# ELK1-induced up-regulation of KIF26B promotes cell cycle progression in breast cancer

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**Research Article**

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# Abstract

**Purpose:** KIF26B is a member of the kinesin superfamily that is up-regulated in various tumors, including breast cancer (BC), which can promote tumor progression. This study aimed to investigate the potential function of KIF26B in BC, and the underlying mechanisms, focusing mainly on cell proliferation.

**Methods:** KIF26B expression was examined in BC tissue samples obtained from 99 patients. We performed MTS, EdU and flow cytometry assays to detect cell proliferation, and western blotting to measure the expression of cell cycle-related proteins in MDA-MB-231 and MDA-MB-468 cells following KIF26B knockdown. Promoter analysis was used to study the upstream regulatory mechanism of KIF26B.

**Results:** KIF26B was upregulated in BC tissue samples. High expression of KIF26B was associated with clinicopathological parameters, such as positive lymph node metastasis, higher tumor grade and high proliferative index in BC tissue. Furthermore, knockdown of KIF26B expression inhibited MDA-MB-231 and MDA-MB-468 cell proliferation, arresting cells in the $G_1$ phase of the cell cycle *in vitro*. Similarly, KIF26B silencing decreased the expression levels of Wnt, β-catenin and cell cycle-related proteins such as c-Myc, cyclin D1 and cyclin-dependent kinase 4, whilst increasing the expression of p27. Moreover, ELK1 could bind to the core promoter region of KIF26B and activate its transcription.

**Conclusion:** KIF26B acts as an oncogene in BC that can promote development of BC by regulating multiple proteins involved in the cell cycle. ELK1 activates KIF26B transcription.


# Introduction

Breast cancer (BC) is one of most common malignant tumors and has become the second risk factor for cancer-related mortality in women worldwide. Among multiple causes, metastasis and uncontrolled proliferation of tumor cells are two of the most important factors [1, 2]. Thus, in this study, we examine the mechanisms associated with the proliferation of BC cells, with the aim of identifying a promising target for the treatment of patients with BC.

KIF26B is a member of the kinesin family (KIF) composed of 2,108 amino acids. KIF26B cannot hydrolyze ATP [3]. Accumulating evidence demonstrates that KIF26B can regulate cytoskeleton-driven processes, such as cell migration, polarization, mitosis and adhesion and plays an important role in the development of the kidney and the nervous system [4-6]. More recent studies have suggested that KIF26B plays an important role in the oncogenesis or progression of many human cancer types [7, 8]. High KIF26B expression is strongly linked to poor prognosis in patients with BC or colorectal cancer [9-11]. However, the role of KIF26B in BC, especially the relationship between high KIF26B expression and cell proliferation, has rarely been reported.

The present study aims to clarify the biological function of KIF26B in BC. In this study, we demonstrate the effect KIF26B on BC cell proliferation and cell cycle progression and explore the upstream transcriptional mechanism of KIF26B.

# Materials And Methods

## Human BC samples

A total of 99 paraffin-embedded BC tissue samples were collected from patients undergoing mammectomy and needle biopsy at Qilu Hospital of Shandong University during 2005-2010. Pathomorphological observation was performed according to the criteria established by the World Health Organization. This study was approved by the Ethical Committee of Shandong University, China (code 2012028).

## Immunohistochemistry (IHC)

Paraffin-embedded sections were immunostained with antibodies against KIF26B

(1:100, Proteintech, Wuhan, China, 17422-1-AP), ER (MaxVision, Fujian, China, Kit-0012), PR (MaxVision, Fujian, China, Kit-0013), HER2 (MaxVision, Fujian, China, Kit-0012) and Ki67 (Maxvision, Fujian, China, Kit-0002), and were assessed by two experienced pathologists. For negative controls, the primary antibody was replaced with PBS. IHC was scored using a semi-quantitative scoring system. Intensity was scored as 1, 2 and 3 for weak, moderate, and strong staining, respectively. Percentage (P) and intensity (I) were multiplied to generate numerical score (S=P*I). The median score was used as cut-off to distinguish between KIF26B high and low expression.

## RNA extraction and RT-qPCR

Total RNA was extracted from cultured cells using TRIzol (Invitrogen) according to manufacturer's instructions, then reverse transcribed into cDNA using ReverTraAce qPCR RT Kit (Toyobo, Japan). RT-qPCR was then performed using SYBR Green PCR Kit (Roche Diagnostic GmbH, Mannheim, Germany). Relative expression was evaluated using the $2^{-\triangle Ct}$ method using GAPDH as the reference gene. Primers for RT-qPCR were as follows: KIF26B forward, 5'-AGGCCATGTGCTTAATGCAAA-3' and reverse 5'-ATCCAGCATCAGATACTGTTTGGT-3'; GAPDH forward 5'-AGAAGGCTGGGGCTCATTTG-3' and reverse 5'-AGGGGCCATCCACAGTCTTC-3'.

## Cell lines

Human BC cell lines MDA-MB-231, MDA-MB-468, MDA-MB-453, MCF-7, SKBR3, MCF-10A and 293T cells were purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). BC cell lines were cultured in Leibovitz's L15 medium (Gibco) containing 10% fetal bovine serum (FBS; Gibco). 293T cells were cultured in DMEM (Gibco) containing 10%FBS. All cells were cultured in a humidified atmosphere at 37°C with 5% $CO_2$.

## SiRNA and cell transfection

KIF26B small interfering RNA (si-KIF26B) and negative control (si-NC) were synthesized by RiboBio (Guangzhou, China). The sequence of si-KIF26B was 5'-CGGACAGCCTCTCCTATTA-3' and the si-NC sequence was 5'-TTCTCCGAACGTGTCACGT-3' [10]. After reaching 90% confluence, MDA-MB-231 and MDA-MB-468 cells were seeded in 6- or 12-well plates and transiently transfected with siRNA using the X-treme GENE transfection reagent (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's instructions. The examination of KIF26B expression level and subsequent experiments were performed 48 h later.

### MTS assay

MDA-MB-231 and MDA-MB-468 cells transfected with si-KIF26B or si-NC for 24 h were seeded in 96-well plates. MTS (5 mg/ml, Promega, Madison, WI, USA) was added into the culture medium at 24, 48,72 and 96 h, respectively, followed by an incubation of 2 h. The absorbance at 490 nm was then read. Three independent experiments were performed.

### EdU assay

MDA-MB-231 andMDA-MB-468 cells transfected with si-KIF26B or si-NC were cultured in 6-well plates for 24 h. The cells were then collected and cultured in 12-well plates for 24 h. The EdU assay was performed in according to the manufacturer's instructions (RiboBio, Guangzhou, China). Cells were visualized under a fluorescent microscope (Olympus, Tokyo, Japan). The experiment was repeated three times.

### Flow cytometry

MDA-MB-231 and MDA-MB-468 cells transfected with si-KIF26B or si-NC were cultured in 6-well plates for 48 h. For the cell cycle assay, the harvested cells were stained with propidium iodide (PI, Beyotime). For apoptosis detection, cells were double-stained with annexin V-fluorescein isothiocyanate (FITC) and PI using an FITC annexin V Apoptosis Detection kit (401003, BestBio, China). The cells were immediately examined by flow cytometry (FACScan; BD Biosciences), according to the manufacturer's protocol. Each experiment was performed in triplicate.

### Dual-luciferase reporter assays

293T cells were seeded in 24-well plates overnight, then co-transfected using 0.5 μg/well of the KIF26B promoter regions (-2,000/0, -1,043/0, -530/0 and -110/0) overexpression plasmids and pGL3-basic vector and 0.01 μg of pRL-TK plasmid (as the internal control) using Lipofectamine 2000 (11668019, Invitrogen). About 48 h after transfection, the cells were subjected to luciferase activity analysis using a Dual-Luciferase Reporter Assay Systems (Promega) to decide the core promoter of KFI26B gene according to the manufacturer's instructions. Similaly, the plasmids with decided core promoter of KFI26B gene and alternative transcription factor overexpression plasmids(ELK1, STAT4, JUN, pCDNA3.1 basic vector) and 0.01 μg of pRL-TK plasmid were co-transfected to 293T cells to establish the relevant transcriptional factor of KIF26B. Each experiment was performed in triplicate.

### Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were carried out using the EZ-Magna ChIP Chromatin Immunoprecipitation Kit (17-10086, Merck). Briefly, the MDA-MB-231 and MDA-MB-468 cells were crosslinked with 1% formaldehyde for 10 min at room temperature before immunoprecipitation reaction. After incubation with 5 μl anti-ELK1 antibody (ab32106, Abcam) or IgG antibody (rabbit anti- IgG, as the negative control) and protein A/G magnetic beads overnight, immunoprecipitation was performed in accordance with the manufacturer's instructions. Co-precipitated DNA was quantified using qPCR. A volume of 10 μl chromatin was used as input control prior to immunoprecipitation. This experiment was repeated three times.

### Vector construction

The KIF26B promoter regions (-2,000/0, -1,043/0, -530/0 and -110/0) were amplified from genomic DNA extracted from MDA-MB-231 cells, then cloned into the pGL3-Basic vector (Promega). The plasmids containing ELK1, STAT4, JUN, and pcDNA3.1 vector originated from our research group.

### Western blotting

Western blotting was carried out using standard protocols. The primary antibodies used were as follows: KIF26B (1:500, 17422-1-AP, Proteintech), ELK1 (1:500, ab32106, Abcam), Wnt6 (1:1,500, ab50030, Abcam), GAPDH (1:1,000, #5174, CST), β-catenin (1:2,000, ab32532, Abcam), c-Myc (1:1,000, #18583, CST), cyclin D1 (1:1,000, #55506, CST), CDK4 (1:1,000, #12790, CST), p27 (1:1,000, #3686, CST)□cyclin E1 (1:1,000, #20808, CST), CDK2 (1:1,000, #18048, CST), p21 (1:1,000, #2947, CST, CDK6 (1:1,000, #13331, CST), p53 (1:1,000, #9282, CST), p-Rb (1:1,000, #8516, CST). Each experiment was performed in triplicate.

### Statistical analysis

Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Differences between the two groups were analyzed using Student's t test. The $\chi^2$ test and Spearman's correlation were used to evaluate the associations between KIF26B expression levels and clinicopathological parameters in BC. Survival analysis was performed using the Kaplan-Meier method and log-rank test. Data are presented as the mean ± SD. $P<0.05$ was considered statistically significant.

## Results

### KIF26B is up-regulated in BC tissues and cell lines

To study the role of KIF26B in BC, IHC was carried out to detect KIF26B expression in 99 samples from patients with BC with follow-up data. We found that KIF26B was significantly up-regulated in ductal carcinoma *in situ* (DCIS) and invasive ductal carcinoma (IDC) compared with adjacent normal tissues ($p<0.0001$). However, no significant difference was observed between IDC and DCIS ($p=0.3524$). In

addition, the staining intensity of KIF26B increased with the grade of DCIS and IDC (Figure 1 a-h). All samples were then assigned to a high or low KIF26B expression group, using median KIF26B expression as cut-off point. High expression of KIF26B was markedly associated with clinicopathological parameters such as positive lymph node metastasis, higher tumor grade and high proliferative index (Table 1). However, survival analysis showed that high KIF26B expression was not associated with overall survival and disease-free survival (Figure1 i-j).

We also examined the expression of KIF26B in cell lines using RT-qPCR. KIF26B was expressed at low levels in an immortalized breast cell line MCF-10A, but expressed at high levels in BC cell lines, especially in cells with a higher degree of malignancy, such as MDA-MB-231 and MDA-MB-468 (Figure 1k). Additionally, Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) suggested that KIF26B expression was significantly increased in BC tumor tissue, compared with normal tissue (Figure 2a). Moreover, the expression levels of KIF26B were not associated with overall survival and disease-free survival (Figure 2b-c). Thus, KIF26B expression was significantly up-regulated in BC tissue samples and cell lines, and KIF26B may be associated with cancer progression.

## ELK1 activates KIF26B transcription

To examine the upstream regulatory molecule of KIF26B, we performed a promoter analysis. The sequence of the human KIF26B promoter from -2,000 bp to 0 bp (the first base of KIF26B cDNA, i.e. the transcription start site, is designated +1) was acquired from the UCSC Genome Browser (http://genome.ucsc.edu/). We constructed a series of truncated fragments from -2,000/-1,043/-530/-110 to 0, which were then cloned into the pGL3-basic vector (Figure 3a). After transient transfection in 293T cell for 48 h, luciferase activity was decreased distinctly in the -530 to -110 bp region ($p<0.001$), suggesting that this region may be a core promoter of the human KFI26B gene (Figure 3b). Using the online JASPAR program, we identified potential transcription factors that might bind to the -530 to -110 bp region from the (Figure S1), then validated them using dual-luciferase report assays. ELK1 markedly activated the KIF26B -530 to 0 promoter region, compared with the negative control ($p<0.01$, Figure 3c). RT-qPCR and western blot analysis further indicated that ELK1 could promote KIF26B gene expression (Figure 3d-f). These findings suggested that ELK1 specifically bound to the core promoter of KIF26B and activated its transcription. To further test this hypothesis, we performed a ChIP assay. Using the online JASPAR program, the ELK1 binding site within the core promoter region of KIF26B was identified as 5'-GTCAGGAAG-3' (Figure 3g). PCR products amplified using primers specific for the ELK1-binding sites immunoprecipitated in the input and anti-ELK1 antibody groups, but not in the anti-IgG group (Figure 3i). Additionally, in the anti-ELK1 group, an approximate 3-fold and 60-fold enrichment of the promoter amplicons of the KIF26B binding site were observed in MDA-MB-231 and MDA-MB-468 cells, respectively (Figure 3h). Collectively, these findings demonstrated that ELK1 activated the transcription of KIF26B by directly binding to its core promoter region.

## Knockdown of KIF26B expression inhibits cell proliferation in vitro

To examine the biological function of KIF26B in BC, we performed MTS, EdU and flow cytometry in MDA-MB-231 and MDA-MB-468 cells following KIF26B siRNA silencing. First, we used western blot analysis to detect knockdown efficiency of KIF26B at 48 h following siRNA transient transfection. Knockdown of KIF26B reduced KIF26B protein expression levels (Figure 4a-b). MTS assays demonstrated that KIF26B knockdown group inhibited proliferation compared with the control group ($p<0.01$, Figure 4c-d). Furthermore, the EdU assay showed a similar effect on cell proliferation following KIF26B silencing ($p<0.01$, Figure 4e-f). Flow cytometry analysis suggested that downregulation of KIF26B had no effect on apoptosis of MDA-MB-231 and MDA-MB-468 ($p>0.05$, Figure 4g). Nevertheless, KIF26B knockdown led to a significant increase in the frequency of cells in the $G_1$ phase of the cell cycle, and a decrease in cells in the S phase relative to the control group, both in MDA-MB-231 and MDA-MB-468 cells ($p<0.05$, $p<0.01$, Figure 4h). These findings indicated that KIF26B upregulated cell proliferation *in vitro*, which was likely achieved by promoting cell cycle progression.

### KIF26B promotes cell proliferation through regulating cell cycle related protein expression *in vitro*

We then detected the expression of several cycle-related proteins following KIF26B siRNA knockdown to further explore the mechanism through which KIF26B promotes cell cycle progression. The results revealed that the levels of c-Myc cyclin D1, CDK4, and p-Rb were decreased whereas p27 was increased. The levels of other proteins such as CyclinE1, CDK2, CDK6, p53 and p21 remain unchanged. We also examined the expression levels of Wnt6, β-catenin the key regulatory molecules of c-Myc, which were found to be down-regulated following KIF26B knockdown (Figure 5a).

## Discussion

BC is the second highest risk factor for women's cancer-related mortality following lung cancer and is a highly heterogeneous disease involving various genetic and epigenetic alterations [2]. Numerous gene abnormalities associated with the carcinogenesis and progression of BC have been characterized. For example, kinesin family (KIF) has been reported to play an important role in tumor development, metastasis and drug resistance for BC patients [12-14]. Among these, KIF26B has only recently been reported and is poorly characterized. Our research group has proposed that KIF26B acted as a novel oncogene to promote cell proliferation and metastasis by activating the VEGF pathway in gastric cancer [8]. Other studies reported that KIF26B was abnormally expressed in colorectal and ovarian cancer [15, 16]. However, the potential role and underlying regulatory mechanism of KIF26B in human BC remains unclear.

In the present study, we found that KIF26B was significantly upregulated in BC tissues and high-malignancy cell lines. In addition, high KIF26B expression levels were markedly associated with clinicopathological parameters, such as positive lymph node metastasis, high tumor grade and high proliferative index. This was consistent with a previous report [11]. Survival analysis indicated that patients with high KIF26B expression appeared to have poor prognosis, although this was not statistically significant. This finding requires further validation by expanding the sample size. Gu *et al* found that

knockdown of KIF26B expression inhibited cell proliferation and migration in the MCF-7 and MDA-MB-231 BC cell lines [17]. Accordingly, our results showed that KIF26B silencing inhibited cell proliferation by MTS and EDU assay in MDA-MB-468 and MDA-MB-231 cells. In our study, flow cytometry assays also demonstrated that KIF26B knockdown arrested the cell cycle in the $G_1$ phase, indicating that KIF26B promoting BC proliferation may be associated with cell-cycle transition from the G1 to S phase. In contrast to the study by Gu *et al*, we found that KIF26B had no effect on apoptosis of BC cells. In addition, we investigated the mechanism through which KIF26B promoted cell cycle progression, which had not been addressed previously.

The mechanism underlying abnormal KIF26B expression has never been reported. In the current study, we carried out a transcriptional regulation analysis. We screened and identified the core promoter region of KIF26B using a dual-luciferase reporter assay. Furthermore, ELK1 was identified and found to activate KIF26B transcription by directly binding to the core promoter region of KIF26B. The Ets proteins form a large family of transcription factors with diverse functions. They regulate gene expression by direct or indirect DNA binding. ELK1 along with ELK4/SAP-1 and ELK3/SAP-2/Net make up the ternary complex factor (TCF) subfamily of ETS-domain transcription factors, which bind to a GGAA/T motif and activate target gene transcription [18, 19]. Demir *et al* reported that Elk-1 constantly moved during different stages of the cell cycle and co-localized with kinesin as well as kinesin-like proteins Eg5 and MKLP-1 during mitosis in brain tumor cell lines [20]. However, few studies have evaluated kinesin and ELK1 transcriptional regulation. ELK1 is an element of the Ets family of transcription factors that is associated with malignant progression of BC by integrative bioinformatics analysis of transcriptional regulatory programs [21]. Liu *et al* reported that higher ELK1 mRNA expression was also associated with worse recurrence-free survival in patients with triple-negative by survival analysis [22]. Moreover, ELK-1 has been found to co-localize with kinesin as well as kinesin-like proteins Eg5 and MKLP-1 during mitosis and was presumed to play a synergistic role in the cell-cycle process [20]. Additionally, it was reported that ELK3 knockdown, similar to ELK1 both bind to variants of the GGAA/T motif, suppressed MDA-MB-231 BC cell proliferation with accumulation at the $G_1$ cell cycle phase [20, 23]. Our results showed that ELK1 bound to KIF26B through the GTCA**GGAA**G motif, which verified the conservative combination of ELK3 and ELK1 with their target genes. Therefore, we hypothesized ELK1 might promote the proliferation of BC cells through the same mechanism as ELK3 and could cooperate with KIF26B in the cell cycle. This hypothesis requires further validation. Our study described the mechanism underlying transcriptional activation of ELK1 on KIF26B for the first time.

To identify the mechanism underlying cancer cell proliferation, it is necessary to measure the expression of cell cycle-related proteins, such as cyclins, CDKs, and CKIs. Classically, the cell cycle is involved in at least three concomitant mechanisms: activation of cyclins and CDKs, suppression of the CDK inhibitors p15 and p21 and the degradation of p27 [24]. The CDK2-cyclin E and CDK4/CDK6-cyclin D complexes are required for cell-cycle transition from the $G_1$ to S phase [25]. The activities of CDKs and cyclins in the $G_1$-S phase could be inhibited by CDK inhibitors, particularly p27 [26]. In this study, western blot analysis

showed that KIF26B silencing not only reduced cyclin D1 and CDK4 and p-Rb levels, but also upregulated p27 expression *in vitro*.

Myc induces cell proliferation by promoting $G_1$ to S phase transition during cell cycle progression Myc-p27 antagonism in several human solid tumors and leukemia [24]. Myc expression is regulated by several signaling pathways including Wnt/β-catenin, Notch, and TGF-β pathways, and Myc expression levels are strongly correlated with the activation of Wnt signaling molecules in BC [27].Previous studies have revealed that KIF26B links Wnt5a-Ror signaling to the control of cell and tissue morphogenetic behavior[28, 29]. However, the relationship between KIF26B and the classical Wnt/β-catenin signaling pathway is largely unknown. Our previous gene expression profile microarray showed that several Wnt signaling components were abnormally expressed following KIF26B knockdown. The levels of Wnt6 and cell membrane-binding molecules of the Wnt pathway, such as FZD and LRP, decreased following KIF26B knockdown. By contrast, the Wnt pathway inhibitors DKK2, ANKRD, NKD, and one of the β-catenin degradation complex molecules APC were upregulated (fold change ≥2, $p$<0.05, accession number GSE72307, supplementary figure 2). As a result, the Wnt/β-catenin pathway was inactivated.

In our study, Wnt β-catenin and c-Myc expression levels decreased after KIF26B knockdown, and p27 was up-regulated. Hence, we propose that the transcriptional factor ELK1 regulates KIF26B expression by directly binding to its core promoter region. KIF26B, in turn, modulates Wnt/β-catenin signaling to up-regulate c-Myc and regulate the levels of cell cycle-related protein, including cyclin D1, CDK4 and p27 to promote cell cycle progress (Figure 5b).

# Conclusions

Overall, these findings indicate that KIF26B is an important oncogene that can promote the proliferation of BC cells and cycle progression via Wnt/β-catenin signaling. Moreover, we report for the first time that ELK1 activates KIF26B transcription and enhances KIF26B expression. Thus, KIF26B may represent a potential therapeutic target for the future treatment of patients with BC.

# Declarations

### Conflicts of interest

None.


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### Author contributions

SuXia Wang conducted the experiments and wrote the manuscript. Hui Zhang and HaiTing Liu provided research design. XiangYu Guo and RanRan Ma gave the experimental guidance. WenJie Zhu contributed to the samples collection. P Gao designed and directed the whole research. All authors approved the final manuscript.

### Ethics approval

All procedures were performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and the Ethical Committee of Shandong University, China (code 2012028). This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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# Figures

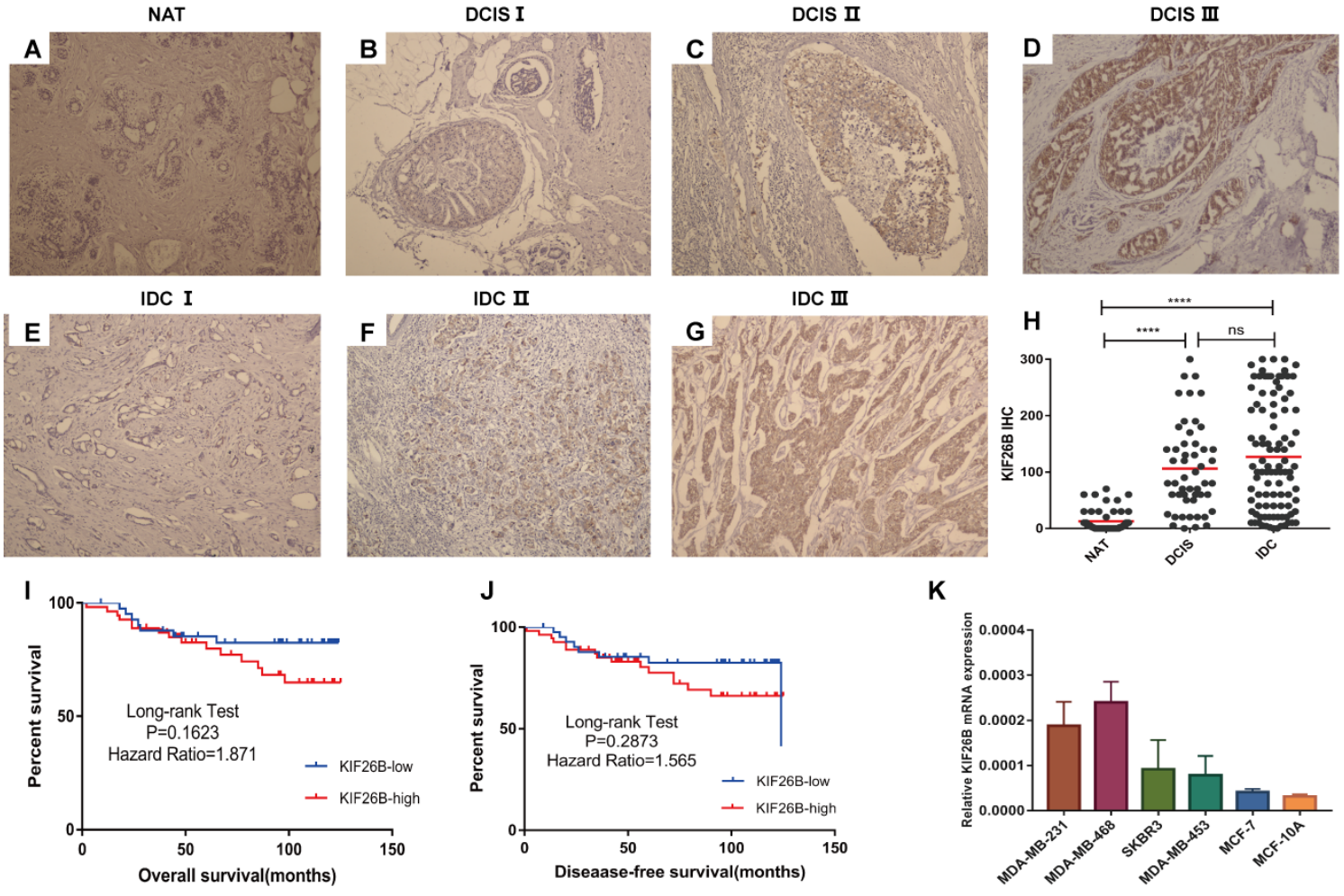
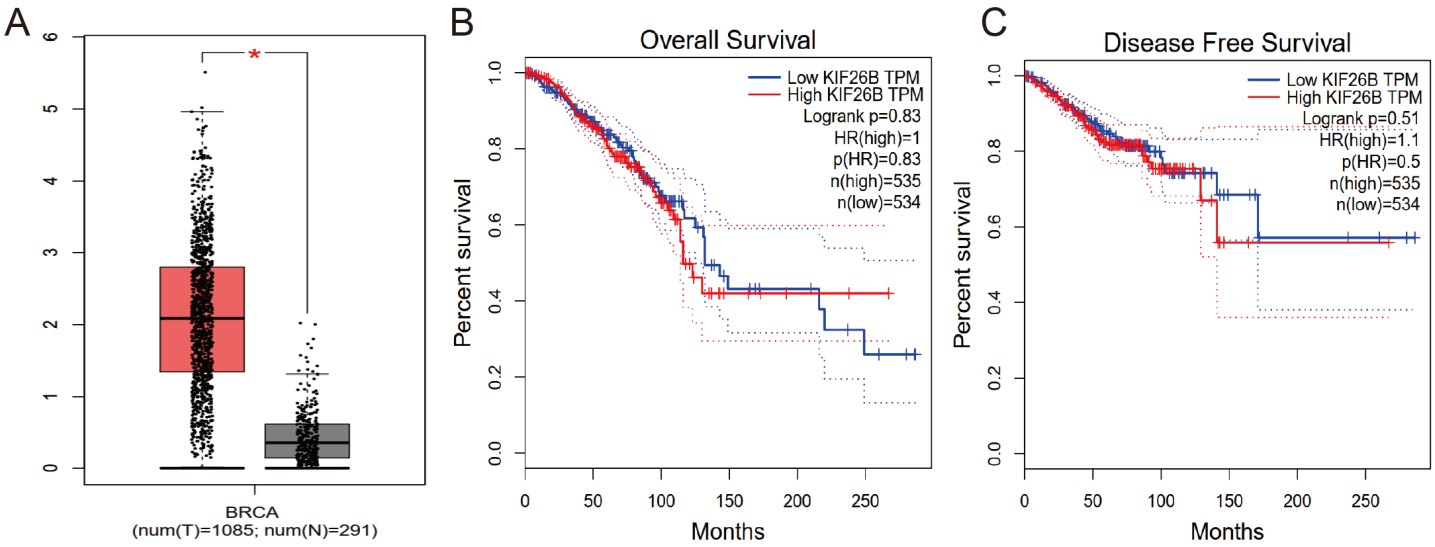



**Figure 1**

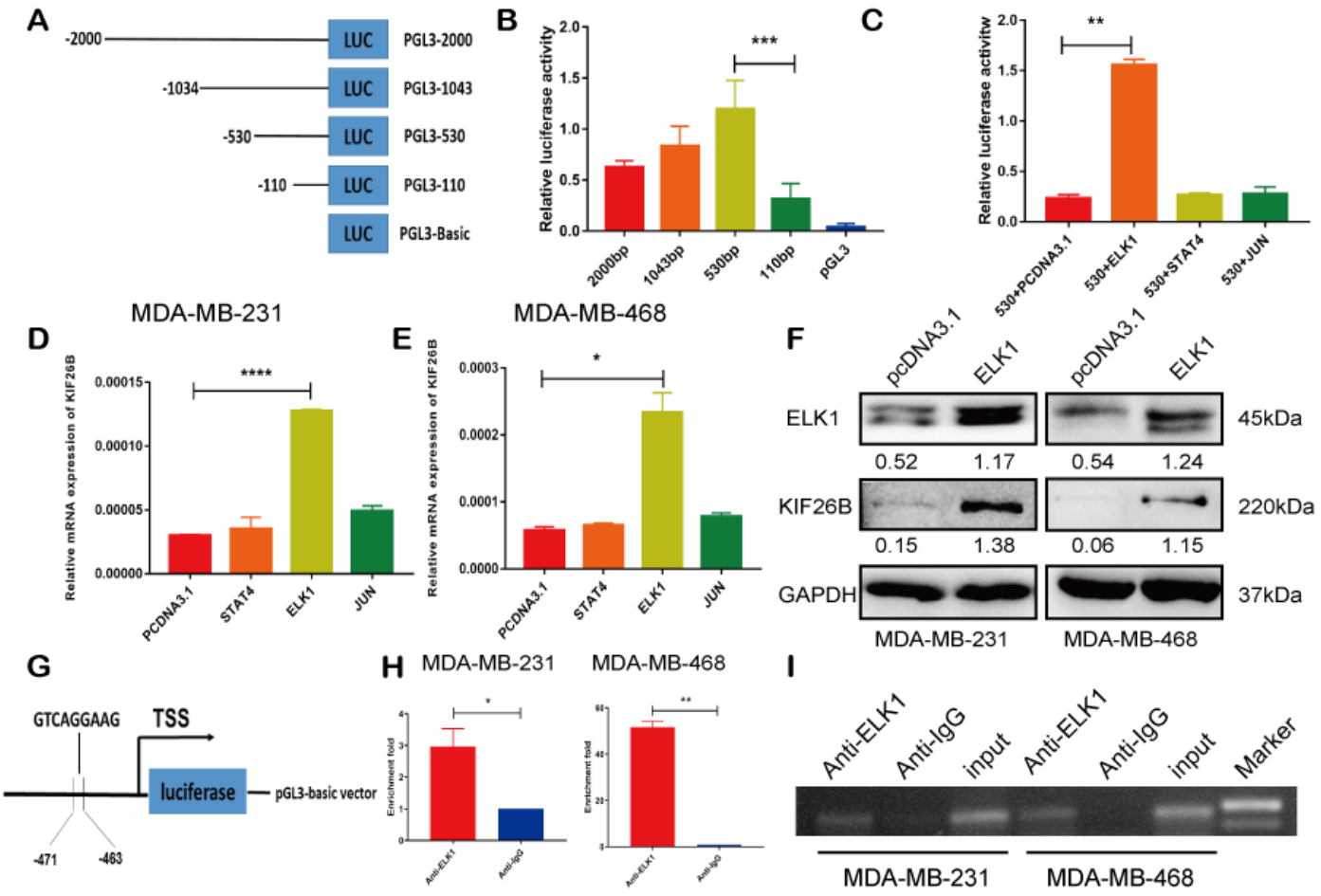
KIF26B is upregulated in human BC tissues and cell lines (a-h) IHC was used to detect the expression of KIF26B in 99 BC tissue samples. Compared with adjacent normal tissues, KIF26B was significantly up-regulated in IDC and DCIS ($p<0.0001$). However, there was no significant difference between IDC and

DCIS (p=0.3524). IHC was scored using a semi-quantitative scoring system. Intensity was scored as 1, 2 and 3 for weak, moderate, and strong staining, respectively. Percentage (P) and intensity (I) were multiplied to generate numerical score (S=P*I). (i-j) Kaplan-Meier curves showed that patients with high KIF26B expression had poorer overall survival and disease-free survival, but without statistical significance (I log-rank, p=0.1623; J log-rank, p=0.2873). (k) The relative expression levels of KIF26B in a panel of breast cell lines. KIF26B expression was weakly detectable in the immortalized BC line MCF-10A, but its expression was markedly increased in BC cell lines, especially in poorly differentiated cell lines MDA-MB-231 and MDA-MB-468.




## Figure 2

KIF2B expression in BRCA in Gene Expression Profiling Interactive Analysis (GEPIA) (a)KIF26B expression was significantly increased in BRCA tumor tissues, compared with normal tissues. (b,c)The expression levels of KIF26B were note associated with overall survival and disease-free survival (http://gepia.cancer-pku.cn/).




## Figure 3

ELK1 activates KIF26B transcription (a) Schematic plot of the KIF26B promoter fragments spanning from -2,000/-1,043/-530/-110 to 0. These promoter fragments were cloned upstream of the firefly luciferase reporter gene in the pGL3-basic vector. (b) Transcriptional activity analysis of the KIF26B promoter fragments in 293T cells showed that luciferase activity significantly decreased in the -530 to -110 region ($p<0.001$), which may be a core promoter of the human KFI26B gene. (c) Luciferase activity assay

indicated that ELK1 signally enhanced promoter activities of pGL3-530/0. (d-f) RT-qPCR and western blot analysis showed ELK1 increased the expression levels of KIF26B in MDA-MB-231 cells and MDA-MB-468 cells. (g) Schematic plot of the luciferase reporter construct including the KIF26B core promoter with presumed ELK1 binding site GTCAGGAAG. (h) ChIP-PCR analysis showed higher enrichment of promoter amplicons of ELK1 in the anti-ELK1 antibody group than in the IgG group in MDA-MB-231 cells and MDA-MB-468 cells, indicating that ELK1 could directly bind to the KIF26B core promoter region. (i) ChIP-PCR analysis indicated that ELK1 directly combined with the KIF26B core promoter region in MDA-MB-231 cells and MDA-MB-468 cells. The input DNA group showed a specific strong band of the predicted size, while the chromatin complex precipitated by antibody against IgG and ELK1 displayed no band or a very weak band, respectively. The primers were specific for the ELK1 binding sites. Three independent experiments were performed, and data are presented as the mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

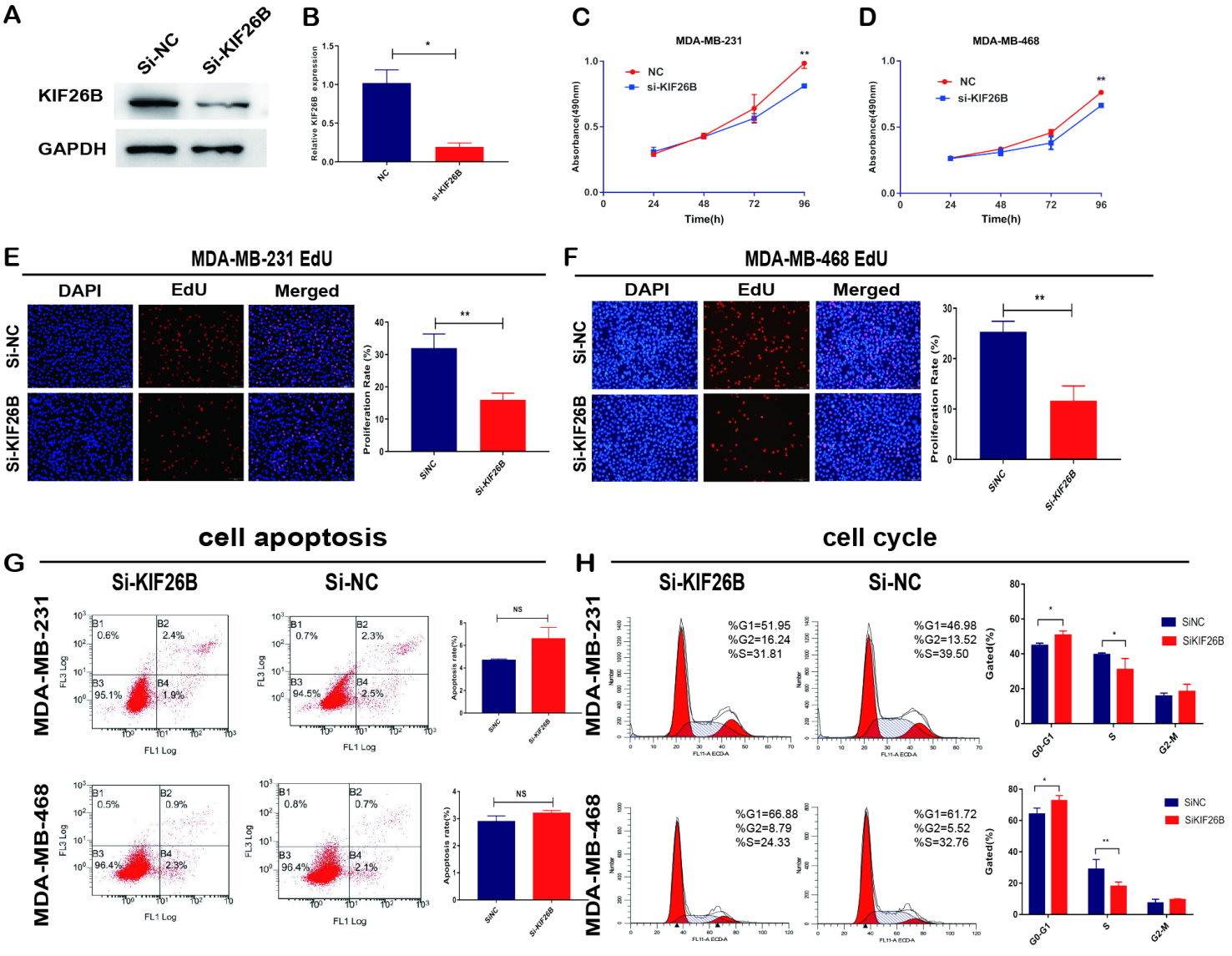



Figure 4

KIF26B promotes BC cell proliferation in vitro (a,b) The KIF26B expression levels were reduced by small interfering RNA (siRNA) targeting KIF26B with western blot analysis. KIF26B expression levels were normalized to GAPDH. The proteins expression levels were quantified as the densitometry value and analyzed by GraphPad Software. (c,d) Cell growth rates were evaluated with MTS assay in MDA-MB-231 and MDA-MB-468 cells following KIF26B interference and the results showed that KIF26B knockdown significantly inhibited the growth of BC cells. (e,f) EdU assay equally showed that decreased expression of KIF26B inhibited the proliferation activity in MDA-MB-231 and MDA-MB-468 cells. (g,h) Flow cytometry analysis was performed to detect the effect of KIF26B on the cell apoptosis and cell cycle. (g) Downregulation of KIF26B did not induce cell apoptosis compared with the negative control group. (h) Downregulation of KIF26B showed significant G1 phase increase and S phase decrease in MDA-MB-231 and MDA-MB-468 cells. Three independent experiments were performed, and data are presented as mean± SD. *$p<0.05$, **$p<0.01$.

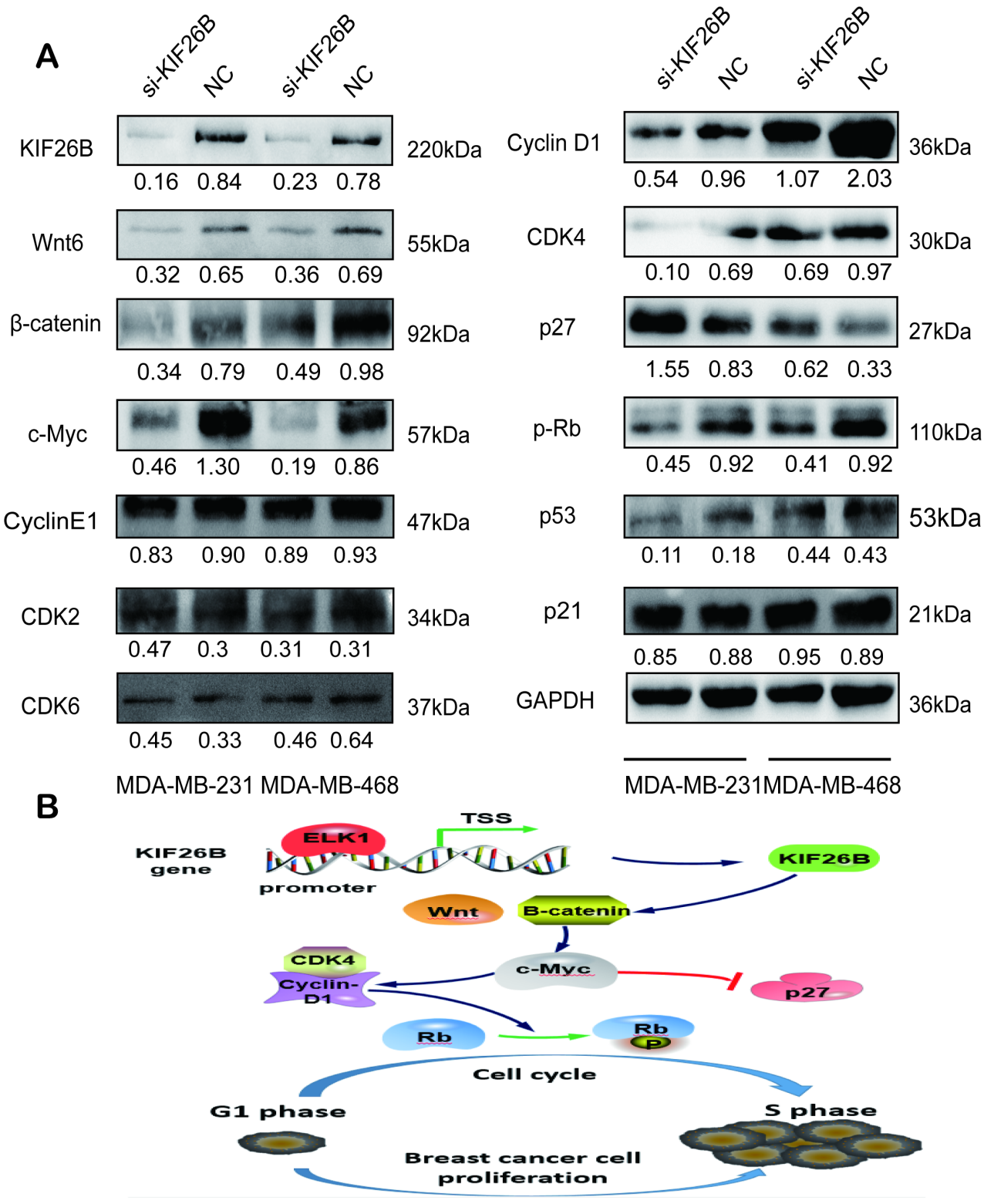



**Figure 5**

KIF26B promotes cell proliferation by modulated the levels of cell cycle-related proteins in MDA-MB-231 and MDA-MB-468 cells (a)Western blot analysis showed that the expression levels of Wnt, β-catenin, c-Myc, cyclinD1, CDK4, p-Rb decreased and p27 increased in both MDA-MB-231 cells and MDA-MB-468 cells after KIF26B silencing, while cyclin E1, CDK2, CDK6, p53 and p21 remained unchanged. (b) Proposed functional action of KIF26B in regulating BC tumorigenesis. The transcriptional factor ELK1

promoted KIF26B expression by directly binding to its core promoter region, and KIF26B acted on the Wnt/ β-catenin signaling pathway to up-regulate c-Myc and regulate the cell cycle-related protein expression levels, including cyclin D1, CDK4, p-Rb and p27 to promote cell cycle progression.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryinfomation.pdf