Table S1. List of RT-PCR primers

PCR Primer	Sequence (5'-3')	Product size(bp)
ZBTB28F	CTACGTCCGCGAGTTCACTC	170bp
ZBTB28R	CCCGGAAAATTGAATAGAAG	
VIMF	TGCCAACCTTTACAGACCTA	390bp
VIMR	CTCATCTCCCTCCTCACTCA	
EcadF	CCTCCGTTTCTGGAATCCAA	282bp
EcadR	GTTCTCTATCCAGAGGCTCT	
IFNαF	GCCTCGCCCTTTGCTTTACT	89bp
IFNαR	CTGTGGGTCTCAGGGAGATCA	
IFNβF	GCTTGGATTCCTACAAAGAAGCA	166bp
IFNβR	ATAGATGGTCAATGCGGCGTC	
IFNγF	TCGGTAACTGACTTGAATGTCCA	93bp
IFNγR	TCGCTTCCCTGTTTTAGCTGC	
IFNL1F	CACATTGGCAGGTTCAAATCTCT	386bp
IFNL1R	CCAGCGGACTCCTTTTTGG	
IFNL3F	TAAGAGGGCCAAAGATGCCTT	205bp
IFNL3R	CTGGTCCAAGACATCCCCC	
SIRPαF	GCCCTCTACCTCGTCCGAAT	193bp
SIRPαR	CATACTCCGTGTGGTTGTTGG	
Siglec10F	AAGGGACTCATCTCAACGGC	114bp
Siglec10R	CCGTCTCTTCGGTAGAATCTTCA	
β-actinF	TCCTGTGGCATCCACGAAACT	315bp
β-actinR	GAAGCATTTGCGGTGGACGAT	

Table S2. List of qRT-PCR Primers

PCR Primer		Sequence (5'-3')	Product size (bp)	Annealing temperature (℃)
	iNOSF	CAGGATGACCTTCAGTATCACA	106bp	60
	iNOSR	CATCCAGCTTGACCAGAGAT		
	CD80F	AAACTCGCATCTACTGGCAAA	87bp	60
	CD80R	GGTTCTTGTACTCGGGCCATA		
	CD86F	CTGCTCATCTATACACGGTTACC	133bp	60
	CD86R	GGAAACGTCGTACAGTTCTGTG		
	CD40F	ACTGAAACGGAATGCCTTCCT	181bp	60
	CD40R	CCTCACTCGTACAGTGCCA		
	IL-1bF	ACAGTGGCAATGAGGATG	129bp	60
	IL-1bR	TGTAGTGGTGGTCGGAGA		
	ARG1F	GTGGAAACTTGCATGGACAAC	76bp	60
	ARG1R	AATCCTGGCACATCGGGAATC		
	MRC1F	TCCGGGTGCTGTTCTCCTA	211bp	60
	MRC1R	CCAGTCTGTTTTTGATGGCACT		
	IL-10F	GCCAAGCCTTGTCTGAGATGA	80bp	60
	IL-10R	CTTGATGTCTGGGTCTTGGTTCT		
	IFNAR1F	ATTTACACCATTTCGCAAAGCTC	120bp	60
	IFNAR1R	TCCAAAGCCCACATAACACTATC		
	IFNAR2F	TCATGGTGTATATCAGCCTCGT	143bp	60
	IFNAR2R	AGTTGGTACAATGGAGTGGTTTT		
qRT-	IFNGR1F	AGCAGGAAGTCGATTATGATCCC	137bp	60
PCR	IFNGR1R	CTGGCACTGAATCTCGTCACA		
	IFNGR2F	CTCCTCAGCACCCGAAGATTC	136bp	60
	IFNGR2R	GCCGTGAACCATTTACTGTCG		
	IFIT1F	AGCCATTTTCTTTGCTTCCC	205bp	60
	IFIT1R	ACAGAGCCTTTTCTTCGGTA		
	IFIT2F	AAGCACCTCAAAGGGCAAAAC	147bp	60
	IFIT2R	TCGGCCCATGTGATAGTAGAC		
	IFIT3F	GGAAACTACGCCTGGGTC	180bp	60
	IFIT3R	CACCTTCGCCCTTTCATT		
	OAS1F	CTGACCTGGTTGTCTTCC	137bp	60
	OAS1R	GACCTCAAACTTCACGGA		
	OAS2F	TGAAGCCCTACGAAGAAT	176bp	60
	OAS2R	ACTGAAGAAGAGGACAAGG		
	OAS3F	GAAGGAGTTCGTAGAGAAGGCG	114bp	60
	OAS3R	CCCTTGACAGTTTTCAGCACC		
	SAMD9LF	GCTAGAAGCTCTGAGAGCAGA	116bp	60
	SAMD9LR	TGCTGCAGTAGGAAGGCATA		
	XAF1F	GTTGGGTGTACGATGTGTCA	200bp	60

	XAF1R	ATGCGGTGCATGATGAACTG		
	RARRES3F	ATGGCTACGTGATCCATCTG	151bp	60
	RARRES3R	AAGCTGTTGTTGACCCGATAG		
	ASCL1F	AACTTCAGCGGCTTTGGCTAC	302bp	60
	ASCL1R	ATGGAGTTCAAGTCGTTGGAG		
	CD24F	AAGTAACTCCTCCCAGAGTACT	120bp	60
qRT-	CD24R	GAGAGAGTGAGACCACGAAG		
PCR	CD47F	CCAGAGAAGG TGAAACGATC	122bp	60
	CD47R	AAACTGTCCCCAGAACAGGA		
	IFNβF	ATGACCAACAAGTGTCTCCTCC	88bp	60
	IFNβR	GGAATCCAAGCAAGTTGTAGCTC		
	β-actinF1	GTCTTCCCCTCCATCGTG	113bp	60
	β-actinR1	AGGGTGAGGATGCCTCTCTT		

Table S3. List of ChIP-PCR Primers

Primer	Sequence (5'-3')	Product size (bp)	Annealing temperature (℃)
chip IFNAR1 F	AAAGTGGTGTTCTGGGTCCT	249bp	60
chip IFNAR1 R	AATCCTGGCCACACTTAGCT		
chip IFNAR2 F1	CAAAACTGCACTTGTACCCC	99bp	60
chip IFNAR2 R1	AAGTGATCTGAAGATGAAGGCA		
chip IFNAR2 F2	GCAGGAAGTCGCAAACTCAT	232bp	60
chip IFNAR2 R2	GAGGAAGAAAGCGTGTTAGGAG		
chip IFNAR2 F3	CTCCTAACACGCTTTCTTCCTC	131bp	60
chip IFNAR2 R3	GGAATGTCCTCAGAGGCAATTG		
chip CD24 F1	GCCGCGTTCCCCTTTCCTCT	226bp	60
chip CD24 R1	TTTCCCGGGACCTGCCATCTTA		
chip CD24 F2	TAAGATGGCAGGTCCCGGGAAA	164bp	60
chip CD24 R2	TGCTGGTACCCGGCTGGTAT		
chip CD24 F3	ATACCAGCCGGGTACCAGCA	221bp	60
chip CD24 R3	AAAGCCACAATAGCCGTGACGT		
chip CD24 F4	ACGTCACGGCTATTGTGGCTTT	161bp	60
chip CD24 R4	CACCATTGCTCTGCCCATGT		
chip CD24 F5	ACATGGGCAGAGCAATGGTG	200bp	60
chip CD24 R5	AAGATTCTCTCCCGGTCCCT		
chip CD47 F1	ATGCCTGTTTGCGACAATGCTC	172bp	60
chip CD47 R1	TACTCGCTCTGCTCTTCCCTAT		
chip CD47 F2	ATAGGGAAGAGCAGAGCGAGTA	185bp	60
chip CD47 R2	GACACCTAGGCTTTCACCA		
chip CD47 F3	TGGTGAAAGCCTAGGTGTC	150bp	60
chip CD47 R3	CCACTGTCCTCTCTCTCTACTT		
chip CD47 F4	AAGTAGAGAGAGAGGACAGTGG	177bp	60
chip CD47 R4	TTCCAGGTCACGTCCTGT		
chip CD47 F5	ACAGGACGTGACCTGGAA	257bp	60
chip CD47 R5	TCACAGGCAGGACCCACT		
chip CD47 F6	AGTGGGTCCTGCCTGTGA	178bp	60
chip CD47 R6	TTCTCTTCCCTCTTTCTCACCG		

Table S4. *ZBTB28* methylation and clinicopathological features of breast tumors

Clinicopathological features	Number (***n***=174)	Methylated status		***p*** value
		methylated	unmethylated	
Age				
<45	57	36	21	**0.032**
≥45	116	91	25	
Unknown	1	1		
Tumor size				
≤5.0 cm	142	108	34	0.125
>5.0 cm	17	10	7	
Unknown	15	10	5	
Stage (AJCC)				
I-II	112	84	28	0.840
III-IV	51	39	12	
Unknown	11	5	6	
Lymph node metastasis				
Positive	84	58	26	0.205
Negative	81	63	18	
Unknown	9	7	2	
Distant metastasis				
Positive	4	3	1	0.670
Negative	125	90	35	
Unknown	45	35	10	
ER				
Positive	87	59	28	0.162
Negative	72	56	16	
Unknown	15	13	2	
PR				
Positive	70	47	23	0.263
Negative	85	64	21	
Unknown	19	17	2	
HER2				
Positive	115	83	32	0.956
Negative	46	33	13	
Unknown	13	12	1	
P53				
Positive	80	58	22	0.908
Negative	67	48	19	
Unknown	27	22	5	
Ki-67				
Positive	135	100	35	0.883
Negative	9	7	2	
Unknown	30	21	9	

Figure S1.

(A, B) Down-regulated *ZBTB28* mRNA expression and methylation of *ZBTB28* promoter were accessed via The Cancer Genome Atlas and MethHC databases. (C) Multiple targeted bisulfite enrichment sequencing (MethTarget) of 5 BrCa cases and 9 controls. (D) Cells were exposed to demethylating agent 5-Aza. *ZBTB28* expression was detected with qRT-PCR and demethylation was measured by qMSP. (E) The relationship between *ZBTB28* methylation and survival of breast cancer patients were illustrated through Survivalmeth database.

Figure S2.

(A) For colony formation assay, pictures were taken on the 14th day. (B) Representative pictures of AO/EB staining assay. (C) Flow cytometry analysis of cell cycle. (D) The localization of ZBTB28 and E-cad or Vimentin in the nucleus of MB231 was analyzed by immunofluorescence. (E) Phase contrast microscopy obtained the changes of morphological for gain-of-function breast cancer cell lines. (F) Pictures were taken at 24h after seeding during transwell assay. (G) Photographs were captured at 0 h and 24 h, then the ratio of wound healing was calculated. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Figure S3.

(A) 48 hours after transfection with siZBTB28 or control siRNA, the expression of ZBTB28 were evaluated by RT-PCR. β-actin was used as control. (B) BT549 cells were transfected with siZBTB28 and control siRNA. Cell viability was assessed by CCK8 assay. (C, D) Pictures were taken at 48 h after transfecting, the relative ratio of

migration and invasion cells per field was shown.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure S4.

(A) RT-PCR results of the regulation of IFNs by ZBTB28. (B) qRT-PCR confirmed that ZBTB28 upregulated IFN-γ. (C) Ectopic expression of ZBTB28 in breast cancer cells influenced the expression of IFNGR1 and IFNGR2. (D) Cancer cells were pretreatment with/without anti-IFNGR2 mAb for 8 hours, then co-culture with THP-1 macrophages for another 24 hours. Protein levels of IFNGR2 and IFN-γ were measured by western blotting; β-actin was used as a loading control.

Figure S5.

(A) Association of *ZBTB28* and *BCL6* expression in breast cancer which was obtained from TCGA cancer dataset (www.cbioportal.org). (B, C) qRT-PCR of CD24 or CD47 mRNA in MCF7 and MB231 underwent with pcDNA 3.1, pcDNA-ZBTB28, siRNA of BCL6, and pcDNA-ZBTB28 with or without siRNA of BCL6. (D) The expression of CD24 and CD47 was detected by qRT-PCR in BCL6-overexpression, ZBTB28-overexpression, BCL6+ZBTB28 overexpression and control group in K562 cells. (E) The expression of SIRPα and Siglec10 were detected by RT-PCR in THP-1 cells and PMA treated THP-1 macrophages.

(B) Figure S6.

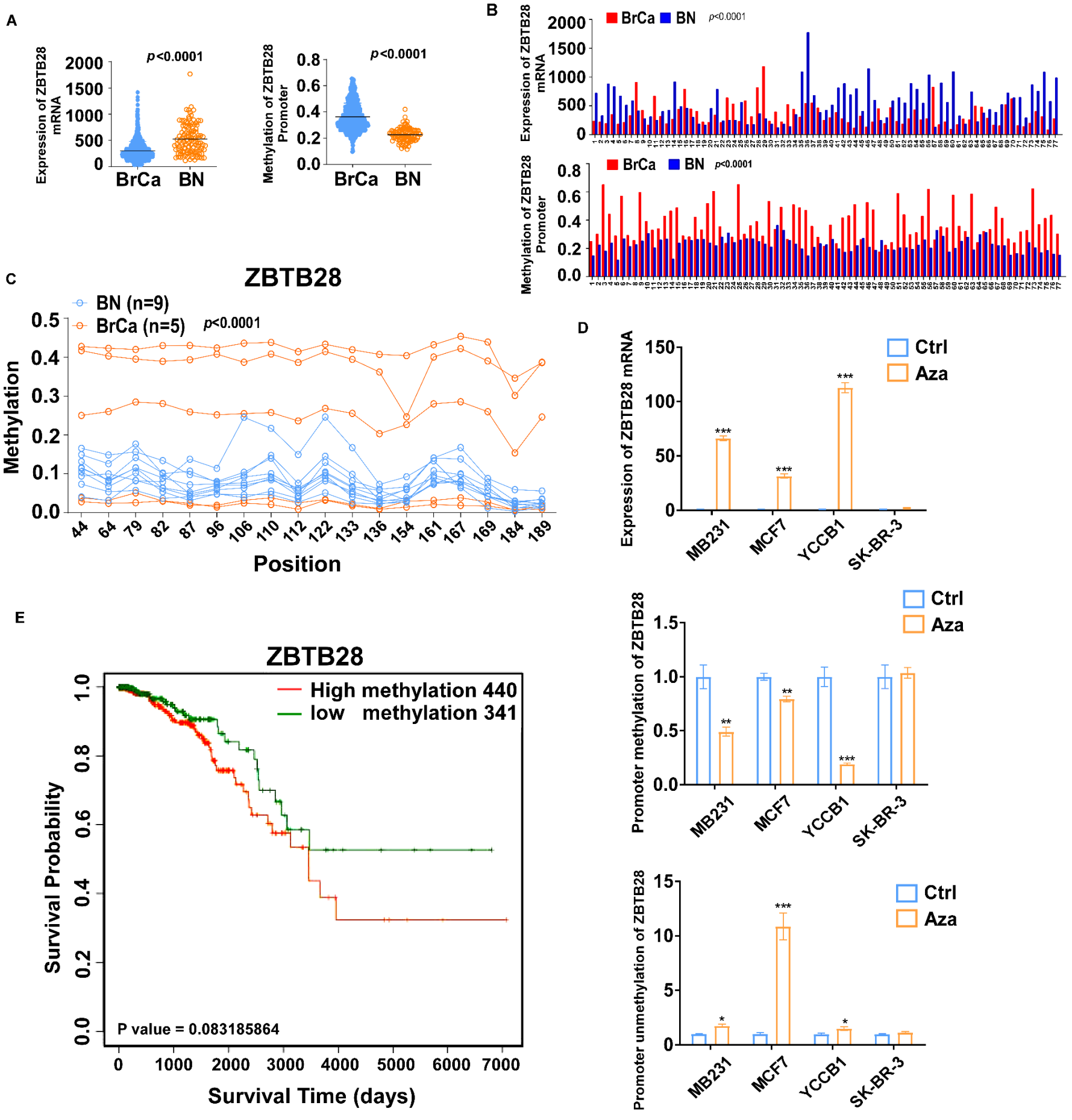
(A) Representative flow-cytometry plots of the phagocytosis of MCF7 cells treated with different treatment. (B) Phagocytosis efficiency was shown as a bar graph. (C) Phagocytosis images of THP-1 macrophages engulfing MCF7 cells. The white arrows

indicate macrophages that engulfed cancer cells.

Figure S7.

(A, B) Representative plots showed the percentage of THP-1 macrophages phagocytosing cancer cells in vitro. (C) Statistical analysis was shown as a bar graph.

Figure S1.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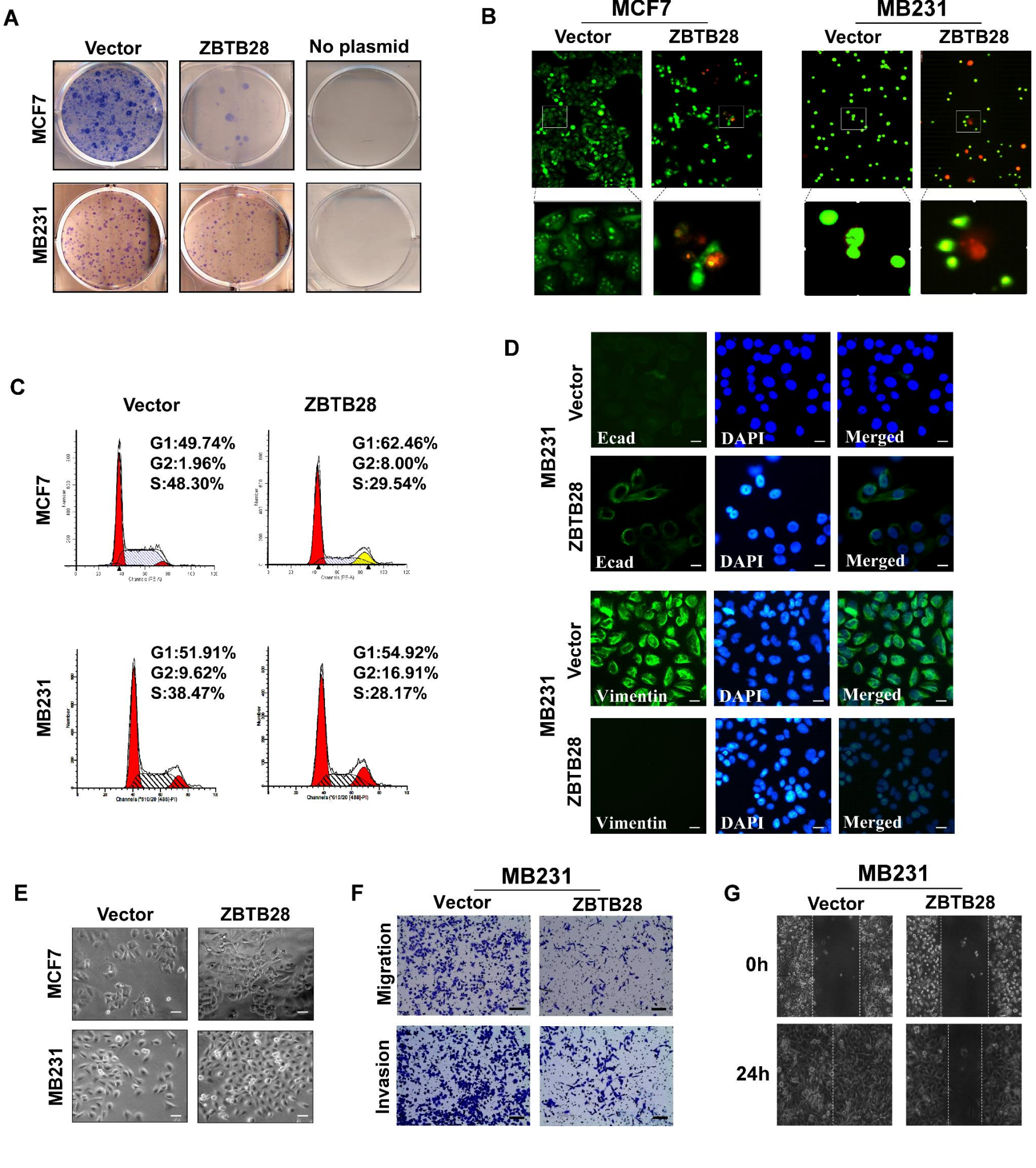
Expression of ZBTB28 mRNA
p<0.0001
BrCa
BN
Methylation of ZBTB28 Promoter
p<0.0001
B
BrCa BN p<0.0001
Expression of ZBTB28 mRNA
Methylation of ZBTB28 Promoter
C
ZBTB28
BN (n=9)
BrCa (n=5)
p<0.0001
Methylation
Position
D
Ctrl
Aza
Expression of ZBTB28 mRNA
Promoter methylation of ZBTB28
Promoter unmethylation of ZBTB28
MB231
MCF7
YCCB1
SK-BR-3
E
ZBTB28
High methylation 440
low methylation 341
Survival Probability
P value = 0.083185864
Survival Time (days)

Figure S2.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Vector
ZBTB28
No plasmid
MCF7
MB231
B
MCF7
MB231
Vector
ZBTB28
Vector
ZBTB28
C
Vector
ZBTB28
MCF7
G1:49.74%
G2:1.96%
S:48.30%
G1:62.46%
G2:8.00%
S:29.54%
MB231
G1:51.91%
G2:9.62%
S:38.47%
G1:54.92%
G2:16.91%
S:28.17%
D
Vector
ZBTB28
MB231
Ecad
DAPI
Merged
Vimentin
E
Vector
ZBTB28
MCF7
MB231
F
MB231
Vector
ZBTB28
Migration
Invasion
G
MB231
Vector
ZBTB28
0h
24h

Figure S3.

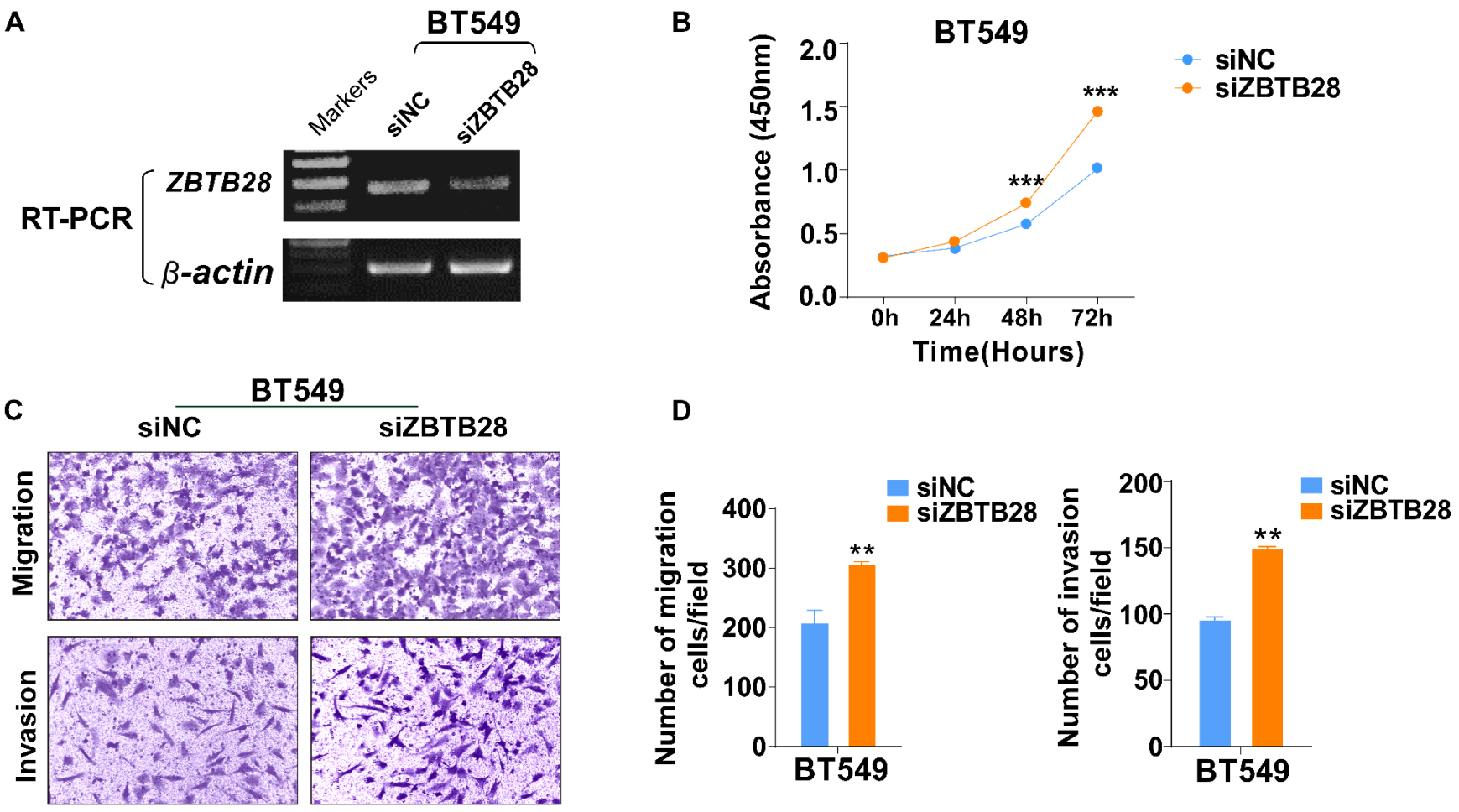
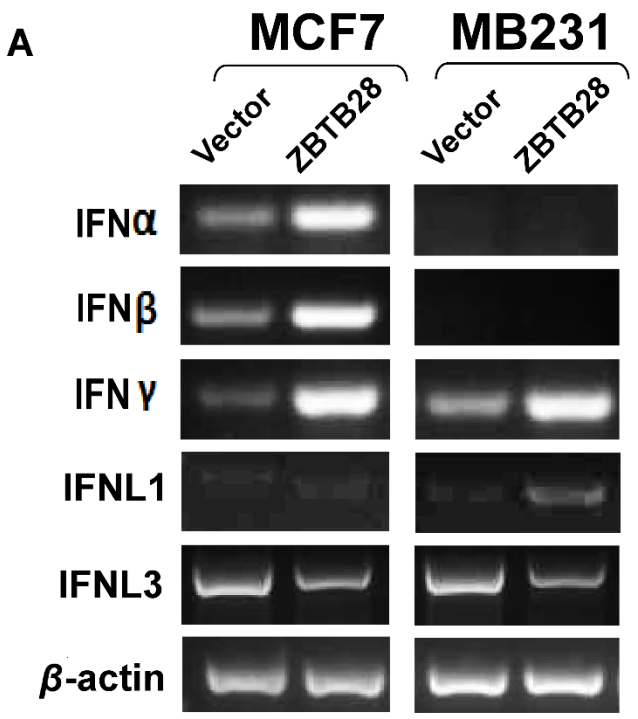

A
BT549
Markers
siNC
siZBTB28
RT-PCR
ZBTB28
β-actin
B
BT549
Absorbance (450nm)
2.0
1.5
1.0
0.5
0.0
0h
24h
48h
72h
Time(Hours)
***
***
siNC
siZBTB28
C
BT549
siNC
siZBTB28
Migration
Invasion
D
Number of migration cells/field
400
300
200
100
0
**
siNC
siZBTB28
BT549
Number of invasion cells/field
200
150
100
50
0
**
siNC
siZBTB28
BT549

Figure S4.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MB231
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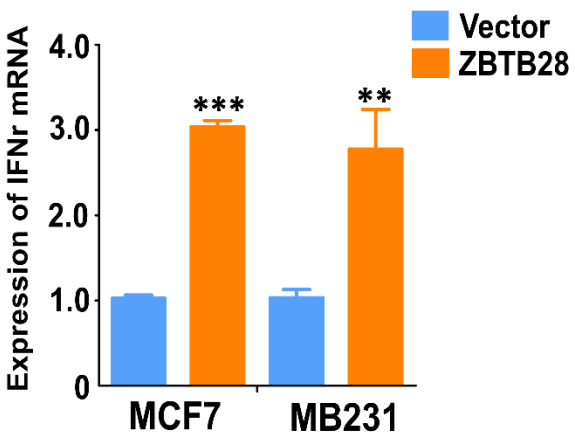
ZBTB28
Vector
ZBTB28
IFNα
IFNβ
IFNγ
IFNL1
IFNL3
β-actin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ZBTB28
Expression of IFNr mRNA
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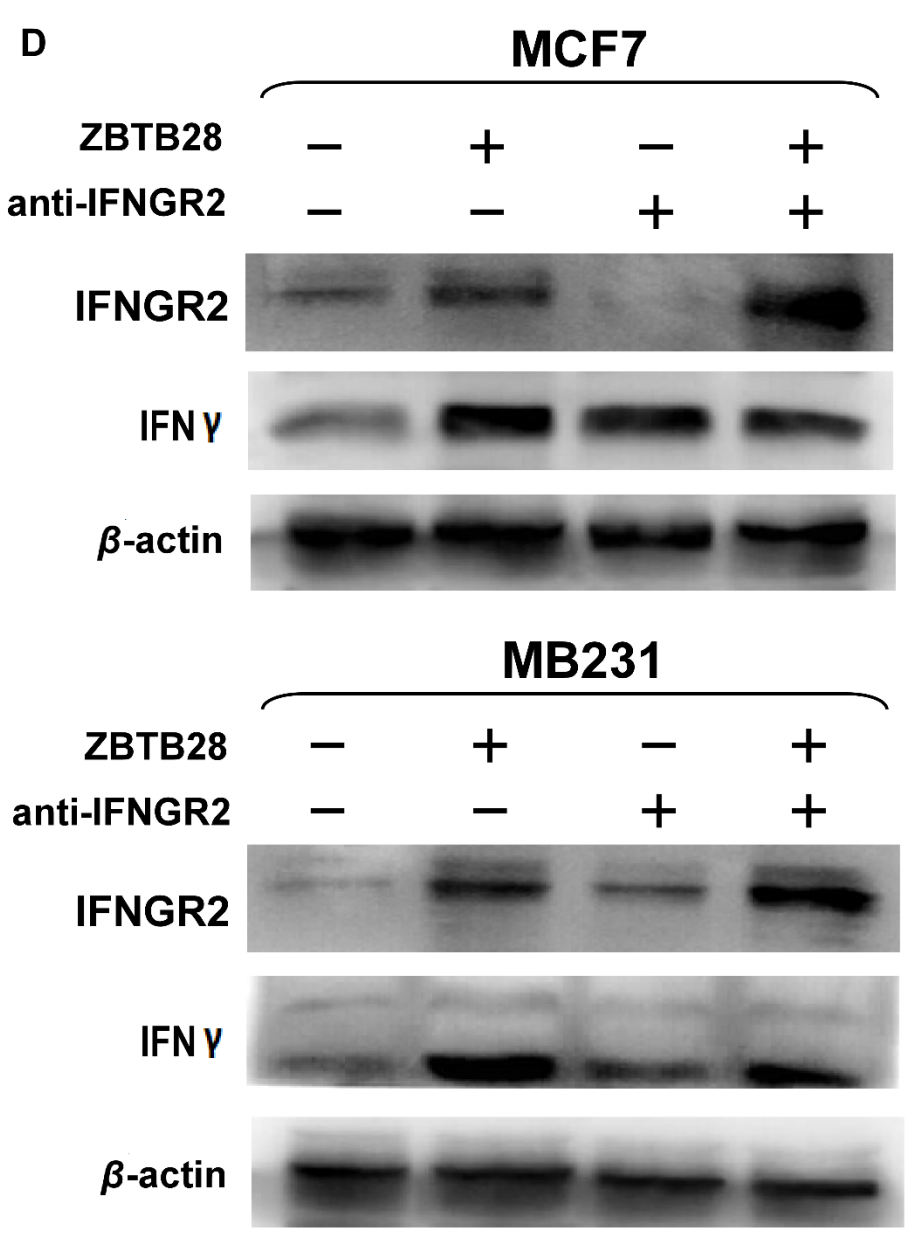
3.0
2.0
1.0
0
***
**
MCF7
MB231


D
MCF7
ZBTB28
anti-IFNGR2
IFNGR2
IFNγ
β-actin
MB231
ZBTB28
anti-IFNGR2
IFNGR2
IFNγ
β-actin

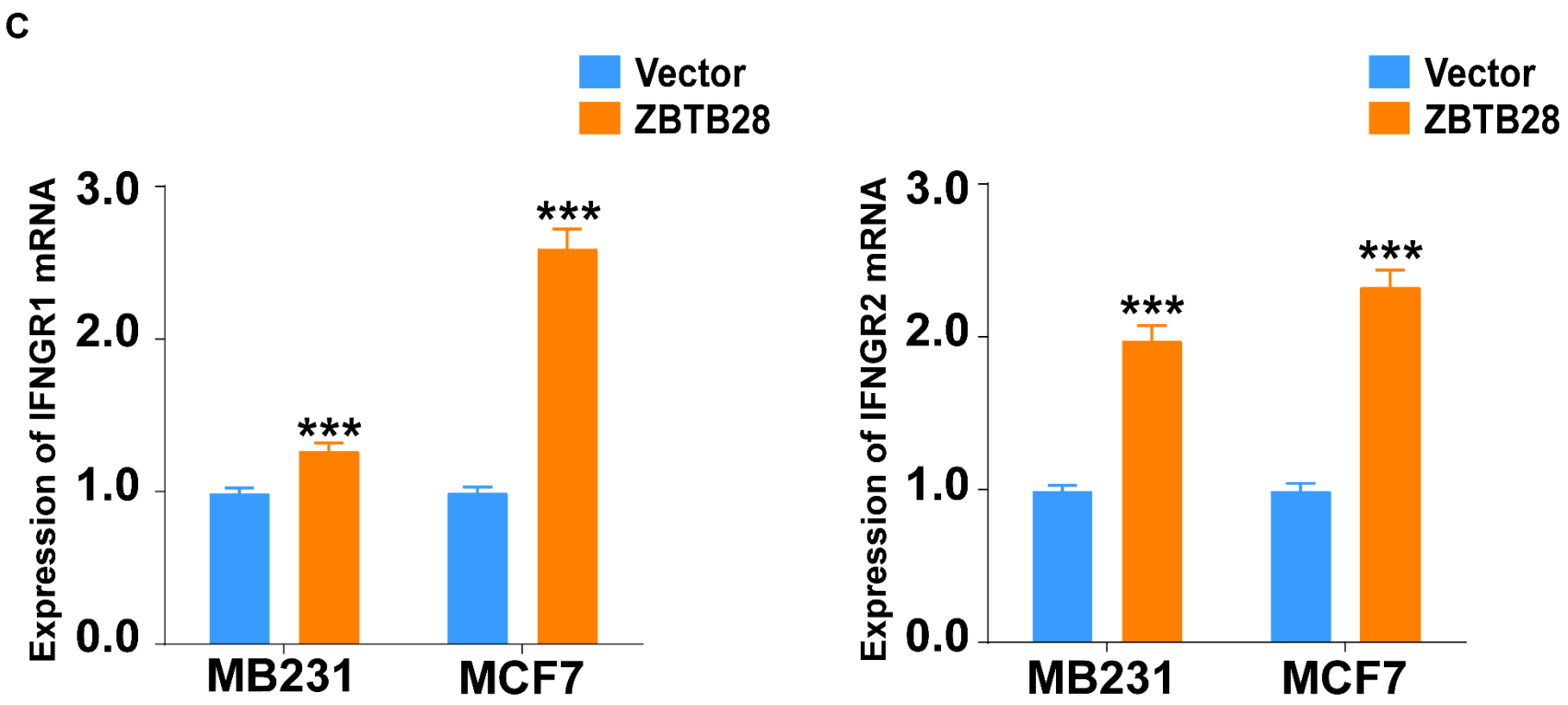
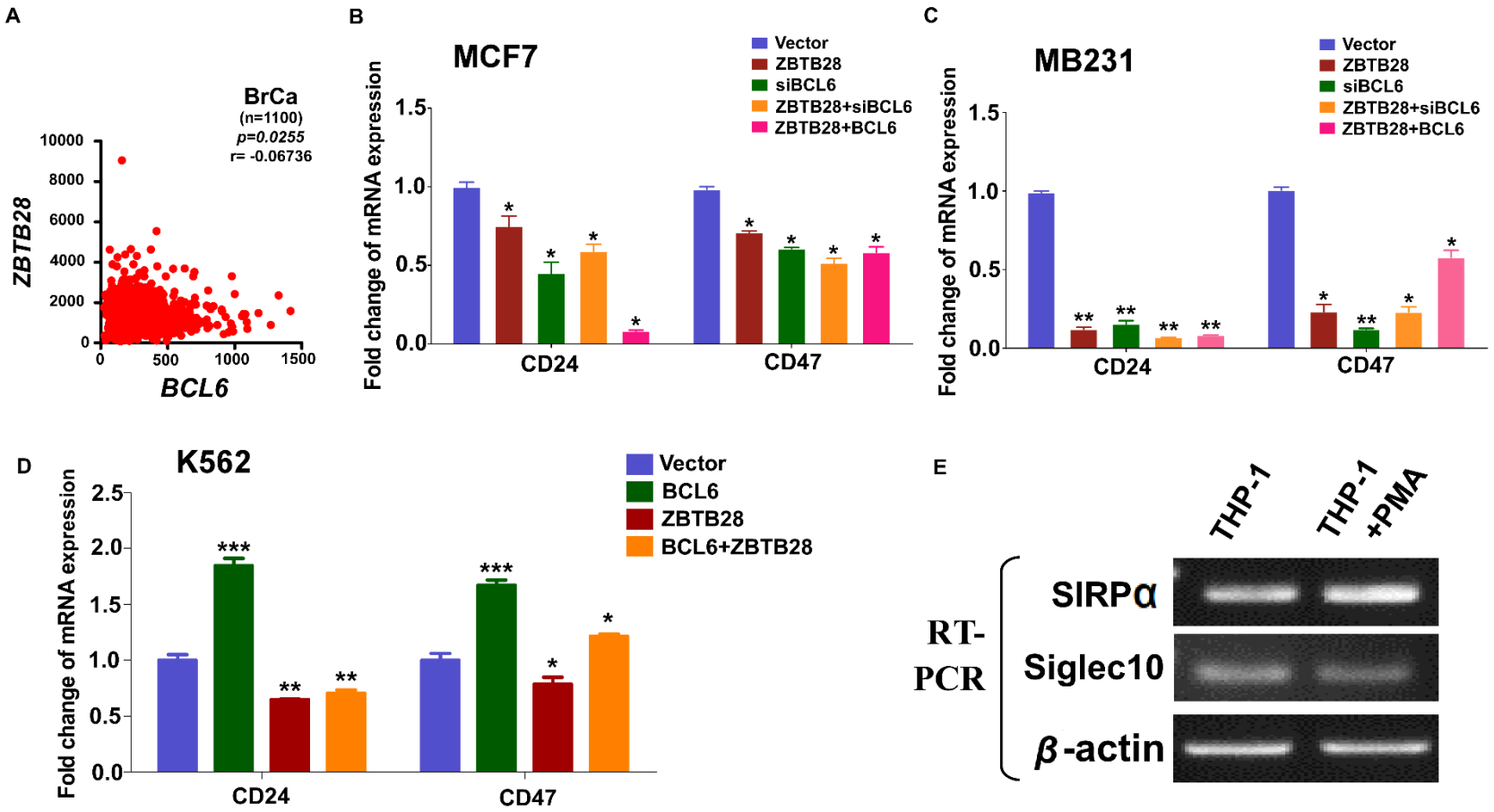

C
Vector
ZBTB28
Expression of IFNGR1 mRNA
3.0
2.0
1.0
0.0
***
***
MB231
MCF7
Vector
ZBTB28
Expression of IFNGR2 mRNA
3.0
2.0
1.0
0.0
***
***
MB231
MCF7

Figure S5.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p=0.0255
r= -0.06736
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BCL6
B
MCF7
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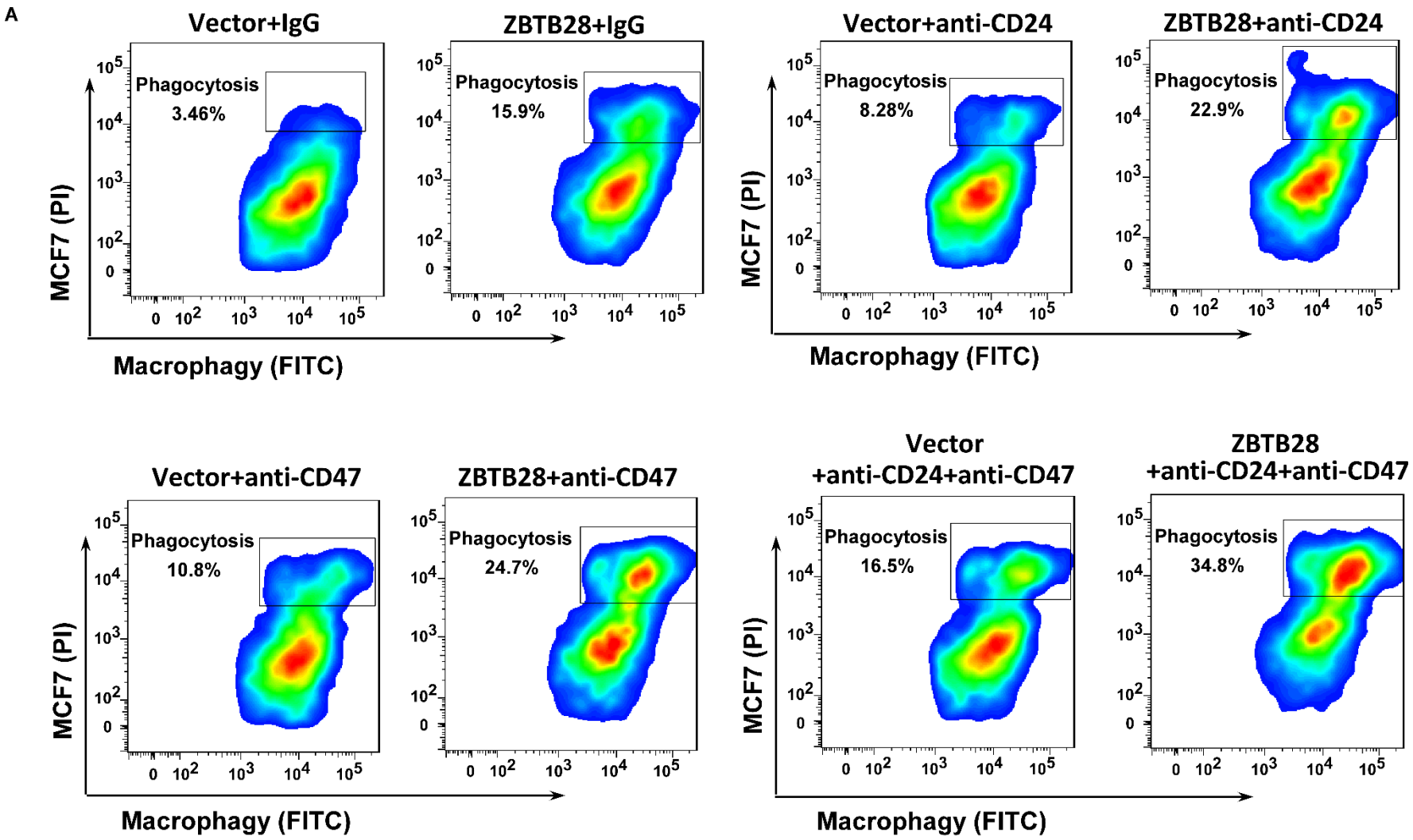
siBCL6
ZBTB28+siBCL6
ZBTB28+BCL6
Fold change of mRNA expression
CD24
CD47
C
MB231
D
K562
Vector
BCL6
ZBTB28
BCL6+ZBTB28
E
THP-1
THP-1 +PMA
RT-PCR
SIRPα
Siglec10
β-actin

Figure S6.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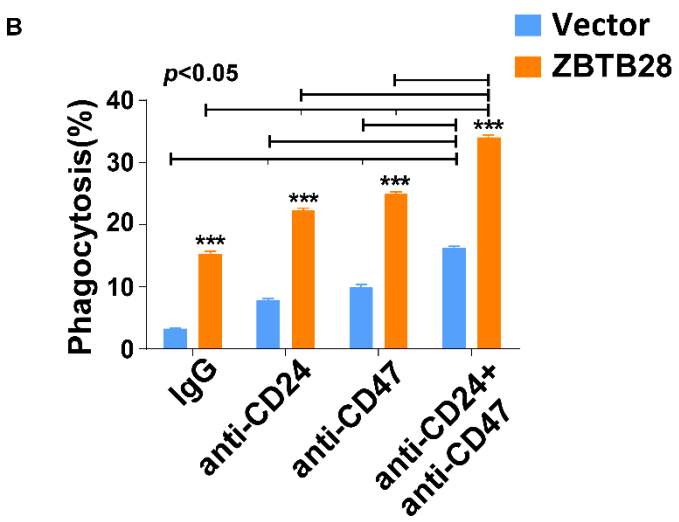
ZBTB28+IgG
Vector+anti-CD24
ZBTB28+anti-CD24
Vector+anti-CD47
ZBTB28+anti-CD47
Vector
+anti-CD24+anti-CD47
ZBTB28
+anti-CD24+anti-CD47
Phagocytosis
3.46%
15.9%
8.28%
22.9%
10.8%
24.7%
16.5%
34.8%
MCF7 (PI)
Macrophagy (FITC)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Vector
ZBTB28
p<0.05
Phagocytosis(%)
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IgG
anti-CD24
anti-CD47
anti-CD24+
anti-CD47

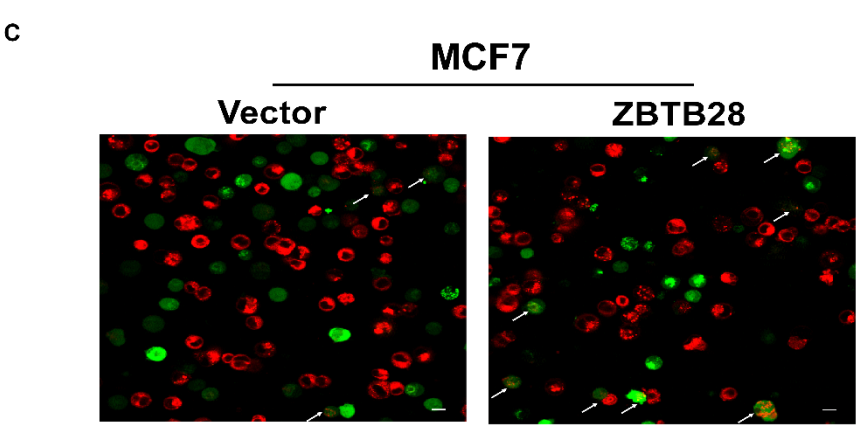
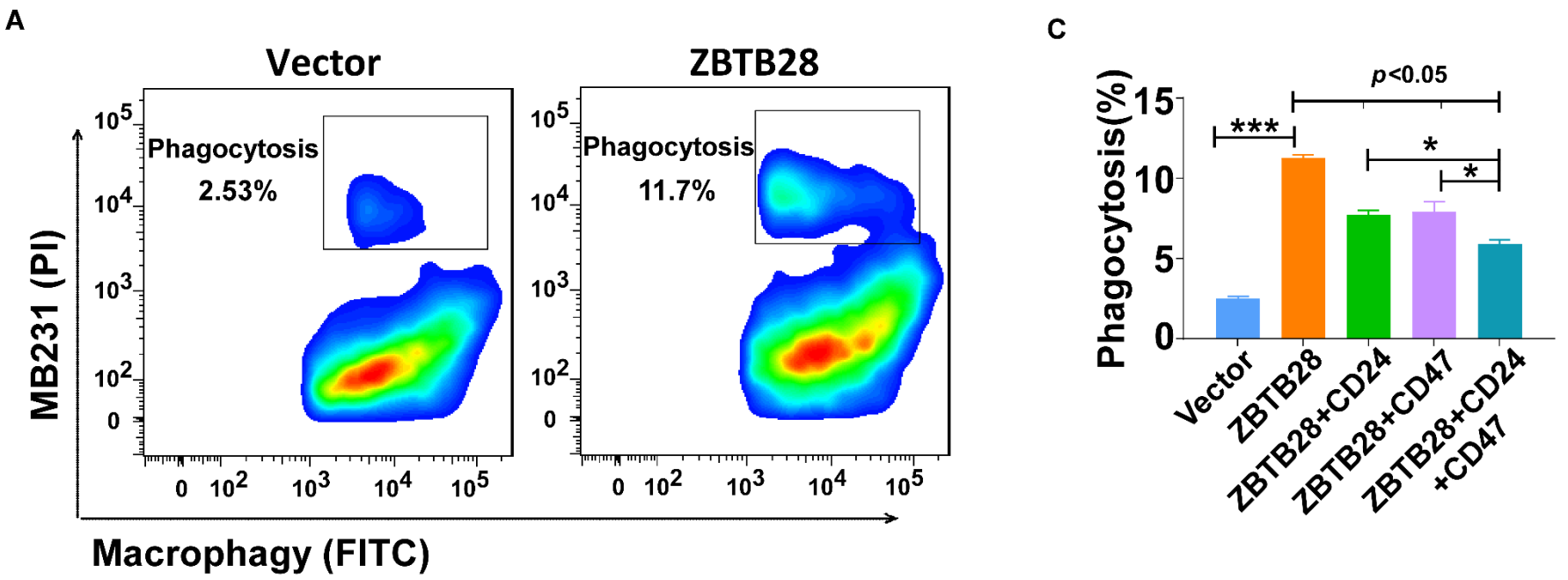

C
MCF7
Vector
ZBTB28

Figure S7.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Vector
ZBTB28
Phagocytosis
2.53%
Phagocytosis
11.7%
MB231 (PI)
Macrophagy (FITC)
C
p<0.05
***
*
*
Phagocytosis(%)
Vector
ZBTB28
ZBTB28+CD24
ZBTB28+CD47
ZBTB28+CD24+CD47
B
ZBTB28+CD24
ZBTB28+CD47
ZBTB28+CD24+CD47
Phagocytosis
8.33%
Phagocytosis
7.94%
Phagocytosis
6.14%
MB231 (PI)
Macrophagy (FITC)