Figure 1

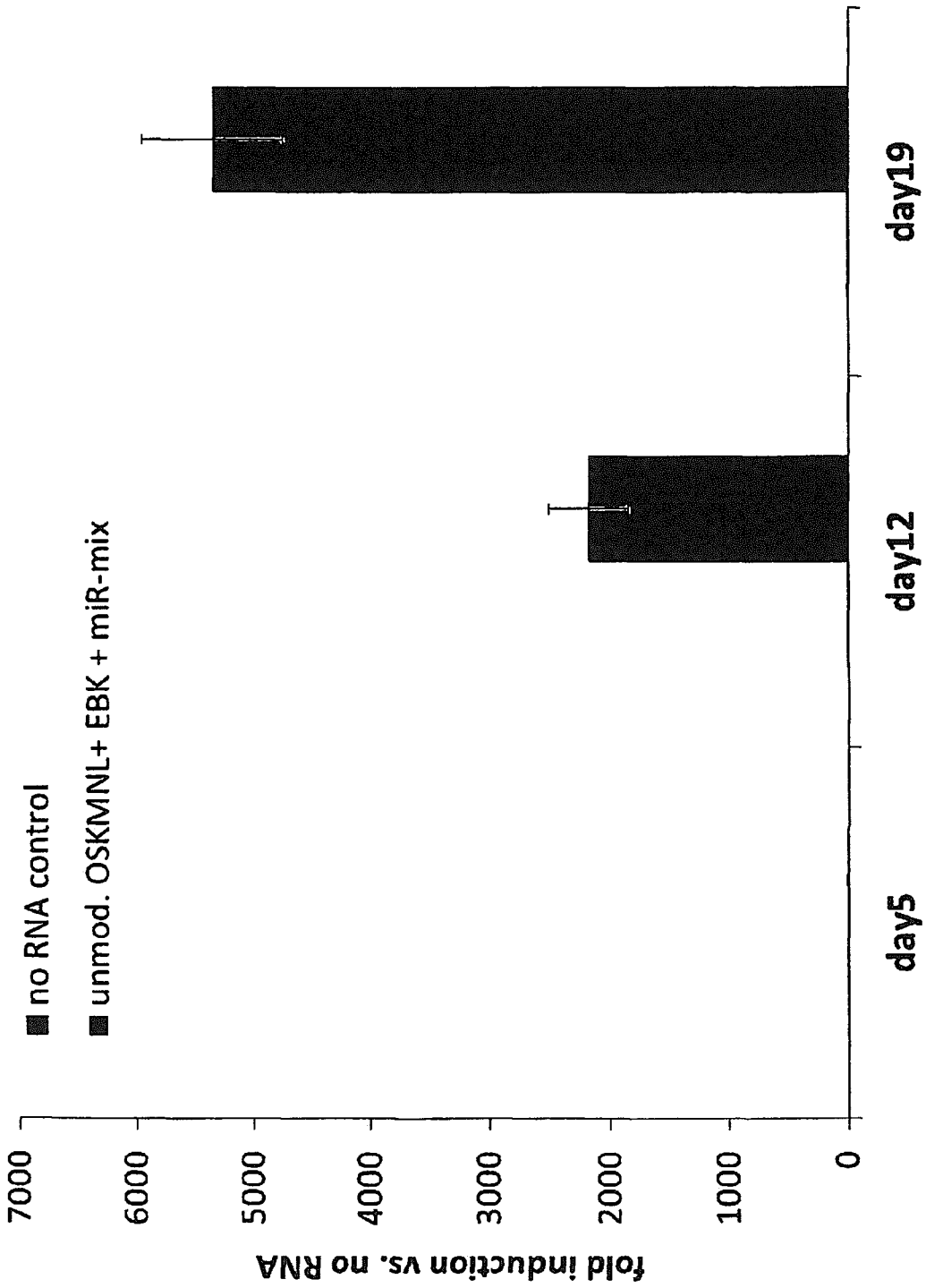

Expression of CLDN6 in iPS cells
no RNA control
unmod. OSKMNL+ EBK + miR-mix
fold induction vs. no RNA
7000
6000
5000
4000
3000
2000
1000
0
day5
day12
day19

Figure 2

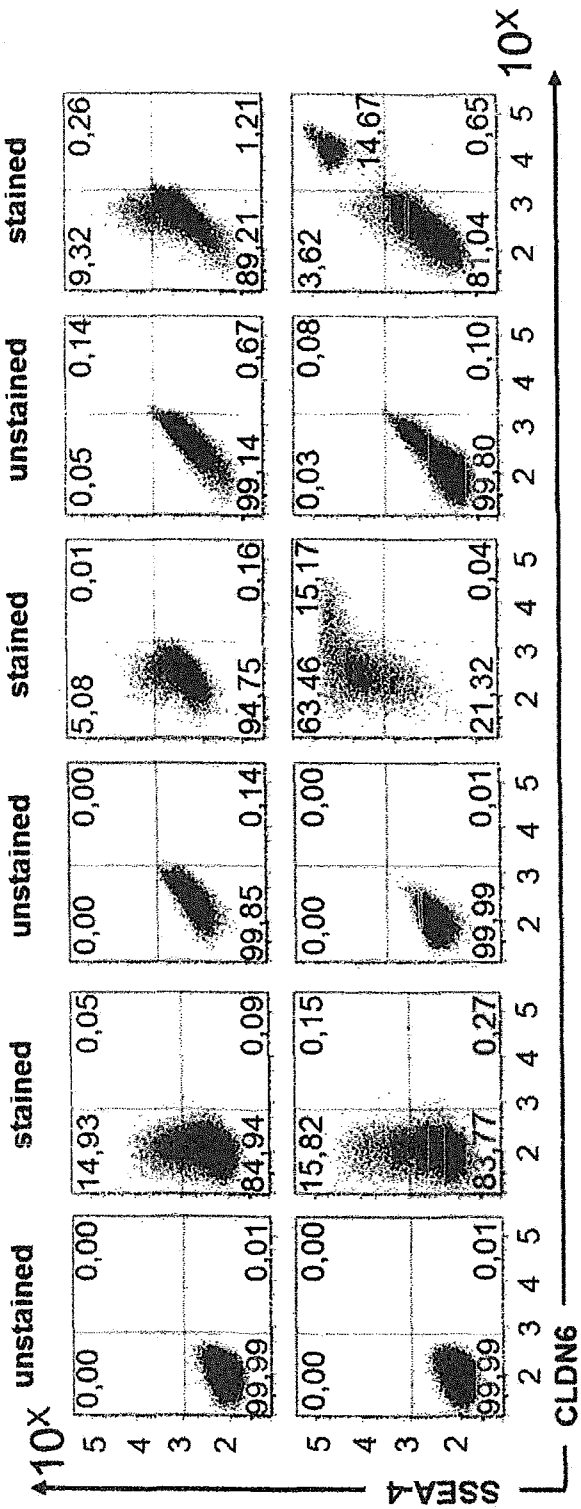
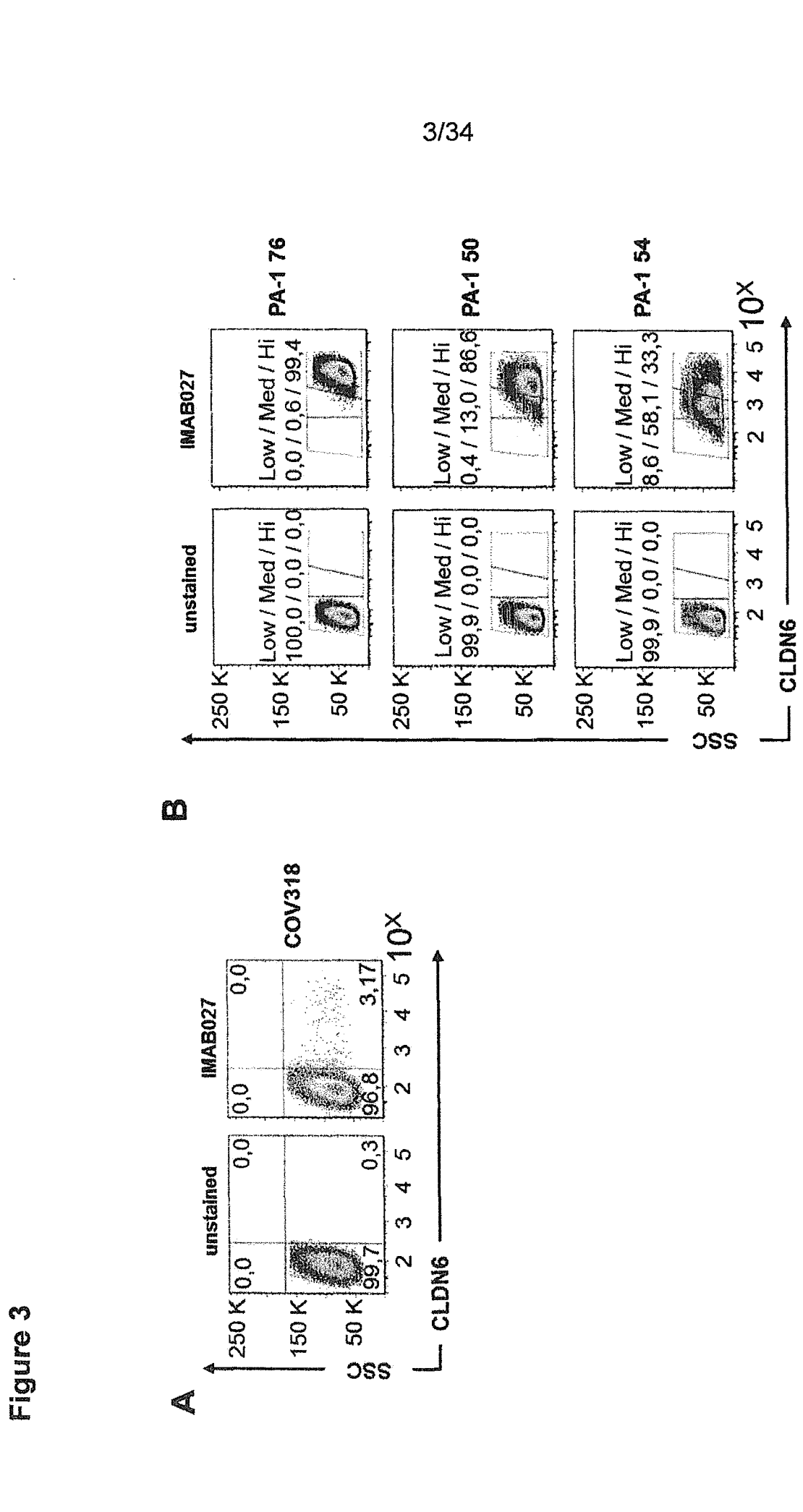
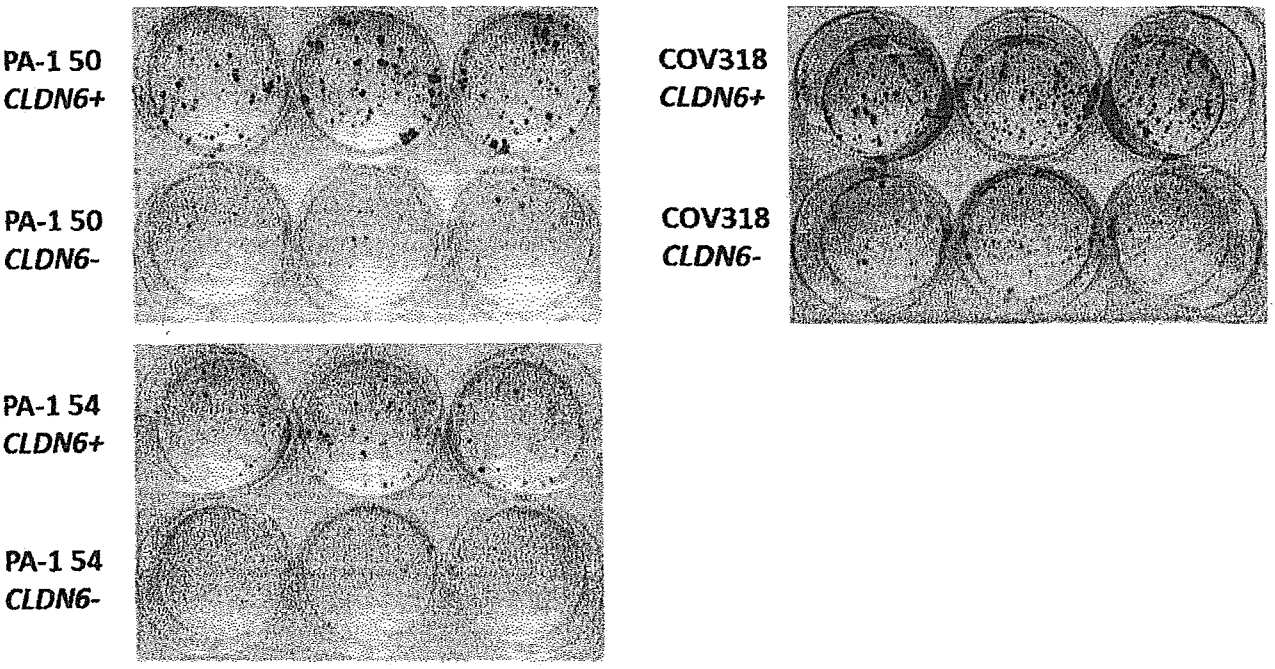

Day 5
Day 12
Day 19
unstained
stained
unstained
stained
unstained
stained
No RNA control
Reprogramming cocktail
SSEA-4
CLDN6
10^X
0,00
0,00
99,99
0,01
14,93
0,05
84,94
0,09
0,00
0,00
99,85
0,14
5,08
0,01
94,75
0,16
0,05
0,14
99,14
0,67
9,32
0,26
89,21
1,21
0,00
0,00
99,99
0,01
15,82
0,15
83,77
0,27
0,00
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
0,0
0,0
0,0
0,0
99,7
0,3
96,8
3,17
250 K
150 K
50 K
SSC
2 3 4 5 10X
CLDN6
B
unstained
IMAB027
Low / Med / Hi
100,0 / 0,0 / 0,0
Low / Med / Hi
0,0 / 0,6 / 99,4
PA-1 76
Low / Med / Hi
99,9 / 0,0 / 0,0
Low / Med / Hi
0,4 / 13,0 / 86,6
PA-1 50
Low / Med / Hi
99,9 / 0,0 / 0,0
Low / Med / Hi
8,6 / 58,1 / 33,3
PA-1 54
250 K
150 K
50 K
SSC
2 3 4 5 10X
CLDN6

A


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

B

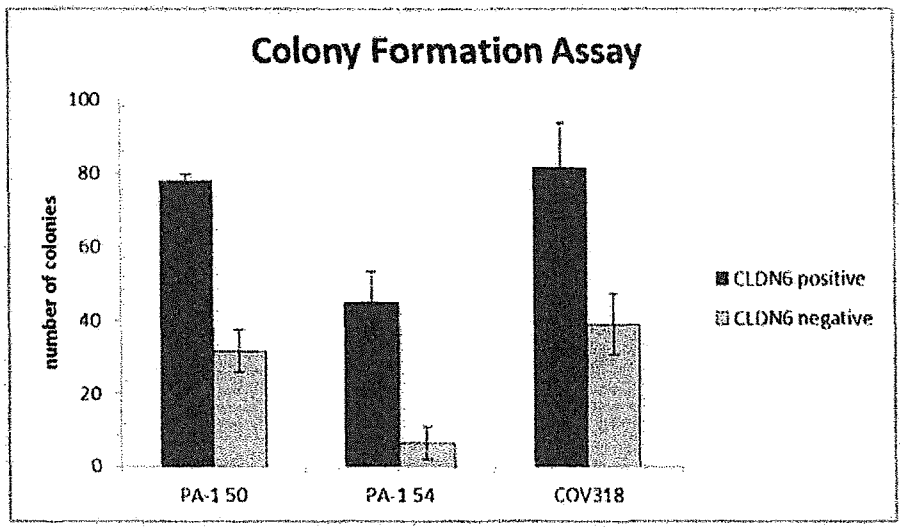

Colony Formation Assay
number of colonies
100
80
60
40
20
0
PA-1 50
PA-1 54
COV318
CLDN6 positive
CLDN6 negative

Figure 4

Figure 5

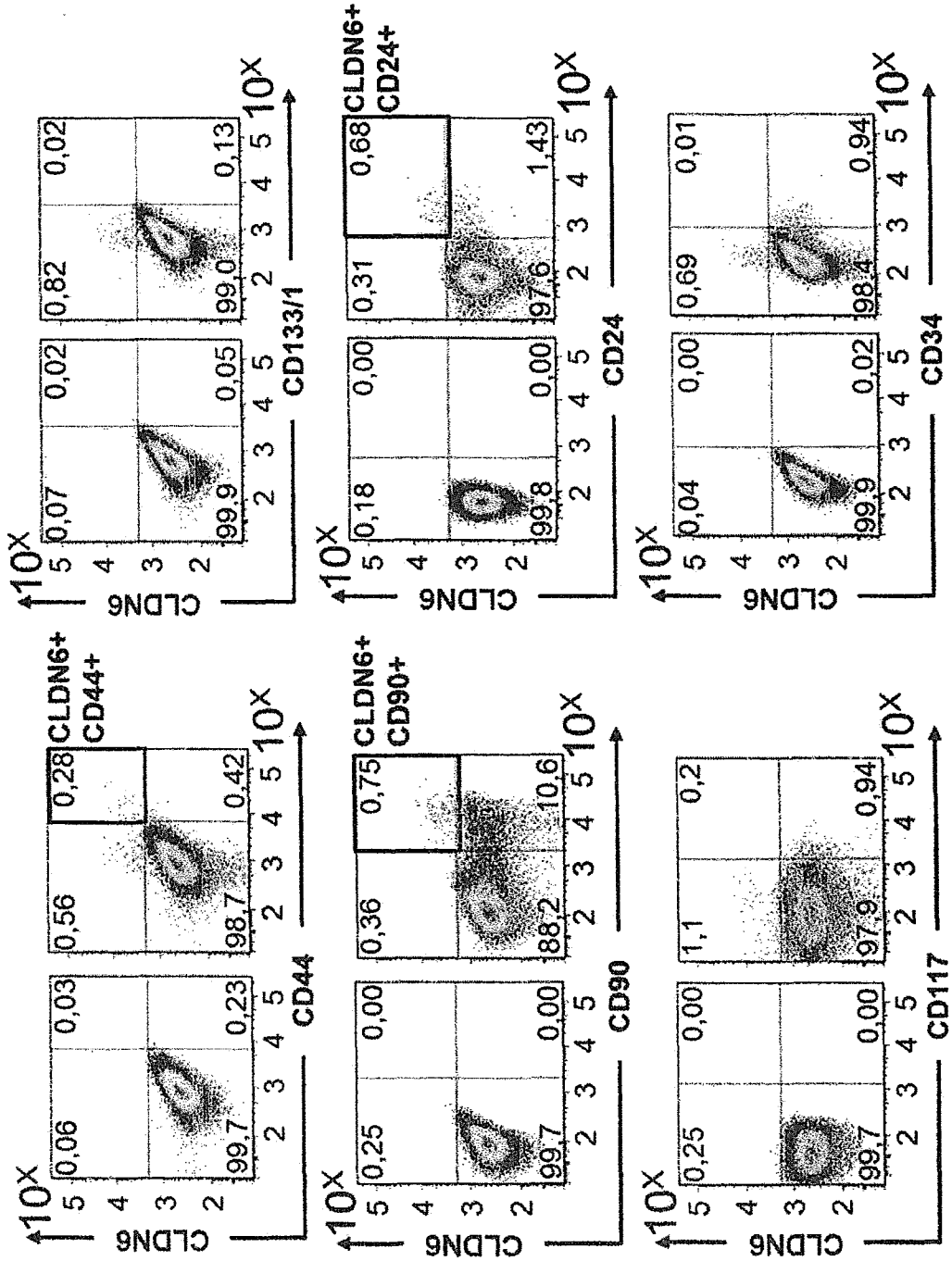

A
unstained
CSC-Panel
unstained
CSC-Panel
CLDN6
CD44
CD133/1
CD90
CD24
CD34
CD117
CLDN6+ CD44+
CLDN6+ CD90+
CLDN6+ CD24+
0,06
0,03
0,23
99,7
0,56
0,28
0,42
98,7
0,25
0,00
0,00
99,7
0,36
0,75
10,6
88,2
0,25
0,00
0,00
99,7
1,1
0,2
0,94
97,9
0,07
0,02
0,05
99,9
0,82
0,02
0,13
99,0
0,18
0,00
0,00
99,8
0,31
0,68
1,43
97,6
0,04
0,00
0,02
99,9
0,69
0,01
0,94
98,4

Figure 5

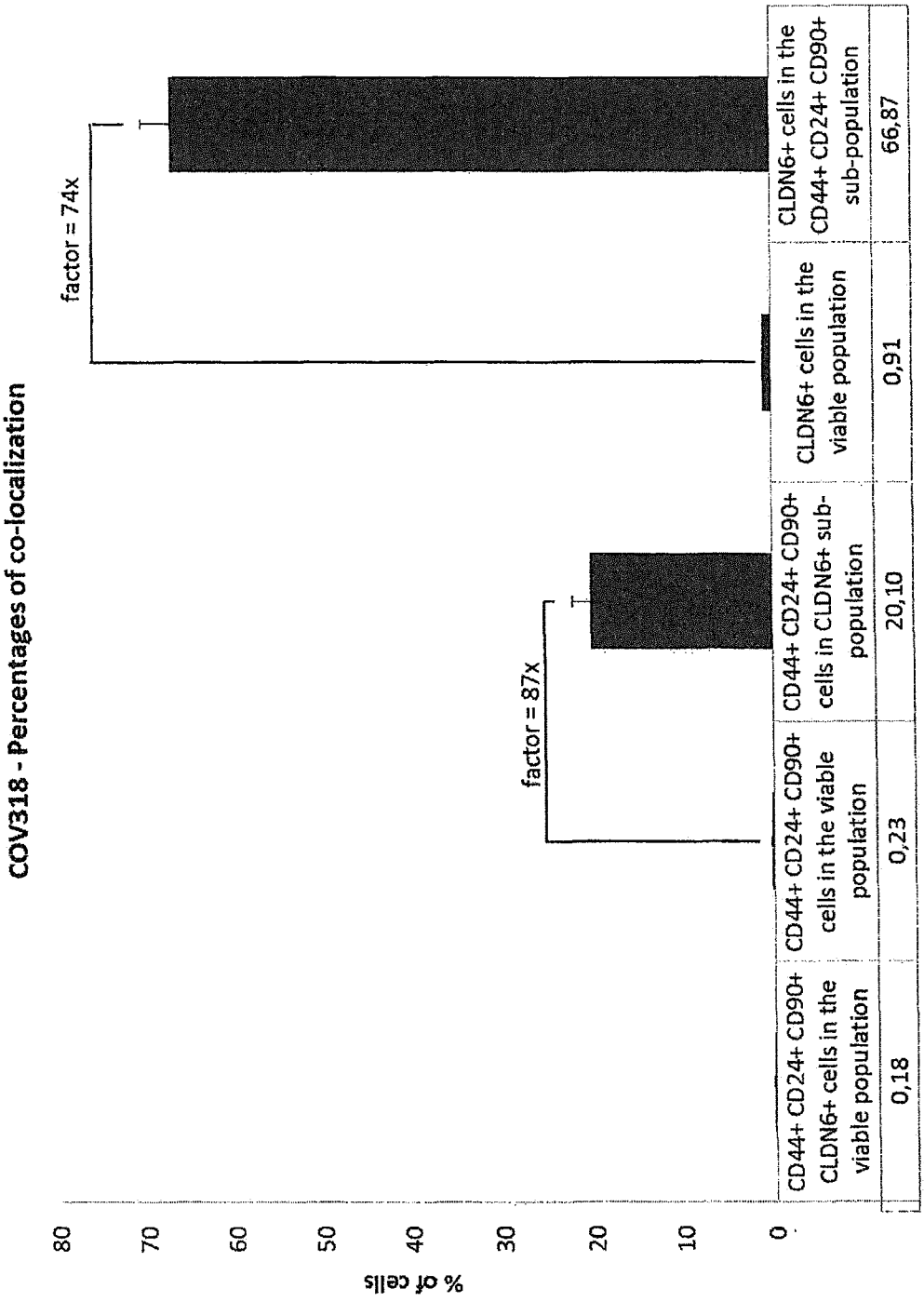

B
COV318 - Percentages of co-localization
% of cells
0
10
20
30
40
50
60
70
80
factor = 87x
factor = 74x
CD44+ CD24+ CD90+ CLDN6+ cells in the viable population
CD44+ CD24+ CD90+ cells in the viable population
CD44+ CD24+ CD90+ cells in CLDN6+ sub-population
CLDN6+ cells in the viable population
CLDN6+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

Figure 6

A

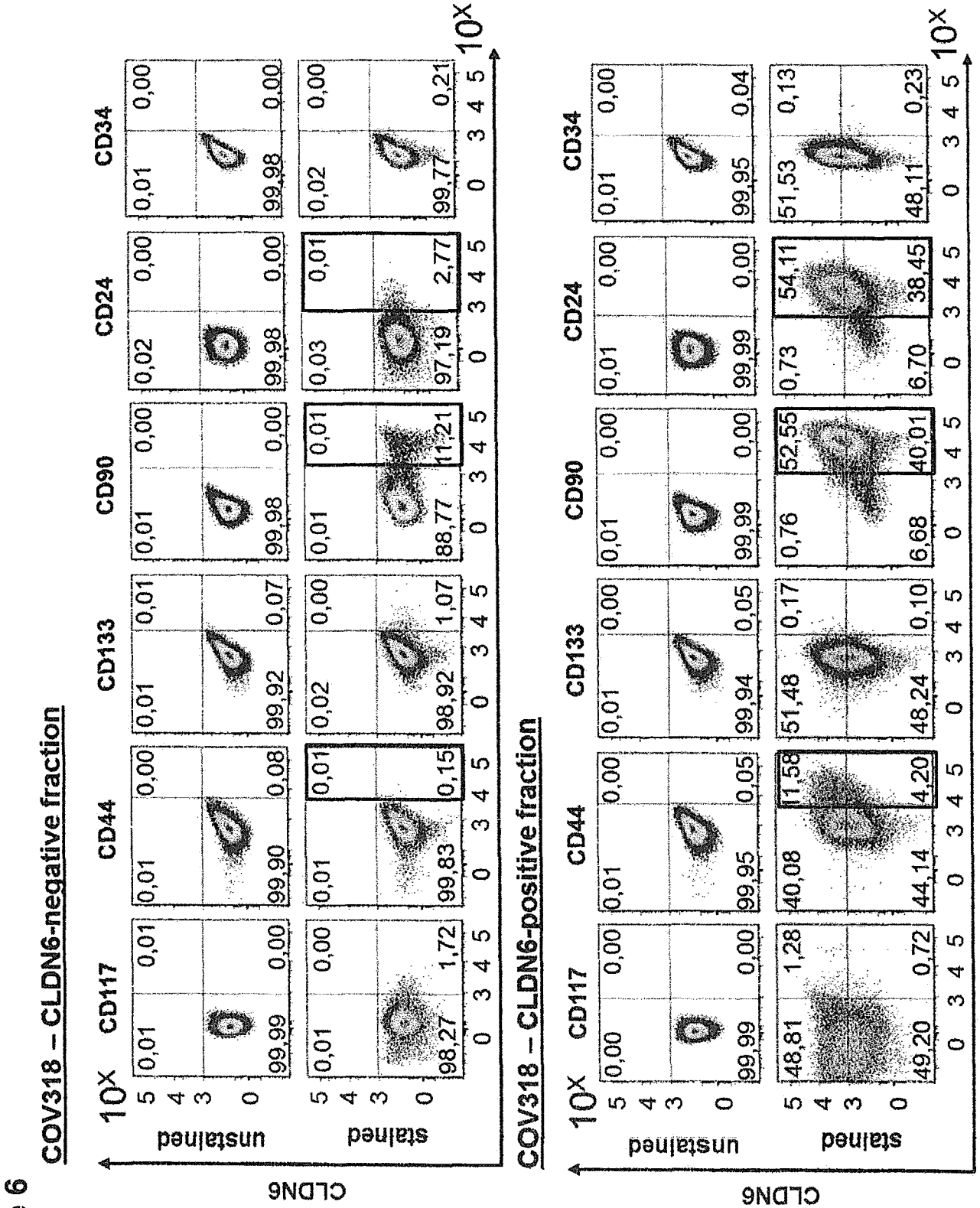
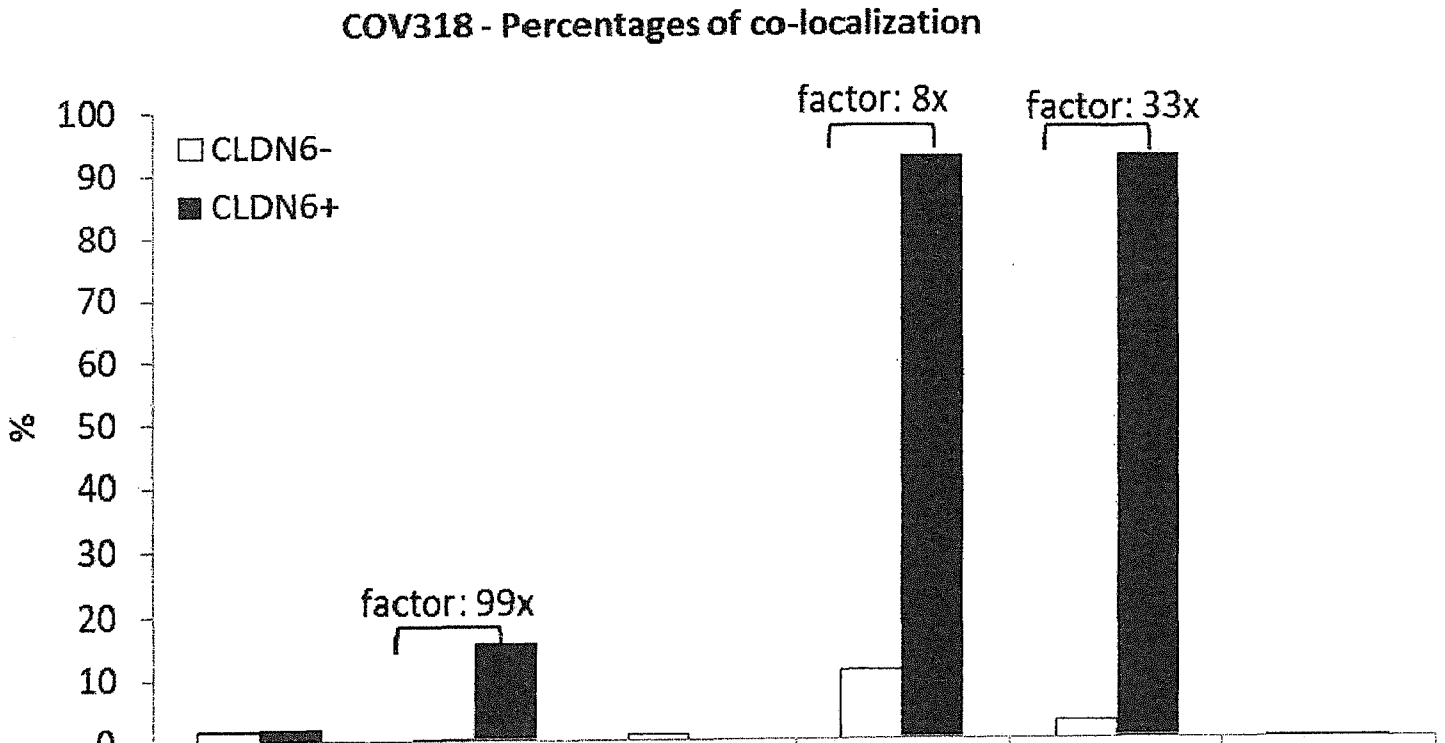

COV318 – CLDN6-negative fraction
COV318 – CLDN6-positive fraction
CD117
CD44
CD133
CD90
CD24
CD34
unstained
stained
CLDN6
10X

Figure 6

B




| | 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

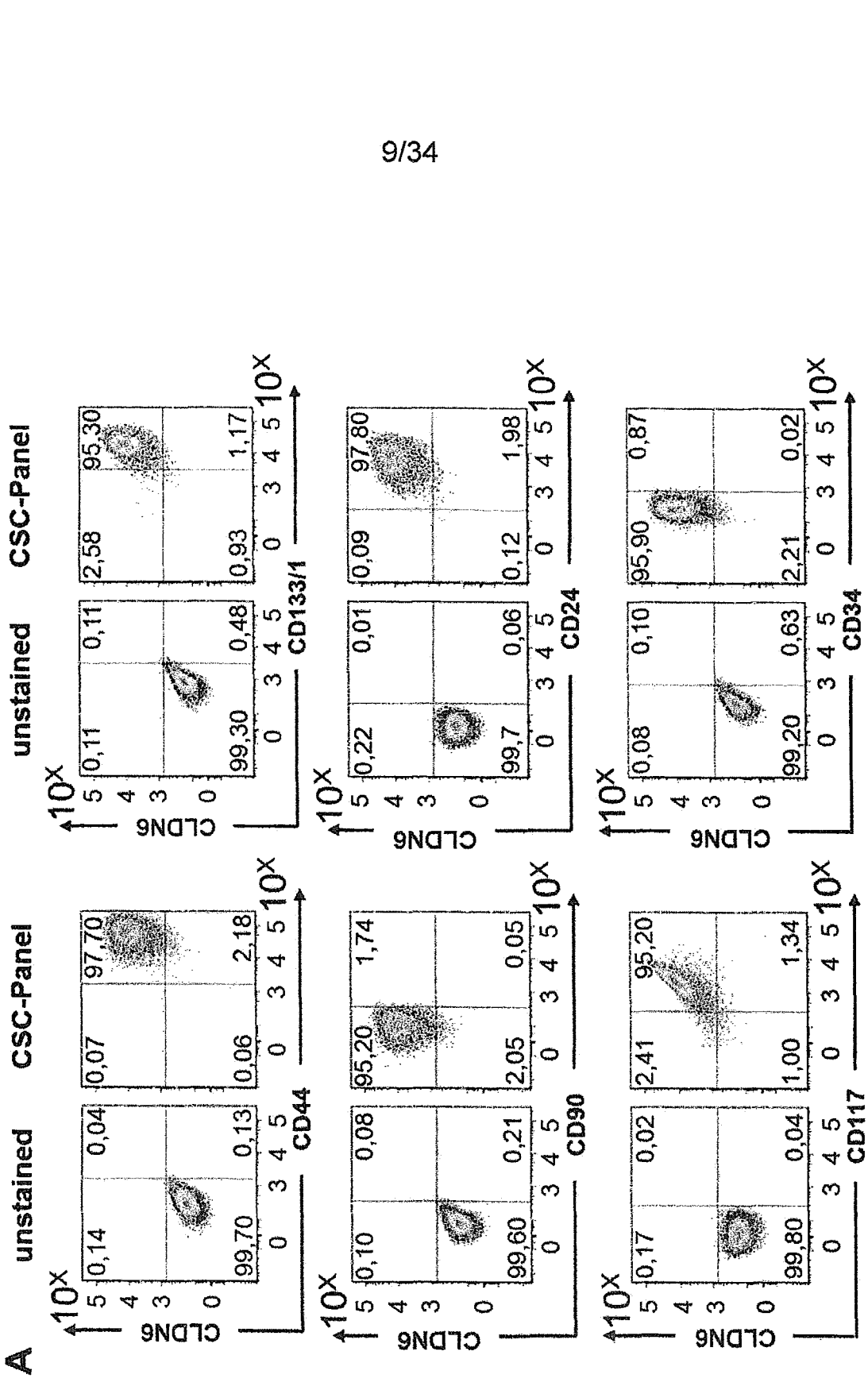

A
unstained
CSC-Panel
unstained
CSC-Panel
CLDN6
CD44
CD133/1
CD90
CD24
CD117
CD34
0,14
0,04
99,70
0,13
0,07
97,70
0,06
2,18
0,11
0,11
99,30
0,48
2,58
95,30
0,93
1,17
0,10
0,08
99,60
0,21
95,20
1,74
2,05
0,05
0,22
0,01
99,7
0,06
0,09
97,80
0,12
1,98
0,17
0,02
99,80
0,04
2,41
95,20
1,00
1,34
0,08
0,10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

Figure 7

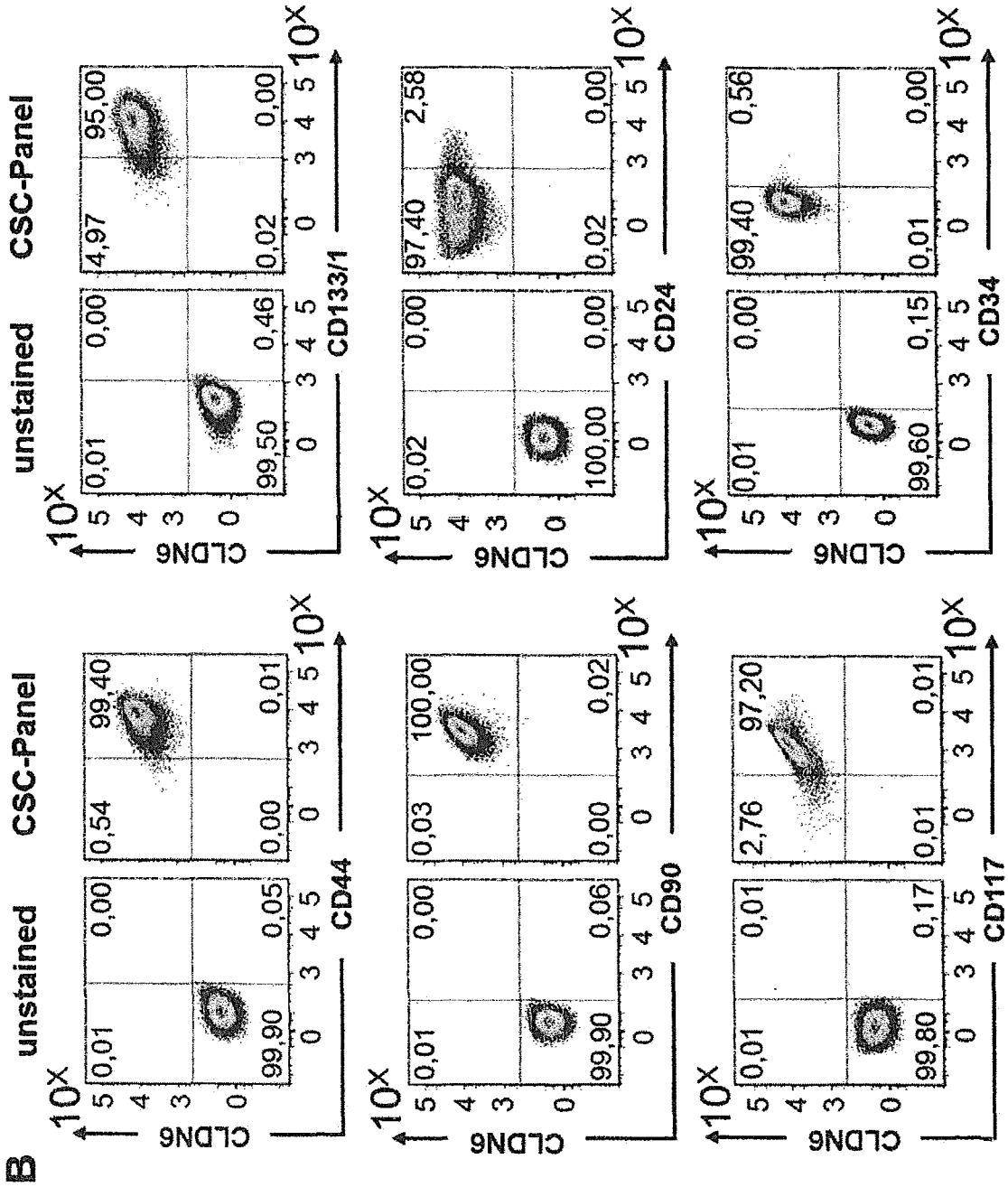

B
unstained
CSC-Panel
CLDN6
CD133/1
CD44
CD24
CD90
CD34
CD117
10X
0,01
0,00
0,54
99,40
99,90
0,05
0,01
0,03
100,00
0,02
99,80
2,76
97,20
0,17
4,97
95,00
99,50
0,46
0,02
97,40
2,58
0,15
99,60
0,56

Figure 7

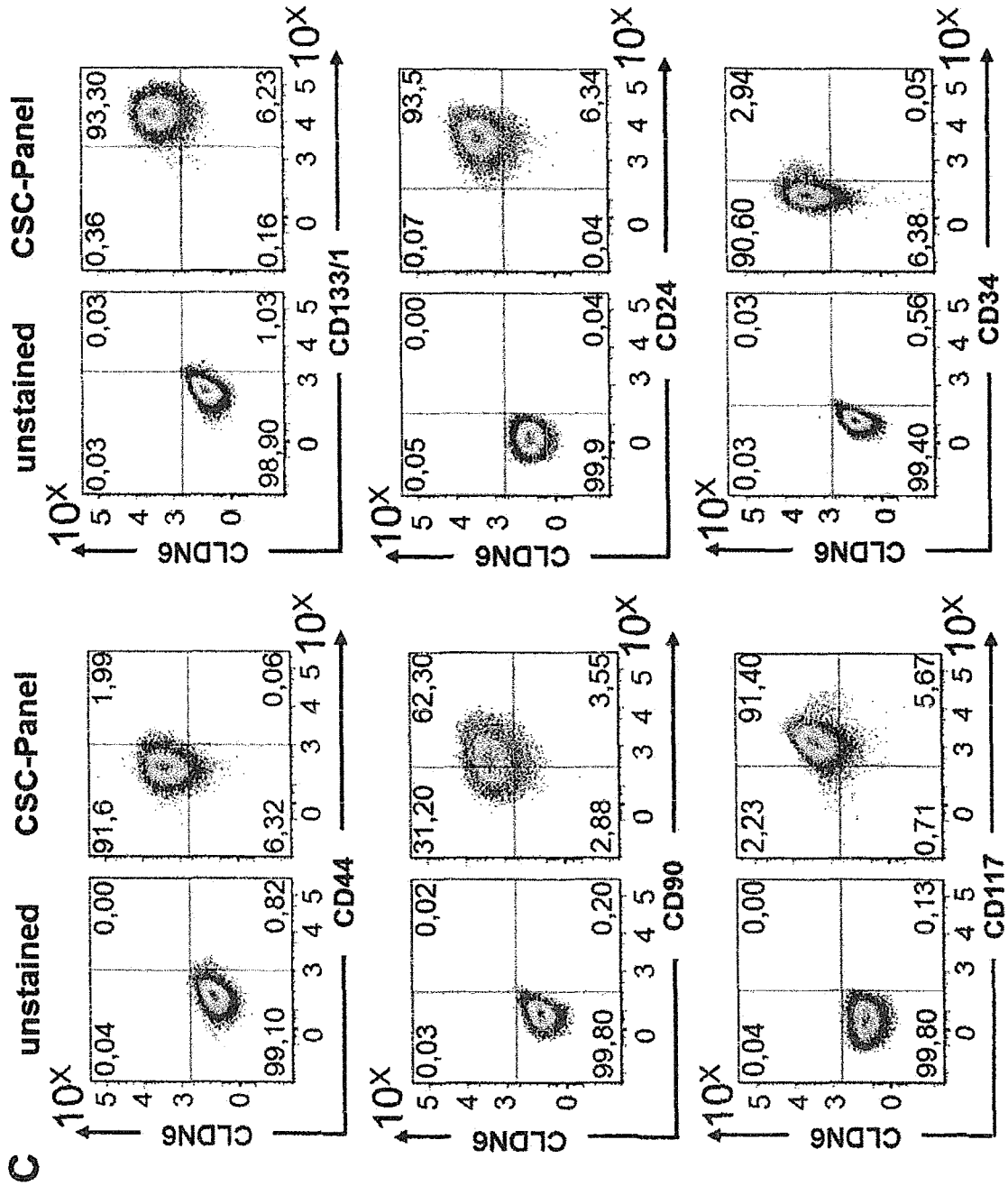

C
unstained
CSC-Panel
unstained
CSC-Panel
CLDN6
CD44
CD133/1
CD90
CD24
CD117
CD34
10X
0,04
0,00
91,6
1,99
99,10
0,82
6,32
0,06
0,03
0,02
31,20
62,30
99,80
0,20
2,88
3,55
0,04
0,00
2,23
91,40
99,80
0,13
0,71
5,67
0,03
0,03
0,36
93,30
98,90
1,03
0,16
6,23
0,05
0,00
0,07
93,5
99,9
0,04
0,04
6,34
0,03
0,03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

Figure 7

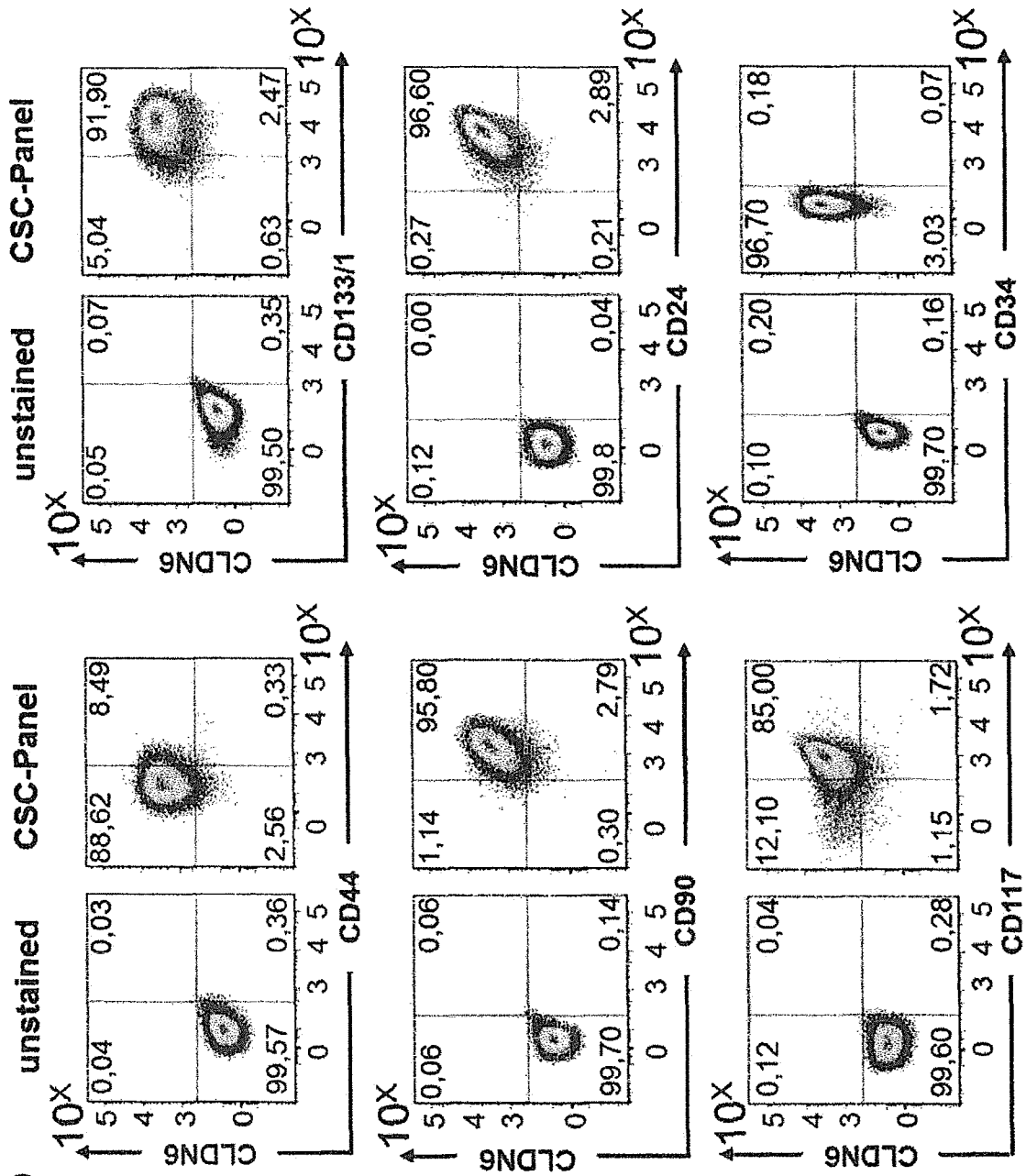

D
unstained
CSC-Panel
unstained
CSC-Panel
CLDN6
CD44
CD133/1
CD90
CD24
CD117
CD34
10X
0,04
0,03
99,57
0,36
88,62
8,49
2,56
0,33
0,05
0,07
99,50
0,35
5,04
91,90
0,63
2,47
0,06
0,06
99,70
0,14
1,14
95,80
0,30
2,79
0,12
0,00
99,8
0,04
0,27
96,60
0,21
2,89
0,12
0,04
99,60
0,28
12,10
85,00
1,15
1,72
0,10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

Figure 8

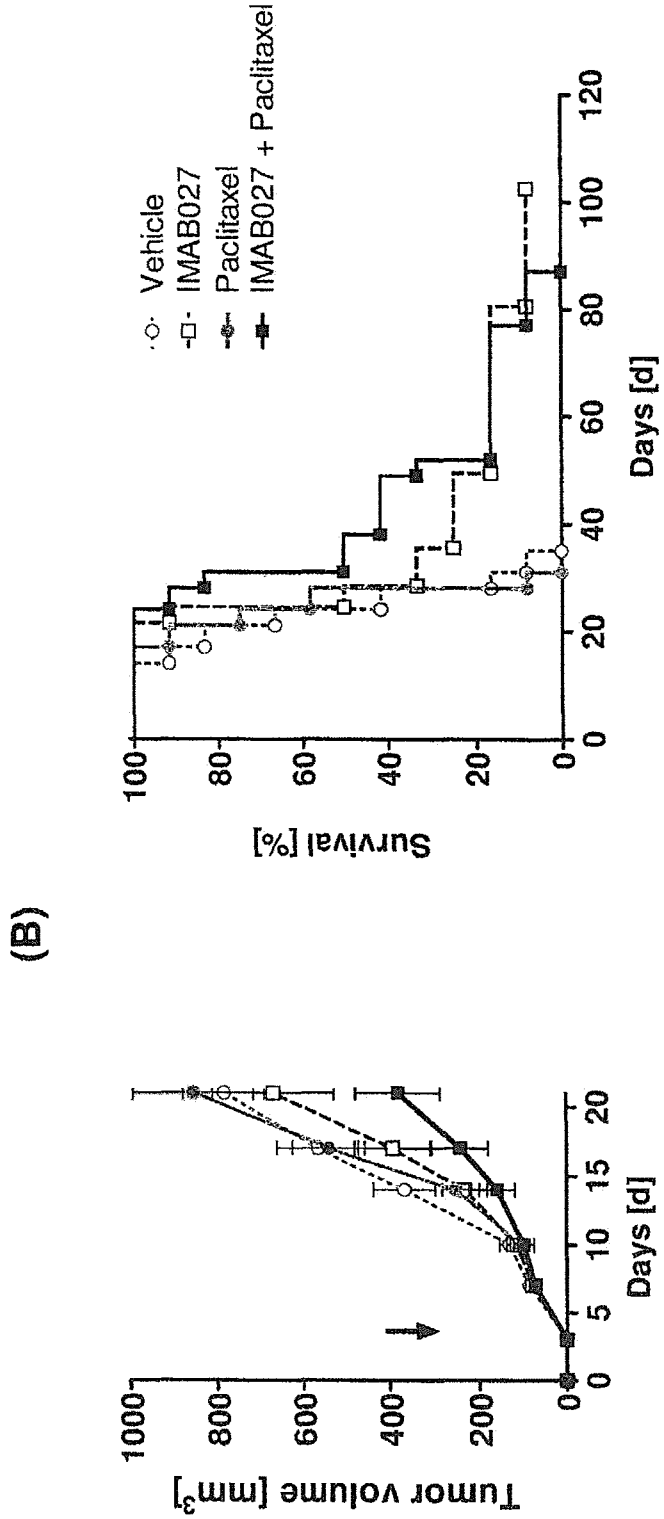

(A)
Tumor volume [mm³]
1000
800
600
400
200
0
0
5
10
15
20
Days [d]
(B)
Survival [%]
100
80
60
40
20
0
0
20
40
60
80
100
120
Days [d]
Vehicle
IMAB027
Paclitaxel
IMAB027 + Paclitaxel

Figure 9

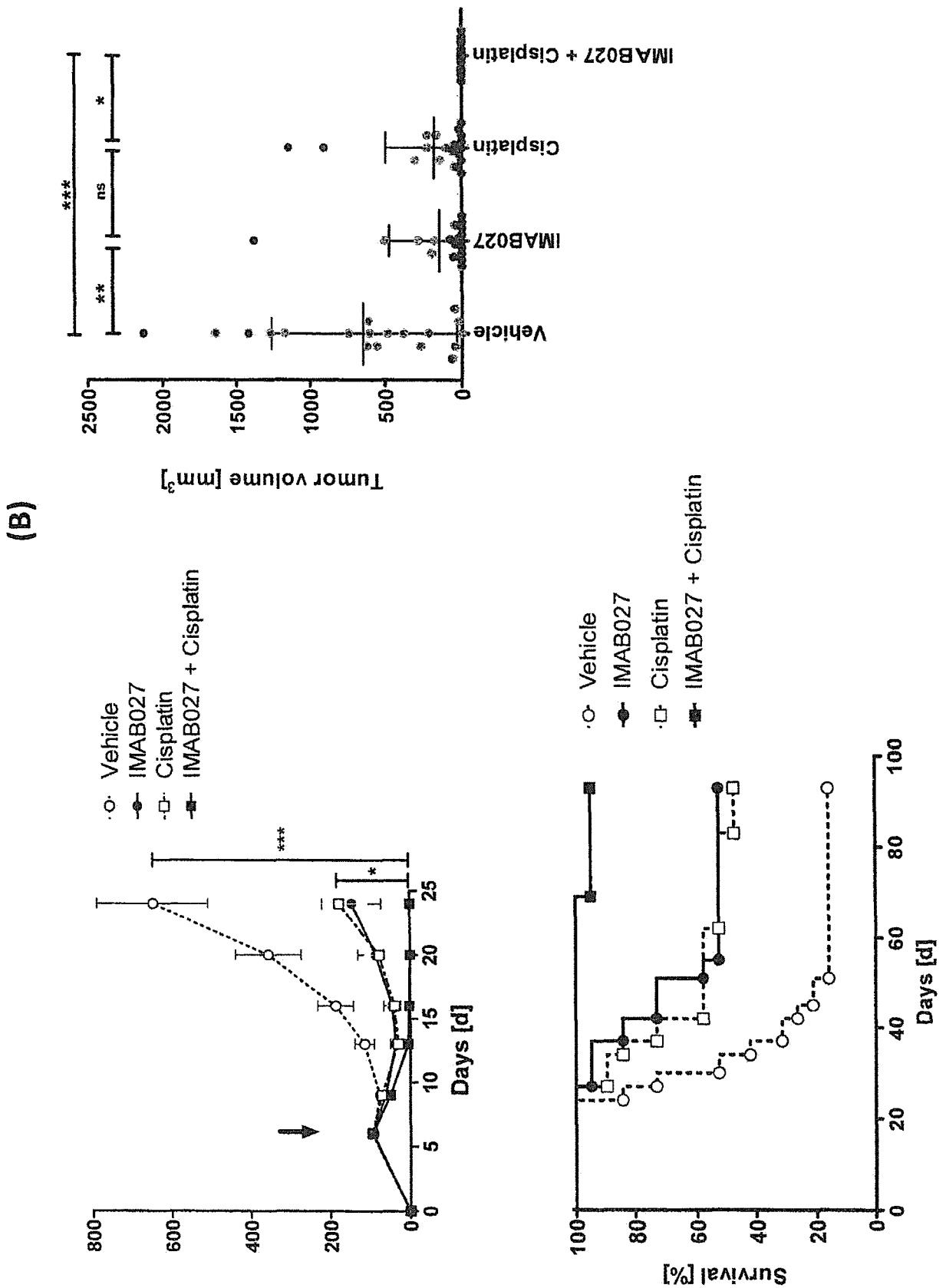
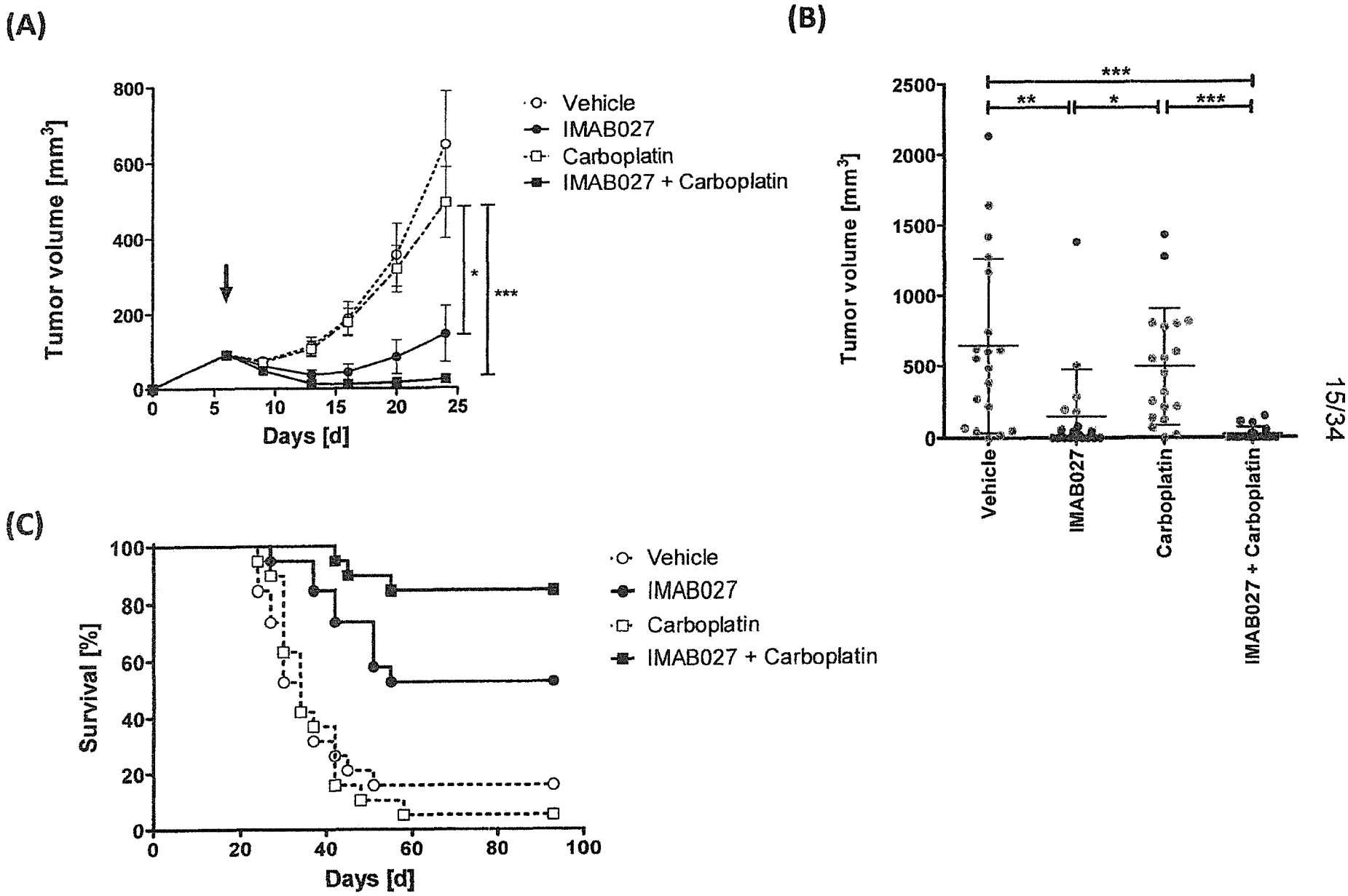

(A)
Tumor volume [mm³]
Days [d]
Vehicle
IMAB027
Cisplatin
IMAB027 + Cisplatin
***
*
(B)
Tumor volume [mm³]
**
ns
***
*
Vehicle
IMAB027
Cisplatin
IMAB027 + Cisplatin
(C)
Survival [%]
Days [d]
Vehicle
IMAB027
Cisplatin
IMAB027 + Cisplatin

Figure 10


(A)
Tumor volume [mm3]
800
600
400
200
0
0
5
10
15
20
25
Days [d]
Vehicle
IMAB027
Carboplatin
IMAB027 + Carboplatin
*
***
(B)
Tumor volume [mm3]
2500
2000
1500
1000
500
0
***
**
*
***
Vehicle
IMAB027
Carboplatin
IMAB027 + Carboplatin
(C)
Survival [%]
100
80
60
40
20
0
0
20
40
60
80
100
Days [d]
Vehicle
IMAB027
Carboplatin
IMAB027 + Carboplatin

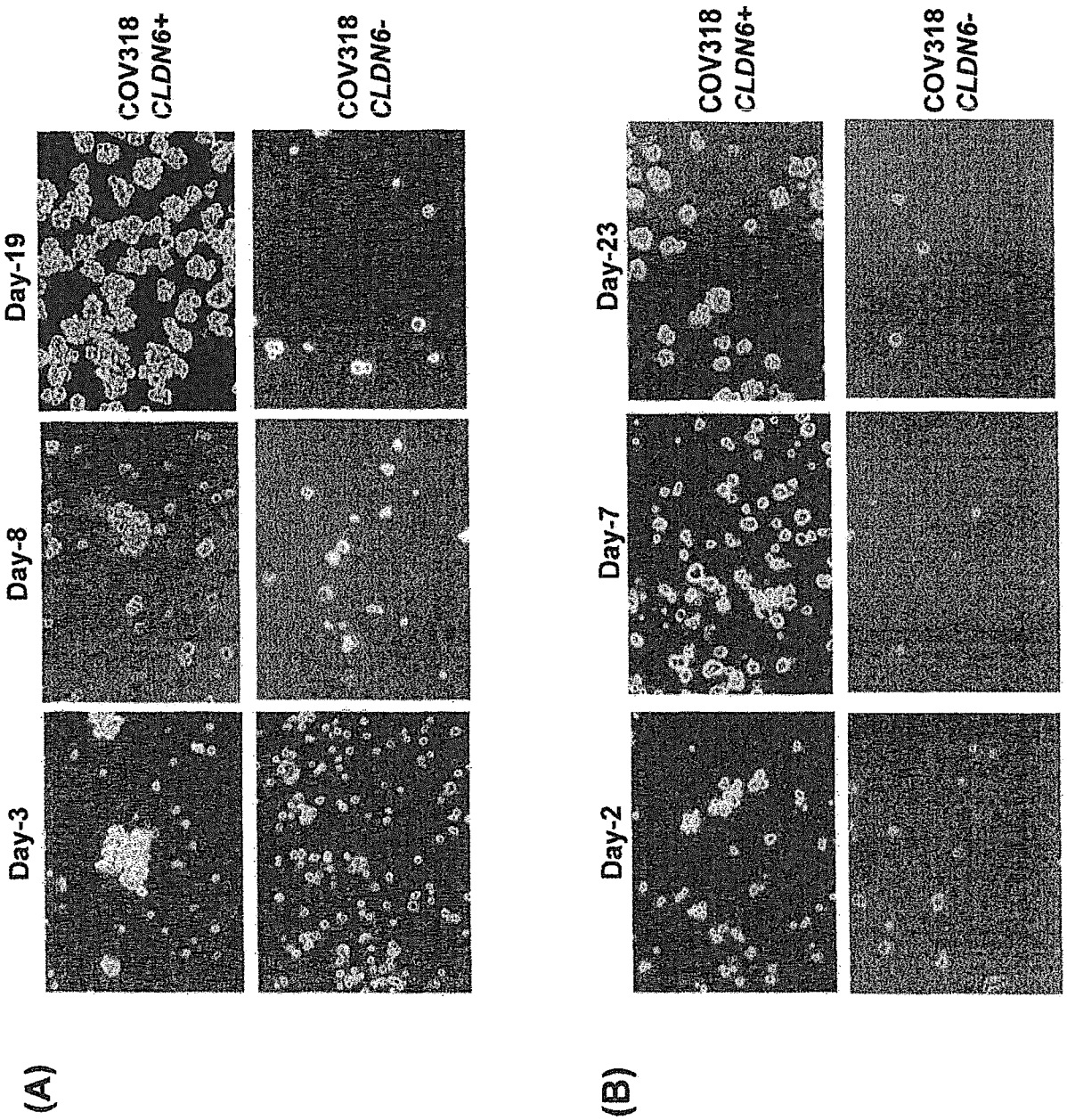

(A)
Day-3
Day-8
Day-19
COV318 CLDN6+
COV318 CLDN6-
(B)
Day-2
Day-7
Day-23
COV318 CLDN6+
COV318 CLDN6-

Figure 11

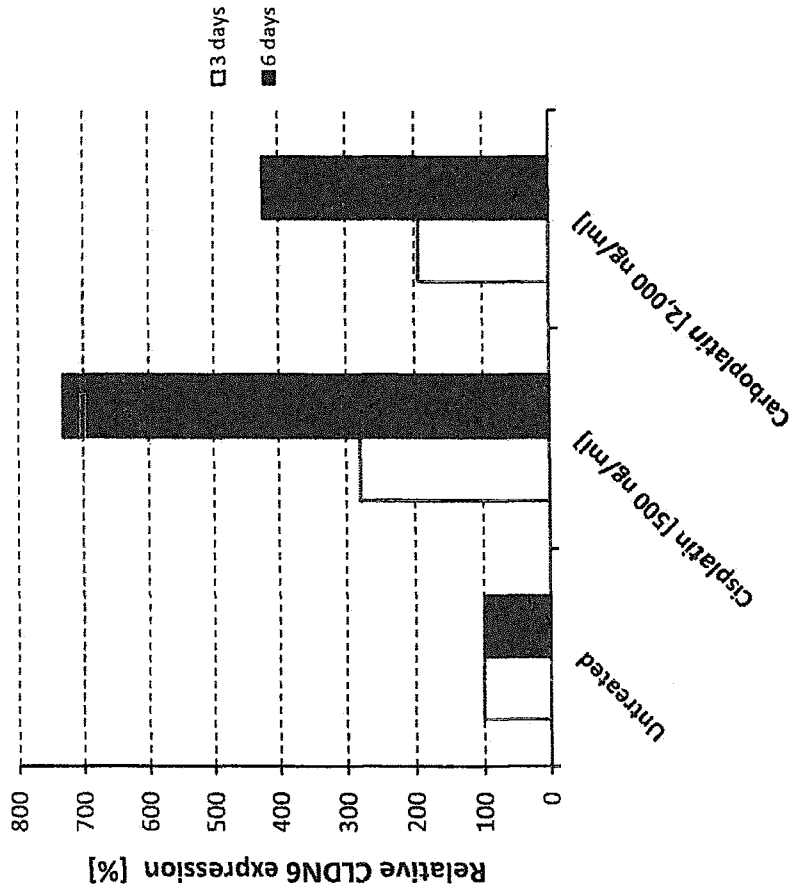
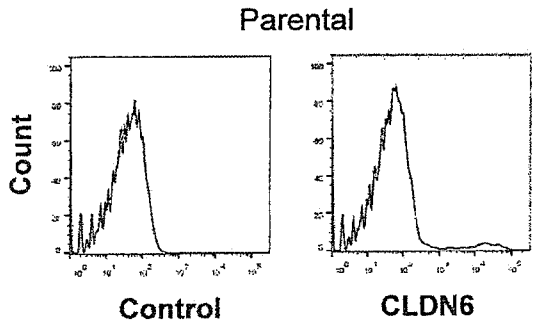

3 days
6 days
Relative CLDN6 expression [%]
800
700
600
500
400
300
200
100
0
Untreated
Cisplatin [500 ng/ml]
Carboplatin [2,000 ng/ml]

Figure 12

Figure 13

(A)


Parental
Count
Control
CLDN6

(B)

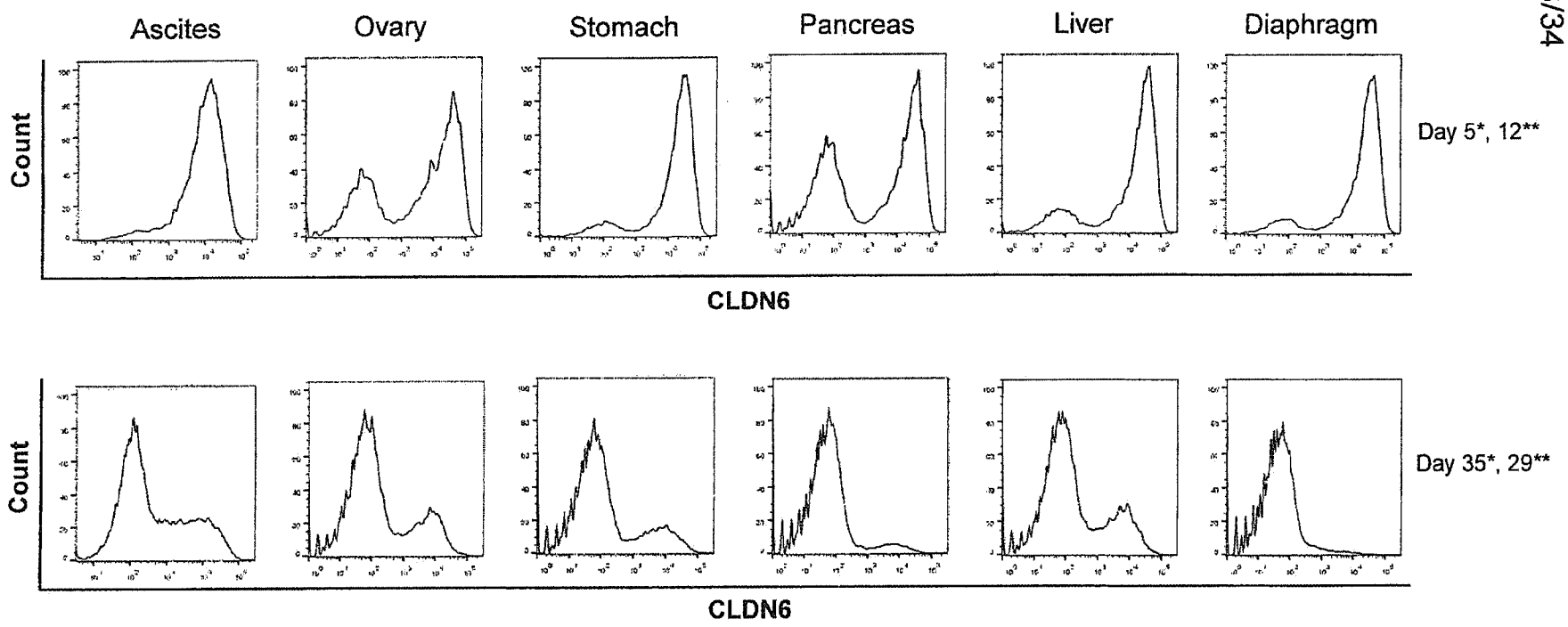
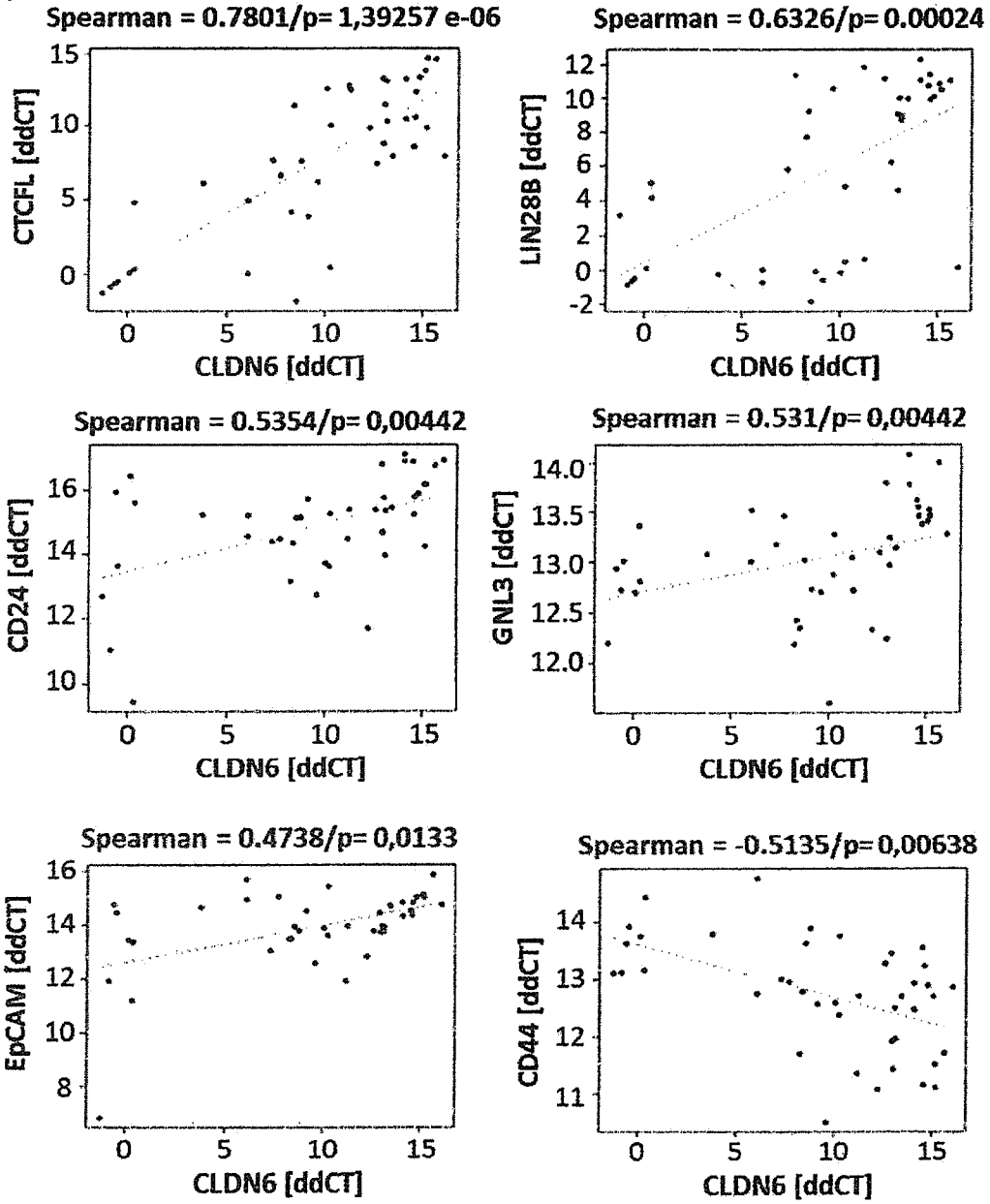

Ascites
Ovary
Stomach
Pancreas
Liver
Diaphragm
Count
CLDN6
Day 5*, 12**
Count
CLDN6
Day 35*, 29**

Figure 14

(A)




(B)

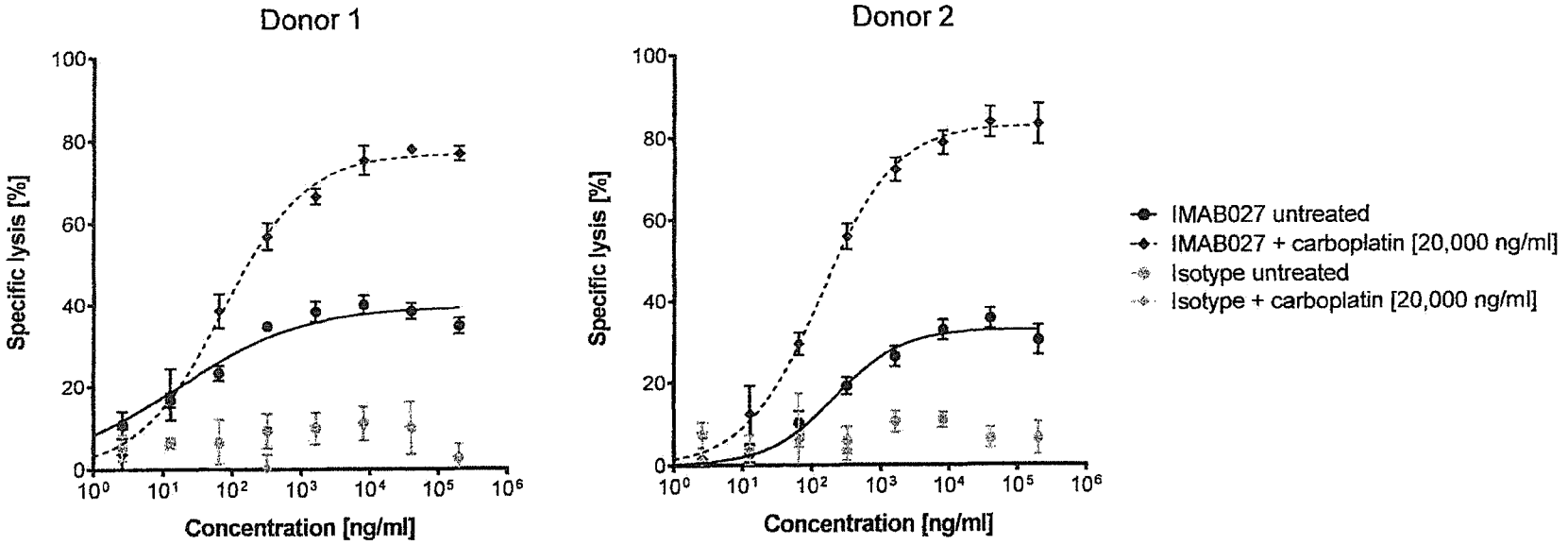
| positive correlation | | |
|---|---|---|
| *marker* | *spearman r* | *p-value* |
| CTCFL | 0,7801 | 1,39E-06 |
| LIN28B | 0,6326 | 0,0002 |
| CD24 | 0,5354 | 0,00441744 |
| GNL3 | 0,531 | 0,00441744 |
| EPCAM | 0,4738 | 0,0132667 |

| 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
Specific lysis [%]
Concentration [ng/ml]
IMAB027 untreated
IMAB027 + carboplatin [20,000 ng/ml]
Isotype untreated
Isotype + carboplatin [20,000 ng/ml]

(B)

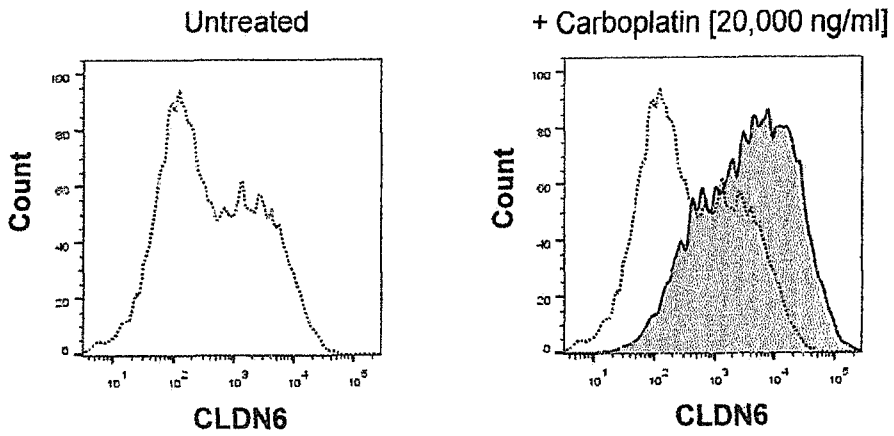

Untreated
+ Carboplatin [20,000 ng/ml]
Count
CLDN6

Figure 15

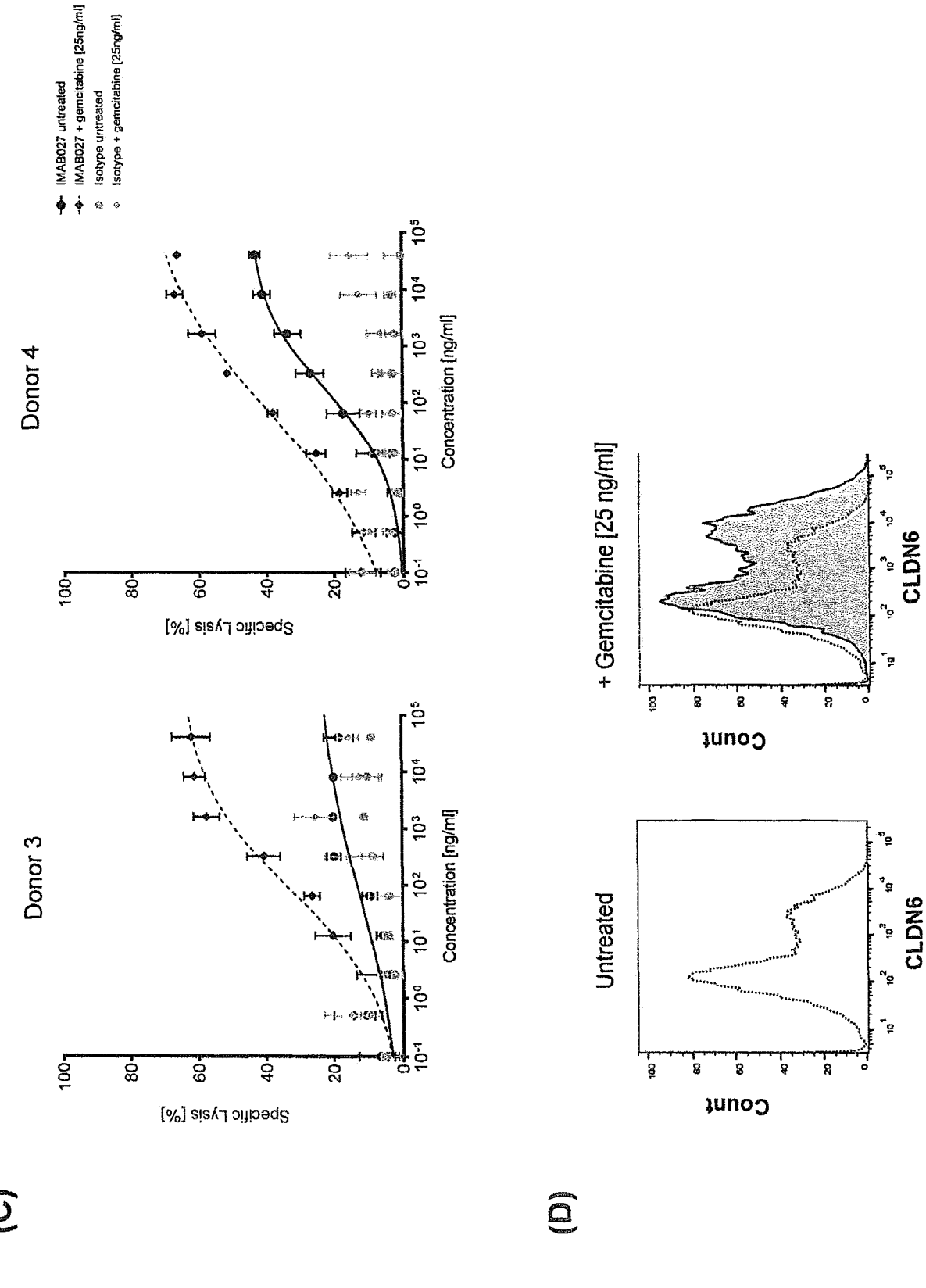
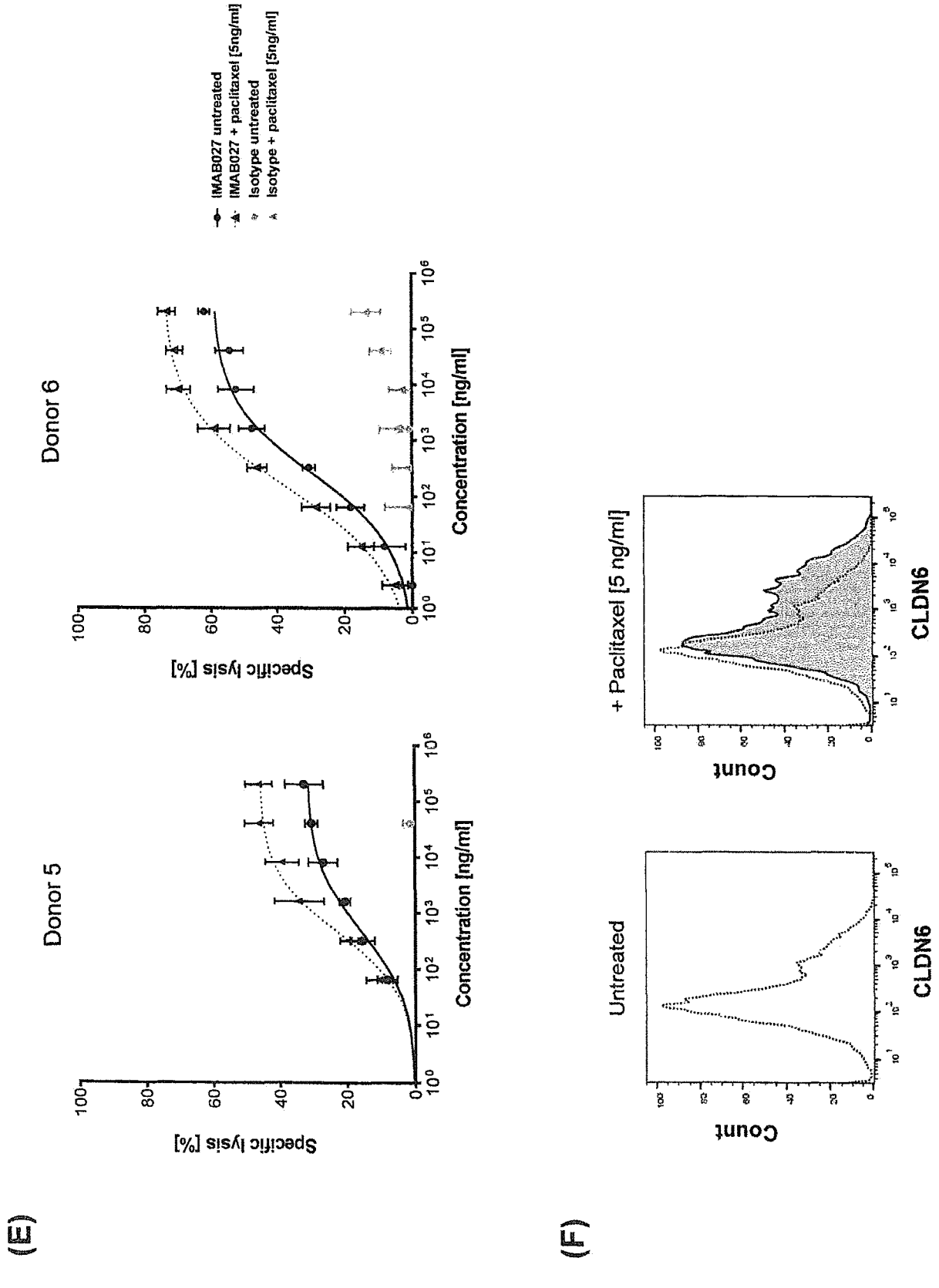
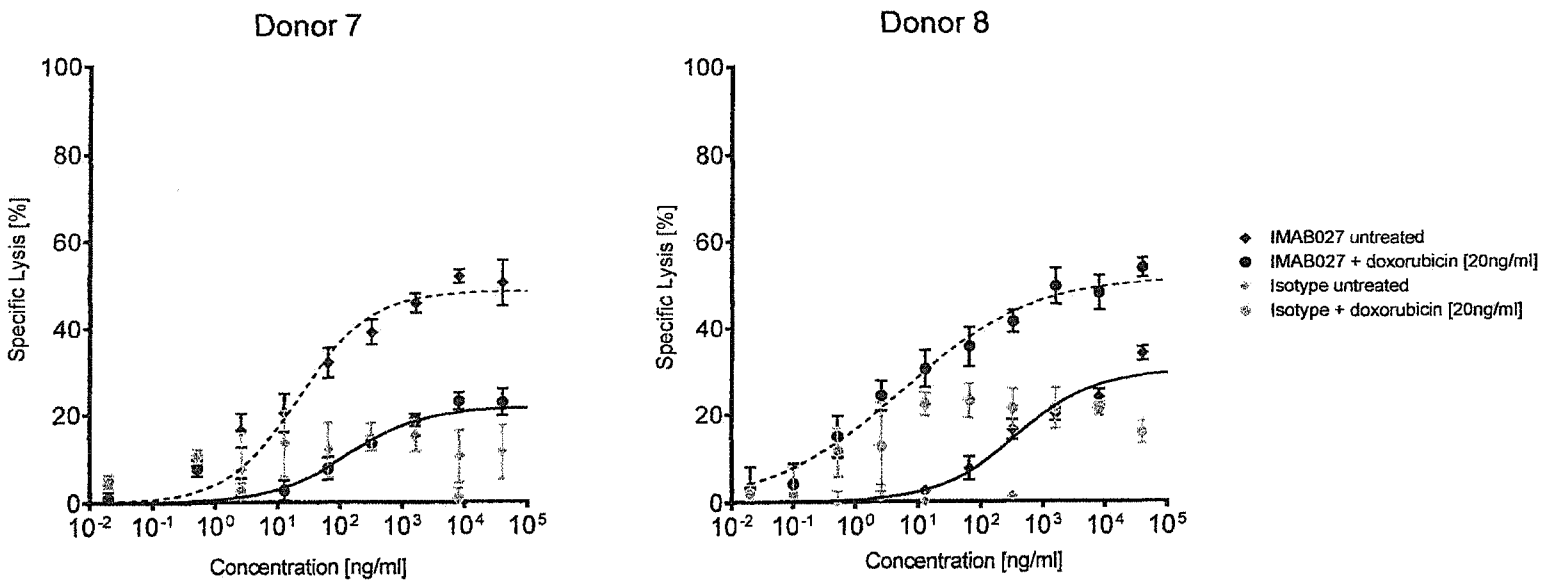

(C)
Donor 3
Donor 4
IMAB027 untreated
IMAB027 + gemcitabine [25ng/ml]
Isotype untreated
Isotype + gemcitabine [25ng/ml]
Specific Lysis [%]
Concentration [ng/ml]
(D)
Untreated
+ Gemcitabine [25 ng/ml]
Count
CLDN6

Figure 15

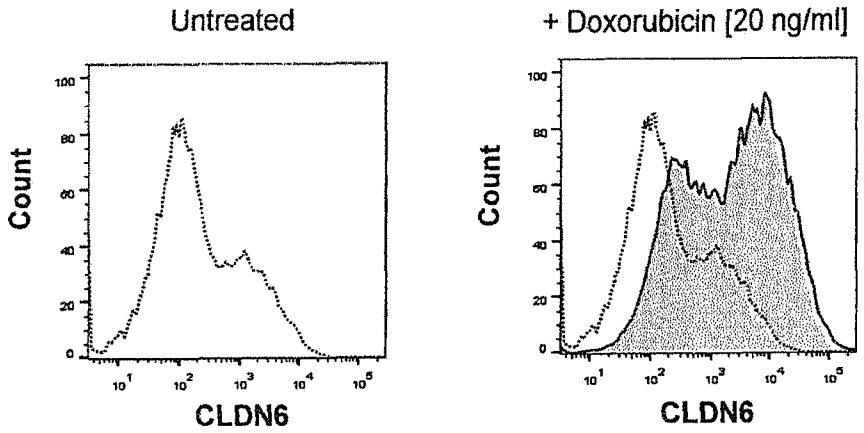

(E)
Donor 5
Donor 6
Specific lysis [%]
Concentration [ng/ml]
IMAB027 untreated
IMAB027 + paclitaxel [5ng/ml]
Isotype untreated
Isotype + paclitaxel [5ng/ml]
(F)
Untreated
+ Paclitaxel [5 ng/ml]
Count
CLDN6

# Figure 15

(G)

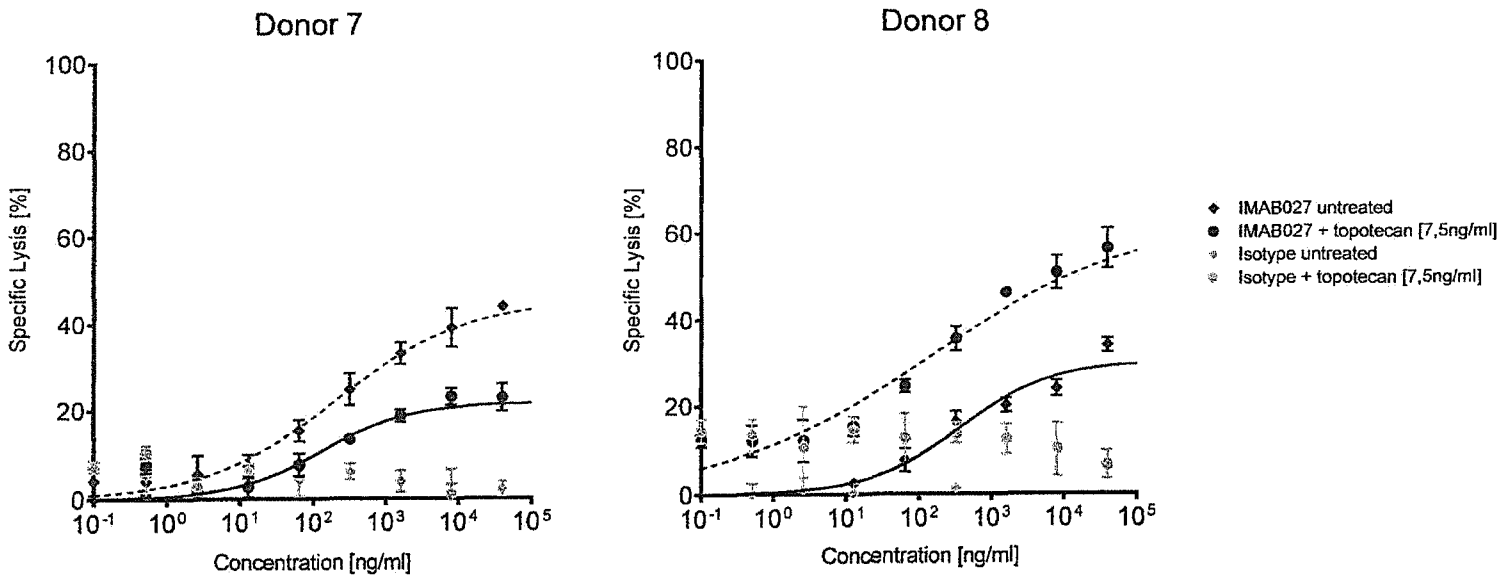

Donor 7
Donor 8
Specific Lysis [%]
Concentration [ng/ml]
IMAB027 untreated
IMAB027 + doxorubicin [20ng/ml]
Isotype untreated
Isotype + doxorubicin [20ng/ml]

(H)


Untreated
+ Doxorubicin [20 ng/ml]
Count
CLDN6

# Figure 15

(I)


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

(J)

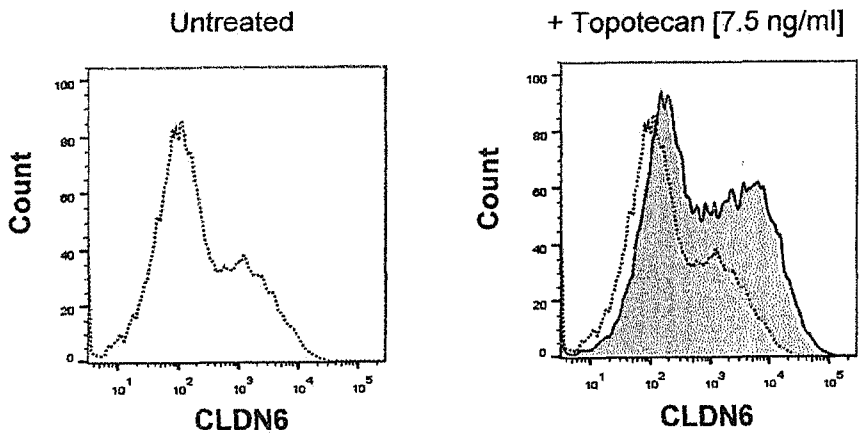

Untreated
+ Topotecan [7.5 ng/ml]
Count
CLDN6

Figure 16

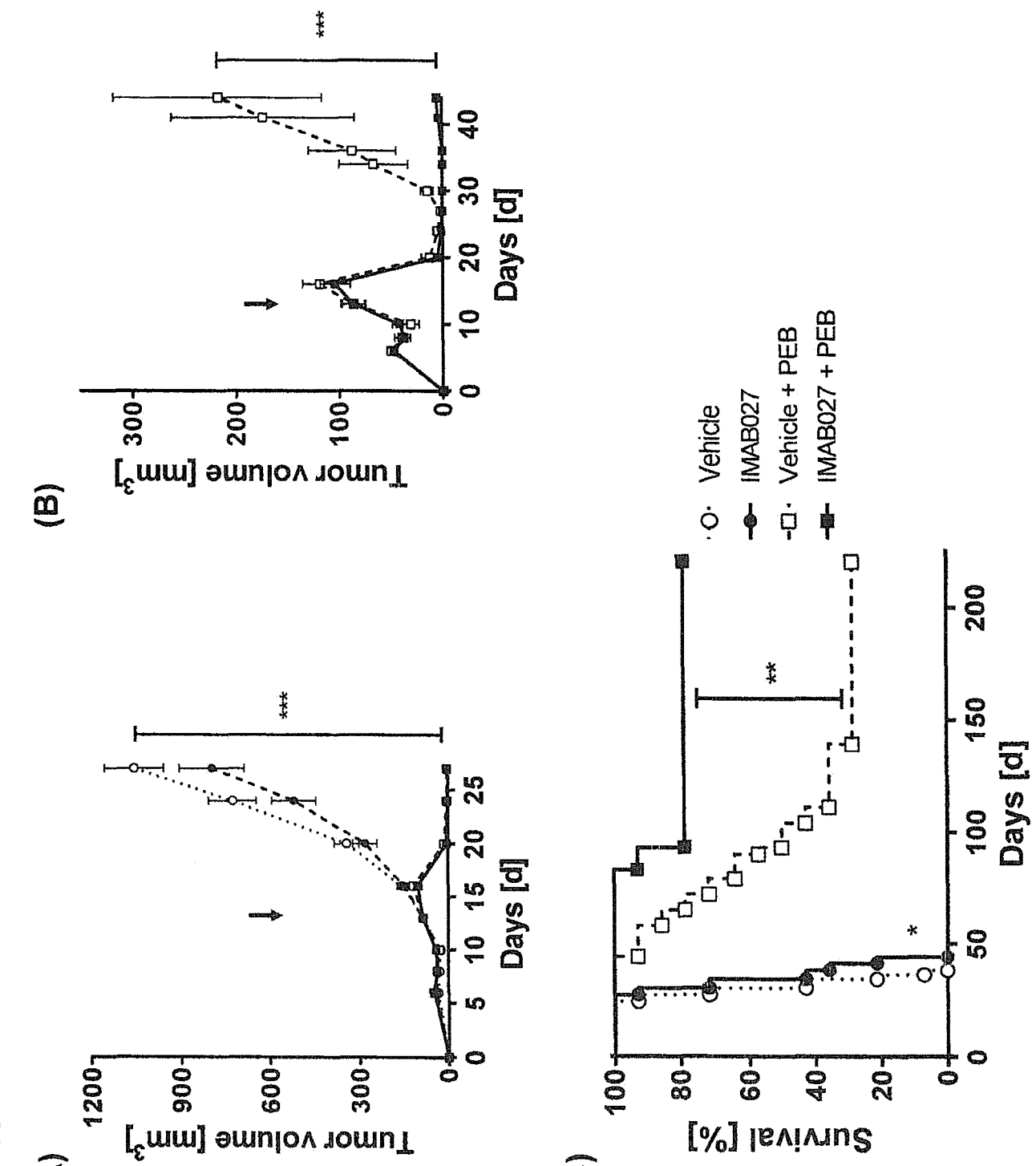
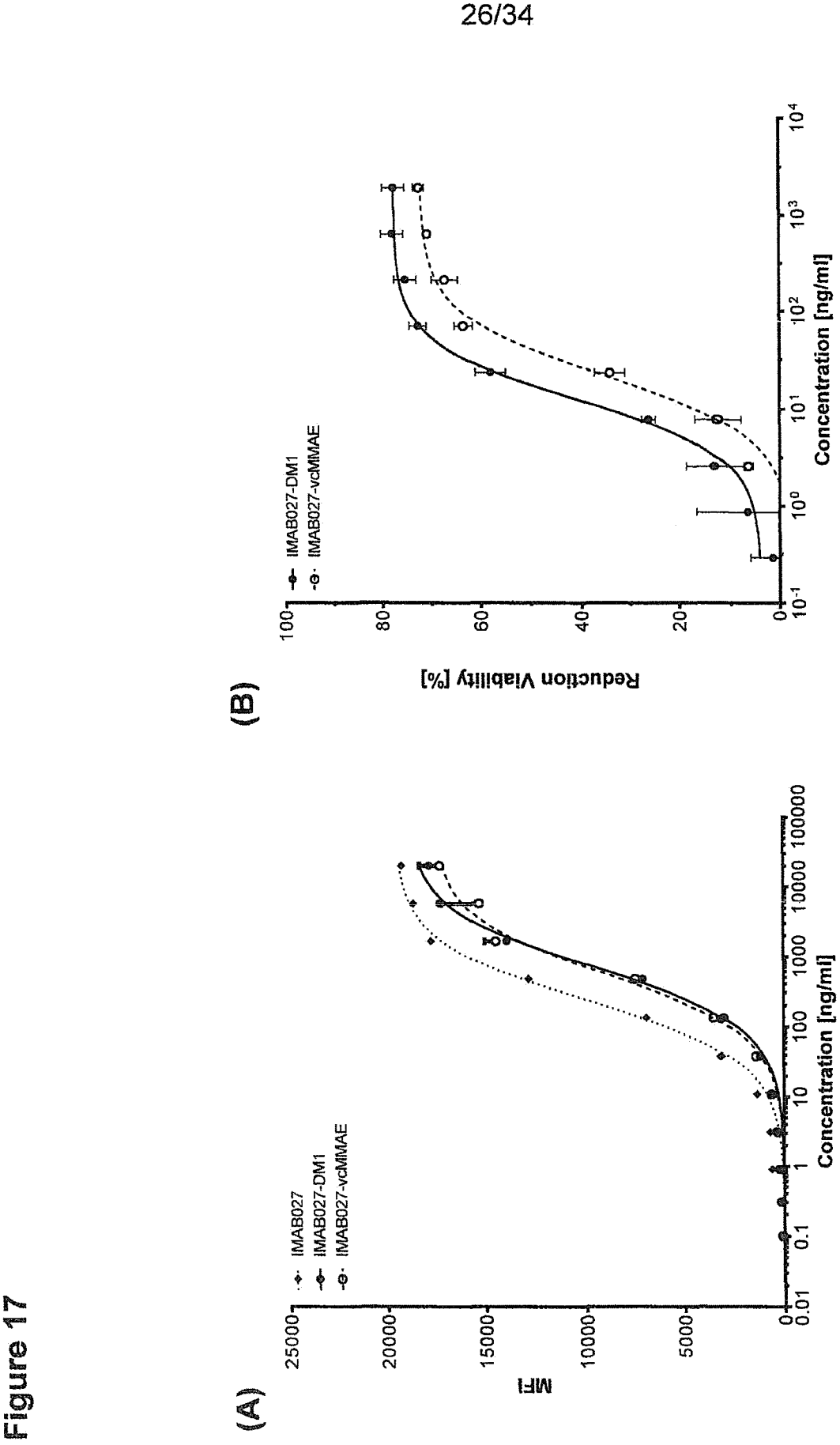

(A)
Tumor volume [mm³]
1200
900
600
300
0
0 5 10 15 20 25
Days [d]
***
(B)
Tumor volume [mm³]
300
200
100
0
0 10 20 30 40
Days [d]
***
(C)
Survival [%]
100
80
60
40
20
0
0 50 100 150 200
Days [d]
Vehicle
IMAB027
Vehicle + PEB
IMAB027 + PEB
**
*

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

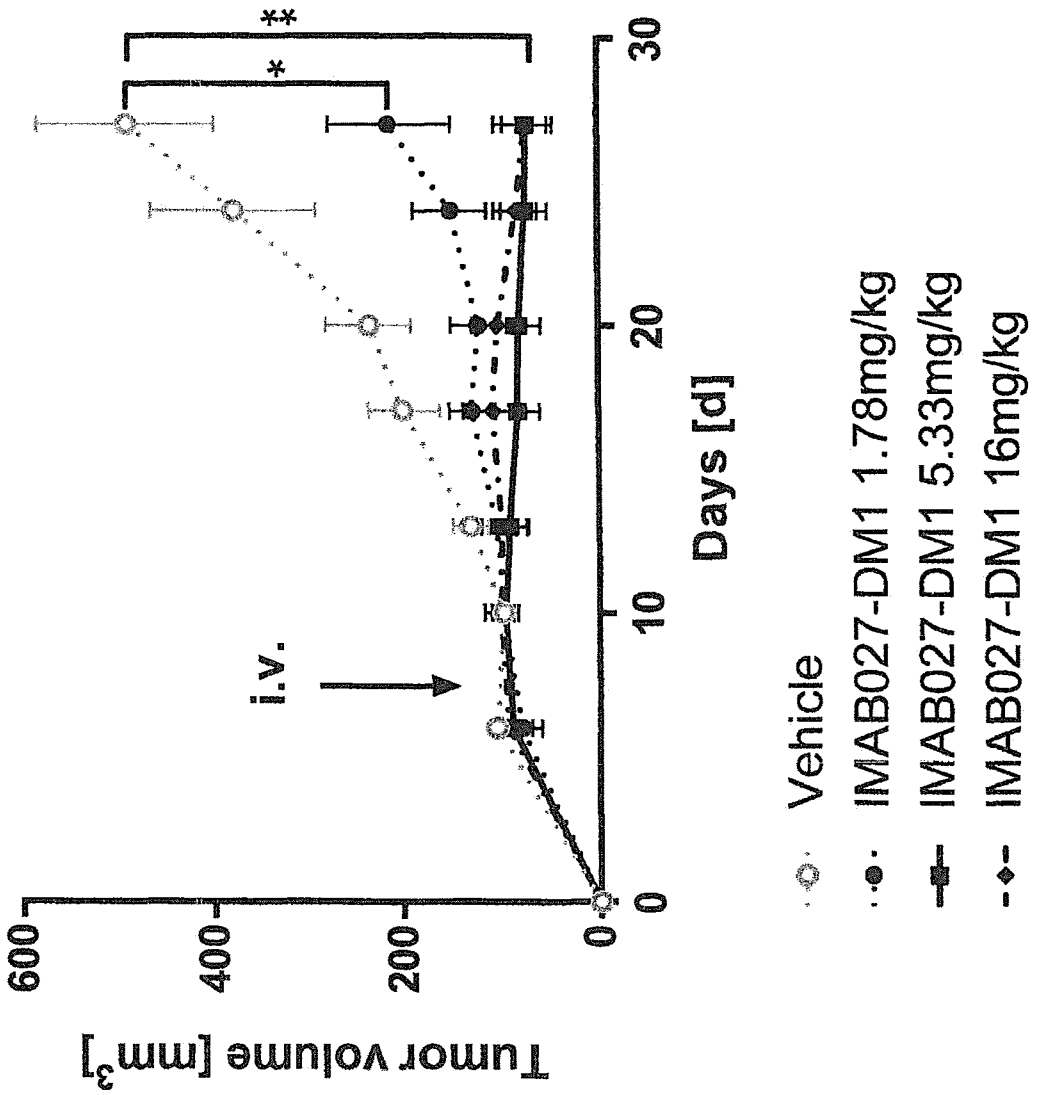

Tumor volume [mm³]
600
400
200
0
0
10
20
30
Days [d]
i.v.
*
**
Vehicle
IMAB027-DM1 1.78mg/kg
IMAB027-DM1 5.33mg/kg
IMAB027-DM1 16mg/kg

Figure 18

Figure 19

(A)

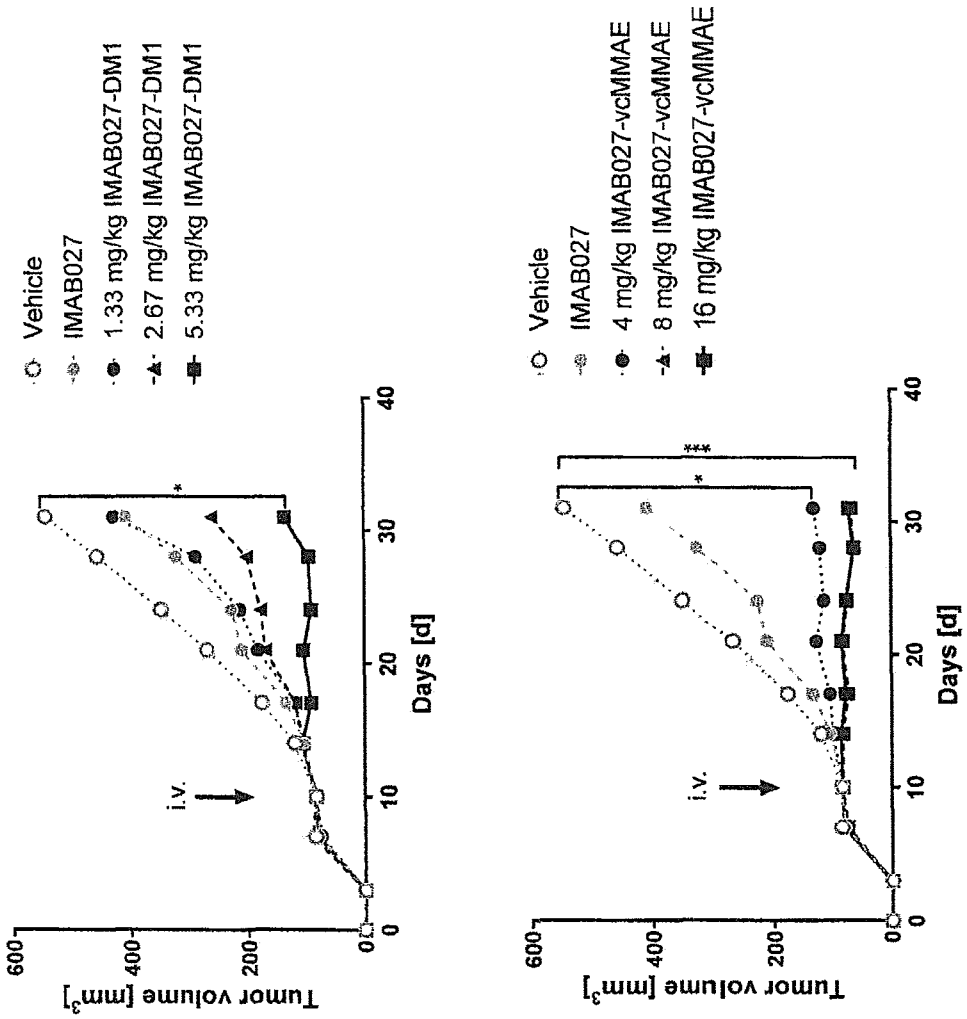

Vehicle
IMAB027
1.33 mg/kg IMAB027-DM1
2.67 mg/kg IMAB027-DM1
5.33 mg/kg IMAB027-DM1
Tumor volume [mm³]
Days [d]
i.v.
*
Vehicle
IMAB027
4 mg/kg IMAB027-vcMMAE
8 mg/kg IMAB027-vcMMAE
16 mg/kg IMAB027-vcMMAE
Tumor volume [mm³]
Days [d]
i.v.
***
*

Figure 19

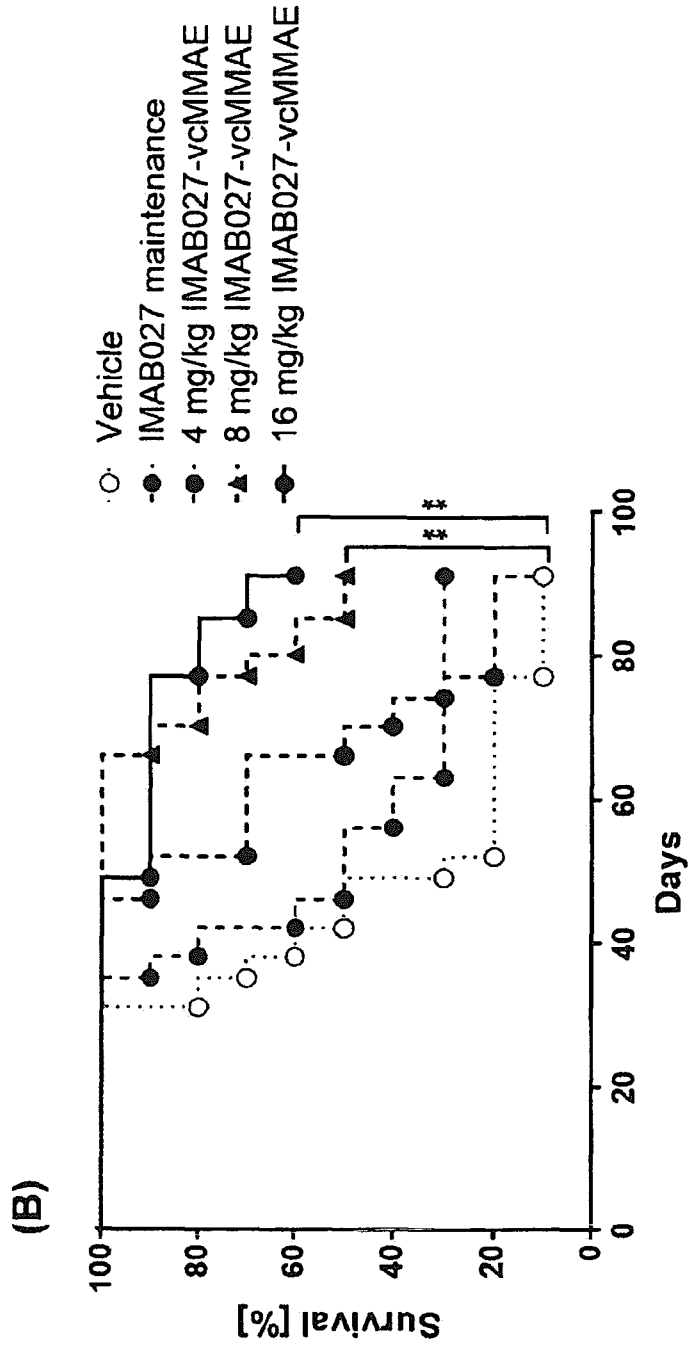

(B)
Vehicle
IMAB027 maintenance
4 mg/kg IMAB027-vcMMAE
8 mg/kg IMAB027-vcMMAE
16 mg/kg IMAB027-vcMMAE
**
**
Survival [%]
100
80
60
40
20
0
0
20
40
60
80
100
Days

Figure 20

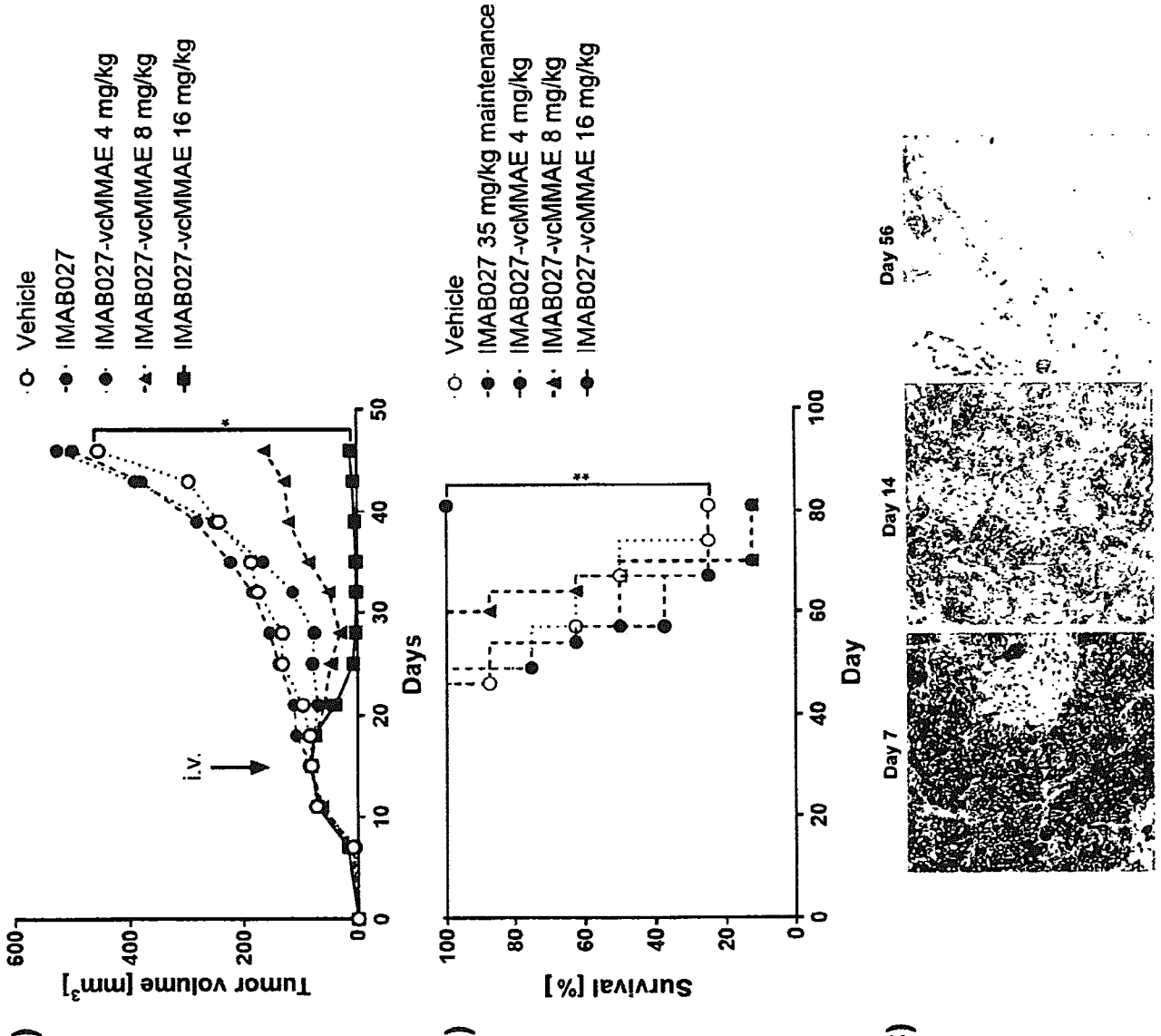

(A)
Vehicle
IMAB027
IMAB027-vcMMAE 4 mg/kg
IMAB027-vcMMAE 8 mg/kg
IMAB027-vcMMAE 16 mg/kg
Tumor volume [mm³]
600
400
200
0
i.v.
0
10
20
30
40
50
Days
*
(B)
Vehicle
IMAB027 35 mg/kg maintenance
IMAB027-vcMMAE 4 mg/kg
IMAB027-vcMMAE 8 mg/kg
IMAB027-vcMMAE 16 mg/kg
Survival [%]
100
80
60
40
20
0
0
20
40
60
80
100
Day
**
(C)
Day 7
Day 14
Day 56

Figure 21

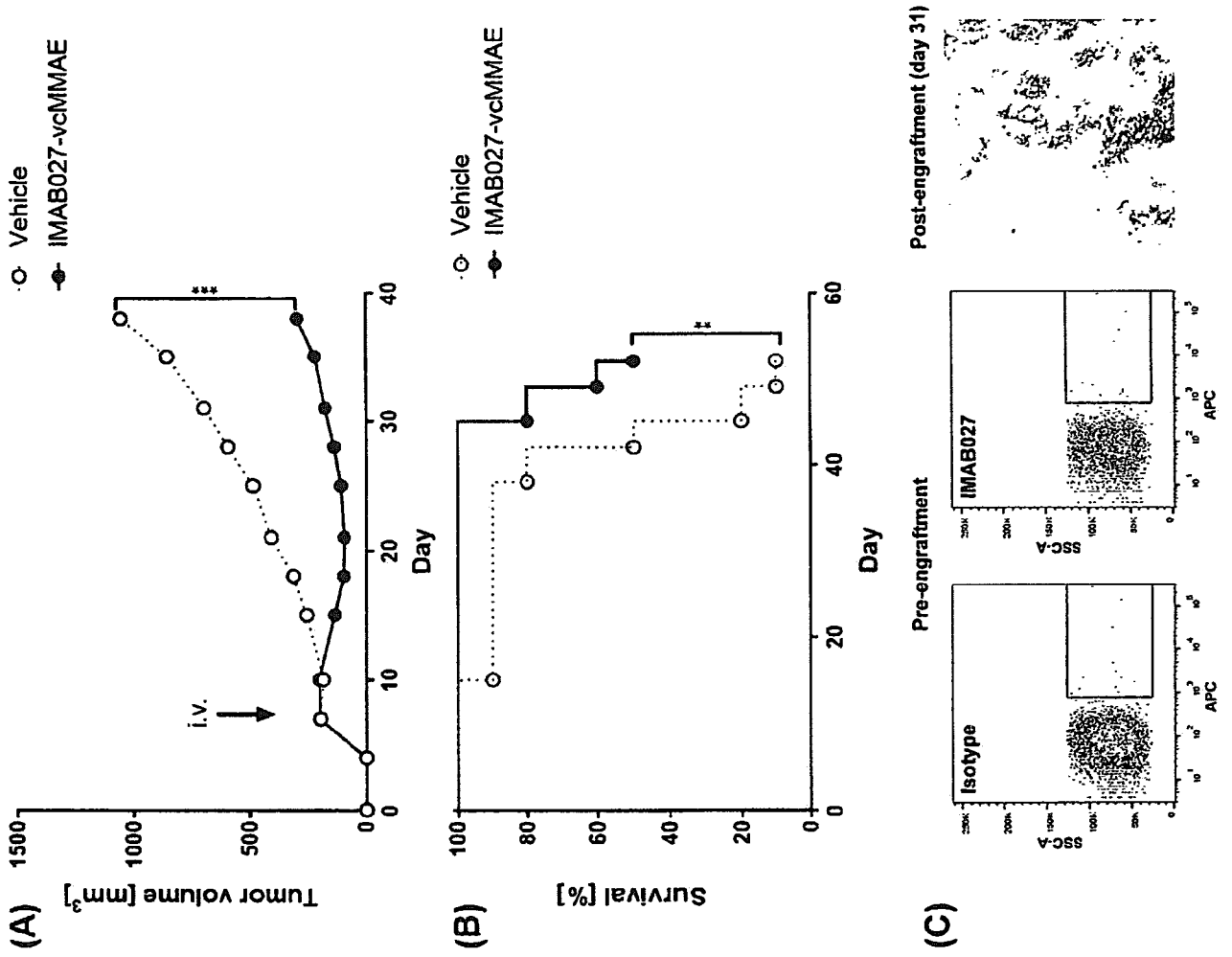

(A)
Vehicle
IMAB027-vcMMAE
Tumor volume [mm³]
1500
1000
500
0
0
10
20
30
40
Day
i.v.
***
(B)
Vehicle
IMAB027-vcMMAE
Survival [%]
100
80
60
40
20
0
0
20
40
60
Day
**
(C)
Pre-engraftment
Isotype
IMAB027
SSC-A
APC
Post-engraftment (day 31)

Figure 22

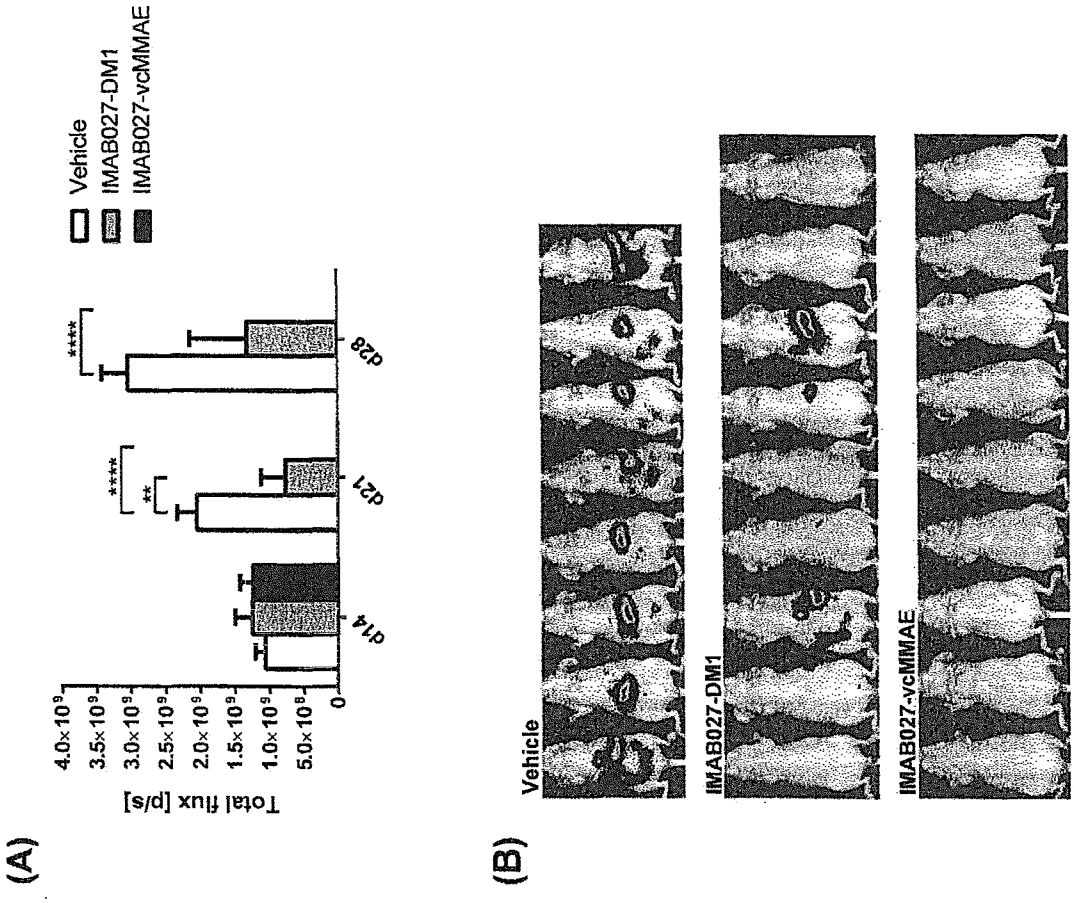

(A)
Total flux [p/s]
4.0×10^9
3.5×10^9
3.0×10^9
2.5×10^9
2.0×10^9
1.5×10^9
1.0×10^9
5.0×10^8
0
d14
d21
d28
****
**
****
Vehicle
IMAB027-DM1
IMAB027-vcMMAE
(B)
Vehicle
IMAB027-DM1
IMAB027-vcMMAE

Figure 23

(A)

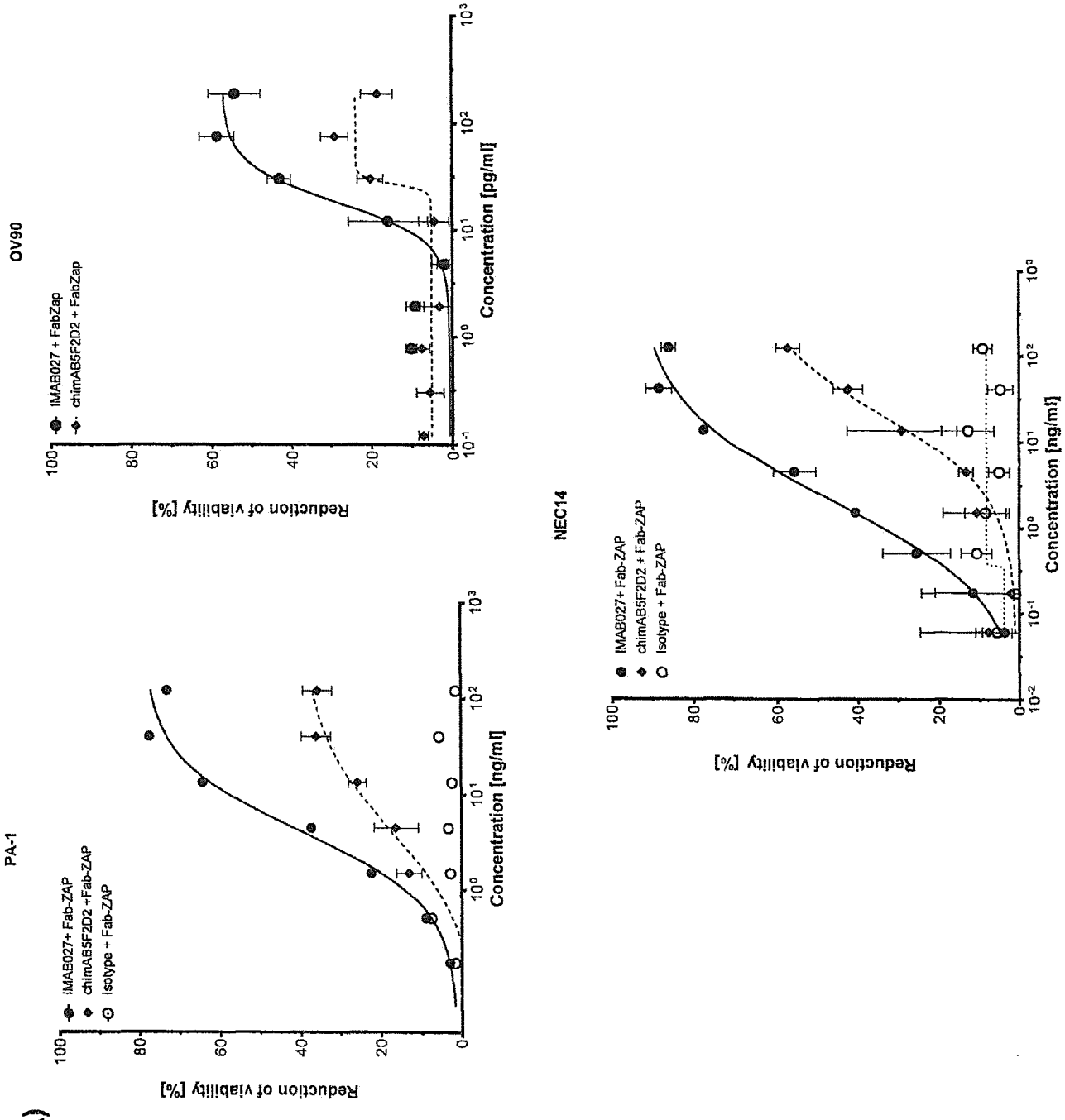

PA-1
IMAB027+ Fab-ZAP
chimAB5F2D2 +Fab-ZAP
Isotype + Fab-ZAP
Reduction of viability [%]
Concentration [ng/ml]
OV90
IMAB027 + FabZap
chimAB5F2D2 + FabZap
Reduction of viability [%]
Concentration [pg/ml]
NEC14
IMAB027+ Fab-ZAP
chimAB5F2D2 + Fab-ZAP
Isotype + Fab-ZAP
Reduction of viability [%]
Concentration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