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Research Article

Keywords: circCOQ10A, MAPK6, neutrophil, gastric cancer, metastasis

Posted Date: October 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10794029/v1>

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Additional Declarations: No competing interests reported.

**m⁶A modification of circCOQ10A potentiates N2 neutrophil polarization to
accelerate gastric cancer progression and cisplatin resistance by regulating
MAPK6/CXCL2 signaling**

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Running title: The role of circCOQ10A in gastric cancer.

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Abstract

Background Circular RNAs (circRNAs) are well-recognized as critical diagnostic biomarkers and functional regulators in various malignancies, including gastric cancer (GC). N6-methyladenosine (m⁶A) RNA modification represents the most prevalent post-transcriptional modification and exerts pivotal regulatory functions in tumor progression. However, the functional implications and molecular mechanisms of m⁶A-modified circRNAs in GC malignant progression remain poorly elucidated. Herein, this study aimed to investigate the biological role and underlying mechanism of m⁶A modification on circCOQ10A in GC progression and cisplatin (DDP) resistance.

Methods The m⁶A modification status of hsa_circ_0026967 (circCOQ10A) was verified through quantitative real-time PCR, methylated RNA immunoprecipitation, and Western blot assays. Gain- and loss-of-function experiments combined with *in vivo* tumorigenesis and metastasis models were performed to validate the oncogenic role of circCOQ10A in GC progression and DDP resistance. RNA immunoprecipitation and dual-luciferase reporter assays were conducted to confirm the direct binding interaction between circCOQ10A and miR-144-3p. Western blot analysis, flow cytometry, and cell co-culture systems were further utilized to clarify the regulatory effects of circCOQ10A on MAPK6/CXCL2 signaling and subsequent N2 neutrophil polarization. Fluorescence in situ hybridization was applied to detect the tissue localization of circCOQ10A and evaluate its clinical prognostic significance in GC patients.

Results Our results demonstrated that circCOQ10A underwent robust m⁶A modification

mediated by METTL14. METTL14 knockdown diminished the m⁶A modification level of circCOQ10A while elevating its endogenous expression. Functional experiments revealed that circCOQ10A knockdown suppressed GC cell proliferation, metastasis, and DDP resistance both *in vitro* and *in vivo*, whereas circCOQ10A overexpression yielded the opposite oncogenic effects. Further mechanistic validation indicated that circCOQ10A functioned as a competing endogenous RNA to sponge miR-144-3p, thereby upregulating MAPK6 expression and triggering the activation of the CXCL2/CXCR2 signaling axis. This activated signaling cascade ultimately facilitated N2-like neutrophil polarization and GC cell invasion. Clinically, circCOQ10A was significantly upregulated in GC tissues, and its high expression was closely correlated with unfavorable clinical prognosis in GC patients.

Conclusions The present study reveals that METTL14-mediated m⁶A modification of circCOQ10A promotes N2 neutrophil polarization, thereby driving GC malignant progression and DDP resistance through the activation of the MAPK6/CXCL2 signaling pathway.

Keywords: circCOQ10A; MAPK6; neutrophil; gastric cancer; metastasis

Introduction

Gastric cancer (GC) remains one of the most prevalent malignant tumors worldwide, contributing substantially to global cancer-related morbidity and mortality (Yeoh et al. 2022). Current clinical therapeutic strategies for GC include early endoscopic resection, radical surgical resection, and systemic chemotherapy

represented by cisplatin (DDP)-based regimens (Kim et al. 2025). Despite continuous advancements in diagnostic and therapeutic approaches, a considerable proportion of GC patients develop distant metastasis and acquired chemotherapy resistance, which constitute the primary causes of treatment failure and poor clinical outcomes (Zhai et al. 2019). Therefore, identifying reliable early diagnostic biomarkers and novel therapeutic targets is urgently required to improve the prognosis of GC patients.

Circular RNAs (circRNAs) are a unique class of endogenous non-coding RNAs characterized by covalently closed continuous loop structures with high stability and tissue specificity (Wei et al. 2020). Accumulating evidence has demonstrated that circRNAs exert diverse biological functions, including microRNA (miRNA) sponging, protein scaffolding, translational regulation, and modulation of mRNA stability, thereby participating in the malignant progression of multiple cancers (Tao et al. 2021). For instance, exosomal circSHKBP1 facilitates GC progression via regulating the miR-582-3p/HUR axis (Xie et al. 2020). CircDLG1 drives GC cell invasion and anti-PD-1 immunotherapy resistance by sponging miR-141-3p (Chen et al. 2021). Moreover, circTADA2A interacts with TRIM28 to stabilize p53 protein, thus mediating FLT3-ITD inhibition in acute myeloid leukemia (Li et al. 2025). CircPETH-encoded peptide circPETH-147aa remodels the tumor immunosuppressive microenvironment by promoting glycolysis and metastasis in hepatocellular carcinoma (Lan et al. 2025). Consistent with these findings, our previous studies have verified that circORC5 and circUGGT2 promote GC metastasis and DDP resistance through the miR-30c-2-3p/AKT1S1 and miR-186-3p/MAP3K9 signaling axes, respectively (Fan et al. 2022;

Chen et al. 2024). Nevertheless, the functional roles and molecular mechanisms of novel circRNAs in regulating GC malignant phenotypes and chemoresistance remain to be further explored.

N6-methyladenosine (m⁶A) is the most abundant and functionally critical post-transcriptional RNA modification in eukaryotic transcripts, which is dynamically and reversibly regulated by a set of core regulatory proteins. Specifically, m⁶A methylation is catalyzed by methyltransferases (writers) including METTL3 and METTL14, while demethylases (erasers) such as ALKBH5 and FTO can reverse this modification to maintain m⁶A homeostasis (Chen et al. 2019; Li et al. 2022). Dysregulated expression and function of m⁶A regulatory factors are closely associated with tumor progression, metastasis, and chemotherapy resistance across various cancers (Zhai et al. 2023; Chen et al. 2025; Su et al. 2020; Yang et al. 2020; Chen et al. 2021; Li et al. 2022; Zhang et al. 2023). METTL14-mediated m⁶A modification modulates the stability of target mRNAs can either recruit specific m⁶A reader proteins to enhance RNA stability (Zhuang et al. 2024), or trigger RNA decay signaling to reduce transcript abundance (Wang et al. 2023). Our previous studies further complemented the regulatory network of m⁶A modification in GC: the m⁶A reader YTHDF1 accelerates GC cell proliferation and invasion by enhancing the translation efficiency of ubiquitin-specific peptidase 14 (Chen et al. 2021), whereas METTL14-mediated m⁶A modification of circORC5 and circUGGT2 fuels GC progression and DDP resistance (Fan et al. 2022; Chen et al. 2024). However, the regulatory interplay between m⁶A modification and novel circRNAs in the GC tumor microenvironment (TME) remains poorly defined.

Tumor-associated neutrophils (TANs) are pivotal immune components in the TME and play dual regulatory roles in tumorigenesis, metastasis, and immune suppression (Zhang et al. 2018; Shaul et al. 2019). Activated neutrophils can secrete reactive oxygen species to induce epithelial DNA damage and initiate oncogenic transformation, while releasing neutrophil elastase and prostaglandin E2 to facilitate tumor cell proliferation (Houghton et al. 2010; Antonio et al. 2015). Additionally, TANs remodel the TME by degrading extracellular matrix components (Wculek & Malanchi 2015; Spicer et al. 2012; Rayes et al. 2015) and secreting pro-tumor factors including MMP-9 and VEGF, ultimately promoting tumor angiogenesis and distant metastasis (Casbon et al. 2015; Zhou et al. 2016; Mishalian et al. 2014; Spiegel et al. 2016; Kuang et al. 2011). Clinically, elevated tumor-infiltrating neutrophils and a high neutrophil-to-lymphocyte ratio are well-established adverse prognostic indicators for multiple malignancies (Mi et al. 2024). Notably, TANs exhibit distinct functional phenotypes depending on TME cues: N1-type TANs induced by IFN- β possess anti-tumor activity, while TGF- β -dominated N2-type TANs exert pro-tumorigenic and immunosuppressive functions. Multiple cytokines, chemokines and paracrine signals in the TME are responsible for TAN phenotypic polarization (Shaul et al. 2019). MAPK6/CXCL2 signaling acts as a core paracrine regulatory axis governing TAN phenotypic polarization in the TME. Tumor-activated MAPK6 signaling triggers robust CXCL2 secretion, which binds to CXCR2 and drives neutrophils toward the pro-tumor N2 phenotype to fuel tumor invasion and metastasis (Zhang et al. 2018; Coffelt et al. 2015). Targeted neutrophil intervention has been proven to suppress tumor metastasis, representing a promising

therapeutic strategy (Gregory & Houghton 2011). However, the upstream molecular mechanism governing N2 TAN polarization in GC, especially circRNA-related m⁶A regulatory mechanisms, requires further in-depth elucidation.

In the present study, we identified a novel circRNA hsa_circ_0026967 derived from the linear COQ10A gene, termed circCOQ10A. We further systematically elucidated that METTL14-mediated m⁶A modification of circCOQ10A facilitated N2 neutrophil polarization, thereby driving GC progression and DDP resistance via activation of the MAPK6/CXCL2 signaling axis. Our findings uncover a novel m⁶A-circRNA-immune regulatory network in GC, providing potential biomarkers and therapeutic targets for overcoming GC chemoresistance.

Materials and Methods

Clinical specimen collection and ethical approval

A commercial tissue microarray (TMA, XT18-003, Shanghai Outdo Biotech) containing 90 paired GC and matched normal gastric tissues was used, alongside 10 fresh frozen GC specimens resected in our hospital. All samples were consecutively collected from Jan 2018 to Dec 2020. Inclusion criteria: histologically verified primary gastric adenocarcinoma, paired tumor/adjacent tissues, complete clinical and survival data, no preoperative chemo-radiotherapy. Exclusion criteria: metastatic gastric lesions, neoadjuvant treatment history, incomplete data, or degraded tissue samples. The commercial TMA was constructed from fully de-identified residual surgical tissues with patient informed consent obtained by the vendor under ethical supervision. All specimen-related experiments were approved by the Ethics Committee of Shanghai

Sixth People's Hospital (Approval No. 2022-YS-056), consistent with the Declaration of Helsinki. Written informed consent was acquired from all patients who provided fresh frozen tissues in our center.

Fluorescence in situ hybridization (FISH)

FISH assays were performed to detect the expression levels and subcellular localization of circCOQ10A and miR-144-3p in 90 pairs of GC and matched normal tissues using TMA samples. The specific probe sequences were designed and synthesized as follows: circCOQ10A probe, 5'-AGTACAAACAGCTTCACACTGTCATTAAAA-biotin-3'; miR-144-3p probe, 5'-AGTACATCATCTATACTGTATAAAAA-DIG-3'. The FISH experimental procedure was strictly implemented in accordance with a previously published protocol (Fan et al. 2022).

Cell culture and DDP resistance cell line establishment

Human GC cell lines MKN-45 (CCTCC-GDC080), HGC-27 (CCTCC-GDC170), AGS (CCTCC-GDC160), 293T (CCTCC-G1107), HL-60 neutrophil precursor cells (CCTCC-GDC142) and normal gastric epithelial GES-1 cells (CCTCC-GDC230) were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (CCTCC). All cells were cultured in RPMI 1640 medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Gibco, Waltham, MA, USA), 100 U/mL penicillin, and 100 μ g/mL streptomycin (Penicillin-Streptomycin-Glutamine; Gibco), and maintained in a humidified incubator with 5% CO₂ at 37°C. To construct the DDP-resistant GC cell line, MKN-45 cells were continuously treated with gradually increased DDP concentrations (Sigma-Aldrich, St. Louis, MO, USA) for six

months until stable DDP-resistant MKN-45/DDP cells that tolerated 1 µg/mL DDP were successfully established. Prior to each functional experiment, MKN-45/DDP cells were cultured in drug-free complete RPMI 1640 medium for two weeks to eliminate residual drug effects.

RNA extraction and real-time quantitative PCR (RT-qPCR)

Total RNA was isolated from GC tissues and cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). First-strand complementary DNA (cDNA) was subsequently synthesized via a reverse transcription kit (Takara, Tokyo, Japan). RT-qPCR was performed using a SYBR Green PCR Kit (Accurate Biotechnology, Hunan, China) on an ABI PRISM 7500 Fast Sequence Detection System (Applied Biosystems, Shanghai, China). The thermal cycling parameters were set as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. The relative gene expression levels were quantified using the $2^{-\Delta\Delta C_t}$ method (Fan et al. 2022). GAPDH and U6 were used as internal reference genes for mRNA and miRNA detection, respectively. All primer sequences used in this study are listed in Supplementary Table S1.

Western blotting analysis

Western blotting was performed to detect the protein expression levels in GC cells and tissues. Total cellular protein was extracted from cell lysates, centrifuged at 12,000 rpm for 5 min at 4°C, and the supernatant was collected for protein quantification using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Equal amounts of protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene

difluoride (PVDF) membranes (Millipore, Boston, MA, USA). The membranes were blocked with 5% non-fat milk at room temperature for 1 h, incubated with primary antibodies overnight at 4 °C, and then incubated with corresponding secondary antibodies at room temperature for 1 h. The primary antibodies used in this study included anti-METTL14 (1:1000, bs-17608R; Bioss, Beijing, China), anti-MAPK6 (1:1000, AF7907; Affinity Biosciences, Cincinnati, OH, USA), anti-p-MAPK6 (1:1000, AF7407; Affinity Biosciences), anti-p-ERK1/2 (1:2000, 28733-1-AP; Proteintech, Wuhan, China), anti-MMP-2 (1:500, 10373-2-AP; Proteintech), anti-PCNA (1:10000, 10205-2-AP; Proteintech), anti-TGF- β (1:2000, 21898-1-AP; Proteintech), anti-CXCL2 (1:500, 26791-1-AP; Proteintech), anti-CXCR2 (1:1000, DF7095; Affinity Biosciences), anti-iNOS (1:2000, 18985-1-AP; Proteintech), anti-TNF- α (1:1000, BA0131; BOSTER), anti-IL-1 β (1:5000, 16806-1-AP; Proteintech), anti-Arg-1 (1:20000, 16001-1-AP; Proteintech), anti-CD206 (1:1000, 18704-1-AP; Proteintech), and anti-GAPDH (1:50000, 6006-1-Ig; Proteintech). GAPDH was used as the internal loading control.

Plasmids, shRNA, and miRNA transfection

All lentiviral shRNA plasmids (sh-METTL14, sh-circCOQ10A and non-target sh-NC) as well as miR-144-3p mimic and inhibitor were designed, synthesized and sequence-verified by GenePharma (Shanghai, China). Prior to synthesis, NCBI Nucleotide BLAST was applied to exclude potential off-target homologous sequences across human transcriptome. Full sense and antisense hairpin sequences of all shRNAs are summarized in Supplementary Table S2. GC cells (MKN-45, HGC-27, AGS) were

seeded in 6-well plates and cultured to 50–60% confluence. Cell transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocols, and subsequent functional experiments were conducted 48h after transfection.

RNA immunoprecipitation (RIP) and MeRIP assays

RIP and methylated RIP (MeRIP) assays were performed using an RNA-Binding Protein Immunoprecipitation Kit (17-700; Millipore) combined with a HiScript II qRT SuperMix Kit (R223; Vazyme, Nanjing, China). The specific antibodies applied for immunoprecipitation included anti-m⁶A (68055-1-Ig; Proteintech), anti-METTL14 (26158-1-AP; Proteintech), and anti-Ago2 (67934-1-Ig; Proteintech), which were used to verify m⁶A modification level, METTL14-circCOQ10A binding, and circCOQ10A-miRNA interaction, respectively.

Bioinformatic analysis

Multiple public databases were utilized for bioinformatic prediction in this study. The miRBase database (<http://www.mirbase.org/index.shtml>) was used to screen the potential binding miRNAs of circCOQ10A. TargetScanHuman 7.1 (http://www.targetscan.org/vert_71/) was applied to predict the downstream target genes of miR-144-3p. The TISIDB database (<http://cis.hku.hk/TISIDB/browse.php?gene=MAPK6>) was employed to analyze the correlation between MAPK6 expression and tumor immune microenvironment characteristics. The open reading frame (ORF) of circCOQ10A was predicted using the NCBI ORFfinder tool (<https://www.ncbi.nlm.nih.gov/orffinder/>).

Dual-luciferase reporter assay

The dual-luciferase reporter assay was performed to verify the direct binding relationship between circCOQ10A and miR-144-3p, as well as between miR-144-3p and MAPK6. Wild-type (circCOQ10A-WT, MAPK6-WT) and mutant-type (circCOQ10A-Mut, MAPK6-Mut) reporter plasmids were constructed. 293T cells were seeded in 96-well plates and co-transfected with the above plasmids together with miR-144-3p mimic or miR-NC. After 48 h of routine culture, firefly and Renilla luciferase activities were detected using a Dual-Luciferase Reporter Assay Kit (RG027; Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions.

Cell co-culture system and flow cytometry analysis

A Transwell co-culture system was established to explore the regulatory effect of circCOQ10A on neutrophil polarization. HL-60 neutrophil cells were seeded in the upper Transwell chamber, while AGS cells stably transfected with sh-circCOQ10A or sh-NC lentivirus were plated in the lower chamber. After 72 h of co-culture, neutrophils were collected and stained with CD11b antibody (0.5 μ g, 982614; BioLegend, San Diego, CA, USA). Neutrophil polarization phenotypes were analyzed using a BD LSR Fortessa X-20 flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA), and data were further processed with FlowJo software (Stanford University, Stanford, CA, USA).

In vivo tumorigenesis assay and lung metastasis models

Six- to eight-week-old female SPF-grade BALB/c nude mice (18–20 g) were purchased from the Shanghai Experimental Animal Center (Shanghai, China) for *in vivo* animal experiments. For the xenograft tumor model, MKN-45 cells stably transfected with sh-circCOQ10A or sh-NC were resuspended in sterile phosphate-buffered saline (PBS),

and 1×10^7 cells (200 μ L) were subcutaneously injected into the right axilla of each mouse. Starting on day 10 after cell inoculation, mice were intraperitoneally injected with 0, 3, or 6 mg/kg DDP every three days for 7 consecutive administrations. One month after modeling, all mice were anesthetized with isoflurane and euthanized, and xenograft tumors were dissected and photographed. Tumor volume was calculated using the formula: $\text{Volume} = (\text{length} \times \text{width}^2)/2$.

For the lung metastasis model, 1×10^7 stably transfected MKN-45 cells were injected into the tail vein of nude mice. Lung metastatic lesions were continuously observed and recorded for 28 days. At the experimental endpoint, nude mice were deeply anesthetized with 4% isoflurane, then humanely euthanized via cervical dislocation, complying with IACUC guidelines. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Shanghai Sixth People's Hospital (Approval No. 2022-0099) and performed in accordance with institutional ethical guidelines.

Additional functional experiments

Colony formation assay, Transwell invasion and migration assay, m⁶A dot blot assay, CCK-8 cell proliferation assay, RNase R digestion assay, nuclear-cytoplasmic fractionation assay, hematoxylin-eosin (HE) staining, and immunohistochemistry (IHC) staining were all performed strictly in accordance with a previously reported experimental method (Fan et al. 2022).

Statistical analysis

All statistical analyses were performed using SPSS 16.0 (IBM, Armonk, NY, USA),

GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA), and Social Pharmacology Research Statistical Software Package v.204 (2024). All quantitative data are presented as mean $\pm$ standard deviation (SD). Student's t-test was used for comparisons between two groups, and one-way analysis of variance (ANOVA) with Tukey's post hoc test and two-way ANOVA with Bonferroni post hoc test were performed for multi-group comparisons, respectively. Kaplan-Meier survival analysis combined with log-rank test was used to evaluate survival differences. Pearson correlation analysis was performed to assess the correlation between circCOQ10A and miR-144-3p expression. The half-maximal inhibitory concentration (IC₅₀) values were calculated via four-parameter logistic regression fitting in GraphPad Prism 8. A *P*-value < 0.05 was considered statistically significant for all analyses.

Results

METTL14 mediated m⁶A modification of circCOQ10A in GC cells

Consistent with our previous findings and m⁶A-circRNA microarray data, hsa_circ_0026967 (circCOQ10A) was identified as a key circRNA with significantly reduced m⁶A modification following METTL14 knockdown in GC cells (fold change = 2.64, *P* = 0.0013) (10) (Figure 1A). Subsequent RT-qPCR, Western blotting, and MeRIP assays further validated the regulatory relationship between METTL14 and circCOQ10A m⁶A modification. In MKN-45 and AGS GC cells, METTL14 overexpression markedly elevated the m⁶A modification level of circCOQ10A while reducing its cellular transcript abundance (Figure 1B, C). In contrast, METTL14 knockdown in HGC-27 cells decreased circCOQ10A m⁶A levels but upregulated

circCOQ10A expression (Figure 1D, E). RIP assays further confirmed the direct interaction between METTL14 and circCOQ10A. Compared with the IgG control group, circCOQ10A was prominently enriched in METTL14-bound RNA fragments in MKN-45 and AGS cells (Figure 1F).

Elevated circCOQ10A expression correlated with unfavorable clinical prognosis in patients with GC

To characterize the molecular properties of circCOQ10A, we first verified its circular stability and subcellular localization. After RNase R digestion for 48 h, linear COQ10A mRNA was significantly degraded, whereas circCOQ10A exhibited strong resistance to RNase R treatment, confirming the stable circular structure of circCOQ10A (Figure 1G). Nuclear-cytoplasmic fractionation and RT-qPCR assays revealed that circCOQ10A was predominantly distributed in the cytoplasm rather than the nucleus of MKN-45 and AGS cells (Figure 1H). FISH analysis further validated the cytoplasmic localization of circCOQ10A in human GC tissues (Figure 1I). Clinically, RT-qPCR detection of 10 paired clinical specimens showed that circCOQ10A expression was upregulated in GC tissues relative to adjacent normal tissues (Figure S1A). Consistently, quantitative FISH analysis of 90 pairs of GC tissue microarray samples further verified the increased circCOQ10A expression in GC lesions (Figure 1J). *In vitro* expression detection revealed that circCOQ10A was highly expressed in MKN-45 and AGS cell lines compared with the normal gastric epithelial GES-1 cells (Figure S1B). Kaplan-Meier survival analysis demonstrated that GC patients with high circCOQ10A expression exhibited shorter overall survival than those with low circCOQ10A

expression (Figure 1K). These findings indicated that circCOQ10A was upregulated in GC and served as a poor prognostic biomarker for GC patients.

CircCOQ10A accelerated GC malignant progression and reversed METTL14-induced tumor-suppressive effects

To clarify the functional interaction between METTL14 and circCOQ10A in GC progression, we constructed cell models with METTL14/circCOQ10A single or combined overexpression or knockdown. First, circCOQ10A-overexpressing (circCOQ10A-OE) HGC-27 cells were successfully constructed and verified by RT-qPCR (Figure 2A). Functional experiments confirmed that METTL14 overexpression inhibited the viability, colony formation ability, and invasive capacity of HGC-27 cells. However, circCOQ10A overexpression promoted GC malignant phenotypes and effectively abrogated the tumor-suppressive effects induced by METTL14 upregulation (Figure 2B–D). Furthermore, stable circCOQ10A-knockdown (circCOQ10A-KD) cell models were established in MKN-45 and AGS cells via lentiviral transfection, with knockdown efficiency validated by RT-qPCR (Figure 2E). Functional assays showed that METTL14 knockdown enhanced cell proliferation, colony formation, and invasion, while circCOQ10A depletion suppressed these malignant behaviors and counteracted the oncogenic effects caused by METTL14 deficiency (Figure 2F–H, Figure S2). Taken together, these results indicated that METTL14 inhibited GC progression predominantly through m⁶A modification-mediated downregulation of circCOQ10A.

CircCOQ10A drove DDP resistance in GC cells

To explore the potential role of circCOQ10A in GC chemotherapy resistance, we

detected circCOQ10A expression in DDP-resistant clinical GC tissues and DDP-resistant MKN-45/DDP cell line. The results showed that circCOQ10A was significantly upregulated in both DDP-resistant GC tissues and MKN-45/DDP cells (Figure 3A). To further verify the regulatory effect of circCOQ10A on DDP resistance, stable circCOQ10A-knockdown MKN-45/DDP cell lines were constructed. CCK-8 assays revealed that circCOQ10A depletion reduced the half-maximal inhibitory concentration (IC_{50}) of DDP in MKN-45/DDP cells (Figure 3B). Moreover, circCOQ10A knockdown markedly suppressed colony formation and invasive abilities of DDP-resistant GC cells (Figure 3C, D).

We further established subcutaneous xenograft tumor models using stably transfected MKN-45 cells to validate the *in vivo* effect of circCOQ10A on DDP chemosensitivity. Consistent with our previous study (Chen et al. 2024), nude mice were intraperitoneally administered with 0, 3, or 6 mg/kg DDP on days 10, 13, 16, 19, 22, 25, and 28 after tumor inoculation. *In vivo* results showed that under DDP treatment, the xenograft tumors in the circCOQ10A-KD group exhibited smaller volume and lower weight compared with the sh-NC control group (Figure 3E). H&E staining and Ki-67 IHC staining further confirmed that circCOQ10A knockdown inhibited tumor proliferation and enhanced the chemotherapeutic effect of DDP *in vivo* (Figure 3F). In summary, these data demonstrated that circCOQ10A contributed to the development of DDP resistance in GC.

CircCOQ10A functioned as a ceRNA to sponge miR-144-3p in GC cells

Given the predominant cytoplasmic distribution of circCOQ10A, we next explored its

potential molecular mechanism as a non-coding RNA. NCBI ORFfinder prediction indicated that circCOQ10A lacks a valid open reading frame, suggesting that it cannot encode functional proteins and may exert regulatory functions via non-coding mechanisms. Subsequent miRBase database prediction screened miR-144-3p as a potential downstream target miRNA of circCOQ10A (Figure S3), and the putative binding sites between circCOQ10A and miR-144-3p were illustrated in Figure 4A. Dual-luciferase reporter assays were performed to verify the direct binding relationship. The results showed that miR-144-3p mimic reduced the luciferase activity of wild-type circCOQ10A reporter vector, while miR-144-3p inhibitor increased its luciferase activity; no significant changes were observed in the mutant circCOQ10A group (Figure 4B). RT-qPCR results revealed that miR-144-3p expression was elevated in METTL14-overexpressing or circCOQ10A-knockdown MKN-45 and AGS cells, whereas it was decreased in METTL14-knockdown or circCOQ10A-overexpressing HGC-27 cells (Figure 4C, D). Notably, intervention of miR-144-3p expression had no significant effect on circCOQ10A levels in GC cells (Figure S4A, B), indicating that miR-144-3p acts as a downstream target of circCOQ10A.

Ago2-RIP assays further confirmed the endogenous interaction between circCOQ10A and miR-144-3p. The results showed that both circCOQ10A and miR-144-3p were significantly enriched in Ago2-bound precipitates compared with the input group (Figure 4E). FISH assays verified the co-localization of circCOQ10A and miR-144-3p in the cytoplasm of GC tissue cells (Figure 4F). Quantitative FISH analysis of clinical samples further revealed a negative correlation between circCOQ10A and miR-

144-3p expression in GC tissues (Figure 4G). Functional rescue experiments demonstrated that miR-144-3p mimic effectively inhibited the proliferation and invasion of HGC-27 cells and reversed the oncogenic effects induced by circCOQ10A overexpression (Figure 4H, Figure S4C). In contrast, miR-144-3p inhibitor markedly promoted the malignant phenotypes of MKN-45 and AGS cells and rescued the tumor-suppressive effects caused by circCOQ10A knockdown (Figure 4I, Figure S4D). Collectively, these results confirmed that circCOQ10A acted as a competing endogenous RNA to sponge miR-144-3p and modulate GC progression.

CircCOQ10A activated MAPK6/ERK1/2 signaling pathway via miR-144-3p sponging in GC cells

TargetScanHuman 7.1 predicted around 1048 potential targets of miR-144-3p. We screened top 30 candidates by cross-referencing evolutionary conservation, GC oncogenic phenotypes, and correlation with neutrophil polarization (Supplementary Table S3), among which MAPK6 was selected as the core functional target for subsequent verification. The potential binding sites between miR-144-3p and the 3'-UTR of MAPK6 are shown in Figure 5A. Dual-luciferase reporter assays verified that miR-144-3p mimic suppressed the luciferase activity of wild-type MAPK6 reporter, with no obvious effect on the mutant MAPK6 vector (Figure 5B). RT-qPCR and Western blotting assays were performed to validate the regulatory relationship among circCOQ10A, miR-144-3p, and MAPK6. The results showed that miR-144-3p mimic downregulated MAPK6 expression and abolished circCOQ10A overexpression-induced MAPK6 upregulation in HGC-27 cells. Conversely, miR-144-3p inhibitor

increased MAPK6 expression and reversed the inhibitory effect of circCOQ10A knockdown on MAPK6 in MKN-45 and AGS cells (Figure 5C-E). Further signaling pathway detection revealed that circCOQ10A overexpression promoted the phosphorylation of MAPK6 and ERK1/2, and upregulated the expression of downstream proliferation and metastasis-related proteins (MMP-2, PCNA); these activating effects were completely reversed by miR-144-3p mimic transfection (Figure 5F). In contrast, circCOQ10A knockdown inhibited MAPK6/ERK1/2 signaling activation and reduced MMP-2 and PCNA expression, while miR-144-3p inhibitor treatment rescued these inhibitory phenotypes (Figure 5G). These data demonstrated that circCOQ10A activated the MAPK6/ERK1/2 signaling pathway by negatively regulating miR-144-3p in GC cells.

CircCOQ10A knockdown suppressed *in vivo* tumorigenesis and lung metastasis of GC. To validate the *in vivo* oncogenic role of circCOQ10A, we constructed subcutaneous xenograft tumor models and tail vein lung metastasis models using stably transfected MKN-45 cells. In the xenograft model, 28 days after cell inoculation, the tumors formed by circCOQ10A-knockdown MKN-45 cells were smaller and lighter than those in the sh-NC control group (Figure 6A, B). Tumor growth curve analysis confirmed that circCOQ10A depletion retarded tumor growth in a time-dependent manner (Figure 6B). H&E and IHC staining results showed that the expression levels of proliferation marker Ki-67 and key effector protein MAPK6 were prominently decreased in circCOQ10A-knockdown xenograft tumor tissues (Figure 6C). In the lung metastasis model, histological observation revealed that the number of pulmonary metastatic nodules was

reduced in the circCOQ10A-KD group compared with the control group (Figure 6D). Moreover, H&E and IHC staining of metastatic lung tissues verified that circCOQ10A knockdown downregulated the expression of MAPK6 and metastasis-related VEGF protein (Figure 6E). These *in vivo* results consistently confirmed that circCOQ10A facilitated GC tumorigenesis and distant lung metastasis.

CircCOQ10A promoted N2 neutrophil polarization and GC invasion via activating the MAPK6/CXCL2/CXCR2 signaling axis

To further explore the immune regulatory mechanism of circCOQ10A/MAPK6 axis in the GC tumor microenvironment, we analyzed the correlation between MAPK6 and tumor immune characteristics using the TISIDB database. Bioinformatic predictions indicated that MAPK6 expression was closely associated with neutrophil polarization status in stomach adenocarcinoma (STAD) and exhibited a significant positive correlation with CXCL2 and CXCR2 expression (Figure S5A, B). Clinical specimen validation further confirmed that the expression of MAPK6, CXCL2, and CXCR2 was markedly upregulated in GC tissues compared with paired adjacent normal tissues (Figure 7A-C). *In vitro* functional validation showed that MAPK6 overexpression increased the mRNA and protein expression levels of CXCL2 in AGS cells (Figure 7D). To simulate the tumor microenvironment and explore neutrophil phenotypic transformation, we established a GC cell-neutrophil co-culture system (Figure 7E). HL60 neutrophils were co-cultured with AGS cells with circCOQ10A knockdown or combined MAPK6 overexpression. The results showed that circCOQ10A knockdown reduced the expression of N2 neutrophil polarization markers (TGF- β , Arg-1, CD-206)

and chemokines (CXCL2, CXCR2) while elevating the expression of N1 neutrophil polarization markers (iNOS, TNF- α , IL-1 β) in co-cultured tumor-associated neutrophils (TANs). However, MAPK6 overexpression effectively reversed these inhibitory effects induced by circCOQ10A depletion (Figure 7F and Figure S5C). The similar results were verified in MKN-45/DDP cells and miR-144-3p reversed circCOQ10A knockdown-induced these effects (Figure 7G). Flow cytometry and Transwell invasion assays further demonstrated that MAPK6 overexpression promoted N2-type neutrophil polarization and enhanced the invasive capacity of co-cultured GC cells. In contrast, circCOQ10A knockdown suppressed N2 neutrophil polarization and GC cell invasion, and this suppressive phenotype was fully rescued by MAPK6 upregulation (Figure S5D, E). In conclusion, these findings revealed that circCOQ10A facilitated N2 neutrophil polarization and GC malignant invasion by activating the MAPK6/CXCL2/CXCR2 signaling cascade.

Discussion

N6-methyladenosine (m⁶A) represents the most prevalent and functionally vital post-transcriptional RNA modification in eukaryotic transcripts, which extensively modulates the biological functions of both coding and non-coding RNAs, including circular RNAs (circRNAs) and long non-coding RNAs (Lu et al. 2025; He et al. 2020). Accumulating evidence has demonstrated that m⁶A modification profoundly affects the binding affinity between circRNAs and their downstream target miRNAs, thereby reprogramming the expression of cancer-associated genes and driving malignant biological behaviors (Lu et al. 2025). Dysregulated m⁶A modification on circRNAs has

been widely reported to trigger aberrant cell proliferation, invasive metastasis, and chemotherapy resistance in multiple human malignancies (Li et al. 2022; Zhang et al. 2023). Our previous study confirmed that METTL14-mediated m⁶A modification of circORC5 fuels GC progression (Fan et al. 2022). Nevertheless, the precise molecular mechanism by which m⁶A-modified circRNAs regulate GC metastasis and chemoresistance remains poorly defined. In the current study, we systematically elucidated a novel oncogenic signaling axis wherein METTL14-mediated m⁶A modification of circCOQ10A facilitated N2-type TAN polarization, thereby accelerating GC progression and DDP resistance via modulation of the miR-144-3p/MAPK6/CXCL2 signaling cascade. These findings uncover a novel m⁶A-circRNA-immune regulatory network and provide a promising therapeutic target for overcoming GC chemoresistance.

CircRNAs have been well-established as critical dual-functional regulators in GC tumorigenesis and chemotherapy resistance, acting as either oncogenic drivers or tumor suppressors (Xiu et al. 2024; Zheng et al. 2023). Consistent with this notion, our previous studies have characterized the distinct roles of multiple circRNAs in GC: circUGGT2 functions as an oncogene to promote GC progression, whereas circLARP4 and circYAP1 serve as tumor suppressors to restrain malignant phenotypes (Chen et al. 2024; Zhang et al. 2017; Liu et al. 2018). In this work, we identified a novel METTL14-modified circRNA, circCOQ10A, which exerts robust pro-tumorigenic functions in GC. *In vitro* and *in vivo* functional assays validated that circCOQ10A overexpression promotes GC cell proliferation, invasion, lung metastasis, and DDP chemoresistance,

while circCOQ10A knockdown effectively abrogates the malignant phenotypes induced by METTL14 deficiency. Collectively, these results confirm that METTL14-mediated m⁶A modification of circCOQ10A is a key driver of GC progression and DDP resistance, further enriching the functional spectrum of m⁶A-modified circRNAs in GC malignancy.

Mechanistically, the competing endogenous RNA regulatory network represents the most classic functional pattern of circRNAs in modulating GC development, whereby circRNAs sponge specific miRNAs to modulate the expression of downstream target genes (Lu et al. 2025). Our previous studies have verified that circORC5 and circUGGT2 promote GC growth and metastasis via sponging miR-30c-2-3p and miR-186-3p, respectively (Fan et al. 2022; Chen et al. 2024). Expanding on these findings, the present study further complemented the circRNA-miRNA regulatory axis in GC. We verified that circCOQ10A was upregulated in clinical GC tissues and DDP-resistant GC specimens, and high circCOQ10A expression was closely correlated with unfavorable clinical prognosis in GC patients. Mechanistic validation confirmed that circCOQ10A predominantly localized in the cytoplasm and directly bound to miR-144-3p to act as its endogenous sponge. Previous studies have reported that miR-144-3p functions as a tumor suppressor in multiple cancers, including hepatocellular carcinoma and thyroid cancer (Xiu et al. 2024; Xing et al. 2023). In line with these findings, our results demonstrated that miR-144-3p was downregulated in GC tissues, exhibited a strong negative expression correlation with circCOQ10A, and effectively reversed the oncogenic effects induced by circCOQ10A overexpression. These data solidify the

ceRNA mechanism of circCOQ10A, demonstrating that m⁶A-modified circCOQ10A drives GC progression through targeted sponging of miR-144-3p.

Mitogen-activated protein kinase 6 (MAPK6), also termed ERK3, is an atypical member of the MAPK family and participates in diverse cellular biological processes (Hansen et al. 2008; Julien et al. 2003; Coulombe et al. 2003). Emerging clinical and experimental evidence has revealed aberrant upregulation of MAPK6 in multiple human malignancies, which acts as an oncogene to facilitate tumor progression in breast cancer, GC, and non-small cell lung cancer (Morazzo et al. 2025; Bogucka et al. 2020; Cai et al. 2021). In the present study, MAPK6 was identified as a direct and functional downstream target of miR-144-3p in GC cells. Mechanistically, miR-144-3p directly bound to the 3'-UTR of MAPK6 and inhibited its post-transcriptional expression, whereas circCOQ10A-mediated miR-144-3p sponging efficiently reversed this inhibitory effect, leading to robust MAPK6 upregulation. Elevated MAPK6 further triggered the phosphorylation of ERK1/2 and upregulated the expression of proliferation and metastasis-associated proteins (MMP2, PCNA), thereby activating the oncogenic MAPK6/ERK1/2 signaling pathway and driving GC tumorigenesis and progression. These findings clarify the molecular mechanism by which circCOQ10A regulates downstream oncogenic signaling via the miR-144-3p/MAPK6 axis in GC.

The tumor microenvironment (TME) and immune cell phenotypic polarization are critical determinants of tumor metastasis, immune escape, and chemotherapy resistance. Tumor-secreted chemokines, particularly CXCL2, are well documented to induce neutrophil polarization toward the pro-tumor N2 phenotype, and N2-type TANs further

promote tumor invasion, distant metastasis, and immunosuppressive TME formation (Zhang et al. 2018; Coffelt et al. 2015; Vazquez et al. 2017; Chao et al. 2016). In this study, we further explored the immune regulatory mechanism of the circCOQ10A/MAPK6 axis in the GC TME. Our results demonstrated that MAPK6 activation in GC cells markedly upregulated the secretion of TGF- β , CXCL2, and CXCR2, which recruited peripheral neutrophils and induced their polarization into pro-tumor N2-type TANs, ultimately facilitating GC cell invasion and malignant progression. CircCOQ10A knockdown suppressed MAPK6/CXCL2/ CXCR2 signaling activation, inhibited N2 neutrophil polarization, and impaired GC invasive capacity, while exogenous MAPK6 overexpression could rescue these inhibitory phenotypes (Figure 8) (Jiang et al. 2025). Collectively, these findings reveal a novel immune regulatory mechanism, demonstrating that circCOQ10A promotes N2 neutrophil polarization and GC malignant progression via activation of the MAPK6/CXCL2/CXCR2 signaling axis. The present findings support the N2-like polarization phenotype associated with the circCOQ10A/MAPK6/CXCL2 axis rather than definitive proof of neutrophil lineage reprogramming.

Despite the comprehensive *in vitro* and *in vivo* validation in this study, several limitations remain. First, our MeRIP and reciprocal rescue assays confirm METTL14 modulates circCOQ10A levels via m⁶A modification. However, without wild-type and m⁶A-site-mutant circCOQ10A comparative experiments, we cannot definitively conclude that circCOQ10A's oncogenic functions intrinsically depend on its m⁶A modification. The current evidence only supports that METTL14 suppresses GC

progression largely by downregulating circCOQ10A through m⁶A deposition, and targeted site-mutant assays are needed in future studies to resolve this question. Second, multiple canonical RRACH m⁶A motifs are predicted on circCOQ10A, and METTL14 mediates its m⁶A modification to reduce circCOQ10A stability. As a well-documented m⁶A reader triggering RNA degradation (Cai et al. 2026), YTHDF2 is predicted to bind m⁶A-labeled circCOQ10A and facilitate its decay. However, this regulatory axis is only supported by indirect data here; direct experiments including YTHDF2-RIP and m⁶A site mutation are required in future work for solid validation. Third, although we detected multiple N1/N2 signature markers in HL-60 neutrophil-like cells, this simplified *in vitro* model cannot fully recapitulate authentic TAN subtypes. Further validation using primary neutrophils and *in vivo*-isolated TANs will be essential to consolidate these results and improve translational relevance. MAPK6 acts as one vital intermediate effector within the circCOQ10A/miR-144-3p cascade, yet it is not the sole regulator of neutrophil phenotypic remodeling or GC malignant behaviors. Multiple distinct pathways cooperatively drive GC progression, and additional downstream effectors remain to be characterized in follow-up research. Fourth, our *in vitro* and *in vivo* phenotypic assays confirm that circCOQ10A reduces cisplatin sensitivity. However, we did not perform dedicated experiments to investigate canonical chemoresistance mechanisms, including DDP-induced DNA damage repair, drug efflux, and apoptosis regulation. and further functional tests are required to uncover intrinsic molecular pathways of DDP resistance. Last but not the least, only 10 pairs of fresh GC specimens were used to preliminarily detect MAPK6 and CXCL2 expression, which

lacks adequate statistical power for systematic clinical immune correlation analysis. Further validation based on large independent clinical cohorts with complete immune phenotyping is required to fully confirm the clinical translatability of the circCOQ10A/MAPK6/CXCL2 axis.

In summary, the present study demonstrates that METTL14-mediated m⁶A modification elevates circCOQ10A expression and oncogenic activity in GC. Mechanistically, circCOQ10A functions as a ceRNA to sponge miR-144-3p, thereby upregulating MAPK6 expression and activating the downstream CXCL2/CXCR2 signaling cascade. This signaling axis further promotes N2-like neutrophil polarization, remodels the pro-tumor immune microenvironment, and ultimately accelerates GC malignant progression and DDP resistance. Our findings highlight the critical role of the circCOQ10A/MAPK6/CXCL2 regulatory network in GC, providing a novel prognostic biomarker and a promising potential therapeutic target for improving the clinical efficacy of GC chemotherapy.

Authors' contributions

Huining Fan: Writing – original draft, Visualization, Software, Funding acquisition, Data curation, Conceptualization. Yan Su: Investigation, Formal analysis. Yi Yu: Formal analysis. Zhaoyu Chen: Formal analysis. Renzheng Huang: review & editing, Validation, Supervision, Resources, Project administration. Jing Zhang: Writing--original draft, Validation, Resources, Project administration, Methodology, Funding acquisition.

Funding Statement

Our work was supported by the grants from National Natural Science Foundation of China (No. 82203784, 82074161, 82405090), Shanghai Natural Science Foundation Project (No.25ZR1401288, 21ZR1448700). Shanghai Municipal Health Commission Program (No.20224Y0140), Interdisciplinary Program of Shanghai Jiao Tong University (project number YG2021QN104) and Shanghai Sixth People's Hospital Program (No. ynqn202117).

Data Availability Statement

The data generated in this study are available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shanghai Sixth People's Hospital (Ethic Number: 2022-YS-056). Written informed consent was obtained from each patient included in the study. The study conformed to recognized ethical standards as set by Declaration of Helsinki. All animal experiments were approved by the Institutional Animal Care and Use Committee of Shanghai Sixth People's Hospital (Ethic Number: QR-Ani-E002-5.0). All animal experiments carried out in this project complied with the WMA Statement on animal use in biomedical research.

Consent for publication

Written patient consent for publication of all related clinical data and images included in this manuscript has been fully obtained prior to submission.

Competing Interest

The authors declare no conflicts of interest.

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Figure and legends

Figure 1. METTL14 mediated the m⁶A modification of circCOQ10A in GC cells. (A) Cluster mapping of the differentially expressed circRNA m⁶A levels using an m⁶A-circRNA epitranscriptomic microarray between control and si-METTL14 groups. (B)

The mRNA and protein expression levels of METTL14 in METTL14 OE-transfected MKN-45 and AGS cells. (C) RT-qPCR (left) and MeRIP (right) analyses of the effects of METTL14 OE on circCOQ10A expression levels and its m⁶A levels in MKN-45 and AGS cells. (D) The mRNA and protein expression levels of METTL14 in METTL14 KD-transfected HGC-27 cells. (E) RT-qPCR (left) and MeRIP (right) analysis of the effects of METTL14 KD on circCOQ10A expression levels and its m⁶A levels in HGC-27 cells. (F) RIP analysis of the effects of METTL14 OE on the enrichment levels of circCOQ10A in METTL14-interacting mRNAs in MKN-45 and AGS cells. (G) RT-qPCR analysis of circCOQ10A stability after exposure to treatment with RNase R exonuclease in MKN-45 and AGS cells. (H) RT-qPCR analysis of the cytoplasmic or nuclear location of circCOQ10A in MKN-45 and AGS cells. (I) FISH assessment of circCOQ10A localization in GC tissues. (J) FISH analysis of the expression levels of circCOQ10A in 90 paired GC tissue samples. (K) Kaplan–Meier plotter of the association of circCOQ10A with overall survival in patients with GC. Data are presented as the means ± SEM of three experiments.

Figure 2. CircCOQ10A facilitated GC cell growth and invasion and mitigated METTL14-induced antitumor effects. (A) RT-qPCR analysis of the expression of circCOQ10A in circCOQ10A OE-transfected HGC-27 cells. (B) CCK-8 analysis of the effects of co-transfection with METTL14 OE and/or circCOQ10A OE on GC cell viability. (C) Colony formation and (D) Transwell analyses of the effects of co-transfection with METTL14 OE and/or circCOQ10A OE on colony formation ability and GC cell invasion. (E) RT-qPCR analysis of the expression levels of circCOQ10A

in circCOQ10A KD-transfected MKN-45 and AGS cells. (F) CCK-8 analysis of the effects of co-transfection with METTL14 KD and/or circCOQ10A KD on GC cell viability. (G) Colony formation and (H) Transwell analyses of the effects of co-transfection with METTL14 KD and/or circCOQ10A KD on colony formation ability and GC cell invasion. Data are presented as the means $\pm$ SEM of three experiments. Scale bars: 50 μ m.

Figure 3. CircCOQ10A promoted the acquisition of DDP resistance in GC cells. (A) RT-qPCR analysis of the expression of circCOQ10A in DDP-sensitive and -resistant patients with GC (n = 10), as well as in the DDP-resistant MKN-45 cells. (B) RT-qPCR analysis of the expression levels of circCOQ10A after transfection with sh-circCOQ10A in MKN-45/DDP cells, and CCK-8 analysis of the IC₅₀ values after transfection with sh-circCOQ10A in MKN-45/DDP cells (shown on the right). (C) Colony formation and (D) Transwell analyses of the effects of circCOQ10A KD on the colony formation ability and cell invasion of MKN-45/DDP cells. (E) Under intraperitoneal injection with 0, 3, or 6 mg/kg DDP every three days for six times, the volumes and weights of tumors in nude mice subcutaneously inoculated with sh-circCOQ10A- or sh-NC-transfected MKN-45 cells at the end of the experiment are shown (n = 5/group). Tumor volumes of the sh-circ/sh-NC groups were calculated every two days. (F) H&E and IHC analyses of cell proliferation and Ki-67 expression levels in each group. Scale bars: 50 μ m.

Figure 4. CircCOQ10A acted as a sponge of miR-144-3p in GC cells. (A) Schematic representation of potential binding sites between the circCOQ10A 3'-UTR and miR-

144-3p. (B) Luciferase activity of the WT or MUT luc-circCOQ10A 3'-UTR after transfection with miR-144-3p mimics or an inhibitor in 293T cells. (C) RT-qPCR analysis of the effects of METTL14 OE or circCOQ10A KD on miR-144-3p expression levels in MKN-45 and AGS cells. (D) RT-qPCR analysis of the effects of METTL14 KD or circCOQ10A OE on miR-144-3p expression levels in HGC-27 cells. (E) RIP analysis of the enrichment levels of circCOQ10A and miR-144-3p in Ago2-interacting mRNAs in MKN-45 and AGS cells. (F) FISH analysis of the co-localization of circCOQ10A with miR-144-3p in the cytoplasm of GC tissues. (G) RT-qPCR analysis of the expression levels of miR-144-3p and its correlation with circCOQ10A expression in 10 paired GC tissues. (H) Transwell analysis of the effects of co-transfection with circCOQ10A OE and/or miR-144-3p mimics on cell invasion in HGC-27 cells. (I) Transwell analysis of the effects of co-transfection with circCOQ10A KD/or miR-144-3p inhibitors on cell invasion in MKN-45 and AGS cells. Data are presented as the means $\pm$ SEM of three experiments. Scale bars: 50 μ m.

Figure 5. CircCOQ10A mediated miR-144-3p to activate MAPK6/Erk1/2 signaling in GC cells. (A) Schematic representation of potential binding sites between miR-144-3p and MAPK6. (B) Luciferase activity of the WT or MUT MAPK6 3'-UTR after transfection with miR-144-3p mimics in 293T cells. (C) RT-qPCR analysis of the effects of co-transfection with circCOQ10A OE and/or miR-144-3p mimics on MAPK6 expression levels in HGC-27 cells. (D, E) RT-qPCR analysis of the effects of circCOQ10A KD and/or miR-144-3p inhibitors on MAPK6 expression levels in MKN-45 and AGS cells. (F) Western blotting analysis of the effects of co-transfection with

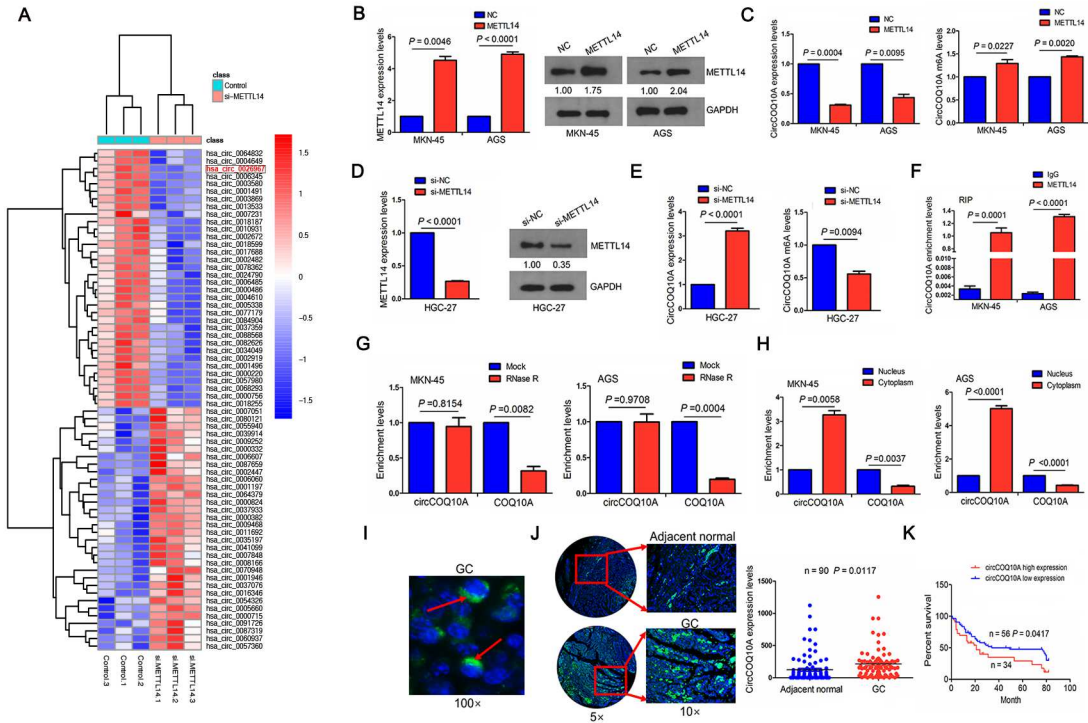
circCOQ10A OE and/or miR-144-3p mimics on the expression levels of MAPK6, p-MAPK6, p-ERK1/2, MMP2, and PCNA in HGC-27 cells. (G) Western blotting analysis of the effects of circCOQ10A KD and/or miR-144-3p inhibitors on the expression levels of MAPK6, p-MAPK6, p-ERK1/2, MMP2, and PCNA in MKN-45 and AGS cells. Data are presented as the means $\pm$ SEM of three experiments.

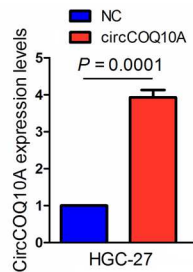
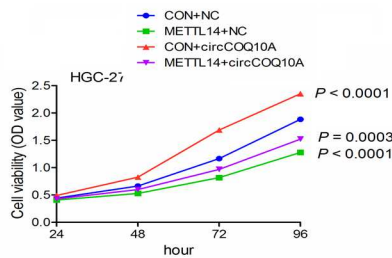
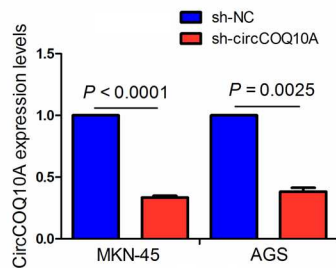
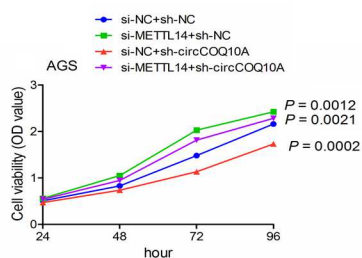
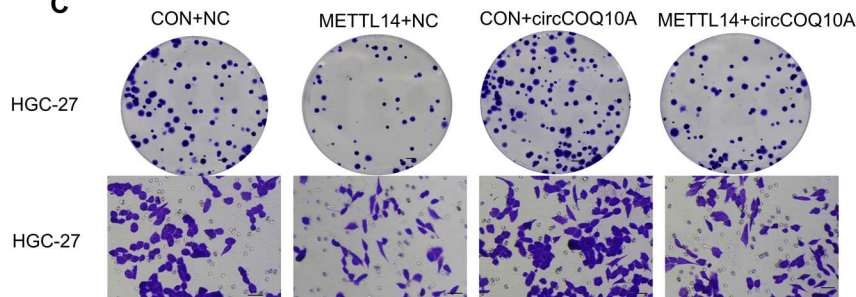
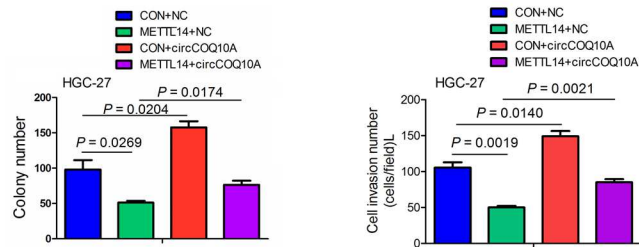
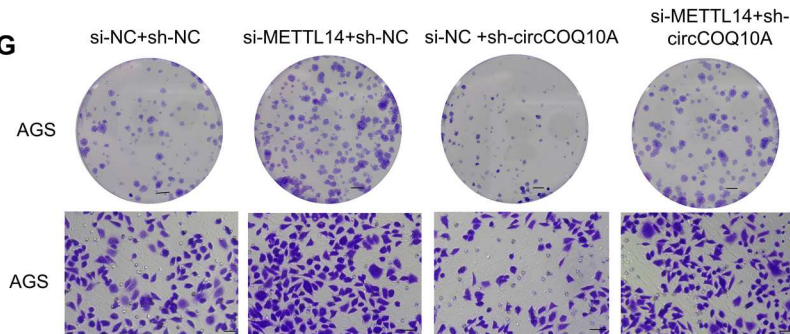
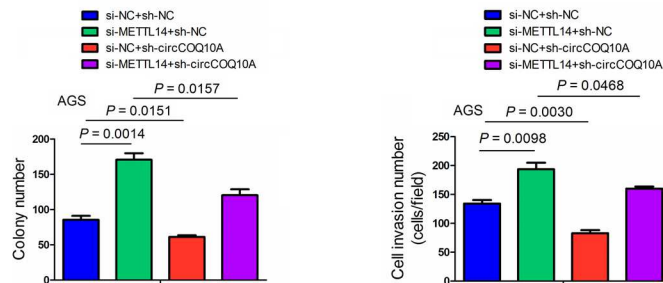
Figure 6. Knockdown of circCOQ10A repressed *in vivo* tumor growth and lung metastasis. (A) Schematic representation of the tumor size between sh-circCOQ10A- and sh-NC-transfected groups. (B) Comparison of the tumor volume and weight between sh-circCOQ10A- and sh-NC-transfected groups. (C) H&E and IHC analyses of cell proliferation and Ki-67 and MAPK6 expression levels in sh-circCOQ10A- and sh-NC-transfected groups. (D) Schematic representation and comparison of the number of metastatic lung tumors between sh-circCOQ10A- and sh-NC-transfected groups. (E) H&E and IHC analyses of cell metastasis and MAPK6 and VEGF expression levels in metastatic lung tissues in sh-circCOQ10A- and sh-NC-transfected groups. Data are presented as the means $\pm$ SEM of five experiments. Scale bars: 50 μ m.

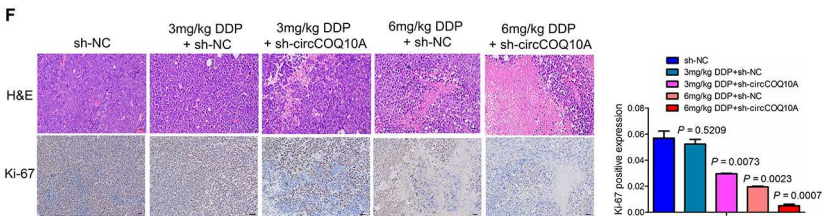
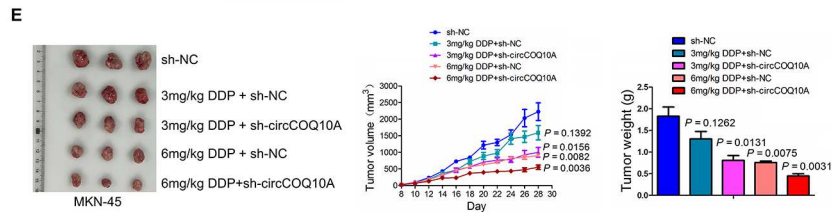
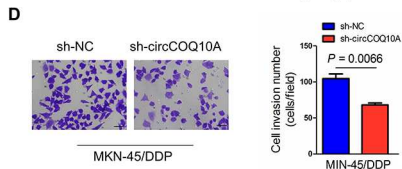
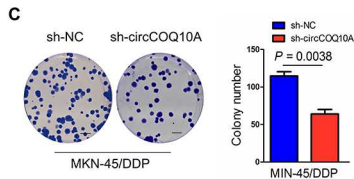
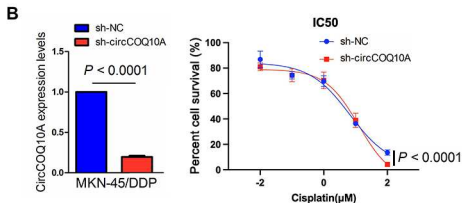
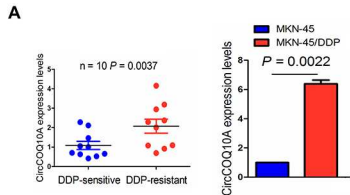
Figure 7. CircCOQ10A mediated MAPK6/CXCL2/CXCR2 signaling to promote N2 neutrophil polarization and GC cell invasion. (A–C) IHC analysis of the expression levels of MAPK6, CXCL2, and CXCR2 in 10 paired GC tissue samples. (D) RT-qPCR and western blotting analysis of the effect of MAPK6 OE on MAPK6 and CXCL2 expression in AGS cells. (E) DMSO-stimulated (1%) neutrophil HL60 cells were co-cultured with AGS cells for 48 h to form *in vitro* TANs, which were treated with sh-circCOQ10A and/or MAPK6 plasmids. (F) Western blotting analysis of the effect of

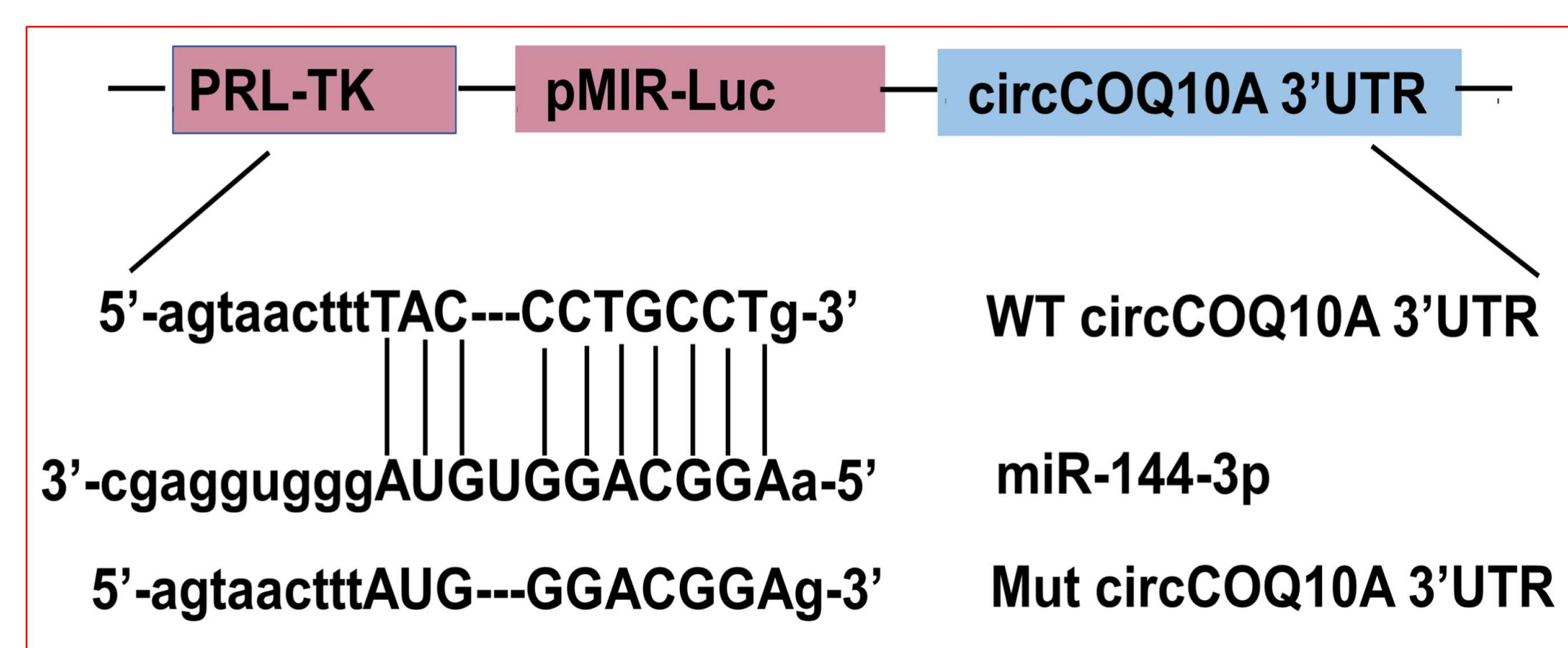
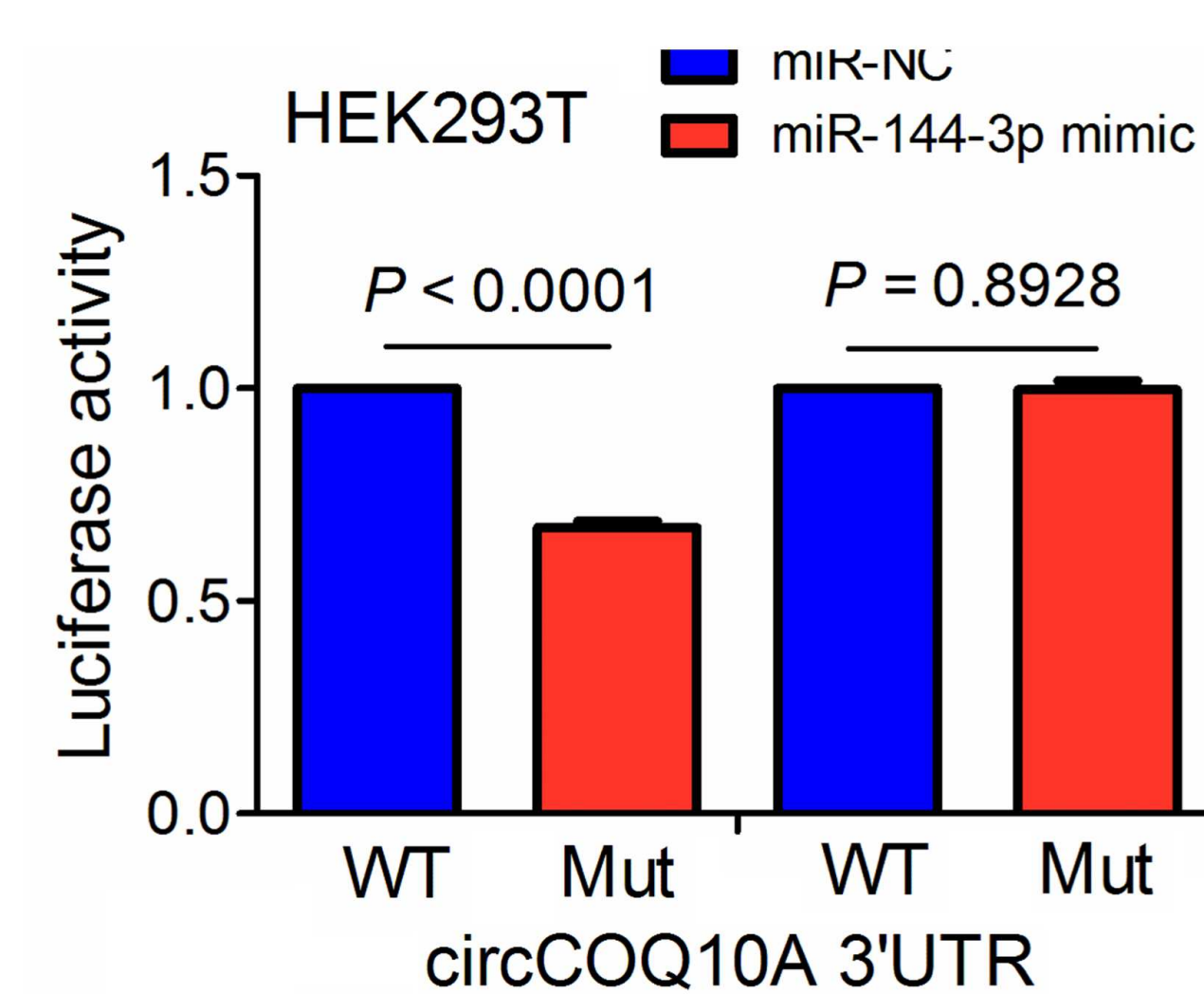
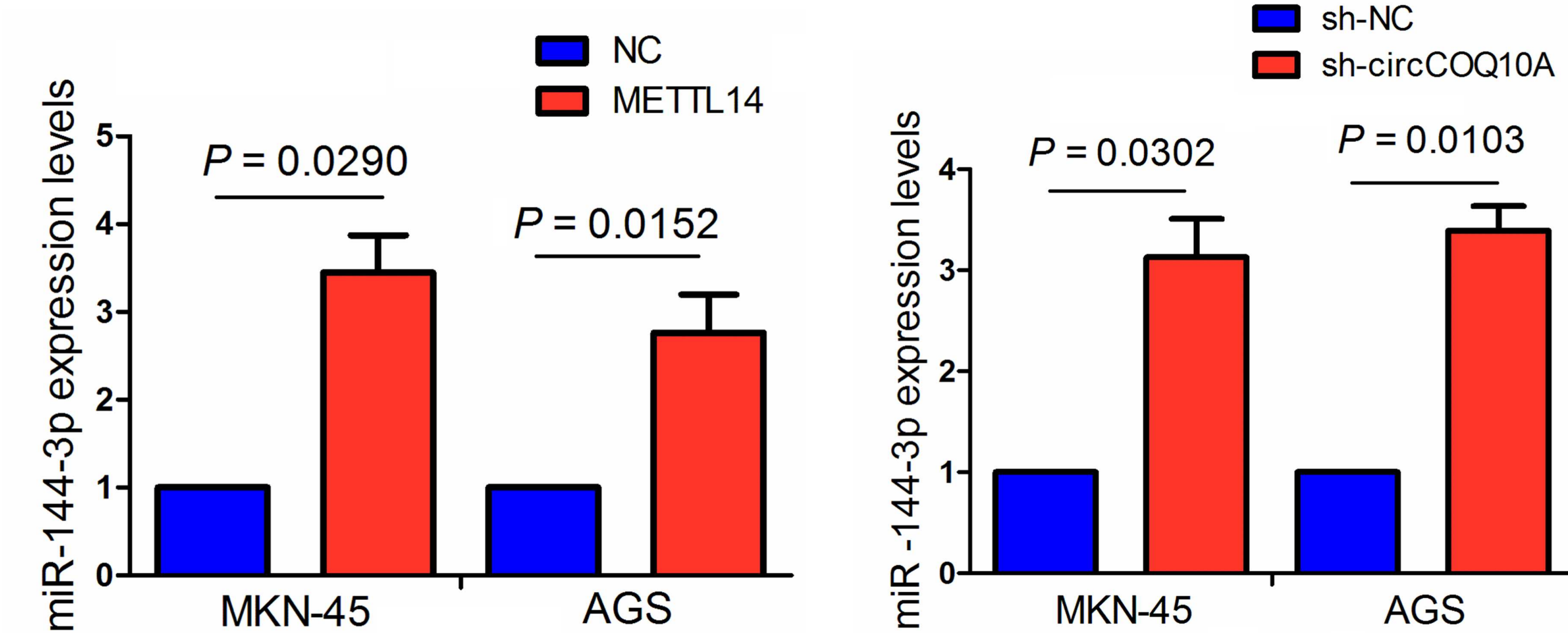
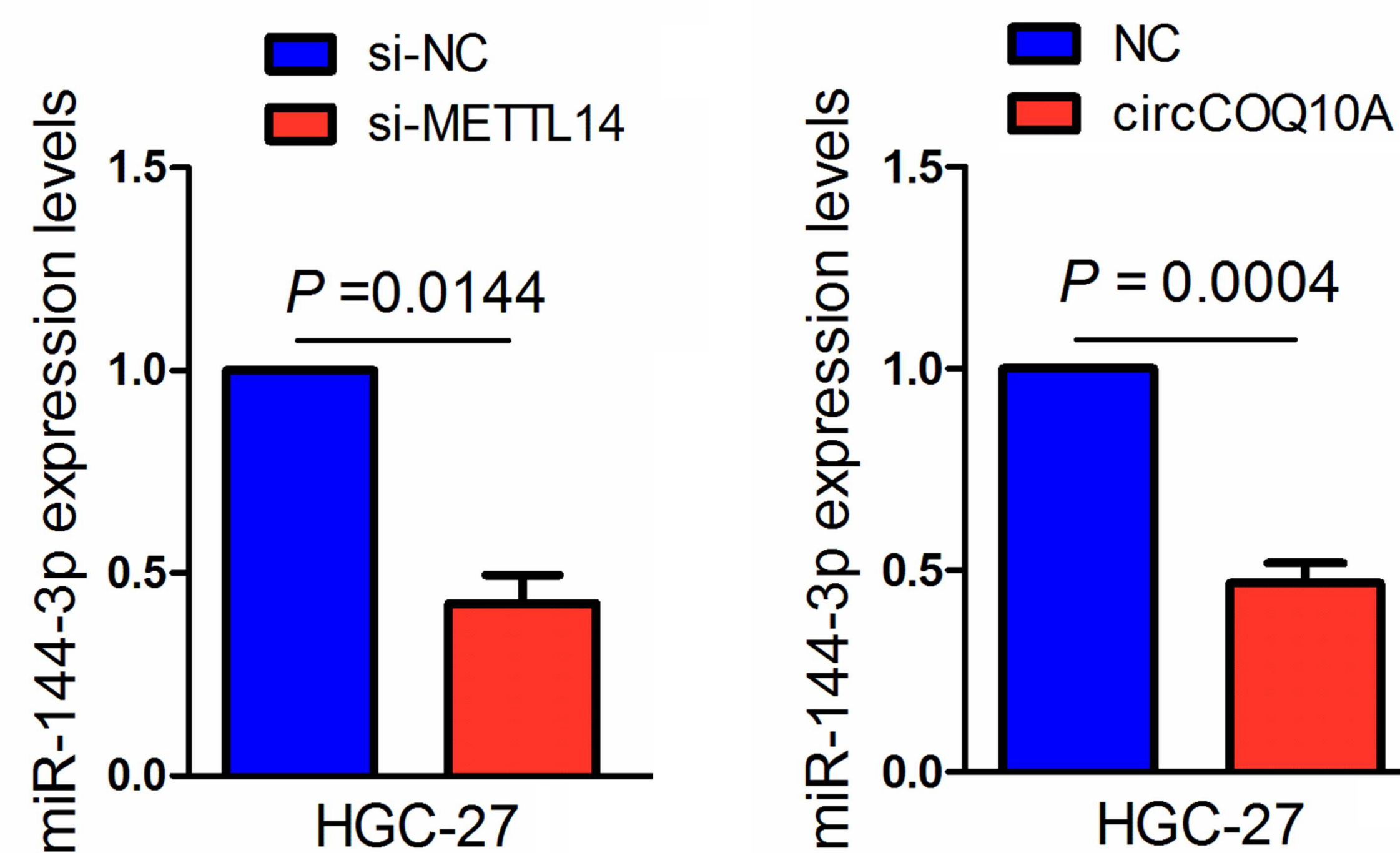
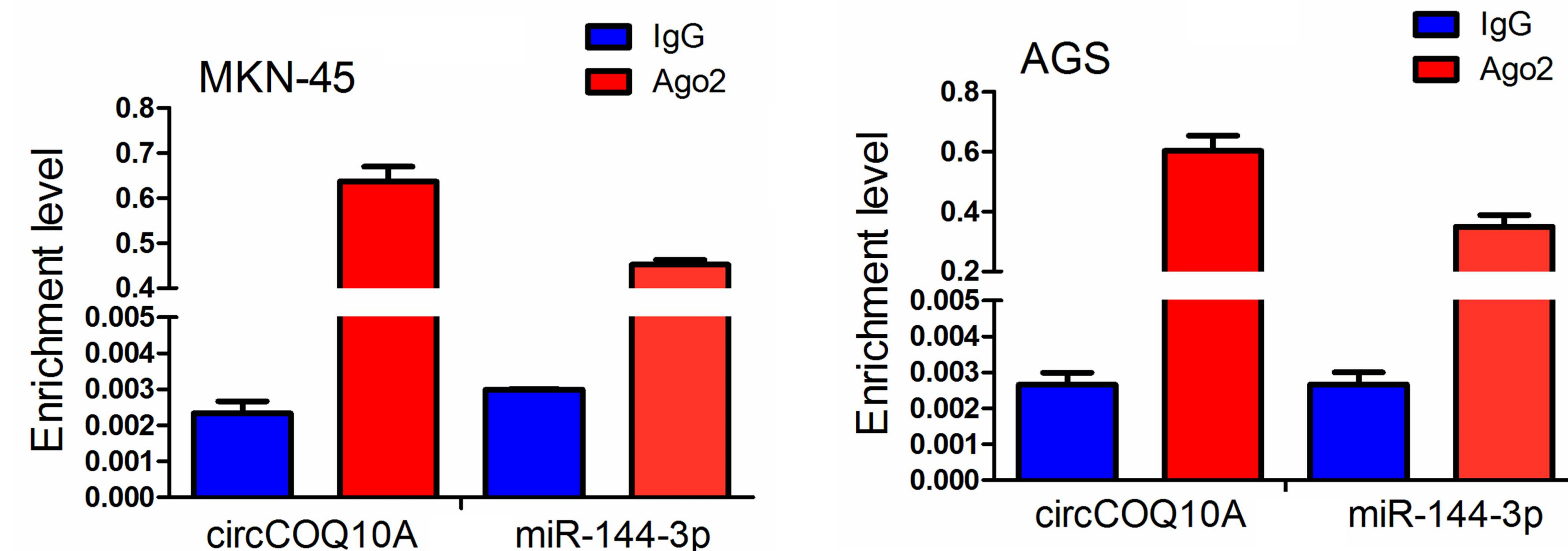
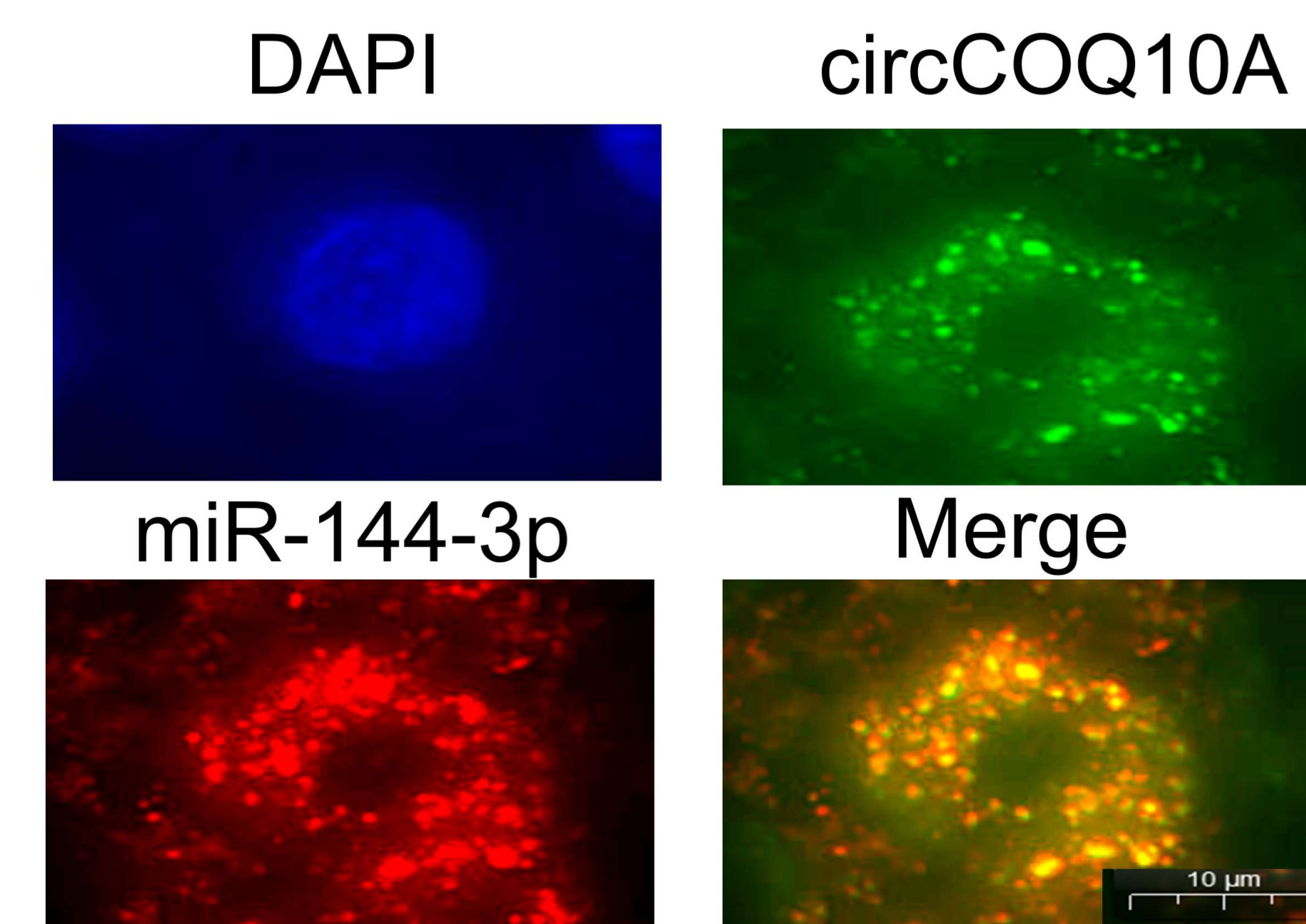
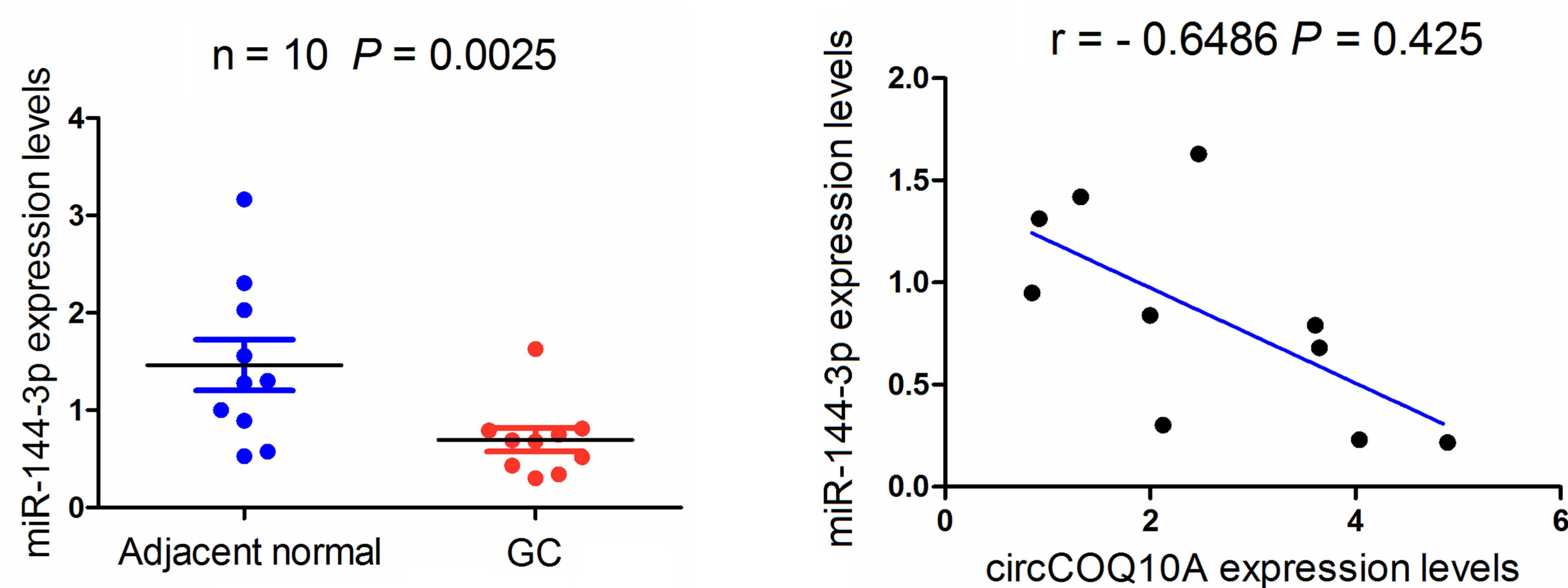
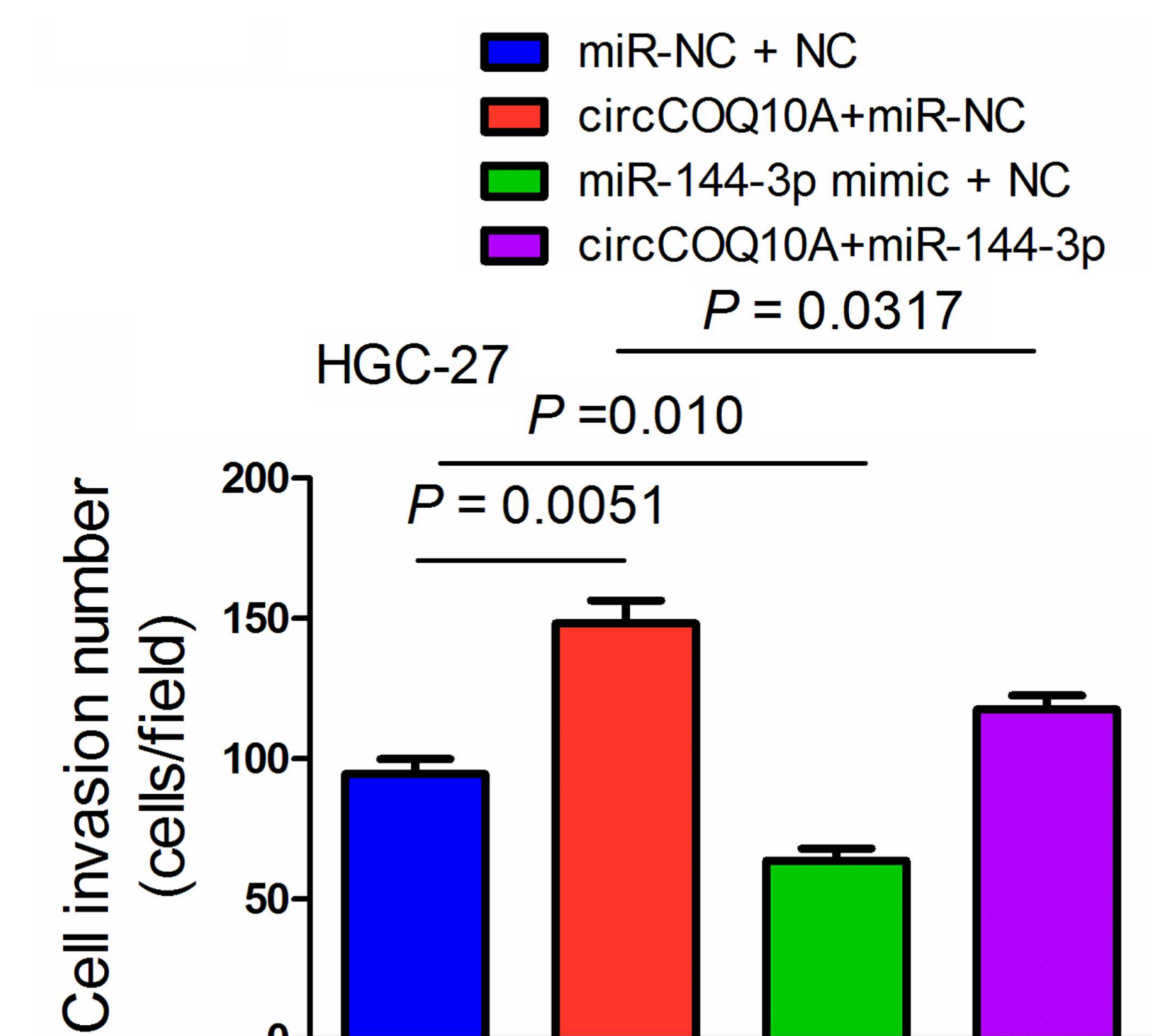
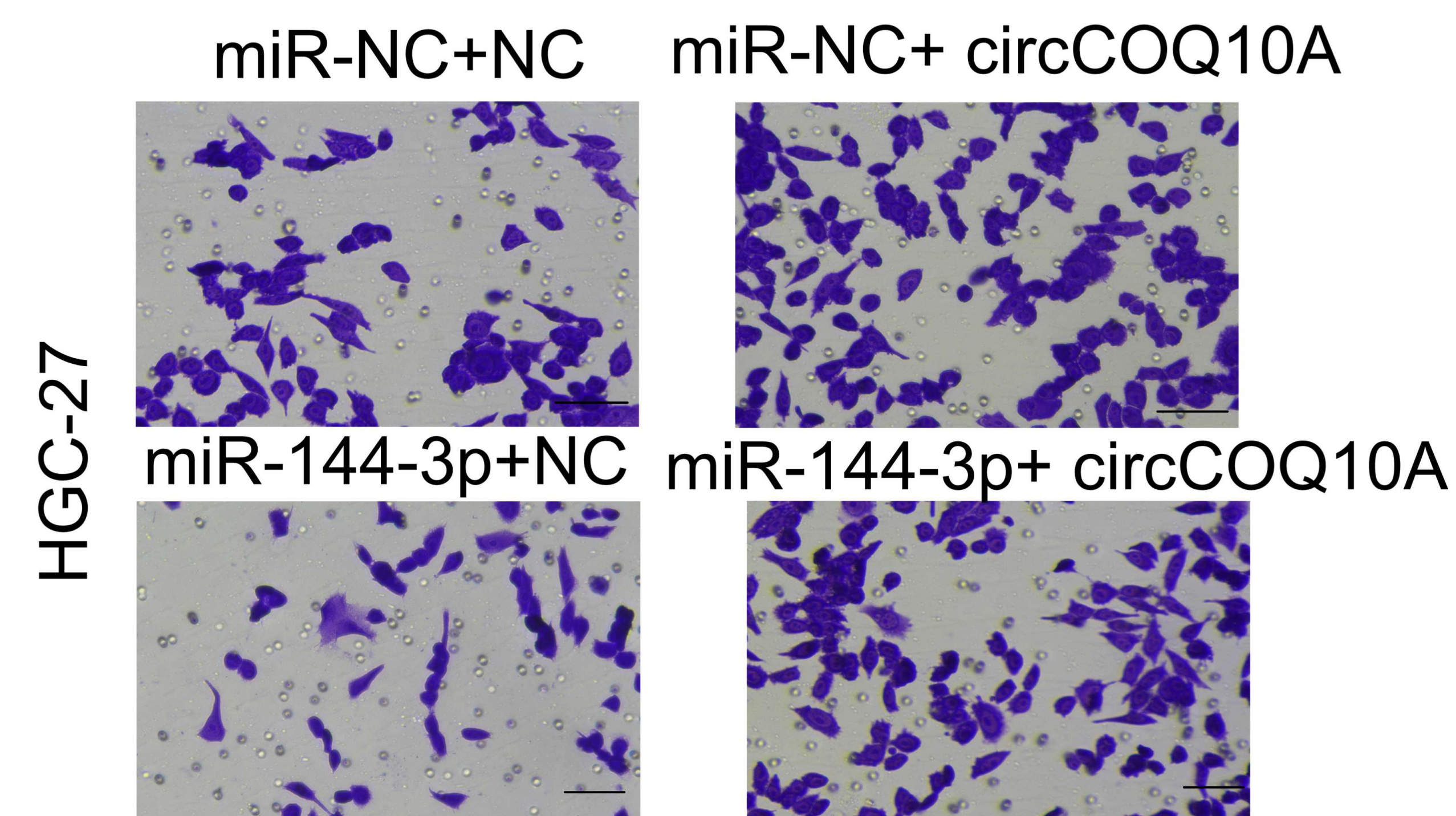
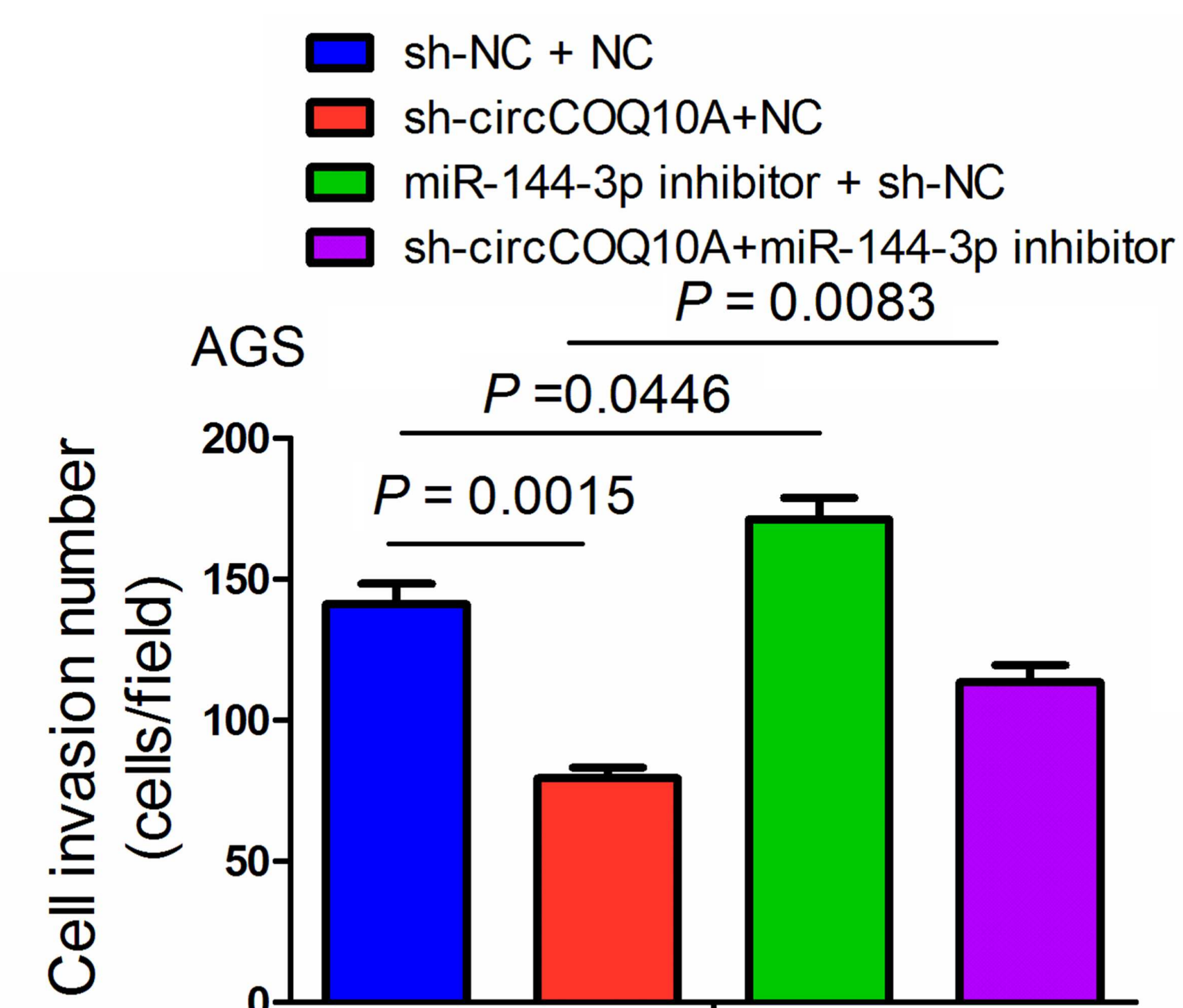
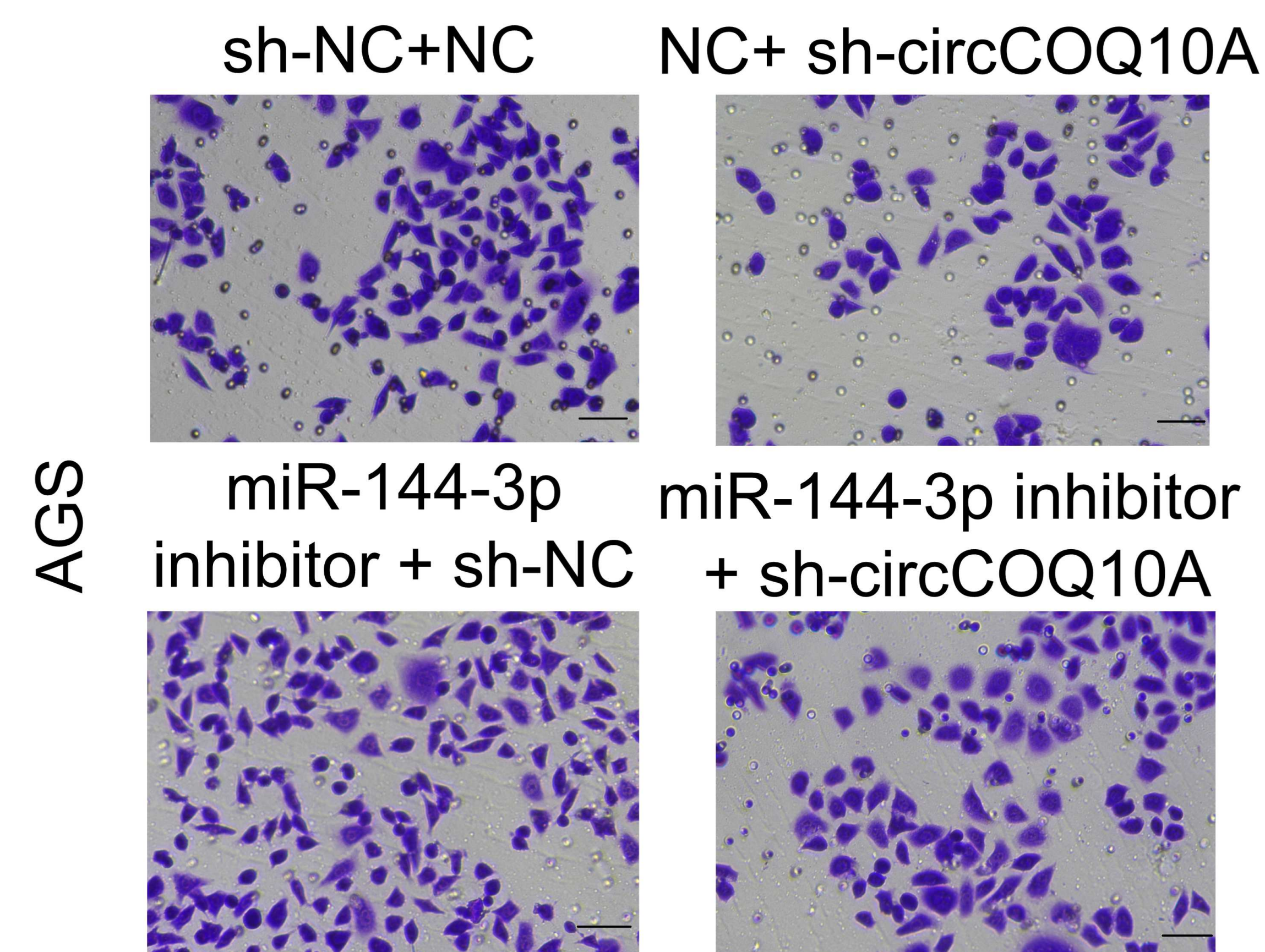
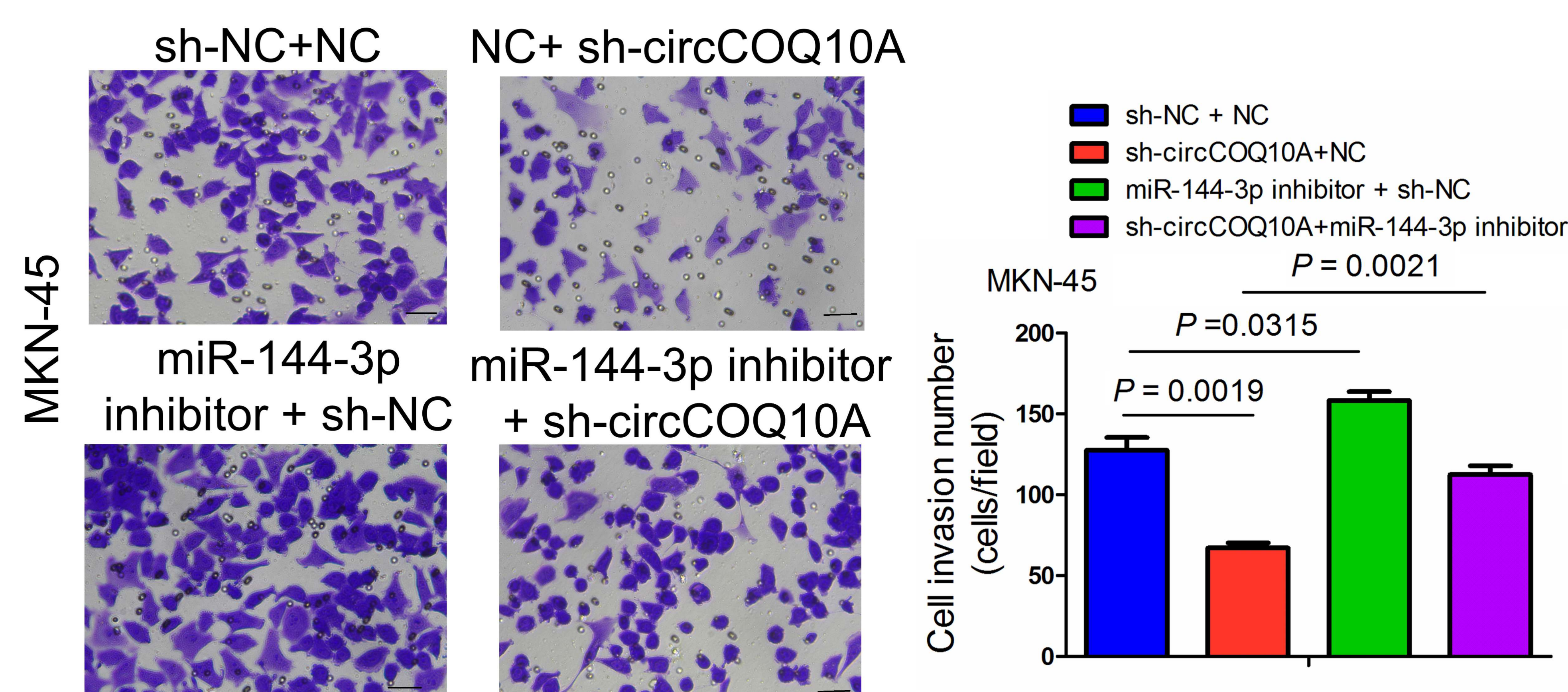
co-treatment with sh-circCOQ10A and/or MAPK6 plasmids on the expression levels of TGF- β , CXCL2, CXCR2, iNOS, TNF- α , IL-1 β , Arg-1 and CD206 in TANs. (G) Western blotting analysis of the effect of co-treatment with sh-circCOQ10A and/or miR-144-3p inhibitor on the expression levels of MAPK6, TNF- α , IL-1 β , Arg-1 and CD206 in MKN-45/DDP cells. Data are presented as the means $\pm$ SEM of three experiments. Scale bars: 50 μ m.

Figure 8 Schematic representation of the proposed mechanism of circCOQ10A in GC progression. METTL14 increased the m⁶A levels of circCOQ10A but decreased circCOQ10A expression levels; circUGGT2 acts as a miR-144-3p sponge to upregulate MAPK6, which activated CXCL2/CXCR2 signaling to promote N2 neutrophil polarization and GC progression.

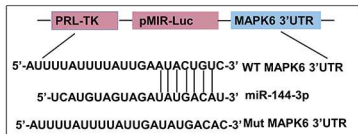


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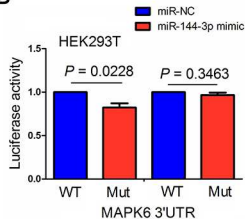


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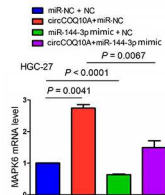
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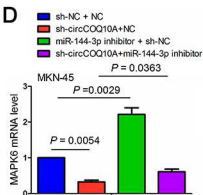
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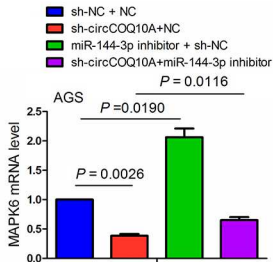
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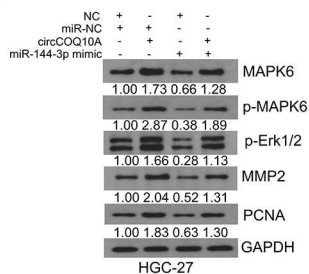
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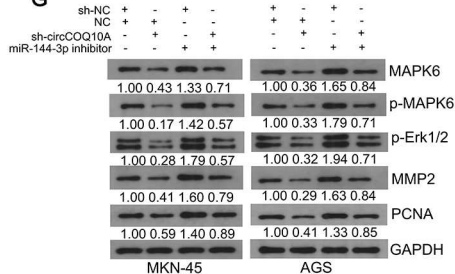
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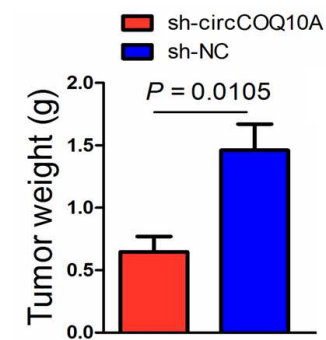
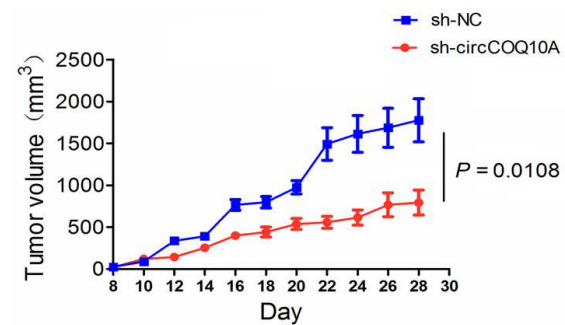
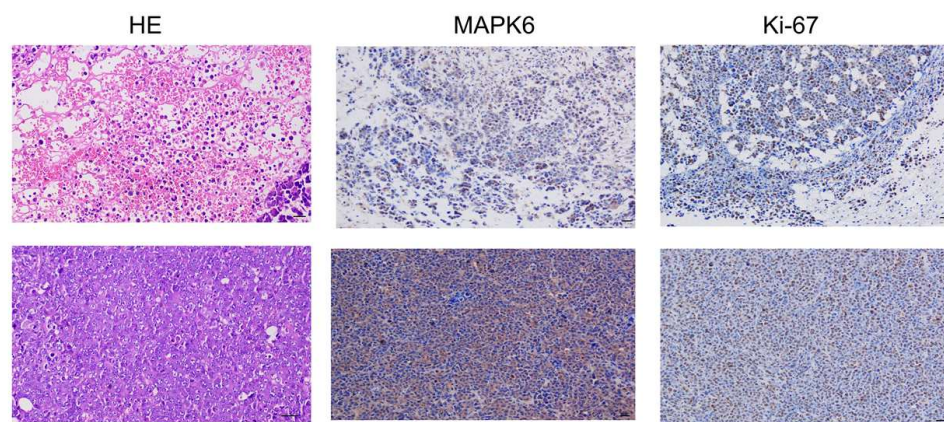


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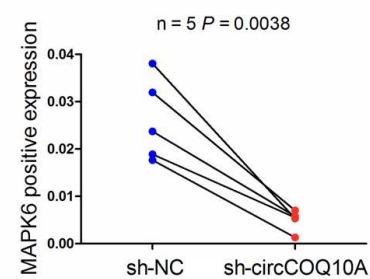


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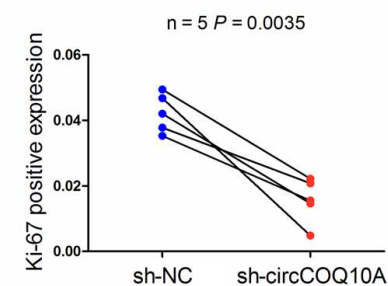
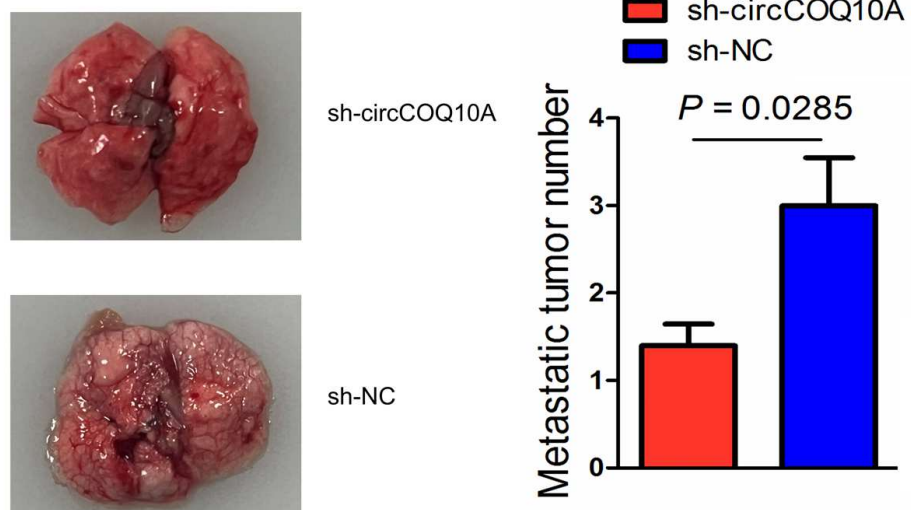
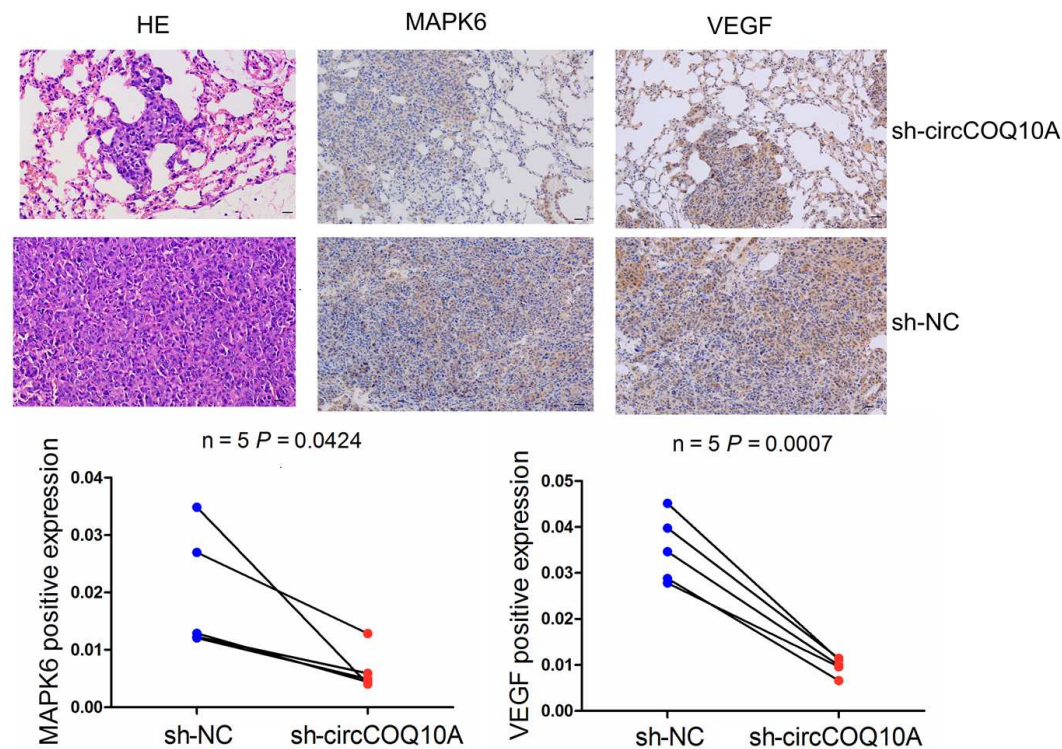


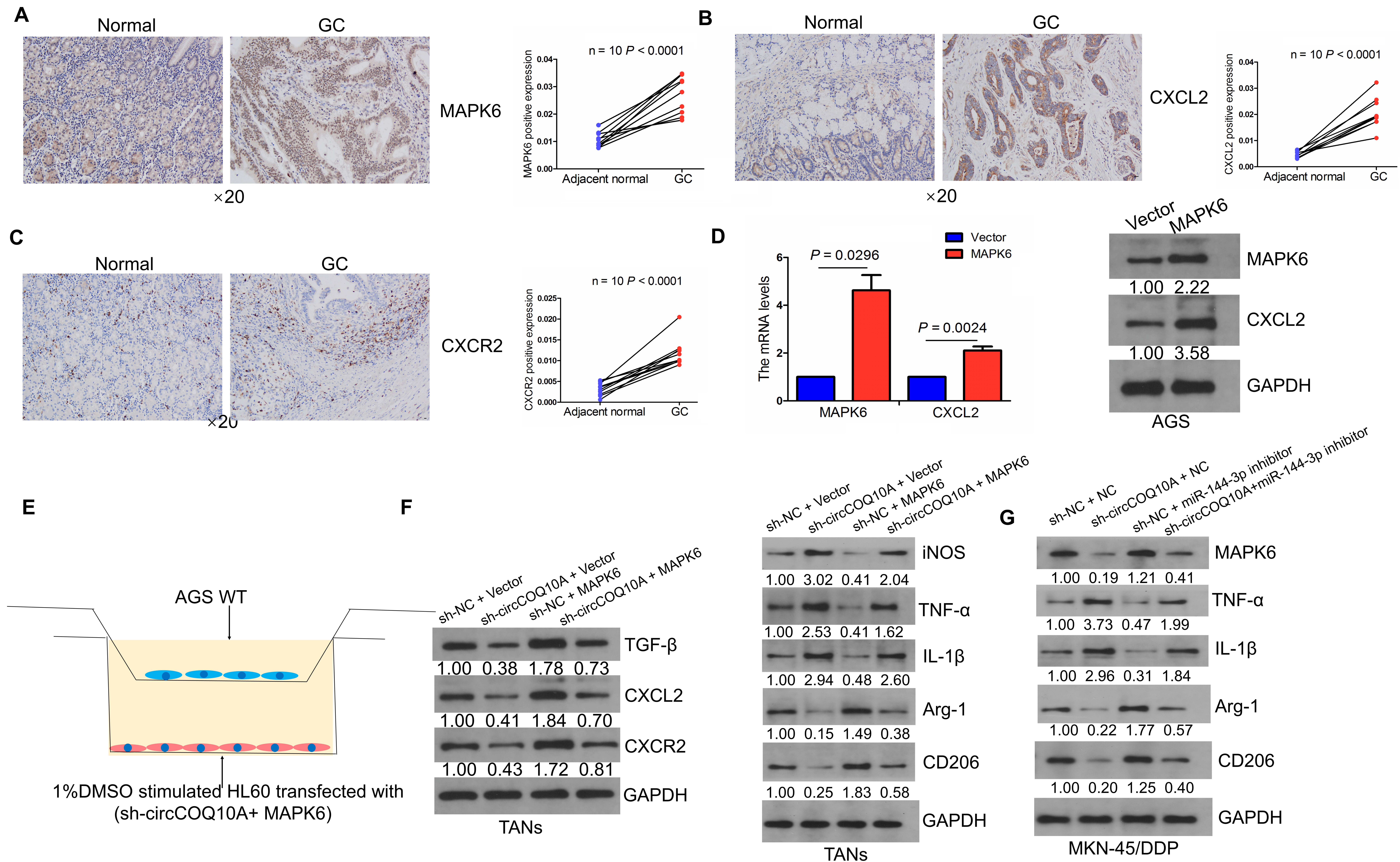
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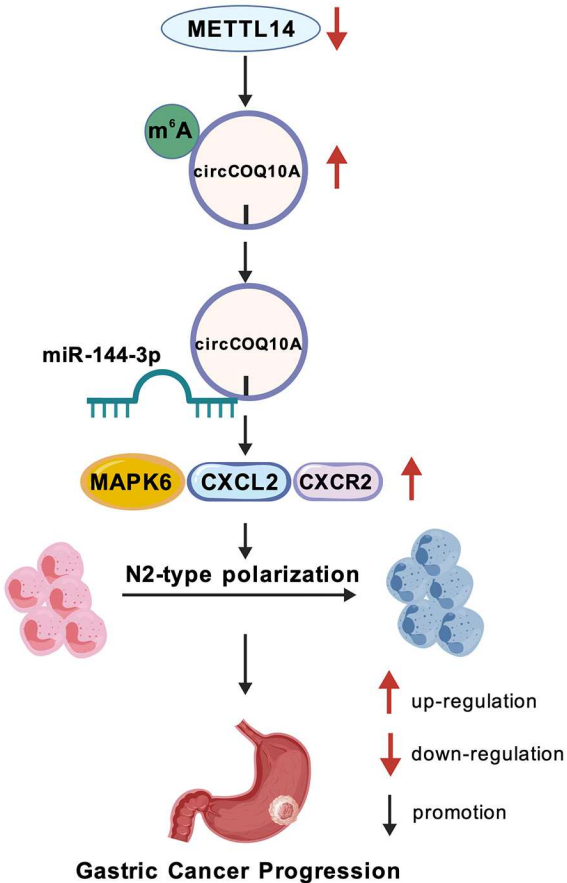
sh-circCOQ10A



sh-NC

**D****E**





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