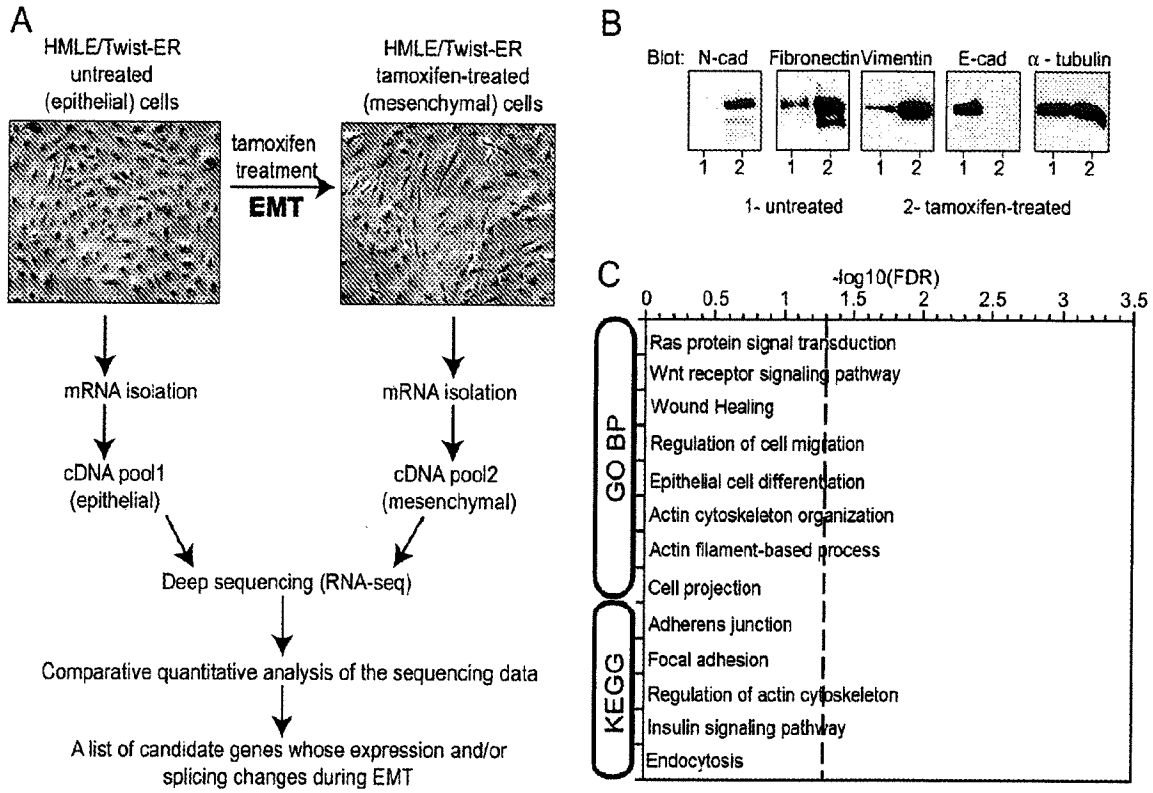



D

| Event Type | | Known Events | Both Isoforms detected | Regulated FDR<0.05 \|ΔPsi\|>=0.1 | Regulated FDR<0.05 \|ΔPsi\|>=0.3 |
|---|---|---|---|---|---|
| Skipped exon | | 38,514 | 10,422 | 481 | 163 |
| Mutually exclusive exon (MXE) | | 6,063 | 2,891 | 276 | 69 |
| Alternative to 5'splice site (A5SS) | | 11,380 | 2,537 | 65 | 22 |
| Alternative to 3'splice site (A3SS) | | 13,781 | 3,022 | 64 | 23 |
| Alternative first exon (AFE) | | 31,474 | 8,266 | 699 | 337 |
| Alternative last exon (ALE) | | 11,244 | 4,640 | 390 | 94 |
| Retained Intron | | 18,673 | 6,014 | 271 | 120 |
| Tandem 3' UTR | pA pA | 5,093 | 4,345 | 1,004 | 550 |
| Total | | 136,222 | 42,137 | 3,250 | 1,378 |

Fig. 1A-1D

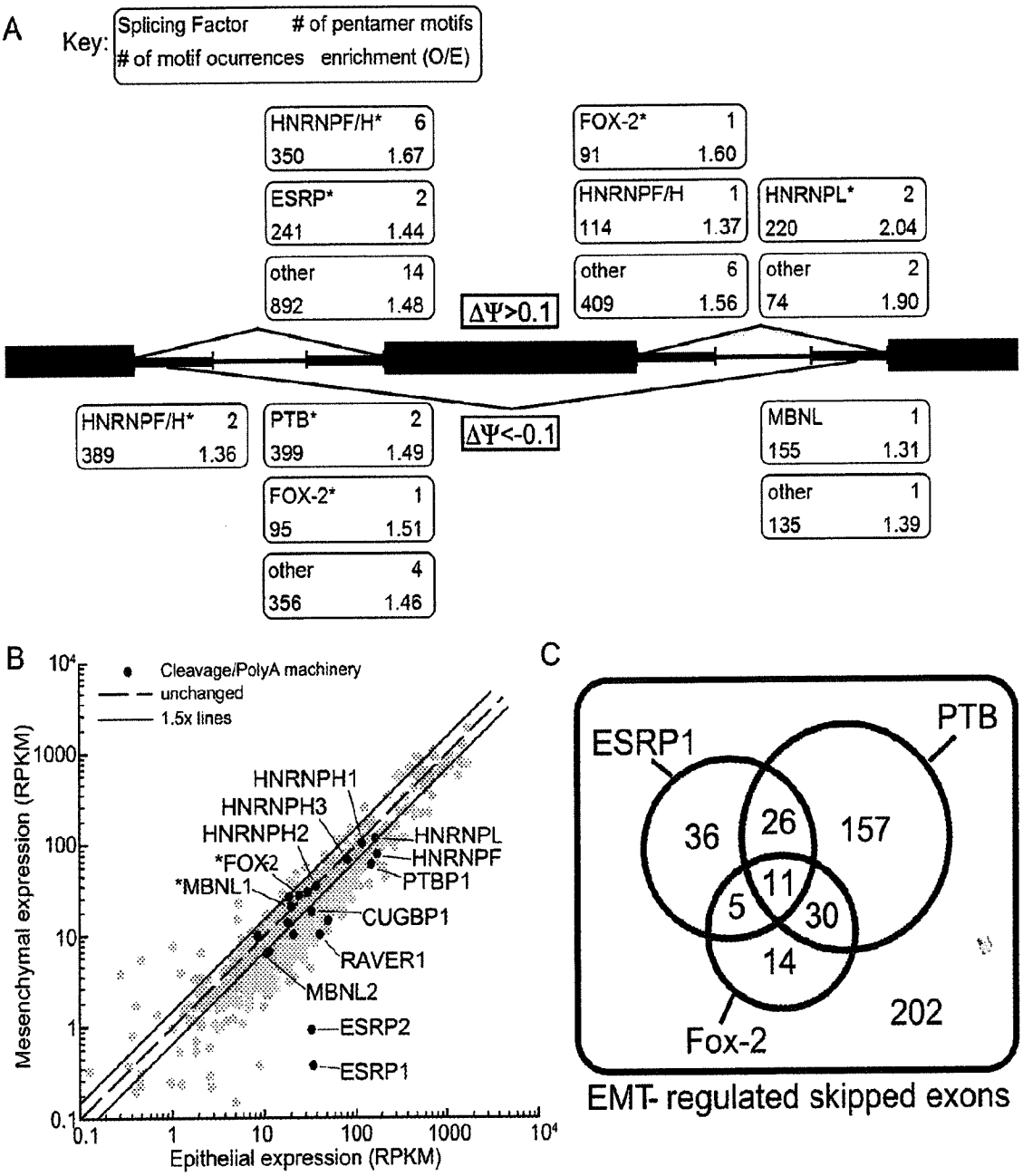

A
Key: Splicing Factor # of pentamer motifs
# of motif ocurrences enrichment (O/E)
HNRNPF/H* 6 350 1.67
ESRP* 2 241 1.44
other 14 892 1.48
FOX-2* 1 91 1.60
HNRNPF/H 1 114 1.37
other 6 409 1.56
HNRNPL* 2 220 2.04
other 2 74 1.90
ΔΨ>0.1
ΔΨ<-0.1
HNRNPF/H* 2 389 1.36
PTB* 2 399 1.49
FOX-2* 1 95 1.51
other 4 356 1.46
MBNL 1 155 1.31
other 1 135 1.39
B
Cleavage/PolyA machinery
unchanged
1.5x lines
Mesenchymal expression (RPKM)
Epithelial expression (RPKM)
HNRNPH1
HNRNPH3
HNRNPH2
*FOX2
*MBNL1
HNRNPL
HNRNPF
PTBP1
CUGBP1
RAVER1
MBNL2
ESRP2
ESRP1
C
ESRP1
PTB
Fox-2
36
26
157
11
5
30
14
202
EMT- regulated skipped exons

Fig. 2A-2C

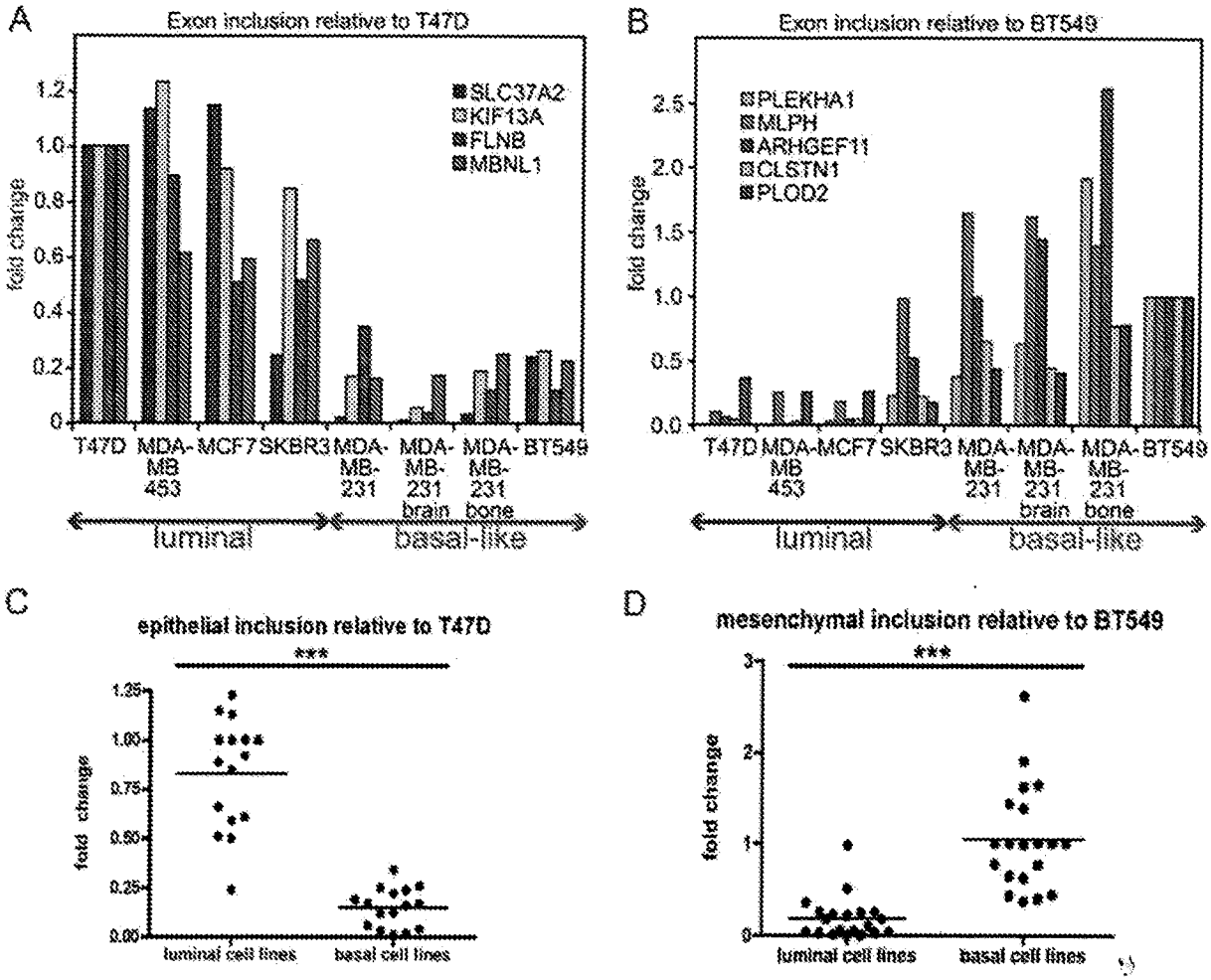
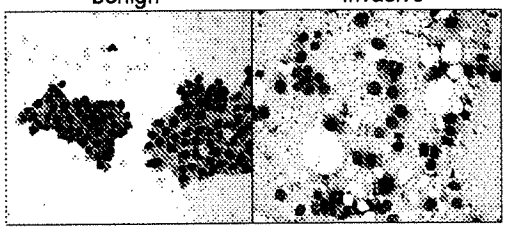

A
Exon inclusion relative to T47D
fold change
SLC37A2
KIF13A
FLNB
MBNL1
T47D
MDA-MB 453
MCF7
SKBR3
MDA-MB-231
MDA-MB-231 brain
MDA-MB-231 bone
BT549
luminal
basal-like
B
Exon inclusion relative to BT549
fold change
PLEKHA1
MLPH
ARHGEF11
CLSTN1
PLOD2
T47D
MDA-MB 453
MCF7
SKBR3
MDA-MB-231
MDA-MB-231 brain
MDA-MB-231 bone
BT549
luminal
basal-like
C
epithelial inclusion relative to T47D
***
fold change
luminal cell lines
basal cell lines
D
mesenchymal inclusion relative to BT549
***
fold change
luminal cell lines
basal cell lines

Fig. 3A-3D

A




B

| Gene name | Function | $\Delta\Psi = \Psi_M - \Psi_E$ | Proposed alternative exon function |
|---|---|---|---|
| ENAH (Mena) | regulation of actin dynamics | -0.38 | likely alters actin-binding properties of Mena |
| SLC37A2 | glycerol-3-phosphate transporter | -1 | introduces a premature stop codon thus altering cytoplasmic C-terminus |
| MBNL1 | muscle blind-like 1, splicing factor | -0.46 | affects intracellular localization of the protein |
| FLNB | F-actin cross-linking protein | -0.35 | affects flexible hinge-1 region and may alter intracellular localization |
| MLPH | a member of Rab effector protein family | 0.58 | likely alters MyoVa-binding domain |
| ARHGEF11 | Rho guanine nucleotide exchange factor | 0.62 | affects C-terminus of the protein which inhibits its transforming activity |

C

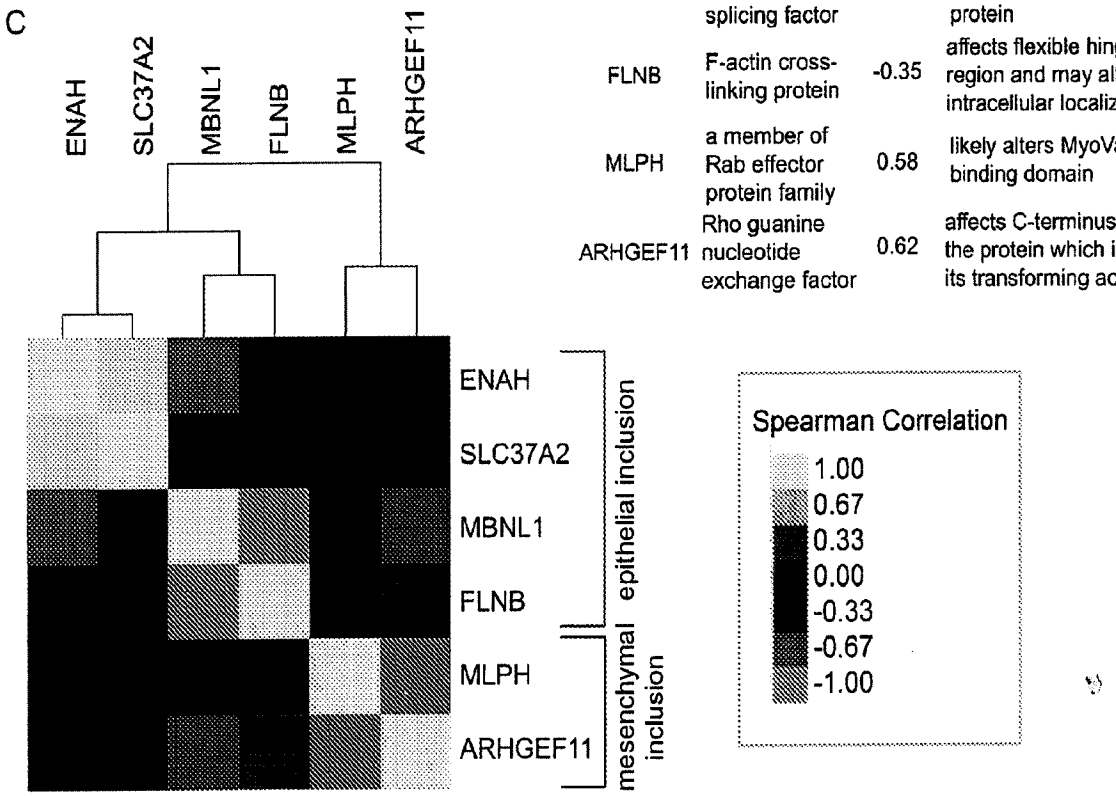



Fig. 4A-4C

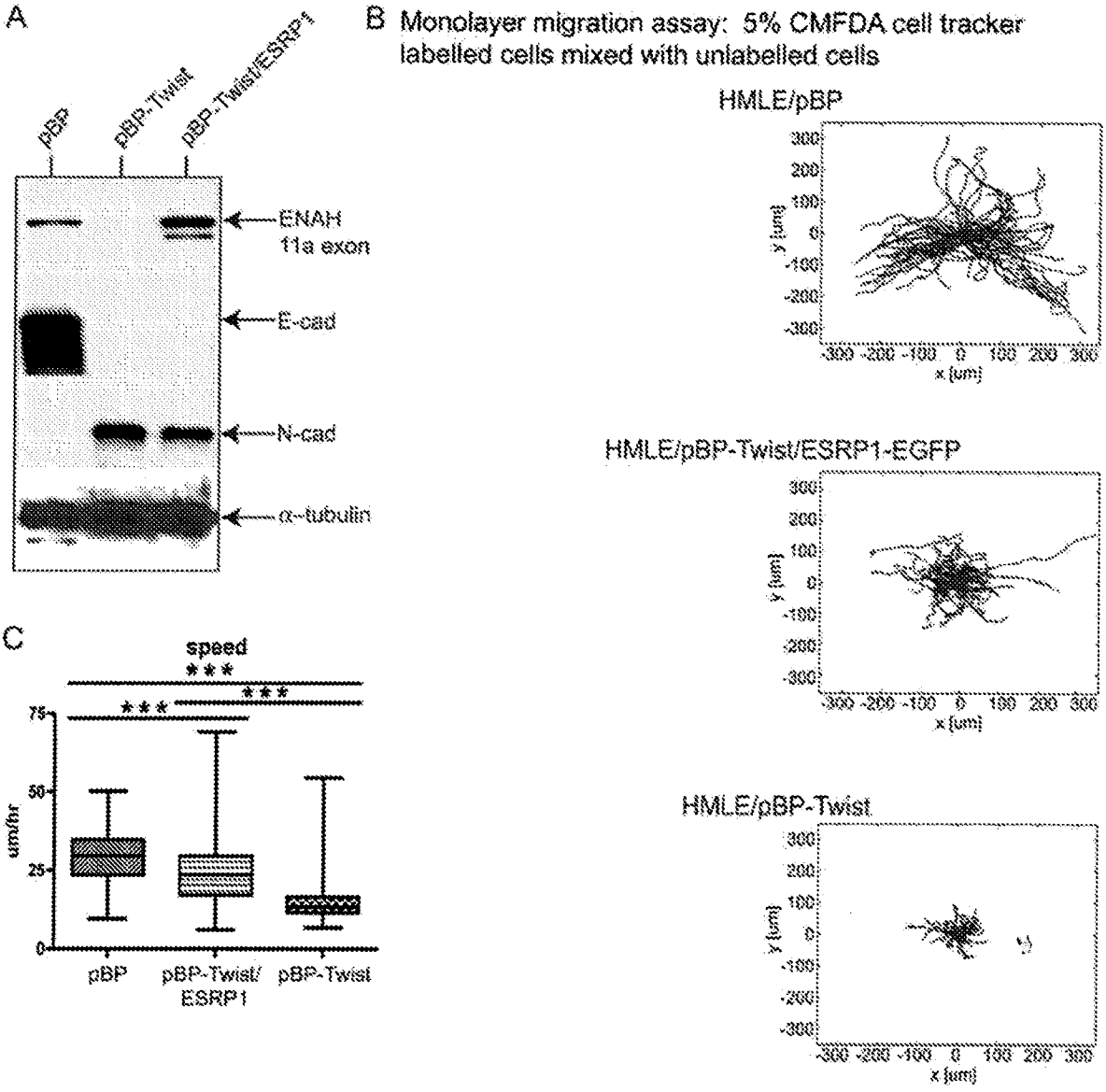

A
pBP
pBP-Twist
pBP-Twist/ESRP1
ENAH
11a exon
E-cad
N-cad
α-tubulin
B Monolayer migration assay: 5% CMFDA cell tracker labelled cells mixed with unlabelled cells
HMLE/pBP
HMLE/pBP-Twist/ESRP1-EGFP
HMLE/pBP-Twist
y [um]
x [um]
C
speed
***
um/hr
pBP
pBP-Twist/ ESRP1
pBP-Twist

Fig. 5A-5C

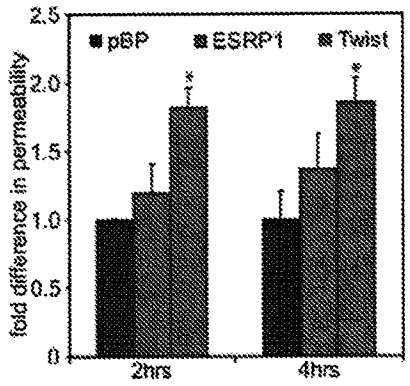
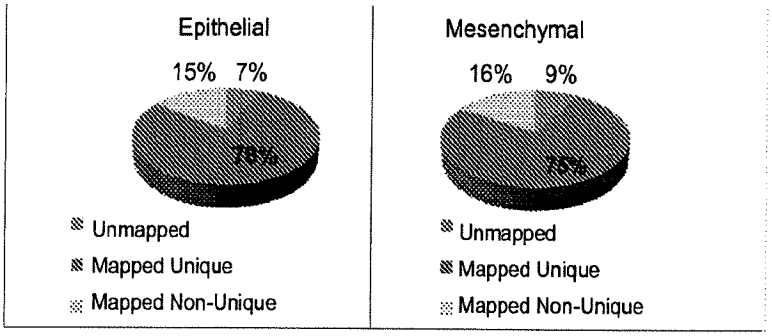

2.5
2.0
1.5
1.0
0.5
0
fold difference in permeability
pBP
ESRP1
Twist
*
*
2hrs
4hrs

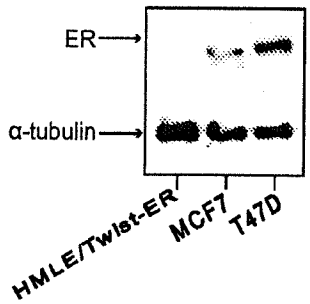
Fig. 6

A

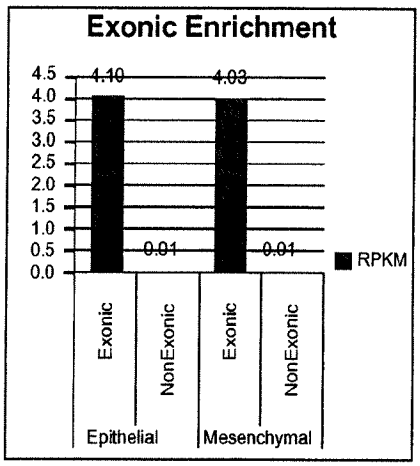
| Sample | Total Reads | Mapped | Unique | Unmapped |
|---|---|---|---|---|
| Epithelial | 30,359,326 | 28,380,409 (93%) | 24,105,519 | 1,978,917 |
| Mesenchymal | 27,530,375 | 25,135,736 (91%) | 20,804,085 | 2,384,639 |




B




C




D

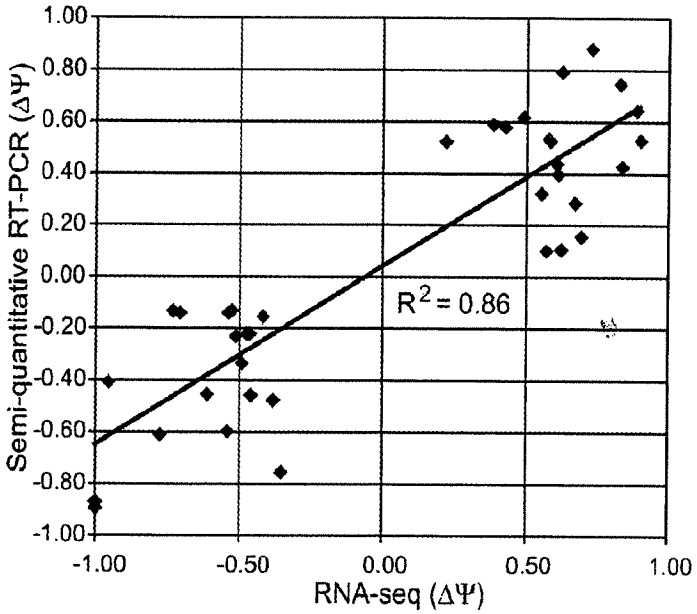
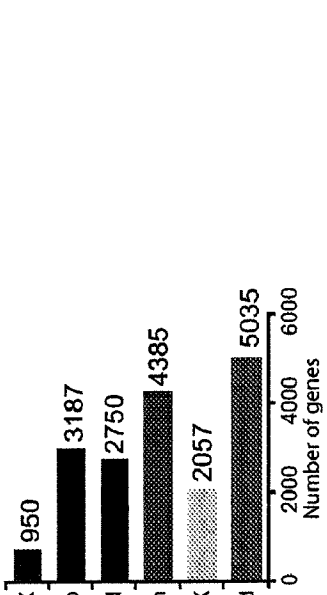
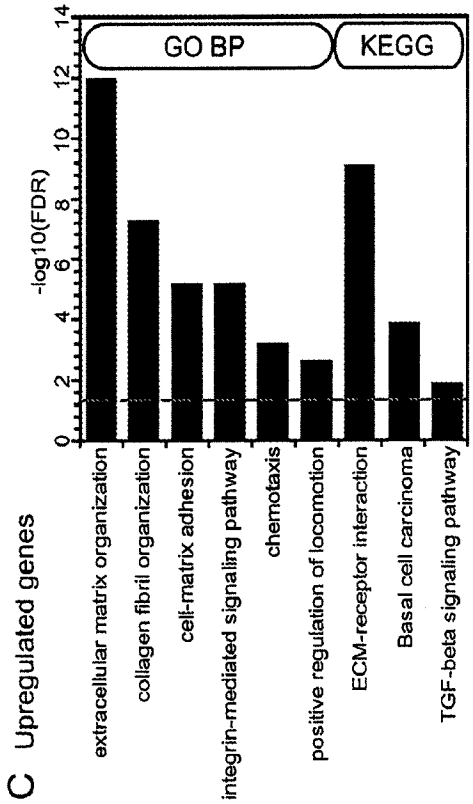



Fig. 7A-7D



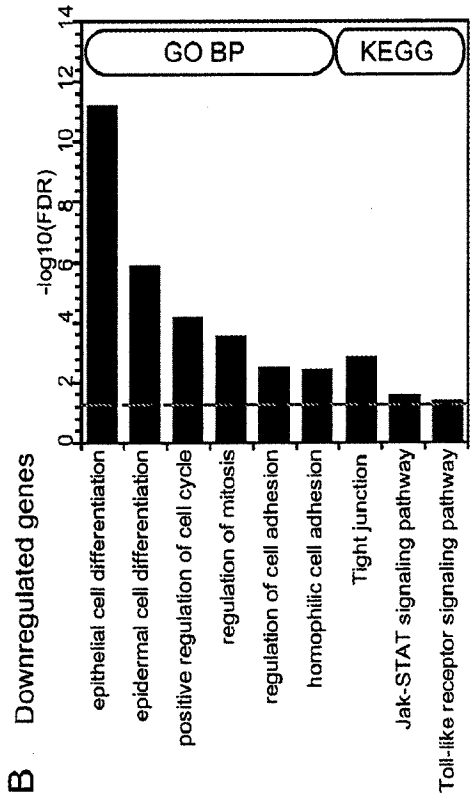




Fig. 8A-8C

504324.2

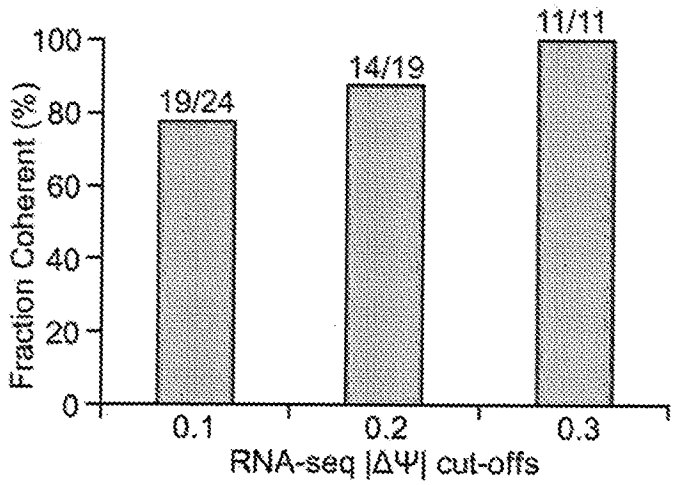

Fraction Coherent (%)
100
80
60
40
20
0
19/24
14/19
11/11
0.1
0.2
0.3
RNA-seq |ΔΨ| cut-offs

Fig. 9

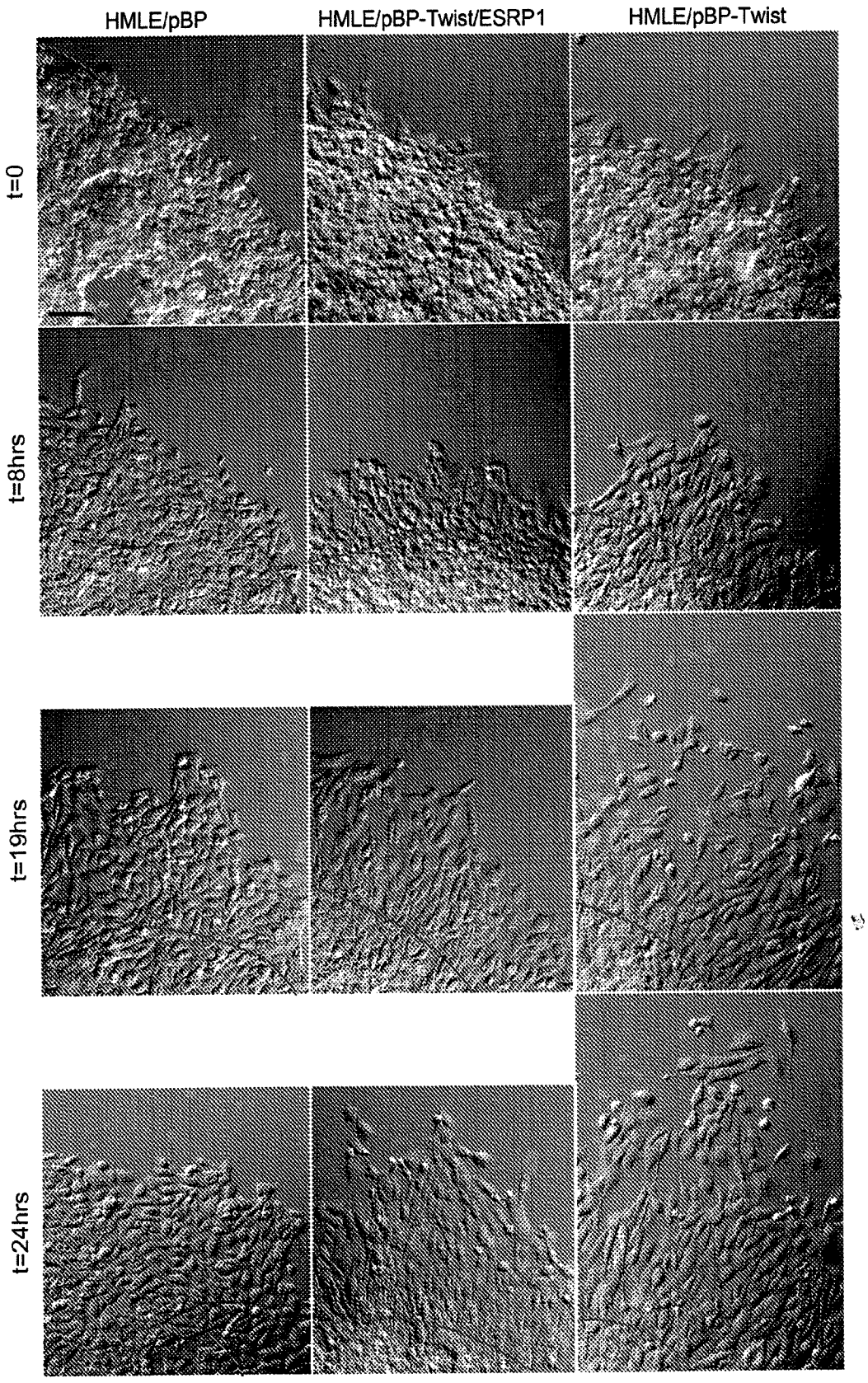

HMLE/pBP
HMLE/pBP-Twist/ESRP1
HMLE/pBP-Twist
t=0
t=8hrs
t=19hrs
t=24hrs

Fig. 10

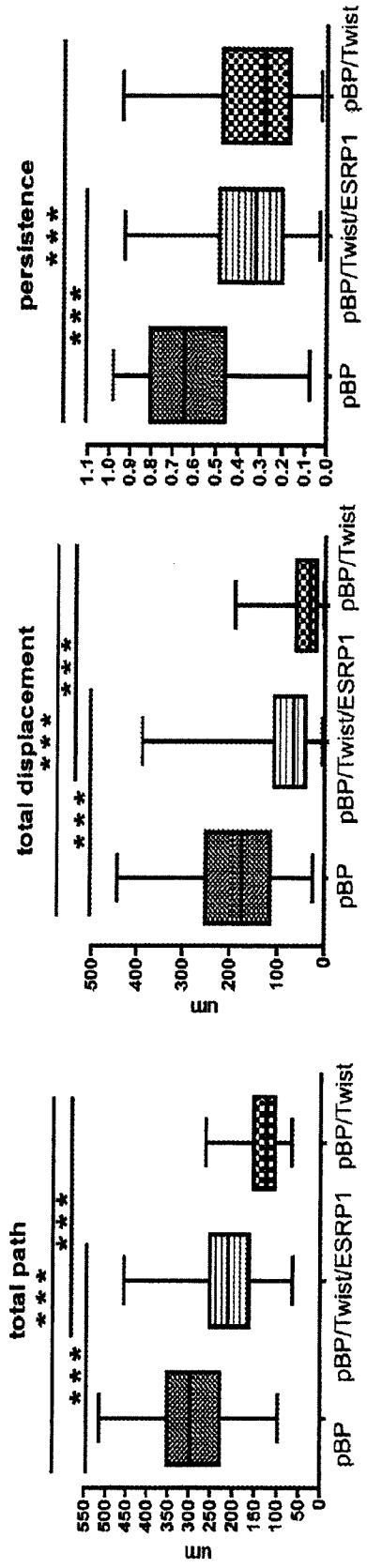



Fig. 11

504324.2

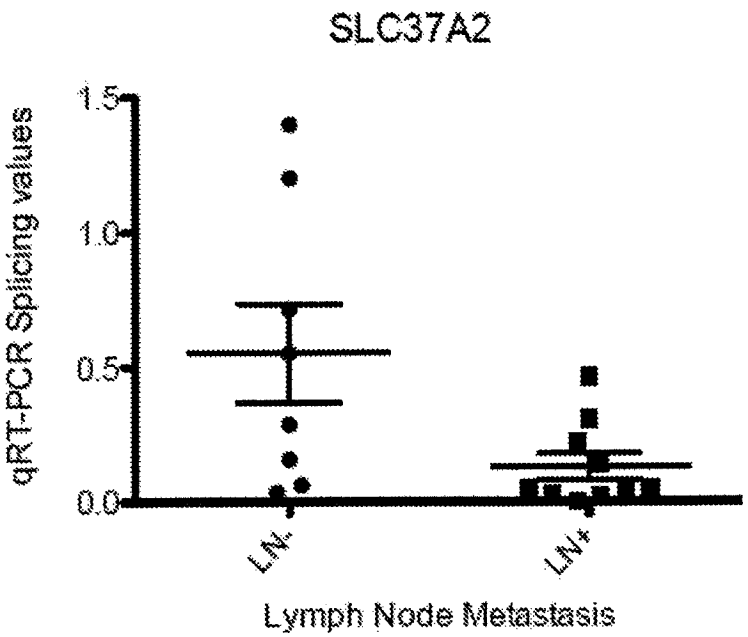

SLC37A2
qRT-PCR Splicing values
1.5
1.0
0.5
0.0
LN-
LN+
Lymph Node Metastasis

Fig. 12

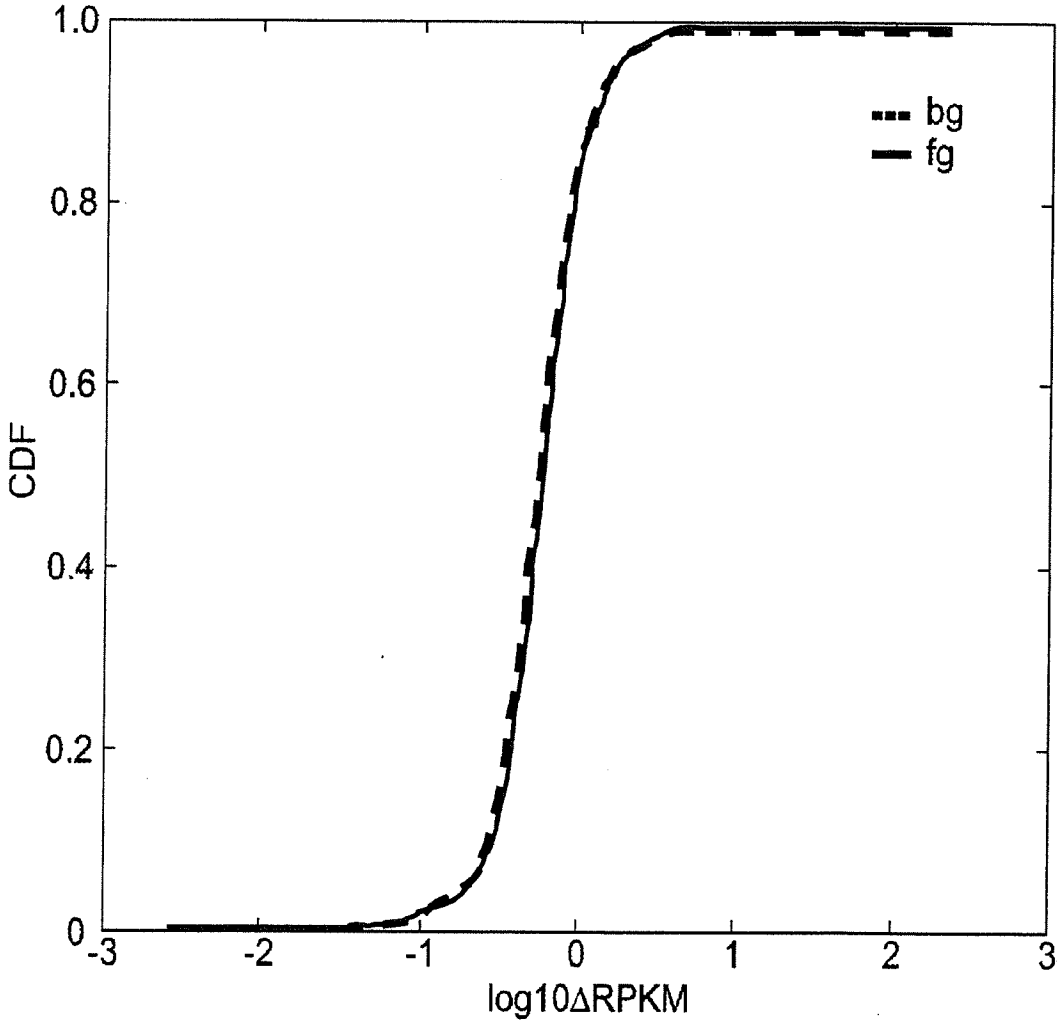



Fig. 13

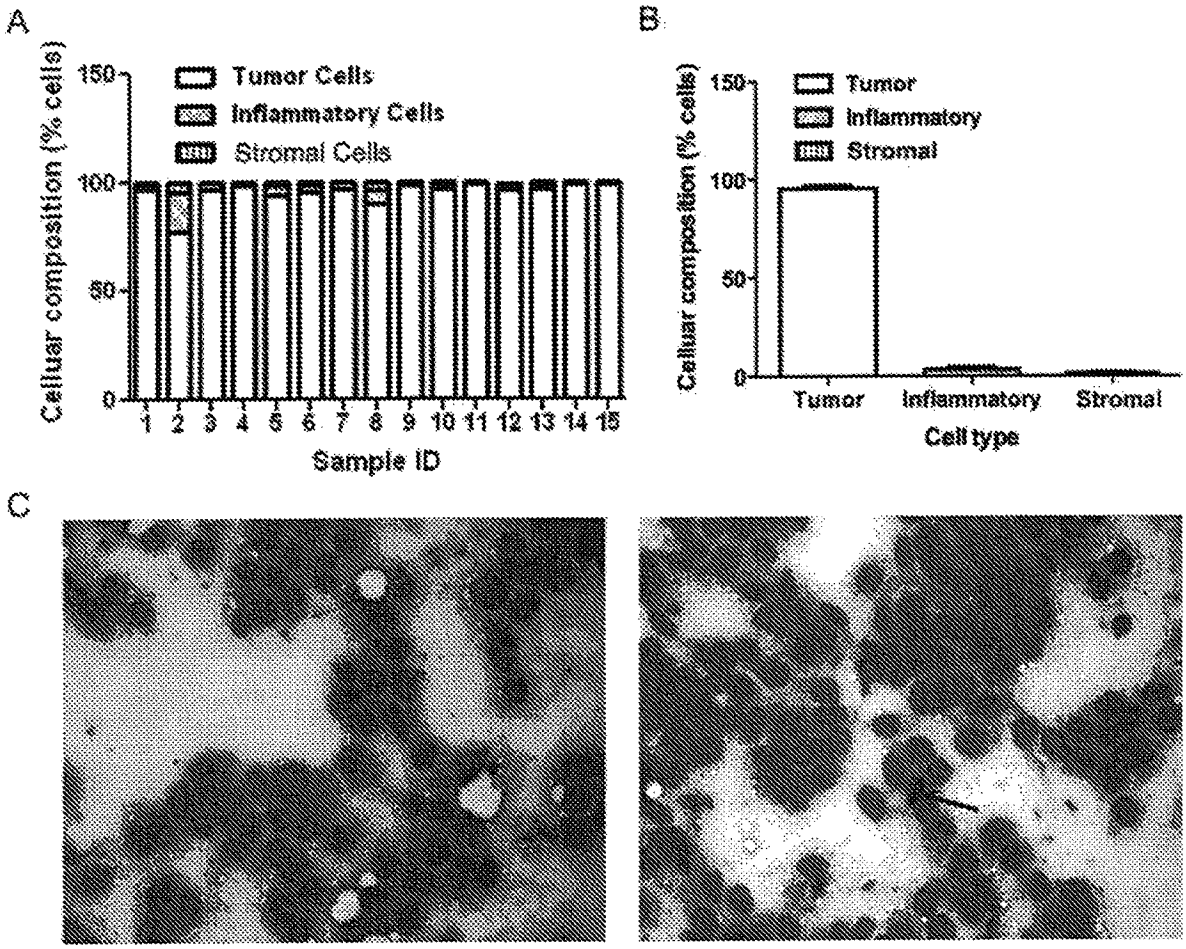

A
Cellular composition (% cells)
150
100
50
0
Tumor Cells
Inflammatory Cells
Stromal Cells
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sample ID
B
Cellular composition (% cells)
150
100
50
0
Tumor
Inflammatory
Stromal
Tumor
Inflammatory
Stromal
Cell type
C

Fig. 14A-14C

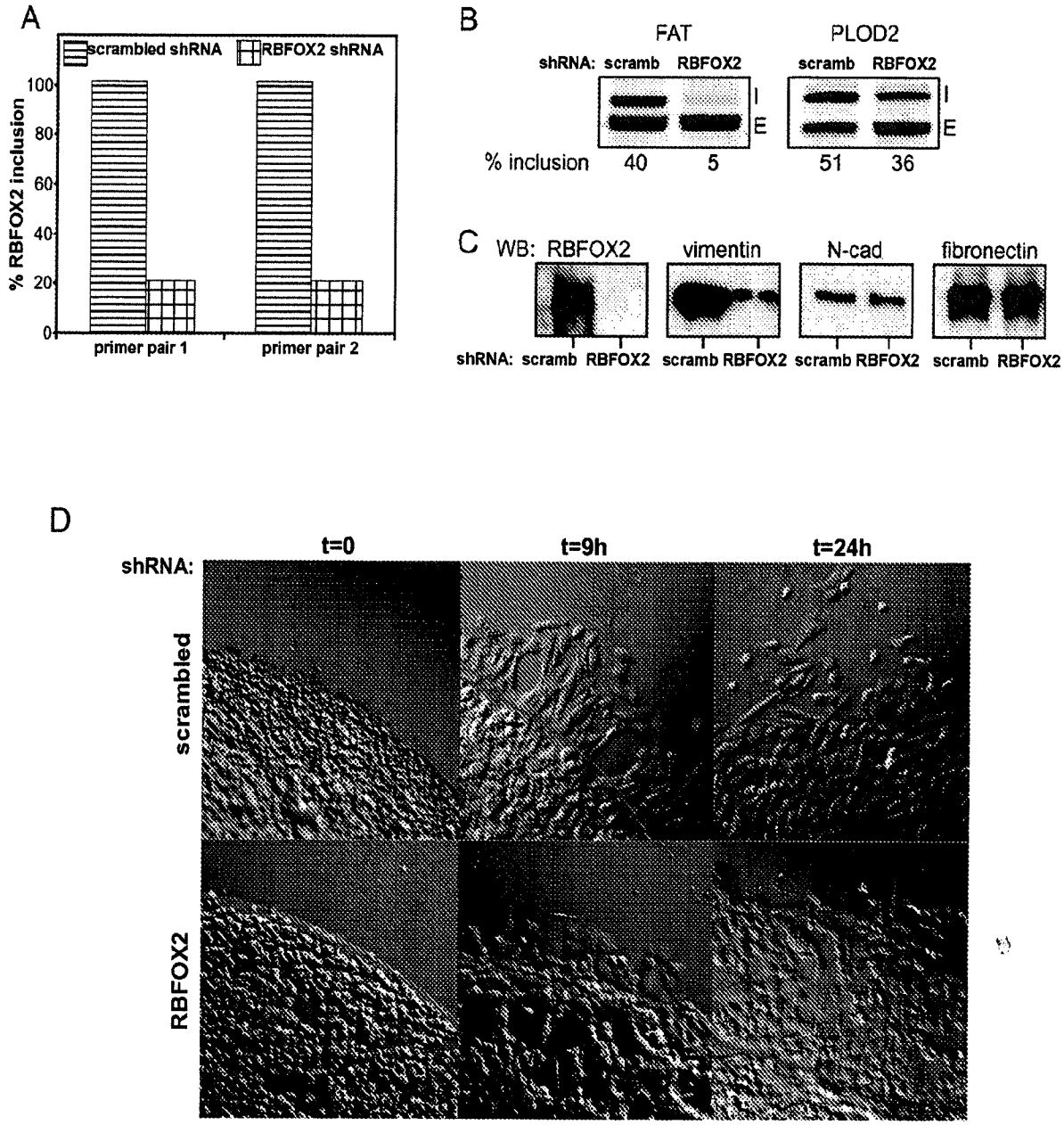

A
scrambled shRNA
RBFOX2 shRNA
% RBFOX2 inclusion
100
80
60
40
20
0
primer pair 1
primer pair 2
B
FAT
PLOD2
shRNA: scramb RBFOX2
scramb RBFOX2
I
E
% inclusion 40 5 51 36
C
WB: RBFOX2
vimentin
N-cad
fibronectin
shRNA: scramb RBFOX2 scramb RBFOX2 scramb RBFOX2 scramb RBFOX2
D
t=0
t=9h
t=24h
shRNA:
scrambled
RBFOX2

Fig. 15A-15D

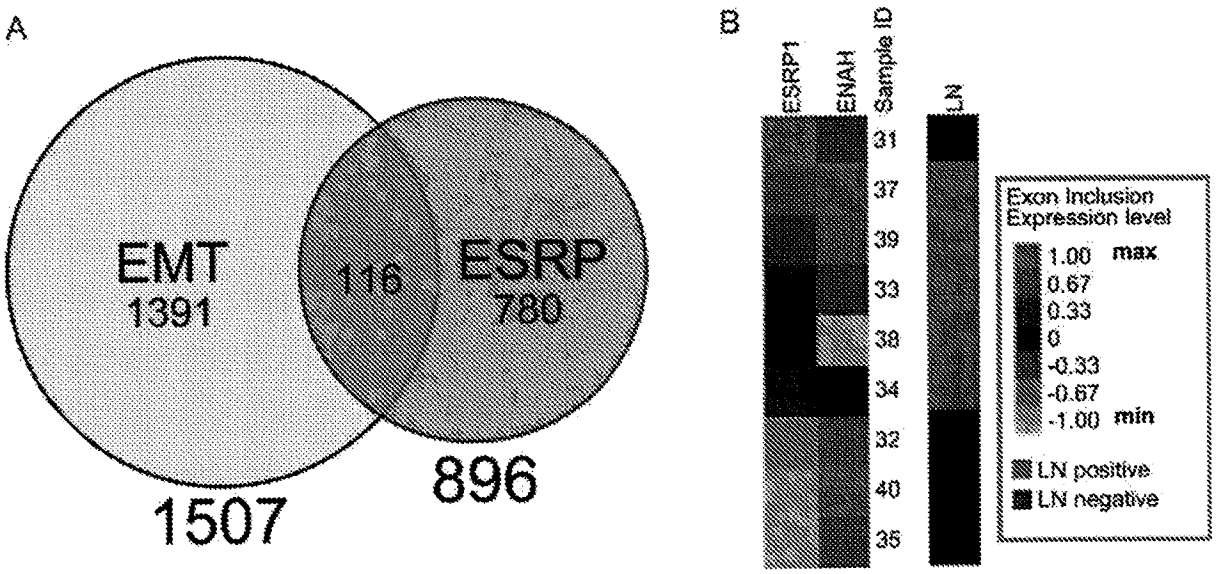

A
EMT
1391
116
ESRP
780
1507
896
B
ESRP1
ENAH
Sample ID
LN
31
37
39
33
38
34
32
40
35
Exon Inclusion
Expression level
1.00 max
0.67
0.33
0
-0.33
-0.67
-1.00 min
LN positive
LN negative

Fig. 16