

# miR-103 promotes the progression of non-Hodgkins lymphoma by inhibiting OTUD7B expression

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## Primary research

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# Abstract

## Background

MicroRNAs (miRNAs) are vital for regulating the malignant phenotypes of tumor cells. The purpose of this work is to investigate the function and downstream mechanism of miR-103 in the progression of non-Hodgkin lymphoma (NHL).

## Methods and Materials

Quantitative real-time polymerase chain reaction (qRT-PCR) was employed to detect miR-103 and OTUD7B mRNA expressions in NHL tissues and cells. Immunohistochemistry and Western blot were used to detect the expression of OTUD7B in NHL tissues and cells. CCK-8 experiment, flow cytometry analysis, and Transwell experiment were used to detect the role of NHL cell proliferation, apoptosis, migration and invasion. Bioinformatics, qRT-PCR, Western blot and dual-luciferase reporter assays were used to validate the targeting relationship between miR-103 and OTUD7B. NF- $\kappa$ B p65 luciferase reporter assay and Western blot were applied to determine NF- $\kappa$ B activity and the expression of NF- $\kappa$ B targeted genes.

## Results

Compared to normal tissues and cells, miR-103 expression levels were remarkably up-regulated in NHL tissues and cell lines. The up-regulation of miR-103 dramatically promoted the proliferation, migration and invasion of NHL cells and inhibited apoptosis. Conversely, down-regulating miR-103 significantly inhibited malignant phenotypes of the NHL cells. Additionally, OTUD7B was identified as a target gene of miR-103, and miR-103 increased NF- $\kappa$ B activity indirectly via repressing OTUD7B.

## Conclusion

The miR-103/OTUD7B/NF- $\kappa$ B axis is involved in NHL progression.

## 1 Background

Non-Hodgkin lymphoma (NHL) is one of the most common hematological malignancies in the world (1, 2). Due to the lack of specificity, current chemotherapeutics leads to side effects, and chemoresistance indicates poor prognosis (3). Further investigation of the mechanism of NHL progression has great significance to explore novel therapy targets.

MicroRNAs (miRNAs), about 19–25 nucleotides in size, are a class of small endogenous single-stranded non-protein-encoding RNAs, which bind to the 3' untranslated region (3' UTR) of mRNA, inducing mRNA degradation or inhibiting mRNA translation, thereby regulating target gene expression (4). So far, accumulating studies have indicated that miRNAs are implicated in a variety of physiological and pathological activities, including tumorigenesis (5, 6). For example, miR-760 inhibits gastric cancer cell

proliferation and induces apoptosis by negatively regulating the expression of GIT1 (7). miR-21 promotes angiogenesis, migration, and invasion of renal clear cell carcinoma by regulating the PDCD4/c-Jun (AP-1) signaling pathway (8). miR-103 plays a cancer-promoting role in various tumors, such as breast cancer, bladder cancer, and gastric cancer (9–11). However, the expression of miR-103 in NHL and its mechanism remain unclear.

Deubiquitylases are a class of proteins involved in post-translational modification of proteins, whose main function is to remove the ubiquitination modification of the target protein, thereby regulating the stability and localization of the target protein (12, 13). Deubiquitylases are involved in preventing the protein from degradation, removing non-degradable ubiquitin signals, maintaining stable ubiquitin level, and mediating ubiquitin signal conversion (14). Ovarian tumor-associated protease 7B (OTU deubiquitinase 7B, OTUD7B, also known as Cezanne) belongs to the deubiquitinylases family, which is related to the tumorigenesis (15). The role of OTUD7B in different tumors is different. For example, OTUD7B can mediate the deubiquitination of epidermal growth factor receptor in breast cancer cells to promote the proliferation and survival of breast cancer cells (16). Differently, the expression level of OTUD7B in hepatocellular carcinoma is significantly lower than that in adjacent liver tissues; patients with liver cancer with a lower expression of OTUD7B have adverse prognosis (17). However, the role of OTUD7B in NHL is unclear.

Nuclear factor-Kappa B (NF- $\kappa$ B) is a well known transcription factor associated with almost all aspects of life activity (18). It consists of five subunits, namely c-Rel, Rel A (p65), Rel B, NF- $\kappa$ B1 (p105/p50), and NF- $\kappa$ B2 (p100/p52) (19). NF- $\kappa$ B plays a very important role in the progression of tumors by regulating multiple downstream targeted genes (19). A lot of studies indicated that NF- $\kappa$ B signaling is abnormally activated in NHL (20–22). However, the upstream mechanisms that cause abnormal activation of NF- $\kappa$ B need to be further explored.

In this study, it was demonstrated that miR-103 expression was significantly up-regulated in NHL. Gain- and loss-of-function experiments confirmed the role of miR-103 in promoting the malignant phenotype of NHL cells. Additionally, it was demonstrated that miR-103 participated in promoting NHL progression by regulating the OTUD7B/NF- $\kappa$ B axis. This study is expected to provide new clues for the treatment of NHL.

## **2 Materials And Methods**

### **2.1 Ethical statement and organization of specimens**

This study is approved by the Ethics Review Committee of First Affiliated Hospital of Zhengzhou University. All patients enrolled in this study were diagnosed with NHL for the first time, and tumor tissue and normal tissues were collected by biopsy, and the procedures during the tissues collection is in accordance with the Declaration of Helsinki. All patients signed a written informed consent form prior to participating in the study. Immediately after collection, the tissues were quickly frozen at -80 °C for further experiments and analysis.

## 2.2 Cell culture

Human NHL cell lines (Raji, NK-92MI, RL, Mino cells) and germinal center-B (GC-B) cells were procured from China Center for Type Culture Collection (CCTCC, Wuhan, China). All cells were cultured in Dulbeccos modified Eagles medium/Hams Nutrient mixture F12 (DMEM/F12, Gibco, Grand Island, NY, USA), which contained 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA), 100 U/mL penicillin and 0.1 mg/mL streptomycin (Sigma, St. Louis, MO, USA). The cells were cultured in an incubator at 37 °C, in 5% CO<sub>2</sub> with saturated humidity.

## 2.3 Cell transfection

Raji and Mino cells in the logarithmic growth phase were selected and seeded in 6-well plates with a cell density of  $5 \times 10^5$ /well. After cultured for 23 h, transfection was performed according to the instructions of Lipofectamine® 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA). miR-103 mimics (miR-103: 5'-AGCAGCAUUGUACAGGGCUAUGA-3'), miR-103 inhibitors (miR-103 in: 5'-UCAUAGCCCUGUACAAUGCUGCU-3'), over-expressed OTUD7B plasmid, shRNAs targeting OTUD7B (shOTUD7B#1: GCTGCGGAAAGCTTTGTATGC; shOTUD7B#2: TTCTCCGAACGTGTCACGT) and their negative controls were transfected into NHL cells, respectively. The cells were collected after 48 h after transfection, and RNA or protein expression was detected by RT-PCR and Western blot to confirm successful transfection.

## 2.4 Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total tissue or cell RNA was extracted using TRIzol reagent (Invitrogen, Waltham, MA, USA). Nanodrop-spectrophotometer was used to detect RNA concentration and purity. According to the manufacturer's protocol, the PrimeScript-RT Kit (Madison, WI, USA) was used to synthesize complementary DNA (cDNA) from 1 µg of total RNA, and then SYBR Green Premix Ex Taq II (TaKaRa, Dalian, China) and ABI 7500 system ( Applied Biosystems, Foster City, CA, USA) was applied for qRT-PCR. The total volume of the PCR system was 30 µL and each sample contained 300 ng of cDNA. GAPDH was the internal reference for OTUD7B, and U6 was the internal reference for miR-103. The primer sequence information was shown in Table 1.

Table 1  
Sequences used for qRT-PCR.

Primers Sequences	
miR-103	F: CCCGCCAAGCCCTTACC R: GCCGTCGGTGATGCTTTTTTTGG
U6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT
OTUD7B	F: TGGCTACCCTGGGGACTTTACTA R: ACTGTCTGGGGAAGGTGGCATA
GAPDH	F: TGTTGCCATCAATGACCCCTT R: TGTTGCCATCAATGACCCCTT
Abbreviation: qRT-PCR, reverse transcription-quantitative PCR; miR-103, microRNA-103; OTUD7B, OTU deubiquitinase 7B; U6: snRNA, small nuclear RNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; F, forward; R, reverse.	

## 2.5 Immunohistochemistry (IHC)

NHL tissues and normal tissues were fixed in 10% formaldehyde and embedded in paraffin. The sections were dewaxed and hydrated. Before immunohistochemical staining, the dewaxed sections were baked at 37 °C for 2 h, the endogenous peroxides were blocked by 1% H<sub>2</sub>O<sub>2</sub> for 5 min and the sections were washed for 3 times, and the sections were blocked using immunostaining blocking solution for 1 h, then incubated with anti-OTUD7B antibody (1: 200 dilution, Abcam, Cambridge, UK, ab118387) overnight at 4 °C. Then the sections were washed with PBS and then incubated with biotin-linked antiserum for 1 h at room temperature. Next, the sections were washed again and stained with 3,3-diaminobenzidine (DAB) for 1 min. Then the sections were washed with double distilled water, stained with hematoxylin for 1 min, and observed under a microscope. Ultimately, the staining was scored by pathologists.

## 2.6 Cell proliferation assay

The cells of each group in the exponential growth phase were collected and prepared into single cell suspension ( $1 \times 10^4$ /ml), then inoculated on 96-well plates with 100  $\mu$ L cell suspension per well, with 3 replicate wells in each group. Subsequently, 10  $\mu$ L of CCK-8 solution (Beyotime Biotechnology, Shanghai, China) were added to the wells, and a blank control well only containing the medium and CCK-8 solution was set. After 2 h of incubation, a microplate reader at a wavelength of 450 nm was employed to determine and record the absorbance (A) values of each well. Ultimately, the plate was measured at intervals of 24 h for 5 days.

## 2.7 Apoptosis Experiment

Annexin V-fluoroisothiocyanate (FITC)/PI double staining kit (Sungene Biotech, TianJin, China) was used to detect the apoptosis of NHL cells. 24 h after cell transfection, the cells were collected, and the cell density was adjusted to  $2 \times 10^6$  cells/well, and the culture was continued for 24 h. After that the cells were centrifuged and the supernatant was discarded, and the cells were washed with pre-cooled PBS twice, and the cells were resuspended with  $1 \times$  Binding buffer. Following that, 5  $\mu$ L AnnexinV-FITC and 5  $\mu$ L PI solution were added to the cell suspension, mixed thoroughly and incubated at room temperature for 15 min. Finally, flow cytometry analyses (Cytomics FC 500; Beckman Coulter) was employed to detect the apoptosis rate according to the instructions of the kit.

## 2.8 Transwell experiment

Transwell experiment was carried out using Transwell chamber (Millipore, Billerica, USA). After that, the Matrigel was added to the Transwell chamber membrane and the chambers were placed in a 24-well plate for cell invasion experiments (in cell migration experiments, Matrigel was not used). NHL cells were harvested and adjusted to a cell density of  $1 \times 10^5$ /mL with serum-free medium, and 200  $\mu$ L cell suspension was added into the Transwell chamber. Meanwhile, 800  $\mu$ L medium containing 10% FBS was added to the wells in the incubator, and the cells were cultured at 37 °C for 24 h. Thereafter, the chamber was removed, and the cells migrated or invaded in the wells of the 24-well plate were counted.

## 2.9 Western blot

Cells were lysed with a RIPA lysis (Pierce, Rockford, IL, USA), and then centrifuged to collect the supernatant. The protein samples were separated by SDS-PAGE and then electrophoretically transferred to a nitrocellulose (NC) membrane. The proteins were then blocked with 5% skim milk at room temperature for 2 h, and then the primary antibody was added to incubate the NC membrane at 4 °C overnight. After that the membrane was washed with TBST for 3 times, and the secondary antibody was added to incubate the NC membrane at room temperature for 2 h. Next, the membrane was washed with TBST for 3 times, and ECL chemiluminescence reagent (Millipore, Bedford, MA, USA) was used to develop the bands. The antibodies used in this study was purchased from Abcam (Cambridge, UK): anti-OTUD7B antibody (1: 500, ab118387), anti-p65 antibody (1: 1000, ab16502), and anti-Bcl-2 antibody (1: 1000, ab185002), anti-XIAP antibody (1: 500, ab28151), anti-CIAP2 (1: 1000, ab32059), anti-Livin antibody (1: 500, ab97350), anti-survivin (1: 1000, ab97350).

## 2.10 Dual luciferase reporter assay

Bioinformatics was utilized to predict the potential binding sites between 3'-UTR of OTUD7B and miR-103. The OTUD7B 3'-UTR wild sequence or OTUD7B 3'-UTR mutant sequence was inserted into the pmiRGLO dual-luciferase miRNA target expression reporter (Promega, Madison, WI, USA). 293T cells were seeded on 24-well plates ( $5 \times 10^5$  cells / well) and 24 h later, the cells were co-transfected with luciferase reporters and miR-103 mimic or negative control mimic using with Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA). The luciferase activity was detected 48 h after transfection with Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions.

NF- $\kappa$ B luciferase reporter plasmid (Beyotime Institute of Biotechnology, Nantong, Jiangsu, China) with OTUD7B plasmid or OTUD7B shRNA were co-transfected into NHL cells using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. After 24 h, luciferase activity was measured using the luciferase kit (Beyotime, Hangzhou, China).

## 2.11 Data analysis

All experiments were repeated 3 times, and the data obtained were expressed as  $x \pm s$ . SPSS17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Independent sample t test was employed to compare the two groups.  $P < 0.05$  indicated that the difference was statistically significant.

## 3 Results

### 3.1 The characteristics of miR-103 expression NHL

In order to study the expression characteristics of miR-103 in NHL tissues, tumor tissues and normal lymph node tissues of 37 NHL patients were collected, and then miR-103 expression was measured. qRT-PCR results showed that miR-103 expression was significantly up-regulated in NHL tissues compared to non-tumor tissues (Fig. 1A). Furthermore, compared with germinal center (GC) -B cells, it was found that the expression levels of miR-103 in four NHL cells (Raji, NK-92MI, RL, Mino cells) were also significantly increased (Fig. 1B). These research data suggested that miR-103 could probably play an oncogenic role in NHL.

### 3.2 miR-103 promoted the proliferation, migration and invasion of NHL cells and inhibited the apoptosis

To investigate the function of miR-103 in NHL cells, miR-103 mimics were transfected with Mino cells, and miR-103 inhibitors were transfected into Raji cells respectively (Fig. 2A). Subsequently CCK-8 experiments and flow cytometry analysis revealed that compared with the control group, miR-103 overexpression promoted Mino cell proliferation and inhibited apoptosis (Fig. 2B&C). In addition, Transwell assays confirmed that miR-103 overexpression significantly enhanced the migration and invasion of Mino cells (Fig. 2D & E). On the contrary, the inhibition of miR-103 in Raji cells showed the opposite effect in the malignant phenotypes of the cells described above (Fig. 2B-E). These studies indicated that miR-103 exerted a cancer-promoting effect in the progression of NHL.

### 3.3 miR-103 directly targeted OTUD7B

To probe the downstream mechanism of miR-103 in NHL cells, two online analysis tools (microRNA and miRDB) were used to search for candidate targets for miR-103. A potential binding site was found between miR-103 and the 3'UTR of OTUD7B (Fig. 3A). To verify the interaction of miR-103 with OTUD7B

3'-UTR, dual luciferase reporter assay was performed. The results suggested that miR-103 overexpression significantly reduced the luciferase activity of WT-OTUD7B 3'-UTR reporter, but did not significantly change the luciferase activity of MUT-OTUD7B 3'-UTR reporter (Fig. 3B). Additionally, Pearson correlation analysis showed a significant negative correlation between miR-103 expression and OTUD7B expression in NHL tissues (Fig. 3C). Furthermore, qRT-PCR and Western blot also demonstrated that miR-103 mimics significantly inhibited mRNA and protein expression of OTUD7B, while down-regulating miR-103 induced increased expression of OTUD7B in NHL cells (Fig. 3D & E). In summary, these studies suggested that OTUD7B was a downstream target of miR-103 in NHL cells and was negatively regulated by the latter.

### **3.4 OTUD7B was down-regulated NHL tissues and cells**

The experiments mentioned above confirmed that OTUD7B was one of the downstream targets of miR-103, so immunohistochemistry were performed to detect the expression characteristics of OTUD7B in NHL tissues and normal tissues. Representative images of the normal and NHL immune groups were shown (Fig. 4A). The statistical results implied that compared with non-tumor tissues, the positive rate of OTUD7B low expression was significantly increased in NHL tissues (Fig. 4B). In addition, qRT-PCR results showed that OTUD7B mRNA expression levels were significantly reduced in NHL tissues compared to non-tumor tissues (Fig. 4C). Consistent with expectations, OTUD7B expression levels were significantly reduced in the four NHL cells compared to GC-B cells (Fig. 4D). The data suggested that OTUD7B probably inhibited NHL progression.

### **3.5 OTUD7B inhibited the proliferation, migration and invasion of NHL cells and promoted the apoptosis**

In order to explore the role of OTUD7B axis in NHL, Raji cells were transfected with OTUD7B overexpressing plasmid, and Mino cells were transfected with OTUD7B shRNA. Transfection efficiency was confirmed by qRT-PCR and Western blot (Fig. 5A & B). The results of CCK-8 experiments, flow cytometry, and Transwell experiments suggested that compared to the control group, overexpression of OTUD7B significantly inhibited Raji cell proliferation, migration, and invasion and promoted the apoptosis (Fig. 5C-F). On the contrary, knocking down OTUD7B promoted the above-mentioned malignant phenotypes of Mino cells (Fig. 5C-F). These results indicated that OTUD7B was involved in inhibiting the malignant biological behaviors of NHL cells.

### **3.6 OTUD7B inhibited NF- $\kappa$ B signaling pathway in NHL**

It is worth noting that previous studies have reported that OTUD7B can inhibit the activation of NF- $\kappa$ B (23–24). Therefore, we made a hypothesis that OTUD7B could inhibit NHL progression by regulating the NF- $\kappa$ B pathway. As we expected, luciferase reporter assay indicated that overexpression of OTUD7B significantly inhibited the p65 NF- $\kappa$ B luciferase reporter activity, while knockdown of OTUD7B promoted the p65 NF- $\kappa$ B luciferase reporter activity (Fig. 6A). In addition, OTUD7B significantly reduced the expression level of p65 protein (an indicator of NF- $\kappa$ B transcriptional activity) (Fig. 7B). Consistently, the

NF- $\kappa$ B target genes were also suppressed by OTUD7B in Raji cells, such as Bcl-2, XIAP, CIAP2, Livin and survivin (Fig. 6B). Conversely, knockdown of OTUD7B expression induced an increase in these proteins in Mino cells (Fig. 6A, B). Hence, it was concluded that OTUD7B inhibited lymphoma progression by impeding the NF- $\kappa$ B signaling pathway.

### **3.7 miR-103/OTUD7B axis was involved in regulating the malignant phenotypes of NHL cells**

Subsequently, miR-103 mimics and OTUD7B overexpression plasmid or its negative control were co-transfected into Mino cells, and miR-103 inhibitors and sh-OTUD7B or its negative control were co-transfected into Raji cells (Fig. 6A & B). As shown, restoring the expression of OTUD7B reversed the effect of miR-103 overexpression on cell proliferation, migration, invasion and apoptosis (Fig. 6C-F). Similarly, knocking down OTUD7B partially weakened the cancer-promoting effect of miR-103 inhibitors in NHL. Therefore, it was confirmed that the miR-103/OTUD7B axis was involved in promoting NHL cell proliferation, migration, and invasion.

## **4 Discussion**

This study reported for the first time that miR-103 was up-regulated in NHL tissues and cells. miR-103 can promote cancer cell proliferation, invasion, migration, and inhibit apoptosis. This work also indicated that miR-103 can promote NHL progression by suppressing the expression of OTUD7B, which was a negative regulator of NF- $\kappa$ B signaling pathway.

Recent studies have shown that multiple miRNAs are dysregulated in malignancies, including lymphomas (25, 26). Clarifying the relationship between miRNA and lymphoma will probably provide new biomarkers and therapy targets for this diseases (27). For example, miR-374b is highly expressed in T-cell lymphoblastic lymphoma and can be used as an independent indicator of relapse and survival (28). Additionally, compared to healthy individuals, miR-21 expression levels in tissues of B-NHL patients were significantly up-regulated; compared with stage I/II B-NHL, the expression level of miR-21 in stage III/IV patients was significantly higher (29). These reports indicate that miRNA can provide a new basis for assessing lymphoma progression, monitoring treatment efficacy and predicting the prognosis. As an important member of the miRNA family, miR-103 plays a pro-cancer role in multiple tumors. For example, miR-103 promotes the stemness of colorectal cancer cells by activating the Wnt/ $\beta$ -catenin signaling pathway, thereby promoting tumorigenesis and metastasis (30). miR-103 is involved in regulating the AKT/mTOR signaling pathway to promote the progression of prostate cancer (31). In this study, for the first time, miR-103 expression level in NHL was investigated and it was proved to be significantly increased in NHL tissues and cells. In vitro experiments confirmed that up-regulating miR-103 significantly promoted NHL cell proliferation, migration, and invasion and inhibited the apoptosis. Conversely, miR-103 inhibitors significantly inhibited the progression of NHL cells. This suggests that miR-103 is a potential therapy target for NHL.

OTUD7B, an important deubiquitinase, specifically regulates the ubiquitination level of substrate proteins by targeting K11-linked polyubiquitin chains, and is implicated in cell proliferation, cell survival, inflammatory responses, and other physiological and pathological processes (15–17). It plays an important role in the development of malignancies including lung cancer, breast cancer and liver cancer (16, 17, 32–34). OTUD7B mediates EGFR deubiquitination through its OTU domain, inhibits EGFR degradation, promotes constant activation of EGFR downstream molecules, enhances AKT signal transduction, and promotes lung cancer cell proliferation, migration and angiogenesis (32). Interestingly, another study reports OTUD7B in non-small cell lung cancer cell line H157 inhibits nuclear translocation of NF- $\kappa$ B, reduces the expression of IL-8 and ICAM-1, and thus inhibits the progression of non-cellular lung cancer (24). These studies suggest that the expression pattern, function and mechanism of OTUD7B in cancer biology are controversial. In this study, it was found that compared with normal tissues, the expression level of OTUD7B in NHL tissues was significantly reduced. Gain-of-function and loss-of-function experiments confirmed that overexpression of OTUD7B significantly inhibited NHL cell proliferation, migration, and invasion. On the contrary, compared with the control group, knocking down OTUD7B significantly promoted NHL progression. Furthermore, it was also found that OTUD7B significantly inhibited the p65 NF- $\kappa$ B luciferase reporter activity. In addition, OTUD7B could also inhibit the expression of p65 protein and the targeted genes of NF- $\kappa$ B signaling. It is confirmed that OTUD7B suppresses the progression of NHL by inhibiting the NF- $\kappa$ B signaling pathway. Additionally, in this work, it was demonstrated that OTUD7B was a target gene of miR-103, and overexpression of OTUD7B reversed the function of miR-103 on NHL cells. These demonstrations not only partly explained the reason of OTUD7B dysregulation in NHL, but also help clarify the downstream mechanism of miR-103.

There are some limitations in this work. Firstly, animal models and in vivo experiments are essential to further validate the oncogenic role of miR-103 and tumor-suppressive role of OTUD7B in NHL progression. Additionally, it's interesting to study whether miR-103 can increase the activity of NF- $\kappa$ B via repressing OTUD7B in the following studies. Additionally, more patients from different centers should be enrolled, and the pathological indexes and prognosis of the patients should be analyzed based on the expression levels of miR-103 and OTUD7B, which is crucial to evaluate the potential of miR-103 and OTUD7B as biomarkers.

## Conclusion

In summary, for the first time, this study verified that miR-103 was up-regulated in NHL tissues and cells, and functioned as a oncogenic miRNA. It is also demonstrated that OTUD7B, a tumor suppressor in NHL, was a novel target gene of miR-103. This work provides new clues for the diagnosis and treatment of NHL.

## Abbreviations

NHL: non-Hodgkin lymphoma; MiRNAs: microRNAs; OTUD7B: OTU deubiquitinase 7B; NF-KB: nuclear factor-Kappa B; GC-B: germinal center -B; qRT-PCR: quantitative reverse transcription-polymerase chain

reaction; IHC: immunohistochemistry; DAB: diaminobenzidine; CCK-8: Cell Counting Kit-8; FBS: fetal bovine serum; PBS: phosphate buffer saline; WT: wild type; MUT: mutant type; UTR: untranslated regions.

## **Declarations**

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

Our study was approved by the Ethics Review Board of The First Affiliated Hospital of Zhengzhou University.

### **Consent for publication**

The publication of this worked has received the permission of all authors.

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

The data used to support the findings of this study are available from the corresponding author upon request.

### **Competing interests**

The authors declare that they have no competing interests.

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### **Authors' contributions**

Conceived and designed the experiments: CW, YTW, YFL;

Performed the experiments: CW, MYL, SJW;

Statistical analysis: CW, YTW, SJW, YFL;

Wrote the paper: CW, YTW, YFL.

All authors read and approved the final manuscript.

### **Authors' information**

Not applicable.

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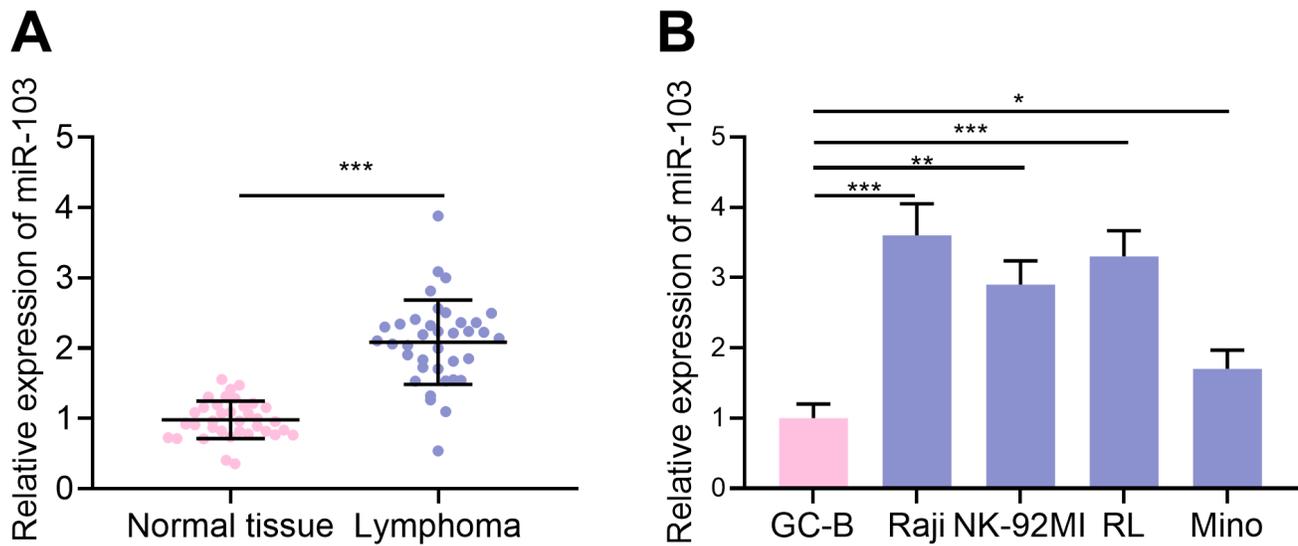
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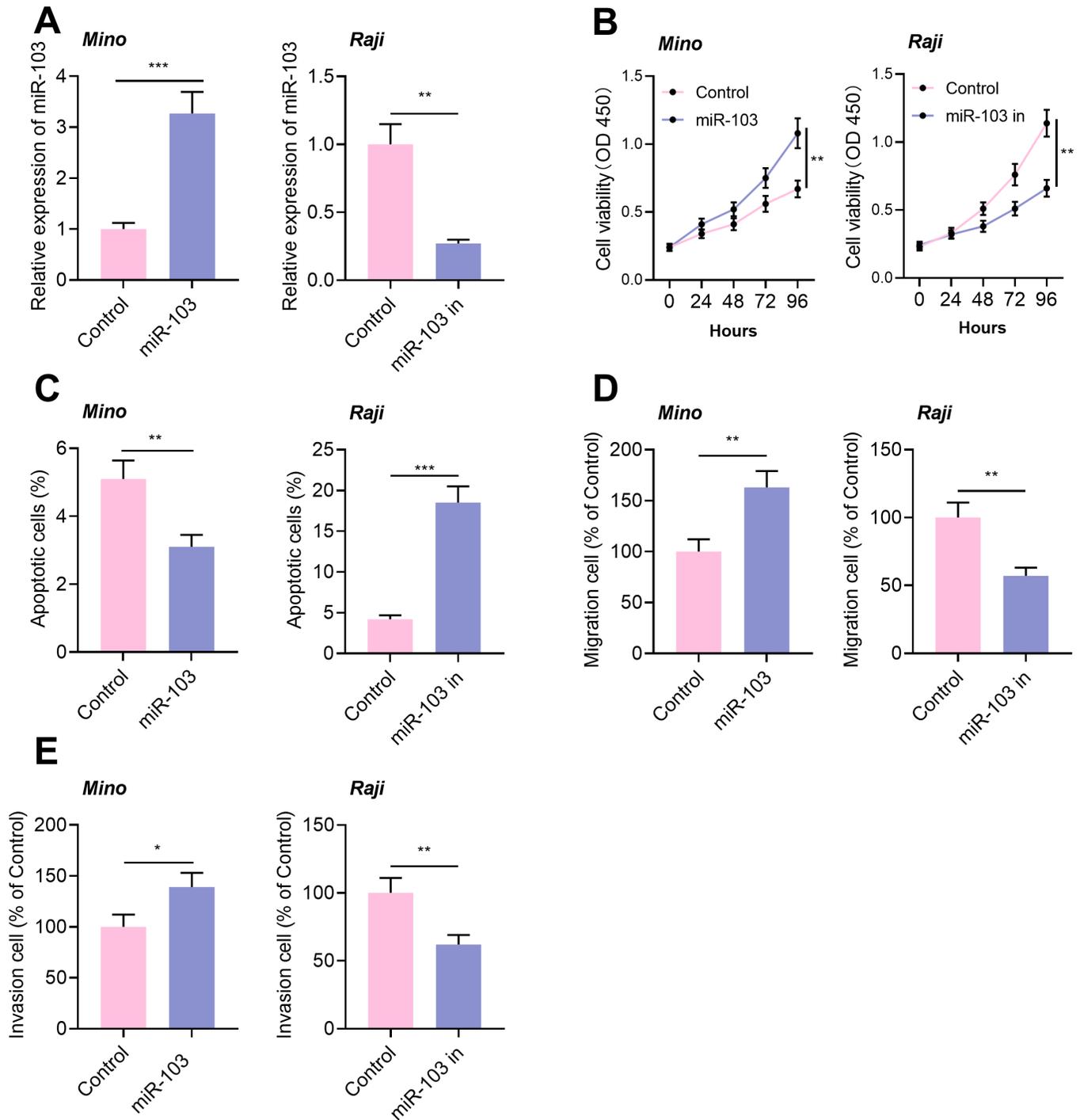
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## Figures



**Figure 1**

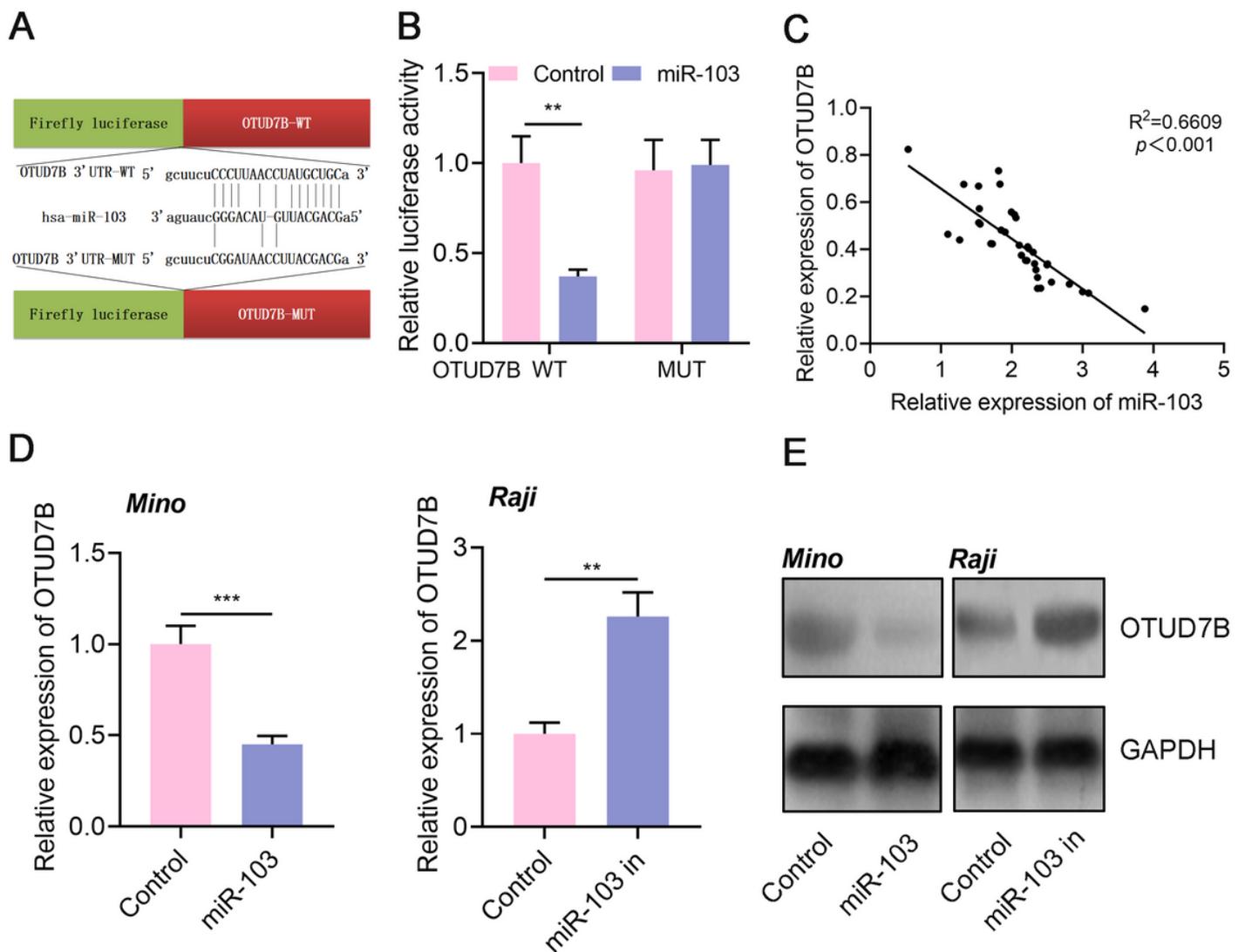
miR-103 expression was significantly up-regulated in NHL. (A) qRT-PCR was used to detect the expression level of miR-103 in NHL tissues and normal lymph node tissues (n=37). (B) qRT-PCR was employed to detect the expression level of miR-103 in human NHL cell lines and normal cells. \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001.



**Figure 2**

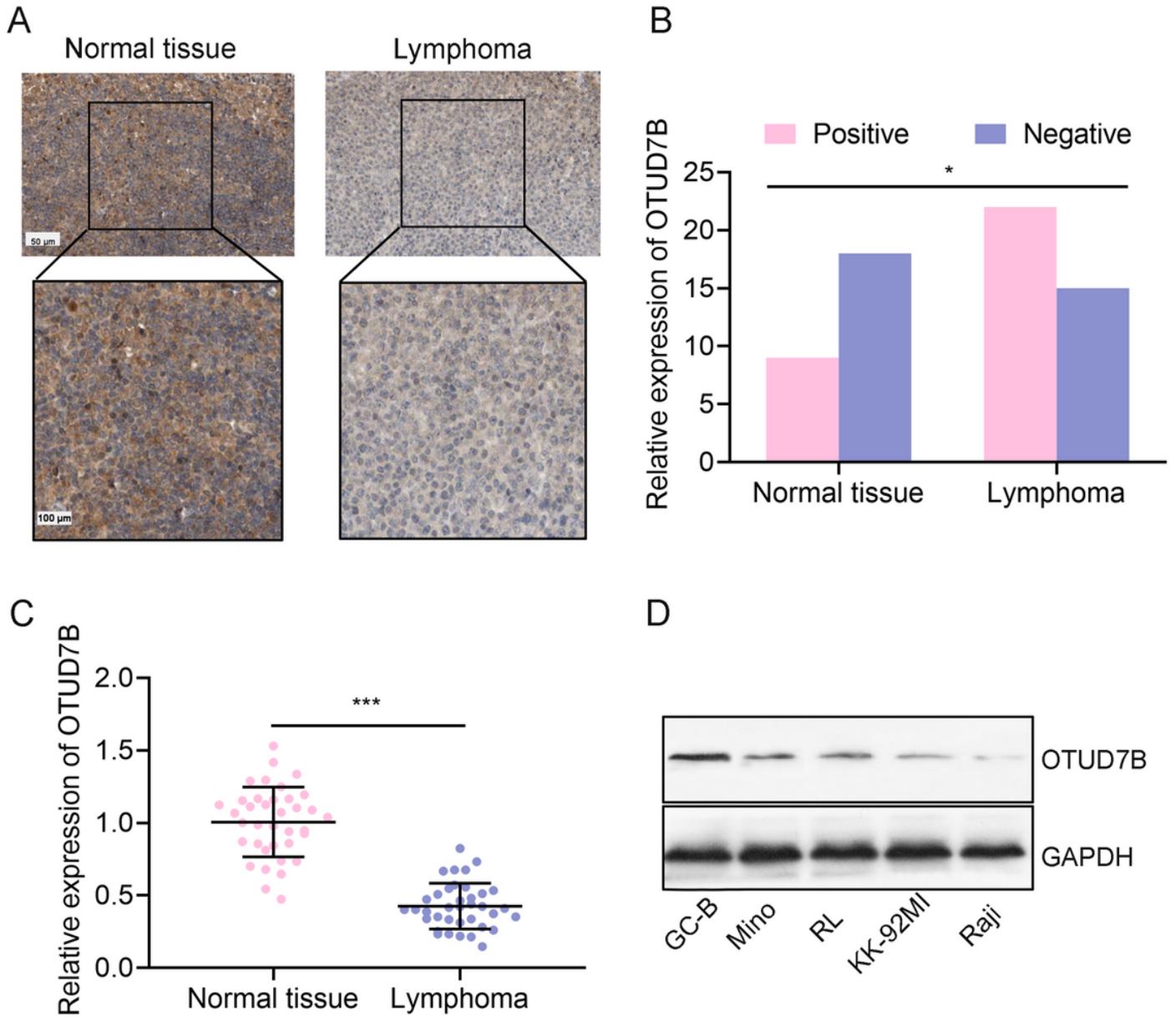
miR-103 promoted the proliferation, migration, and invasion of NHL cells and inhibited the apoptosis. (A) miR-103 and its negative control were transfected into Mino cells; miR-103 inhibitors and its negative control were transfected into Raji cells. Then qRT-PCR was used to detect the expression of miR-103 in Mino and Raji cells. (B) CCK-8 experiments confirmed that up-regulating miR-103 promoted Mino cell proliferation, whereas down-regulating miR-103 inhibited Raji cell proliferation. (C) Flow cytometry

analysis revealed that miR-103 mimics inhibited apoptosis of Mino cells, and miR-103 inhibitors induced apoptosis in Raji cells. (D, E) Transwell experiments showed that overexpression of miR-103 significantly promoted the migration and invasion of Mino cells, while inhibition of miR-103 inhibited the migration and invasion of Raji cells. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



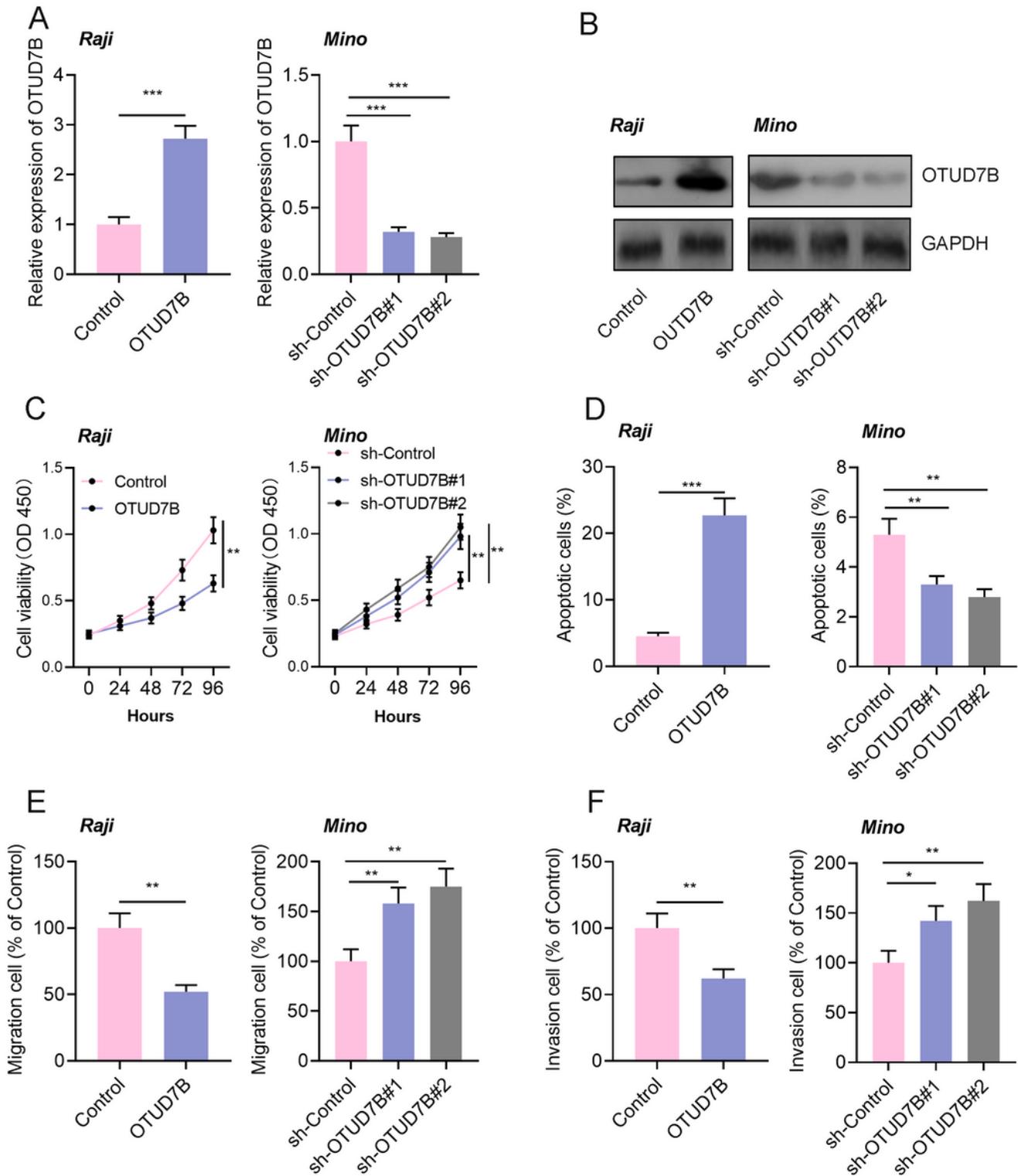
**Figure 3**

OTUD7B was a downstream target of miR-103 in NHL. (A) Bioinformatics was used to predict the potential binding site between miR-103 and the 3'UTR of OTUD7B. (B) Dual luciferase reporter gene assay confirmed that miR-103 mimics significantly inhibited the luciferase activity of wild-type OTUD7B 3' UTR, but did not change the luciferase activity of mutant OTUD7B 3' UTR. (C) Pearson correlation analysis showed that miR-103 and OTUD7B expression in NHL tissues were significantly and negatively correlated. (D, E) qRT-PCR and Western blot results showed that miR-103 mimics inhibited the expression of OTUD7B in Mino cells, while miR-103 inhibitors induced a significant increase in the expression of OTUD7B in Raji cells. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



**Figure 4**

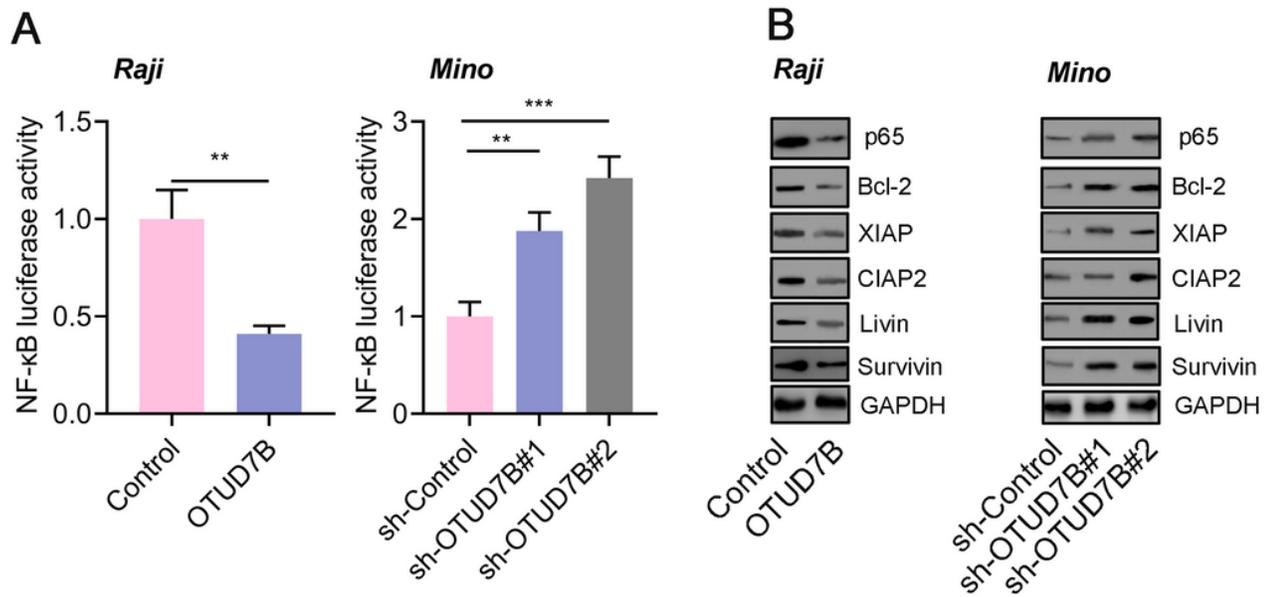
OTUD7B expression was significantly reduced in NHL. (A) Representative figures of immunohistochemistry showed OTUB7D expression in NHL tissues and normal tissues. (B) Chi-square analysis showed that the positive rate of OTUD7B in NHL increased significantly. (C) qRT-PCR results showed that the expression of OTUD7B mRNA in NHL was significantly reduced. (D) Western blot results showed that the expression levels of the 4 NHL cell lines were significantly lower than GC-B cells. \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .



**Figure 5**

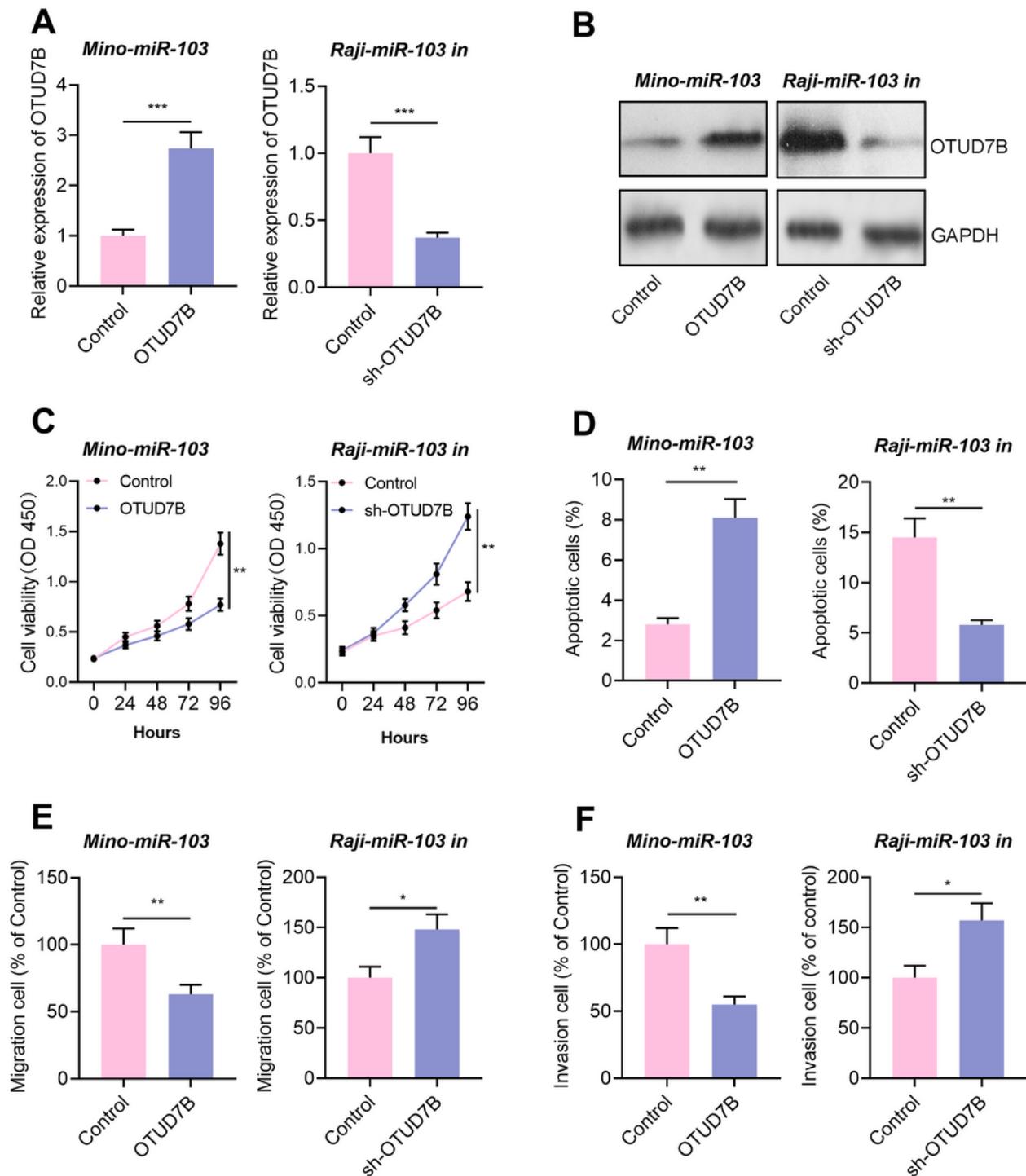
OTUD7B significantly inhibited NHL cell proliferation, migration, and invasion and promoted the apoptosis. (A, B) OTUD7B overexpression plasmid and OTUD7B shRNAs were transfected into Raji and Mino cells, respectively. qRT-PCR and Western blot confirmed the successful transfection. (C) CCK-8 experiments confirmed that OTUD7B overexpression inhibited cell proliferation, while knocking down OTUD7B promoted cell proliferation. (D) Flow cytometry suggested that upregulating OTUD7B promoted

the apoptosis, while knocking down OTUD7B inhibited the apoptosis. (E, F) Transwell results showed that overexpression of OTUD7B inhibited cell migration and invasion, and knockdown of OTUD7B promoted cell migration and invasion. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



**Figure 6**

OTUD7B inhibited NF-κB signaling pathway. (A) Luciferase reporter assay implied that OTUD7B inhibited the NF-κB activity. (B) Western blot showed that OTUD7B inhibited the expression of p65 and downstream genes of NF-κB \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



**Figure 7**

miR-103/OTUD7B axis was involved in regulating the malignant phenotype of NHL cells. (A, B) Mino cells were co-transfected with OTUD7B overexpression plasmid and miR-103 mimics. OTUD7B shRNA and miR-103 inhibitors were co-transfected into Raji cells. qRT-PCR and Western blot confirmed that the transfection was successful. (C) CCK-8 experiments confirmed that overexpression of OTUD7B reversed the effect of miR-103 on promoting cell proliferation, and knocking down OTUD7B weakened the

inhibitory effect of miR-103 inhibitors on cell proliferation. (D) Flow cytometry showed that up-regulating OTUD7B counteracted the inhibitory effect of miR-103 on apoptosis, and down-regulating OTUD7 partially abolished the effect of miR-103 inhibitors on apoptosis. (E, F) Transwell results showed that over-expression of OTUD7B reversed miR-103s promotion of cell migration and invasion, and knockdown of OTUD7B weakened miR-103 inhibitors inhibition of cell migration and invasion. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .