FIG. 1A

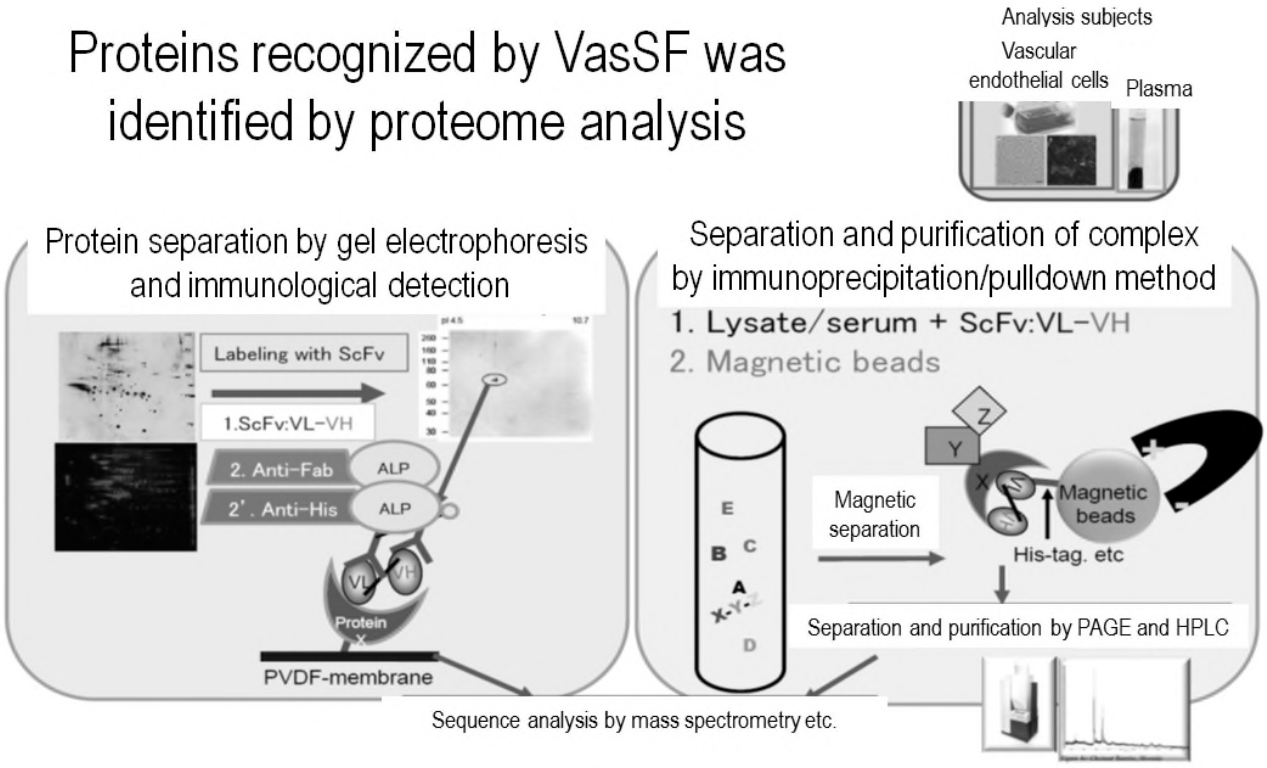

Proteins recognized by VasSF was identified by proteome analysis
Analysis subjects
Vascular endothelial cells
Plasma
Protein separation by gel electrophoresis and immunological detection
Labeling with ScFv
1.ScFv:VL-VH
2. Anti-Fab
ALP
2'. Anti-His
ALP
VL
VH
Protein X
PVDF-membrane
Separation and purification of complex by immunoprecipitation/pulldown method
1. Lysate/serum + ScFv:VL-VH
2. Magnetic beads
Magnetic separation
Magnetic beads
His-tag. etc
Separation and purification by PAGE and HPLC
Sequence analysis by mass spectrometry etc.
Identification of target protein by database search

FIG. 1B

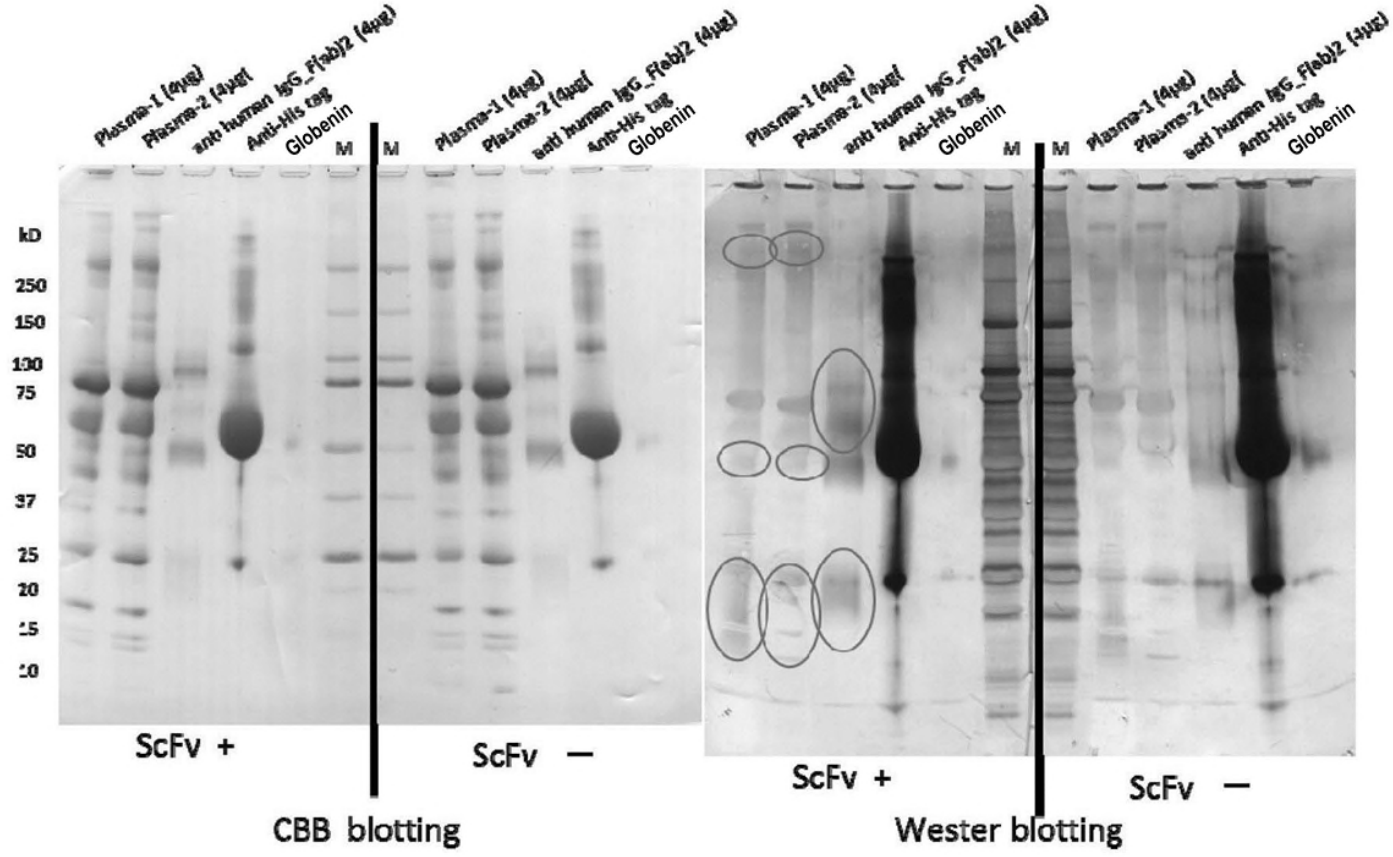

Plasma-1 (4μg)
Plasma-2 (4μg)
anti human IgG_F(ab)2 (4μg)
Anti-His tag
Globenin
M
kD
250
150
130
75
50
37
25
20
15
10
ScFv +
ScFv —
CBB blotting
Wester blotting

FIG. 1C

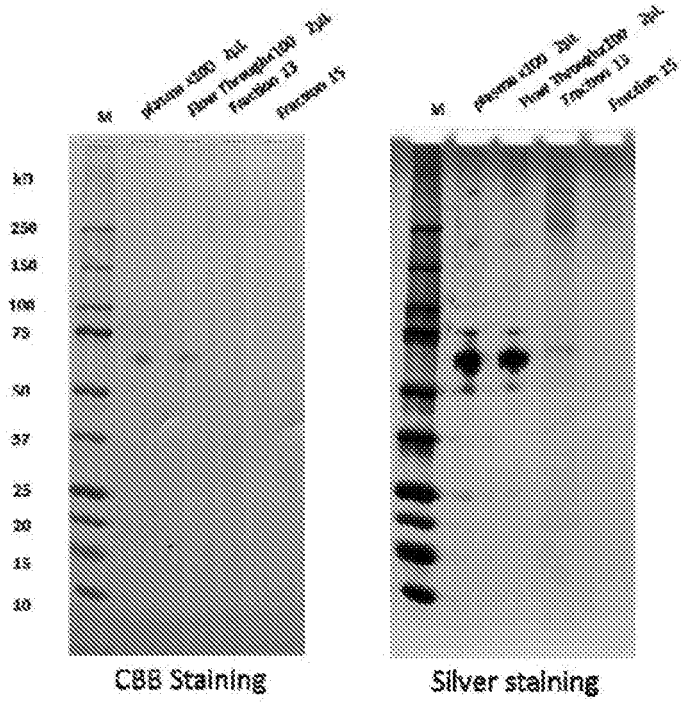

M
Plasma x500 4μL
Flow Through 13μL
Fraction 13
Fraction 15
kD
250
150
100
75
50
37
25
20
15
10
CBB Staining
Silver staining

FIG. 2

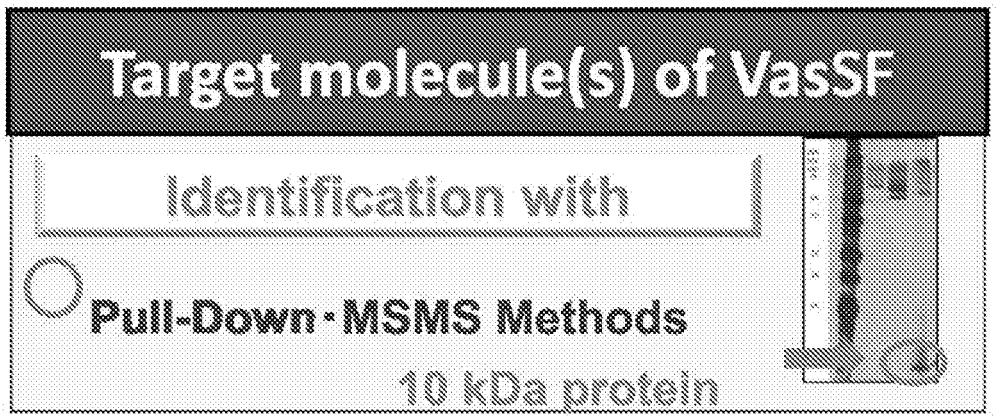
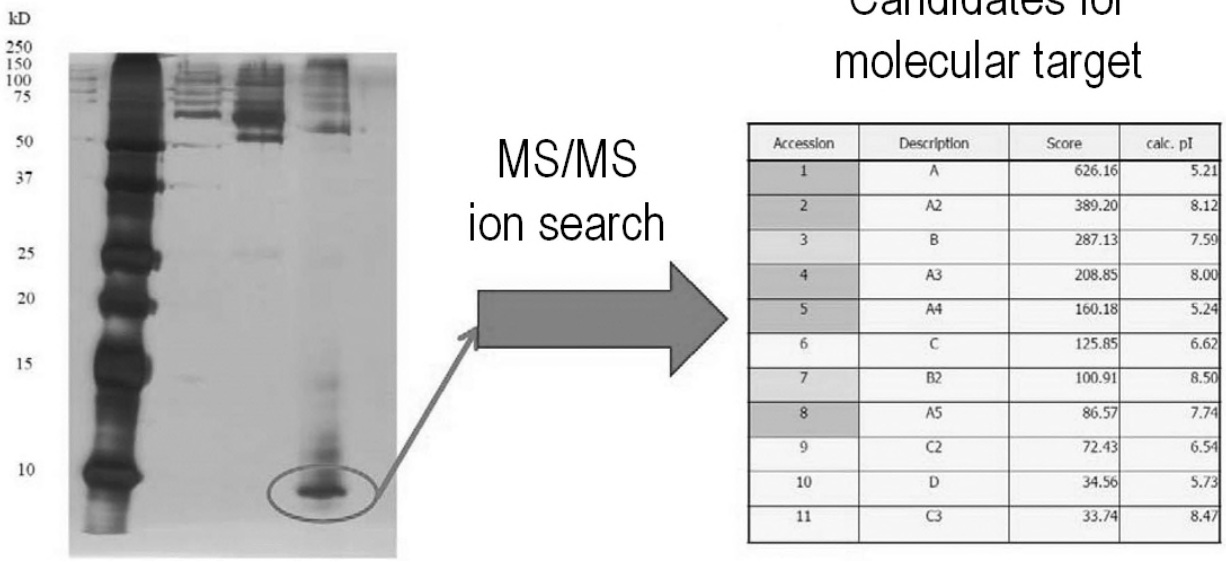

Target molecule(s) of VasSF
Identification with
Pull-Down・MSMS Methods
10 kDa protein

FIG. 3



Accession	Description	Score	calc. pI
1	A	626.16	5.21
2	A2	389.20	8.12
3	B	287.13	7.59
4	A3	208.85	8.00
5	A4	160.18	5.24
6	C	125.85	6.62
7	B2	100.91	8.50
8	A5	86.57	7.74
9	C2	72.43	6.54
10	D	34.56	5.73
11	C3	33.74	8.47

FIG. 4

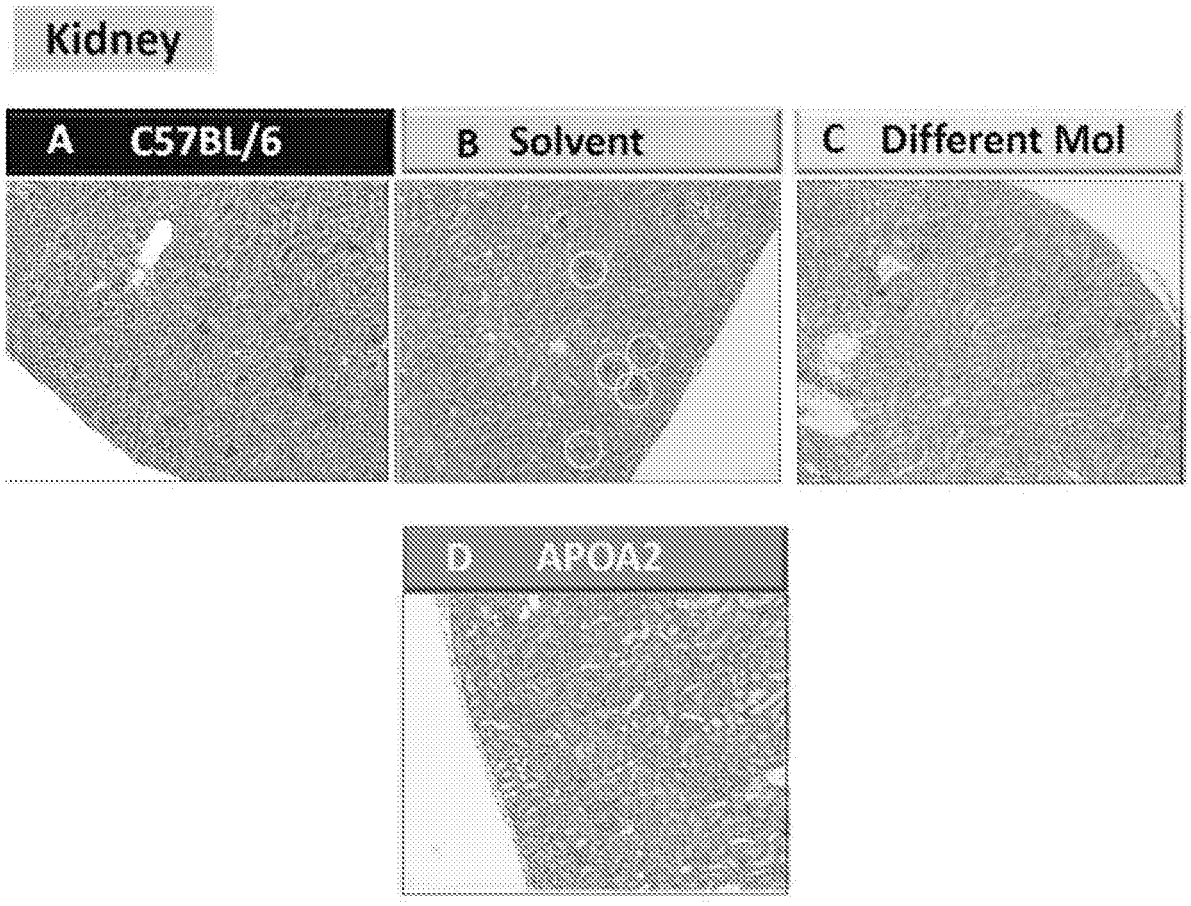


FIG. 5

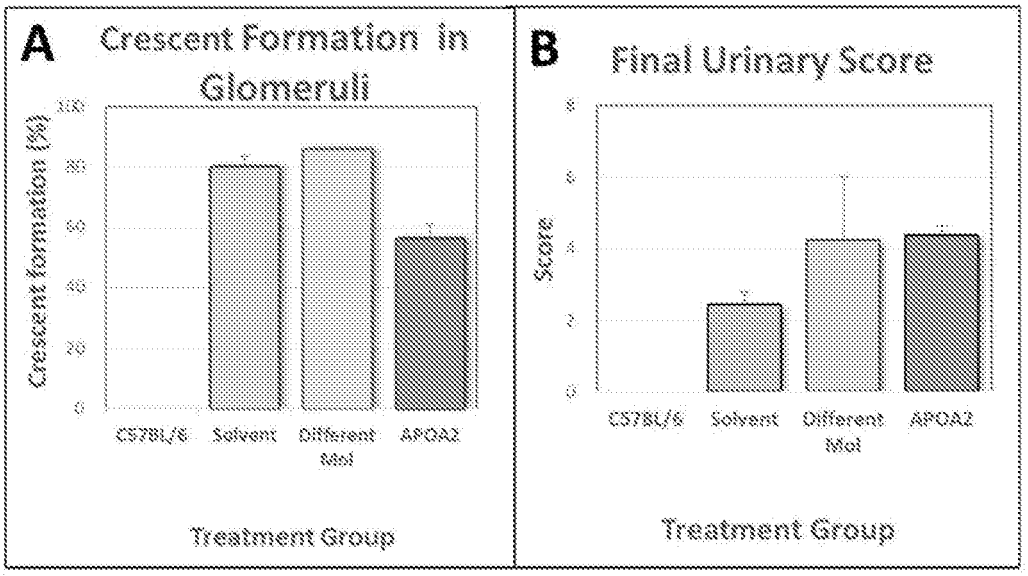

A
Crescent Formation in Glomeruli
Crescent formation (%)
100
80
60
40
20
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group
B
Final Urinary Score
Score
8
6
4
2
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group

FIG. 6

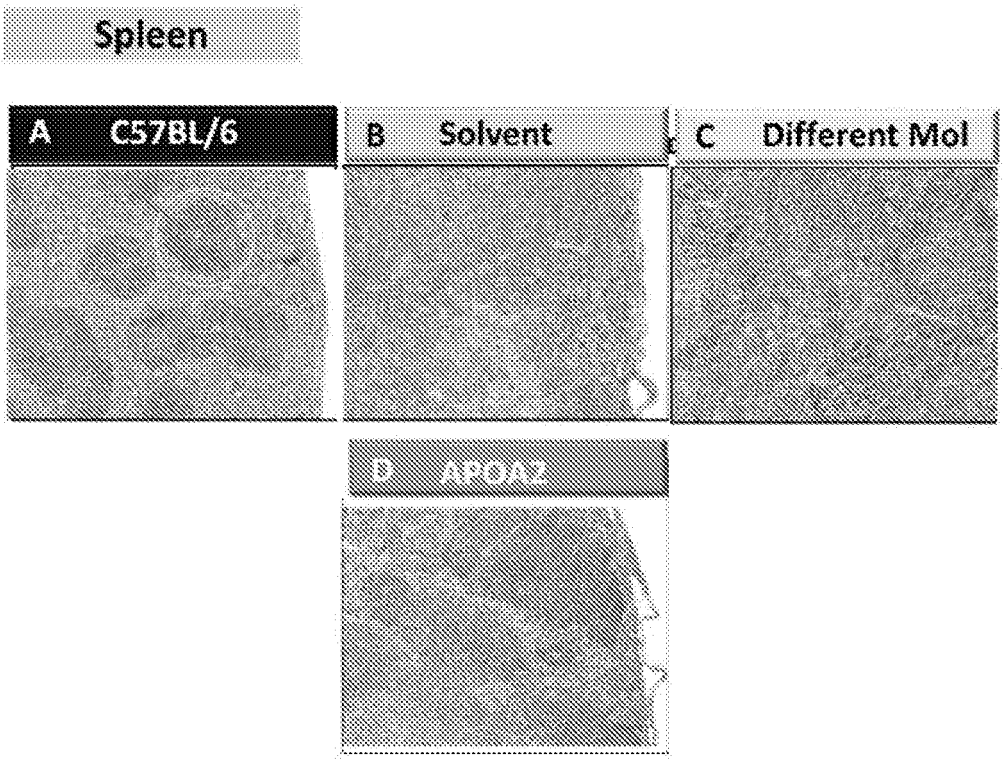

Spleen
A C57BL/6
B Solvent
C Different Mol
D APOA2

FIG. 7

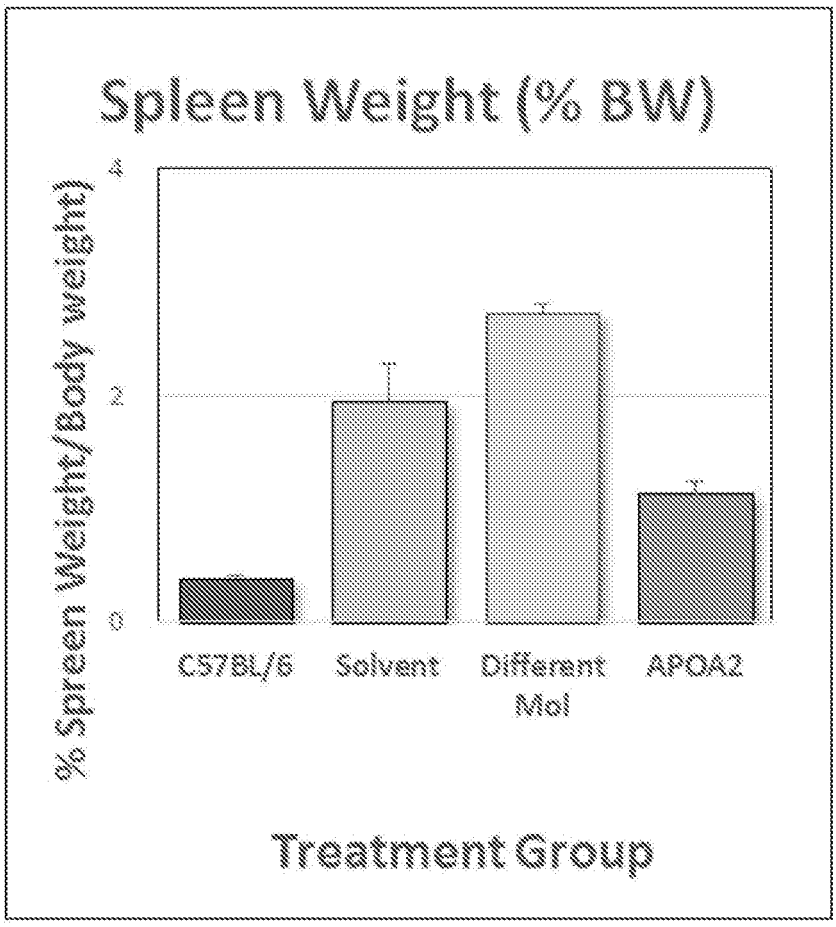

Spleen Weight (% BW)
% Spleen Weight/Body weight)
4
2
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group

FIG. 8

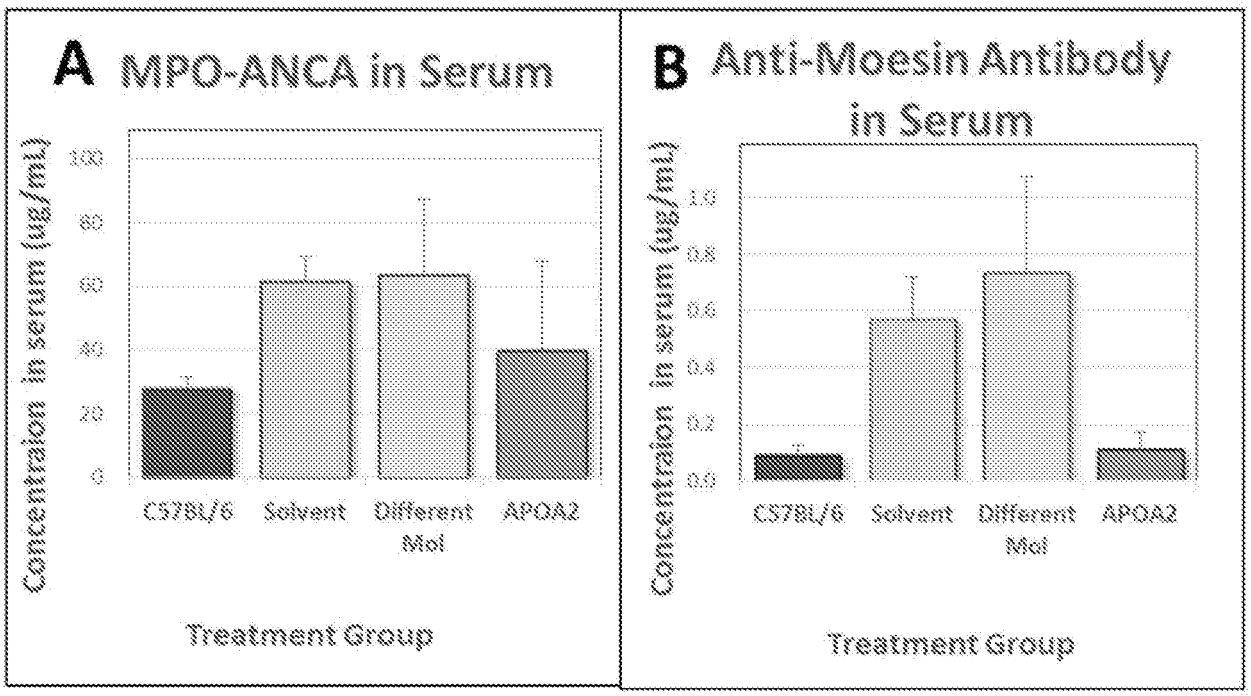

A MPO-ANCA in Serum
Concentraion in serum (ug/mL)
100
80
60
40
20
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group
B Anti-Moesin Antibody in Serum
Concentraion in serum (ug/mL)
1.0
0.8
0.6
0.4
0.2
0.0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group

FIG. 9

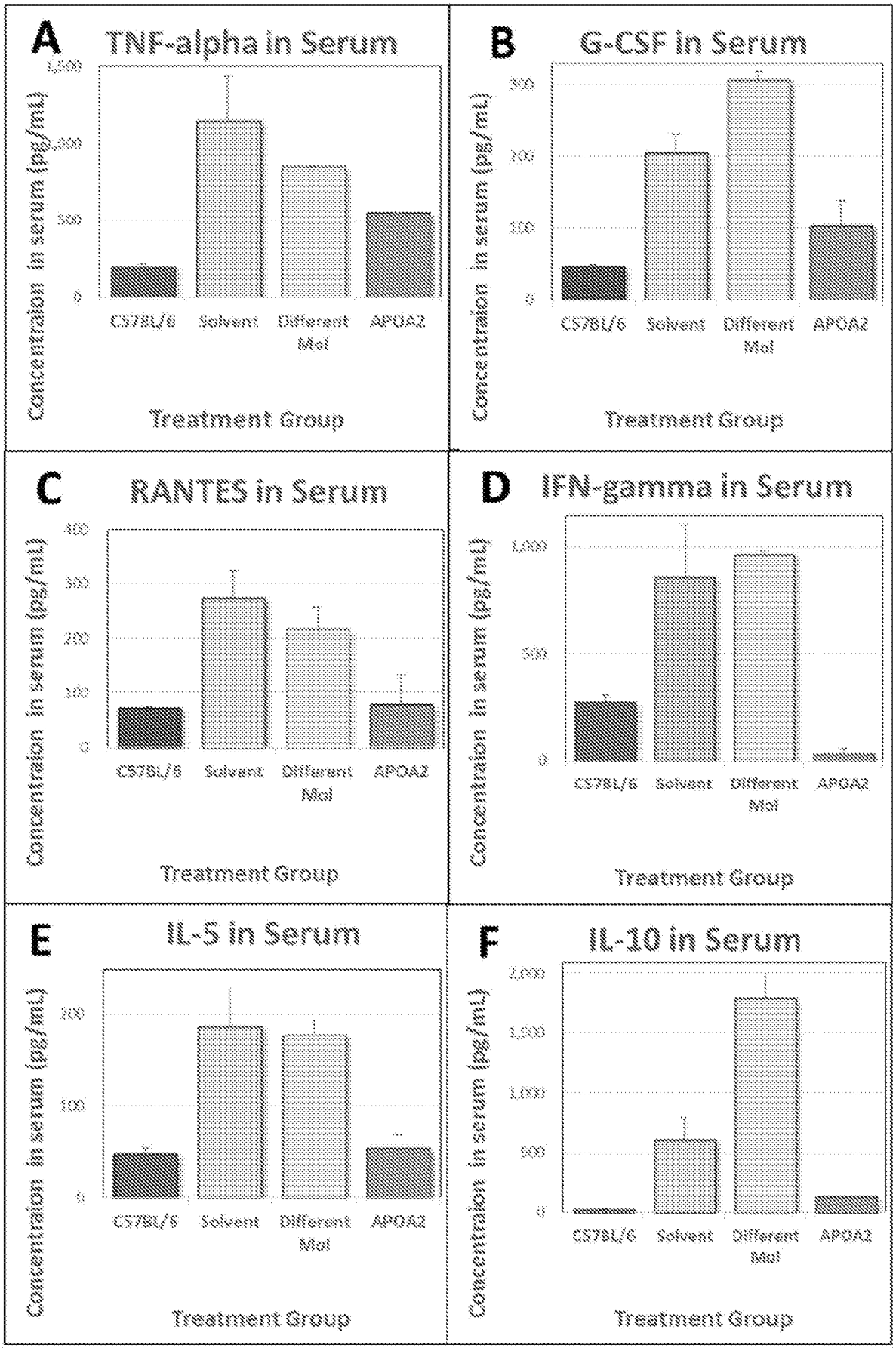

A
TNF-alpha in Serum
Concentration in serum (pg/mL)
1,500
1,000
500
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group
B
G-CSF in Serum
Concentration in serum (pg/mL)
300
200
100
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group
C
RANTES in Serum
Concentration in serum (pg/mL)
400
300
200
100
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group
D
IFN-gamma in Serum
Concentration in serum (pg/mL)
1,000
500
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group
E
IL-5 in Serum
Concentration in serum (pg/mL)
200
100
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group
F
IL-10 in Serum
Concentration in serum (pg/mL)
2,000
1,500
1,000
500
0
C57BL/6
Solvent
Different Mol
APOA2
Treatment Group

FIG. 10

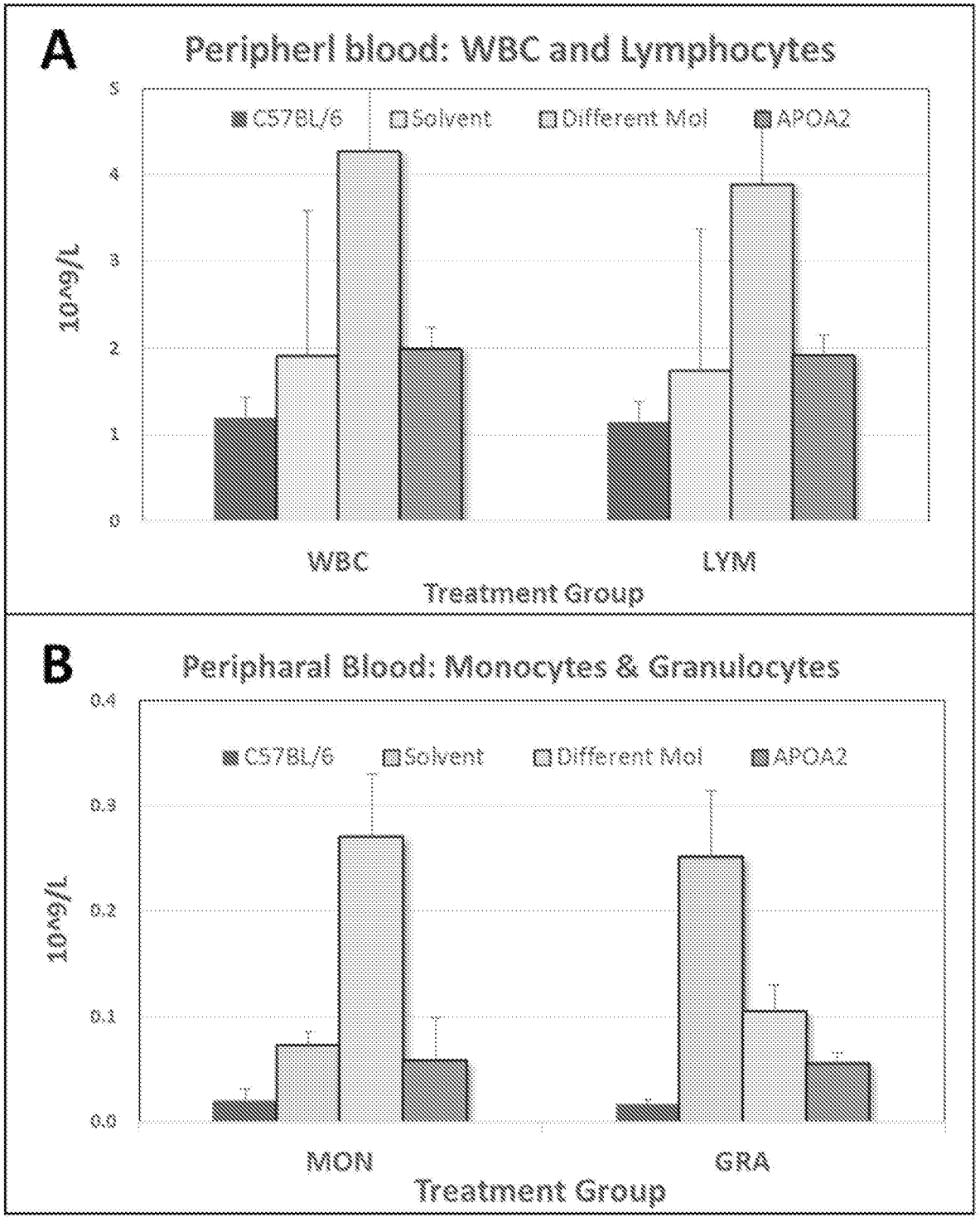

A
Peripherl blood: WBC and Lymphocytes
C57BL/6
Solvent
Different Mol
APOA2
10^9/L
0
1
2
3
4
5
WBC
LYM
Treatment Group
B
Peripharal Blood: Monocytes & Granulocytes
C57BL/6
Solvent
Different Mol
APOA2
10^9/L
0.0
0.1
0.2
0.3
0.4
MON
GRA
Treatment Group

FIG. 11

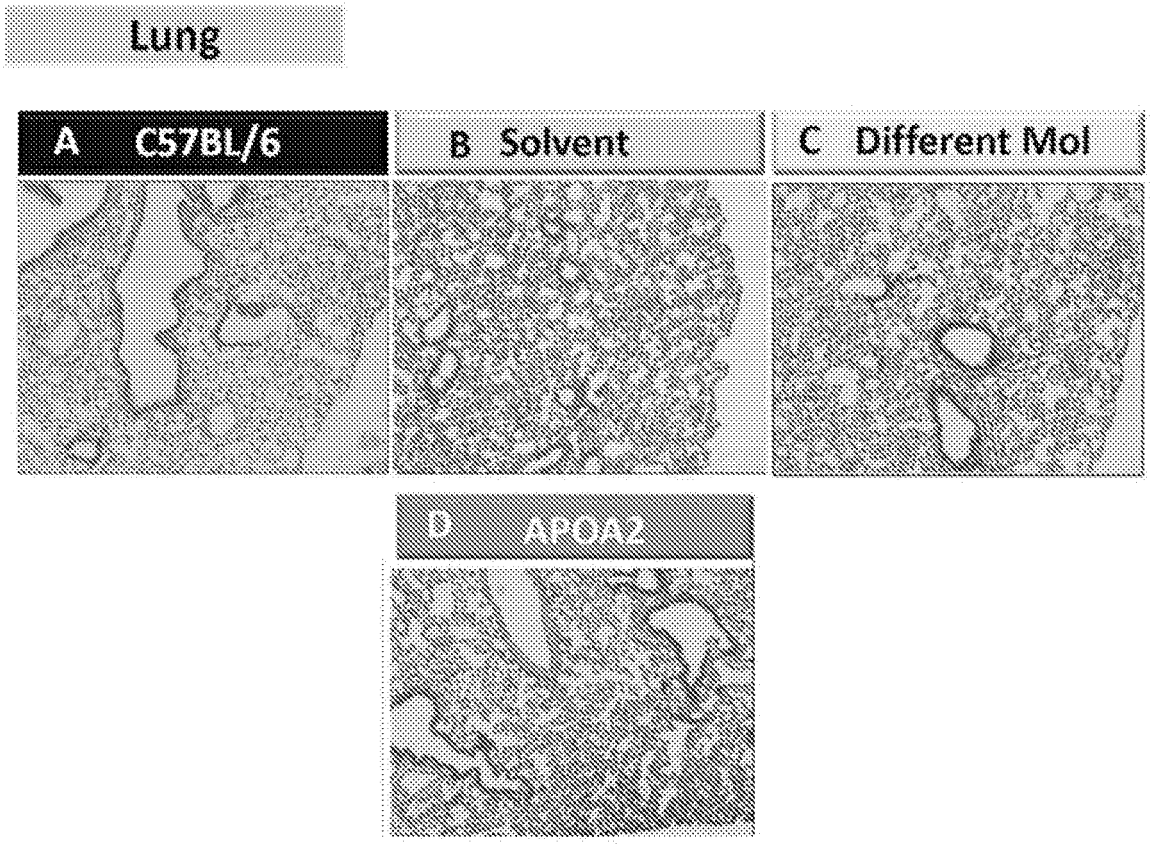

Lung
A C57BL/6
B Solvent
C Different Mol
D APOA2

FIG. 12

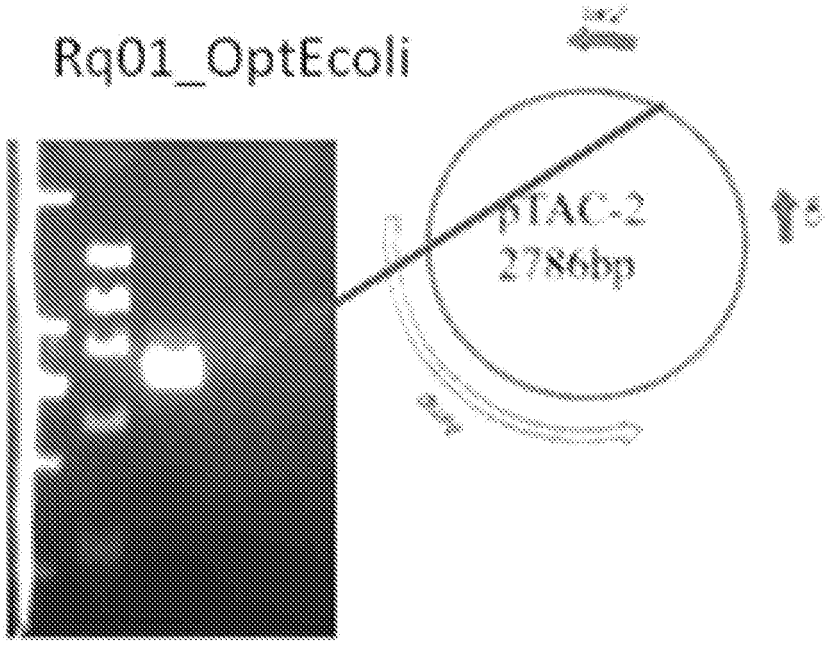

Rq01_OptEcoli
pTAC-2
2786bp

FIG. 13

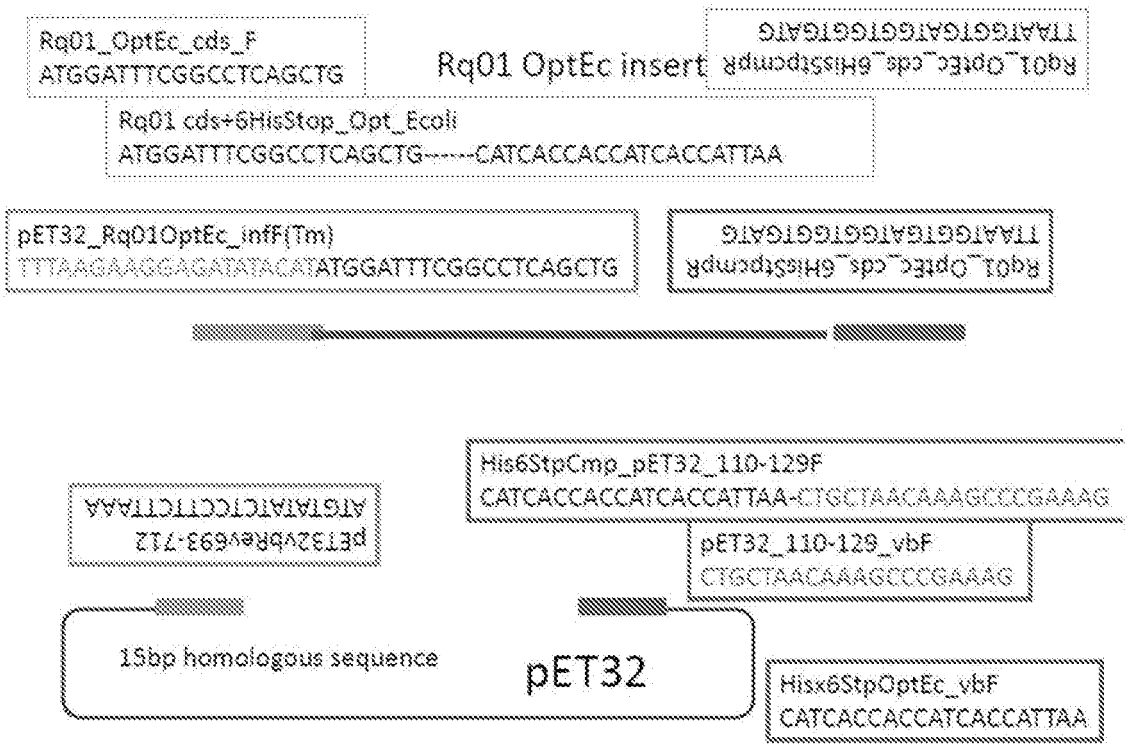

Rq01_OptEc_cds_F
ATGGATTTCGGCCTCAGCTG
Rq01 OptEc insert
Rq01 cds+6HisStop_Opt_Ecoli
ATGGATTTCGGCCTCAGCTG-----CATCACCACCATCACCATTAA
pET32_Rq01OptEc_infF(Tm)
TTTAAGAAGGAGATATACATATGGATTTCGGCCTCAGCTG
His6StpCmp_pET32_110-129F
CATCACCACCATCACCATTAA-CTGCTAACAAAGCCCGAAAG
pET32_110-129_vbF
CTGCTAACAAAGCCCGAAAG
15bp homologous sequence
pET32
Hisx6StpOptEc_vbF
CATCACCACCATCACCATTAA

FIG. 14

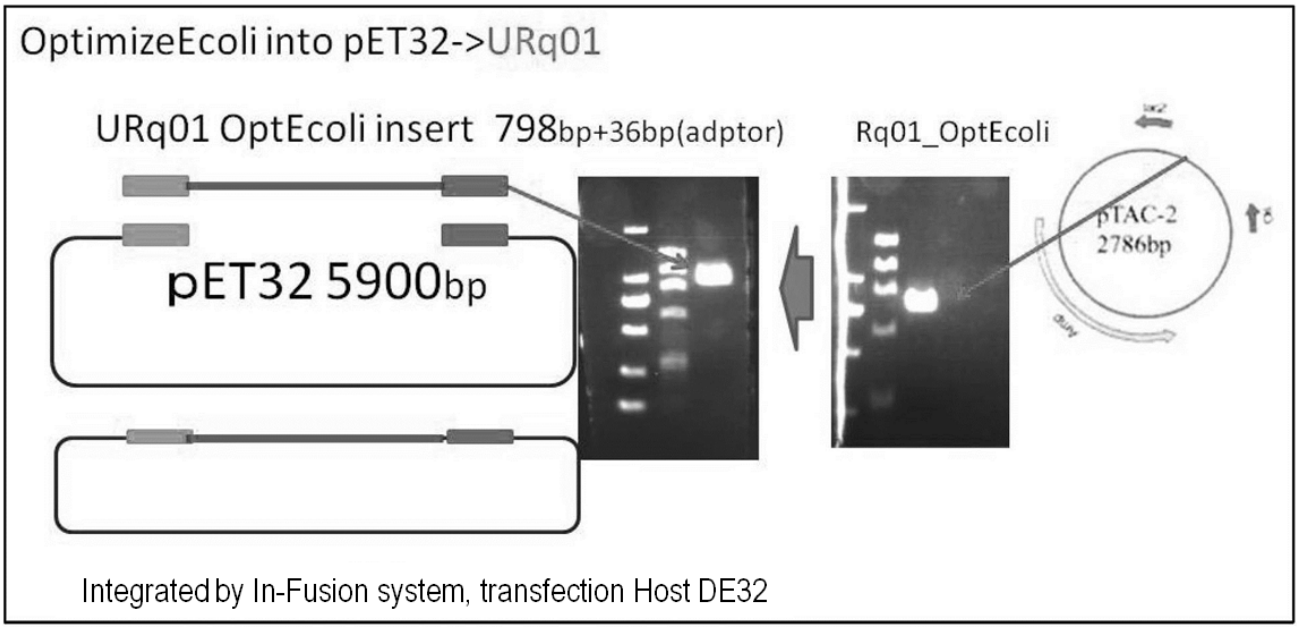

OptimizeEcoli into pET32->URq01
URq01 OptEcoli insert 798bp+36bp(adptor)
Rq01_OptEcoli
pET32 5900bp
pTAC-2
2786bp
Integrated by In-Fusion system, transfection Host DE32

FIG. 15

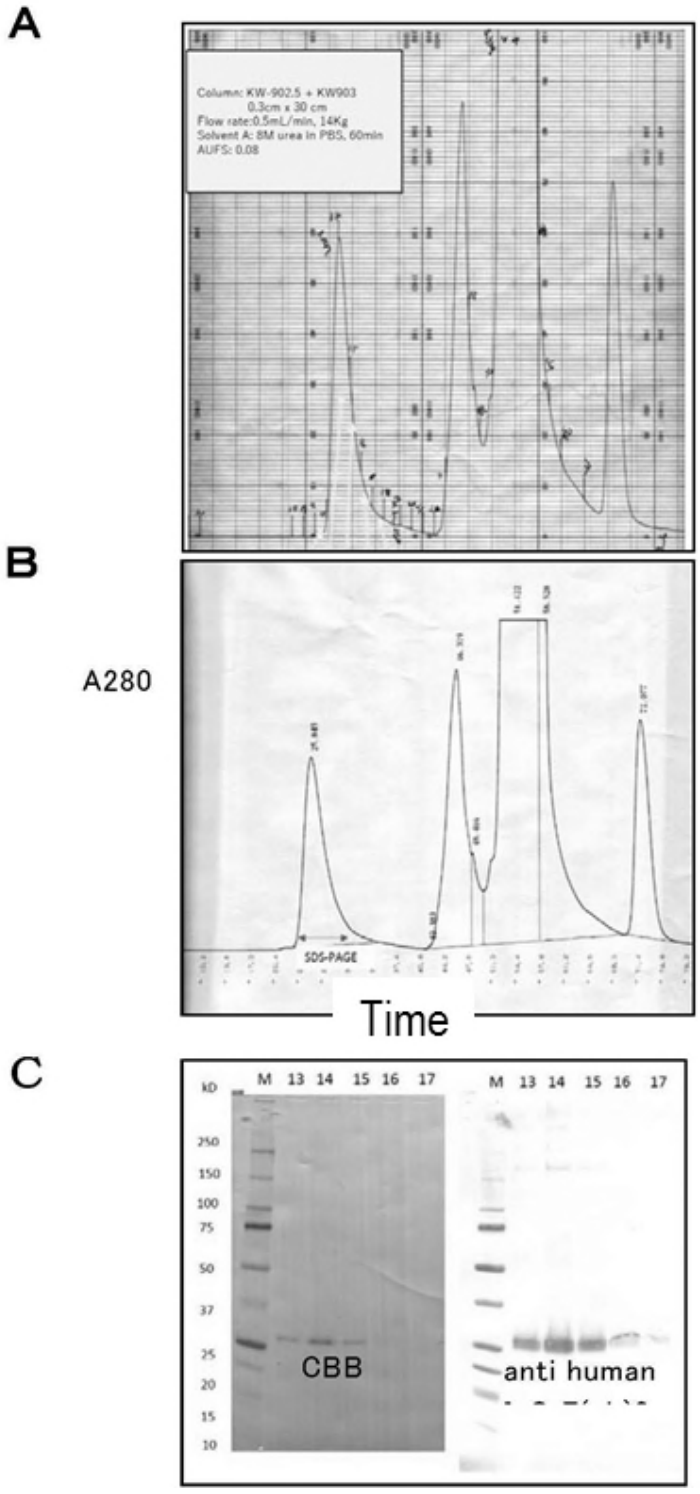

A
Column: KW-902.5 + KW903
0.3cm x 30 cm
Flow rate:0.5mL/min, 14Kg
Solvent A: 8M urea in PBS, 60min
AUFS: 0.08
B
A280
SDS-PAGE
Time
C
kD
M 13 14 15 16 17
250
150
100
75
50
37
25
20
15
10
CBB
M 13 14 15 16 17
anti human

FIG. 16

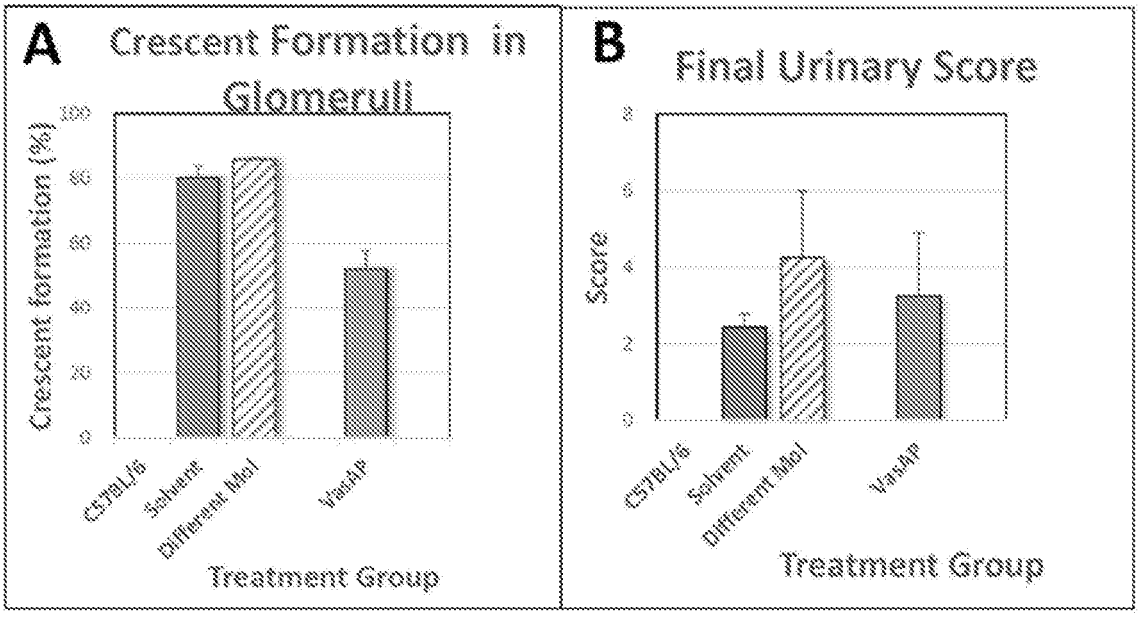

A
Crescent Formation in Glomeruli
Crescent formation (%)
100
80
60
40
20
0
C57BL/6
Solvent
Different Mol
VasAP
Treatment Group
B
Final Urinary Score
Score
8
6
4
2
0
C57BL/6
Solvent
Different Mol
VasAP
Treatment Group

FIG. 17

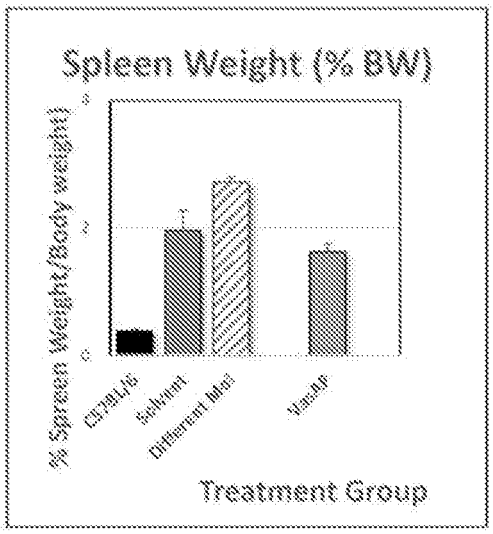

Spleen Weight (% BW)
% Spleen Weight/Body weight)
3
2
1
0
C57BL/6
Solvent
Different Mol
VasAP
Treatment Group

FIG. 18

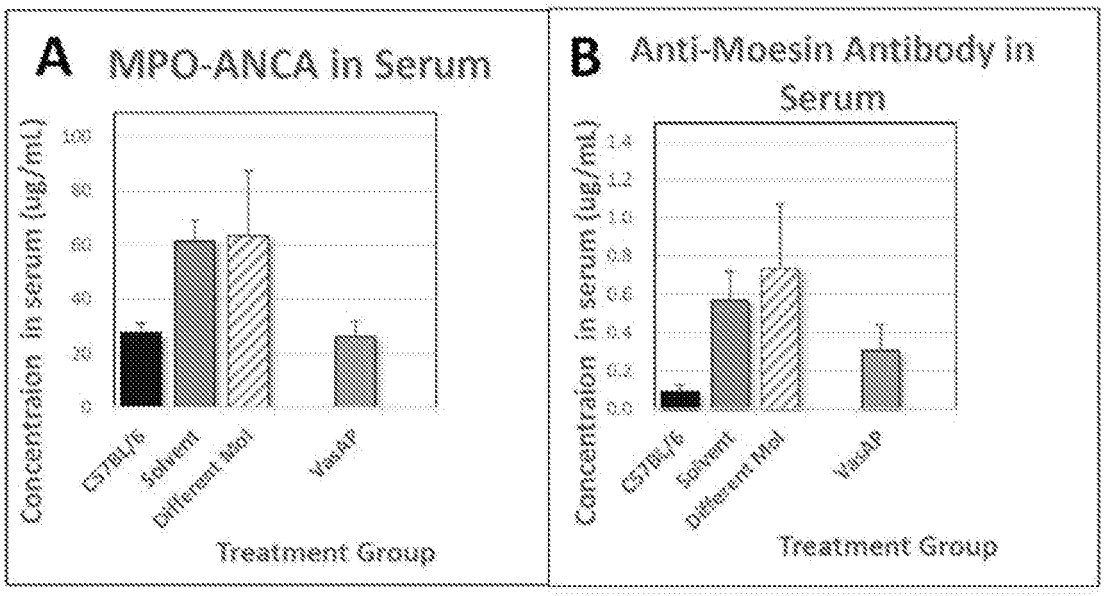

A
MPO-ANCA in Serum
Concentration in serum (ug/mL)
100
80
60
40
20
0
C57BL/6
Solvent
Different Mod
VasAP
Treatment Group
B
Anti-Moesin Antibody in Serum
Concentration in serum (ug/mL)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C57BL/6
Solvent
Different Mod
VasAP
Treatment Group

FIG. 19

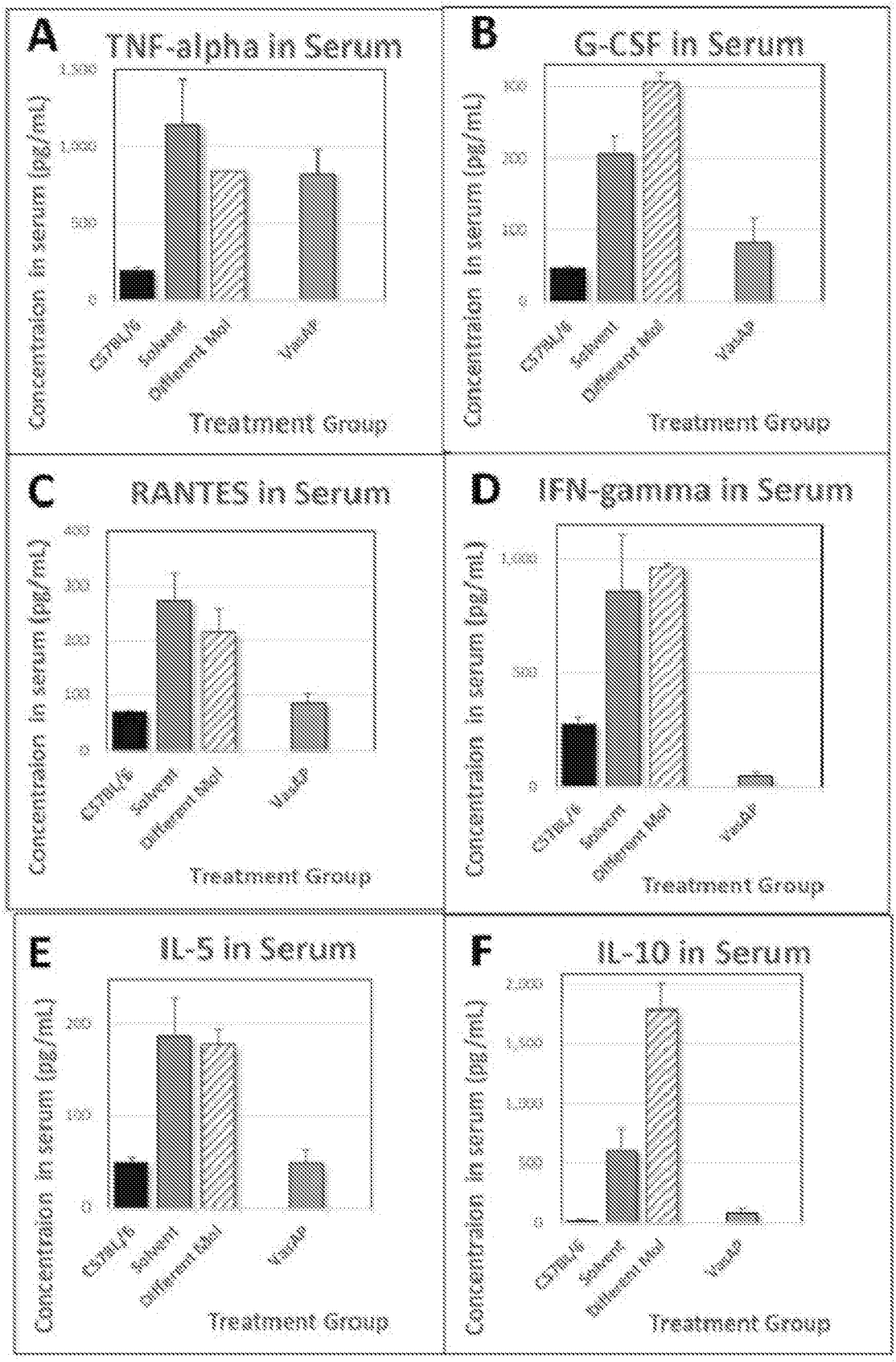

A
TNF-alpha in Serum
Concentraion in serum (pg/mL)
Treatment Group
B
G-CSF in Serum
Concentraion in serum (pg/mL)
Treatment Group
C
RANTES in Serum
Concentraion in serum (pg/mL)
Treatment Group
D
IFN-gamma in Serum
Concentraion in serum (pg/mL)
Treatment Group
E
IL-5 in Serum
Concentraion in serum (pg/mL)
Treatment Group
F
IL-10 in Serum
Concentraion in serum (pg/mL)
Treatment Group

FIG. 20

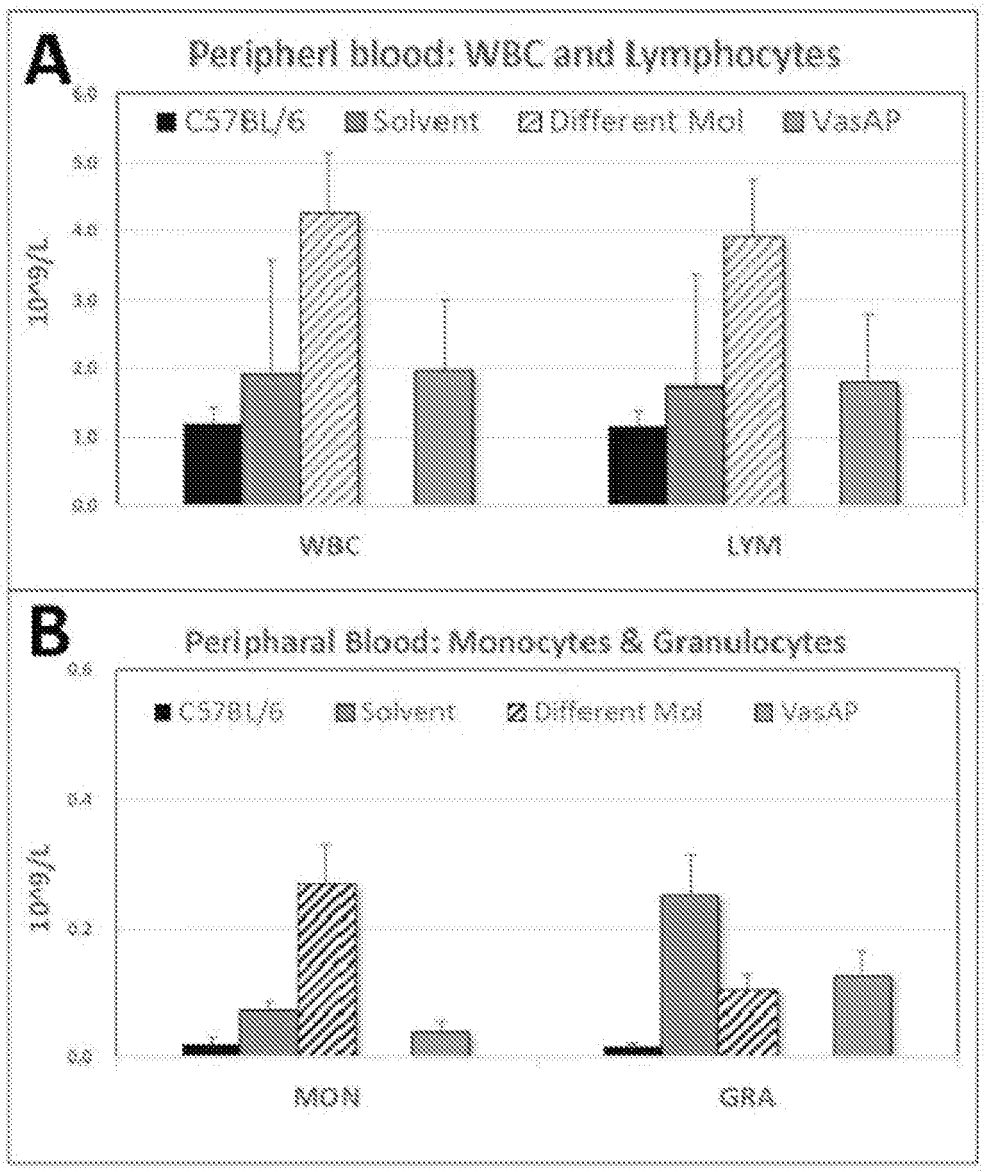

A
Peripherl blood: WBC and Lymphocytes
C57BL/6
Solvent
Different Mol
VasAP
10^9/L
WBC
LYM
B
Peripharal Blood: Monocytes & Granulocytes
C57BL/6
Solvent
Different Mol
VasAP
10^9/L
MON
GRA

FIG. 21

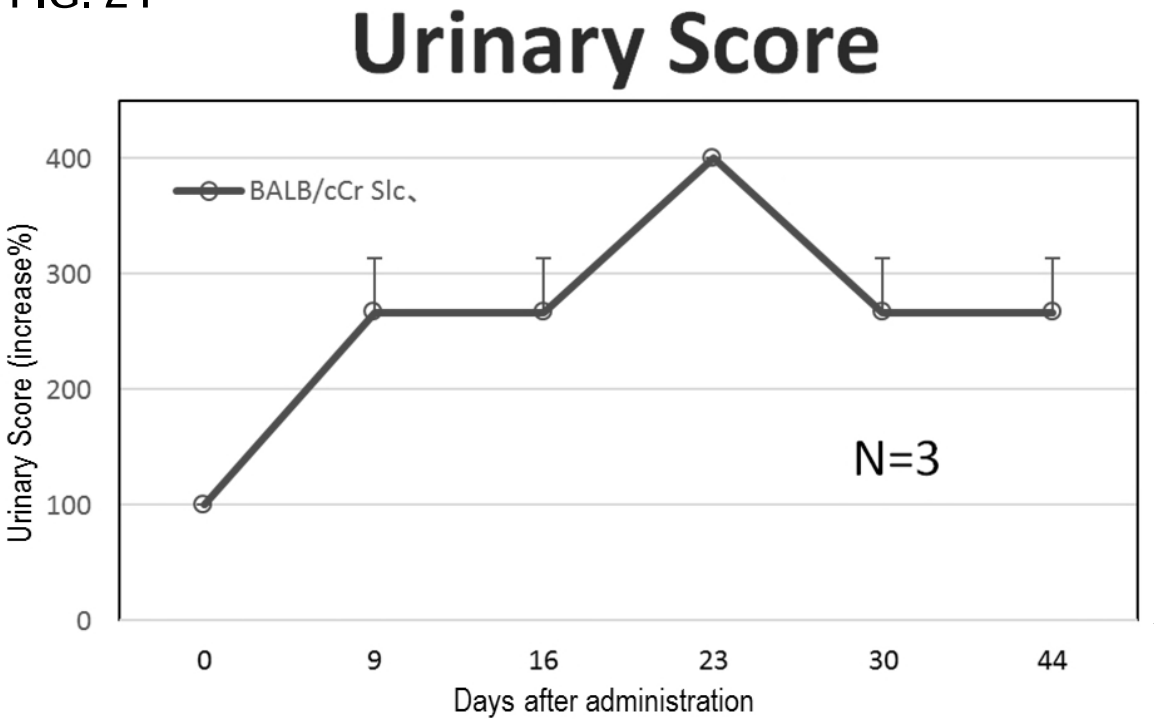

Urinary Score
BALB/cCr Slc、
Urinary Score (increase%)
400
300
200
100
0
N=3
0
9
16
23
30
44
Days after administration

FIG. 22

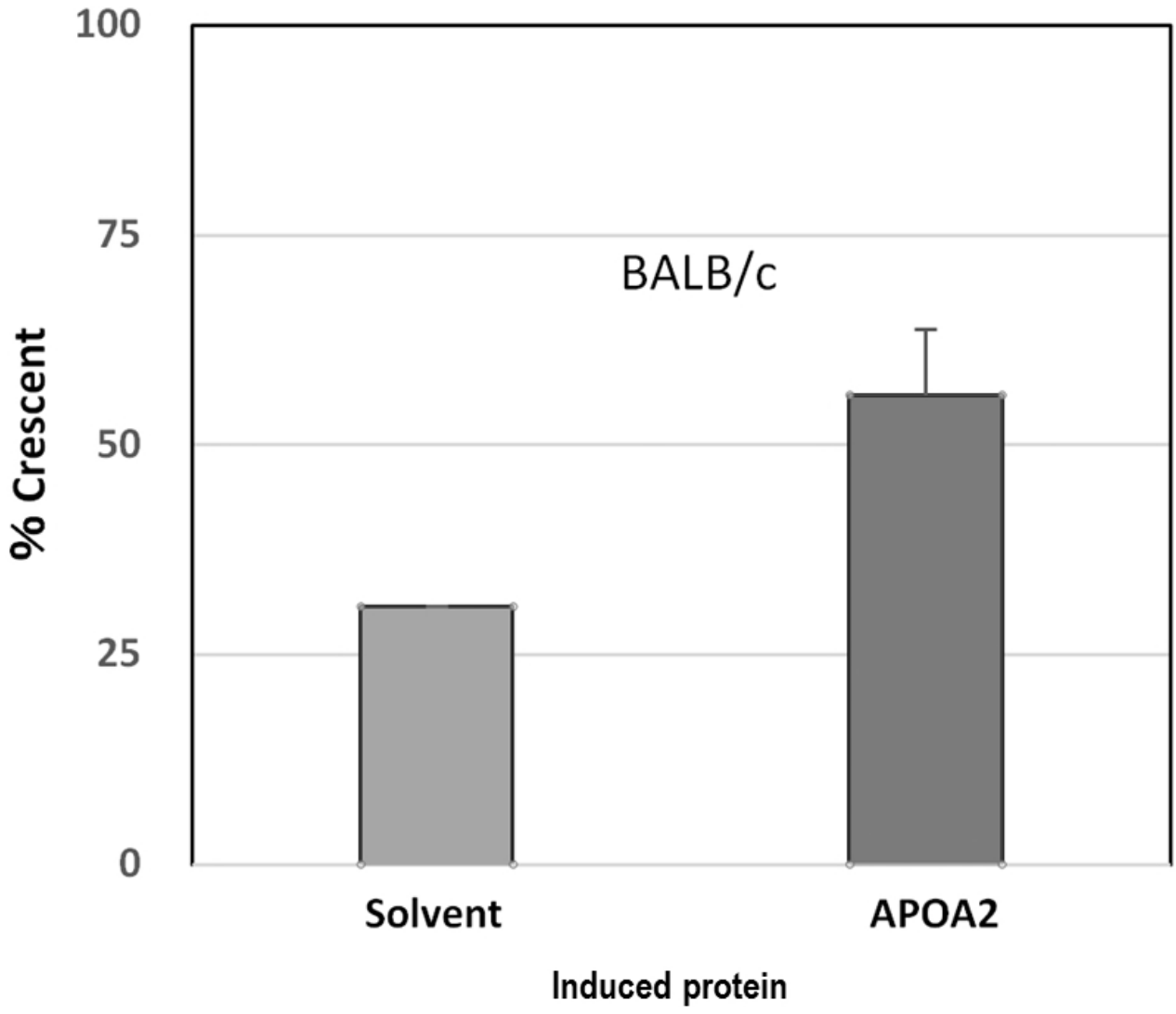

100
75
50
25
0
% Crescent
BALB/c
Solvent
APOA2
Induced protein

FIG. 23

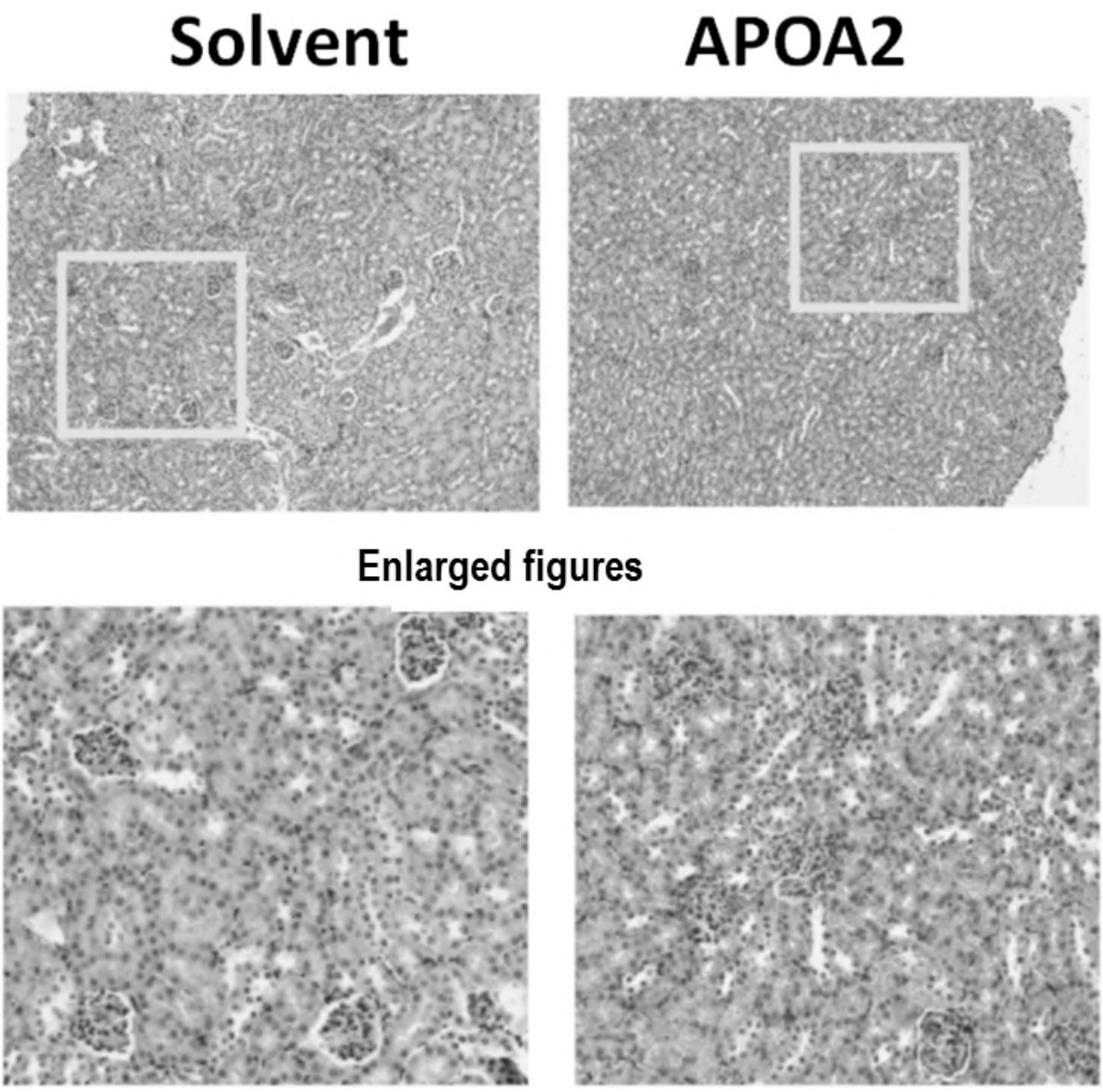


FIG. 24

