

# IFITM3 influences the invasion and metastasis of hepatocellular carcinoma by regulating NCAPG through phosphorylation

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## Research

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# Abstract

**Background:** Several studies have demonstrated that the expressions of IFITM3 and NCAPG are closely related to the prognosis of various tumors. However, the mechanism of action of these two is not yet clear. In this study, we have explored the mechanism of action of IFITM3 and NCAPG in the promotion of the invasion and metastasis of hepatocellular carcinoma (HCC).

**Methods:** Specimens of liver cancer and adjacent tissues from 55 HCC patients at the Department of Hepatobiliary Surgery, Second Affiliated Hospital of Nanchang University were collected, and the expressions of NCAPG and IFITM3 were determined by qRT-PCR and Western blotting. Through the analysis of multiple databases, the relationship between IFITM3 and NCAPG was established by the CO-IP method. SiRNA and plasmids were used to downregulate and upregulate IFITM3, and the expression of STAT3/CDK1, NCAPG mRNA, and protein was observed. After downregulating and upregulating the expression of IFITM3 and NCAPG, the ability of HCC cells to invade and metastasize was determined using a scratch test and Transwell assays. After using pathway inhibitors and activators, the expression of NCAPG was observed.

**Results:** According to the database, both IFITM3 and NCAPG were highly expressed in liver hepatocellular carcinoma. We also confirmed that IFITM3 and NCAPG were upregulated in HCC tissues and cells. Furthermore, the bioinformatics analysis and CO-IP indicated that there was a protein interaction between IFITM3 and NCAPG, and that IFITM3 could regulate NCAPG by phosphorylating it. We further confirmed our observations by retrospective experiments. The reuse of pathway inhibitors and activators indicated that IFITM3 could regulate NCAPG through STAT3/CDK1 to promote the invasion and metastasis of HCC. Finally, the animal experiments confirmed that the results were also reproducible *in vivo*.

**Conclusion:** IFITM3 can regulate NCAPG through STAT3/CDK1 to promote the invasion and metastasis of HCC.

## 1. Background

Liver cancer is a common malignancy, and hepatocellular carcinoma (HCC) is the most common type of liver cancer [1, 2]. HCC has a poor prognosis, as it metastasizes rapidly, and by the time diagnosis is made, most of it is already in the advanced stage of cancer [3, 4]. Therefore, the identification of the biomarkers of liver cancer and understanding the mechanism of invasion and metastasis of liver cancer is imperative.

Interferon-induced transmembrane protein 3 (IFITM3) is a member of interferon-stimulating gene (ISG) family [5, 6]. Previous studies have reported that IFITM3 is closely related to several viral infections [7–9]. For example, studies have shown that restriction of viral entry by IFITM3 inhibits the infectivity of iris virus and Noda virus [10]. Sun et al. have also shown that IFITM3 prevents acute influenza in mice [11]. Recent research has also reported that IFITM3 is closely related to the development of COVID-19 disease [12, 13]. The BAT SARS WIV1 coronavirus uses the ACE2 in a variety of animals as the receptor and

evades IFITM3 through the activation of the membrane fusion protein TMPRSS2[14]. Moreover, the expression of IFITM3 is significantly positively correlated with the prognosis of several tumors[13, 15–17]. For example, IFITM3 upregulates the expression of c-myc through the ERK1/2 signaling pathway to promote the proliferation of liver cancer cells[8]. Although IFITM3 has been proven to be a key gene affecting the progression of several disease, its influence in liver cancer has been studied relatively scarcely, and its specific mechanism remains to be understood.

The non-SMC condensin I complex subunit G (NCAPG), an antigen that organizes the coiled topology of a single chromatid, is overexpressed in various types of cancer[18–20]. It helps reorganize chromatin into rod-shaped mitotic chromosomes and ensures the separation of sister chromatids during cell division[21, 22]. Initially, NCAPG was identified as a breeding gene in dairy cows. Subsequent research reported that NCAPG was associated with the progression of various diseases[23, 24]. For example, NCAPG was identified as a risk factor for psoriasis. It is also involved as a key gene in the progression of various tumors[25–27]. For example, NCAPG is overexpressed in colorectal cancer and prostate cancer tissues and has a close relationship with its prognosis. Recent research has reported that NCAPG plays an extremely important role in HCC, but the specific mechanism of action is unknown.

Protein phosphorylation is an important step in the post-translational modification of proteins and plays an important role in determining the activity of enzymes and other important functional molecules, the delivery of secondary messengers, and the cascade of enzymes[28, 29]. STAT3 is a member of the STAT family. It is an important factor that is involved in the modification of phosphorylation and is an important nuclear transcription factor[30, 31]. STAT3 can also be activated by HBV, HCV, and various oncogene proteins[32]. An unregulated STAT/SOCS signal can also lead to the activation of STAT3, which then regulates the transcription of downstream genes[31, 33, 34]. Hepatocellular carcinoma (HCC) tissues show a significant overexpression of STAT3, which can result in malignant transformation of hepatocytes, causing cancer. Furthermore, activation of the STAT3 signal and c-Myc, EGFR, TGF, survivin, and VEGF is closely related to the occurrence and development of HCC[32, 35]. Cyclin-dependent kinases (CDK) represent a Ser/Thr kinase system that corresponds to the cell cycle process. It is not only regulated by phosphorylation and dephosphorylation but is also affected by oncogenes and tumor suppressor genes. Therefore, identifying and clarifying the relationship between STAT3/CDK1 and HCC is crucial for the diagnosis and treatment of liver cancer.

In our research, we observed that both IFITM3 and NCAPG played an irreplaceable role in the invasion and metastasis of HCC, and IFITM3 could positively regulate NCAPG. Importantly, we discovered for the first time that IFITM3 could influence the invasion and metastasis of HCC by changing the level of NCAPG phosphorylation modification.

## **2. Materials And Methods**

### **2.1. Tissue specimen**

The study was carried out on 55 patients with HCC diagnosed between 2015 and 2019. Only those patients who had undergone hepatectomy without any treatment before surgery, including radiotherapy or chemotherapy, were included in the study. The liver cancer and adjacent tissue specimens were placed in liquid nitrogen immediately upon collection. The study was approved by the Ethics Review Committee of the Second Affiliated Hospital of Nanchang University. The procedure followed the ethical standards of the Human Experiment Responsibility Committee (institution and country) and the 1975 “Helsinki Declaration” (revised in 2008). Informed consent was obtained from all the patients before enrollment.

## **2.2. Cell culture**

The hepatocyte cell line (HL-7702) and four HCC cell lines (SMMC7721, MHCC97H, HCCLM3, and Huh-7) used in this study were purchased from the Shanghai Institute of Cell Biology (Shanghai, China). All cell lines were cultured in Dulbecco’s Modified Eagle Medium (DMEM) (Solarbio, Beijing, China) supplemented with 10% FBS (Biological Industries, Beit-Haemek, Israel), 100 µg/mL streptomycin, and 100 U/mL penicillin, and incubated under 5% carbon dioxide conditions. All the experiments used cells in the logarithmic phase of growth.

## **2.3. Cell transfection**

The SiRNA and plasmids were obtained from Ruibo (Guangzhou), Transfect the SiRNA into the cells first, and then perform qRT-PCR to select the one with the best effect(Fig S1).and the HCC cells were transfected using Lipofectamine 3000 (Thermo Fisher Scientific, Inc), according to the manufacturer’s instructions. All the transfected cells were incubated in complete medium for at least 24 h prior to transfection and were rinsed with phosphate buffered saline (PBS, pH 7.4) before transient transfection.

## **2.4. RNA extraction and qRT-PCR**

Total RNA was isolated from the cells using Trizol reagent (Invitrogen), according to the manufacturer’s instructions. Reverse transcription (RT) and qRT-PCR were performed using PrimeScript RT kit (Dalian, China, Treasure) and SYBR Prime Script RT PCR kit (Dalian, China, Treasure). The sequences of IFITM3, NCAPG, STAT3, CDK1, and GAPDH are provided in the supplementary information. The result was calculated using the  $2^{-\Delta\Delta Ct}$  method.

## **2.5. Western blotting (WB)**

The proteins were separated by polyacrylamide sodium dodecyl sulphate gel electrophoresis (SDS-PAGE), transferred to a nitrocellulose membrane (Amersham, USA), and blocked with 5% skim milk at room temperature. The membrane was then treated with reagents containing the rabbit polyclonal antibody IFITM3 (ab109429, 1:1000, Abcam, Cambridge, UK), NCAPG rabbit polyclonal antibody (ab226805, 1:2000, Abcam, Cambridge, UK), the STAT3 polyclonal rabbit antibody (ab68153, 1:1000, Abcam, Cambridge, UK), CDK1 rabbit polyclonal antibody (ab18, 1:10000, Abcam, Cambridge, UK), and GAPDH rabbit polyclonal antibody (ab9485, 1:2500, Abcam, Cambridge, UK). The treated membrane was incubated overnight at 4 °C, after which it was washed thrice with PBST buffer (PBS buffer containing 0.1% Tween-20) for 10 min. Horse peroxidase-labeled anti-rabbit IgG secondary antibody (ab6721, 1:2000, Abcam, Cambridge, UK) was added to the membrane, followed by incubation for 1 h at room

temperature. The membrane was washed thrice with PBST buffer for 10 min. A photometer (GE, USA) was used to detect immune activity.

## **2.6. Transwell migration analysis**

Tests for cell migration and invasion were performed in a Transwell chamber (Corning Inc., Corning, NY, USA) with a polycarbonate membrane. In the Transwell migration analysis,  $1 \times 10^5$  cells of both the experimental cell lines MHCC-97H and HCC-LM3 were seeded into the upper chamber of DMEM without serum, while 10% FBS was added to the lower chamber. After incubation for about 18–36 h, the cells in the upper chamber were wiped off, and those in the lower chamber were stained with 1% crystal violet at 25 °C for 1 min. The stained cells were observed and counted under an optical microscope (Nikon). The procedure of the Transwell invasion assay was the same as above, except that the upper chamber was coated with 20 µg extracellular matrix gel (Sigma-Aldrich; Merck KGaA).

## **2.7. Scratch test**

MHCC-97H and HCC-LM3 cells were used for the scratch experiment. First, the cells were seeded in a six-well plate and transfected in the six-well plate. While transfecting and changing the medium, the six-well plate was scratched using a 200 µl sterile tip, marked as 0 h, and a picture was taken. After 24 h, another picture was taken at the same marked location. The blank distance of the two pictures was compared statistically.

## **2.8. RNA Seq**

In addition to analyzing gene expression level, RNA Seq can also find new transcripts, SNP, splice variants, and provide allele specific gene expression. It can quickly obtain almost all transcripts sequence information of a specific tissue or organ of a species in a certain state. It has been widely used in basic research, clinical diagnosis and drug development. The experimental flow is total RNA extraction mRNA separation library building reagent quantitative Du library recovery bridge amplification computer sequencing; project analysis process is data output data = data De hybridization transcriptome splicing SSR Analysis and SNP analysis gene function annotation gene expression difference analysis differential gene expression pattern clustering differential gene enrichment analysis

## **2.9. Co-immunoprecipitation (CO-IP)**

The cells were first transfected and then harvested to prepare the protein samples, using the following steps: 1. Cells were harvested 24–48 h after transfection by adding an appropriate amount of cell lysis buffer (containing protease inhibitors), lysing them on ice for 30 min, and then centrifuging at 12000 rpm for 30 min. 2. A microcentrifuge tube was taken for Western blot analysis, 1 µg of the corresponding antibody was added to the remaining lysate, which was added to the cell lysate, and then incubated at 4 °C overnight. 3. An aliquot of 10 µL of protein A agarose beads was taken, and an appropriate amount of lysis buffer was used. The solution was washed thrice by centrifuging at 3,000 rpm for 3 min each time. 4. An aliquot of 10 µL of protein A agarose beads pretreated with the cell lysate was incubated overnight with the antibody and then incubated at 4 °C for 2–4 h for coupling of the Protein A agarose beads. 5. After the immunoprecipitation reaction, the mixture was centrifuged at 3,000 rpm for 3 min at

4 °C to settle the agarose beads at the bottom of the tube, after which the supernatant was carefully aspirated, and agarose beads were washed 3–4 times with 1 mL of lysis buffer. Finally, 15 µL of 2x SDS loading buffer was added and boiled for 5 min. 6. The proteins were then separated by SDS-PAGE and subjected to Western blot analysis.

### **2.10. *In vivo* experiments**

For the *in vivo* invasion and metastasis assays, first we divided the nude mice into seven groups and then injected HCC-LM3 cells from different treatments into the tail vein. Afterward,  $1 \times 10^7$  cells in 100 µL PBS were injected subcutaneously into the sides of male BALB/c-nu/nu mice (6–8 weeks old; n = 6 per group) (Hunan SJA Experimental Laboratory Animal Company) (Hunan, China). After six weeks, the mice were sacrificed (Using cervical dislocation method) and lung tissues were collected for HE staining and immunohistochemistry (IHC). All the animal experiments were approved by the Animal Experiment Ethics Committee of the Second Affiliated Hospital of Nanchang University and were carried out in accordance with the guidance of the British Animal (Scientific Procedures) Act, 1986, and the EU Directive 2010/63/EU.

### **2.11. HE staining and immunohistochemistry (IHC)**

The prepared tissues were fixed with tissue fixative, placed in paraffin blocks, and sectioned using a microtome. The tissue sections were first dewaxed with xylene and then dehydrated with gradient ethanol. The sections were stained with H&E to identify any changes in their morphology and then rehydrated and microwaved in sodium citrate buffer (10 mmol/L, pH 6.0) to recover the antigen. The sections were incubated with 0.3% hydrogen peroxide/PBS for 30 min and then blocked with serum. Subsequently, the tissue was incubated overnight at 4 °C with 1:200 diluted rabbit monoclonal antibody NCAPG (ab226805, Abcam, Cambridge, MA, USA). Subsequently, it was washed with PBS every 5 min for three times and then incubated with the secondary antibody at 37 °C for 30 min. The sections were stained with diaminobenzidine (DAB) and hematoxylin dye, after which the excess dye was washed under running water, rehydrated with gradient alcohol, and sealed with neutral resin. Finally, the sections were observed under an inverted microscope and images were taken.

### **2.12. Statistical Analysis**

Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software Inc.). All the data were expressed as mean  $\pm$  standard deviation (SD). The two groups were compared using the t-test. Pearson's  $\chi^2$  test was used to analyze the relationship between IFITM3 expression and NCAPG. All the experiments in the study were repeated thrice. P values of < 0.05 or < 0.01 were considered statistically significant.

## **3. Results**

### **3.1. High expression of IFITM3 and NCAPG is associated with poor HCC prognosis.**

In order to evaluate the expression of IFITM3 and NCAPG in HCC, we first analyzed the documented expression of IFITM3 and NCAP in HCC from the TCGA database. We observed that both IFITM3 and NCAPG were highly expressed in HCC, and both high and low levels of expression were associated with poor HCC prognosis (Fig. 1A-1D). We then performed PCR and Western blotting on the liver cancer specimens and the adjacent tissues from the 55 patients enrolled in our study (Fig. 1E,1F,1G), and observed that the results were consistent with those in the database. We also performed PCR analysis on liver cell line HL-7702 and the hepatoma cell lines MHCC-97H, HCC-LM3, SMCC-7721, and Huh-7, and these results also indicated that the expression of IFITM3 and NCAPG in HCC cells was upregulated (Fig. 1H and 1I). These results suggest that the expression levels of IFITM3 and NCAPG are upregulated in HCC tissue cells, which affects the prognosis of HCC patients.

### **3.2. The relationship between IFITM3 and NCAPG in HCC**

In order to further understand the relationship between IFITM3 and NCAPG, we carried out bioinformatics analysis on multiple databases available online and observed that both IFITM3 and NCAPG were highly expressed in liver cancer tissues, with a positive correlation between the two (Fig. 2A). This was consistent with the results of qRT-PCR analysis on the tissues (Fig. 2E). Furthermore, we constructed volcano maps of RNA Seq in HCC tissues and adjacent tissues, and found that IFITM3 and ncapg were highly expressed (Fig. 2C), and predicted the target genes downstream of IFITM3 using the mirDIP and starBase databases (Fig. 2D). We then predicted on the String website that there may be a phosphorylation modification between IFITM3 and NCAPG (Fig. 2B). Finally, using CO-IP, we confirmed the presence of protein interactions between IFITM3 and NCAPG (Fig. 2F).

### **3.3. IFITM3 can positively regulate NCAPG**

After understanding the relationship between IFITM3 and NCAPG, in order to further regulate the relationship between the two, we transfected the interfering fragments of IFITM3 (i.e., SiRNA) and plasmids into MHCC-97H and HCC-LM3, and observed the mRNA and protein expressions of NCAPG. Our findings confirmed that IFITM3 caused positive regulation of NCAPG; thus, downregulation of IFITM3 reduced the mRNA and protein levels of NCAPG (Fig. 3A-3C), and upregulation of IFITM3 increased the mRNA and protein levels of NCAPG (Fig. 3D-3F). This indicated that IFITM3 regulated the expression of NCAPG positively.

### **3.4. IFITM3 regulates NCAPG and affects the invasion and metastasis of HCC**

After determining that IFITM3 positively regulates NCAPG, we conducted a retrospective experiment to corroborate our experimental results further. We transfected the interfering fragments and plasmids of IFITM3, interfering fragments and plasmids of NCAPG, interfering fragments of IFITM3 plus plasmids of NCAPG, and plasmids of IFITM3 plus interference fragments of NCAPG simultaneously, and compared them with the control group. We observed that when the expression of IFITM3 was upregulated, NCAPG expression also enhanced, and NCAPG was upregulated, IFITM3 was also enhanced (Fig. 4A-4D). Simultaneous transfection of the IFITM3 interference fragment and NCAPG plasmid or the IFITM3 plasmid and NCAPG interference fragment resulted in levels of NCAPG expression that were close to those of the control group. Regarding the relationship between IFITM3 and NCAPG in terms of the

invasion and metastasis ability of HCC cells, we observed through cell scratch experiments and Transwell experiments that the expression of IFITM3 and NCAPG was positively correlated with the ability of cell invasion and metastasis (Fig. 4E-4H). The upregulation of IFITM3 and NCAPG enhanced the ability of invasion and metastasis of the cells, while downregulation of both showed an opposite effect. On the other hand, simultaneous downregulation of IFITM3 and upregulation of NCAPG, or upregulation of IFITM3 and downregulation of NCAPG showed no significant difference in the invasion and metastasis ability compared to the control group. This indicated that IFITM3 regulated NCAPG to influence the invasion and metastasis of HCC cells.

### **3.5. IFITM3 regulates NCAPG through phosphorylation modification**

Regarding the specific mechanism of action between IFITM3 and NCAPG, our data showed that there may be phosphorylation modifications between IFITM3 and NCAPG, and IFITM3 could regulate NCAPG through the STAT3/CDK1 pathway (Fig. 2B). Therefore, we upregulated the expression of IFITM3 and observed the expression of STAT3/CDK1. We observed that the downregulation of the expression of IFITM3 decreased the expression of STAT3/CDK1 (Fig. 6A-6F), while the upregulation of IFITM3 increased the expression of STAT3/CDK1. Furthermore, we observed that the expression of NCAPG was upregulated after the use of STAT3/CDK1 activators (A18479) and downregulated after the use of inhibitors (A12231) (Fig. 6G-6L). This indicated that IFITM3 regulated NCAPG via STAT3/CDK1.

### **3.6. Confirmation of results through animal experiments**

*In vitro* cell experiments indicated that IFITM3 regulated NCAPG to influence the invasion and metastasis of HCC. In order to further verify our findings, we performed experiments on animals. We observed that the degree of lung metastasis in mice injected with cells containing upregulated IFITM3 and NCAPG was significantly higher than that in the control group and that in mice injected with cells containing downregulated IFITM3 and NCAPG was significantly lower, while injection with cells containing simultaneously upregulated IFITM3 and downregulated NCAPG, or downregulated IFITM3 and upregulated NCAPG, showed no significant difference (Fig. 7A-7B). Thus, *in vivo* experiments reiterated that IFITM3 could affect the expression of NCAPG through phosphorylation, which in turn influenced the invasion and metastasis of HCC (Fig. 7C).

## **4. Discussion**

In this study, we demonstrated that both IFITM3 and NCAPG were highly expressed in HCC tissues and cells, and their expression was closely related to the prognosis and recurrence of liver cancer. We also demonstrated for the first time that IFITM3 regulated NCAPG through phosphorylation to affect the invasion and metastasis of HCC.

Several reports have suggested the involvement of IFITM3 in the development of cancer [36, 37]. For example, studies have reported that IFITM3 upregulated the expression of c-myc through the ERK1/2 signaling pathway and promoted the proliferation of liver cancer cells. Studies have also demonstrated that IFITM3rs12252-CC is related to the poor differentiation and progression of HCC. Recently, IFITM3

was reported to play an irreplaceable role in the development of novel coronaviruses [7, 10, 13, 38]. In our research as well, IFITM3 was observed to play a critical role in HCC. The expression of IFITM3 was directly related to the prognosis of HCC patients and could affect the invasion and metastasis of HCC. Western blot and qRT-PCR of the collected liver cancer specimens and adjacent tissues revealed that IFITM3 was abnormally expressed in liver cancer tissues. After silencing IFITM3, the invasion and metastatic ability of MHCC-97H and HCC-LM3 decreased, while the opposite was observed after the upregulation of IFITM3. IFITM3 is highly likely to be a novel marker of liver cancer, which represents an important breakthrough for the diagnosis and treatment of liver cancer.

NCAPG has been shown to play a vital role in biological growth and development[19, 21, 23, 39]. For instance, NCAPG plays a role in promoting the recombination of smooth muscle chromosomes and can be used as a breeding gene to increase fertility in cows. NCAPG may also be used as a therapeutic target for the treatment of T2DM. Recent studies have also reported that NCAPG plays a vital role in the genesis and development of tumors. Studies have demonstrated that NCAPG acts as an oncogene in HCC and promotes cell proliferation and anti-apoptosis by activating the PI3K/AKT/FOXO4 pathway[40–42]. Silencing NCAPG inhibits the proliferation of hepatoma cells and induces their apoptosis. We also confirmed by Western blotting and qRT-PCR that NCAPG was abnormally expressed in HCC, and was closely related to the invasion and metastasis of liver cancer. Thus, NCAPG can be used as a marker for the diagnosis and treatment of liver cancer.

Phosphorylation is currently the most widely known post-translational modification[28, 30, 32]. It is estimated that nearly one-third of the proteins in eukaryotes are phosphorylated at any given point of time. At present, 518 kinds of kinases have been identified in human cells, while the plants *Arabidopsis* and rice are estimated to possess 1000 and 1467 kinases, respectively. Based on the available databases, about 1–2 million proteins and sites have been estimated to be phosphorylated[33–35, 43]. In actual research, with the help of mass spectrometry, about 62,679 phosphosites in 11,632 proteins are known to be phosphorylated in mammals. Thus, we know that phosphorylation is a highly common form of a post-translational modification. The protein encoded by the STAT3 gene is a member of the STAT protein family. STAT family members are phosphorylated by receptor-associated kinases. Activated STAT3 directly affects the activity of its active proteins, which in turn affects the downstream genes. In our research, we observed that IFITM3 activated STAT3 through phosphorylation. The activated STAT3 promoted the phosphorylation of CDK1 and then regulated NCAPG, thereby affecting the invasion and metastasis of HCC cells.

In conclusion, our study demonstrated that IFITM3 and NCAPG were highly expressed in HCC, and the upregulation of both indicate poor postoperative prognosis. More importantly, we observed that IFITM3 could regulate NCAPG through STAT3 to affect the invasion and metastasis of HCC. Therefore, IFITM3 and NCAPG may be used as biomarkers for the diagnosis and treatment of HCC.

## 5. Conclusion

We observed that the expressions of IFITM3 and NCAPG were significantly upregulated in HCC tissues and cells and were strongly associated with the prognosis. Downregulation of IFITM3 and NCAPG decreased the invasion and metastasis ability of HCC cells, while their upregulation showed the opposite effect. More importantly, we could confirm that phosphorylation modification occurs between IFITM3 and NCAPG. IFITM3 regulates the expression of NCAPG through STAT3/CDK1, which in turn affects the invasion and metastasis of HCC.

## **Abbreviations**

IFITM3: Interferon-induced transmembrane protein 3. NCAPG: The non-SMC condensin I complex subunit G. HCC: hepatocellular carcinoma. CO-IP: Co-immunoprecipitation.

## **Declarations**

### **Author contribution**

Project design: WL, LW. data analysis: RZ, EL. article drafting, and revision: WL, EL. and all agreed to the publication of the final version.

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### **Availability of data and materials**

This article contains data to support the findings of this study. According to reasonable requirements, the data and materials in this study can be obtained from the corresponding author.

### **Competing interest**

The author declares no conflicts of interest.

### **Consent for publication**

Not applicable.

### **Ethics approval and consent to participate**

All experiments strictly followed the panel's specific guidelines for animal feeding, treatment, and euthanasia. All animal experiments were approved in writing by the Animal Experiment Ethics Committee of the Second Affiliated Hospital of Nanchang University, and all were implemented strictly in accordance with the "Guidelines for the Care and Use of Laboratory Animals."

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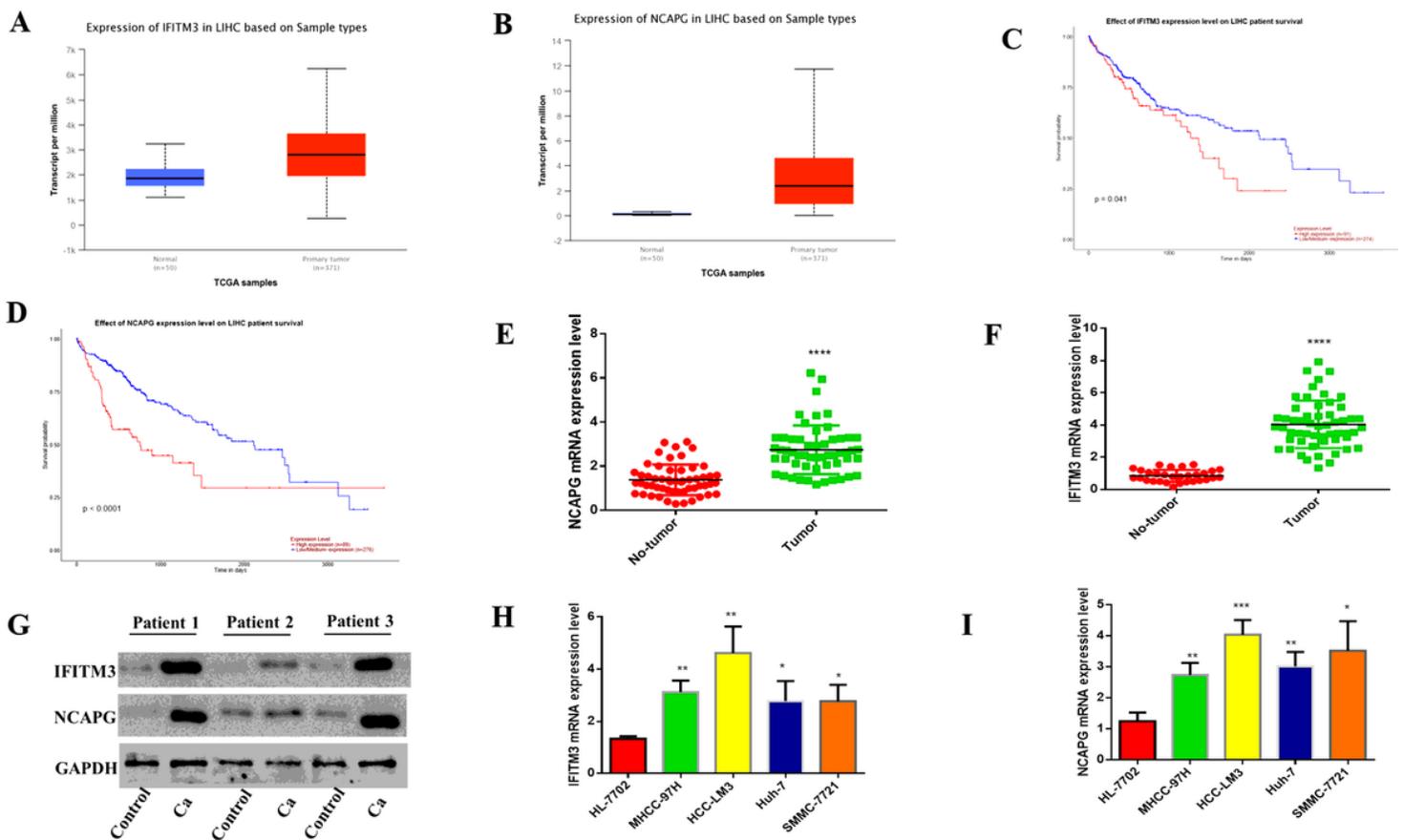
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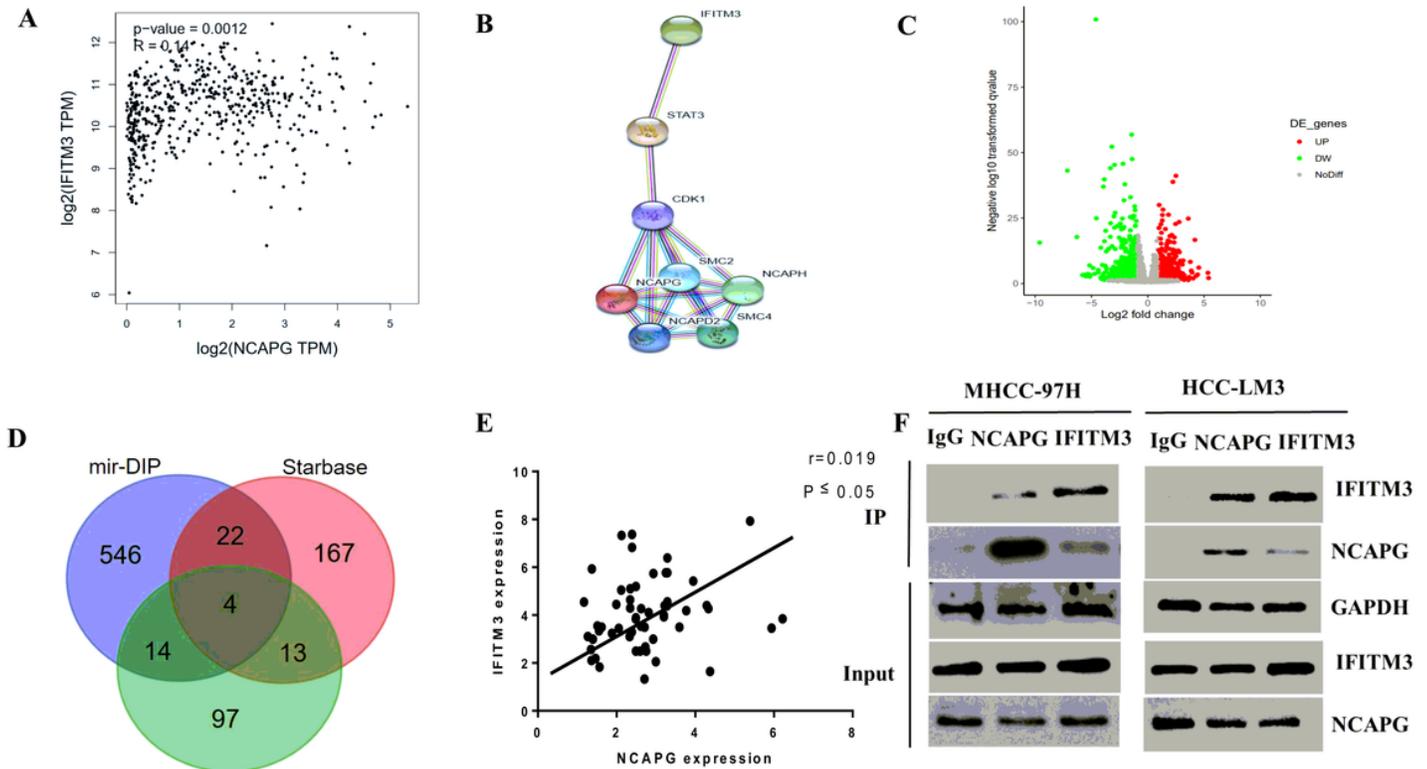
## Figures



**Figure 1**

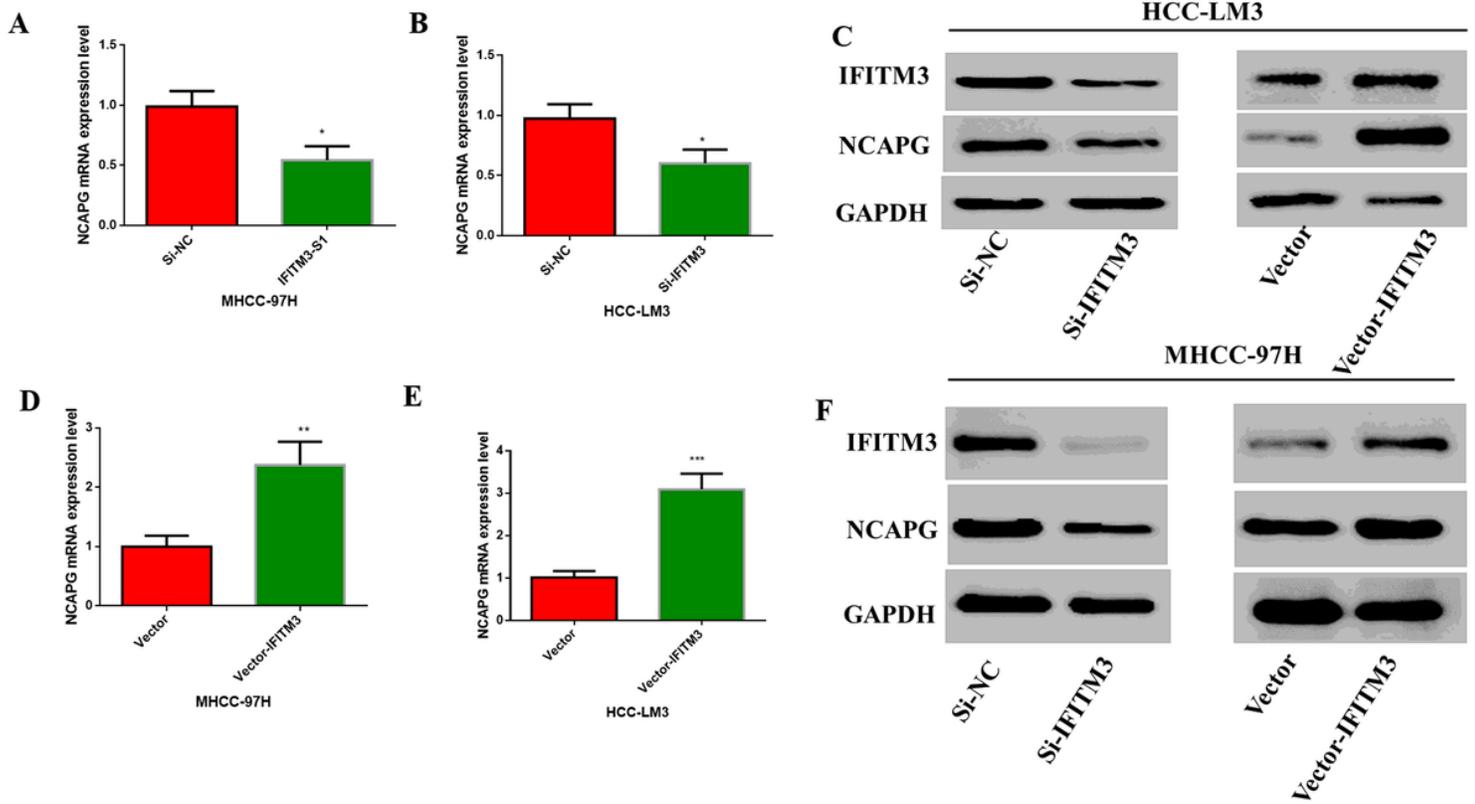
The upregulation of IFITM3 and NCAPG in HCC is related to poor prognosis. A, B: Expression of IFITM3 and NCAPG in HCC in the TCGA database. C, D: Relationship between the expression of IFITM3 and NCAPG in the database and the prognosis of HCC. E, F: Expression of IFITM3 and NCAPG mRNA in liver cancer tissues and adjacent tissues. G: Abnormal expression of IFITM3 and NCAPG protein in liver cancer tissues and adjacent tissues. H, I: Expression of IFITM3 and NCAPG mRNA in liver cancer cell lines

(MHCC-97H, HCC-LM3, SMCC-7721, Huh-7) and liver cell lines (HL-7702). Note: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .



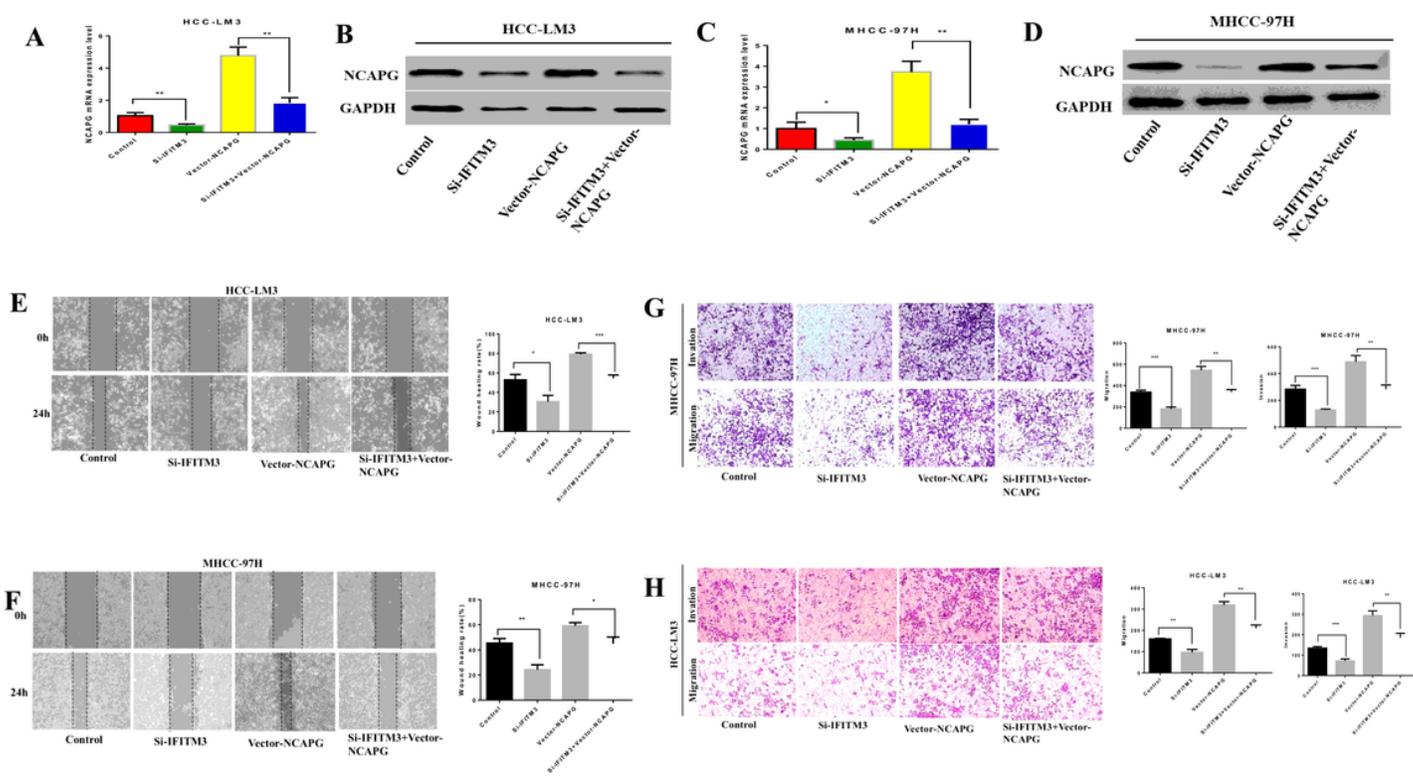
**Figure 2**

Relationship between IFITM3 and NCAPG. A: XY analysis of IFITM3 and NCAPG in the database. B: String Shengxin analysis of the relationship between IFITM3 and NCAPG. C: Volcano graphs of the expression of IFITM3 and NCAPG in HCC analyzed by Shengxin. D: Analysis of the VENE diagram of downstream genes of IFITM3 from the database. E: Relationship between IFITM3 and NCAPG expression in liver cancer specimens. F: Co-immunoprecipitation of IFITM3 and NCAPG in HCC-LM3 and MHCC-97H hepatocellular carcinoma cells. Note: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .



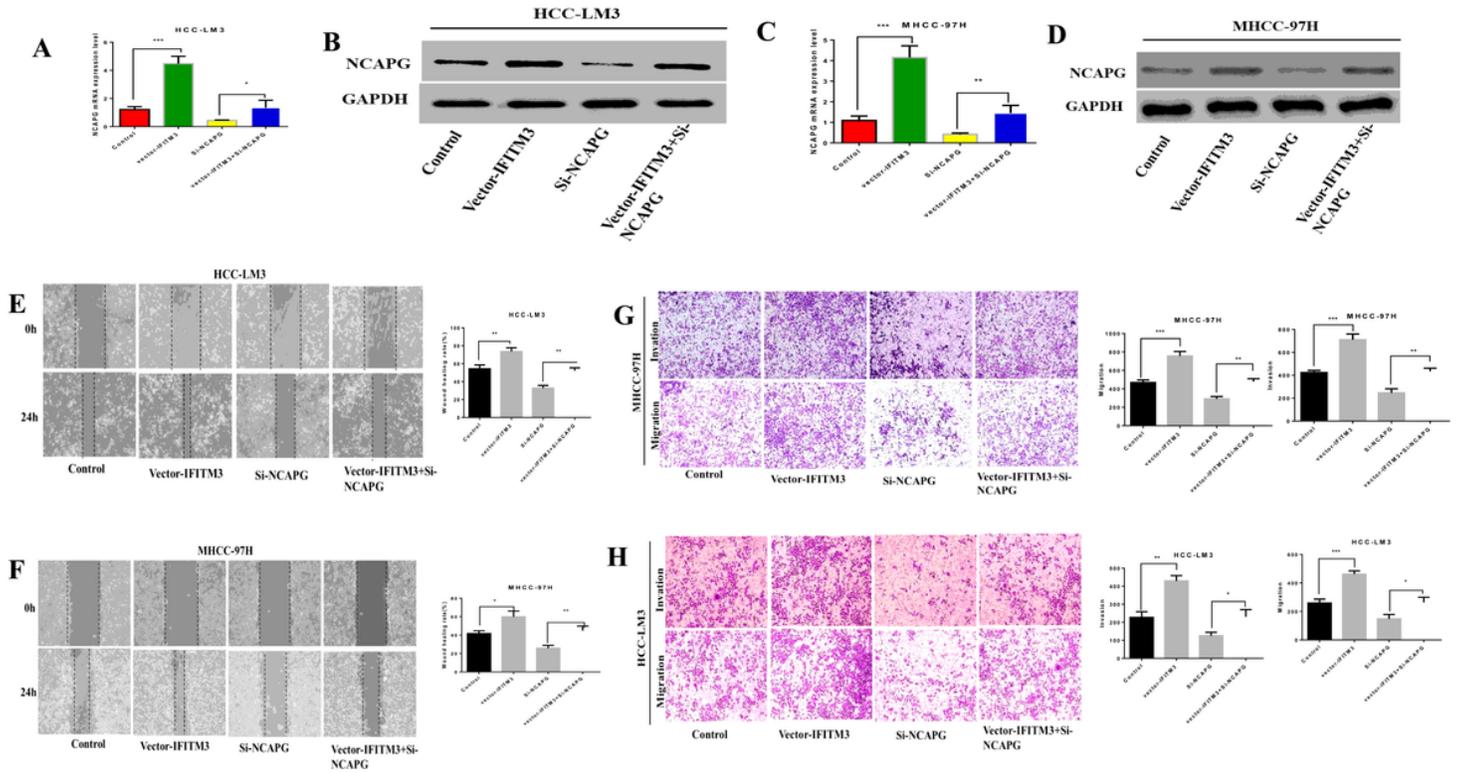
**Figure 3**

Positive regulation of NCAPG by IFITM3 in MHCC-97H and HCC-LM3 as the experimental cell lines. A, B, C, D: Expression of NCAPG mRNA after downregulation of IFITM3 expression. E, F: Expression of NCAPG protein after downregulation of IFITM3 expression. Note: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .



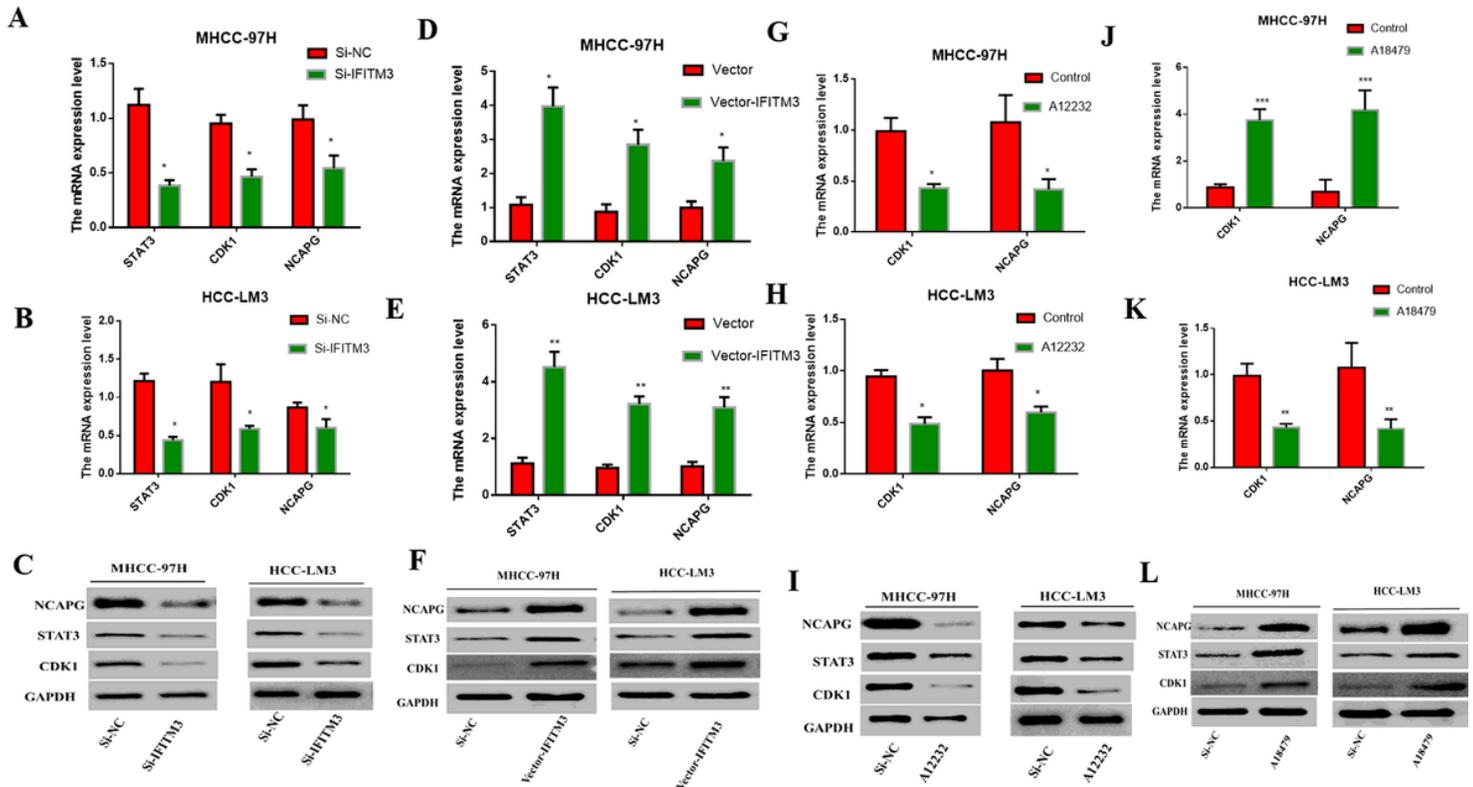
**Figure 4**

IFITM3 regulates NCAPG and affects the invasion and metastasis of HCC. A, B, C, D: The interference fragment transfected with IFITM3 and the plasmid of NCAPG were used to observe the expression of NCAPG. E, F, G, H: Scratch experiments proved that the downregulation of IFITM3 reduced the invasion and metastasis ability of HCC cells, while the upregulation of NCAPG enhanced the invasion and metastasis ability of HCC cells. Note: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .



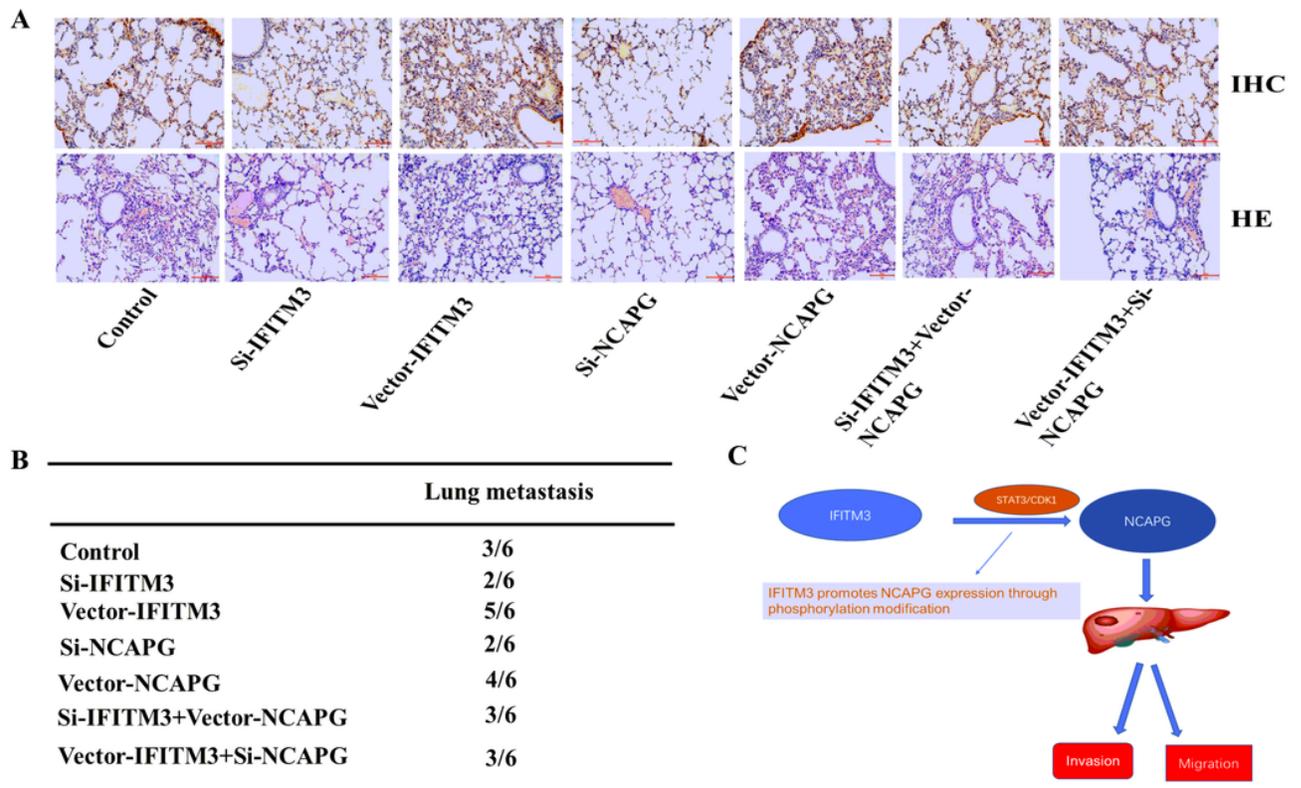
**Figure 5**

IFITM3 regulates NCAPG and thus affects the invasion and metastasis of HCC. A, B, C, D: Plasmid transfected with IFITM3 and interference fragment of NCAPG were used to observe the expression of NCAPG. E, F, G, H: Scratch experiments proved that the upregulation of IFITM3 enhanced the invasion and metastasis ability of HCC cells, while the downregulation of NCAPG reduced the invasion and metastasis ability of HCC cells. Note: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .



**Figure 6**

IFITM3 regulates NCAPG through phosphorylation modification. A, B, C: After downregulating IFITM3, the expression of STAT3, CDK1, and NCAPG changes. D, E, F: After upregulating IFITM3, the expression of STAT3, CDK1, and NCAPG changes. H, I, J: Expression of CDK1 and NCAPG after using pathway inhibitors (A12232). K, L, M: Changes of CDK1 and NCAPG expression after using pathway activator (A18479). Note: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .



**Figure 7**

In vitro experiments confirm that IFITM3 regulates NCAPG to promote HCC invasion and metastasis. A: HE staining and IHC of lung tissue of nude mice injected with HCC-LM3 cells. B: The number of lung metastases of nude mice in each group. C: Simulation diagram of the effect in this experiment. Note: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

## Supplementary Files

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