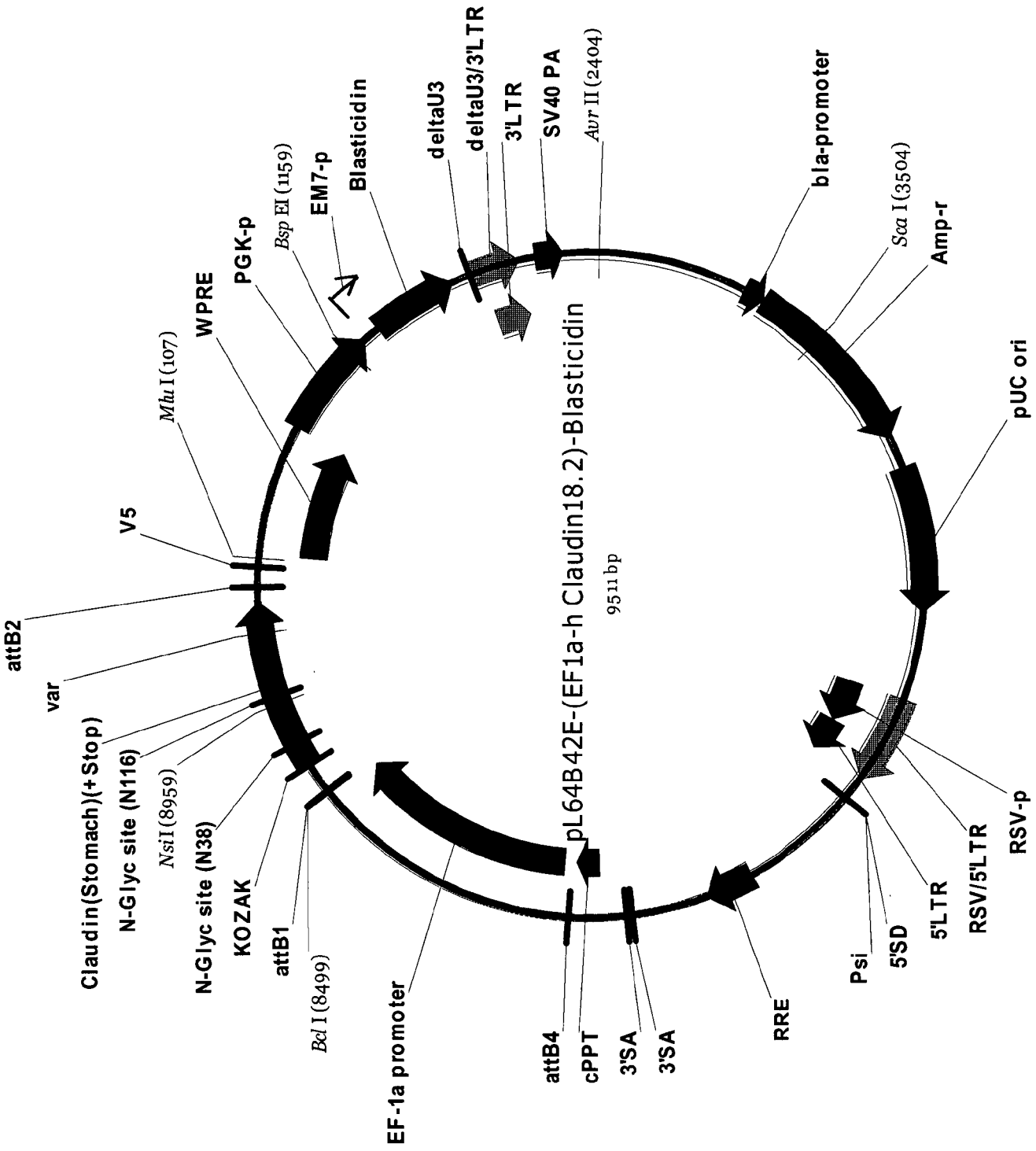

pL64B42E-(EF1a-h Claudin18.2)-Blasticidin
9511 bp
attB2
var
Claudin(Stomach)(+Stop)
N-Glyc site (N116)
NsiI(8959)
N-Glyc site (N38)
KOZAK
attB1
BclI(8499)
EF-1a promoter
attB4
cPPT
3'SA
3'SA
RRE
Psi
5'SD
5'LTR
RSV/5'LTR
RSV-p
pUC ori
Amp-r
ScaI(3504)
bla-promoter
AvrII(2404)
SV40 PA
3'LTR
deltaU3/3'LTR
deltaU3
Blasticidin
EM7-p
BspEI(1159)
PGK-p
WPRE
MluI(107)
V5

Figure 1

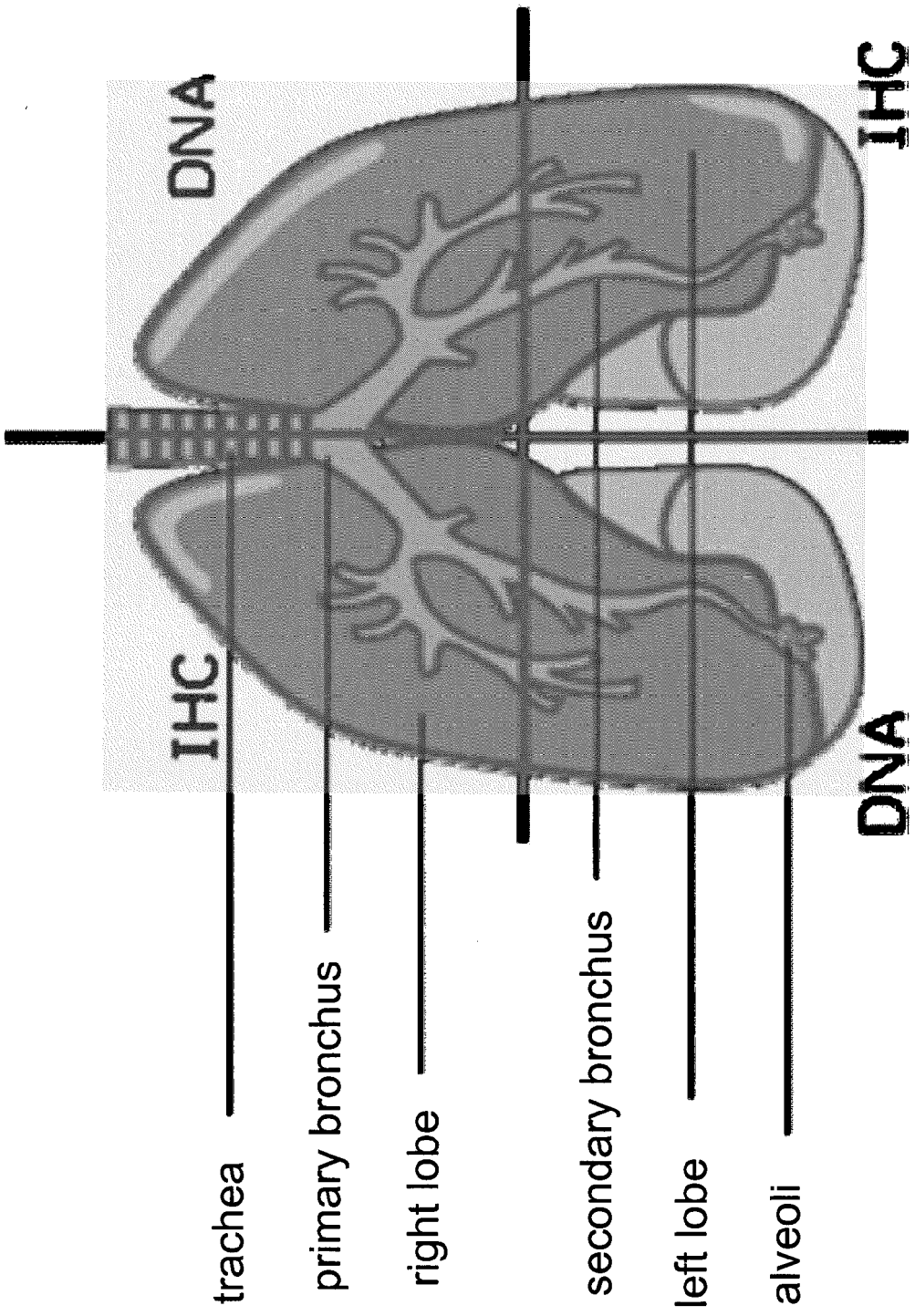

DNA
IHC
IHC
DNA
trachea
primary bronchus
right lobe
secondary bronchus
left lobe
alveoli

Figure 2

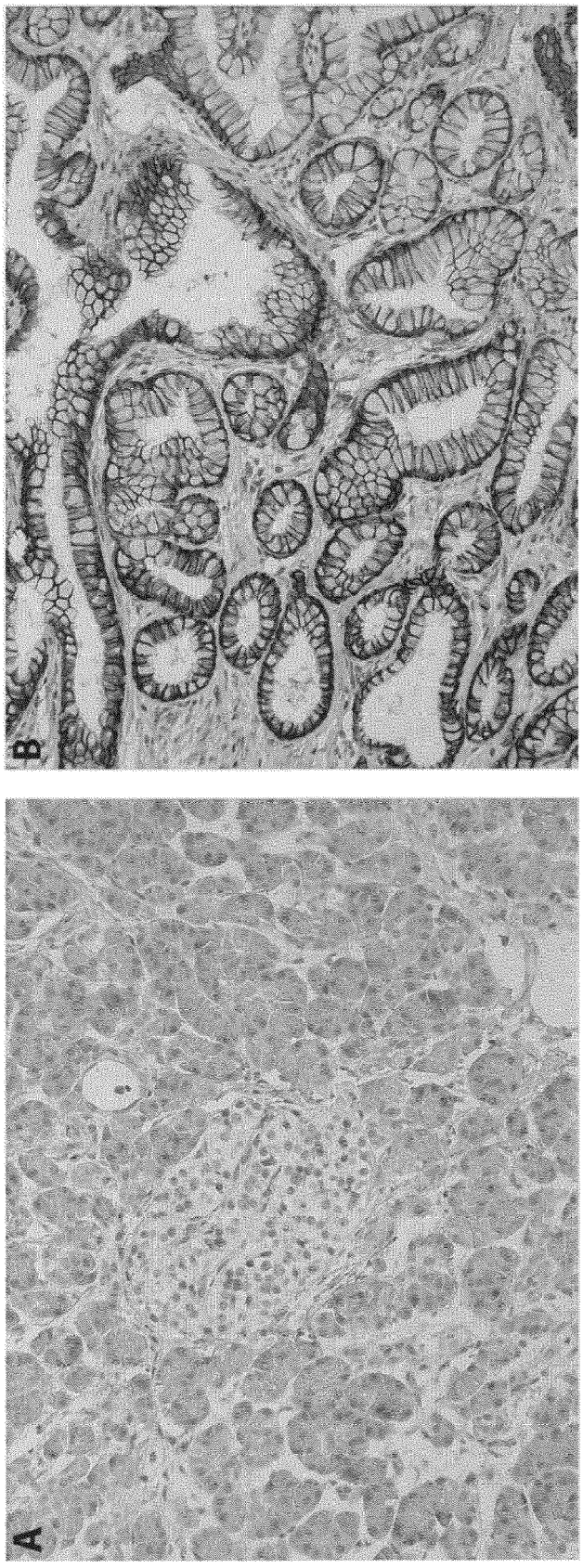


Figure 3

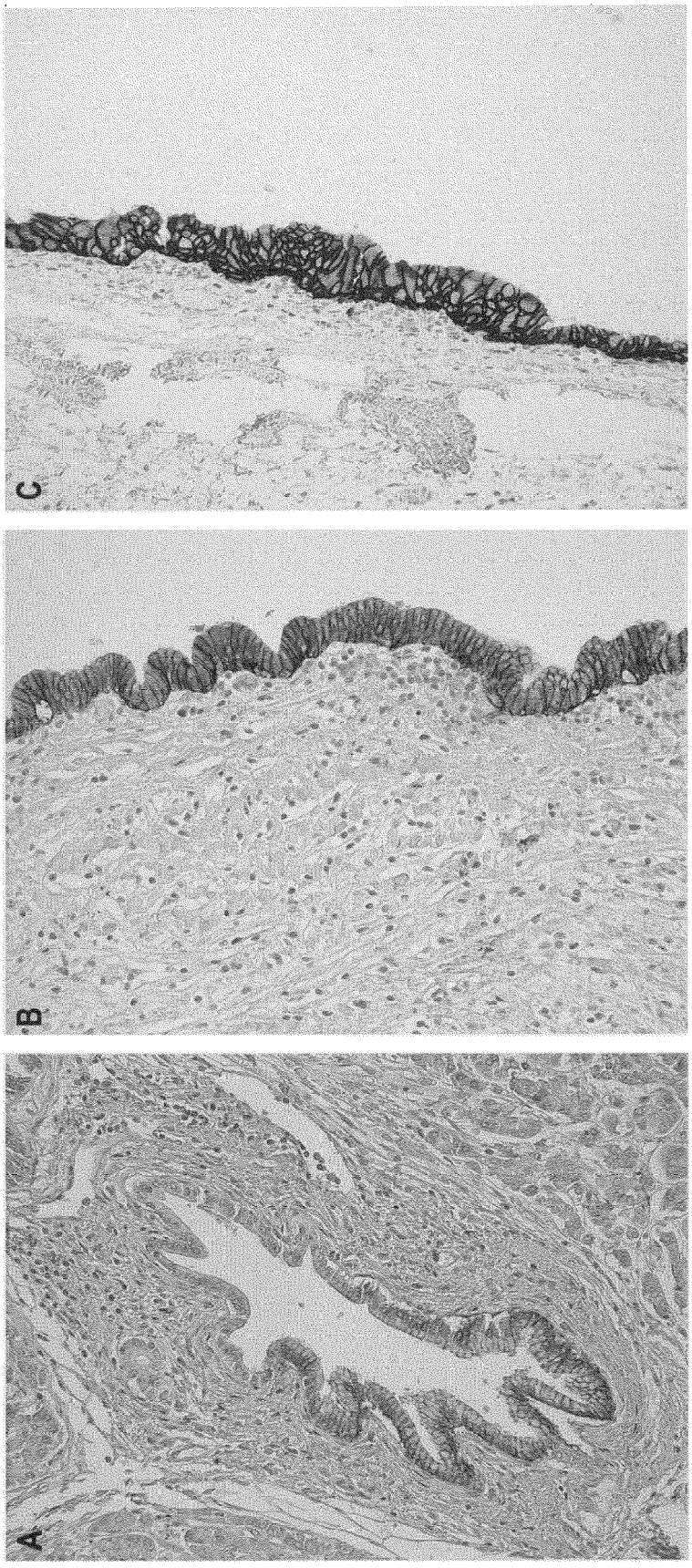


Figure 4

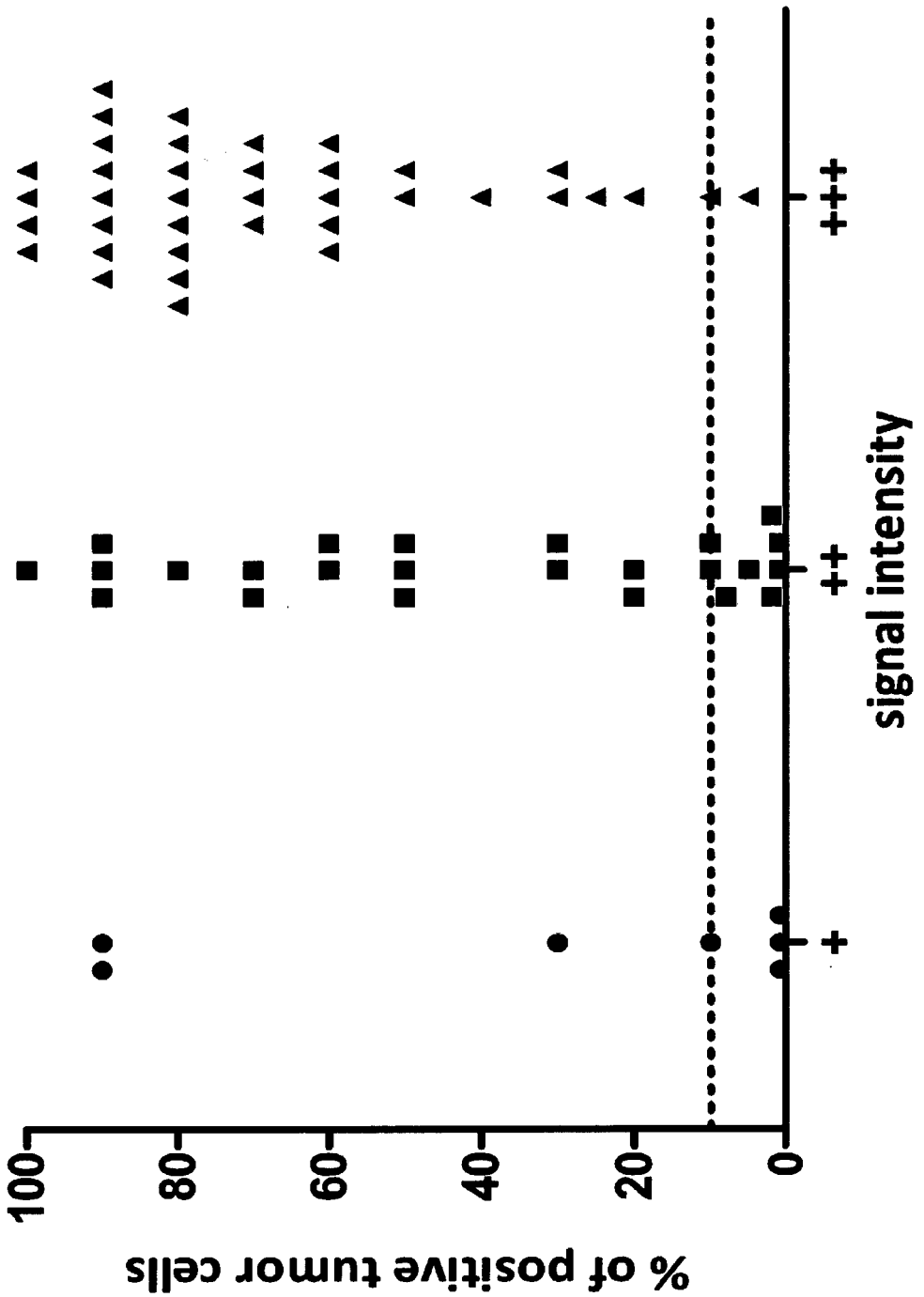


Figure 5

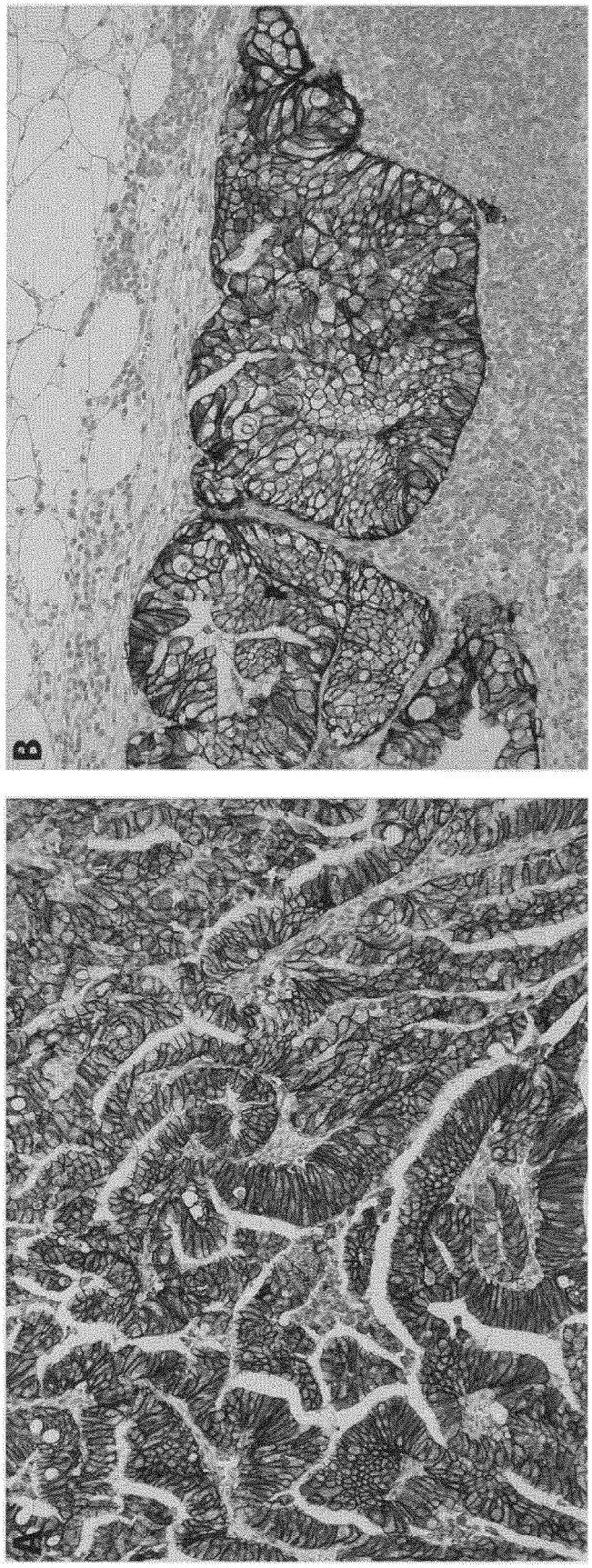


Figure 6

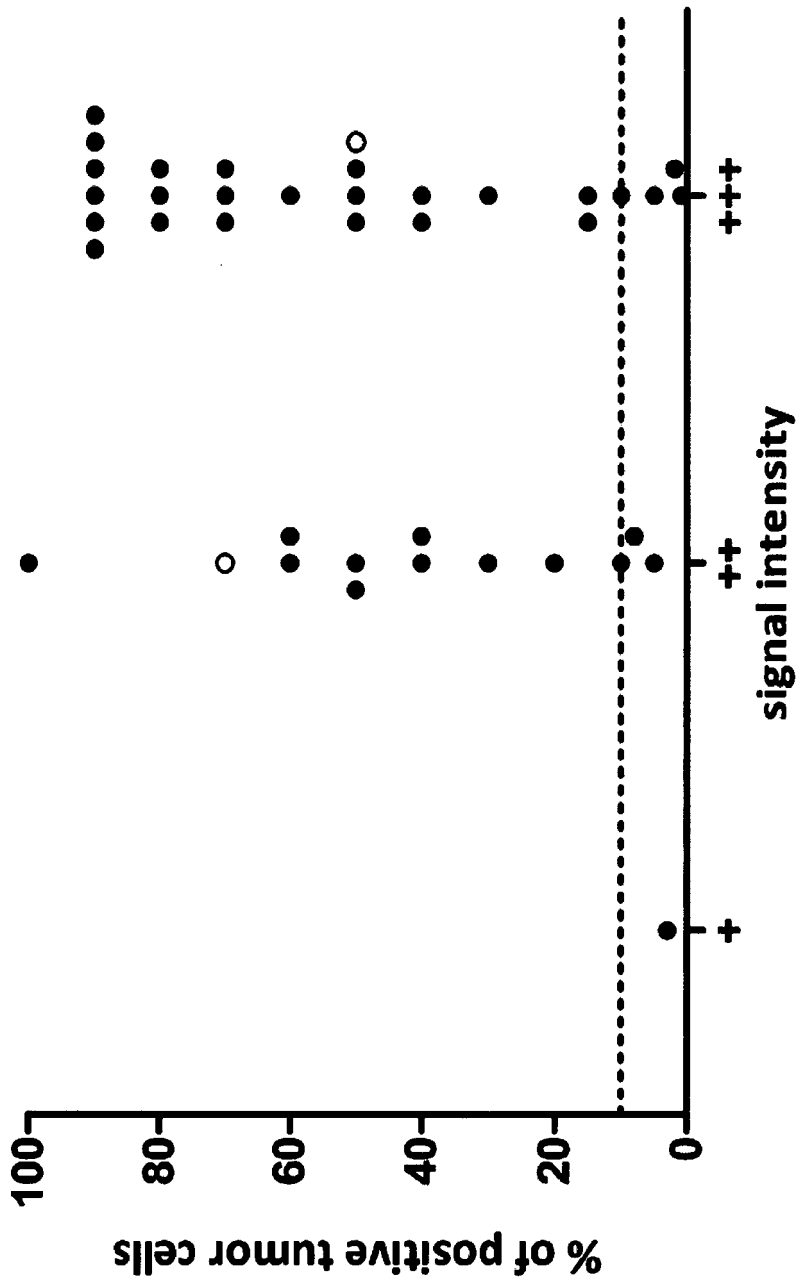


Figure 7

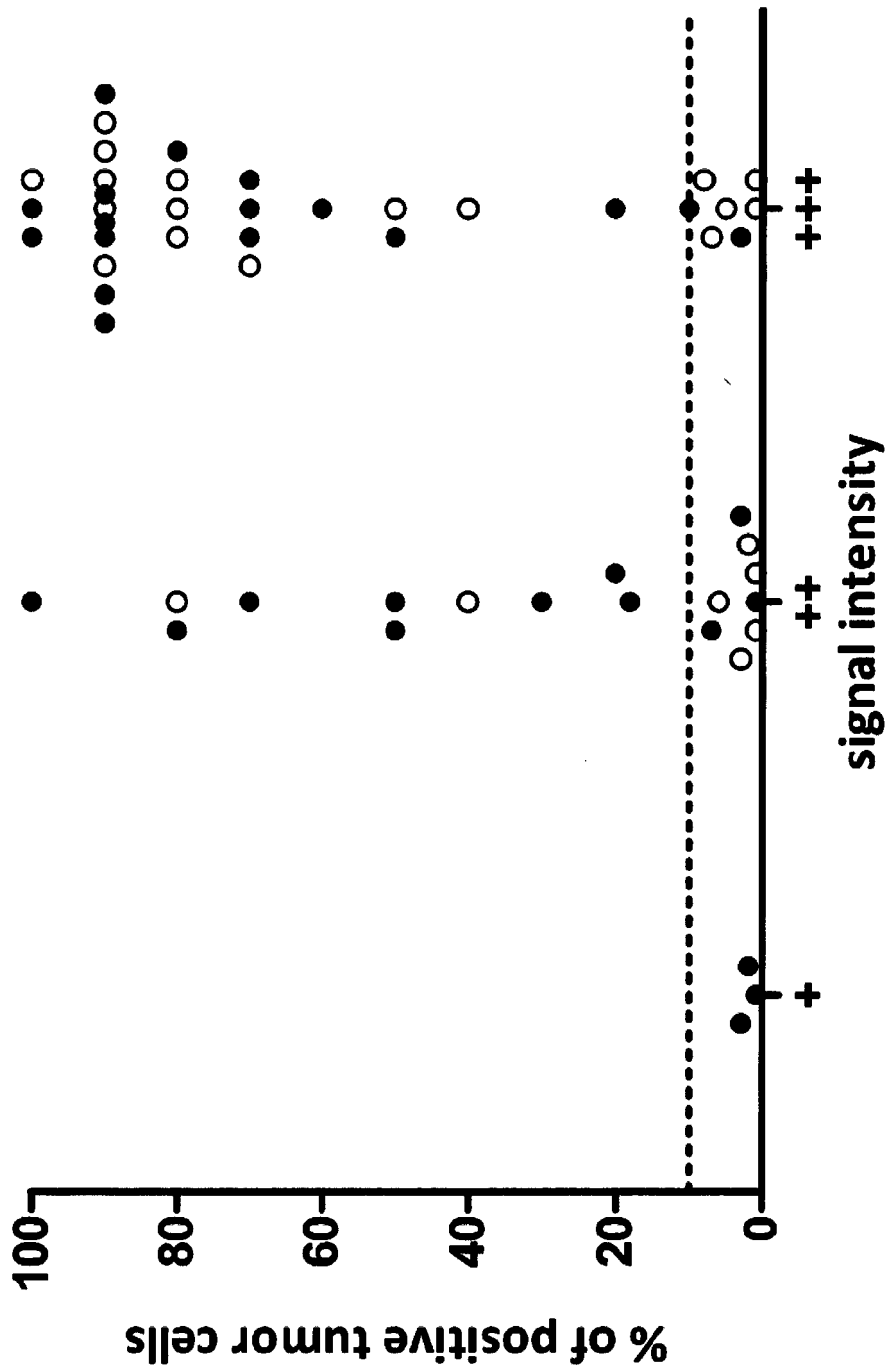


Figure 8

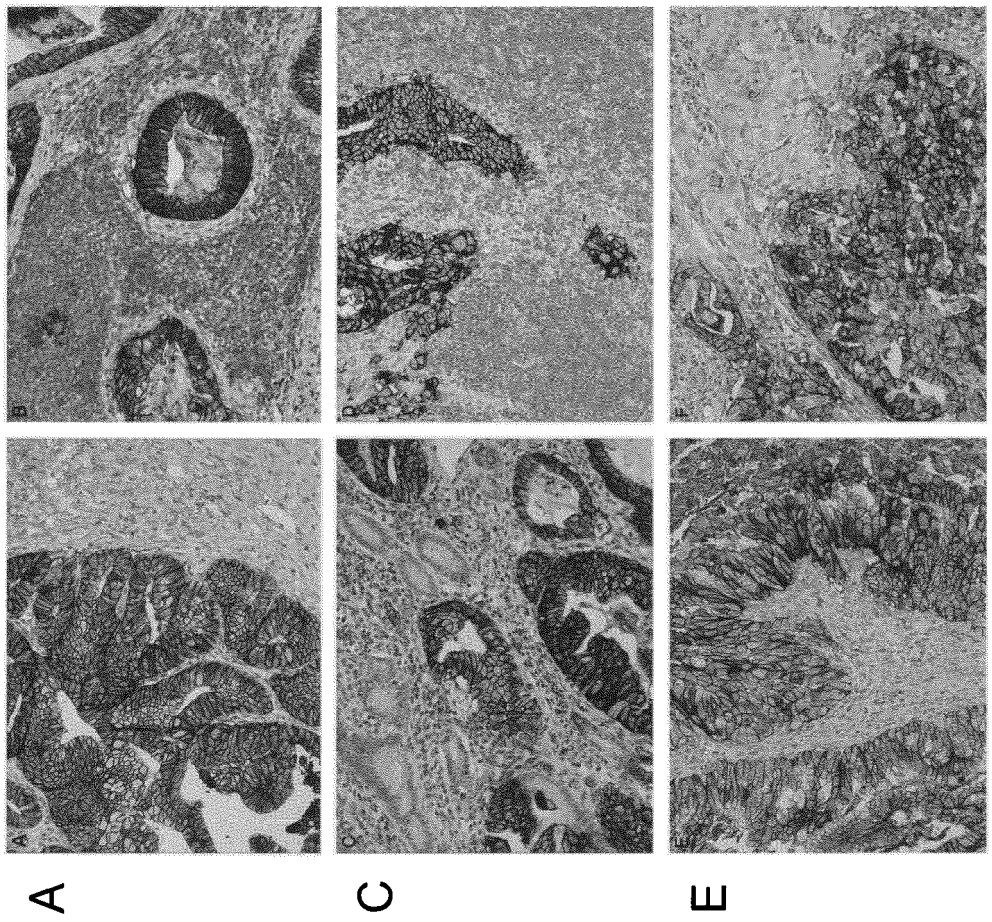


Figure 9

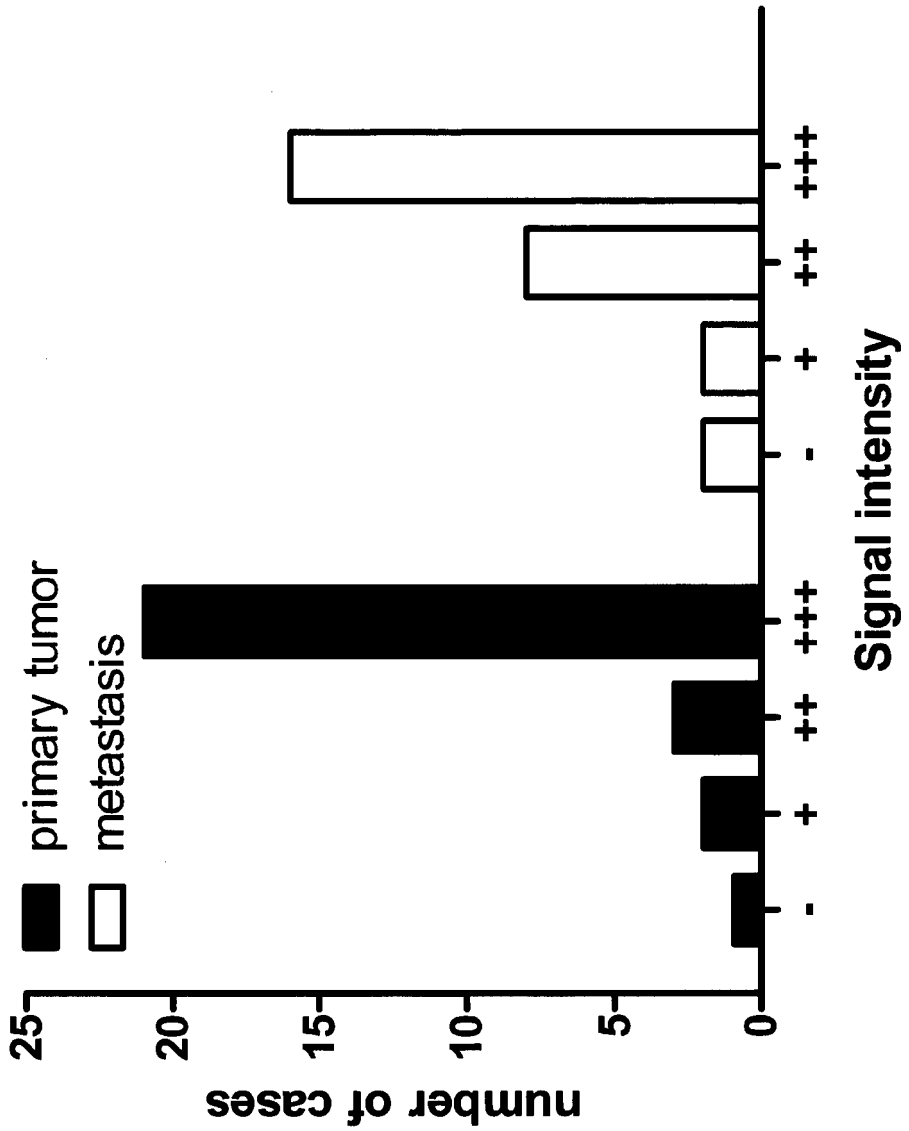

primary tumor
metastasis
number of cases
0
5
10
15
20
25
-
+
++
+++
-
+
++
+++
Signal intensity

Figure 10

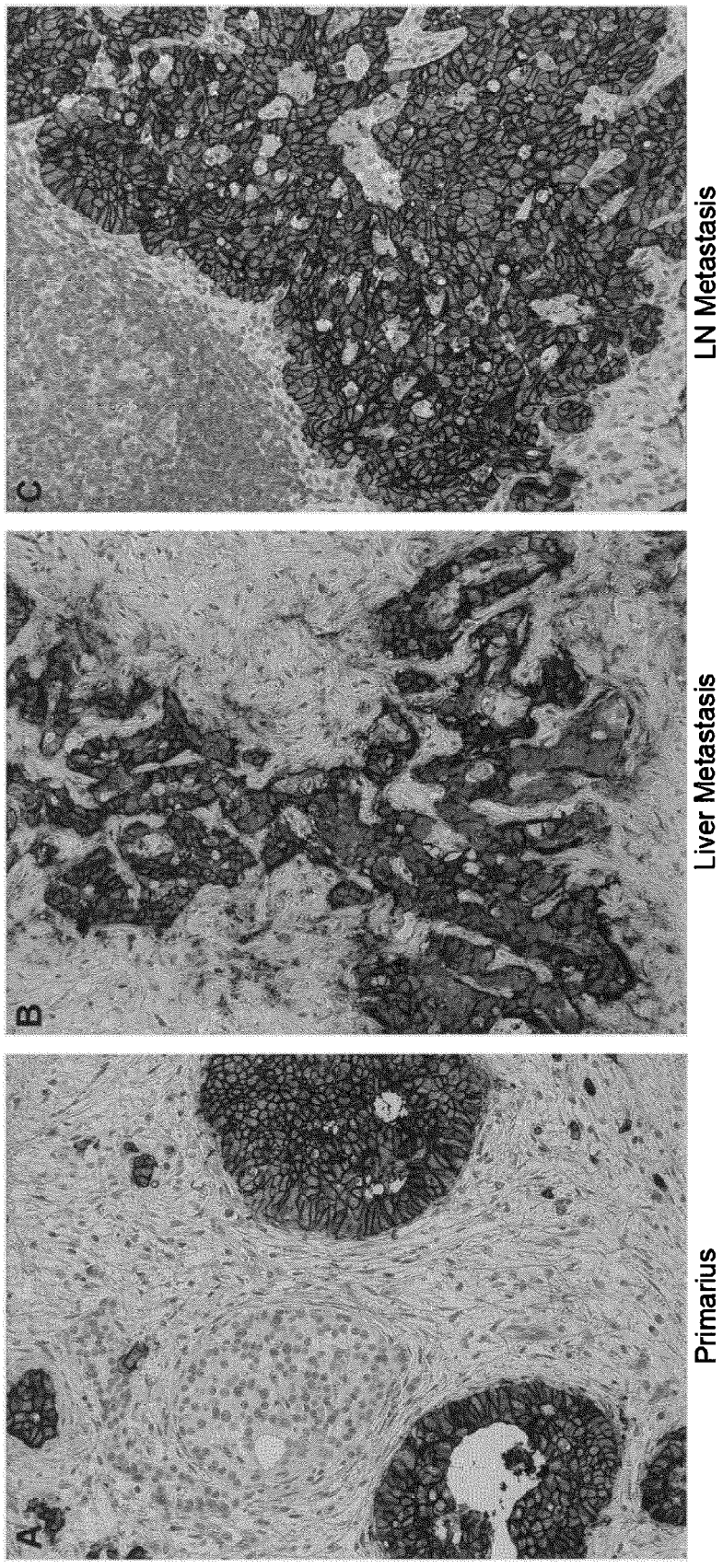


Figure 11

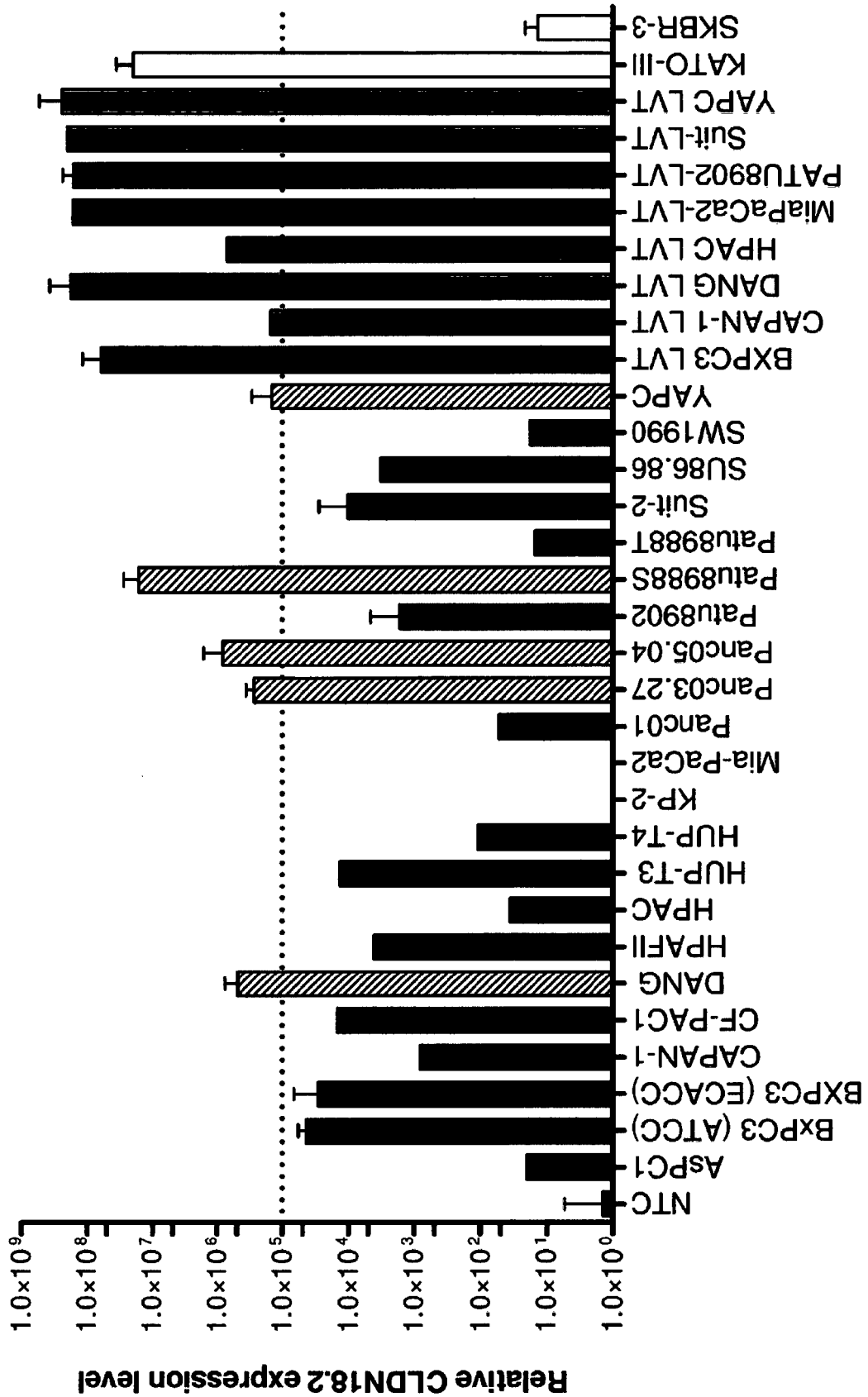

A
Relative CLDN18.2 expression level
1.0×10⁹
1.0×10⁸
1.0×10⁷
1.0×10⁶
1.0×10⁵
1.0×10⁴
1.0×10³
1.0×10²
1.0×10¹
1.0×10⁰
NTC
AsPC1
BxPC3 (ATCC)
BXPC3 (ECACC)
CAPAN-1
CF-PAC1
DANG
HPAFII
HPAC
HUP-T3
HUP-T4
KP-2
Mia-PaCa2
Panc01
Panc03.27
Panc05.04
Patu8902
Patu8988S
Patu8988T
Suit-2
SU86.86
SW1990
YAPC
BXPC3 LVT
CAPAN-1 LVT
DANG LVT
HPAC LVT
MiaPaCa2-LVT
PATU8902-LVT
Suit-LVT
YAPC LVT
KATO-III
SKBR-3

Figure 12

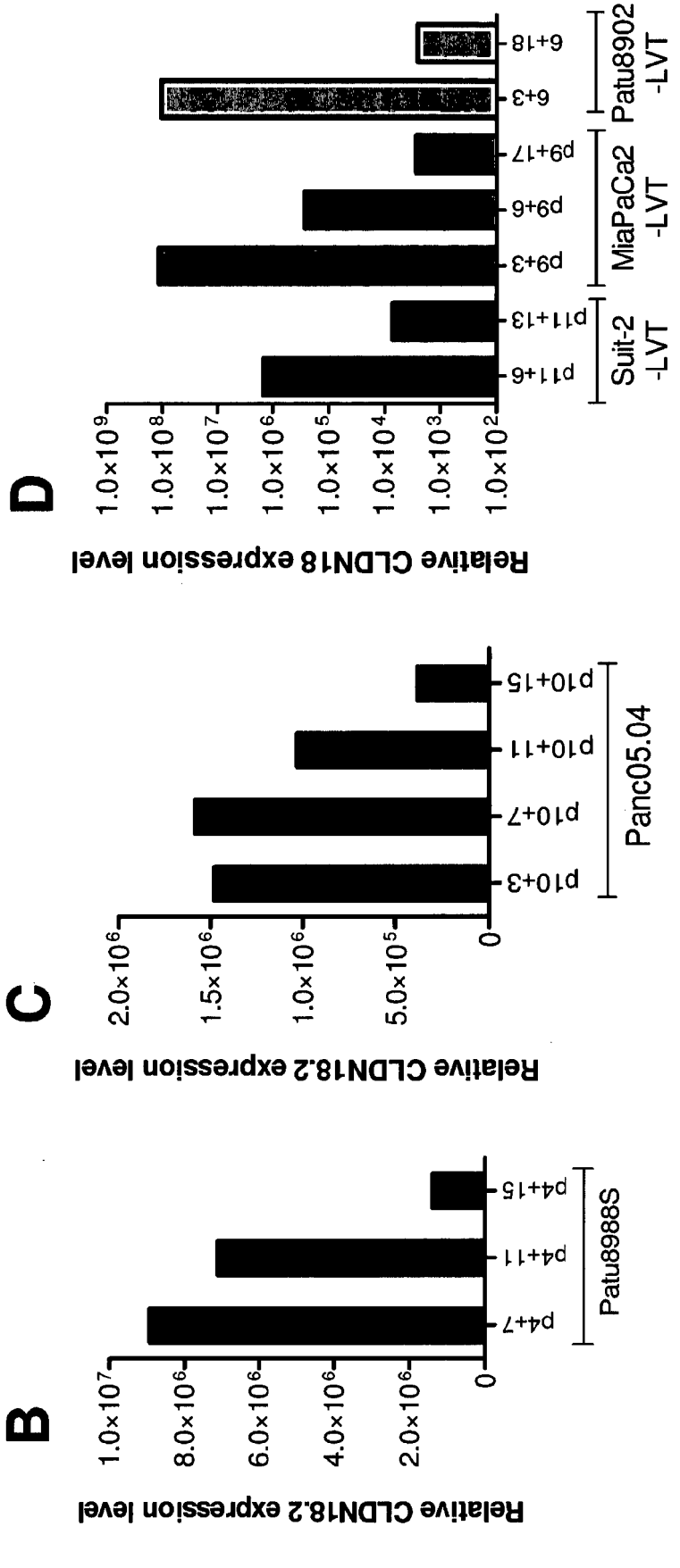

B
Relative CLDN18.2 expression level
1.0×10^7
8.0×10^6
6.0×10^6
4.0×10^6
2.0×10^6
0
p4+7
p4+11
p4+15
Patu8988S
C
Relative CLDN18.2 expression level
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0
p10+3
p10+7
p10+11
p10+15
Panc05.04
D
Relative CLDN18 expression level
1.0×10^9
1.0×10^8
1.0×10^7
1.0×10^6
1.0×10^5
1.0×10^4
1.0×10^3
1.0×10^2
p11+6
p11+13
Suit-2 -LVT
p9+3
p9+6
p9+17
MiaPaCa2 -LVT
6+3
6+18
Patu8902 -LVT

Figure 12

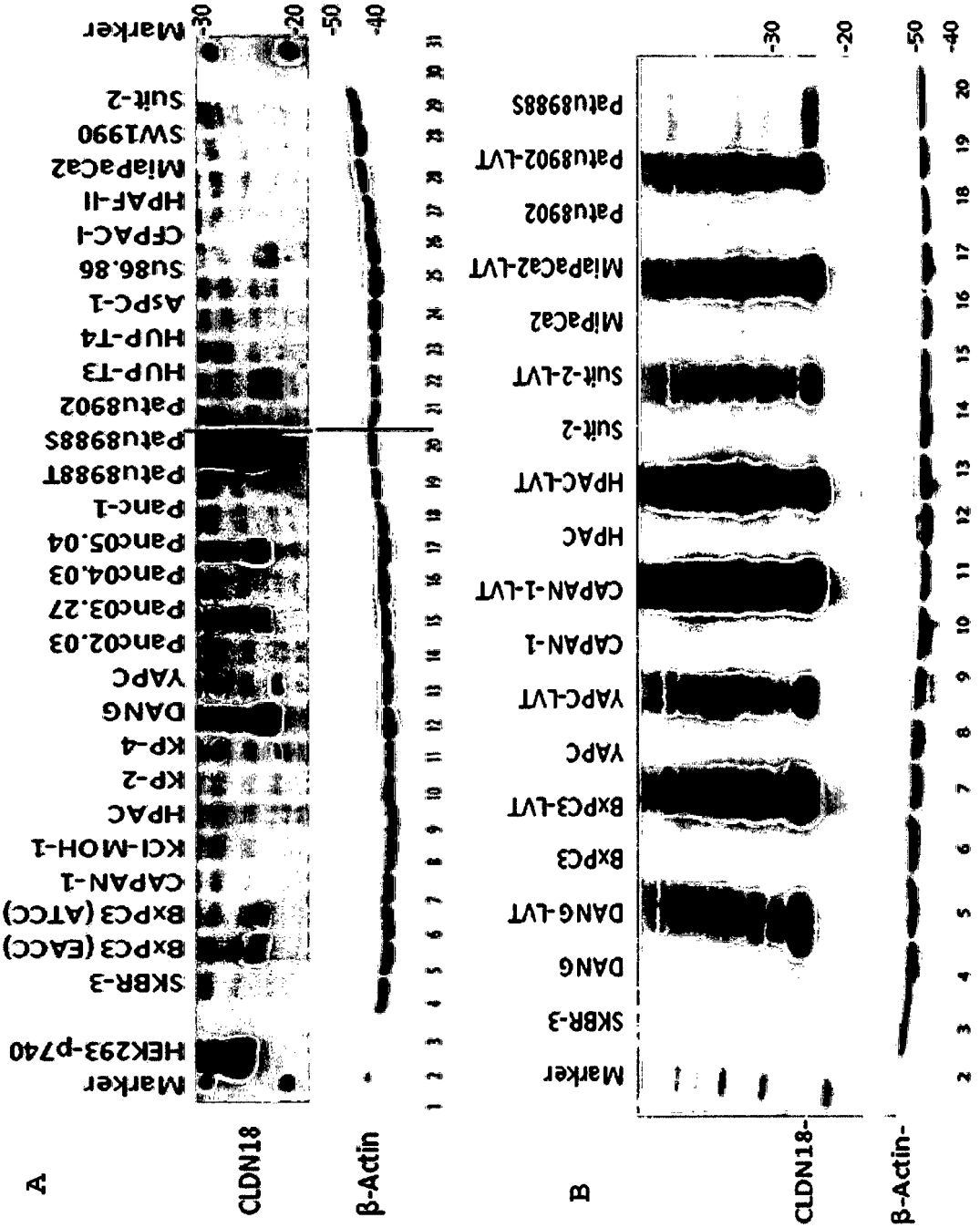

A
CLDN18
β-Actin
Marker
HEK293-p740
SKBR-3
BxPC3 (EACC)
BxPC3 (ATCC)
CAPAN-1
KCI-MOH-1
HPAC
KP-2
KP-4
DANG
YAPC
PancO2.03
PancO3.27
PancO4.03
PancO5.04
Panc-1
Patu8988T
Patu8988S
Patu8902
HUP-T3
HUP-T4
AsPC-1
Su86.86
CFPAC-I
HPAF-II
MiaPaCa2
SW1990
Suit-2
B
CLDN18-
β-Actin-
Marker
SKBR-3
DANG
DANG-LVT
BxPC3
BxPC3-LVT
YAPC
YAPC-LVT
CAPAN-1
CAPAN-1-LVT
HPAC
HPAC-LVT
Suit-2
Suit-2-LVT
MiPaCa2
MiaPaCa2-LVT
Patu8902
Patu8902-LVT
Patu8988S

Figure 13

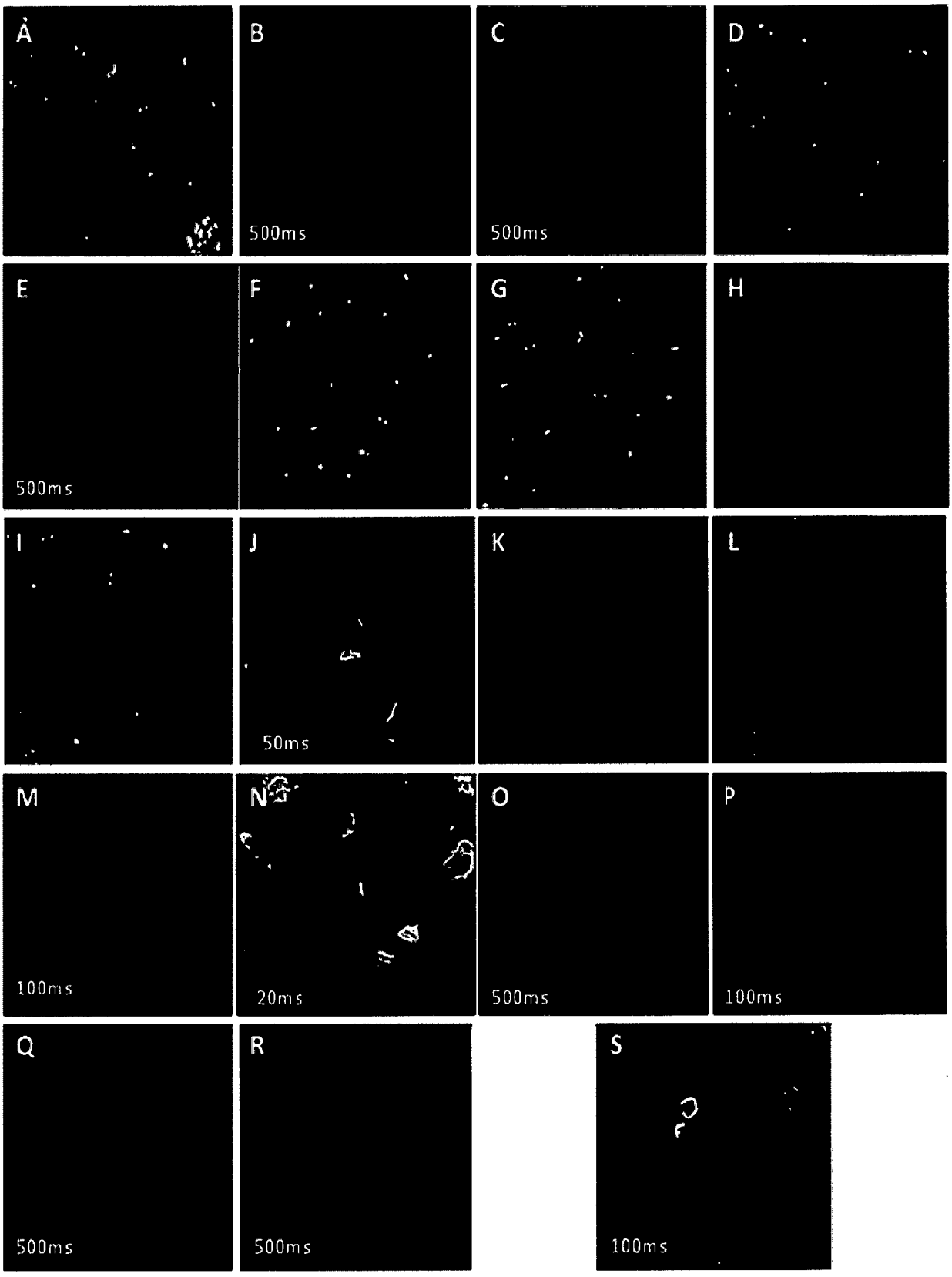

A
B
500ms
C
500ms
D
E
500ms
F
G
H
I
J
50ms
K
L
M
100ms
N
20ms
O
500ms
P
100ms
Q
500ms
R
500ms
S
100ms

Figure 14

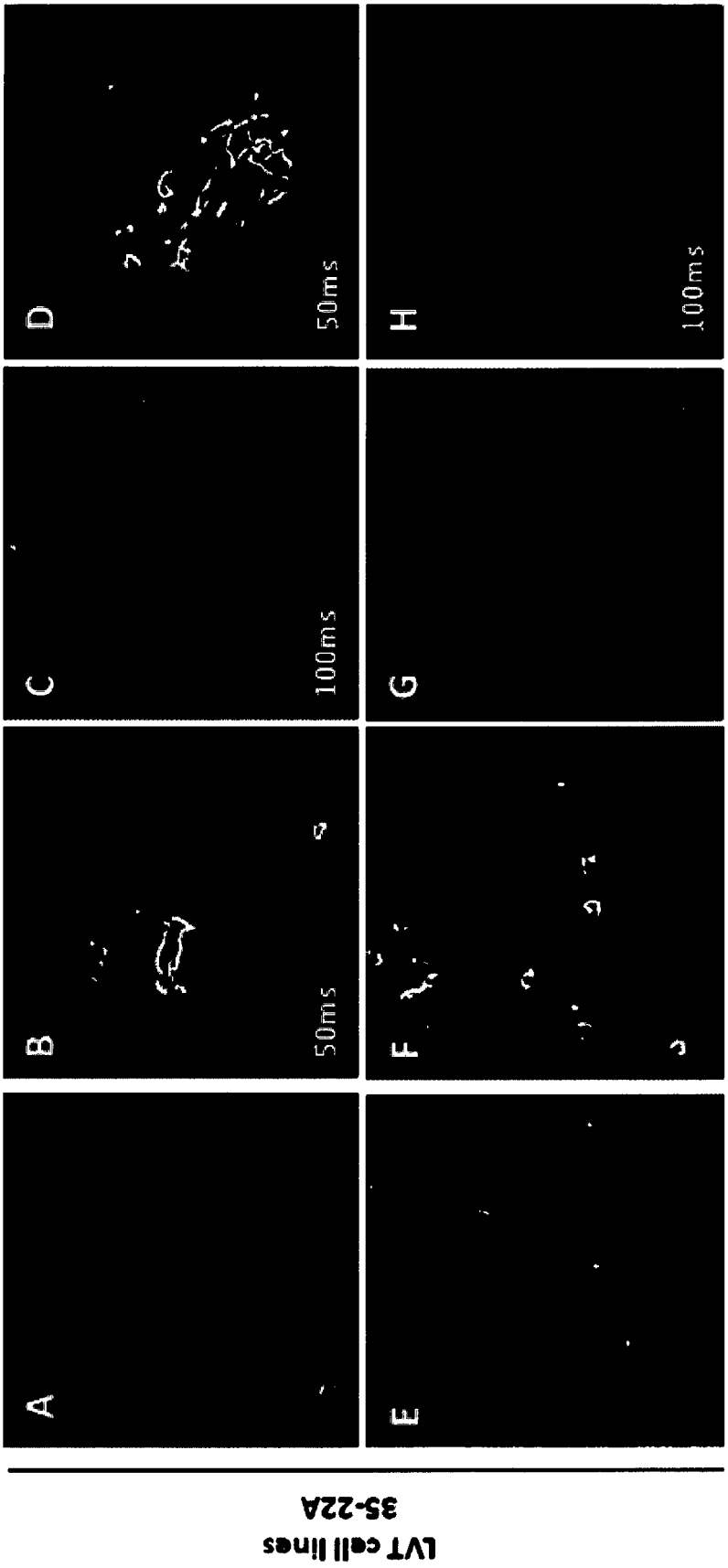


Figure 15

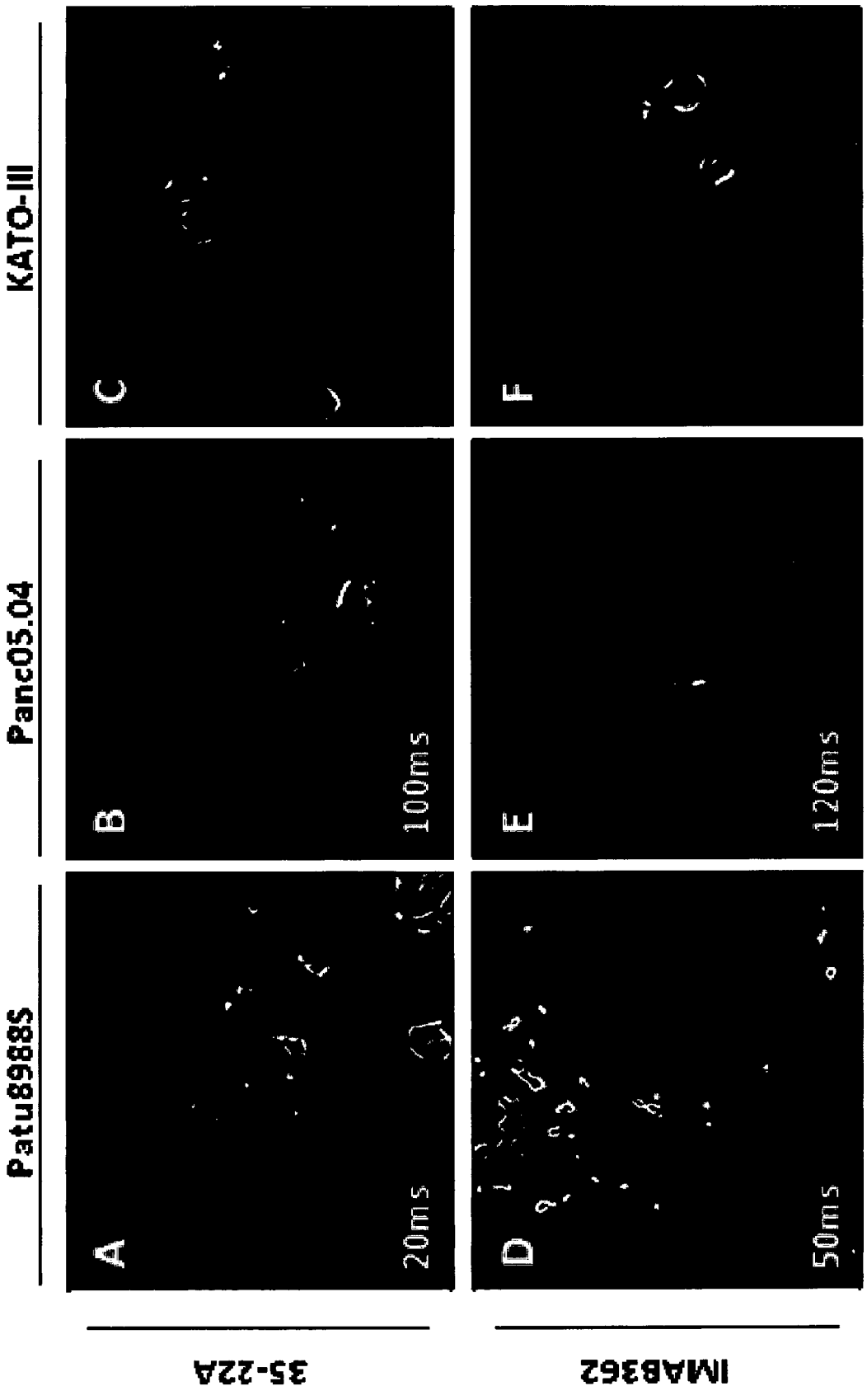

35-22A
IMAB362
Patu8988S
Panc05.04
KATO-III
A
B
C
D
E
F
20ms
100ms
50ms
120ms

Figure 16

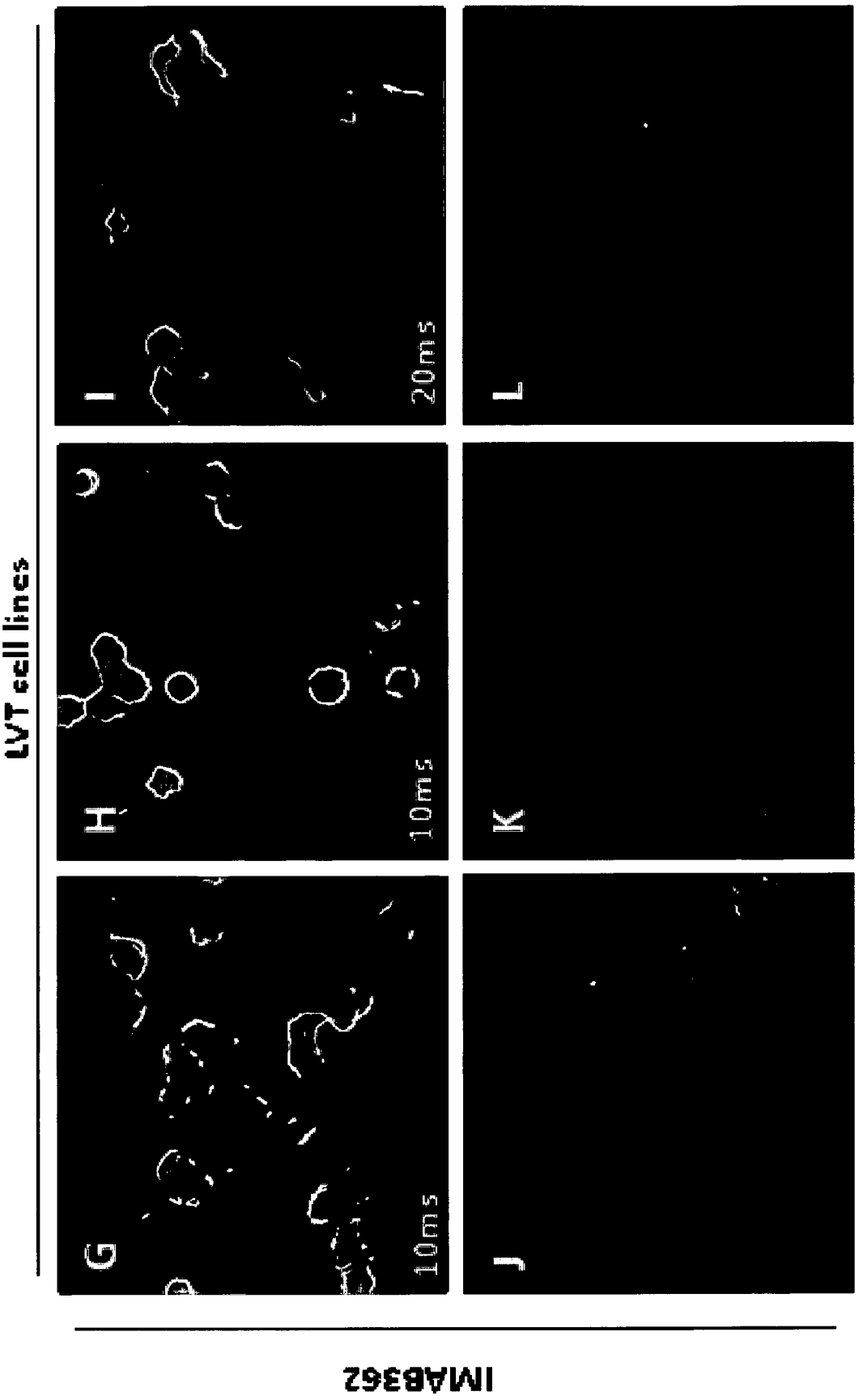


Figure 16

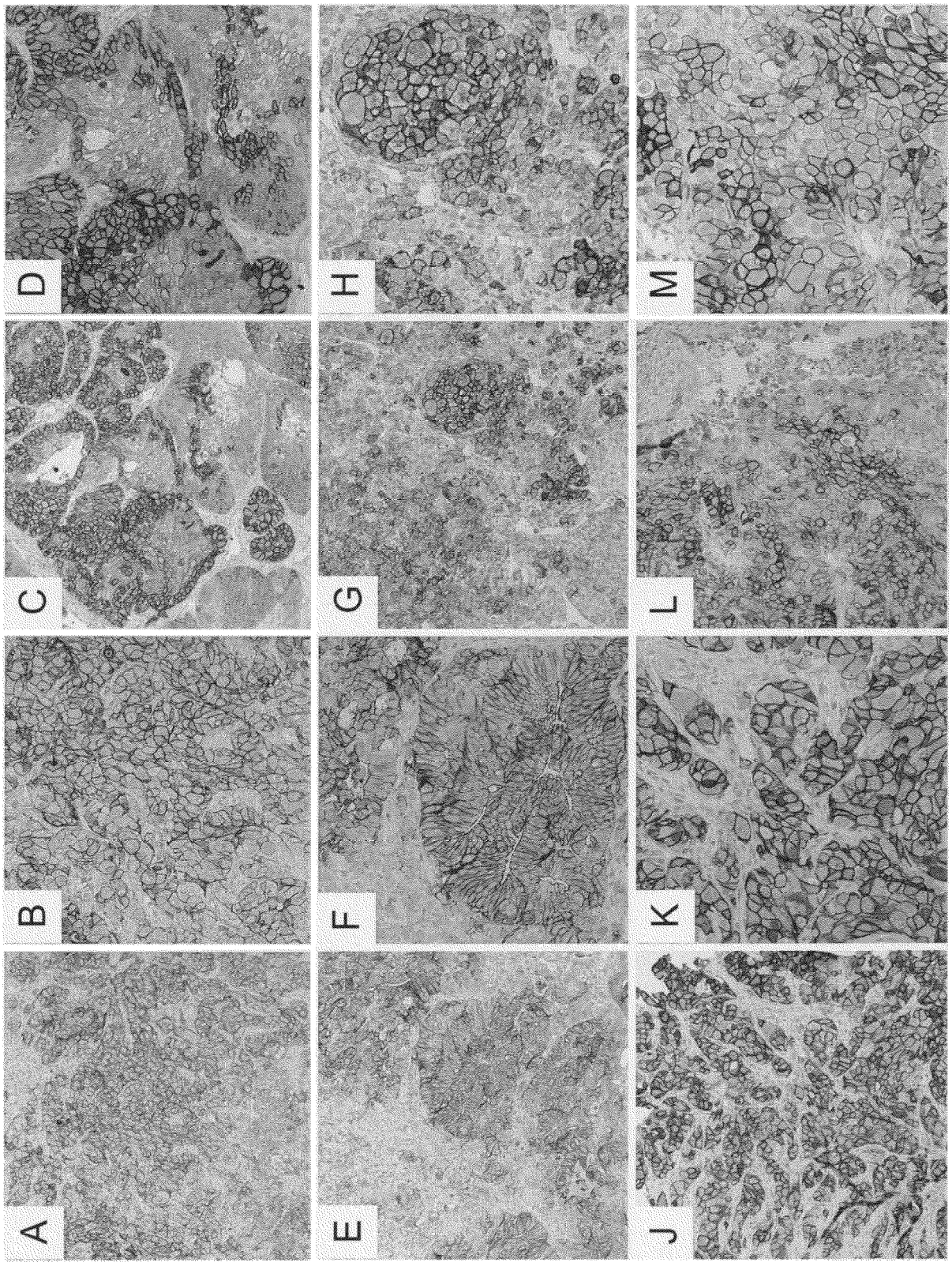


Figure 17

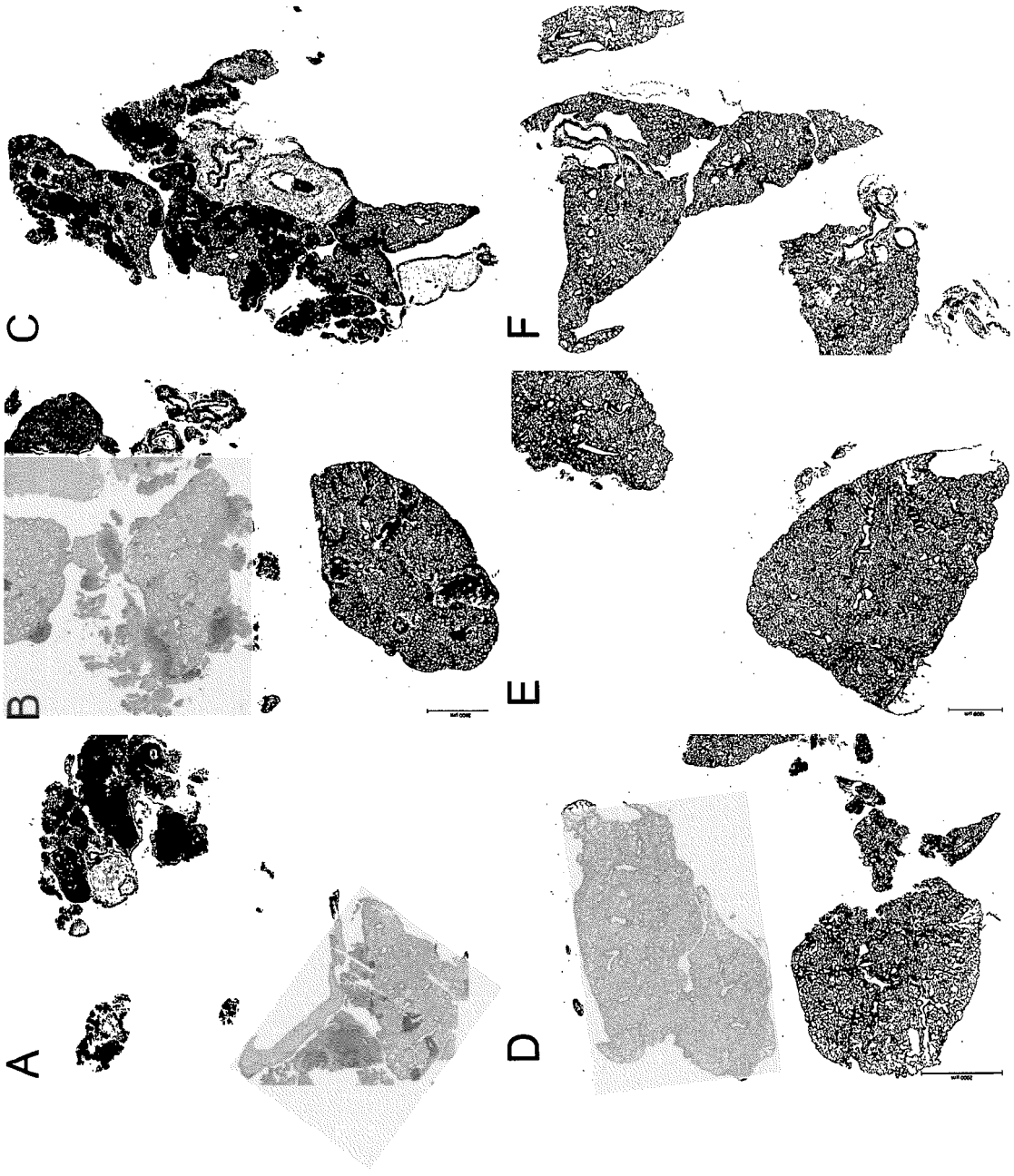
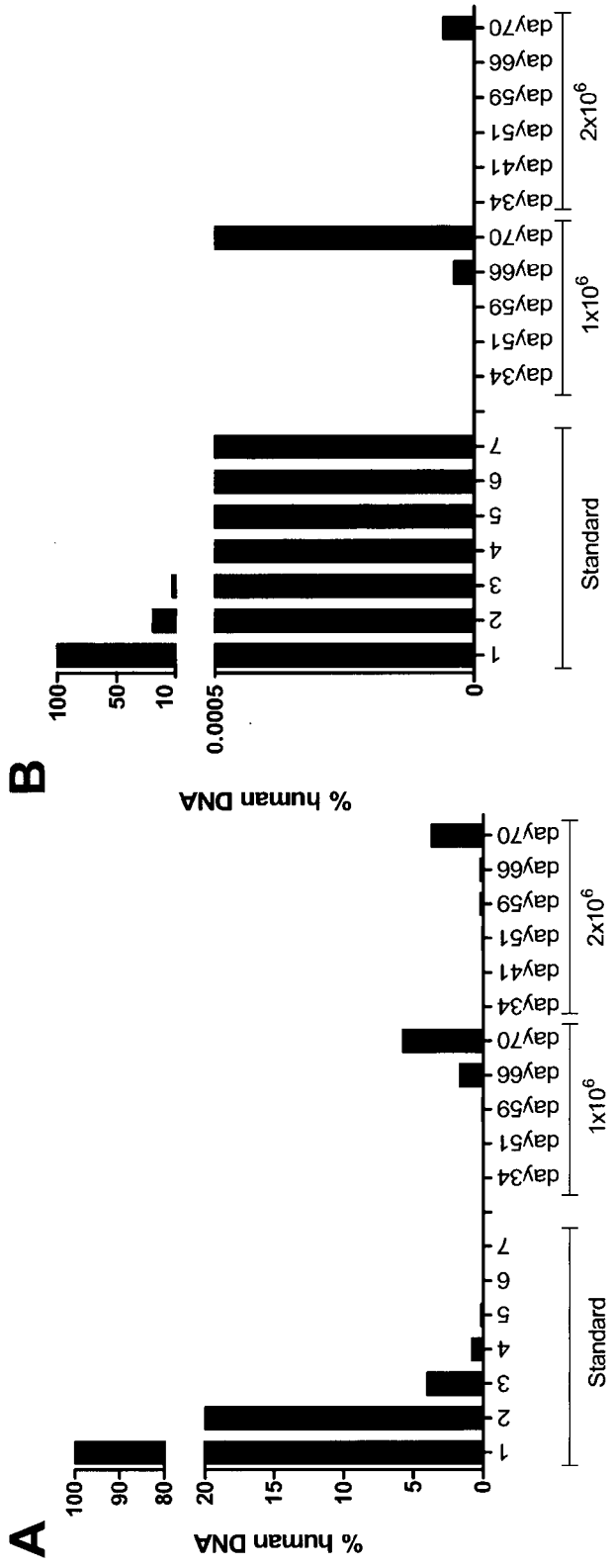


Figure 18

Figure 19


A
% human DNA
100
90
80
20
15
10
5
0
1
2
3
4
5
6
7
Standard
day34
day41
day51
day59
day66
day70
1x10^6
day34
day41
day51
day59
day66
day70
2x10^6
B
% human DNA
100
50
10
0.0005
0
1
2
3
4
5
6
7
Standard
day34
day41
day51
day59
day66
day70
1x10^6
day34
day41
day51
day59
day66
day70
2x10^6

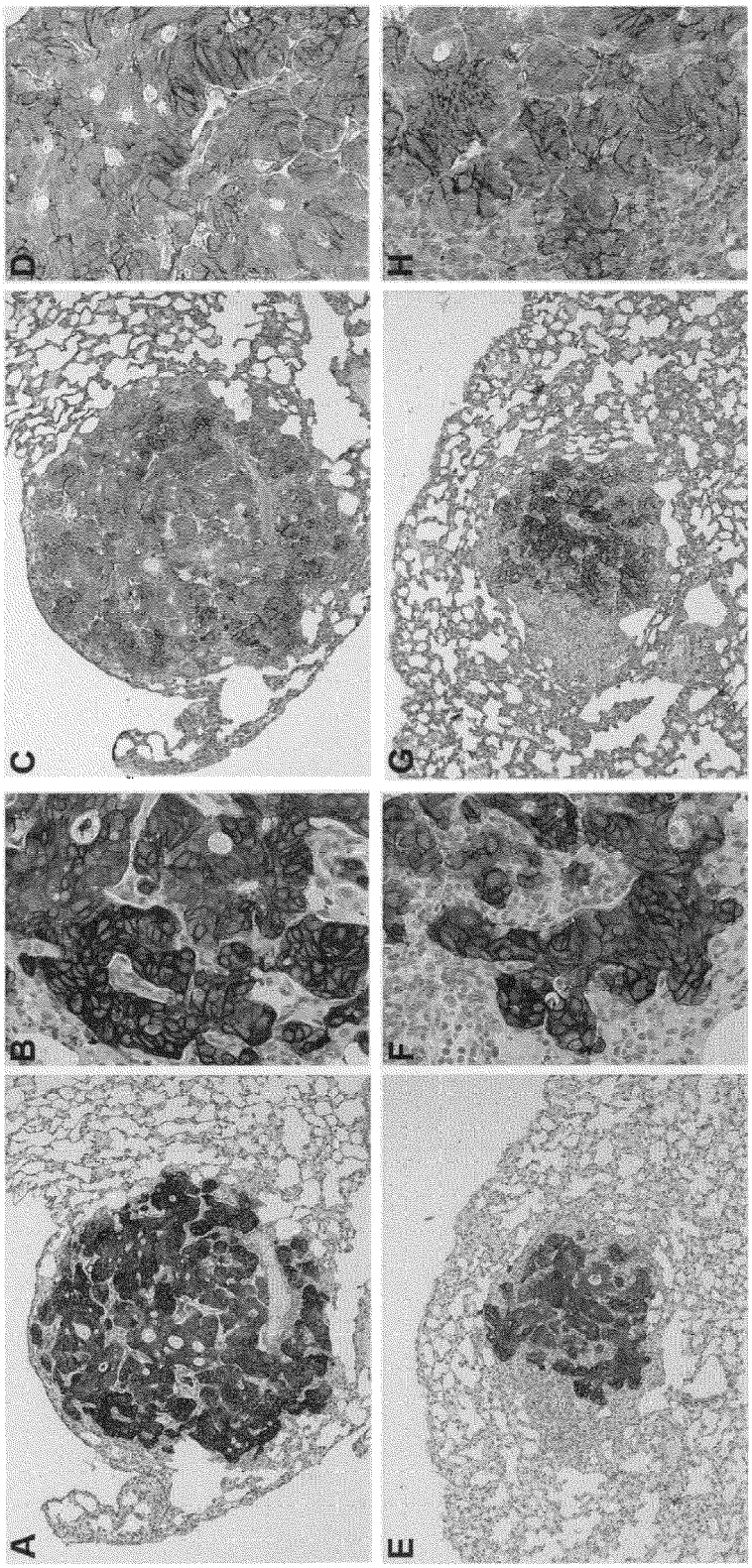


Figure 20

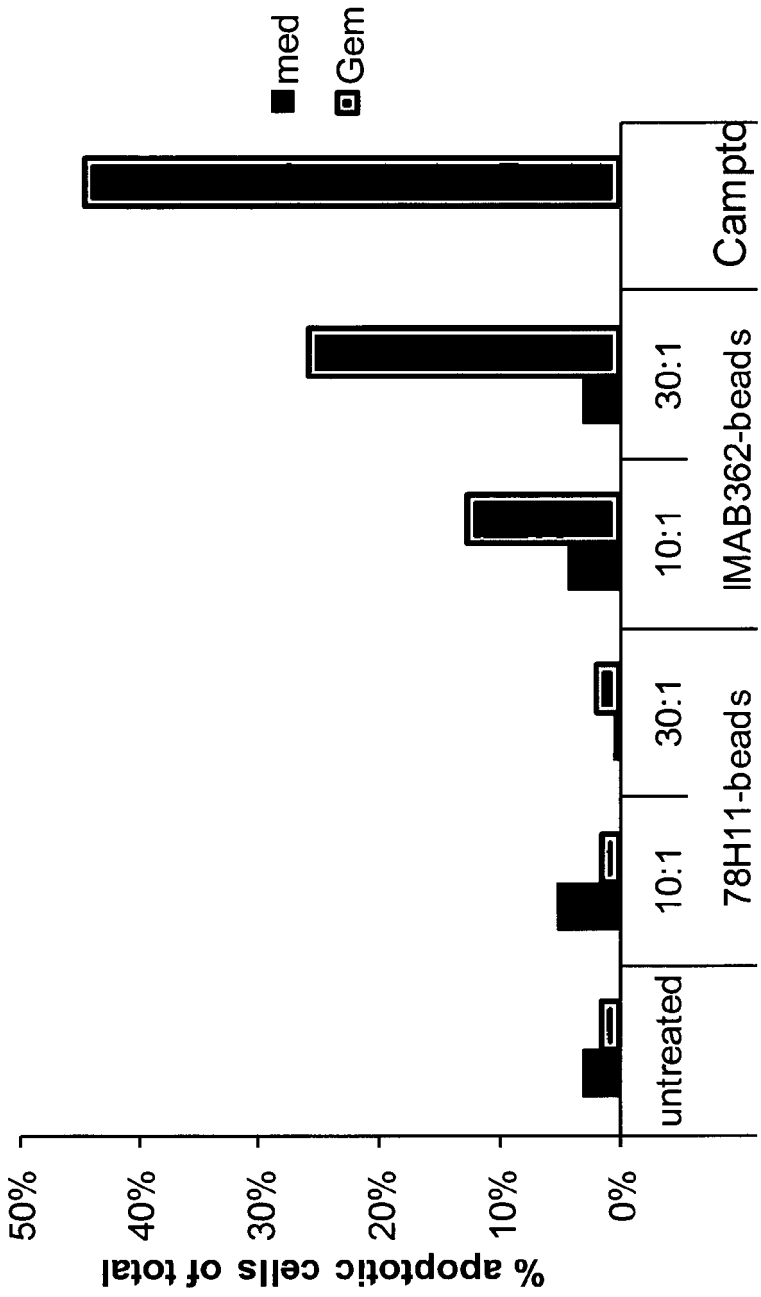
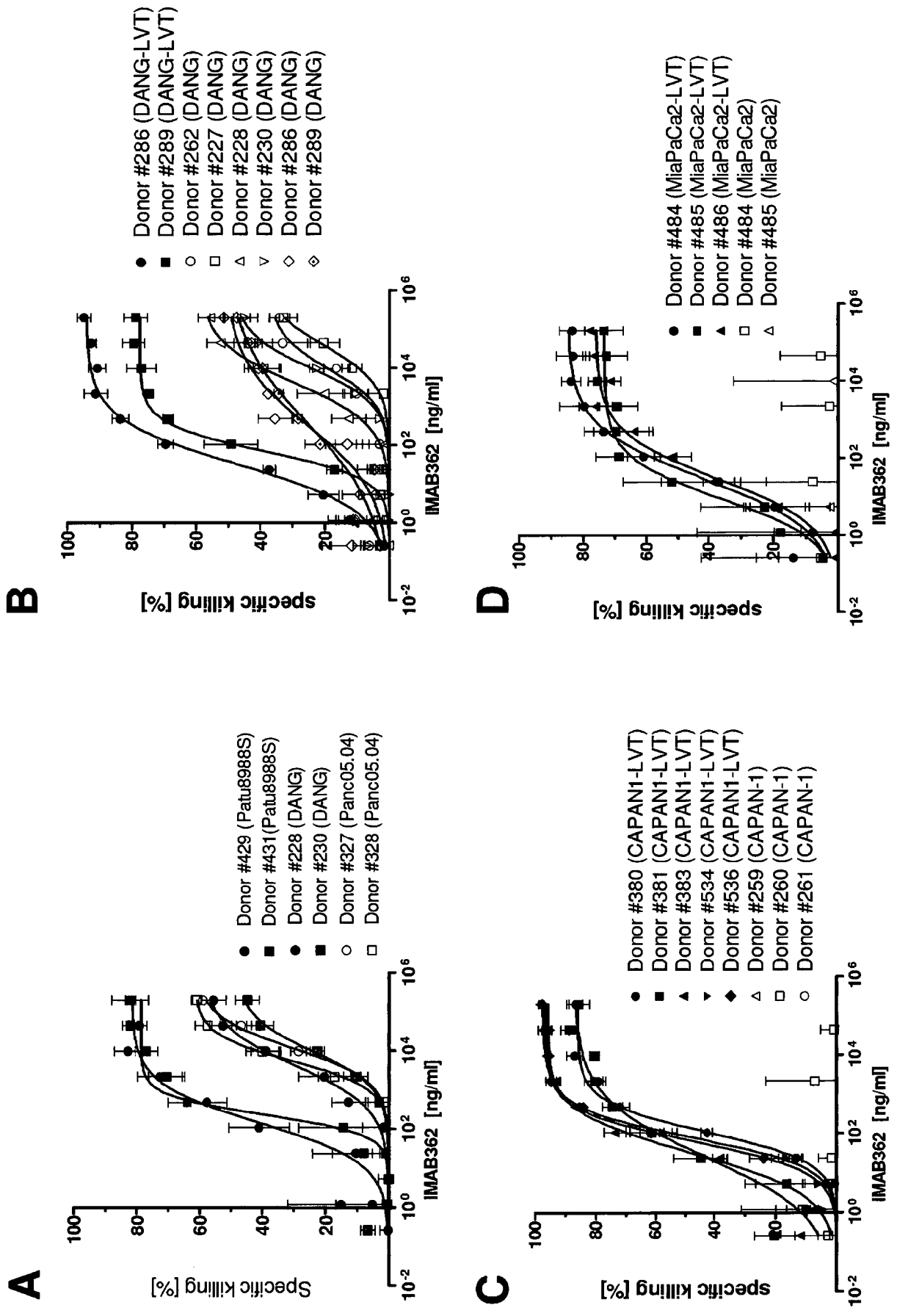

med
Gem
% apoptotic cells of total
50%
40%
30%
20%
10%
0%
untreated
10:1
30:1
78H11-beads
10:1
30:1
IMAB362-beads
Camptq

Figure 21

Figure 22


A
Specific killing [%]
IMAB362 [ng/ml]
Donor #429 (Patu8988S)
Donor #431(Patu8988S)
Donor #228 (DANG)
Donor #230 (DANG)
Donor #327 (Panc05.04)
Donor #328 (Panc05.04)
B
specific killing [%]
IMAB362 [ng/ml]
Donor #286 (DANG-LVT)
Donor #289 (DANG-LVT)
Donor #262 (DANG)
Donor #227 (DANG)
Donor #228 (DANG)
Donor #230 (DANG)
Donor #286 (DANG)
Donor #289 (DANG)
C
specific killing [%]
IMAB362 [ng/ml]
Donor #380 (CAPAN1-LVT)
Donor #381 (CAPAN1-LVT)
Donor #383 (CAPAN1-LVT)
Donor #534 (CAPAN1-LVT)
Donor #536 (CAPAN1-LVT)
Donor #259 (CAPAN-1)
Donor #260 (CAPAN-1)
Donor #261 (CAPAN-1)
D
specific killing [%]
IMAB362 [ng/ml]
Donor #484 (MiaPaCa2-LVT)
Donor #485 (MiaPaCa2-LVT)
Donor #486 (MiaPaCa2-LVT)
Donor #484 (MiaPaCa2)
Donor #485 (MiaPaCa2)

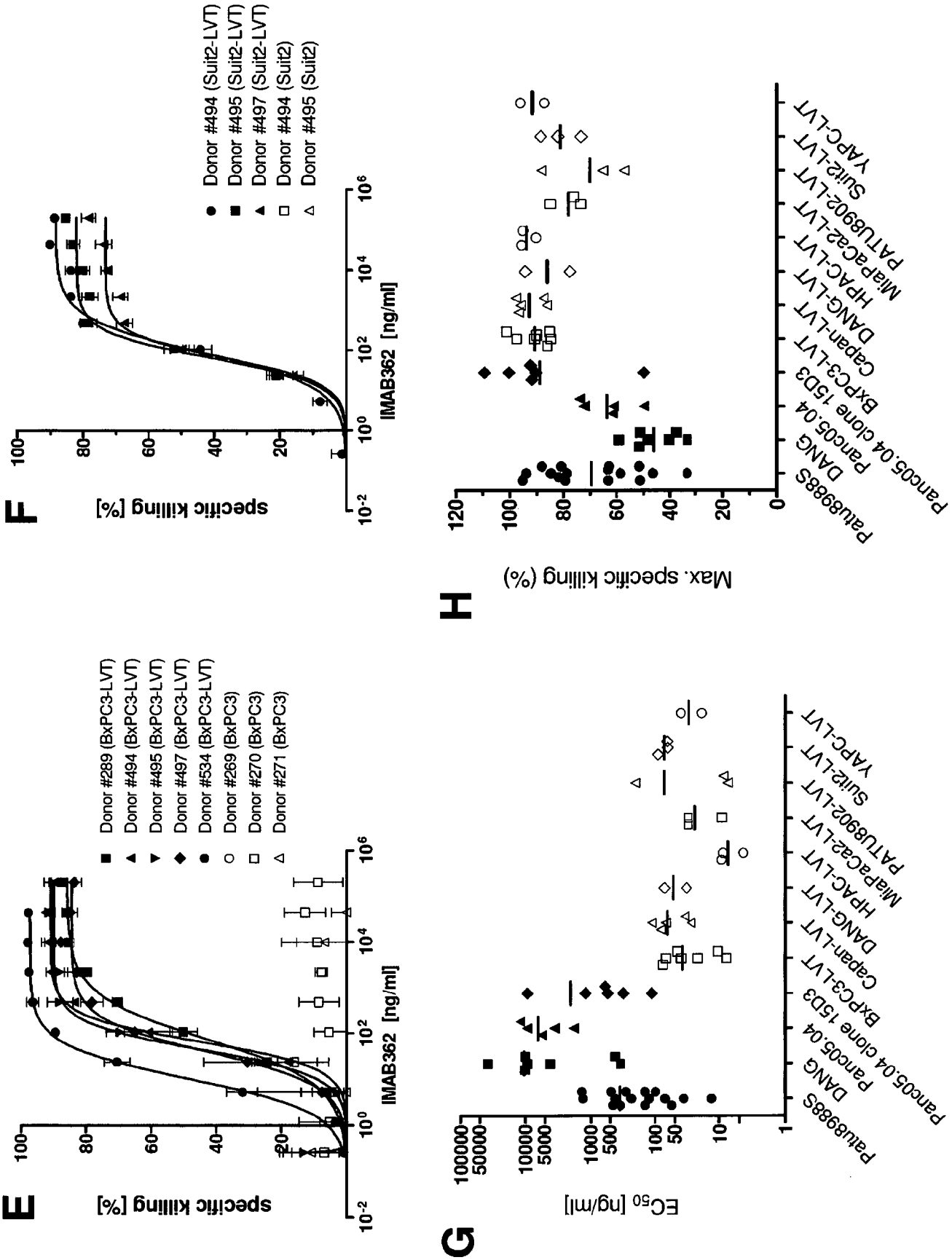
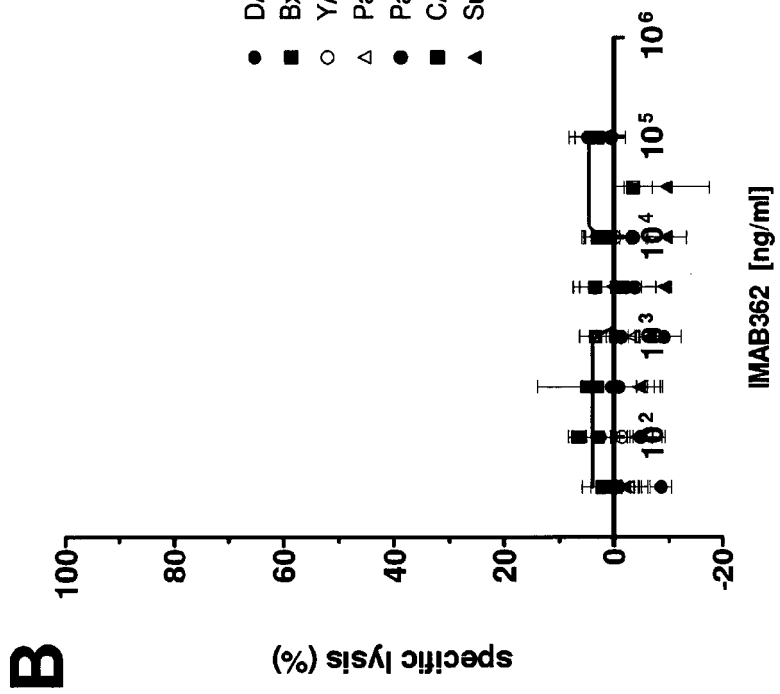

F
specific killing [%]
IMAB362 [ng/ml]
Donor #494 (Suit2-LVT)
Donor #495 (Suit2-LVT)
Donor #497 (Suit2-LVT)
Donor #494 (Suit2)
Donor #495 (Suit2)
H
Max. specific killing (%)
Pali8988S
DANG
Panc05.04
Panc05.04 clone 15D3
BxPC3-LVT
Capan-LVT
DANG-LVT
HPAC-LVT
MiaPaCa2-LVT
PATU8902-LVT
Suit2-LVT
YAPC-LVT
E
specific killing [%]
IMAB362 [ng/ml]
Donor #289 (BxPC3-LVT)
Donor #494 (BxPC3-LVT)
Donor #495 (BxPC3-LVT)
Donor #497 (BxPC3-LVT)
Donor #534 (BxPC3-LVT)
Donor #269 (BxPC3)
Donor #270 (BxPC3)
Donor #271 (BxPC3)
G
EC50 [ng/ml]
Pali8988S
DANG
Panc05.04
Panc05.04 clone 15D3
BxPC3-LVT
Capan-LVT
DANG-LVT
HPAC-LVT
MiaPaCa2-LVT
PATU8902-LVT
Suit2-LVT
YAPC-LVT

Figure 22


B
specific lysis (%)
100
80
60
40
20
0
-20
IMAB362 [ng/ml]
DANG
BxPC3
YAPC
Patu8988S
Panc05.04
CAPAN1
Suit2

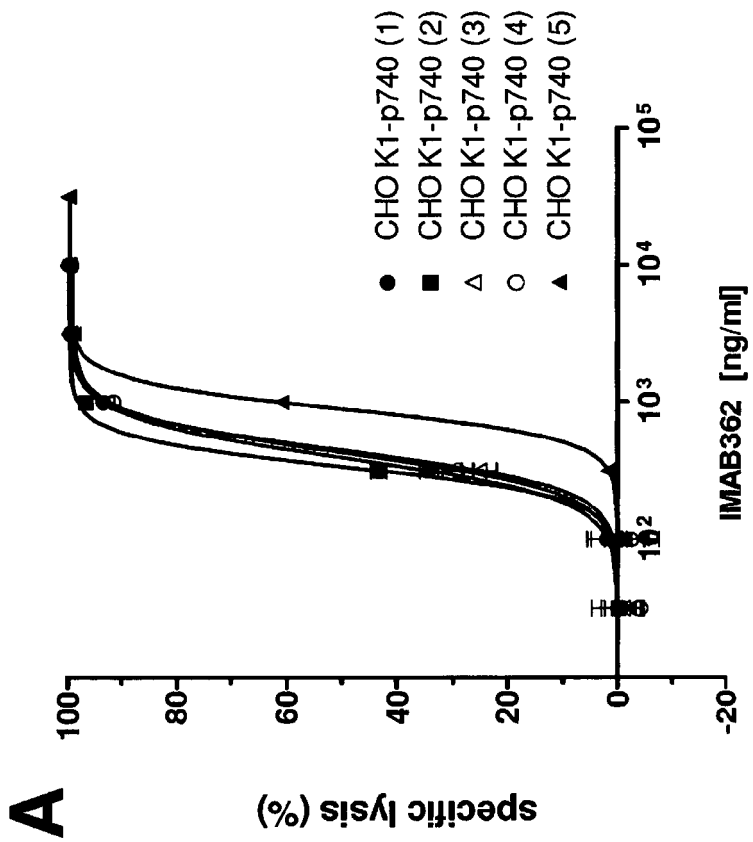

A
specific lysis (%)
100
80
60
40
20
0
-20
IMAB362 [ng/ml]
CHO K1-p740 (1)
CHO K1-p740 (2)
CHO K1-p740 (3)
CHO K1-p740 (4)
CHO K1-p740 (5)

Figure 23

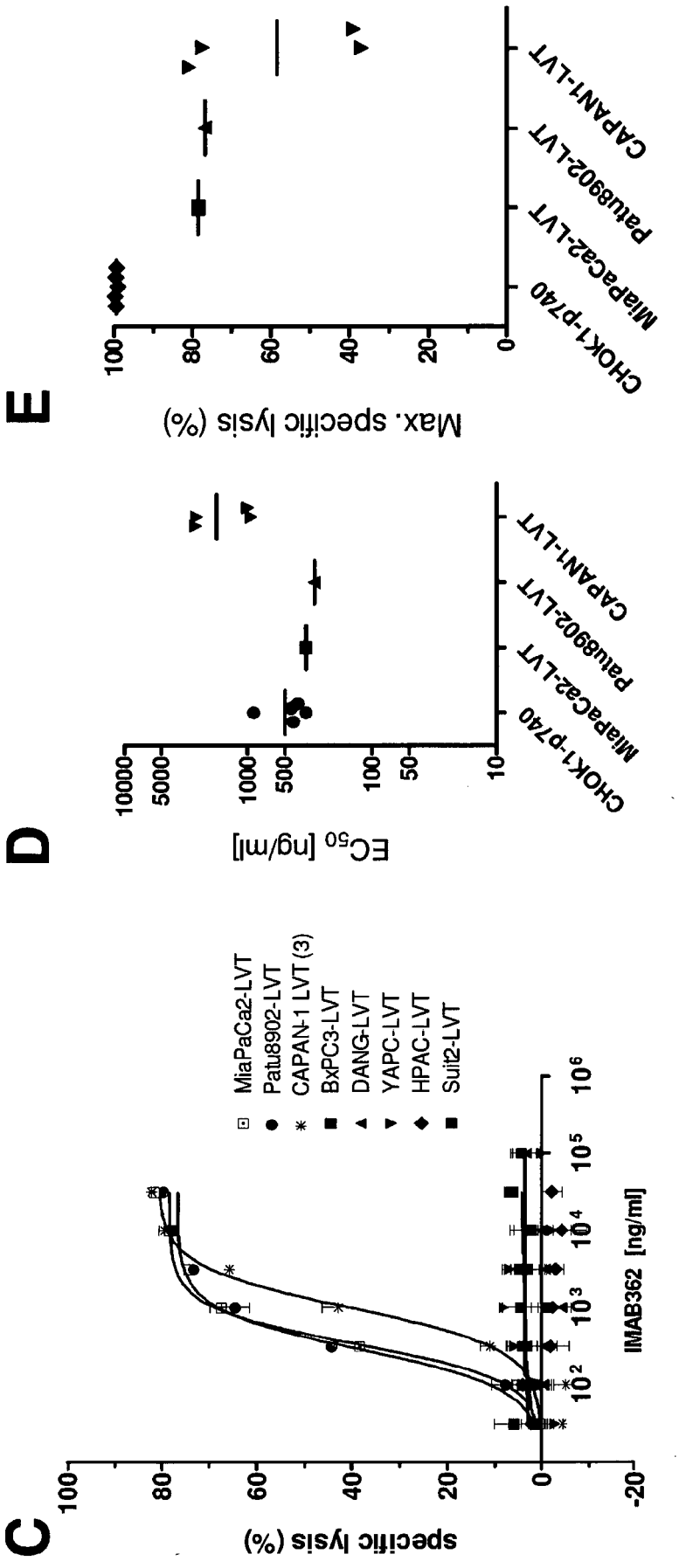

C
specific lysis (%)
100
80
60
40
20
0
-20
IMAB362 [ng/ml]
10^2
10^3
10^4
10^5
10^6
MiaPaCa2-LVT
Patu8902-LVT
CAPAN-1 LVT (3)
BxPC3-LVT
DANG-LVT
YAPC-LVT
HPAC-LVT
Suit2-LVT
D
EC_{50} [ng/ml]
10000
5000
1000
500
100
50
10
CHOK1-p740
MiaPaCa2-LVT
Patu8902-LVT
CAPAN1-LVT
E
Max. specific lysis (%)
100
80
60
40
20
0
CHOK1-p740
MiaPaCa2-LVT
Patu8902-LVT
CAPAN1-LVT

Figure 23

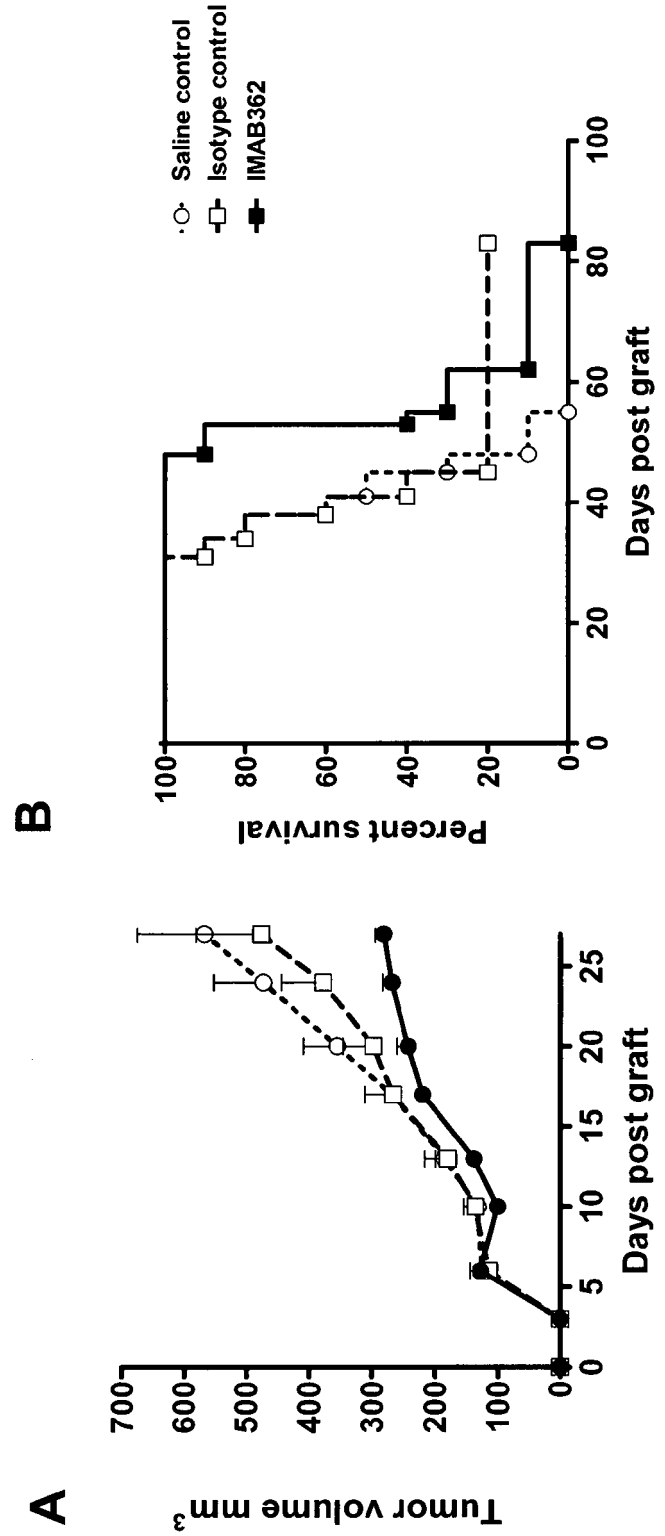

A
Tumor volume mm³
700
600
500
400
300
200
100
0
0
5
10
15
20
25
Days post graft
B
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Days post graft
Saline control
Isotype control
IMAB362

Figure 24

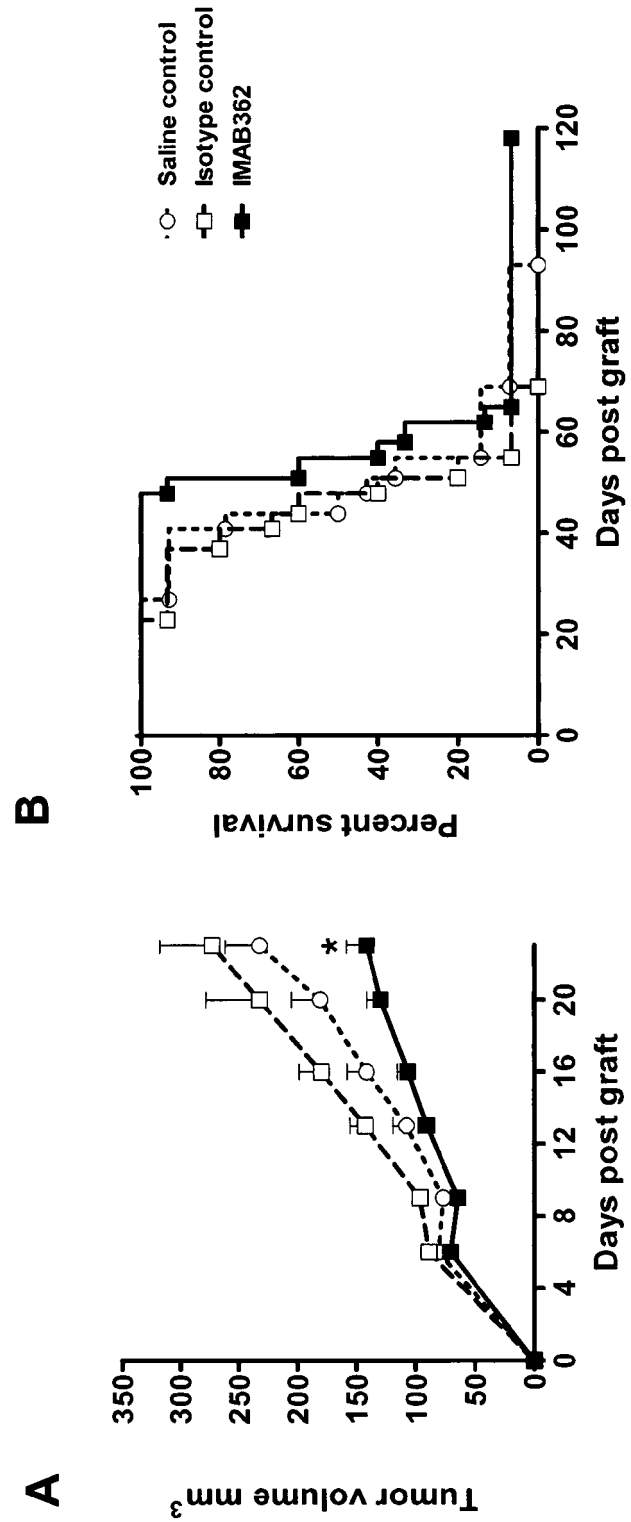

A
Tumor volume mm3
350
300
250
200
150
100
50
0
0
4
8
12
16
20
Days post graft
*
B
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
120
Days post graft
Saline control
Isotype control
IMAB362

Figure 25

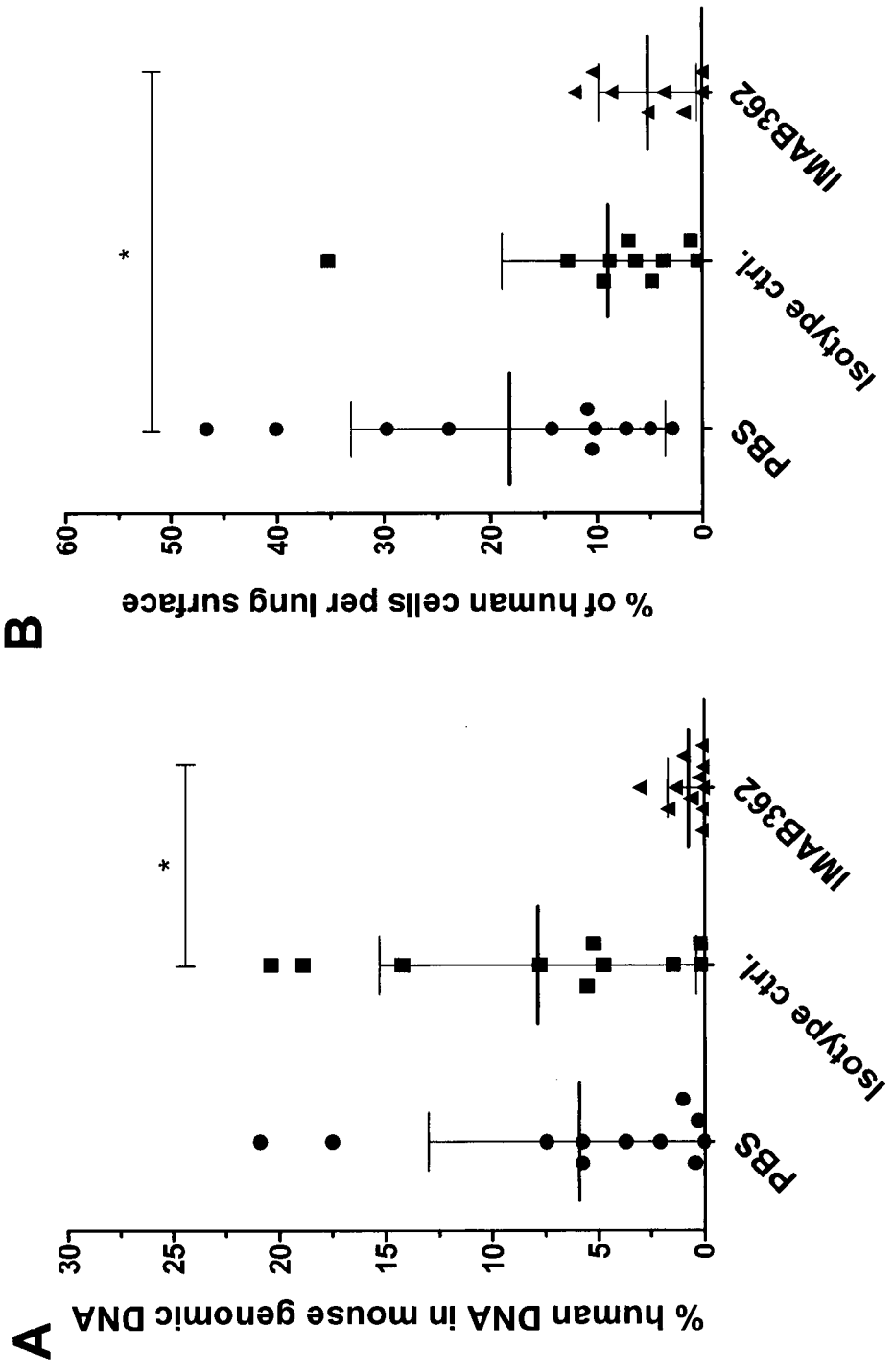
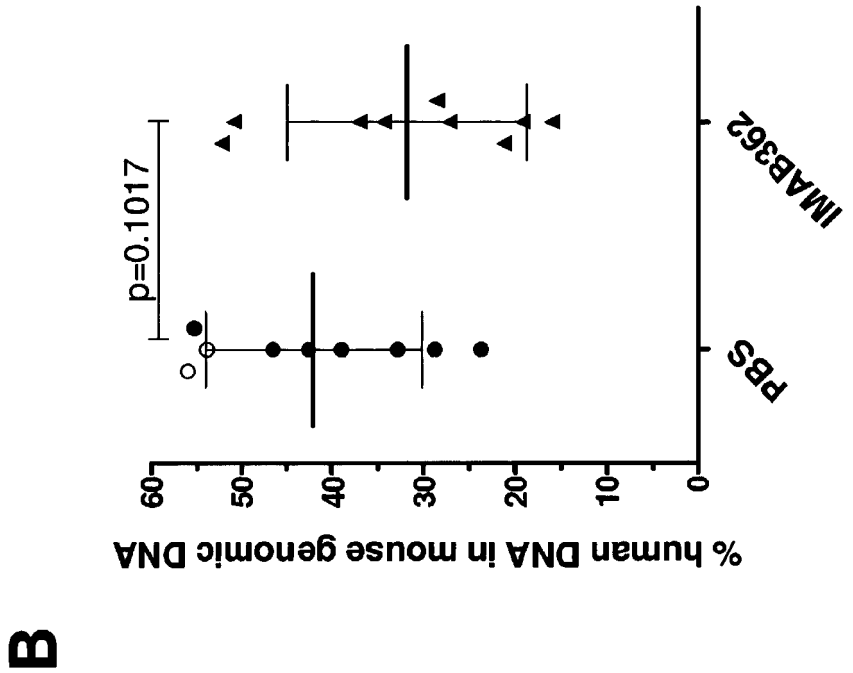

A
% human DNA in mouse genomic DNA
0
5
10
15
20
25
30
*
PBS
Isotype ctrl.
IMAB362
B
% of human cells per lung surface
0
10
20
30
40
50
60
*
PBS
Isotype ctrl.
IMAB362

Figure 26


B
% human DNA in mouse genomic DNA
0
10
20
30
40
50
60
p=0.1017
PBS
IMAB362

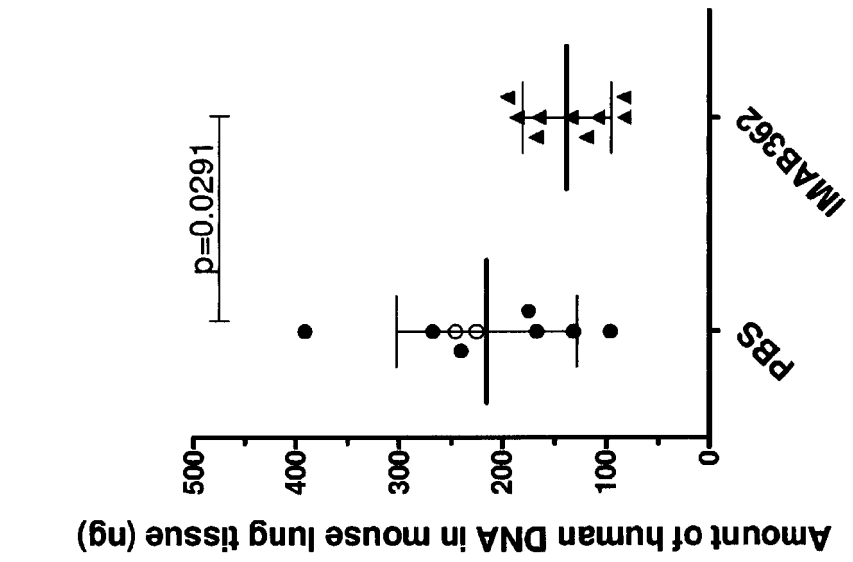
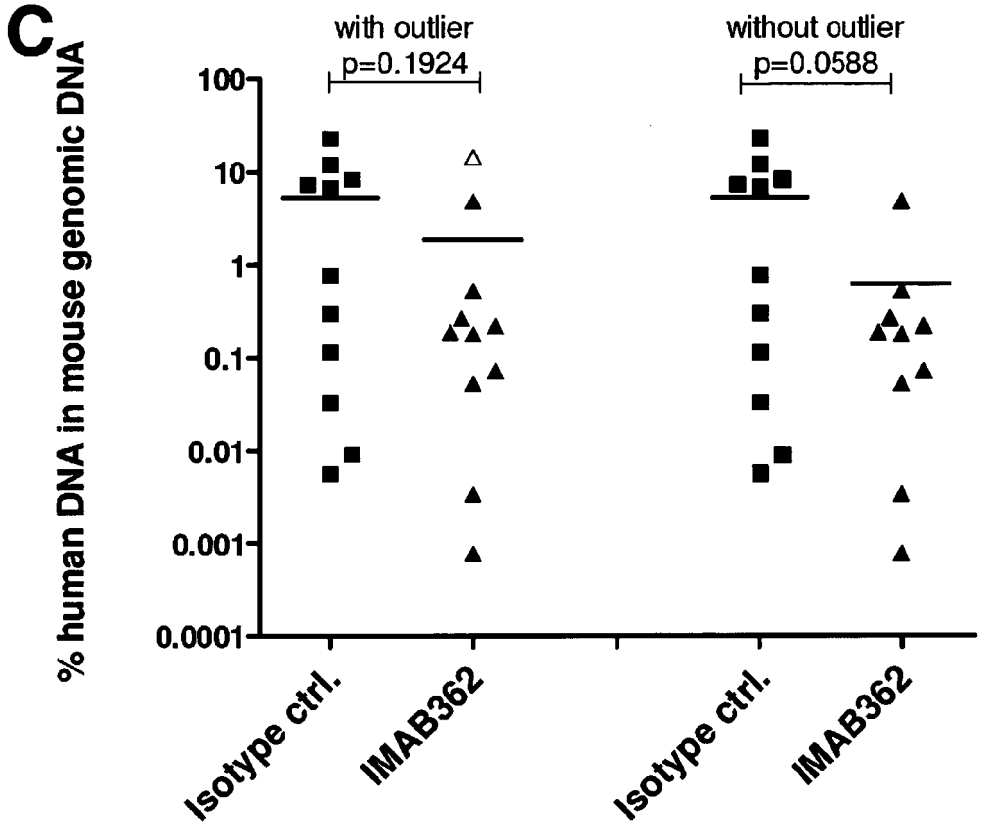

A
Amount of human DNA in mouse lung tissue (ng)
0
100
200
300
400
500
p=0.0291
PBS
IMAB362

Figure 27


C
% human DNA in mouse genomic DNA
with outlier
p=0.1924
without outlier
p=0.0588
100
10
1
0.1
0.01
0.001
0.0001
Isotype ctrl.
IMAB362
Isotype ctrl.
IMAB362

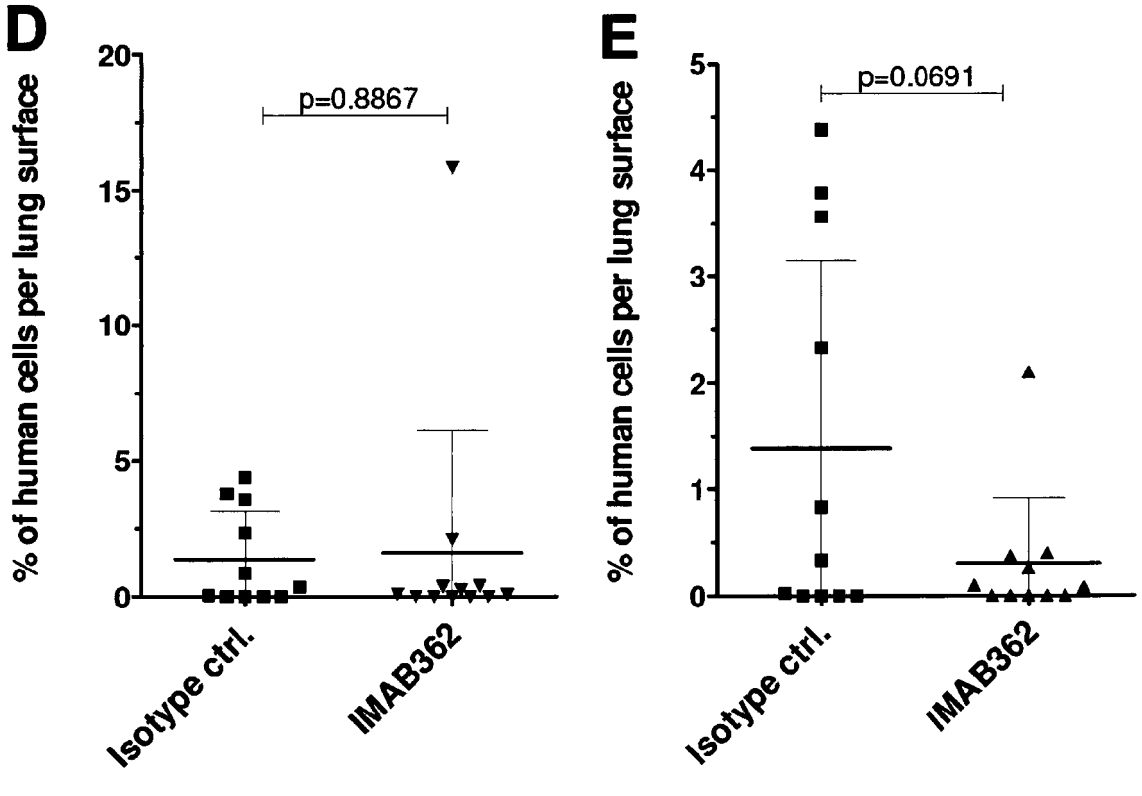

D
% of human cells per lung surface
p=0.8867
20
15
10
5
0
Isotype ctrl.
IMAB362
E
% of human cells per lung surface
p=0.0691
5
4
3
2
1
0
Isotype ctrl.
IMAB362

Figure 27

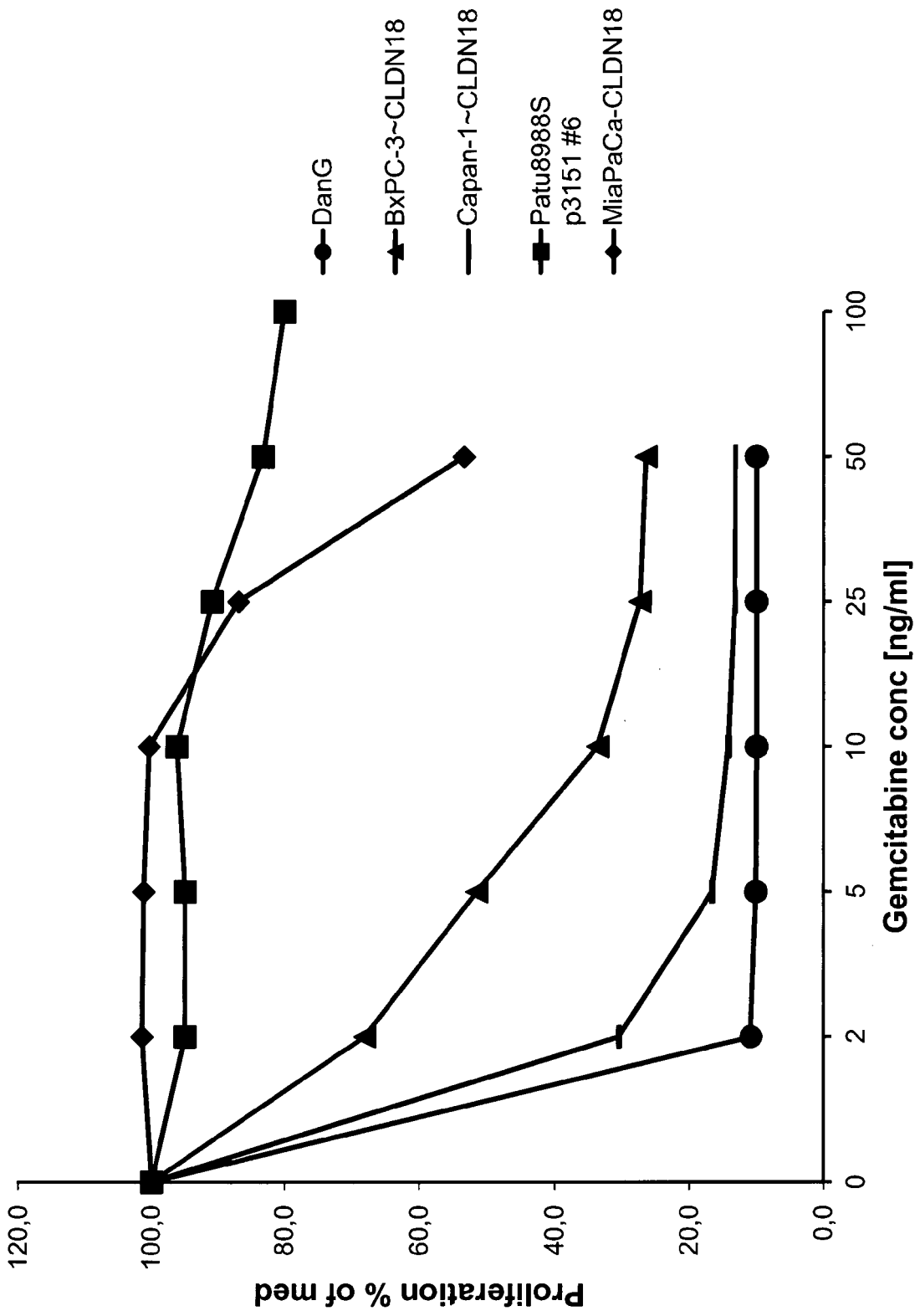

DanG
BxPC-3~CLDN18
Capan-1~CLDN18
Patu8988S p3151 #6
MiaPaCa-CLDN18
Proliferation % of med
120,0
100,0
80,0
60,0
40,0
20,0
0,0
0
2
5
10
25
50
100
Gemcitabine conc [ng/ml]

Figure 28

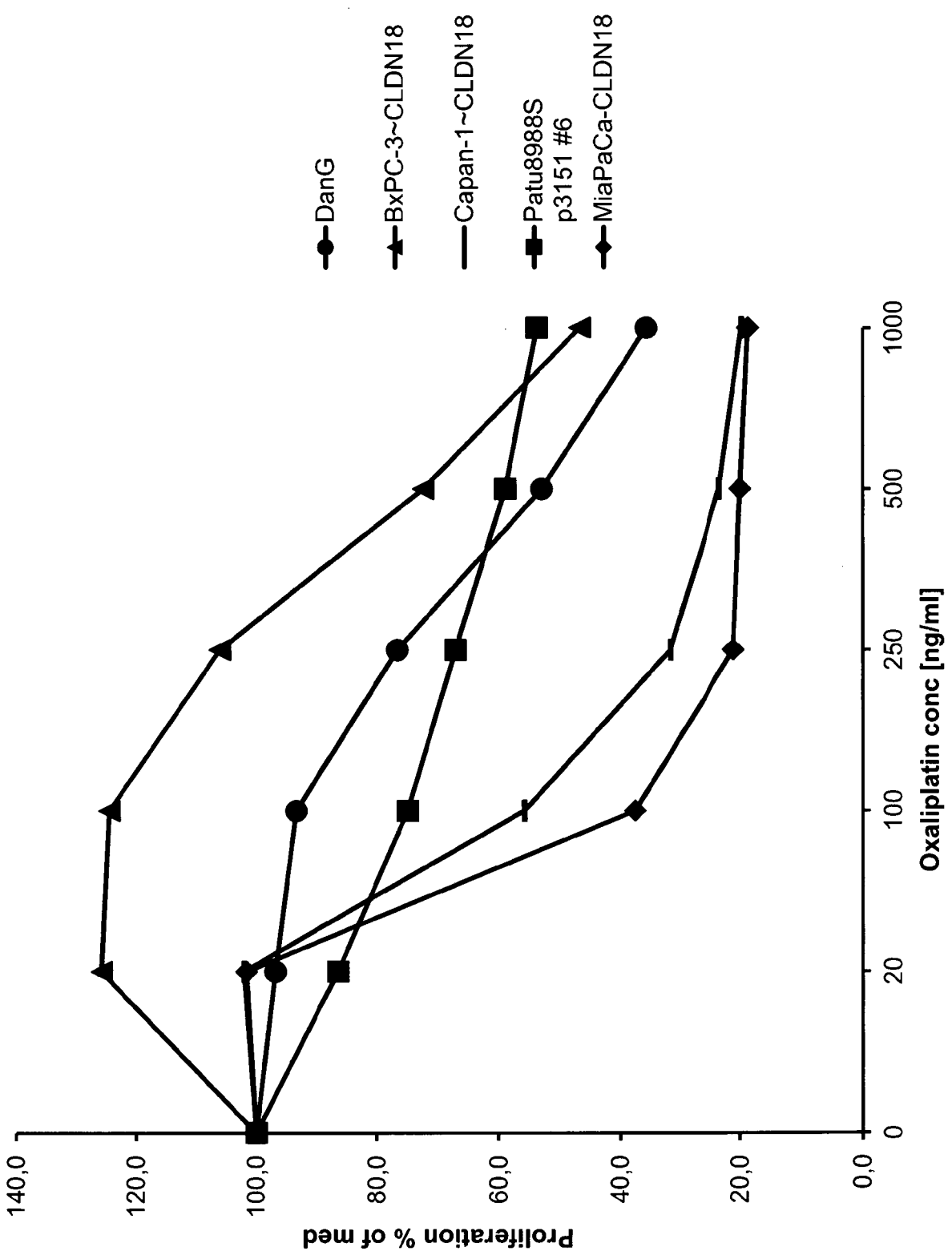

DanG
BxPC-3~CLDN18
Capan-1~CLDN18
Patu8988S p3151 #6
MiaPaCa-CLDN18
Proliferation % of med
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
0
20
100
250
500
1000
Oxaliplatin conc [ng/ml]

Figure 29

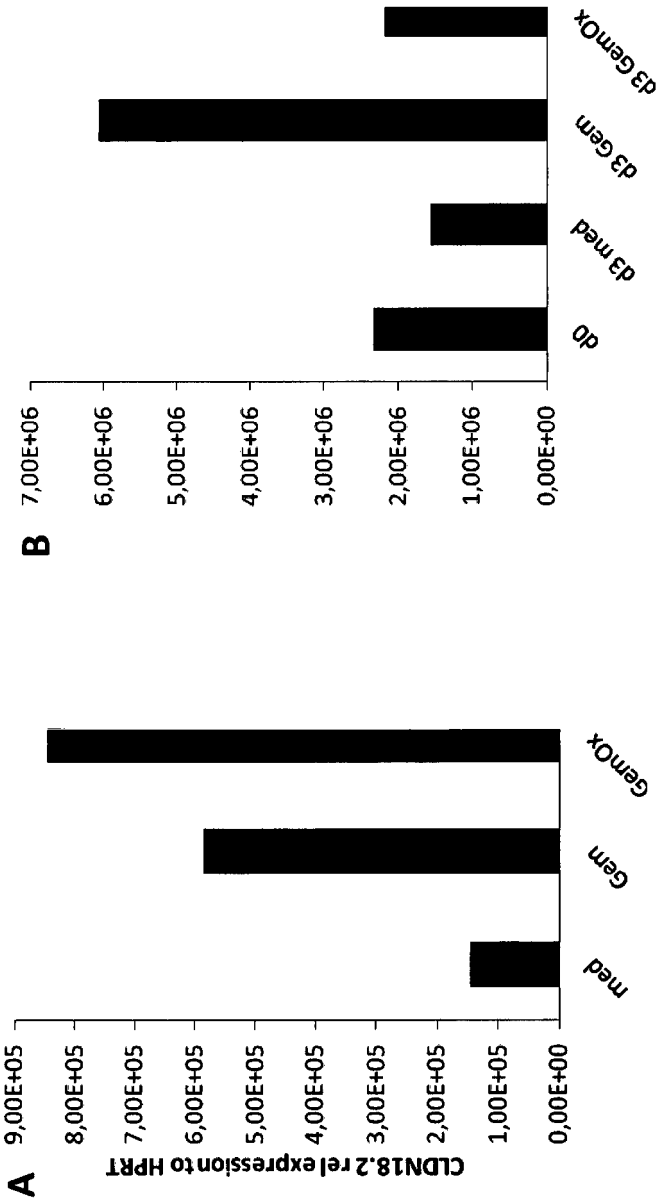

A
CLDN18.2 rel expression to HPRT
9,00E+05
8,00E+05
7,00E+05
6,00E+05
5,00E+05
4,00E+05
3,00E+05
2,00E+05
1,00E+05
0,00E+00
med
Gem
GemOx
B
7,00E+06
6,00E+06
5,00E+06
4,00E+06
3,00E+06
2,00E+06
1,00E+06
0,00E+00
d0
d3 med
d3 Gem
d3 GemOx

Figure 30

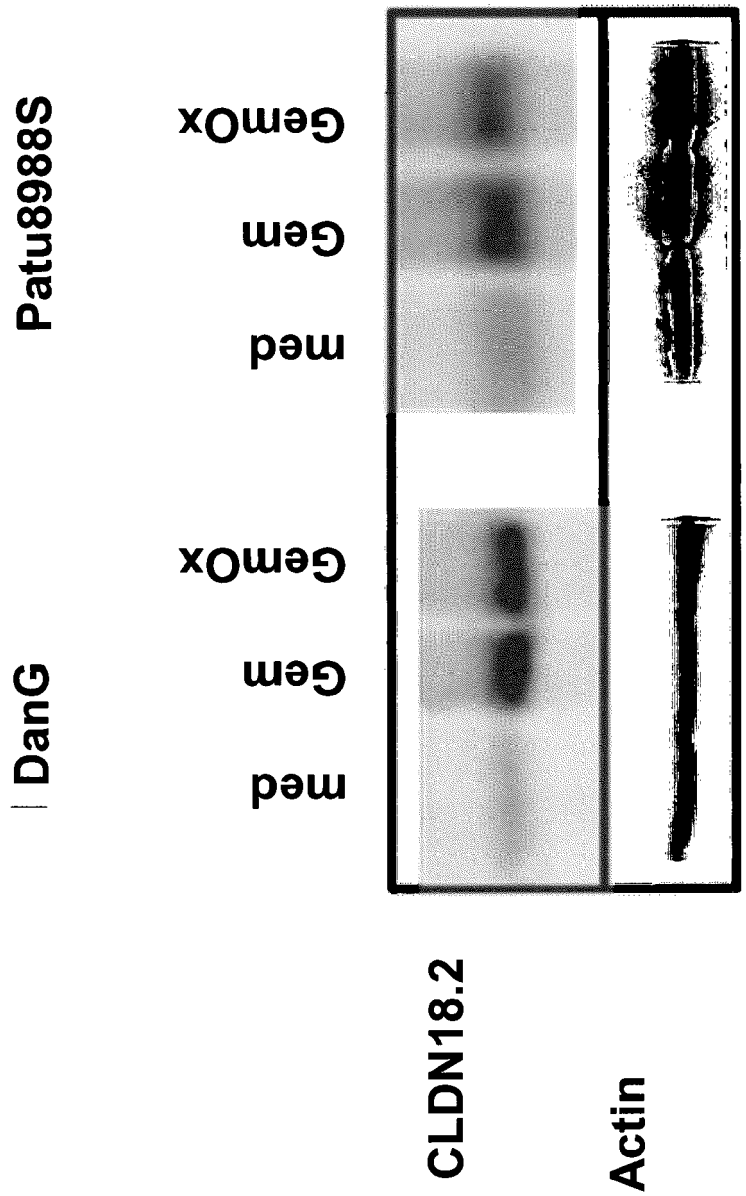


Figure 31

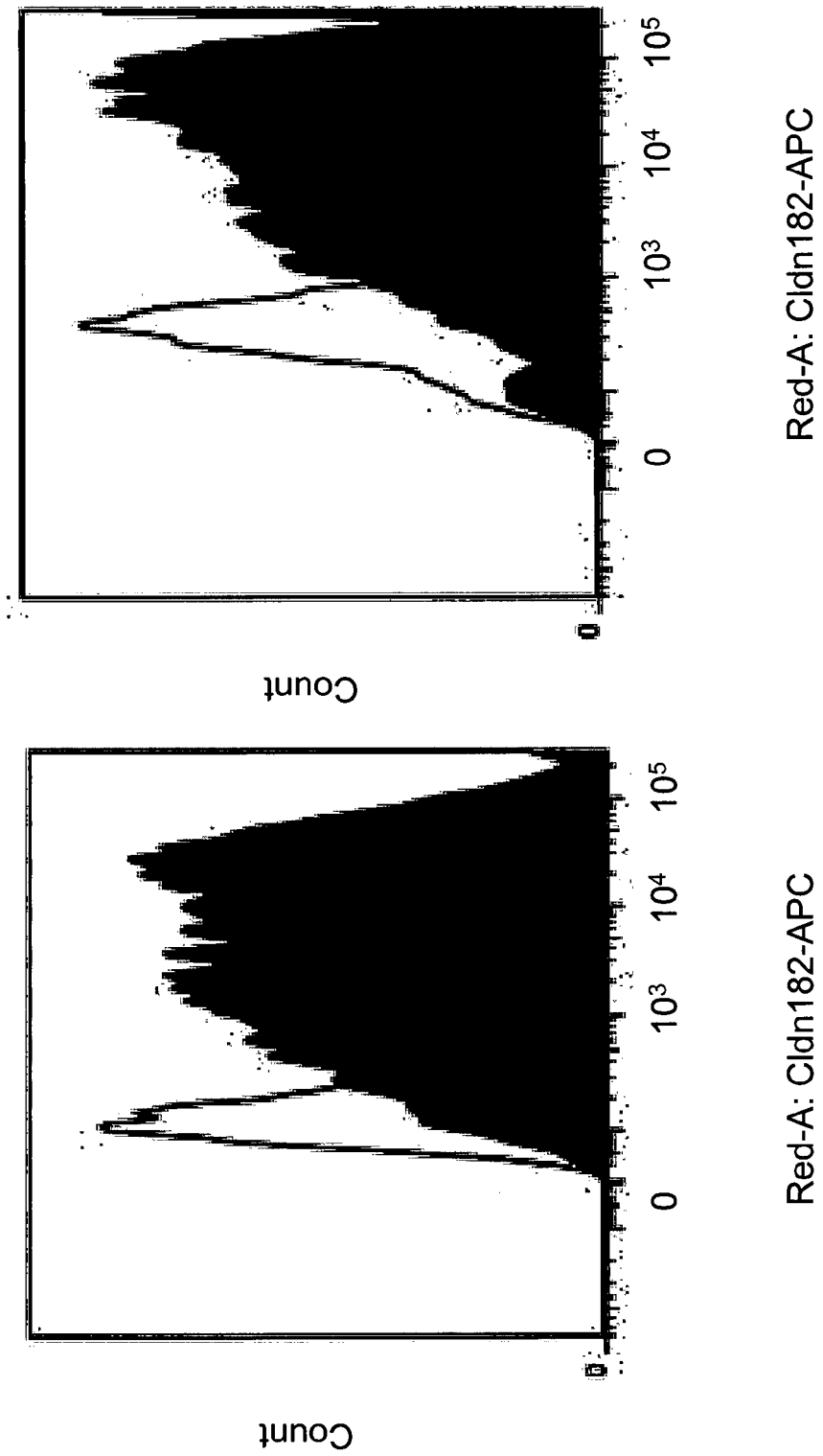
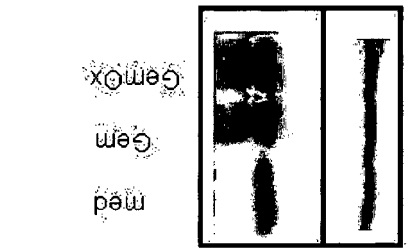
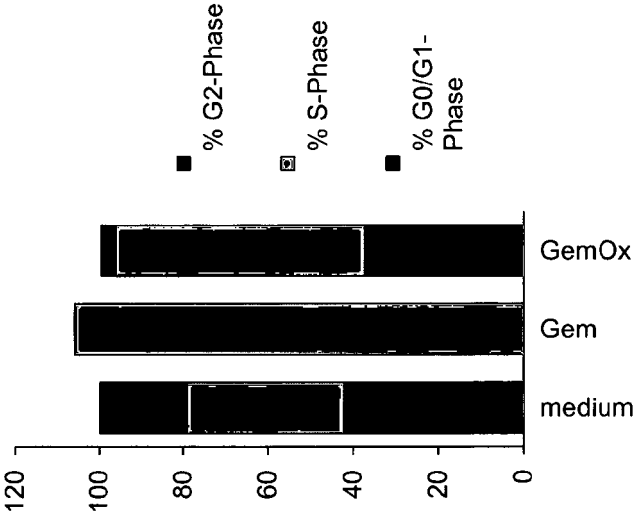

Count
0
10^3
10^4
10^5
Red-A: Cldn182-APC
Count
0
10^3
10^4
10^5
Red-A: Cldn182-APC

Figure 32


med
Gem
GemOx
CLDN18.2
Actin

B


% G2-Phase
% S-Phase
% G0/G1-Phase
GemOx
Gem
medium
120
100
80
60
40
20
0

A

Figure 33

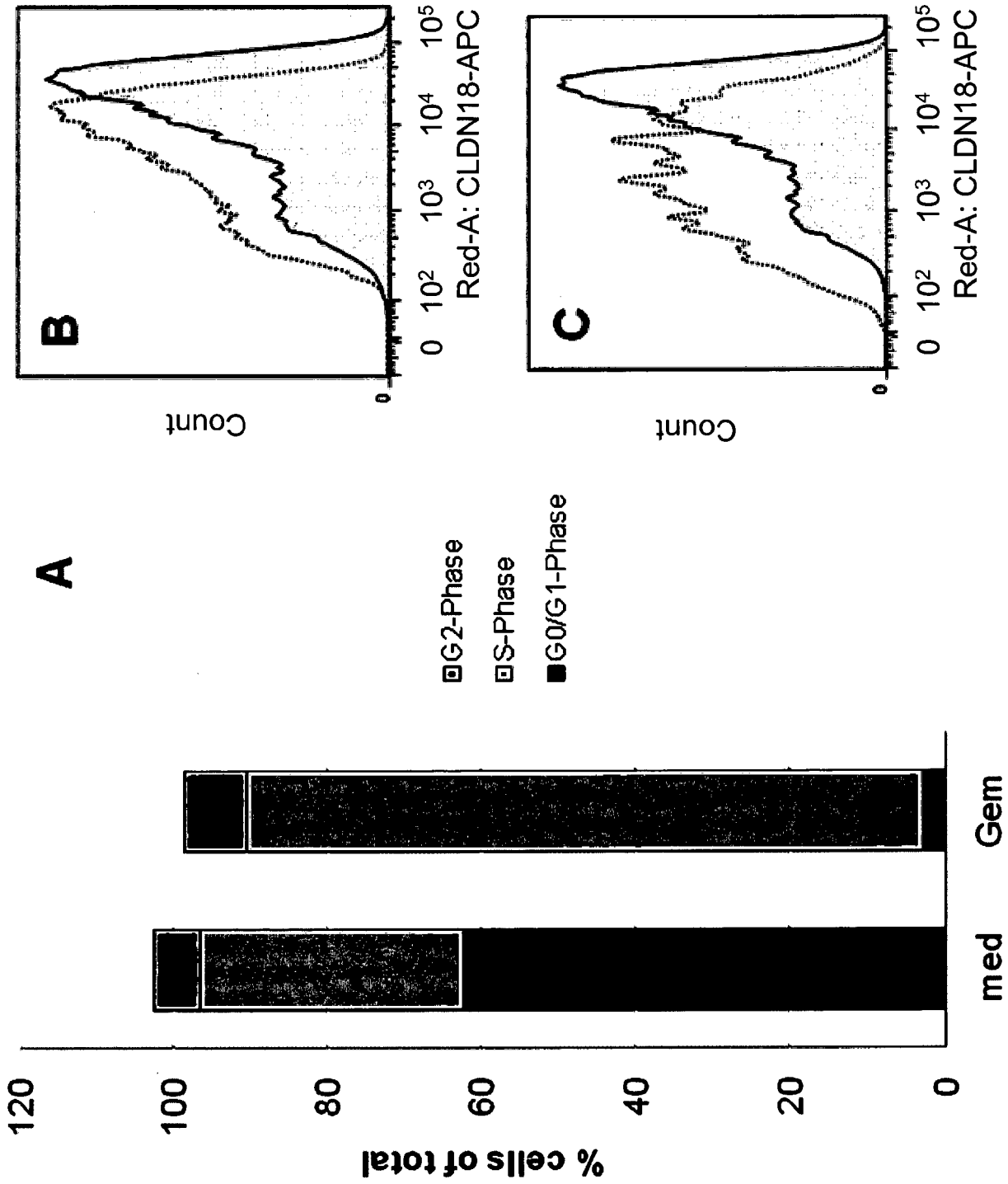
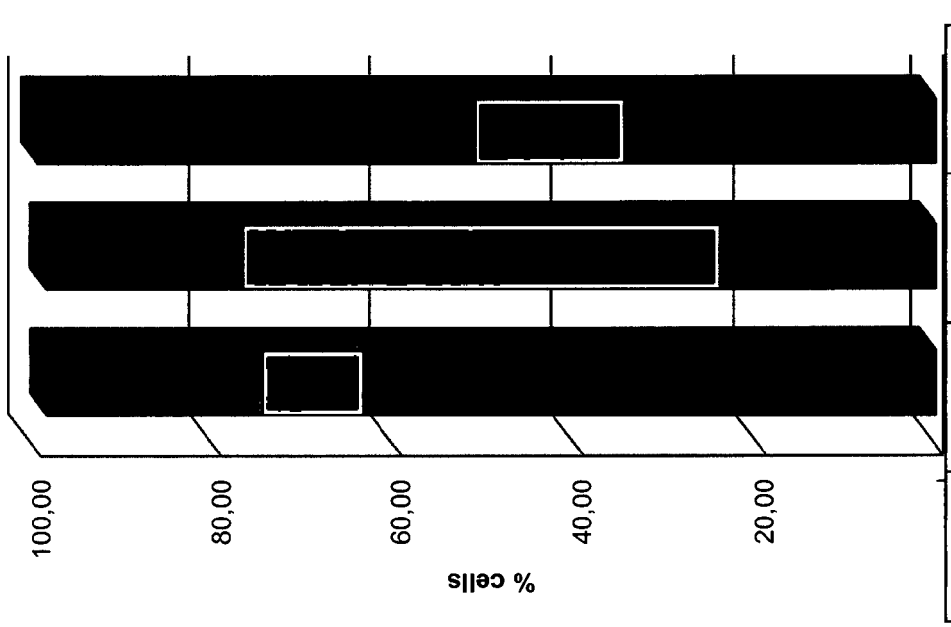

A
B
C
Count
Red-A: CLDN18-APC
0 10² 10³ 10⁴ 10⁵
G2-Phase
S-Phase
G0/G1-Phase
% cells of total
120
100
80
60
40
20
0
med
Gem

Figure 34

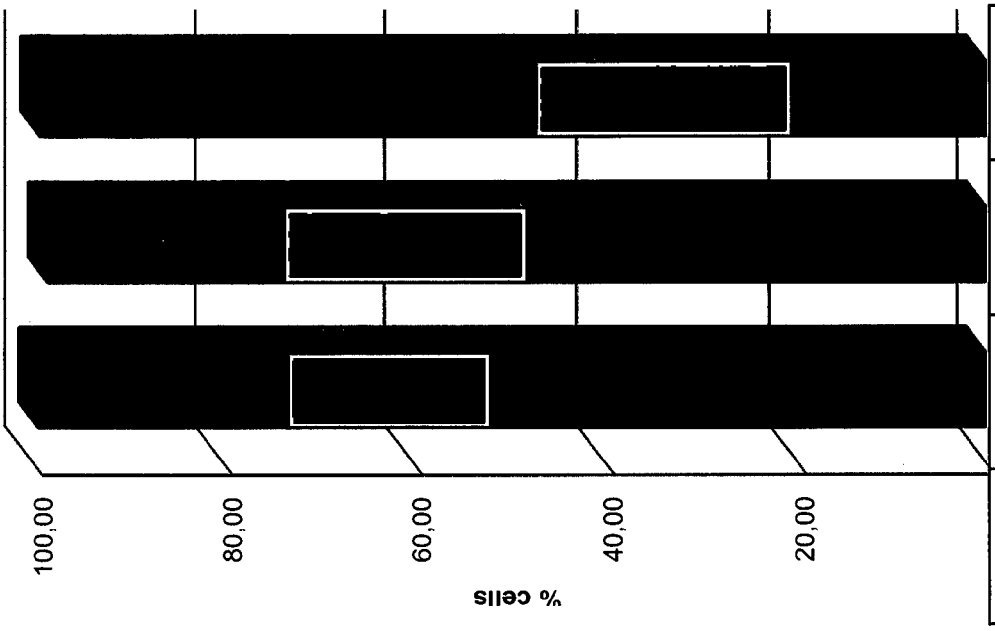
Figure 35

a

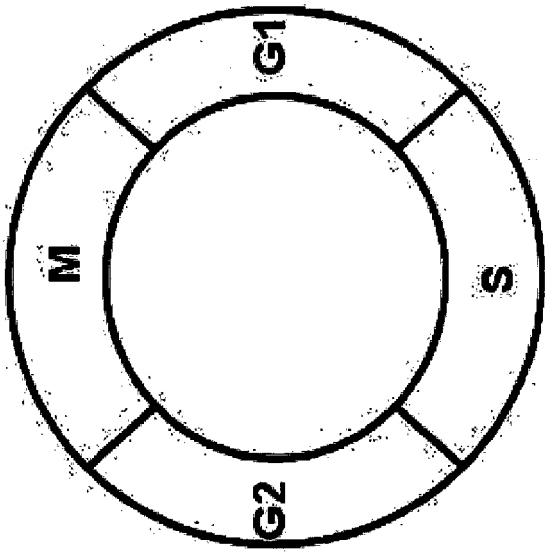


Phase	medium	100 ng/ml 5-FU	500 ng/ml Oxali
G2	23.78	21.75	48.37
S	11.25	52.41	16.37
G1	63.40	24.35	34.74

b



Phase	medium	100 ng/ml 5-FU	500 ng/ml Oxali
G2	26.25	24.94	52.12
S	21.30	25.48	26.46
G1	51.94	48.11	20.75



a/b continued

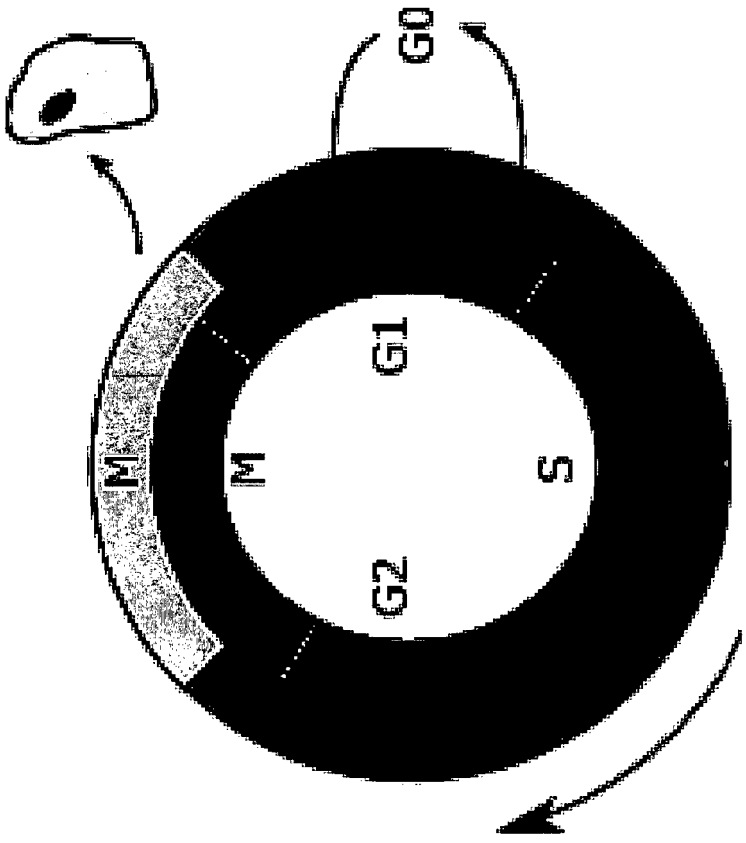
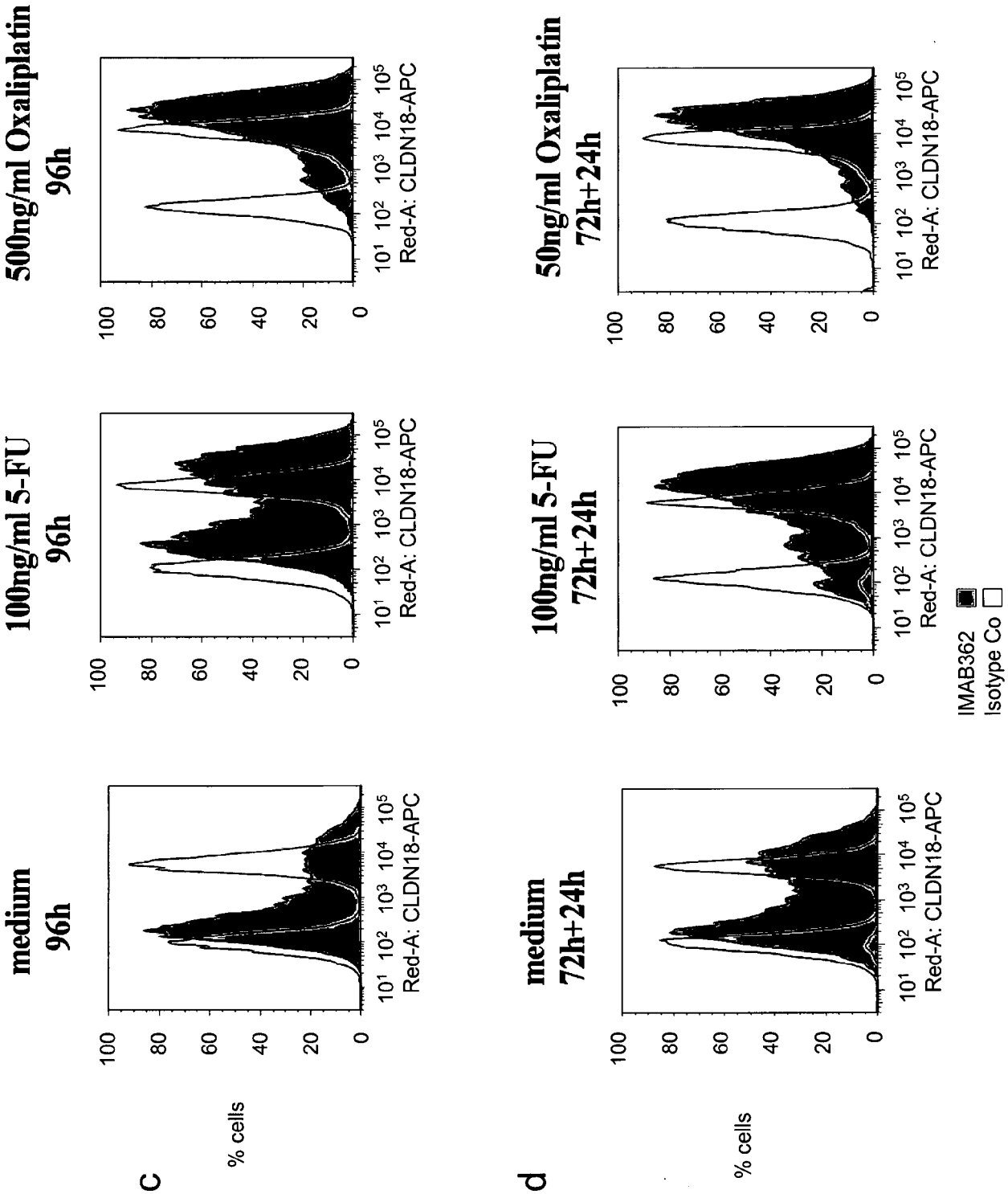


Figure 35

Figure 35


c
d
% cells
medium 96h
100ng/ml 5-FU 96h
500ng/ml Oxaliplatin 96h
medium 72h+24h
100ng/ml 5-FU 72h+24h
50ng/ml Oxaliplatin 72h+24h
Red-A: CLDN18-APC
IMAB362
Isotype Co

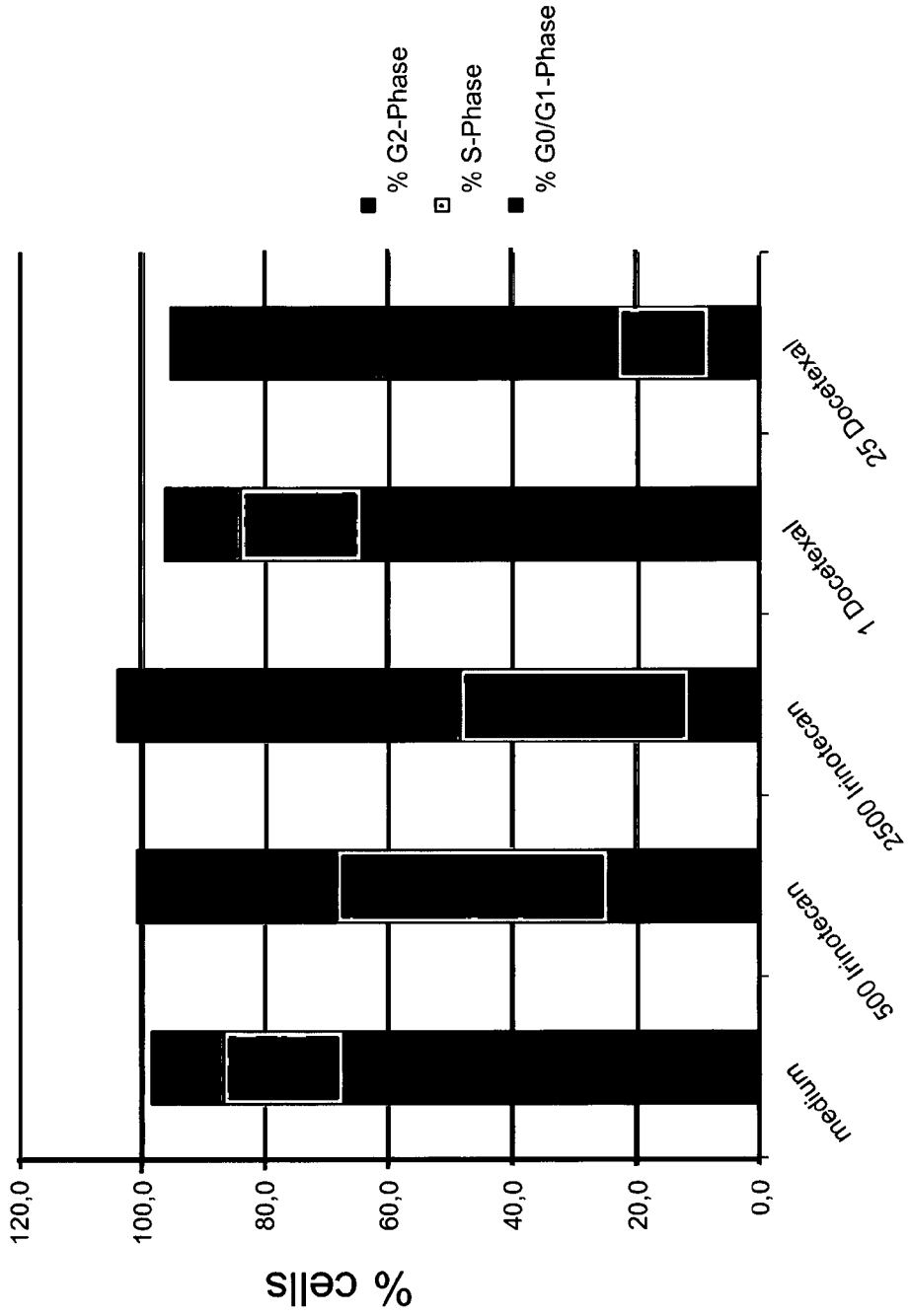
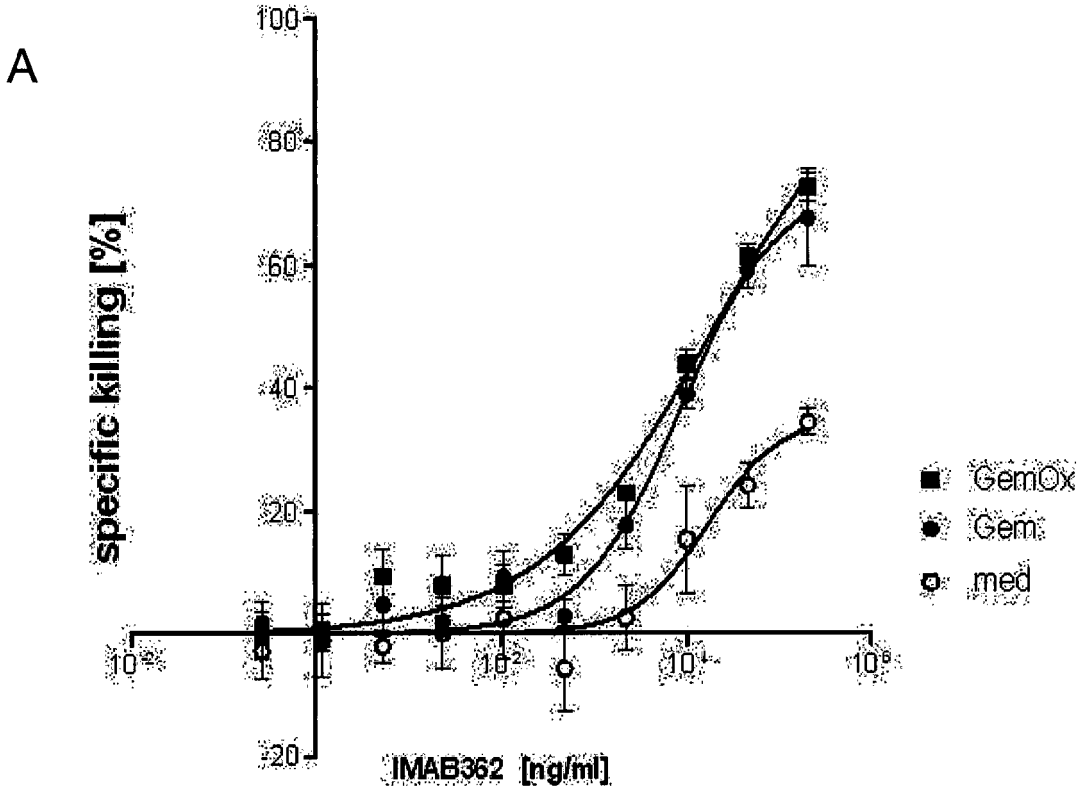

% G2-Phase
% S-Phase
% G0/G1-Phase
medium
500 Irinotecan
2500 Irinotecan
1 Docetaxal
25 Docetaxal
120,0
100,0
80,0
60,0
40,0
20,0
0,0
% cells

Figure 36


A
specific killing [%]
100
80
60
40
20
-20
IMAB362 [ng/ml]
GemOx
Gem
med

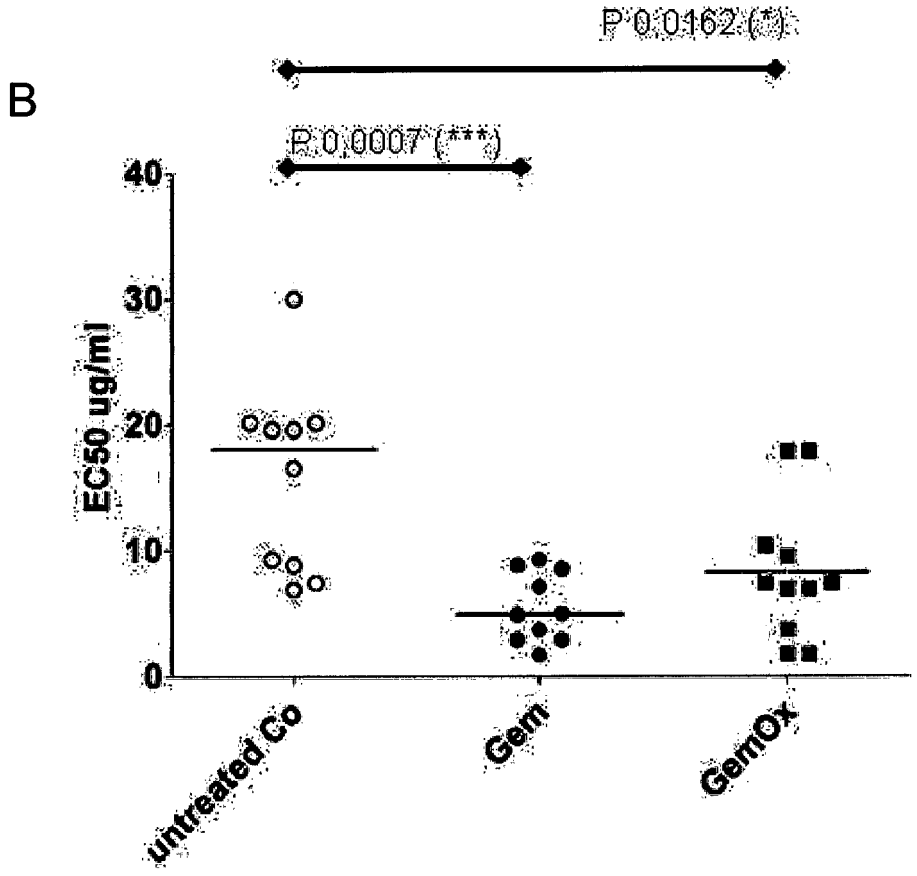
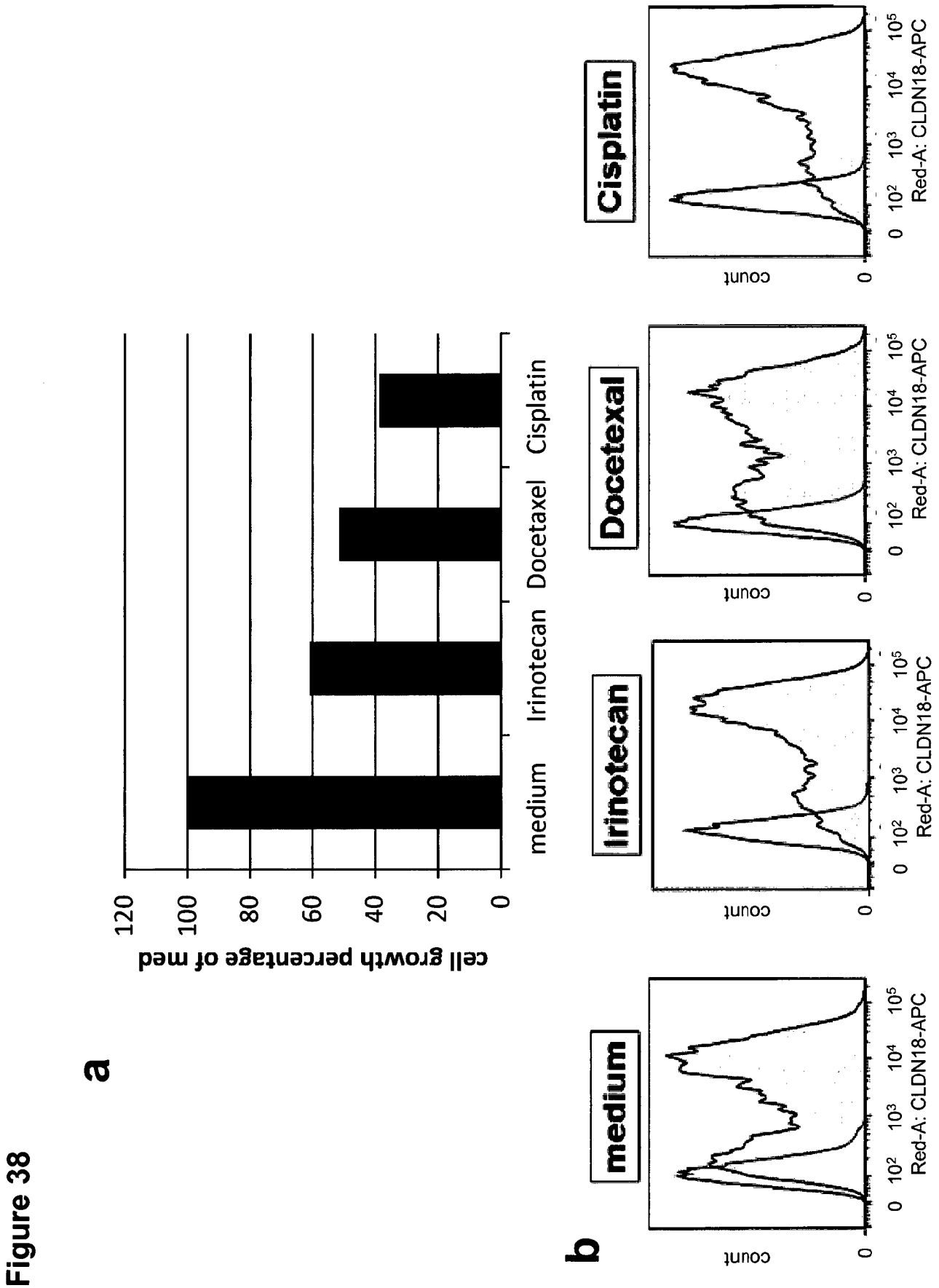

B
P 0.0162 (*)
P 0.0007 (***)
EC50 ug/ml
40
30
20
10
0
untreated Co
Gem
GemOx

Figure 37

Figure 38


a
cell growth percentage of med
120
100
80
60
40
20
0
medium
Irinotecan
Docetaxel
Cisplatin
b
medium
Irinotecan
Docetexal
Cisplatin
count
0
0 10² 10³ 10⁴ 10⁵
Red-A: CLDN18-APC

c

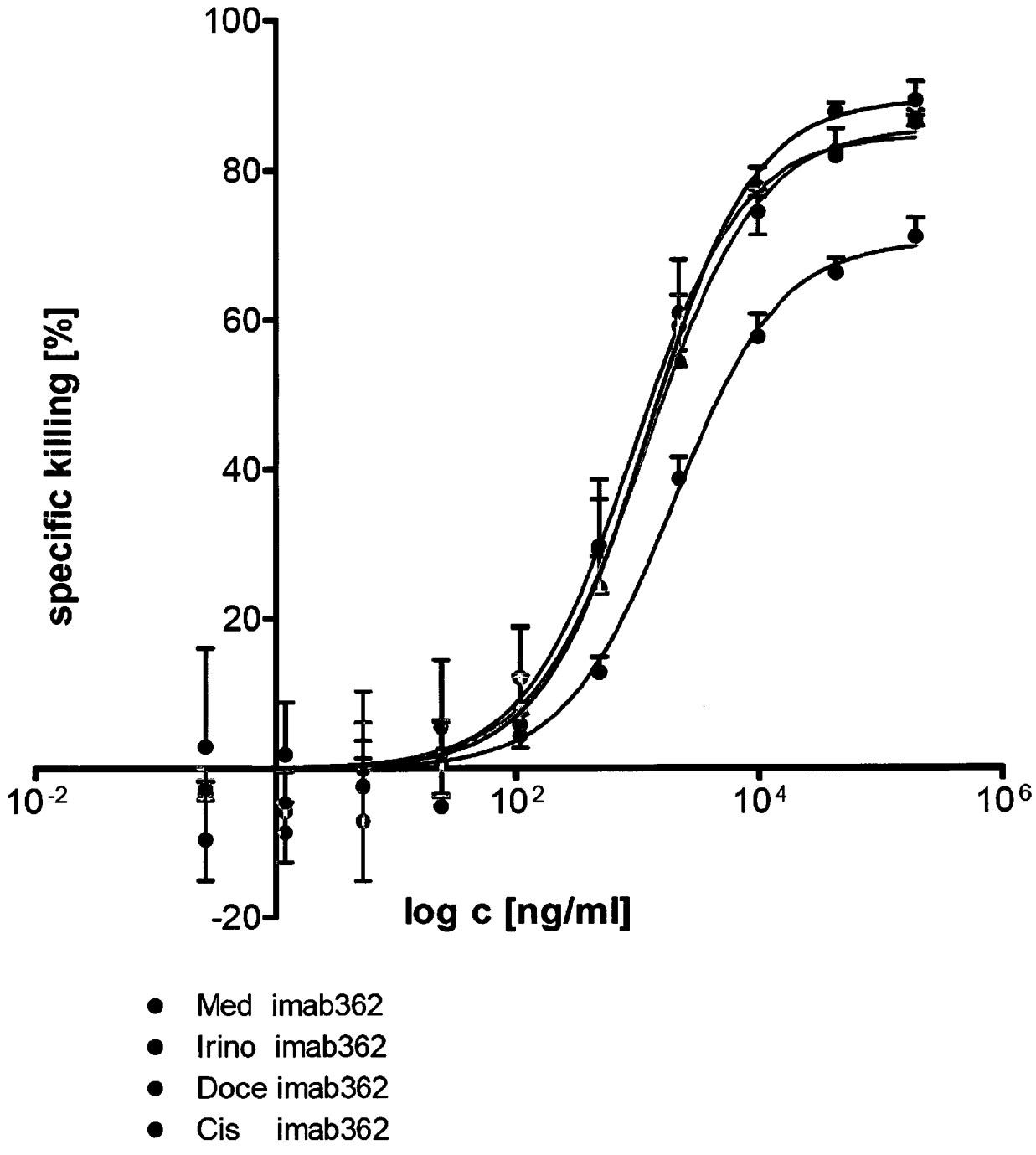
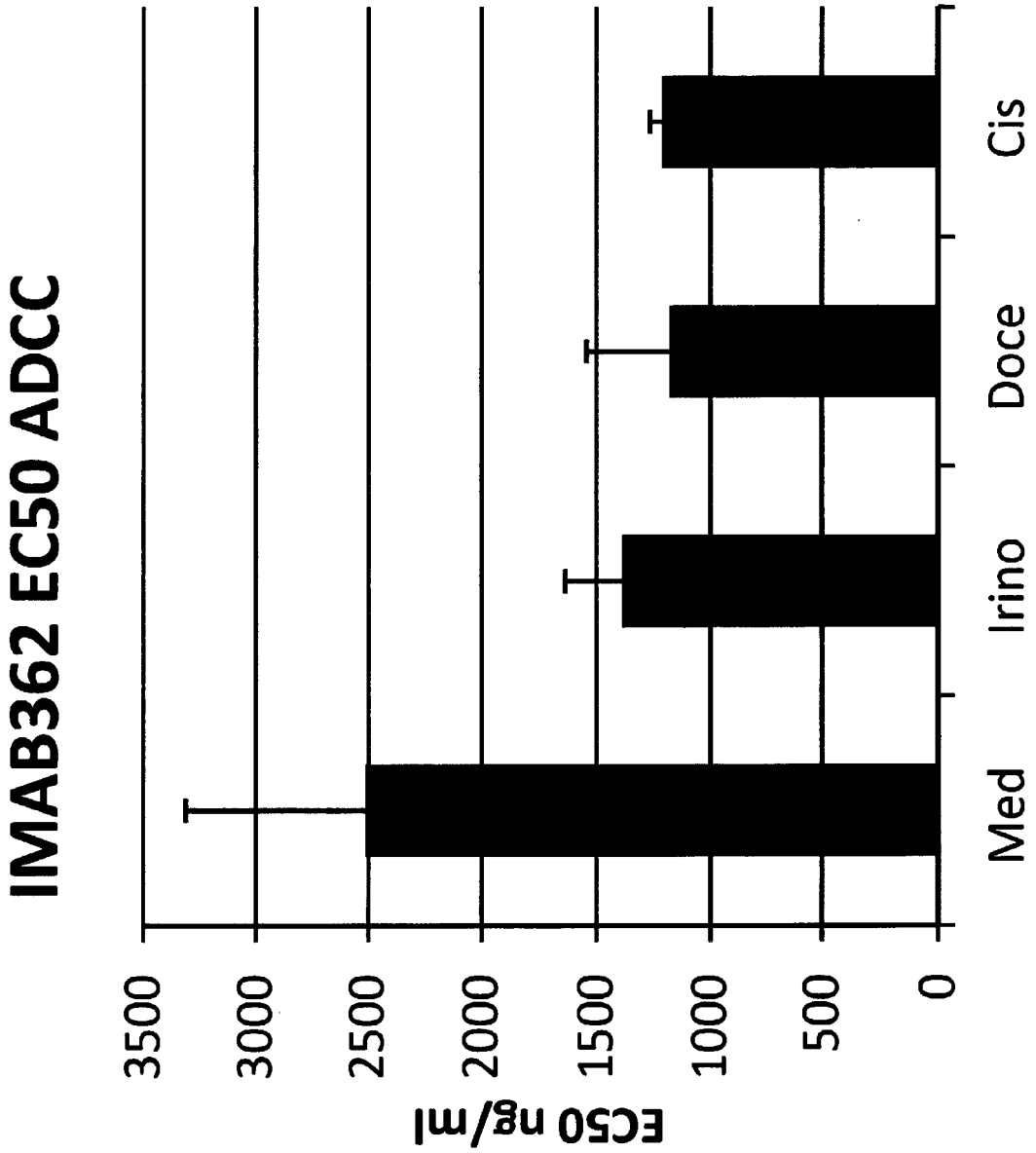

100
80
60
40
20
-20
specific killing [%]
10^{-2}
10^{2}
10^{4}
10^{6}
log c [ng/ml]
Med imab362
Irino imab362
Doce imab362
Cis imab362

Figure 38


IMAB362 EC50 ADCC
EC50 ng/ml
3500
3000
2500
2000
1500
1000
500
0
Med
Irino
Doce
Cis

d

Figure 38

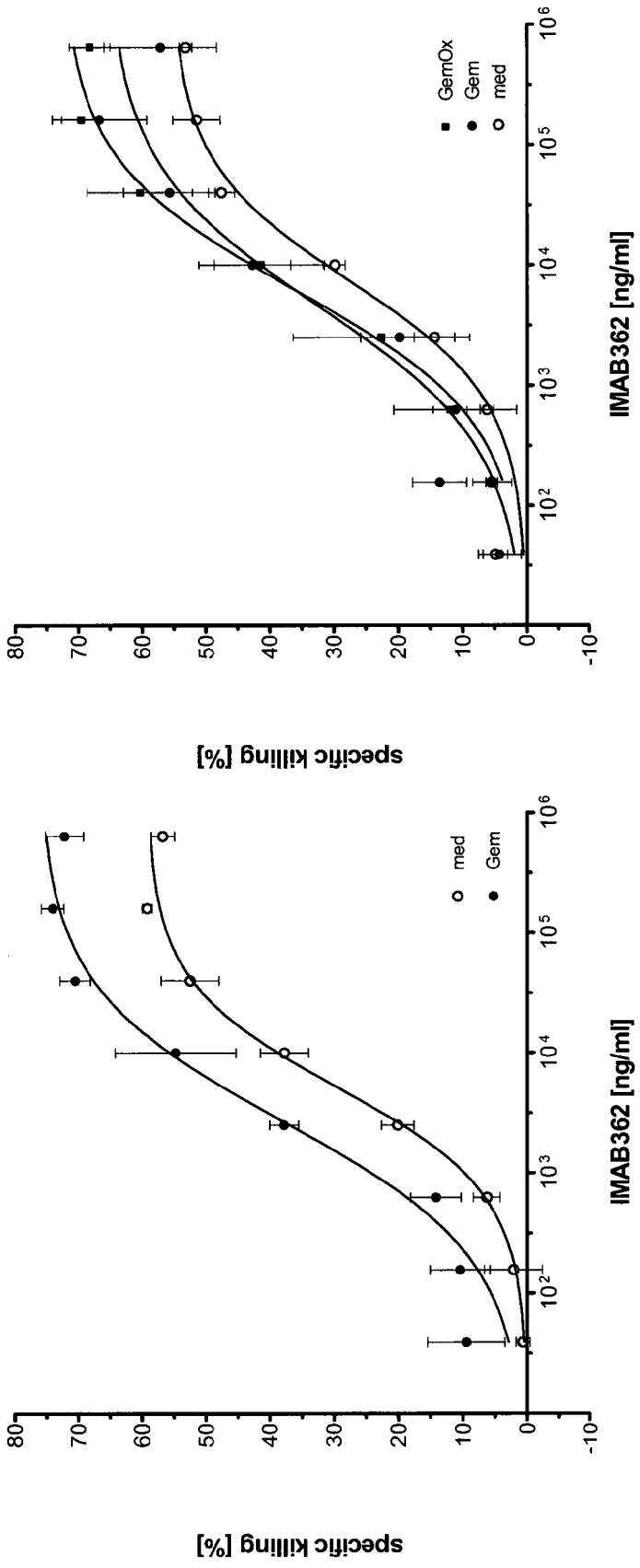

GemOx
Gem
med
specific killing [%]
IMAB362 [ng/ml]
med
Gem
specific killing [%]
IMAB362 [ng/ml]

Figure 39

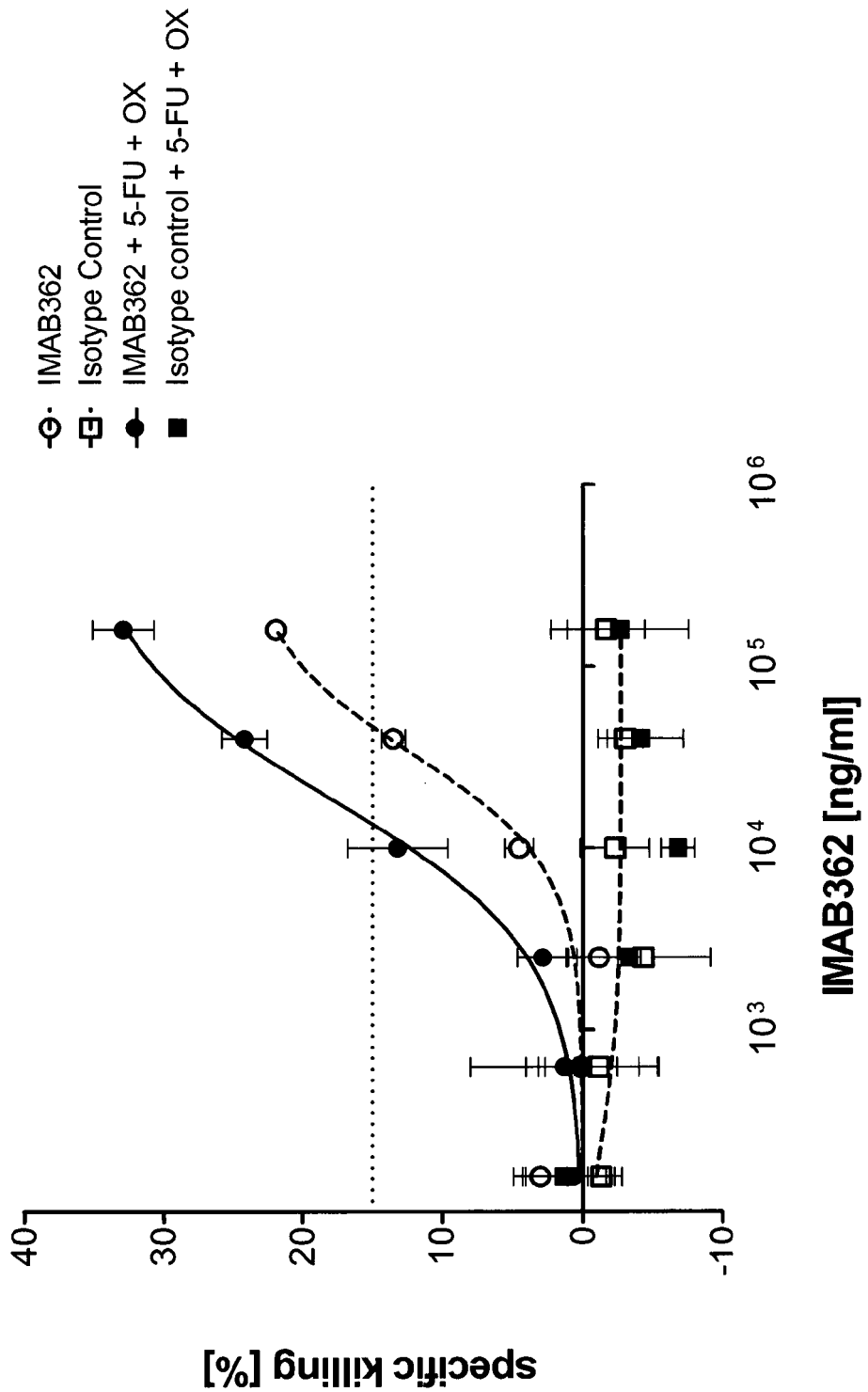

IMAB362
Isotype Control
IMAB362 + 5-FU + OX
Isotype control + 5-FU + OX
specific killing [%]
40
30
20
10
0
-10
10^3
10^4
10^5
10^6
IMAB362 [ng/ml]

Figure 40

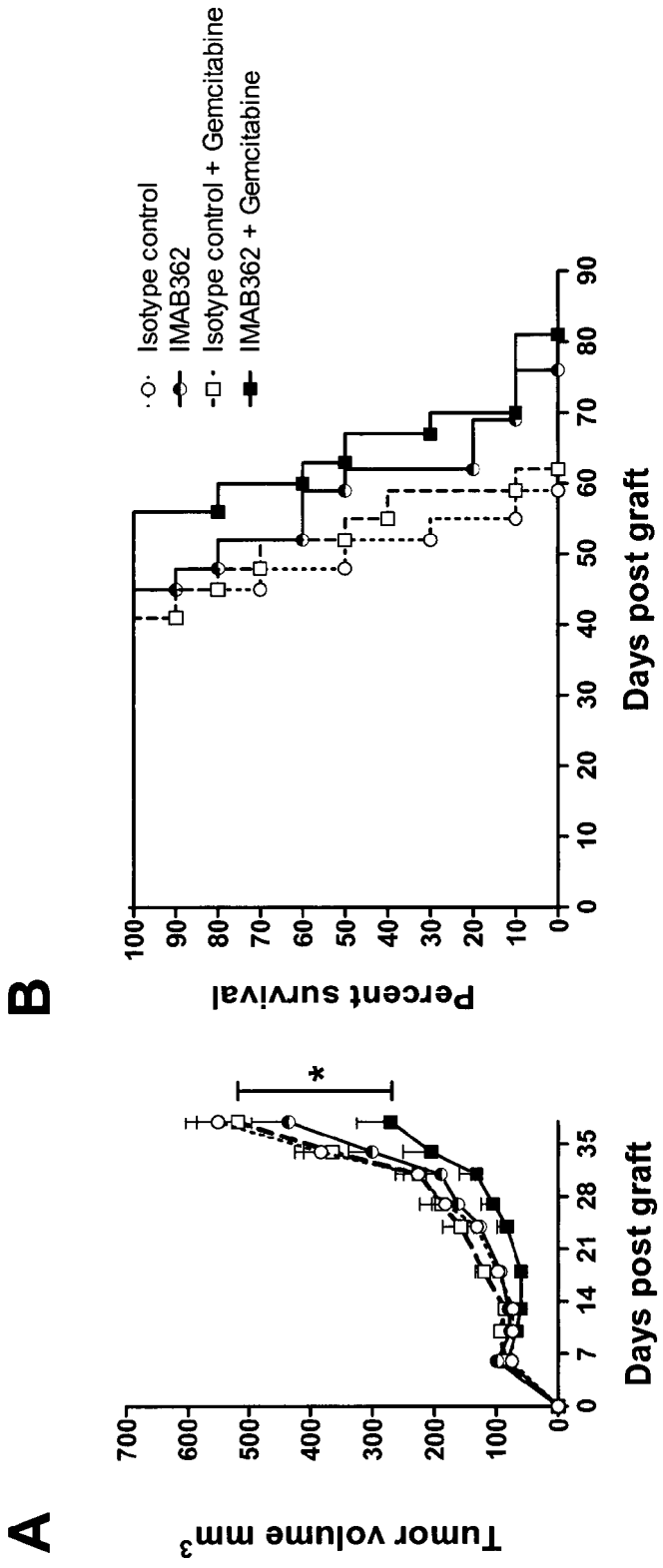

A
Tumor volume mm3
700
600
500
400
300
200
100
0
0 7 14 21 28 35
Days post graft
*
B
Percent survival
100 90 80 70 60 50 40 30 20 10 0
0 10 20 30 40 50 60 70 80 90
Days post graft
Isotype control
IMAB362
Isotype control + Gemcitabine
IMAB362 + Gemcitabine

Figure 41

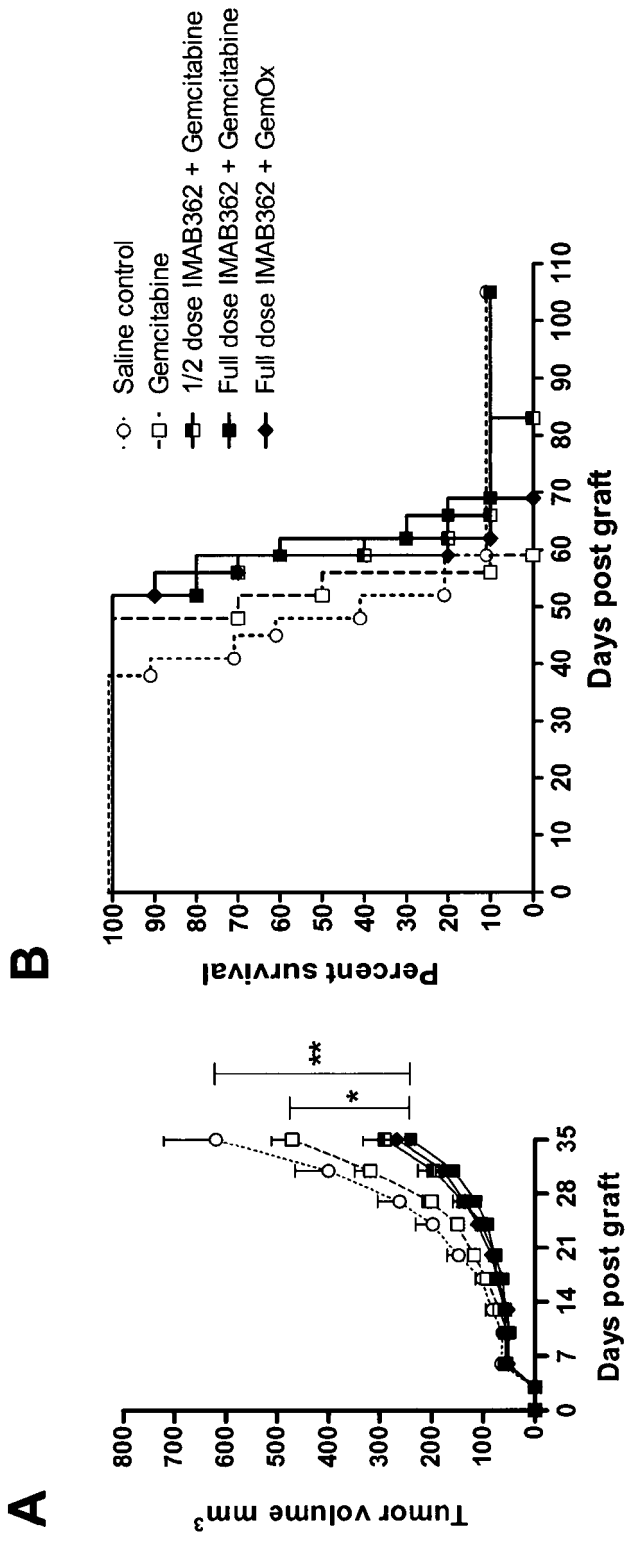

A
Tumor volume mm³
800
700
600
500
400
300
200
100
0
0
7
14
21
28
35
Days post graft
**
*
B
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
110
Days post graft
Saline control
Gemcitabine
1/2 dose IMAB362 + Gemcitabine
Full dose IMAB362 + Gemcitabine
Full dose IMAB362 + GemOx

Figure 42

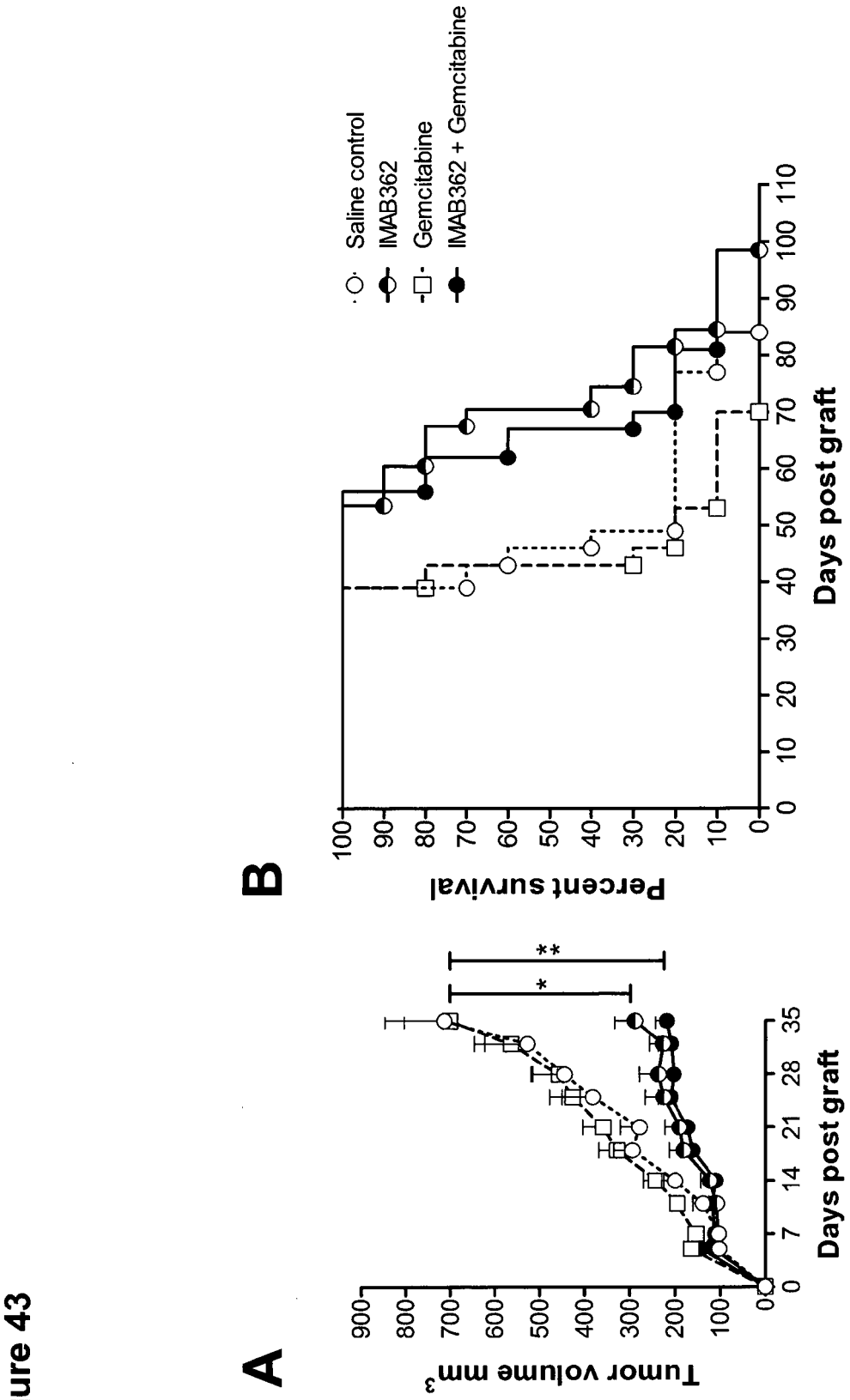

A
Tumor volume mm³
900
800
700
600
500
400
300
200
100
0
0
7
14
21
28
35
Days post graft
**
*
B
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
110
Days post graft
Saline control
IMAB362
Gemcitabine
IMAB362 + Gemcitabine

Figure 43

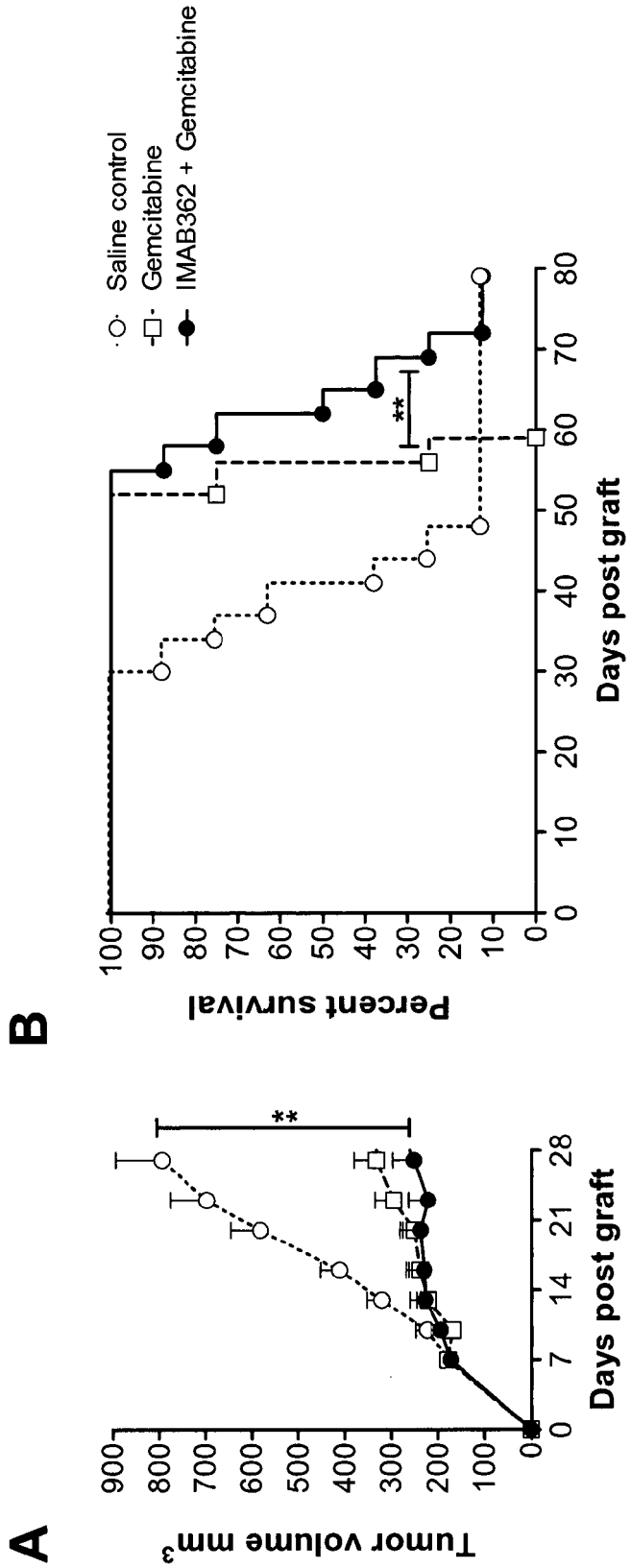

A
Tumor volume mm3
900
800
700
600
500
400
300
200
100
0
0
7
14
21
28
Days post graft
**
B
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
Days post graft
**
Saline control
Gemcitabine
IMAB362 + Gemcitabine

Figure 44

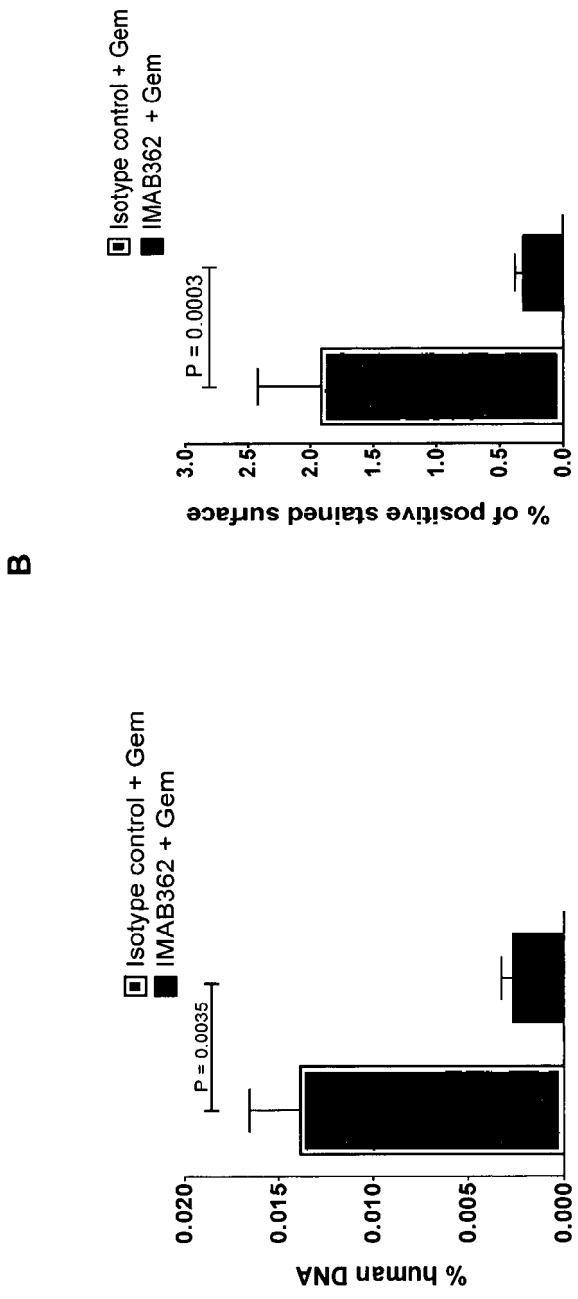
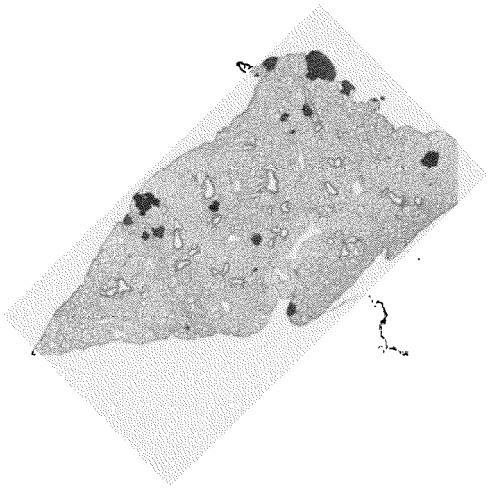

A
% human DNA
0.020
0.015
0.010
0.005
0.000
P = 0.0035
Isotype control + Gem
IMAB362 + Gem
B
% of positive stained surface
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P = 0.0003
Isotype control + Gem
IMAB362 + Gem

Figure 45



D



C

Figure 45