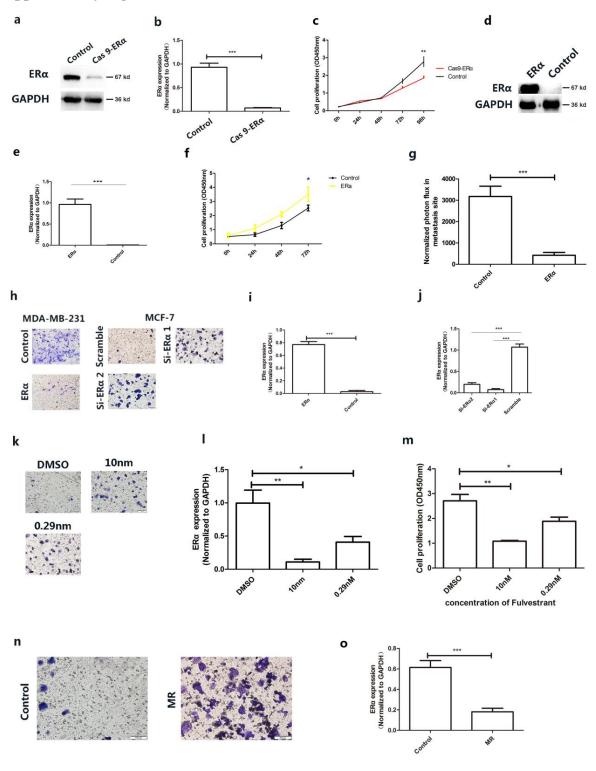
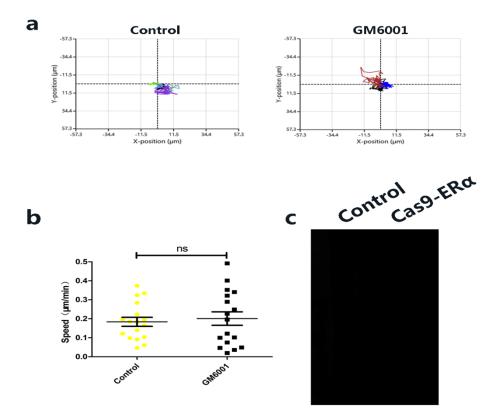
#### **Supplementary Figures:**



**Supplementary Figure 1** 

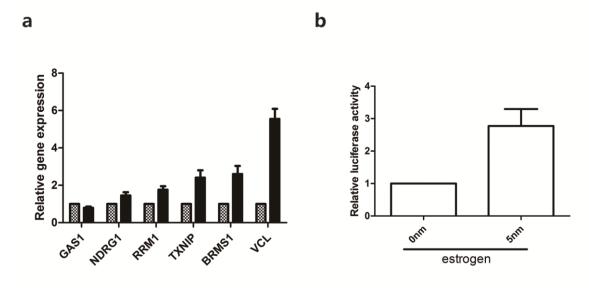
Supplementary Figure 1: ERα inhibits breast cancer metastasis *in vivo* and *in vitro*. Related to figure 2. (a) Western blot assay for detecting the expression levels

of ERa in control and Cas9-ERa MCF-7 cells. (b) Quantification of ERa expression in control and Cas9-ERa MCF-7 cells normalized to GAPDH (n=3). (c) Cell proliferation assay for detecting the proliferative capability of control and Cas9-ERa MCF-7 cells (n=3). (d) Western blot assay for detecting the expression levels of ER $\alpha$ in MDA-MB-231-luc2-vector or MDA-MB-231-luc2-ERa cells. (e) Quantification of ER $\alpha$  expression in the two cell lines normalized to GAPDH (n=3). (f) Cell proliferation assay for detecting the proliferative capability of control and ER $\alpha$ -expressing MDA-MB-231 cells (n=3). (g) Luciferase counts at the metastasis sites of mice in Figure 2c at week 4. (h) A transwell assay was performed to determine the effect of ERa on cell invasion by gain or loss of ERa in MDA-MB-231 or MCF-7 cells. (i, j) Quantification of the ERa expression in MDA-MB-231 or MCF-7 cells from Figure 2g (n=3). (k) A transwell assay was performed to determine the effect of fulvestrant on the invasive capability of MCF-7 cells. (I) Quantification of ER $\alpha$  expression in MCF-7 cells treated with different concentrations of fulvestrant (n=3). (m) Cell proliferation assay (96 h) for detecting the proliferative capability of MCF-7 cells treated with different concentrations of fulvestrant (n=3). (n) A transwell assay was performed to determine the invasive capability of parental MCF-7 cells and MR cells. (o) Quantification of ER $\alpha$  expression in MCF-7 cells and MR cells (n=3). (**b**, **c**, **e**, **f**, **g**, **i**, **j**, **l**, **m**, **o**) Graphs show mean  $\pm$  s.e.m. \**P*<0.05 \*\**P*<0.01\*\*\**P*<0.001. (**b**, **c**, **e**, **f**, **g**, **i**, **o**) Unpaired *t*-test; (**j**, **l**, **m**) ANOVA with Dunnett *t* test.



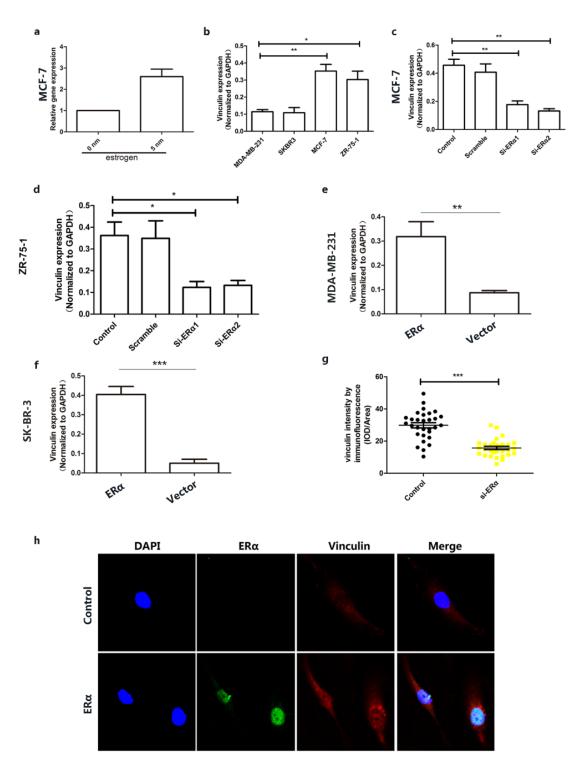
**Supplementary Figure 2** 

Supplementary Figure 2: Loss of ER $\alpha$  induces the amoeboid migration of MCF-7 cells. Related to figure 3. (a) Representative tracks of control MCF-7 cells and GM6001 treated MCF-7 cells (b) The migration speed of control MCF-7 cells and GM6001 treated MCF-7 cells in the 3D matrix (n=17 cells). (c) Conditioned serum-free medium of control or Cas9-ER $\alpha$  MCF-7 cells was collected and used for MMP-2 and MMP-9 activity assessment. (b) Graphs show mean ± s.e.m. ns *P*>0.05 (b) Unpaired *t*-test.



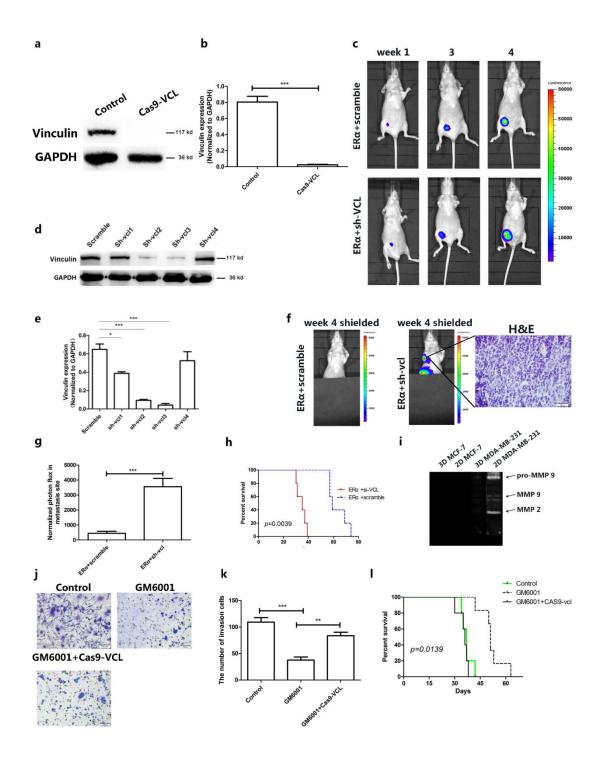
**Supplementary Figure 3** 

**Supplementary Figure 3:** ERα is a transcriptional promoter of vinculin. Related to figure 4. (a) Real-time PCR was performed to identify gene expression. The main metastasis-associated genes from RNA-seq sequencing were verified by Real-time PCR assay. (b) Luciferase activity of vinculin promoter was measured in MCF-7 cells that were treated with 0nm or 5nm estrogen.



**Supplementary Figure 4** 

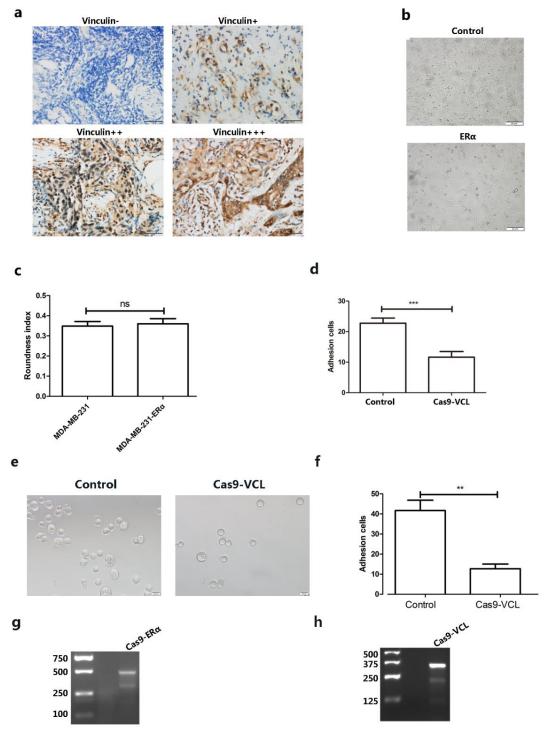
**Supplementary Figure 4: ERα up-regulates the expression of vinculin in breast cancer cells. Related to figure 5. (a)** Real-time PCR detecting the transcription levels of vinculin in MCF-7 cells that were treated with 0nm or 5nm estrogen. The results were normalized to GAPDH (n=3). (b) Quantification of vinculin expression in four breast cancer cell lines normalized to GAPDH (n=3). (c) Quantification of vinculin expression in MCF-7 cells normalized to GAPDH (n=3). (d) Quantification of vinculin expression in ZR-75-1 cells normalized to GAPDH (n=3). (e) Quantification of vinculin expression in MDA-MB-231 cells normalized to GAPDH (n=3). (f) Quantification of vinculin expression in SK-BR-3 cells normalized to GAPDH (n=3). (g) Quantification of vinculin expression levels from confocal images of fig.5f (n = 30 cells). (h) Confocal assay for ER $\alpha$  localization and vinculin expression in MDA-MB-231 (control or ER $\alpha$ -overexpressing) cells. (a, b, c, d, e, f, g) Graphs show mean ± s.e.m. \**P*<0.05 \*\**P*<0.01 \*\*\**P*<0.001. (b, c, d) ANOVA with Dunnett *t* test; (e, f, g) Unpaired *t*-test.





Supplementary Figure 5: Vinculin, downstream of ER $\alpha$ , is important for breast cancer metastasis. Related to figure 6. (a) Western blot assay for detecting the expression of vinculin in control or Cas9-VCL MCF-7-Luc2 cells. (b) Quantification of vinculin expression normalized to GAPDH (n=3). ER $\alpha$ -overexpressing

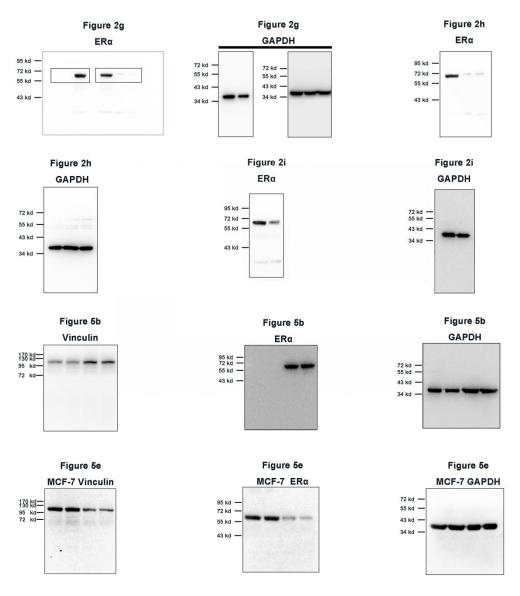
MDA-MB-231-luc2 cells infected with lentivirus containing vinculin sh-RNA (sh-vcl) or scrambled RNA (scramble) were injected into nude mice to generate xenografts (n=5). (c) Bioluminescence imaging at different time points was used to evaluate tumor progression. (d) Western blot assay for testing the interference efficiency of vinculin shRNAs. (e) Quantification of vinculin expression normalized to GAPDH (n=3). (f) Representative images of the scramble group or the sh-vcl group at week 4 are shown after shielding the primary tumor. The lymphatic metastases were determined by H&E staining. (g) Luciferase counts in the metastasis sites of (c) at week 4. (h) The lifetime of mice injected with scramble or sh-vcl cells. (i) Conditioned serum-free 2D or 3D medium of MDA-MB-231 cells was collected and used for MMP-2 and MMP-9 activity assessment; that of MCF-7 cells was used as a negative control. (j) A transwell assay was performed in MDA-MB-231 cells, GM6001-treated MDA-MB-231 cells GM6001-treated or and CRISPR/Cas9-mediated VCL deleted MDA-MB-231 cells. (k) Quantification of invasive cells from (j) (n=3). (l) The lifetime of mice injected with control, GM6001-treated control or Cas9-vinculin MDA-MB-231-luc2 cells. (b, e, g, k) Graphs show mean  $\pm$  s.e.m. \**P*<0.05 \*\**P*<0.01 \*\*\**P*<0.001. (**b**, **g**) Unpaired *t*-test; (**e**) ANOVA with Dunnett t test; (k) ANOVA with Tukey's post hoc test; (h, l) Log-rank test.



**Supplementary Figure 6** 

**Supplementary Figure 6: (a)** Immunohistochemistry was performed using a specific antibody against vinculin. Representative images of vinculin expression levels were shown. Scale bars represent 50  $\mu$ m (40×) (**b**) Optical microscope was used to observe

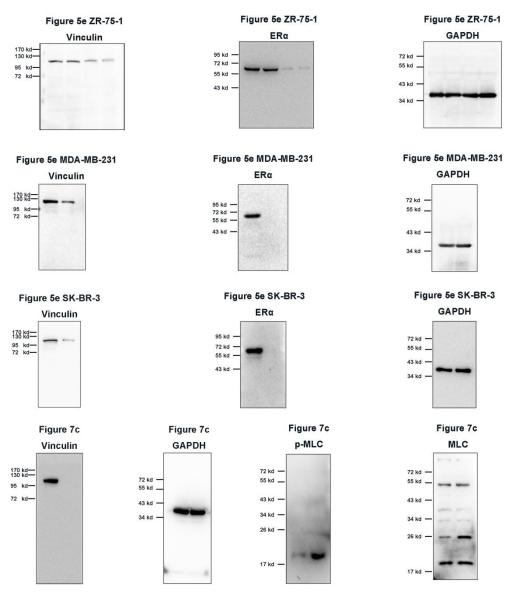
the morphology of MDA-MB-231 cells or MDA-MB-231-ER $\alpha$  in 2D substrate. Scale bar represents 50 µm (40×). (c) Cell morphology (roundness index) of control or ER $\alpha$ -expressing MDA-MB-231 cells (n=100 cells). (d) Quantification of breast cancer cell adhesion to Matrigel after vinculin deletion (n=3). (e) Representative images of control MCF-7 cells and Cas9-vinculin MCF-7 cells adhering to E-cadherin-coated substrates from optical microscope. Scale bars represent 20 µm (f) Quantification of MCF-7 cells adhering to E-cadherin-coated substrates (n=3). (g) Validation of the sgRNA directed against the exon of *ESR1* gene using the Knockout and Mutation Detection Kit. (h) Validation of the sgRNA directed against the exon of *VCL* gene using the Knockout and Mutation Detection Kit. (c, d, f) Graphs show mean  $\pm$  s.e.m. ns *P*>0.05, \*\* *P*<0.01, \*\*\**P*<0.001; (c, d, f) Unpaired *t*-test.

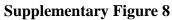


**Supplementary Figure 7** 

Supplementary Figure 7: Original full scans of Western blots related to respective

figures as indicated.





Supplementary Figure 8: Original full scans of Western blots related to respective

figures as indicated.

### Supplementary Tables:

# Supplementary Table 1 The expression level of ERa in primary tumor and lymph node metastasis.

ERa Expression level	-	+	++	+++
Primary tumor	0	35	49	40
Lymphatic metastasis	68	21	18	17

Variables	Total	Loss of ERa in ly	ymphatic metastasis	P-value
		+	-	
	N=124	N=68	N=56	
Age(year)				
>50	68	32	36	0.055
$\leq 50$	56	36	20	
Total	124	68	56	
Tumor size				
>2 cm	68	36	32	0.640
$\leq 2 \text{ cm}$	56	32	24	
Total	124	68	56	
Clinical stages				
AJCC I	20	6	14	0.007
AJCC II	53	28	25	
AJCC III	51	34	17	
Total	124	68	56	
No.of node metastasis				
N1: 1 to 3	73	33	40	0.011
N2: 4 to 9	37	25	12	
N3:≥10	14	10	4	
Total	124	68	56	
PR status				
PR+	96	48	48	0.045
PR-	28	20	8	
Total	124	68	56	
HER2 status				
HER2+	92	52	40	0.523
HER2-	32	16	16	
Total	124	68	56	

# Supplementary Table 2 Association between loss of ERa expression and breast carcinoma characteristics

Statistical analysis of "Age, Tumor size, PR status, HER2 status" was performed with the chi-square test; Statistical analysis of "Clinical stages, No.of node metastasis" was performed with the Wilcoxon rank sum test.

Symbol	GeneID	Locus	FPKM	FPKM	fold_change (T	p_value	fdr	Full Name	Biological	Cellular	Molecular
			(T1+T2/2)	((C1+C2/2)	/ S)				_Process	_Component	_Function
GAS1	2619	chr9:869	3.4752	1.3227	2.627353141	0.0093653	0.999958	growth	GO0000019: negative	GO:0000033:	GO0000033:
		44362-86						arrest-specific	regulation of protein processing	Plasmid membrane	negative
		947189						1	GO0000019: cellular		regulation of
									response to vascular endothelial		mitotic cell
									growth factor stimulus		cycle  GO000
									GO0000019: regulation of ER		0037: cell
									to Golgi vesicle-mediated		cycle arrest
									transport		
NDRG1	10397	chr8:134	13.0272	4.9679	2.622275006	0.0080597	0.999958	N-myc	GO:0010038:response to metal	GO:0005634:nucleu	GO:0005515:
		249413-1						downstream	ion    GO:0030330:DNA	s	protein
		3430954						regulated 1	damage response, signal	GO:0005737:cytopl	binding
		7							transduction by p53 class	asm	GO:0008017:
									mediator	GO:0005813:centros	microtubule
									GO:0032287:peripheral	ome	binding
									nervous system myelin	GO:0005829:cytosol	GO:0017137:
									maintenance	Ш	Rab GTPase
									GO:0045576:mast cell	GO:0005874:microt	binding
									activation	ubule	GO:0043015:
									GO:0071456:cellular response	GO:0005886:plasma	gamma-tubuli
									to hypoxia	membrane	n binding $\parallel$
									GO:0090232:positive	GO:0005913:cell-ce	GO:0045296:
									regulation of spindle	ll adherens junction	cadherin

Supplementary Table 3 The main significantly altered metastasis-associated genes in RNA-seq

									checkpoint		binding
										GO:0015630:microt	
										ubule cytoskeleton	
										GO:0048471:perinu	
										clear region of	
										cytoplasm	
										GO:0055038:recycli	
										ng endosome	
										membrane	
RRM1	6240	chr11:41	59.7513	18.3331	3.259203299	0.0238978	0.999958	ribonucleotid	GO:0006260:DNA replication	GO:0005654:nucleo	GO:0004748:r
		15923-41						e reductase	GO:0006260:DNA replication	plasm	ibonucleoside-
		60106						M1	GO:0009263:deoxyribonucleoti	GO:0005829:cytosol	diphosphate
									de biosynthetic process		reductase
									GO:0015949:nucleobase-contai	GO:0005971:ribonu	activity,
									ning small molecule	cleoside-diphosphat	thioredoxin
									interconversion	e reductase complex	disulfide as
									GO:0044281:small molecule		acceptor
									metabolic process		GO:0004748:r
									GO:0051290:protein		ibonucleoside-
									heterotetramerization		diphosphate
									GO:0055086:nucleobase-contai		reductase
									ning small molecule metabolic		activity,
									process		thioredoxin
											disulfide as
											acceptor
											GO:0005515:

											protein
											binding
											GO:0005524:
											ATP binding
TXNIP	10628	chr1:145	119.403	38.3675	3.112087053	0.0116484	0.999958	thioredoxin	GO:0000122:negative	GO:0005634:nucleu	GO:0004857:
		438461-1						interacting	regulation of transcription from	s	enzyme
		4544262						protein	RNA polymerase II promoter	GO:0005737:cytopl	inhibitor
		8							GO:0006351:transcription,	asm	activity
									DNA-dependent	GO:0005758:mitoch	GO:0005515:
									GO:0006606:protein import	ondrial	protein
									into nucleus    GO:0007049:cell	intermembrane	binding
									cycle    GO:0009612:response	space	GO:0031625:
									to mechanical stimulus	GO:0005829:cytosol	ubiquitin
									GO:0009749:response to		protein ligase
									glucose stimulus		binding
									GO:0030216:keratinocyte		
									differentiation		
									GO:0032355:response to		
									estradiol stimulus		
									GO:0032570:response to		
									progesterone stimulus		
									GO:0035872:nucleotide-bindin		
									g domain, leucine rich repeat		
									containing receptor signaling		
									pathway		
									GO:0042127:regulation of cell		

RMS1     2855     ch1166     47.023     3.9689     11.84819471     0.00877     0.999958     hreat carce     60.00043051;nanoprino     sill     ordining       RMMS1     2855     ch11256     47.023     3.9689     11.84819471     0.008077     0.999958     hreat carce     60.00043051;nanoprino     sill     ordining       RMMS1     104803-6     6112582     47.023     3.9689     11.84819471     0.008077     0.999958     hreat carce     60.00043051;nanoprino     60.0005173;rapprino     ordining       RMMS1     ch11258     47.0243     3.9689     11.84819471     0.008077     0.999958     hreat carce     60.000631;ranoprino     60.0005073;rcput     ordining     GO:000573;rcput     binding       RMMS1     104803-6     6112582     47.0243     3.9689     11.84819471     0.008075     prest carce     GO:000631;ranoprino     GO:000573;rcput     binding       GO:000512:apoptici proces     ch1166     6112582     47.0243     3.9689     11.84819471     A     A     A     A     A     Prestararara     GO:00067												
BRMS1     2855     ch11:66     47.0243     3.9689     11.84819471     0.0080737     0.999958     braca cancer     GO-0002551transcription, ergulation of accor activity II     GO-0002551transcription, GO-000251transcription, ergulation of response II     GO-0002551transcription, GO-000251transcription, ergulation of cell division II     GO-000251transcription, ergulation of cell division II     GO-000251transcription, GO-000251transcription, ergulation of cell division II     GO-0005251transcription, ergulation of cell division II     SI     MO-000551transcription, ergulation of cell division II     Forein       BRMS1     104803-6     6112582     SI     SI     SI     Forein       GO-000521transcription, ergulation of K-kappaB     sI     SI     Forein     SI     Forein       GO-000551transcription, ergulation of Tk-kappaB     sI     Forein     SI     Forein       GO-000551transcription, ergulation of Tk-kappaB     si     Forein     Forein										proliferation		
BRMS1     2855     Harla     47.0243     3.9689     11.8481947     0.0080737     0.999958     imentantiania										GO:0042493:response to drug		
BRMS1     2855     chrl166     47.0243     3.9689     11.84819471     0.0080737     0.999958     breast cancer     GO:0005151:proseptiotic process     GO:0005051:proseptiotic       BRMS1     104803-6     6112582     47.0243     3.9689     11.84819471     0.0080737     0.999958     breast cancer     GO:0005151:proseptiotic process     growth factor receptor     signaling     calcium ion        calcium ion    <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>GO:0042542:response to</td> <td></td> <td></td>										GO:0042542:response to		
BRMS1     25855     chrl166     47.0243     3.9689     11.84819471     0.0080737     0.999958     preast-carcer     GO:0005032rmage-carcer     60:0005737rmage-carcer										hydrogen peroxide		
BRMS1     25855     chr11:66     47.0243     3.9689     11.84819471     0.0080737     0.099958     break cancel     GO:00045087:Innact immune     signaling pathway        GO:0005159:response to     calcium ion										GO:0043065:positive		
BRMS1     25855     chr11:66     47.0243     3.9689     11.84819471     0.0080737     0.99958     breast cancer     GO:00045015:1ranscription, do:0005157:response to calcium ion    GO:00051722:resplatelet-derived     s:       90000531:rranscription, do:0000531:rranscription, do:000531:rranscri										regulation of apoptotic process		
BRMS1     2585     chr11:66     47.0243     3.9689     11.84819471     0.0080737     0.999958     breat cancer     GO:00050512:response to calcium ion    GO:0051128:cellular response to tumor cell     GO:00050531:response to calcium ion    GO:0007128:cellular response     GO:00050531:response to calcium ion    GO:0007128:cellular response     GO:00050531:response to calcium ion    GO:00051552:response to calcium ion    GO:00051782:response to calcium ion    GO:0005051: mital setting inding    GO:0005051: metatatais     GO:0005054:										GO:0045087:innate immune		
BRMS1     2585     chr11:66     47.0243     3.9689     11.84819471     0.0080737     0.999958     beast carcer     G0:00051512;nesponse to calcium ion    G0:007122;scellular response to tumor cell     G0:0005631:transcription, G0:000531:transcription, to tumor cell     G0:0005634:muclen Suppressor 1     G0:0005737:cytop     Breast carcer     G0:0006351:transcription, G0:0005737:cytop     G0:0005737:cytop     Binding    G0:001573:citopapatic transcription factor activity    G0:0032088:negative regulation of NF-kappaB     s    Frank     South factor receptor     Sime 1     Sime 1 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>response   </td> <td></td> <td></td>										response		
BRMS1     2585     chr11:66     47.0243     3.9689     11.84819471     0.0080737     0.999958     breast cancer     GO:0005152:response to calcium ion    GO:0051782:negative regulation of cell division    GO:0071228:cellular response     GO:000534:nucle     GO:0005152:response to calcium ion    GO:0071228:cellular response       BRMS1     25855     chr11:66     47.0243     3.9689     11.84819471     0.0980737     0.999958     breast cancer     GO:000631:transcription, metastasis     GO:000503:transcription, GO:000503:transcription, inding    GO:0032088:negative regulation of NF-kappaB     s       optioning    GO:0051052:       NF-kappaB     inding    GO:004582:negative regulation of transcription, GO:004582:negative regulation of transcription, GO:0054592:negative regulation of transcription, GO:0054592:negative regulation of transcription, GO:0051052:     MF-kappaB										GO:0048008:platelet-derived		
BRMS1     25855     chr11:66     47.0243     3.9689     11.84819471     0.0080737     0.999958     breast cancer interstant     GO:00051592:response to calcium ion    GO:00051782:negative regulation of cell division    GO:000531:transcription,     GO:0005031:transcription,     GO:0005031:										growth factor receptor		
BRMS1   25855   chr11:66   47.0243   3.9689   11.84819471   0.0080737   0.999958   breast cancer   GO:0005173:negative regulation of cell division      60:0005737:cegative regulation of NF-kappaB   s      protein     BRMS1   Lister of the cegative regulation of NF-kappaB   inding      GO:0005737:cegative regulation of NF-kappaB   smart   GO:00051059:     BRMS1   Lister of the cegative regulation of th										signaling pathway		
Image: series of the series										GO:0051592:response to		
Image: state   Image: state <th< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>calcium ion   </td><td></td><td></td></th<>										calcium ion		
Image: state   Image: state <th< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>GO:0051782:negative</td><td></td><td></td></th<>										GO:0051782:negative		
Image: Normal Single										regulation of cell division		
BRMS1   25855   chr11:66   47.0243   3.9689   11.84819471   0.0080737   0.999958   breast cancer   GO:0006351:transcription,   GO:0005634:nucleu   GO:0005634:nucleu   protein     104803-6   6112582   6112582   F <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>GO:0071228:cellular response</td><td></td><td></td></td<>										GO:0071228:cellular response		
104803-6   104803-6   s     protein     6112582   6112582   s     s     protein     104803-6   12582   104803-6   s     s     protein     104803-6   12582   104803-6   104803-6   s     s     protein     104803-6   12582   104803-6   104803-6   104803-6   104803-6   suppressor1   GO:0006915:apoptotic process   GO:0005737:cytopl   binding        104803-6   1449										to tumor cell		
6112582   6112582     6112582   6112582     6112582   6112582     6112582   6112582     6112582   6112582     6112582   6112582     6112582   6112582     6112582   Figure 1     6112582   Figure 1 <	BRMS1	25855	chr11:66	47.0243	3.9689	11.84819471	0.0080737	0.999958	breast cancer	GO:0006351:transcription,	GO:0005634:nucleu	GO:0005515:
Image: Construction of the second			104803-6						metastasis	DNA-dependent	s	protein
Image: state stat			6112582						suppressor 1	GO:0006915:apoptotic process	GO:0005737:cytopl	binding
Image: Second										GO:0032088:negative	asm	GO:0051059:
Image: Second										regulation of NF-kappaB		NF-kappaB
GO:0045892:negative regulation of transcription,												
regulation of transcription,												Ũ
										_		
										DNA-dependent		

									GO:0090312:positive		
									regulation of protein		
									deacetylation		
									GO:2000210:positive		
									regulation of anoikis		
VCL	7414	chr10:75	35.7643	12.5256	2.855296353	0.00804989	0.999952	vinculin	GO:0002009:morphogenesis of	GO:0001725:stress	GO:0002162:
	,	757871-7	5517615	1210200	2.000270000	0100001707	0.77702	, mounn	an epithelium	fiber	dystroglycan
		5879914							GO:0002576:platelet	GO:0005576:extrace	binding
		5077714							degranulation	llular region	GO:0003779:
									GO:0006928:cellular	GO:0005829:cytosol	actin binding
										U0.0003829.cytosof	GO:0005198:s
									component movement		
									GO:0006936:muscle	GO:0005856:cytosk	tructural
									contraction    GO:0007155:cell	eleton	molecule
									adhesion	GO:0005884:actin	activity
									GO:0007160:cell-matrix	filament	GO:0005515:
									adhesion    GO:0007596:blood	GO:0005886:plasma	protein
									coagulation	membrane	binding
									GO:0030032:lamellipodium	GO:0005911:cell-ce	GO:0008013:
									assembly	ll junction	beta-catenin
									GO:0030168:platelet activation	GO:0005912:adhere	binding
									GO:0030336:negative	ns junction	GO:0017048:
									regulation of cell migration	GO:0005913:cell-ce	Rho GTPase
									GO:0034333:adherens junction	ll adherens junction	binding
									assembly	- 	GO:0045294:
									GO:0034394:protein	" GO:0005916:fascia	alpha-catenin
									localization to cell surface	adherens	binding
											onung II

				GO:0043297:apical junction	GO:0005925:focal	GO:0045296:
				assembly	adhesion	cadherin
				GO:0090136:epithelial cell-cell	GO:0005925:focal	binding
				adhesion	adhesion	
					GO:0030055:cell-su	
					bstrate junction	
					GO:0043034:costam	
					ere	
					GO:0043034:costam	
					ere	
					GO:0043234:protein	
					complex	

			Vinculin								
	Primary tumor	-	+	++	+++	Total					
	+	5	19	8	3	35					
ED	++	1	17	26	5	49					
ERα	+++	1	5	9	25	40					
	Total	7	41	43	33	124					

# Supplementary Table 4 The expression level of ERa was positively correlated with the vinculin expression level in the primary tumor

Spearman rank correlation analysis was used. (P < 0.001,  $R^2 = 0.528$ )

## Supplementary Table 5 The expression level of ERa was positively correlated with the vinculin expression level in the lymph node metastasis

			Vinculin							
	lymphatic metastasis	-	+	++	+++	Total				
	-	17	28	13	10	68				
	+	3	13	4	1	21				
ERα	++	0	3	11	4	18				
	+++	1	3	3	10	17				
	Total	21	47	31	25	124				

Spearman rank correlation analysis was used. (P < 0.001,  $R^2 = 0.366$ )

ID	sense ( 5'-3' )	antisense ( 5'-3' )
ESR1-homo-1	5' CAGGCCAAAUUCAGAUAAUTT 3'	5' AUUAUCUGAAUUUGGCCUGTT 3'
ESR1-homo-2	5' GGUCCACCUUCUAGAAUGUTT 3'	5' ACAUUCUAGAAGGUGGACCTT 3'
ESR1-homo-3	5' GAGGGAGAAUGUUGAAACATT 3'	5' UGUUUCAACAUUCUCCCUCTT 3'
Negative control	5' UUCUCCGAACGUGUCACGUTT 3'	5' ACGUGACACGUUCGGAGAATT 3'

### Supplementary Table 6 The sequence of ERa siRNAs

### Supplementary Table 7 The sequence of vinculin shRNAs

ID	5'	stem	loop	stem	3'
VCL-RNAi(1)-a	Ccgg	gcACAGATAAACGGATTAGAA	CTCGAG	TTCTAATCCGTTTATCTGTGC	TTTTTg
VCL-RNAi(1)-b	aattcaaaaa	gcACAGATAAACGGATTAGAA	CTCGAG	TTCTAATCCGTTTATCTGTGC	
VCL-RNAi(2)-a	Ccgg	ccCTGGAAATCAAGCTGCTTA	CTCGAG	TAAGCAGCTTGATTTCCAGGG	TTTTTg
VCL-RNAi(2)-b	aattcaaaaa	ccCTGGAAATCAAGCTGCTTA	CTCGAG	TAAGCAGCTTGATTTCCAGGG	
VCL-RNAi(3)-a	Ccgg	cgGTTGGTACTGCTAATAAAT	CTCGAG	ATTTATTAGCAGTACCAACCG	TTTTTg
VCL-RNAi(3)-b	aattcaaaaa	cgGTTGGTACTGCTAATAAAT	CTCGAG	ATTTATTAGCAGTACCAACCG	
VCL-RNAi(4)-a	Ccgg	gcTCGAGATTATCTAATTGAT	CTCGAG	ATCAATTAGATAATCTCGAGC	TTTTTg
VCL-RNAi(4)-b	aattcaaaaa	gcTCGAGATTATCTAATTGAT	CTCGAG	ATCAATTAGATAATCTCGAGC	

### Supplementary Table 8 The sequence of relative sgRNA

sgRNA	sequence
ESR1	CCATCCCAGATGCTTTGGTG
VCL	CCTGCTCCTTACCTTCGATG

#### Supplementary Table 9 The sequence of primer sets flanking related putative ERα binding sites in the promoter region of vinculin

ID	sense ( 5'-3' )	antisense ( 5'-3' )
Primer 1	5' GTT CAC GCC ATT GTC CTG 3'	5' TTA TCC AGT TCC CTC ACG 3'
Primer 2	5' CAA CCC AAG TCC ATG AGT 3'	5' CTG GGT GTG TAG CCT TCT 3'
Primer 3	5' GTT TAC GTG AAT GGG ACG 3'	5' GTT TGA TGA GAC CAA GGG 3'
Primer 4	5' GAG CAT CTC GAA AAG GGA 3'	5' AGA GAC AGA CTG TGC AGC 3'

#### Supplementary Table 10 The sequence of primer sets for real-time PCR assay

ID	Forward ( 5'-3' )	Reverse ( 5'-3' )
ESR1	5' TCTTGGACAGGAACCAGGGA 3'	5' CAGAGACTTCAGGGTGCTGG 3'
Vinculin	5' CACCGTGAAAGAGTTGCTGC 3'	5' TGGCTTCAGTGTCCTTGCTG 3'
GAPDH	5' GTCAAGGCTGAGAACGGGAA 3'	5' AAATGAGCCCCAGCCTTCTC 3'