

MiR-375 gene therapy attenuates drug resistance of hepatocellular carcinoma cells by inhibiting cell autophagy

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Research

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Abstract

Objective

To probe into the regulatory mechanism of miR-375 in hepatocellular carcinoma (HCC) cells under sorafenib treatment.

Methods

Western blotting and qRT-PCR were applied to measure the expressions of miR-375 and SIRT5 in parental HCC cells (HepG2 and Huh7) and sorafenib-resistant HCC cells (HepG2/so and Huh7/so). HepG2/so cells were accordingly transfected with miR-375 mimic, miR-375 inhibitor, sh-SIRT5, pcDNA3.1-SIRT5 or negative control. Western blotting measured the expressions of p62, LC3I and LC3II in HCC cells. CCK-8 and flow cytometry assessed the survivability and apoptosis of HCC cells, respectively. Bioinformatics techniques and dual-luciferase reporter assay predicted and verified the targeting relationship between miR-375 and SIRT5.

Results

MiR-375 was under-expressed and SIRT5 was over-expressed in HCC cells. Autophagy inhibitor impaired the survival of HepG2/so cells transfected with miR-375 inhibitor. Autophagy activator enhanced the drug resistance of HepG2/so cells transfected with miR-375 mimic. MiR-375 suppressed the drug resistance of HepG2/so cells by inhibiting autophagy. SIRT5 enhanced the drug resistance of HepG2/so cells by promoting autophagy and it could be targeted by miR-375.

Conclusion

MiR-375 suppresses autophagy to attenuate the drug resistance of HCC cells by regulating SIRT5. The findings of this study may provide new therapeutic targets for treating hepatocellular carcinoma.

Introduction

Liver cancer belongs to the commonest deadly cancers worldwide. There were estimated 854,000 incident cases and 810,000 liver cancer deaths globally in 2015, and the occurrence of liver cancer had a substantial increase from 1990 to 2015 [1]. Hepatocellular carcinoma (HCC) accounts for 90% of all cases of primary liver cancer which can be caused by cirrhosis, infection of hepatitis B or C virus, alcohol abuse and other risk factors [2]. Prognosis and treatment assignment of HCC greatly depend on the tumor grade, and the Barcelona Clinic Liver Cancer (BCLC) system is the most recommended staging system for HCC [3]. Sorafenib is a multikinase inhibitor which can act as an antitumor agent by affecting the proliferation, angiogenesis and apoptosis of tumor cells [4]. Sorafenib is now the first treatment

option and standard therapy for advanced-stage HCC (BCLC C) [5]. In spite of the broad use of sorafenib, “sorafenib resistance” has emerged as a hot-spot issue in dealing with the efficacy of sorafenib.

More and more researchers have made efforts to unveil the mechanisms of sorafenib resistance. Autophagy is a metabolic process that is unavoidably altered in cancers and it can be well manipulated to improve the clinical outcomes of cancer patients [6]. Inhibiting autophagy could enhance sorafenib-induced HCC cell death via the regulation of endoplasmic reticulum stress-mediated apoptosis [7]. Autophagy is suggested to be an important participant in mediating sorafenib resistance and increasing studies have probed into the molecular mechanism involved in autophagy-mediated sorafenib resistance in HCC. For instance, ADRB2 signaling promoted sorafenib resistance in HCC by impeding autophagic degradation of HIF1 α [8]. CD24 could activate autophagy to decrease sorafenib sensitivity of HCC cells [9].

In recent years, microRNAs (miRNAs) have shown great promise as therapeutic targets for cancer treatment. These regulatory genes function in diverse cellular activities including autophagy. MiRNA-375 (miR-375) is known to be an autophagy inhibitor in many disease conditions. As an example, miR-375 facilitated inflammation and apoptosis of acinar cells in severe acute pancreatitis by inhibiting the ATG7/autophagy signal [10]. MiR-375 inhibited autophagy to facilitate the sorafenib therapy for HCC [11]. However, the mechanism of miR-375 in regulating sorafenib resistance-activated autophagy in HCC remains largely unknown.

SIRT5 was overexpressed in non-small cell lung cancer (NSCLC) and protected NSCLC cells against anti-tumor therapy [12]. Polletta et al. reported that SIRT5 activation could impair the survivability of tumor cells in response to chemotherapy or other stresses by suppressing ammonia-induced autophagy [13]. The interaction between miR-375 and SIRT5 has not been investigated yet. The online software jefferson predicted that SIRT5 could be targeted by miR-375 on the 3'UTR region. Based on experimental data, this study verifies that miR-375 can reduce sorafenib resistance of HCC cells by down-regulating SIRT5-mediated autophagy.

Materials And Methods

Cell cultivation

Human HCC cell lines (HepG2 and Huh7) were purchased from American Type Culture Collection. These cells were cultivated in 10% fetal bovine serum-based DMEM in a moist environment which was maintained at 37 °C with 5% CO₂.

Establishment of sorafenib-resistant HCC cell models

Sorafenib treatment was given to HepG2 and Huh7 cells that were at logarithmic phase. The initial dose of sorafenib was set at 1 μ mol/L. The culture medium was renewed every 24 hours, in which the sorafenib concentration increased by 0.25 μ mol/L each time until reaching a total of 12 μ mol/L. The

sorafenib-resistant HepG2 and Huh7 cells (HepG2/so and Huh7/so) were continuously treated with sorafenib to enhance the drug resistance. The cells were observed and photographed under an inverted microscope.

Cell transfection

miR-375 mimic, miR-375 inhibitor, sh-SIRT5, pcDNA3.1-SIRT5 or their negative control (mimic NC, inhibitor NC, sh-NC or pcDNA3.1) was transfected into HepG2/so cells via Lipofectamine 2000 (Invitrogen, California, USA). The plasmids and RNAs were provided by GenePharma (Shanghai, China).

CCK-8 assay

The assay was applied to measure the half maximal inhibitory concentration (IC₅₀) of parental and sorafenib-resistant HCC cells. The survival rates of HCC cells were measured 24 hours after treatment with sorafenib of different concentrations (1, 2, 4, 8, 16, 32 μmol/L). The influence of miR-375 or SIRT5 to the survival of sorafenib-resistant HCC cells was also assessed. Cells of each group were cultured in a 96-well plate where every well contained 1.5×10^4 cells. Every portion of cells was incubated with 10 μl of CCK-8 reagent at 37 °C for 3 hours. The absorbance value was measured at 450 nm.

Flow cytometry

Cells in each group were made into suspension for centrifugation at 2000 r/min. The cells were washed with PBS for twice and then resuspended in binding buffer. The cell suspension (195 μL, about 1×10^5 cells) was mixed with 5 μL of Annexin-V-FITC and PI solution. After 10-minute incubation in the dark, the apoptosis rate was measured by a flow cytometer (BD Biosciences, Suzhou, China).

qRT-PCR

The total RNA of HCC cells was extracted using a TRIzol kit. cDNA reversely transcribed from the RNA was used as the template for qRT-PCR. The reaction was performed according to the instruction of SYBR Prime Script RT-PCR kit. The total reactants consisted of 0.8 μl of cDNA, 5.0 μl of SYBR Primix Ex Taq, 1.0 μl of primers and 3.2 μl of RNase H₂O. The thermal cycling was set as follows: 5 minutes at 95 °C; 30 cycles of 15 seconds at 95 °C, 30 seconds at 95 °C and 40 seconds at 72 °C. GAPDH served as a reference gene. Each sample had three duplicates. The results were analyzed according to the $2^{-\Delta\Delta Ct}$ method: $\Delta\Delta Ct = (Ct_{\text{target gene}} - Ct_{\text{reference gene}})_{\text{experimental group}} - (Ct_{\text{target gene}} - Ct_{\text{reference gene}})_{\text{control group}}$. Primer sequences of the primers used in PCR are presented in Table 1.

Table 1
Primer sequences

Name of primer	Sequences
miR-375-F	CACAAAATTTGTTTCGTTCCGGCT
miR-375-R	GTGCAGGGTCCGAGGT
SIRT5-F	ACAATGGCTCGTCCAAGTTC
SIRT5-R	CCAGTAACCTCCTGCTCCTCT
<i>GAPDH</i> F	GTGGCTGGCTCAGAAAAGG
<i>GAPDH</i> R	GGGGAGATTCAGTGTGGTGG
Note: F, forward; R, reverse.	

Western blotting

After two PBS washes, the cells were lysed in lysis buffer on ice for 45 minutes and shaken at 15-minute intervals. After quantification by a BCA kit, proteins extracted from the cells were mixed with loading buffer and transferred onto a PVDF membrane after SDS-PAGE electrophoresis. Non-specific proteins were blocked in room-temperature 5% skim milk powder for 2 hours. After that, proteins on the membrane were incubated with primary antibodies of SIRT5 (#8779, 1:1000), LC3 α/β (#12741, 1:1000) and P62 (#88588, 1:1 000) (Cell Signaling Technology, Beverly, MA, USA) at 4 °C overnight and washed with PBST for 3 × 15 minutes. The proteins were then incubated with the secondary antibody (ab6728, 1:2000, Abcam, Cambridge, MA, USA) for 1 hour. The protein expressions were reflected by chemiluminescence. GAPDH (Cell Signaling, #5174, 1:1000) acted as a reference protein.

Dual-luciferase reporter assay

MiR-375 was found to have binding site on the 3'UTR region of SIRT5 mRNA based on the analysis of jefferson (<https://cm.jefferson.edu/>). Wild and mutated SIRT5-3'UTR regions (WT-SIRT5 and MUT-SIRT5) were synthesized and cloned to luciferase reporter vectors and then co-transfected with miR-375 mimic or mimic NC into HEK-293T cells. Luciferase activities in the cells were assessed 48 hours after transfection using a fluorescent luminescence detector based on the instruction of the dual-luciferase reporter assay kit (Beyotime, Shanghai, China). The relative luciferase activity = Firefly luciferase activity/Renilla luciferase activity.

Statistical analysis

SPSS 18.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad Software Inc.) were applied for statistical analysis. Data were finally presented as mean ± standard deviation (SD). *T* test and One-way analysis of variance were used to analyze the differences of two groups and multi-groups, respectively. Differences were deemed statistically significant when $P < 0.05$.

Results

MiR-375 is under-expressed in sorafenib-resistant HCC cells

The parental strains of HCC cells (HepG2 and Huh7) were continuously cultured in sorafenib of increasing concentrations to obtain sorafenib-resistant HCC cells (HepG2/so and Huh7/so). The morphology of HepG2 and Huh7 cells under the inverted microscope were changed from plump, pebble-like shape (epithelial phenotype) into spindle shape (mesenchymal phenotype) (Fig. 1A). CCK-8 assay detected that the IC50 of sorafenib in HepG2/so and Huh7/so cells was higher than in HepG2 and Huh7 cells (Fig. 1B, $P < 0.05$), suggesting that HepG2/so and Huh7/so cells had stronger tolerance to sorafenib.

MiR-375 was under-expressed in HepG2/so and Huh7/so cells compared to HepG2 and Huh7 cells (Fig. 1C, $P < 0.05$). To investigate the potential effect of miR-375 on sorafenib resistance, HepG2/so were transfected with miR-375 mimic or miR-375 inhibitor. MiR-375 was up-regulated in the miR-375 mimic group and down-regulated in the miR-375 inhibitor group (Fig. 1D, $P < 0.05$, vs the mimic NC and inhibitor NC group respectively), suggesting successful cell transfection. After the transfection, the cells were exposed to sorafenib for 24 hours. Sorafenib treatment impeded the survival of HepG2/so cells ($P < 0.05$). The survival of HepG2/so cells was further damaged by miR-375 mimic while improved by miR-375 inhibitor (Fig. 1E, $P < 0.05$). The results of the flow cytometry assessment of apoptosis showed that miR-375 mimic promoted sorafenib-induced apoptosis of HepG2/so cells while miR-375 inhibitor attenuated the apoptosis (Fig. 1F, $P < 0.05$).

Autophagy acts on miR-375-mediated drug resistance of HCC cells

The autophagy level was inhibited by 3-MA or activated by RAP in HepG2/so cells. CCK-8 assay detected that 3-MA treatment impaired the survivability of the cells with down-regulated miR-375 while RAP treatment enhanced the survivability of cells with over-expressed miR-375 (Fig. 2A, $P < 0.05$, vs the miR-375 inhibitor group and miR-375 mimic group respectively). The cell apoptosis was reduced in the miR-375 inhibitor group while enhanced in the miR-375 inhibitor + 3-MA group (Fig. 2B, $P < 0.05$, vs the inhibitor NC group and miR-375 inhibitor group respectively). The apoptosis promoted by miR-375 mimic was attenuated by RAP in the HepG2/so cells (Fig. 2B, $P < 0.05$).

Meanwhile, according to the Western blotting measurement of the expressions of p62, LC3I and LC3II, the autophagy was inhibited in the miR-375 inhibitor + 3-MA group while enhanced in the miR-375 mimic + RAP group (Fig. 2C, $P < 0.05$, vs the miR-375 inhibitor group and miR-375 mimic group respectively).

MiR-375 attenuates sorafenib resistance by mediating autophagy in HCC cells

According to the analysis of the cell survivability measured by CCK-8, HepG2/so cells were less susceptible to sorafenib than HepG2 cells ($P < 0.05$). The drug resistance of HepG2/so cells was

enhanced by miR-375 inhibitor and reduced by miR-375 mimic (Fig. 3A, $P < 0.05$). The apoptosis rates of HepG2/so cells were significantly reduced compared to HepG2 cells ($P < 0.05$). The number of apoptotic HepG2/so cells was decreased after transfection of miR-375 inhibitor while increased after transfection of miR-375 mimic (Fig. 3B, $P < 0.05$).

Furthermore, the LC3II/LC3I ratio was increased and p62 was decreased in HepG2/so cells compared to HepG2 cells ($P < 0.05$). The expression trends of p62, LC3I and LC3II in HepG2/so cells were promoted by miR-375 inhibitor while perturbed by miR-375 mimic (Fig. 3C, $P < 0.05$).

SIRT5 is targeted and down-regulated by miR-375

SIRT5 was detected to be a downstream target of miR-375 based on the bioinformatic analysis of Jefferson (Fig. 4A). Dual-luciferase reporter assay was designed to confirm the potential regulation between miR-375 and SIRT5. miR-375 mimic attenuated the relative luciferase activity of WT-SIRT5 (Fig. 4B, $P < 0.01$), but did not affect that of MUT-SIRT5. In cells, the expression of SIRT5 was negatively correlated with that of miR-375 (Fig. 4C-D, $P < 0.01$).

SIRT5 is over-expressed in sorafenib-resistant HCC cells and reverses the suppressive effect of miR-375 on sorafenib resistance

According to the results of qRT-PCR and Western blotting, HepG2/so and Huh7/so cells had higher expressions of SIRT5 compared to HepG2 and Huh7 cells (Fig. 5A-B, $P < 0.05$). To investigate the effect of SIRT5 on sorafenib resistance, SIRT5 was either knocked down or over-expressed in HepG2/so cells via transfection of sh-SIRT5 or pcDNA3.1-SIRT5 (Fig. 5C, $P < 0.05$). SIRT5 inhibition aggravated the damage to the survival of HepG2/so cells whereas SIRT5 overexpression improved the survival (Fig. 5D, $P < 0.05$). The suppressive or promotive effect of SIRT5 inhibition or overexpression on sorafenib resistance of HepG2/so cells was reversed by miR-375 inhibitor or miR-375 mimic (Fig. 5D, $P < 0.05$). SIRT5 inhibition exacerbated sorafenib-induced apoptosis of HepG2/so cells, which was later attenuated by miR-375 inhibitor (Fig. 5E, $P < 0.05$). SIRT5 overexpression ameliorated the cell death while the apoptosis rate was increased in the pcDNA3.1-SIRT5 + miR-375 mimic group (Fig. 5E, $P < 0.05$).

Meanwhile, the LC3II/LC3I ratio was reduced and p62 was up-regulated in the sh-SIRT5 group (vs the sh-NC group) and pcDNA3.1-SIRT5 + miR-375 mimic group (vs the pcDNA3.1-SIRT5 + mimic NC group); different expression patterns of LC3II, LC3I and p62 were found in the pcDNA3.1-SIRT5 group (vs the pcDNA3.1 group) and sh-SIRT5 + miR-375 inhibitor group (vs sh-SIRT5 + inhibitor NC group) (Fig. 5F, $P < 0.05$).

The above experiment data exhibited that SIRT5 knockdown inhibited autophagy to augment the susceptibility of HCC cells to sorafenib, and miR-375 down-regulation abolished the assistance of SIRT5 knockdown to sorafenib treatment. In addition, SIRT5 overexpression enhanced the resistance to sorafenib via autophagy activation in HCC cells, while miR-375 up-regulation made SIRT5 overexpressed HCC cells more sensitive to sorafenib. Taken together, miR-375 mediated the tolerance of HCC cells to sorafenib by regulating SIRT5.

Discussion

HCC is the predominant primary liver cancer caused by both intrinsic and extrinsic risk factors [14]. As the first-line treatment option for HCC, sorafenib significantly improves the overall survival of HCC patients but the drug efficacy has been greatly limited by high resistance rate [15]. Some biological processes in tumor microenvironment, including angiogenesis, inflammation, fibrosis, autophagy and viral reactivation, are associated with sorafenib resistance [16]. To further explore the molecular mechanisms underlying regulation of these sorafenib resistance-related biological processes would help amplify the benefits of sorafenib. The present paper elucidates the regulatory mechanism of miR-375 in autophagy-mediated sorafenib resistance of HCC cells.

First of all, sorafenib-resistant HCC cells (HepG2/so and Huh7/so) were established. HepG2/so and Huh7/so cells obtained increased cell viability and decreased apoptosis compared to HepG2 and Huh7 cells. MiR-375 was found to be down-regulated in HepG2/so and Huh7/so cells compared with parental HepG2 and Huh7 cells. Sorafenib impaired the survival of HepG2/so cells and miR-375 overexpression further enhanced sorafenib-induced cell death. To confirm the potential regulation of endogenous miR-375 in HCC cells under sorafenib treatment, the expression of miR-375 was either up-regulated or down-regulated in HepG2/so cells. According to the measurement of cell survivability and apoptosis, miR-375 inhibition made HepG2/so cells more resistant to sorafenib while miR-375 overexpression increased the drug sensitivity. The assistance of miR-375 in anti-tumor therapy has already been uncovered. MiR-375 augmented the susceptibility of HCC cells to sorafenib by targeting the autophagy-related gene, ATG14 [17]. MiR-375 also inhibited the survival of fulvestrant-resistant breast cancer cells by restraining autophagy [18].

Numerous existed scientific reports have authenticated that autophagy inhibition results in suppression on sorafenib resistance of HCC cells. For instance, SNHG16 promoted sorafenib resistance by enhancing autophagy via the miR-23b-3p/ EGR1 axis in HCC [19]. The METTL3/FOXO3 axis regulated autophagy-induced sorafenib resistance in HCC through the methylation of N6-methyladenosine [20]. The LC3II/LC3I ratio was increased and p62 was decreased in HepG2/so cells in comparison with HepG2 cells, suggesting activated autophagy in sorafenib-resistant HCC cells. After transfection of miR-375 inhibitor or miR-375 mimic, the autophagy level was promoted or inhibited in HepG2/so cells. Autophagy activated or inhibited in HepG2/so cells by miR-375 inhibitor or miR-375 mimic was thereafter inhibited by 3-MA or promoted by RAP. The results of CCK-8 and apoptosis detection showed that autophagy manipulation counteracted with the effect of miR-375 on sorafenib resistance. Therefore, miR-375 inhibited sorafenib resistance by regulating autophagy in HCC cells.

Based on the bioinformatic analysis and dual-luciferase reporter assay, SIRT5 was found to be targeted and down-regulated by miR-375. SIRT5 was up-regulated in HepG2/so and Huh7/so cells compared with parental cells. SIRT5 overexpression improved cell survivability and reduced apoptosis of HepG2/so cells while SIRT5 knockdown had opposite effects. SIRT5 is identified as a vital metabolic regulator [21]. SIRT5 could mediate the deacetylation of LDHB to promote autophagy, thereby accelerating the tumorigenesis

of colorectal cancer [22]. Therefore, it was reasonable to consider that SIRT5 might also regulate autophagy in sorafenib resistance. In the present study, SIRT5 inhibition suppressed autophagy and increased apoptosis of HepG2/so cells while SIRT5 overexpression had opposite effects. Down-regulation of miR-375 reversed the effects of SIRT5 inhibition, promoting sorafenib resistance of HepG2/so cells. On the other, up-regulation of miR-375 inhibited autophagy activated by SIRT5 overexpression and suppressed sorafenib resistance of HepG2/so cells.

Collectively, miR-375 mediates autophagy to enhance the sorafenib resistance of HCC cells by directly down-regulating SIRT5. Sorafenib resistance exists in most cases of HCC treatment. Despite this unsatisfactory outcome, sorafenib is still a potent drug for advanced HCC. The miR-375/SIRT5 axis discovered in the present study may serve as a new target for increasing the sorafenib sensitivity and therefore improve the outcomes of HCC treatment.

Abbreviations

HCC: Hepatocellular carcinoma; BCLC:Barcelona Clinic Liver Cancer; NSCLC:non-small cell lung cancer; SD:standard deviation

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors agree the publication.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors report no relationships that could be construed as a conflict of interest.

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Authors' contributions

YJB and WD conceived the ideas. WD designed the experiments. YJB and WD performed the experiments. YJB analyzed the data. WD provided critical materials. YJB and WD wrote the manuscript. YJB supervised the study. All the authors have read and approved the final version for publication.

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Figures

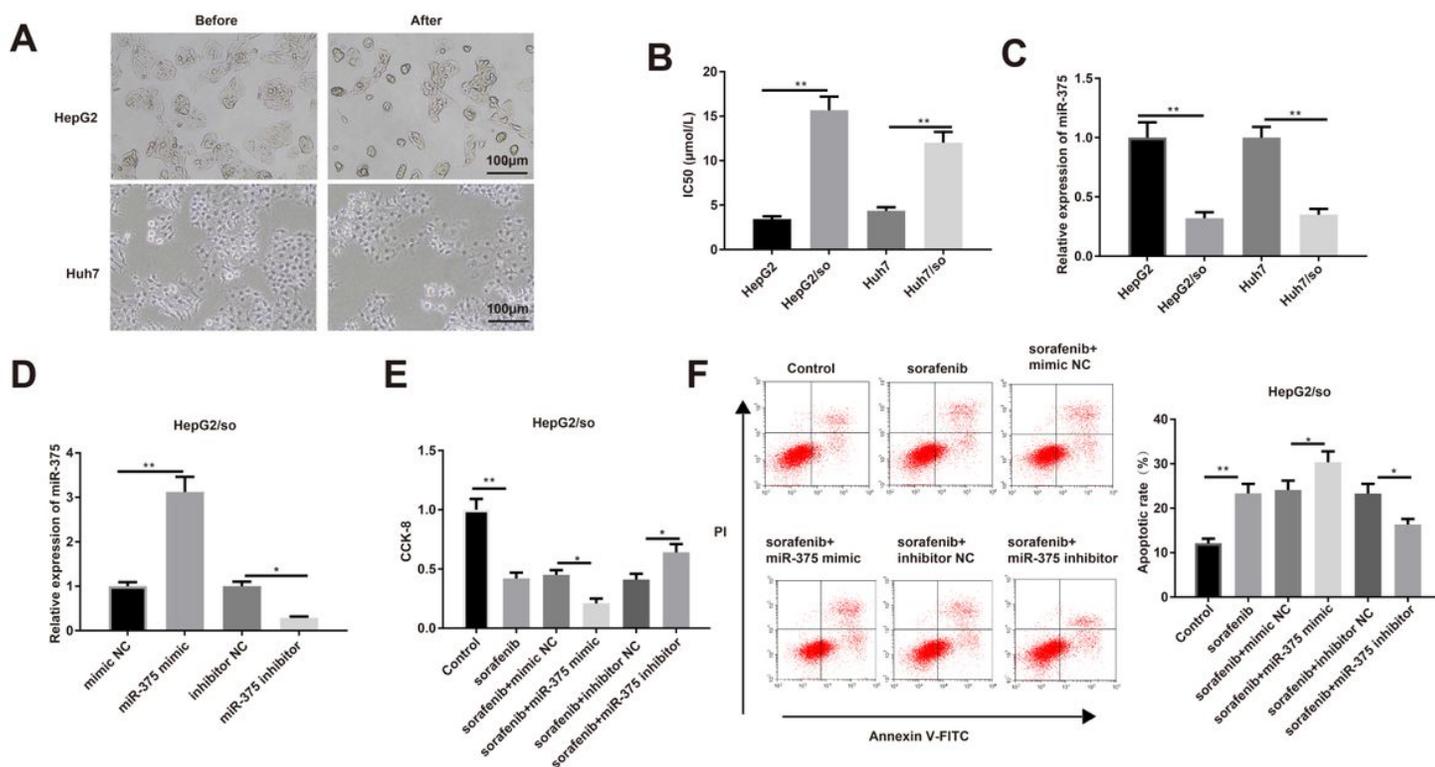


Figure 1

MiR-375 is under-expressed in sorafenib-resistant HCC cells Notes: (A) The morphology of HepG2 and Huh7 cells under an inverted microscope; (B) CCK-8 assay measured the sorafenib IC₅₀ of HepG2, Huh7, HepG2/so and Huh7/so cells; (C) the expression of miR-375 in HepG2, Huh7, HepG2/so and Huh7/so cells. After HepG2/so cells were transfected with miR-375 mimic or miR-375 inhibitor and exposed to sorafenib, (D) qRT-PCR detected the expression of miR-375 in HepG2/so cells; (E) CCK-8 assay assessed the survival of HepG2/so cells; (F) Annexin-V-FITC/PI staining and flow cytometry assessed the apoptosis of HepG2/so cells. N = 3; *P < 0.05, **P < 0.01, *** P < 0.001; data were presented as mean ± SD; T test was for comparison between two groups; One-way analysis of variance was for multi-group comparison; the Tukey test was for post hoc multiple comparisons after ANOVA; HCC, hepatocellular carcinoma cell.

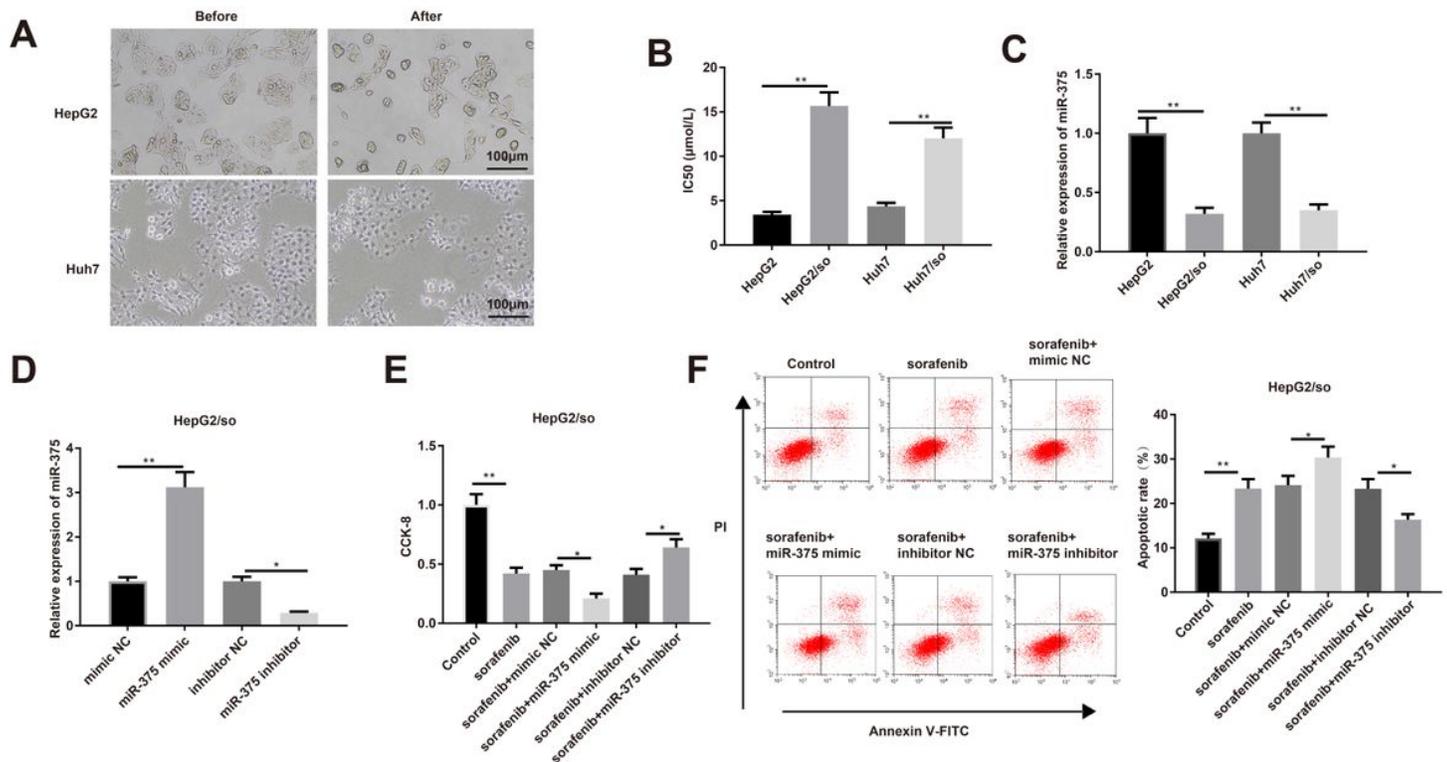


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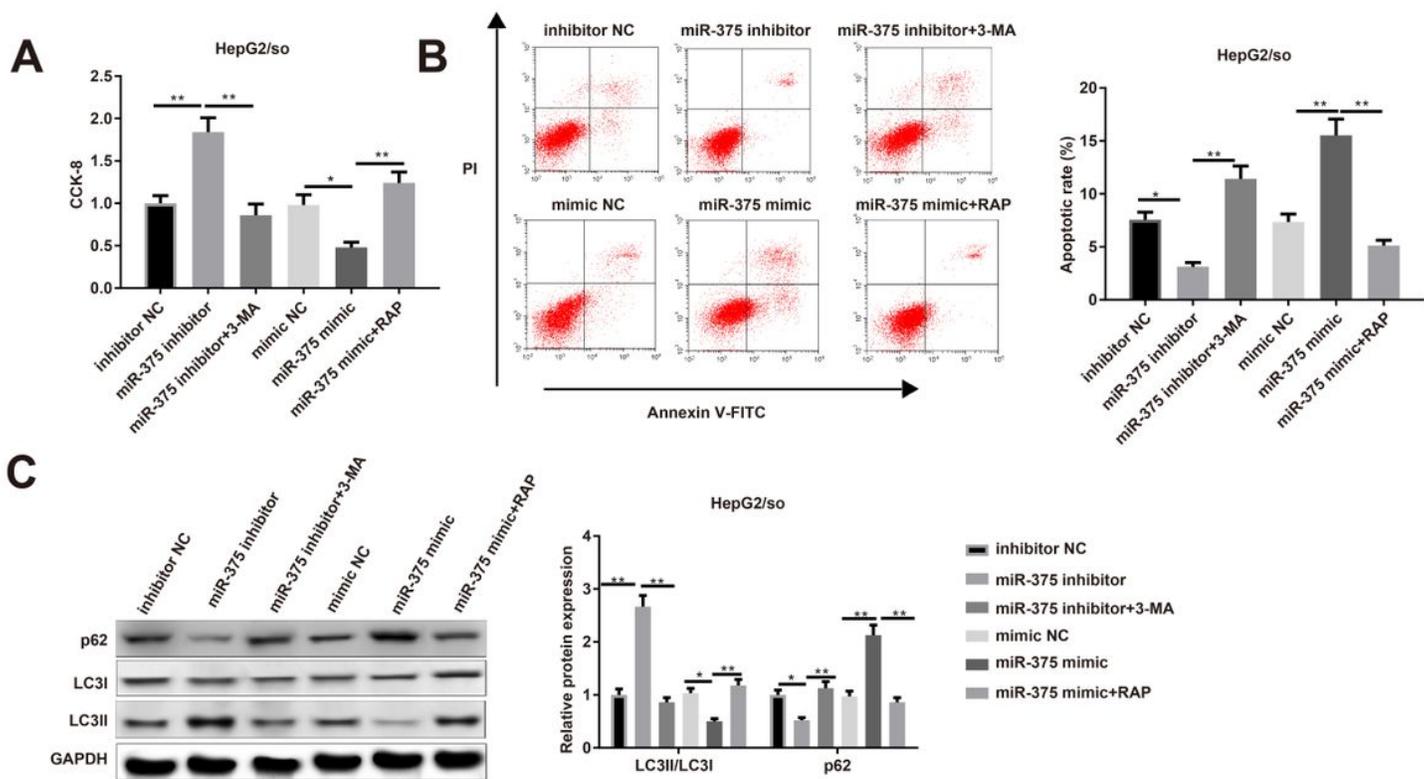


Figure 2

Autophagy acts on miR-375-mediated drug resistance of HCC cells Notes: (A) CCK-8 assay assessed the survivability of HepG2/so cells; (B) Annexin-V-FITC/PI staining and flow cytometry assessed the apoptosis of HepG2/so cells; (C) Western blotting measured the expressions of p62, LC3I and LC3II in HepG2/so cells. N = 3; *P < 0.05, **P < 0.01, *** P < 0.001; data were presented as mean \pm SD; T test was for comparison between two groups; One-way analysis of variance was for multi-group comparison; the Tukey test was for post hoc multiple comparisons after ANOVA; HCC, hepatocellular carcinoma cell.

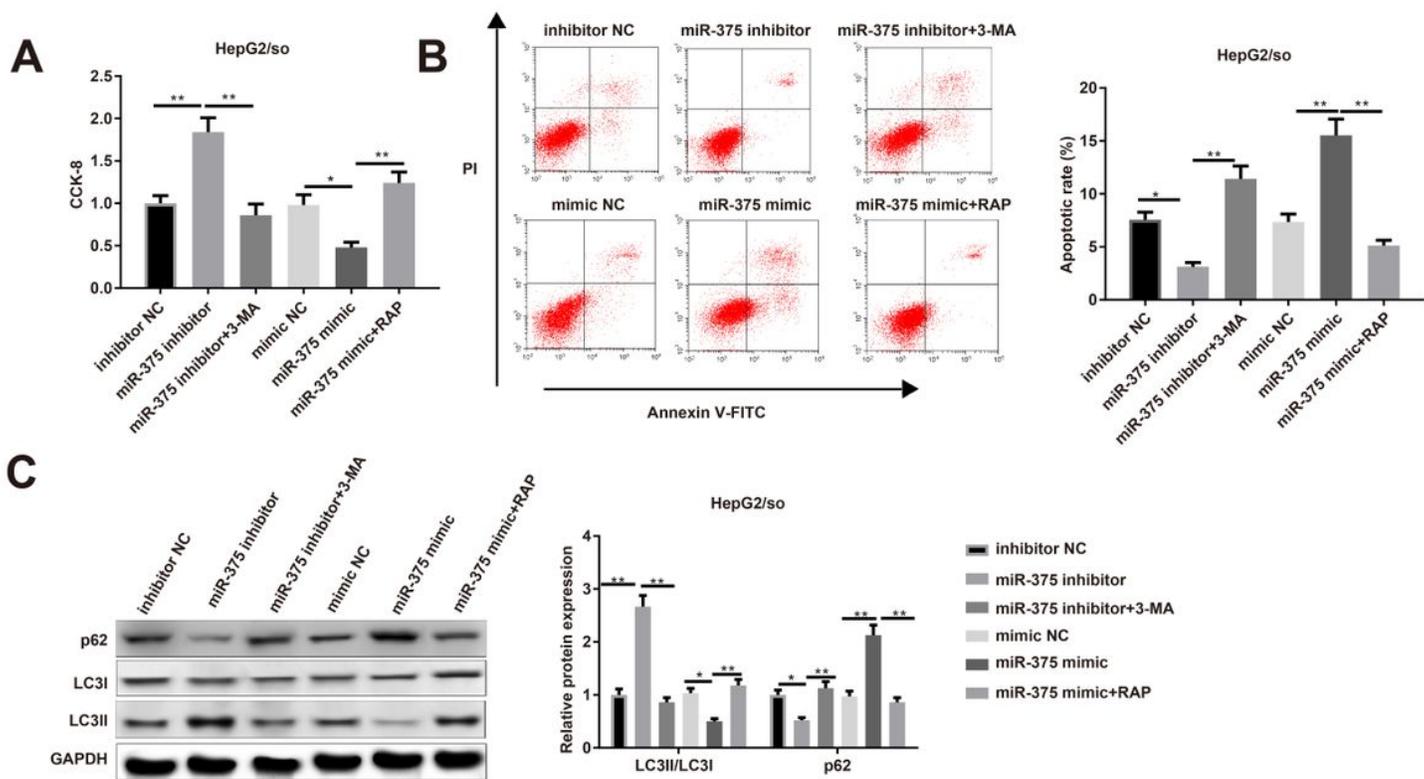


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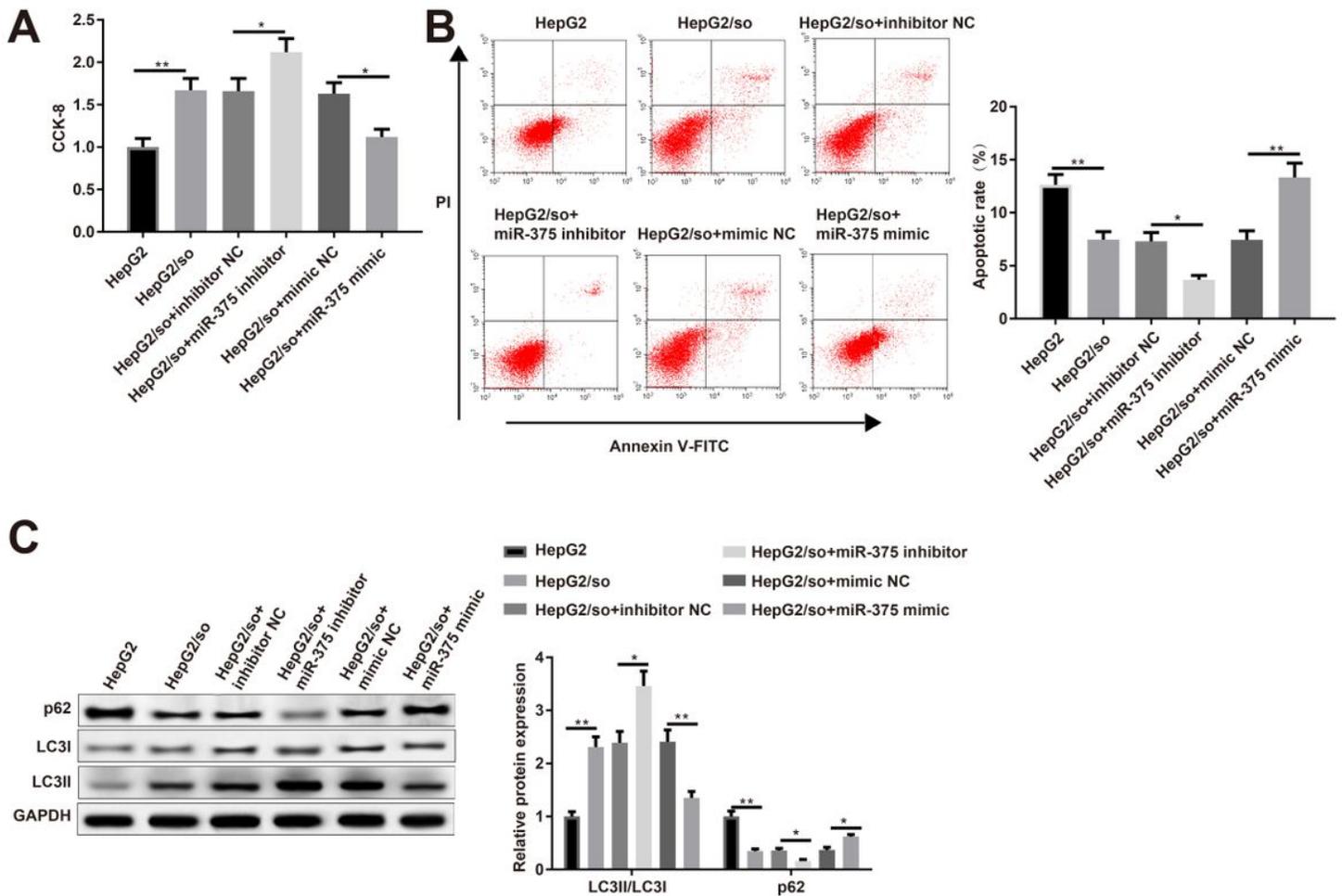


Figure 3

MiR-375 attenuates sorafenib resistance by mediating the autophagy in HCC cells Notes: (A) The survival rates of HepG2 and HepG2/so cells; (B) the apoptosis rates of HepG2 and HepG2/so cells; (C) the levels of p62, LC3I and LC3II in HepG2 and HepG2/so cells. N = 3; *P < 0.05, **P < 0.01, *** P < 0.001; data were presented as mean \pm SD; T test was for comparison between two groups; One-way analysis of variance was for multi-group comparison; the Tukey test was for post hoc multiple comparisons after ANOVA; HCC, hepatocellular carcinoma cell.

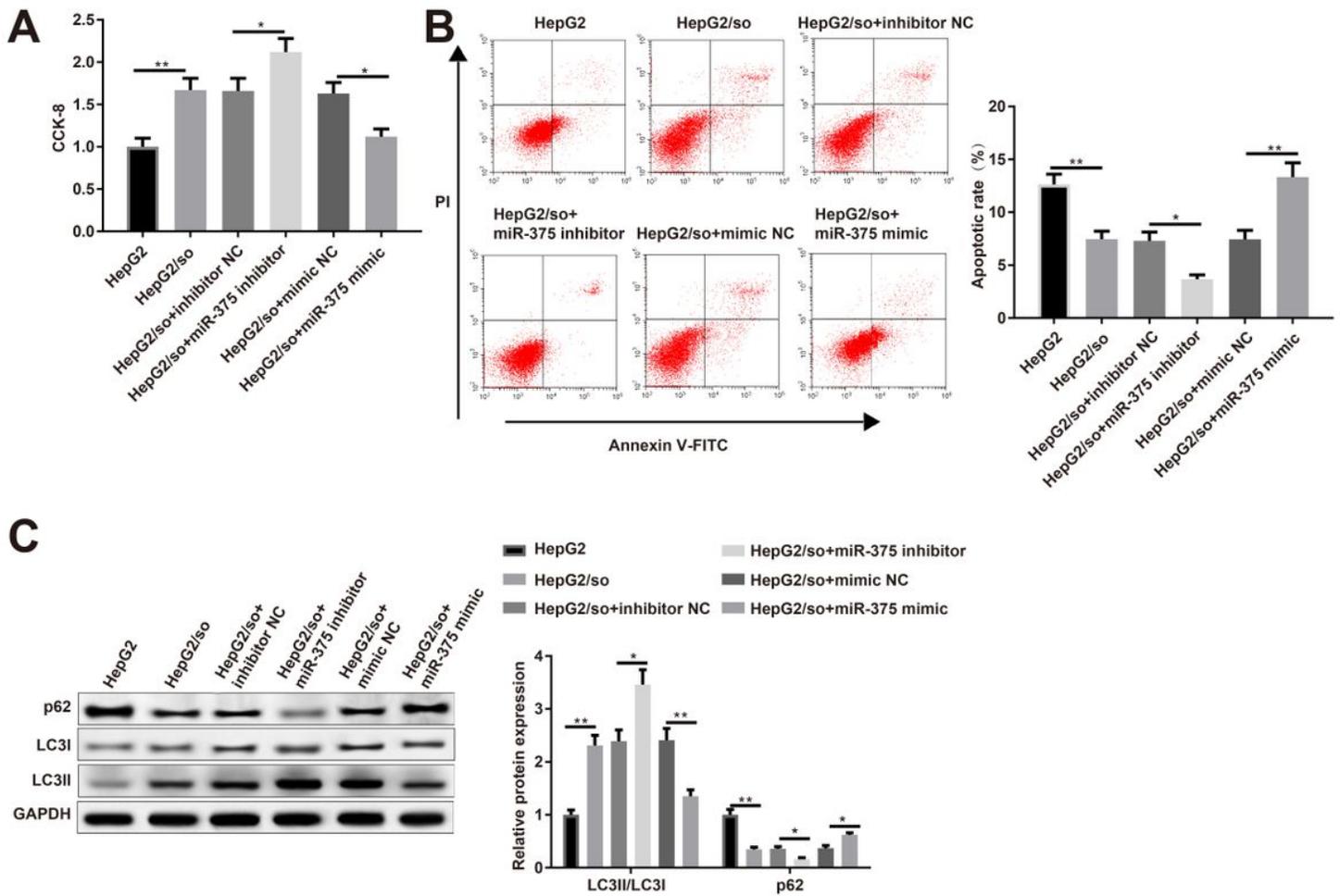


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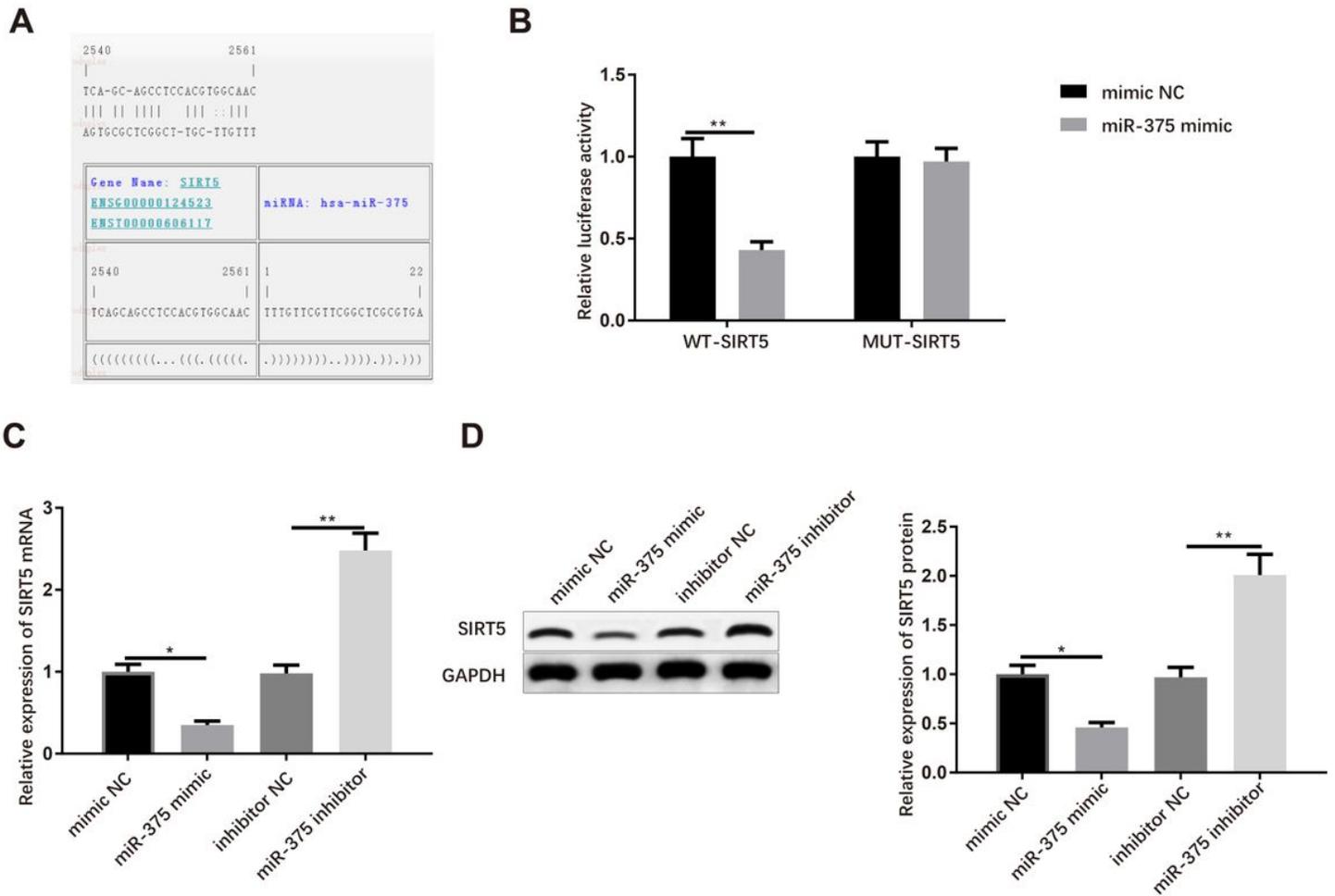


Figure 4

SIRT5 is targeted and down-regulated by miR-375 Notes: (A) The binding sites between miR-375 and SIRT5 were predicted by jefferson; (B) dual-luciferase reporter assay verified the binding between miR-375 and SIRT5; (C-D) the expression of SIRT5 in cells transfected with miR-375 mimic or miR-375 inhibitor. N = 3; *P < 0.05, **P < 0.01, *** P < 0.001; data were presented as mean \pm SD; One-way analysis of variance was for multi-group comparison; the Tukey test was for post hoc multiple comparisons after ANOVA.

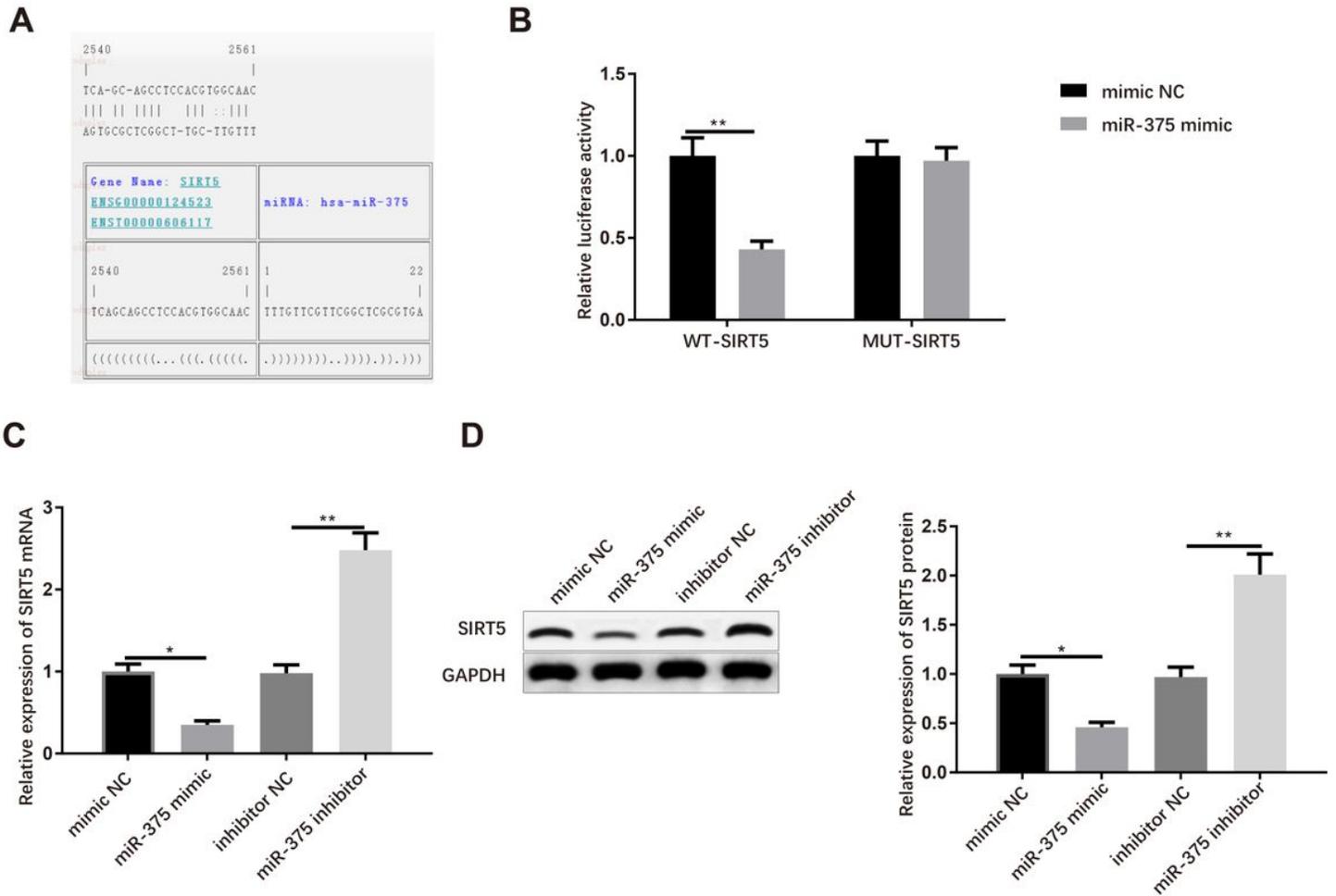


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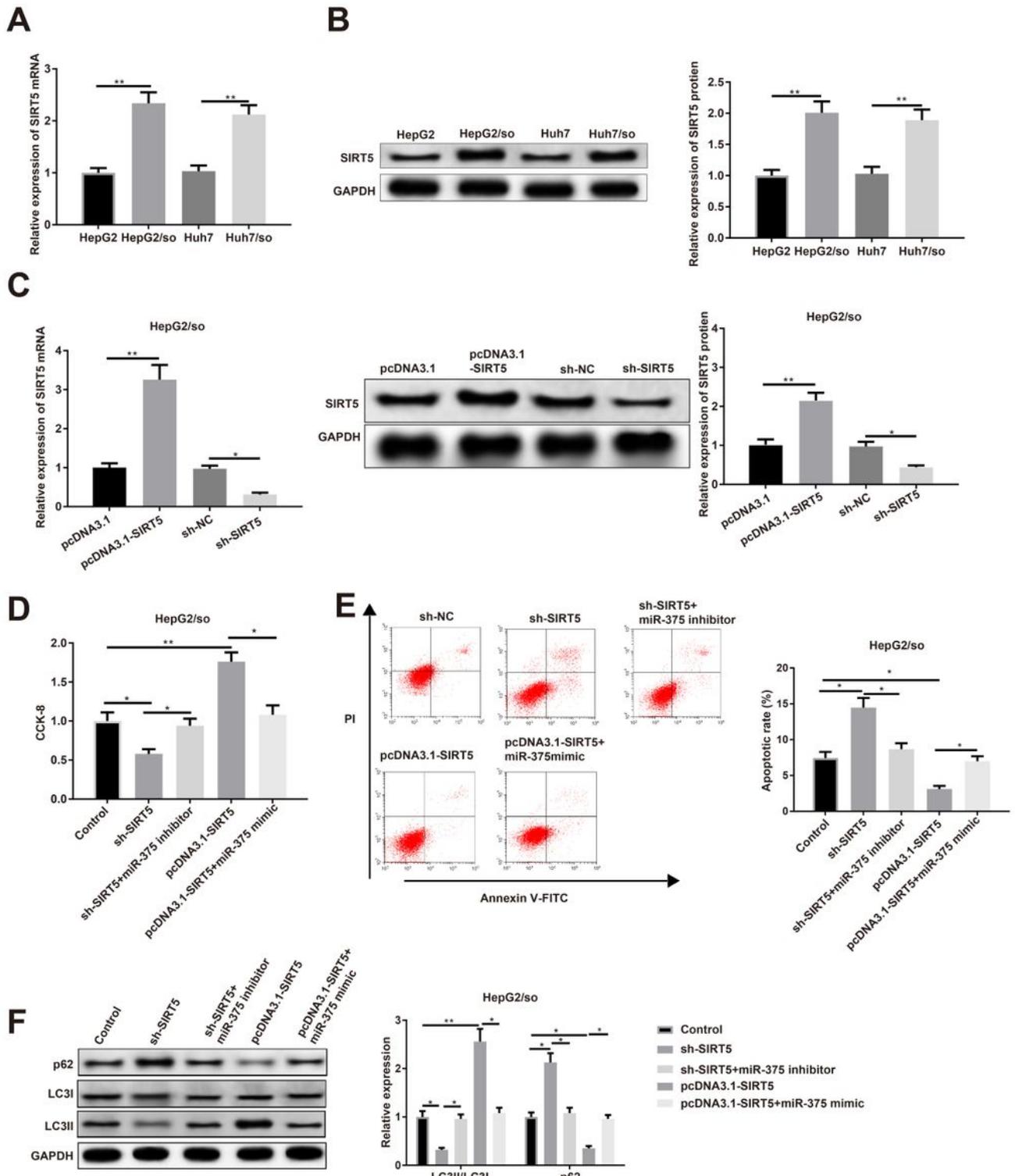


Figure 5

SIRT5 is over-expressed in sorafenib-resistant HCC cells and reverses the suppressive effect of miR-375 on sorafenib resistance. Notes: (A-B) The expression of SIRT5 in HepG2, Huh7, HepG2/so and Huh7/so cells. After HepG2/so cells were transfected with pcDNA3.1-SIRT5 or sh-SIRT5, (C) qRT-PCR measured the expression of SIRT5 in HepG2/so cells; (D) CCK-8 assessed the survival rate of HepG2/so cells; (E) Annexin-V-FITC/PI staining and flow cytometry assessed the apoptosis of HepG2/so cells; (F) the

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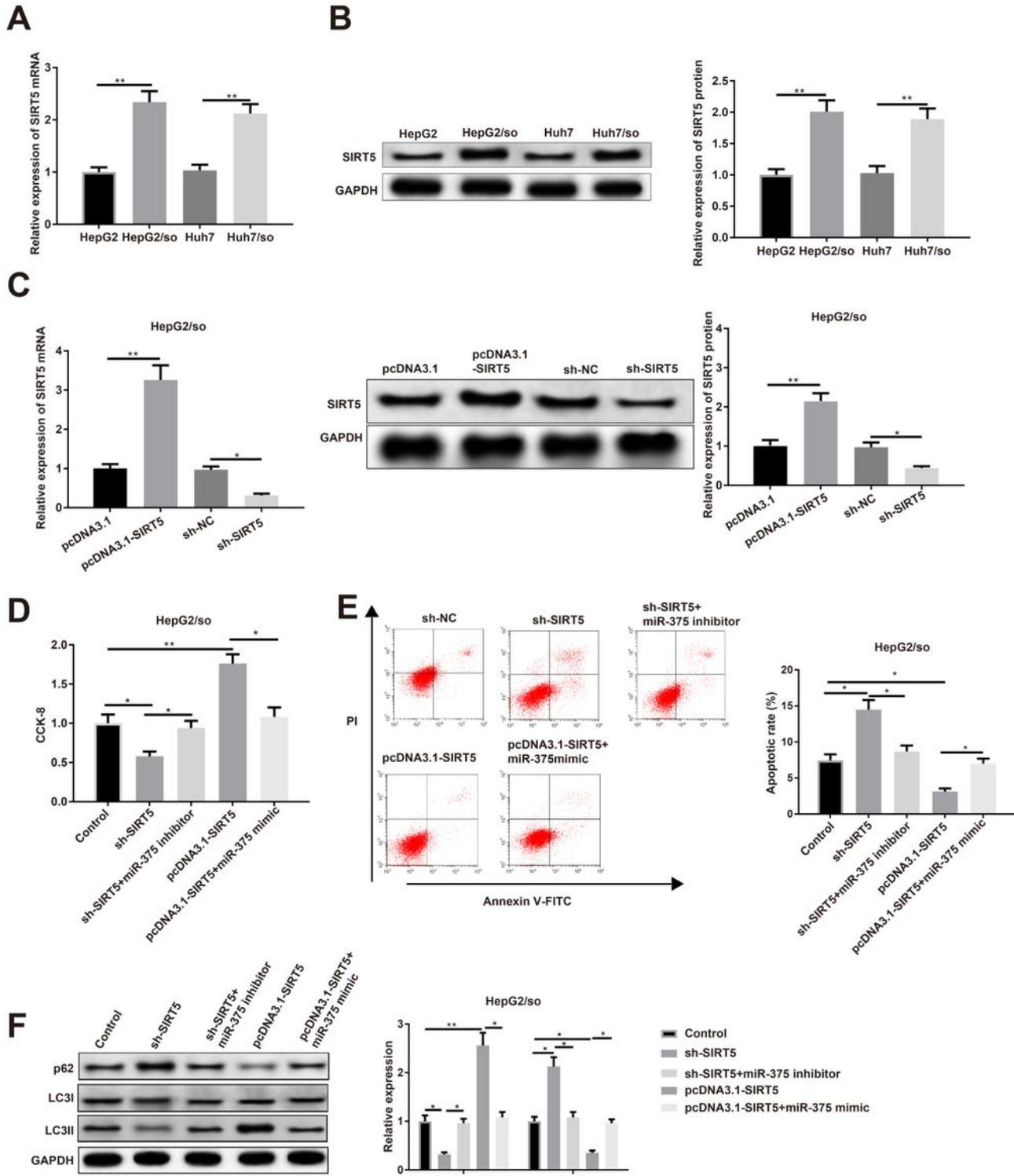


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