

# KIF3A Inhibits NPC Proliferation, Migration and Invasion By Interacting With $\beta$ -Catenin To Suppress Its Nuclear Accumulation

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## Research Article

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# Abstract

## Background

Nasopharyngeal carcinoma (NPC) is a malignant epithelial tumour that is prevalent in Southern China and other Southeast Asian countries. In previous studies, Kinesin Family Member 3A (KIF3A) was shown to play a dual role in cancers. However, the biological role of KIF3A in NPC and the underlying mechanism have not been reported.

## Methods

The KIF3A mRNA and protein expressions in NPC were analyzed by qRT-PCR (Quantitative real-time polymerase chain reaction), Western blotting and immunohistochemistry. CCK-8, EDU incorporation assay, Colony formation, cell cycle assay, Wound-Healing Assay, Transwell verified KIF3A regulates the proliferation, migration, invasion of NPC cells. The in vivo effect of KIF3A on proliferation was elucidated with a xenograft mouse model. COIP (Co-immunoprecipitation) assay showed KIF3A interacts with  $\beta$ -catenin. Confocal microscopy colocalization assay confirmed colocalization of KIF3A and  $\beta$ -catenin in NPC cells. Nuclear and cytoplasmic extraction assays were performed to analyze the distribution of  $\beta$ -catenin in the nuclei and cytoplasm.

## Results

In this study, we found that KIF3A interacts with  $\beta$ -catenin, suppressing the intranuclear aggregation of  $\beta$ -catenin, then inactivating the Wnt/ $\beta$ -catenin signaling pathway as well as the downstream cell cycle factors and EMT signal to inhibit NPC proliferation, migration, and invasion.

## Conclusions

These findings suggest that KIF3A interacts with  $\beta$ -catenin and attenuates the malignant progression of NPC by inhibiting  $\beta$ -catenin intranuclear aggregation. KIF3A may be a promising therapeutic target for NPC patients.

## Background

Nasopharyngeal carcinoma (NPC) is a malignancy of the head and neck that originates from epithelial cells; NPC is mainly distributed in Southern China and the Southeast Asia region and is closely related to Epstein-Barr virus infection, the environment and genetic factors[1–6]. There were 133354 new cases of NPC worldwide in 2020, accounting for 0.7% of new cancer cases worldwide[7]. Although patients with early stage NPC can be cured by radiation therapy alone, patients with advanced-stage NPC have a poor 5-year survival rate due to recurrence and distant metastasis. Therefore, the discovery of novel molecular

diagnostic markers and the exploration of the potential molecular mechanisms underlying the progression of NPC are still urgent.

KIF3A, a member of the kinesin protein family located on human chromosome 5q31.1, plays an essential role in intraflagellar transport (IFT) and assembly and mammalian cilia maintenance[8]. In addition, KIF3A is involved in a variety of cellular processes; KIF3A functions as a molecular motor that transports organelles and macromolecules along microtubules and plays a critical role in early development, cell migration and tumorigenesis[9–11].

To date, KIF3A has been reported to function as a tumour promoter and to enhance the proliferation and metastasis of prostate cancer, triple-negative breast cancer, and bladder cancer[12–14]. Additionally, silencing the KIF3A gene in thyroid cancer cell lines leads to defective ciliogenesis, which in turn promotes mitochondria-dependent apoptosis[15]. However, KIF3A interacts with  $\beta$ -arrestin to function as a tumour suppressor in lung cancer, inhibiting the Wnt/ $\beta$ -catenin pathway[16]. A subsequent study also demonstrated a tumour suppressive role of KIF3A in lung cancer[17]. The above studies indicate that KIF3A may perform diverse functions in different types of tumours; however, to date, the biological role of KIF3A in NPC and the underlying mechanism have not been elucidated.

In this study, we first observed that KIF3A expression was downregulated in NPC, suggesting that KIF3A may suppress the malignant process of NPC. Further experiments indicated that KIF3A suppresses NPC growth and migration by interacting with  $\beta$ -catenin to inhibit the Wnt/ $\beta$ -catenin pathway and downstream epithelial-mesenchymal transition (EMT) signalling and cell cycle factors. Our results reveal that KIF3A might be a potential tumour suppressor in NPC.

## Materials And Methods

### IHC Staining

The expression levels of KIF3A were evaluated by immunohistochemistry (IHC) staining. We performed the streptavidin-peroxidase-conjugated method according to the reagent instructions.

### Cell Culture and Tissue specimens

The HONE1, SUNE1, NP69 and 5-8F NPC cell lines were obtained from the Cancer Research Institute of Southern Medical University (Guangzhou, China). The NPC cell lines were cultured in RPMI 1640 (Biological Industries) supplemented with 10% foetal calf serum (FBS; Biological Industries, USA), and the cell lines were cultured in a humidified chamber with 5% CO<sub>2</sub> at 37°C. 106 NPC and 22 normal nasopharyngeal specimens were obtained from Nan Fang Hospital, China. Consent from the patients and approval from the Ethics Committee of Nan Fang Hospital were obtained before these clinical samples were used for study purposes.

### qRT-PCR

A total RNA kit was used to isolate RNA from the NPC cell lines. cDNA was generated with a reverse transcription kit (TaKaRa Company) in the Bio-Rad T100 Thermal Cycler; subsequently, the cDNA was used as a template and amplified with specific primers in the Roche LightCycler. The fold changes in KIF3A gene expression were analysed by the  $2^{-\Delta\Delta Ct}$  method.

## **In Vivo Tumorigenesis in Nude Mice**

All the in vivo studies were performed following a protocol approved by the Animal Care and Use Committee of Southern Medical University. Approximately  $4 \times 10^6$  SUNE1 cells in the logarithmic phase of growth that had been transfected with negative control or KIF3A-overexpressing cDNA (N=7 per group) were subcutaneously injected into 4-week-old female nude mice (BALB/c, nu/nu). After 15 days, the nude mice were sacrificed, and the tumour tissues were removed and weighed.

## **Western blotting analysis**

NPC cell lysates were generated, total protein was extracted, and a BCA protein assay kit (TIANGEN, Beijing) was used to measure the protein concentration. The antibodies included anti-KIF3A (1:1000), anti- $\beta$ -catenin (1:1000), anti-N-cadherin (1:1000), anti-Vimentin (1:1000), anti-c-Myc (1:1000), anti-CCND1 (1:1000), anti- $\beta$ -actin (1:5000), and anti-GAPDH (1:5000).

## **Transfection**

The siRNAs targeting KIF3A and relevant negative control was synthesized by RiboBio (Guangzhou, China). KIF3A and  $\beta$ -catenin plasmids were purchased from WZ Biosciences Inc. (Shandong, China). Twelve hours before transfection, NPC cells were plated in six-well plates at 50%-60% confluence. Subsequently, according to the manufacturer's protocol, plasmids or siRNAs were transfected into cells using Lipofectamine TM 3000 (Invitrogen, Guangzhou, China). After 24-72 hours, the cells were harvested for further experiments.

## **Cell proliferation analysis and EdU incorporation assay**

Cell proliferation was measured by CCK-8 cell Counting Kit (Vazyme, Nanjing, China). NPC cells were seeded in 96-well plates (1500 cells/well). After the cells attached, 10  $\mu$ L CCK-8 reagent was added to the wells and incubated with the NPC cells at 37°C for 1.5 h. The optical density (OD) was assayed at 450 nm from Day 1 to Day 4. An EdU incorporation assay was conducted with Cell-Light EdU Apollo 567 (RiboBio, Guangzhou, China). NPC cells in the logarithmic phase of growth were seeded in 96-well plates, and after the cells attached, EDU A solution was incubated with the NPC cells for 2 h. Subsequently, 4% paraformaldehyde was used to fix the NPC cells, and then, 0.2% Triton X-100 was used to permeabilize the cells. Apollo solution was added and incubated for 30 min after permeabilization to stain the cell nuclei, and then, DAPI was used to stain the cell nuclei for 10 min. The cells were imaged under an inverted fluorescence microscope.

## **Colony formation and cell cycle progression**

For colony formation, 100 NPC cells were seeded in six-well plates and cultured in a humidified chamber with 5% CO<sub>2</sub> at 37°C. The culture medium was renewed every 72 h. After 2 weeks of incubation, the cells were washed with PBS and fixed with 4% paraformaldehyde for 10 minutes. Then, the NPC cells were stained with crystal violet. Cell cycle analysis was performed according to the instructions of the cell cycle and apoptosis kit (Leagene, Beijing, China).

## **Cell migration assay**

A Transwell assay was performed to measure the migration capability of NPC cells. The cells were mixed with serum-free RPMI 1640 and were added to the upper chamber of Transwell plates. Then, RPMI 1640 supplemented with 10% foetal bovine serum was added to the lower chamber of the Transwell plates. After 10-24 h, the chamber was fixed with paraformaldehyde and stained with Giemsa for 5 min. Then, deionized water was used to wash the chamber, and the chamber was photographed with a microscope after air drying.

## **Wound-Healing Assay**

For wound-healing assays, NPC cells were grown to approximately 100% confluence in 6-well plates. A medium-sized pipette tip was used to scrap artificial wound tracks. The NPC cells were cultured with serum-free medium. To analyse the migration capability of the cells, the wound width was imaged under an inverted microscope at 0 and 36 h.

## **Co-immunoprecipitation (COIP)**

Briefly, a COIP assay was conducted with protein A/G immunoprecipitation beads (Bimake, Shanghai, China). Total protein extracted from NPC cells was coincubated with anti-IgG, anti- $\beta$ -catenin, and anti-KIF3A antibodies overnight at 4°C. The beads were incubated with the antigen-antibody complexes for 45 min at 37°C after the beads were washed twice with wash buffer. Finally, the mixed example was eluted in buffer and boiled for five minutes at 95°C. The immune complexes were subjected to Western blotting assay.

## **Immunofluorescent Staining**

NPC cells were seeded in 35-mm glass bottom cell culture dishes (SORFA LIFE SCIENCE, Zhejiang) and cultured overnight. The NPC cells were fixed with 4% paraformaldehyde, and then, 0.2% Triton X-100 was used to permeabilize the cells. Subsequently, the permeabilized cells were incubated with the indicated antibodies overnight at 4°C. Finally, the NPC cells were incubated with fluorescein-labelled secondary antibodies at 37°C for 60 minutes, and DAPI was used to stain the cell nuclei. The cells were photographed under a confocal microscope.

## **Nuclear and cytoplasmic extraction assay**

Nuclear and cytoplasmic extraction assays were performed with NE-PER Nuclear and Cytoplasmic Extraction reagents (Thermo Scientific, UK) according to reagent instructions. In short, NPC cell pellets were obtained by trypsin digestion and added to ice-cold CER I for a 10-minute incubation at 4°C. After

the incubation, ice-cold CER II was added to the complex and incubated for 1 minute at 4°C. Then, the lysed mixture was centrifuged at 16000 ×g for 5 minutes. The supernatant (cytoplasmic extract) was transferred into a new centrifuge tube and stored at -80°C. The wash buffer was added to the pellet, and after washing, the sample was recentrifuged. Then, the pellet was resuspended by mixing with ice-cold NER and incubated for 40 minutes at 4°C. Finally, the mixture was centrifuged at 16000 ×g for 15 minutes and transferred to a new centrifuge tube for storage at -80°C.

## Statistical Analysis

GraphPad Prism 9 software was used for statistical analysis. The data are expressed as the mean ± SD from at least three independent experiments, A Student's t test was performed to compare two groups, the comparisons among multiple groups used one-way analysis of variance (ANOVA). A P value of less than 0.05 was considered to indicate statistically significant differences, which are labelled as follows: \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

## Decreased KIF3A expression correlates with unfavourable outcome

To explore the basic expression of KIF3A in NPC, we used qRT-PCR and Western blotting to detect KIF3A mRNA and protein expression, respectively, in NPC cells. The results indicated that KIF3A expression was obviously downregulated in NPC cells compared with NP69 cells. Then, we conducted immunohistochemistry to detect KIF3A expression in 106 NPC tissues and 22 nasopharyngeal epithelial tissues. Consistent with the results described above, the expression of KIF3A was significantly reduced in NPC tissues compared with nasopharyngeal epithelial tissues (P=0.018) (Table1). Subsequently, we found that decreased KIF3A expression positively correlated with T stage (T1-T2 vs. T3-T4, P=0.035) and M stage (M0 vs. M1, P=0.039) but did not correlate with other clinicopathological characteristics (Table2). In addition, survival analysis indicated that NPC patients with low KIF3A expression had shorter survival times than patients with high KIF3A expression (P=0.033).

Decreased KIF3A expression correlates with unfavourable outcomes. (A-B) qRT-PCR and Western blotting were performed to determine the mRNA and protein expression of KIF3A in nasopharyngeal epithelium and NPC cells. (C) IHC staining was used to evaluate KIF3A expression in nasopharyngeal epithelium and NPC samples. a: Strong staining of KIF3A in NP tissues; b: low expression of KIF3A in NPC tissues; c: high expression of KIF3A in NPC tissues. (D) Kaplan-Meier survival analysis of the survival rate of NPC patients based on KIF3A expression. Log-rank test was used to calculate P values.

## KIF3A suppresses NPC proliferation in vitro and in vivo

Western blotting assays were used to determine the expression level of KIF3A after transfection of the KIF3A plasmid into HONE1 and SUNE1 cells. The results indicated that the KIF3A protein expression in KIF3A-overexpressing cells was obviously increased compared with that in negative control cells. To determine the biological function of KIF3A in NPC, a Cell Counting Kit-8 assay and colony formation assay were conducted, and the results indicated that the growth of KIF3A-overexpressing HONE1 and

SUNE1 cells was markedly attenuated compared with that of negative control cells. Furthermore, EdU (5-ethynyl-2'-deoxyuridine) incorporation assays and cell cycle analysis showed that the cell cycle progression of KIF3A-overexpressing cells was significantly inhibited compared with that of NC cells. To further demonstrate the role of KIF3A in carcinogenesis in vivo, we performed a subcutaneous tumorigenesis study by inoculating LV-NC-GFP SUNE1 cells or LV-KIF3A-GFP SUNE1 cells into nude mice. After 15 days, the nude mice inoculated with LV-KIF3A-GFP SUNE1 cells exhibited lower tumour weights than the nude mice inoculated with LV-NC-GFP SUNE1 cells. The results described above demonstrate that KIF3A overexpression suppresses NPC proliferation in vivo and in vitro.

KIF3A suppresses NPC proliferation in vitro and in vivo. (A) Western blotting was used to measure KIF3A protein expression after the transfection of the negative control empty plasmid or KIF3A plasmid into HONE1 and SUNE1 cells. CCK-8 assay (B), colony-formation assay (C), EdU incorporation assay (D) and flow cytometry analysis (E) were performed to evaluate changes in the proliferation and cell cycle progression of HONE1 and SUNE1 cells after transfection with the KIF3A plasmids or LV-GFP-KIF3A. Student's t test. Mean  $\pm$  SD, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . (F) The in vivo effect of KIF3A on proliferation was elucidated with a xenograft mouse model in which mice were inoculated with SUNE1-LV-NC and SUNE1-LV-KIF3A cells. Each group included 7 mice. Student's t test, Mean  $\pm$  SD, \*\* $p < 0.01$ .

## **Knockdown of KIF3A expression reverses NPC proliferation**

After transfection of specific small interfering RNA (siRNA) targeting KIF3A, Western blotting assays showed that simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected HONE1 and SUNE1 cells reversed the changes in KIF3A protein expression. In addition, the CCK-8 assay showed that simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected NPC cells reversed the growth inhibition caused by upregulated KIF3A expression. The EdU assay also showed that EdU staining was enhanced after the simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected HONE1 and SUNE1 cells. Together, these data show that knockdown of KIF3A expression in oe-KIF3A-transfected NPC cells restored NPC growth and cell cycle progression.

Knockdown of KIF3A expression reverses NPC proliferation. (A) Western blotting was used to evaluate silencing efficiency after the simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected HONE1 and SUNE1 cells. (B-C) CCK-8 and EdU incorporation assays after the simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected HONE1 and SUNE1 cells. Student's t test. Mean  $\pm$  SD, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

## **KIF3A suppresses NPC migration, and knockdown of KIF3A expression reverses this phenotype**

To investigate the biological effect of KIF3A on NPC cell migration capability, a wound healing assay was conducted, and we observed that KIF3A-overexpressing HONE1 and SUNE1 cells had significantly suppressed wound healing abilities compared with NC HONE1 and SUNE1 cells. Subsequently, Transwell assays showed that overexpression of KIF3A significantly reduced the numbers of migrated HONE1 and

SUNE1 cells compared with the NC. Furthermore, recovery experiments indicated that simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected NPC cells reversed the inhibitory effect on metastasis mediated by upregulated KIF3A expression.

KIF3A suppresses NPC migration, and knockdown of KIF3A expression reverses this phenotype. (A-B) Wound healing assays and Transwell assays were performed to evaluate the migration capability of NPC cells transfected with the KIF3A plasmid. Student's t test. Mean  $\pm$  SD, \* $p < 0.05$ , \*\* $p < 0.01$ . (C-D) Wound healing and Transwell assays after simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected HONE1 and SUNE1 cells, Student's t test. Mean  $\pm$  SD, \* $p < 0.05$ , \*\* $p < 0.01$ .

## **KIF3A suppresses NPC proliferation and migration via the wnt/ $\beta$ -catenin signalling pathway**

To explore the potential mechanism by which KIF3A functions, we used Western blotting to analyse the expression of  $\beta$ -catenin, cell cycle-associated proteins, and EMT markers. We observed that the expression of CCND1, c-Myc, N-cadherin, vimentin, and  $\beta$ -catenin was downregulated in KIF3A-overexpressing HONE1 cells and SUNE1 cells compared with the NC cells. In addition, recovery experiments indicated that interfering with KIF3A expression significantly restored the levels of CCND1, c-Myc, N-cadherin, vimentin, and  $\beta$ -catenin. Finally, we determined whether  $\beta$ -catenin was involved in KIF3A-regulated NPC proliferation and migration. Simultaneous upregulation of  $\beta$ -catenin expression in oe-KIF3A-transfected NPC cells reversed changes in CCND1, c-Myc, N-cadherin, and vimentin protein expression mediated by KIF3A overexpression.

KIF3A suppresses NPC proliferation and migration via the wnt/ $\beta$ -catenin signalling pathway. (A) Changes in the expression levels of KIF3A, N-cadherin, Vimentin, CCND1, and c-Myc were detected by Western blotting after transfection with the KIF3A plasmid in HONE1 and SUNE1 cells.  $\beta$ -actin was used as a loading control. (B) Changes in the expression levels of KIF3A, N-cadherin, Vimentin, CCND1, c-Myc were detected by Western blotting after transfecting the KIF3A plasmid or KIF3A plasmid + siRNA targeting KIF3A in HONE1 and SUNE1 cells. GAPDH was used as a loading control. (C) Changes in the expression levels of N-cadherin, Vimentin, CCND1, c-Myc were detected by Western blotting after transfection with the KIF3A plasmid or KIF3A +  $\beta$ -catenin plasmids in HONE1 and SUNE1 cells. GAPDH was used as a loading control.

## **$\beta$ -catenin reverses the inhibitory effect exerted by KIF3A overexpression in NPC**

To further explore the role of  $\beta$ -catenin in KIF3A-mediated NPC proliferation and metastasis, we transfected KIF3A or KIF3A +  $\beta$ -catenin plasmids into HONE1 and SUNE1 cells. Using CCK-8 assays, EdU incorporation assays and Transwell assays, we observed that simultaneous upregulation of  $\beta$ -catenin expression in oe-KIF3A-transfected HONE1 and SUNE1 cells reversed the inhibitory effect on proliferation, migration, and invasion caused by upregulated KIF3A expression.

$\beta$ -catenin reverses the inhibitory effect exerted by KIF3A overexpression in NPC. (A-B) CCK-8 and EdU incorporation assays were conducted after transfection with the KIF3A plasmid or  $\beta$ -catenin+KIF3A plasmids in HONE1 and SUNE1 cells. (C) A wound healing assay was conducted to assess the migration capability of NPC cells treated with the KIF3A plasmid or  $\beta$ -catenin+KIF3A plasmids. Student's t test. Mean  $\pm$  SD, \*p < 0.05, \*\*p<0.01.

## **KIF3A interacts with $\beta$ -catenin and suppresses $\beta$ -catenin nuclear translocation**

To explore the potential molecular mechanisms by which KIF3A represses the Wnt/ $\beta$ -catenin pathway, we used the BioGRID database to predict potential proteins that interact with KIF3A. Interestingly, we found that  $\beta$ -catenin may be a potential candidate interacting protein. Furthermore, a study reported that KIF3A interacts with  $\beta$ -catenin during spermatogenesis in *Eriocheir sinensis*, but this interaction has not been reported in *H. sapiens*. Therefore, we explored whether there was an interaction between KIF3A and  $\beta$ -catenin.

Endogenous coimmunoprecipitation (CO-IP) was performed and confirmed that KIF3A interacts with  $\beta$ -catenin. In addition, double colocalization by immunofluorescence demonstrated the colocalization of KIF3A and  $\beta$ -catenin in NPC cells. Nuclear and cytoplasmic extraction assays were performed to analyse the distribution of  $\beta$ -catenin in the nuclei and cytoplasm, and the results indicated that overexpression of KIF3A suppresses the levels of  $\beta$ -catenin in the cytoplasm and nuclei. These results demonstrate that KIF3A interacts with  $\beta$ -catenin in NPC and suppresses  $\beta$ -catenin nuclear translocation.

KIF3A interacts with  $\beta$ -catenin and suppresses  $\beta$ -catenin nuclear translocation. (A) A CO-IP assay was performed to detect the interactive relationship between KIF3A and  $\beta$ -catenin in NPC cells. (B) Colocalization of KIF3A and  $\beta$ -catenin by immunofluorescence in HONE1 and SUNE1 cells. (C) Western blotting was conducted to detect changes in  $\beta$ -catenin protein expression in the nucleus and cytoplasm. GAPDH was used as a loading control in the cytoplasm, and histone was used as a loading control in the nucleus.

## **Discussion**

In previous studies, KIF3A played a dual role in cancers, but the role of KIF3A and the underlying molecular mechanism have never been reported in NPC. Here, we first experimentally showed by Western blotting and immunochemical staining assays that KIF3A expression is downregulated in NPC cell lines and tissues compared to normal nasopharyngeal epithelial cells and tissues. Moreover, high expression of KIF3A was associated with a good prognosis for NPC patients, and decreased KIF3A expression was positively correlated with T stage and M stage. These data were similar to observations in lung cancer but contrary to observations in prostate cancer, triple-negative breast cancer, and bladder cancer.

Our preliminary results suggested KIF3A plays a tumour-suppressive role in NPC. To determine the possible role of KIF3A, we first performed CCK-8 assays, colony formation assays and in vivo tumour

growth assays after transfection of the KIF3A plasmid or cDNA. The results indicated that overexpression of KIF3A attenuated NPC proliferation *in vivo* and *in vitro*. We then used wound-healing assays and Transwell assays and observed that upregulated KIF3A expression inhibited NPC migration and invasion. Finally, we knocked down KIF3A expression and found that the proliferation and migration of NPC cell lines were obviously reversed. The data described above indicated that KIF3A functions as a tumour suppressor in NPC. In previous studies, KIF3A functioned as a tumour promotor in prostate cancer, triple-negative breast cancer and bladder cancer but acted as a tumour suppressor in lung cancer. Our results support the previous conclusion in lung cancer but are contrary to the conclusions in prostate cancer, triple-negative breast cancer, and bladder cancer.

EMT (epithelial-mesenchymal transition), which is downstream of Wnt/ $\beta$ -catenin signalling, has been considered to be essential for tumour progression, invasion, metastasis, and resistance[18, 19], especially for EMT-mediated tumour metastasis, and the changes to tumours in this process are mainly reflected by changes in cell adhesiveness and cytoskeleton[20–22]. In our study, we performed Western blotting and observed that the expression of the EMT-related proteins N-cadherin and Vimentin was downregulated after overexpressing KIF3A. However, in triple-negative breast cancer, knockdown of KIF3A expression reduced Vimentin protein expression and enhanced E-cadherin protein expression, which is contrary to our conclusion[13].

It is well known that cell cycle factors are crucial for modulating tumour growth[23]. In a previous study, inhibition of KIF3A decreased the expression of the Wnt/ $\beta$ -catenin downstream molecule CCND1 in prostate cancer. In addition, downregulated KIF3A expression also reduced CCND1 expression in triple-negative breast cancer. However, KIF3A negatively modulated CCND1 expression in lung cancer. In our study, we observed that overexpression of KIF3A inhibited CCND1 protein expression, which was consistent with the conclusion in lung cancer. In addition, c-myc, a classic oncogenic factor upstream of CCND1, was negatively modulated by KIF3A in our study.

In a previous mechanistic study, KIF3A activated the Wnt/ $\beta$ -catenin pathway by enhancing DVL2 phosphorylation, activating  $\beta$ -catenin, and increasing the expression of the cell cycle factor CCND1, which is downstream of the wnt/ $\beta$ -catenin pathway, in prostate cancer[12]. A subsequent lung cancer study indicated that KIF3A stabilizes  $\beta$ -catenin levels by interacting with  $\beta$ -arrestin to modulate the Wnt/ $\beta$ -catenin pathway, and high expression of KIF3A was positively correlated with a reduction in CCND1 expression[16]. In our investigation, we observed that KIF3A suppresses NPC cell proliferation and metastasis by inhibiting the Wnt/ $\beta$ -catenin pathway. The above studies revealed that the Wnt/ $\beta$ -catenin pathway was a potential downstream target regulated by KIF3A.

$\beta$ -catenin has been recognized as a critical functional protein that participates in the Wnt/ $\beta$ -catenin signalling pathway. Numerous studies have indicated that Wnt/ $\beta$ -catenin signalling not only regulates cell differentiation, proliferation, transcription, organogenesis, metastasis and stemness but also is closely related to the cancer process[24–30]. In the Wnt/ $\beta$ -catenin pathway, the  $\beta$ -catenin degradation complex is composed of GSK3 $\beta$ , APC and AXIN. This complex induces the phosphorylation of  $\beta$ -catenin,

phosphorylated  $\beta$ -catenin is subsequently labelled by ubiquitin, and then,  $\beta$ -catenin is degraded by the proteasome system[31], resulting in its decreased accumulation in the cytosol, which in turn inhibits its nuclear translocation.

To further explore the molecular mechanism by which KIF3A inhibits the Wnt/ $\beta$ -catenin signalling pathway, we used the BioGRID database to screen proteins that interact with KIF3A. Interestingly, there may be an interaction between KIF3A and  $\beta$ -catenin. Subsequently, we found by consulting the literature that there was an interaction between KIF3A and  $\beta$ -catenin during spermatogenesis in *Eriocheir sinensis*, but there were no reports of interactions between KIF3A and  $\beta$ -catenin in *H. sapiens*[32].

We then performed COIP to validate the interaction between KIF3A and  $\beta$ -catenin. Fortunately, the results showed that KIF3A bound to  $\beta$ -catenin in NPC. Subsequently, a confocal microscopy colocalization assay showed that KIF3A and  $\beta$ -catenin colocalize in the cytosol and in the nucleus. In addition, we performed nuclear and cytoplasmic extraction assays to determine the nuclear-cytoplasmic distribution of  $\beta$ -catenin after KIF3A overexpression. Western blotting assays indicated that the protein level of  $\beta$ -catenin was downregulated in both the cytoplasm and nucleus. This suggests that KIF3A overexpression reduced the accumulation of  $\beta$ -catenin in the cytosol, which in turn attenuated the nuclear translocation of  $\beta$ -catenin.

## Conclusions

In summary, our work demonstrated that KIF3A functions as a tumour suppressor and that its decreased expression results in poor prognosis for NPC patients. Additionally, mechanistic analysis showed that KIF3A interacts with  $\beta$ -catenin and inactivates the Wnt/ $\beta$ -catenin signalling pathway, then further inhibits  $\beta$ -catenin-induced downstream cell cycle factor expression and EMT signalling and thus attenuates NPC proliferation, migration, and invasion. Our study demonstrated the vital role of the KIF3A/ $\beta$ -catenin complex in NPC pathogenesis and provided a promising therapeutic target for NPC patients.

## Abbreviations

### **NPC**

Nasopharyngeal carcinoma

### **IHC**

Immunohistochemistry

### **EMT**

Epithelial-Mesenchymal Transition

### **Kinesin Family Member 3A**

KIF3A

### **Co-immunoprecipitation**

COIP

### **qRT-PCR**

Quantitative real-time polymerase chain reaction

**NC**

Negative control

**PBS**

Phosphate buffer saline

## **Declarations**

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

All animal studies were performed following a protocol approved by the Animal Care and Use Committee of Southern Medical University and endeavor to minimize the suffering of the animals. Consent from the patients and approval from the Ethics Committee of Nanfang Hospital were obtained before these clinical samples were used for study purposes.

### **Consent for publication**

Not applicable

### **Competing interests**

The authors declare that they have no competing interests

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### **Authors' contributions**

ZH wrote the manuscript and YX revised the manuscript. The experimental was performed by ZH, JLM, HBC. NM, XJG, XJL are responsible for the experimental record and analyze . All authors read and approved the final manuscript.

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### **Availability of data and material**

The data analyzed in this study are available from the corresponding author upon reasonable request.

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## Tables

**Table 1** The expression of KIF3A in NPC and compared to non-cancerous Nasopharyngeal tissues

Group	Cases(n)	KIF3A expression		$\chi^2$ value	P value
Low	High				
NPC	106	63	43	5.607	0.018
Nasopharyngeal epithelium	22	7	15		

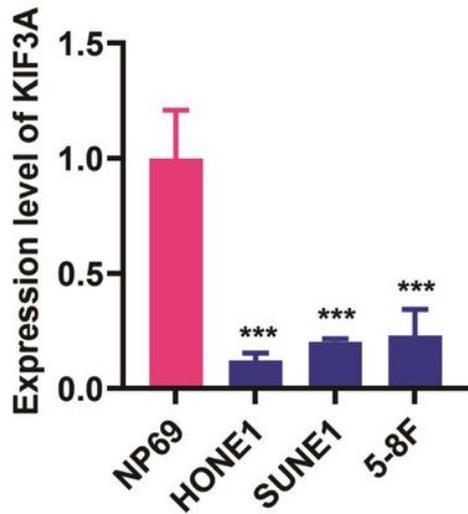
$\chi^2$  test was used to access the expression of KIF3A in NPC and normal Nasopharyngeal tissues.

**Table 2** The relation between the clinicopathologic characteristics and KIF3A expression in NPC

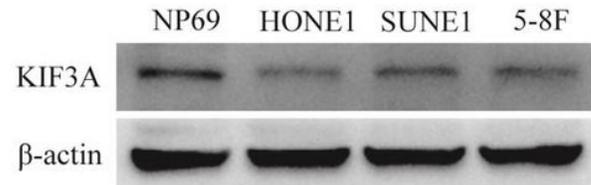
Factors		n	KIF3A expression		$\chi^2$	P value
			Low	High		
Age (y)	<50	52	34	18	1.499	0.221
	$\geq$ 50	54	29	25		
Gender	Male	73	45	28	0.475	0.491
	Female	33	18	15		
Clinical stage	I-II	34	23	11	1.4	0.237
	III-IV	72	40	32		
T stage	T1-T2	51	25	26	4.422	0.035
	T3-T4	55	38	17		
N stage	N0-N1	62	37	25	0.004	0.952
	N2-N3	44	26	18		
M stage	M0	96	54	42	4.279	0.039
	M1	10	9	1		

## Figures

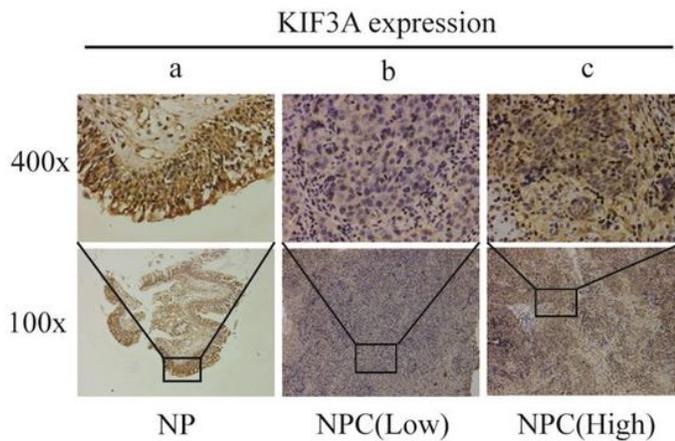
A



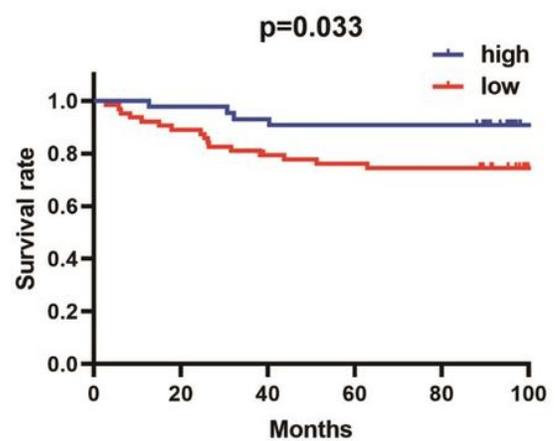
B



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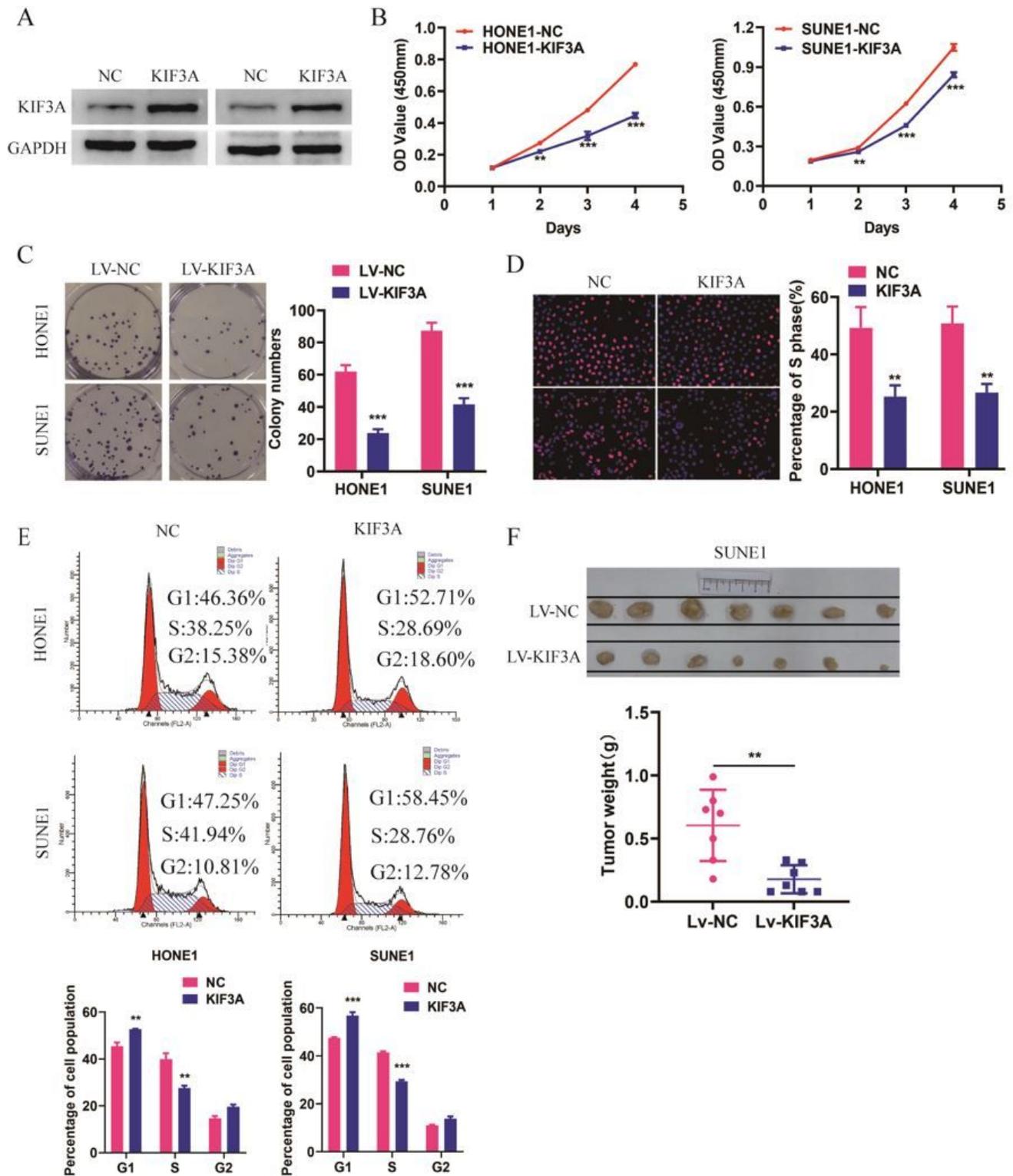


D



## Figure 1

Decreased KIF3A expression correlates with unfavourable outcomes. (A-B) qRT-PCR and Western blotting were performed to determine the mRNA and protein expression of KIF3A in nasopharyngeal epithelium and NPC cells. (C) IHC staining was used to evaluate KIF3A expression in nasopharyngeal epithelium and NPC samples. a: Strong staining of KIF3A in NP tissues; b: low expression of KIF3A in NPC tissues; c: high expression of KIF3A in NPC tissues. (D) Kaplan-Meier survival analysis of the survival rate of NPC patients based on KIF3A expression. Log-rank test was used to calculate P values.

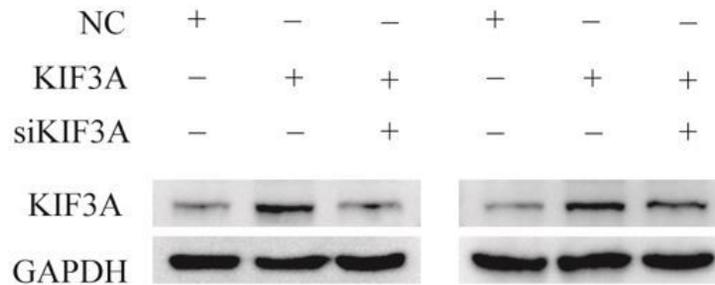


**Figure 2**

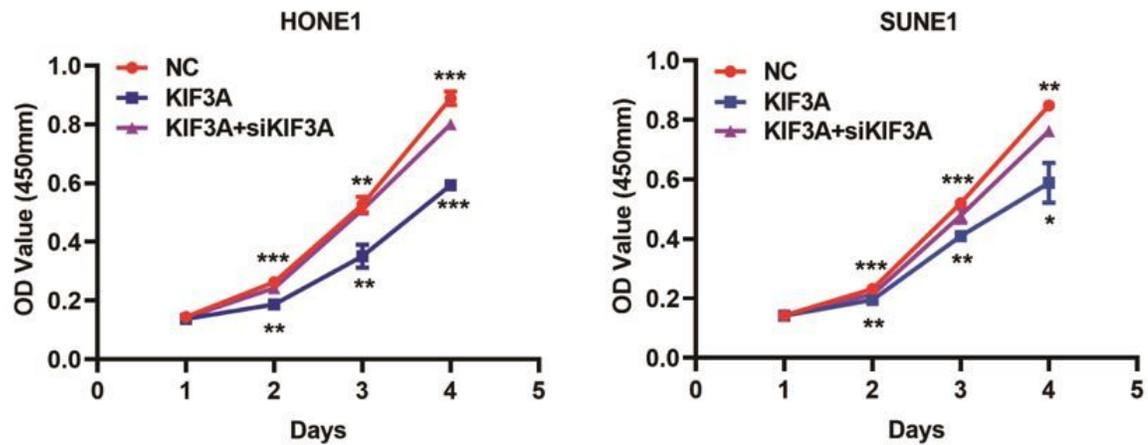
KIF3A suppresses NPC proliferation in vitro and in vivo. (A) Western blotting was used to measure KIF3A protein expression after the transfection of the negative control empty plasmid or KIF3A plasmid into HONE1 and SUNE1 cells. CCK-8 assay (B), colony-formation assay (C), EdU incorporation assay (D) and flow cytometry analysis (E) were performed to evaluate changes in the proliferation and cell cycle progression of HONE1 and SUNE1 cells after transfection with the KIF3A plasmids or LV-GFP-KIF3A.

Student's t test. Mean  $\pm$  SD, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . (F) The in vivo effect of KIF3A on proliferation was elucidated with a xenograft mouse model in which mice were inoculated with SUNE1-LV-NC and SUNE1-LV-KIF3A cells. Each group included 7 mice. Student's t test, Mean  $\pm$  SD, \*\* $p < 0.01$ .

A



B



C

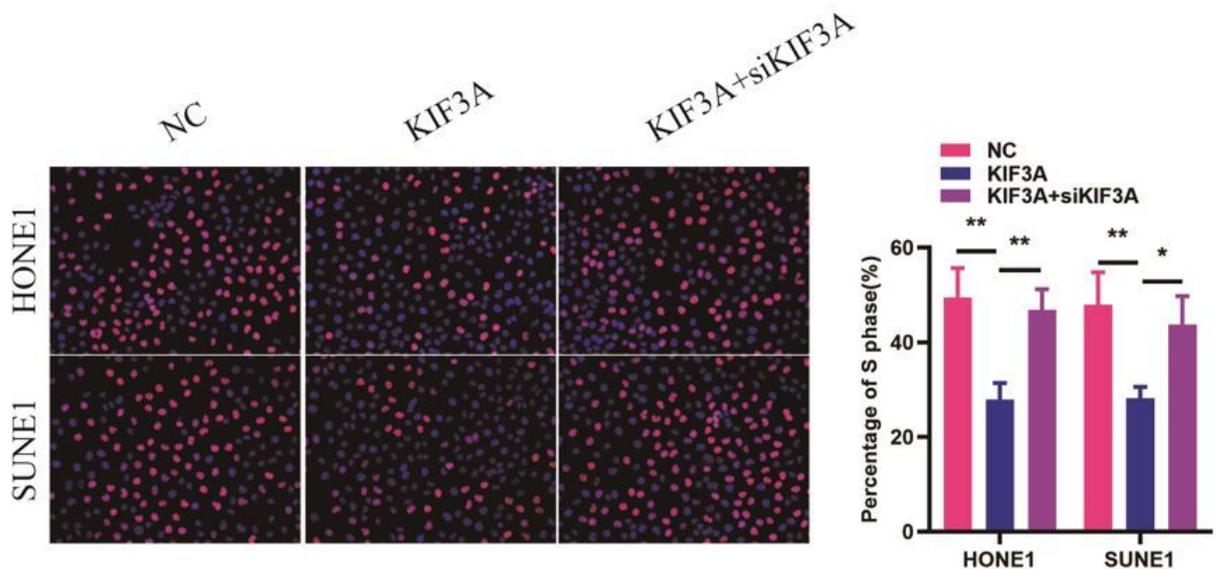
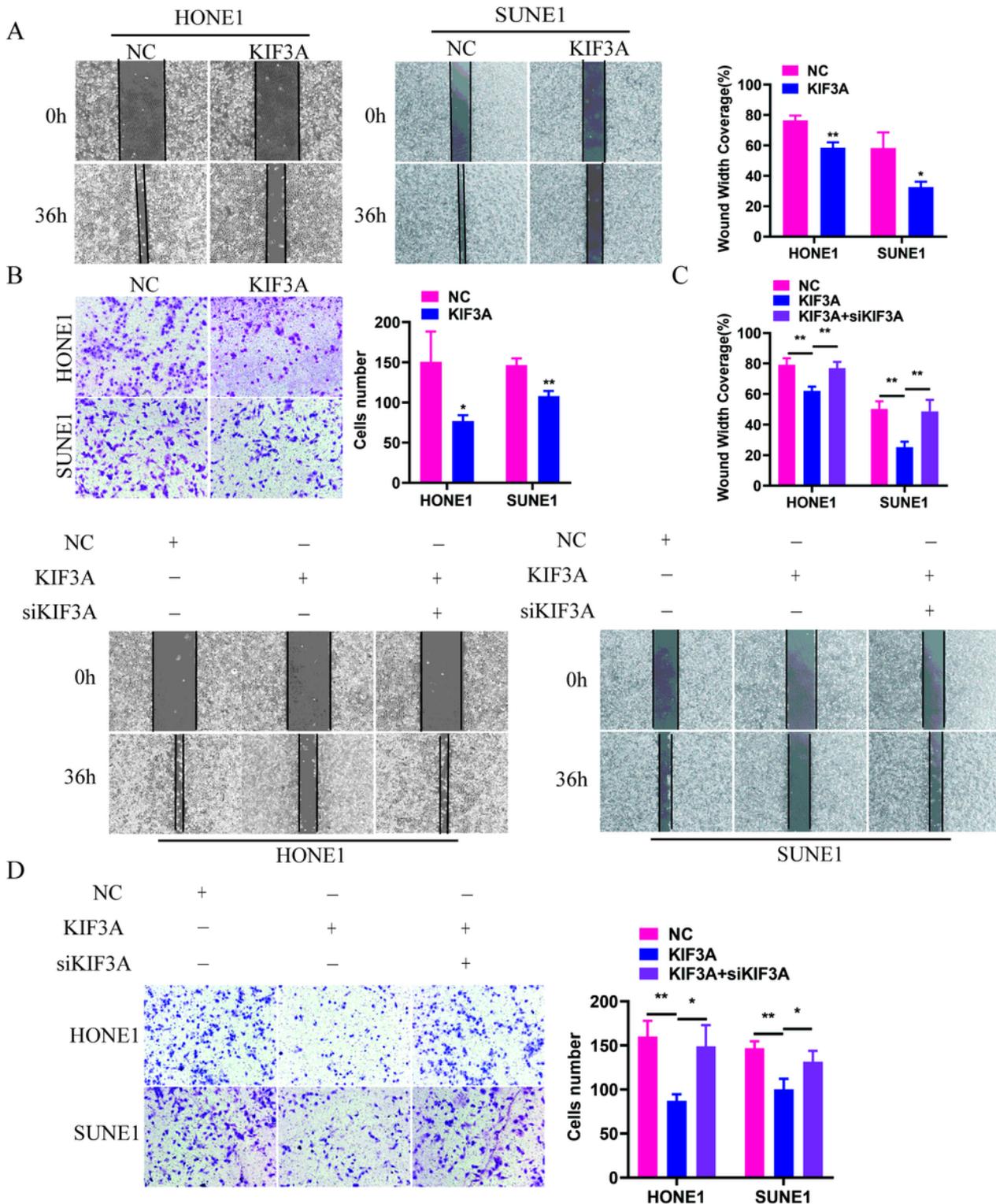


Figure 3

Knockdown of KIF3A expression reverses NPC proliferation. (A) Western blotting was used to evaluate silencing efficiency after the simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected HONE1 and SUNE1 cells. (B-C) CCK-8 and EdU incorporation assays after the simultaneous knockdown

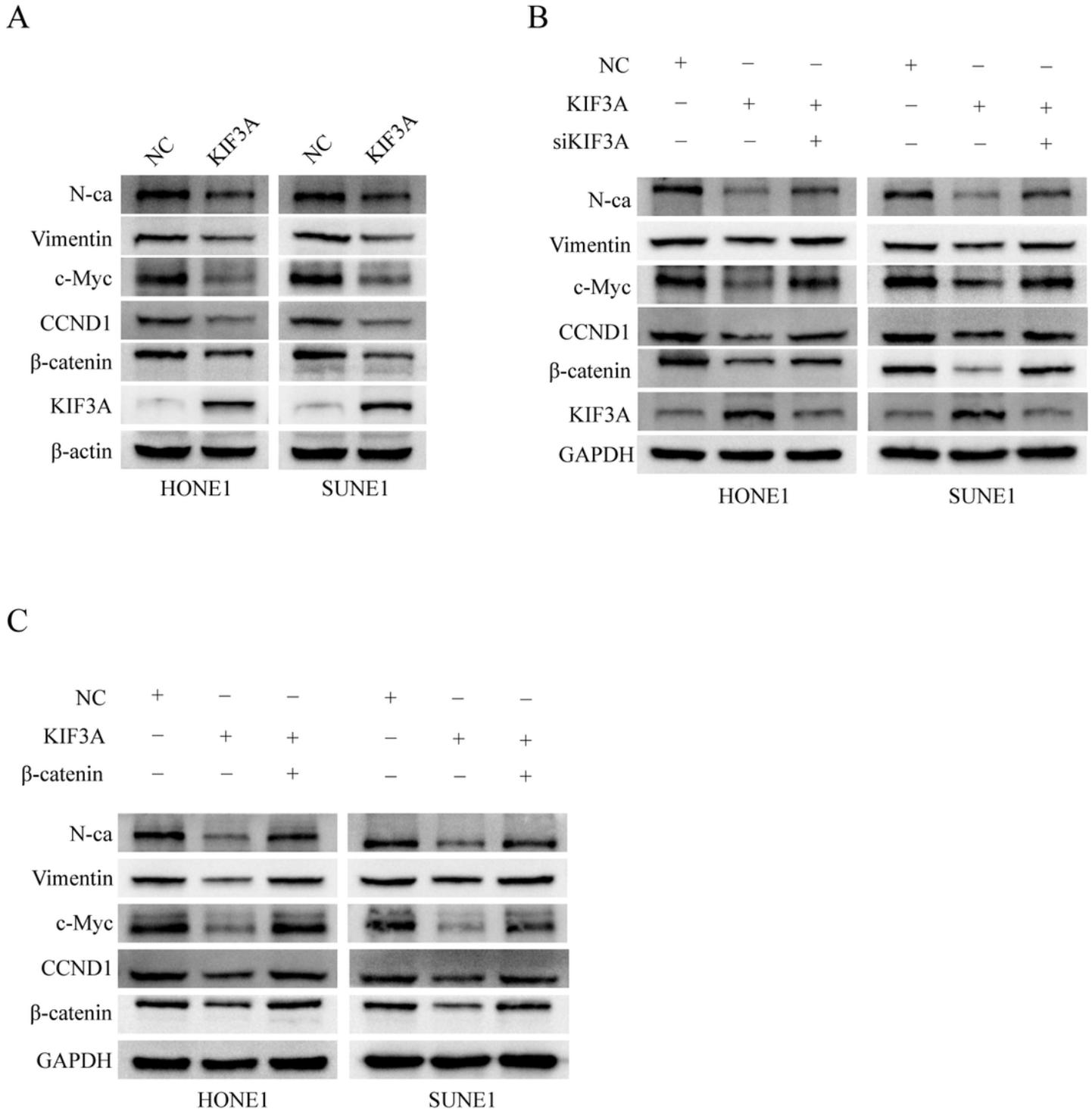
of KIF3A expression in oe-KIF3A-transfected HONE1 and SUNE1 cells. Student's t test. Mean  $\pm$  SD, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**Figure 4**

KIF3A suppresses NPC migration, and knockdown of KIF3A expression reverses this phenotype. (A-B) Wound healing assays and Transwell assays were performed to evaluate the migration capability of NPC cells transfected with the KIF3A plasmid. Student's t test. Mean  $\pm$  SD, \* $p < 0.05$ , \*\* $p < 0.01$ . (C-D) Wound

healing and Transwell assays after simultaneous knockdown of KIF3A expression in oe-KIF3A-transfected HONE1 and SUNE1 cells, Student's t test. Mean  $\pm$  SD, \* $p < 0.05$ , \*\* $p < 0.01$ .

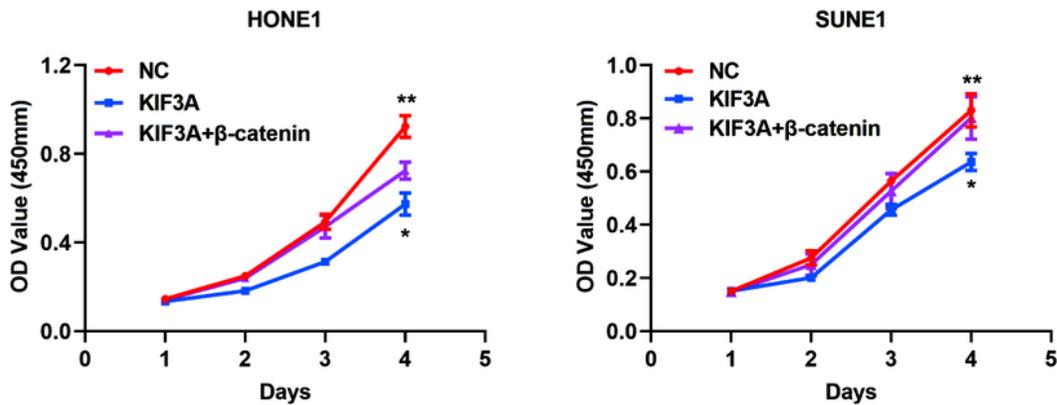


**Figure 5**

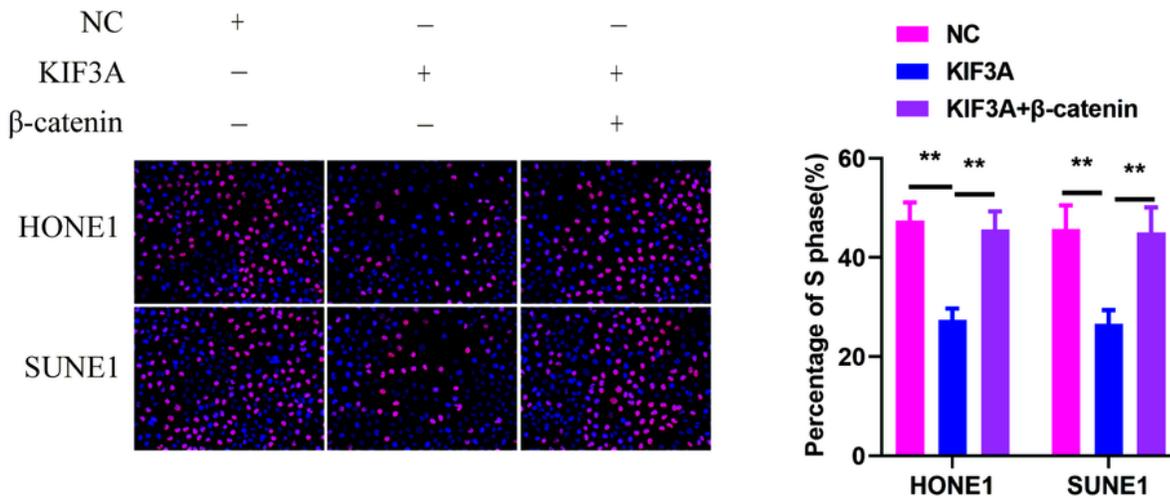
KIF3A suppresses NPC proliferation and migration via the wnt/ $\beta$ -catenin signalling pathway. (A) Changes in the expression levels of KIF3A, N-cadherin, Vimentin, CCND1, and c-Myc were detected by Western blotting after transfection with the KIF3A plasmid in HONE1 and SUNE1 cells.  $\beta$ -actin was used as a

loading control. (B) Changes in the expression levels of KIF3A, N-cadherin, Vimentin, CCND1, c-Myc were detected by Western blotting after transfecting the KIF3A plasmid or KIF3A plasmid + siRNA targeting KIF3A in HONE1 and SUNE1 cells. GAPDH was used as a loading control. (C) Changes in the expression levels of N-cadherin, Vimentin, CCND1, c-Myc were detected by Western blotting after transfection with the KIF3A plasmid or KIF3A + $\beta$ -catenin plasmids in HONE1 and SUNE1 cells. GAPDH was used as a loading control.

A



B



C

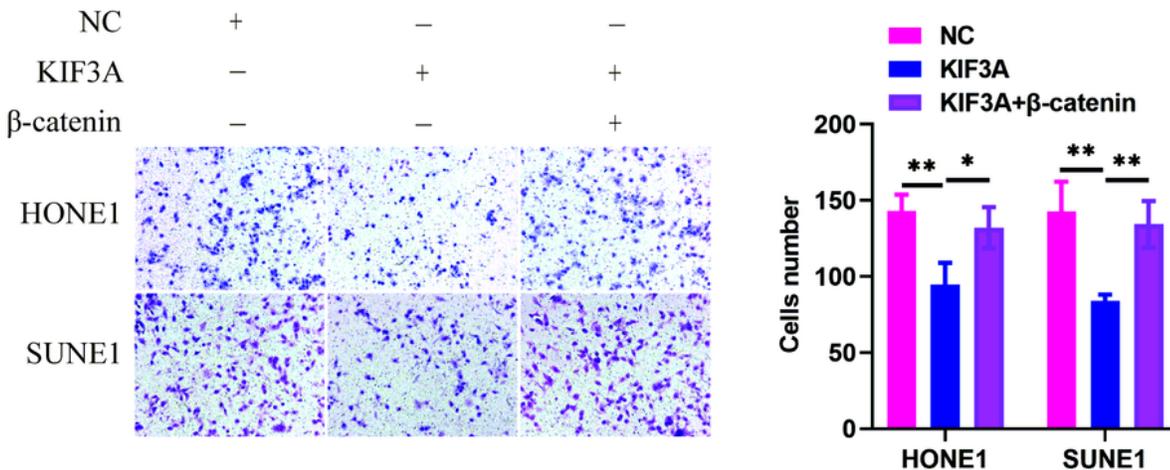


Figure 6

$\beta$ -catenin reverses the inhibitory effect exerted by KIF3A overexpression in NPC. (A-B) CCK-8 and EdU incorporation assays were conducted after transfection with the KIF3A plasmid or  $\beta$ -catenin+KIF3A plasmids in HONE1 and SUNE1 cells. (C) A wound healing assay was conducted to assess the migration capability of NPC cells treated with the KIF3A plasmid or  $\beta$ -catenin+KIF3A plasmids. Student's t test. Mean  $\pm$  SD, \* $p < 0.05$ , \*\* $p < 0.01$ .

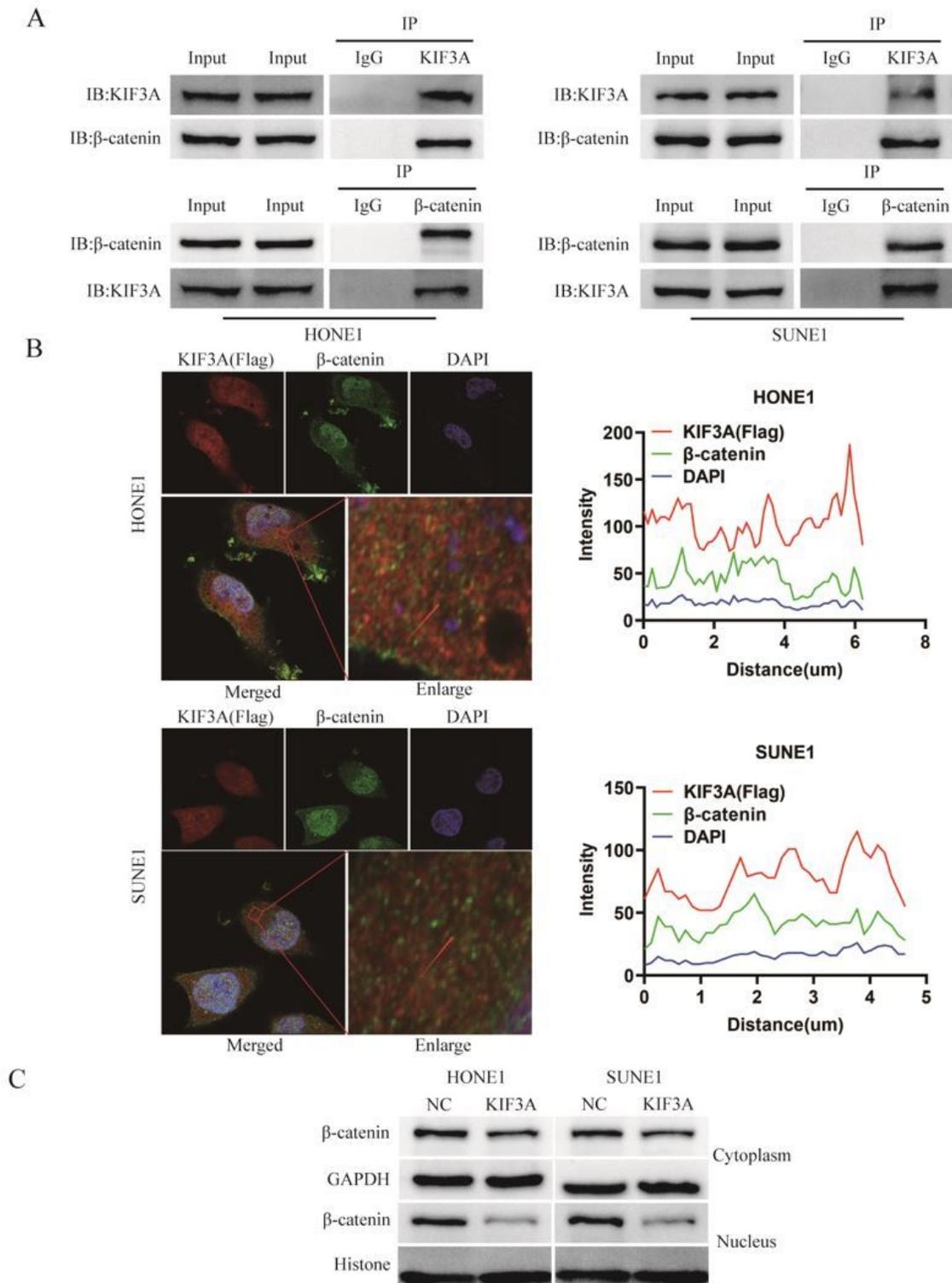


Figure 7

KIF3A interacts with  $\beta$ -catenin and suppresses  $\beta$ -catenin nuclear translocation. (A) A CO-IP assay was performed to detect the interactive relationship between KIF3A and  $\beta$ -catenin in NPC cells. (B) Colocalization of KIF3A and  $\beta$ -catenin by immunofluorescence in HONE1 and SUNE1 cells. (C) Western blotting was conducted to detect changes in  $\beta$ -catenin protein expression in the nucleus and cytoplasm. GAPDH was used as a loading control in the cytoplasm, and histone was used as a loading control in the nucleus.

## Supplementary Files

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