LncRNA MYLK-AS1 facilitates tumor progression and angiogenesis by targeting miR-424-5p/E2F7 axis and activating VEGFR-2 signaling pathway in hepatocellular carcinoma

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Research

Keywords: MYLK antisense RNA 1, Hepatocellular carcinoma, E2F transcription factor 7, competing endogenous RNA, VEGFR-2 signaling pathway

Posted Date: September 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-68878/v1

Version of Record: A version of this preprint was published on November 9th, 2020. See the published version at https://doi.org/10.1186/s13046-020-01739-z.

Abstract

Background

Long non-coding RNA is essential for the metastasis, invasion, angiogenesis and progression of hepatocellular carcinoma (HCC). However, their specific mechanisms are still controversial. Here, we found that Lnc-MYLK-AS1 is a potential oncogene. We systematically analyzed the clinical significance and mechanism of Lnc-MYLK-AS1 in HCC metastasis, invasion and angiogenesis.

Methods

Determine research goals through bioinformatics analysis. The expression of MYLK-AS1 in matched tumor and non-tumor tissues of 156 HCC patients was detected by quantitative reverse transcription PCR. The in vitro and in vivo biological functions of MYLK-AS1 were examined through the loss of function and function gain experiments. The use of dual luciferase reporter gene analysis, quantitative PCR, Western blotting, and fluorescence in situ hybridization (FISH) clarified the underlying mechanism of this competitive endogenous RNA (ceRNA).

Results

MYLK-AS1 is up-regulated in HCC cell lines and tumor tissues, which is related to tumor progression and enhancement of angiogenesis. Overexpression of MYLK-AS1 promotes the proliferation, metastasis, invasion, and angiogenesis of HCC cells, while down-regulation of MYLK-AS1 can reverse these effects in vivo and in vitro. Dual analysis of luciferase and RNA immunoprecipitation showed that microRNA miR-424-5p is the direct target of MYLK-AS1, and MYLK-AS1 acts as ceRNA, which can regulate angiogenesis in HCC. Mechanism studies have shown that miR-424-5p specifically targets E2F transcription factor 7 (E2F7), while the complex MYLK-AS1/miR-424-5p activates VEGFR2 signaling through E2F7, thereby promoting tumor proliferation and angiogenesis.

Conclusion

The up-regulation of MYLK-AS1 is related to tumor cell proliferation, increased angiogenesis and poor prognosis in HCC patients. MYLK-AS1 regulates E2F7 expression and VEGFR2 signaling by acting as a ceRNA of miR-424-5p.

Full Text

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Figures

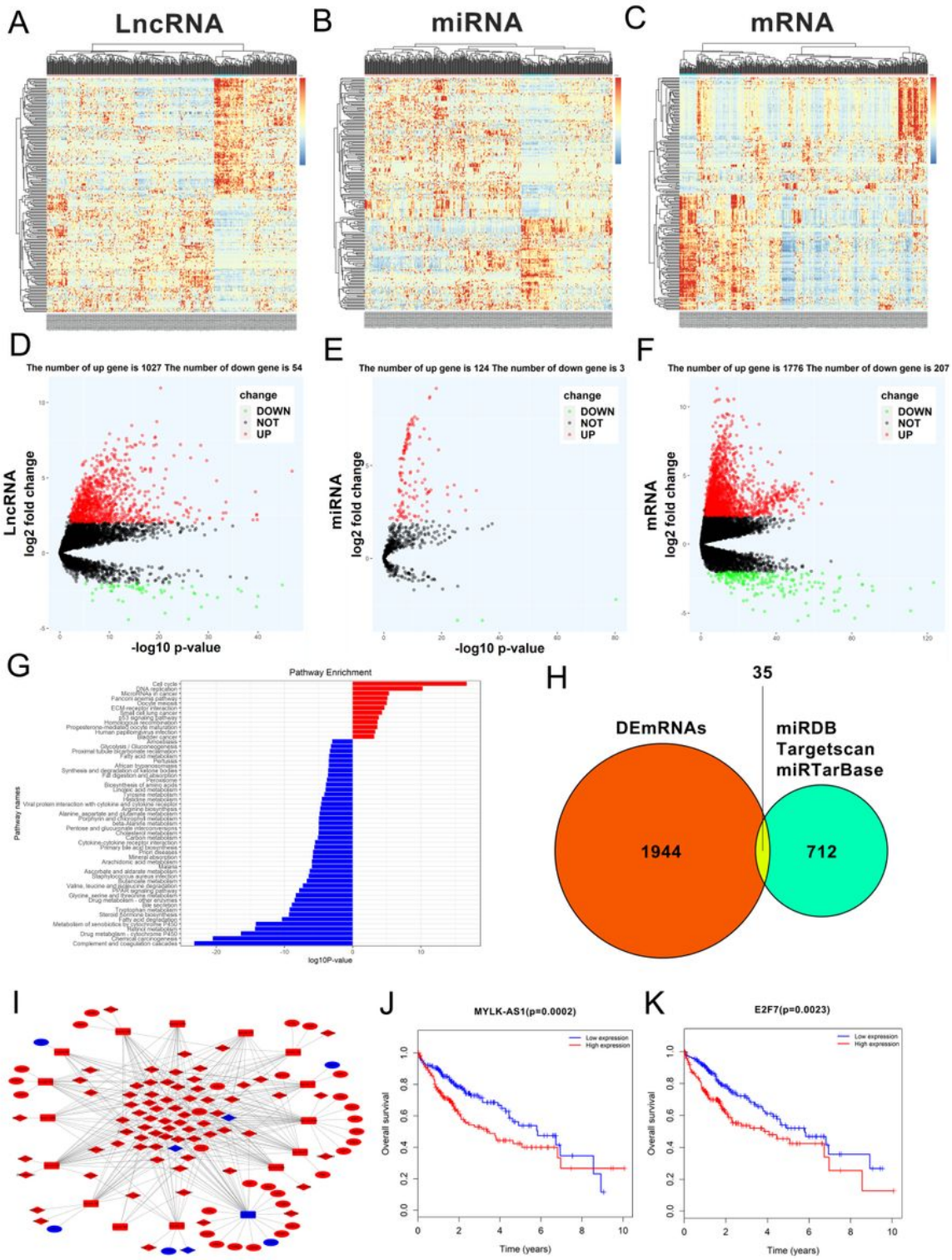

Figure 1

Fig.1 RNA-seq data analysis of hepatocellular carcinoma in TCGA database. A-C 2 Clustered heat maps of the differentially expressed RNAs in hepatocellular carcinoma 3 tissues and adjacent non-tumor liver tissues. Rows represent RNAs, whereas columns represent hepatocellular carcinoma tissues and adjacent non-tumor liver tissue samples. Differentially expressed lncRNAs, miRNAs, and mRNAs in hepatocellular carcinoma tissues and adjacent non-tumor liver tissue samples. log2FC>1.5 and FDR<0.05. FC: folds

change; FDR: false discovery rate. D-F Volcano plots were used to visualize and 8 assess the variation of (D) long non-coding RNAs, (E) microRNAs, and (F) mRNAs expression between hepatocellular carcinoma tissues and adjacent non-tumor liver tissues. The values of the x- and y-axes indicate the averaged normalized signal values of the group (log scaled). G All enriched KEGG pathways for differentially expressed mRNAs (the bar plot shows the enrichment scores of the significantly enriched KEGG pathways). KEGG, Kyoto Encyclopedia of Genes and Genomes. H Identification of 712 commonly changed targeted mRNAs of 127 DEmiRNAs from the three publicly profile datasets (miRDB, Targetscan and miRTarBase). The cross areas meant the number of commonly changed mRNAs between DEmRNAs and target mRNAs is 35, which includes E2F7. I The lncRNA-miRNA-mRNA ceRNA network. Blue squares, downregulated miRNAs; blue circles, downregulated mRNAs; blue diamonds, downregulated lncRNAs. Red squares, upregulated miRNAs; red circles, upregulated mRNAs; red diamonds, upregulated lncRNAs. J-K Kaplan-Meier survival curves for MYLK-AS1 and E2F7 associated with overall survival.

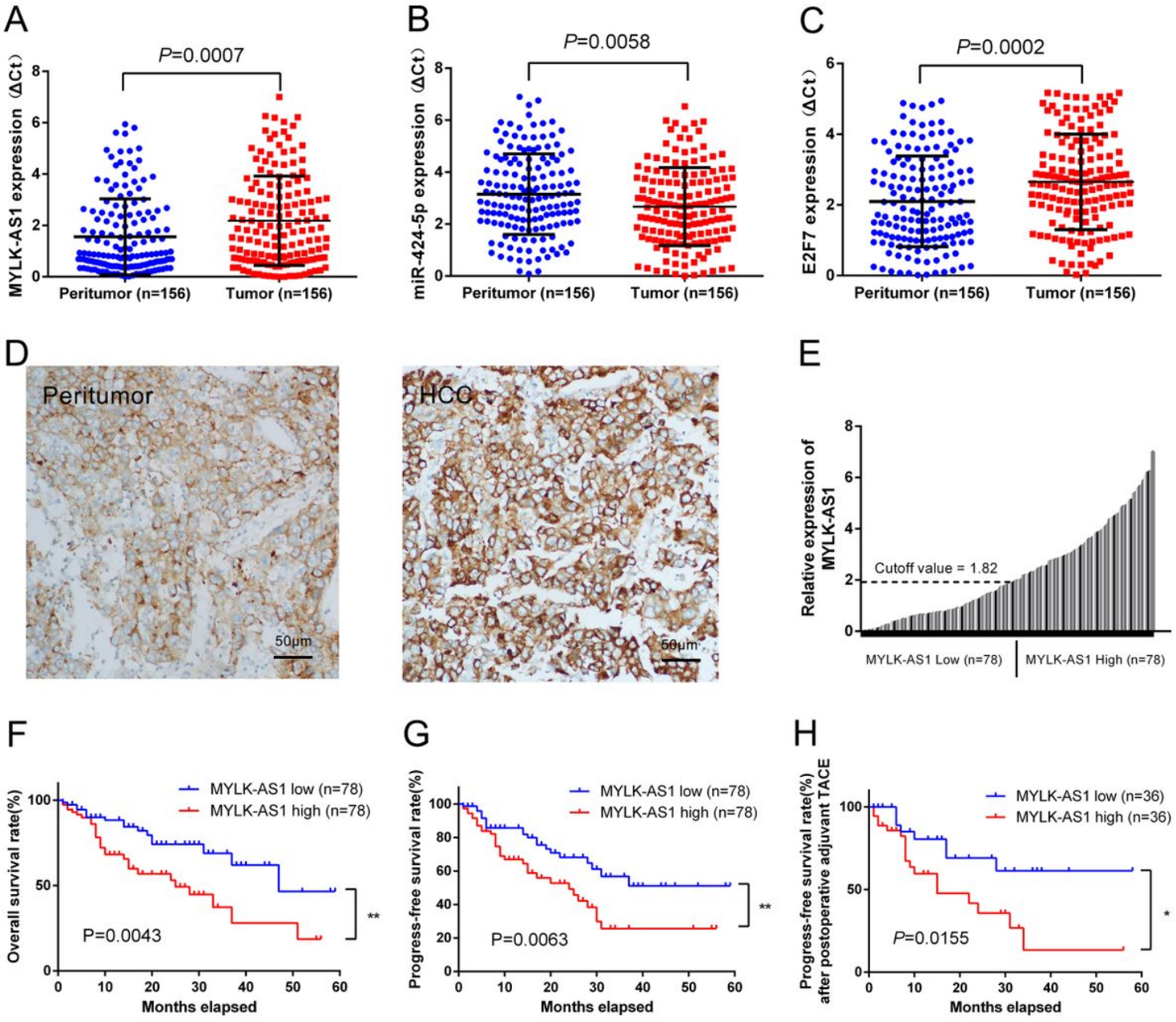

Figure 2

MYLK-AS1 and E2F7 overexpression is positively correlated with HCC progression and poor prognosis. A-C Relative expression of MYLK-AS1, miR-424- 5p and E2F7 detected by qRT-PCR in 156 paired HCC cancer tissues and matched normal tissues. Results are presented as Δ cycle threshold (ΔCt) in tumor tissues relative to normal tissues. D Expression of MYLK-AS1 in peritumoral tissues and HCC tissues by ISH. E qRT-PCR was performed to examine MYLK-AS1 expression in 156 HCC cancer tissues. Relative expression of MYLK-AS1 was presented as log2 (fold change of ΔCt value) in tumor tissue to that of matched normal tissues. HCC patients were 1 divided into high (n = 78) and low (n = 78) groups according to the median value (0.50). F-G Kaplan-Meier plots of the OS and PFS of HCC patients with high (n = 78) and low (n = 78) levels of MYLK-AS1. Data are presented as the mean ± SD. H Kaplan-Meier plots of the PFS of HCC patients after postoperative adjuvant TACE with high (n = 78) and low (n = 78) levels of MYLK-AS1. Data are presented as the mean ± SD.

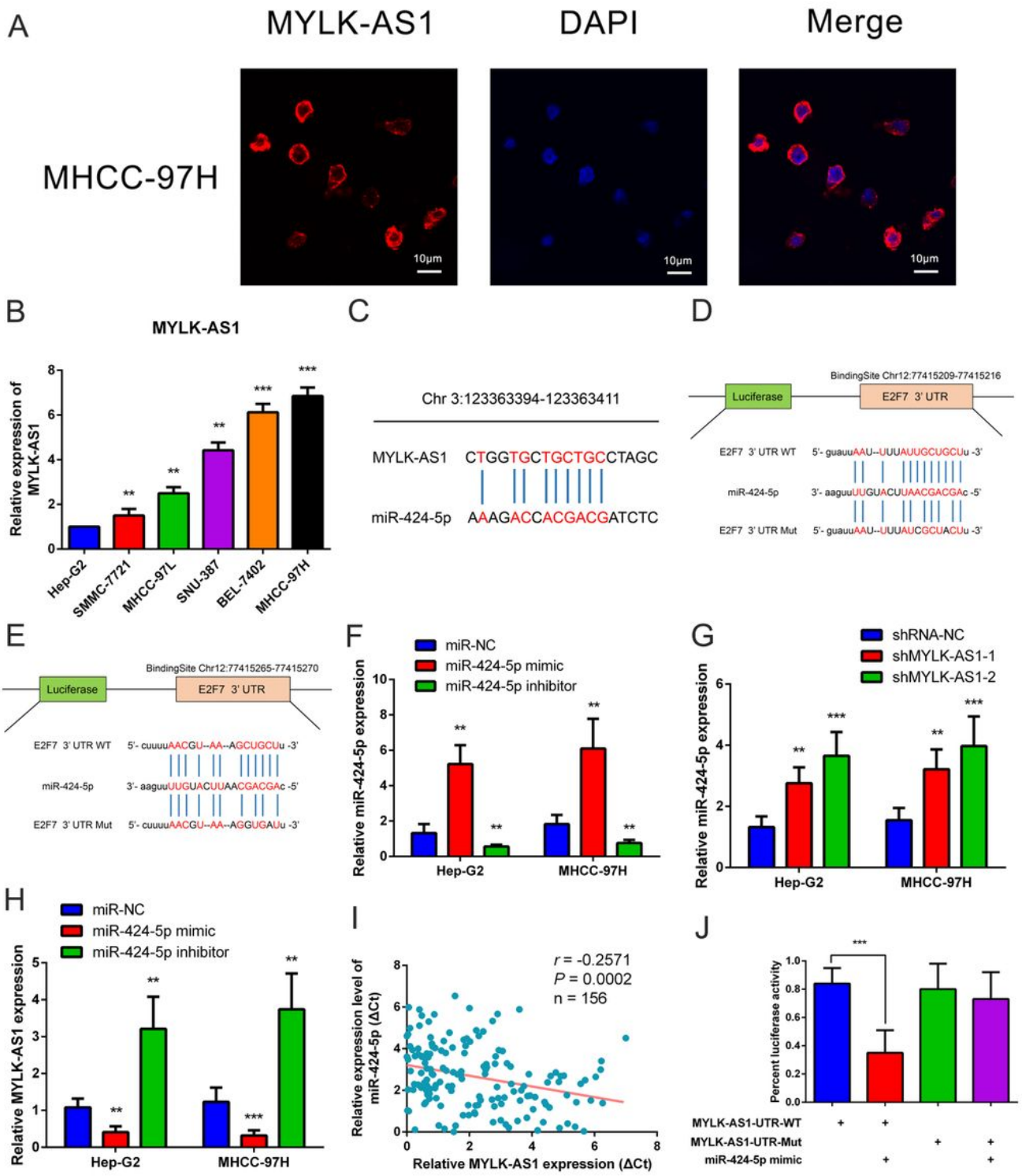

Figure 3

MYLK-AS1 is a ceRNA of miR-424-5p in HCC. A FISH detection for MYLK-8 AS1 (red) was performed in MHCC-97H cells. The nucleus was counterstained with DAPI (blue). B qRT-PCR analysis of the relative expression of MYLK-AS1 in six HCC cell lines. C Schematic representation of the predicted binding site for miR-424-5p in MYLK-AS1 by online database Mircode Predicted algorithm. The numbers indicate the positions of the nucleotides in the reference wild-type sequence of MYLK-AS1 (Ensembl version:

ENSG00000239523). D-E Schematic representation of the predicted miR-424-5p target site within the 3'-UTR of E2F7. The predicted target site for miR- 424-5p is located at the proximal portion of the E2F7 3'-UTR. Two nucleotides complementary to the seed sequence of miR-424-5p were mutated in the E2F7 mutant plasmid. The number indicates the position of the nucleotides in the reference wild-type sequence of E2F7 (NM_203394.3). F Relative expression of miR-424-5p in Hep-G2 and MHCC-97H cells transfected with miR-424-5p mimic or inhibitor. G Relative expression of miR-424-5p in Hep-G2 and MHCC-97H cells after transfection with shMYLK-AS1 or scramble sequence. H Relative expression of MYLK-AS1 in Hep-22 G2 and MHCC-97H cells transfected with miR-424-5p mimic or inhibitor. I Correlation analysis between MYLK-AS1 and miR-424-5p expression in 156 HCC tumor tissues. J Relative luciferase activities of wild type (WT) and mutated (Mut) MYLK-AS1 reporter plasmid in human embryonic kidney (HEK) 293FT cells co-transfected with miR-424-5p mimic. Error bars: mean ± SD. n.s, not significant, *P < 0.05 , **P < 0.01 and ***P < 0.001

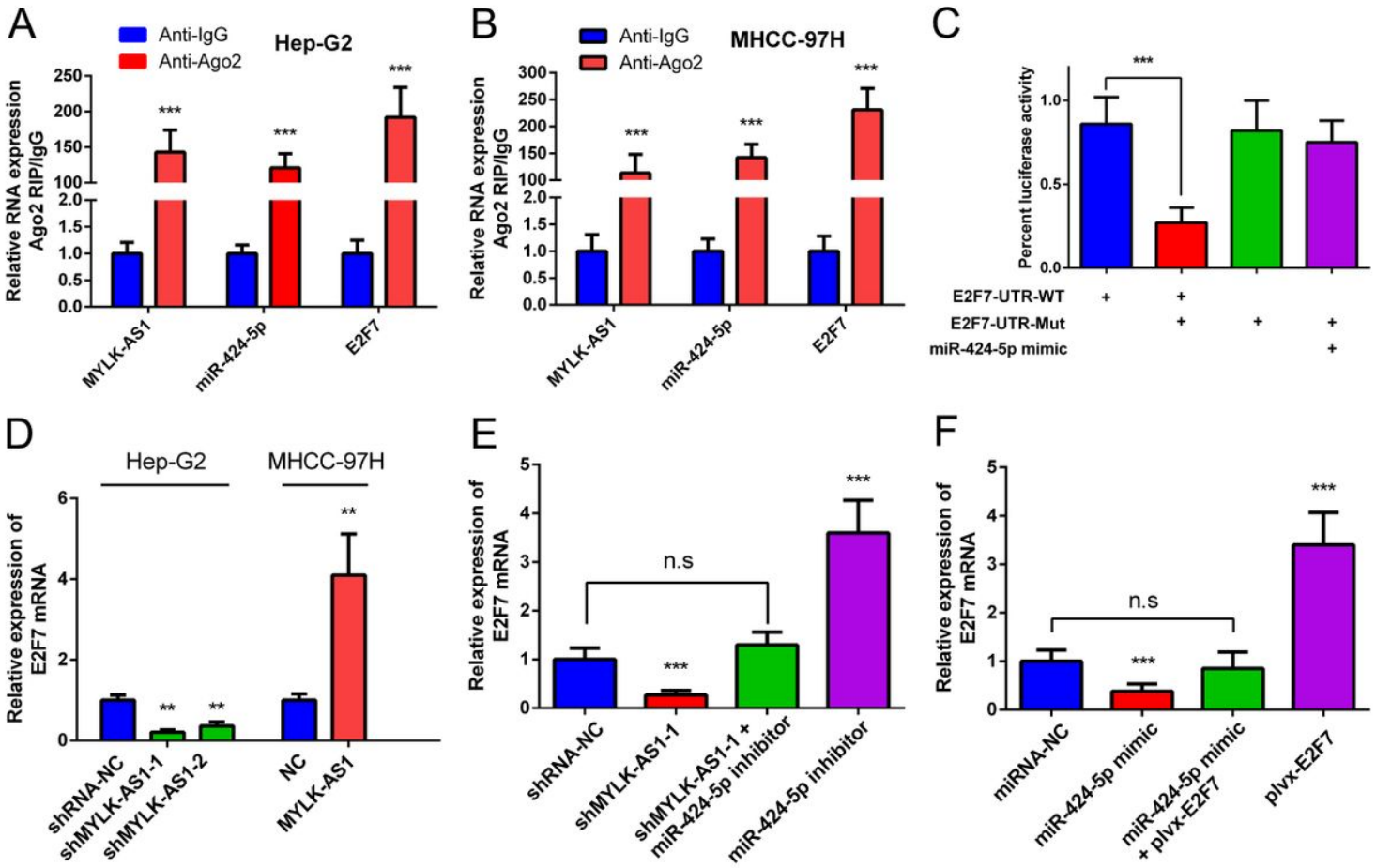

Figure 4

MYLK-AS up-regulates E2F7 expression by competitively binding miR-1 424-5p in HCC. A-B RIP assay to detect the binding capacity of MYLK-AS1, miR-2 424-5p, and E2F7 to anti-Ago2 in Hep-G2 and MHCC-97H (anti-igG was used as control). C Luciferase reporter assay in HEK-293FT cells co-transfected with wide type (WT) or mutated (Mut) E2F7 3'-UTR reporter vector and miR-424-5p mimic. D Relative expression of E2F7 mRNA in Hep-G2 cells with MYLK-AS1 knockdown or MHCC-97H cells with MYLK-AS1

overexpression. E qRT-PCR was conducted to evaluate the mRNA expression of E2F7 gene in MHCC-97H cells following reduced expression of MYLK-AS1 and/or inhibition of miR-424-5p. F qRT-PCR was performed to access the mRNA expression of E2F7 gene in MHCC-97H cells following the ectopic expression of miR-424-5p and/or plvx-E2F7 expression vector lacking the 3'-UTR.

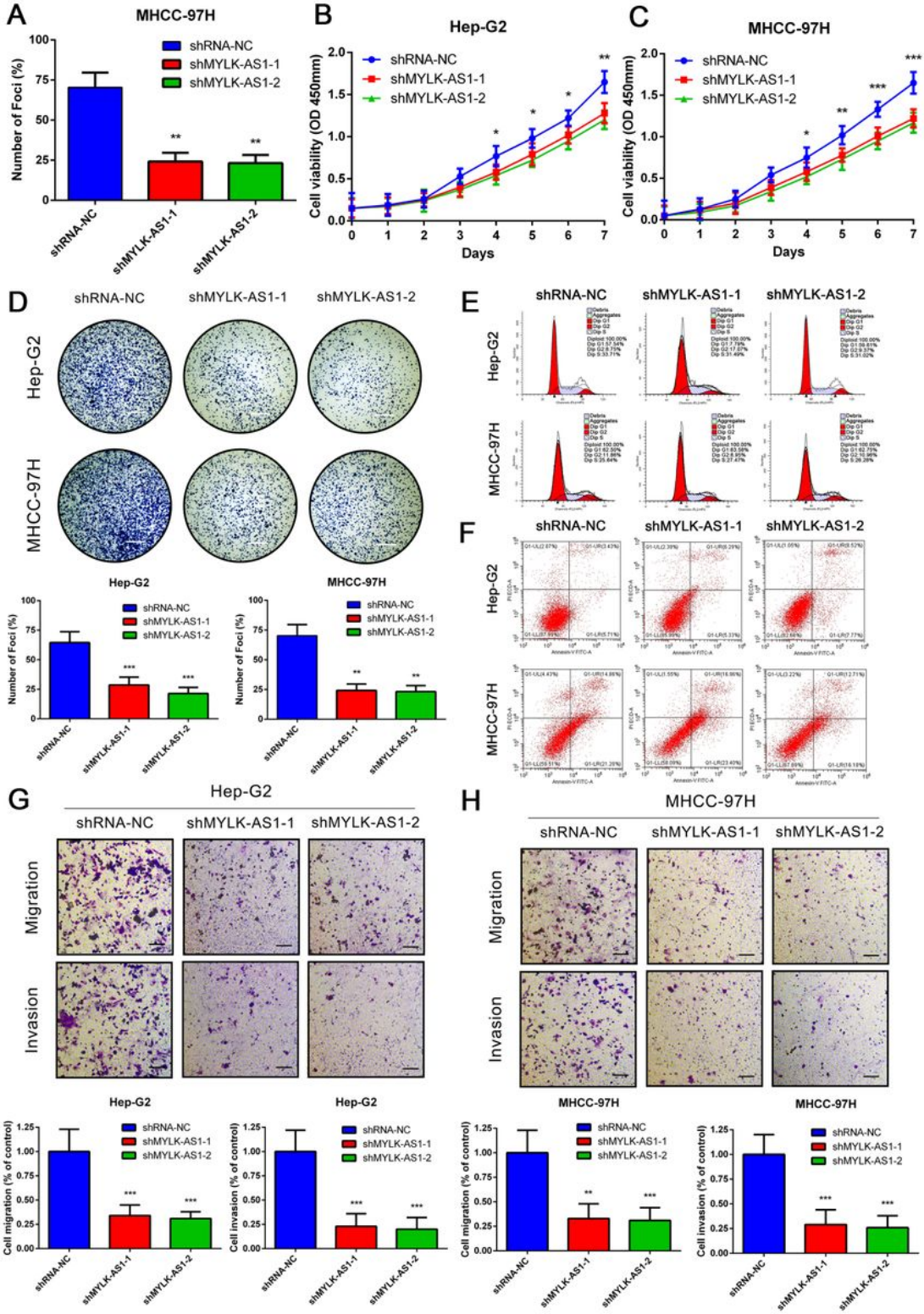

Figure 5

Reduced expression of MYLK-AS1 decreases proliferation, migration and invasion in HCC cells. A qRT-PCR was conducted to verify the relative expression of MYLK-AS1 in Hep-G2 and MHCC-97H cells transfected with two independent shRNAs targeting MYLK-AS1. B-C CCK-8 assay of Hep-G2 and MHCC-97H cells after knockdown of MYLK-AS1. D-H Representative results of the colony formation (scale bar = 500μm), cell cycle assay, apoptosis assays, and transwell (scale bar = 100μm), and of Hep-G2 and MHCC-97H cells after shMYLK-AS1–1 or shMYLK- AS1–2 transfection.

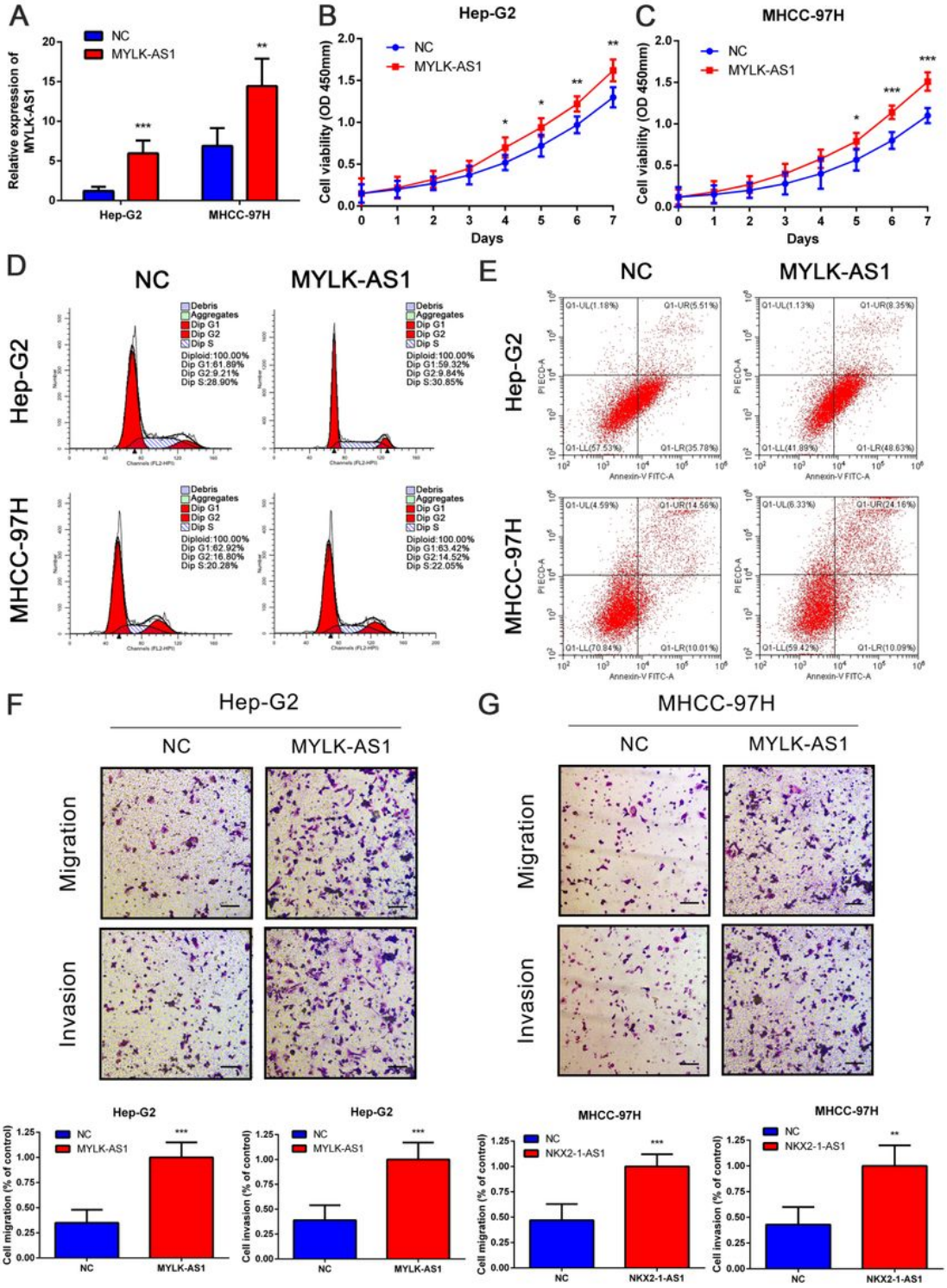

Figure 6

Overexpression of MYLK-AS1 promotes proliferation, migration and invasion in HCC cells. A Relative expression of MYLK-AS1 confirmed by qRT-PCR in Hep-G2 and MHCC-97H cells with MYLK-AS1 overexpression. B Proliferation assays of Hep-G2 and MHCC-97H cells with MYLK-AS1 overexpression by the CCK- 8 assay. D-E Representative results of the cell cycle and apoptosis assays of Hep-G2 and MHCC-97H cells after MYLK-AS1 overexpression. F-G Migration and invasion assays of Hep-G2 and MHCC-97H cells with MYLK-AS1 overexpression by the transwell assay (scale bar = 100μm).

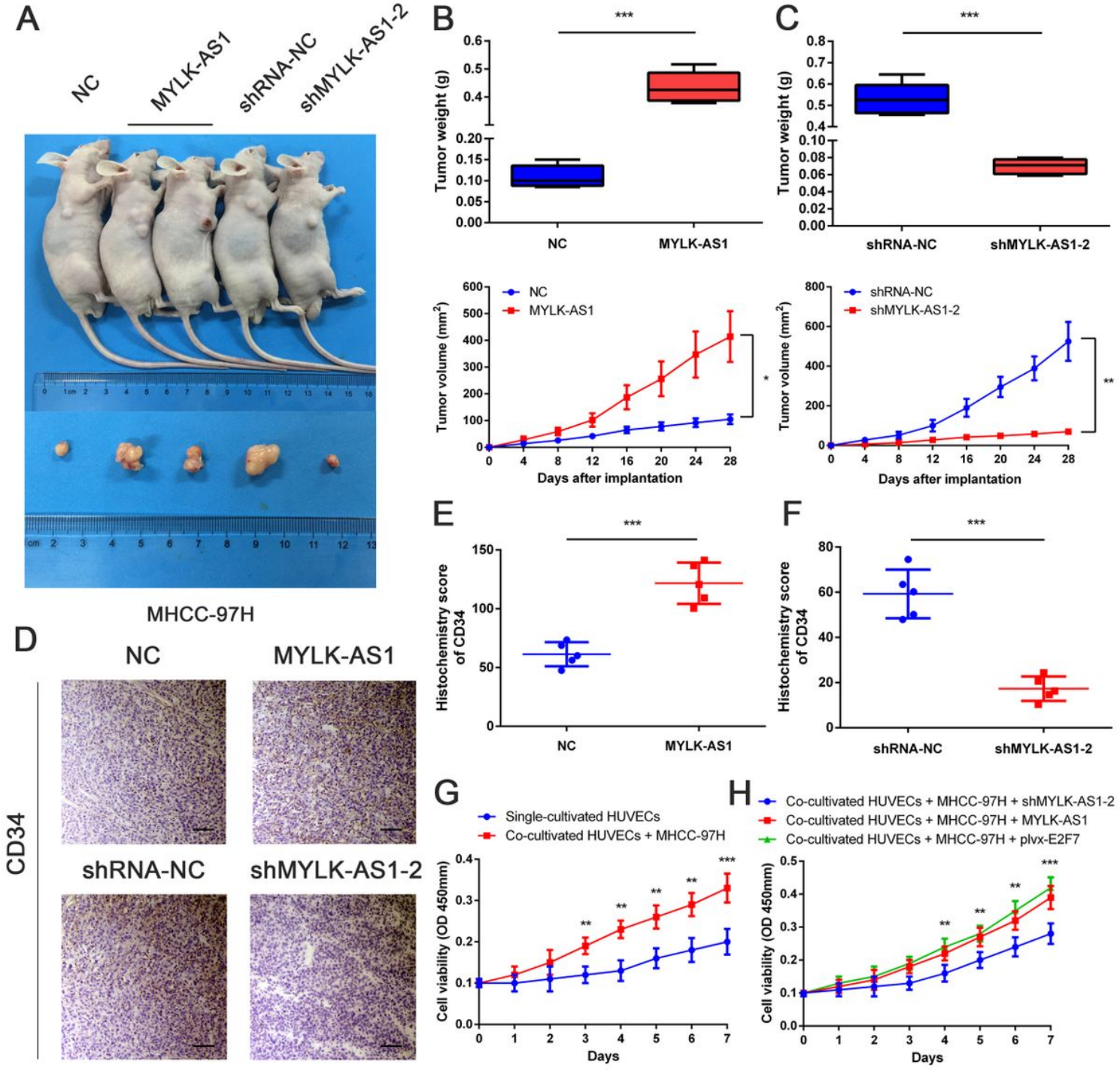

Figure 7

MYLK-AS1 regulates HCC cell proliferation and angiogenesis in vivo and vitro. A The right armpit was injected with MHCC-97H cells transfected with empty vector or MYLK-AS1 expression vector and shMYLK-AS1-NC or shMYLK-AS1-2 in upper panel. Representative images of xenograft tumors are indicated in the bottom panel. B-C Tumor weight and volume of the xenograft in MYLK-AS1 overexpression groups and control group or MYLK-AS1 knowdown group and control group. D 7 Representative IHC staining results of CD34 in corresponding xenografts (scale bar = 8 50μm). E-F Statistical analysis of H-score of CD34 in corresponding xenografts. Error bars: mean ± SD from three independent experiments. G The CCK-8 experiment was used to detect the cell proliferation activity of HUVECs cells when cultured alone and co-cultured with MHCC-97H cells. H MYLK-AS1 knocked down or overexpressed or E2F7 overexpressed MHCC-97H cells were co-cultured with HUVECs cells, CCK-8 experiment was used to detect HUVECs cell proliferation activity.

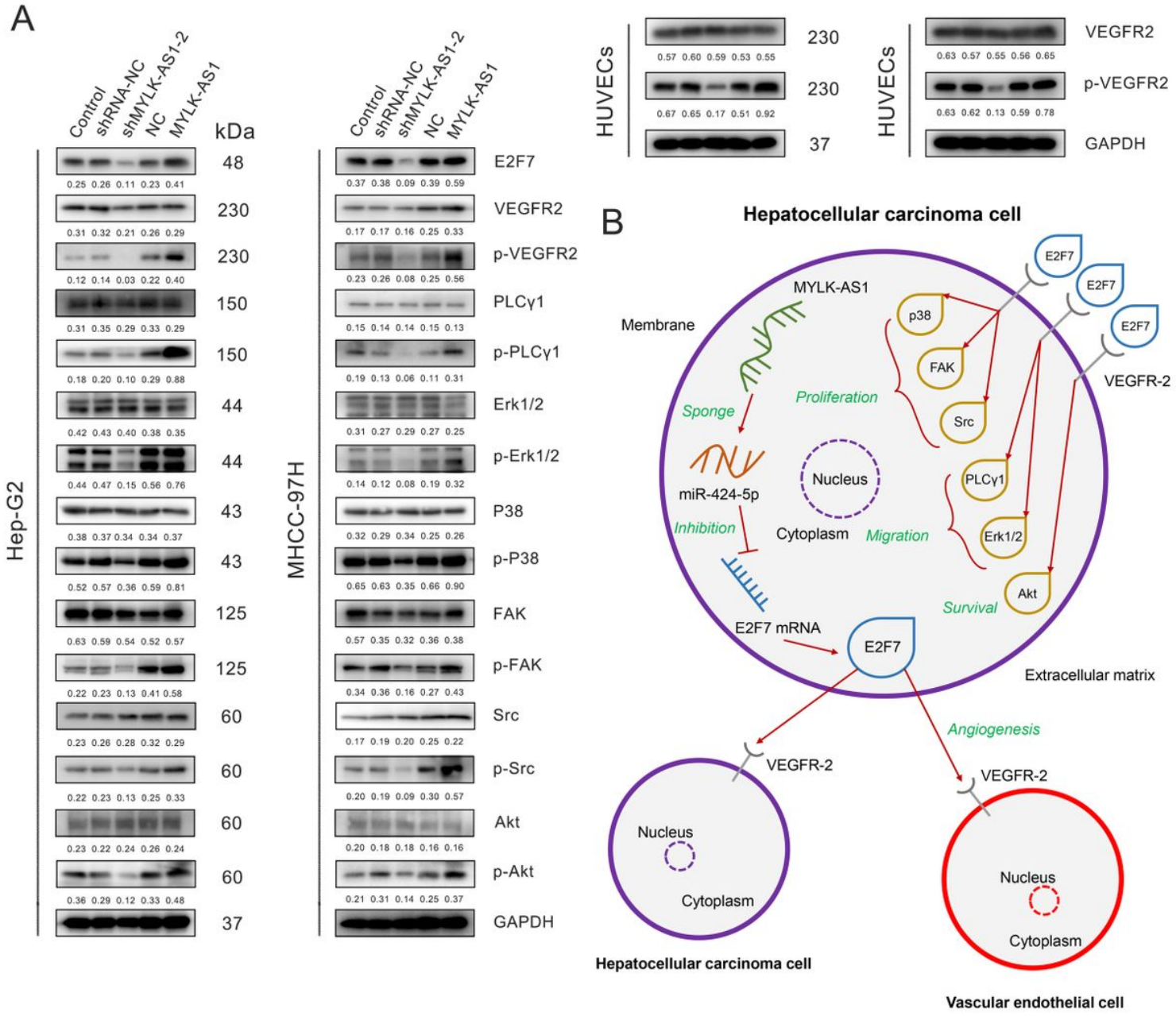

Figure 8

MYLK-AS1/miR-424-5p/E2F7 axis positively regulates HCC metastasis, invasion, and angiogenesis via the VEGFR-2 signaling pathway. A Immunoblot assay of E2F7, VEGFR-2, p-VEGFR-2, PLC-λ, p-PLC-λ, Erk1/2, p-Erk1/2, P38, p-P38, FAK, p-FAK, Src, p-Src, Akt, and p-Akt proteins in Hep-G2 or MHCC-97H cells transfected with shMYLK-AS1–2 and/or MYLK-AS1. Numbers showed quantification of relative protein amount. GAPDH was used as an internal control. After HUVECs cells were co-cultured with corresponding Hep-G2 or MHCC-97H cells for 7 days, the content of VEGFR-2 and p-VEGFR-2 protein was detected. GAPDH serves as an internal reference. B Schematic diagram of the regulatory mechanism of MYLK-AS1/miR-424-5p/E2F7 axis in the promotion of HCC proliferation, metastasis and angiogenesis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- table1.pdf
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- Figs1.jpg