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Supplemental Information

miR372 Promotes Progression of Liver Cancer Cells by Upregulating erbB-2 through Enhancement of YB-1

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Supplemental Data

Results

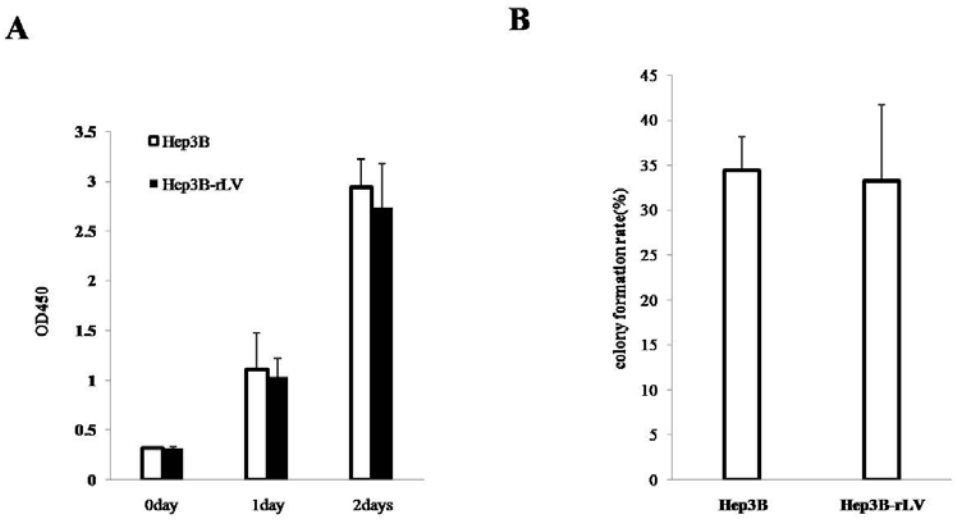
miR372 accelerates growth of liver cancer cells Huh7

We first constructed two stable Huh7 cell lines by infecting with rLV or rLV-miR372. As shown in **FigureS2A**, mature miR372 were significantly overexpressed in rLV- -miR372 groupcompared to rLV group and Huh7 group respectively. As shown in **FigureS2B**, excessive miR372 significantly increased the growth ability of liver cancer cell Huh7 compared to the rLV group and Huh7 group($P<0.01$).And there is no significant difference between rLV group and Huh7 group($P>0.05$). Moreover, the BrdU positive rate was significantly increased in rLV-miR372 group compared to rLV group (61±7.79% versus 28.23±4.83%,$P=0.008<0.01$) and Huh7 group (61±7.79% versus 30.83±2.83%,$P=0.00743<0.01$). However, there is no significant difference between Huh7 group and rLV group(28.23±4.83% versus 30.83±2.83%, $P=0.0779>0.05$) (**FigureS2C**). Furthermore, we performedcolony formation assay and observed a significant increase in colony formation efficiency rate in rLV-miR372 group compared to rLV group (65.95±8.11% versus 41.2±4.48%,$P=0.0083<0.01$) and Huh7 group (65.95±8.11% versus 40.3±2.63%,$P=0.0095<0.01$). However, there is no significant difference between Huh7 group and rLV group (40.3±2.63% versus 41.2±4.48%,$P=0.389>0.05$) (**FigureS2D**). To further explore the effect of miR372 on liver cancer cells Huh7 in vivo, the two stable Huh7 were injected subcutaneously into athymic Balb/C mice. As shown in FigureS 2Ea, the xenograft tumor weight

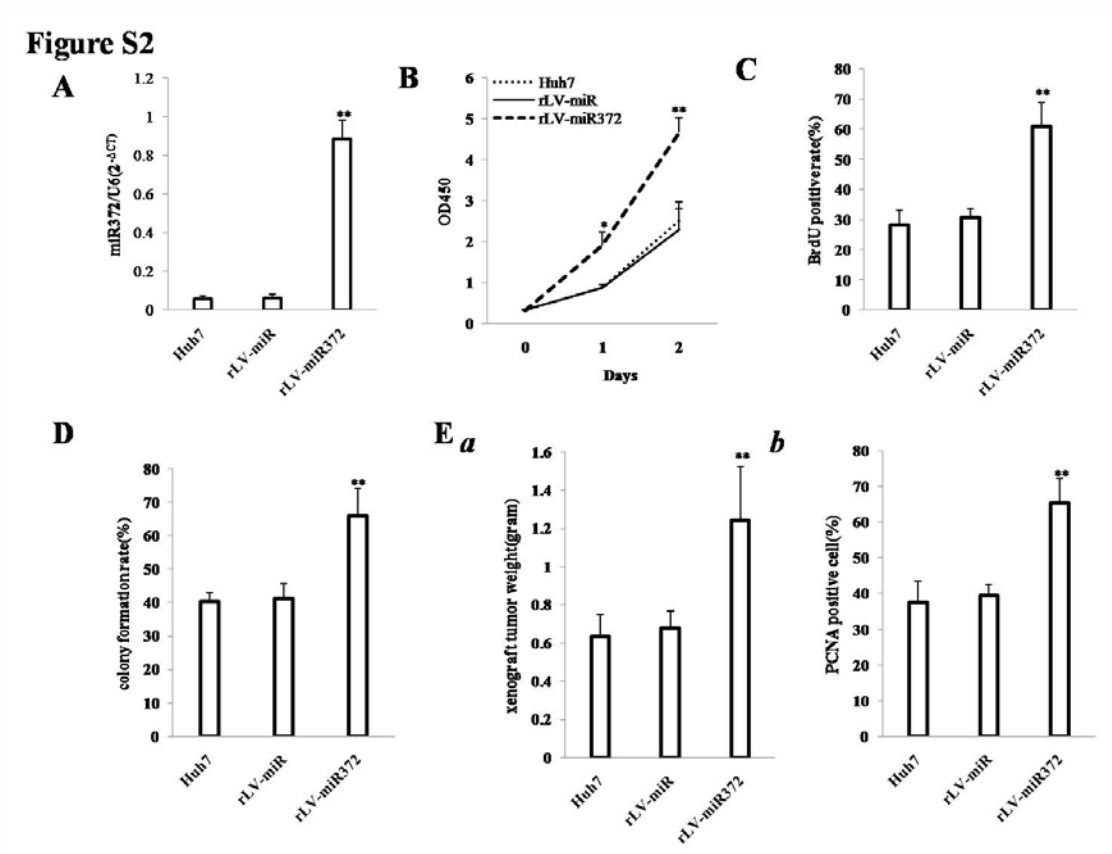
was significantly increased in rLV-miR372 group compared to rLV group (1.243±0.278 gram versus 0.68±0.089 gram, P=0.00353<0.01) and Huh7 group (1.243±0.278 gram versus 0.637±0.107,P=0.00068<0.01). However, there is no significant difference between Huh7 group and rLV group (0.68±0.089 gram versus 0.637±0.107 gram, P=0.2089>0.05). Moreover, PCNA positive rate was significantly increased in rLV- miR372 group compared to rLV group (65.35±6.94% versus 37.63±5.67%,P=0.00065<0.01) and Huh7 group (65.35±6.94% versus 39.55±3.08%,P=0.000012<0.01). However , there is no significant difference between Huh7 group and rLV group (versus 37.63±5.67%, versus 39.55±3.08%, P=0.1506>0.05) (FigureS2Eb).

Taken together, these findings demonstrate that miR372 accelerates malignant growth of liver cancer cells Huh7.

Figure S1



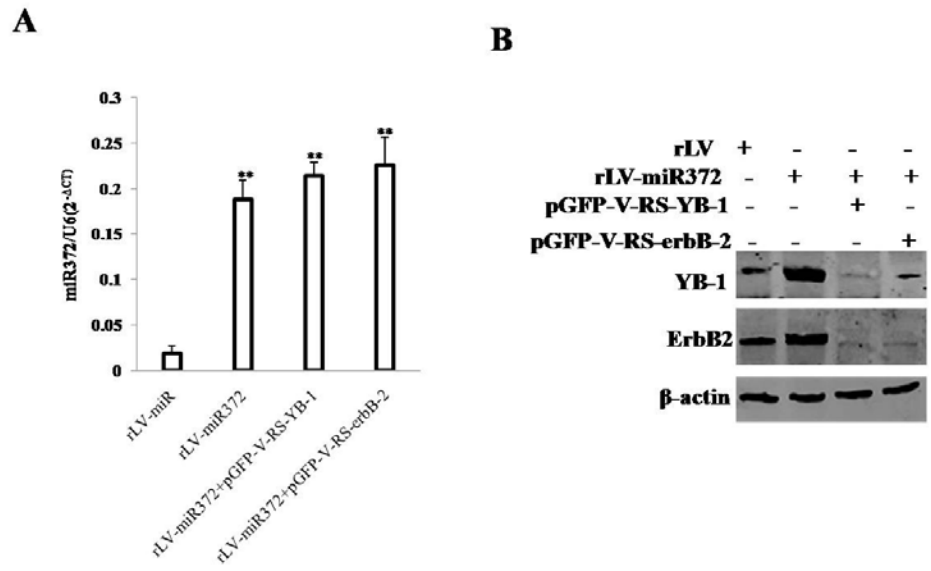
FigureS1: Cell growth assay *in vitro*.**A.** cells proliferation assay using CCK8 in Hep3B cell and Hep3B cells infected with rLV, respectively. Each value was presented as mean±standard error of the mean (SEM).**B.** cells colony formation ability assay in in Hep3B cell and Hep3B cells infected with rLV, respectively.Each value was presented as mean±standard error of the mean (SEM).



FigureS2: Cell growth assay *in vitro and in vivo*in human liver cancer cell Huh7.**A.**The real-time RT-PCR analysis of mature miR372 in human liver cancer cell line Huh7 and Huh7cells infected with rLVand rLV-miR372, respectively. U6 as internal control.Each value was presented as mean±standard error of the mean (SEM).**B.** cells proliferation assay using CCK8 in human liver cancer cell line Huh7 and Huh7 cells infected with rLV and rLV-miR372, respectively. Each value was presented as mean±standard error of the mean (SEM).**C.** cells BrdU assay.Each value was presented as mean±standard error of the mean (SEM).**D.** cells colony formation ability assay in human liver cancer cell line Huh7 and huh7 cells infected with rLVand rLV-miR372, respectively.Each value was presented as mean±standard error of the mean (SEM).**E.** Tumorigenesis test *in vivo* .a.The wet weight of each tumor was determined for each mouse. Each value was presented as mean±standard error of

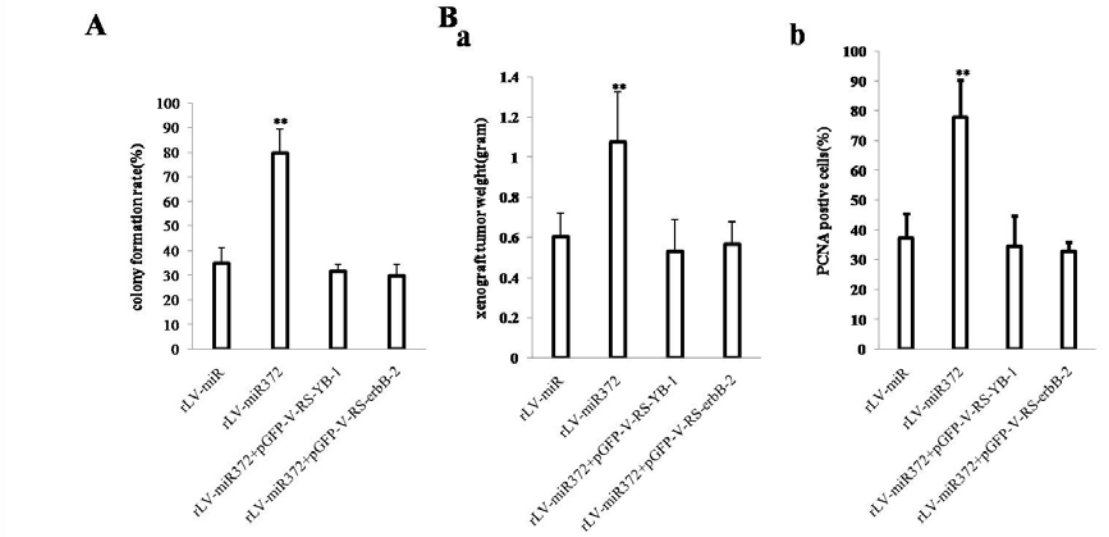
the mean (SEM). **b.**A portion of each tumor was fixed in 4% paraformaldehyde and embedded in paraffin for histological hematoxylin-eosin(HE) staining. Each value was presented as mean±standard error of the mean (SEM).

Figure S3



FigureS3:miR372 influences on the expression of YB-1 and erbB-2in human liver cancer cell Huh7.**A.**The real-time RT-PCR analysis of mature miR372 in human liver cancer cell line Huh7 ,including rLV, rLV-miR372,rLV-miR372 plus pGFP-V-RS-YB-1,and rLV-miR372 plus pGFP-V-RS-erbB-2,respectively. U6 as internal control.Each value was presented as mean±standard error of the mean (SEM).Western blotting with anti-YB-1and anti- erbB-2in human liver cancer cell line Huh7 ,including rLV, rLV-miR372,rLV-miR372 plus pGFP-V-RS-YB-1,and rLV-miR372 plus pGFP-V-RS-erbB-2,respectively.β-actin was used as an internal control.

Figure S4



FigureS4:YB-1 knockdown or erbB-2 knockdown abrogated the functions of miR372 in human liver cancer cell line Huh7. **A.** cells colony formation ability assay in human liver cancer cell line Huh7 , including rLV, rLV-miR372,rLV-miR372 plus pGFP-V-RS-YB-1, and rLV-miR372 plus pGFP-V-RS-erbB-2,respectively.Each value was presented as mean±standard error of the mean (SEM).**B.** Tumorigenesis test *in vivo* in human liver cancer cell line Huh7includingrLV, rLV-miR372,rLV-miR372 plus pGFP-V-RS-YB-1, and rLV-miR372 plus pGFP-V-RS-erbB-2,respectively. a. The wet weight of each tumor was determined for each mouse. Each value was presented as mean±standard error of the mean (SEM). **b.**A portion of each tumor was fixed in 4% paraformaldehyde and embedded in paraffin for histological hematoxylin-eosin(HE) staining. Each value was presented as mean±standard error of the mean (SEM).