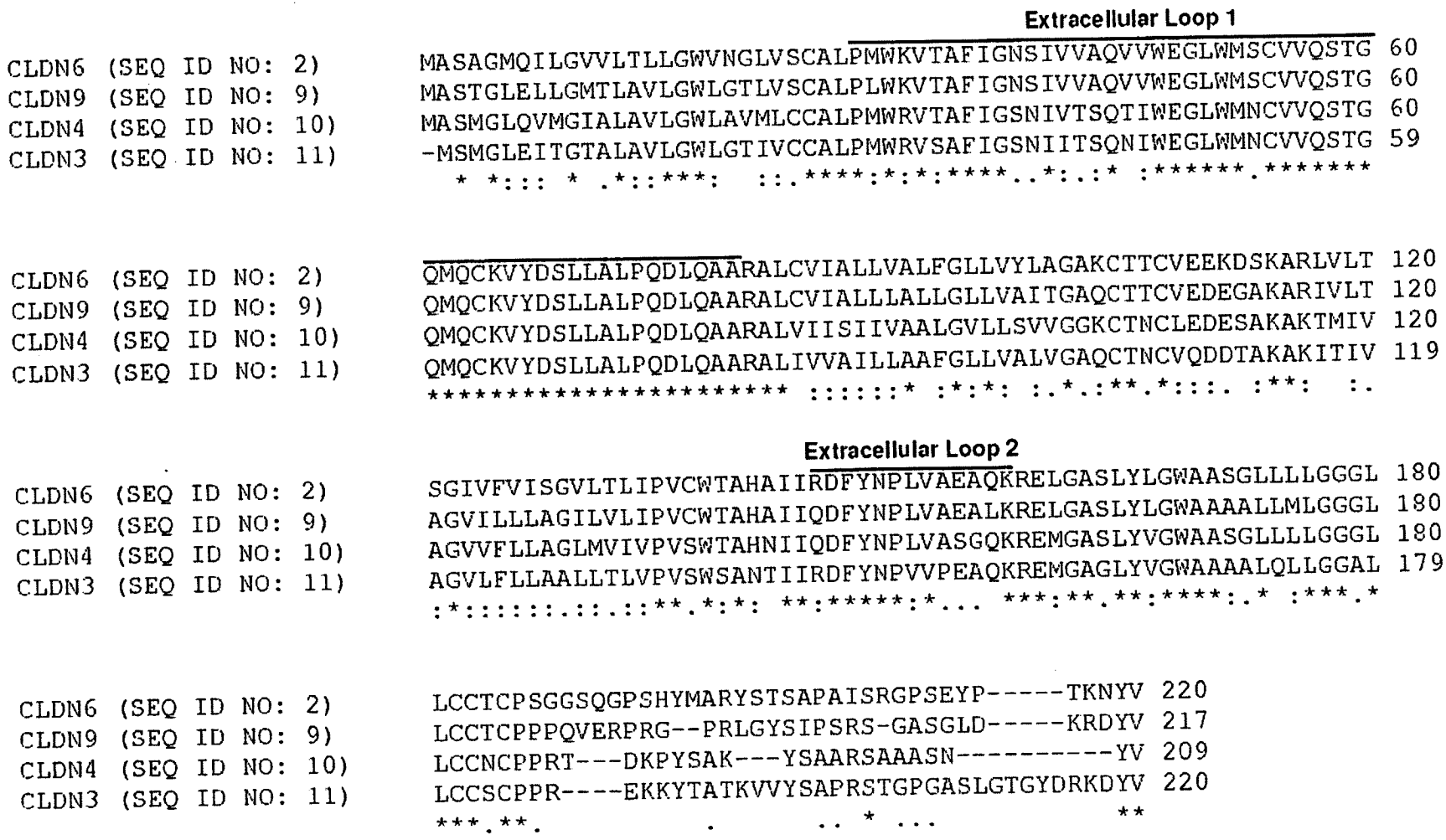
Fig. 1



WO 2012/156018 PCT/EP2012/001721

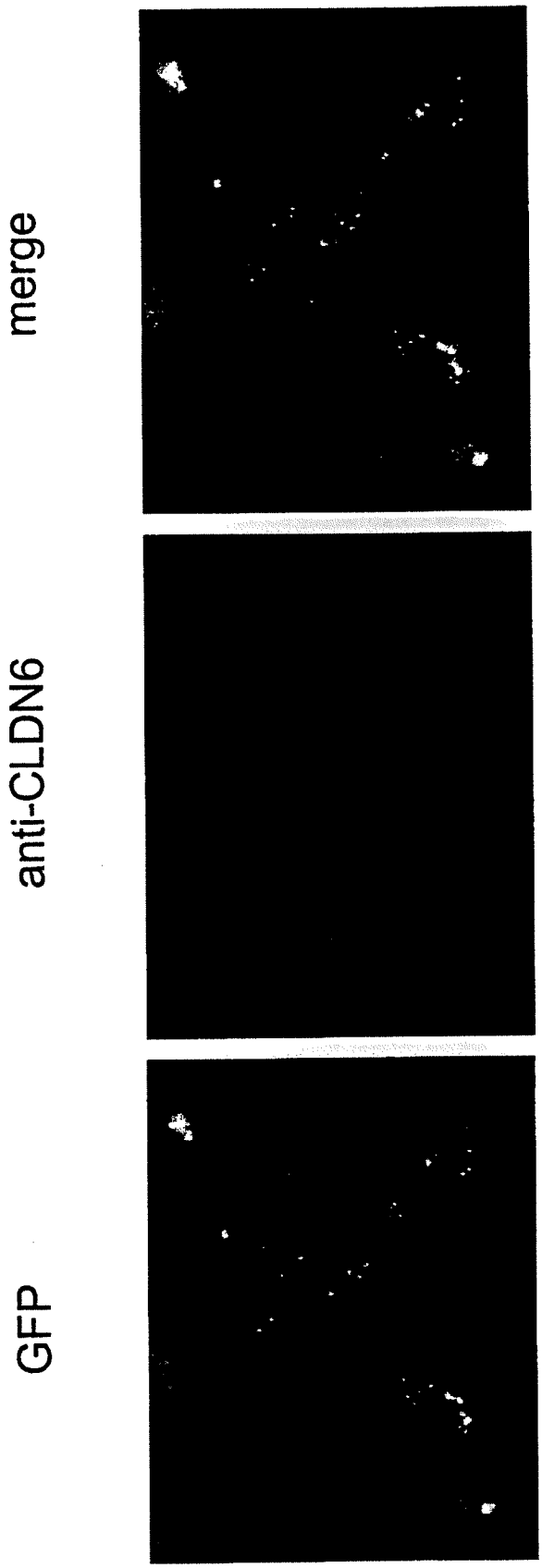


Fig. 2A

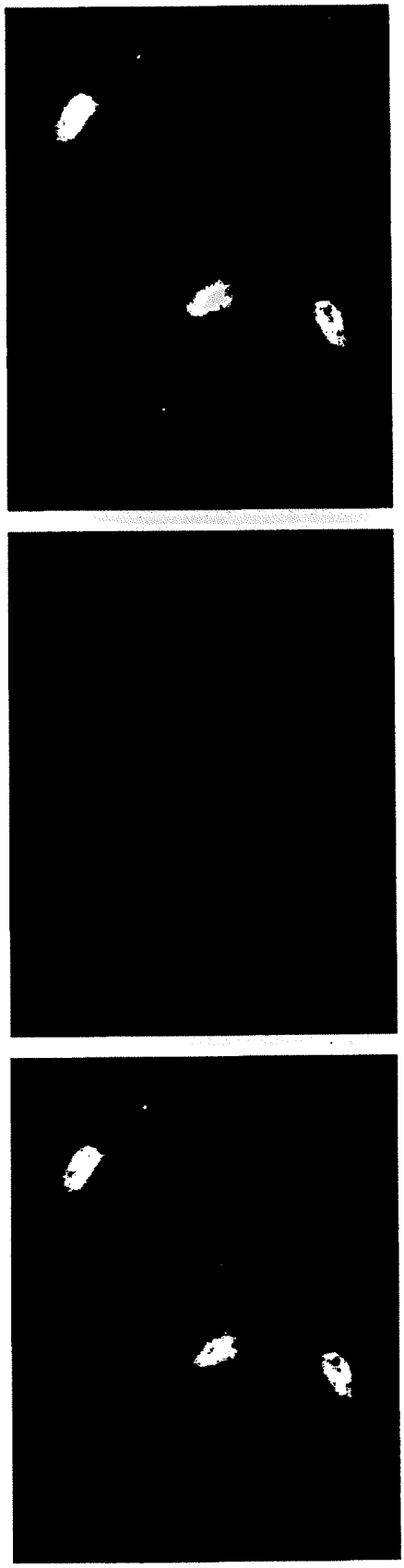


Fig. 2B

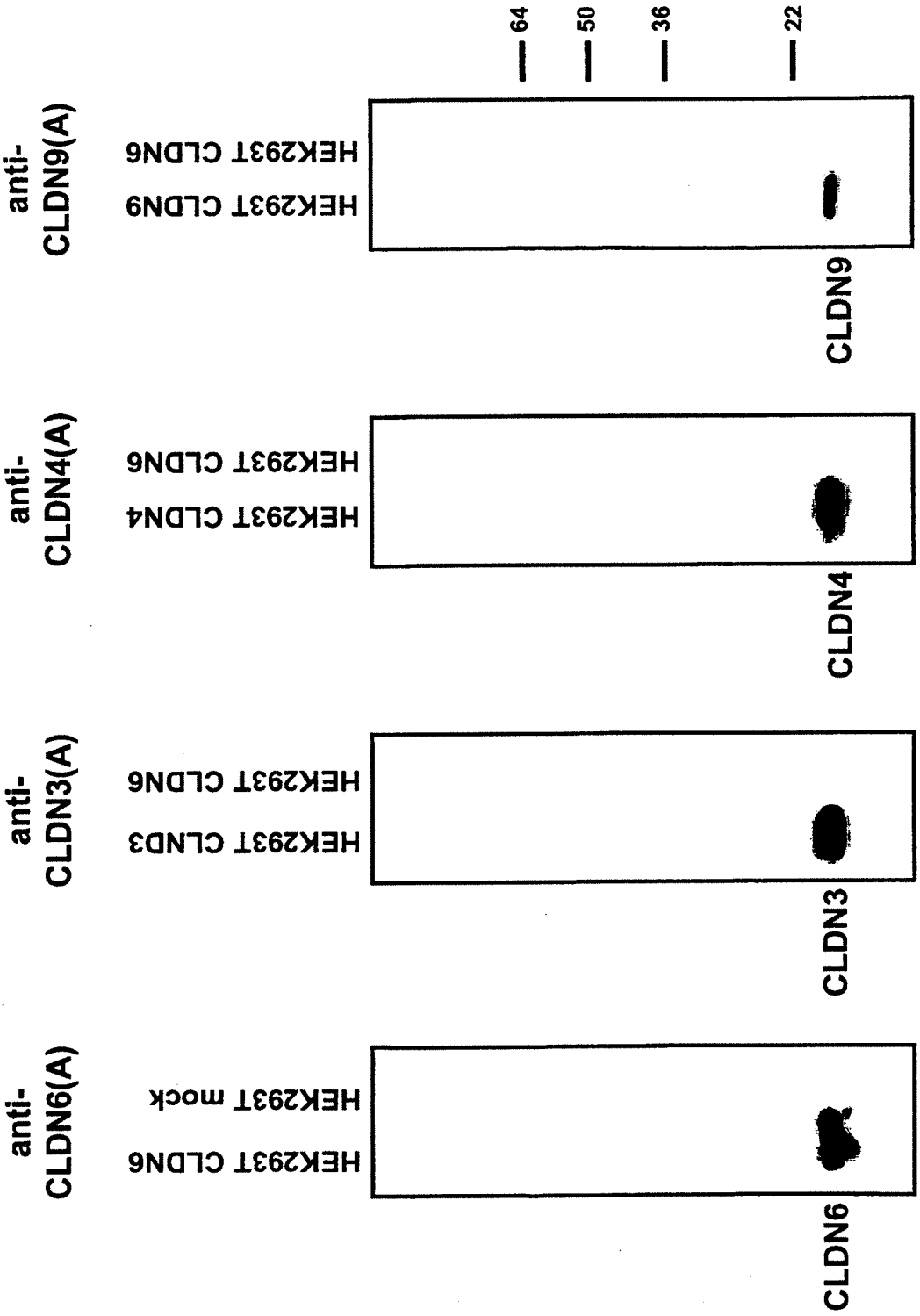

64
50
36
22
anti-CLDN9(A)
HEK293T CLDN6
HEK293T CLDN9
CLDN9
anti-CLDN4(A)
HEK293T CLDN6
HEK293T CLDN4
CLDN4
anti-CLDN3(A)
HEK293T CLDN6
HEK293T CLND3
CLDN3
anti-CLDN6(A)
HEK293T mock
HEK293T CLDN6
CLDN6

Fig. 3

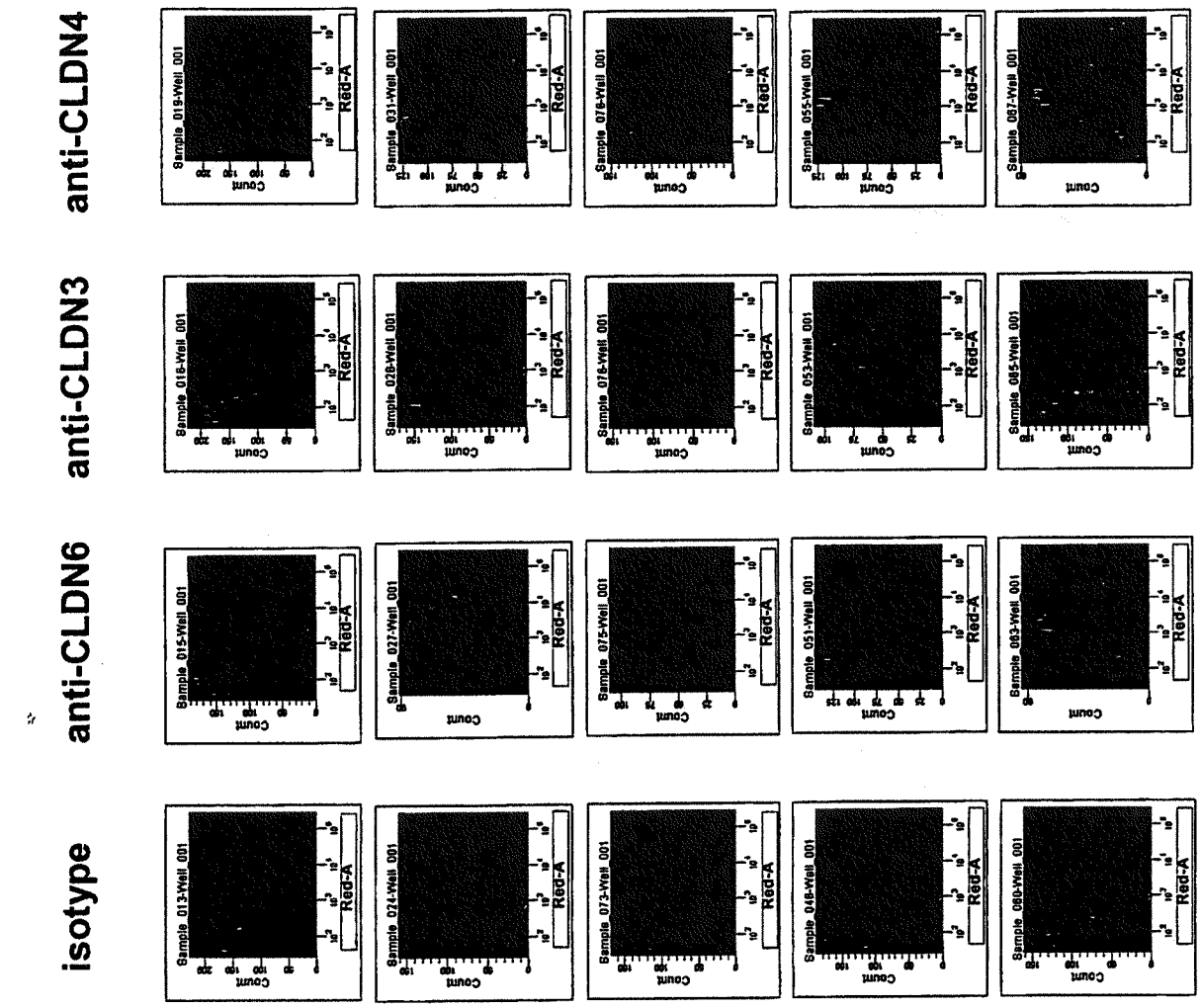

isotype
anti-CLDN6
anti-CLDN3
anti-CLDN4
non-transfected
CLDN6-transfected
CLDN9-transfected
CLDN3-transfected
CLDN4-transfected

Fig. 4

Fig. 5A

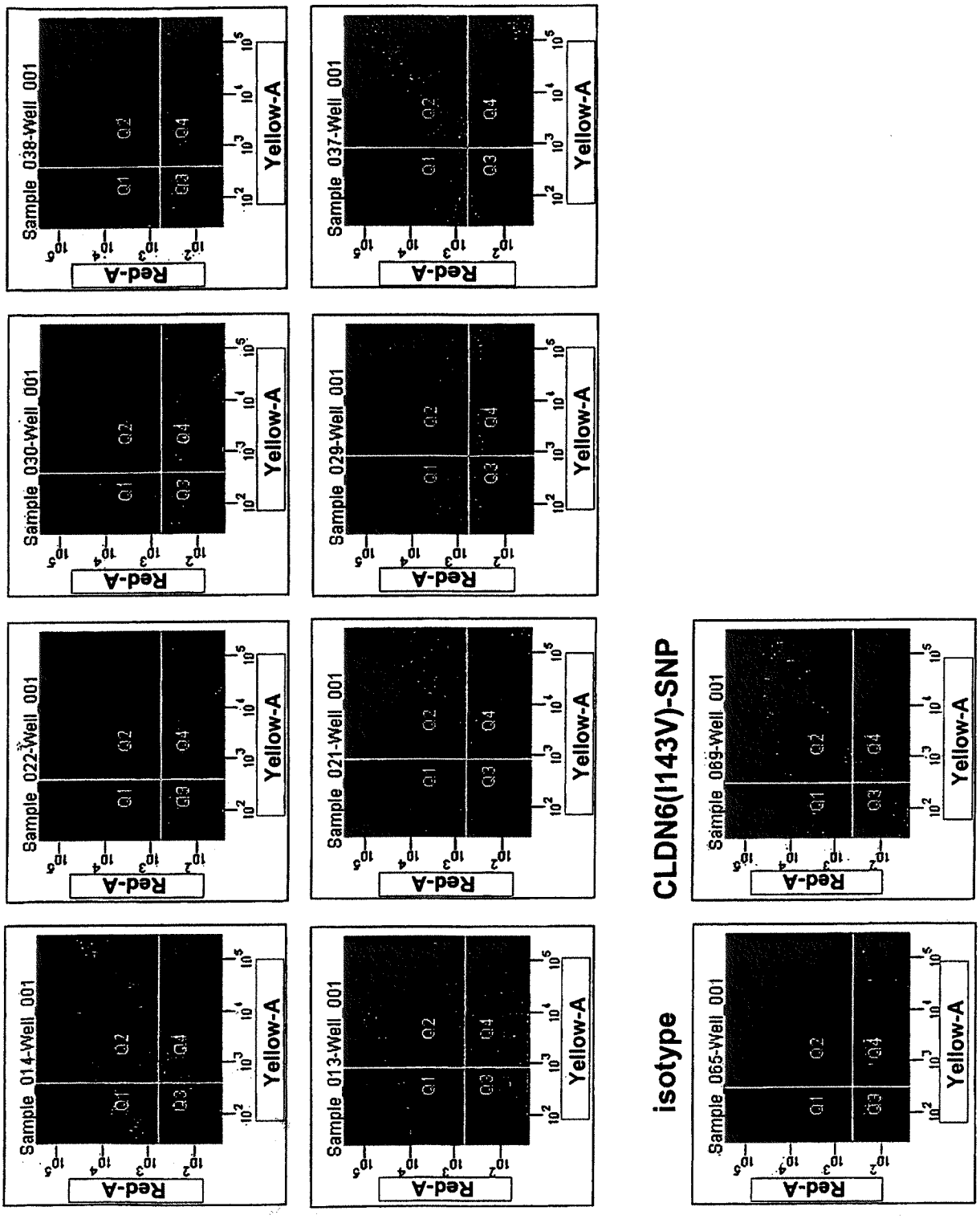

CLDN6
CLDN3
CLDN4
CLDN9
CLDN6(I143V)-SNP
isotype
F3-6C3-H8
F4-4F7-F2
F3-6C3-H8
Red-A
Yellow-A
Q1
Q2
Q3
Q4

Fig. 5B

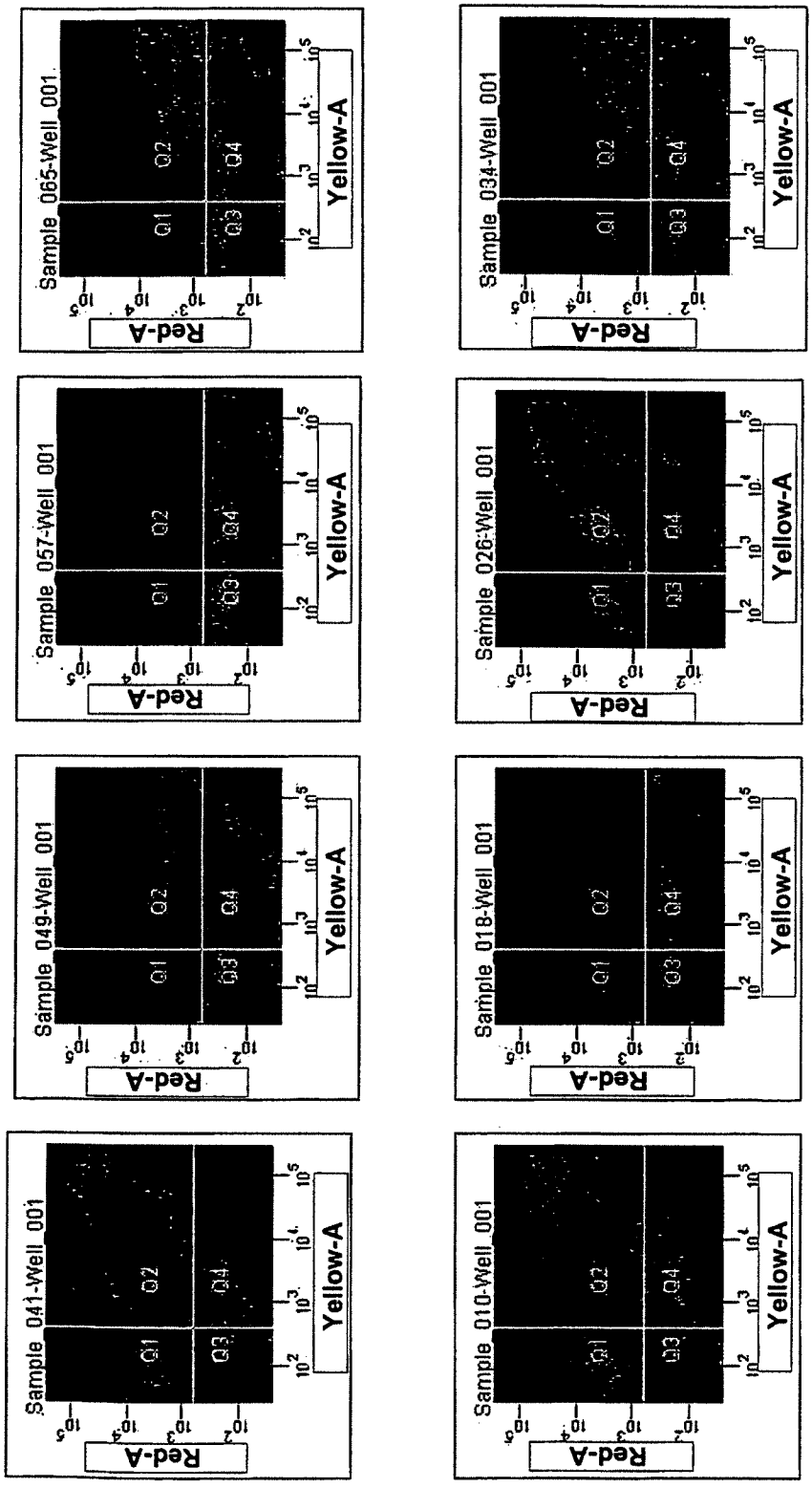
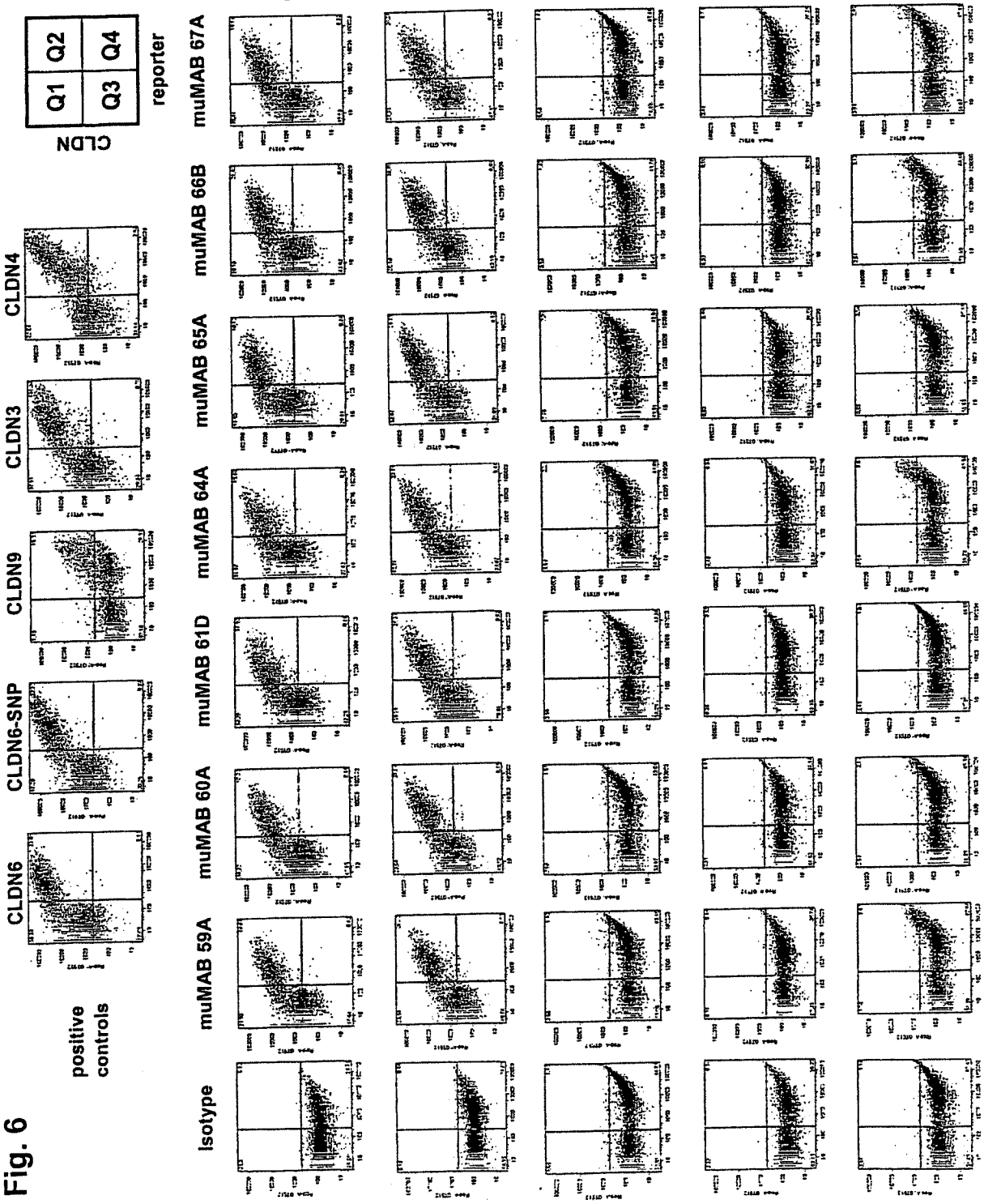

CLDN6
CLDN3
CLDN4
CLDN9
F3-7B3-B4
F3-3F7-A5
Sample_041-Well_001
Sample_049-Well_001
Sample_057-Well_001
Sample_065-Well_001
Sample_010-Well_001
Sample_018-Well_001
Sample_026-Well_001
Sample_034-Well_001
Red-A
Yellow-A
Q1
Q2
Q3
Q4

Fig. 6


CLDN6
CLDN6-SNP(I143V)
CLDN9
CLDN3
CLDN4
muMAB 67A
muMAB 66B
muMAB 65A
muMAB 64A
muMAB 61D
muMAB 60A
muMAB 59A
Isotype
positive controls
CLDN6
CLDN6-SNP
CLDN9
CLDN3
CLDN4
CLDN
Q1
Q2
Q3
Q4
reporter

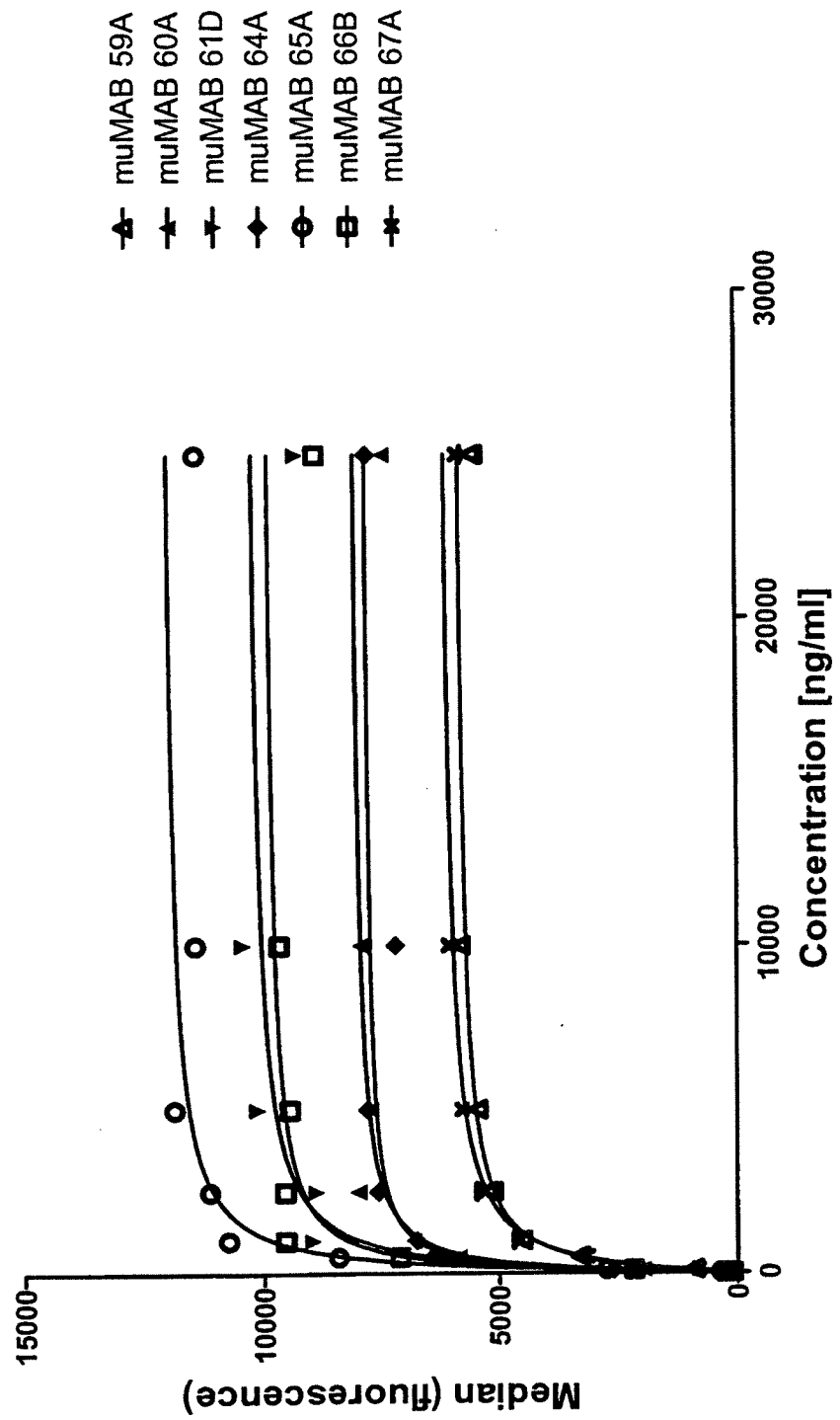

muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Median (fluorescence)
15000
10000
5000
0
0
10000
20000
30000
Concentration [ng/ml]

Fig. 7

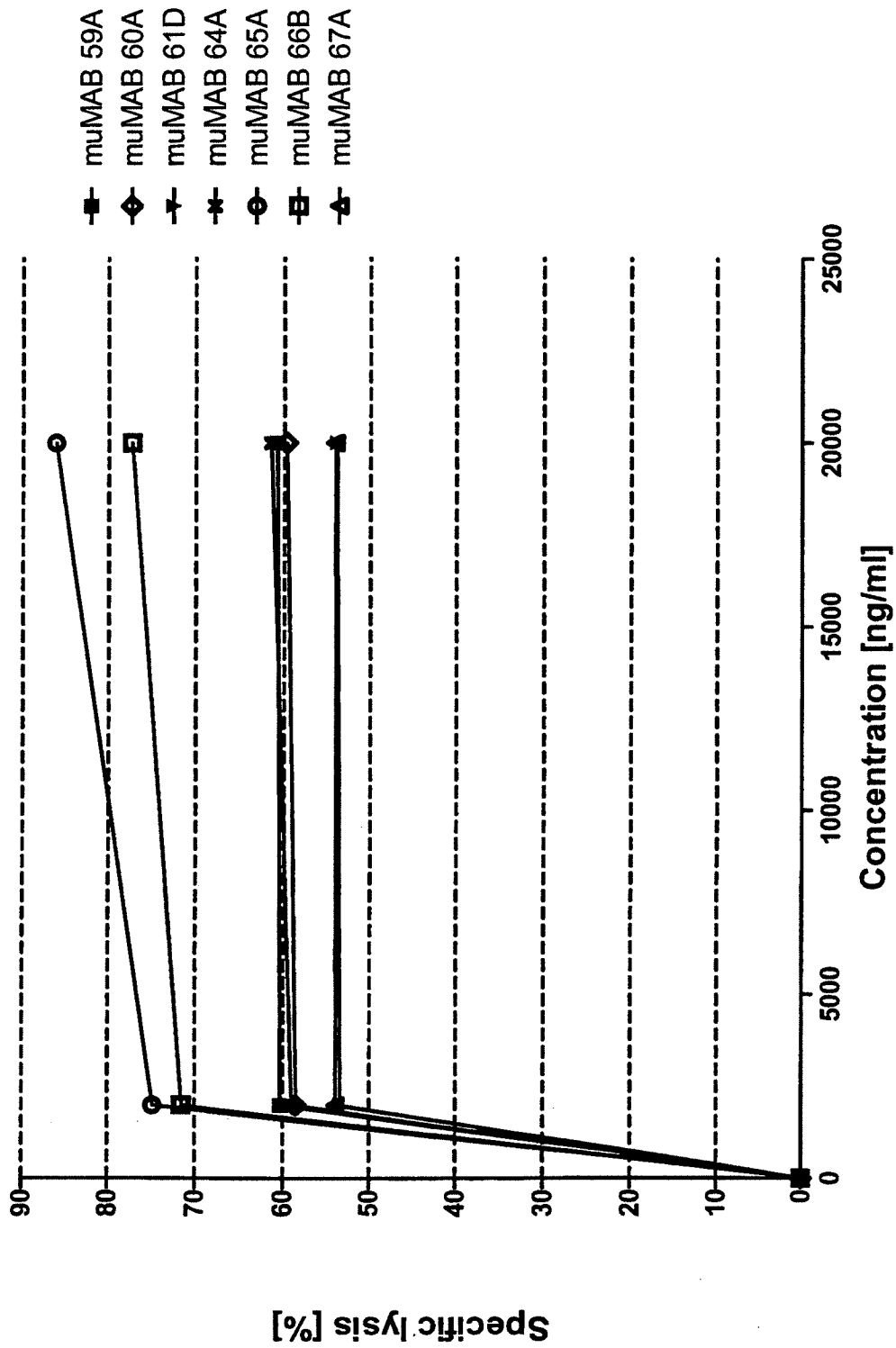

muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Specific lysis [%]
0
10
20
30
40
50
60
70
80
90
0
5000
10000
15000
20000
25000
Concentration [ng/ml]

Fig. 8

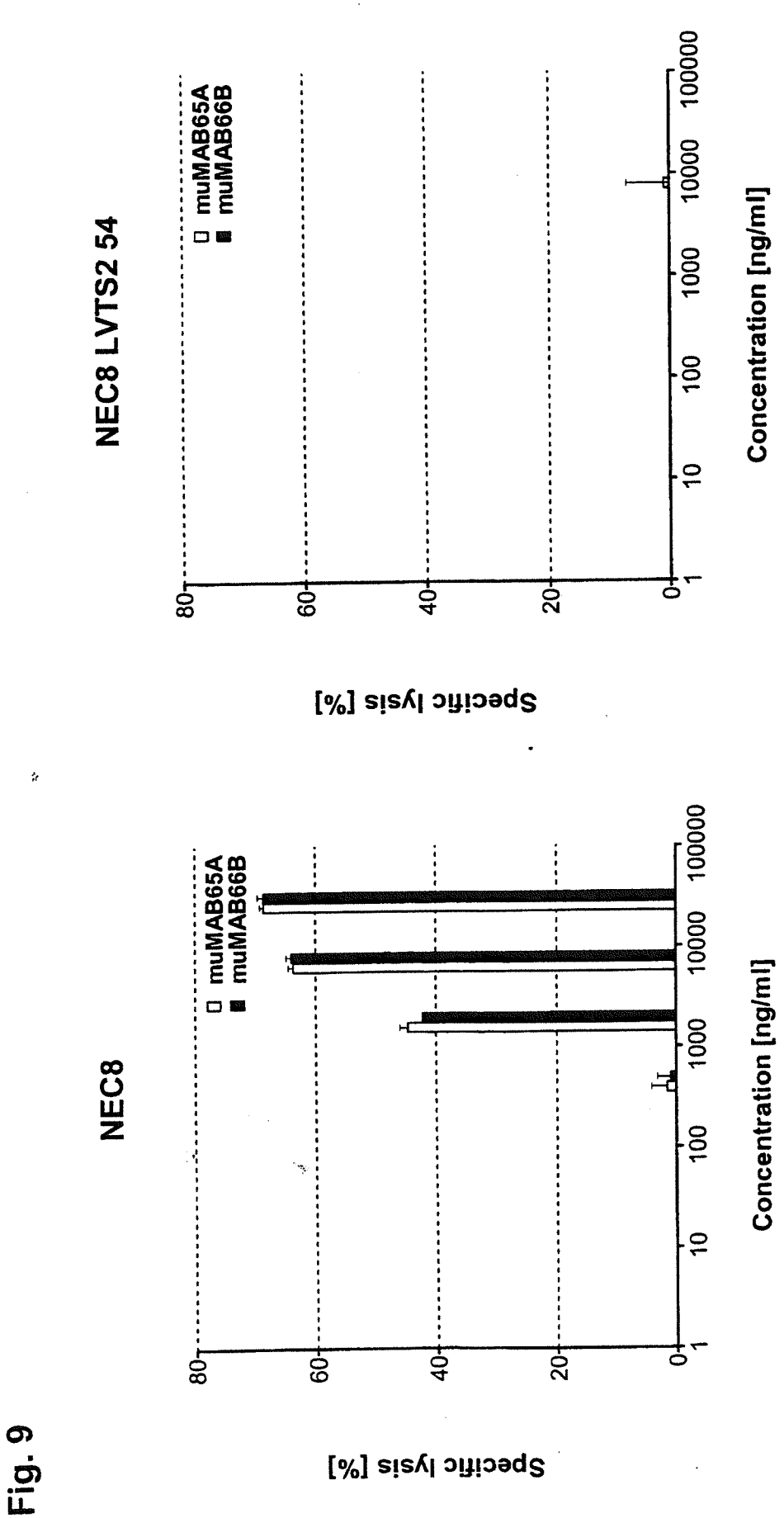

NEC8
muMAB65A
muMAB66B
Specific lysis [%]
0
20
40
60
80
1
10
100
1000
10000
100000
Concentration [ng/ml]
NEC8 LVTS2 54
muMAB65A
muMAB66B
Specific lysis [%]
0
20
40
60
80
1
10
100
1000
10000
100000
Concentration [ng/ml]
error bars: SEM

Fig. 9

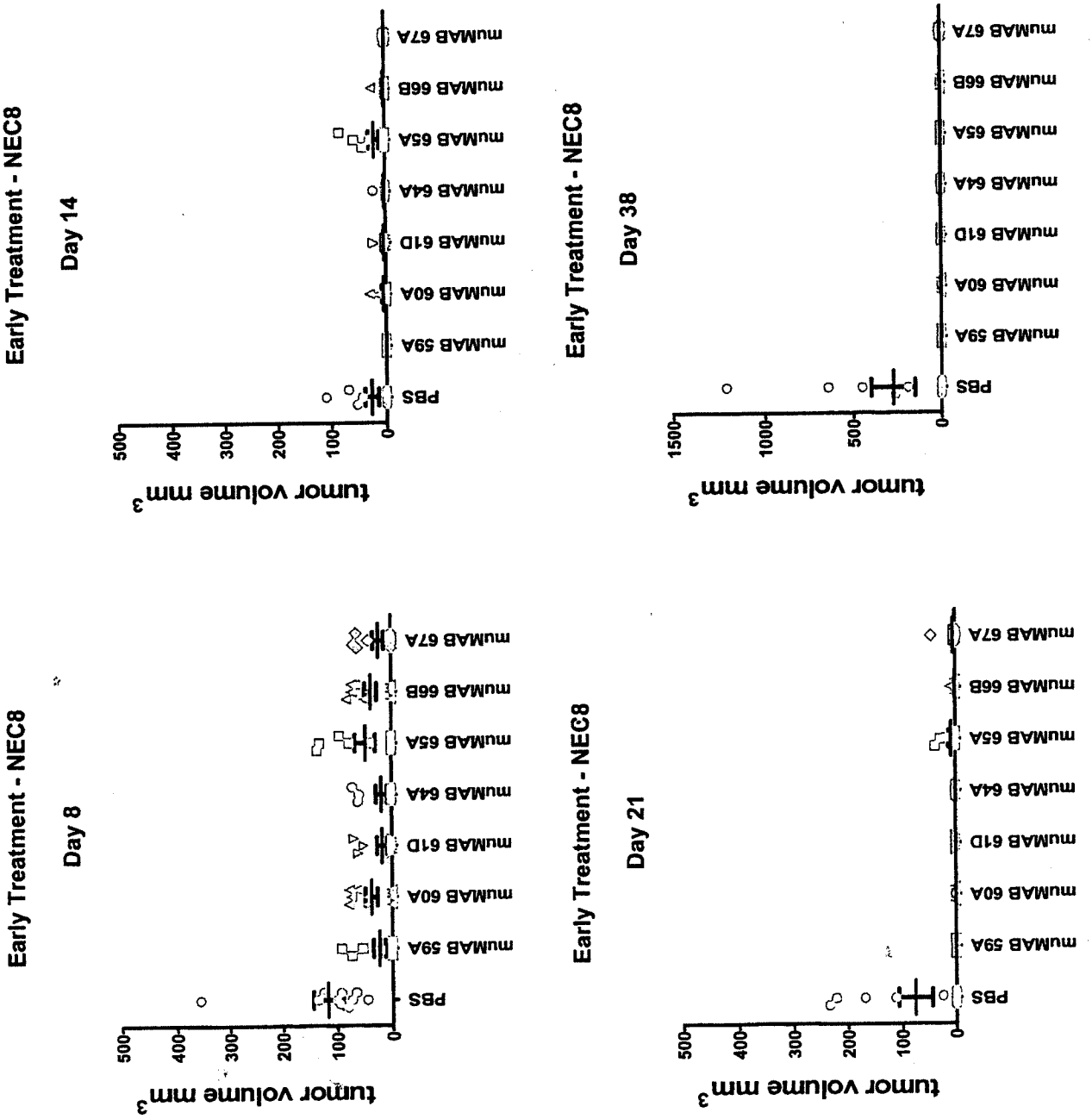

Early Treatment - NEC8
Day 14
tumor volume mm3
0
100
200
300
400
500
PBS
muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Early Treatment - NEC8
Day 38
tumor volume mm3
0
500
1000
1500
PBS
muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Early Treatment - NEC8
Day 8
tumor volume mm3
0
100
200
300
400
500
PBS
muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Early Treatment - NEC8
Day 21
tumor volume mm3
0
100
200
300
400
500
PBS
muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A

Fig. 10

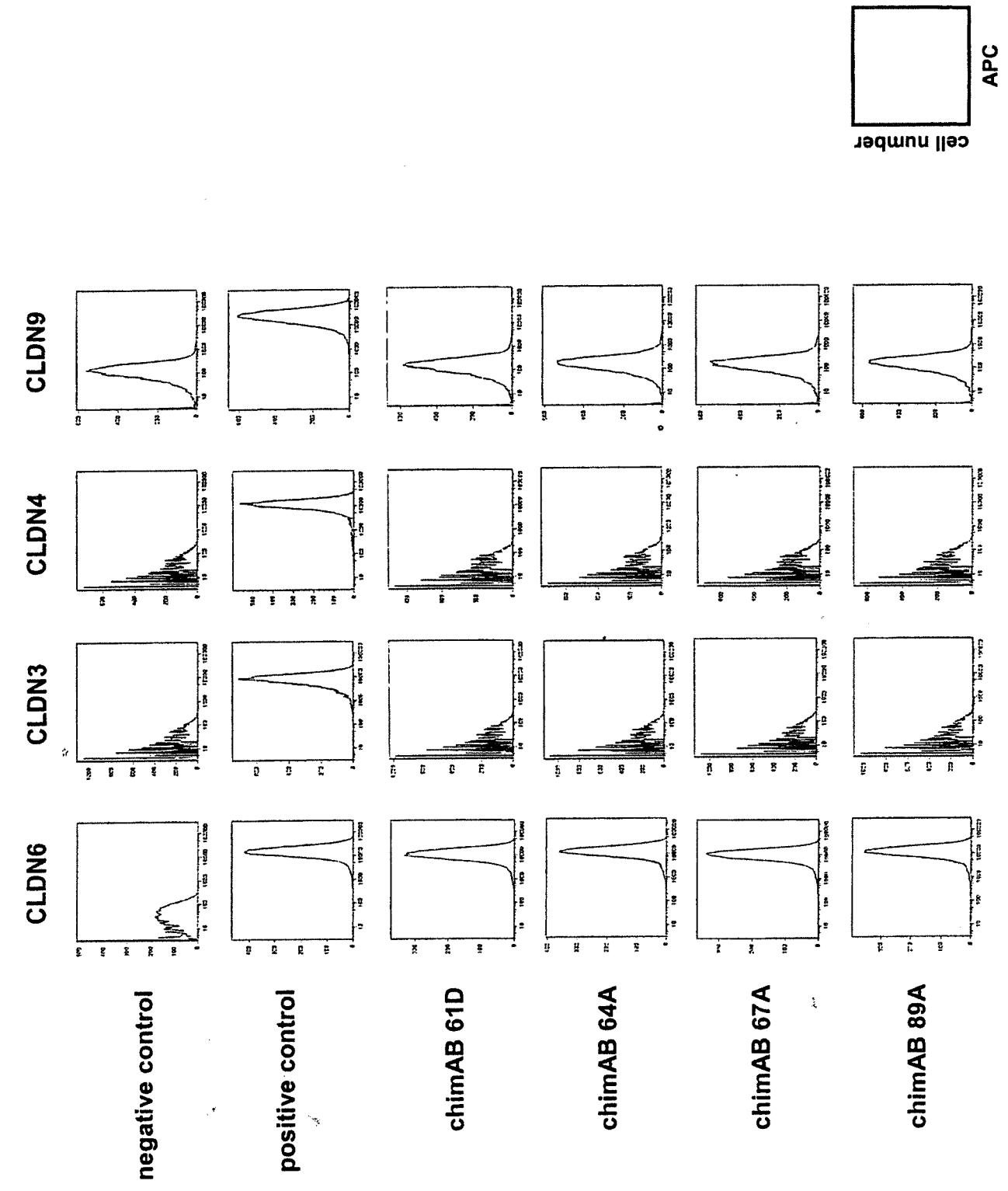

cell number
APC
CLDN6
CLDN3
CLDN4
CLDN9
negative control
positive control
chimAB 61D
chimAB 64A
chimAB 67A
chimAB 89A

Fig. 11

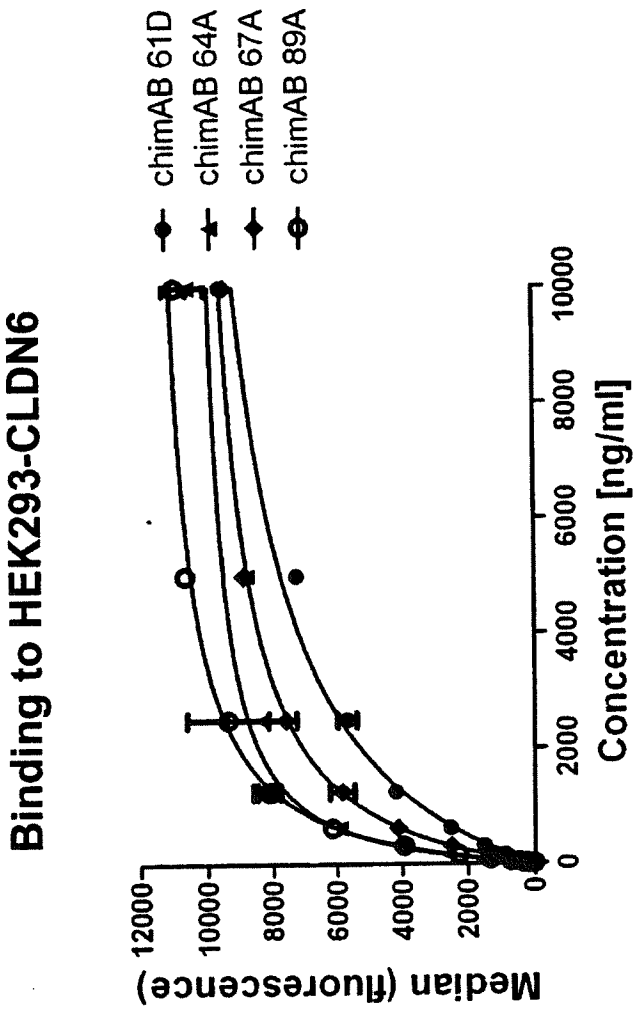

Binding to HEK293-CLDN6
chimAB 61D
chimAB 64A
chimAB 67A
chimAB 89A
Median (fluorescence)
0
2000
4000
6000
8000
10000
12000
0
2000
4000
6000
8000
10000
Concentration [ng/ml]

Fig. 12

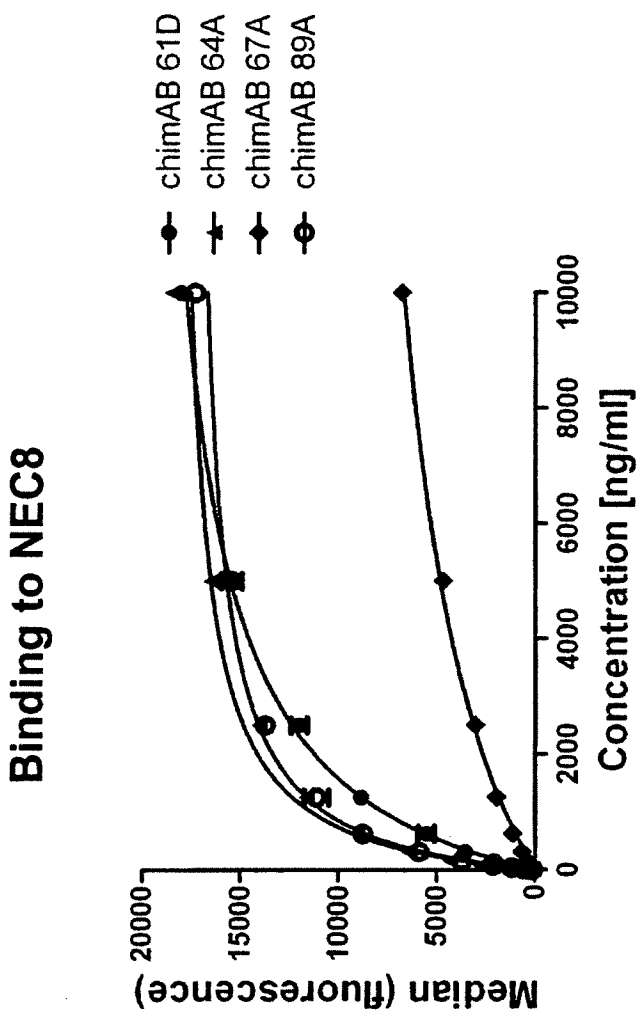

Binding to NEC8
chimAB 61D
chimAB 64A
chimAB 67A
chimAB 89A
Median (fluorescence)
20000
15000
10000
5000
0
0
2000
4000
6000
8000
10000
Concentration [ng/ml]

Fig. 13

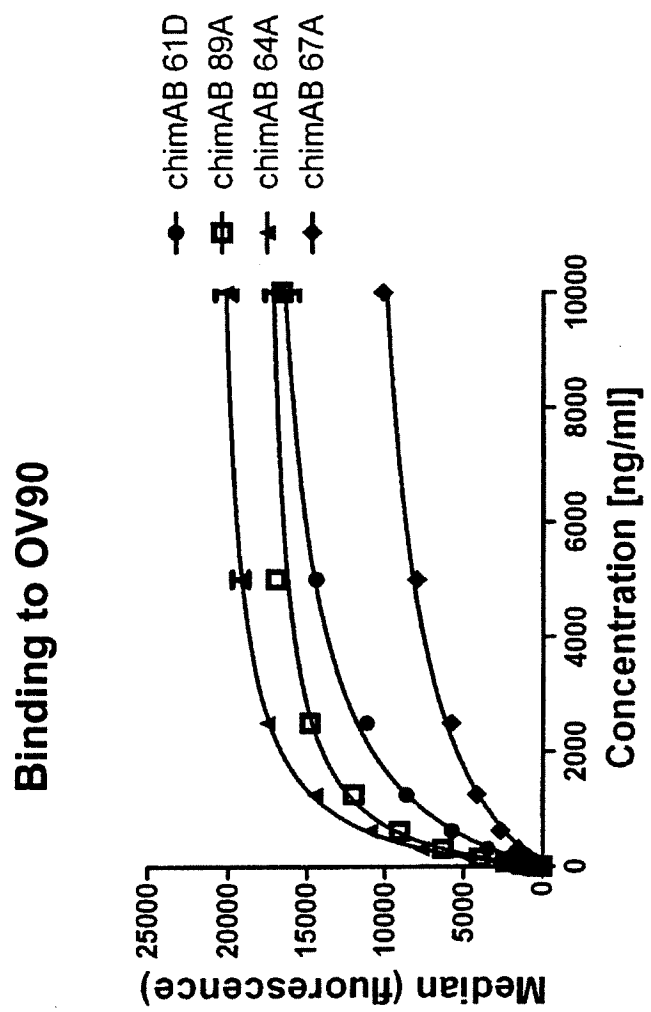

Binding to OV90
chimAB 61D
chimAB 89A
chimAB 64A
chimAB 67A
Median (fluorescence)
25000
20000
15000
10000
5000
0
0
2000
4000
6000
8000
10000
Concentration [ng/ml]

Fig. 14

Fig. 15

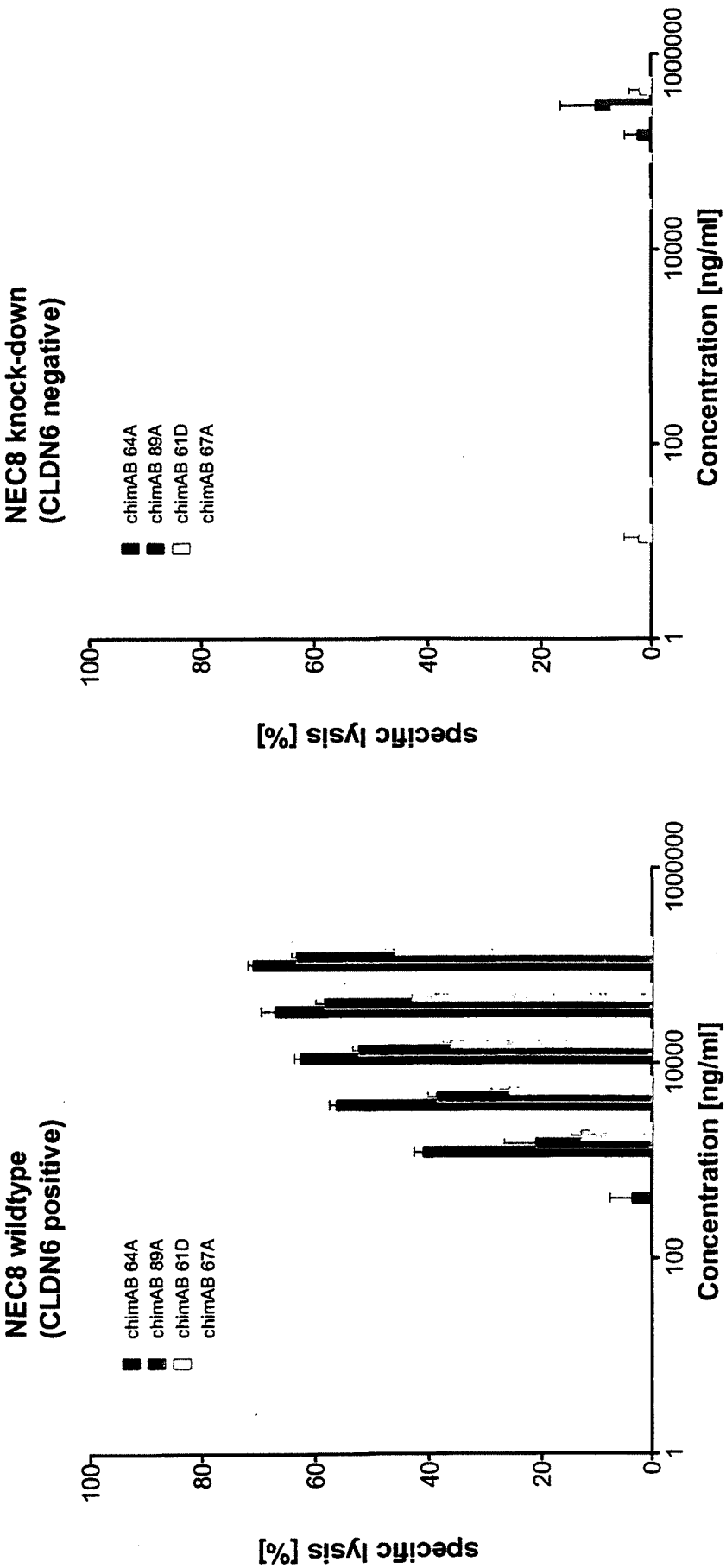

NEC8 knock-down
(CLDN6 negative)
chimAB 64A
chimAB 89A
chimAB 61D
chimAB 67A
specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration [ng/ml]
NEC8 wildtype
(CLDN6 positive)
chimAB 64A
chimAB 89A
chimAB 61D
chimAB 67A
specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration [ng/ml]

Fig. 16

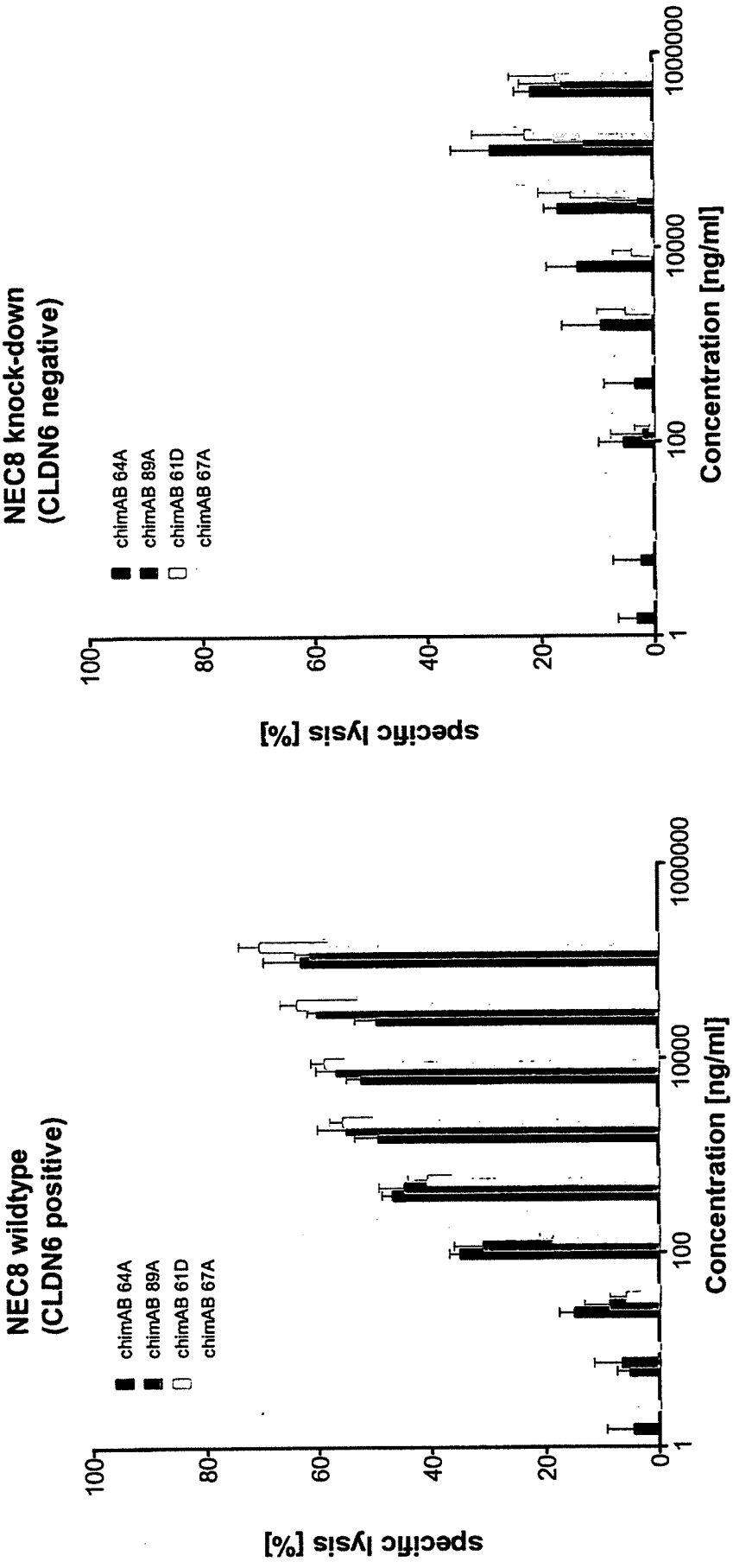

NEC8 knock-down
(CLDN6 negative)
chimAB 64A
chimAB 89A
chimAB 61D
chimAB 67A
specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration [ng/ml]
NEC8 wildtype
(CLDN6 positive)
chimAB 64A
chimAB 89A
chimAB 61D
chimAB 67A
specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration [ng/ml]

Fig. 17

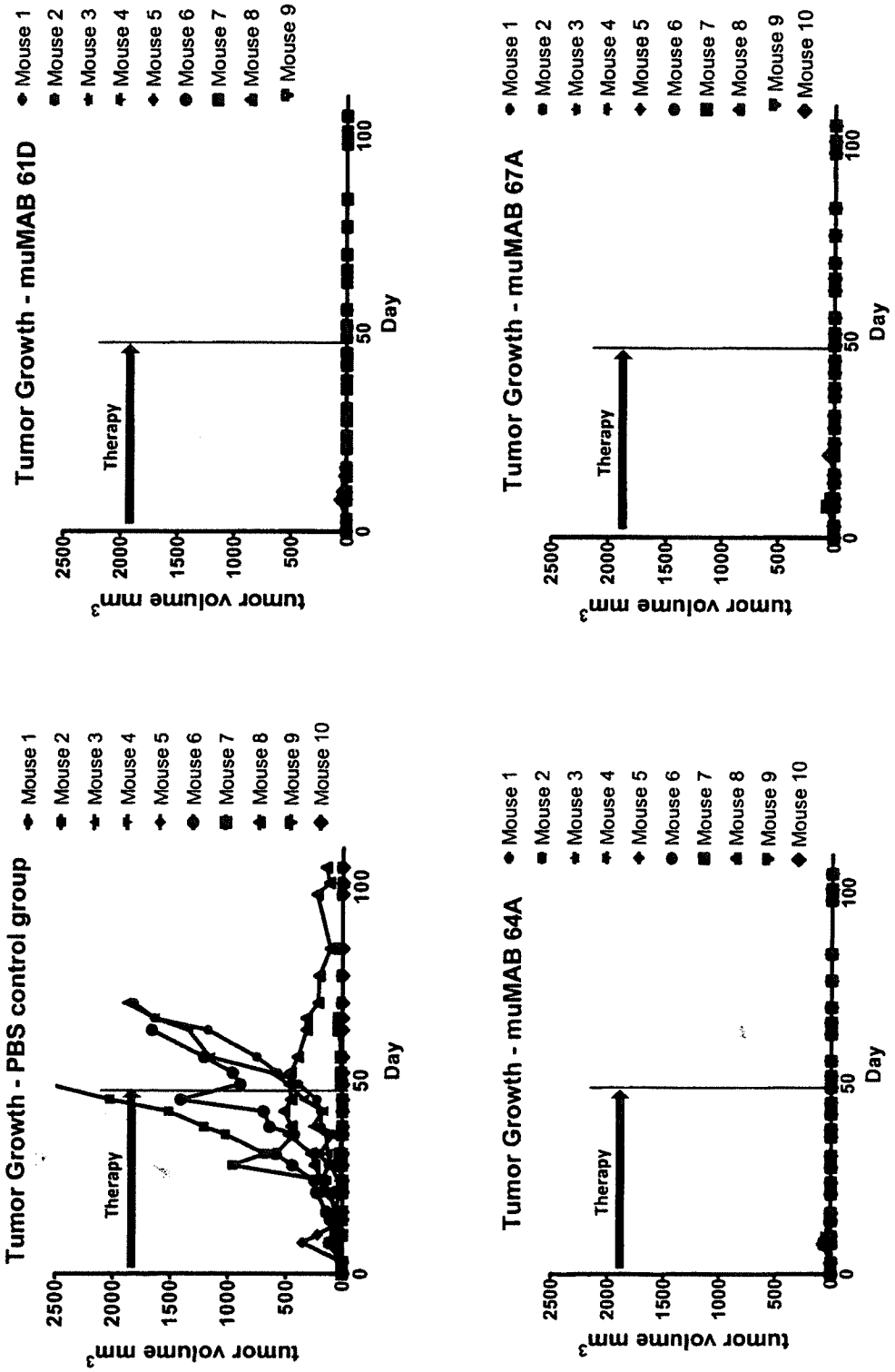
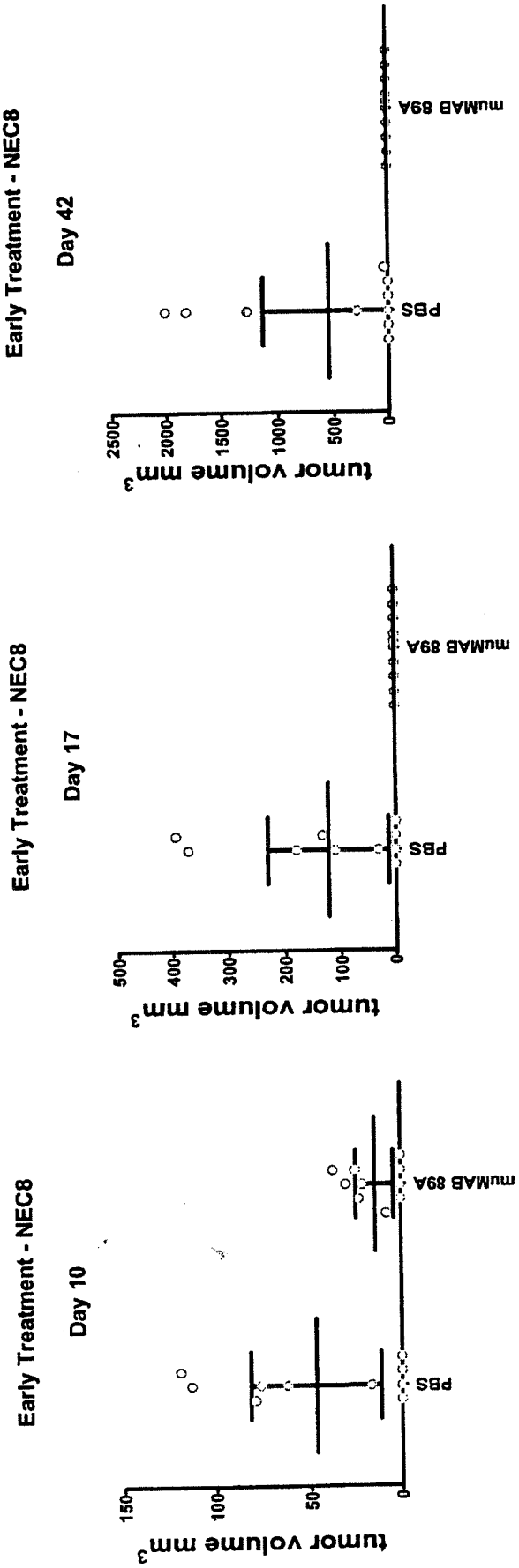

Tumor Growth - PBS control group
Tumor Growth - muMAB 61D
Tumor Growth - muMAB 64A
Tumor Growth - muMAB 67A
Therapy
Day
tumor volume mm³
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10
0
50
100
500
1000
1500
2000
2500

Fig. 18

(A)


Early Treatment - NEC8
Day 10
tumor volume mm3
PBS
muMAB 89A
Early Treatment - NEC8
Day 17
tumor volume mm3
PBS
muMAB 89A
Early Treatment - NEC8
Day 42
tumor volume mm3
PBS
muMAB 89A

(B)

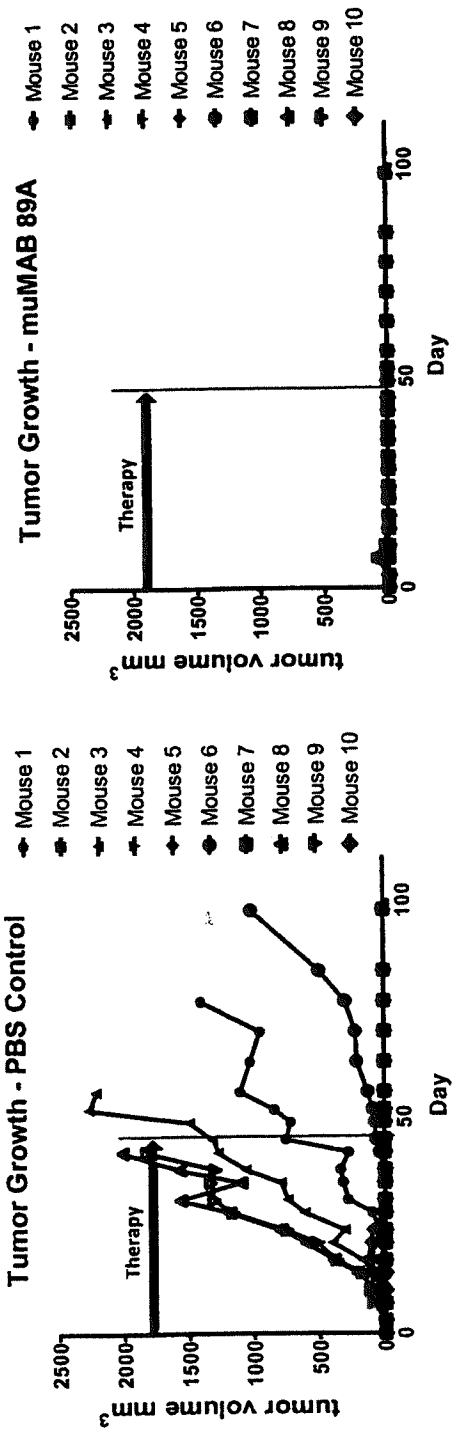

Tumor Growth - PBS Control
Therapy
tumor volume mm3
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10
Tumor Growth - muMAB 89A
Therapy
tumor volume mm3
Day

Fig. 19

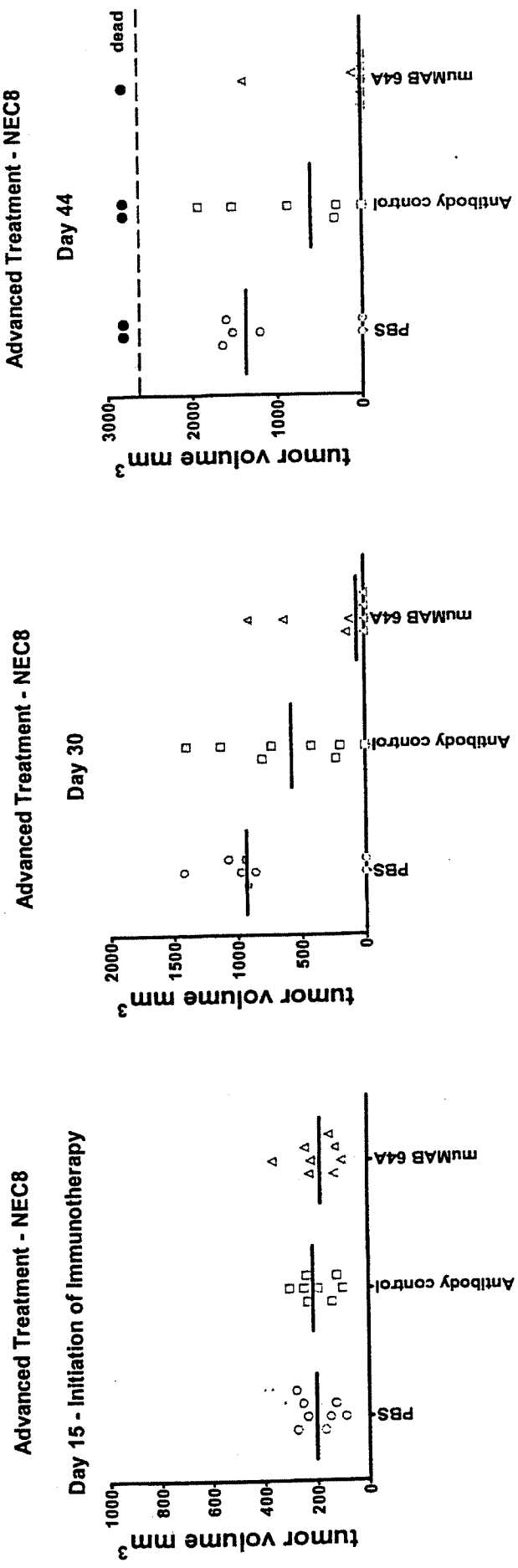
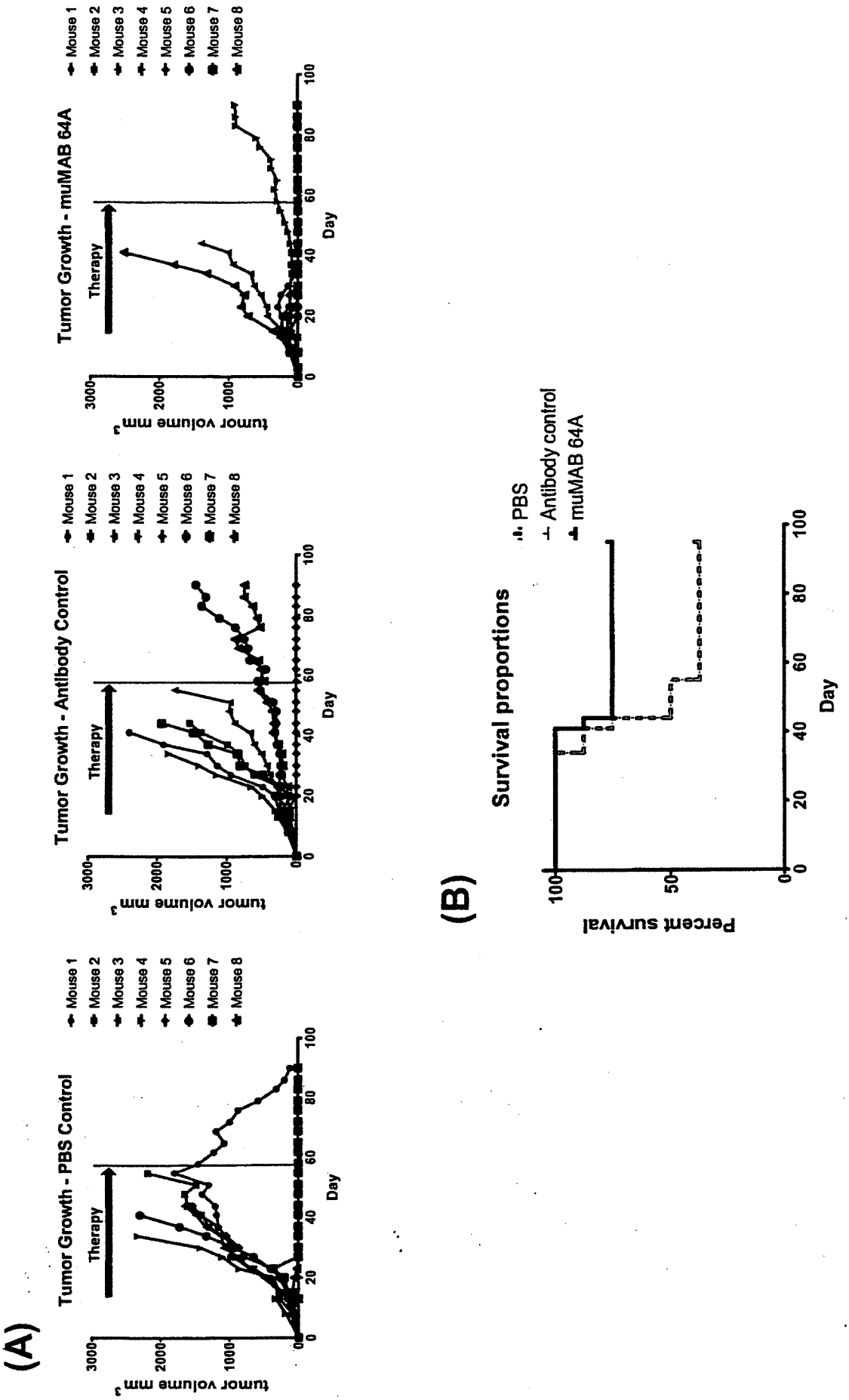

Advanced Treatment - NEC8
Day 15 - Initiation of Immunotherapy
tumor volume mm3
1000
800
600
400
200
0
PBS
Antibody control
muMAB 64A
Advanced Treatment - NEC8
Day 30
tumor volume mm3
2000
1500
1000
500
0
PBS
Antibody control
muMAB 64A
Advanced Treatment - NEC8
Day 44
tumor volume mm3
3000
2000
1000
0
dead
PBS
Antibody control
muMAB 64A

Fig. 20


(A)
Tumor Growth - PBS Control
Tumor Growth - Antibody Control
Tumor Growth - muMAB 64A
Therapy
tumor volume mm³
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
(B)
Survival proportions
PBS
Antibody control
muMAB 64A
Percent survival
Day

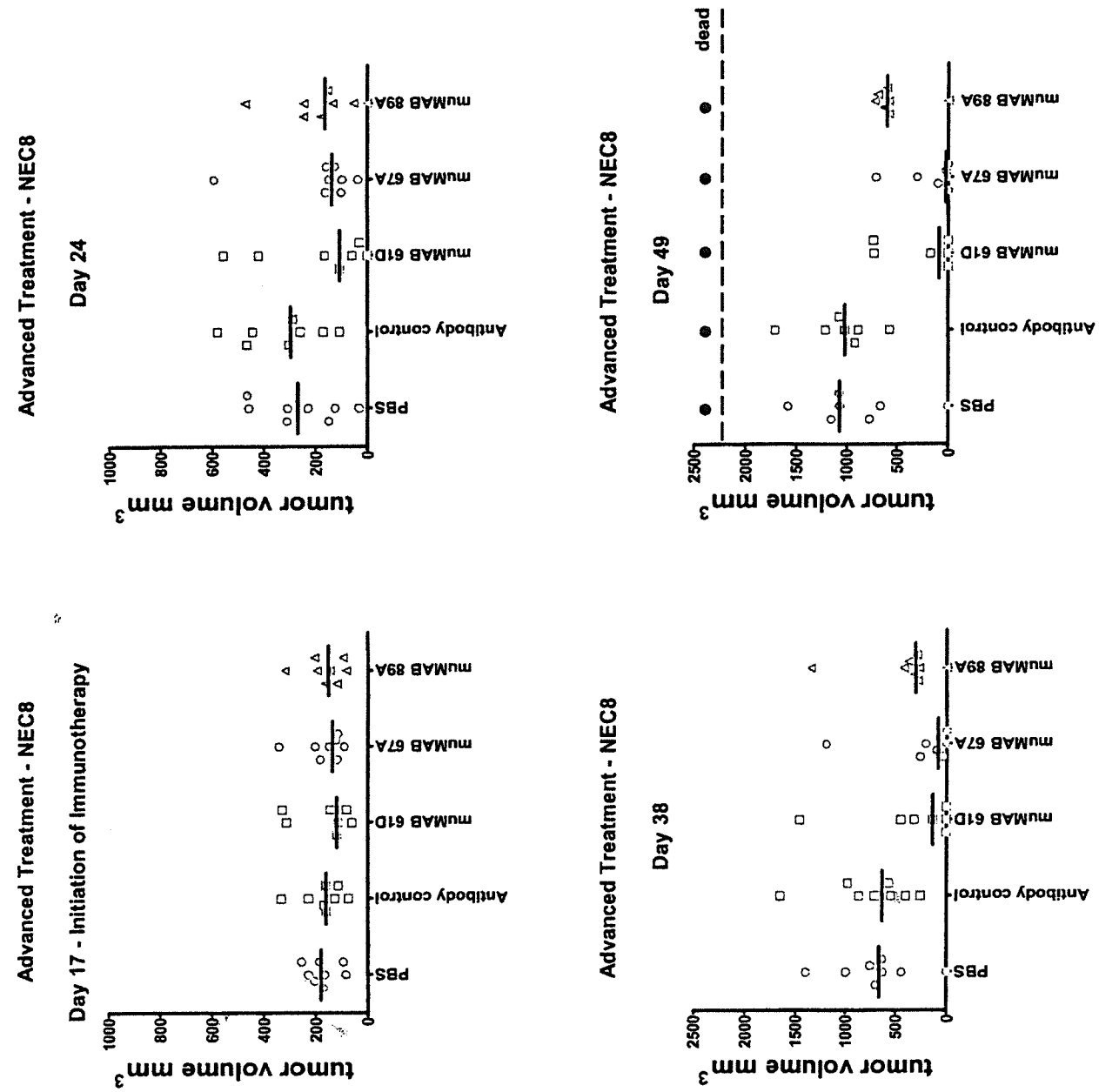
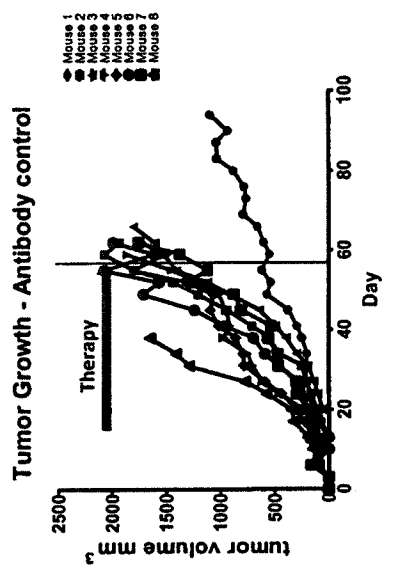
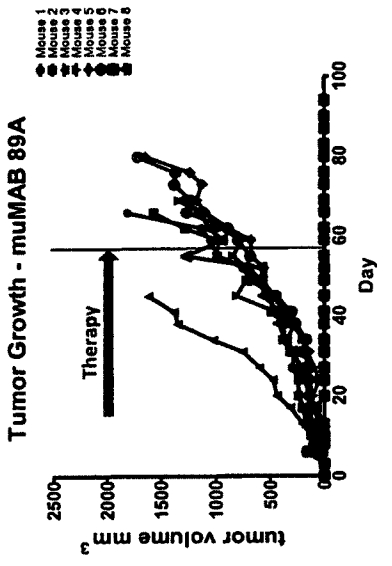

Advanced Treatment - NEC8
Day 24
tumor volume mm3
0
200
400
600
800
1000
PBS
Antibody control
muMAB 61D
muMAB 67A
muMAB 89A
Advanced Treatment - NEC8
Day 49
dead
tumor volume mm3
0
500
1000
1500
2000
2500
PBS
Antibody control
muMAB 61D
muMAB 67A
muMAB 89A
Advanced Treatment - NEC8
Day 17 - Initiation of Immunotherapy
tumor volume mm3
0
200
400
600
800
1000
PBS
Antibody control
muMAB 61D
muMAB 67A
muMAB 89A
Advanced Treatment - NEC8
Day 38
tumor volume mm3
0
500
1000
1500
2000
2500
PBS
Antibody control
muMAB 61D
muMAB 67A
muMAB 89A

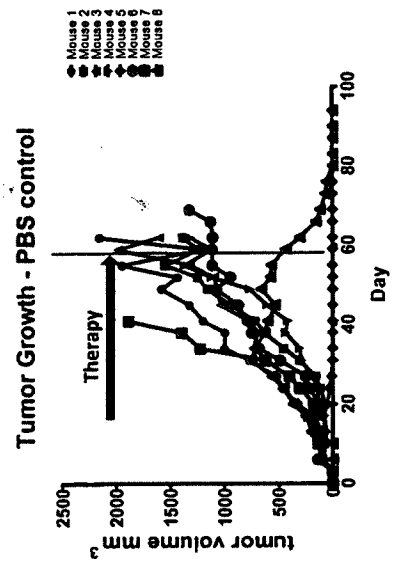
Fig. 21

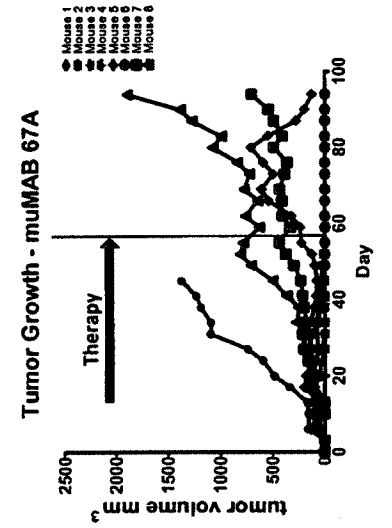
Fig. 22

(A)


Tumor Growth - Antibody control
Therapy
Day
tumor volume mm³
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8


Tumor Growth - muMAB 89A
Therapy
Day
tumor volume mm³
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8


Tumor Growth - PBS control
Therapy
Day
tumor volume mm³
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8


Tumor Growth - muMAB 67A
Therapy
Day
tumor volume mm³
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8

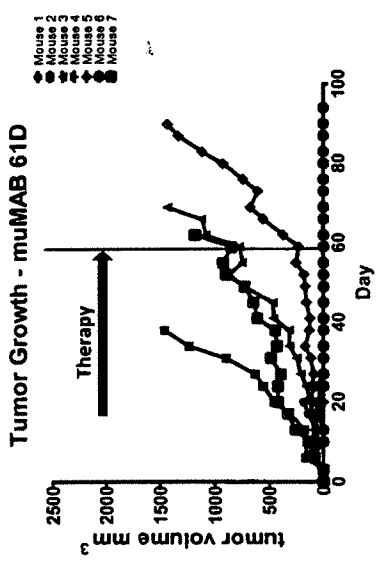

Tumor Growth - muMAB 61D
Therapy
Day
tumor volume mm³
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7

Fig. 22

(B)

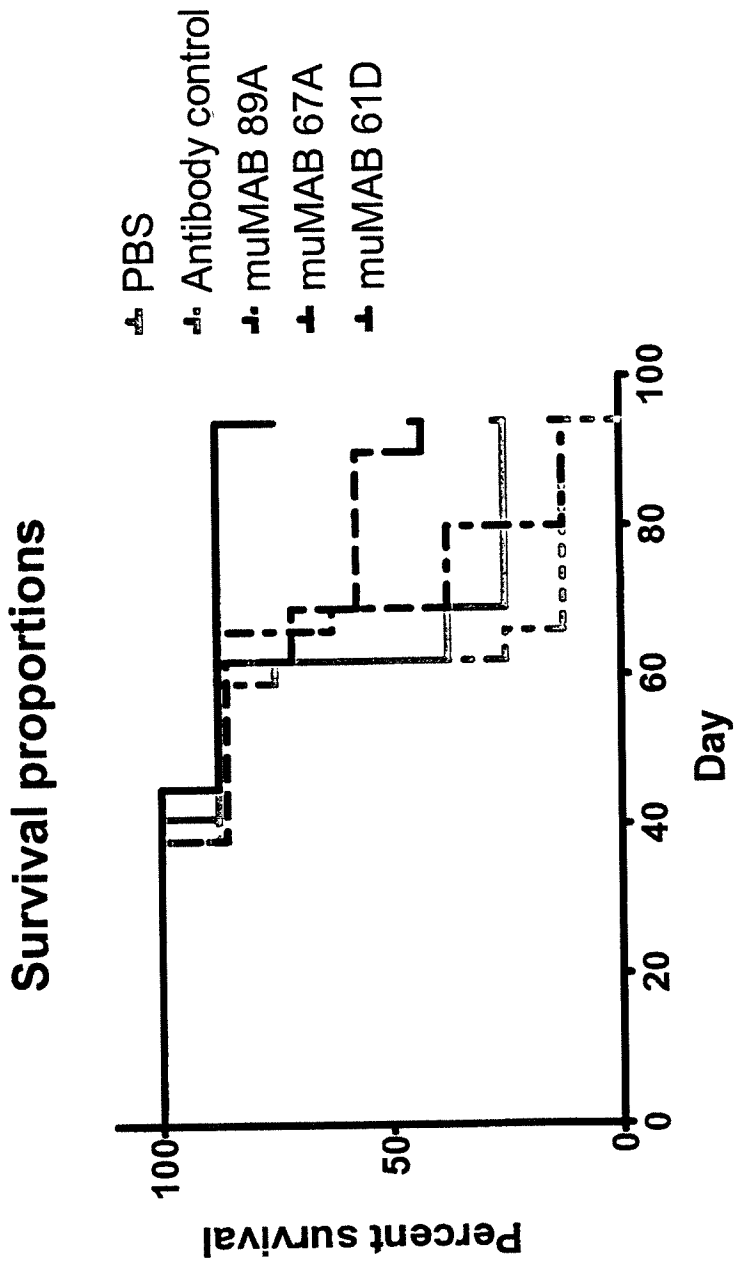

Survival proportions
PBS
Antibody control
muMAB 89A
muMAB 67A
muMAB 61D
Percent survival
100
50
0
0
20
40
60
80
100
Day

Fig. 23

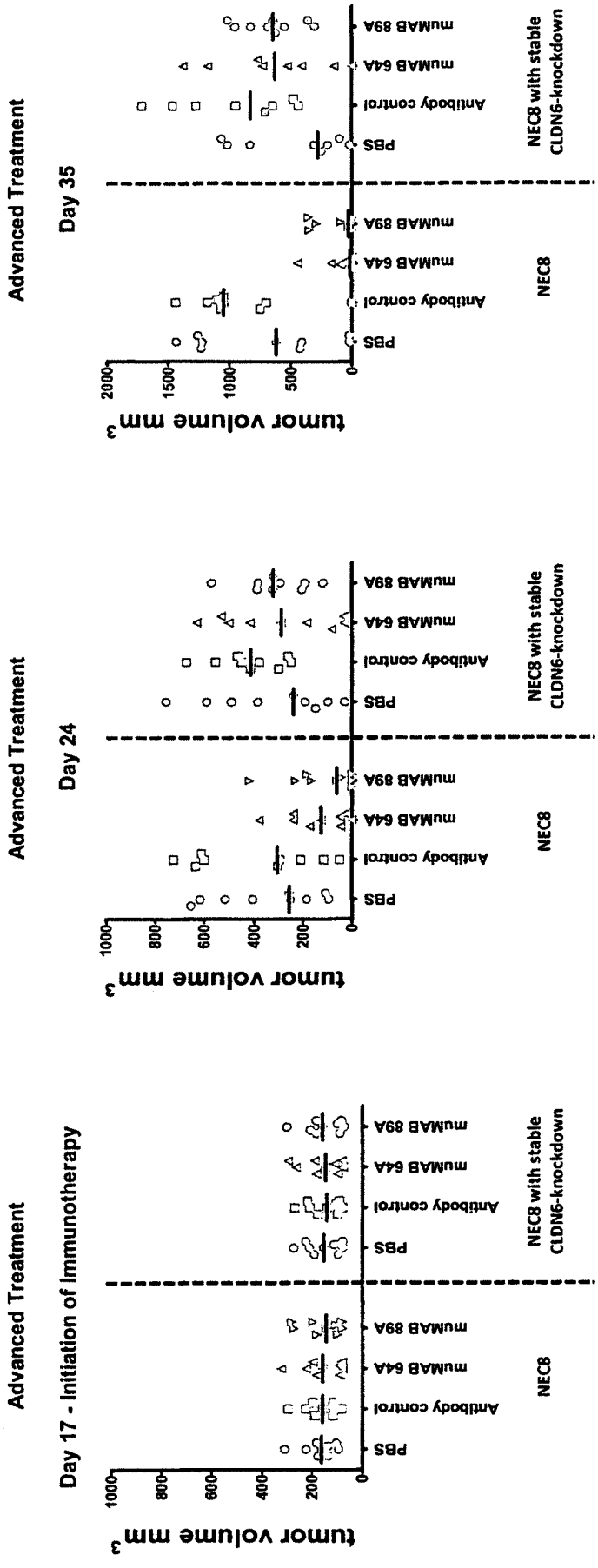

Advanced Treatment
Day 17 - Initiation of Immunotherapy
Day 24
Day 35
tumor volume mm3
0
200
400
600
800
1000
500
1000
1500
2000
PBS
Antibody control
muMAB 64A
muMAB 89A
NEC8
NEC8 with stable CLDN6-knockdown

Fig. 24

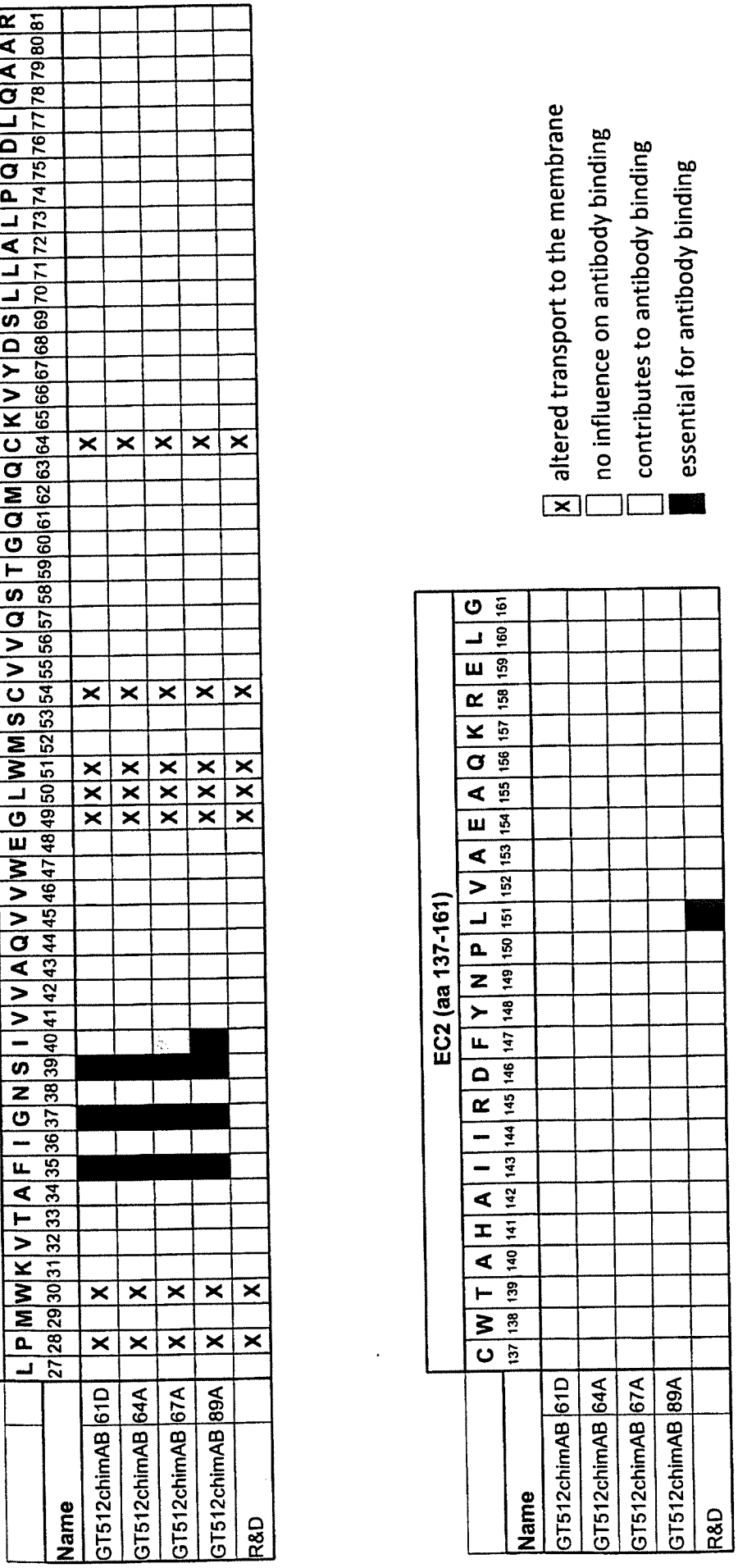

EC1 (aa 27-81)
Name
GT512chimAB 61D
GT512chimAB 64A
GT512chimAB 67A
GT512chimAB 89A
R&D
EC2 (aa 137-161)
altered transport to the membrane
no influence on antibody binding
contributes to antibody binding
essential for antibody binding

Fig. 25

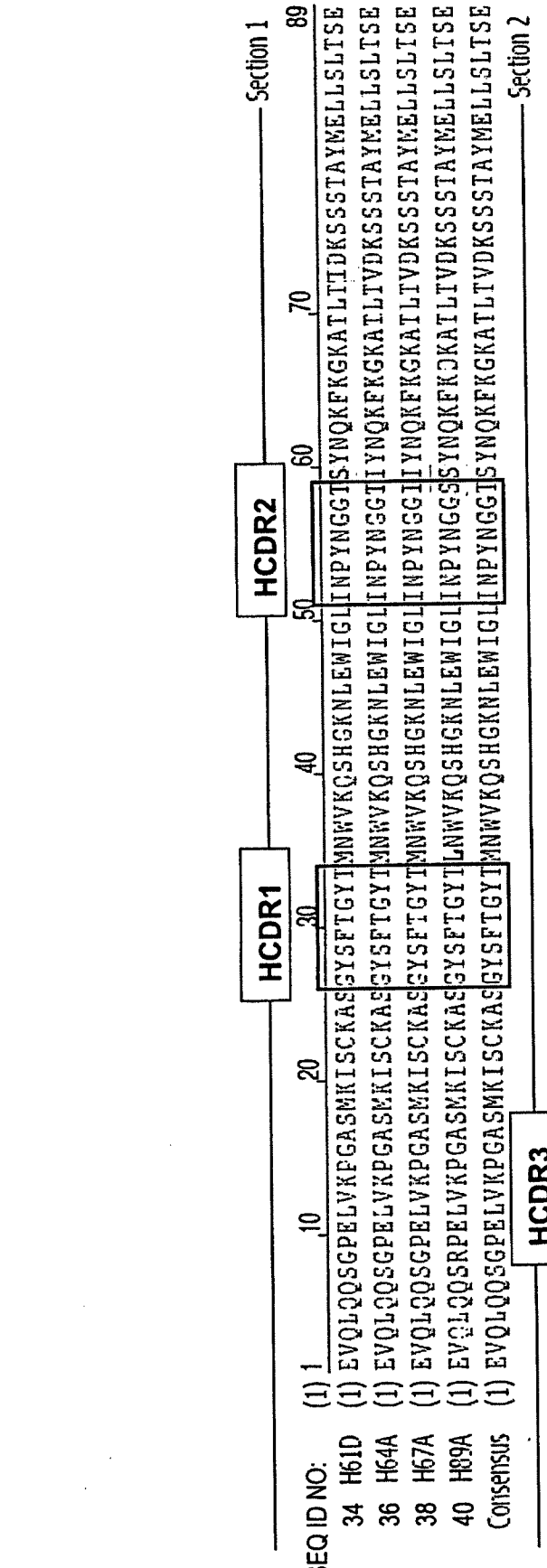
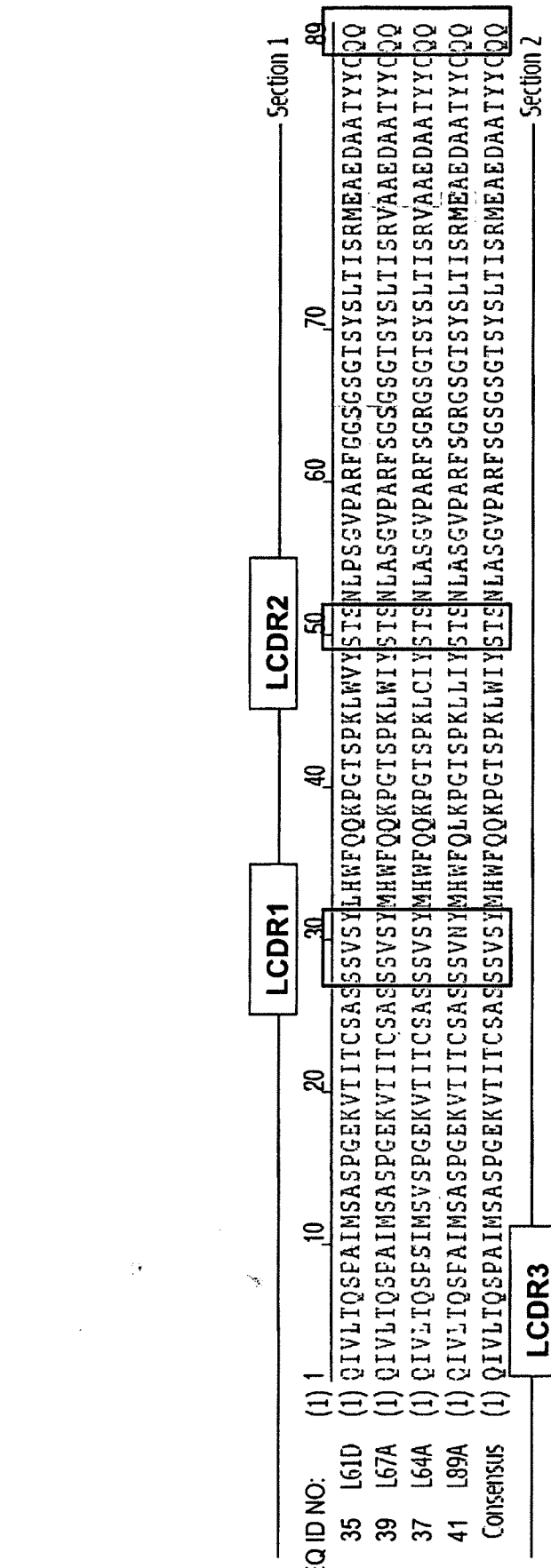

HCDR1
HCDR2
HCDR3
Section 1
Section 2
SEQ ID NO:
34 H61D
36 H64A
38 H67A
40 H89A
Consensus
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGTSYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGTSYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGTSYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGSSYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(1) EVQLQQSRPELVKPGASMKISCKASGYSFTGYTLNWVKQSHGKNLEWIGLINPYNGGSSYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGTSYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(911) DSAVYYCARDYGYVLDYWGQGTTLTVSS
(911) DSAVYYCARCYGFVLDYWGQGTTLTVSS
(911) DSAVFYCARCFGYVLDYWGQGTTLTVSS
(911) DSAVYYCARDYGYVFDYWGQGTTLTVSS
(911) DSAVYYCARDYGYVLDYWGQGTTLTVSS

Fig. 26


LCDR1
LCDR2
LCDR3
Section 1
Section 2
SEQ ID NO:
35 L61D
39 L67A
37 L64A
41 L89A
Consensus

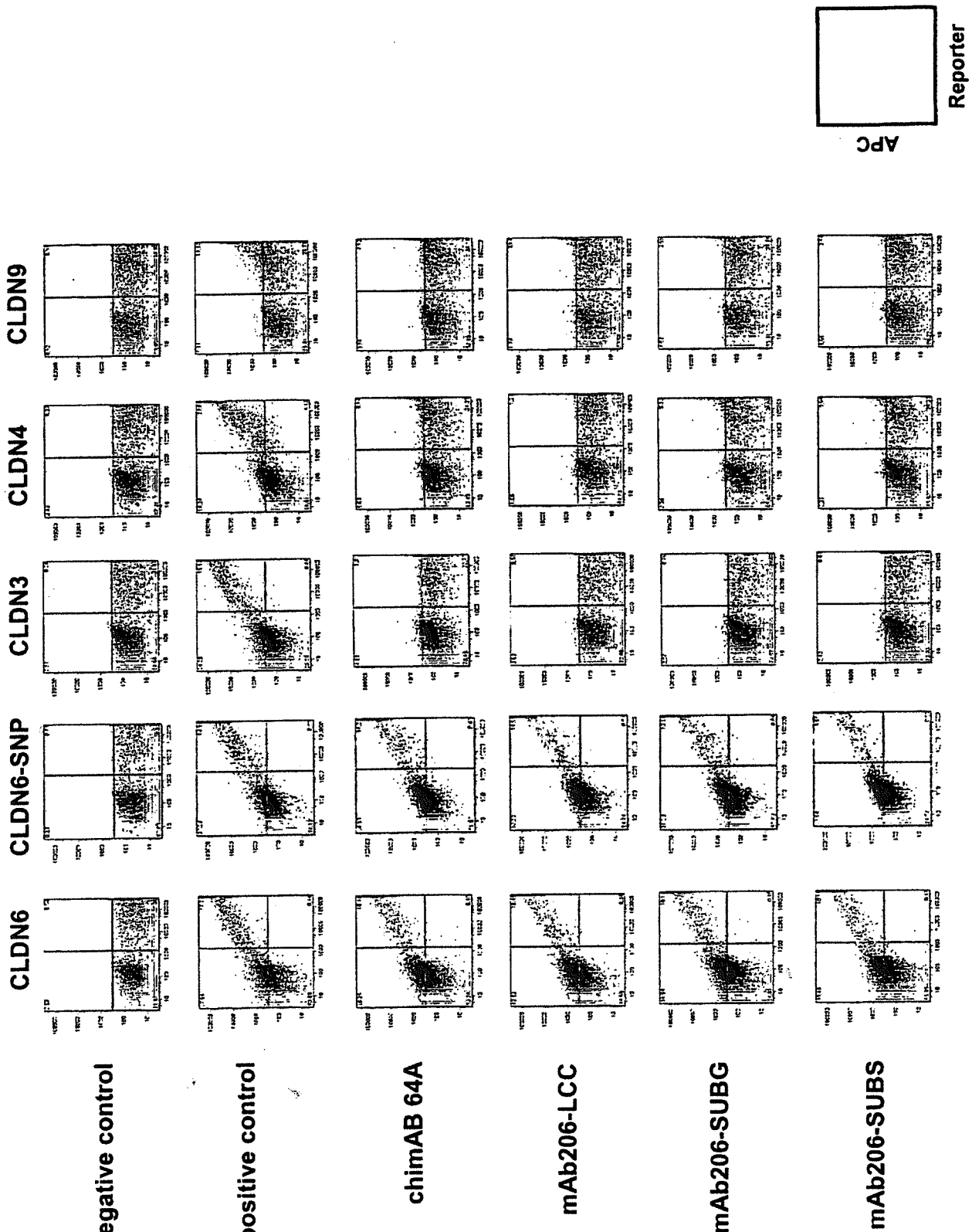

CLDN6
CLDN6-SNP
CLDN3
CLDN4
CLDN9
negative control
positive control
chimAB 64A
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
APC
Reporter

Fig. 27

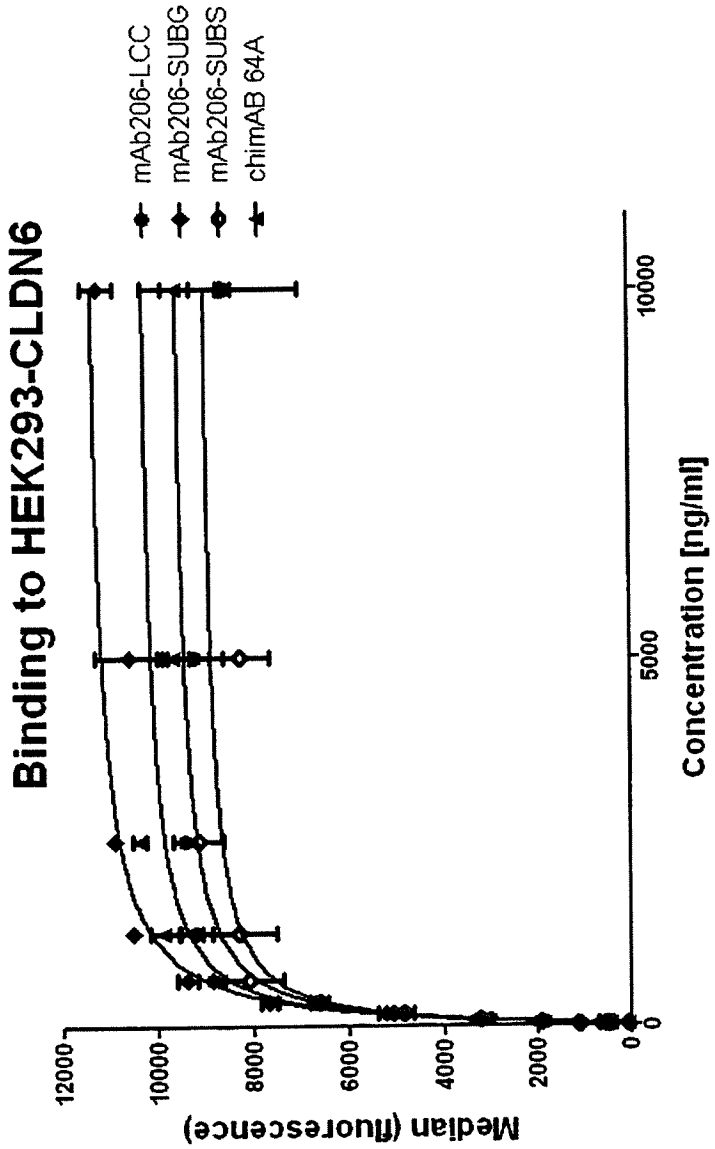

Binding to HEK293-CLDN6
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
chimAB 64A
Median (fluorescence)
12000
10000
8000
6000
4000
2000
0
0
5000
10000
Concentration [ng/ml]

Fig. 28

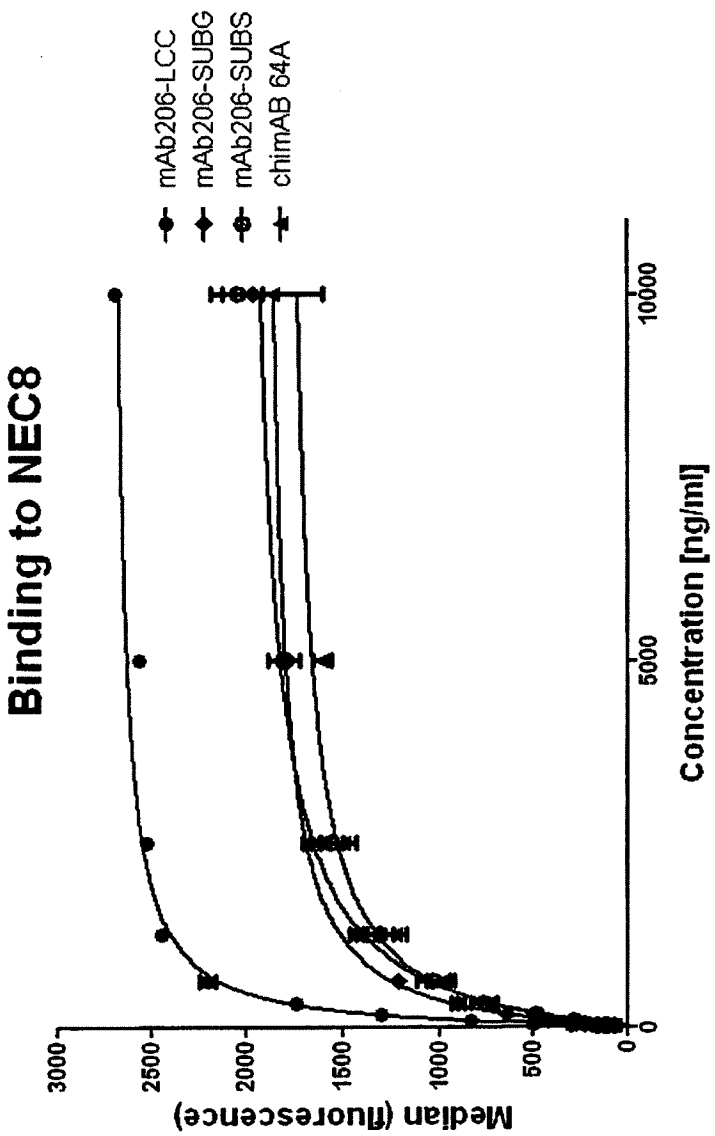

Binding to NEC8
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
chimAB 64A
Median (fluorescence)
3000
2500
2000
1500
1000
500
0
0
5000
10000
Concentration [ng/ml]

Fig. 29

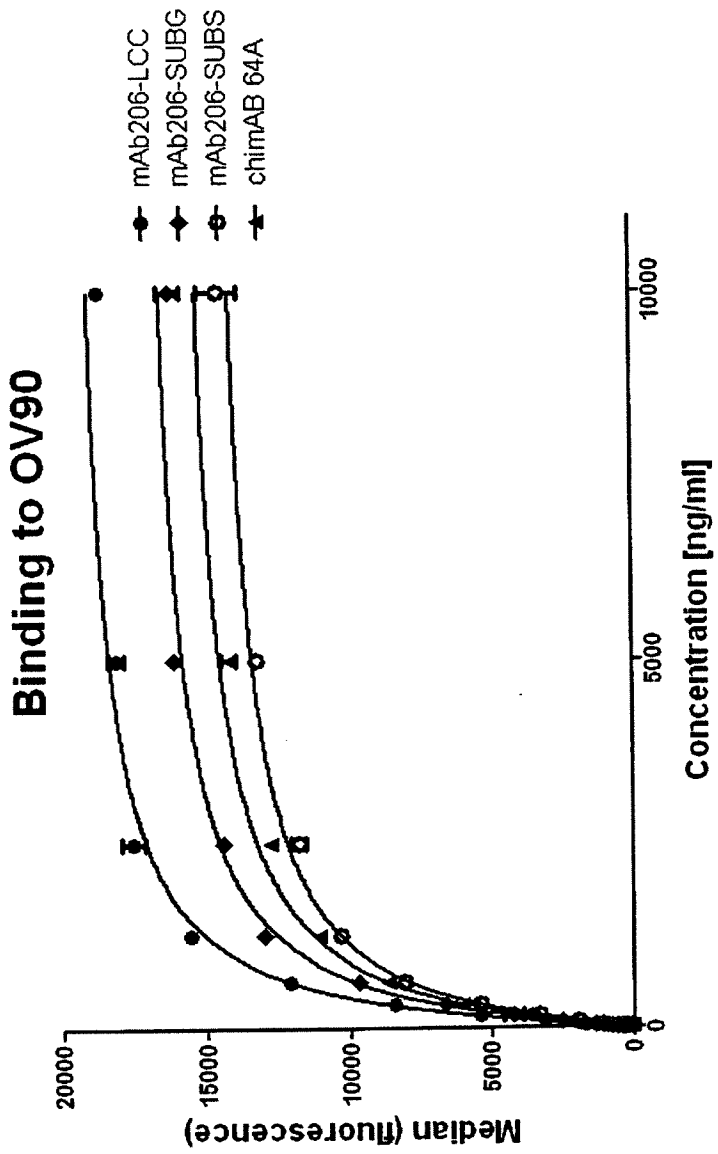
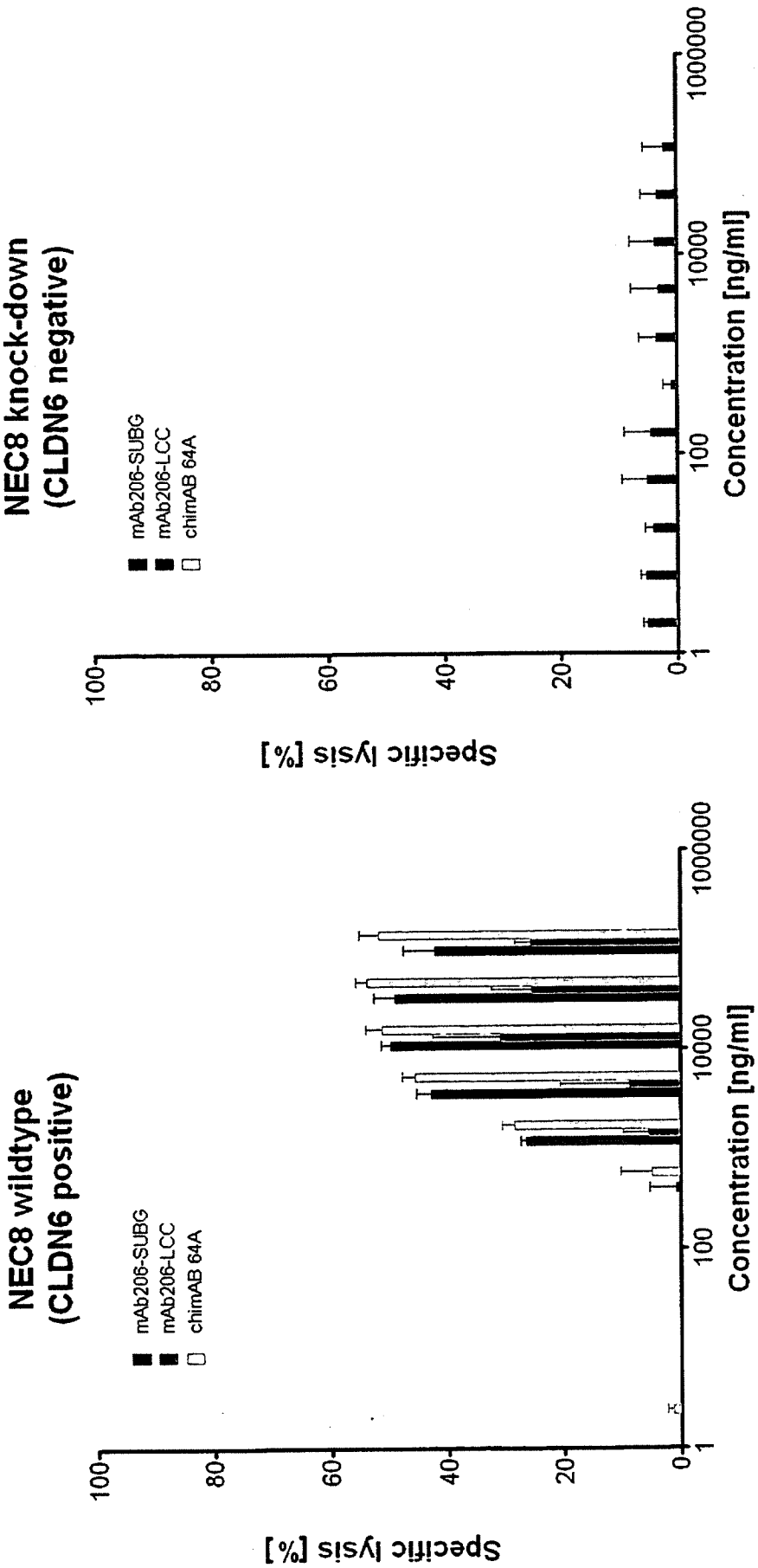

Binding to OV90
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
chimAB 64A
Median (fluorescence)
20000
15000
10000
5000
0
0
5000
10000
Concentration [ng/ml]

Fig. 30

Fig. 31a


NEC8 knock-down
(CLDN6 negative)
mAb206-SUBG
mAb206-LCC
chimAB 64A
Specific lysis [%]
Concentration [ng/ml]
NEC8 wildtype
(CLDN6 positive)
mAb206-SUBG
mAb206-LCC
chimAB 64A
Specific lysis [%]
Concentration [ng/ml]

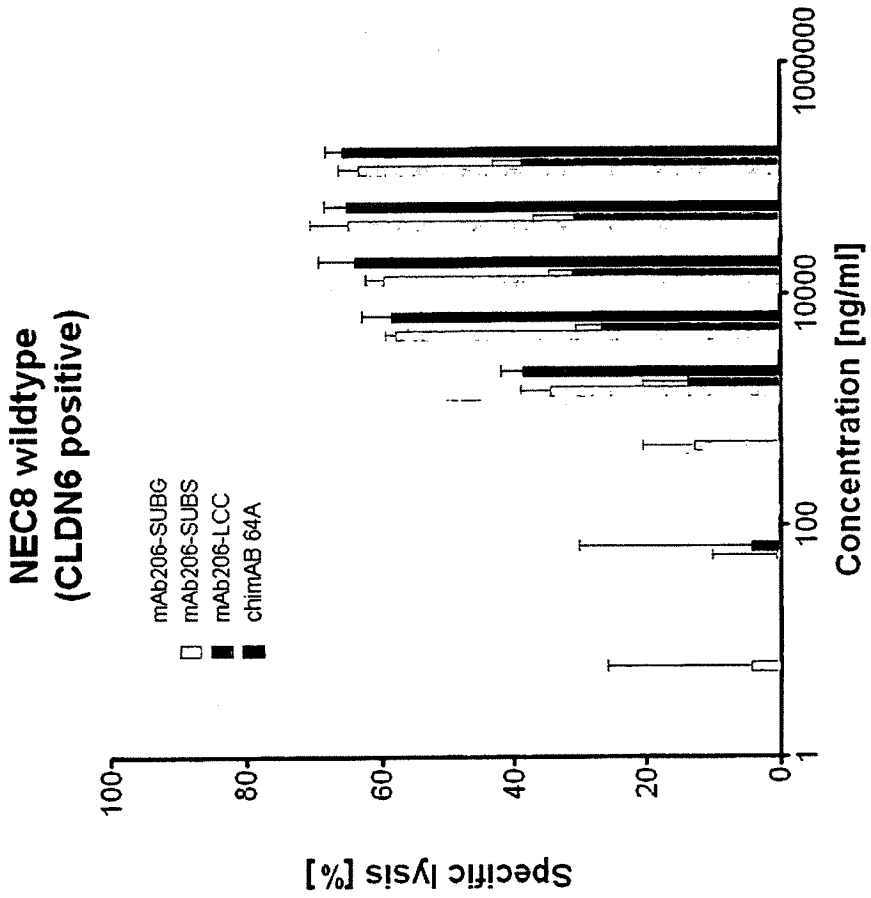

NEC8 wildtype
(CLDN6 positive)
mAb206-SUBG
mAb206-SUBS
mAb206-LCC
chimAB 64A
Specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration [ng/ml]

Fig. 31b

Fig. 32a

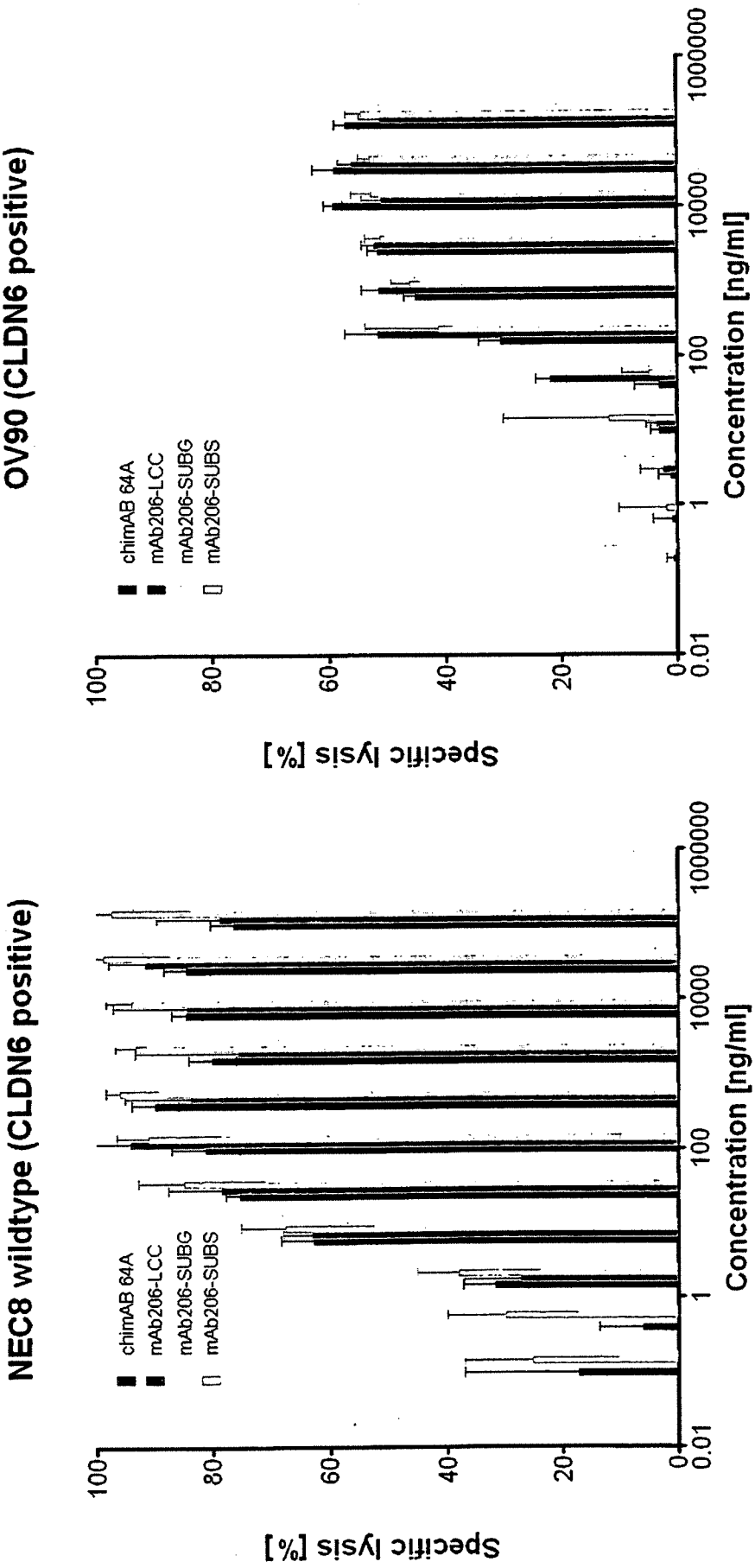

NEC8 wildtype (CLDN6 positive)
OV90 (CLDN6 positive)
chimAB 64A
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
Specific lysis [%]
Concentration [ng/ml]
0
20
40
60
80
100
0.01
1
100
10000
1000000

Fig. 32b

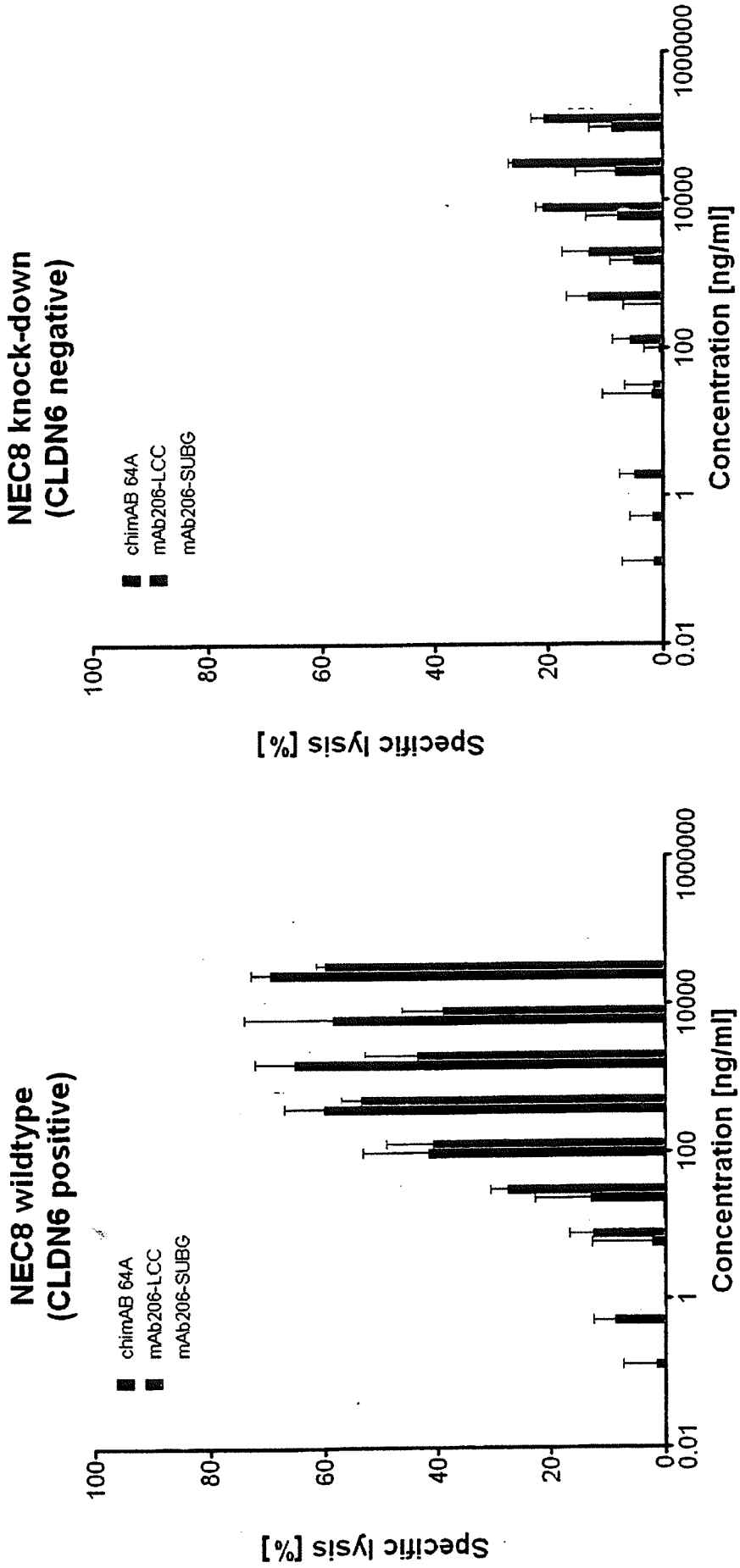

NEC8 knock-down
(CLDN6 negative)
chimAB 64A
mAb206-LCC
mAb206-SUBG
Specific lysis [%]
Concentration [ng/ml]
0
20
40
60
80
100
0.01
1
100
10000
1000000
NEC8 wildtype
(CLDN6 positive)
chimAB 64A
mAb206-LCC
mAb206-SUBG
Specific lysis [%]
Concentration [ng/ml]

Fig. 33

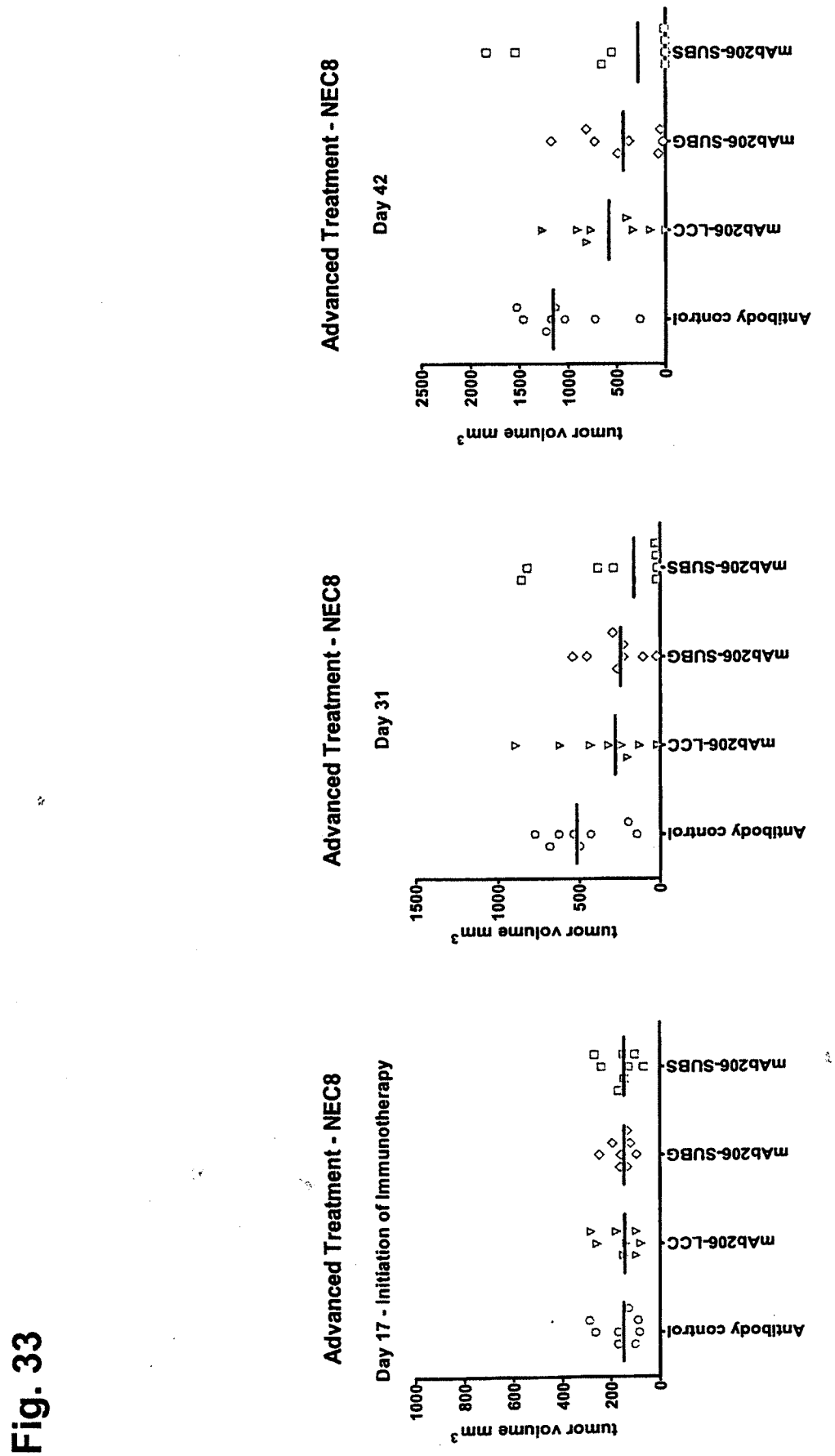
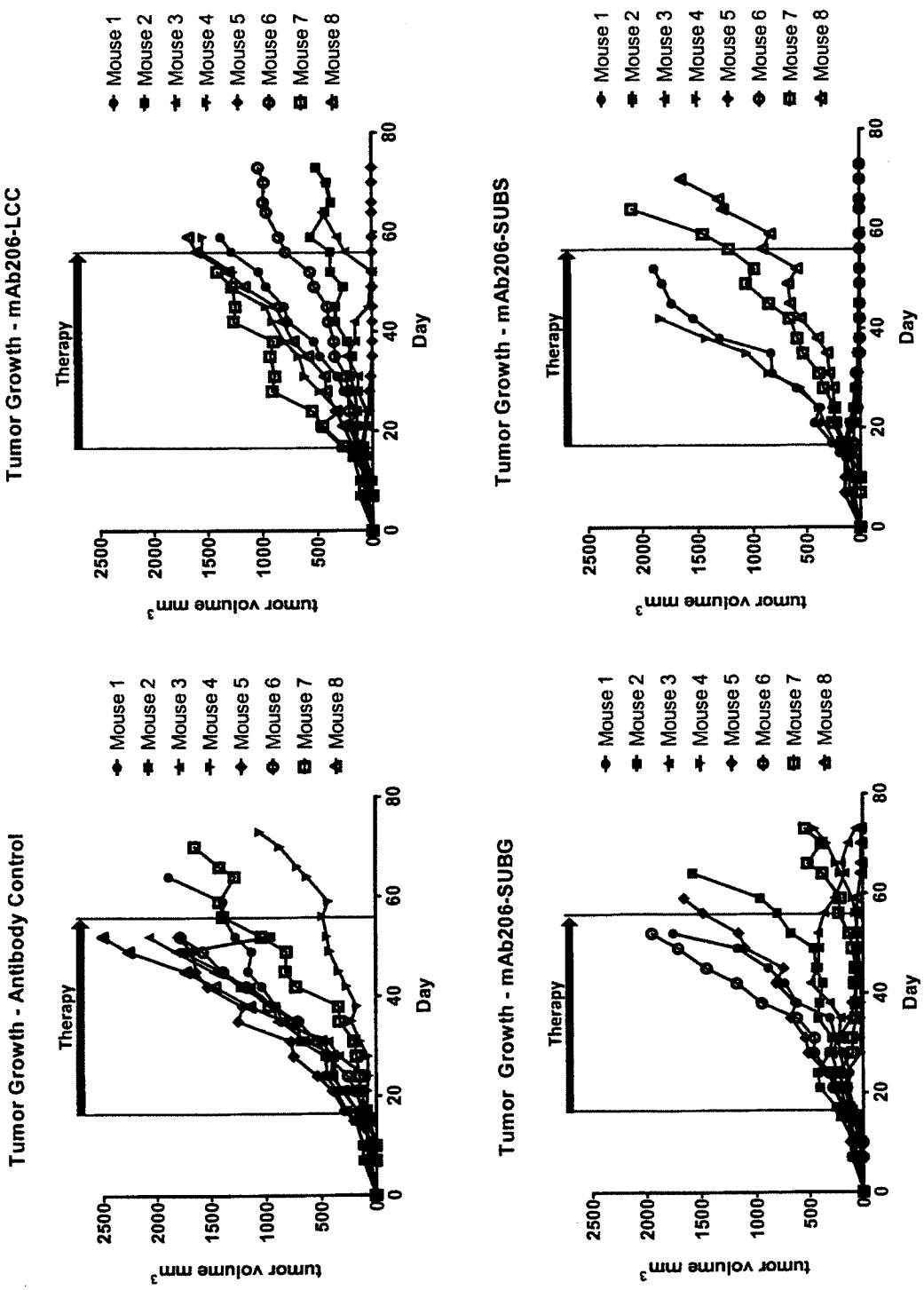

Advanced Treatment - NEC8
Day 17 - Initiation of Immunotherapy
tumor volume mm³
1000
800
600
400
200
0
Antibody control
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
Advanced Treatment - NEC8
Day 31
tumor volume mm³
1500
1000
500
0
Antibody control
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
Advanced Treatment - NEC8
Day 42
tumor volume mm³
2500
2000
1500
1000
500
0
Antibody control
mAb206-LCC
mAb206-SUBG
mAb206-SUBS

Fig. 34a


Tumor Growth - Antibody Control
Tumor Growth - mAb206-LCC
Tumor Growth - mAb206-SUBG
Tumor Growth - mAb206-SUBS
Therapy
Day
tumor volume mm³
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
0
500
1000
1500
2000
2500
20
40
60
80

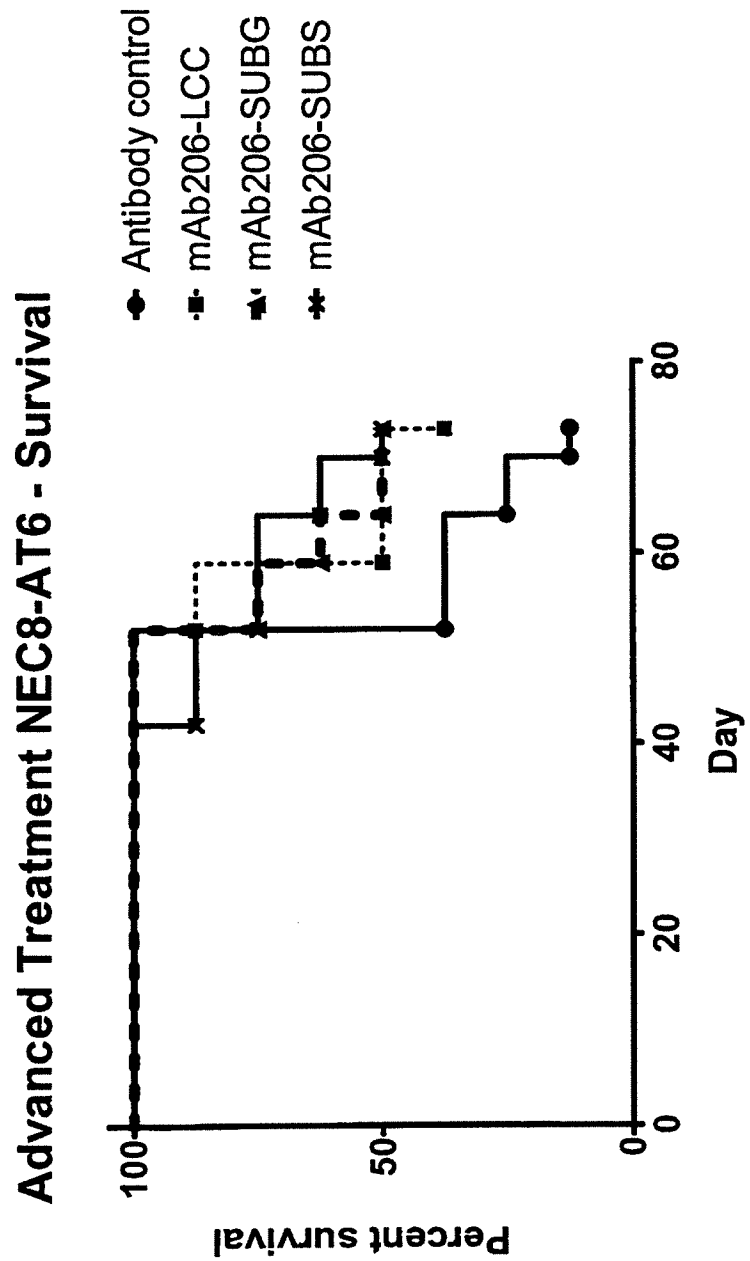
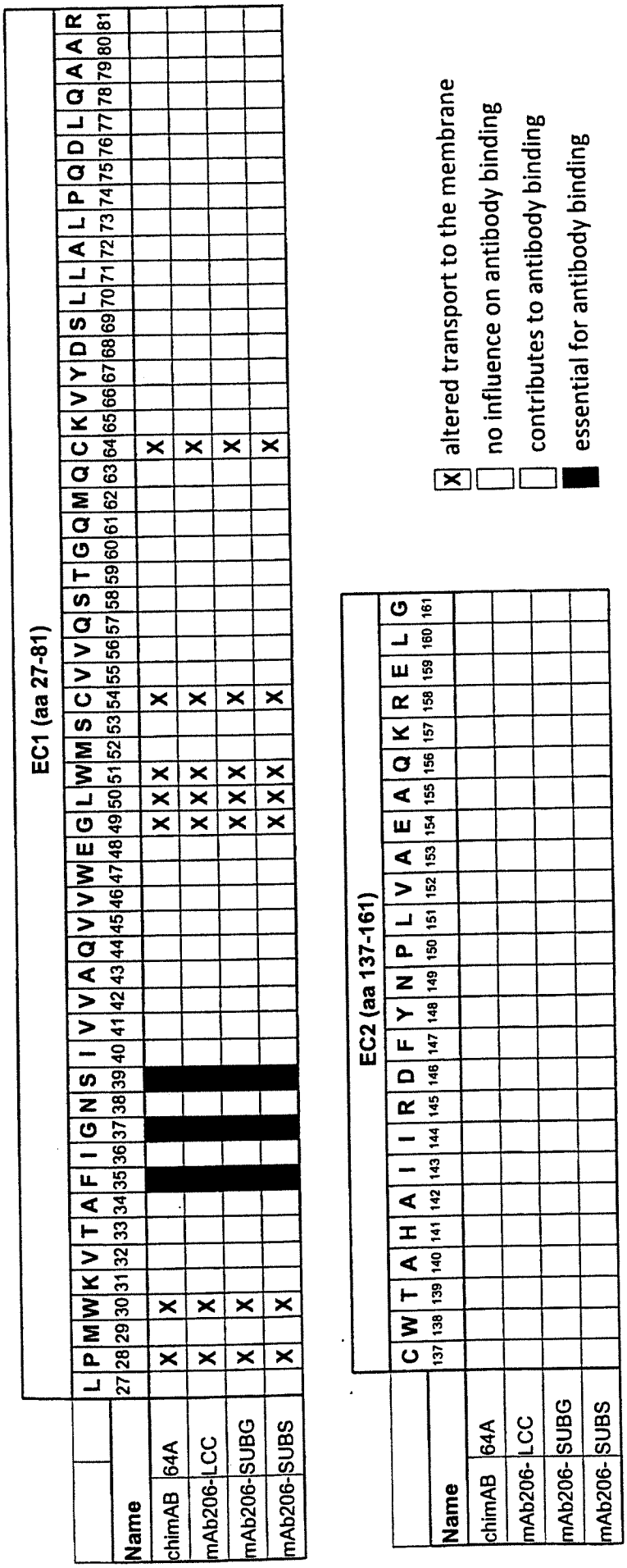

Advanced Treatment NEC8-AT6 - Survival
Antibody control
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
Percent survival
100
50
0
0
20
40
60
80
Day

Fig. 34b

Fig. 35


EC1 (aa 27-81)
EC2 (aa 137-161)
Name
chimAB 64A
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
altered transport to the membrane
no influence on antibody binding
contributes to antibody binding
essential for antibody binding

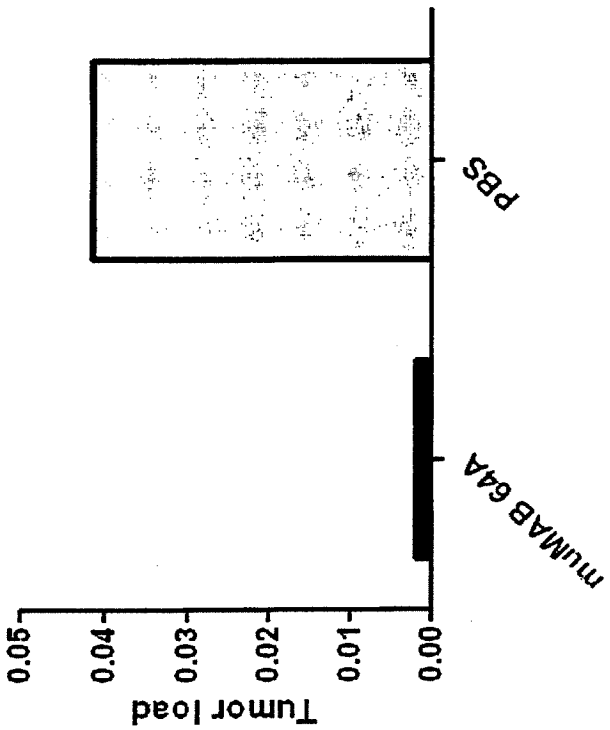


Fig. 36

Fig. 37

	mAb206-LCC	mAb206-SUBG	muMAB 64A
Cancer tissues			
OvarCA	2/2	2/2	2/2
TestisCA	1/1	1/1	4/4
Normal tissues			
Breast	0/1	0/1	-
Colon	0/1	0/1	0/4
Duodenum	0/1	0/1	-
Gall bladder	0/1	0/1	-
Heart	0/1	0/1	0/1
Kidney	0/1	0/1	0/4
Liver	0/1	0/1	0/2
Lung	0/1	0/1	-
Lymphnode	0/1	0/1	-
Oesophagus	0/1	0/1	0/1
Ovar	0/1	0/1	-
Pancreas	0/1	0/1	0/2
Placenta	0/1	0/1	-
Prostate	0/1	0/1	0/2
Sceletal muscle	0/1	0/1	-
Spleen	0/1	0/1	-
Stomach	0/1	0/1	0/2
Testis	0/4	0/4	0/2
Tube	0/1	0/1	-
Thyroidea	0/1	0/1	-
Uterus	0/2	0/2	-