SUPPLEMENTARY INFORMATION

The C/EBPδ protein is stabilized by estrogen receptor α activity, inhibits *SNAI2* expression, and associates with good prognosis in breast cancer

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SUPPLEMENTARY MATERIALS AND METHODS

Reagents and Antibodies. Antibodies were obtained from Cell Signaling technology (SNAI2, C19G7#9585; CDKN1A, #2947; pGSK-3β (S9), #9336S; GSK-3β, #9315S; pAKT (S473), #9271S; AKT, #4691P; β-catenin, #9562S), Santa Cruz Biotechnology (ERα, sc-543; C/EBPδ, sc-135733 and sc-636; C/EBPβ, sc-7962), Rockland (Tubulin, #600-401-880), Abcam (β-actin, ab6276; FBXW7, ab12292), Millipore (H2AX, AB10021), and BD Transduction Laboratories (Aurora A, #610938). The anti-progesterone receptor mouse monoclonal antibody 1294/H9 was kindly provided by Dr. Dean P. Edwards (Baylor College of Medicine, Houston, Texas). Reagents were purchased from Sigma-Aldrich, USA (Fulvestrant, #I4409; 4-hydroxy Tamoxifen #H6278), EMD Biosciences-Calbiochem (MG132, #474990; Puromycin, #540411), and Selleckchem (Bortezomib, #PS-341),

Cell lines and culture. MCF-7 and T47D cells were obtained from ATCC. MCF-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) and T47D cells in ATCC-formulated RPMI-1640 Medium (Catalog No. 30-2001) with 0.2 Units/ml bovine insulin (Sigma-Aldrich, #I0516); each supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 µg/ml streptomycin and 1 mM sodium pyruvate. The Cama-1 cell line was obtained from Dr. Stanley Lipkowitz (NCI) and cultured in DMEM supplemented with 10% FBS and penicillin and streptomycin as above. All cells were grown in a humidified incubator at 37°C and 5% CO2. All cell lines were authenticated in May 2014 through amplification of nine tetranucleotides (GenePrint®10, Promega) by the Protein Expression Laboratory, Leidos Biomedical Research, Inc. Frederick National Laboratory for Cancer Research). All treatments were done in complete medium.

MCF-7 cells with stable silencing of C/EBPδ expression were generated by transfection with expression constructs for two *CEBPD*-specific shRNAs or shGFP as control as described [1] followed by selection with G418.

Transient transfections and RNA interference. Cells were seeded 24 h before transfection at 70% confluence. The next day, $2x10^6$ cells were nucleofected with 400 nM of siRNAs per 100 µl Nucleofector V solution (Amaxa Biosystems/Lonza) according to the manufacturer's instructions. To silence C/EBPδ and FBXW7 two siRNA oligonucleotides were used at 1:1 ratio unless indicated otherwise. Control siRNAs directed against EGFP with the sequence 5'-CAAGCTGACCCTGAAGTTC-3' were used in all experiments as negative controls. The effect of siRNA on protein/RNA expression was assessed two days after nucleofection unless indicated otherwise.

The sequences of silencing oligonucleotides were as follows:
CEBPD siRNA-1-sense 5'-rUrCrGrCrCrGrArCrCrUrCrUrUrCrArArCrArGTT-3'
CEBPD siRNA-1-antisense 5'-rCrUrGrUrUrGrArArGrAr GrGrUrCrGrGrCrGrATT-3'

CEBPD siRNA2-sense 5'-rCrCrArCrUrArArArCrUrGrCrGrArGr ArGrArATT-3'
CEBPD siRNA2-antisense 5'-rCrUrGrUrUrGr ArArGr ArGrGrUrCrGrGrCrGrATT-3'
PGR siRNA-sense 5'- rGrGrUrGrUrUrGrUrCrCrCrCrGrCrUrCrAr UrGTT-3'
hPGR siRNA-antisense 5'- rCrArUrGrArGrGrCrGrGrGrGrArCrArArCrArCrCTT-3'
Fbxw7 siRNA1-sense: 5'-GGGCAGCAGCGGCGGAGGAdTdT-3'
Fbxw7 siRNA1-antisense 5'-UCCUCCGCCGCUGCUGCCCdTdT-3'
Fbxw7 siRNA2-sense: 5'- GCACAGAAUUGAUACAACTT-3'
Fbxw7siRNA2-antisense: 5'-GUUAGUAUCAAUUCUGUGCTG-3'.
To silence the following genes, siRNAs were purchased from Santa Cruz biotechnology:
ERα (sc-29305), SNAI2/Slug (sc-38393), and SIAH2 (sc-37497).
For the data in Figure S8E, the following oligonucleotides were used to silence SNAI2:
SNAI2 siRNA1-sense: 5'- CACACUGAGUGACGCAAUCdTdT-3'
SNAI2 siRNA1-antisense 5'- GAUUGCGUCACUCAGUGUGdTdT-3'
SNAI2 siRNA2-sense: 5'-CACUCCGAAGCCAAAUGACdTdT-3'
SNAI2 siRNA2-antisense: 5'-GUCAUUUGGCUUCGGAGUGdTdT-3'.

Cell migration and invasion assays. To assess cell migration, cells (approximately 5×10^4 cells/100 ul) were seeded in culture inserts (Ibidi) and allowed to adhere for 16 h in full medium (DMEM). Culture inserts were removed using sterile tweezers and filled with fresh medium, and 8 h later the cells were photographed and the width of the "wound" was measured using ImageJ. Experiments were performed in triplicates and a representative image is shown.

Cells were analyzed for invasion through matrigel using Corning biocoat-growth factor reduced 24 well plates according to the manufacturer's protocol (Corning, U.S., Cat. 354483). Briefly, cells were transfected with siRNAs and after 48 h were placed in Matrigel inserts at 2.5×10^4 cells/0.5ml in serum-free medium and were allowed to migrate for 36 h at 37°C. Non-migrating cells were removed from the top of the filter by scrubbing with a cotton swab. Cells that migrated were fixed and stained with DAPI (ProLong Gold, Invitrogen). Cells that migrated through the membrane were counted manually. Data are presented as mean ± standard deviation (S.D.).

To assess formation of colonies in soft agar, cells (5×10^3) were suspended in DMEM containing 0.3% agarose. The cell mixture was then seeded on a layer of 0.5% bottom agar in a 24-well plate and allowed to grow for 2 weeks. The culture medium was changed every two days. After 2 weeks one representative view from duplicate experiments were photographed at a 4-fold magnification. Colonies that were greater than 15 pixel were counted using ImageJ for each of three independent experiments (n=6).

Cell growth/viability assay. Cell proliferation/viability was assessed by AlamarBlue (Invitrogen). Briefly, 5000 cells/100 ul were seeded in 96-well plates, the next day 10 µl of AlamarBlue was added to the media and after 4 h incubation at 37°C the fluorescence was measured in a NOVOstar (BMG Labtech) plate reader at 560/590 with a gain of 1500. Data are presented as fluorescent units for each time point. Experiments were performed in triplicates.

Western Analysis. All Western data are from whole cell lysates unless indicated otherwise. Cells were washed once with cold PBS, scraped into 2x Laemmli sample buffer (Bio-rad) and heated at 100°C for 5 min prior to loading onto 10% SDS polyacrylamide gels. Whole cell and nuclear extracts were prepared as described [2]. After electrophoresis, proteins were transferred to nitrocellulose membrane and blocked with 5% non-fat dry milk in Tris Buffer Saline (TBS). Immunodetection was performed with the indicated primary antibodies for overnight incubation at 4°C. Following washing and incubation with the appropriate HRP-conjugated secondary antibodies, signals were visualized by an enhanced chemiluminescence (ECL) kit (Pierce, Rockford, IL) or LumiGLO Reserve kit (KPL, Gaithersburg, MD). Quantification was done by ImageJ 1.48v (NIH, USA). All western analysis data are representative of at least three independent biological replicates.

Quantitative real time PCR (QPCR). Total RNA was purified by RNAeasy (Qiagen), and cDNA was synthesized using superscript reverse transcriptase III (RT) according to manufacturer's instructions (Invitrogen). PCR was conducted with Fast SYBR Green master mix (Applied Biosystems, Foster city, USA) using the 7500 Fast Real-Time PCR instrument (Applied Biosystems). Unless indicated otherwise, data are from three independent biological replicates, each assayed as triplicates. The relative expression levels were measured using the relative quantitation $\Delta\Delta Ct$ method and normalized to RPLO expression. The primers were as shown in **Table S2**.

Chromatin Immunoprecipitation Assay. The chromatin immunoprecipitation assays were performed with the pre-made assay kit "EZ-ChIP" (Millipore Inc, 17-371) following the manufacturer's instructions. Briefly, MCF-7 cell chromatin was cross-linked and then sonicated to an average size of 800 bp. The DNA fragments were incubated with antibodies specific to C/EBPδ (M-17 sc-636, Santa Cruz Biotechnology, Inc) or control rabbit immunoglobulin G (IgG) at 4°C overnight. After reversal of the cross-linking, the DNA was purified and analyzed by QPCR using promoter-specific primers as follows (amplicon size in bp): SNAI1-S1, forward: AACAGCCCATTTTGAACCAGA, reverse: GAAGTCACCCGGCTCCTTTA, 186 bp; SNAI2-S2, forward TCACAGGCGCCTTTGTCTT, reverse: GGCTTCCAGATGTGGTGCA, 217 bp; N.S. (-Ctrl), forward: AGCCCACAGCCTAGTTCACA, reverse: TTCCTCCTCCCCATTTCATT, 247 bp; TLR4, forward: TTAAGCCACCCAGTCTGTGGT, reverse: TAGTCATCTGACCTCTGCCTGG, 181 bp.

mRNA-Seq Analysis. Total RNAs from MCF-7 cells were purified after 48 h silencing with siRNAs against *CEBPD* or control by using RNAeasy kit (Qiagen). Library preparation was done by following standard protocol: IlluminaTruSeq protocol FC-122-1001. **Primary Analysis of RNA-Seq data:** Four (4) mRNA samples were run as paired-end (PE) 100 bp read length

cycles each on 1 lane of HiSeq2000. Basecalling was done with RTA 1.12.4.2 and alignment with Illumina Casava 1.8.2. All samples showed excellent yield ranging from 337 – 400 million pass filtered paired end reads and excellent quality with over 92% of the bases having qualities Q30 or above. Samples were aligned to the reference human genome hg19 with good alignment ranging from 84 – 85% for both reads and very low mismatch rates <0.35%. RNA mapping statistics calculated using Picard software reported mapping of samples to 86 % mRNA bases, 63-65% unique reads, and <2.6% ribosomal reads. **Secondary Analysis of RNA-Seq data:** The aligned BAM files were imported into Partek Genome Studio v6.4 following the RNA-Seq workflow. Briefly, metadata about the samples was added followed by normalizing counts and doing differential expression analysis with ANOVA for the siNS vs siCEBPD contrasts. Differential gene list were created based on a p-value cutoff of <0.05 and fold-change of >1.5 or < -1.5. **Tertiary Analysis of RNA-Seq data**: To identify those biological processes altered by CEBPD, we used Ingenuity Pathway Analysis software (Ingenuity Systems, Qiagen), a bioinformatic tool for visualizing expression data in the context of KEGG-defined biological pathways. The mRNA-Seq data are available at the NCBI Gene Expression Omnibus under accession number GSE69604 (link for reviewers: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=yvsjaqkgfdsrfmf&acc=GSE69604

ONOCMINE™ analysis: Oncomine v4.5 at www.oncomine.com [3] was used to understand the significance of overlap and direction of expression of the CEBP-activated and CEBPD-repressed genes to human breast cancer sets available on this comprehensive database. Genes overexpressed or underexpressed in the CEBPD-silenced cells were loaded into the Oncomine Premium Research Edition. To narrow the number of relevant datasets to compare to, the following filters were applied: mRNA as data type, breast cancer, and ER status as Analysis Type. Association of the mapped signatures with the database signatures was tested using Fisher's exact test, and was considered significant for Odds Ratio>2, and p-val<0.005.

Immunohistochemistry. Immunohistochemistry in cells or commercially available human tissues (BRC961C_F, Pantomics, Inc) was performed with monoclonal antibodies from Santa Cruz Inc. against C/EBPδ (sc-135733) and C/EBPβ (sc-7962). The protocol for C/EBPδ was as follows: antigen retrieval (30 s at 121°C, then 10 s at 90°C with Citrate buffer), blocking (20 min with 2% normal horse serum), 1st antibody at 1:250 for 30 min, 2nd antibody (biotinylated Horse anti-mouse IgG; Vector Labs) at 1:100 in 1.5% Serum for 30 min, followed by VECTASTAIN Elite ABC kit. Staining was visualized with 3, 3'-diaminobenzidine (DAB) substrate and counterstained with Hematoxylin. The C/EBPδ antibody (sc-135733) was initially provided by BD Biosciences Pharmingen through a Antibody Co-development Collaboration between the NCI and BD Bioscience.

TMA-1: Following deparaffinization and rehydration of the TMA slide, immunohistochemistry for C/EBPδ using the antibody at 1:400 was conducted with a BondMaX auto-immunostainer (Leica Microsystems, Bucks, UK), a BOND polymer detection kit (Leica

Microsystems, Bucks, UK) and 3,3'-diaminobenzidine as a chromogen. Antigen was retrieved by heating with pH 9 Tris-EDTA buffer.

TMA-2: Four-micrometer TMA sections were deparaffinized, rehydrated, and treated in a microwave for 5 + 5 minutes in a citrate buffer (pH 6.0) before they were processed in an automatic immunohistochemistry staining machine according to standard procedures (TechMate500; DakoCytomation, Glostrup, Denmark) using the C/EBPδ specific antibody.

Supplementary References:

1 Balamurugan K, Wang JM, Tsai HH, Sharan S, Anver M, Leighty R *et al*. The tumour suppressor C/EBPdelta inhibits FBXW7 expression and promotes mammary tumour metastasis. Embo J 2010; 29: 4106-4117.

2 Balamurugan K, Sharan S, Klarmann KD, Zhang Y, Coppola V, Summers GH *et al*. FBXW7alpha attenuates inflammatory signalling by downregulating C/EBPdelta and its target gene Tlr4. Nature communications 2013; 4: 1662.

3 Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D *et al*. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 2004; 6: 1-6.

4 Ringner M, Fredlund E, Hakkinen J, Borg A, Staaf J. GOBO: gene expression-based outcome for breast cancer online. PLoS One 2011; 6: e17911.

Supplementary Table S1: Analysis of breast cancer patients' risk of progression (disease free survival) in association with *CEBPD*, *CEBPB*, and *IL6* mRNA expression by BreastMark*

Tumor Type	Gene(s)		*P*-value	HR	CI-lower	CI-upper	n	Events
ER(+):	CEBPD		0.93	0.99	0.81	1.21	1589	509
		IL6	**0.001**	**0.68**	0.54	0.85	1648	536
	CEBPD +	**IL6**	**0.0008**	**0.48**	0.31	0.74	1589	509
	CEBPB		**0.015**	**1.29**	1.05	1.59	1648	536
	CEBPB +	IL6	0.29	0.79	0.52	1.22	1648	536
ER(-):	CEBPD		0.45	1.12	0.83	1.50	517	239
		IL6	0.55	1.08	0.83	1.40	518	240
	CEBPD +	IL6	0.76	1.06	0.73	1.54	517	239
	CEBPB		0.13	0.82	0.64	1.06	518	240
	CEBPB +	IL6	0.81	1.04	0.76	1.42	518	240
ER/PR(+):	CEBPD		0.75	1.06	0.75	1.50	553	162
		IL6	**0.037**	**0.67**	0.46	0.98	602	184
	CEBPD +	**IL6**	**0.032**	**0.44**	0.21	0.95	553	162
	CEBPB		**0.037**	**1.42**	1.02	1.99	602	184
	CEBPB +	IL6	0.7	0.88	0.46	1.67	602	184

* The indicated parameters were used for custom analysis with "high" cut-off at "http://glados.ucd.ie/BreastMark". HR, Hazard ration; CI, confidence interval.

Supplementary TABLE S2: Primers used for QPCR analysis

Human-specific primers			
GENE	5'-Forward-3'	5'-Reverse-3'	Amplicon (bp)
AASS	CGCTTCCTGCCTTTAGACCT	CCCAAGTAAGCCCAACCAT	173
ALPL	AGCCCTTCACTGCCATCCTGT	ATTCTCTCGTTCACCGCCCAC	68
ANXA1	GGCCTTGGAACTGATGAAGATAC	TGTCTTTGGCCAGATCTCTCTT	118
ARHGDIB	GGACAGAGACGTGAAGCACTGA	GCTTGCTGTCCAGCTCATCA	100
AURKA	GCCTCCTGTGAAGACACCAT	ACTTTGTAACAGAGGGAGCC-	471
BIRC3	ACTTGAACAGCTGCTATCCACATC	GTTGCTAGGATTTTTCTCTGAACTG TC	195
CCR4	ACCGGGTCCTTCTTAGCATC	GGTGTCTGCTATATCCGTGGG	164
CDKN1A	ACCATGTGGACCTGTCACTGT	TTAGGGCTTCCTCTTGGAGAA	170
CEBPD	GCCATGTACGACGACGAGA	TTGCTGTTGAAGAGGTCGG	116
CGA	TGCCCAGAATGCACGCTA	CCCCCATTACTGTGACCCTG	199
CTNNB1	ACAAACTGTTTTGAAAATCCA	CGAGTCATTGCATACTGTCC	298
CTSZ	ACCATGTCGTTTCTGTGGCT	TGGCCTTAAACGATGGGG	199
EGR1	CACCTGACCGCAGAGTCTTT	AAAGCGGCCAGTATAGGTGAT	117
FAM129A	GTCCACATGCAAACCAGAGC	ACTGGGCAAGGCCATGTTAT	193
FBXO27	GGCCAGAACTGCTGGATAGTG	GCAGGCATTGTTGTTCCACT	179
FYB	ATCACAACCACCAGTCCCA	AGTGCGTGACACCATCTTGA	101
GDF7	CACCACTTCATGATGTCGCTTT	TTAAGGCTGGACACGTCGAA	182
GEM	GCTCAGCACTGGGATTTTCTG	GGGCTCTTTCTGGACCATCA	141
GPRC5A	GCTGCTCACAAAGCAACGA	CATAGAGCGTGTCCCCTGTC	155
IL18R1	CTCCAGAAGGCAAATGGCA	TGAAGCTTTTGGTGTCTGTGC	123
INHBA	AGTCGGGGAGAACGGGTAT	TCTTCCTGGCTGTTCCTGACT	122
JUND	CAGAGTGTTCGATTCTGCCCTA	GAGCGAGATCGAGGAAAGG	141
MVP	CCAAGGCAGGGTGAGAGTTC	GAAGATGACTGGTAGGTGGGG	110
NUAK1	GACATGGTTCACATCAGACGAG	CAATAGTGCACAGCAGAGACGA	221
NUPR1	GGAAAGCAGAGACAGACAAAGC	GGGCATAGGCATGATGAGAG	198
OSM	ATGGGGGTACTGCTCACACA	GATACGTATATAGGGGTCCAGGAG TC	186
PGR	CAGTGGGCGTTCCAAATG	TGGTGGAATCAACTGTATGTCTTG	83
RASD1	CGCCTCTCCATCCTCACAG	CCACCTCGCGGTAGAAGTC	125
RPLPO	GCAATGTTGCCAGTGTCTGTC	GCCTTGACCTTTTCAGCAAGT	142
S100A9	ATCATCAACACCTTCCACCAATA	TTAGCCTCGCCATCAGCA	217
SERPINB5	CACAGTGGACTAATCCCAGCA	AGCCTTGGGATCAATCATCTTT	92
SHF	CCCCATTCCTGGATGCTC	GGGCTGTGGTGATGATGAGA	81
SNAI2	GCATATTCGGACCCACACATTA	ATTTGACCTGTCTGCAAATGCT	154
STAT4	CGTCGGAGTGAGCGGAC	TGGACTTGATTCCACTGAGACA	183
TAT	AGTCAGCGCATTTTGGGAC	CCAACGCCCCATAACAGAG	136
TERT	CGGAAGAGTGTCTGGAGCAA	GGATGAAGCGGAGTCTGGA	145
TFF1	GTCCCCTGGTGCTTCTATCC	GACTAATCACCGTGCTGGGG	125
TGM2	GACGTCTTTGCCCACATCAC	AAGGCAGTCACGGTATTTCTCA	198
TP53	ACAGCACATGACGGAGGTTG	GCTCATAGGGCACCACCAC	167
TWIST1	AGCAAGATTCAGACCCTCAAGC	CTCCATCCTCCAGACCGAGA	150

Mouse-specific primers			
GAPDH	TGTGTCCGTCGTGGATCTG	CCTGCTTCACCACCTTCTTG	77
P21	GCAGATCCACAGCGATATCC	AACTGCTCACTGTCCACGG	129
SNAI2	GCCTCCAAGAAGCCCAACT	TGCCGACGATGTCCATACA	153

SUPPLEMENTARY FIGURES LEGENDS

Figure S1: *CEBPD* mRNA expression is highest in low-grade tumors and normal-like tumors. *CEBPD* mRNA expression across breast cancer samples analyzed by "Gene-expression based outcome for BC online (GOBO; http://co.bmc.lu.se/gobo/ [4])" according to tumor grades 1-3 (left panel; n=1411, P=0.02) and cancer subtype (n=1881, P<0.00001). Numbers above the plots indicate the number of samples per subgroup.

Figure S2: Validation of antibody for human C/EBPδ immunohistochemistry.
a) Immunostaining of C/EBPδ and C/EBPβ in formalin-fixed, paraffin embedded cell pellets of HEK293T cells that had been transfected with either vector control or expression constructs for human C/EBPδ or the closely related protein C/EBPβ as indicated.
b) Western analysis of cell extracts from the cells shown in panel A confirming expression of both C/EBPδ and C/EBPβ.

Figure S3: Analysis of C/EBPδ expression in human breast cancer tissues.
a) Distribution (%) of TMA-1 specimen across Allred scores (see Methods) for C/EBPδ staining. For further analyses, samples were dichotomized to C/EBPδ-negative (0) and C/EBPδ-positive (>0=1).
b) *CEBPD* mRNA expression in breast cancer tissues by ER status, analyzed with the ONCOMINE tool (oncomine.org) in the TCGA and Curtis-Breast databases. Number of samples per subgroup are indicated above the graph.

Figure S4: ERα supports C/EBPδ protein stability.
a) Western and QPCR analysis of CAMA-1 cells treated with β-estradiol (E2, 1nM) for the indicated times (*P<0.05, ** P<0.01, n=3).
b) Western analysis of C/EBPδ and ERα protein in MCF-7 cells transfected with siRNA against ERα (siESR1) or non-specific control (-) and treated 48 h later with MG132 (20 μM) for the indicated times. DMSO was used as solvent control.

Figure S5: Analysis of the role of SIAH2 and FBXW7 in C/EBPδ stability.
a) Representative Western analysis (top panel) of MCF-7 cells treated with Tamoxifen (1 μM) or ethanol as solvent control (EtOH) and 48 h later with puromycin (15 μg/ml) for the indicated times (h). Tubulin is shown as loading controls. The lower panel shows quantification of C/EBPδ protein expression normalized to the non-specific band from three independent experiments (* P<0.05 and ** P<0.01).
b) Western analysis of C/EBPδ and ERα protein levels in MCF-7 cells 48 h after nucleofection with siRNAs against ERα (siESR1), SIAH2 (siSIAH2), or non-specific control (-,siNS);
c) QPCR analysis of *SIAH2* and *CEBPD* mRNA levels in cells as described in panel A. (n=3, * P<0.05 and ** P<0.01 compared to siNS).

d) Western analysis of the indicated proteins in whole cell extracts from MCF-7 cells transfected with siRNA against ERα (siESRA) or non-specific control (-), or treated with fulvestrant for 48 h.
e) Western analysis and quantification of three independent experiments of C/EBPδ and ERα protein in whole cell extracts from MCF-7 cells transfected with siRNA against FBXW7 (siFBXW7), or non-specific control (-) and treated the next day with fulvestrant for another 48 h (mean±S.E.M., * $P<0.05$ and ** $P<0.01$).
f) Western analysis of the indicated proteins in *nuclear* extracts from MCF-7 cells treated as in panel E.
g) QPCR analysis of Aurora A Kinase (*AURKA*), β-catenin (*CTNNB1*), *CEBPD* and *FBXW7* from cells as in Figure 2i. (mean±S.E.M., n=3, * $P<0.05$ and ** $P<0.01$ compared to non-specific control).

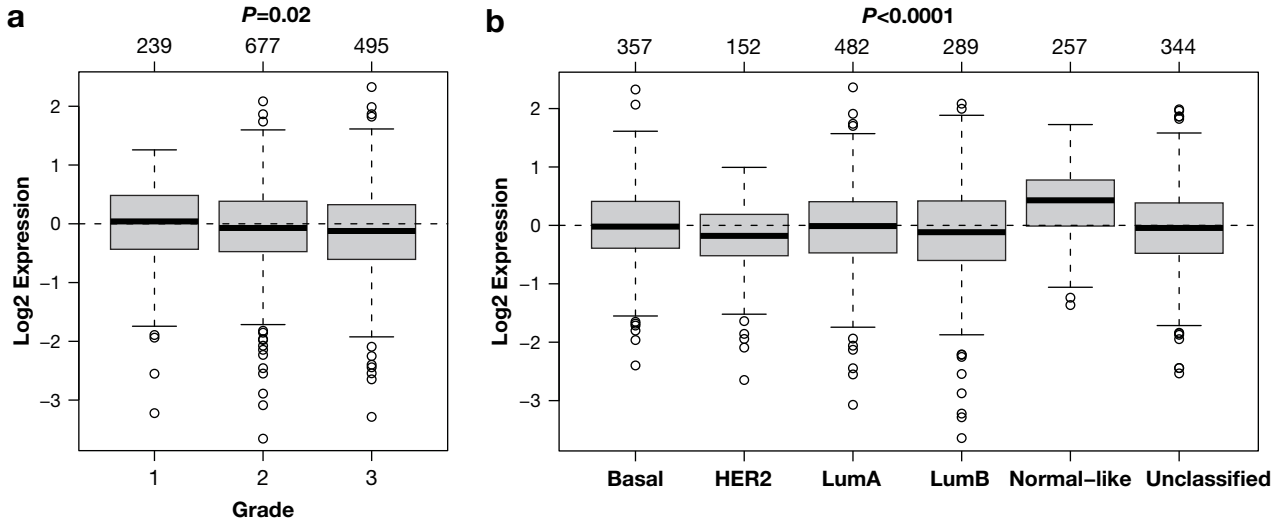
Figure S6. Identification and analysis of C/EBPδ regulated genes in MCF-7 cells by mRNA-Seq.
a-b) Validation of mRNA-Seq results: QPCR analysis of a selection of differentially expressed genes (DEGs) from panel A using independently generated RNA samples from cells as in panel A and (B) a combination of two *CEBPD*-specific siRNA oligonucleotides or (C) each individually (n=3, * $P<0.05$).
c) Analysis by ONCOMINE.org for enrichment of genes that were inhibited by C/EBPδ in MCF-7 cells in ER- tumors compared to ER+ , invasive ductal carcinoma (IDC) compared to ductal carcinoma in situ (DCIS), triple negative (ERBB2/ER/PR-) compared to other cancers, and non-metastatic compared to metastatic cancers. The specific enrichment per subgroup is shown below the indicated datasets.
d) Analysis by ONCOMINE.org for enrichment of genes that were activated by C/EBPδ in MCF-7 cells in ER+ tumors compared to ER- tumors. The specific enrichment per subgroup is shown below the indicated datasets.

Figure S7. C/EBPδ attenuates migration, invasion and proliferation of MCF-7 cells.
a) Wound-healing assay with MCF-7 cells 48 h after transfection with siRNAs against C/EBPδ (siCEBPD) or non-specific control (siNS). Cells were grown with culture inserts until confluent. Representative images are shown from the indicated times after removal of the insert along with quantification of wound closure from three independent experiments each performed in triplicates (** $P<0.01$).
b) Soft-agar colony assay with MCF-7 cells as in panel A evaluated after 13 days of culture. Pictures of colonies (upper panel) were quantified by ImageJ (lower panel and left graph) for the number of colonies >15 pixel size (n=6, ** $P<0.01$).
c) Analysis of cell population growth/viability by vital dye staining (Alamar blue) of MCF-7 cells with stable knockdown of C/EBPδ (shCEBPD) or control (shNS) (data are from two

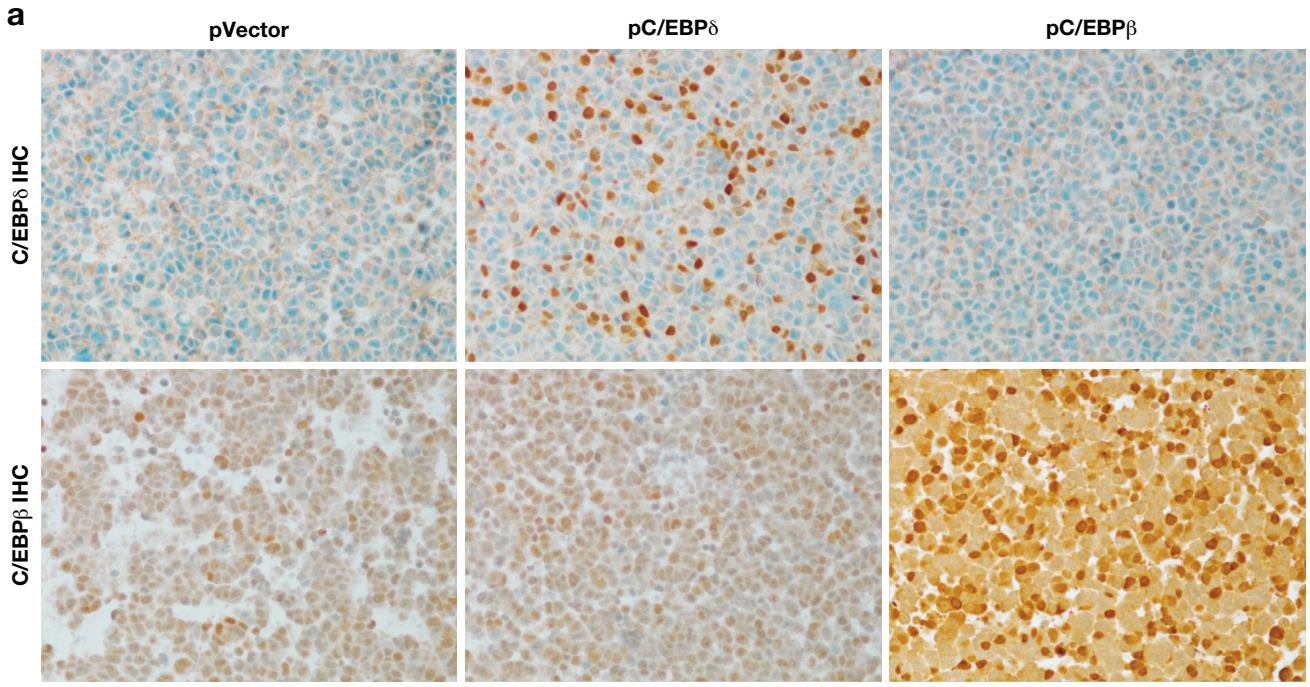
biological replicates done in triplicate each, ** $P<0.01$). The inset shows a representative Western analysis of C/EBPδ levels in these stable cells.

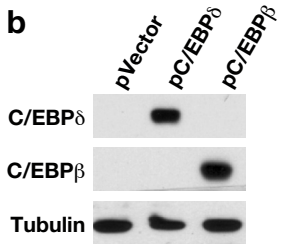
Figure S8: Analysis of SNAI2 as a mediator of C/EBPδ signaling.
a) Western (left panel) and QPCR (right panel) analysis of the indicated protein/mRNA levels in T47D cells 48 h after nucleofection with siRNAs against C/EBPδ (siCEBPD), SNAI2 (siSNAI2) or non-specific control (siNS) as indicated; (n=3, * $P<0.05$, ** $P<0.01$ compared to siNS). Expression of *TP53* mRNA is shown as a negative control.
b) QPCR analysis of the mRNA expression levels of the indicated genes in T47D cells transfected with siRNA as described in panel A (n=3, * $P<0.05$, ** $P<0.01$ compared to siNS).
c) QPCR analysis of the mRNA expression levels of *SNAI2* and CDKN1A in MCF-7 cells 48 h after nucleofection with either one of two independent siRNAs against *CEBPD* or non-specific control (siNS); (n=3, * $p<0.05$, ** $p<0.01$ compared to siNS).
d) Analysis of cell population growth/viability by vital dye staining (Alamar blue) of MCF-7 cells with transient knockdown of SNAI2 with two independent siRNAs along with si RNA against C/EBPδ (siCEBPD) or control (siNS) (n=3, * $P<0.05$, ** $P<0.01$ compared to siNS alone; # $P<0.05$, ## $P<0.01$ compared to siCEBPD alone).
e) Analysis of the indicated mRNA expression levels in the same sets of cells shown in panel D, 48 h after transfection with siRNAs (equivalent to day 1 of the growth curves).

Supplementary Figure 1



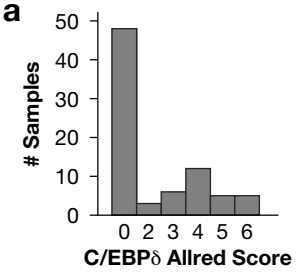
Supplementary Figure 2





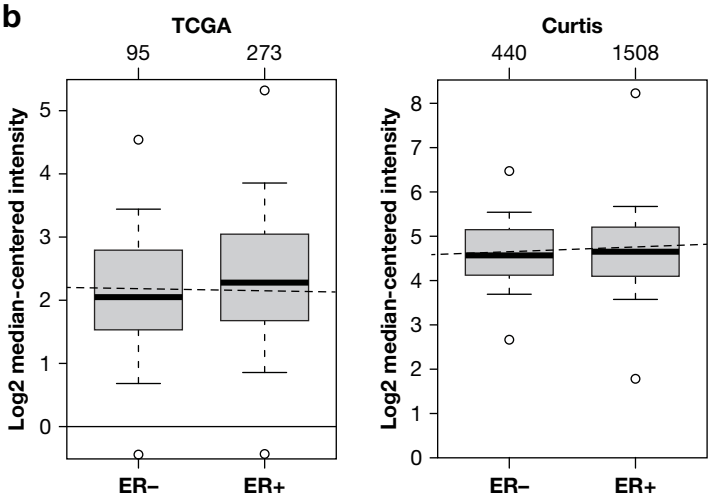
Supplementary Figure 3

a

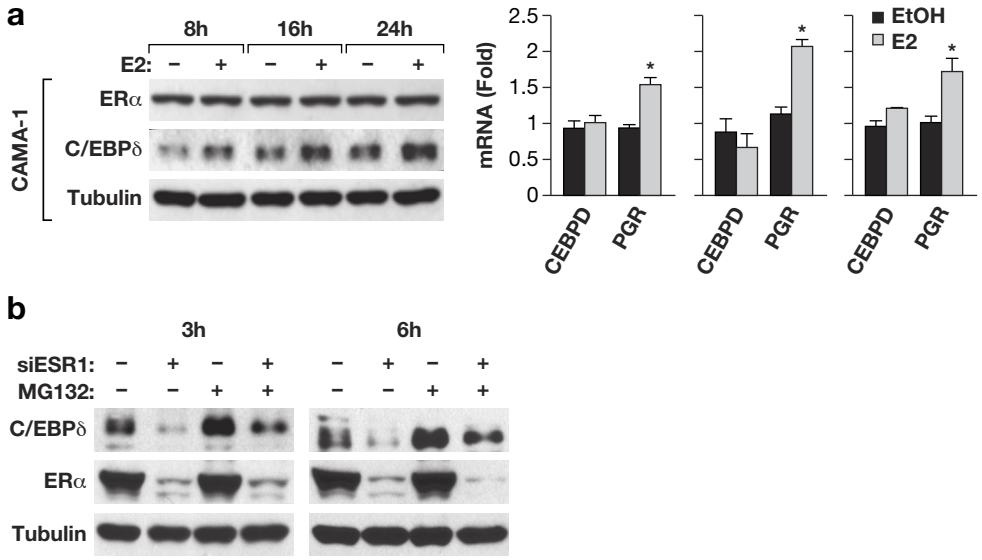


C/EBPδ	n	%
Negative	48	61
Positive	31	39
Total	79	100

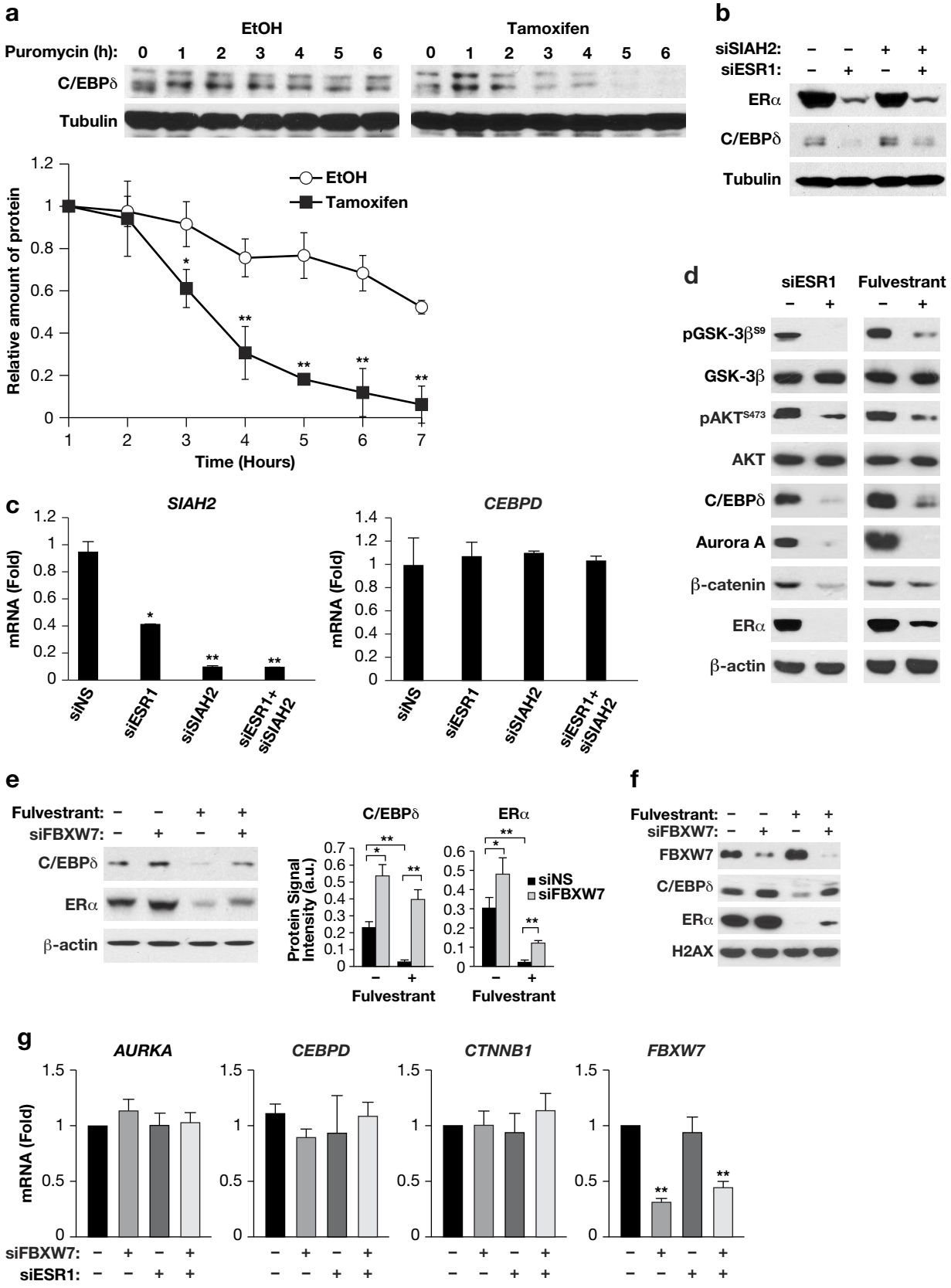
b



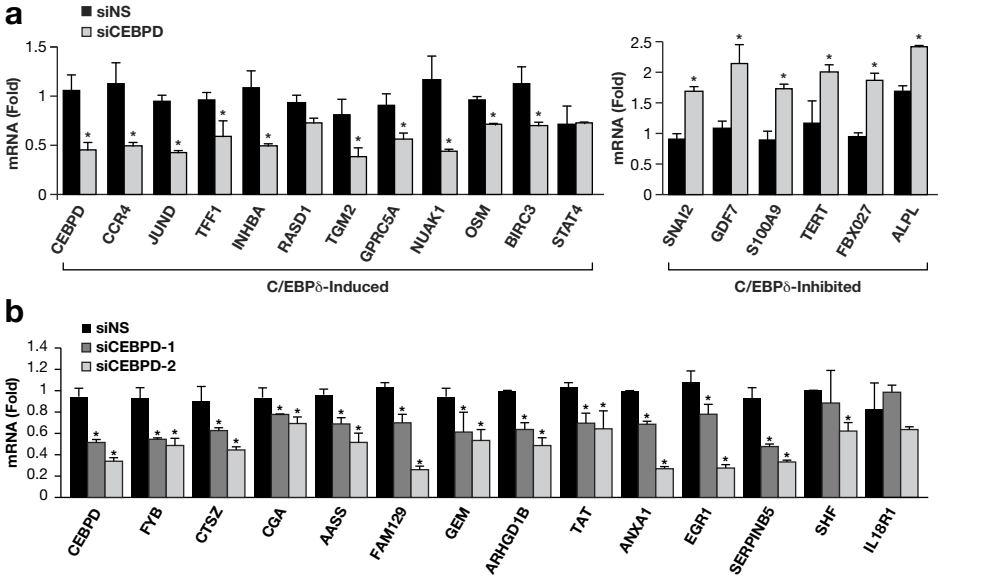
Supplementary Figure 4

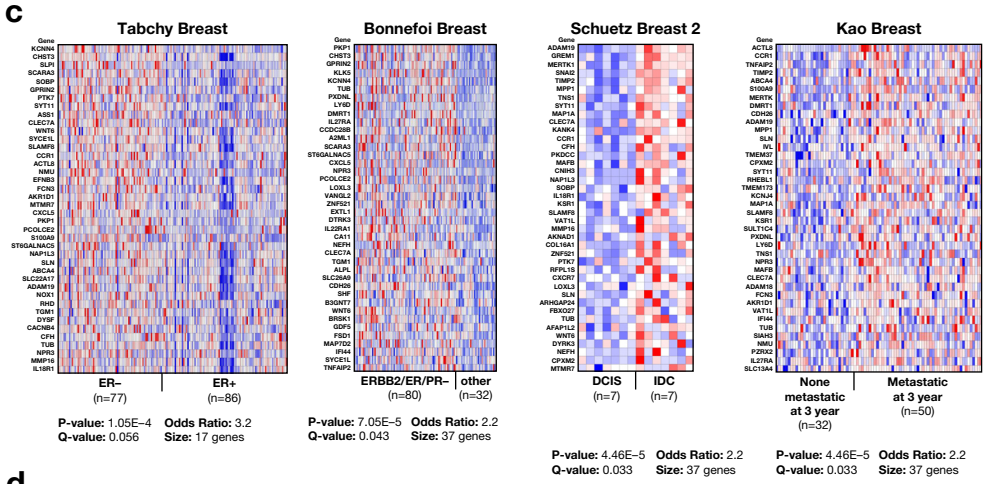


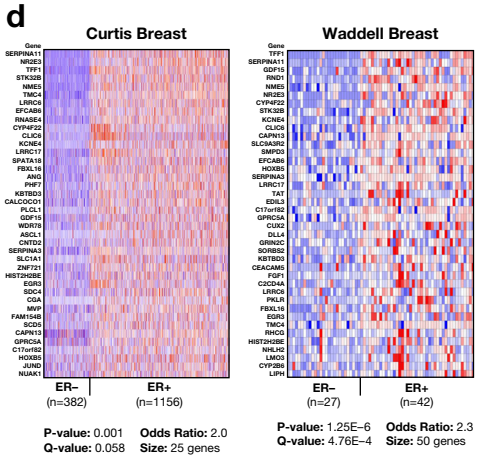
Supplementary Figure 5



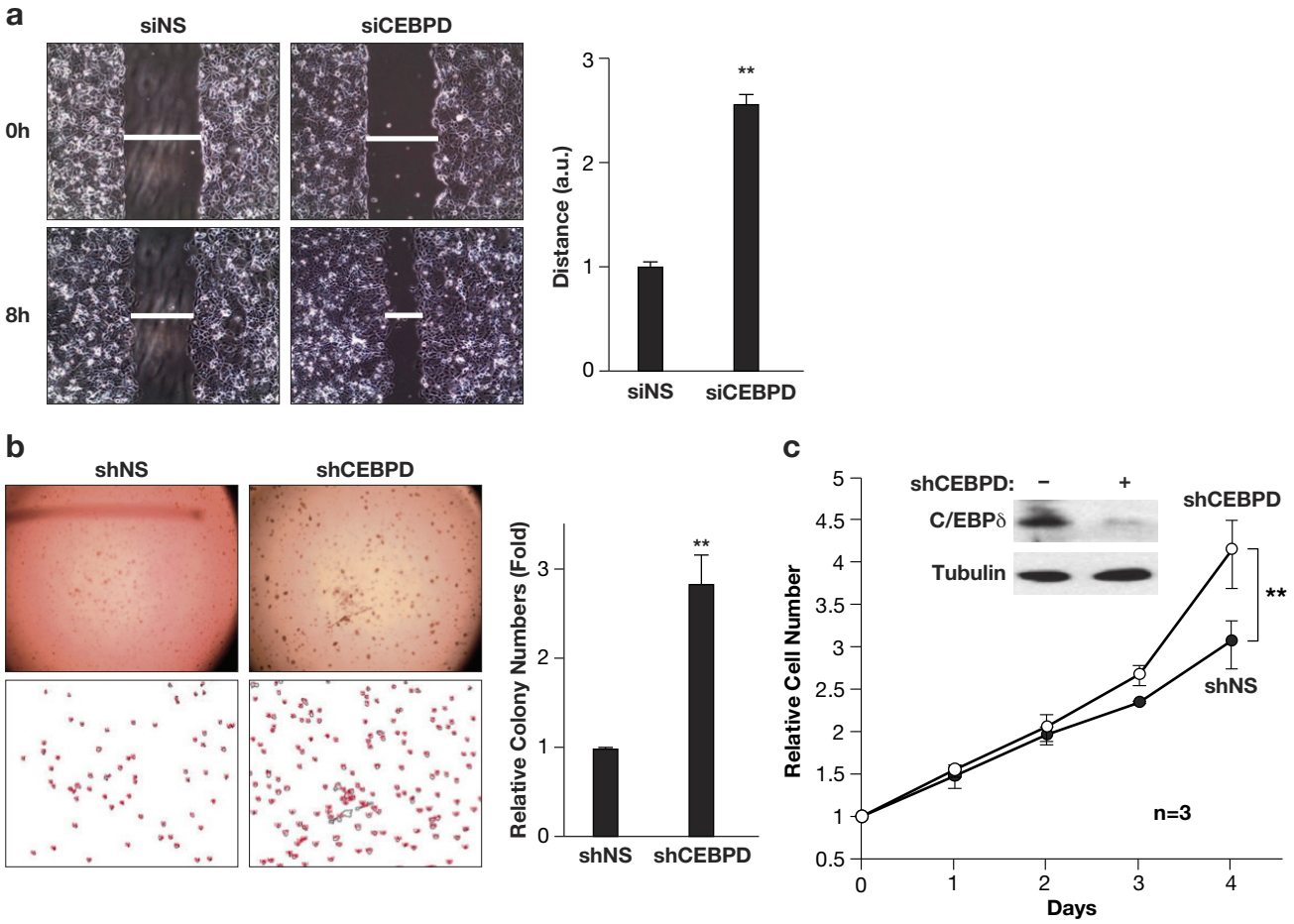
Supplementary Figure 6







Supplementary Figure 7



Supplementary Figure 8