

miR-17-5p promotes the invasion and migration of colorectal cancer by regulating HSPB2

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Abstract

MicroRNA (miRNA) can affect tumor progression by regulating cell proliferation, apoptosis and metastasis. A significant upregulation of miR-17-5p expression was found in colorectal cancer (CRC) tissues by miRNA microarray chip analysis. However, the underlying mechanism of miR-17-5p in CRC is still unclear. The mRNA expression of miR-17-5p was significantly higher in CRC tissues than in adjacent normal tissues. In CRC group, the expression of miR-17-5p in cancer tissues with lymph node metastasis was higher compared with those without lymph node metastasis. The biological function of miR-17-5p was demonstrated through CCK-8, colony formation, flow cytometry and transwell assays. Overexpression of miR-17-5p inhibited CRC cell apoptosis, as well as promoting proliferation, migration and invasion. Transcriptome sequencing and miRNA target prediction software suggested that HSPB2 might be a target gene of miR-17-5p and luciferase reporter detection validated for the first time that miR-17-5p binds directly to the 3'-UTR of HSPB2. In the rescue experiment, the tumor suppressive effect of HSPB2 was detected and miR-17-5p could promote cell proliferation, migration and invasion by targeting HSPB2. Taken together, miR-17-5p promotes invasion and migration by inhibiting HSPB2 in CRC, thereby implicating its potential as a novel diagnostic biomarker and therapeutic target for CRC.

Introduction

Colorectal cancer (CRC) is the third common malignancy worldwide associated with increased morbidity and mortality¹. Five-year survival rate for patients with early CRC is 90%, as compared with 12% for advanced CRC². Although several oncogenes and tumor suppressor genes have been reported to be related to CRC, the molecular mechanism of CRC development remains poorly understood.

MicroRNAs (miRNA) are non-coding RNA species, generally 19–22 nucleotides in length, and can induce translation inhibition or cause mRNA degradation by specifically binding to the target gene^{3–6}. It has been reported that miRNA regulates multiple vital cellular processes, including cell growth, differentiation, cycle progression, and apoptosis, and can be an essential biomarker for diagnosis and treatment of tumors^{7–10}. miR-17-5p, a member of the miR-17-92 cluster, is located in the third intron on chromosome 13, which is an amplified genomic site in many cancers^{11,12}. Previously, miR-17-5p was found to be abnormally expressed in multiple cancers and is involved in the tumorigenesis and progression by inhibiting apoptosis and promoting proliferation and metastasis^{13–20}. The previous analysis of miRNA microarray chips by our team has revealed a significant upregulation of miR-17-5p expression in CRC tissues than adjacent normal tissues²¹. However, the precise molecular mechanism through which miR-17-5p influences CRC progression remains largely unclear.

In this study, the expression level of miR-17-5p in CRC and adjacent normal tissues were analyzed, and its biological effects on CRC cells were investigated. To our knowledge, this is the first report to confirm that HSPB2, as a downstream target gene of miR-17-5p, plays an oncogenic miRNA role in the pathogenesis of CRC by regulating HSPB2. These findings suggest its potential as a novel diagnostic biomarker and target for future CRC treatments.

Results

miR-17-5p was upregulated in most of the CRC tissues and cells. We first examined the expression of miR-17-5p in 47 paired samples of CRC and the adjacent normal tissues. We found that miR-17-5p levels was significantly upregulated in CRC tissues compared to adjacent normal tissues (Fig. 1A), which was consistent with our previous results by microarray chips in 4 pairs of CRC tissues. In the CRC group, the expression of miR-17-5p in cancer tissues with lymph node metastasis was higher compared to those without lymph node metastasis (Fig. 1B), but it was not associated with clinicopathological features such as degree of differentiation, patient age, sex, tumor location and invasion (Table 1). In addition, high expression of miR-17-5p was found in most CRC cell lines (Fig. 1C).

Overexpression of miR-17-5p inhibited CRC cell apoptosis, and promoted proliferation, invasion and migration. To determine whether miR-17-5p overexpression modulated CRC tumorigenesis, HCT116 cells with a certain miR-17-5p expression were transfected with miR-17-5p mimic, and the overexpression effect was confirmed by qRT-PCR (Fig. 2A). A flow cytometry assay showed that HCT116 cells overexpressing miR-17-5p had a significant reduction of apoptotic rate compared to control group (Fig. 2B), as evident from the CCK8 assay that miR-17-5p overexpression induced the viability of CRC cells (Fig. 2C). And colony formation assay revealed that the number of colonies was increased in cells overexpressing miR-17-5p (Fig. 2D). Moreover, Transwell assay showed that over-expression of miR-17-5p resulted in a significant increase in the number of migrating and invading cells compared with control cells (Fig. 2E).

Silencing of miR-17-5p inhibited CRC cell proliferation, invasion and migration. HCT116 cells were subsequently transfected with inhibitor that could endogenously suppress the expression of miR-17-5p, and a decrease expression of miR-17-5p was confirmed via qRT-PCR (Fig. 3A). Apoptotic rate was not significantly decreased after the expression of miR-17-5p was suppressed, as compared with the control cells (Fig. 3B). The CCK8 assay indicated that silencing endogenous miR-17-5p reduced the viability of CRC cells (Fig. 3C). And colony formation assay showed that the number of colonies in miR-17-5p silenced cells was significantly reduced (Fig. 3D). In addition, the silencing of endogenous miR-17-5p reduced cell migration and invasion compared to the control cells in the Transwell assay (Fig. 3E).

HSPB2 was a direct target of miR-17-5p. In order to gain insight into the mechanism by which miR-17-5p acts as an oncogenic miRNA in CRC cells, we performed a transcriptome sequencing analysis to profile differentially expressed genes in HCT116 infected with miR-17-5p mimic and inhibitor. Significant difference revealed by RNA-seq analysis indicated global alterations in gene expression (Fig. 4A). The mRNA expression of 647 and 419 genes was altered by overexpression or knockdown of miR-17-5p, respectively, of which 27 genes were regulated by both (Fig. 4B). Many upregulated or downregulated genes might also be indirect targets, as they were affected as part of the regulatory network. Then we used three bioinformatics algorithms (TargetScan, microRNA.org, and mirtarbase) to screen whether there were potential miR-17-5p binding sites in these 27 genes. We found HSPB2 and miR-17-5p had opposite expression pattern changes according to the transcriptome sequencing analysis and qRT-PCR (Fig. 4C-D).

In addition, there are potential binding sites for miR-17-5p in the 3'-UTR region of HSPB2 to regulate translation or expression (Fig. 5A). Therefore, these findings suggested that HSPB2 was a strong candidate for a direct and functional target gene of miR-17-5p in CRC. Then a luciferase reporter gene assay was performed using the firefly reporter plasmids containing the binding site for miR-17-5p. Compared to control group, the overexpression of miR-17-5p resulted in a significant reduction in luciferase reporter activity at three sites (Fig. 5B), whereas miR-17-5p inhibitor led to a significant increase in reporter activity (Fig. 5C).

The HSPB2 mRNA was downregulated in CRC. We analyzed the expression levels of HSPB2 mRNA in 51 paired CRC and adjacent normal tissues. The results showed that the expression of HSPB2 in CRC tissues was significantly reduced compared to normal adjacent tissues (Fig. 6A). Moreover, compared with normal tissues, the expression of HSPB2 mRNA in cancer tissues was significantly reduced according to the CRC dataset of TCGA, the COAD and READ datasets of GEPIA (Fig. 6B-D).

Interaction mechanism of miR-17-5p and HSPB2 in CRC. Next, we performed a rescue experiment in HCT116 and LOVO cells. When miR-17-5p expression was exogenously increased, we verified that overexpressed miR-17-5p could enhance cell viability, colony formation (Fig. 7C-D), migration and invasion (Fig. 8) as compared with the control group. When miR-17-5p was endogenously silenced, it was reconfirmed that compared to the control group, knockdown of miR-17-5p could reduce cell viability, colony formation (Fig. 9C-D), migration, and invasion (Fig. 10). We overexpressed HSPB2 in HCT116 and LOVO (Fig. 7A-B). CCK8 and colony formation assay revealed that HSPB2 overexpression inhibited CRC cell viability and colony formation compared to the control group (Fig. 7C-D). The Transwell assay showed that overexpression of HSPB2 significantly reduced the number of migrating and invading cells compared to the control group (Fig. 8). Then HSPB2 expression was knocked down (Fig. 9A-B). Compared with the control cells, reduced HSPB2 promoted cell viability and colony formation (Fig. 9C-D), and increased the number of migrating and invading cells (Fig. 10). In addition, we found that overexpression of miR-17-5p suppressed HSPB2 at both the mRNA and protein levels (Fig. 7A-B) and silencing of endogenous miR-17-5p increased HSPB2 at both the mRNA and protein levels (Fig. 9A-B). When miR-17-5p and HSPB2 expression were increased simultaneously, the reintroducing HSPB2 caused by co-overexpression attenuated the tumor-promoting effect of overexpressed miR-17-5p, as shown by reduced proliferation (Fig. 7C-D), migration and invasion (Fig. 8). Simultaneous knockdown of miR-17-5p and HSPB2 revealed that reduced HSPB2 could partially reverse the suppression of proliferation (Fig. 9C-D), migration and invasion (Fig. 10) caused by miR-17-5p inhibitor.

Discussion

In 2018, the International Agency for Research on Cancer (IARC) estimated that there would be more than 521,000 new cases and more than 248,000 new deaths of CRC in China, making it the major public health issue²². The clinical process of CRC is caused by multi-level interactions²³, among which the genetic changes of oncogenes or tumor suppressor genes are the most important drivers of cancer occurrence and development. MiRNAs are short endogenous non-coding RNA that can regulate the expression of

related genes at the post-transcriptional level²⁴. And it has been reported that some miRNAs could exert a vital influence on pathogenesis of CRC²⁵⁻³¹. We previously used microarray chip analysis to screen differentially expressed miRNAs in 4 pairs of CRC tissues. It was found that the expression of miR-17-5p was increased in CRC compared to normal tissues²¹, suggesting that miR-17-5p had the potential to promote CRC tumorigenesis. The effects that miR-17-5p could exert on tumorigenesis and CRC development, as well as the downstream target genes and interaction mechanisms of miR-17-5p are our prime concerns.

miR-17-5p was reported to be a critical oncogenic miRNA, and the increased expression of miR-17-5p was found to be involved in the development of multiple tumors, including hepatocellular carcinoma¹⁵, gastric cancer³², prostate cancer³³, ovarian cancer³⁴, and breast cancer³⁵. However, miR-17-5p overexpression was also found to inhibit cell proliferation, invasion, and tumor metastasis in certain cancer cell lines³⁶. In this study, we detected the expression of miR-17-5p in 47 pairs of CRC tissue and adjacent normal tissue samples, and the results showed that miR-17-5p was significantly upregulated in CRC tissues and was associated with lymph node metastasis. Moreover, we found that overexpression of miR-17-5p promoted the proliferation, anti-apoptosis, migration and invasion of CRC cells, whereas knockdown of miR-17-5p inhibited cell proliferation, migration and invasion but had no significant effect on apoptosis. Taken together, these results suggest that miR-17-5p may contribute to the development of CRC by promoting certain malignant biological characteristics such as growth and metastasis.

In order to investigate the target genes of miR-17-5p in CRC, we first performed transcriptome sequencing to obtain differentially expressed genes affected by expression of miR-17-5p. It was noteworthy that 647 differentially expressed genes were affected by miR-17-5p overexpression, 419 differentially expressed genes regulated by miR-17-5p knockdown, and 27 differentially expressed genes regulated by both. To further focus on potential target genes, we used three bioinformatics algorithms (TargetScan, microRNA.org, and mirtarbase) to predict the potential binding site for miR-17-5p in these 27 genes. We identified HSPB2 as a strong candidate gene based on the above data. Subsequently, the luciferase reporter assay was used to validate that miR-17-5p directly binds to the 3'-UTR region of HSPB2 in two CRC cell lines. To our knowledge, HSPB2 was proposed for the first time as a direct target gene of miR-17-5p.

HSPB2, a novel and unique member of the Heat shock proteins (HSPs) family, is located on the 11q22-q23 chromosome^{37,38}. HSPs can interact with its substrate to keep the denatured protein in a folded state and transform it into a functional conformation as a molecular chaperone^{39,40}. Furthermore, HSPs have been reported to be involved in cancer cell proliferation, survival, and apoptosis⁴¹⁻⁴³. Studies have shown that expression of HSPB2 is reduced in esophageal squamous cell carcinoma⁴⁴ and pancreatic cancer, which can inhibit the progression of pancreatic cancer⁴⁵. HSPB2 also inhibits the apoptotic pathway by inhibiting the activation of caspases-8 in breast cancer⁴⁶. Moreover, studies have revealed that HSPB2 is a novel target of p53⁴⁷, which can restore the transcriptional activity of mutant p53 and inhibit tumor progression to a certain extent⁴⁵. However, there have been no reports to date concerning the effect of

HSPB2 on CRC as a downstream of miR-17-5p. We analyzed the expression of HSPB2 mRNA in 51 paired samples of CRC and adjacent normal tissues, and the results showed that the expression of HSPB2 in CRC was significantly reduced. Because no studies have reported the expression of HSPB2 in CRC, we checked the expression of HSPB2 in the network data set. Compared to normal tissues, the expression of HSPB2 in cancer tissues was significantly reduced according to the CRC dataset of TCGA, the COAD and READ datasets of GEPIA. This suggests the potential role of HSPB2 as a CRC suppressor gene and makes our study clinically relevant. In subsequent rescue experiments, the biological function of HSPB2 was tested in two CRC cell lines (HCT116 and LOVO). The results showed that overexpressed HSPB2 inhibited cell proliferation, migration and invasion in CRC. To our knowledge, we showed for the first time that HSPB2 inhibits the progression of CRC.

To further verify the direct relationship between miR-17-5p and HSPB2 and the underlying mechanism, a rigorous rescue experiment was performed. When miR-17-5p was overexpressed or silenced, HSPB2 expression was decreased or increased at the mRNA and protein levels. This suggests that miR-17-5p inhibits expression of HSPB2 in CRC cells and a strong miRNA-mRNA interaction. Next, we co-overexpressed miR-17-5p and HSPB2, and subsequent biological functional tests revealed that reintroducing HSPB2 could partially reverse the miR-17-5p-mediated promotion of cell proliferation, migration, and invasion. After simultaneous knockdown of miR-17-5p and HSPB2, as expected, reduced HSPB2 was rescued by miR-17-5p inhibitors from suppressing tumors such as proliferation, migration, and invasion. Taken together, our findings uncover a novel mechanism for the first time through which miR-17-5p inhibits its expression through the miR-17-5p-HSPB2 axis, thereby promoting CRC tumorigenesis by inhibiting HSPB2, a downstream target of miR-17-5p.

In summary, this study unravels a novel mechanism for tumor progression through which miR-17-5p promotes CRC cell proliferation, migration and invasion by targeting HSPB2. This suggests that miR-17-5p can be used as a diagnostic and prognostic biomarker for CRC patients. Future studies on miR-17-5p and HSPB2 are warranted to develop novel molecular therapeutic avenues.

Materials And Methods

Patients and specimens. Ninety-eight paired specimens of CRC tissues and adjacent normal tissues were provided by Clinical Biobank in the First Hospital of Hebei Medical University. Written informed consent was obtained from all patients. CRC and normal tissues were collected from CRC resection specimens, and none of patients had received radiotherapy or chemotherapy prior to surgery. Study protocols were approved by the Ethics Committee of the First Hospital of Hebei Medical University and adhered to the principles of the Declaration of Helsinki.

Cell lines culture. Human CRC cell lines HCT116, SW480, LOVO, SW1116, Caco2, and SW1463 cell lines were obtained from Prof. Jun Yu (The Chinese University of Hong Kong, Hong Kong, China). HCT116 were cultured in McCoy's 5A medium (Gibco, Gaithersburg, MD, USA) and the other cells lines were cultured in DMEM medium (Gibco, Gaithersburg, MD, USA, supplemented with 10% fetal bovine serum

(FBS; Gibco, Gaithersburg, MD, USA) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA) in a 37°C humidified incubator with 95% air and 5% CO₂.

Transfection and plasmid construction. The hsa-miR-17-5p mimic, inhibitor, HSPB2 overexpression plasmid or shRNA-HSPB2 and a respective negative control (GeneCopoeia, MD, USA) were transfected into cells in 6-well plates using Lipofectamine 2000 (Invitrogen, Carlsbad, USA) when the cells were approximately 60–80% confluent. After the cells were cultured in medium without FBS for 6 h, the medium was replaced by that supplemented with 10% FBS. Cells were harvested 48 h after transfection and the effect of transfection was assessed by real-time quantitative polymerase chain reaction (qRT-PCR).

RNA extraction and qRT-PCR. Total RNA was extracted from the transfected cells and tissues using Trizol Reagent (Invitrogen, Carlsbad, USA). To quantify miR-17-5p, All-in-One miRNA qRT-PCR kits (GeneCopoeia, MD, USA) were used with miR-17-5p-specific primers (HmiRQP0230, GeneCopoeia, MD, USA) according to the manufacturer's instructions. To quantify HSPB2, cDNA was reverse transcribed using PrimeScript RT reagent kit (Takara, Beijing, China). Then, a qRT-PCR was performed by using SYBR Green Master Mix (Vazyme, NJ, USA). U6 was used as internal control of miRNA, and GAPDH was used as internal control of mRNA. The primer sequences were as follows: HSPB2(forward): 5'-ATGTCGGGCCGCTCAGTGCC-3'; HSPB2(reverse): 5'-GTCACCTCGTCTGGGGTAAA-3' (22); GAPDH (forward): 5'-GAGTCAACGGATTTGGTCGT-3'; GAPDH (reverse) 5'-CATGGGTGGAATCATATTGGA-3'. All the experiments were performed in triplicate on an ABI7500 Sequence Detection System, and the mean cycle threshold (CT) data were obtained. The relative amount normalized to the internal control was calculated with the Eq. $2^{-\Delta\Delta CT}$.

Cell apoptosis assay. The transfected cells were stained using the Annexin V-FITC/ PI Apoptosis Detection Kit (Beyotime, Jiangsu, China) in accordance with the manufacturer's instructions. 1×10^5 cells were analyzed for apoptosis using a flow cytometry (BD, MA, USA), and the percentage of the apoptotic cells was quantified using Cell Quest software.

Cell proliferation assay. The transfected cells were plated at 2×10^3 cells per well in 96-well plates and incubated overnight in medium supplemented with 10% FBS. Cell proliferation was measured using a Cell Counting Kit-8 (Dojindo, Tokyo, Japan) at 24, 48, and 72 h post-transfection following the manufacturer's instruction. The absorbance at 450 nm was measured using a Promega GloMax Luminescence detector (Promega, MD, USA).

Colony formation assay. 1×10^3 transfected cells were plated in cultured dishes and grown for 2 weeks. Subsequently, the cells were washed twice with phosphate buffer saline (PBS), fixed with 4% paraformaldehyde and stained with 0.5% crystal violet for 1 h. The dishes were scanned and the average number of colonies was achieved.

Transwell invasion and migration assay. Invasion and migration assay were performed using Transwell chambers with 8- μ m pores (Corning, MA, USA). For invasion assay, 2×10^5 transfected cells suspended in 500 μ l serum-free medium were placed in the upper chambers coated with Matrigel, and medium containing 10% FBS was added to the lower chamber as a chemoattractant. For migration assay, cells in 100 μ l serum-free medium were added to the upper chambers, and medium containing 10% FBS was placed in the lower chamber as a chemoattractant. Following incubation at 37°C for 24 h, cells on the upper surface of the chamber were gently wiped off with cotton swabs. The cells were stained using a Diff-Quick stain kit according to the manufacturer's protocol and counted blindly (five random fields per chamber).

RNA sequencing and data analysis. Total RNA was extracted from cells transfected with hsa-miR-17-5p mimic or inhibitor and the negative control cells, and then subjected to commercial RNA-sequencing (RNA-seq) analysis (Novogene). Preparation of library and sequencing of transcriptome were performed using NEB Next Ultra RNA Library Prep Kit for Illumina (Novogene, Beijing, China) following the manufacturer's protocols, and the attribute sequence of each sample was added with an index code. The clustering of the index-coded samples was achieved on a cBot Cluster Generation System. After cluster generation, 125-bp paired-end reads to genes were mapped using HTSeq v0.6.0 software. Afterwards, fragments per kilobase of transcript per million fragments mapped (FPKM) were also calculated. Analysis of differential expression was performed using the DEGSeq R package through the one scaling normalized factor. Corrected P values of 0.005 and log₂ (fold changes) of 1 were set as the threshold for significantly differential expression after adjusted using the Benjamini-Hochberg method. Moreover, the hierarchical clustering and heatmaps were generated to show the normalized expression among the samples.

Luciferase reporter assay. Four sets of luciferase reporter plasmids were constructed, and each set of plasmids contained a 60–80 bp fragment of HSPB2 3'-untranslational region (3'-UTR) with a conserved miR-17-5p binding site (Sangon Biotech, Shanghai, China). For the luciferase reporter assays, HCT116 and LOVO cells were cultured in 96-well plates, and each well was co-transfected with firefly luciferase reporter plasmids, miR-17-5p mimic, inhibitor or the negative control using Lipofectamine 2000 (Invitrogen, Carlsbad, USA). The firefly luciferase and renilla luciferase activities were evaluated using the Dual Luciferase Reporter Assay Kit (Promega, MD, USA) following the manufacturer's instructions after transfection for 48 h.

Western blot analysis. Total protein was isolated from the cultured cells using RIPA lysis buffer (Beyotime, Shanghai, China). Proteins were separated by 12% SDS-PAGE (Bio-Rad) and transferred onto polyvinylidene difluoride membranes (Millipore, MA, USA). The immunoreactive protein bands were detected by the Odyssey Scanning System (Li-Cor, Lincoln, USA) after using antibodies against HSPB2 (Proteintech, Wuhan, China) and β -actin (Solarbio, Beijing, China).

Databases analysis. We used the online databases TargetScan⁴⁸, microRNA.org⁴⁹ and mirtarbase⁵⁰ to predict target genes of miR-17-5p, and confirmed the specific binding sites for miR-17-5p on the 3'-UTR

region of the target gene. In addition, the online database The Cancer Genome Atlas (TCGA) and Gene Expression Profiling Interactive Analysis (GEPIA) were used to further ascertain the mRNA expression of the target gene.

Statistical analysis. The results of normal distribution data are expressed as mean \pm SD and the results of non-normal distribution data are expressed as median and quartile spacing. Student's t-test, one-way ANOVA and two-way ANOVA were used in this study. All statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, CA, USA) and SPSS 21 (IBM, NY, USA). $P < 0.05$ was considered as statistically significant.

Declarations

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Authors contributions

Conception and design of study: Weifang Yu and Jia Wang. Performed the experiments: Weifang Yu, Jia Wang, Chao Li, Mingda Xuan, Shuangshuang Han. Data analysis and interpretation: Yingfu Zhang, Pengfei Liu. Manuscript writing: Weifang Yu and Jia Wang. Project supervision: Zengren Zhao. Final approval of manuscript: All authors.

Competing Interests

The authors declare that they have no conflict of interest.

Availability of data and materials

The data used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethical approval

The study protocol was approved by the Ethics Committee of the First Hospital of Hebei Medical University and adhered to the principles of the Declaration of Helsinki.

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Tables

Table I. The relationship between the relative expression level of miR-17-5p and clinicopathological variables in 47 patients with CRC.

Clinicopathological data	n	miR-17-5p relative expression	U	P
Gender				
Male	31	6.7371 (3.1734, 10.0152)	233	0.558
Female	16	7.0274 (3.9014, 14.7741)		
Age[years]				
≥68	24	6.7974 (3.2270, 10.4221)	239	0.827
<68	23	7.0274 (3.2852, 9.7473)		
Position				
Colon	22	5.3080 (2.8689, 7.7239)	327.5	0.218
Rectum	25	8.8520 (3.9014, 14.2116)		
Differentiated degree				
Poorly differentiated	8	9.1324 (8.1624, 10.2390)	86	0.230
Moderately and Highly differentiated	39	6.1988 (3.1362, 10.4831)		
Invasive status				
Penetrating serosa	29	7.1514 (3.5859, 9.7126)	213.5	0.929
Non-penetrating serosa	18	6.1988 (3.0022, 12.6758)		
Lymph node metastasis				
Absence	29	5.5983 (2.9601, 8.8104)	128.5	0.018*
Presence	18	8.9922 (5.7549, 14.5365)		

Figures

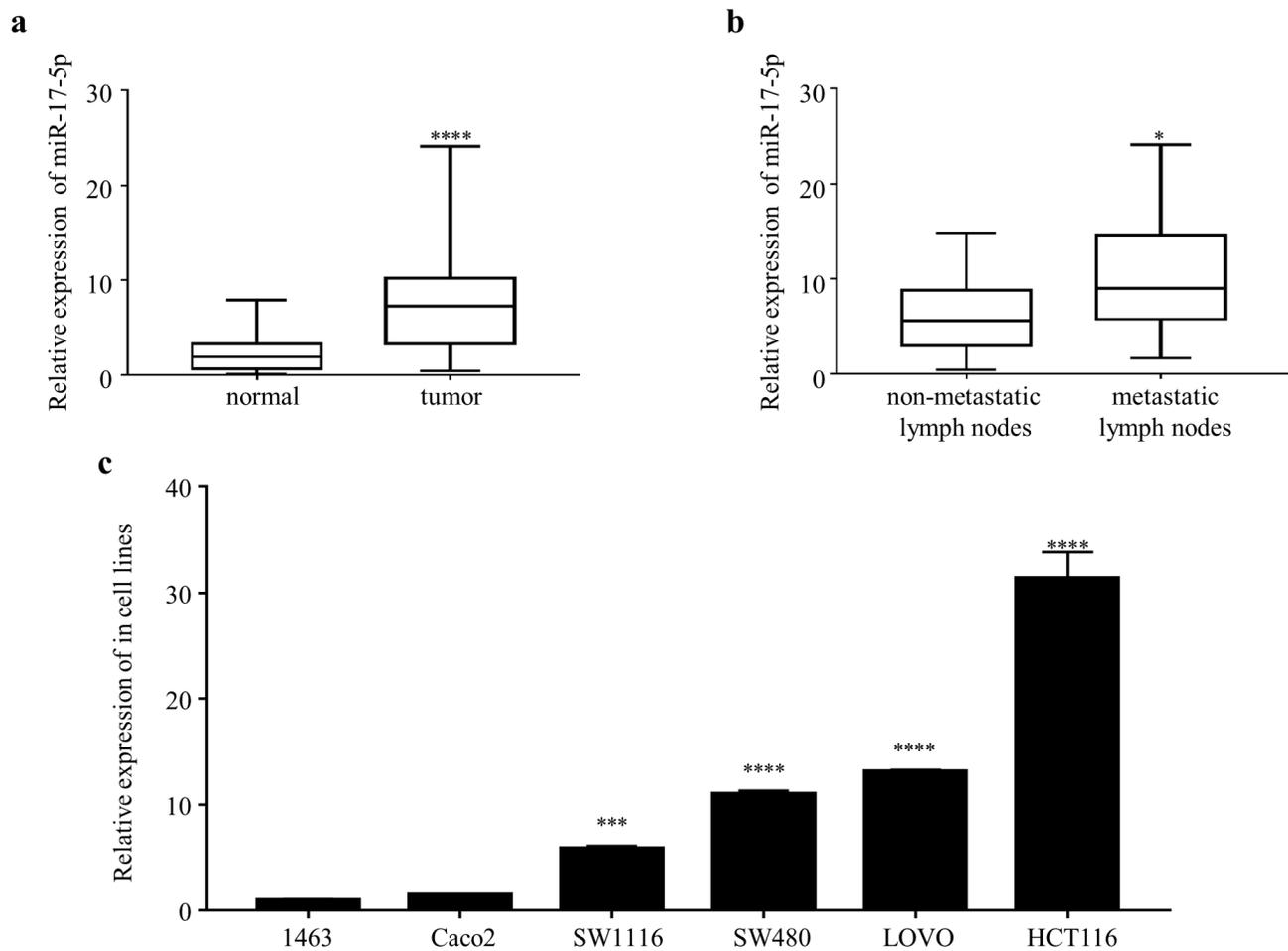


Figure 1

The expression of miR-17-5p is elevated in CRC patient samples and cell lines. (A) The expression level of miR-17-5p in 47 paired of CRC and adjacent normal tissues by qRT-PCR. (B) qRT-PCR analysis of miR-17-5p expression in CRC tissues with (n=18) and without lymph node metastasis (n=29). (C) Expression levels of miR-17-5p in 6 CRC cell lines. * P <0.05; *** P <0.001; **** P <0.0001.

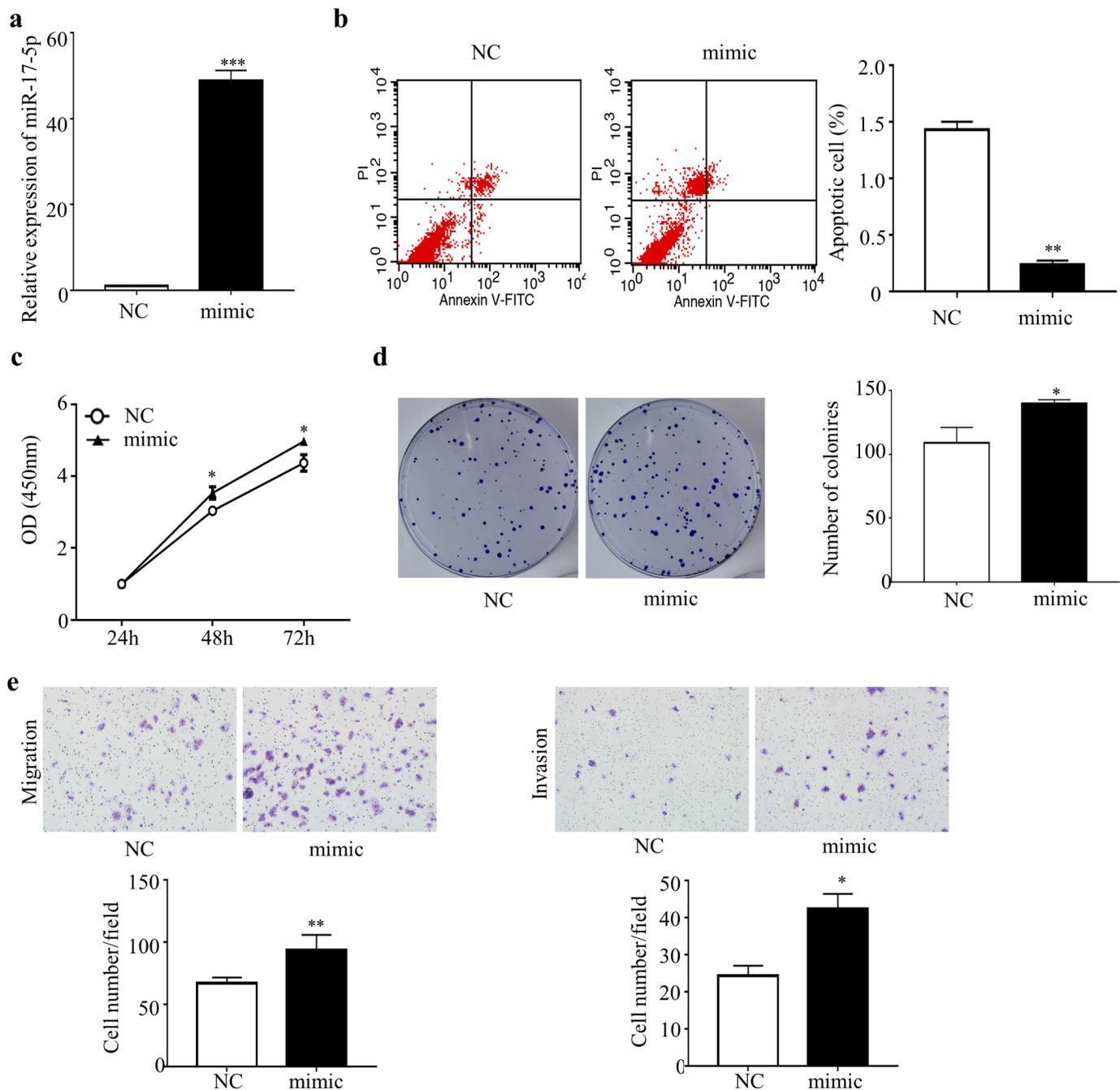


Figure 2

Overexpressed miR-17-5p inhibited CRC cell apoptosis, promoted the proliferation, invasion and migration. (A) The overexpression miR-17-5p was detected in HCT116 cells by qRT-PCR. (B) Flow cytometry was used to evaluate the cell apoptosis after increasing miR-17-5p expression. (C, D) CCK8 assay and colony formation assay were used to determine the effect of miR-17-5p overexpression on cell proliferation. (E) Transwell assay was performed to assess the migration and invasion potency after the expression of miR-17-5p was up-regulated. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

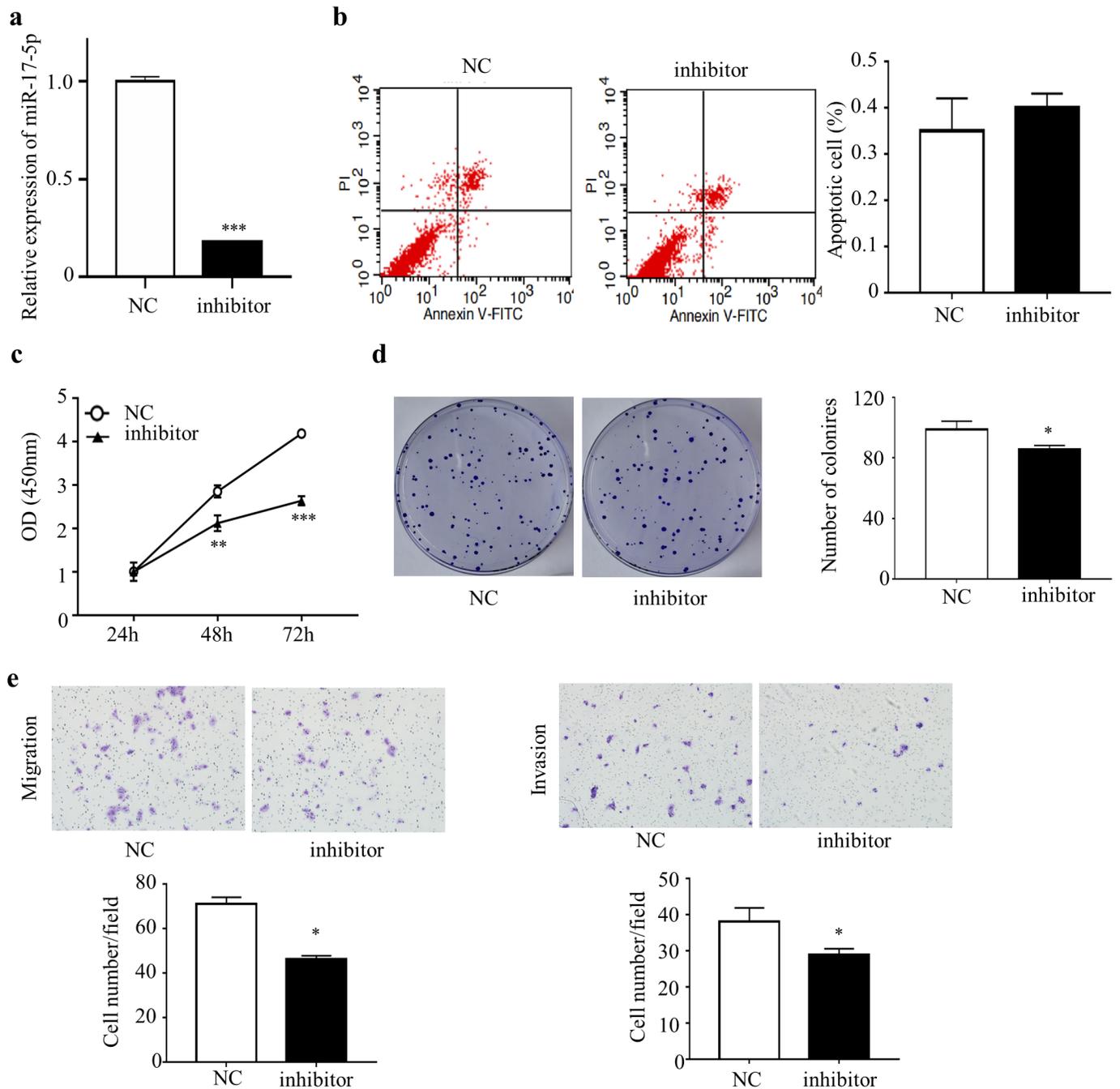


Figure 3

Overexpressed miR-17-5p inhibited CRC cell apoptosis, promoted the proliferation, invasion and migration. (A) The overexpression miR-17-5p was detected in HCT116 cells by qRT-PCR. (B) Flow cytometry was used to evaluate the cell apoptosis after increasing miR-17-5p expression. (C, D) CCK8 assay and colony formation assay were used to determine the effect of miR-17-5p overexpression on cell proliferation. (E) Transwell assay was performed to assess the migration and invasion potency after the expression of miR-17-5p was up-regulated. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

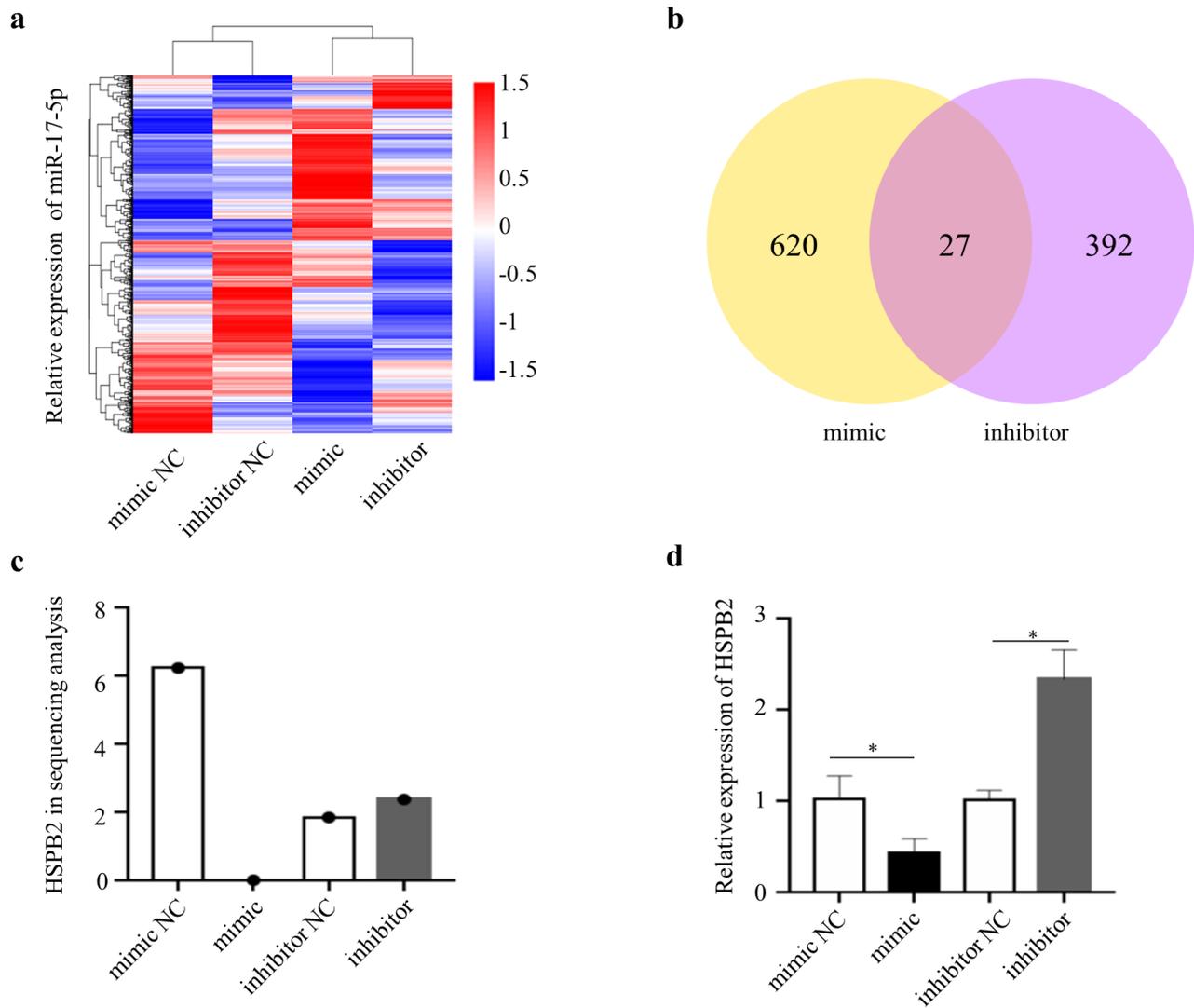


Figure 4

HSPB2 is predicted to be the target of miR-17-5p. (A) Hierarchical clustering heat map of RNA sequencing show all differential genes in up and down regulated miR-17-5p cells. (B) Venn diagram depicting the overlap differential genes in affected by miR-17-5p overexpression and knockdown. (C) Analysis data of HSPB2 expression in sequencing samples. (D) The relative expression of HSPB2 mRNA in sequencing samples by qRT-PCR. * P < 0.05

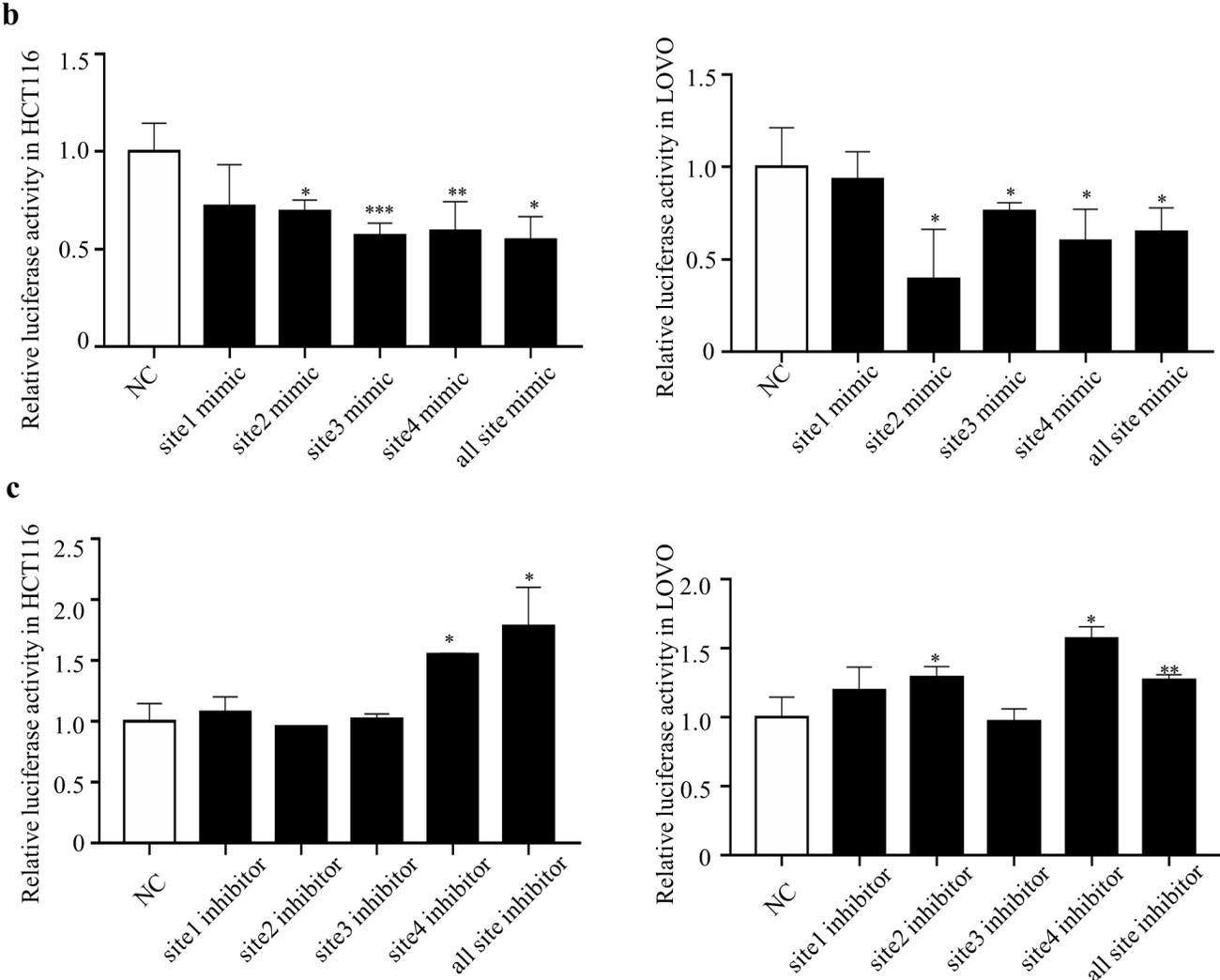
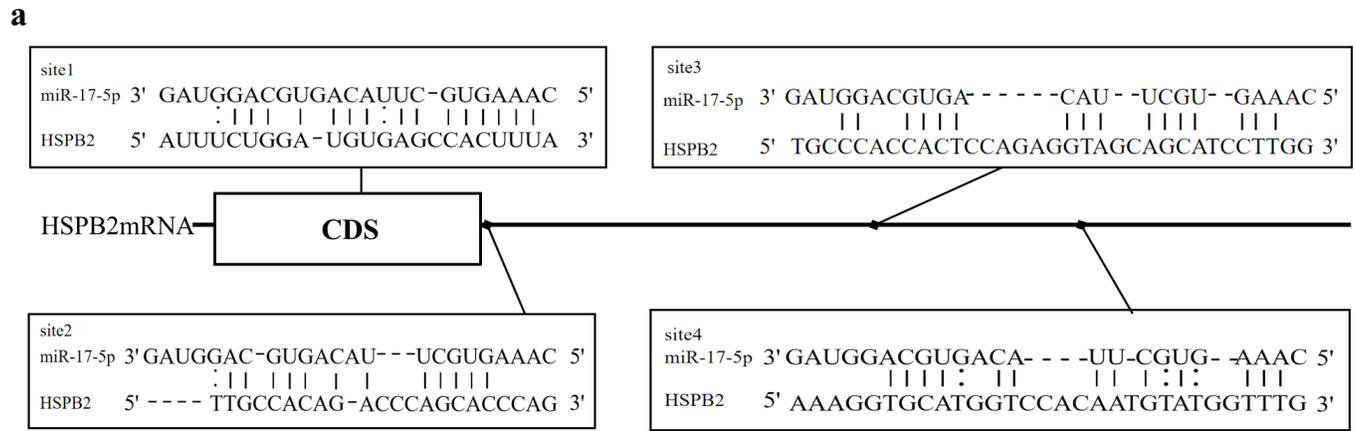


Figure 5

HSPB2 is identified as a direct target of miR-17-5p. (A) Predicted binding sites of miR-17-5p at the 3'-UTR of HSPB2 mRNA. (B) Relative luciferase activity in HCT116 and LOVO cells co-transfected with miR-17-5p mimics together with reporter vectors carrying HSPB2 3'-UTR binding sites. (C) Relative luciferase activity in HCT116 and LOVO cells co-transfected with miR-17-5p inhibitor together with reporter vectors carrying HSPB2 3'-UTR binding sites. * P <0.05; ** P <0.01; *** P <0.001.

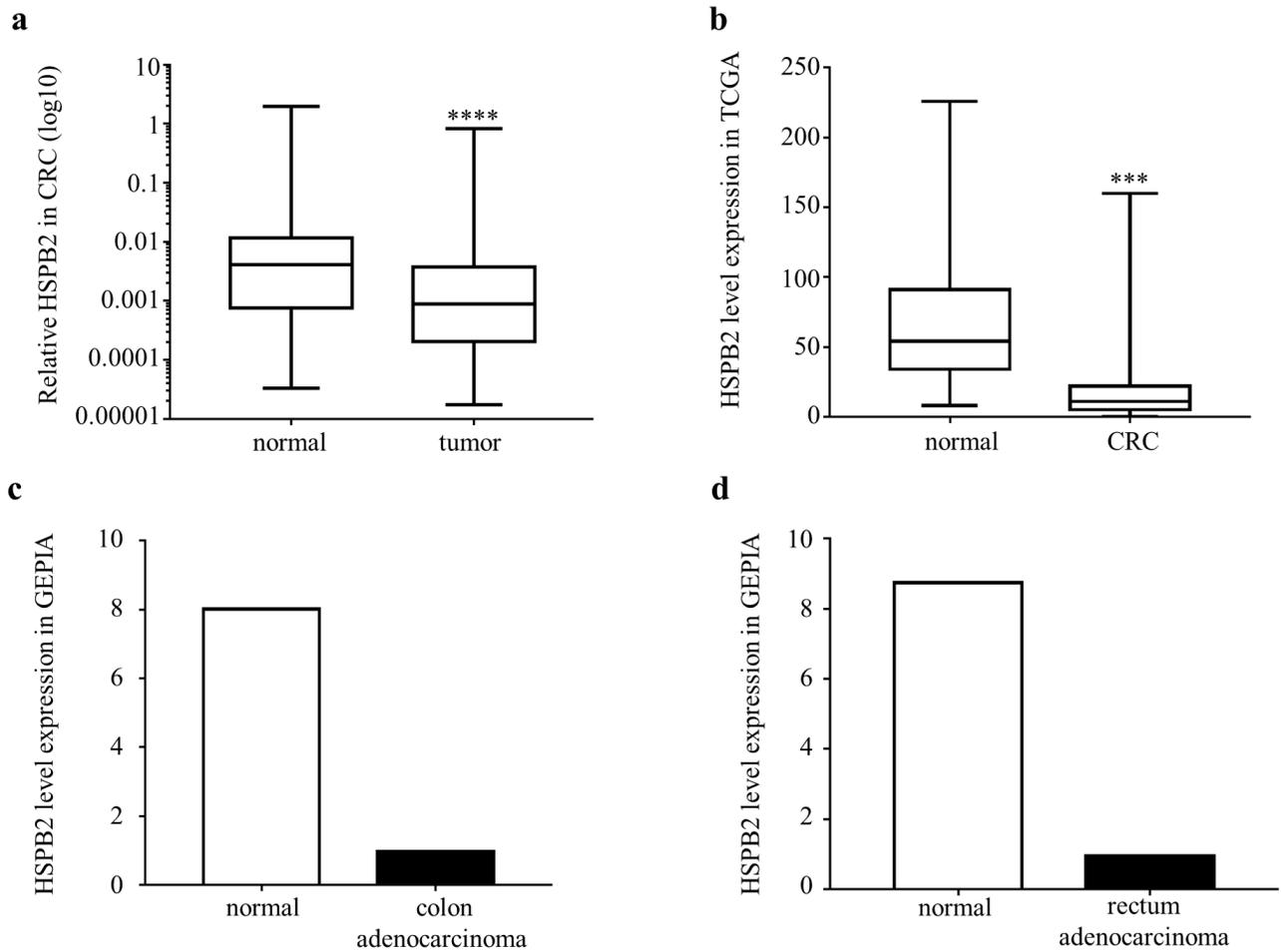


Figure 6

The expression of HSPB2 mRNA is down-regulated in CRC tissues. (A) The expression level of HSPB2 in 51 paired of CRC and adjacent normal tissues by qRT-PCR. (B) The HSPB2 expression profile of CRC tissues (n=647) and normal tissues (n=51) in CRC dataset of TCGA. (C) The HSPB2 expression profile of colon adenocarcinoma tissues (n=275) and normal tissues (n=349) in COAD dataset of GEPIA. (D) The HSPB2 expression profile of rectum adenocarcinoma tissues (n=92) and normal tissues (n=318) in READ dataset of GEPIA. *** P <0.001; **** P <0.0001.

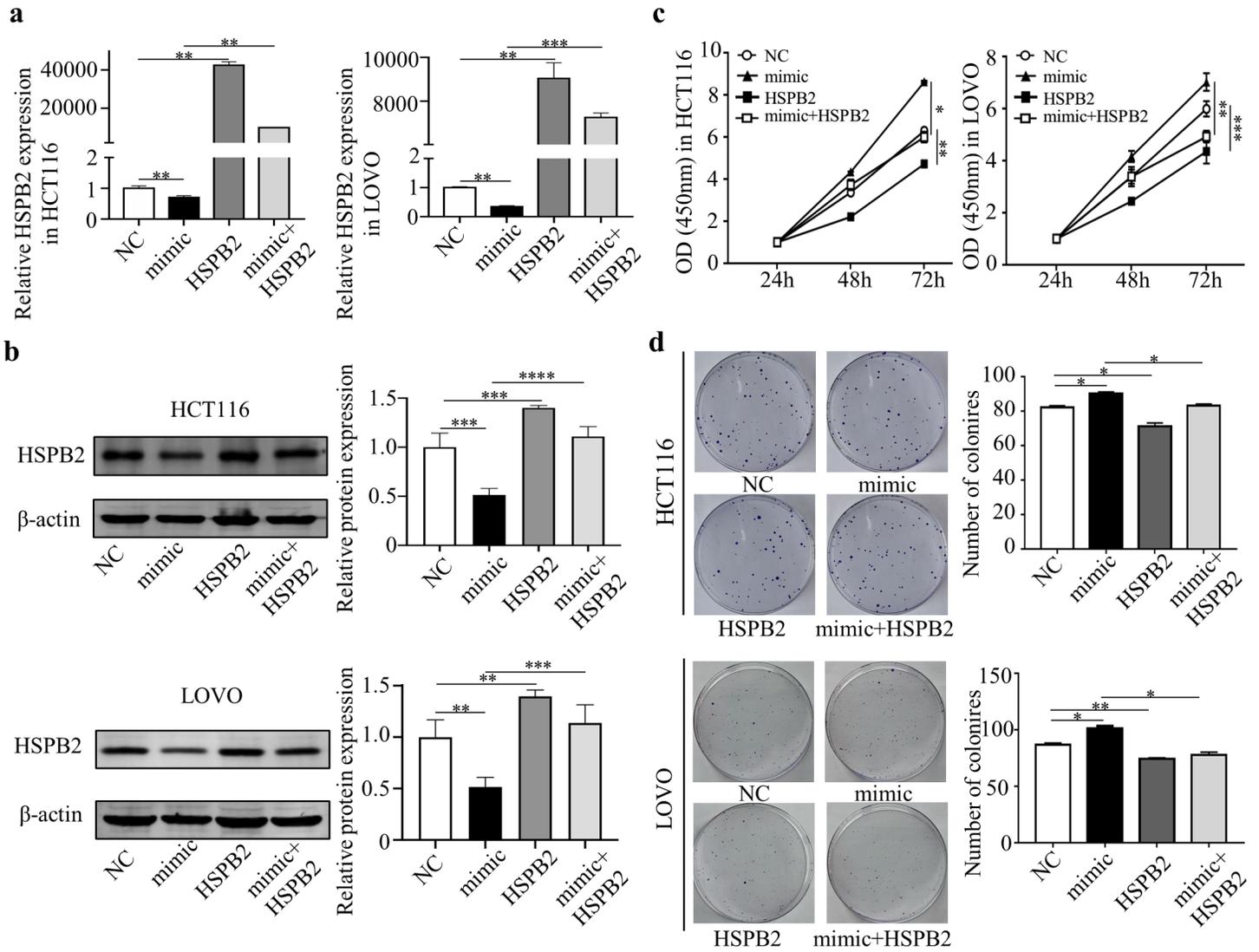


Figure 7

Reintroduction of HSPB2 could reverse the promotion of miR-17-5p in cell proliferation. (A, B) The overexpression of miR-17-5p and HSPB2 alone or simultaneously in HCT116 and LOVO cells confirmed by qRT-PCR and western blot analysis. (C, D) CCK8 analysis and colony formation assay were used to determine the effect of miR-17-5p and HSPB2 overexpression alone or simultaneously on cell proliferation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

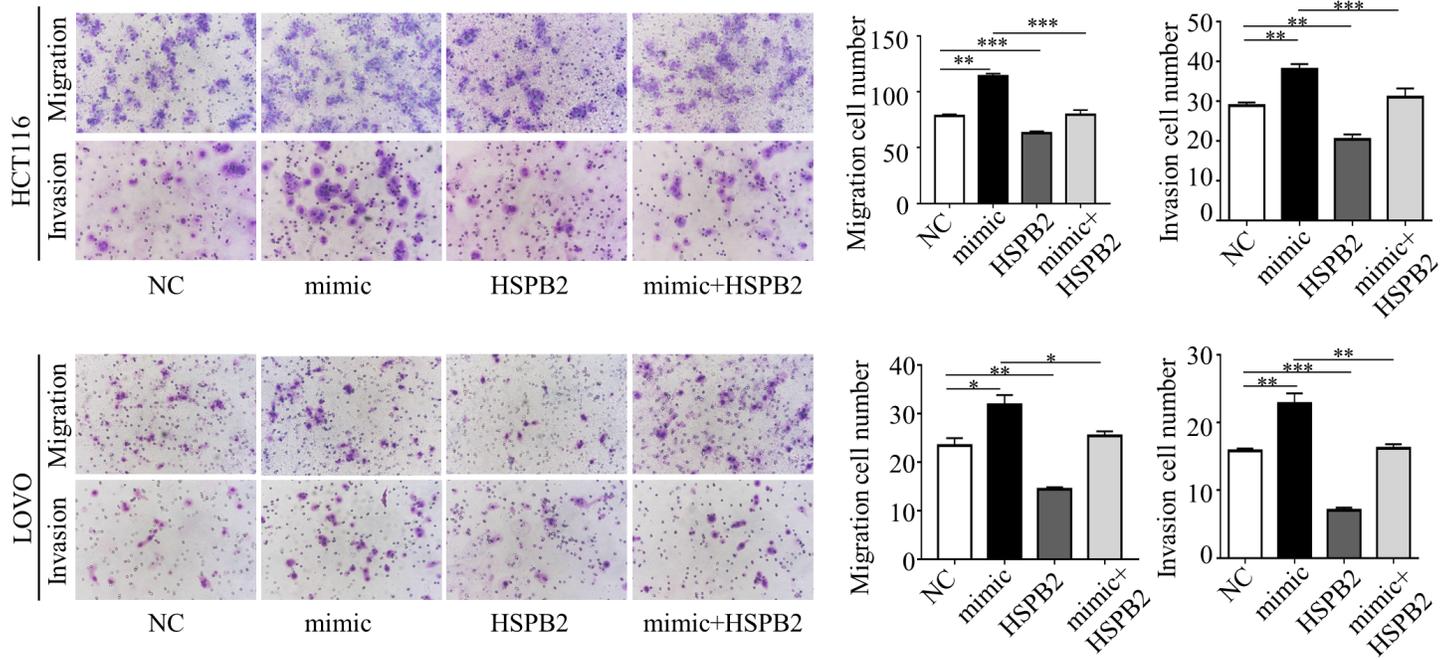


Figure 8

Transwell assay was performed to assess the migration and invasion potency after the expression of miR-17-5p and HSPB2 was up-regulated alone or simultaneously. * P <0.05; ** P <0.01; *** P <0.001.

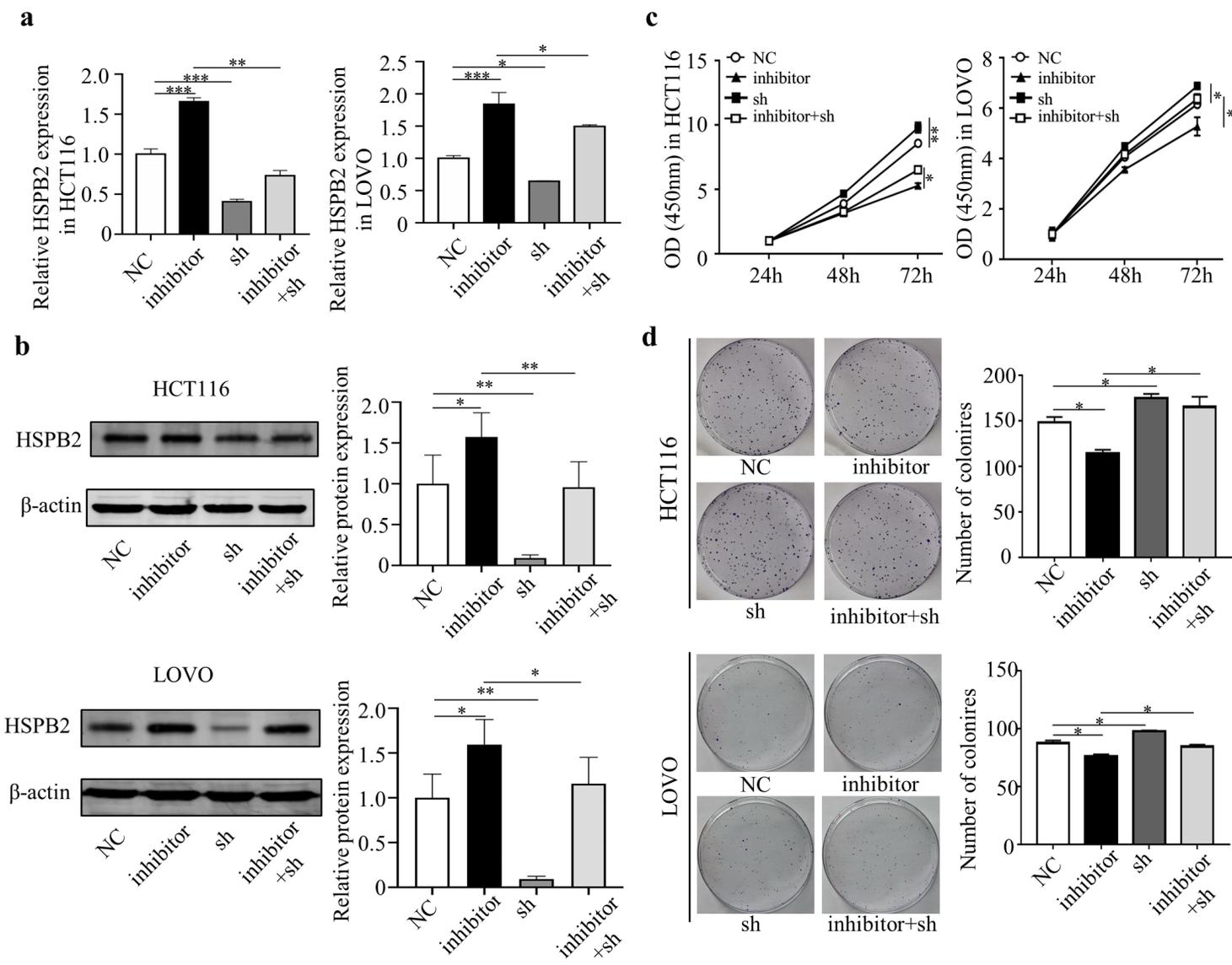


Figure 9

Reduced HSPB2 could rescue the suppression of miR-17-5p inhibitor in cell proliferation. (A, B) The decrease expression of miR-17-5p and HSPB2 alone or simultaneously in HCT116 and LOVO cells confirmed by qRT-PCR and western blot analysis. (C, D) CCK8 analysis and colony formation assay were used to determine the effect of knockdown miR-17-5p and HSPB2 alone or simultaneously on cell proliferation. * P < 0.05; ** P < 0.01; *** P < 0.001.

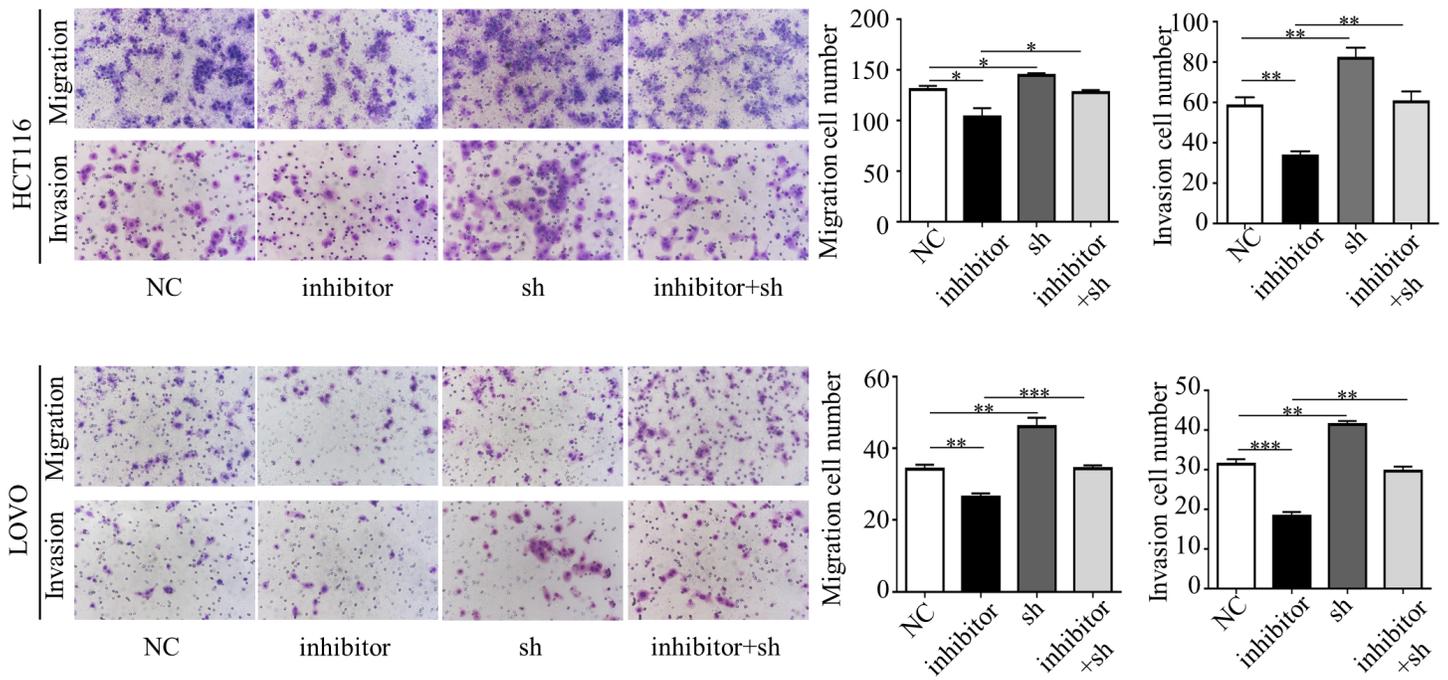


Figure 10

Transwell assay was performed to assess the migration and invasion potency after the expression of miR-17-5p and HSPB2 was down-regulated alone or simultaneously. * P <0.05; ** P <0.01; *** P <0.001.

Supplementary Files

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- [SupplementaryMaterials.pdf](#)