

Molecular Mechanism into CDR1as-Mediated miR-7 Sponging in the Regulation of Insulinoma Cell Proliferation and Apoptosis

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Article

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Abstract

Pancreatic neuroendocrine tumors (PNETs) are rare malignancies with incompletely understood molecular mechanisms. Emerging evidence implicates non-coding RNAs in tumorigenesis, including circular RNA ciRS-7, which functions as a competitive endogenous RNA by sponging miR-7—a known tumor suppressor. Here, we report that ciRS-7 is significantly upregulated, while miR-7 is downregulated, in the human insulinoma cell line NES2Y. Knockdown of ciRS-7 suppressed cell proliferation (assessed by CCK-8 and colony formation assays) and induced apoptosis (measured by flow cytometry), accompanied by increased expression of pro-apoptotic proteins Bax and Caspase-3. Conversely, ciRS-7 overexpression enhanced proliferative capacity and upregulated the miR-7 targets Myrip and Pax6. Mechanistic studies confirmed that ciRS-7 modulates Myrip and Pax6 expression via miR-7 sequestration. Notably, silencing Myrip reversed ciRS-7-mediated effects, inhibiting proliferation and promoting apoptosis. These findings demonstrate that ciRS-7 promotes insulinoma cell growth by acting as a miR-7 sponge and activating the Myrip/Pax6 signaling axis, highlighting its potential as a therapeutic target in PNETs.

Introduction

Pancreatic Neuroendocrine Tumors (PNETs) are a heterogeneous group of neoplasms arising from neuroendocrine cells within the pancreas, representing approximately 3% of all pancreatic tumors [1]. Although the incidence of PNETs remains relatively low, epidemiological studies have demonstrated a steady increase in the diagnosis of PNETs over the past two decades, likely due to advances in imaging techniques and increased clinical awareness [2, 3]. The clinical presentation of PNETs varies widely, ranging from asymptomatic cases to severe hormone-related syndromes, and patient outcomes can differ significantly. Conventional treatment strategies include surgical resection, chemotherapy, and targeted therapy [4, 5]. However, therapeutic efficacy for advanced or metastatic disease remains suboptimal [6]. Insulinomas are a subtype of pancreatic neuroendocrine tumors (PNETs) that arise from the insulin-secreting β -cells of the pancreatic islets. These neoplasms are characterized by the autonomous and excessive production of insulin, which leads to recurrent episodes of hypoglycemia in affected patients [7]. Insulinomas, being functional PNETs, provide a unique opportunity to explore the molecular underpinnings of tumor development due to their well-defined biological and clinical characteristics.

Non-coding RNAs (ncRNAs) play critical regulatory roles in gene expression and have been widely associated with the development and progression of various cancers [8]. Among them, circular RNAs (circRNAs) represent a unique class of ncRNAs characterized by their covalently closed loop structures formed through back-splicing events, which render them resistant to degradation by exonucleases and thus highly stable and conserved across species [9]. Unlike linear RNAs, circRNAs are resistant to degradation by exonucleases due to their unique loop structure, making circRNAs remarkably stable and abundant in various tissues and body fluids, including blood and saliva [10]. CircRNAs participate in the regulation of gene expression through multiple mechanisms, including acting as microRNA (miRNA)

sponges to modulate post-transcriptional gene regulation [11], interacting with RNA-binding proteins (RBPs) to influence RNA processing [12] and even regulating transcription and translation processes [13]. MicroRNA-7 (miR-7) is a widely expressed microRNA that has been shown to exert either tumor-suppressive or oncogenic functions depending on the cancer type and cellular context [14]. Importantly, downregulation of miR-7 has been observed in both type 2 diabetes and PNETs, suggesting its protective role against islet cell dysregulation [15]. In many cancers, miR-7 suppresses tumor cell proliferation, migration, and invasion by targeting key oncogenes such as epidermal growth factor receptor (EGFR), phosphatidylinositol 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) [16]. However, the expression and function of miR-7 are regulated by multiple factors, among which circRNAs serving as miRNA sponges represent a crucial regulatory mechanism [17]. CiRS-7, also known as CDR1as, was the first extensively studied circRNA capable of sequestering miR-7 through abundant binding sites, thereby relieving the inhibitory effects of miR-7 on its downstream target genes and promoting tumor progression [18]. Given the critical roles of ciRS-7 and miR-7 in tumor development and progression, as well as the essential functions of Myrip and Pax6 in insulin secretion and pancreatic development [19], the objective of this study is to elucidate the involvement of the ciRS-7/miR-7 regulatory axis in pancreatic islet cell tumorigenesis by modulating the expression of Myrip and Pax6. Our findings indicate that knockdown or overexpression of the circular RNA ciRS-7 suppresses cell growth in pancreatic islet cell tumor cells by targeting miR-7 and modulating the Myrip and Pax6 signaling pathways, ultimately inducing apoptosis. These results suggest that ciRS-7 may serve as a potential therapeutic target for pancreatic islet cell tumors.

Materials and methods

Quantitative reverse transcription polymerase chain reaction (qRT–PCR)

Quantitative reverse transcription polymerase chain reaction can reliably detect and quantitatively measure the products generated in each cycle of the PCR process [20]. Total RNA was extracted from HPDE6-C7 and NES2Y cells via TRIzol reagent (15596026, Thermo, USA) following the manufacturer's instructions. MiRNA was extracted from HPDE6-C7 and NES2Y cells via TRIzol reagent (Life Technologies, Carlsbad, USA) following the manufacturer's instructions. Total RNA was isolated, and an mRNA reverse transcription kit (CW2569, CWBIO, China) was used to reverse transcribe total RNA into cDNA. The expression levels of miR-7, ciRS-7, Myrip, Pax6, Bax and Caspase-3 were quantified via qRT–PCR, and specific primers were used to perform qRT–PCR. U6 or GAPDH was selected as the internal reference for analysis of target gene expression. The combination of Primer 8.0 software (Premier Biosoft, Palo Alto, USA) and Sangon Biotech (Shanghai, China) was used to design all the primers (Table 1). We used the $2^{-\Delta\Delta CT}$ method to calculate the relative expression levels of each gene.

Table 1
Primer sequences for qRT–PCR.

Primers	Forward (5–3)	Reverse (5–3)
ciRS-7	TAGTACGTCGTGCCCTGA	CACTTGACGTGCAGCATC
miR-7	CCACGTTGGAAGACTAGTGATTT	TATGGTTGTTCTGCTCTCTGTCTC
GAPDH	ACACCCACTCCTCCACCTTT	TTACTCCTTGGAGGCCATGT
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
Myrip	ACCTTCCTCGTCAACACCAAG	GTAGAACCATTCCAGAGATTGGG
Pax6	TGGGCAGGTATTACGAGACTG	ACTCCCGCTTATACTGGGCTA
Bax	GATGCGTCCACCAAGAAGCTGAG	CACGGCGGCAATCATCCTCTG
Caspase-3	GTGGAGGCCGACTTCTTGTATGC	TGGCACAAAGCGACTGGATGAAC

Cell culture and transfection

The HPDE6-C7 (NO.BNCC359453) cell line from BeNa Culture Collection of Beijing and NES2Y (No.BFN607200613) cell lines were obtained from Biochannel Biotechnology of Nanjing. HPDE6-C7 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. NES2Y cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Small interfering RNAs (siRNAs) (siRNAs, siciRS-7-NC, siciRS-7-1 and siciRS-7-2, siMyrip-NC, siMyrip-1 and siMyrip-2) were designed by Kidan Biotech of Guangzhou (Table 2). ciRS-7 and Myrip were knocked down by siRNA, and the empty vector was used as a negative control. All the cells were subsequently transfected with Lipofectamine RNA iMAX (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions.

Table 2
Primer names and sequences

Primers	Forward (5–3)	Reverse (5–3)
siciRS-7-NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
siciRS-7-1	GCAAUAUCCAGGGUUUCCGAUTT	AUCGGAAACCCUGGAUUAUUGCTT
siciRS-7-2	UAUCCAGGGUUUCCGAUGCTT	CCAUCGGAAACCCUGGAUATT
siMyrip-NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
siMyrip-1	GUACGAGUUAGCAAUGAAATT	UUUCAUUGCUAACUCGUACTT
siMyrip-2	CGAUAGCGAGGAAGACUUUTT	AAAGUCUUCUCGCUAUCGTT

Cell Counting Kit-8

The cells in the different groups were digested, counted, and seeded in 96-well plates (0030730119, Eppendorf, Germany) at 1×10^4 cells/well (100 μ l per well). Each group was divided into three wells. After the cells had adhered to the wall, the medium was changed, and the intervention factors were added. After 48 hours of treatment, the culture medium was removed, and the cells were incubated with 110 μ l of DMEM containing 10 μ l of CCK-8 solution. After incubation at 37°C and 5% CO₂ for 4 h, the absorbance value at 450 nm was analyzed with a Bio-Tek microplate (MB-530, Heales, China).

Plasmid construction and transfection

The ciRS-7 overexpression plasmid was generated as follows: the full-length human ciRS-7 complementary DNA (cDNA) was inserted into the GV141 vector (Mailgene biosciences, China) in accordance with the manufacturer's protocol. Cells were transfected with the resulting ciRS-7 overexpression plasmid using Lipofectamine™ 3000 reagent (Invitrogen, Carlsbad, USA), following the manufacturer's guidelines. To assess transfection efficiency, ciRS-7 overexpression levels were quantified by quantitative real-time PCR using RNA extracted from cells harvested 48 hours after transfection.

Flow cytometry

For the determination of cell apoptosis, cells were double-stained with Annexin V-fluorescein isothiocyanate (Annexin V-FITC) and propidium iodide (PI) to identify apoptotic populations. Following trypsin digestion without EDTA, the cells were collected, washed with phosphate-buffered saline (PBS), and resuspended in binding buffer. Annexin V-FITC and PI staining were performed according to the manufacturer's instructions (KGA108, KeyGEN Biotech Co., Ltd., Jiangsu, China). Cell apoptosis was then analyzed using an A00-1-11102 flow cytometer (Beckman Coulter, USA).

Colony formation assay

After transfection, the cells (6000/well) were seeded into six-well plates and cultured for 14 days in high-glucose DMEM supplemented with 10% FBS at 37°C and 5% CO₂. The colonies were subsequently fixed in 4% paraformaldehyde and stained with 0.05% crystal violet.

Western blot

Western blot

The total proteins were extracted with RIPA buffer (P0013B, Fude Biotech, China). The proteins were deposited onto a nitrocellulose filter membrane via polyacrylamide gel electrophoresis. The membranes were blocked with 5% (w/v) nonfat dry milk for 90 min before being incubated with primary antibodies

overnight at 4°C. The primary antibodies used included those against GAPDH (CST 5174s, 1:1000), Myrip (Abcam ab10149, 1:1000), Pax6 (CST 60433, 1:1000), Bax (Proteintech 60267-1-Ig, 1:1000), and Caspase-3 (GeneTex GTX110543, 1:1000). The sections were then incubated with secondary anti-goat IgG (FDG007, 1:5000), anti-mouse IgG (FDM007, 1:5000) and anti-rabbit IgG (CST 7074s, 1:5000) antibodies for 1.5 h at room temperature. We used an ECL chromogenic substrate to visualize the protein bands.

Statistical analysis

Statistical analysis of functional outcomes was carried out using Student's t-test for two-group comparisons and two-way ANOVA for multi-group comparisons. Data are presented as mean $\pm$ SEM. Significance levels are denoted as: NS, non-significant; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Results

Differential Expression of ciRS-7 and miR-7 in Pancreatic Neuroendocrine Tumors.

Accumulating evidence has highlighted the involvement of non-coding RNAs, particularly circRNAs and miRNAs, in the initiation and progression of various cancers [21]. Given their emerging regulatory roles in tumorigenesis, we focused on the potential dysregulation of ciRS-7 and miR-7 in pancreatic neuroendocrine tumors (PNETs). To investigate the expression profiles of ciRS-7 and miR-7 in PNET cells, we performed RT-qPCR analysis in a normal human pancreatic ductal epithelial cell line (HPDE6-C7) and a pancreatic neuroendocrine tumor cell line (NES2Y). The results revealed that ciRS-7 was significantly upregulated in NES2Y cells compared to HPDE6-C7 cells (Fig. 1A). Conversely, miR-7 expression was markedly downregulated in the tumor cell line relative to the normal control (Fig. 1B). These findings suggest that ciRS-7 may exert oncogenic functions, whereas miR-7 may act as a tumor suppressor in the progression of pancreatic neuroendocrine tumors.

Knockdown of ciRS-7 suppresses tumor cell proliferation and induces apoptosis

CiRS-7 (also known as CDR1as) is a well-characterized circRNA containing multiple conserved binding sites for miR-7, allowing it to specifically sequester and inhibit this miRNA [22]. To validate the functional role of ciRS-7, we designed a small interfering RNA (siRNA) targeting ciRS-7 to silence its expression in pancreatic islet cell tumor cells. RT-qPCR analysis confirmed that si-ciRS-7 effectively reduced ciRS-7 expression levels (Fig. 2A), indicating efficient transfection. Subsequently, CCK-8 and colony formation assays were performed to evaluate the impact of ciRS-7 silencing on cell proliferation. The CCK-8 assay revealed a significant decrease in cell viability following ciRS-7 knockdown (Fig. 2B). Consistently, the colony formation assay showed that the clonogenic ability of tumor cells was markedly impaired after ciRS-7 suppression (Fig. 2C). These results indicate that ciRS-7 promotes cell proliferation in pancreatic islet cell tumor cells.

To further investigate the effect of ciRS-7 silencing on cell apoptosis, flow cytometric analysis was performed. Our results showed that knockdown of ciRS-7 significantly increased the apoptotic rate in NES2Y cells (Fig. 2D). Consistently, RT-qPCR and Western blot analyses revealed that the expression levels of the apoptosis-related genes Bax and Caspase-3 were markedly upregulated following ciRS-7 suppression (Fig. 2E-2H). Collectively, these data indicate that Silencing of ciRS-7 suppresses the growth of NES2Y cells, suggesting that ciRS-7 may function as a pro-proliferative factor in promoting tumor cell proliferation.

Overexpression of ciRS-7 enhanced tumor cell proliferation and suppressed apoptosis

To investigate the functional role of ciRS-7 in NES2Y cells, we overexpressed ciRS-7 via plasmid transfection and subsequently assessed cell proliferation using CCK-8 assay and colony formation assays. Transfection successfully elevated ciRS-7 expression by more than 1.5-fold compared to control cells (Fig. 3A). At 48 hours after ciRS-7 transfection, NES2Y cell proliferation was induced due to ciRS-7 overexpression (Fig. 3B). Cells transfected with the ciRS-7 plasmid formed significantly more colonies than those transfected with the control vector (Fig. 3C). Subsequently, flow cytometric analysis was performed to evaluate apoptosis in insulinoma cells following ciRS-7 overexpression. The results showed that overexpression of ciRS-7 significantly attenuated apoptosis in insulinoma cells (Fig. 3D). These results indicate that overexpression of ciRS-7 significantly enhances the growth activity of insulinoma cells and suppresses apoptosis, which is consistent with the findings observed upon ciRS-7 knockdown.

CiRS-7 regulates the expression of Myrip and Pax6 by modulating miR-7

Given the established role of ciRS-7 as a potent molecular sponge for miR-7 [23], we further investigated the functional consequences of ciRS-7 silencing on miR-7 bioavailability and its downstream targets. RT-qPCR analysis revealed that knockdown of ciRS-7 significantly increased miR-7 expression, while markedly decreasing the mRNA levels of Myrip and Pax6 (Fig. 4A-4D). Conversely, overexpression of ciRS-7 significantly reduced miR-7 expression and upregulated the mRNA levels of Myrip and Pax6 (Fig. 4E-4H). Consistent with the transcriptional results, Western blot analysis revealed corresponding changes in MYRIP and PAX6 protein levels. (Fig. 4I-4J). These results suggest that ciRS-7 may regulate Myrip and Pax6 expression through modulating miR-7.

Myrip is regulated by ciRS-7 and promotes cell proliferation while inhibiting apoptosis in NES2Y cells.

To further elucidate the regulatory relationship between Myrip, ciRS-7, miR-7, and Pax6, we designed a small interfering RNA (siRNA) targeting Myrip to silence its expression in pancreatic islet cell tumor cells. Figures 5A and 5B validated the knockdown efficiency of si-Myrip using RT-qPCR and Western blot analysis, respectively. Myrip is an interacting partner of the small GTPase Rab27, playing a role in the

trafficking and exocytosis of secretory granules [24]. To further investigate the regulatory effects of Myrip on its upstream and downstream genes in pancreatic neuroendocrine tumor cells, RT-qPCR was performed following Myrip knockdown. The results showed that silencing Myrip significantly reduced the mRNA expression levels of both its upstream regulator ciRS-7 and its downstream target Pax6, while markedly increasing miR-7 expression (Figs. 5C-5G). CCK-8 and colony formation assays demonstrated that Myrip silencing led to a significant inhibition of cell proliferation (Figs. 5H-5I). Flow cytometric analysis revealed a notable increase in apoptosis after Myrip knockdown (Fig. 5J). Moreover, RT-qPCR and Western blot analyses revealed that silencing Myrip significantly upregulated the expression levels of Bax and Caspase-3 (Fig. 5K-5N). Taken together, these results suggest that Myrip knockdown reverses the expression of ciRS-7 and its downstream targets (Myrip and Pax6), thereby inhibiting tumor cell proliferation and promoting apoptosis.

Discussion

The pathogenesis of pancreatic neuroendocrine tumor is multifactorial, involving dysregulation of oncogenes, tumor suppressors, and intricate non-coding RNA networks[25]. In this study, we investigated the role of ciRS-7 in islet cell tumorigenesis and uncovered a critical regulatory mechanism mediated by the ciRS-7/miR-7/Myrip/Pax6 signaling axis in modulating tumor cell proliferation and apoptosis. Our results demonstrate that ciRS-7 is highly expressed in pancreatic neuroendocrine tumor cells, whereas its target miR-7 is downregulated. This finding is consistent with the well-established function of ciRS-7 as a molecular sponge for miR-7 [26]. Specifically, ciRS-7 binds to and sequesters miR-7, thereby reducing its effective concentration and alleviating miR-7-mediated suppression of downstream target genes [27]. We further demonstrated that knockdown of ciRS-7 significantly suppressed proliferation and induced apoptosis in pancreatic neuroendocrine tumor cells, whereas ciRS-7 overexpression produced the opposite effects—promoting proliferation and attenuating apoptosis. Together, these findings indicate that ciRS-7 acts as an oncogene in this context. Notably, miR-7 expression was upregulated following ciRS-7 knockdown, which is consistent with its established function as a molecular sponge for miR-7. More importantly, we observed that the expression levels of Myrip and Pax6 were markedly reduced upon ciRS-7 silencing. Myrip, also known as Myosin Va and Rab-interacting protein, is involved in intracellular trafficking and cytoskeletal dynamics, processes that are essential for cell motility and functional polarization [28]. Pax6 has been widely recognized as a master regulator of pancreatic islet development and β -cell function, with critical implications in both normal physiology and pathological conditions such as tumorigenesis [29, 30]. Our findings suggest that ciRS-7 may regulate the expression of Myrip and Pax6 through modulating miR-7, thereby influencing the biological behavior of pancreatic neuroendocrine tumor cells. Functionally, Myrip depletion recapitulated the tumor-suppressive effects of ciRS-7 knockdown, including reduced cell viability and increased apoptotic activity, highlighting its pro-tumorigenic role in pancreatic neuroendocrine tumors. Furthermore, we observed that silencing Myrip led to a significant downregulation of ciRS-7 and Pax6 expression, while miR-7 expression was upregulated. Our findings suggest that Myrip is not only regulated by the ciRS-7/miR-7 axis, but may also participate in this complex regulatory network and

influence the expression of both ciRS-7 and Pax6. These results provide a foundation for further investigation into the clinical utility of the ciRS-7/miR-7/Myrip/Pax6 axis as a diagnostic marker or therapeutic target in pancreatic neuroendocrine tumors.

Conclusion

Through in vitro investigations, this study elucidates the mechanistic role of ciRS-7 in pancreatic islet cell tumors. We demonstrate that ciRS-7 is markedly upregulated in pancreatic neuroendocrine tumor cells and functions as a molecular sponge for miR-7. This interaction alleviates miR-7-mediated suppression of Myrip and Pax6, consequently promoting tumor cell proliferation while inhibiting apoptosis. CDR1as functions as a miR-7 sponge and regulates proliferation and apoptosis in insulinoma cells by modulating the expression of Myrip and Pax6. This finding provides novel insights into the molecular pathogenesis of pancreatic neuroendocrine tumors and reveals a potential therapeutic and diagnostic target for future development.

Declarations

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Contributions

Donghui Lu and Yuan Lin supported and supervised the study. Chen Liu, Fushi Piao and Zheng Feng responded to the study design. Zhenlin Tan conducted most experiments in vitro. Meihui Li, Minli Hu, Dan Wu and Zhimei Luo contributed to data collection. Minli Hu prepared the manuscript. Donghui Lu reviewed the manuscript.

Ethics approval and consent to participate

This study does not involve animal experiments or clinical research involving human subjects; therefore, no animal or clinical ethics approval was required.

Consent for publication

Not applicable.

Competing interests

The authors report no potential conflicts of interest, including financial or personal relationships that could influence the findings presented in this study.

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Figures

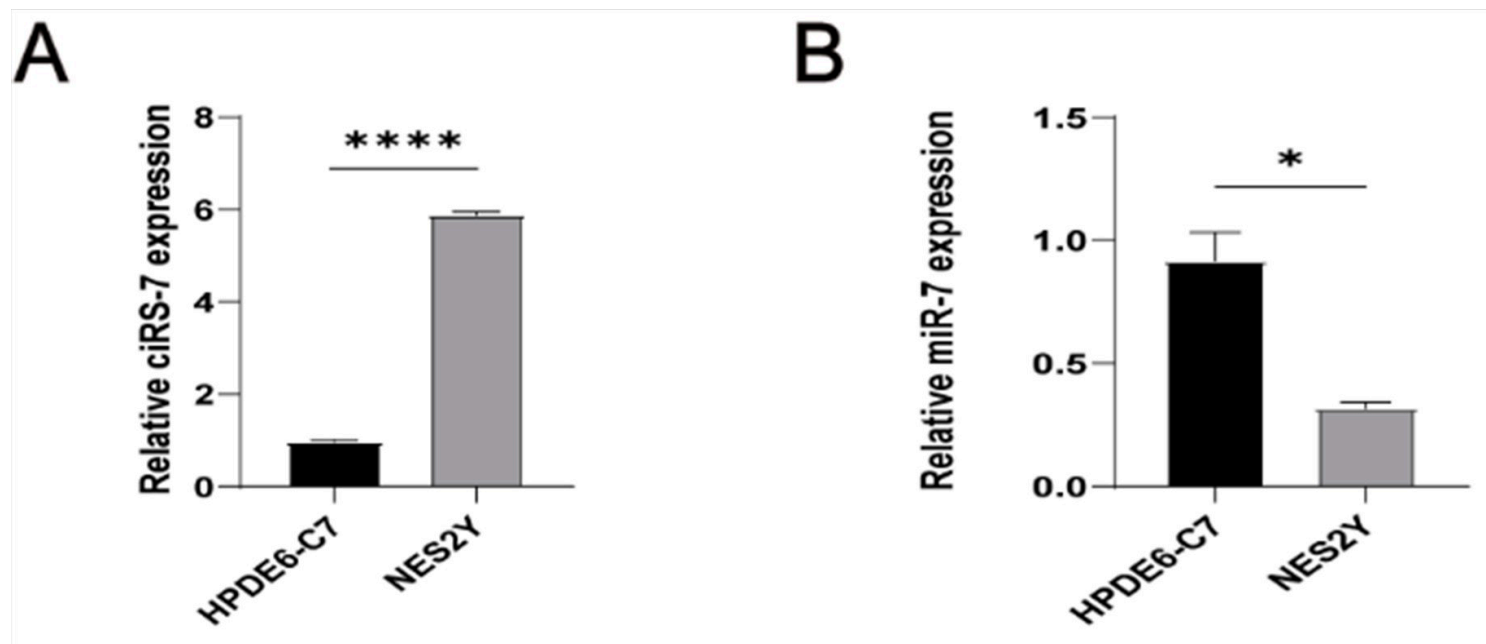


Figure 1

Expression levels of ciRS-7 and miR-7 in pancreatic neuroendocrine tumor cell lines. (A) ciRS-7 expression in HPDE6-C7 and NES2Y cells. (B) miR-7 expression in HPDE6-C7 and NES2Y cells. Data are presented as mean $\pm$ SEM from three independent experiments. Statistical significance was determined by Student's t-test; * $P < 0.05$, **** $P < 0.0001$.

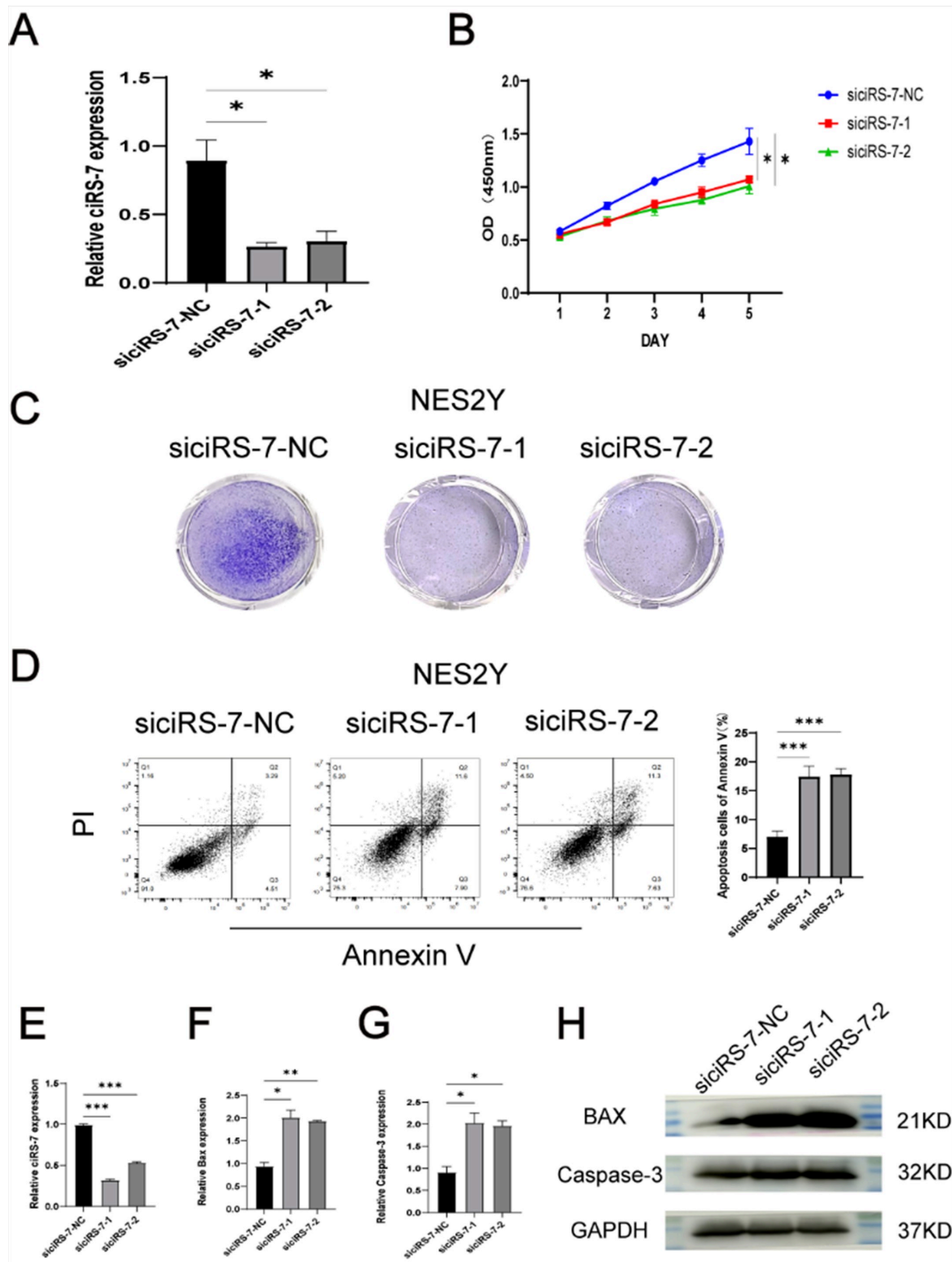


Figure 2

Silencing of ciRS-7 inhibits cell proliferation and induces apoptosis. (A) RT-qPCR confirms effective knockdown of ciRS-7 by si-ciRS-7. (B) CCK-8 assay shows reduced cell viability after ciRS-7 silencing. (C) Colony formation assay demonstrates decreased clonogenic capacity upon ciRS-7 downregulation. (D) Flow cytometry analysis reveals increased apoptosis following ciRS-7 knockdown. (E-H) RT-qPCR and

Western blot show upregulation of Bax and Caspase-3 expression after ciRS-7 suppression. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

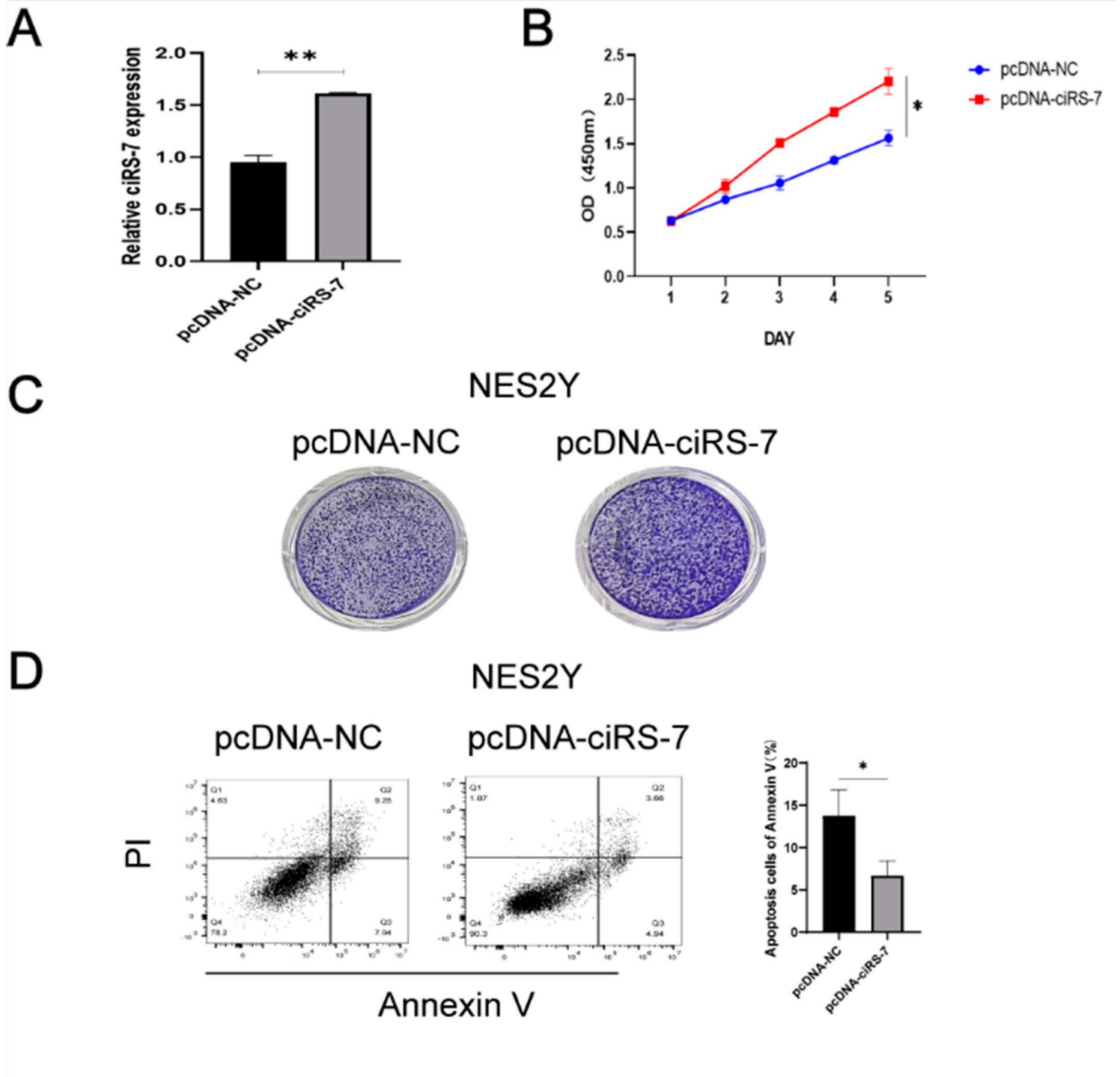


Figure 3

Effects of ciRS-7 overexpression via plasmid transfection on proliferation and apoptosis in NES2Y cells.

(A) ciRS-7 mRNA was analyzed by quantitative real-time PCR in NES2Y cells. (B) In NES2Y cells, cell proliferation was assessed by the CCK-8 assay in ciRS-7-overexpressing cells and control cells. (C) Colony formation assay revealed that overexpression of ciRS-7 enhanced clonogenic capacity. (D) Flow cytometry analysis showed that overexpression of ciRS-7 reduced cell apoptosis. $*P < 0.05$, $**P < 0.01$.

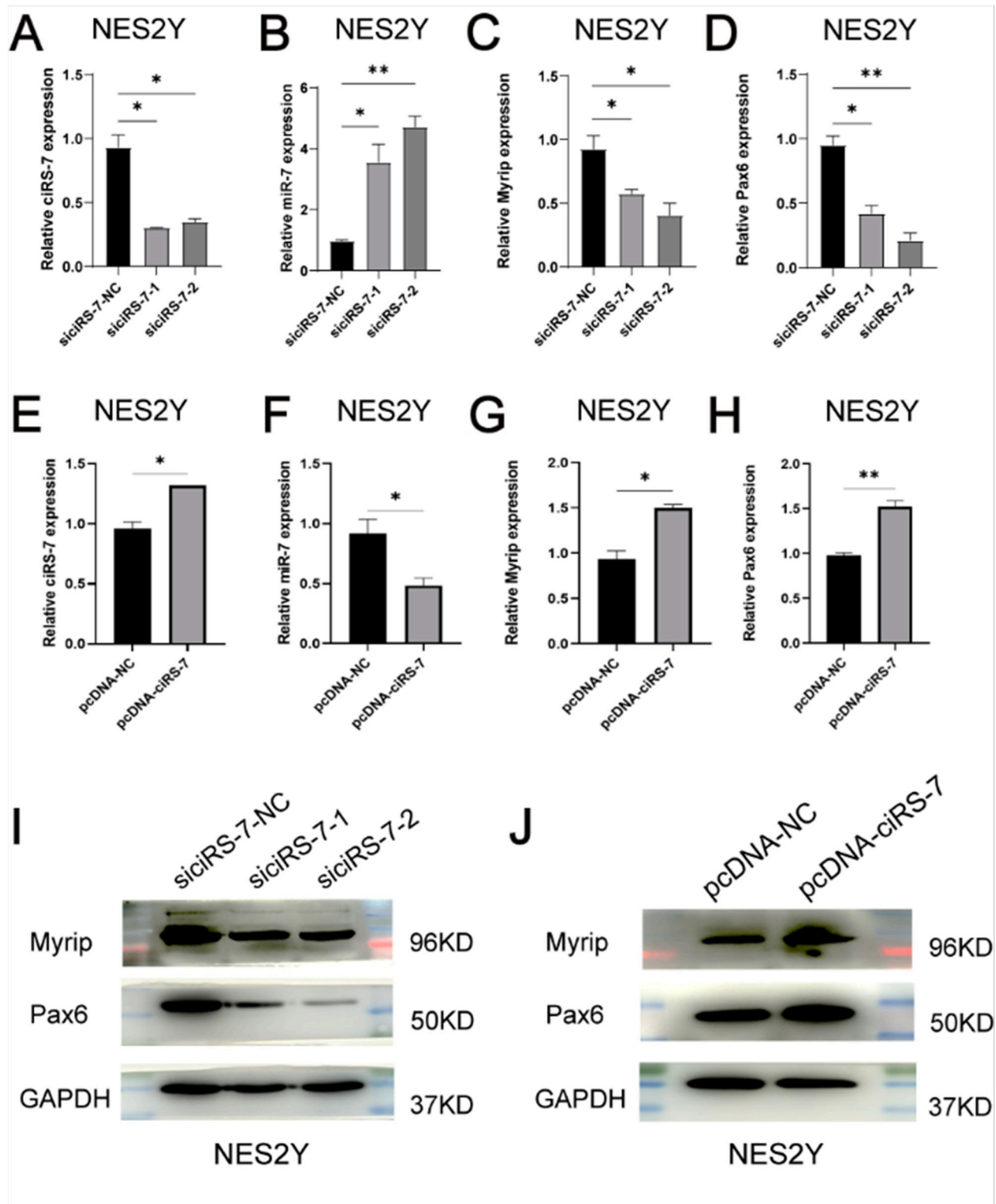


Figure 4

CiRS-7 regulates Myrip and Pax6 expression via modulation of miR-7. (A-D) RT-qPCR shows that silencing ciRS-7 increases miR-7 expression and decreases Myrip and Pax6 mRNA levels. (E-H) RT-qPCR shows that overexpressing ciRS-7 decreases miR-7 expression and increases Myrip and Pax6 mRNA levels. (I) Western blot analysis was performed to validate the changes in MYRIP and PAX6 protein expression following ciRS-7 knockdown and overexpression. * $P < 0.05$, ** $P < 0.01$.

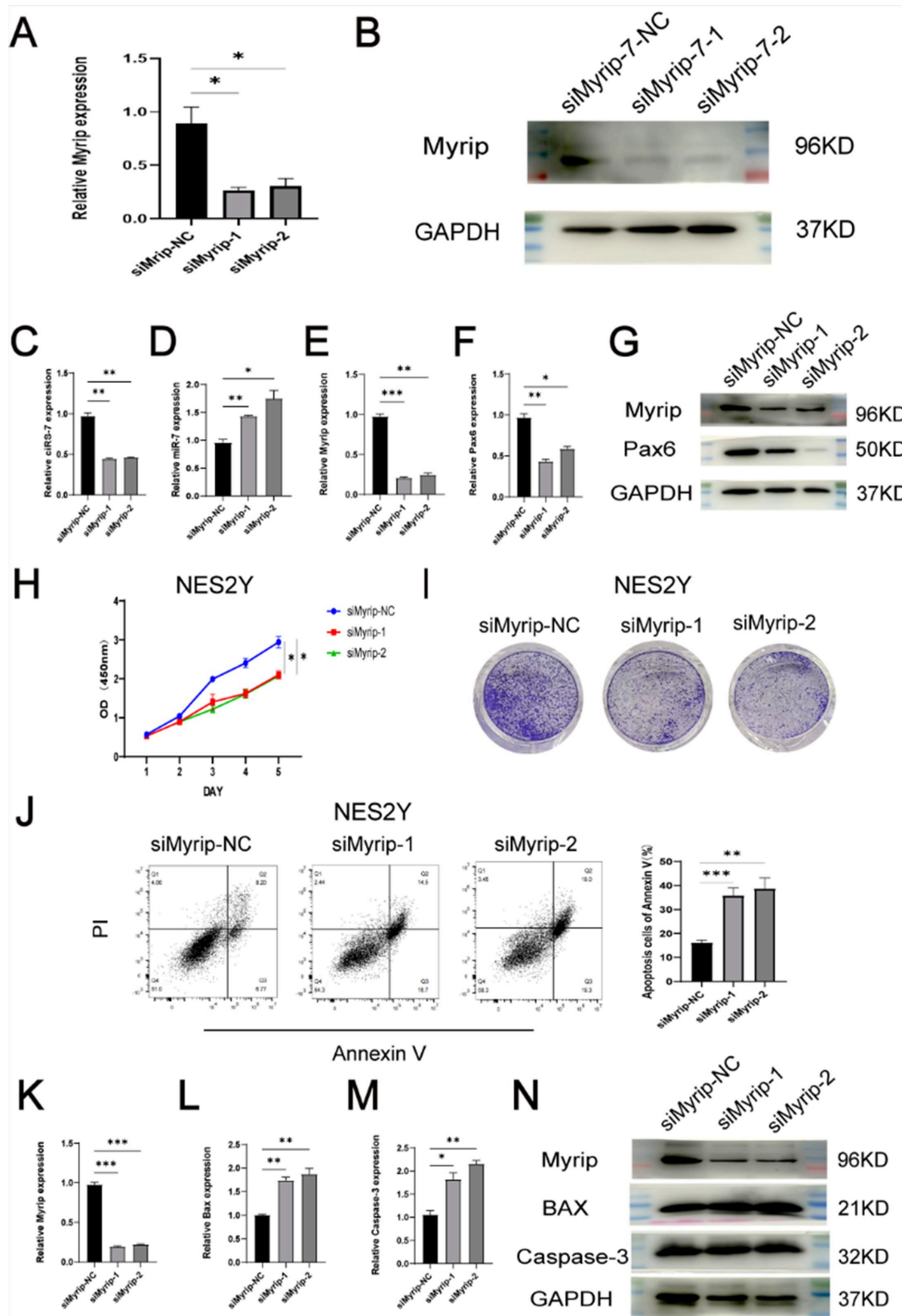


Figure 5

Functional characterization of Myrip in NES2Y cells. (A, B) RT-qPCR and Western blot confirm efficient knockdown of Myrip. (C-F) mRNA Expression of Myrip-Related Genes in NES2Y Cells After Myrip Silencing. (G) Protein levels of upstream and downstream genes following Myrip silencing. (H, I) CCK-8 and colony formation assays show that Myrip knockdown inhibits cell proliferation. (J) Flow cytometry

reveals increased apoptosis after Myrip silencing. (K-M) RT-qPCR and Western blot demonstrate upregulation of Bax and Caspase-3 expression upon Myrip knockdown. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [1MGpLVCirhsacirc0001946.docx](#)