

Table S1. Correlation between Notch3 expression and clinicopathological factors in patients with breast cancer

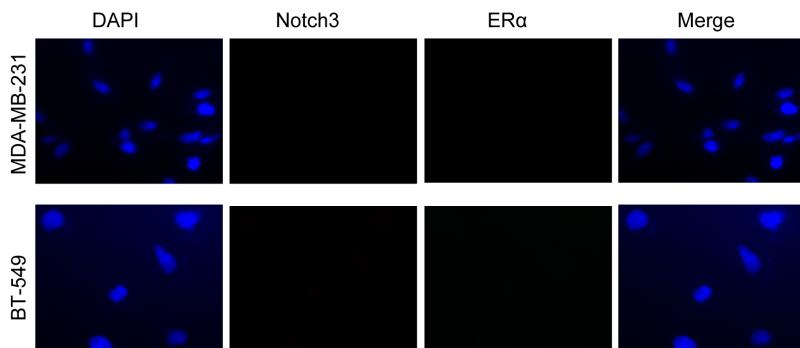
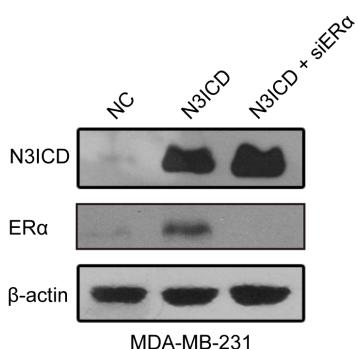
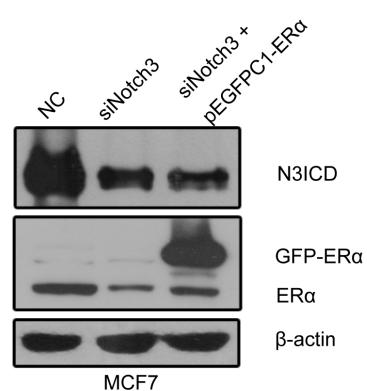
Clinicopathological features	Notch3 (n=60)		χ^2	<i>P</i> value
	Negative (n=17)	Positive (n=43)		
Age (year)				
<50	11	19	2.052	0.252
≥50	6	24		
Menstrual conditions				
premenopausal	11	27	0.019	1
postmenopausal	6	16		
Tumor size (cm)				
<2	0	3	2.144	0.37
≥2, <5	12	24		
≥5	4	15		
LN metastasis				
Negative	2	18	4.966	0.034*
Positive	15	25		
Stage				
I	0	1	1.071	0.871
II	6	20		
III	9	21		
IV	0	1		
ER expression				
Negative	11	13	6.033	0.020*
Positive	6	30		
PR expression				
Negative	12	13	8.163	0.008**
Positive	5	30		
HER-2 expression				
Negative	15	26	3.573	0.111
Positive	2	15		

Table S2. Oligonucleotide sequences for siRNA constructs used in real-time PCR and ChIP and EMSA assays

Assay	Sequences (5' to 3')		Amplicon (bp)
RT-PCR			
Notch3	F ATGCAGGATAGCAAGGAGGA	R AAGTGGTCCAACAGCAGCTT	86
ERα	F CTCTCCCACATCAGGCACA	R CTTTGGTCCGTCTCCTCCA	157
E-cadherin	F AAAGGCCATTTCCTAAAAACCT	R TGCGTTCTCTATCCAGAGGCT	172
Vimentin	F GACGCCATCAACACCGAGTT	R CTTTGTGTTGGTTAGCTGGT	238
β-actin	F GAGACCTTCAACACCCCAGCC	R AATGTCACGCACGATTCCC	264
siRNA			
siNotch3 #1	UAUAGGUGUUGACGCCAUCCACGCA		
siNotch3 #2	GAGCCAAUAAGGACAUGCA		
siERα	CGAGUAUGAUCCUACCAGA		
siNC	UUCUCCGAACGUGUCACGU		
ChIP			
ERαP1	F CAGTTCCCCAGCTGCTAAA	R GGCTTCTCTAAATGTGCTGCCTTA	
ERαP2	F GGGCACATAAGGCAGCACAT	R CCCTGACATTGGCTTAAACATCAC	
NC	F AACTGAGGTCTGGCAGGTT	R CGTTGGCTAGAAATACGTAGTG	
EMSA			
probe or competitor 1 (P1)	AACGAGGAGGGGGAAATCAAACAG		
P1 mutant	AACGAGGAGGTGTCTTCAAACAG		
probe or competitor 1 (P2)	TAAAGTTCAAGGAAAGCTGCTCTTT		
P2 mutant	TAAAGTTCAATAGCGCTGCTCTTT		

Table S3. Proteins and description of corresponding antibodies

Antibodies	Vendor	Source	Catalog NO.
Notch1	CST	Rabbit/mono	3439
Notch2	CST	Rabbit/mono	4530
Notch3	CST	Rabbit/mono	5276
Notch4	CST	Mouse/mono	2423
ER α	CST	Rabbit/mono	8644
E-cadherin	CST	Rabbit/mono	3195
Vimentin	CST	Rabbit/mono	5741
P27	Santa Cruz	Rabbit/mono	3686
β -actin	Santa Cruz	Mouse/mono	Sc-47778
ER α	Santa Cruz	Mouse/mono	Sc-8002
Notch3	Santa Cruz	Rabbit/mono	Sc-5593

A**B****C**

Supplementary Figure 1 (A) Immunofluorescence staining for Notch3 and ER α in MDA-MB-231 (upper panel) and BT-549 (bottom panel) cells. Nuclei were stained with DAPI (4', 6-diamidino-2-phenylindole). A merged image was obtained by combining Notch3, ER α and DAPI fluorescent staining images. **(B)** Western blot showing the protein levels of N3ICD and ER α after overexpressing N3ICD and transfecting ER α RNAi in MDA-MB-231 cells. **(C)** Western blot showing the protein levels of Notch3 and ER α after transfecting Notch3 RNAi and overexpressing pEGFPC1-ER α in MCF-7 cells.