

Supplementary Table 1. Pearson's r correlation analysis using NKI and UNC data sets identified 34 genes the most inversely correlated with ZBTB4 expression

Gene Symbol	Description	r (NKI, N=295)	p value	r (UNC, N=500)	p value
BIRC5	Baculoviral IAP repeat-containing 5 (survivin)	-0.598	0	-0.481	0
BLM	Bloom syndrome	-0.527	0	-0.506	0
BM039	Uncharacterized bone marrow protein BM039	-0.538	0	-0.532	0
BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	-0.518	0	-0.572	0
CCNA2	Cyclin A2	-0.577	0	-0.536	0
CCNB1	Cyclin B1	-0.503	0	-0.484	0
CCNB2	Cyclin B2	-0.588	0	-0.587	0
CDC2	Cell division cycle 2, G1 to S and G2 to M	-0.479	0	-0.54	0
CDC20	CDC20 cell division cycle 20 homolog (S. cerevisiae)	-0.572	0	-0.548	0
CDC45L	CDC45 cell division cycle 45-like (S. cerevisiae)	-0.563	0	-0.516	0
CDC6	CDC6 cell division cycle 6 homolog (S. cerevisiae)	-0.475	0	-0.465	0
CDKN3	Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	-0.477	0	-0.473	0
CENPA	Centromere protein A, 17kDa	-0.58	0	-0.539	0
CHEK1	CHK1 checkpoint homolog (S. pombe)	-0.491	0	-0.605	0
DC13	DC13 protein	-0.477	0	-0.452	0
DKFZp762E1312	Hypothetical protein DKFZp762E1312	-0.58	0	-0.484	0
E2F1	E2F transcription factor 1	-0.58	0	-0.494	0
EXO1	Exonuclease 1	-0.537	0	-0.563	0
EZH2	Enhancer of zeste homolog 2 (Drosophila)	-0.47	0	-0.456	0
FLJ10156	Hypothetical protein FLJ10156	-0.474	0	-0.476	0
GTSE1	G-2 and S-phase expressed 1	-0.473	0	-0.484	0
LRP8	Low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	-0.488	0	-0.507	0
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	-0.578	0	-0.474	0
MYBL2	V-myb myeloblastosis viral oncogene homolog (avian)-like 2	-0.559	0	-0.497	0
ORC6L	Origin recognition complex, subunit 6 homolog-like (yeast)	-0.588	0	-0.47	0
PTTG2	Pituitary tumor-transforming 2	-0.589	0	-0.501	0
RAD51	RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)	-0.514	0	-0.462	0
RAD54L	RAD54-like (S. cerevisiae)	-0.493	0	-0.469	0
RANBP1	RAN binding protein 1	-0.488	0	-0.461	0
RRM2	Ribonucleotide reductase M2 polypeptide	-0.52	0	-0.51	0
SNRPC	Small nuclear ribonucleoprotein polypeptide C	-0.453	0	-0.454	0
STK6	Serine/threonine kinase 6	-0.479	0	-0.495	0
TRIP13	Thyroid hormone receptor interactor 13	-0.55	0	-0.458	0
TTK	TTK protein kinase	-0.556	0	-0.568	0

Supplementary Table 2. Common 163 genes regulated after Sp1 and EZH2 knockdown in MDA-MB-231 cells

Gene Symbol	Description	Sp1 KO/Fold Change	EZH2 KO/ Fold Change
TNFSF18	tumor necrosis factor (ligand) superfamily, member 18 (TNFSF18).	0.42	0.74
CENPA	centromere protein A (CENPA).	0.45	0.71
TYMS	thymidylate synthetase (TYMS).	0.47	0.72
LTB	lymphotoxin beta (TNF superfamily, member 3) (LTB).	0.47	0.63
IL6	interleukin 6 (interferon, beta 2) (IL6).	0.49	0.58
EVI2A	ecotropic viral integration site 2A (EVI2A).	0.50	0.55
PLOD2	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2).	0.51	0.54
DMBT1	deleted in malignant brain tumors 1 (DMBT1).	0.52	0.69
ADD3	adducin 3 (gamma) (ADD3).	0.56	0.75
CDC25C	cell division cycle 25 homolog C (S. pombe) (CDC25C).	0.57	0.64
LXN	latexin (LXN).	0.58	0.71
GSTM2	glutathione S-transferase M2 (muscle) (GSTM2).	0.58	0.79
ARHGDI1B	Rho GDP dissociation inhibitor (GDI) beta (ARHGDI1B).	0.59	0.79
TMPO	thymopoietin (TMPO).	0.61	0.67
EIF5	eukaryotic translation initiation factor 5 (EIF5).	0.62	0.73
CAT	catalase (CAT).	0.63	0.48
AKAP12	A kinase (PRKA) anchor protein (gravin) 12 (AKAP12).	0.63	0.73
INSIG1	insulin induced gene 1 (INSIG1).	0.64	0.67
DUSP6	dual specificity phosphatase 6 (DUSP6).	0.65	0.68
ALDOA	aldolase A, fructose-bisphosphate (ALDOA).	0.65	0.68
KRT18	keratin 18 (KRT18).	0.65	0.79
NFE2L3	nuclear factor (erythroid-derived 2)-like 3 (NFE2L3).	0.65	0.80
IGFBP7	insulin-like growth factor binding protein 7 (IGFBP7).	0.66	0.72
TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase) (TGM2).	0.66	0.67
HERPUD1	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1 (HERPUD1).	0.66	0.78
GABPB2	GA binding protein transcription factor, beta subunit 2 (GABPB2).	0.66	0.76
HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4 (HSD17B4).	0.67	0.79
EZH2	enhancer of zeste homolog 2 (Drosophila) (EZH2).	0.67	0.40
MAT2B	methionine adenosyltransferase II, beta (MAT2B).	0.67	0.63
VAMP4	vesicle-associated membrane protein 4 (VAMP4).	0.67	0.79
PCNA	proliferating cell nuclear antigen (PCNA).	0.67	0.77
MCL1	myeloid cell leukemia sequence 1 (BCL2-related) (MCL1).	0.67	0.62
RRM2	ribonucleotide reductase M2 polypeptide (RRM2).	0.67	0.65
CALD1	caldesmon 1 (CALD1).	0.68	0.70
SERPINI1	serpin peptidase inhibitor, clade I (neuroserpin), member 1 (SERPINI1).	0.68	0.76
CKAP2	cytoskeleton associated protein 2 (CKAP2).	0.69	0.68
CRIM1	cysteine rich transmembrane BMP regulator 1 (chordin-like) (CRIM1).	0.69	0.57
ZNF187	zinc finger protein 187 (ZNF187).	0.69	0.71
HMBS	hydroxymethylbilane synthase (HMBS).	0.69	0.68
ITM2B	integral membrane protein 2B (ITM2B).	0.69	0.66
IDH1	isocitrate dehydrogenase 1 (NADP+), soluble (IDH1).	0.70	0.53
TGFB2	transforming growth factor, beta 2 (TGFB2).	0.70	0.77
IFNGR1	interferon gamma receptor 1 (IFNGR1).	0.70	0.78
SREBF1	sterol regulatory element binding transcription factor 1 (SREBF1).	0.70	0.78
CTH	cystathionase (cystathionine gamma-lyase) (CTH).	0.70	0.67
EHF	ets homologous factor (EHF).	0.70	0.64
IL8	interleukin 8 (IL8).	0.71	0.60
MAP4K2	mitogen-activated protein kinase kinase kinase kinase 2 (MAP4K2).	0.71	0.73
CTGF	connective tissue growth factor (CTGF).	0.71	0.72
RFC3	replication factor C (activator 1) 3, 38kDa (RFC3).	0.71	0.66
TJP2	tight junction protein 2 (zona occludens 2) (TJP2).	0.71	0.74
RAB22A	RAB22A, member RAS oncogene family (RAB22A).	0.72	0.69
FUCA1	fucosidase, alpha-L- 1, tissue (FUCA1).	0.72	0.77
BMPR2	bone morphogenetic protein receptor, type II (serine/threonine kinase) (BMPR2).	0.72	0.70

Supplementary Table 2. continued

SH3BGR1	SH3 domain binding glutamic acid-rich protein like (SH3BGR1).	0.72	0.66
DAB2	disabled homolog 2, mitogen-responsive phosphoprotein (<i>Drosophila</i>) (DAB2).	0.72	0.80
CACYBP	calyculin binding protein (CACYBP).	0.72	0.65
RCN2	reticulocalbin 2, EF-hand calcium binding domain (RCN2).	0.72	0.68
SSFA2	sperm specific antigen 2 (SSFA2).	0.73	0.65
SNX5	sorting nexin 5 (SNX5).	0.73	0.53
LBR	lamin B receptor (LBR).	0.73	0.60
IKBK1	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma (IKBK1).	0.73	0.79
PE1	peroxisomal D3,D2-enoyl-CoA isomerase (PE1).	0.73	0.73
AUH	AU RNA binding protein/enoyl-Coenzyme A hydratase (AUH), nuclear gene encoding mitochondrial protein.	0.73	0.68
LAMP2	lysosomal-associated membrane protein 2 (LAMP2).	0.74	0.66
RFC4	replication factor C (activator 1) 4, 37kDa (RFC4).	0.74	0.72
PSMA1	proteasome (prosome, macropain) subunit, alpha type, 1 (PSMA1).	0.75	0.69
MCRS1	microspherule protein 1 (MCRS1).	0.75	0.73
PAWR	PRKC, apoptosis, WT1, regulator (PAWR).	0.75	0.67
YME1L1	YME1-like 1 (<i>S. cerevisiae</i>) (YME1L1), nuclear gene encoding mitochondrial protein.	0.75	0.78
F3	coagulation factor III (thromboplastin, tissue factor) (F3).	0.75	0.67
HNMT	histamine N-methyltransferase (HNMT).	0.76	0.58
IGFBP1	insulin-like growth factor binding protein 1 (IGFBP1).	0.76	0.38
CLIC4	chloride intracellular channel 4 (CLIC4), nuclear gene encoding mitochondrial protein.	0.76	0.63
NR4A2	nuclear receptor subfamily 4, group A, member 2 (NR4A2).	0.76	0.58
ULK1	omo sapiens unc-51-like kinase 1 (<i>C. elegans</i>) (ULK1).	0.76	0.80
FDFT1	farnesyl-diphosphate farnesyltransferase 1 (FDFT1).	0.76	0.63
SOD2	superoxide dismutase 2, mitochondrial (SOD2), nuclear gene encoding mitochondrial protein.	0.77	0.77
NEDD1	neural precursor cell expressed, developmentally down-regulated 1 (NEDD1).	0.77	0.73
FIGN1	fidgetin-like 1 (FIGN1).	0.77	0.69
ACTG1	actin, gamma 1 (ACTG1).	0.77	0.40
OIP5	Opa interacting protein 5 (OIP5).	0.77	0.65
EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1).	0.77	0.73
MRPL3	mitochondrial ribosomal protein L3 (MRPL3), nuclear gene encoding mitochondrial protein.	0.78	0.69
BIRC2	baculoviral IAP repeat-containing 2 (BIRC2).	0.78	0.75
CRIP1	cysteine-rich PDZ-binding protein (CRIP1).	0.78	0.71
PTP4A2	omo sapiens protein tyrosine phosphatase type IVA, member 2, transcript variant 9 (PTP4A2).	0.78	0.78
BMP4	bone morphogenetic protein 4 (BMP4).	0.78	0.61
RBBP9	retinoblastoma binding protein 9 (RBBP9).	0.78	0.80
PHYH	phytanoyl-CoA 2-hydroxylase (PHYH).	0.78	0.76
EBAG9	estrogen receptor binding site associated, antigen, 9 (EBAG9).	0.78	0.53
CEPT1	choline/ethanolamine phosphotransferase 1 (CEPT1).	0.79	0.73
ITGB1	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) (ITGB1).	0.79	0.63
PSMC1	proteasome (prosome, macropain) 26S subunit, ATPase, 1 (PSMC1).	0.79	0.63
IDS	iduronate 2-sulfatase (Hunter syndrome) (IDS).	0.79	0.76
FER1L3	fer-1-like 3, myoferlin (<i>C. elegans</i>) (FER1L3).	0.79	0.74
NUP54	nucleoporin 54kDa (NUP54).	0.79	0.64
SHMT1	serine hydroxymethyltransferase 1 (soluble) (SHMT1).	0.79	0.76
ABC9	ATP-binding cassette, sub-family B (MDR/TAP), member 9 (ABC9).	0.79	0.80
MST1	macrophage stimulating 1 (hepatocyte growth factor-like) (MST1).	0.79	0.76
MGAT2	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT2).	0.80	0.60
ATP2C1	ATPase, Ca ⁺⁺ transporting, type 2C, member 1 (ATP2C1).	0.80	0.64
XBP1	X-box binding protein 1 (XBP1).	0.80	0.67
PLEK2	pleckstrin 2 (PLEK2).	1.26	1.46
PPP2R2A	protein phosphatase 2 (formerly 2A), regulatory subunit B, alpha isoform (PPP2R2A).	1.26	1.38
SAA1	serum amyloid A1 (SAA1).	1.26	1.30
NDUF2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa (NDUF2), nuclear gene encoding mitochondrial protein.	1.26	1.26
IL1RL1	interleukin 1 receptor-like 1 (IL1RL1).	1.26	1.41
S100A13	S100 calcium binding protein A13 (S100A13).	1.27	1.45

Supplementary Table 2. continued

ZNF275	zinc finger protein 275 (ZNF275).	1.27	1.26
CDS2	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2 (CDS2).	1.28	1.33
RNF24	ring finger protein 24 (RNF24).	1.29	1.38
CCT6A	chaperonin containing TCP1, subunit 6A (zeta 1) (CCT6A).	1.30	1.30
SRP68	signal recognition particle 68kDa (SRP68).	1.30	1.38
RBM3	RNA binding motif (RNP1, RRM) protein 3 (RBM3).	1.31	1.54
PLAGL2	pleiomorphic adenoma gene-like 2 (PLAGL2).	1.32	1.53
ACADVL	acyl-Coenzyme A dehydrogenase, very long chain (ACADVL).	1.32	1.25
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC).	1.33	1.68
MKNK1	MAP kinase interacting serine/threonine kinase 1 (MKNK1).	1.33	1.49
ABCD1	ATP-binding cassette, sub-family D (ALD), member 1 (ABCD1).	1.33	1.53
ELAVL1	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R) (ELAVL1).	1.34	1.37
TNRC15	trinucleotide repeat containing 15 (TNRC15).	1.34	1.48
SLC29A1	solute carrier family 29 (nucleoside transporters), member 1 (SLC29A1), nuclear gene encoding mitochondrial protein.	1.35	1.36
SUPT5H	suppressor of Ty 5 homolog (<i>S. cerevisiae</i>) (SUPT5H).	1.35	1.98
VHL	von Hippel-Lindau tumor suppressor (VHL).	1.36	1.51
PKD1	polycystic kidney disease 1 (autosomal dominant) (PKD1).	1.36	1.92
WDR6	WD repeat domain 6 (WDR6).	1.37	1.59
SLC5A6	solute carrier family 5 (sodium-dependent vitamin transporter), member 6 (SLC5A6).	1.38	1.50
HSBP1	heat shock factor binding protein 1 (HSBP1).	1.38	1.37
GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4 (GNB4).	1.38	1.31
BNIP1	BCL2/adenovirus E1B 19kDa interacting protein 1 (BNIP1).	1.38	1.36
NXF1	nuclear RNA export factor 1 (NXF1).	1.39	1.45
KIAA0427	KIAA0427 (KIAA0427).	1.39	1.43
EPHB4	EPH receptor B4 (EPHB4).	1.40	1.38
RBMS1	RNA binding motif, single stranded interacting protein 1 (RBMS1).	1.42	1.44
HIVEP1	human immunodeficiency virus type I enhancer binding protein 1 (HIVEP1).	1.44	1.39
TXNL2	thioredoxin-like 2 (TXNL2).	1.44	1.37
KIAA1545	omo sapiens KIAA1545 protein (KIAA1545).	1.45	1.37
PEMT	phosphatidylethanolamine N-methyltransferase (PEMT), nuclear gene encoding mitochondrial protein.	1.45	1.27
MAT2A	methionine adenosyltransferase II, alpha (MAT2A).	1.47	1.45
MTF1	metal-regulatory transcription factor 1 (MTF1).	1.47	1.71
ZNF35	zinc finger protein 35 (ZNF35).	1.48	1.30
PABPN1	poly(A) binding protein, nuclear 1 (PABPN1).	1.48	1.37
EIF2C2	eukaryotic translation initiation factor 2C, 2 (EIF2C2).	1.50	1.26
POLRMT	polymerase (RNA) mitochondrial (DNA directed) (POLRMT), nuclear gene encoding mitochondrial protein.	1.53	1.42
SEC24C	SEC24 family, member C (<i>S. cerevisiae</i>) (SEC24C).	1.54	1.39
ATOX1	ATX1 antioxidant protein 1 homolog (yeast) (ATOX1).	1.54	1.39
SRI	sorcin (SRI), transcript variant 1, mRNA.	1.54	0.78
FLNC	filamin C, gamma (actin binding protein 280) (FLNC).	1.55	1.43
ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein (ID3).	1.57	1.55
ZIC2	Zic family member 2 (odd-paired homolog, Drosophila) (ZIC2).	1.57	1.32
FZD4	frizzled homolog 4 (Drosophila) (FZD4).	1.58	1.40
WHSC2	Wolf-Hirschhorn syndrome candidate 2 (WHSC2).	1.60	1.63
LHFPL2	lipoma HMGIC fusion partner-like 2 (LHFPL2).	1.64	1.45
FGFRL1	fibroblast growth factor receptor-like 1 (FGFRL1).	1.67	1.27
SPHK1	sphingosine kinase 1 (SPHK1).	1.76	1.36
IFITM3	interferon induced transmembrane protein 3 (1-8U) (IFITM3).	1.85	1.29
IFIT1	interferon-induced protein with tetratricopeptide repeats 1 (IFIT1).	1.86	1.43
CH25H	cholesterol 25-hydroxylase (CH25H).	2.01	1.32
PI3	peptidase inhibitor 3, skin-derived (SKALP) (PI3).	2.04	1.60
HSPA8	heat shock 70kDa protein 8 (HSPA8).	2.17	1.26
CDC25A	cell division cycle 25 homolog A (<i>S. pombe</i>) (CDC25A).	2.26	1.62
RPL28	ribosomal protein L28 (RPL28).	3.04	1.37

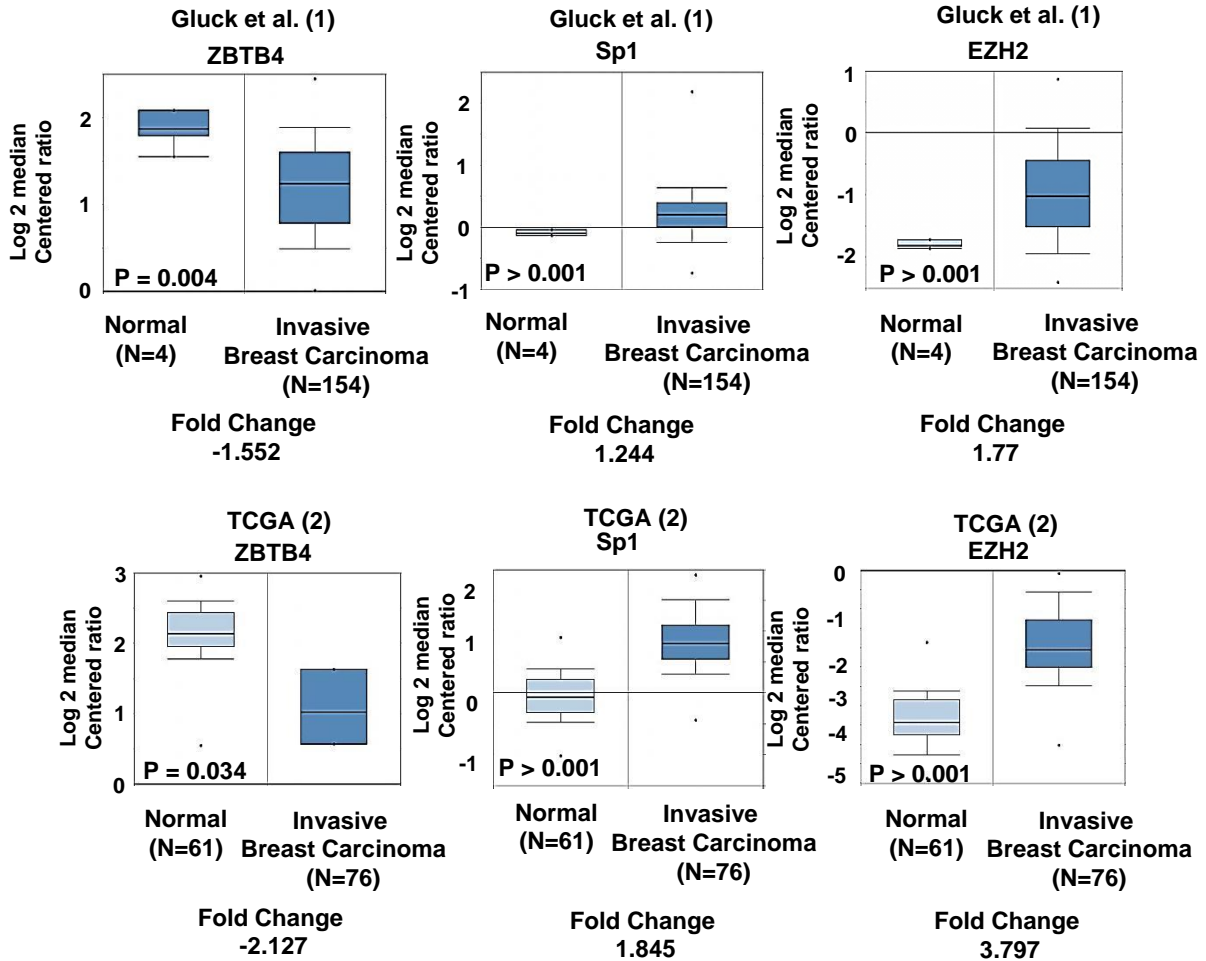
Supplementary Table 3. Functional categories by DAVID analysis using Sp1/EZH2 common 163 gene signature.

Term	Count	P Value	Fold Enrichment	Bonferroni	Benjamini	FDR
GO:0010941~regulation of cell death	22	1.17E-04	2.55	0.16	0.08	0.19
GO:0042981~regulation of apoptosis	21	2.74E-04	2.47	0.34	0.10	0.45
GO:0043067~regulation of programmed cell death	21	3.12E-04	2.45	0.37	0.09	0.52
GO:0010033~response to organic substance	20	1.91E-04	2.62	0.25	0.09	0.32
GO:0042127~regulation of cell proliferation	19	0.0015	2.28	0.89	0.17	2.44
GO:0010604~positive regulation of macromolecule metabolic process	16	0.0354	1.77	1.00	0.57	45.01
GO:0016265~death	15	0.0199	1.96	1.00	0.50	28.38
GO:0007049~cell cycle	15	0.0334	1.83	1.00	0.56	43.09
GO:0008219~cell death	14	0.0391	1.84	1.00	0.58	48.42
GO:0008285~negative regulation of cell proliferation	13	4.20E-04	3.41	0.47	0.10	0.70

Supplementary Table 4. The result from seven different prediction algorithm analysis

Prediction Algorithm	1NN	3NN	NC	SVM	BAS	CCP	LDA
p Value	0.00503	0.0113	0.000351	0.0451	0.112	0.214	0.252

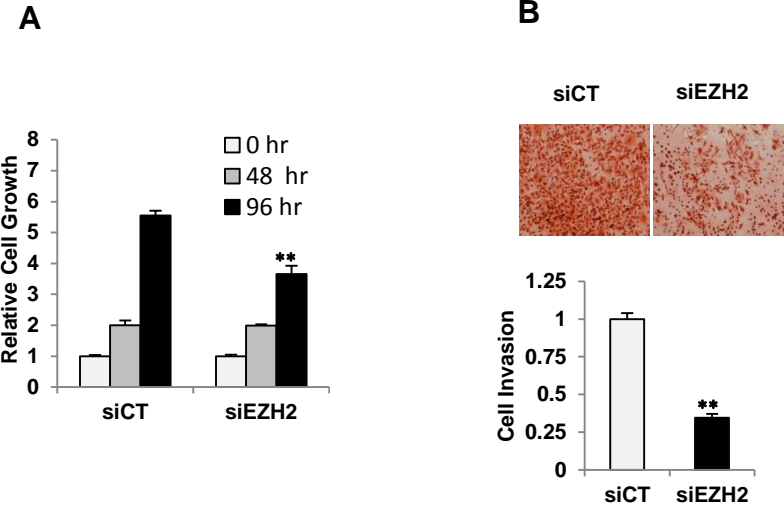
Supplementary Figure 1. Both Sp1 and EZH2 are highly expressed in invasive breast carcinoma vs. normal breast whereas ZBTB4 expression is low in two independent breast cancer data sets.



1. Glück S, Ross JS, Royce M, McKenna EF Jr, Perou CM, Avisar E, Wu L. TP53 genomics predict higher clinical and pathologic tumor response in operable early-stage breast cancer treated with docetaxel-capecitabine ± trastuzumab. *Breast Cancer Res Treat.* 2012 Apr;132(3):781-91.

2. The Cancer Genome Atlas - Invasive Breast Carcinoma Gene Expression Data (TCGA). The Cancer Genome Atlas, Office of Cancer Genomics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. (<http://tcga-data.nci.nih.gov/tcga/>).

Supplementary Figure 2. Knockdown of EZH2 by RNAi (siEZH2) decreased MDA-MB-231 cell growth (A) and invasion (B) compared to cells transfected with a control non-specific oligonucleotide (siCT). Significantly ($p < 0.05$) decreased responses are indicated (**).



Supplementary Figure 3. GC-rich Sp1 sites in EZH2 promoter region.
Sp1 consensus sequence shown as red color.

EZH2 promoter

Wild type

Sp1 Sp1

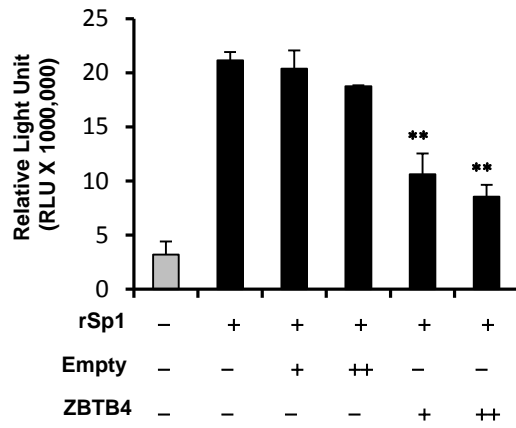
- 458 ACTTGGCTTCCAGCACCCGCCCCGCCCCTCCCCCGCCCCGGGAACTCTGCGGCGCCG - 382
 TGAACCGAAGGTCGTGGGCGGGGCGGGGAGGGGGCGGGCCCTTGAGACGCCGCGGC

Mutant

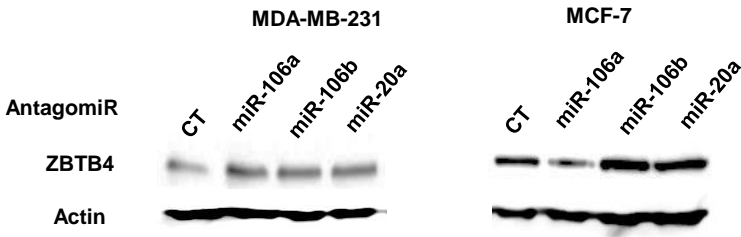
Sp1 Sp1

- 458 ACTTGGCTTCCAGCACCCGAAAAGCTTCTCAACGTTGCGGAACTCTGCGGCGCCG - 382
 TGAACCGAAGGTCGTGGGCTTTTCGAAGAGGTTGCAAGCCCTTGAGACGCCGCGGC

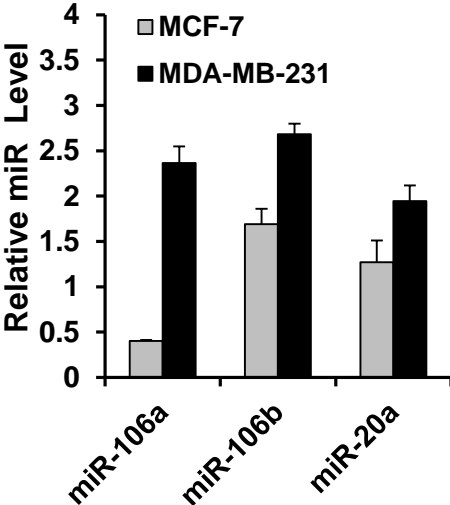
Supplementary Figure 4. Competition between Sp1 and ZBTB4 to GC-rich Sp1 sites in EZH2 promoter region.



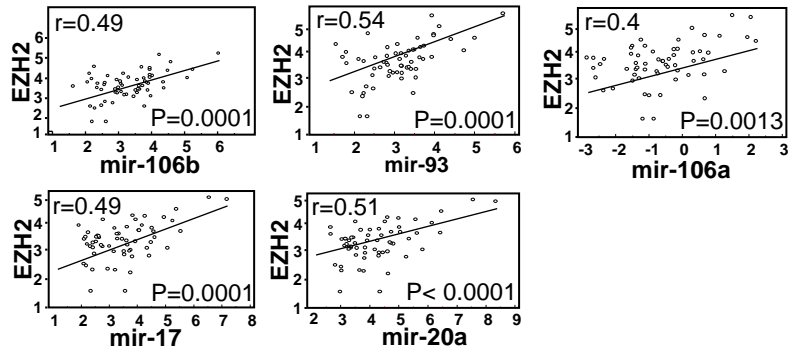
Supplementary Figure 5. Increased expression of ZBTB4 after transfection of antagomiRs in MCF-7 and MDA-MB-231 cells.



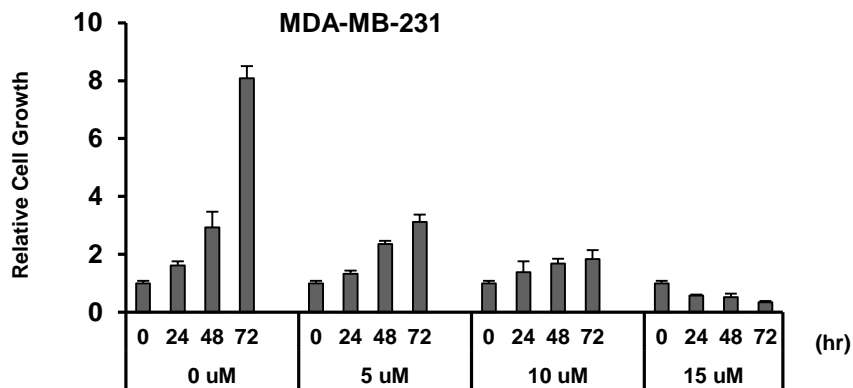
Supplementary Figure 6. Different expression Levels of miR-106a, miR-106b and miR-20a in MCF-7 and MDA-MB-231 cells.



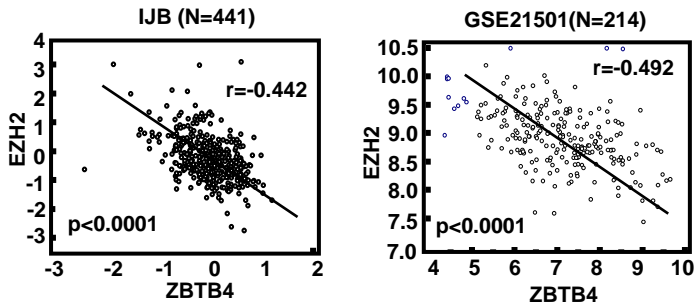
Supplementary Figure 7. Pierson's correlation analysis using NCI60 cell line miR and mRNA data sets (<http://discover.nci.nih.gov/cellminer/home.do>) (r = coefficient value). Positive correlation between EZH2 and oncogenic miRs expressions.



Supplementary Figure 8. Inhibitory effects of betulinic acid on MDA-MB-231 cell growth.



Supplementary Figure 9. Pearson's correlation analysis using two independent breast cancer patient data sets. EZH2 expression is inversely correlated with ZBTB4 expression.



Supplementary Figure 10. Kaplan-Meier Survival analysis(EZH2^{high}ZBTB4^{low} vs. EZH2^{low}ZBTB4^{high} groups). The EZH2^{high}ZBTB4^{low} group exhibit more significantly poor clinical outcome than EZH2^{low}ZBTB4^{high} Patient group.

