

## Supplemental Table 1

**Supplemental Table 1.** Expression of ZBTB4 in human breast tumors.

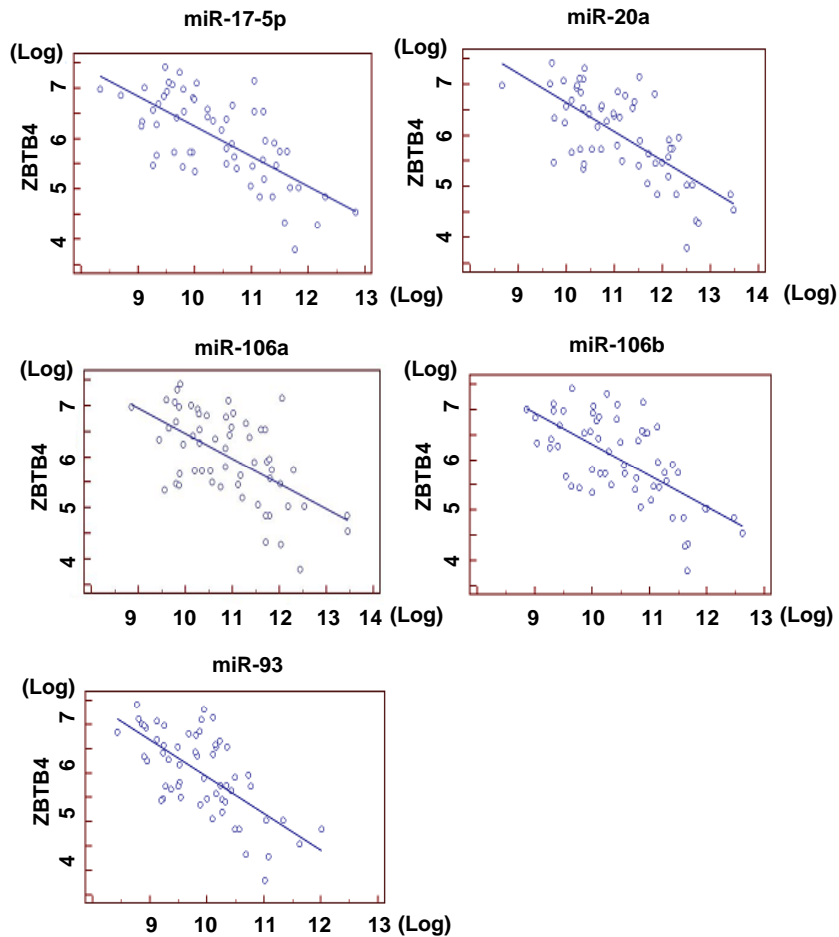
Sample ID	Clinical Characteristics	Normal Tissue Present?	Normal Tissue Labeled?	Label in Tumor: 0=weak or undetectable 1=similar to normal tissue 2=present but not nuclear
513798	IDC, grade 1	Yes	Yes	1
514199	IDC, grade 1	Yes	Yes	0
511661	IDC, grade 2	Yes	Yes	0
513764	IDC, grade 2	Yes	Yes	2
514488	IDC, grade 2	Yes	Yes	1
517732	IDC, grade 2	Yes	Yes	0
511632	IDC, grade 3	Yes	Yes	0
513959	IDC, grade 3	Yes	Yes	0
514782	IDC, grade 3	Yes	Yes	1
514834	IDC, grade 3	Yes	Yes	0
515134	IDC, grade 3	Yes	Yes	1
520565	IDC, grade 3	Yes	Yes	0
520619	IDC, grade 3	Yes	Yes	0
520937	IDC, grade 3	Yes	Yes	0
520970	IDC, grade 3	Yes	Yes	1
521026	IDC, grade 3	Yes	Yes	0
521134	IDC, grade 3	Yes	Yes	0
521265	IDC, grade 3	Yes	Yes	0
521715	IDC, grade 3	Yes	Yes	0
521813	IDC, grade 3	Yes	Yes	0
521837	IDC, grade 3	Yes	Yes	1
522086	IDC, grade 3	Yes	Yes	1

ICD = Invasive Duct Carcinoma

## Supplemental Table 2

**Table S2. Pierson's correlation analysis between ZBTB4 and miRs.** ZBTB4 expression is inversely correlated with multiple miRs derived from miR-17-92 cluster and its paralogues using mRNA and miR gene profiling data sets of NCI-60 cell lines (Top). Several examples of scattered plot show an inverse relationship between miR and ZBTB4 (Bottom).

miRNA	Correlation R Value	p Value
miR-17-5p	-0.582	<0.00001
miR-20a	-0.642	<0.00001
miR-93	-0.579	<0.00001
miR-106b	-0.621	<0.00003
miR-106a	-0.552	<0.00001
miR-20b	-0.53	<0.00002
miR-19	-0.514	<0.00003



### Supplemental Table 3

**Table S3.** Sp1 gene promoter CHIP Primer Sets.

CHIP Primer Set	Forward	Reverse
1 (-2738 ~ -2502)	5' CCAGCCTTCTTGGTGTGTTT 3'	5' CTACTCCCAGGACGGATCAA 3'
2 (-2161 ~ -1897)	5' CTCCAAAATGCTGGGATTA 3'	5' GCTTGAGCCCAGGAGTTAAA 3'
3 (-1877 ~ -1415)	5' CGGCAGTTTAATTCCTCAA 3'	5' CCGGCCTTAATAGCTTGTC A 3'
4 (-1331 ~ -1091)	5' TTCGTGATTGCAAAAAGCTG 3'	5' GGGACGAGATCTGGTGACAG 3'
5 (-948 ~ -688)	5' TAGTGTTGATGCGGAACTGC 3'	5' ACTTGAGTGGCAGAGGAGA 3'
6 (-626 ~ -386)	5' TGC GTCCTTCTGTCTCTT 3'	5' GATGATTGGCTTGAAGGAA 3'
7 (-261 ~ +26)	5' CTTGAGAGCAAGCGAGTCT 3'	5' GGACTCATCTTACCGCTCA 3'
8 (+152 ~ +450)	5' GGAGGGAAGGGAGGGAGAC 3'	5' GGGAAATCTACGAAAGTGG 3'
9 (+682 ~ +882)	5' TGAGACGGAGTTTTCGCTCT 3'	5' GAGACCAGCCTGACCAACAT 3'
10 (+1107 ~ +1438)	5' CATTTCCTATCCCAAAGCA 3'	5' TTGCCACCACTCCTTTTC 3'
11 (+1864 ~ +2299)	5' TGCAGCAGAATTGAGTCACC 3'	5' TTGGTTGCACCTGGTATGA 3'

## Supplemental Table 4

**Table S4.** Primer Sets for ZBTB33, ZBTB41 and ZBTB7A.

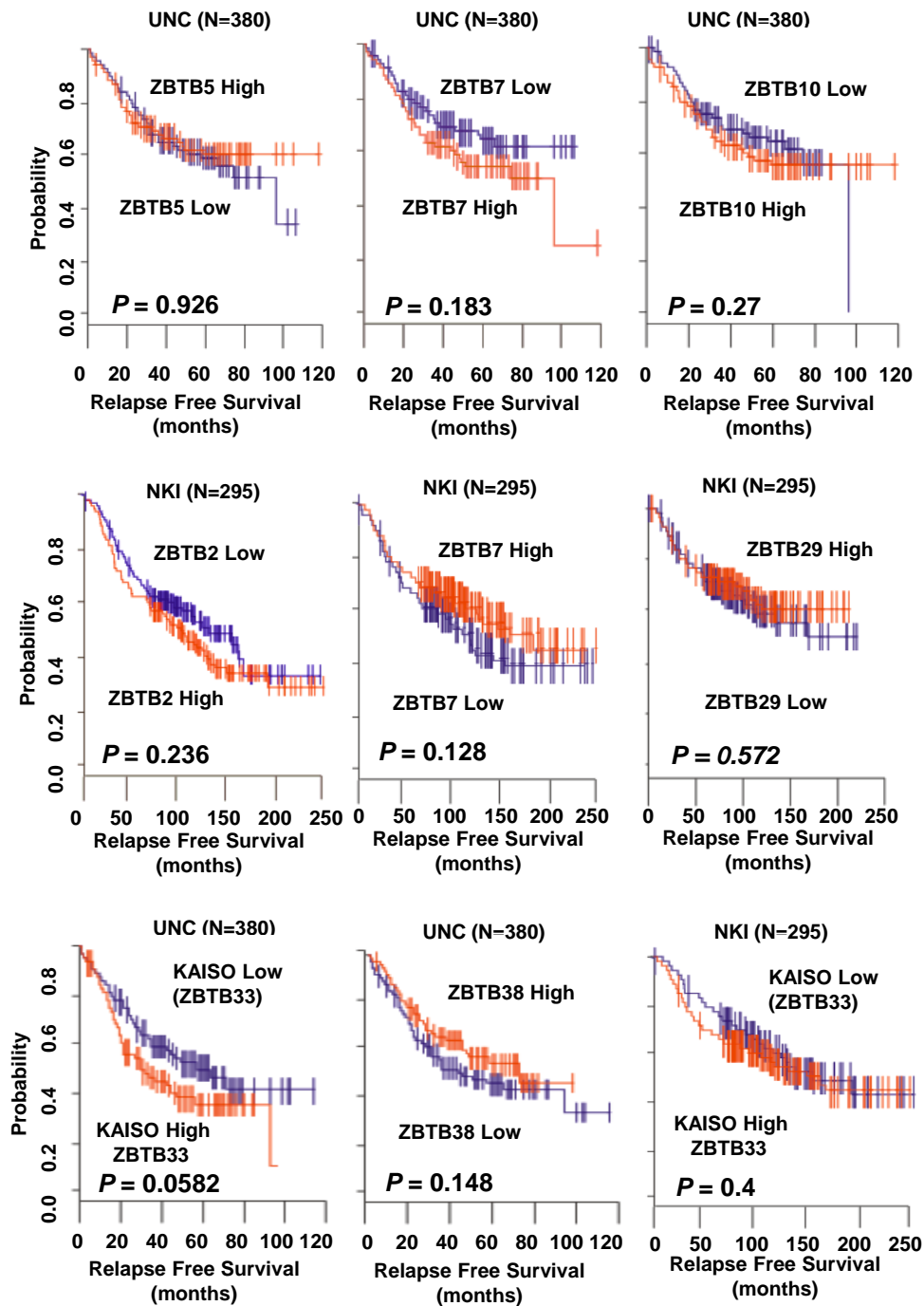
<b>PrimerSet</b>	<b>Forward</b>	<b>Reverse</b>
ZBTB33	TGCAAGGTTTATGCAAATATCG	ATACGTTTGTTTGCCATCTCG
ZBTB41	TTAGGAAACATCAGACAACCAA	TTTGCACTTGTTTTTCAGTCTCA
ZBTB7A	CCAGCAGAACGTGTACGAGA	GGTTGCTCTGGAAGAACTCG

## Supplemental Table 5

**Table S5.** Primer Sets for VEGF165, VEGF121, VEGFR1 and sVEGFR1.

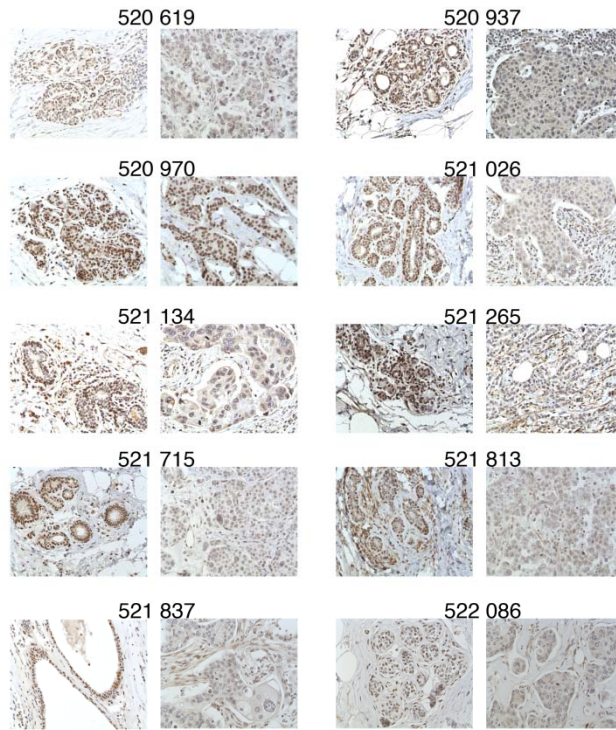
<b>PrimerSet</b>	<b>Forward</b>	<b>Reverse</b>
VEGF165	AAGAAAATCCCTGTGGGCCTT	TGGTGAGAGATCTGCAAGTACGTT
VEGF121	AAGGCCAGCACATAGGAGAG	TTCCTGGTGAGAGATTTTTCTTG
VEGFR1	TGGCTGCGACTCTCTCTG	CAAAGGAACTTCATCTGGGTCC
sVEGFR1	ACAGCCTTTTTGTTGCAGTGC	TTCAGGCACCTATGCCTGCAC

## Supplemental Figure 1



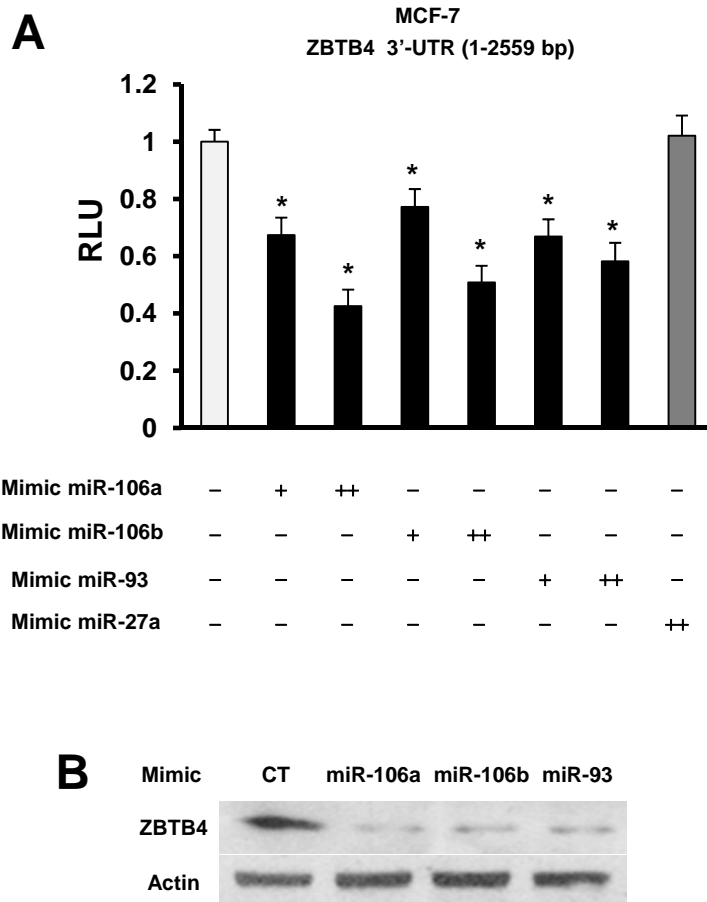
**Figure S1. Survival Analysis of ZBTB genes.** Not all the ZBTB genes exhibit significant correlation ( $p$  value  $< 0.05$ ) with relapse free patient survival. Kaplan-Meier survival analysis of ZBTB mRNAs previously known to interact with methylated or non-methylated GC-rich sequences (ZBTB2, ZBTB5, ZBTB7, and ZBTB10), to be closely related to ZBTB4 (KAISO; ZBTB33 and ZBTB38), or to be tumor suppressive (HIC1; ZBTB29)

## Supplemental Figure 2



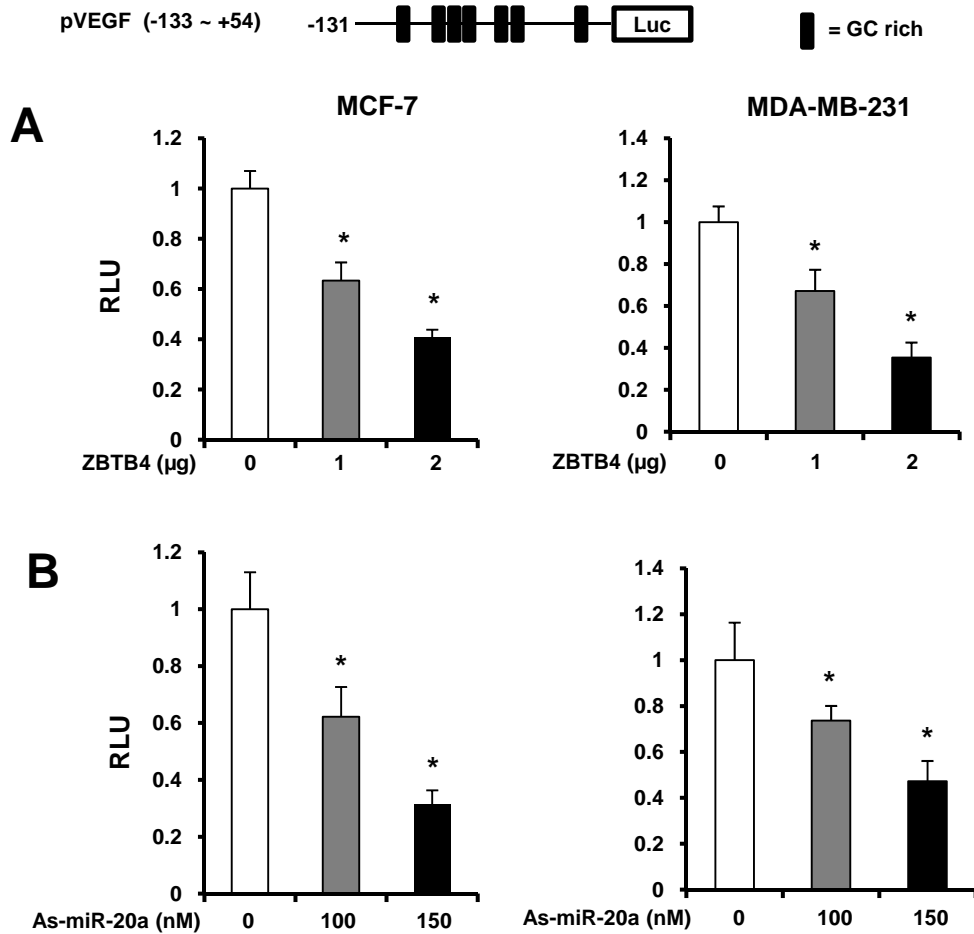
**Figure S2.** Immunostaining of tumor (right) and non-tumor (left) tissue from breast cancer patients with invasive ductal carcinoma (see Fig. 1B and Supplemental Table 1).

### Supplemental Figure 3



**Figure S3.** Targeting of ZBTB4 by other miRs derived from miR-17-92 cluster paralogues, miR-106b-95 and miR-106a-363 cluster. MiR-106a, miR-106b and miR-93 derived from miR-17-92 cluster paralogues (miR-106a-363 and miR-106b-93 clusters) also interact with 3'-UTR region of ZBTB4 region (A). Transfection of mimics of miR-106a, miR-106b and miR-93 downregulates ZBTB4 protein (B). \*, Significantly ( $p < 0.05$ ) decreased.

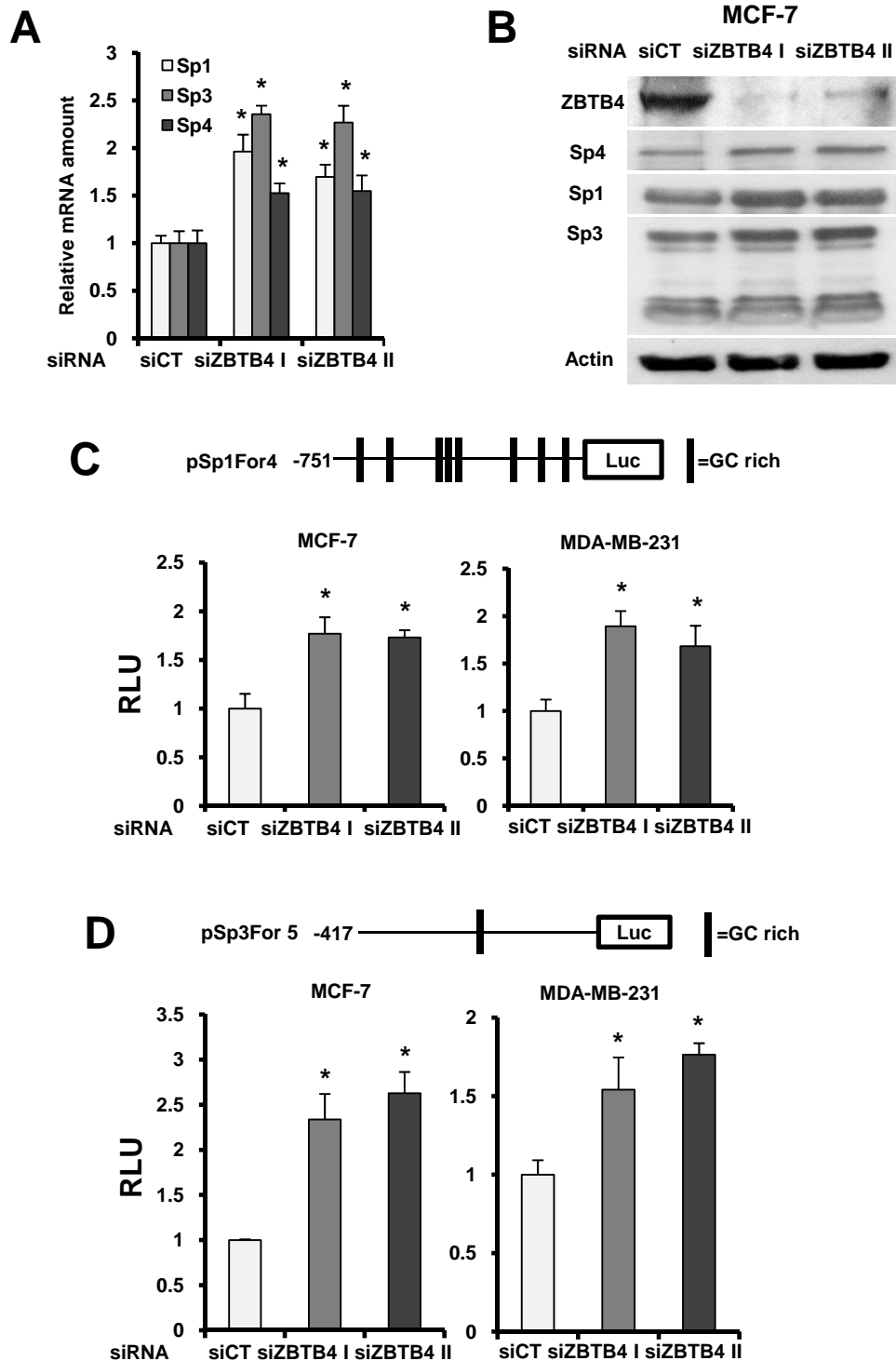
## Supplemental Figure 4



**Figure S4. ZBTB4 and as-miR-20a inhibit VEGF promoter luciferase activity.** Either ZBTB4 (A) or as-miR-20a (B) transfection decreased luciferase activity in cells cotransfected with pVEGF (-133 ~ +54) reporter luciferase containing multiple GC-rich sequences. \*, Significantly ( $p < 0.05$ ) decreased.

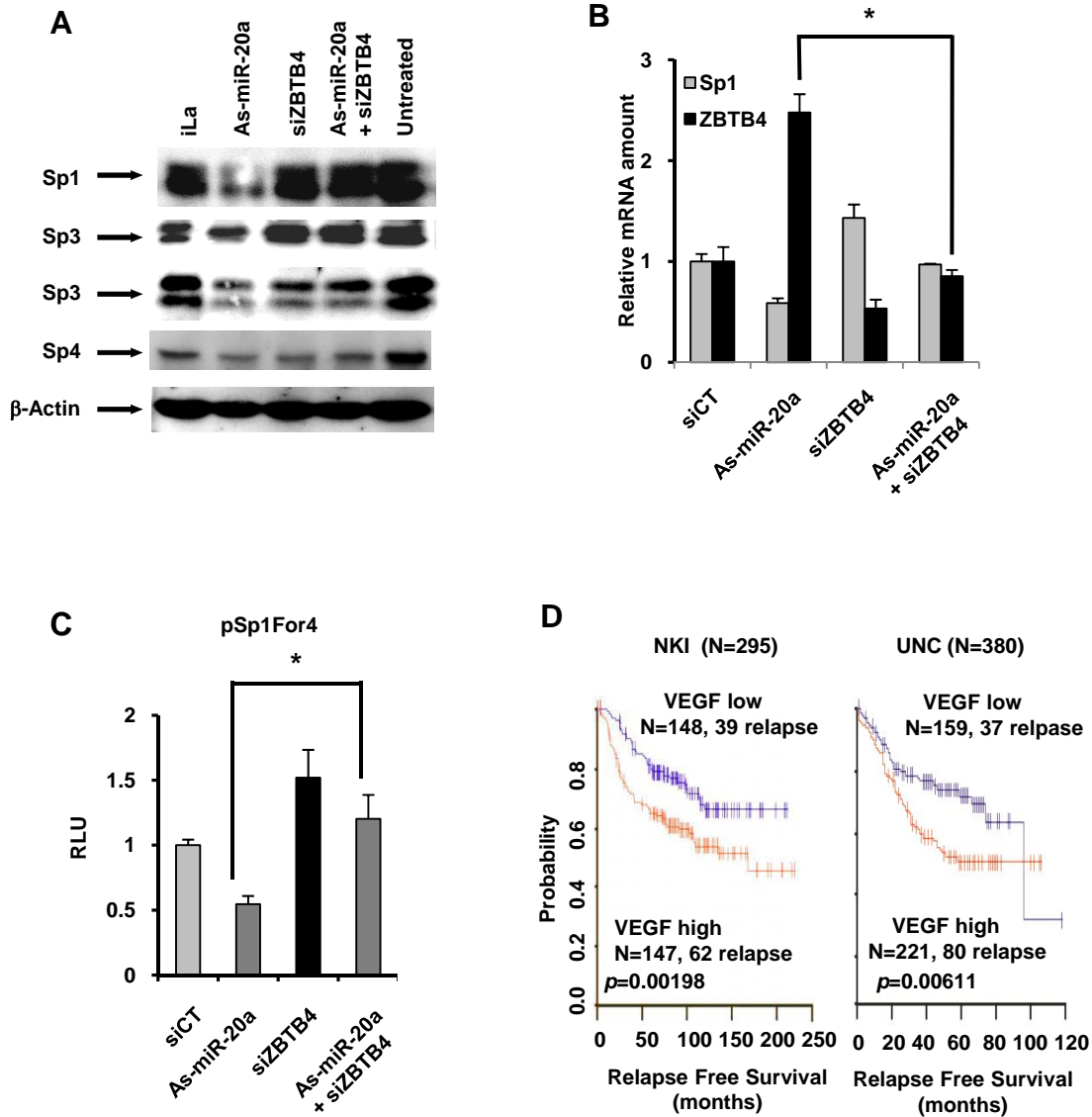


## Supplemental Figure 5



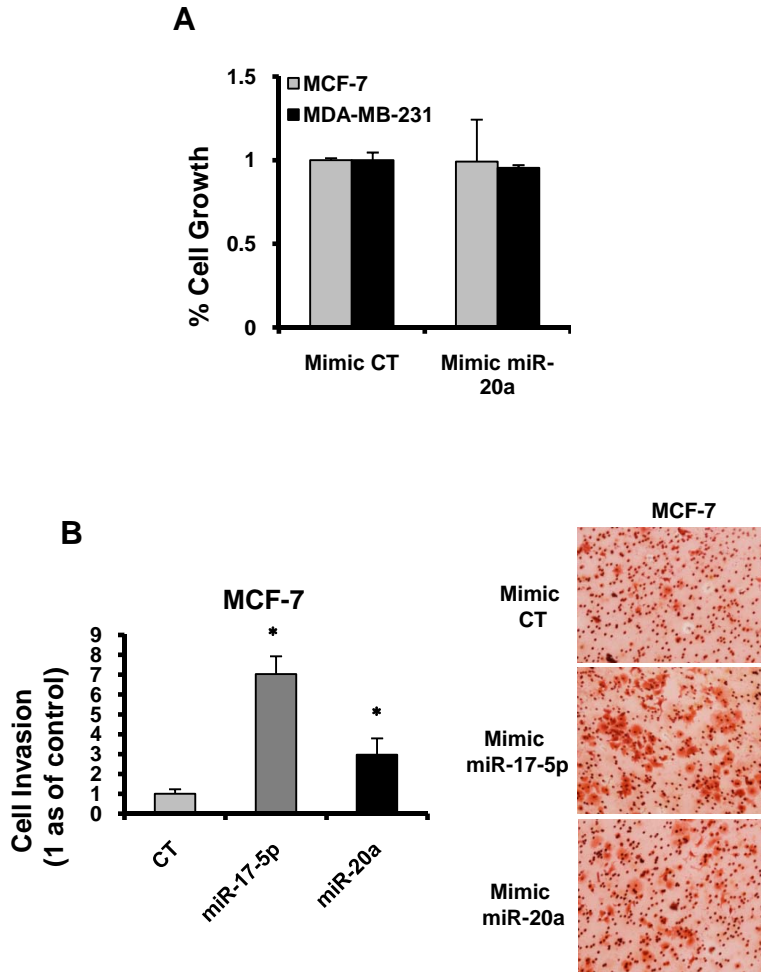
**Figure S5. The Effects of ZBTB4 knockdown in Sp mRNA and protein expression.** ZBTB4 depletion resulted from by ZBTB4 siRNA transfection increased Sp1, Sp3 and Sp4 mRNA (A) and protein level (B). Consistently, pSp1FOR4 and pSp3FOR5 luciferase activities, containing Sp1 and Sp3 gene promoter region, were increased after ZBTB4 knockdown. \*, Significantly ( $p < 0.05$ ) increased.

## Supplemental Figure 6



**Figure S6. Rescue experiments and prognostic value of VEGF.** Rescue of as-miR-20a-mediated downregulation of Sp proteins (A), Sp1 mRNA (B), and luciferase activity (C) in MDA-MB-231 cells. Cells were treated as indicated, and protein, mRNA and luciferase activity were determined as described in the Materials and Methods. Prognostic activity of VEGF (D). Correlation of VEGF expression level with relapse free survival of breast cancer patients using publicly-available data sets (van de Vijver et al, 2002; Oh et al, 2006). \*, Significant ( $p < 0.05$ ) rescue by siZBTB4.

## Supplemental Figure 7



**Figure S7. Effects of miR mimic and ZBTB4 overexpression in breast cancer cells.** Cell proliferation (A) and invasion (B). Cells were transfected with miR mimics (150 nM) for 72 hr and cell numbers were determined and, 24 hr after transfection, cells were transferred to a Boyden Chamber and cell invasion ( $p < 0.05$ ) was determined as described.