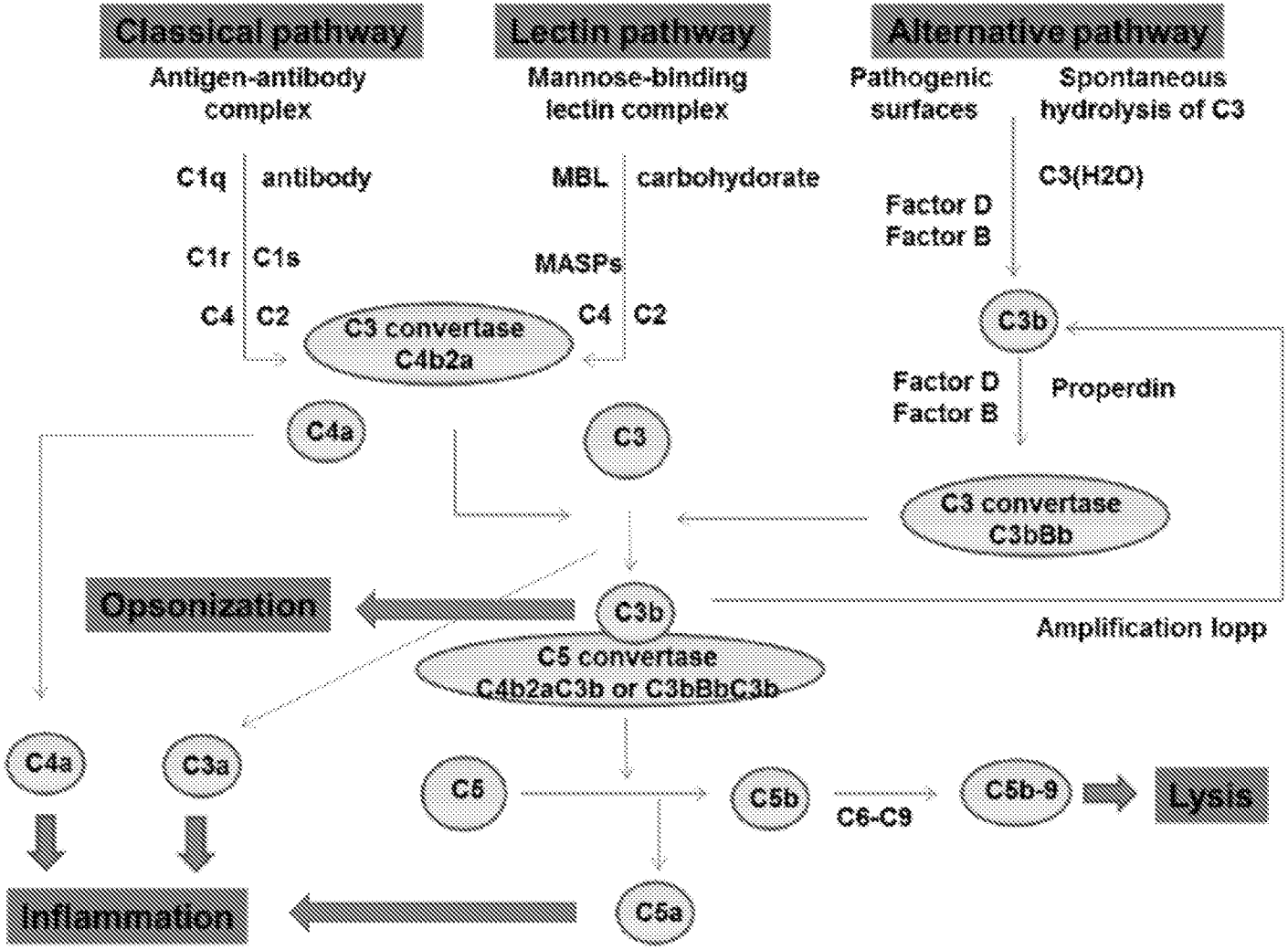

Classical pathway
Antigen-antibody complex
Lectin pathway
Mannose-binding lectin complex
Alternative pathway
Pathogenic surfaces
Spontaneous hydrolysis of C3
C1q
antibody
MBL
carbohydorate
C3(H2O)
Factor D
Factor B
C1r
C1s
MASPs
C4
C2
C4
C2
C3 convertase C4b2a
C3b
Factor D
Factor B
Properdin
C4a
C3
C3 convertase C3bBb
Opsonization
C3b
Amplification lopp
C5 convertase C4b2aC3b or C3bBbC3b
C4a
C3a
C6
C5b
C6-C9
C5b-9
Lysis
Inflammation
C5a

Figure 1

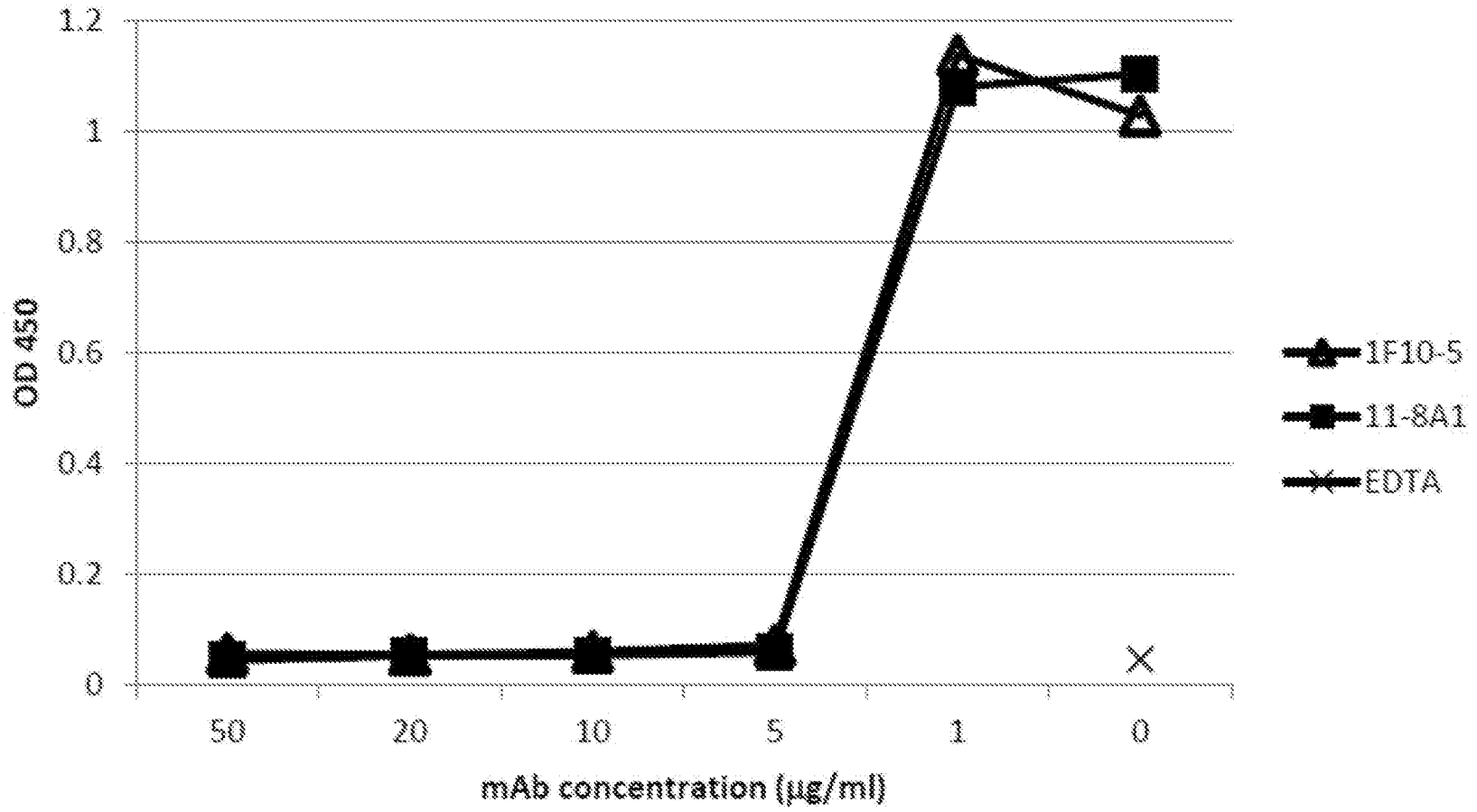

1.2
1
0.8
0.6
0.4
0.2
0
OD 450
50
20
10
5
1
0
mAb concentration (µg/ml)
1F10-5
11-8A1
EDTA

Figure 2

CTCAAGGTCCTTACAatgaaatgcagctgggttatcgtcttcctgatggcagtggttaca

L K V L T M K C S W V I V F L M A V V T

ggggtcaattcagaggttcagctgcagcagtctggggcagaccttgtgaggccagggggcc

G V N S E V Q L Q Q S G A D L V R P G A

tcagtcaagttgtcctgcacaacttctggcttcaacattaaagacacctatgtgcactgg

S V K L S C T T S G **F N I K D T Y V H** W CDR1 **(SEQ ID NO: 3)**

gtgaaacagaggcctgaacagggcctggaatggattggaaggattgatcctgcgaatggt

V K Q R P E Q G L E **W I G R I D P A N G** CDR2 **(SEQ ID NO: 4)**

cttactacatttgatccgaggttccaggccaaggccactataacagcagacacatcctcc

L T T F D P R F Q A K A T I T A D T S S

aataccgcctacctgcagctcagcagcctgacatctgaggacactgccgtctattactgt

N T A Y L Q L S S L T S E D T A V Y Y C

acatatgctatggaatattggggtcaaggaacctcagtcaccgtctcctcagccaaaacg

T Y A M E Y W G Q G T S V T V S S A K T CDR3 **(SEQ ID NO: 5)**

acaccccatctgtctatccactggcccc **(SEQ ID NO: 1)**

T P P S V Y P L A **(SEQ ID NO: 2)**

Figure 3A

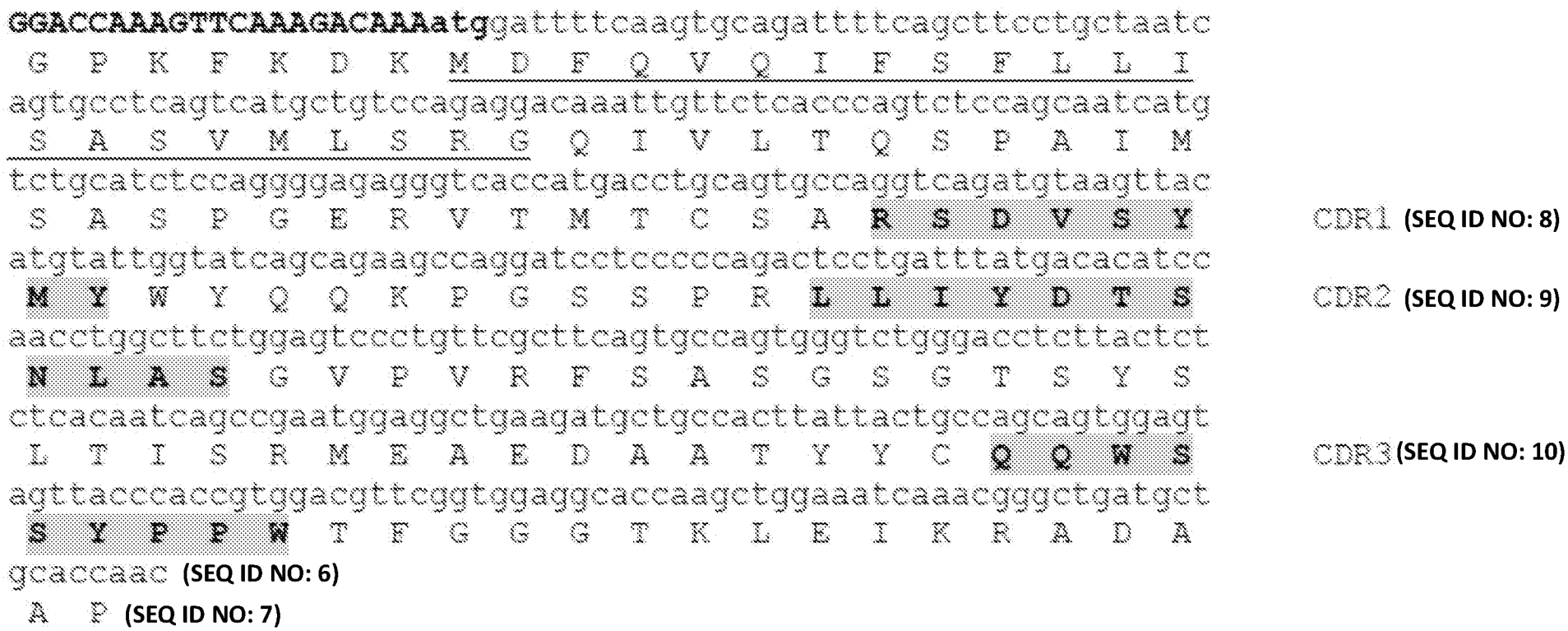

GGACCAAAGTTCAAAGACAAAatggattttcaagtgcagattttcagcttcctgctaatc
G P K F K D K M D F Q V Q I F S F L L I
agtgcctcagtcatgctgtccagaggacaaattgttctcacccagtctccagcaatcatg
S A S V M L S R G Q I V L T Q S P A I M
tctgcatctccaggggagagggtcaccatgacctgcagtgccaggtcagatgtaagttac
S A S P G E R V T M T C S A R S D V S Y
CDR1 (SEQ ID NO: 8)
atgtattggtatcagcagaagccaggatcctcccccagactcctgatttatgacacatcc
M Y W Y Q Q K P G S S P R L L I Y D T S
CDR2 (SEQ ID NO: 9)
aacctggcttctggagtccctgttcgcttcagtgccagtgggtctgggacctcttactct
N L A S G V P V R F S A S G S G T S Y S
ctcacaatcagccgaatggaggctgaagatgctgccacttattactgccagcagtggagt
L T I S R M E A E D A A T Y Y C Q Q W S
CDR3 (SEQ ID NO: 10)
agttacccaccgtggacgttcggtggaggcaccaagctggaaatcaaacgggctgatgct
S Y P P W T F G G G T K L E I K R A D A
gcaccaac (SEQ ID NO: 6)
A P (SEQ ID NO: 7)

Figure 3B

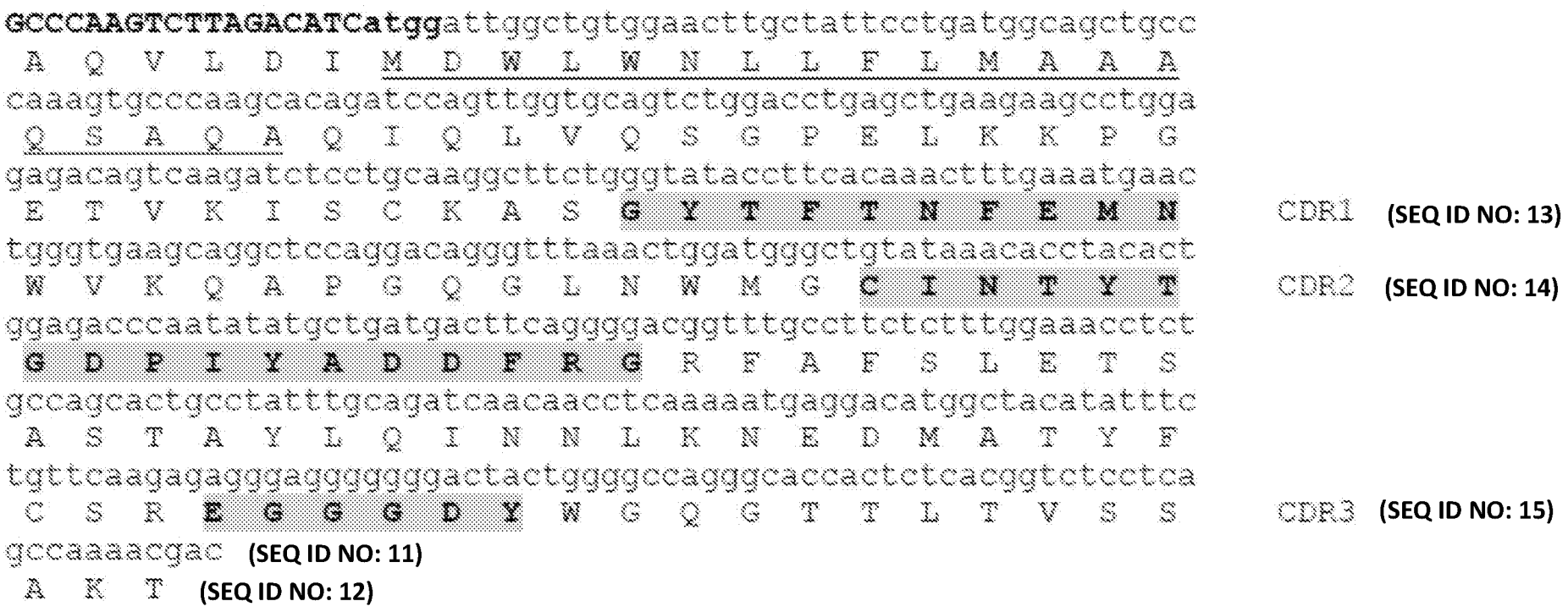

GCCCAAGTCTTAGACATCatggattggctgtggaacttgctattcctgatggcagctgcc
A Q V L D I M D W L W N L L F L M A A A
caaagtgcccaagcacagatccagttggtgcagtctggacctgagctgaagaagcctgga
Q S A Q A Q I Q L V Q S G P E L K K P G
gagacagtcaagatctcctgcaaggcttctgggtataccttcacaaactttgaaatgaac
E T V K I S C K A S G Y T F T N F E M N
CDR1 (SEQ ID NO: 13)
tgggtgaagcaggctccaggacagggtttaaactggatgggctgtataaacacctacact
W V K Q A P G Q G L N W M G C I N T Y T
CDR2 (SEQ ID NO: 14)
ggagacccaatatatgctgatgacttcaggggacggtttgccttctctttggaaacctct
G D P I Y A D D F R G R F A F S L E T S
gccagcactgcctatttgcagatcaacaacctcaaaaaatgaggacatggctacatatttc
A S T A Y L Q I N N L K N E D M A T Y F
tgttcaagagagggagggggggactactgggggccagggcaccactctcacggtctcctca
C S R E G G G D Y W G Q G T T L T V S S
CDR3 (SEQ ID NO: 15)
gccaaaacgac (SEQ ID NO: 11)
A K T (SEQ ID NO: 12)

Figure 4A

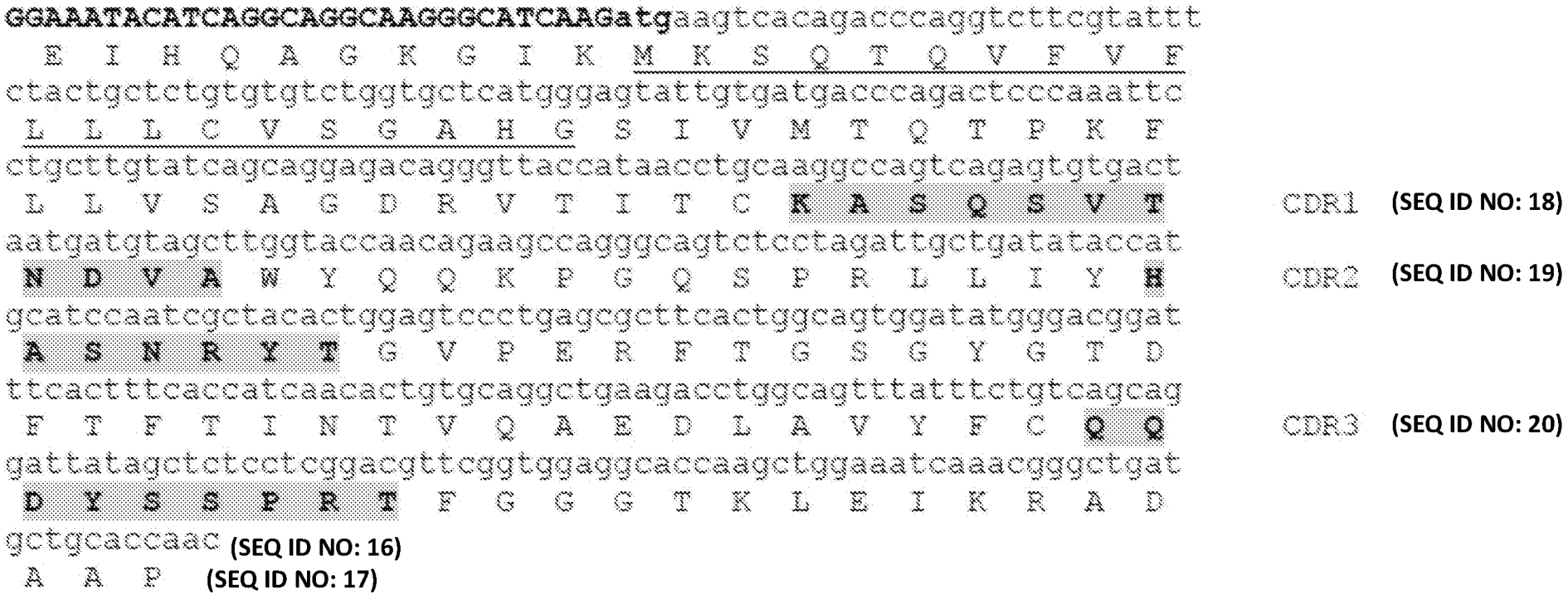

GGAAATACATCAGGCAGGCAAGGGCATCAAGatgaagtcacagacccaggtcttcgtattt
E I H Q A G K G I K M K S Q T Q V F V F
ctactgctctgtgtgtctggtgctcatgggagtattgtgatgacccagactcccaaattc
L L L C V S G A H G S I V M T Q T P K F
ctgcttgtatcagcaggagacagggttaccataacctgcaaggccagtcagagtgtgact
L L V S A G D R V T I T C K A S Q S V T
CDR1 (SEQ ID NO: 18)
aatgatgtagcttggtaccaacagaagccagggcagtctcctagattgctgatataccat
N D V A W Y Q Q K P G Q S P R L L I Y H
CDR2 (SEQ ID NO: 19)
gcatccaatcgctacactggagtccctgagcgcttcactggcagtggatatgggacggat
A S N R Y T G V P E R F T G S G Y G T D
ttcactttcaccatcaacactgtgcaggctgaagacctggcagtttatttctgtcagcag
F T F T I N T V Q A E D L A V Y F C Q Q
CDR3 (SEQ ID NO: 20)
gattatagctctcctcggacgttcggtggaggcaccaagctggaaatcaaacgggctgat
D Y S S P R T F G G G T K L E I K R A D
gctgcaccaac (SEQ ID NO: 16)
A A P (SEQ ID NO: 17)

Figure 4B


OD450
1.4
1.2
1
0.8
0.6
0.4
0.2
0
20
5
1
0
Guinea pig
Human
Mouse
Rhesus
Cyno
OD450
1.4
1.2
1
0.8
0.6
0.4
0.2
0
50
20
10
5
1
0
Guinea pig
Human
Mouse
Rhesus
Cyno

Figure 5

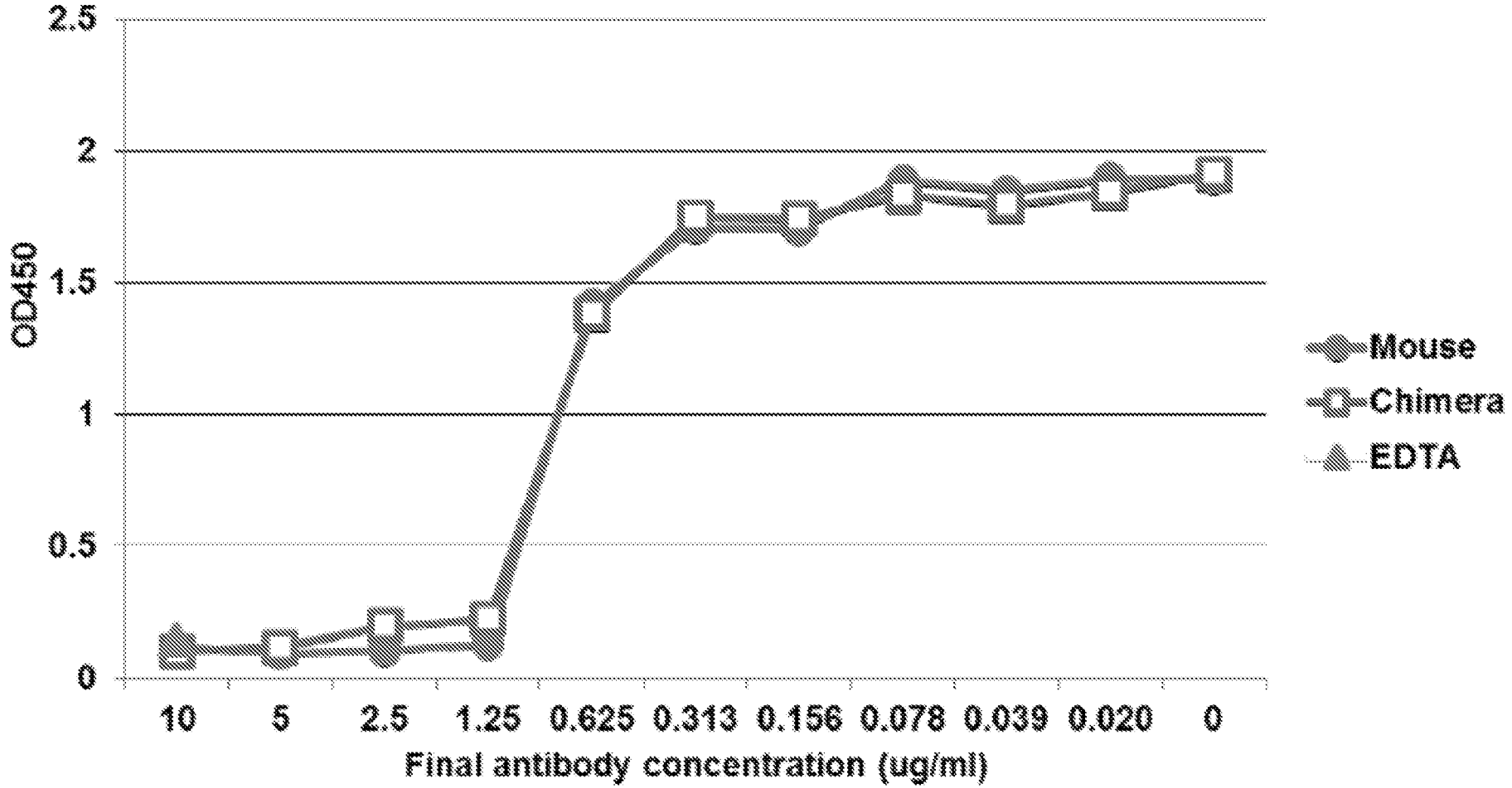

2.5
2
1.5
1
0.5
0
OD450
10
5
2.5
1.25
0.625
0.313
0.156
0.078
0.039
0.020
0
Final antibody concentration (ug/ml)
Mouse
Chimera
EDTA

Figure 6

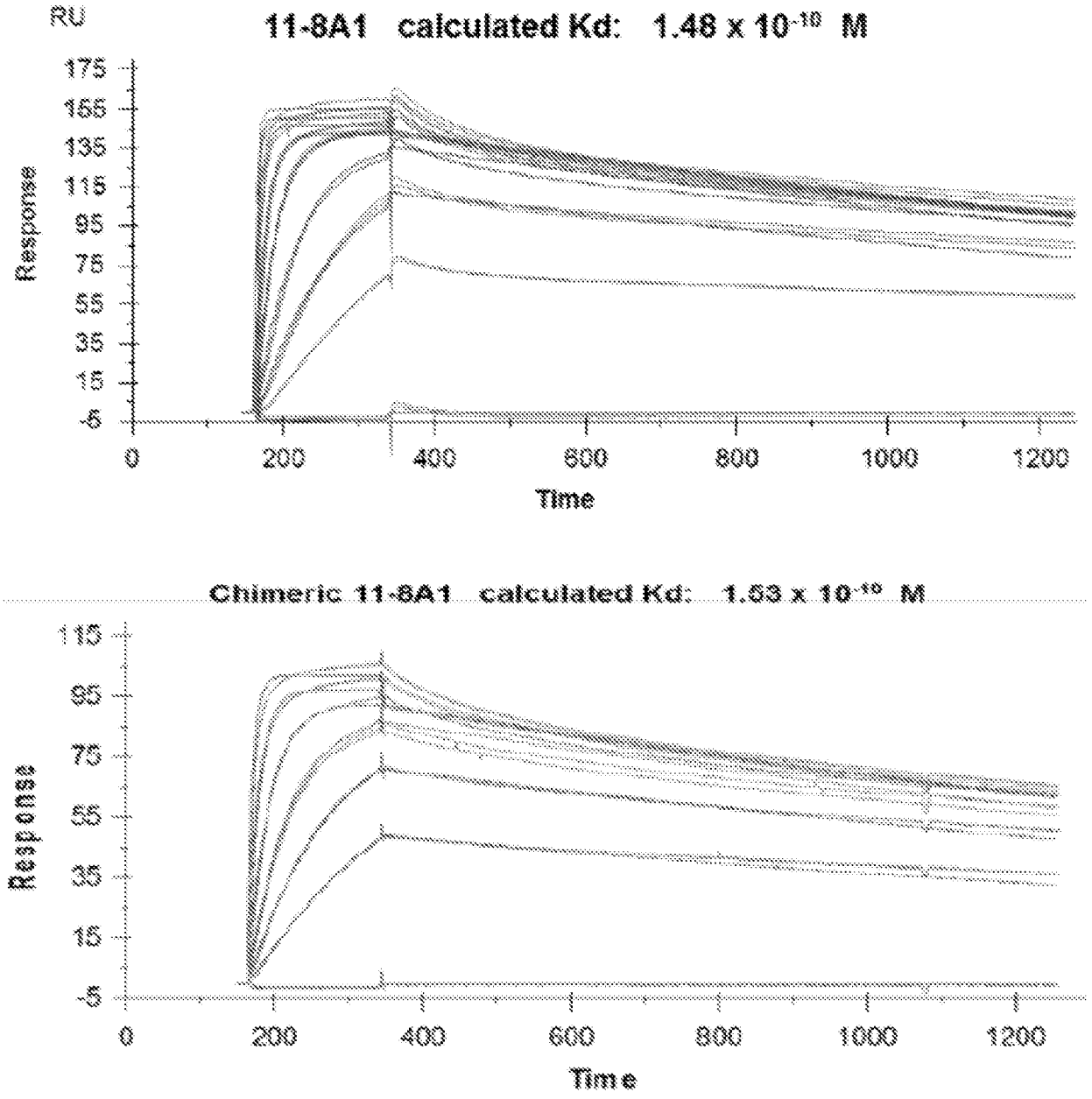

RU
11-8A1 calculated Kd: 1.48 x 10-10 M
Response
175
155
135
115
95
75
55
35
15
-5
0
200
400
600
800
1000
1200
Time
Chimeric 11-8A1 calculated Kd: 1.53 x 10-10 M
Response
115
95
75
55
35
15
-5
0
200
400
600
800
1000
1200
Time

Figure 7

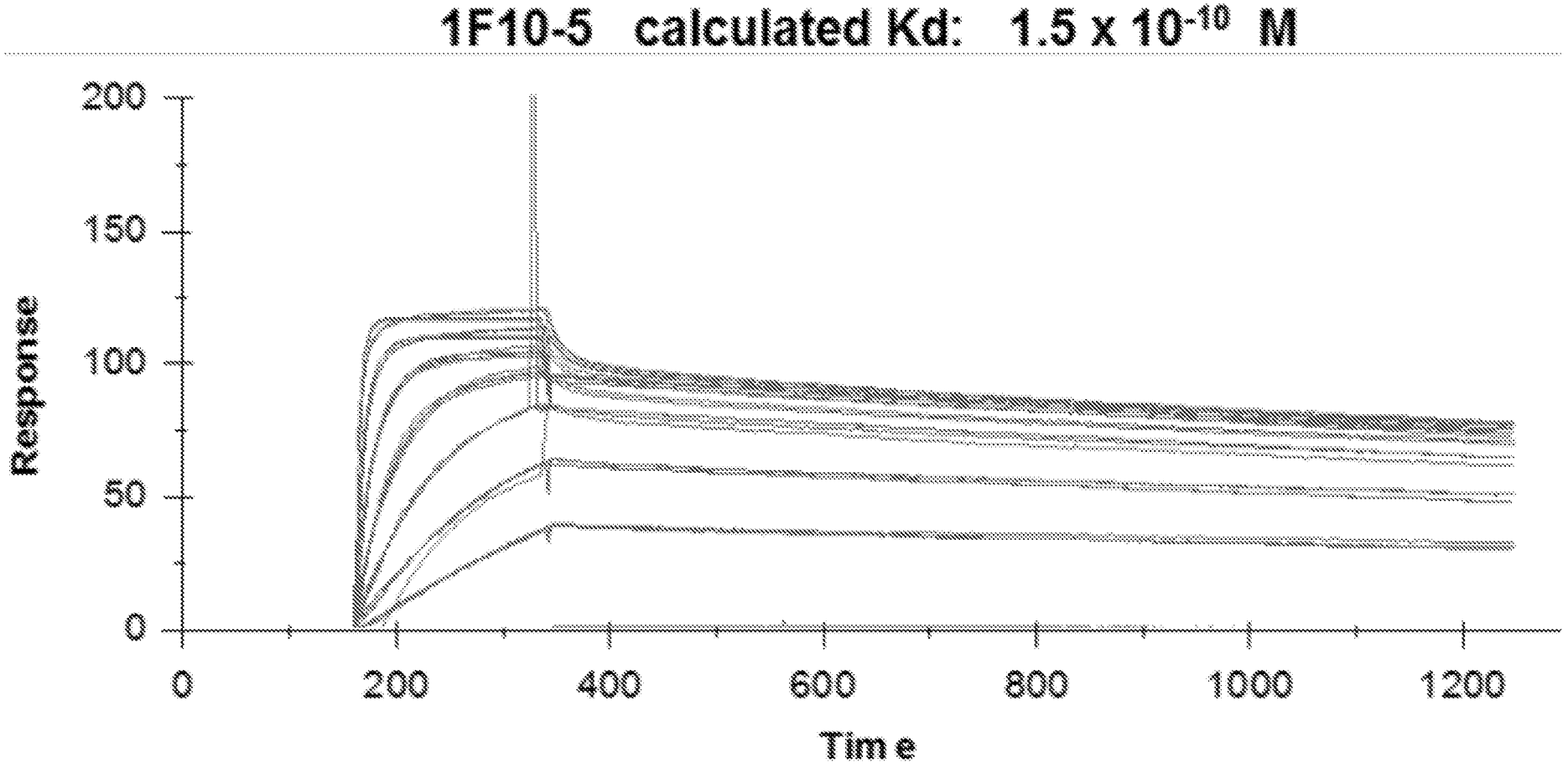

1F10-5 calculated Kd: 1.5 x 10-10 M
Response
200
150
100
50
0
0
200
400
600
800
1000
1200
Time

Figure 8

A

ILGGREAEAHARPYMASVQLNGAHLCGGVLVAEQWVLSAAHCLEDAADGKVQVLLG
AHSLSQPEPSKRLYDVLRAVPHPDSQPDTIDHDLLLLQLSEKATLGPAVRPLPWQRVDR **(SEQ ID NO: 21)**
DVAPGTLCDVAGWGIVNHAGRRPDSLQHVLLPVLDRATCNRRTHHDGAITERLMCAES **(SEQ ID NO: 22)**
NRRDSCKGDSGGPLVCGGVLEGVVTSGSRVCGNRKKPGIYTRVASYAAWIDSVLA

B

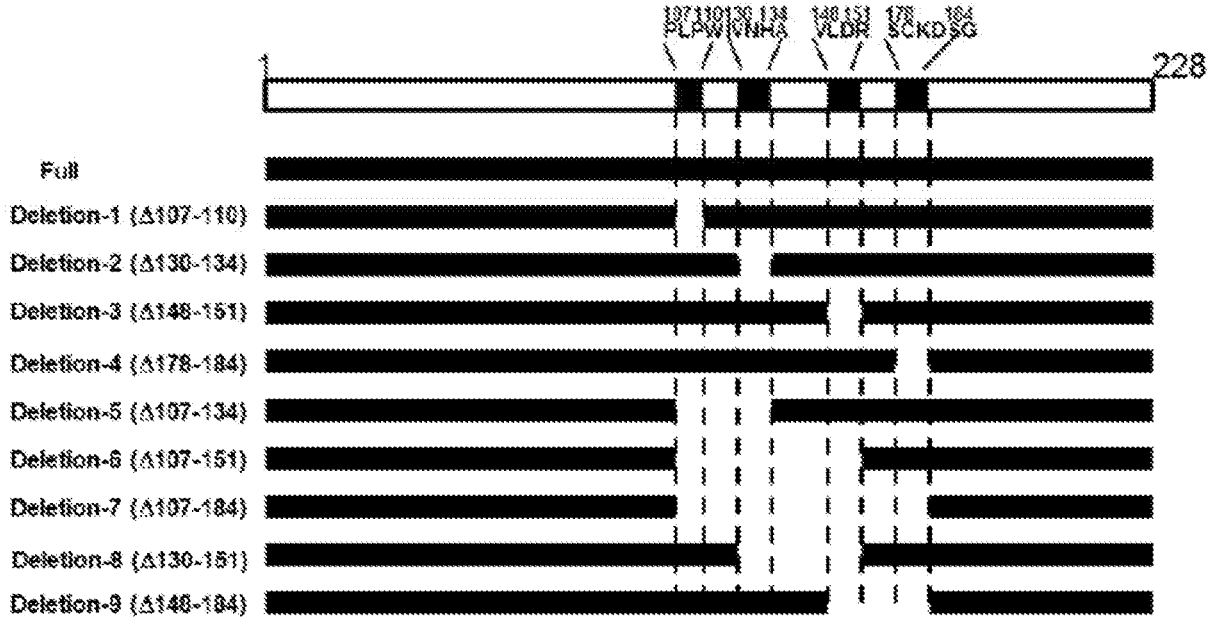
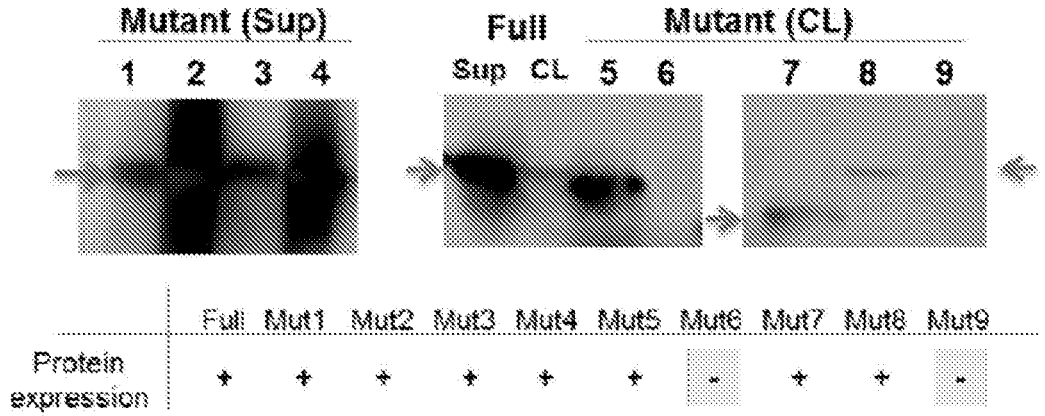


Figure 9A and Figure 9B

C



D

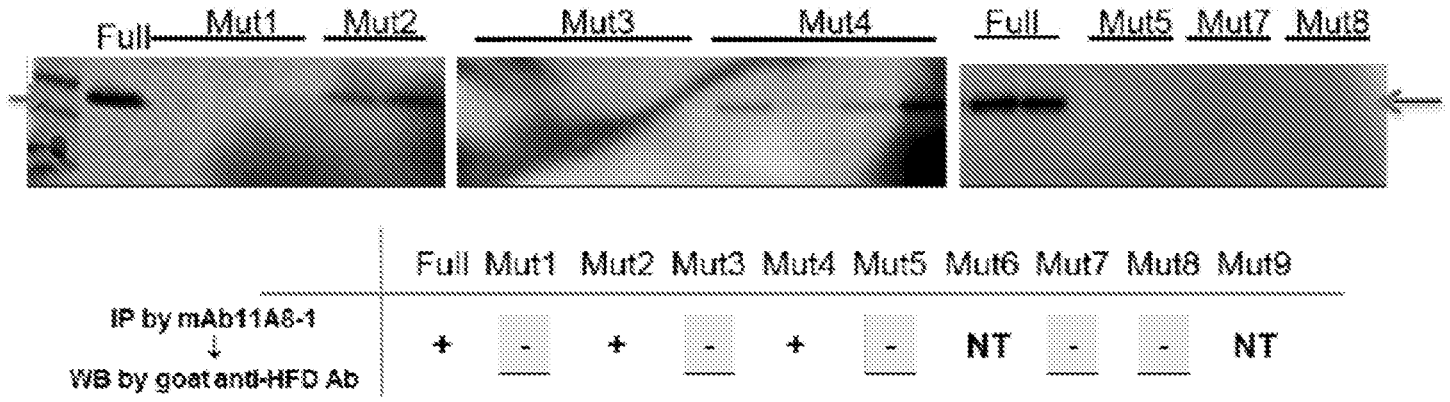


Figure 9C and Figure 9D

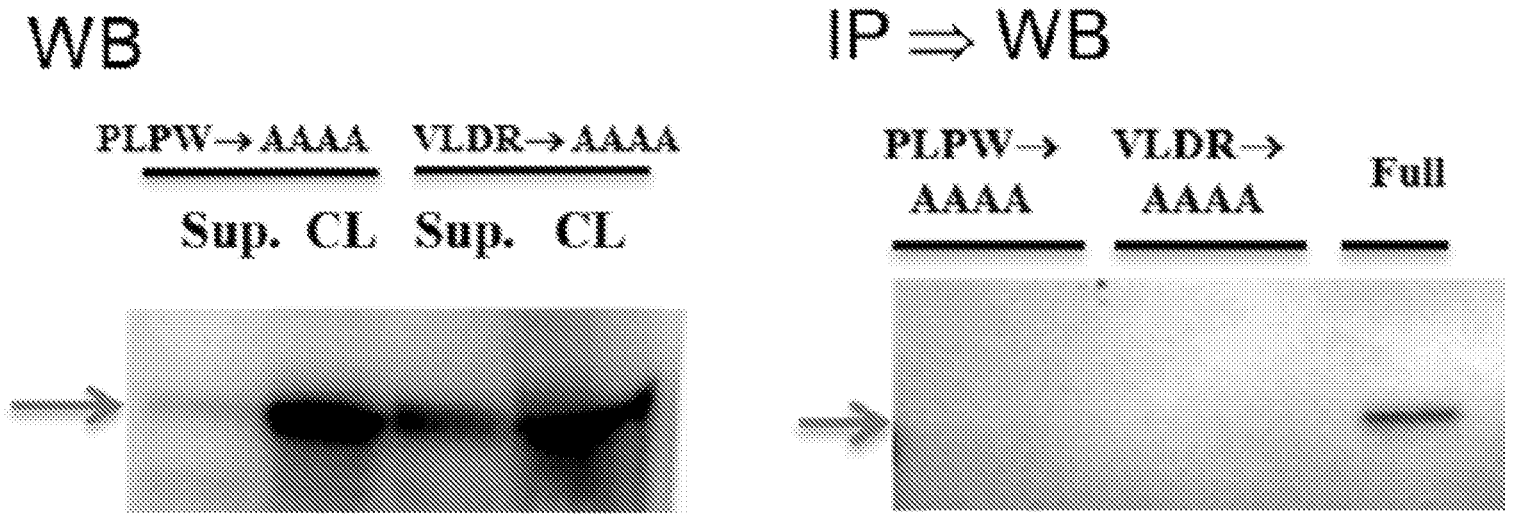

WB
PLPW→AAAA
VLDR→AAAA
Sup. CL Sup. CL
IP ⇒ WB
PLPW→ AAAA
VLDR→ AAAA
Full

Figure 10

A

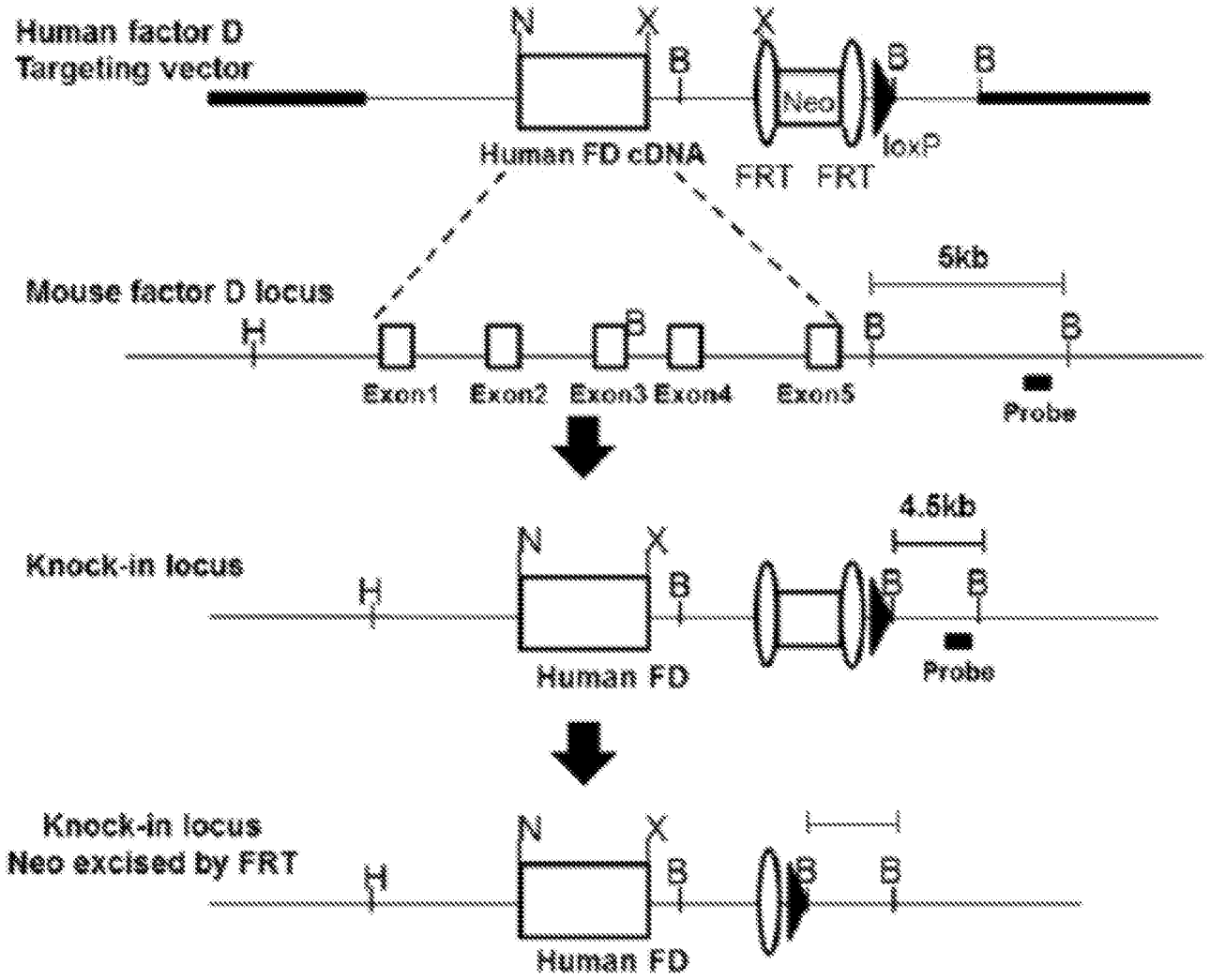

Human factor D
Targeting vector
N
X
B
X
B
B
Neo
loxP
Human FD cDNA
FRT
FRT
Mouse factor D locus
5kb
H
B
B
B
Exon1
Exon2
Exon3
Exon4
Exon5
Probe
Knock-in locus
4.5kb
N
X
H
B
B
B
Human FD
Probe
Knock-in locus
Neo excised by FRT
N
X
H
B
B
B
Human FD

Figure 11A

B

(SEQ ID NO: 25)

GTGTCTCAGCCACAGCGGCTTCACCATGCACAGCTGGGAGCGCCTGGCAGTTCTGGTCCTCCTAGGAGCG
GCCGCCTGCGCGGCGCCGCCCCGTGGTCGGATCCTGGGCGGCAGAGAGGCCGAGGCGCACGCGCGGCCCT
ACATGGCGTCGGTGCAGCTGAACGGCGCGCACCTGTGCGGCGGCGTCCTGGTGGCGGAGCAGTGGGTGCT
GAGCGCGGCGCACTGCCTGGAGGACGCGGCCGACGGGAAGGTGCAGGTTCTCCTGGGCGCGCACTCCCTG
TCGCAGCCGGAGCCCTCCAAGCGCCTGTACGACGTGCTCCGCGCAGTGCCCCACCCGGACAGCCAGCCCG
ACACCATCGACCACGACCTCCTGCTGCTACAGCTGTCGGAGAAGGCCACACTGGGCCCTGCTGTGCGCCC
CCTGCCCTGGCAGCGCGTGGACCGCGACGTGGCACCGGGAACTCTCTGCGACGTGGCCGGCTGGGGCATA
GTCAACCACGCGGGCCGCCGCCCGGACAGCCTGCAGCACGTGCTCTTGCCAGTGCTGGACCGCGCCACCT
GCAACCGGCGCACGCACCACGACGGCGCCATCACCGAGCGCTTGATGTGCGCGGAGAGCAATCGCCGGGA
CAGCTGCAAGGGTGACTCCGGGGGCCCGCTGGTGTGCGGGGGCGTGCTGGAGGGCGTGGTCACCTCGGGC
TCGCGCGTTTGCGGCAACCGCAAGAAGCCCGGGATCTACACCCGCGTGGCGAGCTATGCGGCCTGGATCG
ACAGCGTCCTGGCCTAGgggacaccagagacacgtggctcagggaaacaCTCGAGagagacacgtggct
cacaataaatgcatgcatctgagccctgtgcatctttctttttttttttttttaagatttatttattgtta
tatgtatctacactatagctgtcttcagacacaccagaagagggagtcagatcttattacagatggttgt
gagccaccatgtggttgctgggatttgaactcagggccttcggaagagcagtcagtgctcttaaccactg
agccatctcaccagcccgccctgtgcatctttattgagtgcctgttgcatacacacctgaaggcgggtgt
tttcacgcttggccacacgggggcgcgcgtgctgttttagaattgtgaggcggggctcattcctatccta
ctgaggtcctgctgtacccaggactgttgtctgggctcagacagctcttactttgagccttaccacataa
atactaaaagggaaaactgaggcacagagcggccatgaacaagactggattccaacccagagcccttcct
ggtttactgaagagggagttggtaatgtctttgtcacaggctggaaccctgtgggtgacaatgggcagaa
cctggacccaaaccctgtgtctttctaccaggagaacaggacactgaggcaaagggagtcttctggcttc
ctgtaggtattggagactaaggatcttagaggtgaaaccactgaggaccctgctgtgaaagaggatagaa
ctggatcctgggtctaaactttgtctctgctaggcaggttcagcttggagtcatctttatgaacaagctg
ccaccaggctcatggagagggatagccacagacacaggccaggcccagctctactccctcgggaggctag
tgcttagcaccacttgtcccacaatcccagagtgctccgcacctaaacagaggagcctgggacgccactc
agctgcgtctagcacgtcccctagatggcttgatatgcatgtctatgcacggccagcactctgcagagag
tggaccggagcagtcttccaggactgccaccctggtggccatgtttccggctatgggaggtcaggaagtc
gagcttgtgcatggccaggatgctgcaccaccatgctgctggcttctgttggcagaggaggtgctctagg
ggcagcagtgggcagggcaaagggaagtcacaaaactctgaaggggtataccagttcccaggcacaacat
gcctgcctcttccagcactgaggagaaggtagttcaaagctgggatgctcacctgggactgttaccctgt
ctgactgtagctccctcacactgcggtggagtacatcgataatt

Figure 11B

Figure 11C and Figure 11D

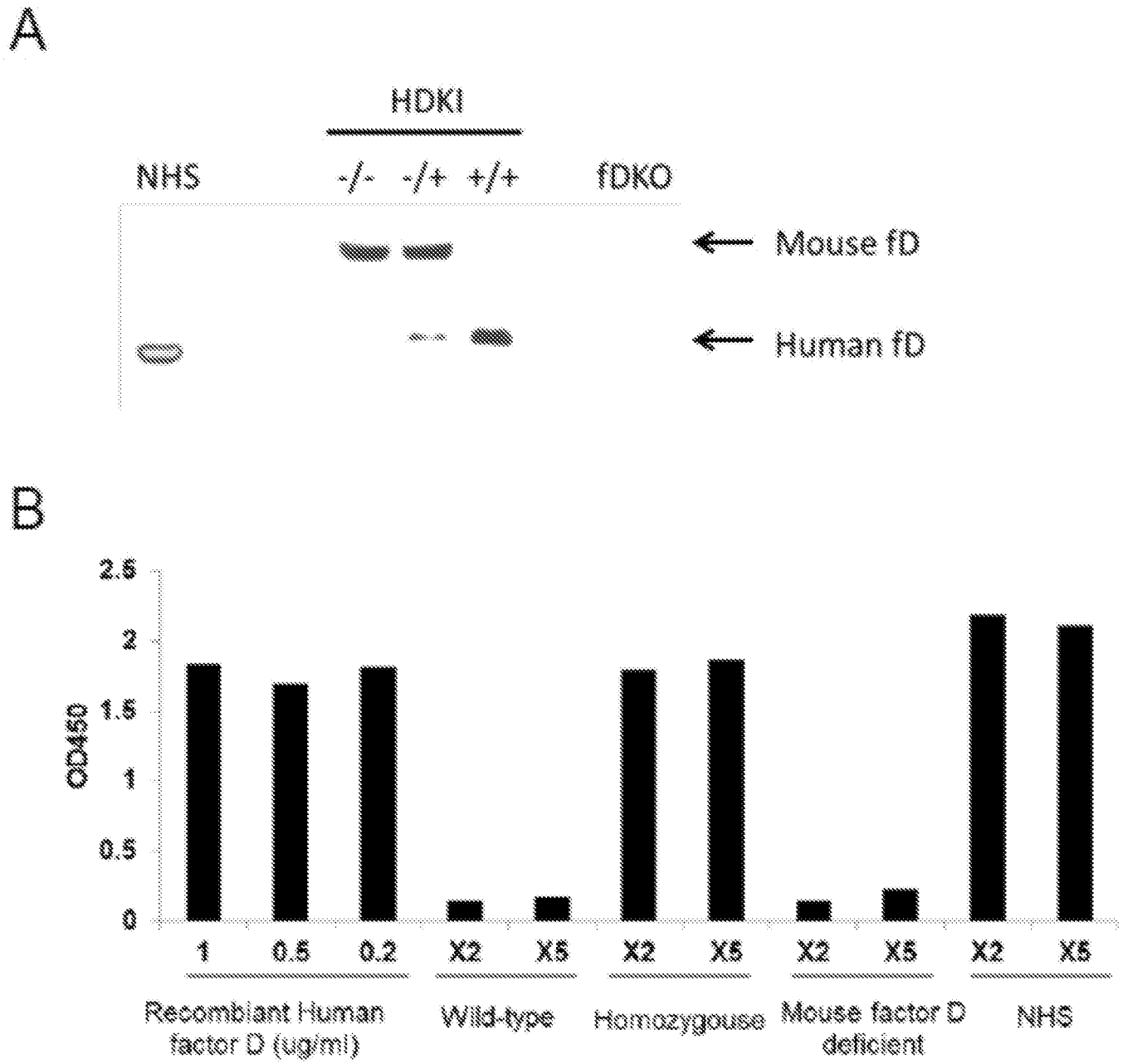


Figure 12A and Figure 12B

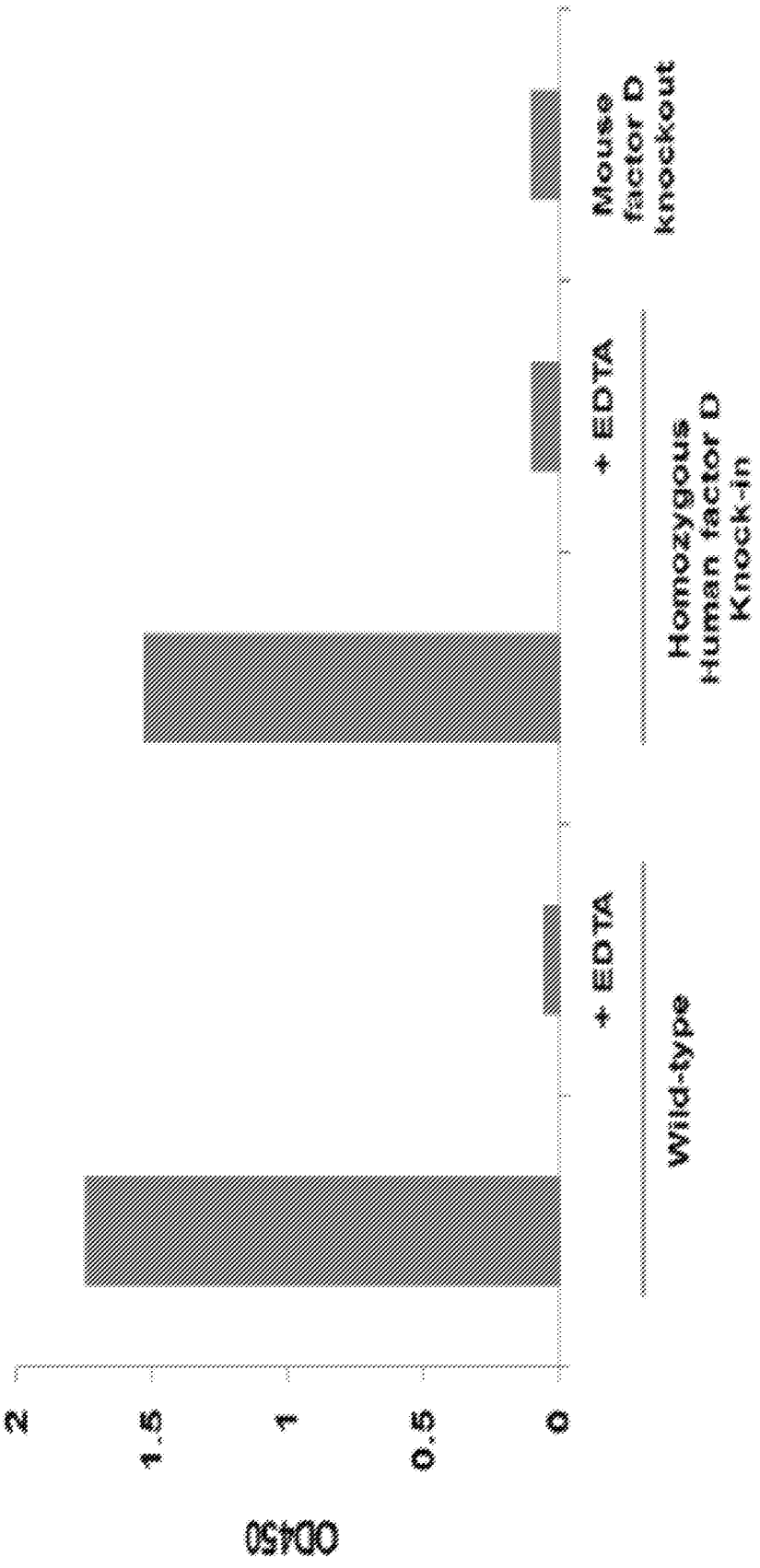

OD450
2
1.5
1
0.5
0
Wild-type
+ EDTA
Homozygous
Human factor D
Knock-in
+ EDTA
Mouse
factor D
knockout

Figure 13

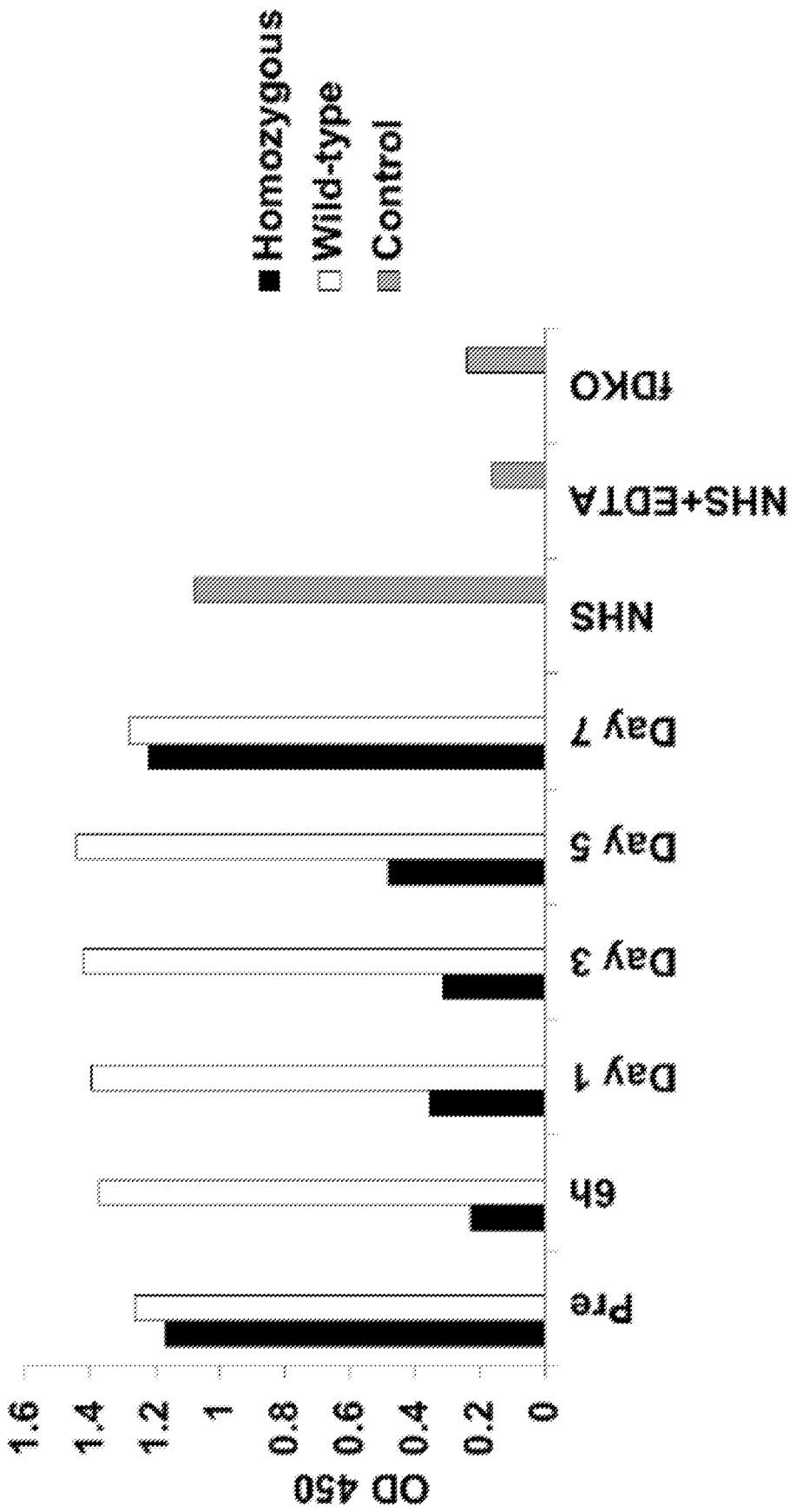
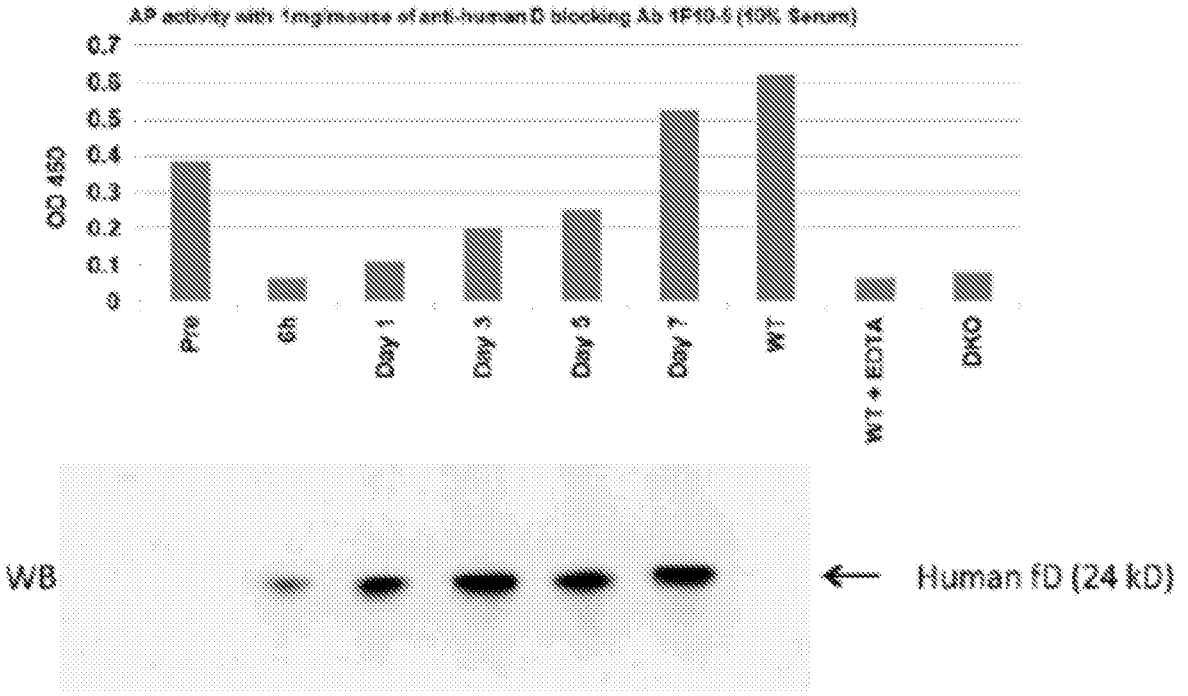

Homozygous
Wild-type
Control
OD 450
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Pre
6h
Day 1
Day 3
Day 5
Day 7
NHS
NHS+EDTA
fDKO

Figure 14


AP activity with 1mg/mouse of anti-human D blocking Ab 1F10-5 (10% Serum)
OD 450
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
PNS
6h
Day 1
Day 3
Day 5
Day 7
WT
WT + EDTA
DKO
WB
Human fD (24 kD)

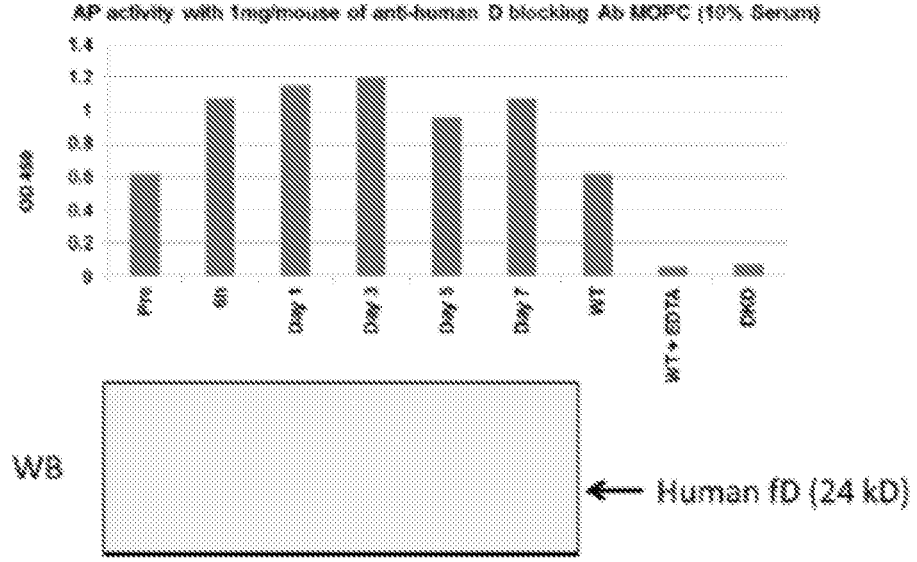

AP activity with 1mg/mouse of anti-human D blocking Ab MOPC (10% Serum)
WB
Human fD (24 kD)

Figure 15

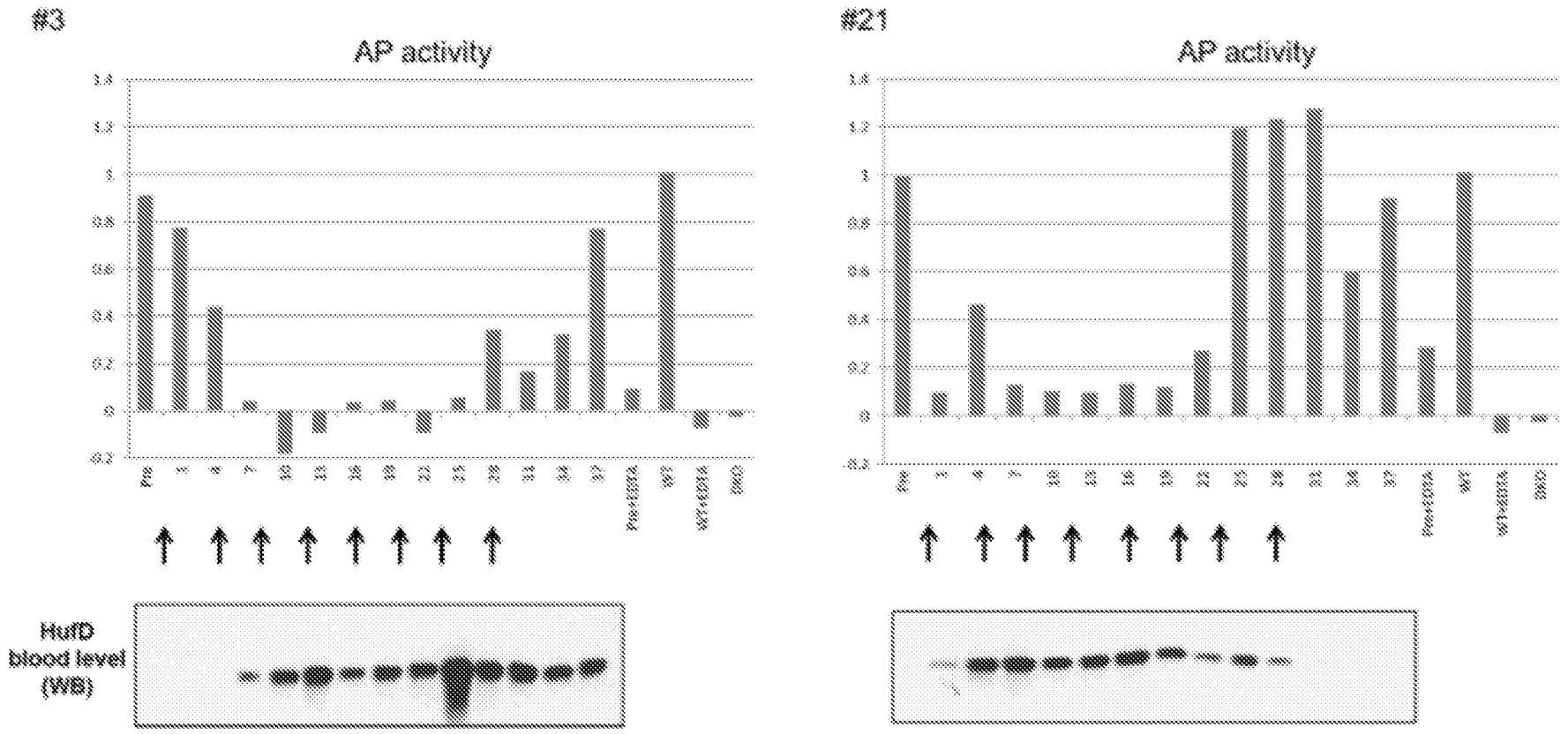


Figure 16

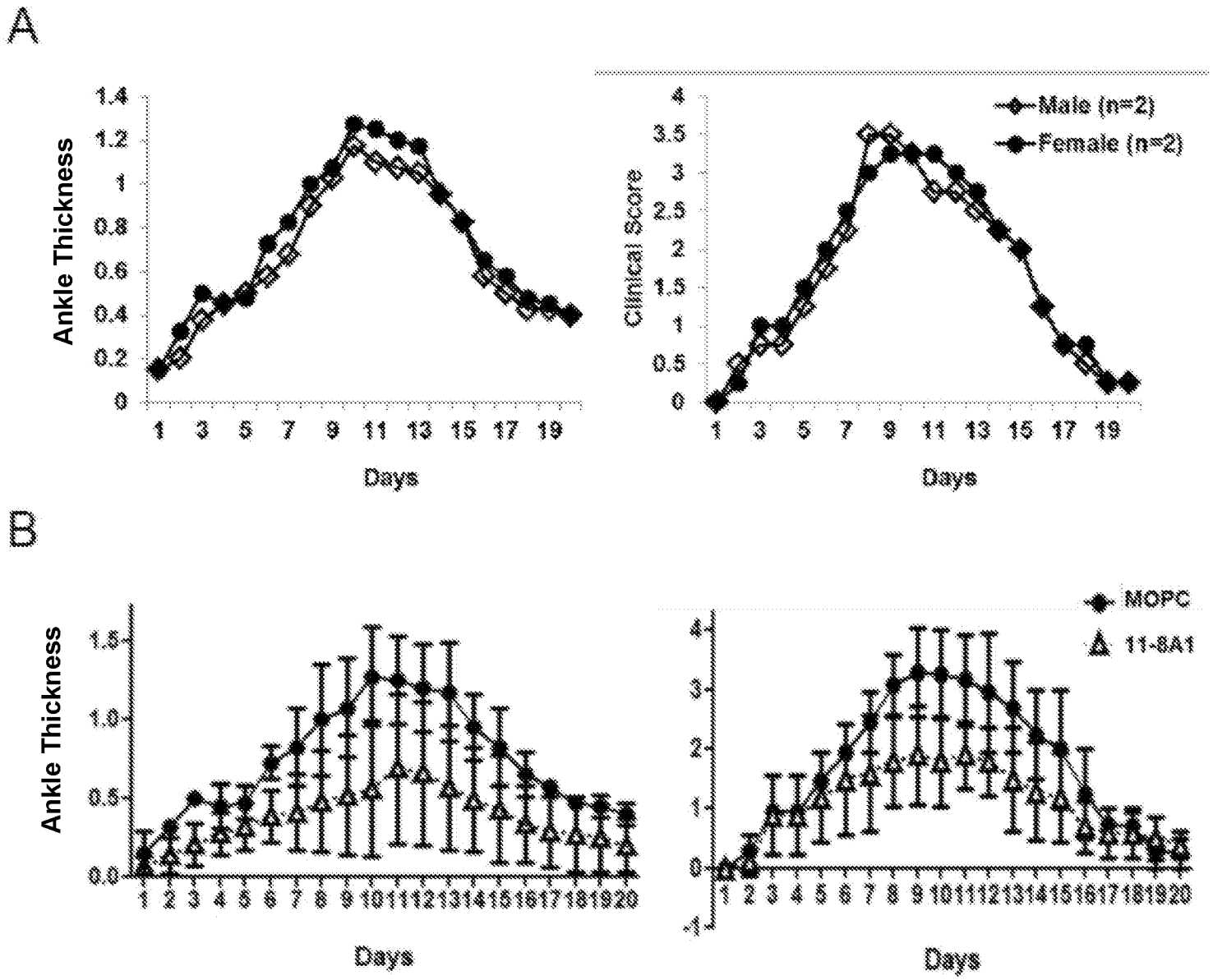

A
Ankle Thickness
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1 3 5 7 9 11 13 15 17 19
Days
Clinical Score
4
3.5
3
2.5
2
1.5
1
0.5
0
1 3 5 7 9 11 13 15 17 19
Days
Male (n=2)
Female (n=2)
B
Ankle Thickness
1.5
1.0
0.5
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days
4
3
2
1
0
-1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days
MOPC
11-8A1

Figure 17

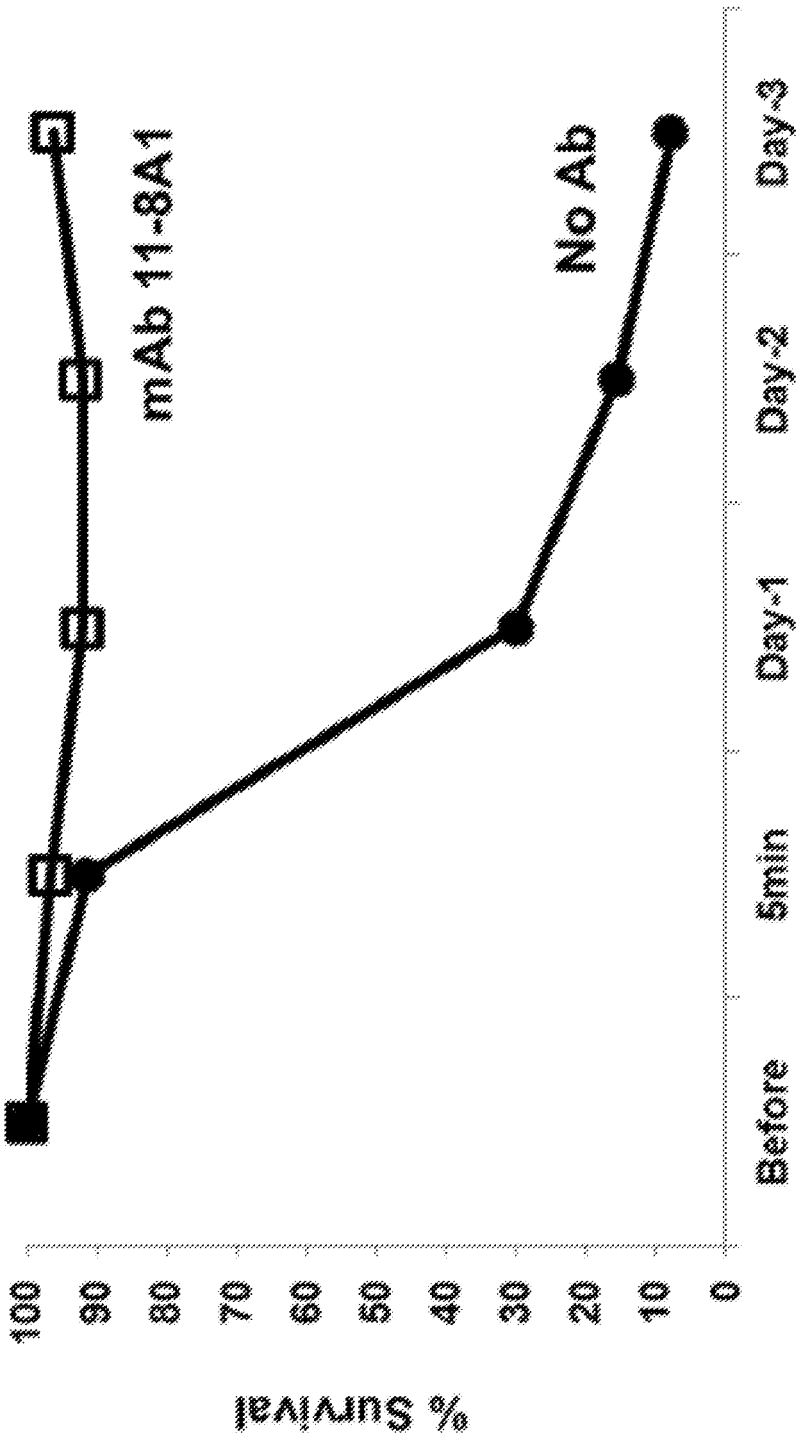

% Survival
100
90
80
70
60
50
40
30
20
10
0
Before
5min
Day-1
Day-2
Day-3
mAb 11-8A1
No Ab

Figure 18

A

ILGGREAEAHARPYMASVQLNGAHLCGGVLVAEQWVLSAAHCLEDAADGKVQVLLG
AHSLSQPEPSKRLYDVLRAVPHPDSQPDTIDHDLLLLQLSEKATLGPAVRPLPWQRVDR
DVAPGTLCDVAGWGIVNHAGRRPDSLQHVLLPVLDRATCNRRTHHDGAITERLMCAES
NRRDSCKGDSGGPLVCGGVLEGVVTSGSRVCGNRKKPGIYTRVASYAAWIDSVLA

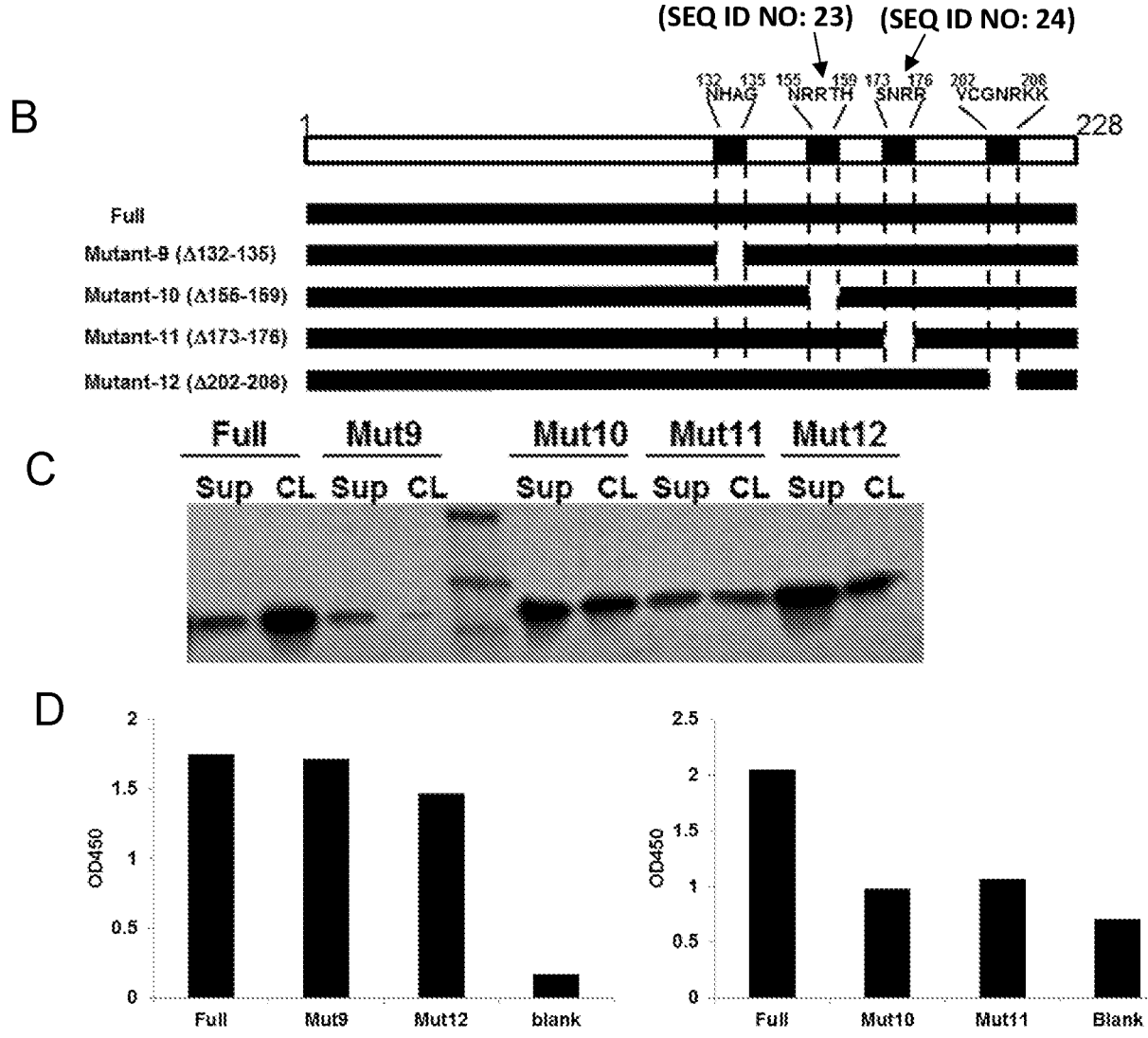


Figure 19

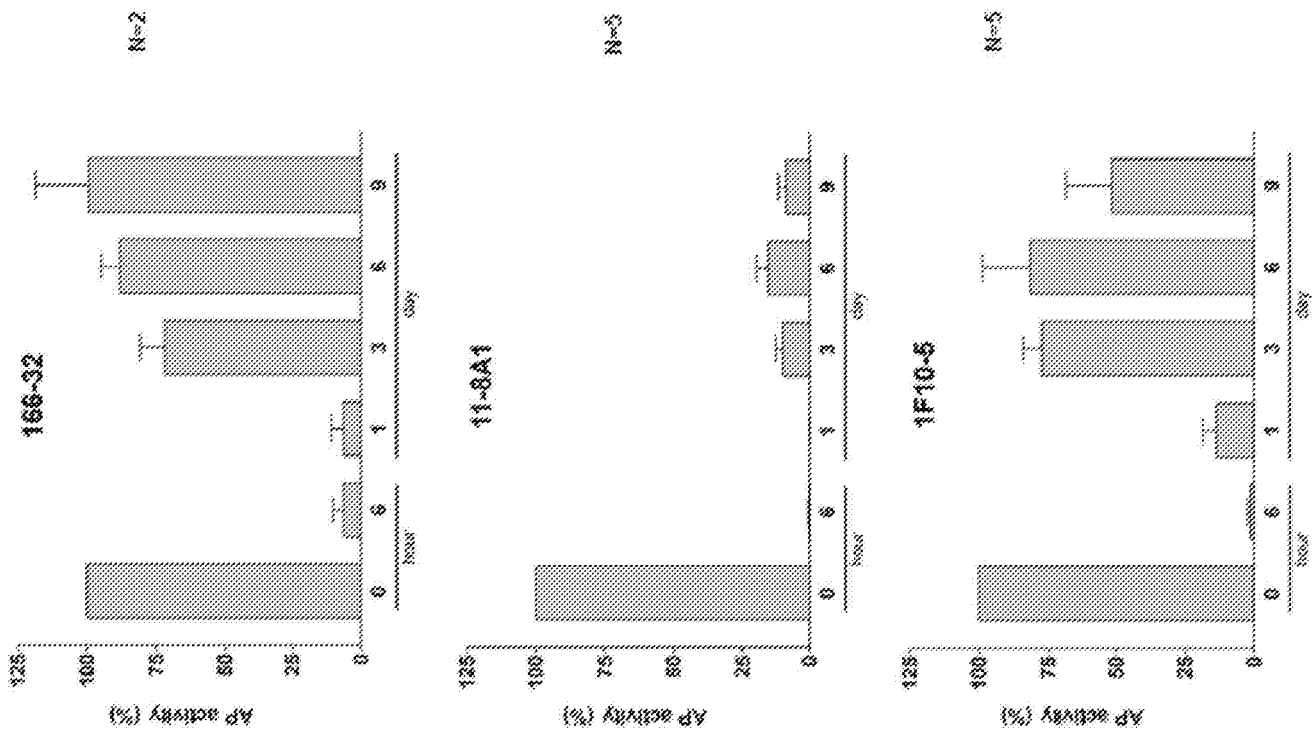

166-32
N=2
11-8A1
N=3
1F10-5
N=5
AP activity (%)
125
100
75
50
25
0
hour
day

Figure 20

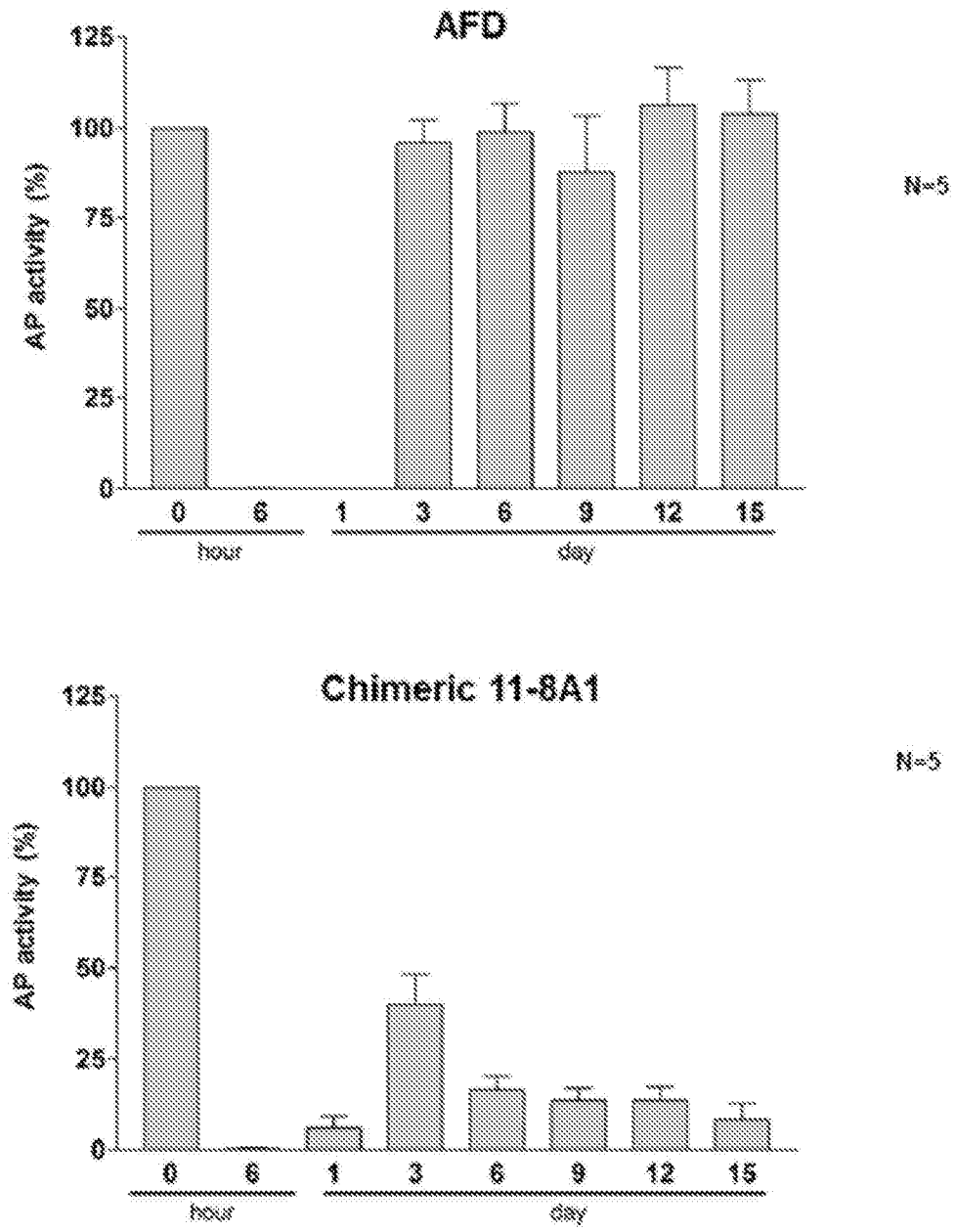

AFD
AP activity (%)
125
100
75
50
25
0
0
6
1
3
6
9
12
15
hour
day
N=5
Chimeric 11-8A1
AP activity (%)
125
100
75
50
25
0
0
6
1
3
6
9
12
15
hour
day
N=5

Figure 21