

# The circBVES Acts as a Sponge of miR-145-5p to Promote Gastric Cancer Glycolysis and Progression Through Regulating HMGB3 Expression

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## Research

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# Abstract

**Background:** CircRNA is a new type of non-coding RNA that has attracted much attention for involvement in the development and progression of various human diseases, especially cancer. The most reported role of circRNA in many tumors is 'MiRNA sponge'. We aimed to investigate the role of circBVES in the proliferation and glycolysis of gastric cancer cells and its molecular mechanisms.

**Methods:** In this study, higher CircBVES expression in gastric cancer tissues was detected by RNA sequencing. Real-time quantitative polymerase chain reaction (qRT-PCR) was used to detect the expression of CircBVES in gastric cancer tissues, and the relationship between the expression of CircBVES and prognosis was further analyzed. Then, the effects of CircBVES on the growth and glycolysis of gastric cancer cells were investigated through in vitro and in vivo functional experiments. The interaction between CircBVES and miR-145-5p was detected by bioinformatics analysis, luciferase activity assay and RNA immunoprecipitation.

**Results:** We found that the expression of CircBVES in gastric cancer tissues was evidently up-regulated, and its level was closely correlated with the prognosis of patients with gastric cancer. Inhibition of CircBVES decreased cell proliferation and glycolysis in vitro. Low expression of CircBVES inhibited tumor growth in vivo. Mechanism analysis showed that CircBVES may serve as a competitive endogenous RNA of miR-145-5p to reduce the expression of miR-145-5p in gastric cancer cells, and relieve the repressive effect of miR-145-5p on target genes HMGB3 and cycle-related proteins CCNE1 and CDK2.

**Conclusions:** Our results suggest that CircAGFG1 may promote the progress of gastric cancer through the CircBVES / miR-145-5p / HMGB3 axis, providing a new target for the treatment of gastric cancer cells.

## Background

Gastric cancer (GC) is one of the most common malignancies in the world and the fourth most frequent cancer and the third leading cause of cancer mortality worldwide. Although recent advances in medical diagnosis and treatment, the prognosis of patients with gastric cancer is still poor, and the 5-year survival rate is below 20% [1, 2]. Recurrence and metastasis are the main causes of death in patients with gastric cancer. Early diagnosis and treatment can reduce the death rate of patients with gastric cancer. However, the diagnosis and treatment rate of early gastric cancer is still low. Most patients have advanced or late stage at the time of diagnose and missed the optimal operation period. Even if these patients receive radical surgery and chemotherapy, postoperative recurrence and mortality rate remains high [3]. Gastric cancer is a multifactorial disease, which involves a series of complex processes such as activation of proto-oncogenes, inactivation of tumor suppressor genes and abnormal expression of relevant signaling pathways [4]. Therefore, it is of great significance for the early diagnosis and treatment of gastric cancer to further study the molecular mechanism of the occurrence and development of gastric cancer, search for new markers and therapeutic targets, and explore its function and mechanism of action in gastric cancer.

Circular RNAs (circRNAs) is a new type of non-coding RNA molecule that is widely discovered in the cytoplasm of eukaryotic cells. In recent years, a large number of studies have shown that circular RNA is a competitive endogenous RNA molecule, which plays a key role in the regulation of gene expression and the development of tumors [5, 6]. As we all known, circular RNA is not the same as linear RNA. During mRNA splicing, the 5' -terminus of the upstream exon and the 3' -terminus of the downstream exon are spliced together to forming a covalently closed loop structure, which without the 5'-cap structure and the 3'-poly A tail. At the same time, the circular RNA is highly stable, and its circular structure can avoid the decomposition of ribonuclease in the blood [7, 8]. In fact, the functions of circRNAs are diverse, which including regulating splicing or transcription, binding proteins, and transporting RNA [9]. In recent years, numerous of reports revealed that circRNAs can play an important regulatory role in different diseases through various signal transduction pathways. Hansen TB et al. found that circRNA circs-7 is a key pathogenic factor for a variety of neurological and brain tumor diseases [10]. Vausort M et al found that for patients with myocardial infarction, the expression of circular RNA MICRA in their peripheral blood specimens often showed a low expression state, and its expression level was also significantly negatively correlated with the incidence of left ventricular dysfunction in patients [11]. In addition, the most studied is the 'miRNA sponge', also known as the competitive endogenous RNA (ceRNA) mechanism. CircRNAs can sponge miRNAs and then suppress their functions and thus impact the expression of downstream target genes, which playing a role in promoting or suppressing cancer [12]. Such as, Lu et al. illustrated that circSLC8A1 acts as a sponge of miR-130b/miR-494 in suppressing bladder cancer progression via regulating PTEN. Song et al. found that circRNA\_101996 increases cervical cancer proliferation and invasion through activating TPX2 expression by restraining miR-8075 [13, 14].

MiRNA is a type of single-stranded non-coding RNA with a size of 18–25 nucleotides. It is a key molecule that can be widely used in organisms to regulate gene expression. It is well known that miRNAs can participate in progression of varies of cancers, including gastric cancer [15, 16]. For example, Zheng et al. found that miRNA-154-5p Inhibits Proliferation, Migration and Invasion by Targeting E2F5 in Prostate Cancer Cell Lines [17].

In the current study, we using RNA-seq and identified a novel GC-related circRNA circBVES with a circBase ID of hsa\_circ\_077527. Then, we explored the circBVES expression in GC, and investigated its biological role in vitro and in vivo and underlying molecular mechanism of circBVES in GC development and progression. The results showed that circBVES up-regulated in GC tissues and positively correlated with HMGB3 expression. Further investigations revealed that down-regulated circBVES can inhibit the proliferation and glycolysis in vitro and vivo by acting as a sponge for the miR-145-5p to relieve microRNA repression for target gene HMGB3. In summary, circBVES is a promising target for treatment.

## Materials And Methods

### Patients and tissues

In this study, a total of 80 pairs of GC tissues and adjacent tissues were collected from patients who were diagnosed with GC at the First People's Hospital of Neijiang and the Affiliated Hospital of Guizhou Medical University. All patients signed an informed consent form before the operation and did not suffer from other malignant tumors or receive any preoperative chemotherapy or radiotherapy. The tissue samples after surgical were immediately snap-frozen in liquid nitrogen, and then transferred to -80°C refrigerator for long-term storage for until used. In addition, the tissue were identified by two senior pathologists. At the same time, our research was also approved by the Ethics Committee of the First People's Hospital of Neijiang City and the Affiliated Hospital of Guizhou Medical University.

## Cell culture and treatment

The gastric cancer cell lines HGC-27, MGC-803, BGC-823, SGC-7901, MKN45 and normal gastric epithelial cell line GES-1 were purchased from the Cell Center of Shanghai Institutes for Biological Sciences. The human gastric cell lines were cultured in RPMI 1640 medium (Gibco, Carlsbad, CA, USA), which supplemented with 10% FBS (Invitrogen) and 1% streptomycin (Gibco, USA). Cells were incubated at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>. The cells are replaced with culture medium every other day or every day according to the state of the cells. When cells are passaged, 0.25% trypsin is used to digest the cells.

## Plasmid construction and cell transfection

To silence circ-BVES expression, small interfering RNA (siRNA) against circ-BVES (sh-circ)(5'-ATGTCCTCTCTTCGTAAAGAT-3') and (5'-AGCATGTCCTCTCTTCGTAAA-3'), miR-145-5p mimic (5'-GUCCAGUUUCCCAGGAAUCCCU-3'), mimic negative control (miR-NC) (5'-UCACAGCCUCCUAGACAGACUAUA-3'), miR-145-5p inhibitor (inh-145-5p) (5'-CAGGTCAAAGGGTCCTTAGGGA-3') and negative control (inh-NC) (5'-UUCUCGGAUCGUGACACGUTT-3') were generated from Geneseeed (Guangzhou, China). When cells grow in log phase, SGC-7901 and MKN45 cells were transfected using Lipofectamine 3000 (Invitrogen; USA) following the manufactures' instructions. After cultured for 24–48 h, SGC-7901 and MKN45 cells were collected for subsequent experiment.

## Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolation from tissues or cells and reverse transcribed into cDNA using Prime Script RT Reagent Kit (Takara, Japan) following the manufacturer's protocols. qRT-PCR was conducted on a Bio-Rad CFX96 system (Bio-Rad, CA, USA) with TB Green Premix Ex Taq (Takara, Japan). GAPDH and U6 were respectively selected as internal controls for circRNA and miRNA. The relative expression levels were calculated according to the  $2^{-\Delta\Delta Ct}$  method. The primers sequences were as follows: circBVES (Forward, 5'-TGGAACATCAGCTCAGCCTC-3'; Reverse, 5'-TCAATCTTTACGAAGAGAGGACA-3'); miR-145-5p (Forward, 5'-GTCCAGTTTTCCAGGAATCCCT-3'; Reverse, 5'-CAGGTCAAAGGGTCCTTAGGGA-3'); U6 (Forward, 5'-CTCGCTTCGGCAGCACA-3'; Reverse, 5'-AACGCTTCACGAATTTGCGT-3').

## Cell Counting Kit-8 (CCK-8) assay

The proliferation of GC cells was detected by CCK8 assay (Dojindo Laboratories, Kumamoto, Japan). Cells in the logarithmic growth were collected and seeded into 96-well plates at a concentration of  $1 \times 10^3$  cells/ml. After culture for 24, 48, 72, and 96 h, 10  $\mu$ l of CCK8 solution was added into each well and further cultured for another 2 h. Finally, the optical density of each hole was measured at a wavelength of 450 nm.

## **Detection Of Extracellular Acidification Rate (ECAR) And Cellular Oxygen Consumption Rate (OCR)**

ECAR and OCR were detected by the Seahorse Bioscience XF96 Extracellular Flux Analyzer, following the manufacturer's instructions of Seahorse XF Cell Mito Stress Test Kit and Glycolysis Stress Test Kit (Seahorse Bioscience, Billerica, MA, USA). Cells were seeded in XF96 Cell Culture Microplates (Seahorse Bioscience) at a density of  $1 \times 10^4$  cells/well. For ECAR detection, glucose, oligomycin, and 2-deoxyglucose were automatically added to each well. For OCR measurement, oligomycin, FCCP (Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone) and rotenone were added into XF96 Cell Culture Microplates. The data of ECAR and OCR analyzed by the XF-96 wave software.

## **Glucose consumption, Adenosine Triphosphate (ATP) Synthesis and Lactate Production**

The Glucose Assay kit, Lactate Assay Kit and ATP Assay kit (Invitrogen, USA) were used to determine the GC cells glucose consumption, Adenosine Triphosphate (ATP) Synthesis and Lactate Production. All measurements followed manufacturer's instructions and were normalized for the number of cells in each experiment.

## **Animal experiments**

5–8 weeks old male BALB/c mice were performed using in vivo tumor growth assays. In short, SGC-7901 cells stably transfected with empty vector or si-circBVES (JiKai, Shanghai, China) and selected with puromycin. Then the cells harvested and resuspended in culture medium ( $2 \times 10^6$  cells/ml). For xenograft experiments, subcutaneously inoculated the cells in the concentration into female BALB/c mice. The tumor volume was measured once a week and calculated to be  $0.5 \times \text{length} \times \text{width}^2$ . The mice were sacrificed after 6 weeks, and tumor tissues were taken for further analysis.

## **Immunohistochemical (IHC) analysis**

Mouse tumor tissues were fixed with 10% formalin, dehydrated, paraffin-embedded and cut into 4  $\mu$ m specimens. Subsequently, the samples were dewaxed with xylene and rehydrated with graded alcohol. The specimens were repaired with sodium citrate, blocked with hydrogen peroxide, and bovine serum albumin. After incubating with Ki67 and PCNA at 4 °C overnight, the sections were washed twice and coupled with HRP-polymer at room temperature. After washing, 3,3'-diaminobenzidine (DAB) substrate (Sigma) was added to the tissue section, waiting for the color to change. The sections were counterstained with hematoxylin and observed through a microscope.

# RNA-binding protein immunoprecipitation assay

All the steps of RNA binding protein immunoprecipitation (RIP) detection are performed following to the previous method according to the manufacturer's instructions of Magna RIP Kit (MilliPore). The interaction between miR-145-5p and circ-BVES in SGC-7901 and MKN45 cells was verified by biotin labeled miR-145-5p probes. The probe was designed and obtained from Sangon Biotech(Shanghai, China). The SGC-7901 and MKN45 cells were lysed with RIP lysis buffer, and the magnetic beads were incubated at 4 °C overnight. The enrichment of Circ-BVES was analyzed by RT-QPCR.

## Dual-luciferase reporter assay

In order to confirm the interaction between circ-BVES and miR-145-5p, miR-145-5p and target gene HMGB3, we constructed circBVES-WT, circBVES-Mut, HMGB3 3'UTR-WT and HMGB3 3'UTR- Mut recombinant plasmid. All plasmids were confirmed by sequencing. The relative luciferase activity of the treated SGC-7901 and MKN45 cells were examined using the dual luciferase assay kit (Promega, Madison, WI, USA) according to the manufacturer's protocol.

## Western blot assay

Total protein was extracted from the treated SGC-7901 and MKN45 cells using a protein extraction kit (Key Gene,China) according to the manufacturer's instructions. The concentration of each sample was quantitated by BCA kit (Servicebio). The equivalent proteins of each group were separated by 10% SDS-PAGE under 90 V voltage according to molecular weight, and the separated proteins were transferred to PVDF membranes (microwells, USA). After blocking with 5% skim milk for 2 h, they were incubated with primary antibodies against CCNE1 (1:1000), CDK2 (1:1000), HMGB3 (1:1000) (Abcam, USA), GAPDH (1:1000) at 4 °C overnight. After washing and removing the free antibody with TBST buffer, incubate with the secondary antibody at 1:2000 dilution at room temperature for 2 h. Finally, the enhanced chemiluminescence (ECL) kit (Pierce, Waltham, MA, U.S.A.) was used to observe the immunoreactive bands.

## Statistical analysis

All statistical analyses in the present study were performed using SPSS 17.0 and GraphPad Prism 7.0 software. Data were showed as mean  $\pm$  standard deviation (SD). Comparisons between two groups were conducted using the twin tailed Student's t-test. The correlation between groups was analyzed by Pearson correlation. P value < 0.05 was considered as statistically significant.

## Results

### circBVES was upregulated in gastric cancer tissues and cell lines

CircRNA microarray was used to detect the expression profile of circRNA in gastric cancer tissues and adjacent normal tissues. A total of 32 circRNAs was identified to be differentially expressed between the two groups, including upregulated (n = 21) and downregulated (n = 11) circRNAs (Additional file 1). We selected up-regulated circBVES as the research object, and qRT-PCR was used to detect the expression level of circBVES in gastric cancer tissues and cell lines, so as to determine whether circBVES is involved in the occurrence and development of gastric cancer. Moreover, the overview of the circBVES was displayed (Fig. 1a and b). The qRT-PCR results displayed that circBVES was significantly up-expressed in gastric cancer (Fig. 1c). Similarly, our data also showed the expression level of circBVES was higher in the GC tissues provided by patients with stage III–IV disease (TNM staging) than the patients with stage I–II disease (Fig. 1d). We further confirmed that, compared with GES-1 cells, the expression level of circBVES was higher in HGC-27, MGC-803, BGC-823, SGC-7901 and MKN45 cell lines (Fig. 1f). The SGC-7901 and MKN45 cell lines with the highest expression levels were selected for subsequent studies. In addition, GC patients with high circBVES expression exhibited a worse prognosis than those with low circBVES expression (Fig. 1e).

### **Inhibition of circBVES suppresses the cell proliferation and glycolysis in vitro.**

To study the biological function of circBVES in GC, we inhibit the expression of circBVES by transfecting SGC-7901 and MKN45 cells with circBVES (Fig. 2a and b). The CCK-8 assay indicated that knockdown of circBVES significantly inhibited cell viability in SGC-7901 and MKN45 cells (Fig. 2c and d). It is generally believed that proliferating solid cancer cells convert their glucose metabolism pattern to hypoxic glycolysis. To investigate whether the effect of circBVES on cell proliferation was due to its effect on metabolism, we measured the effect of circBVES on cell glycolysis, which provides ATP to cancer cells. As is shown that, ECAR was significantly reduced in SGC-7901 and MKN45 cells silenced by circBVES, reflecting the positive role of circBVES glycolysis in gastric cancer cells (Fig. 2e and f). The oxygen consumption of cells reflects the respiration of mitochondria. During aerobic glycolysis, the OCR of cells is decreased. As the same as ECAR results, we observed significant increases in OCR in SGC-7901 and MKN45 cells knocked out by circBVES, which enhanced the positive effect of aerobic glycolysis (Fig. 2g and h). In addition, glucose uptake rate, lactic acid production and ATP synthesis were significantly reduced in SGC-7901 and MKN45 cells with circBVES knockdown (Fig. 2i-k). Therefore, circBVES positively regulated aerobic glycolysis and proliferation in GC cells.

## **CircBVES promotes tumor growth in vivo**

To investigate the effect of circBVES knockdown on tumor growth, the stable cell lines were constructed by using lentivirus mediated knockdown of circBVES in MKN45 cells. The cells were injected into the flanks subcutaneous of nude mice. The results showed that the tumor weight and volume of the two groups of MKN45 cells with low circBVES expression was significantly slower and smaller than that of the sh-NC group (Fig. 3a–c). In addition, the weight of mice with low circBVES expression decreased slowly over time (Fig. 3d). In addition, immunohistochemical results showed that the expression of KI67 and PCNA in tumors with low expression of circBVES was significantly lower than that in sh-NC group

(Fig. 3e and f). The qRT-PCR analysis revealed that the expression of circBVES was significantly decreased in tumors formed by sh-circBVES cells (Fig. 3g).

## **CircBVES functions as molecular sponge for miR-145-5p in GC cells**

In order to further explore the potential mechanism of circBVES affecting GC cell proliferation and glycolysis, according to the analysis of STARBASE (<http://starbase.sysu.edu.cn>), miR-145-5p was predicted to bind circBVES, which was verified by luciferase reporter assay (Fig. 4a and b). Moreover, starBase also showed that miR-145-5p was significantly downregulated in cancer tissues compared with their normal counterparts in GC and we verified it by qRT-PCR (Fig. 4c and d). The RIP assay showed that a specific enrichment of circBVES and miR-145-5p in the miR-145-5p probe group compared with the scramble group in both SGC-7901 and MKN45 cells (Fig. 4e and f). In Fig. 4g and h, the results showed that circBVES inhibited the expression of miR-145-5p in GC cells and a significantly inverse correlation between the expression of circBVES and miR-145-5p. In order to determine whether miR-145-5p is involved in the biological effects of circBVES-mediated GC cells, sh-circBVES-1 and inh-145-5p were co-transfected into GC cells. We found that the suppression effects on cell proliferation and glycolysis by circBVES knockdown could be partially rescued by miR-145-5p inhibitor (Fig. 4i-q).

## **MiR-145-5p inhibited cell proliferation and glycolysis through regulating HMGB3 expression**

Then the target mRNA of miR-145-5p was explored by bioinformatics analysis. According to the Targetscan ([http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)) analysis, HMGB3 was predicted as the target of miR-145-5p (Fig. 5a). Moreover, starBase also showed that miR-145-5p is negatively correlated with HMGB3. And it highly expressed in GC tissues compared with their normal counterparts (Fig. 5b and c). The interaction between miR-145-5p and HMGB3 was detected by luciferase report experiment. The results show that miR-145-5p mimics can significantly reduce the activity of the luciferase reporter vector with HMGB3 3'UTR-WT sequence (Fig. 5d). Then, qRT-PCR and western blot were used to detect the RNA expression and protein level of HMGB3 in SGC-7901 and MKN45 cells with miR-145-5p mimics or miR-145-5p inhibitors (Fig. 5e-h). Then the rescued experiments showed that proliferation and glycolysis of GC cells was significantly promoted by miR-145-5p inhibitors compared with the NC group. While HMGB3 knockdown rescued the anti-tumor effect of miR-145-5p on GC cells (Fig. 5i-q).

## **HMGB3 is indirectly regulated by circBVES and the downstream of it**

To validate whether circBVES could regulate the expression of HMGB3 in GC cells, we found that knockdown of circBVES markedly decreased the expressions of HMGB3. Moreover, the decrease of HMGB3 induced by circBVES knockdown could be reversed by miR-145-5p inhibitors (Fig. 6a). In addition,

we found a significantly positive association between the expression of circBVES and HMGB3 in GC tissues (Fig. 6b). By using bioinformatics analysis, we found that HMGB3 is associated with cell cycle (Fig. 6c). Then the western blot demonstrated that knockdown of circBVES decreased the protein levels of HMGB3 and downstream targets CCNE1 and CDK2, while the effects caused by silencing circBVES could be reversed by miR-145-5p inhibitors (Fig. 6d-f). In summary, these data suggest that circBVES might serve as a ceRNA for miR-145-5p to regulate HMGB3 expression, which leading to GC cell proliferation and glycolysis (Fig. 6g).

## Discussion

In recent years, more and more circRNAs have been discovered due to the rapid development of molecular biology technology. Because of its unique circular structure with excellent stability, it can avoid being decomposed by RNA exonuclease. Due to the advantage, circRNAs may become an ideal biomarker for cancer diagnosis [18]. A growing body of evidence indicates that circRNA is associated with heart failure, Alzheimer's disease, Parkinson's disease and other human diseases, especially the occurrence of malignant tumors [19–21]. Some circRNAs have been reported to play a role in promoting or suppressing cancer in bladder cancer, thyroid cancer, pancreatic cancer, hepatocellular carcinoma and other types of cancer [22–25]. But this is just the tip of the iceberg, and there are still a lot of circRNAs waiting for us to study.

In our study, we applied RNA sequencing technology to obtain the expression profile of circRNA in GC tissues and adjacent tissues. Then, we discovered a new circRNA, named circBVES, which was significantly upregulated in GC tissues and related to the clinical stage of GC and overall poor survival of patients. Further in vitro and in vivo functional experiments showed that circBVES significantly affected the proliferation and glycolysis of GC cells. These study suggest that circBVES plays an oncogene role in the occurrence and development of GC and potential to be a new diagnostic and prognostic marker or therapeutic target for GC patients.

At present, the function of circRNAs as ceRNA is the most widely studied, because circRNAs can combine with miRNA, thus preventing the effect of miRNA on target genes and indirectly affecting the expression of target genes. Moreover the role of ceRNA depends on the number of miRNA binding sites in each transcript, while circRNAs have a considerable number of miRNA binding sites, so many miRNA can be enriched. In other words, the more miRNA binding sites a circRNA has, the stronger the miRNA sponge will be, which playing a more important role in the development of tumors. Therefore, circRNAs can act as sponges for miRNAs and play a role in many human diseases including cancer [26]. For example, it was reported that circRNA\_100290 plays a role in oral cancer by functioning as a sponge of the miR-29 family [27]. Besides, circRNA-002178 act as a ceRNA to promote PDL1/PD1 expression in lung adenocarcinoma [28]. Moreover, circ-CSPP1 promotes proliferation, invasion and migration of ovarian cancer cells by acting as a miR-1236-3p sponge [29]. In our study, we found that circBVES contained MRE of miR-145-5p through bioinformatics analysis. Therefore, we speculated that circBVES might play a carcinogenic role in GC through the adsorption of miR-145-5p by sponging. Further, dual-luciferase reporting and RNA pull-

down experiments confirmed that circBVES could directly interact with miR-145-5p. We also found that miR-145-5p was significantly down-regulated in GC tissues. Consistent with our results, miR-145-5p has been reported to be significantly down-regulated in the tissues of gastric cancer patients, and negatively correlated with the malignancy of gastric cancer [30]. At the same time, we also found that miR-145-5p is down-regulated in colorectal cancer, cervical cancer, prostate cancer and liver cancer, and may act as an inhibitor in the occurrence and development of tumors [31–34]. Our findings demonstrated that circBVES serves as an oncogene by sponging miR-145-5p in GC and revealed the significance of interaction between circBVES and miR-145-5p in tumorigenesis and development of GC.

According to miRNA sponge hypothesis, circRNAs could competitively combine with free miRNAs. By reducing the number of corresponding free miRNAs, circRNAs can inhibit their binding to targeted mRNAs, thus indirectly promoting the expression of target genes [26]. In our study, we found that HMGB3 is one of the potential targets of miR-145-5p by TargetScan. Then, double luciferase reporting experiments confirmed our prediction. In addition, the up-regulation of miR-145-5p resulted in the down-regulation of HMGB3 at mRNA and protein levels, while the down-regulation of miR-145-5p showed the opposite effect. We also found that HMGB3 was over-expressed in GC and negatively correlated with miR-145-5p. Consistent with our results, it has been reported that HMGB3 is highly expressed in breast cancer, colorectal cancer and glioblastoma, and can regulate the development of tumors [35–37]. To verify the relationship between circBVES and HMGB3, we found that low expression of circBVES inhibited HMGB3 expression at mRNA and protein levels. In addition, miR-145-5p inhibitors partially eliminate these effects, which further supports our hypothesis, that circBVES acts as a sponge of miR-145-5p promote HMGB3-mediated proliferation and glycolysis in GC.

## Conclusions

In conclusion, our results suggest that circBVES abnormal expression in gastric cancer, and can affect the proliferation and glycolysis of gastric cancer cells. We firstly demonstrated that circBVES might sponge miR-145-5p to modulate HMGB3 expression, leading to tumorigenesis and development of GC. Our findings suggested that circBVES may be a valuable prognostic indicator and therapeutic target for GC. Moreover, the regulatory network of the circBVES/ miR-145-5p /HMGB3 axis may contribute to a better understanding of the occurrence and development mechanism of GC.

## Abbreviations

GC

Gastric cancer; qRT-PCR:Quantitative real-time polymerase chain reaction; RIP:RNA Immunoprecipitation; HMGB3:High mobility group box 3; CCNE1:Cyclin E1; CDK2:Cyclin Dependent Kinase 2

## Declarations

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## **Authors' contributions**

Lu Jin and Wei Huang designed the study and wrote the manuscript. Zhiwei He and Changhao Zhu performed all of the experiments. Zhiwei He provided research budget. Data curation: Guoliang Xiao; Software: Xiao Chen. Investigation: Xianjin Yang. All authors read and approved the final manuscript.

## **Competing interests**

The authors declare that they have no competing interests.

## **Availability of data and materials**

Please contact author for data request.

## **Consent for publication**

All authors consent this manuscript to be published.

## **Ethics approval and consent to participate**

The present study was approved by the Ethics Committee.

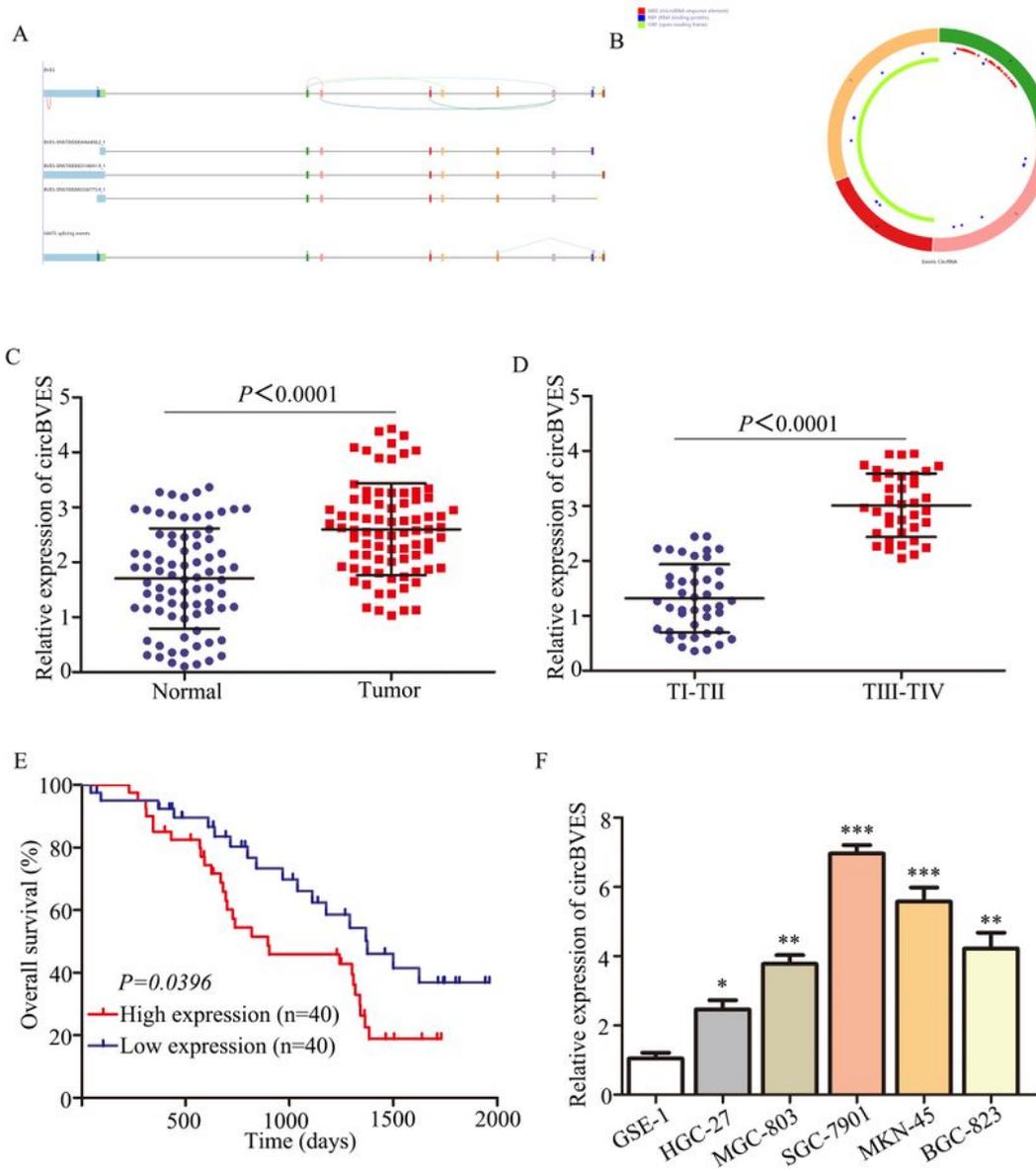
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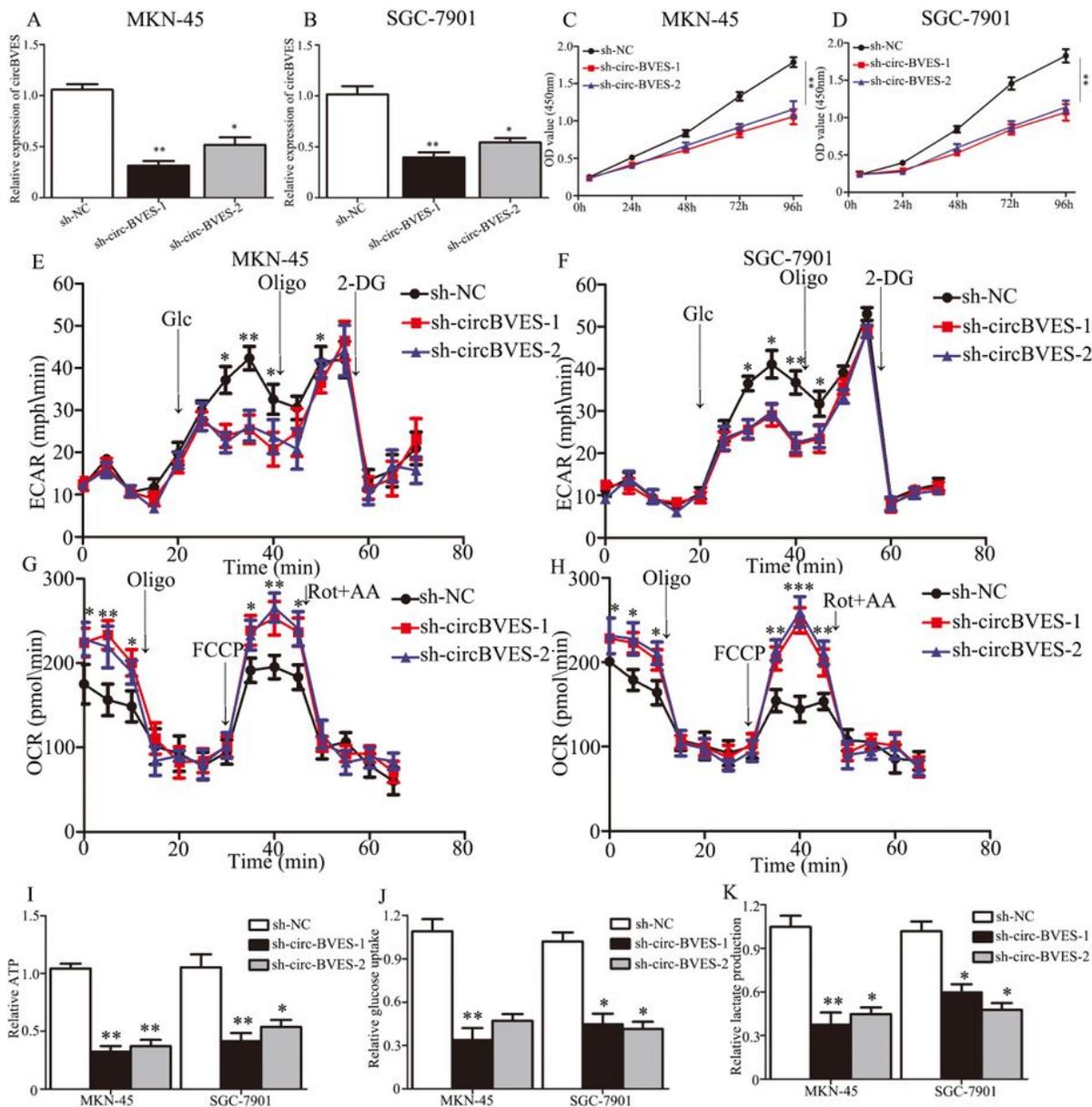
# Figures



**Figure 1**

The expression of circBVES in gastric cancer tissues and cell lines. a-b The overview of the circBVES. c Relative expression of circBVES in GC tissues (Tumor) and adjacent non-tumor tissues (Normal) was detected by qRT-PCR (n= 80). d CircBVES expression in different stages of GC (TNM staging) was

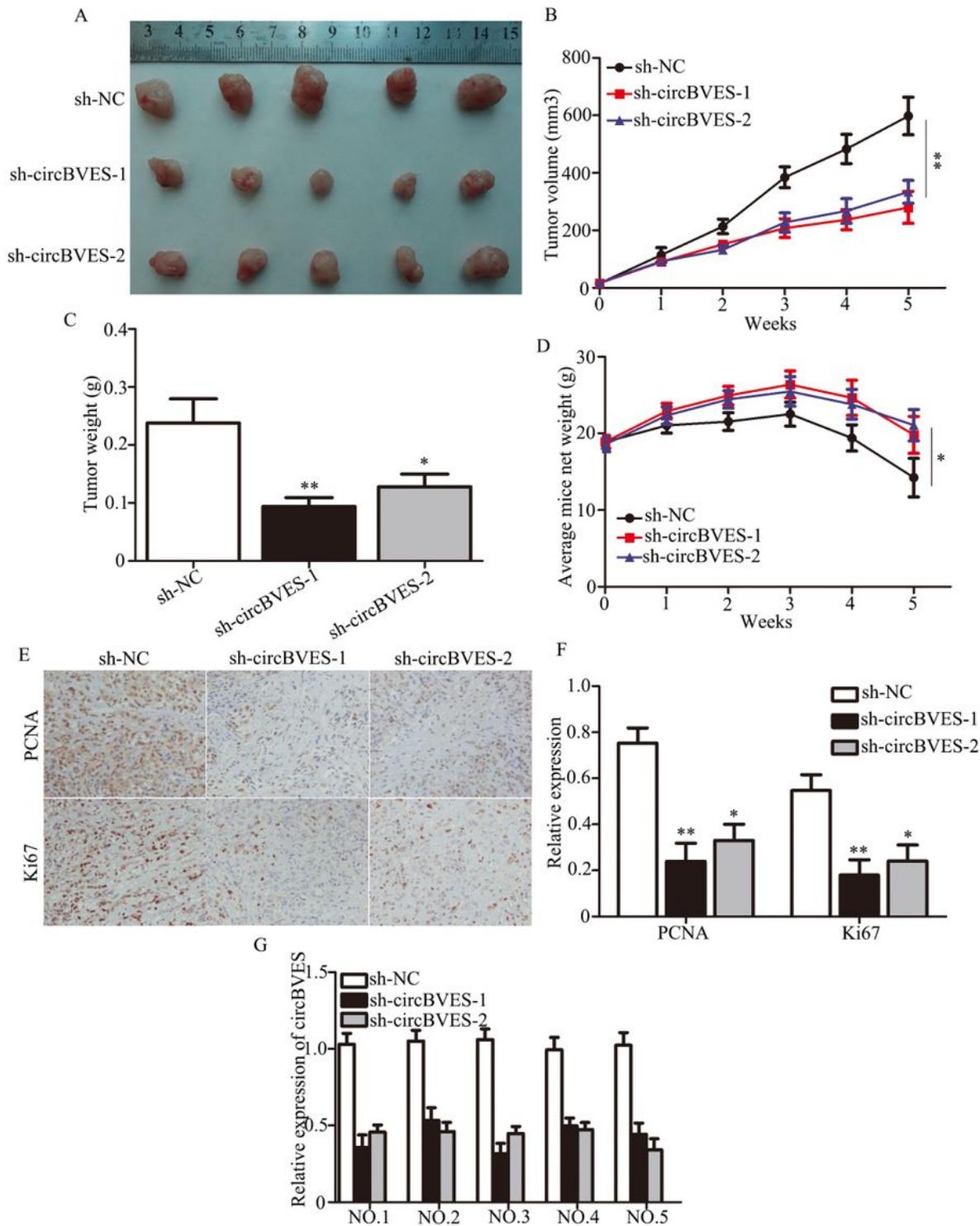
examined by qRT-PCR. e The correlation between the expression of circBVES and overall survival times of patients with gastric cancer. f Relative expression of circBVES in five GC cell lines and GSE-1 cells was determined by qRT-PCR. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



**Figure 2**

Knockdown of circBVES suppresses GC cell proliferation and glycolysis in vitro. a-b qRT-PCR analysis of the relative expression of circBVES in MKN-45 and SGC-7901 cells after transfection with sh-circ or sh-NC.

c-d The effect of circBVES knockdown on the proliferation of GC cells were evaluated by CCK8 assays. e-f Representative image of ECAR measurement in circBVES silencing and control MKN-45 and SGC-7901 cells. g-h Chart of OCR measurement in circBVES-silenced MKN-45 and SGC-7901 cells. i-k ATP synthesis, glucose uptake and lactate production were measured in MKN-45 and SGC-7901 cells stably knockdown circBVES or NC. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



**Figure 3**

CircBVES facilitates tumorigenesis of GC cells in vivo. a Representative images of xenograft tumors of the circBVES silencing and control group. b Growth curves of xenograft tumors which were measured once a week. c The weight of mice was detected each week. d The weight of tumor tissues. e-f The expression of Ki67 and PCNA in circBVES silencing and control tumors was detected through immunohistochemical staining. g Relative expression of circBVES in transplanted tumors in mice with specific number was detected by qRT-PCR. \*P<0.05, \*\*P<0.01.

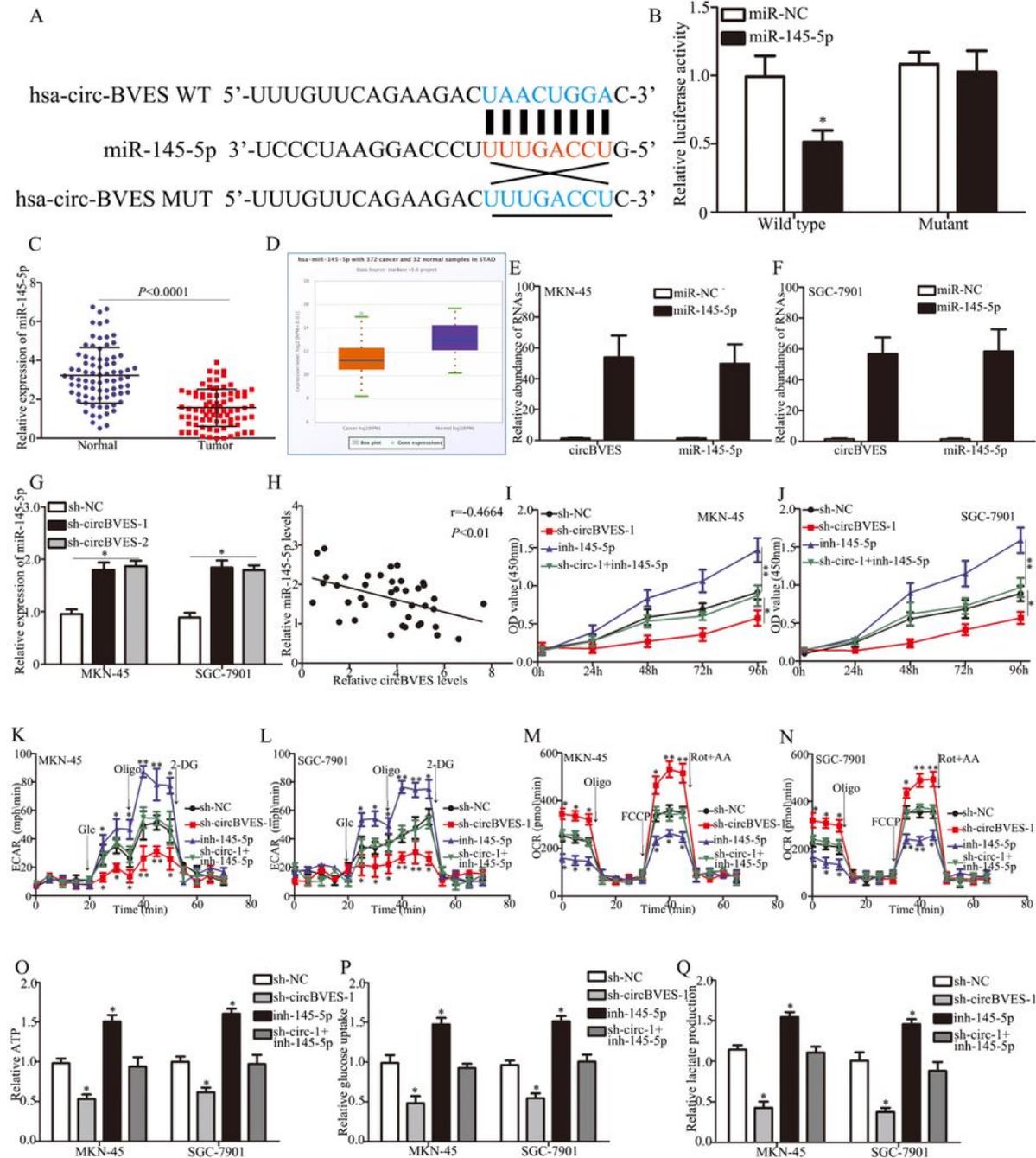
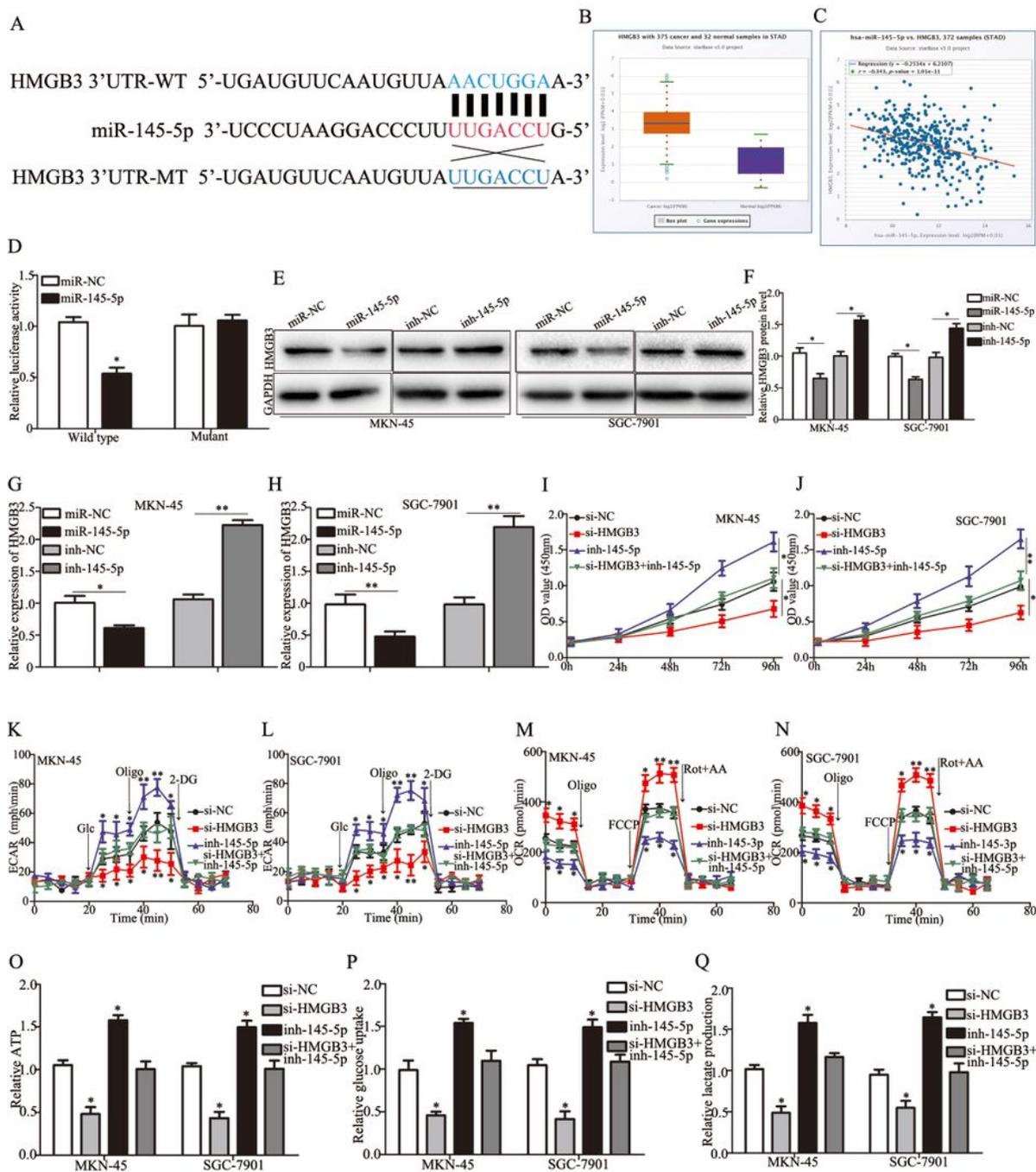


Figure 4

CircBVES functions as a sponge for miR-145-5p. a Schematic representation of the predicted target site for miR-145-5p in circBVES. b Interaction between miR-145-5p and circBVES was verified via dual-luciferase reporter assay. c Relative expression of miR-145-5p in GC tissues (Tumor) and adjacent non-tumor tissues (Normal) was determined by qRT-PCR. d Relative expression of miR-145-5p in GC tissues (Tumor) compared with normal tissue (normal) was analyzed using Starbase. e-f RIP assay was carried out to determine the interaction between miR-145-5p and circBVES in MKN-45 and SGC-7901 cells. g The relative expression of miR-145-5p was detected by qRT-PCR after transfection with sh-circBVES. h Pearson correlation analysis of circBVES and miR-145-5p expression in 40 GC tissues. i-j CCK-8 assays in MKN-45 and SGC-7901 cells after transfected with sh-circBVES-1, inh-145-5p or both. k-n The ECAR and OCR in MKN-45 and SGC-7901 cells treated with sh-circBVES-1, inh-145-5p or both. o-q Glucose uptake, lactate production, and ATP synthesis were measured in MKN-45 and SGC-7901 cells treated with sh-circBVES-1, inh-145-5p or both. \*P<0.05, \*\*P<0.01.



**Figure 5**

The miR-145-5p targeted HMGB3 to regulated proliferation and glycolysis of GC cells. a MiR-145-5p binding sites in HMGB3 predicted by bioinformatics analysis. b-c The expression of miR-145-5p in gastric cancer and its correlation with HMGB3 predicted by bioinformatics analysis. d Interaction between miR-145-5p and HMGB3 was verified via dual-luciferase reporter assay. e-h Relative mRNA and protein levels of HMGB3 were detected in cells after transfected with miR-NC, miR-145-5p, inh-NC and inh-145-5p using

qRT-PCR and western blot, respectively. i-j CCK-8 assays in MKN-45 and SGC-7901 cells after transfected with miR-NC, miR-145-5p, inh-NC and inh-145-5p. k-n The ECAR and OCR in MKN-45 and SGC-7901 cells treated with miR-NC, miR-145-5p, inh-NC and inh-145-5p. o-q Glucose uptake, lactate production, and ATP synthesis were measured in MKN-45 and SGC-7901 cells treated with miR-NC, miR-145-5p, inh-NC and inh-145-5p. \* $P < 0.05$ , \*\* $P < 0.01$ .

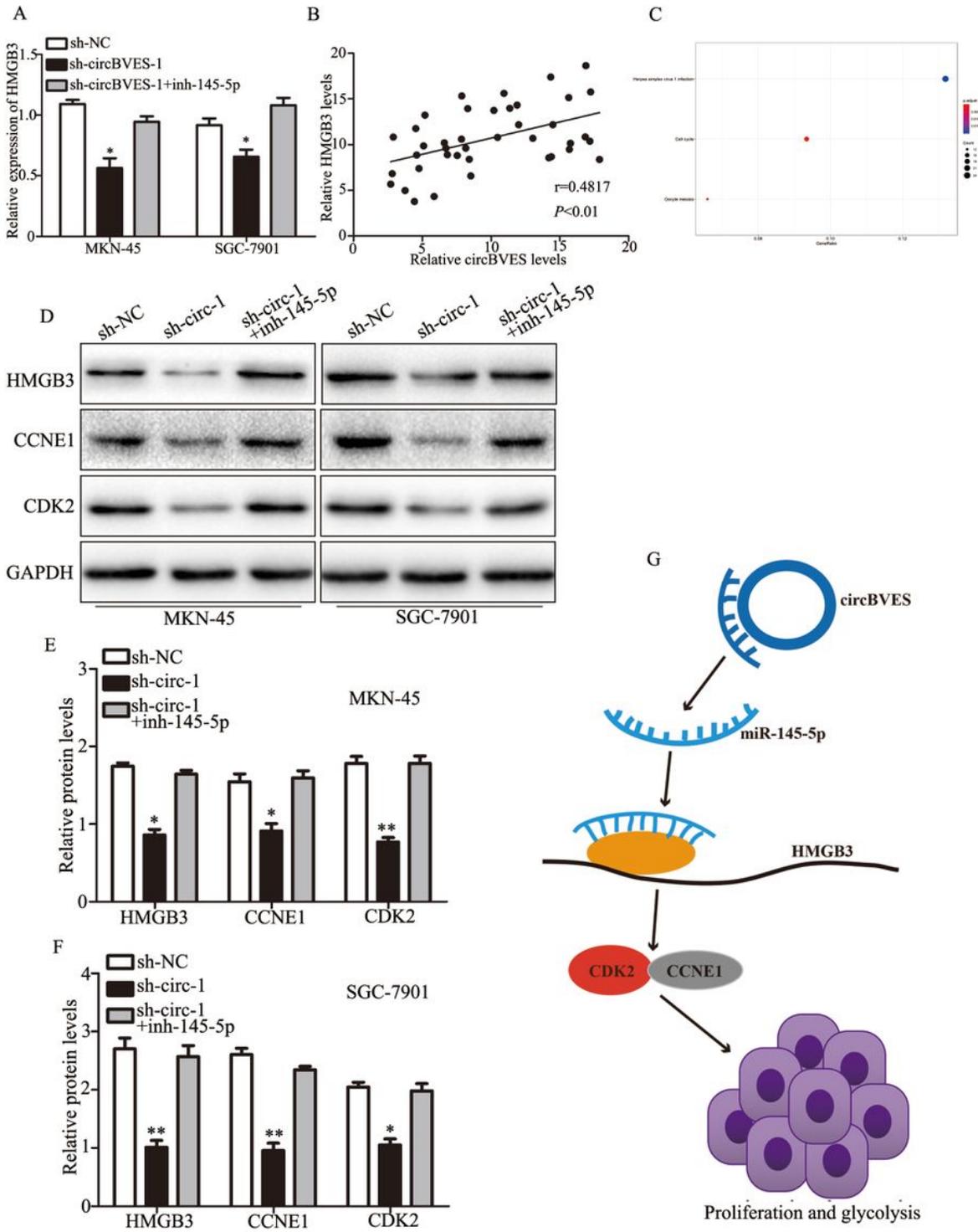


Figure 6

HMGB3 is indirectly regulated by circBVES and circBVES promotes cell proliferation and glycolysis through circBVES /miR-145-5p/ HMGB3 axis. a Relative expression of HMGB3 was detected by qRT-PCR in cells transfected with sh-circBVES-1, inh-145-5p and sh-circBVES-1. b Association analysis of the relationship between circBVES and HMGB3. c The cell cycle is the downstream pathway of HMGB3 predicted by bioinformatics analysis. d-f Relative expression of HMGB3 and downstream cell cycle-related molecules at protein level in cells transfected with sh-NC, sh-circBVES-1, inh-145-5p and sh-circBVES-1 was determined by western blot. f Schematic model shows the results of the study. \*P<0.05, \*\*P<0.01.

## Supplementary Files

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