Cancer Cell, Volume *15*

Supplemental Data

Translational Activation of Snail1 and Other Developmentally Regulated Transcription Factors by YB-1 Promotes an Epithelial-Mesenchymal Transition

Valentina Evdokimova, Cristina Tognon, Tony Ng, Peter Ruzanov, Natalya Melnyk, Dieter Fink, Alexey Sorokin, Lev P. Ovchinnikov, Elai Davicioni, Timothy J. Triche, and Poul H.B. Sorensen

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Transwell Migration Assay and Analysis of Wound Healing by Time-Lapse Microscopy

Cells were starved in serum- and growth factor-free medium for 12 hr and then placed into the top chambers of 96-well Transwell plates (8 μm; Trevigen; 50,000 cells/well). The bottom chambers were filled with growth medium supplemented with 5% horse serum, 20 ng/ml EGF, 100 ng/ml IGF-1 or 100 ng/ml SDF-1. Cultures were maintained for 48 hr, then non-motile cells at the top of the filter were removed and the migrated cells in the bottom chamber were incubated for 1 hr in cell dissociation solution (Trevigen) containing 1 μM of Calcein-AM. Percentages of migrated cells were calculated based on standard curves generated for each cell line. For time-lapse video microscopy, confluent monolayer cells were wounded and then imaged over a 15-hr period at 25 min intervals using a Zeiss Observer-Z1 microscope fitted with O_2/CO_2/temperature modules. Velocity software was used to calculate x- and y-axis migration plots of single cells and the average total length of tracks for each cell line.

Cell Counting, Flow Cytometry (FACS) and BrdU Incorporation

Cell counting and FACS analysis was performed as previously described (Sorokin et al., 2005). 3D-cultured cells were extracted from Matrigel using a neutral protease (Dispase, BD Biosciences) followed by treatment with Accumax (Chemicon) to disrupt clumps and produce single cell suspensions. For BrdU incorporation assay, cells were grown on coverslips, serum starved for 24 hr (-) and re-stimulated with complete growth medium for 1 hr (+). BrdU labeling mixture was added for an additional 1 hr, and then cells were fixed and stained using a BrdU labeling and detection kit (Roche).

Tissue Microarrays (TMAs)

IHC was performed using YB-1 or E-cadherin antibodies, as described above. Results from duplicate cores were combined to give a single result per case. In cases where there was a discrepancy, the higher of the two scores for a particular stain was used. 143 out of the 175 cases were considered interpretable. Scoring of the TMA was completed by a certified pathologist and an independent observer. Statistical significance of correlation between YB-1 and E-cadherin expression was determined using Fisher's exact test. Survival analysis was performed by the generation of Kaplan-Meier curves and the log-rank statistic was used to assess the differences between groups. All analyses were performed using the SPSS software (SPSS Inc., Chicago, Illinois).

RT-PCR and RNase Protection Assay

For semi-quantitative RT-PCR analysis, RNA samples were reverse transcribed using random hexamer primers, and the resulting cDNA was amplified by PCR in the linear range for each transcript (25 cycles) using Taq polymerase (Invitrogen). Quantitative RT-PCR was performed in the iCycler real time PCR machine (BioRad) using the SYBR green/fluorescein PCR Master Mix (SuperArray). All values were normalized to an endogenous human ribosomal protein *L19* (*hRPL19*). Relative mRNA expression was calculated using the comparative $\Delta\Delta C_t$ method. Primer sequences are provided in Table S4. RNase protection assay was done as previously described (Evdokimova et al., 2006b) using the hCYC-1 human cyclin multi-probe template set (BD RiboQuant).

In vitro Transcription and Translation

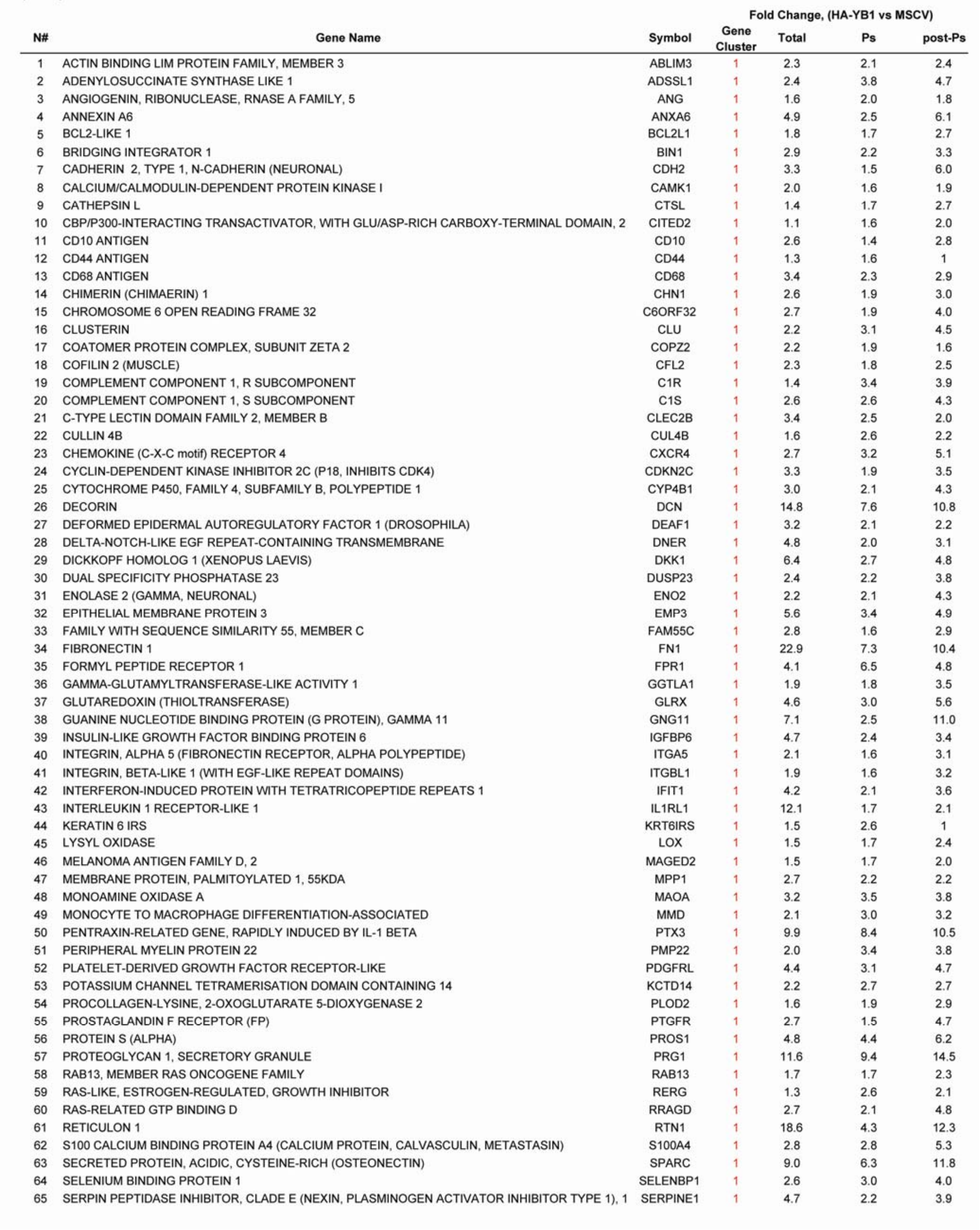
pSP36 coding for the 5’UTR *β-globin*-LUC was obtained from Dr. Wakiyama (RIKEN Genomic Sciences Center, Tsurumi, Japan). *Snail1* 5’UTR was synthesized using overlapping synthetic primers harboring the entire *Snail1* 5’UTR sequence (underlined) (forward: 5’-GATATT**AAGCTT**GGCACGGCCTAGCGAGTGGTTCTTCTGCGCTACTGCTGCGCGAATCGGCGAC-3’ and reverse: 5’-TGCTGCT**CCATGG**AGTGGTCGAGGCACTGGGGTCGCCGATTCGCGCAGCAGTAGCGCAGAA-3’) between the Hind III and Nco I restriction sites (bold), and inserted into pSP36-fLUC (fireflyLUC) in lieu of the *β-globin* 5’UTR. Uncapped mRNAs were synthesized using the RiboMax kit (Promega) and the corresponding plasmids as templates. Capped mRNAs were synthesized in the presence of 200 μM m^7GpppG (New England Biolabs). *In vitro* translation was performed using nuclease-treated rabbit reticulocyte lysate (Promega) according to the manufacturer’s recommendations.

Transient Transfection and Luciferase Assay

MCF10AT-MSCV or MCF10AT-YB-1 cells growing on 6-well plates were transiently transfected with pcDNA-fLUC harboring 5’UTRs of either *β-globin* or *Snail1* (1 μg/well) using FuGENE 6 (Roche). A renilla LUC pRL reporter (Promega) was used as an internal control (100 ng/well). Where indicated, cells were starved before transfection for 16 hr, incubated with transfection mixture for 6 hr, and then starved for another 18 hr in the absence or presence of 50 nM rapamycin. Cells were harvested 24 hr after transfection, lysed and incubated with luciferase substrates as described in the protocol of the Dual Luciferase kit (Promega). Luciferase activity was measured for 10 sec with a luminometer (Tecan). All firefly readouts were normalized to renilla Luc levels. Experiments were performed in duplicate and repeated three times.

Supplemental Tables and Figures

Table S1. Genes differentially expressed between MCF10AT-YB-1 and –MSCV cells in each of Total, Ps and post-Ps fractions (average of two biological replicates >1.5 fold-change, t-test p < 0.05).



N#	Gene Name	Symbol	Gene Cluster	Fold Change, (HA-YB1 vs MSCV) Total	Ps	post-Ps
1	ACTIN BINDING LIM PROTEIN FAMILY, MEMBER 3	ABLIM3	1	2.3	2.1	2.4
2	ADENYLOSUCCINATE SYNTHASE LIKE 1	ADSSL1	1	2.4	3.8	4.7
3	ANGIOGENIN, RIBONUCLEASE, RNASE A FAMILY, 5	ANG	1	1.6	2.0	1.8
4	ANNEXIN A6	ANXA6	1	4.9	2.5	6.1
5	BCL2-LIKE 1	BCL2L1	1	1.8	1.7	2.7
6	BRIDGING INTEGRATOR 1	BIN1	1	2.9	2.2	3.3
7	CADHERIN 2, TYPE 1, N-CADHERIN (NEURONAL)	CDH2	1	3.3	1.5	6.0
8	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE I	CAMK1	1	2.0	1.6	1.9
9	CATHEPSIN L	CTSL	1	1.4	1.7	2.7
10	CBP/P300-INTERACTING TRANSACTIVATOR, WITH GLU/ASP-RICH CARBOXY-TERMINAL DOMAIN, 2	CITED2	1	1.1	1.6	2.0
11	CD10 ANTIGEN	CD10	1	2.6	1.4	2.8
12	CD44 ANTIGEN	CD44	1	1.3	1.6	1
13	CD68 ANTIGEN	CD68	1	3.4	2.3	2.9
14	CHIMERIN (CHIMAERIN) 1	CHN1	1	2.6	1.9	3.0
15	CHROMOSOME 6 OPEN READING FRAME 32	C6ORF32	1	2.7	1.9	4.0
16	CLUSTERIN	CLU	1	2.2	3.1	4.5
17	COATOMER PROTEIN COMPLEX, SUBUNIT ZETA 2	COPZ2	1	2.2	1.9	1.6
18	COFILIN 2 (MUSCLE)	CFL2	1	2.3	1.8	2.5
19	COMPLEMENT COMPONENT 1, R SUBCOMPONENT	C1R	1	1.4	3.4	3.9
20	COMPLEMENT COMPONENT 1, S SUBCOMPONENT	C1S	1	2.6	2.6	4.3
21	C-TYPE LECTIN DOMAIN FAMILY 2, MEMBER B	CLEC2B	1	3.4	2.5	2.0
22	CULLIN 4B	CUL4B	1	1.6	2.6	2.2
23	CHEMOKINE (C-X-C motif) RECEPTOR 4	CXCR4	1	2.7	3.2	5.1
24	CYCLIN-DEPENDENT KINASE INHIBITOR 2C (P18, INHIBITS CDK4)	CDKN2C	1	3.3	1.9	3.5
25	CYTOCHROME P450, FAMILY 4, SUBFAMILY B, POLYPEPTIDE 1	CYP4B1	1	3.0	2.1	4.3
26	DECORIN	DCN	1	14.8	7.6	10.8
27	DEFORMED EPIDERMAL AUTOREGULATORY FACTOR 1 (DROSOPHILA)	DEAF1	1	3.2	2.1	2.2
28	DELTA-NOTCH-LIKE EGF REPEAT-CONTAINING TRANSMEMBRANE	DNER	1	4.8	2.0	3.1
29	DICKKOPF HOMOLOG 1 (XENOPUS LAEVIS)	DKK1	1	6.4	2.7	4.8
30	DUAL SPECIFICITY PHOSPHATASE 23	DUSP23	1	2.4	2.2	3.8
31	ENOLASE 2 (GAMMA, NEURONAL)	ENO2	1	2.2	2.1	4.3
32	EPITHELIAL MEMBRANE PROTEIN 3	EMP3	1	5.6	3.4	4.9
33	FAMILY WITH SEQUENCE SIMILARITY 55, MEMBER C	FAM55C	1	2.8	1.6	2.9
34	FIBRONECTIN 1	FN1	1	22.9	7.3	10.4
35	FORMYL PEPTIDE RECEPTOR 1	FPR1	1	4.1	6.5	4.8
36	GAMMA-GLUTAMYLTRANSFERASE-LIKE ACTIVITY 1	GGTLA1	1	1.9	1.8	3.5
37	GLUTAREDOXIN (THIOLTRANSFERASE)	GLRX	1	4.6	3.0	5.6
38	GUANINE NUCLEOTIDE BINDING PROTEIN (G PROTEIN), GAMMA 11	GNG11	1	7.1	2.5	11.0
39	INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 6	IGFBP6	1	4.7	2.4	3.4
40	INTEGRIN, ALPHA 5 (FIBRONECTIN RECEPTOR, ALPHA POLYPEPTIDE)	ITGA5	1	2.1	1.6	3.1
41	INTEGRIN, BETA-LIKE 1 (WITH EGF-LIKE REPEAT DOMAINS)	ITGBL1	1	1.9	1.6	3.2
42	INTERFERON-INDUCED PROTEIN WITH TETRATRICOPEPTIDE REPEATS 1	IFIT1	1	4.2	2.1	3.6
43	INTERLEUKIN 1 RECEPTOR-LIKE 1	IL1RL1	1	12.1	1.7	2.1
44	KERATIN 6 IRS	KRT6IRS	1	1.5	2.6	1
45	LYSYL OXIDASE	LOX	1	1.5	1.7	2.4
46	MELANOMA ANTIGEN FAMILY D, 2	MAGED2	1	1.5	1.7	2.0
47	MEMBRANE PROTEIN, PALMITOYLATED 1, 55KDA	MPP1	1	2.7	2.2	2.2
48	MONOAMINE OXIDASE A	MAOA	1	3.2	3.5	3.8
49	MONOCYTE TO MACROPHAGE DIFFERENTIATION-ASSOCIATED	MMD	1	2.1	3.0	3.2
50	PENTRAXIN-RELATED GENE, RAPIDLY INDUCED BY IL-1 BETA	PTX3	1	9.9	8.4	10.5
51	PERIPHERAL MYELIN PROTEIN 22	PMP22	1	2.0	3.4	3.8
52	PLATELET-DERIVED GROWTH FACTOR RECEPTOR-LIKE	PDGFRL	1	4.4	3.1	4.7
53	POTASSIUM CHANNEL TETRAMERISATION DOMAIN CONTAINING 14	KCTD14	1	2.2	2.7	2.7
54	PROCOLLAGEN-LYSINE, 2-OXOGLUTARATE 5-DIOXYGENASE 2	PLOD2	1	1.6	1.9	2.9
55	PROSTAGLANDIN F RECEPTOR (FP)	PTGFR	1	2.7	1.5	4.7
56	PROTEIN S (ALPHA)	PROS1	1	4.8	4.4	6.2
57	PROTEOGLYCAN 1, SECRETORY GRANULE	PRG1	1	11.6	9.4	14.5
58	RAB13, MEMBER RAS ONCOGENE FAMILY	RAB13	1	1.7	1.7	2.3
59	RAS-LIKE, ESTROGEN-REGULATED, GROWTH INHIBITOR	RERG	1	1.3	2.6	2.1
60	RAS-RELATED GTP BINDING D	RRAGD	1	2.7	2.1	4.8
61	RETICULON 1	RTN1	1	18.6	4.3	12.3
62	S100 CALCIUM BINDING PROTEIN A4 (CALCIUM PROTEIN, CALVASCULIN, METASTASIN)	S100A4	1	2.8	2.8	5.3
63	SECRETED PROTEIN, ACIDIC, CYSTEINE-RICH (OSTEONECTIN)	SPARC	1	9.0	6.3	11.8
64	SELENIUM BINDING PROTEIN 1	SELENBP1	1	2.6	3.0	4.0
65	SERPIN PEPTIDASE INHIBITOR, CLADE E (NEXIN, PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1), 1	SERPINE1	1	4.7	2.2	3.9

66	SERPIN PEPTIDASE INHIBITOR, CLADE E (NEXIN, PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1), 2	SERPINE2	1	4.7	4.3	7.4
67	SOLUTE CARRIER FAMILY 16 (MONOCARBOXYLIC ACID TRANSPORTERS), MEMBER 6	SLC16A6	1	7.3	2.1	4.8
68	SOLUTE CARRIER FAMILY 2 (FACILITATED GLUCOSE TRANSPORTER), MEMBER 14	SLC2A14	1	1.7	2.1	6.1
69	SOLUTE CARRIER FAMILY 2 (FACILITATED GLUCOSE TRANSPORTER), MEMBER 3	SLC2A3	1	2.0	3.6	8.6
70	SOLUTE CARRIER FAMILY 20 (PHOSPHATE TRANSPORTER), MEMBER 1	SLC20A1	1	2.1	2.1	2.5
71	SOLUTE CARRIER ORGANIC ANION TRANSPORTER FAMILY, MEMBER 4A1	SLCO4A1	1	1.7	2.0	2.3
72	SORTING NEXIN 14	SNX14	1	3.3	2.0	3.8
73	ST3 BETA-GALACTOSIDE ALPHA-2,3-SIALYLTRANSFERASE 5	ST3GAL5	1	3.8	5.5	8.8
74	SULFOTRANSFERASE FAMILY, CYTOSOLIC, 1B, MEMBER 1	SULT1B1	1	10.7	3.4	4.3
75	TIMP METALLOPEPTIDASE INHIBITOR 1	TIMP1	1	2.6	2.5	2.4
76	TIMP METALLOPEPTIDASE INHIBITOR 2	TIMP2	1	1.9	1.5	2.8
77	TRANSCOBALAMIN II; MACROCYTIC ANEMIA	TCN2	1	2.4	2.0	3.0
78	TRANSGELIN	TAGLN	1	4.1	4.3	4.6
79	TRANSMEMBRANE PROTEIN 100	TMEM100	1	8.5	2.0	3.6
80	TRANSMEMBRANE PROTEIN 37	TMEM37	1	3.0	2.9	2.8
81	TRANSMEMBRANE PROTEIN 58	TMEM58	1	1.5	2.7	2.1
82	TSPY-LIKE 3 (PSEUDOGENE)	TSPYL3	1	1.2	2.1	1.4
83	TUMOR NECROSIS FACTOR, ALPHA-INDUCED PROTEIN 6	TNFAIP6	1	7.3	6.7	13.3
84	TWIST HOMOLOG 1 (ACROCEPHALOSYNDACTYLY 3; SAETHRE-CHOTZEN SYNDROME) (DROSOPHIL	TWIST1	1	1.5	1.8	2.1
85	TWISTED GASTRULATION HOMOLOG 1 (DROSOPHILA)	TWSG1	1	1.2	1.6	2.0
86	UDP-N-ACETYL-ALPHA-D-GALACTOSAMINE:POLYPEPTIDE N-ACETYLGALACTOSAMINYLTRANSFER	GALNTL2	1	4.4	1.7	3.9
87	VIMENTIN	VIM	1	3.0	2.4	2.6
88	YIPPEE-LIKE 2 (DROSOPHIILA)	YPEL2	1	1.5	4.7	1.3
89	ACID PHOSPHATASE-LIKE 2	ACPL2	2	-3.3	-2.3	-3.5
90	ACTIN-LIKE 8	ACTL8	2	-2.0	-2.5	-2.1
91	ACTIVATING TRANSCRIPTION FACTOR 3	ATF3	2	-2.8	-2.8	-1.9
92	ACYLTRANSFERASE LIKE 1	AYTL1	2	-3.8	-2.8	-3.2
93	ADAPTOR-RELATED PROTEIN COMPLEX 1, MU 2 SUBUNIT	AP1M2	2	-18.0	-20.1	-18.7
94	ADAPTOR-RELATED PROTEIN COMPLEX 1, SIGMA 3 SUBUNIT	AP1S3	2	-3.9	-2.2	-2.6
95	ADAPTOR-RELATED PROTEIN COMPLEX 3, SIGMA 1 SUBUNIT	AP3S1	2	1.0	-2.1	1.0
96	ADENYLATE KINASE 3-LIKE 1	AK3L1	2	-1.9	-2.3	-2.0
97	ADENYLOSUCCINATE SYNTHASE	ADSS	2	-1.1	-2.1	1.0
98	ADP-RIBOSYLATION FACTOR-LIKE 4A	ARL4A	2	-1.5	-2.6	-1.4
99	ADP-RIBOSYLATION FACTOR-LIKE 4C	ARL4C	2	-2.5	-2.1	-3.7
100	ADP-RIBOSYLTRANSFERASE 3	ART3	2	-51.5	-40.8	-26.9
101	ALDEHYDE DEHYDROGENASE 1 FAMILY, MEMBER A3	ALDH1A3	2	-10.4	-3.1	-13.5
102	ALPHA-2-GLYCOPROTEIN 1, ZINC	AZGP1	2	-7.0	-5.7	-6.3
103	AMINOACYLASE 1	ACY1	2	-1.1	-2.0	-1.1
104	ANDROGEN-INDUCED 1	AIG1	2	-1.9	-2.0	-1.1
105	ANKYRIN REPEAT DOMAIN 22	ANKRD22	2	-24.7	-10.5	-28.9
106	ANNEXIN A3	ANXA3	2	-2.3	-5.5	-2.7
107	ANNEXIN A8	ANXA8	2	-32.0	-14.2	-19.7
108	ANTERIOR GRADIENT 2 HOMOLOG (XENOPUS LAEVIS)	AGR2	2	-11.1	-30.4	-6.0
109	ARGININE VASOPRESSIN-INDUCED 1	AVPI1	2	-2.3	-2.2	-2.0
110	ASPARAGINE SYNTHETASE	ASNS	2	-4.9	-3.7	-2.9
111	ATP BINDING DOMAIN 4	ATPBD4	2	-1.3	-2.1	1.0
112	ATPASE, CA++ TRANSPORTING, CARDIAC MUSCLE, SLOW TWITCH 2	ATP2A2	2	-2.1	-2.0	-1.9
113	ATPASE, NA+/K+ TRANSPORTING, ALPHA 1 POLYPEPTIDE	ATP1A1	2	-1.7	-2.0	-1.8
114	ATPASE, NA+/K+ TRANSPORTING, BETA 1 POLYPEPTIDE	ATP1B1	2	-2.4	-2.0	-1.9
115	BMP AND ACTIVIN MEMBRANE-BOUND INHIBITOR HOMOLOG (XENOPUS LAEVIS)	BAMBI	2	-2.5	-3.1	-2.2
116	BRADYKININ RECEPTOR B1	BDKRB1	2	-1.6	-2.6	-2.8
117	BUTYROBETAINE (GAMMA), 2-OXOGLUTARATE DIOXYGENASE (GAMMA-BUTYROBETAINE HYDROX	BBOX1	2	-34.0	-7.0	-5.8
118	CADHERIN 1, TYPE 1, E-CADHERIN (EPITHELIAL)	CDH1	2	-9.3	-2.5	-22.7
119	CADHERIN 19, TYPE 2	CDH19	2	-7.6	-2.7	-6.3
120	CADHERIN 3, TYPE 1, P-CADHERIN (PLACENTAL)	CDH3	2	-16.6	-6.3	-18.1
121	CALBINDIN 2, 29KDA (CALRETININ)	CALB2	2	-7.4	-2.9	-2.9
122	CALCYPHOSINE	CAPS	2	-3.3	-4.4	-3.0
123	CARBONIC ANHYDRASE II	CA2	2	-46.4	-22.8	-18.0
124	CARBONIC REDUCTASE 4	CBR4	2	-1.6	-2.0	-1.1
125	CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE 1 (BILIARY GLYCOPROTEIN)	CEACAM1	2	-3.3	-1.7	-4.1
126	CARNITINE O-OCTANOYLTRANSFERASE	CROT	2	-1.1	-2.3	-1.9
127	CAS-BR-M (MURINE) ECOTROPIC RETROVIRAL TRANSFORMING SEQUENCE C	CBLC	2	-4.4	-3.5	-3.5
128	CATHEPSIN H	CTSH	2	-3.1	-4.5	-3.5
129	CATHEPSIN L2	CTSL2	2	-3.9	-5.3	-3.5
130	CD24 ANTIGEN (SMALL CELL LUNG CARCINOMA CLUSTER 4 ANTIGEN)	CD24	2	-3.5	-7.6	-6.2
131	CD82 ANTIGEN	CD82	2	-8.8	-2.2	-7.4
132	CDC42 SMALL EFFECTOR 1	CDC42SE1	2	-3.1	-2.6	-1.4
133	CDC42 SMALL EFFECTOR 2	CDC42SE2	2	-2.7	-1.7	-2.4
134	CDC6 CELL DIVISION CYCLE 6 HOMOLOG (S. CEREVISIAE)	CDC6	2	1.5	-1.9	-1.2
135	CELLULAR REPRESSOR OF E1A-STIMULATED GENES 1	CREG1	2	-7.5	-6.1	-4.3
136	CELLULAR RETINOIC ACID BINDING PROTEIN 2	CRABP2	2	-3.4	-4.4	-1.8

137	CHEMOKINE (C-X-C MOTIF) LIGAND 10	CXCL10	2	-9.8	-6.8	-5.4
138	CHEMOKINE (C-X-C MOTIF) LIGAND 11	CXCL11	2	-9.0	-29.4	-17.1
139	CHITINASE 3-LIKE 2	CHI3L2	2	1.6	-2.0	1.1
140	CHLORIDE CHANNEL, CALCIUM ACTIVATED, FAMILY MEMBER 2	CLCA2	2	-26.6	-4.8	-31.1
141	CHROMOSOME 10 OPEN READING FRAME 58	C10ORF58	2	-2.7	-2.5	-2.4
142	CHROMOSOME 14 OPEN READING FRAME 128	C14ORF128	2	-4.0	-2.7	-2.4
143	CHROMOSOME 15 OPEN READING FRAME 48	C15ORF48	2	-3.6	-4.4	-1.8
144	CHROMOSOME 16 OPEN READING FRAME 24	C16ORF24	2	-1.2	-1.9	-1.4
145	CHROMOSOME 20 OPEN READING FRAME 19	C20ORF19	2	-1.4	-2.4	-1.1
146	CHROMOSOME 6 OPEN READING FRAME 115	C6ORF115	2	-1.6	-2.2	-1.4
147	CHROMOSOME 6 OPEN READING FRAME 5	TRAF3IP2	2	-1.9	-2.3	-1.9
148	CHROMOSOME 7 OPEN READING FRAME 24	C7ORF24	2	-2.0	-2.3	-1.6
149	CHROMOSOME 9 OPEN READING FRAME 52	C9ORF52	2	-3.1	-2.0	-2.6
150	CKLF-LIKE MARVEL TRANSMEMBRANE DOMAIN CONTAINING 7	CMTM7	2	-2.9	-2.7	-3.2
151	CLAUDIN 1	CLDN1	2	-23.2	-14.3	-65.6
152	CLAUDIN 4	CLDN4	2	-17.3	-26.9	-21.6
153	CLAUDIN 7	CLDN7	2	-9.3	-4.2	-4.9
154	COENZYME Q3 HOMOLOG, METHYLTRANSFERASE (YEAST)	COQ3	2	-1.1	-3.1	-1.2
155	COILED-COIL DOMAIN CONTAINING 51	CCDC51	2	1.0	-2.3	1.0
156	COLLAGEN, TYPE XVII, ALPHA 1	COL17A1	2	-7.5	-1.9	-11.9
157	CREATINE KINASE, MITOCHONDRIAL 1B	CKMT1A	2	-8.2	-3.1	-6.1
158	CTD (CARBOXY-TERMINAL DOMAIN, RNA POLYMERASE II, POLYPEPTIDE A) SMALL PHOSPHATASE-	CTDSPL	2	-1.4	-1.8	-2.0
159	CYSTATIN A (STEFIN A)	CSTA	2	-2.0	-3.1	-2.3
160	CYSTATIN E/M	CST6	2	-3.2	-3.0	-2.1
161	CYSTEINE AND GLYCINE-RICH PROTEIN 2	CSRP2	2	-1.9	-2.7	-1.8
162	CYTOCHROME B5 REDUCTASE 2	CYB5R2	2	-1.1	-2.9	-1.7
163	D21S2089E	D21S2089E	2	-2.1	-2.3	-1.5
164	DEAD (ASP-GLU-ALA-ASP) BOX POLYPEPTIDE 5	DDX5	2	1.2	-2.5	-1.1
165	DEAFNESS, AUTOSOMAL DOMINANT 5	DFNA5	2	-1.8	-2.5	-1.5
166	DEFECTIVE IN SISTER CHROMATID COHESION HOMOLOG 1 (S. CEREVISIAE)	DCC1	2	-1.1	-2.2	-1.3
167	DEFENSIN, BETA 1	DEFB1	2	-12.6	-7.3	-5.9
168	DEHYDROGENASE/REDUCTASE (SDR FAMILY) MEMBER 3	DHRS3	2	-1.9	-2.2	-2.1
169	DENTICLELESS HOMOLOG (DROSOPHILA)	DTL	2	1.3	-2.1	-1.7
170	DEP DOMAIN CONTAINING 6	DEPDC6	2	-2.1	-2.1	-1.8
171	DESMOCOLLIN 2	DSC2	2	-10.3	-2.2	-18.8
172	DESMOCOLLIN 3	DSC3	2	-19.2	-2.8	-38.6
173	DESMOGLEIN 3 (PEMPHIGUS VULGARIS ANTIGEN)	DSG3	2	-19.8	-3.8	-31.5
174	DESMOPLAKIN	DSP	2	-6.7	-2.0	-4.7
175	DUAL SPECIFICITY PHOSPHATASE 2	DUSP2	2	-2.1	-2.1	-2.5
176	E-1 ENZYME	MASA	2	-1.5	-2.1	-1.4
177	EARLY GROWTH RESPONSE 1	EGR1	2	-7.3	-2.2	-2.9
178	EGF-CONTAINING FIBULIN-LIKE EXTRACELLULAR MATRIX PROTEIN 1	EFEMP1	2	-2.3	-1.9	-1.9
179	ELONGATION FACTOR RNA POLYMERASE II-LIKE 3	ELL3	2	-3.2	-2.6	-2.5
180	ELOVL FAMILY MEMBER 6, ELONGATION OF LONG CHAIN FATTY ACIDS (FEN1/ELO2, SUR4/ELO3-LIKE	ELOVL6	2	-1.9	-2.1	1.0
181	ENGULFMENT AND CELL MOTILITY 3	ELMO3	2	-2.8	-2.1	-2.9
182	EPHRIN-A1	EFNA1	2	-3.0	-2.1	-1.5
183	EPITHELIAL V-LIKE ANTIGEN 1	EVA1	2	-25.0	-11.5	-15.7
184	ETS HOMOLOGOUS FACTOR	EHF	2	-5.0	-2.6	-6.5
185	EXTRAEMBRYONIC, SPERMATOGENESIS, HOMEOBOX 1 HOMOLOG (MOUSE)	ESX1	2	-1.3	-2.4	-1.7
186	F11 RECEPTOR	F11R	2	-4.5	-4.0	-5.0
187	FAMILY WITH SEQUENCE SIMILARITY 79, MEMBER B	FAM79B	2	-3.2	-3.0	-1.6
188	FAMILY WITH SEQUENCE SIMILARITY 84, MEMBER B	FAM84B	2	-4.2	-2.2	-5.2
189	FAR UPSTREAM ELEMENT (FUSE) BINDING PROTEIN 1	FUBP1	2	-1.5	-2.0	-1.2
190	FAT TUMOR SUPPRESSOR HOMOLOG 2 (DROSOPHILA)	FAT2	2	-6.0	-1.6	-13.4
191	FATTY ACID BINDING PROTEIN 6, ILEAL (GASTROTROPIN)	FABP6	2	-2.1	-3.4	-1.4
192	FATTY-ACID-COENZYME A LIGASE, VERY LONG-CHAIN 1	SLC27A2	2	-11.8	-7.3	-8.1
193	F-BOX PROTEIN 25	FBXO25	2	-1.1	-2.1	-1.3
194	FIBROBLAST GROWTH FACTOR BINDING PROTEIN 1	FGFBP1	2	-23.0	-4.7	-3.7
195	FIBRONECTIN LEUCINE RICH TRANSMEMBRANE PROTEIN 3	FLRT3	2	-10.8	-3.6	-7.9
196	FIBULIN 1	FBLN1	2	-2.4	-1.8	-2.8
197	FRIZZLED HOMOLOG 3 (DROSOPHILA)	FZD3	2	-1.4	-2.5	-1.8
198	FXYD DOMAIN CONTAINING ION TRANSPORT REGULATOR 3	FXYD3	2	-25.8	-30.3	-43.9
199	G PROTEIN-COUPLED RECEPTOR 87	GPR87	2	-3.2	-4.7	-2.9
200	GLUTAMATE-AMMONIA LIGASE (GLUTAMINE SYNTHETASE)	GLUL	2	-1.7	-3.2	-2.2
201	GLUTATHIONE PEROXIDASE 2 (GASTROINTESTINAL)	GPX2	2	-3.3	-5.9	-3.1
202	GOLGI TRANSPORT 1 HOMOLOG A (S. CEREVISIAE)	GOLT1A	2	-1.7	-4.1	-1.7
203	GROWTH DIFFERENTIATION FACTOR 15	GDF15	2	-2.0	-2.0	-2.2
204	GROWTH FACTOR RECEPTOR-BOUND PROTEIN 14	GRB14	2	-2.3	-2.4	-2.4
205	GROWTH HORMONE REGULATED TBC PROTEIN 1	GRTP1	2	-2.0	-3.8	-2.9
206	GUANYLATE CYCLASE 1, SOLUBLE, ALPHA 3	GUCY1A3	2	-2.5	-2.1	-5.1
207	HAIRY AND ENHANCER OF SPLIT 1, (DROSOPHILA)	HES1	2	-5.3	-4.3	-4.1

208	HETEROCHROMATIN PROTEIN 1, BINDING PROTEIN 3	HP1BP3	2	-1.1	-1.9	1.0
209	HIGH-MOBILITY GROUP BOX 3	HMGB3	2	-3.5	-2.0	-2.0
210	HISTONE 1, H3A	HIST1H3E	2	-37.9	-123.8	-27.6
211	HIV-1 REV BINDING PROTEIN 2	HRB2	2	-1.7	-2.2	-1.5
212	HRAS-LIKE SUPPRESSOR	HRASLS	2	-1.1	-2.9	-1.2
213	HRAS-LIKE SUPPRESSOR 2	HRASLS2	2	-2.0	-4.6	-2.9
214	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 7	HSD17B7	2	1.1	-2.0	-1.1
215	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 8	HSD17B8	2	-1.2	-2.4	-1.5
216	HYPOTHETICAL PROTEIN BC015148	RP11-484I6.3	2	1.8	-2.0	1.6
217	HYPOTHETICAL PROTEIN FLJ12684	FLJ12684	2	-7.0	-5.6	-7.5
218	HYPOTHETICAL PROTEIN FLJ13984	FLJ13984	2	1.0	-2.2	-1.5
219	HYPOTHETICAL PROTEIN FLJ20152	FLJ20152	2	-2.5	-2.0	-2.4
220	HYPOTHETICAL PROTEIN FLJ22684	GPR110	2	-11.4	-7.9	-14.8
221	HYPOTHETICAL PROTEIN FLJ22684	GPR110	2	-7.2	-4.7	-5.8
222	HYPOTHETICAL PROTEIN FLJ31204	P11-479E16.	2	-8.0	-3.7	-3.0
223	HYPOTHETICAL PROTEIN LOC149086	LOC149086	2	-15.1	-8.3	-10.0
224	HYPOTHETICAL PROTEIN LOC202051	LOC202051	2	1.2	-2.0	1.6
225	IBR DOMAIN CONTAINING 2	IBRDC2	2	-4.8	-2.3	-9.0
226	IMMEDIATE EARLY RESPONSE 2	IER2	2	-6.0	-2.2	-2.1
227	IMP2 INNER MITOCHONDRIAL MEMBRANE PEPTIDASE-LIKE (S. CEREVISIAE)	IMMP2L	2	-1.3	-2.2	-1.2
228	INFLUENZA VIRUS NS1A BINDING PROTEIN	IVNS1ABP	2	1.0	-2.1	-1.4
229	INOSITOL POLYPHOSPHATE-4-PHOSPHATASE, TYPE II, 105KDA	INPP4B	2	-2.5	-2.1	-1.9
230	INOSITOL POLYPHOSPHATE-5-PHOSPHATASE, 145KDA	INPP5D	2	-3.2	-2.3	-5.1
231	INTEGRIN, ALPHA 2 (CD49B, ALPHA 2 SUBUNIT OF VLA-2 RECEPTOR)	ITGA2	2	-3.6	-2.2	-3.1
232	INTEGRIN, ALPHA 6	ITGA6	2	-4.9	-4.2	-4.4
233	INTEGRIN, BETA 6	ITGB6	2	-35.2	-7.7	-27.9
234	INTERFERON REGULATORY FACTOR 6	IRF6	2	-21.7	-6.8	-19.3
235	INTERFERON, GAMMA-INDUCIBLE PROTEIN 30	IFI30	2	-6.2	-4.2	-4.2
236	INTERLEUKIN 1, ALPHA	IL1A	2	-5.1	-5.6	-5.3
237	ISLET CELL AUTOANTIGEN 1, 69KDA	GLCCI1	2	-1.6	-2.1	-1.9
238	ISOCHORISMATASE DOMAIN CONTAINING 1	ISOC1	2	-1.6	-2.0	-1.6
239	JAGGED 1 (ALAGILLE SYNDROME)	JAG1	2	-10.7	-2.2	-9.4
240	KALLIKREIN 5	KLK5	2	-2.0	-2.0	-2.4
241	KALLIKREIN 8 (NEUROPSIN/OVASIN)	KLK8	2	-2.6	-3.0	-2.0
242	KERATIN 15	KRT15	2	-49.7	-41.4	-55.4
243	KERATIN 16 (FOCAL NON-EPIDERMOLYTIC PALMOPLANTAR KERATODERMA)	KRT16	2	-2.1	-2.2	-2.5
244	KERATIN 17	KRT17	2	-18.5	-12.0	-14.5
245	KERATIN 4	KRT4	2	-37.3	-18.0	-25.4
246	KERATINOCYTE ASSOCIATED PROTEIN 3	KRTCAP3	2	-12.3	-13.3	-6.1
247	KH DOMAIN CONTAINING, RNA BINDING, SIGNAL TRANSDUCTION ASSOCIATED 3	KHDRBS3	2	-1.9	-2.4	-2.2
248	KIAA1522	KIAA1522	2	-2.1	1.5	-2.3
249	KIAA1654 PROTEIN	KIAA1654	2	-4.1	-8.7	-2.2
250	LADININ 1	LAD1	2	-3.3	-2.2	-4.8
251	LAMININ, ALPHA 3	LAMA3	2	-3.9	-1.6	-3.8
252	LAMININ, GAMMA 2	LAMC2	2	-1.5	-2.3	-2.6
253	LATEXIN	LXN	2	-5.6	-9.8	-5.6
254	LECITHIN RETINOL ACYLTRANSFERASE (PHOSPHATIDYLCHOLINE--RETINOL O-ACYLTRANSFERASE)	LRAT	2	-5.7	-2.3	-5.7
255	LEM DOMAIN CONTAINING 1	LEMD1	2	-4.8	-3.3	-1.3
256	LEPRECAN-LIKE 1	LEPREL1	2	-4.8	-2.5	-4.1
257	LEUCINE-RICH REPEATS AND IMMUNOGLOBULIN-LIKE DOMAINS 3	LRIG3	2	-5.1	-2.8	-4.4
258	LIPOCALIN 2 (ONCOGENE 24P3)	LCN2	2	-1.4	-2.2	-1.9
259	LIPOIC ACID SYNTHETASE	LIAS	2	-1.1	-2.3	1.0
260	LIPOLYSIS STIMULATED LIPOPROTEIN RECEPTOR	LSR	2	-4.9	-2.8	-5.1
261	LIPOPOLYSACCHARIDE-INDUCED TNF FACTOR	LITAF	2	-6.4	-2.4	-3.7
262	LY6/PLAUR DOMAIN CONTAINING 3	LYPD3	2	-4.8	-2.2	-2.2
263	MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS II, DO BETA	HLA-DOB	2	-6.1	-4.5	-3.7
264	MAL, T-CELL DIFFERENTIATION PROTEIN 2	MAL2	2	-17.6	-18.6	-17.0
265	MATRIX GLA PROTEIN	MGP	2	-9.0	-11.8	-11.0
266	MATRIX METALLOPEPTIDASE 28	MMP28	2	-1.7	-1.6	-2.0
267	MATRIX METALLOPEPTIDASE 7 (MATRILYSIN, UTERINE)	MMP7	2	-2.0	-5.1	-2.8
268	MCM3 MINICHROMOSOME MAINTENANCE DEFICIENT 3 (S. CEREVISIAE)	MCM3	2	1.3	-2.1	1.0
269	MEMBRANE TARGETING (TANDEM) C2 DOMAIN CONTAINING 1	MTAC2D1	2	-9.7	-3.9	-9.5
270	MEMBRANE-ASSOCIATED RING FINGER (C3HC4) 3	3-Mar	2	-1.3	-2.3	-1.4
271	MITOCHONDRIAL RIBOSOMAL PROTEIN L30	MRPL30	2	-1.2	-2.1	1.1
272	MITOGEN-ACTIVATED PROTEIN KINASE 13	MAPK13	2	-2.4	-3.2	-2.6
273	MYC-INDUCED MITOCHONDRIA PROTEIN	MIMITIN	2	1.1	-2.0	1.4
274	MYOSIN IB	MYO1B	2	-5.0	-3.6	-4.2
275	NETRIN 4	NTN4	2	-4.3	-2.8	-3.8
276	NEUREGULIN 4	NRG4	2	-2.5	-2.7	-2.0
277	NEUROTROPHIN 5 (NEUROTROPHIN 4/5)	NTF5	2	-2.4	-3.3	-2.5
278	NIPA-LIKE DOMAIN CONTAINING 3	NPAL3	2	-1.5	-2.0	-1.5

279	NOGGIN	NOG	2	-1.5	-2.5	-1.9
280	NUCLEAR RECEPTOR COACTIVATOR 7	NCOA7	2	-1.9	-2.1	-1.5
281	NUDIX (NUCLEOSIDE DIPHOSPHATE LINKED MOIETY X)-TYPE MOTIF 14	NUDT14	2	-1.2	-2.0	-1.6
282	OLFACTOMEDIN 4	OLFM4	2	-17.9	-1.6	-7.9
283	OTU DOMAIN, UBIQUITIN ALDEHYDE BINDING 2	OTUB2	2	-3.3	-3.1	-5.3
284	P8 PROTEIN (CANDIDATE OF METASTASIS 1)	P8	2	-4.4	-3.0	-1.8
285	PARATHYROID HORMONE-LIKE HORMONE	PTHLH	2	-3.0	-3.4	-3.1
286	PEF PROTEIN WITH A LONG N-TERMINAL HYDROPHOBIC DOMAIN	PEF1	2	1.1	-2.0	1.2
287	PERP, TP53 APOPTOSIS EFFECTOR	PERP	2	-2.2	-2.9	-2.4
288	PHOSPHATIDIC ACID PHOSPHATASE TYPE 2C	PPAP2C	2	-2.8	-2.7	-2.4
289	PHOSPHOGLUCOMUTASE 2	PGM2	2	-1.9	-3.1	-2.2
290	PHOSPHOGLYCERATE DEHYDROGENASE	PHGDH	2	-3.1	-2.2	-1.9
291	PHOSPHOLIPASE A2, GROUP XIIA	PLA2G12A	2	-1.5	-2.3	1.0
292	PIRIN (IRON-BINDING NUCLEAR PROTEIN)	PIR	2	-2.5	-2.5	-1.4
293	PLACENTA-SPECIFIC 8	PLAC8	2	-3.4	-2.5	-2.4
294	PLASMA MEMBRANE PROTEOLIPID (PLASMOLIPIN)	PLLP	2	-3.6	-2.8	-2.1
295	PLASMINOGEN ACTIVATOR, TISSUE	PLAT	2	-14.7	-4.5	-9.1
296	PLECKSTRIN HOMOLOGY DOMAIN CONTAINING, FAMILY F (WITH FYVE DOMAIN) MEMBER 1	PLEKHF1	2	-1.9	-2.1	-2.1
297	PNC1 PROTEIN	MGC4399	2	1.2	-2.0	1.1
298	POLO-LIKE KINASE 2 (DROSOPHILA)	PLK2	2	-2.8	-3.2	-2.4
299	POLYMERASE (DNA DIRECTED), BETA	POLB	2	1.0	-2.1	1.0
300	POLYMERASE (DNA DIRECTED), EPSILON 2 (P59 SUBUNIT)	POLE2	2	-1.3	-3.2	-1.9
301	POTASSIUM CHANNEL TETRAMERISATION DOMAIN CONTAINING 1	KCTD1	2	-1.5	-2.4	-1.8
302	POTASSIUM CHANNEL, SUBFAMILY K, MEMBER 1	KCNK1	2	1.2	-1.9	-1.1
303	POTASSIUM VOLTAGE-GATED CHANNEL, DELAYED-RECTIFIER, SUBFAMILY S, MEMBER 3	KCNS3	2	-1.2	-2.7	-3.3
304	PRKC, APOPTOSIS, WT1, REGULATOR	PAWR	2	-1.4	-2.0	-1.4
305	PROLINE-SERINE-THREONINE PHOSPHATASE INTERACTING PROTEIN 2	PSTPIP2	2	-5.9	-3.2	-4.9
306	PROMETHIN	LOC57146	2	-3.2	-2.5	-1.7
307	PROTEASE, SERINE, 12 (NEUROTRYPSIN, MOTOPSIN)	PRSS12	2	-8.0	-4.2	-11.3
308	PROTEASOME (PROSOME, MACROPAIN) SUBUNIT, BETA TYPE, 9	PSMB9	2	-2.3	-2.7	-1.5
309	PROTEIN ARGININE METHYLTRANSFERASE 6	PRMT6	2	-1.4	-2.5	-1.1
310	PROTEIN KINASE C, DELTA	PRKCD	2	-1.5	-1.6	-2.5
311	PROTEIN PHOSPHATASE 1, REGULATORY (INHIBITOR) SUBUNIT 14C	PPP1R14C	2	-5.3	-3.5	-4.4
312	PROTEIN TYROSINE PHOSPHATASE TYPE IVA, MEMBER 1	PTP4A1	2	-3.2	-2.9	-2.3
313	PRP3 PRE-MRNA PROCESSING FACTOR 3 HOMOLOG (YEAST)	PRPF3	2	-1.4	-1.9	-1.1
314	RAB25, MEMBER RAS ONCOGENE FAMILY	RAB25	2	-58.1	-75.4	-55.6
315	RAB38, MEMBER RAS ONCOGENE FAMILY	RAB38	2	-2.1	-2.7	-1.8
316	RELAXIN 2	RLN2	2	-3.1	-4.3	-2.0
317	RETINOIC ACID RECEPTOR RESPONDER (TAZAROTENE INDUCED) 1	RARRES1	2	-8.0	-10.1	-5.3
318	RHO GDP DISSOCIATION INHIBITOR (GDI) BETA	ARHGDIB	2	-2.7	-2.7	-2.4
319	RHO GTPASE ACTIVATING PROTEIN 8	ARHGAP8	2	-1.9	-2.1	-2.4
320	RIBONUCLEASE P 21KDA SUBUNIT	RPP21	2	1.1	-2.1	1.1
321	RIBOSE 5-PHOSPHATE ISOMERASE A (RIBOSE 5-PHOSPHATE EPIMERASE)	RPIA	2	1.0	-2.4	-1.1
322	RNA BINDING MOTIF PROTEIN 35A	RBM35A	2	-39.4	-12.9	-45.1
323	RNA BINDING PROTEIN WITH MULTIPLE SPLICING	RBPMS	2	-1.8	-2.1	-1.4
324	ROUNDABOUT, AXON GUIDANCE RECEPTOR, HOMOLOG 1 (DROSOPHILA)	ROBO1	2	-2.7	-1.6	-3.6
325	RWD DOMAIN CONTAINING 4A	RWDD4A	2	1.4	-2.0	1.1
326	S100 CALCIUM BINDING PROTEIN A2	S100A2	2	-2.6	-2.2	-2.4
327	S100 CALCIUM BINDING PROTEIN P	S100P	2	-4.7	-3.2	-3.0
328	SECRETED PHOSPHOPROTEIN 1 (OSTEOPONTIN, BONE SIALOPROTEIN I)	SPP1	2	-9.2	-2.5	-1.9
329	SECRETORY LEUKOCYTE PEPTIDASE INHIBITOR	SLPI	2	-1.8	-2.1	-2.3
330	SERINE INCORPORATOR 2	SERINC2	2	-6.2	-4.6	-6.8
331	SERINE PEPTIDASE INHIBITOR, KUNITZ TYPE, 2	SPINT2	2	-14.1	-7.2	-8.6
332	SERINE/THREONINE KINASE 17A (APOPTOSIS-INDUCING)	STK17A	2	-2.0	-2.3	-1.3
333	SERPIN PEPTIDASE INHIBITOR, CLADE B (OVALBUMIN), MEMBER 13	SERPINB13	2	-4.9	-2.6	-8.2
334	SERPIN PEPTIDASE INHIBITOR, CLADE B (OVALBUMIN), MEMBER 3	SERPINB3	2	-32.9	-9.0	-5.5
335	SERPIN PEPTIDASE INHIBITOR, CLADE B (OVALBUMIN), MEMBER 3	SERPINB3	2	-26.1	-4.1	-3.4
336	SERUM/GLUCOCORTICOID REGULATED KINASE	SGK	2	-2.9	-2.5	-2.1
337	SH3 DOMAIN CONTAINING, YSC84-LIKE 1 (S. CEREVISIAE)	SH3YL1	2	-3.1	-4.4	-3.4
338	SIGNAL-TRANSDUCING ADAPTOR PROTEIN-2	STAP2	2	-4.2	-3.3	-2.7
339	SLIT AND NTRK-LIKE FAMILY, MEMBER 6	SLITRK6	2	-24.4	-2.4	-7.5
340	SMALL TRANS-MEMBRANE AND GLYCOSYLATED PROTEIN	LOC57228	2	-1.5	-2.0	-2.0
341	SOLUTE CARRIER FAMILY 2 (FACILITATED GLUCOSE TRANSPORTER), MEMBER 9	SLC2A9	2	-7.7	-4.7	-8.1
342	SOLUTE CARRIER FAMILY 20 (PHOSPHATE TRANSPORTER), MEMBER 2	SLC20A2	2	-1.5	-2.0	-2.6
343	SOLUTE CARRIER FAMILY 25, MEMBER 13 (CITRIN)	SLC25A13	2	1.0	-2.3	-1.4
344	SOLUTE CARRIER FAMILY 3 (ACTIVATORS OF DIBASIC AND NEUTRAL AMINO ACID TRANSPORT), 2	SLC3A2	2	-2.3	-2.3	-2.1
345	SOLUTE CARRIER FAMILY 44, MEMBER 3	SLC44A3	2	-4.2	-3.1	-2.4
346	SOLUTE CARRIER FAMILY 7 (CATIONIC AMINO ACID TRANSPORTER, Y+ SYSTEM), MEMBER 5	SLC7A5	2	-6.6	-3.9	-10.7
347	SPARC RELATED MODULAR CALCIUM BINDING 1	SMOC1	2	-3.7	-2.2	-4.8
348	S-PHASE KINASE-ASSOCIATED PROTEIN 2 (P45)	SKP2	2	-1.4	-2.1	-1.4
349	SPHINGOMYELIN PHOSPHODIESTERASE 2, NEUTRAL MEMBRANE (NEUTRAL SPHINGOMYELINASE)	SMPD2	2	-1.3	-2.0	-1.6

350	START DOMAIN CONTAINING 10	STARD10	2	-2.6	-2.5	-1.7
351	STRATIFIN	SFN	2	-3.0	-4.2	-3.7
352	SULFATASE 2	SULF2	2	-5.2	-2.2	-6.0
353	SUPPRESSION OF TUMORIGENICITY 14 (COLON CARCINOMA)	ST14	2	-9.5	-3.6	-11.5
354	SYNAPTOJANIN 2 BINDING PROTEIN	SYNJ2BP	2	-1.6	-2.2	-1.2
355	SYNAPTOPHYSIN-LIKE 1	SYPL1	2	-1.5	-1.9	-1.4
356	SYNAPTOTAGMIN VIII	SYT8	2	-4.2	-2.8	-4.9
357	TENASCIN C (HEXABRACHION)	TNC	2	-6.7	-1.8	-6.1
358	TETRASPANIN 1	TSPAN1	2	-29.3	-26.7	-29.3
359	TETRASPANIN 13	TSPAN13	2	-4.3	-6.1	-4.6
360	TETRATRICOPEPTIDE REPEAT DOMAIN 12	TTC12	2	-1.1	-2.0	-1.5
361	THO COMPLEX 3	THOC3	2	-1.2	-2.0	-1.2
362	THYROID HORMONE RECEPTOR ASSOCIATED PROTEIN 6	THRAP6	2	1.1	-3.0	-1.1
363	TIMP METALLOPEPTIDASE INHIBITOR 3 (SORSBY FUNDUS DYSTROPHY, PSEUDOINFLAMMATORY)	TIMP3	2	-6.4	-2.3	-8.1
364	TP53 ACTIVATED PROTEIN 1	TP53AP1	2	-1.1	-2.9	-1.4
365	TRANSCOBALAMIN I (VITAMIN B12 BINDING PROTEIN, R BINDER FAMILY)	TCN1	2	-18.3	-26.2	-29.2
366	TRANSMEMBRANE CHANNEL-LIKE 4	TMC4	2	-5.5	-2.0	-5.0
367	TRANSMEMBRANE PROTEIN 27	TMEM27	2	-4.4	-10.5	-4.0
368	TRANSMEMBRANE PROTEIN 40	TMEM40	2	-5.3	-4.5	-3.1
369	TRANSMEMBRANE PROTEIN 54	TMEM54	2	-1.8	-2.6	-1.9
370	TRIPARTITE MOTIF-CONTAINING 22	TRIM22	2	-2.6	-2.7	-2.9
371	TRIPARTITE MOTIF-CONTAINING 29	TRIM29	2	-6.8	-3.5	-11.0
372	TROPONIN I TYPE 2 (SKELETAL, FAST)	TNNI2	2	-3.3	-2.1	-3.2
373	TROPONIN T TYPE 1 (SKELETAL, SLOW)	TNNT1	2	-3.2	-3.8	-2.5
374	TUMOR PROTEIN D52-LIKE 1	TPD52L1	2	-3.3	-2.2	-1.8
375	TUMOR-ASSOCIATED CALCIUM SIGNAL TRANSDUCER 1	TACSTD1	2	-16.5	-14.4	-12.5
376	TUMOR-ASSOCIATED CALCIUM SIGNAL TRANSDUCER 2	TACSTD2	2	-8.3	-6.0	-7.7
377	UDP-GLCNAC:BETAGAL BETA-1,3-N-ACETYLGLUCOSAMINYLTRANSFERASE 3	B3GNT3	2	-3.4	-2.0	-3.1
378	UDP-N-ACETYL-ALPHA-D-GALACTOSAMINE:POLYPEPTIDE N-ACETYLGALACTOSAMINYLTRANSFER.)	GALNT3	2	-6.3	-4.2	-5.1
379	URIDINE PHOSPHORYLASE 1	UPP1	2	-1.6	-2.4	-1.5
380	V-ETS ERYTHROBLASTOSIS VIRUS E26 ONCOGENE HOMOLOG 2 (AVIAN)	ETS2	2	-8.2	-2.9	-6.4
381	VISININ-LIKE 1	VSNL1	2	-5.1	-4.6	-4.0
382	V-MYC MYELOCYTOMATOSIS VIRAL ONCOGENE HOMOLOG (AVIAN)	MYC	2	-2.1	-1.6	-2.0
383	WAP FOUR-DISULFIDE CORE DOMAIN 2	WFDC2	2	-25.1	-14.3	-14.6
384	ZINC FINGER PROTEIN 146	ZNF146	2	-1.5	-2.0	1.3

Table S2. Pathological features and culture conditions of cell lines used in this study, according to ATCC description

Cell Line	**Tumor type**	**Tissue Source, Morphology**	**Culture medium**
MCF10A	breast, FD	fibrocyst; epithelial	DMEM/F12, 5% horse serum, 20 ng/ml EGF, 10 μg/ml inslin, 100 ng/ml cholera toxin, 500 ng/ml hydrocortisone
HMT-3522S	breast, FD	Fibrocyst; epithelial	DMEM/F12, 250 ng/ml insulin, 10 μg/ml transferring; 2.6 ng/ml sodium selenite, 0.1 nM estradiol, 1.4 μM hydrocortisone, 5 μg/ml prolactin, 10 nM EGF
HCC1937	breast, DCIS	primary site; epithelial	RPMI, 10% FBS
MCF7	breast, IDAC	pleural effusion; epithelial	DMEM, 10% FBS
MDA-MB-175VII	breast, IDC	pleural effusion; epithelial	DMEM, 10% FBS
MDA-MB-231	breast, AC	pleural effusion; basal; spindle shaped	DMEM, 10% FBS
MDA-MB-435S	metastatic melanoma	pleural effusion; spindle shaped	DMEM, 10% FBS
MDA-MB-436	breast, AC	pleural effusion; pleomorphic, multinucleated cells	Leibovitz's L15, 10% FBS,
T47D	breast, IDAC	pleural effusion; epithelial	RPMI, 10% FBS
SUM1315MO2	breast, IDAC	metastatic nodule; spindle shaped	Ham's F12, 5% FBS, 10 ng/ml EGF, 5 μg/ml insulin, 10 mM Hepes, pH7.2
HeLa	cervix, AC	primary site; epithelial	DMEM, 10% FBS
PC-3	prostate, AC	bone metastasis; epithelial	DMEM, 10% FBS
EpH4	non-transformed	mouse mammary epithelial	DMEM, 5% CS, 2 mM L-glutamine

AC-adenocarcinoma; DCIS- ductal carcinoma in situ; IDC- invasive ductal carcinoma; FD- fibrocystic disease; IDAC- infiltrating ductal adenocarcinoma

Table S3. List of antibodies, suppliers and concentrations used for immunoblotting, IF or IHC

Antibody	Isotype	Suppliers	Dilution
Actin	goat polyclonal	Santa Cruz Biotech	1:2,000
α-Smooth Muscle Actin (SMA)	mouse IgG2A	Sigma, clone IA4	1:1,000
AKT	rabbit polyclonal	Cell Signaling	1:1,000
p-AKT (Ser 473)	rabbit polyclonal	Cell Signaling	1:1,000
E-cadherin	mouse IgG2A	BD Biosciences	1:1,000
N-cadherin	mouse IgG1	BD Biosciences	1:1,000
CDK4	mouse IgG1	NeoMarkers	1:1,000
CDK6	mouse IgG1	Santa Cruz Biotech	1:1,000
Cyclin A2	rabbit polyclonal	Upstate	1:1,000
Cyclin B1	rabbit polyclonal	Santa Cruz Biotech	1:1,000
Cyclin D1	mouse IgG2A	Cell Signaling	1:1,000
Cyclin D3	mouse monoclonal	Santa Cruz Biotech	1:1,000
Cyclin E	mouse monoclonal	Upstate	1:1,000
Cyclin G1	rabbit polyclonal	Santa Cruz Biotech	1:1,000
Cytokeratin 14 (CK14)	rabbit polyclonal	Covance	1:1,000
Cytokeratin 18 (CK18)	mouse IgG1	Progen Biotechnik	1:1,000
pan-Cytokeratin (pan-CK)	mouse IgG1	Abcam, clone C-11	1:1,000
p-ERK/p44/p42 MAPK (Thr202/Tyr204)	rabbit polyclonal	Cell Signaling	1:1,000
4E-BP1	rabbit polyclonal	Cell Signaling	1:1,000
Fibronectin	mouse IgG1	Santa Cruz Biotech	1:500
Foxo3A	goat polyclonal	Abcam; ab17026	1:1,000
GFP	rabbit polyclonal	Abcam	1:1,000
Gogin-97	mouse IgG1	Molecular Probes	1:1,000
HA.11	mouse monoclonal	Covance, clone 16B12	1:1,000
HA	rabbit polyclonal	Novus Biologicals	1:1,000
HIF-1α	rabbit polyclonal	Cell Signaling	1:500
HoxC6	goat polyclonal	Santa Cruz Biotech	1:1,000
Integrin β1	mouse IgG2B	Chemicon	1:1,1000
Ki-67	mouse monoclonal	Abcam; ab8191	1:500
Laminin 5	rabbit polyclonal	Abcam	1:1,000
Lef-1	rabbit monoclonal	Abcam	1:1,000
MEK	rabbit polyclonal	Cell Signaling	1:1,000
p-MEK (Ser 217/221)	rabbit polyclonal	Cell Signaling	1:1,000
MUC1	mouse IgG1	Upstate	1:1,000
p18	mouse IgG1	Santa Cruz Biotech	1:1,000
p27/KIP1	mouse IgG1	PharMingen International	1:1,000
p38 MAPK	rabbit polyclonal	Cell Signaling	1:1,000
p-p38 (thr180/Tyr182)	rabbit polyclonal	Santa Cruz Biotech	1:1,000
p63	mouse IgG2A	NeoMarkers, clone 4A4	1:500
Pericentrin	rabbit polyclonal	Abcam	1:1,000
p-Smad1/5/8, p-Smad2, p-Smad-3	rabbit polyclonal	Cell Signaling	1:1,000
Snail1	rabbit polyclonal	Abcam	1:1,000
Snail	Rat monoclonal	Cell Signaling	1:1,000
Slug	goat polyclonal	Santa Cruz Biotech	1:1,000
TCF4	rabbit polyclonal	Cell Signaling	1:1,000
Twist	rabbit polyclonal	Santa Cruz Biotech	1:1,000
Vimentin	mouse IgM	Sigma, clone VIM 13.2	1:1,000
YB-1	rabbit polyclonal	home-made against full-length YB1	1:5,000
ZO-1	rabbit polyclonal	Zymed	

Table S4. Primers used for semi-quantitative RT-PCR and quantitative SYBR green RT-PCR

Name	**Gene accession :**	**Forward, 5’-3’**	**Reverse, 5’-3’**	**Product size, bp**
SNAIL1	NM_005985	CACTATGCCGCGCTCTTTC	GCTGGAAGGTAAACTCTGGATTAGA	101
SNAIL2	NM_003068	GGACACATTAGAACTCACACGGG	GCAGTGAGGGCAAGAAAAAGG	51
TWIST	NM_000474	GCAGGGCCGGAGACCTAG	TGTCCATTTTCTCCTTCTCTGGA	52
GAPDH	NM_002046	CACCAGGGCTGCTTTTAACTC	GACAAGCTTCCCGTTCTCAG	151
RPL19	NM_000981	GATGCCGGAAAAACACCTTG	TGGCTGTACCCTTCCGCTT	86
PRICKLE1	NM_153026	TGCCTTTGGAGATGGAGCC	TGGTAAGCAAGCAAAATAGAGC	159
MNT	NM_020310	CGCAGCAACAACAGAGAGC	CTGTTCCTGCTCTCGCTCC	71
HIF1α	NM_001530	GCTTGCTCATCAGTTGCCAC	CATAACAAAACCATCCAAGGC	187
ZNF208	NM_007153	GTCTTCCTGGGTATTGCTGC	TCTTCTATGCCCTGCTCTGG	158
TCF4	NM_001083962	TCTTCTCATATTCCACAGTCCAGC	CTGGAGAATAGATCGAAGCAAGT	271
ATOH1	NM_005172	GCAGGCGAGAGAGCATCC	CCTGCAAAGTGGGAGCC	89
HOXC6	NM_004503	GTCGTGTTCAGTTCCAGCCG	CTGGATACTGGCTTTCTGGTC	183
ZNF286	NM_020652	CCAAGAGAAGAGCACAGAAGAGG	CTTTGTGCAGGATCCAGCTTC	135
TOB2	NM_016272	CAGATTGGTGAGAAGGGAGC	GAATGAAGGTAGGGCTGGGTG	199
LEF1	NM_016269	CCTTGGTGAACGAGTCTGAAATC	GAGGTTTGTGCTTGTCTGGC	70
ZEB2	NM_014795	GCAAACAAGCCAATCCCAG	GTTGGGCACACTAGCTGGAC	158
YB-1	NM_001082785	TACCCATACGACGTCCCAGA	TATAATGGTTACGGTCTGCTG	400
L32	NM_172086	GGCGGAAACCCAGAGGCATTGA	CCTGGCGTTGGGATTGGTGACTCT	250

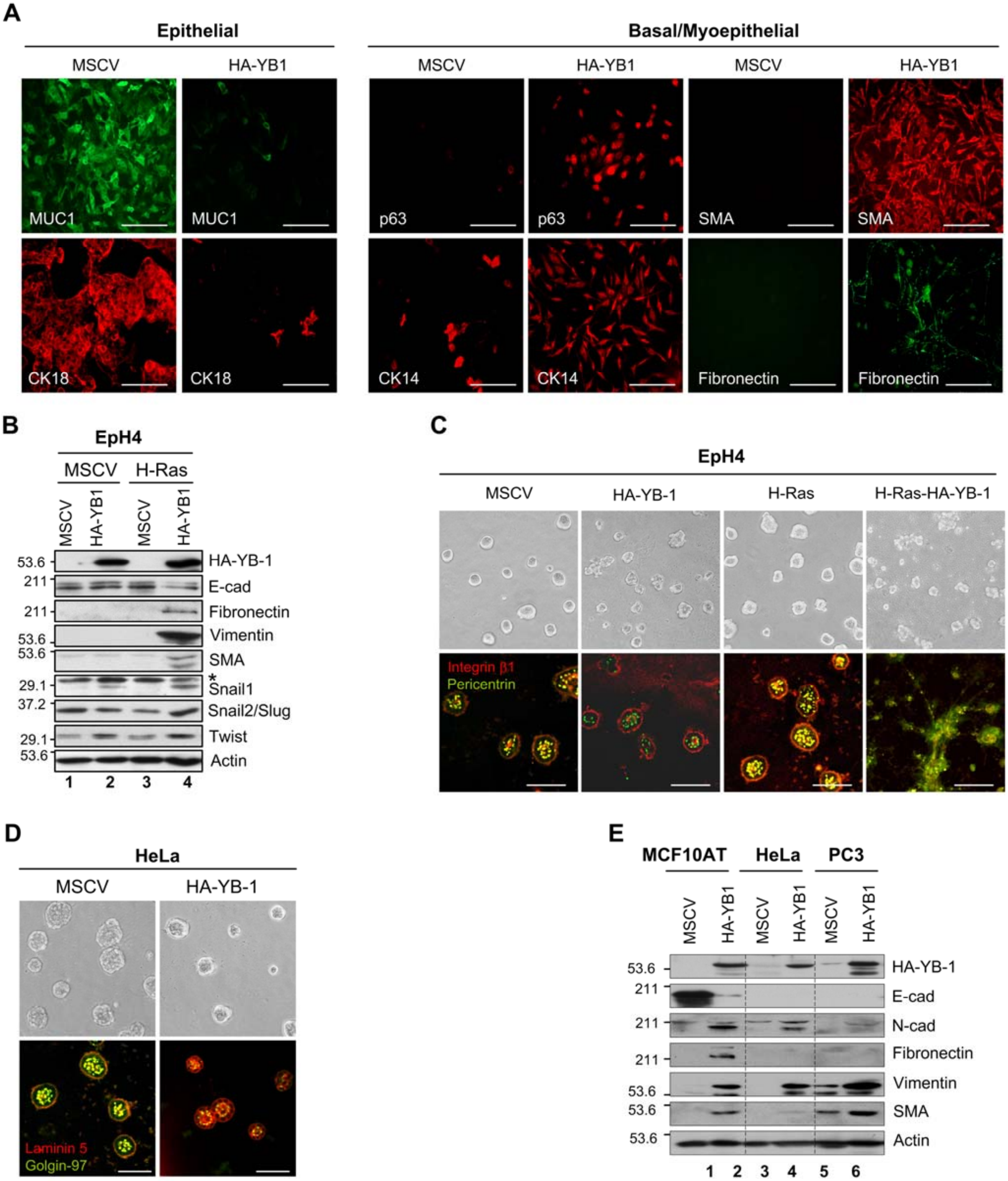


Figure S1. YB-1 Induces EMT-Like Morphological Changes and Expression of Snail1 in Various Carcinoma Cell Lines

(A) Expression and localization of epithelial and mesenchymal markers in MCF10AT cell lines was examined by immunofluorescence microscopy. Scale bars, 200 μm.

(B-C) EpH4 cell lines were grown on Matrigel for 3 days and then examined by immunoblotting (B) or phase contrast (top panels) or confocal (bottom panels) microscopy (C). Star in (B) indicates a non-specific protein cross-reacting with Snail1 antibodies in mouse cell extracts. Pericentrin and integrin β1 in (C) were used as markers of apical and basolateral polarity, respectively. Scale bars, 100 μM.

(D) HeLa cell lines were grown on Matrigel for 5 days and then examined by phase contrast (top panels) or confocal (bottom panels) microscopy. Scale bars, 100 μM.

(E) MCF10AT, HeLa and PC3 cell lines were grown in monolayers and analyzed for the expression of epithelial (E-cadherin) and mesenchymal (N-cadherin, fibronectin, vimentin and SMA) markers by immunoblotting.

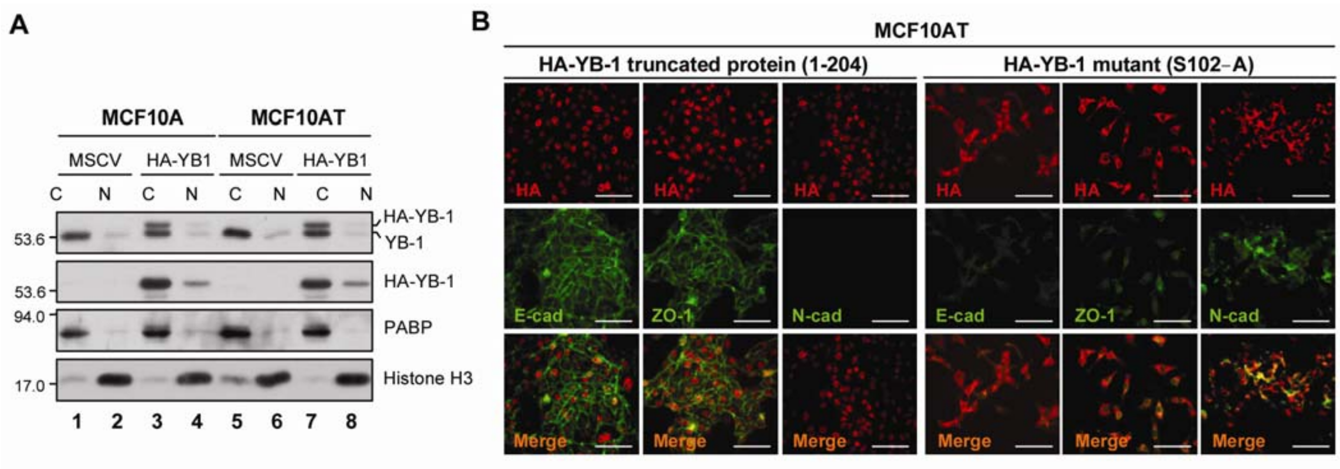


Figure S2. Cytoplasmic Localization of YB-1 Correlates with the EMT Phenotype

(A) YB-1 localization in MCF10A and MCF10AT cell lines was analyzed by immunoblotting. Poly(A)-binding protein (PABP) and histone H3 were used as markers of cytosolic (C) and nuclear (N) fractions, respectively.

(B) MCF10AT parental cells were transiently transfected with the plasmids encoding either the truncated form of YB-1 (1-204) or the YB-1 mutant protein (S102-A) and then analyzed for the expression of epithelial (E-cadherin and ZO-1) or mesenchymal (N-cadherin) proteins by immunofluorescence microscopy. Expression of YB-1 proteins was confirmed using anti-HA antibodies. Scale bars, 100 μm.

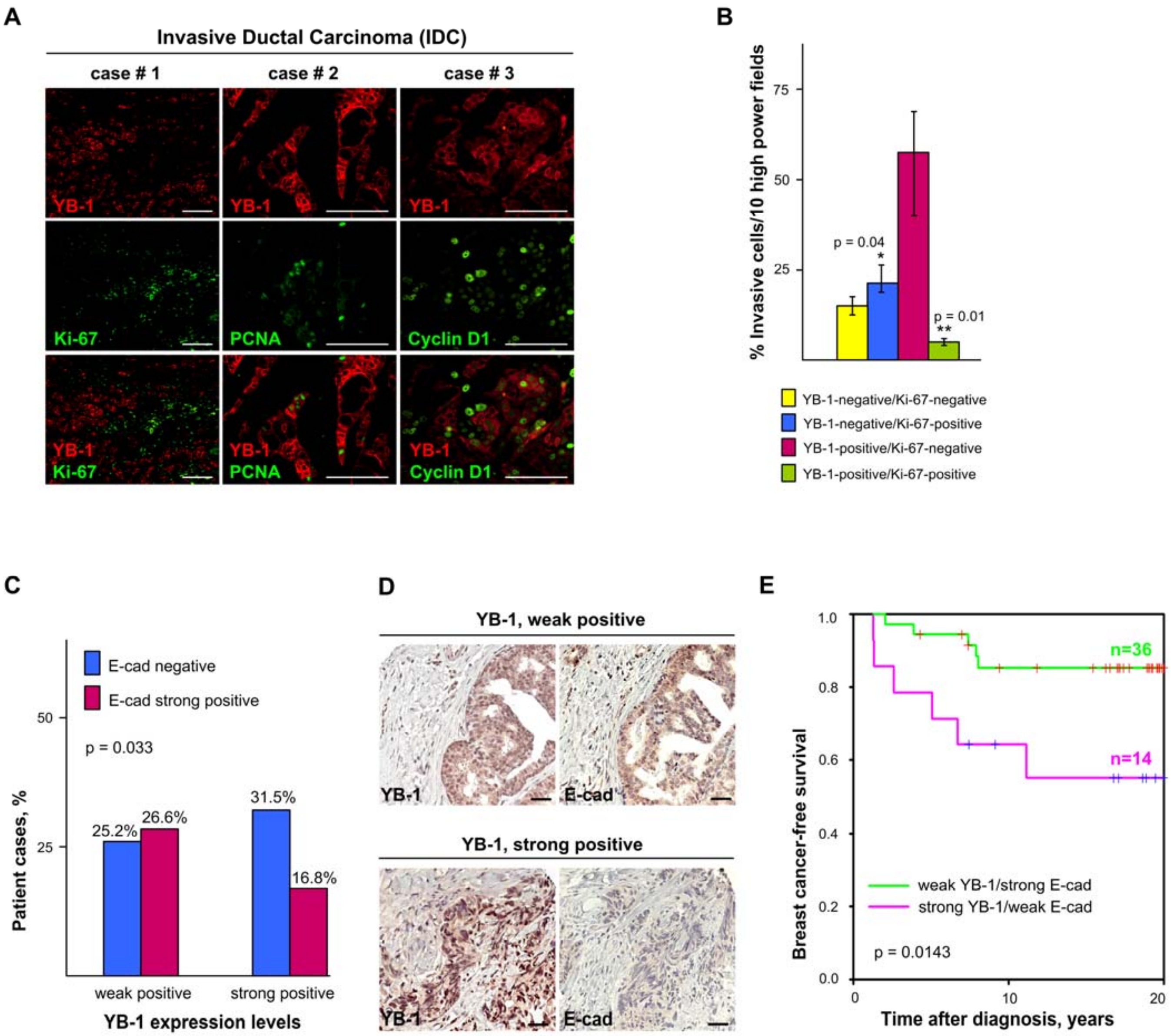


Figure S3. Elevated Expression of YB-1 in Human Breast Cancer Specimens Correlates with Reduced Levels of Proliferation Markers and E-cadherin

(A) Invasive areas detected by morphology in three individual IDC cases were further examined for the expression of YB-1 and proliferation markers, including Ki-67, PCNA, and cyclin D1 by double immunofluorescence microscopy. Representative images are shown. Scale bars, 100 μm.

(B) The percentages of YB-1 and Ki-67-expressing invasive cells shown in (A) were determined relative to total cell populations visualized by DAPI staining (not shown) in ten random high power fields analyzed from each IDC case described in (A). Averaged values across all three cases ± standard deviations are shown. p-values of 0.04 and 0.01 indicate significantly higher proportions of YB-1-positive/Ki-67-negative cells compared with YB-1-negative/Ki-67-positive or YB-1-positive/Ki-67-positive cells, respectively.

(C-D) The expression pattern of YB-1 and E-cadherin was determined using a breast cancer tissue microarray containing 143 breast tumor cases in duplicate (C). Tissues were scored according to YB-1 expression as weakly positive (0&1) or strongly positive (2). Statistical significance was determined by the Fisher's exact test. Immunohistochemical staining for YB-1 and E-cadherin in representative cases of IDC are shown in (D). Scale bars, 50 μm.

(E) Breast cancer-related survival in the strong YB-1/weak E-cadherin subset was compared to the weak YB-1/strong E-cadherin subset by Kaplan-Meier analyses. Vertical ticks indicate censored patients.

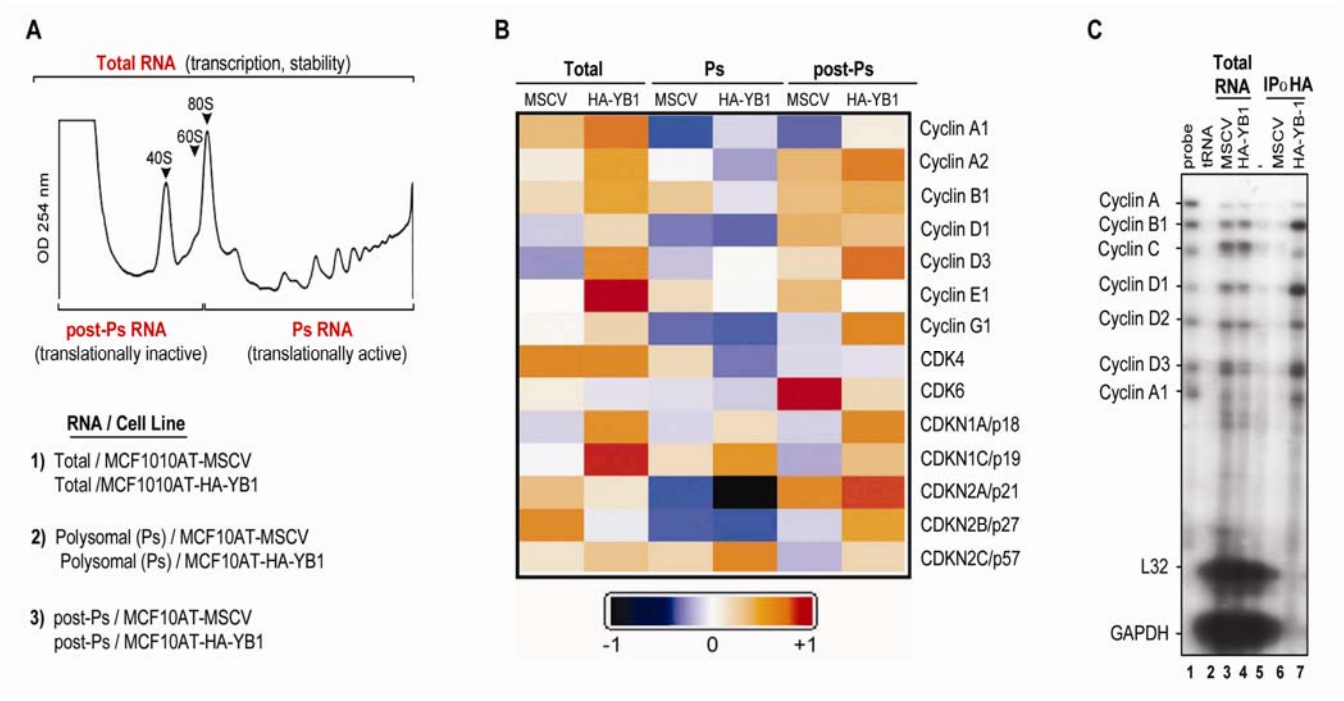


Figure S4. YB-1 is Directly Responsible for Translational Repression of *cyclin B1*, *D1* and *D3* mRNAs

(A) Schematic outline of the experimental design used for microarray analysis. A characteristic fractionation profile of MCF10AT cell lines and the fractions taken for the analysis are shown.

(B) Heat diagram depicting the relative abundance of selected cell cycle-related transcripts in total, Ps and post-Ps RNA populations from MCF10AT cell lines. Genes were normalized to a mean value of 0 and standard deviation of 1 unit on an untransformed data range. Note that in addition to altered expression of certain cyclins and CDKs, the expression profiles of several cell cycle-dependent kinase inhibitors was also significantly changed, with a reduction of *p21/CIP1* mRNA levels and an elevation of *p18*, *p19* and *p57* in the Ps RNA fractions of MCF10AT-YB-1 cells.

(C) Multiprobe RNase protection assay of total cellular mRNAs and those isolated in the complex with HA-YB-1 from post-Ps fractions by immunoprecipitation with anti-HA antibodies. mRNAs encoding cyclin B1, D1 and D3 were immunoprecipitated from MCF10AT-HA-YB-1 but not from MCF10AT-MSCV cells, indicating binding specificity.

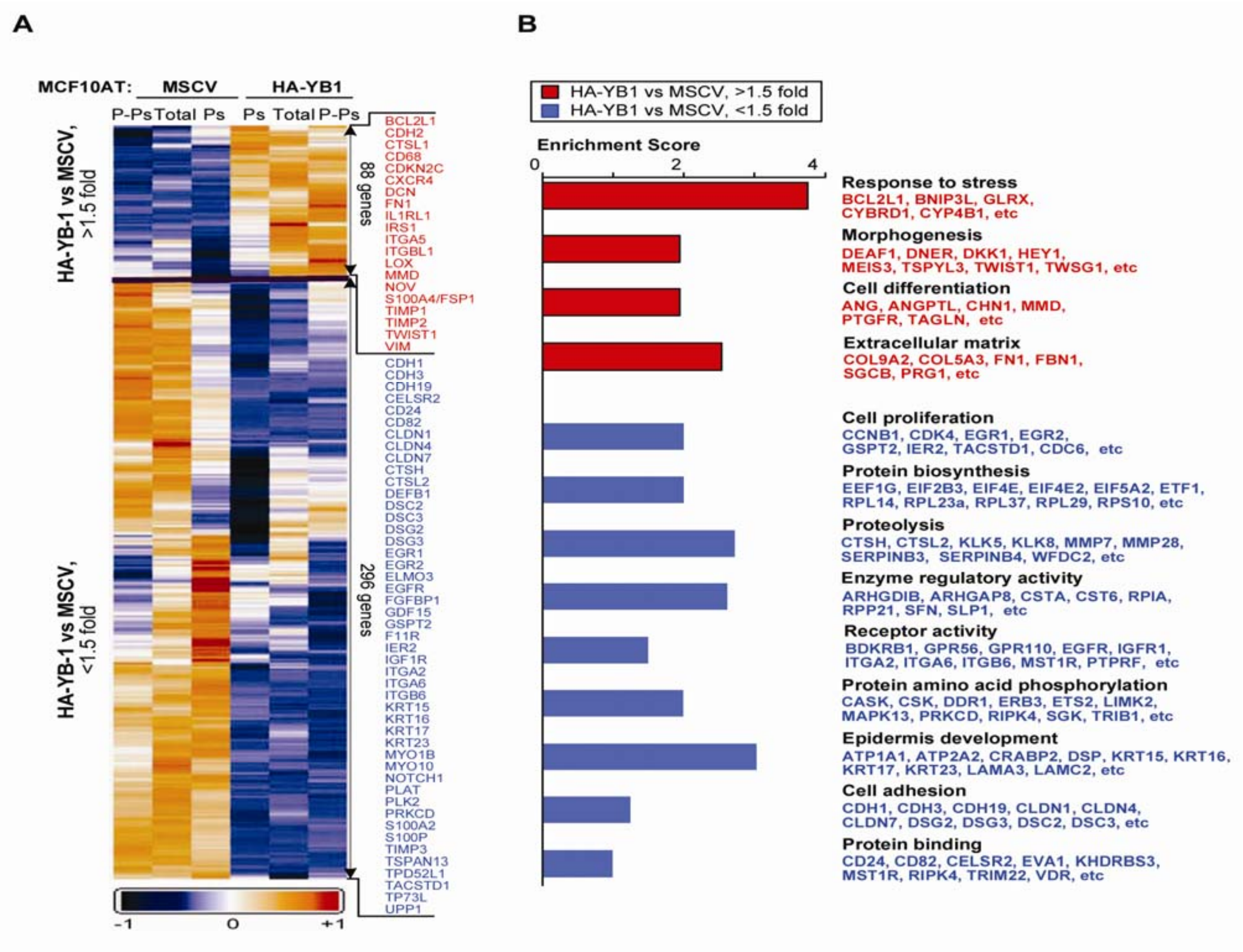


Figure S5. Increase in YB-1 Levels is Associated with Alterations in Gene Expression at Both the Transcriptional and Translational Levels

(A) Transcripts differentially expressed in the polysomal fractions of MCF10AT-MSCV and -HA-YB1 cells (n=384, see Table S1) were used to generate a two-way hierarchical clustering dendrogram and heat map for the averaged sample duplicates from post-Ps, total and Ps mRNA populations. Transcripts were clustered using complete-linkage cluster distances and optimally ordered using Pearson's distance metric. Representative mRNAs from the two main gene clusters, corresponding to the messages whose abundance was increased (*red* branch) or decreased (*blue* branch) in the total and/or Ps fractions of MCF10AT-YB-1 cells, are shown.

(B) The two gene clusters were used in gene ontology overrepresentation analysis with the DAVID Functional Annotation tool (http://david.abcc.ncifcrf.gov) using a $p < 0.05$ cut-off for significance and enrichment scores plotted as the –log p-value. Several significant categories with enrichment scores of > 1.5 in each of the gene clusters are highlighted in the comparison bar-graph for comparison of ontology overrepresentation. Representative mRNAs are listed under each category.

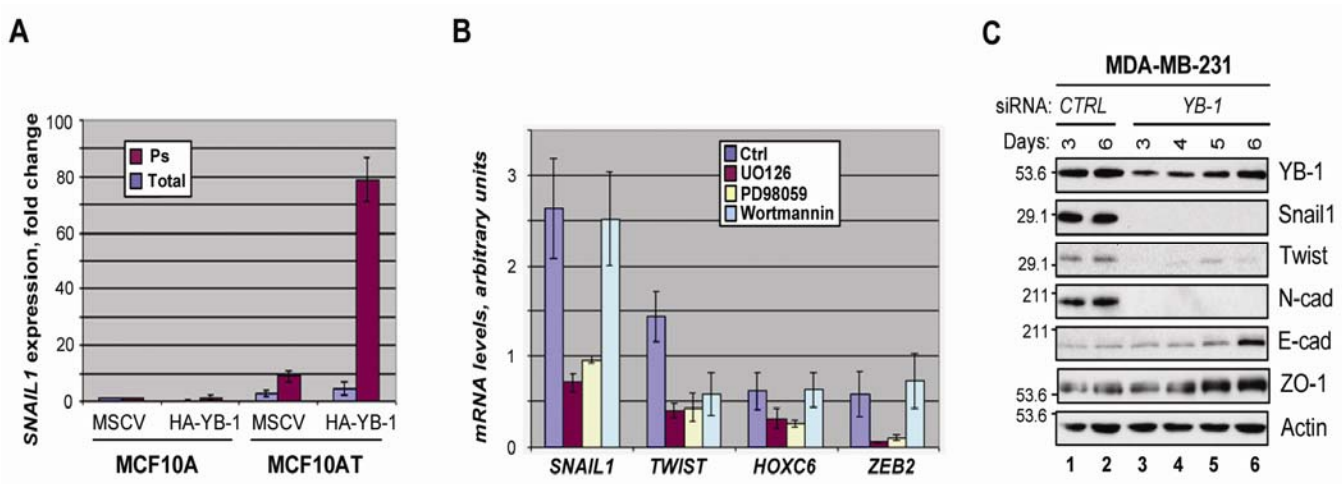


Figure S6. Transcription of EMT-Inducing Genes Depends on Ras-ERK Signaling, and Requires Elevated Levels of YB-1 for Translational Activation

(A) Relative levels of *Snail1* mRNA measured by real-time RT-PCR in total and Ps fractions from MCF10A and MCF10AT cell lines expressing a control vector (MSCV) or HA-YB-1. PCR reactions were performed in triplicate, the data was normalized to an endogenous human ribosomal protein *L19* (*hRPL19*), and presented as a mean ± SD.

(B) Levels of selected candidate mRNAs in total extracts from MCF10AT-YB-1 cells treated with the MEK inhibitors UO126 and PD98059, and the PI3K inhibitor wortmannin as determined by quantitative RT-PCR. The experiment was performed in triplicate and the data is presented as a mean ± SD.

(C) MDA-MB-231 cells were transiently transfected with 25 nM of scrambled (*CTRL*) or a Smart *YB-1* siRNA pool, as indicated. The expression kinetics of YB-1 and its downstream targets were examined between 3 and 6 days post-transfection.

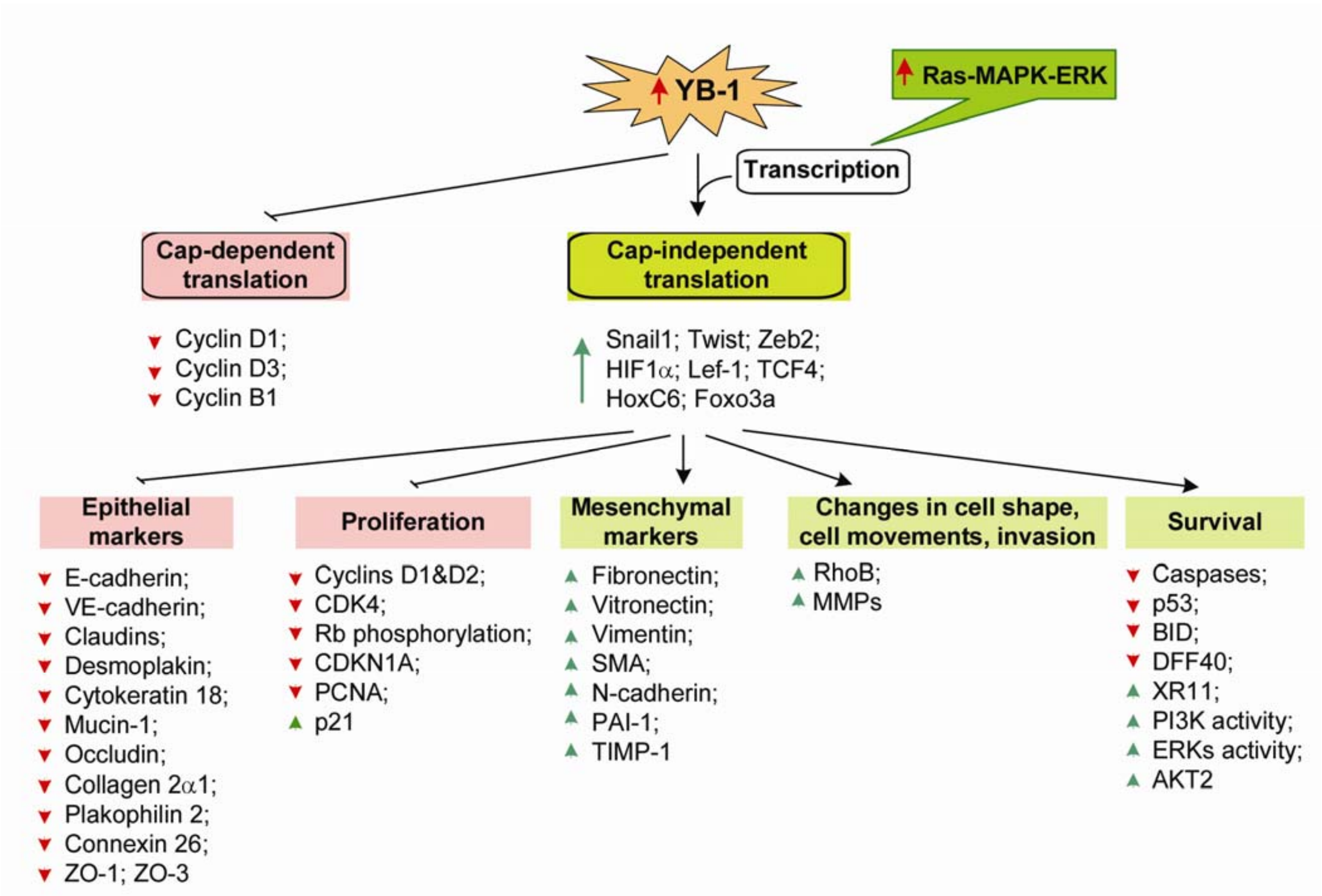


Figure S7. A Hypothetical Model of YB-1-Mediated Translational Regulation

YB-1 is upregulated as a part of an adaptive cellular mechanism directed at overcoming uncontrolled proliferation. It acts as an eIF4E antagonist and inhibits cap-dependent translation, primarily affecting messages that are highly dependent on eIF4E. Conversely, higher levels of YB-1 are required for cap-independent translational activation of another subset of mRNAs including *Snail1* and *Twist*. Transcription of the corresponding genes, normally restricted to the specific stages of early embryonic development, is likely induced in breast cancer cells by hyperactivated Ras-ERK signaling. However, these transcripts remain translationally silenced and require elevated YB-1 levels for efficient translation. YB-1-mediated translational activation of these messages results in the production of transcriptional factors that act in concert to repress epithelial and cell cycle-related genes and to initiate a mesenchymal gene expression program, thereby promoting tumor invasion and metastasis. Epithelial and mesenchymal target genes are listed based on previous reports (Barrallo-Gimeno and Nieto, 2005; Peinado et al., 2007).