# UBE2T Regulates FANCI Monoubiquitination to Promote NSCLC Progression by Activating EMT

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**Primary research**

**Keywords:** FANCI, NSCLC, UBE2T, monoubiquitination, EMT

**Posted Date:** November 2nd, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-977052/v1

# Abstract

**Background:** Fanconi anemia complementation group I (FANCI) acts as a critical protein factor for maintaining DNA stability. However, roles of FANCI in tumors has not been well revealed. In current study, we aimed to explore the function and potential mechanism of FANCI in non-small-cell lung cancer (NSCLC).

**Methods:** To detect the expression of FANCI and UBE2T in NSCLC tissues, quantitative reverse-transcription PCR (qRT-PCR) and Western blot assays were employed. CCK-8, wound healing, Transwell, flow cytometry analysis and tumor xenograft were used to investigate the biological effects of FANCI in NSCLC in vitro and in vivo. FANCI binds with UBE2T was confirmed using coimmunoprecipitation (co-IP) assay. The EMT protein markers were detected via Western blot.

**Results:** FANCI was upregulated in NSCLC tumor tissues compared with adjacent. In A549 and H1299 cells, knockdown of FANCI inhibited cell growth, migration, invasion and cell cycle,as well as epithelial-to-mesenchymal transition (EMT) in vitro. In vivo, the tumor growth was also repressed when FANCI was downregulated. Mechanistically, UBE2T directly bound with FANCI and regulated the monoubiquitination of FANCI. Futhermore, UBE2T restored the inhibitory effects induced by knocking down FANCI in NSCLC cells.

**Conclusion:** FANCI was a putative oncogene in NSCLC, and was monouniubiquitinated by UBE2T to regulate cell growth, invasion and migration. Our findings suggested that FANCI might applied as a predicted biomarker and therapeutic target for NSCLC.


# Background

Lung cancer is responsible for the most cancer-related death in the world, and non-small-cell lung cancer (NSCLC) accounts for the large category of lung cancers(Bray et al. 2018, Chen et al. 2016). Up to date, although some targeted therapies against epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK) or c-rosoncogene 1 receptor tyrosine kinase (ROS1) have been confirmed to bring survival benefits for NSCLC patients, a considerable part of NSCLC is still incurable, mainly due to late diagnosis and drug resistance(Del Re et al. 2019, Doval et al. 2019, Hiley et al. 2016). Combined with above, it’s eagerly to uncover the pathogenesis of NSCLC and develop new treatments for NSCLC.

Fanconi anemia (FA) is a rare recessive disease characterized by anemia and bone marrow failure, which was caused by mutated FA family proteins(Engel et al. 2019, Kelaidi et al. 2019). FA family consists about 19 genes which are associated with cell cycle progression regulation and DNA damage repair(Dong et al. 2015, Katsuki and Takata 2016). FANCI is a member of FA, located in the long arm of chromosome 15. It forms a complex with its molecular chaperone FANCD2 and participates in DNA repair and ribosome biosynthesis, and then is closely related to cell cycle regulation in S phase and G2 phase(Joo et al. 2011, Kottemann and Smogorzewska 2013, Sondalle et al. 2019). Moreover, the core complex of FA, as an E3 ubiquitin ligase, monoubiquitinates FANCD2 and FANCI when DNA is damaged, and stables

both proteins(Meetei et al. 2003, Smogorzewska et al. 2007). Lately, a few studies have found that FANCI participates the occurrence and development of several tumors. For example, FANCI is associated with susceptibility to familial prostate cancer(Paulo et al. 2018). FANCI has also been used as a novel marker for hepatitis B virus associated hepatocellular carcinoma(Xie et al. 2019). In lung adenocarcinoma (LUAD), FANCI was demonstrated to cooperate with IMPDH2 to promote the tumor growth(Zheng and Li 2020). However, our understanding of FANCI functions in tumors is still very limited, especially the underlying mechanism in NSCLC.

Ubiquitin-conjugating enzyme E2T (UBE2T) is an E2 ubiquitin ligase in the FA pathway. It facilitates the monoubiquitination of FANCD2/FANCI through binding to FANCL and then promotes DNA interstrand cross-links (ICLs) repair(Longerich et al. 2009, Machida et al. 2006). More and more evidence demonstrates that UBE2T is involved in multiple cancers. As reported, UBE2T could decrease BRCA1 and promote breast cancer progression(Ueki et al. 2009). For gallbladder cancer, UBE2T was identified as a putative biomarker(Zhu X. et al. 2020). Moreover, UBE2T induced hepatocellular carcinoma radioresistance by modulating H2AX monoubiquitination and activating CHK1(Sun et al. 2020). In NSCLC, UBE2T was found associated with poor prognosis based on the TCGA database(Wu et al. 2020). In addition, UBE2T promoted epithelial mesenchymal transition (EMT) and accelerated NSCLC cells proliferation, migration, and invasion(Yin et al. 2020). In EMT process, epithelial cells transfer to invasive mesenchymal cells due to the lost of apical basal polarity and cell-cell adhesion. EMT has closely relationship with tumors depending on its biological processes of wound healing, cell migration and proliferation(Hay 1995, Thiery 2003, Zhao et al. 2017). Although these studies have found that UBE2T participate in NSCLC, the role of UBE2T in the carcinogenesis of NSCLC is still not well revealed.

In the current study, FANCI was found overexpressed and exerted a critical role in NSCLC by promoting EMT. In terms of mechanism, we confirmed that the stability and monoubiquitination of FANCI were increased by the interaction with UBE2T, contributing to a better understanding of the pathogenesis of NSCLC.

## Materials And Methods

# Samples collection

We collected 32 pairs of fresh NSCLC samples and adjacent normal lung tissues from February 2016 to February 2020 at Fujian Provincial Hospital. This study was approved by the Medical Ethics Committee of Fujian Provincial Hospital and was conducted according to the Declaration of Helsinki. The written informed consent was also obtained from all participants.

# Cell culture and transfection

Five NSCLC cell lines (H1650, H1975, H827, A549 and H1299) and human normal epithelial cell line (HBE) were purchased from The Cell Bank of Type Culture Collection of The Chinese Academy of

Sciences. RPMI-1640 (Thermofisher, USA) medium containing 10% FBS (Gibco, USA) was used to culture cells at the condition of 37°C with 5% $CO_2$ and saturated humidity. Targeting FANCI short hairpin RNA (shRNA) and UBE2T overexpression lentivirus were synthesized by Genepharma (Shanghai, China) and puromycin were applied for cells selection. Lipofectamine 3000 (Thermofisher, USA) was applied for cell transfection based on the manufacturer's protocol.

# Quantitative Reverse-Transcription PCR (qRT-PCR)

TRIzol reagent (ThermoFisher, USA) was applied total RNA isolation from NSCLC tissues and cultured cells. The qPCR-RT Master Mix Kit (Yeasen, China) was used for synthesizing cDNA according to the manufacturers' instructions. For qRT-PCR, SYBR Premix Ex Taq™ II (Takara Biotechnology Co., Ltd.) and TaqMan Universal Master Mix II (ThermoFisher, USA) was employed. QRT-PCR was conducted on a Roche Light Cycler480 system (Roche Diagnostics, Inc.) in accordance with the manufacturer's protocol. Then $2^{-\Delta\Delta T}$ method was used to calculate the relative expression of FANCI and UBE2T. β-actin served as an internal control. Primer sequences used in this study were showed in Table 1.

Table 1

Primer sequences (5'-3')

| Gene | Primer sequences |
|---|---|
| FANCI F | CCACCTTTGGTCTATCAGCTTC |
| FANCI R | CAACATCCAATAGCTCGTCACC |
| UBE2T F | TTGATTCTGCTGGAAGGATTTG |
| UBE2T R | CAGTTGCGATGTTGAGGGAT |
| β-actin F | AGGGGCCGGACTCGTCATACT |
| β-actin R | GGCGGCACCACCATGTACCCT |

# Western blot

Precooled RIPA buffer (Beyotime, China) was applied to extract total protein from cells or tissues. BCA Protein Assay kit (Beyotime, China) was applied for protein density detection. Proteins were then separated by 10% SDSPAGE. Subsequently proteins were transferred onto PVDF membranes (Millipore, USA). 5% fat-free milk was employed to block the membranes at room temperature for 2h. After blockage, the bands were incubated with primary antibodies (anti-FANCI, ab74332; anti-UBE2T, ab179802; anti-E-cadherin, ab40772; anti-N-cadherin, ab76011; anti-Vimentin, ab92547) which were purchased from abcam (USA) at 4°C overnight following with corresponding secondary antibodies incubation for 1 h at room temperature. β-actin (ab8226, abcam, USA) was applied as the internal reference protein.

# Cell proliferation

Cell proliferation was detected using CCK-8 (Dojindo, Japan). About $4\times10^3$ cells were seeded into each well (96-well plate) and maintained for the indicated times. Afterwards, 10 μl of CCK-8 reagent was added into each well for another 2h incubation. Finally, at 450 nm, the absorbance of each well was determined.

# Cell migration

Cell migration was tested by transwell and wound healing assays. Around $5\times10^5$ cells mixed in 200 μl basal medium were added into the upper chamber and the lower chamber was supplemented with 500 μl total medium for transwell assay. Around incubation for 24h, 4% paraformaldehyde was used to fix ccells following with staining using 0.1% crystal violet (Beyotime, China) and finally observed under the light microscope (Olympus Corporation, Japan). Meanwhile, cells were firstly seeded into a 6-well plate for 24 h incubation for scratch assay. After that, a straight line through the bottom of plate was scratched and photos were took at immediately and 24 h later.

# Flow cytometry

Cell cycle and apoptosis were determined via flow cytometry by using the Annexin V-APC/PI Apoptosis Detection Kit and Cell Cycle Detection Kit (Beyotime, China) in accordance to the manufacturer's protocols which was described before(Wang X. et al. 2018).

# Immunoprecipitation Analysis

After cell lysis, 10-20 μl lysis solution was collected as Input for further detection. The rest of cell lysates were treated with anti-UBE2T antibody at 4°C for 12-16h, Afterwards, protein A/G-Sepharose beads (MCE, China) were supplemented for incubation about 4 h and collected finally. After washed three times for obtaining the co-precipitated proteins, western blotting was subsequently conducted with anti-FANCI antibody as described before.

# Coimmunoprecipitation (CO-IP)

Co-IP assay was conducted by employing the Crosslink Magnetic IP/Co-IP Kit (ThermoFisher, USA) based on the manufacturer's instructions.

# Mouse Tumor Xenografts

BALB/c nude mice (n=6, age:6-weeks, gender:female ) were purchased from Gempharmatech (Nanjing, China). Around $1\times10^7$ A549/sh-NC cells or A549/sh-FANCI cells were injected into the mice subcutaneous right flanks (n=3 per group). Tumor volume was recorded every week. 5 weeks later, mice were sacrificed for harvesting tumors. The Animal Care and Use Committee of Fujian Provincial Hospital approved the *in vivo* experiments.

# Immunohistochemistry (IHC)

IHC staining was performed on mice tissue sections embedded in paraffin. Afterwards, the sections were treated with primary anti-FANCI (showed above). By comparison with entire tissue area, the positive staining score was defined as 0 (0%), 1(1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%). If the staining score≥3, it was considered to be highly expressed.

# Statistical Analysis

Data was analysed using GraphPad Prism 6.01 software (GraphPad, USA). Between two or more groups, the differences was assessed with student's *t*-test or one-way analysis of variance (ANOVA), respectively. When *p*-value lower than 0.05, it was considered as statistically significant.

## Results

# FANCI was highly expressed in NSCLC tissues and cell lines

In 32 NSCLC tumor tissues, FANCI was found higher expressed than paired adjacent tissues by qRT-PCR (Fig. 1A). As western blot showed, the protein level was also up-regulated in tumors (Fig. 1B). Moreover, the expression of FANCI was found generally higher expressed in NSCLC cell lines (H1650, H1975, H827, A549, H1299) compared to HBE cells and was higher in A549 and H1299 cells (Fig. 1C). Therefore, H1299 and A549 cells were chosen for subsequent experiments.

# Downregulation of FANCI inhibited NSCLC cell proliferation

For evaluating the cellular functions of FANCI in NSCLC, FANCI was knocked down in A549 and H1299 cells. QRT-PCR showed that sh-FANCI decreased FANCI expression effectively (Fig. 2A). The results of CCK-8 assay indicated that FANCI knockdown restrained cell proliferation obviously compared with control group (Fig. 2B). Cell apoptosis rate was also elevated when FANCI was knocked down (Fig. 2C). Furthermore, as flow cytometry showed that the cell cycle in sh-FANCI group was blocked obviously (Fig. 2D). The above data showed that knockdown of FANCI abated cell growth and led to cycle arrest in NSCLC cells.

# FANCI regulates migration and EMT in NSCLC cells

Next, cell migration and invasion ability was determined with wound healing and Transwell assays. As Figure 3A and B showed, cell migration and invasion were attenuated obviously when FANCI was down-regulated. Furthermore, the EMT markers including E-cadherin, N-cadherin and Vimentin were also detected in A549 and H1299 cells. The western blot results revealed that E-cadherin was increased, but that of N-cadherin and Vimentin were reduced with FANCI knockdown (Fig. 3C). These results indicated that knock down of FANCI prohibited EMT in NSCLC cells.

# UBE2T contributes to the monoubiquitination of FANCI in NSCLC

As reported before, UBE2T induces monoubiquitination of FANCI to active downstream pathway. The RIP assay showed that UBE2T could bind to FANCI in A549 and H1299 cells (Fig. 4A). In NSCLC, UBE2T was also higher expressed in tumor tissues than adjacent tissues at mRNA and protein levels (Fig. 4B and C). The sh-UBE2T knocked down the expression of UBE2T effectively confirmed by qRT-PCR (Fig. 4D). When UBE2T was down-regulated, FANCI mRNA level was not changed significantly (Fig. 4E), but decreased at protein level (Fig. 4F), suggesting UBE2T might regulate the stability of FANCI protein by binding to it. To further explore the monoubiquitination of FANCI by UBE2T, co-IP assay was conducted and ubiquitin was examined. As results showed, the ubiquitin content in A549/sh-UBE2T was downregulated compared with that in A549/NC cells. On the contrary, the abundance was much higher in H1299/UBE2T cells than in the H1299/Vector cells (Fig. 4G), indicating UBE2T affected the monoubiquitination of FANCI in NSCLC.

# UBE2T mitigated the inhibitory effects of FANCI downregulation

To explore whether UBE2T could alleviate the influences of FANCI in NSCLC cells, UBE2T was subsequently overexpressed in FANCI knockdown cells. We found the overexpression of UBE2T promoted the proliferation but repressed the apoptosis of A549 and H1299 cells when FANCI was knocked down (Fig. 5A and B). The FANCI knockdown cell migration and invasion was also enhanced when UBE2T was upregulated (Fig. 5C and D). The EMT markers were detected and as WB showed, although sh-FANCI depressed EMT, overexpressed UBE2T partly rescued this (Fig. 5E). The above results revealed that UBE2T regulated the functions of FANCI in NSCLC.

# Knockdown of FANCI inhibits tumor growth in vivo

The sh-NC/A549 and sh-FANCI/A549 stable expressing cells were injected subcutaneously to nude mice to establish tumor xenografts. The tumor size and weight was smaller in sh-FANCI group compared to sh-

NC group and the tumor volume showed similar pattern (Fig. 6A-C). Additionally, as western blot and IHC showed, FANCI was obviously lowexpressed in sh-FANCI group (Fig. 6D and E), suggesting the knockdown of FANCI significantly inhibits tumor growth in vivo.

# Discussion

The 5-year overall survival rate is still dissatisfied, despite great efforts has been made for treating NSCLC, including surgery, chemotherapy, radiotherapy and targeted therapy(Cao et al. 2018, Giaj-Levra et al. 2020). Elucidating the underlying mechanisms of NSCLC tumorigenesis requires intensive and sustained exploration. Interstrand crosslinks (ICLs) refers to the covalent bond between complementary bases of double stranded DNA into chemical bond, which is one of the most toxic types of DNA damage(Rozelle et al. 2021). FA pathway is responsible for ICLs repair in S phase and maintains genomic stability. FANCL, as an E3 ubiquitin ligase, plays an irreplaceable role in FA pathway activation and ICLs repair. When ICLs occur on DNA, the FA core complex is recruited to the replication fork stagnated, and FANCL, the E3 ubiquitination ligase subunit in the FA-core complex, interacts specifically with UBE2T and then jointly promote the monoubiquitination of FANCI-FANCD2 heterodimer. The ubiquitinated FANCI-FANCD2 complex recruits downstream endonucleases to cut DNA, and then the downstream proteins undergo cross injury synthesis and homologous recombination repair to finally complete the repair of ICLs(Ceccaldi et al. 2016). FANCI was revealed as a biomarker for the poor prognosis of LUAD, however, a previous study found that FANCI was a negative factor of Akt pathway by regulating PHLPP phosphatases and indicated the tumor suppressor role of FANCI(Zhang X. et al. 2016, Zheng and Li 2020). Therefore, the role of FANCI played in tumors remains unclear, and further study is needed. Herein, we discovered FANCI was overexpressed in NSCLC tumor tissues. Knock down of FANCI inhibited NSCLC cells EMT, growth, migration and invasive, as well as the tumor growth. Depending on these results, we proposed FANCI as an oncogene in NSCLC, providing a novel potential therapeutic target for NSCLC.

UBE2T is an E2 enzyme, functions as an oncogene, widely dysregulated in numerous cancers(Liu et al. 2017, Zhang W. et al. 2019). Recently, UBE2T was reported to promote β-catenin nuclear translocation in HCC through MAPK/ERK axis(Lioulia et al. 2021). Moreover, UBE2T promotes the progression of LUAD by regulating autophagy via the p53/AMPK/mTOR signaling pathway(Zhu J. et al. 2021). As a member of E2 family, UBE2T enhances DNA crosslinking-induced damage repair by monoubiquitinating FANCI-FANCD2 (ID2) complex(Nepal et al. 2017). Some studies have pointed out FANCD2 is the preferred substrate for ubiquitination in FANCI-FANCD2 complex(Sato et al. 2012, Smogorzewska et al. 2007), but FANCD2 changes to be a poor substrate for ubiquitination when FANCI is absent(Wang S. et al. 2021). In contrast to FANCD2, the FA-core complex could ubiquitinate the isolation FANCI efficiently(van Twest et al. 2017, Wang S. et al. 2021). It also was reported that the protein abundance of FANCI in U2OS cells was nearly 10 times that of FANCD2, indicating that FANCI may also be ubiquitinated alone in cells to a certain degree(Beck et al. 2011). In present study, we found UBE2T was upregualted in NSCLC tumor tissues. Therefore, in terms of mechanism, we hypothesized FANCI might be regulated by UBE2T during the occurrence and development of NSCLC. According to the co-IP and WB assays, we confirmed that UBE2T interacted with FANCI and then stabilized FANCI protein level. Similarly, FANCI has been reported

to be bound by UBL5 (ubiquitin and ubiquitin-like protein 5) directly and then more stable(Oka et al. 2015). More interestingly, we discovered that UBE2T overexpression raised the monoubiquitination level of FANCI in NSCLC cells. Above results suggested the function of FANCI in NSCLC was probably monitored by UBE2T via monoubiquitination manner. Nevertheless, it's still not well known about how FA-core complex and UBE2T specifically regulate FANCI monoubiquitination and this needs more further research. In addition, the rescue experiments showed overexpressed UBE2T partly reversed the inhibitory effects of EMT, cell growth, migration and invasion caused by FANCI knockdown, indicating the role of FANCI in NSCLC could be regulated by UBE2T.

## Conclusions

In conclusion, we have revealed the oncogenic role of FANCI in promoting proliferation and EMT in NSCLC, which was mediated by UBE2T induced monoubiquitination of FANCI. Based on our study, we suggest UBE2T/FANCI may be a potential target for NSCLC and may serve as a biomarker for poor prognosis prediction.

## Abbreviations

FA: Fanconi anemia; FANCI: Fanconi anemia complementation group I; NSCLC: non-small-cell lung cancer; qRT-PCR: Quantitative reverse-transcription PCR; EMT: epithelial-to-mesenchymal transition; ICLs: interstrand cross-links; UBE2T: Ubiquitin-conjugating enzyme E2T; CCK-8: cell count kit 8.

## Declarations


# Acknowledgements

Not applicable.


# Authors' contributions

Jiguang Zhang and Xing Lin designed the study. Jingdong Wang and Jiguang Zhang performed the experiments. Xing Lin and Jingdong Wang prepared the figures. Jincheng Wu, Jianyuan Huang, and Zhaoxian Lin contributed to the drafting of the manuscript. All authors read and approved the final manuscript.


# Funding

None

# Availability of data and materials

The datasets in the present study are available from the corresponding author on reasonable request.

# Ethics approval and consent to participate

The protocols of animal studies were approved by The Animal Care and Use Committee of Fujian Provincial Hospital.

# Consent for publication

Not applicable.

# Competing interests

The authors confirm no conflicts of interest

## References


1. Beck M, Schmidt A, Malmstroem J, Claassen M, Ori A, Szymborska A, Herzog F, Rinner O, Ellenberg J, Aebersold R. 2011. The quantitative proteome of a human cell line. Mol Syst Biol 7:549.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424.
3. Cao J, Yuan P, Wang Y, Xu J, Yuan X, Wang Z, Lv W, Hu J. 2018. Survival Rates After Lobectomy, Segmentectomy, and Wedge Resection for Non-Small Cell Lung Cancer. Ann Thorac Surg 105:1483–1491.
4. Ceccaldi R, Sarangi P, D'Andrea AD. 2016. The Fanconi anaemia pathway: new players and new functions. Nat Rev Mol Cell Biol 17:337–349.
5. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. 2016. Cancer statistics in China, 2015. CA Cancer J Clin 66:115–132.
6. Del Re M, et al. 2019. Understanding the Mechanisms of Resistance in EGFR-Positive NSCLC: From Tissue to Liquid Biopsy to Guide Treatment Strategy. Int J Mol Sci 20.
7. Dong H, Nebert DW, Bruford EA, Thompson DC, Joenje H, Vasiliou V. 2015. Update of the human and mouse Fanconi anemia genes. Hum Genomics 9:32.

8. Doval DC, Desai CJ, Sahoo TP. 2019. Molecularly targeted therapies in non-small cell lung cancer: The evolving role of tyrosine kinase inhibitors. Indian J Cancer 56:S23-S30.
9. Engel NW, Schliffke S, Schuller U, Frenzel C, Bokemeyer C, Kubisch C, Lessel D. 2019. Fatal Myelotoxicity Following Palliative Chemotherapy With Cisplatin and Gemcitabine in a Patient With Stage IV Cholangiocarcinoma Linked to Post Mortem Diagnosis of Fanconi Anemia. Front Oncol 9:420.
10. Giaj-Levra N, Borghetti P, Bruni A, Ciammella P, Cuccia F, Fozza A, Franceschini D, Scotti V, Vagge S, Alongi F. 2020. Current radiotherapy techniques in NSCLC: challenges and potential solutions. Expert Rev Anticancer Ther 20:387–402.
11. Hay ED. 1995. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel) 154:8–20.
12. Hiley CT, Le Quesne J, Santis G, Sharpe R, de Castro DG, Middleton G, Swanton C. 2016. Challenges in molecular testing in non-small-cell lung cancer patients with advanced disease. Lancet 388:1002–1011.
13. Joo W, Xu G, Persky NS, Smogorzewska A, Rudge DG, Buzovetsky O, Elledge SJ, Pavletich NP. 2011. Structure of the FANCI-FANCD2 complex: insights into the Fanconi anemia DNA repair pathway. Science 333:312–316.
14. Katsuki Y, Takata M. 2016. Defects in homologous recombination repair behind the human diseases: FA and HBOC. Endocr Relat Cancer 23:T19-37.
15. Kelaidi C, et al. 2019. Bone Marrow Failure in Fanconi Anemia: Clinical and Genetic Spectrum in a Cohort of 20 Pediatric Patients. J Pediatr Hematol Oncol 41:612–617.
16. Kottemann MC, Smogorzewska A. 2013. Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. Nature 493:356–363.
17. Lioulia E, Mokos P, Panteris E, Dafou D. 2021. UBE2T promotes beta-catenin nuclear translocation in hepatocellular carcinoma through MAPK/ERK-dependent activation. Mol Oncol.
18. Liu LP, Yang M, Peng QZ, Li MY, Zhang YS, Guo YH, Chen Y, Bao SY. 2017. UBE2T promotes hepatocellular carcinoma cell growth via ubiquitination of p53. Biochem Biophys Res Commun 493:20–27.
19. Longerich S, San Filippo J, Liu D, Sung P. 2009. FANCI binds branched DNA and is monoubiquitinated by UBE2T-FANCL. J Biol Chem 284:23182–23186.
20. Machida YJ, Machida Y, Chen Y, Gurtan AM, Kupfer GM, D'Andrea AD, Dutta A. 2006. UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. Mol Cell 23:589–596.
21. Meetei AR, et al. 2003. A novel ubiquitin ligase is deficient in Fanconi anemia. Nat Genet 35:165–170.
22. Nepal M, Che R, Ma C, Zhang J, Fei P. 2017. FANCD2 and DNA Damage. Int J Mol Sci 18.
23. Oka Y, Bekker-Jensen S, Mailand N. 2015. Ubiquitin-like protein UBL5 promotes the functional integrity of the Fanconi anemia pathway. EMBO J 34:1385–1398.

24. Paulo P, Maia S, Pinto C, Pinto P, Monteiro A, Peixoto A, Teixeira MR. 2018. Targeted next generation sequencing identifies functionally deleterious germline mutations in novel genes in early-onset/familial prostate cancer. PLoS Genet 14:e1007355.
25. Rozelle AL, Cheun Y, Vilas CK, Koag MC, Lee S. 2021. DNA interstrand cross-links induced by the major oxidative adenine lesion 7,8-dihydro-8-oxoadenine. Nat Commun 12:1897.
26. Sato K, Toda K, Ishiai M, Takata M, Kurumizaka H. 2012. DNA robustly stimulates FANCD2 monoubiquitylation in the complex with FANCI. Nucleic Acids Res 40:4553–4561.
27. Smogorzewska A, et al. 2007. Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. Cell 129:289–301.
28. Sondalle SB, Longerich S, Ogawa LM, Sung P, Baserga SJ. 2019. Fanconi anemia protein FANCI functions in ribosome biogenesis. Proc Natl Acad Sci U S A 116:2561–2570.
29. Sun J, Zhu Z, Li W, Shen M, Cao C, Sun Q, Guo Z, Liu L, Wu D. 2020. UBE2T-regulated H2AX monoubiquitination induces hepatocellular carcinoma radioresistance by facilitating CHK1 activation. J Exp Clin Cancer Res 39:222.
30. Thiery JP. 2003. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 15:740–746.
31. Ueki T, Park JH, Nishidate T, Kijima K, Hirata K, Nakamura Y, Katagiri T. 2009. Ubiquitination and downregulation of BRCA1 by ubiquitin-conjugating enzyme E2T overexpression in human breast cancer cells. Cancer Res 69:8752–8760.
32. van Twest S, Murphy VJ, Hodson C, Tan W, Swuec P, O'Rourke JJ, Heierhorst J, Crismani W, Deans AJ. 2017. Mechanism of Ubiquitination and Deubiquitination in the Fanconi Anemia Pathway. Mol Cell 65:247–259.
33. Wang S, Wang R, Peralta C, Yaseen A, Pavletich NP. 2021. Structure of the FA core ubiquitin ligase closing the ID clamp on DNA. Nat Struct Mol Biol 28:300–309.
34. Wang X, Yin H, Zhang H, Hu J, Lu H, Li C, Cao M, Yan S, Cai L. 2018. NF-kappaB-driven improvement of EHD1 contributes to erlotinib resistance in EGFR-mutant lung cancers. Cell Death Dis 9:418.
35. Wu ZH, Zhang YJ, Sun HY. 2020. High ubiquitin conjugating enzyme E2 T mRNA expression and its prognostic significance in lung adenocarcinoma: A study based on the TCGA database. Medicine (Baltimore) 99:e18543.
36. Xie S, Jiang X, Zhang J, Xie S, Hua Y, Wang R, Yang Y. 2019. Identification of significant gene and pathways involved in HBV-related hepatocellular carcinoma by bioinformatics analysis. PeerJ 7:e7408.
37. Yin H, Wang X, Zhang X, Zeng Y, Xu Q, Wang W, Zhou F, Zhou Y. 2020. UBE2T promotes radiation resistance in non-small cell lung cancer via inducing epithelial-mesenchymal transition and the ubiquitination-mediated FOXO1 degradation. Cancer Lett 494:121–131.
38. Zhang W, Zhang Y, Yang Z, Liu X, Yang P, Wang J, Hu K, He X, Zhang X, Jing H. 2019. High expression of UBE2T predicts poor prognosis and survival in multiple myeloma. Cancer Gene Ther 26:347–355.

39. Zhang X, Lu X, Akhter S, Georgescu MM, Legerski RJ. 2016. FANCI is a negative regulator of Akt activation. Cell Cycle 15:1134–1143.
40. Zhao L, Sun H, Kong H, Chen Z, Chen B, Zhou M. 2017. The Lncrna-TUG1/EZH2 Axis Promotes Pancreatic Cancer Cell Proliferation, Migration and EMT Phenotype Formation Through Sponging Mir-382. Cell Physiol Biochem 42:2145–2158.
41. Zheng P, Li L. 2020. FANCI Cooperates with IMPDH2 to Promote Lung Adenocarcinoma Tumor Growth via a MEK/ERK/MMPs Pathway. Onco Targets Ther 13:451–463.
42. Zhu J, Ao H, Liu M, Cao K, Ma J. 2021. UBE2T promotes autophagy via the p53/AMPK/mTOR signaling pathway in lung adenocarcinoma. J Transl Med 19:374.
43. Zhu X, Li T, Niu X, Chen L, Ge C. 2020. Identification of UBE2T as an independent prognostic biomarker for gallbladder cancer. Oncol Lett 20:44.

# Figures

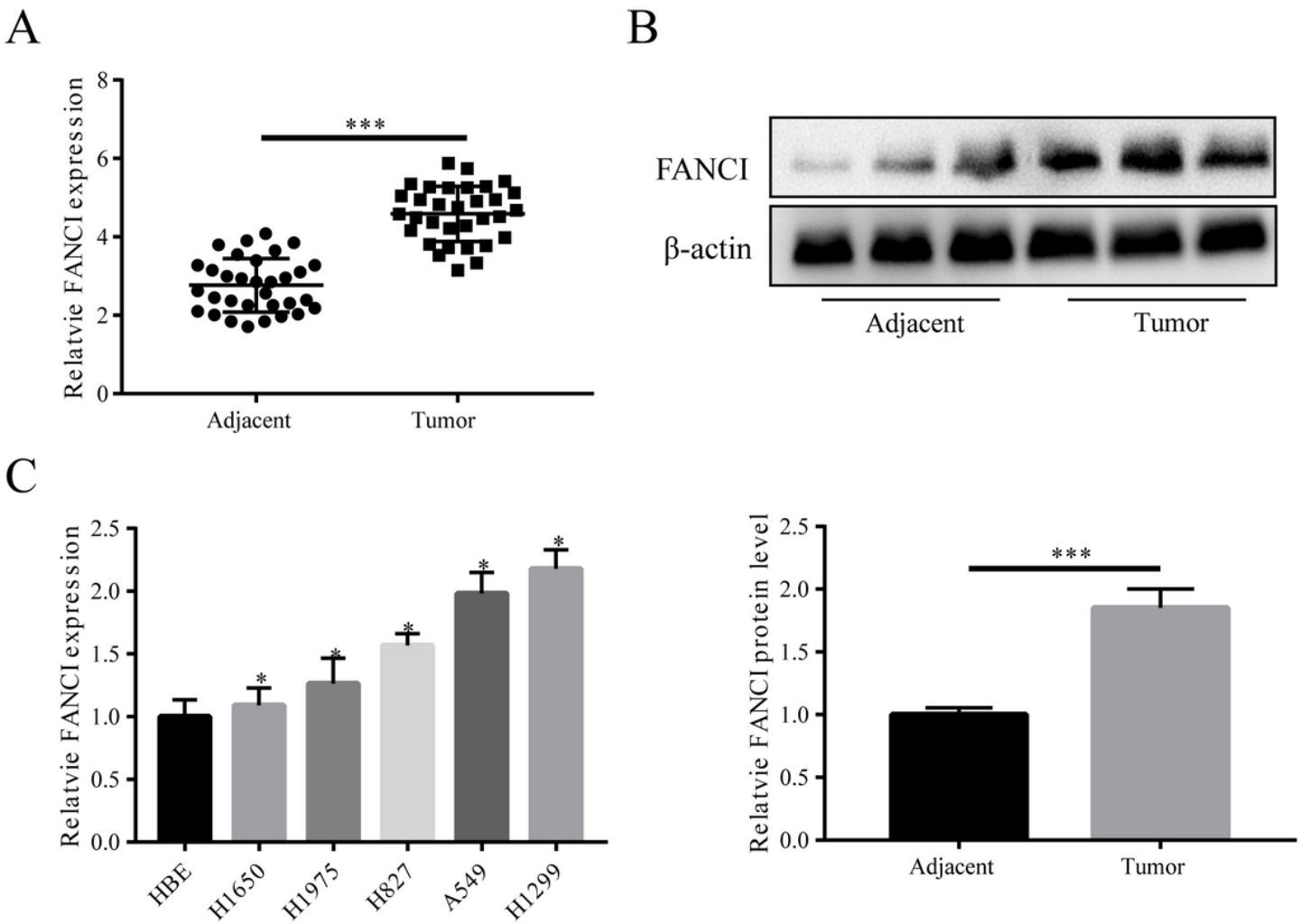
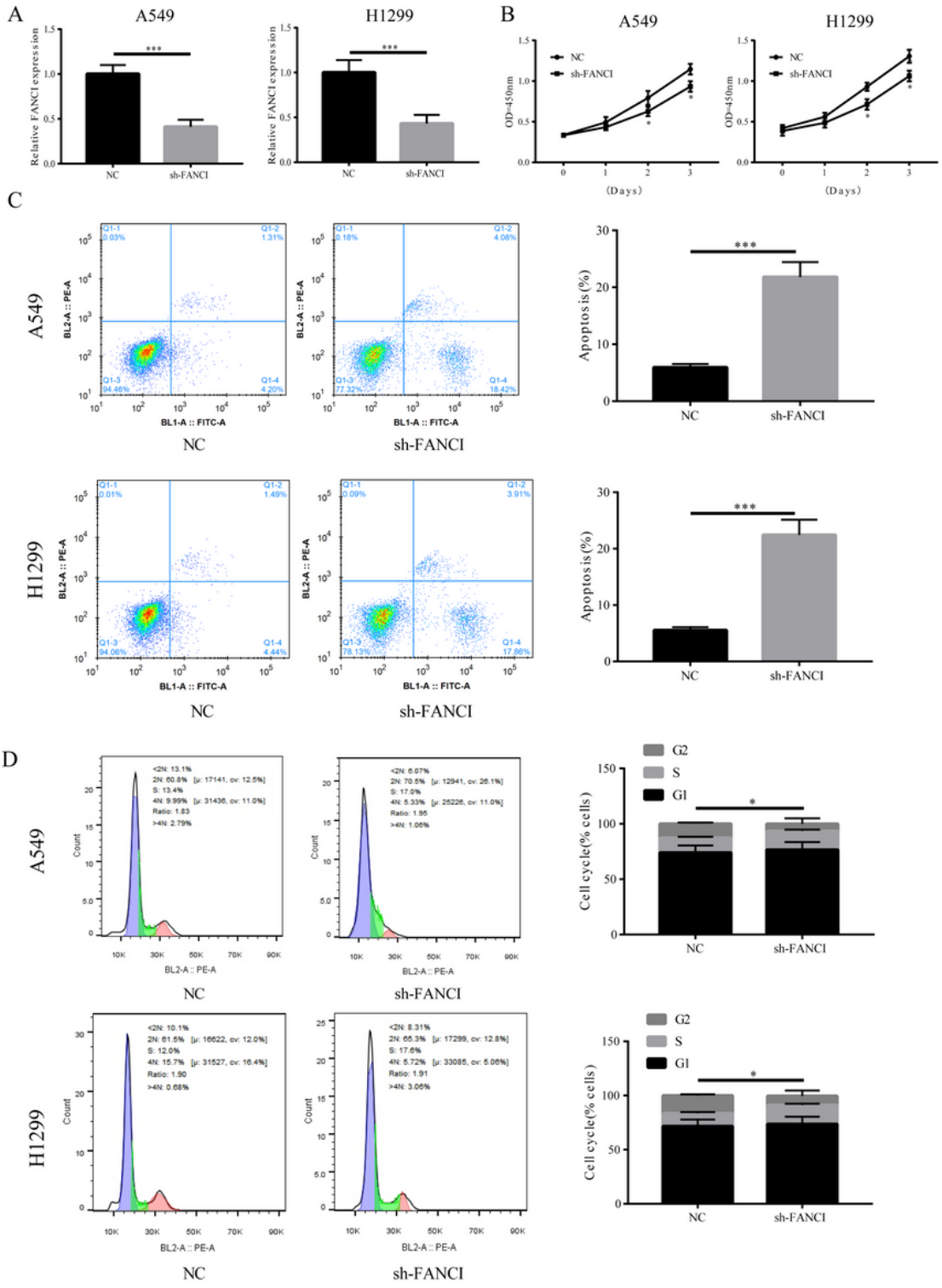



**Figure 1**

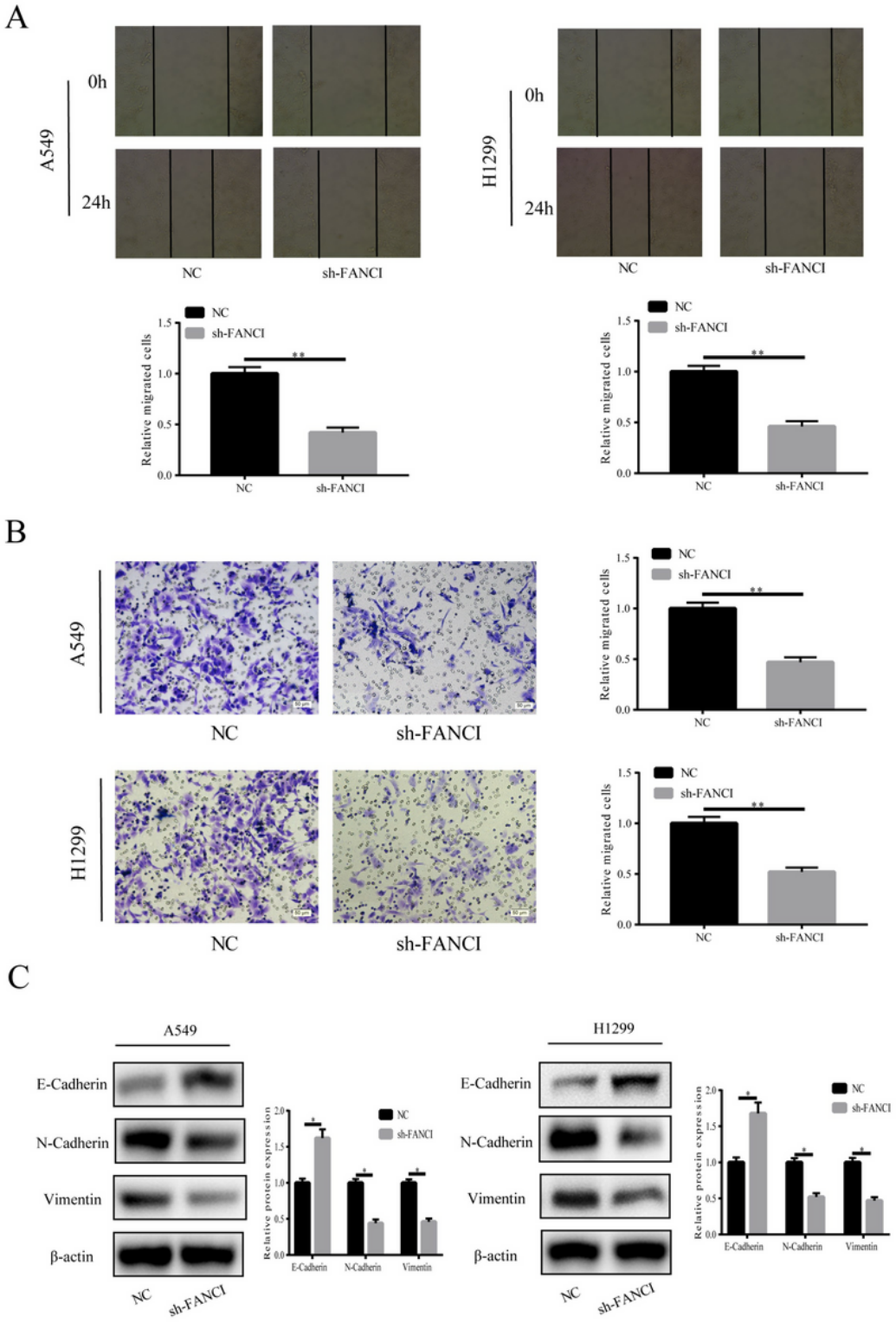
FANCI is overexpressed in NSCLC tissues and cell lines. (A and B) FANCI was up-regulated in NSCLC tumor tissues compared to adjacent tissues at mRNA and protein levels. (C) The expression of FANCI in HBE cell and NSCLC cell lines.




## Figure 2

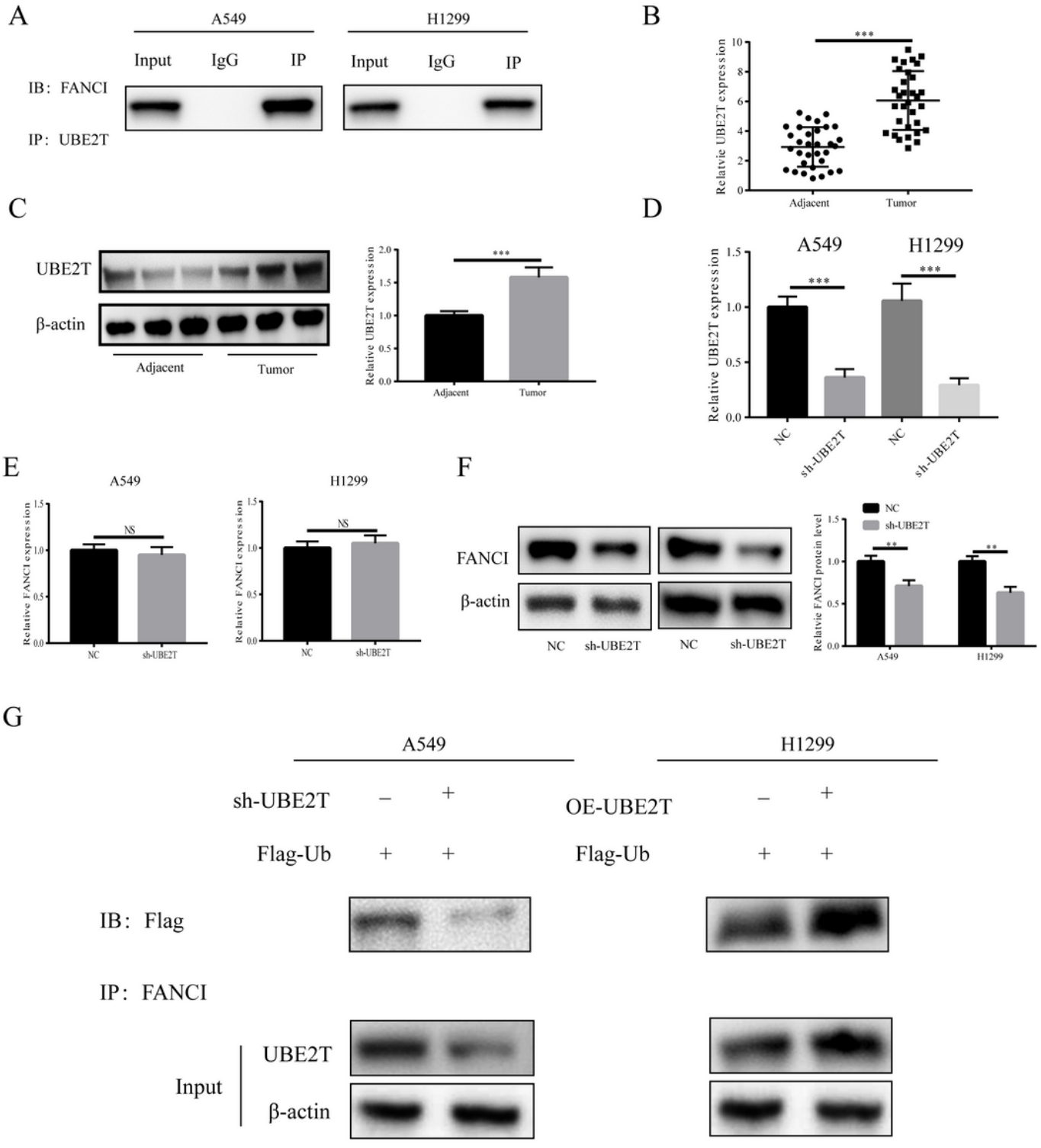
Knockdown of FANCI inhibits NSCLC cell viability. (A) The sh-FANCI knocked down the expression of FANCI obviously.The down-regulation of FANCI inhibited the cell proliferation (B), promoted cell apoptosis

(C) and blocked cell cycle (D) in A549 and H1299 cells.




## Figure 3

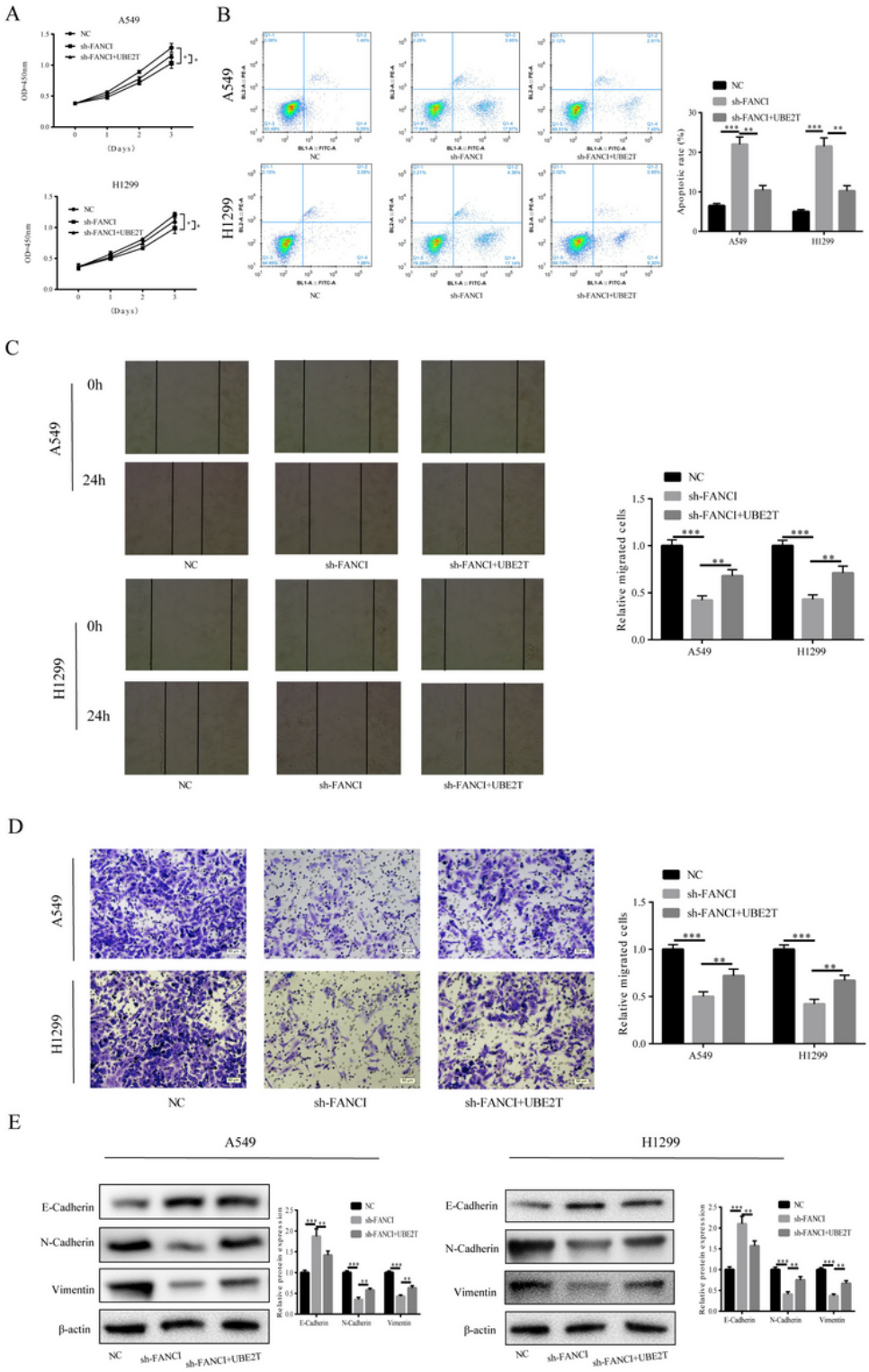
Knockdown of FANCI inhibits EMT in NSCLC cells. (A and B) The wound-healing and transwell assays showed that down-regulation of FANCI prohibits cell migration and invasion in A549 and H1299 cells. (C) Knockdown of FANCI raised E-cadherin, whereas reduced N-cadherin and Vimentin protein expression level.




## Figure 4

UBE2T contributes to the ubiquitination of FANCI. (A) CO-IP showed UBE2T could bind to FANCI. (B and C) UBE2T was overexpressed in NSCLC tumor tissues compared to adjacent tissues at mRNA and protein levels. (D) The transfection efficiency of sh-UBE2T. (E) FANCI mRNA level was not significantly influenced by sh-UBE2T. (F) The down-regulation of UBE2T caused the reduction of FANCI protein. (G) The total FANCI protein in A549 and H1299 cells was obtained using the immunoprecipitation method and the

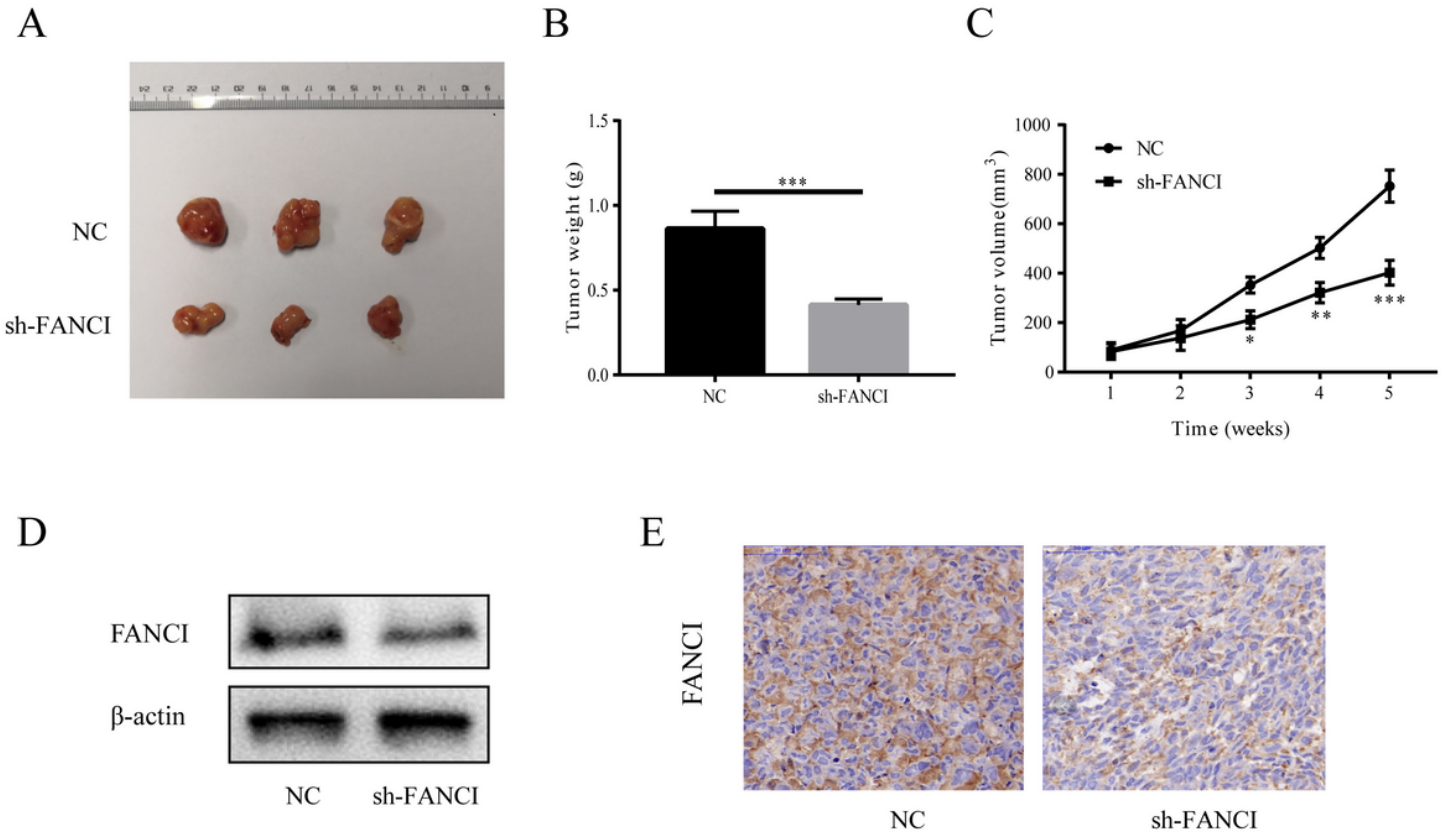
enrichment of ubiquitin was obviously higher than that in the control group after UBE2T was overexpressed.




## Figure 5

UBE2T overexpression alleviates the inhibitory effects of FANCI knockdown in NSCLC cells. (A) CCK-8 cell proliferation assay in sh-FANCI/sh-FANCI+UBE2T groups. (B) Cell apoptosis analysis in sh-FANCI/sh-

FANCI+UBE2T groups.(C and D) The wound-healing and transwell assays in sh-FANCI/sh-FANCI+UBE2T groups.(E) The expression of E-cadherin, N-cadherin and Vimentin were detected by western blot.




## Figure 6

Knockdown of FANCI suppresses the tumor growth in vivo. (A-C) The tumor weights and volumes were decreased sh-FANCI group compared to sh-NC group. (D and E) The western blot and IHC results in tumor tissues showed that FANCI expression was significantly lower in sh-FANCI group.