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Original Article

SP1-IKBIP axis promotes the proliferation and invasion of glioma with Wnt/β-catenin associated epithelial-mesenchymal transition

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Abstract: Glioma is the most aggressive tumor of the central nervous system and is associated with poor prognosis, especially in patients with high-grade gliomas. In this study, we investigated the biological role of inhibitor of kappa B kinase interacting protein (IKBIP) in promoting the progression of glioma. Analysis of the TCGA and GTEx databases revealed that IKBIP is upregulated in both lower-grade gliomas (LGG) and glioblastomas (GBM) compared with normal brain tissues. Kaplan-Meier survival analysis demonstrated that IKBIP upregulation is associated with shorter overall survival (OS) and disease-specific survival (DSS) in patients with LGG. Pan-cancer analysis indicated that IKBIP is aberrantly expressed in various malignant tumors, including gliomas. IKBIP knockdown inhibited the proliferation of glioma cells both *in vitro* and *in vivo*. Additionally, IKBIP knockdown in U251 and U87 glioma cell lines significantly suppressed their invasive capacity. Furthermore, IKBIP knockdown resulted in decreased expression of proteins associated with Wnt/β-catenin/epithelial-mesenchymal transition (EMT) pathway, including β-catenin, ZEB1, ZEB2, N-cadherin, whereas the expression of E-cadherin was increased. Conversely, IKBIP overexpression reduced the level of phosphorylated β-catenin (p-β-catenin) while increasing the expression of total β-catenin in glioma cells. Furthermore, we identified that transcription factor SP1 (Specificity Protein 1), which is also upregulated in glioma tissues and cell lines and is associated with the malignant phenotype of glioma, can bind to the promoter region of IKBIP. Upregulation of SPI in glioma cells significantly increased the expression level of IKBIP, while inhibiting the phosphorylation of β-catenin. These findings collectively suggest that upregulation of IKBIP promotes the proliferation and invasive behaviors of glioma cells by activating the Wnt/β-catenin/EMT pathway. Overall, our findings suggest that SP1-IKBIP axis facilitates the proliferation and invasion of glioma through Wnt/β-catenin-associated EMT, and SP1-IKBIP axis may represent a promising target for the clinical diagnosis and treatment of glioma.

Keywords: SP1-IKBIP axis, glioma, proliferation and invasion, Wnt/β-catenin pathway, epithelial-mesenchymal transition (EMT)

Introduction

Glioma is the most common type of primary brain tumor in adults and is characterized by high invasiveness, rapid proliferation, infiltration into surrounding tissues, and the formation of tumors with poorly defined boundaries [1, 2]. These tumors are most frequently diagnosed in individuals aged 45-70 years. In addition, gliomas exhibit a modest male predominance, with a male-to-female ratio of approximately 1.5:1 [3, 4]. Globally, the incidence of Gliomas is higher in North America and Europe as compared to the Asian countries [5, 6]. Differences in disease prevalence have also been observed among ethnic groups, with Caucasian population showing a higher incidence than Asians and Africans.

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Several factors are associated with an increased risk of developing gliomas, including inherited disorders such as neurofibromatosis type 1 and type 2, genetic alterations involving IDH1/2, TP53 or 1p/19q codeletion [7, 8]. Environmental factors, including exposure to ionizing radiation, have also been implicated in the development of glioma [9, 10]. Glioblastoma, the most malignant subtype of glioma, has a median survival of about 18 months following surgery. Current therapeutic strategies for gliomas mainly include surgical resection, radiotherapy, and chemotherapy [11, 12]. However, despite the use of these conventional therapies, survival outcomes are still suboptimal, with limited improvement over the past two decades. Therefore, the identification of molecular markers specific to tumor subtypes and the development of targeted therapeutic strategies based on these molecular markers represent important and promising directions of further research. Identifying reliable biomarkers that can guide therapeutic target selection may further improve the clinical management of glioma patients.

IκB kinase-interacting protein (IKBIP) is an important protein associated with the regulation of the NF-κB signaling pathway by interacting with the NF-κB protein complex [13, 14]. NF-κB is a key transcription factor that regulates cellular responses to stress, immune responses, inflammation, cell growth, and apoptosis [15-17]. IKBIP specifically inhibit NF-κB activity, thereby preventing its nuclear translocation and subsequent transcriptional activation of target genes that are critical for cell survival and function [18, 19]. In addition, IKBIP is involved in the degradation and regulation of IκB family proteins, which normally suppress NF-κB activity by binding to NF-κB and preventing its nuclear translocation. Through modulation of this process, IKBIP regulates the release and activation of NF-κB signaling [20, 21].

IKBIP plays an essential role in immune regulation, including the activation and proliferation of T and B lymphocytes, enabling them to respond to immune challenges. Additionally, IKBIP is involved in the regulation of inflammatory processes, which is particularly important because NF-κB signaling is implicated in the pathogenesis of multiple inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease. Targeting IKBIP may represent a potential therapeutic strategy for controlling immune response and inflammation.

Beyond immune regulation, IKBIP is also involved in the regulation of cell proliferation and apoptosis. Through the regulation of NF-κB activity, IKBIP can affect cell life and death and thus affect tumorigenesis and cancer progression. Altered expression of IKBIP has been observed in several types of cancers [19, 22]. Given that NF-κB signaling is frequently dysregulated in cancer and contributes to cancer progression, IKBIP is considered as a potential target in cancer research. However, the specific molecular mechanism by which IKBIP regulates the occurrence and development of glioma still needs to be clarified.

Therefore, this study aimed to investigate the role and underlying mechanism of IKBIP in glioma progression, particularly its regulation of the Wnt/β-catenin/EMT pathway and its potential as a prognostic biomarker and therapeutic target.

Materials and methods

Cell culture and transfection

Both human glioma cell lines and normal human astrocytes were cultured in high-glucose DMEM medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA). Cells were cultured at 37°C in a humidified incubator containing 5% CO_2. All cell lines were used within 10-15 passages to ensure experimental consistency and reliability. To knock down IKBIP or SP1, shRNAs provided by Jikai Gene (Shanghai, China) were used. The shRNAs were cloned into a lentiviral expression vector. Lentiviral particles were produced by co-transfecting the recombinant plasmids into 293T cells along with packaging plasmids. The harvested lentiviral particles were subsequently used to infect U251 and U87 glioma cells to establish stable knockdown cell lines. Next, infected cells were selected using puromycin. After 48 hours of transfection, the knockdown efficiency of the target genes was verified through quantitative real-time PCR (qRT-PCR) and Western blotting.

qRT-PCR assay

Total RNA was extracted from glioma cells using TRIzol reagent (Invitrogen, USA). The purity and

Table 1. The qRT-PCR primers used in the manuscript

Genes	Primers
SP1 Forward	5'-TGGCAGCAGTACCAATGGC-3'
SP1 Reverse	5'-CCAGGTAGTCCTGTCAGAACTT-3'
IKBIP Forward	5'-GCTCATCTAAAGCGTCTACAG-3'
IKBIP Reverse	5'-AAGCGTCGTCAGACTGTTGTT-3'
GAPDH Forward	5'-GGAGCGAGATCCCTCCAAAAT-3'
GAPDH Reverse	5'-GGCTGTTGTCATACTTCTCATGG-3'

Table 2. The qRT-PCR reaction system

Reagent	Volume	Final concentration
TB Green Premix Ex Taq II (Tli RNaseH Plus) (2X)	25 μL	1X
PCR Forward Primer (10 μM)	2 μL	0.4 μM
PCR Reverse Primer (10 μM)	2 μL	0.4 μM
ROX Reference Dye II (50X)	1 μL	1X
DNA Template	4 μL	200 ng
ddH_2O	16 μL	
Total	50 μL	

concentration of the extracted RNA were measured using a Nanodrop spectrophotometer to ensure suitability for downstream experiments. cDNAs was then synthesized using a reverse transcription kit (TaKaRa, Japan), which were used as a template for qRT-PCR.

qPCR was performed using TB Green (TaKaRa, Japan) on an Applied Biosystems 7500 real-time PCR system. GAPDH served as the reference gene, and relative gene expression levels were normalized to human GAPDH expression using the $2^{-\Delta\Delta Ct}$ method [23]. The qRT-PCR primers used in this study are listed in **Table 1**, and the reaction system is presented in **Table 2**. The reaction conditions were as follows: initial denaturation at 95°C for 30 s; 40 cycles of denaturation at 95°C for 5 s, and annealing/extension at 60°C for 34 s; and a final melting curve analysis. All qRT-PCR reactions were performed in triplicate.

CCK-8 assay

Cell proliferation was evaluated using a CCK-8 kit (Dojindo, CK04-500T, Japan). U251 and U87 cells were suspended in 200 μl DMEM supplemented with 10% FBS and seeded into a 96-well plate at a density of 1×10^3 cells, and cultured at 37°C with 5% CO_2. Cell proliferation was tested at 24, 48 and 72 h after seeding. At each time point, 10 μL of CCK-8 solution was added to each well, and the plates were incubated at 37°C for 2 hours. The absorbance at 490 nm was measured using a microplate reader to evaluate cell proliferation. Each experiment was repeated three times to ensure data reproducibility, and the obtained values were normalized.

Colony formation assay

For the colony formation experiment, 1×10^3 cells were inoculated into a 60-mm culture dish and incubated at 37°C in a 5% CO_2 incubator. After being cultured in DMEM containing 10% FBS for approximately 7-10 days, the clones were fixed and stained with 0.5% crystal violet staining solution (MCE, USA). The number of colonies was counted, and the data were collected for statistical analysis [24].

Cell migration and invasion assays

For the migration experiment, the cells were cultured in a serum-free DMEM for 24 hours prior to the experiment. A total of 600 uL of complete DMEM medium containing 10% FBS was added to the lower chamber of a 24-well plate. Subsequently, 5×10^4 starved cells were suspended in 100 uL of serum-free DMEM and seeded into the upper chamber (Corning Falcon, USA). The chamber was then cultured at 37°C in an incubator containing 5% CO_2 for 24 hours. The migrated cells on the lower surface of the membrane were fixed with 4% paraformaldehyde (MCE, USA) and stained with 0.5% crystal violet (MCE, USA). After washing with PBS and air-drying, the stained cells were observed and photographed under an Olympus IX73 inverted microscope. Three to five random microscopic fields were captured for each sample, and the number of migrated cells was quantified using ImageJ software. For the invasion experiment, the chamber were precoated with Matrigel before cell inoculation, and the other procedures were identical to those used in the migration assay.

In vivo tumourigenicity assay

Female BALB/C nude mice (weighting 20-22 g, aged 6 weeks) were used for tumor transplantation. To minimize animal suffering, mice were anesthetized by inhalation of isoflurane prior to tumor cell inoculation. Glioma cells were resuspended at a concentration of 1×10^7 cells in 100 μL of 0.9% sterile normal saline (Biosharp, China) and injected subcutaneously into the right axillary region of each mouse using a sterile 27G needle.

After injection, the mice were monitored closely for four weeks, and tumor size was measured on a weekly basis. At the end of the experiment, mice were deeply anesthetized with isoflurane and euthanized by cervical dislocation in accordance with ethical guidelines. Tumors were excised and weighed immediately. The tumor tissues were then subjected to histological processing, including fixation, paraffin embedding, sectioning, and immunohistochemical staining. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Guangdong Medical University (Approval No.: GDY2302676).

Immunohistochemistry (IHC)

Paraffin-embedded tumor tissues were deparaffinized, rehydrated, and subjected to antigen retrieval. After permeabilization and blocking, the sections were incubated overnight at 4°C with primary antibodies against Ki67 (1:8000, proteintech, China), PCNA (1:3000, proteintech, China), and β-catenin (1:10000, proteintech, China).

After reaction with HRP-Goat anti-Rabbit secondary antibodies (Ready to use, proteintech, China), immunoreactivity was visualized using a DAB staining kit (Aladdin, China). Finally, the sections were counterstained with hematoxylin, and digital images were captured using an Olympus IX73 inverted microscope for evaluation of immunohistochemical results.

Western blotting

Cells were lysed using M-PER™ reagent (Thermo Scientific, USA) supplemented with a protease inhibitor cocktail (Thermo Scientific, USA). The lysates were then centrifuged, and the supernatants were collected for protein extraction. Protein concentrations were then measured using a BCA protein assay kit (Thermo Scientific, USA).

Equal amounts of proteins were separated by SDS-PAGE and subsequently transferred onto nitrocellulose membranes. The membranes were then blocked to prevent non-specific binding, and then incubated overnight with primary antibodies: anti-SP1 (1:1000, Abcam, UK), anti-IKBIP (1:1000, Abcam, UK), anti-ZEB2 (1:2000, Proteintech, China), anti-ZEB1 (1:1000, Proteintech, China), anti-β-catenin (1:1000, Cell Signaling Technology, USA), anti-E-cadherin (1:1000, Santa Cruz Biotechnology, USA), anti-N-cadherin (1:1000, Cell Signaling Technology, USA), anti-Slug (1:1000, Cell Signaling Technology, USA), anti-Snail (1:1000, Cell Signaling Technology, USA), Phospho-Beta Catenin (Ser33) (1:5000, Proteintech, China) and anti-β-actin (1:10000, Proteintech, China). After incubation with HRP conjugated secondary antibodies (1:10000, Proteintech, China), protein bands were visualized using an enhanced chemiluminescence detection system. The band intensities was quantified, and the expression levels of target proteins were normalized to β-actin, which served as the internal loading control.

Chromatin immunoprecipitation (ChIP) analysis

ChIP assays were performed using a Millipore EZ ChIP analysis kit (Kaihong, Shandong, China). Glioma U251 or U87 cells ($\sim1\times10^7$) were treated with 1% formaldehyde at 27°C for 15 minutes. Glycine (125 mM) was added to terminate the crosslinking reaction. Then, the cells were washed twice with pre-cooled PBS and lysed in lysis buffer. The cells were then incubated with anti-SP1 (Abcam, USA) and IgG (Abcam, USA) at 4°C overnight, followed by the addition of Protein A/G agarose (Thermo Scientific, USA) and incubation at 65°C for 6 hours. Proteinase K (Thermo Scientific, USA) was added to digest chromatin proteins and antibodies. DNA was purified using the DNA purification kit (TIANGEN, China). qRT-PCR was performed using SYBR Green dye (Promega, USA) to assess SP1 binding to the IKBIP promoter regions. IKBIP primer sequences were forward: 5'-GCCCGCTTATGCAAATAAACAC-3', reverse: 5'-CATCCTCGTTGCTTCACTGAG-3'.

Immunofluorescence assay

Cells were seeded onto glass coverslips and cultured until approximately 70% confluence. The cells were washed three times with PBS and fixed with 4% paraformaldehyde for 20 minutes. After fixation, the cells were washed three times with PBS and permeabilized with 0.2% Triton X-100 at room temperature for 5 minutes. The slides were then blocked with 3% BSA for 1 hour followed by incubation with primary antibody (β-catenin, 1:500; Proteintech, China) for 2 hours at room temperature. After washing with PBS, the cells were incubated with the corresponding fluorophore-conjugated secondary antibody (1:800, Proteintech, China) at room temperature for 1 hour. Finally, the slides were mounted using mounting medium containing DAPI to stain the nuclei. Fluorescence images were observed using Olympus IX73 fluorescence inverted microscope.

Data acquisition

RNA expression data and corresponding clinical information were acquired from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases through systematic data downloads. Additionally, glioma-related datasets from the Chinese Glioma Genome Atlas (CGGA) were retrieved and analyzed using its online platform.

Bioinformatics analysis

Gene expression data and related clinical information, including survival time, survival status, and clinicopathological parameters, were analyzed using R software. Receiver operating characteristic (ROC) curve analysis was initially performed to evaluate the predictive performance of IKBIP expression for patient survival, with the optimal cutoff values determined. Kaplan-Meier survival analysis was then conducted to assess the association between IKBIP expression and patient survival outcomes. Survival curves were generated using the survival and survminer R packages to compare survival probabilities between groups with different levels of IKBIP expression. Univariate Cox proportional hazards regression was applied to evaluate the association between IKBIP expression and overall survival (OS). Subsequently, multivariate Cox regression analysis was performed to determine whether IKBIP expression served as an independent prognostic factor after adjusting for other clinical variables. In addition, ROC curves were generated using the survival ROC R package to evaluate the prognostic value of IKBIP expression for predicting 1-, 3-, and 5-year survival outcomes.

Statistical analysis

All statistical analyses were performed using GraphPad Prism and R software. Data were presented as mean ± standard deviation (SD). Comparisons between two groups were performed using two-tailed Student's t-tests. Differences among multiple groups were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc tests.

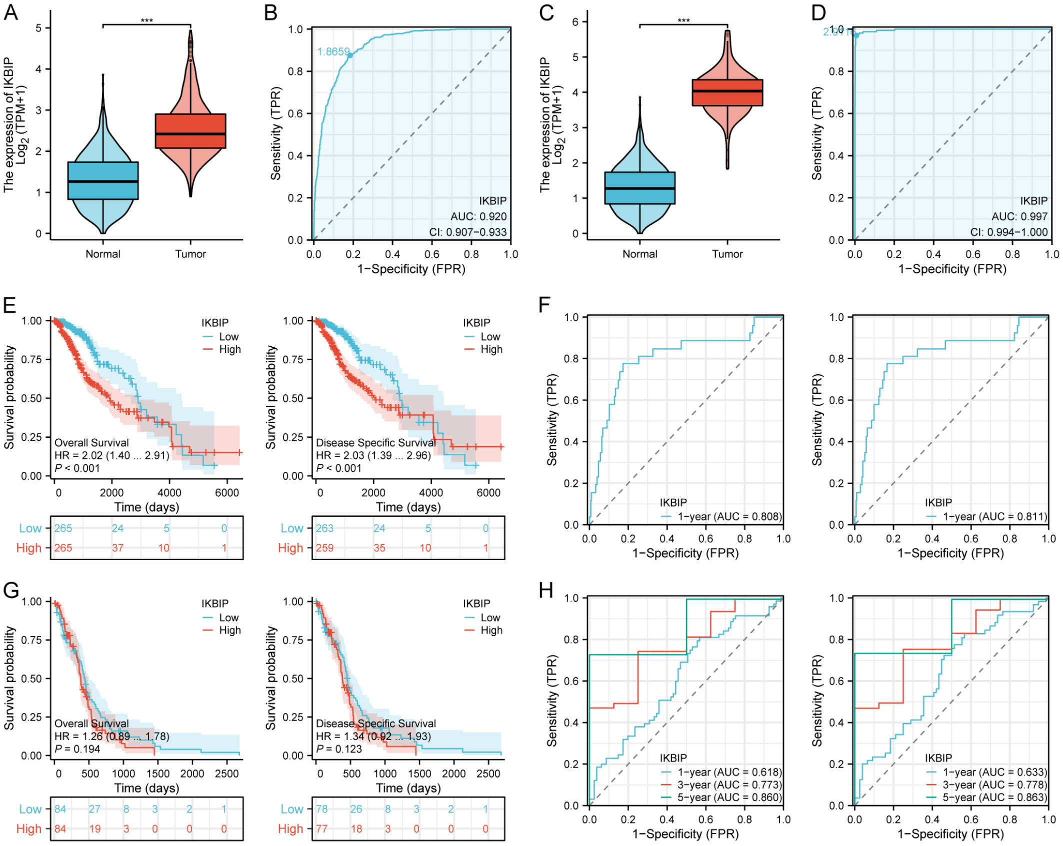
Survival analyses were conducted using the Kaplan-Meier method, and differences in survival curves were evaluated using the log-rank test. Univariate and multivariate Cox proportional hazards regression analyses were performed to identify independent prognostic factors for OS. For experiments involving measurements at multiple time points, repeated-measures ANOVA was applied. A p value < 0.05 was considered statistically significant.

Results

IKBIP is upregulated in glioma tissues and associated with poor prognosis

Bioinformatics analyses of datasets from TCGA and GTEx revealed that the expression level of IKBIP was significantly higher in both LGG and GBM samples, compared to normal brain tissues (**Figure 1A**, **1C**). ROC curve analysis was performed to evaluate the prognostic value of IKBIP expression, demonstrating that IKBIP exhibit favorable predictive performance for survival in both LGG and GBM patients, as reflected by relatively high areas under the curve (AUCs) (**Figure 1B**, **1D**).

Kaplan-Meier survival analysis revealed that higher IKBIP expression was significantly associated with shorter OS and DSS in LGG patients (**Figure 1E**). The time-dependent ROC curve



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Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Gender	530				
Female	238	Reference			
Male	292	1.112 (0.791 - 1.563)	0.542		
Age	530				
<= 40	265	Reference		Reference	
> 40	265	2.898 (2.015 - 4.168)	< 0.001	3.015 (2.052 - 4.429)	< 0.001
IDH status	527				
WT	97	Reference		Reference	
Mut	430	0.184 (0.129 - 0.263)	< 0.001	0.287 (0.190 - 0.432)	< 0.001
1p/19q codeletion	530				
Non-codel	359	Reference		Reference	
Codel	171	0.401 (0.256 - 0.629)	< 0.001	0.493 (0.302 - 0.806)	0.005
IKBIP	530				
Low	265	Reference		Reference	
High	265	2.021 (1.405 - 2.906)	< 0.001	1.446 (0.978 - 2.138)	0.064

Figure 1. IKBIP expression and prognostic significance in gliomas. (A, C) IKBIP is significantly upregulated in LGG (A) and GBM (C) tissues compared to normal brain tissues, as shown by TCGA and GTEx data. (B, D) ROC curve analyses demonstrate high AUC values for IKBIP in distinguishing LGG (B) and GBM (D) from normal tissues. (E, G) Kaplan-Meier survival curves reveal that high IKBIP expression correlates with poorer OS and DSS in LGG patients (E) but not in GBM patients (G). (F, H) Time-ROC curves confirm IKBIP's strong prognostic capability for LGG (F) but not GBM (H). (I) High IKBIP expression in LGG is associated with poor OS, though it does not emerge as an independent prognostic factor in multivariate analysis (P=0.064). Data are expressed as mean ± SD. ***P* < 0.01, ****P* < 0.001, vs. the control group.

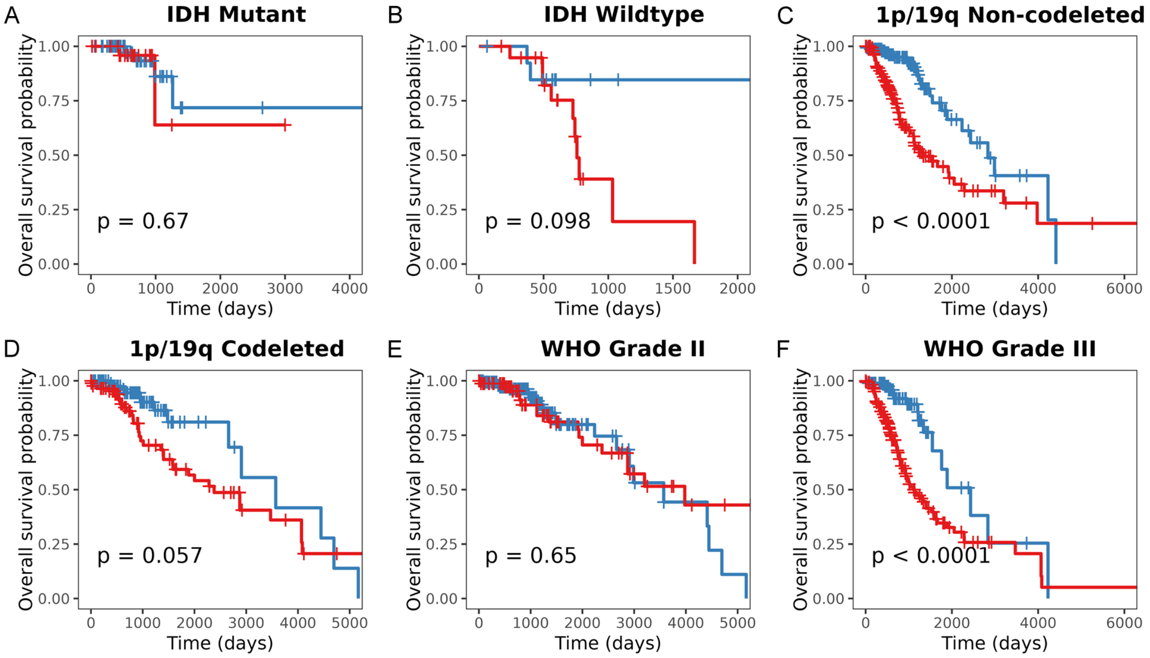


Figure 2. Stratified Kaplan-Meier survival analyses of IKBIP expression in TCGA-LGG cohort. Kaplan-Meier curves showing overall survival of TCGA-LGG patients stratified by IKBIP expression (High vs. Low, median cutoff) within molecular and histological subgroups. A. IDH-mutant tumors (n=91). B. IDH-wildtype tumors (n=34). C. 1p/19q non-codeleted tumors (n=330). D. 1p/19q codeleted tumors (n=195). E. WHO grade II gliomas (n=255). F. WHO grade III gliomas (n=269). IDH, isocitrate dehydrogenase; LGG, lower-grade glioma; WHO, World Health Organization.

analysis further confirmed the prognostic value of IKBIP expression in predicting survival outcomes in LGG patient (**Figure 1F**). However, these associations were not observed in GBM patients (**Figure 1G**, **1H**).

Univariate Cox analysis further demonstrated that elevated IKBIP expression was significantly associated with poorer OS in LGG patients (*P* < 0.001); however, in the multivariate Cox regression model, the association did not remain statistically significant (*P*=0.064) (**Figure 1I**). To further clarify this discrepancy, stratified survival analyses were conducted in the TCGA-LGG cohort according to isocitrate dehydrogenase (*IDH*) mutation status, 1p/19q codeletion status, and WHO grade (**Figure 2**). High IKBIP expression was significantly associated with shorter OS in *IDH*-wildtype tumors (P=0.021), 1p/19q non-codeleted tumors (P=0.002), and WHO grade III gliomas (P=0.007), whereas no significant association was observed in *IDH*-mutant tumors (P=0.1), 1p/19q codeleted tumors (P=0.27), or WHO grade II tumors (P=0.68). These findings indicate that the prognostic impact of IKBIP expression in TCGA-LGG is concentrated in molecularly aggressive subgroups, including IDH-wildtype, non-codeleted, and high-grade tumors. Therefore, the borderline significance observed in the overall multivariate analysis (P=0.064) may reflect confounding effects by these established prognostic markers rather than an absence of biological relevance.

Further validation using the CGGA database revealed consistent findings. Glioma patients with low IKBIP expression had significantly better OS than those with high expression (**Figure 3A**). Time-dependent ROC curve analysis showed that IKBIP expression possessed moderate prognostic predictive ability, with AUC values of 0.791, 0.781, and 0.710 for predicting 1-, 3-, and 5-year survival, respectively (**Figure 3B**). Both univariate and multivariate Cox regression analyses confirmed that IKBIP was an independent prognostic factor for OS in glioma patients (**Figure 3C**, **3D**). Furthermore, IKBIP expression was closely associated with several key clinicopathological characteristics, including age, primary-recurrent-secondary (PRS) type, tumor grade, 1p19q codeletion status, chemotherapy status, *IDH* mutation status, and histological subtype of glioma (**Figure 3E**). These results indicate that the prognostic significance of IKBIP expression in gliomas may be influenced by various clinical and molecular factors.

IKBIP knockdown inhibited the proliferation of glioma cells both in vitro and in vivo

We first examined the expression levels of IKBIP in several glioma cell lines (U87, U251, LN229, TJ905, and LN308) and normal human astrocytes (HEB cells). qRT-PCR and Western blot assays revealed that IKBIP expression was significantly higher in glioma cell lines compared with HEB cells (**Figure 4A**). To further investigate the biological function of IKBIP in glioma, stable IKBIP-knockdown cell lines were established in U251 and U87 cells. The knockdown efficiency was verified using qRT-PCR and Western blot assay, which showed that IKBIP expression at both mRNA and protein levels was reduced by more than 60% (**Figure 4B**).

CCK-8 and colony formation assays revealed that IKBIP knockdown significantly inhibited cell viability of U251 and U87 cells, reflected by reduced cell proliferation and colony formation (**Figure 4C**, **4D**). These results indicate that IKBIP plays a significant role in glioma cell proliferation.

To investigate the effect of IKBIP on tumor growth *in vivo*, a subcutaneous transplanted tumor model was established in mice using U87 cells with stable IKBIP knockdown. Tumors in the IKBIP knockdown group exhibited a significantly slower growth rate compared with those in the control group, and both tumor volume and weight were significantly reduced at endpoint (**Figure 5**).

In addition, IHC staining showed that the expression of Ki-67 and PCNA was significantly reduced in tumor tissues of IKBIP-knockdown group, and IKBIP knockdown was associated with reduced nuclear localization of β-catenin (**Figure 6**). These results indicate that IKBIP knockdown significantly inhibits the proliferation of glioma both *in vitro* and *in vivo*, suggesting that it plays an important role in the progression of glioma.

IKBIP knockdown inhibited glioma invasion via Wnt/β-catenin/EMT pathway

To further investigate the role of IKBIP in glioma cell invasion, Transwell migration and invasion assays were conducted, showing that IKBIP knockdown significantly inhibited the migration and invasion abilities of glioma U251 and U87 cells (**Figure 7A**, **7B**).

To investigate the specific molecular mechanism underlying this inhibitory effect, we examined the expression of key proteins associated with the Wnt/β-catenin signaling pathway and epithelial-mesenchymal transition (EMT). IKBIP knockdown resulted in decreased expression N-cadherin, β-catenin, ZEB1, ZEB2, Snail, and Slug. In contrast, E-cadherin expression was notably increased (**Figure 7C**). These findings indicate that IKBIP knockdown inhibits glioma cell proliferation and invasion by inhibiting activation of the Wnt/β-catenin pathway and reversing EMT process.

SP1 knockdown suppressed IKBIP expression and inhibited the proliferation, migration and invasion of glioma cells

PCR and WB analyses revealed that, compared with normal astrocytes, SP1 expressed was significantly increased in glioma cells (**Figure 8A**). SP1 knockdown significantly suppressed the expression of IKBIP in U251 and U87 glioma cells (**Figure 8B**). In addition, SP1 knockdown markedly decreased the clonogenic capacity, migration and invasion ability of U251 and U87 glioma cells (**Figure 8C-E**).

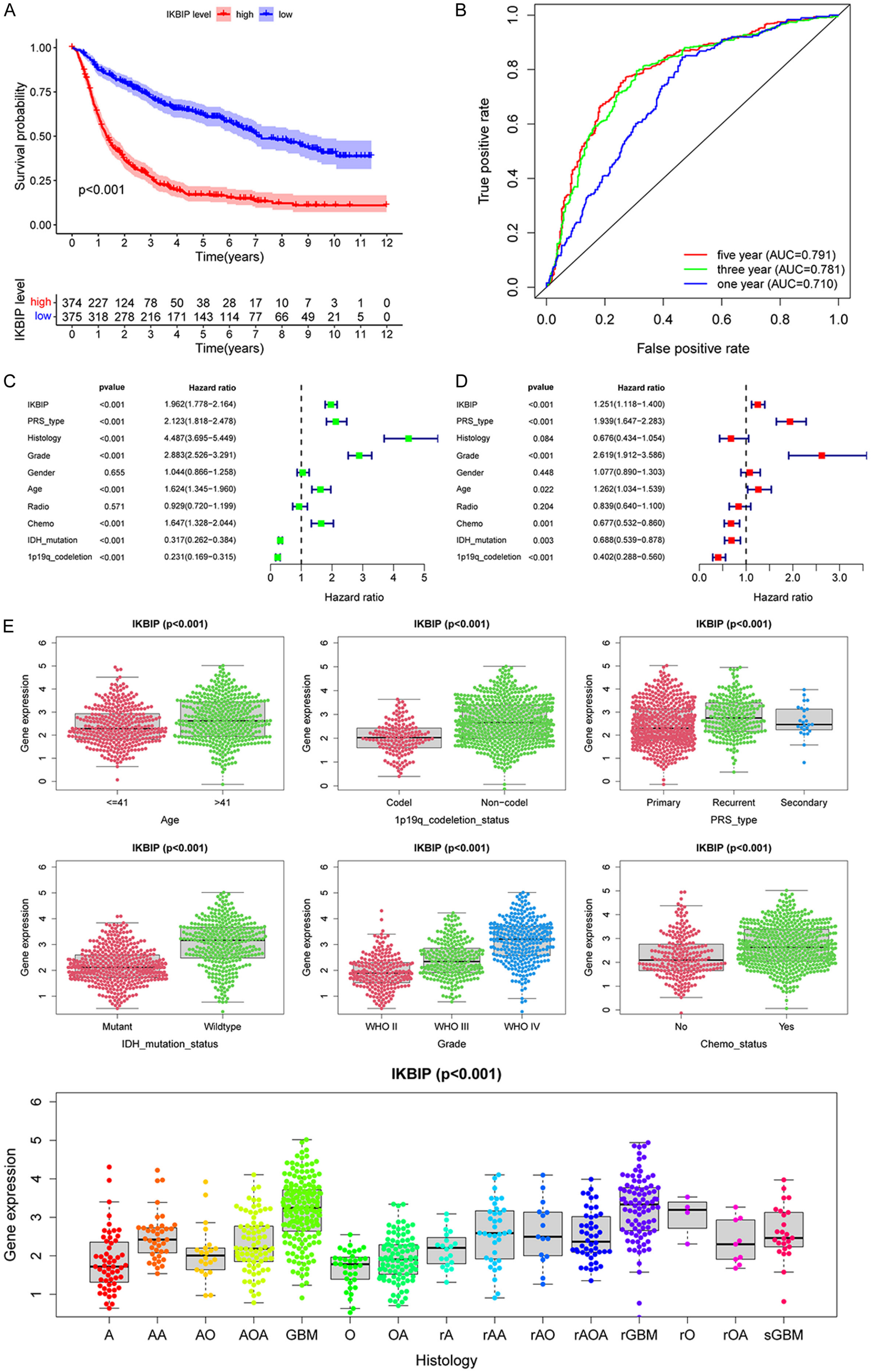

A
IKBIP level
high
low
Survival probability
1.00
0.75
0.50
0.25
0.00
p<0.001
0 1 2 3 4 5 6 7 8 9 10 11 12
Time(years)
IKBIP level
high 374 227 124 78 50 38 28 17 10 7 3 1 0
low 375 318 278 216 171 143 114 77 66 49 21 5 0
Time(years)
B
True positive rate
False positive rate
0.0 0.2 0.4 0.6 0.8 1.0
five year (AUC=0.791)
three year (AUC=0.781)
one year (AUC=0.710)
C
pvalue
Hazard ratio
IKBIP <0.001 1.962(1.778−2.164)
PRS_type <0.001 2.123(1.818−2.478)
Histology <0.001 4.487(3.695−5.449)
Grade <0.001 2.883(2.526−3.291)
Gender 0.655 1.044(0.866−1.258)
Age <0.001 1.624(1.345−1.960)
Radio 0.571 0.929(0.720−1.199)
Chemo <0.001 1.647(1.328−2.044)
IDH_mutation <0.001 0.317(0.262−0.384)
1p19q_codeletion <0.001 0.231(0.169−0.315)
0 1 2 3 4 5
Hazard ratio
D
pvalue
Hazard ratio
IKBIP <0.001 1.251(1.118−1.400)
PRS_type <0.001 1.939(1.647−2.283)
Histology 0.084 0.676(0.434−1.054)
Grade <0.001 2.619(1.912−3.586)
Gender 0.448 1.077(0.890−1.303)
Age 0.022 1.262(1.034−1.539)
Radio 0.204 0.839(0.640−1.100)
Chemo 0.001 0.677(0.532−0.860)
IDH_mutation 0.003 0.688(0.539−0.878)
1p19q_codeletion <0.001 0.402(0.288−0.560)
0.0 1.0 2.0 3.0
Hazard ratio
E
IKBIP (p<0.001)
Gene expression
<=41
>41
Age
IKBIP (p<0.001)
Codel
Non−codel
1p19q_codeletion_status
IKBIP (p<0.001)
Primary
Recurrent
Secondary
PRS_type
IKBIP (p<0.001)
Mutant
Wildtype
IDH_mutation_status
IKBIP (p<0.001)
WHO II
WHO III
WHO IV
Grade
IKBIP (p<0.001)
No
Yes
Chemo_status
IKBIP (p<0.001)
A AA AO AOA GBM O OA rA rAA rAO rAOA rGBM rO rOA sGBM
Histology

Figure 3. Prognostic value of IKBIP in gliomas based on the CGGA cohort. A. Low IKBIP expression is associated with better OS in glioma patients, whereas high expression correlates with worse prognosis. B. Time-dependent ROC curve analysis demonstrates the predictive performance of IKBIP for survival, with AUCs of 0.791, 0.781, and 0.710 at 1, 3, and 5 years, respectively. C, D. Univariate and multivariate Cox regression analyses reveal that IKBIP serves as an independent predictive marker for OS in glioma patients. E. The correlation of IKBIP in glioma with age, primary-recurrent-secondary (PRS) type, tumor grade, 1p19q codeletion status, chemotherapy status, IDH mutation status, and histological subtype of glioma.

SP1 bound to the promoter region of IKBIP and promoted its expression

Bioinformatics analysis of the TCGA database showed that SP1 expression was significantly higher in glioma tissues than in normal brain tissues (**Figure 9A**, **9B**). Elevated SP1 expression was strongly associated with poorer 5-year OS and DSS in LGG patients (**Figure 9C**). Furthermore, both univariate and multivariate Cox regression analyses indicated that high SP1 expression was significantly associated with unfavorable prognosis in LGG patients (**Figure 9D**).

To identify potential SP1 binding sites within the IKBIP promoter region, we performed transcription factor binding prediction using the JASPAR database. A high-scoring SP1 binding motif (TTAGCCACGCCCCGT) was identified within the IKBIP promoter region (-1680 to -1666, positive strand) (**Figure 10A**, **10C**). Pearson correlation analysis revealed a significant positive correlation between IKBIP and SP1 expression in gliomas (**Figure 10B**). Additionally, ChIP assays showed that SP1 directly bound to the promoter region of IKBIP (**Figure 10D**).

To further explore the molecular mechanism by which SP1 regulates IKBIP expression, as well as the biological role of the SP1-IKBIP axis in the development of glioma, stable SP1-overexpressing U251 and U87 cell lines were established. SP1 overexpression significantly increased IKBIP expression in both cell lines (**Figure 11A**, **11B**) and decreased the phosphorylation level of β-catenin (**Figure 11B**), suggesting activation of the Wnt/β-catenin signaling pathway. Functional assays further demonstrated that overexpression of SP1 significantly enhanced the proliferation, migration, and invasion of glioma cells (**Figure 11C-E**).

SP1 overexpression promoted the nuclear translocation of β-catenin and activated the Wnt/β-catenin/EMT pathway

Through the WB experiment, we found that when SP1 and IKBIP were overexpressed, β-catenin was overexpressed in glioma cells, while the expression of p-β-catenin decreased (**Figure 11B**). It was speculated that the overexpression of SP1 and IKBIP inhibited the degradation of β-catenin and subsequently promoted its nuclear translocation to activate the Wnt/β-catenin/EMT pathway, thereby promoting the proliferation and invasive phenotype of glioma cells.

To further verify this hypothesis, immunofluorescence staining was performed, and the results showed that overexpression of SP1 significantly enhanced the nuclear accumulation of β-catenin in glioma cells (**Figure 12**). These findings confirmed our previous hypothesis that SP1 overexpression indeed activated the Wnt/β-catenin/EMT pathway by promoting the nuclear translocation of β-catenin, thereby promoting the proliferation and invasive phenotype of glioma cells.

Pro-tumorigenic effects of SP1 are functionally dependent on IKBIP

To further determine whether the tumor-promoting effect of SP1 was mediated through the regulation of IKBIP expression, we constructed stable U251 and U87 cell lines with simultaneous overexpression of SP1 and knockdown of IKBIP (**Figure 13A**). SP1 overexpression alone significantly enhanced the proliferation and invasion of glioma cells (**Figure 11C-E**), which were notably attenuated by concurrent SP1 overexpression and IKBIP knockdown (**Figure 13B-D**). These observations suggest that the pro-tumorigenic effect of SP1 was counteracted by IKBIP knockdown, supporting that the oncogenic role of SP1 is functionally dependent on IKBIP.

Overexpression of IKBIP promoted glioma proliferation, migration and invasion by activating the Wnt/β-catenin/EMT pathway

To further evaluate the role of IKBIP in glioma progression, we constructed U251 and U87 cell lines with stable IKBIP overexpression (**Figure 14A**). When IKBIP is overexpressed, the

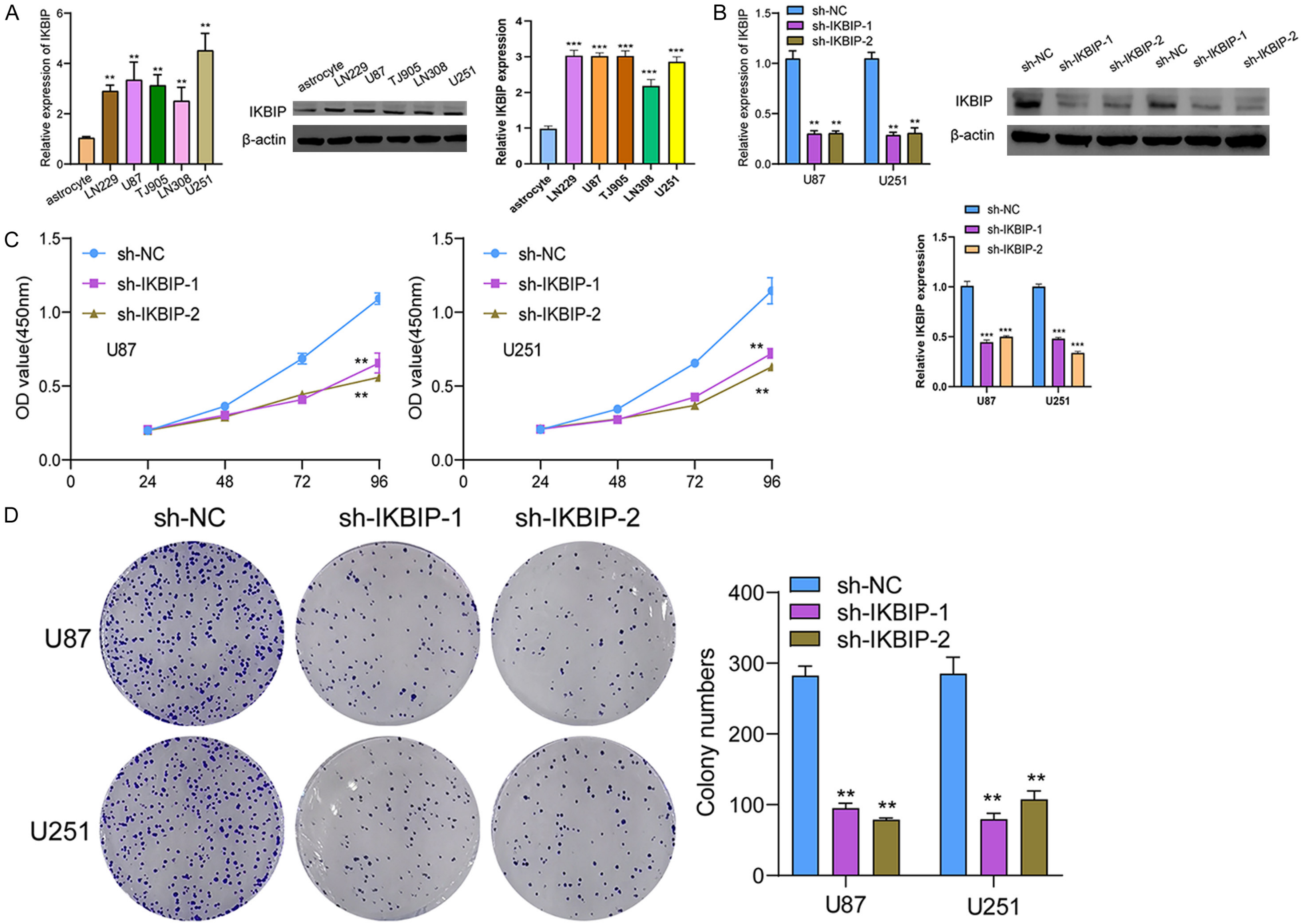


Figure 4. Effect of IKBIP knockdown on glioma cell proliferation. A. qRT-PCR and western blot analysis showing IKBIP expression levels in glioma cell lines (U87, U251, A172, LN308, HEB) and normal astrocytes. B. qRT-PCR and western blot analysis confirming the efficient IKBIP knockdown in U87 and U251 cells. C, D. CCK-8 and clonogenic assays showing the effect of IKBIP knockdown on cell proliferation in U251 and U87 cells. Data are expressed as mean ± SD. ***P* < 0.01, ****P* < 0.001, vs. the control group.

Figure 5. Impact of IKBIP knockdown on glioma tumor growth *in vivo*. A. The subcutaneously transplanted tumors in nude mice; B. IKBIP knockdown significantly inhibited the tumor-forming ability of glioma cells *in vivo.* Data are expressed as mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the control group.

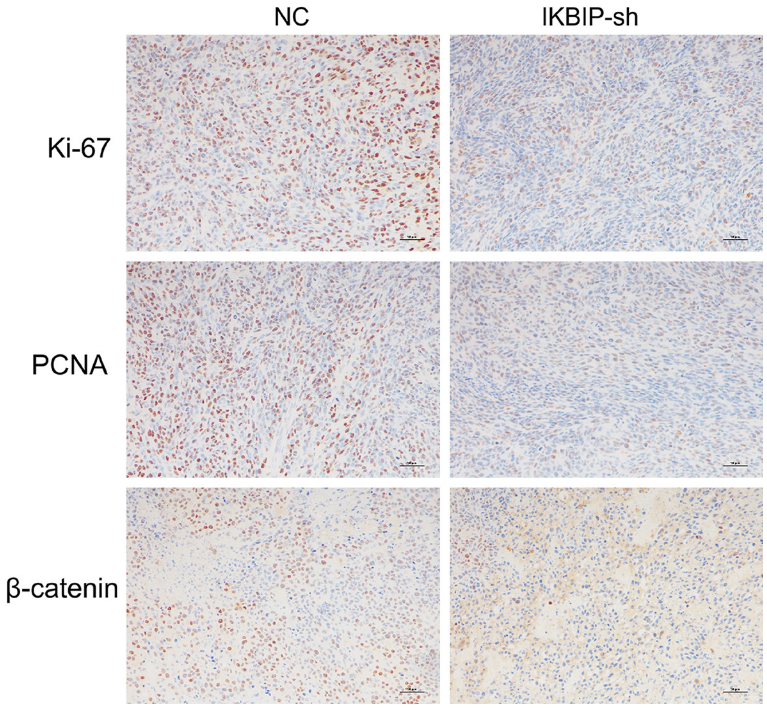


Figure 6. Immunohistochemical analysis of Ki67, PCNA and β-catenin expression in tumor tissues of mice in the IKBIP knockdown group and the control group (scale bar: 50 μm, Magnification 100×).

expressions of β-catenin and N-cadherin significantly increase, while the expressions of P-β-catenin and E-cadherin significantly decrease (**Figure 14B**). This indicates that the overexpression of IKBIP can enhance the expression of β-catenin, which is presumably achieved by inhibiting its phosphorylation and degradation. Additionally, the overexpression of IKBIP can significantly promote the EMT process. Functional assays demonstrated that IKBIP overexpression significantly enhanced glioma cell proliferation, colony formation, migration, and invasion (**Figure 14C-E**). IKBIP overexpression also promoted the nuclear translocation of β-catenin (**Figure 15**). These findings suggest that overexpression of IKBIP activates the Wnt/β-catenin pathway, thereby promoting the EMT process, and subsequently facilitating the proliferation and invasive growth of glioma cells.

Discussion

Glioma is a malignant intracranial tumor that poses a significant threat to human health, especially high-grade gliomas, which are associated with poor clinical outcomes. The prognosis of glioma patients is influenced by multiple factors, including pathological grade and molecular characteristics [25, 26]. Key prognostic markers of glioma include *IDH* mutations, 1p/19q co-deletion, O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, *TP53* mutations, and tumor grade. *IDH* mutations are generally associated with a more favorable prognosis; however, not all patients harbor IDH mutations [27, 28]. TP53 mutations have been associated with increased tumor invasiveness and drug resistance, although their prognostic value may vary across different subtypes [29]. Among these factors, tumor pathological grade remains the most direct prognostic indicator, and high-grade gliomas usually have a poorer prognosis [30, 31]. Therefore, it is urgent to clarify the molecular mechanism underlying glioma pathogenesis and search for new diagnostic and therapeutic targets.

IKBIP modulates the NF-κB signaling pathway by interacting with NF-κB dimers and blocking

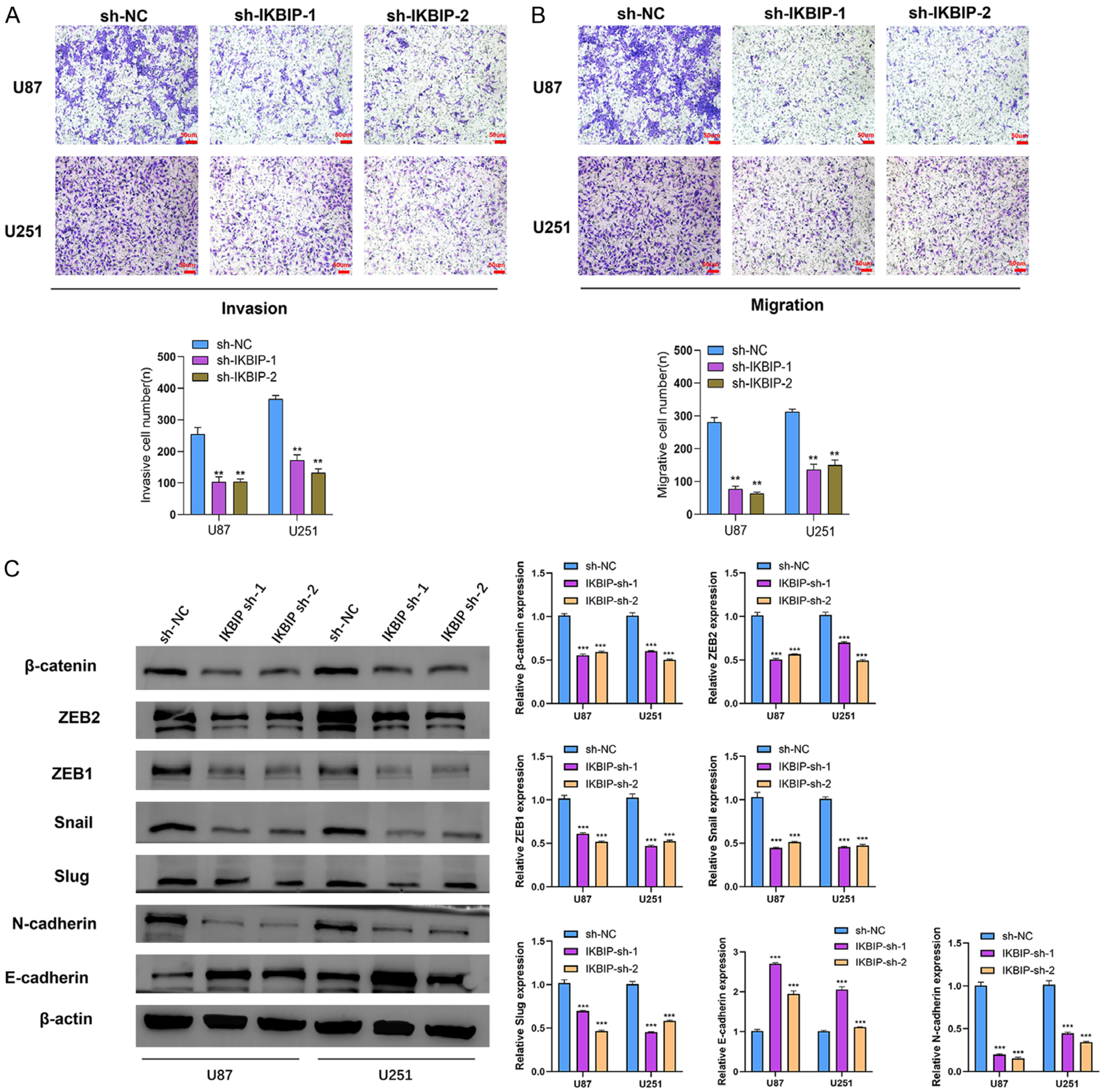


Figure 7. Effect of IKBIP knockdown on glioma cell migration, invasion, and EMT-related protein expression. A, B. Transwell assays showing that IKBIP knockdown significantly inhibited cell migratory and invasive abilities (scale bar: 50 μm, Magnification 100×). C. Western blot analysis showing the expression levels of N-cadherin, β-catenin, ZEB1, ZEB2, Snail, and Slug after IKBIP knockdown. Data are expressed as mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$, vs. the control group.

their translocation to the nucleus [32, 33]. The NF-κB pathway plays a critical role in regulating cell survival and proliferation, and its dysregulation due to aberrant IKBIP expression may contribute to tumor occurrence and progression. This mechanism highlights the importance of IKBIP in the pathological process of cancer [34, 35]. Previous studies have suggested that IKBIP overexpression can enhance NF-κB activity, thereby promoting cell proliferation and inhibiting cell apoptosis. For instance, Bi et al. identified IKBIP as a key regulator of GBM progression, with higher expression correlating with advanced tumor grade and poor prognosis [19]. In addition, IKBIP has been reported to interact with CDK4, preventing its degradation and enhancing its stability, thereby promoting glioblastoma cell proliferation [22]. Consistently, studies have reported that IKBIP is upregulated in gliomas and is correlated with unfavorable prognostic outcomes [19, 20, 36, 37]. Yang *et al.* reported, based on analysis of TCGA and CGGA databases, that IKBIP was associated with more aggressive glioma phe-

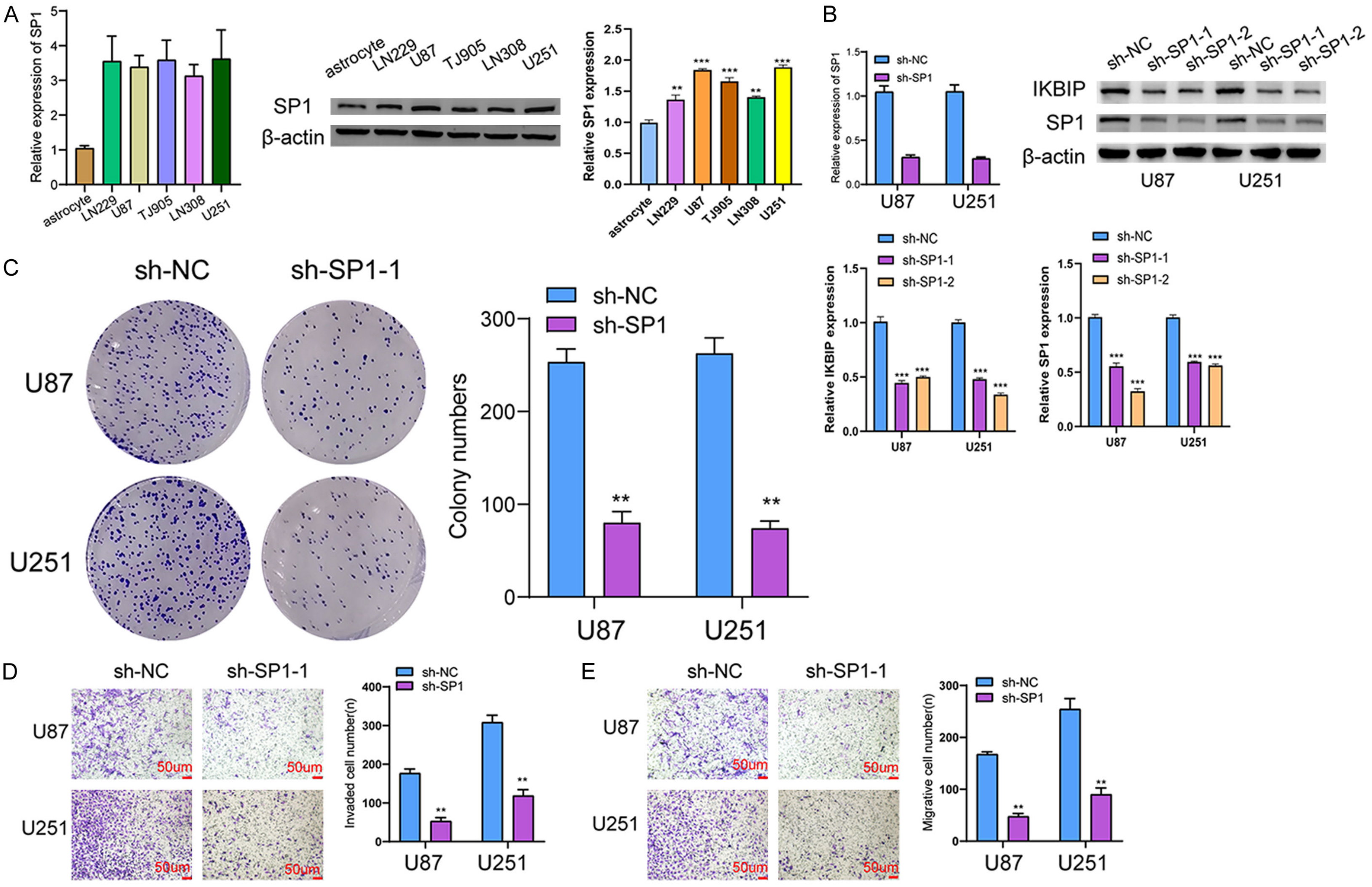
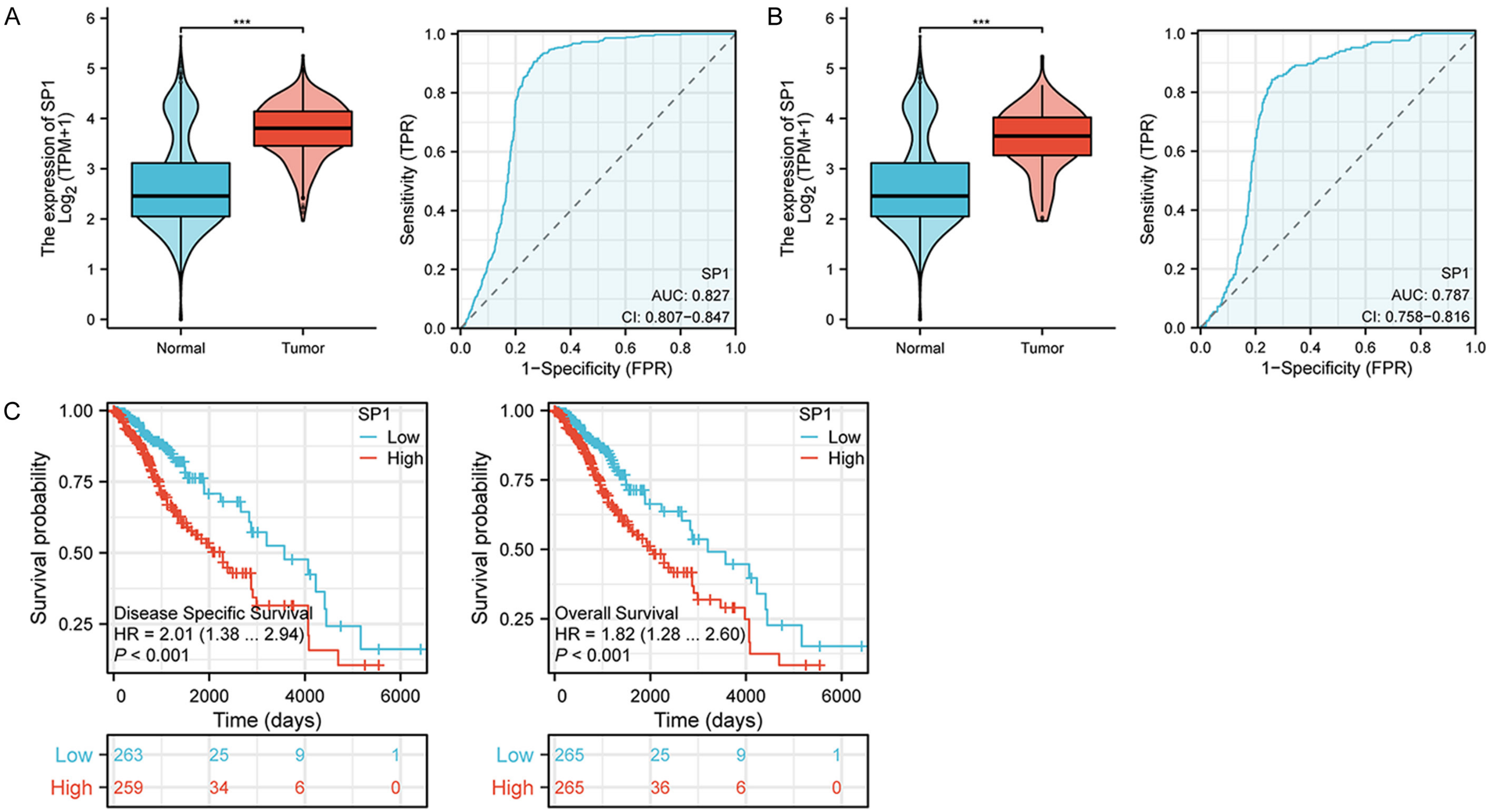
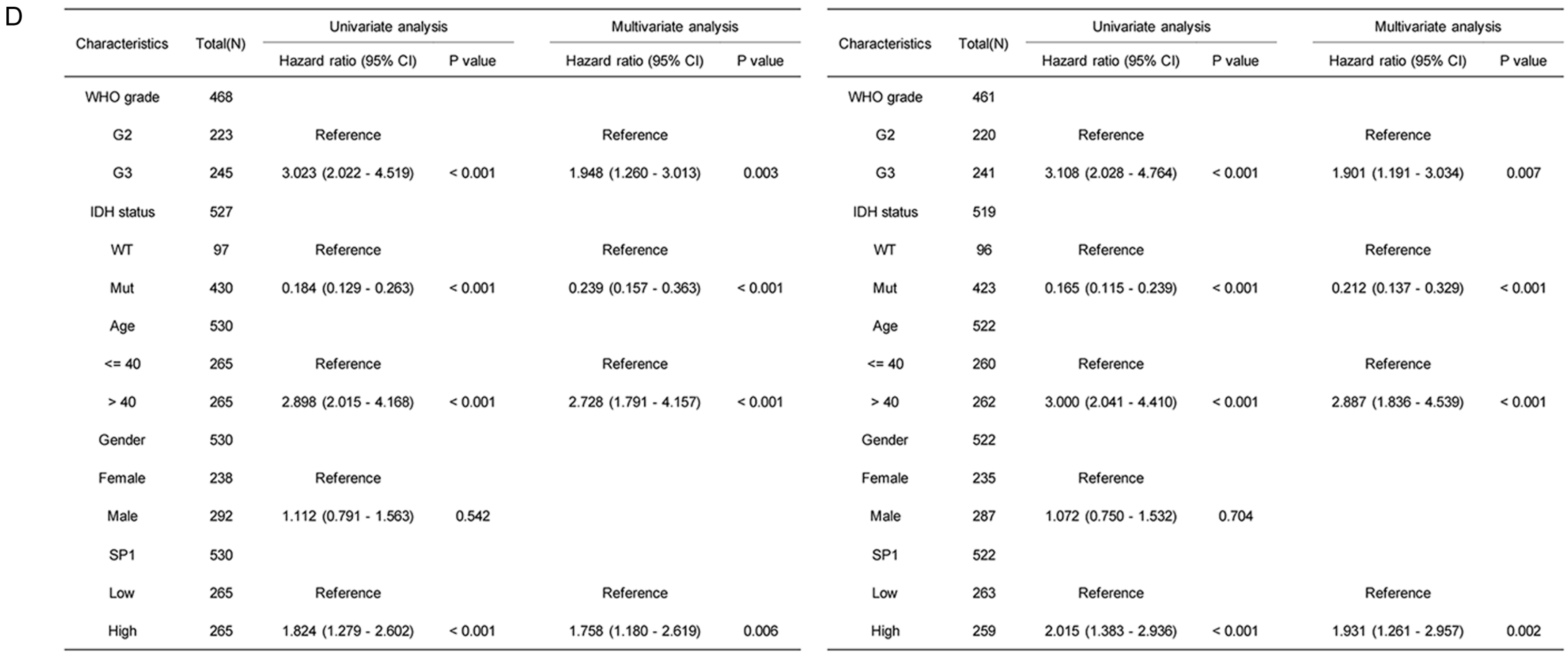


Figure 8. Effect of SP1 knockdown on IKBIP expression and glioma cell behavior. A. qRT-PCR and western blot analysis showing SP1 expression in glioma cell lines (U87, U251, A172, LN308, HEB) and normal controls. B. qRT-PCR and western blot analysis confirming the efficiency of SP1 knockdown in U87 and U251 cells. C. Colony formation assays showing that SP1 knockdown significantly inhibited cell proliferation in U251 and U87 cells. D, E. Transwell assays showing that SP1 knockdown significantly suppressed cell invasion and migration in U251 and U87 cells (scale bar: 50 μm, Magnification 100×). Data are expressed as mean ± SD. **$P < 0.01$, ***$P < 0.001$, vs. the control group.

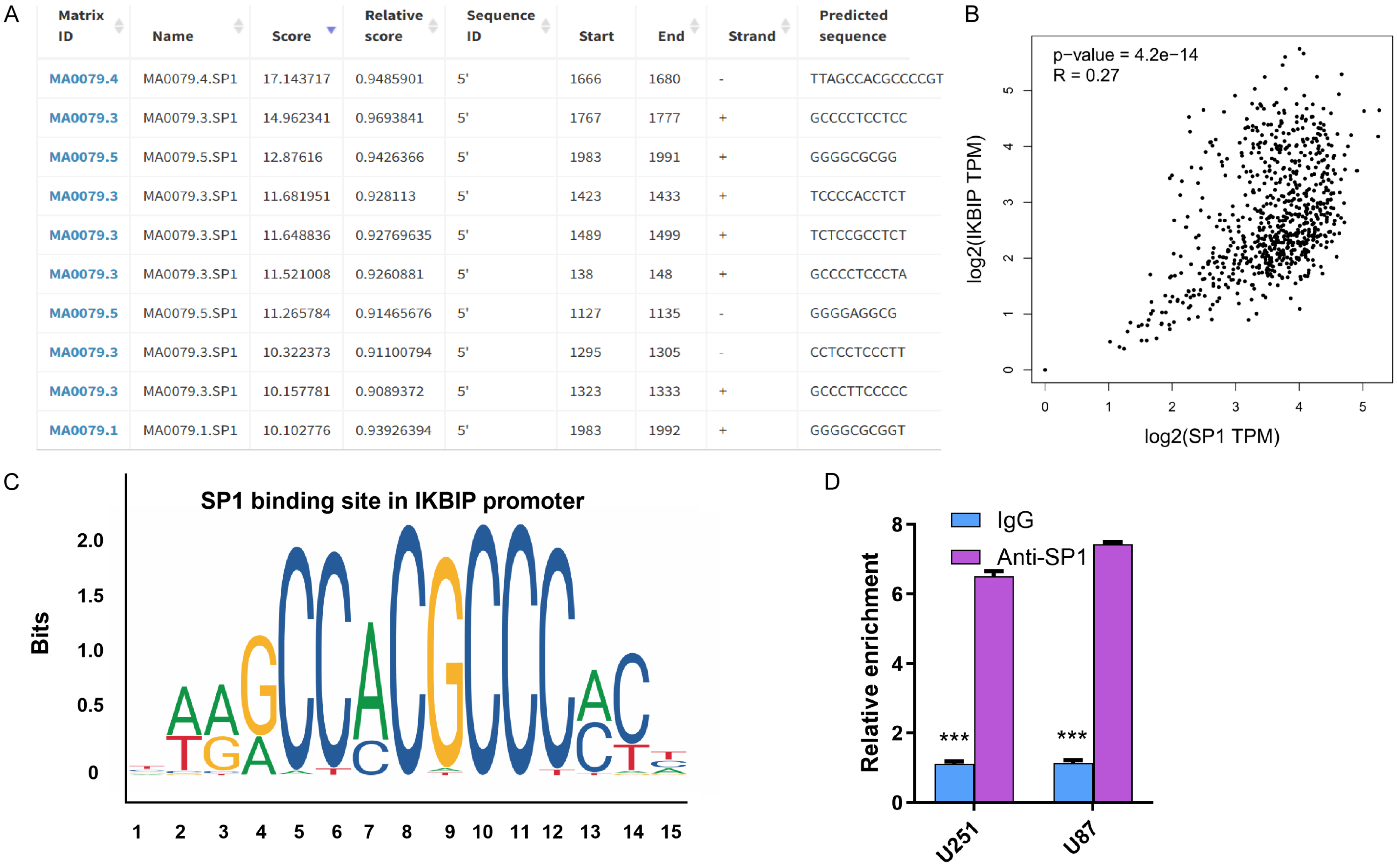

A
The expression of SP1 Log2 (TPM+1)
***
Normal
Tumor
Sensitivity (TPR)
1−Specificity (FPR)
SP1
AUC: 0.827
CI: 0.807−0.847
B
The expression of SP1 Log2 (TPM+1)
***
Normal
Tumor
Sensitivity (TPR)
1−Specificity (FPR)
SP1
AUC: 0.787
CI: 0.758−0.816
C
Survival probability
SP1
Low
High
Disease Specific Survival
HR = 2.01 (1.38 ... 2.94)
P < 0.001
Time (days)
Low 263 25 9 1
High 259 34 6 0
Survival probability
SP1
Low
High
Overall Survival
HR = 1.82 (1.28 ... 2.60)
P < 0.001
Time (days)
Low 265 25 9 1
High 265 36 6 0



Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
WHO grade	468				
G2	223	Reference		Reference	
G3	245	3.023 (2.022 - 4.519)	< 0.001	1.948 (1.260 - 3.013)	0.003
IDH status	527				
WT	97	Reference		Reference	
Mut	430	0.184 (0.129 - 0.263)	< 0.001	0.239 (0.157 - 0.363)	< 0.001
Age	530				
<= 40	265	Reference		Reference	
> 40	265	2.898 (2.015 - 4.168)	< 0.001	2.728 (1.791 - 4.157)	< 0.001
Gender	530				
Female	238	Reference			
Male	292	1.112 (0.791 - 1.563)	0.542		
SP1	530				
Low	265	Reference		Reference	
High	265	1.824 (1.279 - 2.602)	< 0.001	1.758 (1.180 - 2.619)	0.006

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
WHO grade	461				
G2	220	Reference		Reference	
G3	241	3.108 (2.028 - 4.764)	< 0.001	1.901 (1.191 - 3.034)	0.007
IDH status	519				
WT	96	Reference		Reference	
Mut	423	0.165 (0.115 - 0.239)	< 0.001	0.212 (0.137 - 0.329)	< 0.001
Age	522				
<= 40	260	Reference		Reference	
> 40	262	3.000 (2.041 - 4.410)	< 0.001	2.887 (1.836 - 4.539)	< 0.001
Gender	522				
Female	235	Reference			
Male	287	1.072 (0.750 - 1.532)	0.704		
SP1	522				
Low	263	Reference		Reference	
High	259	2.015 (1.383 - 2.936)	< 0.001	1.931 (1.261 - 2.957)	0.002

Figure 9. SP1 expression and its prognostic significance in glioma. A, B. Elevated SP1 expression in glioma tissue samples compared to normal brain tissues. C. High SP1 expression correlates with poorer 5-year OS and DSS in LGG. D. SP1 is an independent prognostic factor for LGG.



Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA0079.4	MA0079.4.SP1	17.143717	0.9485901	5'	1666	1680	-	TTAGCCACGCCCCGT
MA0079.3	MA0079.3.SP1	14.962341	0.9693841	5'	1767	1777	+	GCCCCTCCTCC
MA0079.5	MA0079.5.SP1	12.87616	0.9426366	5'	1983	1991	+	GGGGCGCGG
MA0079.3	MA0079.3.SP1	11.681951	0.928113	5'	1423	1433	+	TCCCCACCTCT
MA0079.3	MA0079.3.SP1	11.648836	0.92769635	5'	1489	1499	+	TCTCCGCCTCT
MA0079.3	MA0079.3.SP1	11.521008	0.9260881	5'	138	148	+	GCCCCTCCCTA
MA0079.5	MA0079.5.SP1	11.265784	0.91465676	5'	1127	1135	-	GGGGAGGCG
MA0079.3	MA0079.3.SP1	10.322373	0.91100794	5'	1295	1305	-	CCTCCTCCCTT
MA0079.3	MA0079.3.SP1	10.157781	0.9089372	5'	1323	1333	+	GCCCTTCCCCC
MA0079.1	MA0079.1.SP1	10.102776	0.93926394	5'	1983	1992	+	GGGGCGCGGT

Figure 10. SP1 binds to the promoter region of IKBIP. A. Predicted SP1 binding sites within the IKBIP promoter region based on JASPAR analysis. B. Correlation analysis between IKBIP expression and candidate transcription factor SP1, based on TCGA-LGG RNA-seq data. C. Predicted binding site and motif of SP1 within the IKBIP promoter region. D. ChIP analysis confirming of binding of SP1 to the IKBIP promoter region. Data are expressed as mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$, vs. the control group.

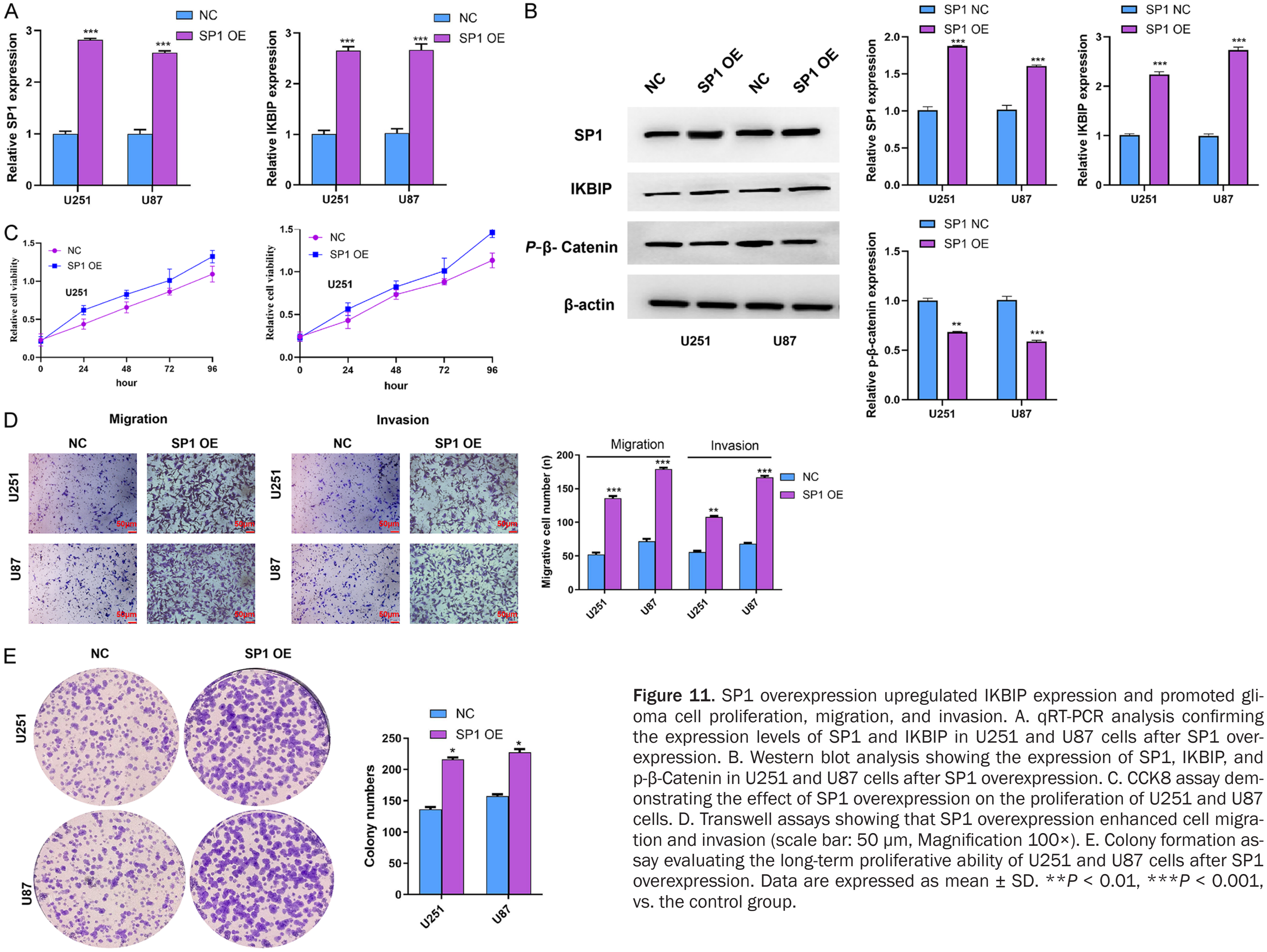


Figure 11. SP1 overexpression upregulated IKBIP expression and promoted glioma cell proliferation, migration, and invasion. A. qRT-PCR analysis confirming the expression levels of SP1 and IKBIP in U251 and U87 cells after SP1 overexpression. B. Western blot analysis showing the expression of SP1, IKBIP, and p-β-Catenin in U251 and U87 cells after SP1 overexpression. C. CCK8 assay demonstrating the effect of SP1 overexpression on the proliferation of U251 and U87 cells. D. Transwell assays showing that SP1 overexpression enhanced cell migration and invasion (scale bar: 50 μm, Magnification 100×). E. Colony formation assay evaluating the long-term proliferative ability of U251 and U87 cells after SP1 overexpression. Data are expressed as mean ± SD. $**P < 0.01$, $***P < 0.001$, vs. the control group.

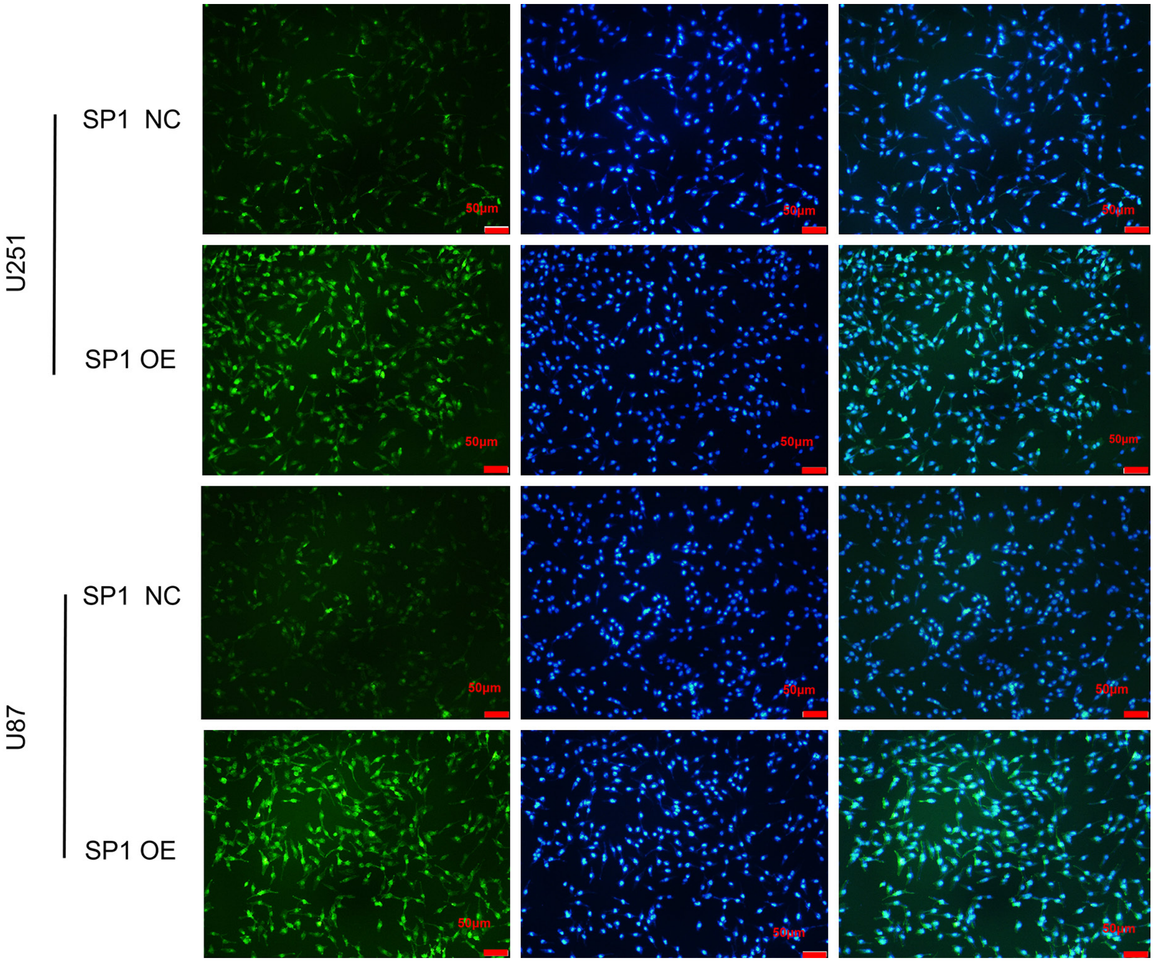


Figure 12. Immunofluorescence analysis of β-catenin expression and subcellular localization following SP1 overexpression (scale bar: 50 μm, Magnification 100×).

notypes and is significantly involved in EMT, serving as an independent prognostic factor in glioma [37]. However, the specific molecular mechanism by which IKBIP activates the Wnt/β-catenin/EMT pathway to promote glioma progression remains unclear. This study confirmed, through public database analyses and both *in vitro* and *in vivo* experiments, that IKBIP is highly expressed in glioma tissues and cells. More importantly, our findings demonstrate that elevated expression of IKBIP is associated with activation of the Wnt/β-catenin-EMT pathway, thereby promoting the proliferative and invasive phenotypes of glioma cells. Mechanistically, overexpression of IKBIP decreased the phosphorylation of β-catenin while promoting nuclear accumulation of β-catenin in glioma cells. These findings suggest that in glioma, overexpression of IKBIP may promote stabilization and nuclear translocation of β-catenin, thereby activating the Wnt/β-catenin signaling pathway and facilitating EMT, ultimately contributing to glioma progression.

Transcription factors are crucial regulatory proteins that control gene expression by binding to specific DNA sequences and modulating transcriptional activity [38-41]. The role of transcription factors is particularly significant as they influence tumor cell growth, differentiation, metastasis, and therapeutic response [42, 43]. For instance, transcription factors such as c-Myc and E2F are overexpressed in various cancers and can promote tumorigenesis by upregulating oncogenic gene expression [44]. In contrast, some transcription factors promote cancer development by inhibiting tumor suppressor genes [45, 46]. Transcription factors are also key drivers of tumor progres-

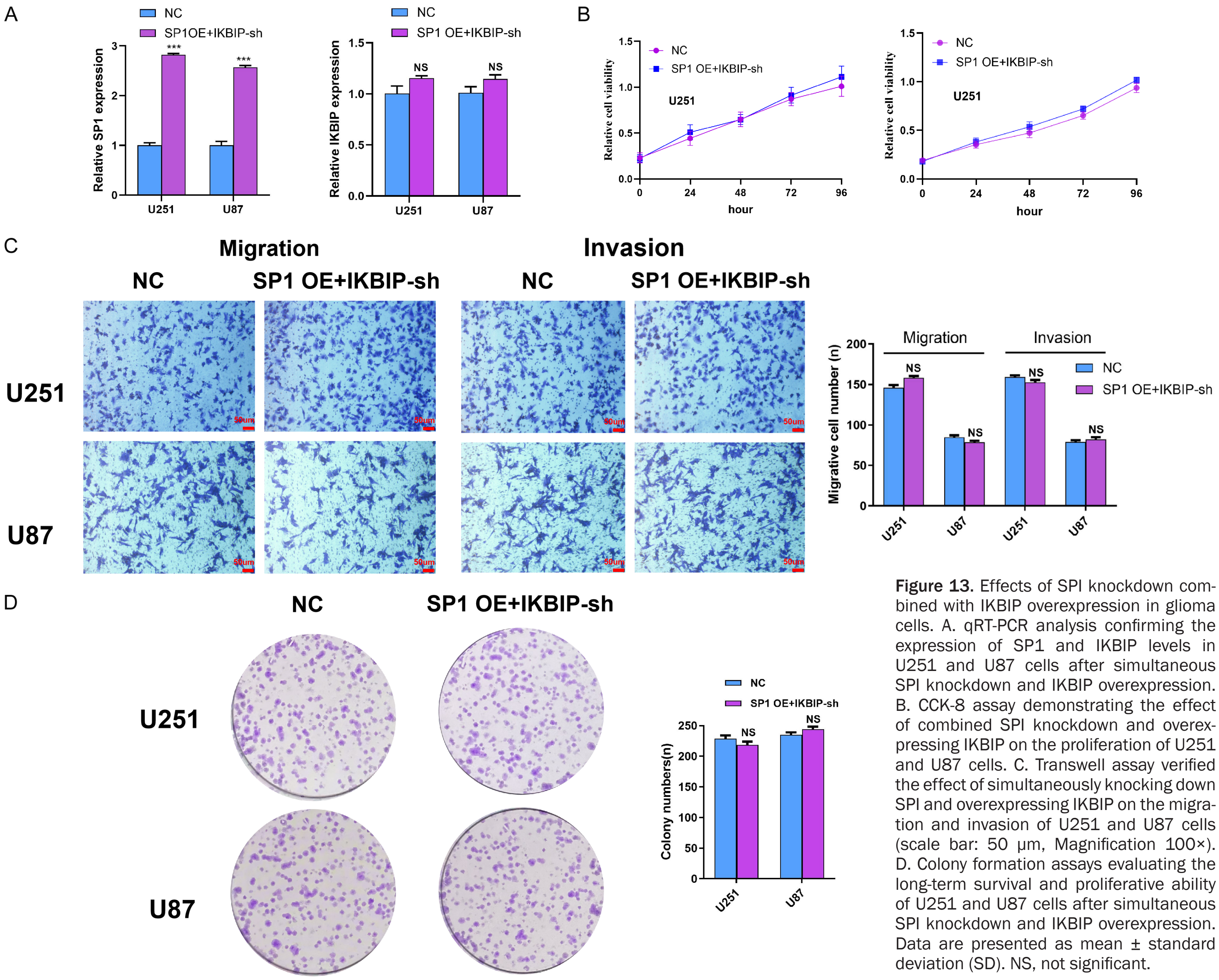


Figure 13. Effects of SPI knockdown combined with IKBIP overexpression in glioma cells. A. qRT-PCR analysis confirming the expression of SP1 and IKBIP levels in U251 and U87 cells after simultaneous SPI knockdown and IKBIP overexpression. B. CCK-8 assay demonstrating the effect of combined SPI knockdown and overexpressing IKBIP on the proliferation of U251 and U87 cells. C. Transwell assay verified the effect of simultaneously knocking down SPI and overexpressing IKBIP on the migration and invasion of U251 and U87 cells (scale bar: 50 μm, Magnification 100×). D. Colony formation assays evaluating the long-term survival and proliferative ability of U251 and U87 cells after simultaneous SPI knockdown and IKBIP overexpression. Data are presented as mean ± standard deviation (SD). NS, not significant.

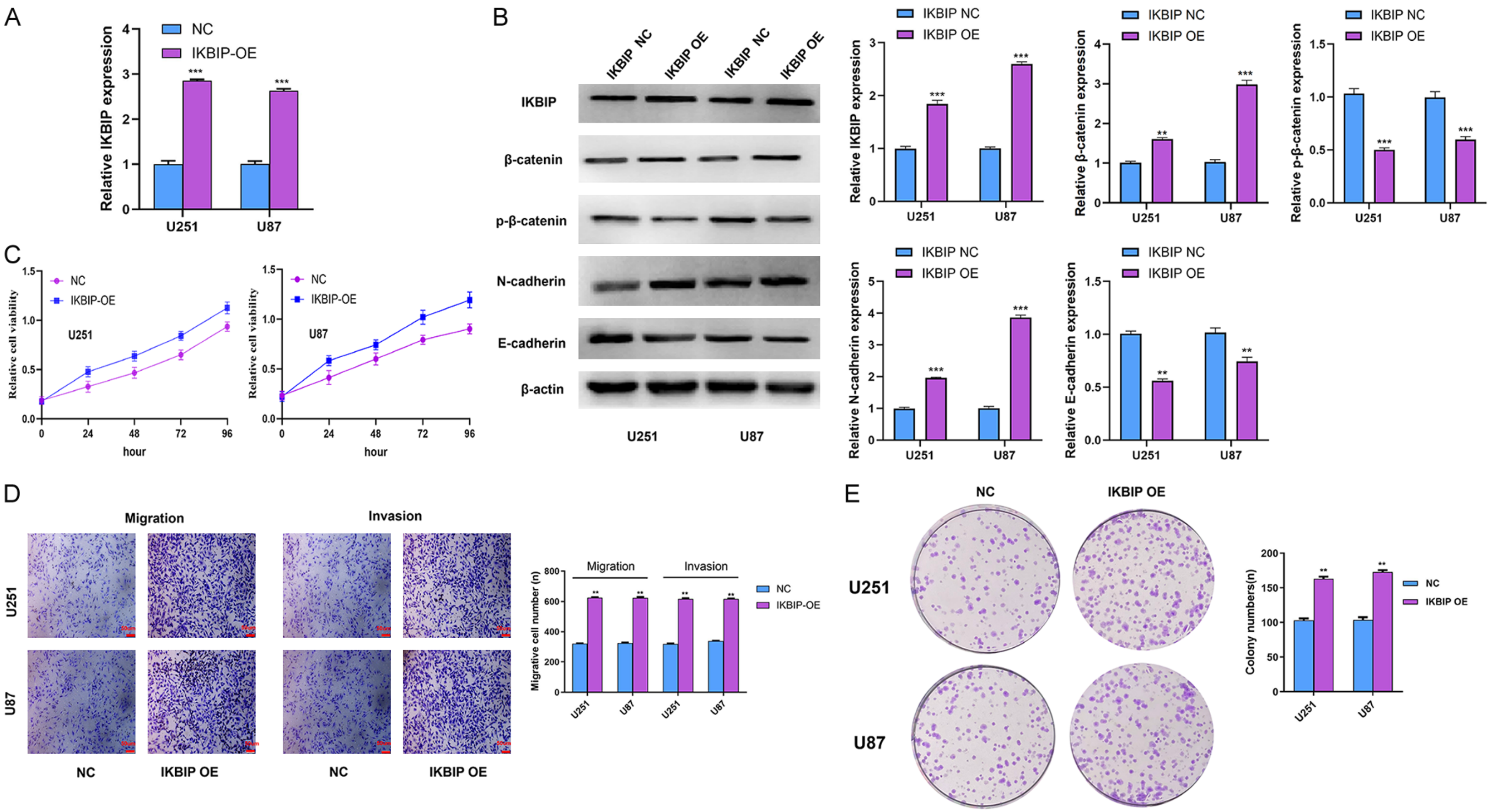


Figure 14. IKBIP overexpression promoted glioma proliferation, migration and invasion via activation of the Wnt/β-catenin/EMT pathway. A. qRT-PCR analysis of IKBIP expression in U251 and U87 cells after IKBIP overexpression. B. Western blot analysis showing the expression of IKBIP, β-Catenin, P-β-Catenin, N-cadherin, and E-cadherin in U251 and U87 cells after IKBIP overexpression. C. CCK-8 assay demonstrating the effect of IKBIP overexpression on the proliferation of U251 and U87 cells. D. Transwell assay showing that IKBIP overexpression enhances migration and invasion of U251 and U87 cells (scale bar: 50 μm, Magnification 100×). E. Colony formation assays evaluating the long-term proliferative capacity of U251 and U87 cells following IKBIP overexpression. Data are expressed as mean ± SD. $**P < 0.01$, $***P < 0.001$, vs. the control group.

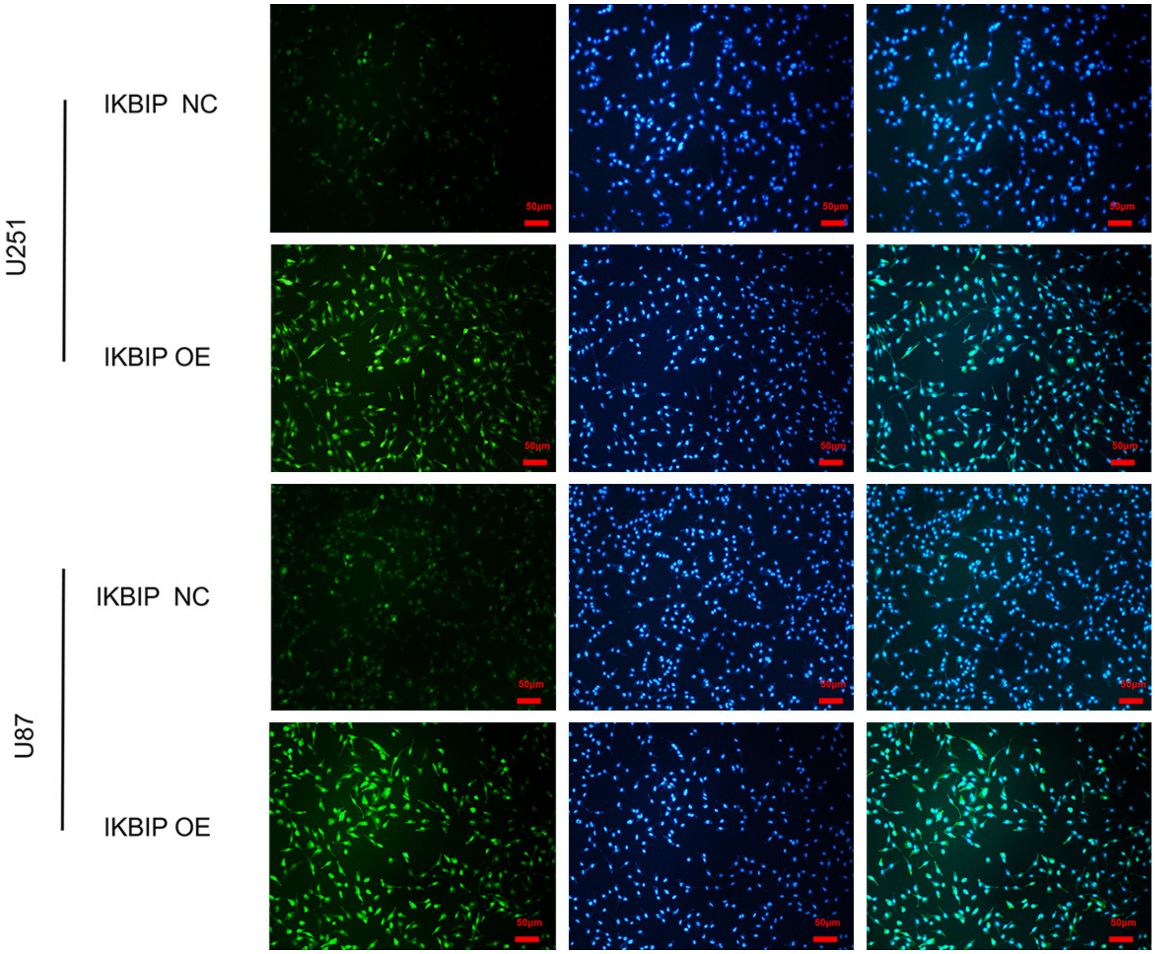


Figure 15. Immunofluorescence analysis of β-catenin expression and and subcellular localization in U251 and U87 cells following IKBIP overexpression (scale bar: 50 μm, Magnification 100×).

sion through promoting cell division and suppressing apoptosis. Conversely, several transcription factors, including Snail and Slug, are critically involved in tumor metastasis by promoting cell motility and invasiveness. Moreover, transcription factors such as Nrf2 have been implicated in the development of drug resistance through the regulation of antioxidant and detoxification-related genes, enabling tumor cells to withstand therapeutic stress [47, 48]. Therefore, investigating the roles of transcription factors is essential for understanding complex tumorigenesis and for identifying novel therapeutic targets. Our study demonstrated that the transcription factor SP1 binds to the promoter region of IKBIP and promotes its transcription, resulting in increased IKBIP expression in glioma. Bioinformatics analysis based on TCGA datasets further demonstrated that SP1 expression is significantly higher in glioma tissues and is associated with poor prognosis. Consistently, functional experiments confirmed that SP1 positively regulates IKBIP expression, as SP1 knockdown led to a notable decrease in IKBIP expression in glioma cells. Furthermore, knockdown of SP1 greatly impaired the clonogenic capacity and invasive growth of glioma cells. These observations underscore the critical role of SP1 in glioma progression by regulating IKBIP expression, thereby influencing cell proliferation, survival, and metastatic potential.

The Wnt/β-catenin signaling pathway is complex yet not fully elucidated molecular mechanism that plays a crucial role in various biological processes, including tissue development, cell differentiation, and the maintenance of tissue homeostasis in different organs [49-51]. This pathway is especially important in regulat-

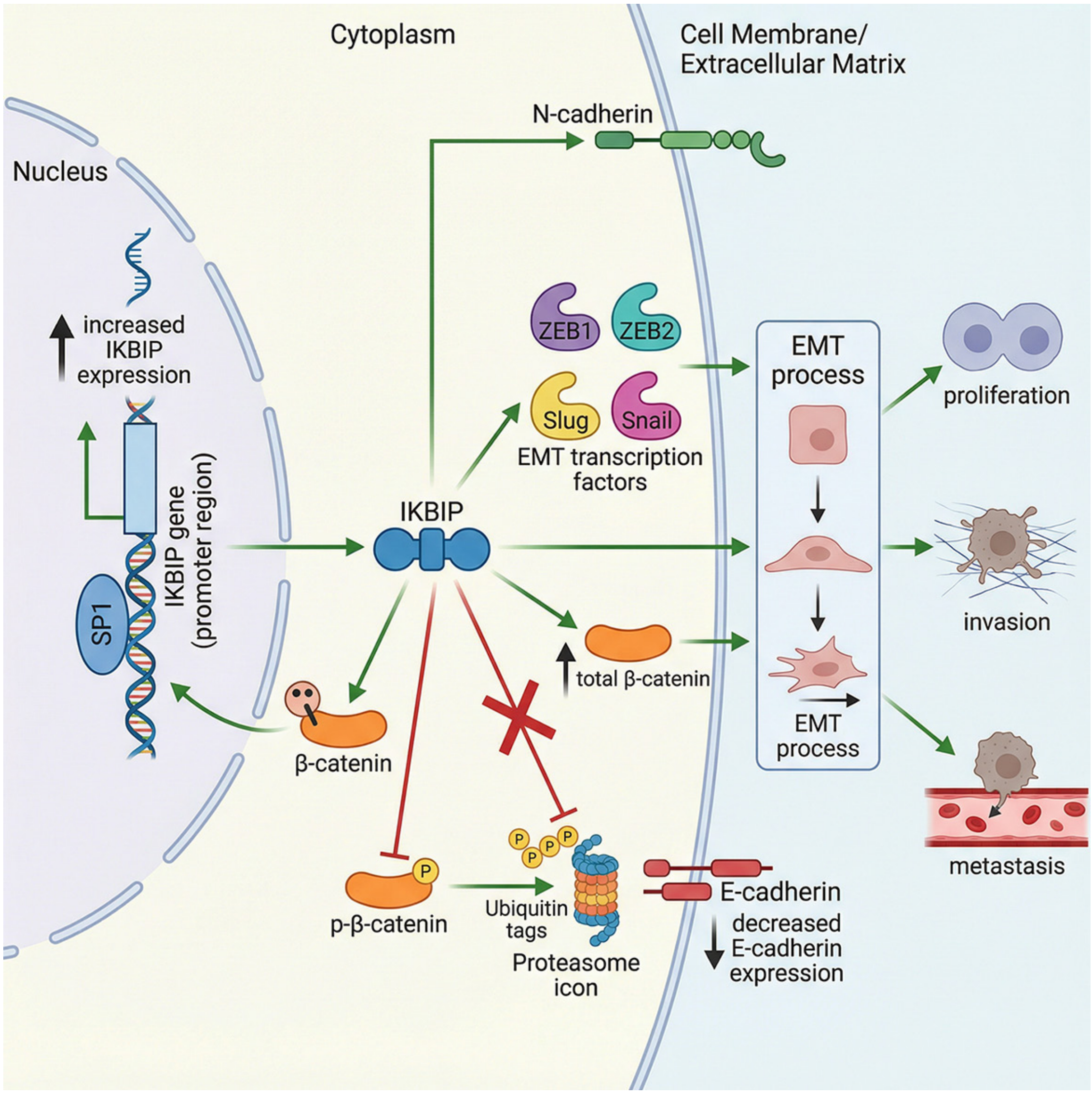


Figure 16. Schematic diagram illustrating the mechanism by which the SP1-IKBIP axis promotes glioma progression via activation of the Wnt/β-catenin/EMT pathway. In glioma cells, SP1 binds to the promoter region of IKBIP and enhances its transcription. Upregulation of IKBIP promotes the expression of mesenchymal markers, including ZEB1, ZEB2, Slug, Snail, N-cadherin, and β-catenin, while suppressing the expression of epithelial marker, E-cadherin. In addition, IKBIP overexpression reduces the expression of phosphorylated β-catenin, thereby inhibiting its degradation, leading to the intracellular accumulation of total β-catenin. The accumulated β-catenin translocates to the nucleus and activates downstream target genes, ultimately facilitating epithelial-mesenchymal transition (EMT) and promoting glioma cell proliferation, migration, and invasion.

ing EMT, a biological process essential for the embryonic development and tumor metastasis. In the absence of Wnt signaling, β catenin is targeted for degradation by a group of proteins including GSK-3β, Axin and APC. This degradation process maintains low intracellular levels of β-catenin and prevents its translocation into the nucleus [52, 53]. In contrast, upon Wnt signaling pathway activation, the activity of the destruction complex is inhibited, leading to the stabilization and accumulation of β-catenin in the cytoplasm. Stabilized β-catenin subsequently translocates to the nucleus, where it binds with transcription factors and regulates the expression of genes involved in cell growth, survival, and differentiation [54, 55]. Activation of the Wnt/β-catenin pathway promotes EMT, during which epithelial cells of strong cell-cell adhesion and low motility acquire mesenchymal-like properties, including increased motility and invasiveness. This phenotypic transition is crucial for cancer cells to detach from the primary site, invade surrounding tissues, and migrate to distant organs. Aberrant activation of the Wnt/β-catenin pathway has been reported in various cancers, including colorectal cancer, breast cancer, and glioma. Constitutive activation of β-catenin signaling results in uncontrolled cell division and tumor progression. Additionally, dysregulation of this pathway plays a pivotal role in EMT-mediated cancer metastasis by enhancing the invasiveness of cancer cells [56-58]. In gliomas, dysregulated Wnt/β-catenin signaling pathway has been implicated in tumor development, progression, and therapeutic failure. This is because increased β-catenin activity in the glioma cells is associated with increased cell proliferation, reduced apoptosis, and enhanced cell motility.

Our study demonstrates that the SP1-IKBIP axis promotes the nuclear translocation of β-catenin, thereby activating the Wnt/β-catenin pathway. This activation further facilitates EMT and enhances the proliferative and invasive phenotypes of glioma cells. Mechanistically, we identified IKBIP as a critical mediator of this pathway, and its knockdown leads to suppressed glioma cell proliferation, migration and invasiveness. Besides, IKBIP silence attenuated the Wnt/β-catenin/EMT signaling pathway, accompanied by reduced glioma cell motility and invasiveness. Moreover, IKBIP knockdown led to decreased expression of β-catenin, N-cadherin, and EMT-related transcription factors, implying inhibition of mesenchymal phenotype and a shift toward a more epithelial-like state. These findings support the role of IKBIP as an important regulator of glioma metastasis through modulation of the Wnt/β-catenin pathway and EMT process. From a clinical perspective, targeted inhibition of IKBIP may represent a potential therapeutic strategy for glioma. Modulation of this pathway could potentially suppress tumor growth and limit metastatic progression, thereby improving patient outcomes.

Conclusion

IKBIP is significantly upregulated in glioma tissues and is associated with poor clinical outcome. Elevated IKBIP expression promotes proliferation, migration, and invasion of glioma cells, whereas its knockdown markedly suppresses these malignant phenotypes. Transcription factor SP1 is highly expressed in glioma and directly binds to the promoter region of IKBIP, functioning as an upstream regulator of IKBIP. Inhibition of SP1 significantly reduces IKBIP expression and impairs glioma cell proliferation and invasion. Mechanistically, the SP1-IKBIP axis promotes glioma progression by activating the Wnt/β-catenin pathway and inducing EMT process (**Figure 16**). Collectively, these findings suggest that the SP1-IKBIP axis may serve as a potential diagnostic biomarker and therapeutic target in the management of gliomas.

Acknowledgements

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Disclosure of conflict of interest

None.

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