# The Low Expression of MEOX2 is Associated With Poorer Survival in Breast Cancer

**Huxia Wang**
The First Affiliated Hospital of Xi'an Jiaotong University
**Yanan Tang**
The First Affiliated Hospital of Xi'an Jiaotong University
**Meixia Wang**
Shenmu Hospital
**Caixia Ding**
Shaanxi Provincial Cancer Hospital
**Xiaomin Yang**
Shaanxi Provincial Cancer Hospital
**Pihua Han**
Shaanxi Provincial Cancer Hospital
**Jing Zhao**
Shaanxi Provincial Cancer Hospital
**Peijun Liu** ( liupeijun@xjtu.edu.cn )
The First Affiliated Hospital of Xi'an Jiaotong University

**Research Article**

**Keywords:** breast cancer, diagnosis, MEOX2, prognosis

**Posted Date:** December 6th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-1128452/v1

# Abstract

The regulation of vertebrate limb myogenesis gene, Mesenchyme Homeobox 2 (MEOX2), has been reported to be associated with most cancer progression closely. However, its role and function in breast cancer are unidentified. Here, we aim to investigate the association of MEOX2 expression with clinicopathological features and the survival probability of breast cancer. The MEOX2 expression in breast cancer was first analyzed from The Cancer Genome Atlas (TCGA) database. Then, the association of MEOX2 with patients' clinicopathological variables and prognostic probability were detected by bioinformatics analysis. Moreover, a high-throughput tissue microarray containing 135 cases of breast cancer was used to further clarify the expression of MEOX2 in breast cancer patients. The expression of MEOX2 is inhibited in breast cancer than in normal tissues, and the lower MEOX2 expression indicates the poorer prognosis of breast cancer patients. In addition, the histological grade of MEOX2 expression is negatively correlated with the Ki67 level. Multivariate COX regression also verified that MEOX2 was an independent prognostic factor in breast cancer patients. Based on our results, we can conclude that lower MEOX2 expression was related to tumor proliferation and could be a new diagnostic and prognostic biomarker of breast cancer.


# Introduction

Breast cancer, as the most common malignancy, threatens women's health seriously [1,2]. This heterogeneous disease is categorized into three basic therapeutic groups according to the molecular characters: the estrogen receptor (ER) positive group; HER2 (also called ERBB2) amplified group and triple-negative breast cancers (TNBCs, lacking expression of ER, progesterone receptor (PR) and HER2) [3]. Although clinical treatment was guided after this molecular typing, there are still many people who die from this disease due to recurrence and metastasis[1,4,5]. This extremely dismal prognosis of breast cancer is primarily because an effective biomarker is still lacking in current. Therefore, further studies to identify new prognostic markers and therapeutic targets are still required.

Mesenchyme Homeobox 2 (MEOX2) is considered a transcription factor involved in numerous biological processes, especially in the regulation of angiogenesis and vasculogenesis[6,7]. For instance, abundant expression of MEOX2 could inhibit the proliferation of vascular smooth muscle and vascular endothelial cells[8,9]. Recently, emerging evidence points out that MEOX2 participates in the progression of most cancer and might become a potential biomarker in malignancy[10,11]. The hepatocellular carcinoma patients with declined MEOX2 expression implied a worse overall survival (OS) rate[12]. Similarly in laryngeal carcinoma and Wilms tumor, the lower expression of MEOX2 is related to the poorer prognosis, which tips it should be a tumor suppressor[13,14]. Despite being reported extensively, the function of MEOX2 in different kinds of cancers was not completely consistent. The functional role of MEOX2 in breast cancer has not been investigated up to date.

In this study, we analyzed the expression pattern of MEOX2 in breast cancer. Meanwhile, we used the UALCAN, bc-GenExMiner, and KM Plotter online tools to reveal the association of MEOX2 with clinicopathological features and prognosis. Subsequently, we tested MEOX2 expression in breast cancer tissues using a high-throughput tissue microarray. Our results give a shred of strong evidence that MEOX2 should be a novel prognostic biomarker of breast cancer.

# Methods

# Online database analysis

UALCAN (http://ualcan.path.uab.edu) is an interactive web-portal to perform to in-depth analyses of TCGA gene expression data[39]. MEOX2 mRNA expression in breast cancer tissues and normal tissues from TCGA database was analyzed using the UALCAN online tool.

Breast Cancer Gene-Expression Miner v4.5 (bc-GenExMiner v4.5) (http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php) is a statistical mining tool of published annotated breast cancer transcriptomic data (DNA microarrays [n = 10716] and RNA-seq [n = 4716]). It offers the possibility to explore gene-expression of genes of interest in breast cancer[40,41]. The data from this website were downloaded on June 22, 2020. The relationship between MEOX2 and pathological features of breast cancer was analyzed by bc-GenExMiner v4.5 online tool.

The main purpose of Kaplan-Meier plotter is to discovery and verification of survival biomarkers based on meta-analysis[42,43]. The values of MEOX2 mRNA expression in prognosis of all breast cancers and different clinicopathologic classifications were analyzed using Kaplan–Meier Plotter online tool.

**Tissue Microarrays, Patients, And Follow-up**

The breast cancer tissue microarray (HBreD140Su07; Shanghai Outdo Biotech Co., Ltd., Shanghai, China) contained 140 cases of breast carcinomas. 135 of them were evaluated, excluding the microarray cores which were either fragmented or lost during the procedure of immunohistochemistry. The average age of available samples were 59.27 ±12.33 years (range: 37–88 years). All the patients were followed up for 5.6 - 10 years. The follow-up data were collected from November 2005 to July 2014. OS was defined as the time from surgery to death or the last known follow-up. Forty-two patients were dead during the follow-up.

**Immunohistochemistry staining and evaluation.**

The microarray slices of breast cancer tissues were dewaxed in xylene, hydrated in gradient ethanol, and then treated with 3%H2O2 solution to block endogenous peroxidase activity. After antigen recovery and goat serum blocking, they were incubated in rabbit monoclonal anti-MEOX2 primary antibody (1:200, Affinity; China) overnight at 4°C. HRP-labeled polymer anti-rabbit secondary antibody (1;5000, Abacm, UK) were then added for 60min at room temperature. The slides were rinsed with phosphate-buffered saline (PBS), stained using the 3, 3’-diaminobenzidine (DAB) to visualize the reaction. The imags were acquired

and examined with a Leica Microsystems slide scanner (SCN 400; Leica, Mannheim, Germany). Three randomly fields of view per section were individually examined, and the intensity of staining was scored as described as follows: 0 (negative), 1 (weakly positive), 2 (moderately positive), or 3 (strongly positive). The protein expression was evaluated according to the percentage of brown areas in the IHC samples: 0 (<10%), 1 (10–40%), 2 (40–70%), and 3 (>70%). The IHC score for each field were expressed as a product of the staining intensity and staining extent (range, 0–9). It was divided into low expression group and high expression group; the cutoff value was median.

# Statistical analysis

Statistical analyses were performed using GraphPad Prism 7 (CA, USA) and SPSS software 22 (IBM Corp., Armonk, NY, USA). Mann–Whitney test and Kruskal–Wallis test were used to determine the difference of MEOX2 expression. The associations between MEOX2 expression and clinicopathological features were analyzed by $\chi 2$ test. Survival analysis was performed using Kaplan-Meie. Log-rank test was used to compare different patient groups. Multivariate analysis was performed using a Cox proportional hazard model to evaluate the effect of clinicopathological features and MEOX2 expression on OS rate [hazard ratios (HRs). 95% confidence intervals (CIs) were calculated. All statistical tests were two-sided. Significance was indicated by *P < .05, **P < .01, ***P < .001.

# Results

### The expression pattern of MEOX2 in breast cancer.

We firstly analyzed the expression pattern of MEOX2 in breast cancer by UALCAN tool. From the expression levels of 1097 breast cancer samples and 114 normal tissues, MEOX2 expression appears an obvious decline in breast cancer compared to normal tissues ($P < 0.01$) (Figure 1). This expression pattern of MEOX2 in breast cancer indicates that MEOX2 might be repressed once breast cancer occurs.

### MEOX2 is associated with the clinicopathological features of breast cancer.

To investigate the correlation between MEOX2 expression and the clinicopathological features and molecular subtypes in breast cancer, bc-GenExMiner v4.5 web tool was used to analyze this relation. The results showed that low expression level of MEOX2 represented apparently the relative lower expression of ER and PR; and the relative infrequent incidence of p53 mutation. While low MEOX2 expression predicted a relative rich expression of HER2 in breast cancer. From the all molecular markers perspective, MEOX2 expressions were significantly lower in ER-negative, PR-negative, HER2-positive, and p53-mutant groups than that in ER-positive, PR-positive, HER2-negative and p53 wild-type groups (Figure 2A-D. $P < 0.0001$). In the molecular subtypes viewpoint, MEOX2 expression were different among groups of Normal breast-like (n=639), Luminal A (n=1433), Luminal B (n=1029), HER2 + (n=736), and Basal like (n=832) (Figure 2E, P□0.0001). The expression of MEOX2 in triple-negative breast cancer (TNBC) group has a lower level than that in non-TNBC group (Figure 2F, P□0.0001). All these results indicated that MEOX2, as a vital molecular marker, is associated with clinicopathological features in breast cancer.

### Lower MEOX2 expression implies poorer survival in breast cancer.

Based on our pre-existing results, we concluded that low expression of MEOX2 indicates poor prognosis of breast cancer. However, we further used KM Plotter to reveal the relationship between MEOX2 expression and the prognosis in different molecular subtypes of breast cancer in this study. Lower expression of MEOX2 was significantly associated with poorer prognosis in Luminal A type (Figure 3A, P = 0.015), while there was no significantly correlation in other molecular subtypes (Figure 3B-D, P > 0.05).

### The protein level of MEOX2 expression further confirms MEOX2 is a vital biomarker

To further pinpoint the relationship between MEOX2 expression and different pathological features as well as prognosis of breast cancer, we confirmed it on the protein expression level by immunohistochemical staining (Figure 4, Table 1). The results indicated that MEOX2 expression was significantly higher in histological grade□/□group, and lower in grade □ group of cancer tissues (Figure 4A and 4B). Meanwhile, it was significantly higher in Ki67 □ 20% group than in Ki67 ≥ 20% group (Figure 4C and 4D). However, there were no significant difference of the relationship among MEOX2 expression and age, pathological type, AJCC stage, ER, PR, HER2, p53, AR and EGFR expression. Furthermore, although it showed a downward trend of MEOX2 expression in TNBC, there was no significant difference among different molecular subtypes. (Figure 4E). In addition, there was a negative correlation between MEOX2 expression and Ki67 expression (r = -0.274, *P* = 0.002). All these results for further description of MEOX2 as a vital molecular marker related with pathological features.

Table 1
The relationships of MEOX2 expression with clinical and pathologic parameters of breast cancer patients.

| variables | n | MEOX2 expression | | $\chi^2$ | p value |
|---|---|---|---|---|---|
| | | Low(%) | High(%) | | |
| Age (year) | | | | 0.286 | 0.593 |
| ≤ 60 | 83 | 47(56.6) | 36(43.4) | | |
| 60 | 52 | 27(51.9) | 25(48.1) | | |
| Pathological type | | | | 0.975 | 0.323 |
| Invasive ductal carcinoma | 109 | 62(56.9) | 47(43.1) | | |
| Other types | 26 | 12(46.2) | 14(53.8) | | |
| Grade | | | | 5.122 | 0.024* |
| I/II | 63 | 29(46.0) | 34(54.0) | | |
| III | 62 | 41(66.1) | 21(33.9) | | |
| T stage | | | | 3.356 | 0.187 |
| T0/T1 | 34 | 16(47.1) | 18(52.9) | | |
| T2 | 82 | 51(62.2) | 31(37.8) | | |
| T3/T4 | 16 | 7(43.8) | 9(56.2) | | |
| N stage | | | | 0.372 | 0.946 |
| N0 | 68 | 38(55.9) | 30(44.1) | | |
| N1 | 35 | 18(51.4) | 17(48.6) | | |
| N2 | 19 | 11(57.9) | 8(42.1) | | |
| N3 | 12 | 6(50.0) | 6(50.0) | | |
| AJCC stage | | | | 0.398 | 0.820 |
| | 20 | 10(50.0) | 10(50.0) | | |
| II | 73 | 42(57.5) | 31(42.5) | | |
| | 37 | 20(54.1) | 17(45.9) | | |
| ER | | | | 0.516 | 0.473 |

Low/high by the sample mean. Pearson χ2 test. *P < 0.05 was considered statistically significant

| variables | | n | MEOX2 expression | | $\chi^2$ | p value |
|---|---|---|---|---|---|---|
| | | | Low(%) | High(%) | | |
| | negative | 52 | 30(57.7) | 22(42.3) | | |
| | positive | 78 | 40(51.3) | 38(48.7) | | |
| PR | | | | | 1.495 | 0.221 |
| | negative | 78 | 46(59.0) | 32(41.0) | | |
| | positive | 52 | 25(48.1) | 27(51.9) | | |
| HER2 | | | | | 0.356 | 0.551 |
| | negative | 90 | 51(56.7) | 39(43.3) | | |
| | positive | 43 | 22(51.2) | 21(48.8) | | |
| Ki67 | | | | | 18.943 | 0.000*** |
| | < 20% | 85 | 34(40.0) | 51(60.0) | | |
| | ≥ 20% | 45 | 36(80.0) | 9(20.0) | | |
| Subtypes | | | | | 3.110 | 0.375 |
| Luminal A | | 27 | 11(40.7) | 16(59.3) | | |
| Luminal B | | 49 | 28(57.1) | 21(42.9) | | |
| HER2-rich | | 26 | 14(53.8) | 12(46.2) | | |
| TNBC | | 25 | 16(64.0) | 9(36.0) | | |
| p53 | | | | | 0.535 | 0.465 |
| | negative | 68 | 35(51.5) | 33(48.5) | | |
| | positive | 64 | 37(57.8) | 27(42.2) | | |
| AR | | | | | 0.848 | 0.357 |
| | negative | 29 | 18(62.1) | 11(37.9) | | |
| | positive | 103 | 54(52.4) | 49(47.6) | | |
| EGFR | | | | | 1.804 | 0.179 |
| | negative | 82 | 41(50.0) | 41(50.0) | | |
| | positive | 50 | 31(62.0) | 19(38.0) | | |

Low/high by the sample mean. Pearson χ2 test. *P < 0.05 was considered statistically significant

### Meox2 Is An Independent Factor Associated With Patient's Survival

Moreover, we got the prognostic data of the microarrays and examined the above association. The results showed that lower MEOX2 expression was significantly related to decreased overall survival time (OS; Figure 5A, P = 0.0011). Survival analysis of different molecular subtypes indicated that low MEOX2 expression was associated with reduced OS in Luminal A and Luminal B groups (Figure 5B, P = 0.0048; Figure 5C, P = 0.0216). However, in HER2-rich and TNBC subtypes, the declining trend of survival existed in the MEOX2 low expression group, there was no statistical difference (Figure 5C, P = 0.4722; Figure 5D, P = 0.1873). Additionally, to evaluate the affection of age, pathological type, grade, T stage, N stage, molecular subtype and MEOX2 expression on OS, multivariate analysis was performed with a Cox proportional hazard model. The results revealed that N stage and MEOX2 expression were two independent factors that significantly associated with survival (*P*=0.005 and *P*□0.001, respectively; Table 2). Hence, our findings indicated MEOX2 is an independent factor associated with patient's survival.

Table 2
The univariate and multivariate survival analysis of 115 breast cancer patients.

| variables | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | HR | 95%CI | *p* value | HR | 95%CI | *p* value |
| Age | 1.324 | 0.623-2.813 | 0.465 | 1.006 | 0.459-2.206 | 0.988 |
| Pathological type | 0.676 | 0.205-2.226 | 0.520 | 0.678 | 0.202-2.268 | 0.528 |
| Grade | 1.321 | 0.651-2.681 | 0.440 | 0.839 | 0.389-1.812 | 0.655 |
| T stage | 1.671 | 0.685-4.076 | 0.259 | 1.064 | 0.423-2.676 | 0.896 |
| N stage | 2.407 | 1.132-5.118 | 0.022* | 3.275 | 1.419-7.561 | 0.005** |
| Subtype | 0.994 | 0.408-2.425 | 0.990 | 1.392 | 0.520-3.727 | 0.510 |
| MEOX2 expression | 0.200 | 0.076-0.520 | 0.001** | 0.167 | 0.062-0.453 | 0.000*** |

# Discussion

Up to date, an effective therapeutic for breast cancer has been lacking in the clinical[3,15]. Despite the different treatments, including surgery, radiotherapy, chemotherapy, and endocrine therapy have been routinely applied for breast cancer[3,16,17]. However, a part of patients will still get recurrence and metastasis. Thus, additional studies on therapeutic targets are still needed to reveal new prognostic markers are of great importance. In our previous study, we found that the MEOX2 expression was significantly repressed in breast cancer. The mRNA level of MEOX2 in breast cancer cells MCF-7 and SUM159PT was significantly lower than that in human mammary epithelial cells MCF-10A. However,

there are still no reports on associations between MEOX2 expression and clinicopathological features of breast cancer.

In this study, we firstly revealed MEOX2 is associated with clinicopathological features and prognosis in breast cancer. The results showed that the MEOX2 mRNA level was obviously higher in normal breast tissues than in breast cancer tissues. Moreover, low MEOX2 expression was associated with ER-negative, PR-negative, HER2 positive, and p53 mutation subtypes of breast cancer. Indeed, MEOX2 expression was lower in a triple-negative group. The decreasing of MEOX2 expression was significantly correlated with the decreased overall survival days by KM Plotter analysis. Furthermore, we used a high-throughput tissue microarray to verify the above results by immunohistochemical staining, which showed MEOX2 was detected in 74.1% of the total 135 breast cancer tissues, including 74 cases of low expression and 61 cases of high expression. MEOX2 was associated with low histological grade and negatively correlated with Ki67 expression level. Low MEOX2 level implied the poorer prognosis of all breast cancer patients and subtypes of Luminal A and Luminal B. Multivariate COX regression revealed that MEOX2 should be an independent prognostic factor in breast cancer.

MEOX2 was firstly discovered in VSMCs, and was cloned by Gorski et al. in 1993[18]. It inhibited the proliferation and regulated the transition of vascular smooth muscle cells[19,20]. Furthermore, MEOX2 could impede endothelial cell angiogenesis by down-regulating the NF-kB downstream target genes via preventing the binding of NF-kB to its target. It activated p21 WAF1/ CIP1 transcription in vascular endothelial cells through direct interaction with upstream A T-rich s sequences to inhibit angiogenesis[21,22]. MEOX2 could also promote cellular senescence via up-regulating of cyclin dependent kinase inhibitors p16 and p21[8]. Besides, TGF-b was reported to regulate MEOX2 expression in epithelial cells through Smad signaling[23].

Notably, instead of a maladaptation role in vasculogenesis, MEOX2 promoted the development of vascular cells in endothelial colony forming cells (ECFCs), and enhanced network formation in DM pregnancies[24]. In Alzheimer's disease, the MEOX2-induced effect on angiogenesis was biphasic, MEOX2 were anti-angiogenic at a high level, while proangiogenic at a moderate level[25,26].

MEOX2 functions a dual role in tumor development and prognosis, however, its specific mechanism was still unclear. MEOX2 expression was significantly suppressed in liver cancer tissues. The decreased expression was associated with edmondson staging, vascular invasion, envelope invasion, and shorter overall survival time and disease-free survival, suggesting that it was an independent prognostic factor in liver cancer[27,28]. Compared with normal laryngeal tissue, MEOX2 expression in laryngeal carcinoma tissue was significantly lower. It was correlated with TNM stage, histological differentiation, and tumor grade. This was due to MEOX2 could inhibit cell viability and promote apoptosis by regulating apoptosis related factors and the PI3K/Akt pathway[13]. Loss of MEOX2 in Wilms Tumor may accelerate angiogenesis and augment signals in Wnt pathway[29]. In this way, MEOX2 was acted as a potential tumor suppressor gene.

On the other hand, MEOX2-GLI1 transcription axis in lung cancer was involved in the migration, diffusion, and resistance to cisplatin of cancer cells. MEOX2-GLI1 was related to poor overall survival in lung cancer patients. Accompanying by epigenetic events constituted, overexpression of MEOX2 might cause a new cancer drug resistance mechanism [30,31]. Another study suggested that the MEOX2 level in lung cancer was decreased, it may be downregulated by DNA methylation[32]. In gastric cancer, the conclusion was contrary to that in lung cancer. Overexpression of E2F1 induced the down regulation of MEOX2, reducing the sensitivity of cells to anti-cancer drugs and inhibiting cell apoptosis. In addition, there were some other similar reports in glioma[33,34], pancreas tumor[35], and colon tumors[36]. Recently, different miRNAs have been found to regulate MEOX2 expression in cancers, such as miR-221[37], miR-301[38] and miR-301a[28].

Our data got similar conclusions to the reports in liver cancer and laryngeal cancer. We confirmed that the decreasing of MEOX2 expression suggested a poor prognosis of breast cancer. Ki67 was a vital index to evaluate the proliferation of tumor cells, and it is inversely correlated with MEOX2 expression, which indicates that MEOX2 might inhibit the malignant behaviors of breast cancer cells.

However, our work also exists certain limitations. First, it was a retrospective analysis article, and the results might be affected by other factors. Secondly, the specific mechanisms of MEOX2 in breast cancer were not investigated, we only revealed an association of MEOX2 with clinicopathological features by immunohistochemical staining.

In conclusion, lower MEOX2 expression was related to tumor proliferation and could be a new diagnostic and prognostic biomarker of breast cancer.

# Declarations


### Funding information

This work was supported by the Health Research Foundation of Shaanxi Province (No.2018D029).


### Competing interests

The authors declare that they have no competing interests.

# References


1. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2019. *Ca-Cancer J Clin*, **69**, 7–34 https://doi.org/doi:10.3322/caac.21551 (2019).
2. Harbeck, N. *et al.* Breast cancer. *Nature reviews. Disease primers*, **5**, 66 https://doi.org/doi:10.1038/s41572-019-0111-2 (2019).
3. Harbeck, N. & Gnant, M. Breast cancer., **389**, 1134–1150 https://doi.org/doi:10.1016/s0140-6736(16)31891-8 (2017).

4. DeSantis, C. E. *et al.* Breast cancer statistics, 2019. *Ca-Cancer J Clin*, **69**, 438–451 https://doi.org/doi:10.3322/caac.21583 (2019).
5. Nakamoto, S. *et al.* Systemic immunity markers associated with lymphocytes predict the survival benefit from paclitaxel plus bevacizumab in HER2 negative advanced breast cancer. *Sci Rep-Uk*, **11**, https://doi.org/doi: 10.1038/s41598-021-85948-2 (2021).
6. Northcott, J. M., Czubryt, M. P. & Wigle, J. T. Vascular senescence and ageing: a role for the MEOX proteins in promoting endothelial dysfunction. *Can J Physiol Pharm*, **95**, 1067–1077 https://doi.org/doi:10.1139/cjpp-2017-0149 (2017).
7. Cantile, M., Schiavo, G., Terracciano, L. & Cillo, C. Homeobox genes in normal and abnormal vasculogenesis. *Nutr Metab Cardiovas*, **18**, 651–658 https://doi.org/doi:10.1016/j.numecd.2008.08.001 (2008).
8. Douville, J. M., Cheung, D. Y. C., Herbert, K. L., Moffatt, T. & Wigle, J. T. Mechanisms of MEOX1 and MEOX2 Regulation of the Cyclin Dependent Kinase Inhibitors p21(ClP1/WAF1) and p16(INK4a) in Vascular Endothelial Cells., **6**, https://doi.org/doi: 10.1371/journal.pone.0029099 (2011).
9. Chen, Y., Rabson, A. B. & Gorski, D. H. MEOX2 regulates nuclear factor-kappa B activity in vascular endothelial cells through interactions with p65 and I kappa B beta. *Cardiovasc Res*, **87**, 723–731 https://doi.org/doi:10.1093/cvr/cvq117 (2010).
10. Liu, Y. *et al.* Identification of tumor microenvironment-related prognostic genes in colorectal cancer based on bioinformatic methods. *Sci Rep-Uk*, **11**, https://doi.org/doi:10.1038/s41598-021-94541-6 (2021).
11. Wang, Z. *et al.* MEOX2 serves as a novel biomarker associated with macrophage infiltration in oesophageal squamous cell carcinoma and other digestive system carcinomas. *Autoimmunity*, **54**, 373–383 https://doi.org/doi:10.1080/08916934.2021.1919880 (2021).
12. Zhou, P. *et al.* Growth arrest-specific homeobox is associated with poor survival in patients with hepatocellular carcinoma. *Med. Oncol*, **29**, 3063–3069 https://doi.org/doi:10.1007/s12032-012-0258-0 (2012).
13. Tian, L. *et al.* Over-expression of MEOX2 promotes apoptosis through inhibiting the PI3K/Akt pathway in laryngeal cancer cells. *Neoplasma*, **65**, 745–752 https://doi.org/doi:10.4149/neo_2018_171218N824 (2018).
14. Ohshima, J. *et al.* Two Candidate Tumor Suppressor Genes, MEOX2 and SOSTDC1, Identified in a 7p21 Homozygous Deletion Region in a Wilms Tumor. *Gene Chromosome Canc*, **48**, 1037–1050 https://doi.org/doi:10.1002/gcc.20705 (2009).
15. Feng, R. M., Zong, Y. N., Cao, S. M. & Xu, R. H. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? *Cancer Commun*, **39**, https://doi.org/doi:10.1186/s40880-019-0368-6 (2019).
16. Arreal, L. *et al.* Targeting PML in triple negative breast cancer elicits growth suppression and senescence. *Cell Death Differ*, **27**, 1186–1199 https://doi.org/doi:10.1038/s41418-019-0407-5 (2020).

17. Yang Fu, N. & Visvader, J. E. Halting triple negative breast cancer by targeting PROCR. *Cell research*, **29**, 875–876 https://doi.org/doi:10.1038/s41422-019-0245-5 (2019).
18. Gorski, D. H. *et al.* Molecular cloning of a diverged homeobox gene that is rapidly down-regulated during the G0/G1 transition in vascular smooth muscle cells. *Mol Cell Biol*, **13**, 3722–3733 https://doi.org/doi:10.1128/mcb.13.6.3722 (1993).
19. Smith, R. C. *et al.* p21CIP1-mediated inhibition of cell proliferation by overexpression of the gax homeodomain gene. *Genes Dev*, **11**, 1674–1689 https://doi.org/doi:10.1101/gad.11.13.1674 (1997).
20. Zheng, H. *et al.* Gax regulates human vascular smooth muscle cell phenotypic modulation and vascular remodeling. *Am J Transl Res*, **8**, 2912–2925 (2016).
21. Patel, S., Leal, A. D. & Gorski, D. H. The homeobox gene Gax inhibits angiogenesis through inhibition of nuclear factor-kappaB-dependent endothelial cell gene expression. *Cancer Res*, **65**, 1414–1424 https://doi.org/doi:10.1158/0008-5472.CAN-04-3431 (2005).
22. Chen, Y., Leal, A. D., Patel, S. & Gorski, D. H. The homeobox gene GAX activates p21 WAF1/CIP1 expression in vascular endothelial cells through direct interaction with upstream AT-rich sequences. *J Biol Chem*, **282**, 507–517 https://doi.org/doi:10.1074/jbc.M606604200 (2007).
23. Valcourt, U., Thuault, S., Pardali, K., Heldin, C. H. & Moustakas, A. Functional role of Meox2 during the epithelial cytostatic response to TGF-beta. *Mol Oncol*, **1**, 55–71 https://doi.org/doi:10.1016/j.molonc.2007.02.002 (2007).
24. Gohn, C. R., Blue, E. K., Sheehan, B. M., Varberg, K. M. & Haneline, L. S. Mesenchyme Homeobox 2 Enhances Migration of Endothelial Colony Forming Cells Exposed to Intrauterine Diabetes Mellitus. *J Cell Physiol*, **232**, 1885–1892 https://doi.org/doi:10.1002/jcp.25734 (2017).
25. Douville, J. M. & Wigle, J. T. Regulation and function of homeodomain proteins in the embryonic and adult vascular systems. *Can J Physiol Pharmacol*, **85**, 55–65 https://doi.org/doi:10.1139/y06-091 (2007).
26. Wu, Z. *et al.* Role of the MEOX2 homeobox gene in neurovascular dysfunction in Alzheimer disease. *Nat Med*, **11**, 959–965 https://doi.org/doi:10.1038/nm1287 (2005).
27. Zhou, P. *et al.* Growth arrest-specific homeobox is associated with poor survival in patients with hepatocellular carcinoma. *Med Oncol*, **29**, 3063–3069 https://doi.org/doi:10.1007/s12032-012-0258-0 (2012).
28. Zhou, P. *et al.* miR-301a is a candidate oncogene that targets the homeobox gene Gax in human hepatocellular carcinoma. *Dig Dis Sci*, **57**, 1171–1180 https://doi.org/doi:10.1007/s10620-012-2099-2 (2012).
29. Ohshima, J. *et al.* Two candidate tumor suppressor genes, MEOX2 and SOSTDC1, identified in a 7p21 homozygous deletion region in a Wilms tumor. *Genes Chromosomes Cancer*, **48**, 1037–1050 https://doi.org/doi:10.1002/gcc.20705 (2009).
30. Armas-Lopez, L. *et al.* Epigenomic study identifies a novel mesenchyme homeobox2-GLI1 transcription axis involved in cancer drug resistance, overall survival and therapy prognosis in lung cancer patients. *Oncotarget*, **8**, 67056–67081 https://doi.org/doi:10.18632/oncotarget.17715 (2017).

31. Avila-Moreno, F. *et al.* Overexpression of MEOX2 and TWIST1 is associated with H3K27me3 levels and determines lung cancer chemoresistance and prognosis., **9**, e114104 https://doi.org/doi:10.1371/journal.pone.0114104 (2014).
32. Cortese, R., Hartmann, O., Berlin, K. & Eckhardt, F. Correlative gene expression and DNA methylation profiling in lung development nominate new biomarkers in lung cancer. *Int J Biochem Cell Biol*, **40**, 1494–1508 https://doi.org/doi:10.1016/j.biocel.2007.11.018 (2008).
33. Tachon, G. *et al.* Prognostic significance of MEOX2 in gliomas. *Mod Pathol*, **32**, 774–786 https://doi.org/doi:10.1038/s41379-018-0192-6 (2019).
34. Vastrad, B., Vastrad, C., Godavarthi, A. & Chandrashekar, R. Molecular mechanisms underlying gliomas and glioblastoma pathogenesis revealed by bioinformatics analysis of microarray data. *Med Oncol*, **34**, 182 https://doi.org/doi:10.1007/s12032-017-1043-x (2017).
35. Permuth-Wey, J. *et al.* A genome-wide investigation of microRNA expression identifies biologically-meaningful microRNAs that distinguish between high-risk and low-risk intraductal papillary mucinous neoplasms of the pancreas., **10**, e0116869 https://doi.org/doi:10.1371/journal.pone.0116869 (2015).
36. Xing, Y. *et al.* Comprehensive analysis of differential expression profiles of mRNAs and lncRNAs and identification of a 14-lncRNA prognostic signature for patients with colon adenocarcinoma. *Oncol Rep*, **39**, 2365–2375 https://doi.org/doi:10.3892/or.2018.6324 (2018).
37. Chen, Y. *et al.* Regulation of the expression and activity of the antiangiogenic homeobox gene GAX/MEOX2 by ZEB2 and microRNA-221. *Mol Cell Biol*, **30**, 3902–3913 https://doi.org/doi:10.1128/MCB.01237-09 (2010).
38. Cao, G. *et al.* Intronic miR-301 feedback regulates its host gene, ska2, in A549 cells by targeting MEOX2 to affect ERK/CREB pathways. *Biochem Biophys Res Commun*, **396**, 978–982 https://doi.org/doi:10.1016/j.bbrc.2010.05.037 (2010).
39. Chandrashekar, D. S. *et al.* A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia*, **19**, 649–658 https://doi.org/doi:10.1016/j.neo.2017.05.002 (2017).
40. Jezequel, P. *et al.* bc-GenExMiner: an easy-to-use online platform for gene prognostic analyses in breast cancer. *Breast Cancer Res Treat*, **131**, 765–775 https://doi.org/doi:10.1007/s10549-011-1457-7 (2012).
41. Jezequel, P. *et al.* bc-GenExMiner 3.0: new mining module computes breast cancer gene expression correlation analyses. *Database (Oxford)* 2013, bas060, doi:10.1093/database/bas060 (2013)
42. Lanczky, A. *et al.* miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. *Breast Cancer Res Treat*, **160**, 439–446 https://doi.org/doi:10.1007/s10549-016-4013-7 (2016).
43. Gyorffy, B. *et al.* An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res Treat*, **123**, 725–731 https://doi.org/doi:10.1007/s10549-009-0674-9 (2010).

# Figures

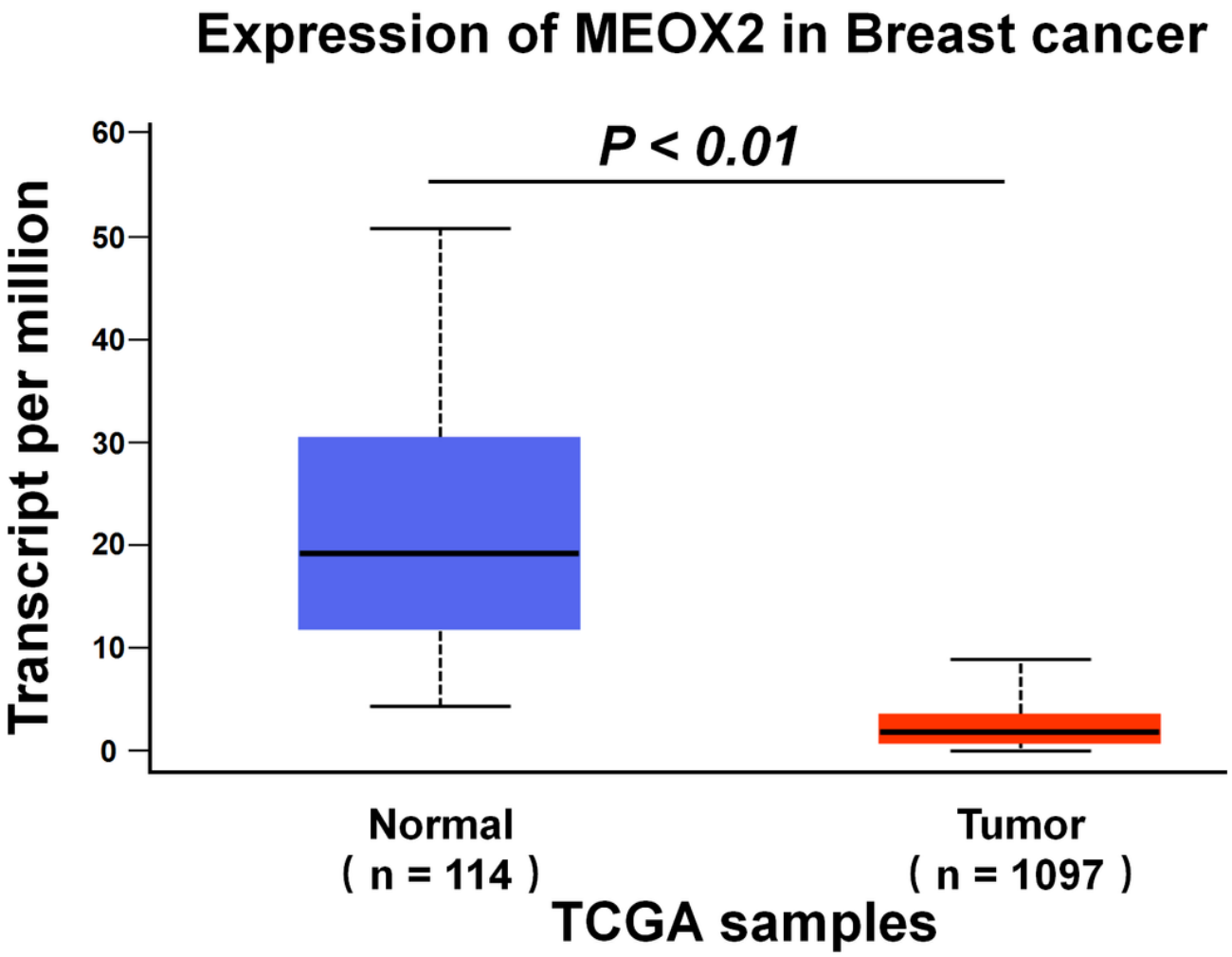
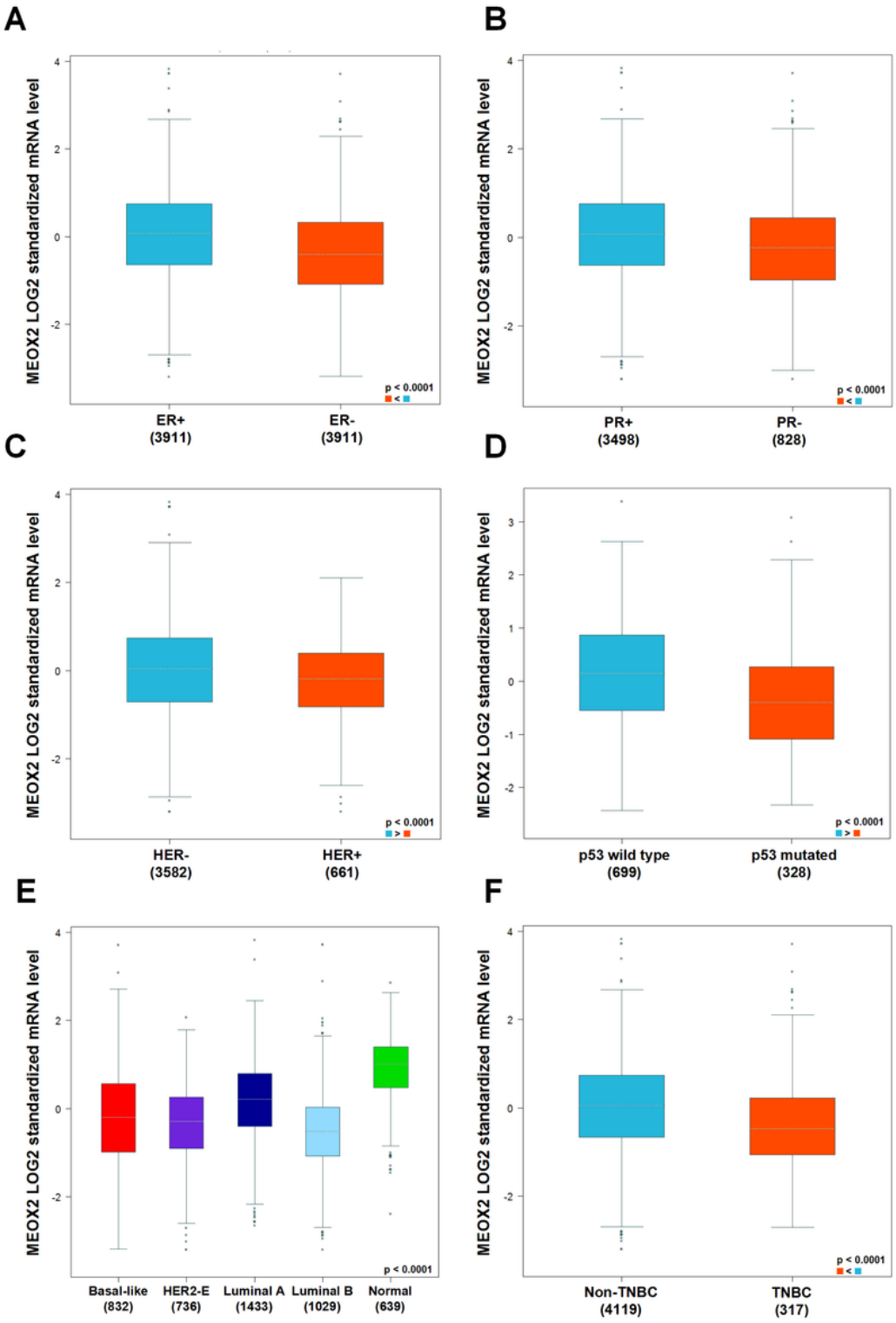


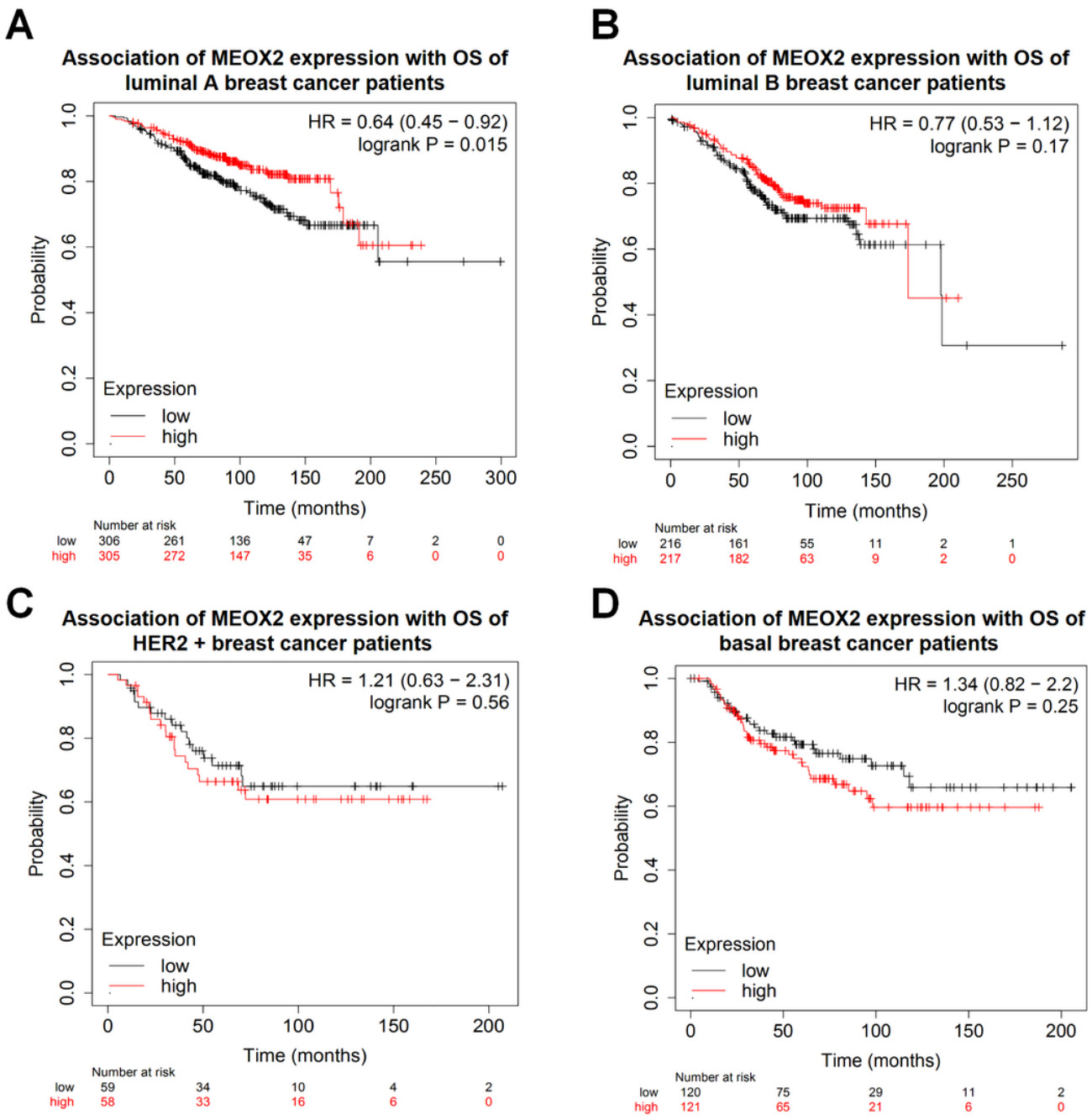

**Figure 1**

Expression of MEOX2 mRNA in breast cancer tissues and normal tissues. Box plot was produced by UALCAN database.



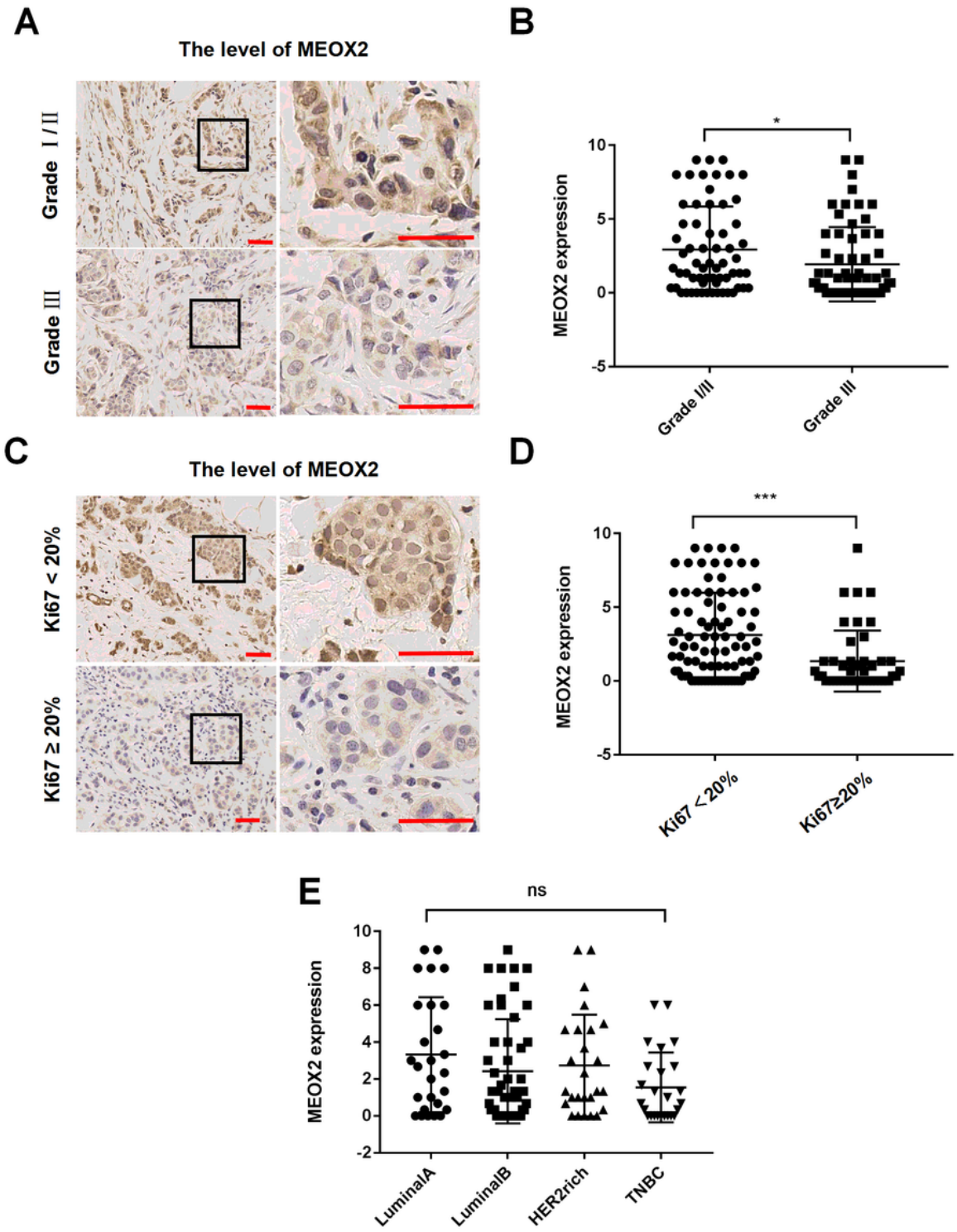

## Figure 2

The association of MEOX2 expression with clinicopathological features in breast cancer. The association of MEOX2 expression with (A) ER expression (n = 4462, P < 0.0001); (B) PR expression (n = 4326, P < 0.0001); (C) HER2 expression (n = 4243, P < 0.0001); (D) mutation of p53 (n = 1027, P < 0.0001); (E) cancer subtype (n = 4669, P□0.0001), and (F) TNBC status (n = 4436, P < 0.0001). Box plots were produced by bc-GenExMinerv4.5. Data was analyzed by unpaired t test.



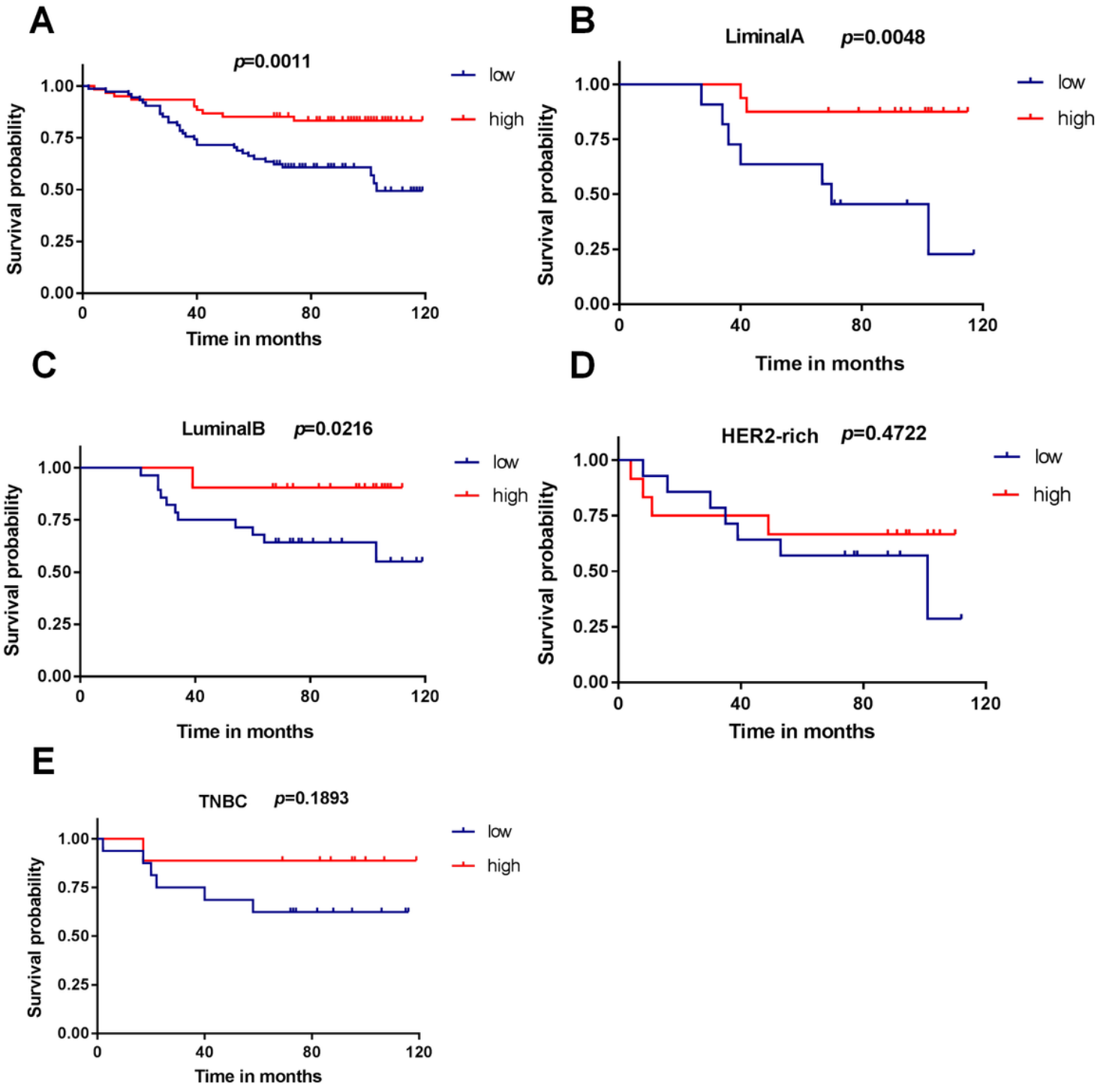

## Figure 3

The association of MEOX2 mRNA expression with survival. The association of MEOX2 expression with overall survival (OS) of (A) Luminal A (P=0.015) (B) Luminal B (P=0.17) (C) HER2-rich (P=0.56) (D) basal-like subtype (P=0.25). Kaplan-Meier curves were produced using KM Plotter.




## Figure 4

The protein level of MEOX2 expression was detected by immunohistochemical staining. Immunohistochemical analysis of MEOX2 expression in breast cancer with (A) (B) different histological grades (P = 0.0152, Mann–Whitney test). (C)(D) different Ki67 expression levels (P = 0.0002, Mann–Whitney test). (E) different molecular subtypes (P = 0.1618, Kruskal–Wallis test) . Immunohistochemical image scale bar = 50μm.




## Figure 5

MEOX2 is an independent factor associated with patient's survival. The association of MEOX2 expression with OS among (A) all breast cancer patients (High expression n = 61, Low expression n = 74; P = 0.0011, log-rank test) (B) Luminal A subtype (High expression n = 16, Low expression n = 11; P = 0.0048, log-rank test) (C) Luminal B subtype (High expression n = 21, Low expression n = 28; P =0.0216, log-rank test) (D)

HER-2 rich subtype (High expression n = 12, Low expression n = 14; P =0.4722, log-rank test) (E) TNBC subtype (High expression n = 9, Low expression n = 16; P = 0.1893, log-rank test).