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ORIGINAL RESEARCH

RETRACTED ARTICLE: LINC00894 Enhances the Progression of Breast Cancer by Sponging miR-429 to Regulate ZEB1 Expression

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Purpose: Long non-coding RNAs (lncRNAs) are known to regulate tumorigenesis. Although breast cancer tissues show a high expression of *LINC00894*, its specific biological role in breast cancer progression is still unknown. In this study, lncRNA microarray was used to analyze the lncRNA expression in breast cancer tissues and *LINC00894* was selected for further analysis.
Materials and Methods: Expression of *LINC00894* in 4[illegible] pairs of breast cancer tissues and normal tissues obtained from patients with breast cancer was assessed by quantitative reverse transcription-PCR, while proliferation and invasion of breast cancer cells were assessed using a Cell Counting Kit-8 (CCK-8), EdU assay, colony formation experiment, and trans-well assays. A dual-luciferase reporter gene assay and bioinformatics analysis were employed to detect potential targets of *LINC00894*. Additionally, RNA Binding Protein Immunoprecipitation (RIP) and Western blot assays were utilized to clarify its interaction and roles in the regulation of breast cancer progression.
Results: High expression of *LINC00894* was observed in breast cancer cells, and its over-expression significantly expedited cell proliferation and invasion. Moreover, *LINC00894* positively regulated the expression of *ZEB1* by competitively binding to miR-429.
Conclusion: Taken together, these results suggest that *LINC00894* competitively binds to miR-429 to mediate *ZEB1* expression; consequently, it is implicated to play a role in the progression of breast cancer.
Keywords: lncRNA, ceRNA, proliferation, invasion, breast cancer

Introduction

Breast cancer is not only the most prevalent malignant tumor in females, but also one of the major causes of cancer-related deaths in females, worldwide.[1,2] About 2.1 million newly diagnosed breast cancer cases were reported worldwide in 2018, accounting for almost 1 in 4 cancer cases among women.[1] Early-stage breast cancer responds well to radiation, drug therapy, and surgical intervention.[3] Nevertheless, there is a trend for breast cancer to metastasize in distal organs (eg, the brain, lungs, liver, and bones), and poor prognosis is observed in patients with distal metastasis. Despite progress in systemic chemotherapy, the median survival of patients with metastatic breast cancer is less than two years.[4] Hence, more studies on effective strategies to diagnose breast cancer at an early stage, inhibit its metastasis and predict prognosis, and reduce mortality of breast cancer patients are needed.

Long non-coding RNAs (lncRNAs) are non-coding RNAs composed of over 200 base pairs.[5] Most of these lncRNAs have a poly A tail and are incapable of

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being translated into proteins unlike mRNAs.[6] lncRNAs were identified earlier as "transcriptional noise" or "dark matter" without any biofunctions.[7] However, many dynamically expressed lncRNAs have been identified in whole genome transcriptome analysis, and many of these lncRNAs participate in various biological activities, for example, lincRNA-RoR manages the reprogramming of human induced pluripotent stem cells.[8] *MYOSLID* is a new lncRNA that depends on serum response factors and may amplify the differentiation of vascular smooth muscle cells.[9] lncRNA is a major regulatory factor in the progression of breast cancer. The TEAD4-regulated lncRNA, *MNX1-AS1*, partially enhances breast cancer progression by suppressing BTG2 and activating BCL2.[10] Moreover, the lncRNA *SOX2OT* enhances breast cancer progression by sponging *miR-194-5p* and regulating AKT2.[11] The breast juice lncRNA is utilized as a marker to screen breast cancer.[12] However, the exact role of *LINC00894* in the progression of breast cancer is unclear. Therefore, in this study, lncRNA microarray was used to analyze the lncRNA expression in breast cancer tissues, and *LINC00894* was selected for further analysis.

The results of our study verified that high levels of *LINC00894* were present in both breast cancer cells and tissues. Overexpressed *LINC00894* could accelerate breast cancer cell invasion and proliferation. In brief, *LINC00894* was confirmed to be involved in breast cancer progression through competitive binding to *miR-429* to mediate transcriptional factor Zinc finger E-box binding homeobox 1 (*ZEB1*) expression.

Materials and Methods

Clinical Tissue Samples

Forty-five pairs of breast cancer tissues and normal tissues were obtained from patients with breast cancer confirmed by pathological tests at the Second People's Hospital of Lianyungang City. Each pair of breast cancer tissue and normal tissue was obtained from the same patient. None of the patients had undergone preoperative chemotherapy or radiotherapy, and they provided informed consent. Immediately after removal, all the samples were frozen in liquid nitrogen to perform further experiments. The experiments were approved by the Ethics Committee of the Second People's Hospital of Lianyungang City. All population-related studies were performed based on the World Medical Association Declaration of Helsinki, and written informed consent forms were obtained from all the participants.

Cell Culture and Transfection

Breast cancer cell lines (CAL-51, MCF-7, BT-20, BT-549, and AU565) and human normal mammary epithelial cells (MCF 10A) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (FBS, Beyotime, Nantong, China), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA), and maintained at 37 °C under 5% CO_2. The *LINC00894* overexpression plasmid, *LINC00894* siRNA, *LINC00894* shRNA, miR-429 inhibitors, and miR-429 mimics were constructed by GenePharma (Shanghai, China). Cell transfection was conducted using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After 48–72 h of culture, the cells were washed and transfected cells were collected for further experiments.

RNA Extraction and Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

The RNAiso Plus kit (Takara, Japan) was employed for the extraction of total RNA from MCF-7 and AU565 cells and PrimeScript RT-PCR kit (Takara, Japan) was used for reverse transcription as per the manufacturers' instructions. Using a Fast Real-time PCR 7500 System (Applied Biosystems), qRT-PCR was conducted using SYBR-green PCR Master Mix in 10 mL reaction mixtures in triplicate. *GAPDH* or *U6* were used as internal controls.[13] Primer sequences were designed as follows: *LINC00894* forward 5′- GCAGGGTCTCTTGAGTTCCT −3′, reverse 5′-TTCCTCAAGCTTCTCCAGGG-3′; *miR-429* forward 5′-ACACTCCAGCTGGGTGCCAAAATGGTCTGT-3′, reverse 5′- CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATTATGAC-3′; *ZEB1* forward 5′-GCCAATAAGCAAACGATTCTG-3′, reverse 5′- TTTGGCTGGATCACTTTCAAG-3′. The $2^{-\Delta Ct}$ method was utilized for calculating relative RNA levels.

Cell Proliferation

Cell Counting Kit-8 (CCK-8, Dojindo, Japan) was used to assess the viability of AU565 and MCF-7 cells as per the manufacturer's instructions within five days after seeding transfected cells in 96-well plates. A multifunctional microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) was employed to measure absorbance at 450 nm after 1 h of incubation.

For the EdU assay, cell culture was performed using the EdU reagent for 2 h, with 15 min of cell fixation in 4% paraformaldehyde, followed by EdU staining as per the manufacturer's protocol.

Colony Formation Assays

For the colony formation assay, 0.5 × 10^3 cells were inoculated into a 12-well plate and cultured for ten days. The original medium was replaced with fresh medium on the 5th day. Following incubation, PBS was used to rinse the cells, which were then immobilized with 4% paraformaldehyde for 5 min and stained with 0.1% crystal violet for 30 s. The experiment was performed thrice.

Transwell Cell Invasion Assay

After suspending cells at a density of 1.0 × 10^5/mL in serum-free medium, a transwell chamber (Corning, NY, USA) precoated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was placed in the 24-well plate. The apical chamber and basolateral chamber contained a 200 μL suspension of cells and 500 μL medium with 10% FBS, respectively. Forty-eight hours later, with the chambers removed, the penetrating cells were fixed in 5% paraformaldehyde and subsequently stained with 0.1% crystal violet for 20 min. A light microscope (Olympus) was utilized for imaging and counting the invasive cells in five randomly selected fields for each field.

Subcellular Distribution

RNA was extracted from cells at the cytoplasmic and nuclear levels using a PARIS Kit (Life Technologies, USA), and the quantification of total RNA in each fraction was carried out using qRT-PCR. Internal references were *U6* and *GAPDH* for the nucleus and cytoplasm, respectively.

Dual-Luciferase Reporter Gene Assay

Mutant-type (*ZEB1* Mut, *LINC00894* Mut) and wild-type plasmids (*ZEB1* Wt, *LINC00894* Wt) were constructed. After seeding HEK293T cells into 24-well plates, Lipofectamine 2000 was used to co-transfect 50 nM miR-429 mimics or negative control and wild- or mutant-type plasmids. Plasmid to vector (pRL-SV40) ratio was 80 ng:5 ng. A dual-luciferase reporter assay kit (Promega, Madison, WI, USA) was used to detect luciferase intensities.

RNA Immunoprecipitation (RIP) Assay

Magna Nuclear RIP™ Nuclear RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA, USA) was used for the RIP assay. Briefly, cell lysis was performed using a complete RIPA buffer and adding RNase inhibitor and protease inhibitor cocktails. After conjugating with human AGO2 antibody or IgG as control (Millipore, Bedford, MA, USA), cell extracts were obtained using RIP buffer containing magnetic beads. Next, protein digestion was carried out to obtain immunoprecipitated RNAs. Finally, purified RNAs were quantified using qRT-PCR. Anti-miR-429 and anti-*LINC00894* procured from Abcam (Cambridge, MA, USA) were used in the RIP assay.

Western Blotting

After extracting and quantifying proteins using the BCA method, the protein samples were separated using SDS-PAGE gel electrophoresis, proteins were transferred to nitrocellulose membranes, and the membranes were blocked with 5% defatted milk. Next, the membranes were incubated with antibody against *ZEB1* (ab203829, Abcam, Shanghai, China) and GAPDH antibody (ab181602, Abcam, Shanghai, China) as primary antibodies and subsequently with corresponding secondary antibodies. Protein bands were visualized using chemiluminescence.

Construction of Tumor Models

Six BALB/c athymic nude mice (five-week-old) obtained from the National Laboratory Animal Center (Beijing, China) were subjected to seven days of acclimation before performing the assay. The North China University of Science and Technology approved all the animal experiments, which were performed following the National Institutes of Health (NIH) guidelines on animal welfare. For the construction of a breast cancer xenograft model, 4 × 10^6 AU565 cells stably transfected with sh-LINC00894 or sh-NC were injected into the dorsal right flank of each mouse. The tumor volume was measured with calipers at two major axes every 1 week and calculated as: $V = 0.5 \times L$ (length) $\times W^2$ (width). Four weeks later, the mice were anesthetized using 40 mg sodium pentobarbital and then sacrificed with 10% formalin perfusion of the central nervous system.[14] Death was confirmed when the heartbeat and breathing completely stopped as well as with the disappearance of the foot

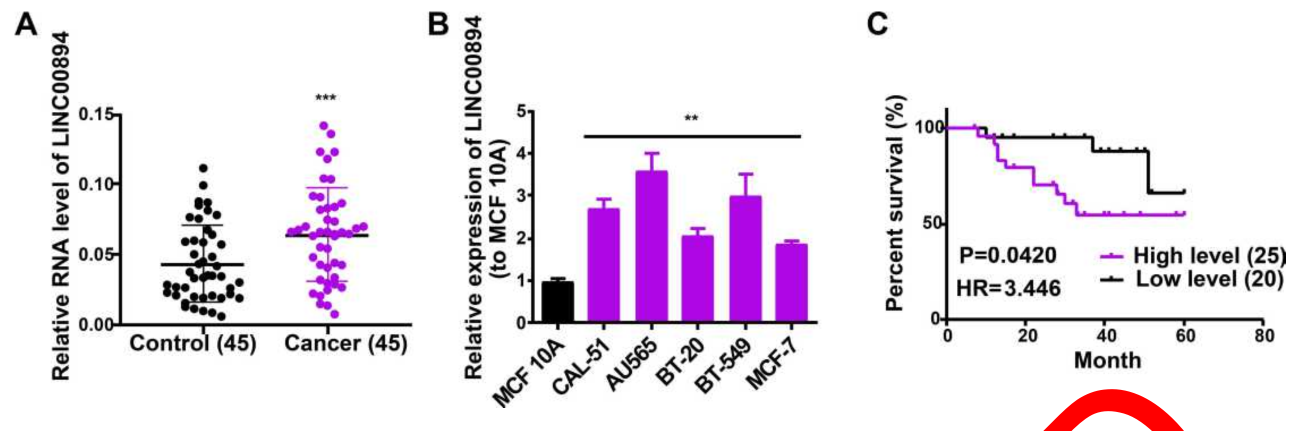

Figure 1 Characteristics and expression of *LINC00894* in breast cancer cells and tissues. (**A**) *LINC00894* expression in breast cancer tissues and paired normal tissue samples (n= 45) using qRT-PCR. (**B**) *LINC00894* expression in breast cancer cell lines (CAL-51, MCF-7, BT-20, BT-549, and AU565) and normal mammary epithelial cells MCF 10A using qRT-PCR. (**C**) Kaplan-Meier analysis of overall survival of breast cancer patients stratified by *LINC00894* expression.
Notes: **P value < 0.01 and ***P value < 0.001.

withdrawal reflex. Tumor tissues were removed and weighed.

Lung Metastasis Assay

Briefly, 1 × 10^6 AU565 cells in 30 µL of 30% Matrigel were injected intravenously through the tail vein of nude mice. After 4 weeks, nude mice were sacrificed, and metastatic nodules in each lung were analyzed.

Statistical Processing

All statistical analyses were conducted using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA) and SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Quantitative data are reported as mean ± SD. A nonparametric test was applied for non-normally distributed data, and *t*-test was conducted for the analysis of normally distributed data. Pearson's correlation coefficient was determined to assess associations among *LINC00894*, miR-429 and ZEB1. Log rank test and Kaplan-Meier method were used to assess survival rates. Data concerning the association of *LINC00894* expression with clinicopathological features of breast cancer were analyzed by chi-squared test or Fisher's exact test. *P*<0.05 indicated statistical differences.

Results

LINC00894 Expression in Breast Cancer

For identifying the correlation between lncRNAs and breast cancer, GSE119233 microarray was employed to analyze the expression profiles of lncRNAs from 20 breast cancer tissues and 10 adjacent normal tissues.[15] Collectively, the top 200 dysregulated lncRNAs, including 100 upregulated and 100 downregulated lncRNAs in breast cancer tissues, were analyzed (Figure S1A and B). High expression of *LINC00894* was observed in breast cancer tissues. Moreover, the high expression of *LINC00894* in 45 pairs of breast cancer tissue samples and breast cancer cell lines (CAL-51, MCF-7, BT-20, BT-549, and AU565) was verified using qRT-PCR (Figure 1A and B). Particularly, among the breast cancer cell lines used for subsequent experiments, AU565 cells showed the highest *LINC00894* expression, whereas MCF-7 cells exhibited the lowest expression. In addition, the survival rate of breast cancer patients with highly expressed *LINC00894* was inferior to that of patients with low *LINC00894* expression (Figure 1C). The average expression level (0.064) of *LINC00894* in breast cancer tissues was taken as the cut off value for high and low levels. Correlation between *LINC00894* expression and clinicopathological parameters of breast cancer patients is shown in Table 1. We found that higher *LINC00894* expression was associated with TNM stage ($P < 0.05$), but there was no association between the expression level of LINC00894 and molecular subtypes in breast cancer ($P > 0.05$).

LINC00894 Functions in Breast Cancer Cells

qRT-PCR was conducted to verify the transfection efficacy of the *LINC00894* overexpression vector and *LINC00894* siRNA in breast cancer cells (Figure 2A). Employing the CCK-8 assay, proliferation of breast cancer cells was observed to be remarkably reduced due to downregulation of *LINC00894* and accelerated by overexpression of *LINC00894* (Figure 2B); these results were similar to those obtained from the EdU assay and colony formation

Table 1 Patient Clinicopathologic Features

Clinicopathologic Features	Number of Cases	LINC00894 Expression		*p* value
		Low (n=20)	High (n=25)	
Age				0.5572
<40	25	10	15	
≥40	20	10	10	
Tumor size				**0.0389***
T1	19	12	7	
T2-T4	26	8	18	
N stages				**0.0010***
N0	21	15	6	
N1-3	24	5	19	
Metastasis				**0.0169***
M0	22	14	8	
M1	23	6	17	
TNM stage				
I/II	15	12	3	**0.0012***
III/IV	30	8	22	
ER status				0.5273
Negative	30	12	18	
Positive	15	8	7	
HER-2 status				[illegible]
Negative	26	14	12	
Positive	19	6	[illegible]	
PR status				0.7600
Negative	18	7	11	
Positive	27	[illegible]	[illegible]	

Notes: Total data from 45 tumor tissues of breast cancer patients were analyzed. For the expression of LINC00894 was assayed by qRT-PCR, the average expression level was used as the cutoff. Data were analyzed by chi-squared test and Fisher's exact test. *P-value in bold indicates statistically significant.

experiments (Figure 2C–F). Furthermore, the transwell assay revealed that invasion of breast cancer cells was enhanced by *LINC00894* overexpression and decreased by *LINC00894* downregulation (Figure 2G and H). Taken together, our findings uncovered that *LINC00894* might play regulatory roles in cell proliferation and invasion of breast cancer cells.

LINC00894 is Targeted by miR-429

Breast cancer cells were fractionated into cytoplasmic and nuclear fractions, with *GAPDH* and *U6* as the respective controls for verifying the cellular location of *LINC00894*. The results of qRT-PCR indicated the presence of *LINC00894* in the cytoplasmic fractions of AU565 and MCF-7 cells (Figure 3A). Hence, it can be concluded that *LINC00894* is implicated in breast cancer progression by post-transcriptional regulation and that *LINC00894* is a possible ceRNA in breast cancer progression. The expression of *miR-429* was reduced in breast cancer cells as observed by qRT-PCR (Figure 3B). In addition, the expressions of miR-429 and *LINC00894* were negatively related in breast cancer tissues (Figure 3C). Starbase prediction identified closely matched sequences in miR-429 to both Exon-5 and Exon-7 of *LINC00894* (Figure 3D). pGL3-*LINC00894* wild type (Wt) and pGL3-*LINC00894* Mut were constructed based on these binding sequences (Figure 3D). HEK293T cells displayed significantly down-regulated luciferase activities following co-transfection with miR-429 mimics and *LINC00894* Wt. However, these activities remained unchanged after the cells were co-transfected with miR-429 mimics and *LINC00894* Mut (Figure 3E). RIP analysis was conducted to determine whether *LINC00894* participated to form ribonucleoprotein complex with RNAs. Results indicated that *LINC00894* was more abundant in anti-AGO2 antibody than in controls, which was similar to miR-429 (Figure 3F). Therefore, it can be speculated that *LINC00894* binds to miR-429.

ZEB1 is a Target of miR-429 Regulated by LINC00894

To explore the potential roles of miR-429 in breast cancer progression, screening for the target gene of miR-429 via bioinformatics prediction resulted in the discovery of *ZEB1*, which was used for subsequent analyses (Figure 4A). miRNA negative control (miR-NC) or miR-429 mimics were selected to co-transfect the constructed luciferase plasmids (*ZEB1* Mut and *ZEB1* Wt) in HEK293T cells. Luciferase activity of the Mut reporter+ miR-429 mimics was unchanged, while that of Wt reporter+ miR-429 mimics group was repressed (Figure 4B). *ZEB1* served as a candidate target of miR-429 according to the above results. qRT-PCR revealed a significant enhancement in the RNA levels of *ZEB1* in breast cancer tissues versus normal tissue samples (Figure 4C). Additionally, increased protein levels of *ZEB1* were observed in breast cancer tissues by Western blot analysis (Figure 4D). Moreover, the expression levels of miR-429 and *ZEB1* were negatively correlated (Figure 4E). However,

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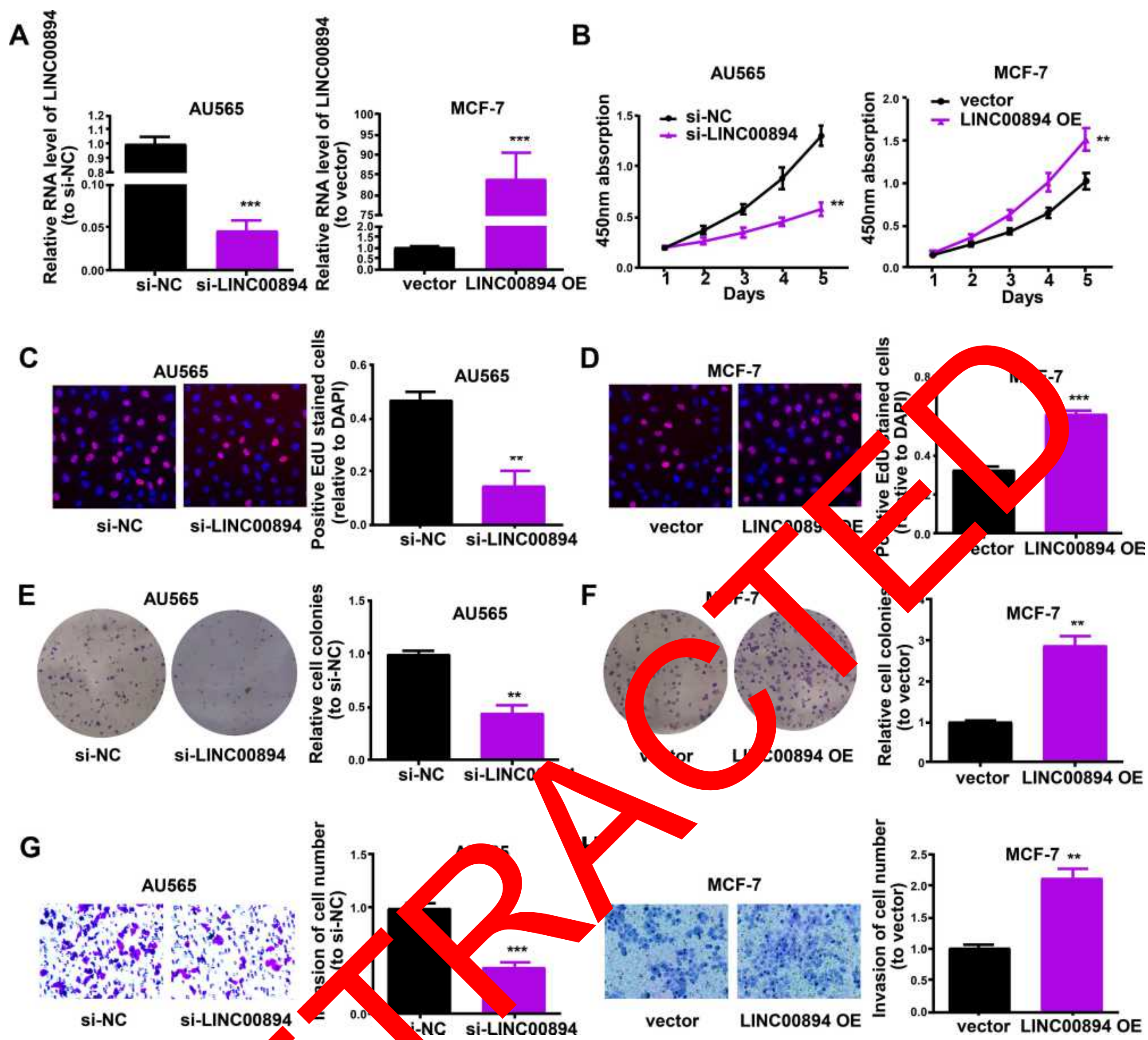

Figure 2 Regulatory effects of *LINC00894* on proliferative and invasive abilities of breast cancer cells. (**A**) qRT-PCR for *LINC00894* expression in cells following transfection with *si-LINC00894* or LINC00894 overexpression (OE) vector. (**B**–**F**) Proliferation of AU565 and MCF-7 cells after transfection with *LINC00894* siRNA and *LINC00894* overexpression (OE) vector, respectively, in CCK-8 assay, EdU assay, and colony formation experiment. (**G** and **H**) Invasion of AU565 and MCF-7 cells following transfection with *LINC00894* siRNA and *LINC00894* OE vector, respectively in a transwell assay. All the data represent three individual experiments and are shown as mean ± SD.
Notes: **P value < 0.01, ***P value < 0.001.
Abbreviations: si, siRNA; NC, negative control; OE, overexpression.

a positive correlation was observed between the expressions of *ZEB1* and *LINC00894* (Figure 4F).

To determine whether *LINC00894* regulates *ZEB1* expression by targeting miR-429, we measured *ZEB1* expression levels after adjusting the content of *LINC00894* and miR-429. The transfection efficacy of miR-429 inhibitor and miR-429 mimic is shown in Figure 5A. Subsequently, upregulated *ZEB1* expression was identified in AU565 cells following transfection with miR-429 inhibitor as indicated by both Western blotting and qRT-PCR. However, this effect was reversed after co-transfection with *LINC00894* siRNA (Figure 5B). Additionally, inhibitory effects of *ZEB1* expression were detected after transfection with *miR-429* mimics in MCF-7 cells; these effects were reversed by co-transfection with the *LINC00894* overexpression plasmid (Figure 5C). Next, the *LINC00894* Wt overexpression plasmid and corresponding mutant overexpression plasmid were transfected in MCF-7 cells prior to determining *ZEB1* expression levels. As indicated by Western blotting and qRT-PCR, overexpression of wild-type *LINC00894* upregulated *ZEB1* expression,

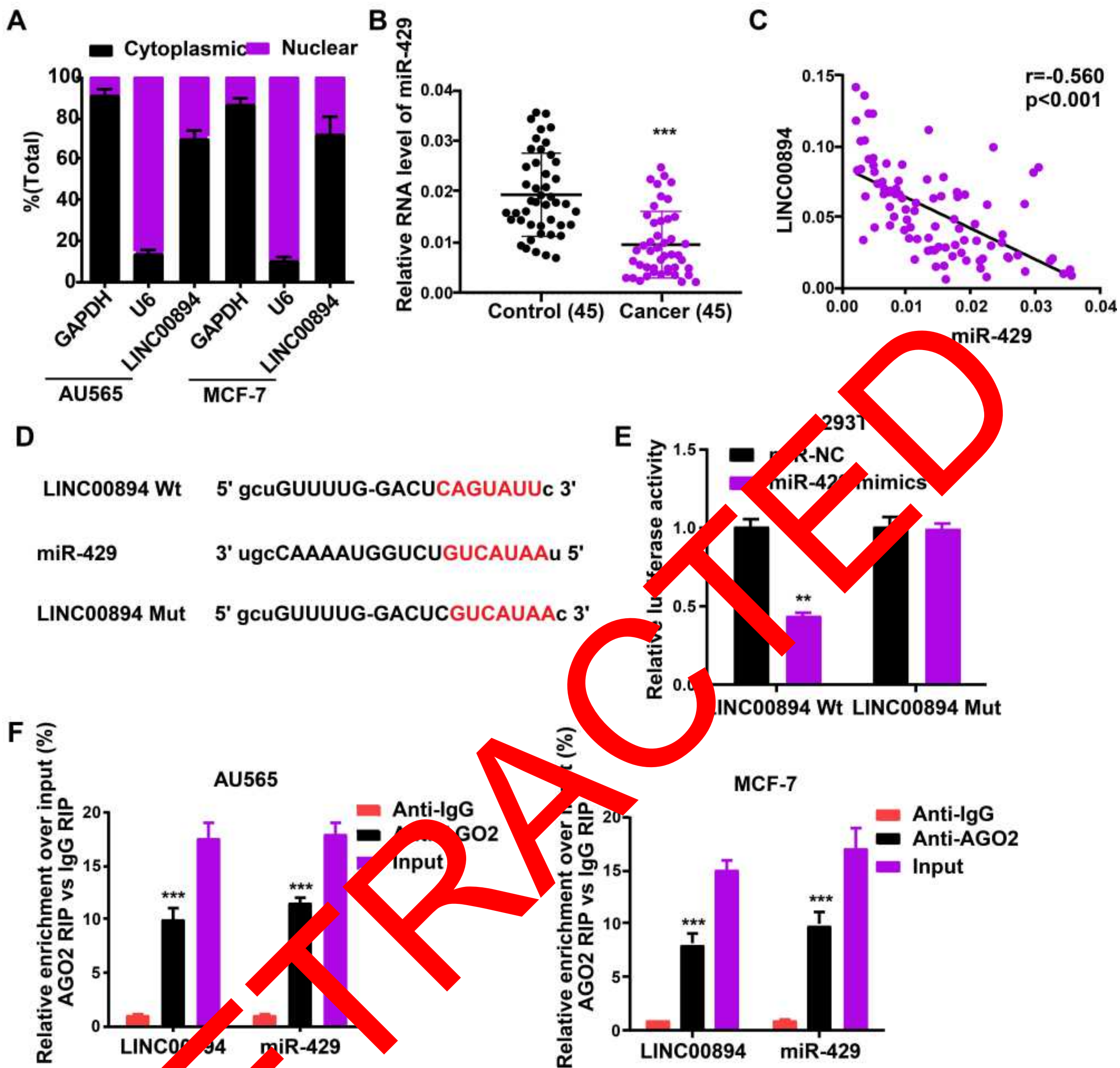

Figure 3 Direct interaction [illegible] *LINC00894* with miR-429. (**A**) *LINC00894* in AU565 and MCF-7 cells at cytoplasmic and nuclear levels using qRT-PCR. (**B**) MiR-429 expression in br[illegible]er tiss[illegible] and adj[illegible]nt non-cancerous tissues. (**C**) Correlation analysis of *LINC00894* and *miR-429* expressions in breast cancer. (**D**) Binding sequences of [illegible]-429 and *LINC008*[illegible] (**E**) [illegible]ual-luciferase reporter gene assay in HEK293T cells. (**F**) Amounts of *LINC00894* and miR-429 in AU565 and MCF-7 cells in the RIP experi[illegible]nt.

Notes: [illegible] < 0.001.

Abbreviation[illegible], miRNA; NC, negative control; Wt, wild-type; Mut, mutant-type.

whereas mutant-type *LINC00894* did not (Figure 5D). In summary, *LINC00894* directly binds to miR-429 to generate positive regulatory effects on *ZEB1* expression.

LINC00894/miR-429 Axis Regulates the Behavior of Breast Cancer Cells

The present study determined whether miR-429 had an impact on the proliferation and invasion abilities of AU565 and MCF-7 cells. The proliferation and invasion abilities were remarkably enhanced via downregulation of miR-429 in AU565 cells versus those of controls, which was partially reversed by *LINC00894* siRNA treatment (Figure 6A–D). In contrast, overexpression of miR-429 restrained the proliferative and invasive abilities of MCF-7 cells, and the overexpression was partially reversed by overexpressed *LINC00894* (Figure 6A–D).

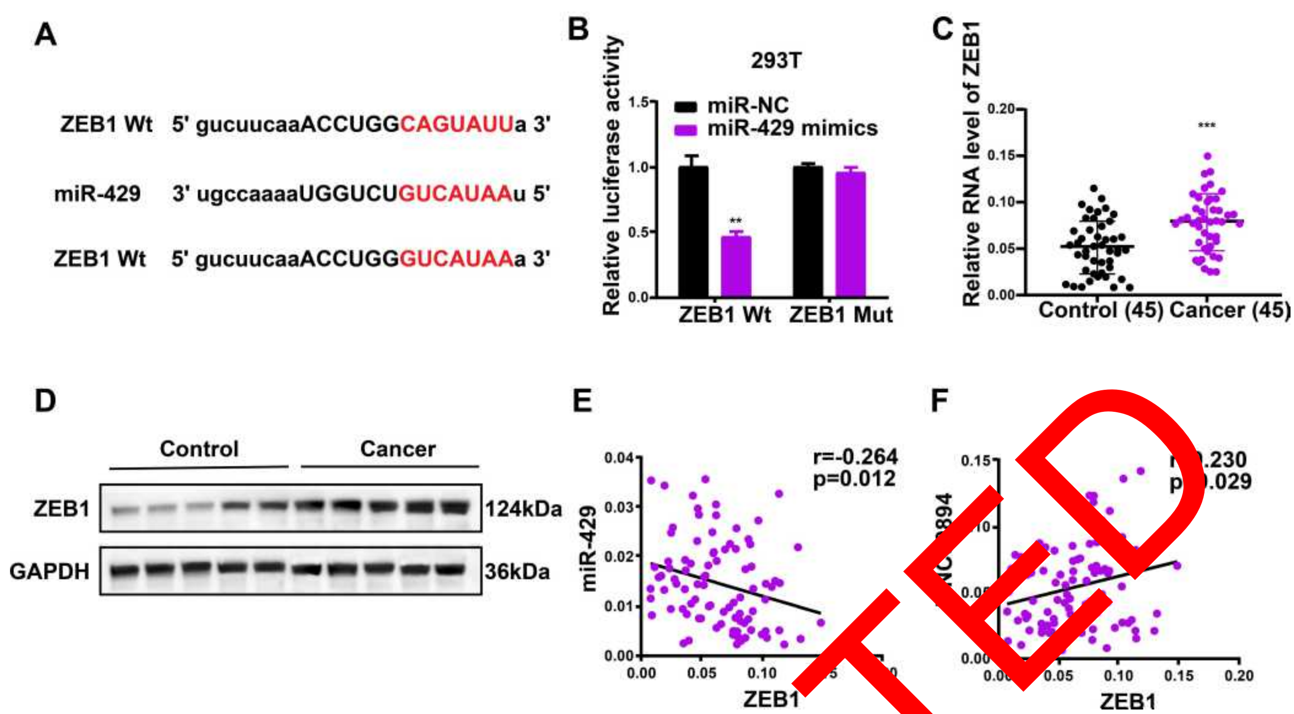

Figure 4 *ZEB1* was directly targeted by miR-429. (**A**) Assumed miRNA binding sites in *ZEB1* sequence. (**B**) Dual-luciferase reporter gene assay. (**C**) Expression of *ZEB1* in breast cancer and normal tissues. (**D**) Protein levels of *ZEB1* in breast cancer tissues and non-cancerous tissues using Western blotting. (**E**) Correlation analysis of *ZEB1* and miR-429 expressions in breast cancer. (**F**) Correlation analysis of *LINC00894* and *ZEB1* expression in breast cancer. All the data represent three individual experiments and are shown as mean ± SD.

Notes: **P value < 0.01, ***P value < 0.001.

Abbreviations: Wt, wild-type; Mut, mutant-type.

Downregulation of LINC00894 Inhibits Breast Cancer Tumor Growth and Migration in vivo

A tumor formation assay revealed that tumor growth in vivo was suppressed by *LINC00894* downregulation and that AU565 cells transduced with *sh-LINC00894* grew at a slower rate after implantation in mice (Figure 7A). In addition, the average volume and weight of xenografts obtained from sh-*LINC00894* transduced cells were markedly lower than those obtained from sh-NC cells (Figure 7B and C). Images of pulmonary metastatic tumors and their stained sections are shown in Figure 7D. The number of lung metastatic nodules was observed to be decreased in the *LINC00894* shRNA group. As shown in Figure 7E and F, the results indicated that knockdown of *LINC00894* increased miR-429 expression and decreased *ZEB1* expression in pulmonary metastatic tumors. Taken together, the above findings indicated that *LINC00894* shRNA inhibited the growth and migration of breast cancer tumors in vivo.

Discussion

Despite dramatic advances in cancer research, breast cancer remains a major health problem worldwide.[16,17] lncRNAs are reported to function as regulatory factors in many cellular processes,[18] and dysregulation of lncRNAs is known to be associated with the development of diseases[19,20] such as Parkinson's disease,[21] pancreatic cancer,[22] and Alzheimer's disease.[23] Moreover, it was demonstrated that several lncRNAs, including lncRNA-ATB,[24] DSCAM-AS1,[25] and lncRNA BCAR4,[26] participated in the occurrence and progression of breast cancer. These studies suggested that lncRNAs may be crucial in the initiation and progression of breast cancer. Therefore, further investigation of the biofunctions and molecular mechanisms underlying the role of lncRNAs in breast cancer may facilitate the development of new target molecules for breast cancer treatment.

Therefore, an lncRNA microarray was performed to analyze lncRNA expression in breast cancer tissues, and *LINC00894* was found to be highly expressed in breast cancer tissues. Subsequently, *LINC00894* was shown to

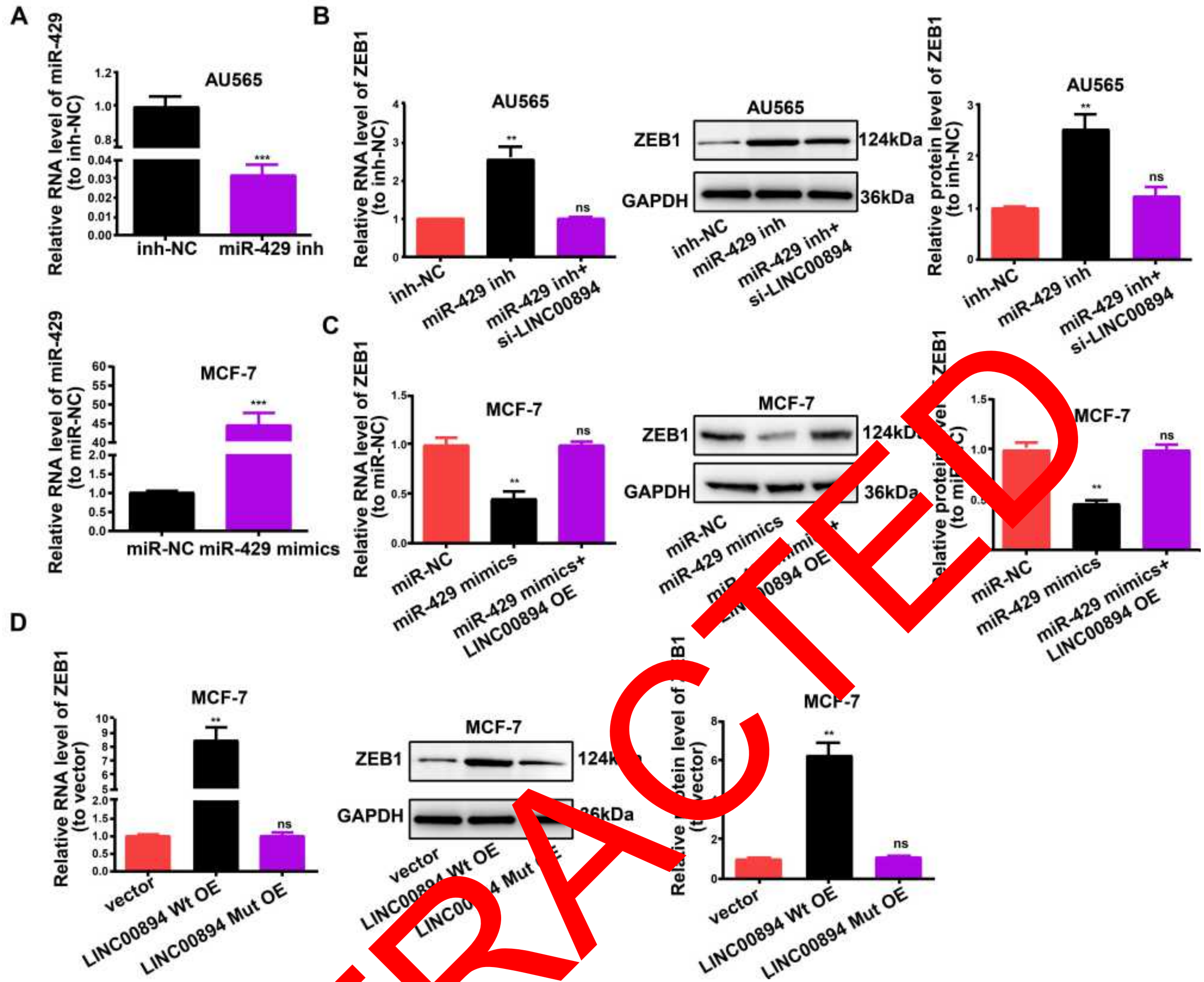

Figure 5 *LINC00894*/miR-429 axis is key to *ZEB1* expression. (**A**) Transfection efficiency of miR-429 inhibitor and miR-429 mimics. (**B**) RNA and protein levels of *ZEB1* in AU565 cells after transfection with miR-429 inhibitor ± *LINC00894* siRNA. (**C**) RNA and protein levels of *ZEB1* in MCF-7 cells after transfection with miR-429 mimics ± *LINC00894* OE plasmid. (**D**) RNA and protein levels of *ZEB1* after transfection with *LINC00894* Mut OE plasmid or *LINC00894* wild type (Wt) OE plasmid. All the data represent three individual experiments and are shown as mean ± SD.
Notes: ** P value < 0.01, *** P value < 0.001.
Abbreviations: ns, no significant difference; Wt, wild-type; Mut, mutant-type; OE, overexpression; inh, inhibitor; NC, negative control; si, siRNA.

promote the proliferative and invasive capacities of AU565 and MCF-7 cells using CCK-8, EdU, and transwell assays. The tumor xenograft model and lung metastasis assay were utilized to verify the effects of *LINC00894* on breast cancer. It was demonstrated that tumor growth and metastasis were suppressed by *LINC00894* knockdown in AU565 cells. Therefore, investigating the effects of *LINC00894* on the proliferation and invasion inhibition of breast cancer cells is important for further studies on the occurrence, progression and metastasis of breast cancer.

LncRNAs may bind to miRNAs and regulate their functions.[27,28] miRNAs (18–22 nucleotides) are a class of non-coding RNAs, and various pathophysiological processes may be regulated by miRNAs, including inflammation and cancer.[29–31] miRNAs could negatively regulate the expression levels of other non-coding transcripts and protein coding genes, and are involved in the post-transcriptional modulation of several genes.[32,33] In our study, miR-429 bound to *LINC00894* and was expressed at low levels in breast cancer cell lines. Importantly, in cell function assays, proliferation and invasion abilities of breast cancer cells were limited by overexpressed miR-429; however, the effects of miR-429 mimics were partially reversed by *LINC00894* overexpression. Besides, miR-429 was

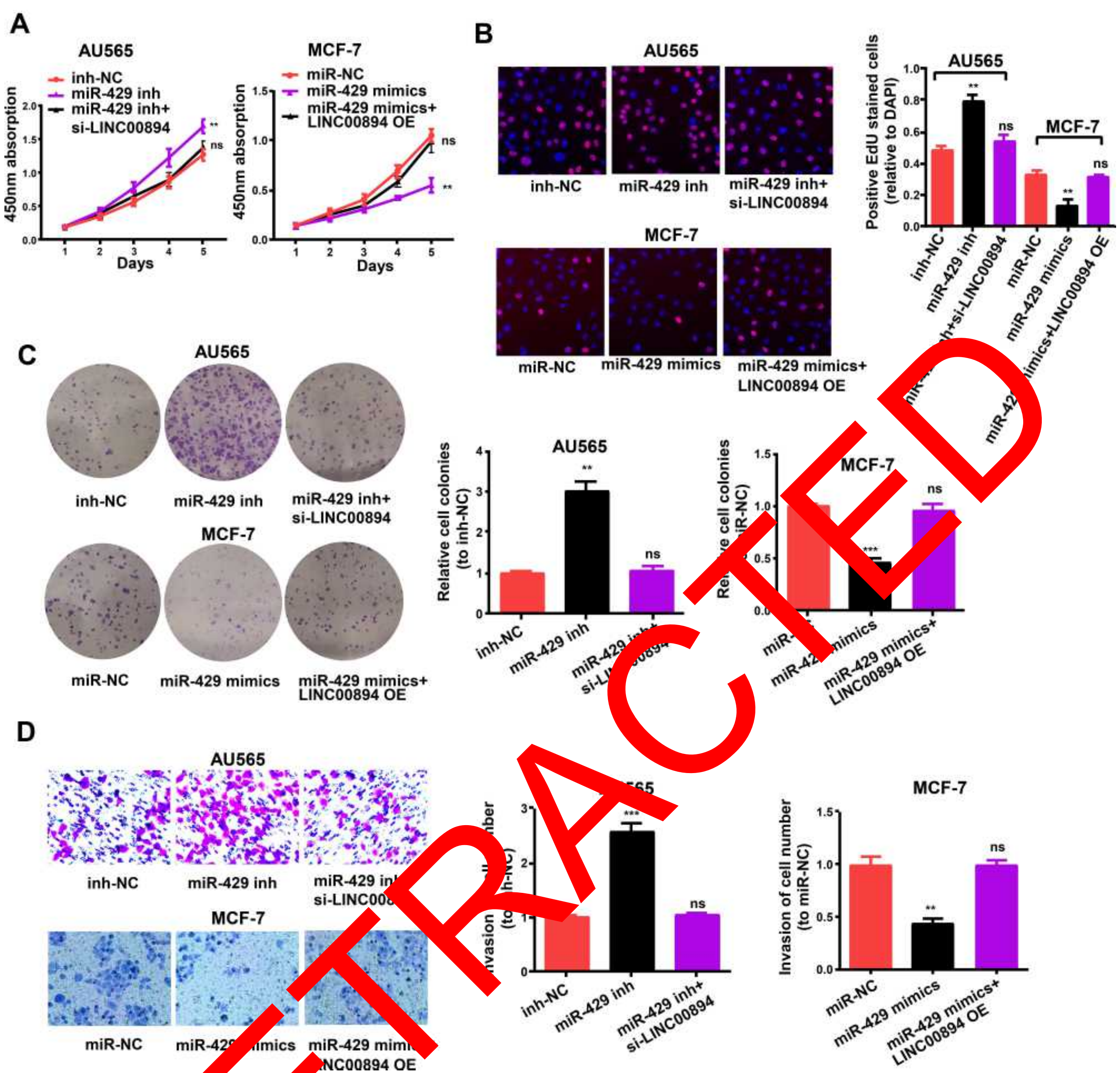

Figure 6 *LINC00894*/miR-429 axis [illegible]s behaviors [illegible] breast cancer cells. (**A**) Proliferative abilities of AU565 and MCF-7 cells in a CCK-8 assay. (**B**) Proliferative abilities of AU565 and MCF-7 c[illegible] EdU as[illegible] (**C**) Pro[illegible]ative abilities of AU565 and MCF-7 cells in a colony formation experiment. (**D**) Invasion by cells following alteration of AU565 and MCF-7 [illegible] lines i[illegible]ifferent [illegible]n experiments. All the data represent three individual experiments and are reported as mean ± SD.

Notes: **P valu[illegible] 0.01, ***P[illegible]alue < 0.001.

Abbreviations: [illegible] no sig[illegible]nce; OE, overexpression; inh, inhibitor; NC, negative control; si, siRNA.

reported to inhibit the pathological processes of cancers.[34,35] For instance, miR-429 was shown to inhibit the invasion and migration of breast cancer cells.[36] Thus, *LINC00894* and miR-429 may contribute to the initiation and progression of breast cancer.

Next, we confirmed that *ZEB1* was a downstream target of *miR-429* and that *LINC00894* adjusted the expression of *ZEB1* by competitively binding *miR-429*. *ZEB1*, as an epithelial mesenchymal transition (EMT) regulator, together with the EMT associated molecules such as SNAIL, SLUG, and TWIST, participates in multiple biological processes related to malignancy such as invasion and metastasis.[37,38] Besides, *ZEB1* is also closely related to hypoxia and chemoresistance of tumors.[39,40] Meanwhile, it has been proven that *ZEB1* can promote the pathophysiological process of tumors, including that of breast cancer.[41–43]

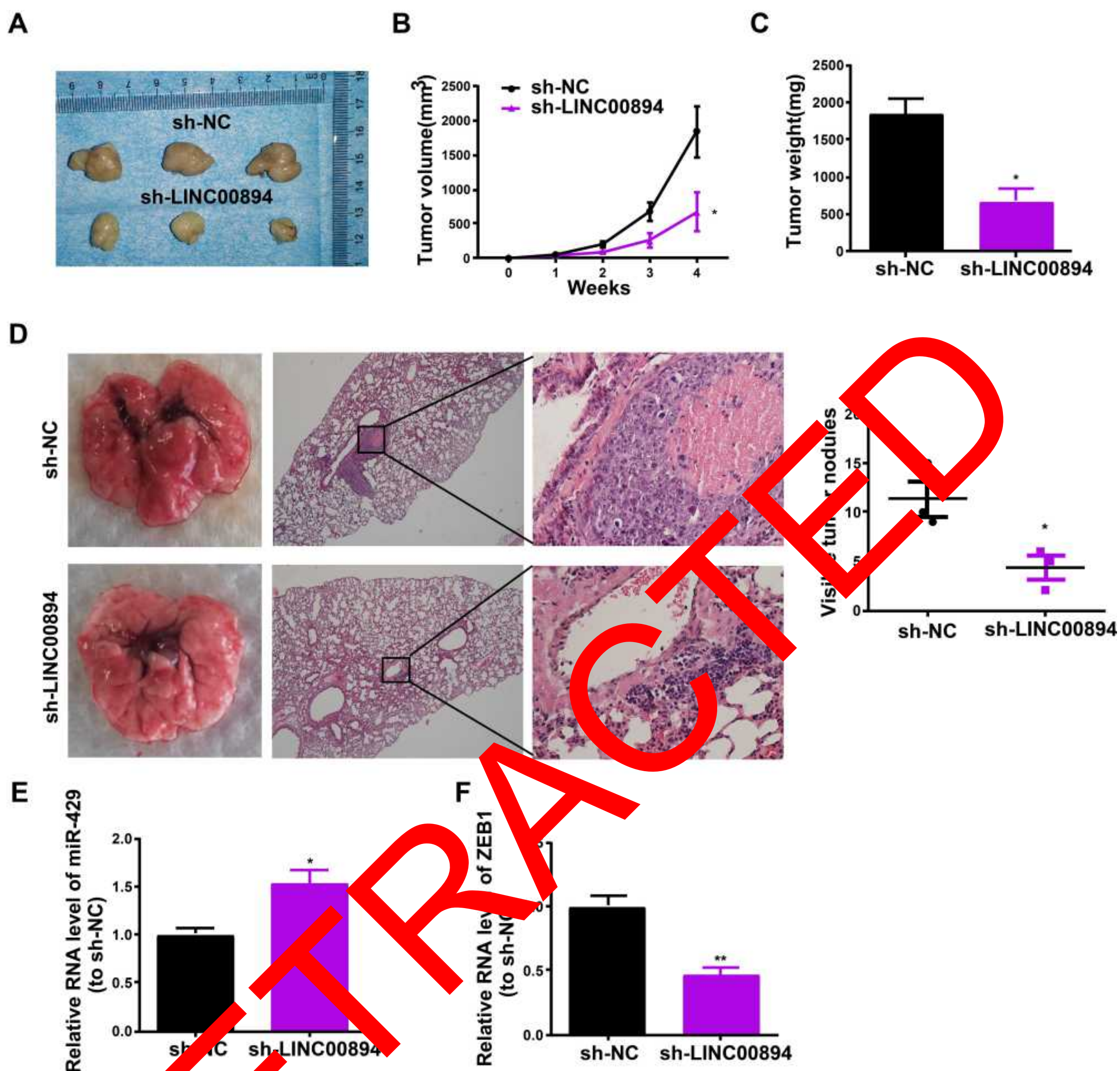

Figure 7 *LINC00894* regulate[illegible]st cancer in [illegible]vo. (**A**) Xenograft tumors (n=3 in each group). (**B**) Tumor volumes in both *LINC00894* knockdown and control groups measured at one [illegible]ervals. [illegible] Tumor [illegible]ights measured after tumor dissection. (**D**) Images of pulmonary metastatic tumors and their stained sections. (**E, F**) The expressions o[illegible]iR-429 [illegible] and ZE[illegible] pulmonary metastatic tumors.
Notes: *P[illegible]lue < 0.05[illegible]P value < 0.[illegible] compared to sh-NC.
Abbrevia[illegible]s: NC[illegible]ol; sh, shRNA.

Taken toget[illegible], *LINC00894* is a competitive endogenous RNA that plays an important role in regulating the expression of *ZEB1* by sponging *miR-429* to regulate breast cancer progression.

Data Sharing Statement

The datasets used or analyzed in the current study are available upon reasonable request.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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