Am J Cancer Res 2024;14(7):3468-3482
www.ajcr.us /ISSN:2156-6976/ajcr0155704

Original Article

TRIM27 promotes the Warburg effect and glioblastoma progression via inhibiting the LKB1/AMPK/mTOR axis

Juexian Xiao[1], Haitao Luo[1], Shikai Gui[1], Wanli Yu[1], Lunshan Peng[1], Jun Huang[2], Qing Wu[3], Meizhen Yao[4], Zujue Cheng[1]

[1]Department of Neurosurgery, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330006, Jiangxi, China; [2]Department of Gastrointestinal Surgery, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China; [3]Department of Orthopaedics, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China; [4]Department of Gynaecology and Obstetrics, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China

Received January 30, 2024; Accepted June 24, 2024; Epub July 15, 2024; Published July 30, 2024

Abstract: Altered protein ubiquitination is associated with cancer. The novel tripartite motif (TRIM) family of E3 ubiquitin ligases have been reported to play crucial roles in the development, growth, and metastasis of various tumors. The TRIM family member TRIM27 acts as a potential promoter of tumor development in a wide range of cancers. However, little is known regarding the biological features and clinical relevance of TRIM27 in glioblastoma (GBM). Here, we report findings of elevated TRIM27 expression in GBM tissues and GBM cell lines. Further functional analysis showed that TRIM27 deletion inhibited GBM cell growth both in vitro and in vivo. Furthermore, we found that TRIM27 promoted the growth of GBM cells by enhancing the Warburg effect. Additionally, the inactivation of the LKB1/AMPK/mTOR pathway was critical for the oncogenic effects of TRIM27 in GBM. Mechanistically, TRIM27 could directly bind to LKB1 and promote the ubiquitination and degradation of LKB1, which in turn enhanced the Warburg effect and GBM progression. Collectively, these data suggest that TRIM27 contributes to GNM pathogenesis by inhibiting the LKB1/AMPK/mTOR axis and may be a promising candidate as a potential diagnostic and therapeutic marker for patients with GBM.

Keywords: Glioblastoma, TRIM27, Warburg effect, LKB1, AMPK

Introduction

Glioblastoma (GBM) is one of the deadliest and most aggressive primary brain tumors. Despite advances in surgical and chemoradiotherapy techniques, the prognosis and survival of GBM patients remain poor [1, 2]. Due to GBM's highly aggressive and heterogeneous nature, traditional surgical resection combined with chemotherapy and radiotherapy often fail to completely remove intracranial tumor tissues, ultimately resulting in therapeutic failure and relapses [3]. Therefore, there is an urgent need for combination therapies that are personalized with specific molecular-targeting therapeutics.

Ubiquitination is a crucial post-translational modification that has been the focus of many studies on eukaryotic signaling modulation under physiological and pathological conditions [4, 5]. Ubiquitination occurs via a multistep enzymatic cascade that facilitates the binding of E1, E2, or E3 ligases to substrates [6]. The TRIM family is a novel family of E3 ubiquitin ligases, whose members are involved in the dynamic regulation of myriad cellular processes such as DNA repair and transfer, transcription, and signal transduction [7, 8]. Accumulating evidence has shown that many TRIM proteins play a vital role in tumorigenesis and are dysregulated in diverse types of cancer [9, 10]. Notably, TRIM27 acts as a cancer-causing gene in many cancer types, including gastric [11], ovarian cancer [12], and breast cancer [13]. And elevated TRIM27 expression appears closely correlated with poor outcomes and an indicator of poor prognosis in colorectal cancer [14]. Additionally, Ma et al. found that TRIM27

contributed to the progression of esophageal cancer by modulating the PTEN/AKT signaling pathway [15], while Liu et al. reported that TRIM27 modulated SIX3-β-catenin signaling thereby functioning as an oncogene that promoted cell proliferation and progression of non-small cell lung cancer [16]. However, little is known about the exact action and potential signaling cascades of TRIM27 in GMB.

In the present study, we provide evidence that TRIM27 was upregulated in GBM tissues and that high TRIM27 levels significantly correlated with poor prognosis of GBM patients. Loss- and gain-of-function studies also indicated that TRIM27 could promote the Warburg effect and the progression of glioblastoma by inhibiting the LKB1/AMPK/mTOR axis. Mechanistically, our findings demonstrate that TRIM27 could directly interact with and ubiquitinate LKB1 for its degradation in GBM cells. Thus, our study offers insight into possible activation mechanisms of the LKB1/AMPK/mTOR axis and identifies TRIM27 as a promising candidate target for GBM therapy.

Materials and methods

Clinical specimens

Primary glioblastoma tissue samples (n=30) and non-neoplastic brain tissue samples (n=6) were obtained from the Department of Neurosurgery at the Second Affiliated Hospital of Nanchang University. Written informed consent was obtained from all adult patients, and the research procedure was approved by the Ethics and Research Committees of the Second Affiliated Hospital of Nanchang University. The clinical characteristics of all patients are summarised in Supplementary Table 1.

Cell lines and culture conditions

The human GBM cell lines U118, U251, LN229, T98G, and U87 were acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% FBS (Thermo Fsher Scientific, Waltham, MA, USA) in a 37°C humidified incubator with 5% CO_2. The PKM2 inhibitor Compound 3K was obtained from Selleck Chem and resuspended as a 15 mM working solution in DMSO.

RNA extraction and qRT-PCR assay

Total RNA was isolated using the standard TRIzol-based protocol and reverse-transcribed using the PrimeScript RT kit (Thermo Fisher Scientific) following the manufacturer's guidelines. qRT-PCR analysis was performed using YBR Premix Ex Taq (TaKaRa, Dalian, China) and the ABI PRISM 7900HT Sequence Detection System. GAPDH was used as an internal control. The primers for qRT-PCR used are as follows: TRIM27, forward: 5'-TACTGCGAGGAGGACCAGAT-3'; reverse: 5'-GGTCGAGCTGGTTCTGGATT-3'. LKB1, forward: 5'-ATCTACACTCAGGACTTCAC-3'; reverse: 5'-CTCTGTGCCGTTCATACACAC-3'. GAPDH, forward: 5'-TGACTAACCCTGCGCTCCT-3'; reverse: 5'-GTGACCAGGCGCCCAATAC-3'.

Western blot analysis and immunohistochemistry (IHC) staining

Standard methods were used to analyze the expression of specific proteins using western blotting. The following antibodies were utilized: TRIM27 (1:1000, Abcam), LKB1 (1:1000, CST), AMPKα1 (1:1000, Abcam), mTOR (1:1000, Abcam), p-AMPKα1 (1:1000, CST), p-mTOR (1:1000, CST), and Tubulin (1:1000, Santa Cruz). Enhanced chemiluminescence (Pierce, USA) was used to detect signals. For immunohistochemistry (IHC) staining, patient and mouse tumor tissues were fixed, embedded, sectioned, and deparaffinized. Subsequently, the sections were blocked in serum-free protein blocking buffer (DAKO, CA, USA) for 30 minutes and stained with anti-TRIM27 (1:200, Abcam) or Ki-67 (1:200, CST) antibodies. Immuno-scoring of all the samples was performed in a blinded manner by one pathologist and one scientist. The intensity score of nuclear and/or cytoplasmic staining was rated as follows: 0, negative; 1, weak; 2, moderate; 3, strong. The proportion of stained tumor cells was expressed as percentages (0-100%). The overall immunostaining score for each specimen was calculated by multiplying the percentage score by the intensity score.

Constructs and plasmids

TRIM27 and LKB1 cDNA expression constructs as well as shRNA (short hairpin RNA)-encoding plasmids targeting TRIM27 or LKB1 were produced by GenePharma (Shanghai, China). The

shRNA sequence for the human TRIM27 gene (#1 CCCAGTTCTCTTGCAACAT; #2 CCCAGTTCTCTTGCAACAT). GBM cells were transfected with shRNA plasmids or overexpression constructs according to the manufacturer's protocol using the Lipofectamine 2000 Transfection Reagent (Invitrogen, USA).

Colony formation, CCK8 assay and EdU assay

For the colony formation assay, GBM cells were seeded in 6-well plates at a density of 1 × 10^3 cells/well and cultured for two weeks. Subsequently, the cells were washed thrice with 1 × PBS, fixed with methanol, and stained with 1% crystal violet. Visible cell clusters that were stained purple were counted. For the CCK-8 assay, cell viability was evaluated with the Cell Counting kit 8 (CCK-8; Sigma-Aldrich). In brief, GBM cells were seeded into a 96-well plate at 5,000 cells per well. At the indicated time points, fresh medium containing 10% CCK-8 was added to each well to replace the primary medium. After incubation for 4 h at 37°C, the cells were analyzed on a plate reader to obtain the OD values at 450 nm. Cell proliferation was assessed by EdU and DAPI staining. The Cell-light EdU luminescence detection kit (RiboBio, Guangzhou, China) was used to monitor DNA replication based on the protocols provided by the manufacturer.

Tumorigenicity assay and bioluminescence imaging

The firefly luciferase gene was used for the transduction of GBM cells (for injection), and tumor development was monitored in vivo by bioluminescence imaging. For in vivo tumorigenicity assays, nude mice (male BALB/c-nu/nu, aged 6-8 weeks) were injected subcutaneously with 1 × 10^6 GBM cells (100 ml 1 × PBS) in the abdomen. To image, mice were anesthetized using isoflurane and monitored using a Lumina Series III IVIS (In Vivo Imaging System; PerkinElmer, MA, USA). All experimental protocols using animal models were approved by the Ethics Committee for Animal Experiments of the Second Affiliated Hospital of Nanchang University.

Glycolysis stress test

The glycolysis stress assay was performed as previously described [9]. Briefly, ECAR was detected using a Seahorse XF24 extracellular flux analyzer (Agilent Seahorse Bioscience). GBM cells (5 × 10^4 cells) transfected with the appropriate constructs were seeded into each well. After incubation in the absence of CO_2 for 1 h, glycolysis was determined using an XF Cell Glycolysis Test Kit (Agilent Technologies). Following baseline recordings, injections of oligomycin (1 μM), glucose (10 mM), or 2-DG (50 mM) were administered sequentially.

Co-IP and in vivo ubiquitination assay

For Co-IP, cell lysates were incubated with appropriate primary antibodies at 4°C overnight and then with protein A/G-Sepharose beads as previously described [17]. Subsequently, co-precipitated proteins were collected and analyzed by immunoblotting with appropriate antibodies. For in vivo ubiquitination experiments, the previously described plasmids were co-transfected into cells. The cells were then treated with the proteasome inhibitor MG132 (50 μg/mL) for 12 hours before being harvested. Subsequently, the cell lysates were used for immunoprecipitation with anti-LKB1 antibodies and an anti-Ub antibody was used to determine LKB1 ubiquitination levels.

Statistical analysis

All numbers were displayed using GraphPad Prism 6 (GraphPad Software, USA) and reported as averages ± standard error of the average. Significant differences were analyzed using two-tailed distribution and Student's t-test. Survival curves were obtained using the Kaplan-Meier technique, and significance was evaluated using the log-rank test. Differences were considered significant at $P<0.05$.

Results

TRIM27 is overexpressed in GBM tissues

To explore whether TRIM27 plays a role in GBM progression, we first examined the expression of TRIM family proteins in the TCGA-GBM, GSE108474 and GSE108474 datasets (**Figure 1A** and Supplementary Table 2). We generated Venn diagrams of differentially expressed TRIM genes in the three datasets and calculated the number of overlapping genes, which included TRIM27 (**Figure 1B**). Our

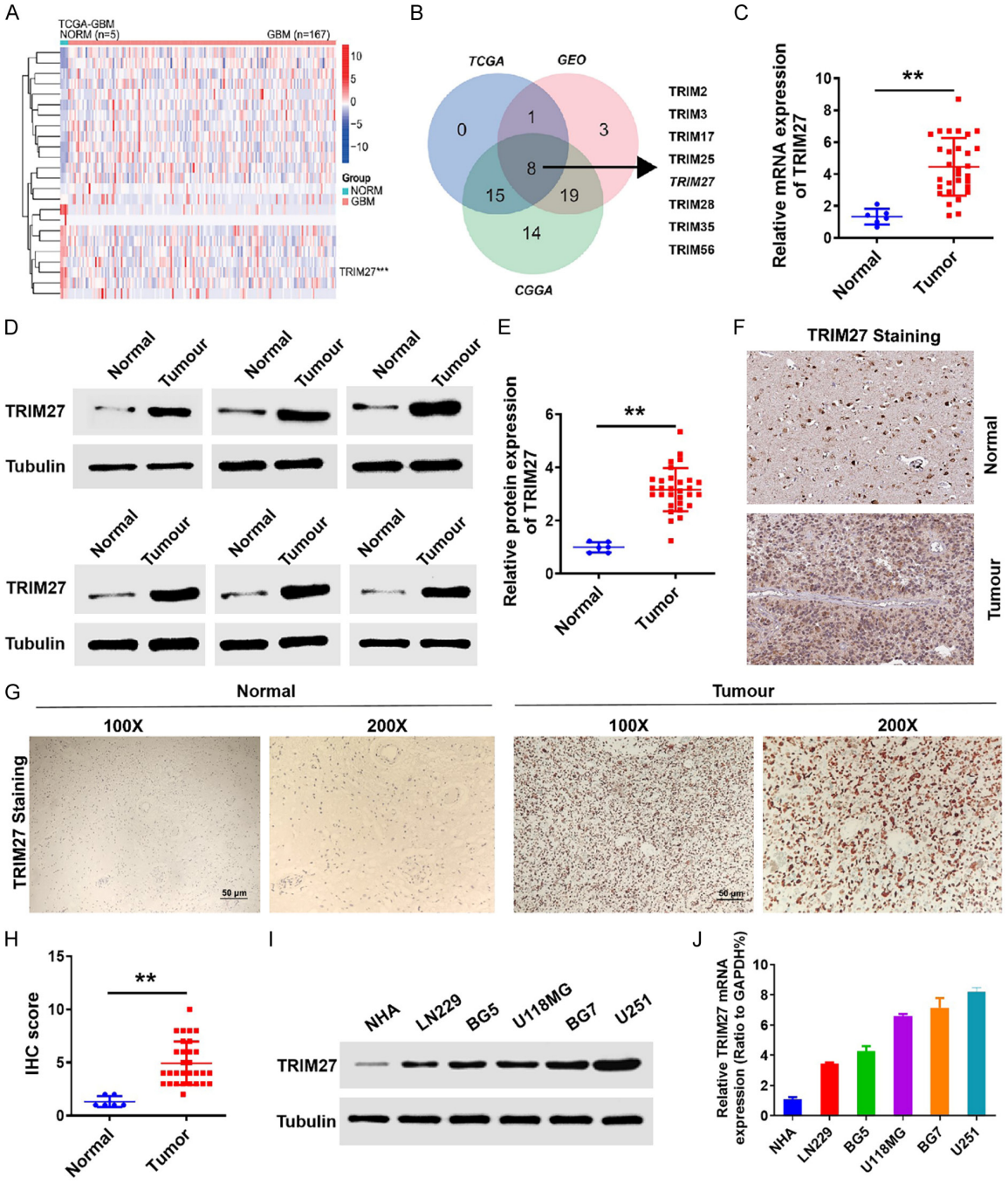


Figure 1. TRIM27 is elevated in GBM tissues. (A) A heat map of differentially expressed TRIM family genes between GBM (n=169) and normal brain tissue (n=5) from the TCGA-GBM dataset. (B) Wayne plots showing eight genes that showed significantly different expression in the TCGA-GBM, GSE108474, and GSE108474 datasets. (C) TRIM27 mRNA expression in 30 GBM tissues and 6 NBT samples was determined by qRT-PCR analysis. $^{**}P<0.01$. (D and E) Analysis (D) and quantification (E) of TRIM27 protein levels in GBM tissues and normal brain tissues by western blotting assay. Tubulin was used as a loading control. $^{**}P<0.01$. (F) Representative immunostaining of TRIM27 protein expression in human glioma and normal brain tissue (NBT) samples (images from the Human Protein Atlas). (G and H) Representative immunostaining (G) and quantification (H) of TRIM27 in human gliomas of different grade and normal brain tissue (NBT) samples (magnification: 100 × and 200 ×). Scale bar, 50 μm. (I and J) TRIM27 protein and mRNA levels were detected in normal human astrocytes (NHAs) and glioma cell lines U251, BG7, U118MG, BG5, and LN229.

results showed that mRNA levels of TRIM27 were markedly elevated in a set of GBM samples (n=30) compared to samples from non-neoplastic brain tissues (NBT, **Figure 1C**; n=6). And the protein level of TRIM27 was low in NBT but increased in GBM tissues (**Figure 1D** and **1E**). Moreover, TRIM27 was overexpressed in GBM tissues compared to that in normal brain tissues (The Human Protein Atlas) (**Figure 1F**). Immunohistochemistry (IHC) results revealed that TRIM27 was also over-expressed in tissue specimens with the highest GBM grade (**Figure 1G** and **1H**). Notably, TRIM27 was also expressed at elevated levels in four human GBM cell lines compared to normal human astrocytes (NHA; **Figure 1I** and **1J**). These findings combined support elevated TRIM27 expression in GBM tissues and suggest a potential tumor-promoting role for TRIM27 in GBM progression.

TRIM27 promotes the growth of GBM cells in vitro and in vivo

To determine the role of TRIM27 in GBM development, we generated U251 and U118G GBM cell lines in which TRIM27 was silenced using TRIM27-targeting shRNA vectors (**Figure 2A-C**). In both EdU and CCK8 assays, TRIM27 knockdown greatly suppressed the proliferation of GBM cells (**Figure 2D-G**). Furthermore, cell proliferation ability was also reduced in TRIM27-silenced GBM cells compared to controls, as evidenced in the colony formation assay (**Figure 2H-K**). Next, we investigated the effect of modulating TRIM27 expression on GBM cell proliferation in vivo using a mouse xenograft model. At 4 weeks after grafting, tumor size and weight were both markedly reduced in TRIM27-silenced groups compared to control groups (**Figure 2L-N**). Ki67 staining indicated that TRIM27 knockdown inhibited the proliferation of GBM cells (**Figure 2O**). Taken together, these data suggest that TRIM27 may play an oncogenic role in GBM development.

TRIM27 enhances aerobic glycolysis of GBM cells

To determine the mechanism by which TRIM27 affects GBM cell progression, we carried out gene set enrichment analysis (GSEA) and found that glycolysis-related genes were dramatically enriched in GBM specimens with high TRIM27 expression (Supplementary Figure 1), suggesting that aerobic glycolytic pathways may be strongly associated with TRIM27 expression in GBM. These observations prompted us to further probe the link between TRIM27 and the glycolytic profile in GBM cells. TRIM27 knockdown appeared to markedly inhibit aerobic glycolysis in GBM cells, as evidenced by decreased glucose-6-phosphate (G6P) production, glucose consumption, and the production of lactate and ATP (**Figure 3A-D**). TRIM27 silencing inhibited ECAR, indicating a global glycolytic flux in GBM cells (**Figure 3E-H**). Furthermore, we found that OCR values, which measure mitochondrial respiration, were also increased in TRIM27-silenced GBM cells (**Figure 3I-L**). Next, to confirm whether TRIM27 function was dependent on aerobic glycolysis in GBM, we treated GBM cells with the glycolysis inhibitor 2-deoxy-D-glucose (2-DG). We found that TRIM27 overexpression facilitated G=BM cell viability and that this oncogenic effect was abolished with 2-DG treatment (**Figure 3M-P**). These results suggest that TRIM27 exerts its oncogenic effects through enhancing aerobic glycolysis in GBM cells.

LKB1/AMPK/mTOR pathway is critical for TRIM27-dependent GBM tumor biology

LKB1 is an upstream kinase of AMPK. Activated AMPK in turn regulates glycolysis through additional phosphorylation events in downstream pathways [18, 19]. We therefore investigated whether TRIM27 controls glycolysis and tumor progression via the LKB1/AMPK signaling pathway. We observed dramatically elevated protein levels of LKB1 and p-AMPK and decreased levels of p-mTOR in TRIM27 knockdown GBM cells (**Figure 4A**). In contrast, overexpression of TRIM27 led to the opposite effects in GBM cells (**Figure 4B**). Furthermore, our results showed that combination knockdown of both TRIM27 and LKB1 markedly increased glycolysis in and proliferation of GBM cells (**Figure 4C-J**). As shown in **Figure 4K** and **4L**, mice bearing TRIM27-silenced cells showed a notable decrease in tumour weight and volume, whereas simultaneous knockdown of both TRIM27 and LKB1 partially abolished the anti-tumour effect of TRIM27 inhibition. In addition, we treated TRIM27 overexpressing GBM cells with the AMPK activator metformin (Met) and found that Met suppressed the promoting effect of TRIM27 over-

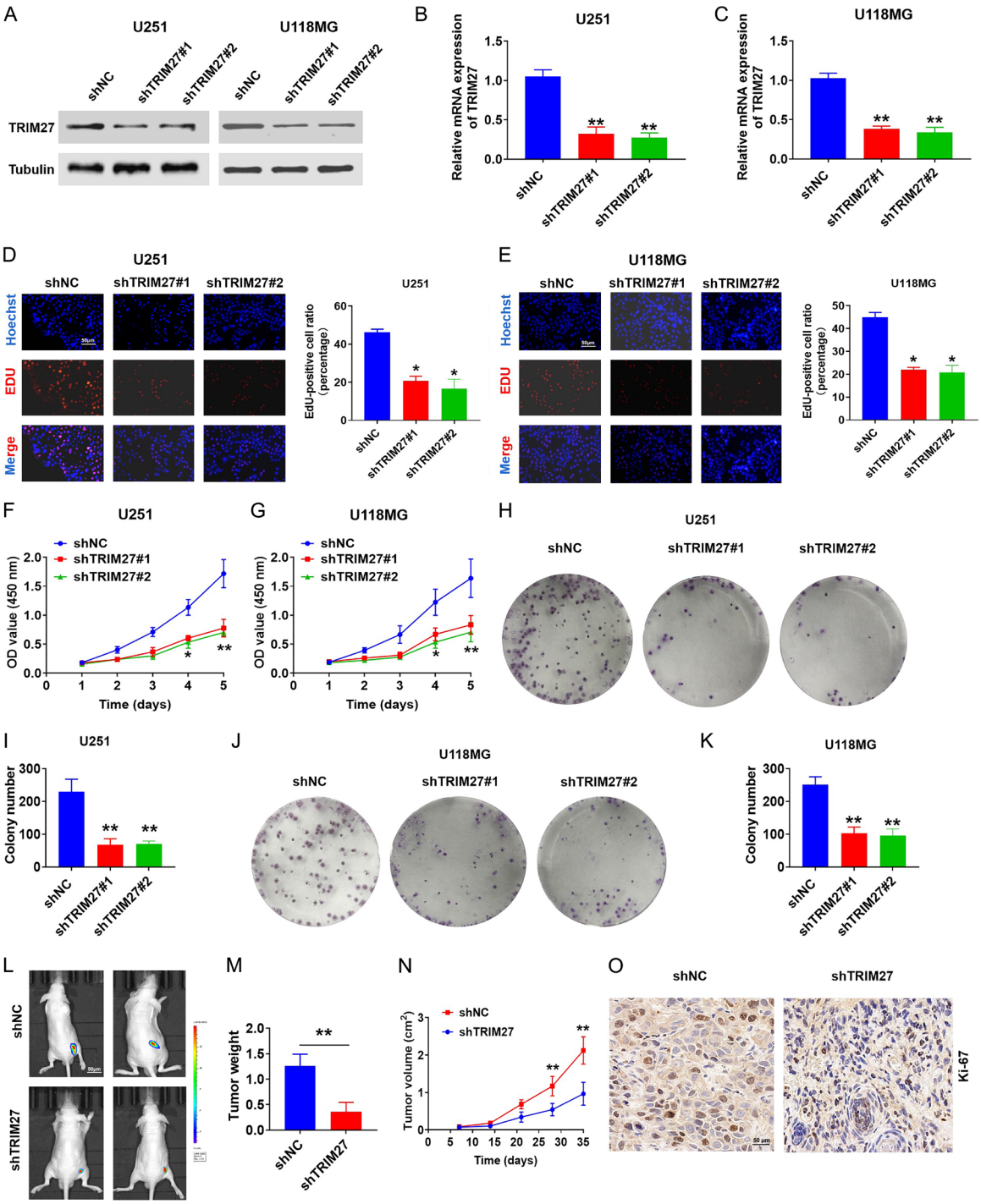
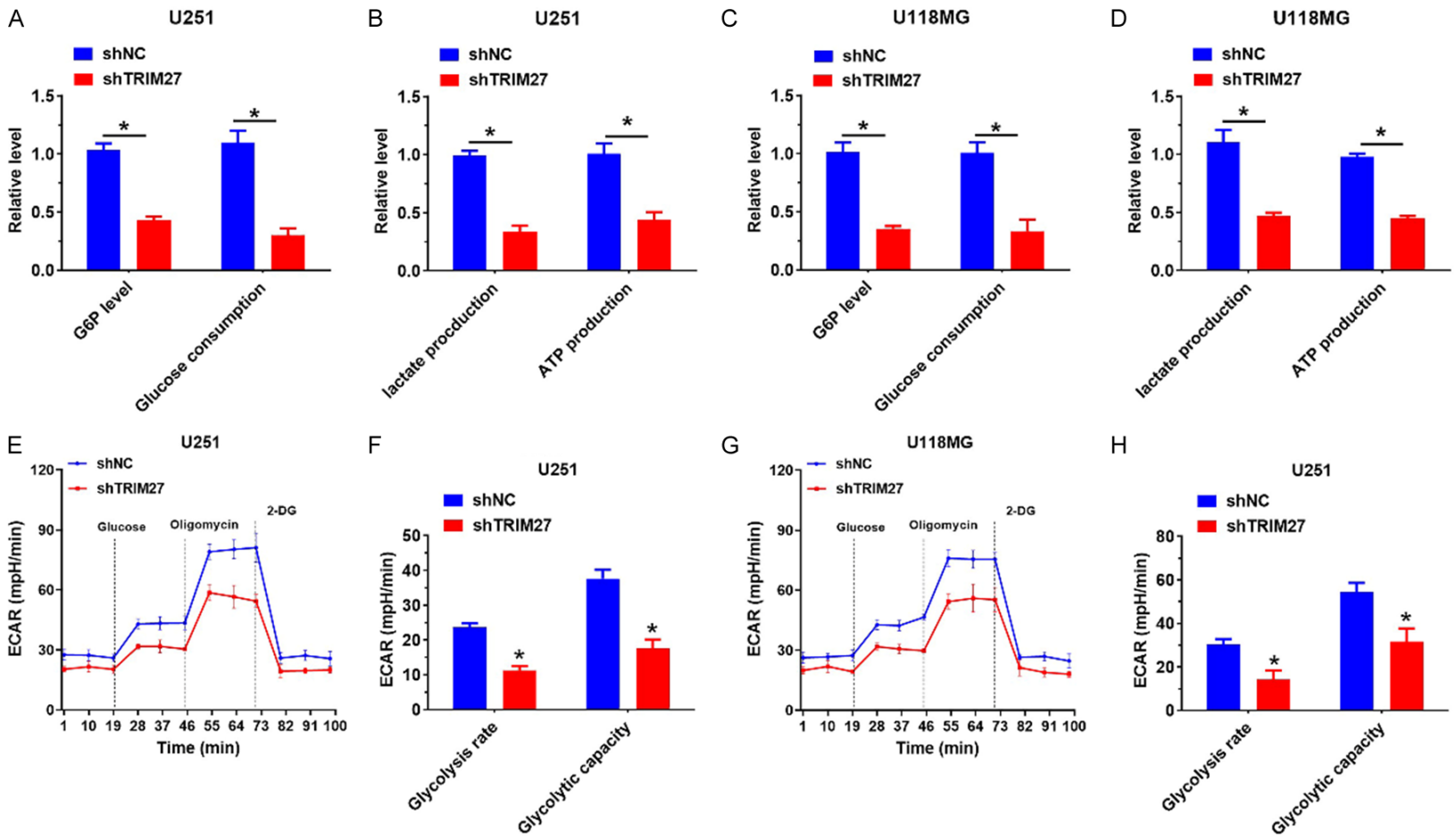


Figure 2. Effects of TRIM27 expression on GBM cell proliferation. (A) Protein levels of TRIM27 were assessed in U251 and U118MG cells transfected with shNC or shTRIM27 plasmids by western blotting assay. (B and C) The mRNA levels of TRIM27 were detected U251 and U118MG cells transfected with shNC or shTRIM27. $^{**}P$<0.01. (D-G) EdU (D and E) and CCK-8 assay (F and G) showing proliferation of GBM cells following knockdown of TRIM27. Scale bar, 50 μm. $^{*}P$<0.05, $^{**}P$<0.01. (H-K) Representative images (H and J) and quantification (I and K) of colony formation assays using GBM cells transfected with shTRIM27 plasmids. $^{*}P$<0.05, $^{**}P$<0.01. (L-N) U251/shTRIM27 cells were subcutaneously injected into nude mice. Tumor volumes were measured on the indicated days. At the experimental endpoint, tumors were dissected, photographed, and weighed. Scale bar, 50 μm. n=6, $^{*}P$<0.05, $^{**}P$<0.01. (O) Ki67 staining using tumor tissues isolated from nude mice injected with TRIM27-silenced cells. Scale bar, 50 μm.


A
U251
shNC
shTRIM27
Relative level
G6P level
Glucose consumption
B
U251
lactate procduction
ATP production
C
U118MG
D
U118MG
E
U251
ECAR (mpH/min)
Glucose
Oligomycin
2-DG
Time (min)
F
U251
Glycolysis rate
Glycolytic capacity
G
U118MG
H
U251

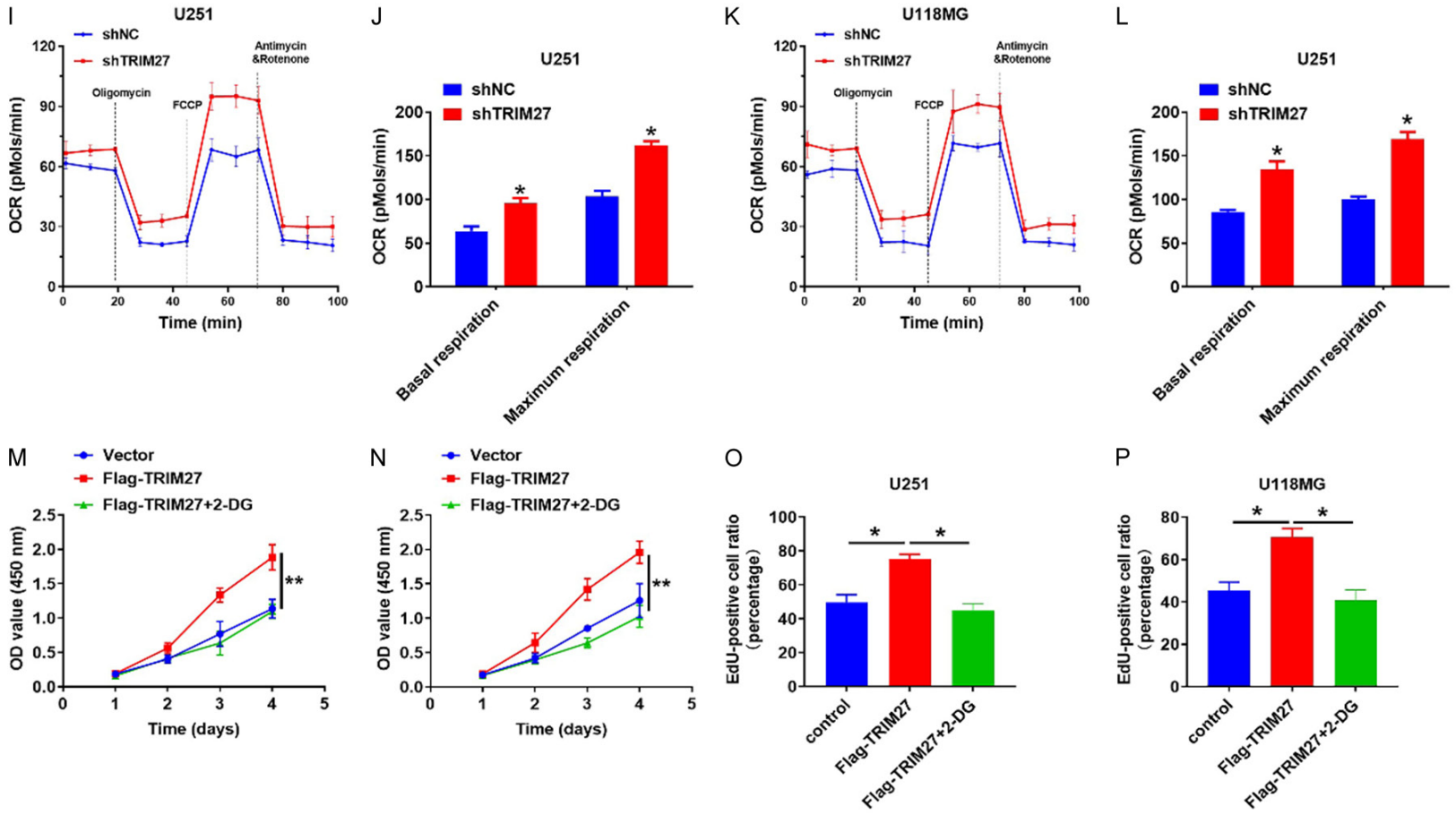
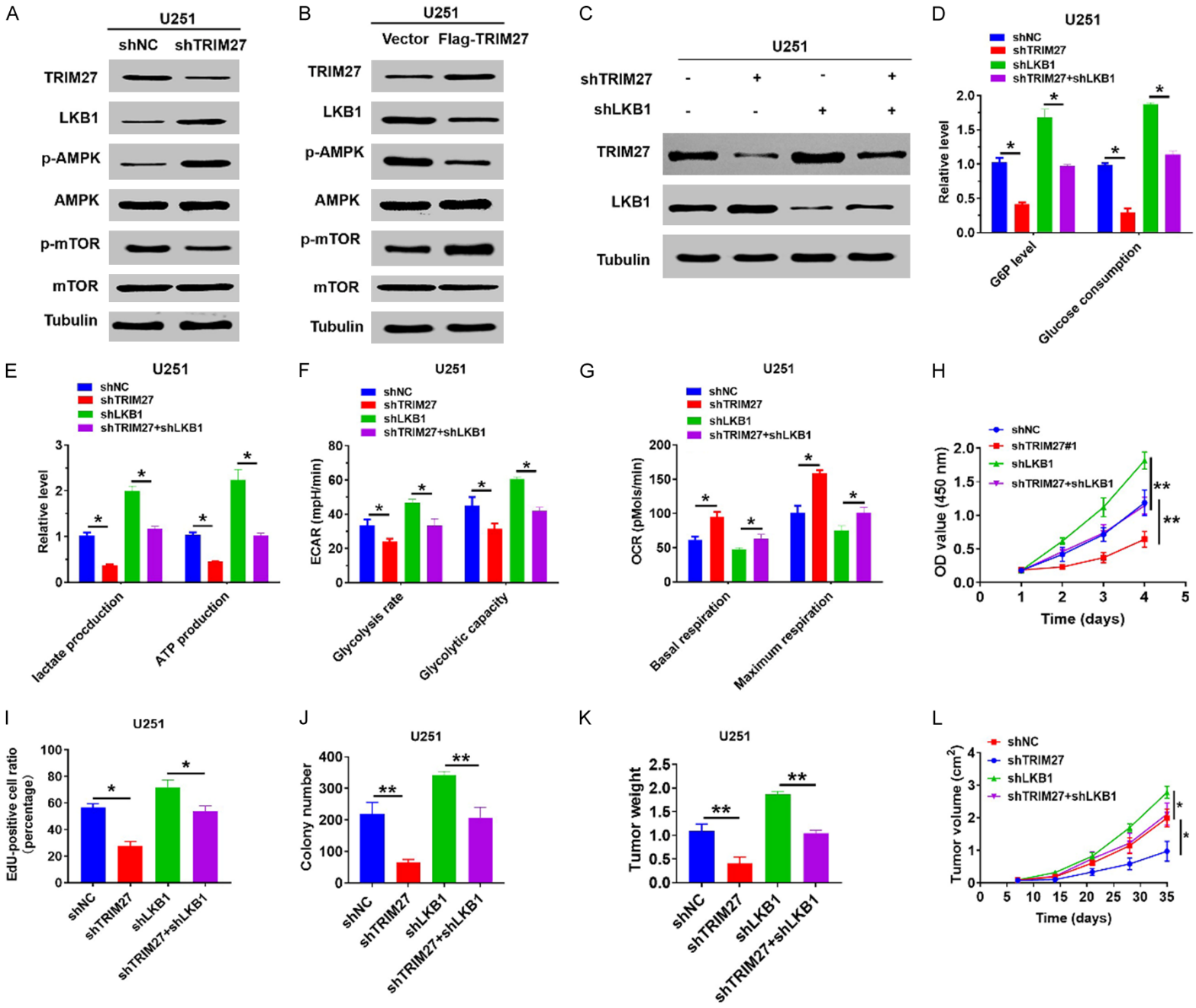


Figure 3. TRIM27 promotes the growth of GBM cells by enhancing glycolysis. A-D. Glucose-6-phosphate (G6P) production, glucose consumption, lactate production, and ATP levels in TRIM27-silenced GBM cells. $^{*}P<0.05$. E-H. ECAR data showing the glycolytic rate and capacity in TRIM27-silenced GBM cells. Glucose (10 mM), the oxidative phosphorylation inhibitor oligomycin (1.0 μM), and the glycolytic inhibitor 2-deoxyglucose (2-DG, 50 mM) were sequentially added to each well at the indicated time points. $^{*}P<0.05$. I-L. OCR results showing basal and maximum respiration in TRIM27-silenced GBM cells. Oligomycin (1.0 μM), the mitochondrial uncoupler carbonyl cyanide p-trifluoromethoxy phenylhydrazone (FCCP, 1.0 μM), and the mitochondrial complex I inhibitor rotenone plus the mitochondrial complex III inhibitor antimycin A (Rote/AA, 0.5 μM) were sequentially added. $^{*}P<0.05$. M-P. Effects of 2-DG on the viability of TRIM27-silenced GBM cells. $^{*}P<0.05$, $^{**}P<0.01$.


A
U251
shNC
shTRIM27
TRIM27
LKB1
p-AMPK
AMPK
p-mTOR
mTOR
Tubulin
B
U251
Vector
Flag-TRIM27
TRIM27
LKB1
p-AMPK
AMPK
p-mTOR
mTOR
Tubulin
C
U251
shTRIM27
shLKB1
TRIM27
LKB1
Tubulin
D
U251
shNC
shTRIM27
shLKB1
shTRIM27+shLKB1
Relative level
G6P level
Glucose consumption
E
U251
shNC
shTRIM27
shLKB1
shTRIM27+shLKB1
Relative level
lactate procduction
ATP production
F
U251
shNC
shTRIM27
shLKB1
shTRIM27+shLKB1
ECAR (mpH/min)
Glycolysis rate
Glycolytic capacity
G
U251
shNC
shTRIM27
shLKB1
shTRIM27+shLKB1
OCR (pMols/min)
Basal respiration
Maximum respiration
H
shNC
shTRIM27#1
shLKB1
shTRIM27+shLKB1
OD value (450 nm)
Time (days)
I
U251
EdU-positive cell ratio (percentage)
shNC
shTRIM27
shLKB1
shTRIM27+shLKB1
J
U251
Colony number
shNC
shTRIM27
shLKB1
shTRIM27+shLKB1
K
U251
Tumor weight
shNC
shTRIM27
shLKB1
shTRIM27+shLKB1
L
shNC
shTRIM27
shLKB1
shTRIM27+shLKB1
Tumor volume (cm3)
Time (days)

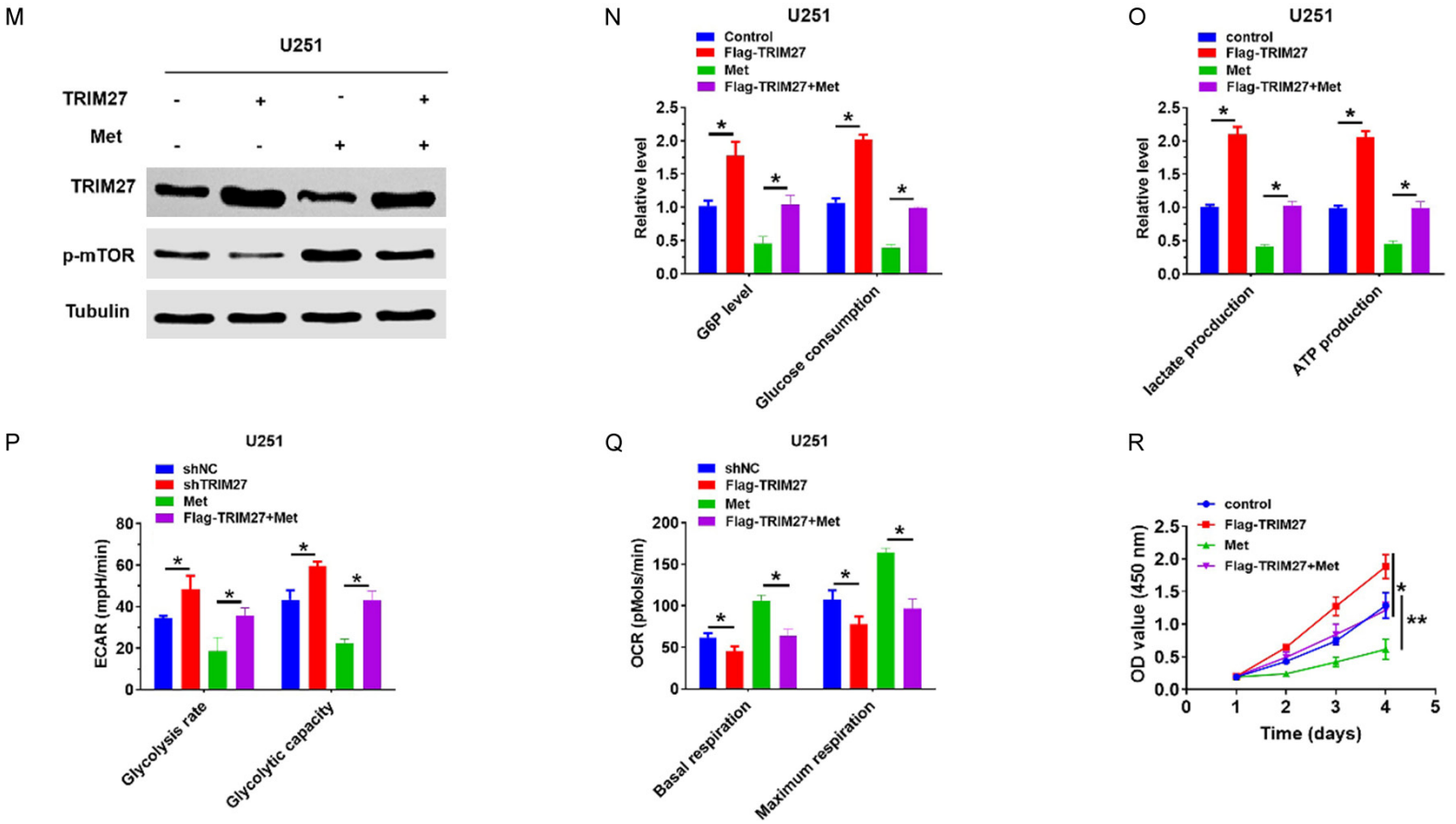


Figure 4. TRIM27 promotes glycolysis by regulating LKB1/AMPK/mTOR pathway. (A and B) Protein levels of LKB1, total AMPK, p-AMPK, total mTOR, and p-mTOR were assessed by western blotting in TRIM27-silenced U251 cells (A) and TRIM27-overexpression U251 cells (B). (C) Western blotting showing the protein expression of TRIM27 and LKB1 in each group. (D and E) Glucose uptake, G6P production, and lactate and ATP levels were measured following knockdown of LKB1 in TRIM27-silenced U251 cells. (F and G) ECAR and OCR were measured after LKB1 knockdown in TRIM27-silenced U251 cells. (H-J) Quantification of CCK8 (H), EdU (I), and colony formation (J) assays using TRIM27-silenced U251 cells transfected with a shLKB1 construct. $^{*}P<0.05$, $^{**}P<0.01$. (K and L) The quantification of tumor weight (K) and tumor volume (L) in different groups. $^{*}P<0.05$, $^{**}P<0.01$. (M) Western blotting showing the protein expression of TRIM27 and p-mTOR in TRIM27-overexpression U251 cells treated with Met. (N and O) Glucose uptake, the production of G6P, and lactate and ATP levels were measured in TRIM27-overexpression U251 cells treated with Met. (P and Q) ECAR and OCR were measured in TRIM27-overexpression U251 cells treated with Met. (R) Quantification for CCK8 assays in TRIM27-overexpression U251 cells treated with Met. $^{*}P<0.05$, $^{**}P<0.01$.

expression on p-mTOR expression (**Figure 4M**). The promotion of cell proliferation and cellular glycolysis by TRIM27 overexpression was also offset by Met activation of AMPK (**Figure 4N-R**). These findings indicate that TRIM27 functions through the LKB1/AMPK/mTOR signaling pathway in regulating GBM cells.

TRIM27 mediates ubiquitination and degradation of LKB1

Ubiquitination is a crucial step in the regulation of LKB1 expression [20]. As an E3 ubiquitin ligase, TRIM27 mediates substrate ubiquitination. Notably, we found that altered TRIM27 expression had no impact on LKB1 mRNA levels in GBM cells (Supplementary Figure 2). We speculated that TRIM27 might mediate the reduction in LKB1 expression by promoting its ubiquitination and subsequent degradation. To this end, we first determined whether TRIM27 could directly bind to LKB1 in GBM cells. As indicated in **Figure 5A** and **5B**, co-IP analysis supported interactions between TRIM27 and LKB1 in GBM cells. Docking analysis revealed potential binding interactions between TRIM27 and LKB1 (**Figure 5C**). Furthermore, treating TRIM27 knockdown GBM cells with cycloheximide CHX prolonged the half-life of LKB1 (**Figure 5D-G**). And LKB1 protein expression was restored in TRIM27-expressing GBM cells treated with the proteasome inhibitor MG132 (**Figure 5H** and **5I**). Additionally, knocking down TRIM27 greatly inhibited LKB1 ubiquitination, whereas overexpression of TRIM27 increased LKB1 ubiquitination in GBM cells (**Figure 5J** and **5K**). Finally, the K48R mutation in Ub, but not the K63R mutation, abolished TRIM27-mediated LKB1 ubiquitination (**Figure 5L**). Collectively, these data indicate that TRIM27 was responsible for the ubiquitination of LKB1, which led to its subsequent proteasome-dependent degradation in GBM cells.

Discussion

Modulating TRIM protein levels can have both positive and negative effects on carcinogenesis, and varied expression of select TRIM family proteins can affect the growth and prognosis of cancer. TRIM proteins have been identified as possible targets for treating solid tumors [21, 22]. Our findings suggest that TRIM27 is upregulated in human gliomas and that elevated levels of TRIM27 are significantly associated with poor prognosis and more malignant phenotypes in individuals with GBM. We also demonstrated that TRIM27 could promote tumor proliferation and progression in vitro and in vivo, which is likely mediated through the inhibition of the LKB1/AMPK pathway.

The Warburg effect is a key future in tumor development. Clinical anticancer treatment studies targeting the Warburg effect should consider all relevant molecular targets [23, 24]. The metabolic sensor AMPK plays a critical role in the modulation of cellular metabolism and growth in response to energy stress and is a well-known cancer marker [25]. Previous studies have reported that the AMPK/mTOR pathway is a critical signaling cascade in modulating the Warburg effect in many cancer cells [18, 26]. We found that TRIM27 knockdown significantly inhibited aerobic glycolysis and inactivated the AMPK/mTOR pathway. Conversely, TRIM27 overexpression significantly enhanced the AMPK/mTOR pathway and the glycolytic activity of GBM cells. Importantly, inhibiting the AMPK/mTOR pathway rescued the reduced glycolysis and malignant phenotypes in TRIM27-silenced GBM cells, suggesting that TRIM27 exerts its oncogenic function through regulating the AMPK/mTOR signaling pathway in GBM cells.

LKB1 is a well-studied tumor suppressor and primary upstream kinase of AMPK [27]. Loss-of-function mutations in LKB1 are frequently detected and contribute significantly to the development and metastasis of many tumors [28], including GBM [29]. Therefore, targeting LKB1 activity is an appealing strategy for facilitating GBM oncogenesis. Several proteins have been reported as targets for TRIM27-mediated proteasome-dependent degradation in various cancers [13, 30]. Consistent with these observations, our findings revealed that TRIM27 functioned as an E3 ubiquitin ligase for LKB1 in GBM cells. In co-IP assays examining endogenous and exogenous proteins, we provide evidence supporting direct interaction between TRIM27 and LKB1. Additionally, TRIM27 could reduce the half-life of LKB1 and promote the proteasome-mediated degradation of LKB1 in GBM cells. Finally, TRIM27 promotes the polyubiquitination of the LKB1-K48

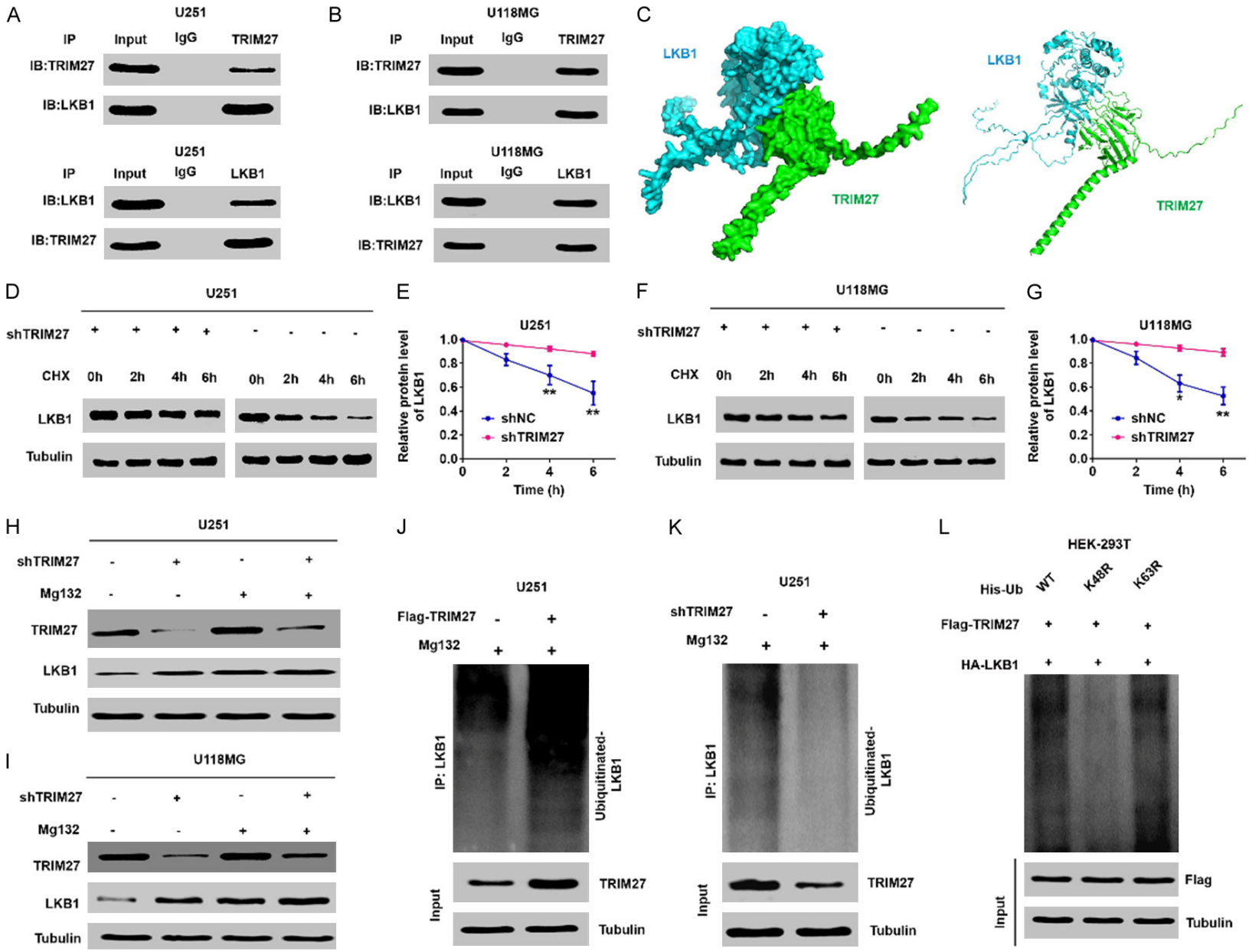

A
U251
IP
Input
IgG
TRIM27
IB:TRIM27
IB:LKB1
U251
IP
Input
IgG
LKB1
IB:LKB1
IB:TRIM27
B
U118MG
IP
Input
IgG
TRIM27
IB:TRIM27
IB:LKB1
U118MG
IP
Input
IgG
LKB1
IB:LKB1
IB:TRIM27
C
LKB1
TRIM27
LKB1
TRIM27
D
U251
shTRIM27
CHX
0h
2h
4h
6h
LKB1
Tubulin
E
U251
Relative protein level of LKB1
shNC
shTRIM27
Time (h)
F
U118MG
shTRIM27
CHX
0h
2h
4h
6h
LKB1
Tubulin
G
U118MG
Relative protein level of LKB1
shNC
shTRIM27
Time (h)
H
U251
shTRIM27
Mg132
TRIM27
LKB1
Tubulin
I
U118MG
shTRIM27
Mg132
TRIM27
LKB1
Tubulin
J
U251
Flag-TRIM27
Mg132
IP: LKB1
Ubiquitinated-LKB1
Input
TRIM27
Tubulin
K
U251
shTRIM27
Mg132
IP: LKB1
Ubiquitinated-LKB1
Input
TRIM27
Tubulin
L
HEK-293T
His-Ub
WT
K48R
K63R
Flag-TRIM27
HA-LKB1
Input
Flag
Tubulin

Figure 5. TRIM27 interacting with LKB1 and destabilising its expression. (A and B) The interaction between TRIM27 and LKB1 was confirmed by co-IP assays in U251 and U118G cells. (C) Docking analysis for the binding of TRIM27 and LKB1. (D-G) Representative (D and F) and quantitative (E and G) results of LKB1 protein levels in TRIM27-silenced GBM cells. The cells were treated with cycloheximide (CHX, 100 mg/ml) at the indicated time points and then harvested for western blot analysis. $^{**}P<0.01$. (H and I) GBM cells transfected with shTRIM27 (H) or TRIM27-overexpression plasmids (I) were treated with 10 μM MG132. The cells were then collected after 6 hours and immunoblotted with the indicated antibodies. (J and K) Lysates from U251 cells transfected with TRIM27-overexpression plasmid (J) or shTRIM27 (K) were immunoprecipitated with the anti-Ub and immunoblotted with the anti-LKB1. (L) Determination of LKB1 ubiquitination type in HEK293 cells.

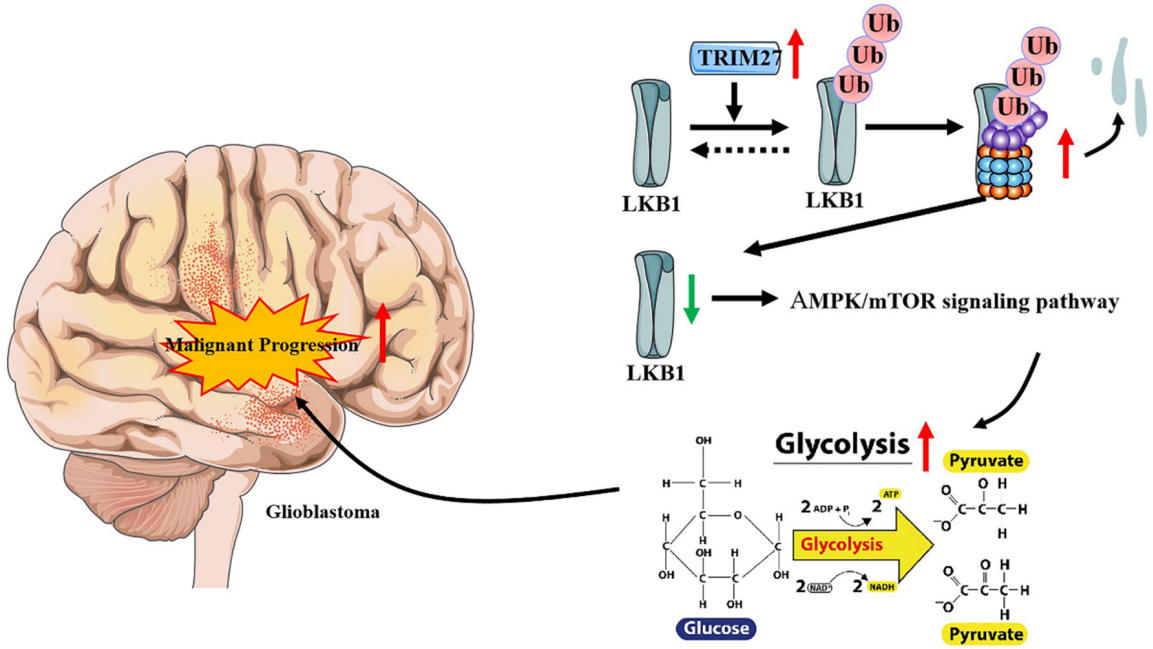


Figure 6. The proposed mechanistic scheme of how TRIM27 promotes GBM progression by enhancing glycolysis via modulating the LKB1/AMPK/mTOR pathway.

linkage. More detailed studies are needed to identify the LKB1 ubiquitination site for TRIM27 polyubiquitination.

Collectively, our results indicate that TRIM27 is an E3 ubiquitin ligase of LKB1 in GBM cells. Our data suggest that TRIM27 interacts with LKB1 and increases its polyubiquitination of LKB1, which in turn destabilizes LKB1 and attenuates the AMPK/mTOR pathway, thereby enhancing glycolysis to promote GBM progression (**Figure 6**). Thus, our findings suggest that pharmaceutical inhibition of TRIM27 or its interaction with the LKB1/AMPK/mTOR pathway may prove a promising strategy in treating human gliomas.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (82060445, 82160587 and 82360475); The Chinese Academy of Medical Sciences (ZZ15-WT-04); Jiangxi Provincial Health Commission science and Technology Plan project (2021-30447); The Health China Buchang Zhiyuan Public welfare projects for heart and brain health under (Grant No. HIGHER2023099).

Disclosure of conflict of interest

None.

Address correspondence to: Zujue Cheng, Department of Neurosurgery, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, No. 1 Min De Road, Nanchang 330006, Jiangxi, China. Tel: +86-791-86297662; Fax: +86-791-86-297662; E-mail: Ndefy05002@ncu.edu.cn; Meizhen Yao, Department of Gynaecology and Obstetrics, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China. E-mail: yaomeizhen076@sina.com

References

[1] Ma R, Taphoorn MJB and Plaha P. Advances in the management of glioblastoma. J Neurol Neurosurg Psychiatry 2021; 92: 1103-1111.

[2] Campos B, Olsen LR, Urup T and Poulsen HS. A comprehensive profile of recurrent glioblastoma. Oncogene 2016; 35: 5819-5825.

[3] Le Rhun E, Preusser M, Roth P, Reardon DA, van den Bent M, Wen P, Reifenberger G and Weller M. Molecular targeted therapy of glioblastoma. Cancer Treat Rev 2019; 80: 101896.

[4] Popovic D, Vucic D and Dikic I. Ubiquitination in disease pathogenesis and treatment. Nat Med 2014; 20: 1242-1253.

[5] Song L and Luo ZQ. Post-translational regulation of ubiquitin signaling. J Cell Biol 2019; 218: 1776-1786.

[6] Asmamaw MD, Liu Y, Zheng YC, Shi XJ and Liu HM. Skp2 in the ubiquitin-proteasome system: a comprehensive review. Med Res Rev 2020; 40: 1920-1949.

[7] Hatakeyama S. TRIM family proteins: roles in autophagy, immunity, and carcinogenesis. Trends Biochem Sci 2017; 42: 297-311.

[8] Pan M, Li X, Xu G, Tian X, Li Y and Fang W. Tripartite motif protein family in central nervous system diseases. Cell Mol Neurobiol 2023; 43: 2567-2589.

[9] Cambiaghi V, Giuliani V, Lombardi S, Marinelli C, Toffalorio F and Pelicci PG. TRIM proteins in cancer. Adv Exp Med Biol 2012; 770: 77-91.

[10] Huang N, Sun X, Li P, Liu X, Zhang X, Chen Q and Xin H. TRIM family contribute to tumorigenesis, cancer development, and drug resistance. Exp Hematol Oncol 2022; 11: 75.

[11] Yao Y, Liu Z, Cao Y, Guo H, Jiang B, Deng J and Xiong J. Downregulation of TRIM27 suppresses gastric cancer cell proliferation via inhibition of the Hippo-BIRC5 pathway. Pathol Res Pract 2020; 216: 153048.

[12] Jiang J, Xie C, Liu Y, Shi Q and Chen Y. Up-regulation of miR-383-5p suppresses proliferation and enhances chemosensitivity in ovarian cancer cells by targeting TRIM27. Biomed Pharmacother 2019; 109: 595-601.

[13] Xing L, Tang X, Wu K, Huang X, Yi Y and Huan J. TRIM27 functions as a novel oncogene in non-triple-negative breast cancer by blocking cellular senescence through p21 ubiquitination. Mol Ther Nucleic Acids 2020; 22: 910-923.

[14] Zhang Y, Feng Y, Ji D, Wang Q, Qian W, Wang S, Zhang Z, Ji B, Zhang C, Sun Y and Fu Z. TRIM27 functions as an oncogene by activating epithelial-mesenchymal transition and p-AKT in colorectal cancer. Int J Oncol 2018; 53: 620-632.

[15] Ma L, Yao N, Chen P and Zhuang Z. TRIM27 promotes the development of esophagus cancer via regulating PTEN/AKT signaling pathway. Cancer Cell Int 2019; 19: 283.

[16] Liu S, Tian Y, Zheng Y, Cheng Y, Zhang D, Jiang J and Li S. TRIM27 acts as an oncogene and regulates cell proliferation and metastasis in non-small cell lung cancer through SIX3-β-catenin signaling. Aging (Albany NY) 2020; 12: 25564-25580.

[17] Cui H, Wang Q, Lei Z, Feng M, Zhao Z, Wang Y and Wei G. DTL promotes cancer progression by PDCD4 ubiquitin-dependent degradation. J Exp Clin Cancer Res 2019; 38: 350.

[18] Bi L, Ren Y, Feng M, Meng P, Wang Q, Chen W, Jiao Q, Wang Y, Du L, Zhou F, Jiang Y, Chen F, Wang C, Tang B and Wang Y. HDAC11 regulates glycolysis through the LKB1/AMPK signaling pathway to maintain hepatocellular carcinoma stemness. Cancer Res 2021; 81: 2015-2028.

[19] Zhang CS, Hawley SA, Zong Y, Li M, Wang Z, Gray A, Ma T, Cui J, Feng JW, Zhu M, Wu YQ, Li TY, Ye Z, Lin SY, Yin H, Piao HL, Hardie DG and Lin SC. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. Nature 2017; 548: 112-116.

[20] Li X, Sun X, Li L, Luo Y, Chi Y and Zheng G. MDM2-mediated ubiquitination of LKB1 contributes to the development of diabetic cataract. Exp Cell Res 2022; 417: 113191.

[21] Zhan W and Zhang S. TRIM proteins in lung cancer: mechanisms, biomarkers and therapeutic targets. Life Sci 2021; 268: 118985.

[22] Li X, Bai Y, Feng K, Chu Z, Li H, Lin Z and Tian L. Therapeutic, diagnostic and prognostic values of TRIM proteins in prostate cancer. Pharmacol Rep 2023; 75: 1445-1453.

[23] Furukawa T, Tabata S, Minami K, Yamamoto M, Kawahara K and Tanimoto A. Metabolic reprograming of cancer as a therapeutic target. Biochim Biophys Acta Gen Subj 2023; 1867: 130301.

[24] Kozal K, Jóźwiak P and Krześlak A. Contemporary perspectives on the Warburg effect inhibition in cancer therapy. Cancer Control 2021; 28: 10732748211041243.

[25] Hsu CC, Peng D, Cai Z and Lin HK. AMPK signaling and its targeting in cancer progression and treatment. Semin Cancer Biol 2022; 85: 52-68.

[26] Li Y, Liu Y, Jin K, Dong R, Gao C, Si L, Feng Z, Zhang H and Tian H. Negatively regulated by miR-29c-3p, MTFR1 promotes the progression and glycolysis in lung adenocarcinoma via the AMPK/mTOR signalling pathway. Front Cell Dev Biol 2021; 9: 771824.

[27] Shackelford DB and Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tu-

mour suppression. Nat Rev Cancer 2009; 9: 563-575.
[28] Li TT and Zhu HB. LKB1 and cancer: the dual role of metabolic regulation. Biomed Pharmacother 2020; 132: 110872.
[29] Zhang K, Wang J, Wang J, Luh F, Liu X, Yang L, Liu YR, Su L, Yang YS, Chu P and Yen Y. LKB1 deficiency promotes proliferation and invasion of glioblastoma through activation of mTOR and focal adhesion kinase signaling pathways. Am J Cancer Res 2019; 9: 1650-1663.
[30] Yu H, Wan L, Tang Z, Yao C, Zhang D, Jiang M, Wang C, Liu Y, Xue C, Wang X, Shi Y, Zhang L, Wang X and Wei Z. TRIM27 regulates the expression of PDCD4 by the ubiquitin-proteasome pathway in ovarian and endometrial cancer cells. Oncol Rep 2022; 48: 120.

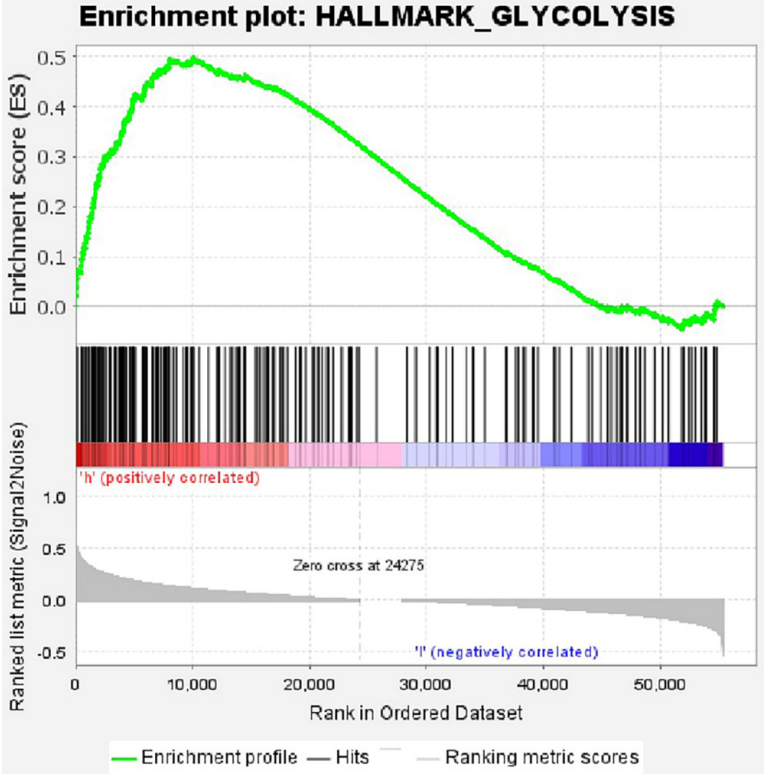
Supplementary Table 1. Basic information of the validation cohort

Parameters	Number	Percentage
Age		
≤60	12	40.00%
>60	18	60.00%
Gender		
Male	14	46.67%
Female	16	53.33%
KPS		
<80	6	20.00%
≥80	24	80.00%
Size		
<3 cm	18	60.00%
≥3 cm	12	40.00%
TRIM27		
Low	4	13.33%
High	26	86.67%
IDH1-R132H		
Low	25	83.33%
High	5	16.67%

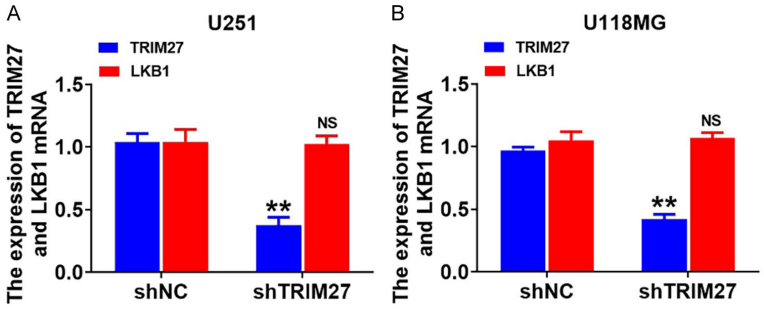
Supplementary Table 2. TRIM family members in TCGA-GBM, CGGA, and GSE108474 datasets

Gene name		
TCGA GBM	CGGA	GSE108474
TRIM14	TRIM11	TRIM10
TRIM16L	TRIM13	TRIM11
TRIM17	TRIM14	TRIM13
TRIM2	TRIM16	TRIM15
TRIM22	TRIM16L	TRIM16
TRIM25	TRIM17	TRIM17
TRIM27	TRIM2	TRIM2
TRIM28	TRIM21	TRIM23
TRIM3	TRIM22	TRIM24
TRIM32	TRIM23	TRIM25
TRIM33	TRIM24	TRIM26
TRIM34	TRIM25	TRIM27
TRIM35	TRIM26	TRIM28
TRIM38	TRIM27	TRIM29
TRIM4	TRIM28	TRIM3
TRIM40	TRIM29	TRIM31
TRIM54	TRIM3	TRIM35
TRIM56	TRIM32	TRIM36
TRIM58	TRIM33	TRIM37
TRIM65	TRIM34	TRIM40
TRIM66	TRIM35	TRIM41
TRIM67	TRIM36	TRIM44
TRIM68	TRIM37	TRIM46
TRIM72	TRIM38	TRIM47

	TRIM39	TRIM55
	TRIM41	TRIM56
	TRIM44	TRIM59
	TRIM45	TRIM63
	TRIM46	TRIM69
	TRIM47	TRIM8
	TRIM50	
	TRIM52	
	TRIM54	
	TRIM55	
	TRIM56	
	TRIM58	
	TRIM59	
	TRIM6	
	TRIM60P18	
	TRIM61	
	TRIM62	
	TRIM63	
	TRIM65	
	TRIM66	
	TRIM67	
	TRIM68	
	TRIM69	
	TRIM7	
	TRIM71	
	TRIM72	
	TRIM73	
	TRIM74	
	TRIM8	
	TRIM9	



Supplementary Figure 1. GSEA comparing the gene sets of glycolysis pathway targets in TRIM27^{high} GBM patients. Data were obtained from TCGA database. NES means normalized enrichment score.



Supplementary Figure 2. The mRNA levels of TRIM27 and LKB1 were detected. A and B. The mRNA levels of TRIM27 and LKB1 assessed by qRT-PCR in GBM cells transfected with shNC or shTRIM27.