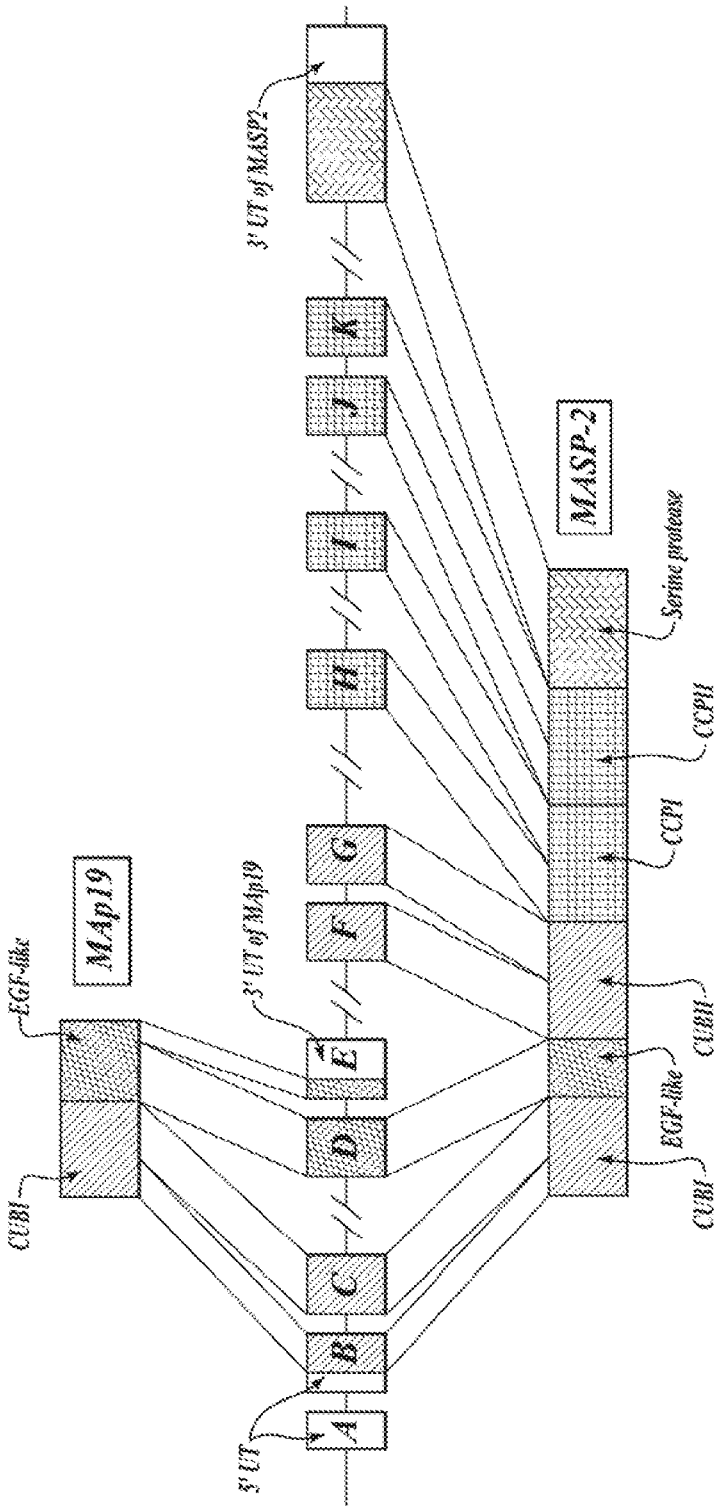
3' UT of MASP2
K
J
I
H
G
F
E
D
C
B
A
5' UT
MASP-2
Serine protease
CCPII
CCPI
CUBII
EGF-like
CUBI
MAp19
EGF-like
3' UT of MAp19
CUBI

FIG. 1

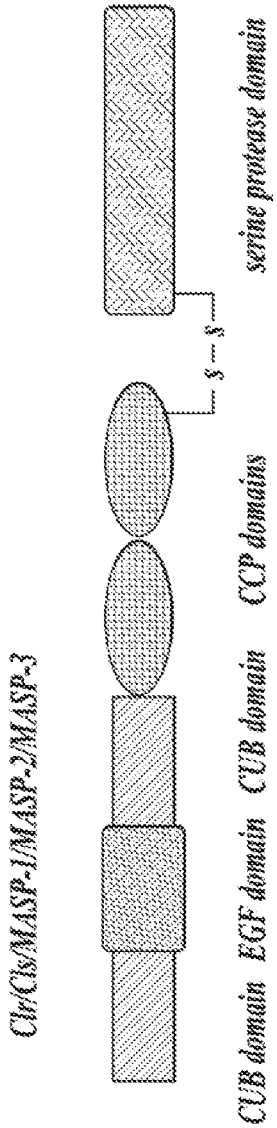
Clr/Cls/MASP-1/MASP-2/MASP-3
CUB domain
EGF domain
CUB domain
CCP domains
S – S
serine protease domain

FIG. 2A

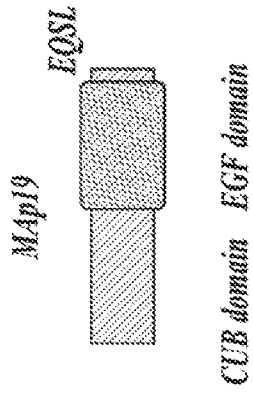
MAp19
EQSL
CUB domain
EGF domain

FIG. 2B

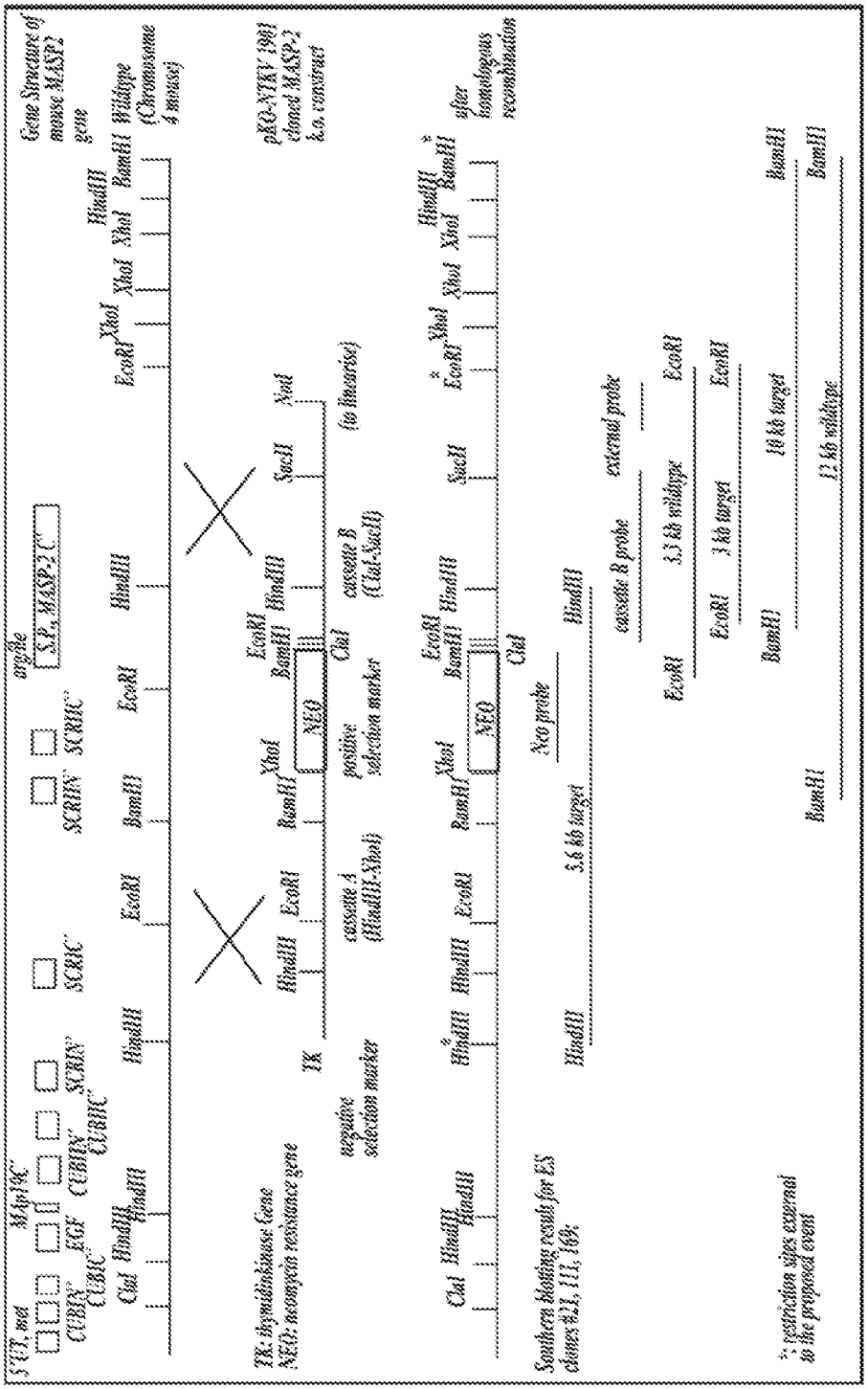
Gene Structure of mouse MASP2 gene
pKO-NTKV 1901 cloned MASP-2 k.o. construct
after homologous recombination
Wildtype (Chromosome 4 mouse)
TK: thymidinkinase Gene
NEO: neomycin resistance gene
negative selection marker
positive selection marker
cassette A (HindIII-XbaI)
cassette B (ClaI-SacII)
(to linearise)
Neo probe
external probe
cassette B probe
Southern blotting result for ES clones #21, 111, 169:
3.6 kb target
3.3 kb wildtype
3 kb target
10 kb target
11 kb wildtype
*: restriction sites external to the proposed event

FIG. 3

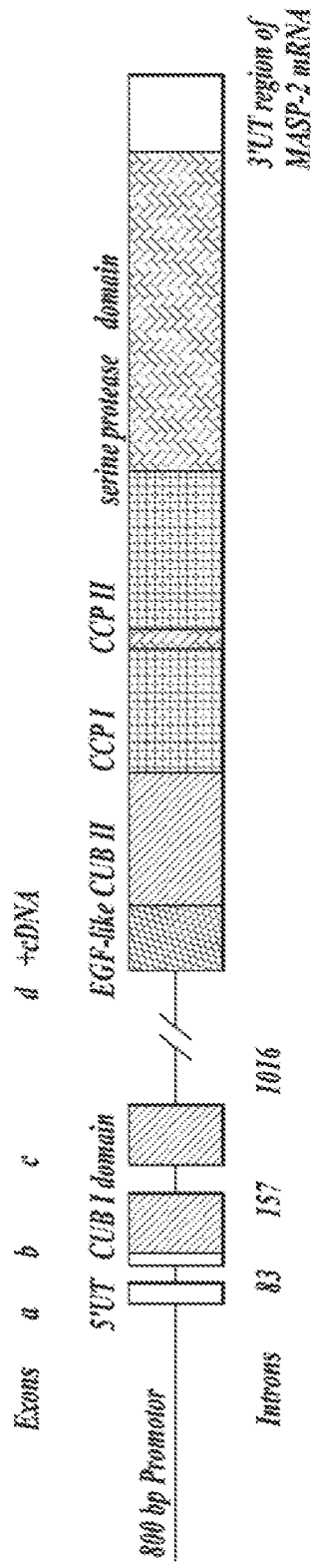
Exons a b c d +cDNA
5'UT CUB I domain EGF-like CUB II CCP I CCP II serine protease domain
3'UT region of MASP-2 mRNA
800 bp Promotor
Introns 83 157 1016

FIG. 4

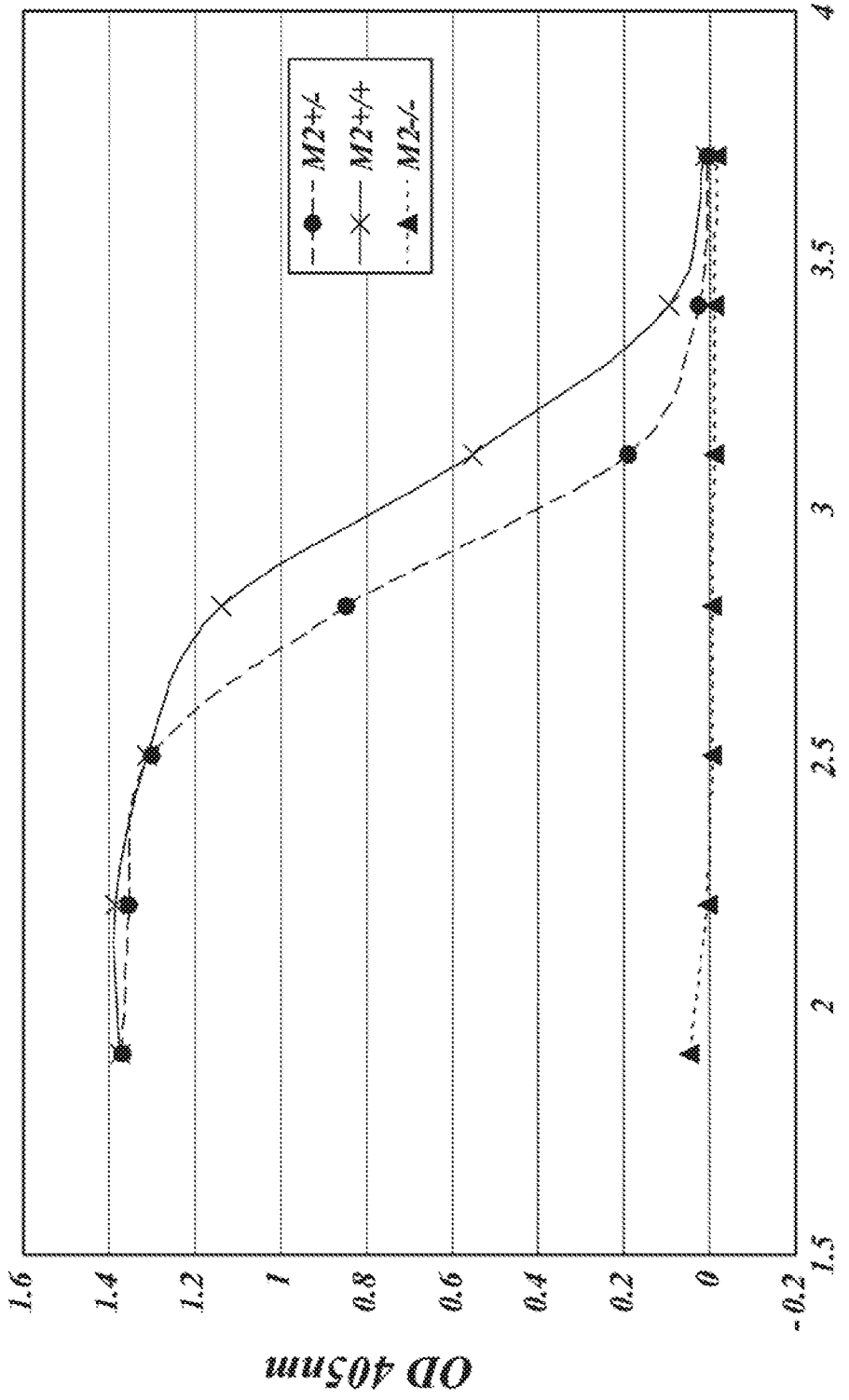
M2+/+
M2+/-
M2-/-
OD 405nm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
- 0.2
1.5
2
2.5
3
3.5
4
log serum dilutions

FIG. 5A

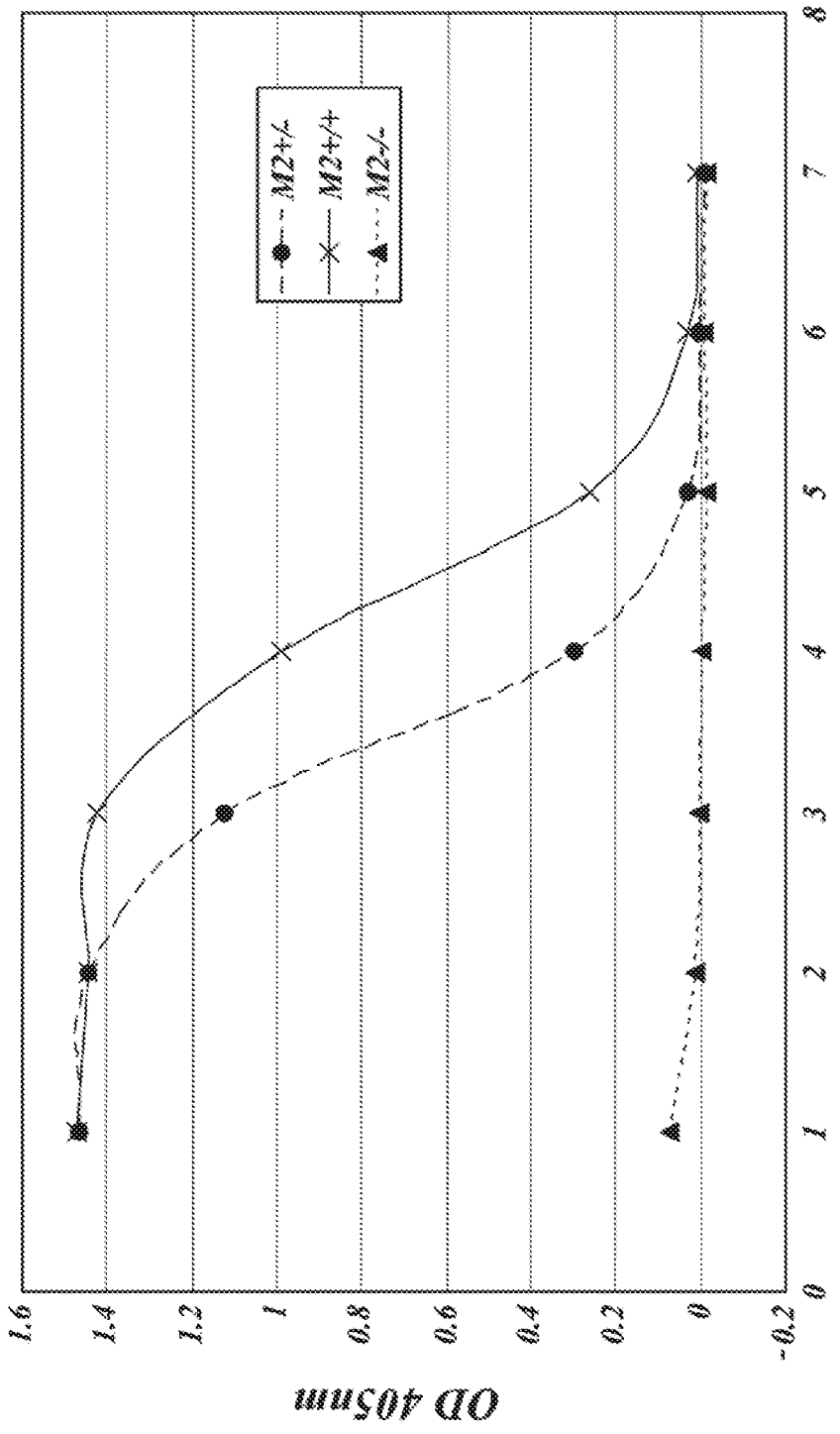
M2+/-
M2+/+
M2-/-
OD 405nm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
1
2
3
4
5
6
7
8
log serum dilutions

FIG. 5B

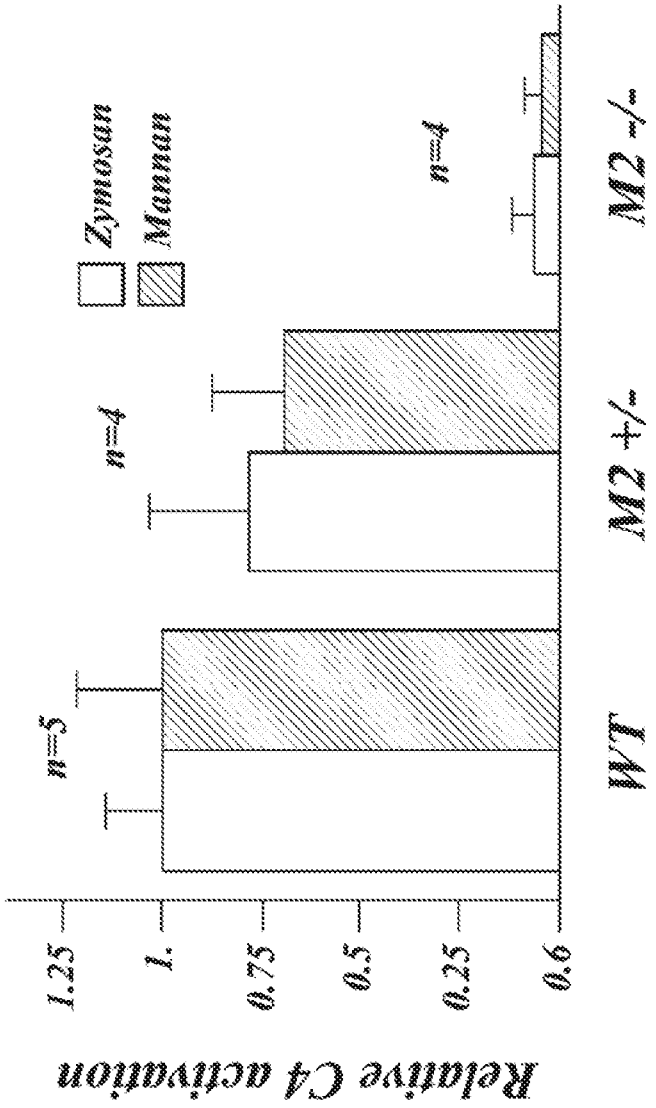
Relative C4 activation
1.25
1.
0.75
0.5
0.25
0.6
n=5
n=4
n=4
Zymosan
Mannan
WT
M2 +/-
M2 -/-

FIG. 5C

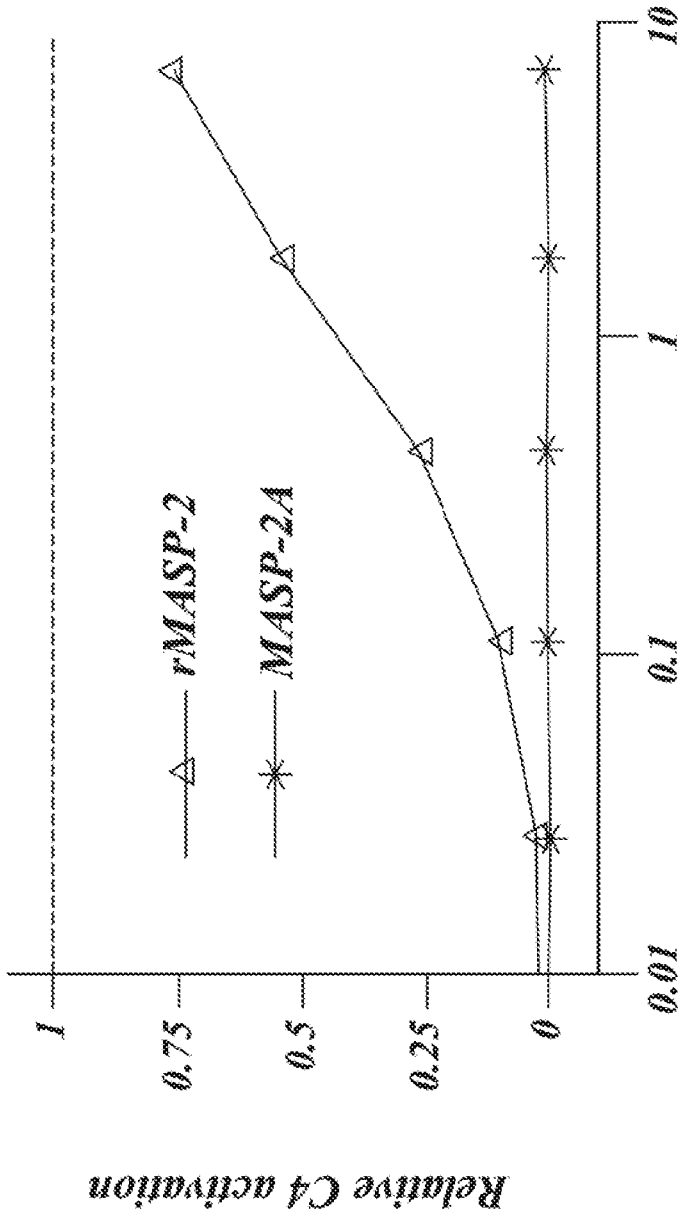
Relative C4 activation
1
0.75
0.5
0.25
0
0.01
0.1
1
10
[Recombinant protein] µg/ml
rMASP-2
MASP-2A

FIG. 6

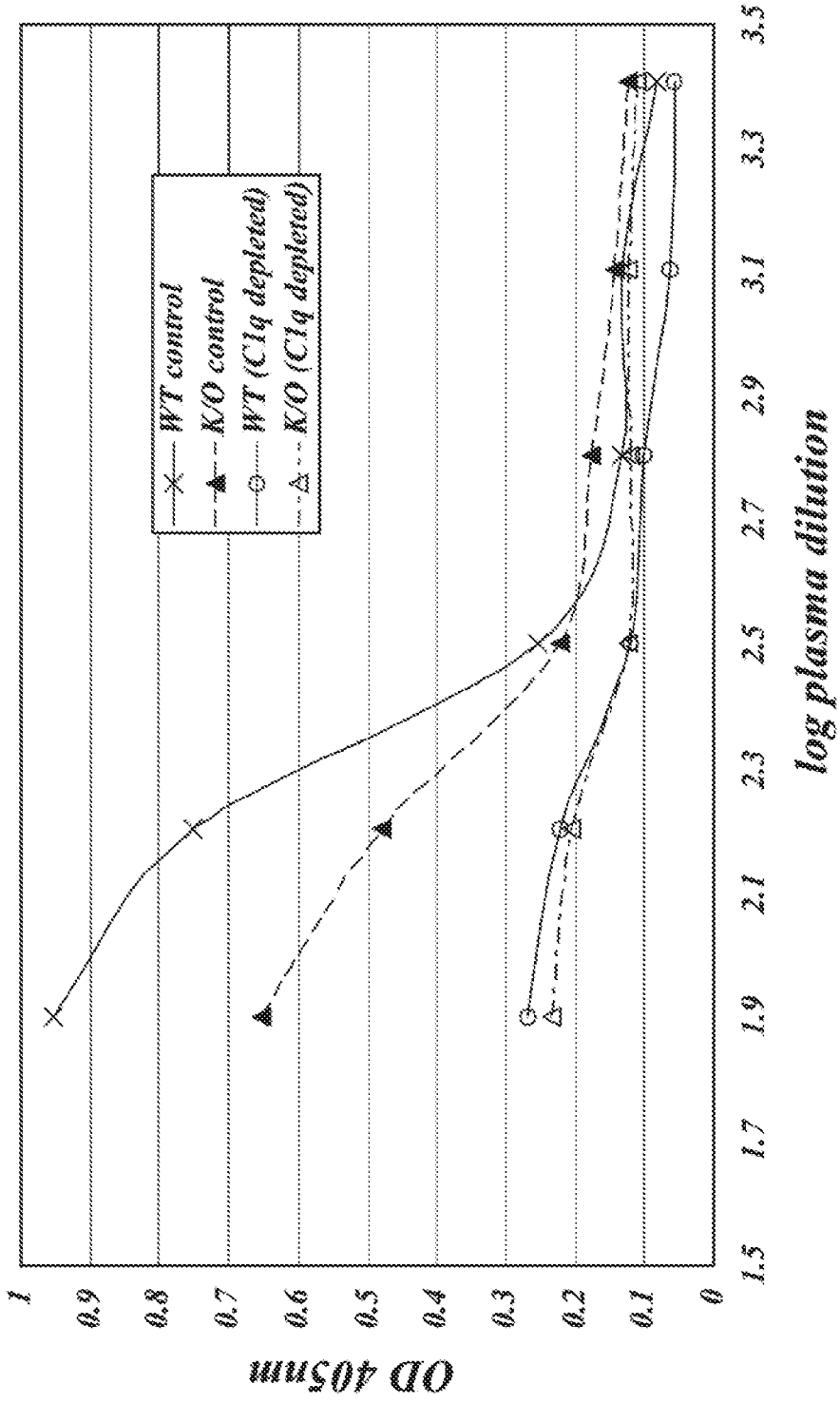
OD 405nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
log plasma dilution
WT control
K/O control
WT (C1q depleted)
K/O (C1q depleted)

FIG. 7

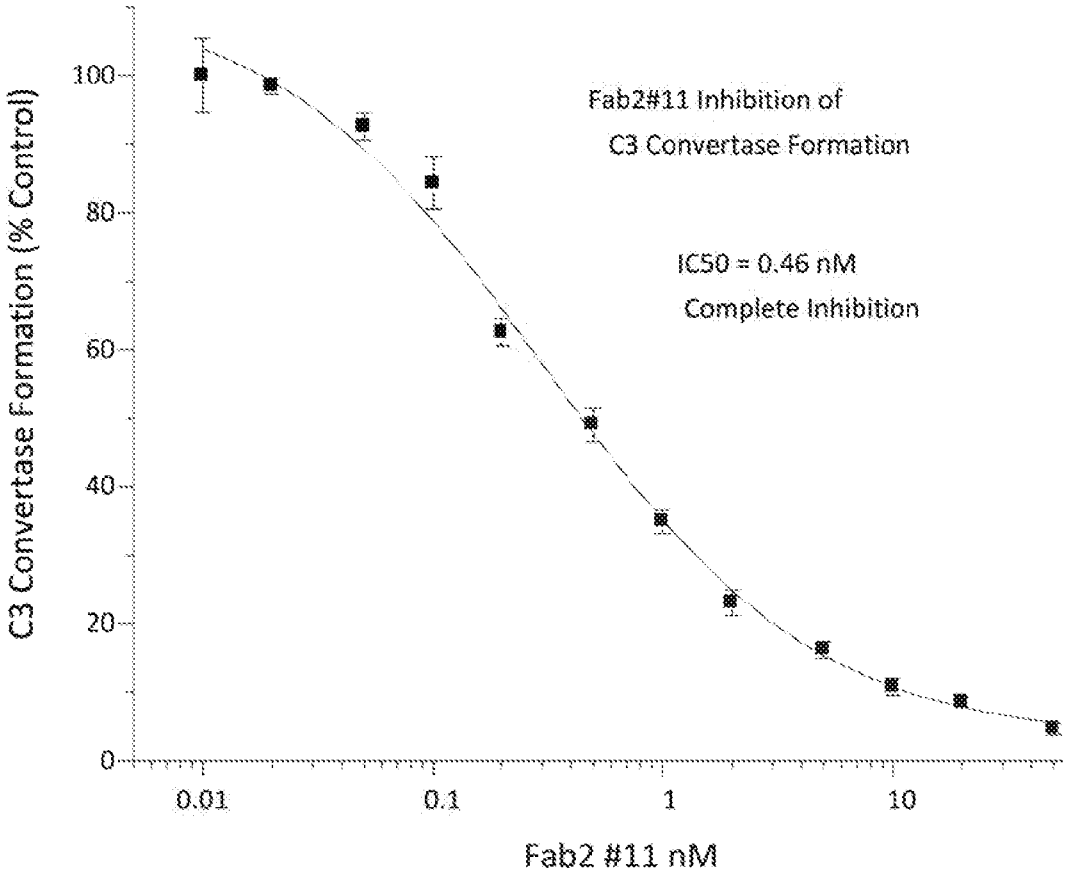
C3 Convertase Formation (% Control)
100
80
60
40
20
0
0.01
0.1
1
10
Fab2 #11 nM
Fab2#11 Inhibition of
C3 Convertase Formation
IC50 = 0.46 nM
Complete Inhibition

FIG. 8A

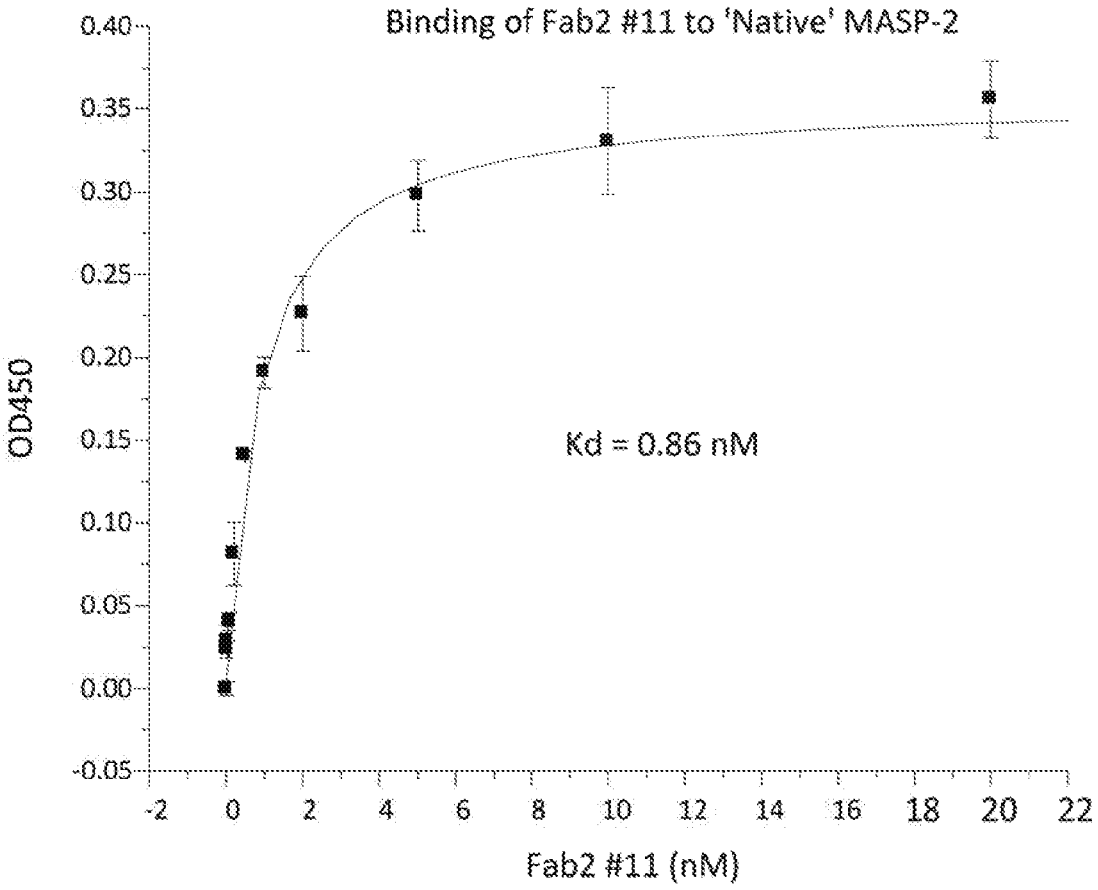
Binding of Fab2 #11 to 'Native' MASP-2
OD450
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-2
0
2
4
6
8
10
12
14
16
18
20
22
Fab2 #11 (nM)
Kd = 0.86 nM

FIG. 8B

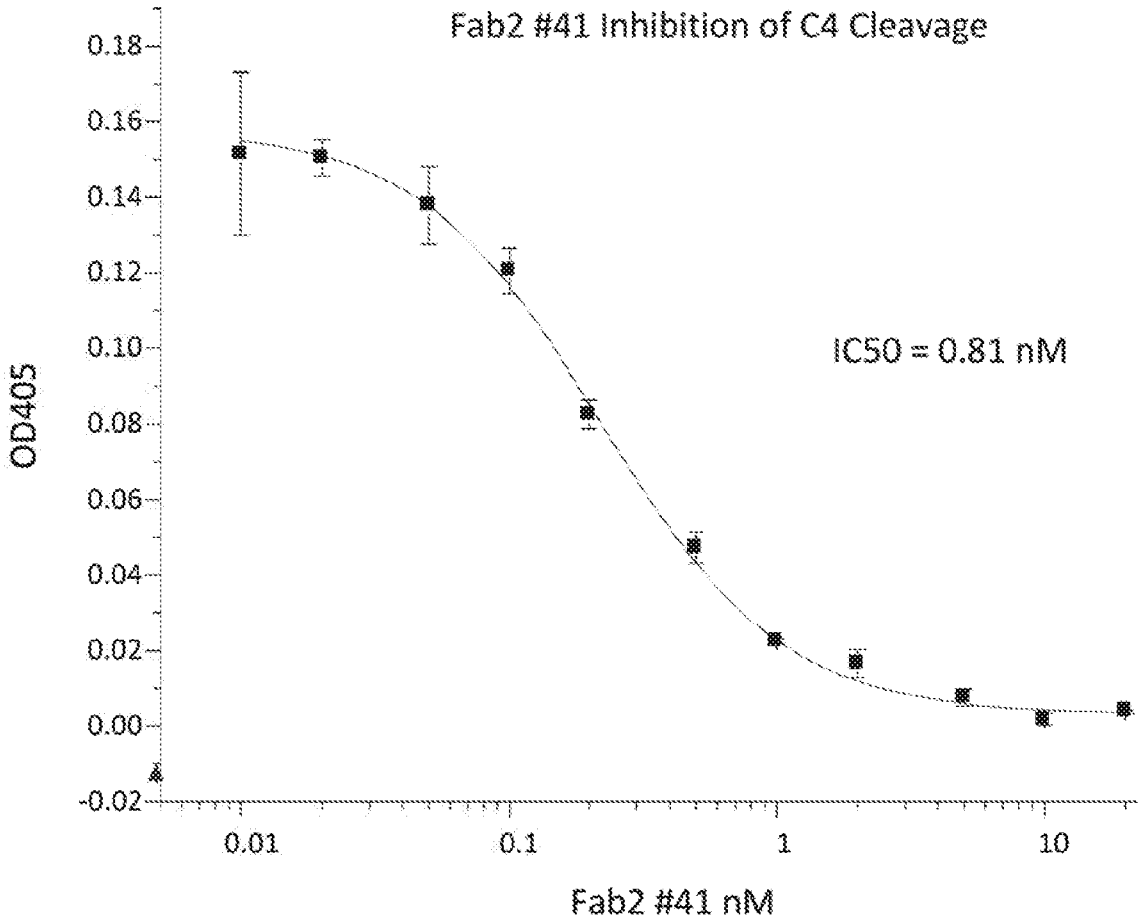
Fab2 #41 Inhibition of C4 Cleavage
IC50 = 0.81 nM
OD405
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.01
0.1
1
10
Fab2 #41 nM

FIG. 8C

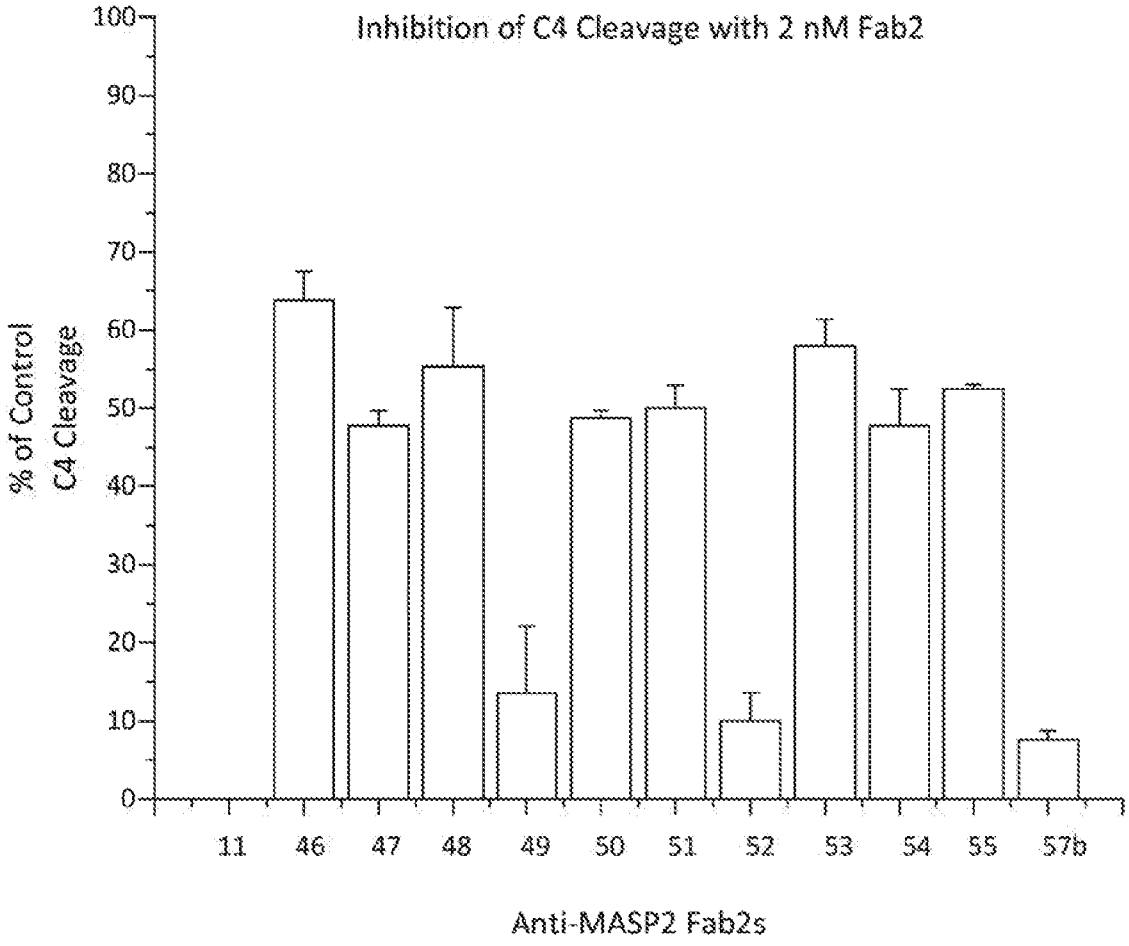
Inhibition of C4 Cleavage with 2 nM Fab2
% of Control C4 Cleavage
100
90
80
70
60
50
40
30
20
10
0
11
46
47
48
49
50
51
52
53
54
55
57b
Anti-MASP2 Fab2s

FIG. 9

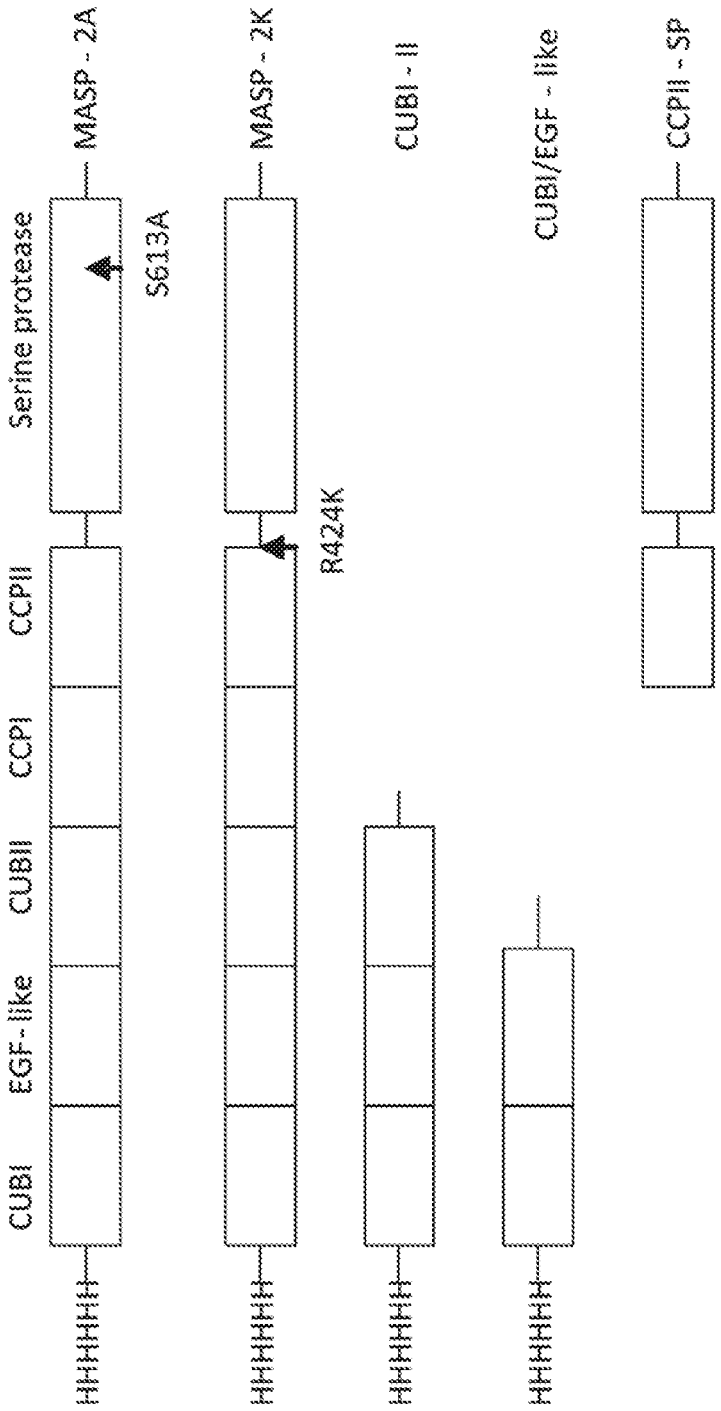
CUBI
EGF-like
CUBII
CCPI
CCPII
Serine protease
MASP - 2A
S613A
MASP - 2K
R424K
CUBI - II
CUBI/EGF - like
CCPII - SP

FIG. 10

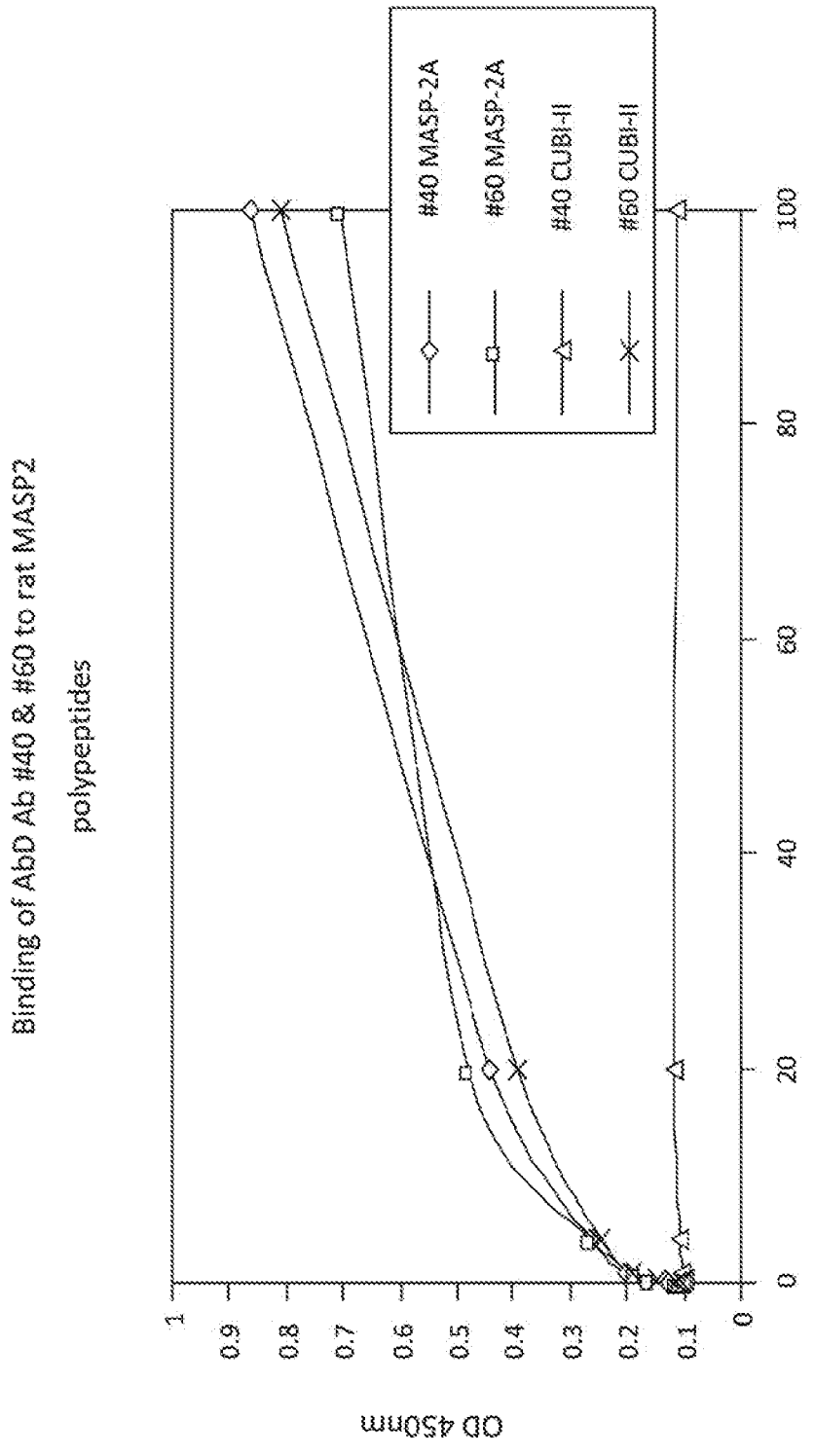
Binding of AbD Ab #40 & #60 to rat MASP2
polypeptides
#40 MASP-2A
#60 MASP-2A
#40 CUBI-II
#60 CUBI-II
OD 450nm
[Ab] nM
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100

FIG. 11

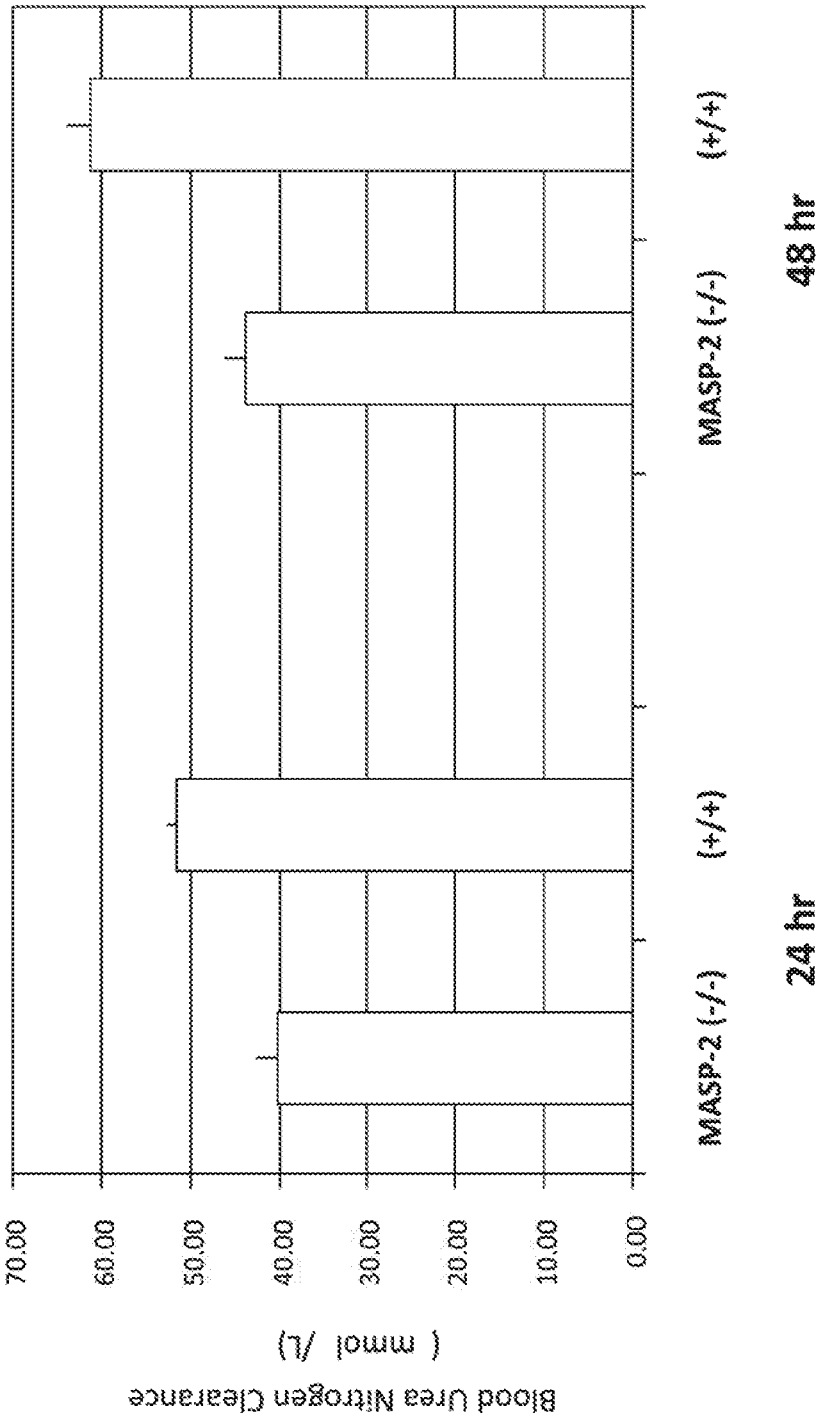
Blood Urea Nitrogen Clearance
(mmol /L)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
MASP-2 (-/-)
(+/+)
24 hr
MASP-2 (-/-)
(+/+)
48 hr

FIG. 12

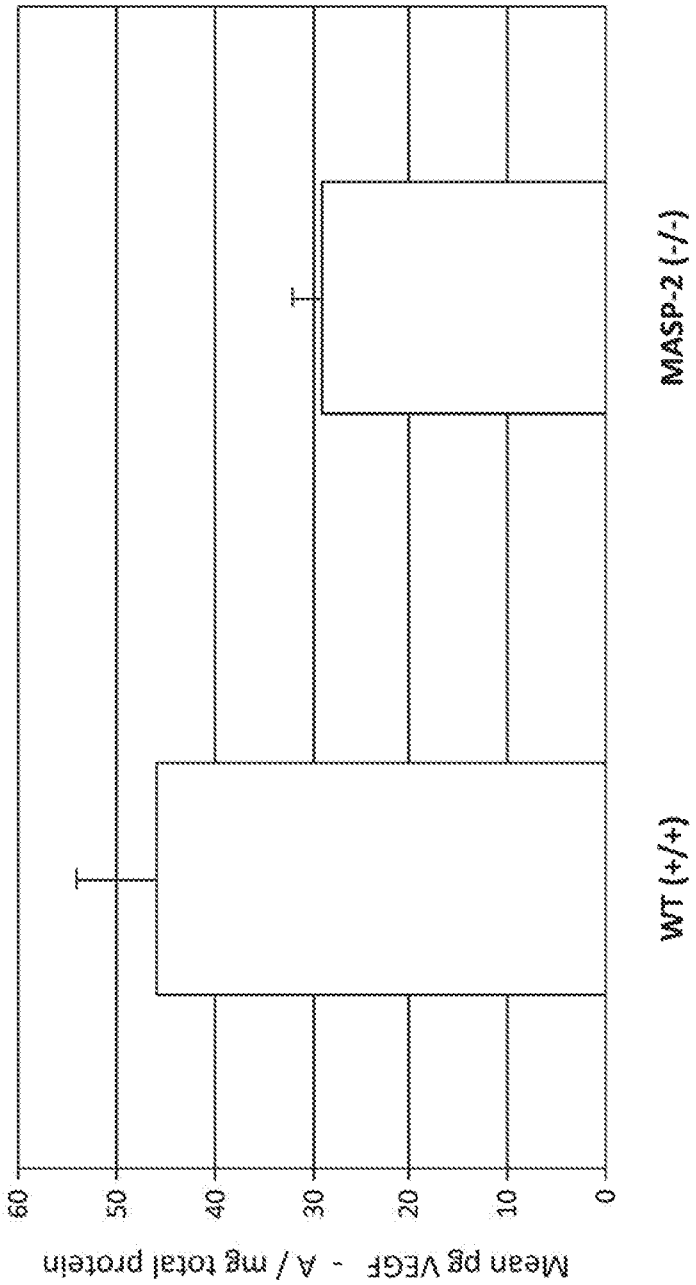

FIG. 13A

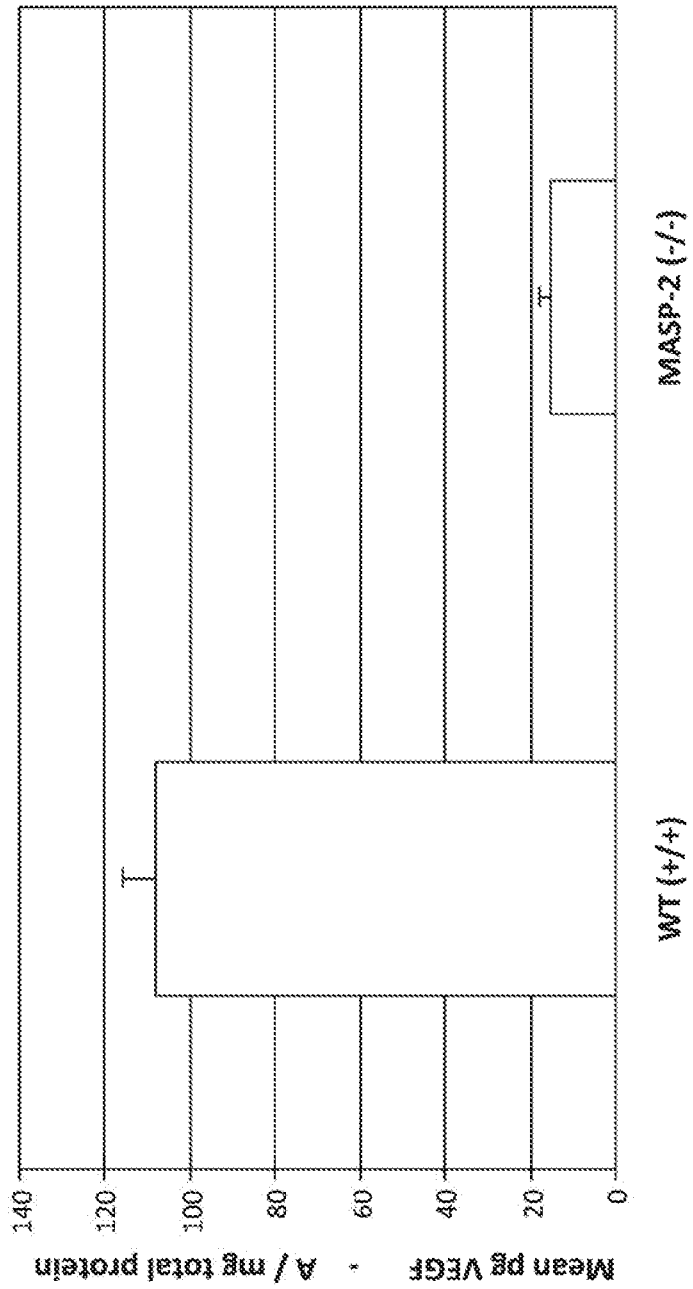
Mean pg VEGF / A / mg total protein
0
20
40
60
80
100
120
140
WT (+/+)
MASP-2 (-/-)

FIG. 13B

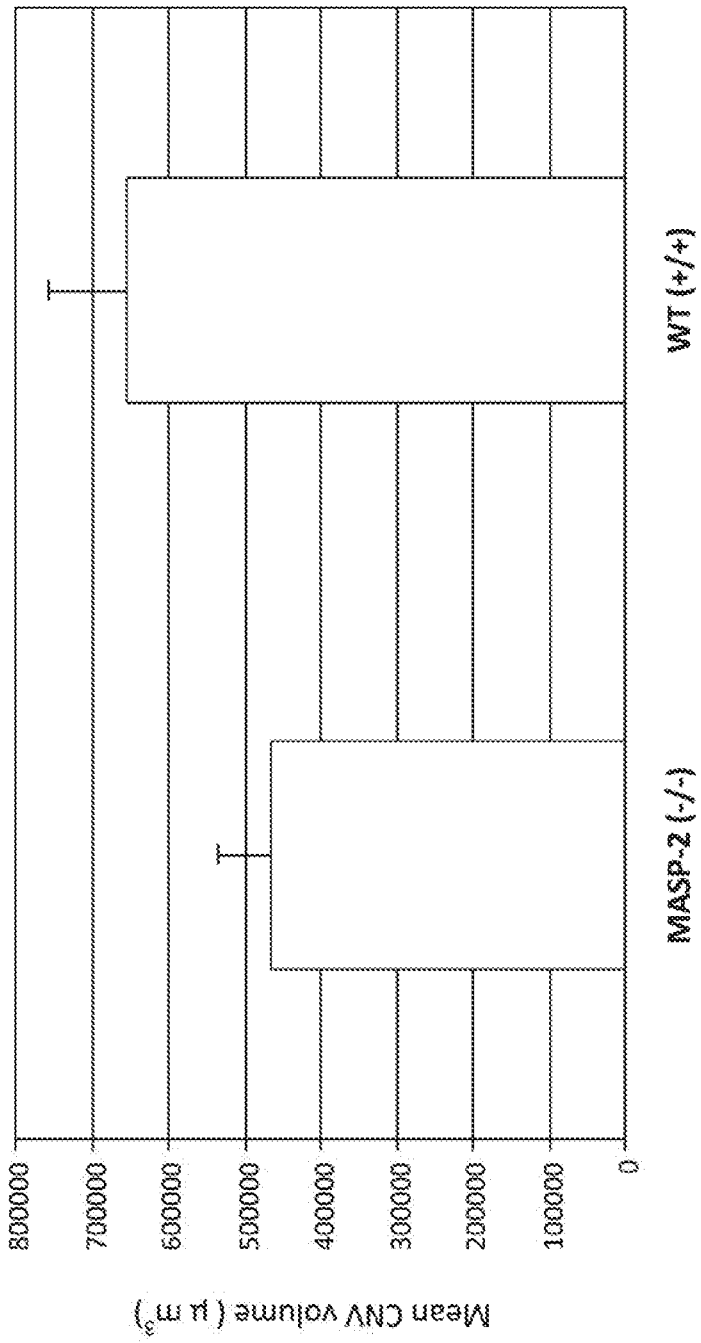
Mean CNV volume (μ m³)
800000
700000
600000
500000
400000
300000
200000
100000
0
WT (+/+)
MASP-2 (-/-)

FIG. 14

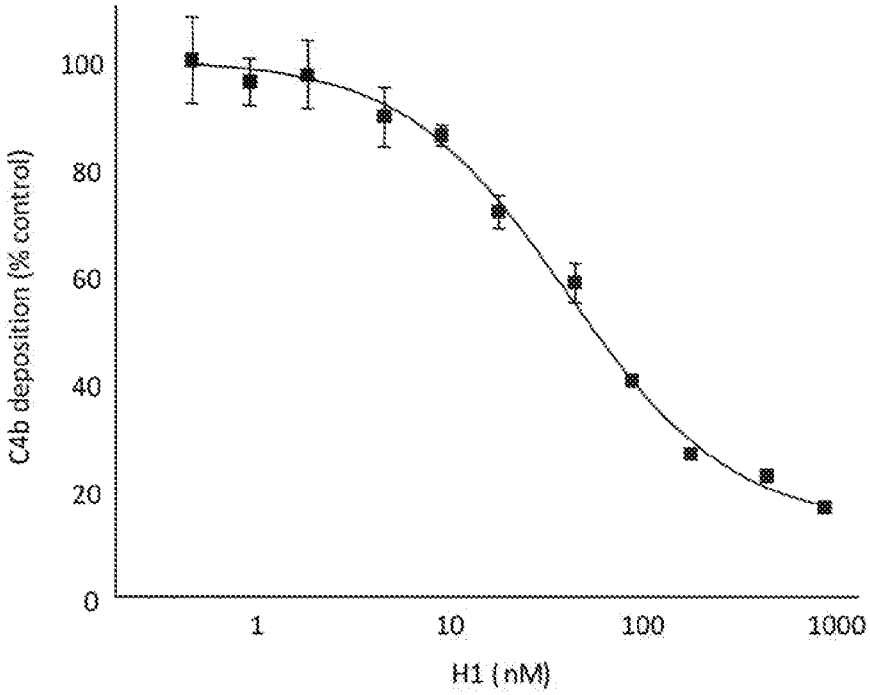

FIG. 15A

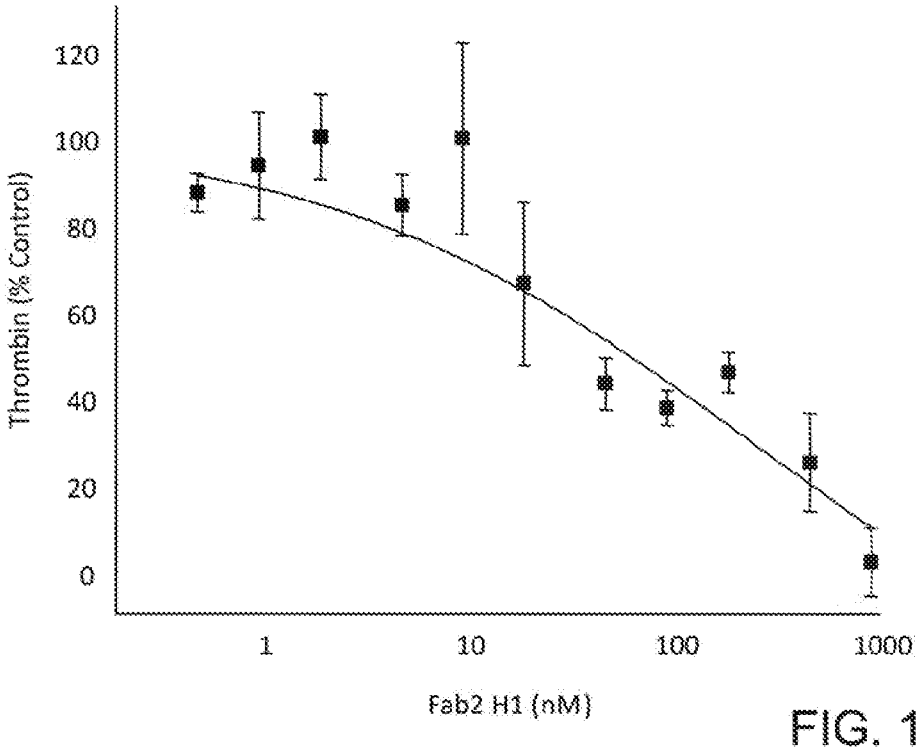

FIG. 15B

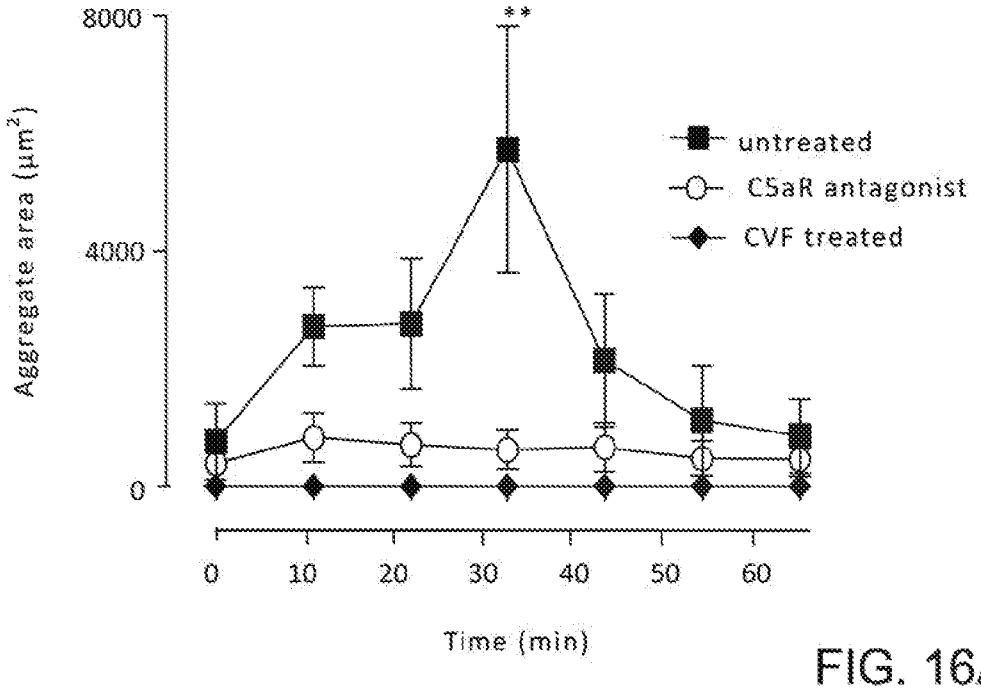

FIG. 16A

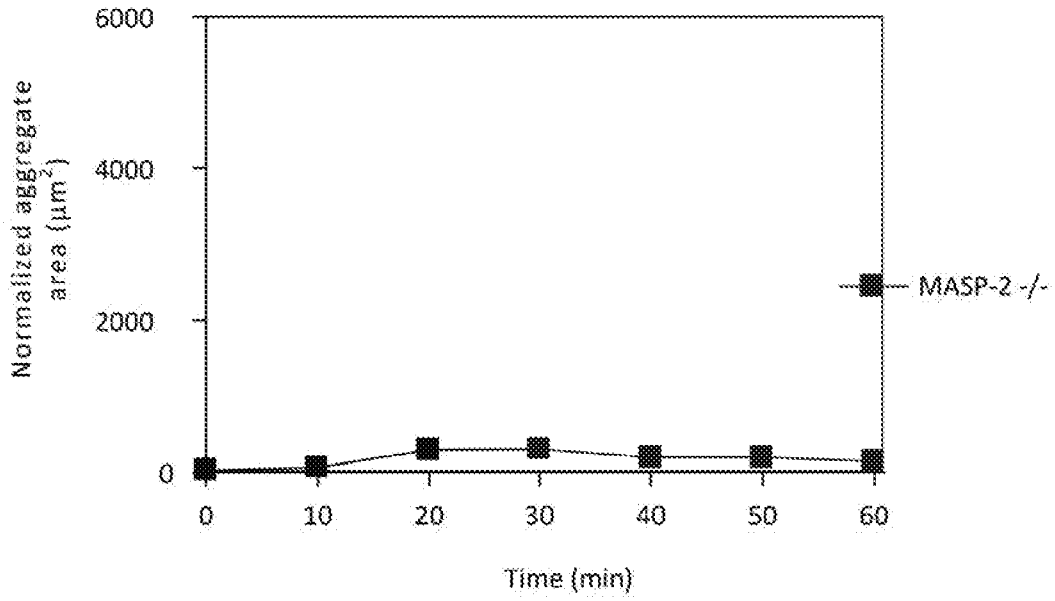

FIG. 16B

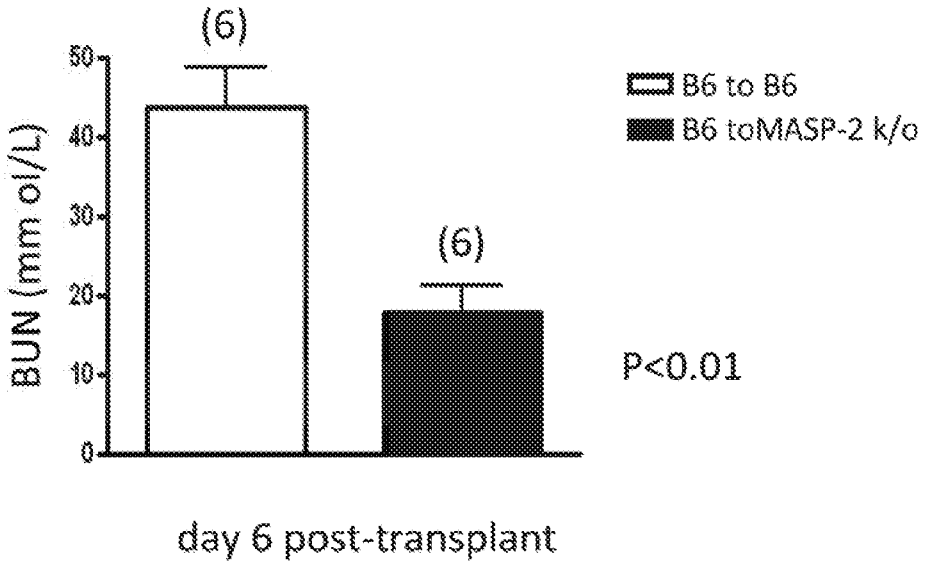
(6)
(6)
BUN (mm ol/L)
50
40
30
20
10
0
B6 to B6
B6 toMASP-2 k/o
P<0.01
day 6 post-transplant

FIG. 17

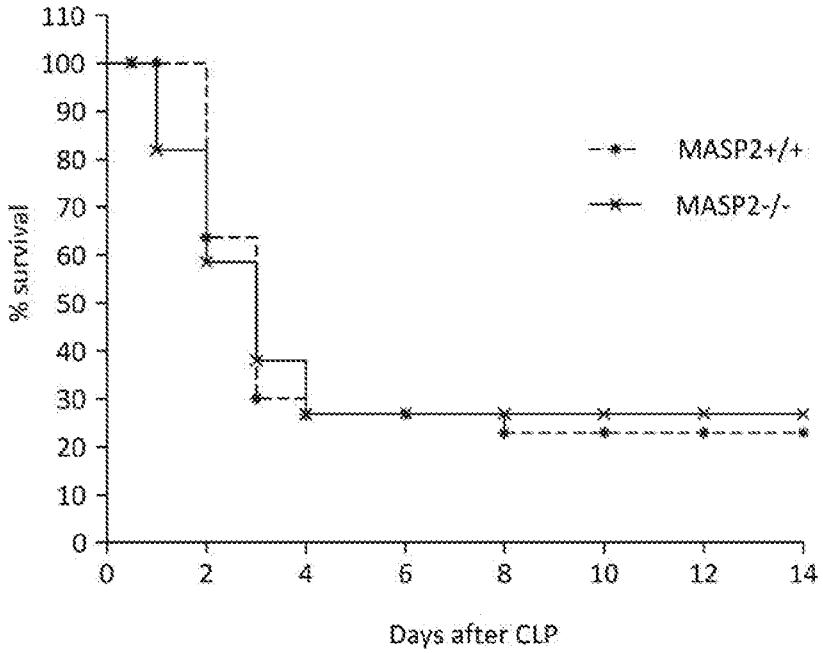
110
100
90
80
70
60
50
40
30
20
10
0
% survival
0
2
4
6
8
10
12
14
Days after CLP
MASP2+/+
MASP2-/-

FIG. 18

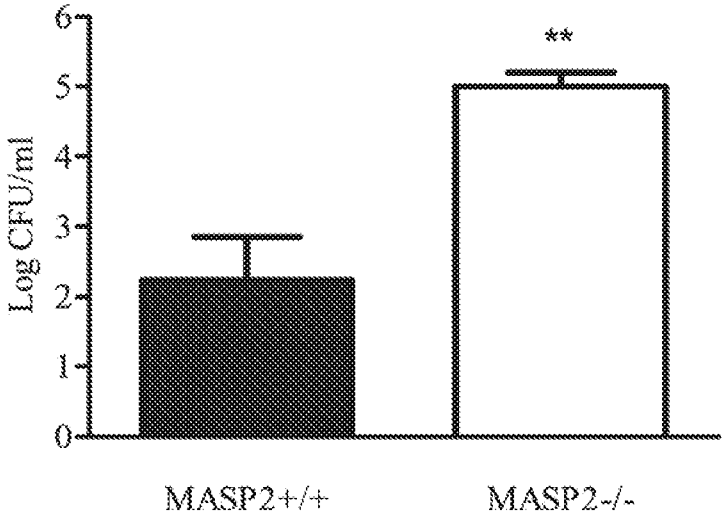
6
5
4
3
2
1
0
Log CFU/ml
**
MASP2+/+
MASP2-/-

FIG. 19

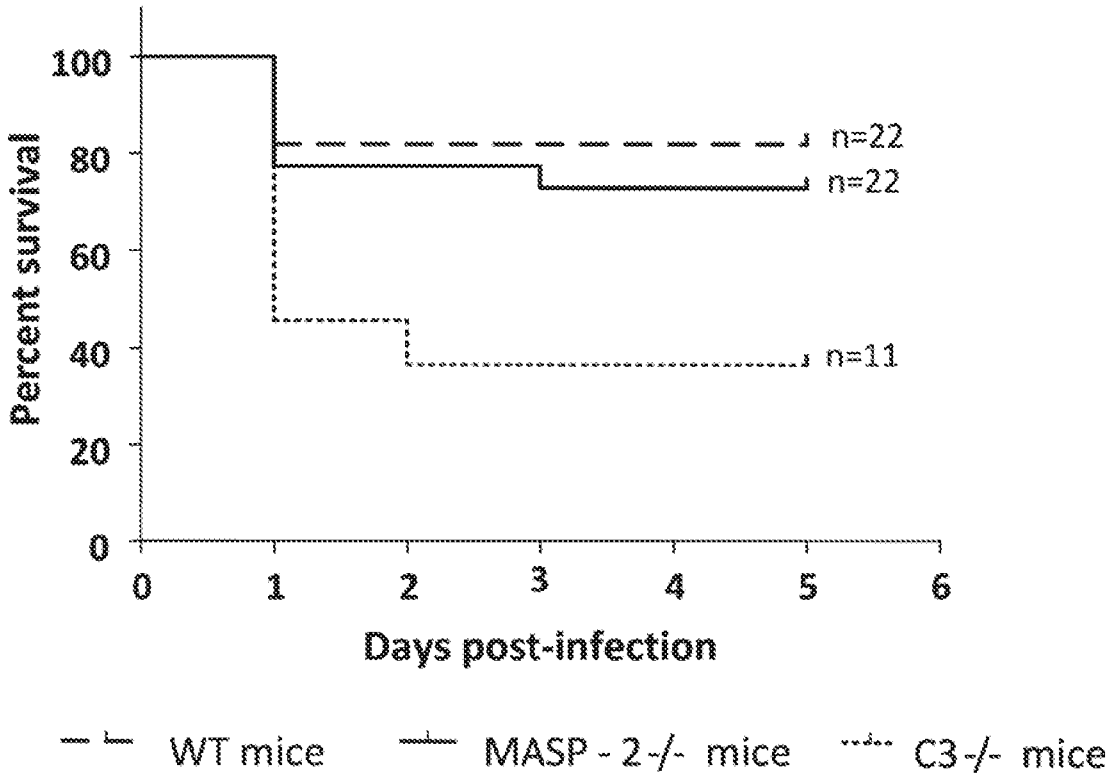
100
80
60
40
20
0
Percent survival
0
1
2
3
4
5
6
Days post-infection
n=22
n=22
n=11
WT mice
MASP - 2 -/- mice
C3 -/- mice

FIG. 20

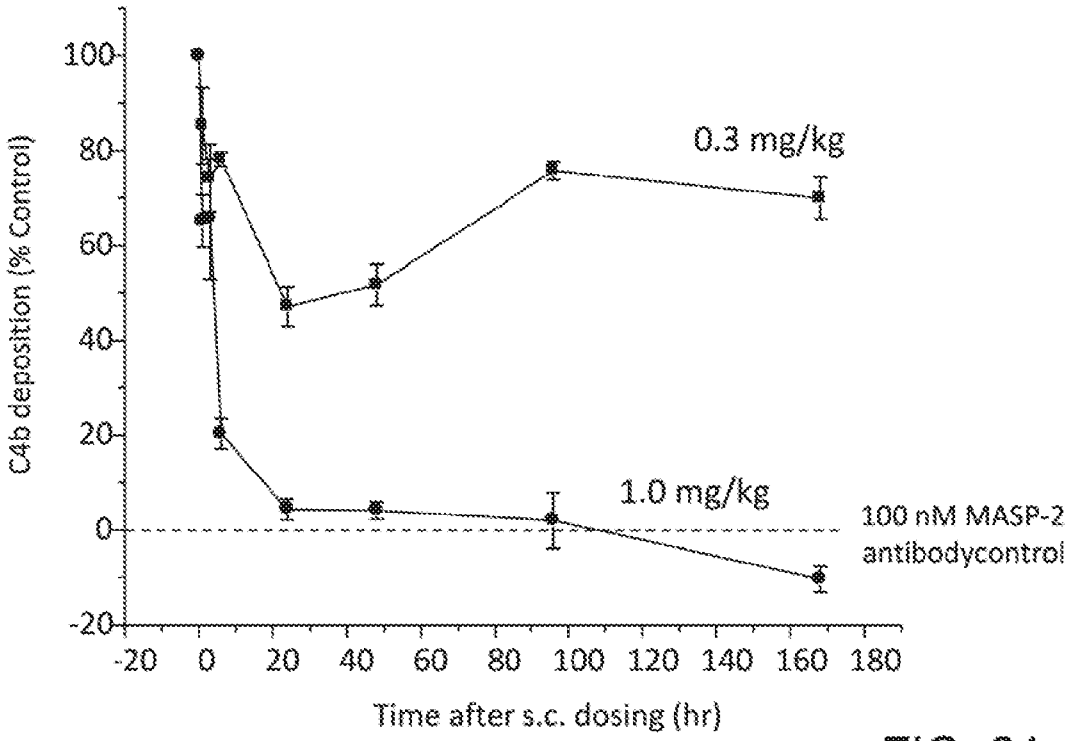
C4b deposition (% Control)
100
80
60
40
20
0
-20
0.3 mg/kg
1.0 mg/kg
100 nM MASP-2
antibodycontrol
-20 0 20 40 60 80 100 120 140 160 180
Time after s.c. dosing (hr)

FIG. 21

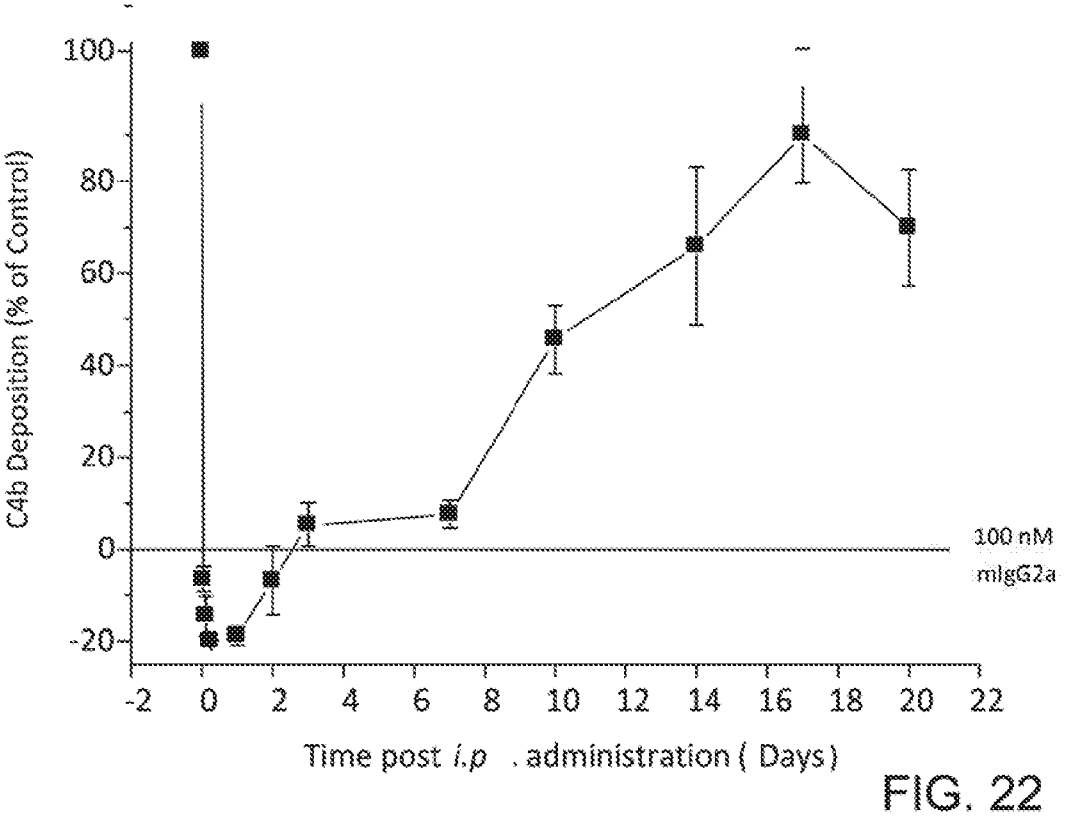
C4b Deposition (% of Control)
100
80
60
40
20
0
-20
100 nM
mIgG2a
-2 0 2 4 6 8 10 12 14 16 18 20 22
Time post i.p . administration (Days)

FIG. 22

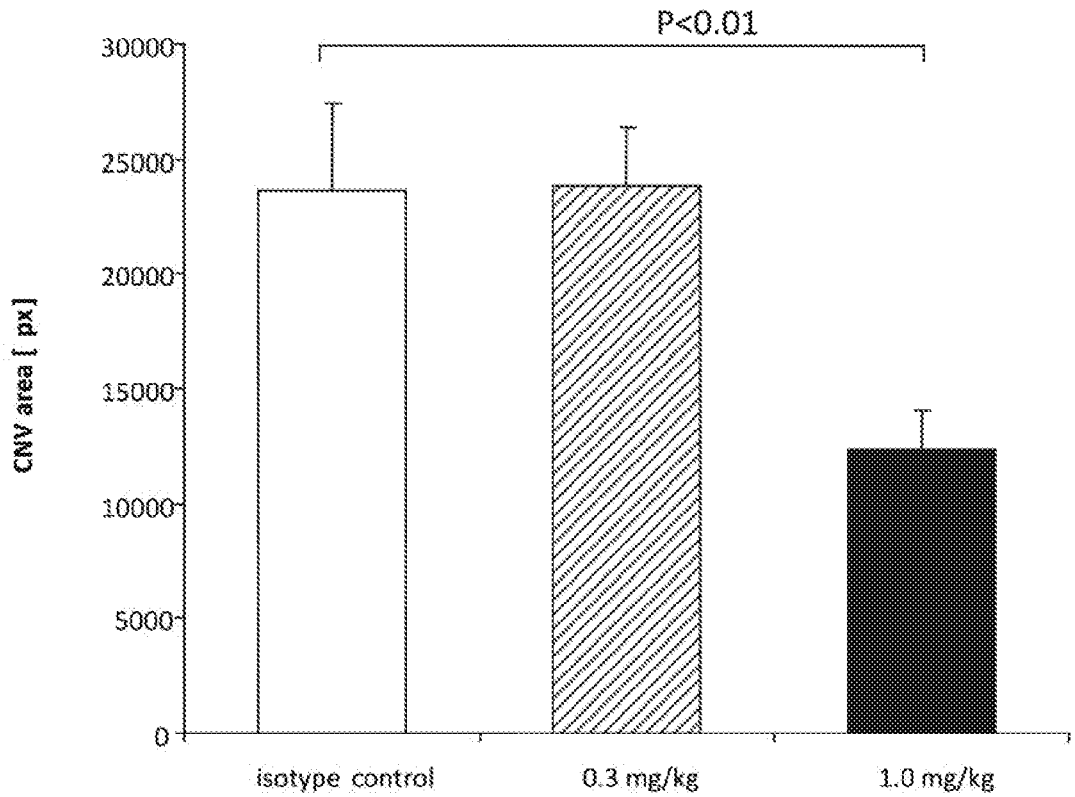
P<0.01
30000
25000
20000
15000
10000
5000
0
CNV area [px]
isotype control
0.3 mg/kg
1.0 mg/kg

FIG. 23

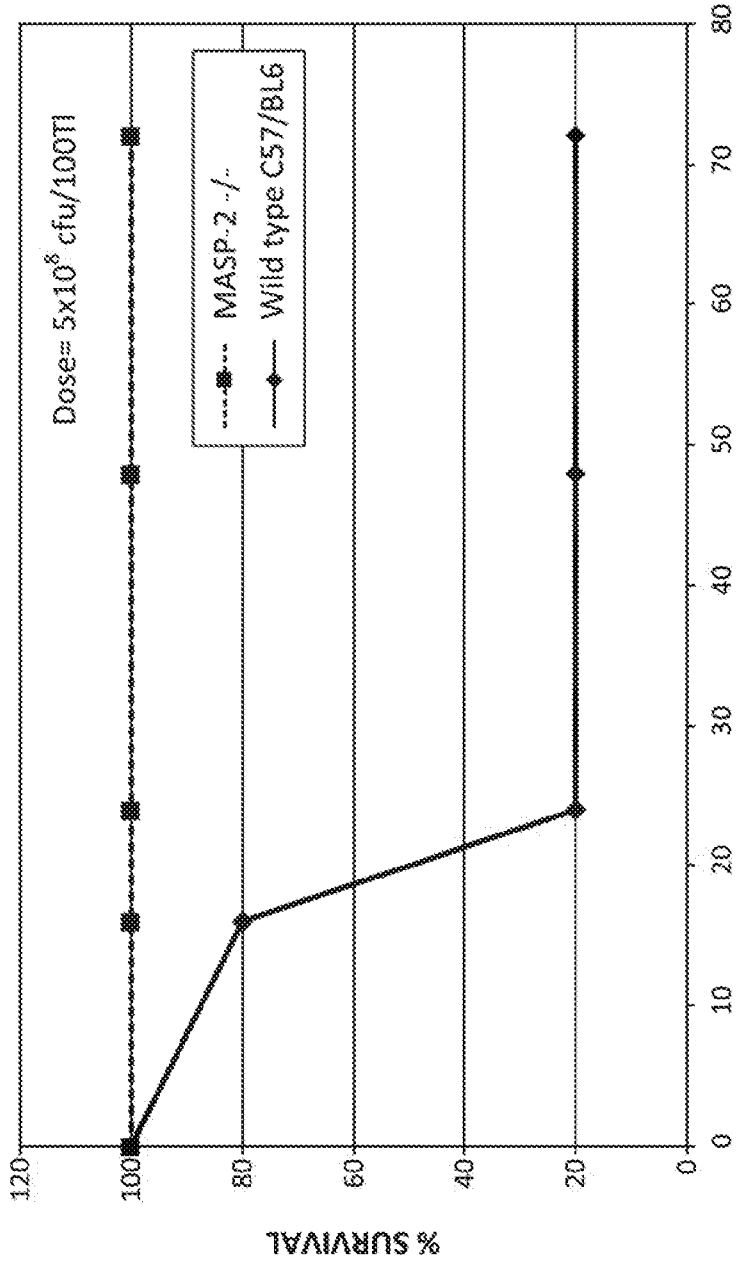
Dose= 5x10^6 cfu/100Tl
MASP-2 -/-
Wild type C57/BL6
% SURVIVAL
TIME (HOURS)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80

FIG. 24A

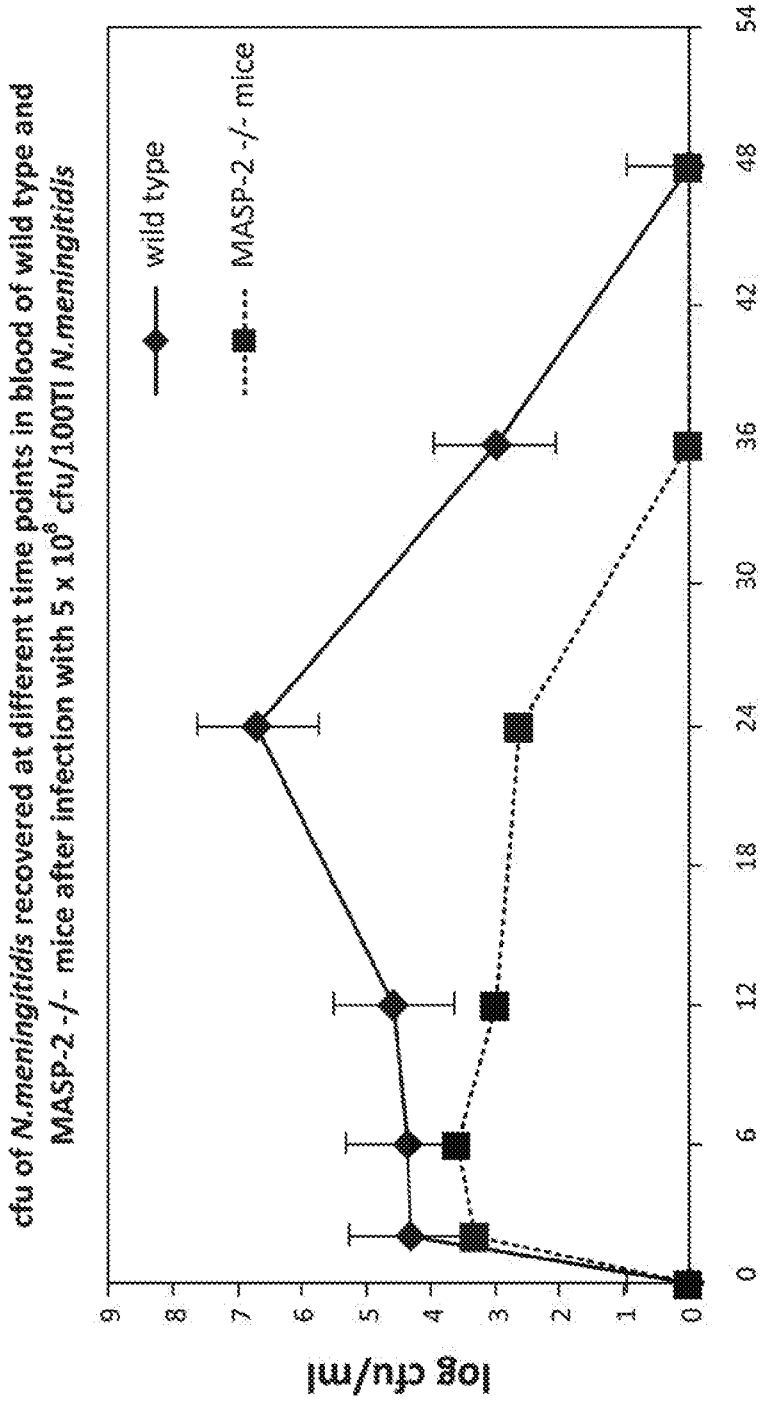
cfu of N.meningitidis recovered at different time points in blood of wild type and MASP-2 -/- mice after infection with 5 x 10^6 cfu/100TI N.meningitidis
wild type
MASP-2 -/- mice
log cfu/ml
TIME (HOURS)
0
1
2
3
4
5
6
7
8
9
0
6
12
18
24
30
36
42
48
54

FIG. 24B

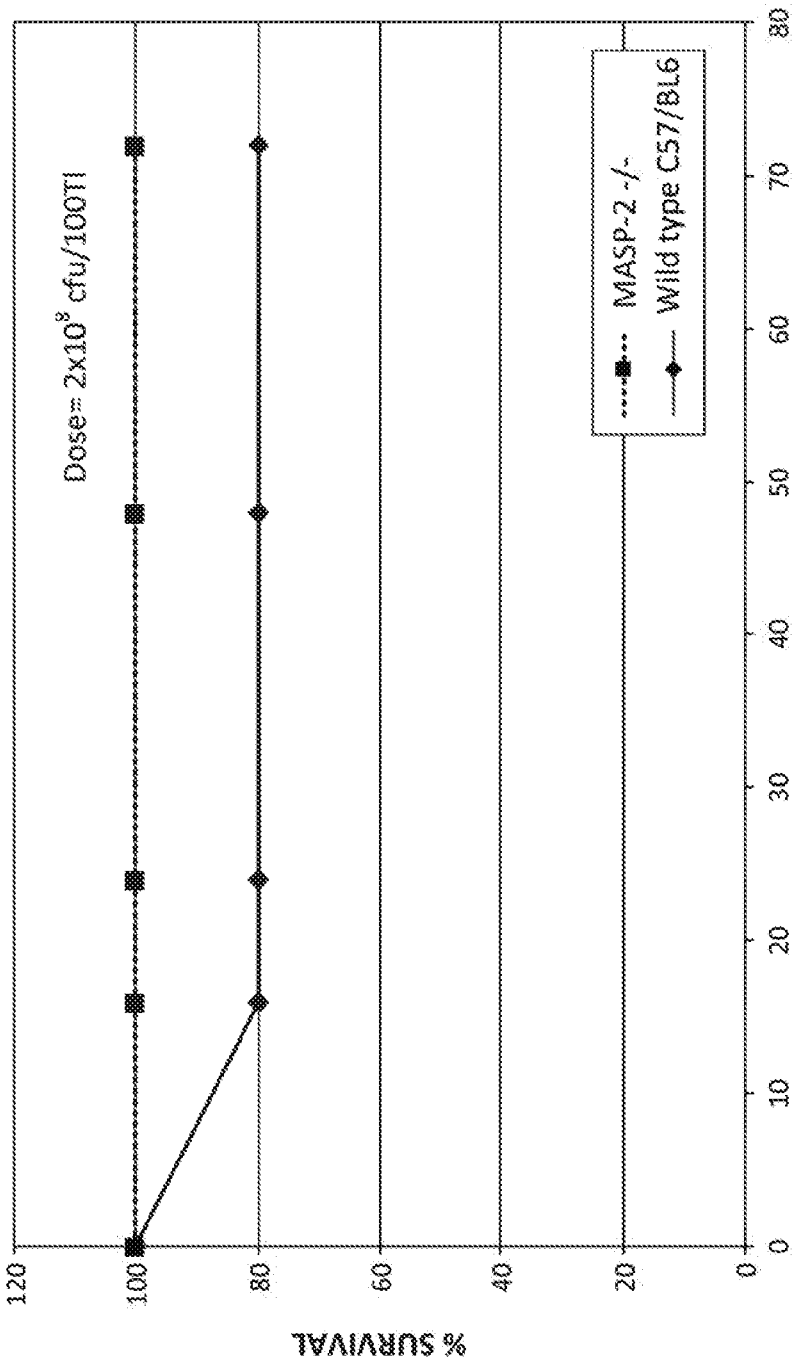
Dose= 2x10^9 cfu/100Tl
MASP-2 -/-
Wild type C57/BL6
% SURVIVAL
TIME (HOURS)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80

FIG. 25A

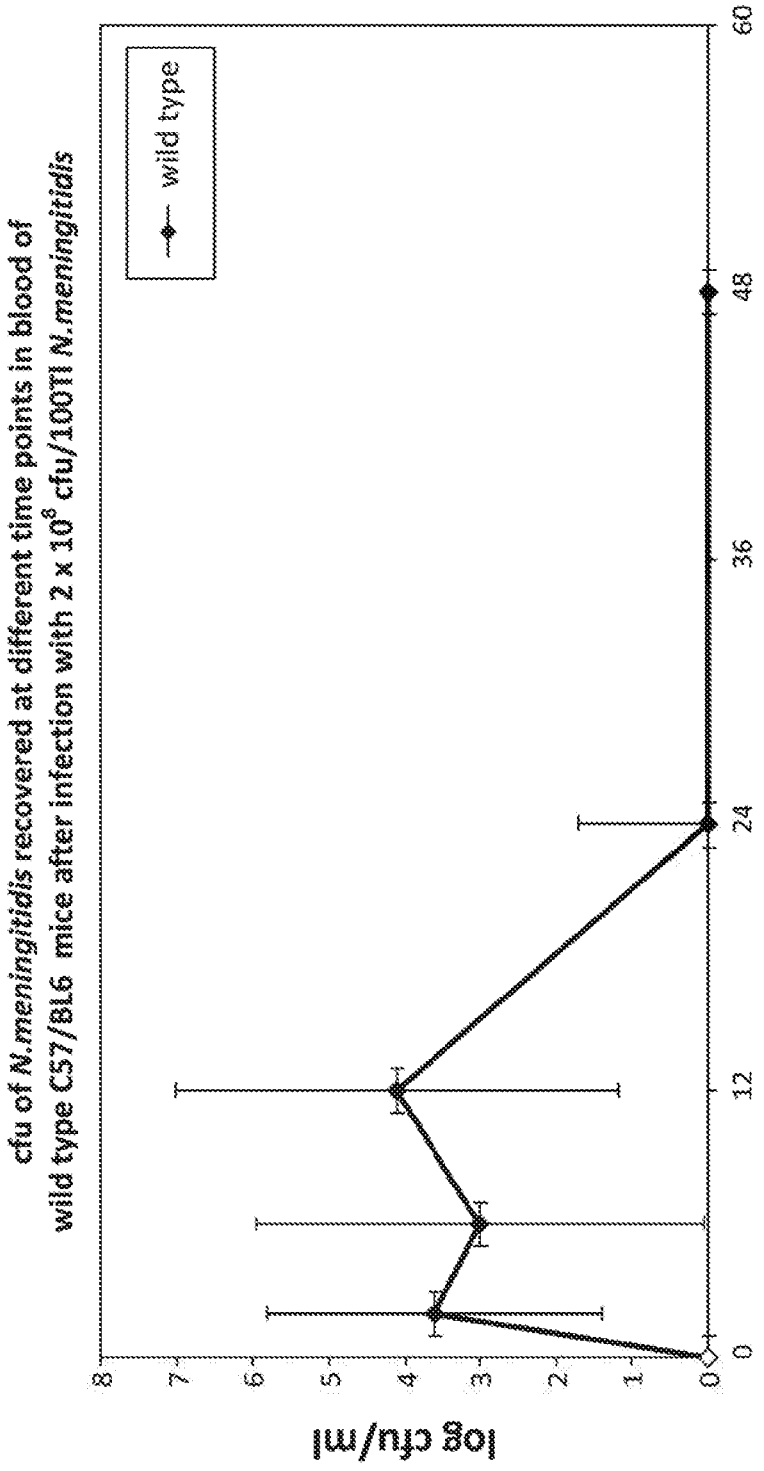
cfu of N.meningitidis recovered at different time points in blood of wild type C57/BL6 mice after infection with 2 x 10^8 cfu/100Tl N.meningitidis
wild type
log cfu/ml
TIME (HOURS)
0
1
2
3
4
5
6
7
8
0
12
24
36
48
60

FIG. 25B

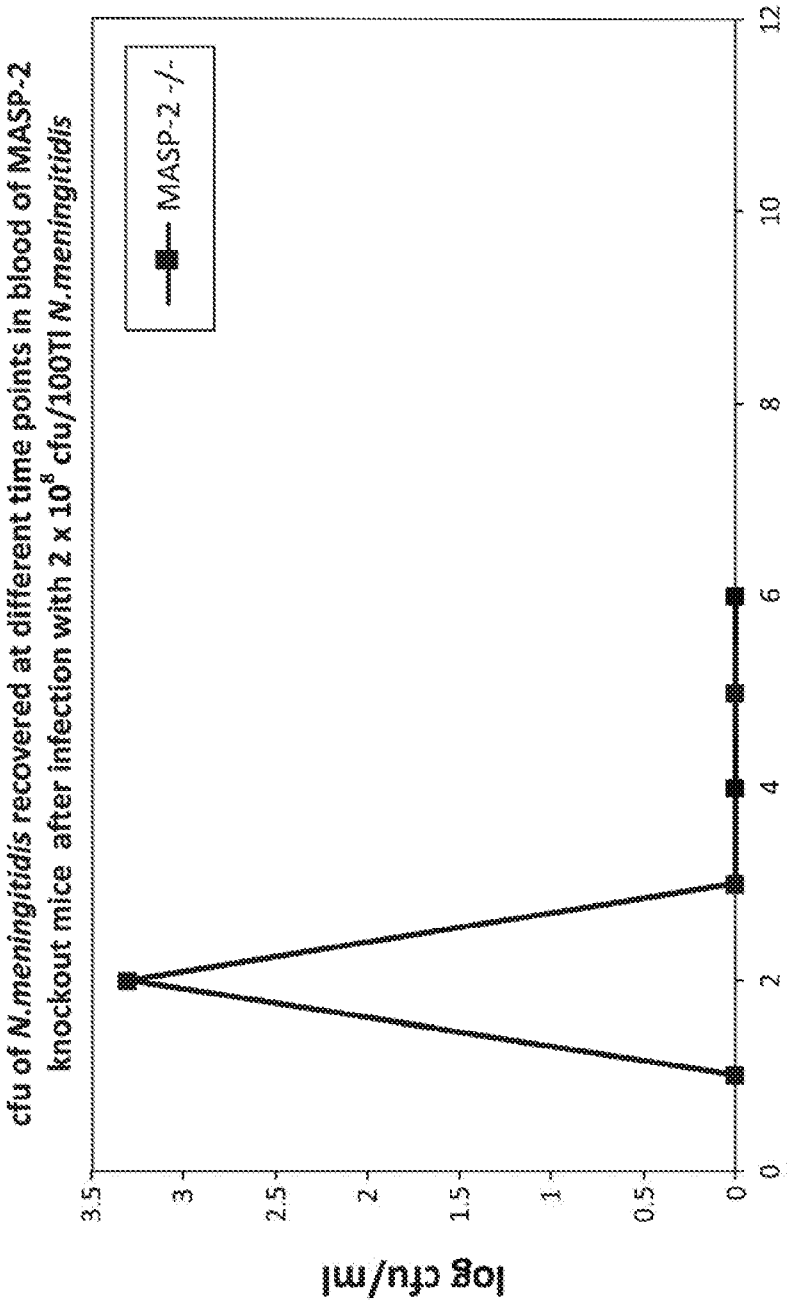
cfu of N.meningitidis recovered at different time points in blood of MASP-2 knockout mice after infection with 2 x 10^8 cfu/100TI N.meningitidis
MASP-2 -/-
log cfu/ml
TIME (HOURS)
0
0.5
1
1.5
2
2.5
3
3.5
0
2
4
6
8
10
12

FIG. 25C

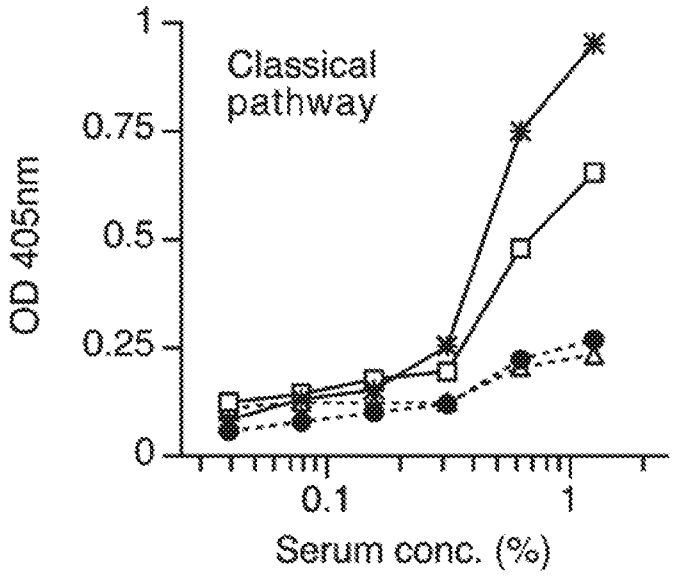

FIG. 26A

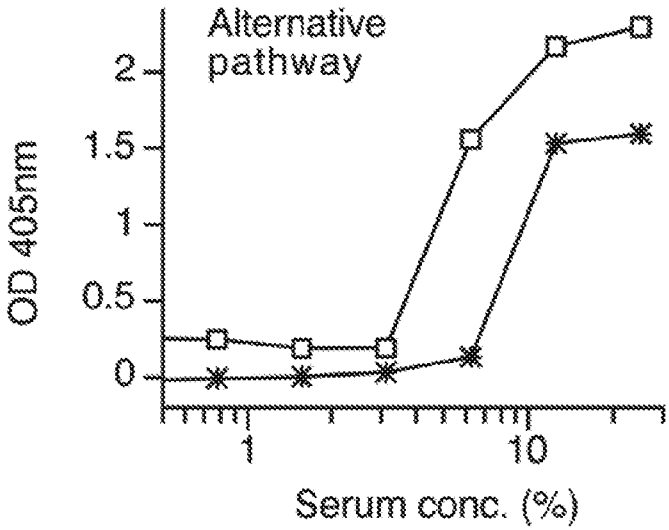

FIG. 26B

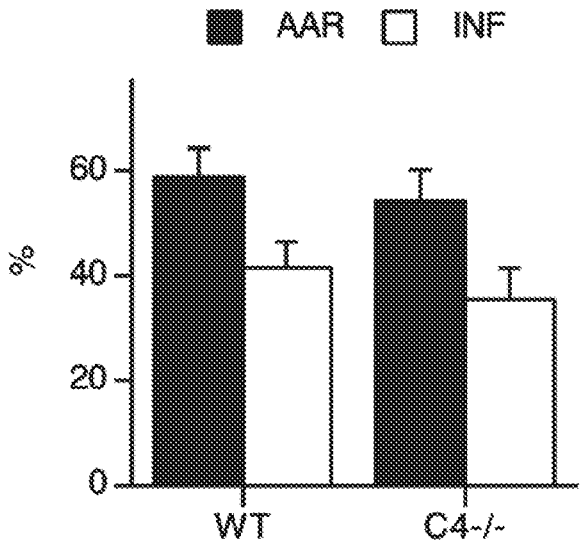

FIG. 27A

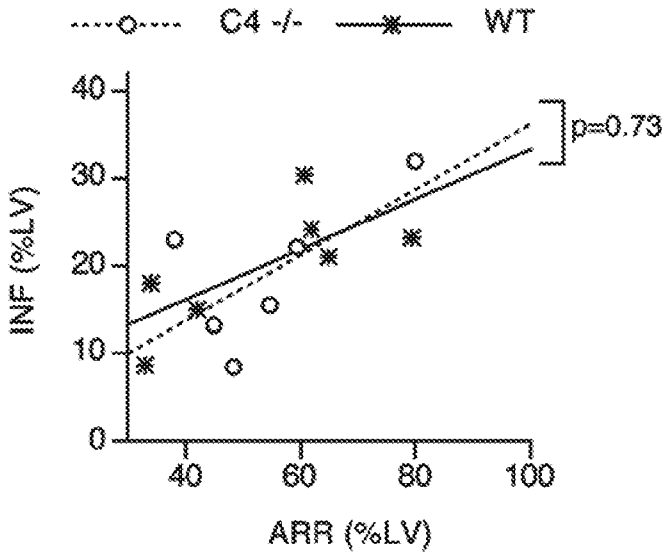

FIG. 27B

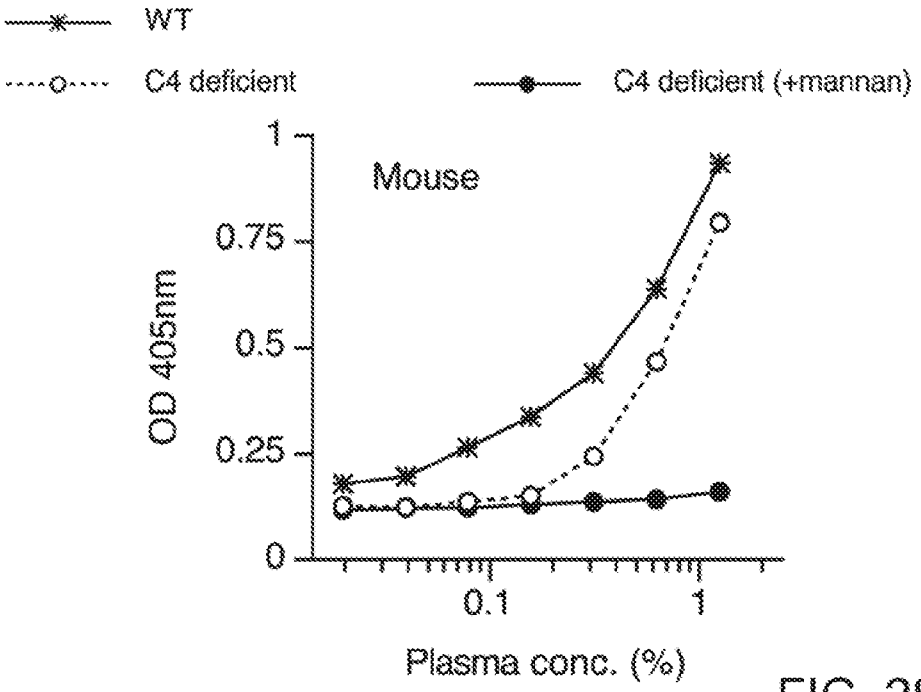

FIG. 28A

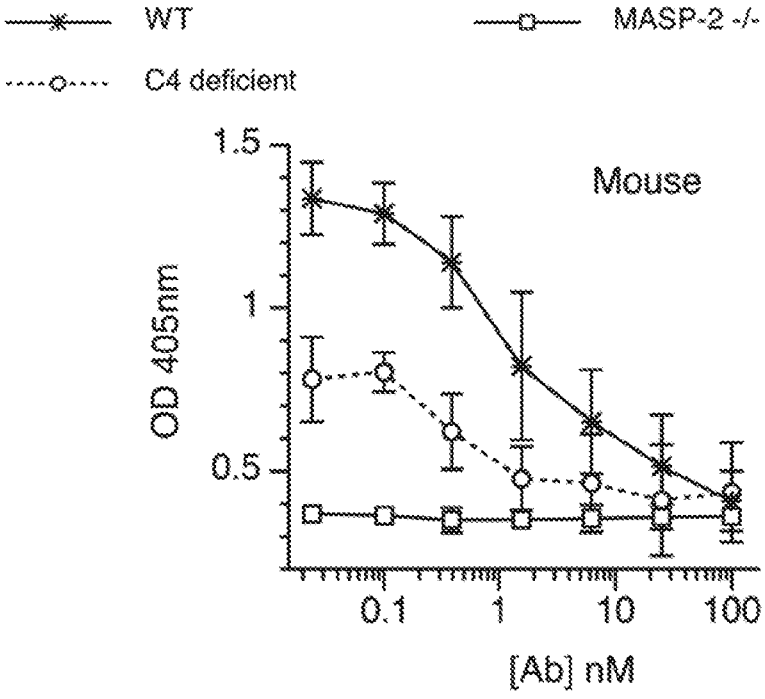

FIG. 28B

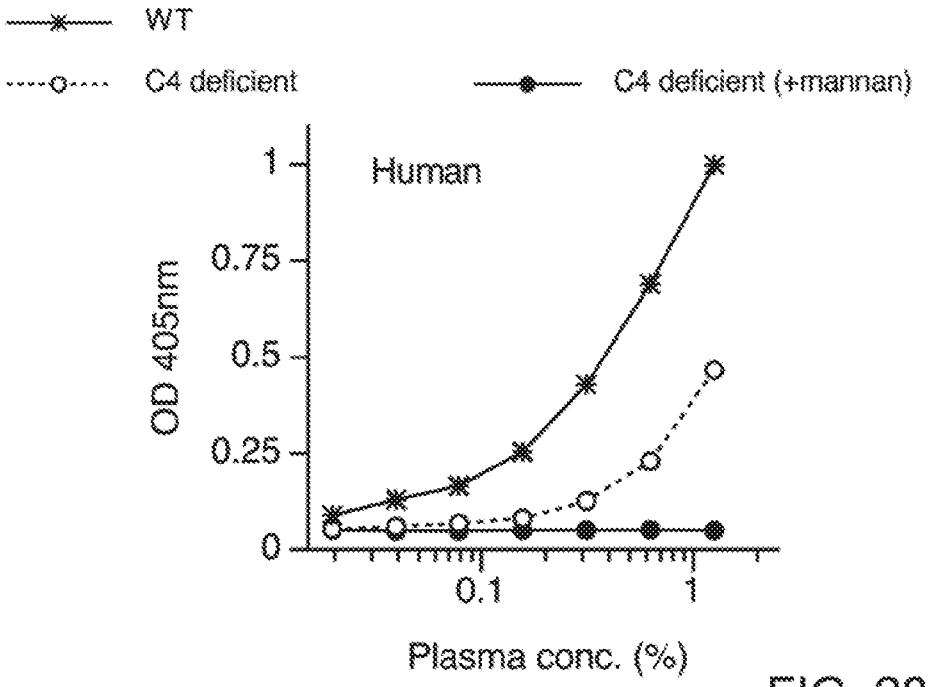

FIG. 28C

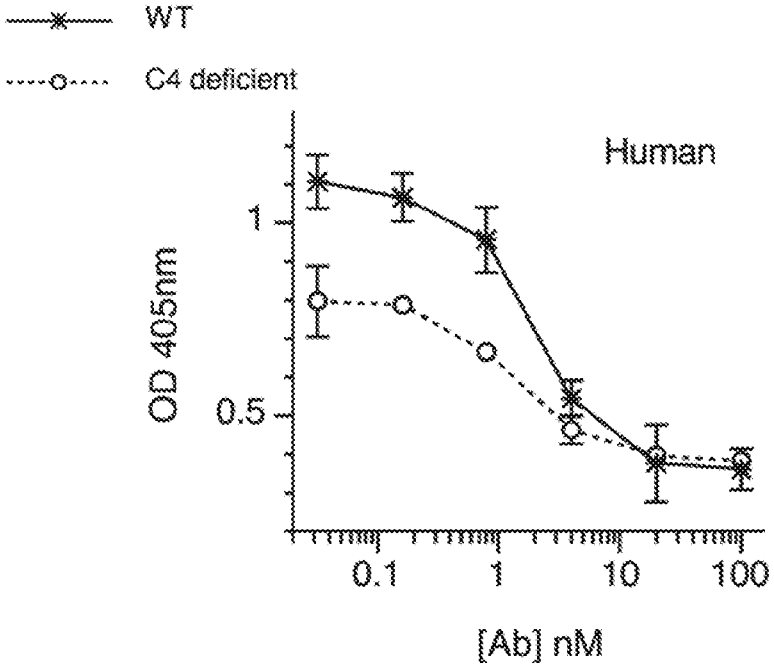

FIG. 28D

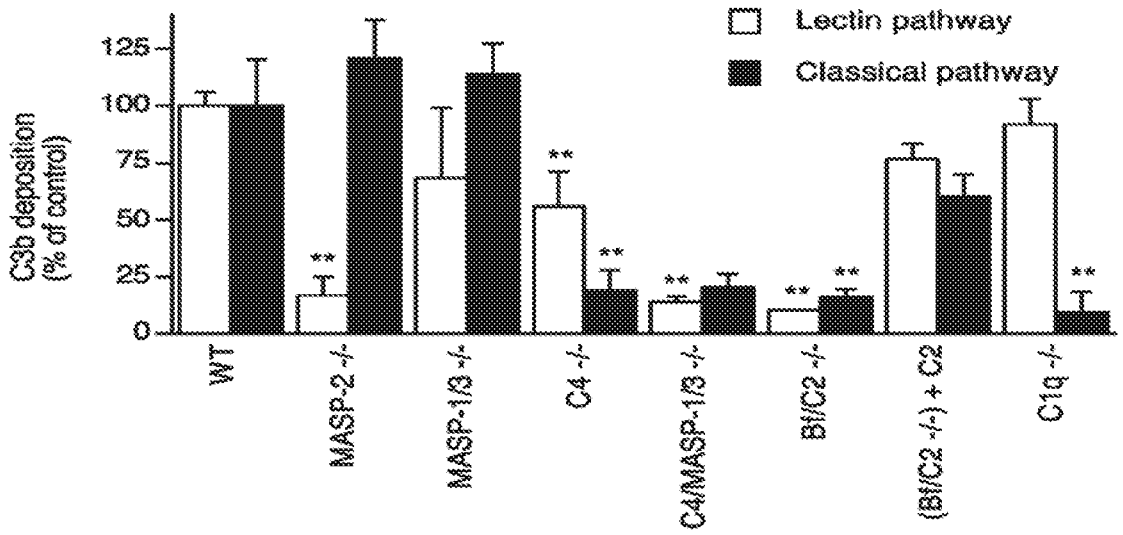

FIG. 29A

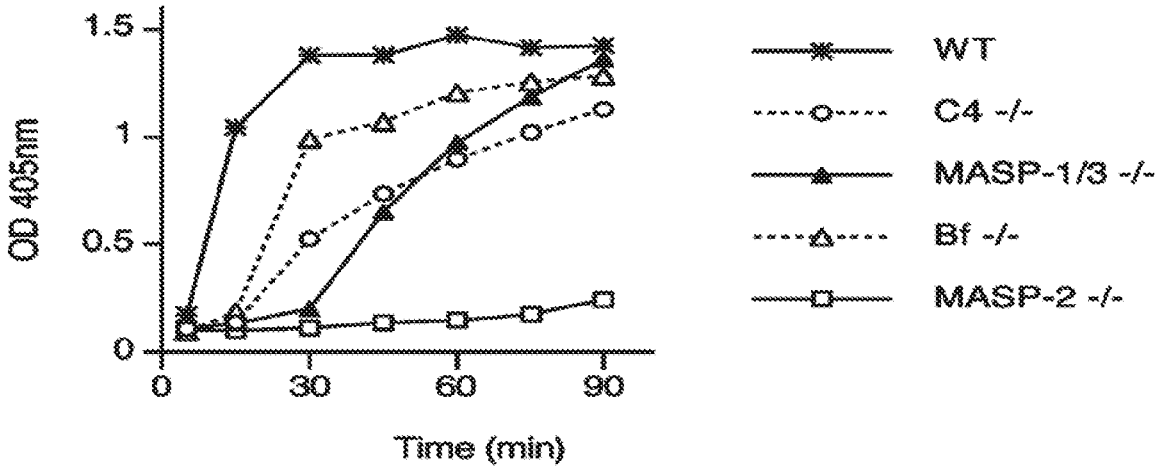

FIG. 29B

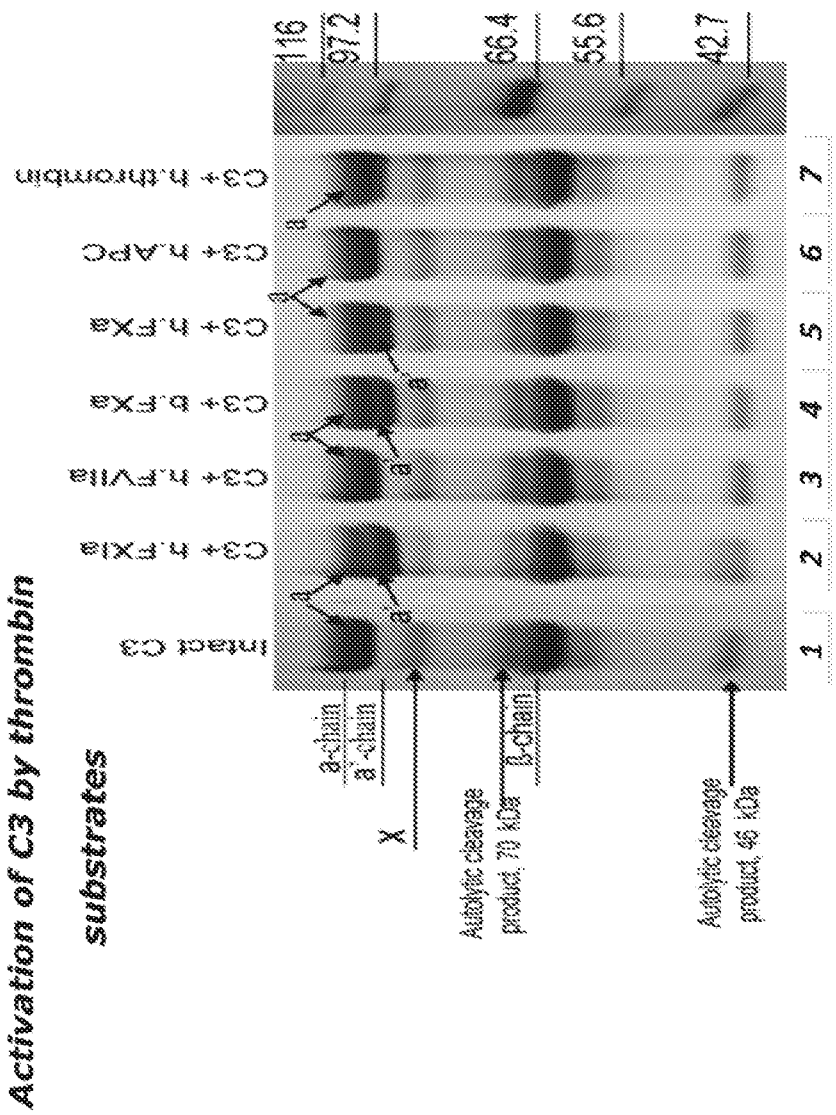
Activation of C3 by thrombin
substrates
intact C3
C3+ h.FXIa
C3+ h.FVIIa
C3+ b.FXa
C3+ h.FXa
C3+ h.APC
C3+ h.thrombin
1
2
3
4
5
6
7
116
97.2
66.4
55.6
42.7
α-chain
α'-chain
β-chain
X
Autolytic cleavage product, 70 kDa
Autolytic cleavage product, 46 kDa

FIG. 30

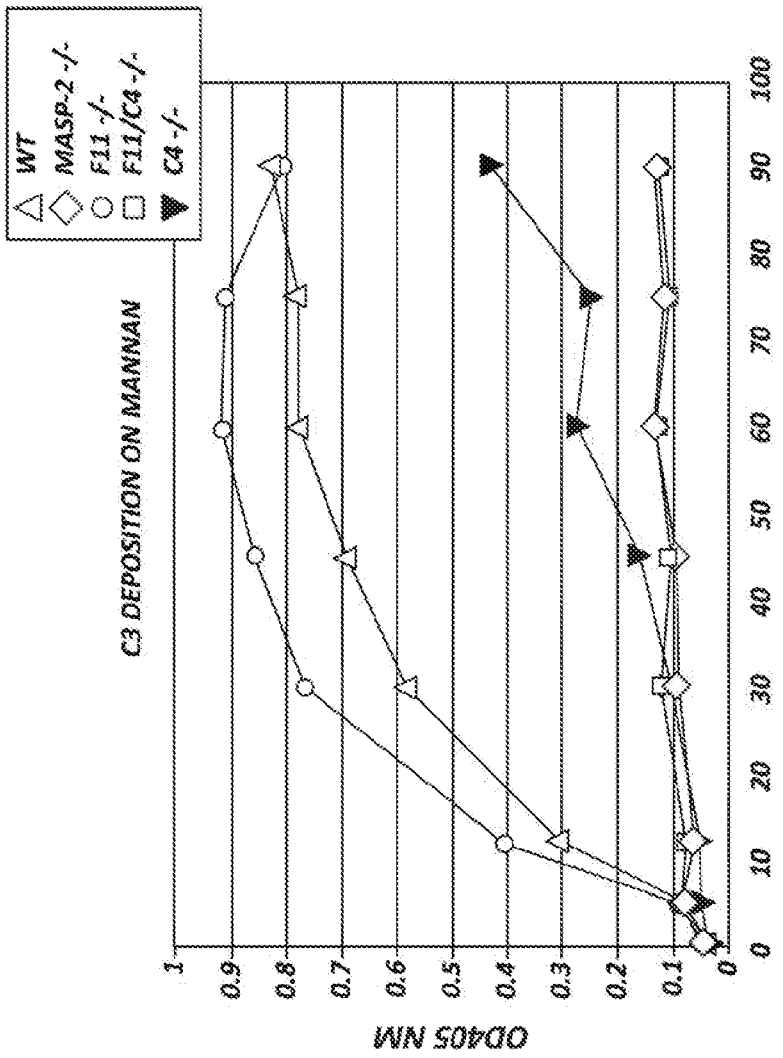
WT
MASP-2 -/-
F11 -/-
F11/C4 -/-
C4 -/-
C3 DEPOSITION ON MANNAN
OD405 NM
TIME (MIN.)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
10
20
30
40
50
60
70
80
90
100

FIG. 31

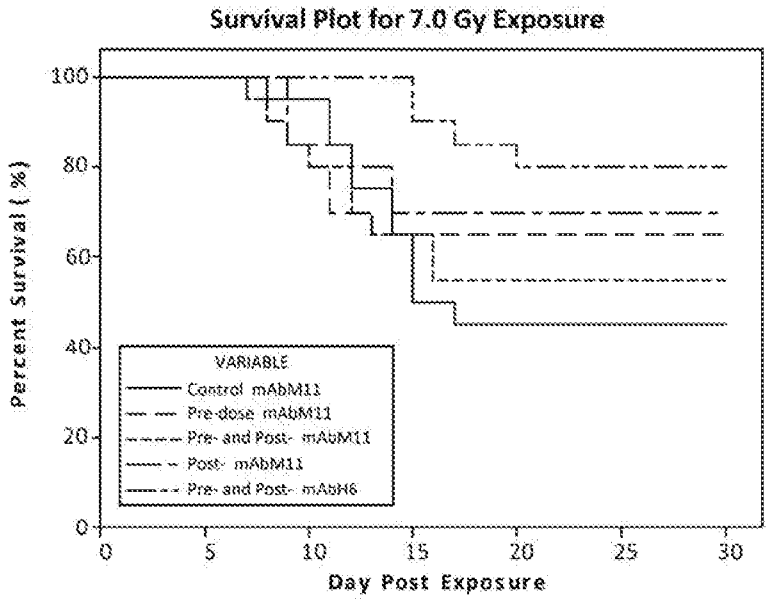

FIG. 32A

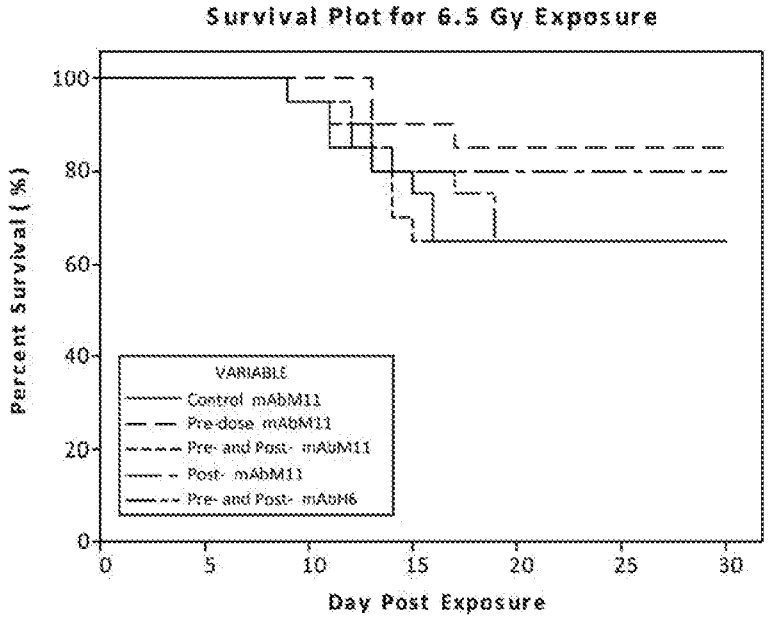

FIG. 32B

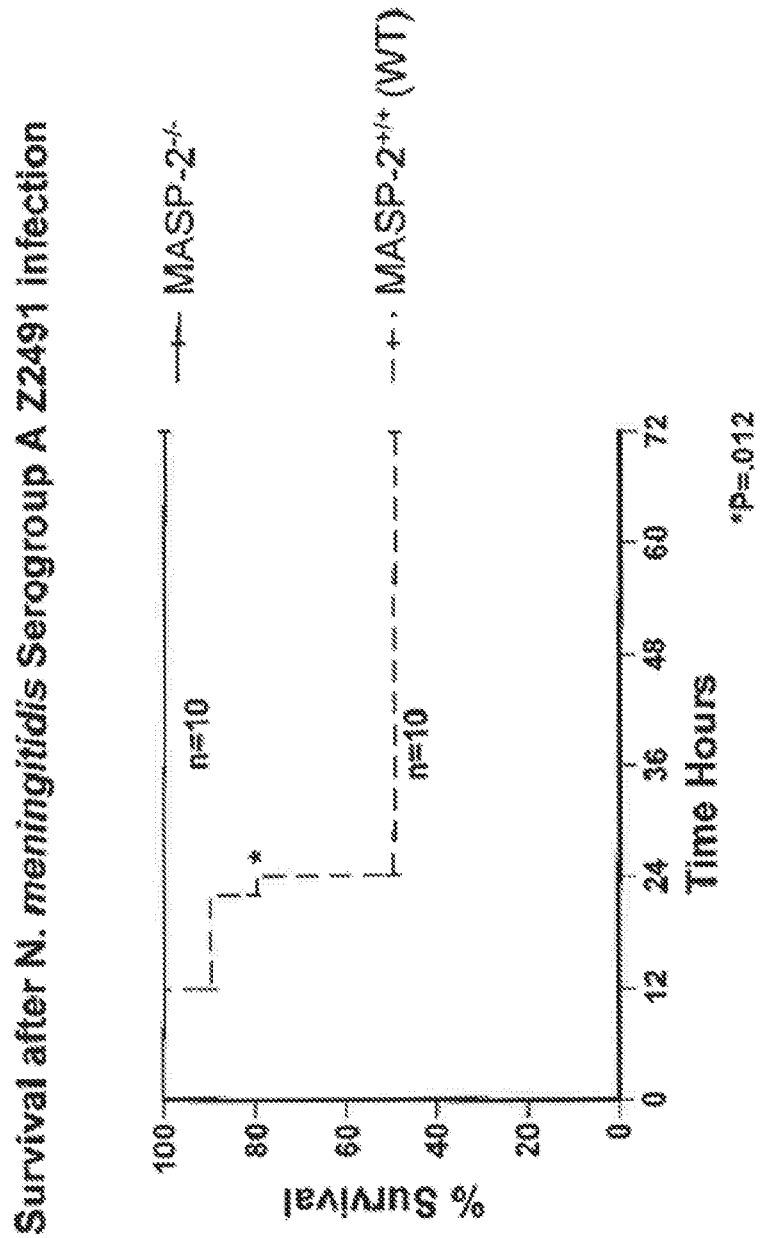
Survival after N. meningitidis Serogroup A Z2491 infection
MASP-2-/-
MASP-2+/+ (WT)
n=10
n=10
*
*P=.012
% Survival
Time Hours
100
80
60
40
20
0
0
12
24
36
48
60
72

FIG. 33

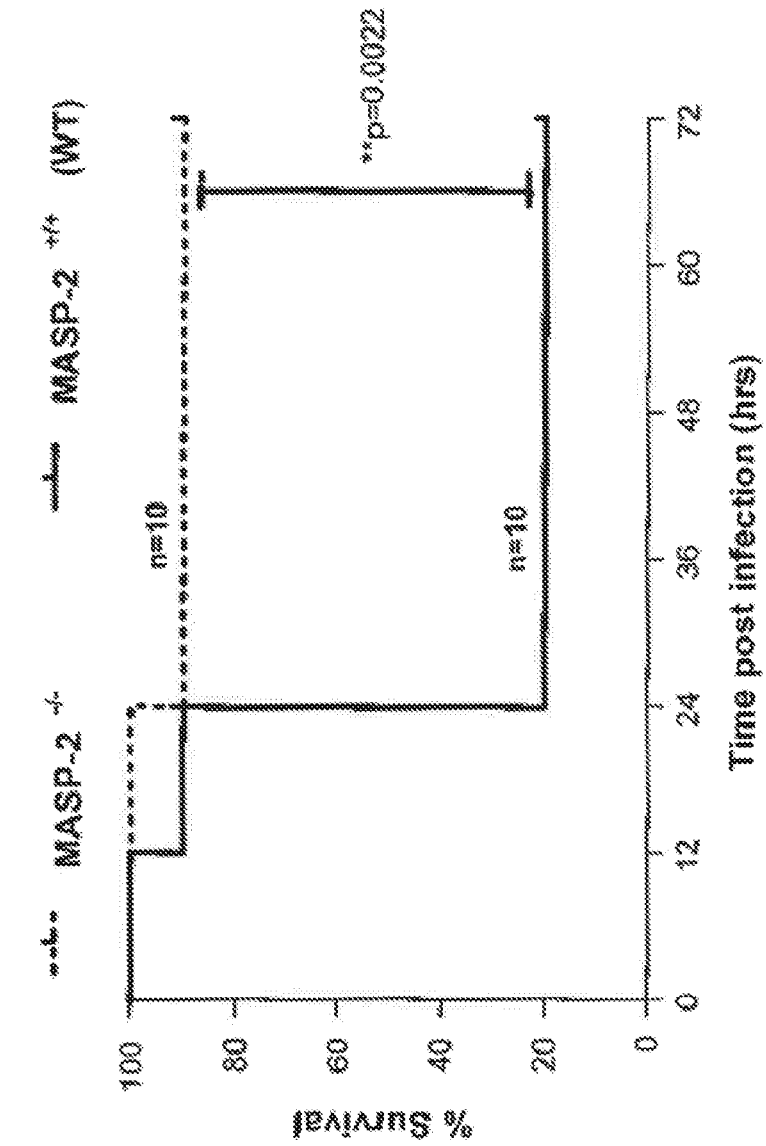
MASP-2 -/-
MASP-2 +/+ (WT)
**p=0.0022
n=10
n=10
% Survival
Time post infection (hrs)
0
20
40
60
80
100
0
12
24
36
48
60
72

FIG. 34

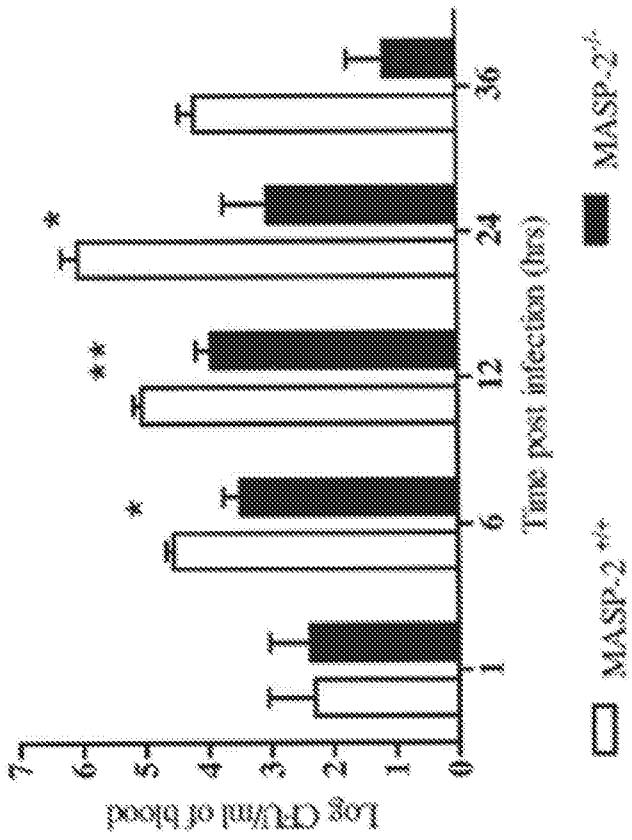
Log CFU/ml of blood
0
1
2
3
4
5
6
7
1
6
12
24
36
Time post infection (hrs)
MASP-2+/+
MASP-2-/-
*
**
*
*

FIG. 35

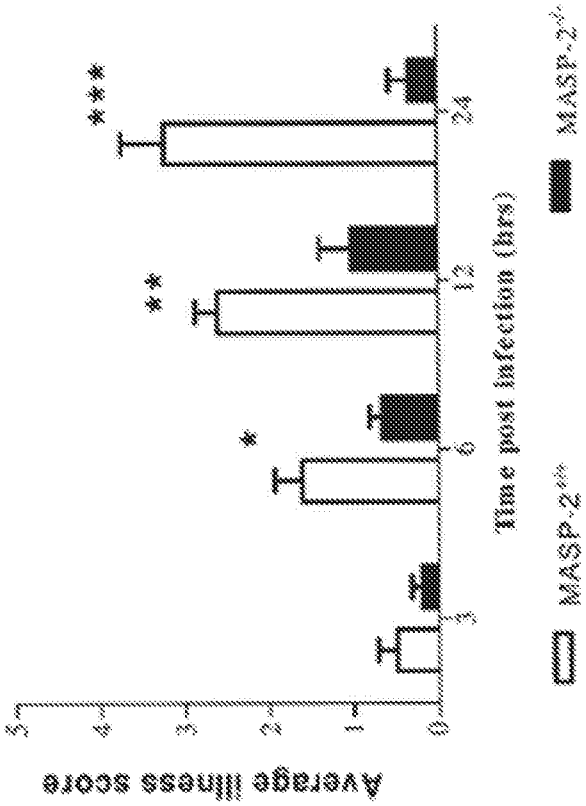
Average illness score
0
1
2
3
4
5
3
6
12
24
Time post infection (hrs)
*
**
***
MASP-2+/+
MASP-2-/-

FIG. 36

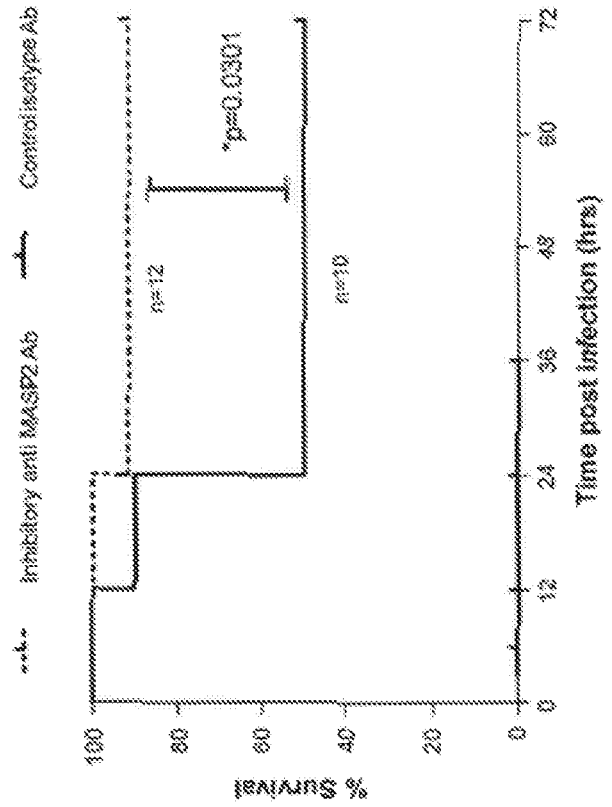
Inhibitory anti MASP2 Ab
Control isotype Ab
% Survival
100
80
60
40
20
0
0
12
24
36
48
60
72
Time post infection (hrs)
n=12
n=10
*p=0.0301

FIG. 37

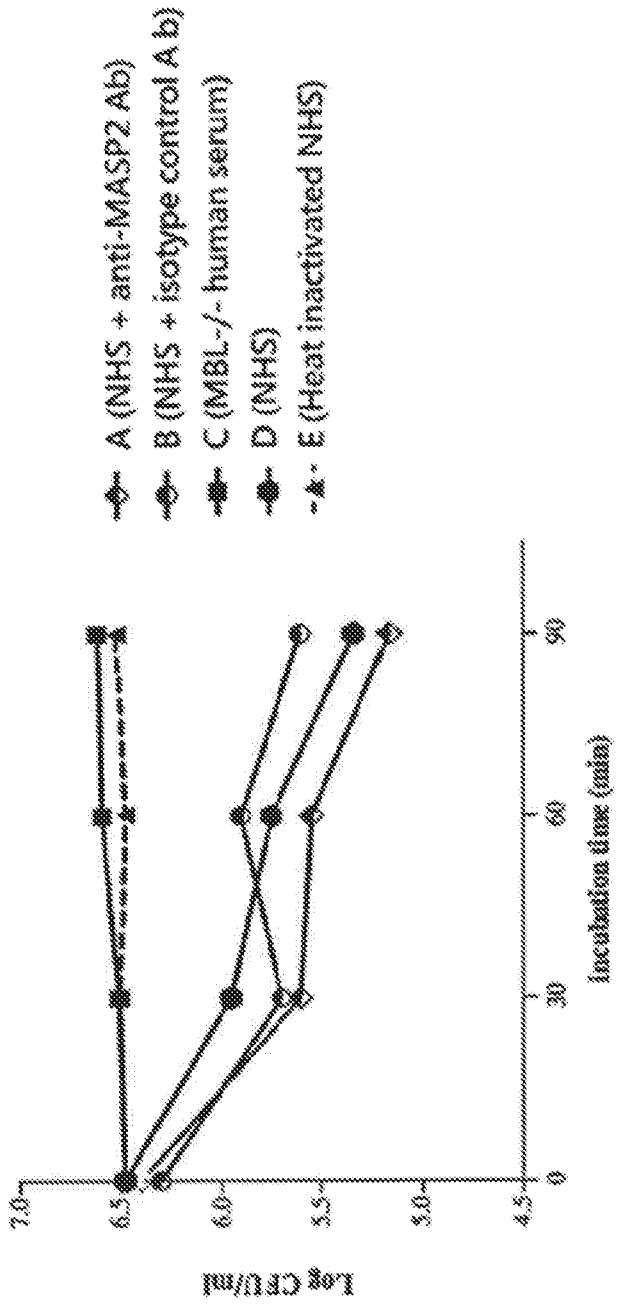
A (NHS + anti-MASP2 Ab)
B (NHS + isotype control A b)
C (MBL-/- human serum)
D (NHS)
E (Heat inactivated NHS)
Log CFU/ml
7.0
6.5
6.0
5.5
5.0
4.5
0
30
60
90
Incubation time (min)

FIG. 38

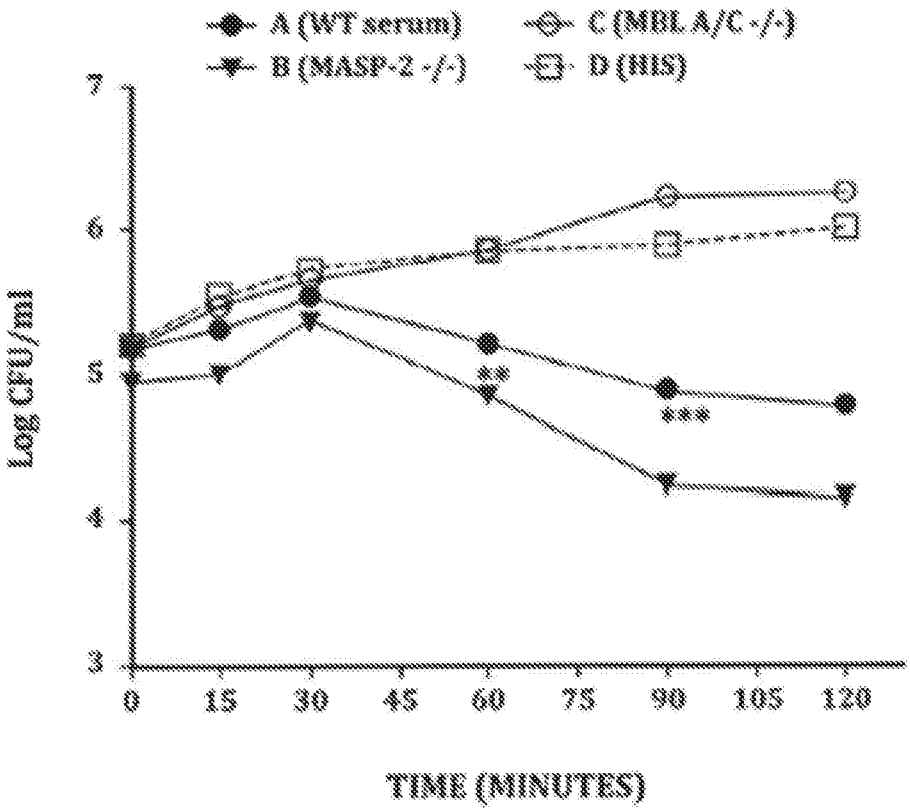
A (WT serum)
B (MASP-2 -/-)
C (MBL A/C -/-)
D (HIS)
Log CFU/ml
7
6
5
4
3
0
15
30
45
60
75
90
105
120
TIME (MINUTES)

FIG. 39

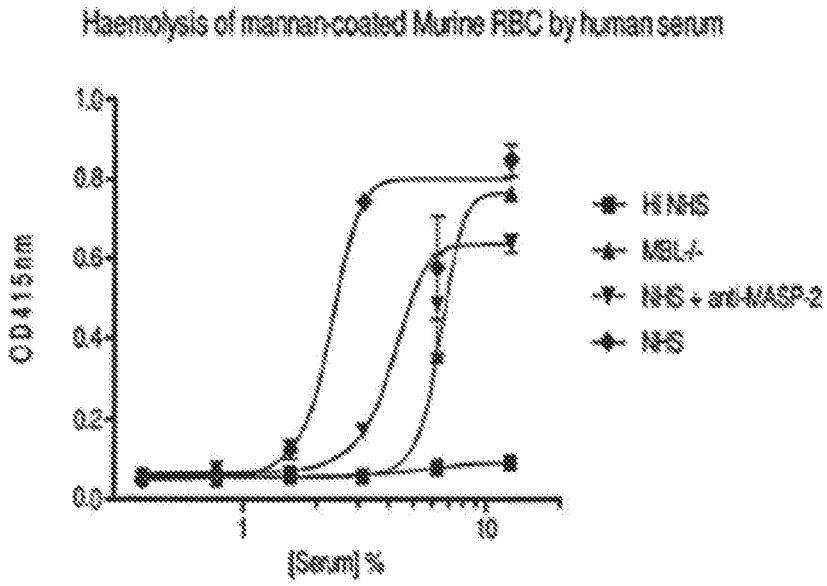
Haemolysis of mannan-coated Murine RBC by human serum
OD 415 nm
1.0
0.8
0.6
0.4
0.2
0.0
1
0
[Serum] %
NHS
MBL-/-
NHS + anti-MASP-2
NHS

FIG. 40

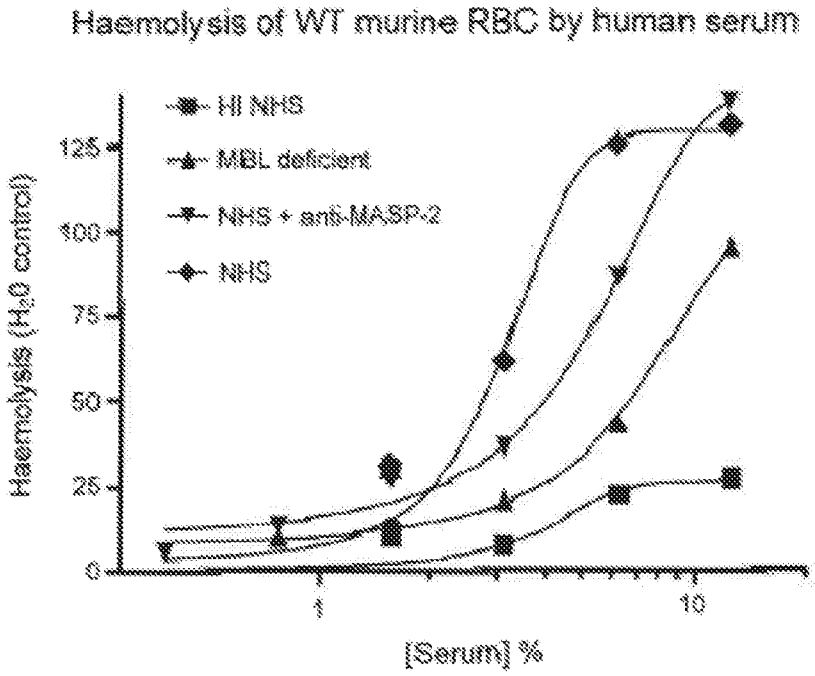
Haemolysis of WT murine RBC by human serum
HI NHS
MBL deficient
NHS + anti-MASP-2
NHS
Haemolysis (H2O control)
125
100
75
50
25
0
1
10
[Serum] %

FIG. 41

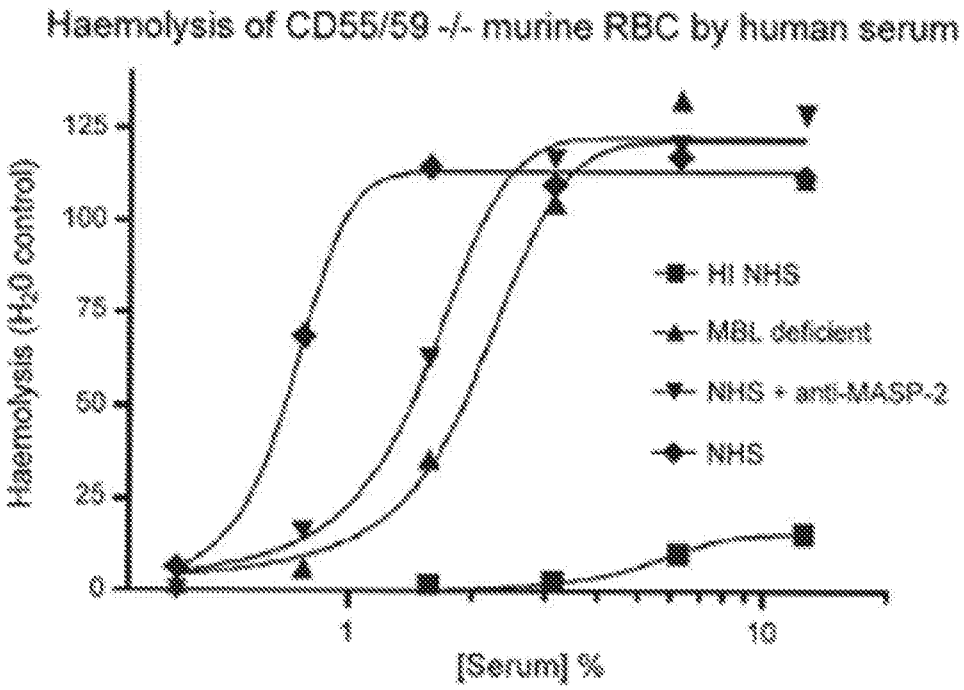
Haemolysis of CD55/59 -/- murine RBC by human serum
Haemolysis (H_2O control)
125
100
75
50
25
0
1
10
[Serum] %
HI NHS
MBL deficient
NHS + anti-MASP-2
NHS

FIG. 42

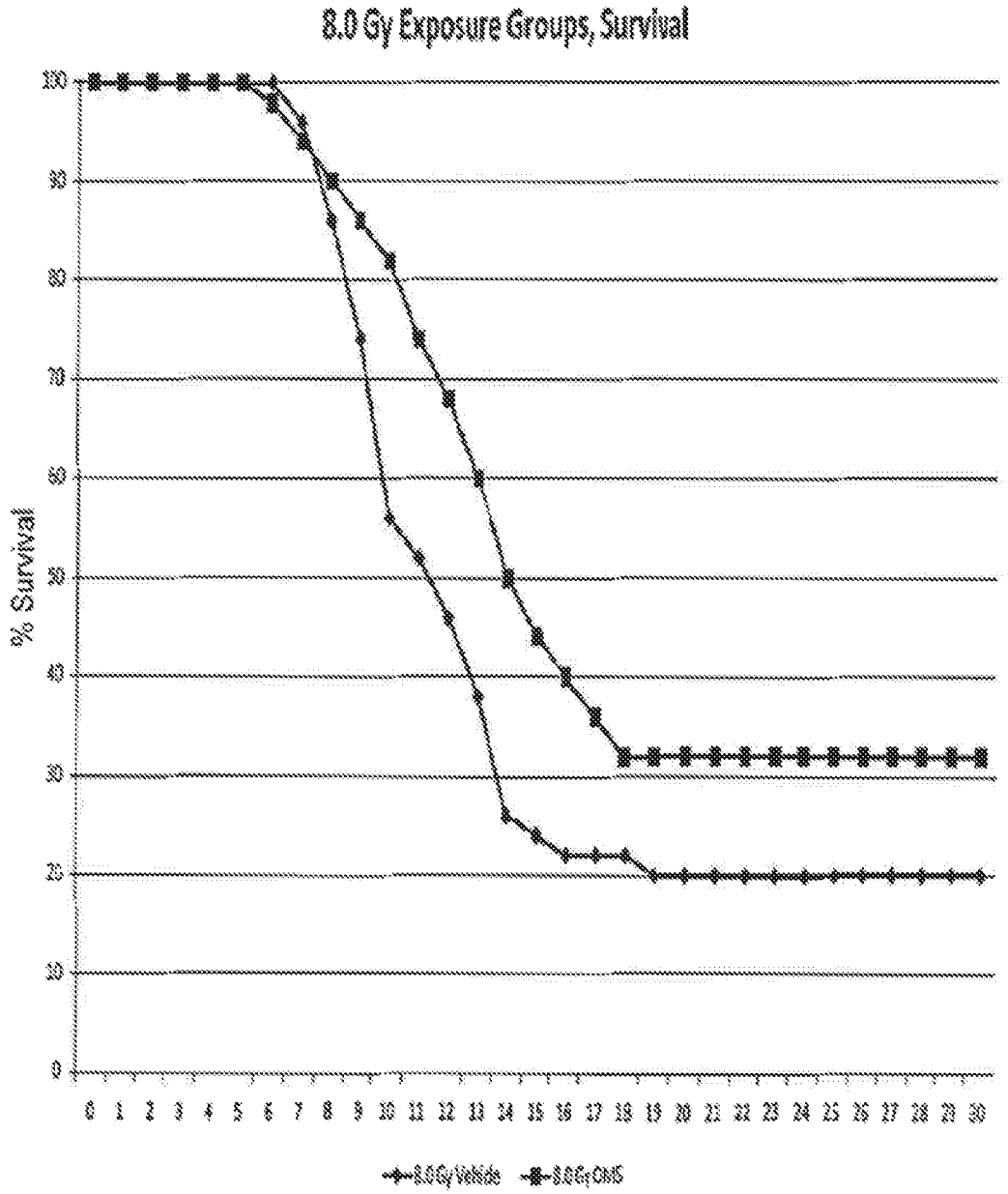
8.0 Gy Exposure Groups, Survival
% Survival
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
8.0Gy/Vehicle
8.0Gy/CM5

FIG. 43

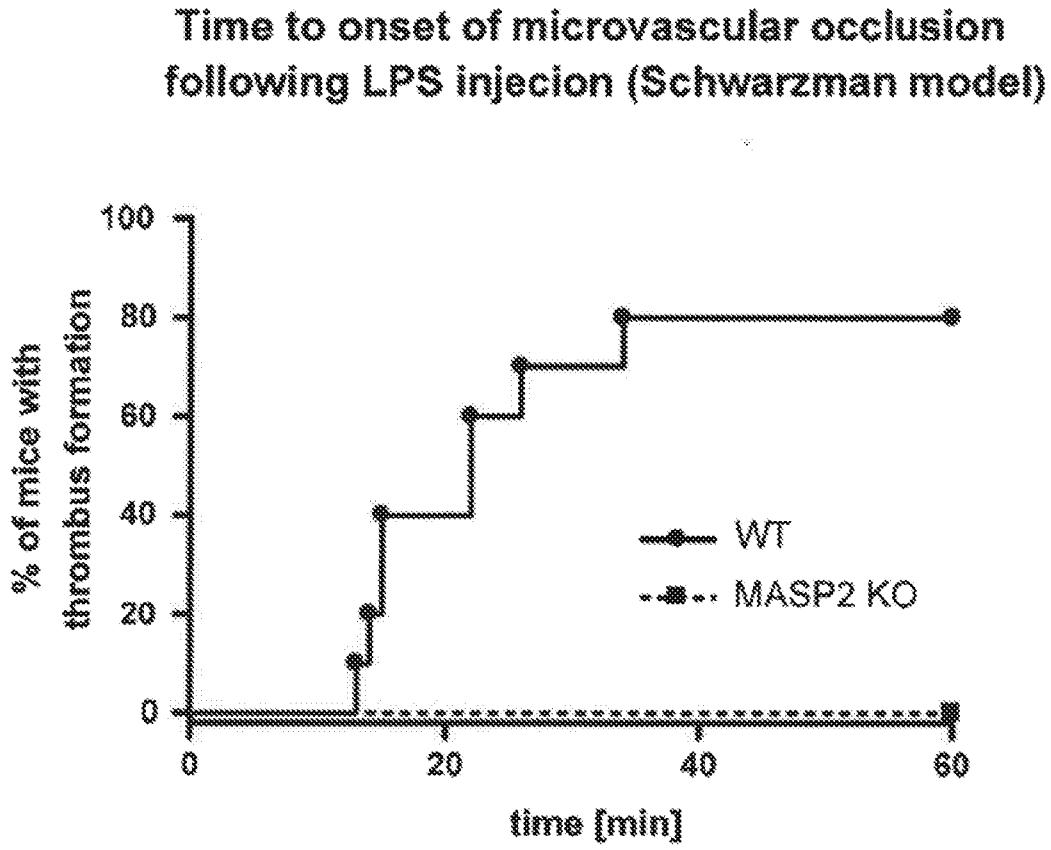
Time to onset of microvascular occlusion following LPS injecion (Schwarzman model)
% of mice with thrombus formation
100
80
60
40
20
0
0
20
40
60
time [min]
WT
MASP2 KO

FIG. 44

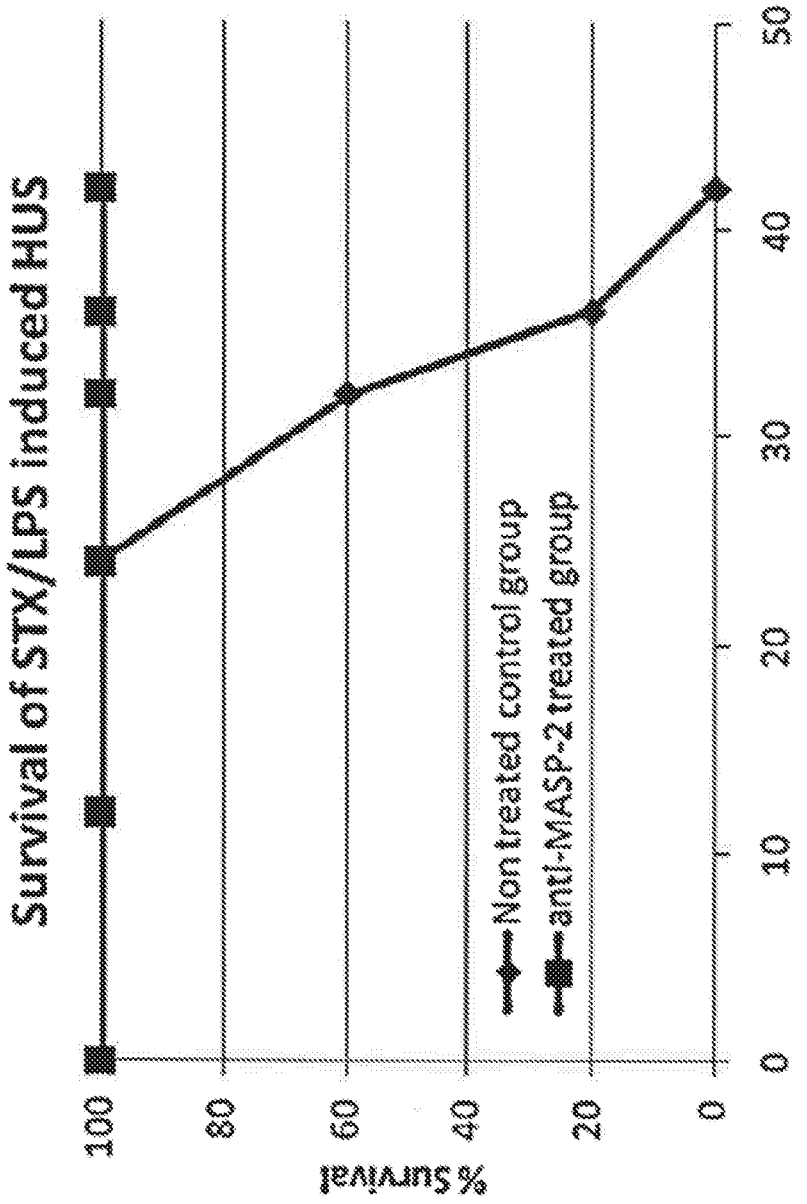
Survival of STX/LPS induced HUS
% Survival
100
80
60
40
20
0
0
10
20
30
40
50
time (hrs)
Non treated control group
anti-MASP-2 treated group

FIG. 45

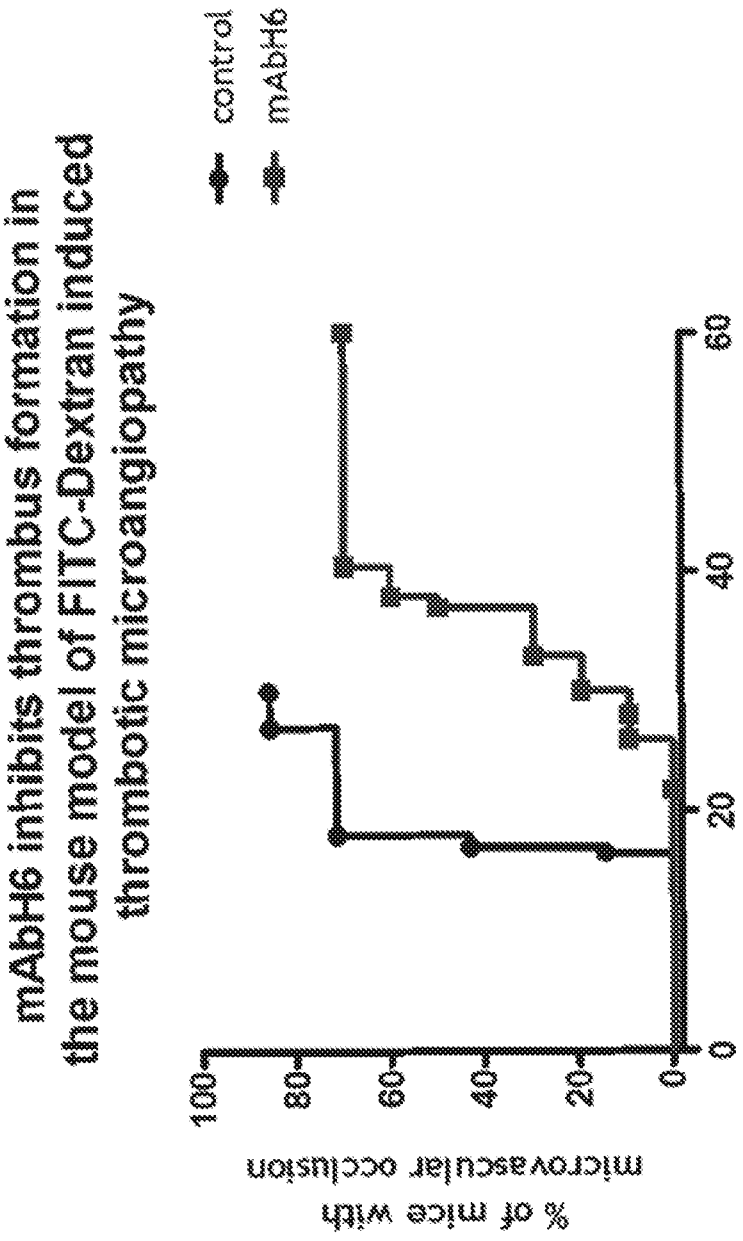
mAbH6 inhibits thrombus formation in the mouse model of FITC-Dextran induced thrombotic microangiopathy
control
mAbH6
% of mice with microvascular occlusion
100
80
60
40
20
0
0
20
40
60
time [min]

FIG. 46

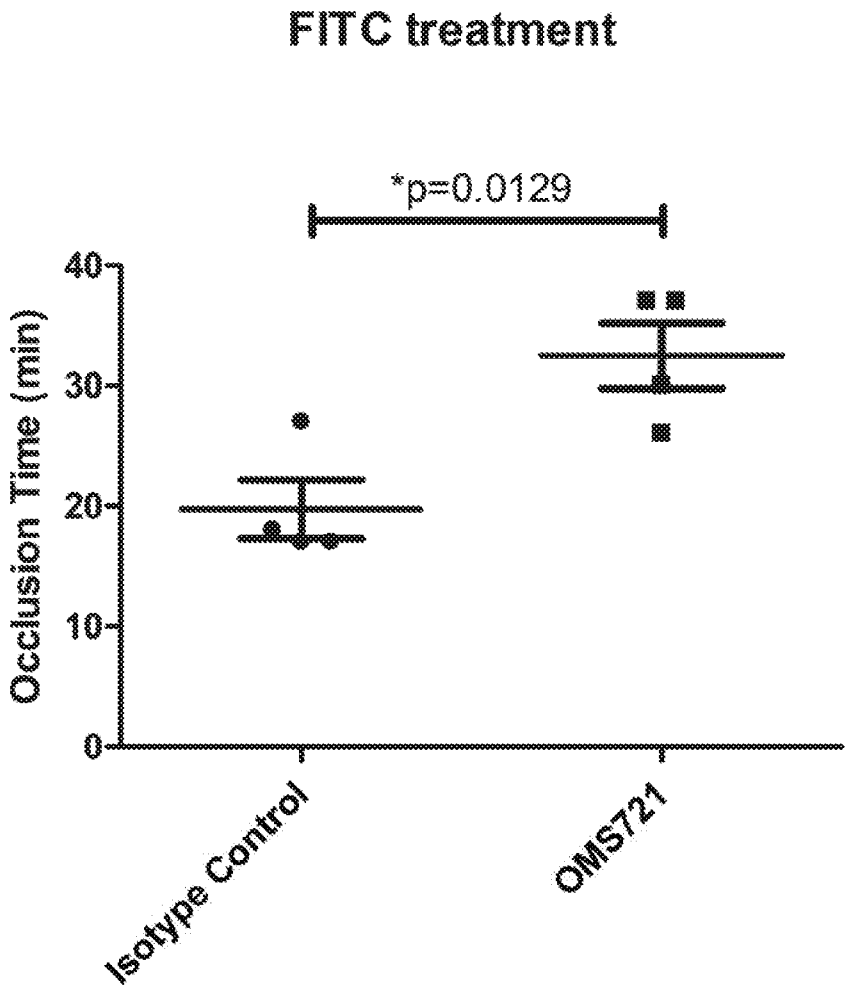
FITC treatment
*p=0.0129
Occlusion Time (min)
40
30
20
10
0
Isotype Control
OMS721

FIG. 47

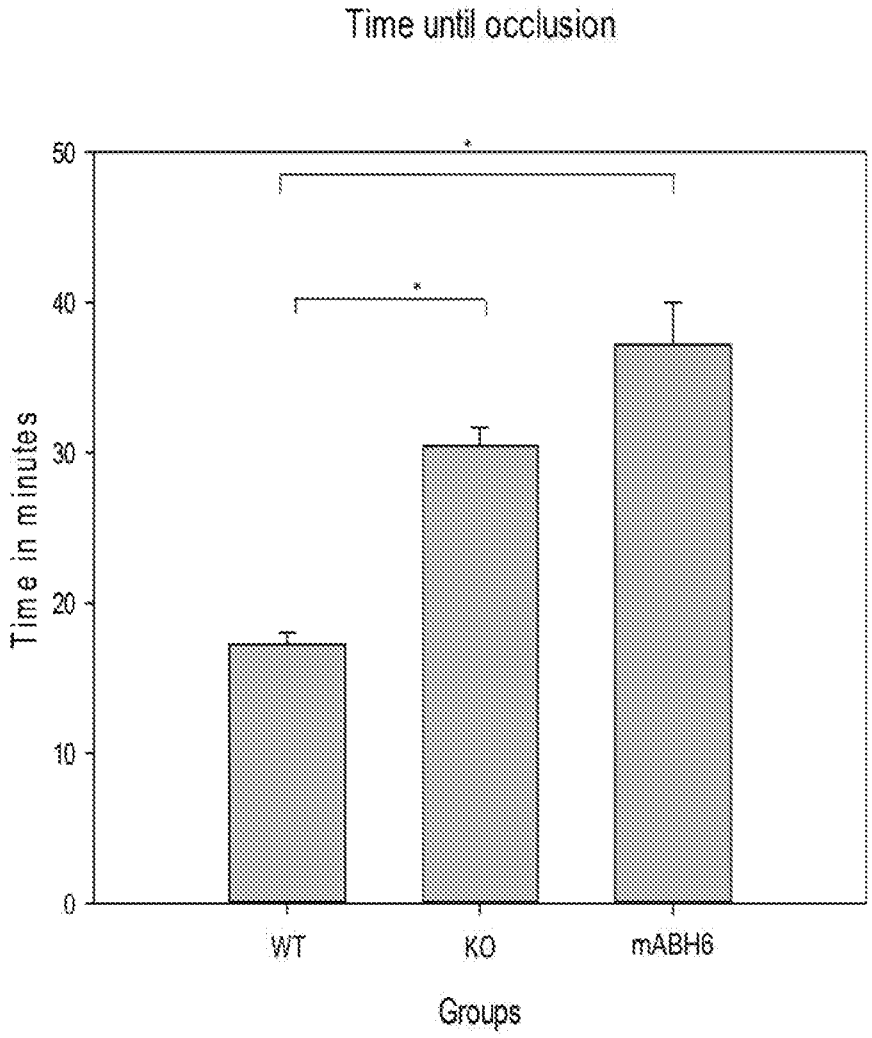
Time until occlusion
Time in minutes
50
40
30
20
10
0
*
*
WT
KO
mABH6
Groups

FIG. 48

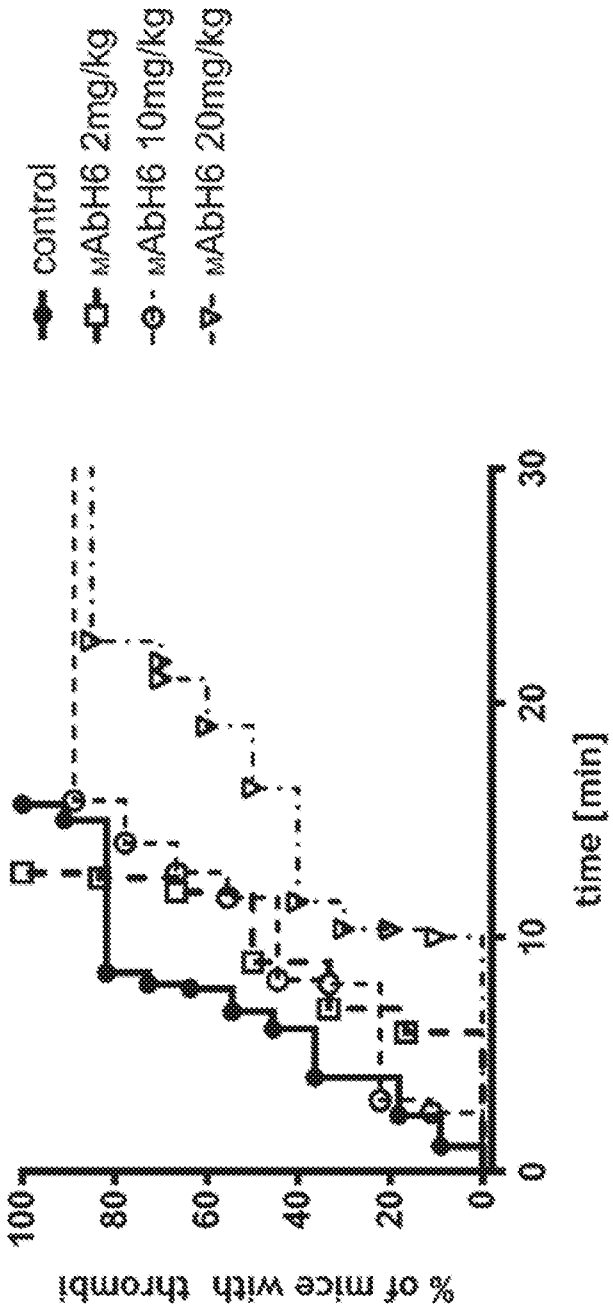
control
mAbH6 2mg/kg
mAbH6 10mg/kg
mAbH6 20mg/kg
% of mice with thrombi
100
80
60
40
20
0
0
10
20
30
time [min]

FIG. 49

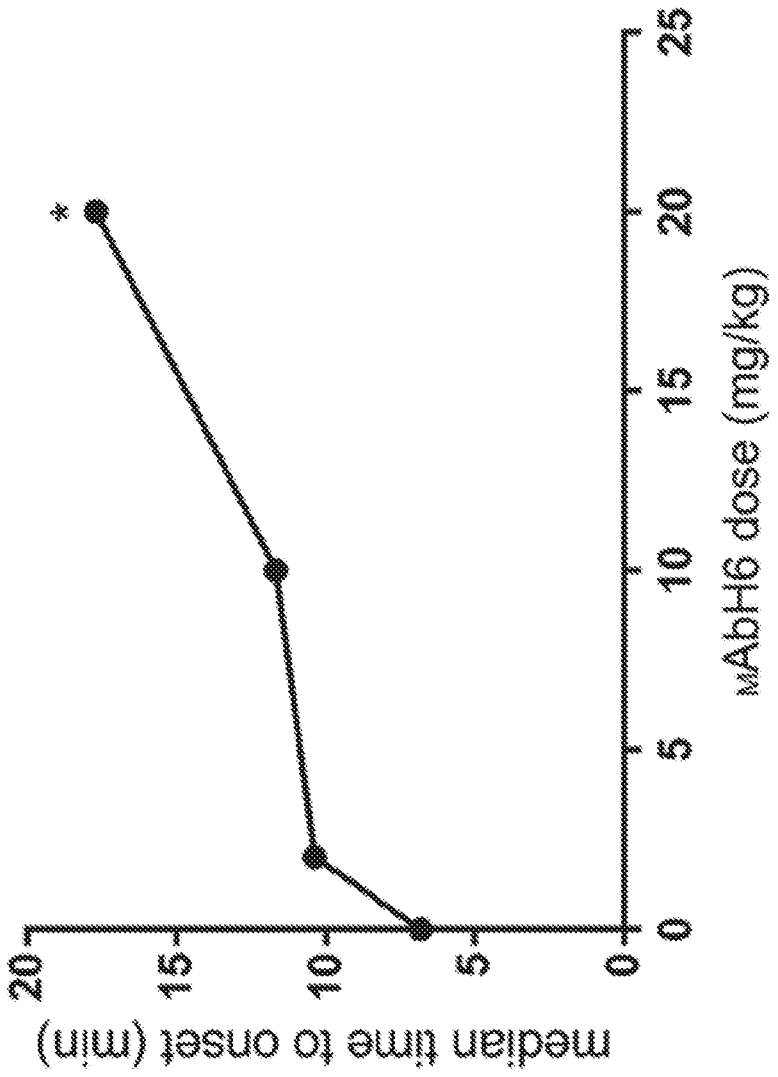
median time to onset (min)
20
15
10
5
0
*
0
5
10
15
20
25
mAbH6 dose (mg/kg)

FIG. 50

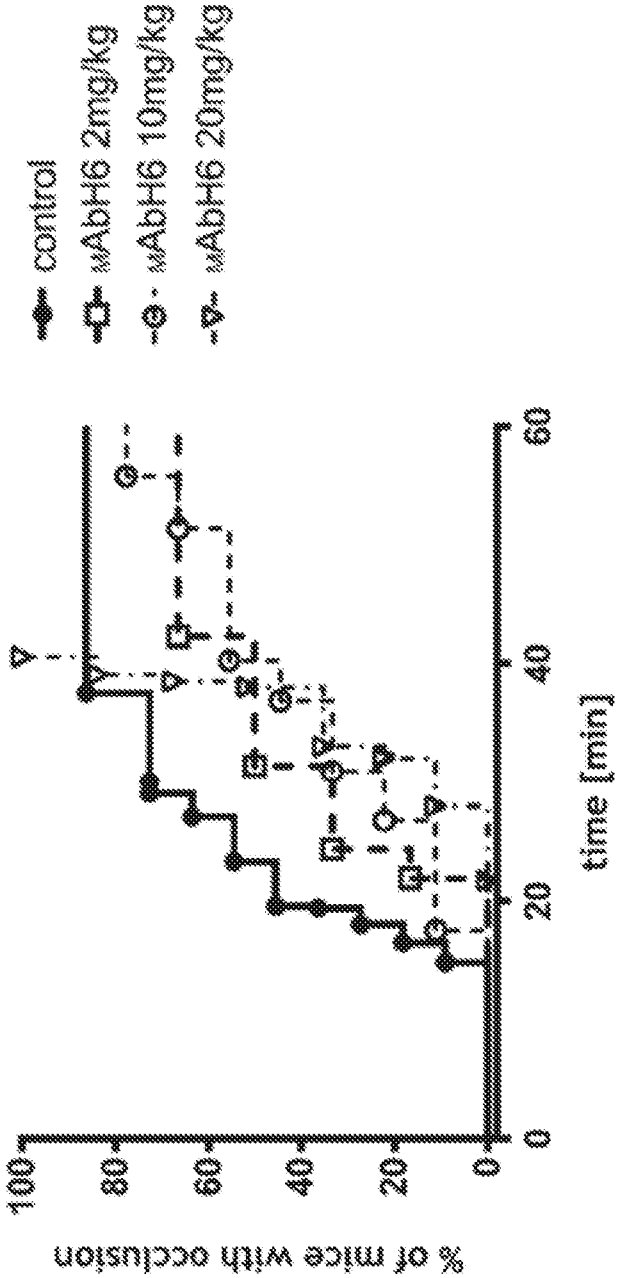
control
αAbH6 2mg/kg
αAbH6 10mg/kg
αAbH6 20mg/kg
% of mice with occlusion
100
80
60
40
20
0
0
20
40
60
time [min]

FIG. 51

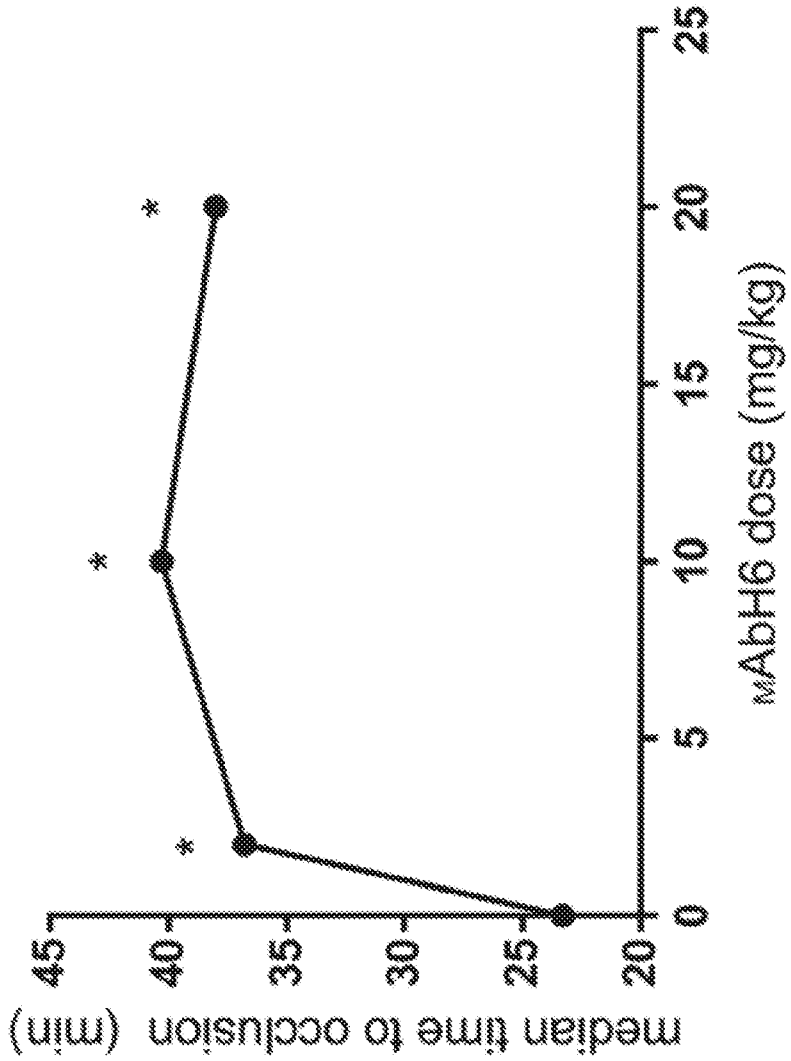
median time to occlusion (min)
45
40
35
30
25
20
0
5
10
15
20
25
mAbH6 dose (mg/kg)
*
*
*

FIG. 52

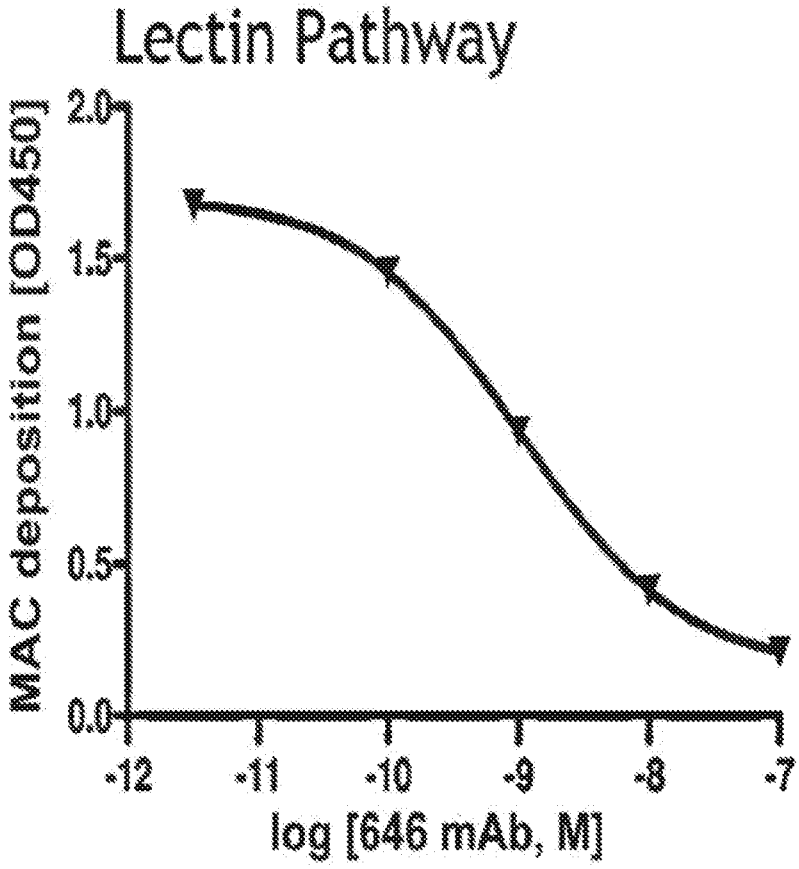
Lectin Pathway
MAC deposition [OD450]
2.0
1.5
1.0
0.5
0.0
-12
-11
-10
-9
-8
-7
log [646 mAb, M]

FIG. 53A

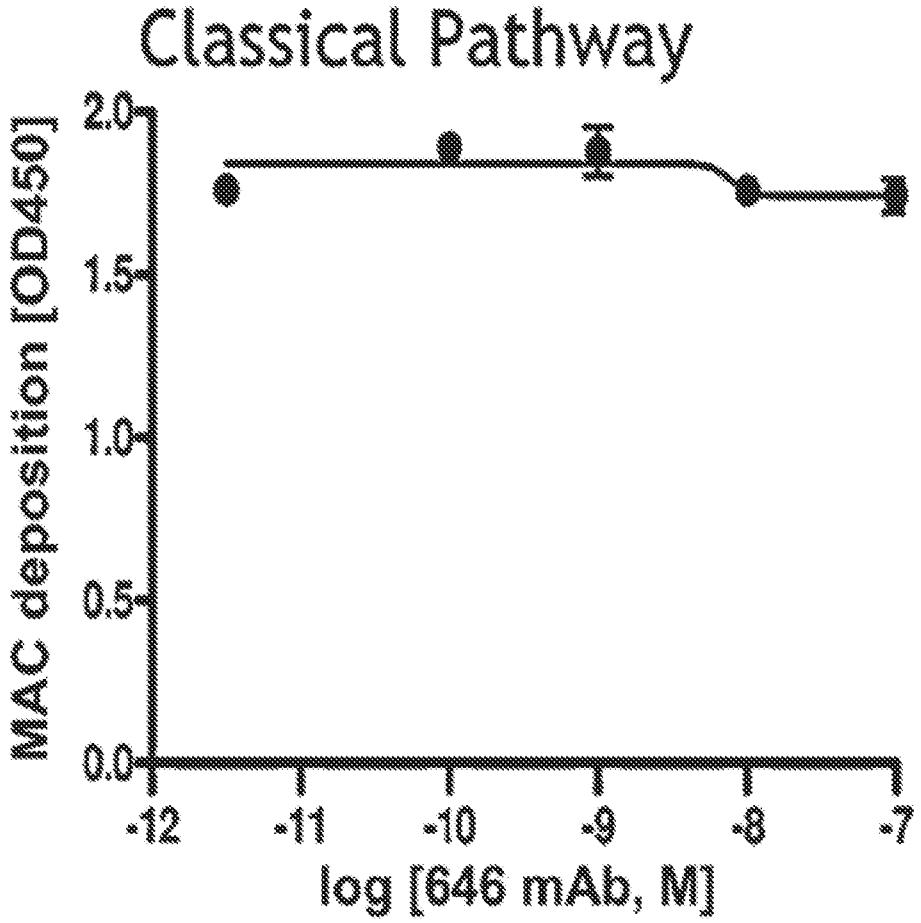
Classical Pathway
MAC deposition [OD450]
2.0
1.5
1.0
0.5
0.0
-12
-11
-10
-9
-8
-7
log [646 mAb, M]

FIG. 53B

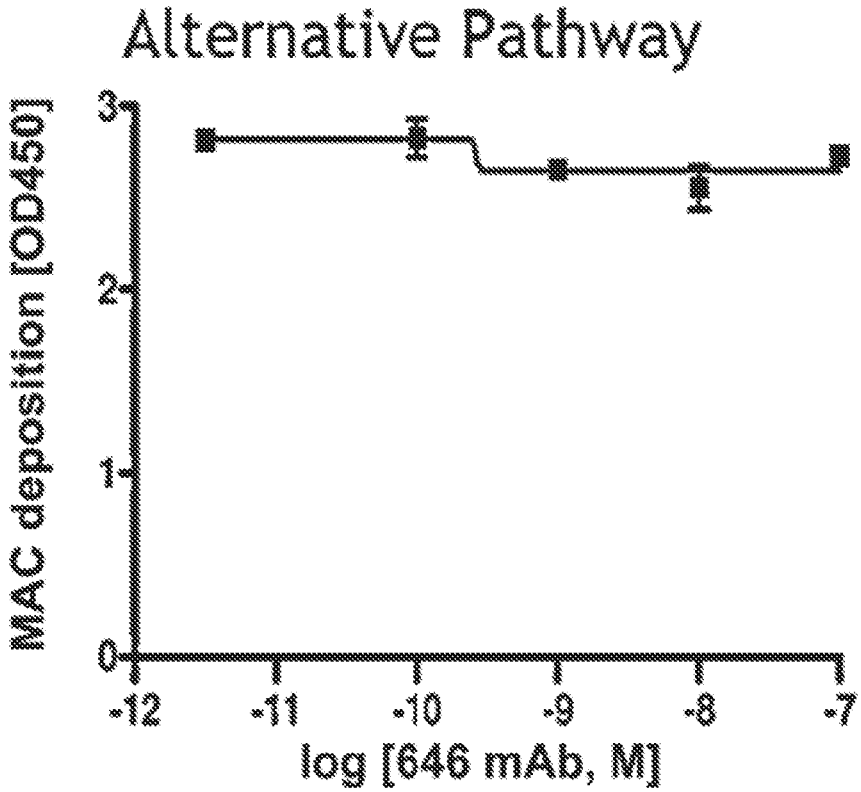
Alternative Pathway
MAC deposition [OD450]
0
1
2
3
-12
-11
-10
-9
-8
-7
log [646 mAb, M]

FIG. 53C

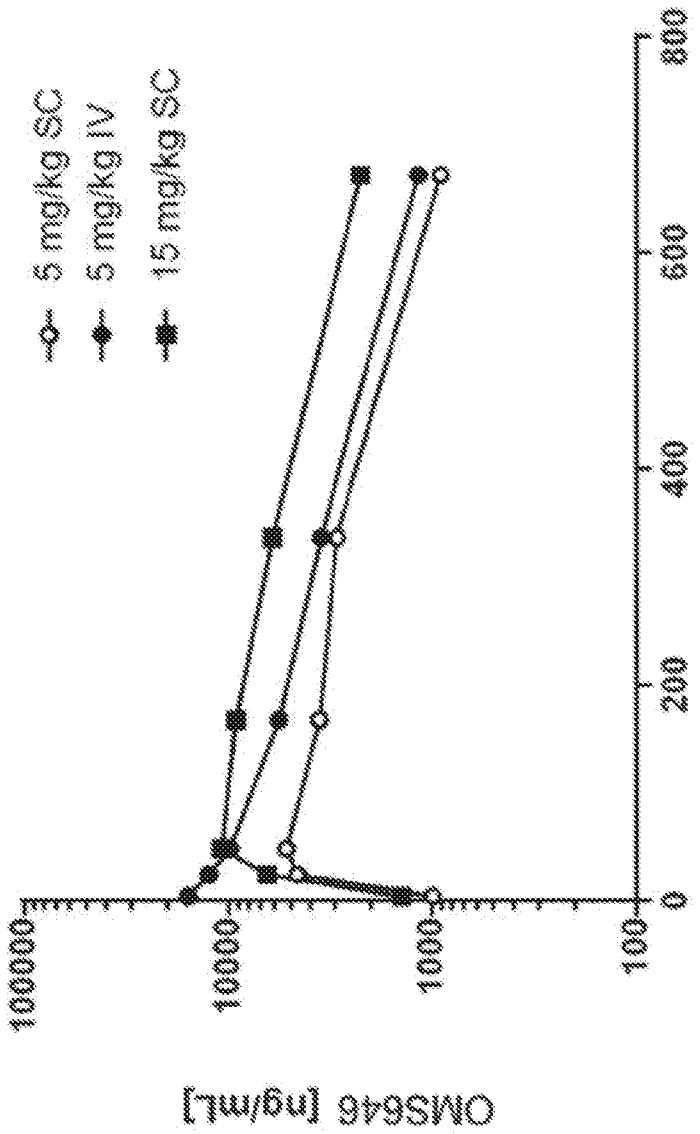
5 mg/kg SC
5 mg/kg IV
15 mg/kg SC
100000
10000
1000
100
0
200
400
600
800
time [hrs]
OMS646 [ng/mL]

FIG. 54

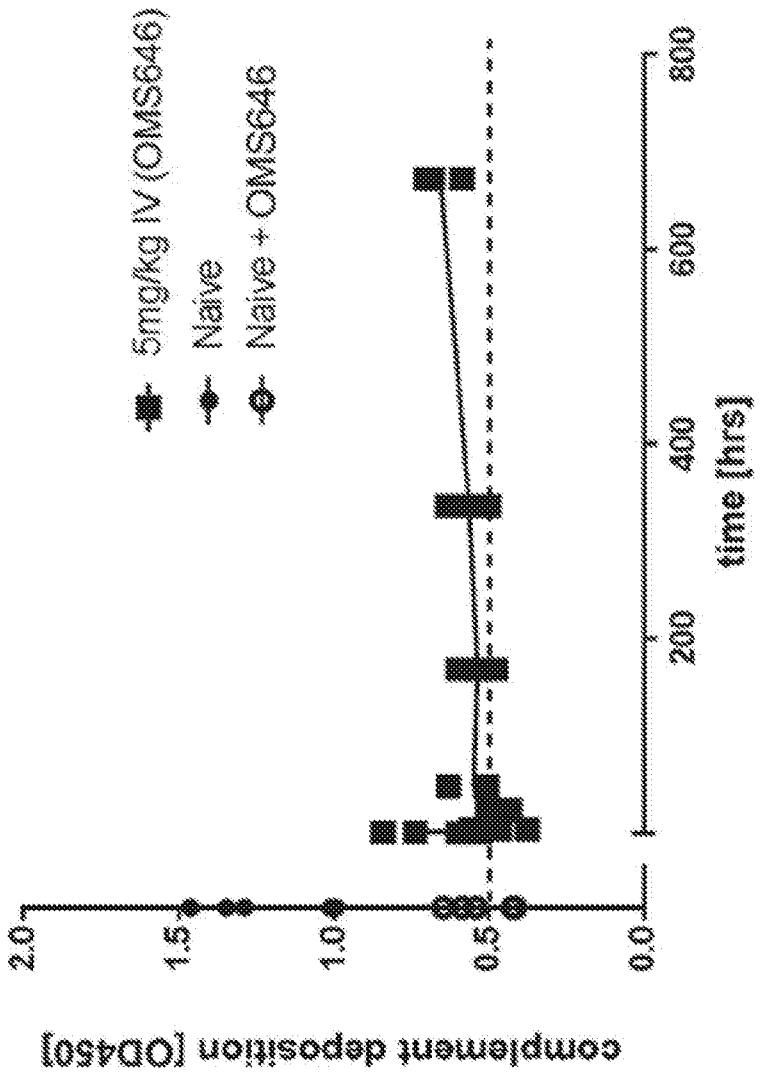
5mg/kg IV (OMS646)
Naive
Naive + OMS646
complement deposition [OD450]
2.0
1.5
1.0
0.5
0.0
200
400
600
800
time [hrs]

FIG. 55A

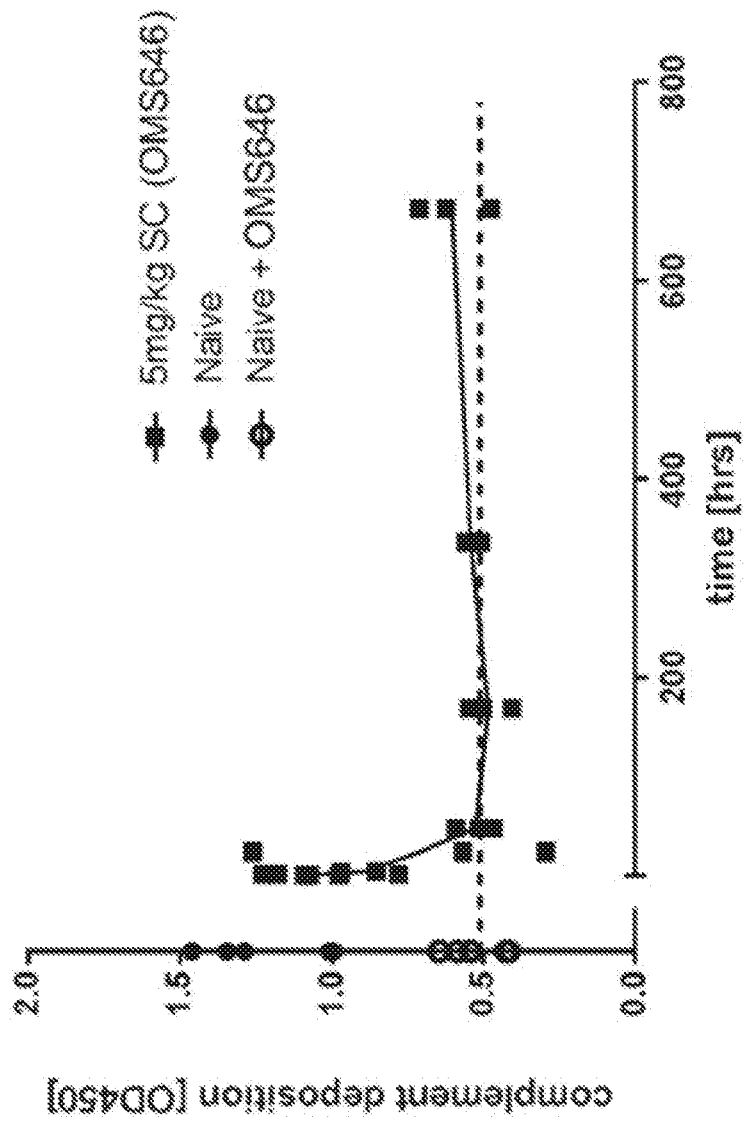
5mg/kg SC (OMS646)
Naive
Naive + OMS646
complement deposition [OD450]
2.0
1.5
1.0
0.5
0.0
200
400
600
800
time [hrs]

FIG. 55B

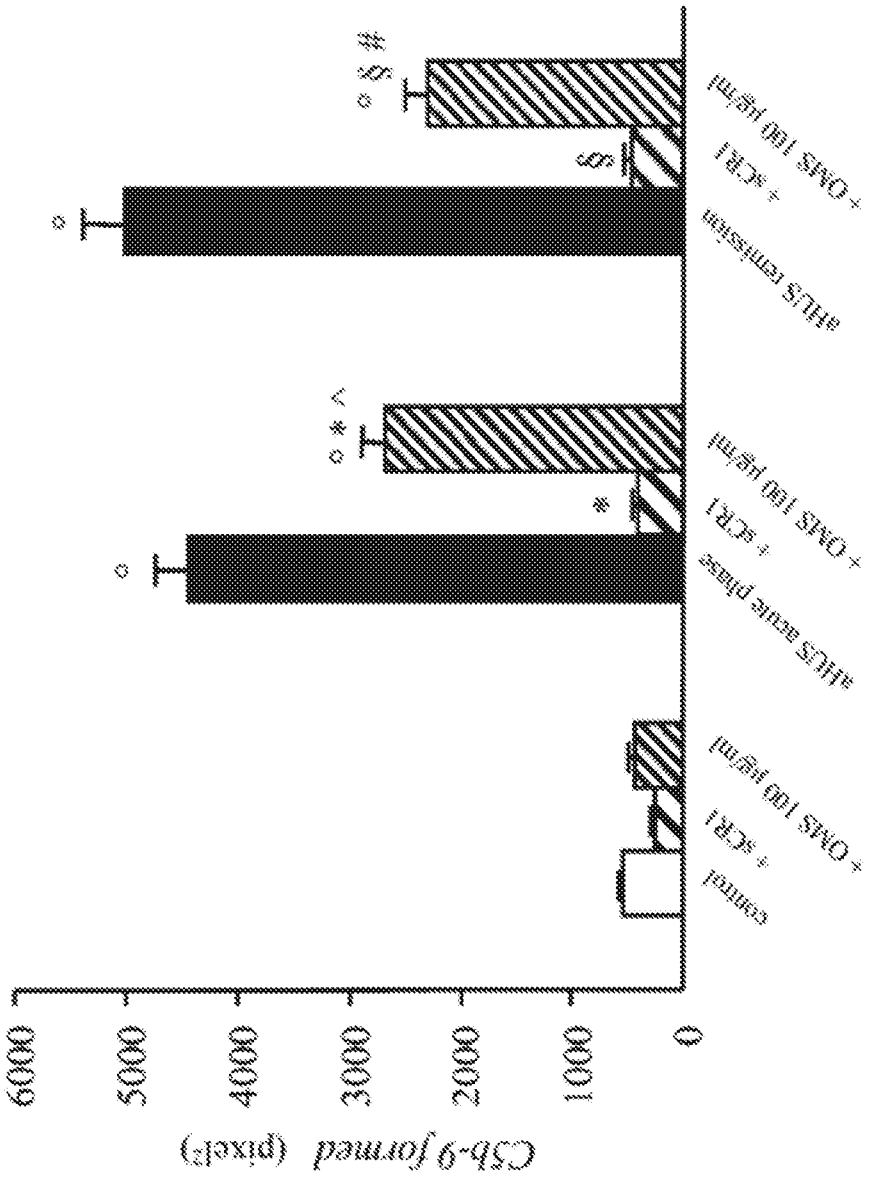
Cfb-9 formed (pixel²)
6000
5000
4000
3000
2000
1000
0
control
+ sCR1
+ DMS 100 µg/ml
ΔHUS acute phase
+ sCR1
+ DMS 100 µg/ml
ΔHUS remission
+ sCR1
+ DMS 100 µg/ml

FIG. 56

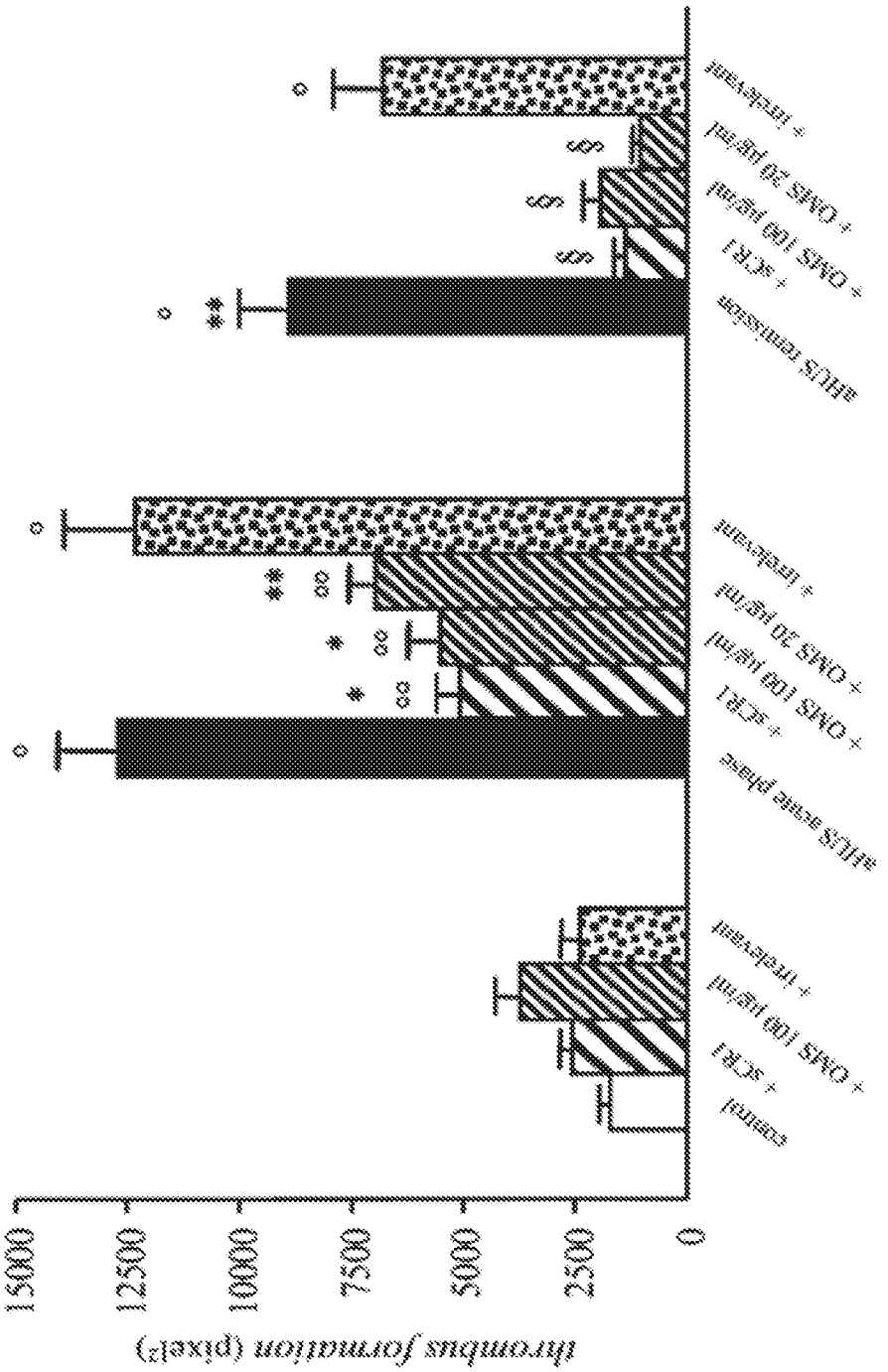
thrombus formation (pixel²)
15000
12500
10000
7500
5000
2500
0
control
+ scR1
+ OMS 100 µg/ml
+ OMS 30 µg/ml
+ vehicle ctrl
ΔHLS smooth phase
+ scR1
+ OMS 100 µg/ml
+ OMS 30 µg/ml
+ vehicle ctrl
ΔHLS constriction
+ scR1
+ OMS 100 µg/ml
+ OMS 30 µg/ml
+ vehicle ctrl

FIG. 57

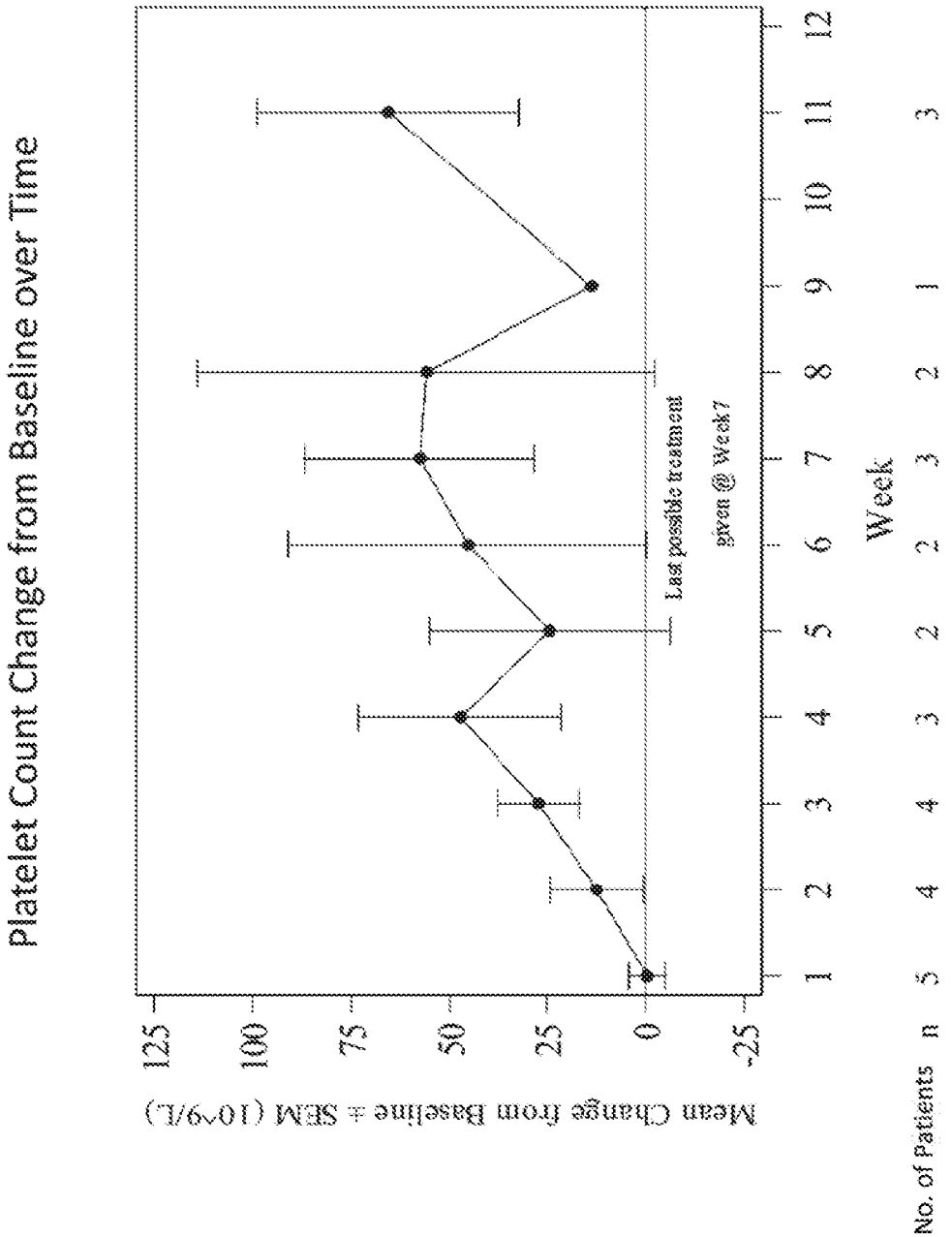
Platelet Count Change from Baseline over Time
Mean Change from Baseline ± SEM (10^9/L)
125
100
75
50
25
0
-25
Last possible treatment given @ Week7
Week
1 2 3 4 5 6 7 8 9 10 11 12
No. of Patients n
5 4 4 3 2 2 3 2 1 3

FIG. 58

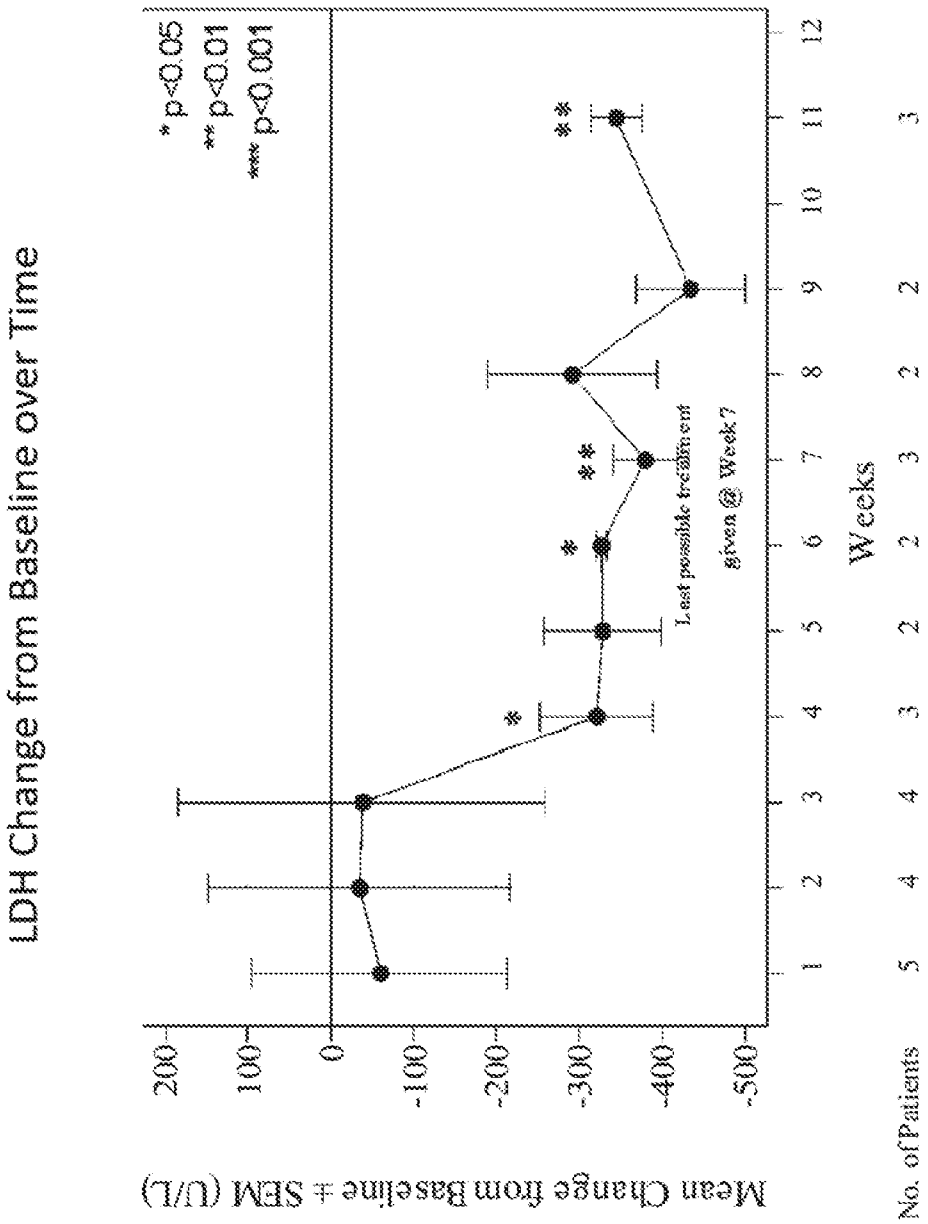
LDH Change from Baseline over Time
* p<0.05
** p<0.01
*** p<0.001
Mean Change from Baseline ± SEM (U/L)
200
100
0
-100
-200
-300
-400
-500
Weeks
1 2 3 4 5 6 7 8 9 10 11 12
No. of Patients
5 4 4 3 2 2 3 2 2 2 2 3
Last possible treatment given @ Week 7

FIG. 59

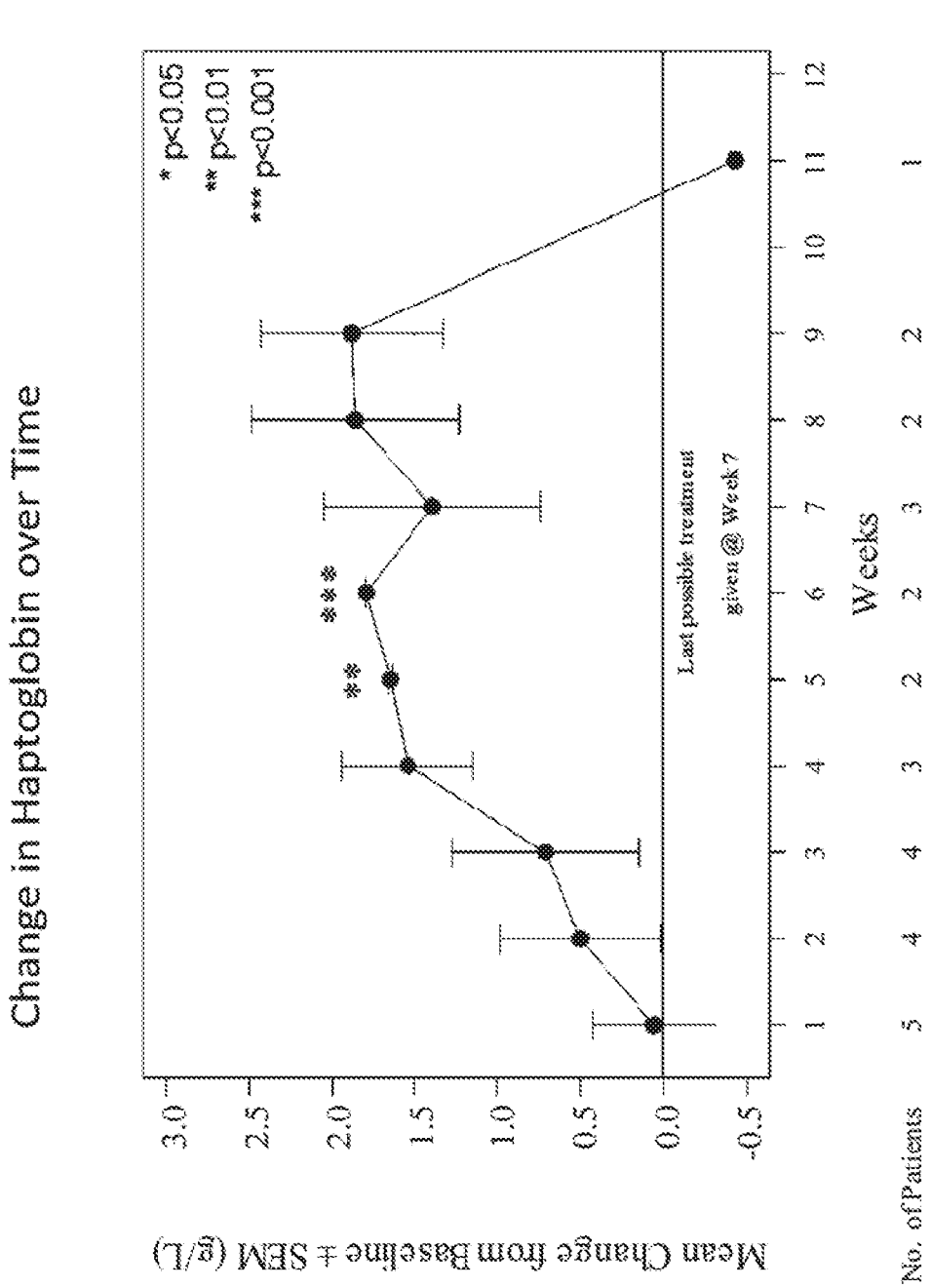
Change in Haptoglobin over Time
* p<0.05
** p<0.01
*** p<0.001
Mean Change from Baseline ± SEM (g/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Last possible treatment given @ Week 7
Weeks
No. of Patients
5 4 4 3 2 2 2 3 2 2 2 1

FIG. 60