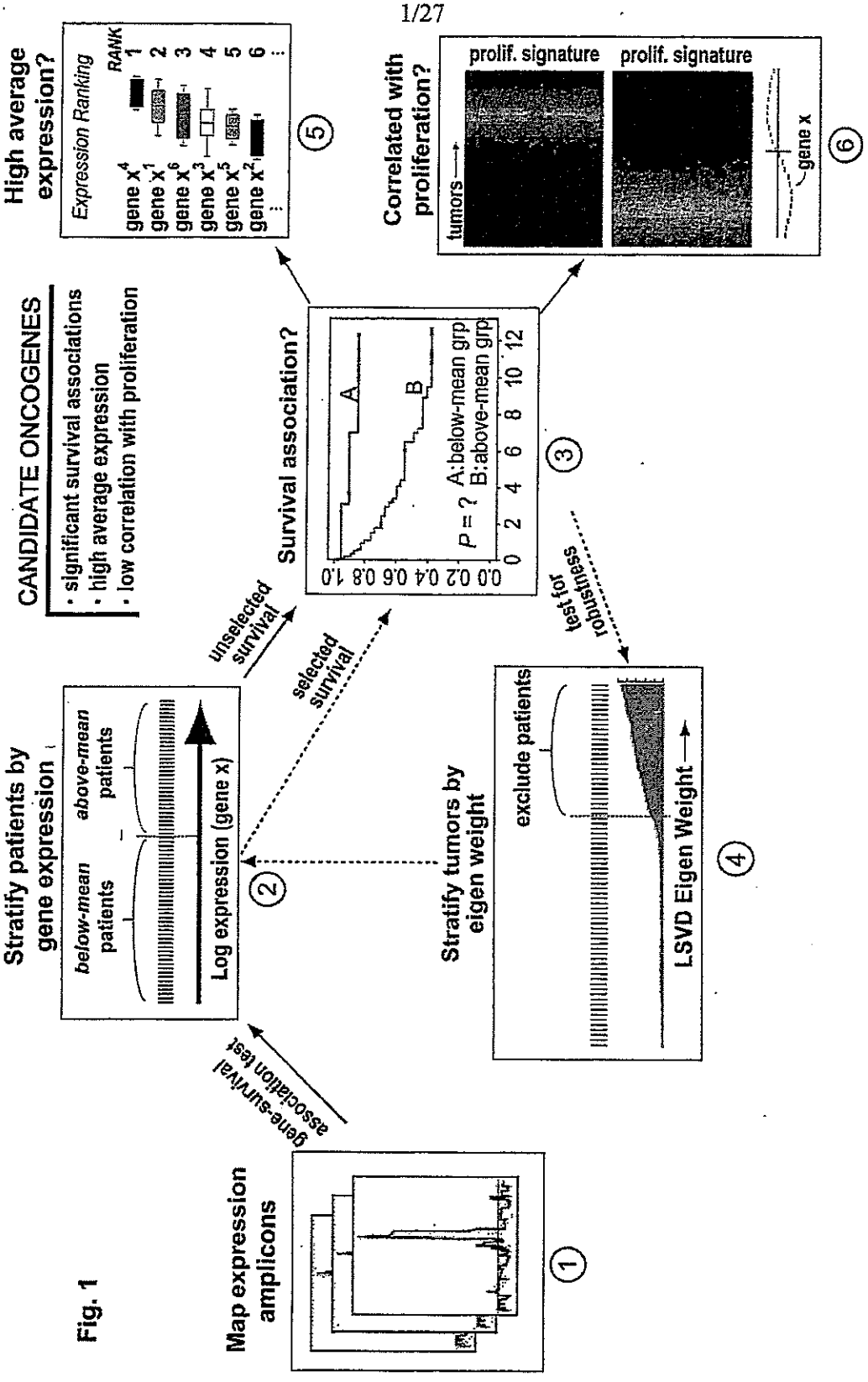

Map expression amplicons
1
gene-survival association test
Stratify patients by gene expression
below-mean patients
above-mean patients
Log expression (gene x)
2
unselected survival
selected survival
Survival association?
A
B
P = ?
A:below-mean grp
B:above-mean grp
0 2 4 6 8 10 12
0.0 0.2 0.4 0.6 0.8 1.0
3
test for robustness
Stratify tumors by eigen weight
exclude patients
LSVD Eigen Weight
4
CANDIDATE ONCOGENES
• significant survival associations
• high average expression
• low correlation with proliferation
High average expression?
Expression Ranking
RANK
gene x4 1
gene x1 2
gene x6 3
gene x3 4
gene x5 5
gene x2 6
5
Correlated with proliferation?
prolif. signature
prolif. signature
tumors
gene x
6

Fig. 1

Fig. 2

characteristics	**Uppsala n=251**	**Stockholm n=159**	**Belgium n=227**	**Singapore n=100**
Age (years)				
Median	64	56	61	51
Range	28-93	31-87	24-86	29-86
Tumor Size				
<2 cm	112 (45%)	64 (40%)	86 (38%)	16 (16%)
2-3 cm	101 (40%)	71 (45%)	93 (41%)	47 (47%)
>3 cm	38 (15%)	22 (14%)	48 (21%)	35 (35%)
Unknown	0 (0%)	2 (1%)	0 (0%)	2 (2%)
Histologic Grade				
Grade 1	67 (27%)	28 (18%)	34 (15%)	11 (11%)
Grade 2	127 (51%)	58 (36%)	91 (40%)	40 (40%)
Grade 3	55 (22%)	61 (38%)	60 (26%)	47 (47%)
Unknown	2 (0.8%)	12 (8%)	42 (19%)	2 (2%)
Lymph Node Status				
Positive	82 (33%)	60 (38%)	38 (17%)	45 (45%)
Negative	160 (64%)	94 (59%)	183 (81%)	53 (53%)
Unknown	9 (4%)	5 (3%)	6 (3%)	2 (2%)
ER Status				
Positive	213 (85%)	130 (82%)	171 (75%)	61 (61%)
Negative	34 (14%)	29 (18%)	48 (21%)	36 (36%)
Unknown	4 (2%)	0 (0%)	8 (4%)	3 (3%)
Metastasis within 5 years				
Yes	42 (17%)	26 (16%)	43 (19%)	15 (15%)
No	183 (73%)	121 (76%)	135 (59%)	35 (35%)
Censored	26 (10%)	12 (8%)	49 (22%)	50 (50%)
Treatment				
No Systemic Therapy	143 (57%)	33 (21%)	77 (34%)	10 (10%)
Endocrine	80 (32%)	96 (60%)	109 (48%)	23 (23%)
Chemo	24 (10%)	12 (8%)	41 (18%)	30 (30%)
Endocrine & Chemo	4 (2%)	18 (11%)	0 (0%)	15 (15%)
Unknown	0 (0%)	0 (0%)	0 (0%)	22 (22%)

Fig. 3

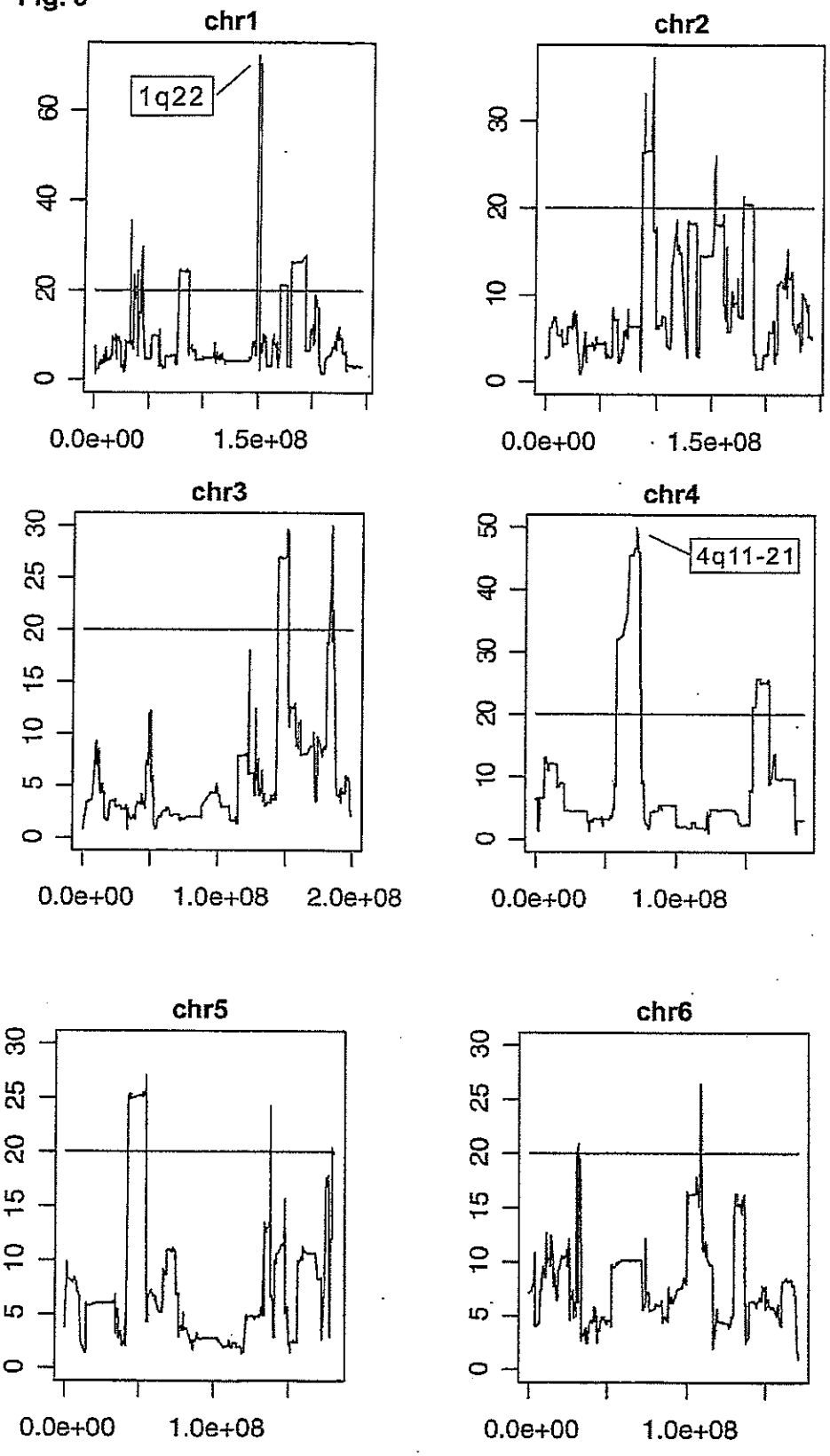

chr1
1q22
0
20
40
60
0.0e+00
1.5e+08
chr2
0
10
20
30
0.0e+00
1.5e+08
chr3
0
5
10
15
20
25
30
0.0e+00
1.0e+08
2.0e+08
chr4
4q11-21
0
10
20
30
40
50
0.0e+00
1.0e+08
chr5
0
5
10
15
20
25
30
0.0e+00
1.0e+08
chr6
0
5
10
15
20
25
30
0.0e+00
1.0e+08

Fig. 3 (cont.)

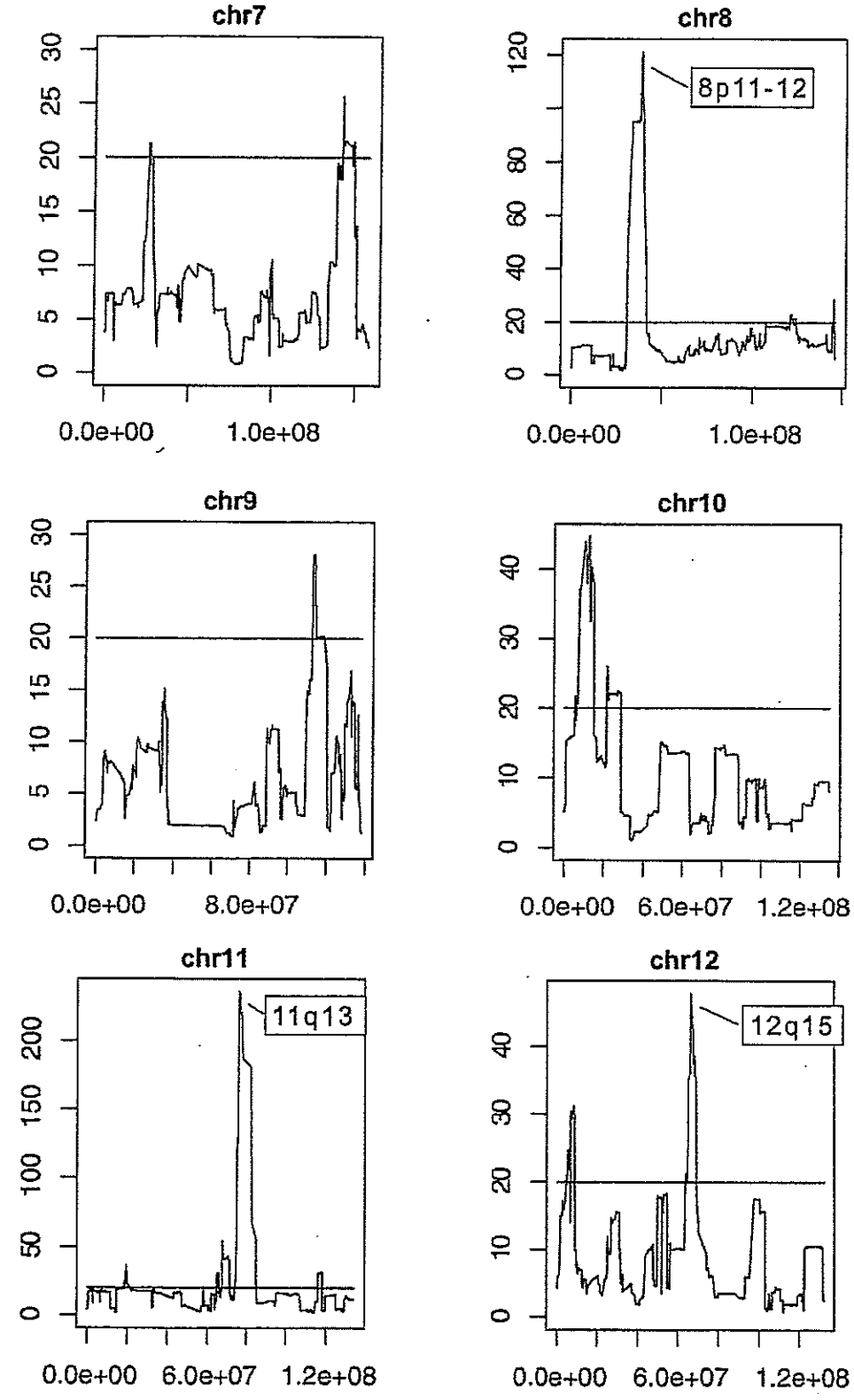

chr7
0
5
10
15
20
25
30
0.0e+00
1.0e+08
chr8
0
20
40
60
80
120
8p11-12
0.0e+00
1.0e+08
chr9
0
5
10
15
20
25
30
0.0e+00
8.0e+07
chr10
0
10
20
30
40
0.0e+00
6.0e+07
1.2e+08
chr11
0
50
100
150
200
11q13
0.0e+00
6.0e+07
1.2e+08
chr12
0
10
20
30
40
12q15
0.0e+00
6.0e+07
1.2e+08

Fig. 3 (cont.)

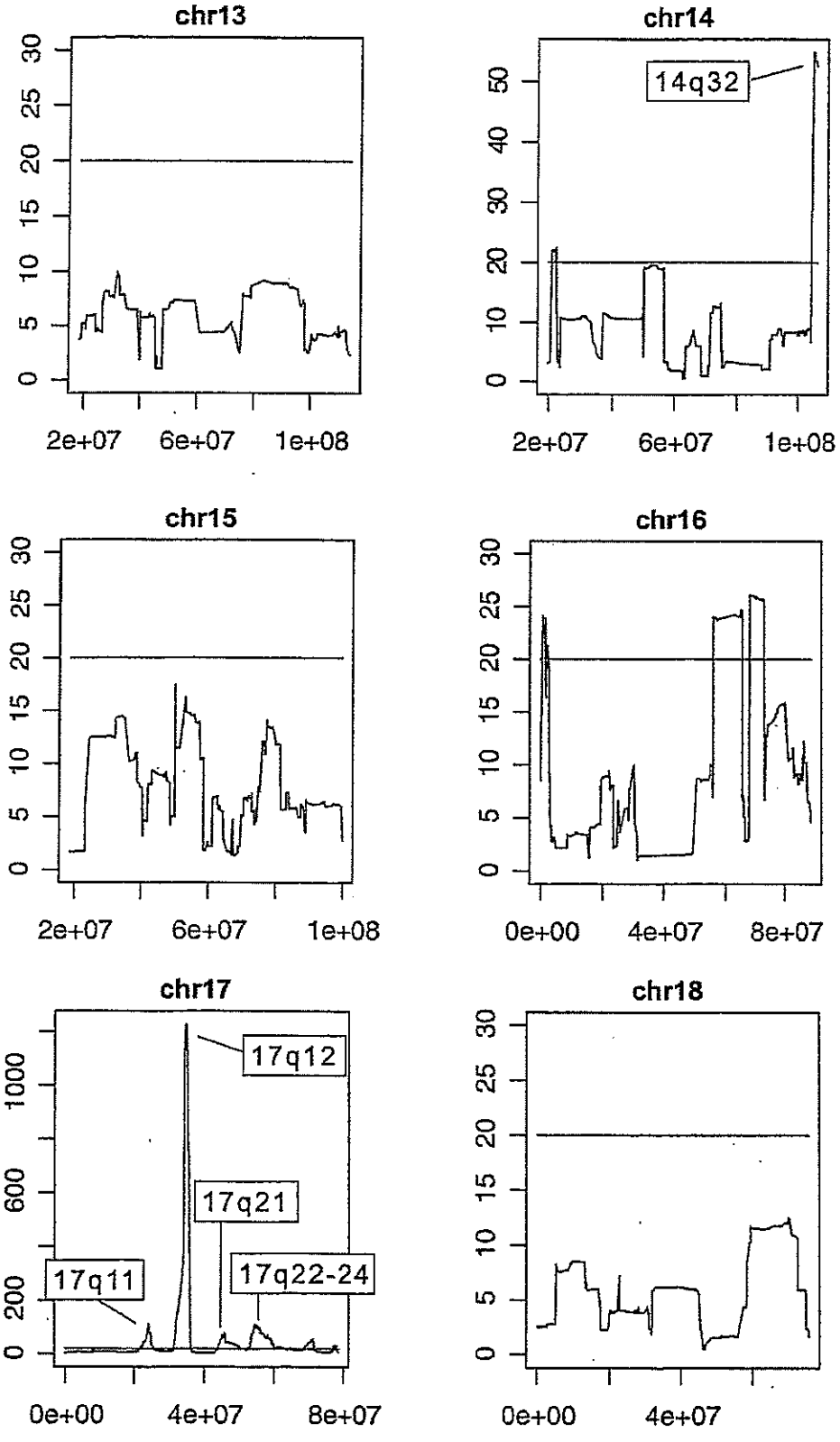
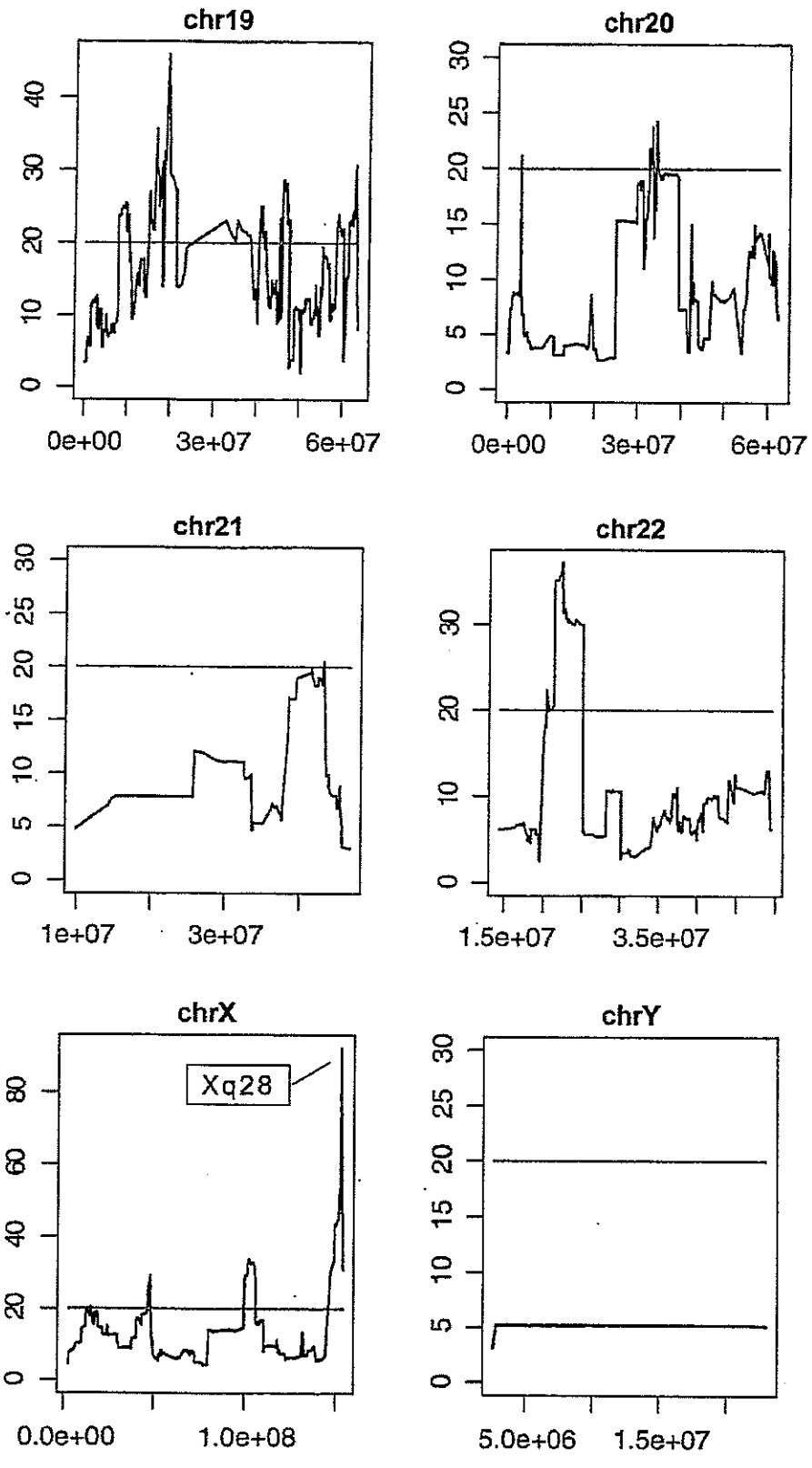

chr13
0
5
10
15
20
25
30
2e+07
6e+07
1e+08
chr14
14q32
0
10
20
30
40
50
2e+07
6e+07
1e+08
chr15
0
5
10
15
20
25
30
2e+07
6e+07
1e+08
chr16
0
5
10
15
20
25
30
0e+00
4e+07
8e+07
chr17
17q12
17q21
17q11
17q22-24
0
200
600
1000
0e+00
4e+07
8e+07
chr18
0
5
10
15
20
25
30
0e+00
4e+07

Fig. 3 (cont.)


chr19
0
10
20
30
40
0e+00
3e+07
6e+07
chr20
0
5
10
15
20
25
30
0e+00
3e+07
6e+07
chr21
0
5
10
15
20
25
30
1e+07
3e+07
chr22
0
10
20
30
1.5e+07
3.5e+07
chrX
Xq28
0
20
40
60
80
0.0e+00
1.0e+08
chrY
0
5
10
15
20
25
30
5.0e+06
1.5e+07

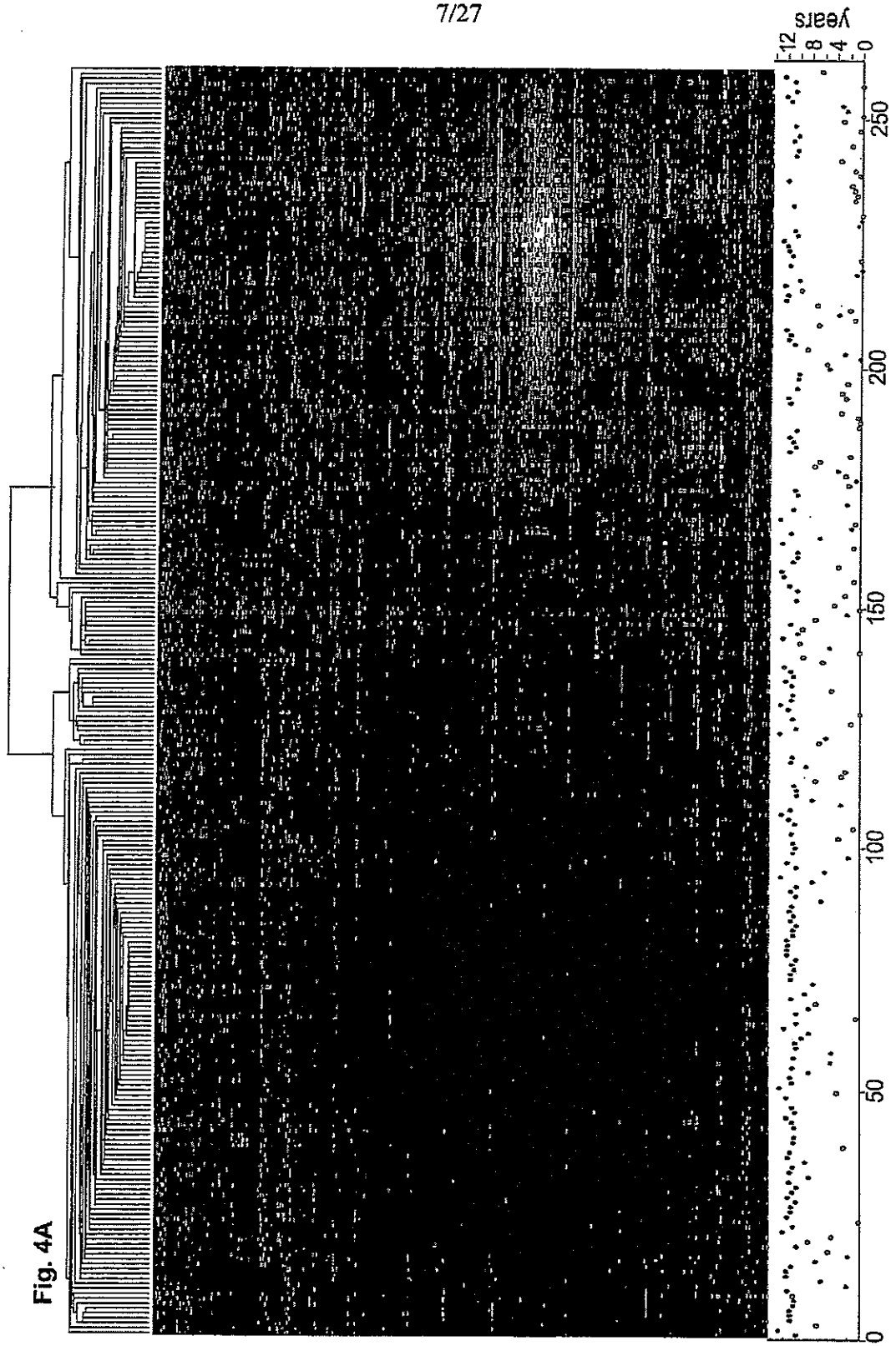


Fig. 4A

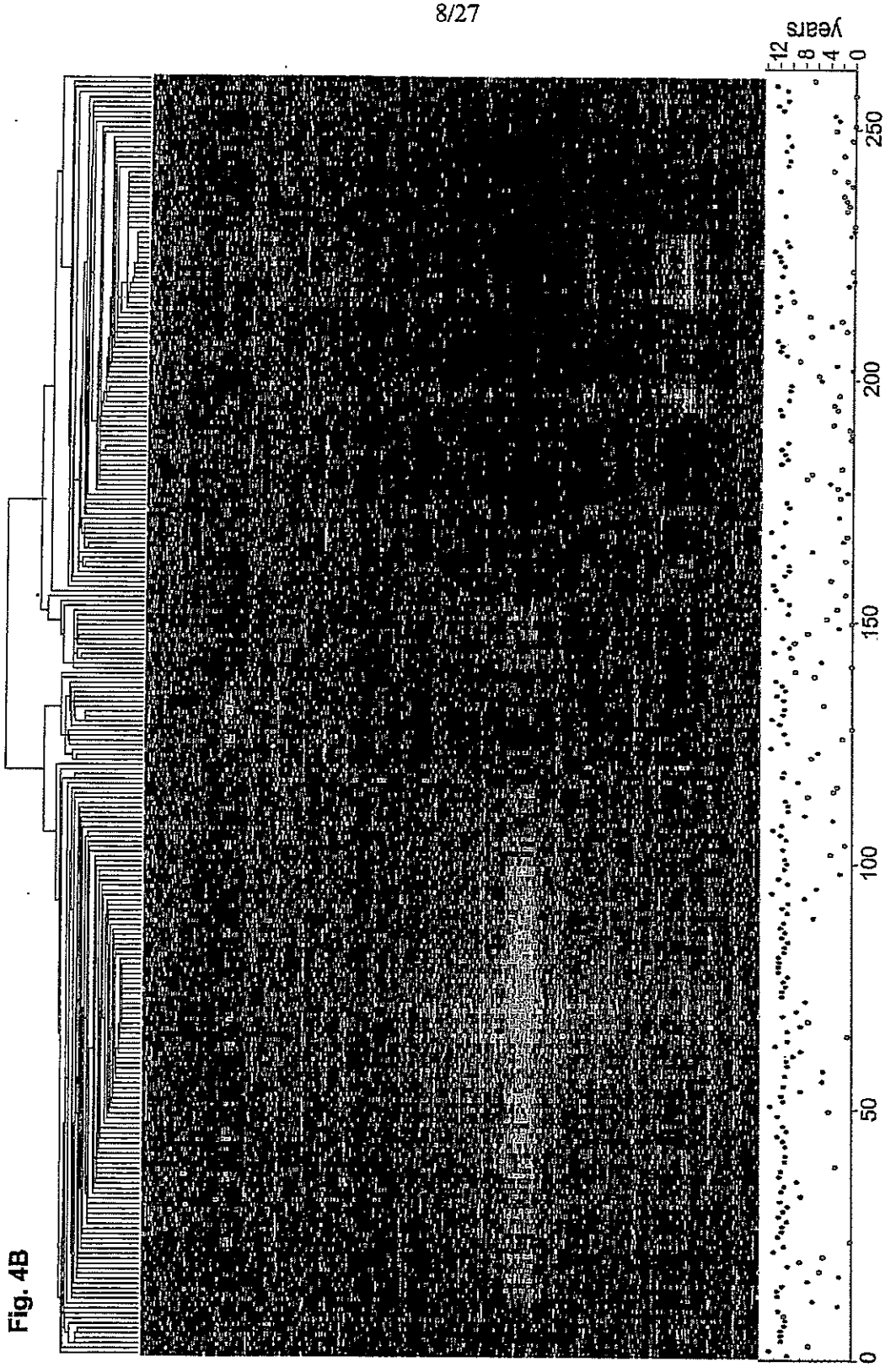


Fig. 4B

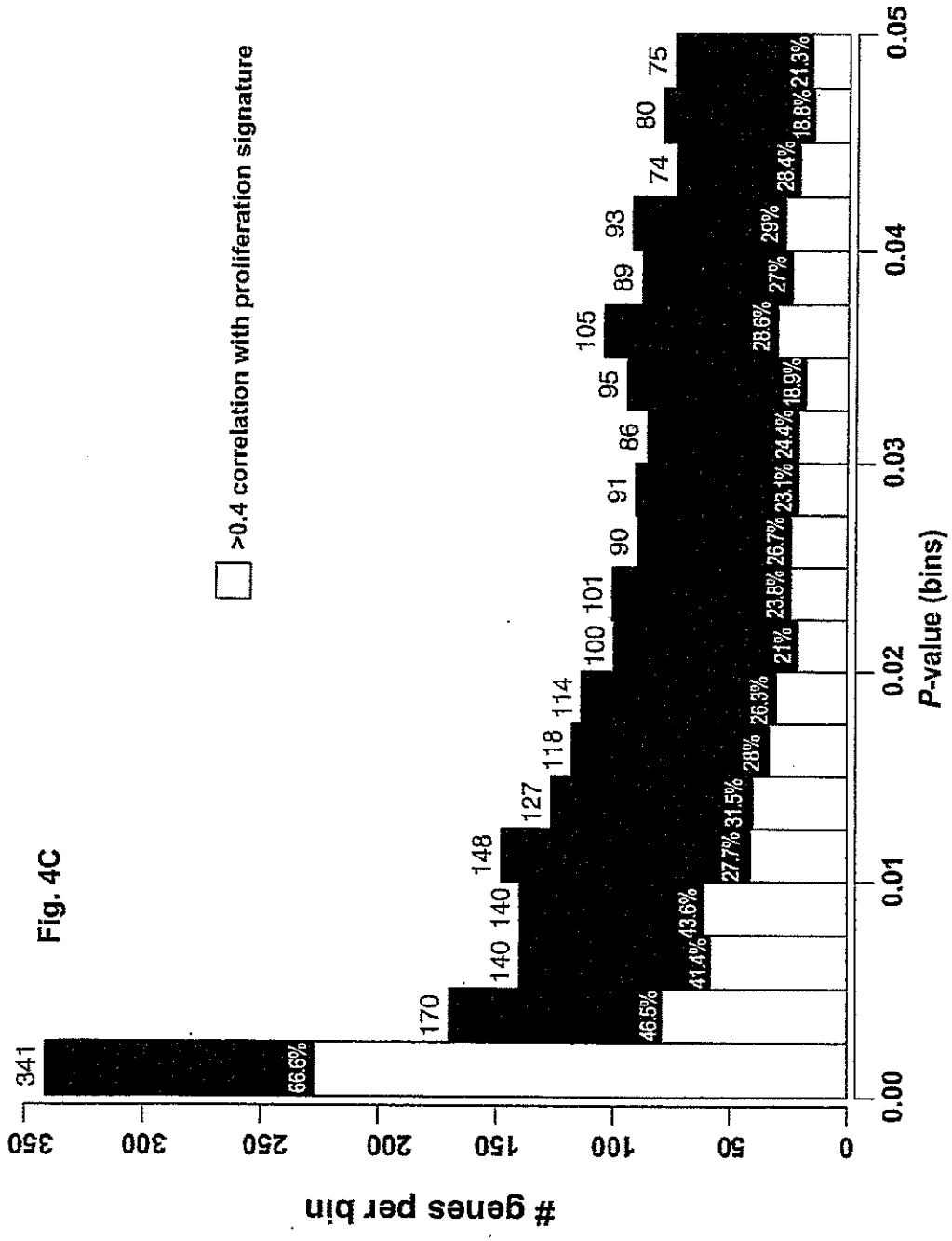
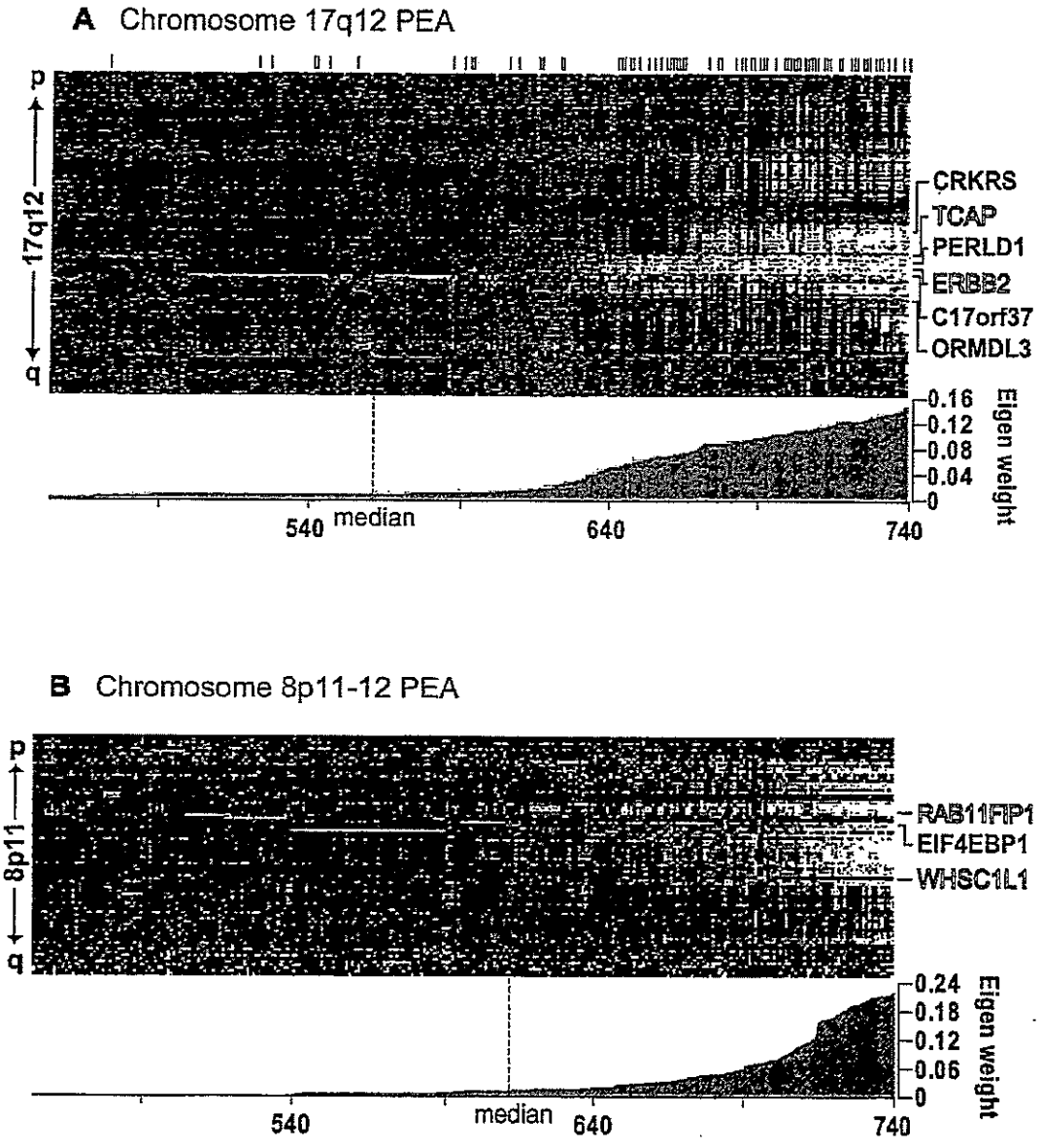

>0.4 correlation with proliferation signature
genes per bin
0
50
100
150
200
250
300
350
P-value (bins)
0.00
0.01
0.02
0.03
0.04
0.05
341
66.6%
170
46.5%
140
41.4%
140
43.6%
148
27.7%
127
31.5%
118
28%
114
26.3%
100
21%
101
23.8%
90
26.7%
91
23.1%
86
24.4%
95
18.9%
105
28.6%
89
27%
93
29%
74
28.4%
80
18.8%
75
21.3%

Fig. 4C

Fig. 5


A Chromosome 17q12 PEA
p
17q12
q
CRKRS
TCAP
PERLD1
ERBB2
C17orf37
ORMDL3
0.16
0.12
0.08
0.04
0
Eigen weight
540
median
640
740
B Chromosome 8p11-12 PEA
p
8p11
q
RAB11FIP1
EIF4EBP1
WHSC1L1
0.24
0.18
0.12
0.06
0
Eigen weight
540
median
640
740

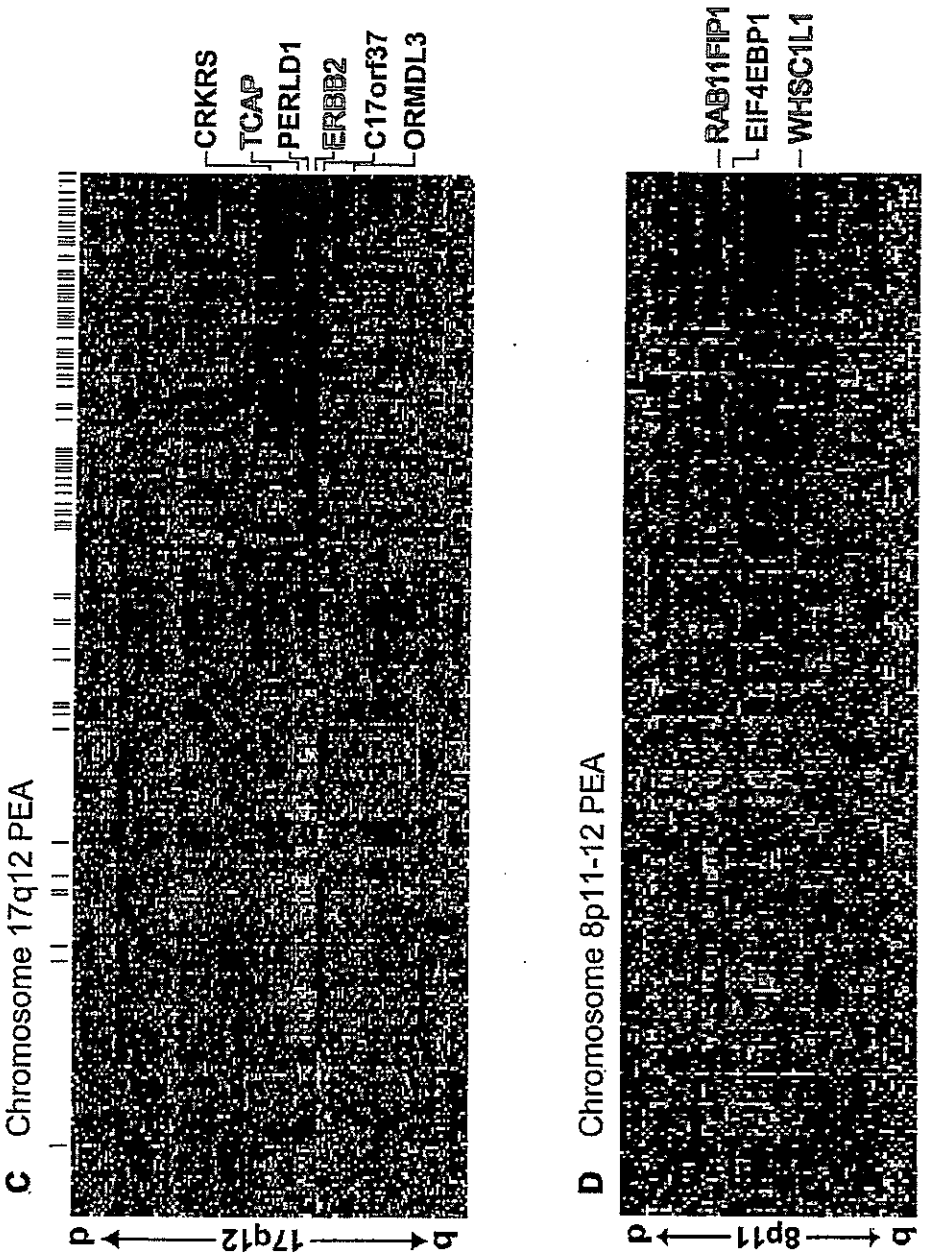

C Chromosome 17q12 PEA
CRKRS
TCAP
PERLD1
ERBB2
C17orf37
ORMDL3
p
17q12
q
D Chromosome 8p11-12 PEA
RAB11FIP1
EIF4EBP1
WHSC1L1
p
8p11
q

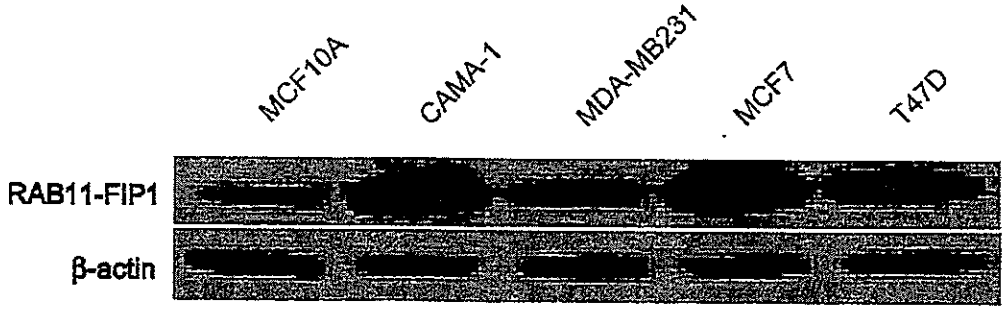
Fig. 5E

TRIAGE output:

TRIAGE steps:	2,3	4	5	6
	unselected survival	selected survival	exp. rank	prolif. corr.
CRKRS	0.006	NS	29	0.45*
TCAP	0.004	0.038	19	0.20
PERLD1	0.039	NS	12	0.17
ERBB2	0.010	0.037	3	0.13
C17orf37	0.006	NS	2	0.42*
ORMDL3	0.011	NS	14	0.18
	unselected survival	selected survival	exp. rank	prolif. corr.
RAB11FIP1	0.0002	0.004	4	0.16
EIF4EBP1	0.006	NS	13	0.58*
WHSC1L1	0.035	0.049	46	0.05

Fig. 6A

Endogenous Expression of RAB11-FIP1 (RCP)



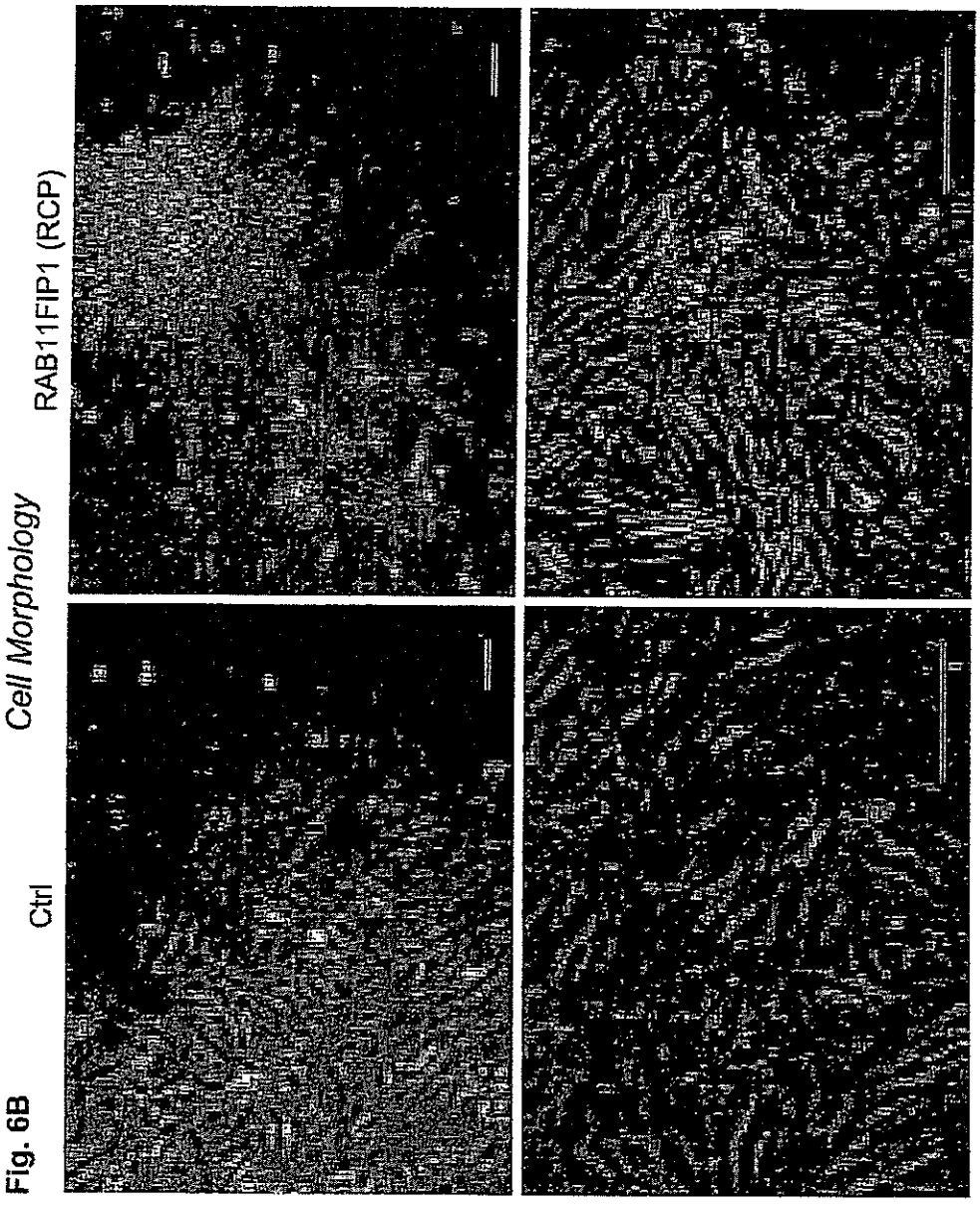


Fig. 6B

Fig. 6C

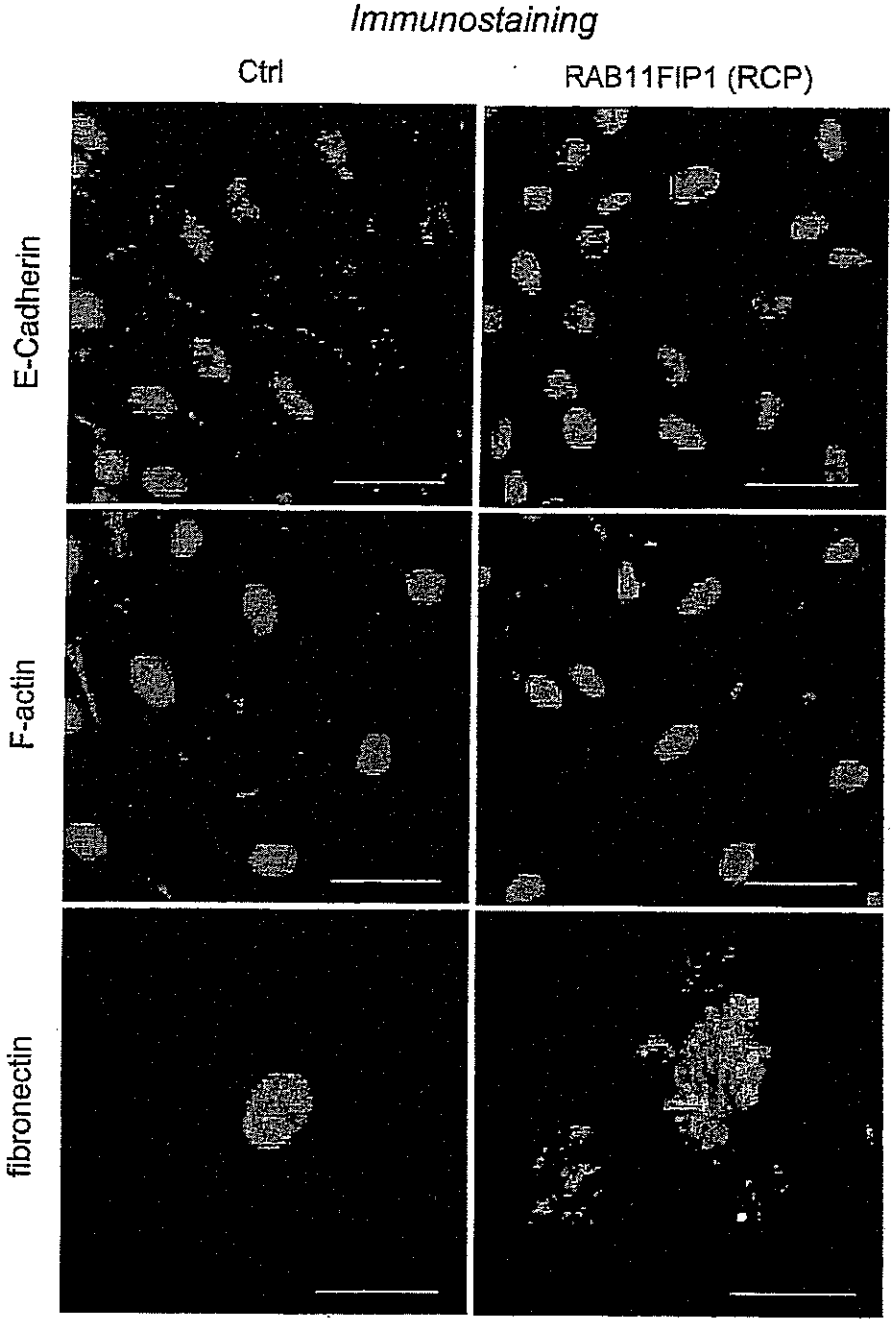

Immunostaining
Ctrl
RAB11FIP1 (RCP)
E-Cadherin
F-actin
fibronectin

Fig. 6D

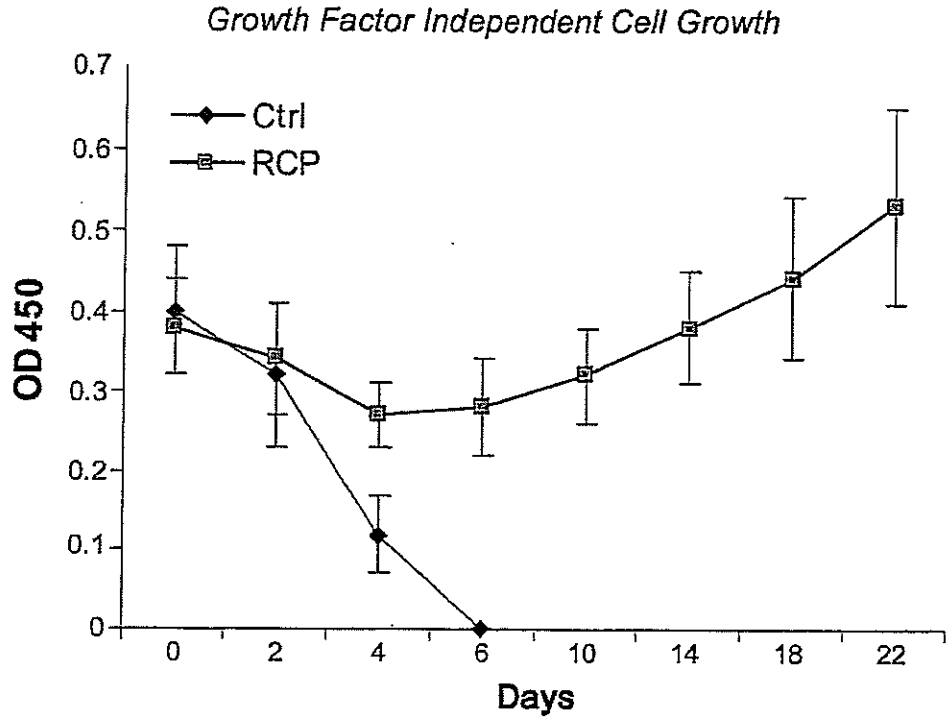
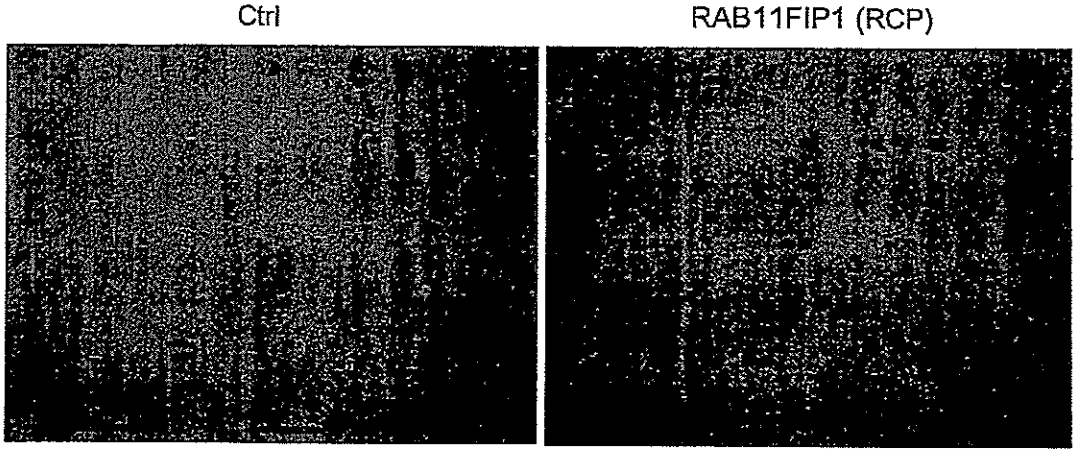


Fig. 6E *Anchorage-Independent Growth*



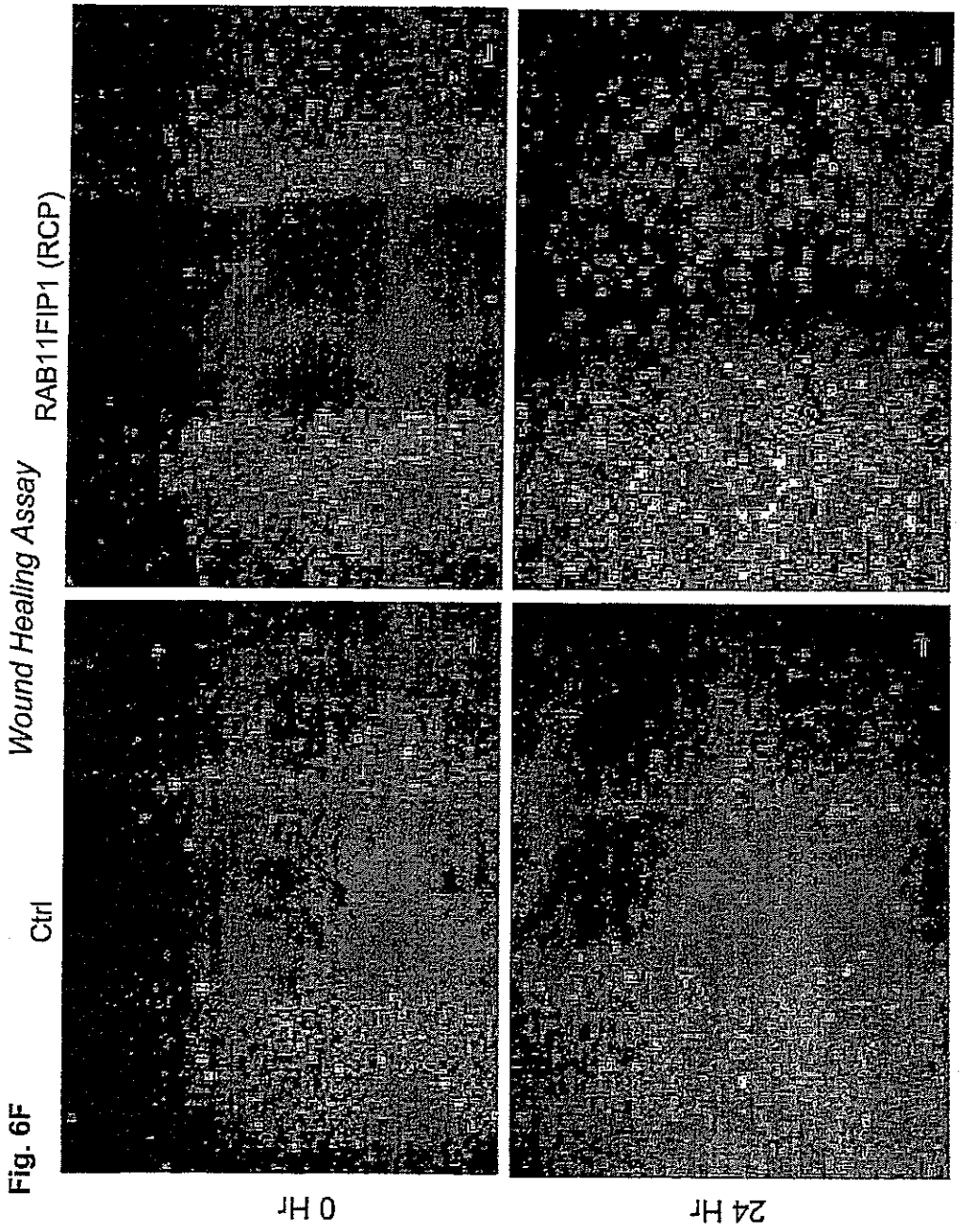
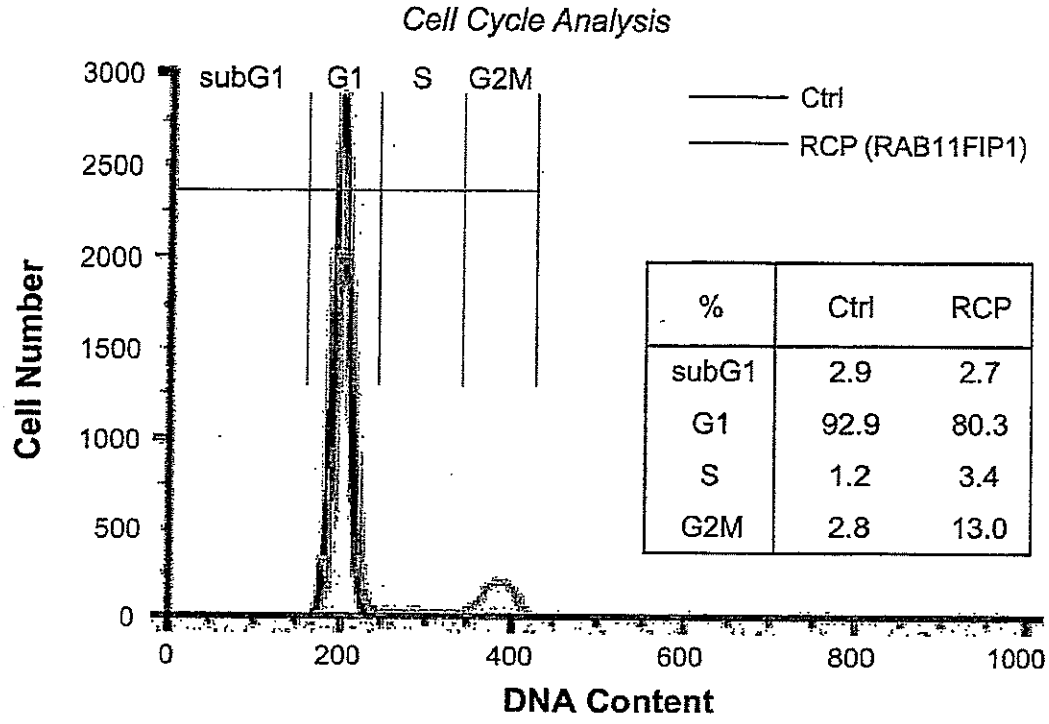


Fig. 6F

Fig. 7A



%	Ctrl	RCP
subG1	2.9	2.7
G1	92.9	80.3
S	1.2	3.4
G2M	2.8	13.0

Fig. 7B

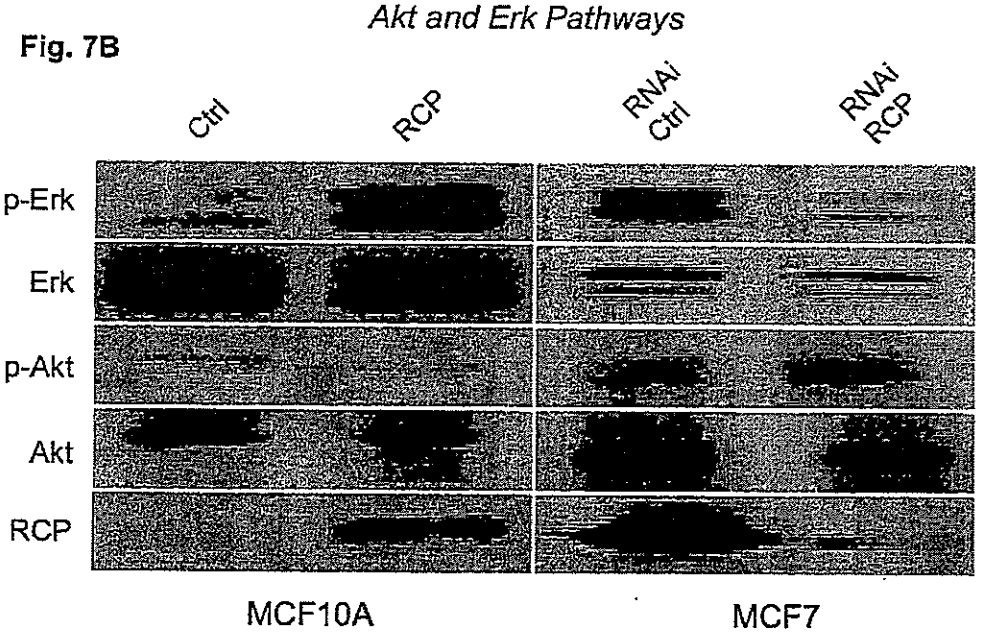


Fig. 7C

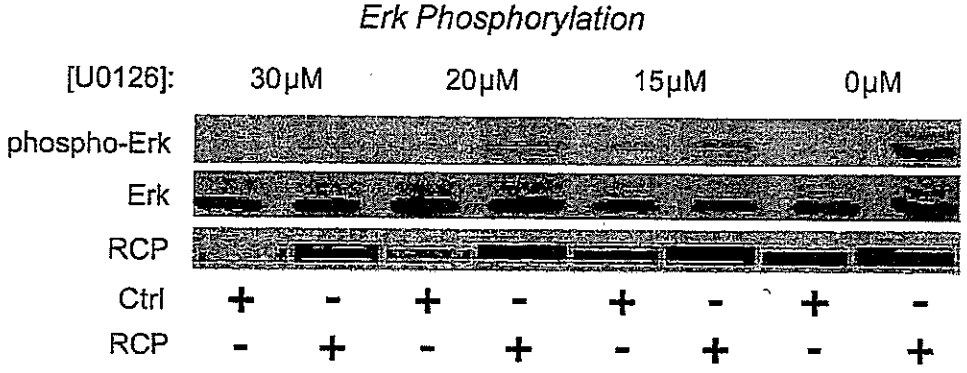

Erk Phosphorylation
[U0126]:
30μM
20μM
15μM
0μM
phospho-Erk
Erk
RCP
Ctrl
RCP
+ - + - + - + -
- + - + - + - +

Fig. 7D

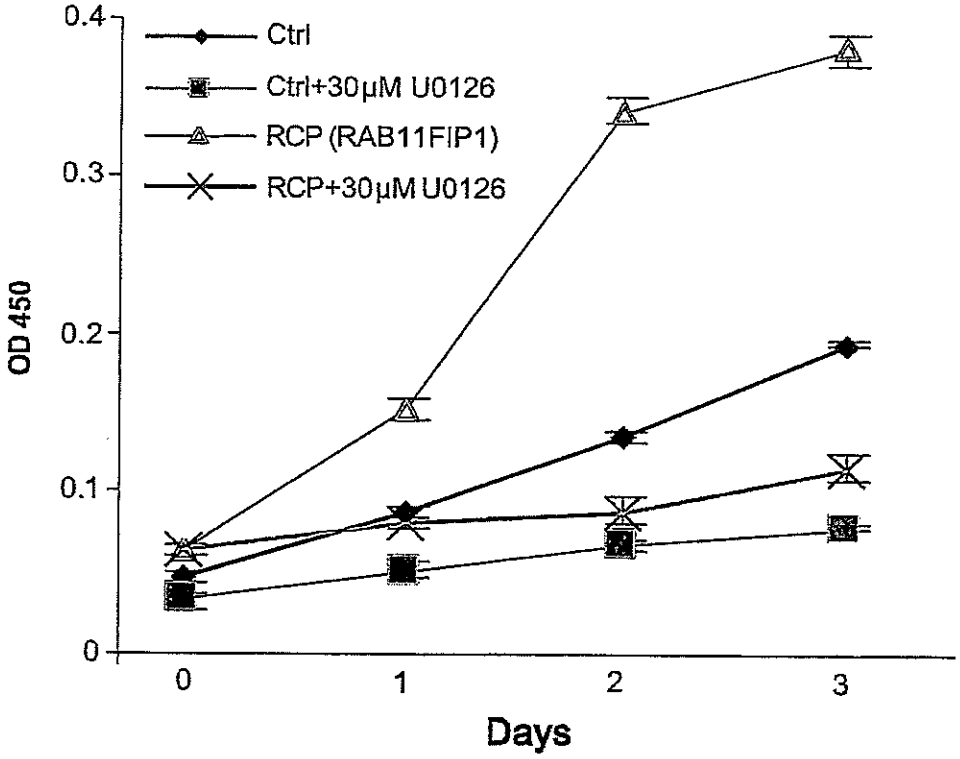

0.4
0.3
0.2
0.1
0
OD 450
Ctrl
Ctrl+30μM U0126
RCP (RAB11FIP1)
RCP+30μM U0126
0
1
2
3
Days

Fig. 8A

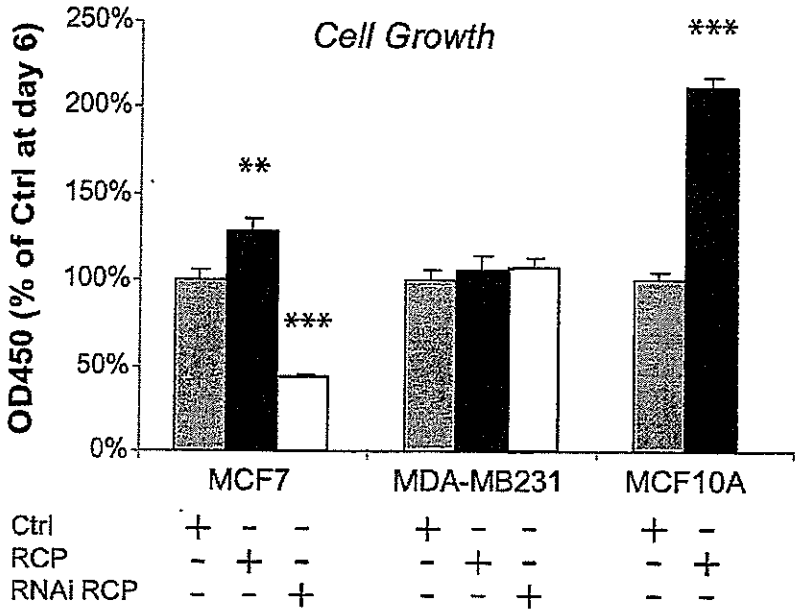

Cell Growth
OD450 (% of Ctrl at day 6)
250%
200%
150%
100%
50%
0%
**
***
***
MCF7
MDA-MB231
MCF10A
Ctrl + - - + - - + -
RCP - + - - + - - +
RNAi RCP - - + - - + - -

Fig. 8B

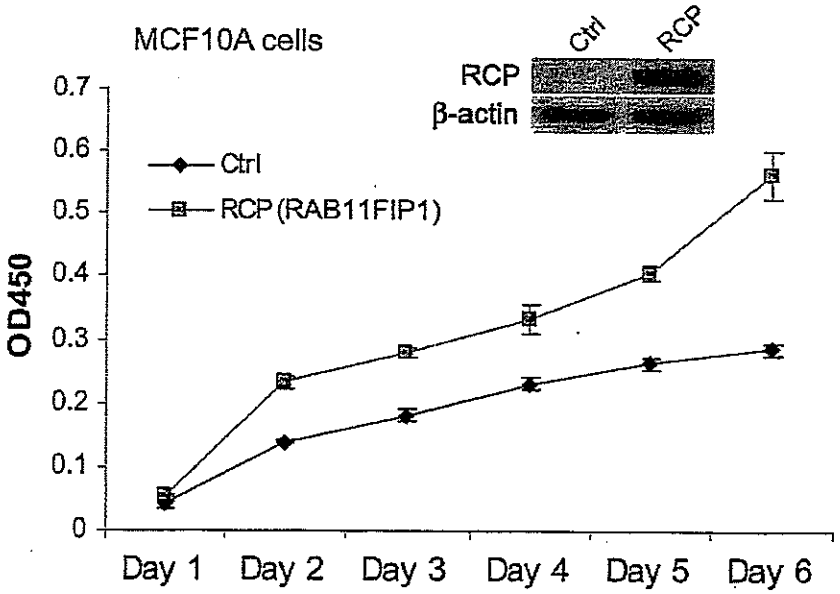

MCF10A cells
Ctrl
RCP
RCP
β-actin
Ctrl
RCP (RAB11FIP1)
OD450
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6

Fig. 8C

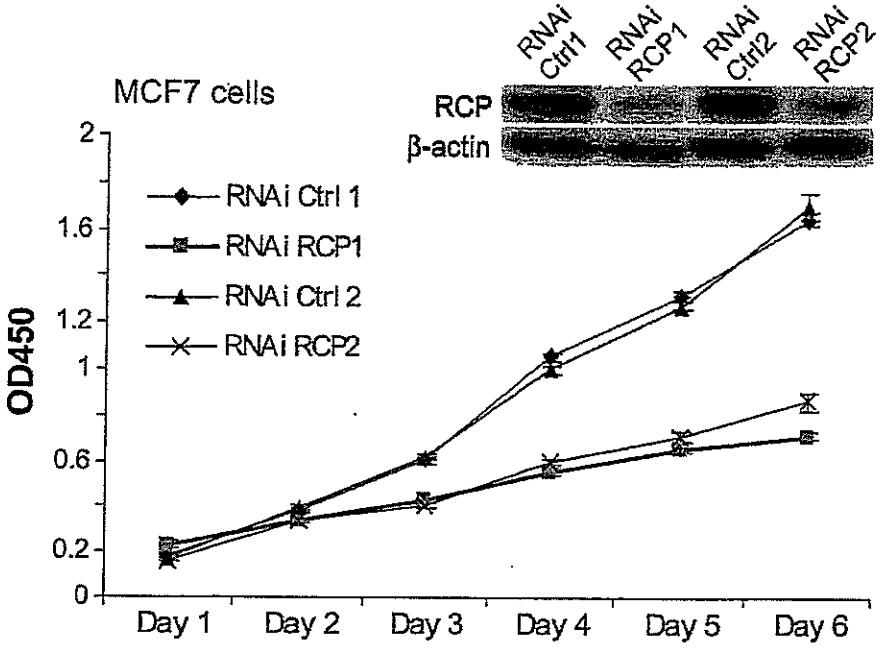

RNAi Ctrl1
RNAi RCP1
RNAi Ctrl2
RNAi RCP2
RCP
β-actin
MCF7 cells
RNAi Ctrl 1
RNAi RCP1
RNAi Ctrl 2
RNAi RCP2
OD450
2
1.6
1.2
1
0.6
0.2
0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6

Fig. 8D

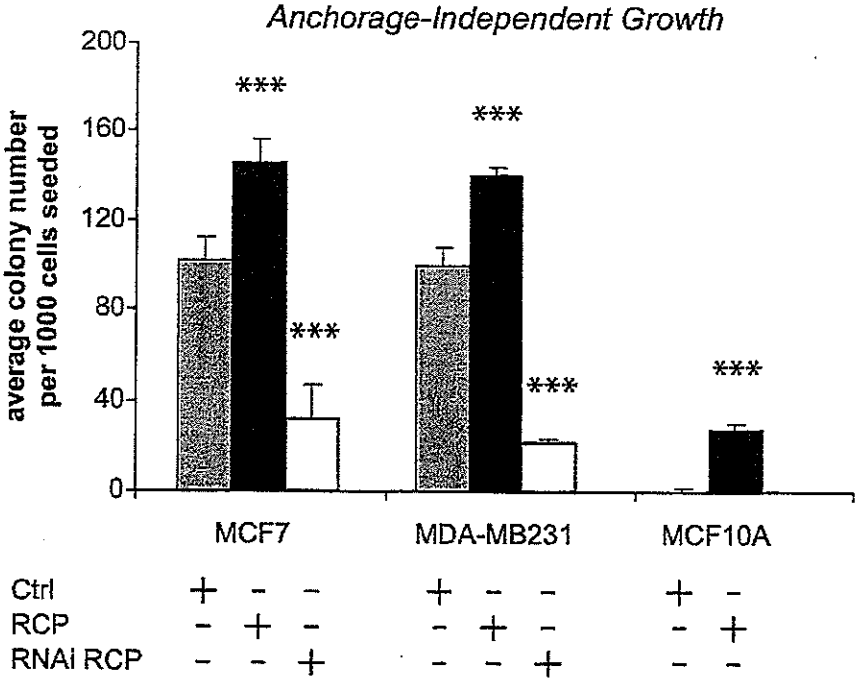

Anchorage-Independent Growth
average colony number per 1000 cells seeded
200
160
120
80
40
0
***
***
***
***
***
MCF7
MDA-MB231
MCF10A
Ctrl
RCP
RNAi RCP

Fig. 8E

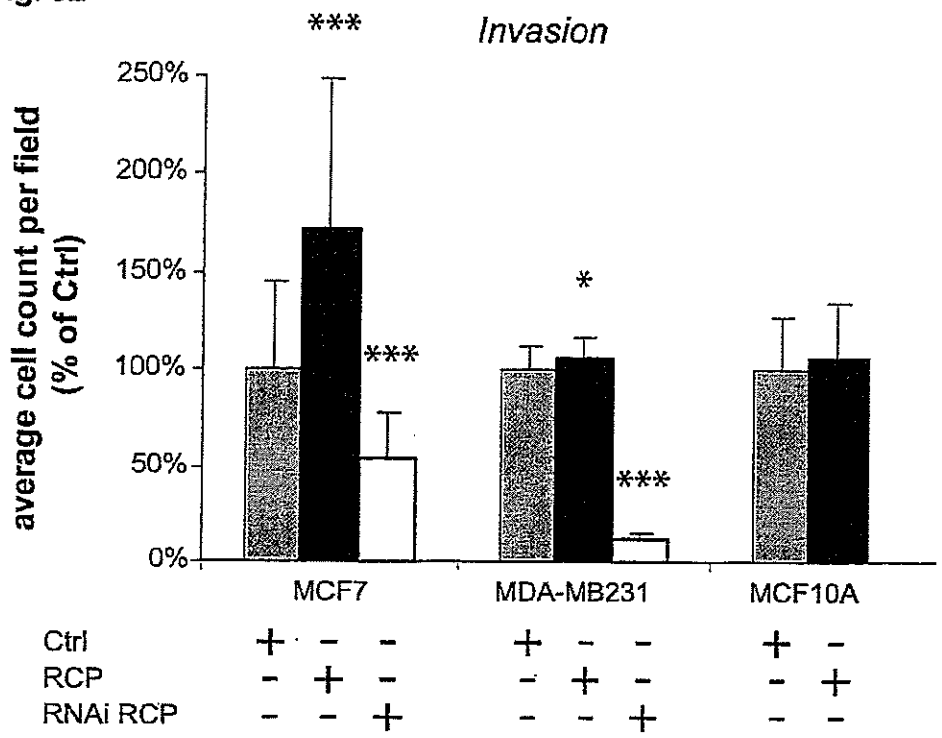

Invasion
***
*
***
***
250%
200%
150%
100%
50%
0%
average cell count per field (% of Ctrl)
MCF7
MDA-MB231
MCF10A
Ctrl + - - + - - + -
RCP - + - - + - - +
RNAi RCP - - + - - + - -

Fig. 8F

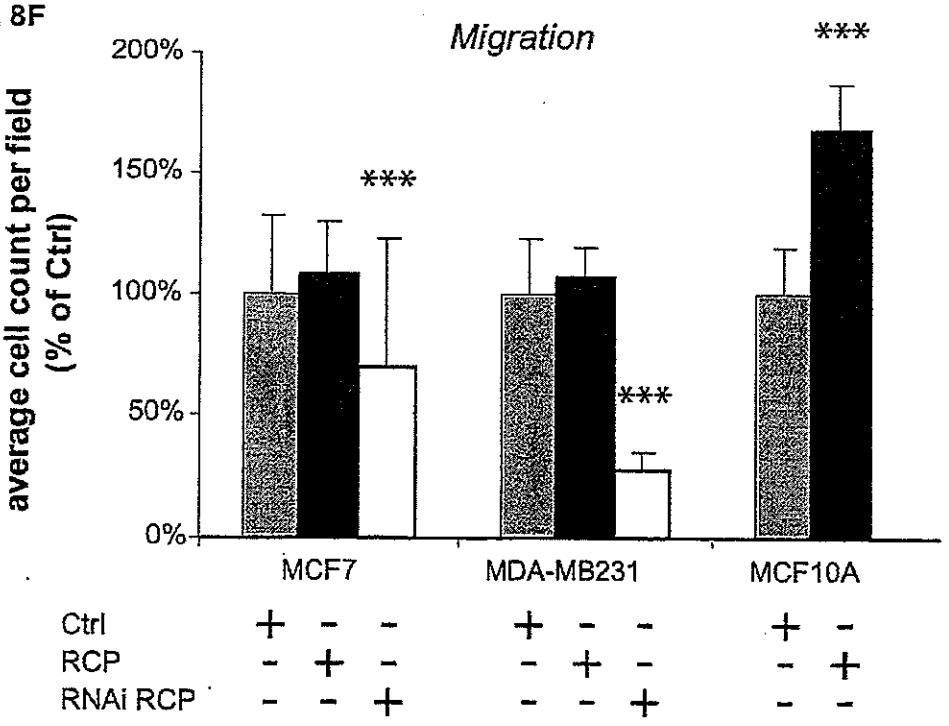

Migration
***
***
***
200%
150%
100%
50%
0%
average cell count per field (% of Ctrl)
MCF7
MDA-MB231
MCF10A
Ctrl + - - + - - + -
RCP - + - - + - - +
RNAi RCP - - + - - + - -

Fig. 9A

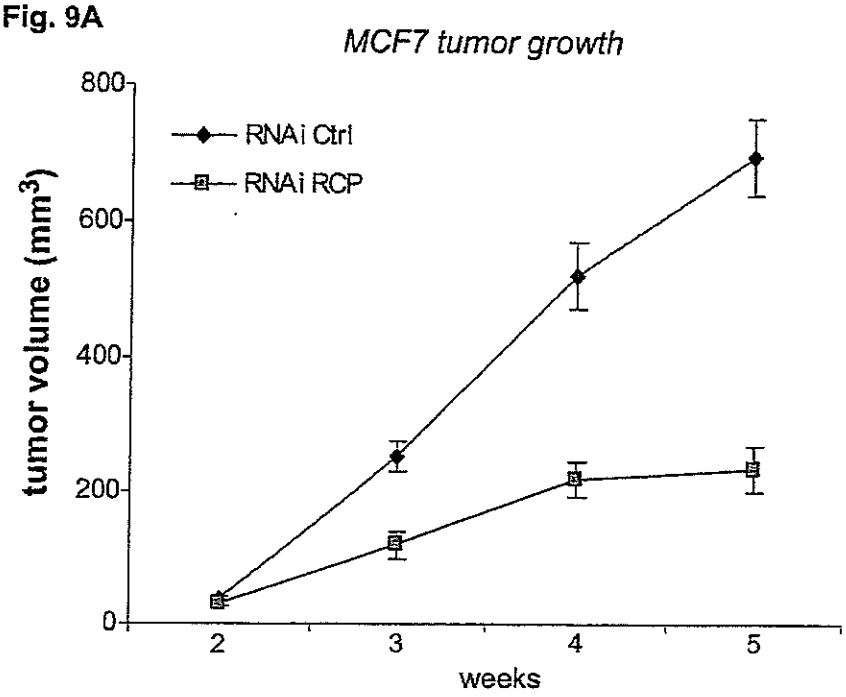

MCF7 tumor growth
800
600
400
200
0
tumor volume (mm3)
RNAi Ctrl
RNAi RCP
2
3
4
5
weeks

Fig. 9B

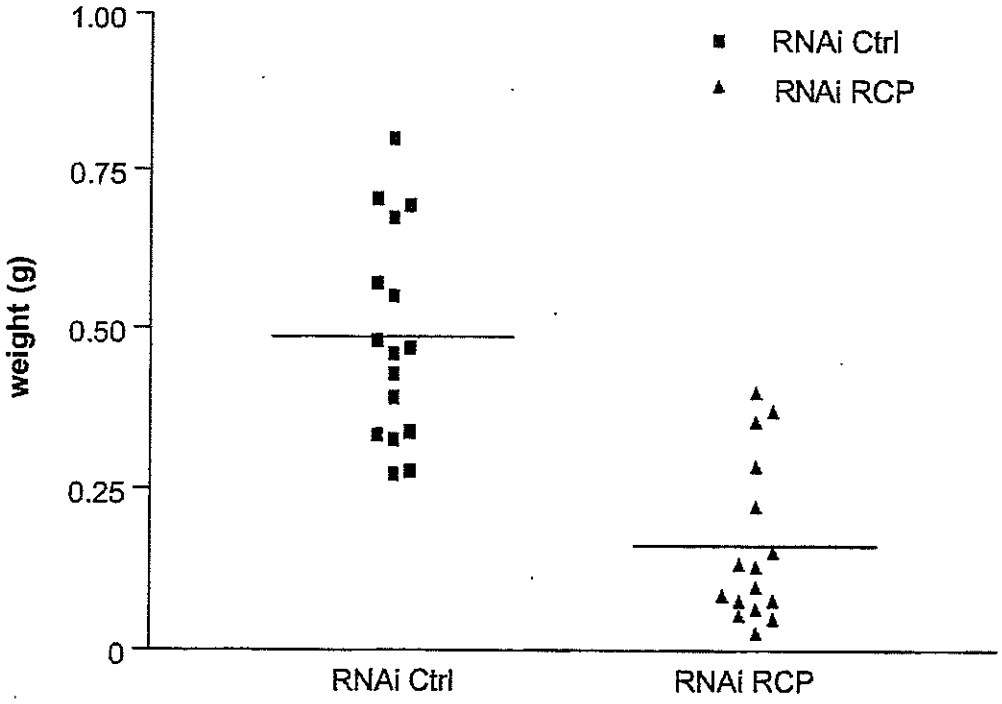

1.00
0.75
0.50
0.25
0
weight (g)
RNAi Ctrl
RNAi RCP
RNAi Ctrl
RNAi RCP

Fig. 9C

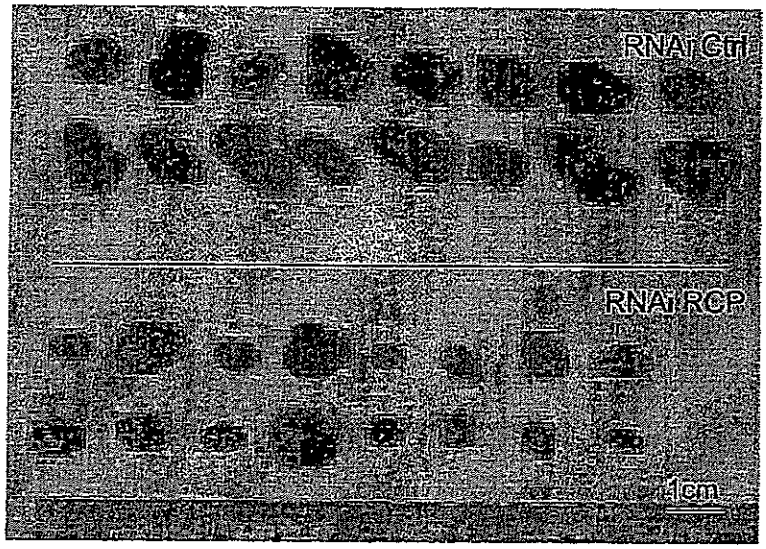

RNAi Ctrl
RNAi RCP
1cm

Fig. 9D

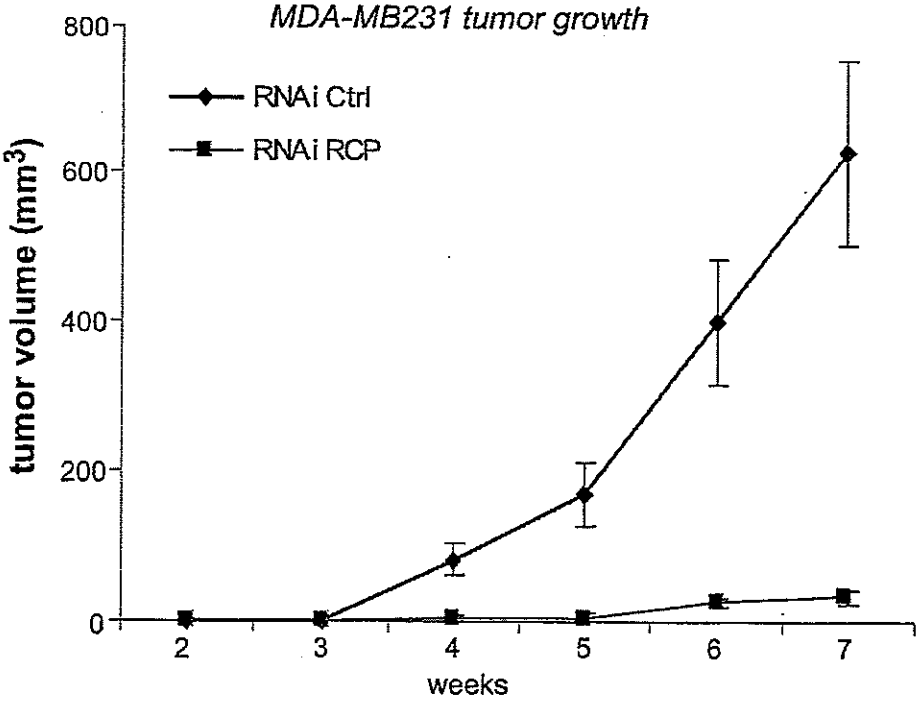

MDA-MB231 tumor growth
RNAi Ctrl
RNAi RCP
tumor volume (mm3)
800
600
400
200
0
2
3
4
5
6
7
weeks

Fig. 9E

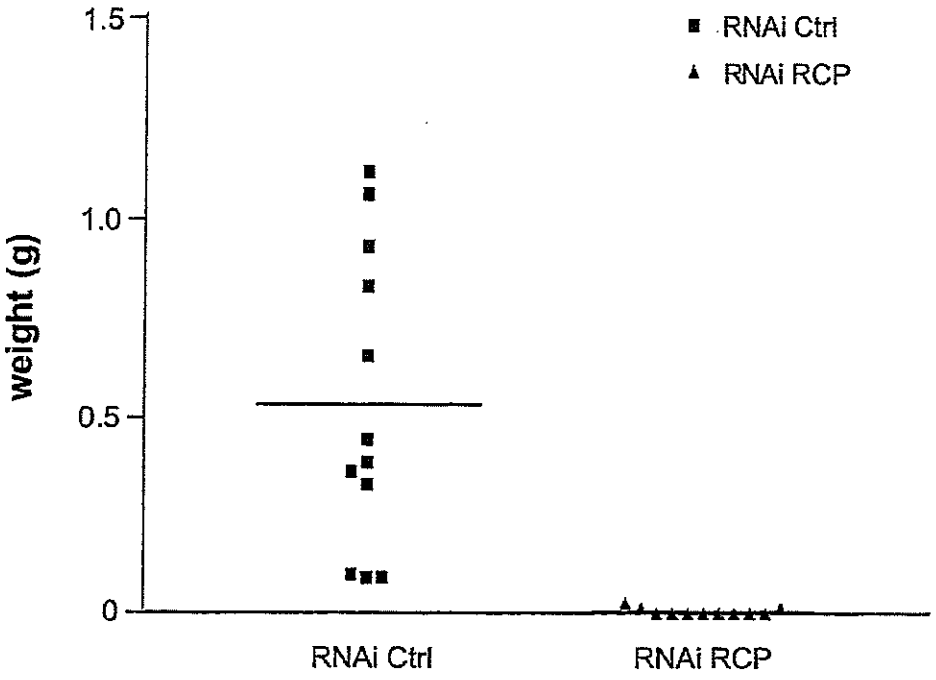

1.5
1.0
0.5
0
weight (g)
RNAi Ctrl
RNAi RCP
RNAi Ctrl
RNAi RCP

Fig. 9F

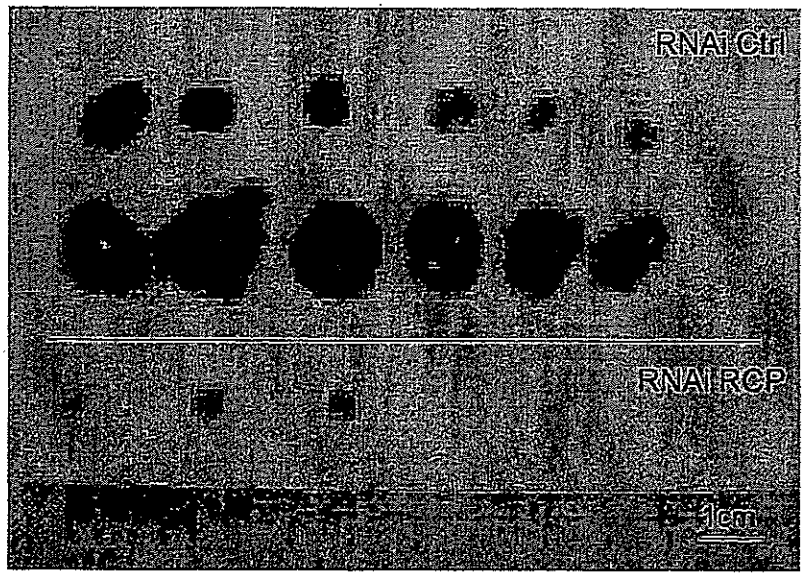

RNAi Ctrl
RNAi RCP
1cm

Fig. 9G

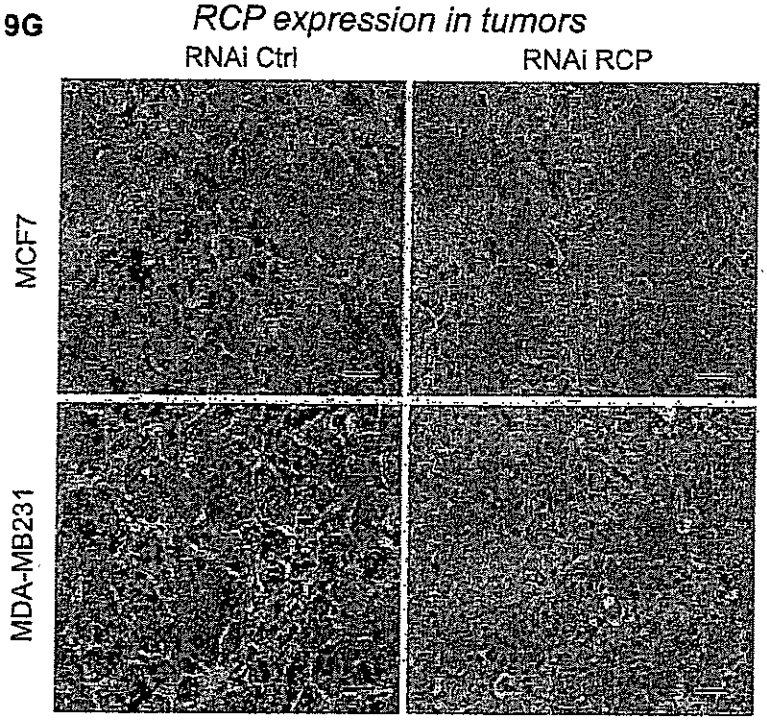
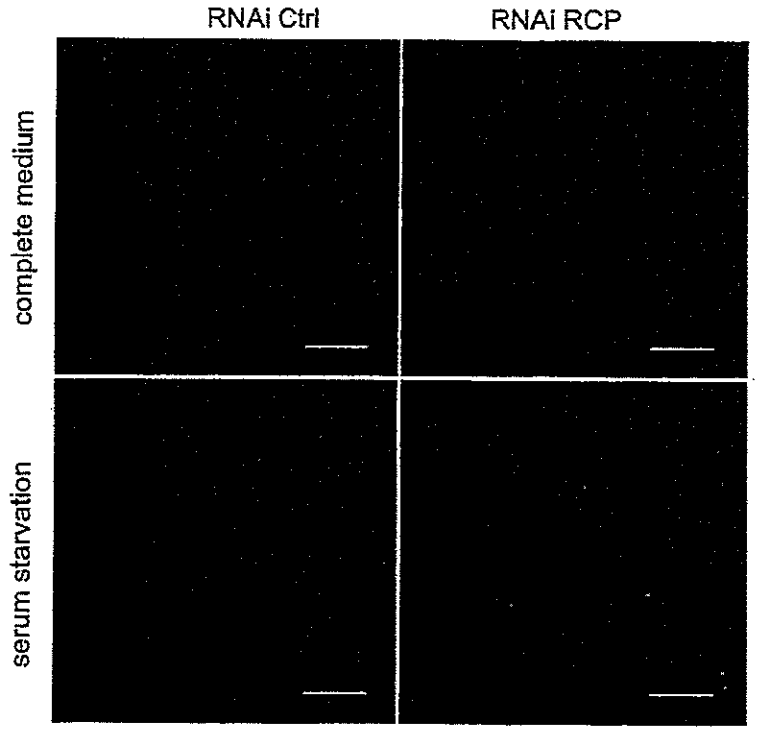

RCP expression in tumors
RNAi Ctrl
RNAi RCP
MCF7
MDA-MB231

Fig. 9H


Apoptosis of MDA-MB231 cells
RNAi Ctrl
RNAi RCP
complete medium
serum starvation

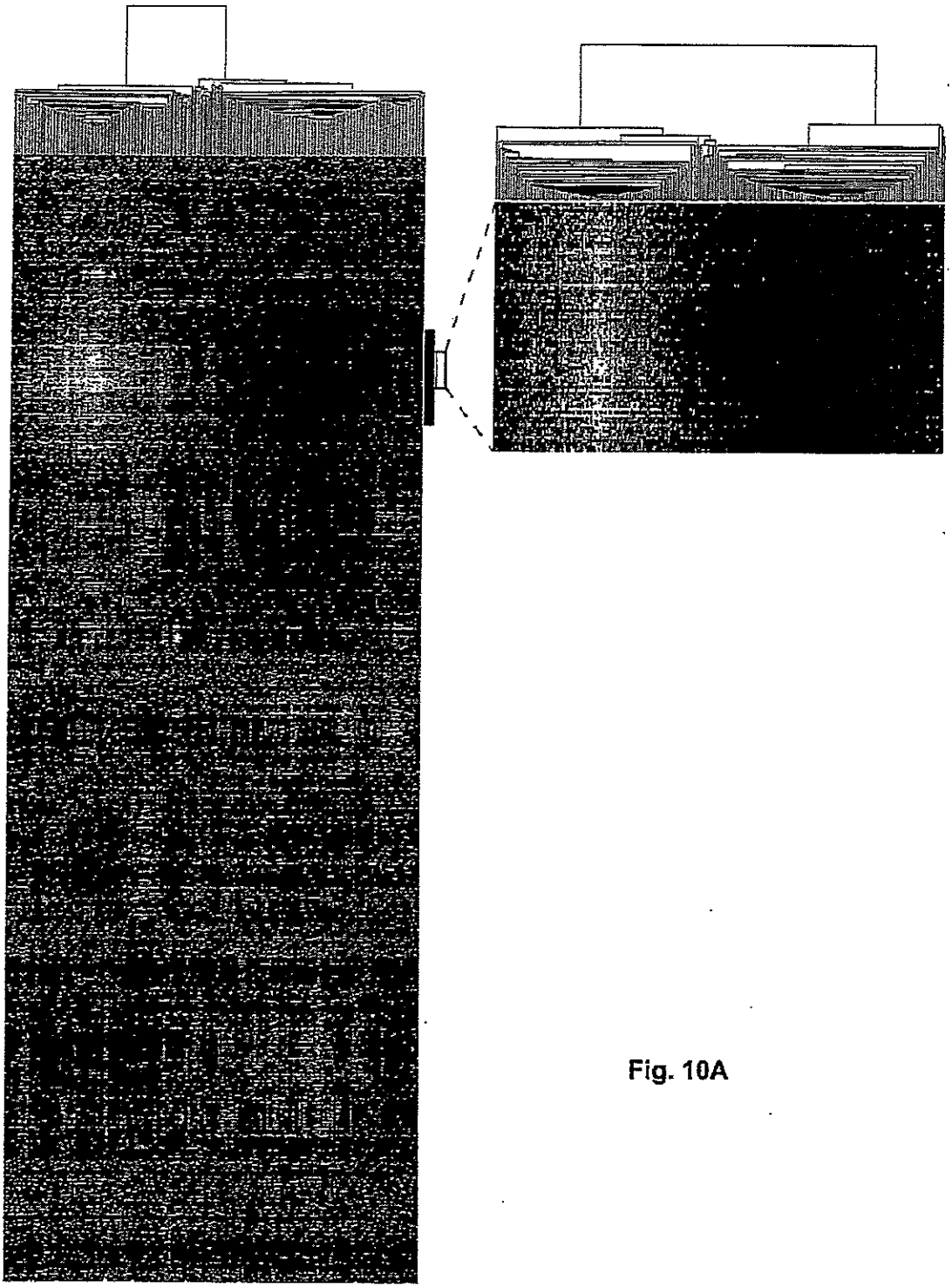
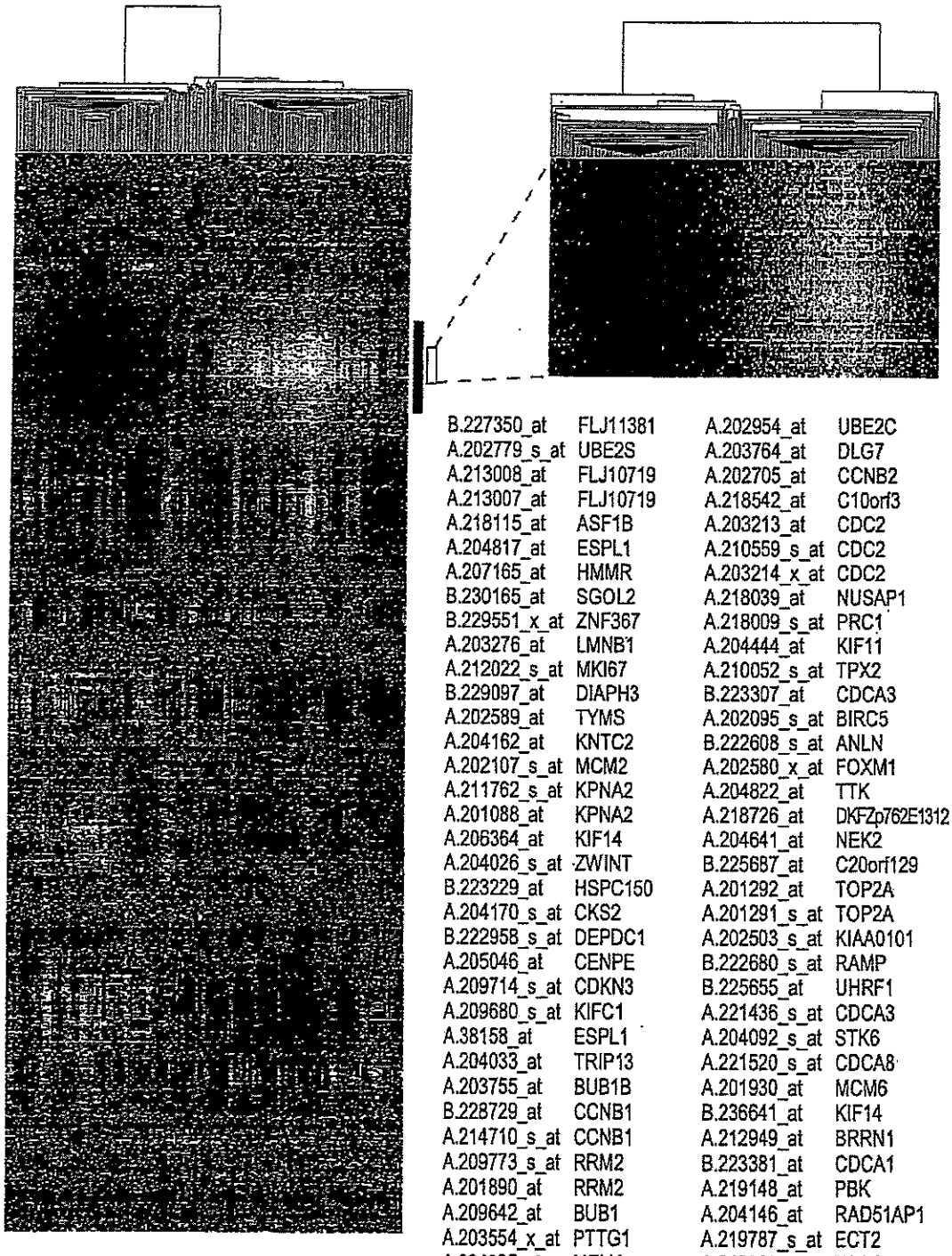


Fig. 10A



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B.228729_at	CCNB1	B.236641_at	KIF14
A.214710_s_at	CCNB1	A.212949_at	BRRN1
A.209773_s_at	RRM2	B.223381_at	CDCA1
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A.203554_x_at	PTTG1	A.219787_s_at	ECT2
A.204825_at	MELK	A.218308_at	TACC3
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Fig. 10B

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