# circ_0035277 Promotes Gastric Cancer Proliferation and Metastasis via miR-576-3p/LIN28B Axis

**Xiaoyan Wang**
The Affiliated Suqian First People's Hospital of Nanjin Medical University
**Rong He**
The Affiliated People's Hospital of Jiangsu University
**Yan Wang**
The Affiliated Suqian First People's Hospital of Nanjin Medical University
**Yunyun Liu**
The Affiliated Suqian First People's Hospital of Nanjin Medical University
**Yuxin Wang**
The Affiliated Suqian First People's Hospital of Nanjin Medical University
**Dandan Chen**
The Affiliated Suqian First People's Hospital of Jiangsu University
**Yu Fan** ( yuf12345@ujs.edu.cn )
The Affiliated People's Hospital of Jiangsu University

**Research Article**

**Keywords:** circ_0035277, miR-576-3p, LIN28B, gastric cancer, metastasis

**Posted Date:** January 6th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1207711/v1

# Abstract

Circular RNAs (circRNAs) regulate biological processes of human tumours. Gastric cancer is a prevalent disease that presents tumours with high metastasis. This study aimed to understand regulatory function and mechanism of circ_0035277 in gastric cancer development. circ_0035277 level in tissues and cells was assessed using RT-qPCR assay. Function of circ_0035277 was evaluated via CCK-8, colony formation and Transwell assays. Location of circ_0035277 was verified with FISH assay. Potential mechanism of circ_0035277 was examined using bioinformatics and luciferase reporter assays. BALB/c nude mice were utilised to construct the gastric cancer metastasis model with subcutaneous injection. Haematoxylin–eosin staining was performed to measure visible tumour nodules in lung tissues. Results showed that circ_0035277 is significantly up-regulated in tissues and cell lines of gastric cancer, accelerates the deterioration of gastric cancer, is significantly located in the cytoplasm and serves as a sponge to participate in gastric cancer by targeting miR-576-3p. Furthermore, lin-28 homolog B (LIN28B) was a direct target of miR-576-3p and reversed the role of circ_0035277/miR-576-3p on gastric cancer metastasis. In vivo studies have shown that knockdown of circ_0035277 suppresses gastric cancer metastasis. Overall, circ_0035277 accelerated gastric cancer progression and metastasis by regulating miR-576-3p/LIN28B axis. These findings can provide a potential target for the treatment of gastric cancer.


# Introduction

Gastric cancer is a typical malignant tumour in the digestive system that presents high morbidity and mortality worldwide [1]. An epidemiological investigation showed that the majority of gastric cancer patients are terminal at the time of diagnosis [2]. Although surgical resection, radiotherapy and immunotherapy are common treatment methods for gastric cancer, an ideal therapy effect is still lacking [3]. Hence, exploring effective methods of diagnosis, treatment and prevention in gastric cancer research is crucial.

Circular RNAs (circRNAs), a kind of endogenous noncoding RNA, are closed-loop formed by the connection of 5' and 3' ends [4] and characterised by stability, tissue specificity, disease specificity and evolutionary conservatism [5]. circRNAs play an important role in human tumours, such as breast cancer [6], colorectal cancer [7], gastric cancer [8] and osteosarcoma [9]. Furthermore, abnormal expression of circRNAs in gastric cancer is closely related to tumour size, differentiation, metastasis and other clinicopathological parameters [10–12]. Previous studies have shown that circRNAs are involved in cancer progression through a variety of regulatory mechanisms [13]. Exon circRNAs exist in the cytoplasm and present specific binding sites of microRNAs (miRNAs) [14]. miRNAs are complementary to 3’untranslated region (3'UTR) of messenger RNA (mRNA) and result in the degradation of mRNA and mediation of gene regulation after transcription [15]. circRNAs serve as a sponge for and block miRNAs whilst targeting messenger RNA (mRNA) binding [16]. For instance, circLARP4 suppresses the development of gastric cancer by sponging miR-424-5p and modulating target LATS1 level [17]. Although circ_0035277 is up-regulated in plasma of gastric cancer patients [18], its regulatory roles in gastric cancer remain unclear.

We aimed to understand the biological role and mechanism of circ_0035277 in gastric cancer in this study. The results revealed that circ_0035277 acts as a tumour facilitator to regulate the development and metastasis of gastric cancer and is specifically bound to miR-576-3p. This finding is consistent with the binding site of lin-28 homolog B (LIN28B) on miR-576-3p. Notably, circ_0035277 promoted gastric cancer metastasis by targeting the miR-576-3p/LIN28B axis.

# Materials And Methods

### Tissues samples collection

Fifteen paired gastric cancer and adjacent normal tissues were collected from The Affiliated Suqian First People's Hospital of Nanjing Medical University from February to June 2017. Characteristics of all participants are provided in Additional file 1. Collected samples were stored at −80°C immediately after surgery. All participants signed a consent agreement and the project was approved by the Ethics Committee of The Affiliated Suqian First People's Hospital of Nanjing Medical University.

### Cell culture and transfection

Human gastric epithelial (GES-1) and gastric cancer (AGS, NCI-N87, MKN-45, HGC-27 and SNU-1) cell lines were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). All cells were cultured in DMEM or RPMI 1640 medium (Gibco, USA) with 10% foetal bovine serum (FBS, Gibco, USA) at 37°C with 5% CO2. Small interfering RNA circ_0035277 (si-circ_0035277, 5'-GCCTCATTCATGTGTGGTGTT-3'), NC mimic (5'-UUUGUACUACACAAAAGUACUG-3'), miR-576-3p mimic (5'-AAGAUGUGGAAAAAUUGGAAUC-3'), NC inhibitor (5'-CAGUCCUUUUGUGUAGUACAA-3') and miR-576-3p inhibitor (5'-GAUUCCAAUUUUUCCACAUCUU-3') were synthesised by GenePharma (Shanghai, China). cDNA of circ_0035277 and LIN28B was cloned into pcDNA3.1 vector for overexpression of circ_0035277 and LIN28B. Cells were transfected with 100 nM si-circ_0035277, miR-576-3p mimic, miR-576-3p inhibitor, pcDNA-3.1-circ_0035277 or pcDNA-3.1-LIN28B using Lipofectamine 2000 (Invitrogen, USA) following the manufacturer's instructions.

### Real-time quantitative PCR assay

The total RNA of tissues and cells was isolated using Trizol reagent (TransGen, China) and reverse-transcribed to cDNA using PrimeScript®RT (Takara, Japan). Reactions were then performed with the ABI Prism 7500 PCR system (Applied Biosystems, USA) utilising SYBR Green Master Mix and TaqMan MicroRNA Assay Kit (Takara, Japan), as recommended by the supplier. U6 and GAPDH were used as internal control. The expression of all genes was calculated using 2 − ΔΔCT. Primer sequences of mRNA and miRNAs are shown in Additional file 2.

### CCK-8 assay

HGC-27 and AGS cells were placed into 96-well plates with 1×104 cells/well. CCK-8 solution (10 μL, Beyotime, China) was then added to cells after 96 hours (h). Cell optical density was detected with a

microplate reader (Bio-Rad, China) at 450 nm after incubation for 1 h at 37°C.

### Colony formation assay

HGC-27 and AGS cells were seeded into 6-well plates with 500 cells/well and incubated with 5% CO2 at 37 ℃ for 14 days. Cells were then fixed with methanol and stained with Giemsa dyeing for 15 minutes. The number of clones was obtained using a light microscope (Olympus, Japan).

### Transwell assay

HGC-27 and AGS cells were seeded into the upper Transwell chamber with 5×104 cells/well and incubated with FBS-free medium at 37 ℃. Cells were then seeded into the upper chamber with precoated Matrigel for invasion. The bottom Transwell chamber was supplied with 500 μL medium with 20% FBS. Cells migrated, invaded and became fixed on the small outdoor surface after 24 h and then stained with crystal violet for 15 minutes. Migrated and invaded cells were counted with a light microscope (Olympus, Japan).

### Fluorescence in situ hybridisation assay

HGC-27 cells were placed into 48-well plates with 1×104 cells/well, fixed in 4% paraformaldehyde and permeabilised with 0.5% Triton X-100 for 5 minutes. HGC-27 cells were then incubated with circ_0035277-labeled fluorescence in situ hybridisation (FISH) probes (RiboBio, China) at 37 ℃ for 12 h. Lastly, DAPI staining (Beyotime, China) was used to reverse-stain nuclei. Images were observed with a fluorescence microscope (Nikon, Japan).

### Bioinformatics analysis

The online database Circular RNA Interactome was utilised to assess miRNAs binding with circRNA. Three online databases, namely, Targetscan, miRDB and miRTarbase, were used to find potential target genes of miRNAs.

### Dual-luciferase reporter assay

Wild-type or mutant 3’UTR of circ_0035277 and LIN28B were cloned into pGL3-Firefly-Renilla vector (Promega, USA) separately. Cells were placed into 24-well plates, cotransfected with pGL3-Firefly-Renilla vector and mimicked or inhibited with Lipofectamine 2000. The luciferase activity was measured using dual-luciferase reporter assay (Promega, USA) according to the manufacturer’s instruction. Lastly, the luciferase activity was normalised to Renilla signals.

### Western blot assay

Tissues and cells were fully lysed with 500 μL protein lysate of RIPA:PMSF=100:1 (Beyotime, China) and then iced for 15 minutes. The protein concentration of samples was determined using the BCA kit (Beyotime, China). Protein samples (20 μg) were added to the loading buffer at 95 ℃ for 5 minutes,

placed in SDS-PAGE gel and shifted into PVDF membranes. Specific primary antibodies anti-LIN28B (ab229629, 1:1000, abcam, USA) and anti-GAPDH (ab9485, 1:1500, abcam, USA) were incubated at 4 ℃ overnight. Membranes were incubated with the secondary antibody (ab150082, 1:10,000, abcam, USA) for 2 h. Lastly, images were collected via ECL luminescence (Beyotime, China) and Western blot imaging system.

### Tumour xenograft model

Six to eight week-old BALB/c nude mice were obtained from Jiangsu Medical Laboratory Animal Centre (Nanjing, China). Mice were raised in a specific pathogen-free room with free sterile food and water. Lentivirus with firefly luciferase-infected HGC-27 cell line was applied to establish a stable luciferase-positive HGC-27 cell line. The HGC-27 cell line was infected with lentivirus si-NC or si-circ_0035277. A 10 mm incision was performed on the mice abdomen after intraperitoneal anaesthesia with 50 mg/kg of sodium pentobarbital. HGC-27 cells (1×106) mixed with Matrigel matrix were injected into the subserosal region of the stomach similar to a previous study [19]. Lastly, the stomach was reinserted into the peritoneal cavity to close the incision. Luciferase activity of fireflies was observed via bioluminescence imaging. BALB/c nude mice were euthanised with 200 mg/kg of pentobarbital. Animal experiments were approved by the Animal Care and Use Committee of The Affiliated Suqian First People's Hospital of Nanjing Medical University.

### Haematoxylin and eosin staining

Lung tissues were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin and sectioned. Lung slices were immersed in 0.5% haematoxylin for 5 minutes and eosin solution for 3 minutes. Images of stained sections were observed under a light microscope (Nikon, Japan).

### Statistical analysis

Data were analysed via GraphPad 8.0 software, presented with mean±SD and repeated at least three times. Student's unpaired t-test was used to compare differences between the two groups. One-way ANOVA, followed by post hoc Bonferroni test, was utilised to assess differences amongst multiple groups. $P < 0.05$ was considered significantly different.

## Results

### circ_0035277 is highly expressed in gastric cancer tissues and cell lines

circ_0035277 in gastric cancer tissues and cell lines was assessed using RT-qPCR assay to investigate its physiological expression. The results indicated that circ_0035277 is significantly up-regulated (mean ratio of 3.43-fold) in gastric cancer tissues compared with adjacent normal tissues (Fig. 1A). circ_0035277 expression consistently and significantly increased in gastric cancer cell lines (AGS, NCI-N87, MKN-45, HGC-27 and SNU-1) compared with the normal epithelial cell line (GES-1) (Fig. 1B). AGS and HGC-27 cells were selected for further experiments given the high ratio of circ_0035277 expression in

AGS and HGC-27 at 3.00-fold and 3.69-fold, respectively. Overall, these results demonstrated that the up-regulation of circ_0035277 in gastric cancer tissues and cell lines indicates its likely relation to the development of gastric cancer.

### circ_0035277 promotes gastric cancer cell proliferation and metastasis

circ_0035277 was knocked down or overexpressed in HGC-27 and AGS cells to explore its possible roles in gastric cancer. The transfection efficiency of circ_0035277 was confirmed via the RT-qPCR assay (Fig. 2A). The CCK-8 assay suggested that inhibition of circ_0035277 significantly suppresses cell viability in HGC-27 (42.21% inhibition) and AGS (48.61% inhibition) cells compared with the control group (Fig. 2A). Overexpression of circ_0035277 enhanced cell viability in HGC-27 and AGS cells at 48.56% and 53.70%, respectively (Fig. 2B). Similarly, knockdown of circ_0035277 reduced the colony formation whereas overexpression of circ_0035277 increased the colony number of HGC-27 and AGS (Figs. 2C and 2D). Knockdown of circ_0035277 suppressed cell migration and invasion, whereas overexpression of circ_0035277 promoted cell migration and invasion (Figs. 2E and 2F). These results showed that circ_0035277 accelerates the deterioration of gastric cancer by mediating cell proliferation, migration and invasion.

### circ_0035277 targets miR-576-3p in gastric cancer

Endogenous circRNAs act as miRNA sponges to exert multiple biological functions [20]. We carried out the FISH assay to assess the localisation of circ_0035277 and understand its mechanism involved in the progression of gastric cancer further. circ_0035277 was markedly located in the cytoplasm (Fig. 3A). The online database Circular RNA Interactome showed that circ_0035277 may bind to miR-1180, miR-136, miR-198, miR-224, miR-510, miR-512-5p, miR-518a-5p, miR-527, miR-576-3p, miR-616, miR-651, miR-659 and miR-892a. In addition, RT-qPCR assay revealed that circ_0035277 negatively regulates the miR-576-3p expression without affecting other miRNAs (Fig. 3B). Accordingly, miR-576-3p was assumed the potential target of circ_0035277. Furthermore, the miR-576-3p expression was down-regulated (mean ratio of 1.85-fold) in gastric cancer tissues (Fig. 3C). The results of Pearson's correlation analysis validated the negative relevance between circ_0035277 and miR-576-3p (Fig. 3D). Similarly, the miR-576-3p level was lower in gastric cancer cell lines compared with GES-1 (Fig. 3E). As shown in Fig. 3F, the circ_0035277 3'-UTR sequence contains predicted binding sites of miR-576-3p. Although the mutant-type circ_0035277 remained unaffected, the miR-576-3p mimic subsequently suppressed the luciferase activity of wild-type circ_0035277 (Fig. 3G). Overall, circ_0035277 negatively modulated the miR-576-3p expression by binding with it.

### circ_0035277 promotes gastric cancer cell proliferation and metastasis by regulating miR-576-3p

Cells were transfected with the overexpression of circ_0035277 and miR-576-3p mimic jointly and alone to understand whether circ_0035277 executed its function via miR-576-3p. The RT-qPCR assay indicated that circ_0035277 significantly suppresses miR-576-3p expression and cotransfects with the miR-576-3p-restored miR-576-3p level (Fig. 4A). The miR-576-3p mimic also partially abolished the promotion effect of circ_0035277 on gastric cancer cell proliferation (Figs. 4B–4D). The Transwell assay results displayed

that circ_0035277 significantly promotes cell migration and invasion ability whilst miR-576-3p mimic reverses this phenomenon (Figs. 4E and 4F). These data revealed that circ_0035277 performs as a tumour facilitator by targeting miR-576-3p in gastric cancer.

### miR-576-3p targets LIN28B in gastric cancer

Bioinformatics analysis was utilised to find targets of miR-576-3p and explore the possible circRNA–miRNA–mRNA loop in gastric cancer. Three online databases, namely, Targetscan, miRDB and miRTarbase, indicated 12 intersecting target genes (Fig. 5A). The RT-qPCR assay was subsequently applied to detect the relevance between miR-576-3p and intersecting target genes. Data showed that miR-576-3p negatively modulates the LIN28B expression (Fig. 5B). The Western blot assay presented that LIN28B is up-regulated in gastric cancer tissues (Figs. 5C and 5D). Potential binding sites of miR-576-3p with 3'UTR of LIN28B are illustrated in Fig. 5E. The miR-576-3p mimic significantly decreased the luciferase activity of wild-type LIN28B rather than the mutant LIN28B (Fig. 5F). The Western blot analysis further showed that miR-576-3p negatively regulates the protein expression of LIN28B (Figs. 5G and 5H). These data indicated that LIN28B is a target of miR-576-3p.

### circ_0035277 promotes gastric cancer cell metastasis via the miR-576-3p/LIN28B axis

HGC-27 cells were transfected with the overexpression of circ_0035277, miR-576-3p and LIN28B to verify whether circ_0035277 regulated gastric cancer cell malignancy through the miR-576-3p/LIN28B axis further. The Western blot showed that circ_0035277 up-regulates the expression of LIN28B and further cotransfects with the miR-576-3p mimic-suppressed LIN28B level in HGC-27 cells (Figs. 6A and 6B). LIN28B functionally promoted cell migration and invasion (Figs. 6C and 6D). Furthermore, the miR-576-3p mimic abolished the migration and invasion of circ_0035277-treated HGC-27 cells and LIN28B reversed these roles of circ_0035277/miR-576-3p (Figs. 6C and 6D). Overall, these results suggested that circ_0035277 participates in gastric cancer progression through the miR-576-3p/LIN28B axis.

### Knockdown of circ_0035277 suppresses gastric cancer metastasis in vivo

HGC-27 cells transfected with lentivirus siRNA-hsa_circ_0035277 were injected into BALB/c nude mice to construct the gastric cancer orthotopic transplantation model and investigate the biological role of circ_0035277 on gastric cancer metastasis. The results indicated that knockdown of circ_0035277 significantly reduces bioluminescence in 35 days (Figs. 7A and 7B). Moreover, HE staining showed that knockdown of circ_0035277 significantly suppresses visible tumour nodules in lung tissues (Figs. 7C and 7D). These findings revealed that knockdown of circ_0035277 suppresses gastric cancer metastasis in vivo.

## Discussion

circRNAs were thought to be the product of faulty base splicing without biological properties in past years [21]. Two studies on circRNAs published in Nature until 2013 have attracted considerable research

attention worldwide [22, 23]. An increasing number of studies have indicated that circRNAs are involved in the formation of various diseases, including gastric cancers [5, 24]. Many circRNAs are abnormally expressed in gastric cancers [25, 26]. For instance, circNHSL1 was elevated in gastric cancers and promoted the occurrence of tumours [27]. circ_0035277 was up-regulated in the plasma of gastric cancer patients according to NCBI GEO databases GSE89143 and GSE93541 [18]. However, the involvement of circ_0035277 in the progression and metastasis of gastric cancer remains unknown. Therefore, this study focused on the potential function and molecular mechanism of circ_0035277 in gastric cancer. Our results revealed that circ_0035277 promotes cell proliferation and metastasis by targeting the miR-576-3p/LIN28B axis in gastric cancer.

Consistent with GEO databases GSE89143 and GSE93541, circ_0035277 was highly expressed in gastric cancer tissues and cells. These data indicated that circ_0035277 may be associated with the development of gastric cancer. Moreover, the functional role showed that circ_0035277 accelerates the development and metastasis of gastric cancer in vivo and in vitro. Intron and exon circRNAs are very important forms of circRNAs [28]. Intron circRNAs are located in the nucleus and affect RNA-mediated genetics and epigenetics [29]. Meanwhile, exon circRNAs are located in the cytoplasm and contain binding sites to miRNAs that sponge for miRNAs [30]. Herein, the abundance of circ_0035277 in the cytoplasm indicated that endogenous circ_0035277 acts as miRNA sponges to participate in gastric cancer and circ_0035277 directly targets miR-576-3p. miR-576-3p acts as a tumour suppressor in lung cancer [31], glioma [32] and bladder cancer [33]. For instance, Greenawalt EJ et al. [31] suggested that miR-576-3p suppresses lung cancer progression by targeting SGK1. The miR-576-3p expression was down-regulated in gastric cancer and miR-576-3p eliminated the promoting effect of circ_0035277 on gastric cancer metastasis in this study.

circRNAs isolate the inhibitory effect of miRNAs on their target genes [34, 35]. Bioinformatics and luciferase reporter assays in this study suggested that miR-576-3p is specifically bound to LIN28B. Lin28B is a highly conserved RNA binding protein that belongs to the Lin28 protein family [36], considered an oncogene and involved in many carcinogenic signalling networks [37, 38]. LIN28B is a new biomarker associated with poor clinical outcomes of gastric cancer [39]. Consistent with the findings of Zhang X [40], our results showed that LIN28B promotes gastric cancer metastasis. Moreover, LIN28B reversed the role of circ_0035277/miR-576-3p on gastric cancer metastasis.

Therefore, circ_0035277 promoted gastric cancer proliferation and metastasis by regulating the miR-576-3p/LIN28B axis. This in-depth understanding of the role and mechanism of circ_0035277 can provide new insights into the treatment of gastric cancer.

# Declarations

### Authors' contributions

The authors declare that all data were generated in-house and that no paper mill was used. All authors contributed to this review with conception and design, literature review, drafting and critical revision,

editing, and approval of the final version.

### Funding

This study was supported in part by Suqian Science and Technology Support Project Fund (K201907), Jiangsu Innovative Team Leading Talent Fund (CXTDC2016006, QNRC2016446), Jiangsu 333 Talent Fund (BRA2020016), Jiangsu Provincial Key Research and Development Special Fund (BE2015666), Jiangsu Six High Peak Talent Fund (WSW-205, WSW236), Zhenjiang Key Research and Development Fund (SH2021038).

### Availability of data and material

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

### Ethics approval and consent to participate

The consent agreement was signed by all participants and the project was approved by the Ethics Committee of The Affiliated Suqian first People's Hospital of Nanjing Medical University.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## References

1. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. Lancet. 2020; 396(10251):635-648.
2. Necula L, Matei L, Dragu D, Neagu AI, Mambet C, Nedeianu S, Bleotu C, Diaconu CC, Chivu-Economescu M. Recent advances in gastric cancer early diagnosis. World J Gastroenterol. 2019; 25(17):2029-2044.
3. Song Z, Wu Y, Yang J, Yang D, Fang X. Progress in the treatment of advanced gastric cancer. Tumour Biol. 2017; 39(7):1010428317714626.
4. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet. 2019; 20(11):675-691.
5. Zhang Z, Yang T, Xiao J. Circular RNAs: Promising Biomarkers for Human Diseases. EBioMedicine. 2018; 34:267-274.
6. Sang Y, Chen B, Song X, Li Y, Liang Y, Han D, Zhang N, Zhang H, Liu Y, Chen T et al. circRNA_0025202 Regulates Tamoxifen Sensitivity and Tumor Progression via Regulating the miR-182-5p/FOXO3a

Axis in Breast Cancer. Mol Ther. 2021; 29(12):3525-3527.

7. Zeng K, Chen X, Xu M, Liu X, Hu X, Xu T, Sun H, Pan Y, He B, Wang S. CircHIPK3 promotes colorectal cancer growth and metastasis by sponging miR-7. Cell Death Dis. 2018; 9(4):417.
8. Shan C, Zhang Y, Hao X, Gao J, Chen X, Wang K. Biogenesis, functions and clinical significance of circRNAs in gastric cancer. Mol Cancer. 2019; 18(1):136.
9. Huang X, Yang W, Zhang Z, Shao Z. Dysregulated circRNAs serve as prognostic and diagnostic markers in osteosarcoma by sponging microRNA to regulate the downstream signaling pathway. J Cell Biochem. 2020; 121(2):1834-1841.
10. Patop IL, Kadener S. circRNAs in Cancer. Curr Opin Genet Dev. 2018; 48:121-127.
11. Huang X, Zhang W, Shao Z. Prognostic and diagnostic significance of circRNAs expression in lung cancer. J Cell Physiol. 2019; 234(10):18459-18465.
12. Huang YS, Jie N, Zou KJ, Weng Y. Expression profile of circular RNAs in human gastric cancer tissues. Mol Med Rep. 2017; 16(3):2469-2476.
13. Han B, Chao J, Yao H. Circular RNA and its mechanisms in disease: From the bench to the clinic. Pharmacol Ther. 2018; 187:31-44.
14. Qi X, Zhang DH, Wu N, Xiao JH, Wang X, Ma W. ceRNA in cancer: possible functions and clinical implications. J Med Genet. 2015; 52(10):710-718.
15. Wang Z, Liu Y. Predicting Functional MicroRNA-mRNA Interactions. Methods Mol Biol. 2017; 1580:117-126.
16. Ebbesen KK, Hansen TB, Kjems J. Insights into circular RNA biology. RNA Biol. 2017; 14(8):1035-1045.
17. Zhang J, Liu H, Hou L, Wang G, Zhang R, Huang Y, Chen X, Zhu J. Circular RNA_LARP4 inhibits cell proliferation and invasion of gastric cancer by sponging miR-424-5p and regulating LATS1 expression. Mol Cancer. 2017; 16(1):151.
18. Li T, Shao Y, Fu L, Xie Y, Zhu L, Sun W, Yu R, Xiao B, Guo J. Plasma circular RNA profiling of patients with gastric cancer and their droplet digital RT-PCR detection. J Mol Med (Berl). 2018; 96(1):85-96.
19. Busuttil RA, Liu DS, Di Costanzo N, Schröder J, Mitchell C, Boussioutas A. An orthotopic mouse model of gastric cancer invasion and metastasis. Sci Rep. 2018; 8(1):825.
20. Yang G, Zhang Y, Yang J. Identification of Potentially Functional CircRNA-miRNA-mRNA Regulatory Network in Gastric Carcinoma using Bioinformatics Analysis. Med Sci Monit. 2019; 25:8777-8796.
21. Patop IL, Wüst S, Kadener S. Past, present, and future of circRNAs. Embo j. 2019; 38(16):e100836.
22. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature. 2013; 495(7441):384-388.
23. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013; 495(7441):333-338.

24. Arnaiz E, Sole C, Manterola L, Iparraguirre L, Otaegui D, Lawrie CH. CircRNAs and cancer: Biomarkers and master regulators. Semin Cancer Biol. 2019; 58:90-99.
25. Lei B, Tian Z, Fan W, Ni B. Circular RNA: a novel biomarker and therapeutic target for human cancers. Int J Med Sci. 2019; 16(2):292-301.
26. Dang Y, Ouyang X, Zhang F, Wang K, Lin Y, Sun B, Wang Y, Wang L, Huang Q. Circular RNAs expression profiles in human gastric cancer. Sci Rep. 2017; 7(1):9060.
27. Zhu Z, Rong Z, Luo Z, Yu Z, Zhang J, Qiu Z, Huang C. Correction to: Circular RNA circNHSL1 promotes gastric cancer progression through the miR-1306-3p/SIX1/Vimentin axis. Mol Cancer. 2020; 19(1):67.
28. Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, Sun W, Dou K, Li H. Circular RNA: A new star of noncoding RNAs. Cancer Lett. 2015; 365(2):141-148.
29. Shang Q, Yang Z, Jia R, Ge S. The novel roles of circRNAs in human cancer. Mol Cancer. 2019; 18(1):6.
30. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L et al. Corrigendum: Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol. 2017; 24(2):194.
31. Greenawalt EJ, Edmonds MD, Jain N, Adams CM, Mitra R, Eischen CM. Targeting of SGK1 by miR-576-3p Inhibits Lung Adenocarcinoma Migration and Invasion. Mol Cancer Res. 2019; 17(1):289-298.
32. Hu Q, Liu F, Yan T, Wu M, Ye M, Shi G, Lv S, Zhu X. MicroRNA-576-3p inhibits the migration and proangiogenic abilities of hypoxia-treated glioma cells through hypoxia-inducible factor-1α. Int J Mol Med. 2019; 43(6):2387-2397.
33. Liang Z, Li S, Xu X, Xu X, Wang X, Wu J, Zhu Y, Hu Z, Lin Y, Mao Y et al. MicroRNA-576-3p inhibits proliferation in bladder cancer cells by targeting cyclin D1. Mol Cells. 2015; 38(2):130-137.
34. Xiong DD, Dang YW, Lin P, Wen DY, He RQ, Luo DZ, Feng ZB, Chen G. A circRNA-miRNA-mRNA network identification for exploring underlying pathogenesis and therapy strategy of hepatocellular carcinoma. J Transl Med. 2018; 16(1):220.
35. Zhang F, Zhang R, Zhang X, Wu Y, Li X, Zhang S, Hou W, Ding Y, Tian J, Sun L et al. Comprehensive analysis of circRNA expression pattern and circRNA-miRNA-mRNA network in the pathogenesis of atherosclerosis in rabbits. Aging (Albany NY). 2018; 10(9):2266-2283.
36. Guo W, Hu Z, Bao Y, Li Y, Li S, Zheng Q, Lyu D, Chen D, Yu T, Li Y et al. A LIN28B Tumor-Specific Transcript in Cancer. Cell Rep. 2018; 22(8):2016-2025.
37. Yuan L, Tian J. LIN28B promotes the progression of colon cancer by increasing B-cell lymphoma 2 expression. Biomed Pharmacother. 2018; 103:355-361.
38. Ren J, Fu J, Ma T, Yan B, Gao R, An Z, Wang D. LncRNA H19-elevated LIN28B promotes lung cancer progression through sequestering miR-196b. Cell Cycle. 2018; 17(11):1372-1380.
39. Xu J, Zhou Y, Yang J, Gu Y, Zhang E, Yuan W, Wang C, Jin G, Ma H, Hu Z. Hypomethylation-activated cancer-testis gene LIN28B promotes cell proliferation and metastasis in gastric cancer. Gene. 2021; 813:146115.

40. Zhang X, Liang W, Liu J, Zang X, Gu J, Pan L, Shi H, Fu M, Huang Z, Zhang Y et al. Long non-coding RNA UFC1 promotes gastric cancer progression by regulating miR-498/Lin28b. J Exp Clin Cancer Res. 2018; 37(1):134.

# Figures

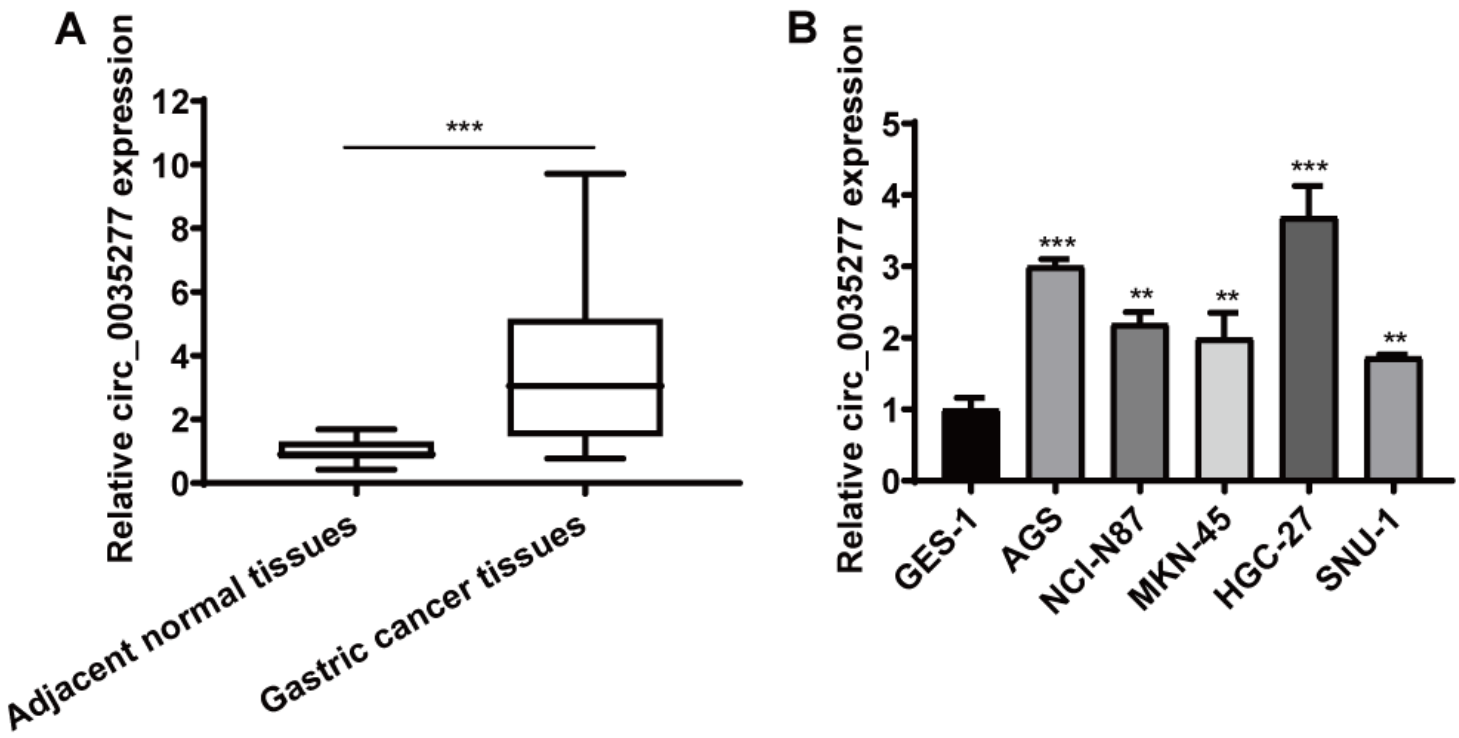
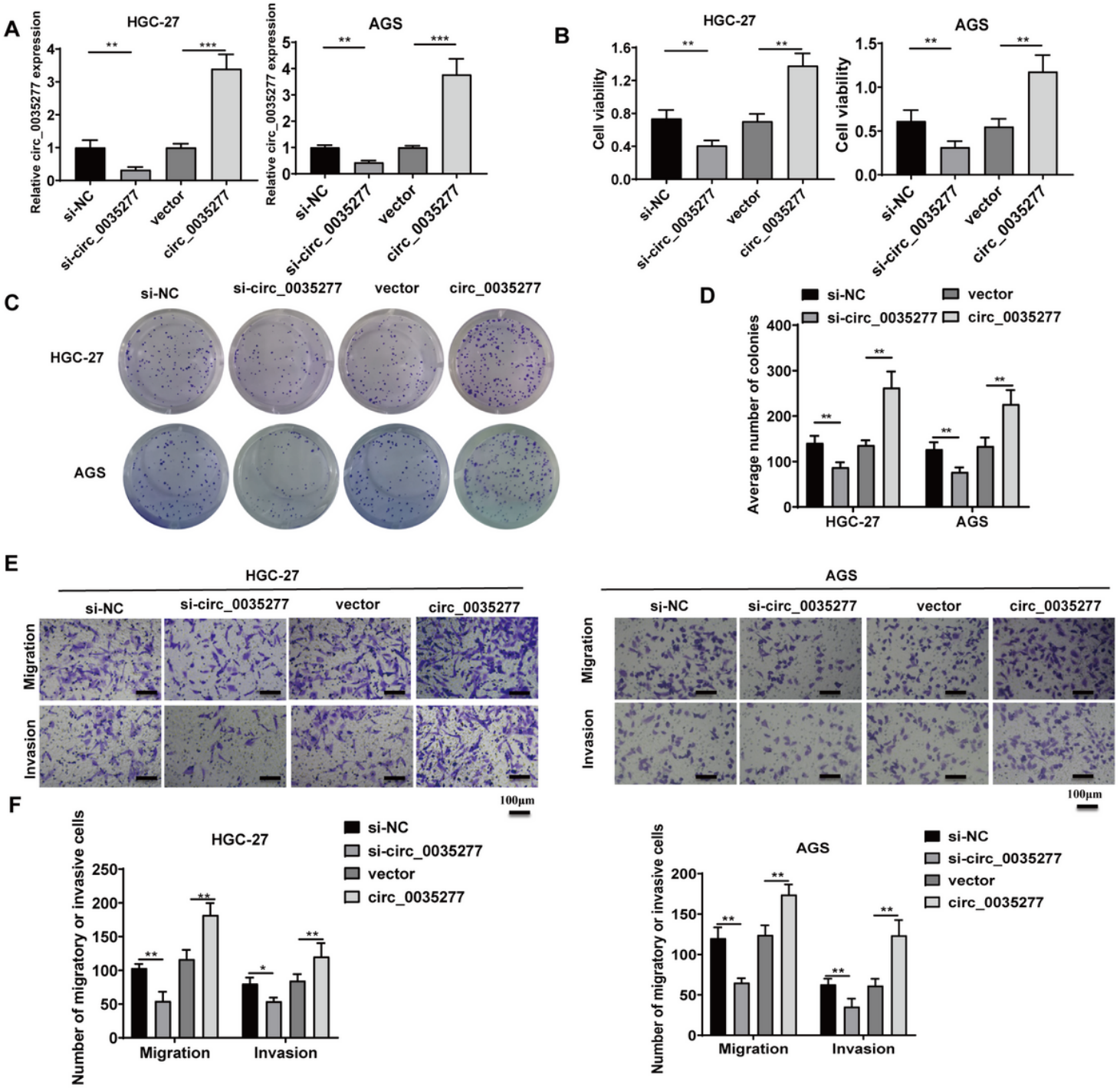


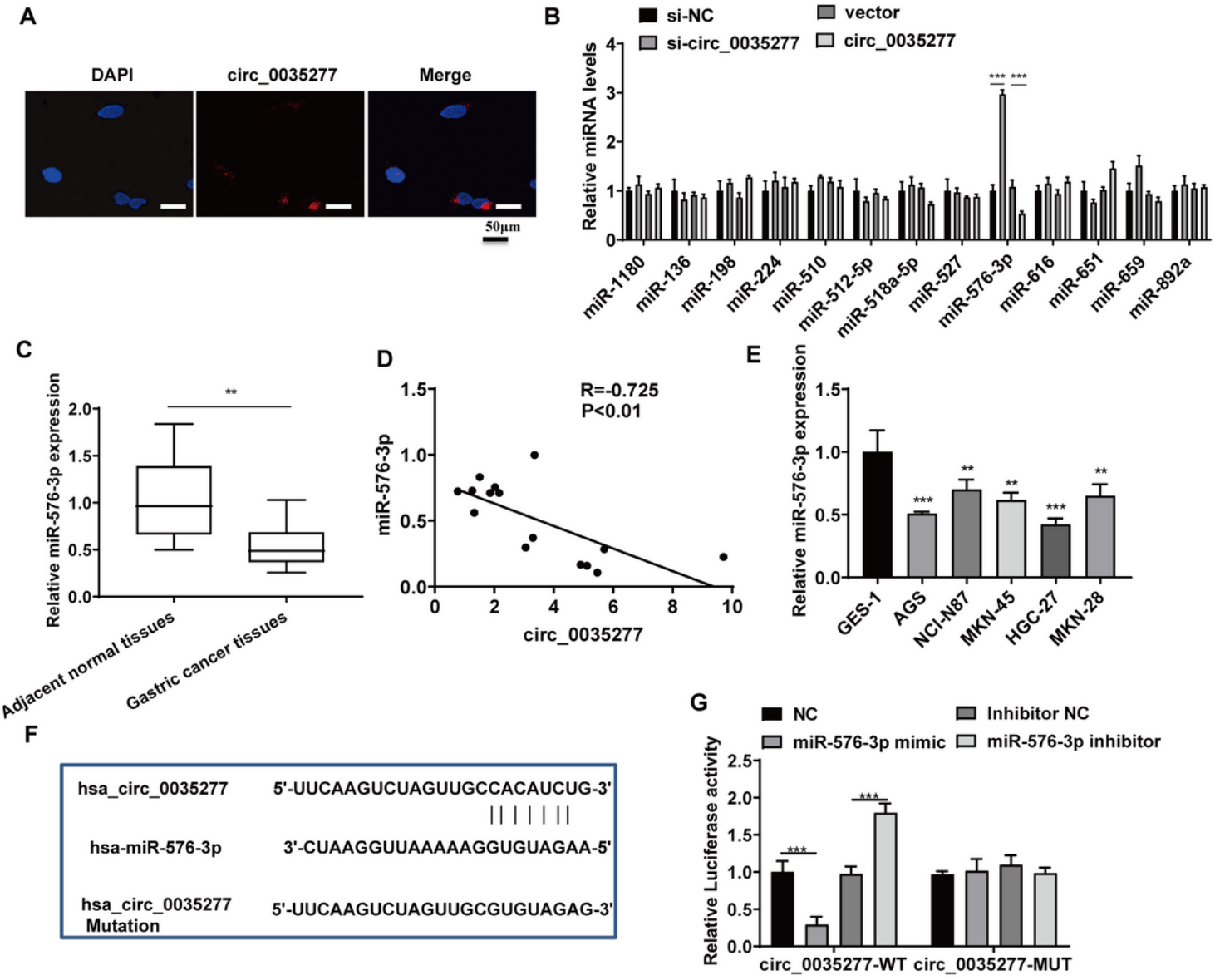

**Figure 1**

**circ_0035277 is highly expressed in gastric cancer tissues and cell lines. A** The relative expression of circ_0035277 in gastric cancer tissues was detected by RT-qPCR assay (n=15). **B** The level of circ_0035277 in gastric cancer cell lines (AGS, NCI-N87, MKN-45, HGC-27, SNU-1) and normal cell line GES-1 was measured by RT-qPCR assay. **P < 0.01, ***P < 0.001, vs. The compared control group.



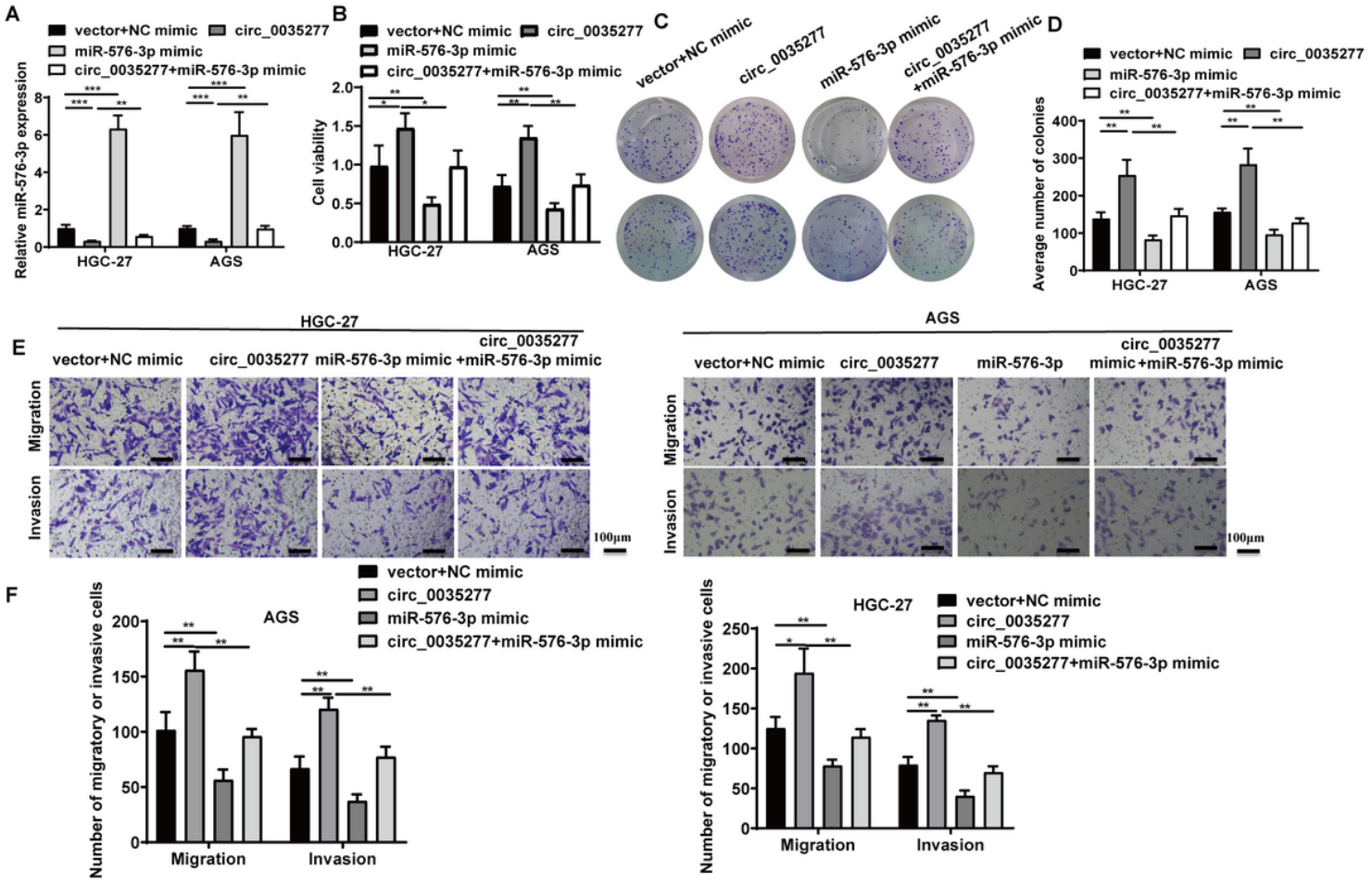

## Figure 2

**circ_0035277 promotes gastric cancer cell proliferation and metastasis.** circ_0035277 was knocked down or overexpressed in HGC-27 and AGS cells. **A** The transfection efficiency of circ_0035277 was confirmed by RT-qPCR assay. **B** CCK-8 assay was used to measure the cell viability of circ_0035277 in HGC-27 and AGS cells. **C-D** Colony formation assays were performed to investigate the proliferation of circ_0035277 in HGC-27 and AGS cells. **E-F** Transwell assay was carried out to assess cell migration and invasion of circ_0035277 in HGC-27 and AGS cells. Scale bar=100 μm. *P < 0.05, **P < 0.01, ***P < 0.001, vs. The compared control group.



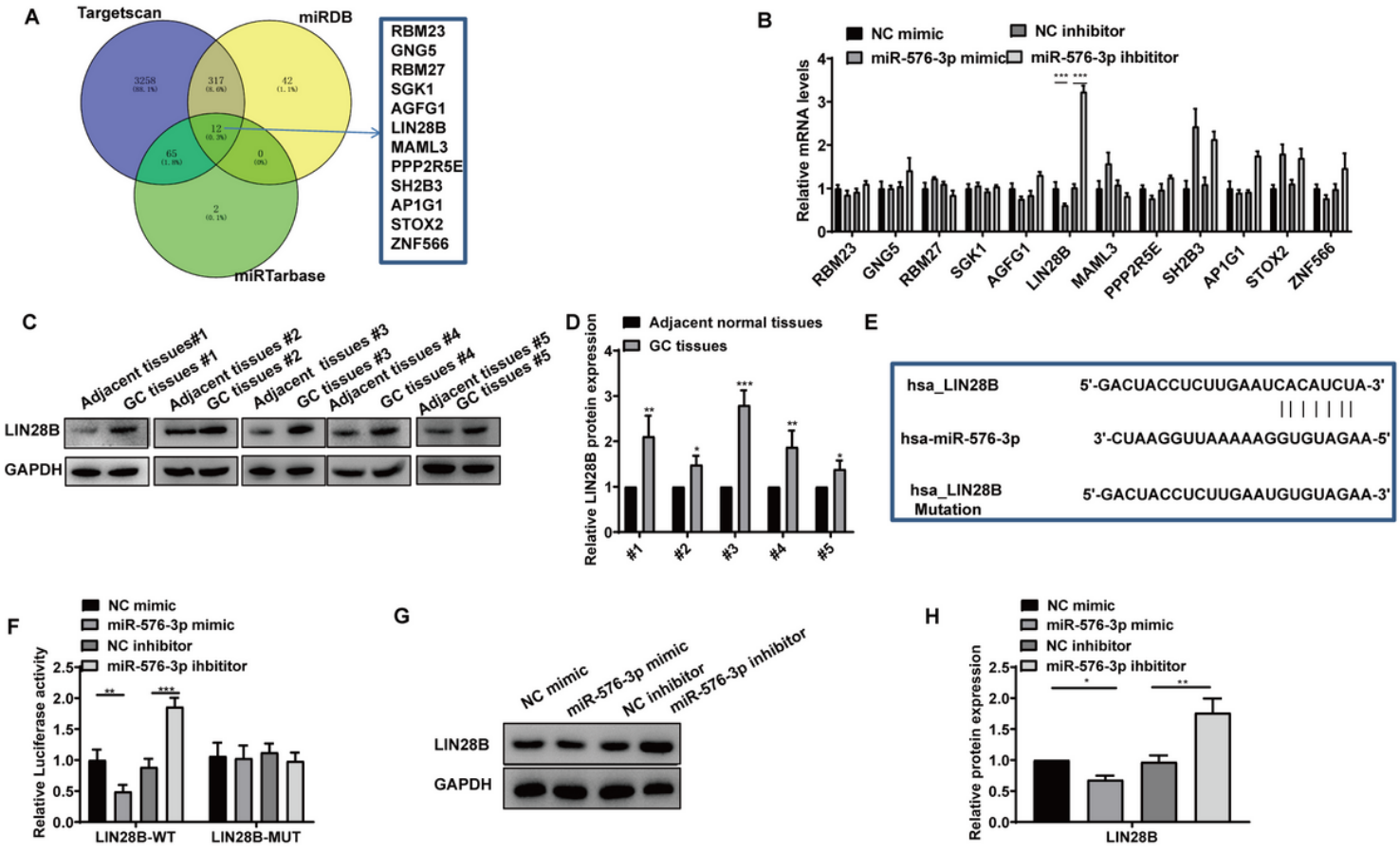

## Figure 3

**circ_0035277 targets miR-576-3p in gastric cancer. A** The localization of circ_0035277 in HGC-27 cells was confirmed by FISH assay. Scale bar=50 μm. **B** The levels of possible miRNAs were detected by RT-qPCR assay when HGC-27 cells transfected with circ_0035277 and si-circ_0035277. **C** MiR-576-3p level in gastric cancer tissues was detected by RT-qPCR assay (n=15). **D** Pearson's correlation analysis was used to assess the relevance between circ_0035277 and miR-576-3p. **E** The level of miR-576-3p in AGS, NCI-N87, MKN-45, HGC-27, MKN-28 and GES-1 was measured by RT-qPCR assay. **F** The promising binding sites between circ_0035277 and miR-576-3p were predicted by online database Circular RNA Interactome. **G** Luciferase reporter assay was carried out to prove the relationship between circ_0035277 and miR-576-3p. **P < 0.01, ***P < 0.001, vs. The compared control group.




## Figure 4

**circ_0035277 promotes gastric cancer cell proliferation and metastasis by regulating miR-576-3p.** HGC-27 and AGS cells were transfected with overexpression of circ_0035277, miR-576-3p mimic, jointly or respectively. **A** The relative expression of miR-576-3p in HGC-27 and AGS cells was detected by RT-qPCR assay. **B** CCK-8 assay was used to measure HGC-27 and AGS cell viability. **C-D** Colony formation assays were performed to investigate HGC-27 and AGS cell proliferation. **E-F** Transwell assay was carried out to assess HGC-27 and AGS cell migration and invasion. Scale bar=100 μm. *P < 0.05, **P < 0.01, ***P < 0.001, vs. The compared control group.




## Figure 5

**miR-576-3p targets LIN28B in gastric cancer. A** Three online databases Targetscan, miRDB, and miRTarbase was performed to predict intersecting target genes. **B** RT-qPCR assay was used to detect intersecting target gene expression. **C-D** The level of LIN28B was proved by western blot assay. **E** The potential binding sites of miR-576-3p with 3'UTR of LIN28B. **F** Luciferase reporter assay was carried out to prove the relationship between miR-576-3p and LIN28B. **G-H** Western blot was used to assess LIN28B expression. *P < 0.05, **P < 0.01, ***P < 0.001, vs. The compared control group.

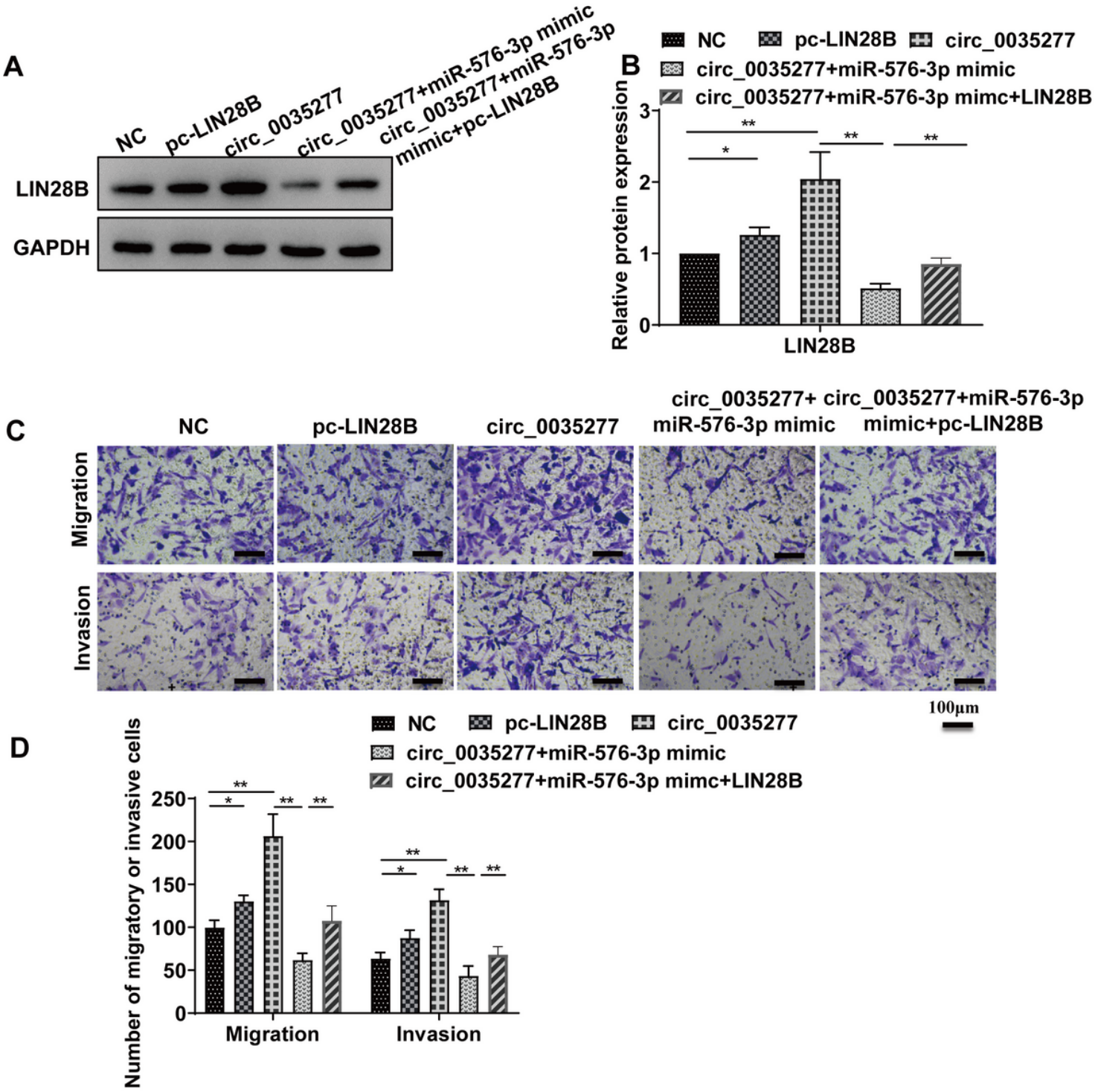



Figure 6

**circ_0035277 promotes gastric cancer cell metastasis by miR-576-3p/LIN28B axis.** HGC-27 cells were transfected with overexpression of circ_0035277, miR-576-3p, and LIN28B. **A-B** Western blot was used to assess LIN28B expression. **C-D** Transwell assay was carried out to assess HGC-27 cell migration and invasion. Scale bar=100 μm. *P < 0.05, **P < 0.01, ***P < 0.001, vs. The compared control group.

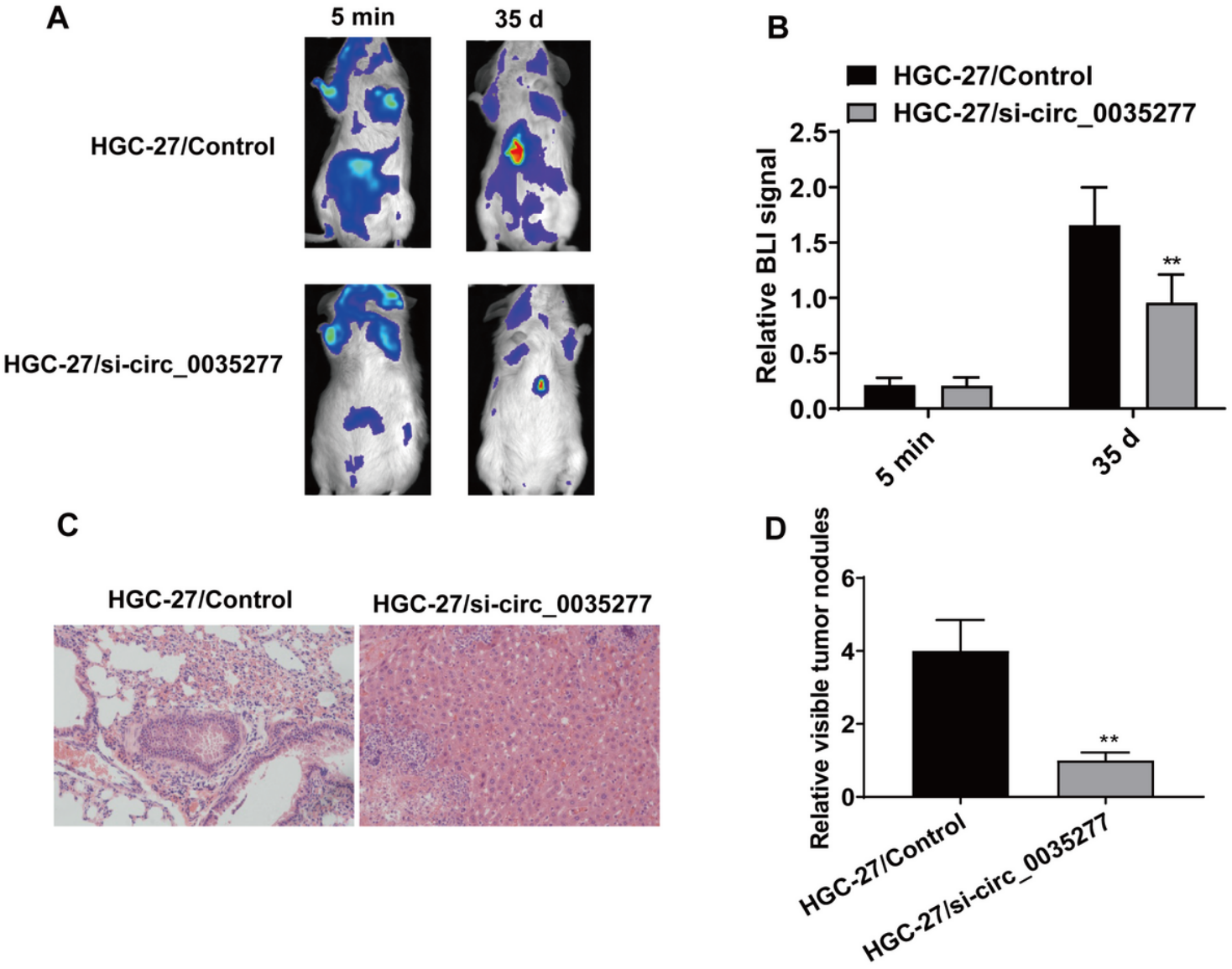



Figure 7

**Knockdown of circ_0035277 suppresses gastric cancer metastasis in vivo. A-B** The bioluminescence images in different groups (HGC-27/Control group, HGC-27/si-circ_0035277 group) were captured. **C-D** HE staining was performed to measure the visible tumor nodules in the lung tissues. Scale bar=200 μm. **P < 0.01, vs. The compared control group.

# Supplementary Files

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

- Additionalfile1.doc
- Additionalfile2.doc