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Supplemental Information

FOXM1-Mediated LINC-ROR Regulates the Proliferation and Sensitivity to Sorafenib in Hepatocellular Carcinoma

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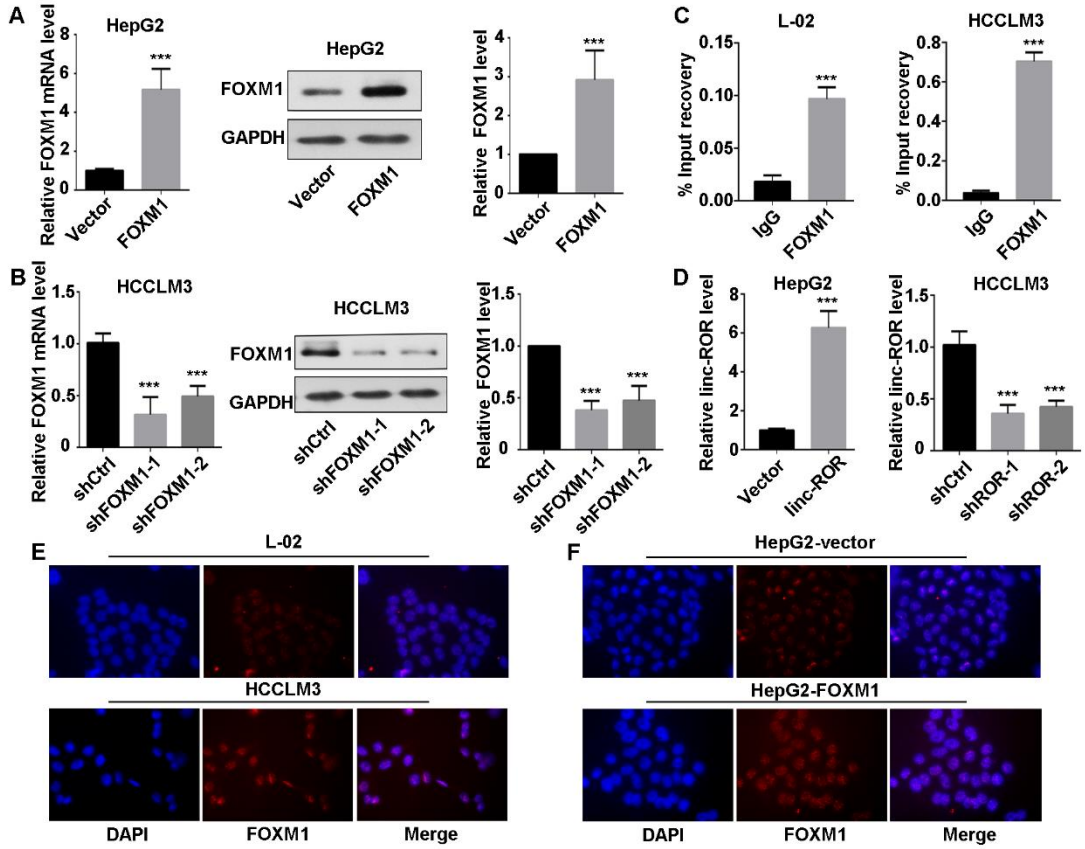
Supplemental Data

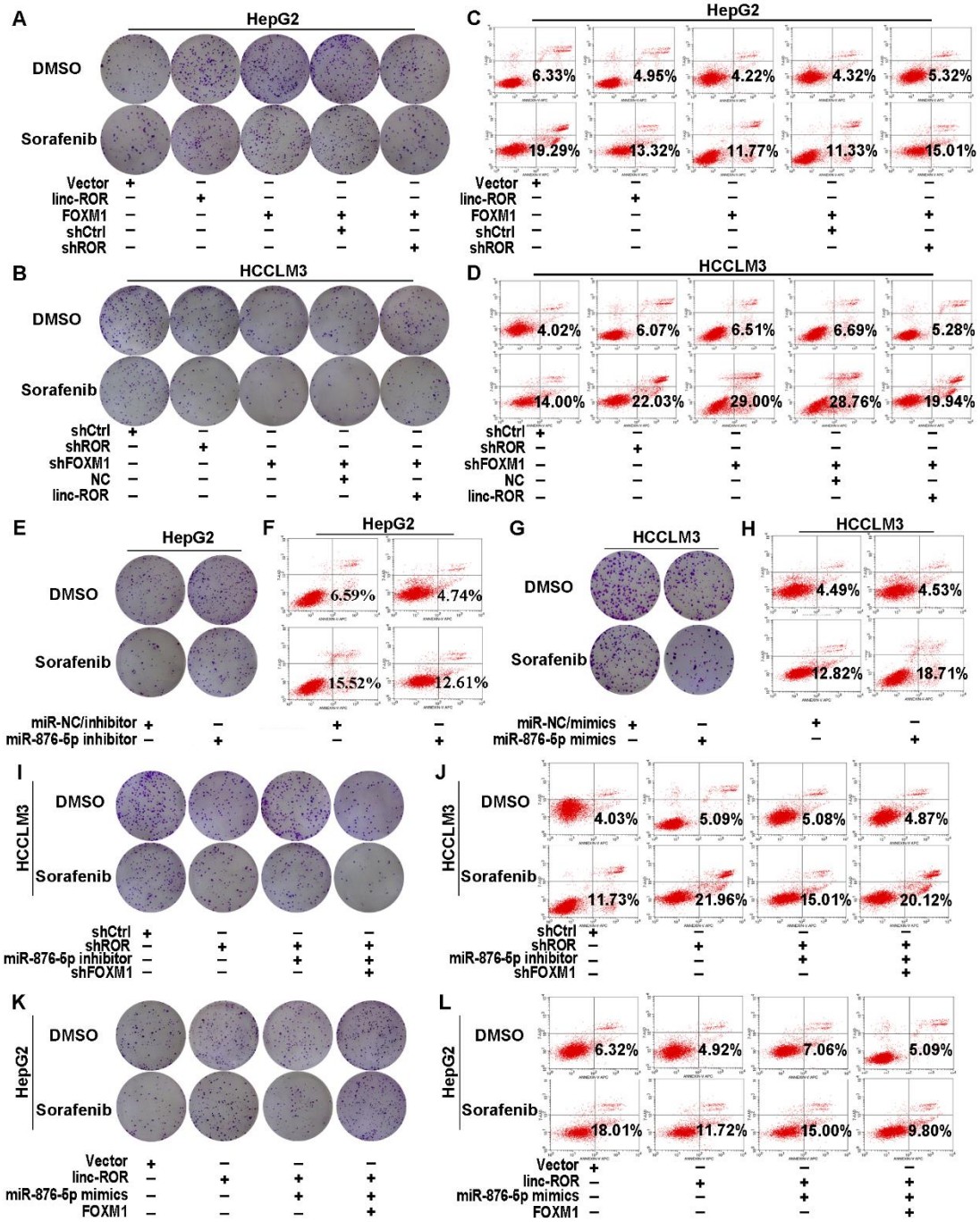
Supplemental Figures and Legends

Figure S1



A. Quantitative real-time PCR and western blot analysis of FOXM1 mRNA or protein level in FOXM1 overexpressed HepG2 cells, normalized to control group. B. Quantitative real-time PCR and western blot result of FOXM1 mRNA or protein level in FOXM1 silenced HCCLM3 cells, compared to control groups. C. CHIP assays using anti-FOXM1 or anti-IgG antibody were applied to determine the affinity of FOXM1 on linc-ROR promoter in L-02 and HCCLM3 cells. Relative enrichment is normalized to IgG as negative control. D. Quantitative real-time PCR analysis of linc-ROR level in linc-ROR overexpressed HepG2 cells and linc-ROR silenced HCCLM3 cells, normalized to control group. E. Immunofluorescence analysis of FOXM1 in L-02 and HCCLM3 cells. F. Immunofluorescence analysis of FOXM1 in HepG2 cells stably transfected with vector or FOXM1 respectively. All data are presented as the mean ± S.D. from three independent experiments. The p-values represent comparisons between groups (**p < 0.01, ***p<0.001).

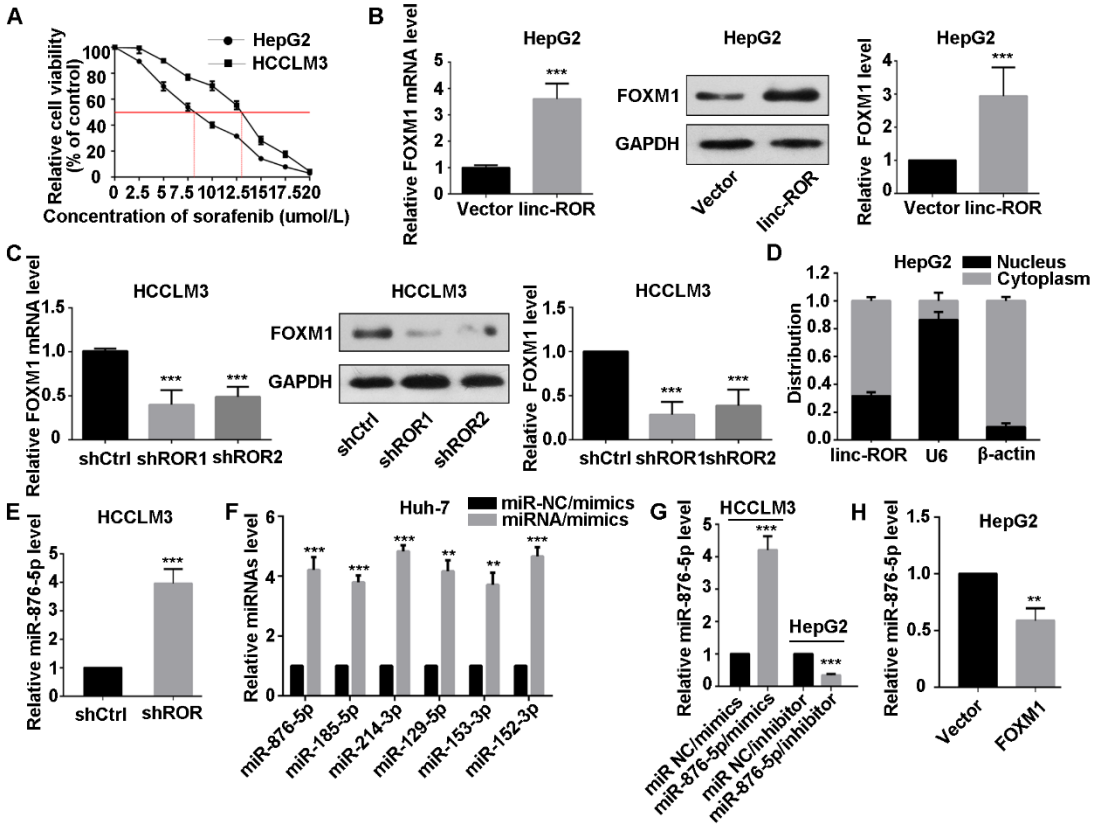
Figure S2



A. Colony formation images of HepG2 cells treated with FOXM1 or linc-ROR or co-treated with FOXM1 and shROR individually, with sorafenib or DMSO added. B. Colony formation images of HCCLM3 cells transfected with shFOXM1 or shROR or co-transfected with shFOXM1 and linc-ROR individually, with sorafenib or DMSO added. C. Flow cytometry analysis of HepG2 cells treated with FOXM1 or linc-ROR or co-treated with FOXM1 and shROR individually, with sorafenib or DMSO added. D. Flow cytometry analysis of HCCLM3 cells transfected with shFOXM1 or shROR or co-transfected with shFOXM1 and linc-ROR individually, with sorafenib or DMSO added. E-F. Colony formation images and flow cytometry analysis of HepG2 cells treated with miR-NC/inhibitor or miR-876-5p/inhibitor, with sorafenib or DMSO added. G-H. Colony formation images and flow cytometry

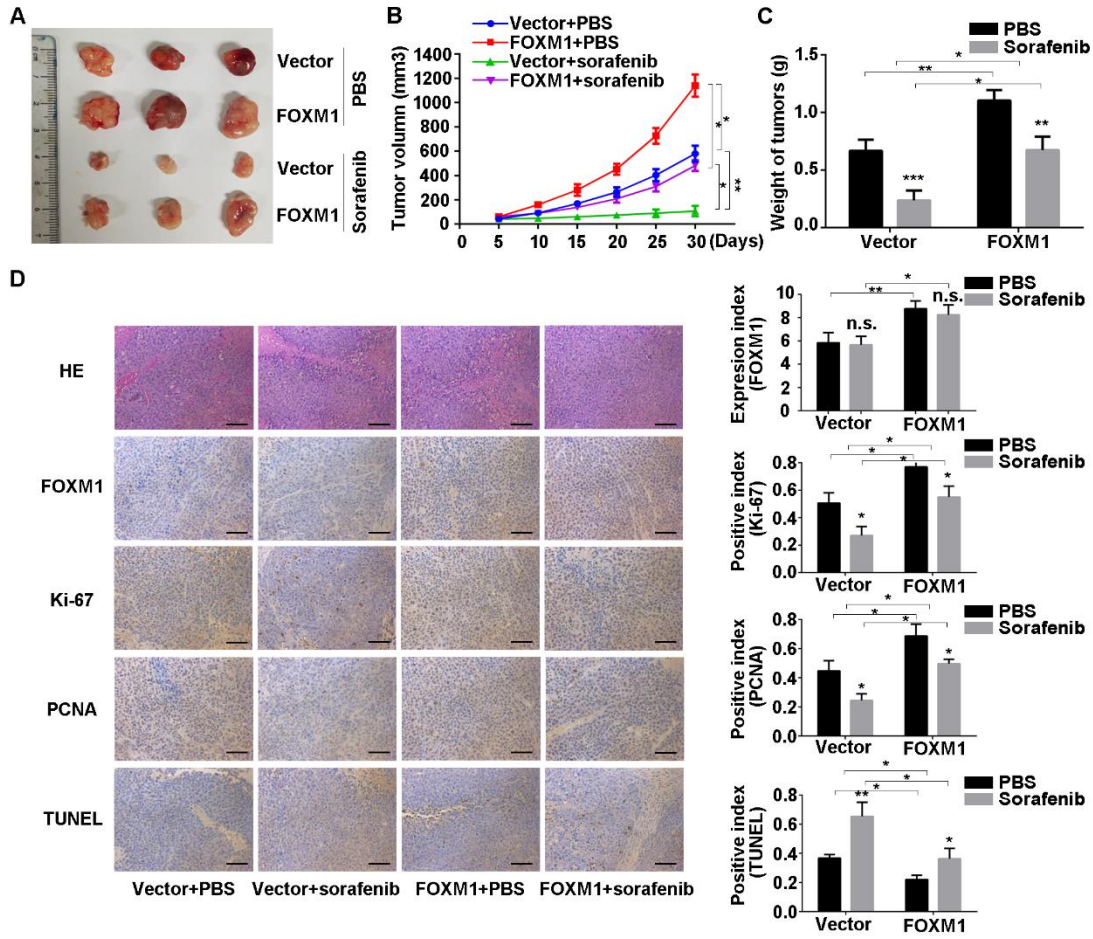
analysis of HCCLM3 cells under transfection of miR-NC/mimics or miR-876-5p/mimics, supplemented with sorafenib or DMSO. I. Colony formation images of HCCLM3 cells treated with shROR or co-transfected with shROR and miR-876-5p inhibitor or co-transfected with shROR, miR-876-5p inhibitor and shFOXM1, with the addition of sorafenib or DMSO. J. Flow cytometry analysis of HCCLM3 cells treated with shROR or co-transfected with shROR and miR-876-5p inhibitor or co-transfected with shROR, miR-876-5p inhibitor and shFOXM1, with the addition of sorafenib or DMSO. The percentage represents a sum of both Annexin positive population and 7-AAD positive population. K. Colony formation images of HepG2 cells transfected with linc-ROR or co-transfected with linc-ROR and miR-876-5p mimics or co-transfected with linc-ROR, miR-876-5p mimics and FOXM1 under sorafenib treatment. L. Flow cytometry analysis of HepG2 cells transfected with linc-ROR or co-transfected with linc-ROR and miR-876-5p mimics or co-transfected with linc-ROR, miR-876-5p mimics and FOXM1, with the addition of sorafenib. The percentage represents a sum of both Annexin positive population and 7-AAD positive population.

Figure S3



A. Cell viability of HepG2 and HCCLM3 cells under different concentration of sorafenib treatment was tested by CCK-8. B. Quantitative real-time PCR or western blotting analysis of FOXM1 mRNA or protein level in linc-ROR overexpressed HepG2 cells, normalized to control group. C. Quantitative real-time PCR or western blot analysis of FOXM1 mRNA or protein level in linc-ROR silenced HCCLM3 cells, normalized to control group. D. The distribution of linc-ROR in HepG2 cells. U6 was used as nuclear RNA marker and β-actin was used as cytoplasmic RNA marker. E. Quantitative real-time PCR analysis of miR-876-5p level in linc-ROR silenced HCCLM3 cells, normalized to control group. F. Quantitative real-time PCR analysis of each miRNA level after transfection of respective miRNA mimics in Huh-7 cells compared with control groups. G. Quantitative real-time PCR analysis of miR-876-5p after transfection of miR-876-5p inhibitor in HepG2 cells or miR-876-5p mimics in HCCLM3 cells. H. Quantitative real-time PCR analysis of miR-876-5p after FOXM1 overexpression in HepG2 cells. All data are presented as the mean ± S.D. from three independent experiments. The p-values represent comparisons between groups (**p < 0.01, ***p<0.001).

Figure S4



A. HepG2-vector and HepG2-FOXM1 cells were implanted into nude mice respectively. When the average tumor volume reached 50 mm^3, the two group mice were subdivided into 4 groups randomly and were given 0 or 60 mg/kg sorafenib or PBS; tumor sizes were calculated every 5 days. Images of subcutaneous xenograft tumors were taken. B. The growth curves of the subcutaneous xenograft tumors were made and the bars indicated the standard deviation (SD). C. The final tumor weights were measured and calculated. D. Representative images of H&E staining, immunohistochemical staining (for FOXM1, Ki-67 and PCNA), and TUNEL staining were exhibited. Scale bar, 50μm (n.s. no significance, *$p < 0.05$, **$p < 0.01$, ***$p<0.001$).

Supplemental Tables

Table S1. The linc-ROR promoter domain binding with FOXM1

>hg19, dna range=chr18:54745025-54745392 5'pad=0 3'pad=0 strand=+ repeatMasking=none
TGCGGAAGAGGACATAGGAGCCGTGGCAGAGGTGTCCCAAGGCCTCTGCCTCTCTGCTG TCCCTTCACAGCGACCTTAACAGGCCCCATGATAGTCATGTGCAGGTGATCGTGACCAAA TCTTGAGATGTGGCTTCCTCCGCCCCTCTCGAGGGCTCCATAAAAGGAAGTCCCCAGGG TACTGTGGTAACACCCTGGTGGCTCCCTCTGGGGTCCCAAAGAGGAAGGCGTGGGTGTG TGCACAGGTTTGTGGTCAGGTCTGCTAGGGTGGCCGGGGAACACCACCCTGTAGAAGTT GTTCCTGGCTCCTGTCCTCTGCACTGTTGGCCACTCTTAGCAAGCATCTGGGGAGAGCAG TGTTGTGCTGAT

Table S2. Sequences of PCR primers used in this study

Linc-ROR	Forward(5'-3')	GAAGGTTCAACATGGAAACTGG
	Reverse(5'-3')	TGAGACCTGCTGATCCCATTC
FOXM1	Forward(5'-3')	CTCAATGGAGAGTGAAAACGCA
	Reverse(5'-3')	GGGCATTTTGAACAGGAAGG
GAPDH	Forward(5'-3')	ACAACTTTGGTATCGTGGAAGG
	Reverse(5'-3')	GCCATCACGCCACAGTTTC
miR-129-5p	Forward(5'-3')	ACACTCCAGCTGGGCTTTTTGCGGTCTGG
	Reverse(5'-3')	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCAAGCCC
miR-214-3p	Forward(5'-3')	ACACTCCAGCTGGGACAGCAGGCACAGACA
	Reverse(5'-3')	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACTGCCTG
miR-152-3p	Forward(5'-3')	ACACTCCAGCTGGGTCAGTGCATGACAGA
	Reverse(5'-3')	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCAAGTTC
miR-153-3p	Forward(5'-3')	ACACTCCAGCTGGGTTGCATAGTCACAAAA
	Reverse(5'-3')	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGATCACTT
miR-185-5p	Forward(5'-3')	ACACTCCAGCTGGGTGGAGAGAAAGGCAGT
	Reverse(5'-3')	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAGGAAC
miR-876-5p	Forward(5'-3')	ACACTCCAGCTGGGTGGATTTCTTTGTGAA
	Reverse(5'-3')	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGGTGATT
U6	Forward(5'-3')	CTCGCTTCGGCAGCACA
	Reverse(5'-3')	AACGCTTCACGAATTTGCGT

Table S3. Sequences of shRNA against specific target and miRNA mimics/inhibitor

sh-ROR-1	5’-3’	GGAGAGGAAGCCTGAGAGT
sh-ROR-2	5’-3’	GCCTCTGCACTCTTATGGAAGGAGGAAAT
sh-FOXM1-1	5’-3’	GGCUGCACUAUCAACAAUATT
sh-FOXM1-2	5’-3’	CCAACAGGAGTCTAATCAA
sh-NC	5’-3’	TTCTCCGAACGTGTCACGT
miR-876-5p mimics	Sense(5’-3’)	UGGAUUUCUUUGUGAAUCACCA
	Antisense(5’-3’)	GUGAUUCACAAAGAAAUCCAUU
miR-876-5p inhibitor	5’-3’	UGGUGAUUCACAAAGAAAUCCA
miR mimics/NC	Sense(5’-3’)	UUCUCCGAACGUGUCACGUTT
	Antisense(5’-3’)	ACGUGACACGUUCGGAGAATT
miR inhibitor/NC	5’-3’	CAGUACUUUUGUGUAGUACAA