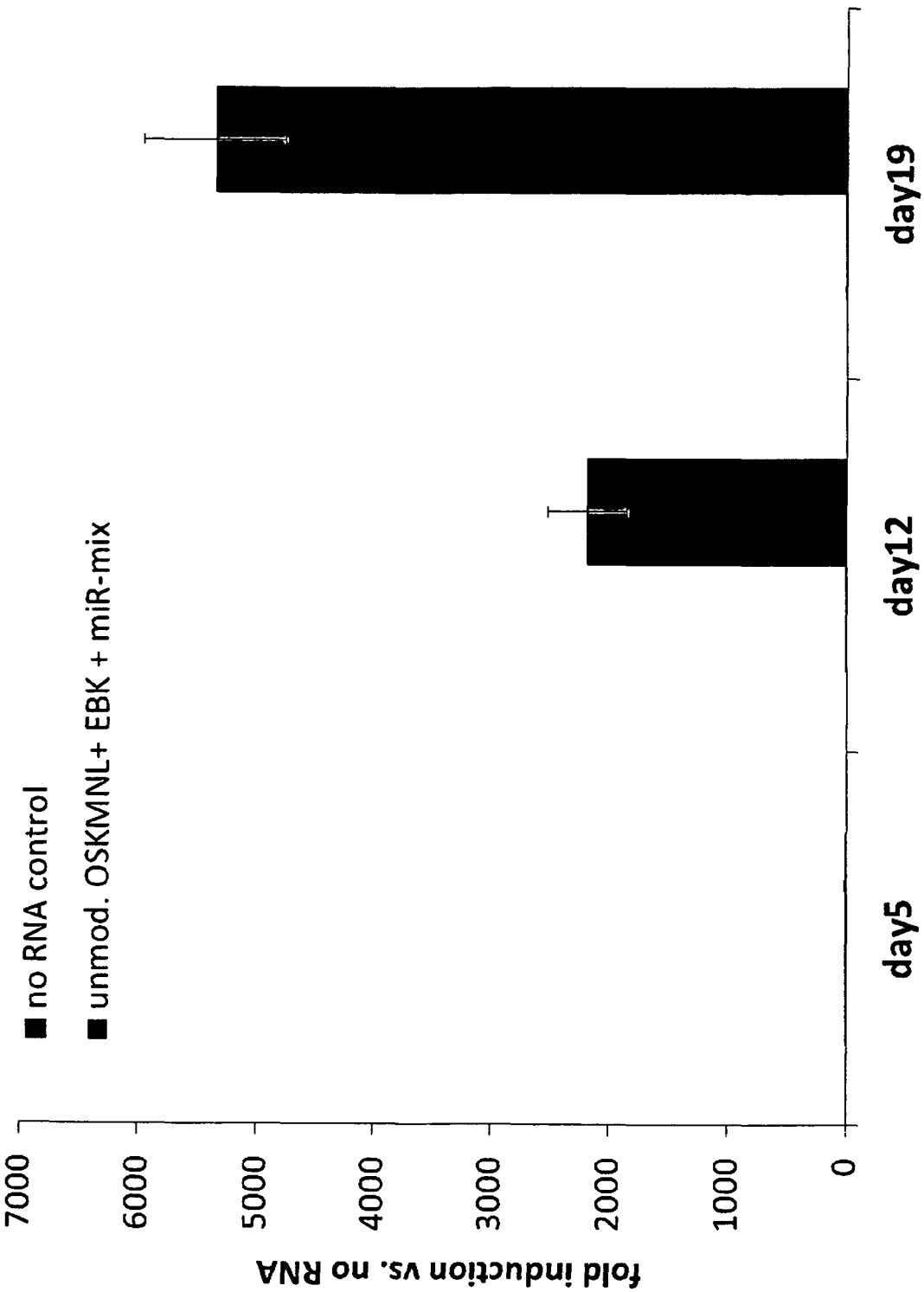

Expression of CLDN6 in iPS cells
no RNA control
unmod. OSKMNL+ EBK + miR-mix
fold induction vs. no RNA
0
1000
2000
3000
4000
5000
6000
7000
day5
day12
day19

Figure 1

Figure 2

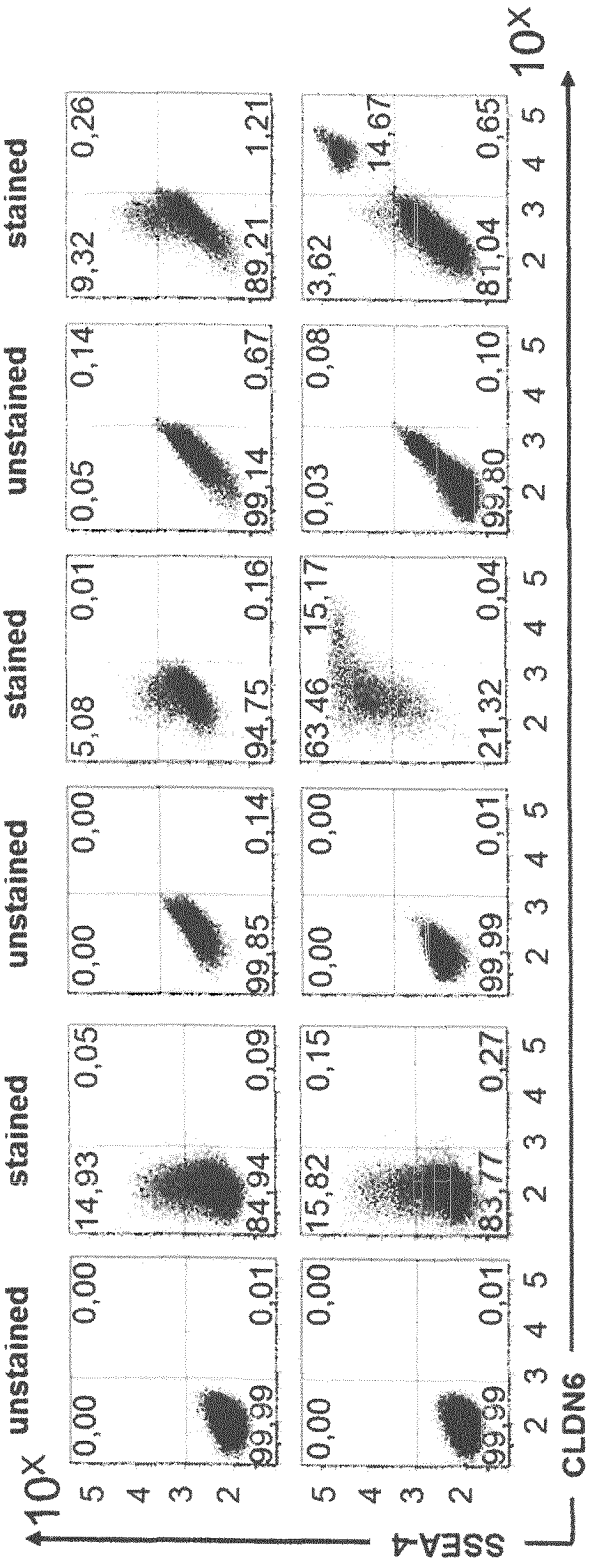
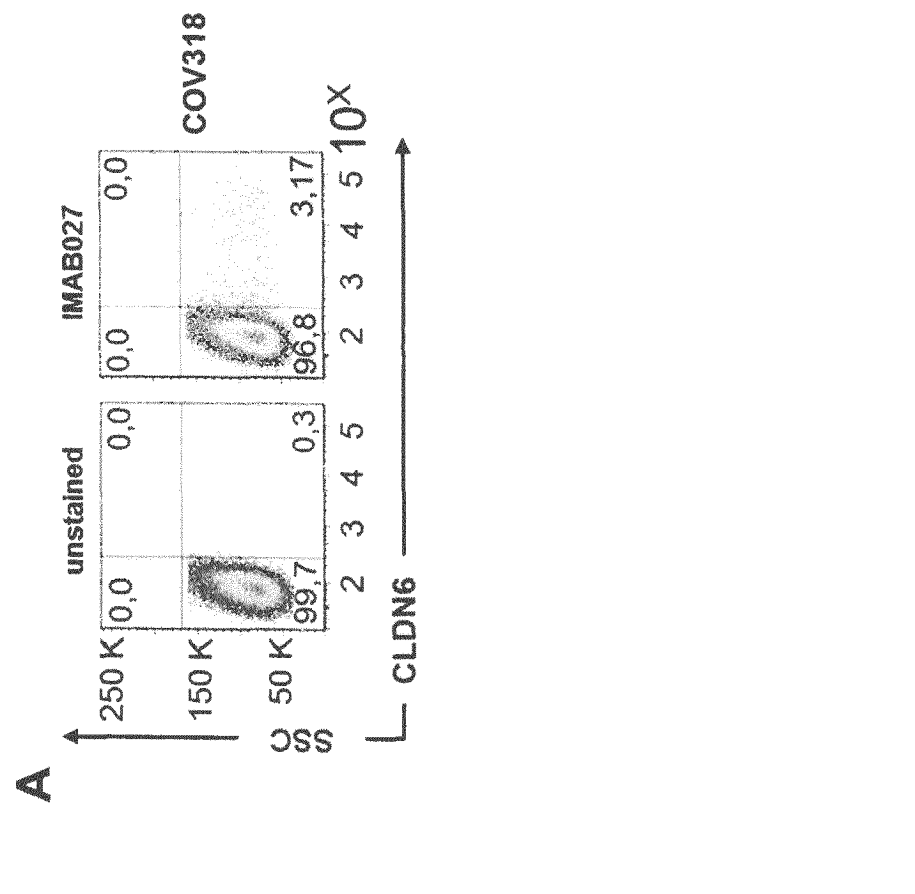
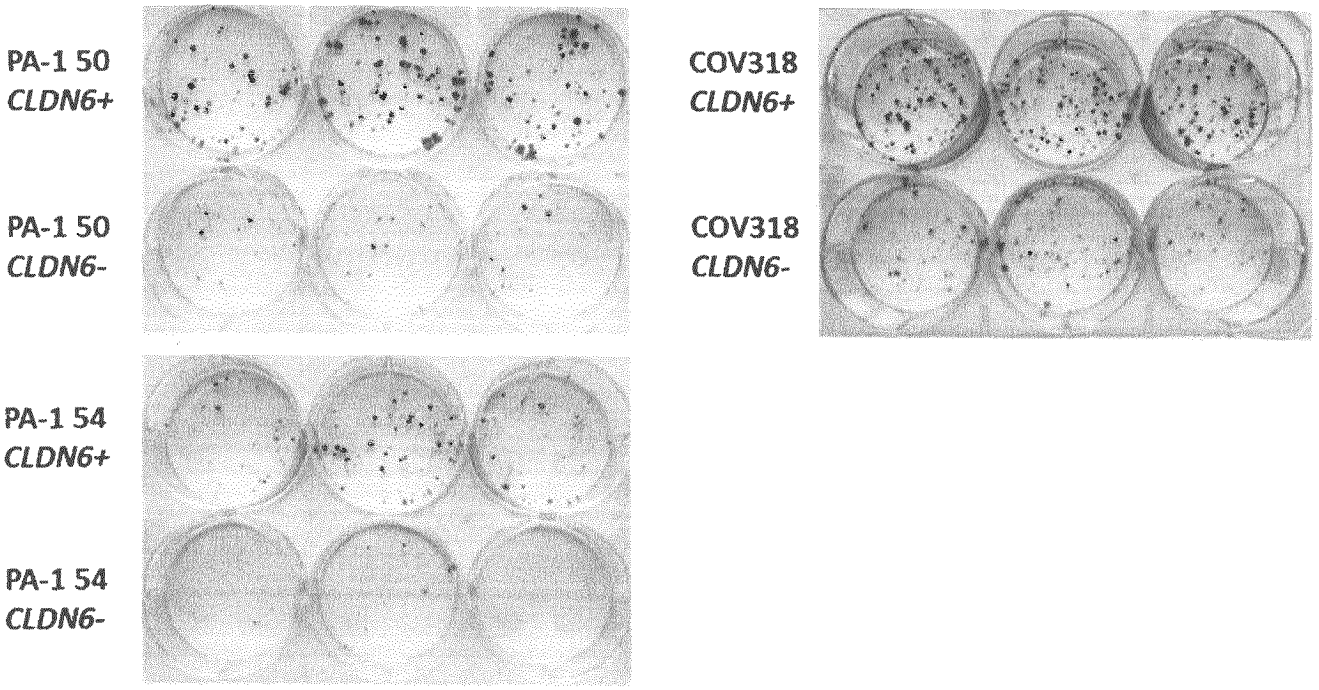

Day 5
Day 12
Day 19
unstained
stained
unstained
stained
unstained
stained
No RNA control
Reprogramming cocktail
SSEA4
CLDN6
10^X
0,00
0,00
99,99
0,01
14,93
0,05
84,94
0,09
0,00
0,00
99,85
0,14
5,08
0,01
94,75
0,16
0,05
0,14
99,14
0,67
9,32
0,26
89,21
1,21
0,00
0,00
99,99
0,01
15,82
0,15
83,77
0,27
0,00
0,00
99,99
0,01
63,46
15,17
21,32
0,04
0,03
0,08
99,80
0,10
3,62
14,67
81,04
0,65

Figure 3


A
unstained
IMAB027
COV318
250 K
150 K
50 K
SSC
0,0
0,0
0,0
0,0
99,7
0,3
96,8
3,17
2 3 4 5
CLDN6
10X
B
unstained
IMAB027
PA-1 76
PA-1 50
PA-1 54
Low / Med / Hi
100,0 / 0,0 / 0,0
0,0 / 0,6 / 99,4
99,9 / 0,0 / 0,0
0,4 / 13,0 / 86,6
99,9 / 0,0 / 0,0
8,6 / 58,1 / 33,3
250 K
150 K
50 K
SSC
2 3 4 5
CLDN6
10X

A


PA-1 50
CLDN6+
PA-1 50
CLDN6-
COV318
CLDN6+
COV318
CLDN6-
PA-1 54
CLDN6+
PA-1 54
CLDN6-

B

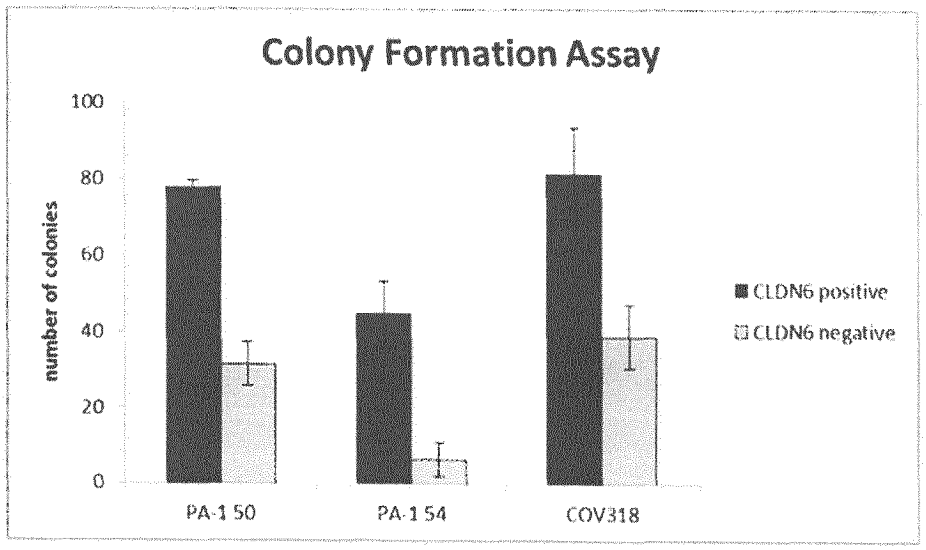

Colony Formation Assay
number of colonies
100
80
60
40
20
0
PA-1 50
PA-1 54
COV318
CLDN6 positive
CLDN6 negative

Figure 4

Figure 5

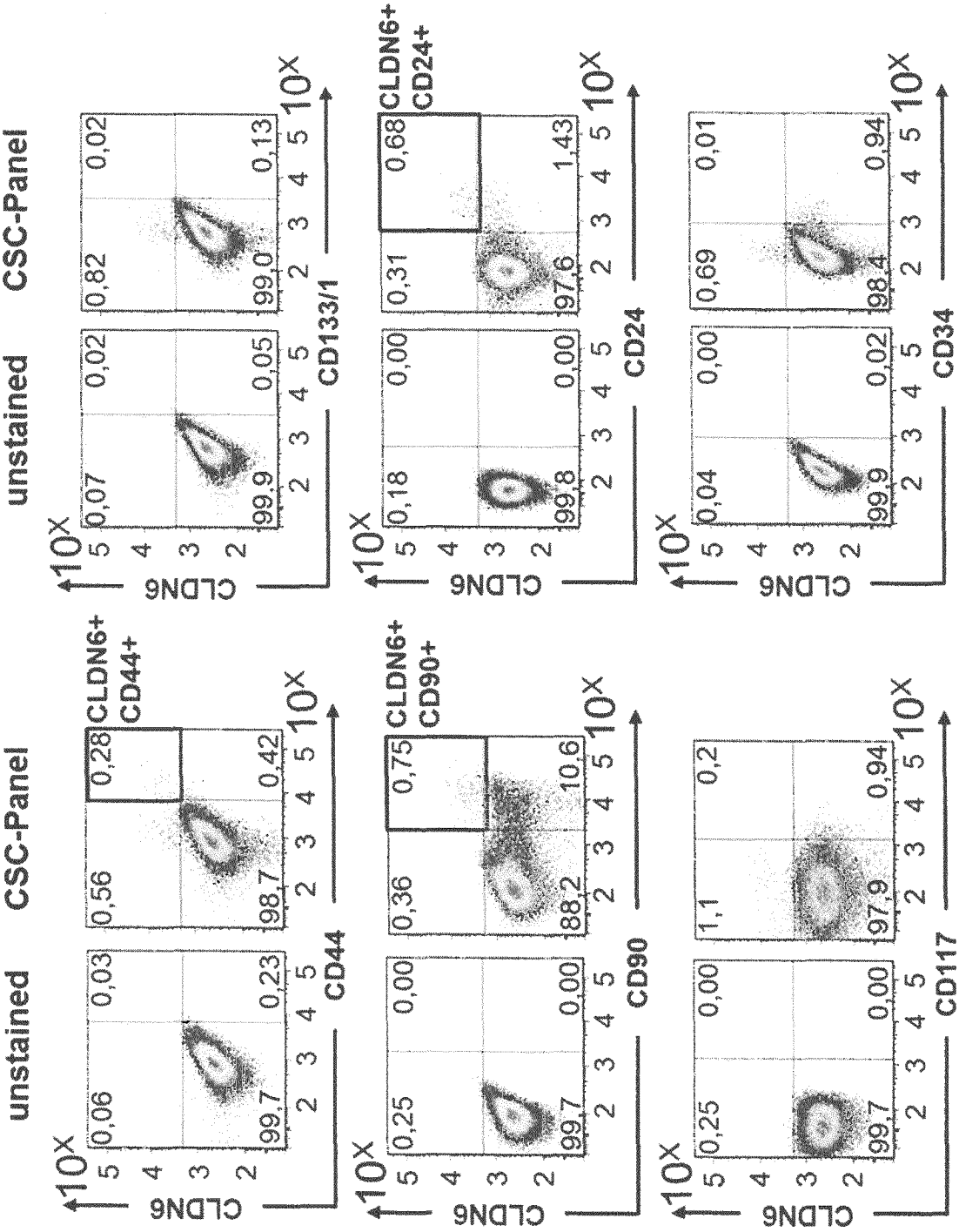

A
unstained
CSC-Panel
unstained
CSC-Panel
CLDN6
CD44
CLDN6+ CD44+
CD133/1
CLDN6+ CD24+
CD90
CLDN6+ CD90+
CD24
CD34
CD117

Figure 5

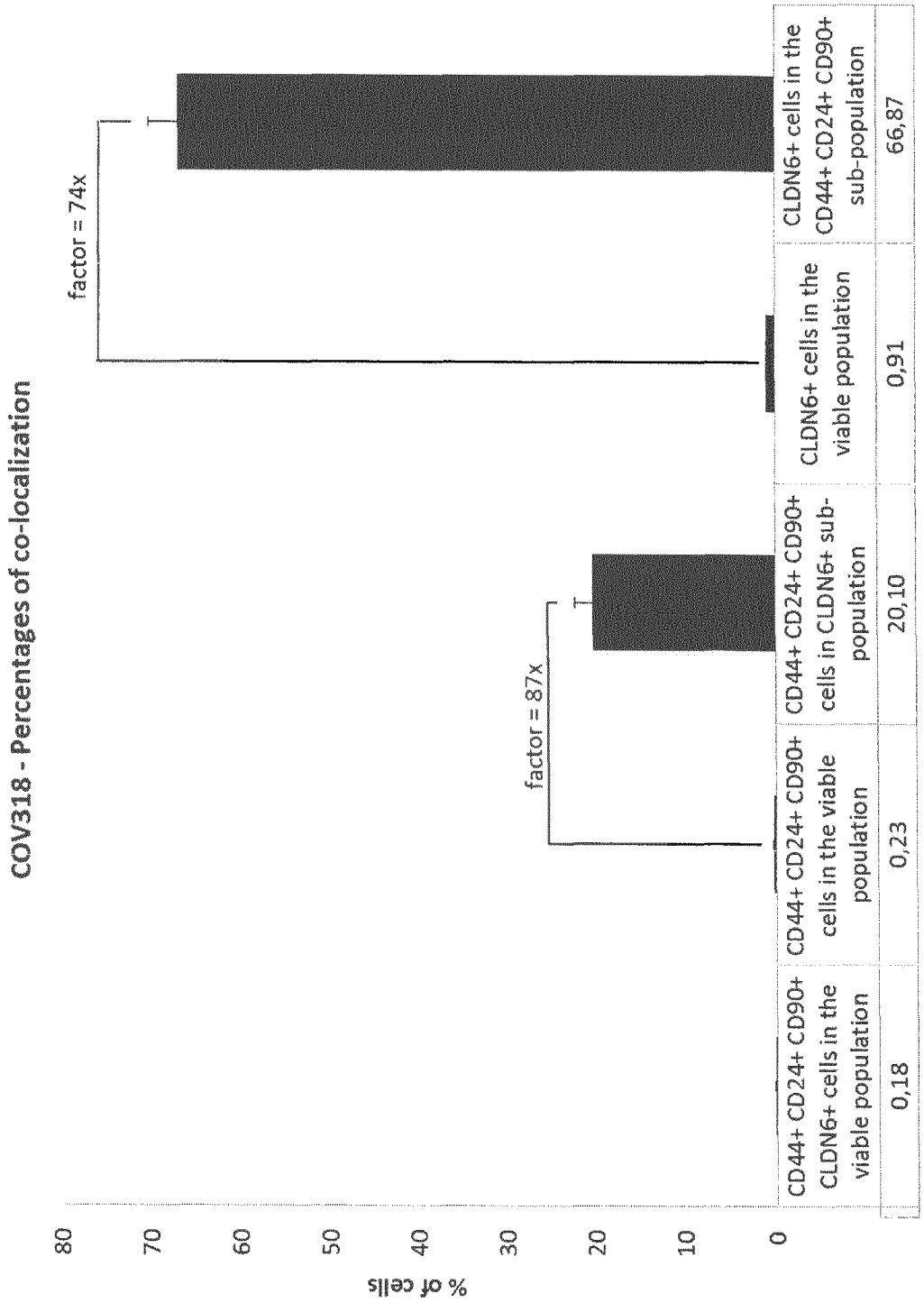

B
COV318 - Percentages of co-localization
% of cells
0
10
20
30
40
50
60
70
80
factor = 87x
factor = 74x
CD44+ CD24+ CD90+ CLDN6+ cells in the viable population
CD44+ CD24+ CD90+ cells in the viable population
CD44+ CD24+ CD90+ cells in CLDN6+ sub-population
CLDN6+ cells in the viable population
CLDN6+ cells in the CD44+ CD24+ CD90+ sub-population
0,18
0,23
20,10
0,91
66,87

Figure 6
A

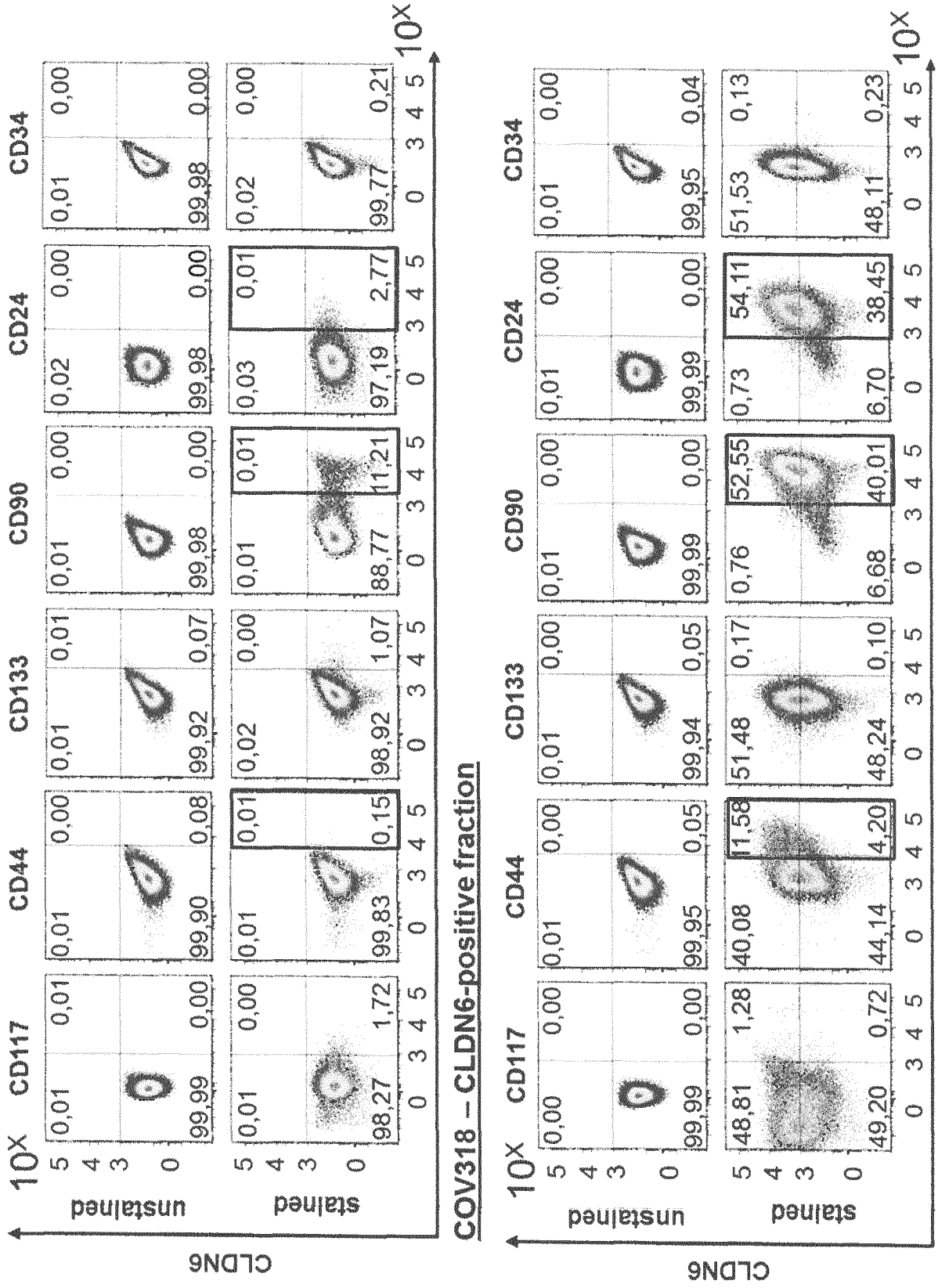

COV318 – CLDN6-negative fraction
COV318 – CLDN6-positive fraction
CD117
CD44
CD133
CD90
CD24
CD34
unstained
stained
CLDN6
10X

Figure 6

B

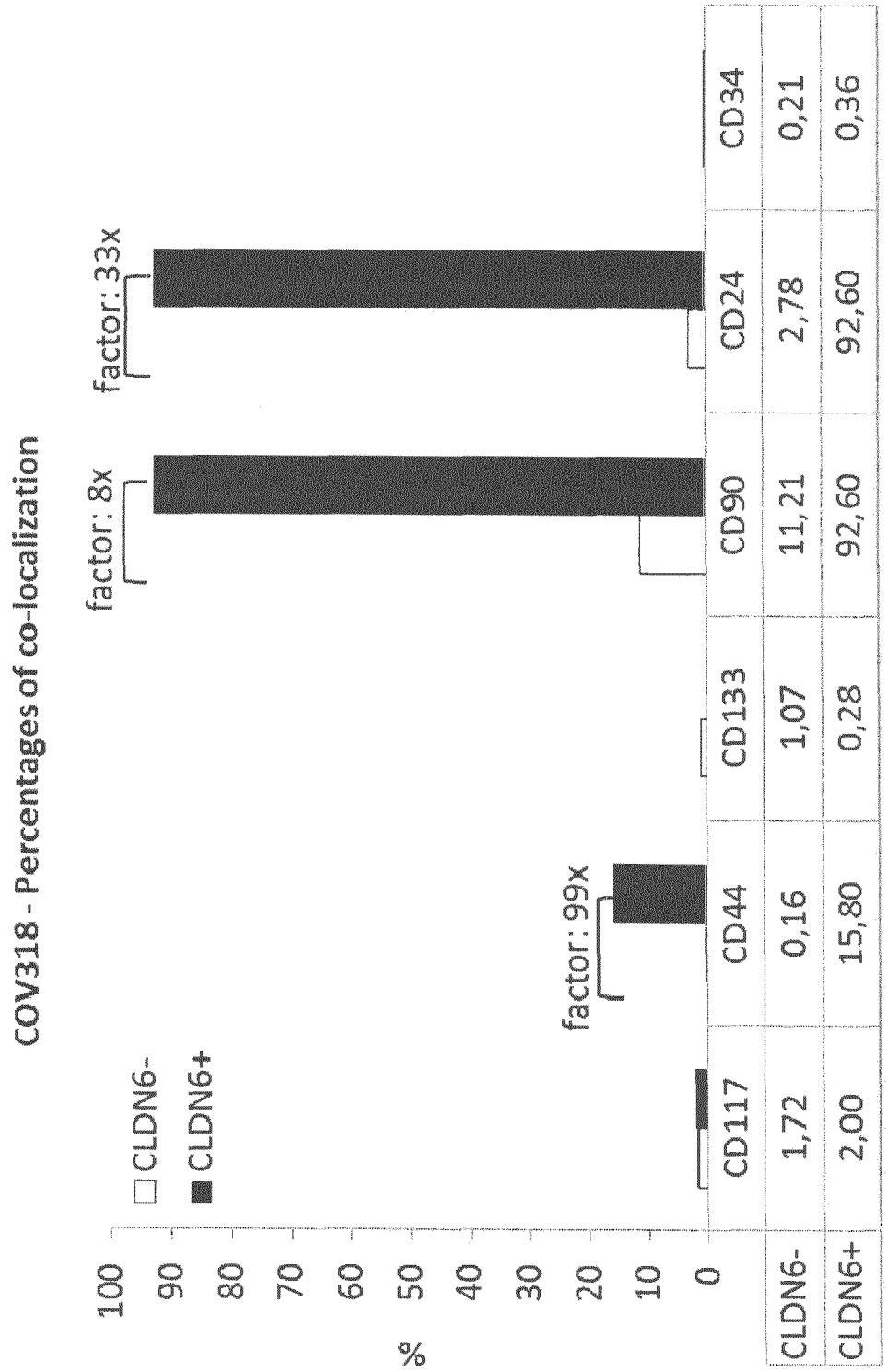

COV318 - Percentages of co-localization
CLDN6-
CLDN6+
%
100
90
80
70
60
50
40
30
20
10
0
factor: 99x
factor: 8x
factor: 33x
CD117
CD44
CD133
CD90
CD24
CD34
CLDN6- 1,72 0,16 1,07 11,21 2,78 0,21
CLDN6+ 2,00 15,80 0,28 92,60 92,60 0,36

Figure 7

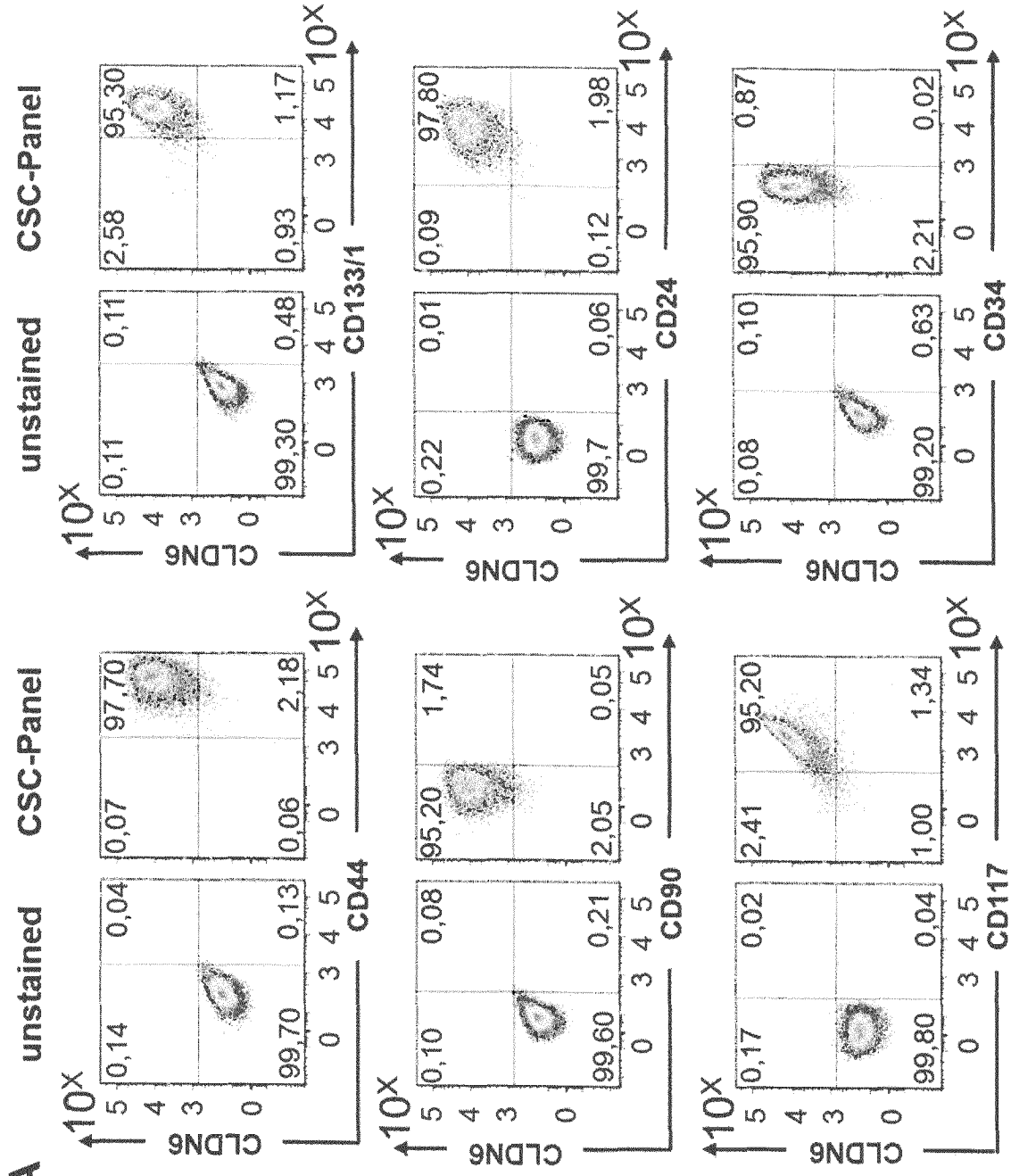

A
unstained
CSC-Panel
unstained
CSC-Panel
CLDN6
CD44
CD133/1
CD90
CD24
CD117
CD34
0,14
0,04
99,70
0,13
0,07
97,70
0,06
2,18
0,11
0,11
99,30
0,48
2,58
95,30
0,93
1,17
0,10
0,08
99,60
0,21
95,20
1,74
2,05
0,05
0,22
0,01
99,7
0,06
0,09
97,80
0,12
1,98
0,17
0,02
99,80
0,04
2,41
95,20
1,00
1,34
0,08
0,10
99,20
0,63
95,90
0,87
2,21
0,02

Figure 7

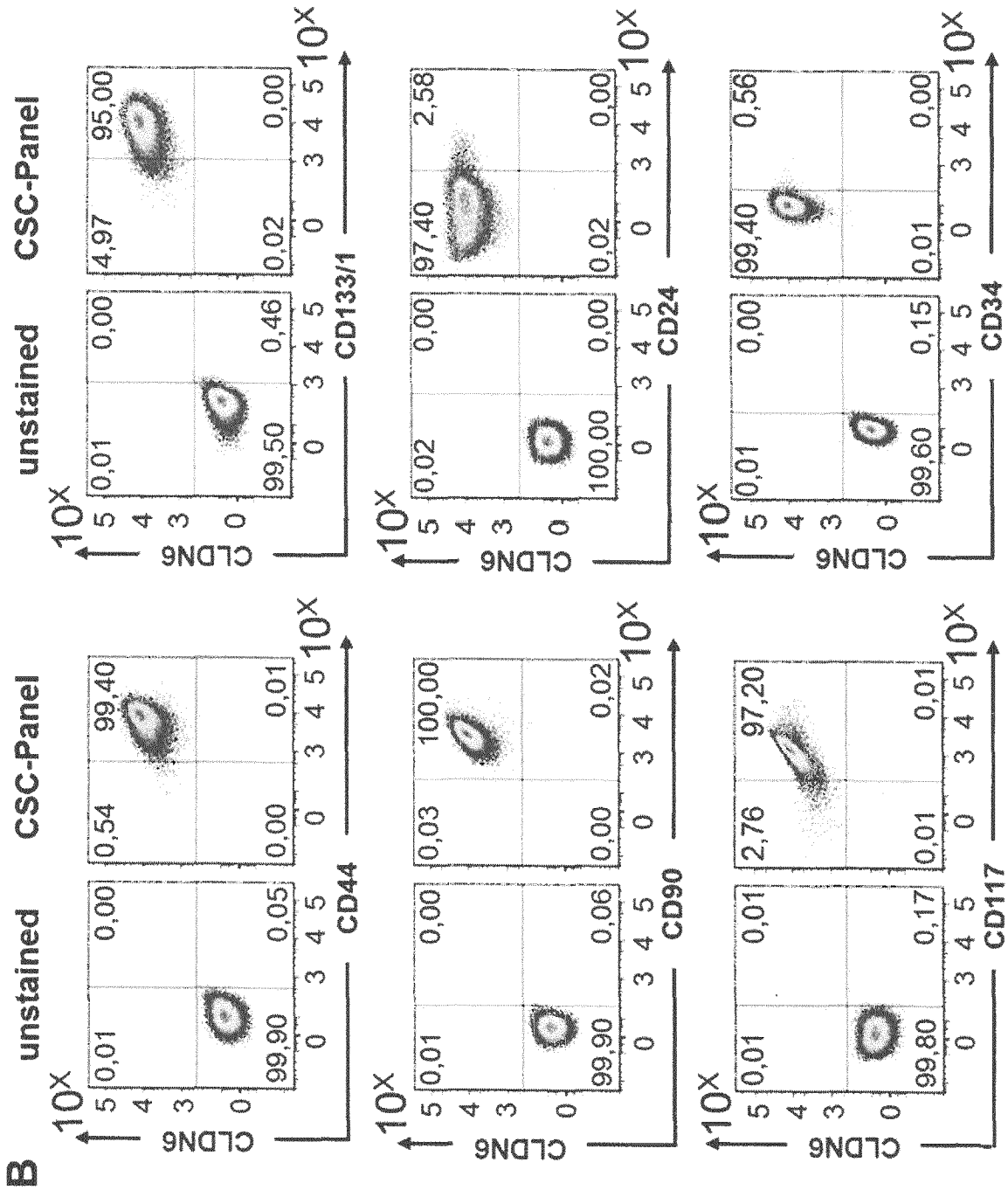

B
unstained
CSC-Panel
CLDN6
CD44
CD133/1
CD90
CD24
CD34
CD117
0,01
0,00
0,54
99,40
0,00
0,01
99,90
0,05
0,06
4,97
95,00
0,02
0,46
99,50
0,03
100,00
0,02
0,02
97,40
2,58
100,00
0,01
0,56
99,40
0,15
99,60
2,76
97,20
0,17
99,80

Figure 7

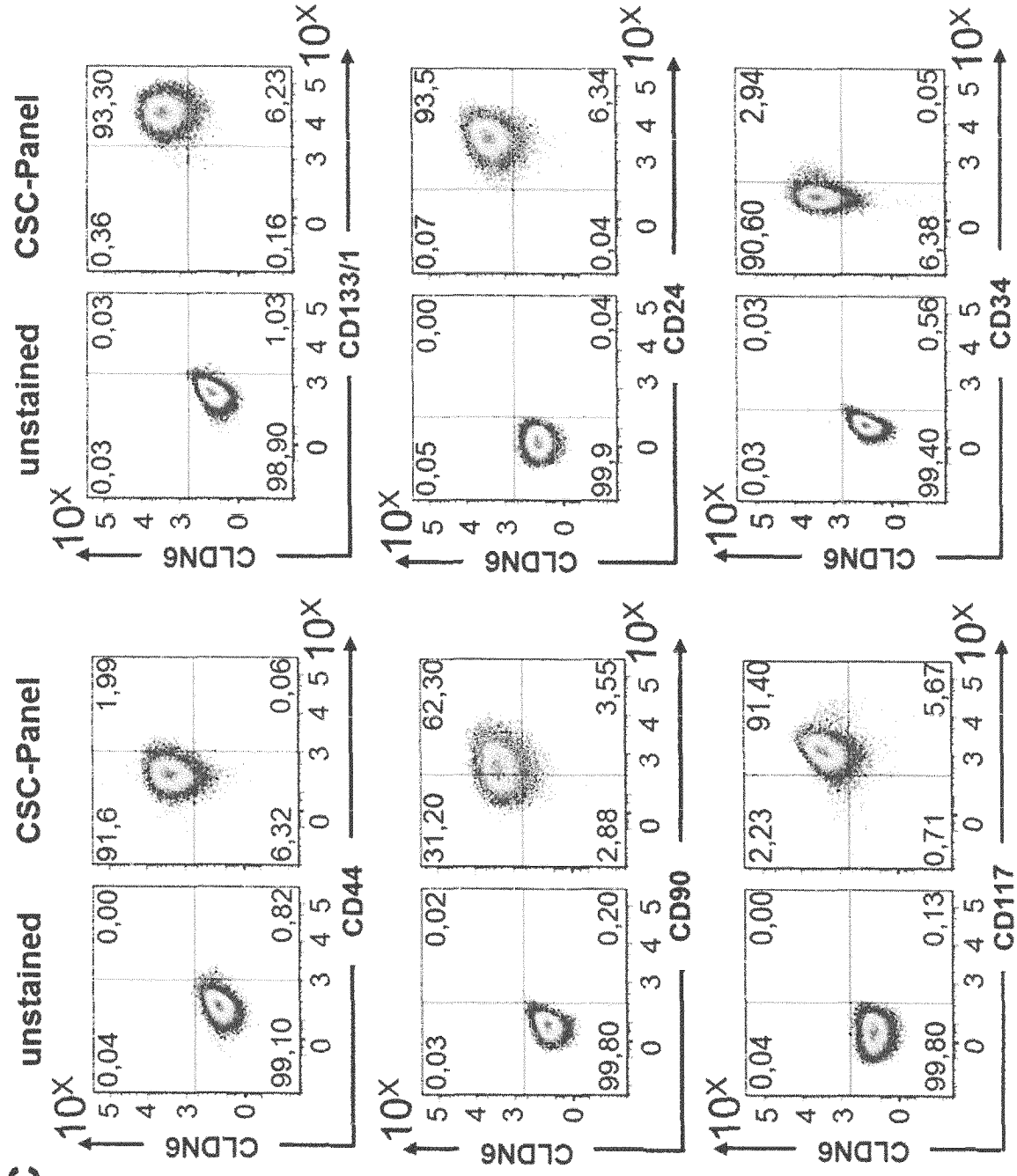

C
unstained
CSC-Panel
CLDN6
CD44
CD133/1
CD90
CD24
CD34
CD117
0,04
0,00
99,10
0,82
91,6
1,99
6,32
0,06
0,03
0,02
99,80
0,20
31,20
62,30
2,88
3,55
0,04
0,13
2,23
91,40
0,71
5,67
0,03
0,00
98,90
1,03
0,36
93,30
0,16
6,23
0,05
0,00
99,9
0,04
0,07
93,5
0,04
6,34
0,03
0,03
99,40
0,56
90,60
2,94
6,38
0,05

Figure 7

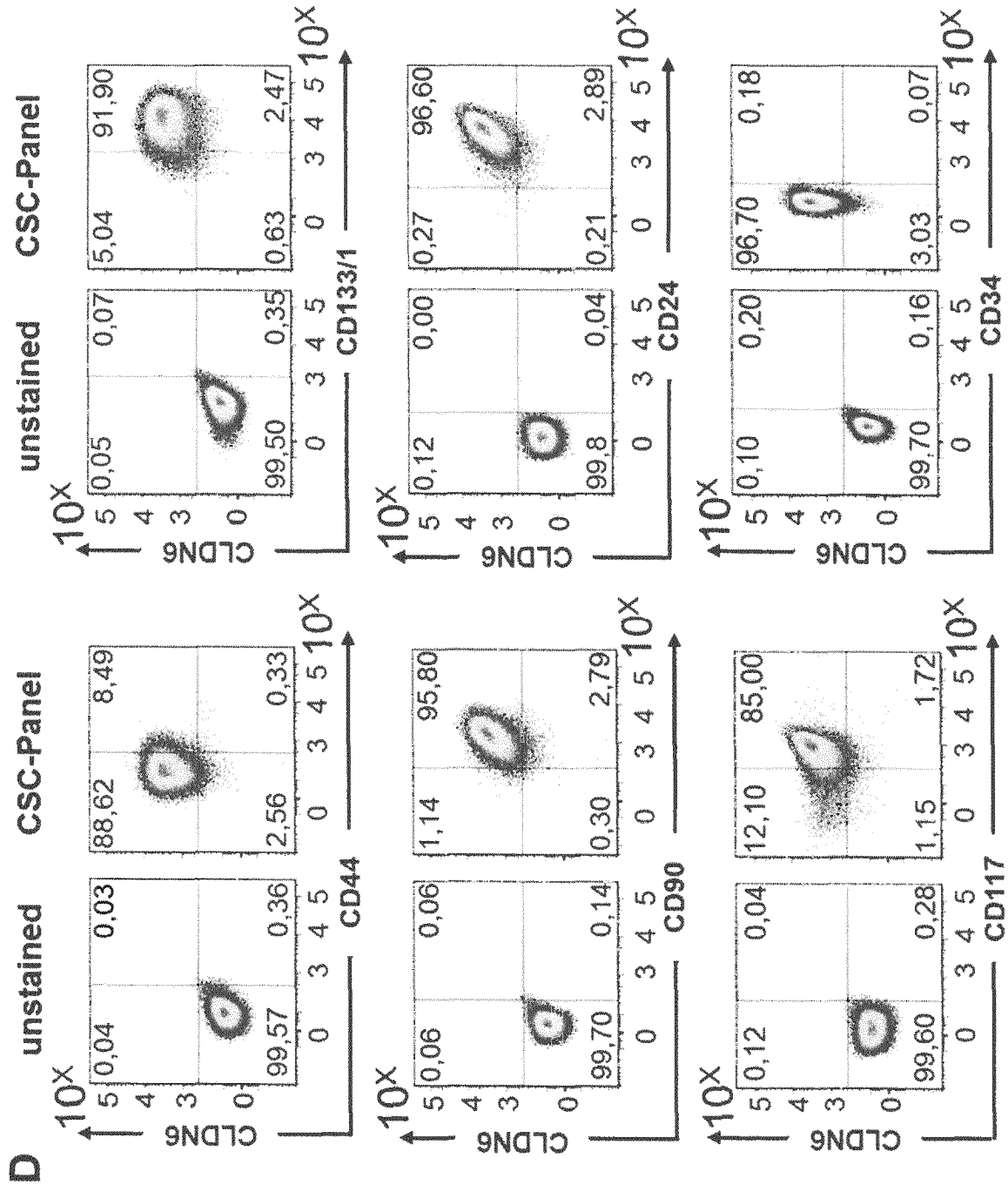

D
unstained
CSC-Panel
CLDN6
CD44
CD133/1
CD90
CD24
CD117
CD34
0,04
0,03
99,57
0,36
88,62
8,49
2,56
0,33
0,05
0,07
99,50
0,35
5,04
91,90
0,63
2,47
0,06
0,14
99,70
1,14
95,80
0,30
2,79
0,12
0,00
99,8
0,04
0,27
96,60
0,21
2,89
0,10
0,20
99,70
0,16
96,70
0,18
3,03
0,07
0,12
0,04
99,60
0,28
12,10
85,00
1,15
1,72

Figure 8

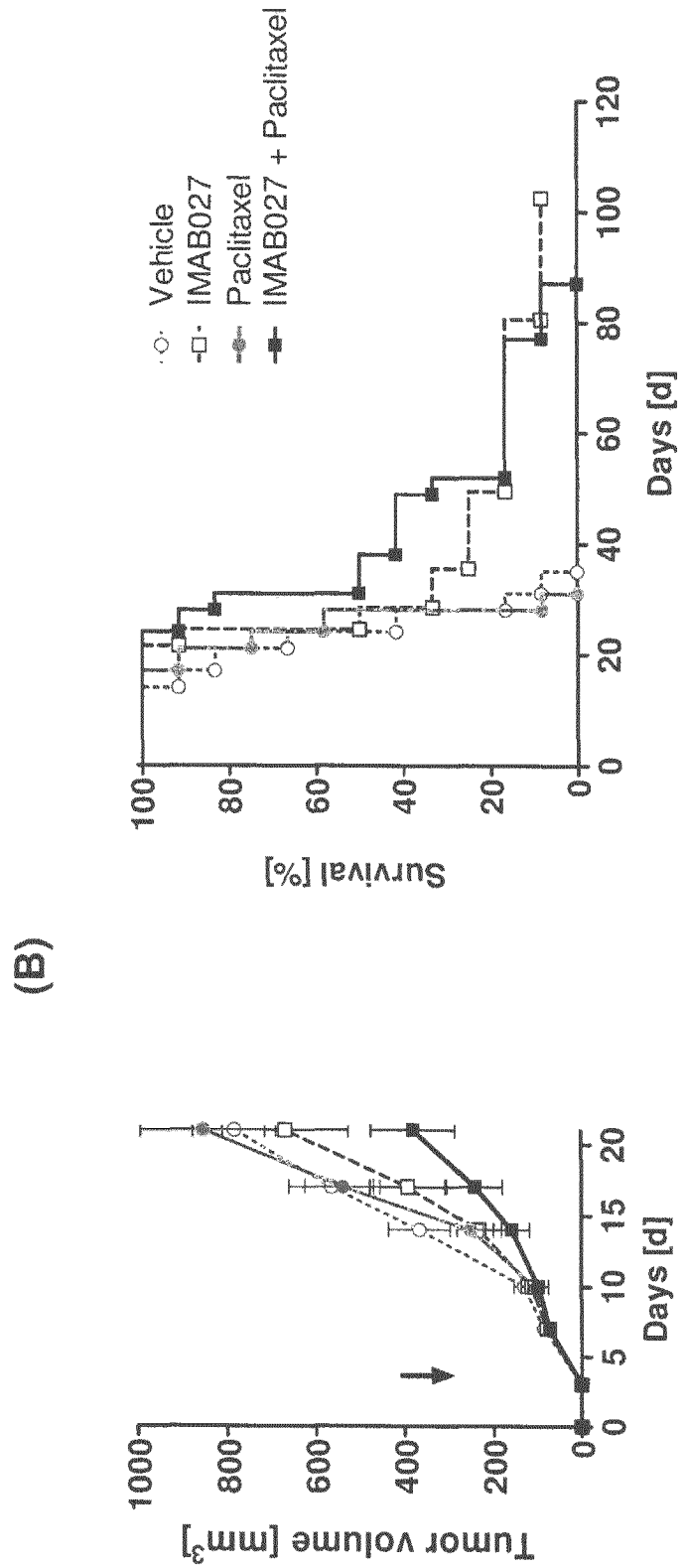

(A)
Tumor volume [mm³]
1000
800
600
400
200
0
0
5
10
15
20
Days [d]
(B)
Survival [%]
100
80
60
40
20
0
0
20
40
60
80
100
120
Days [d]
Vehicle
IMAB027
Paclitaxel
IMAB027 + Paclitaxel

Figure 9

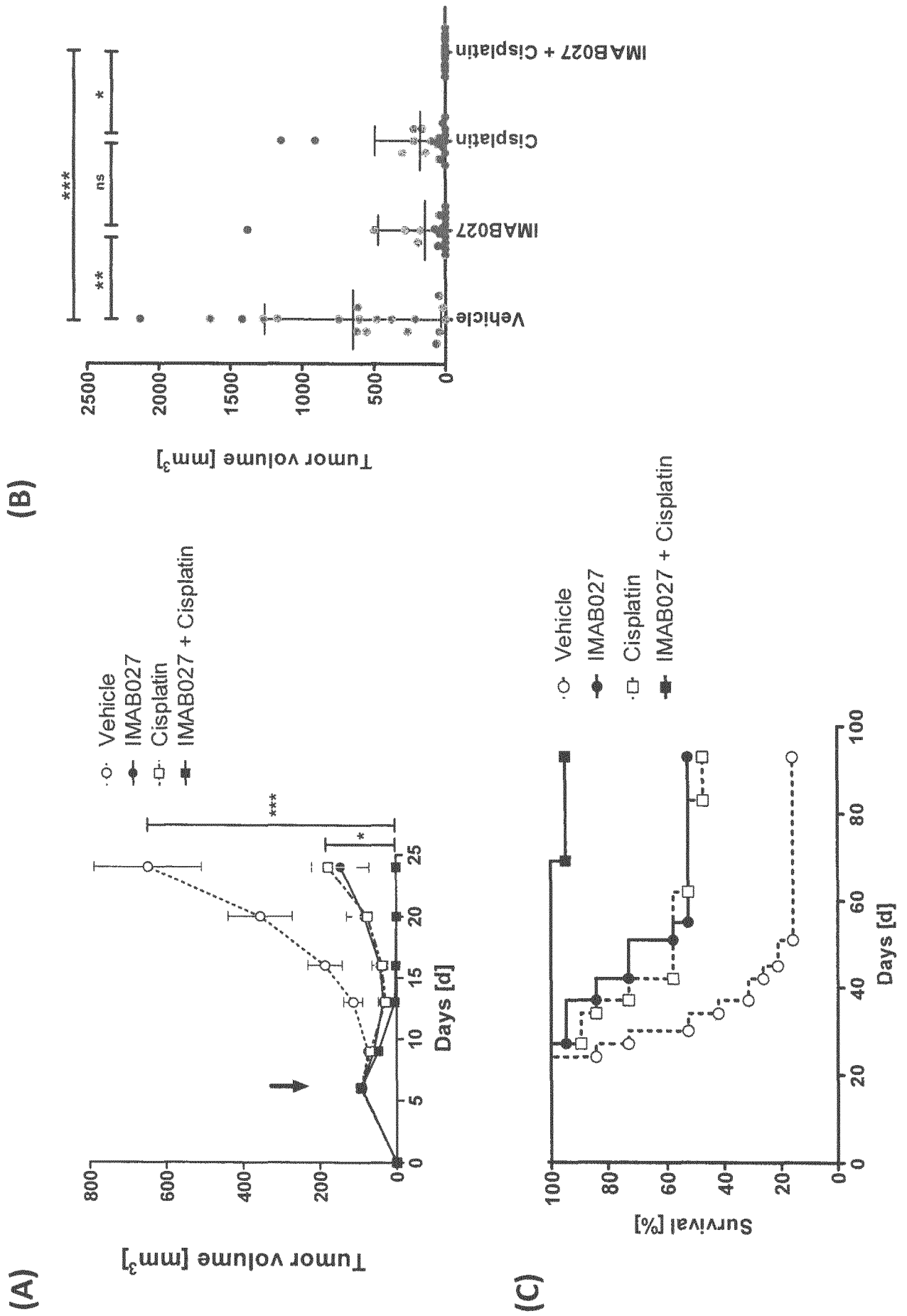
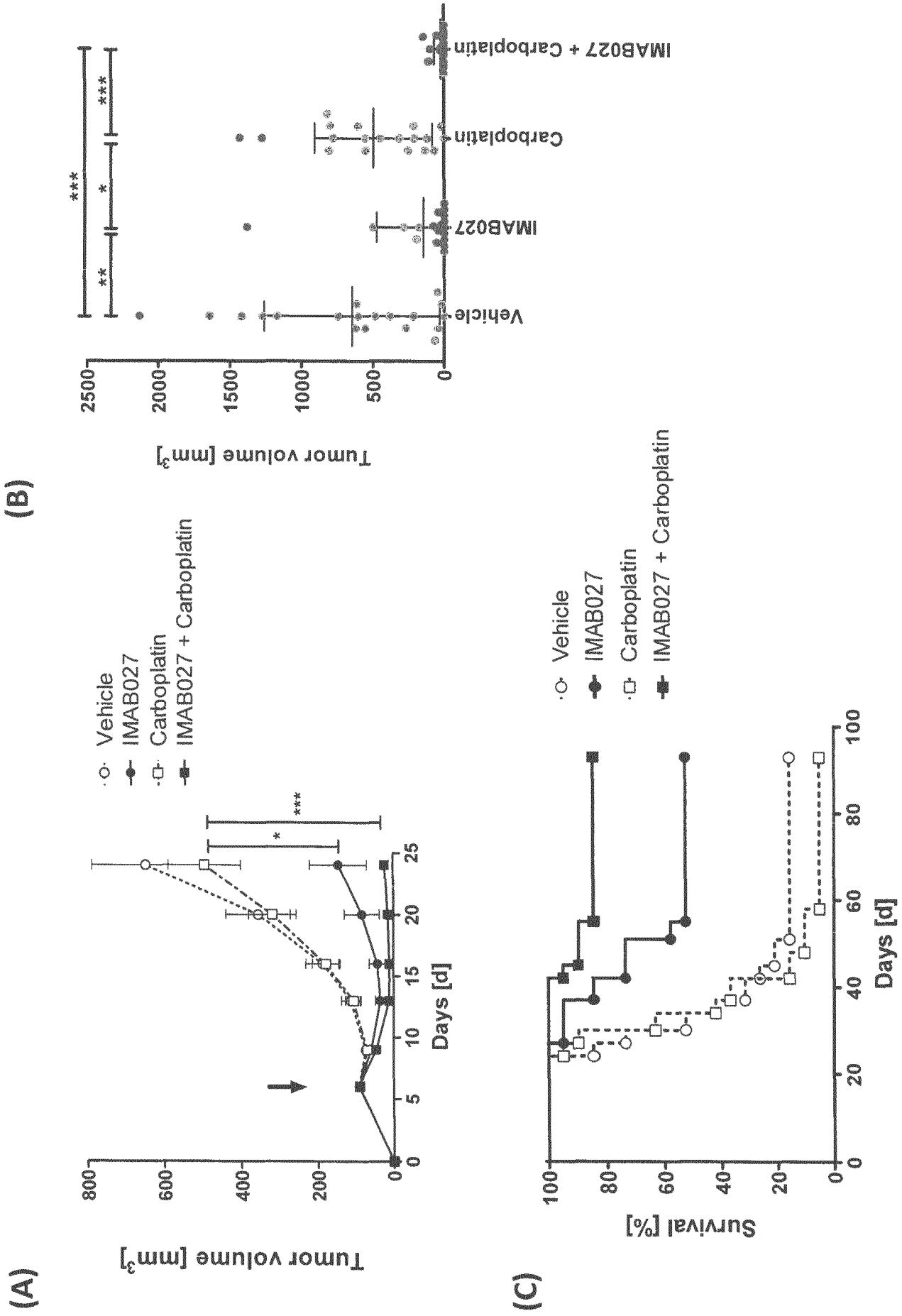

(A)
Tumor volume [mm³]
Days [d]
Vehicle
IMAB027
Cisplatin
IMAB027 + Cisplatin
***
*
(B)
Tumor volume [mm³]
Vehicle
IMAB027
Cisplatin
IMAB027 + Cisplatin
**
ns
*
***
(C)
Survival [%]
Days [d]
Vehicle
IMAB027
Cisplatin
IMAB027 + Cisplatin

Figure 10


(A)
Tumor volume [mm³]
Days [d]
Vehicle
IMAB027
Carboplatin
IMAB027 + Carboplatin
(B)
Tumor volume [mm³]
Vehicle
IMAB027
Carboplatin
IMAB027 + Carboplatin
(C)
Survival [%]
Days [d]
Vehicle
IMAB027
Carboplatin
IMAB027 + Carboplatin

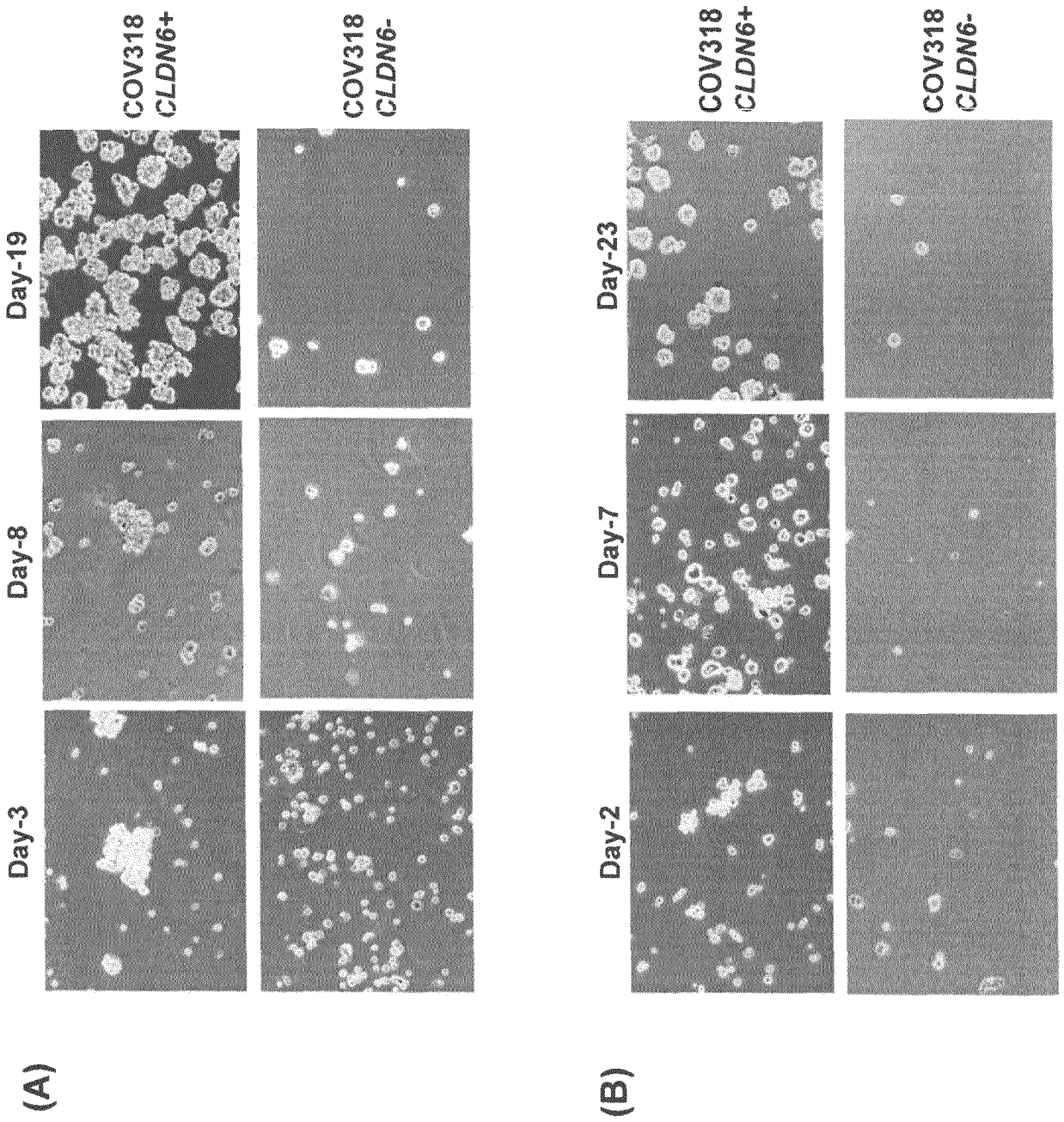

(A)
COV318 CLDN6+
COV318 CLDN6-
Day-19
Day-8
Day-3
(B)
COV318 CLDN6+
COV318 CLDN6-
Day-23
Day-7
Day-2

Figure 11

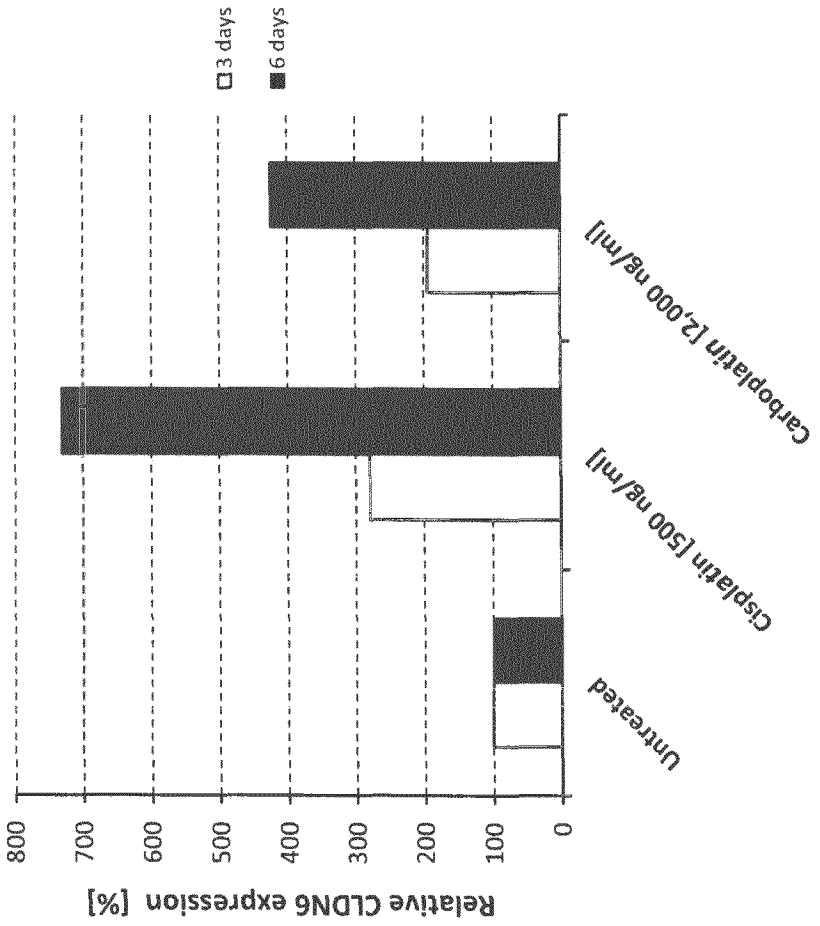

3 days
6 days
Relative CLDN6 expression [%]
800
700
600
500
400
300
200
100
0
Untreated
Cisplatin [500 ng/ml]
Carboplatin [2,000 ng/ml]

Figure 12

Figure 13

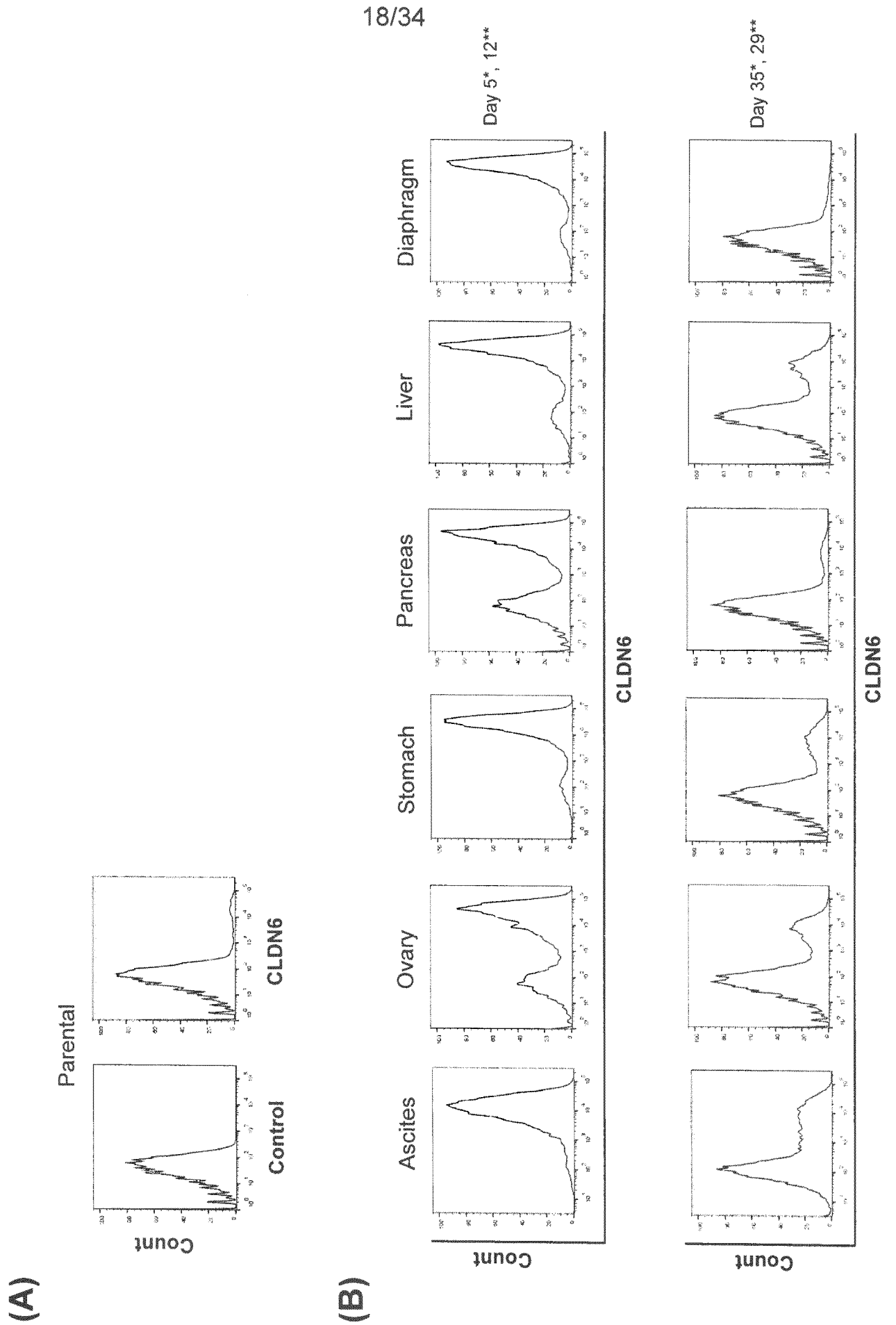
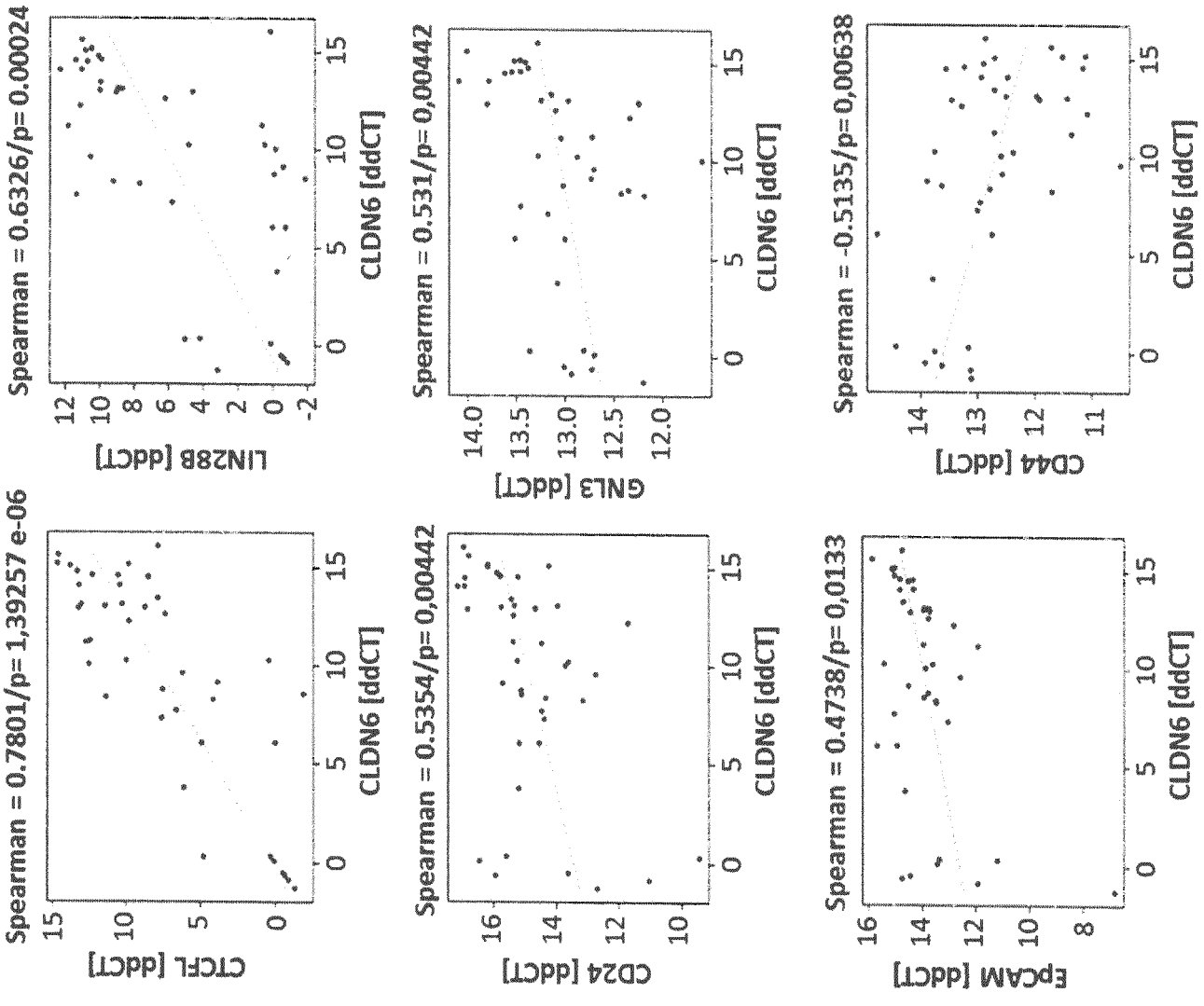

(A)
Parental
Control
CLDN6
Count
(B)
Ascites
Ovary
Stomach
Pancreas
Liver
Diaphragm
Day 5*, 12**
Day 35*, 29**
CLDN6
Count

Figure 14

(A)



(B)

positive correlation		
marker	*spearman r*	*p-value*
CTCFL	0,7801	1,39E-06
LIN28B	0,6326	0,0002
CD24	0,5354	0,00441744
GNL3	0,531	0,00441744
EPCAM	0,4738	0,0132667

negative correlation		
marker	*spearman r*	*p-value*
CD44	-0,5135	0,00638357

no correlation		
marker	*spearman r*	*p-value*
ABCG2	-0,1924	0,477832
ALDH1A1	-0,1792	0,499984
AMACR	0,0101	0,972292
ATXN1	0,1244	0,615194
BMI1	0,2806	0,235788
BMP4	-0,0826	0,763766
CD34	-0,1255	0,615194
CD117	0,0511	0,877142
MYD88	-0,0667	0,818222
NANOG	-0,0978	0,712542
NOTCH1	0,3172	0,189975
POU5F1	-0,1236	0,615194
CD133	-0,133	0,606964
SNAI1	0,0058	0,982996
SOX2	0,2472	0,335274

Figure 15

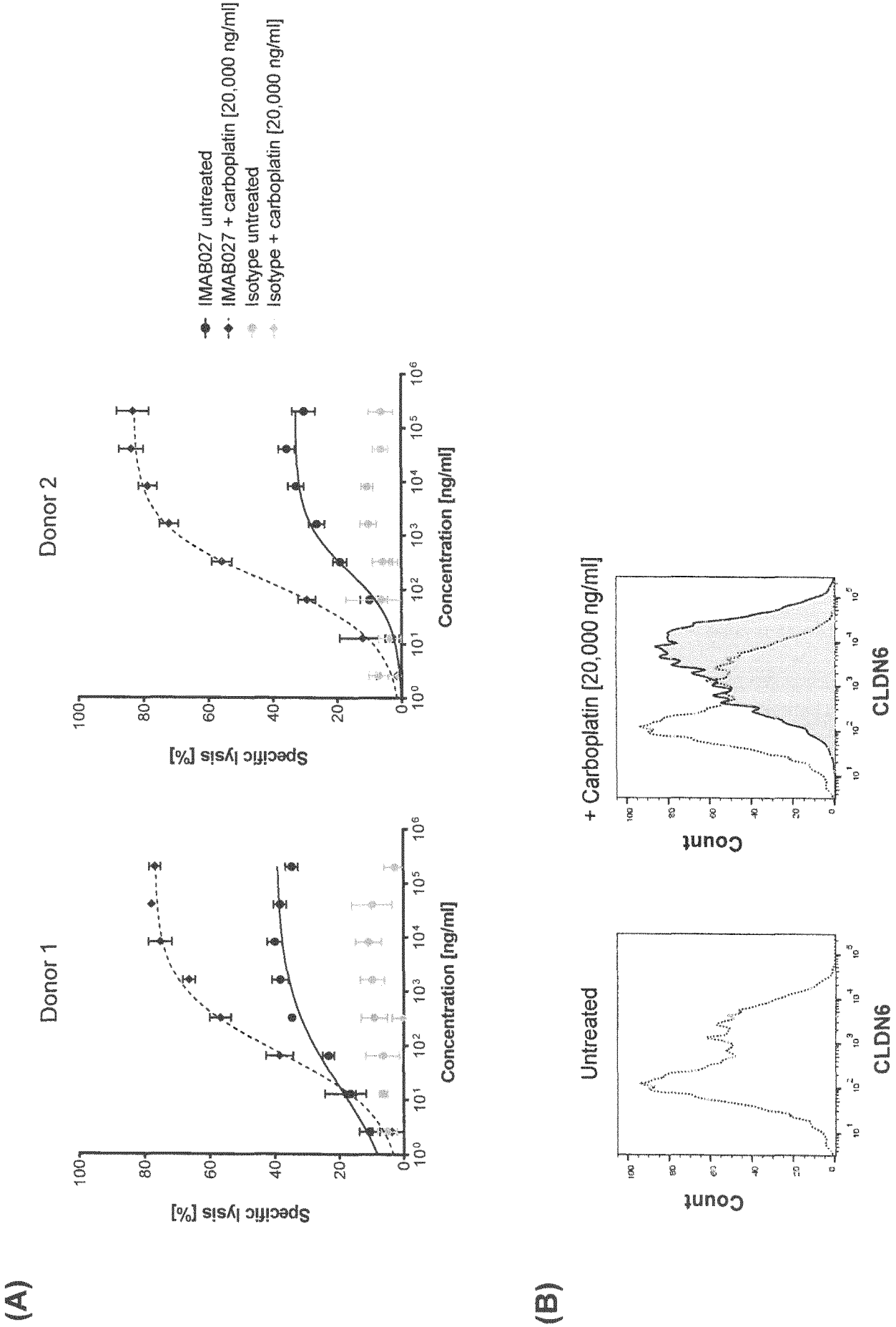
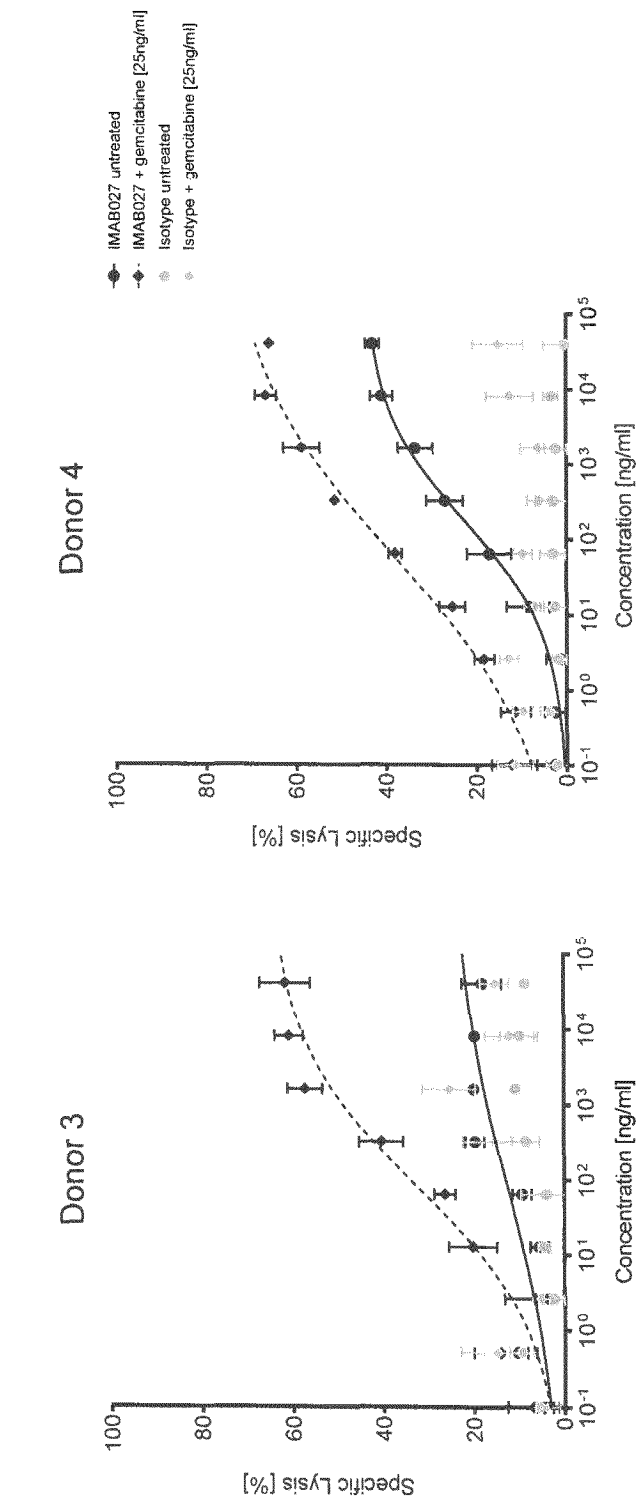

(A)
Donor 1
Donor 2
IMAB027 untreated
IMAB027 + carboplatin [20,000 ng/ml]
Isotype untreated
Isotype + carboplatin [20,000 ng/ml]
Specific lysis [%]
Concentration [ng/ml]
(B)
Untreated
+ Carboplatin [20,000 ng/ml]
Count
CLDN6

Figure 15


(C)
Donor 3
Donor 4
IMAB027 untreated
IMAB027 + gemcitabine [25ng/ml]
Isotype untreated
Isotype + gemcitabine [25ng/ml]
Specific Lysis [%]
Concentration [ng/ml]

(D)
Untreated
+ Gemcitabine [25 ng/ml]
Count
CLDN6

Figure 15

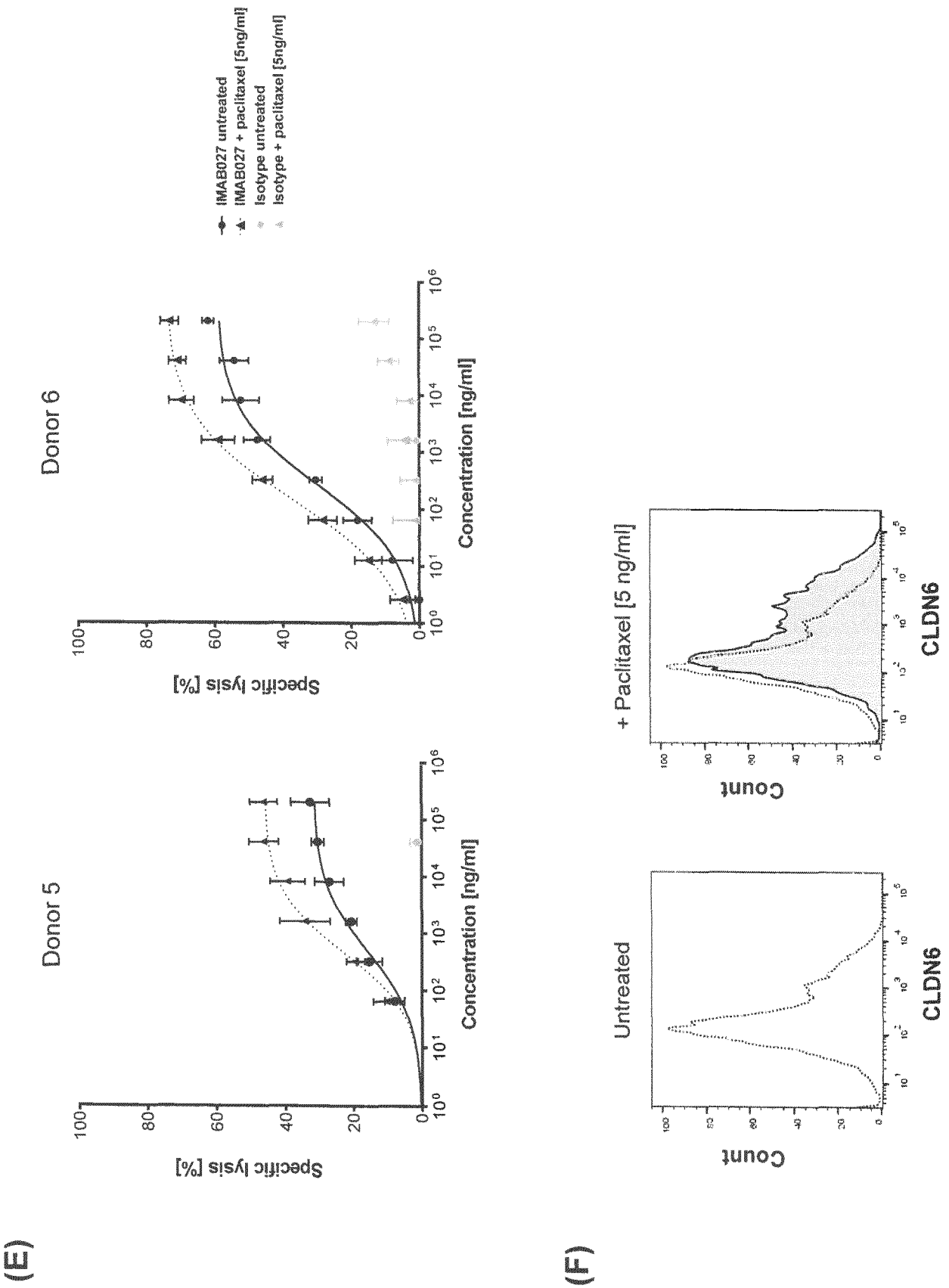
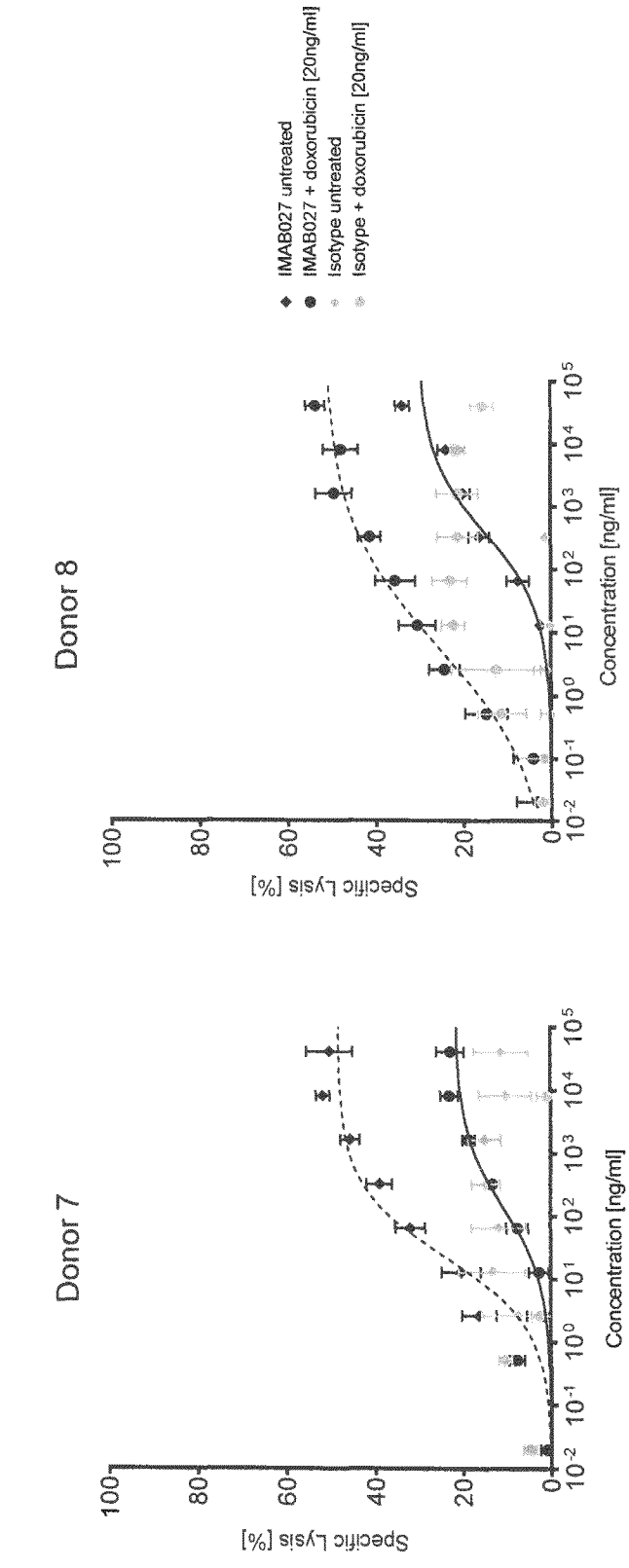

(E)
Donor 6
Donor 5
Specific lysis [%]
Concentration [ng/ml]
IMAB027 untreated
IMAB027 + paclitaxel [5ng/ml]
Isotype untreated
Isotype + paclitaxel [5ng/ml]
(F)
Untreated
+ Paclitaxel [5 ng/ml]
Count
CLDN6

Figure 15


(G)
Donor 7
Donor 8
IMAB027 untreated
IMAB027 + doxorubicin [20ng/ml]
Isotype untreated
Isotype + doxorubicin [20ng/ml]
Specific Lysis [%]
Concentration [ng/ml]

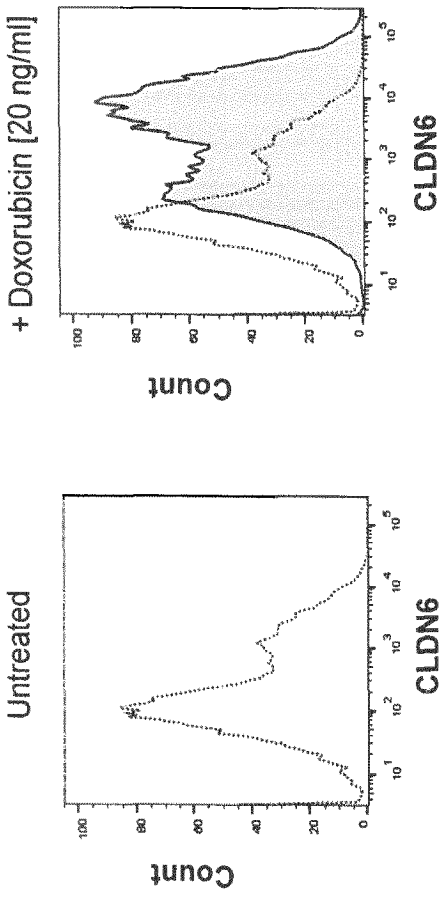
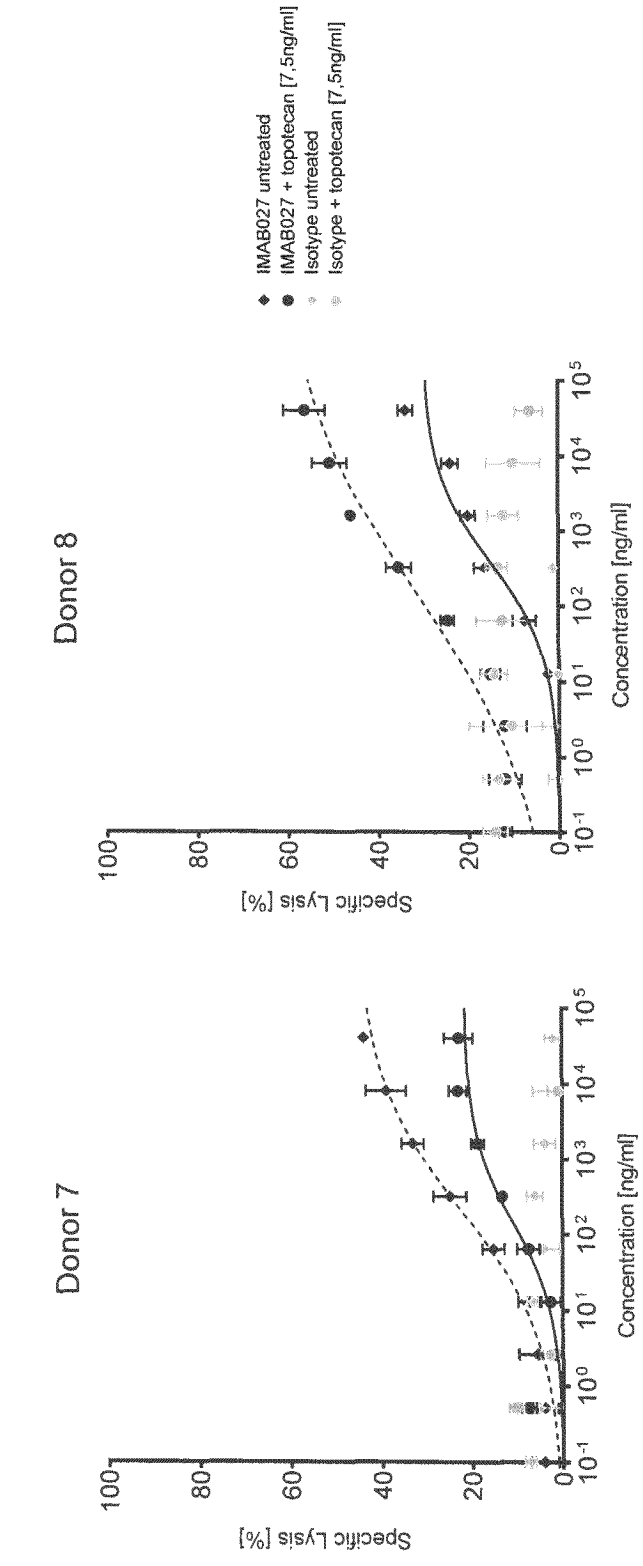

(H)
Untreated
+ Doxorubicin [20 ng/ml]
Count
CLDN6

Figure 15


(I)
Donor 8
Donor 7
IMAB027 untreated
IMAB027 + topotecan [7,5ng/ml]
Isotype untreated
Isotype + topotecan [7,5ng/ml]
Specific Lysis [%]
Concentration [ng/ml]

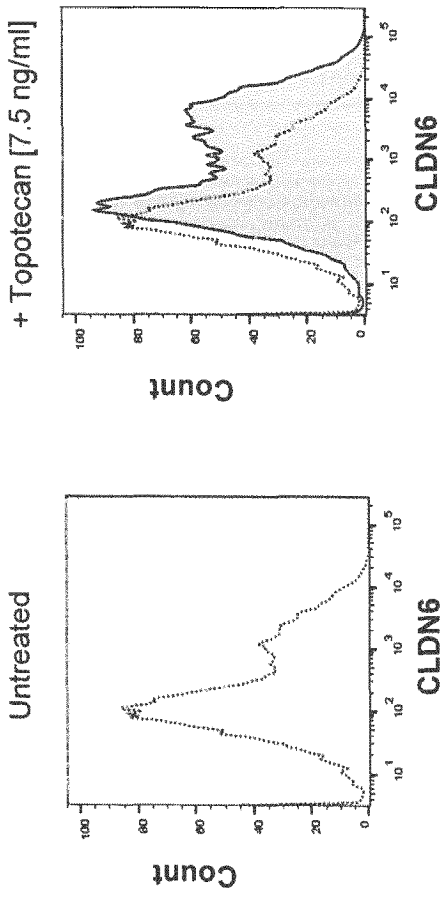

(J)
Untreated
+ Topotecan [7.5 ng/ml]
Count
CLDN6

Figure 16

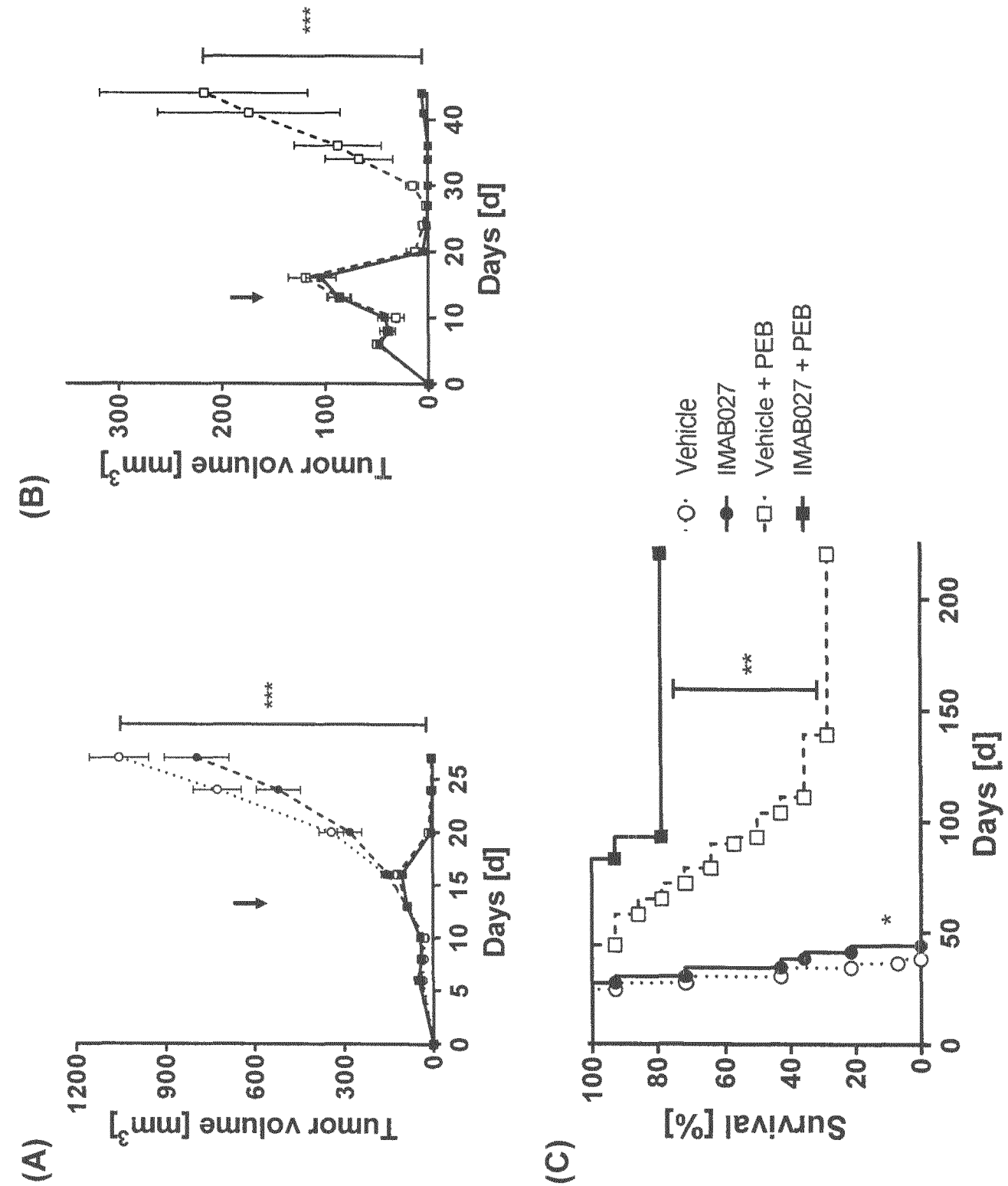
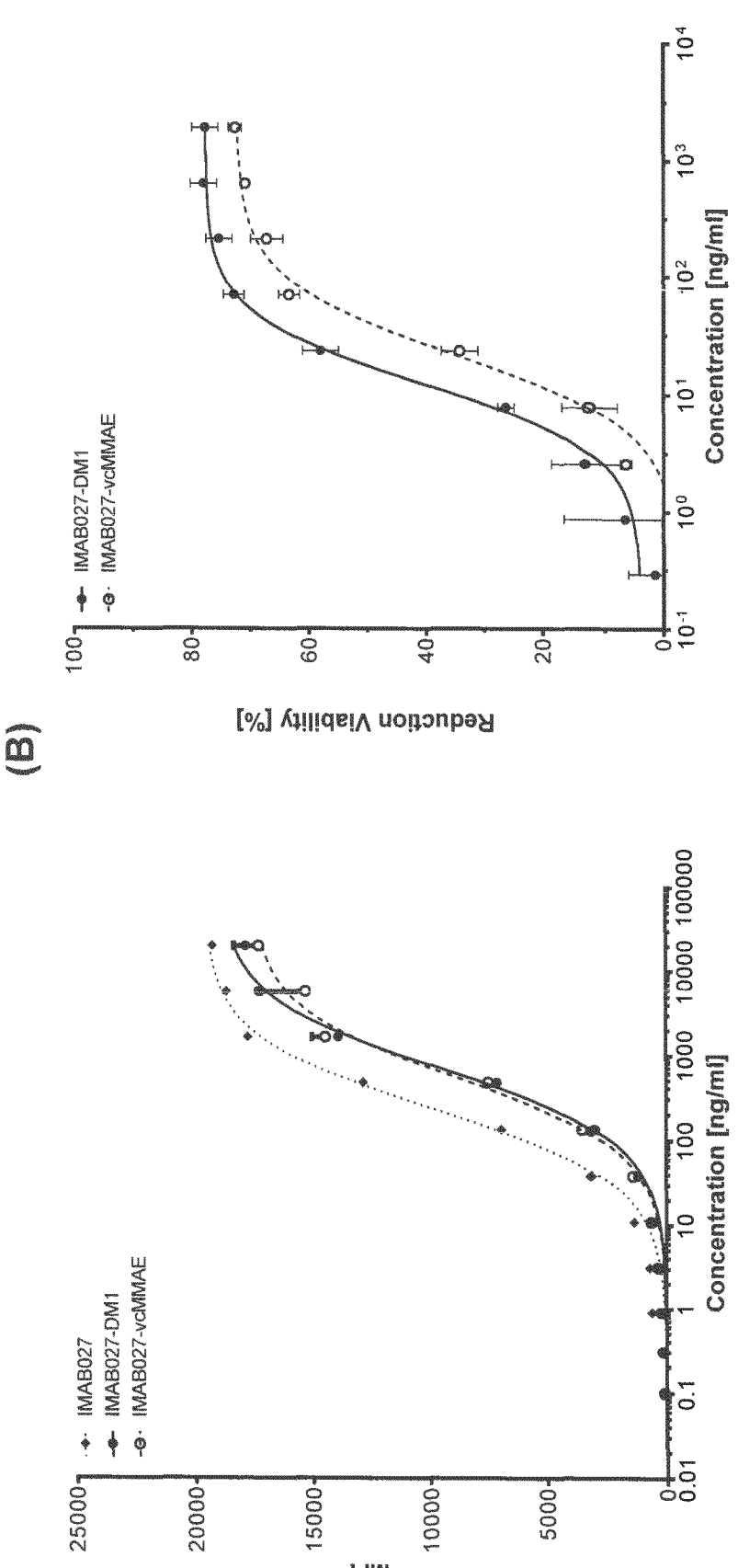

(A)
Tumor volume [mm³]
1200
900
600
300
0
0 5 10 15 20 25
Days [d]
***
(B)
Tumor volume [mm³]
300
200
100
0
0 10 20 30 40
Days [d]
***
(C)
Survival [%]
100
80
60
40
20
0
0 50 100 150 200
Days [d]
Vehicle
IMAB027
Vehicle + PEB
IMAB027 + PEB
**
*

Figure 17


(A)
MFI
25000
20000
15000
10000
5000
0
0.01
0.1
1
10
100
1000
10000
100000
Concentration [ng/ml]
IMAB027
IMAB027-DM1
IMAB027-vcMMAE
(B)
Reduction Viability [%]
100
80
60
40
20
0
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
Concentration [ng/ml]
IMAB027-DM1
IMAB027-vcMMAE

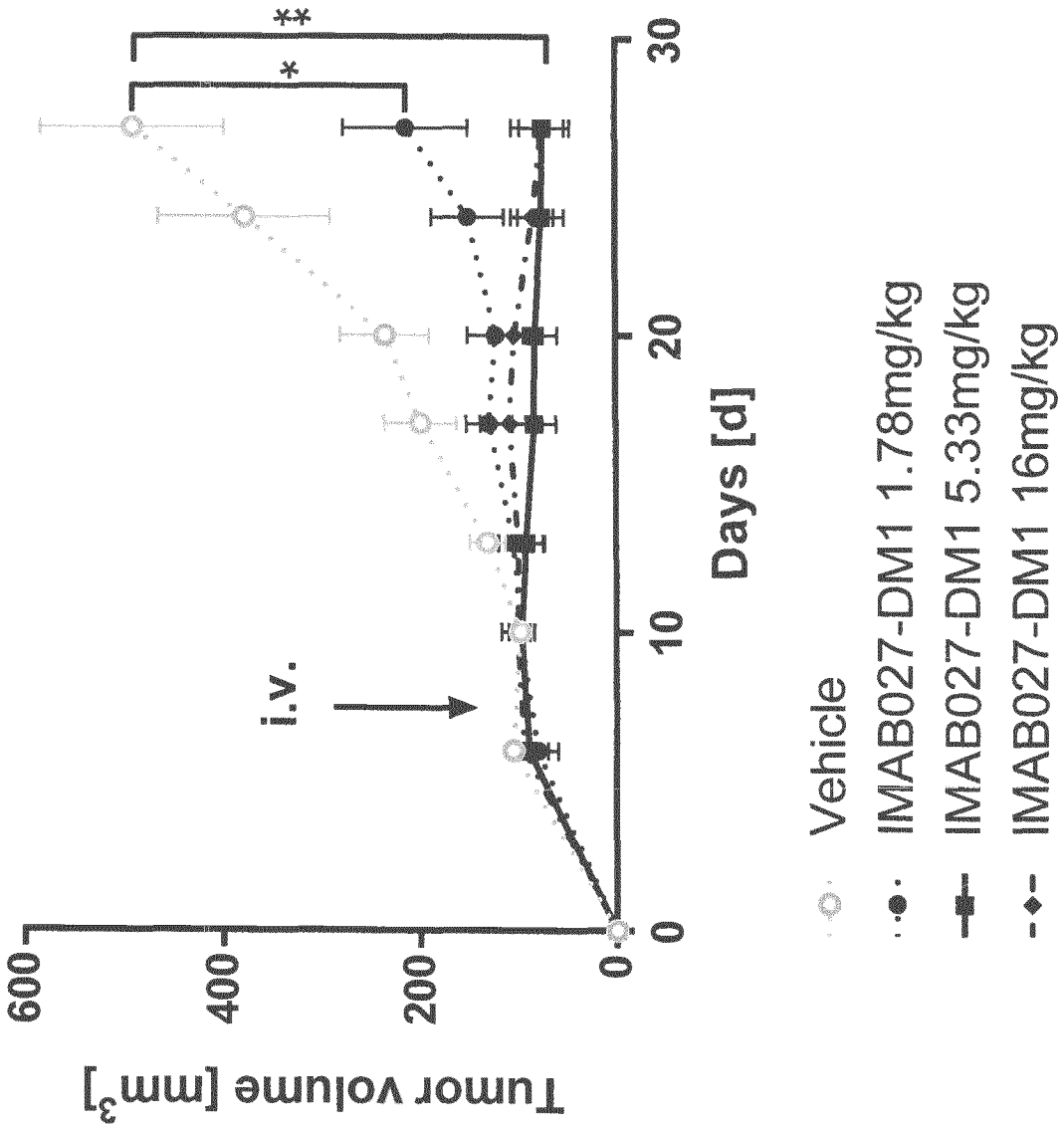
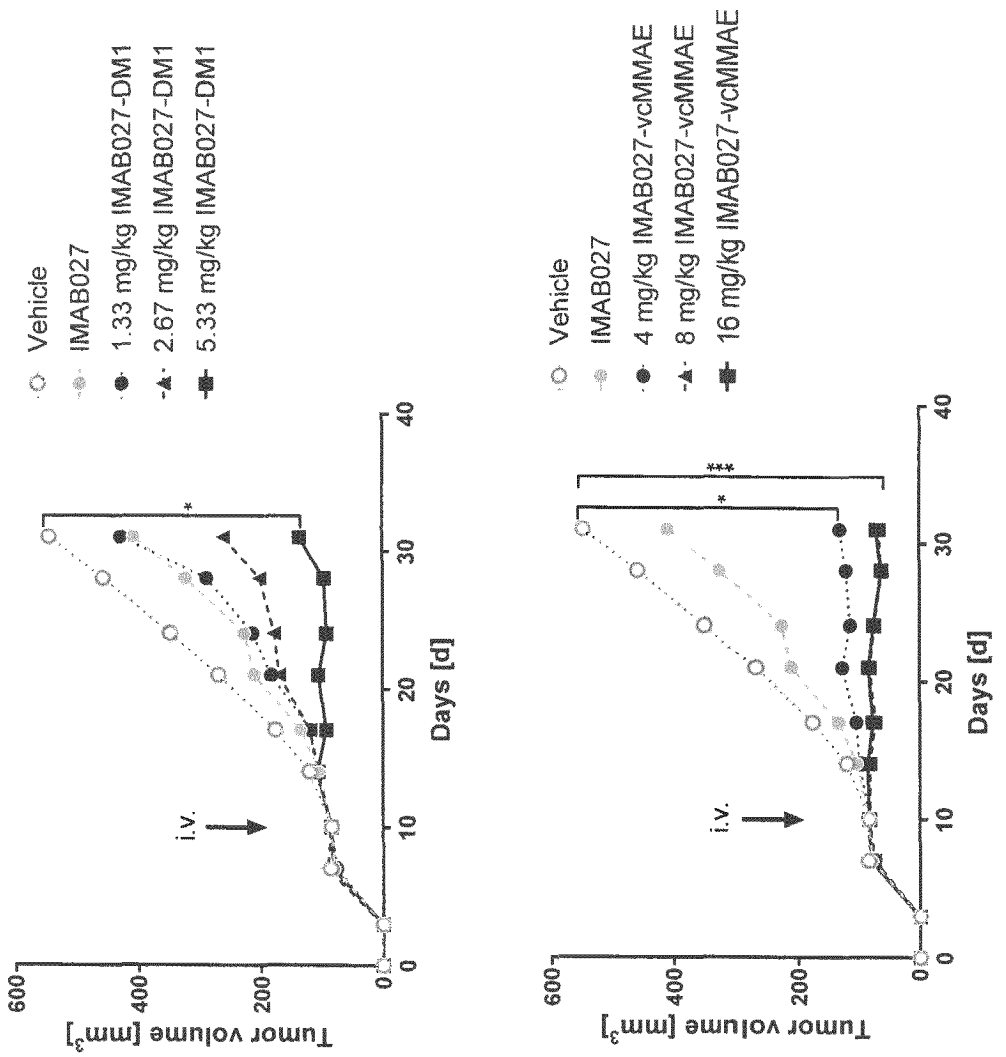

Tumor volume [mm³]
600
400
200
0
0
10
20
30
Days [d]
i.v.
**
*
Vehicle
IMAB027-DM1 1.78mg/kg
IMAB027-DM1 5.33mg/kg
IMAB027-DM1 16mg/kg

Figure 18

Figure 19

(A)


Vehicle
IMAB027
1.33 mg/kg IMAB027-DM1
2.67 mg/kg IMAB027-DM1
5.33 mg/kg IMAB027-DM1
i.v.
*
Tumor volume [mm³]
Days [d]
0
200
400
600
0
10
20
30
40
Vehicle
IMAB027
4 mg/kg IMAB027-vcMMAE
8 mg/kg IMAB027-vcMMAE
16 mg/kg IMAB027-vcMMAE
i.v.
***
*
Tumor volume [mm³]
Days [d]

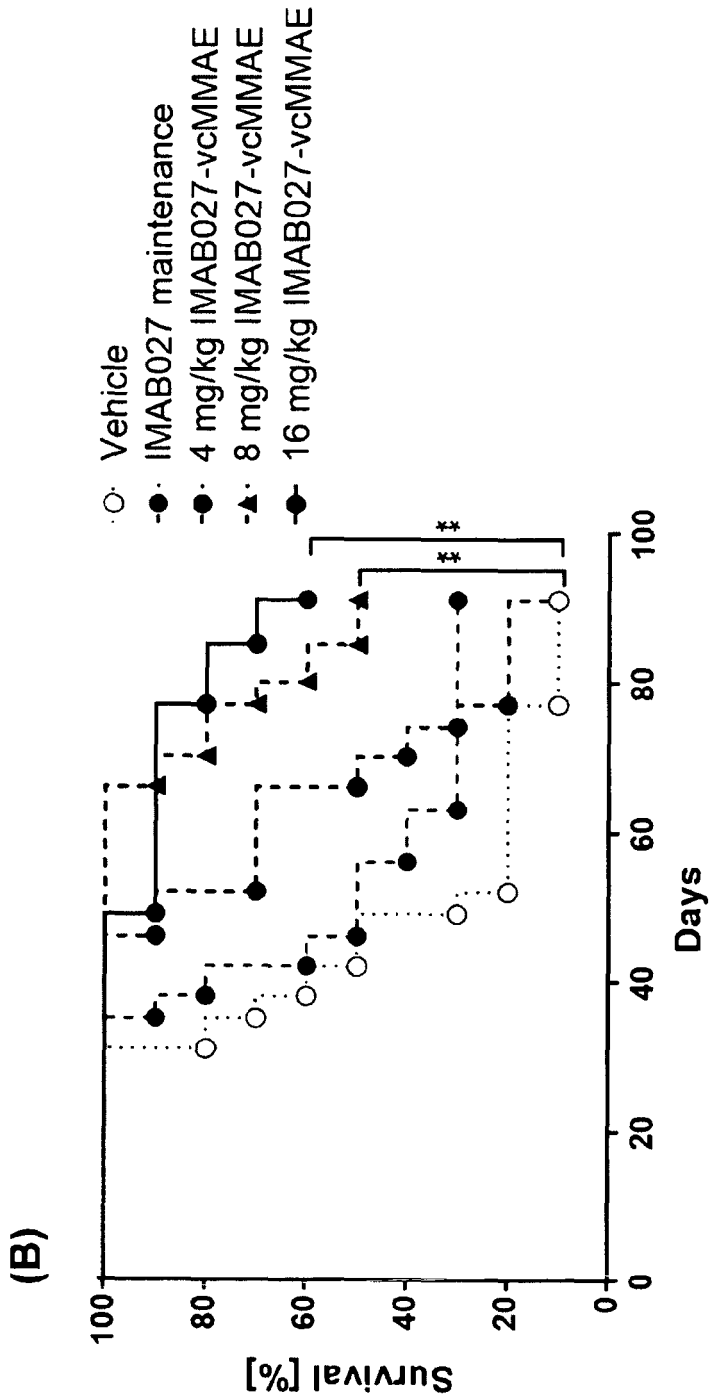

(B)
Survival [%]
100
80
60
40
20
0
0
20
40
60
80
100
Days
Vehicle
IMAB027 maintenance
4 mg/kg IMAB027-vcMMAE
8 mg/kg IMAB027-vcMMAE
16 mg/kg IMAB027-vcMMAE
**
**

Figure 19

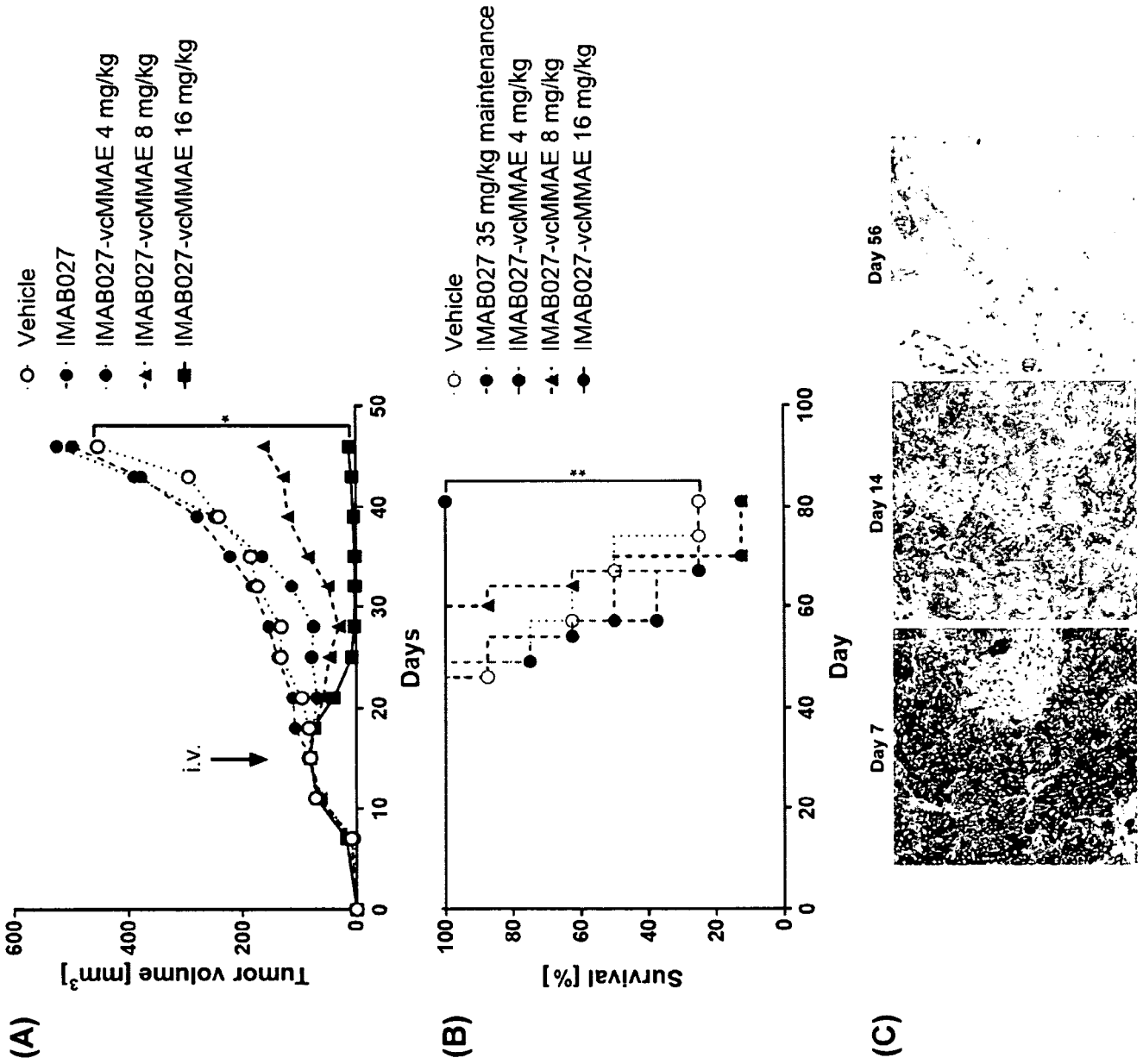

(A)
Tumor volume [mm³]
Vehicle
IMAB027
IMAB027-vcMMAE 4 mg/kg
IMAB027-vcMMAE 8 mg/kg
IMAB027-vcMMAE 16 mg/kg
i.v.
Days
(B)
Survival [%]
Vehicle
IMAB027 35 mg/kg maintenance
IMAB027-vcMMAE 4 mg/kg
IMAB027-vcMMAE 8 mg/kg
IMAB027-vcMMAE 16 mg/kg
Day
(C)
Day 7
Day 14
Day 56

Figure 20

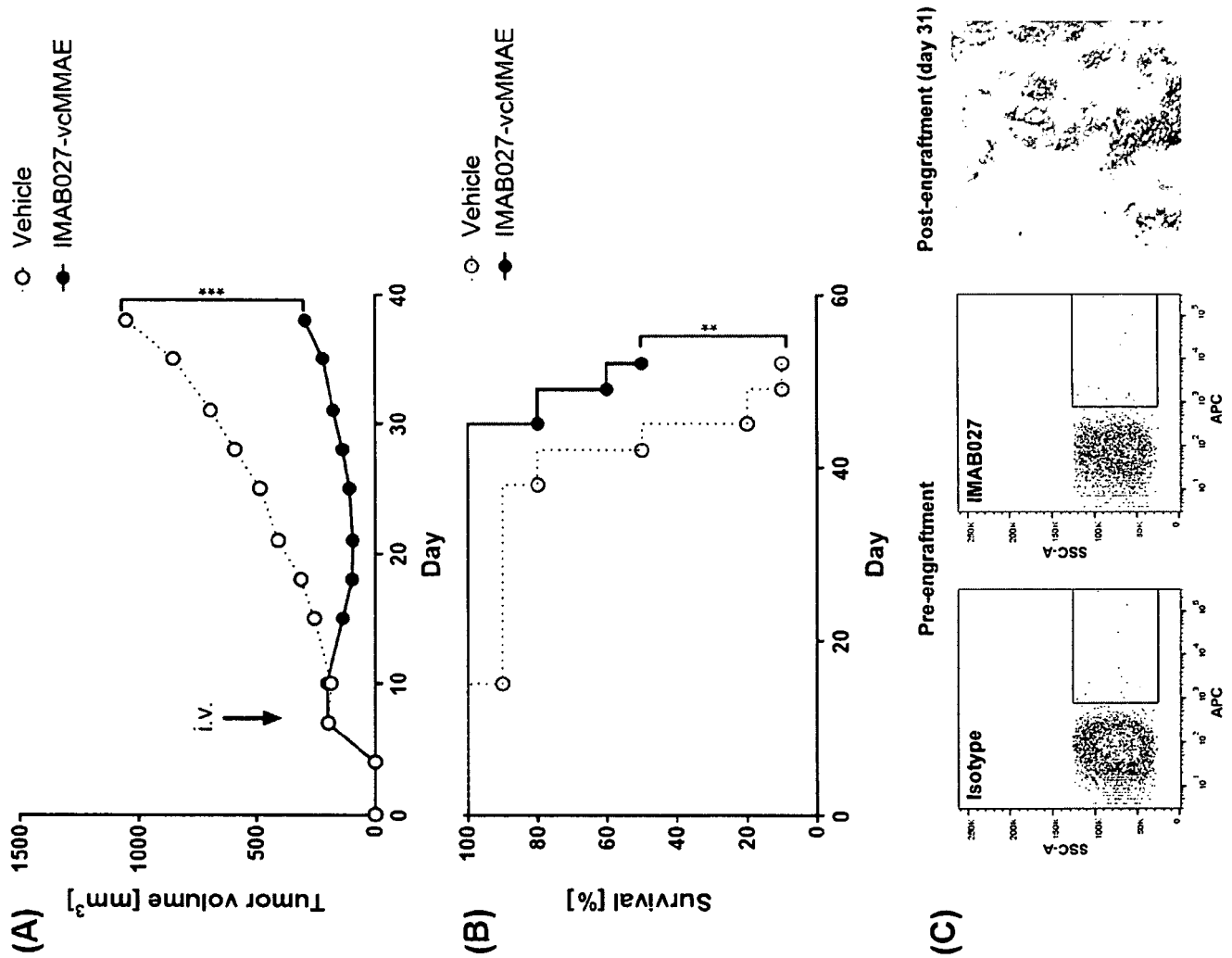

(A)
Tumor volume [mm³]
1500
1000
500
0
0
10
20
30
40
Day
i.v.
***
Vehicle
IMAB027-vcMMAE
(B)
Survival [%]
100
80
60
40
20
0
0
20
40
60
Day
**
Vehicle
IMAB027-vcMMAE
(C)
Pre-engraftment
Post-engraftment (day 31)
Isotype
IMAB027
SSC-A
APC

Figure 21

Figure 22

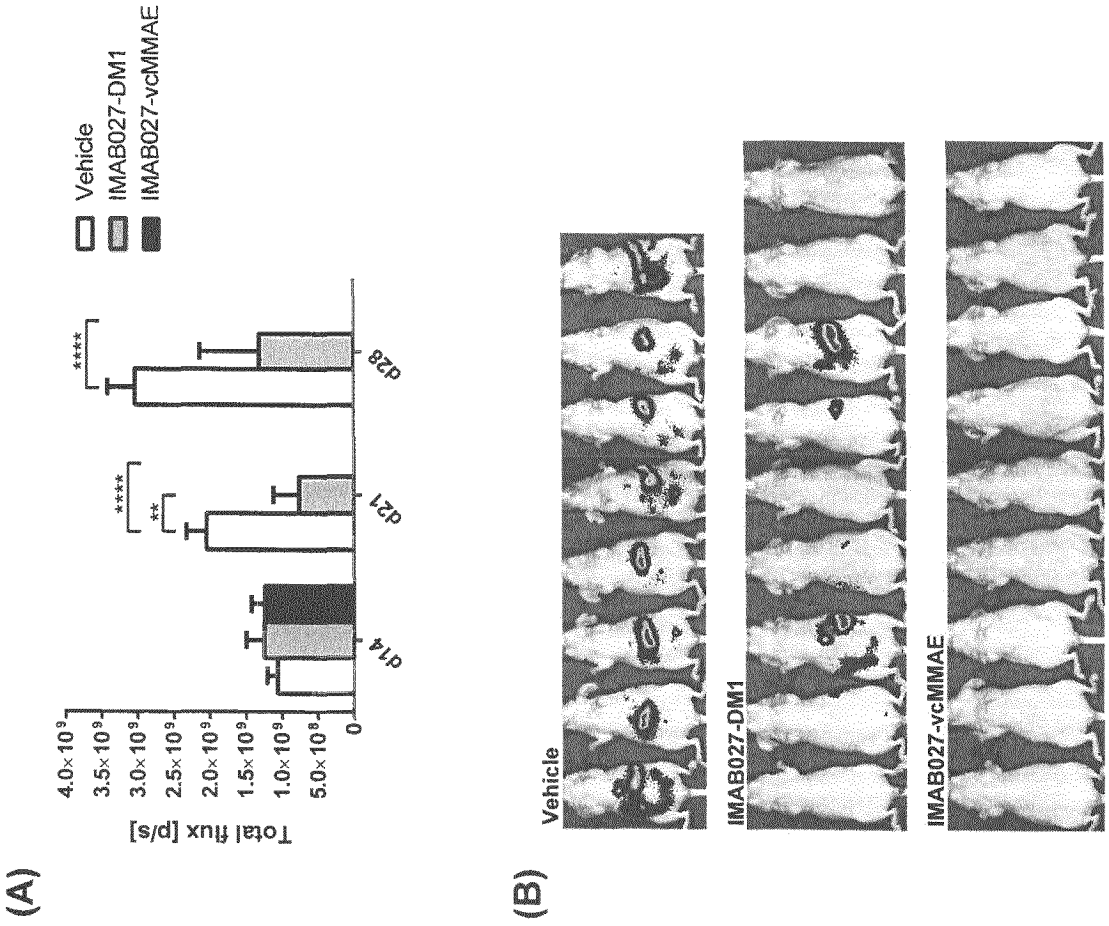

(A)
Vehicle
IMAB027-DM1
IMAB027-vcMMAE
Total flux [p/s]
4.0×10^9
3.5×10^9
3.0×10^9
2.5×10^9
2.0×10^9
1.5×10^9
1.0×10^9
5.0×10^8
0
d14
d21
d28
****
**
****
(B)
Vehicle
IMAB027-DM1
IMAB027-vcMMAE

Figure 23

(A)

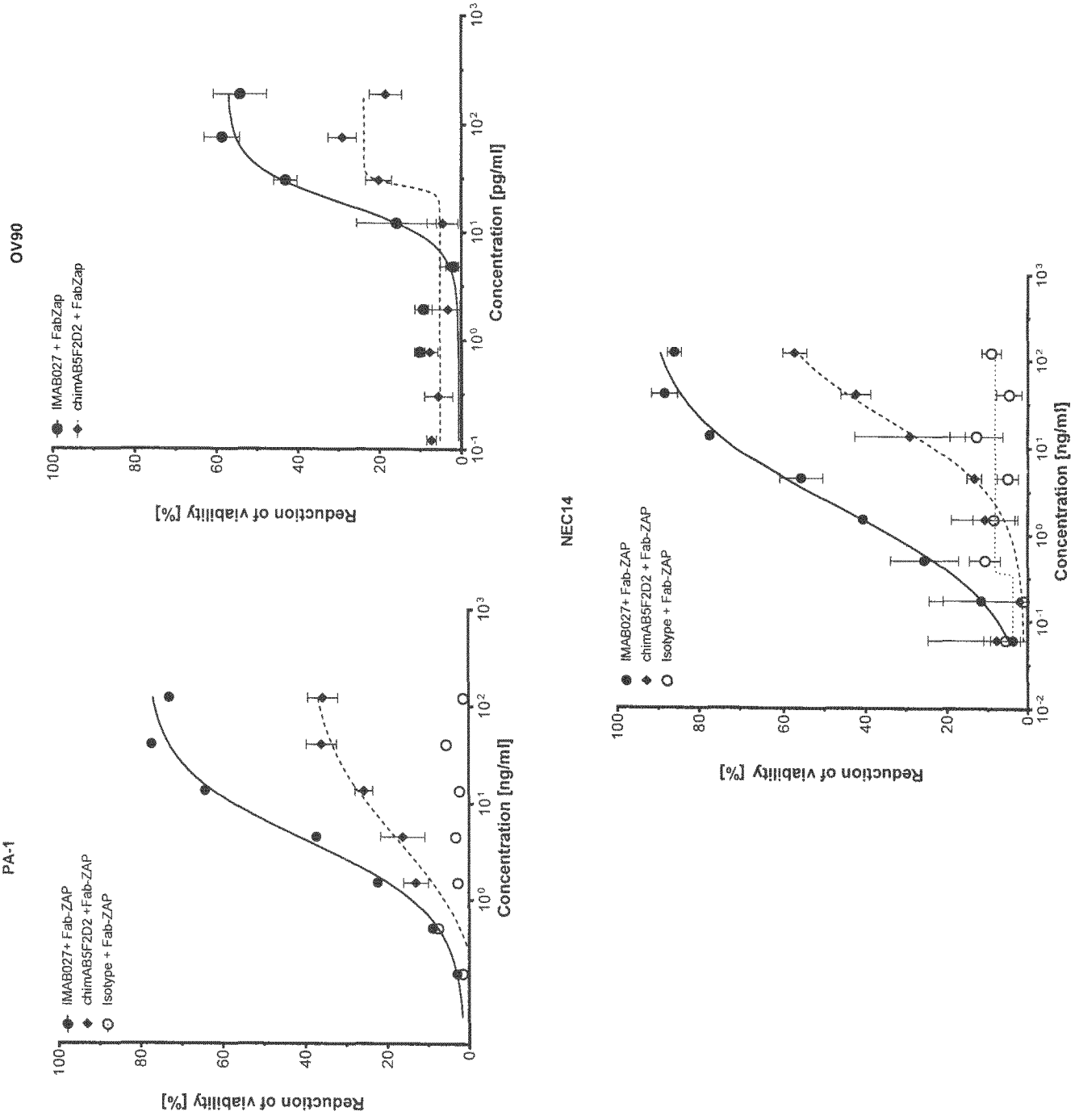

PA-1
IMAB027+ Fab-ZAP
chimAB5F2D2 +Fab-ZAP
Isotype + Fab-ZAP
Reduction of viability [%]
Concentration [ng/ml]
OV90
IMAB027 + FabZap
chimAB5F2D2 + FabZap
Reduction of viability [%]
Concentration [pg/ml]
NEC14
IMAB027+ Fab-ZAP
chimAB5F2D2 + Fab-ZAP
Isotype + Fab-ZAP
Reduction of viability [%]
Concentration [ng/ml]

Figure 23

(B)

Name	PA-1		OV90		NEC14	
	relative EC50	relative maximum	relative EC50	relative maximum	relative EC50	relative maximum
IMAB027 binding	100%	100%	100%	100%	100%	100%
IMAB027 endocytosis	100%	100%	100%	100%	100%	100%
chimAB5F2D2 binding	92%	143%	69%	97%	80%	93%
chimAB5F2D2 endocytosis	102%	50%	237%	39%	980%	71%