Original paper

MiR-21-5p regulates colon adenocarcinoma cell progression and epithelial-mesenchymal transition by negatively regulating Tensin 1

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Colon adenocarcinoma (COAD) is one of the most prevalent forms of cancer in the world. Still, the molecular mechanism of COAD development remains unknown, making it especially important to investigate the molecular mechanism of COAD development and identify new therapeutic targets.

A real-time fluorescence quantification polymerase chain reaction (RT-qPCR) was used to determine the level of miR-21-5p expression in COAD tissues and cell lines. Both miR-21-5p silencing and overexpression were performed in LOVO and T84 cell lines. Cell viability, apoptosis rate, migration, and invasion ability were determined using MTT, flow cytometry, and the Transwell assay, respectively. Western blot was applied to detect the levels of protein expression associated with the epithelial-mesenchymal transition (EMT).

Using a dual luciferase reporter gene, the targeting connection among miR-21-5p and Tensin 1 was validated. Tensin 1 expression was silenced to investigate its effect on miR-21-5p inhibitor activity in COAD cells. Subcutaneous tumor-forming animal experiments in nude mice were used to investigate the effect of miR-21-5p on COAD tumor growth *in vivo*. Ki-67 expression was identified through immunohistochemistry. MiR-21-5p was found in high concentrations in COAD tissues and cells. Overexpression of miR-21-5p increased COAD cell line viability and EMT, facilitated cell migration and invasion, and inhibited apoptosis. Tensin 1 was regulated negatively by miR-21-5p. Tensin 1 silencing reversed the effect of miR-21-5p silencing on COAD cells. Subcutaneous tumor formation experiments in nude mice revealed that inhibiting miR-21-5p expression slowed the growth rate of tumor volume.

According to immunohistochemical results, the percentage of Ki-67-positive cells was significantly lower in the anti-miR-21-5p group. MiR-21-5p levels were upregulated in COAD cells, and reducing miR-21-5p expression inhibited COAD cell viability, migration, invasion, and EMT *in vitro*. Tensin 1 negatively regulated miR-21-5p, which regulated COAD cell and EMT progression.

Key words: miR-21-5p, colon adenocarcinoma, TNS1, EMT procession/progression.

Introduction

Colon adenocarcinoma (COAD) is a common digestive system tumor with high morbidity and mortality rates worldwide [1]. According to global COAD epidemiology in 2020 and projections for 2040, 1.9 million new cases of COAD were reported globally, with approximately 940,000 cases dying as a result of COAD. Globally, the number of new cases of COAD is expected to reach 3.2 million by 2040 [2]. Colon adenocarcinoma is caused by a number of factors, including a low-fiber diet, excessive alcohol consumption, obesity, smoking, and age [3, 4]; the clinical manifestations are typically characterized by abdominal pain, diarrhea, blood in the stools, persistent fatigue, and weight loss. Colon adenocarcinoma treatment is determined by the severity of the disease, the position of the tumor, and the patient's health status. Early COAD treatment generally includes surgical resection, chemotherapy, radiotherapy, immunotherapy, and targeted therapy [5–7]; however, due to the lack of obvious symptoms in early COAD patients and a lack of awareness of physical examination, most COAD patients have already advanced to the middle and late stages, or even metastases, when they are detected, and they lose the chance of surgery. The primary cause of patient death, as well as the focus and difficulty of COAD treatment, is COAD invasion and metastasis [8].

The epithelial-mesenchymal transition (EMT) is a cell biological event in which epithelial cells are converted to mesenchymal cells [9]. Recently, it has been discovered that EMT plays a crucial function in cancer invasion and metastasis. Cancer cells can adapt to constant changes in the microenvironment *via* EMT, resulting in increased migration and invasion ability [10]. It has also been demonstrated that miRNAs (microRNAs) play a role in the regulation of the EMT process [11].

MiRNAs are non-coding RNA molecules with 20–23 nucleotides. They are also known as small molecule RNAs [12]. MiRNAs are important regulators of gene expression in cells. They reduce target protein synthesis by binding to the 3' untranslated region (3' UTR) of the goal messenger RNA (mRNA) and lowering its translation rate; they can also contribute to the target mRNA's degradation and destabilization, resulting in a decrease in the amount of the mRNA; or they can indirectly affect target gene expression by inhibiting transcription [13]. MiRNAs are important in a variety of biological processes, including embryonic growth, control of the cell cycle, immune response, apoptosis, and tumorigenesis [14]. Abnormal miRNA expression has been linked to the pathogenesis of a number of diseases, so studying miRNAs not only contributes to a better understanding of cellular regulatory mechanisms, but it may also open up new avenues for disease diagnosis and treatment [15, 16].

The miR-21-5p gene is a member of the miR-21 family. MiR-21-5p is derived from the 5' end of miR-21 [17], which has important biological functions, particularly in cancer. MiR-21-5p has been found to be overexpressed in many kinds of cancers, including breast, lung, colorectal, and pancreatic cancers [18–21]. MiR-21-5p has the ability to suppress the levels of several oncogenes, thereby promoting cancer cell growth, invasion, and anti-apoptosis. As a result, research on miR-21-5p has received a lot of attention in the fields of cancer biology and molecular medicine. Using the GEPIA database, it was discovered that MiR-21-5p expression increased greatly in COAD tissues. The exact molecular process by which miR-21-5p influences COAD progression, however, remains unknown.

Tensin 1 (TNS1) is a cytoplasmic phosphorylated protein with an estimated molecular mass of 220 kDa which belongs to the Tensin protein family and its function is linked to cell motility, viscosity, and signal transduction [22]. Cancer is a disease that results from the involuntary expansion and spread of malignant cells, often through EMT, which allows them to gain a greater capacity for migration and invasion, allowing them to break through the basement membrane and enter blood vessels or lymphatic vessels, where they can spread elsewhere in the body and form distant metastases. Relevant research has shown that EMT progression is accompanied by TNS1 upregulation during the development of lung adenocarcinoma, influencing cancer progression and treatment [23]. And the mechanism underlying TNS1's role in the development of COAD remains unknown.

The purpose of this study was to look at the expression and biological function of miR-21-5p in human COAD tissues, paracancerous tissues, and COAD cell lines to further clarify miR-21-5p's role in the occurrence and progression of COAD. Cell function experiments were performed to investigate the effects of miR-21-5p expression on COAD cell viability, migration, invasion, and EMT. This study identifies new targets and treatment strategies for COAD.

Material and methods

Obtaining tissue specimens

The Ethics Committee of the School of Clinical Medicine, Jiangxi Medical College approved this study. Sixty-one COAD patients who underwent surgical treatment at the School of Clinical Medicine, Jiangxi Medical College had their COAD tissues and corresponding paracancerous tissue specimens pathologically confirmed. Following dissociation, the specimens were quickly immersed in liquid nitrogen for transport, then frozen and stored in a –80°C refrigerator. Preoperative radiotherapy or biologic therapy were not administered to any of the patients, and everyone signed a well-informed approval/consent form.

Methods for culturing and transfecting cells

Procell Life Science and Technology Co., Ltd. (Wuhan, China) provided three COAD cell lines, SW1116, LOVO, and T84. Fuxiang Biotechnology Co., Ltd. (Shanghai, China) provided the human normal colon epithelial cells NCM-460. DMEM basal medium (R20163, Yuanye, Shanghai, China) was used to culture the cells. Complete medium was made by mixing DMEM basal medium, 10% fetal bovine serum and penicillin/streptomycin (1%, S17034, Yuanye). All cells were cultured at 37°C in a 5% CO_2 incubator. Fulen Gen (Guangzhou, China) provided miR-21-5p mimics (mimics) and negative controls (mimics-NC), miR-21-5p inhibitors (inhibitors) and negative controls (inhibitors-NC), TNS1 silencing expression vectors (si-TNS1) and negative controls (si-NC), miR-21-5p inhibitors *in vivo* (anti-miR-21-5p) and negative controls (anti-NC). The vectors listed above were transmitted into LOVO and T84 cells with Lipofectamine 3000 (L3000001, Invitrogen, Austin, TX, USA), respectively.

RNA extraction and polymerase chain reaction for real-time fluorescence quantification

After extracting RNA from cells and tissues with the NcmZol reagent (R40033, Yuanye), reverse transcription was carried out using the RT Master Mix for qPCR II kit (HY-K0510A, MedChem Express, Monmouth Junction, NJ, USA). SYBR Green (HY-K0501, MedChem Express) for qPCR reactions. The following primer sequences were examined in this experiment: *miR-21-5p*'F: 5′-TCGGCAG GTAGCTTATCAGACTGA-3′, R:5′-CAGTGCAGG-GTCCGAGGTAT-3′; *U6*: F: 5′-CTCGCTTCGGCA GCACA-3′, R:5′-AACGCTTCACGAATTTGCGT-3′: *TNS1*: F: 5′-ACCGAGGCAGGATAGGAGTT-3′, R:5′-CACGTGGTGCAGAAACAAGG-3′; β-*actin*: F:5′-GCACCACACCTTCTACAATGAG-3′, R:5′-GA-TAGCACAGCCTGGATAGCA-3′.

Method of Western blot detection

Total proteins were extracted from cells and tissues using RIPA lysate, respectively, and protein concentration was quantified using a BCA kit (R21250, Yuanye). They were then separated using an SDS-PAGE gel kit (R21148, Yuanye), PVDF transmembrane, skimmed milk powder sealing, primary and secondary antibody incubation, and finally, ECL luminescent solution (PE0010, Solarbio, Beijing, China) was added, and the target bands were detected using a ChemDocTM XRS + System instrument and analyzed using Image J software for gray value. The experiment was repeated three times. The following antibodies were used in the experiments: anti-epithelial cell calmodulin (E-cadherin) antibody (ab227639, 1 : 100, Abcam, Waltham, MA, USA), anti-N-cadherin antibody (ab70611, 1 : 5000, Abcam), anti-Vimentin antibody (ab92547, 1 : 1000, Abcam), anti-actin antibody (ab8227, 1 : 1000, Abcam), anti-Snail antibody (ab216347, 1 : 1000, Abcam), anti-zinc finger E-box binding homeobox 1 (ZEB1) antibody (ab303480, 1 : 1000, Abcam), and anti-TNS1 (ab233133, 1 : 1000, Abcam) primary antibody. After adding various primary antibodies, the mixture was incubated for an entire night at 4°C. Next, Goat Anti-Rabbit IgG H and L (HRP), the secondary antibody, was added, and the mixture was incubated for 2 hours at room temperature.

MTT detection technique

Cell viability was determined by performing the MTT assay, in which cells in the logarithmic growth phase were prepared as a suspension, then inoculated in a 96-well plate and incubated in an incubator until the cells became adherent to the wall. Following the completion of the incubation, MTT solution (ST316, Beyotime Biotechnology, Shanghai, China) was added to each well and incubated, followed by the removal of the medium and the addition of dimethyl sulfoxide (ST1276, Beyotime Biotechnology) to each well. An enzyme meter was used to measure the absorbance (OD) at 490 nm.

Flow cytometry analysis

Following transfection, apoptosis was observed after the cells in every group were cultured for 24 h. After aspirating the medium in the cell culture flask, the cells were washed with phosphate-buffered saline (PBS) solution, then PBS was aspirated, trypsin was added and incubated, and when the cytoplasm began to retract and the cells were dispersed, trypsin was discarded and fresh medium was added. The cell mass was broken up and thoroughly mixed to create a single cell suspension. Prior to centrifugation, the cell suspension underwent two rounds of washing in PBS solution. After discarding the supernatant, the cells were resuspended in a propidium iodide working solution (S19136, Yuanye). Finally, flow cytometry (BD FACSLyric, BD Biosciences, San Jose, CA, USA) was used to detect the cells after they had been incubated for 15 min.

Dual luciferase reporter assay

Tensin 1 wild-type (WT) and mutant (MUT) luciferase vectors were created (TNS1-WT and TNS1-MUT). TNS1-WT/TNS1-MUT cells were inoculated and cultured in 12-well plates, and TNS1-WT/TNS1-MUT cells were co-transfected with *miR-21-5p* mimic by Lipofectamine 3000 (L3000001, Invitrogen), with mimic-NC as the control group. The dual-luciferase reporter gene assay kit's instructions (RG027, Beyotime Biotechnology) were followed to perform the firefly luciferase activity assay 48 hours after transfection.

Tumor formation in nude mice

Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) provided BALB/c male nude mice, 6–8 weeks old, SPF grade, weighing 18–20 g. The right axillary skin of nude mice was selected as the injection site, and different treatment groups of LOVO cell suspensions (LOVO cells transfected with anti-*miR-21-5p* and LOVO cell suspensions transfected with anti-NC) were inoculated at 1.5×10^6 cells/mice, respectively, and the nude mice continued to be placed in the sterile laminar flow room after inoculation. Tumor growth was observed and photographed at 7-day intervals following surgery, and the size of the subcutaneous tumors was measured using vernier calipers. At the same time, the expression level of *miR-21-5p* in tumor tissues of mice was detected by RT-qPCR every 7 days. On the 35th day, the mice were killed by dislocation of the neck and the tumor masses were weighed.

Immunohistochemical staining

Routine dewaxing, hydration, citric acid high-pressure antigen repair, H_2O_2 closure of endogenous peroxidase activity, normal goat serum closure, and overnight incubation with anti-Ki-67 antibody (ab16667, 1 : 200, Abcam) were performed. Following that, a dropwise addition of HRP (ab6721, 1 : 1000, Abcam) secondary antibody was performed and incubated at room temperature for 1 hour, DAB (DA1010, Solarbio, Beijing, China) was used for color development, hematoxylin was used for re-staining, and neutral gum was used to seal the sections. The sections were examined microscopically with an Olympus BX51 microscope (Olympus, Tokyo, Japan), and images were captured and analyzed.

Statistical analysis

In this experiment, SPSS 21.0 (IBM Corporation, USA) and GraphPad-Prism 9.0 (GraphPad Software, USA) software were used for statistical analysis. To compare the differences between the two groups, the *t*-test was used, and the ANOVA was applied to compare multiple groups. * $p < 0.05$,** $p < 0.01$, *** $p < 0.001$ were deemed statistically significant.

Results

MiR-21-5p was highly expressed in colon adenocarcinoma tissues and cells

This study used RT-qPCR to examine the expression of *miR-21-5p* in cancer and paracancerous tissues in order to clarify the expression of *miR-21-5p* in COAD. The level of *miR-21-5p* was found to be higher in cancer tissues from COAD patients (Figure 1A). *MiR-21-5p* expression was also found to be lower in patients with TNM stages I~II than in patients with stages III~V (Figure 1B). Following that, the expression of *miR-21-5p* in COAD cell lines and normal human colonic epithelial cells was examined, and the findings revealed that *miR-21-5p* levels were higher in COAD cell lines, including higher expression in LOVO and T84 cells (Figure 1C).

The effect of *miR-21-5p* overexpression on cells

The expression of *miR-21-5p* was significantly increased in LOVO and T84 cells after transfection with *miR-21-5p* mimics, according to RT-qPCR results (Figure 2A). The cell viability was significantly increased after transfection with miR-21-5p mimics, according to MTT results (Figure 2B). Flow cytometry results revealed that transfection with miR-21-5p mimics significantly reduced the apoptosis rate of LOVO and T84 cells (Figure 2C, D). The migration and invasion abilities of LOVO cells and T84 cells were significantly increased after transfection with miR-21-5p mimics, according to Transwell assay results (Figure 2E–H). Epithelial-mesenchymal transition marker protein E-cadherin expression levels were significantly reduced after transfer with miR-21-5p mimics, whereas N-cadherin, Vimentin, Snail and ZEB1 expression levels were substantially elevated (Figure 2I–N). High levels of miR-21-5p improved viability, migration, invasion, and EMT while inhibiting mortality in A549 and H1299 cells.

The effects of suppressing miR-21-5p expression on cells

The expression of *miR-21-5p* in LOVO and T84 cells was significantly reduced after transfection with an inhibitor of miR-21-5p (Figure 3A), cell viability was significantly lowered (Figure 3B), and the apoptosis rate in LOVO and T84 cells was significantly increased (Figure 3C, D). Transwell assay results revealed that miR-21-5p inhibitor transfection significantly reduced the migration and invasion abilities of LOVO and T84 cells (Figure 3E–H). The expression levels of the EMT marker protein E-cadherin were significantly upregulated, while N-cadherin, Vimentin, Snail and ZEB1 were significantly downregulated (Figure 3I–N). The results show that down-regulation of miR-21-5p inhibited the viability, migration, invasion, and EMT processes of LOVO and T84 cells and promoted apoptosis.

Tensin 1 was negatively regulated by miR-21-5p.

We used the TargetScan (https://www.targetscan.org/vert_80/), miRWalk (https://www.uni-heidelberg.de/en) and GEPIA (http://cancer-pku.cn/) databases to predict the target genes of *miR-21-5p*'s action/activity, and the results indicated that TNS1 was the target gene (Figure 4A, 4B). Tensin 1 was found to be less expressed in COAD tissues and cell

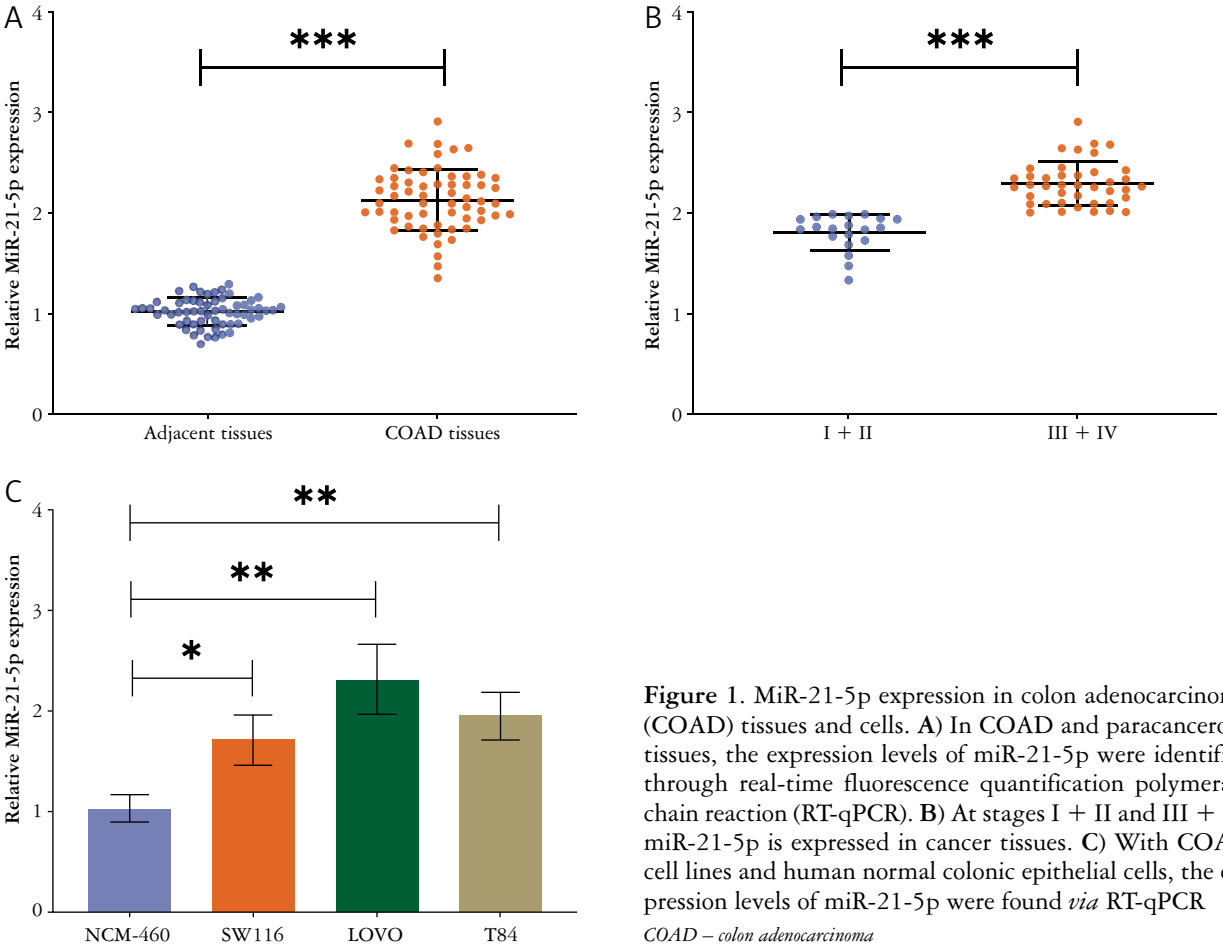


Figure 1. MiR-21-5p expression in colon adenocarcinoma (COAD) tissues and cells. **A**) In COAD and paracancerous tissues, the expression levels of miR-21-5p were identified through real-time fluorescence quantification polymerase chain reaction (RT-qPCR). **B**) At stages I + II and III + IV, miR-21-5p is expressed in cancer tissues. **C**) With COAD cell lines and human normal colonic epithelial cells, the expression levels of miR-21-5p were found *via* RT-qPCR

COAD – colon adenocarcinoma

lines, according to RT-qPCR and Western blot results (Figure 4C–F). The dual luciferase reporter gene results showed that luciferase activity was significantly reduced in cells co-transfected with *miR-21-5p* mimics and TNS1-WT-3'UTR plasmid (Figure 4G, H), confirming the direct targeting connection between TNSI and *miR-21-5p*. *MiR-21-5p* and TNS1 had a linearly negative correlation, according to a Pearson analysis (Figure 4I).

Silencing Tensin 1 expression reduced the inhibitory effect of miR-21-5p silencing on cells

Tensin 1 expression was significantly reduced in LOVO and T84 cells after si-TNS1 transfection, according to Western blot results (Figure 5A). MiR-21-5p inhibitor dramatically reduced cell viability, according to MTT and flow cytometry data, but si-TNS1 lessened the inhibitory effect of miR-21-5p inhibitor on COAD cells (Figure 5B–D). Transwell assays demonstrated that si-TNS1 transfection greatly boosted LOVO and T84 cells' capacity for migration and invasion (Figure 5E–H). In COAD cells, si-TNS1 transfection resulted in a significant down-regulation of E-cadherin and TNS1, and an up-regulation of N-cadherin, Vimentin, Snail and ZEB1 levels (Figure 5I–O). These results imply that TNS1 silencing lessens the miR-21-5p inhibitors' inhibitory effects on the migration, invasion, and viability of LOVO and T84 cells.

The effect of inhibiting miR-21-5p expression on tumor growth *in vivo*

In this study, to create a nude mouse xenograft model, LOVO cells from various treatment groups were injected into the right axilla of the naked/nude mice. The results of RT-qPCR every 7 days demonstrated that compared to the anti-NC group, the expression level of *miR-21-5p* in the tumor tissues of the anti-miR-21-5p group was significantly decreased (Figure 6A), indicating that miR-21-5p was effectively silenced throughout the experiment. The findings demonstrated that compared to the anti-NC group, subcutaneous tumors in mice with LOVO cells in the anti-miR-21-5p group grew more slowly (Figure 6B–D). The IHC study's findings demonstrated that the anti-miR-21-5p group had a significantly smaller number of Ki-67-positive cells in tumor tissues formed by LOVO cells than the anti-NC group (Figure 6E), indicating that tumor cells' proliferation ability was reduced, according to immunohistochemical staining results.

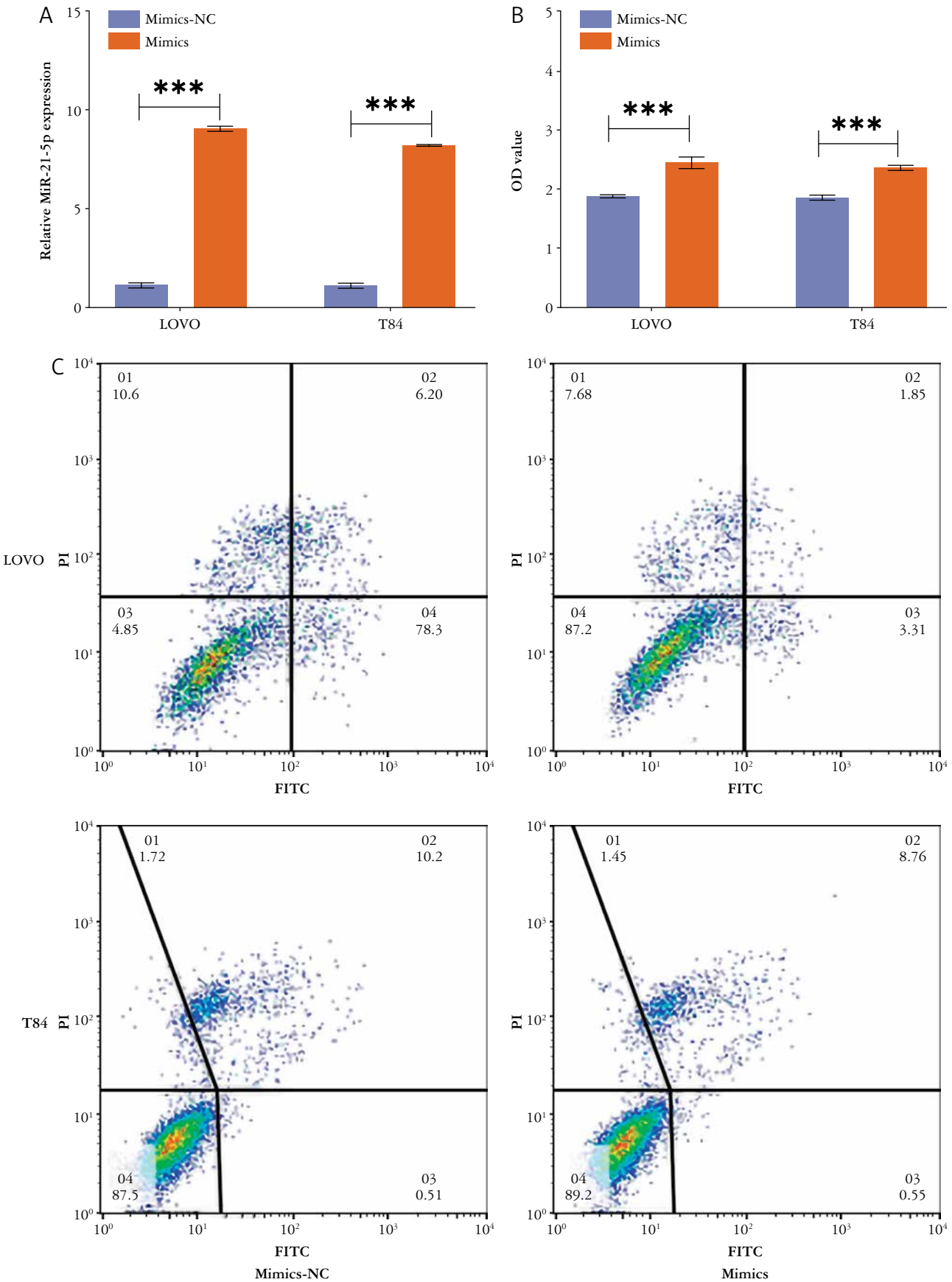
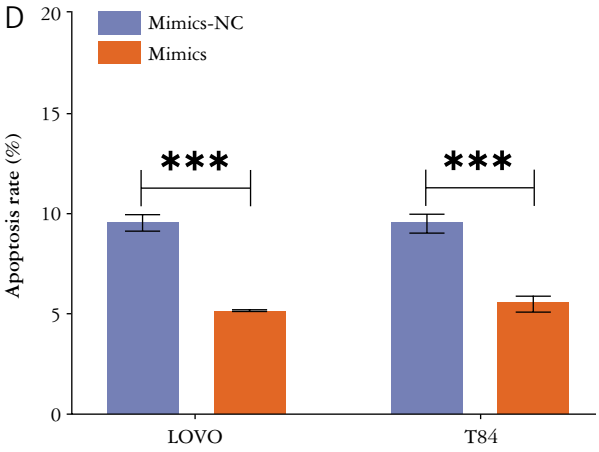
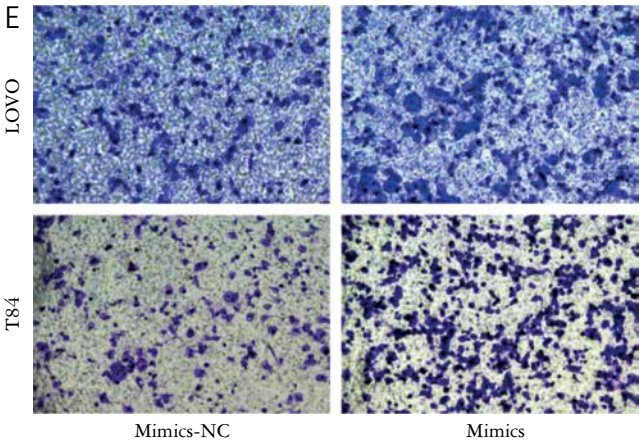
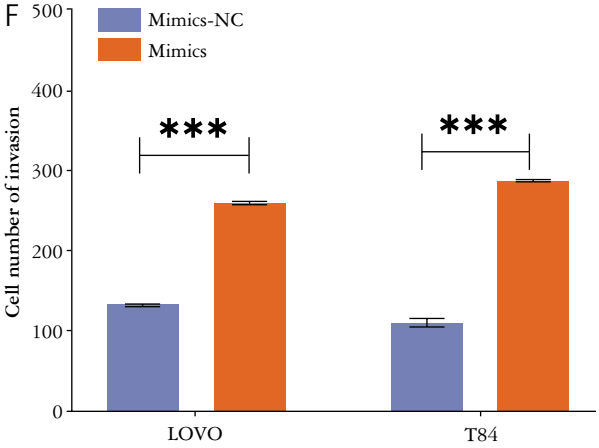
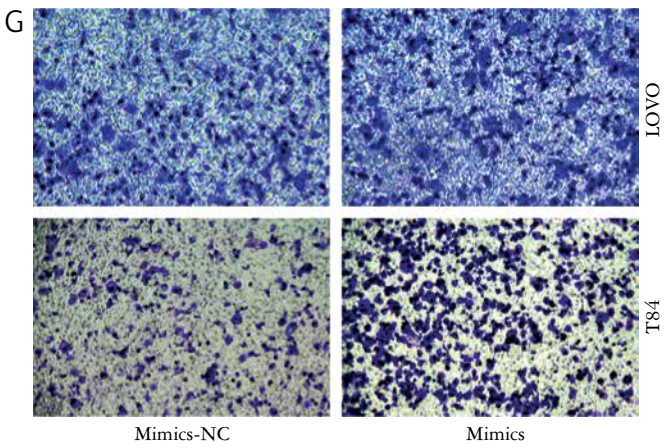


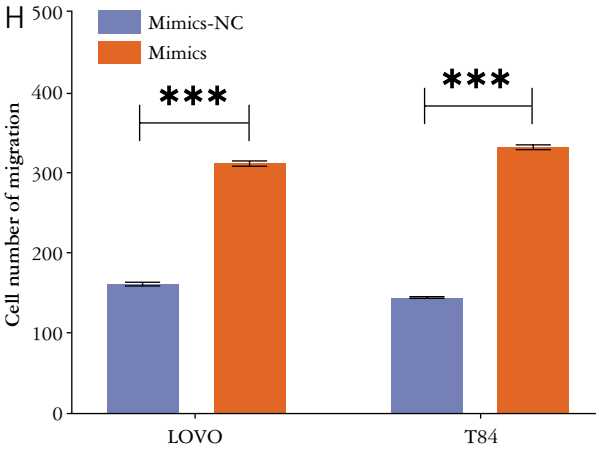
Figure 2. Cellular effects of miR-21-5p overexpression. **A**) MiR-21-5p expression was measured in LOVO and T84 cells using real-time fluorescence quantification polymerase chain reaction. **B**) The MTT assay is used to detect cell viability. **C, D**) Assay for detecting apoptosis using flow cytometry. **E–H**) A Transwell assay is used to detect cell invasion and migration. **I–N**) Epithelial-mesenchymal transition marker gene expression (E-cadherin, N-cadherin, Vimentin, Snail and ZEB1) was analyzed using Western blot

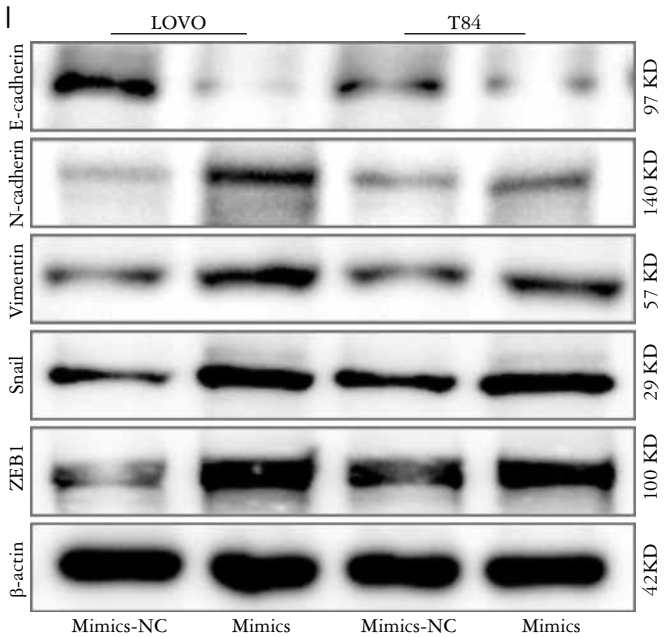


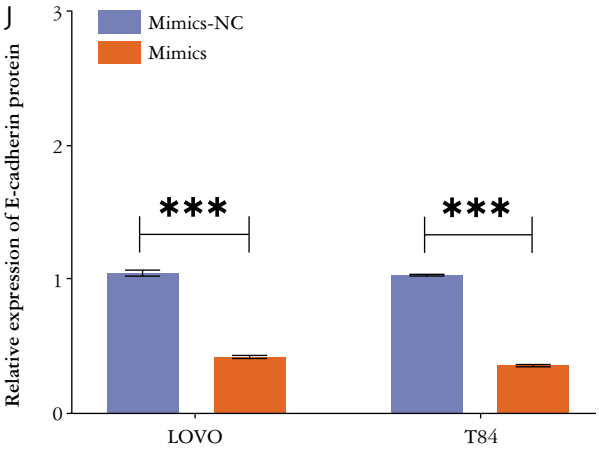












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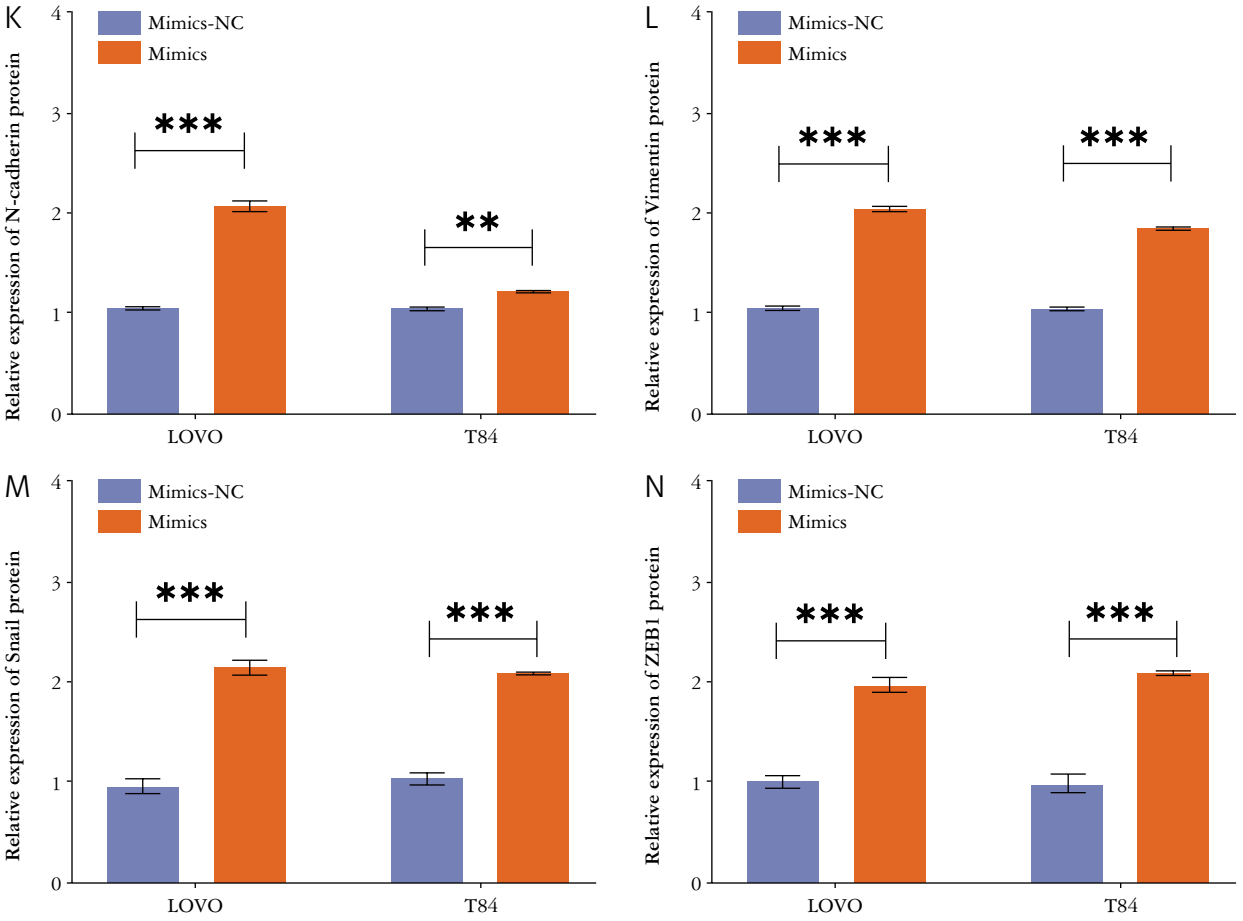

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Discussion

Colon adenocarcinoma is one of the most common malignant tumors in China and around the world, and it affects women more than men [24]. Although the incidence and mortality rates of COAD have been decreasing over the last 20 years, there are some differences in the incidence and mortality rates among different countries, particularly in developing countries where the incidence of COAD is on the rise [25]. Tumor metastasis is the primary cause of COAD pathogenicity and mortality, and tumor cell EMT is essential for tumor metastasis.

Epithelial-mesenchymal transition is the process by which cells change from being epithelial to being mesenchymal. Significant alterations in cell morphology, cellular connectivity, cytoarchitecture and gene expression are all part of this process [26]. Among these, the expression of E-cadherin is down-regulated while that of Vimentin, N-cadherin, Snail, ZEB1, *etc.* is up-regulated [27]. Presently, an increasing number of studies have shown that EMT contributes to the metastatic process of COAD. Wang *et al*. [28] discovered that by controlling EMT in tumor cells, the proprotein convertase subtilisin/kexin type 9 (PCSK9) may encourage the metastasis of COAD cells. Astragalus inhibited COAD metastasis, according to Liu *et al*., by upregulating E-cadherin expression during EMT and downregulating Vimentin and N-cadherin expression [29]. Investigating the connection between novel gene functions and the occurrence of EMT in COAD is crucial because tumorigenesis EMT is a process regulated by multiple genes.

According to recent research, miRNAs have the ability to target and regulate genes and signaling pathways linked to EMT in cells [30, 31]. Different miRNAs may contribute in different ways to different illnesses. Wang *et al*., for instance, discovered that miR-19a/b can stimulate EMT in glioma cells, which results in tumor cell invasion and proliferation [32]. Human retinal pigment epithelium is shielded from EMT by miR-302d, as demonstrated by Hu *et al*. [33]. The first finding of this study was the high expression of miR-21-5p in COAD, which indicated that miR-21-5p aided in the progression of COAD. Following that, we created miR-21-5p silencing and overexpression vectors and transfected them to COAD cells. It was discovered that overexpression of miR-21-5p increased the expression of N-cadherin and Vimentin while downregulating the expression of E-cadherin. Thus, miR-21-5p may facilitate COAD's EMT process. Comparable to what we dis-

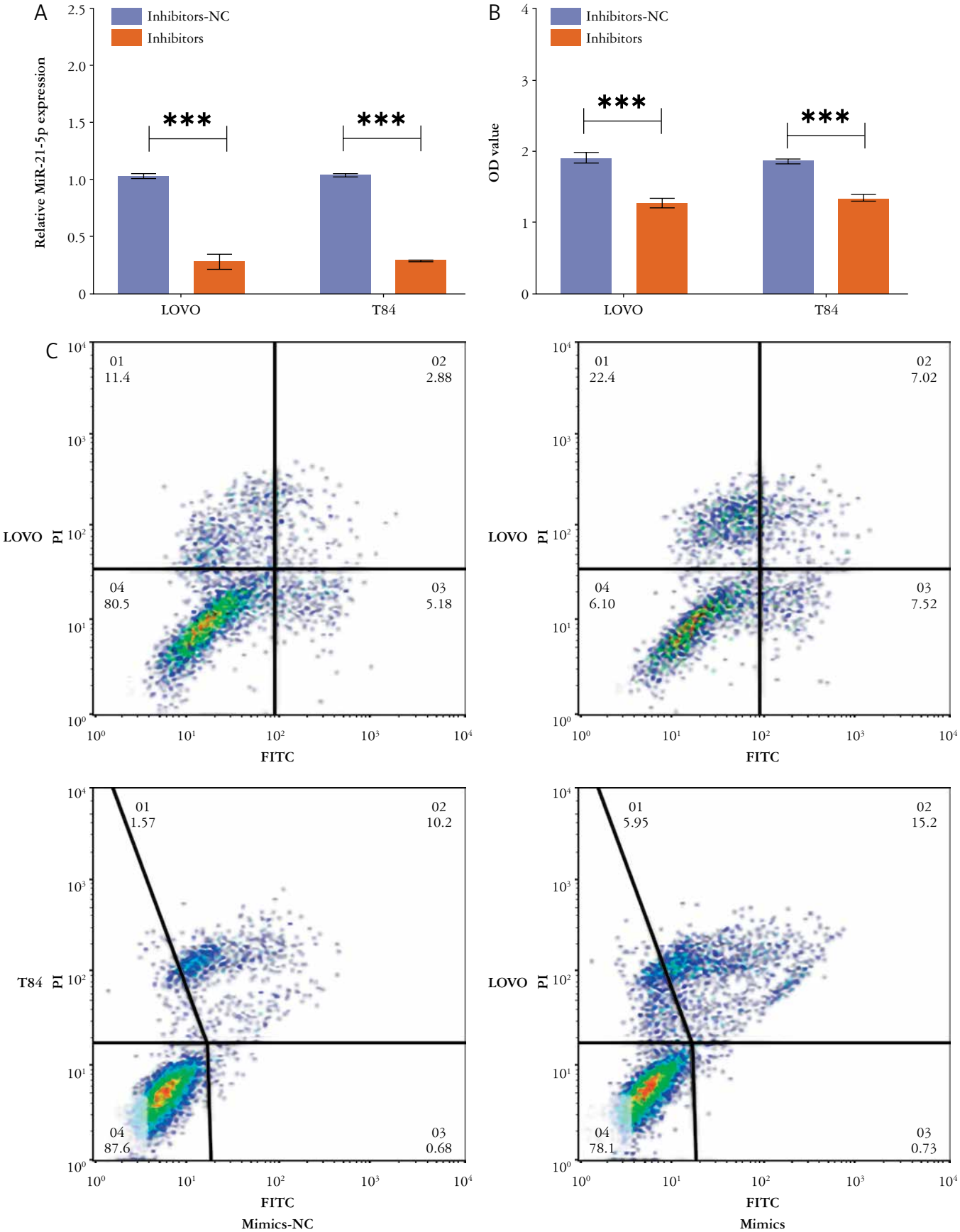
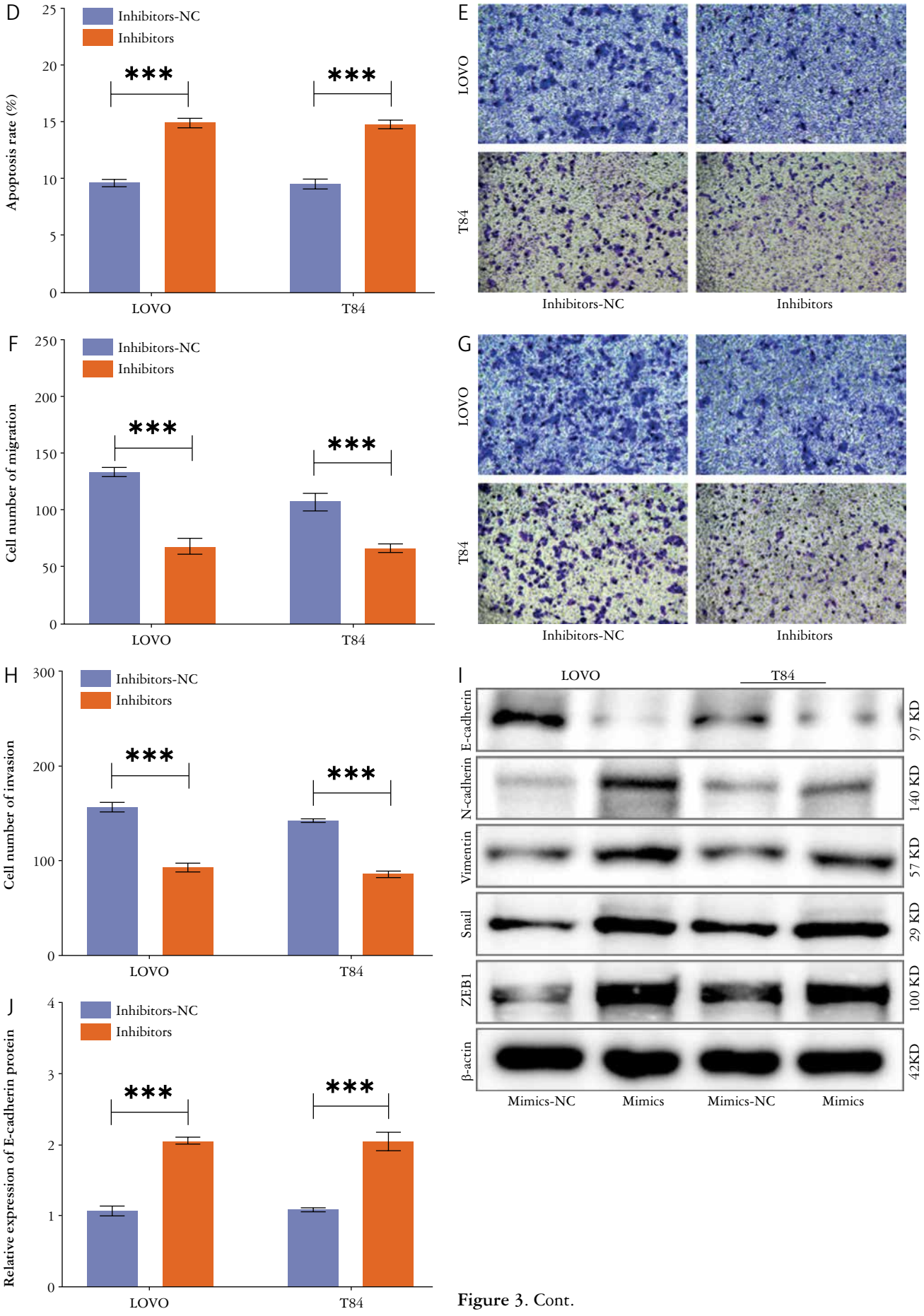


Figure 3. Cellular effects of silencing miR-21-5p expression. **A**) MiR-21-5p expression was measured in LOVO and T84 cells using real-time fluorescence quantification polymerase chain reaction. B) The MTT assay is used to detect cell viability. **C, D**) Assay for detecting apoptosis using flow cytometry. **E–H**) A Transwell assay is used to detect cell invasion and migration. **I–N**) Epithelial-mesenchymal transition marker gene expression (E-cadherin, N-cadherin, Vimentin, Snail and ZEB1) was analyzed using Western blot



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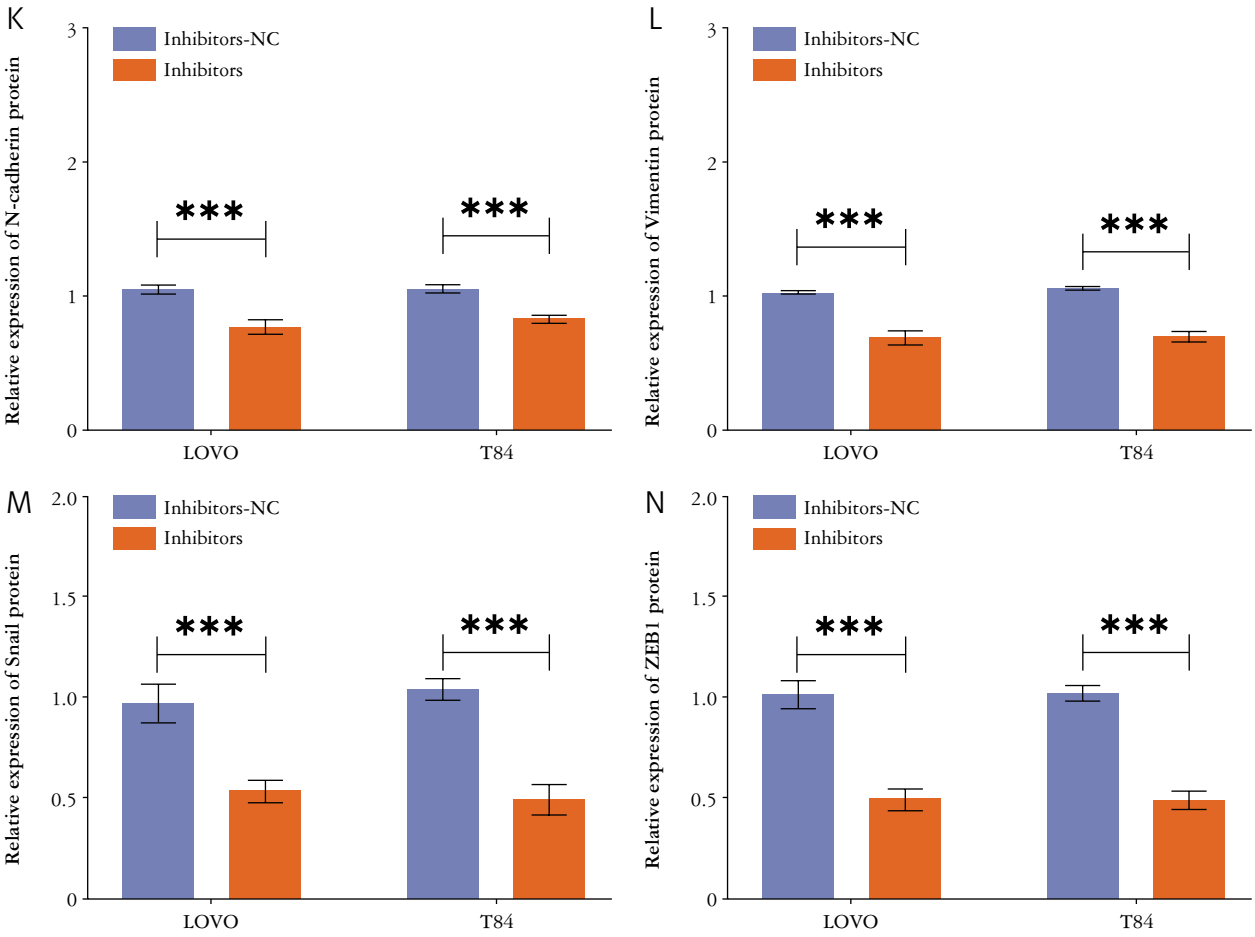

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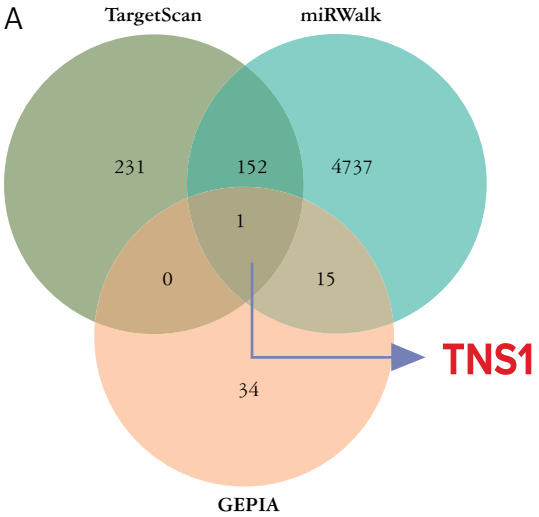
covered, miR-21-5p expression levels were markedly increased in hepatocellular, laryngeal, and esophageal cancers, and they facilitated the growth and metastasis of tumor cells by encouraging EMT [34–36]. Given that miRNAs bind to particular target genes to accomplish a variety of tasks, we postulated that miR-21-5p target genes would play a role in the regulation of EMT mechanism. Studying the target genes of miR-21-5p's action/activity is crucial to improving the precision of COAD diagnosis and treatment, as the target genes that govern the development of EMT in COAD remain unknown.

Using a gene database, we predicted that TNS1 would be the target gene of miR-21-5p, the two were confirmed to be targeted by the dual luciferase reporter gene. A transmembrane junctional protein called TNS1 is located between the cytoskeleton and the extracellular matrix. Tensin 1 proteins interact



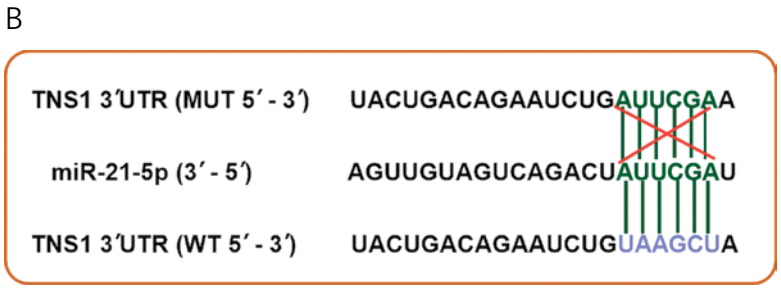
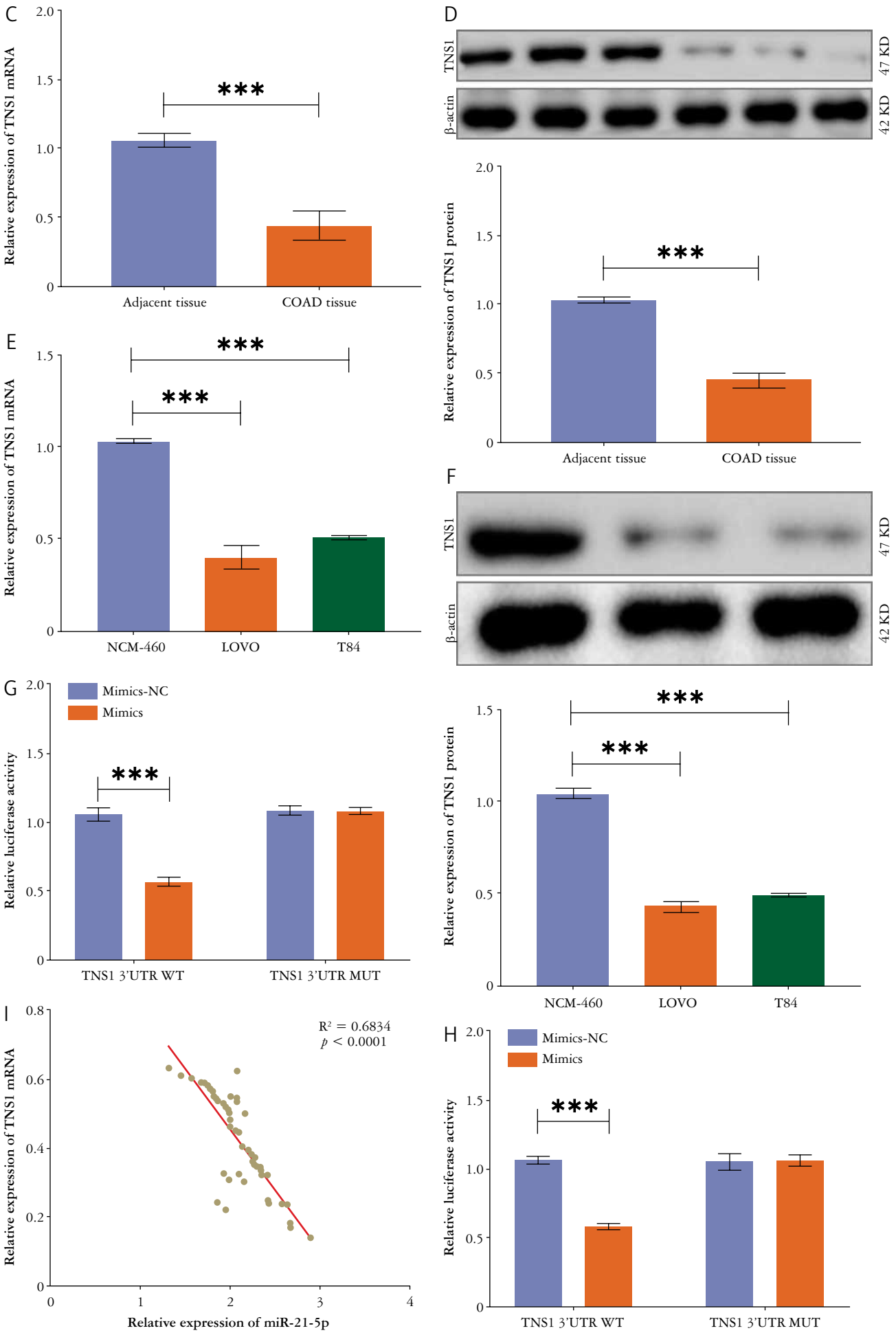


Figure 4. MiR-21-5p-targeted negative regulation of Tensin 1 (TNS1). **A**) Prediction of MiR-21-5p downstream target genes. **B**) MiR-21-5p binding site on TNS1. **C–F**) TNS1 expression was measured in tissues and cells using qRT-PCR and Western blot. **G, H**) A dual luciferase reporter assay confirms miR-21-5p targeting to TNS1. **I**) TNS1 and wmiR-21-5p have a linear relationship



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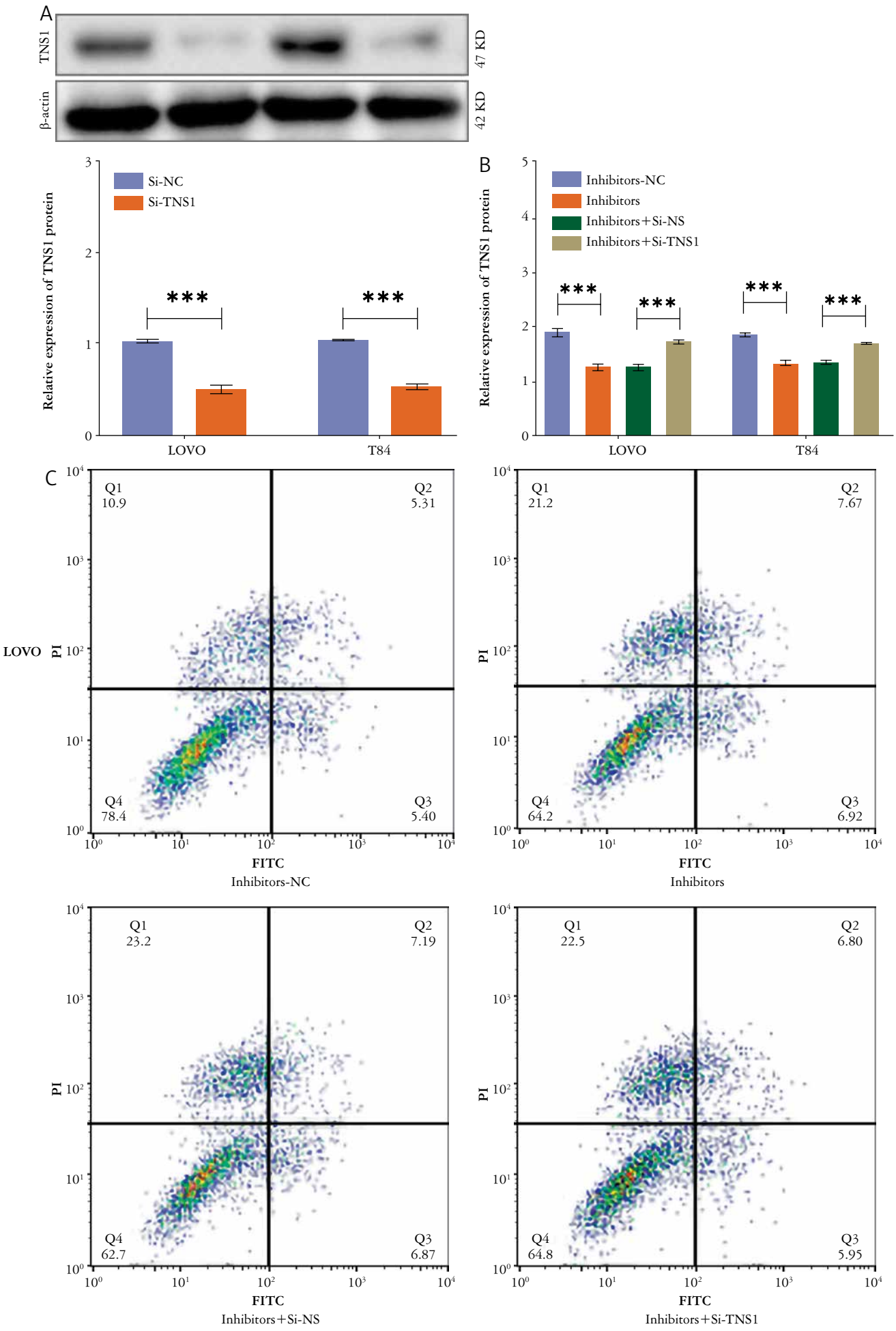
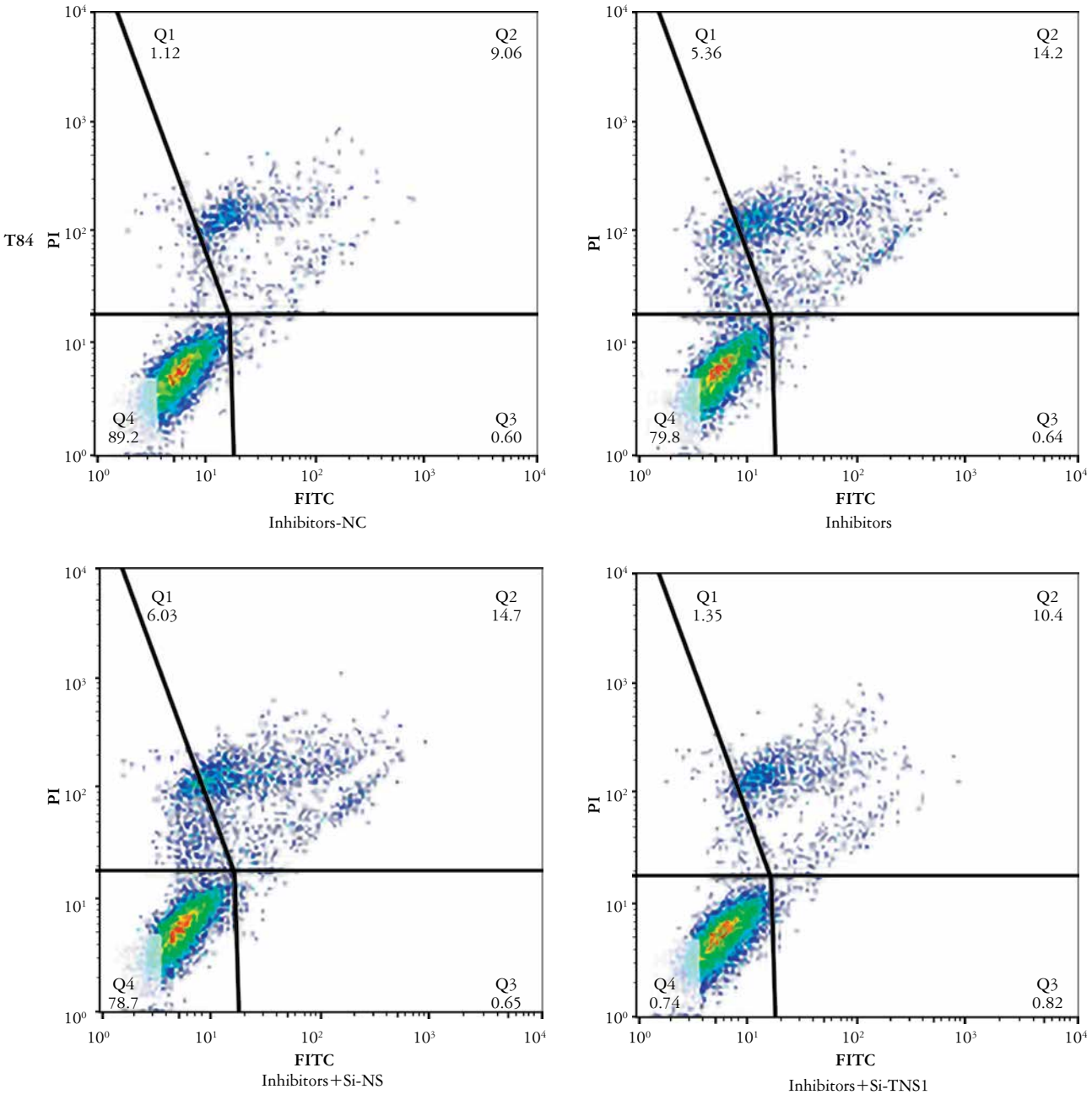
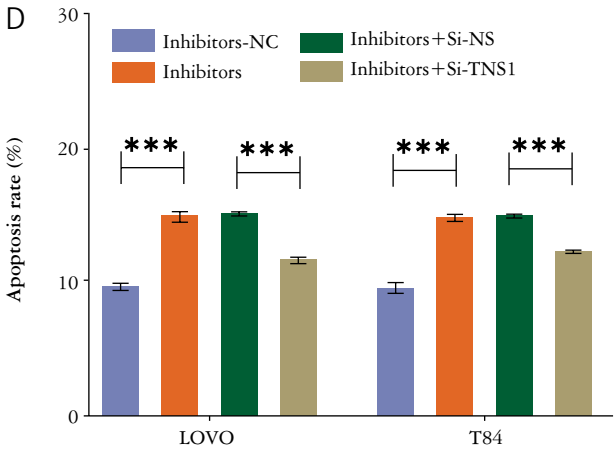


Figure 5. Silencing Tensin 1 (TNS1) expression reduced the inhibitory effect of miR-21-5p silencing on cells. **A**) TNS1 protein expression levels were determined using a Western blot after TNS1 silencing. **B**) MTT assay for cell viability detection. **C, D**) Assay for detecting apoptosis using flow cytometry. E–H) A Transwell assay is used to detect cell invasion and migration. **I–O**) Epithelial-mesenchymal transition marker protein expression (E-cadherin, N-cadherin, Vimentin, Snail and ZEB1) and TNS1 expression were analyzed using Western blot





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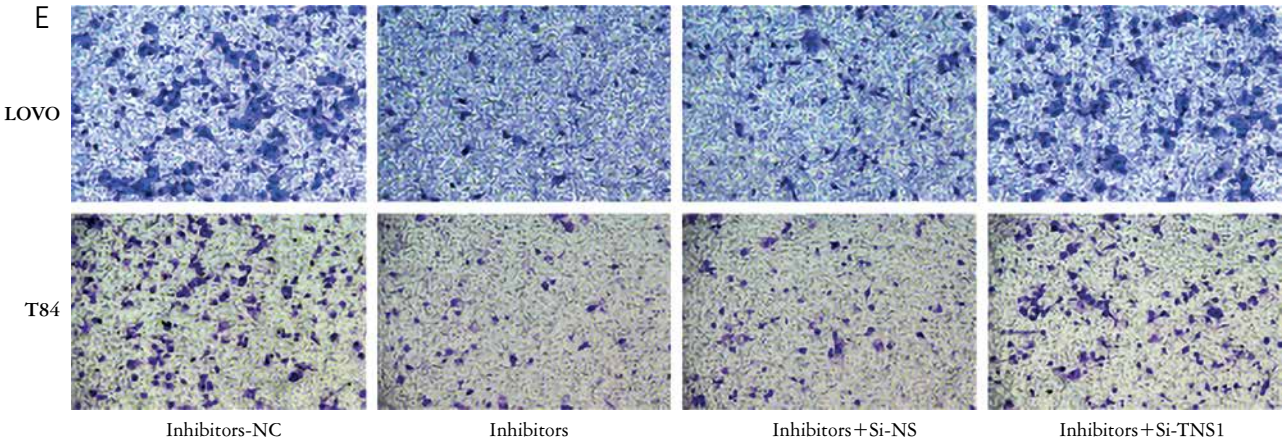

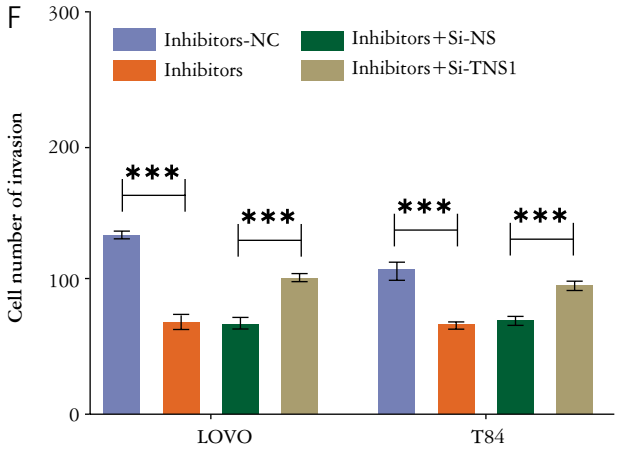

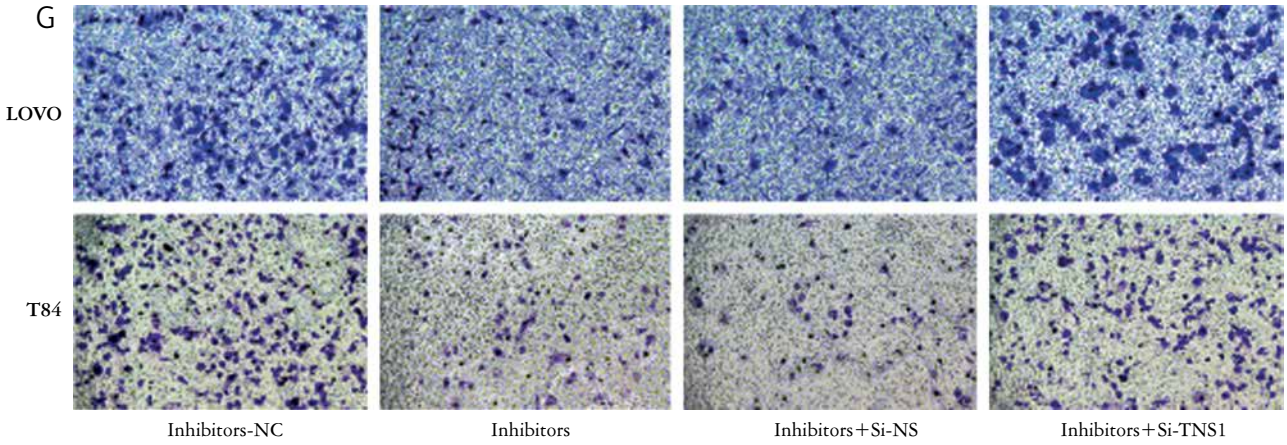

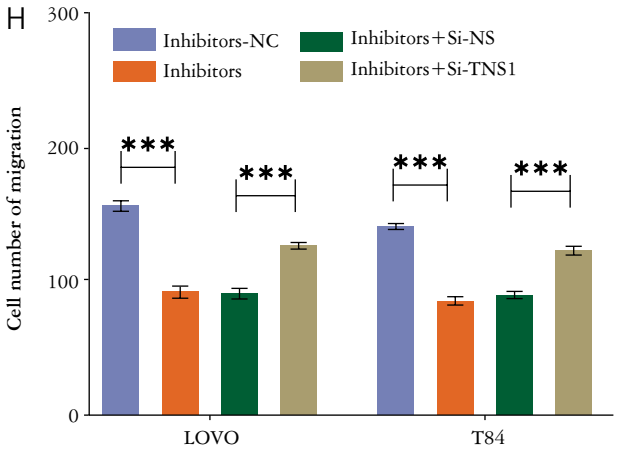

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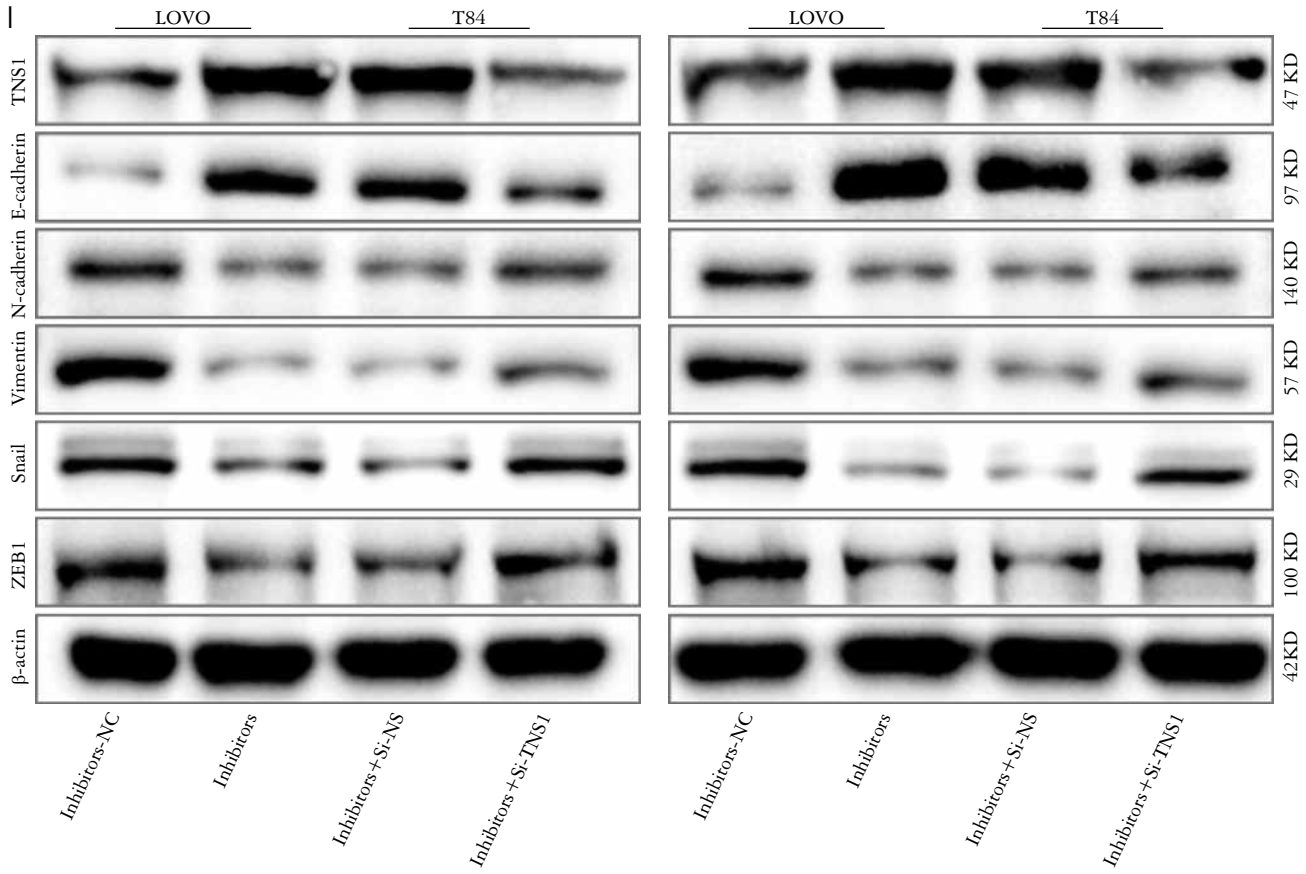

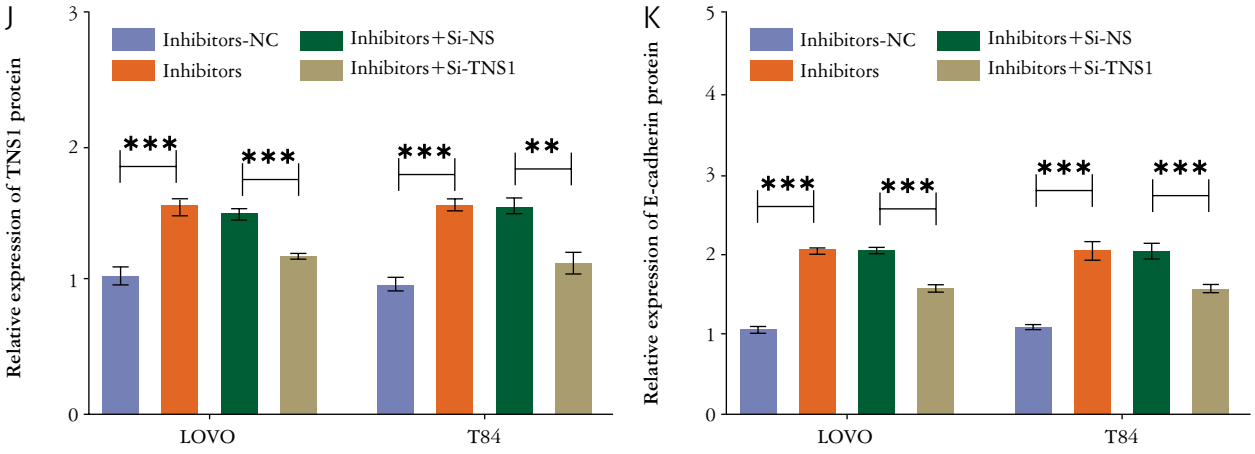

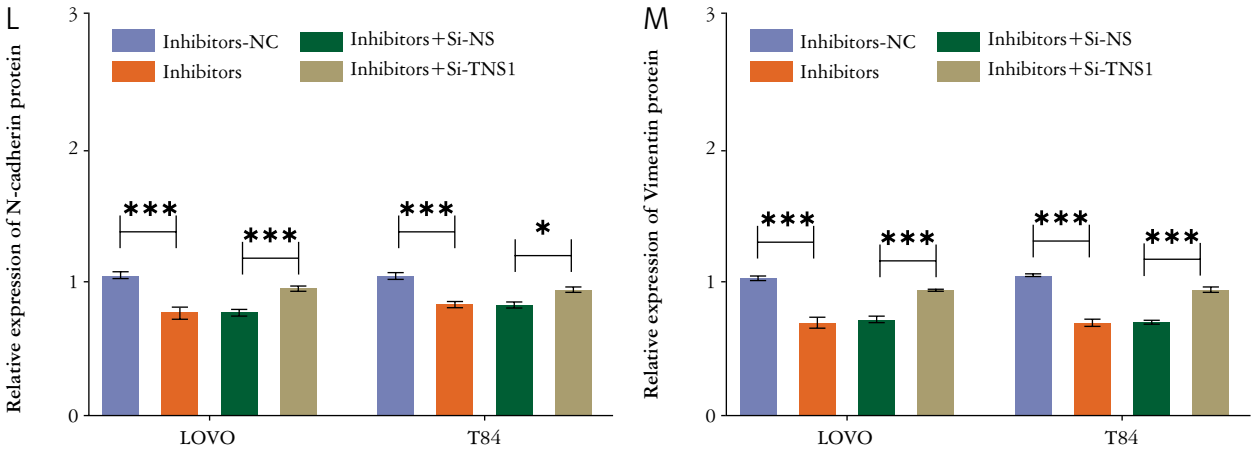

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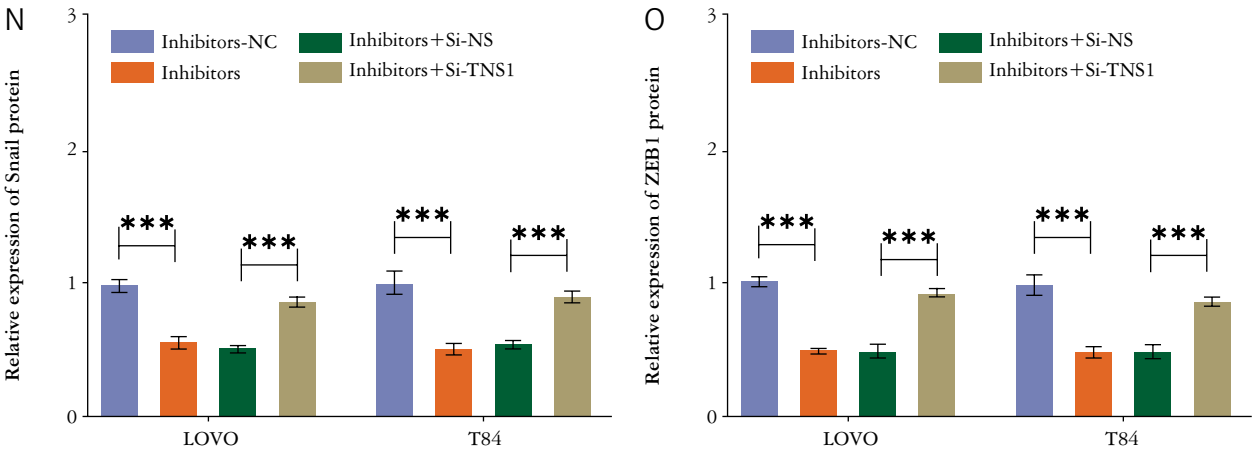

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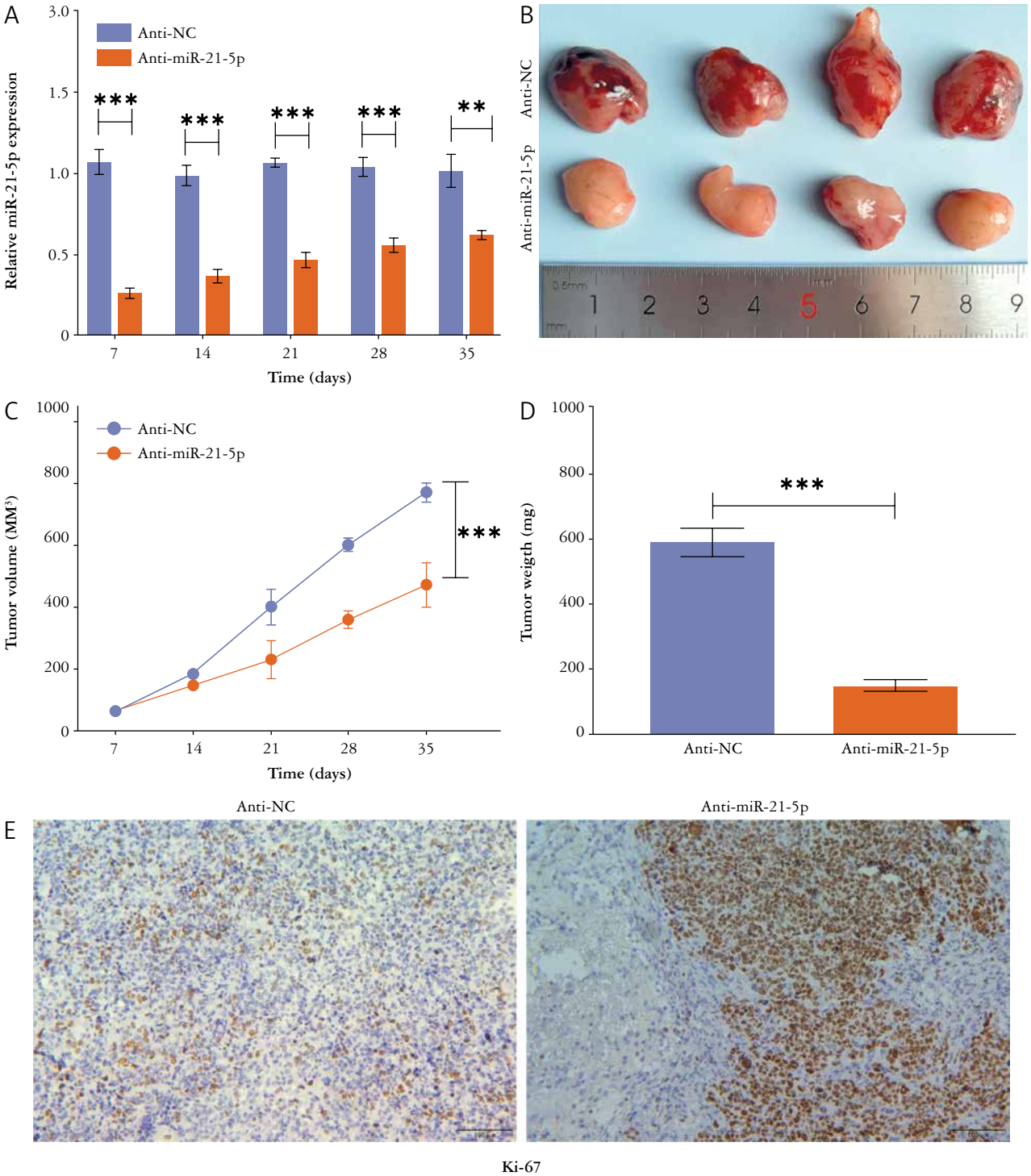

Figure 6. The effect of inhibiting miR-21-5p expression on tumor growth *in vivo*. **A**) The expression level of miR-21-5p in tumor tissues was detected by real-time fluorescence quantification polymerase chain reaction. **B**) Images of subcutaneous tumors in nude mice. **C**) Subcutaneous tumor growth curves. **D**) The histogram of tumor weight. **E**) Ki-67 levels in tumor tissues from nude mice were detected using IHC staining

with extracellular matrix proteins and cell adhesion molecules to support cellular adhesion and morphology as well as stable cellular junctions [37]. Tensin 1 also interacts with cytoskeletal components such as actin and microfilament-associated proteins, and is involved in cell structure and movement regulation [38]. Tensin 1 has been found to play various roles in cancer in recent studies. Jiang *et al*. discovered that TNS1 is overexpressed in gastric cancer and that inhibition of TNS1 expression inhibits gastric cancer cell proliferation [39]. Tang *et al*. [40] discovered that TNS1 was lowly expressed in bladder cancer patients. These results were in line with our earlier discoveries that TNS1 expression is low in COAD patients and that blocking TNS1 expression lessens the limiting impact of miR-21-5p silencers on the invasion, migration, EMT, and viability of COAD cells.

In summary, we discovered that miR-21-5p has a high expression level in COAD tissues and cells by detecting and analyzing the expression level of miR-21-5p in COAD tissues and cells, and inhibition of miR-21-5p expression was found to be able to reduce the growth rate of tumors through *in vivo* xenograft experiments. Furthermore, silencing miR-21-5p expression decreased COAD cell viability, migration, invasion, and EMT while increasing COAD cell apoptosis, whereas miR-21-5p had a negative target-regulatory relationship with TNS1, and silencing TNS1 reversed the effects of miR-21-5p inhibitors. Theoretically, our experimental results pave the way for the creation of therapeutic drugs that target COAD.

Conclusions

MiR-21-5p expression was increased in COAD cells and tissues. *In vitro*, inhibiting miR-21-5p expression reduced the viability, migration, invasion, and EMT of COAD cells. MiR-21-5p suppressed TNS1 expression to influence COAD cell progression and the EMT process, thereby reducing tumor development.

Disclosures

1. Institutional review board statement: This study was approved by the Ethics Committee of the School of Clinical Medicine, Jiangxi Medical College.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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