LINC00152 Acts as a Potential Marker in Gliomas and Promotes Tumor Proliferation and Invasion Through The LINC00152/miR-107/RAB10 Axis

qing liu (liuqingdr@csu.edu.cn)
Xiangya Hospital Central South University https://orcid.org/0000-0003-1357-4866
gang peng
Xiangya Hospital Central South University
Jun Su
Xiangya Hospital Central South University
zeyou wang
Second Xiangya Hospital
songhua xiao
Sun Yat-Sen University

Research Article

Keywords: long non-coding RNA, LINC00152/MIR-107/RAB10 axis, glioblastoma, proliferation, invasion, prognosis

Posted Date: June 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-615132/v1

Abstract

Aberrant expression of long noncoding RNAs plays a pivotal role in tumorigenesis. Recently, several studies have showed that the *LINC00152* gene is upregulated in a variety of tumors and plays an oncogene role; however, its underlying molecular mechanisms in glioblastoma remain unclear. In this study, we found that *LINC00152* was upregulated in gliomas and its expression was significantly associated with high tumor aggressiveness and poor outcomes for glioma patients through bioinformatics analysis. Functionally, the knockdown of *LINC00152* not only inhibited malignant behaviors of glioma, such as proliferation and invasion of glioma cells and induced apoptosis *in vitro* but also suppressed tumorigenesis *in vivo*. Mechanistically, results of the bioinformatics analysis and experimental studies confirmed that *LINC00152* and *RAB10* as the targets of *miR-107*, and *LINC00152* might act as a sponge for *miR-107* to regulate the expression of *RAB10* in glioblastoma. Additionally, silencing *miR-107* reversed the effects induced by *LINC00152* knockdown on glioblastoma cells both *in vitro* and *in vivo*. Taken together, our data suggested that *LINC00152* is a candidate prognostic marker of glioma, and that the *LINC00152/MIR-107/RAB10* axis plays a pivotal role in regulation of the glioma malignancy, and therefore, targeting the axis might be an effective therapeutic strategy to treat glioma.

Introduction

Glioma, the most common and aggressive primary brain tumor arising from glial cells in the central nervous system, is highly invasive and resistant to traditional therapies. It accounts for approximately 80% of primary malignant brain tumors(Ricard et al. 2012;Lathia et al. 2015;Wang et al. 2015;Siegel et al. 2017). Glioblastoma multiforme (GBM) is the most frequent and severe subtype of glioma accounting for 50% of diffuse gliomas(Furnari et al. 2015;Lathia et al. 2015;Yuan et al. 2015). Despite multimodal treatments, including surgery, radiotherapy, and chemotherapy, which are constantly being improved, the average survival time of GBM patients is only approximately 15 months from diagnosis(Omuro and DeAngelis 2013). Therefore, it is necessary to completely understand the molecular mechanisms underlying GBM invasion and metastasis and to develop novel therapeutic strategies.

Long non-coding RNAs (LncRNAs) are a group of endogenous small RNAs of over 200 nucleotides long that do not have a protein-coding capacity(Huarte 2015;Beermann et al. 2016;Deguchi et al. 2017;Fan et al. 2017;Gomes et al. 2017). Over the past decade, lncRNAs have been described in a variety of processes including evolution(Gummalla et al. 2014), embryonic development(Floris et al. 2017), metabolism(Huarte 2015;Beermann et al. 2016), and oncogenesis(Fang et al. 2015;Gomes et al. 2017). Mechanistically, lncRNAs with aberrant expression levels play critical roles at the transcriptional and post-transcriptional levels in cancer biology(Fang et al. 2015;Huarte 2015;Beermann et al. 2016). Recently, many lncRNAs have been reported to be aberrantly expressed and involved in tumor initiation and progression of glioma. For example, *miR-155* host gene (*miR155HG*) is highly expressed in glioblastoma cells and facilitates glioma progression(Wu et al. 2017). *HOTAIR*, a cell cycle-associated lncRNA, is preferentially expressed in classical and mesenchymal glioma cells and is involved in the regulation of cell proliferation in glioblastoma(Zhang et al. 2013;Pastori et al. 2015). Other lncRNAs, such as

CCAT2(Guo et al. 2016), *HULC*(Zhu et al. 2016), and *H19*(Jia et al. 2016) regulate angiogenesis and behavior of glioma cells. *TUG1* promotes glioma stem cell (GSC) self-renewal and growth and inhibit GSC differentiation(Katsushima et al. 2016). In addition, these lncRNAs are potential therapeutic targets for glioblastoma cells. *HOTAIR* regulates the permeability of blood-tumor barrier (BTB) via binding to *miR-148b-3p*, which further targets *USF1* in glioma microvascular endothelial cells, thus, *HOTAIR/miR-148b-3p/USF1* axis is a potential novel drug target for glioma treatment(Sa et al. 2017). *XIST* lncRNA increases BTB permeability and inhibits glioma angiogenesis by targeting *miR-137* and might be a potential therapeutic target in glioma samples(Yu et al. 2017).

Long intergenic non-coding RNA 152 (*LINC00152*) is 828 nucleotides long and is located on chromosome 2p11.2 in an intergenic region between the pseudogenes platelet-activating factor acetylhydrolase 1b regulatory subunit 1 pseudogene 1 (*PAFAH1B1P1*) and *LOC107985796*(Teng et al. 2017;Yu et al. 2017). During the past few years, accumulating evidence have indicated that *LINC00152* is upregulated and plays an oncogene role in several carcinomas, including promoting cell proliferation and metastasis and inhibiting apoptosis(Ji et al. 2015;Chen et al. 2017;Feng et al. 2017). Besides, *LINC00152* might also act as a diagnostic and prognostic biomarker for different cancers(Chen et al. 2017;Feng et al. 2017). The abnormal expression of *LINC00152* in glioma tissues and GSCs has been reported(Yu et al. 2017). However, the biological role and underlying molecular mechanisms of *LINC00152* in glioblastoma cells is still unclear.

In this study, we combined bioinformatics analysis and experimental studies to investigate the expression pattern of *LINC00152*, its biological function, and the underlying mechanism in gliomas.

Materials And Methods

Cell lines and cultures

Human glioma cell lines (U251 and U87) were obtained from the American Type Culture Collection (Manassas, VA, USA). All cell lines were subjected to a short tandem repeat test before this study. Every cell line was passaged less than 10 times during the experiments. Individual cell lines were maintained according to the supplier's instructions. Cells were cultured in medium supplemented with 10 % fetal bovine serum and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) and were incubated at 37 °C in a humidified incubator with 5% CO_2.

Human tissue samples

Patients with glioma who were newly diagnosed, treated, and followed at the Department of Neurosurgery, Xiangya Hospital, Central South University, Hunan, China were enrolled for this study. We obtained frozen tissue samples from 73 gliomas and 78 normal brain tissues between March 2008 and November 2010. This study was approved by the hospital institutional review board and written informed consent was obtained from all patients. All the protocols were reviewed by the Joint Ethics Committee of the Central South University Health Authority and were performed following national guidelines. Tissue

samples were collected during surgery and diagnosed using the World Health Organization (WHO) criteria by two pathologists who were blinded to patient data. Tissues were frozen in RNAlater (Ambion) in liquid nitrogen and stored until total RNA or protein were extracted. Clinical data, including gender, age, follow-up, and outcome, were obtained from medical records.

Cells transfection

Cell transfection was performed using Lipofectamine 2000 (Invitrogen–Life Technologies, Carlsbad, CA, USA) as per the manufacturer's instructions. Cells were seeded in cell culture dishes or plates and were grown overnight. On the following day, the cells were transfected with miRNA mimics/NC using Lipofectamine 2000 and incubated. After 72 h incubation, these cells were subjected to western blot analysis and also other assays.

Vector construction

The pLKO.1-puro vector used for the stable expression of shRNA against *LINC00152* (sh-linc) contained a puromycin resistance gene. The scrambled control shRNA (sh-ctr) sequence had no homology to any human genomic sequences. The cultured cells (3×10^5 cells/well) were seeded in 6-well culture plates and maintained in DMEM medium containing 10% FBS for 24 h before transfection. Cell transfection was performed using Lipofectamine 2000 (Invitrogen–Life Technologies, Carlsbad, CA, USA) as per the manufacturer's instructions. For screening, puromycin (1 μg/mL) was added to the medium 72 h after transfection. The medium was replaced every 2 d for 2-3 weeks. U251 and U87 cells with high endogenous *LINC00152* expression were selected for silencing. The expression levels of *LINC00152* was confirmed by qRT-PCR.

Quantitative real-time PCR (qRT-PCR) assays

RNA was isolated from harvested cells or human tissues with Trizol reagent according to the manufacturer's instructions (Invitrogen, CA, USA). One μg of total RNA was reverse transcribed to cDNA using a Reverse Transcription Kit (Thermo Fisher Scientific, MA, USA). qRT-PCR was performed using SYBR Premix DimerEraser kit (Takara, Dalian, China) on a CFX96 Real-Time PCR Detection System (Bio-Rad, CA, USA) to determine the relative expression levels of target genes. Expression of each gene was quantified by measuring Ct values and normalized using the $2^{-\Delta\Delta ct}$ method. U6 small nuclear (snRNA) and GAPDH mRNA were used as an internal control for mRNA and mature miRNA, respectively. The primers used were showed as follows: *LINC00152*: F: 5'-AAAATCACGACTCAGCCCCC-3' and R: 5'-AATGGGAAACCGACCAGACC-3'; *miR-107*: F: 5'-GGAGCAGCATTGTACAGG-3' and R: 5'-CAGTGCGTGTCGTGGA-3'; *RAB10*: F: 5'-TTTCACACCATCACAACCTCC-3' and R: 5'-GGTACAACTCTTTTGTCGTCC-3'; U6: F: 5'-CTCGCTTCGGCAGCACA-3' and R: 5'-AACGCTTCACGAATTTGCGT-3; and *GAPDH*: F: 5'-GGGAGCCAAAAGGGTCAT -3' and R: 5'-GTCCTTCCACGATACCAA-3'.

Cell Counting kit-8 assay

Cell proliferation of GBM cells was measured by Cell Counting Kit-8 (CCK-8; Sigma-Aldrich, Shanghai, China) according to the manufacturer's instructions. Briefly, U251 and U87 cells transfected with sh-linc or sh-ctr were seeded at a density of 2×10^3 cells per well in 96-well plates. The cells were incubated for 24, 48, 72, and 96 h after transfection. At each of the desired time points, CCK-8 solution was added (10 μL/well) to the cells and incubated for 2 h at 37 °C, followed by absorbance measurements at 420 nm using a microplate reader (Model 680 microplate reader, Bio-Rad Laboratories). Each assay was performed in five replicates.

Colony formation assay

After transfection, the cells were digested using 0.25% trypsin, and then cells were suspended in medium for counting. Cells were seeded onto a 6-well plate at 1×10^3 cells per well and were incubated at 37 °C with 5% CO_2 containing saturated humidity for 14 d and the growth medium was replaced once in three days. After clone formation, the supernatant was discarded, and the plate was carefully immersed twice with PBS. The cells were fixed by adding 4% paraformaldehyde and incubation for 15 min. Then, the fixing solution was removed and appropriate amounts of Crystal Violet Staining Solution was added and incubated for 30 min and then the staining solution was slowly washed away using running water. After air drying, the resulting colonies were then counted.

Flow cytometric analysis of apoptosis

Cellular apoptosis was assessed by Annexin V/propidium iodide (PI) staining and flow cytometry was performed using an Annexin V-fluorescein Isothiocyanate Apoptosis Detection Kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions. Briefly, the cells were digested by trypsin, centrifuged, and washed twice with phosphate buffered saline (PBS). Then the harvested cells ($5´ 10^5$) were resuspended in Annexin V binding buffer. After staining with Annexin V and PI, the cells were analyzed with the help of a flow cytometer. All experiments were repeated at least three times.

Cell Matrigel invasion assay

Matrigel invasion migration was evaluated using a Transwell migration assay. Briefly, filters coated with Matrigel in the upper compartment were loaded with 200 μL of serum-free medium containing 5×10^4 transfected cells, and the lower compartment was filled with 20% FBS. After 24 h, cells migrated to the bottom surface were fixed with 100% methanol and were counted after staining with 0.5% crystal violet. The number of invaded cells were counted in six randomly selected fields under a microscope and the average value was calculated. Each experiment was conducted in triplicate.

Western blot analysis

Western blot was performed as described previously(Wang et al. 2016). Briefly, cells were lysed and the total proteins were extracted and quantified. Equivalent amounts of protein from each sample were

separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, USA). The membrane was blocked with 5% skim milk at room temperature for 1 h, followed by incubation with primary antibody (*RAB10* with 1:1000 dilution, *GAPDH* with 1:5000 dilution) at 4 °C overnight and detected by chemiluminescence. Antibodies against *RAB10* (#4262) were obtained from Cell Signaling Technology (Beverly, MA, USA) and antibodies against *GAPDH* (sc-32233) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Luciferase reporter assays

Luciferase reporter assays were performed as described previously(Peng et al. 2015). Briefly, the luciferase reporter plasmids containing wild-type (wt) or mutant (mut) *LINC00152* genes were purchased from GeneChem (Shanghai, China). phRL-TK plasmid was used as an internal control. Glioma cells (2×10^5) were plated in 24-well plates, and then cotransfected with either wildtype or mut*LINC00152* luciferase reporter plasmid, together with *miR-107* mimics or miR-NC. After incubation for 24 h, luciferase activities were measured using a Dual-Luciferase Reporter Assay System kit (Promega) according to the manufacturer's instructions.

Wound healing assay

Glioma cells were cultured in 6-well plates until 80% confluency was reached. Subsequently, cells were then transfected with *LINC00152* shRNA or control shRNA. Cultures were scratched using a 10 Ml tip 6-h post transfection to form wound gaps. The wound gaps were photographed 12, 24, and 36 h following the scratch. Fields containing wounds were visualized and the distance migrated by cells was measured from five different areas for each wound.

Mouse xenograft models

All the animal procedures were performed in accordance with institutional guidelines. Ethical approval was obtained from the Institute Research Ethics Committee of Central South University. For subcutaneous implantation, U251 cells stably transfected with sh-linc or sh-ctr were collected and suspended in PBS buffer at a concentration of 1×10^7 cells/ml. Tumor growth was monitored by caliper measurement once or twice a week. After 35 d, mice were euthanized, and tumors were extracted for immunohistochemical analysis of Ki-67.

To assay the effects of *LINC00152* on tumor formation after *miR-107* knockdown, U251 cells stably transfected with sh-linc (10 mice) or sh-ctr (5 mice) were collected and suspended in PBS buffer at a concentration of 1×10^7 cells/ml. Aliquots of 40 µl PBS containing 1 µg of miR-NC were directly injected into the tumors of the sh-ctr group mice. The sh-linc group mice were divided into two groups. Aliquots containing 40 µl PBS with 1 µg of *miR-107* inhibitors or miR-NC were directly injected into the tumor. Tumor growth was monitored by caliper measurement once or twice a week for at least 5 weeks. After 35 d, mice were euthanized, and tumors extracted.

Bioinformatics analysis

Three publicly available databases, including The TCGA lower grade glioma and glioblastoma (GBMLGG) dataset (https://xenabrowser.net/), the Gene Expression Omnibus (GEO) GSE16011 (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi) and CGGA dataset (http://www.cgga.org.cn/), were used in this study. Gene expression analysis and Survival analysis were performed, and the detail of them was described in previous publications(Su et al. 2019;Su et al. 2019).

Statistical analysis

All experiments were performed three times and data were analyzed using GraphPad Prism 5 (La Jolla, CA, USA). Statistical differences between groups were analyzed using Student's t-test, one-way ANOVA, and χ^2 tests, using the SPSS 17.0 program and RStudio. A *p*-value of < 0.05 was considered to indicate a statistically significant result.

Results

LINC00152 is upregulated in glioma tissues and its expression is associated with glioma patient outcomes

LINC00152 is an oncogene that promotes cell proliferation, invasion, and migration in various tumors(Yu et al. 2017). To investigate the expression of *LINC00152* in gliomas and normal brain tissues, the GSE16011 database was used. The result showed that the expression of *LINC00152* was significantly upregulated in gliomas compared to levels in normal tissue ($p < 0.05$, Fig. 1a). Furthermore, we analyzed the expression of *LINC00152* in different grades of glioma and found that its level increases with WHO grades based on the GSE16011 database (Fig. 1a), which was well validated in TCGA and CGGA datasets (Fig. 1b and supplementary Fig. 1a). Moreover, *LINC00152* expression was significantly correlated with MGMT promoter status and IDH status of glioma. *LINC00152* expression in IDH-Wt gliomas was markedly higher than that in IDH-Mut gliomas based on TCGA database (Fig. 2c), consistent with result obtained from the CGGA database (Supplementary Fig. 1b). *LINC00152* expression in gliomas with MGMT promoter unmethylation was significantly higher than that in gliomas with MGMT promoter methylation based on TCGA database (Fig. 2d). In addition, *LINC00152* expression was significantly upregulated in the mesenchymal and classical subtype compared with other two respective molecular subtypes in the TCGA dataset (Fig. 1e).

Since *LINC00152* expression significantly associated with WHO grade and molecular subtype in gliomas, we further investigated its prognostic value and the Kaplan–Meier survival analysis was performed. According the median of *LINC00152*, the glioma patients were grouped into high group and low group. Based on the TCGA database, the result indicated that high expression of *LINC00152* is significantly associated with poor prognosis in glioma patients (Fig. 1f), which was well validated in CGGA dataset (supplementary Fig. 1c). To investigate whether *LINC00152* could be an independent prognostic marker for glioma, we simultaneously performed univariate and multivariate Cox regression analysis based on

the TCGA dataset. Univariate Cox analysis showed that *LINC00152* expression, patient age at diagnosis, WHO grade, MGMT promoter status, IDH status and transcriptome subtype were significantly associated with overall survival of glioma patients. According to multivariate Cox analysis, the LINC00152 expression was still a significant predictive factor after adjusting for the aforementioned clinical factors (Table 1).

Table 1
Univariate and multivariable Cox regression analysis of overall survival based on TCGA dataset

	Univariate analysis		**Multivariate analysis**	
	HR (95% CI for HR)	P	HR (95% CI for HR)	P
LINC00152	1.645 (1.551–1.745)	***	1.206 (1.081–1.345)	***
gender male VS Female	1.225 (0.955–1.572)	0.110	-	-
Age ≤ 40 VS > 40	0.231 (0.165–0.324)	***	0.3947 (0.2565–0.6074)	***
grade WHO II VS WHO III + IV	0.178 (0.121–0.262)	***	0.4497 (0.2768–0.7306)	**
IDH status WT VS MT	10.050 (7.524–13.43)	***	3.205 (1.863–5.514)	***
MGMT promoter status Methylation VS Unmethylation	3.228 (2.447–4.259)	***	-	-
Transcriptome subtype NE + PN VS CL + ME	0.152 (0.113–0.203)	***	1.012 (0.6335–1.618)	0.959
Abbreviations: HR, hazard ratio, CI, Confidence interval.				

Taken together, these results indicated that the expression of *LINC00152* was significantly upregulated in GBM and significantly correlated with WHO grade, IDH status, MGMT promoter status and transcriptome subtype of gliomas. Importantly, *LINC00152* is an independent prognostic marker for glioma patients and high expression indicates poor clinical prognosis.

The role of LINC00152 **in glioma cell proliferation, cell migration, and cell apoptosis**

Cell proliferation and invasion are significantly processed in cancer progression(Fang et al. 2015;Huarte 2015;Lathia et al. 2015). To explore the functions of *LINC00152* in glioma, we used a short hairpin RNA (shRNA) to decrease the expression levels of *LINC00152* in U251 and U87 cells. qRT-PCR analysis showed that this shRNA can downregulate the expression of *LINC00152* in both the cell lines with high efficiency (Fig. 2a). As a functional assay, CCK-8 assay was performed to monitor the effects of *LINC00152* on cell proliferation and the results showed that knockdown of *LINC00152* expression in U251 and U87 cells caused significant inhibition on cell proliferation (Fig. 2b). On the other hand, *LINC00152* knockdown can significantly diminish colony formation and invasion abilities of U251 and U87 cells (Fig. 2c and 2d). Furthermore, flow cytometric analysis demonstrated that *LINC00152* silencing in U251 and U87 cells induced cell apoptosis and the percentage of apoptotic cells was significantly increased in the *LINC00152* knockdown group when compared to that in the control group which were transfected with control shRNA (Fig. 2e). Taken together, these results suggest that *LINC00152* plays an important role in the regulation of cell proliferation, invasion, and apoptosis in glioma cells.

LINC00152 **regulates tumor growth** in vivo

To confirm the functional effects of *LINC00152* on glioma cells *in vivo*, we subcutaneously injected U251 cells with stable *LINC00152* knockdowns, or U251 cells were transfected with sh-ctr into the right flanks of 4–6 week-old nude mice. After 35 d, tumors were visible and all the mice were euthanized to harvest xenografts. There was a significant reduction in tumor growth in xenografts with *LINC00152* knockdown compared to negative controls and the relative tumor weight in the *LINC00152* knockdown group was significantly lighter than that in the control group (Fig. 3a and 3b). H&E staining showed that the tumor tissues were isolated from xenograft mice model (Fig. 3c). In addition, immunohistochemistry (IHC) analysis revealed that the expression of Ki-67 in *LINC00152* knockdown group was lower than that in the control group (Fig. 3d). Taken together, these data verify that *LINC00152* promotes the growth of GBM cells *in vivo*.

LINC00152 **is a target of** miR-107

LncRNAs are known to act as ‘sponges’ to sequester endogenous miRNAs to regulate miRNA targets. Therefore, StarBase v2.0 software (http://starbase.sysu.edu.cn/index.php) was used to identify all potential miRNAs that can bind to *LINC00152* gene and the software returned 6 hits, including *miR-107*, *miR-376c-3p*, *miR-193b-3p*, *miR-193a-3p*, *miR-103a-3p*, and *miR-155-5p*. Besides, it has been previously reported that *miR-107* is downregulated in glioma and overexpression of *miR-107* could inhibit proliferation of glioma(Chen et al. 2016), which compelled us to investigate whether *LINC00152* was a real target of *miR-107*. The site on *LINC00152* that *miR-107* could potentially bind is shown in Fig. 4a. We also analyzed the expression levels of *miR-107* in glioma and normal tissues and further investigated the correction factors between *LINC00152* expression and *miR-107* expression. The result showed that the expression of *miR-107* negatively correlated with the expression of *LINC00152* ($r = -0.447$, $p < 0.05$, Fig. 4b). Luciferase reporter assays showed that *miR-107* mimics significantly suppressed luciferase activity in U251 and U87 cells that carried plasmids with wildtype rather than mutant 3'-UTR of

LINC00152 (Fig. 4c), which revealed that *LINC00152* was a direct target of *miR-107*. In addition, *LINC00152* silencing could upregulate *miR-107* expression in both U251 and U87 cells (Fig. 4d). Taken together, these findings demonstrated that *LINC00152* was a direct target of *miR-107* and *LINC00152* could suppress expression levels of *miR-107* in glioma cells.

RAB10 **is a target of** miR-107

Next, we aimed at identifying the target genes of *miR-107* via TargetScan (http://www. targetscan.org/), and observed that *RAB10* was predicted as a potential target of *miR-107*. *RAB10*, a member of the Ras small GTPase superfamily, is a protein-coding gene with GTP and GDP binding domains. Indeed, recent studies demonstrated that *RAB10* is closely associated with the genesis and development of certain cancers(Jiang et al. 2016;Wang et al. 2017). Thus, dual luciferase reporter assays were performed to confirm whether *miR-107* binds to the putative *miR-107* binding site at the 3'-UTR of *RAB10* (Fig. 5a). The results showed that luciferase intensity was significantly attenuated by co-transfected *miR-107* mimics and *RAB10*-WT vectors, but not in the mutant vector lacking the putative *miR-107* binding site in both U251 and U87 cells. Also, miR-NC also could not affect the luciferase intensity of *RAB10*-WT/MT vectors (Fig. 5b). These results suggested that *RAB10* is a direct target of *miR-107*. Then, qRT-PCR and western blotting were performed to assess whether *miR-107* could negatively regulate the expression of *RAB10* at both the mRNA and protein levels in GBM cell lines, respectively. As expected, *miR-107* mimics decreased *RAB10* mRNA levels, and conversely, *miR-107* inhibitor increased *RAB10* mRNA levels (Fig. 5c). Similar results were observed by using western blot analysis (Fig. 5d). These results indicated that *RAB10* is a direct binding target of *miR-107* and its expression can be regulated by *miR-107* levels in glioma samples.

- LINC00152 **regulates the expression of** RAB10 **depending on** miR-107

Since, *LINC00152* harbors an identical *miR-107* binding site as *RAB10*, we wanted to know whether *LINC00152* might regulate the expression of *RAB10* through *miR-107*. Firstly, the expression of *LINC00152* and *RAB10* in glioma tissues were measured and correlation analysis was performed to detect the potential association between them. The results showed that the expression of *RAB10* was not only upregulated in glioma tissues, but also had significantly positive correlation with the expression of *LINC00152* (Fig. 6a and 6b). Furthermore, knockdown of *LINC00152*, in U251 and U87 cell lines, decreased the mRNA levels of *RAB10* (Fig. 6c) and diminished the expression of *RAB10* at the protein level (Fig. 6d). However, overexpression of *miR-107* attenuated the effect of *LINC00152* silencing on the regulation of *RAB10* expression (Fig. 6c and 6d). In summary, these results indicate that *LINC00152* can regulate expression of *RAB10* depending on *miR-107* in GBM cells.

- miR-107 **reverses the functions of** LINC00152 **in glioma**

To further validate the interactions between *LINC00152* and *miR-107*, we investigated whether *miR-107* silencing can rescue the effects caused by *LINC00152* knockdown, including cell proliferation, clone formation, invasion abilities *in vitro*, and tumor growth *in vivo*. As expected, CCK-8 and colony formation assays demonstrated that *miR-107* silencing could weaken the suppressive effects of *LINC00152*

knockdown on cell proliferation and clone formation in U87 and U251 cells (Fig. 7a-b). Moreover, matrigel transwell assays showed that *LINC00152* knockdown and inhibition of *miR-107* caused opposite effects on the ability of GBM cell invasion. However, *miR-107* inhibitor could partially reverse the effect of *LINC00152* knockdown on cell invasion of GBM (Fig. 7c). Furthermore, by *in vivo* assays, tumor growth in xenografts with co-transfected sh-*LINC00152* and *miR-107*-NC clones was decreased compared with that in negative control group, whereas *miR-107* silencing eliminated the suppressor effect induced by *LINC00152* knockdown on tumor growth (Fig. 7d-e). Collectively, these results suggest that the suppressive effects of *LINC00152* knockdown on GBM could be reversed by *miR-107* silencing both *in vitro* and *in vivo*.

Discussion

Glioblastoma multiforme (GBM) is the most prevalent and most lethal primary intrinsic brain tumor. It accounts for 50% of malignant glioma cases and is characterized histologically by considerable cellularity and mitotic activity, vascular proliferation, and necrosis(Omuro and DeAngelis 2013;Lathia et al. 2015). Although targeted therapies or immunotherapies have been used to treat GBM, maximal surgical resection followed by concurrent radiation therapy with temozolomide (TMZ) and subsequent additional adjuvant temozolomide (TMZ) therapy remains the standard therapy for GBM (Ricard et al. 2012;Omuro and DeAngelis 2013). Although accepted the standard therapy, GBM patient prognosis is still clinically frustrating (Wen and Kesari 2008;Stupp et al. 2009). To better understand and to find more effective treatments for this disease, it is vital to identify novel biomarkers and therapeutic targets. Combining bioinformatics analysis and biological experiments, our present study revealed that *LINC00152* is a potential prognostic marker for glioma patients and play an oncogene role in GBM.

LINC00152 is one 828-bp lncRNA and locates at chromosome 2p11.2. Increasing researches indicate *LINC00152* plays an oncogene role in many cancers and may act as a diagnostic and prognostic biomarker for them (Yu et al. 2017). For example, Wu *et al (*Wu et al. 2016) *LINC00152* was significantly upregulated in clear cell renal cell carcinoma, may serve as an independent predictor of overall survival and can promote cell proliferation and invasion, inhibit cell cycle and apoptosis. In this study, we found that the expression of *LINC00152* is not only dramatically upregulated, also significantly associated with WHO grade, IDH status, MGMT promoter status and transcriptome subtype in glioma. The promoter methylation of MGMT is clinically used as a biomarker of response to alkylating agents for glioma (Esteller et al. 2000). Thus, *LINC00152* may be related to the sensitivity of glioma to TMZ chemotherapy, which needs to be confirmed by further study. Furthermore, this study indicated that *LINC00152* is an independent prognostic factor for glioma patients and that low levels of *LINC00152* expression predict better prognosis. Functionally, through loss-of-function approaches, we fund that knocked down the expression of *LINC00152* significantly inhibited cell proliferation, colony formation, invasion, and induced cell apoptosis in vitro and decreased tumor growth in vivo, which was consistent with previous studies(Cai et al. 2018;Liu et al. 2018). Taken together, *LINC00152* is a potential prognostic marker and therapeutic target for glioma patient and acts as an oncogene in GBM.

Certain lncRNAs have been proposed to function as “miRNA sponges”(Ebert and Sharp 2010;Beermann et al. 2016), they contain miRNA response elements (MREs) that can sequester miRNAs, thereby, preventing the miRNAs from binding to their target genes (Ebert and Sharp 2010;Beermann et al. 2016;Bhan et al. 2017). For example, lncRNA *LINC00673*, regulates non-small cell lung cancer proliferation, migration, invasion, and epithelial-mesenchymal transition by sequestering *miR-150-5p*(Lu et al. 2017). LncRNA *SPRY4-IT1* acts as a sponge RNA to sequester *miR-101-3p* to promote proliferation and metastasis of bladder cancer cells through upregulation of *EZH2*(Liu et al. 2017). As a member of lncRNAs, *LINC00152* is also reported that it can function as “miRNA sponges” to many miRNAs, including miR-497, miR-608, miR-153-3p, miR-138 and so on(Cai et al. 2017;Liu et al. 2019;Ouyang et al. 2019;Sun et al. 2019). It is not difficult to find that the one lncRNA can regulate multiple miRNAs. Here, we confirmed that *LINC00152* acted as a miRNA sponge for *miR-107* and regulated the expression of *miR-107*. Chen et al reported that *miR-107* is downregulated in glioma and overexpression of *miR-107* could inhibit proliferation of glioma(Chen et al. 2016). Besides, our results showed that *LINC00152* plays oncogenic role depending *miR-107* in vitro and in vivo.

RAB10, a member of the Ras small GTPase superfamily, is a protein-coding gene with GTP and GDP binding domains(Just and Peranen 2016;Jaldin-Fincati et al. 2017). *RAB10* participates in the insulin-stimulated translocation of *GLUT4* in adipocytes(Sano et al. 2011), basement membrane secretion(Lerner et al. 2013), and the formation and maintenance of the endoplasmic reticulum (English and Voeltz 2013). As a member of the RAS oncogene family, recent studies have showed that *RAB10* is closely related to tumorigenesis and cancer development. In hepatocellular carcinoma, *RAB10* overexpression promotes tumor growth through multiple oncogenic pathways, cell stress, and apoptosis pathways and indicates a poor prognosis for HCC patients(Wang et al. 2017). Jiang *et al* identified *RAB10* as a target of *miR-329* and found that *miR-329* was able to inhibit osteosarcoma cell proliferation, promote apoptosis, and induce G0/G1 cell cycle arrest via *RAB10*(Jiang et al. 2016). In this study, our study showed that *RAB10* was a target of *miR-107* and further revealed the regulation mechanism among *LINC00152*, *miR-107* and *RAB10*. Additionally, we also fund that *RAB10* is upregulated in glioma, which suggests it may be an oncogene for glioma.

The mechanism of linc00152 in glioma is complex. Cai *et al*(Cai et al. 2018) found that *LINC00152* acted as a miRNA sponge for *miR-612* in GBM cells, negatively regulated *miR-612* releases, which resulted in the elevated *AKT2*, activated NF-κB pathway to promote proneural–mesenchymal transition(Cai et al. 2018). Liu *et al* reported that overexpression of *LINC00152* suppressed *miR-107* expression in U87 cells and enhanced the expression of *HMGA2*, a direct target gene of *miR-107*(Liu et al. 2018). In this study we revealed the new regulatory network of *LINC00152*, *miR-107*, and *RAB10*, which may enrich our understanding of the mechanism of *LINC00152* in glioma and provide novel strategy for the treatment of glioma.

Conclusion

LINC00152 is aberrantly upregulated in gliomas and may be a valuable prognostic marker for glioma patients. The *LINC00152* plays a pivotal role in regulation of the glioma malignancy via *LINC00152/MIR-107/RAB10* axis. Therefore, targeting this axis might be an effective therapeutic strategy to treat glioma.

Declarations

Acknowledgments

The authors would like to thank the support of the National Nature Science Foundation of China (No 81801908 Gang Peng), the Science and Technology Planning Project of Guangzhou (No 201803010013, Songhua Xiao) and like to thank Andrew V. Yang for providing help with writing this manuscript.

Funding

This study was supported by grants from the National Nature Science Foundation of China (No 81801908) and the Science and Technology Planning Project of Guangzhou (No 201803010013).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Code availability

Not applicable

Authors' contributions

GP mainly performed the experiments, analyzed the data and wrote the paper. JS performed construction of vectors and cells culture and transfection. ZW performed western blot and prepared the samples. QL and SX carried out the experiment design, manuscript drafting, and revision. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all participants included in this study.

Patient consent for publication

Written informed consent for publication was obtained from all participants.

Competing interests

The authors declare that they have no competing financial interests.

References

1. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. Physiol Rev. 2016;96:1297–325.
2. Bhan A, Soleimani M, Mandal SS. Long Noncoding RNA and Cancer: A New Paradigm. Cancer Res. 2017;77:3965–81.
3. Cai J, Zhang J, Wu P, Yang W, Ye Q, Chen Q, Jiang C. (2018). Blocking LINC00152 suppresses glioblastoma malignancy by impairing mesenchymal phenotype through the miR-612/AKT2/NF-kappaB pathway. J Neurooncol.
4. Cai Q, Wang Z, Wang S, Weng M, Zhou D, Li C, Wang J, Chen E, Quan Z. (2017). Long non-coding RNA LINC00152 promotes gallbladder cancer metastasis and epithelial-mesenchymal transition by regulating HIF-1alpha via miR-138. Open Biol 7.
5. Chen L, Li ZY, Xu SY, Zhang XJ, Zhang Y, Luo K, Li WP. Upregulation of miR-107 Inhibits Glioma Angiogenesis and VEGF Expression. Cell Mol Neurobiol. 2016;36:113–20.
6. Chen QN, Chen X, Chen ZY, Nie FQ, Wei CC, Ma HW, Wan L, Yan S, Ren SN, Wang ZX. Long intergenic non-coding RNA 00152 promotes lung adenocarcinoma proliferation via interacting with EZH2 and repressing IL24 expression. Mol Cancer. 2017;16:17.
7. Deguchi S, Katsushima K, Hatanaka A, Shinjo K, Ohka F, Wakabayashi T, Zong H, Natsume A, Kondo Y. Oncogenic effects of evolutionarily conserved noncoding RNA ECONEXIN on gliomagenesis. Oncogene. 2017;36:4629–40.
8. Ebert MS, Sharp PA. Emerging roles for natural microRNA sponges. Curr Biol. 2010;20:R858–61.
9. English AR, Voeltz GK. Rab10 GTPase regulates ER dynamics and morphology. Nat Cell Biol. 2013;15:169–78.
10. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med. 2000;343:1350–4.
11. Fan C, Tang Y, Wang J, Xiong F, Guo C, Wang Y, Zhang S, Gong Z, Wei F, Yang L, He Y, Zhou M, Li X, Li G, Xiong W, Zeng Z. Role of long non-coding RNAs in glucose metabolism in cancer. Mol Cancer. 2017;16:130.
12. Fang XY, Pan HF, Leng RX, Ye DQ. Long noncoding RNAs: novel insights into gastric cancer. Cancer Lett. 2015;356:357–66.
13. Feng S, Zhang J, Su W, Bai S, Xiao L, Chen X, Lin J, Reddy RM, Chang AC, Beer DG, Chen G. Overexpression of LINC00152 correlates with poor patient survival and knockdown impairs cell proliferation in lung cancer. Sci Rep. 2017;7:2982.
14. Floris G, Zhang L, Follesa P, Sun T. Regulatory Role of Circular RNAs and Neurological Disorders. Mol Neurobiol. 2017;54:5156–65.
15. Furnari FB, Cloughesy TF, Cavenee WK, Mischel PS. Heterogeneity of epidermal growth factor receptor signalling networks in glioblastoma. Nat Rev Cancer. 2015;15:302–10.

16. Gomes CC, De Sousa SF, Calin GA, Gomez RS. The emerging role of long noncoding RNAs in oral cancer. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;123:235–41.
17. Gummalla M, Galetti S, Maeda RK, Karch F. Hox gene regulation in the central nervous system of Drosophila. Front Cell Neurosci. 2014;8:96.
18. Guo H, Hu G, Yang Q, Zhang P, Kuang W, Zhu X, Wu L. Knockdown of long non-coding RNA CCAT2 suppressed proliferation and migration of glioma cells. Oncotarget. 2016;7:81806–14.
19. Huarte M. The emerging role of lncRNAs in cancer. Nat Med. 2015;21:1253–61.
20. Jaldin-Fincati JR, Pavarotti M, Frendo-Cumbo S, Bilan PJ, Klip A. Update on GLUT4 Vesicle Traffic: A Cornerstone of Insulin Action. Trends Endocrinol Metab. 2017;28:597–611.
21. Ji J, Tang J, Deng L, Xie Y, Jiang R, Li G, Sun B. LINC00152 promotes proliferation in hepatocellular carcinoma by targeting EpCAM via the mTOR signaling pathway. Oncotarget. 2015;6:42813–24.
22. Jia P, Cai H, Liu X, Chen J, Ma J, Wang P, Liu Y, Zheng J, Xue Y. Long non-coding RNA H19 regulates glioma angiogenesis and the biological behavior of glioma-associated endothelial cells by inhibiting microRNA-29a. Cancer Lett. 2016;381:359–69.
23. Jiang W, Liu J, Xu T, Yu X. MiR-329 suppresses osteosarcoma development by downregulating Rab10. FEBS Lett. 2016;590:2973–81.
24. Just WW, Peranen J. Small GTPases in peroxisome dynamics. Biochim Biophys Acta. 2016;1863:1006–13.
25. Katsushima K, Natsume A, Ohka F, Shinjo K, Hatanaka A, Ichimura N, Sato S, Takahashi S, Kimura H, Totoki Y, Shibata T, Naito M, Kim HJ, Miyata K, Kataoka K, Kondo Y. Targeting the Notch-regulated non-coding RNA TUG1 for glioma treatment. Nat Commun. 2016;7:13616.
26. Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CL, Rich JN. Cancer stem cells in glioblastoma. Genes Dev. 2015;29:1203–17.
27. Lerner DW, Mccoy D, Isabella AJ, Mahowald AP, Gerlach GF, Chaudhry TA, Horne-Badovinac S. A Rab10-dependent mechanism for polarized basement membrane secretion during organ morphogenesis. Dev Cell. 2013;24:159–68.
28. Liu D, Gao M, Wu K, Zhu D, Yang Y, Zhao S. LINC00152 facilitates tumorigenesis in esophageal squamous cell carcinoma via miR-153-3p/FYN axis. Biomed Pharmacother. 2019;112:108654.
29. Liu D, Li Y, Luo G, Xiao X, Tao D, Wu X, Wang M, Huang C, Wang L, Zeng F, Jiang G. LncRNA SPRY4-IT1 sponges miR-101-3p to promote proliferation and metastasis of bladder cancer cells through up-regulating EZH2. Cancer Lett. 2017;388:281–91.
30. Liu X, Yidayitula Y, Zhao H, Luo Y, Ma X, Xu M. LncRNA LINC00152 promoted glioblastoma progression through targeting the miR-107 expression. Environ Sci Pollut Res Int. 2018;25:17674–81.
31. Lu W, Zhang H, Niu Y, Wu Y, Sun W, Li H, Kong J, Ding K, Shen HM, Wu H, Xia D, Wu Y. Long non-coding RNA linc00673 regulated non-small cell lung cancer proliferation, migration, invasion and epithelial mesenchymal transition by sponging miR-150-5p. Mol Cancer. 2017;16:118.

32. Omuro A, Deangelis LM. Glioblastoma and other malignant gliomas: a clinical review. JAMA. 2013;310:1842–50.
33. Ouyang T, Zhang Y, Tang S, Wang Y. Long non-coding RNA LINC00052 regulates miR-608/EGFR axis to promote progression of head and neck squamous cell carcinoma. Exp Mol Pathol. 2019;111:104321.
34. Pastori C, Kapranov P, Penas C, Peschansky V, Volmar CH, Sarkaria JN, Bregy A, Komotar R, St Laurent G, Ayad NG, Wahlestedt C. The Bromodomain protein BRD4 controls HOTAIR, a long noncoding RNA essential for glioblastoma proliferation. Proc Natl Acad Sci U S A. 2015;112:8326–31.
35. Peng G, Yuan X, Yuan J, Liu Q, Dai M, Shen C, Ma J, Liao Y, Jiang W. miR-25 promotes glioblastoma cell proliferation and invasion by directly targeting NEFL. Mol Cell Biochem. 2015;409:103–11.
36. Ricard D, Idbaih A, Ducray F, Lahutte M, Hoang-Xuan K, Delattre JY. Primary brain tumours in adults. Lancet. 2012;379:1984–96.
37. Sa L, Li Y, Zhao L, Liu Y, Wang P, Liu L, Li Z, Ma J, Cai H, Xue Y. The Role of HOTAIR/miR-148b-3p/USF1 on Regulating the Permeability of BTB. Front Mol Neurosci. 2017;10:194.
38. Sano H, Peck GR, Kettenbach AN, Gerber SA, Lienhard GE. Insulin-stimulated GLUT4 protein translocation in adipocytes requires the Rab10 guanine nucleotide exchange factor Dennd4C. J Biol Chem. 2011;286:16541–5.
39. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017;67:7–30.
40. Stupp R, Hegi ME, Mason WP, Van Den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO, European Organisation For R, Treatment of Cancer Brain T, Radiation Oncology G, and National Cancer Institute of Canada Clinical Trials G (2009). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10: 459–466.
41. Su J, Long W, Ma Q, Xiao K, Li Y, Xiao Q, Peng G, Yuan J, Liu Q. Identification of a Tumor Microenvironment-Related Eight-Gene Signature for Predicting Prognosis in Lower-Grade Gliomas. Front Genet. 2019;10:1143.
42. Su J, Ma Q, Long W, Tang H, Wu C, Luo M, Wang X, Xiao K, Li Y, Xiao Q, Zhang C, Li H, Liu Q. LCTL Is a Prognostic Biomarker and Correlates With Stromal and Immune Infiltration in Gliomas. Front Oncol. 2019;9:1083.
43. Sun Z, Guo X, Zang M, Wang P, Xue S, Chen G. Long non-coding RNA LINC00152 promotes cell growth and invasion of papillary thyroid carcinoma by regulating the miR-497/BDNF axis. J Cell Physiol. 2019;234:1336–45.
44. Teng W, Qiu C, He Z, Wang G, Xue Y, Hui X. Linc00152 suppresses apoptosis and promotes migration by sponging miR-4767 in vascular endothelial cells. Oncotarget. 2017;8:85014–23.

45. Wang W, Jia WD, Hu B, Pan YY. RAB10 overexpression promotes tumor growth and indicates poor prognosis of hepatocellular carcinoma. Oncotarget. 2017;8:26434–47.
46. Wang Z, Guo Q, Wang R, Xu G, Li P, Sun Y, She X, Liu Q, Chen Q, Yu Z, Liu C, Xiong J, Li G, Wu M. The D Domain of LRRC4 anchors ERK1/2 in the cytoplasm and competitively inhibits MEK/ERK activation in glioma cells. J Hematol Oncol. 2016;9:130.
47. Wang Z, Yang J, Xu G, Wang W, Liu C, Yang H, Yu Z, Lei Q, Xiao L, Xiong J, Zeng L, Xiang J, Ma J, Li G, Wu M. Targeting miR-381-NEFL axis sensitizes glioblastoma cells to temozolomide by regulating stemness factors and multidrug resistance factors. Oncotarget. 2015;6:3147–64.
48. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med. 2008;359:492–507.
49. Wu X, Wang Y, Yu T, Nie E, Hu Q, Wu W, Zhi T, Jiang K, Wang X, Lu X, Li H, Liu N, Zhang J, You Y. Blocking MIR155HG/miR-155 axis inhibits mesenchymal transition in glioma. Neuro Oncol. 2017;19:1195–205.
50. Wu Y, Tan C, Weng WW, Deng Y, Zhang QY, Yang XQ, Gan HL, Wang T, Zhang PP, Xu MD, Wang YQ, Wang CF. Long non-coding RNA Linc00152 is a positive prognostic factor for and demonstrates malignant biological behavior in clear cell renal cell carcinoma. Am J Cancer Res. 2016;6:285–99.
51. Yu H, Xue Y, Wang P, Liu X, Ma J, Zheng J, Li Z, Li Z, Cai H, Liu Y. Knockdown of long non-coding RNA XIST increases blood-tumor barrier permeability and inhibits glioma angiogenesis by targeting miR-137. Oncogenesis. 2017;6:e303.
52. Yu M, Xue Y, Zheng J, Liu X, Yu H, Liu L, Li Z, Liu Y. Linc00152 promotes malignant progression of glioma stem cells by regulating miR-103a-3p/FEZF1/CDC25A pathway. Mol Cancer. 2017;16:110.
53. Yu Y, Yang J, Li Q, Xu B, Lian Y, Miao L. (2017). LINC00152: A pivotal oncogenic long non-coding RNA in human cancers. Cell Prolif 50.
54. Yuan J, Xiao G, Peng G, Liu D, Wang Z, Liao Y, Liu Q, Wu M, Yuan X. MiRNA-125a-5p inhibits glioblastoma cell proliferation and promotes cell differentiation by targeting TAZ. Biochem Biophys Res Commun. 2015;457:171–6.
55. Zhang JX, Han L, Bao ZS, Wang YY, Chen LY, Yan W, Yu SZ, Pu PY, Liu N, You YP, Jiang T, Kang CS, Glioma Cooperative C G. HOTAIR, a cell cycle-associated long noncoding RNA and a strong predictor of survival, is preferentially expressed in classical and mesenchymal glioma. Neuro Oncol. 2013;15:1595–603.
56. Zhu Y, Zhang X, Qi L, Cai Y, Yang P, Xuan G, Jiang Y. HULC long noncoding RNA silencing suppresses angiogenesis by regulating ESM-1 via the PI3K/Akt/mTOR signaling pathway in human gliomas. Oncotarget. 2016;7:14429–40.

Tables

Table 1: Univariate and multivariable Cox regression analysis of overall survival based on TCGA dataset

	Univariate analysis		Multivariate analysis	
	HR (95% CI for HR)	P	HR (95% CI for HR)	P
LINC00152	1.645 (1.551-1.745)	***	1.206 (1.081-1.345)	***
gender male VS Female	1.225 (0.955-1.572)	0.110	-	-
Age ≤40 VS >40	0.231 (0.165-0.324)	***	0.3947 (0.2565-0.6074)	***
grade WHO II VS WHO III + IV	0.178 (0.121-0.262)	***	0.4497 (0.2768-0.7306)	**
IDH status WT VS MT	10.050 (7.524-13.43)	***	3.205 (1.863-5.514)	***
MGMT promoter status Methylation VS Unmethylation	3.228 (2.447-4.259)	***	-	-
Transcriptome subtype NE + PN VS CL+ME	0.152 (0.113-0.203)	***	1.012 (0.6335-1.618)	0.959

Abbreviations: HR, hazard ratio, CI, Confidence interval.

Figures

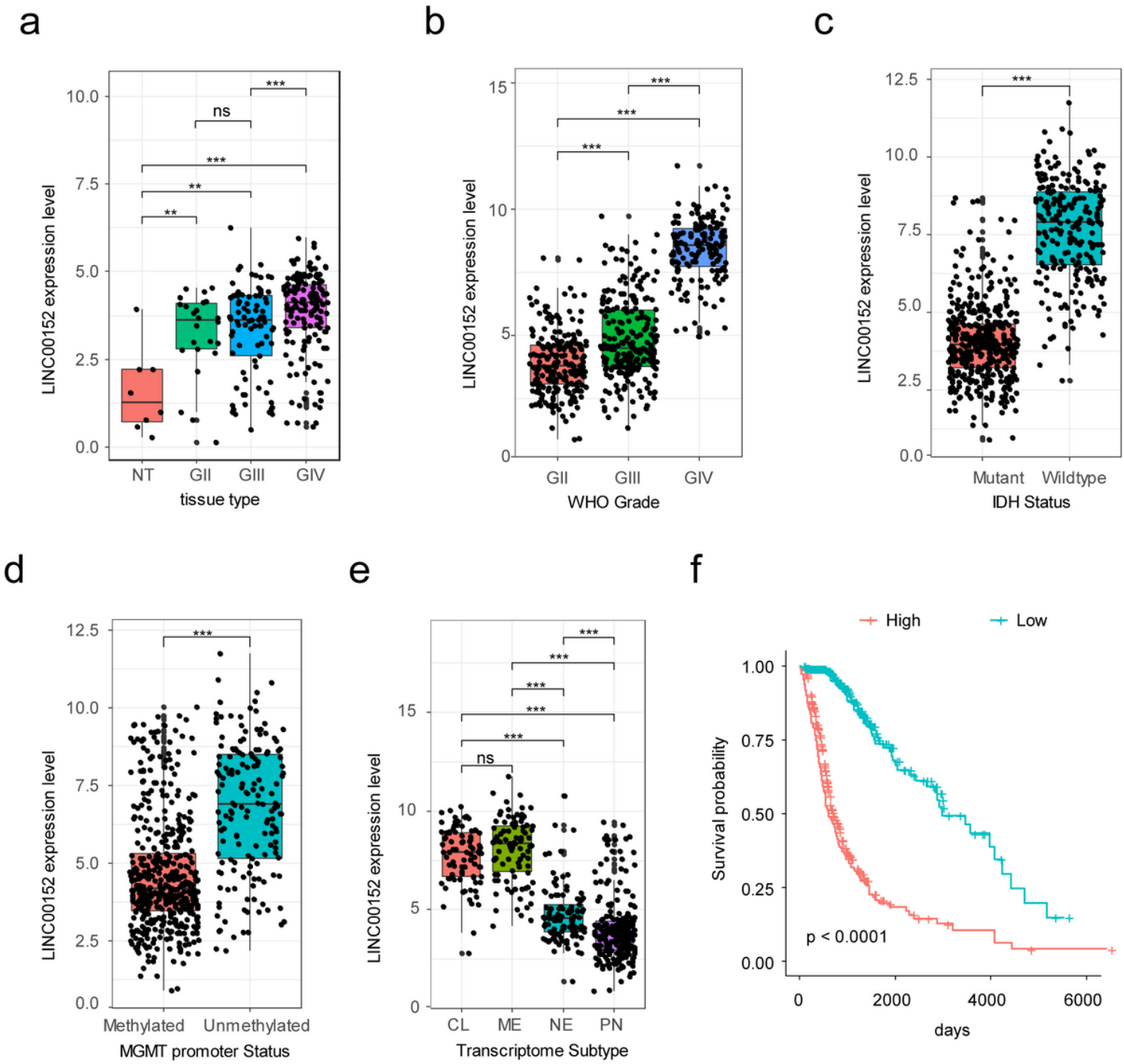

Figure 1

LINC00152 was significantly upregulated in gliomas and a prognostic factor for glioma patients a. The expression of LINC00152 between glioma tissues and normal brain tissues based on GSE16011 dataset. b. LINC00152 expression in glioma of WHO grade Ⅱ-Ⅳ based on the TCGA dataset. c. The expression of LINC00152 is significantly higher in IDH-Wildtype gliomas than that in IDH-Mutant disease based on the TCGA dataset. d. The expression of LINC00152 is significantly higher in gliomas with MGMT promoter unmethylation than that in gliomas with MGMT promoter methylation based on the TCGA dataset. e. LINC00152 expression pattern in different molecular subtypes of glioma (CL, ME, NE, PN) in the TCGA dataset. f. Kaplan-Meier curves for overall survival of patients with glioma with respect to levels of

LINC00152 expression, which showed that high LINC00152 expression predicts poor prognosis for glioma patients based on the TCGA dataset. CL: classical, ME: mesenchymal, NE: neural, PN: proneural, NT: normal brain tissue, GII: grade II, GIII: grade III, GIV: grade IV, * P<0.05; ** P<0.01; *** P<0.001

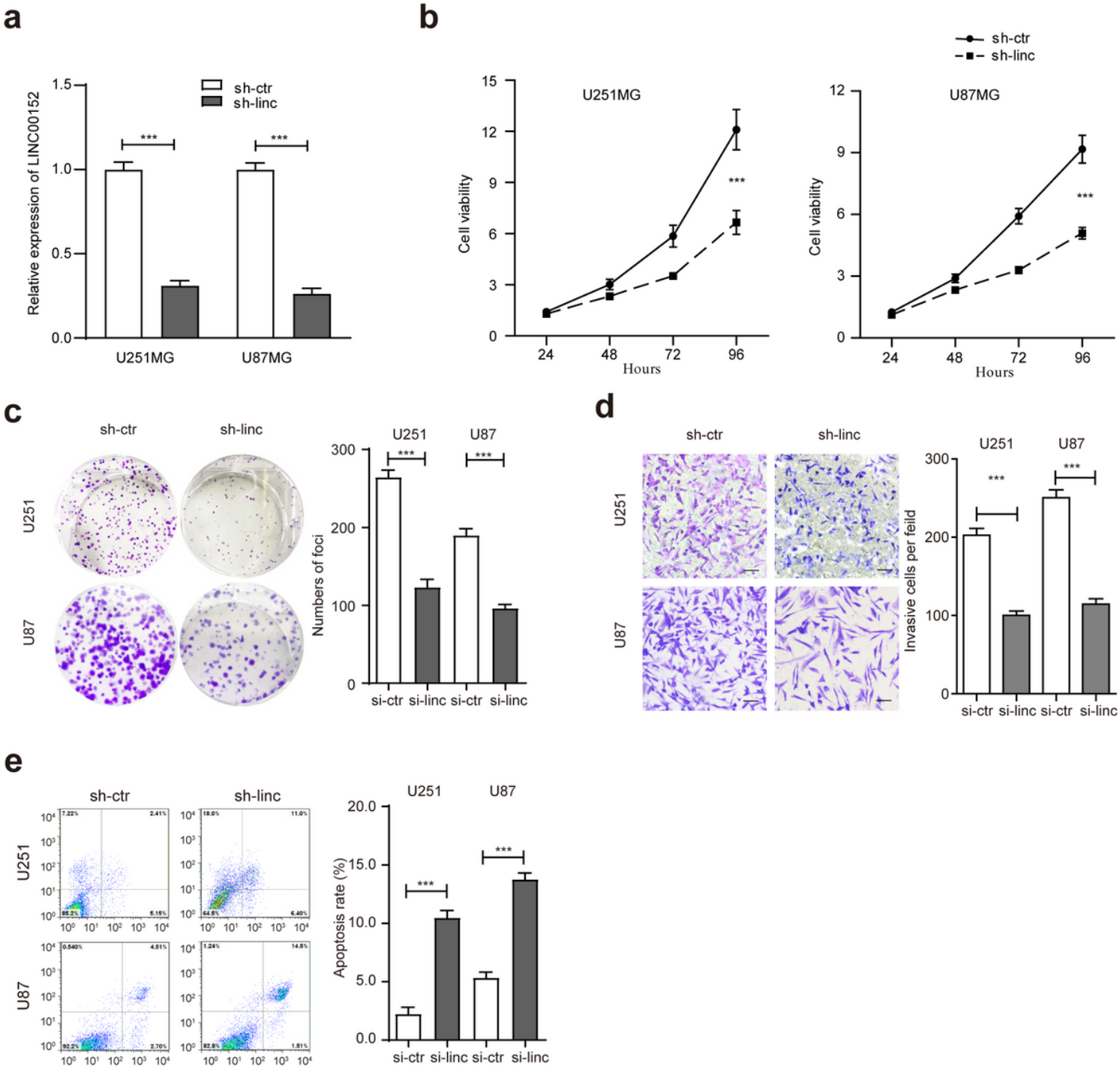

Figure 2

Knockdown of LINC00152 significantly inhibited proliferation, migration and invasion and induces apoptosis in vitro. a. Knockdown of LINC00152 expression using shRNA. shRNA dramatically suppressed LINC00152 expression at the RNA level compared with control shRNA (sh-ctr) in U251 and U87 cell lines, as determined by qRT-PCR. b. CCK-8 show reduced proliferation of U251 and U87 cells transfected with

shRNA LINC00152 (sh-linc) compared with sh-ctr. c. Knockdown of LINC00152 inhibited colony formation ability in vitro. d. Matrigel chamber invasion assay showed reduced invasion of U251 and U87 cells after transfection with shRNA LINC00152 compared with control shRNA. e. Flow cytometry analysis of apoptosis showed that the knockdown of LINC00152 in U251 and U87 cells increased the percentage of apoptotic cells compared with sh-ctr. * P<0.05; ** P<0.01; *** P<0.001

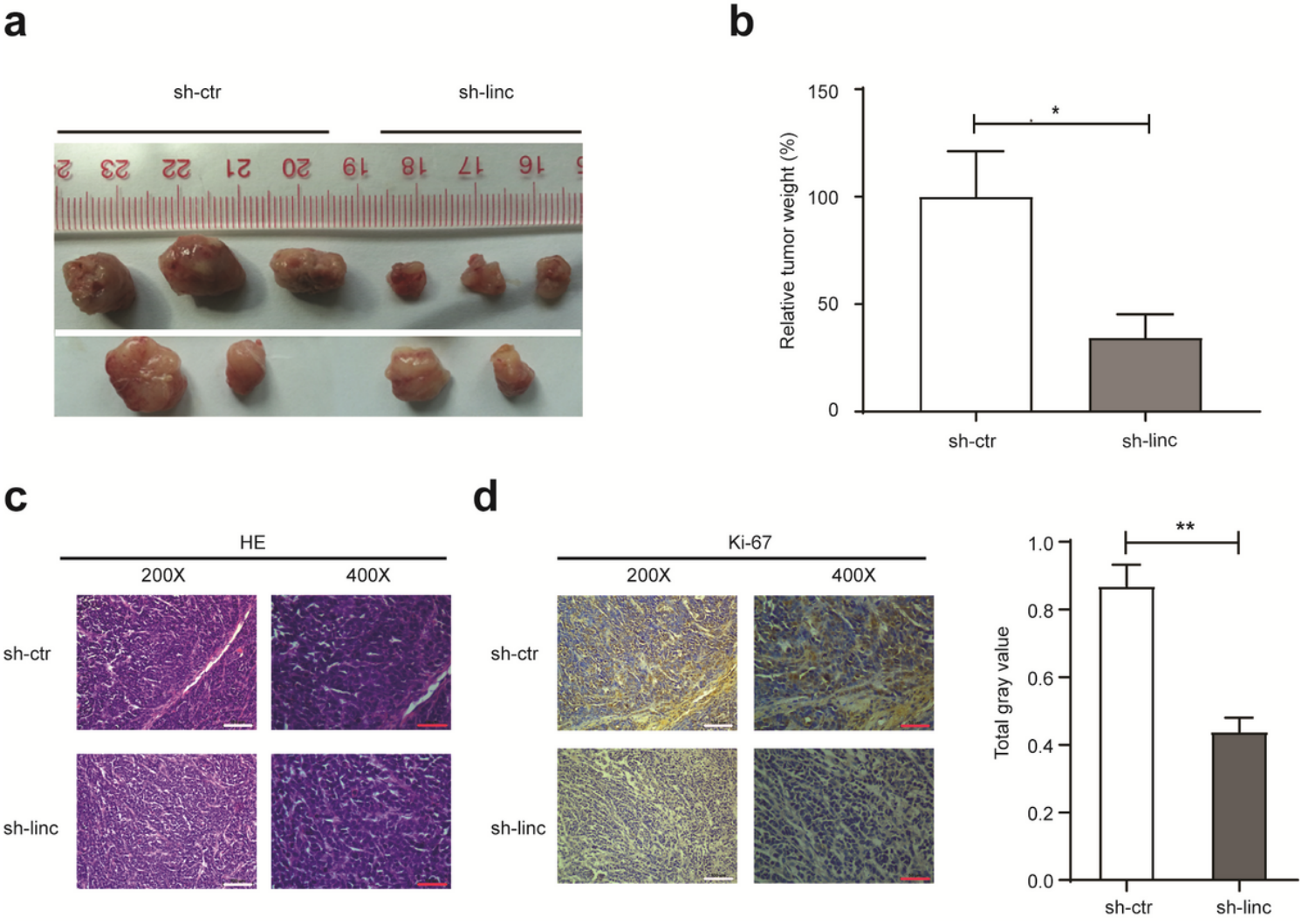

Figure 3

Knockdown of LINC00152 gene suppressed tumor growth in vivo. a. A representative picture of tumor xenograft morphology 35 d after injection. b. Tumor weights were measured after dissection from euthanized mice. c. H&E staining of xenograft tumors isolated from mice that had been injected with U251 cells transfected with sh-ctr or sh-linc (×200 magnification). Metastasis nodules are indicated by arrows. d. Representative images of Ki-67 immunohistochemistry from xenograft tumors (×200 magnification). * P<0.05; ** P<0.01; *** P<0.001; value of red bar and white bar were 50μm and 100μm respectively.

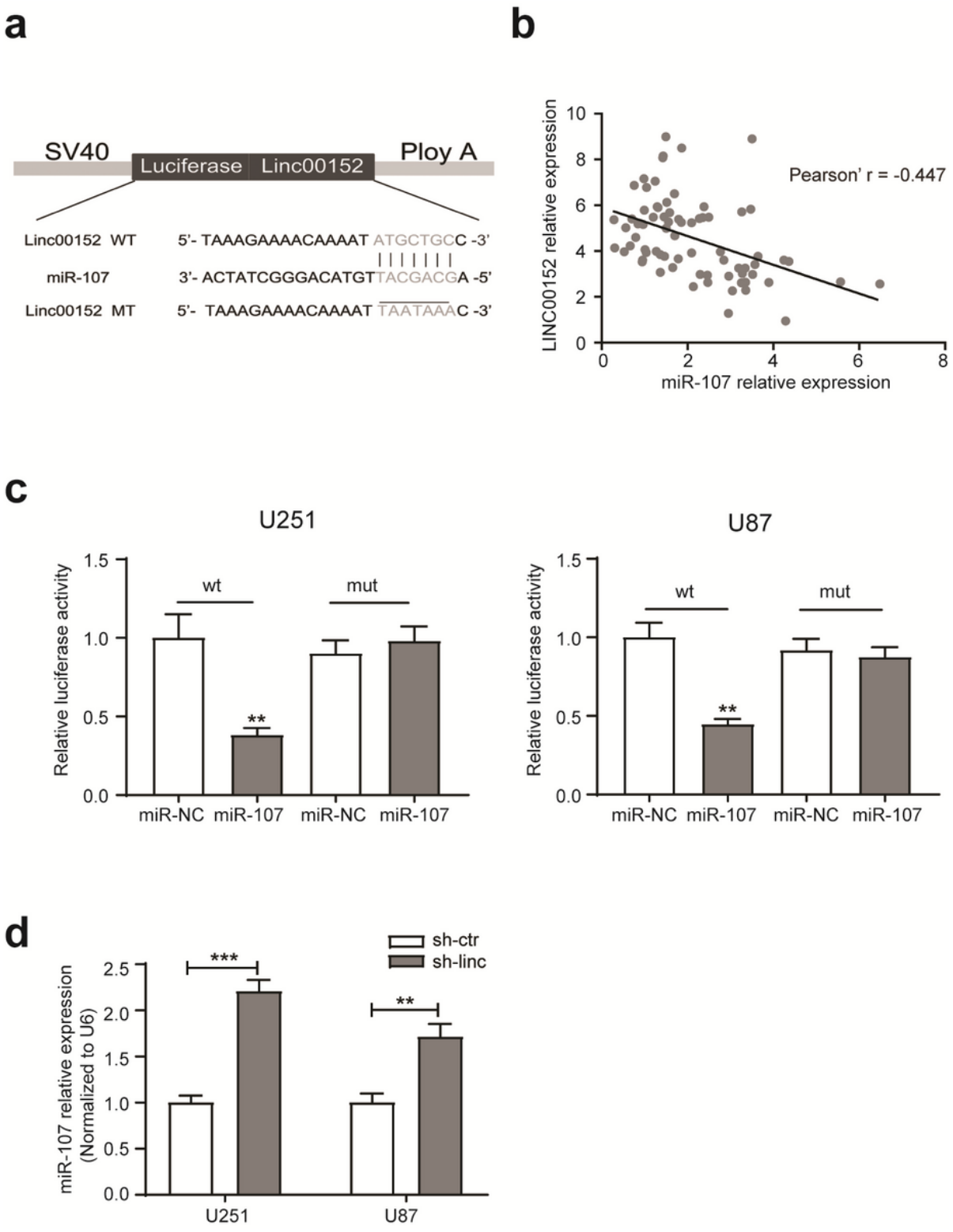

Figure 4

LINC00152 was a direct target of miR-107. a. Depiction of putative miR-107 binding sites on LINC00152 sequence. LINC00152 cDNA containing the putative miRNA recognition sites was cloned downstream of luciferase gene. b. Correlation analysis of the expression of LINC00152 and MIR-107 in glioma tissues. c. Luciferase assays of U251 and U87 cells co-transfected with either pMIR-REPORT-WT/MT LINC00152

and miR-107 or negative control as indicated. d. mRNA levels of miR-107 in U251 and U87 cells transfected with LINC00152 shRNA or control shRNA. * P<0.05; ** P<0.01; *** P<0.001.

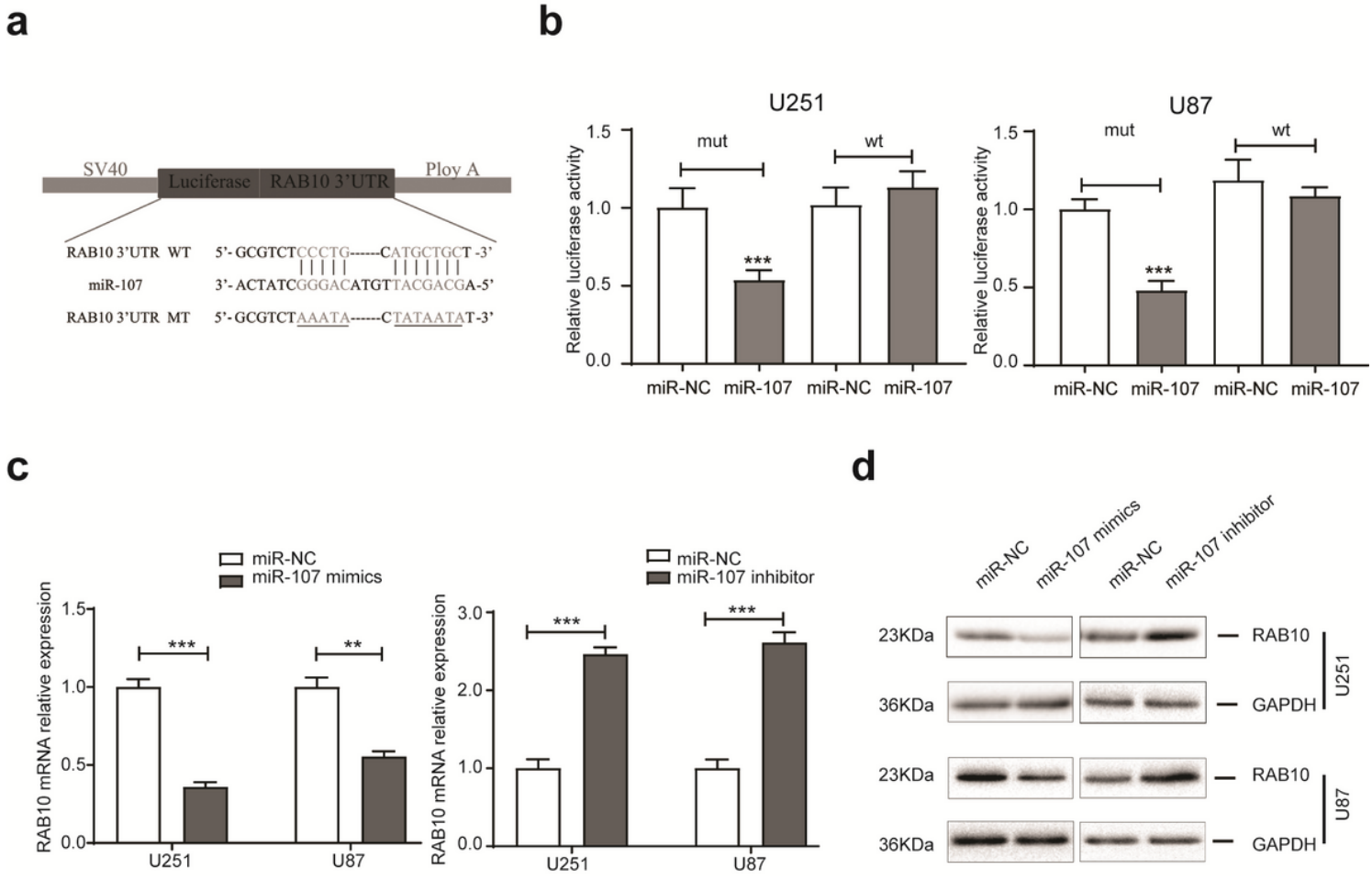

Figure 5

RAB10 was a direct target of miR-107. a. Depiction of miR-107 interaction sites in the 3'-UTR of RAB10. The 3'-UTR of a RAB10 cDNA containing miR-107 putative recognition sites was cloned 3' downstream of firefly luciferase gene. b. Luciferase assays of U251 and U87 cells co-transfected with either pMIR-REPORT-WT/MT MALAT1 and miR-107 or negative controls as indicated. c. qRT-PCR showing mRNA levels of RAB10 24 h after miR-107 mimics were transfected into U251 and U87 cells. d. Western blot analysis showing protein levels of RAB10 24 h after miR-107 mimics or inhibitor were transfected into glioma cell lines. * P<0.05; ** P<0.01; *** P<0.001

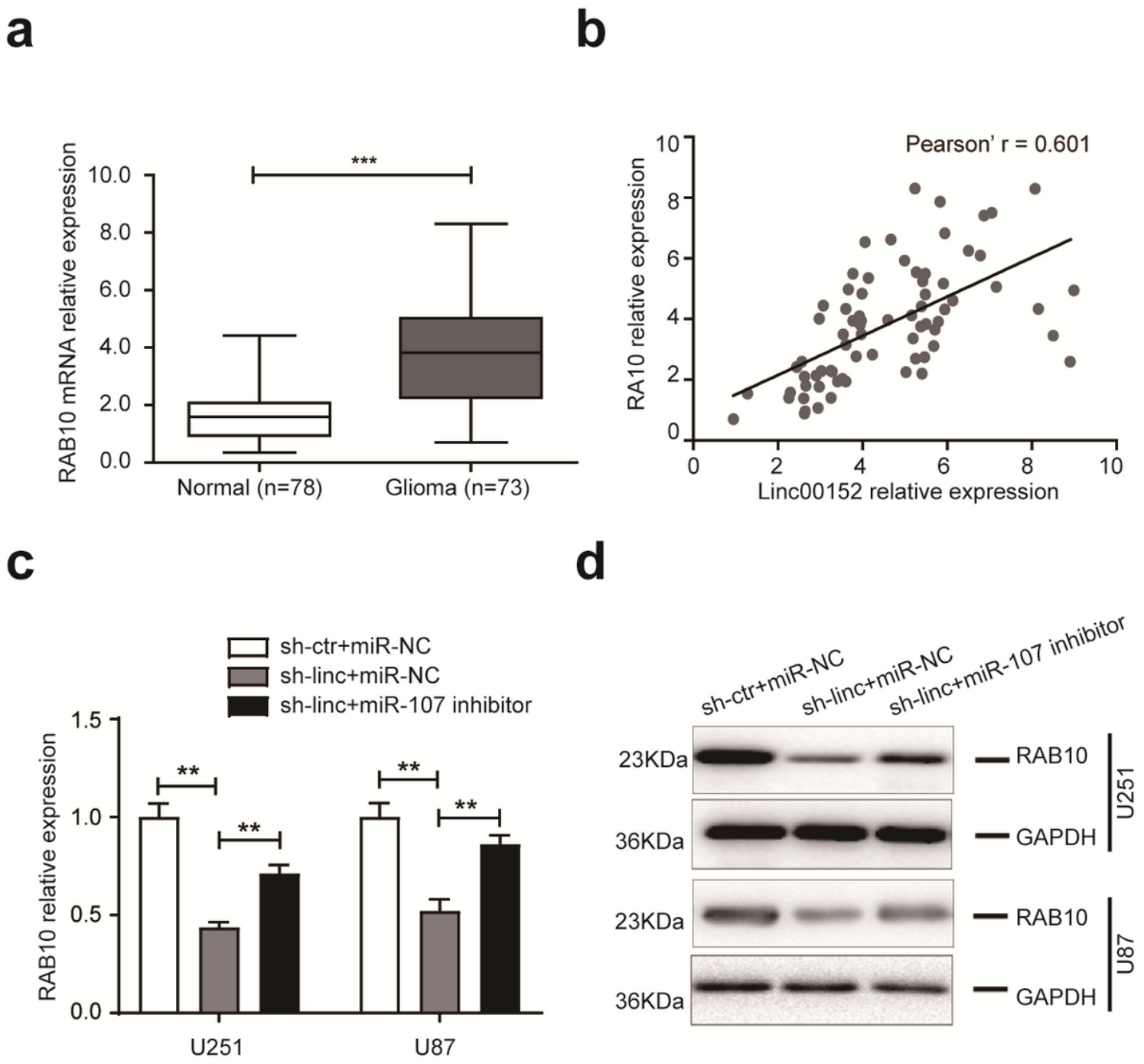

Figure 6

LINC00152 regulated expression of RAB10 gene through miR-107. a. Relative expression of RAB10 in glioma tissues (n=73) compared with that in normal brain tissues (n=78). RAB10 expression is determined using qRT-PCR and normalized to GAPDH expression levels. b. Correlation analysis of the expression of LINC00152 and RAB10 in glioma tissues. c. qRT-PCR showing mRNA levels of RAB10. Down-regulation of LINC00152 expression in glioma cell lines decreased mRNA levels of RAB10 and miR-107 inhibitor reversed this effect. d. Western blot analysis showing RAB10 protein levels. The addition of miR-107 inhibitor reversed the effects of LINC00152 shRNA in U251 and U87 cells. * P<0.05; ** P<0.01; *** P<0.001

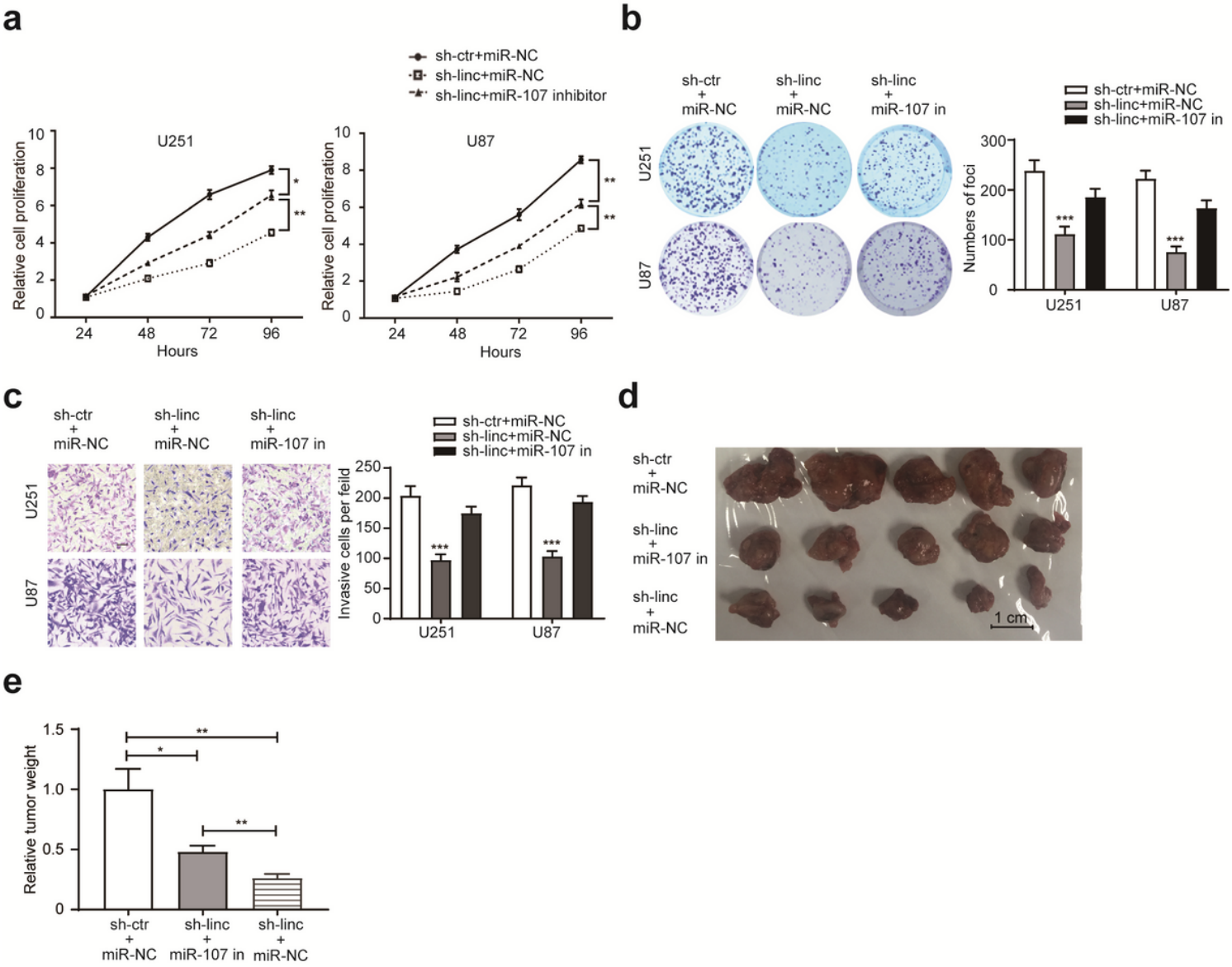

Figure 7

The effects induced by LINC00152 knockdown were reversed by miR-107 inhibition. a. CCK-8 assay showed that down-regulation of miR-107 partially reversed the inhibitory effect of proliferation induced by LINC00152 shRNA on U251 and U87 cell lines. b. Colony formation assay showed that down-regulation of miR-107 reversed the effects of LINC00152 shRNA on U251 and U87 cell lines. The miR-107 inhibitor increased the colony formation of U251 and U87 cells and reversed the effect of LINC00152 shRNA. c. Matrigel chamber invasion and migration assay showing reduced invasion of U251 and U87 cell lines after transfection with LINC00152 shRNA. The miR-107 inhibitor increased the invasion of U251 and U87 cells and reversed the effect of LINC00152 shRNA. d. A representative picture of tumor xenograft morphology 35 d after injection. e. Tumor weights were measured after dissection from euthanized mice. * P<0.05; ** P<0.01; *** P<0.001

Supplementary Files

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

- OnlineSupplementaryFig1.png