Fig. 1

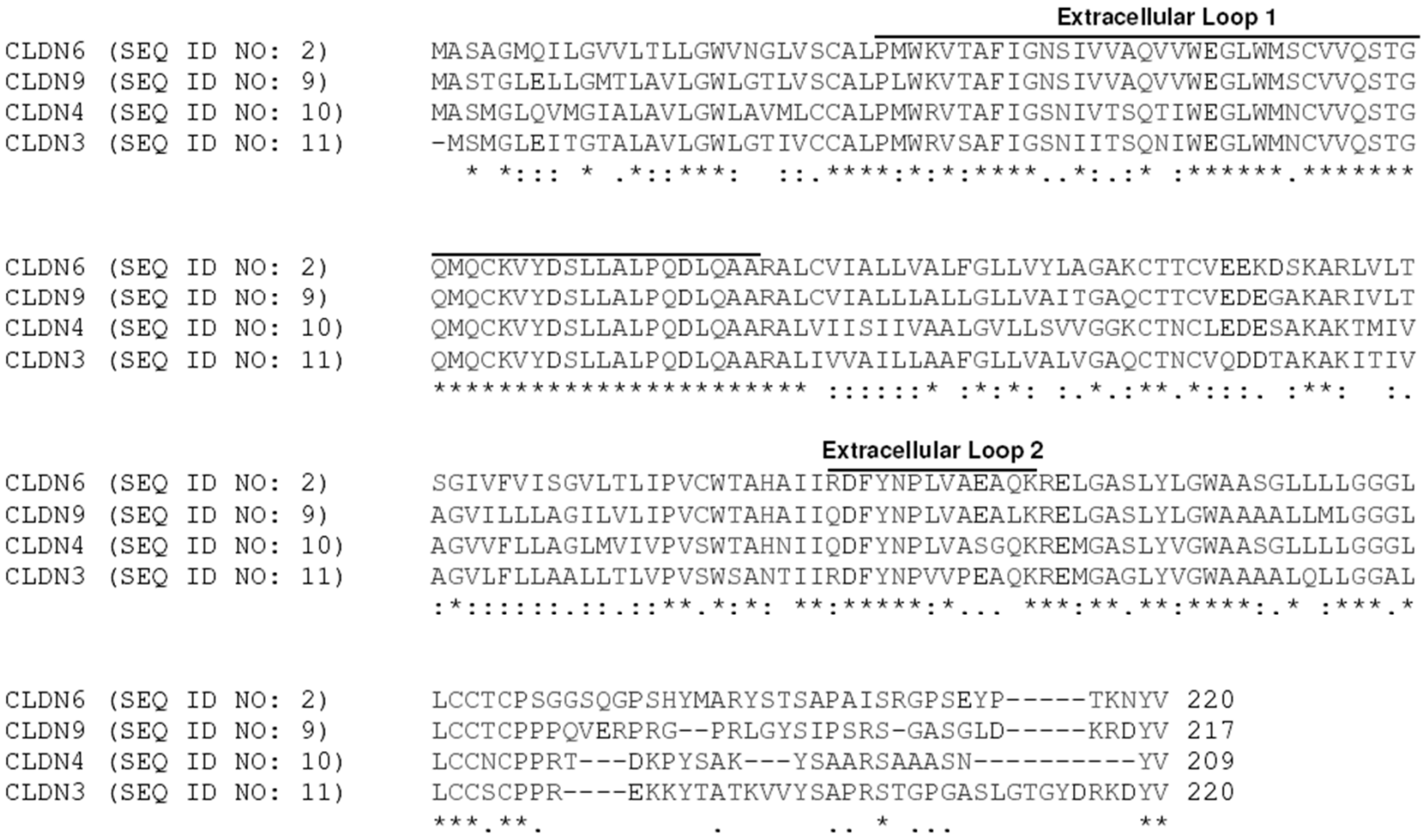

Extracellular Loop 1
CLDN6 (SEQ ID NO: 2) MASAGMQILGVVLTLLGWVNGLVSCALPMWKVTAFIGNSIVVAQVVWEGLWMSCVVQSTG 60
CLDN9 (SEQ ID NO: 9) MASTGLELLGMTLAVLGWLGTLVSCALPLWKVTAFIGNSIVVAQVVWEGLWMSCVVQSTG 60
CLDN4 (SEQ ID NO: 10) MASMGLQVMGIALAVLGWLAVMLCCALPMWRVTAFIGSNIVTSQTIWEGLWMNCVVQSTG 60
CLDN3 (SEQ ID NO: 11) -MSMGLEITGTALAVLGWLGTIVCCALPMWRVSAFIGSNIITSQNIWEGLWMNCVVQSTG 59
QMQCKVYDSLLALPQDLQAARALCVIALLVALFGLLVYLAGAKCTTCVEEKDSKARLVLT 120
QMQCKVYDSLLALPQDLQAARALCVIALLLALLGLLVAITGAQCTTCVEDEGAKARIVLT 120
QMQCKVYDSLLALPQDLQAARALVIISIIVAALGVLLSVVGGKCTNCLEDESAKAKTMIV 120
QMQCKVYDSLLALPQDLQAARALIVVAILLAAFGLLVALVGAQCTNCVQDDTAKAKITIV 119
Extracellular Loop 2
SGIVFVISGVLTLIPVCWTAHAIIRDFYNPLVAEAQKRELGASLYLGWAASGLLLLGGGL 180
AGVILLLAGILVLIPVCWTAHAIIQDFYNPLVAEALKRELGASLYLGWAAAALLMLGGGL 180
AGVVFLLAGLMVIVPVSWTAHNIIQDFYNPLVASGQKREMGASLYVGWAASGLLLLGGGL 180
AGVLFLLAALLTLVPVSWSANTIIRDFYNPVVPEAQKREMGAGLYVGWAAAALQLLGGAL 179
LCCTCPSGGSQGPSHYMARYSTSAPAISRGPSEYP-----TKNYV 220
LCCTCPPPQVERPRG--PRLGYSIPSRS-GASGLD-----KRDYV 217
LCCNCPPRT---DKPYSAK---YSAARSAAASN----------YV 209
LCCSCPPR----EKKYTATKVVYSAPRSTGPGASLGTGYDRKDYV 220

Fig. 2A

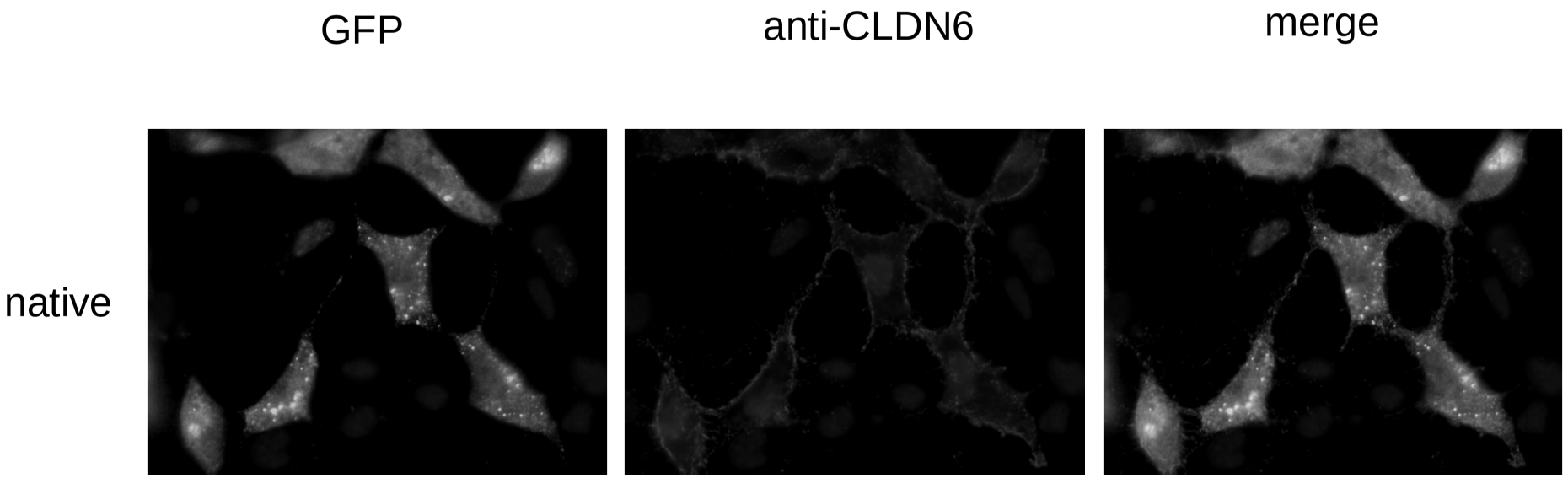


Fig. 2B

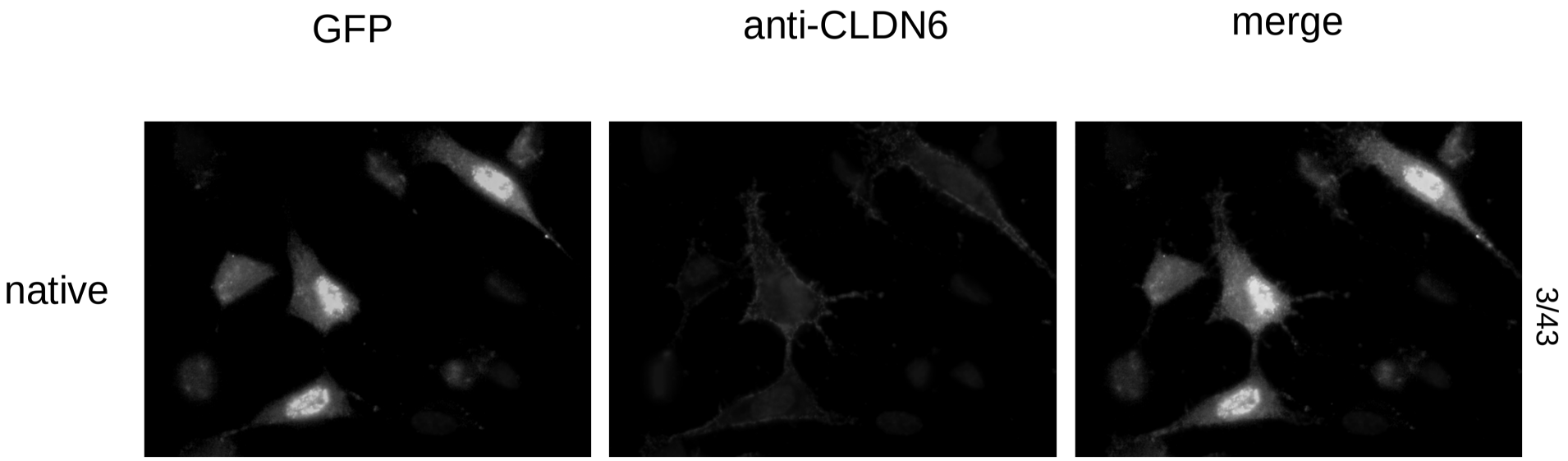


Fig. 3

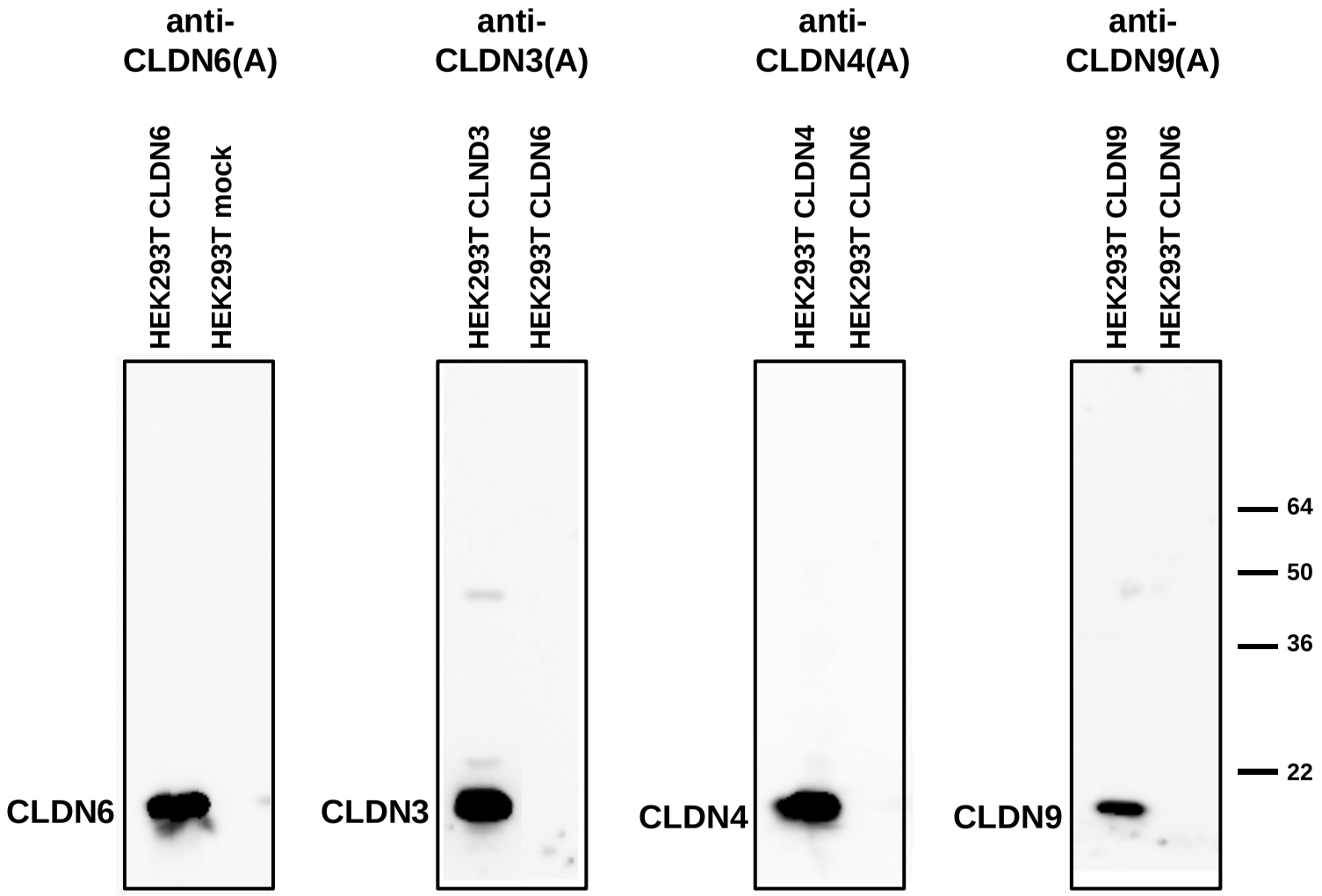

anti-
CLDN6(A)
HEK293T CLDN6
HEK293T mock
CLDN6
anti-
CLDN3(A)
HEK293T CLND3
HEK293T CLDN6
CLDN3
anti-
CLDN4(A)
HEK293T CLDN4
HEK293T CLDN6
CLDN4
anti-
CLDN9(A)
HEK293T CLDN9
HEK293T CLDN6
CLDN9
64
50
36
22

Fig. 4

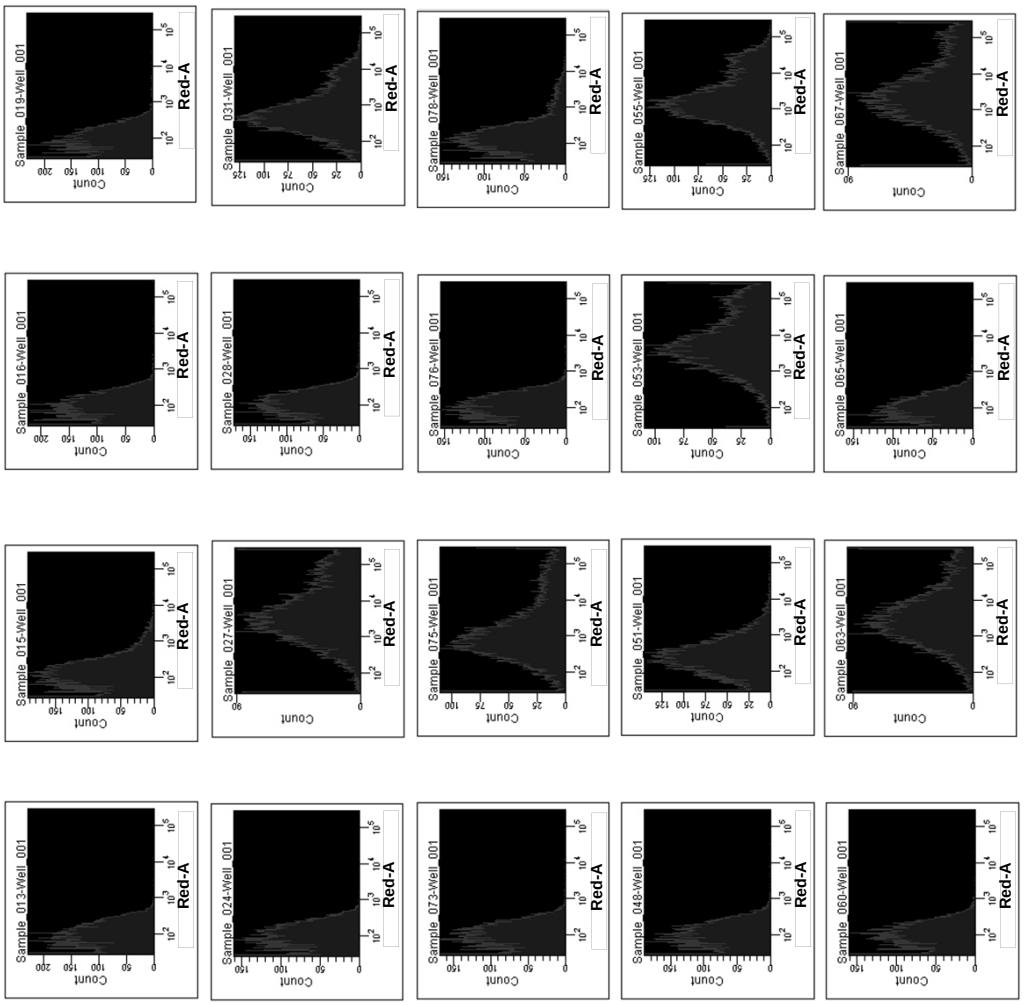

isotype
anti-CLDN6
anti-CLDN3
anti-CLDN4
non-transfected
CLDN6-transfected
CLDN9-transfected
CLDN3-transfected
CLDN4-transfected
Sample_013-Well_001
Sample_015-Well_001
Sample_016-Well_001
Sample_019-Well_001
Sample_024-Well_001
Sample_027-Well_001
Sample_028-Well_001
Sample_031-Well_001
Sample_073-Well_001
Sample_075-Well_001
Sample_076-Well_001
Sample_078-Well_001
Sample_048-Well_001
Sample_051-Well_001
Sample_053-Well_001
Sample_055-Well_001
Sample_060-Well_001
Sample_063-Well_001
Sample_065-Well_001
Sample_067-Well_001
Count
Red-A

Fig. 5A

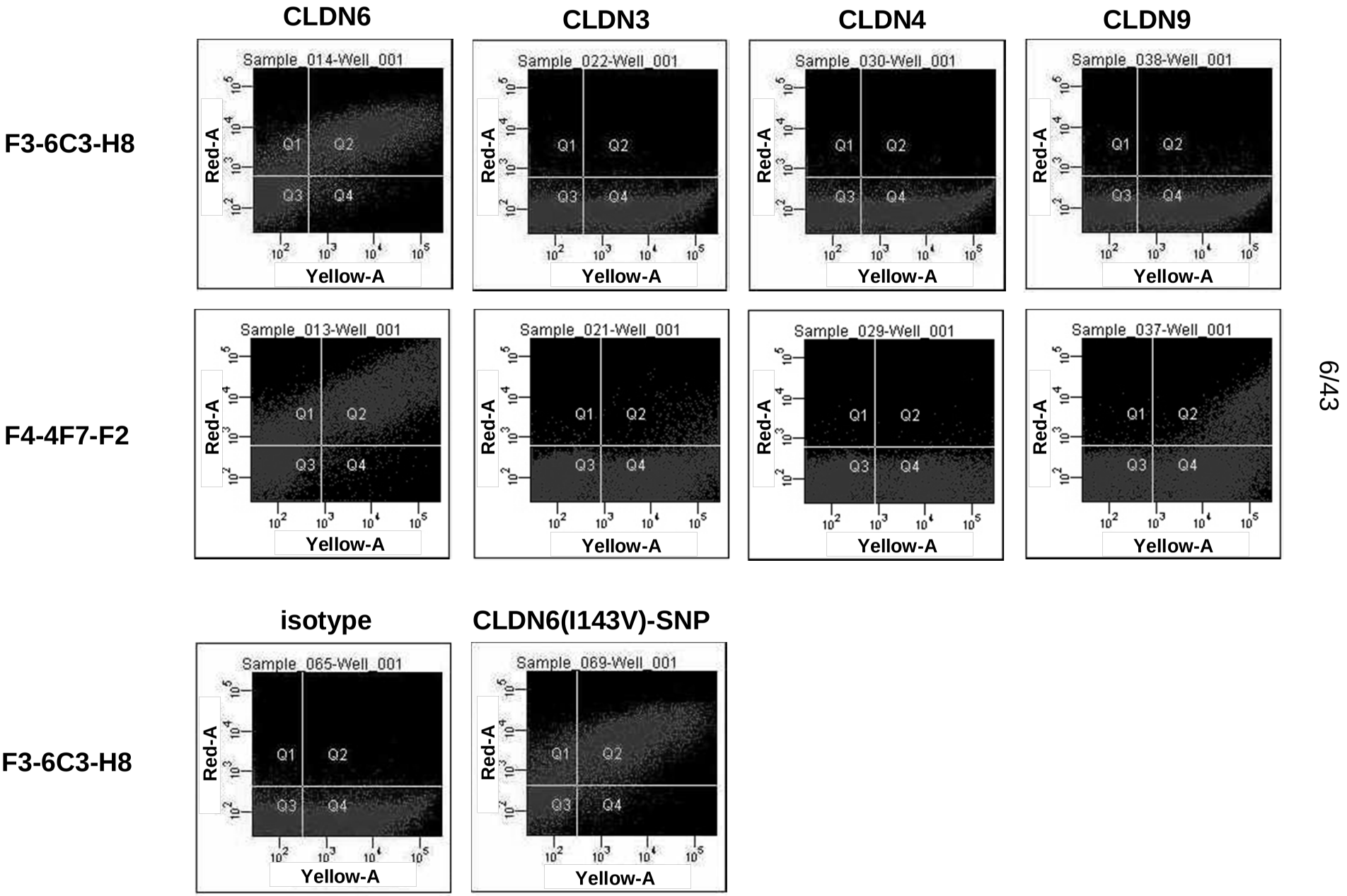

CLDN6
CLDN3
CLDN4
CLDN9
F3-6C3-H8
F4-4F7-F2
isotype
CLDN6(I143V)-SNP
F3-6C3-H8
Sample_014-Well_001
Sample_022-Well_001
Sample_030-Well_001
Sample_038-Well_001
Sample_013-Well_001
Sample_021-Well_001
Sample_029-Well_001
Sample_037-Well_001
Sample_065-Well_001
Sample_069-Well_001
Red-A
Yellow-A
Q1
Q2
Q3
Q4

Fig. 5B

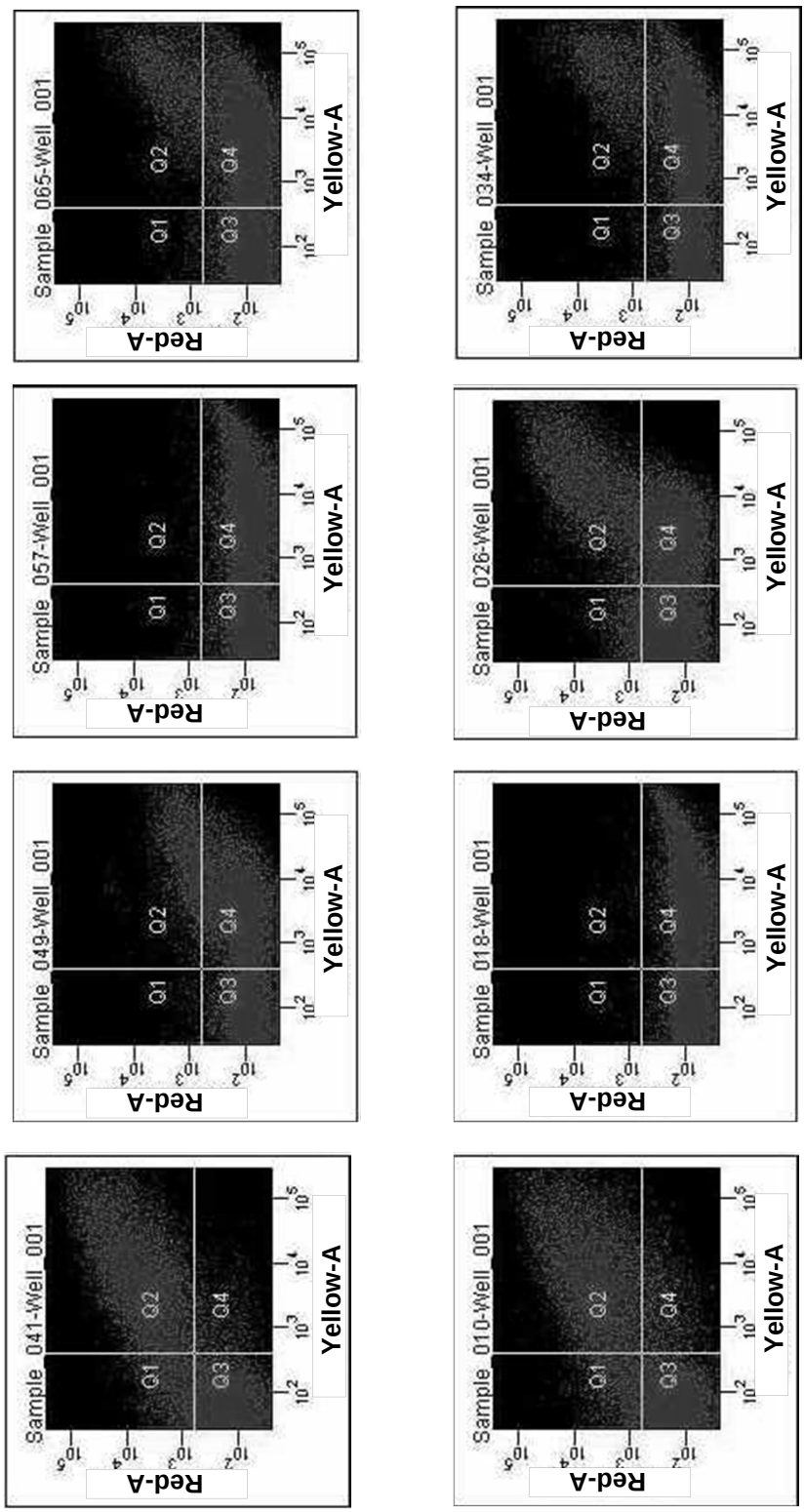
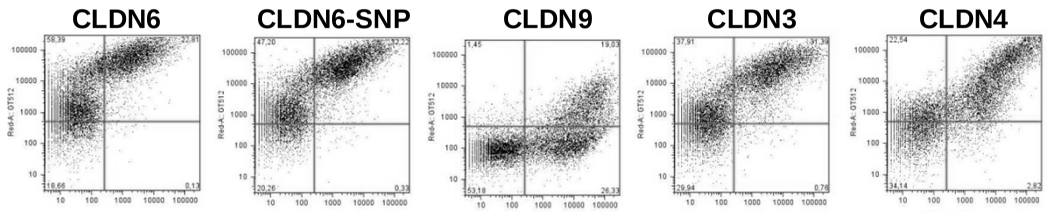
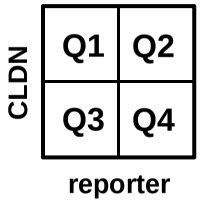
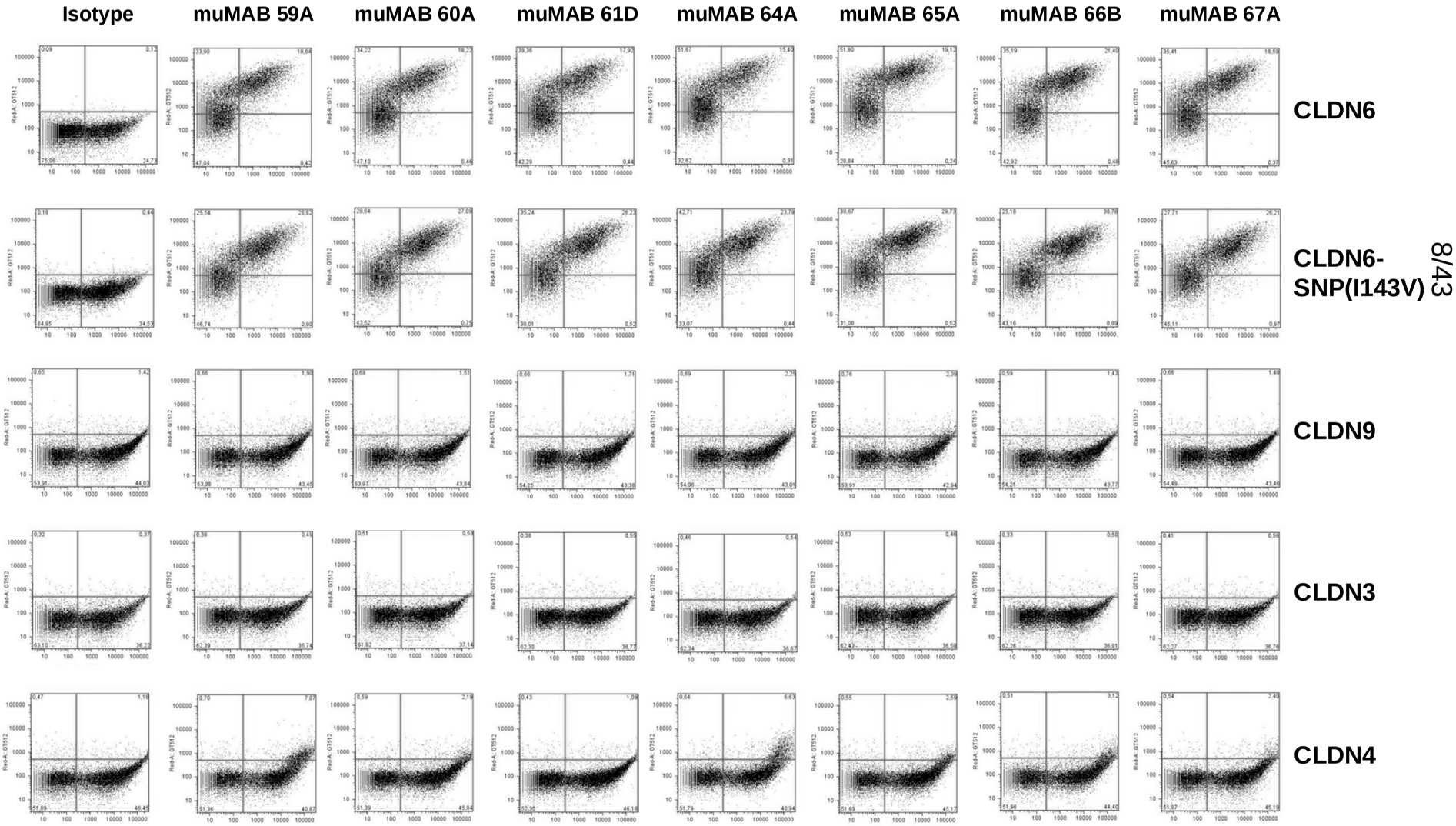

CLDN6
CLDN3
CLDN4
CLDN9
F3-7B3-B4
F3-3F7-A5
Sample_041-Well_001
Sample_049-Well_001
Sample_057-Well_001
Sample_065-Well_001
Sample_010-Well_001
Sample_018-Well_001
Sample_026-Well_001
Sample_034-Well_001
Q1
Q2
Q3
Q4
Red-A
Yellow-A

Fig. 6

positive controls







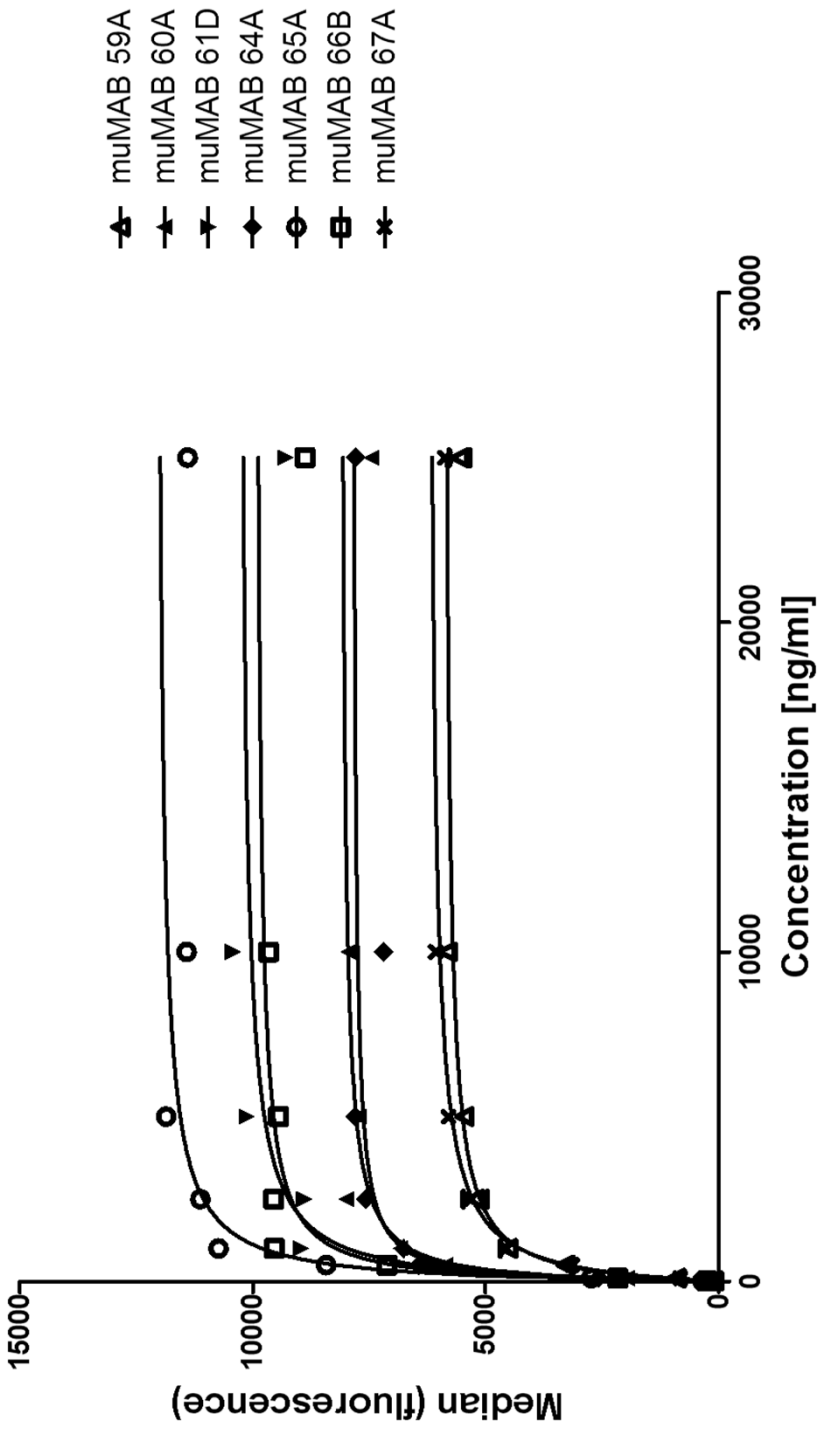

muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Median (fluorescence)
15000
10000
5000
0
0
10000
20000
30000
Concentration [ng/ml]

Fig. 7

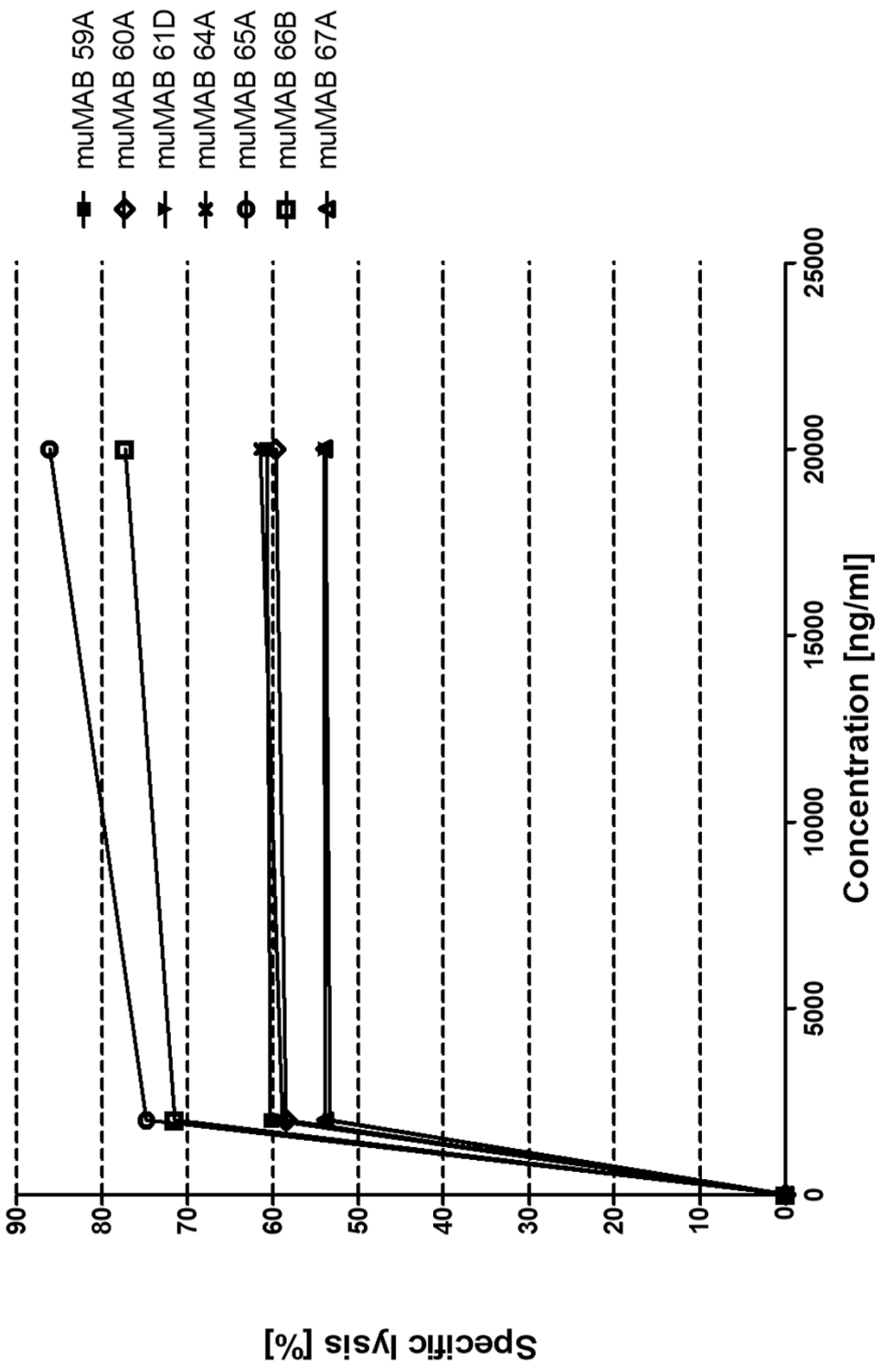
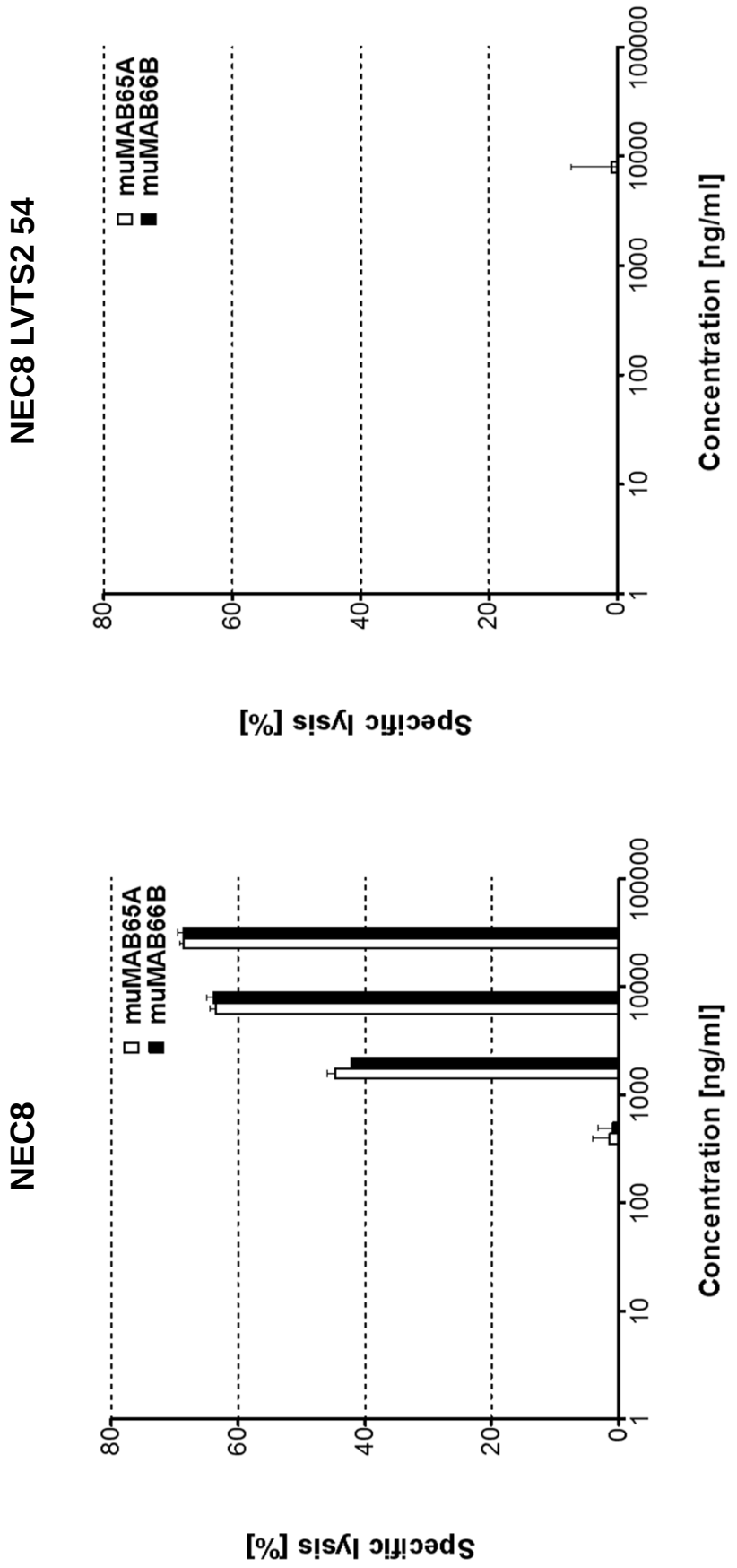

muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Specific lysis [%]
0
10
20
30
40
50
60
70
80
90
0
5000
10000
15000
20000
25000
Concentration [ng/ml]

Fig. 8

Fig. 9



error bars: SEM

Fig. 10

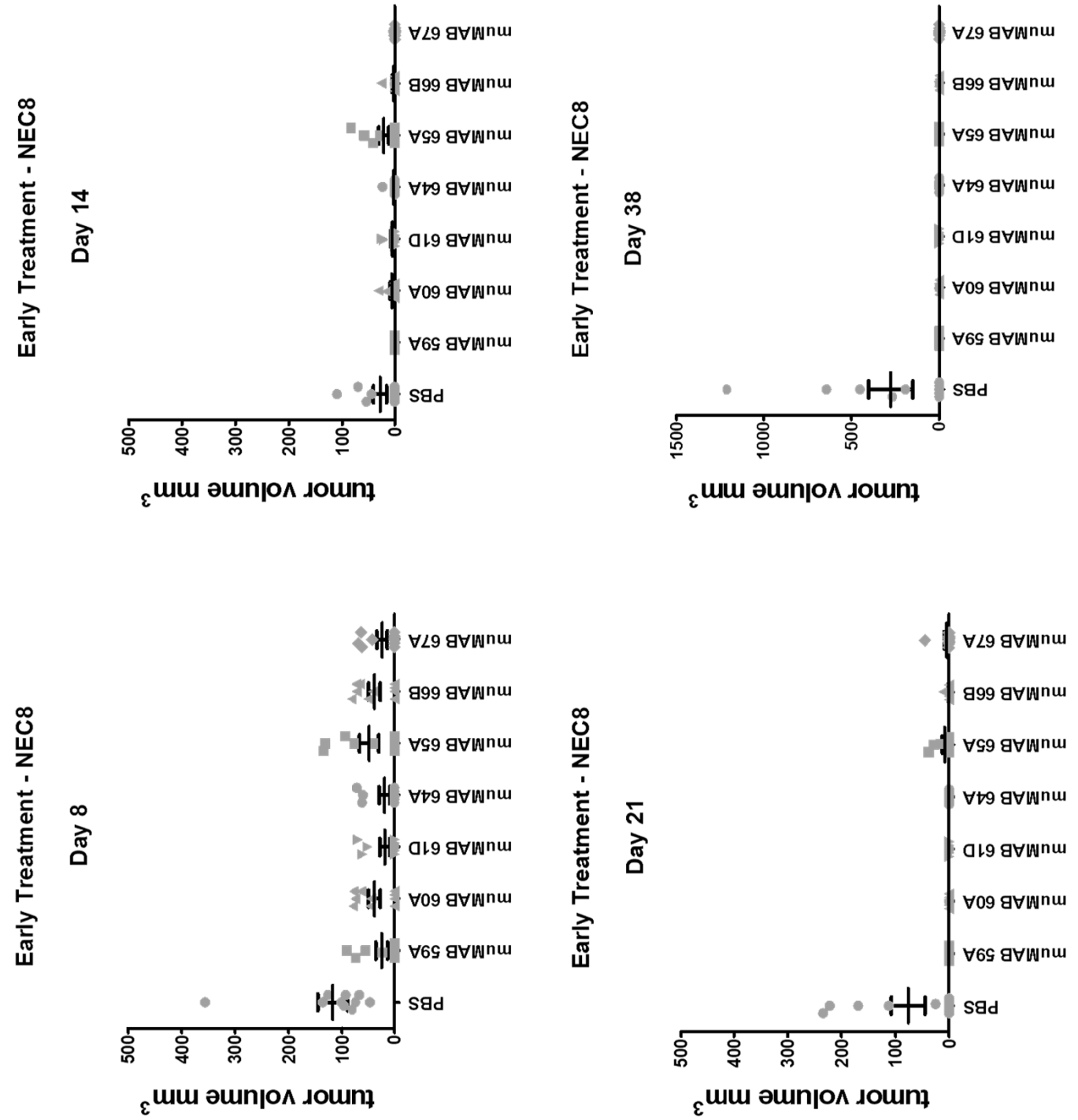
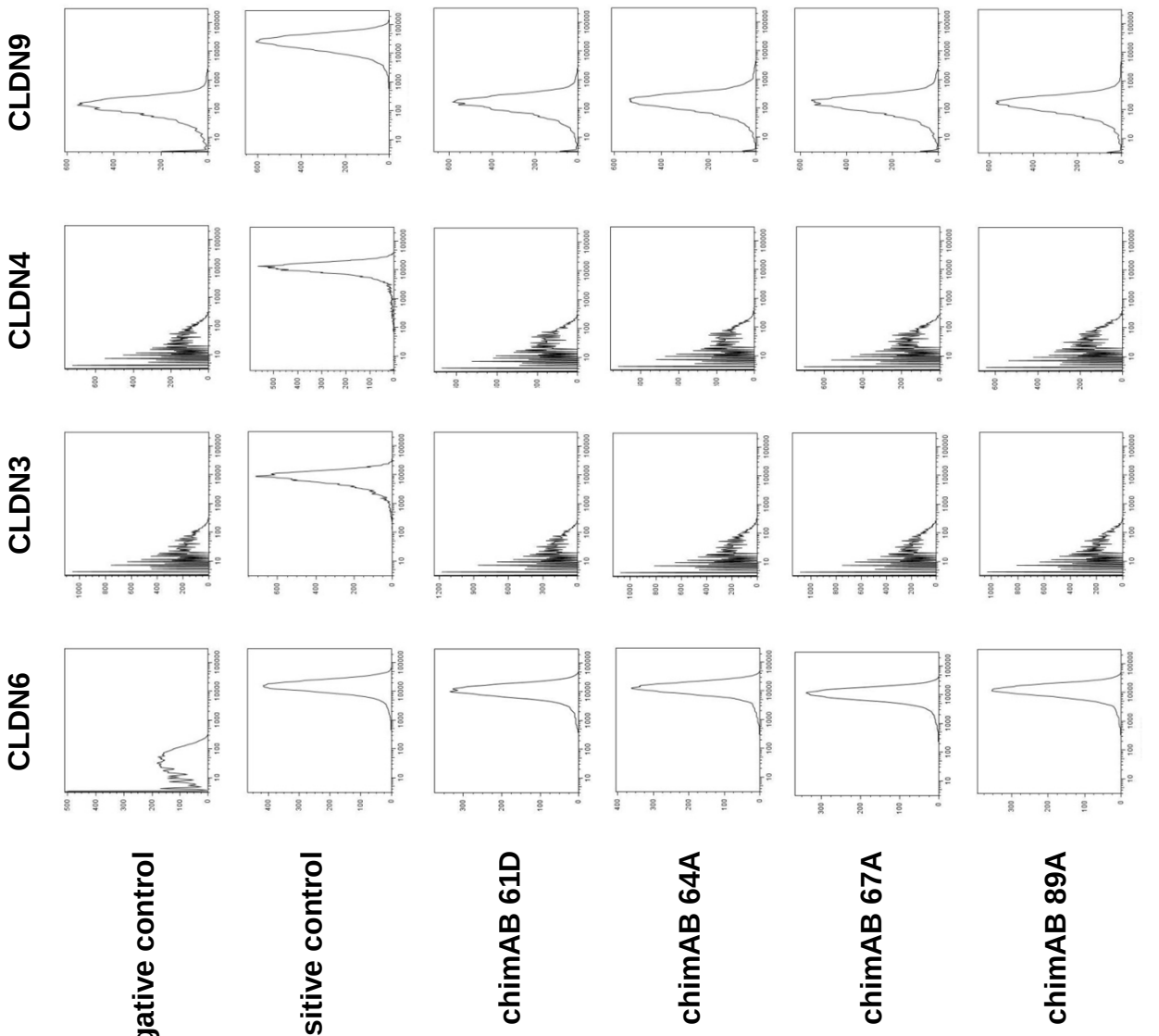

Early Treatment - NEC8
Day 8
tumor volume mm3
0
100
200
300
400
500
PBS
muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Early Treatment - NEC8
Day 14
tumor volume mm3
0
100
200
300
400
500
PBS
muMAB 59A
muMAB 60A
muMAB 61D
muMAB 64A
muMAB 65A
muMAB 66B
muMAB 67A
Early Treatment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

Fig. 11


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

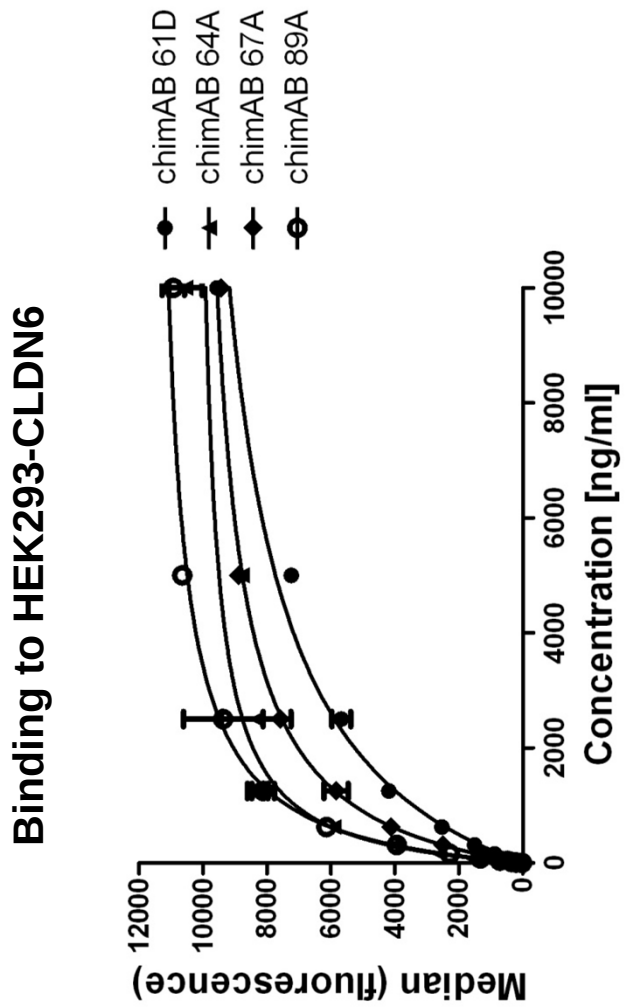

Binding to HEK293-CLDN6
chimAB 61D
chimAB 64A
chimAB 67A
chimAB 89A
Median (fluorescence)
0
2000
4000
6000
8000
10000
12000
0
2000
4000
6000
8000
10000
Concentration [ng/ml]

Fig. 12

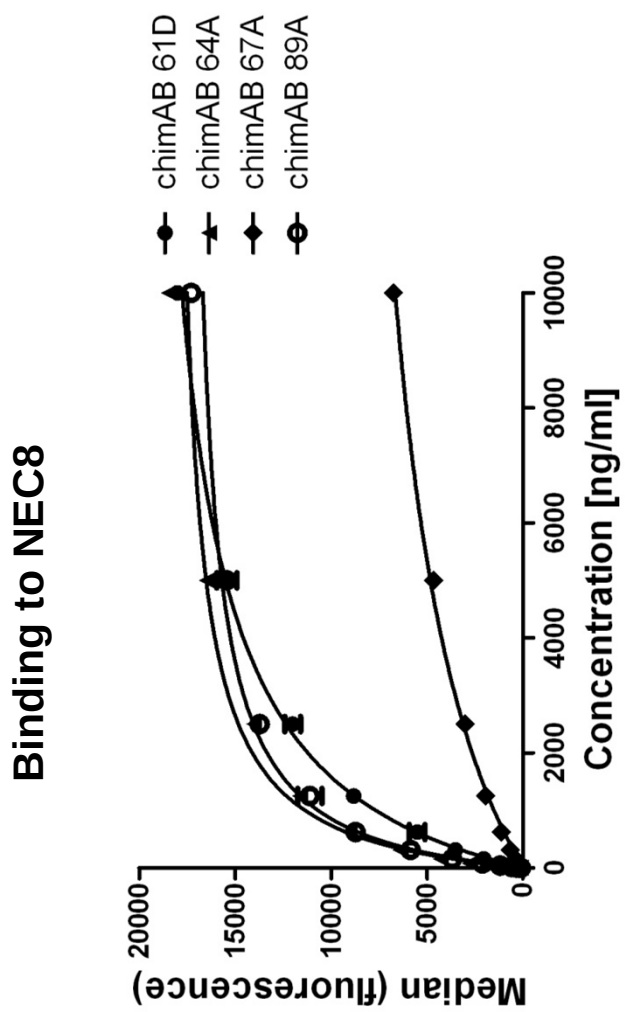

Binding to NEC8
chimAB 61D
chimAB 64A
chimAB 67A
chimAB 89A
Median (fluorescence)
20000
15000
10000
5000
0
0
2000
4000
6000
8000
10000
Concentration [ng/ml]

Fig. 13

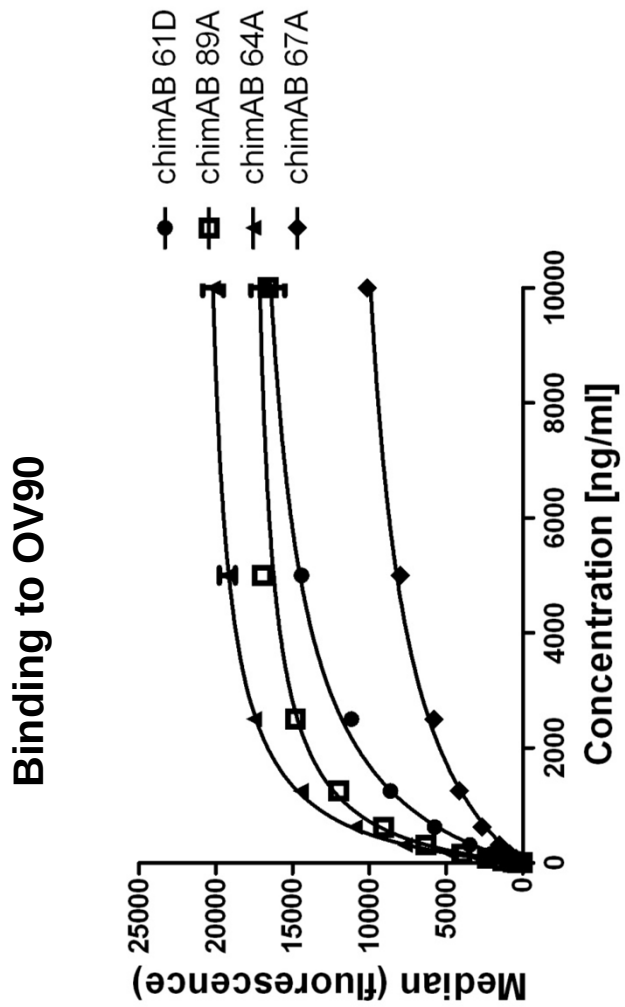

Binding to OV90
chimAB 61D
chimAB 89A
chimAB 64A
chimAB 67A
Median (fluorescence)
25000
20000
15000
10000
5000
0
0
2000
4000
6000
8000
10000
Concentration [ng/ml]

Fig. 14

Fig. 15

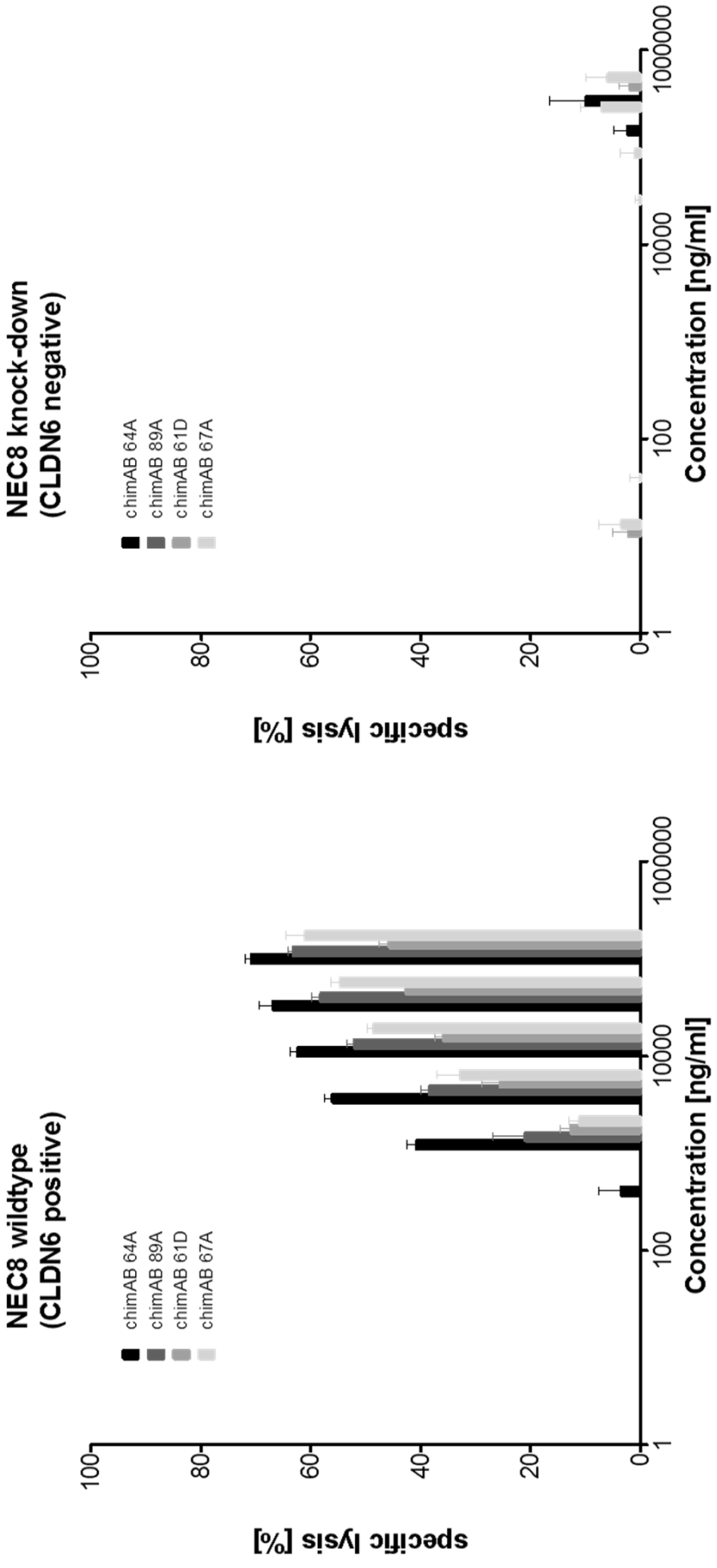

NEC8 wildtype
(CLDN6 positive)
chimAB 64A
chimAB 89A
chimAB 61D
chimAB 67A
specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration [ng/ml]
NEC8 knock-down
(CLDN6 negative)
chimAB 64A
chimAB 89A
chimAB 61D
chimAB 67A
specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration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

Fig. 16

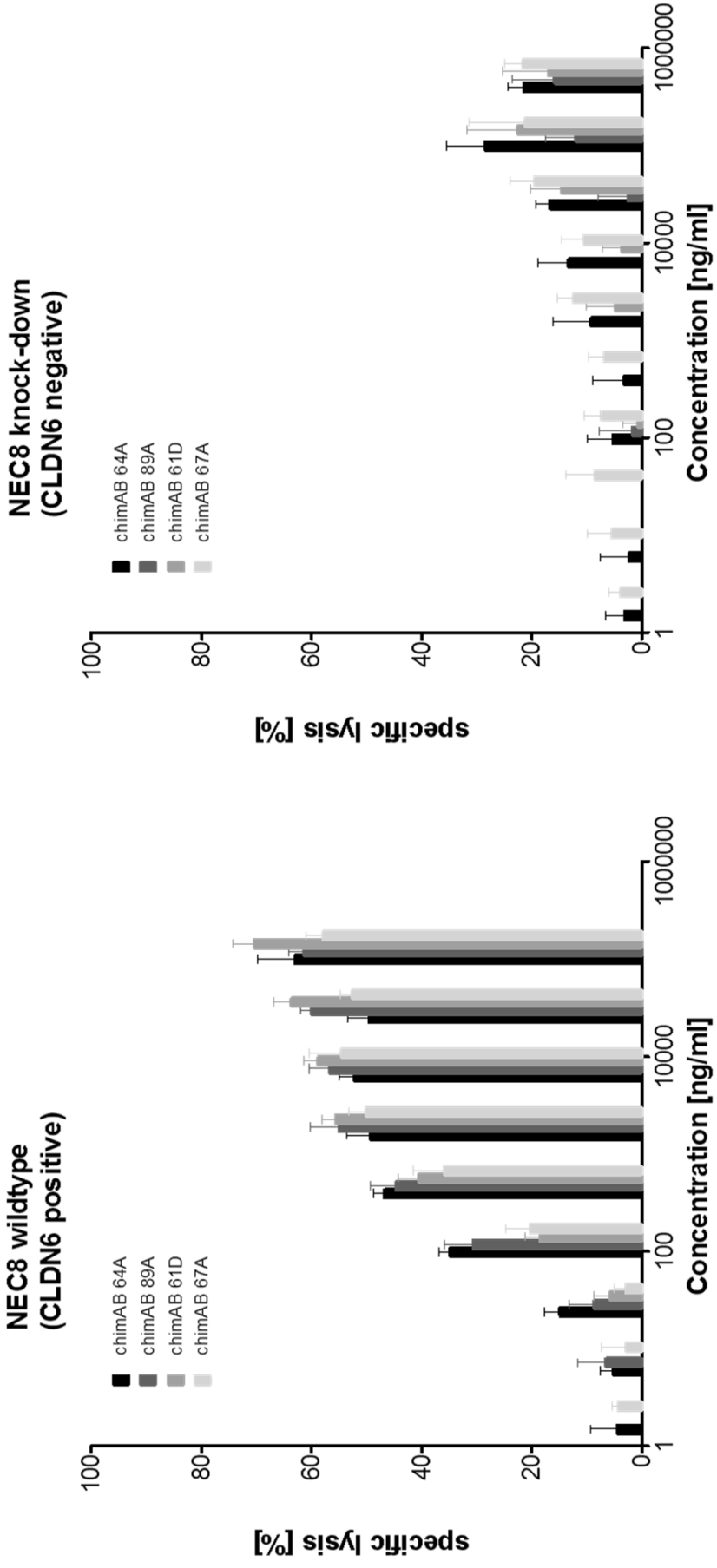

NEC8 wildtype
(CLDN6 positive)
chimAB 64A
chimAB 89A
chimAB 61D
chimAB 67A
specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration [ng/ml]
NEC8 knock-down
(CLDN6 negative)
chimAB 64A
chimAB 89A
chimAB 61D
chimAB 67A
specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration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

Fig. 17

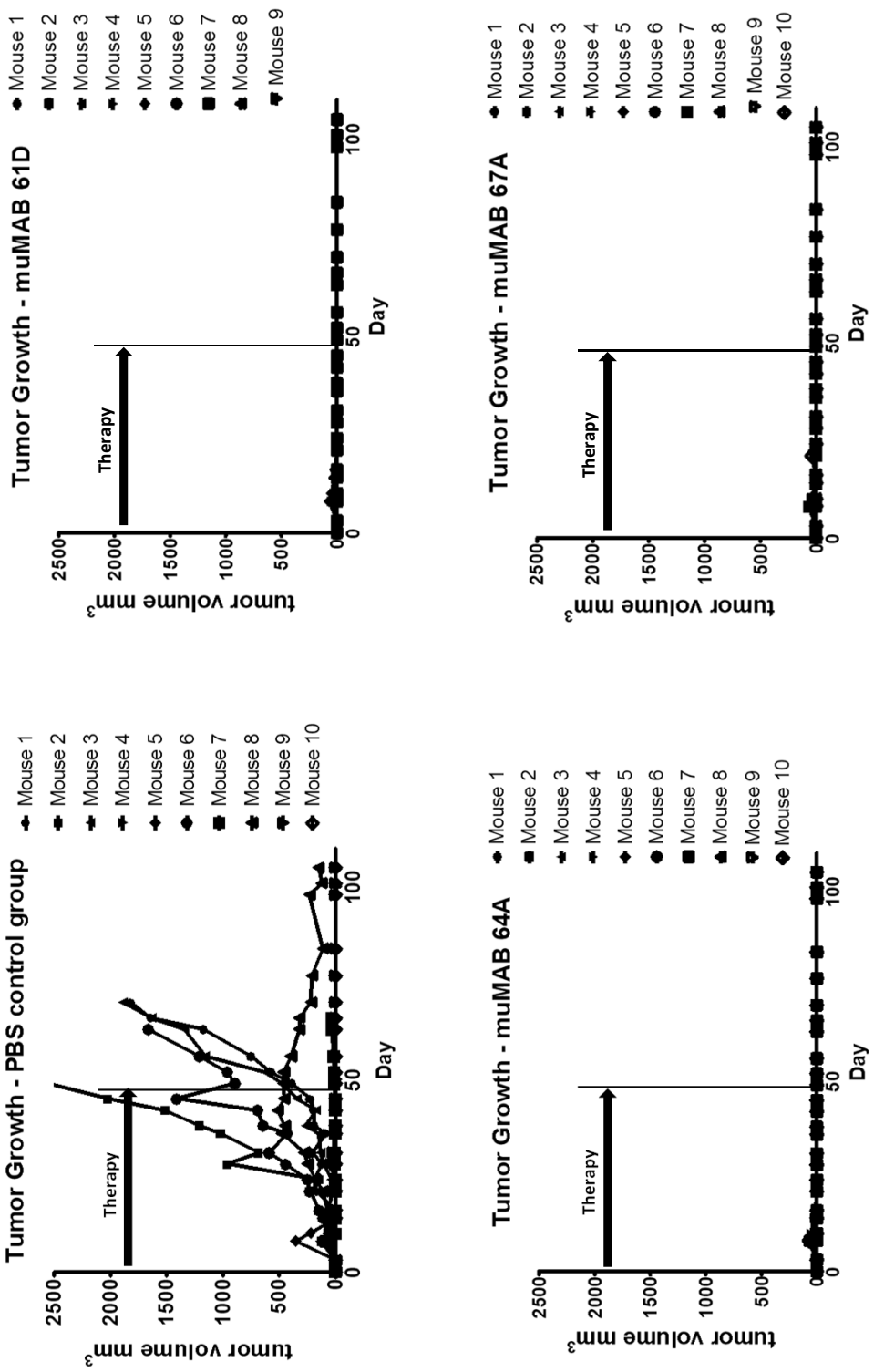

Tumor Growth - PBS control group
Therapy
tumor volume mm³
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10
Tumor Growth - muMAB 61D
Tumor Growth - muMAB 67A
Tumor Growth - muMAB 64A

Fig. 18

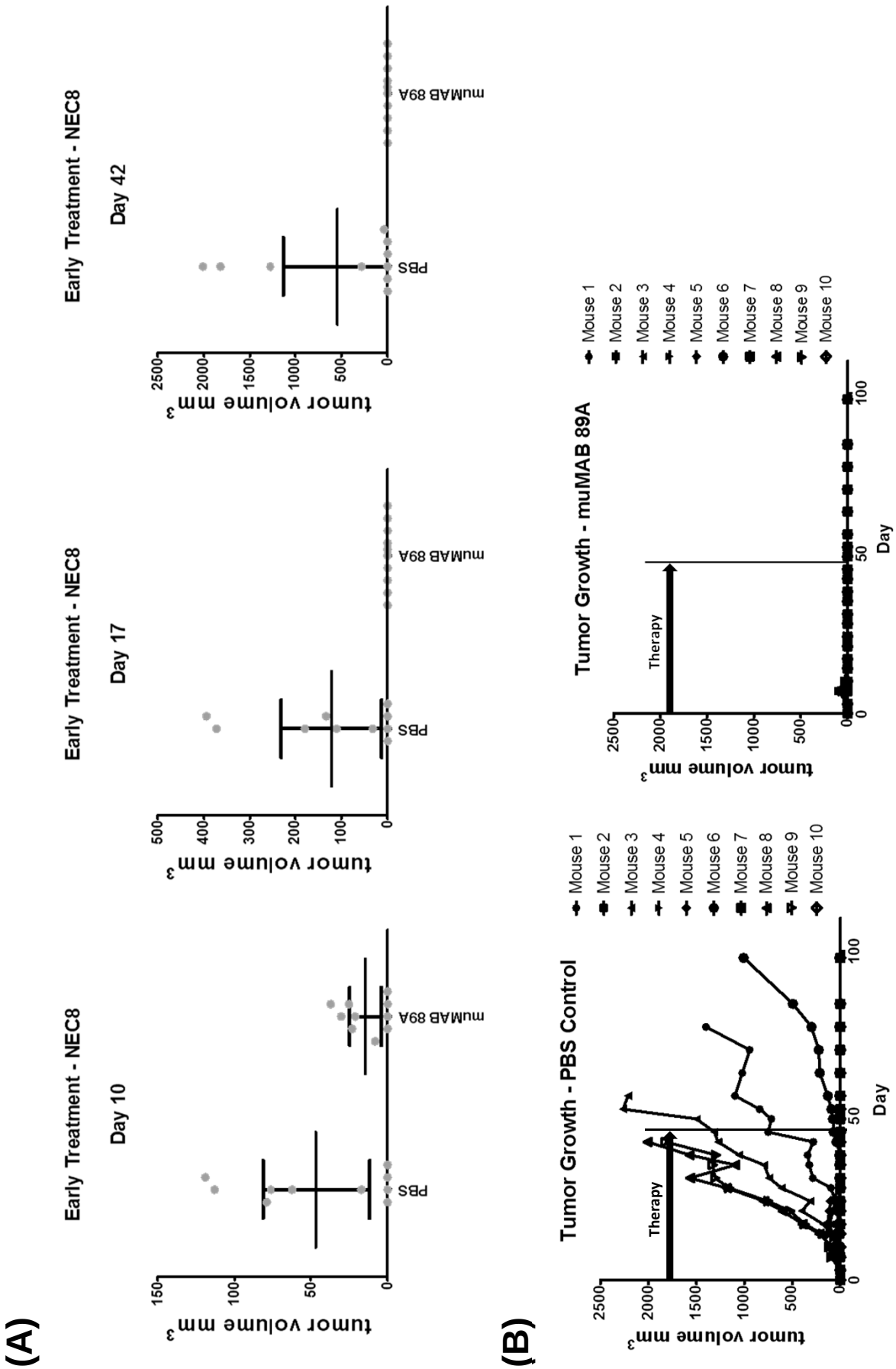

(A)
Early Treatment - NEC8
Day 10
tumor volume mm³
PBS
muMAB 89A
Early Treatment - NEC8
Day 17
tumor volume mm³
PBS
muMAB 89A
Early Treatment - NEC8
Day 42
tumor volume mm³
PBS
muMAB 89A
(B)
Tumor Growth - PBS Control
Therapy
tumor volume mm³
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10
Tumor Growth - muMAB 89A
Therapy
tumor volume mm³
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10

Fig. 19

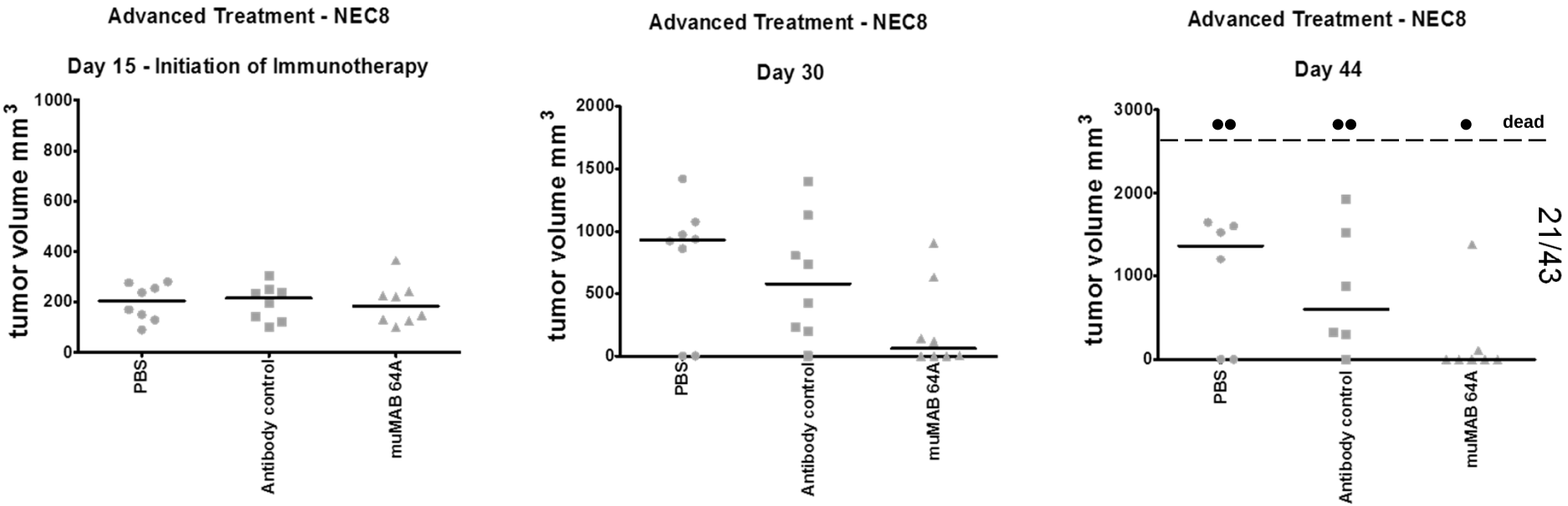
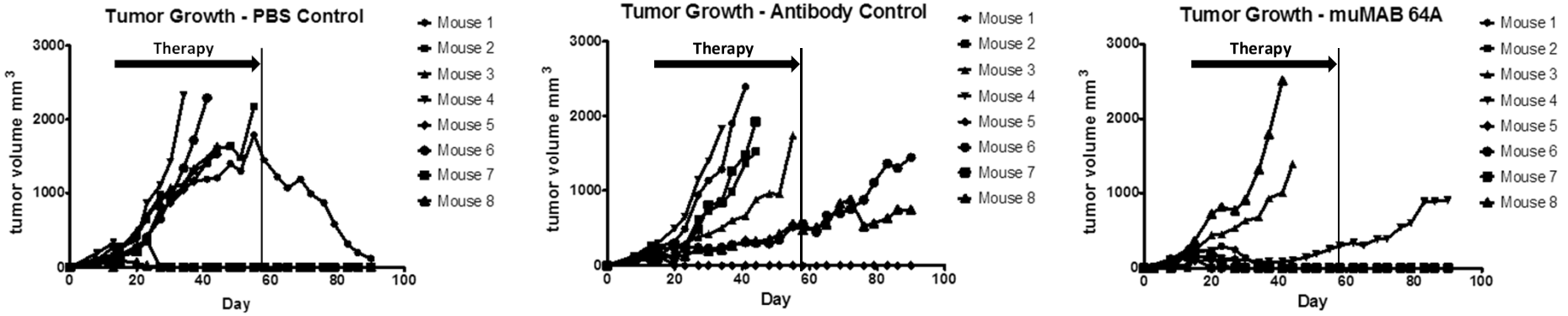

Advanced Treatment - NEC8
Day 15 - Initiation of Immunotherapy
tumor volume mm3
1000
800
600
400
200
0
PBS
Antibody control
muMAB 64A
Advanced Treatment - NEC8
Day 30
tumor volume mm3
2000
1500
1000
500
0
PBS
Antibody control
muMAB 64A
Advanced Treatment - NEC8
Day 44
tumor volume mm3
3000
2000
1000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

Fig. 20

(A)


Tumor Growth - PBS Control
Therapy
tumor volume mm3
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Tumor Growth - Antibody Control
Therapy
tumor volume mm3
Day
Tumor Growth - muMAB 64A
Therapy
tumor volume mm3
Day

(B)

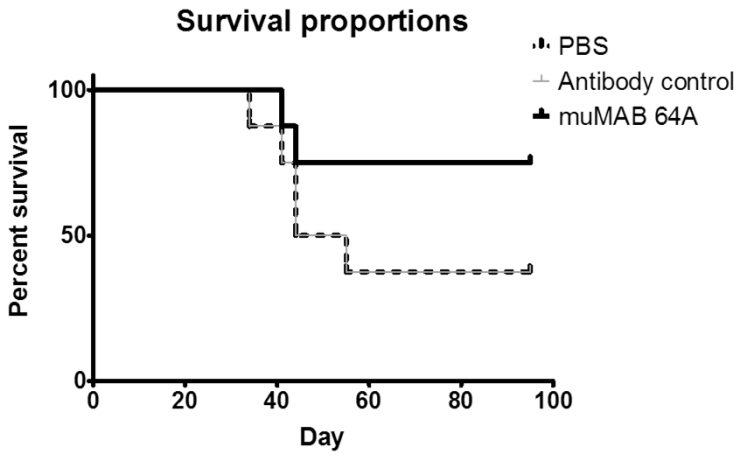

Survival proportions
PBS
Antibody control
muMAB 64A
Percent survival
Day

Fig. 21

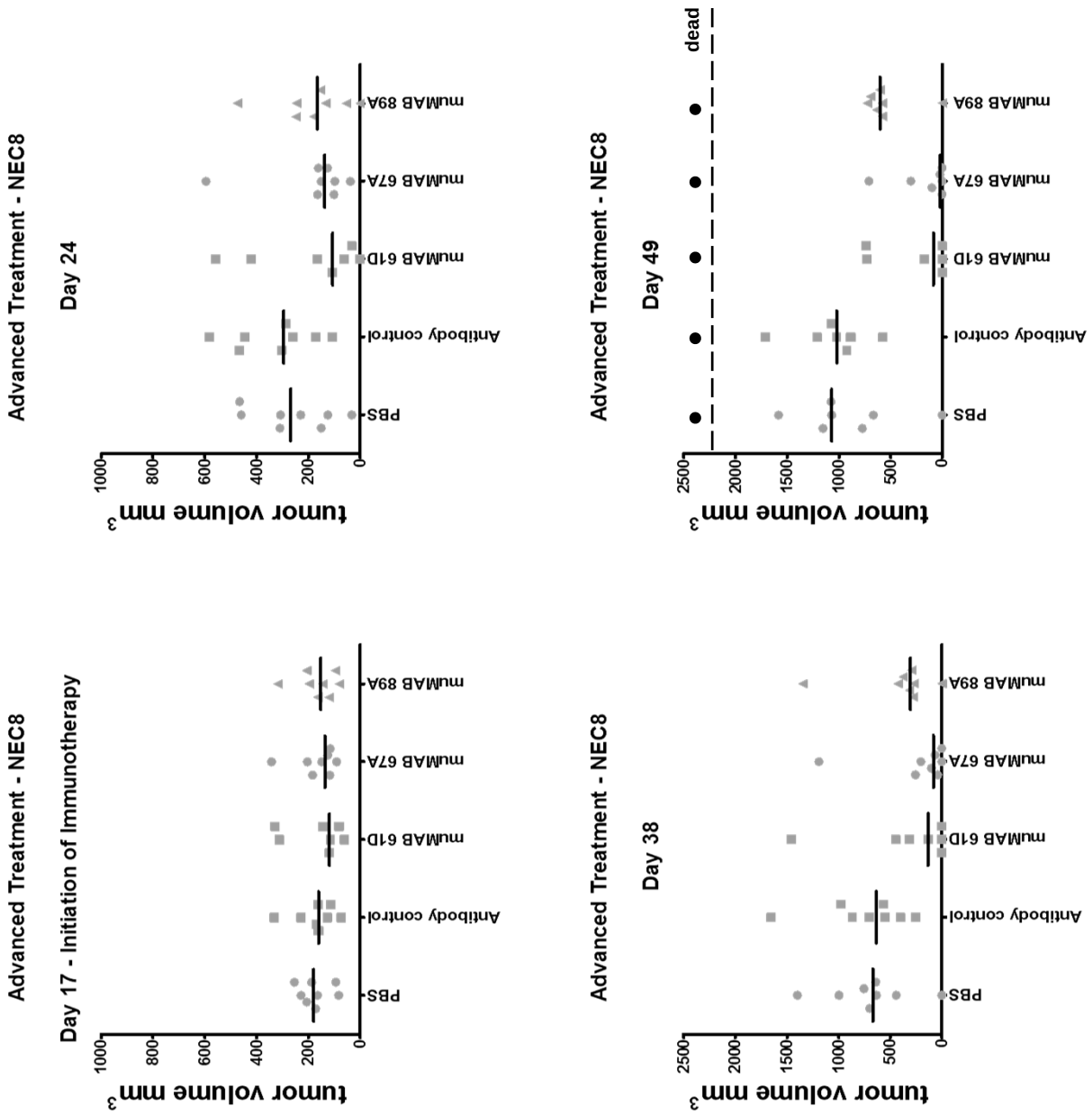
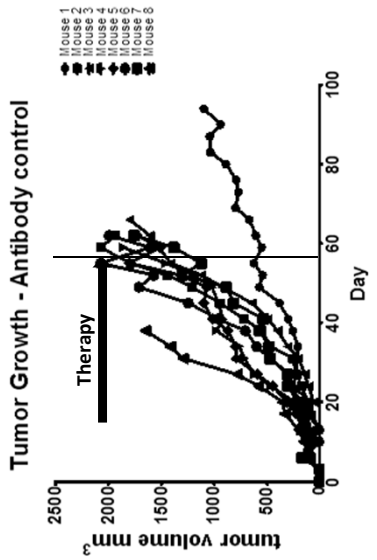
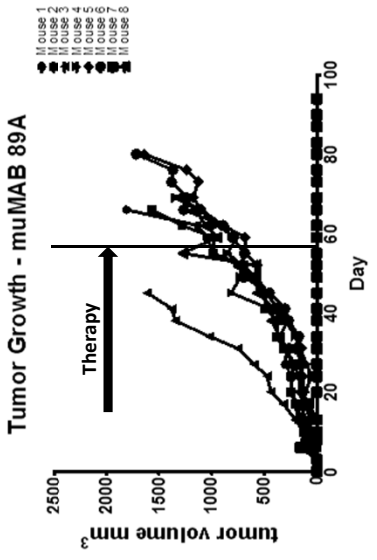
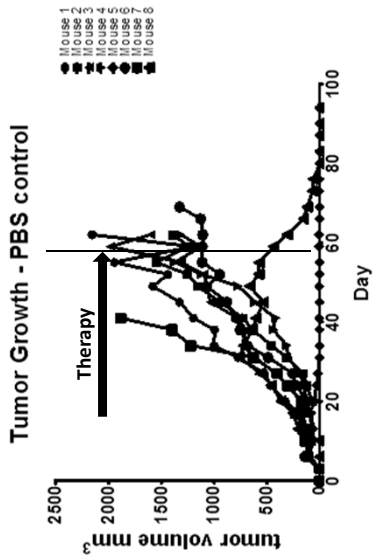

Advanced Treatment - NEC8
Day 17 - Initiation of Immunotherapy
Day 24
Day 38
Day 49
dead
tumor volume mm3
0
200
400
600
800
1000
500
1000
1500
2000
2500
PBS
Antibody control
muMAB 61D
muMAB 67A
muMAB 89A

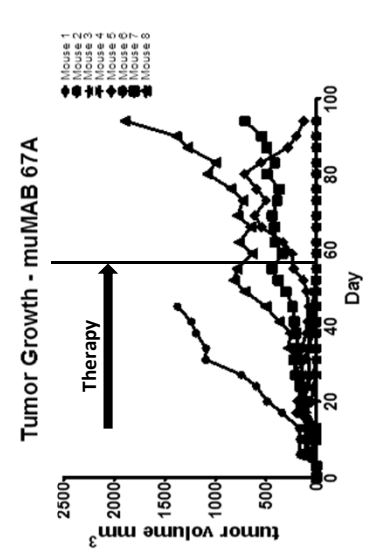
Fig. 22

(A)


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


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


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


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

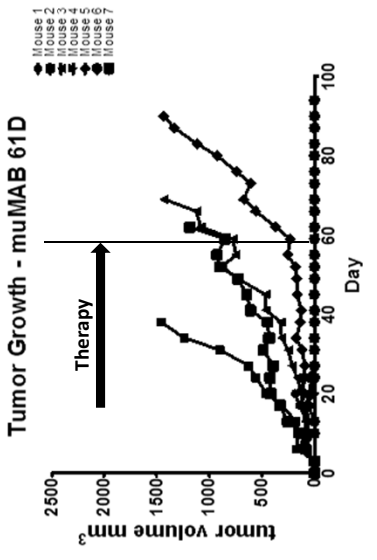

Tumor Growth - muMAB 61D
Therapy
tumor volume mm3
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7

Fig. 22

(B)

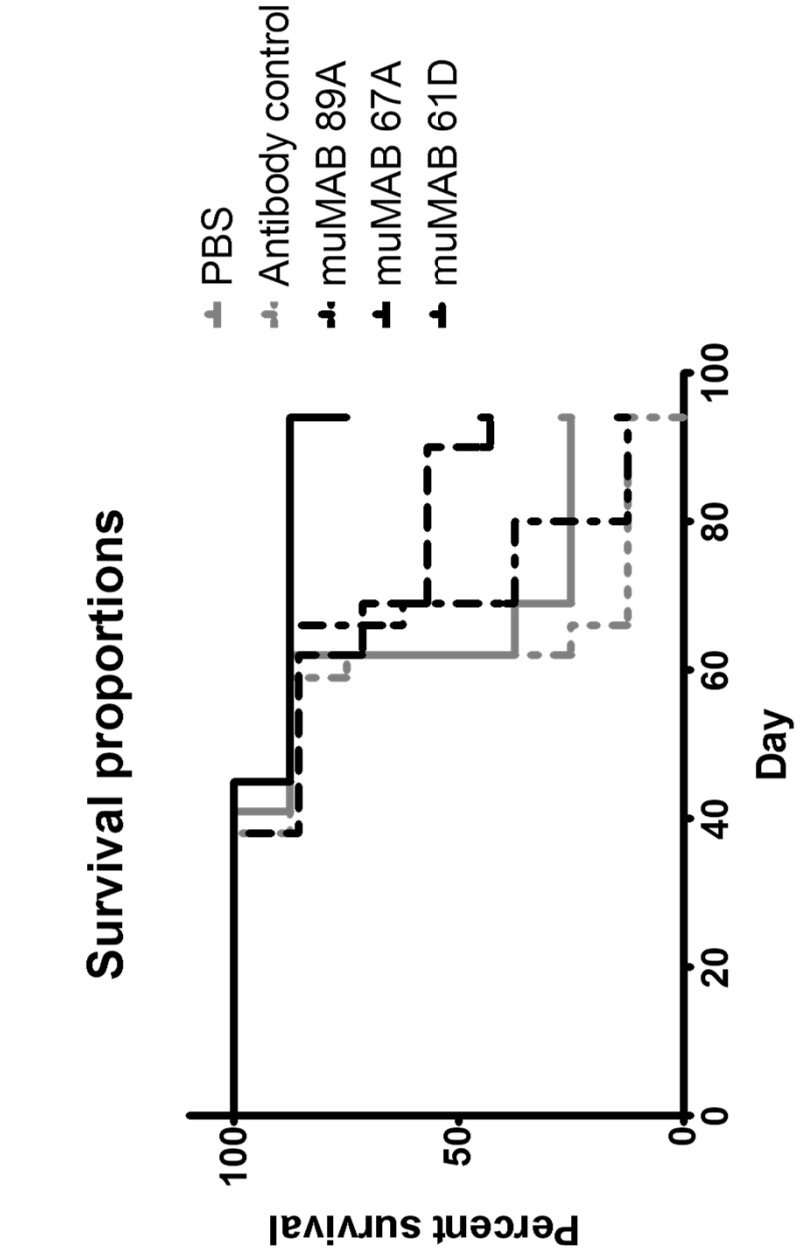

Survival proportions
PBS
Antibody control
muMAB 89A
muMAB 67A
muMAB 61D
Percent survival
100
50
0
0
20
40
60
80
100
Day

Fig. 23

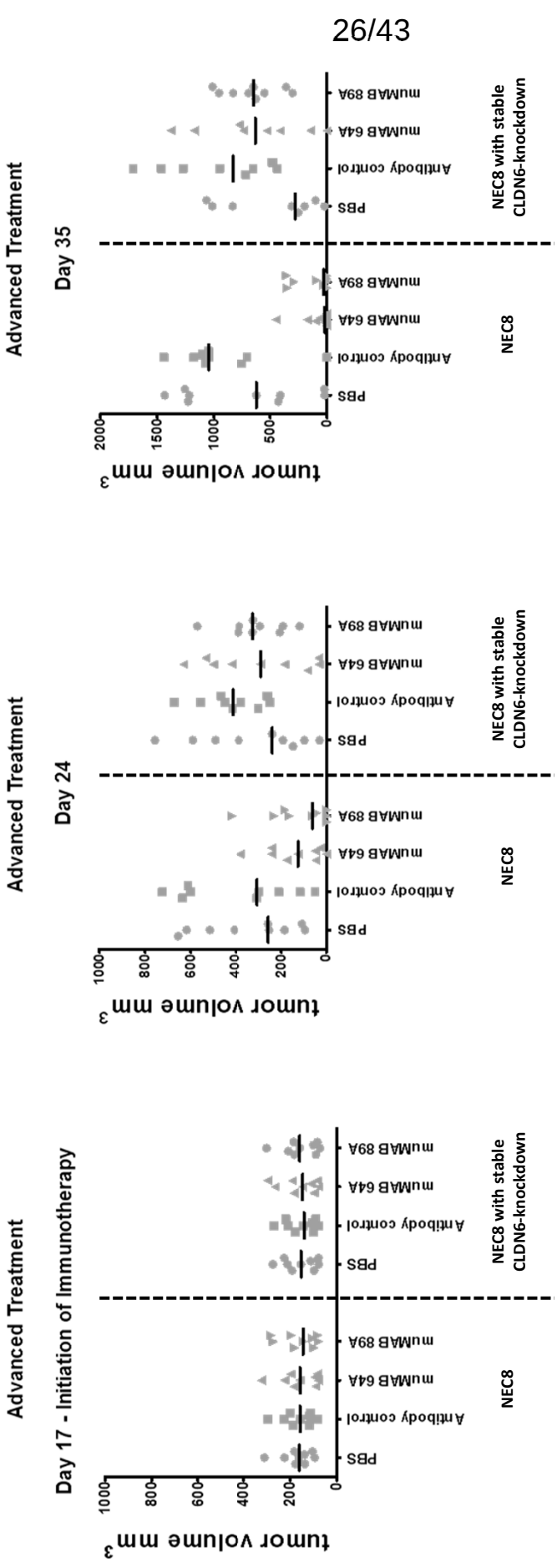

Advanced Treatment
Day 17 - Initiation of Immunotherapy
Day 24
Day 35
tumor volume mm3
0
200
400
600
800
1000
500
1000
1500
2000
PBS
Antibody control
muMAB 64A
muMAB 89A
NEC8
NEC8 with stable CLDN6-knockdown

Fig. 24

	EC1 (aa 27-81)																																																						
Name	L 27	P 28	M 29	W 30	K 31	V 32	T 33	A 34	F 35	I 36	G 37	N 38	S 39	I 40	V 41	V 42	A 43	Q 44	V 45	V 46	W 47	E 48	G 49	L 50	W 51	M 52	S 53	C 54	V 55	V 56	Q 57	S 58	T 59	G 60	Q 61	M 62	Q 63	C 64	K 65	V 66	Y 67	D 68	S 69	L 70	L 71	A 72	L 73	P 74	Q 75	D 76	L 77	Q 78	A 79	A 80	R 81
GT512chimAB 61D		X		X																			X	X	X			X										X																	
GT512chimAB 64A		X		X																			X	X	X			X										X																	
GT512chimAB 67A		X		X																			X	X	X			X										X																	
GT512chimAB 89A		X		X																			X	X	X			X										X																	
R&D		X		X																			X	X	X			X										X																	

	EC2 (aa 137-161)																								
Name	C 137	W 138	T 139	A 140	H 141	A 142	I 143	I 144	R 145	D 146	F 147	Y 148	N 149	P 150	L 151	V 152	A 153	E 154	A 155	Q 156	K 157	R 158	E 159	L 160	G 161
GT512chimAB 61D																									
GT512chimAB 64A																									
GT512chimAB 67A																									
GT512chimAB 89A																									
R&D																									

x altered transport to the membrane
no influence on antibody binding
contributes to antibody binding
essential for antibody binding

Fig. 25

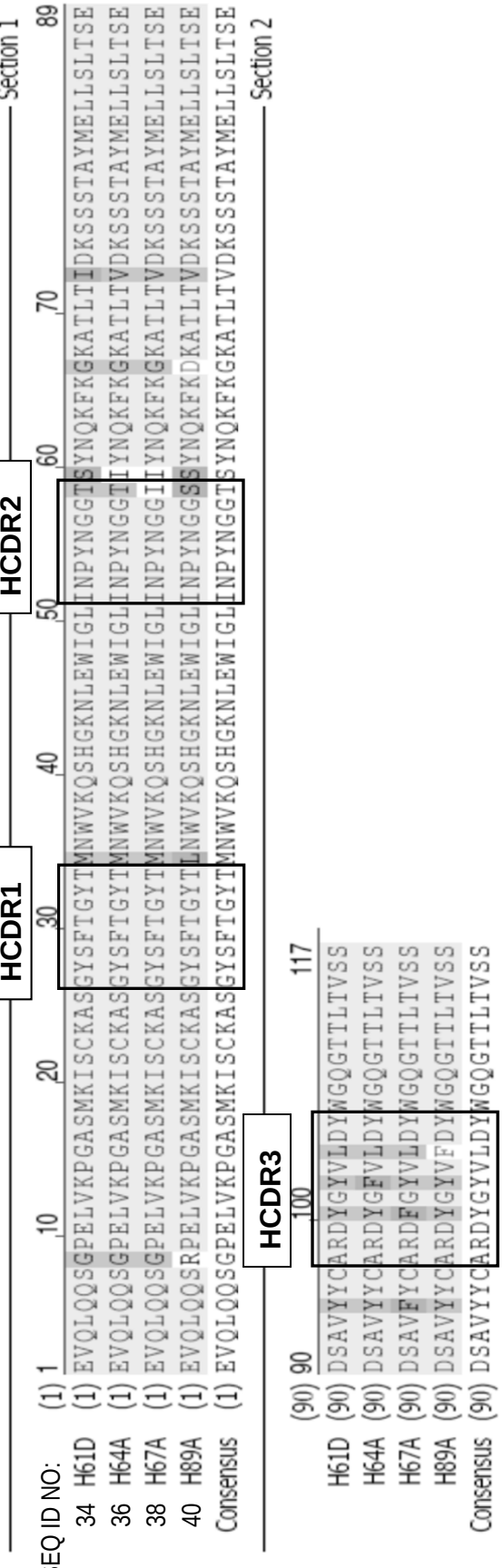

Section 1
Section 2
HCDR1
HCDR2
HCDR3
SEQ ID NO:
34 H61D
36 H64A
38 H67A
40 H89A
Consensus
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGTSYNQKFKGKATLTIDKSSSTAYMELLSLTSE
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGTIYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGIIYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(1) EVQLQQSRPELVKPGASMKISCKASGYSFTGYTLNWVKQSHGKNLEWIGLINPYNGGSSYNQKFKDKATLTVDKSSSTAYMELLSLTSE
(1) EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNGGTSYNQKFKGKATLTVDKSSSTAYMELLSLTSE
(90) DSAVYYCARDYGYVLDYWGQGTTLTVSS
(90) DSAVYYCARDYGFVLDYWGQGTTLTVSS
(90) DSAVFYCARDFGYVLDYWGQGTTLTVSS
(90) DSAVYYCARDYGYVFDYWGQGTTLTVSS
(90) DSAVYYCARDYGYVLDYWGQGTTLTVSS

Fig. 26

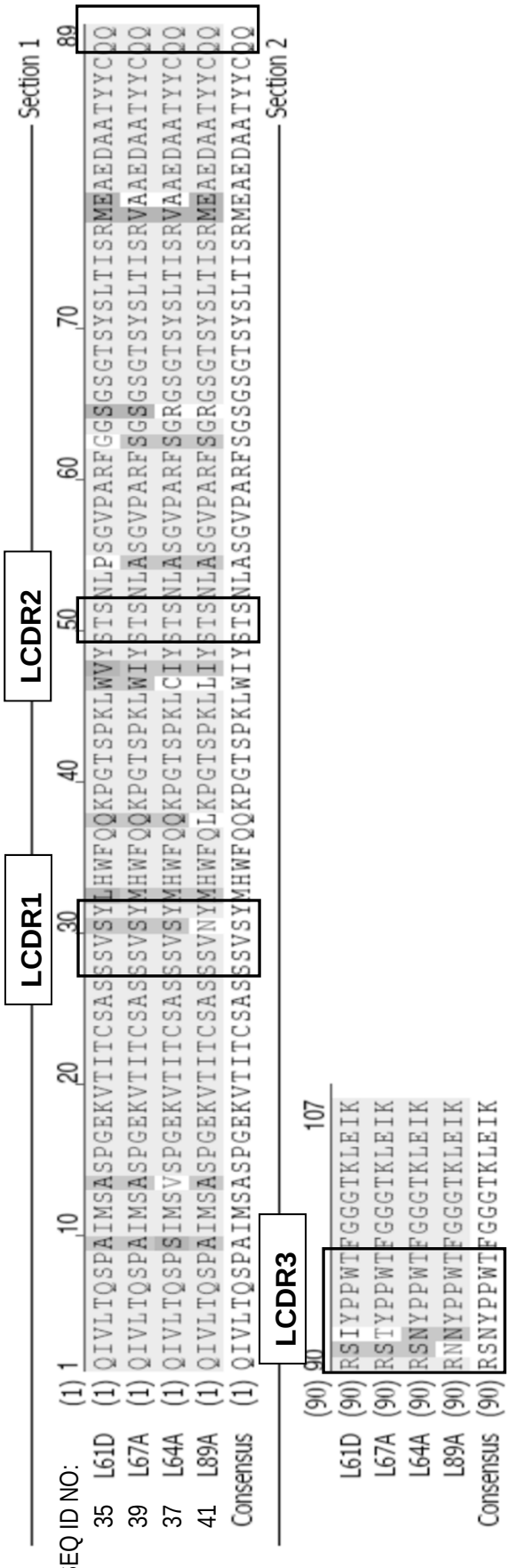
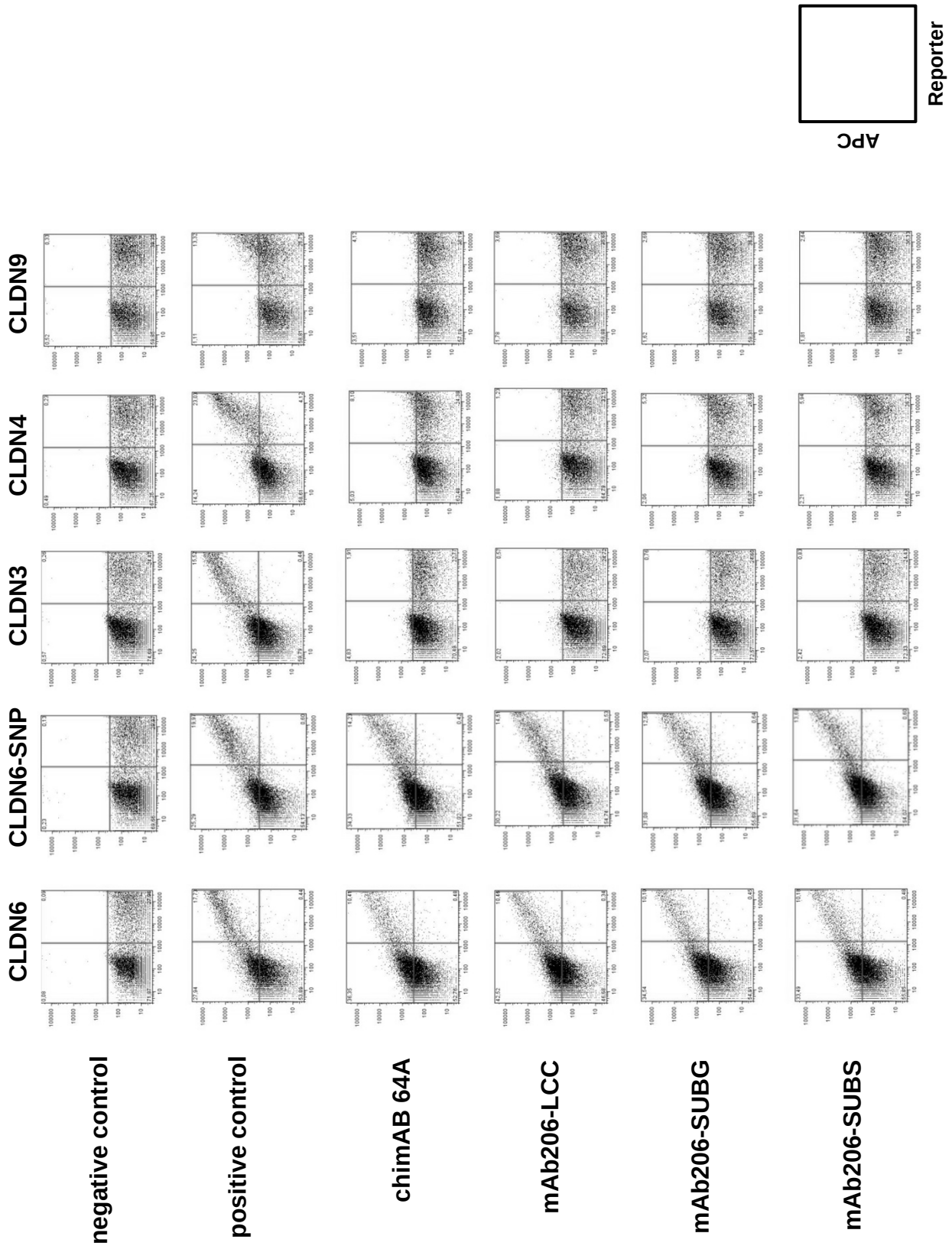

Section 1
Section 2
LCDR1
LCDR2
LCDR3
SEQ ID NO:
35 L61D
39 L67A
37 L64A
41 L89A
Consensus
(1) QIVLTQSPAIMSASPGEKVTITCSASSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISRMEAEDAATYYCQQ 89
(90) RSIYPPWTFGGGTKLEIK 107
(90) RSTYPPWTFGGGTKLEIK
(90) RSNYPPWTFGGGTKLEIK
(90) RNNYPPWTFGGGTKLEIK
(90) RSNYPPWTFGGGTKLEIK

Fig. 27


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

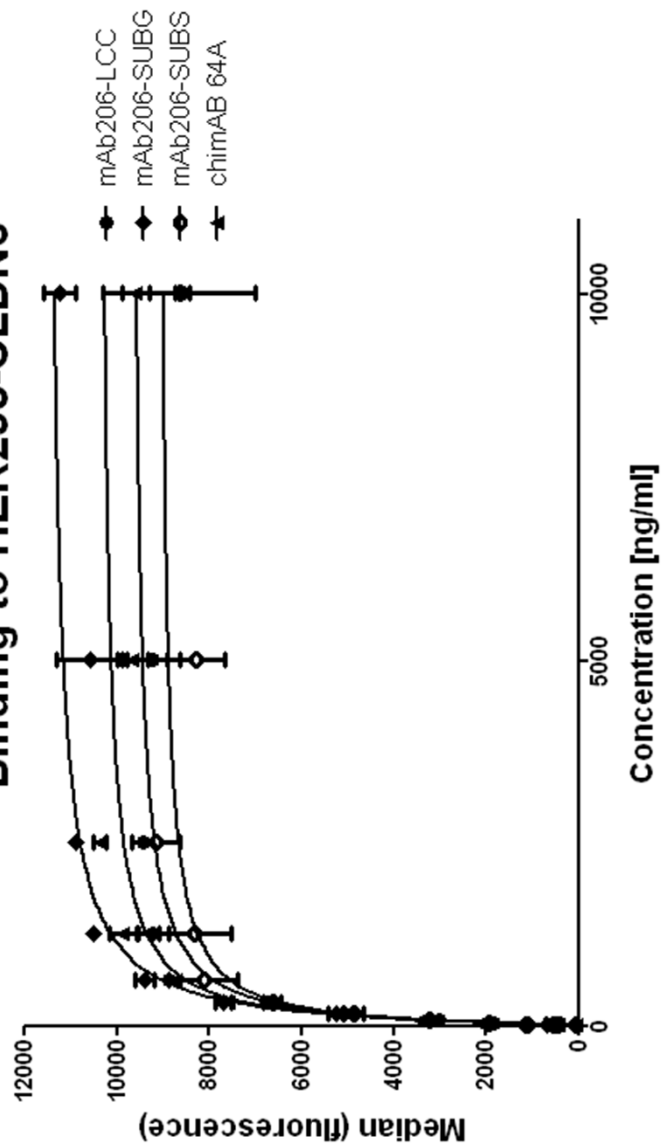

Binding to HEK293-CLDN6
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
chimAB 64A
Median (fluorescence)
12000
10000
8000
6000
4000
2000
0
0
5000
10000
Concentration [ng/ml]

Fig. 28

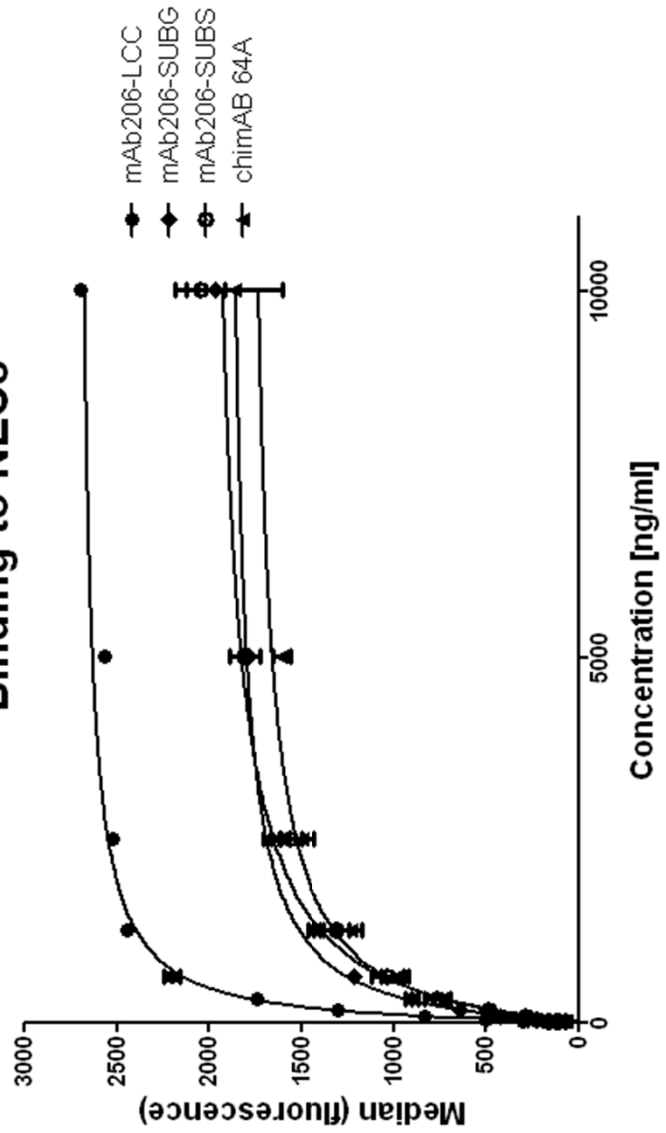

Binding to NEC8
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
chimAB 64A
Median (fluorescence)
0
500
1000
1500
2000
2500
3000
0
5000
10000
Concentration [ng/ml]

Fig. 29

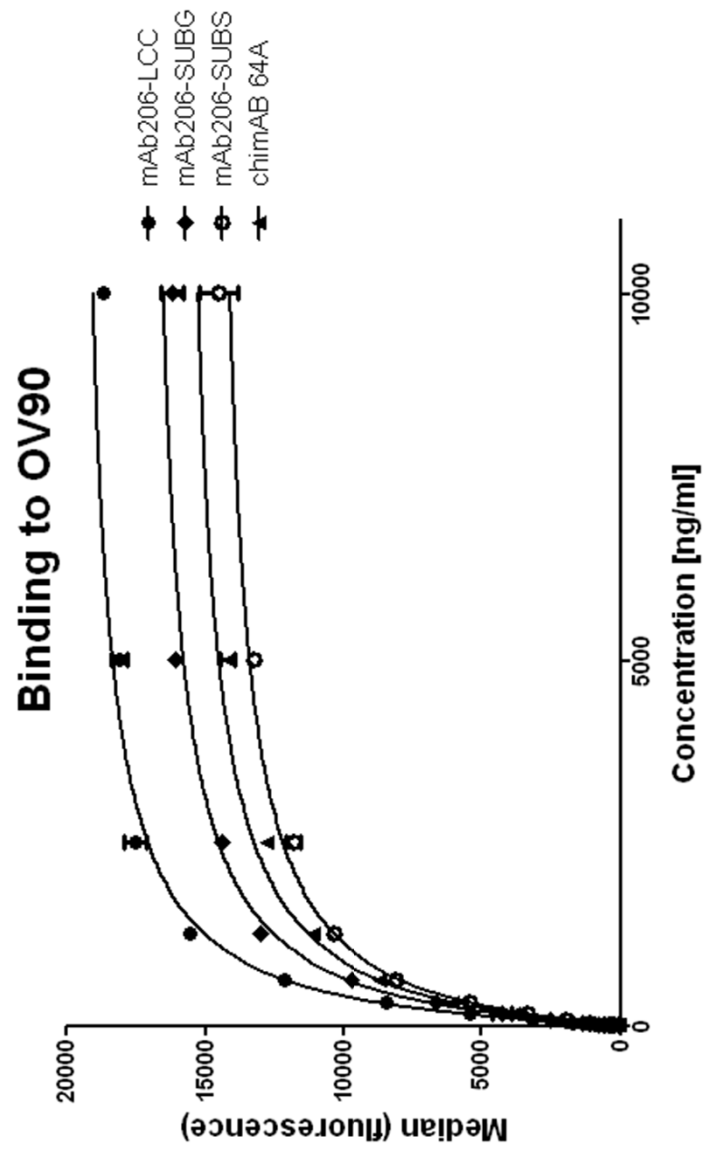
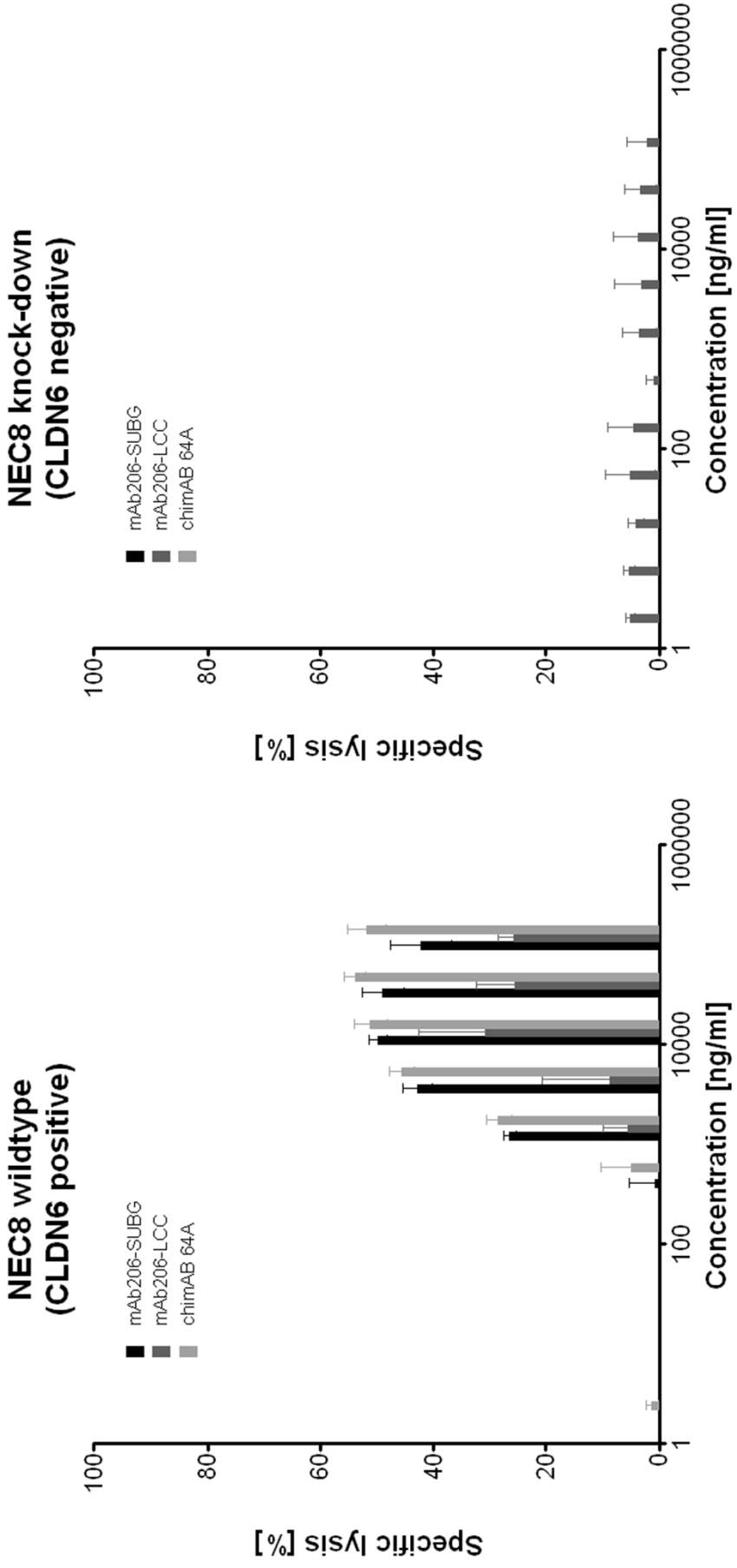

Binding to OV90
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
chimAB 64A
Median (fluorescence)
20000
15000
10000
5000
0
0
5000
10000
Concentration [ng/ml]

Fig. 30

Fig. 31a


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

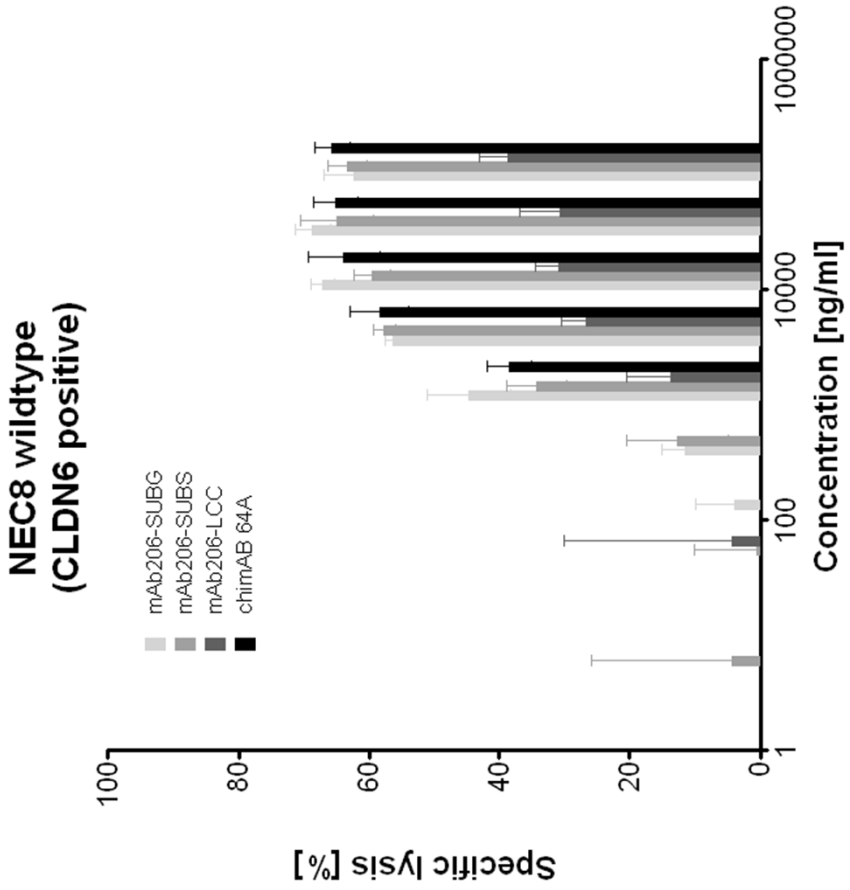

NEC8 wildtype
(CLDN6 positive)
mAb206-SUBG
mAb206-SUBS
mAb206-LCC
chimAB 64A
Specific lysis [%]
0
20
40
60
80
100
1
100
10000
1000000
Concentration [ng/ml]

Fig. 31b

Fig. 32a

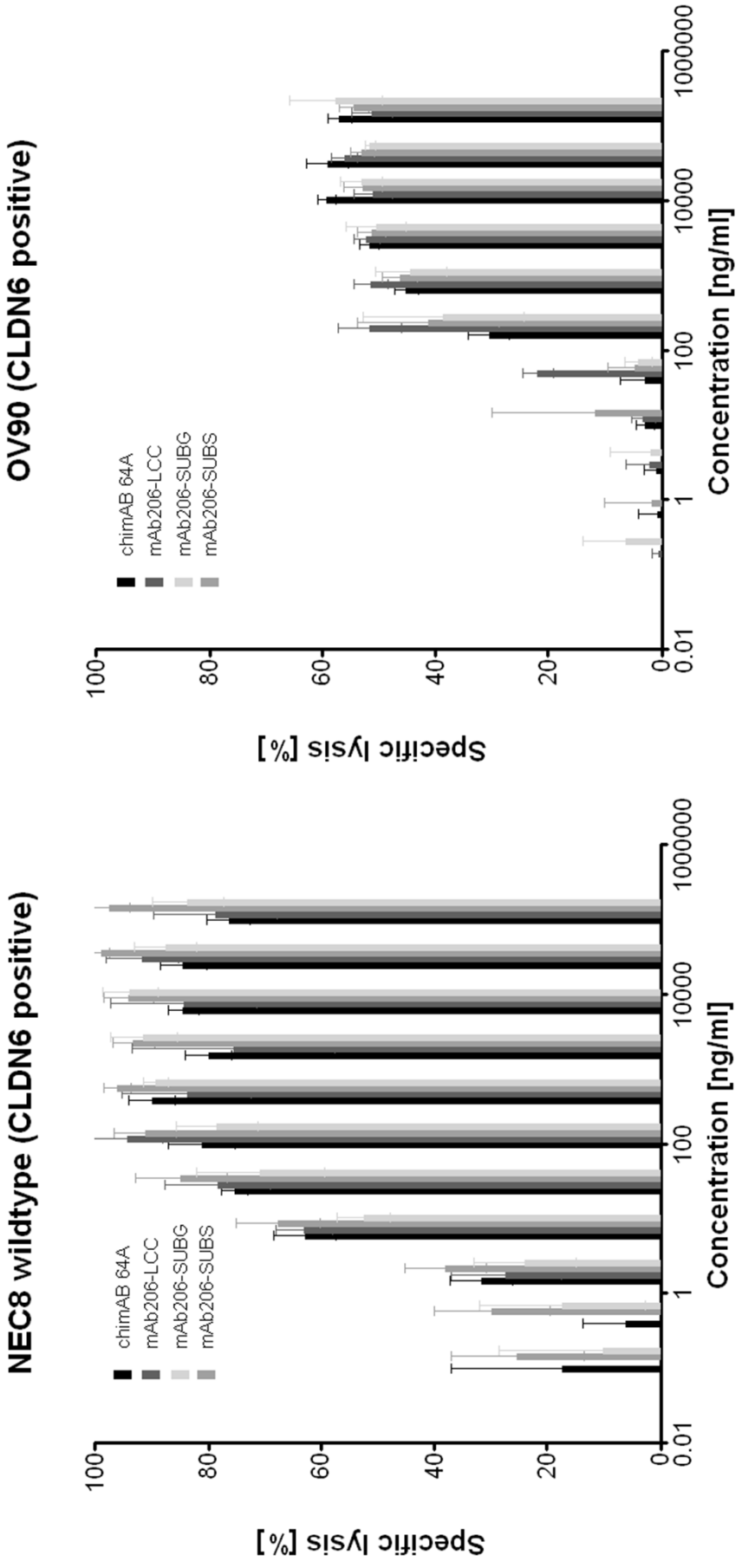

NEC8 wildtype (CLDN6 positive)
OV90 (CLDN6 positive)
chimAB 64A
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
Specific lysis [%]
Concentration [ng/ml]
0
20
40
60
80
100
0.01
1
100
10000
1000000

Fig. 32b

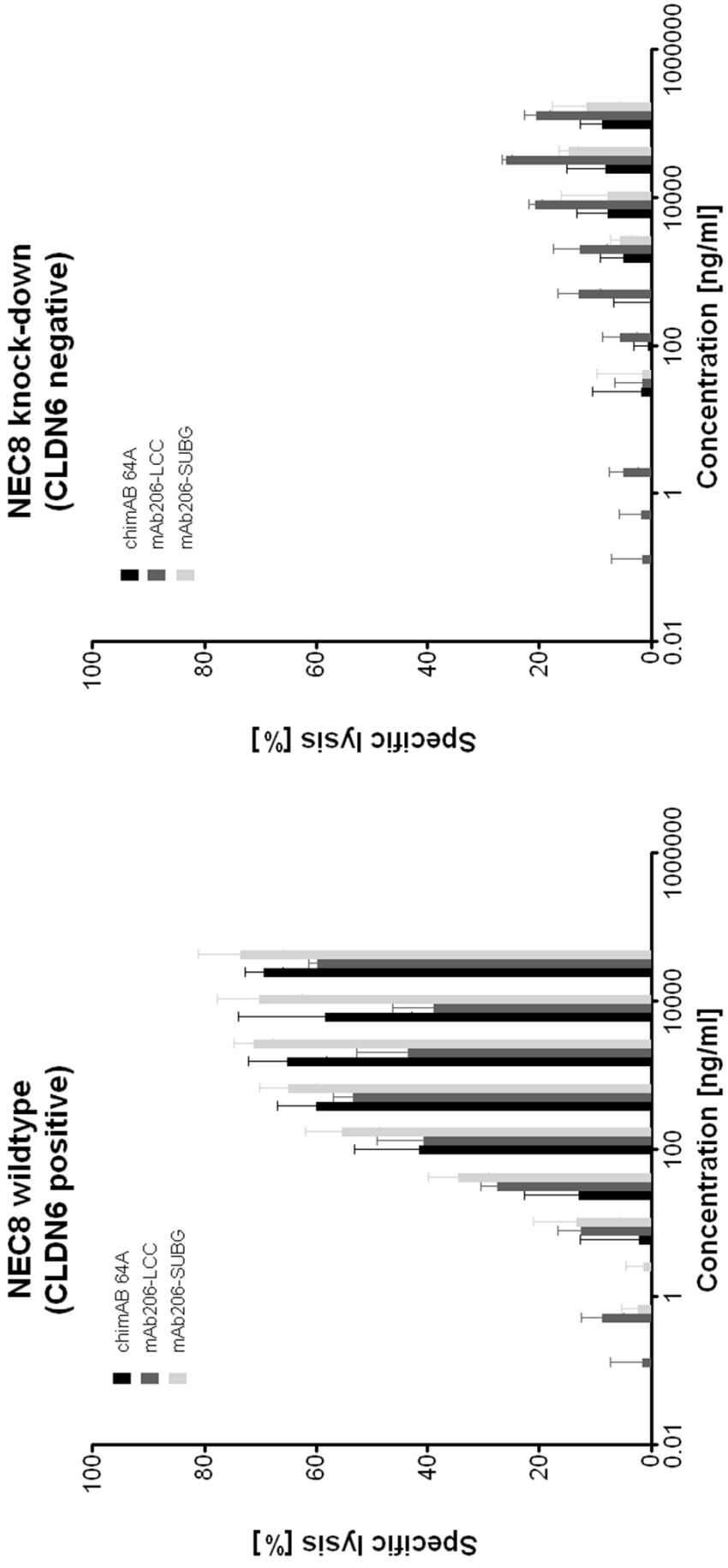

NEC8 wildtype
(CLDN6 positive)
chimAB 64A
mAb206-LCC
mAb206-SUBG
Specific lysis [%]
Concentration [ng/ml]
NEC8 knock-down
(CLDN6 negative)
chimAB 64A
mAb206-LCC
mAb206-SUBG
Specific lysis [%]
Concentration [ng/ml]

Fig. 33

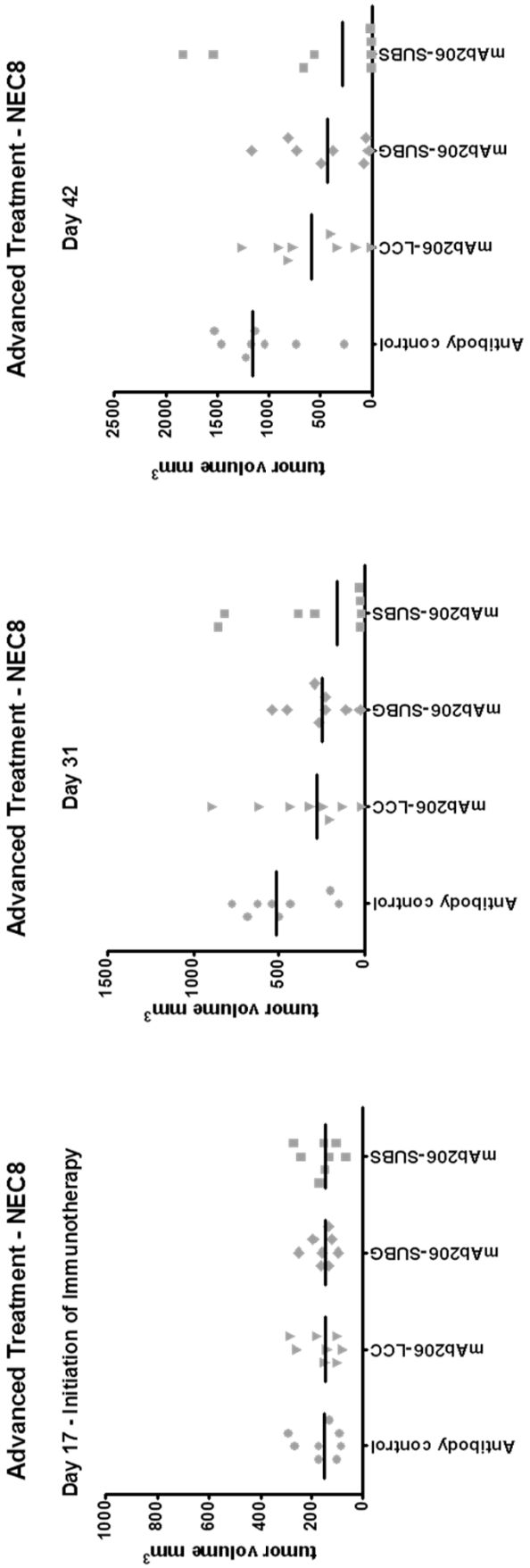
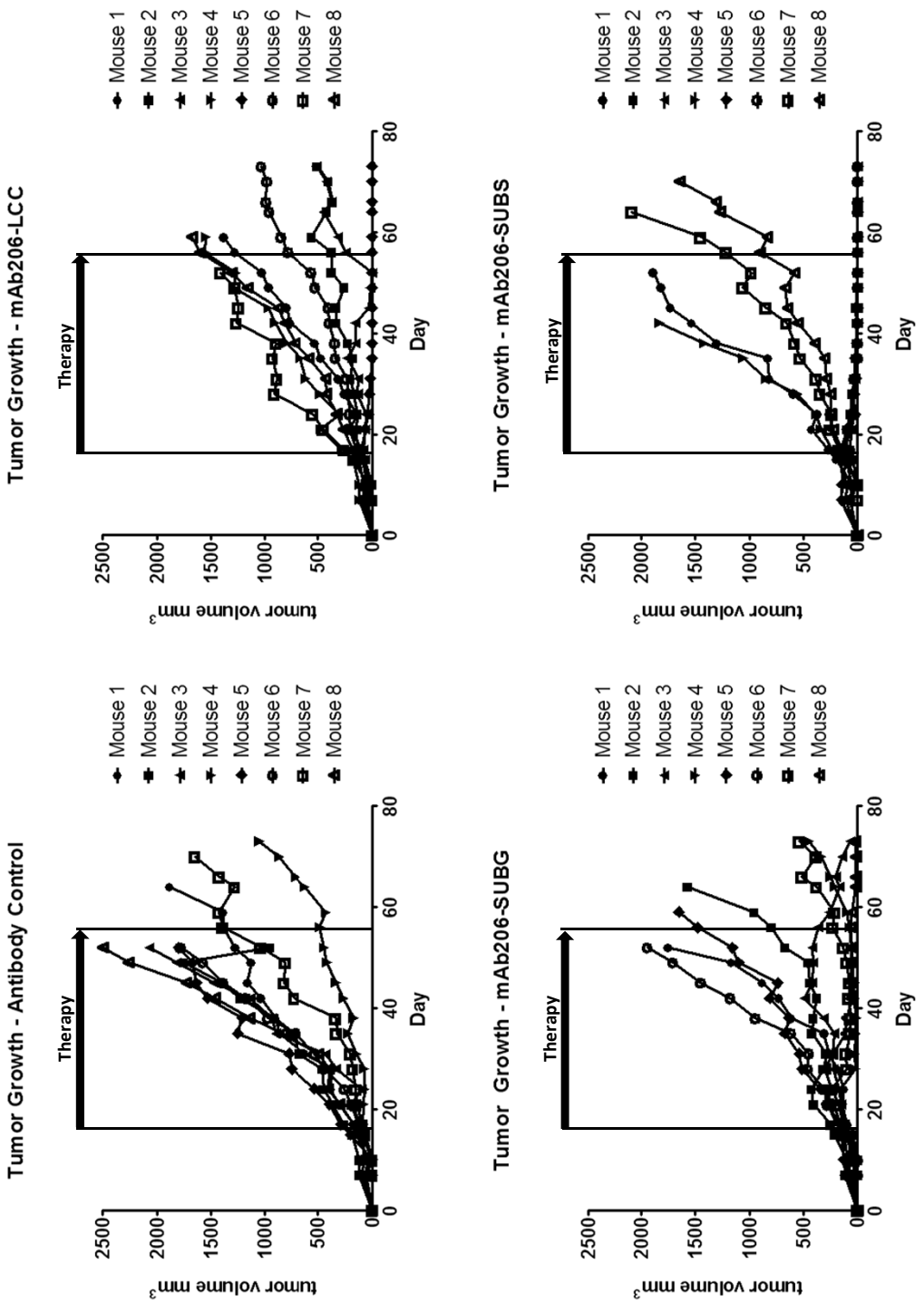

Advanced Treatment - NEC8
Day 17 - Initiation of Immunotherapy
tumor volume mm3
1000
800
600
400
200
0
Antibody control
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
Advanced Treatment - NEC8
Day 31
tumor volume mm3
1500
1000
500
0
Antibody control
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
Advanced Treatment - NEC8
Day 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-SUBS
Tumor Growth - mAb206-SUBG
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
0
20
40
60
80

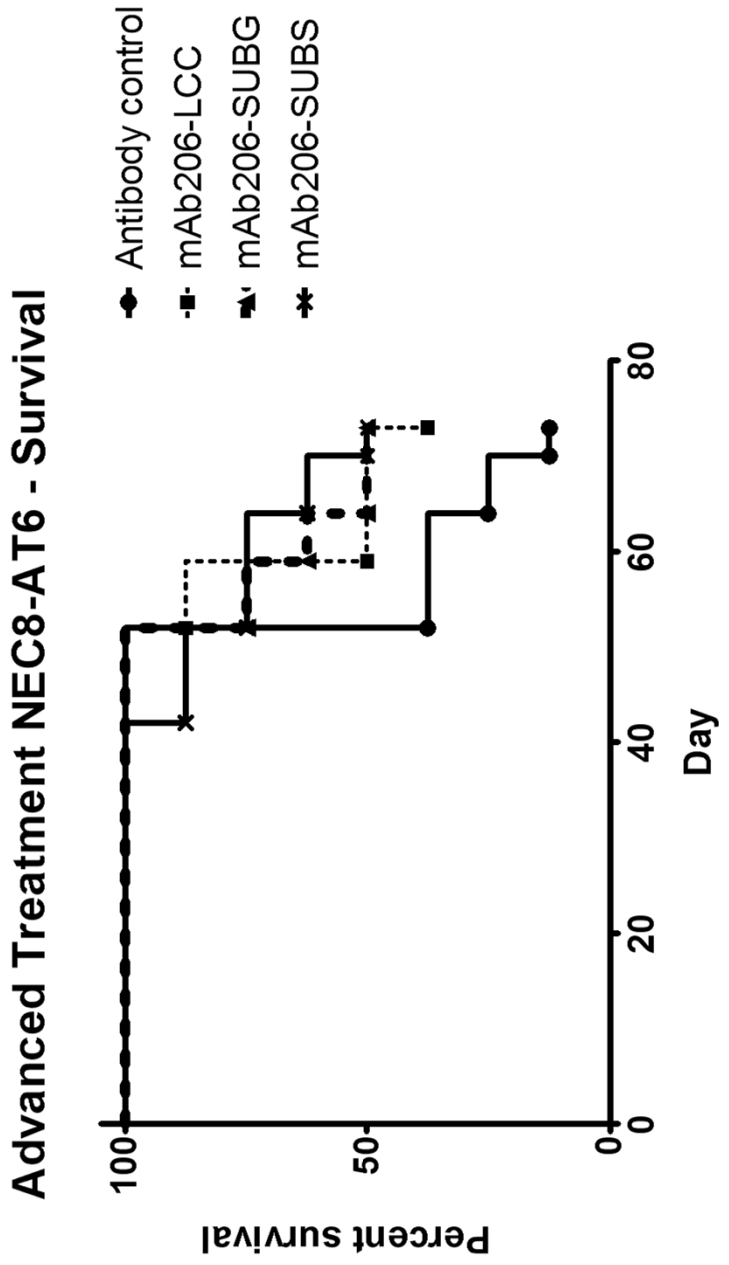

Advanced Treatment NEC8-AT6 - Survival
Antibody control
mAb206-LCC
mAb206-SUBG
mAb206-SUBS
Percent survival
100
50
0
0
20
40
60
80
Day

Fig. 34b

Fig. 35

EC1 (aa 27-81)

Name	L 27	P 28	M 29	W 30	K 31	V 32	T 33	A 34	F 35	I 36	G 37	N 38	S 39	I 40	V 41	V 42	A 43	Q 44	V 45	V 46	W 47	E 48	G 49	L 50	W 51	M 52	S 53	C 54
chimAB 64A		X		X																			X	X	X			X
mAb206- LCC		X		X																			X	X	X			X
mAb206- SUBG		X		X																			X	X	X			X
mAb206- SUBS		X		X																			X	X	X			X

Name	V 55	V 56	Q 57	S 58	T 59	G 60	Q 61	M 62	Q 63	C 64	K 65	V 66	Y 67	D 68	S 69	L 70	L 71	A 72	L 73	P 74	Q 75	D 76	L 77	Q 78	A 79	A 80	R 81
chimAB 64A										X																	
mAb206- LCC										X																	
mAb206- SUBG										X																	
mAb206- SUBS										X																	

EC2 (aa 137-161)

Name	C 137	W 138	T 139	A 140	H 141	A 142	I 143	I 144	R 145	D 146	F 147	Y 148	N 149	P 150	L 151	V 152	A 153	E 154	A 155	Q 156	K 157	R 158	E 159	L 160	G 161
chimAB 64A																									
mAb206- LCC																									
mAb206- SUBG																									
mAb206- SUBS																									

X altered transport to the membrane

no influence on antibody binding

contributes to antibody binding

essential for antibody binding

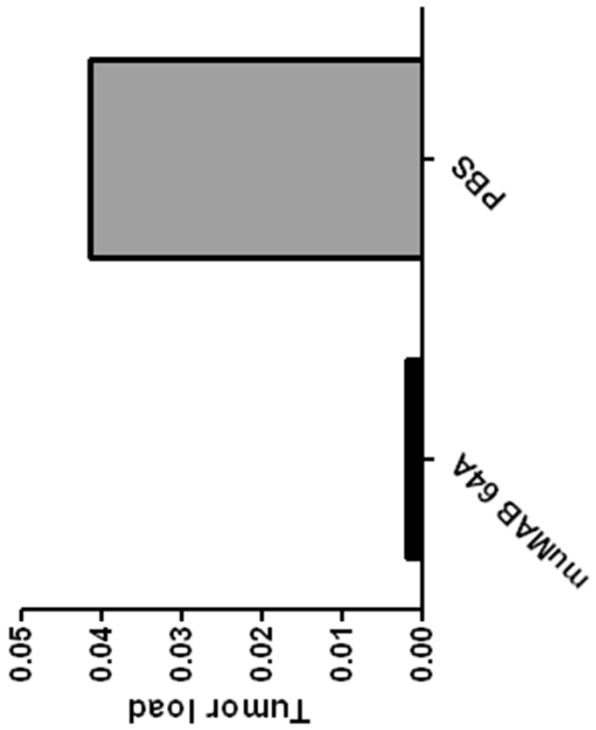


Fig. 36

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	-