# LINC02418 promotes colon cancer progression by suppressing apoptosis via interaction with miR-34b-5p/BCL2 axis

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## Abstract

# Background

LncRNAs have been demonstrated to be functional regulators in tumor progression through interaction with various signaling pathways in multiple cancer types. However, the effect of LINC02418 on CRC progression still remains unclear.

# Methods

LncRNA expression profile in CRC tissues was explored by using the TCGA database. The expressional level of LINC02418 in CRC patients was confirmed by qRT-PCR. Kaplan-Meier analyses was used to investigate the correlations between LINC02418 and OS of patients with CRC. After stably transducing sh-LINC02418 and sh-NC into HCT116 and LoVo cells, cell proliferative, migratory and invasive abilities were detected by CCK-8 assay, colony formation assay and trans-well assay, respectively. The binding between LINC02418 and miR-34b-5p, and the interaction between miR-34b-5p and BCL2 were determined by dual-luciferase assay. Western blot experiments were conducted to further explore the effect of miR-34b-5p on BCL2 signaling pathway. Rescue experiments were performed to uncover the role of LINC02418 /miR-34b-5p/ BCL2 axis in CRC progression.

# Results

LINC02418 was upregulated in human colon cancer samples and its high expression correlated with poor prognosis. LINC02418 promoted cancer progression by enhancing tumor growth, cell mobility and invasiveness of colon cancer cells. Additionally, LINC02418 could physically bind to miR-34b-5p and subsequently interact with BCL2 signaling pathway. Down-regulation of LINC02418 reduced cell proliferation, but transfection of miR-34b-5p inhibitor or BCL2 in LINC02418-silenced colon cancer cells significantly promoted cell growth.

# Conclusions

LINC02418 was upregulated in human colon cancer samples and could be used as indicator for prediction of prognosis. LINC02418 acted as a tumor driver by negatively regulating cell apoptosis through LINC02418 /miR-34b-5p/ BCL2 axis and in colorectal cancer.

## Background

Colorectal cancer (CRC), also known as colon cancer, is one of the most common malignancies worldwide. Although improvements have been made in the diagnosis and treatment, the colon cancer remains the top leading cause of cancer-related death [1, 2]. The metastasis usually occurs in late stage

of CRC in which tumor cells detach from the primary tumor, invade into surrounding tissue, migrate and colonize at distant organs such as liver and lung. Metastasis is the main cause of CRC-related death, thus uncovering the molecular mechanisms underlying and identification of new diagnostic markers is emerging need in current CRC studies. The progress of normal intestinal epithelial to CRC is a multi-stage and complicated process which is associated with the accumulation of both genetic and epigenetic changes. The aberration, mutations of several suppressive genes or oncogenes, and epigenetic alteration contribute to the progression of CRCs [3–6].

Non-coding RNA is the most abundant transcripts of genome, which are further divided into small ncRNA and long non-coding RNA (lncRNAs) according to transcripts length. LncRNAs are defined as a class of RNA transcripts with length over 200 nucleotides and lack of protein-coding capacity, many of which exhibit specific cell-type and developmental-stage expression pattern[7, 8]. Emerging studies have found that lncRNA plays crucial roles in a variety of cellular events, including transcriptional regulation of genes, cell proliferation, cell differentiation, cell cycle and apoptosis[9–11]. To date, mounting literatures have reported that lncRNAs level is dysregulated in development and progression of numerous cancer, which implicated that lncRNAs can serve as indicators for diagnosis and patient outcomes, and targets for therapeutic strategies[12–15].

MicroRNAs are single-strand RNAs (18 ~ 22nt), which bind to seed sequences of 3’-untraslated regions (UTRs) of target genes to mediate translation inhibition[16]. Accumulating researches have demonstrated that miRNAs participate in cell apoptosis, differentiation and metabolism. They also have both positive and negative effect on tumor invasion and metastasis [17–19].

Growing documents proved that the aberrant lncRNA expression is involved in carcinogenesis, tumor metastasis in diverse cancer types. The mechanism study revealed that lncRNAs exert their role in gene expression network by affecting chromatin modification, mRNA transcription and interacting with RNA binding proteins [20–22]. In addition, lncRNAs also act as “miRNA sponges” and sequester miRNAs to modify miRNAs target genes transcription, which has been identified as the lncRNA-miRNA-mRNA regulatory network in cancer tumorigenesis and progression [23, 24]. Although several of these lncRNAs have been annotated in the past decades, the role and potential regulatory mechanisms of lncRNAs in colon cancer still need to be clearly clarified [25].

In the present study, we investigated lncRNAs expression profile in colon cancer by RNA sequencing (RNA-seq) based on Cancer Genome Atlas (TCGA) and characterized the role of long non-coding RNA LINC02418 as a novel oncogene in colon cancer. Mechanistic analysis revealed that LINC02418 acts as competing endogenous RNA (ceRNA) for miR-34b-5p to prevent degradation of BCL gene. Our results highlighted LINC02418 /miR-34b-5p/ BCL2 axis might be a promising therapeutic target for CRC treatment.

# Materials And Methods

# Clinical specimens

Tumor tissues and adjacent tissues from CRC patients ($n$ = 20) were collected from China-Japan Union Hospital of Jilin University and all the participants signed the consent forms. The project was authorized by the Ethical and Scientific Committee of China-Japan Union Hospital of Jilin University. All samples were stored at -80 °C until subsequent analysis.

# Cell culture

Human CRC cell lines including SW460, HCT116, HT-29, LoVo, Colo205, SW480 and normal colon epithelial cell line NCM460 were obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in RPMI 1640 medium supplmented with 10% fetal bovine serum (Gibco, Carlsbad, CA), 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were maintained at 37 °C in an incubator with 5% $CO^2$.

# Cells transfection

The sequence of BCL2 was inserted into vector pcDNA3.1 (Santa Cruz, Dallas, TX) for its ectopic expression in HCT116 and LoVo cell lines. Constructions, miR-34b-5p mimic, miR-34b-5p inhibitor miR-NC mimic were delivered into CRC cells by Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) according to manufactures instructions.

# Establishment of stable cell lines

Three shRNAs sequences targeting LINC02418 and negative control sequence were inserted into HuSH shRNA GFP Lenti Cloning Vector (Origene, Rockville, MD) following commercial guidance. The lentivirus was packaged with usage of 293T cells following common protocol.

# Cell counting kit-8 analysis and colony formation assay

For CCK-8 experiment, the cells were seeded at density of $5 \times 10^4$ cells per well on 48-well plate. Cells were harvested at 12 h, 24 h, 48 h and 72 h, and cell proliferation was assessed by using cell counting kit-8 (Beyotime, Shanghai, CHA) according to the manufacturer's instructions. The optical density (OD) at 450 nm was measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA).

For colony formation assay. Total number of 3000 cells were seeded in 6-well plates and maintained in RPMI 1640 medium containing 10% FBS. After culture of 14 days, cells were fixed with 4% paraformaldehyde for 15 min and then stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO) for another 5 min till manually counting of visible colonies.

# Xenograft assay

Male BALB/c nude mice of 5 weeks old were maintained under specific pathogen-free facility and were manipulated according to protocols approved by the China-Japan Union Hospital of Jilin University. HCT116-sh-LINC02418, HCT116-sh-NC, LoVo-sh-LINC02418 and LoVo-sh-NC cells ($2 \times 10^6$cells) were

inoculated subcutaneously in the right flank of the nude mice. After 7 days post injection, tumor size was measured every 3 days and the tumor volumes were recorded. After 21 days post injection, mice were sacrificed by cervical dislocation.

# Transwell migration and invasion assay

Cells migration assay was performed in a 24-well transwell chambers (CoStar, Badhoevedorp, Netherlands). Cells were plated and allowed to migrate through 8 μm-pore sized polycarbonate membrane. The chamber for invasion assay was pre-coated with 1 mg/ml Matrigel (Sigma-Aldrich, St. Louis, MO). A number of $5 \times 10^4$ cells were added to the upper chamber of the transwells and the lower chamber was filled with 500 μl RPMI 1640 medium containing 10% FBS. After 24 h incubation, the cells were fixed by 4% formaldehyde for 10 min, stained by 0.1% crystal violet for 20 min. Images were captured under microscope.

# Quantitative real time PCR (qRT-PCR) assay

Total RNA was extracted from clinical tissue and CRC cells by TRIzol reagent (Invitrogen, Carlsbad, CA). RNA reverse transcription was performed using Prime ScriptTM RT Master Mix and qPCR was carried out by SYBR Premix Ex Taq II (TaKaRa Biotechnology, Dalian, China). Applied Biosystems 7900 Real-Time PCR System (Applied Biosystems, Foster City, CA) was used for RNA quantification assay. U6 and GAPDH were used as internal control of miRNAs and mRNAs, respectively. Fold change was calculated by the $2^{-\triangle\triangle Ct}$ method.

# Luciferase assay

The fragments containing the binding sites or the mutated sites were synthesized and inserted into a pGL3-basic vector for dual luciferase assay. HCT116 and LoVo Cells were seeded in a 12-well plate and co-transfected with reporter plasmids and miR-NC/miR-34b-5p mimic. After 48 hours, cells were harvested, dual-luciferase reporter assays were performed according to the protocol by using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI) on a GloMax 20/20 luminometer (Promega, Madison, WI).

# Western Blot

Protein samples were separated on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride membranes (PVDF) (Millipore, Billerica, MA). The membranes were blocked in 5% nonfat milk. Primary antibodies including anti-GAPDH, anti-BCL2, anti-Caspase 9, anti-cleaved-Caspase 9, anti-Caspase 3, and anti-cleaved-Caspase 3 were used for incubation overnight at 4 °C. The membranes were incubated with the secondary antibody at room temperature the next day and the signal was detected by Pierce Fast Western Blot Kit (Thermo Fisher Scientific, Waltham, MA).

# Statistical analysis

Statistical data analysis was conducted using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA). Experiments were carried out in triplicate and the data was displayed as mean ± SD. Statistical analysis was conducted using Student's *t*-test or one-way analysis of variance. Statistical significance was considered when $p < 0.05$.

# Results

### LINC02418 expression is upregulated and associated with poor prognosis in CRC patients

To identify the LncRNA expression profile in CRC patients, we examined the expressional level of LncRNAs in human CRC samples and normal intestinal tissue. By using the Cancer Genome Atlas (TCGA) database, we found LINC02418 abundance was significantly up-regulated in CRC samples ($n = 478$) when compared with normal tissue ($n = 41$) (Fig. 1a).To validate the results, LINC02418 level in 20 pairs of CRC samples and adjacent tissue were examined by RT-qPCR. Similarly, quantification test showed LINC02418 was highly expressed in 19 out of the 20 CRC patients (Fig. 1b). These results implied that expression of LINC02418 in CRC tissues was markedly higher than that in normal tissues. Moreover, the expression of LINC02418 is increased by 2–6 folds in SW620, HCT116, LoVo, Colo205, HT-29 and SW480 cell line when compared with normal colon epithelial cell line NCM460 (Fig. 1c). The HCT116 and LoVo cell lines were chosen for further experiments, because they had the highest level of LINC02418.

Next, to study the clinical significance of LINC02418 in CRC, the correlation between LINC02418 expression and clinicopathologic characteristics was assessed by Kaplan-Meier survival assay. As shown in Fig. 1d and e, in both early (Stage 1–2) and late stages (Stage 3–4), CRC patients with high expression of LINC02418 had poorer overall survival (OS) rate, which indicate LINC02418 is one potential indicator for prognosis prediction and LINC02418 may exert promoter role in CRC progress.

### Knock-down of LINC02418 inhibits tumor growth, cell mobility and cell invasion

In order to dissect the effect of LINC02418 on the biologic activity of CRC cell lines, HCT116 and LoVo cells stably transduced with shLINC02418 were established. Based on the endogenous level of LINC02418, HCT116-sh-LINC02418#1 and LoVo-sh-LINC02418#1 were selected for further analysis (Fig. 2a). The CCK-8 assay revealed that compared with sh-NC transduced CRC cells and blank control (without shRNA transducing) group, stable knockdown of LINC02418 statistically inhibited the proliferation of HCT116 and LoVo cells (Fig. 2b). Colony formation assay was carried out to further analyze the effect of LINC02418 on tumor cell growth. As revealed in Fig. 2c, stable down-regulation of LINC02418 significantly reduced the proliferation of HCT116 and LoVo cells.

To evaluate whether LINC02418 affect CRC growth in vivo, subcutaneous tumor formation experiment was set up in nude mice. HCT116 and LoVo cells transduced with sh-LINC02418 or sh-NC were subcutaneously injected into the nude mice for 21 days and tumor size was recorded at indicated days. As shown in Fig. 2d and e, tumor size of xenograft tumor from sh-LINC02418-transduced HCT116 and

LoVo cells were obviously less than that in sh-NC-transduced cells, which was consistent with *in vitro* experiments.

The transwell experiment showed the stable inhibition of LINC02418 significantly repressed mobility and invasiveness of HCT116 and LoVo cells (Fig. 2f). Taken together, these data suggested that LINC02418 contributed to CRC cell growth and metastasis *in vivo* and *in vitro*.

### LINC02418 acts as a ceRNA for miR-34b-5p in CRC cells

It is documented that LncRNAs could exert their function trough acting as ceRNA of miRNAs [26]. The lncRNA-miRNA-mRNA regulatory network has been proved participate in cancer malignization. To assess whether LINC02418 interacted with other miRNAs in CRC, the potential binding partner for LINC02418 was analyzed by online software StarBase v2.0. As shown in Fig. 3a, LINC02418 contained putative binding sequence for miR-34b-5p.

To confirm the binding between LINC02418 and miR-34b-5p, dual luciferase assays were performed in both HCT116 and LoVo cell lines. The luciferase activity was markedly inhibited when LINC02418-WT and miR-34b-5p mimic were co-transfected into HCT116 and LoVo cells. Co-transfection of LINC02418-WT and miR-NC and co-transfection of LINC02418-MUT and miR-34b-5p mimic had no statistical impact on luciferase activity (Fig. 3b). In addition, knockdown of LINC02418 in HCT116 and LoVo cells greatly enhanced the expression level of miR-34b-5p (Fig. 3c).Taken together, it could be found that LINC02418 negatively regulated miR-34b-5p expression in CRC cells.

Subsequently, we detected the relationship between LINC02418 and miR-34b-5p in clinical samples. In 20 pairs of CRC samples, LINC02418 expressional level negatively correlated with miR-34b-5p level (Fig. 3d, e).

### BCL2 is the target of miR-34b-5p in CRC cells

BCL2, the gatekeeper for cell apoptosis, was identified as one possible target for miR-34b-5p by using software TargetScan (Fig. 4a). Interestingly, previous articles reported that miR-34b-5p regulates multiple cellular processes including cell apoptosis and cell proliferation through participating in a several critical signal pathway like VEGF-A, BCL2 and Notch [27]. To determine the interaction between BCL2 and miR-34b-5p, wild type 3'UTR (containing miR-34b–5p recognition site) and the mutant 3'UTR of *BCL2* were cloned into luciferase reporter plasmid. Dual-luciferase assay showed that miR-34b-5p mimic transfection reduced luciferase activity in wild type construction but not in mutant type construction (Fig. 4b, c). In parallel, miR-34b-5p transfection reduced endogenous protein level of BCL2, while miR-34b-5p inhibitor addition significantly restored BCL protein expression in both HCT116 and LoVo cells (Fig. 4d). These results proved that BCL2 was one of the target genes of miR-34b-5p. Moreover, quantification of BCL2 in 20 pairs of CRC samples revealed that BCL2 expressional level was positively correlated with the amount LINC02418 (Fig. 4e, f).

### LINC02418 promotes colon cancer cell proliferation by upregulating BCL2 via sponging miR-34b-5p

To elucidate the effect of LINC02418 on miR-34b-5p/BCL2 axis, protein level of several apoptotic markers were detected in HCT116 and LoVo cells. Western blot analysis showed that knockdown of LINC02418 greatly enhanced the amount of cleaved-Caspase 9 and cleaved-Caspase 3, and strongly reduced BCL2 expression. Transfection of miR-34b-5p inhibitor into cells with defective expression of LINC02418 not only reduced the level of cleaved forms of Caspase 9 and Caspase 3, but also promoted protein expression of BCL2.Overexpression of BCL2 also reduced protein level of cleaved-Caspase 9 and cleaved-Caspase 3 (Fig. 5a). Subsequently, CCK-8 and colony formation assays showed inhibition of LINC02418 repressed cell growth in HCT116 and LoVo cells. However, transfection of miR-34b-5p inhibitor and overexpression of BCL2 abolished the effect of LINC02418 depletion on cell proliferation (Fig. 5b, c). These findings demonstrated that LINC02418 regulated colon cancer cell proliferation through upregulating BCL2 expression via sponging miR-34b-5p.

# Discussion

Colon cancer is one common malignancies of digestive tract and has been one of the most serious healthy threaten worldwide. There is urgent need to explore more effective early diagnostic indicators and treatment strategies.

In this study, with analyzing of TCGA database, we found LINC02418 level was up-regulated in CRC samples and cell lines and was associated with prognosis of CRC (Fig. 1). Subsequently, we identified the effect of LINC02418 on CRC cells growth, migration and invasion (Fig. 2). Through further bioinformatic screening and dual-luciferase assay, miR-34b-5p was found that could bind to both LINC02418 and *BCL2* gene and was negatively correlated with the amount of LINC02418 (Fig. 3 and Fig. 4). Finally, protein detection cell growth and colony formation experiments displayed that LINC02418 regulated CRC cells proliferation by regulating BCL2 level via sponging miR-34b-5p (Fig. 5).

Long non-coding RNAs and microRNAs play multiple roles in tumor progression in almost all organs. The tissue and cell expression specificity and high throughput detection technology make lncRNAs and miRNAs become potential diagnostic and therapeutic targets for clinical treatment [7, 23–25]. In CRC patients, the high expression of LINC02418 correlated with poor prognosis of CRC and negatively correlated with the amount of miR-34b-5p, indicating LINC02418 quantification could be considered as candidate indicator for CRC diagnosis and prognosis. If the expression of LINC02418 can be analyzed together with other clinical indicators, such as gender and age for comprehensive analysis, it may be possible to improve the accuracy of LINC02418 as a predictor in CRC.

Knockdown of LINC02418 decreased the CRC cell growth *in vitro* and *in vivo* and limited cell migration and invasion ability, indicating that LINC02418 was able to promote CRC progress and might be involved in tumor development-associated signaling pathways. MicroRNAs are a group of small non-coding RNAs which bind to cognate mRNA via base pairing and decrease the level of target gene either by translational repression or mRNA degradation[16]. Profound evidences suggested that miRNAs are dysregulated in a variety of cancer tissues and may play distinct roles depending on the type of cancer, the stage of the

disease, or the molecules with which it interacts [17–19, 28]. Through competitively binding to microRNAs, lncRNAs attenuate the regulatory effect of microRNAs on target genes. The lncRNA/microRNA/mRNA network has already been proved to be critical for cancer occurrence and development. Bioinformatic analysis revealed that LINC02418 and 3'UTR region of *BCL2* contained complementary sequence of miR-34b-5p, implying miR-34b-5p could bind to LINC02418 and *BCL2* gene. Dual-luciferase activity assays confirmed the interaction between miR-34b-5p and LINC02418 (Fig. 3) or 3'UTR region of *BCL2* (Fig. 4). Moreover, quantification assay determined that expressional level of miR-34b-5p was negatively correlated with the amount of LINC02418 and *BCL2* in CRC patients (Fig. 3), indicating the regulatory function of LINC02418/miR-34b-5p/BCL2 axis in CRC.

BCL2 is believed to suppress apoptosis in a variety of tissues and cancers. BCL2 can inhibit the release of cytochrome c and pro-apoptotic factors, so that the relevant factors are not able to reach the downstream caspase pathway to activate caspase 9 and caspase3.[29]. The positive correlation between LINC02418 and BCL2 expression level suggested dysregulation of apoptosis might be associated with the contribution of LINC02418 in CRC progression. Western blot experiments showed in the presence of sh-LINC02418, protein level of cleaved-Caspase 9 and cleaved-Caspase 3 in CRC cells significantly increased but BCL2 expression was inhibited, indicating silence of LINC02418 could improve cell apoptosis and reduce colon cancer cells growth (Fig. 5a). However, protein level of BCL2 in cells with less LINC02418 was restored by miR-34b-5p inhibitor transfection. The CRC cells growth was also compensated either by down-regulation of mir-34b-5p or ectopic expression of BCL2 protein (Fig. 5b, c).

Combining all the evidences from the study, we speculated that in normal intestinal epithelial tissue, LINC02418 stays in a low level which leads to the expression of miR-34b-5p as well as low expression of BCL2. As a consequence, cell apoptosis is activated. However, in human CRC cells, LINC02418 expression is upregulated and the expression of mir-34b-5p and BCL2 are affected by increased LINC02418 level. Thus, cell apoptosis is inhibited, allowing cancer cells escaping from cell death and re-entering abnormal cell cycles.

# Conclusion

Briefly, our present study demonstrated LINC02418 was upregulated in colon cancer, which promoted tumor cells growth and migration. LINC02418 could bind to miR-34b-5p to sequester the binding between miR-34b-5p and its target gene *BCL2*. Hence, LINC02418 positively regulated tumorigenesis through LINC02418/miR-34b-5p/BCL2 axis and might be indicated as a biomarker for CRC.

# Abbreviations

lncRNAs, long non-coding RNAs; CRC, colorectal cancer; miRNA, microRNA; CCK-8,  Cell Counting Kit-8; BCL2, B-cell lymphoma-2 (BCL2); ceRNA, competing endogenous RNA

# Declarations


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### Data Sharing Statement

The datasets used during the present study are available from the corresponding author on reasonable request

### Authors' contributions

JT and PC performed the experiments. CSL and JT were responsible for manuscript writing and revision. YFL offered assistance in clinical sample collection and analysis. XQY, XYW and ZRW provided technical assistance for the current study. All authors read and approved the manuscript for submission.

### Ethics approval and consent to participate

The present study was approved by the Ethical and Scientific Committee of China-Japan Union Hospital of Jilin University. All patients and healthy volunteers signed informed consent.

### Competing interests

There are no conflicts of interest.


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### Consent for publication

Not applicable.

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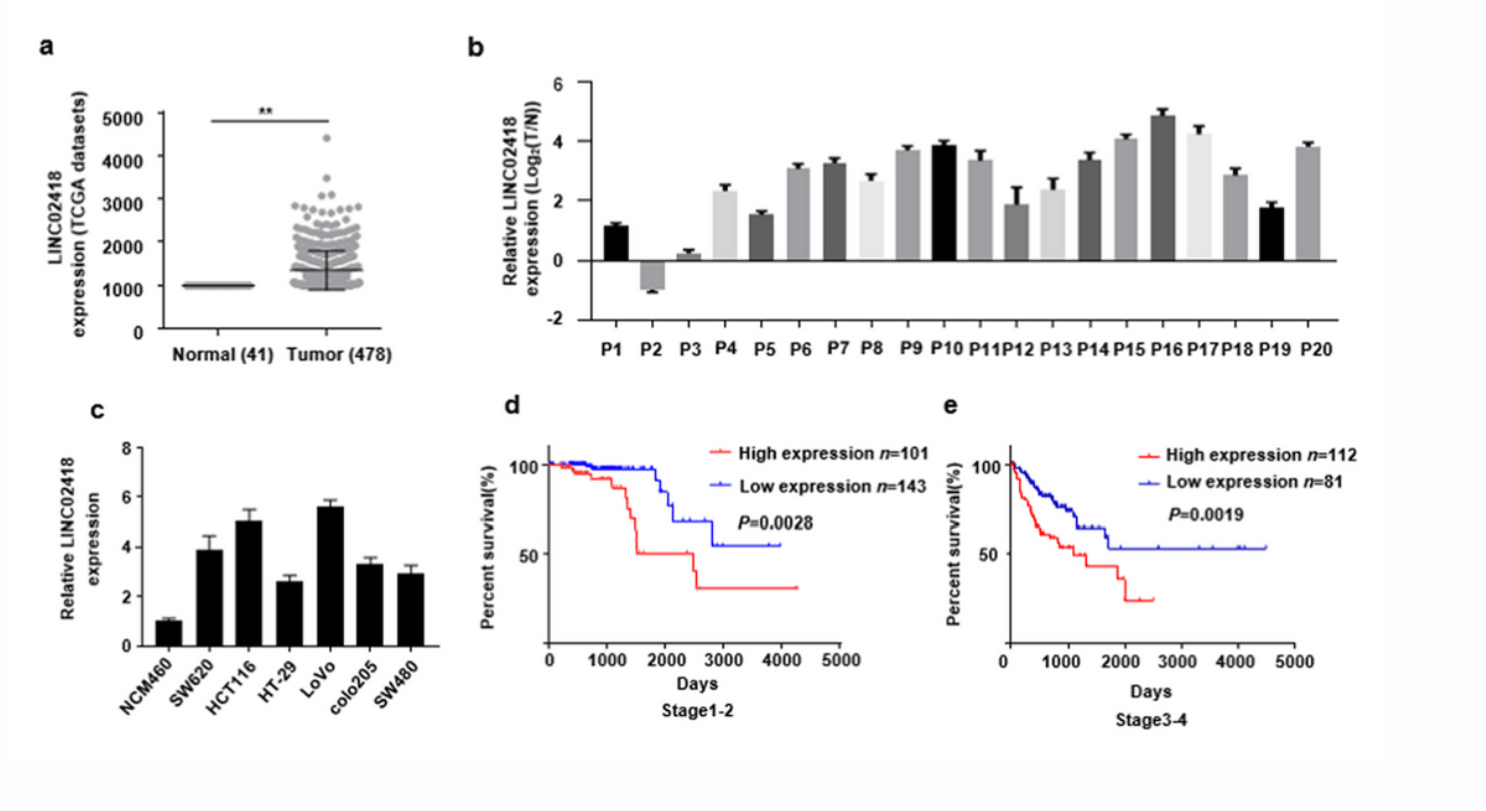
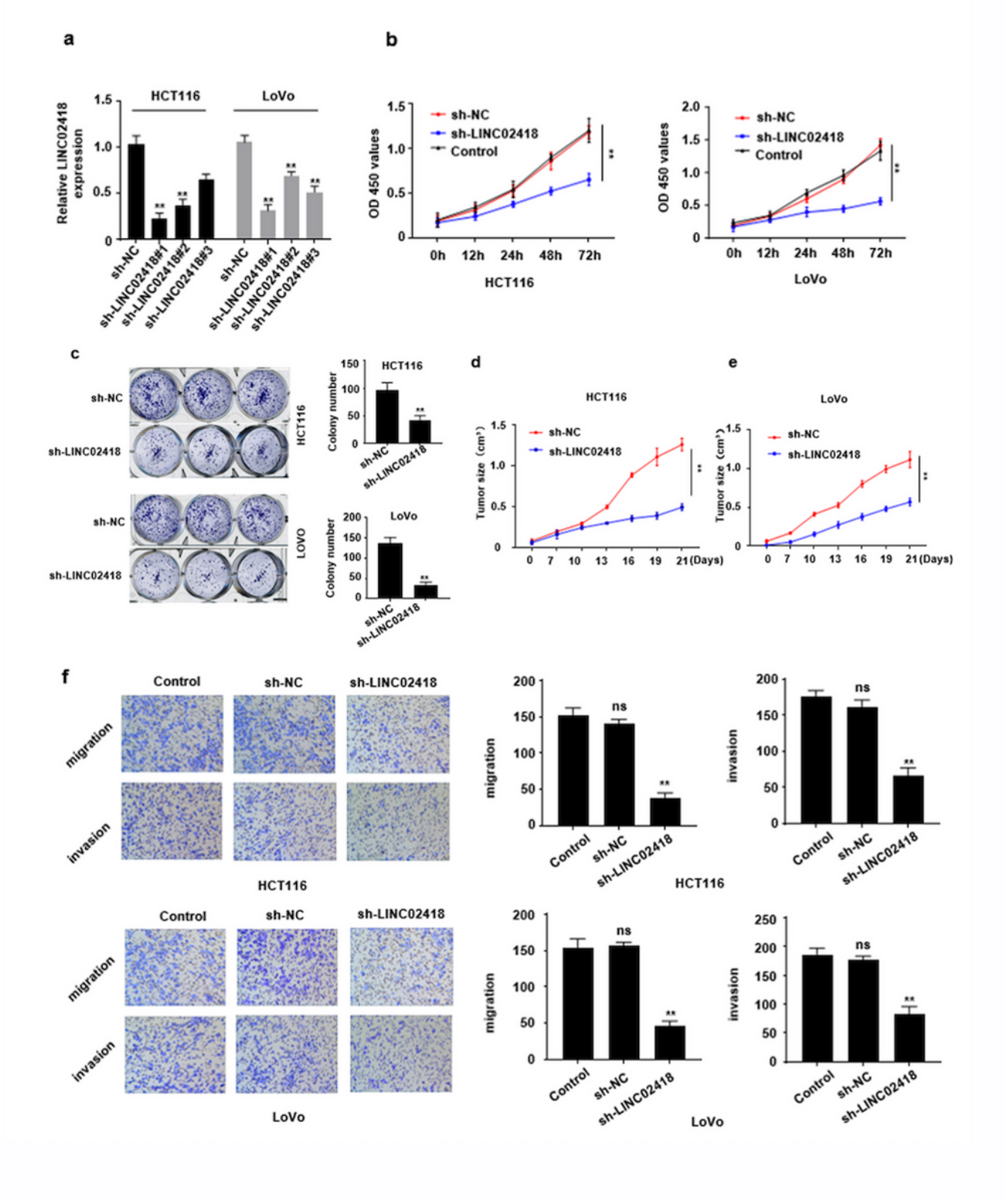
# Figures




## Figure 1

LINC02418 expression in in CRC tissues and cell lines a The differential expression of LINC02418 in CRC samples (n=478) and adjacent normal colon tissues (n=41) was analyzed based on TCGA; **, P<0.01, compared to adjacent tissue group. b Quantification analysis of LINC02418 level was conducted in 20 pairs of CRC samples and adjacent normal colon tissue. c LINC02418 levels in SW620, HCT116, LoVo, Colo205, HT-29, SW480 and normal colon epithelial cell line NCM460 were determined by qRT-PCR; **, P<0.01, compared to NCM460. d Kaplan-Meier analyses of the correlations between expression of LINC02418 and overall survival of patients with CRC in stage 1-2 and stage 3-4. The error bars stand for standard deviation (SD). Data were representatives of three independent experiments with similar results.




## Figure 2

Down-regulation of LINC02418 inhibited tumor growth, cell mobility and cell invasion. a HCT116 and LoVo cells were transfected with three shRNAs targeting LINC02418 and control sh-NC. The cells were harvested at 48h post transfection and qRT-PCR was conducted to measure LINC02418 level. b-c Cell proliferation of HCT116 and LoVo cells were determined via CCK-8 assays (b) and colony formation assays (c). d-e Tumor volume curve of mice subcutaneously injected with HCT116 (d) and LoVo (e) cells

which were stably transduced with sh-LINC02418 or sh-NC. f Transwell assay were performed to determine the effect of LINC02418 on the migration and invasion ability of HCT116 and LoVo cells. The results were expressed as the number of invaded cells per field. The error bars stand for standard deviation (SD). Data were representatives of three independent experiments with similar results; **, P<0.01, compared to sh-NC group; ns, no difference, compared to control group. Down-regulation of LINC02418 inhibited tumor growth, cell mobility and cell invasion a HCT116 and LoVo cells were transfected with three shRNAs targeting LINC02418 and control sh-NC. The cells were harvested at 48h post transfection and qRT-PCR was conducted to measure LINC02418 level. b-c Cell proliferation of HCT116 and LoVo cells were determined via CCK-8 assays (b) and colony formation assays (c). d-e Tumor volume curve of mice subcutaneously injected with HCT116 (d) and LoVo (e) cells which were stably transduced with sh-LINC02418 or sh-NC. f Transwell assay were performed to determine the effect of LINC02418 on the migration and invasion ability of HCT116 and LoVo cells. The results were expressed as the number of invaded cells per field. The error bars stand for standard deviation (SD). Data were representatives of three independent experiments with similar results; **, P<0.01, compared to sh-NC group; ns, no difference, compared to control group.

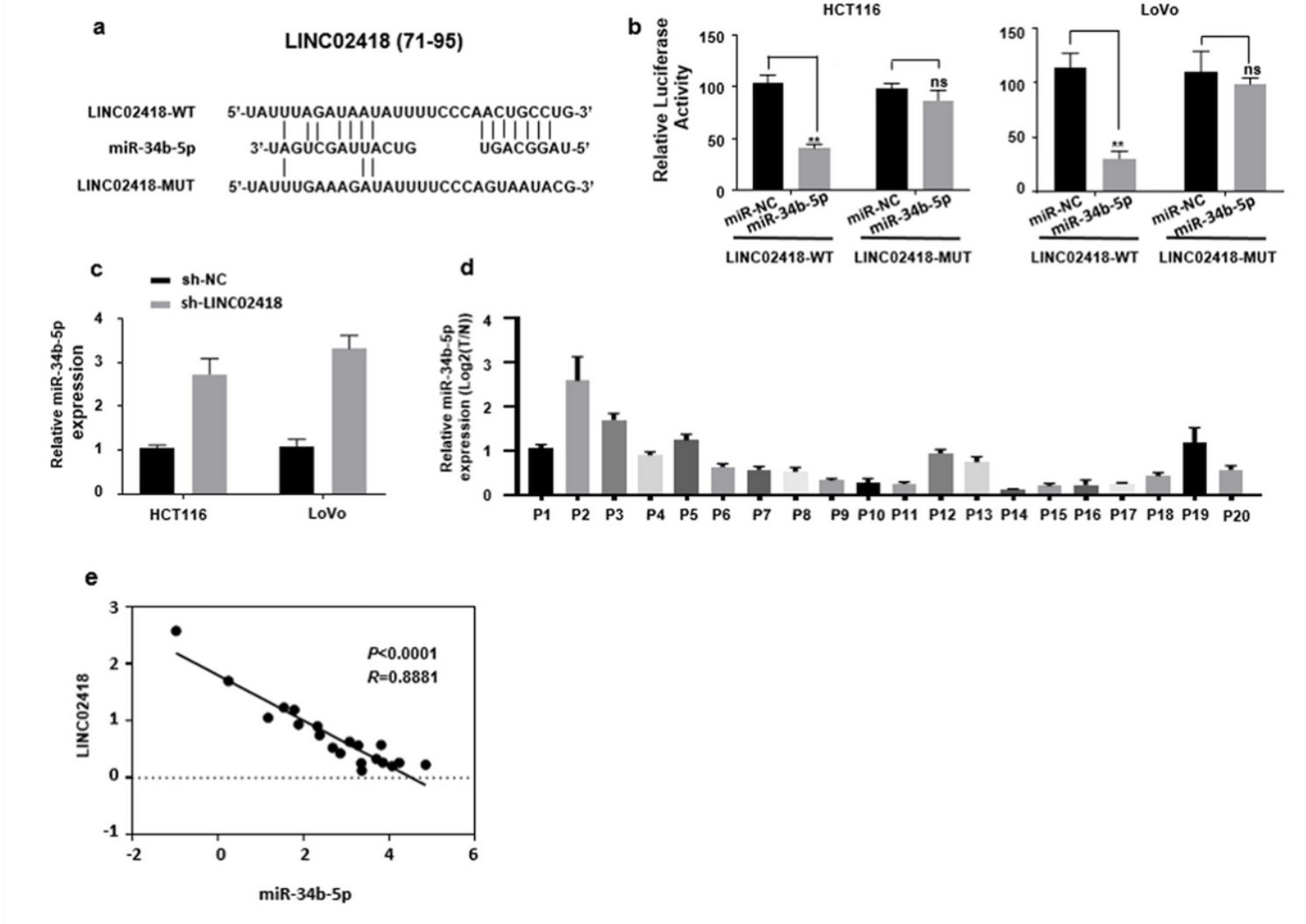
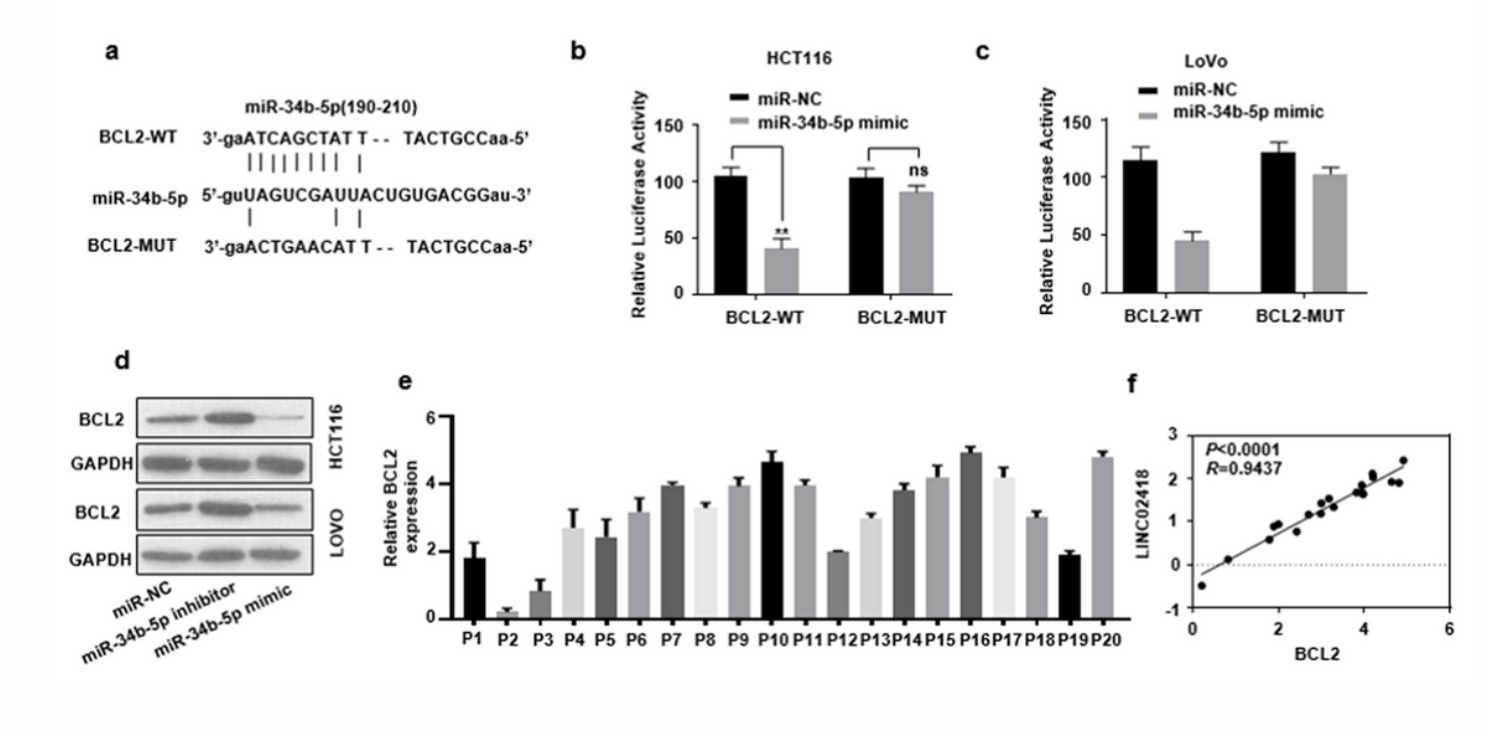


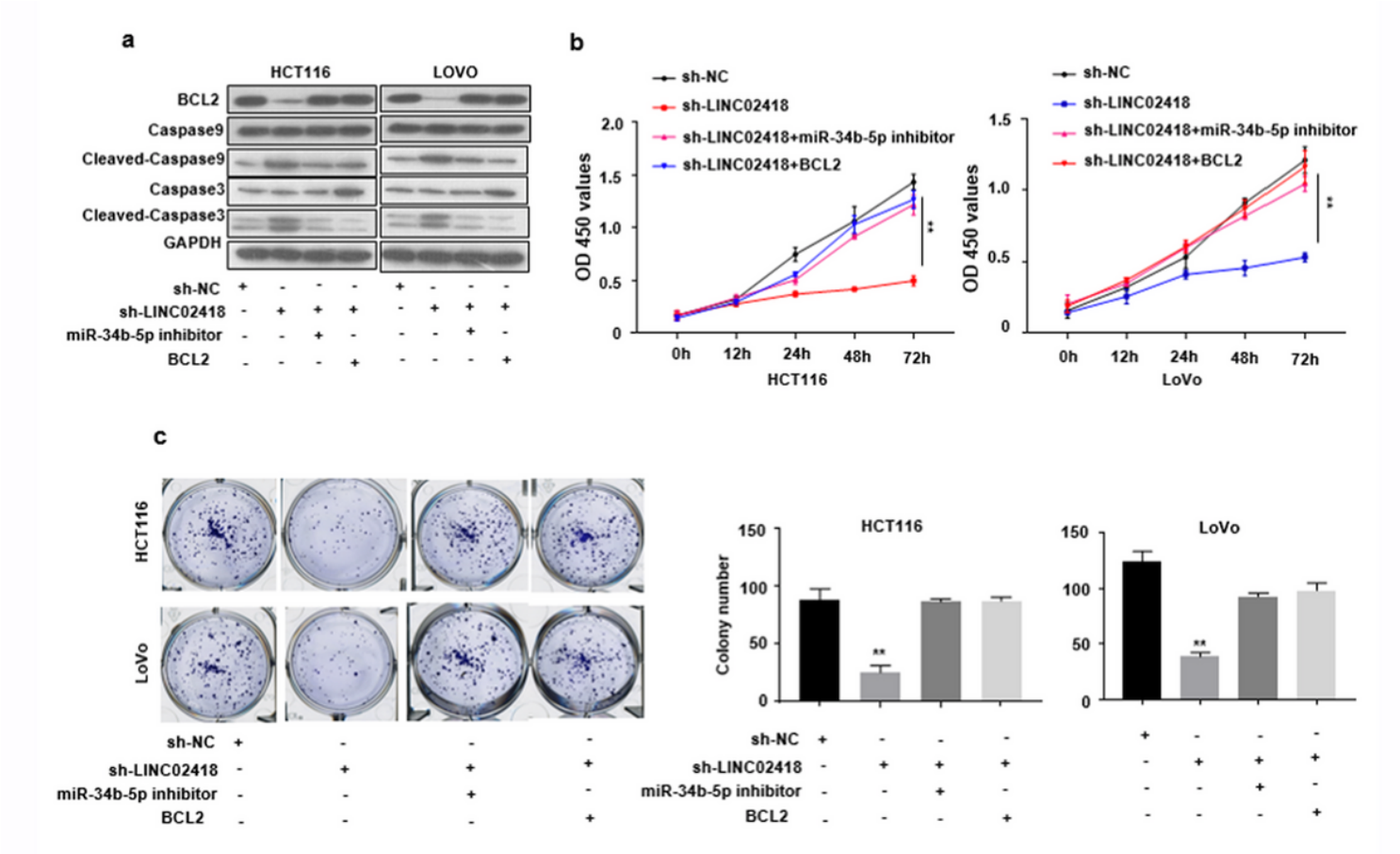

Figure 3

LINC02418 was a ceRNA for miR-34b-5p in CRC cells. a The putative binding sites of miR-34b-5p to wild type LINC02418 (LINC02418-WT) and the mutant LINC02418 (LINC02418-MUT). b Luciferase activity of HCT116 and LoVo cells co-transfected with miR-34b-5p mimic/miR-NC and luciferase reporters harboring LINC02418-WT or LINC02418-MUT were measured by dual luciferase assays; **, P<0.01; ns, no difference, compared to miR-NC group. c The effect of knockdown of LINC02418 on miR-34b-5p expression in CRC cells were quantified by qRT-PCR; **, P<0.01, compared to sh-NC group. d The differential expression of miR-34b-5p in CRC and normal tissues was analyzed by qRT-PCR. e The negative correlation between miR-34b-5p and LINC02418 levels was presented by Pearson's correlation curve. The error bars stand for standard deviation (SD). Data were representatives of three independent experiments with similar results.




## Figure 4

BCL2 was the target of miR-34b-5p in CRC cells. a The putative binding sites of miR-34b-5p to wild type BCL2 (BCL2-WT) and the mutant BCL2 (BCL2-MUT). b-c Luciferase activity detection of HCT116 (b) and LoVo (c) cells co-transfected with miR-34b-5p mimic/miR-NC and luciferase reporters expressing BCL2-WT or BCL2-MUT. d Endogenous protein level of BCL2 in HCT116 and LoVo cells transfected with miR-NC, miR-34b-5p inhibitor or miR-34b-5p mimic was detected by western blotting. e The expressional level of BCL2 mRNA in CRC and normal tissues was analyzed by qRT-PCR. f Pearson's correlation curve revealed the positive relevance between BCL2 and LINC02418 levels. The error bars stand for standard deviation (SD). Data were representatives of three independent experiments with similar results; **, P<0.01; ns, no difference, compared to miR-NC group.




## Figure 5

LINC02418 indirectly regulated BCL2 expression through sponging miR-34b-5p. a Endogenous protein level of BCL2, caspase 9, caspase 3 and cleaved-caspase 9, cleaved-caspase 3 were detected in HCT116 and LoVo cells by western blotting. b-c Cell growth of HCT116 and LoVo cells were determined by CCK-8 assays and colony formation assays. The error bars stand for standard deviation (SD). Data were representatives of three independent experiments with similar results; **, P<0.01, compared to sh-NC group.