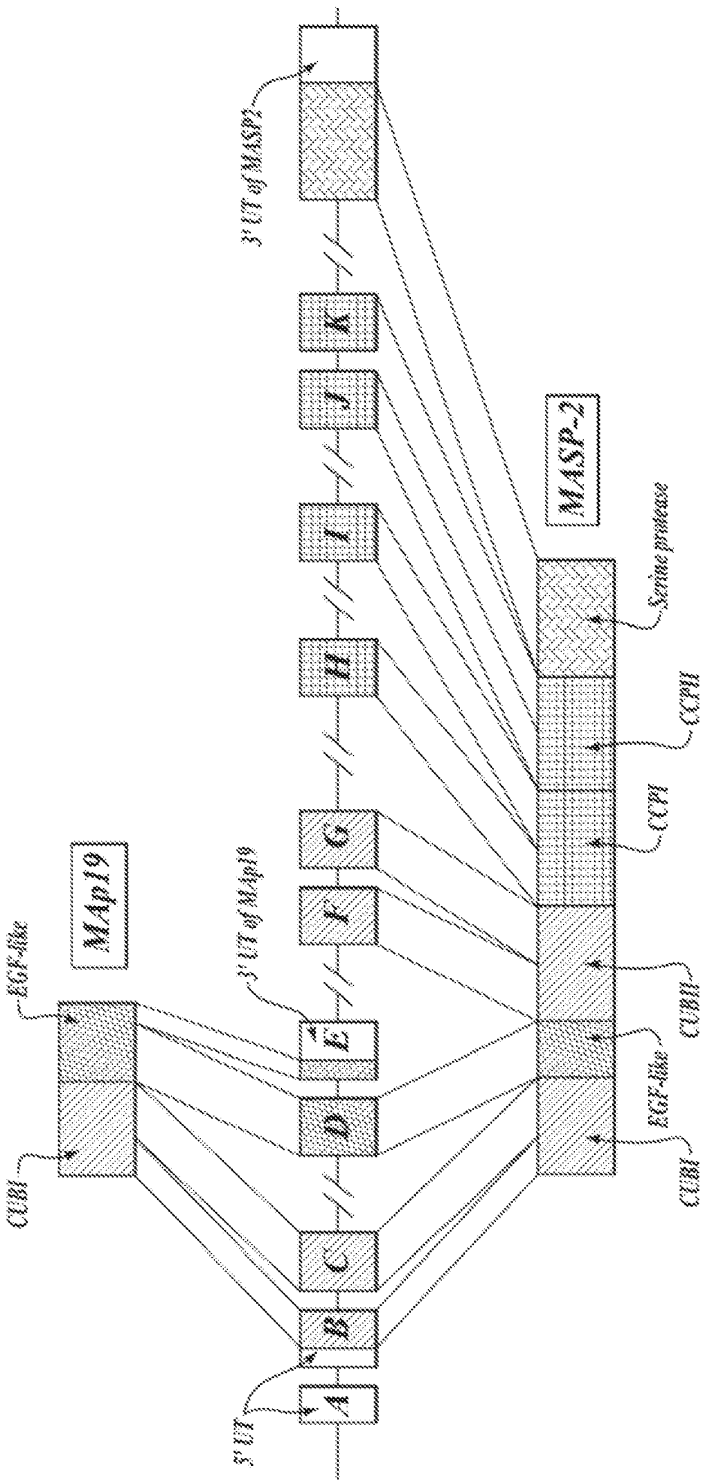

3' UT of MASP2
K
J
I
H
G
F
E
D
C
B
A
5' UT
MASP-2
Serine protease
CCPII
CCPI
CUBII
EGF-like
CUBI
MAp19
3' UT of Map19
EGF-like
CUBI

FIG. 1

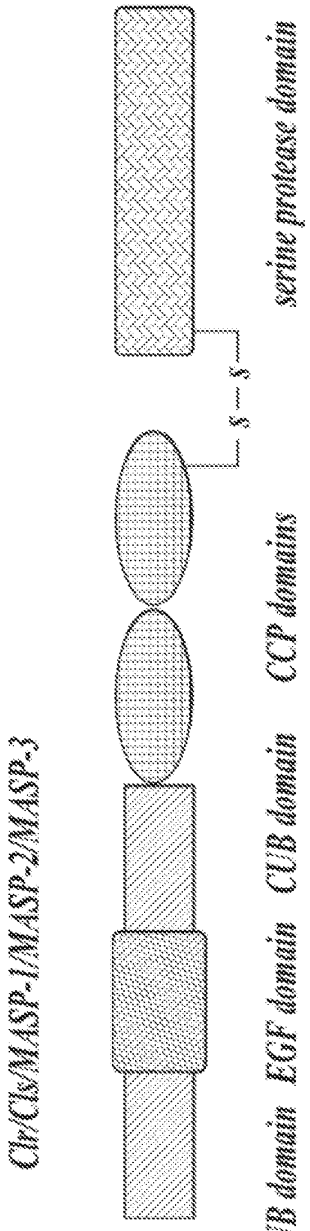

Cr/Cs/MASP-1/MASP-2/MASP-3
CUB domain
EGF domain
CUB domain
CCP domains
S — S
serine protease domain

FIG. 2A

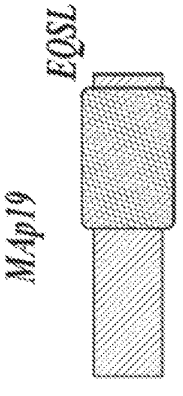

MAp19
EQSL
CUB domain
EGF domain

FIG. 2B

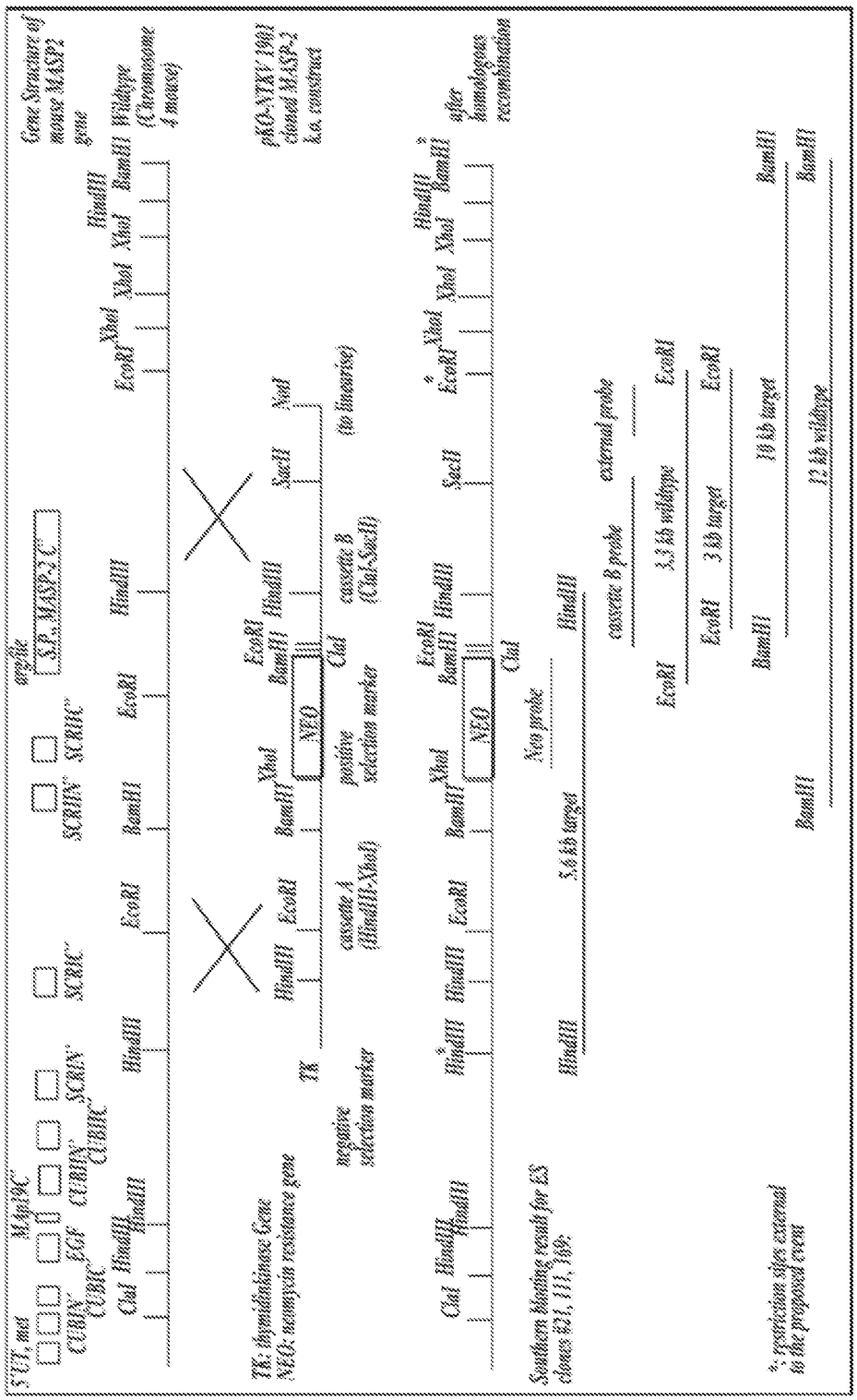

Gene Structure of mouse MAP2 gene
wild type
S.P. MAP2.C
SCRIV'
SCRIC'
CUBIV'
CUBIC'
EGF
MapIVC'
5'UT, met
Cla1 HindIII
HindIII
EcoRI
BamHI
HindIII
EcoRI
HindIII
XbaI
BamHI
HindIII
EcoRI
XbaI
XbaI
HindIII
BamHI
Wildtype (Chromosome 4 mouse)
pKO-NTKV 1901 cloned MAP-2 k.o. construct
TK: thymidinkinase Gene
NEO: neomycin resistance gene
TK
HindIII
EcoRI
BamHI
XbaI
NEO
EcoRI
BamHI
HindIII
SacII
NotI
negative selection marker
cassette A (HindIII-XbaI)
positive selection marker
Cla1
cassette B (Cla1-SacII)
(to linearize)
Cla1 HindIII
HindIII
HindIII*
HindIII
EcoRI
BamHI
XbaI
NEO
EcoRI
BamHI
HindIII
SacII
EcoRI*
XbaI
XbaI
HindIII
BamHI*
after homologous recombination
Cla1
Southern blotting result for ES clones #21, 111, 300:
Neo probe
HindIII
5.6 kb target
HindIII
cassette B probe
external probe
EcoRI
3.3 kb wildtype
EcoRI
EcoRI
3 kb target
EcoRI
BamHI
10 kb target
BamHI
BamHI
12 kb wildtype
BamHI
*: restriction sites external to the proposed event

FIG. 3

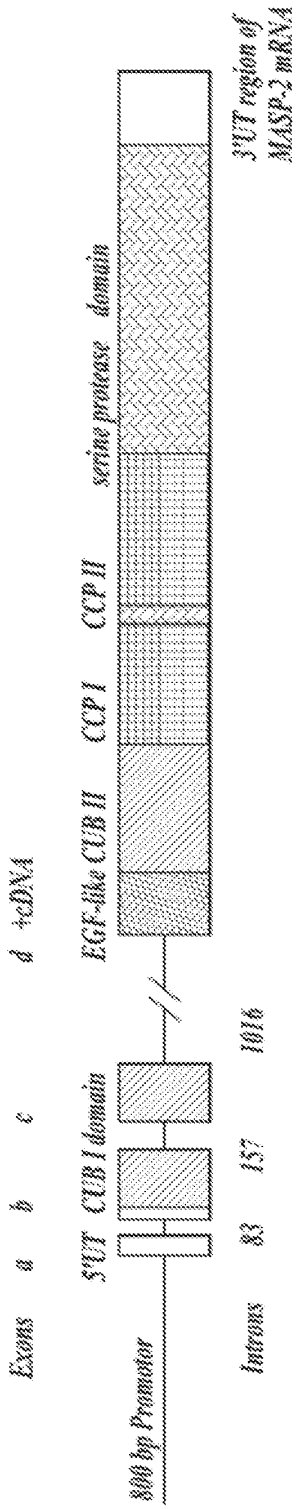

Exons a b c d cDNA
5'UT CUB I domain EGF-like CUB II CCP I CCP II serine protease domain
3'UT region of MASP-3 mRNA
800 bp Promotor
Introns 83 157 1016

FIG. 4

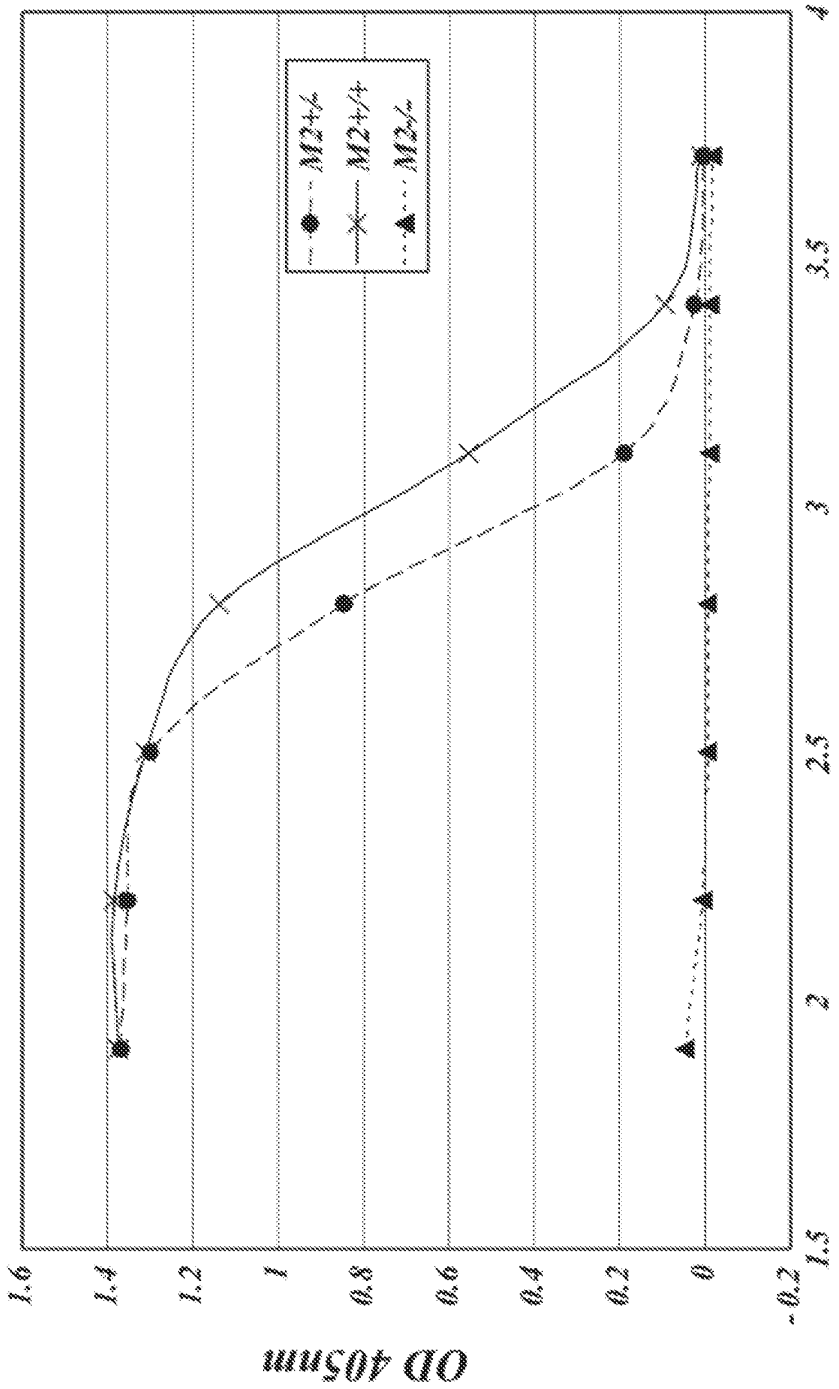

M2+/+
M2+/-
M2-/-
OD 405nm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
1.5
2
2.5
3
3.5
4
log serum dilutions

FIG. 5A

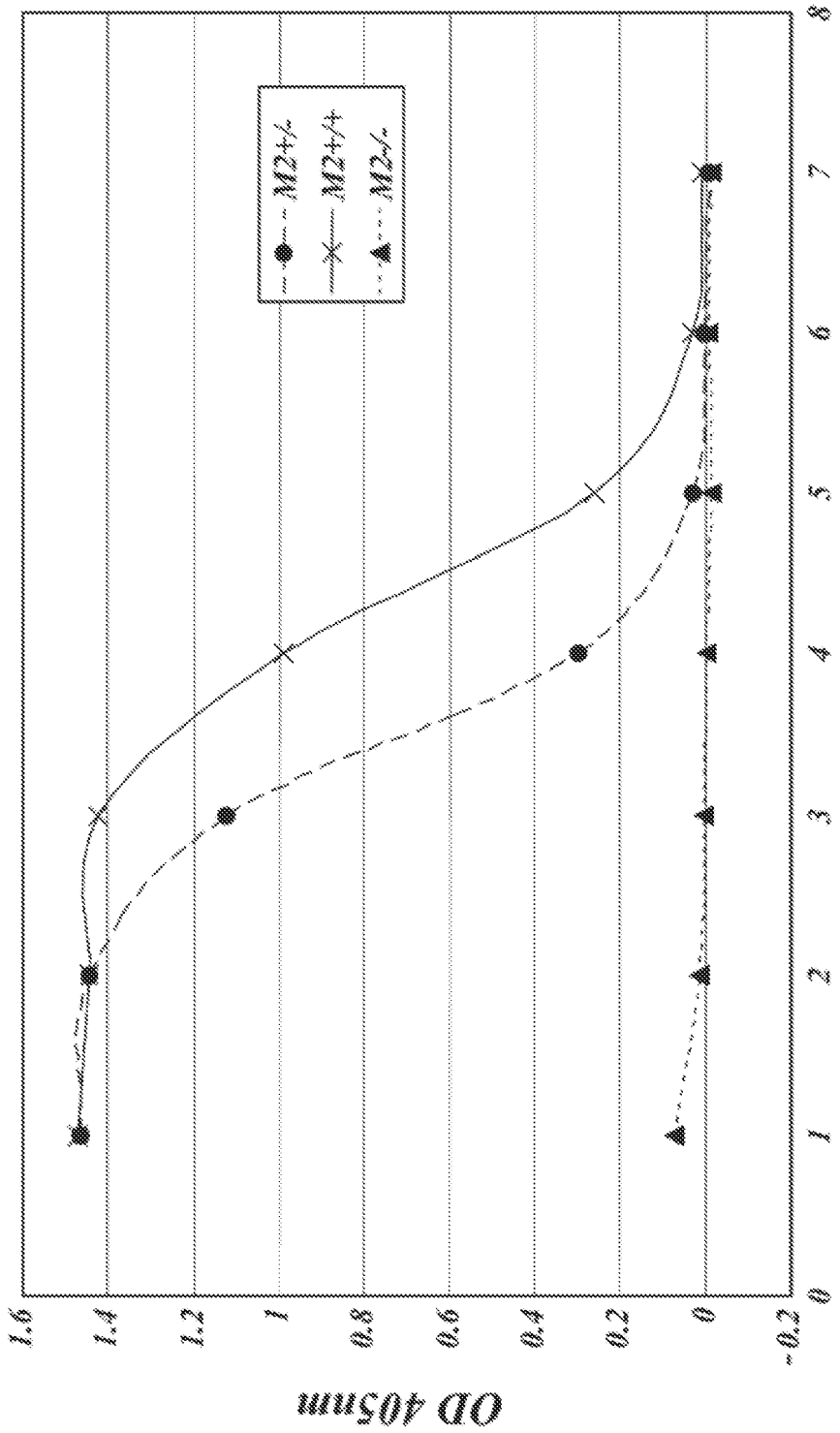

M2+/-
M2+/+
M2-/-
OD 405nm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
1
2
3
4
5
6
7
8
log serum dilutions

FIG. 5B

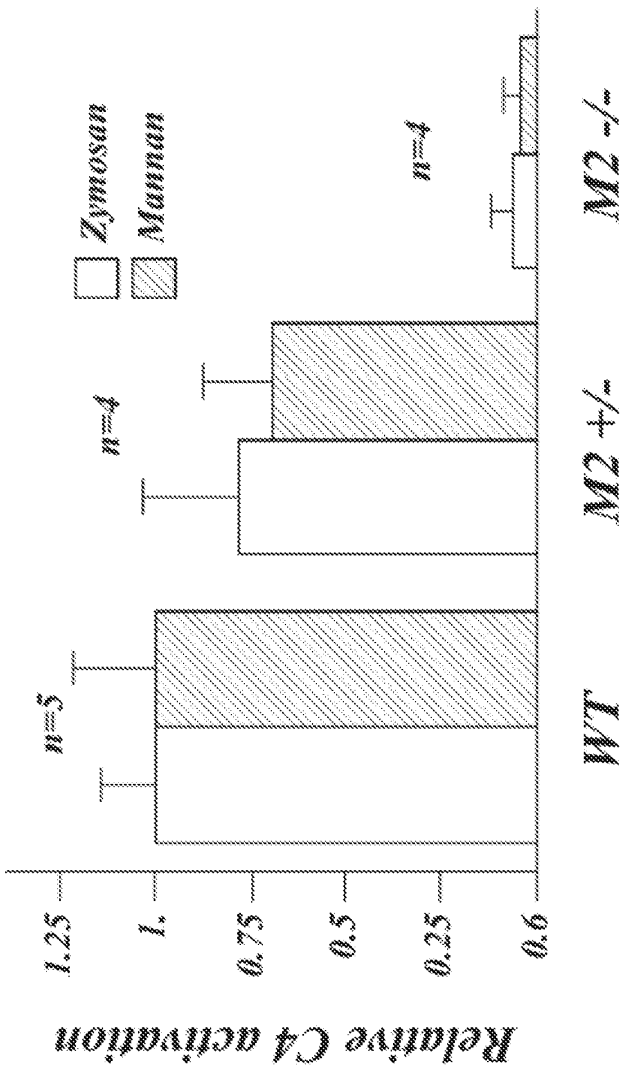

Relative C4 activation
1.25
1.
0.75
0.5
0.25
0.6
n=5
n=4
n=4
Zymosan
Mannan
WT
M2 +/-
M2 -/-

FIG. 5C

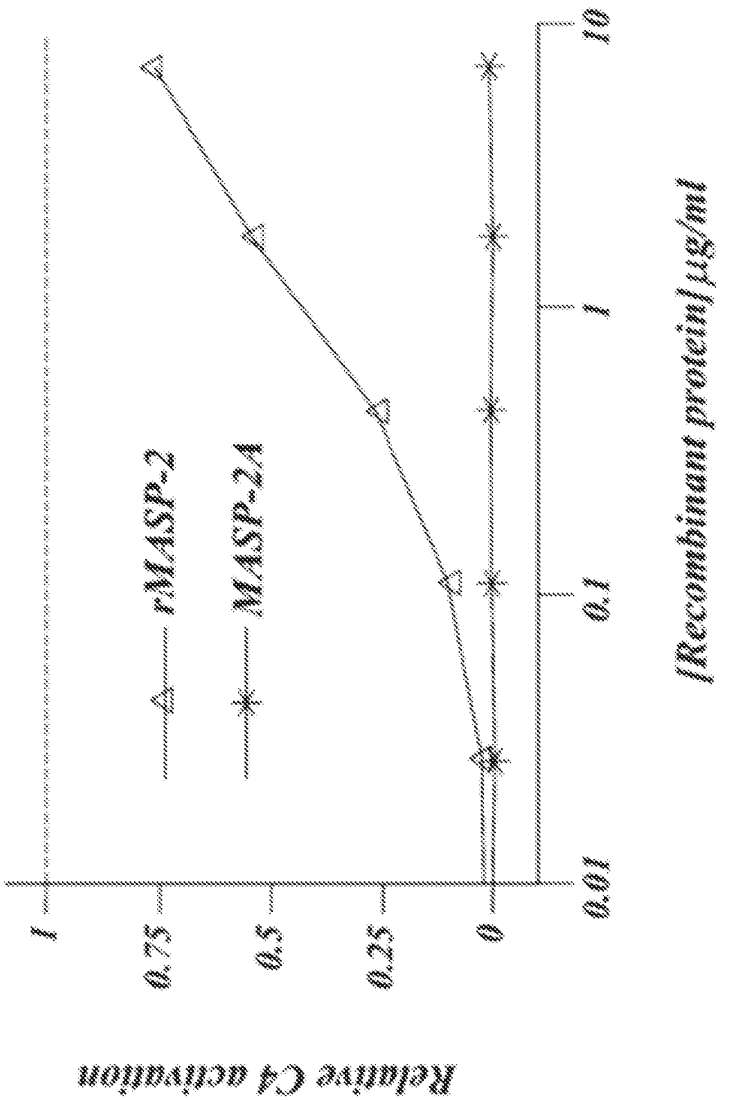

Relative C4 activation
1
0.75
0.5
0.25
0
0.01
0.1
1
10
[Recombinant protein] μg/ml
rMASP-2
MASP-2A

FIG. 6

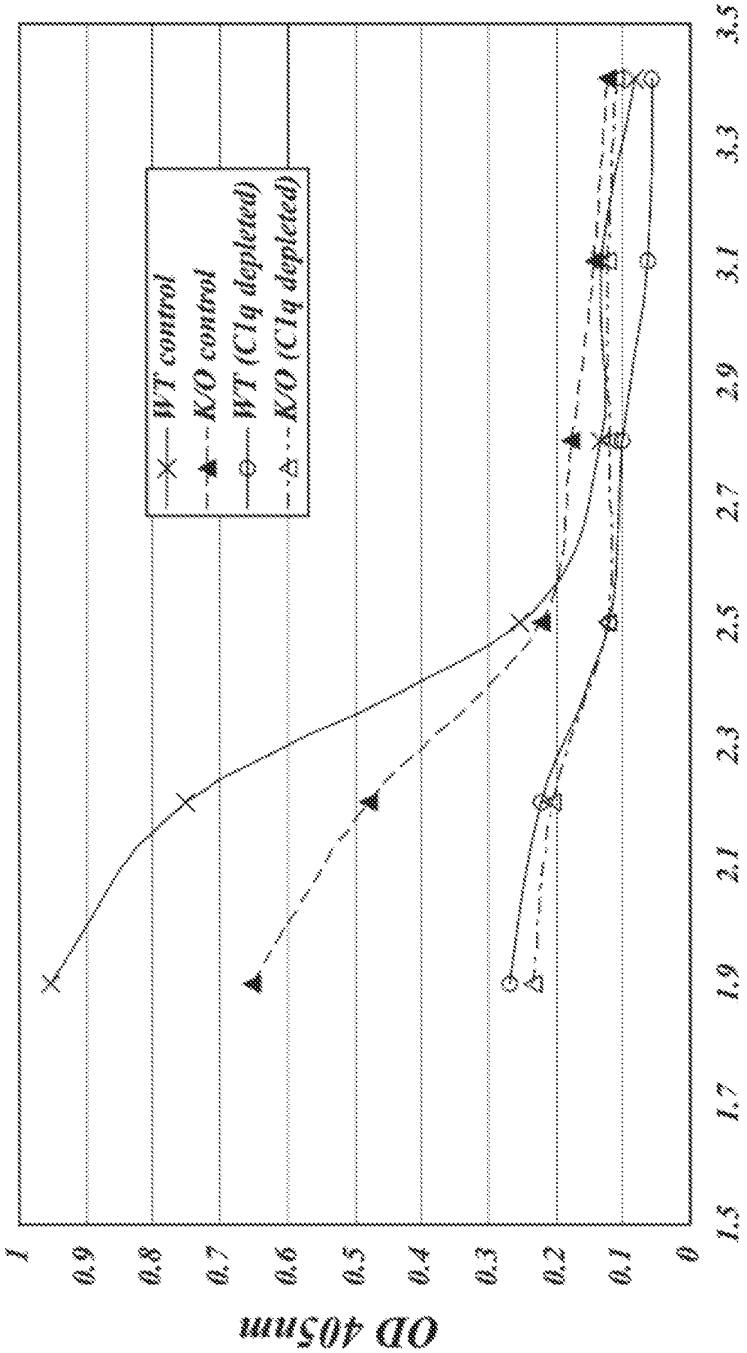

OD 405nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
log plasma dilution
WT control
K/O control
WT (C1q depleted)
K/O (C1q depleted)

FIG. 7

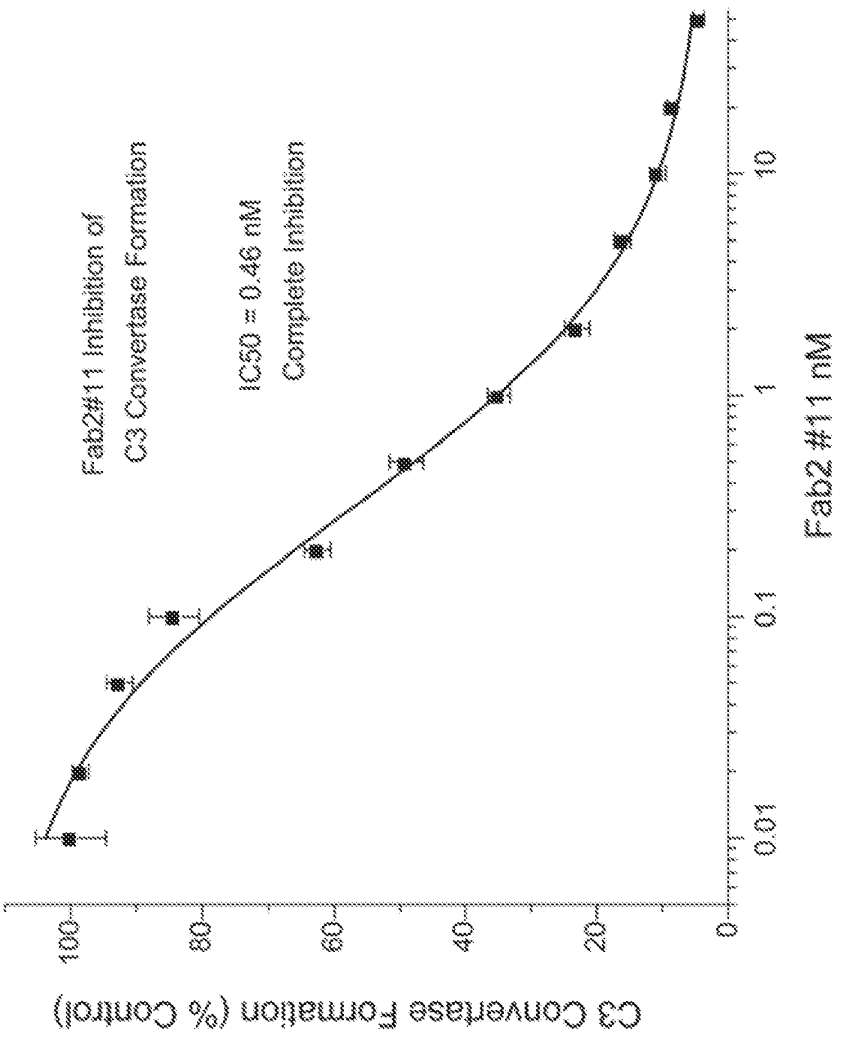

Fab2#11 Inhibition of
C3 Convertase Formation
IC50 = 0.46 nM
Complete Inhibition
C3 Convertase Formation (% Control)
100
80
60
40
20
0
0.01
0.1
1
10
Fab2 #11 nM

FIG. 8A

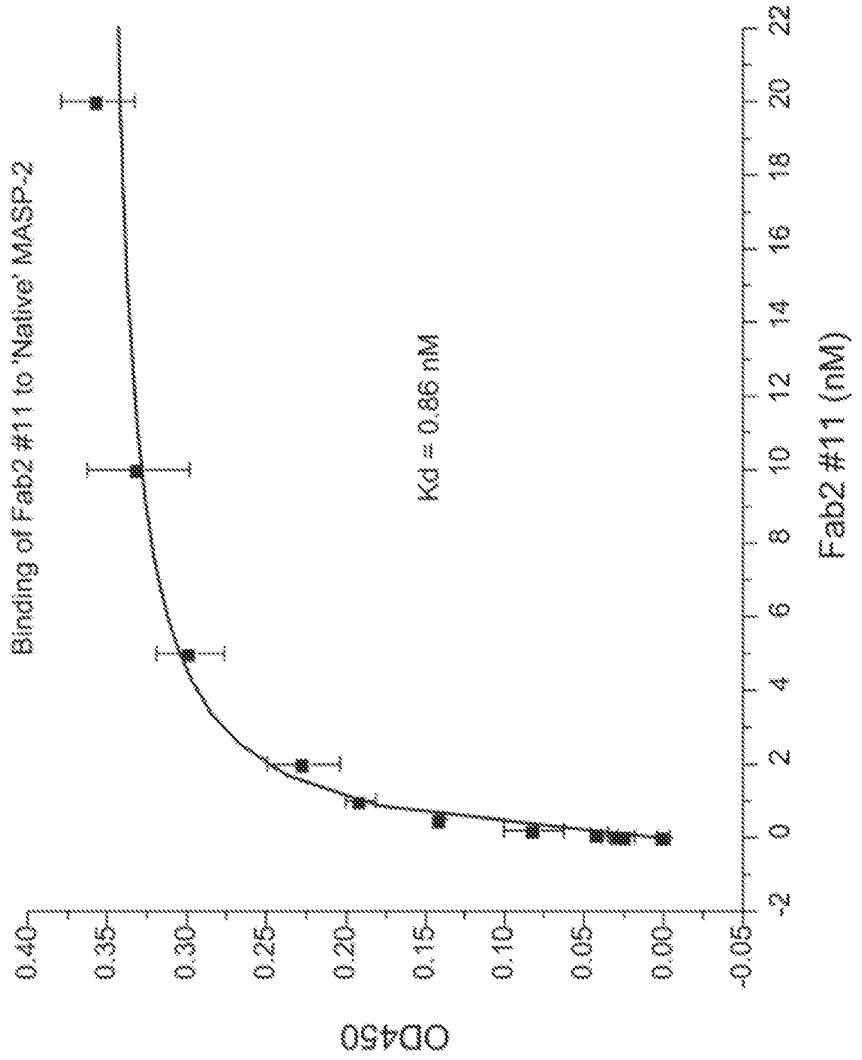

Binding of Fab2 #11 to 'Native' MASP-2
Kd = 0.86 nM
OD450
Fab2 #11 (nM)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-2
0
2
4
6
8
10
12
14
16
18
20
22

FIG. 8B

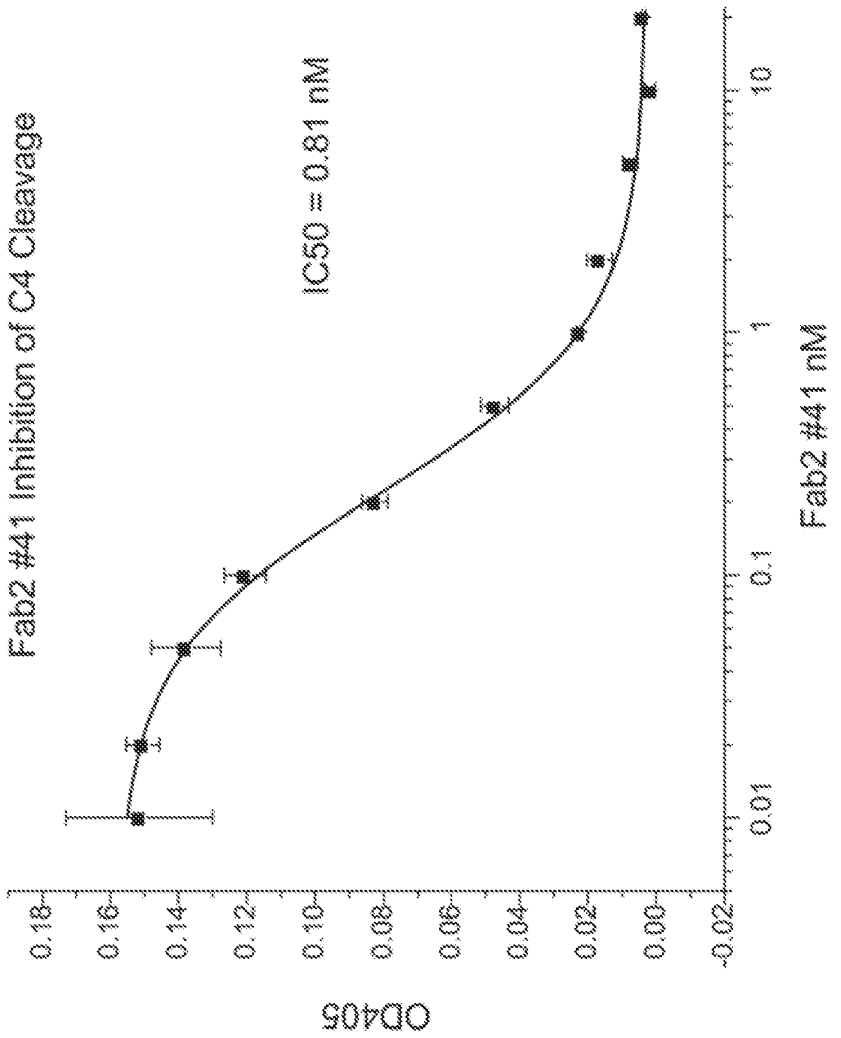

Fab2 #41 Inhibition of C4 Cleavage
IC50 = 0.81 nM
OD405
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.01
0.1
1
10
Fab2 #41 nM

FIG. 8C

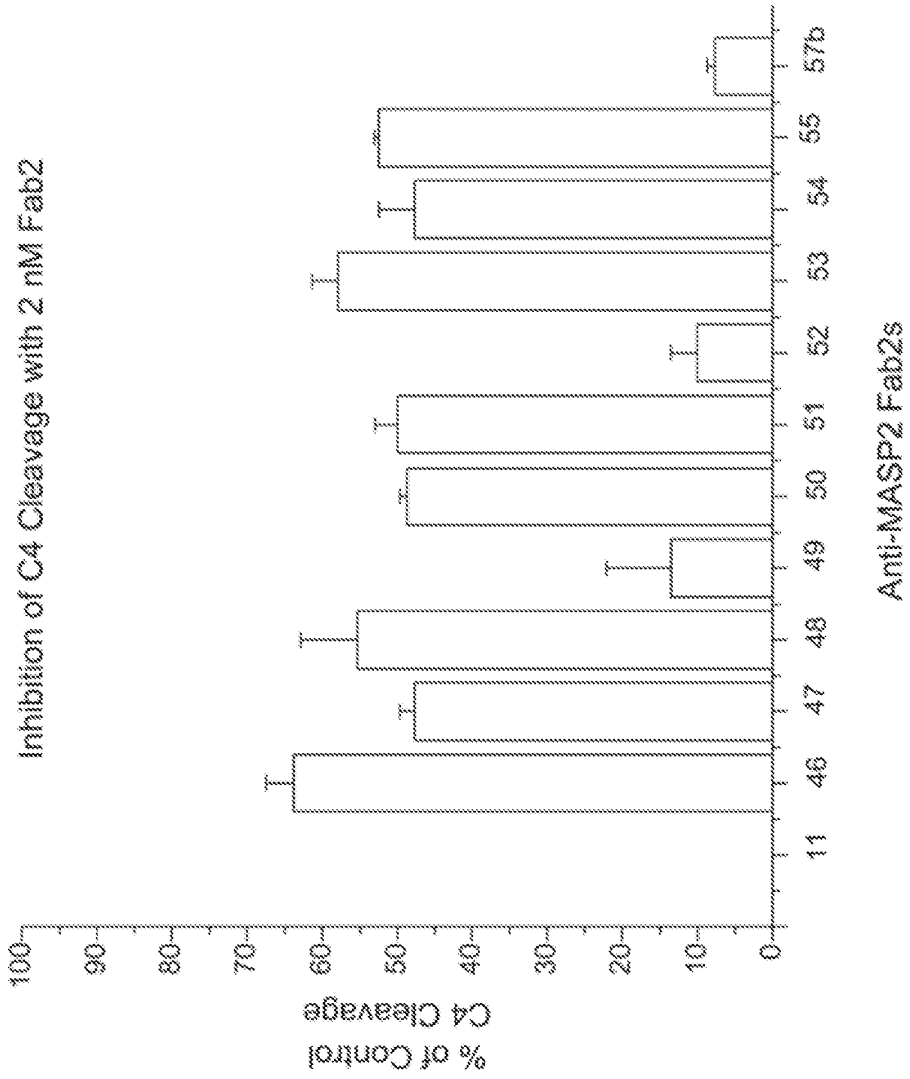

Inhibition of C4 Cleavage with 2 nM Fab2
% of Control C4 Cleavage
100
90
80
70
60
50
40
30
20
10
0
11
46
47
48
49
50
51
52
53
54
55
57b
Anti-MASP2 Fab2s

FIG. 9

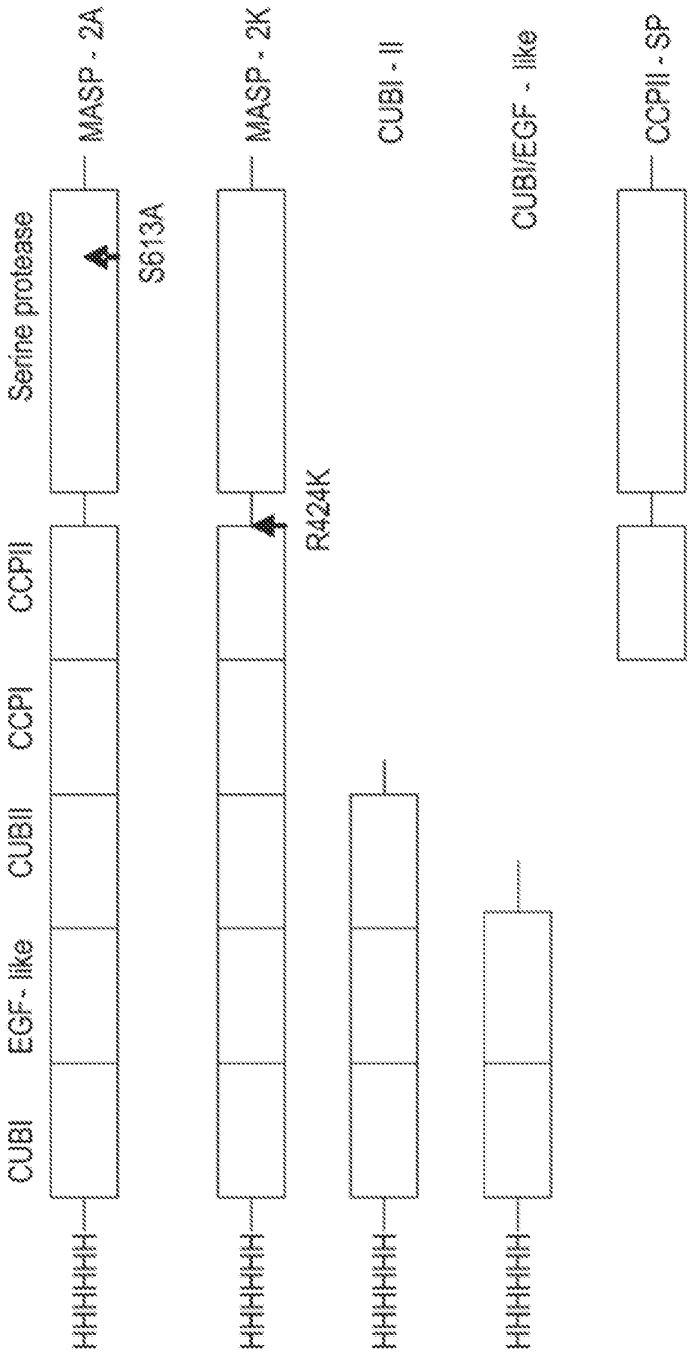

MASP - 2A
MASP - 2K
CUBI - II
CUBII/EGF - like
CCPII - SP
Serine protease
S613A
R424K
CCPII
CCPI
CUBII
EGF- like
CUBI
HHHHHH

FIG. 10

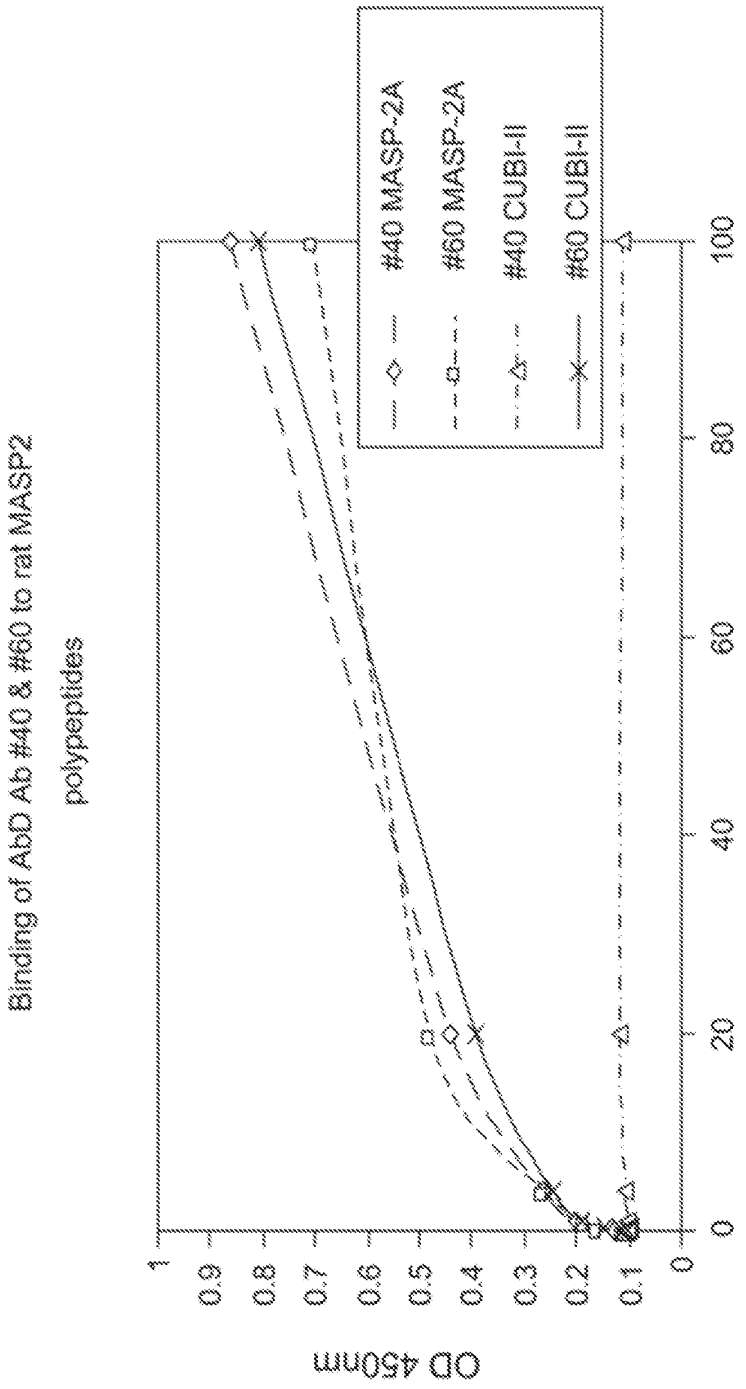

Binding of AbD Ab #40 & #60 to rat MASP2 polypeptides
#40 MASP-2A
#60 MASP-2A
#40 CUBI-II
#60 CUBI-II
OD 450nm
[Ab] nM
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100

FIG. 11

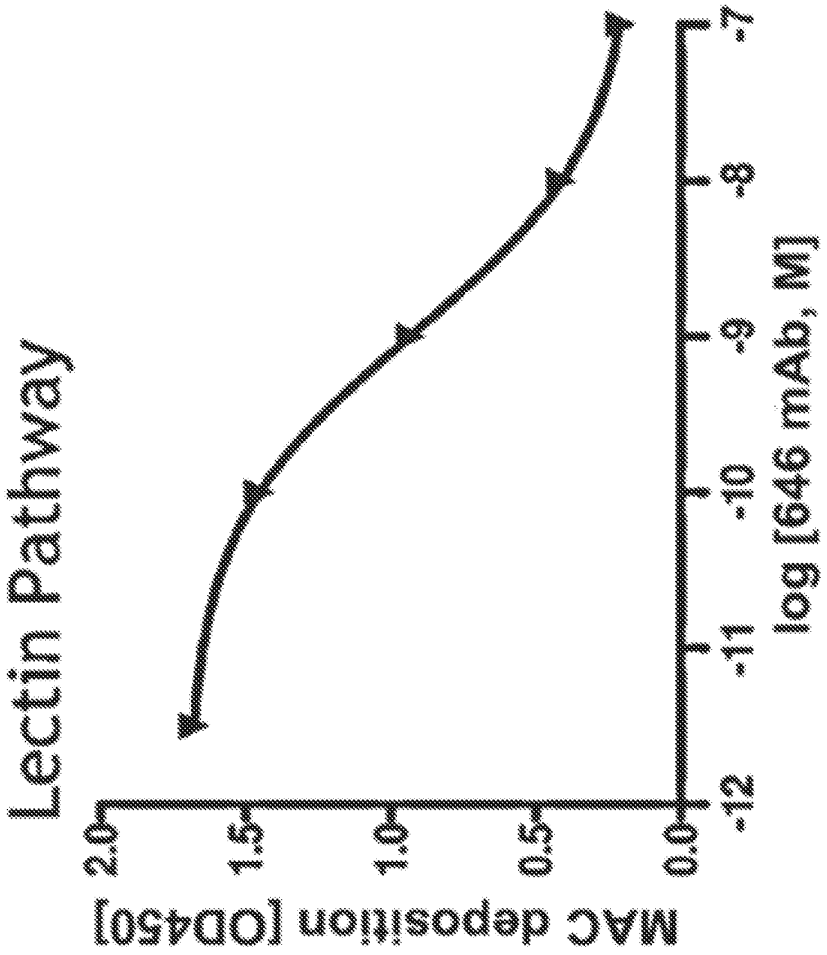

Lectin Pathway
MAC deposition [OD450]
2.0
1.5
1.0
0.5
0.0
-7
-8
-9
-10
-11
-12
log [646 mAb, M]

FIG. 12A

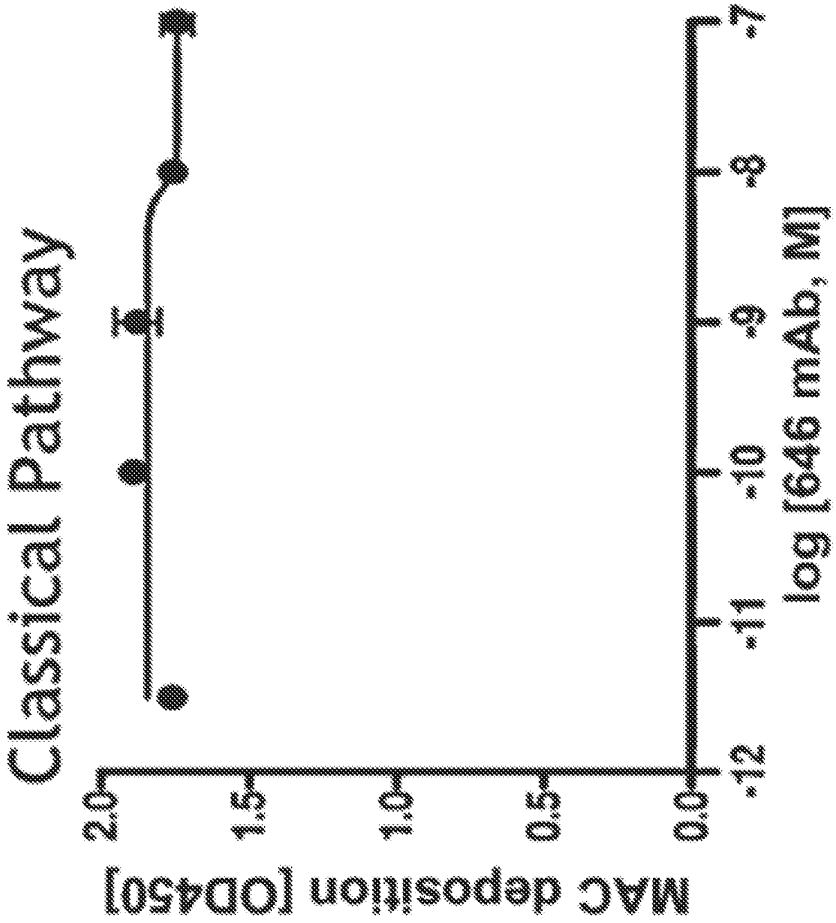

Classical Pathway
MAC deposition [OD450]
2.0
1.5
1.0
0.5
0.0
-7
-8
-9
-10
-11
-12
log [646 mAb, M]

FIG. 12B

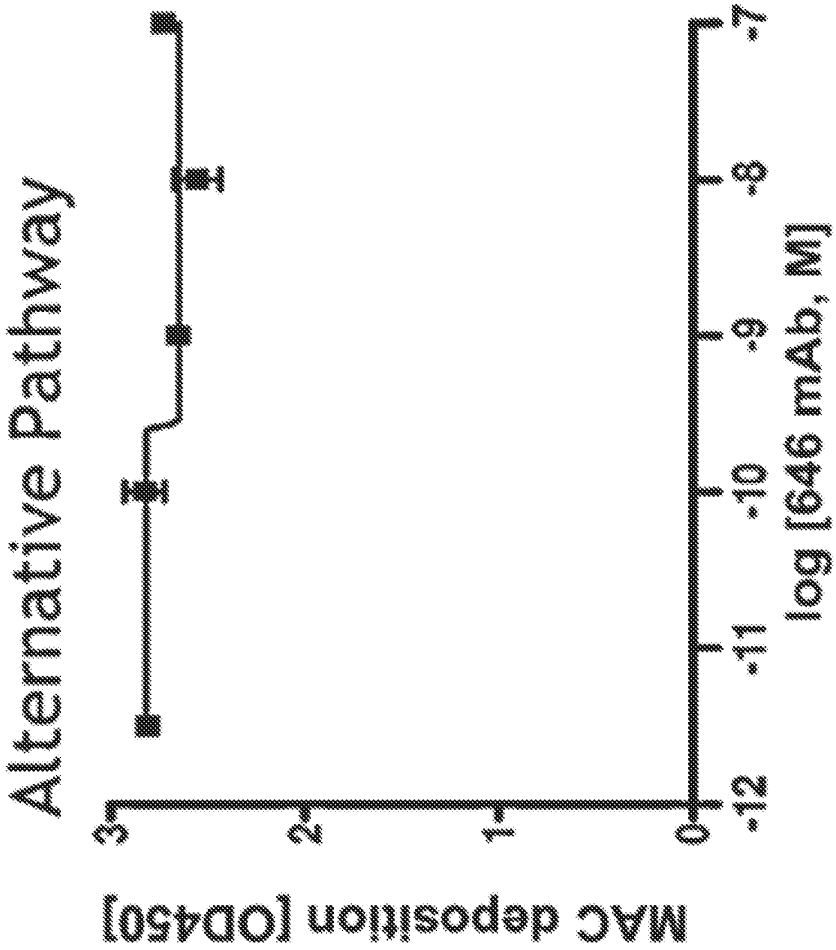

Alternative Pathway
MAC deposition [OD450]
3
2
1
0
-7
-8
-9
-10
-11
-12
log [646 mAb, M]

FIG. 12C

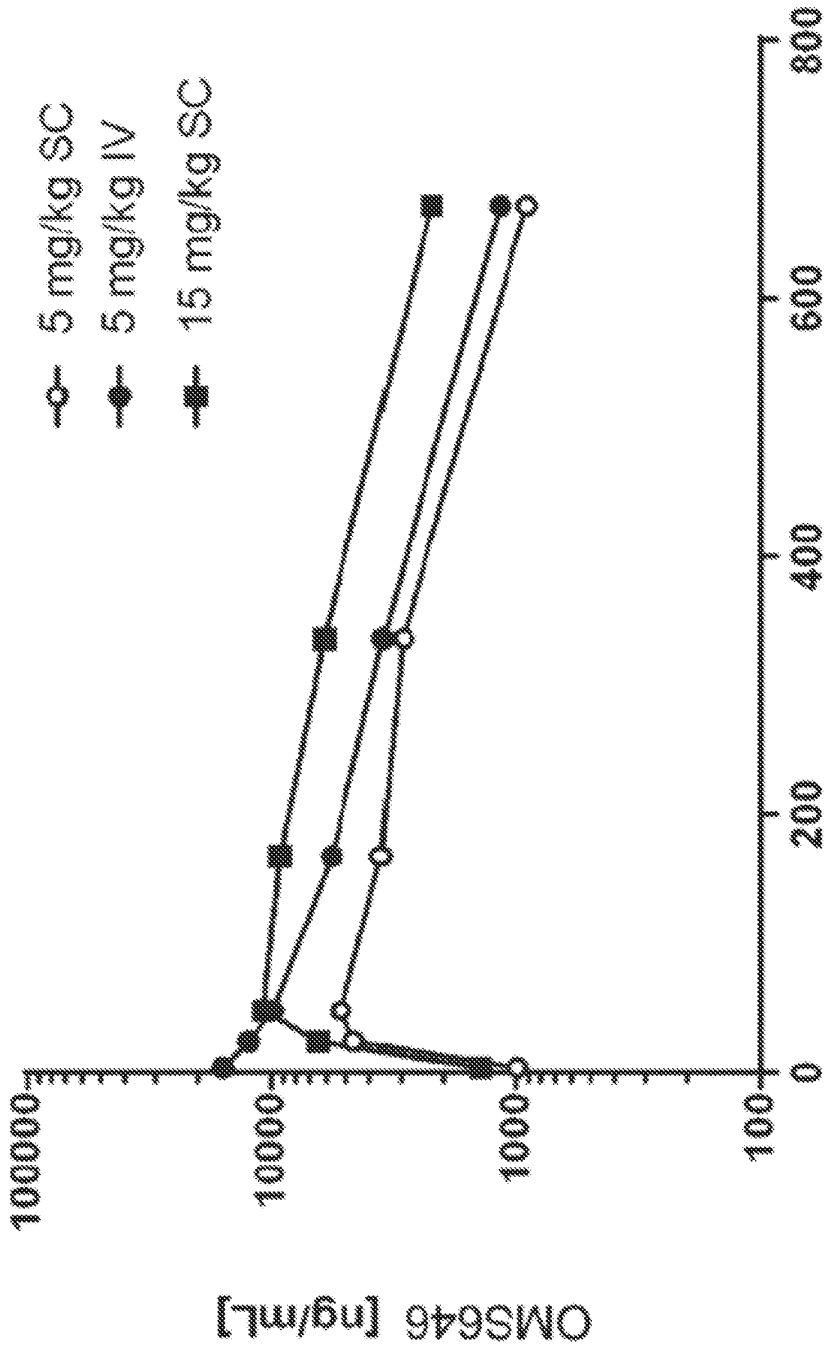

5 mg/kg SC
5 mg/kg IV
15 mg/kg SC
100000
10000
1000
100
0
200
400
600
800
time [hrs]
OMS646 [ng/mL]

FIG. 13

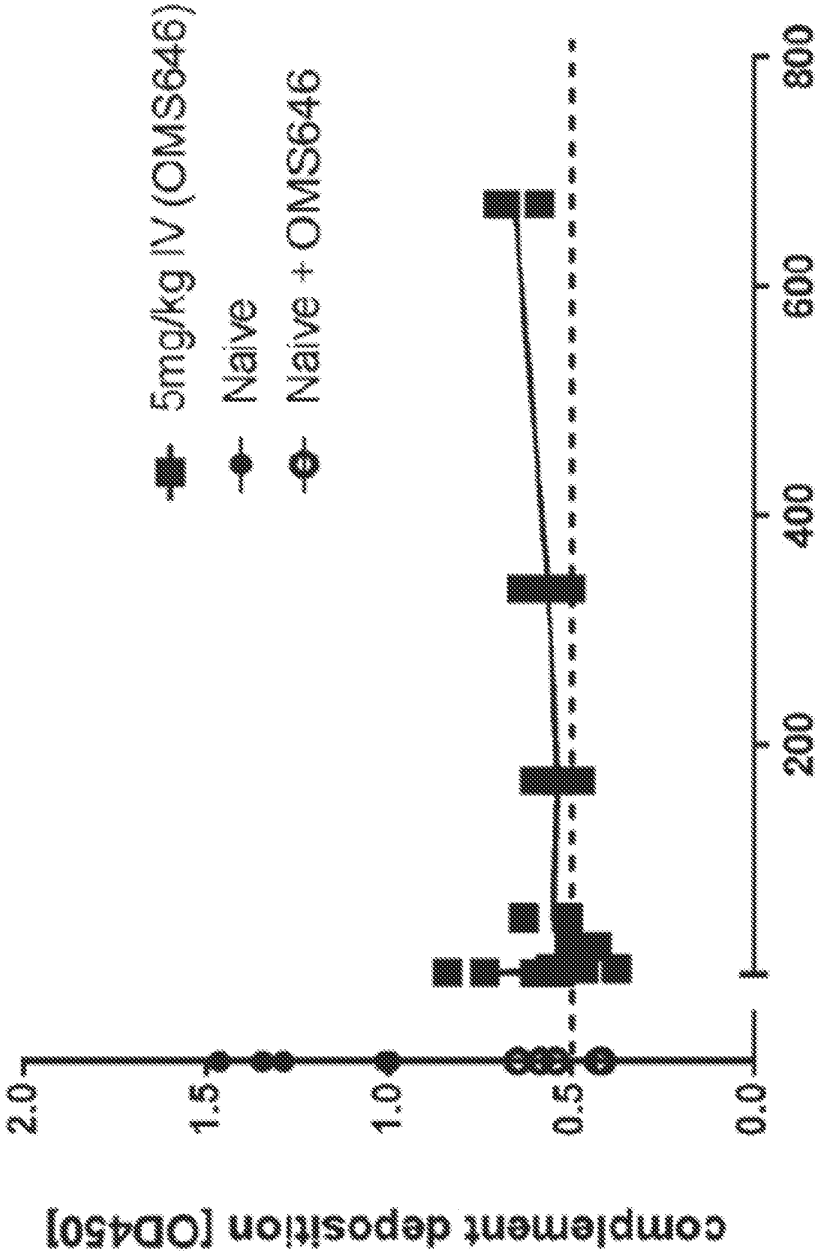

5mg/kg IV (OMS646)
Naive
Naive + OMS646
complement deposition [OD450]
2.0
1.5
1.0
0.5
0.0
200
400
600
800
time [hrs]

FIG. 14A

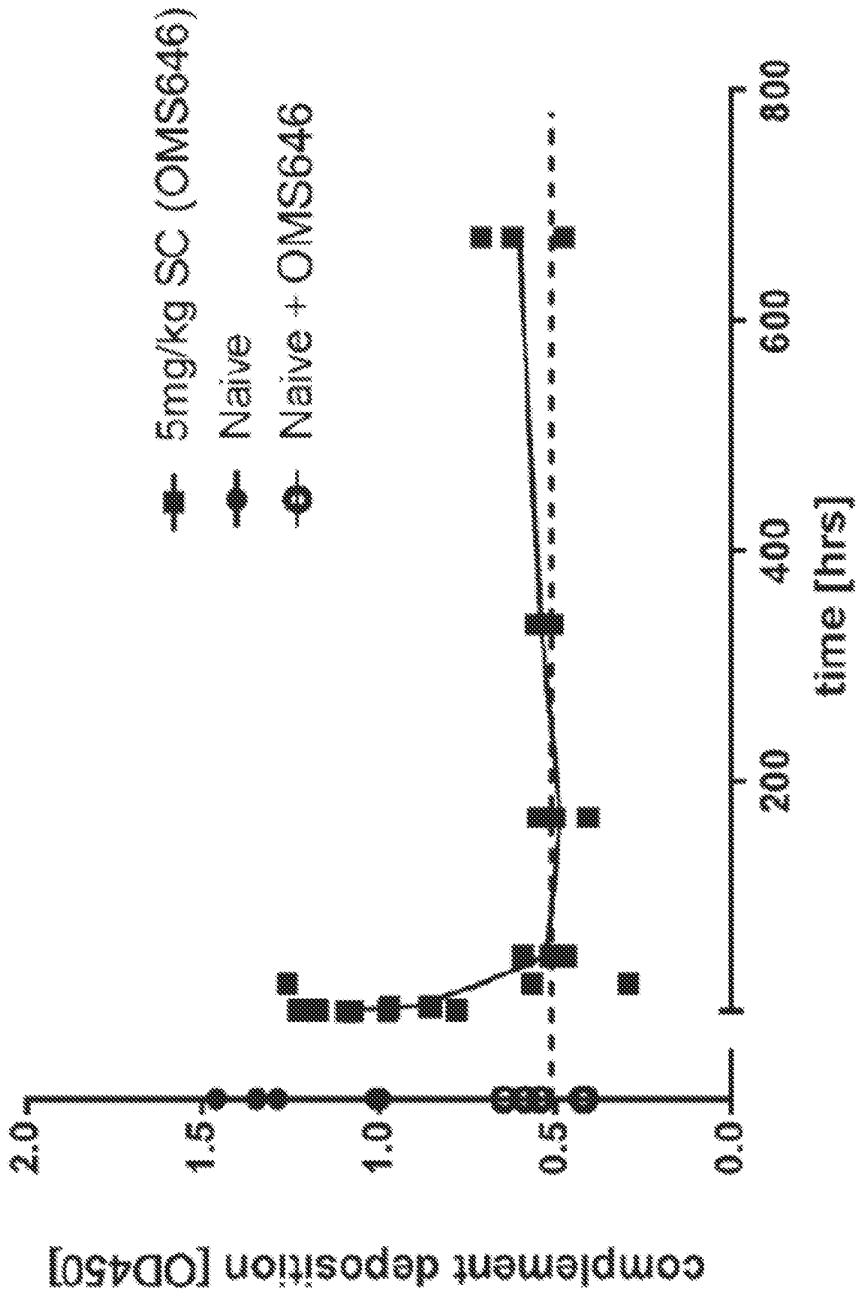

5mg/kg SC (OMS646)
Naive
Naive + OMS646
complement deposition [OD450]
2.0
1.5
1.0
0.5
0.0
200
400
600
800
time [hrs]

FIG. 14B

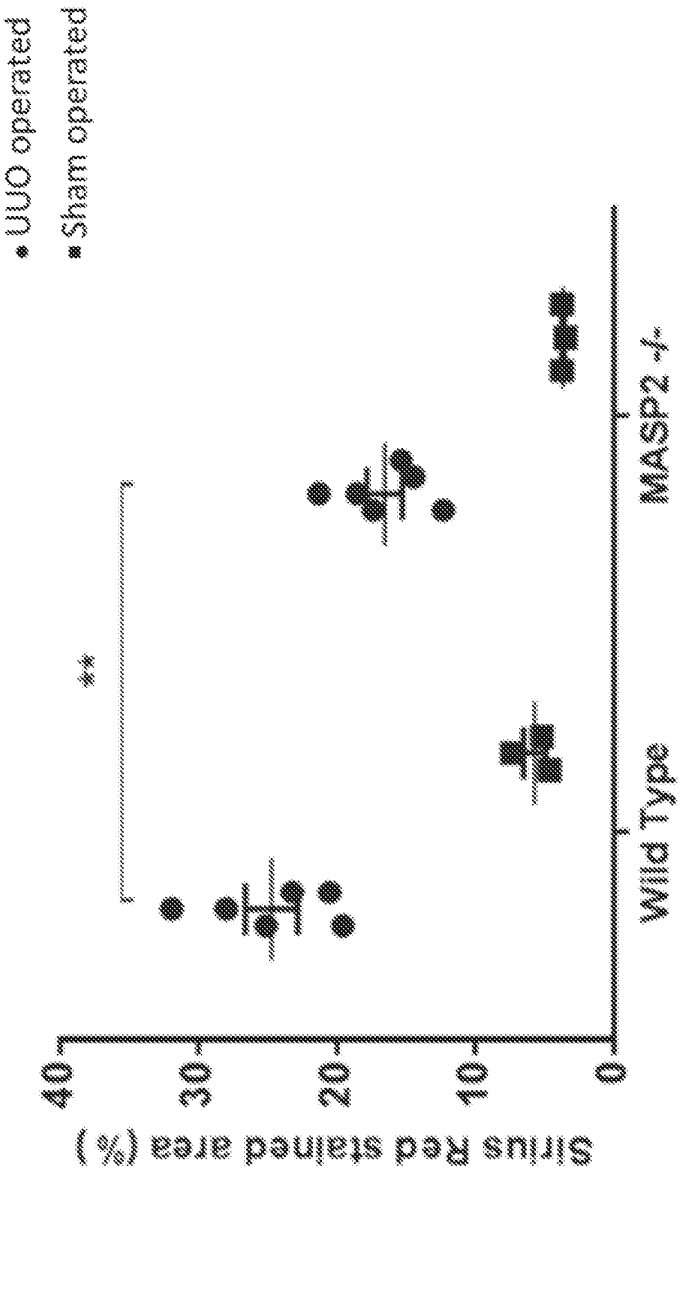

UUO operated
Sham operated
**
Sirius Red stained area (%)
40
30
20
10
0
Wild Type
MASP2 -/-
** p= 0.0096

FIG. 15

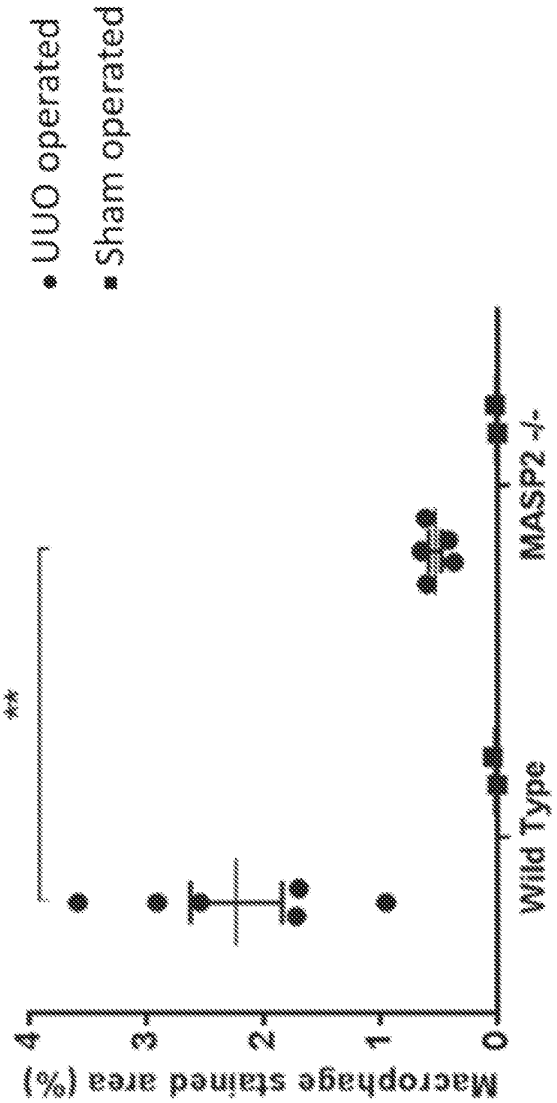

UUO operated
Sham operated
**
Macrophage stained area (%)
0
1
2
3
4
Wild Type
MASP2 -/-
**p=0.0035

FIG. 16

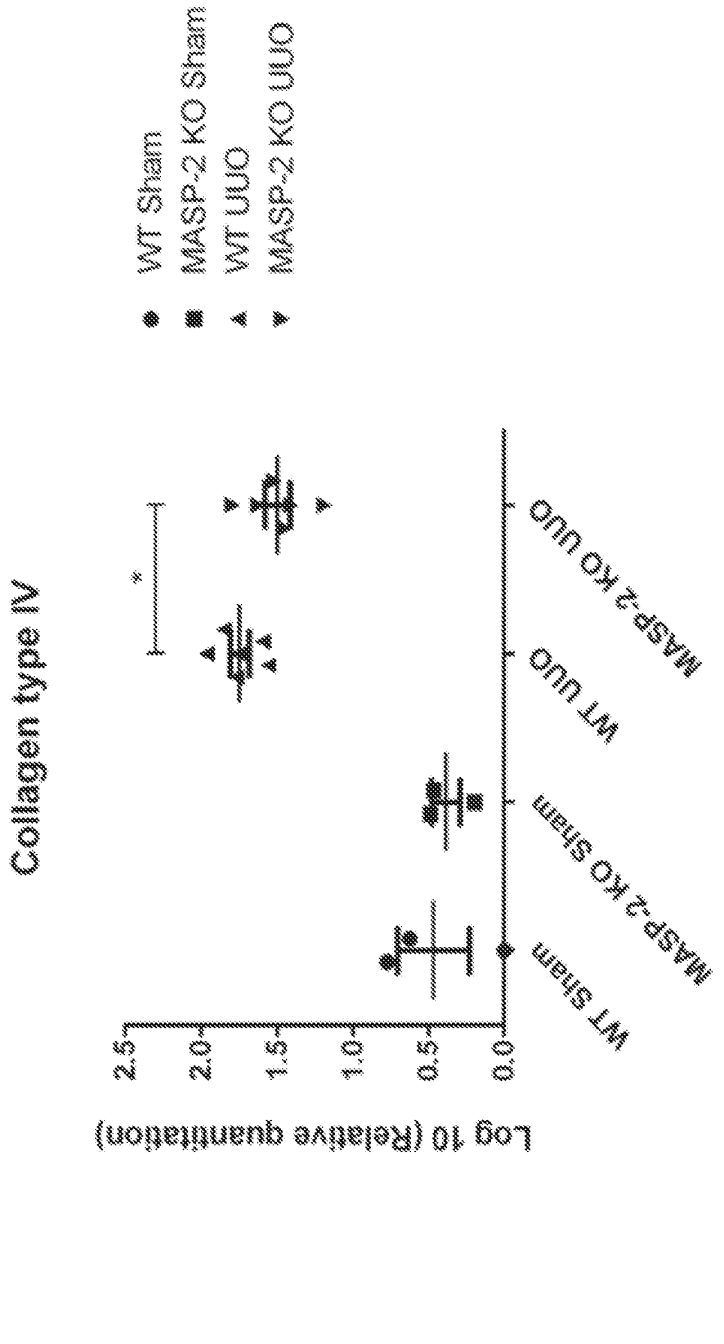

Collagen type IV
WT Sham
MASP-2 KO Sham
WT UUO
MASP-2 KO UUO
*
Log 10 (Relative quantitation)
2.5
2.0
1.5
1.0
0.5
0.0
WT Sham
MASP-2 KO Sham
WT UUO
MASP-2 KO UUO
Mouse group
*p=0.0388

FIG. 17

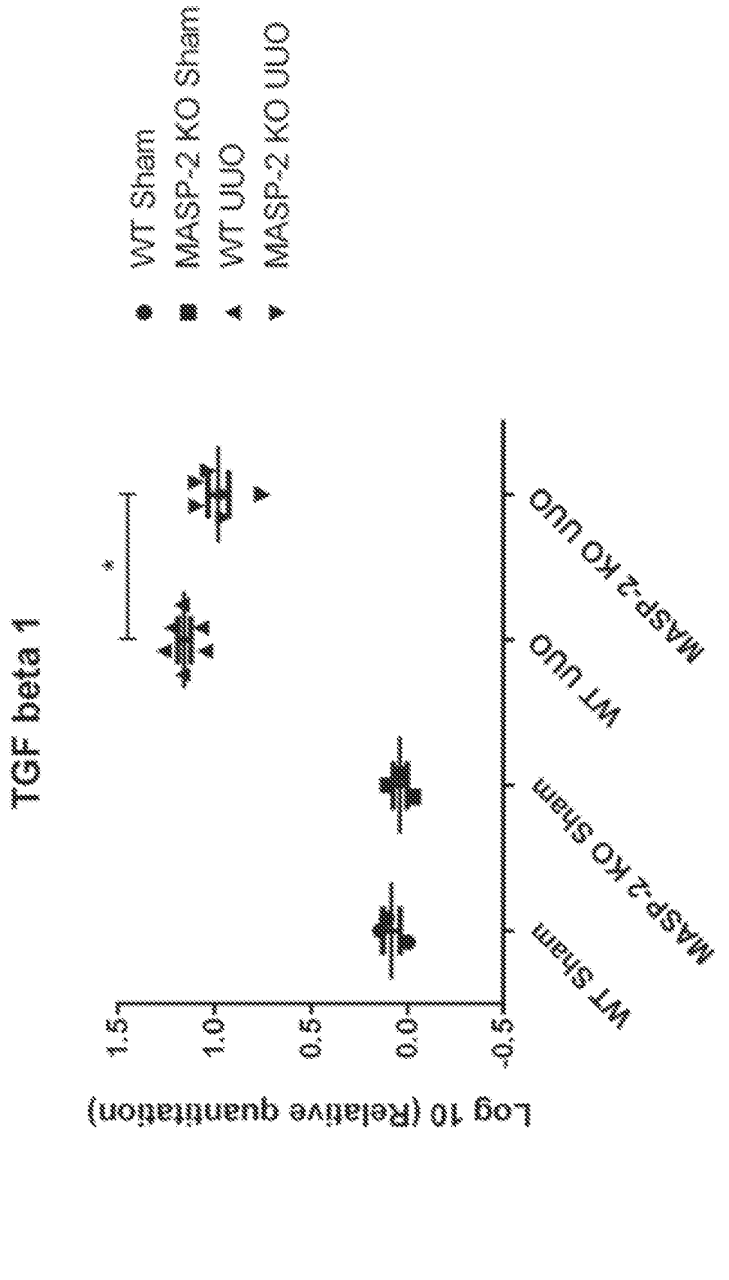

TGF beta 1
WT Sham
MASP-2 KO Sham
WT UUO
MASP-2 KO UUO
*
1.5
1.0
0.5
0.0
-0.5
Log 10 (Relative quantitation)
WT Sham
MASP-2 KO Sham
WT UUO
MASP-2 KO UUO
Mouse group
*p=0.0174

FIG. 18

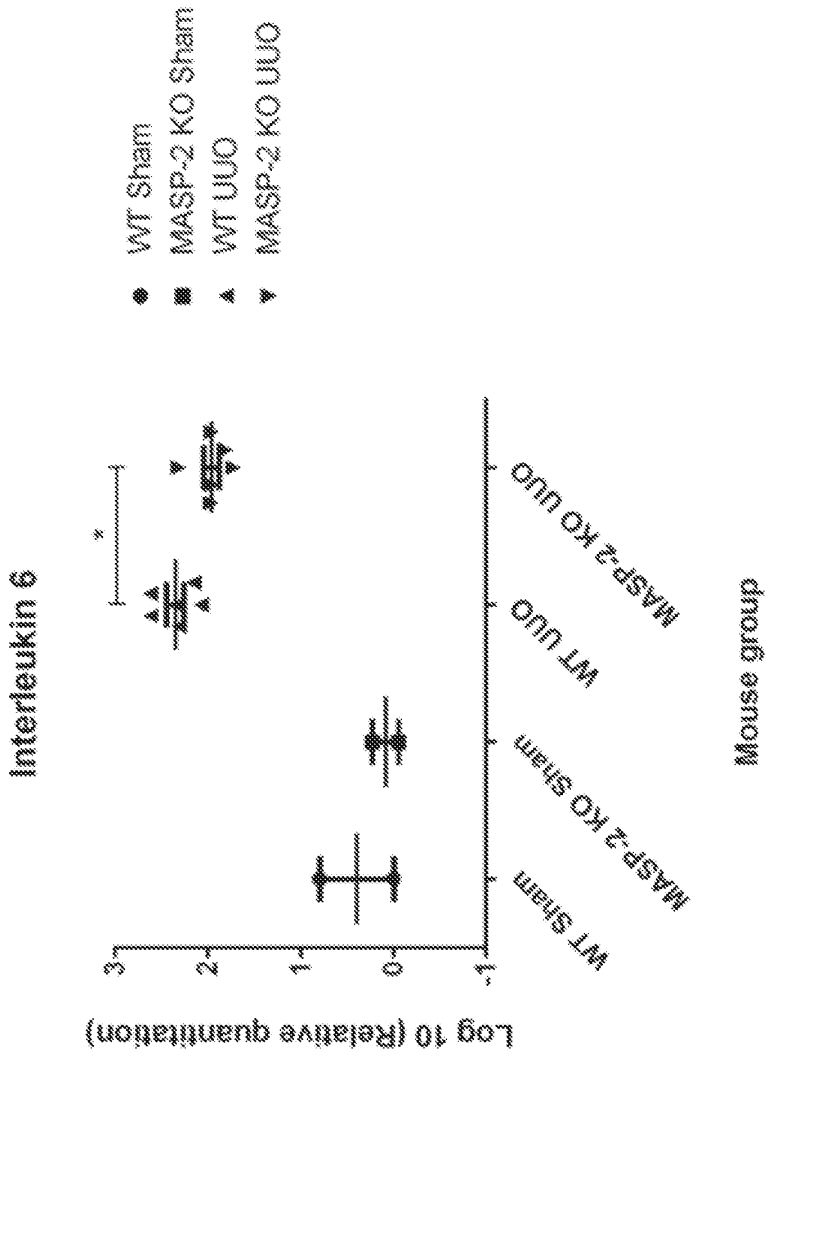

WT Sham
MASP-2 KO Sham
WT UUO
MASP-2 KO UUO
Interleukin 6
*
Log 10 (Relative quantitation)
3
2
1
0
-1
WT Sham
MASP-2 KO Sham
WT UUO
MASP-2 KO UUO
Mouse group
*p=0.0109

FIG. 19

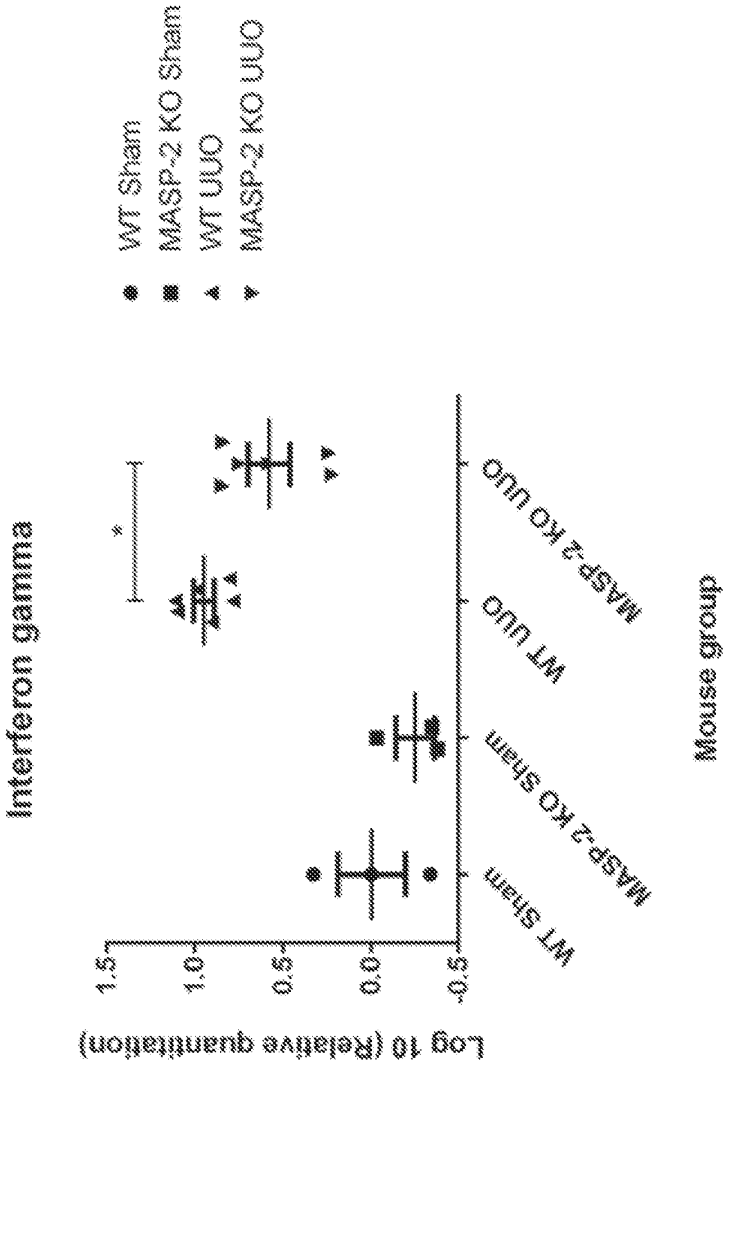


FIG. 20

*p=0.0182

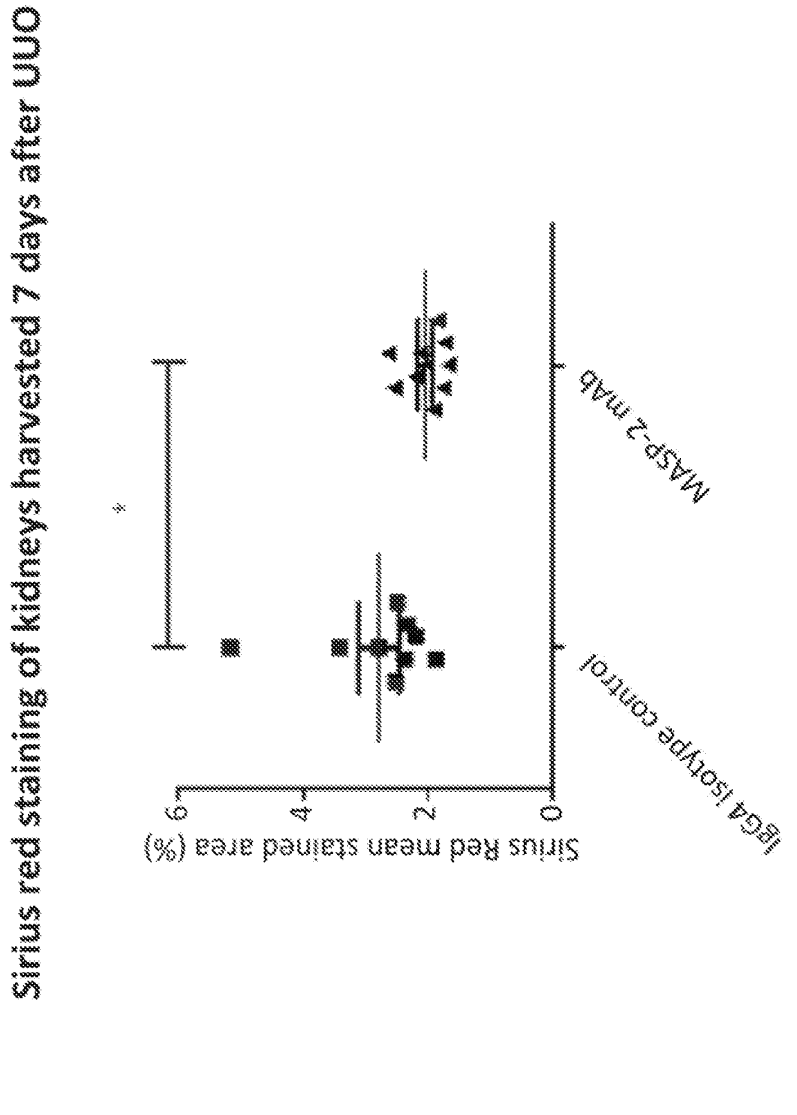

Sirius red staining of kidneys harvested 7 days after UUO
Sirius Red mean stained area (%)
0
2
4
6
IgG4 isotype control
MASP-2 mAb
*
*p=0.0477

FIG. 21

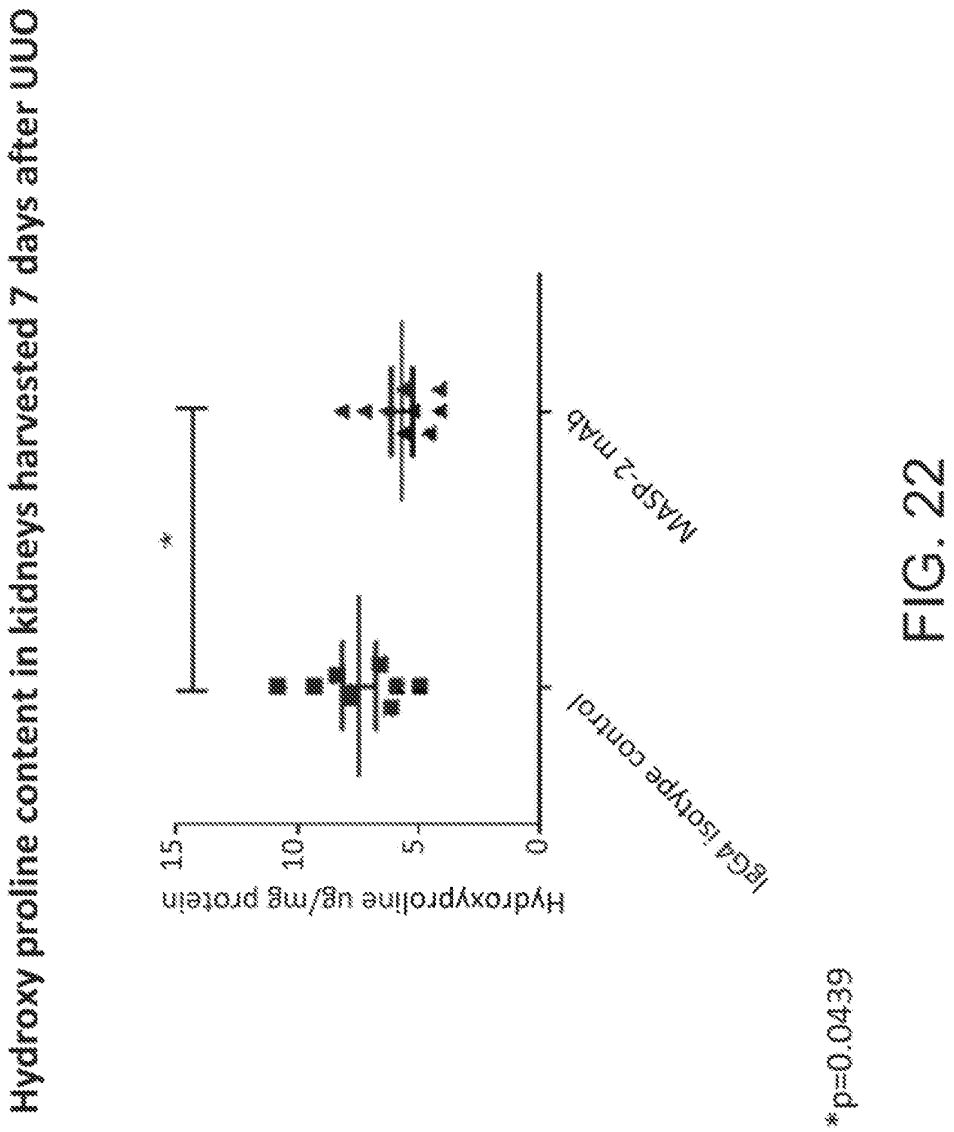

Hydroxy proline content in kidneys harvested 7 days after UUO
Hydroxyproline ug/mg protein
15
10
5
0
*
IgG4 isotype control
MASP-2 mAb
*p=0.0439

FIG. 22

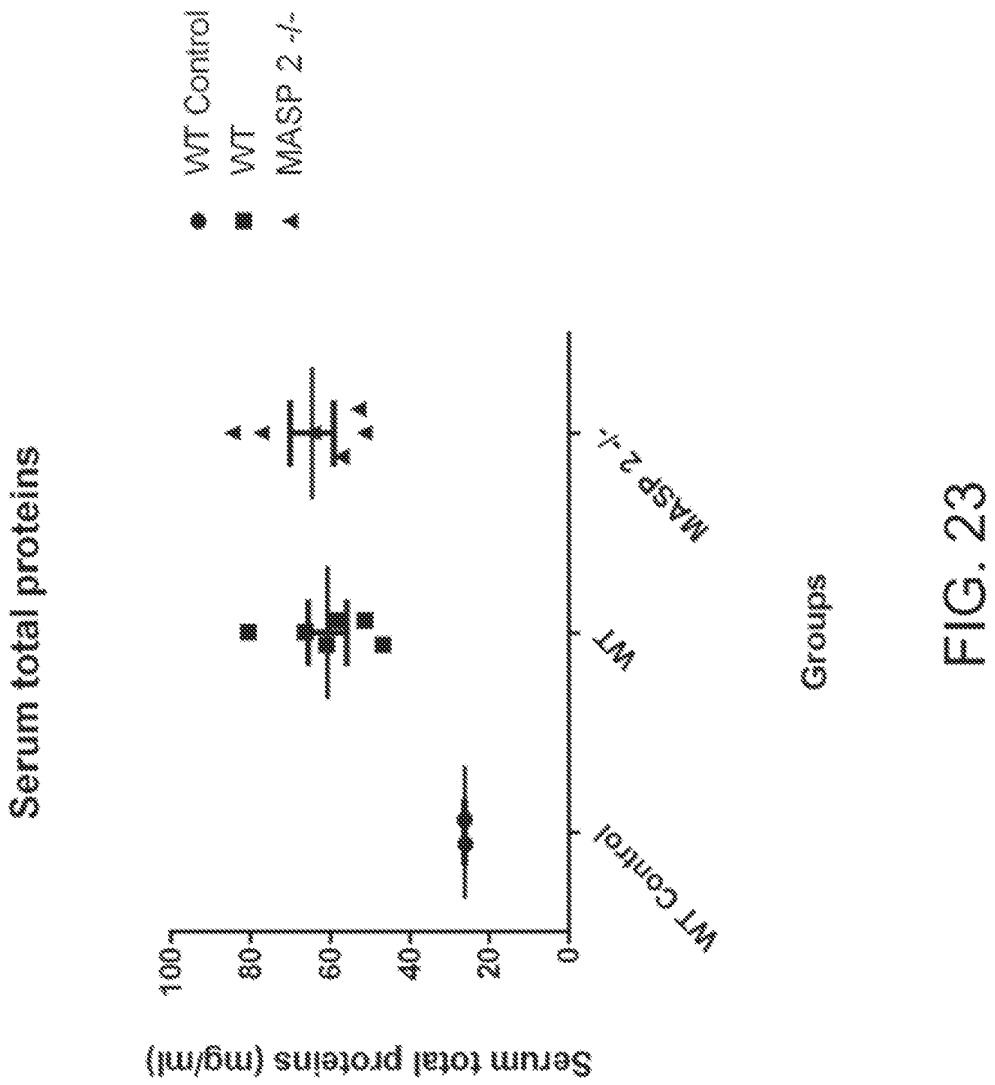

Serum total proteins
WT Control
WT
MASP 2 -/-
Serum total proteins (mg/ml)
100
80
60
40
20
0
WT Control
WT
MASP 2 -/-
Groups

FIG. 23

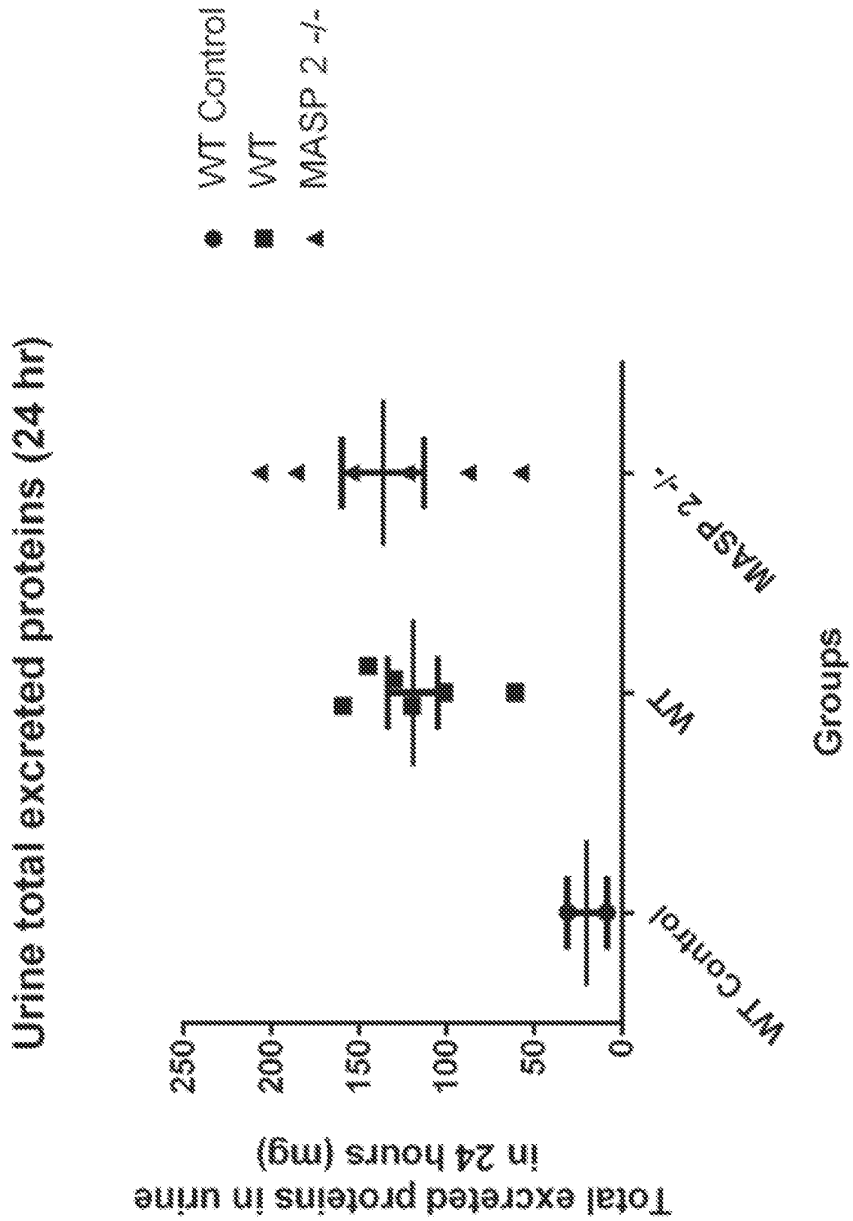

WT Control
WT
MASP 2 -/-
Urine total excreted proteins (24 hr)
Total excreted proteins in urine
in 24 hours (mg)
250
200
150
100
50
0
WT Control
WT
MASP 2 -/-
Groups

FIG. 24

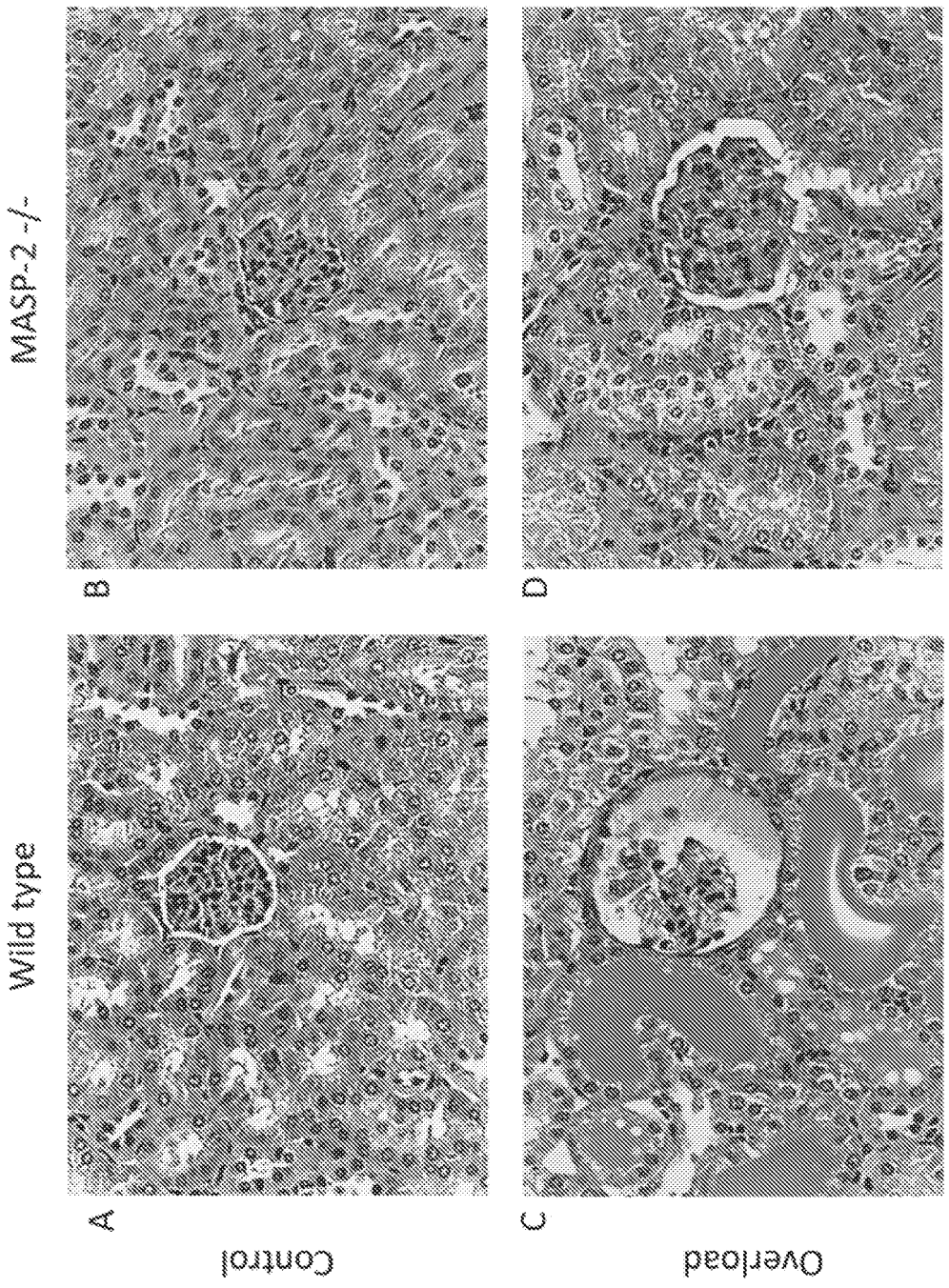


FIG. 25

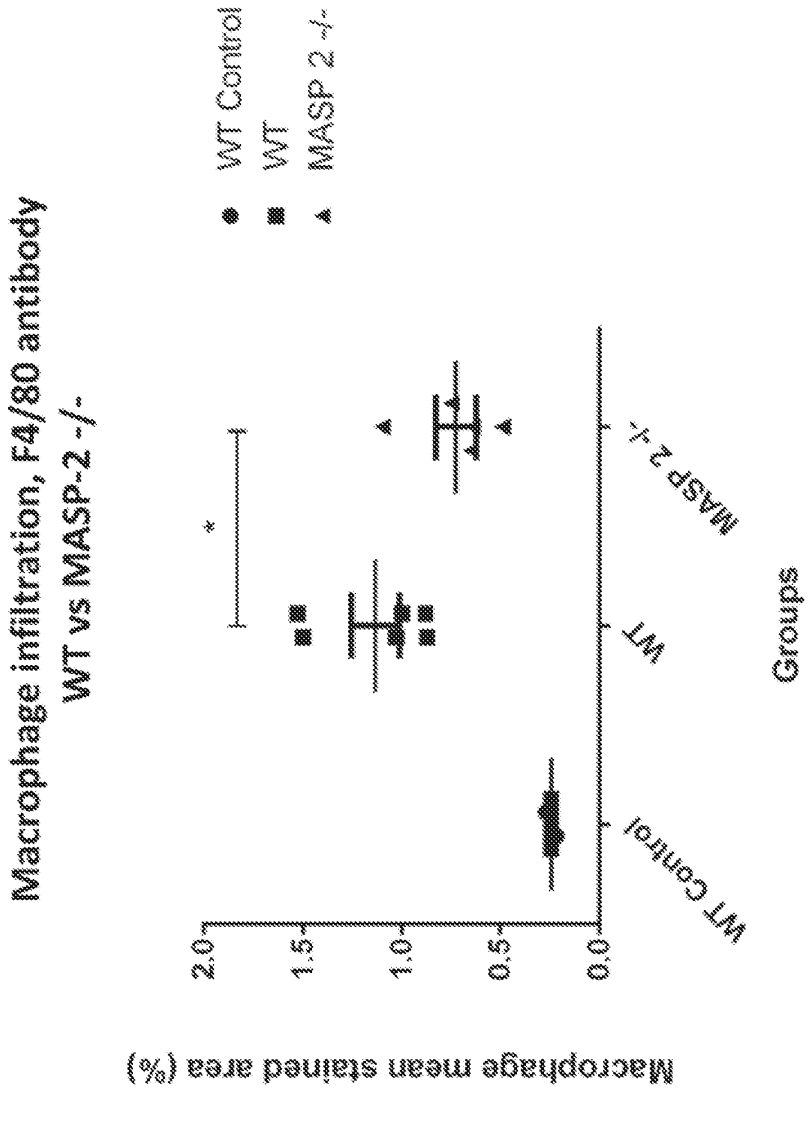

Macrophage infiltration, F4/80 antibody
WT vs MASP-2 -/-
WT Control
WT
MASP 2 -/-
*
2.0
1.5
1.0
0.5
0.0
Macrophage mean stained area (%)
WT Control
WT
MASP 2 -/-
Groups
*p=0.035

FIG. 26

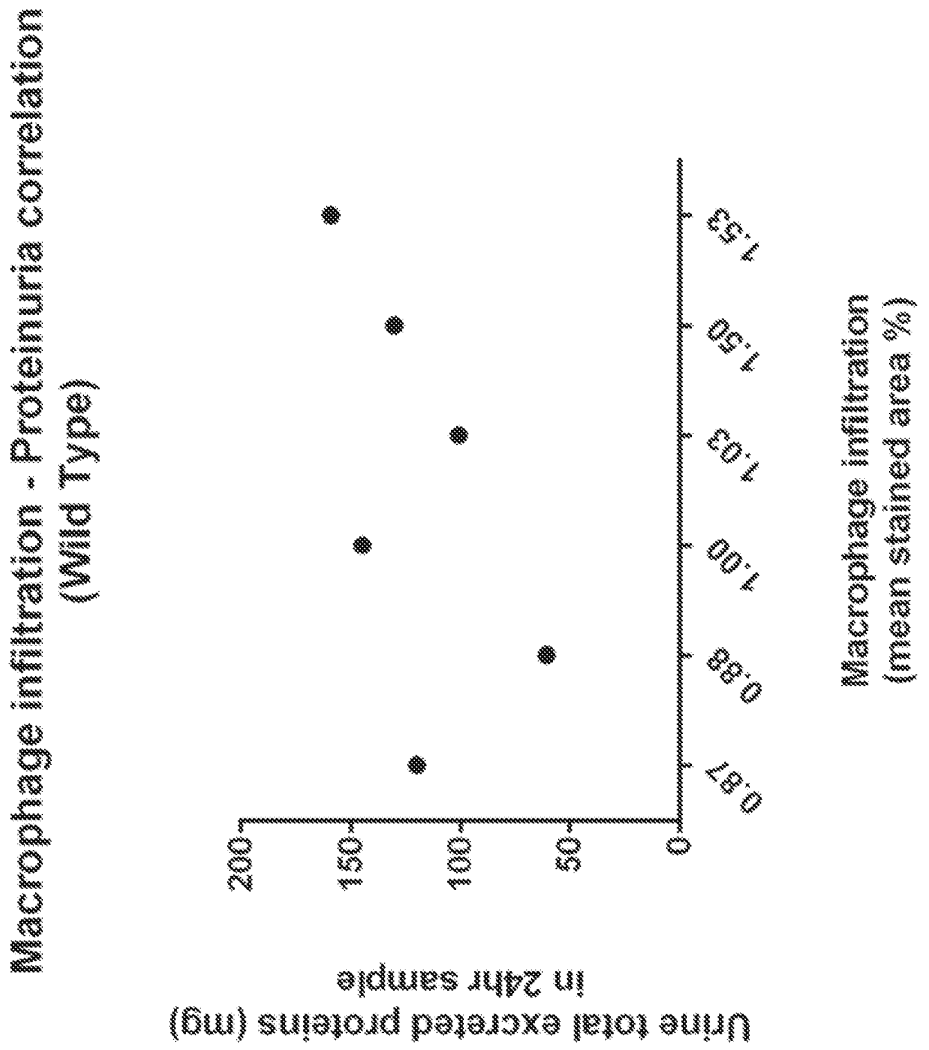

Macrophage infiltration - Proteinuria correlation
(Wild Type)
Urine total excreted proteins (mg)
in 24hr sample
200
150
100
50
0
0.81
0.88
1.00
1.03
1.50
1.53
Macrophage infiltration
(mean stained area %)

FIG. 27A

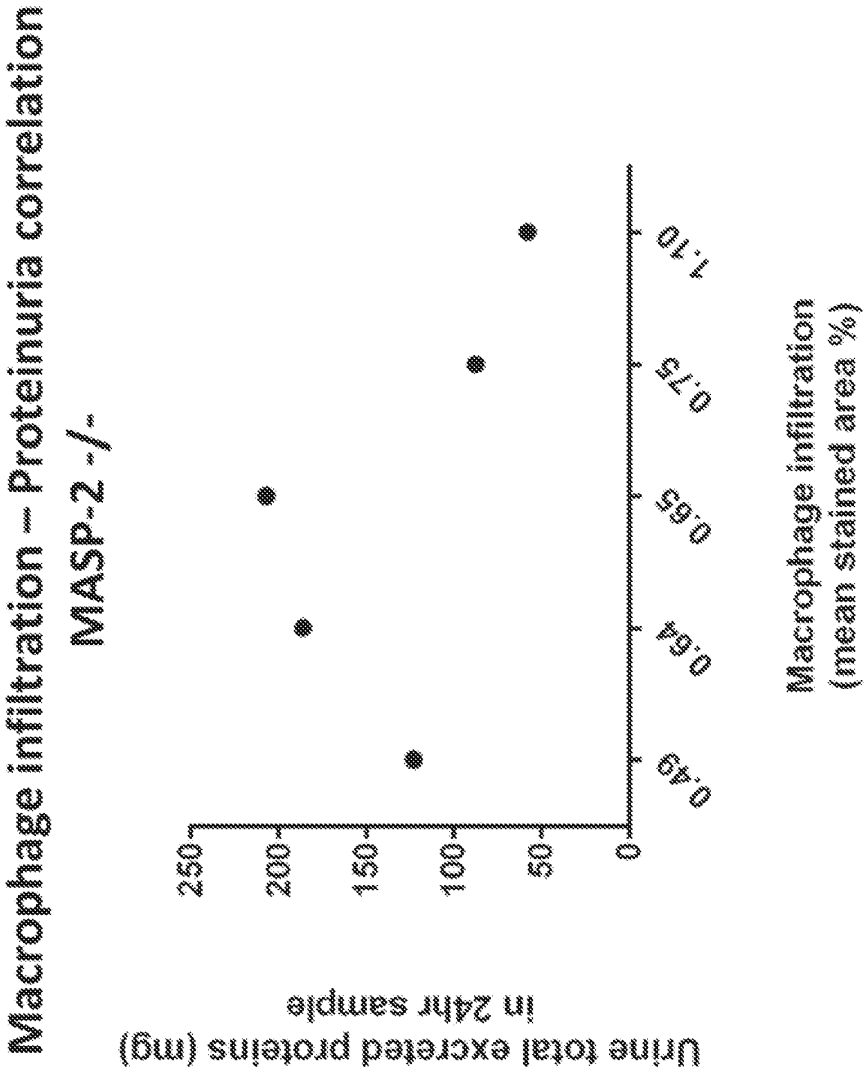

Macrophage infiltration – Proteinuria correlation
MASP-2 -/-
Urine total excreted proteins (mg)
in 24hr sample
250
200
150
100
50
0
0.49
0.64
0.65
0.75
1.10
Macrophage infiltration
(mean stained area %)

FIG. 27B

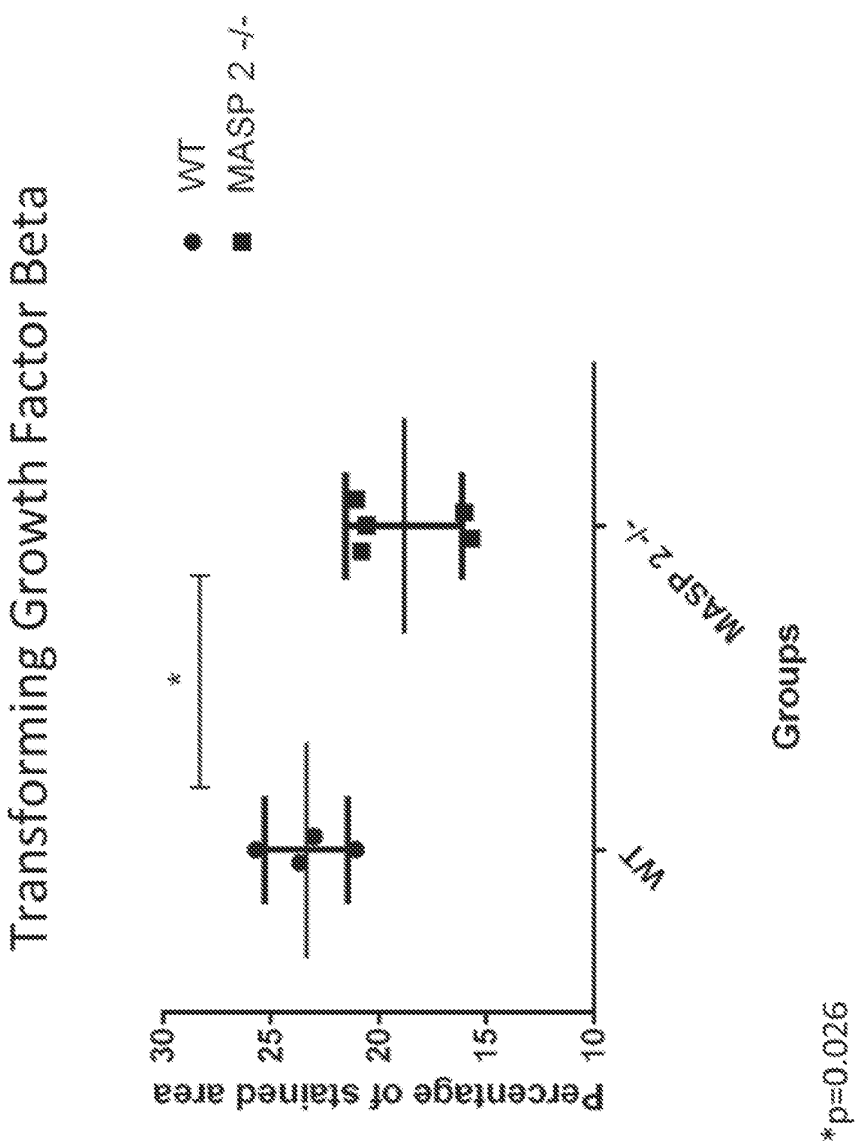

Transforming Growth Factor Beta
WT
MASP 2 -/-
*
Percentage of stained area
30
25
20
15
10
WT
MASP 2 -/-
Groups
*p=0.026

FIG. 28

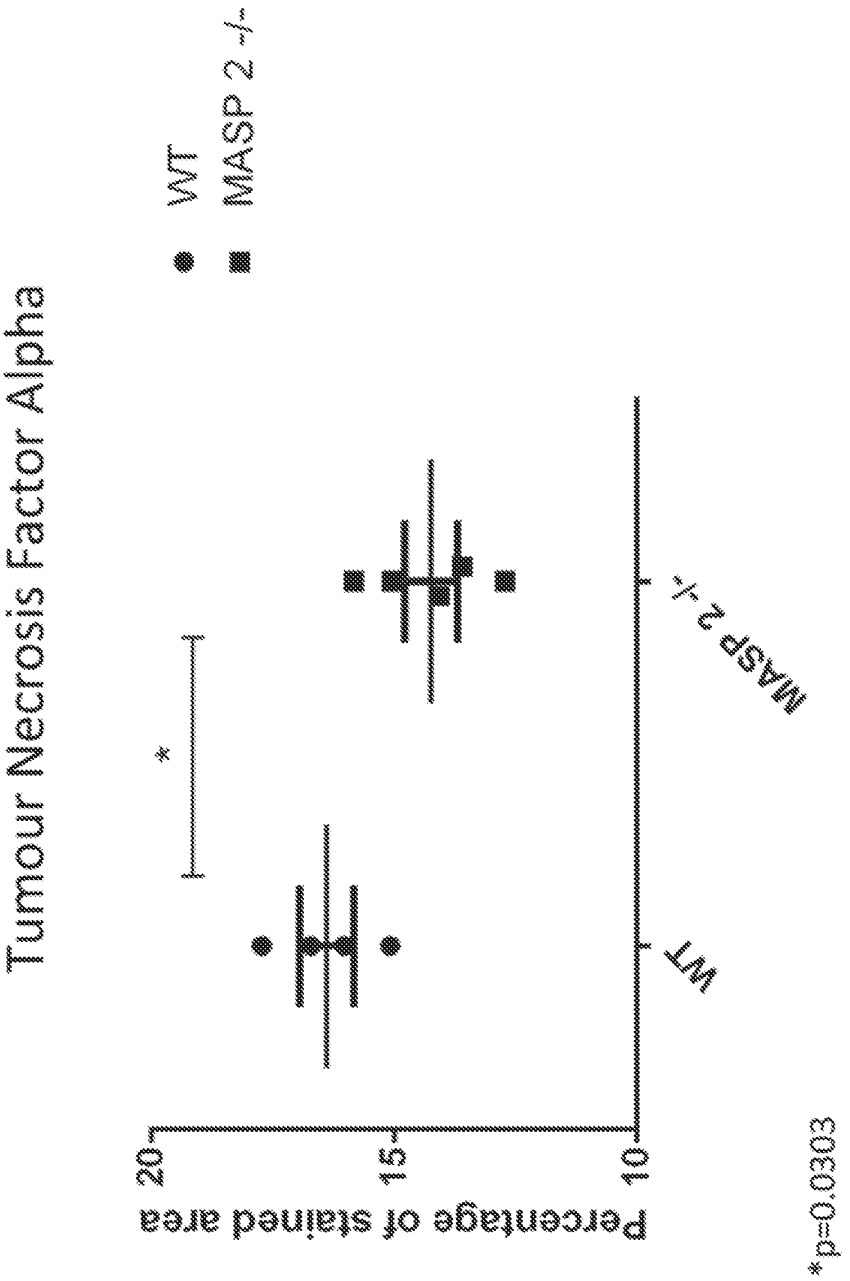

Tumour Necrosis Factor Alpha
WT
MASP 2 -/-
*
Percentage of stained area
20
15
10
WT
MASP 2 -/-
*p=0.0303

FIG. 29

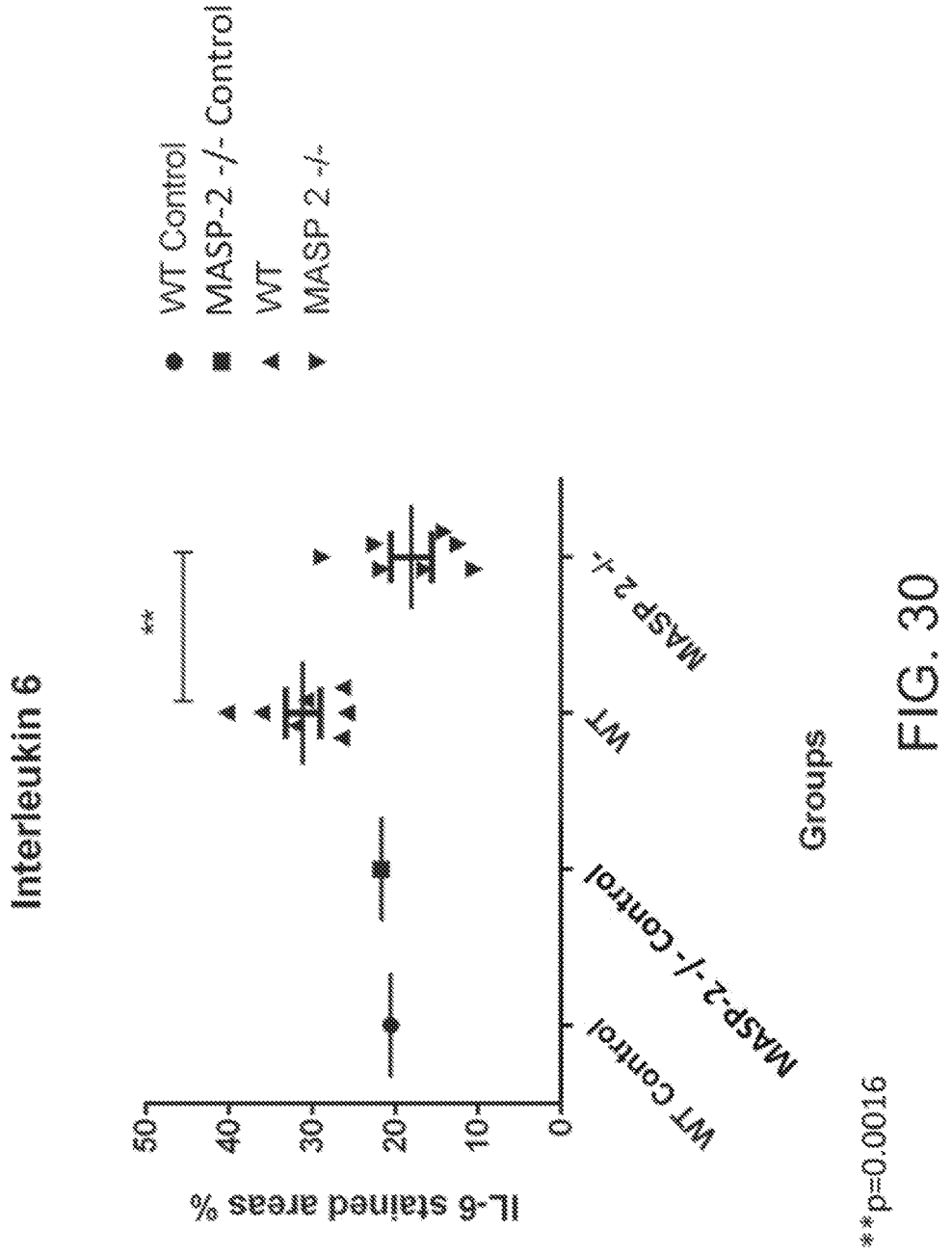

Interleukin 6
WT Control
MASP-2 -/- Control
WT
MASP 2 -/-
**
IL-6 stained areas %
50
40
30
20
10
0
WT Control
MASP-2 -/- Control
WT
MASP 2 -/-
Groups
** p=0.0016

FIG. 30

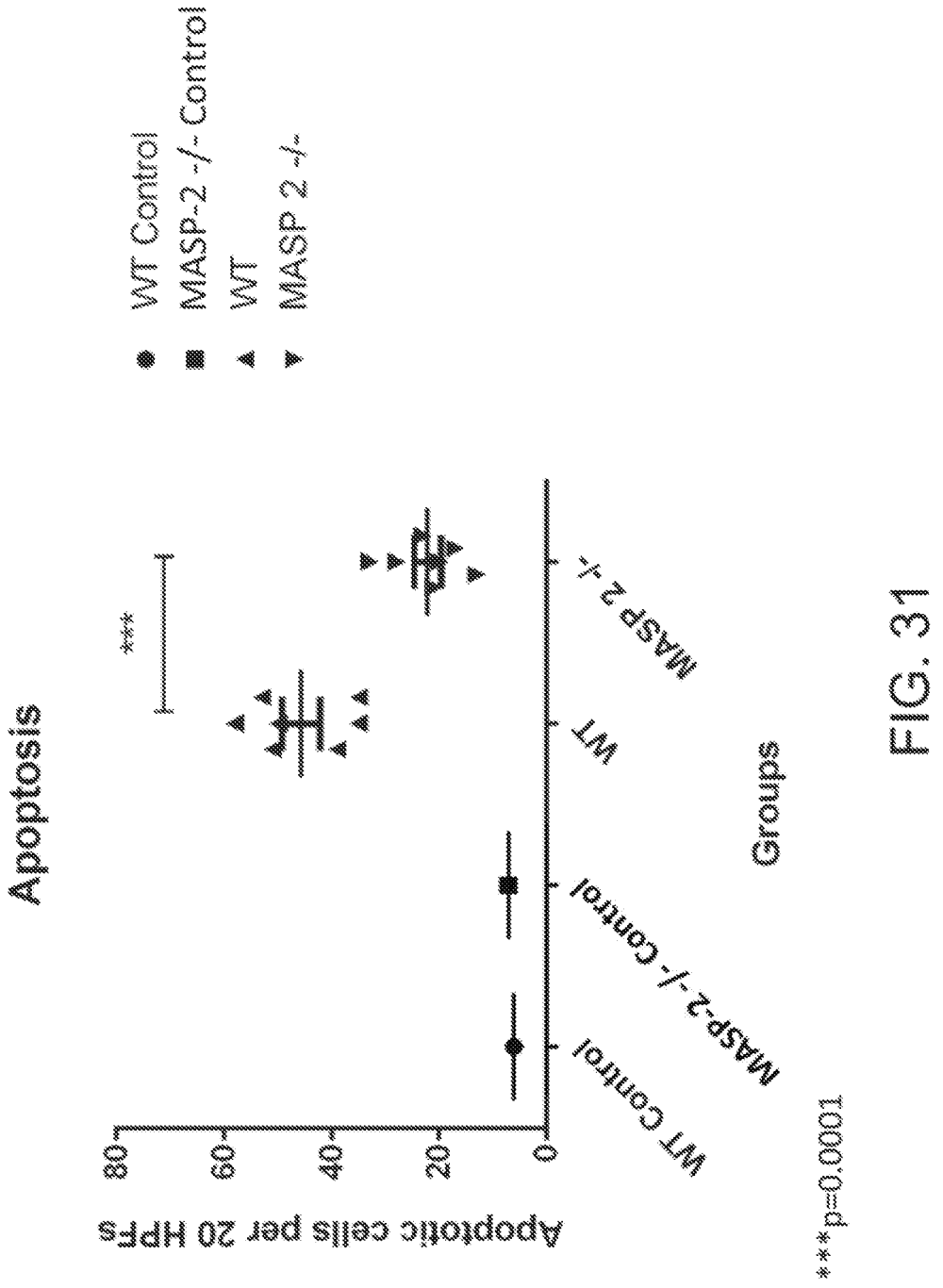

WT Control
MASP-2 -/- Control
WT
MASP 2 -/-
Apoptosis
***
Apoptotic cells per 20 HPFs
0
20
40
60
80
WT Control
MASP-2 -/- Control
WT
MASP 2 -/-
Groups
***p=0.0001

FIG. 31

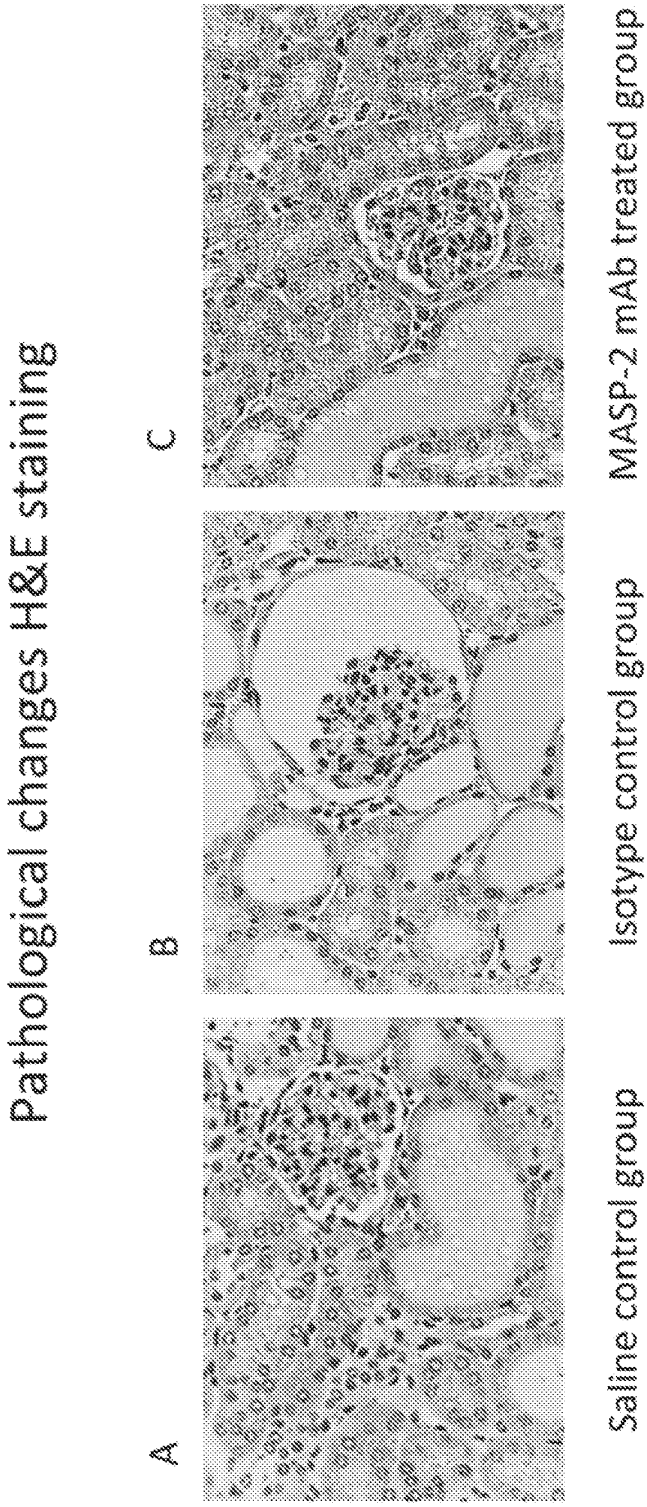

Pathological changes H&E staining
A
Saline control group
B
Isotype control group
C
MASP-2 mAb treated group

FIG. 32

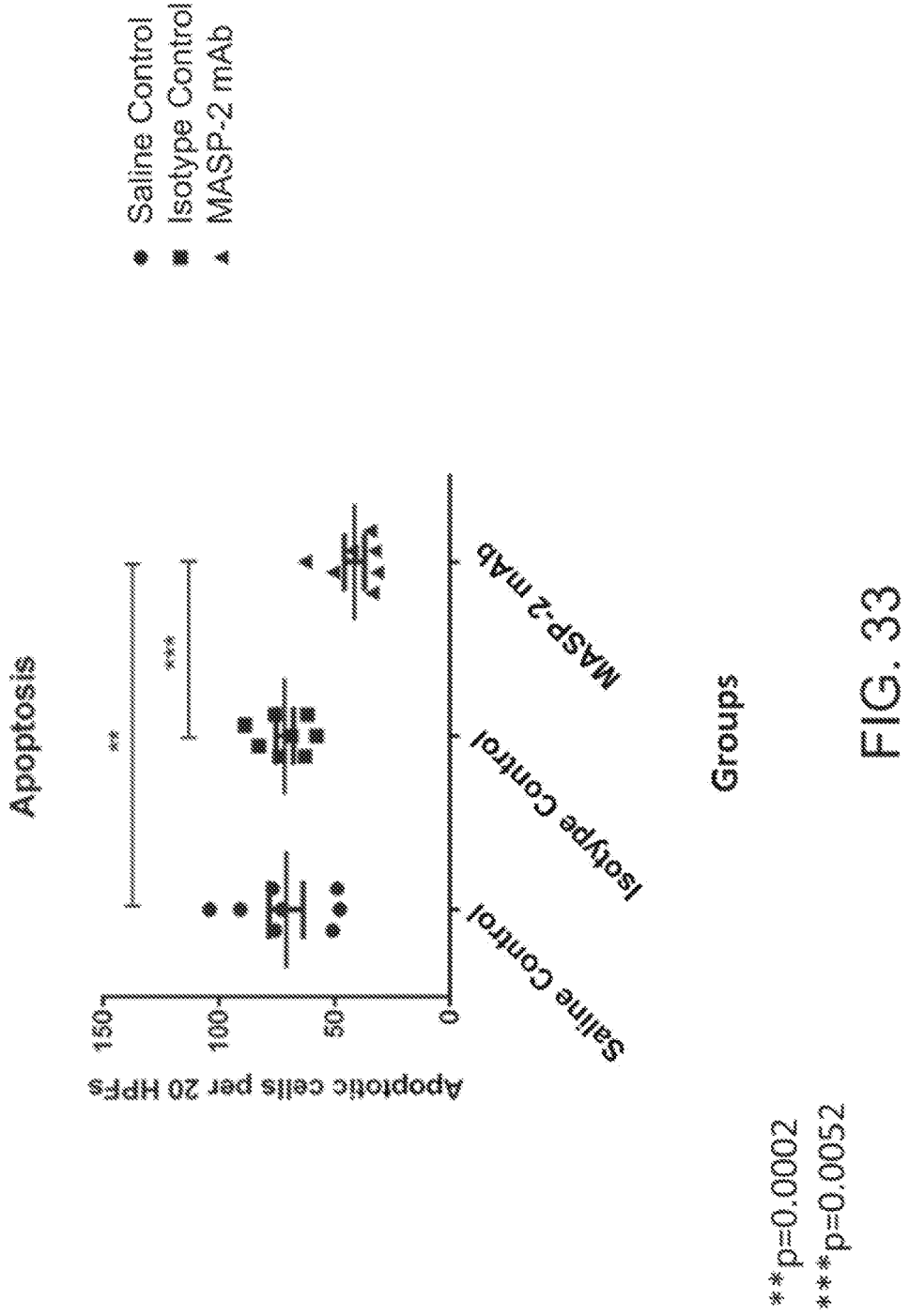

Saline Control
Isotype Control
MASP-2 mAb
Apoptosis
Apoptotic cells per 20 HPFs
150
100
50
0
**
***
Saline Control
Isotype Control
MASP-2 mAb
Groups
**p=0.0002
***p=0.0052

FIG. 33

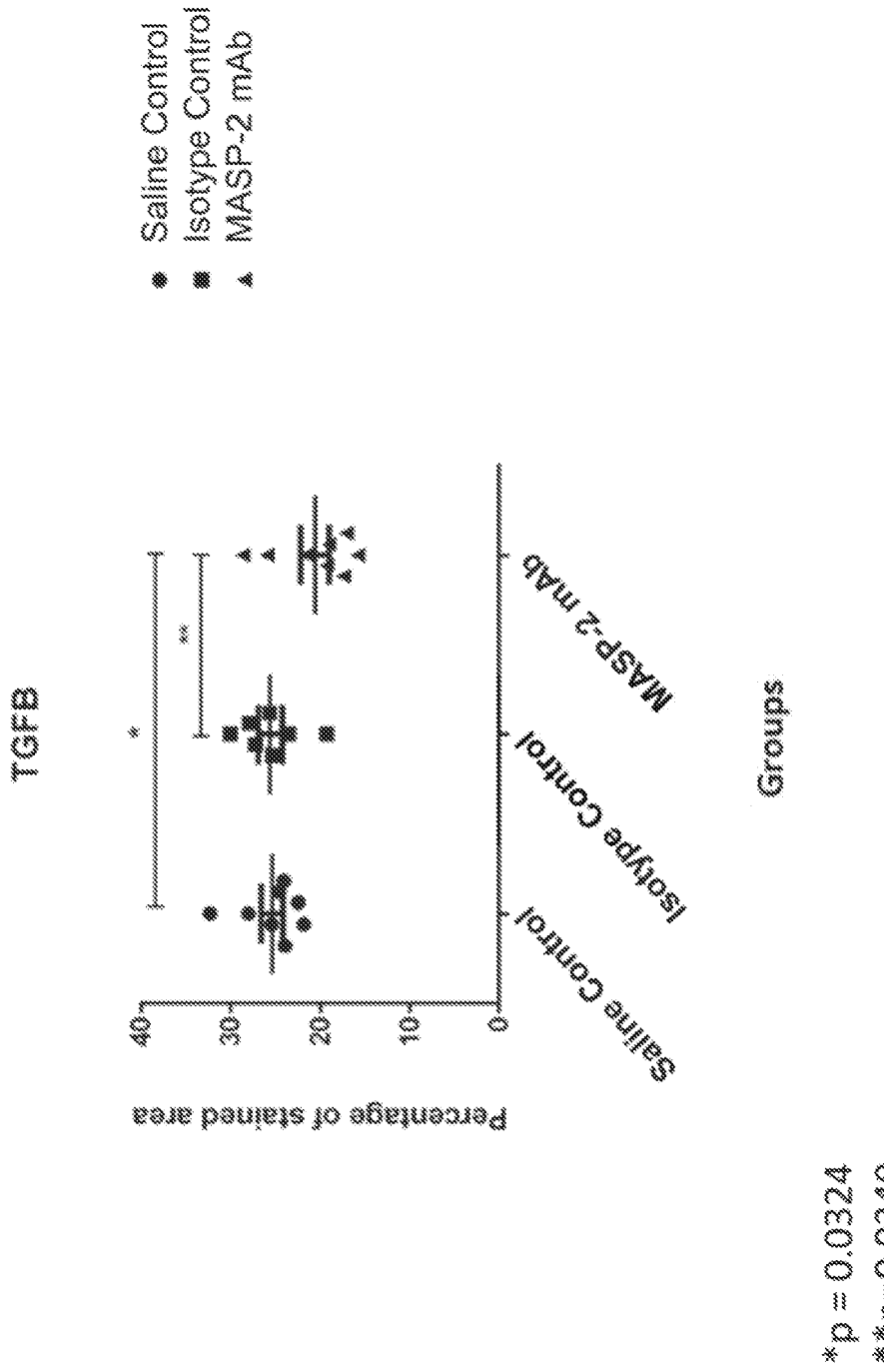

Saline Control
Isotype Control
MASP-2 mAb
TGFB
Percentage of stained area
0
10
20
30
40
*
**
Saline Control
Isotype Control
MASP-2 mAb
Groups
*p = 0.0324
**p=0.0349

FIG. 34

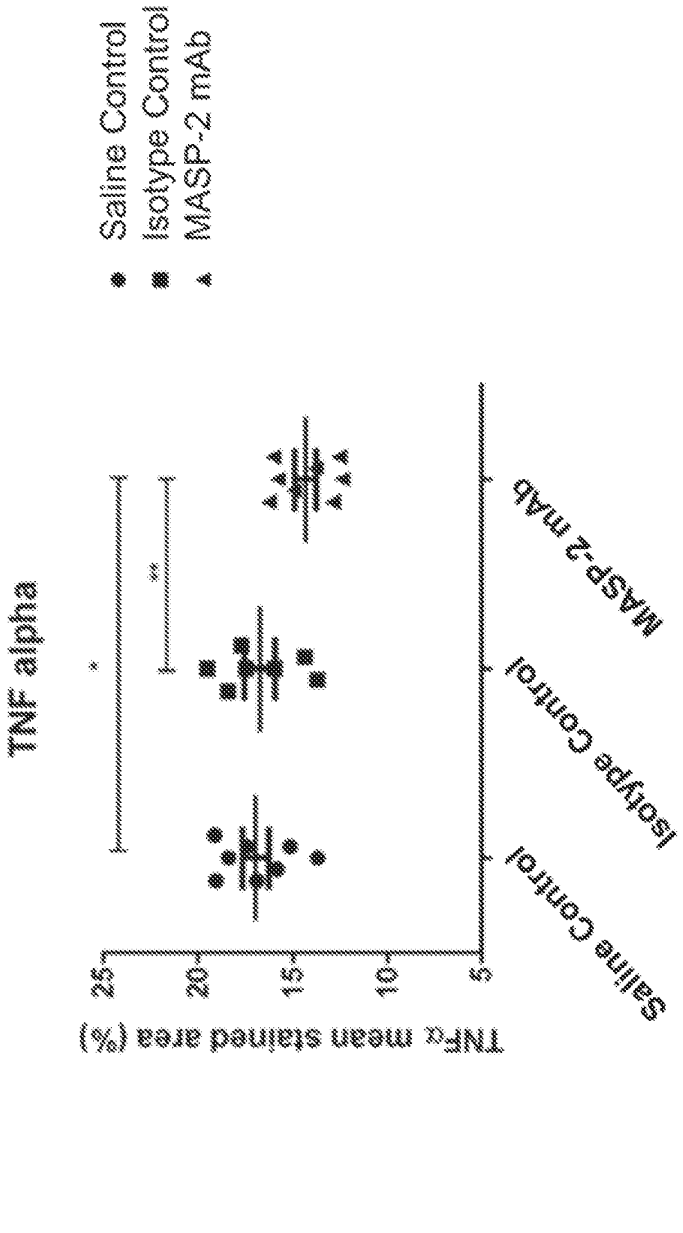

Saline Control
Isotype Control
MASP-2 mAb
TNF alpha
*
**
25
20
15
10
5
TNFα mean stained area (%)
Saline Control
Isotype Control
MASP-2 mAb
Mouse Groups
*p=0.011
**p=0.0285

FIG. 35

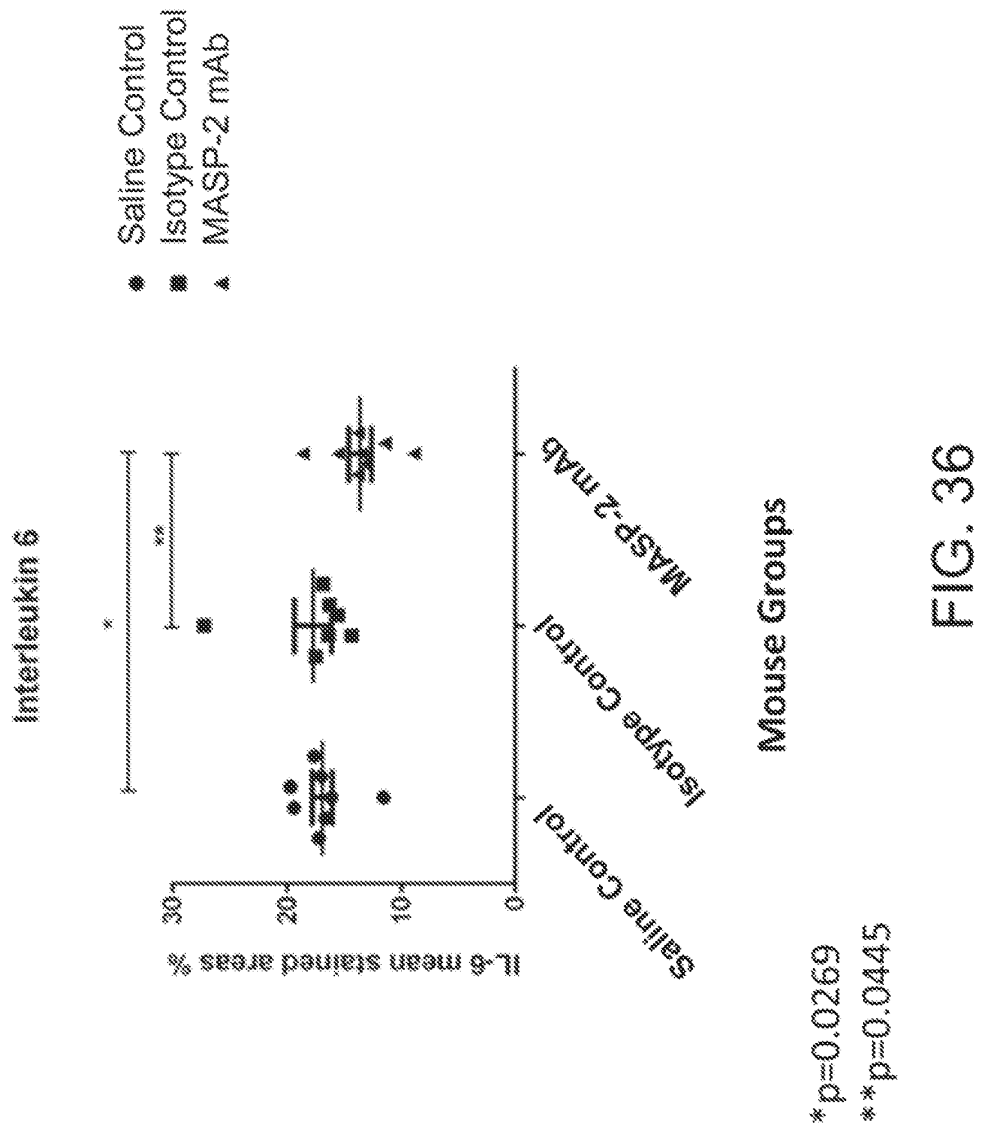

Interleukin 6
Saline Control
Isotype Control
MASP-2 mAb
IL-6 mean stained areas %
30
20
10
0
*
**
Saline Control
Isotype Control
MASP-2 mAb
Mouse Groups
*p=0.0269
**p=0.0445

FIG. 36

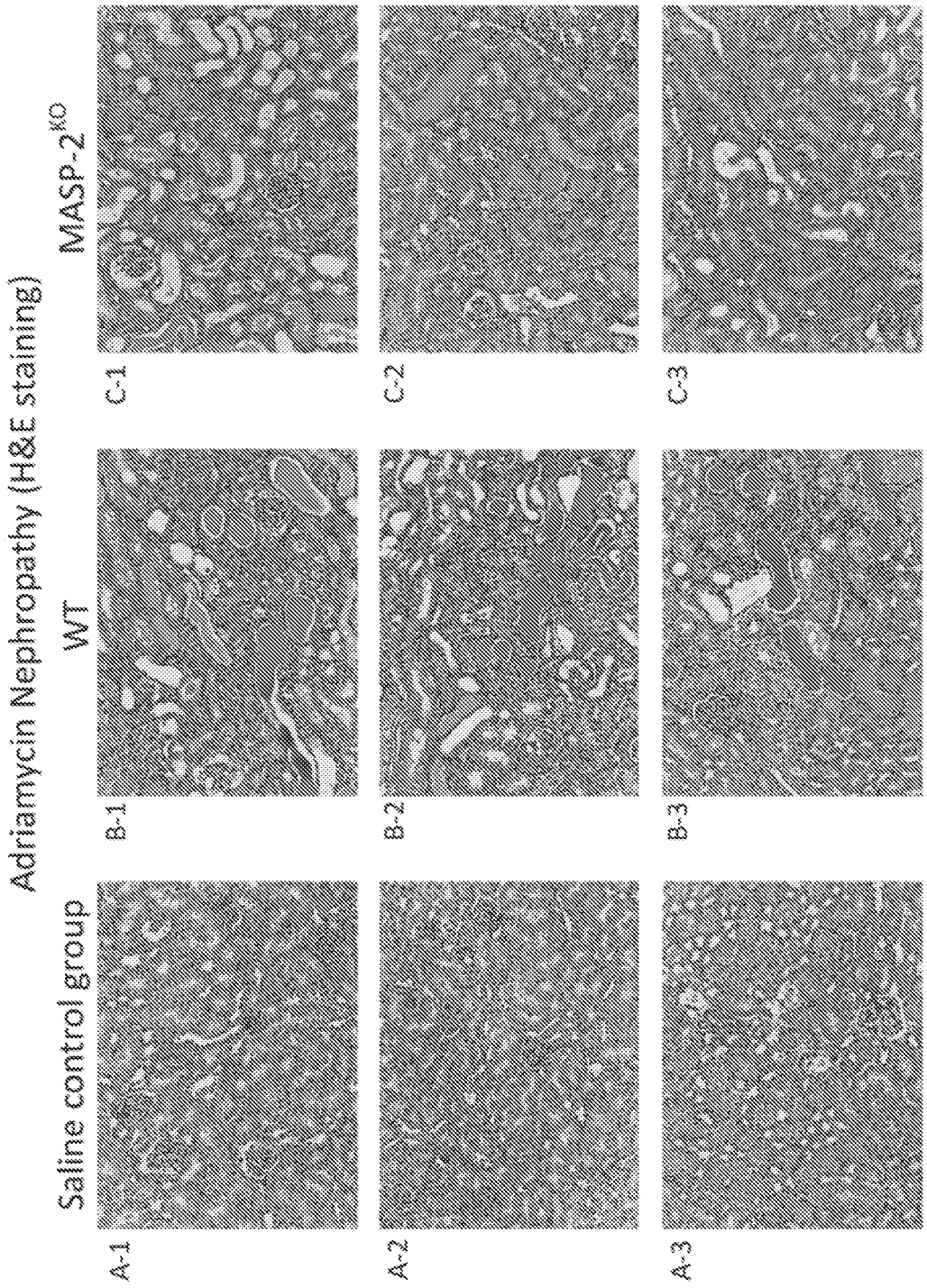

Adriamycin Nephropathy (H&E staining)
Saline control group
WT
MASP-2KO
A-1
A-2
A-3
B-1
B-2
B-3
C-1
C-2
C-3

FIG. 37

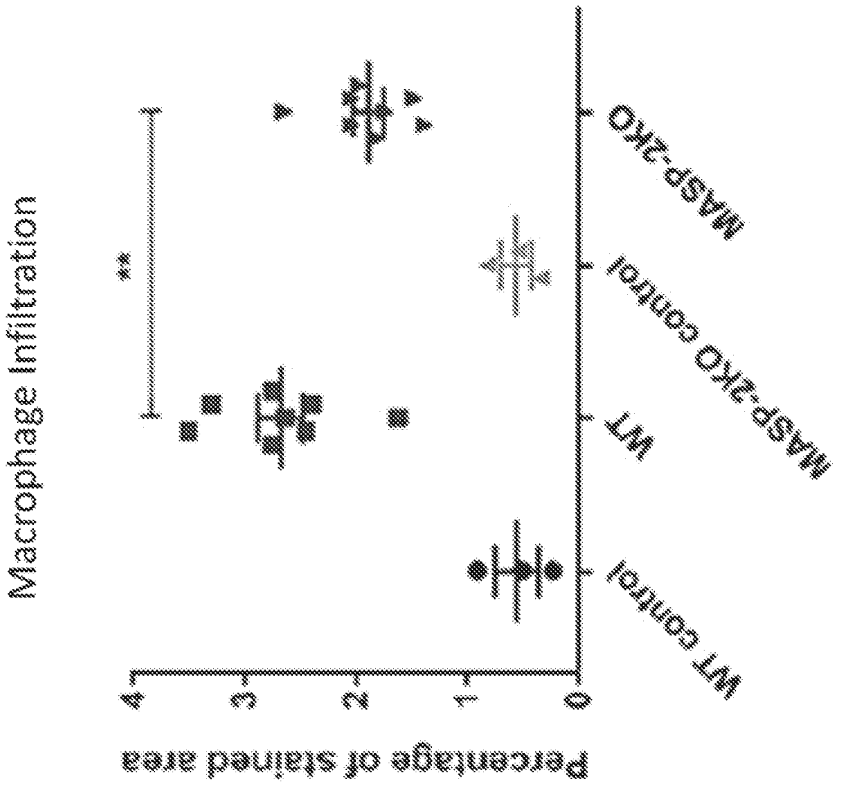

Macrophage Infiltration
Percentage of stained area
0
1
2
3
4
**
WT control
WT
MASP-2KO control
MASP-2KO

FIG. 38

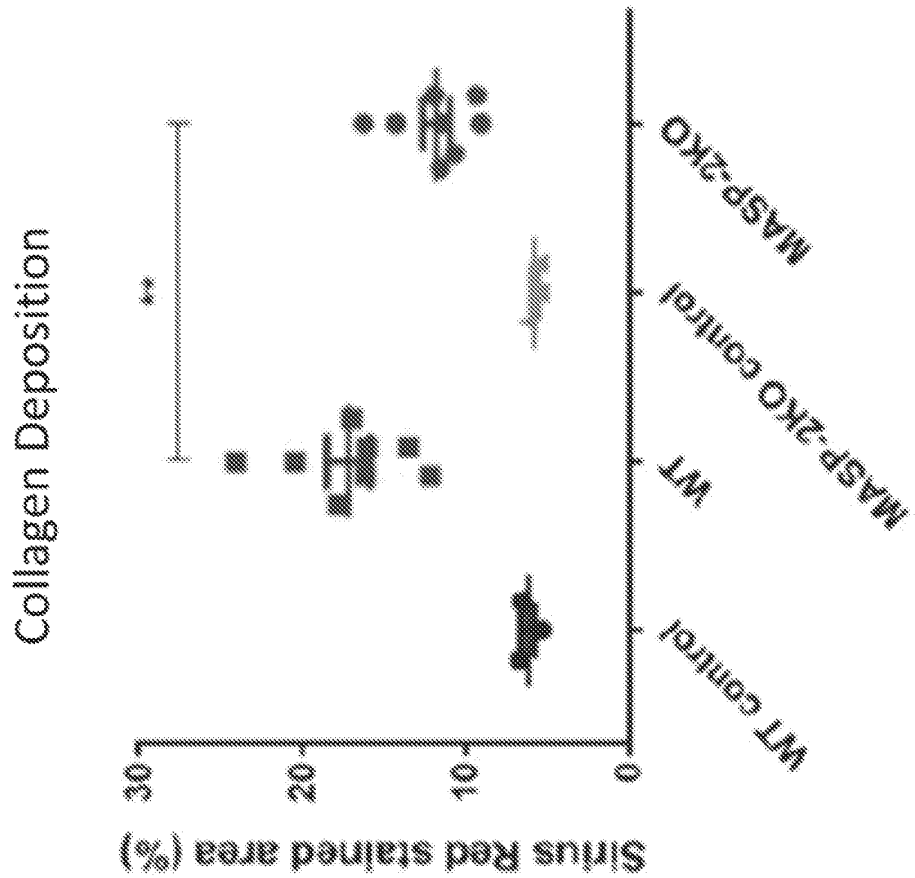

Collagen Deposition
**
Sirius Red stained area (%)
30
20
10
0
WT control
WT
MASP-2KO control
MASP-2KO

FIG. 39

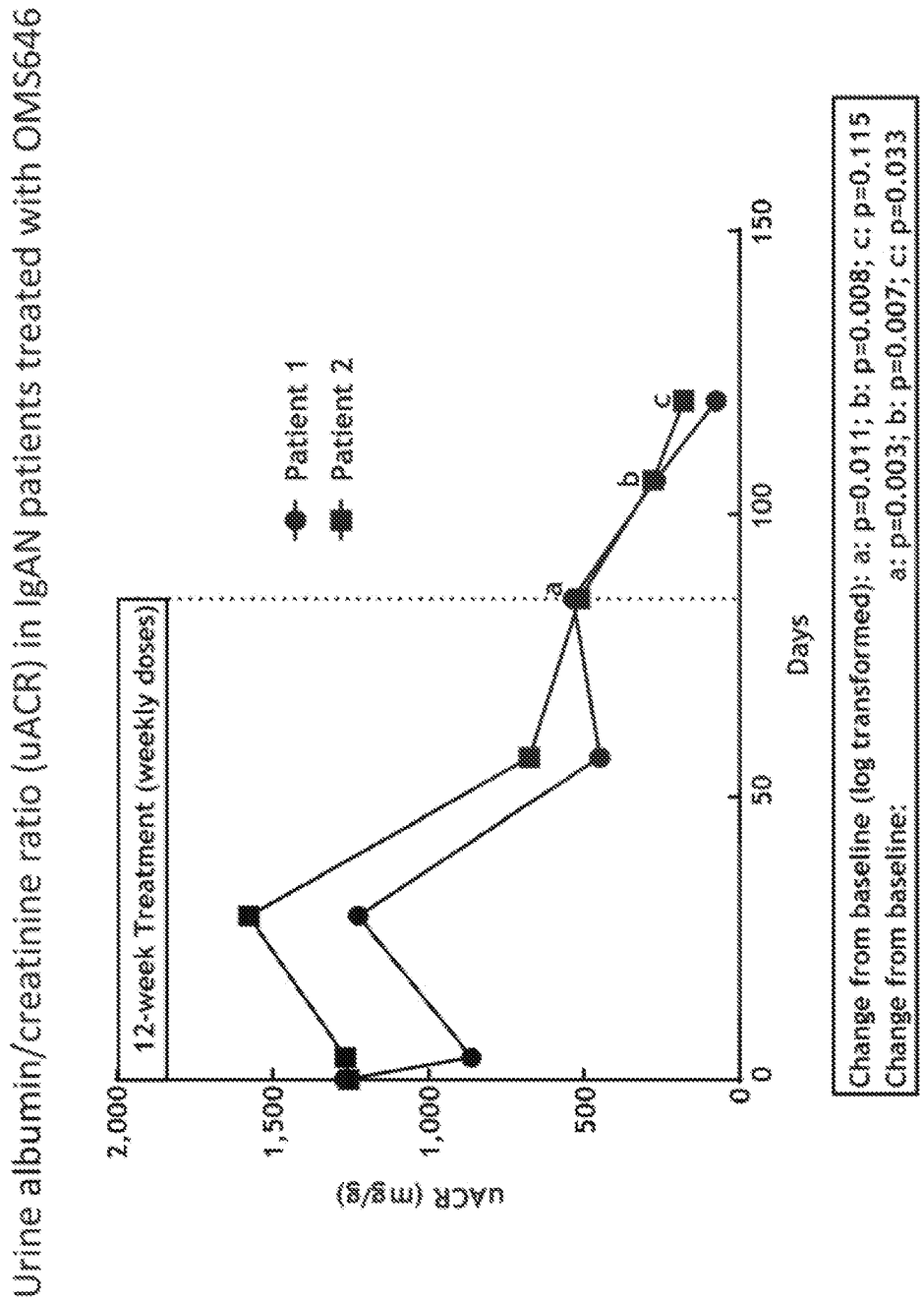

Urine albumin/creatinine ratio (uACR) in IgAN patients treated with OMS646
12-week Treatment (weekly doses)
Patient 1
Patient 2
uACR (mg/g)
2,000
1,500
1,000
500
0
0
50
100
150
Days
a
b
c
Change from baseline (log transformed): a: p=0.011; b: p=0.008; c: p=0.115
Change from baseline: a: p=0.003; b: p=0.007; c: p=0.033

FIG. 40

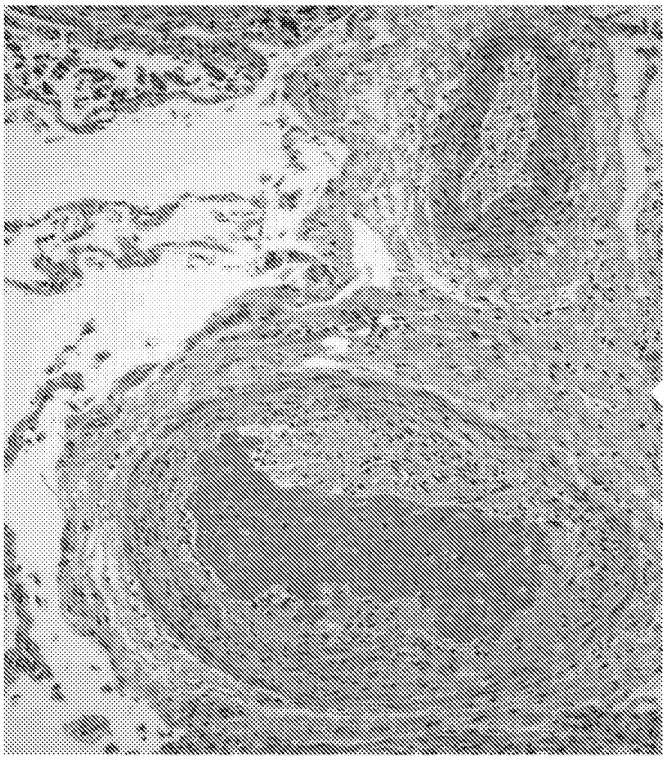


FIG. 41A

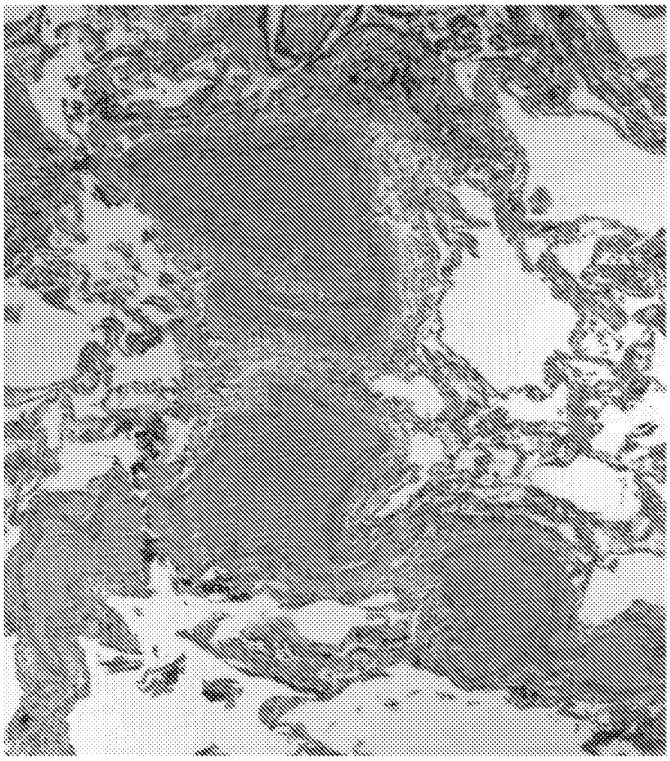


FIG. 41B

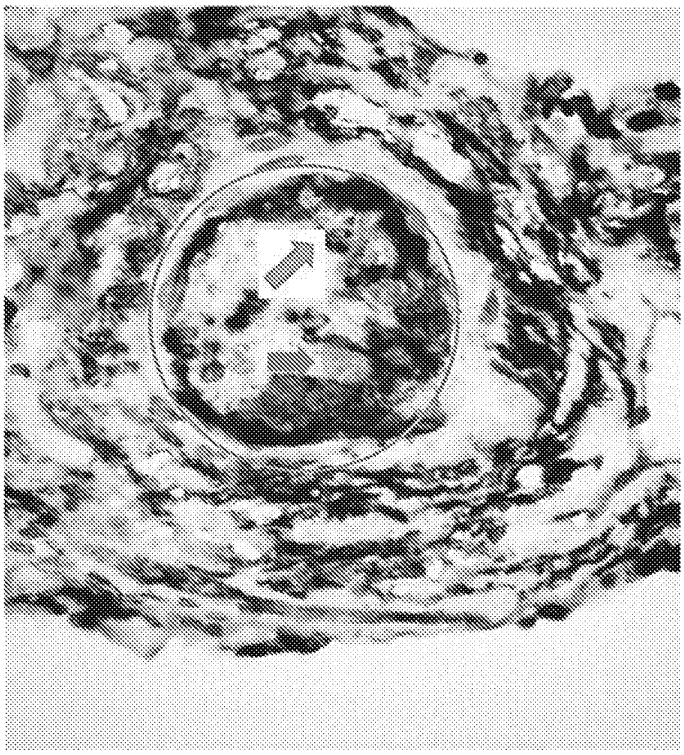


FIG. 41C

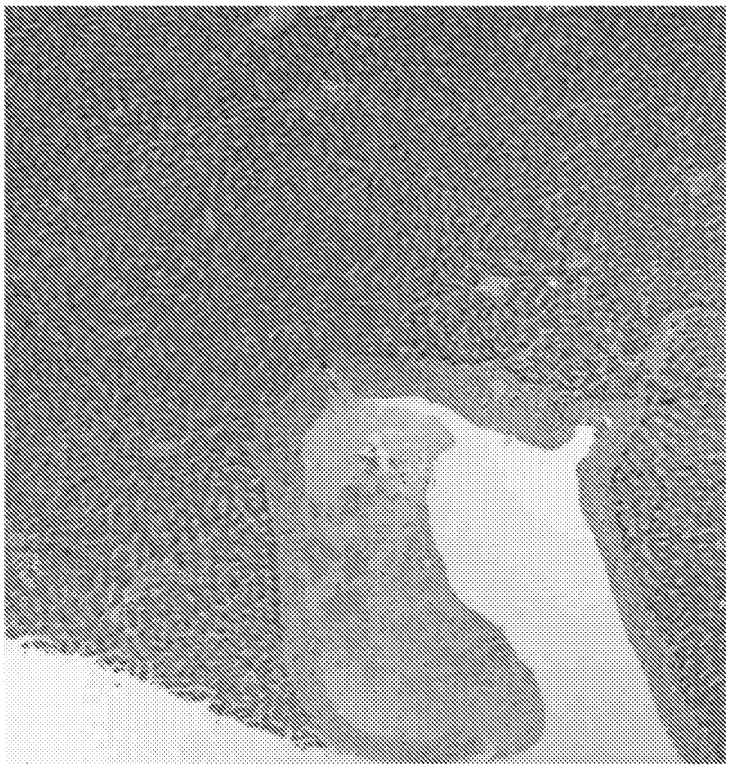


FIG. 41D

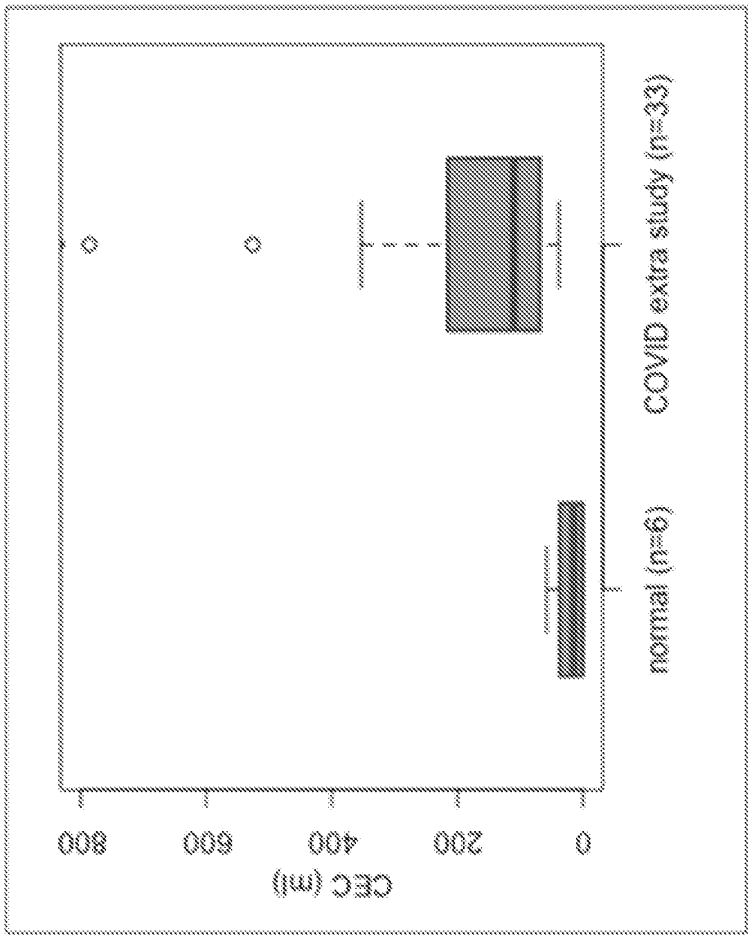


FIG. 42A

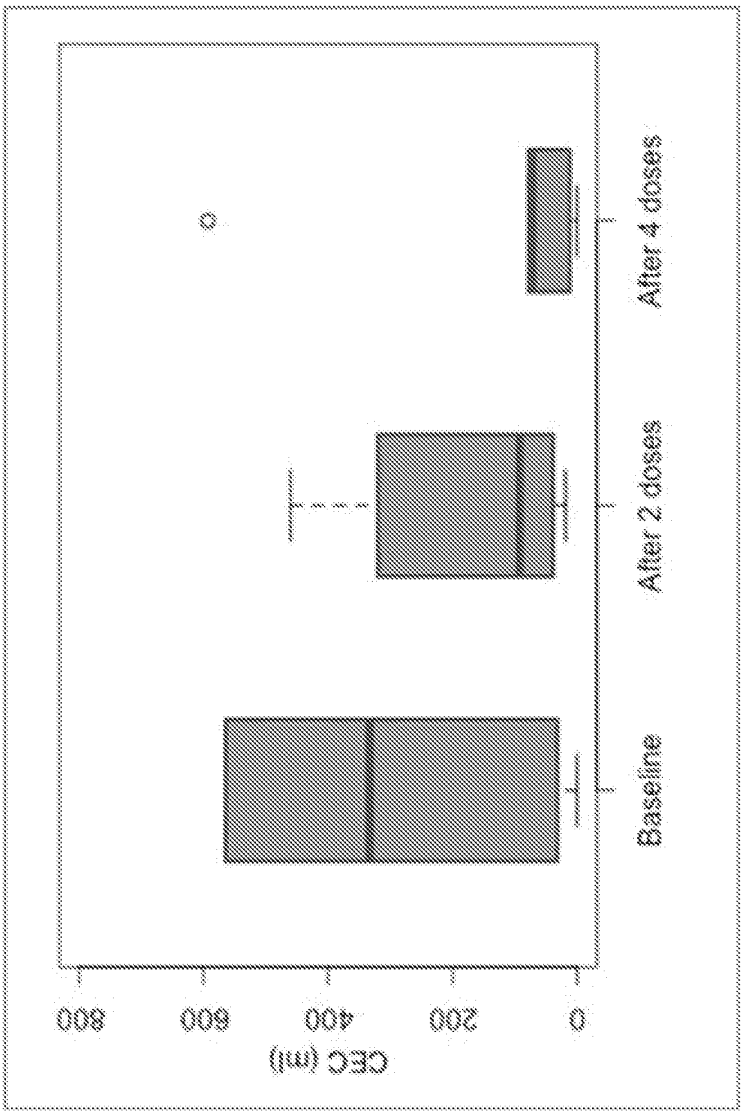


FIG. 42B

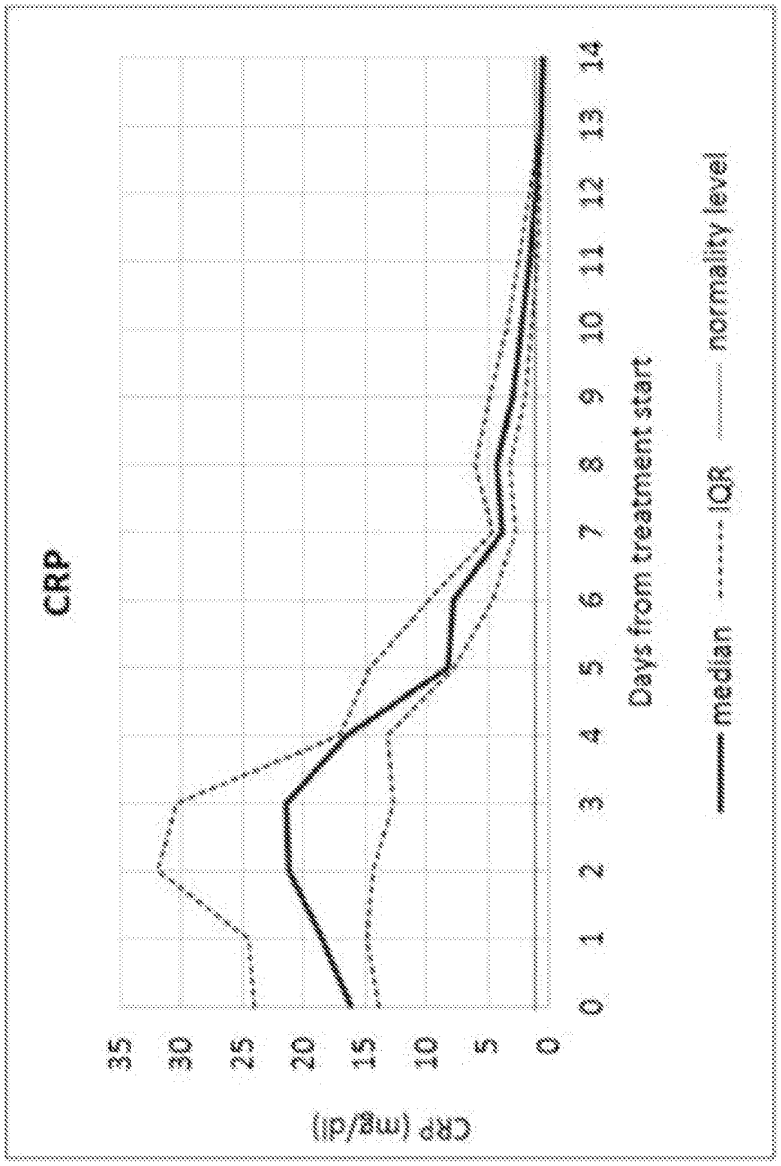

CRP
CRP (mg/dl)
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days from treatment start
median
IQR
normality level

FIG. 43

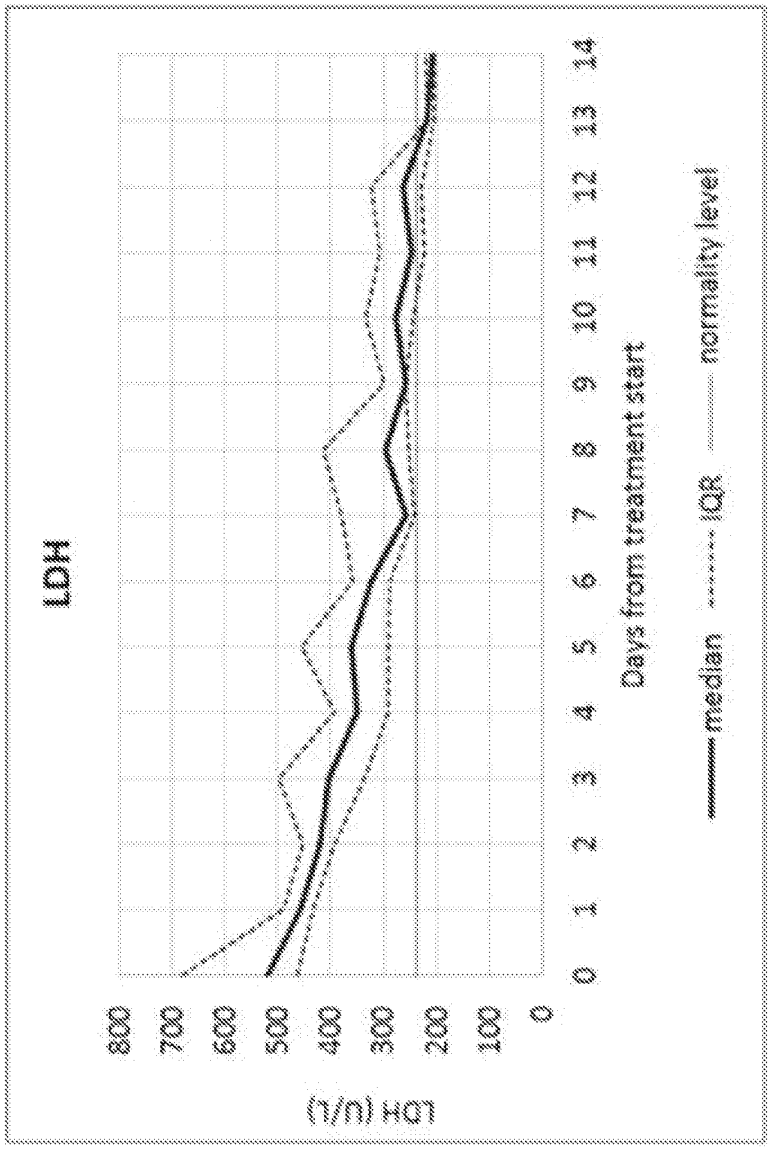

LDH
LDH (U/L)
800
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days from treatment start
median
IQR
normality level

FIG. 44

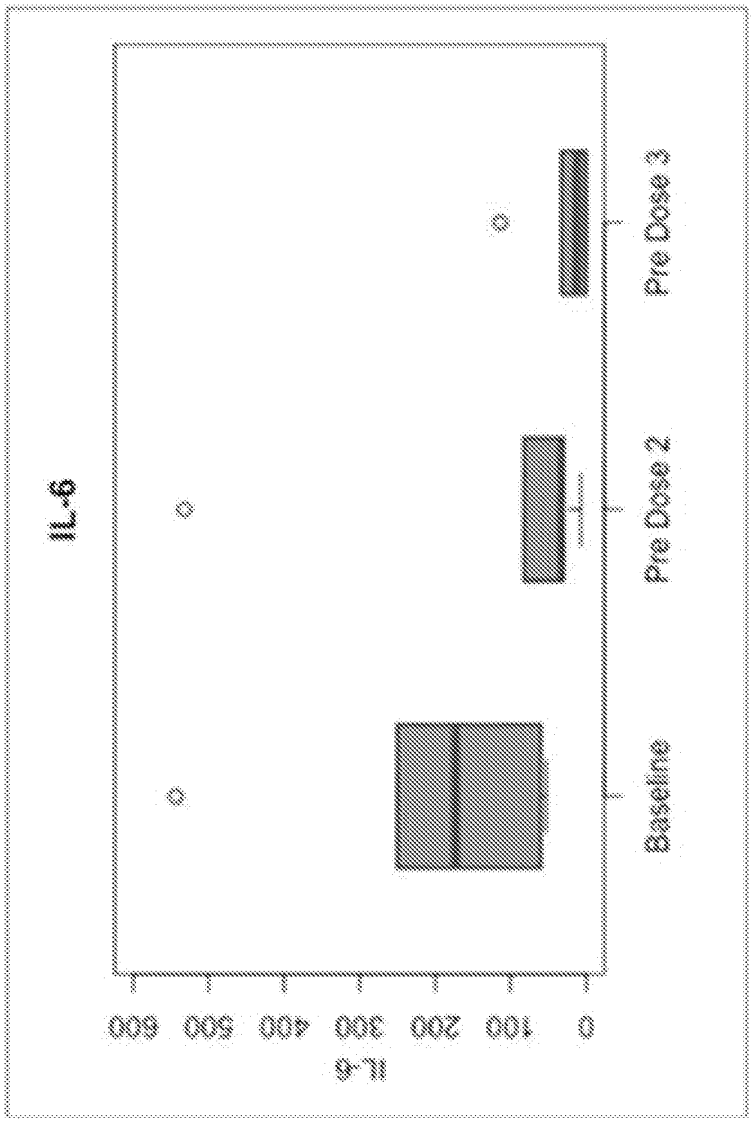


FIG. 45

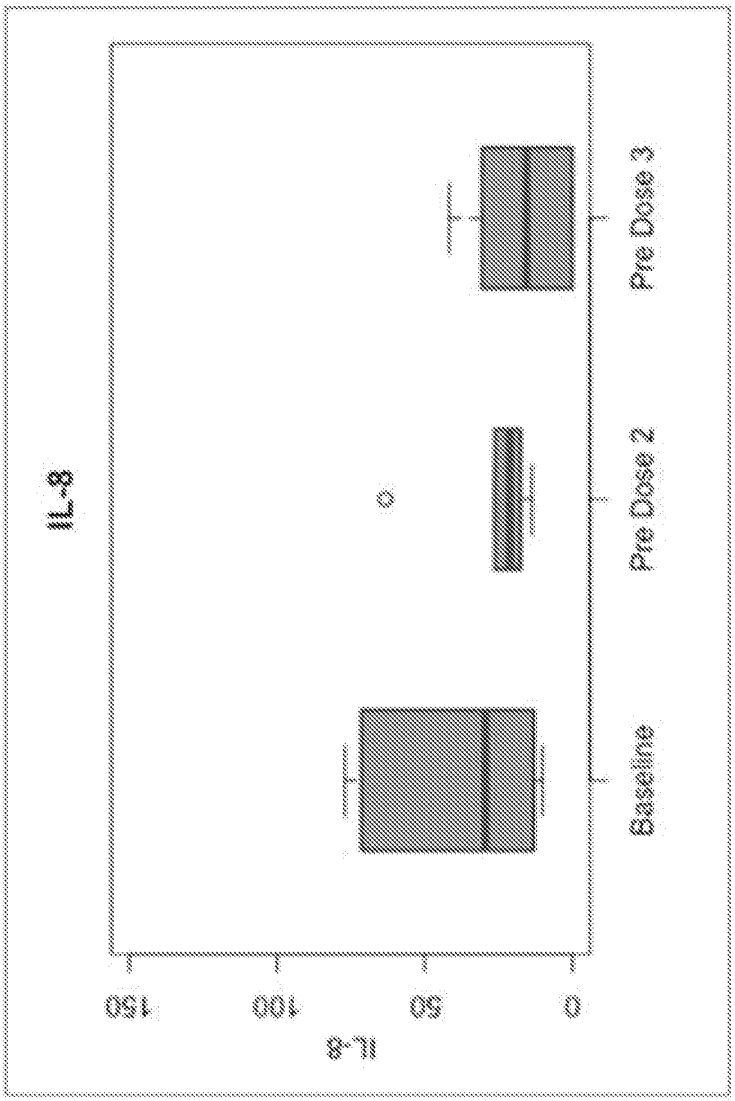


FIG. 46

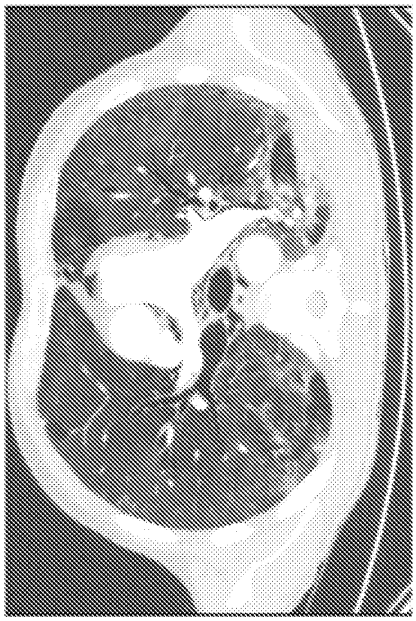


FIG. 47B



FIG. 47A

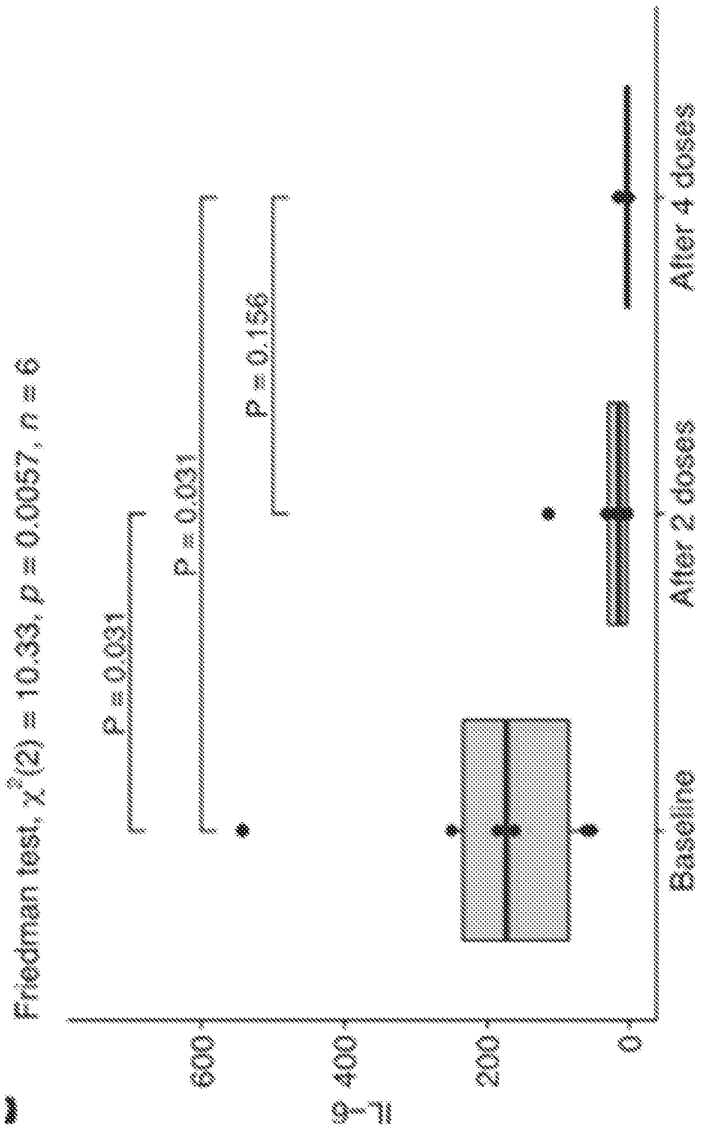

Friedman test, $\chi^2(2) = 10.33$, $p = 0.0057$, $n = 6$
P = 0.031
P = 0.031
P = 0.156
600
400
200
0
IL-7
Baseline
After 2 doses
After 4 doses

FIG. 48

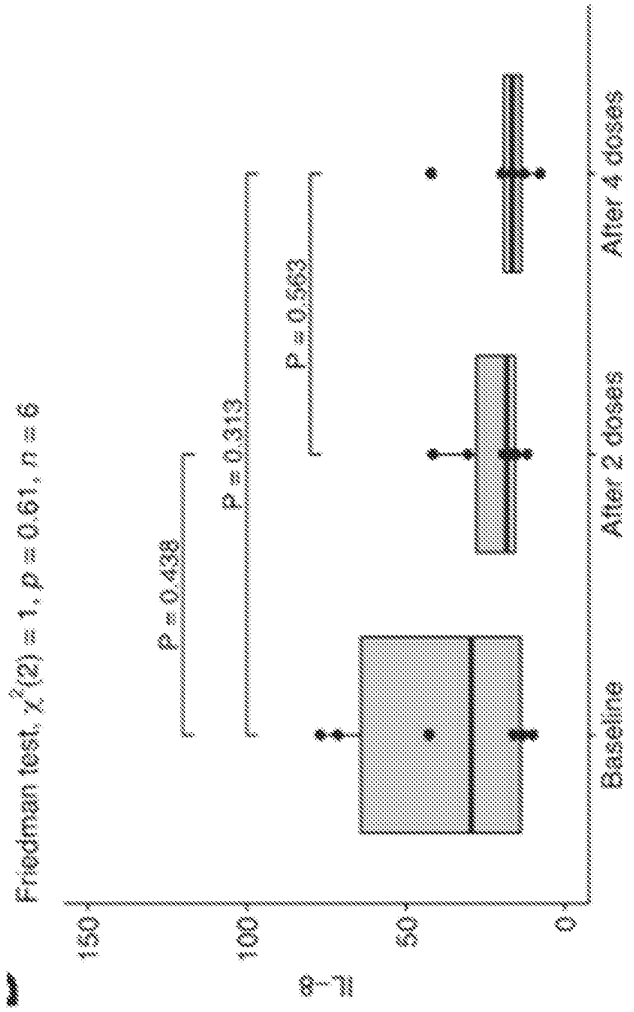

Friedman test, $\chi^2(2) = 1$, $p = 0.61$, $n = 6$
P = 0.438
P = 0.313
P = 0.563
150
100
50
0
Baseline
After 2 doses
After 4 doses

FIG. 49

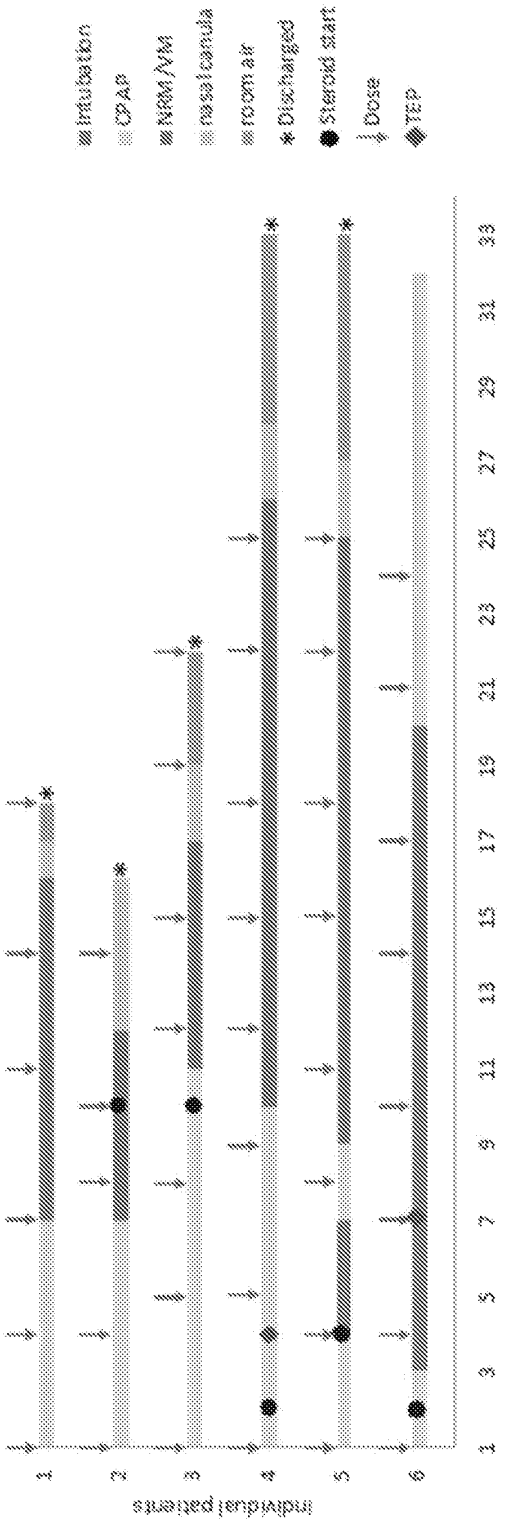

Intubation
CPAP
NRM/NM
nasal cannula
room air
Discharged
Steroid start
Dose
TEP
Individual patients
1
2
3
4
5
6
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
Days from treatment start

FIG. 50

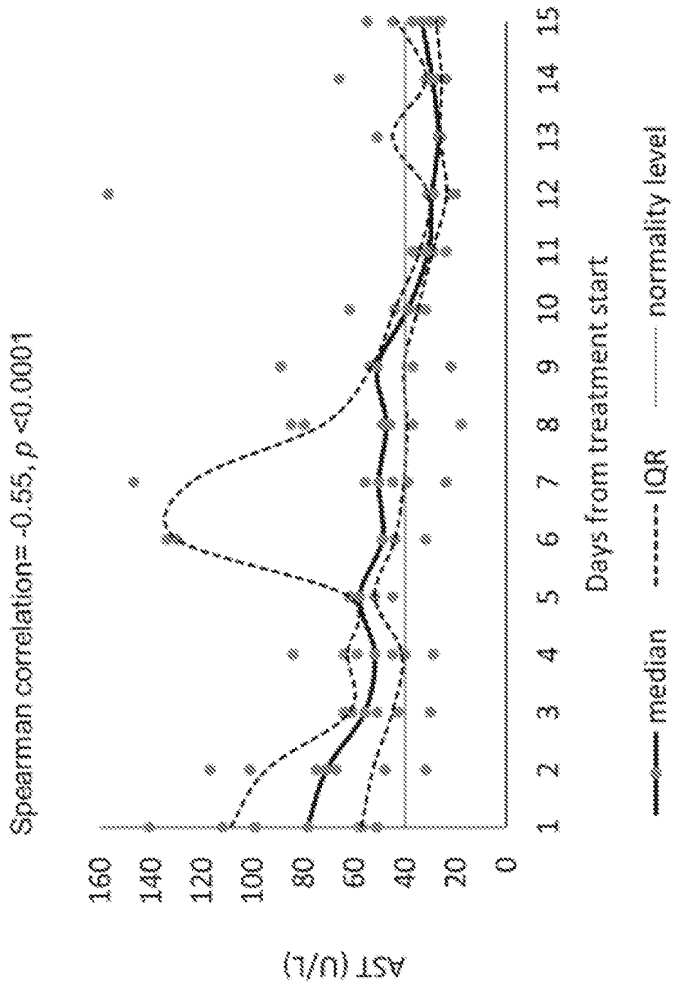

Spearman correlation= -0.55, p <0.0001
AST (U/L)
160
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days from treatment start
median
IQR
normality level

FIG. 51A

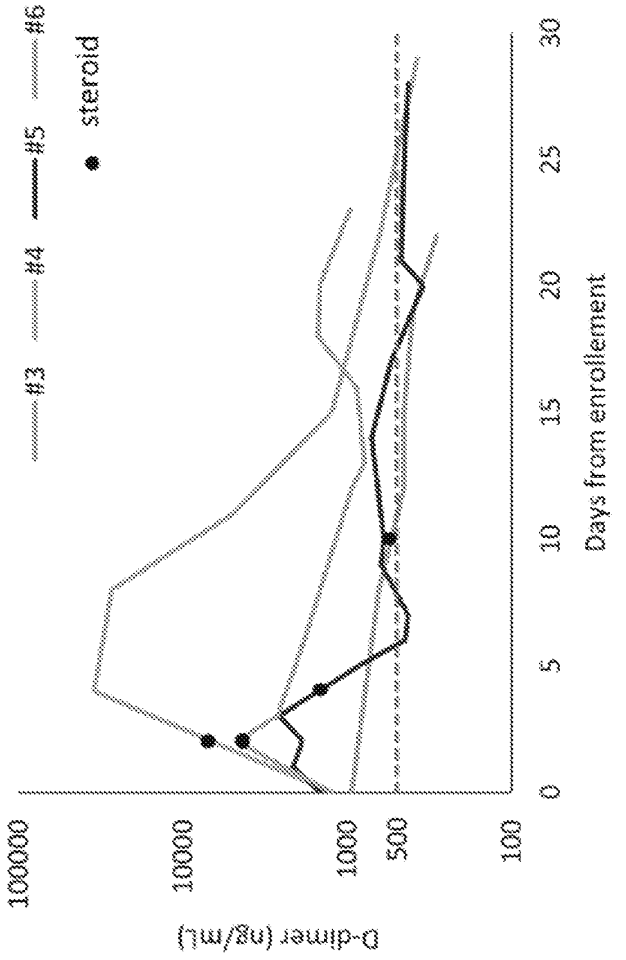

#3
#4
#5
#6
steroid
D-dimer (ng/mL)
100000
10000
1000
500
100
0
5
10
15
20
25
30
Days from enrollement

FIG. 51B

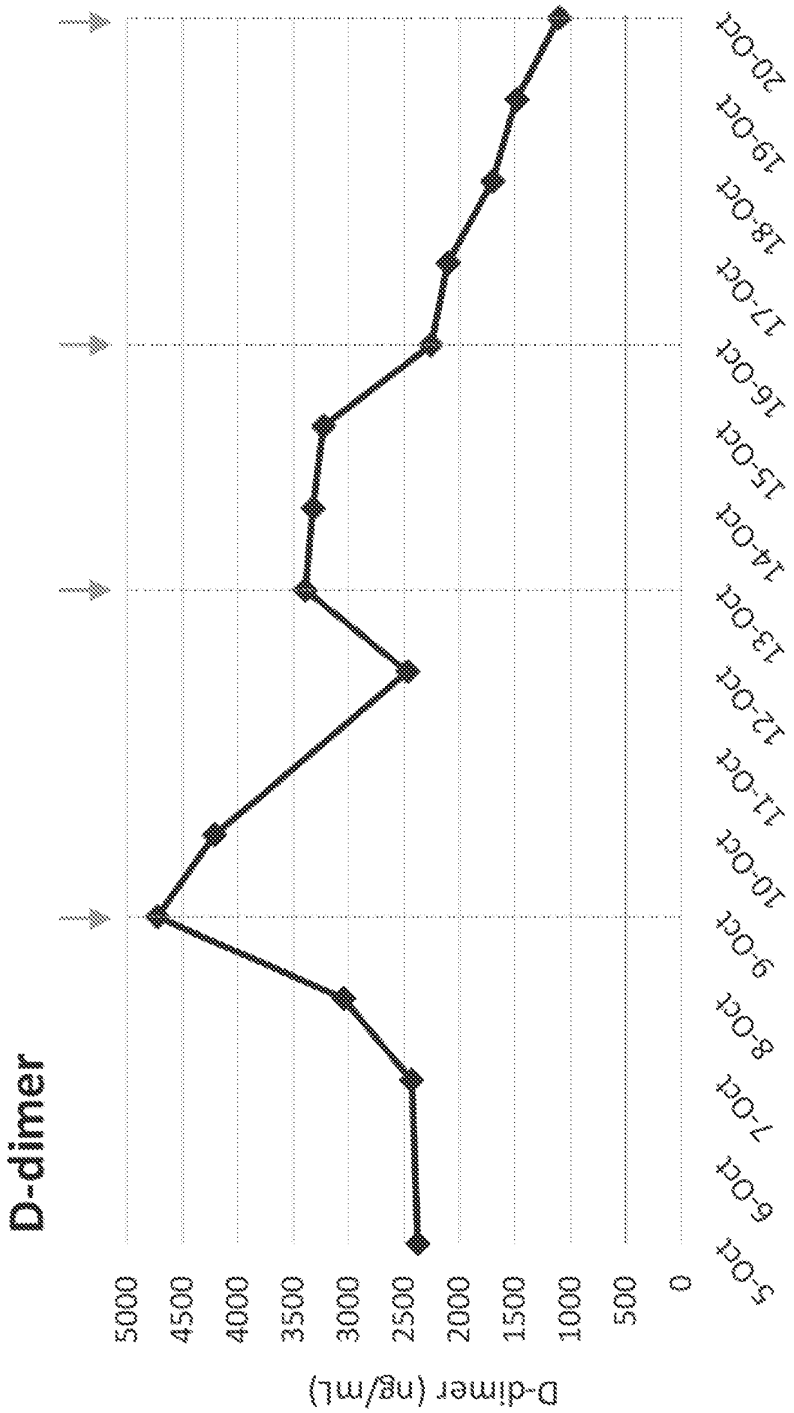

D-dimer
D-dimer (ng/mL)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
5-Oct
6-Oct
7-Oct
8-Oct
9-Oct
10-Oct
11-Oct
12-Oct
13-Oct
14-Oct
15-Oct
16-Oct
17-Oct
18-Oct
19-Oct
20-Oct

FIG. 52A

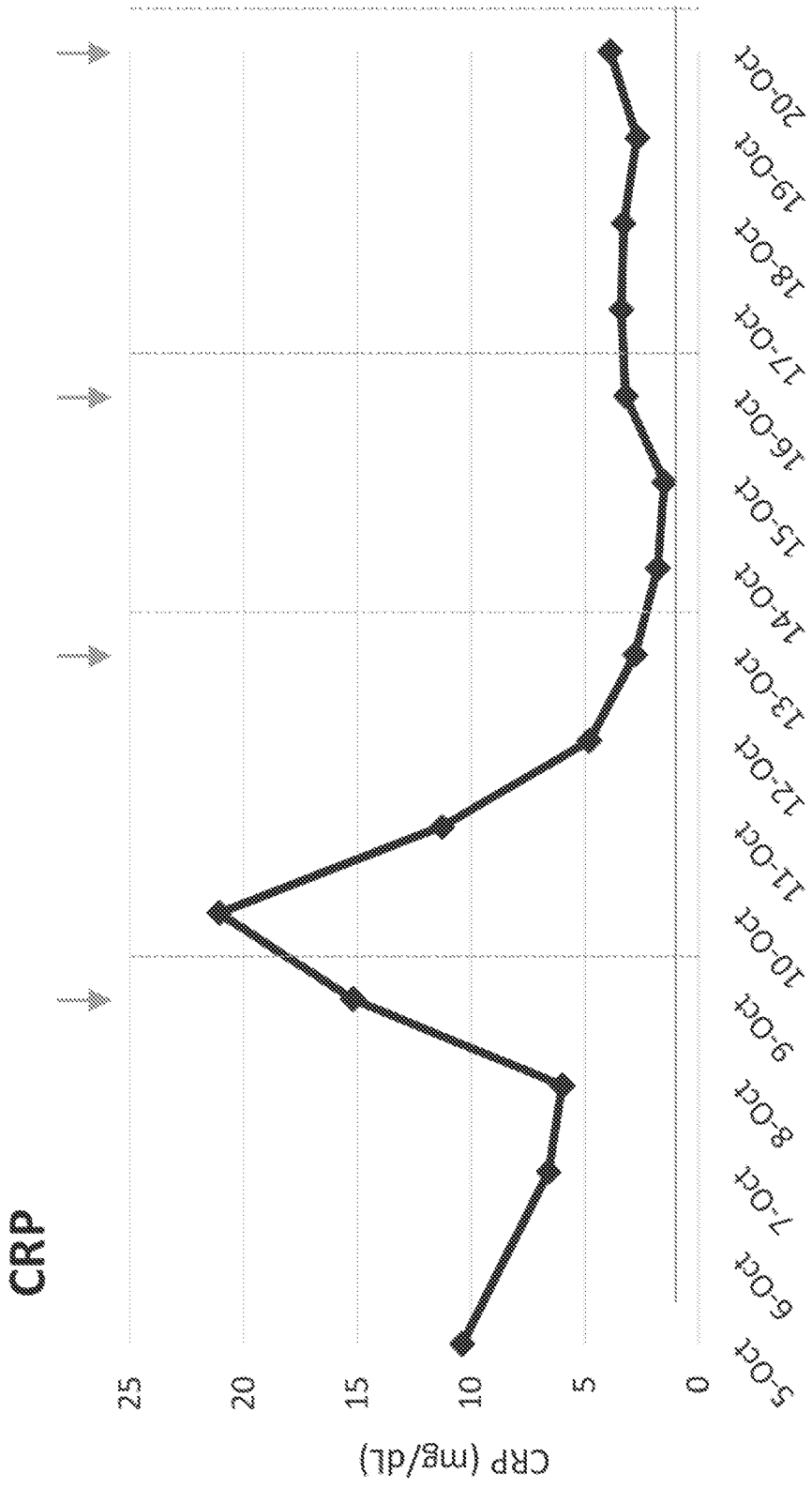

CRP
CRP (mg/dL)
25
20
15
10
5
0
5-Oct
6-Oct
7-Oct
8-Oct
9-Oct
10-Oct
11-Oct
12-Oct
13-Oct
14-Oct
15-Oct
16-Oct
17-Oct
18-Oct
19-Oct
20-Oct

FIG. 52B

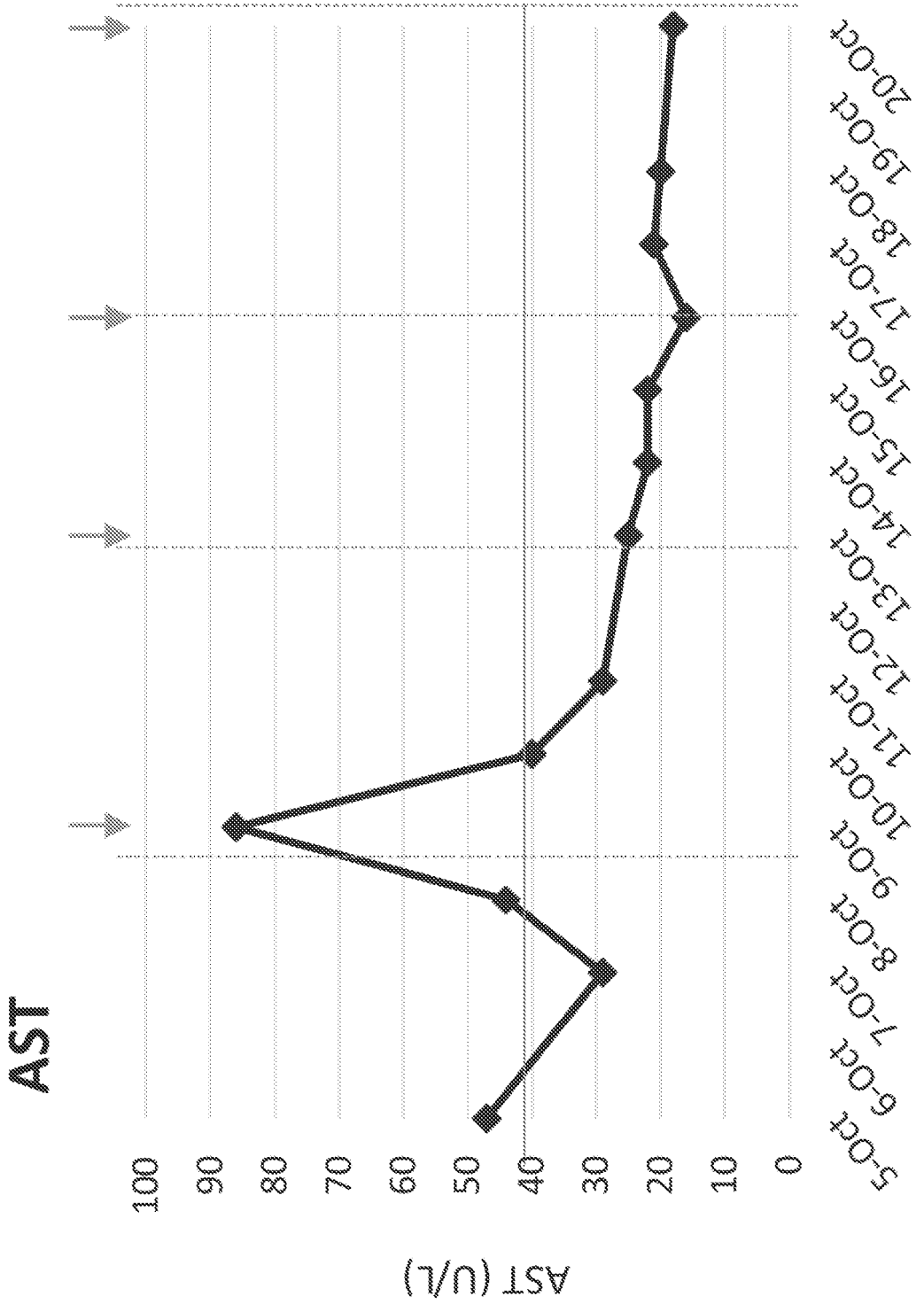

AST
AST (U/L)
100
90
80
70
60
50
40
30
20
10
0
5-Oct
6-Oct
7-Oct
8-Oct
9-Oct
10-Oct
11-Oct
12-Oct
13-Oct
14-Oct
15-Oct
16-Oct
17-Oct
18-Oct
19-Oct
20-Oct

FIG. 52C

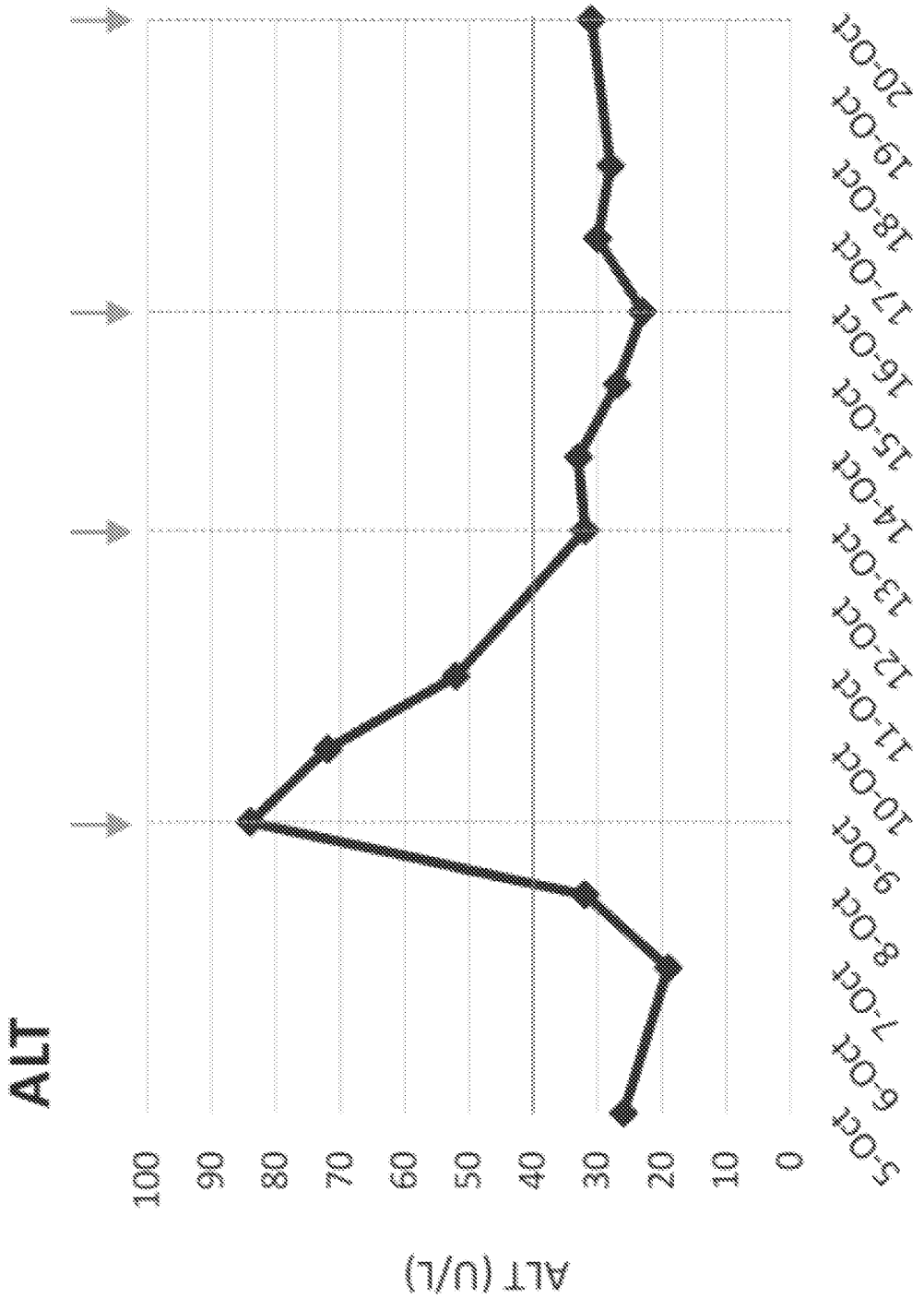

ALT
ALT (U/L)
100
90
80
70
60
50
40
30
20
10
0
5-Oct
9-Oct
7-Oct
8-Oct
9-Oct
10-Oct
11-Oct
12-Oct
13-Oct
14-Oct
15-Oct
16-Oct
17-Oct
18-Oct
19-Oct
20-Oct

FIG. 52D

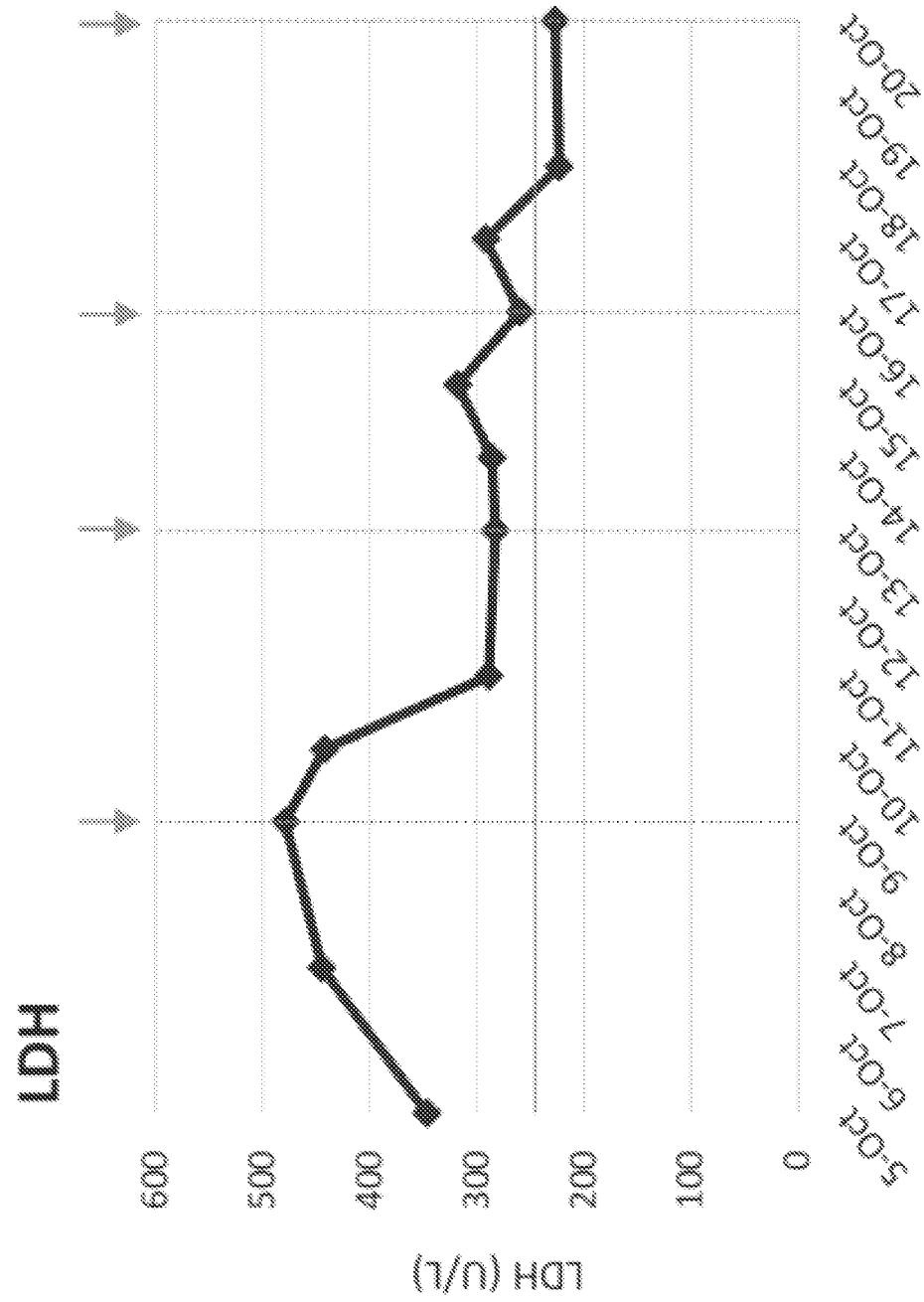

LDH
LDH (U/L)
600
500
400
300
200
100
0
-5
-9
-7
-8
-6
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20

FIG. 52E

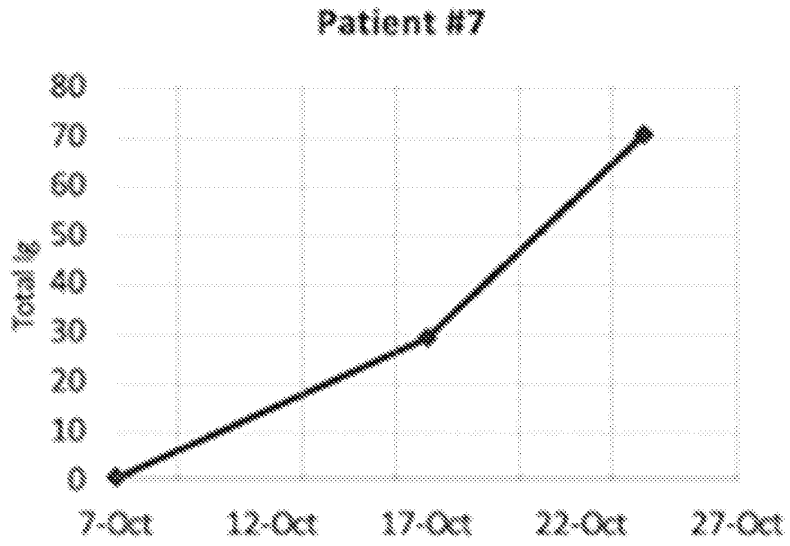

Patient #7
Total %
80
70
60
50
40
30
20
10
0
7-Oct
12-Oct
17-Oct
22-Oct
27-Oct

FIG. 53