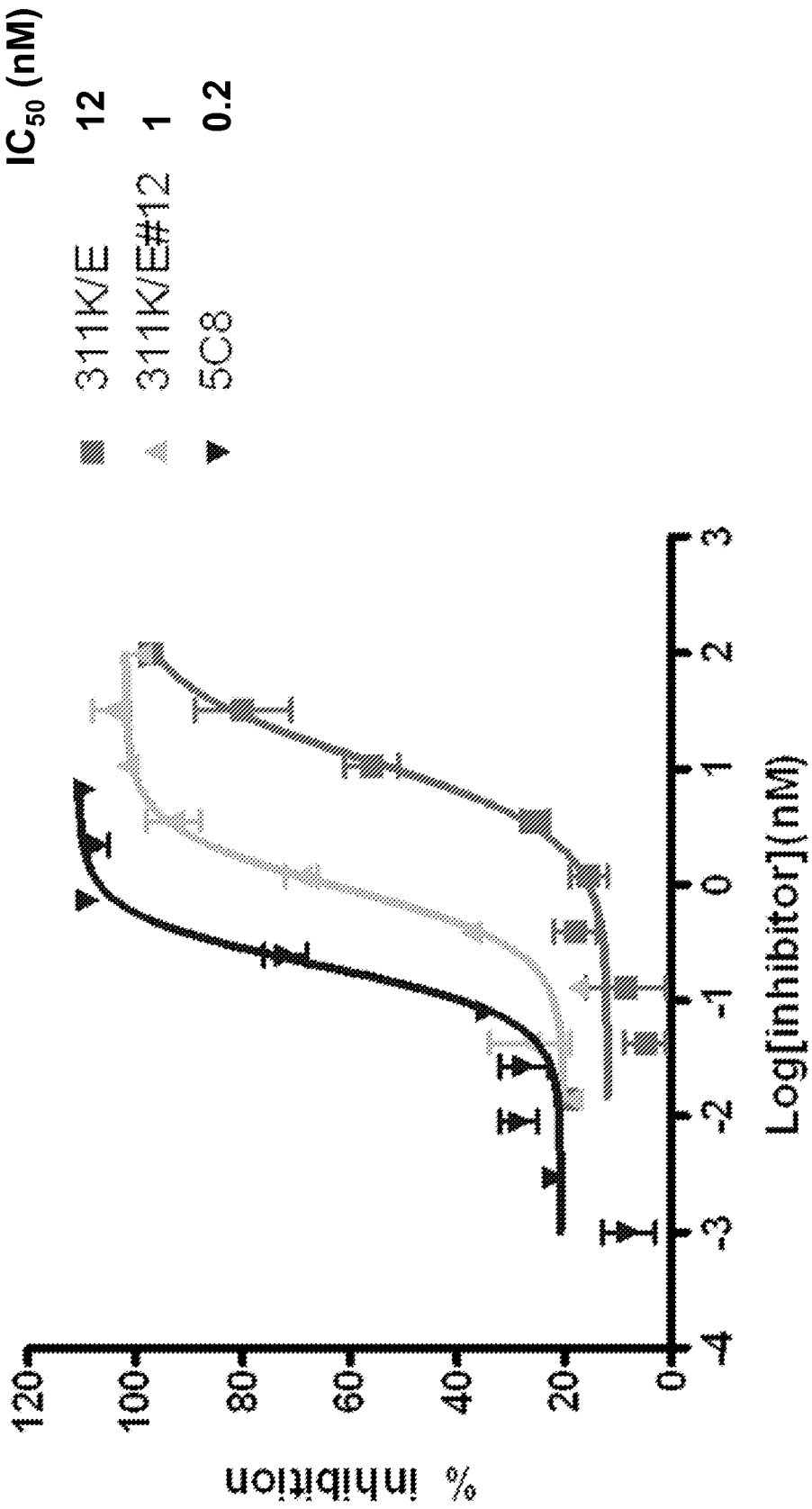

IC50 (nM)
311K/E 12
311K/E#12 1
5C8 0.2
% inhibition
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
3
Log[inhibitor](nM)

Fig. 10D

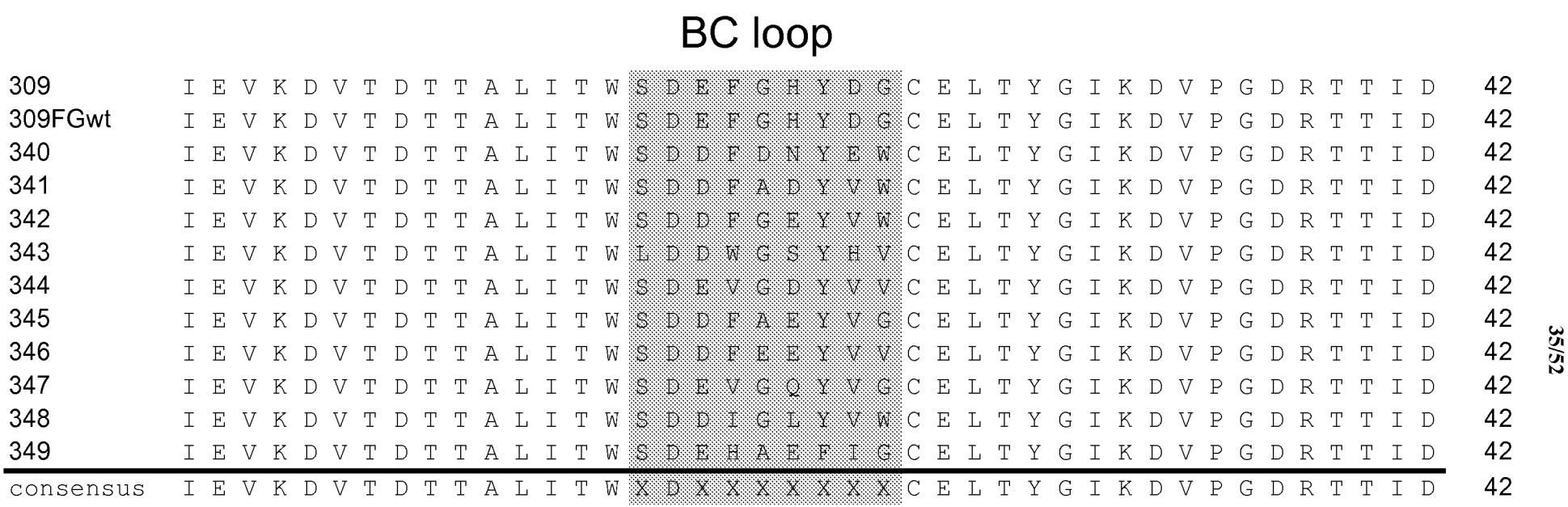

BC loop
309 I E V K D V T D T T A L I T W S D E F G H Y D G C E L T Y G I K D V P G D R T T I D 42
309FGwt I E V K D V T D T T A L I T W S D E F G H Y D G C E L T Y G I K D V P G D R T T I D 42
340 I E V K D V T D T T A L I T W S D D F D N Y E W C E L T Y G I K D V P G D R T T I D 42
341 I E V K D V T D T T A L I T W S D D F A D Y V W C E L T Y G I K D V P G D R T T I D 42
342 I E V K D V T D T T A L I T W S D D F G E Y V W C E L T Y G I K D V P G D R T T I D 42
343 I E V K D V T D T T A L I T W L D D W G S Y H V C E L T Y G I K D V P G D R T T I D 42
344 I E V K D V T D T T A L I T W S D E V G D Y V V C E L T Y G I K D V P G D R T T I D 42
345 I E V K D V T D T T A L I T W S D D F A E Y V G C E L T Y G I K D V P G D R T T I D 42
346 I E V K D V T D T T A L I T W S D D F E E Y V V C E L T Y G I K D V P G D R T T I D 42
347 I E V K D V T D T T A L I T W S D E V G Q Y V G C E L T Y G I K D V P G D R T T I D 42
348 I E V K D V T D T T A L I T W S D D I G L Y V W C E L T Y G I K D V P G D R T T I D 42
349 I E V K D V T D T T A L I T W S D E H A E F I G C E L T Y G I K D V P G D R T T I D 42
consensus I E V K D V T D T T A L I T W X D X X X X X X X C E L T Y G I K D V P G D R T T I D 42



**Fig. 11A**

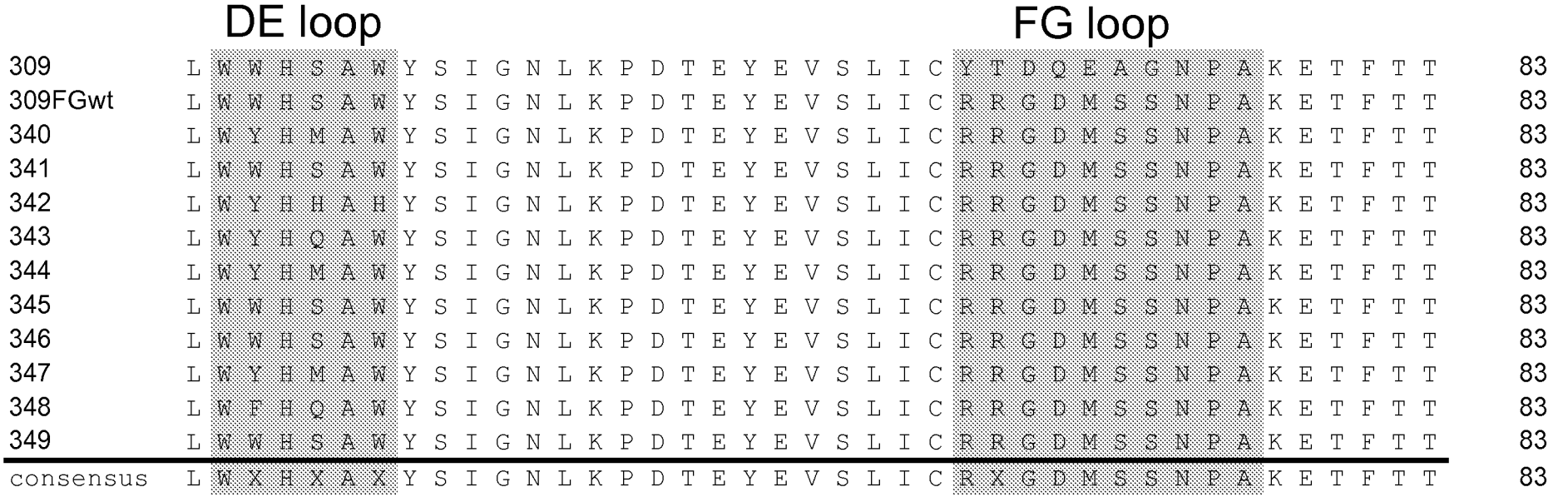

DE loop
FG loop
309 L W W H S A W Y S I G N L K P D T E Y E V S L I C Y T D Q E A G N P A K E T F T T 83
309FGwt L W W H S A W Y S I G N L K P D T E Y E V S L I C R R G D M S S N P A K E T F T T 83
340 L W Y H M A W Y S I G N L K P D T E Y E V S L I C R R G D M S S N P A K E T F T T 83
341 L W W H S A W 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 83
342 L W Y H H A H 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 83
343 L W Y H Q A W 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 83
344 L W Y H M A W 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 83
345 L W W H S A W 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 83
346 L W W H S A W 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 83
347 L W Y H M A W 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 83
348 L W F H Q A W 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 83
349 L W W H S A W 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 83
consensus L W X H X A X Y S I G N L K P D T E Y E V S L I C R X G D M S S N P A K E T F T T 83



**Fig. 11B**

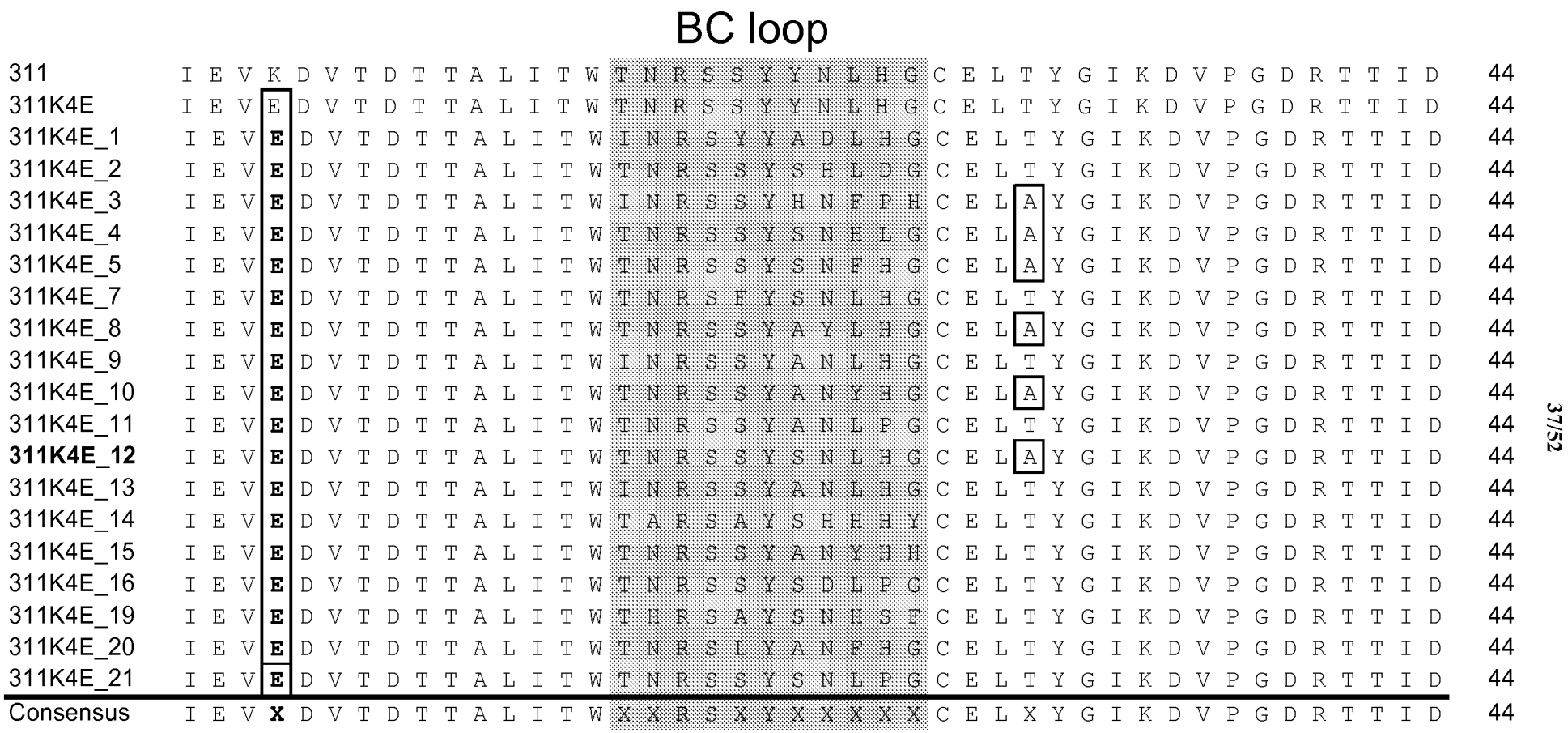

BC loop
311 I E V K D V T D T T A L I T W T N R S S Y Y N L H G C E L T Y G I K D V P G D R T T I D 44
311K4E I E V E D V T D T T A L I T W T N R S S Y Y N L H G C E L T Y G I K D V P G D R T T I D 44
311K4E_1 I E V E D V T D T T A L I T W I N R S Y Y A D L H G C E L T Y G I K D V P G D R T T I D 44
311K4E_2 I E V E D V T D T T A L I T W T N R S S Y S H L D G C E L T Y G I K D V P G D R T T I D 44
311K4E_3 I E V E D V T D T T A L I T W I N R S S Y H N F P H C E L A Y G I K D V P G D R T T I D 44
311K4E_4 I E V E D V T D T T A L I T W T N R S S Y S N H L G C E L A Y G I K D V P G D R T T I D 44
311K4E_5 I E V E D V T D T T A L I T W T N R S S Y S N F H G C E L A Y G I K D V P G D R T T I D 44
311K4E_7 I E V E D V T D T T A L I T W T N R S F Y S N L H G C E L T Y G I K D V P G D R T T I D 44
311K4E_8 I E V E D V T D T T A L I T W T N R S S Y A Y L H G C E L A Y G I K D V P G D R T T I D 44
311K4E_9 I E V E D V T D T T A L I T W I N R S S Y A N L H G C E L T Y G I K D V P G D R T T I D 44
311K4E_10 I E V E D V T D T T A L I T W T N R S S Y A N Y H G C E L A Y G I K D V P G D R T T I D 44
311K4E_11 I E V E D V T D T T A L I T W T N R S S Y A N L P G C E L T Y G I K D V P G D R T T I D 44
311K4E_12 I E V E D V T D T T A L I T W T N R S S Y S N L H G C E L A Y G I K D V P G D R T T I D 44
311K4E_13 I E V E D V T D T T A L I T W I N R S S Y A N L H G C E L T Y G I K D V P G D R T T I D 44
311K4E_14 I E V E D V T D T T A L I T W T A R S A Y S H H H Y C E L T Y G I K D V P G D R T T I D 44
311K4E_15 I E V E D V T D T T A L I T W T N R S S Y A N Y H H C E L T Y G I K D V P G D R T T I D 44
311K4E_16 I E V E D V T D T T A L I T W T N R S S Y S D L P G C E L T Y G I K D V P G D R T T I D 44
311K4E_19 I E V E D V T D T T A L I T W T H R S A Y S N H S F C E L T Y G I K D V P G D R T T I D 44
311K4E_20 I E V E D V T D T T A L I T W T N R S L Y A N F H G C E L T Y G I K D V P G D R T T I D 44
311K4E_21 I E V E D V T D T T A L I T W T N R S S Y S N L P G C E L T Y G I K D V P G D R T T I D 44
Consensus I E V X D V T D T T A L I T W X X R S X Y X X X X X C E L X Y G I K D V P G D R T T I D 44

Fig. 12A

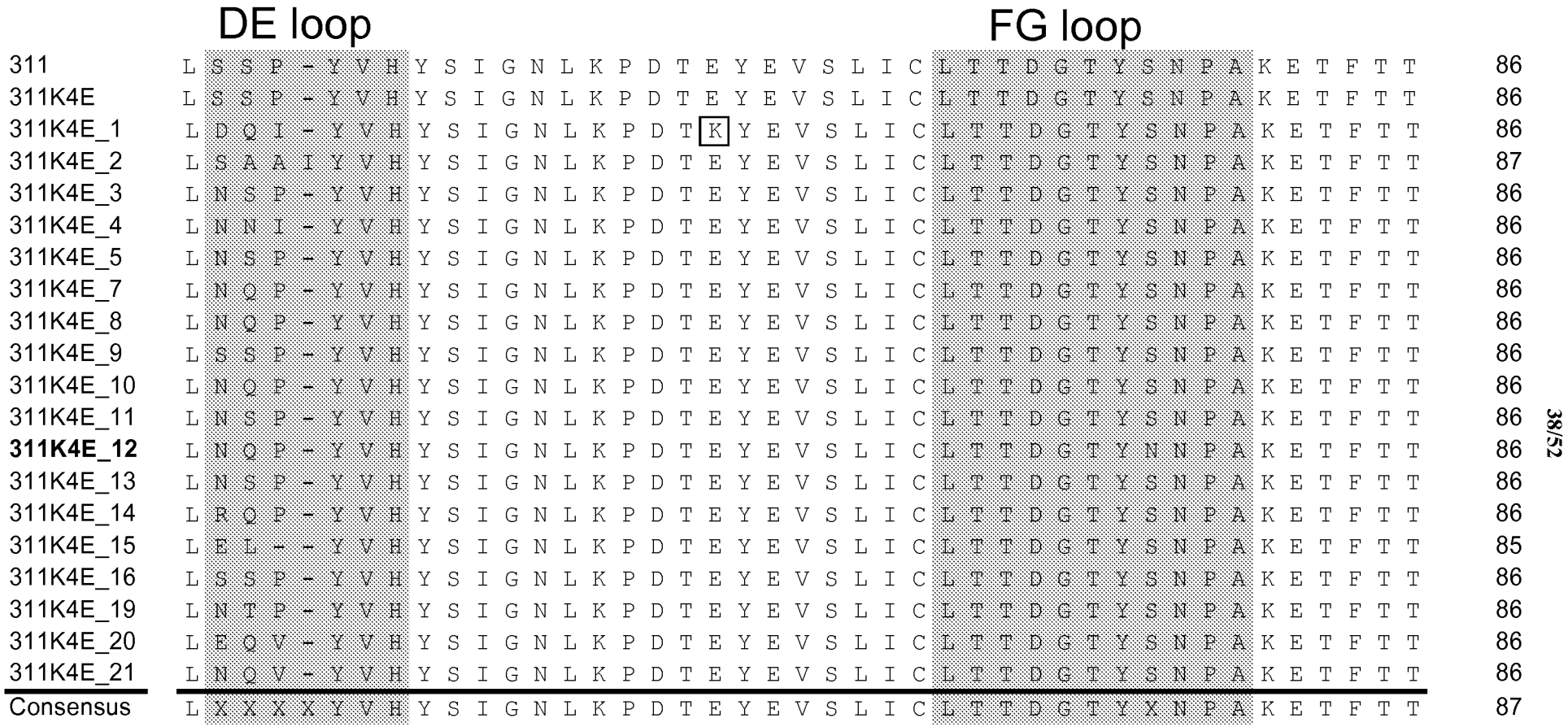

DE loop
FG loop
311 L S S P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E L S S P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_1 L D Q I - Y V H Y S I G N L K P D T K Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_2 L S A A I Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 87
311K4E_3 L N S P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_4 L N N I - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_5 L N S P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_7 L N Q P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_8 L N Q P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_9 L S S P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_10 L N Q P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_11 L N S P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_12 L N Q P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y N N P A K E T F T T 86
311K4E_13 L N S P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_14 L R Q P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_15 L E L - - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 85
311K4E_16 L S S P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_19 L N T P - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_20 L E Q V - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
311K4E_21 L N Q V - Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y S N P A K E T F T T 86
Consensus L X X X X Y V H Y S I G N L K P D T E Y E V S L I C L T T D G T Y X N P A K E T F T T 87



**Fig. 12B**

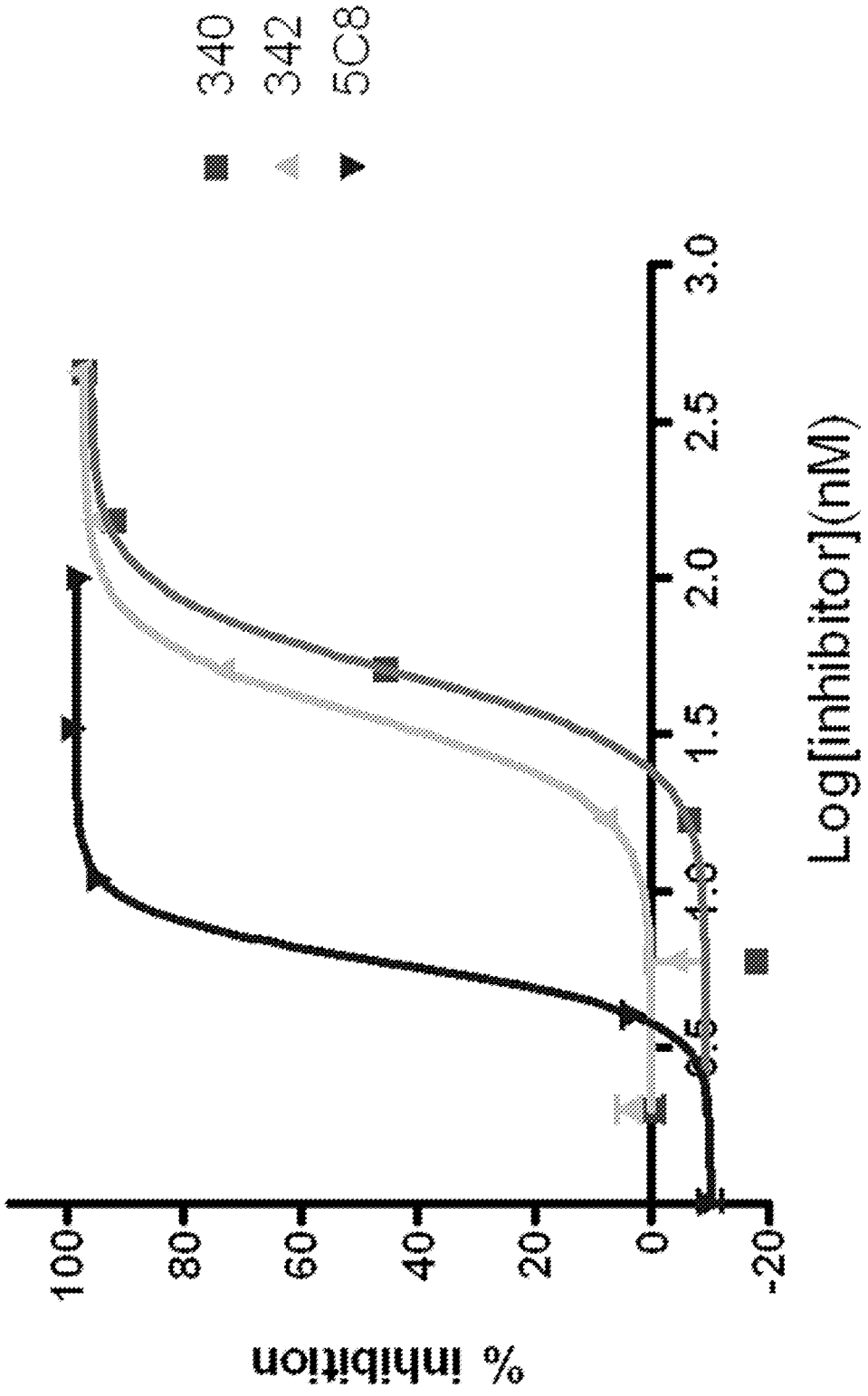

340
342
5C8
% inhibition
100
80
60
40
20
0
-20
0.5
1.0
1.5
2.0
2.5
3.0
Log[inhibitor](nM)

Fig. 13

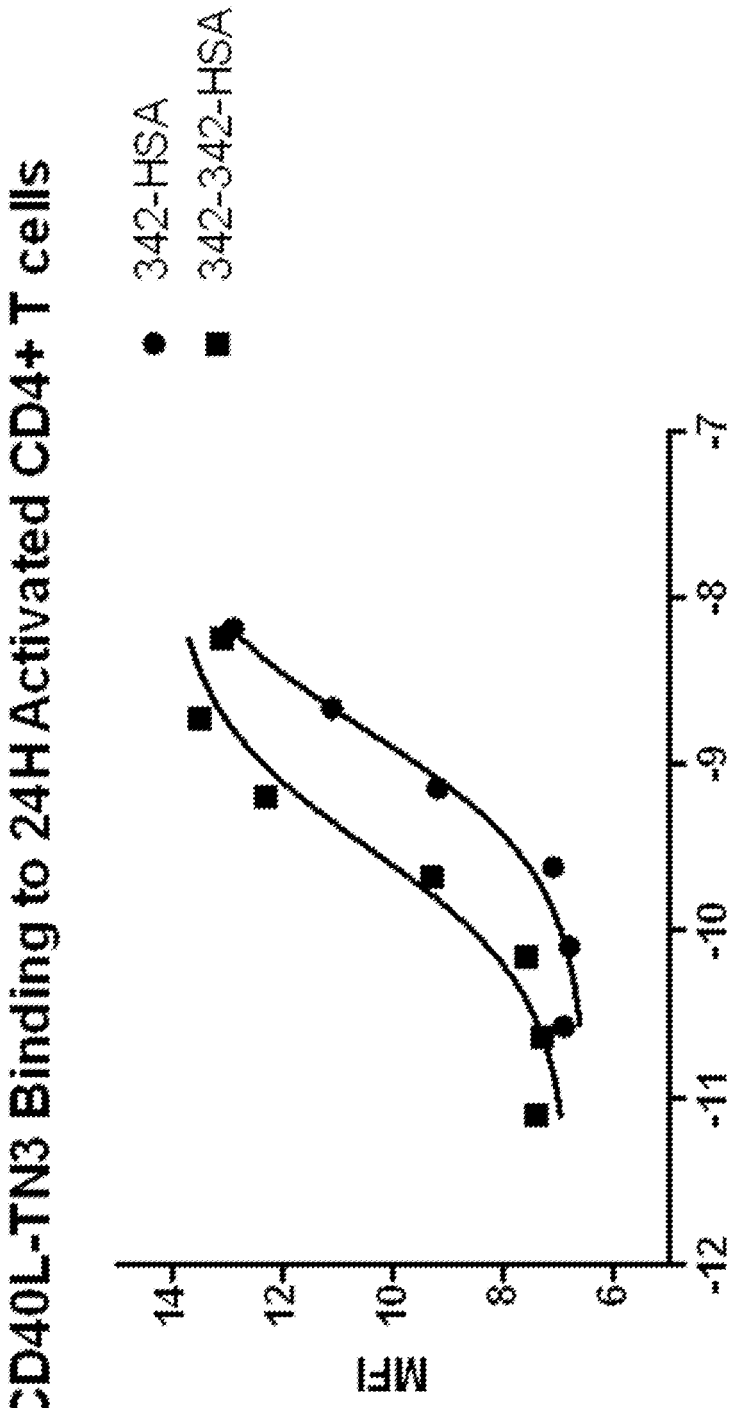
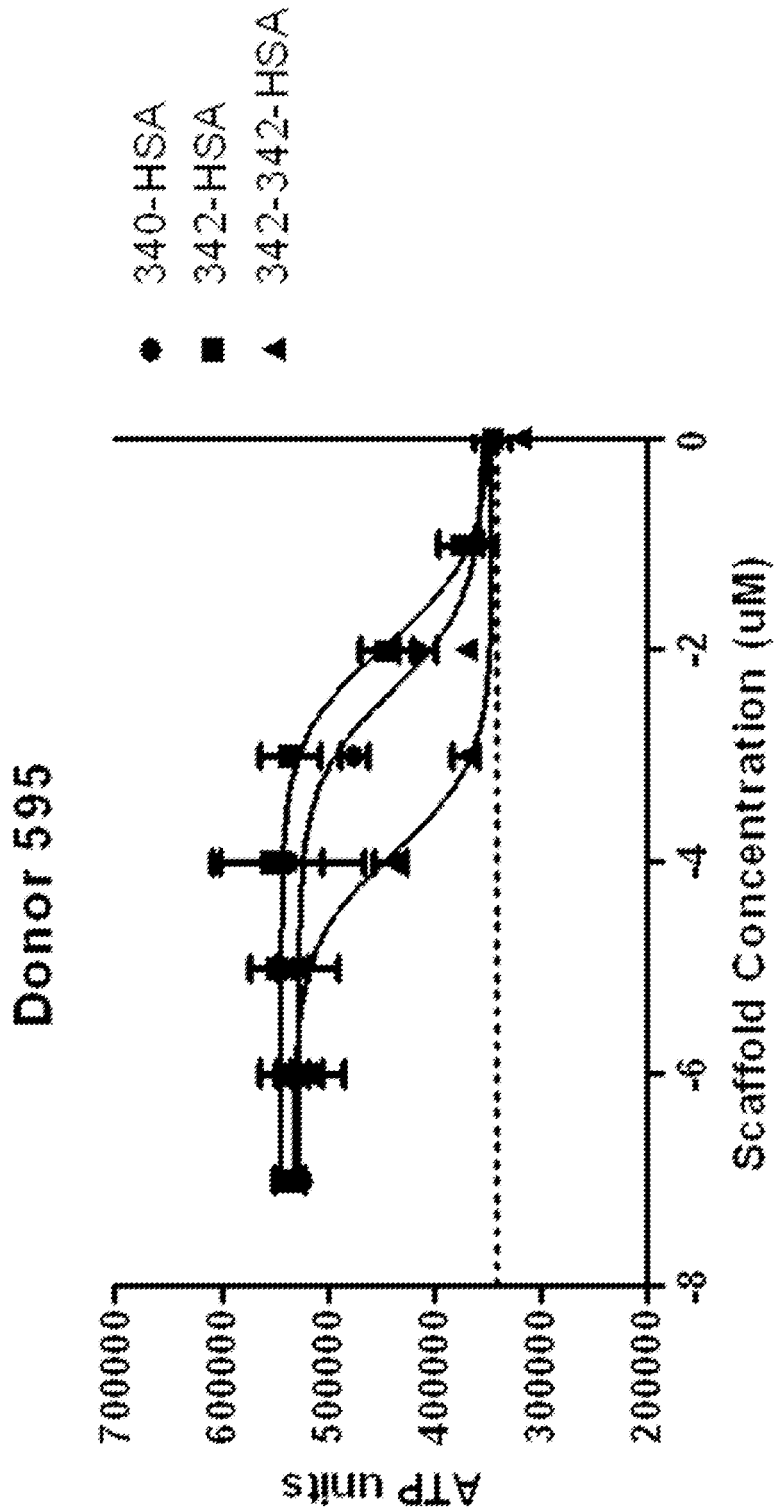

Anti-CD40L-TN3 Binding to 24H Activated CD4+ T cells
342-HSA
342-342-HSA
MFI
14
12
10
8
6
-12
-11
-10
-9
-8
-7
Log [M] CD40L-TN3

Fig. 14




| | 340-HSA | 342-HSA | 342-342-HSA |
|---|---|---|---|
| IC50 | 0.004054 | 0.01102 | 0.0001205 |

Fig. 15

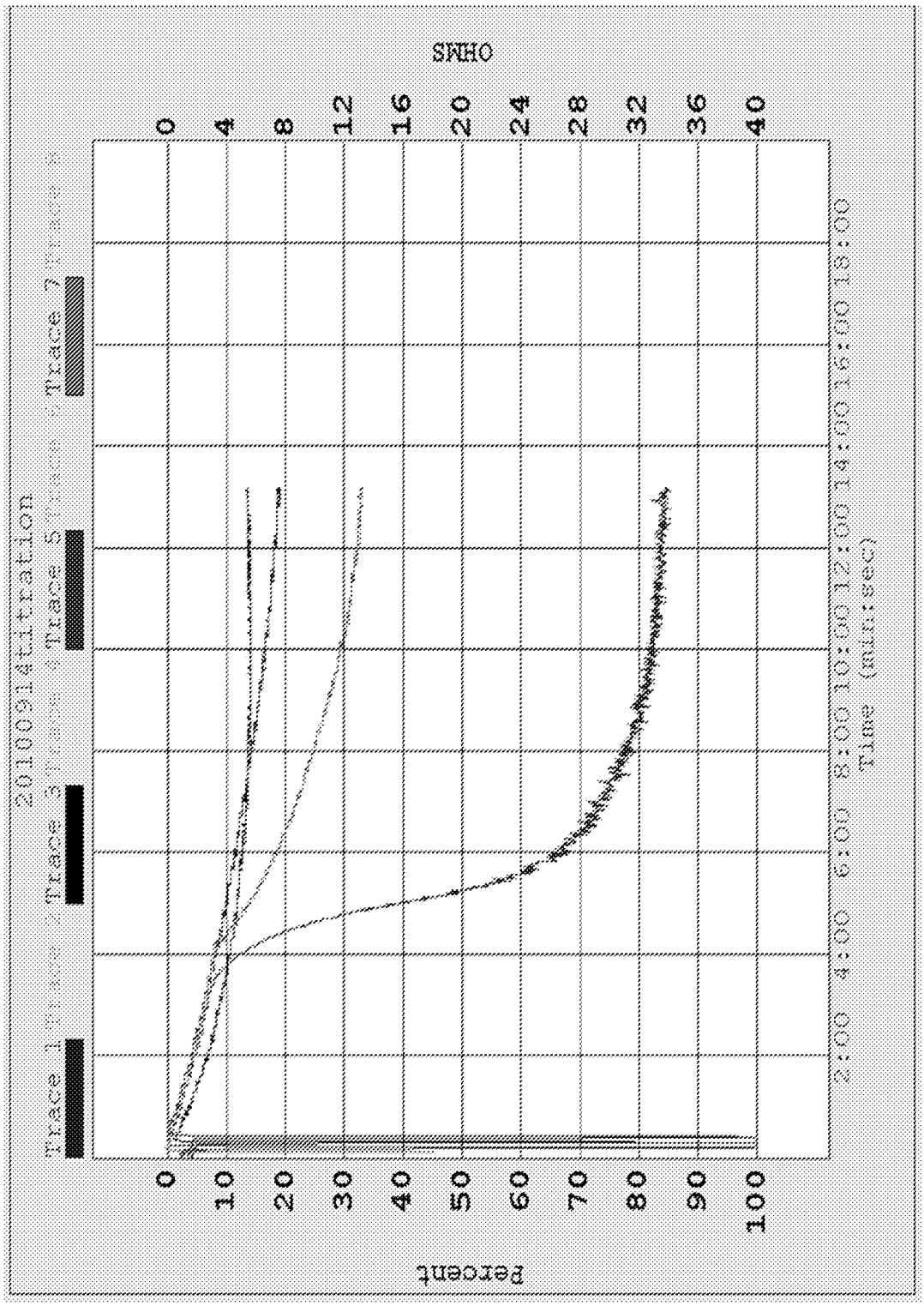

20100914titration
Trace 1
Trace 3
Trace 5
Trace 7
OHMS
0
4
8
12
16
20
24
28
32
36
40
percent
0
10
20
30
40
50
60
70
80
90
100
2:00
4:00
6:00
8:00
10:00
12:00
14:00
16:00
18:00
Time (min:sec)

Fig. 16A

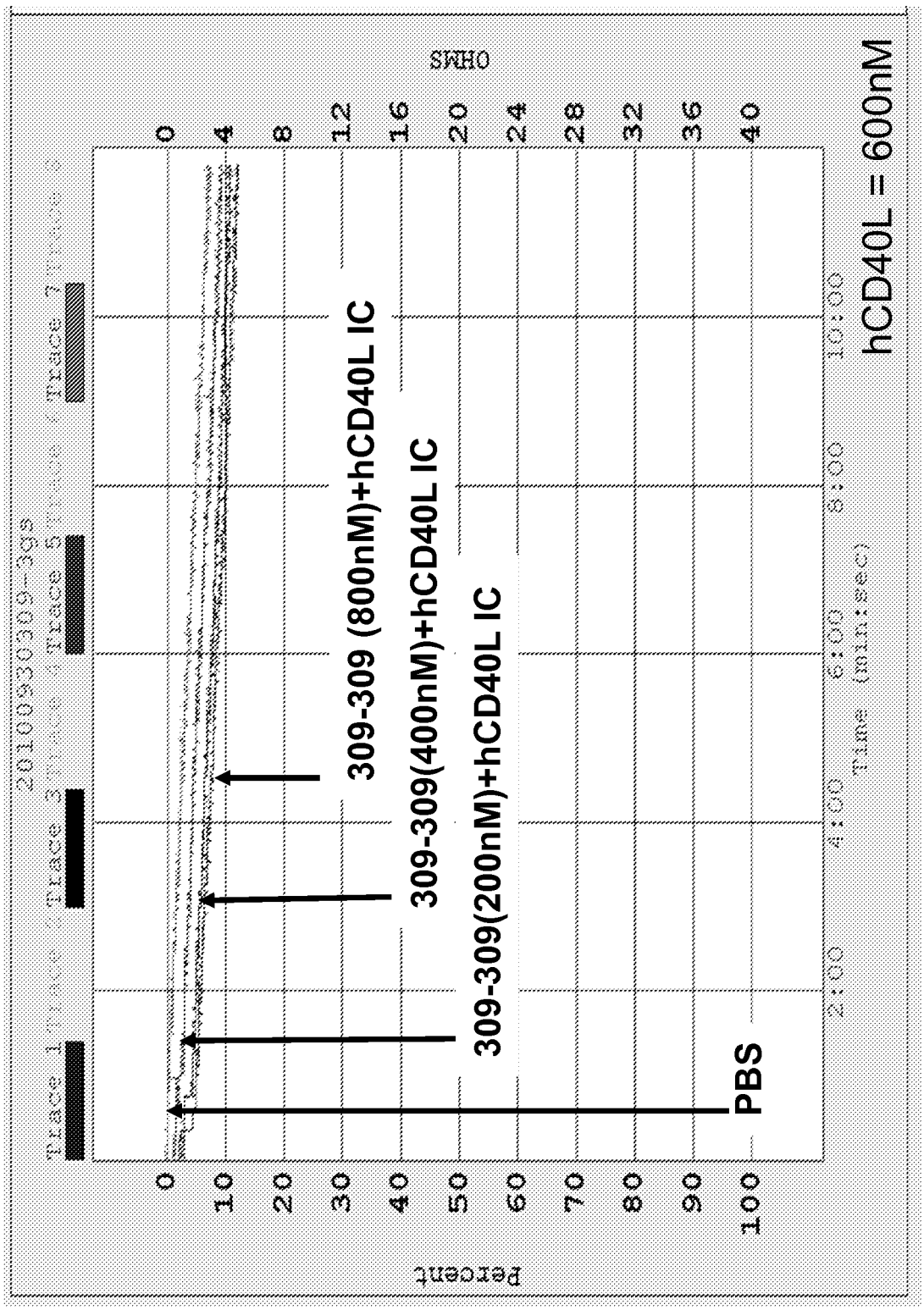

20100930309-395
Trace 1
Trace 3
Trace 5
Trace 7
OHMS
0
4
8
12
16
20
24
28
32
36
40
Percent
0
10
20
30
40
50
60
70
80
90
100
2:00
4:00
6:00
8:00
10:00
Time (min:sec)
309-309 (800nM)+hCD40L IC
309-309(400nM)+hCD40L IC
309-309(200nM)+hCD40L IC
PBS
hCD40L = 600nM

Fig. 16B

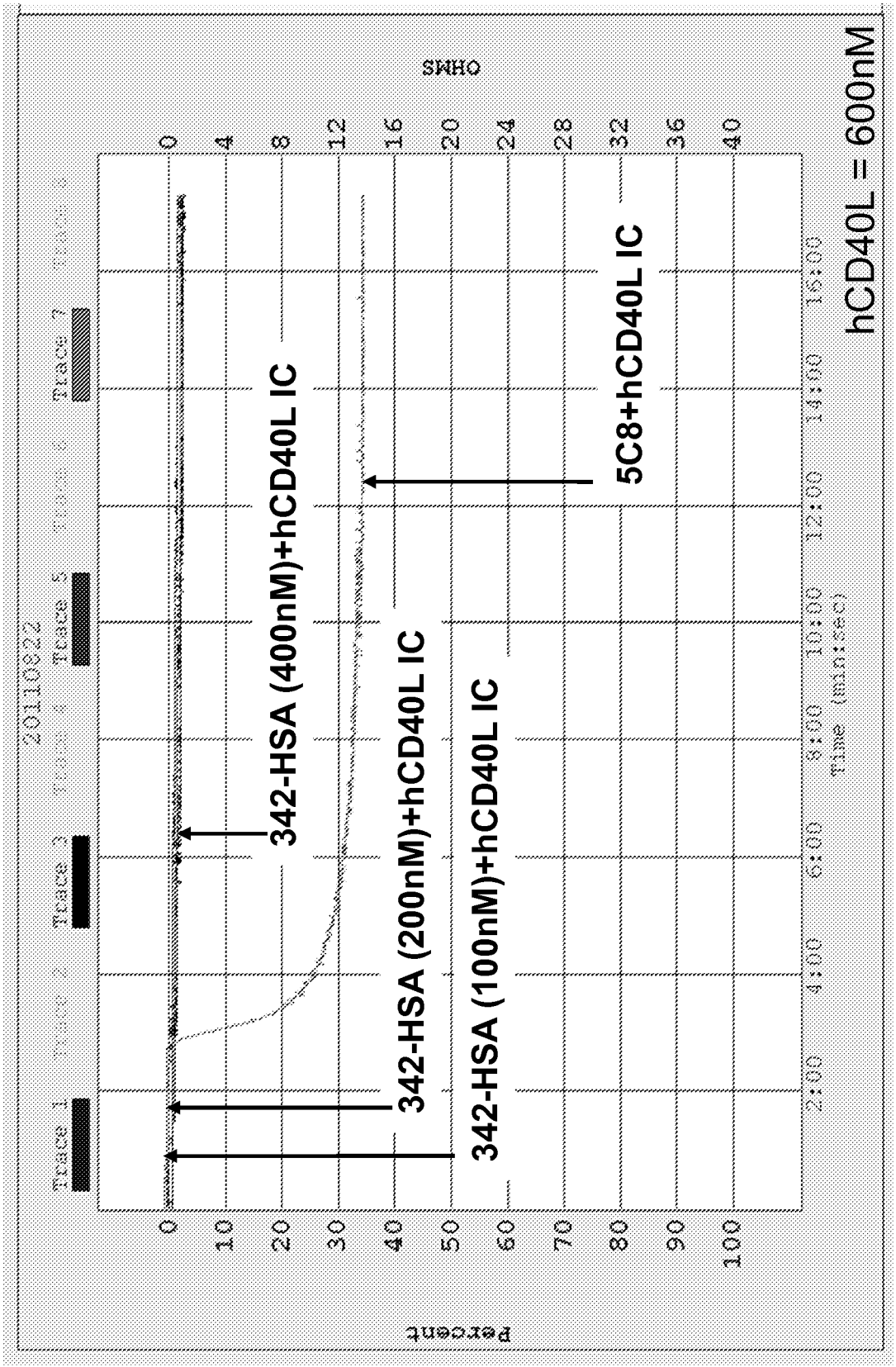

342-HSA (400nM)+hCD40L IC
342-HSA (200nM)+hCD40L IC
342-HSA (100nM)+hCD40L IC
5C8+hCD40L IC
hCD40L = 600nM
Percent
OHMS
Time (min:sec)
Trace 1
Trace 3
Trace 5
Trace 7

Fig. 16C




| CD40L | 309 | loop |
|---|---|---|
| 1:H249N | S47O | DE |
| 1:T251N | D17OD1 | BC |
| 1:V247O | W49NE1 | DE |
| 2:R203NH1 | E18OE1 | BC |
| 2:I204N | E18OE2 | BC |
| 2:S185OG | D70OD2 | FG |

Fig. 17A

| CD40L | 311K4E-12 | Loop |
|---|---|---|
| 1:Y146OH | N17N | BC |
| 1:E142O | N17N | BC |
| 1:E142OE1 | R18N | BC |
| 1:Y146OH | R18N | BC |
| 1:M148O | R18NH1 | BC |
| 1:E142OE1 | S19N | BC |
| 1:E142OE2 | S19N | BC |
| 1:Y146O | H51NE2 | DE |
| 1:Y146OH | H15O | BC |
| 1:L155N | S19OG | BC |
| 1:N151ND2 | S22O | BC |
| 2:E230OE1 | H51N | DE |
| 2:E230OE2 | V50N | DE |
| 2:R200NH1 | T7OG1 | AB |
| 2:R200NH1 | D8OD1 | AB |
| 2:R200NH1 | T10OG1 | AB |
| 2:R200NH1 | T10OG1 | AB |
| 2:R203NH1 | E4OE1 | AB |
| 2:R203NH1 | D5OD1 | AB |

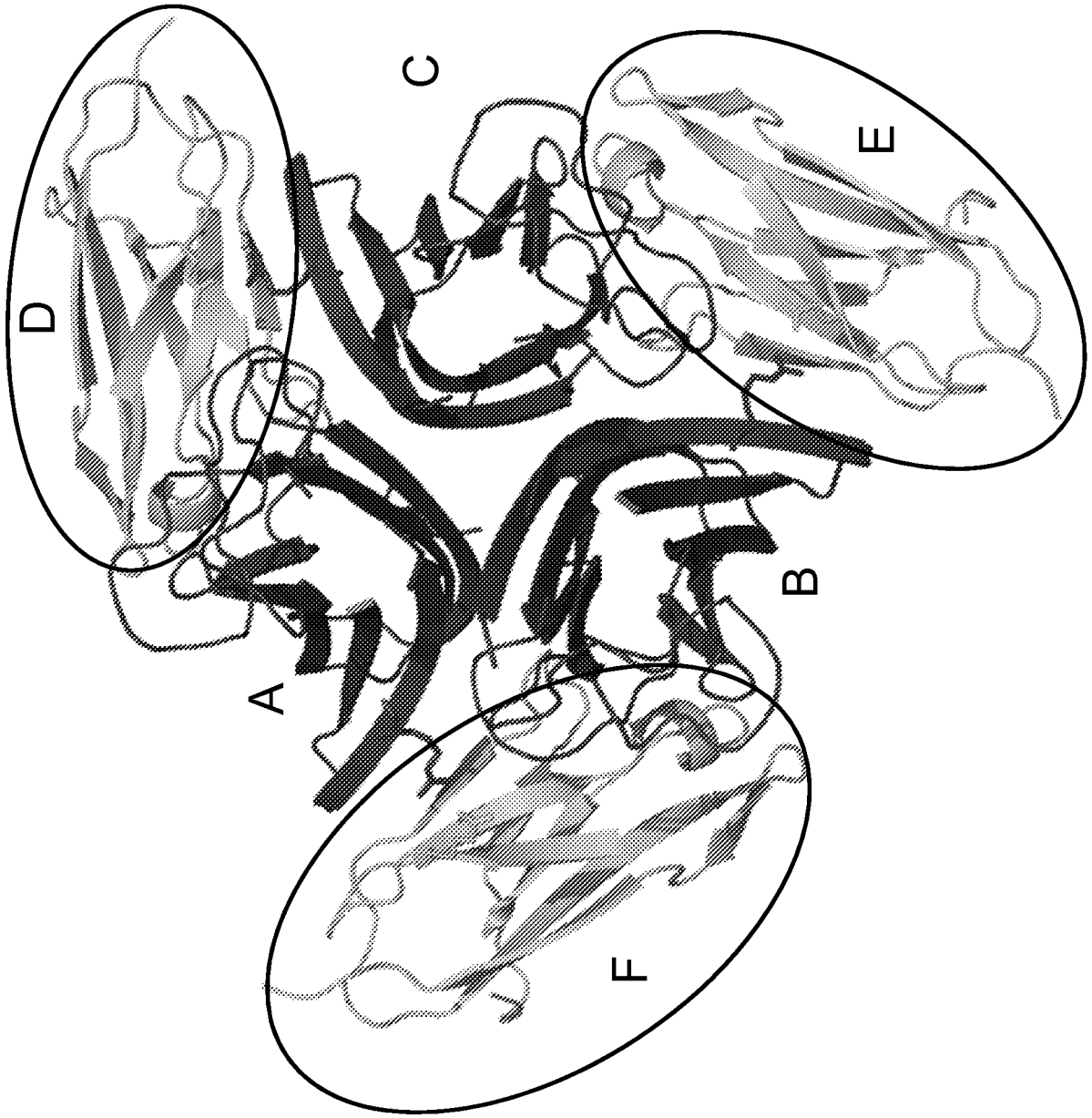



Fig. 17B

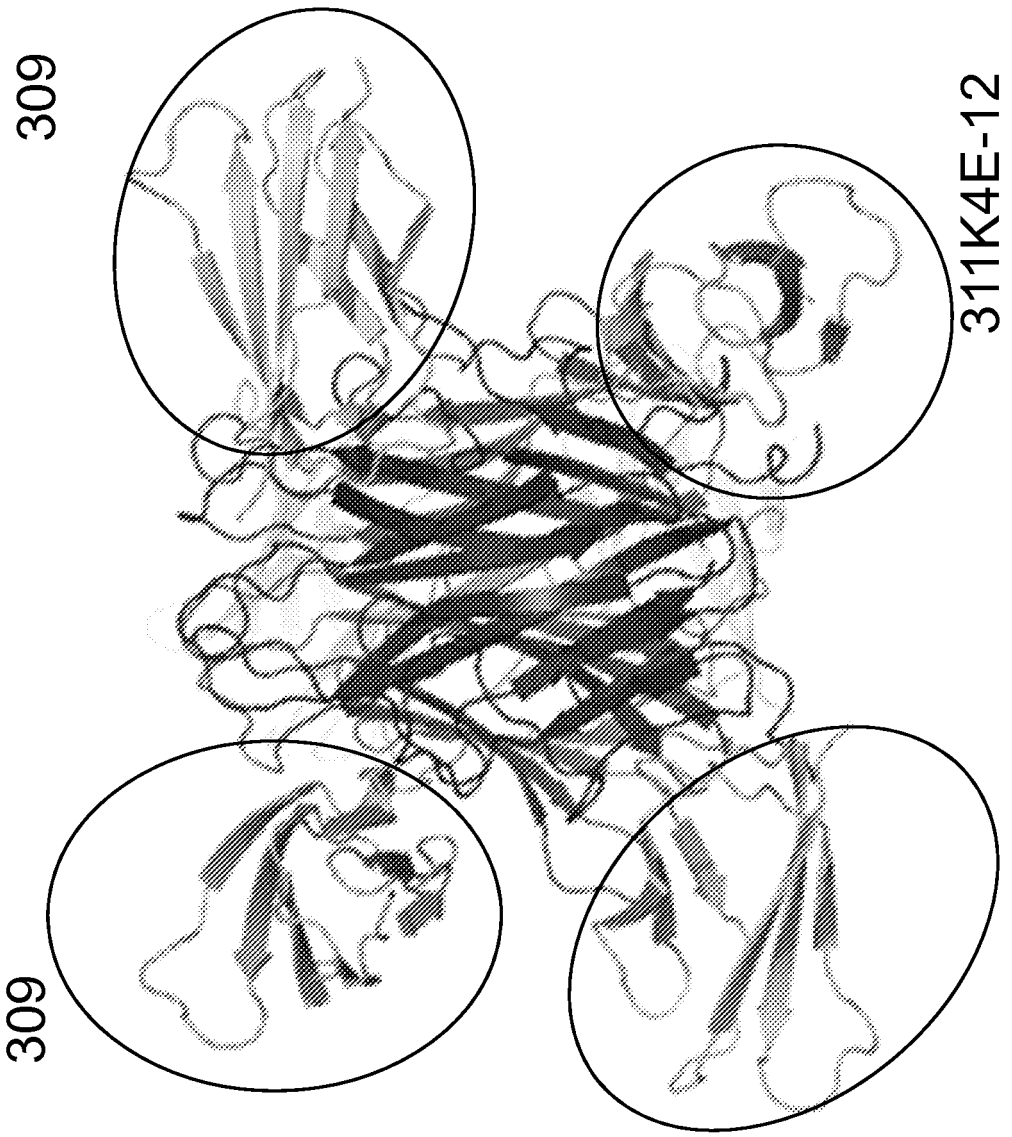



Fig. 17C


CD40L
342
CD40L
A:THR 251[ OG1]
A:THR 251[ N ]
A:HIS 249[ N ]
A:HIS 249[ N ]
A:SER 245[ O ]
A:VAL 247[ O ]
A:SER 246[ OG ]
342
D:ASP 18[ OD1]
D:ASP 18[ OD1]
D:HIS 47[ O ]
D:HIS 48[ O ]
D:HIS 48[ NE2]
D:HIS 50[ NE2]
D:HIS 48[ ND1]

FIG. 17D




FIG. 17E

MIETYNQTSP RSAATGLPIS MKIFMYLLTV FLITQMIGSA LFAVYLHRRL DKIEDERNLH 60

EDFVFMKTIQ RCNTGERSLS LLNCEEIKSQ FEGFVKDIML NKEETKKENS FEMQKGDQNP 120

QIAAHVISEA SSKTTSVLQW AEKGYYTMSN NLVTLENGKQ LTVKRQGLYY IYAQVTFCSN 180

REASSQAPFI ASLCLKSPGR FERILLRAAN THSSAKPCGQ QSIHLGGVFE LQPGASVFVN 240

VTDPSQVSHG TGFTSFGLLK L 261



Fig. 18A

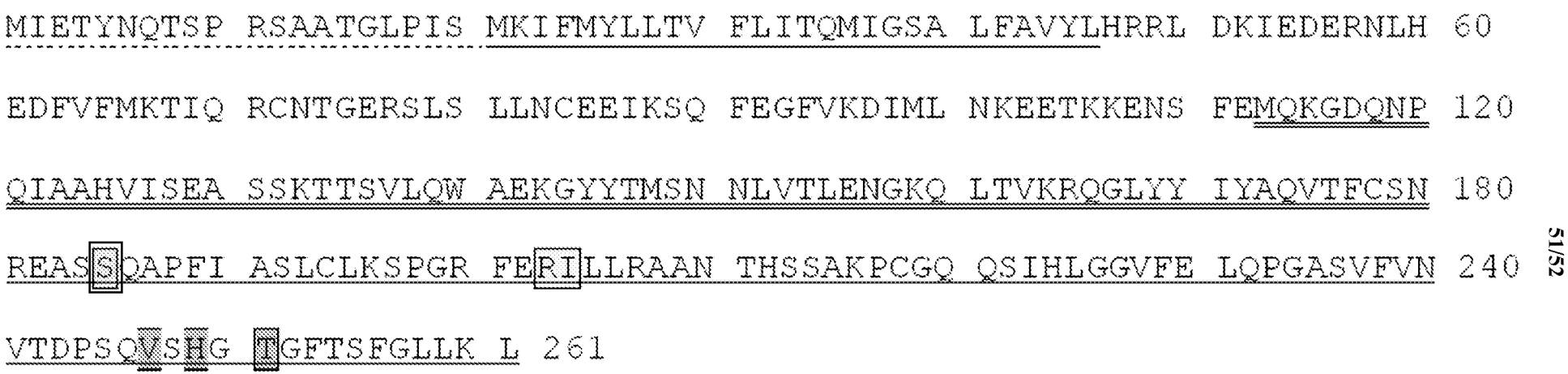

MIETYNQTSP RSAATGLPIS MKIFMYLLTV FLITQMIGSA LFAVYLHRRL DKIEDERNLH 60
EDFVFMKTIQ RCNTGERSLS LLNCEEIKSQ FEGFVKDIML NKEETKKENS FEMQKGDQNP 120
QIAAHVISEA SSKTTSVLQW AEKGYYTMSN NLVTLENGKQ LTVKRQGLYY IYAQVTFCSN 180
REASSQAPFI ASLCLKSPGR FERILLRAAN THSSAKPCGQ QSIHLGGVFE LQPGASVFVN 240
VTDPSQVSHG TGFTSFGLLK L 261



**Fig. 18B**

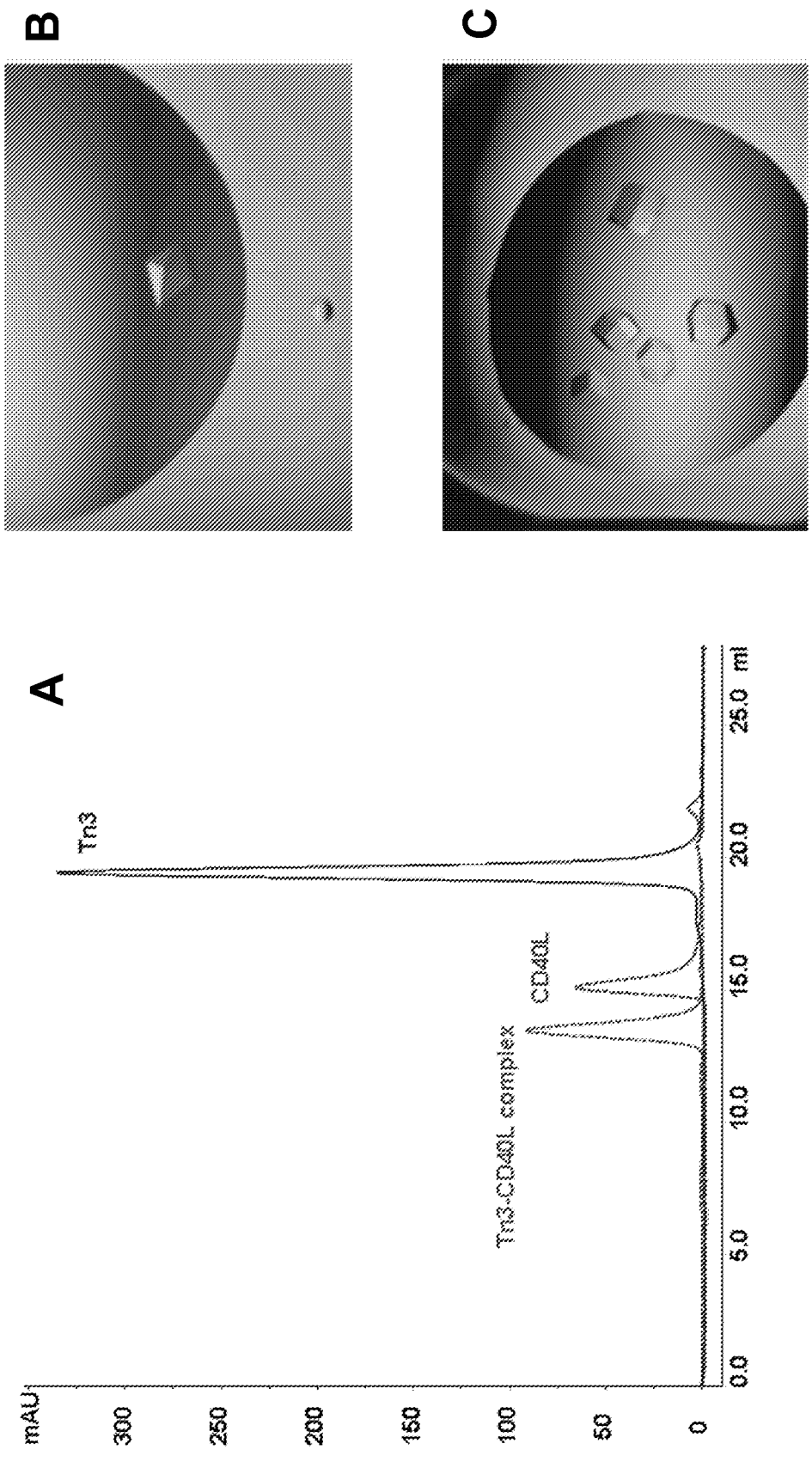

A
B
C
mAU
300
250
200
150
100
50
0
0.0
5.0
10.0
15.0
20.0
25.0 ml
Tn3
CD40L
Tn3-CD40L complex

FIG. 19