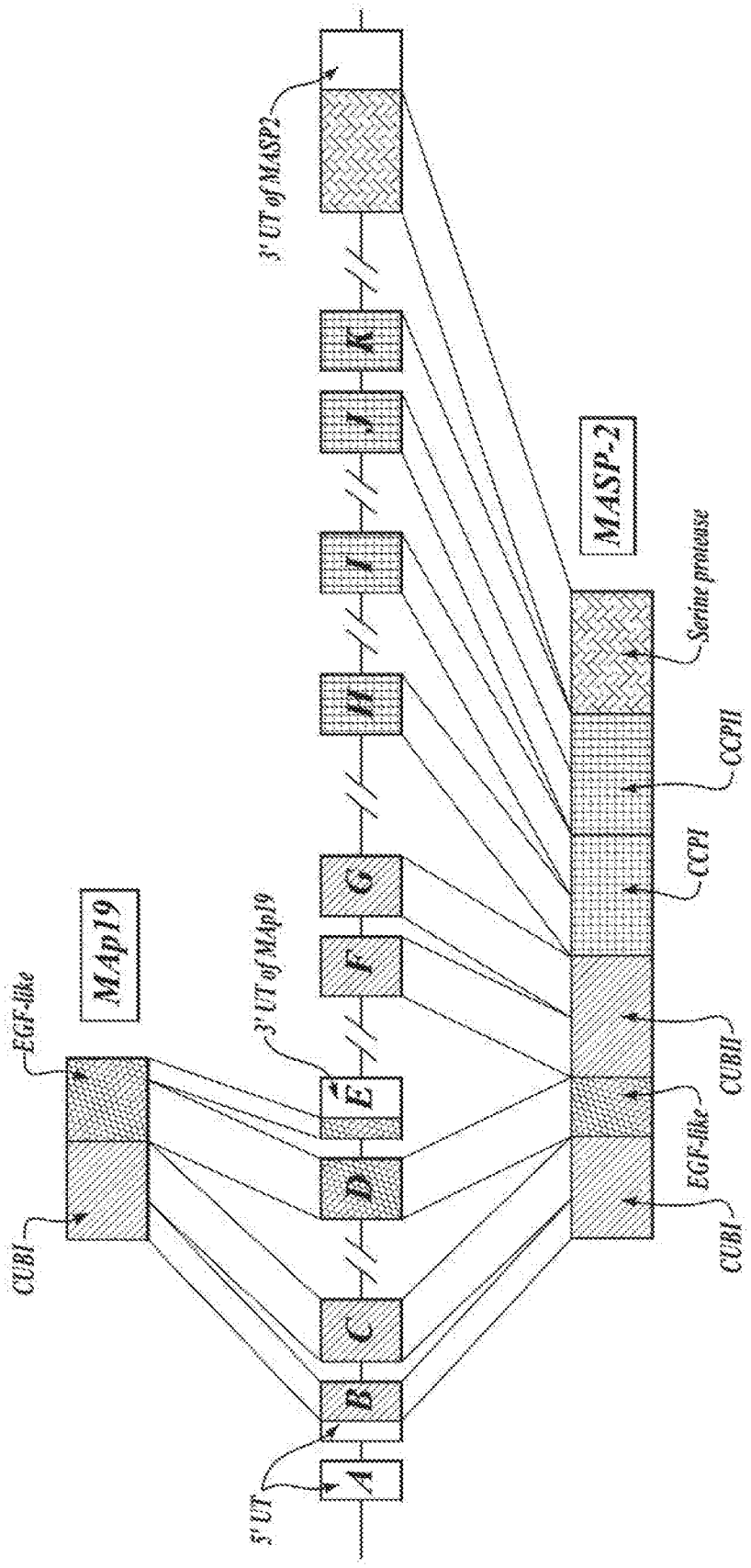
MAp19
EGF-like
CUBI
3' UT of Map19
5' UT
A
B
C
D
E
F
G
H
I
J
K
3' UT of MASP2
MASP-2
Serine protease
CCPII
CCPI
CUBII
EGF-like
CUBI

Fig. 1.

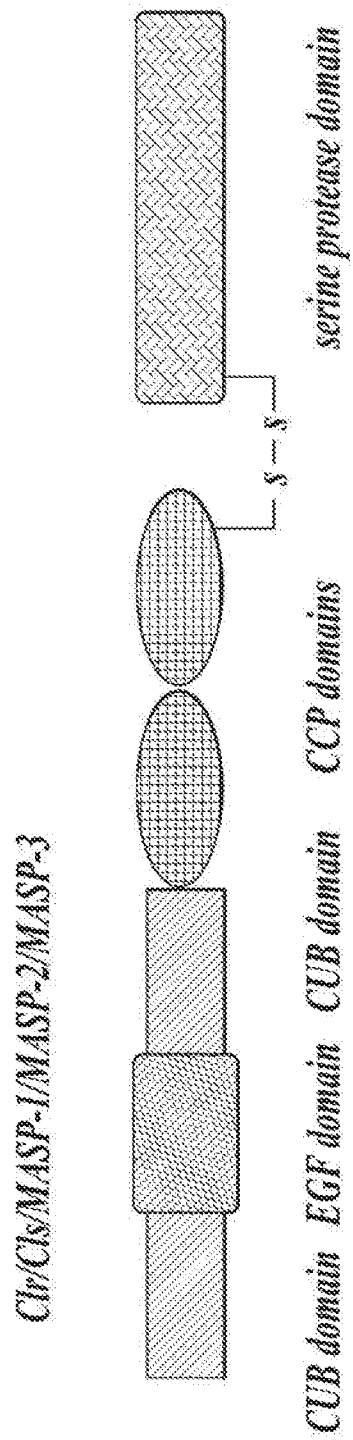
C1r/C1s/MASP-1/MASP-2/MASP-3
CUB domain
EGF domain
CUB domain
CCP domains
S — S
serine protease domain

Fig. 2A.

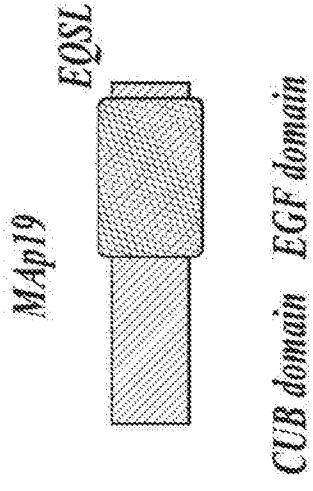
MAp19
EQSL
CUB domain
EGF domain

Fig. 2B.

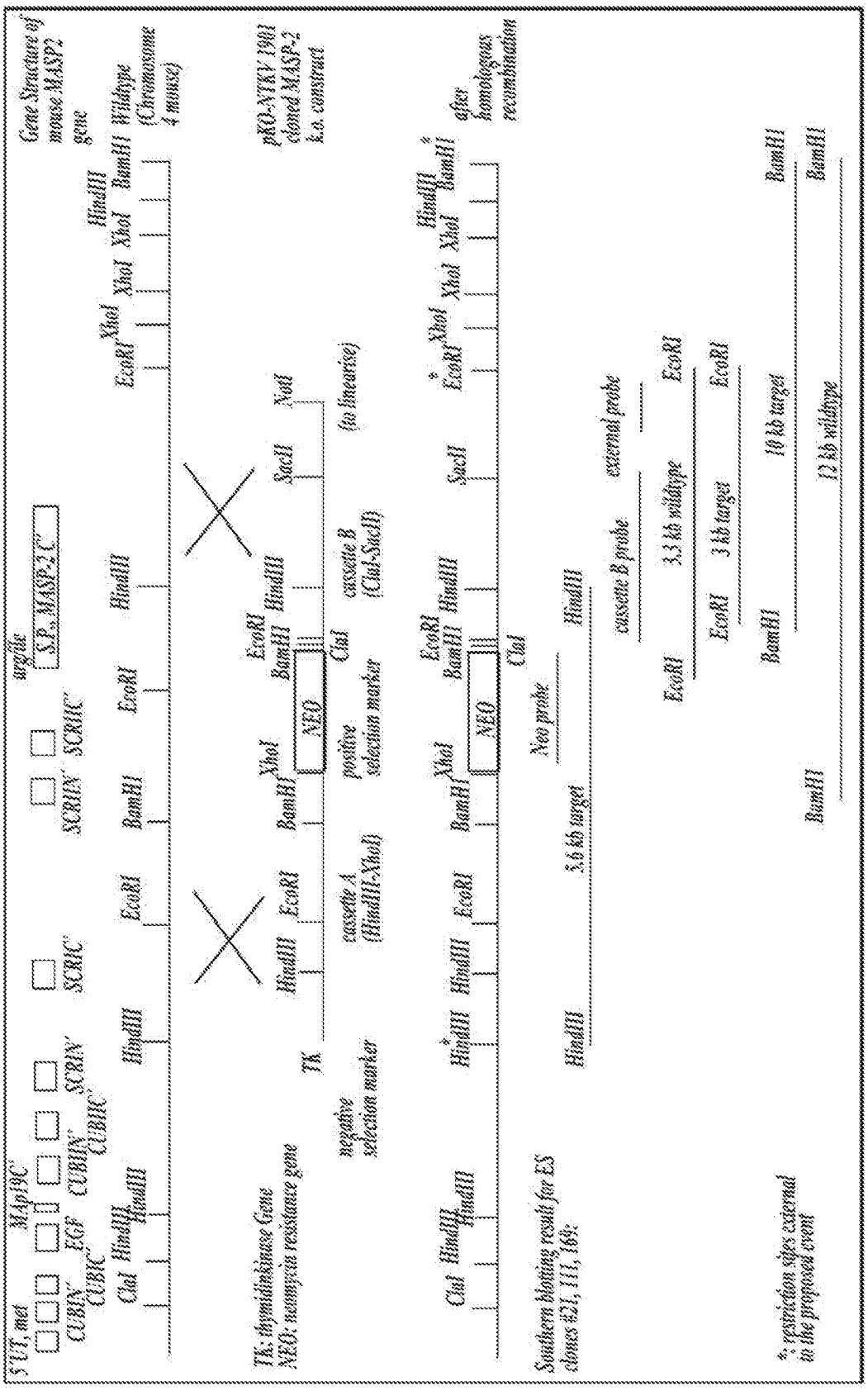
5'UT, met
Map1BC'
CUBIN'
EGF
CUBIC'
CUBIN'
CUBIC'
SCBIN'
SCBIC'
SCBIN'
SCBIC'
urgtlle
S.P. MASP-2 C'
Gene Structure of mouse MASP2 gene
ClaI HindIII HindIII
HindIII
EcoRI
BamH1
EcoRI
HindIII
EcoRI XhoI XhoI HindIII BamH1 Wildtype (Chromosome 4 mouse)
TK: thymidinkinase Gene
NEO: neomycin resistance gene
TK
negative selection marker
HindIII
EcoRI
cassette A (HindIII-XhoI)
BamH1
XhoI
positive selection marker
NEO
ClaI
EcoRI BamH1 HindIII
cassette B (ClaI-SacII)
SacII
NotI
(to linearise)
pKO-NTKV 1901 cloned MASP-2 k.o. construct
ClaI HindIII HindIII
HindIII*
HindIII
EcoRI
BamH1
XhoI
NEO
ClaI
EcoRI BamH1 HindIII
SacII
* EcoRI XhoI XhoI HindIII BamH1 *
after homologous recombination
Southern blotting result for ES clones #23, 111, 168:
HindIII
3.6 kb target
Neo probe
HindIII
cassette B probe
external probe
EcoRI
3.3 kb wildtype
EcoRI
EcoRI
3 kb target
EcoRI
BamH1
10 kb target
BamH1
BamH1
12 kb wildtype
BamH1
*: restriction sites external to the proposed event

Fig.3.

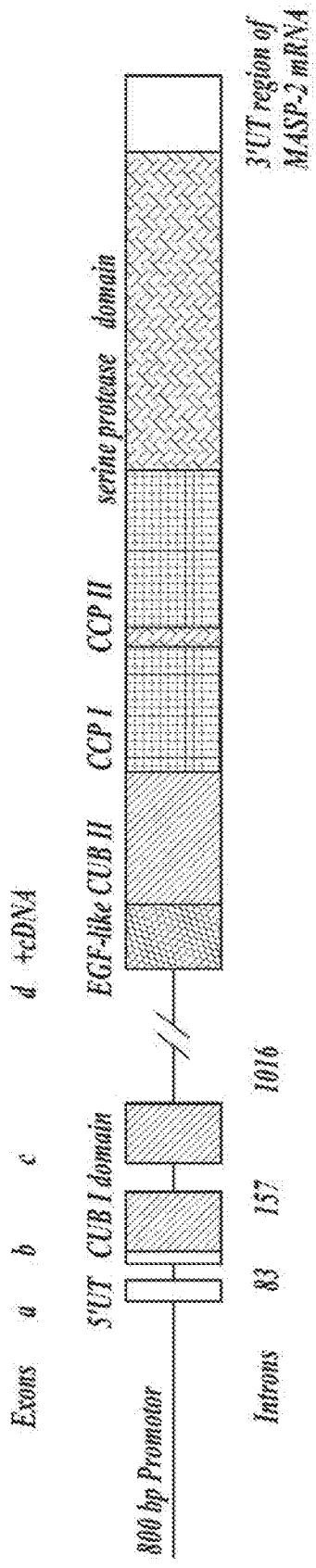
Exons a b c d +cDNA
5'UT CUB I domain EGF-like CUB II CCP I CCP II serine protease domain
3'UT region of MASP-2 mRNA
800 bp Promotor
Introns 83 157 1016

Fig.4.

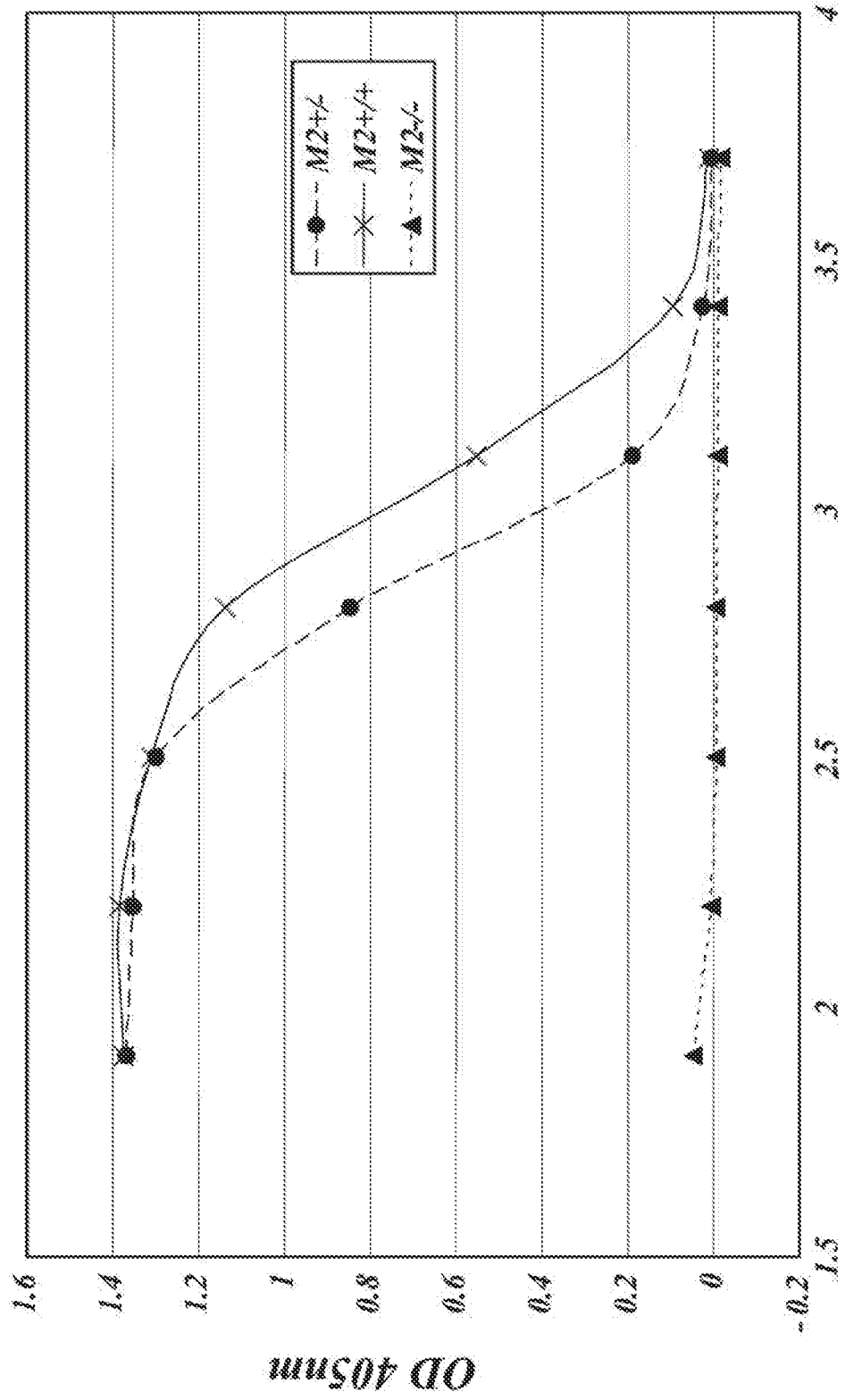
M2+/-
M2+/+
M2-/-
OD 405nm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
1.5
2
2.5
3
3.5
4
log serum dilutions

Fig. 5A.

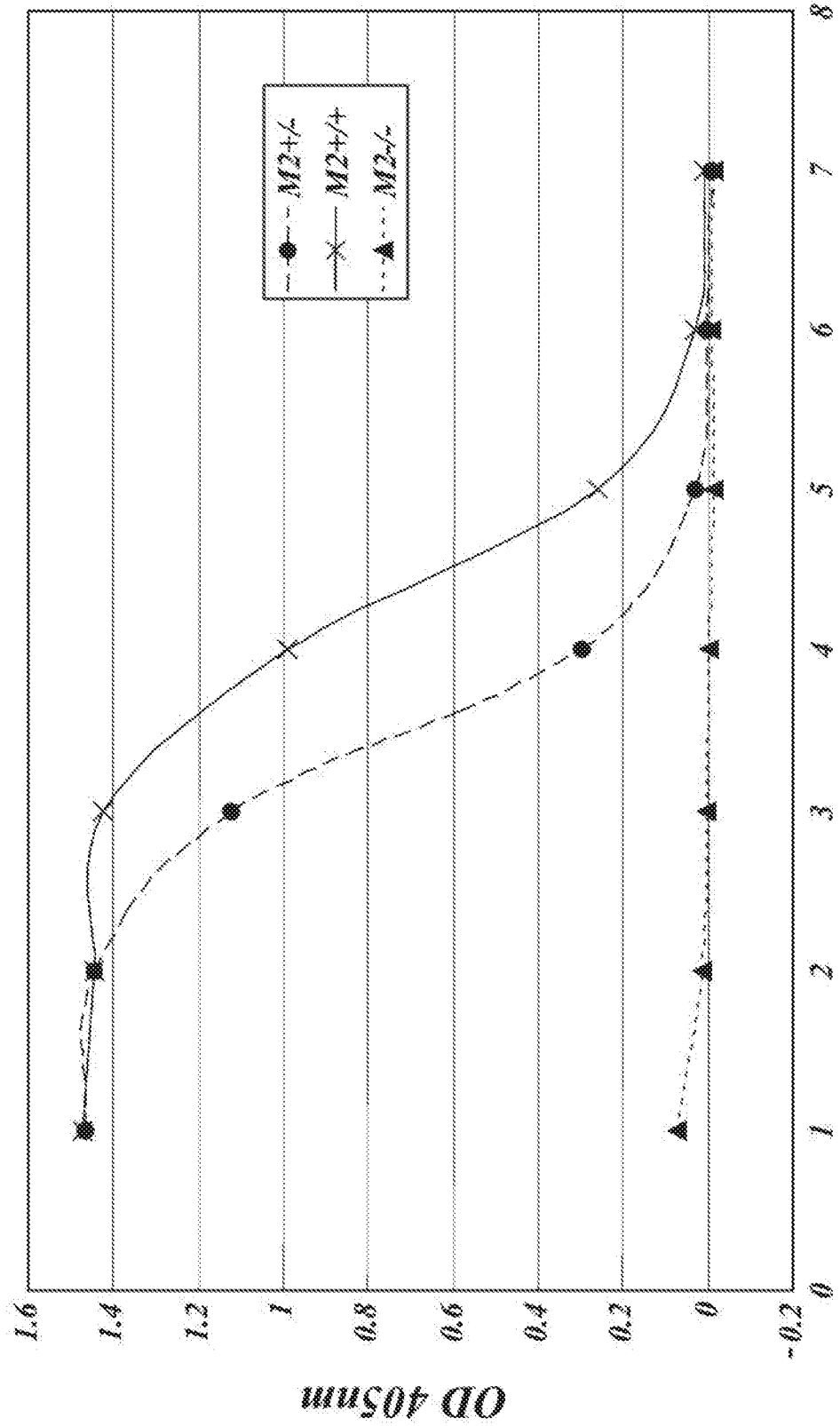
M2+/-
M2+/+
M2-/-
OD 405nm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
- 0.2
0
1
2
3
4
5
6
7
8
log serum dilutions

Fig.5B.

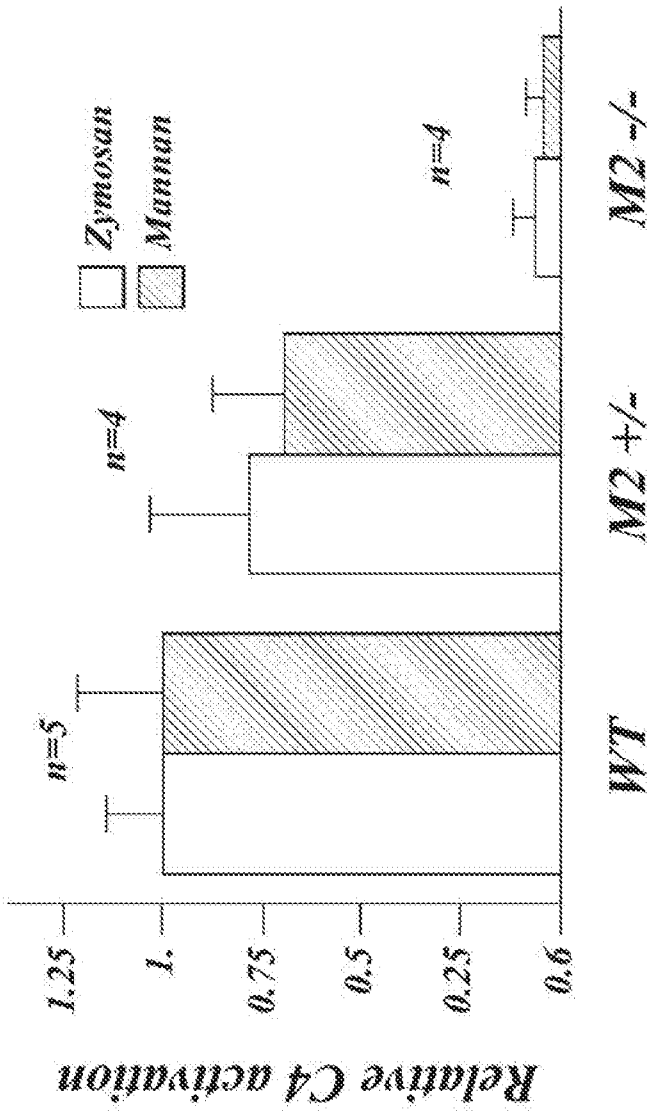
Relative C4 activation
1.25
1.
0.75
0.5
0.25
0.6
n=5
n=4
n=4
Zymosan
Mannan
WT
M2 +/-
M2 -/-

Fig. 5C.

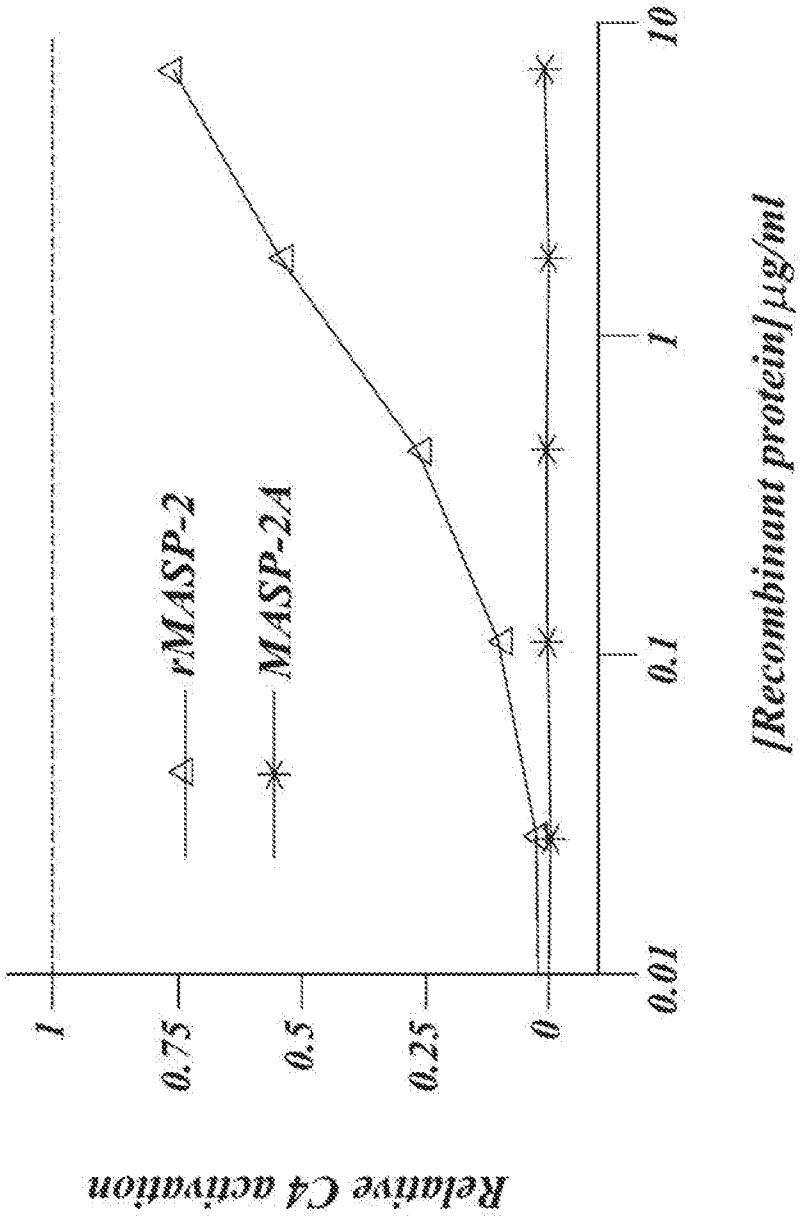
Relative C4 activation
1
0.75
0.5
0.25
0
0.01
0.1
1
10
[Recombinant protein] μg/ml
rMASP-2
MASP-2A

Fig. 6.

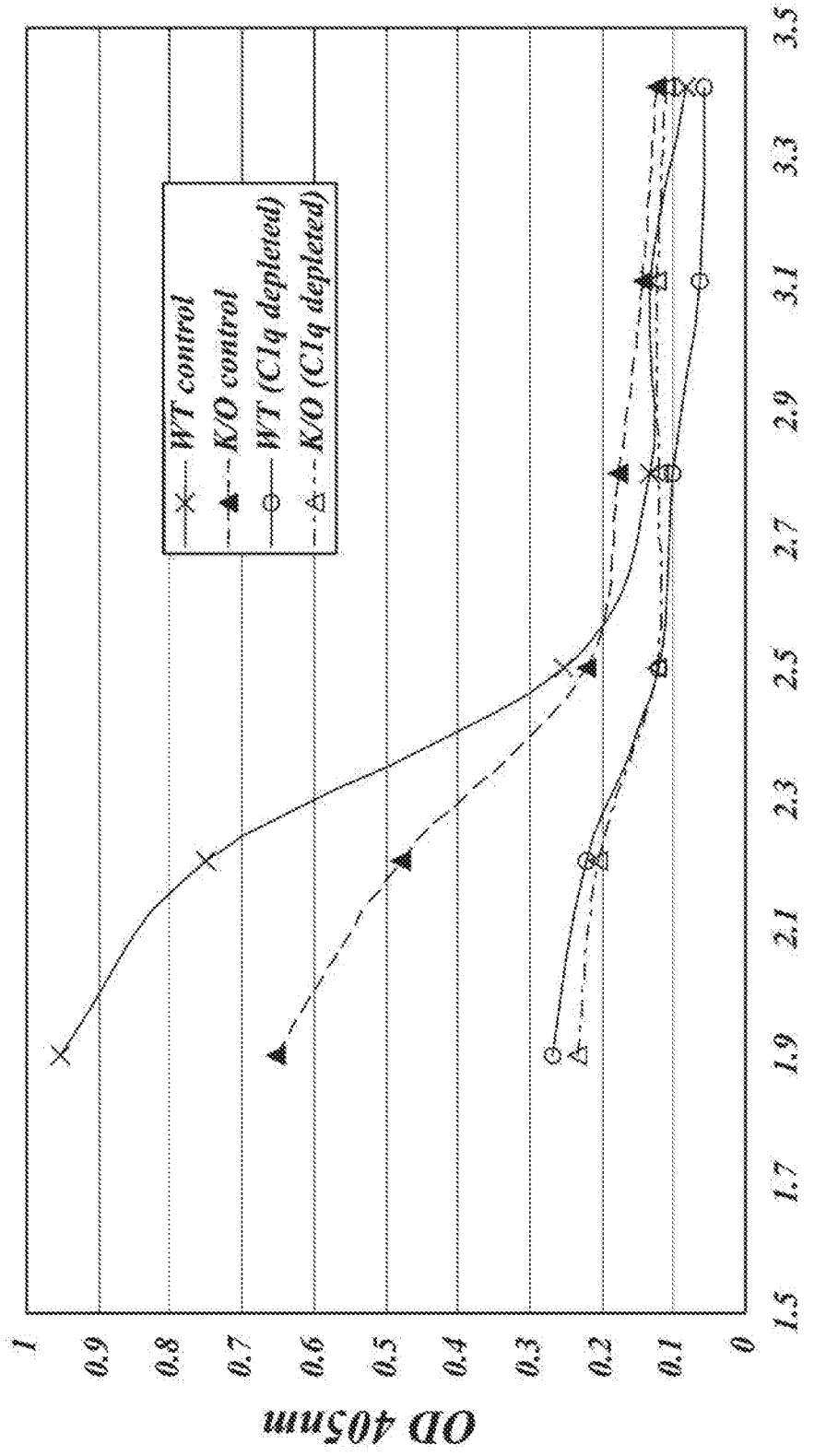
OD 405nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
log plasma dilution
WT control
K/O control
WT (C1q depleted)
K/O (C1q depleted)

Fig. 7.

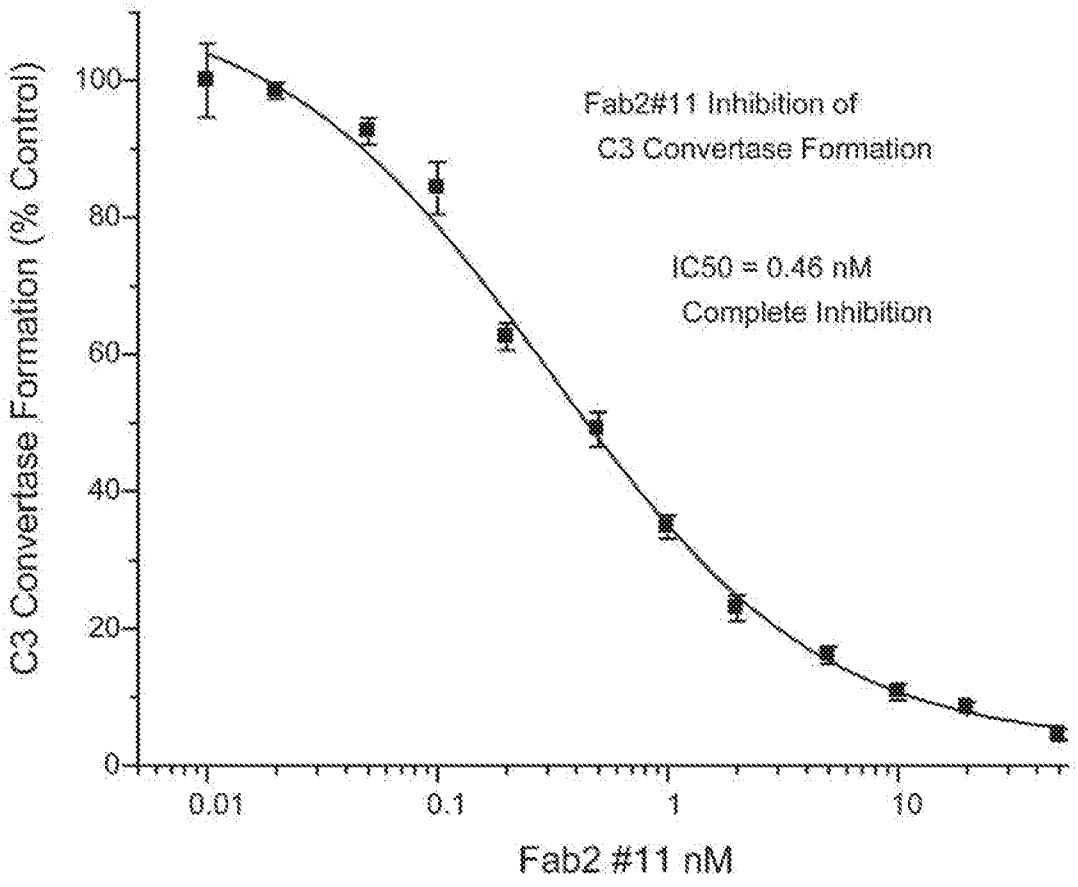
C3 Convertase Formation (% Control)
100
80
60
40
20
0
0.01
0.1
1
10
Fab2 #11 nM
Fab2#11 Inhibition of
C3 Convertase Formation
IC50 = 0.46 nM
Complete Inhibition

Fig.8A.

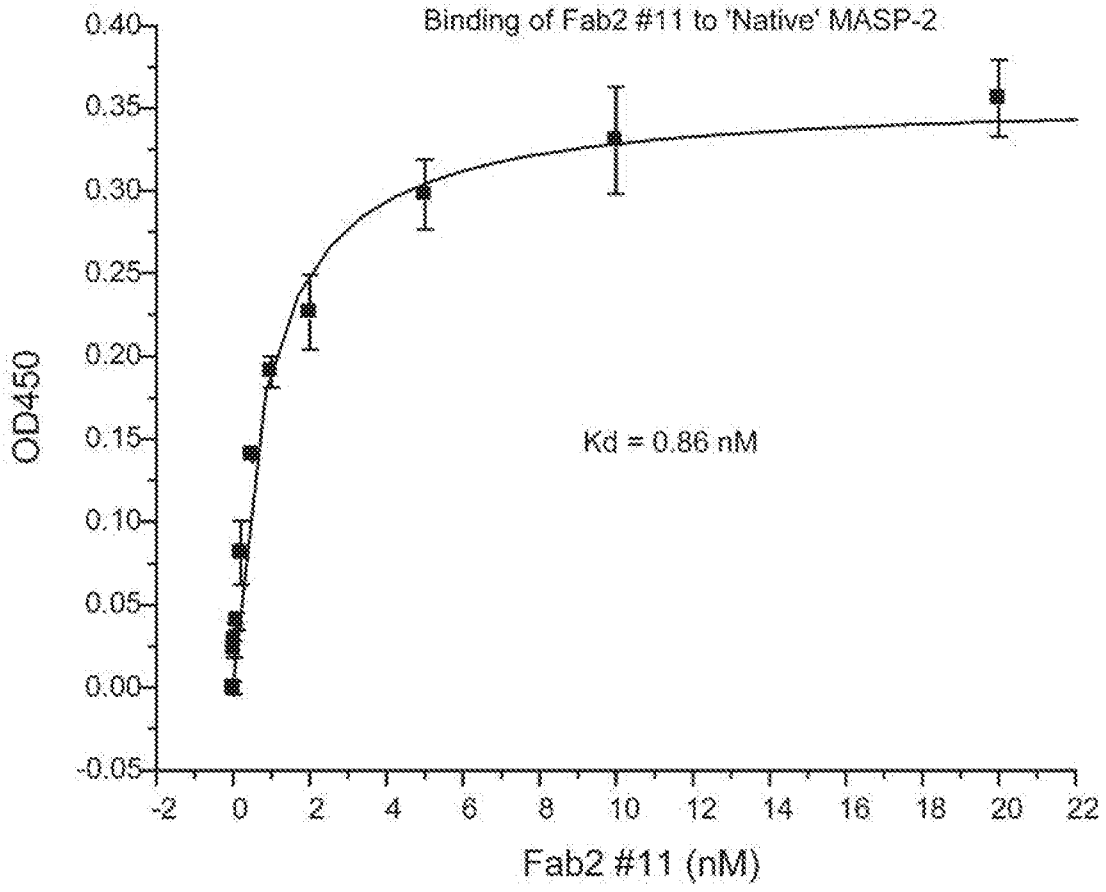
Binding of Fab2 #11 to 'Native' MASP-2
OD450
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
Kd = 0.86 nM
-2
0
2
4
6
8
10
12
14
16
18
20
22
Fab2 #11 (nM)

Fig.8B.

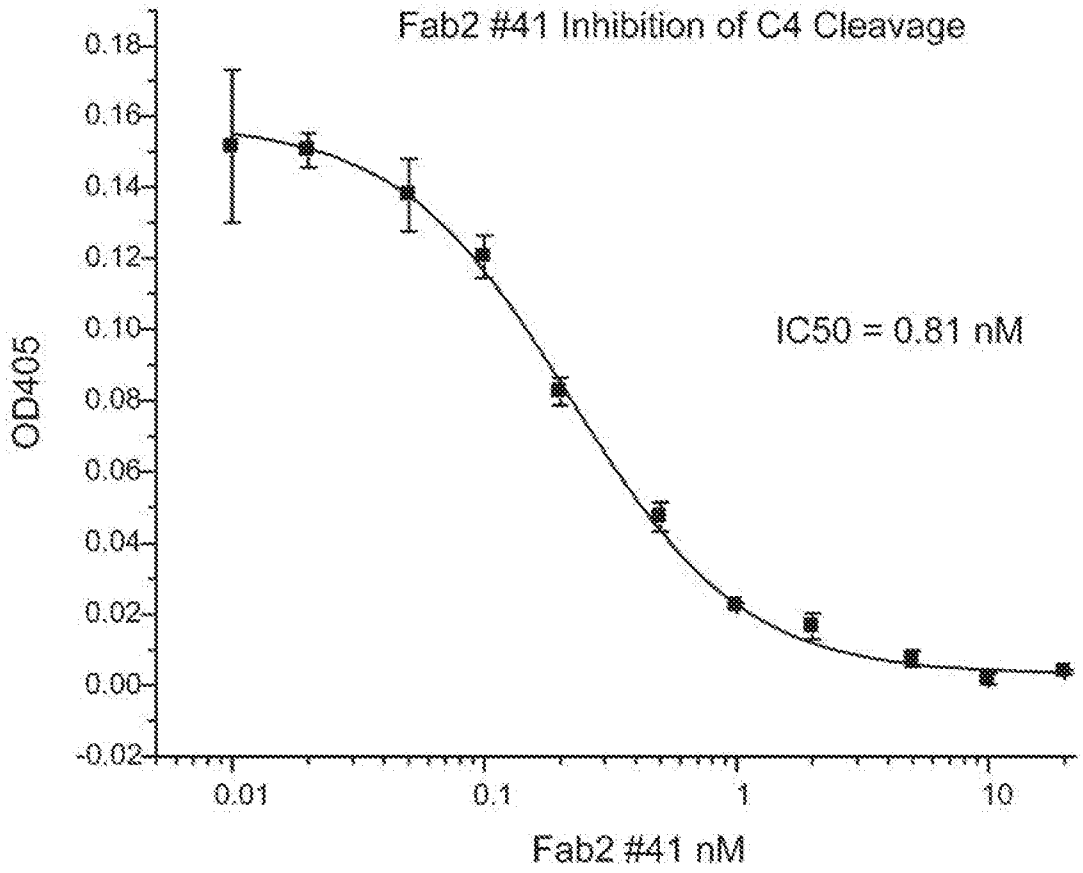
Fab2 #41 Inhibition of C4 Cleavage
IC50 = 0.81 nM
OD405
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.01
0.1
1
10
Fab2 #41 nM

Fig.8C.

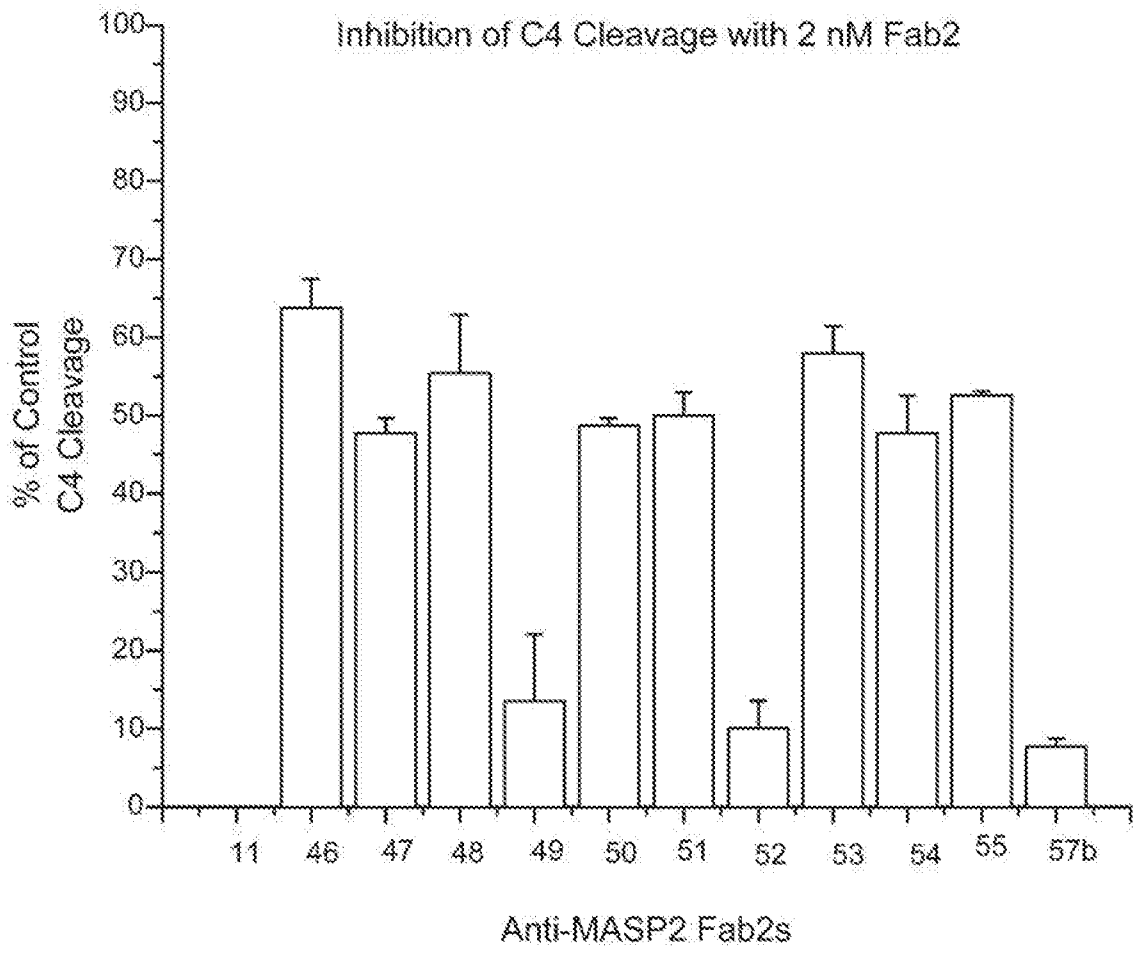
Inhibition of C4 Cleavage with 2 nM Fab2
% of Control C4 Cleavage
100
90
80
70
60
50
40
30
20
10
0
11
46
47
48
49
50
51
52
53
54
55
57b
Anti-MASP2 Fab2s

Fig. 9.

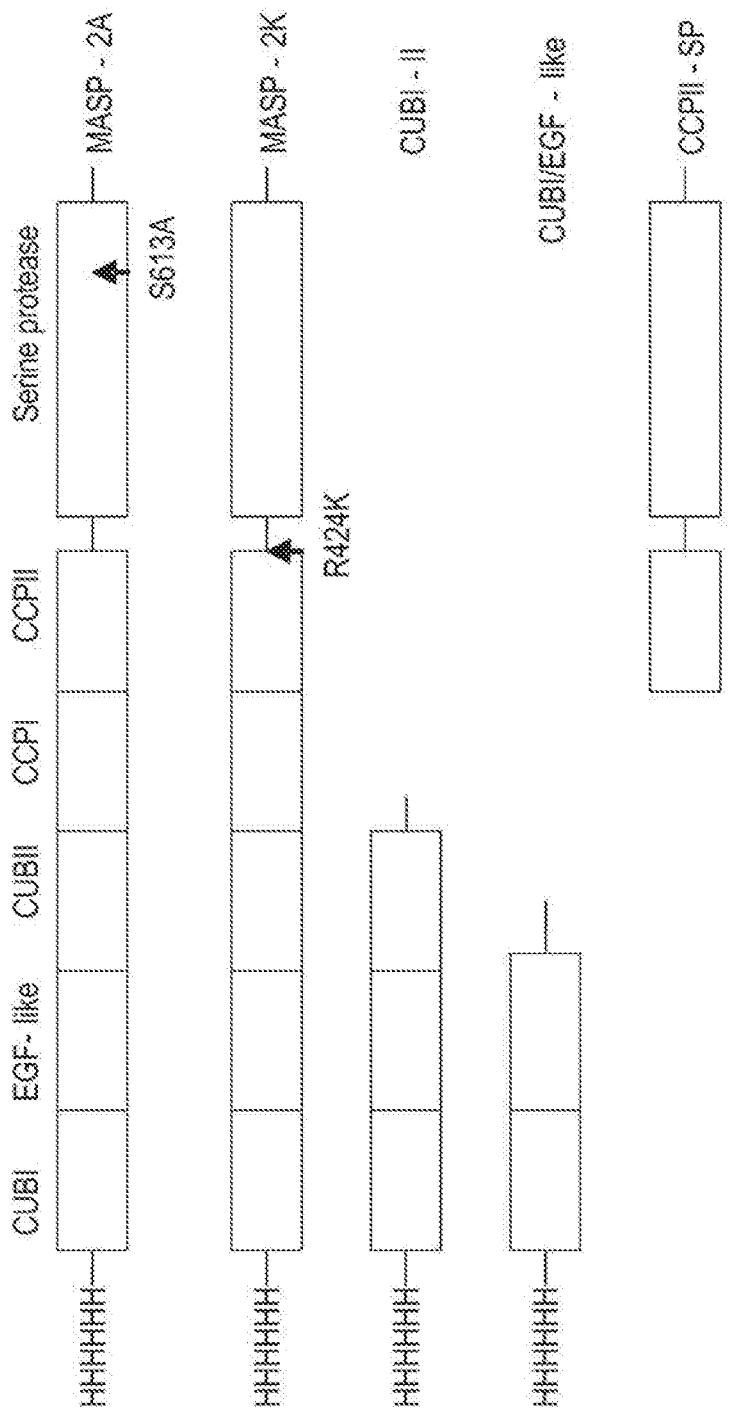
CUBI
EGF- like
CUBII
CCPI
CCPII
Serine protease
MASP - 2A
S613A
HHHHHH
MASP - 2K
R424K
HHHHHH
CUBI - II
HHHHHH
CUBI/EGF - like
HHHHHH
CCPII - SP

Fig. 10.

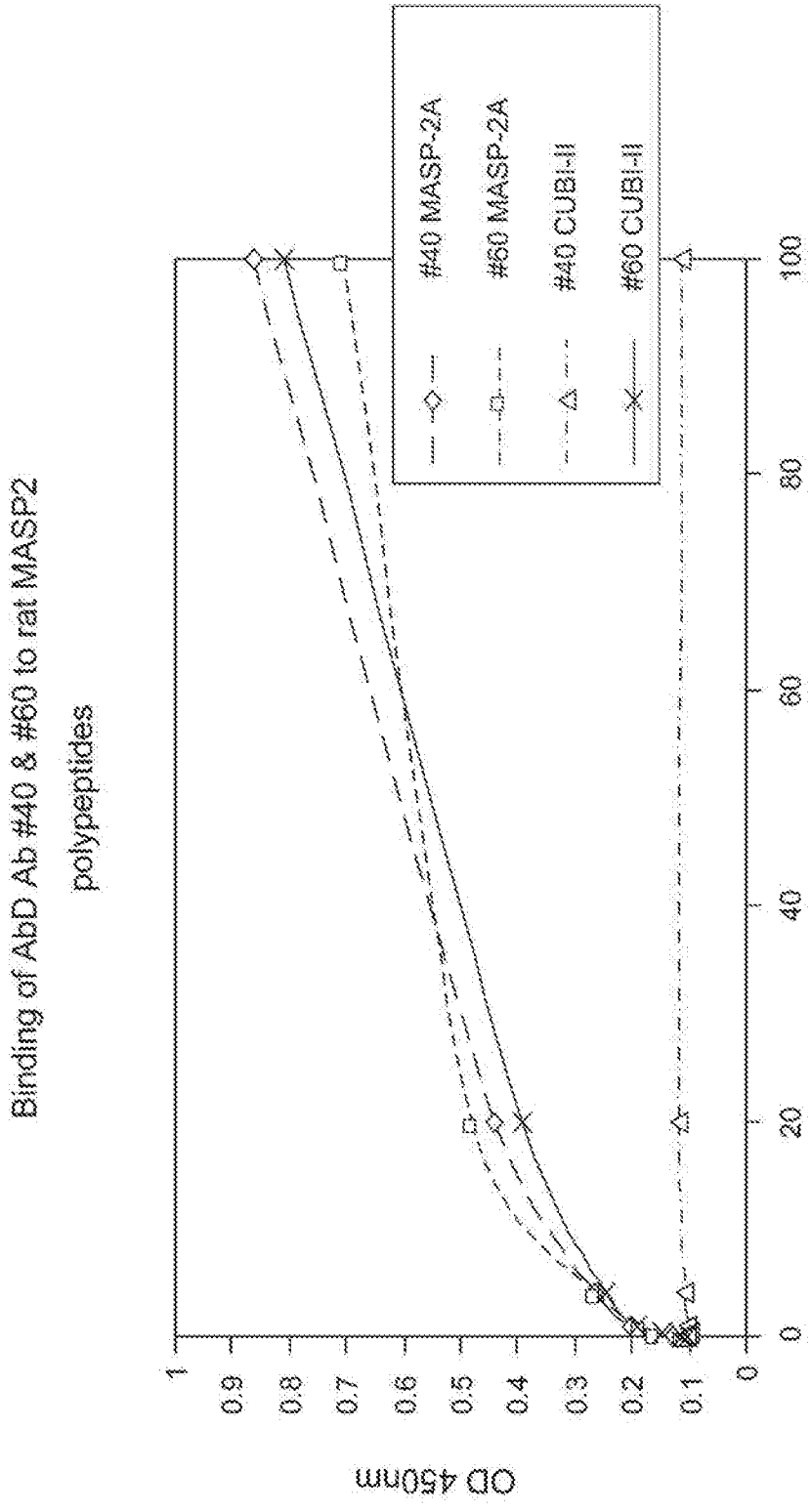
Binding of AbD Ab #40 & #60 to rat MASP2
polypeptides
#40 MASP-2A
#60 MASP-2A
#40 CUBI-II
#60 CUBI-II
OD 450nm
[Ab] nM
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100

Fig. 11.

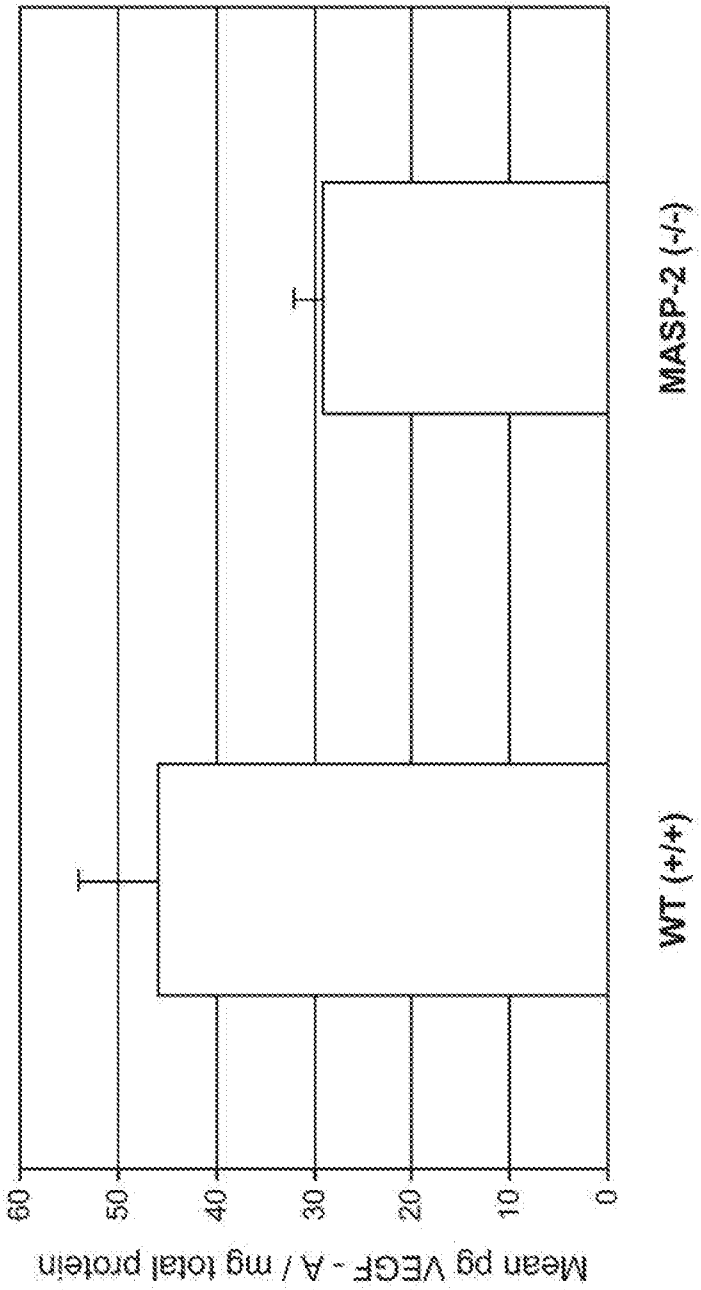

Fig. 12A.

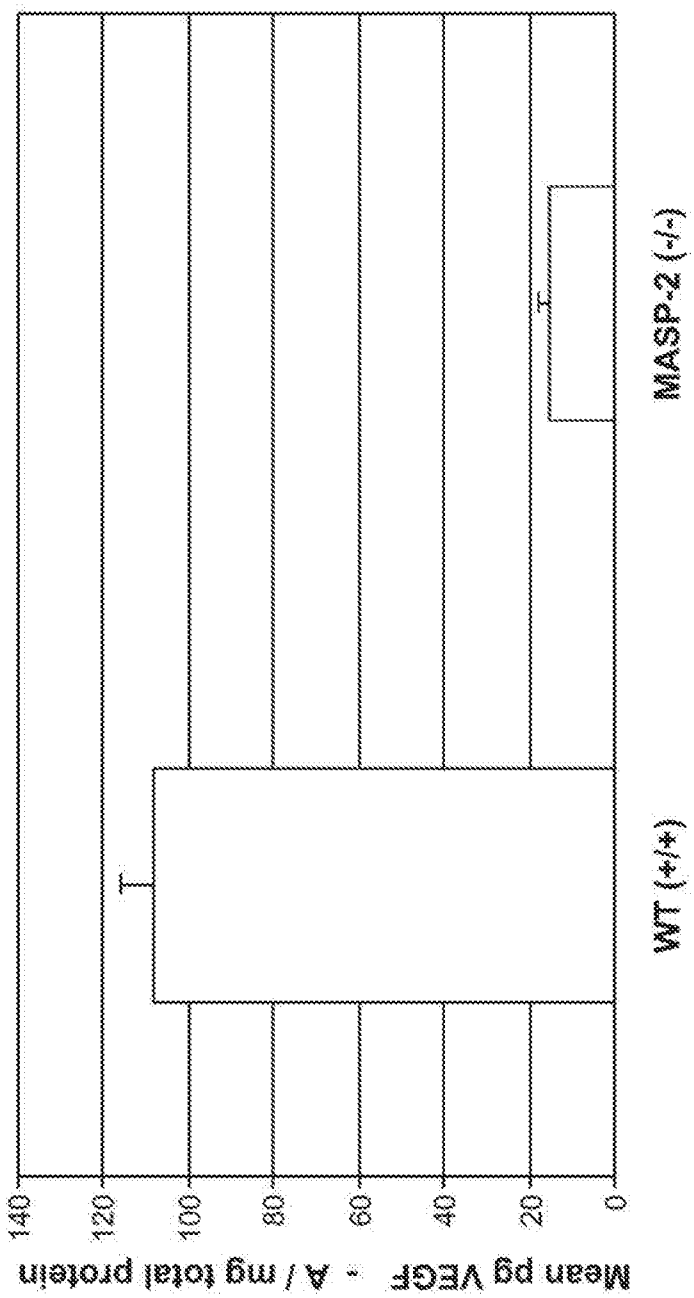
Mean pg VEGF - A / mg total protein
0
20
40
60
80
100
120
140
WT (+/+)
MASP-2 (-/-)

Fig. 12B.

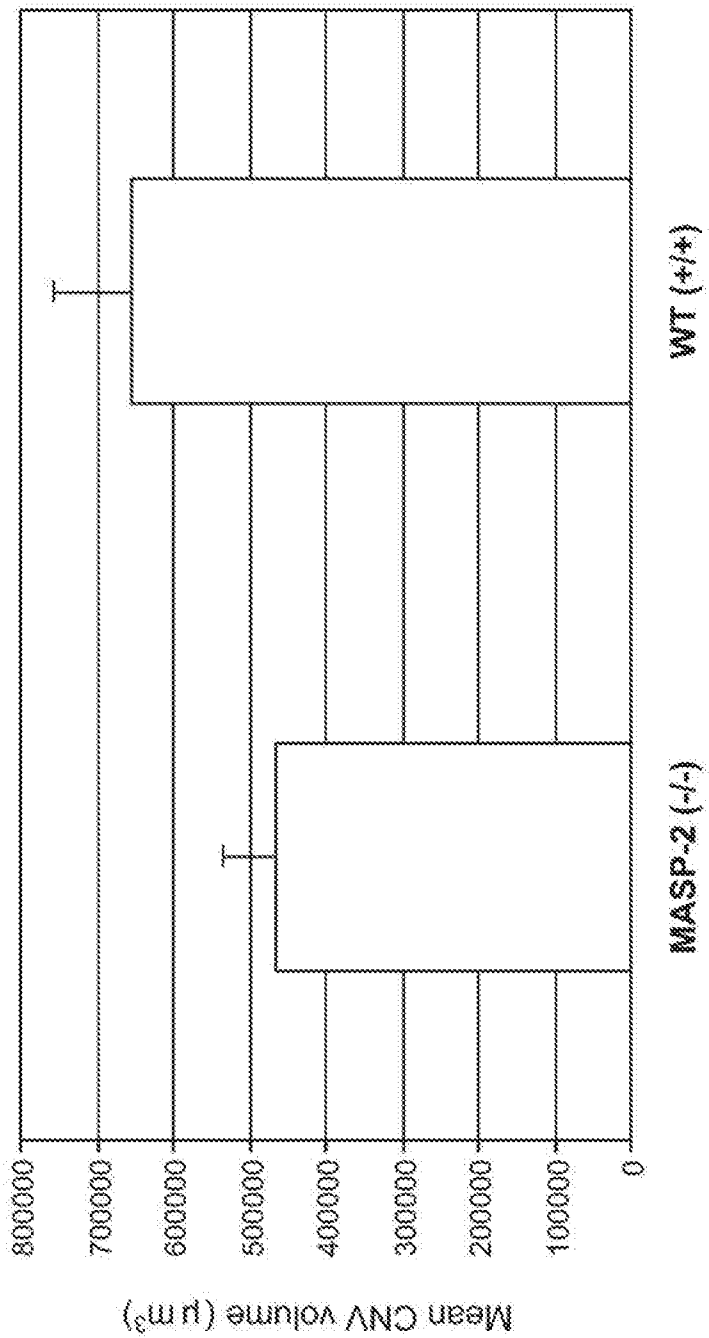
Mean CNV volume (μm³)
800000
700000
600000
500000
400000
300000
200000
100000
0
MASP-2 (-/-)
WT (+/+)

Fig. 13.

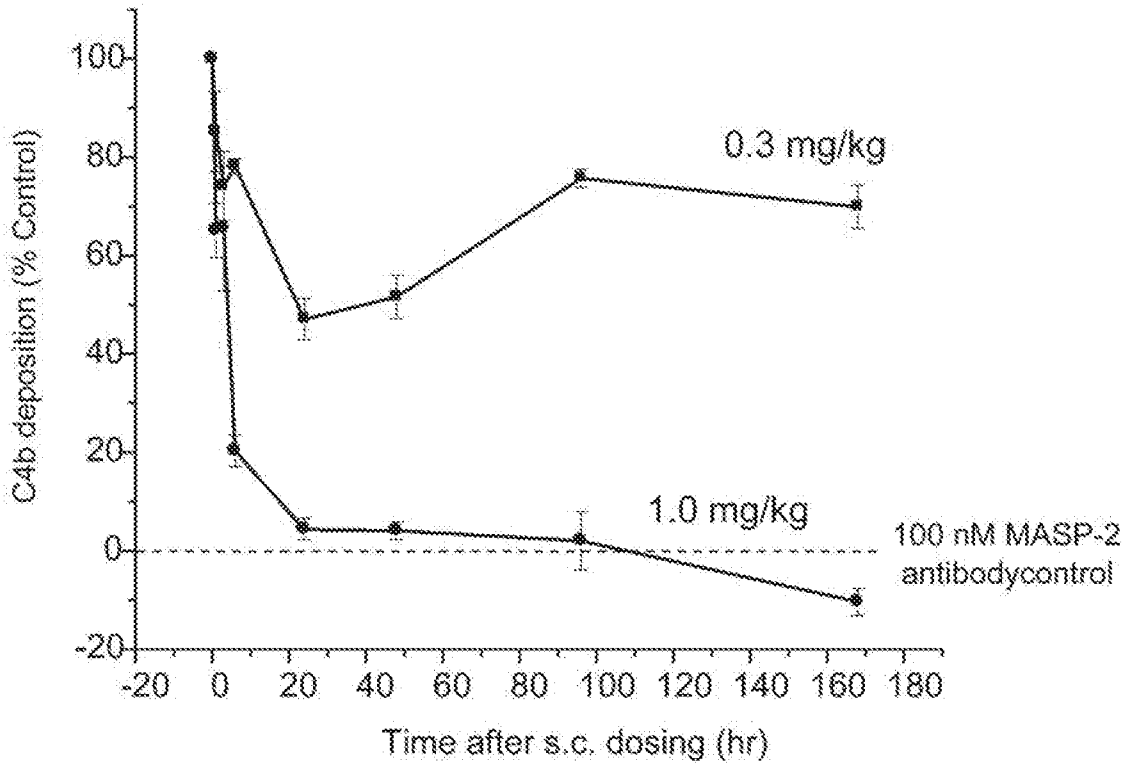
C4b deposition (% Control)
100
80
60
40
20
0
-20
0.3 mg/kg
1.0 mg/kg
100 nM MASP-2
antibodycontrol
-20 0 20 40 60 80 100 120 140 160 180
Time after s.c. dosing (hr)

Fig.14.

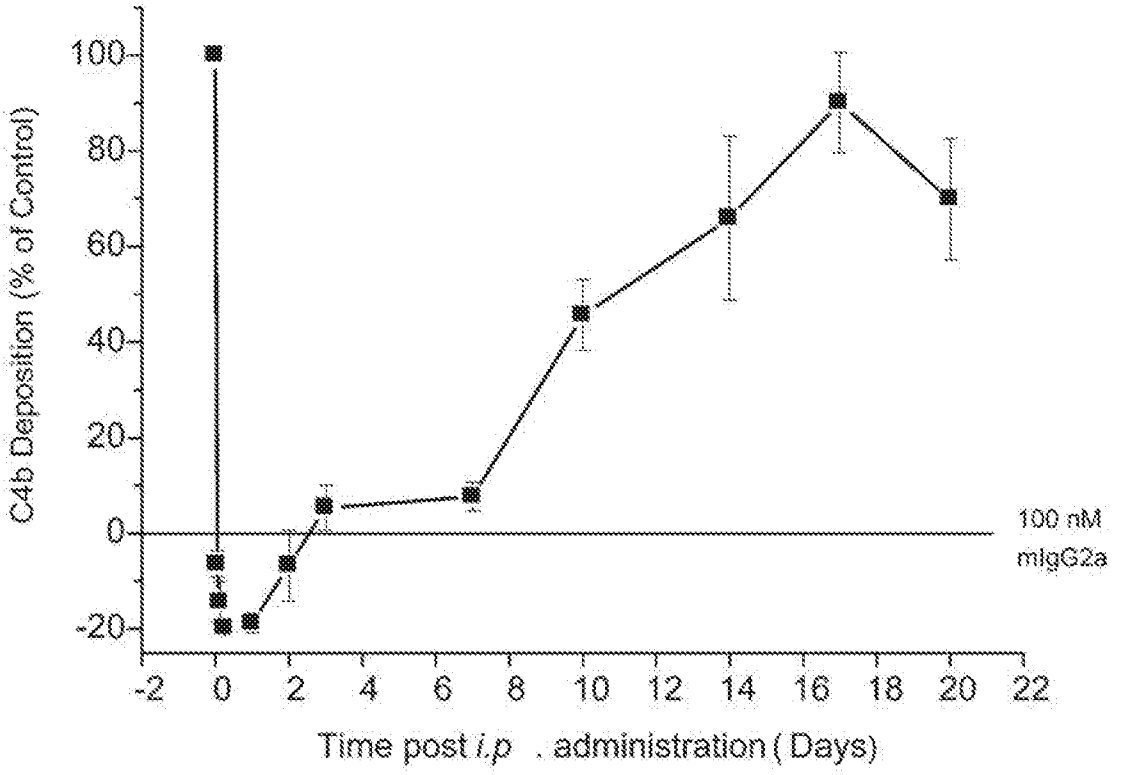
C4b Deposition (% of Control)
100
80
60
40
20
0
-20
100 nM
mIgG2a
-2 0 2 4 6 8 10 12 14 16 18 20 22
Time post *i.p* . administration (Days)

Fig.15.

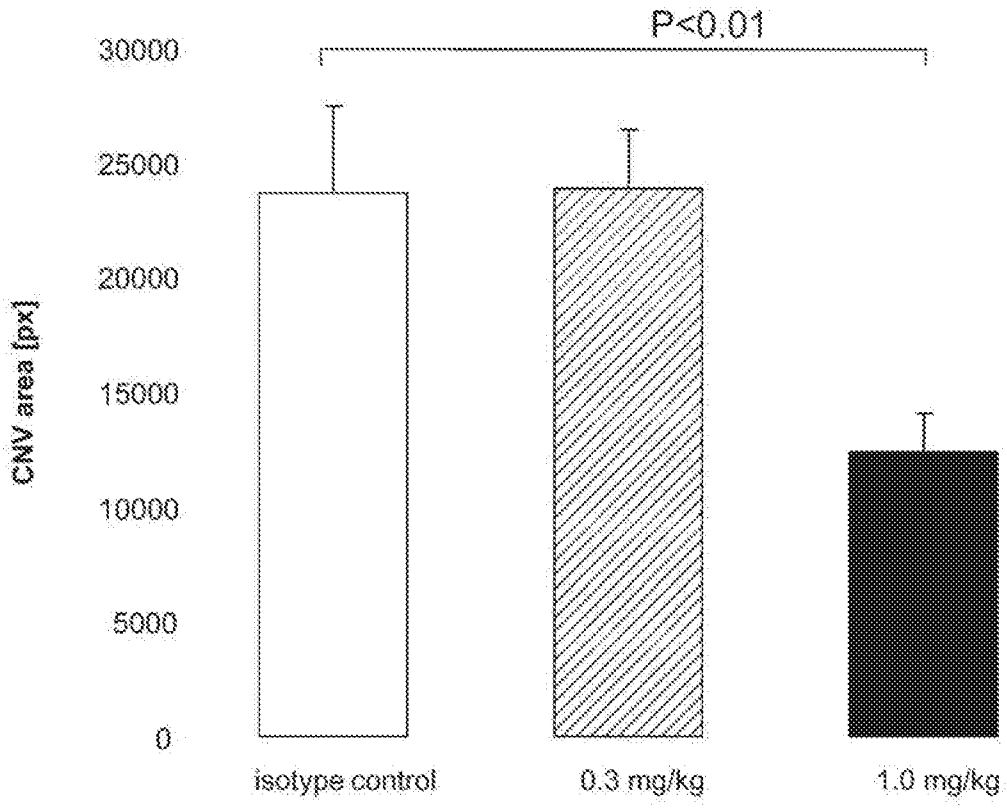
P<0.01
30000
25000
20000
15000
10000
5000
0
CNV area [px]
isotype control
0.3 mg/kg
1.0 mg/kg

Fig. 16.

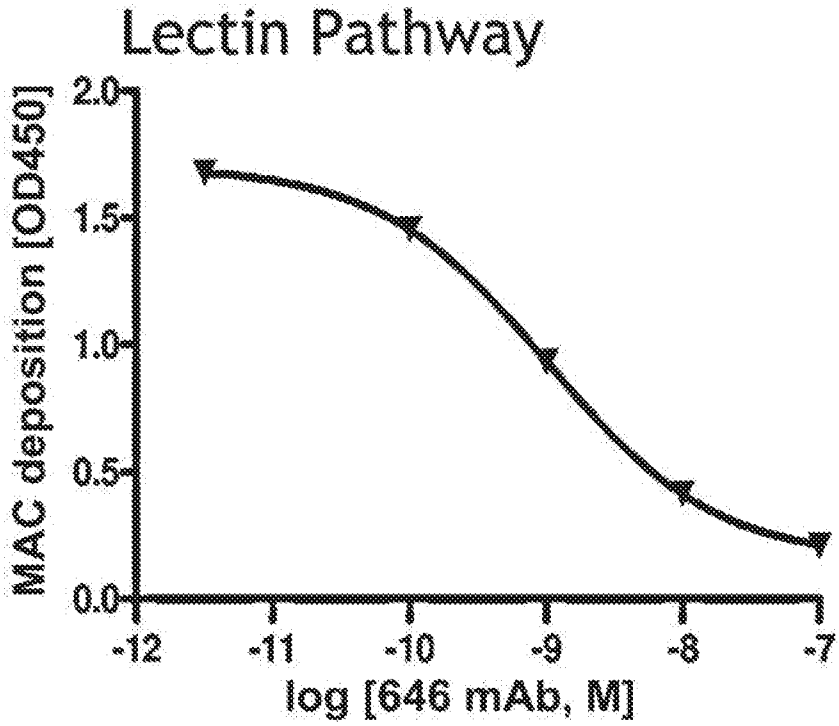

Fig.17A.

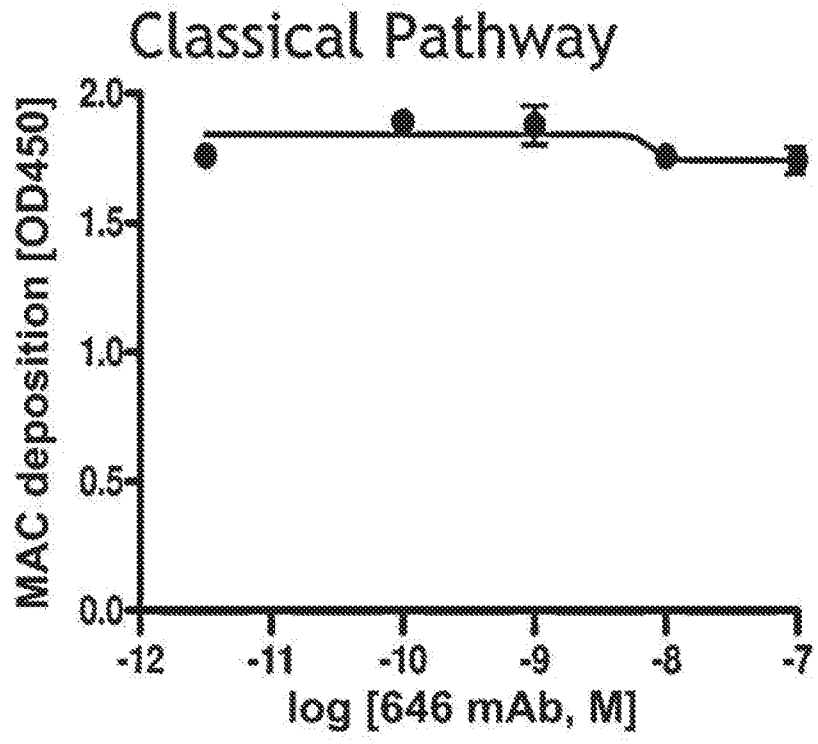

Fig.17B.

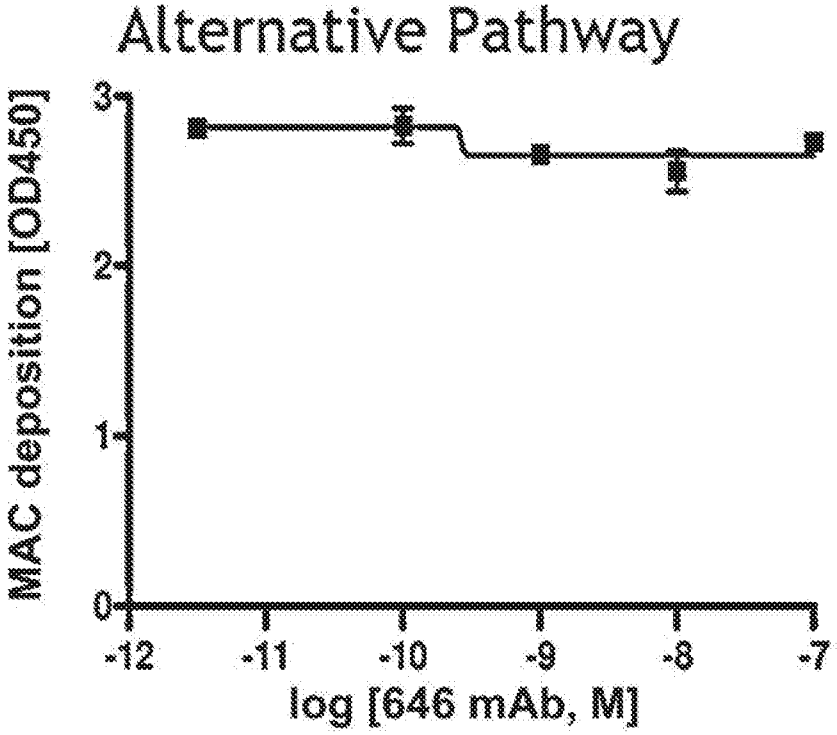
Alternative Pathway
MAC deposition [OD450]
0
1
2
3
-12
-11
-10
-9
-8
-7
log [646 mAb, M]

Fig.17C.

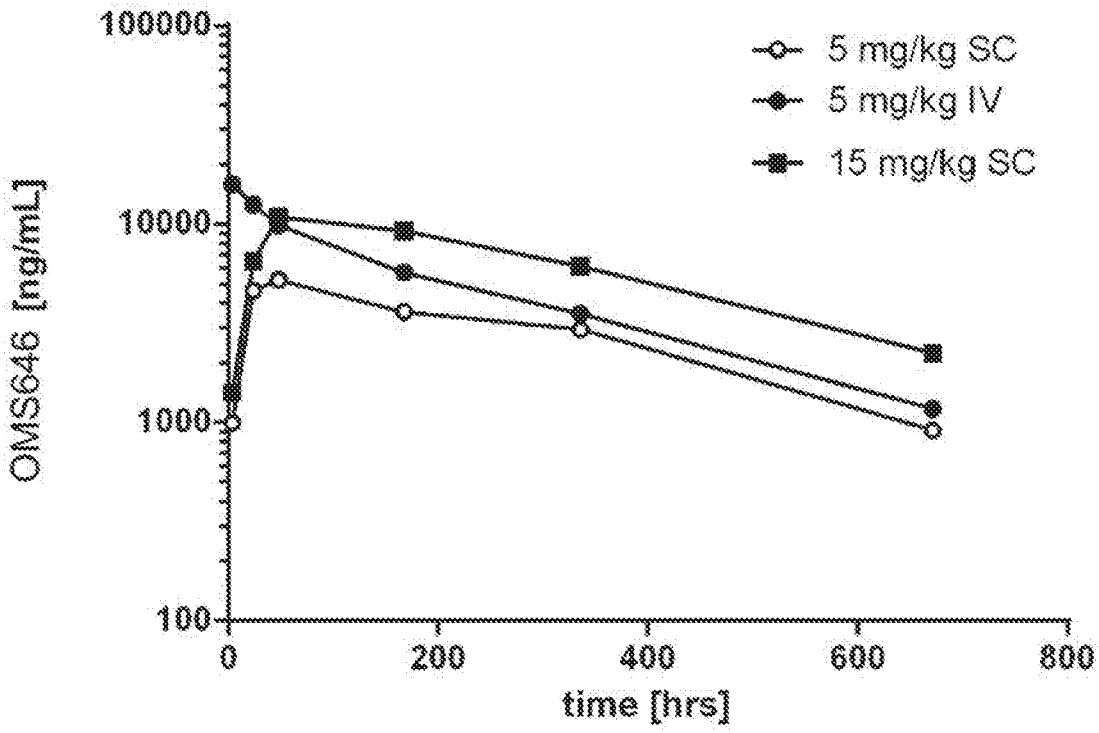
100000
10000
1000
100
OMS646 [ng/mL]
0
200
400
600
800
time [hrs]
5 mg/kg SC
5 mg/kg IV
15 mg/kg SC

Fig. 18

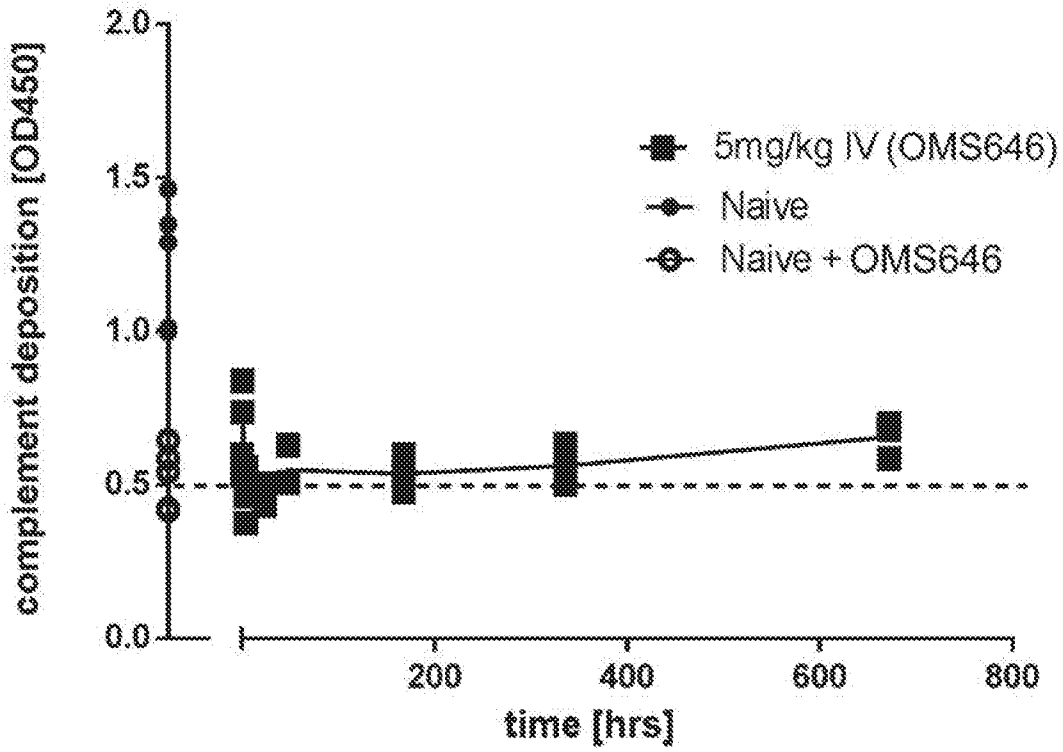

Fig.19A.

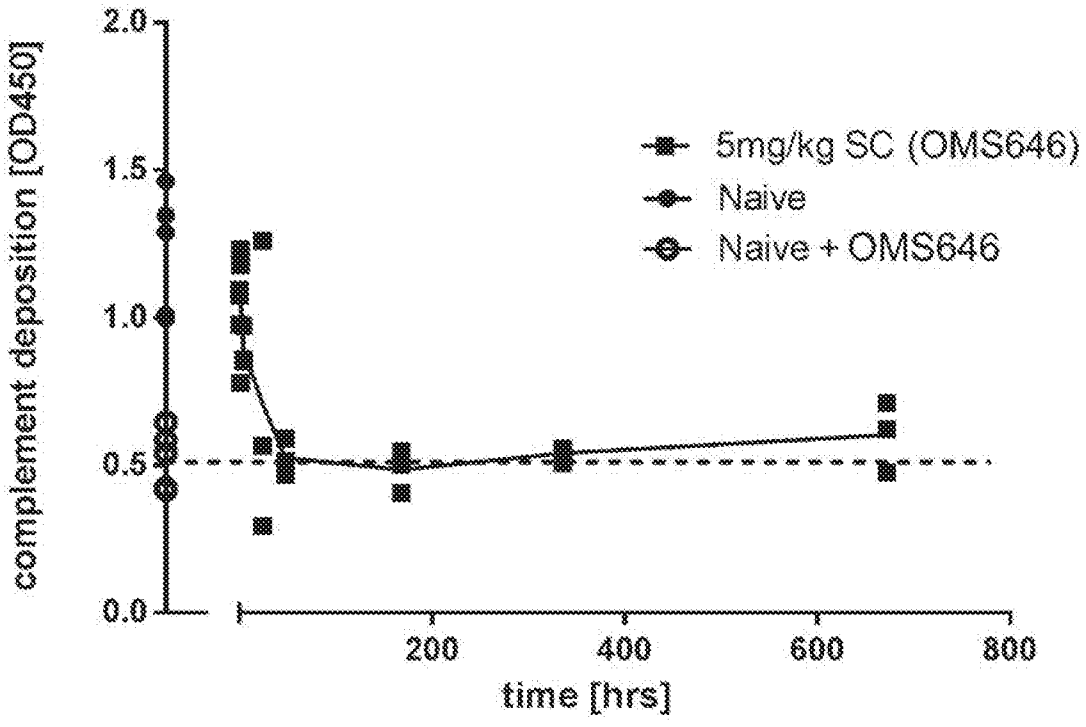

Fig.19B.

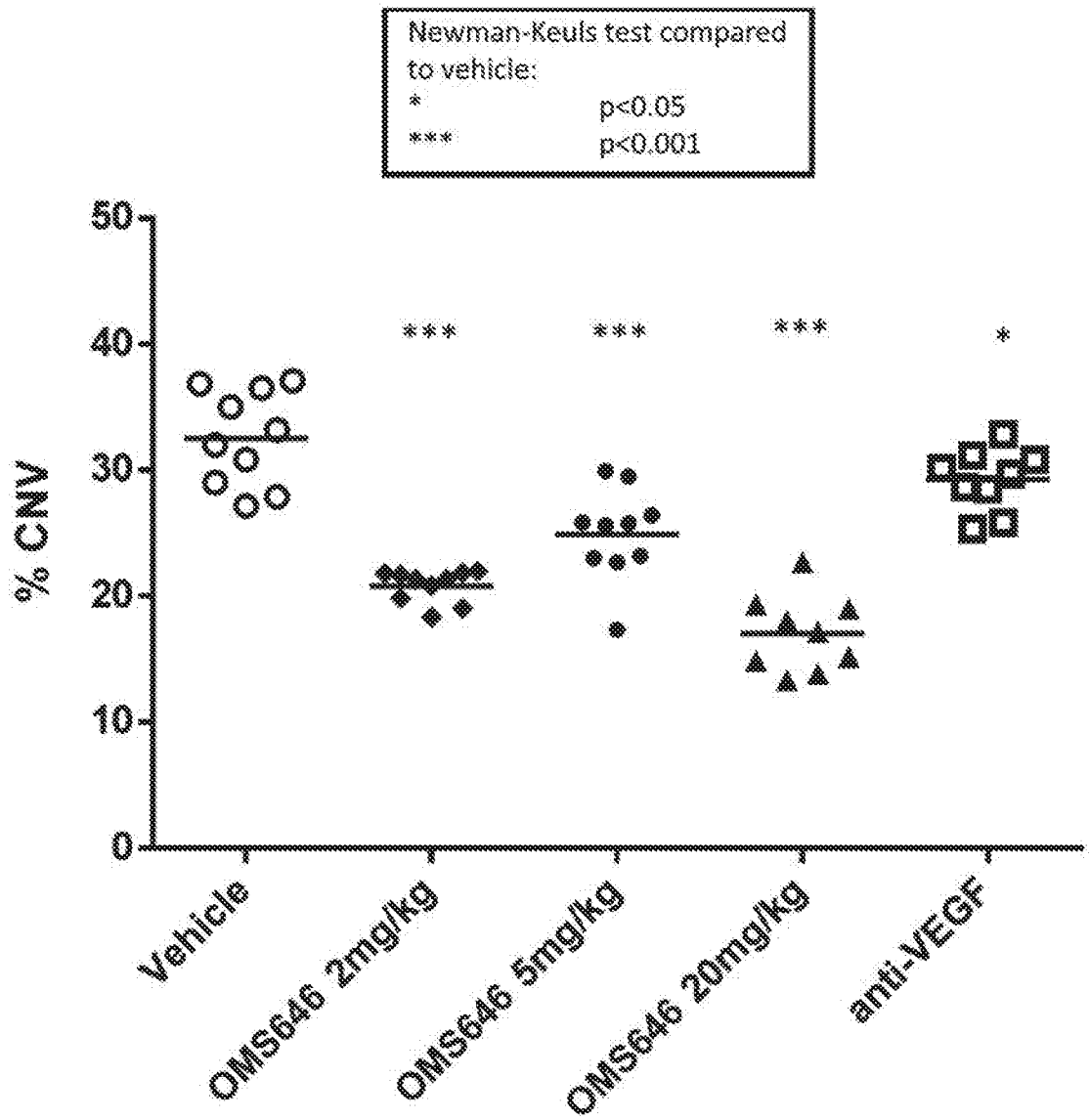
Newman-Keuls test compared to vehicle:
* p<0.05
*** p<0.001
% CNV
50
40
30
20
10
0
***
***
***
*
Vehicle
OMS646 2mg/kg
OMS646 5mg/kg
OMS646 20mg/kg
anti-VEGF

Fig. 20.