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RESEARCH ARTICLE

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YY1/circCTNNB1/miR-186-5p/YY1 positive loop aggravates lung cancer progression through the Wnt pathway

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ABSTRACT

Lung cancer is one familiar cancer that threatens the lives of humans. circCTNNB1 has been disclosed to have regulatory functions in some diseases. However, the functions and related regulatory mechanisms of circCTNNB1 in lung cancer remain largely indistinct. The mRNA and protein expression levels were examined through real-time polymerase chain reaction (RT-qPCR) and western blot. The cell proliferation was tested through CCK-8 assay. The cell migration and invasion were confirmed through Transwell assays. The cell senescence was evaluated through SA-β-gal assay. The binding ability between miR-186-5p and circCTNNB1 (or YY1) was verified through luciferase reporter and RIP assays. In this study, the higher expression of circCTNNB1 was discovered in lung cancer tissues and cell lines and resulted in poor prognosis. In addition, circCTNNB1 facilitated lung cancer cell proliferation, migration, invasion, and suppressed cell senescence. Knockdown of circCTNNB1 retarded the Wnt pathway. Mechanism-related experiments revealed that circCTNNB1 combined with miR-186-5p to target YY1. Through rescue assays, YY1 overexpression could rescue decreased cell proliferation, migration, invasion, increased cell senescence, and retarded Wnt pathway mediated by circCTNNB1 suppression. Furthermore, YY1 acts as a transcription factor that can transcriptionally activate circCTNNB1 to form YY1/circCTNNB1/miR-186-5p/YY1 positive loop. Through in vivo assays, circCTNNB1 accelerated tumour growth in vivo. All findings revealed that a positive loop YY1/circCTNNB1/miR-186-5p/YY1 aggravated lung cancer progression by modulating the Wnt pathway

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Introduction

Lung cancer, as a familiar tumour, has the highest lethality [1,2]. In lung cancer, non-small cell lung cancer (NSCLC) takes up about 85% [3]. In recent years, targeted therapies have been developed to lengthen the survival time of lung cancer patients, but the five-year survival rate has remained low [4,5]. Exploring the latent tumorigenic mechanisms for better bio-targets for lung cancer treatment is imperative.

Circular RNAs (circRNAs) are a kind of non-coding RNA, which is featured by covalently closed loops and participate in the tumorigenesis and development of cancers [6,7]. CircRNAs can be expressed, making them to be latent biomarkers for cancer progression, including lung cancer. circPOLA2 modulates miR-326/GNB1 axis to facilitate stemness in lung cancer [8]. circBANP aggravates the progression of lung cancer through the miR-503/LARP1 axis [9]. CircNEIL3 combines miR-1184 to enhance PIF1 expression, thereby regulating pyroptosis to affect radiotherapy in lung adenocarcinoma [10]. Besides, circRNA C190 modulates the EGFR/ERK pathway to accelerate non-small cell lung cancer [11]. CircCTNNB1 serves as a novel circRNA, and owns regulatory effects on some diseases. circCTNNB1 interacts with RBM15 to affect m6A modification, modulating aerobic glycolysis in osteosarcoma [12]. Moreover, circCTNNB1 binds miR-96-5p to strengthen scavenger receptor class B type 1 (SRB1) expression, improving cerebral ischaemia/reperfusion injury [13]. In spinal cord injury, circCTNNB1 modulates the Wnt/β-catenin pathway to affect neuronal injury [14].

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Furthermore, circCTNNB1 regulates DDX3-triggered transactivation of YY1 to exacerbate cancer progression [15]. However, no report has investigated the role and related regulatory mechanisms of circCTNNB1 in lung cancer.

The miRNAs are another cluster of small non-coding RNAs (18□22 nucleotides) that are important mediators regulated by lncRNAs or circRNAs to join in many biological processes in lung cancer progression. MiR-186-5p has been uncovered to be a suppressor in diversified cancers, including lung cancers. miR-186-5p suppresses cisplatin resistance by targeting SIX1 in NSCLC [16]. miR-186 targets SIRT6 to retard lung cancer progression [17]. In addition, lncRNA XIST sponges miR-186-5p to modulate NSCLC cell proliferation and invasion [18]. Circ_0076305 combines miR-186-5p to strengthen cisplatin (DDP) resistance in NSCLC [19]. Reports have focused on the interactions between circRNAs and miRNAs in lung cancer. However, the relationship between circCTNNB1 and miR-186-5p in lung cancer remains unknown.

This study was focused on studying the functions and regulatory mechanisms of competing endogenous RNAs (ceRNAs) of circCTNNB1 in lung cancer. This study uncovered that circCTNNB1 exhibited higher expression, resulted into poor prognosis in lung cancer, and formed the positive loop YY1/circCTNNB1/miR-186-5p/YY1 to aggravated lung cancer progression through the Wnt pathway. This study may provide new light for bio-markers in lung cancer treatment.

Materials and methods

Tissue samples

The lung cancer ($n = 40$) and adjacent normal ($n = 40$) tissues were collected from lung cancer patients at The Affiliated Tumor Hospital of Xinjiang Medical University, Xinjiang, China. The collected tissues were kept in liquid nitrogen right away for experiments. This study was acquired with the approval of the Ethics Committee of The Affiliated Tumor Hospital of Xinjiang Medical University (K-2022041). All lung cancer patients with no treatment have signed the informed consent.

Cell lines and culture

The normal bronchial epithelial cell line (BEAS-2B) and lung cancer cell lines (H1299, A549, PC9, and H1975) were bought from the American Tissue Culture Collection (ATCC, USA). The incubation of these cells in Dulbecco's modified Eagle's medium (DMEM; Gibco, New York, NY, USA), including 10% foetal bovine serum (FBS, Gibco, USA), was performed in a wet incubator (5% CO_2, 37°C).

RNase R and actinomycin D treatment

After RNase R (Sigma, Kawasaki, Kanagawa, Japan) treatment, the circular or linear CTNNB1 levels were examined by real-time quantitative polymerase chain reaction (RT-qPCR).

After actinomycin D (5 μg/mL, Sigma, Kawasaki, Kanagawa, Japan) treatment, at 0, 10, 20, and 30 h, the levels of circular or linear CTNNB1 were measured by RT-qPCR.

RT-qPCR

The RNAs from lung cancer tissues or cells were separated through TRIzol reagent (Invitrogen, USA). The transcription of RNAs to complementary DNA (cDNA) was made through the PrimeScript™ RT Master Mix kit (Takara, Dalian, China). Then, qRT-PCR was conducted through the SYBR Premix Ex Taq™ (Takara, Shanghai, China). The relative expressions were figured up through the $2^{-\Delta\Delta Ct}$ method (GAPDH serves as the internal reference).

The primers were shown:
circ-CTNNB1:
Forward5'-TTTTCTTTTACATGCCCCCTCT-3,'
Reverse5'-GGAAGAAGAGAGTTTTTGTGTCC TT-3;'
miR-186-5p:
Forward5'-AAGAATTCTCCTTTTGGGCT-3,'
Reverse5'-GTGCGTGTCGTGGAGTCG-3;'
YY1:
Forward5'-GGAGGAATACCTGGCATT GACC-3,'
Reverse5'-CCCTGAACATCTTTGTGCAGCC-3;'
U6:
Forward5'-GCUUCGGCAGCACAUAUACUAA AAU-3,'

Reverse5'-CGCUUCACGAAUUUGCGUG UCAU-3;'
GAPDH:
Forward5'-GCCAAGGTCATCCATGACAAC-3,'
Reverse5'-ACCACTGACACGTTGGCAGTG-3;'

Subcellular fraction

H1299 and A549 cells were mixed with fractionation buffer and next centrifugated (500×g, 5 min). The supernatant (cytoplasm) and the precipitation (nucleus) were separated. CircCTNNB1 expression in the nucleus or cytoplasm was evaluated through RT-qPCR. GAPDH or U6 (for cytoplasm or nucleus) acted as an internal reference.

Cell transfection

The pcDNA3.1 targeting YY1 (pcDNA3.1/YY1) with negative control (Vector), shRNAs targeting circCTNNB1 (sh-circCTNNB1#1, #2, #3) or YY1 (sh-YY1) with negative control (sh-NC), miR-186-5p mimic with negative control (NC mimic) were constructed and purchased by GenePharma (Shanghai, China). The transfection of these plasmids into H1299 and A549 cells was made with Lipofectamine 2000 (Invitrogen, Waltham, MA, USA).

Cell counting kit-8 (CCK-8) assay

H1299 and A549 cells (1×10^4 cells/well) were plated into the 96-well plate. Each well was mixed with CCK-8 solution (10 μL, Dojindo Laboratories, Kumamoto, Japan) at 0, 24, 48, 72 and 96 h. Post incubation for another 4 h, one spectrophotometer (Thermo Fisher Scientific, MA, USA) was employed to measure the absorbance (450 nm).

Transwell assay

Transwell chambers pre-coated with (or without) Matrigel (BD Biosciences, MD, USA) were employed. The addition of serum-free DMEM medium was made into the upper chamber, and the addition of DMEM medium with 20% FBS was made into the lower chamber. The staining with crystal violet was conducted. The migrated and invaded cells were manually calculated through one microscope (Olympus Corporation, Tokyo, Japan).

SA-β-Gal assay

At pH 6.0, senescent cells had senescence-associated β-galactosidase (SA-β-Gal) activity. The artificial substrate X-Gal was utilized to stain cells to examine SA-β-Gal activity. The SA-β-Gal Kit (Beyotime Institute of Biotechnology, Shanghai, China) was employed. The cytoplasm (blue) stained with SA-β-Gal was deemed as positive.

Luciferase reporter assay

The pmirGLO dual-luciferase vectors (Promega, Madison, WI, USA) were inserted with wild-type or mutant-type sequences of circCTNNB1 (or YY1) to build the circCTNNB1 (or YY1)-WT and circCTNNB1 (or YY1)-MUT reporter vectors. H1299 and A549 cells were co-transfected with these above reporter vectors and miR-186-5p mimic/NC mimic through Lipofectamine 2000. After 48 h, the luciferase activity was evaluated through the dual-luciferase reporter assay system (Promega, Madison, WI, USA).

For evaluating the interaction ability between YY1 and the circCTNNB1 promoter, the pGL3 luciferase reporter vectors (Promega, Madison, WI, USA) were inserted with the circCTNNB1 promoter region (WT or MUT) to construct the circCTNNB1 promoter-WT and circCTNNB1 promoter-MUT reporter vectors. These constructed reporter vectors and pcDNA3.1/YY1 (or Vector) plasmids were co-transfected into H1299 and A549 cells.

RIP assay

H1299 and A549 cell lysates were mixed with magnetic beads conjugated with anti-Ago2, anti-IgG, or anti-YY1 antibodies. The negative control was the IgG group, and the positive control was the input group. After immunoprecipitation, the relative enrichment of circCTNNB1, miR-186-5p, and YY1 was assessed by RT-qPCR.

Western blot

The proteins were extracted from H1299 and A549 and were performed to 10% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The moving of proteins to PVDF membranes (Beyotime, Shanghai, China) was done. After blocking, the PVDF membranes were added with the primary antibodies for 12 h at 4°C and the appropriate secondary antibodies for another 2 h. β-actin was set as the internal reference. The chemiluminescence detection kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was utilized to detect blots.

The primary antibodies were as follows: WNT3A (ab116222, 1 μg/mL, Abcam, China, Shanghai); β-catenin (ab223075, 1 μg/mL); c-myc (ab32072, 1/1000); YY1 (ab109228, 1/1000); β-actin (ab8226, 1 μg/mL).

In vivo assay

This assay was acquired with the approval of the Animal Care and Use Committee of Beijing Viewsolid Biotechnology Co. LTD (VS212601463). The male BALB/c nude mice (one-month-old, total $n = 10$, randomly $n = 5$ for each group) were obtained from the Vital River company (Beijing, China). The injection of transfected A549 cells (sh-NC, sh-circCTNNB1) into mice was performed. After 28 days, the mice were euthanized. The tumour size, volume, and weight were measured.

Immunohistochemistry (IHC) assay

The dewaxing and re-hydration for the 4 μm paraffin-embedded tumour tissue sections were done. After blocking, sections were cultured with Ki-67 antibodies at 4°C overnight and mixed with secondary antibodies (1:1000, ab7090, Abcam, Shanghai, China). The dyeing by diaminobenzidine (DAB) and re-dyeing by haematoxylin for sections were performed. Images were captured through one microscope (Nikon, Tokyo, Japan).

Statistical analysis

The data were displayed as the mean ± standard deviation (SD). The GraphPad Prism software, version 8.0 (GraphPad Software, La Jolla, CA), was utilized for statistical analysis. Each experiment was done in triplicate. The Student's *t*-test (for two groups) or one-way analysis of variance (ANOVA, for multiple groups) was adopted to make comparisons. $p < 0.05$ was considered statistically significant.

Results

The higher expression of circCTNNB1 was discovered in lung cancer

As shown in Figure 1(a), circCTNNB1 expression was up-regulated in lung cancer tissues compared with the normal tissues. Higher circCTNNB1 expression resulted in poor prognosis in lung cancer patients (Figure 1(b)). circCTNNB1 exhibited higher expression in lung cancer cell lines (H1299, A549, PC9 and H1975) than in the bronchial epithelial cell line (BEAS-2B) (Figure 1(c)). Due to the higher expression of circCTNNB1 in H1299 and A549 cells, these two cell lines were collected for further experiments. The RNase R assay illustrated that CTNNB1 was degraded, whereas circCTNNB1 could resist RNase R digestion (Figure 1(d)). The half-life of circCTNNB1 was longer than that of CTNNB1 transcript after Actinomycin D treatment, indicating that circCTNNB1 was more stable than CTNNB1 (Figure 1(e)). These data indicated that circCTNNB1 was a circle RNA. Furthermore, circCTNNB1 was found to be located in the cytoplasm (Figure 1(f)). CircCTNNB1 expression was up-regulated in lung cancer.

CircCTNNB1 facilitated lung cancer cell proliferation, migration, invasion, and suppressed cell senescence

The knockdown efficiency of circCTNNB1 was confirmed in Figure 2(a), and sh-circCTNNB1#1 was chosen for the next experiments due to its' higher knockdown efficiency (sh-circCTNNB1). The cell proliferation was weakened after silencing circCTNNB1 (Figure 2(b)). The cell migration and invasion abilities were attenuated after circCTNNB1 knockdown (Figure 2(c,d)). The cell senescence was enhanced after circCTNNB1

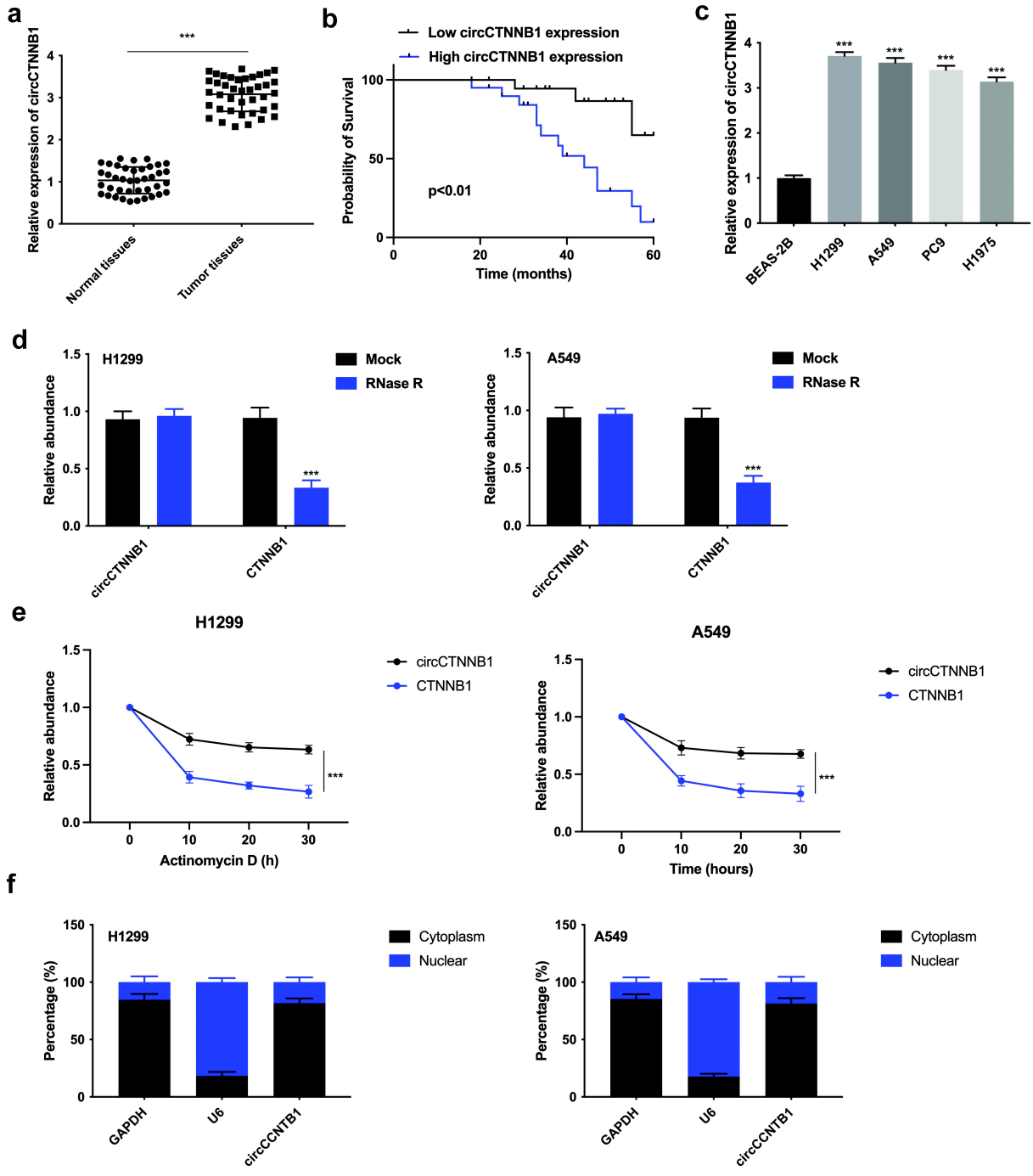

Figure 1. The higher expression of circCTNNB1 was discovered in lung cancer.

(a) The mRNA expression of circCTNNB1 was examined in lung cancer tissues ($n = 40$) and normal tissues ($n = 40$) through RT-qPCR. (b) The prognosis of lung cancer patients with higher ($n = 20$) or lower ($n = 20$) circCTNNB1 expression was verified. (c) The mRNA expression of circCTNNB1 was determined in human bronchial epithelial cell line (BEAS-2B) and lung cancer cell lines (H1299, A549, PC9, and H1975) through RT-qPCR. (d) The relative abundance of circCTNNB1 and CTNNB1 was evaluated after RNase R treatment through RT-qPCR. (e) The relative abundance of circCTNNB1 and CTNNB1 was assessed after Actinomycin D treatment through RT-qPCR. (F) The location of circCTNNB1 in cytoplasm or nuclear was verified through RT-qPCR. ***$p < 0.001$.

suppression (Figure 2e). Taken together, circCTNNB1 facilitated lung cancer cell proliferation, migration, invasion, and suppressed cell senescence.

Knockdown of circCTNNB1 retarded the Wnt pathway

β-catenin transcribed by CTNNB1 is a key regulator in the Wnt pathway. So, circCTNNB1, which affects

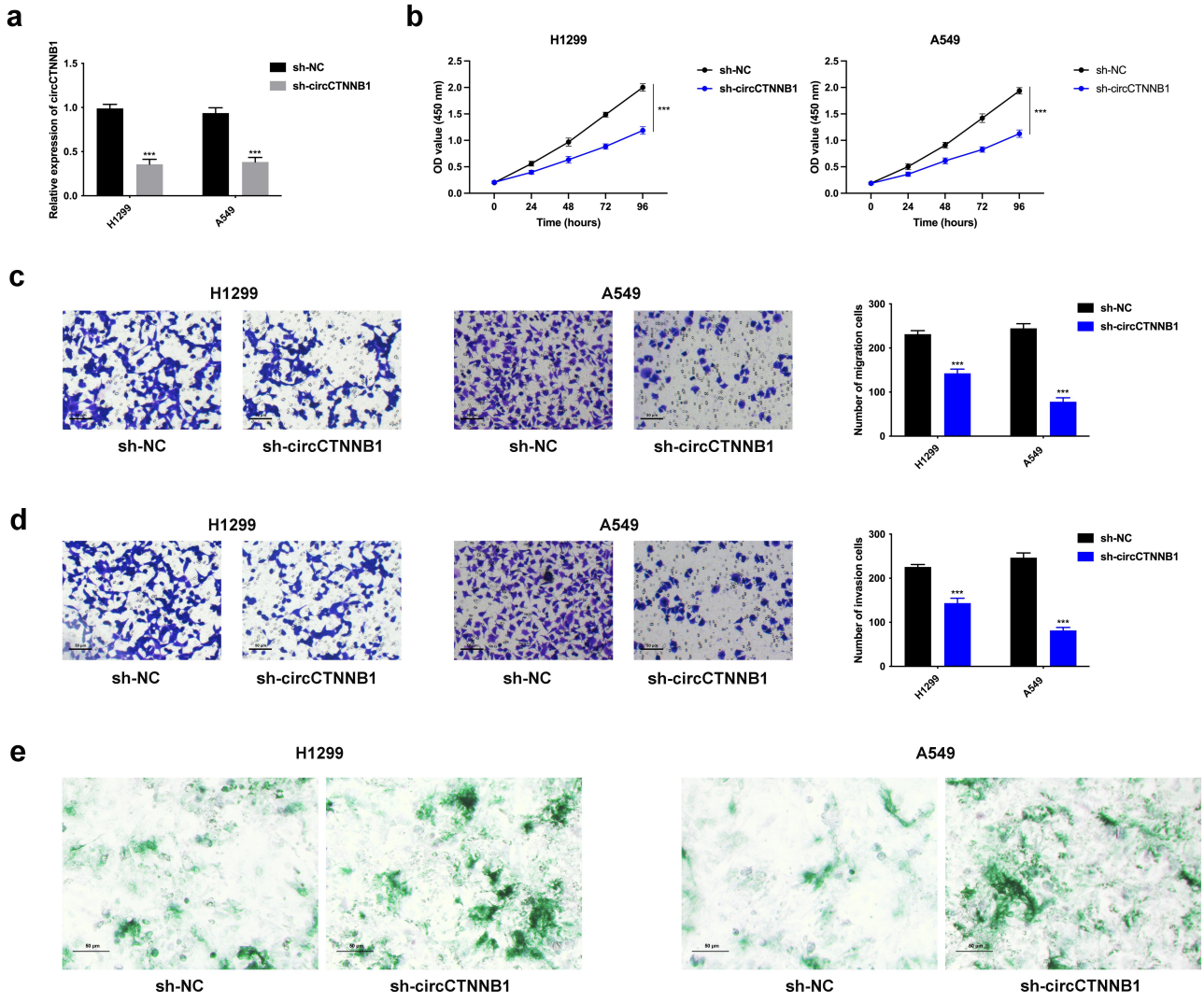

Figure 2. CircCTNNB1 facilitated lung cancer cell proliferation, migration, invasion, and suppressed cell senescence.

(a) The knockdown efficiency of circCTNNB1 was confirmed through RT-qPCR in the sh-NC, sh-circCTNNB1#1, sh-circCTNNB1#2, and sh-circCTNNB1#3 groups. (b) The cell proliferation was tested through CCK-8 assay in the sh-NC and sh-circCTNNB1 groups. (c-d) The cell migration and invasion were examined through Transwell assay in the sh-NC and sh-circCTNNB1 groups. (e) The cell senescence was evaluated through SA-β-gal assay in the sh-NC and sh-circCTNNB1 groups. ***$p < 0.001$.

the Wnt pathway in lung cancer, needs further investigation. The nuclear translocation of β-catenin was decreased after circCTNNB1 inhibition (Figure 3(a)). The Wnt pathway-related proteins (WNT3A, β-catenin, and c-myc) were reduced after silencing circCTNNB1 (Figure 3(b)). The knockdown of circCTNNB1 retarded the Wnt pathway.

CircCTNNB1 combined with miR-186-5p to target YY1

The related ceRNA mechanisms of circCTNNB1 were investigated. Under the screening condition (CLIP-seq Type: other CLIP), 13 miRNAs (miR-1294; miR-25-3p; miR-32-5p; miR-367-3p; miR-92b-3p; miR-363-3p; miR-223-3p; miR-944; miR-186-5p; miR-552-3p; miR-374b-3p; miR-668-3p; miR-526b-5p) were obtained. Through RT-qPCR, among these miRNAs, miR-186-5p was markedly elevated after circCTNNB1 knockdown (Figure S1). Thus, miR-186-5p was chosen for further experiments. Through the starBase online database, the binding sites between circCTNNB1 and miR-186-5p (YY1) were presented (Figure 4(a)). circCTNNB1 combined with miR-186-5p and miR-186-5p bound with YY1 through luciferase reporter and RIP assay

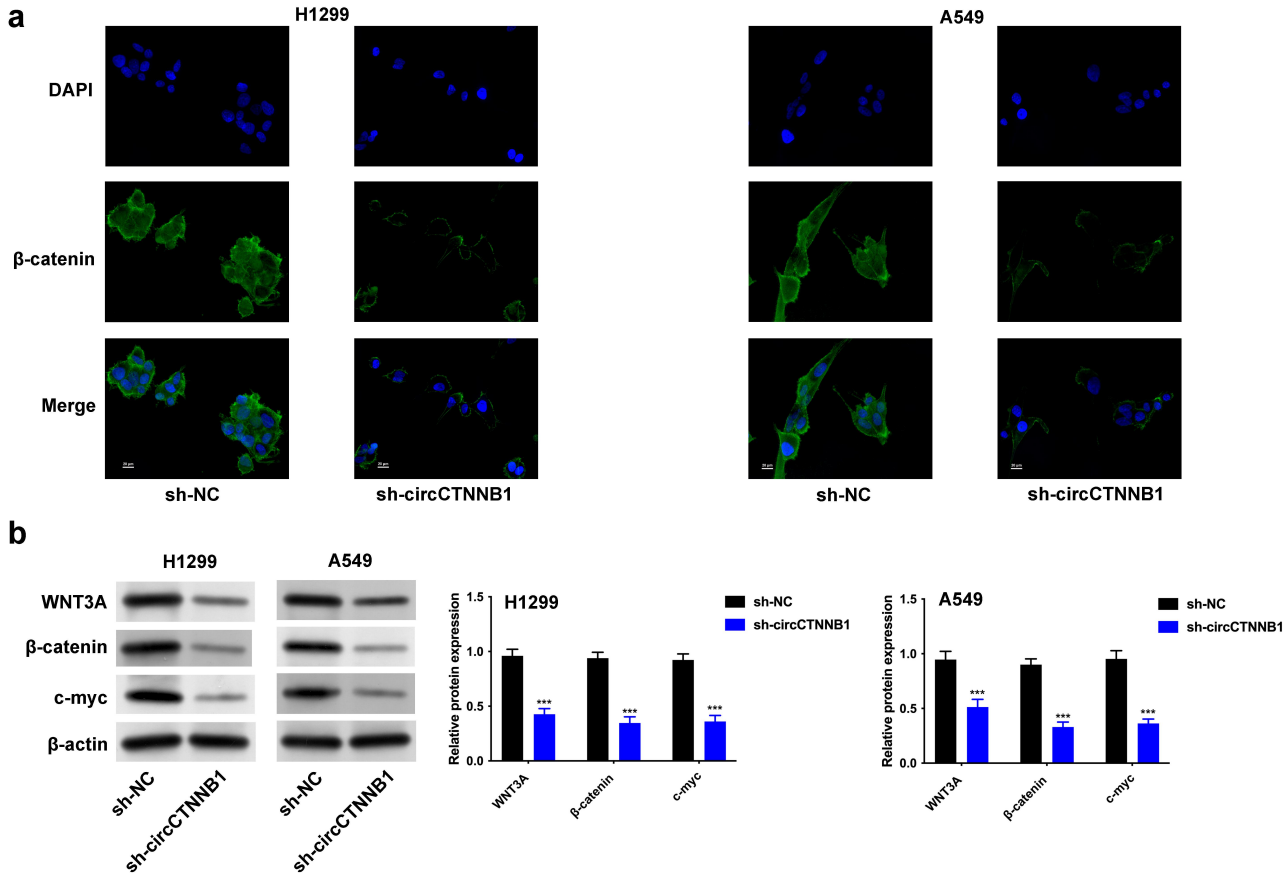

Figure 3. Knockdown of circCTNNB1 retarded the Wnt pathway.
(a) The nuclear translocation of β-catenin was notarized through IF assay in the sh-NC and sh-circCTNNB1 groups. (b) The protein expressions of WNT3A, β-catenin, and c-myc were determined through western blot in the sh-NC and sh-circCTNNB1 groups. ***$p < 0.001$.

(Figure 4(b,c)). The miR-186-5p expression was enhanced after circCTNNB1 knockdown, but YY1 mRNA and protein expression levels were reduced after circCTNNB1 knockdown or miR-186-5p mimic (Figure 4(d,e)). miR-186-5p expression was lower, and YY1 expression was higher in lung cancer tissues and cell lines (Figure 4(f,g)). circCTNNB1 combined with miR-186-5p to target YY1.

YY1 overexpression rescued the decreased cell proliferation, migration, invasion, and increased cell senescence mediated by circCTNNB1 suppression

The cell proliferation was weakened after circCTNNB1 knockdown, but this effect was reversed after YY1 overexpression (Figure 5(a)). In addition, the decreased cell migration and invasion stimulated by circCTNNB1 suppression were effectively alleviated after YY1 up-regulation (Figure 5(b,c)). The increased cell senescence induced by circCTNNB1 inhibition was relieved after YY1 overexpression (Figure 5(d)). These findings uncovered that YY1 overexpression could rescue the decreased cell proliferation, migration, and invasion and increased cell senescence mediated by circCTNNB1 suppression.

YY1 overexpression rescued the retarded Wnt pathway mediated by circCTNNB1 knockdown

The decreased nuclear translocation of β-catenin mediated by circCTNNB1 knockdown was rescued after YY1 amplification (Figure 6(a)). The reduced protein expressions of WNT3A, β-catenin, and c-myc triggered by circCTNNB1 inhibition were reversed after YY1 overexpression (Figure 6(b)). YY1 overexpression could rescue the retarded Wnt pathway mediated by circCTNNB1 knockdown.

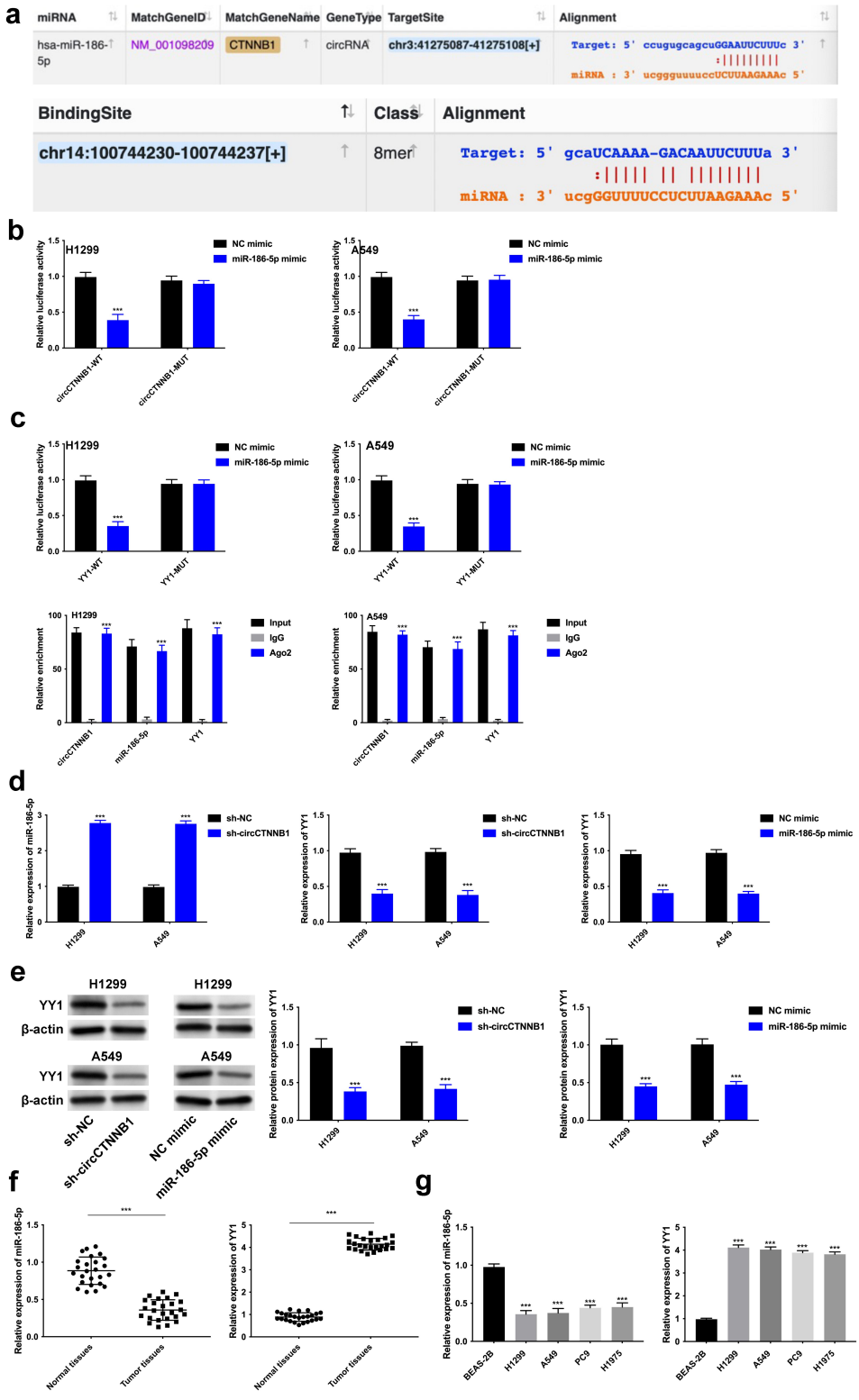

Figure 4. CircCTNNB1 combined with miR-186-5p to target YY1.

(a) The binding sites between circCTNNB1 and miR-186-5p (YY1) were displayed. (b-c) The binding ability between circCTNNB1 and miR-186-5p (YY1) was confirmed through luciferase reporter and RIP assays. (d) The expressions of miR-186-5p and YY1 were detected through RT-qPCR. (e) The protein expression of YY1 was assessed after circCTNNB1 knockdown or miR-186-5p mimic through western blot. (f) The expressions of miR-186-5p and YY1 were confirmed in lung cancer tissues and normal tissues through RT-qPCR. (g) The expressions of miR-186-5p and YY1 were tested in lung cancer cell lines and normal bronchial epithelial cell line (BEAS-2B) through RT-qPCR. ***$p < 0.001$.

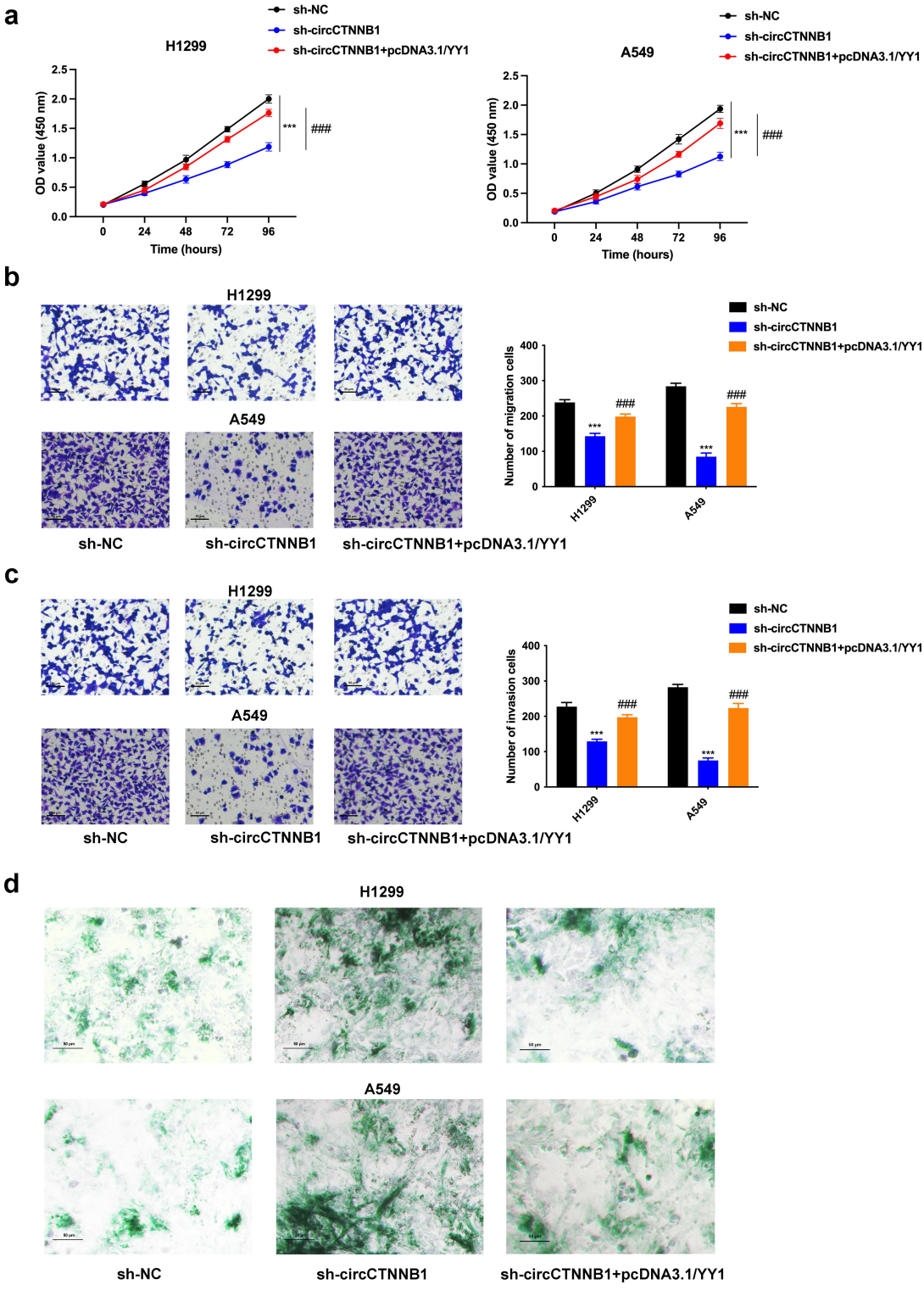

Figure 5. YY1 overexpression can rescue the decreased cell proliferation, migration, invasion, and the increased cell senescence mediated by circCTNNB1 suppression.

Groups were divided into the sh-NC, sh-circCTNNB1, and sh-circCTNNB1+pcDNA3.1/YY1 groups. (a) The cell proliferation was measured through a CCK-8 assay. (b-c) The cell migration and invasion were assessed through Transwell assay. (d) The cell senescence was confirmed through SA-β-gal assay. ***$p < 0.001$ vs the sh-NC group; ###p < 0.001 vs the sh-circCTNNB1.

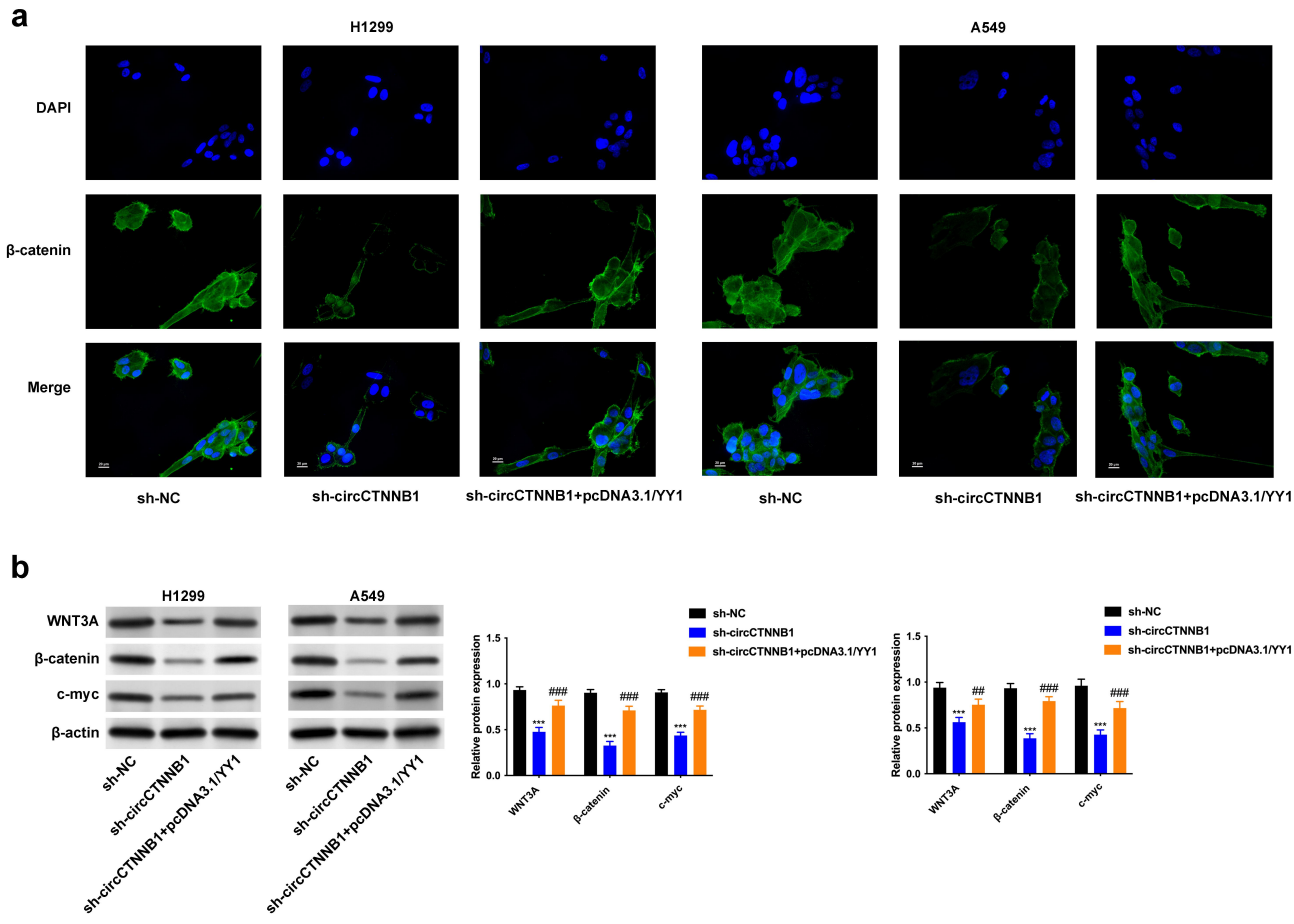

Figure 6. YY1 overexpression can rescue the retarded Wnt pathway mediated by circCTNNB1 knockdown.

Groups were divided into the sh-NC, sh-circCTNNB1, and sh-circCTNNB1+pcDNA3.1/YY1 groups. (a) The nuclear translocation of β-catenin was examined through IF assay. (b) The protein expressions of WNT3A, β-catenin, and c-myc were determined through western blot. $^{**}p < 0.01$, $^{***}p < 0.001$ vs the sh-NC group; ##p < 0.01, ###p < 0.001 vs the sh-circCTNNB1.

YY1 ranscriptionally activated circCTNNB1 to form YY1/circCTNNB1/miR-186-5p/YY1 loop

Whether YY1 can regulate the transcription of circCTNNB1 in lung cancer needs further investigation. The circCTNNB1 expression was positively correlated to YY1 expression in lung cancer tissues (Figure 7(a)). The expression of circCTNNB1 was reduced after YY1 inhibition (Figure 7(b)). The binding sites of YY1 are shown in Figure 7c. YY1 was verified to combine with the promoter of circCTNNB1 (Figure 7(d-e)).

CircCTNNB1 accelerated tumor growth in vivo

The xenograft mouse models were adopted to determine the regulatory effects of circCTNNB1 on tumorigenesis *in vivo*. The A549 cells transfected with sh-circCTNNB1 (or sh-NC) plasmids were inoculated into mice. The tumour size, volume, and weight were reduced after circCTNNB1 suppression (Figure 8(a-c)). The Ki67 expression level was decreased after circCTNNB1 inhibition (Figure 8(d)). circCTNNB1 accelerated tumour growth *in vivo*.

Discussion

circRNAs exert regulatory functions in diversified ways, such as affecting proteins or miRNAs [20,21]. CircCTNNB1 has been discovered to participate in some diseases [12–15], but its regulatory roles in lung cancer remain vague. In this study, the higher expression of circCTNNB1 was in lung cancer tissues and cell lines and resulted in poor prognosis. In addition, circCTNNB1 facilitated lung cancer cell proliferation, migration, invasion, and suppressed cell senescence.

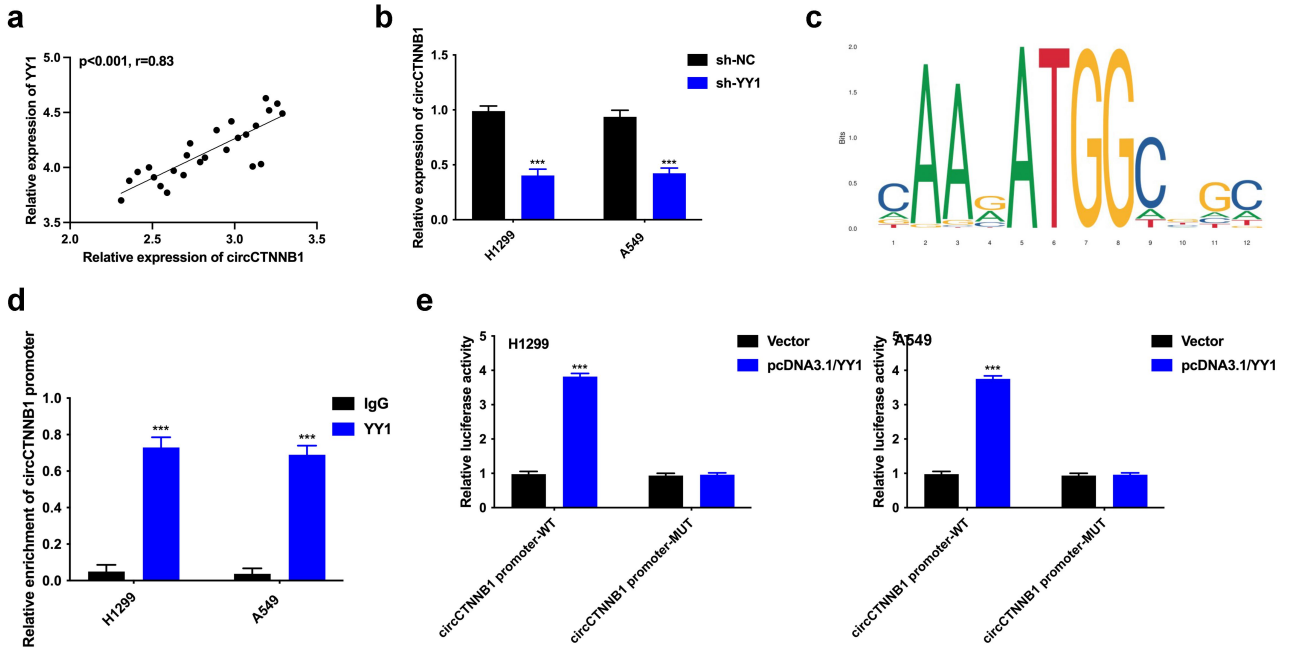

Figure 7. YY1 can transcriptionally activate circCTNNB1 to form YY1/circCTNNB1/miR-186-5p/YY1 loop.
(a) The correlation between circCTNNB1 and YY1 was verified. (b) The expression of circCTNNB1 was tested after YY1 knockdown through RT-qPCR. (b) The binding site of YY1 was displayed. (d-e) The binding ability between YY1 and the promoter of circCTNNB1 was measured through RIP and luciferase reporter assays. ***$p < 0.001$.

The Wnt pathway has been disclosed to be a vital pathway in cancer progression [22,23]. The Wnt pathway exists in the regulation of lung cancer. lncRNA PKMYT1AR activates the Wnt pathway in non-small cell lung cancer to facilitate cancer stem cell maintenance [24]. CircEIF3I affects the Wnt/β-catenin pathway to strengthen cell proliferation and invasion by targeting the miR-1253/NOVA2 axis in lung cancer [25]. Cigarette smoking strengthens the Wnt/β-catenin pathway to stimulate tumorigenesis in lung cancer [26]. Besides, MARVELD3 retards the Wnt/β-catenin pathway to suppress the epithelial-mesenchymal transition progress in non-small cell lung cancer [27]. circCTNNB1 has been reported to activate Wnt signalling and up-regulate β-catenin protein expression [14]. Besides, β-catenin transcribed by CTNNB1 is a key regulator in the Wnt pathway. So, circCTNNB1 affects the Wnt pathway in lung cancer and needs further investigation. Our results also revealed that the knockdown of circCTNNB1 retarded the Wnt pathway.

circRNAs affect cellular processes in cancers by sponging specific miRNAs to release mRNAs, forming a ceRNA axis [28,29]. For example, circ_0007401 sponges miR-6509-3p to target fli1, affecting gemcitabine resistance in pancreatic cancer [30]. Furthermore, circ_0072088 regulates miR-1225-5p/WT1 axis to aggravate malignant behaviour in non-small cell lung cancer [31]. Circ_0003747 targets the miR-338-3p/PLCD3 axis to intensify thyroid cancer progression [32]. circSCN8A combines miR-1290 to release ACSL4, inhibiting malignant progression in non-small cell lung cancer [33]. This ceRNA axis has also been confirmed to participate in lung cancer progression. However, the related ceRNA mechanisms of circCTNNB1 in lung cancer need more exploration. In this study, further mechanism-related experiments revealed that circCTNNB1 combined with miR-186-5p to target YY1. Through rescue assays, YY1 overexpression can rescue the decreased cell proliferation, migration, invasion, increased cell senescence, and the retarded Wnt pathway mediated by circCTNNB1 suppression.

YY1 is a common transcription factor that affects circRNA, lncRNA, or mRNA expression in cancers. YY1-induced circFIRRE contributes to the progression of osteosarcoma [34].

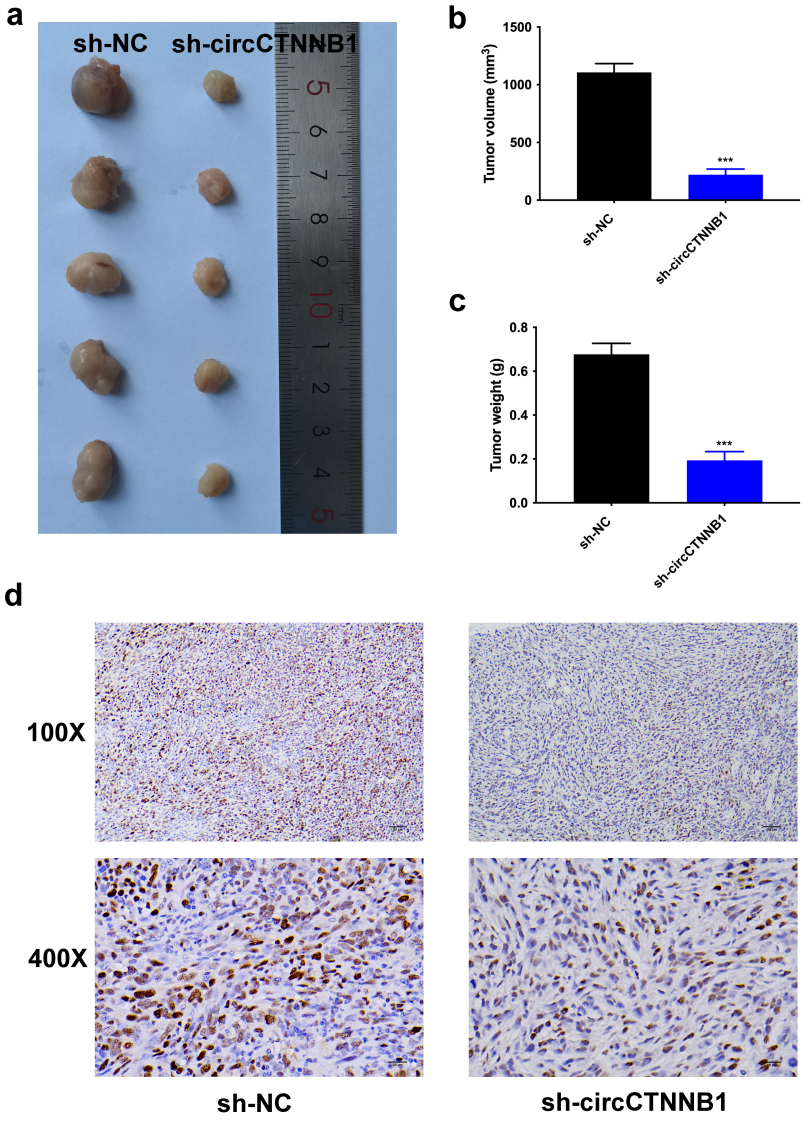

Figure 8. CircCTNNB1 accelerated tumour growth in vivo.

(a-c) The tumour size, volume, and weight were evaluated in the sh-NC and sh-circCTNNB1 groups. (d) The Ki67 expression was examined through IHC assay in the sh-NC and sh-circCTNNB1 groups. ***$p < 0.001$.

YY1-stimulated up-regulation of lncRNA LINC00466 aggravates glioma progression through targeting miR-508/CHEK1 axis [35]. Moreover, YY1-triggered lncRNA MCM3AP-AS1 targets the miR-340-5p/KPNA4 axis to strengthen angiogenesis in lung cancer [36]. Besides, in tongue squamous cell carcinoma, YY1-mediated PTEN dephosphorylation relieves IR-stimulated DNA repair [37]. Whether YY1 can regulate the transcription of circCTNNB1 in lung cancer needs further investigation. Similar to previous studies, YY1 can transcriptionally activate circCTNNB1, forming the YY1/circCTNNB1/miR-186-5p/YY1 loop. Besides, circCTNNB1 accelerated tumour growth *in vivo*.

A positive loop YY1/circCTNNB1/miR-186-5p/YY1 aggravates lung cancer progression through the Wnt pathway. This discovery may supply useful therapeutic bio-targets for lung cancer therapeutic strategies important in clinical diagnosis and treatment. This work also has some limitations for the influences of circCTNNB1 on lung cancer progression, such as lacking more human samples and more cellular processes (autophagy, exosomes, mitochondrial dysfunction, immune escape). In the future, more experiments will be carried out on lung cancer progression for further investigations.

Abbreviations

competing endogenous RNAs	ceRNAs
non-small cell lung cancer	NSCLC
Circular RNAs	circRNAs
scavenger receptor class B type 1	SRB1

Disclosure statement

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Author contributions

YLS, YY, YYZ and CLL designed the study. YZ and SN collected the data. YYZ and CLL analysed the data. YLS and YY wrote the manuscript. All authors read and approved the final manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author.

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