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RESEARCH PAPER

Transcription factor NFIC functions as a tumor suppressor in lung squamous cell carcinoma progression by modulating lncRNA CASC2

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ABSTRACT

Nuclear factor I (NFI) family is emerging found playing oncogenic or tumor-suppressive potential in cancers. However, the function and underlying mechanisms of NFIC, in the progression of Lung Squamous Cell Carcinoma (LUSC) remain unclear. Therefore, this study aims to probe into the function of NFIC in the development of LUSC. In the present study, we reported that NFIC was low expressed in human LUSC tissues and cell lines. NFIC inhibited LUSC cell proliferation and promoted cell apoptosis *in vitro* and *in vivo*. Moreover, NFIC also inhibited LUSC cell migration and invasion. Furthermore, we found that there were binding sites between lncRNA cancer susceptibility candidate 2 (CASC2) and NFIC, whose relationship was confirmed by the luciferase reporter assay. The expression of CASC2 and the expression of NFIC were positively correlated, and the function of CASC2 overexpression is similar to that of NFIC overexpression, which suggested that CASC2 may play a key role in LUSC development. Our study provided a new perspective for NFIC acting as an antioncogene in LUSC tumorigenesis, and NFIC and CASC2 may serve as novel potential targets for the treatment of LUSC.

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Background

Lung cancer is the most frequent cause of cancer-related deaths worldwide, and an estimated 1.6 million people die of this disease every year [1–3]. Although progress in clinical and experimental oncology has been made in recent years, 5-year survival in patients with lung cancer varies from 4–17% depending on stage and subgroup differences [1]. Lung Squamous Cell Carcinoma (LUSC) is the most common type of non-small-cell lung cancer (NSCLC) that accounts for more than 80% of lung cancers [4]. Therefore, elucidating the mechanisms underlying the development and progression of LUSC is essential for diagnosis and treatment for individuals with lung cancer.

Nuclear factor I C (NFIC) belongs to the nuclear factor I family that consists of additional three members: NFIA, NFIB, NFIX [5–7]. NFIC binds to DNA sequence with its conserved DNA-binding domain at N-termini, which regulates cell proliferation and differentiation via the transcriptional control of the target genes [8–10]. More recently, NFIC has been found implicated in various cancers, and its oncogenic and tumor-suppressive potential are varied in different cancer types [8,11,12]. For instance, NFIC functioned as a tumor suppressor in glioma based on insertions mutation within *Nfic* in mouse models [13]. However, NFIC, as a pro-oncogene, promoted cell migration and metastasis via suppression of an integrin subunit in melanoma [14]. Given the different potential roles of NFIC in cancers, functions of NFIC in lung cancer, especially LUSC needed to be elucidated.

Long noncoding RNAs (lncRNAs) are identified as transcripts of more than 200 nucleotides, many of which are involved in cancer progression [15–17]. Numerous researchers have revealed that lncRNA cancer susceptibility candidate 2 (CASC2), acting as a tumor suppressor lncRNA, is commonly absent in human cancer, including glioma, breast cancer, colorectal cancer, lung cancer, ovarian cancer, and liver cancer [18–21]. Studies have suggested that CASC2 suppresses tumorigenesis through inhibiting cell proliferation and promoting cell apoptosis [18].

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He *et.al*. revealed that CASC2 was significantly decreased in NSCLC, and the expression level of CASC2 may serve as an independent predictor for overall survival of NSCLC [22]. Upregulation of CASC2 significantly decreased the cell proliferation of human NSCLC cells and suppressed the growth of xenograft NSCLC tumors in mice [22]. In the study, we aimed to explore the role of the transcription factor NFIC and lncRNA CASC2 in the development and progression of lung squamous cell carcinoma.

Materials and methods

Clinical specimens and cell lines LUSC tissues and paired normal tissues were collected from LUSC patients in the third affiliated Hospital of Chongqing medical university (CQMU). The study was approved by the Human Research Ethics Committee of CQMU Human Bronchial Epithelial.

Cell culture 16HBE and human LUSC cell lines (H1703, H520, SK-MES-1and H2170) were purchased from American type culture collection (ATCC, Maryland, USA.). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (11,965,092, Gibco, USA) medium supplemented with 10% fetal bovine serum (16,140,071, Gibco, USA), 100X penicillin and 100X streptomycin (10,378,016, Gibco, USA) at 37°C with 5% CO_2.

Plasmids and interfering RNAs Human full-length NFIC or CASC2 cDNA was inserted into the vector pcDNA3.1 to overexpression NFIC or CASC2 in LUSC cells. As for the Dual-Luciferase Report assay, the WT or mutated CASC2 promoter sequence was cloned into pGL3 vector and verified by sequencing. Small interfering RNAs (siRNAs) specifically targeting human NGFIC were purchased from GenePharma, (Shanghai, China). All the expression vectors were transduced into cells using Lipofectamine 2000 according to the manufacturer's instructions.

Immunohistochemical staining (IHC) Briefly, sections were stained with anti-NFIC rabbit monoclonal primary antibody (1:2000, ab200829, Abcam, UK) or anti-ki67 mouse monoclonal primary antibody (1:2000, ab238020, Abcam, UK). TUNEL detection was performed using a TUNEL kit (ab206386, Abcam, UK) according to the manufacturer's instructions.

Real-time PCR Total RNAs were extracted from both lung cancer cell lines and human samples using TRIzol reagent (15,596,026, Invitrogen, USA). cDNA was obtained by reverse transcribing using RT reagent kit (205,111, Qiagen, German). Quantitative (real-time) PCR was performed using the SYBR® Premix Ex TaqTM II kit (DRR820A, Takara, Japan). The results were normalized to the expression level of GADPH and all RT-qPCR data were evaluated using the $2^{-\Delta\Delta Ct}$ method. The gene-specific primers as follows: NFIC forward, 5′-TGGCGGCGATTACTACACTTCG-3′ and reverse, 5′-GGCTGTTGAATGGTGACTTGTCC-3′; CASC2 forward, 5′- GCACATTGGACGGTGTT TCC-3′ and reverse, 5′- CCCAGTCCTTCACAGG TCAC-3′; GADPH forward, 5′- GGAGCGAGATC CCTCCAAAAT-3′ and reverse, 5′-GGCTGTTGT CATACTTCTCATGG-3′.

Western blot Tissue or cells were lysed on ice in RIPA buffer (Thermo, USA) containing antiprotease mixture (78,425, Thermo, USA). Soluble proteins were subjected to SDS–PAGE gel electrophoresis. Proteins were electro-phoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Mil- lipore, Billerica, MA, USA) and immunoblotted with anti-NFIC (ab200829, 1:1000), anti-GAPDH (ab8245, 1:2000), anti-cyclin D1 (ab16663, 1:1000), anti-p21 (ab109520, 1:2000), anti-Bax (ab32503, 1:1000), anti-Bcl-2 (ab182858, 1:2000), anti-cleaved-caspase3 (ab32042, 1:1000), anti-cleaved-caspase9 (ab2324, 1:2000), anti-cox-2 (ab179800, 1:2000), anti-MMP -2 (ab92536, 1:1000), anti-MMP-9 (ab76003, 1:2000). The secondary antibodies were anti-mouse/Rabbit antibody (ab205719, ab205718, 1:1000). Immunoreactive proteins were visualized using ECL Western Blotting Substrate (W1001, Promega, USA). All antibodies were purchased from Abcam (UK).

Cell proliferation assay Cell proliferation rate was measured at 0, 24, 48, and 72 h using a CCK8 assay kit (ab228554, Abcam, UK,) according to the manufacturer's instructions. Cell viability was assessed by reading absorbance at 450 nm using a microplate reader.

Migration, invasion assays Migration and invasion assay were performed in 24-well chambers

(Corning, USA) without/with Matrigel-coated membranes, respectively. Briefly, 5×10^4 cells/well LUSC cells were seeded with serum-free medium into the upper chambers. Medium with 10% FBS was added to the bottom chambers. After 48-h incubation, and subsequently, cells penetrating to the lower surface of the membrane were fixed by 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet.

Wound healing assays In brief, cells were plated to confluence in six-well plates and then a scratch wound was generated using a 10 µl blunt pipette tip. Progression of migration was observed and photographed at 0 and 24 h after wounding. The distance between the two edges of the scratch was measured and calculated.

Cell cycle and apoptosis detection For cell apoptosis assay, cells were stained with annexin V-fluorescein isothiocyanate and propidium iodide (FITC/PI) using an apoptosis kit (40302ES20, Yeasen, USA) and analyzed with a flow cytometer (BD FACSCalibur, San Jose, CA). For cell cycle analysis, cells were stained with PI for 10 min at room temperature in the dark. Then analyzed by flow cytometry and the percentages of cells in G0/G1, S, and G2/M phases were counted and analyzed.

Luciferase reporter assay pGL3-basic reporter gene vector containing WT or mutated CASC2 promoter sequence were transfected into 293 T cells were by Lipofectamine 2000 in six-well plates followed by dual-luciferase reporter detection kits (LUC1, Sigma, German). Renilla was used as the internal control for transfection efficiency. The results were expressed as the ratio of firefly luciferase activity to Renilla luciferase activity.

Chromatin immunoprecipitation (ChIP) ChIP assays were performed using a ChIP assay kit according to the manufacturer's instruction (17–295, Sigma, German). Briefly, SK-MES-1 was ultrasonically cleaved to obtain chromatin fragments and incubated overnight with anti-NFIC antibodies or normal rabbit IgG. Real-time PCR analysis was performed using SYBR Green (RR820A, Takara, Japan) in an ABI 7500 fast fluorescence temperature cycler. Normal rabbit IgG was used as NC. The primers used for ChIP are as follows: NFIC forward, 5′-TGGCGGCGATTACTACACTTCG-3′ and reverse, 5′-GGCTGTTGAATGGTGACTTGTCC-3. The results were calculated as a percentage relative to the input DNA.

Tumor xenograft experiment Six-week-old Balb/c athymic nude mice (Vitalriver, Beijing, China) were maintained under specific pathogen-free conditions. The mice were subcutaneously injected in the right flank with 1×10^6 LUSC cells transfected with pc-NFIC or pc-NC (n = 3). Once tumors were formed, tumor volumes were calculated as $0.5 \times \text{length} \times \text{width}^2$ every 5 days. After 30 days, mice were killed, and tumors were excised, weighed, and immediately frozen at −80°C for future use. All animal experiments were conducted in conformance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Statistical methods Student's *t*-test or one-way ANOVA were used for statistical analysis when appropriate. All statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). A two-tailed value of $P < 0.05$ was considered statistically significant.

Result

Transcriptional factor NFIC is low expressed in LUSC tissues and cell lines

To investigate the expression level of NFIC in LUSC, tumor tissue and its matched non-tumor tissue were collected from 20 patients. Immunohistochemical (IHC) staining showed that NFIC was lower expressed in tumor tissue than non-tumor tissue (Figure 1 A). At the same time, Real-time PCR showed that NFIC was significantly low expressed in lung cancer tissues as compared to normal tissues (Figure 1b). Four pairs of tumor and adjacent tissue were randomly selected for western blot detection. Compared to normal tissue, the protein level of NFIC was downregulated in LUSC tissue (Figure 1c). As shown in Figure 1 C, NFIC was significantly downregulated in lung cancer cell lines, especially in SK-MES-1and H2170 cell lines, compared with 16HBE (Figure 1d). So, SK-MES-1and H2170 cell lines were selected for further research. In

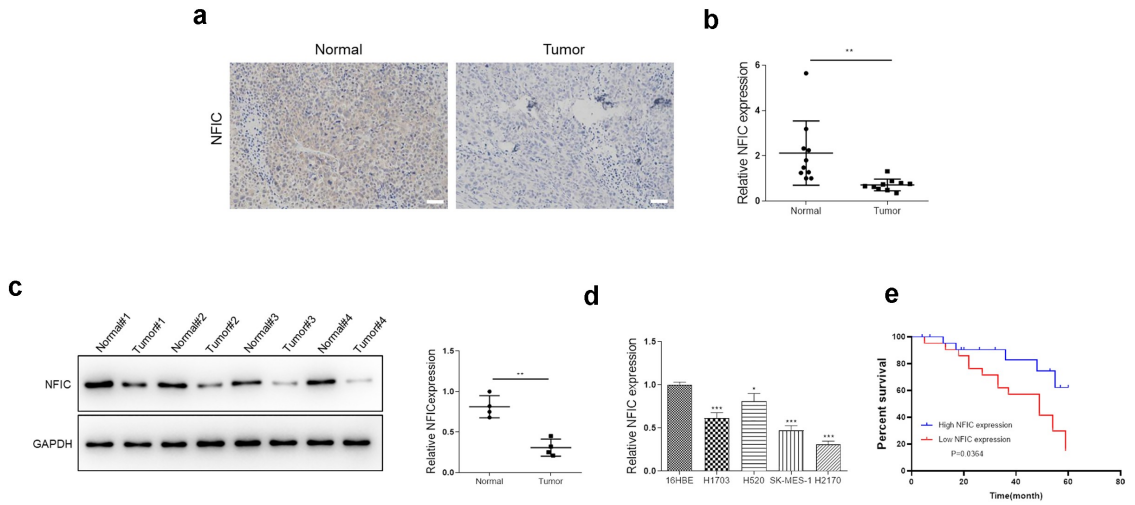

Figure 1. Transcriptional factor NFIC is low expressed in LUSC tissues and cell lines. (a) The protein level of NFIC was assessed in LUSC tissue and paired normal tissue by IHC staining (n = 20). (b) The relative RNA level of NFIC was downregulated in LUSC tumor tissue(n = 20). (c) 4 pairs of tumor and adjacent tissue were randomly selected for western blot detection and normalized to the level of GADPH. (d) The mRNA expression level of NFIC was measured by RT-PCR in the Human Bronchial Epithelial Cell Line, 16HBE lung cancer cell lines including H1703, H520, SK-MES-1and H2170. (e) Kaplan–Meier survival curve of NFC-high and NFIC-low lung cancer patients (n = 45). *P < 0.05, *P < 0.05, **P < 0.01, and ***P < 0.001 by Student's t-test.

addition, the Kaplan–Meier demonstrated that survival of patients in the NFIC high expression group was longer than in the low NFIC expression group (Figure 1e).

Overexpression of NFIC inhibits the proliferation and promotes the apoptosis of LUSC cell

To further investigate the role of NFIC in LUSC *in vitro*, plasmid pc-NFIC, as well as control plasmid pc-NC, were transfected to SK-MES -1and H2170 cell lines. RT-PCR was performed to measure the transfection efficiency. Compared with the control group, the expression level of NFIC was dramatically upregulated in SK-MES -1and H2170 cells transfected with pc-NFIC (Figure 2a). Next, the proliferation of SK-MES -1 and H2170 cells transfected with pc-NFIC were significantly decreased when compared with the control by CCK8 assays (Figure 2b). Equally to the CCK8 assay, the percentage of EdU positive cells in SK-MES-1 and H2170 cells transfected with pc-NFIC were observably reduced (Figure 2c). In addition, flow cytometry revealed that the percentage of cells in S and G2/M phase were decreased significantly, while the percentage of cells in G0/G1 phase increased in SK-MES-1 cells (Figure 2d). A similar result was observed in H2170 cells (Figure 2d). Meanwhile, the cell cycle-related protein level of cyclin D1 and p21 were markedly increased in pc-NFIC transfected LUSC cells (Figure 2e). What more, the pc-NFIC mediated overexpression of NFIC dramatically promoted apoptosis of SK-MES-1 and H2170 cells (figure 2f). Western blot analysis observed that proapoptotic proteins Bax, cleaved-caspase3 and cleaved-caspase9 were upregulated, whereas Bcl-2 was downregulated (Figure 2g).

Overexpression of NFIC inhibits the migration and invasion of LUSC cell

Wound healing assay and transwell assay revealed that the enforced expression of NFIC markedly suppresses cell migration of SK-MES-1 and H2170 cells compared with the control (Figures 3A, 3B). As shown in Figure 3b, the number of invasion cells were decreased in pc-NFIC groups compared with control. Next, as

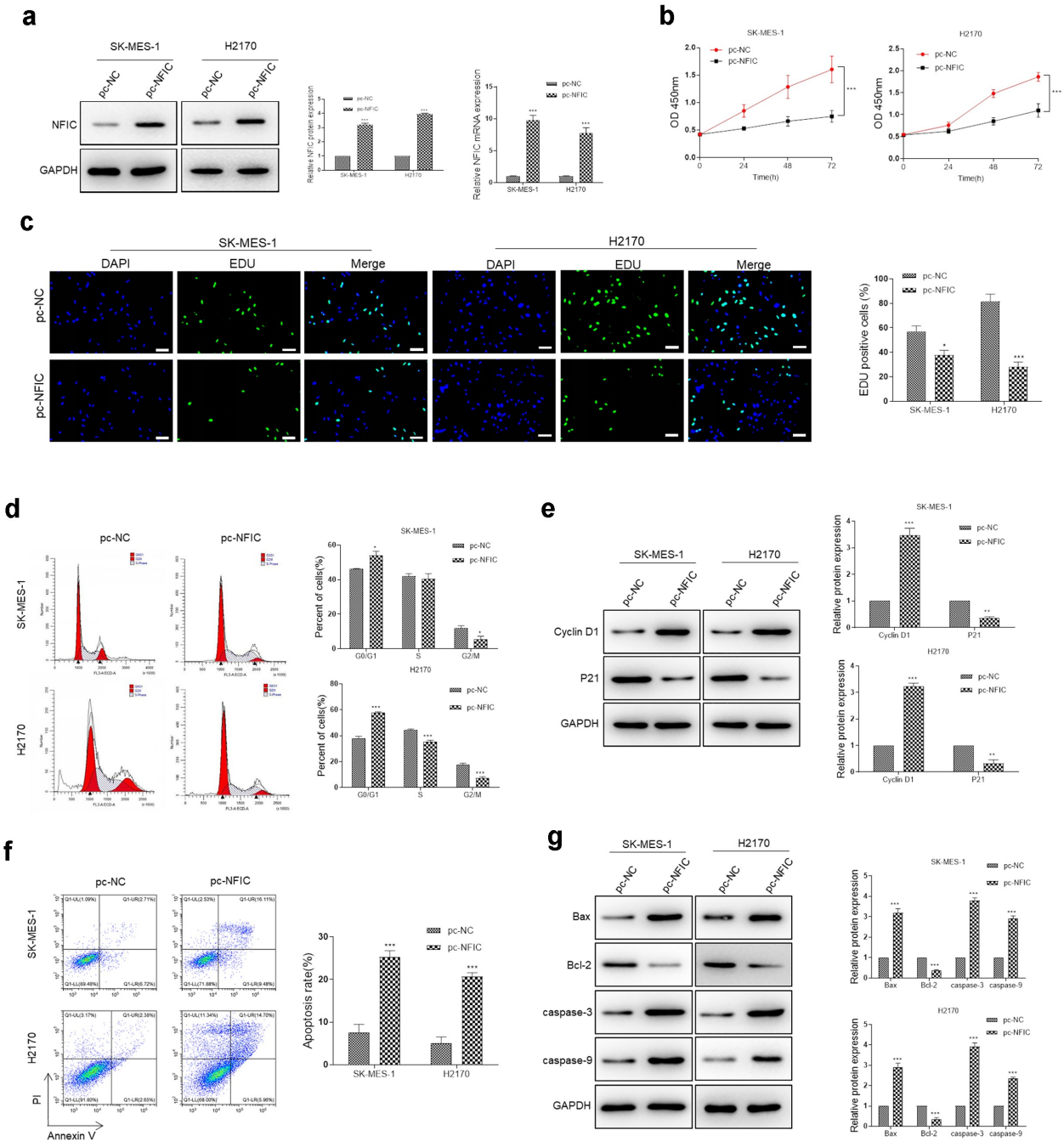

Figure 2. Overexpression of NFIC inhibits cell proliferation and promotes cell apoptosis of LUSC. (a) The NFIC expression in SK-MES-1and H2170 cells transfected with pc-NFIC as well as pc-NC by Western blot and RT-PCR analysis. (b and c) Cell proliferation was measured using CCK-8 assay (b) and EdU staining assay (c) in SK-MES-1and H2170 cells. (d and f) Cell cycle (d) and apoptosis (f) was measured by Flow Cytometry in SK-MES-1and H2170 cells. (e and g) Western blot was used to detect the expression of cell cycle related protein (E; cyclin D1, p21) and apoptosis related protein (G; Bax, Bcl-2, cleaved-caspase3 and cleaved-caspase9) normalized to the level of GADPH. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ by Student's *t*-test.

expected, the migration- and invasion-related protein level of cox-2, MMP-2, MMP-9 were all reduced by overexpression of NFIC compared with control group (Figure 3c). Taken together, those data above demonstrated that NFIC inhibited cell migration and invasion in LUSC.

NFIC positively regulates CASC2 expression by binding to its promoter region

To explore the molecular mechanisms by which NFIC contributed to the phenotypes of LUSC cells, the target gene of transcription factor NFIC was

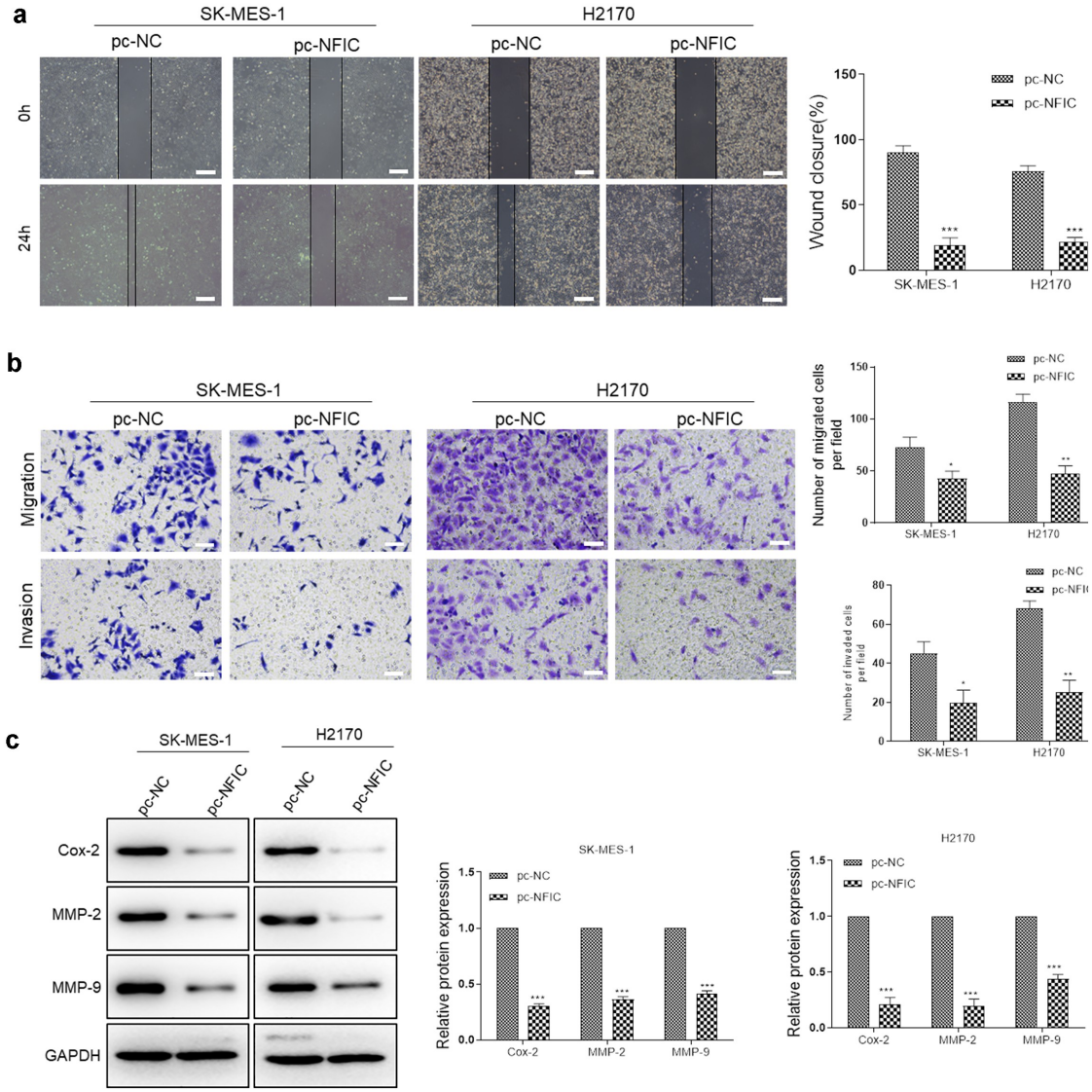

Figure 3. Overexpression of NFIC inhibits the migration and invasion of LUSC cell. (a-b) NFIC promoted SK-MES-1and H2170 migration and invasion as determined by a Wound healing assay and Transwell migration assay. (c) migration related proteins cox-2, MMP-2, MMP-9 were measured by western blot in SK-MES-1and H2170. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ by Student's t-test.

predicted using the software database. We found that the most conserved binding site of NFIC was "CAATCCAA" which located at the promoter region of CASC2 (Figure 4a). Subsequently, luciferase reporter assay found that the promoter activity of CASC2 WT was significantly increased in the NFIC overexpressed LUSC cells, whereas, the changes were not observed for CASC2 MUT (Figure 4b). Consistently, the CASC2 promoter region was markedly amplified from the NFIC-immunoprecipitated DNA, however, that was not observed in IgG-immunoprecipitated chromatins (Figure 4 C). As showed in Figure 4d, CASC2 was reduced significantly in LUSC tissues compared to normal tissues, which showed a positive relation with NFIC expression (Figure 4e). Consistently, CASC2 expression was reduced when using siRNA to suppress NFIC expression in SK-MES -1and H2170 cells (figure 4f), whereas, the expression of CASC2 was increased when overexpressing NFIC (Figure 4g). These results indicated that NFIC may inhibit the progress of LUSC by regulating CASC2.

Overexpression of CASC2 inhibits the proliferation, migration and invasion, and promotes the apoptosis

Owing to our findings above, we speculated that CASC2 might play an important role in suppressing

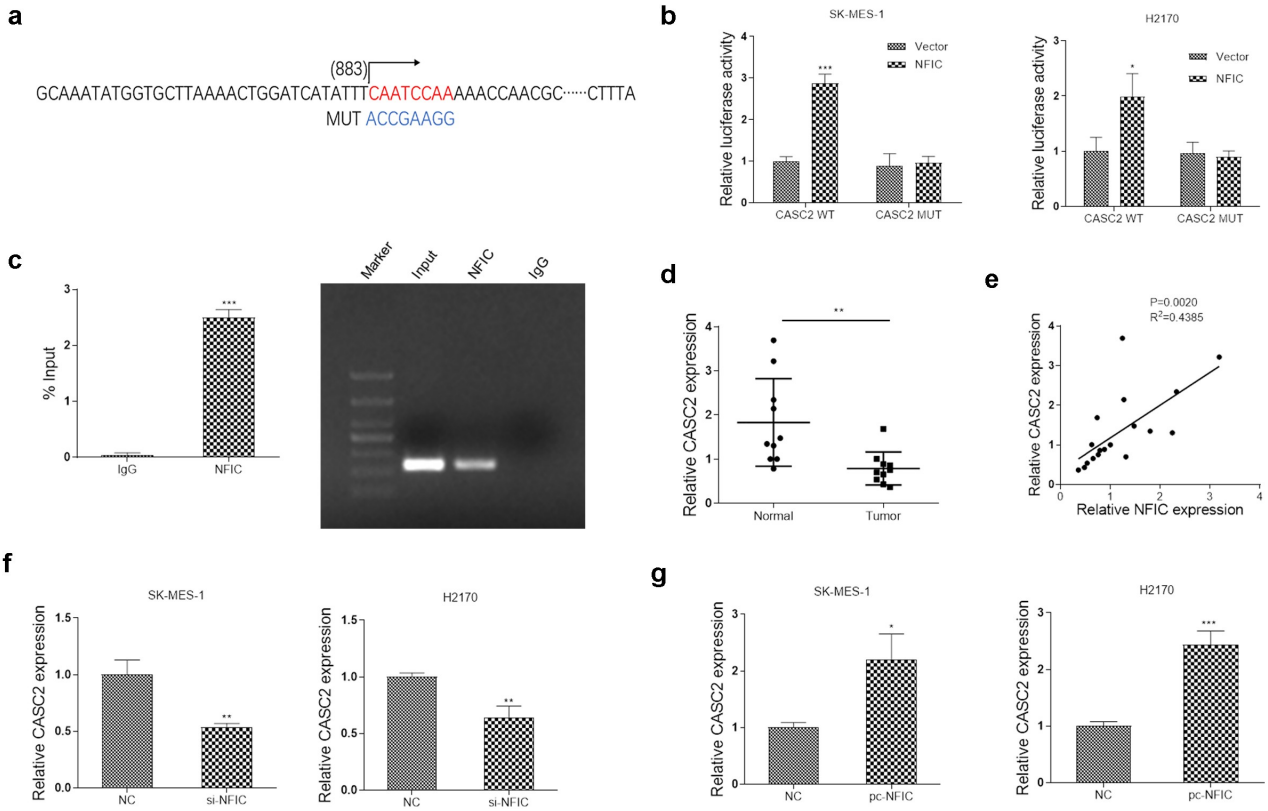

Figure 4. NFIC positively regulates CASC2 expression by binding to its promoter region. (a) Sequence of the CASC2 promoter, with the predicted binding sites of NFIC by A. Mutations were introduced into the conserved binding sites to impede NFIC binding. (b) Dual-luciferase report assay was performed in SK-MES-1and H2170 cells after transfection of the WT or mutant constructs together with the pc-NFIC or pc-NC. (c) ChIp analysis of NFIC at the CASC2 promoter region in SK-MES-1 cells. (d) The relative RNA level of CASC2 was downregulated in LUSC tumor tissue compared paired normal tissue (n = 20). (e) CASC2 and NFIC have a positive relation at mRNA level in LUSC. (f-g) RT-PCR was used to investigate the mRNA expression of CASC2 in the NFIC suppressing or over-expressing LUSC cells (SK-MES-1and H2170). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ by Student's t-test.

LUSC progression. To further demonstrate our hypothesis, the gene of CASC2 was introduced into vector pc. DNA and plasmid pcDNA-CASC2 as well as empty vector were transfected LUSC cell lines. RT-PCR showed that the relative expression level of CASC2 was remarkedly increased in SK-MES-1 and H2170 cells transfected with pcDNA-CASC2 (Figure 5a). Next, we found that the ability of cell proliferation was depressed in pcDNA-CASC 2 transfected LUSC cells compared with control (Figure 5b). Consistent with CCK-8 assay results, the number of cell colony formation was also significantly reduced in SK-MES-1and H2170 cells transfected with pcDNA-CASC2 (Figure 5c). Besides, flow cytometry detection showed that the percentage of cells in S and G2/M phase was strongly reduced, however, G0 phase cells were increased in pcDNA-CASC2 transfected LUSC cells (Figure 5d). Furthermore, wound healing assay and Transwell assay revealed that compared with control, the degree of wound closure and the number of migration/invasion cells were both decreased when CASC2 was up-regulated (Figure5eandf). Compared with control, overexpression of CASC2 significantly increased the percentage of the Annexin V-positive cells (Figure 5g). Western blot was analysis illustrated that proapoptotic proteins Bax, cleaved-caspase3 and cleaved-caspase9 were upregulated whereas Bcl-2was downregulated (Figure 5h).

Besides, to prove that NFIC regulate lung squamous cell carcinoma progression through lncRNA CASC2, rescue experiment was carried out in the supplementary Figure. We found that knockdown

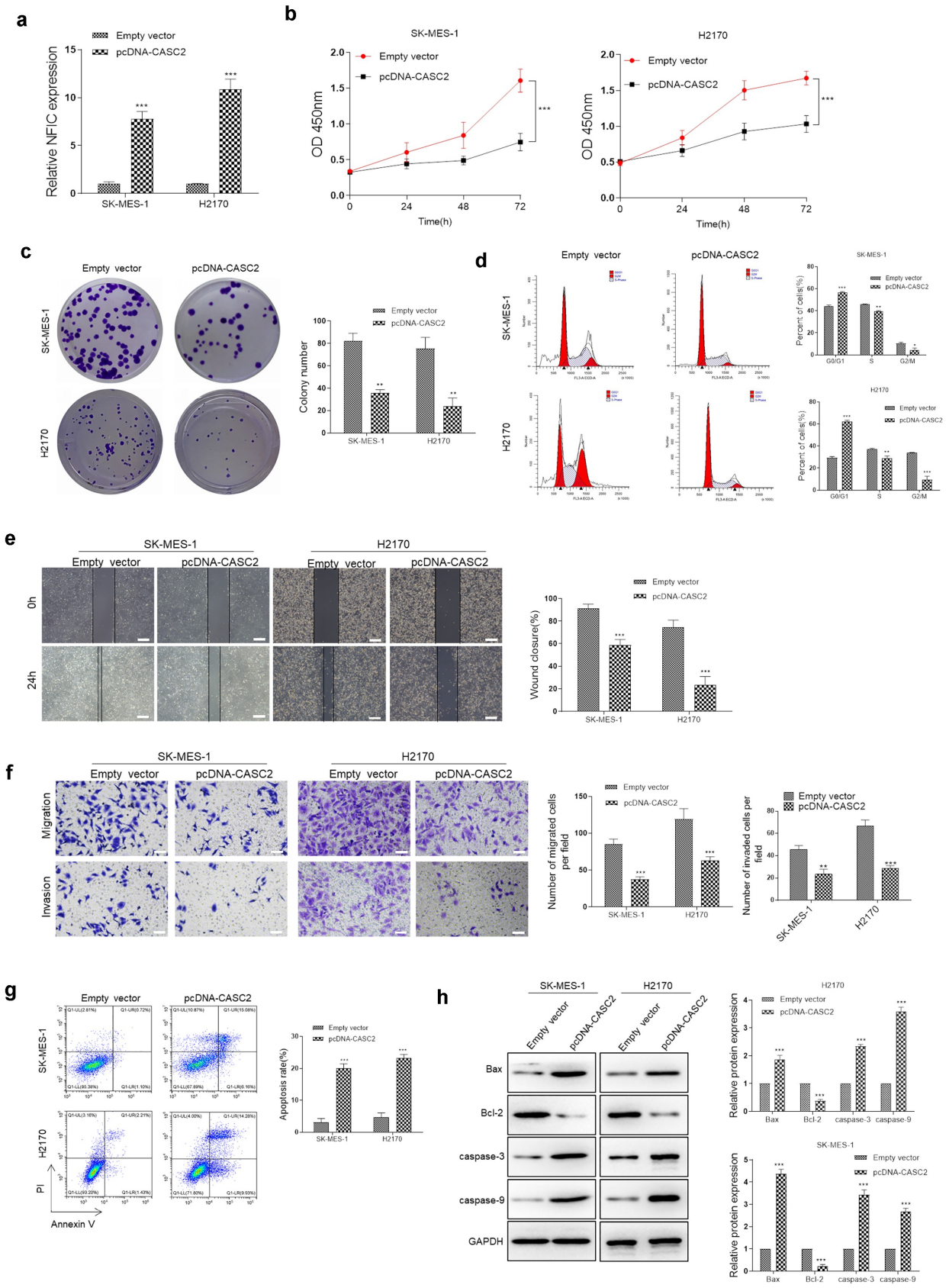

Figure 5. Overexpression of CASC2 inhibits cell proliferation, migration and invasion, and promotes cell apoptosis. (a) RT-PCR analysis of CASC2 expression in SK-MES-1and H2170 cells transfected with pcDNA-CASC2 or empty vector. (b and c) Cell proliferation was measured using CCK-8 assay (b) and colony formation assay (c) in SK-MES-1and H2170 cells. (d) Cell cycle was measured by Flow Cytometry in SK-MES-1and H2170 cells. (e-f) Wound healing assay and Transwell migration assay to detection the ability of migration and invasion in SK-MES-1and H2170 cells. (g) SK-MES-1and H2170 cells apoptosis were investigated after overexpressing CASC2. (h) Western blot was performed to detect apoptosis related protein (G; Bax, Bcl-2, cleaved-caspase3 and cleaved-caspase9) normalized to the level of GADPH. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ by Student's t-test.

of CASC2 reversed the functions of NFIC overexpression on the lung squamous cell carcinoma progression (Fig. S1).

Effects of NFIC expression on tumorigenesis in vitro

To investigate whether NFIC involved tumor growth *in vivo*, LUSC cells transfected with pc-NFIC or control vector pc-NC were subcutaneously injected in nude mice respectively (n = 3) (Figure 6a). Consistent with the i*n vitro* results, tumor growth in the NFIC group was smaller than that in the control group (Figure 6b). The average tumor weight and tumor volume were markedly lower in NFIC group than in the control group (Figure 6b and c). PCR assay revealed that the expression of CASC2 was significantly upregulated in the pc-NFIC groups (Figure 6d). Importantly, the expression of Ki67 immunostaining analysis revealed that tumors from the NFIC group had lower Ki-67 expression than those from control group. However, more apoptotic cells were detected in tumors of NFIC group compared with control (Figure 6e). Furthermore, the expression of cox-2, MMP-2, MMP-9 were decreased in the pc-NFIC compared with control group (figure 6f).

Discussion

The present study demonstrated that NFIC was downregulated in LUSC tissues and was closely associated with patient's survival. Specifically, we observed that NFIC exhibited tumor suppressor roles by inhibiting cell proliferation, migration, invasion and facilitating apoptosis of LUSC cells through regulating CASC2. These results indicated that NFIC played an antioncogenic role in LUSC tumorigenesis.

The nuclear factor I (NFI) transcription factors play important roles during normal development and have been associated with cancer progression [23]. NFIC has been reported participating in the development of glioma, breast cancer, hematopoietic tumor, and melanoma [11,14,24,25]. However, the function of NFIC in lung cancers has not been widely studied and is still unclear. Herein, we found that NFIC was involved in the suppression of LUSC by regulating CASC2. However, the other members of the NFI family have different roles in lung cancer subtypes. NFIB is another member of NFI

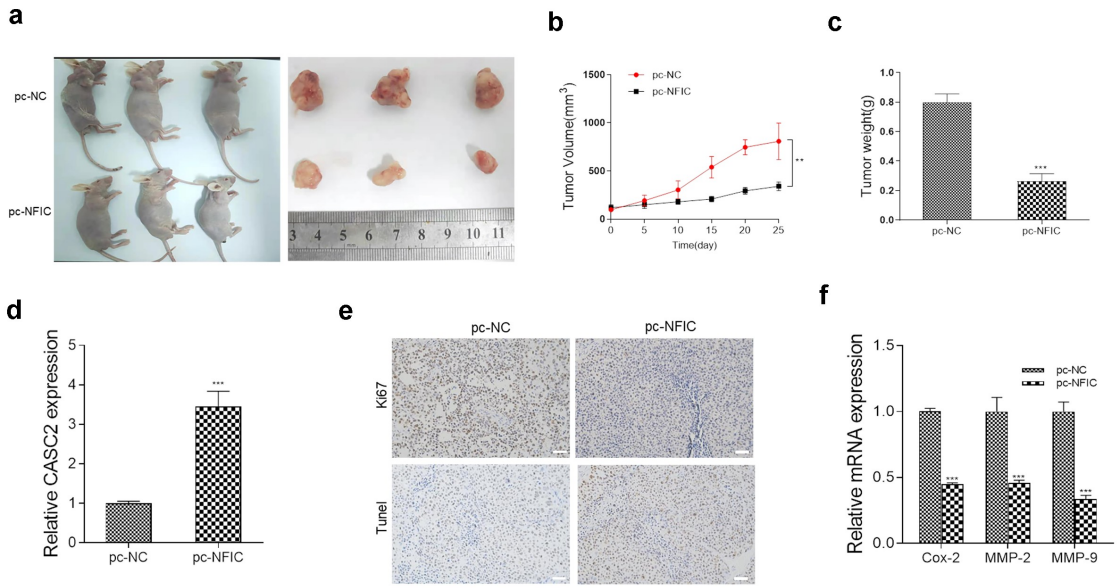

Figure 6. Effects of NFIC expression on tumorigenesis *in vitro*. (a-c) Tumor samples(a), tumor volumes(b) and tumor weights(c) from respective groups are represented. (d) The expression of CASC2 detected by PCR. (e) Ki-67 and TUNEL staining was performed in xenograft tumors to further determine the effect of NFIC on cell proliferation and apoptosis. (f) The expression of migration related proteins cox-2, MMP-2, MMP-9 were measured by PCR. $^{**}P < 0.01$, and $^{***}P < 0.001$ by Student's t-test.

transcription factors and is showing tumor suppressor role and oncogene role in small cell lung cancer (SCLC) and in non-small cell lung carcinoma (NSCLC) respectively [26,27]. High expression NFIB has been reported in SCLC tumors and cell lines, knockdown of NFIB induced the reducing SCLC cell proliferation, and increasing cell apoptosis [7,26,28]. Whereas, contrary to its role as an oncogene in SCLC, NFIB was downregulated and restrains the process of epithelial-to-mesenchymal transition (EMT) in NSCLC [29]. In our study, we observed that NFIC inhibited tumor process by repressing cell proliferation, migration, invasion and facilitating apoptosis of LUSC cells. Importantly, further studies were required to determine the bio-function of NFI transcription factors in the development of lung cancer.

Presumably, given that NFIC functioned as a transcription factor, LncRNA CASC2 as a target gene of NFIC was uncovered using software. NFIC interacted with the promoter region of CASC2 and then regulated the expression of that. In the present data, CASC2 showed a positive correlation with NFIC in human LUSC tissue and cell lines. Consistent with previous reports, CASC2 was showed a tumor suppressor role and markedly reduced in lung cancers [22]. Moreover, overexpression of CASC2 significantly inhibited NSCLC cell proliferation both *in vitro* and *in vivo* [22]. As expected, the LUSC cells transfected with pcDNA CASC2 showed dramatically attenuating proliferation in the CCK8 and clone formation assay. Amazingly, our data indicated that CASC2 inhibited cell migration and invasion. However, more further investigations needed to better elucidate mechanisms of CASC2 in lung cancer.

Here, we testified about the function of NFIC *in vivo*. Inconsistent with the results of *in vitro*, NFIC depressed the growth of xenograft tumors induced by LUSC cells in mice. And the cell proliferation of tumors was significantly reduced. Importantly, the effect of NFIC in LUSC metastasis *in vivo* should be further investigated.

In summary, our study found NFIC was remarkedly reduced in human LUSC tissue and cell lines, and its downregulation indicating shorter survival time of LUSC patients. Then, we observed that NFIC inhibited the process of LUSC by positively regulating CASC2. The present data may improve the understanding of a new target for the treatment of patients with LUSC.

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Disclosure statement

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