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Circular RNA circCRIM1 suppresses lung adenocarcinoma cell migration, invasion, EMT, and glycolysis through regulating miR-125b-5p/BTG2 axis

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Abstract. – OBJECTIVE: The present studies indicate that circRNAs play pivotal roles in human cancers. Lung adenocarcinoma (LUAC), one of lung cancer types, has high metastasis rate. Herein, we focused our study on the function and mechanism of circular RNA circCRIM1 in LUAC development.

PATIENTS AND METHODS: Quantitative Real-time polymerase chain reaction (qRT-PCR) was performed to detect the levels of circCRIM1, miR-125b-5p, and BTG anti-proliferation factor 2 (BTG2). Transwell assay was carried out to assess cell migration and invasion. The protein levels of BTG2, EMT markers, and HK2 were measured by Western blot. Glycolysis was analyzed through determining glucose consumption and lactate production. Furthermore, targets of circCRIM1 and miR-125b-5p were predicted and verified by starBase and the dual-luciferase reporter assay, respectively. Also, we explored circCRIM1 affecting tumor growth in vivo using mouse xenograft.

RESULTS: CircCRIM1 and BTG2 were downregulated, and miR-125b-5p was upregulated in LUAC tissues and cells. CircCRIM1 upregulation inhibited LUAC cell migration, invasion, epithelial-mesenchymal transition (EMT), glycolysis, and tumor growth. Moreover, circCRIM1 regulated LUAC cell development through targeting miR-125b-5p. MiR-125b-5p affected LUAC cell growth via targeting to BTG2. Also, circCRIM1 promoted BTG2 expression by inhibiting miR-125b-5p in LUAC cells.

CONCLUSIONS: CircCRIM1 was lowly expressed in LUAC. Moreover, circCRIM1 functioned as a sponge of miR-125b-5p to improve BTG2 expression, thereby suppressing LUAC development. Our finding indicated that circCRIM1 could be considered as a biomarker and target for the diagnosis and therapy of LUAC patients.

Key Words:
CircCRIM1, MiR-125b-5p, BTG2, Cell mobility, Glycolysis, Lung adenocarcinoma.

Introduction

Lung adenocarcinoma (LUAC), a cancer with high incidence and mortality, is a type of lung cancer that is one of the leading causes for cancer related deaths in the world[1,2]. Although advanced technology has applied for the diagnosis and treatment of patients with lung cancer, the prognosis and 5-year survival rate of lung cancer patients is still unsatisfactory[3,4]. Therefore, the analysis of lung cancer development mechanism is essential for the treatment of the patients.

Circular RNAs (circRNAs), a new group of endogenous non-coding RNAs, mainly exist in cytoplasm and exert function as miRNA sponges[5]. CircRNAs are related to numerous cellular processes, including proliferation, migration, invasion, autophagy, and apoptosis[6,7]. CircRNA plays a pivotal role in the development of many human cancers, including LUAC[8,9]. CircCRIM1, also known as circ_0002346, was reported to repress LUAC cell invasion and metastasis[10], meaning that circCRIM1 was involved in the development of LUAC. However, the molecular mechanism of circCRIM1 in LUAC is still largely unknown.

MicroRNAs (miRNAs), identified as non-coding transcripts with about 22 nucleotides, have been reported to regulate cell growth through modulating translation or degradation of message RNA (mRNA) in human cancers [11,12]. Therefore, it is important to investigate miRNA function in cancer cells. It was shown that miR-125b-5p acted as anti-cancer gene in various cancers. For example, Liu et al[13] demonstrated that miR-125b-5p inhibited bladder cancer cell viability as well as migration, and induced apoptosis through modulating phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway. Hua et al[14] confirmed that miR-125b-5p negatively modulated

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the development of hepatocellular carcinoma by binding to thioredoxin reductase 1 (TXNRD1). But an emerging evidence indicated that miR-125b-5p was highly expressed in patients with LUAC[15], meaning that miR-125b-5p might serve as an oncogene. However, the function of miR-125b-5p in LUAC cells is unclear.

In this study, we found that circCRIM1 likely interacted with miR-125b-5p, and BTG anti-proliferation factor 2 (BTG2) was a potential target of miR-125b-5p. Subsequently, their levels and functions were investigated in LUAC. Furthermore, circCRIM1 was verified as a tumor suppressor, and a new mechanism that circCRIM1 regulated miR-125b-5p/BTG2 axis to affect cell growth was reported in LUAC cells.

Patients and Methods

Tissues and Cell Culture

LUAC and adjacent normal tissues (N=30) were collected from the patients in Huaihe Hospital of Henan University. Informed consent was provided by each patient, and this research was approved by the Ethics Review Committee of Huaihe Hospital of Henan University.

BEAS-2B cell line and two LUAC cell lines (A549 and PC-9) were provided by Shanghai Institutes for Biological Science (Shanghai, China) and kept in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) under 5% CO_2 as well as 37°C incubation. 10% fetal bovine serum (FBS; Gibco) and 100 U/mL penicillin-streptomycin (Gibco) were introduced into DMEM for cell culture.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was obtained with the application of RNAiso Plus (TaKaRa, Dalian, China). Next, complementary DNA (cDNA) was generated with Reverse Transcription Kit (TaKaRa), as well as qRT-PCR was conducted with SYBR Premix Ex Taq (TaKaRa). Relative RNA levels of circCRIM1, miR-125b-5p, and BTG2, normalized to U6 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used were listed in Table I.

Cell Transfection

MiR-125b-5p mimic (miR-125b-5p), miR-125b-5p inhibitor (anti-miR-125b-5p), small interfering RNA against BTG2 (si-BTG2), and their control (miR-NC, anti-miR-NC, and si-NC) were provided by GenePharma (Shanghai, China). CircCRIM1 full length was constructed into the pcDNA3.1 vector (GenePharma) for its overexpression. Transfection assay was conducted with the use of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA).

Transwell Assay

Transwell chamber (BD Biosciences, Franklin Lakes, NJ, USA) was employed to examine the migratory and invasive abilities in line with user's manual. Firstly, LUAC cells were transfected and then incubated. Next, the cells fixed with serum-free medium were introduced into the upper chamber, and the lower chamber was placed with medium containing 10% FBS. 24 h later, non-migrated cells were removed, and migrated cells were analyzed using a microscope. The steps were carried out for cell invasion assay when the insert of the chamber was coated with Matrigel (BD Biosciences).

Western Blot Assay

Western blot was conducted as previously described [16], the proteins were obtained using lysis buffer (Thermo Fisher Scientific, Waltham, USA), exposed to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Next, the membranes were blocked using 5% non-fat skim, incubated by primary antibodies (1:1,000), and then incubated with secondary antibodies (1:2000). Finally, protein signal was measured by chemiluminescence (ECL) kit (Bio-Rad, Hercules, CA, USA). Primary antibodies against matrix metallopeptidase 9 (MMP-9) (ab137867), N-cadherin (ab18203), E-cadherin (ab15148), hexokinase 2 (HK2) (ab227198), BTG2

Table I. The primers used for qRT-PCR.

Gene name	Primer sequence (5′-3′)
circCRIM1	F: GTCCCCCGGACAGCTATGAA
	R: CAAAGGGATTGCTGCAGGTTC
miR-125b-5p	F: TCCCTGAGACCCTAACTTGTGA
	R: AGTCTCAGGGTCCGAGGTATTC
BTG2	F: CATCATCAGCAGGGTGGC
	R: CCCAATGCGGTAGGACAC
U6	F: CTCGCTTCGGCAGCACA
	R: AACGCTTCACGAATTTGCGT
GAPDH	F: GGGCTGCTTTTAACTCTGGT
	R: TGATTTTGGAGGGATCTCGC

(ab197362), GAPDH (ab128915), and secondary antibodies (ab205718) were obtained from Abcam (Cambridge, MA, USA).

The Measurement of Glucose Consumption and Lactate Production

For the determination of glucose consumption, transfected LUAC cells were cultured for 24 h and then incubated with phenol-red free medium containing 1% FBS for 3 days. Next, glucose in media was assessed by a colorimetric glucose assay kit (BioVision, Inc., Milpitas, CA, USA) in line with the recommended protocol. In addition, lactate assay kit (Sigma-Aldrich; Merck KGaa, Darmstadt, Germany) was employed to detect lactate production based on the user's manual.

The Dual-Luciferase Reporter Assay

The circCRIM1-binding sites or BTG2-binding sites of miRNA were predicted by bioinformatics tool starBase. Every fragment or its mutant was inserted into the pGL3-basic vector (Promega, Madison, WI, USA). Next, the recombinant luciferase vector and miR-125b-5p or miR-NC were transfected into LUAC cells. Finally, luciferase activity was determined by Dual-Luciferase Assay (Promega) based on the recommended instruction.

Xenograft Assay

Here, we used female BALB/c nude mice (4 weeks old) to perform xenograft assay. Briefly, A549 cells transfected with circCRIM1 or Vector were subcutaneously injected into the mice. 7 d later, tumor size (length×width^2/2) was calculated every 4 d. 27 d later, the tumor was removed and tumor weight was analyzed. This study was approved by the Animal Ethics Committee of Huaihe Hospital of Henan University.

Statistical Analysis

Student's *t*-test was used to analyze statistical significance. All results were from at least three independent experiments and were processed as the means ± standard deviation (SD). Statistical difference was considered with $p<0.05$.

Results

CircCRIM1 was Downregulated in LUAC Tissues and Cells

Firstly, qRT-PCR was applied to determine the level of circCRIM1 in LUAC tissues and normal tissues. The data suggested that circCRIM1 was significantly downregulated in LUAC tissues (Figure 1A). Furthermore, the level of circCRIM1 was explored in LUAC cells. As shown in Figure 1B, circCRIM1 level was lower in LUAC cells (A549 and PC-9) than in normal cells (BEAS-2B). Therefore, circCRIM1 acted as an oncogene in LUAC development.

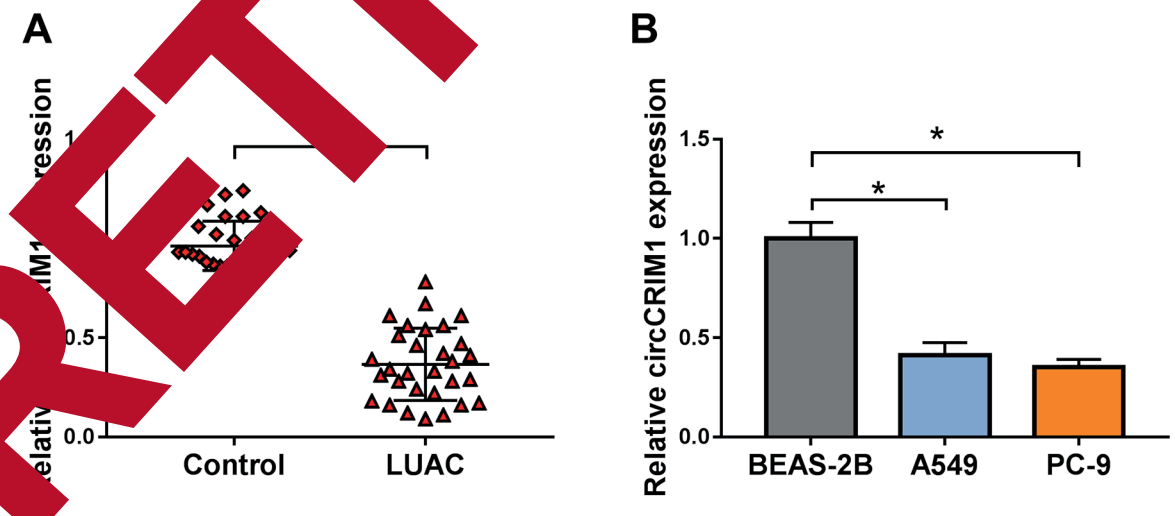

Figure 1. High circCRIM1 expression in LUAC. **(A)** CircCRIM1 level was detected by qRT-PCR in LUAC tissues and normal tissues. **(B)** CircCRIM1 level was determined in LUAC cells and normal cells. *$p<0.05$.

CircCRIM1 Overexpression Suppressed LUAC Cell Migration, Invasion, EMT, and Glycolysis

To explore the function of circCRIM1 in LUAC cells, circCRIM1 level was overexpressed through transfecting circCRIM1 into A549 and PC-9 cells, and Vector was used as negative control. QRT-PCR assay suggested that circCRIM1 level was remarkably increased due to the transfection with circCRIM1 (Figure 2A). Next, transwell assay was used to analyze cell mobility. As demonstrated in Figure 2B, circCRIM1 overexpression significantly suppressed cell migration and invasion. Moreover, the levels of three EMT markers, MMP9, N-cadherin, and E-cadherin, were detected using Western blot. The results indicated that the levels of MMP9 and N-cadherin were downregulated, and the level of E-cadherin was upregulated by circCRIM1 overexpression in A549 and PC-9 cells (Figure 2C and D). Next, we analyzed the effect of circCRIM1 on glycolysis, and found that circCRIM1 overexpression decreased glucose consumption and lactate production (Figure 2E and F). Similarly, HK2, a glycolysis-related gene, was lowly expressed in CRIM1-upregulated A549 and PC-9 cells that in control cells (Figure 2G). Taken together, circCRIM1 upregulation repressed LUAC growth.

CircCRIM1 Was a Sponge for miR-125b-5p

StarBase was used to analyze the target genes of circCRIM1 in LUAC cells, the data demonstrated that miR-125b-5p was a target of circCRIM1 (Figure 3A). Then, circCRIM1 or its mutant was constructed into luciferase reporter plasmid, and then co-transfected into A549 and PC-9 cells with miR-125b-5p or miR-NC. The analysis of luciferase activity showed that the luciferase density of A549 and PC-9 cells was downregulated by co-transfection with miR-125b-5p and circCRIM1, while showed no change after co-transfection with miR-125b-5p and circCRIM1-MUT (Figure 3B and C). These data indicated that circCRIM1 targeted miR-125b-5p. Next, we determined the level of miR-125b-5p in LUAC and found that miR-125b-5p level was significantly higher in LUAC tissues/cells than that in normal tissues/cells (Figure 3D and E). Also, whether circCRIM1 affected the expression of miR-125b-5p was investigated. As shown in Figure 3F, miR-125b-5p expression was significantly downregulated by circCRIM1 overexpression. Taken together, circCRIM1 targeted miR-125b-5p and then downregulated its level in LUAC cells.

CircCRIM1 Repressed miR-125b-5p Expression to Affect LUAC Cell Growth

In this study, we found that circCRIM1 expression was negatively correlated with miR-125b-5p expression (Figure 4A). To investigate whether miR-125b-5p could reverse the function of circCRIM1 in LUAC cells, A549 and PC-9 cells were transfected with Vector, circCRIM1, circCRIM1+miR-NC, or circCRIM1+miR-125b-5p, respectively. QRT-PCR displayed that miR-125b-5p expression was reduced by circCRIM1 overexpression, and subsequently increased due to the transfection with miR-125b-5p (Figure 4B). Next, transwell assay and Western blot indicated that circCRIM1 overexpression inhibited cell migration, invasion, and EMT, whereas this action was impaired by miR-125b-5p upregulation (Figure 4C-F). Furthermore, we analyzed the glycolysis of these cells, and found that miR-125b-5p upregulation weakened the effect of circCRIM1 overexpression on the glycolysis of A549 and PC-9 cells (Figure 4G-I). Thus, circCRIM1 repressed miR-125b-5p expression to affect LUAC cell development.

MiR-125b-5p Was a Sponge for BTG2

To explore the functional mechanism of miR-125b-5p, starBase was employed to predict the potential targets of miR-125b-5p. The results demonstrated that miR-125b-5p had a potential to target BTG2 (Figure 5A). Then, the dual-luciferase reporter assay was performed to verify this interaction. As shown in Figure 5B and C, only in the cells transfected with miR-125b-5p and BTG2 3'UTR-WT, the luciferase activity was repressed, confirming the interaction between miR-125b-5p and BTG2. Furthermore, the level of BTG2 in LUAC tissues and cells was investigated. The data suggested that BTG2 level in LUAC tissues/cells was significantly lower than that in normal tissues/cells (Figure 5D-G). In addition, we analyzed the effect of miR-125b-5p on BTG2 expression through transfecting anti-miR-125b-5p into A549 and PC-9 cells. After confirmed that anti-miR-125b-5p significantly suppressed miR-125b-5p expression (Figure 5H), our results demonstrated that BTG2 expression was upregulated due to miR-125b-5p knockdown (Figure 5I-J). Taken together, miR-125b-5p bound to BTG2 and then downregulated its level.

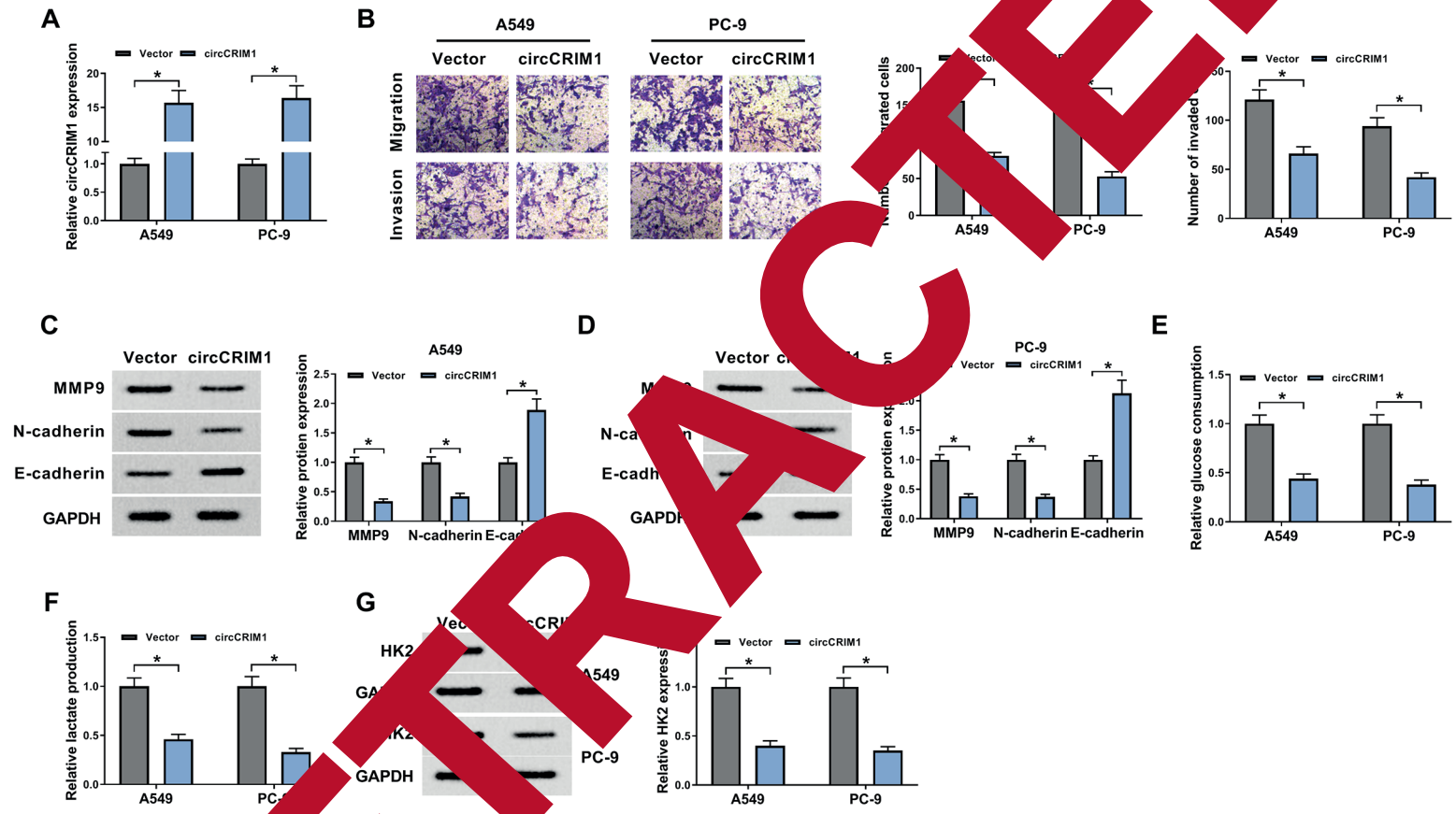

Figure 2. The effect of cir M1 on C cell mobility, EMT, and glycolysis. **(A)** CircCRIM1 expression was measured in A549 and PC-9 cells transfected with Vector or circCRIM1. **(B)** well a was performed to assess cell migratory and invasive abilities. **(C** and **D)** The levels of MMP-9, N-cadherin, and E-cadherin were measured by Western b and **F** cose consumption and lactate production were determined using related kits. **(G)** Western blot assay was carried out to analyze the lev of HK2. 05.

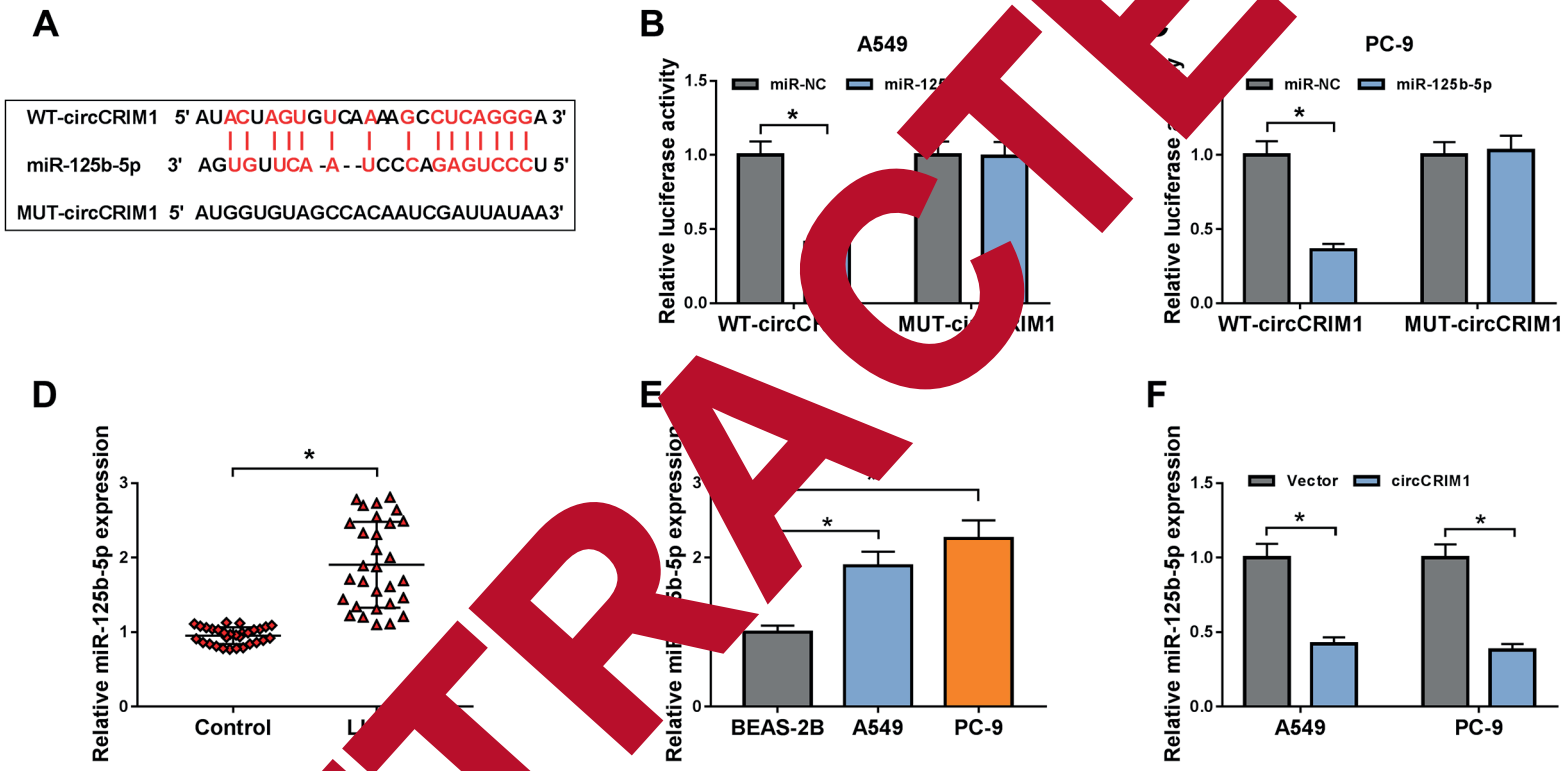

Figure 3. CircCRIM1 functio[n] sponge for mi[R]-5p. **(A)** The interaction between circCRIM1 and miR-125b-5p was predicted by starBase. **(B** and **C)** The luciferase activity was determ[ined] in A549 and PC-9 ce[lls] transfected with WT-circCRIM1 or MUT-circCRIM1 and miR-125b-5p or miR-NC. **(D** and **E)** The level of miR-125b-5p was investig[ated] in LUA[C] and normal tissues **(D)** as well as LUAC and normal cells **(E)**. **(F)** MiR-125b-5p expression was detected in A549 and PC-9 cells transfected with Ve[ctor] or circ[CRIM1]. *$p<0.05$.

MiR-125b-5p Repressed BTG2 Expression to Affect LUAC Cell Growth

Using qRT-PCR, we found that BTG2 expression was negatively correlated with miR-125b-5p expression (Figure 6A). To determine whether miR-125b-5p regulating LUAC cell progression via repressing BTG2 expression, anti-miR-NC, anti-miR-125b-5p, anti-miR-125b-5p + si-NC, or anti-miR-125b-5p + si-BTG2 was transfected into A549 and PC-9 cells. QRT-PCR presented that miR-125b-5p knockdown-upregulated BTG2 level was downregulated by the transfection with si-BTG2 (Figure 6B and C). Next, transwell assay and Western blot were used to assess cell mobility and the levels of EMT markers, respectively. As demonstrated in Figure 6D-G, the effect of miR-125b-5p knockdown on cell migration, invasion, and EMT was impaired by BTG2 depletion. On the other hand, glucose consumption, lactate production, and HK2 expression were suppressed by miR-125b-5p knockdown, and then partly rescued by BTG2 depletion in A549 and PC-9 cells (Figure 6H-J). Therefore, miR-125b-5p mediated LUAC cell progression through repressing BTG2 expression.

CircCRIM1 Inhibited miR-125b-5p Expression to Increase BTG2 Level

Using qRT-PCR, we analyzed the relations between the levels of BTG2 an RIM1 a found that BTG2 expressio pe ely cor related with circCRIM1 e ssion (ure 7A). Then, whether circCRI ffectin pression through mo lati 25b-5p pression was inve ted. A onstrated in Figure 7B and C G2 expressi s signifi cantly upregul CRIM1 ove pression, but this effect as wea by miR-125b-5p up regulatio erefore, circ 1 repressed miR-125b-5 pression to promo TG2 expression in L cells.

Circ ttenu Tumor Growth Vivo

explo er circCRIM1 affects tumo owth *in o*, female BALB/c nude mice w taneously injected with A549 cells sfec th circCRIM1 or Vector. Then, tumor size and weight were analyzed. As shown Figure 8A and B, tumor size and weight in ci IM1 group mice were smaller than that in control mice. Furthermore, the levels of circCRIM1, miR-125b-5p, BTG2 were determined. As expected, circCRIM1 and BTG2 levels were increased, and miR-125b-5p level was reduced in circCRIM1 group mice (Figure 8C and D). Therefore, circCRIM1 attenuated tumor growth *in vivo*.

Discussion

Lung adenocarcinoma (LUAC) ommon lung cancer type which threa s peop alth[1]. Nowadays, surgical treatm and radio therapy are used for the apy of UAC, b outcome is poor[17]. In ent de s, circRNAs have been considered as ic kers an gets for the diagnosis the LUA . Here, we demonstra at circCR a dogenous circRNA, s d the devel t of LUAC through r latin P-125b-5p/BTG2 axis. Furthermore, circCRIM be considered as a biomar target for the nosis and therapy of L patients.

ircRNAs ha been showed to be involved e growth of AC cells. Qiu et al[20] reported t ircPRKCI erexpression accelerated cell pro ion ell as tumorigenesis of LUAC via ta miR-545/miR-589. Yao et al[21] demonstrated that circ_0006427 upregulation d LUAC cell proliferation, migration, as en nvasion through binding to miR-6783-3p. Yao et al[22] revealed that circ_0001946 enhanced LUAC development through modulating miR-135a-5p level. These results indicated that circRNAs exerted important function in LUAC development. In this study, we found that circRNA circCRIM1 was significantly downregulated in LUAC tissues and cells, suggesting the involvement of circCRIM1 in LUAC progression. Moreover, EMT has been pointed to play an important role in the tumorigenic process[23], and glutaminolysis has been acknowledged as an indispensable metabolic process that supports cancer progression[24]. In this paper, circCRIM1 overexpression inhibited LUAC cell migration, invasion, EMT, glycolysis, implying that circCRIM1 could repress metastasis and growth of LUAC cells. Similarly, the inhibitory action of circCRIM1 on metastasis had been reported in previous paper[10]. Besides, our results also disclosed that circCRIM1 could suppress LUAC tumor growth *in vivo*. Therefore, circCRIM1 acted as a suppressor in LUAC development.

CircRNA was considered as a potential target for the therapy of human cancers since it regulated cell growth as suppressor or oncogene[25]. Likewise lncRNAs, circRNA could serve as com-

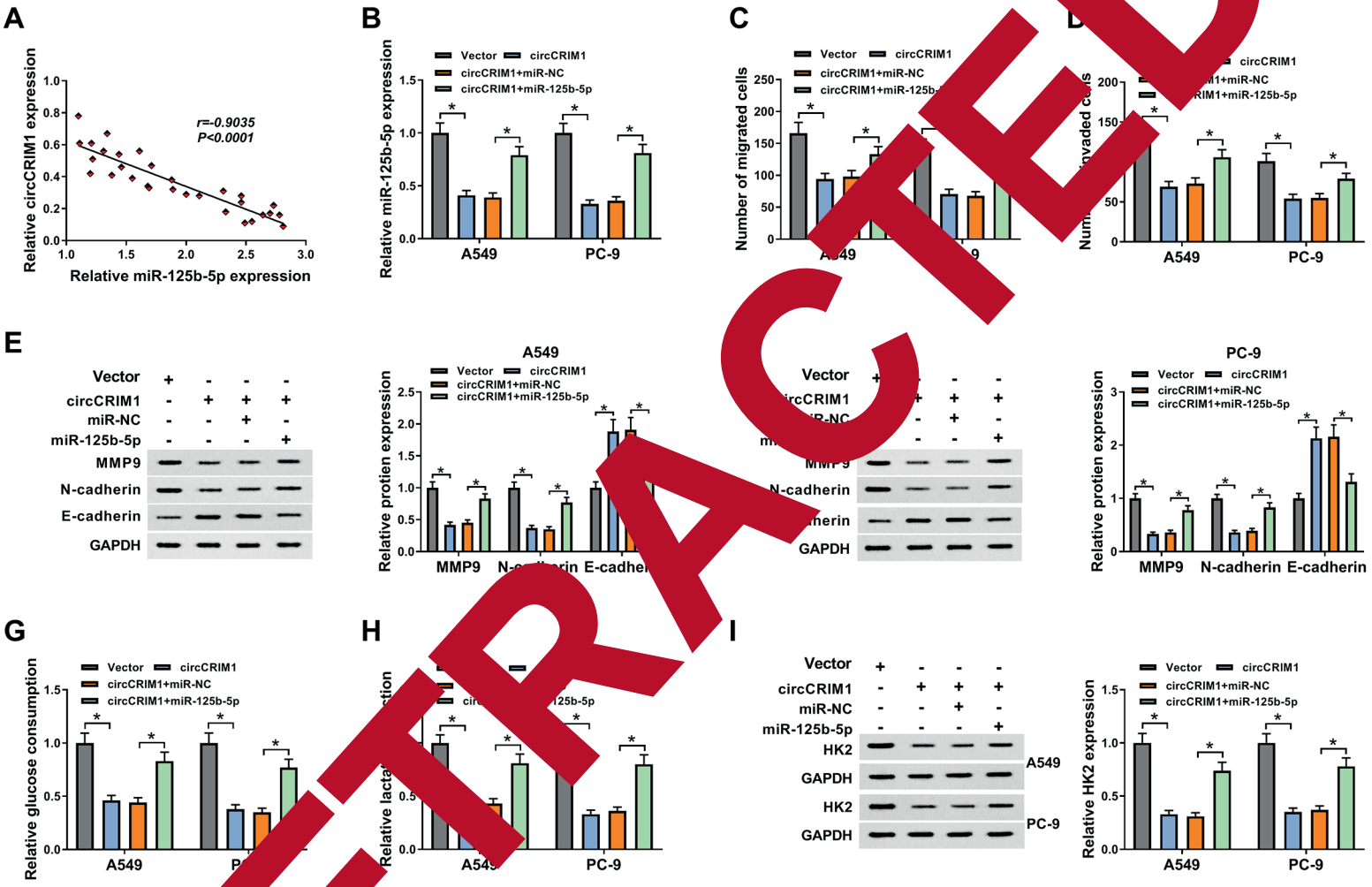

Figure 4. MiR-125b-5p re[illegible]ffect of [illegible]CRIM1 on LUAC cells. **(A)** The relationship between circCRIM1 expression and miR-125b-5p expression was analyzed. **(B)** MiR-125b-5p exp[illegible] was m[illegible]ed in A549 and PC-9 cells transfected with Vector, circCRIM1, circCRIM1 + miR-NC, or circCRIM1 + miR-125b-5p, respectively. **(C)** Cell m[illegible]y. [illegible]nvasive abilities were assessed by transwell assay. **(E** and **F)** Western blot assay was applied to determine the levels of MMP-9, N-c[illegible]rin, a[illegible]-cadher[illegible]d **H)** Glucose consumption and lactate production were investigated using related kits. **(I)** HK2 level was measured by Western blot[illegible]g. *$p<0.0$

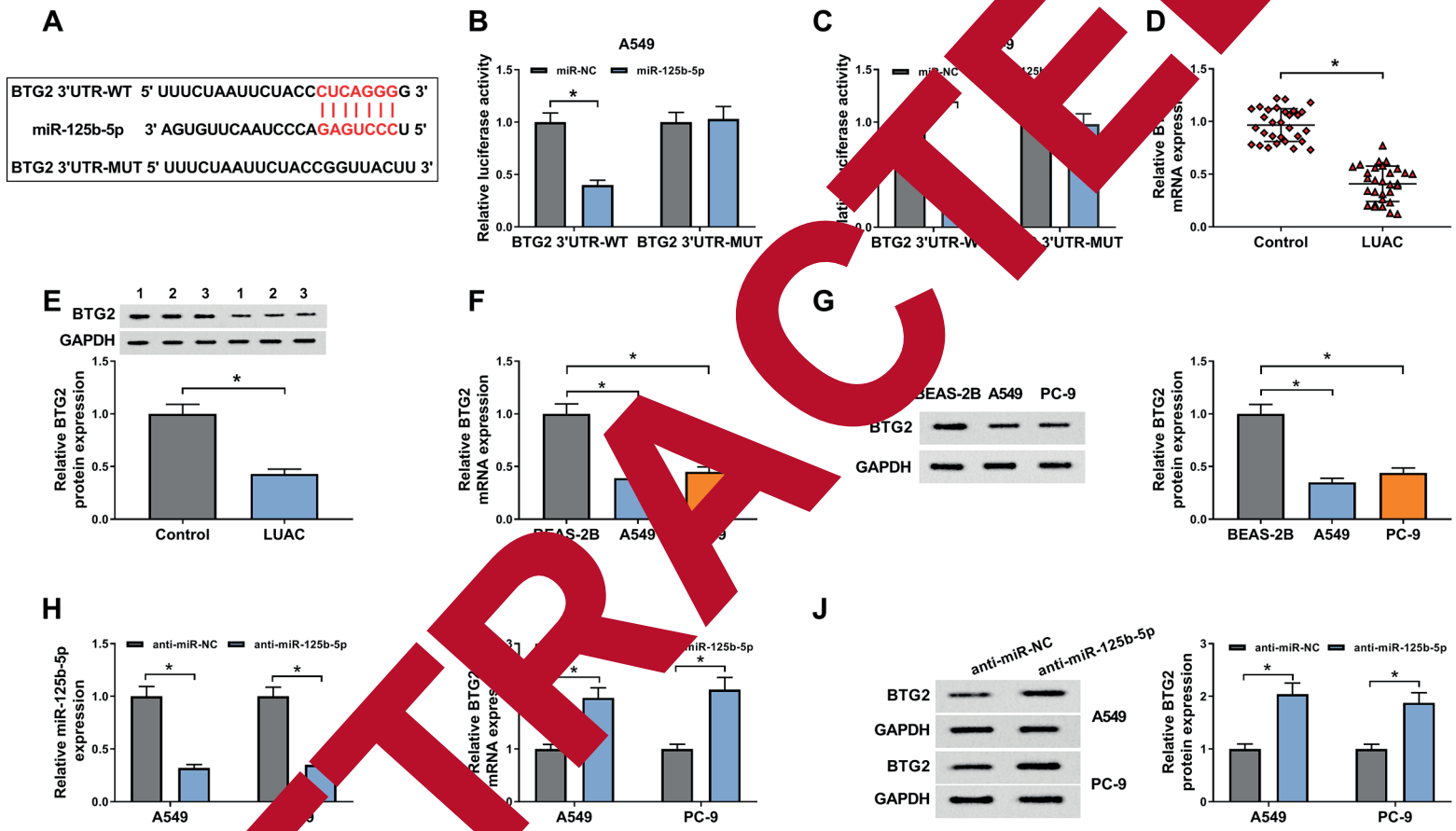

Figure 5. MiR-125b-5p ion as nge for BTG2. **(A)** The interaction between miR-125b-5p and BTG2 was predicted by starBase. **(B** and **C)** The luciferase activity was determine 549 an 9 cells transfected with BTG2 3'UTR-WT or BTG2 3'UTR-MUT and miR-125b-5p or miR-NC. **(D-G)** MiR-125b-5p level was analyzed in LUAC an ssues **(D E)** as well as LUAC and normal cells **(F** and **G)**. **(H-J)** MiR-125b-5p level **(H)** and BTG2 level **(I** and **J)** were investigated in A549 and PC-9 ansfect th anti-miR-NC or anti-miR-125b-5p. *p<0.05.

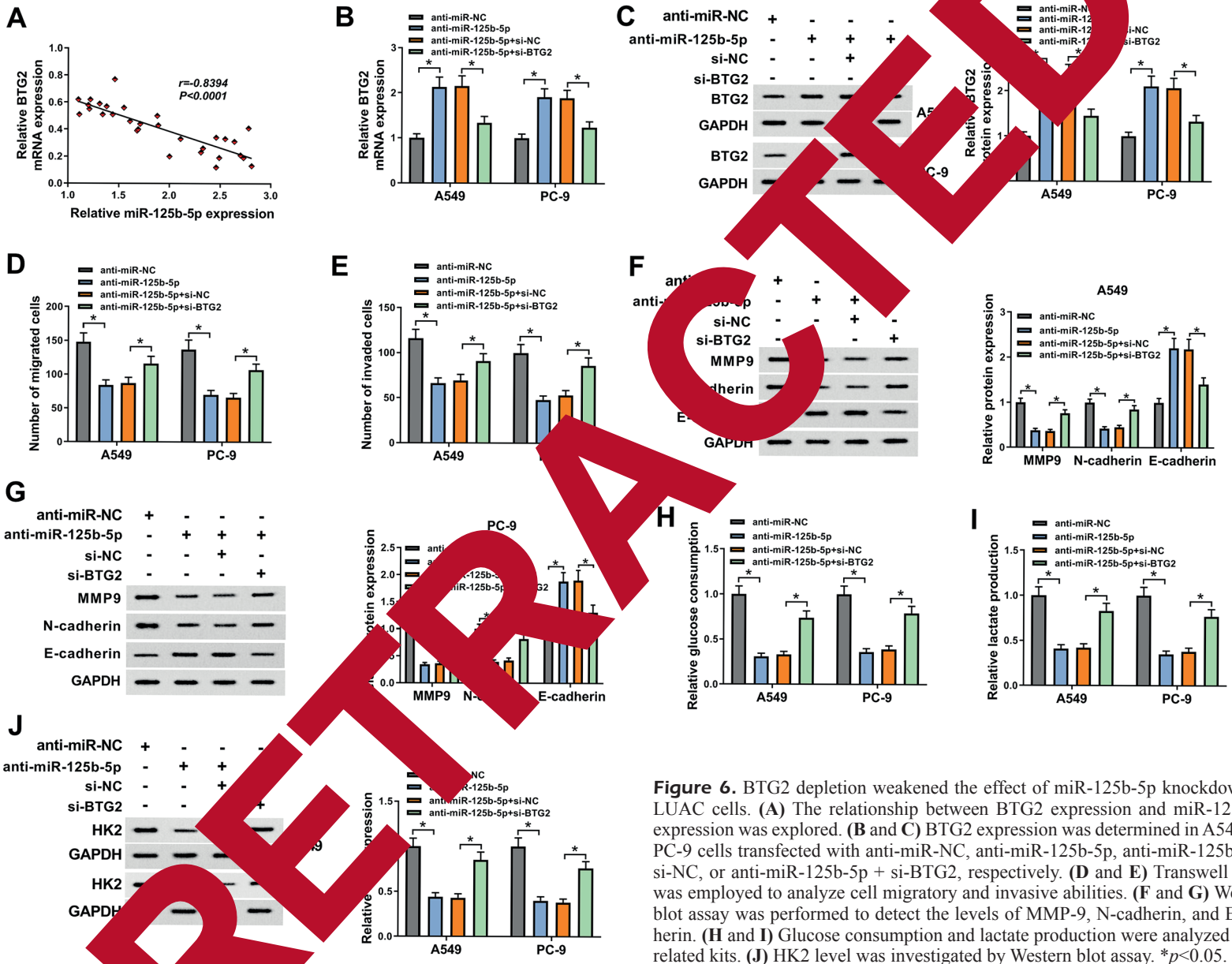

Figure 6. BTG2 depletion weakened the effect of miR-125b-5p knockdown on LUAC cells. **(A)** The relationship between BTG2 expression and miR-125b-5p expression was explored. **(B** and **C)** BTG2 expression was determined in A549 and PC-9 cells transfected with anti-miR-NC, anti-miR-125b-5p, anti-miR-125b-5p + si-NC, or anti-miR-125b-5p + si-BTG2, respectively. **(D** and **E)** Transwell assay was employed to analyze cell migratory and invasive abilities. **(F** and **G)** Western blot assay was performed to detect the levels of MMP-9, N-cadherin, and E-cadherin. **(H** and **I)** Glucose consumption and lactate production were analyzed using related kits. **(J)** HK2 level was investigated by Western blot assay. *p<0.05.

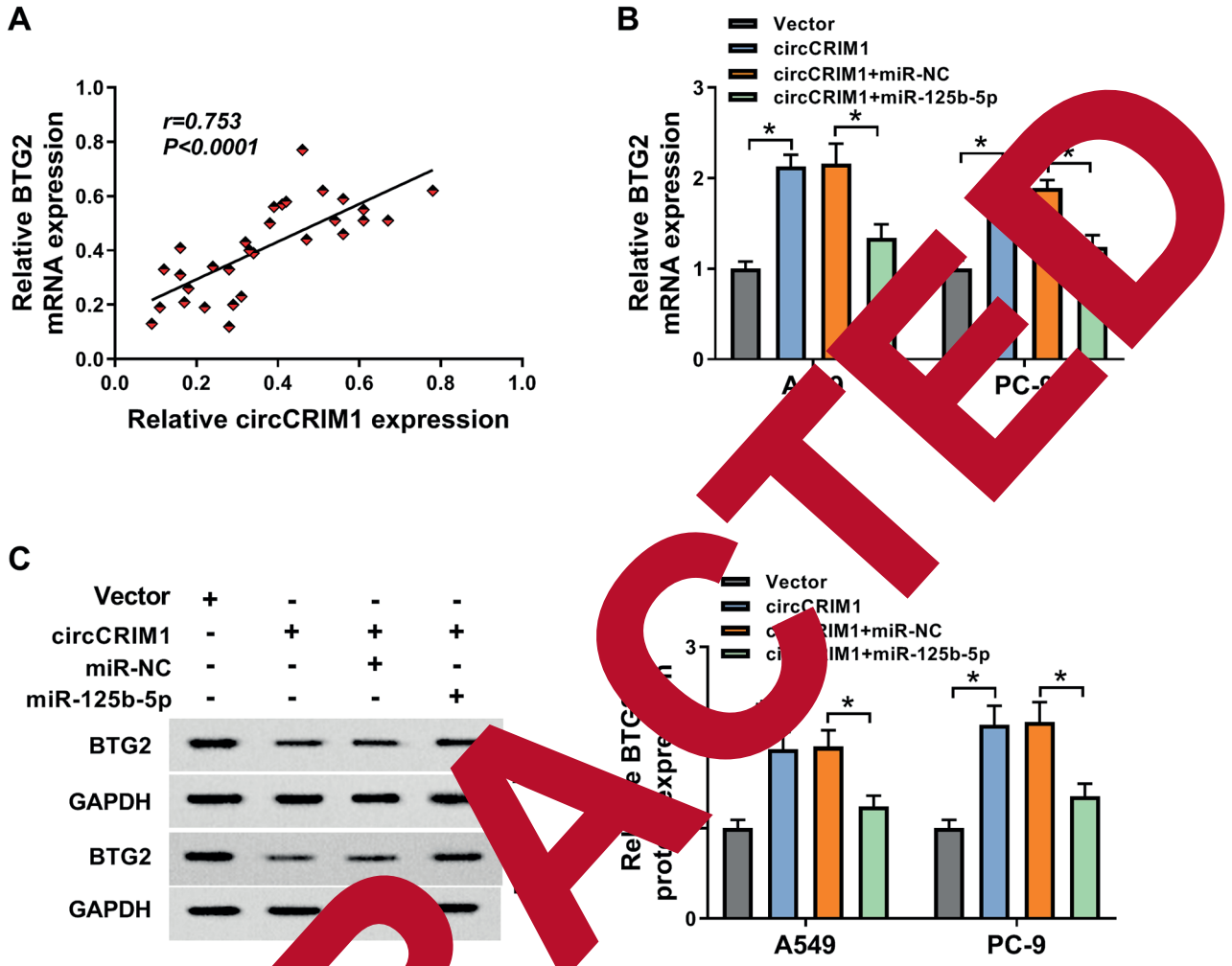

Figure 7. Association of circ miR- TG2 in LUAC cells. **(A)** The relationship between BTG2 expression and circCRIM1 expression s i and G2 expression was determined in A549 and PC-9 cells transfected with Vector, circCRIM1 RIM NC, or circCRIM1 + miR-125b-5p, respectively. *p<0.05.

peting endoge us RN RNA) of miRNAs to impact m As expressi human cancers[26]. Of not rc-MTO1 regula UAC develop-ment binding miR-17[27]. The previous evi-de owed t miR-93 and miR-182 were two target CRIM1 played crucial roles circ regu LUAC cell progression. miR- another new target of circ-CR , was ied. Moreover, miR-125b-5p le ignificantly increased in LUAC tissues ext, we proved that miR-125b-5p erexpression reversed the inhibitory effect of CRIM1 on LUAC cell development. There-fo iR-125b-5p exerted an oncogenic role in LUAC. However, miR-125b-5p suppressed cell growth in most human cancers, such as gallblad-der cancer[28], breast cancer[29], esophageal squa-mous cell carcinoma[30], and osteosarcoma[31]. Taken together, miR-125b-5p exerted different function in various cancers, and negatively modulated cell development in LUAC. Furthermore, miR-125b-5p was a downstream component of circCRIM1 in LUAC.

MiRNA, a small non-coding RNA, modulat-ed many cellular phenotypes through targeting mRNA in various cancers[32]. MiR-125b-5p sup-pressed the growth of keratinocyte via targeting AKT serine/threonine kinase 3 (Akt3)[33]. Further-more, Rasheed et al[34] showed that miR-125b-5p modulated the growth of osteoarthritic chondro-cytes through targeting TNF receptor associated factor 6 (TRAF6). Mechanistically, our results indicated that miR-125b-5p targeted BTG2 and repressed its expression in LUAC cells. Also, we

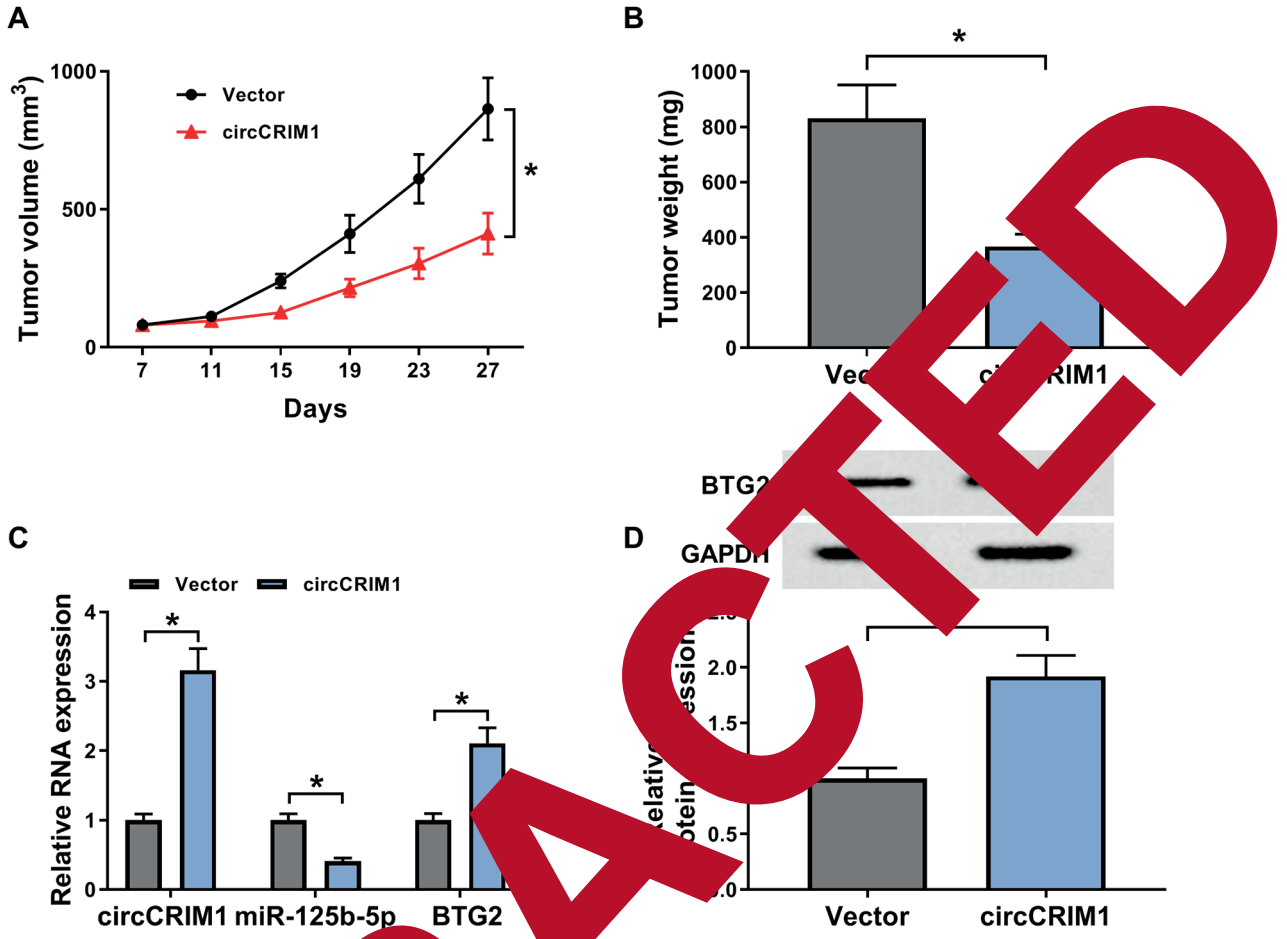

Figure 8. The effect of circCRIM1 [illegible] on tumo[illegible]wth *in vivo*. **(A** and **B)** Tumor volume and weight were calculated in mice overexpressed with [illegible]RIM1 c[illegible]control. **(C** [illegible] **D)** The levels of circCRIM1, miR-125b-5p, and BTG2 were determined. *$p<0.05$.

testified that [illegible]R-125b[illegible]nockdown repressed LUAC ce[illegible]obility and [illegible]lysis through up-regulati[illegible]of BTG2 expre[illegible], proving that miR[illegible]-5p c[illegible] regulate growth and metastas[illegible] LUA[illegible]ells through targeting BTG2. Furth[illegible]TG2 [illegible] gene that exerted an [illegible] pro[illegible]ve ro[illegible]d promoted apoptosis in [illegible] cells[illegible], identified as a suppressor, wa[illegible]wly exp[illegible]d in various cancers, including he[illegible]ular carcinoma[36], gastric cancer[37], and [illegible]. Consistent with these data, our [illegible]sults showed that BTG2 was downregulated in [illegible]C tissues and cells. Taken together, BTG2, a[illegible]ownstream gene of miR-125b-5p, inhibited LUAC cell growth and metastasis. Furthermore, circCRIM1 repressed miR-125b-5p expression to promote BTG2 expression, supporting that circCRIM1 functioned as a ceRNA of miR-125b-5p to affect BTG2 expression.

Conclusions

These results demonstrated that circCRIM1 was downregulated and inhibited migration, invasion, EMT, and glycolysis in LUAC cells. The mechanism analysis revealed that circCRIM1 could act as ceRNA of miR-125b-5p to upregulate BTG2 expression, thereby hindering the development of LUAC. Therefore, our findings provided theoretical basis for the therapy of LUAC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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