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Original Article

Long non-coding RNA *CYTOR* enhances gastric carcinoma proliferation, migration and invasion via the miR-136-5p/HOXC10 axis

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Abstract: Known as long non-coding RNAs (lncRNAs), they are essential in regulating tumour metastasis. In gastric carcinoma (GC), lncRNA cytoskeleton regulator (*CYTOR*) keeps at high levels, but its influences on GC cell proliferation, migration and invasion need further investigation. Hence, the role played by lncRNA *CYTOR* in GC was explored in this study. We employed quantitative reverse transcription PCR (RT-qPCR) to determine lncRNA *CYTOR* and microRNA (miR)-136-5p levels in GC, Western blot analysis to measure Homeobox C10 (*HOXC10*), and Flow cytometry, transwell, and cell counting kit-8 (CCK-8) assays to evaluate the roles played by miR-136-5p and lncRNA *CYTOR* in GC cells. Furthermore, bioinformatics analysis and Luciferase assay were carried out to identify the target genes of the two. LncRNA *CYTOR* was found to be upregulated in GC cells, and its knockdown inhibited GC cell growth. MiR-136-5p, underexpressed in GC cells, was identified as a target of *CYTOR* in modulating GC progression. Moreover, *HOXC10* was miR-136-5p's downstream target. Finally, *CYTOR* participated in GC progression in vivo. Collectively, *CYTOR* modulates the miR-136-5p/HOXC10 axis to accelerate GC progression.

Keywords: Cytoskeleton regulator, miR-136-5p, HOXC10, gastric carcinoma, proliferation, migration, invasion

Introduction

In recent decades, gastric carcinoma (GC) has become a common health burden worldwide [1]. The leading causes of GC include nitrite intake and Helicobacter pylori infection, while the main reason for the increased mortality of GC patients is tumour metastasis [2]. However, the molecular pathways causing GC metastases are still unclear. With genomic transcripts > 200 nucleotides in length, long non-coding RNAs (lncRNAs) are known to participate in multiple bioprocesses such as tumour growth, metastasis, and differentiation [3]. LncRNAs can regulate tumour-related gene expression by epigenetic regulation [4]. In addition to interacting with the transcription pre-initiation complex in the promoter region, lncRNAs can function as precursors for microRNAs (miRNAs) and competitive endogenous RNAs (ceRNAs) to participate in signal pathway regulation [5-7]. Additionally, LncRNAs have been implicated in the development and sensitivity to the medication of GCs. For example, the lncRNA *HOTAIR* serves as a miR-331-3p sponge to promote GC cell growth, migration, and invasiveness [8]. In addition, the lncRNA *PCAT-1* enhances cisplatin resistance in GC cells by suppressing PTEN expression [9]. Moreover, the lncRNA*TLN2-4:1* was reported to inhibit GC cell migration and invasiveness by modulating Talin protein expression [10]. Therefore, exploring the impact of lncRNA expression on GC onset and development is of great clinical significance.

The lncRNA cytoskeleton regulator (*CYTOR*) is an essential regulator of cancer [11, 12]. Even so, the function of lncRNA *CYTOR* in GC is still obscure. We discovered that lncRNA *CYTOR* is widely expressed in GC tissues by bioinformat-

ics analysis of public data and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) validation of clinical specimens. Therefore, we investigated the biological role of lncRNA *CYTOR* using GC cell lines and mouse tumour models. MiRNA target online software (StarBase) was used to confirm lncRNA *CYTOR*--miR-136-5p and miR-136-5p--HOXC10 interactions. MiR-136-5p can inhibit GC metastasis and trigger GC cell apoptosis by targeting *HOXC10* and AEG-1 [13]. Overexpression of *HOXC10* promotes migration, invasion, and tumour growth by upregulating proinflammatory cytokines [14]. Therefore, we hypothesized that lncRNA *CYTOR* plays a role by modulating the miR-136-5p/HOXC10 axis. For confirmation, we verified the impact of lncRNA *CYTOR* expression on miR-136-5p/HOXC10 axis and GC cell lines by RT-qPCR assay, cell motility assay, western blot assay, cell viability assay and GC subcutaneous tumour model in nude mice assay.

Materials and methods

Bioinformatics

The TCGA database (URL: https://portal.gdc.cancer.gov/) was introduced to observe lncRNA *CYTOR* expression in GC patients. Kaplan-Meier (KM) survival curves were used to clarify differences in disease-free survival (DFS) in patients with high and low lncRNA *CYTOR* expression.

Prediction of lncRNA targets

The StarBase (v2.0, URL: http://starbase.sysu.edu.cn/) was introduced to predict lncRNA *CYTOR*'s potential miRNA targets. TargetScan website (URL: http://www.targetscan.org/) was then used to determine miRs' downstream target genes. The expression of downstream target genes was observed by the TCGA database, and the correlation between lncRNA *CYTOR* and downstream target gene expression was detected by Spearman correlation analysis.

Cell culture and transfection

MKN-45 and Hs-746T cells were received from the National Authenticated Cultures Collection (Shanghai, China) and placed in penicillin/streptomycin (1%) and FBS (10%; Gibco, Waltham, MA, United States)-supplemented RPMI 1640 medium for routine culture. Sangon from Shanghai, China, was commissioned to synthesize plasmids. We prepared the plasmid by introducing the shRNA targeting lncRNA *CYTOR* (5'-CAGUCUCUAUGUGUCUUAATT-3') into pcDNA3.1, and sh-NC (5'-ACAAAGUUCUGUGAUGCACUGA-3') served as a negative control. This was followed by the cloning of lncRNA *CYTOR* cDNA fragments comprising wild type (WT) or mutant (MUT) miRNA binding site into pmirGLO (Promega, Madison, WI, United States). Also from Sangon, Shanghai, China, the mimic, inhibitor, and controls of miR-136-5p were obtained. Transfection was then carried out as per the Lipofectamine 2000 instructions (Qiagen, Hilden, Germany). Additionally, a nude mouse tumor xenotransplantation model was constructed by selecting stable cell lines using lentiviral vectors carrying si-Lnc-CYTOR or pcDNA-Lnc-CYTOR.

Luciferase reporter assay

We examined the association between miR-136-5 and lncRNA *CYTOR* or *HOXC10* by using dual luciferase reporter (DLR) analysis based on the predicted targets in the bioinformatics database. The lncRNA *CYTOR* MUT or WT bound to miR-136-5p were carried by pGL3 basic vectors. Thereafter, co-transfection of MUT- or WT-lncRNA *CYTOR* with miR-136-5p into GC cells was carried out. In addition, miR-136-5p was treated with co-transfection with MUT-*HOXC10* or WT-*HOXC10*. Luciferase activity measurement was conducted at 48 h post-transfection using a DLR assay system ordered from Promega Corporation, Fitchburg, WI, United States.

RT-qPCR

Total RNA extraction with RNAisoPlus (TAKARA, Japan) was performed as per the manufacturer's recommended protocol. Then came the cDNA synthesis with a Prime Script RT Master Mix cDNA Synthesis System from Bio-Rad, Hercules, CA, United States and the preparation of RT-qPCR reactions using SYBR Green (Bio-Rad). The target genes' expression, normalized to *GAPDH*, was calculated by $2^{-\Delta\Delta Ct}$. Primers for qPCR are presented in **Table 1**.

Cell motility analysis

We performed migration and invasion assays [15] using transwell chambers with or without

Table 1. Primer sequence

Primer		Sequence
miR-136-5p	F	3'-ACTCCATTTGTTTTGATGAT-5'
U6	F	3'-CTCGCTTCGGCAGCACA-5'
U6	R	3'-AACGCTTCACGAATTTGCGT-5'
HOXC10	F	3'-GTTCTGGGGCCAACGACTTC-5'
HOXC10	R	3'-GCTGAGGCGATTCCAGATGT-5'
Lnc-CYTOR	F	3'-CCCCTGGAAACCTCTTGACTC-5'
Lnc-CYTOR	R	3'-ACGGAGGTTGGAATGTGGAT-5'
E-cadherin	F	3'-GGGGTTAAGCACAACAGCAA-5'
E-cadherin	R	3'-CAAAATCCAAGCCCGTGGTG-5'
N-cadherin	F	3'-AGGTTTGCCAGTGTGACTCC-5'
N-cadherin	R	3'-ATGATGCAGAGCAGGATGGC-5'
α-SMA	F	3'-TGGTCAGAAGACGGTTGCAT-5'
α-SMA	R	3'-CGGACAGATTTTGCTCCTCTCT-5'
TGFβ1	F	3'-ATTGAGCACCTTGGGCACTGT-5'
TGFβ1	R	3'-TCTGGGCTTGTTTCCTCACCTT-5'
MMP9	F	3'-CGCGCTGGGCTTAGATCATT-5'
MMP9	R	3'-GATGCCATTCACGTCGTCCT-5'
β-actin	F	3'-GTCATTCCAAATATGAGATGCGT-5'
β-actin	R	3'-GCTATCACCTCCCCTGTGTG-5'

Matrigel (Thermo Fisher Scientific, Waltham, MA, United States). In brief, 2×10^4 transfected cells in serum-free medium and a complete medium (added 10% FBS) were placed into the upper and lower chamber, respectively, for 48 h of cultivation in a humidified, 5% CO_2 and 37°C environment. Following removal of cells on the upper surface of the filter, the cells on the lower surface were treated with 70% methanol immobilization (30 min) and 0.2% crystal violet staining.

Western blot analysis

Following 30 min of ice incubation in a radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology, Shanghai, China) comprising a phosphatase and protease inhibitor cocktail (Thermo Fisher Scientific), cells were treated with a 15-minute centrifugation (10,000×*g*) at four °C and protein concentration determination with a bicinchoninic acid protein assay kit (Beyotime). Samples with equal amounts of proteins were then separated with 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes for one h at 100 V. After room temperature blocking for one h with non-fat milk (5%) in Tris-buffered saline and Tween 20 (TBST), the membranes were kept overnight at four °C with anti-matrix metalloproteinase 9 (MMP-9; 1:1,000, ab76003; Abcam), anti-transforming growth factor (TGF)-β1 (1:1,000, ab215715; Abcam), anti-N-cadherin (1:5,000, ab76011; Abcam), anti-E-cadherin (1:5,000, ab76319; Abcam), anti-HOXC10 (1:5,000, ab153904; Abcam), and anti-alpha smooth muscle actin (α-SMA; 1:5,000, ab17263; Abcam) primary antibodies. Following thrice TBST rinses, they were treated with one h room temperature incubation with the respective secondary antibodies. The membranes were incubated with an ECL reagent (Millipore) to visualize proteins. Quantification of the immunoblots was performed with ImageJ 1.51p.

Cell viability testing

We used a Cell Counting Kit-8 (CCK-8/WST-8) assay kit (Solarbio, Beijing, China) for cell viability measurement. GC cells were inoculated into a 96-well culture plate for 24 h of cultivation. A microplate reader was utilized to read the absorbance at 450 nm after the addition of CCK-8 reagents to GC cells and 20 min of room temperature incubation.

GC subcutaneous tumour model in nude mice

To prove the specificity of lncRNA *CYTOR* action in GC tumour growth, an ectopic (subcutaneous) GC model in nude mice was generated. The lentiviral vector stably expressing lncRNA *CYTOR* was introduced into MKN-45 and a stably-expressing cell strain was generated by screening with puromycin. BALB/c nude mice, all males aged 4-6 weeks supplied by Guangxi Medical University's Animal Experimental Center, were housed in institutional animal facilities. All protocols have obtained approval from Guangxi Medical University's Animal Care and Ethics Committee (Number: 202101203). Then subcutaneous implantation of lncRNA *CYTOR*-overexpressing MKN-45 into the mouse right armpit area was carried out to establish a GC tumour model. Nineteen days after cell implantation, the animals were euthanized with 5% Isoflurane inhalation. Tumours were surgically removed, weighed, and photographed.

Statistical analysis

Protein levels are shown as normalized fold change relative to the control value. The statis-

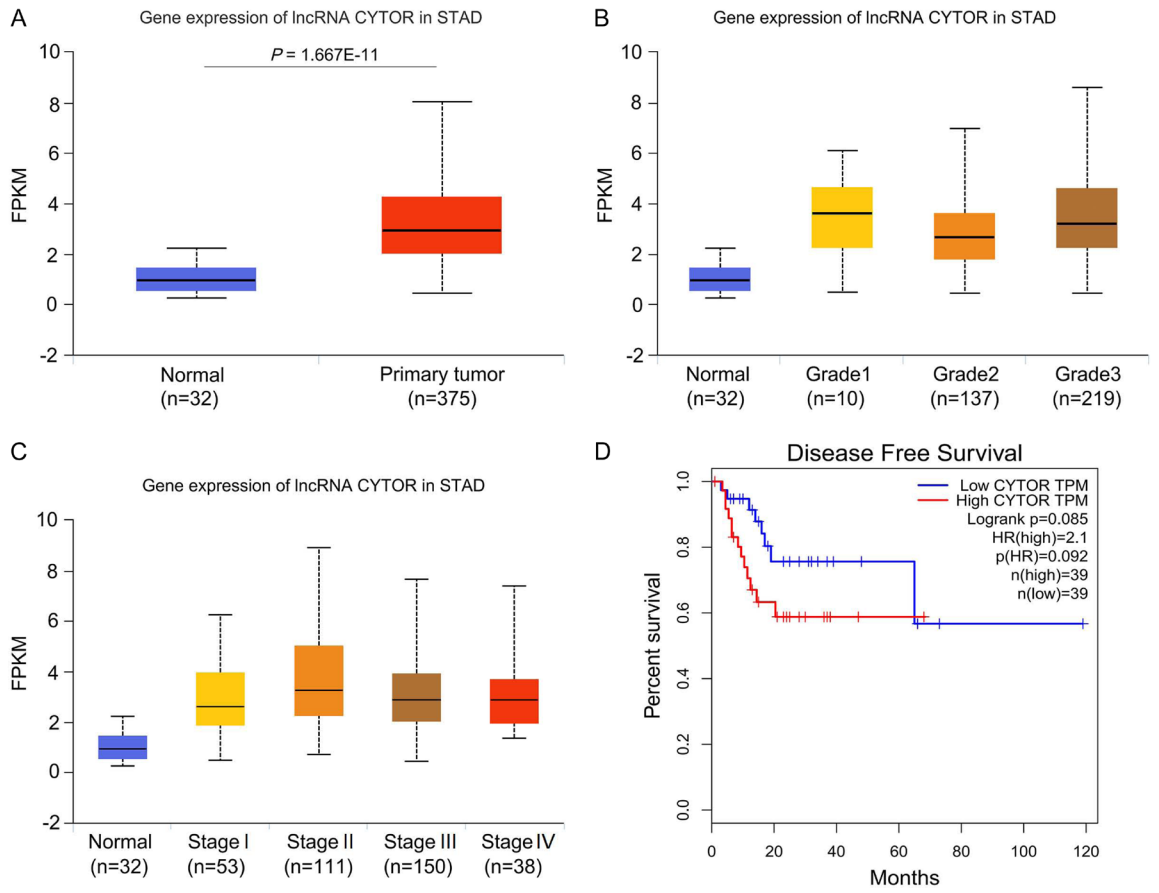

Figure 1. Long non-coding RNA (lncRNA) cytoskeleton regulator (*CYTOR*) expression. A. LncRNA *CYTOR* expression in gastric carcinoma (GC) tissues and normal tissues. B. LncRNA *CYTOR* in different tumor grades. C. LncRNA CYTOR in different tumor stages. D. Disease-free survival (DFS) of GC patients with high and low lncRNA CYTOR levels.

tical analysis was used, followed by a Tukey's post-hoc test, with $P < 0.05$ as the threshold of significance.

Results

High lncRNA CYTOR expression in GC

Higher lncRNA *CYTOR* expression in GC tissues versus normal counterparts was observed ($P < 0.05$, **Figure 1A**). However, lncRNA *CYTOR* was not significantly different in different tumour stages, as shown in **Figure 1B** and **1C**. According to the 90% and 10% quantile of lncRNA *CYTOR* expression value, GC patients were assigned to high or low expression group. The KM survival curve analysis of DFS revealed a hazard ratio (HR) of 2.1 in the high expression group, $P = 0.092$ (**Figure 1D**).

Bioinformatics prediction

LncRNAs can serve as competing ceRNAs to regulate mRNA expression by competing for their targeting miRNAs. We first predicted the possible targets of *CYTOR* using the Starbase database (URL: https://starbase.sysu.edu.cn/index.php). Among the candidate target genes of *CYTOR* predicted by bioinformatics databases, we focused on miR-136-5p, which is involved in regulating GC metastasis by targeting *HOXC10* [13]. The prediction result from Targetscan also identified *HOXC10* as miR-136-5p's direct target. Moreover, we observed highly expressed *HOXC10* in GC tissues from the TCGA database (**Figure 2A-C**) and poorer DFS in patients with high *HOXC10* expression versus those with low *HOXC10* expression (*HR* = 2.0, $P = 0.019$, **Figure 2D**). An obvious positive

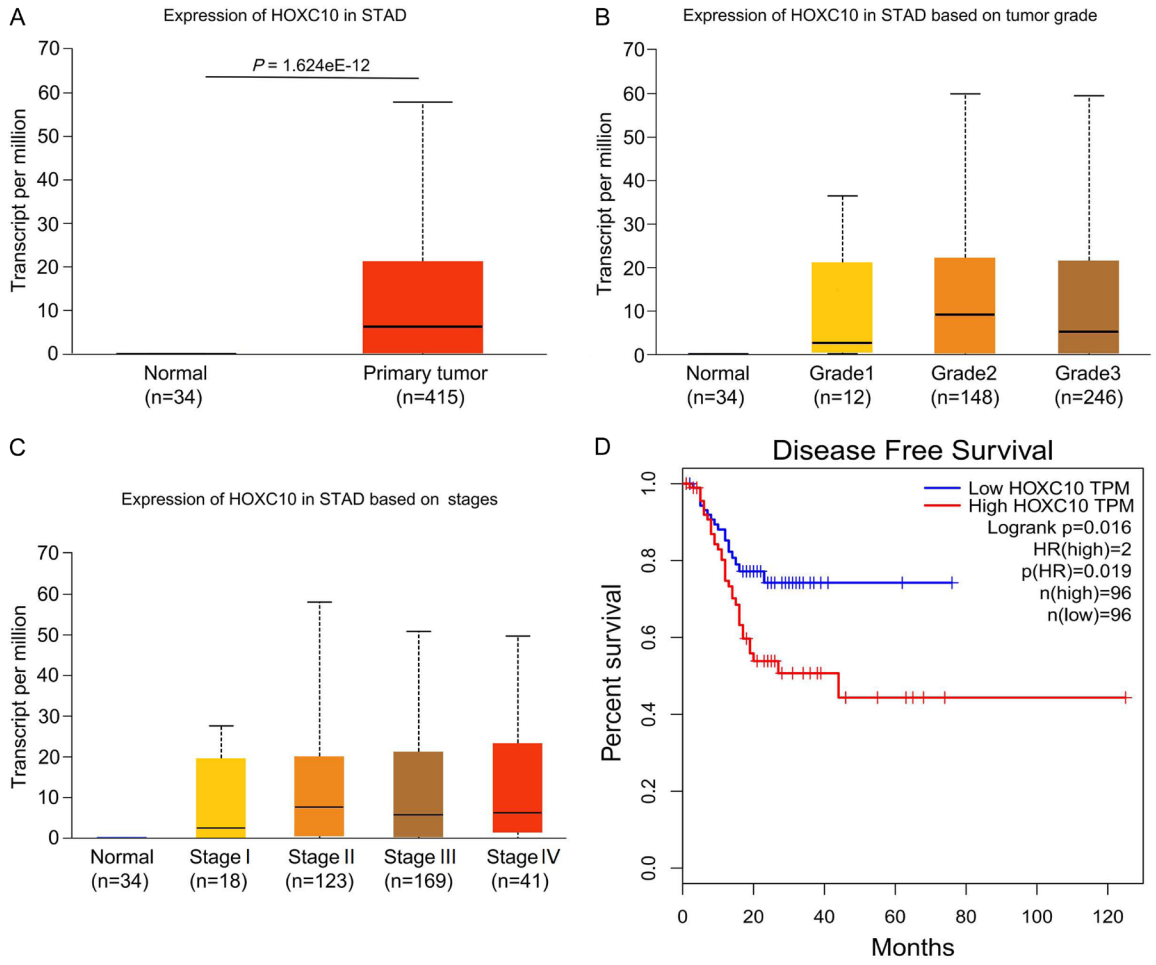

Figure 2. Homeobox C10 (*HOXC10*) expression. A. *HOXC10* in gastric carcinoma (GC) tissues and normal tissues. B. *HOXC10* in different tumor grades. C. *HOXC10* in different tumor stages. D. Disease-free survival (DFS) of GC patients with high and low *HOXC10* levels.

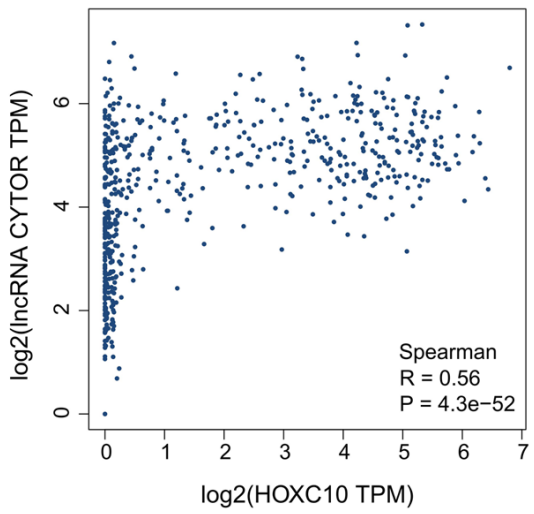

Figure 3. Correlation between CYTOR and HOXC10 expression.

correlation was also determined between lncRNA *CYTOR* and *HOXC10* expression levels (R = 0.56, P < 0.05, as shown in **Figure 3**). Therefore, we investigated whether *CYTOR* exerts its effect through the *CYTOR*-miR-136-5p-*HOXC10* network.

LncRNA CYTOR is a sponge for miR-136-5p

As indicated by DLR assays, the miR-136-5p mimic inhibited the Lnc-CYTOR-WT activity but not Lnc-CYTOR-MUT (**Figure 4A**). The luciferase activity was inhibited in transfected cells with the miR-136-5p mimic fused to the 3'-UTR of *HOXC10* compared with the control groups (**Figure 4B**). According to qPCR, overexpressing lncRNA *CYTOR* suppressed miR-136-5p, whereas lncRNA *CYTOR* knockdown had the opposite effect (**Figure 4C**). Furthermore, the

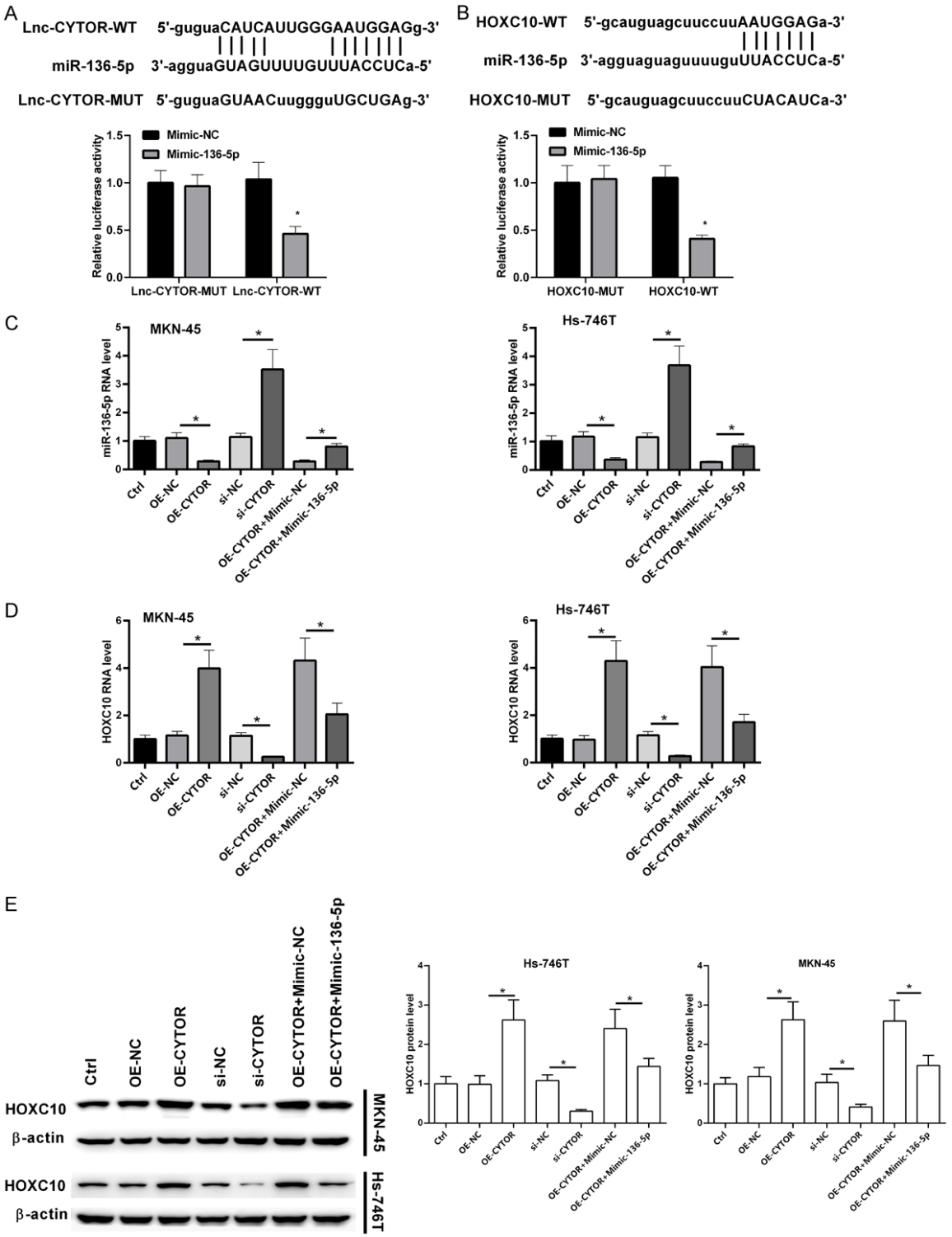

Figure 4. The role of long non-coding RNA (lncRNA) cytoskeleton regulator (*CYTOR*) as a miR-136-5p sponge. A. Diagram of the putative binding site in *CYTOR* with miR-136-5p and strategy for construction of the Lnc-CYTOR mutant vector. WT: wild-type; MUT: mutant. B. The *HOXC10* 3'-untranslated region (UTR) harbours potential miR-136-5p binding loci. MKN-45 and Hs-746T cells were mock-transfected or transfected with pcDNA-NC (OE-NC), pcDNA-Lnc-CYTOR (OE-CYTOR), siRNA-negative control (si-NC), or siRNA-CYTOR (si-CYTOR) and co-transfected with pcDNA-Lnc-CYTOR, miR-136-5p negative control (Mimic-NC), or miR-136-5p mimic. C. miR-136-5p RNA was measured by qPCR. D. *HOXC10* mRNA was measured by qPCR. E. HOXC10 protein was quantified by western blot. The mean ± SD was used to describe the data. *P < 0.05.

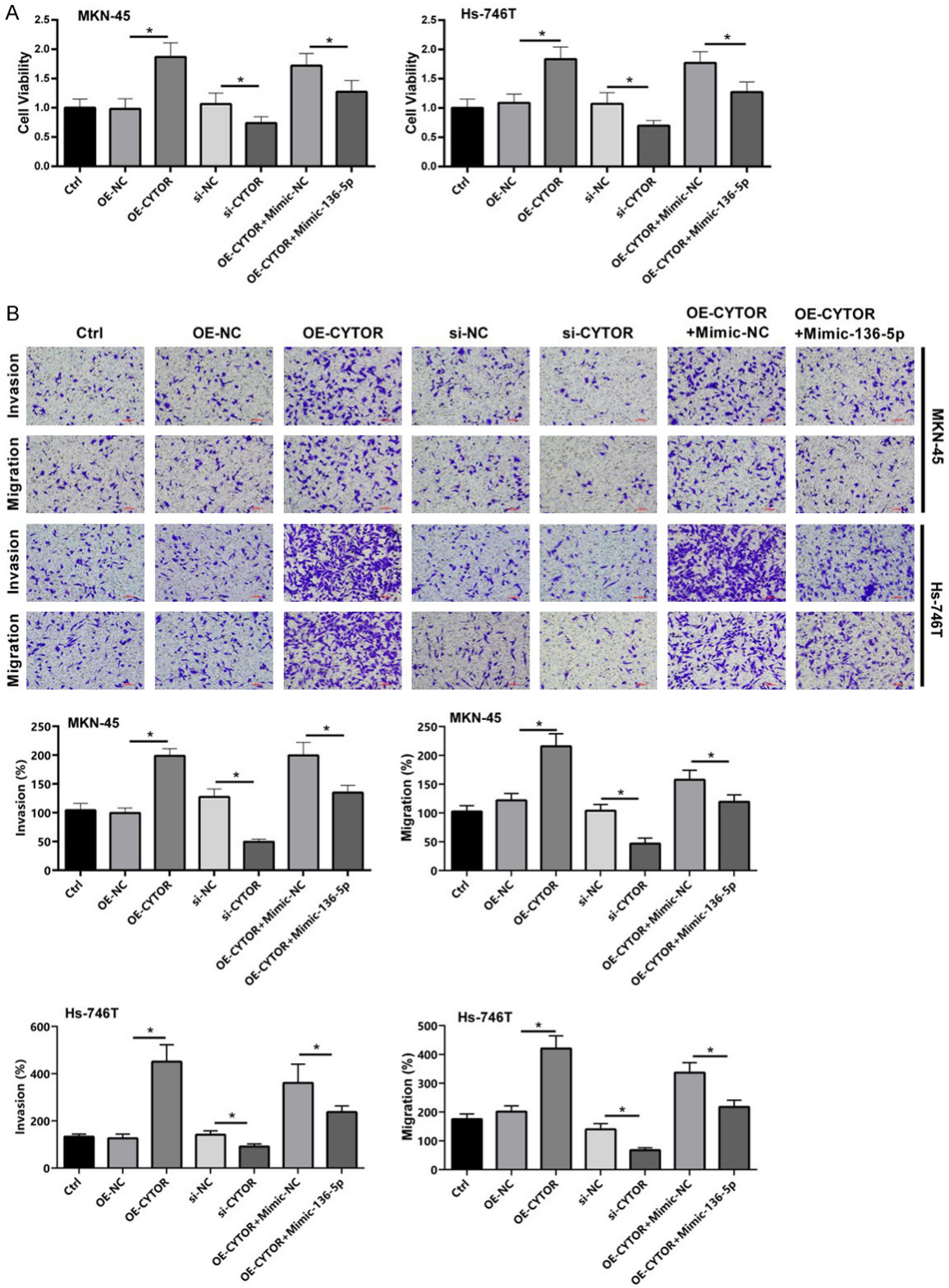

Figure 5. Overexpression of the lncRNA *CYTOR* promotes gastric carcinoma (GC) cell invasion and migration. MKN-45 and Hs-746T cells were mock-transfected or transfected with pcDNA-NC (OE-NC), pcDNA-Lnc-CYTOR (OE-CYTOR), siRNA-negative control (si-NC), or siRNA-CYTOR (si-CYTOR), and co-transfected with pcDNA-Lnc-CYTOR, miR-136-5p negative control (Mimic-NC), or miR-136-5p mimic. A. CCK-8/WST-8 assays were carried out to determine cell viability. B. Transwell/matrigel assays were conducted to measure cell invasion and migration. The mean ± SD was used to describe the data. *$P < 0.05$.

HOXC10 expression level increased in lncRNA *CYTOR*-overexpressing cells and decreased in lncRNA *CYTOR*-knockdown cells (**Figure 4D** and **4E**). These data indicate that lncRNA *CYTOR* regulates *HOXC10* expression by competing for miR-136-5p.

Overexpression of lncRNA CYTOR enhanced GC cell invasiveness and migration via miR-136-5p/HOXC10

Next, the role played by lncRNA *CYTOR* in GC cell invasiveness and migration was examined. Overexpression of lncRNA *CYTOR* increased GC cell viability, whereas lncRNA *CYTOR* knockdown had the opposite effect. However, upregulating miR-136-5p expression attenuated this impact of lncRNA *CYTOR* (**Figure 5A**). The transwell assay demonstrated that suppressing lncRNA *CYTOR* inhibited GC cell invasion and migration. When the lncRNA *CYTOR* level was elevated relative to the control group, GC cell invasiveness and migration were boosted, an effect suppressed by miR-136-5p overexpression (**Figure 5B**). Furthermore, flow cytometry analysis of cell apoptosis demonstrated that lncRNA *CYTOR* overexpression reduced apoptosis of GC cells, whereas lncRNA *CYTOR* knockdown did the opposite. The effects of lncRNA *CYTOR* overexpression were inhibited by the co-transfection with miR-136-5p (Figure S1). Overall, lncRNA *CYTOR* increases GC cell migration and invasiveness through miR-136-5p.

CYTOR regulates GC cell epithelial-mesenchymal transition (EMT) via miR-136-5p

RT-qPCR results demonstrated that overexpression of lncRNA *CYTOR* decreased E-cadherin (an epithelial marker) and elevated N-cadherin, α-SMA, and MMP-9 (mesenchymal marker proteins) and TGF-β1 levels in GC cells (**Figure 6A**). However, the opposite trend was observed for cells with lncRNA *CYTOR* knocked down. Moreover, miR-136-5p overexpression attenuated these effects of lncRNA *CYTOR*. Similar results were obtained by western blot analysis for mesenchymal and epithelial markers (**Figure 6B**). These results indicated that the upregulation of EMT markers by lncRNA *CYTOR* is attributed to miR-136-5p expression.

CYTOR enhances GC xenograft tumour growth in vivo

At day 19 after injection of lncRNA *CYTOR*-overexpressed or lncRNA *CYTOR*-silenced GC cells in nude BALB/c mice, *CYTOR*-overexpressing cell-derived xenograft tumours were more significant than control cell-derived tumours. Conversely, lncRNA *CYTOR*-knockdown tumours were smaller than control tumours (**Figure 7A**). The mouse weight, tumour growth curve, and average tumour weight are shown in Figure S2. Western blotting and RT-qPCR also revealed that overexpression of lncRNA *CYTOR* decreased miR-136-5p and E-cadherin and increased *HOXC10*, N-cadherin, α-SMA, MMP-9, and TGF-β1 in GC xenograft tumours (**Figure 7B** and **7C**). However, lncRNA *CYTOR*-knockdown tumours showed the opposite trend. Collectively, lncRNA *CYTOR* overexpression enhanced the tumorigenicity of GC cells.

Rescue experiment

GC cells were treated with lncRNA CYTOR overexpression or transfection of si-HOXC10 or overexpression of *HOXC10*. Based on qPCR, *HOXC10* gene expression was markedly inhibited after transfection of si-HOXC10 or miR-136-5p mimic in lncRNA *CYTOR*-overexpressing GC cells (**Figure 8A**, **8B**) ($P < 0.01$). However, miR-136-5p mimic and *HOXC10* co-transfection led to markedly reversed inhibition of miR-136-5p mimic on the *HOXC10* gene ($P < 0.01$). MTT assay (**Figure 8C**, **8D**) significantly inhibited viability of GC cells overexpressing lncRNA *CYTOR* after si-HOXC10 or miR-136-5p mimic transfection ($P < 0.01$). However, significantly reversed inhibiting effects of miR-136-5p mimic on cell activity was observed following miR-136-5p mimic and *HOXC10* co-transfection ($P < 0.01$).

GC cell migration ability was determined by Transwell without Matrigel. GC cells overexpressing lncRNA *CYTOR* were found to have a significant reduction in the count of cells that migrated after transfection with si-HOXC10 or miR-136-5p ($P < 0.01$). However, miR-136-5p mimic and *HOXC10* co-transfection significantly reversed the inhibition of miR-136-5p mimic on the number of cell migration ($P < 0.01$), as shown in **Figure 9A**, **9B**. Transwell supplemented with matrigel was utilized for GC cell inva-

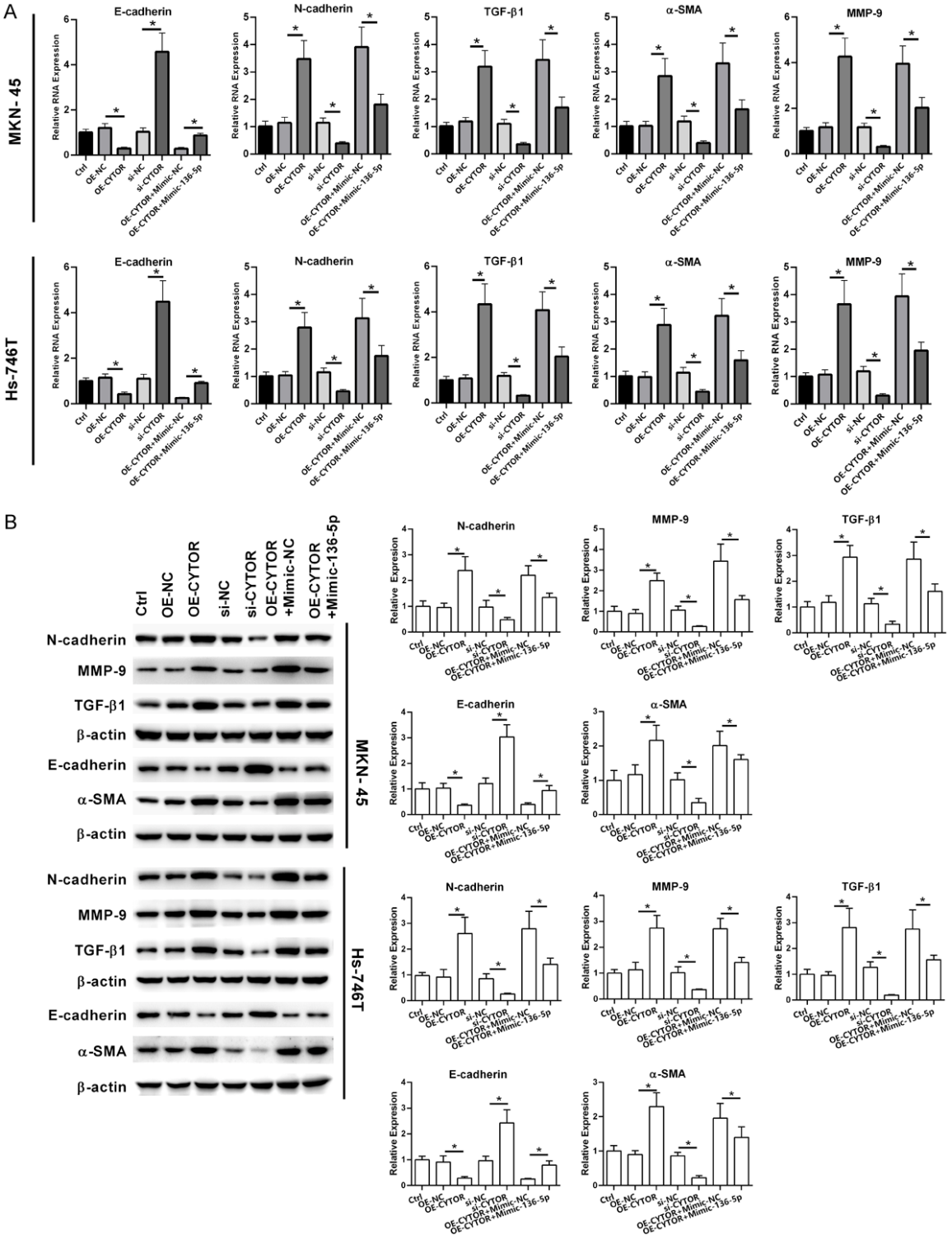

Figure 6. Overexpressing lncRNA *CYTOR* promotes GC cell epithelial-mesenchymal transition (EMT). MKN-45 and Hs-746T were mock-transfected or transfected with pcDNA-NC (OE-NC), pcDNA-Lnc-CYTOR (OE-CYTOR), siRNA-negative control (si-NC), or siRNA-CYTOR (si-CYTOR) and co-transfected with pcDNA-Lnc-CYTOR, miR-136-5p negative control (Mimic-NC), or miR-136-5p mimic. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was carried out to quantify E-cadherin, N-cadherin, TGF-β1, α-SMA, and MMP-9 mRNA (A). Western blotting was performed to measure E-cadherin, N-cadherin, TGF-β1, α-SMA, and MMP-9 protein levels (B). The mean ± SD was used to describe the data. *$P < 0.05$.

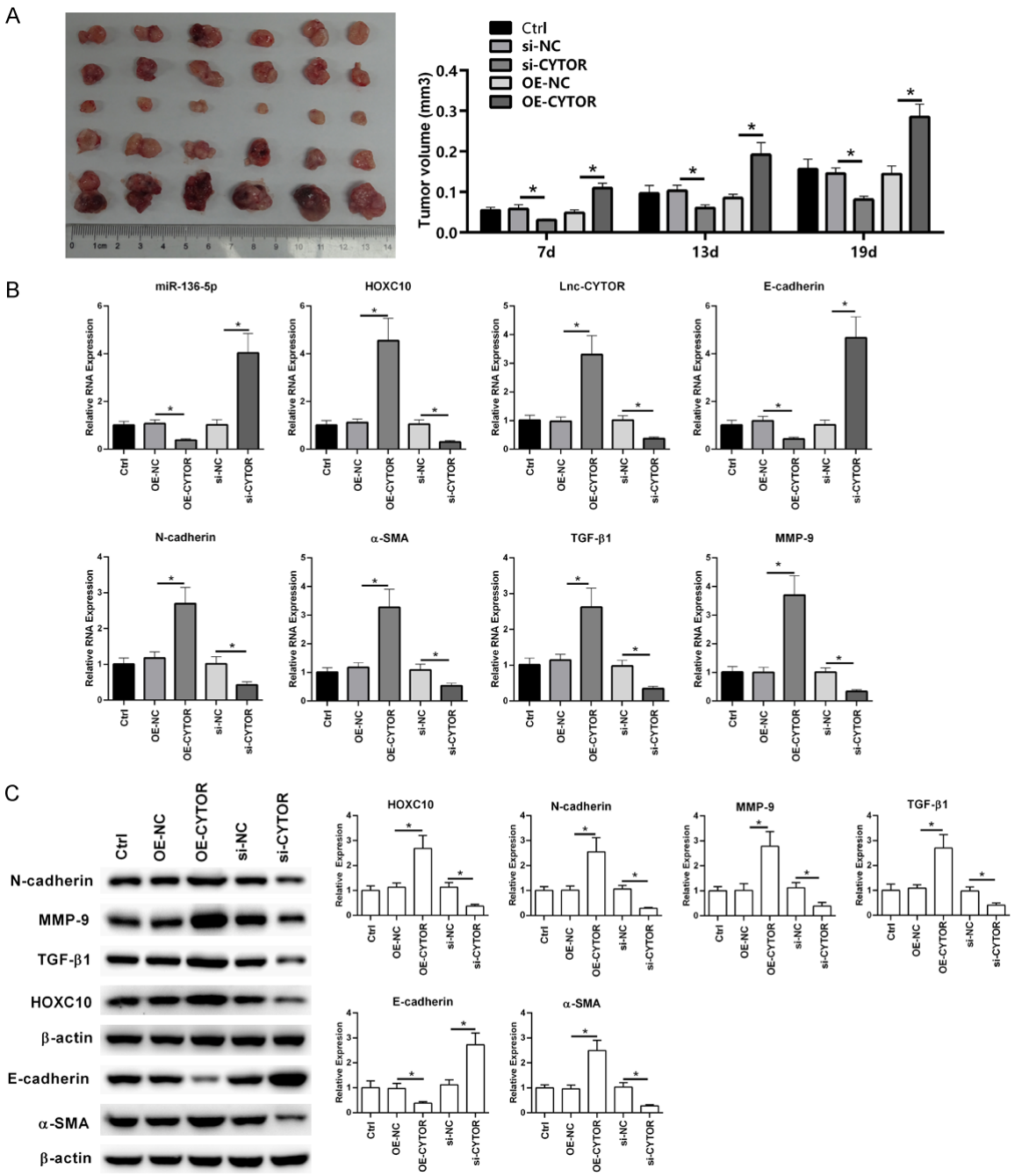

Figure 7. Overexpression of the lncRNA *CYTOR* promotes GC tumour growth *in vivo*. A. Tumour weight and volume measurements (representative tumours from each treatment group). B and C. E-cadherin, N-cadherin, TGF-β1, α-SMA, HOXC10, and MMP-9 mRNA and protein levels in GC tumours by RT-qPCR and western blotting, respectively.

sion assay. A markedly reduced count of GC cells overexpressing lncRNA *CYTOR* that underwent invasion *was identified* after si-HOXC10 or miR-136-5p mimic transfection ($P < 0.01$). However, the inhibition of miR-136-5p mimic on cell invasion was significantly reversed after miR-136-5p mimic and *HOXC10* co-transfection ($P < 0.01$, **Figure 9C**, **9D**).

EMT-related gene levels in lncRNA *CYTOR*-overexpressing Hs 746T cells transfected with si-HOXC10 or overexpressing *HOXC10* were detected by qPCR (**Figure 10A-E**). EMT-related genes N-cadherin, α-SMA, TGFβ-1 and MMP-9 showed statistically inhibited levels in GC cells overexpressing lncRNA *CYTOR* after si-HOXC10 or miR-136-5p mimic transfection, while

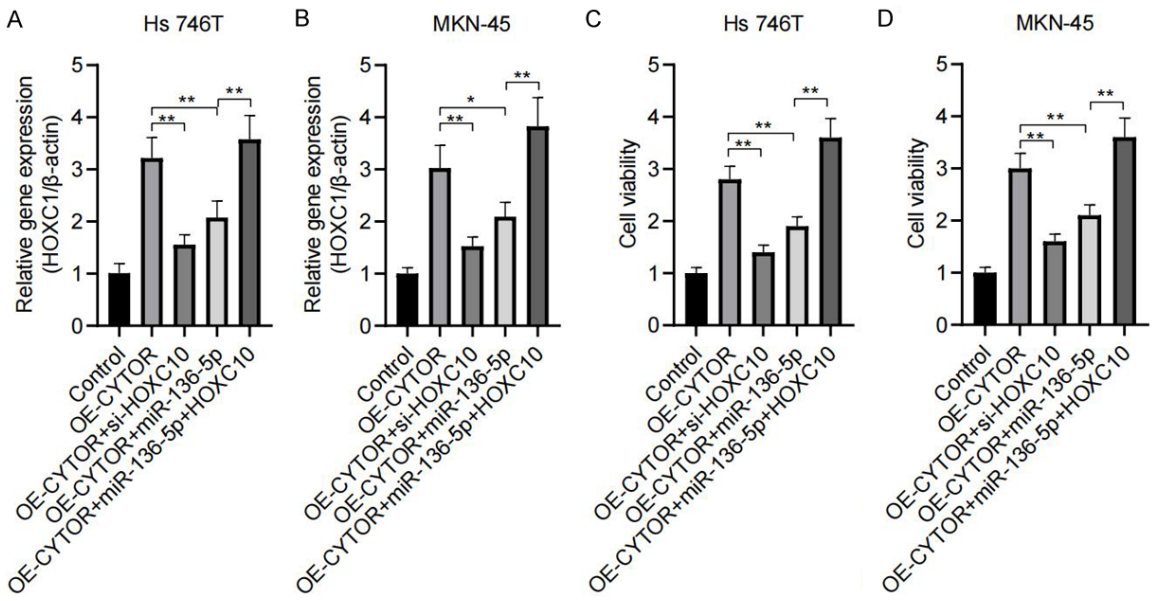

Figure 8. Regulation of lncRNA CYTOR/miR-136-5p/HOXC10 gene expression levels on gastric cancer cell activity. A, B. HOXC10 gene expression by qPCR. C, D. Cell viability by MTT assay. **P < 0.01, *P < 0.05.

E-cadherin had increased expression (P < 0.01). However, miR-136-5p mimic and *HOXC10* co-transfection markedly reduced the inhibition of miR-136-5p mimic on EMT-related genes (P < 0.01). Changes in EMT-related genes mentioned above were also observed during qPCR in MKN-45 cells (**Figure 10F-J**).

Immunoblotting experiments quantified EMT-related proteins in Hs 746T cells overexpressing lncRNA *CYTOR* after si-HOXC10 transfection or *HOXC10* overexpression (as shown in **Figure 11A-L**). EMT-related proteins N-cadherin, α-SMA, TGFβ-1 and MMP-9 were found to be significantly decreased in GC cells overexpressing lncRNA *CYTOR* after si-HOXC10 or miR-136-5p mimic transfection, while E-cadherin protein was increased (P < 0.01). However, miR-136-5p mimic and *HOXC10* co-transfection contributed to markedly reversed inhibition of miR-136-5p mimic on EMT-related proteins (P < 0.01). The above-mentioned changes in EMT-related proteins were also observed during western blot analysis of MKN-45 (as shown in **Figure 12A-L**). In short, lncRNA *CYTOR* increases GC cell migration and invasion through miR-136-5p/HOXC10.

Discussion

In East Asia, Central and South America, and Eastern Europe, GC ranks first in incidence among gastrointestinal cancers [16]. In most patients, GC is diagnosed at a late stage, and a successful treatment strategy is limited, resulting in high mortality [17]. Several recent studies have revealed that hundreds of lncRNAs were aberrantly expressed during GC development. Therefore, lncRNAs are considered potential biomarkers for achieving early GC detection. For instance, the lncRNA *GMAN* presents high expression in GC tissues, with its upregulated expression being strongly linked to meagre overall survival rates in such patients [18]. Zhang et al. [19] reported the ability of lncRNA *HOXC-AS3* to regulate GC cell proliferation and migration by interacting with *YBX1*. Earlier, we found up-regulated lncRNA *CYTOR* in GC tissues and the correlation of high lncRNA *CYTOR* expression with GC patients' RFS, but the mechanism has not been elucidated. Thus, the function of the lncRNA *CYTOR* in GC is discussed in this research. Overexpressing lncRNA *CYTOR* was found to enhance GC cell invading and migrating capacities and elevate viability. EMT refers to the process by which epithelial cells acquire mesenchymal signatures, which participates in cancer cell migration and invasion [20]. During EMT, cells undergo a series of biochemical changes, which lead to a decline in E-cadherin (an epithelial cell marker) and a concomitant increase in α-SMA, MMP-9, and N-cadherin (mesenchymal markers) [21]. Evidence suggests that *H. pylori* infection-induced

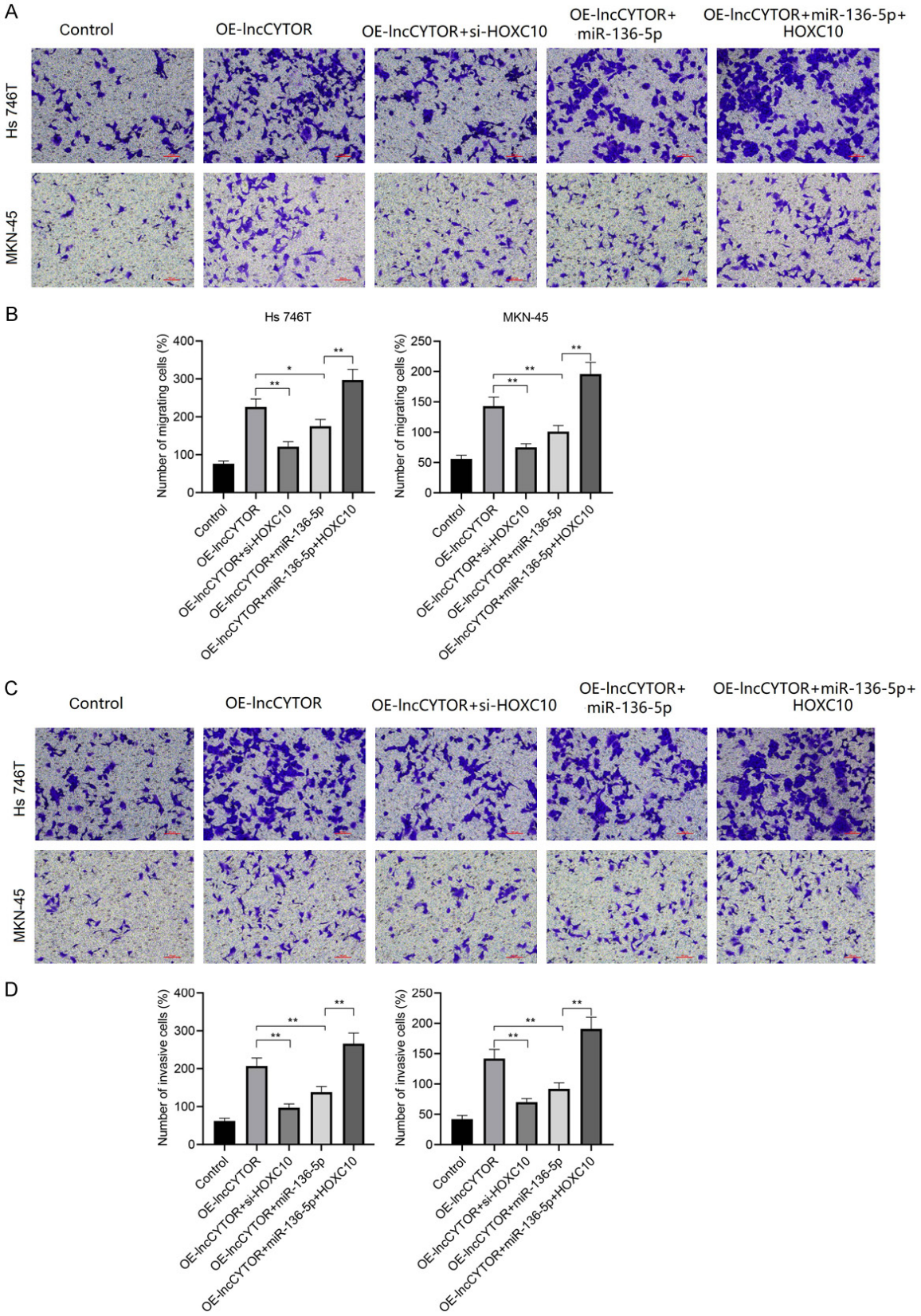

Figure 9. Effect of lncRNA CYTOR/miR-136-5p/HOXC10 axis on gastric carcinoma cell migration and invasion abilities. A. Migration ability detection by Transwell chamber without matrigel; B. Quantitative statistical results of the migration number of Hs 746T and MKN-45. C. Invasion ability detection by Transwell chamber without matrigel; D. Quantitative statistical results of the invasion number of Hs 746T and MKN-45 cells. **P < 0.01, *P < 0.05.

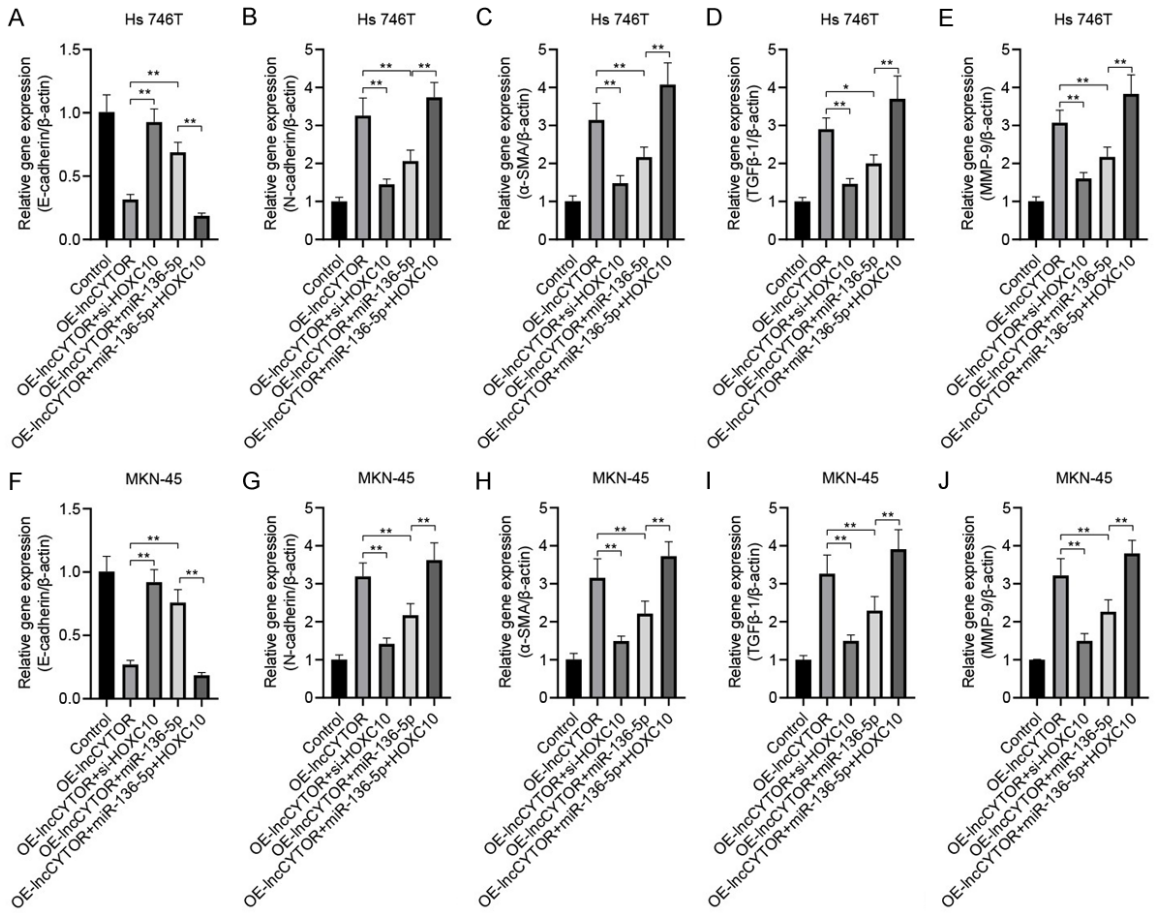

Figure 10. LncRNA CYTOR promotes EMT marker gene expression in gastric carcinoma cells through miR-136-5p/HOXC10 axis. A-E. EMT-related genes' levels in Hs 746T were detected by qPCR. F-J. EMT-related genes' levels in MKN-45 were detected by qPCR. $^{**}P < 0.01$, $^{*}P < 0.05$.

EMT progression increases the probability of gastric carcinogenesis [22]. Our data indicated that overexpression of lncRNA *CYTOR* boosted N-cadherin, α-SMA, and MMP-9 expression and reduced concomitant E-cadherin expression, suggesting that lncRNA *CYTOR* enhances EMT in GC cells. We also observed that overexpression of lncRNA *CYTOR* increases the level of TGF-β1. Relevant studies have shown that TGF-β1 was one of the most relevant EMT inducers [23]. All these suggest the involvement of overexpressed lncRNA *CYTOR* in GC cell invasion and migration.

LncRNA *CYTOR* has been indicated to promote pancreatic cancer cell migration and proliferation [24], indicating that this lncRNA may function as an oncogenic factor. Tumour xenograft experiments demonstrated that knockdown of lncRNA *CYTOR* suppressed GC tumour growth in xenografted mice, whereas overexpression of lncRNA *CYTOR* promoted tumour formation and growth. Thus, our findings revealed that lncRNA *CYTOR* has a pro-oncogenic role in GC metastasis.

LncRNAs modulate the de-repression of miRNA targets and exert additional post-transcriptional regulation by acting as a sponge for miRNAs. We searched bioinformatics platforms to address the underlying mechanism by which lncRNA *CYTOR* inhibited GC metastasis and identified miR-136-5p as a candidate. Furthermore, a luciferase reporter assay demonstrated the link between lncRNA *CYTOR* and miR-136-5p, with the former being a negative regulator of the latter. Numerous studies have examined miR-136-5p expressing profiles and functions in various human cancer cell lines. It has been reported to be a tumour suppressor gene by suppressing migration and invasion enhancer 1 (MIEN1) expression in osteosarco-

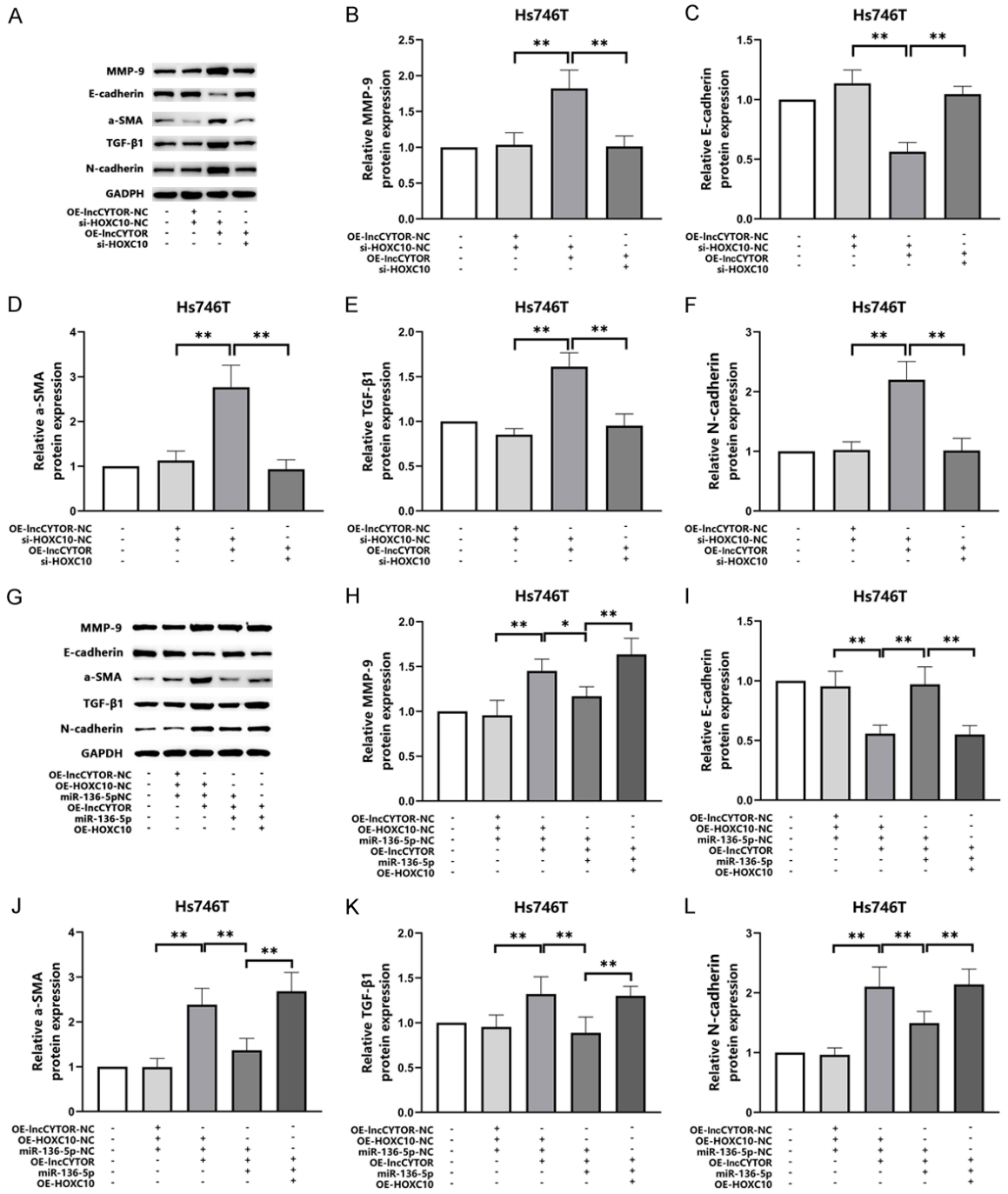

Figure 11. LncRNA CYTOR promotes EMT-marker protein expression in gastric carcinoma cells through the miR-136-5p/HOXC10 axis. A-L. Western blotting determination of EMT-related protein levels in Hs 746T. **P < 0.01, *P < 0.05.

ma cells [25]. Moreover, it showed lower expression in hepatocellular carcinoma than in normal counterparts, suggesting that miR-136-5p may act as an anti-carcinoma miRNA [26]. Additionally, miR-136-5p targets AEG-1 and BCL2 to induce GC cell apoptosis [27]. MiR-136 was also reported to inhibit the TGF-β1/SMAD3 signalling pathway in diabetic rats [28]. TGF-β1 is known to be crucial in cell proliferation, migration, and differentiation of epithelial-mesenchymal cell metastasis [29]. Therefore, lncRNA *CYTOR* may mediate TGF-β1 expression

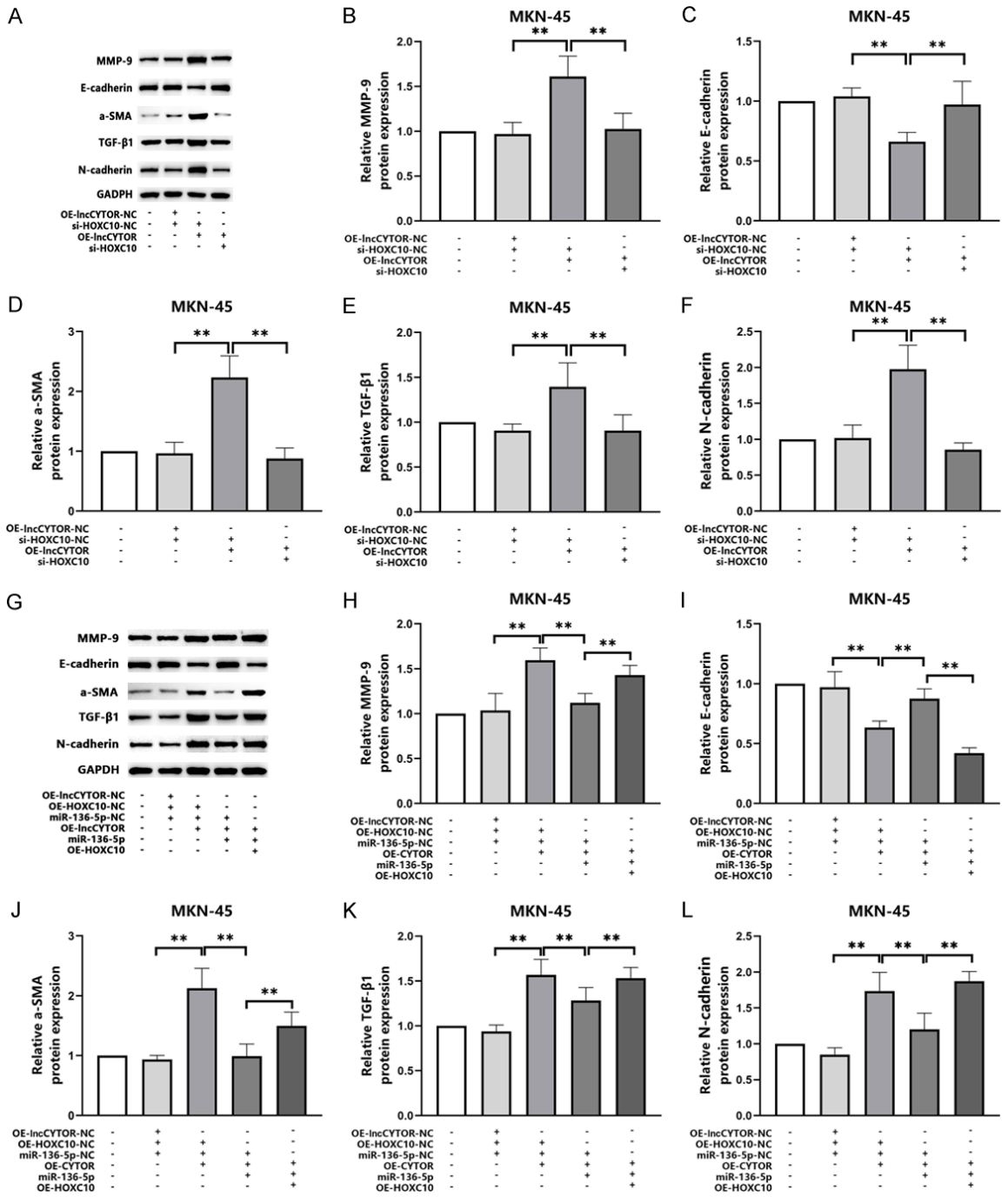

Figure 12. LncRNA CYTOR promotes EMT-marker protein expression in gastric carcinoma cells through the miR-136-5p/HOXC10 axis. A-L. Western blotting detection of EMT-related protein levels in MNK-45. **P < 0.01, *P < 0.05.

via downregulating miR-136-5p, leading to enhanced GC cell invasion and migration capacities. As reported by Zheng et al. [13], miR-136 suppresses GC-specific peritoneal metastases through targeting *HOXC10*. In the TCGA database, we observed highly expressed *HOXC10* in GC tissues and the significant relationship between high *HOXC10* expression and GC patients' RFS. In addition, a positive connection was identified between *HOXC10* and lncRNA *CYTOR* expression. For verification, in vitro cellular experiments were performed, and our data showed lncRNA *CYTOR* overexpression led to reduced miR-136-5p and higher

HOXC10 expression, suggesting that lncRNA *CYTOR* enhanced *HOXC10* expression via inhibition of miR-136-5p. *HOXC10* is a member of the *HOX* gene family, which is required for physiological processes such as limb morphology, angiogenesis, and fat metabolism [30]. Carlson et al. [31] found that *HOXC10* was expressed as short (Hoxc10S) or long (Hoxc10L) transcripts during development and regeneration. The former presents low expression in developing tail tips, hindlimbs, and forelimbs, while the latter has a similar expression pattern except that its expression cannot be found in the developing forelimb. *HOXC10* has also been shown to enhance human glioma cell angiogenesis by upregulating VEGF-A [32]. And a significant correlation of *HOXC10* mRNA with body fat content was reported by Brune et al. [33]. Evidence has also linked *HOXC10* expression imbalance to various tumours. For example, *HOXC10* expression is upregulated in colorectal cancer tissues [34]. Kim et al. [35] indicated that *HOXC10* enhances GC cell proliferation and metastasis through direct binding to the CST1 promoter region. Subsequently, rescue experiments showed that *HOXC10* silencing attenuated the effects of lncRNA *CYTOR* on EMT and GC tumour growth, suggesting a positive correlation between lncRNA *CYTOR* and *HOXC10* expression. The above suggests that lncRNA *CYTOR* may promote GC invasiveness and metastasis through the miR-136-5p/*HOXC10* axis. However, the value of the lncRNA *CYTOR*/miR-136-5p/*HOXC10* axis in predicting GC patients' prognoses needs further clinical validation. Going forward, we will continue to address these key issues in depth. This study demonstrates that lncRNA *CYTOR* promotes GC growth in xenograft models, but it has not been proven to promote GC metastasis in xenograft models. Therefore, we will continue to explore the impact of lncRNA *CYTOR* on GC metastasis in future research using a metastasis model.

Conclusion

This work has examined the role played by lncRNA *CYTOR* in GC growth, migration and invasion. The correlation analysis has uncovered the positive correlation of lncRNA *CYTOR* with *HOXC10* and its inverse correlation with miR-136-5p. Conclusively, *CYTOR* promotes growth and development via the miR-136-5p/*HOXC10* axis in GC. These findings suggest the potential of lncRNA *CYTOR* as a novel target for GC therapy.

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Disclosure of conflict of interest

None.

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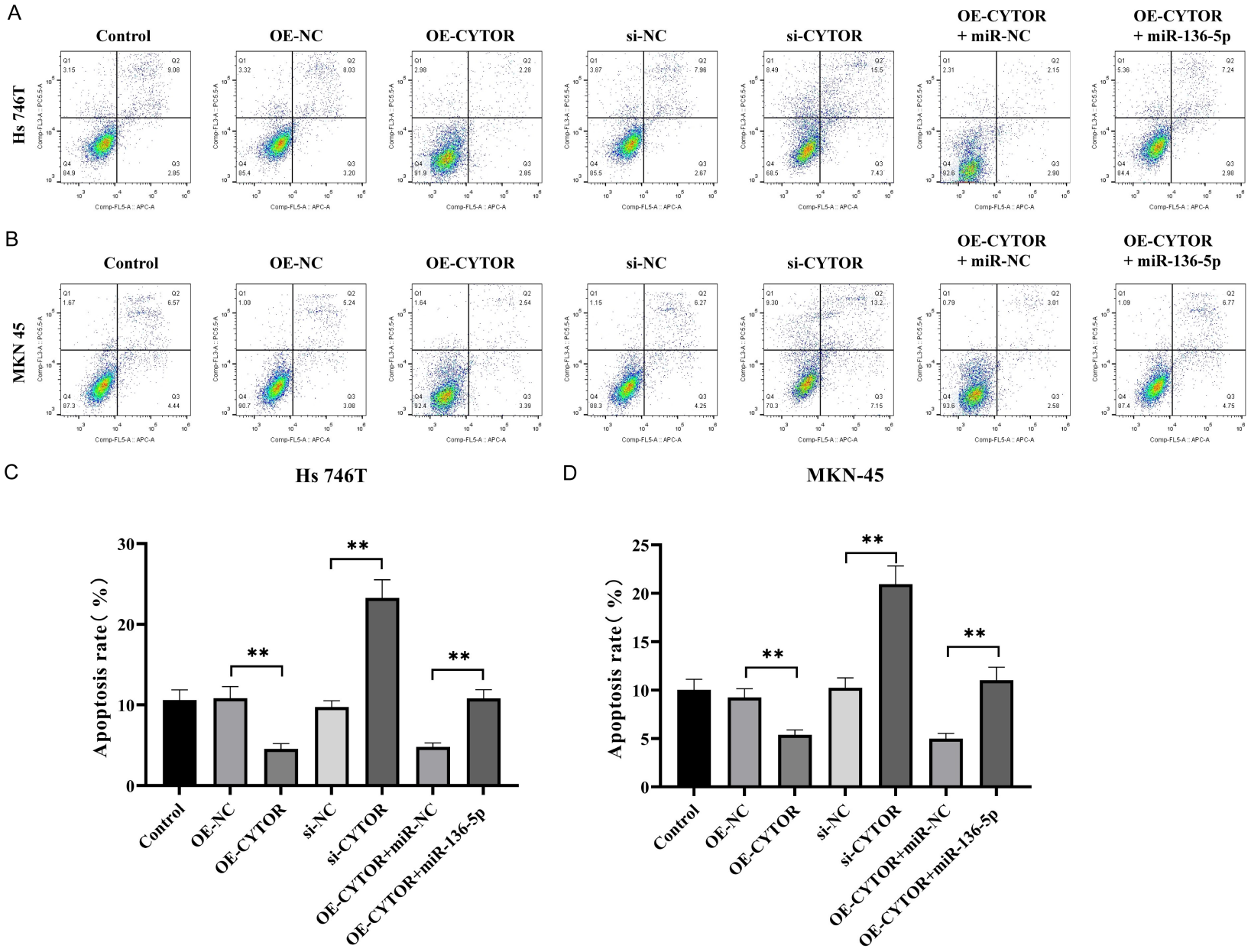
A
Control
OE-NC
OE-CYTOR
si-NC
si-CYTOR
OE-CYTOR + miR-NC
OE-CYTOR + miR-136-5p
Hs 746T
B
MKN 45
C
Hs 746T
D
MKN-45
Apoptosis rate (%)
Control
OE-NC
OE-CYTOR
si-NC
si-CYTOR
OE-CYTOR+miR-NC
OE-CYTOR+miR-136-5p

Figure S1. A-D. MKN-45 and Hs-746T cells were mock-transfected or transfected with pcDNA-NC (OE-NC), pcDNA-Lnc-CYTOR (OE-CYTOR), siRNA-negative control (si-NC), or siRNA-CYTOR (si-CYTOR) and co-transfected with pcDNA-Lnc-CYTOR, miR-136-5p negative control (Mimic-NC), or miR-136-5p mimic. Cell apoptosis determination was made by flow cytometry.

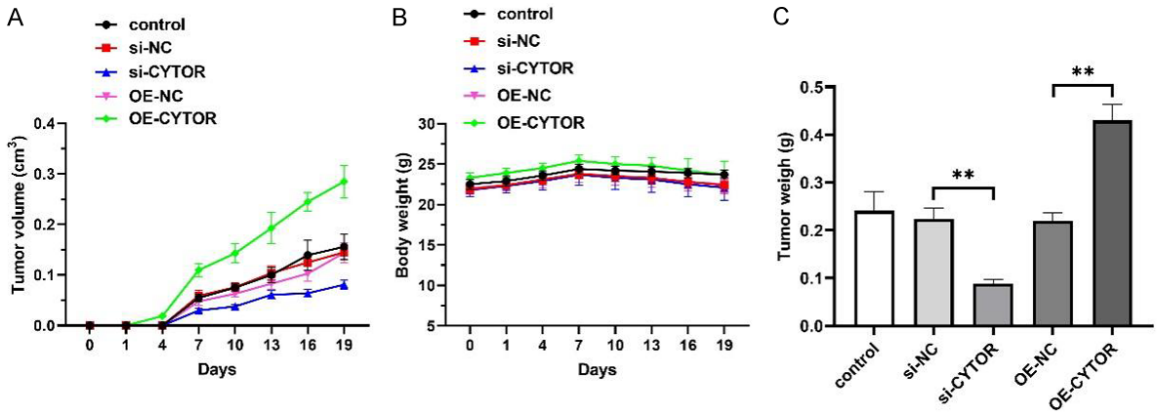

Figure S2. A. Tumour growth curves. B. Mouse body weight curves. C. Tumour weight quantification. The mean ± SD was used to describe the data. **P < 0.01.