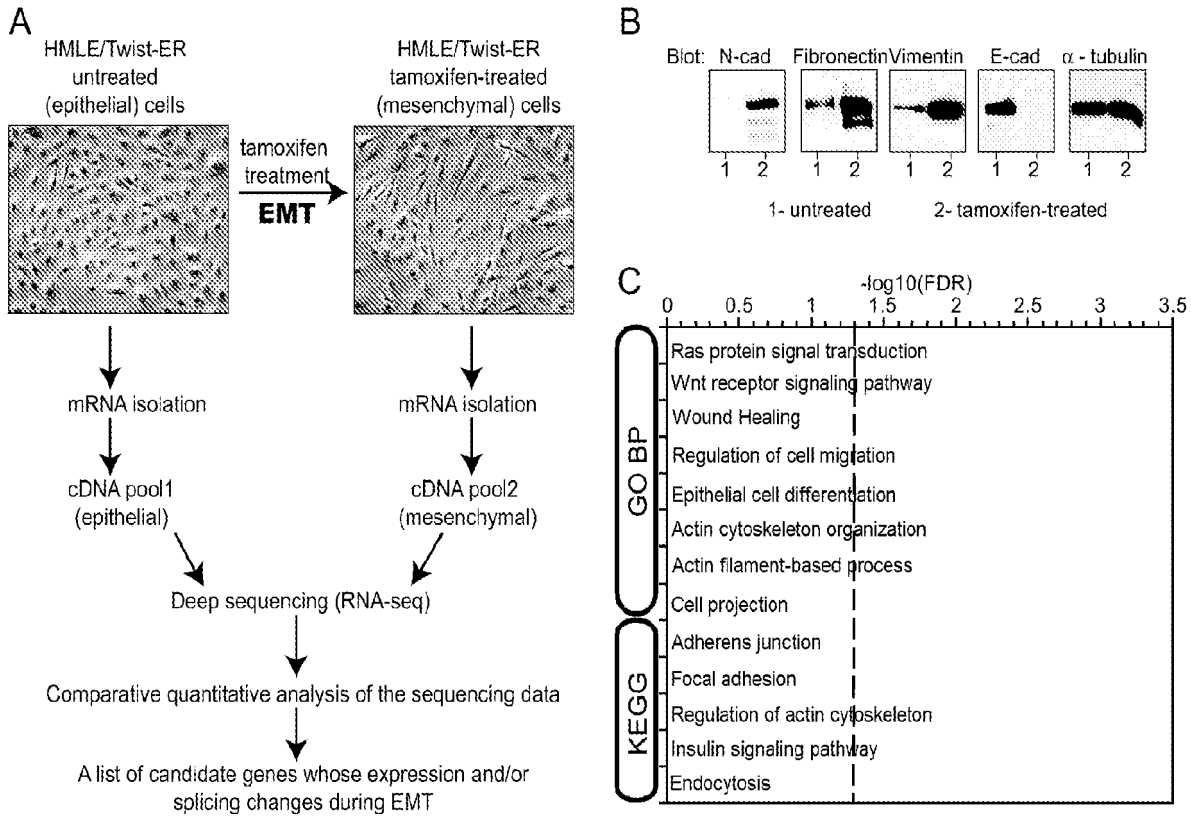


D

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

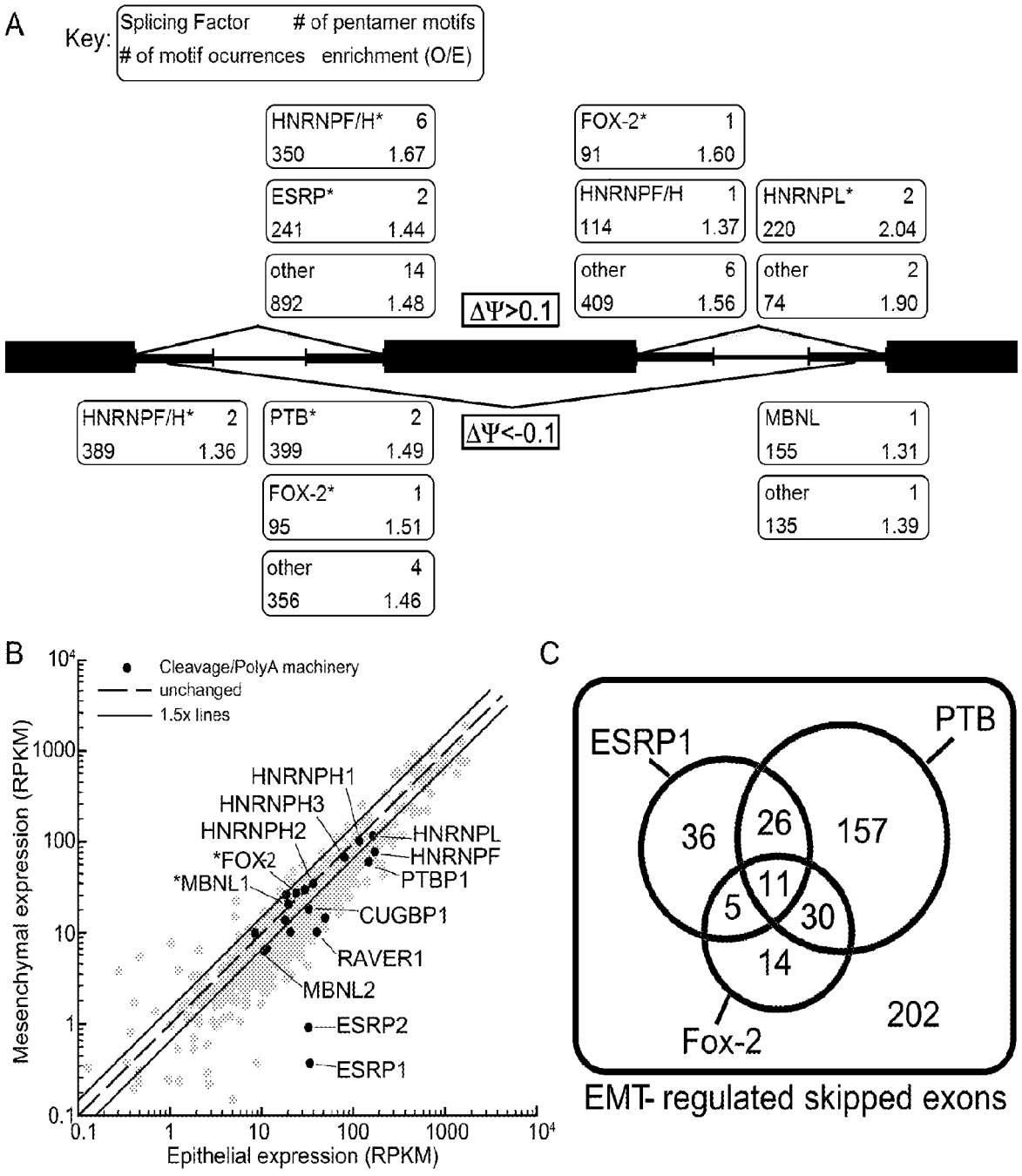

A
Key:
Splicing Factor # of pentamer motifs
# of motif ocurrences enrichment (O/E)
HNRNPF/H* 6 350 1.67
ESRP* 2 241 1.44
other 14 892 1.48
FOX-2* 1 91 1.60
HNRNPF/H 1 114 1.37
other 6 409 1.56
HNRNPL* 2 220 2.04
other 2 74 1.90
ΔΨ>0.1
ΔΨ<-0.1
HNRNPF/H* 2 389 1.36
PTB* 2 399 1.49
FOX-2* 1 95 1.51
other 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

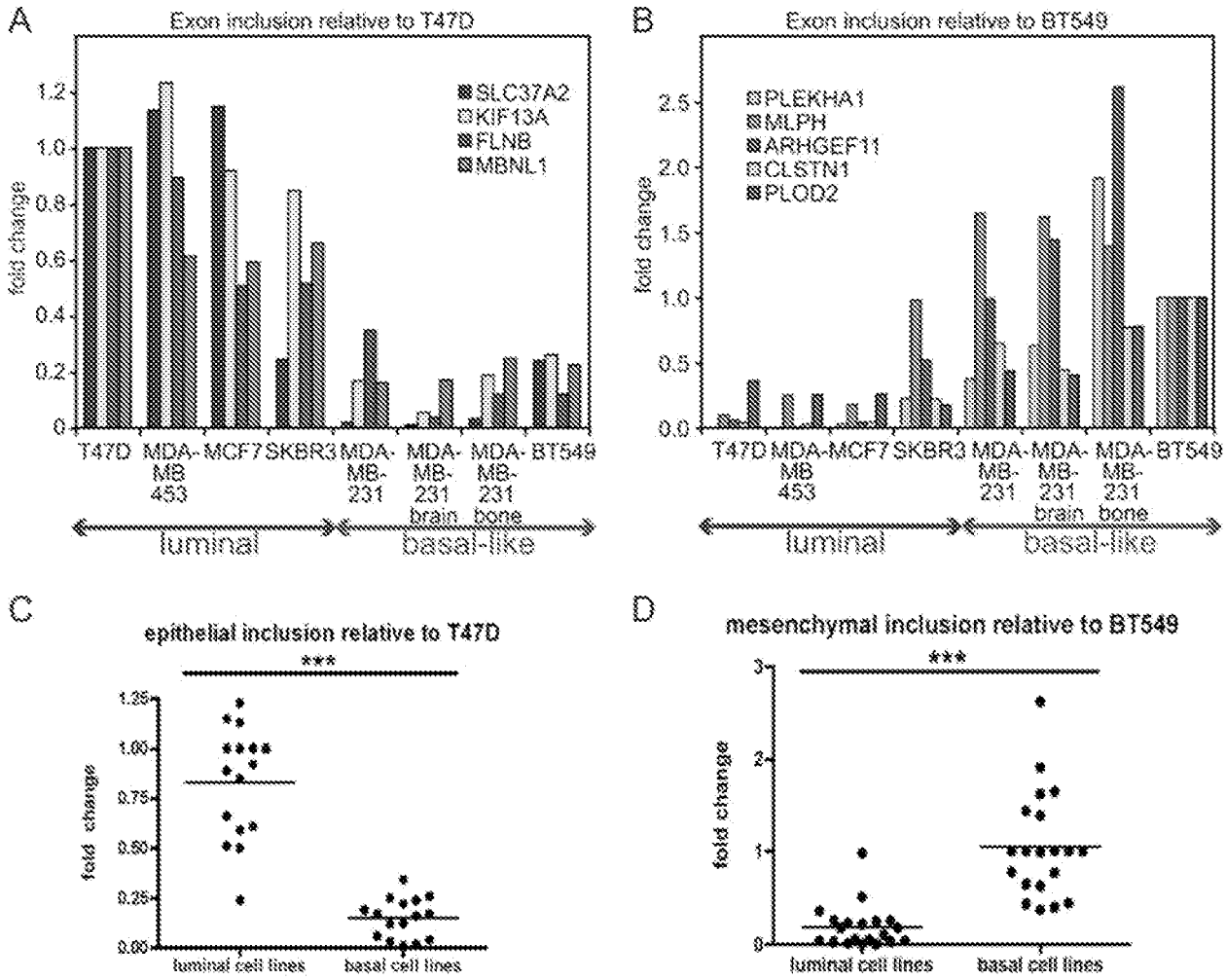
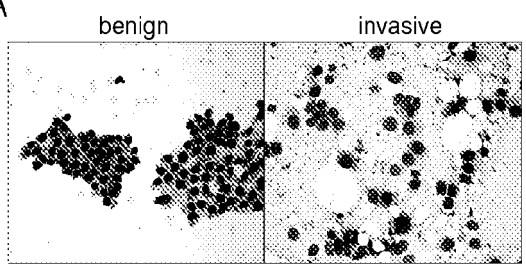

A
Exon inclusion relative to T47D
fold change
SLC37A2
KIF13A
FLNB
MBNL1
T47D
MDA-MB 453
MCF7
SKBR3
MDA-MB-231
MDA-MB-231 brain
MDA-MB-231 bone
BT549
luminal
basal-like
B
Exon inclusion relative to BT549
fold change
PLEKHA1
MLPH
ARHGEF11
CLSTN1
PLOD2
C
epithelial inclusion relative to T47D
***
fold change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

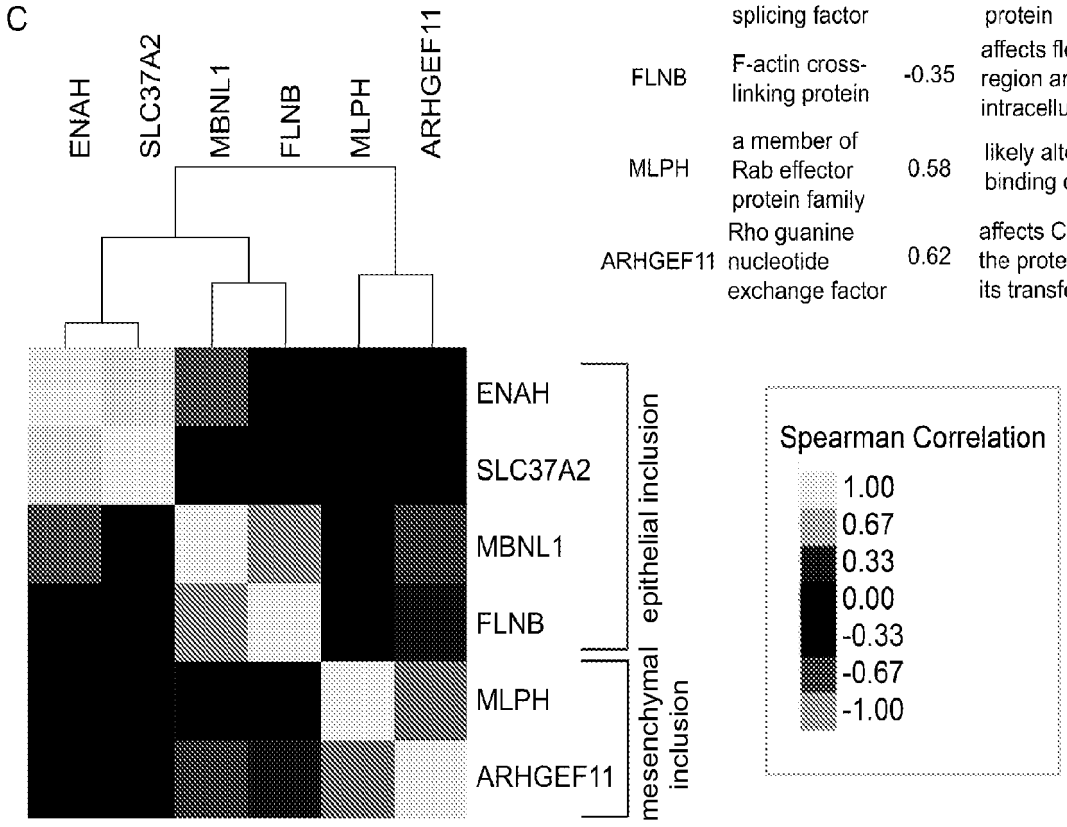


Fig. 4A-4C

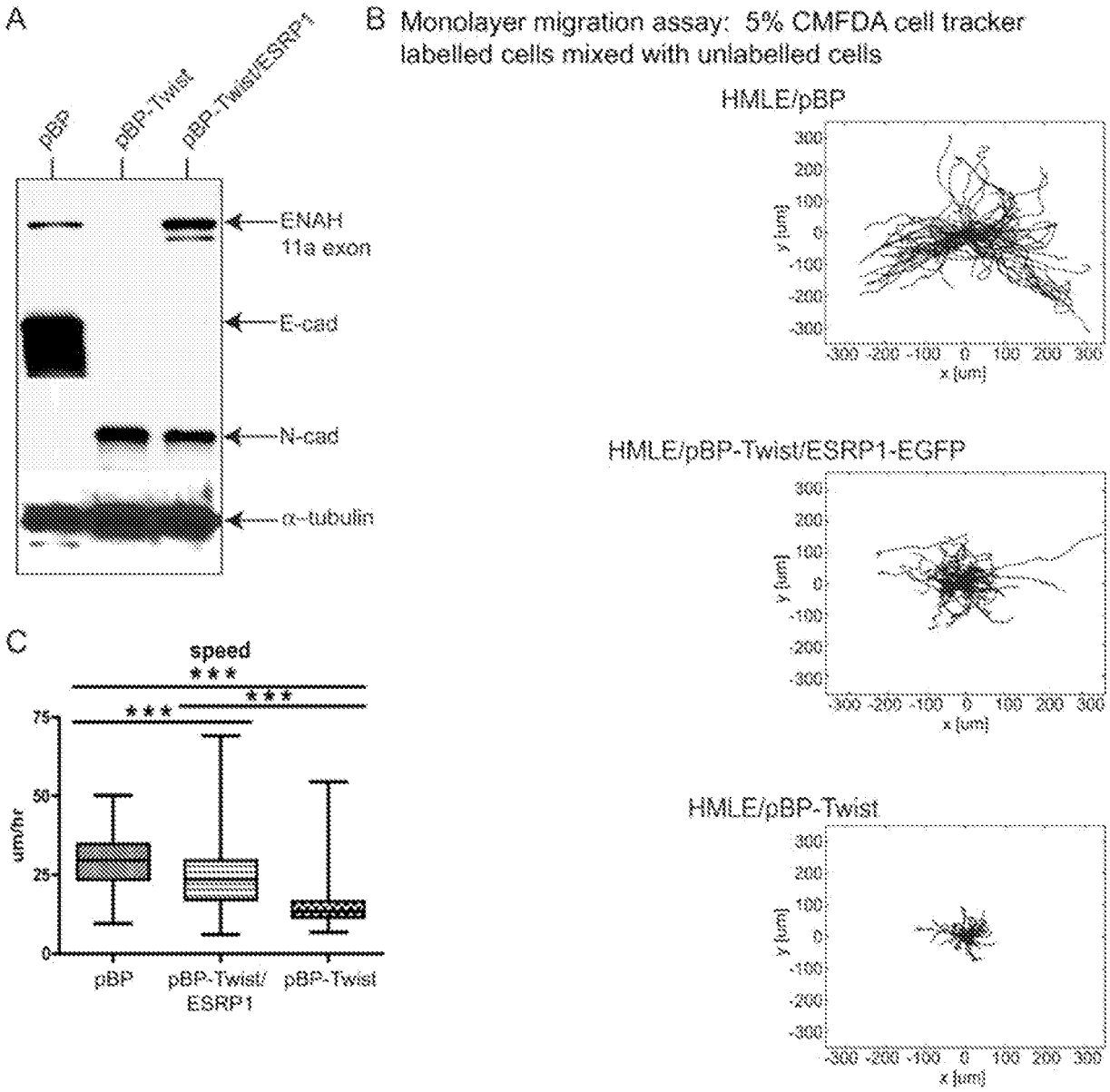

A
pBP
pBP-Twist
pBP-Twist/ESRP1
ENAH
11a exon
E-cad
N-cad
α-tubulin
B Monolayer migration assay: 5% CMFDA cell tracker labelled cells mixed with unlabelled cells
HMLE/pBP
HMLE/pBP-Twist/ESRP1-EGFP
HMLE/pBP-Twist
y (um)
x (um)
C
speed
***
um/hr
pBP
pBP-Twist/ ESRP1
pBP-Twist

Fig. 5A-5C

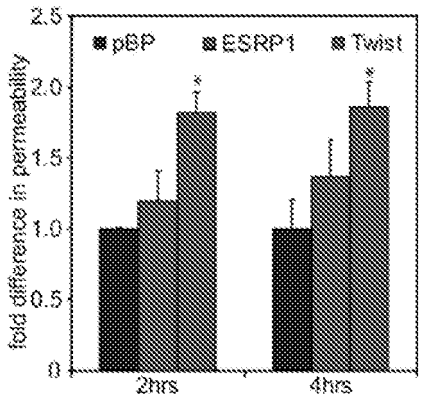
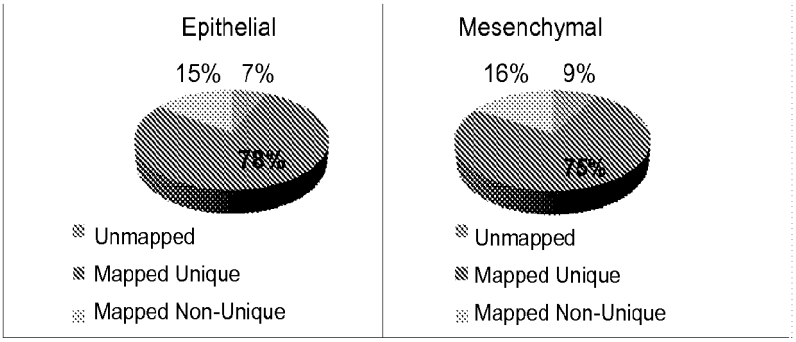

2.5
2.0
1.5
1.0
0.5
0
fold difference in permeability
pBP
ESRP1
Twist
*
*
2hrs
4hrs

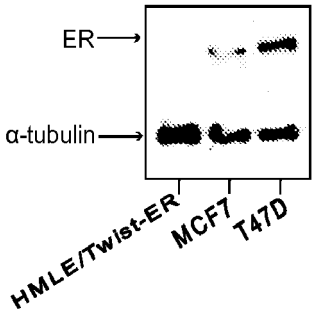
Fig. 6

A

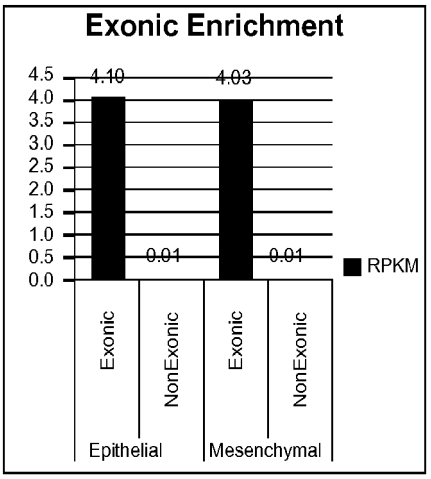
Sample	Total Reads	Mapped	Unique	Unmapped
Epithelial	30,359,326	28,380,409 (93%)	24,105,519	1,978,917
Mesenchymal	27,530,375	25,135,736 (91%)	20,804,085	2,384,639



B



C



D

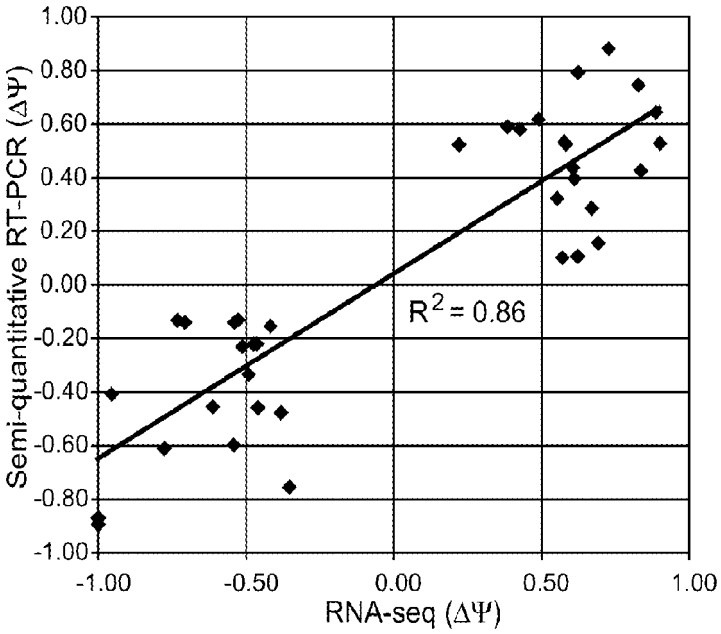


Fig. 7A-7D

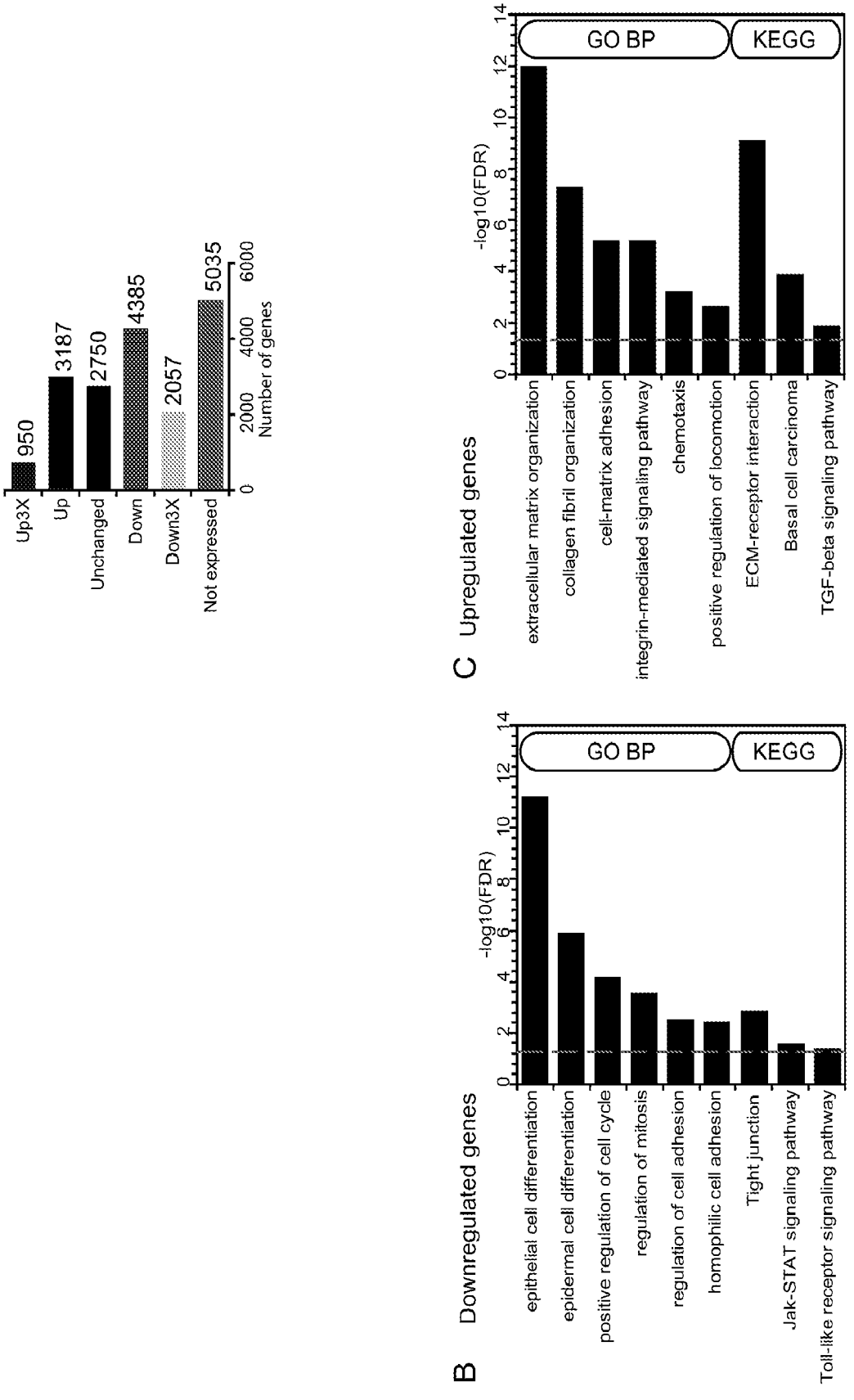


Fig. 8A-8C

504324.2

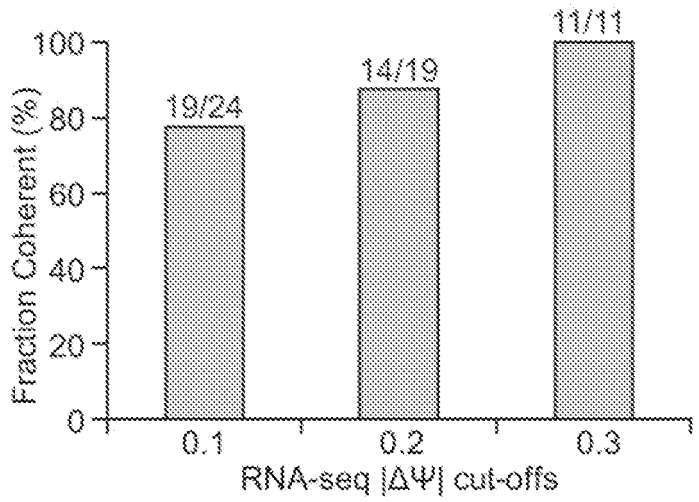

Fraction Coherent (%)
100
80
60
40
20
0
19/24
14/19
11/11
0.1
0.2
0.3
RNA-seq |ΔΨ| cut-offs

Fig. 9

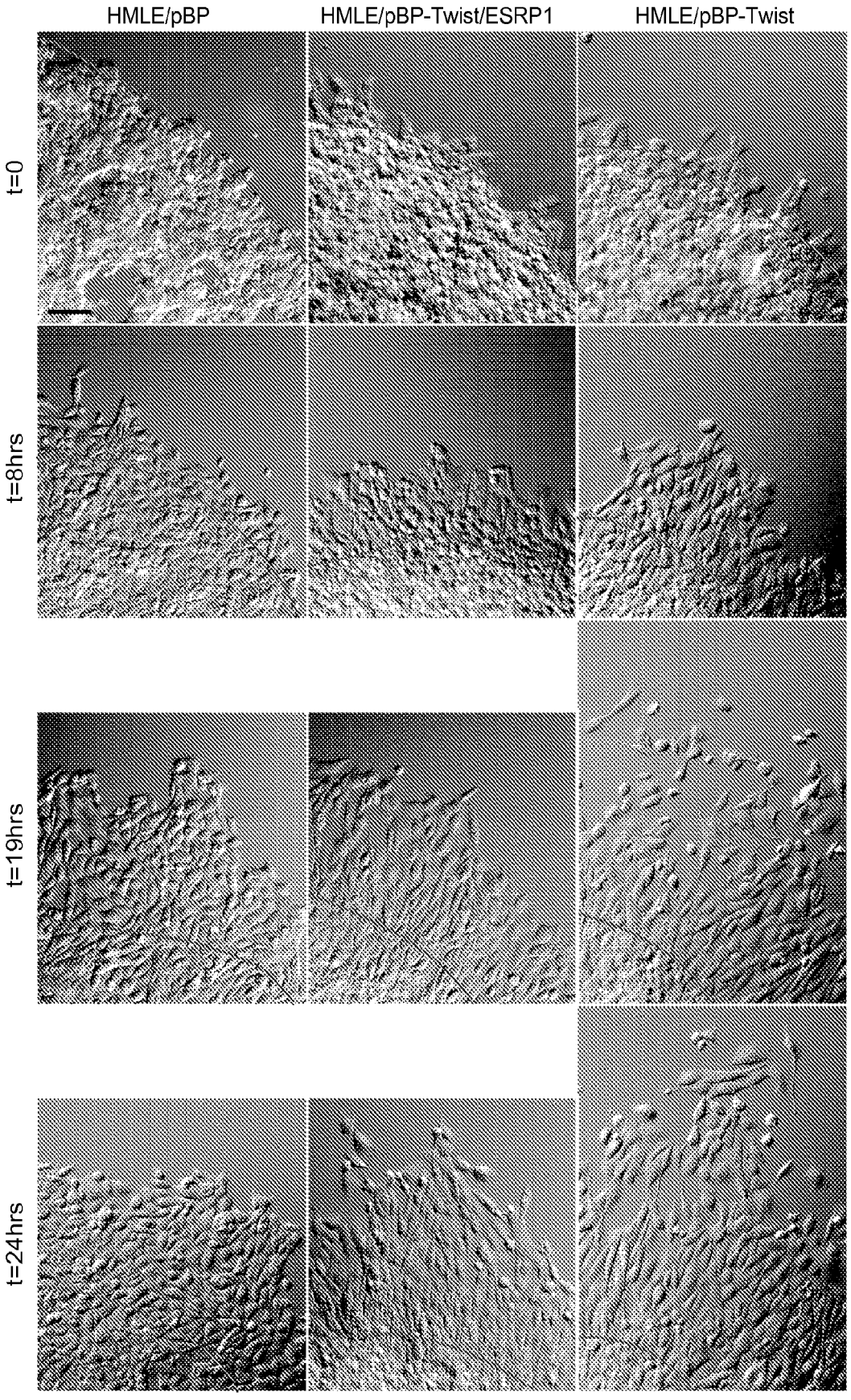

HMLE/pBP
HMLE/pBP-Twist/ESRP1
HMLE/pBP-Twist
t=0
t=8hrs
t=19hrs
t=24hrs

Fig. 10

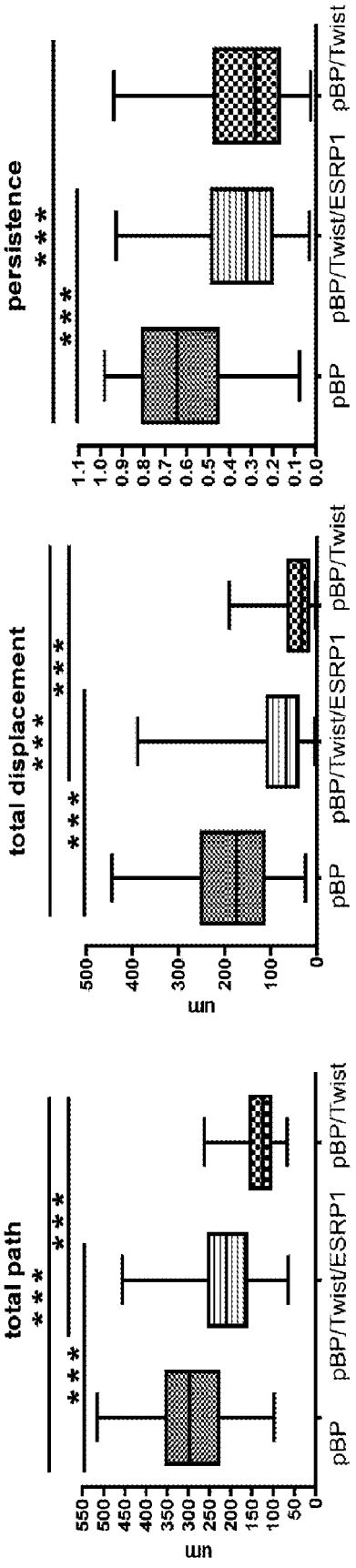

total path
total displacement
persistence
um
pBP
pBP/Twist/ESRP1
pBP/Twist

Fig. 11

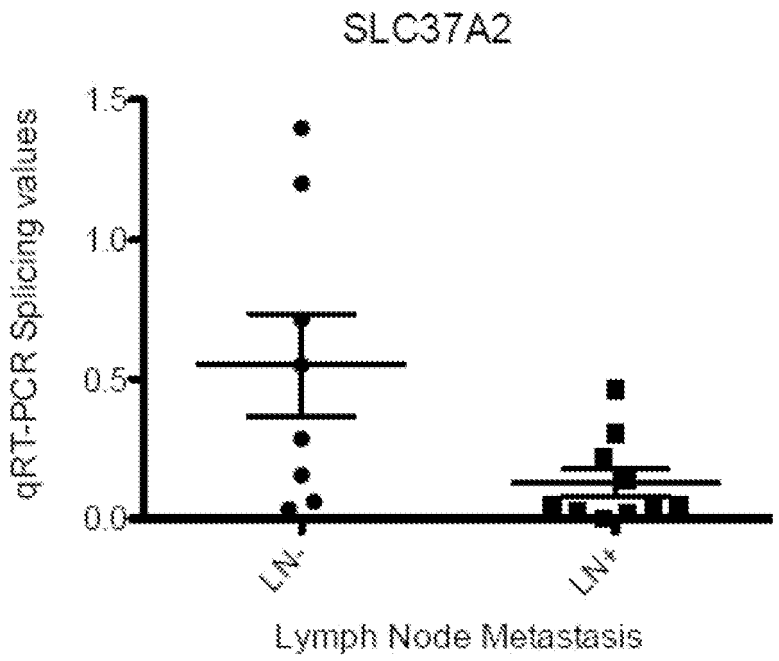

SLC37A2
qRT-PCR Splicing values
1.5
1.0
0.5
0.0
LN-
LN+
Lymph Node Metastasis

Fig. 12

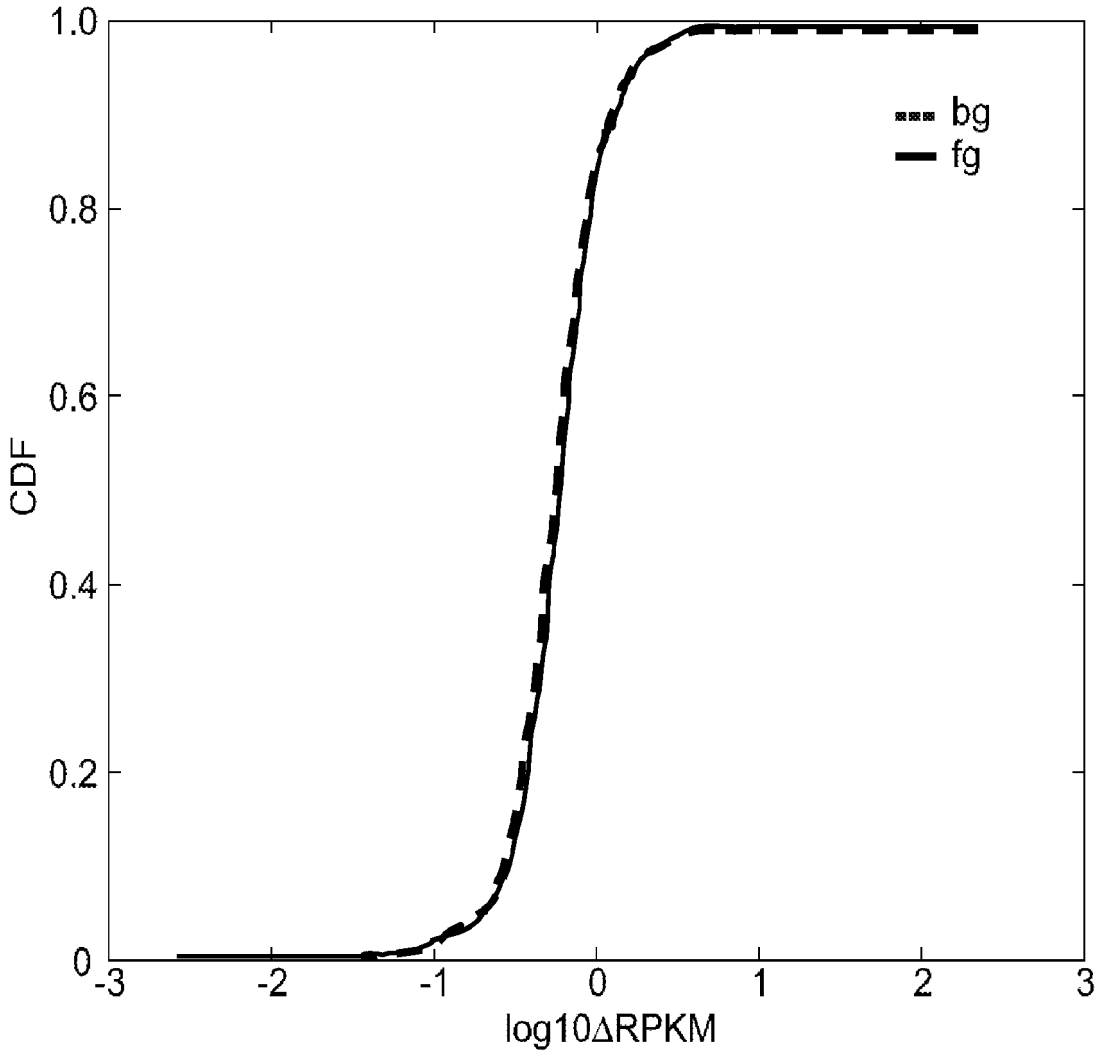


Fig. 13

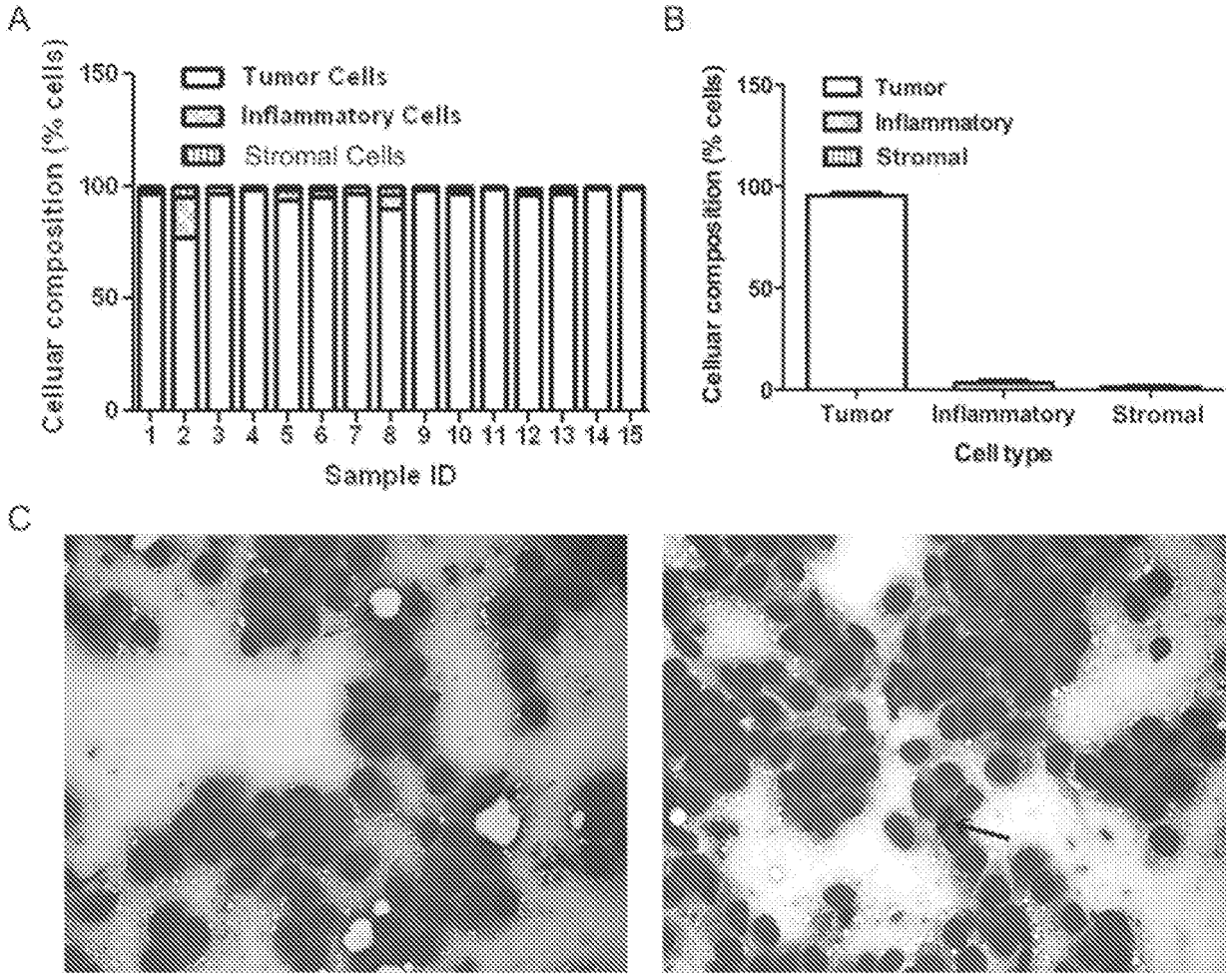

A
Cellular composition (% cells)
150
100
50
0
Tumor Cells
Inflammatory Cells
Stromal Cells
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sample ID
B
Cellular composition (% cells)
150
100
50
0
Tumor
Inflammatory
Stromal
Tumor
Inflammatory
Stromal
Cell type
C

Fig. 14A-14C

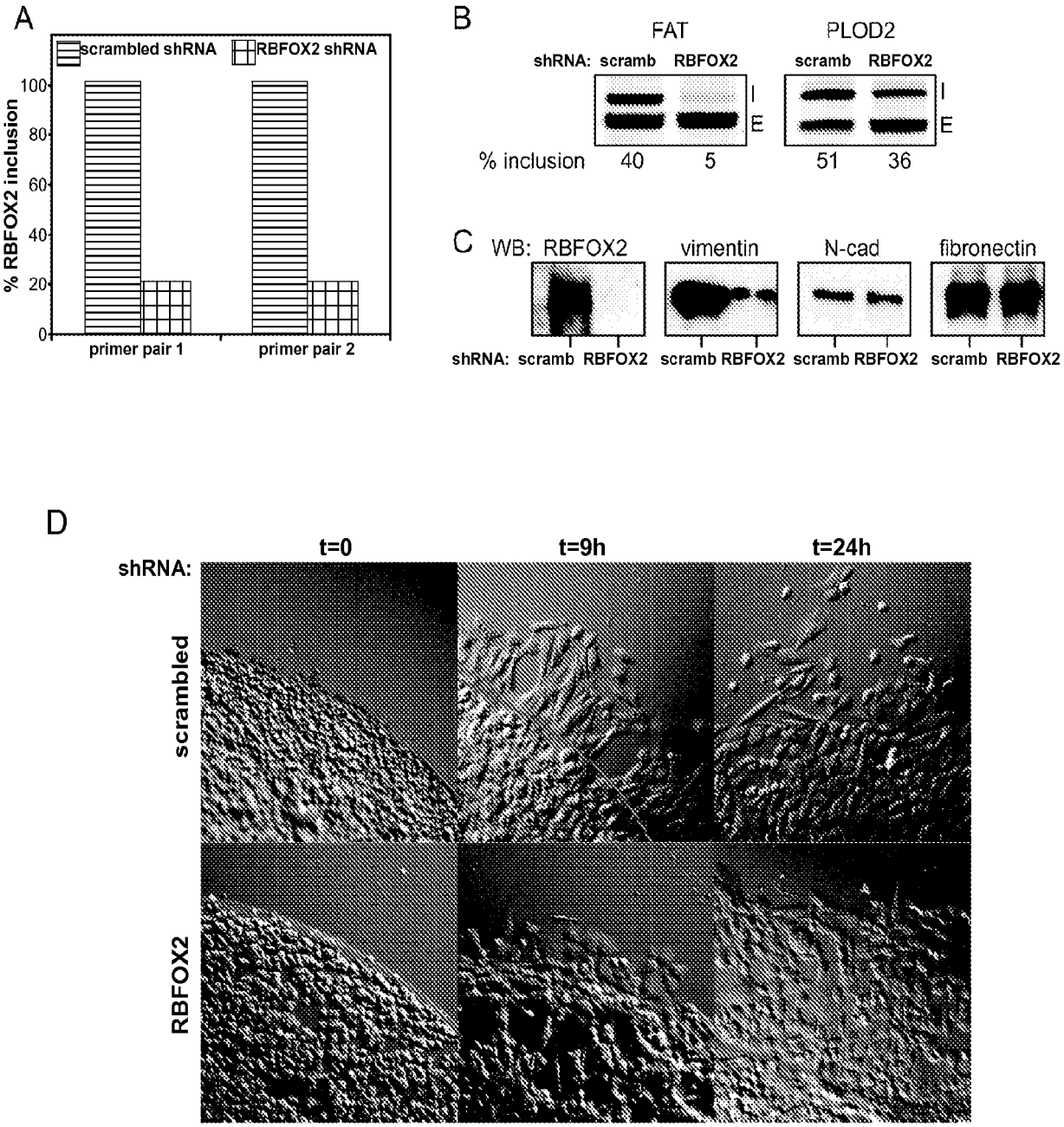

A
scrambled shRNA
RBFOX2 shRNA
% RBFOX2 inclusion
100
80
60
40
20
0
primer pair 1
primer pair 2
B
FAT
PLOD2
shRNA: scramb RBFOX2
scramb RBFOX2
I
E
% inclusion 40 5 51 36
C
WB: RBFOX2
vimentin
N-cad
fibronectin
shRNA: scramb RBFOX2 scramb RBFOX2 scramb RBFOX2 scramb RBFOX2
D
t=0
t=9h
t=24h
shRNA:
scrambled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

Fig. 15A-15D

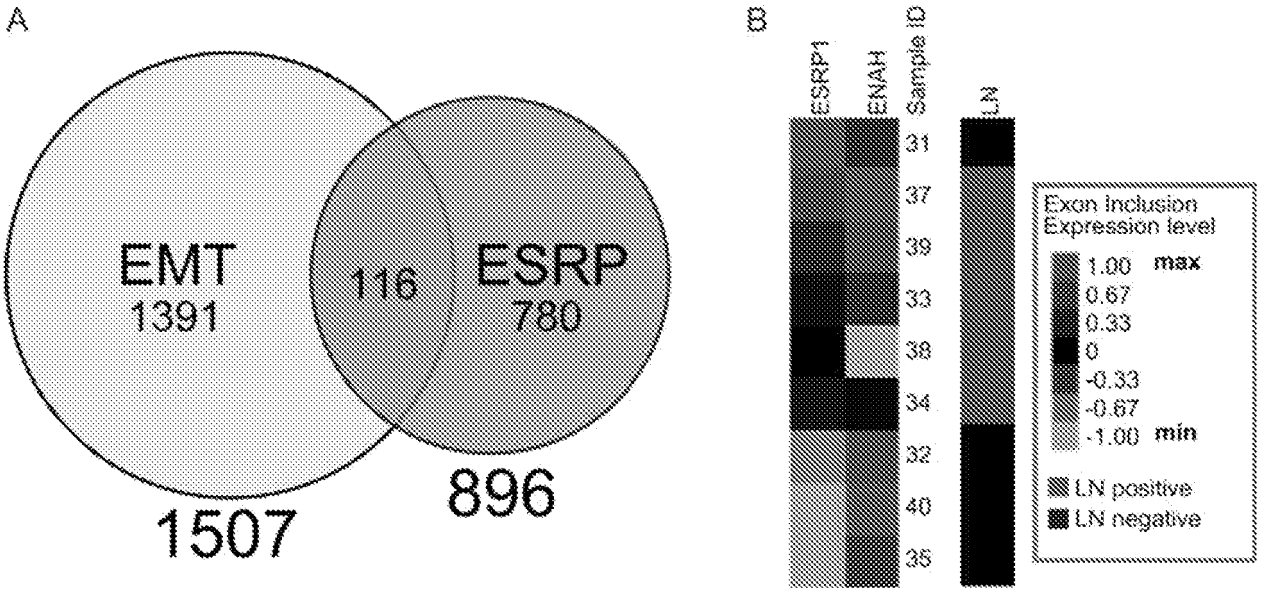

A
EMT
1391
116
ESRP
780
1507
896
B
ESRP1
ENAH
Sample ID
31
37
39
33
38
34
32
40
35
LN
Exon Inclusion
Expression level
1.00 max
0.67
0.33
0
-0.33
-0.67
-1.00 min
LN positive
LN negative

Fig. 16