Supplementary Material

MicroRNA-197 Promotes Metastasis of Hepatocellular Carcinoma by Activating Wnt/β-Catenin Signaling

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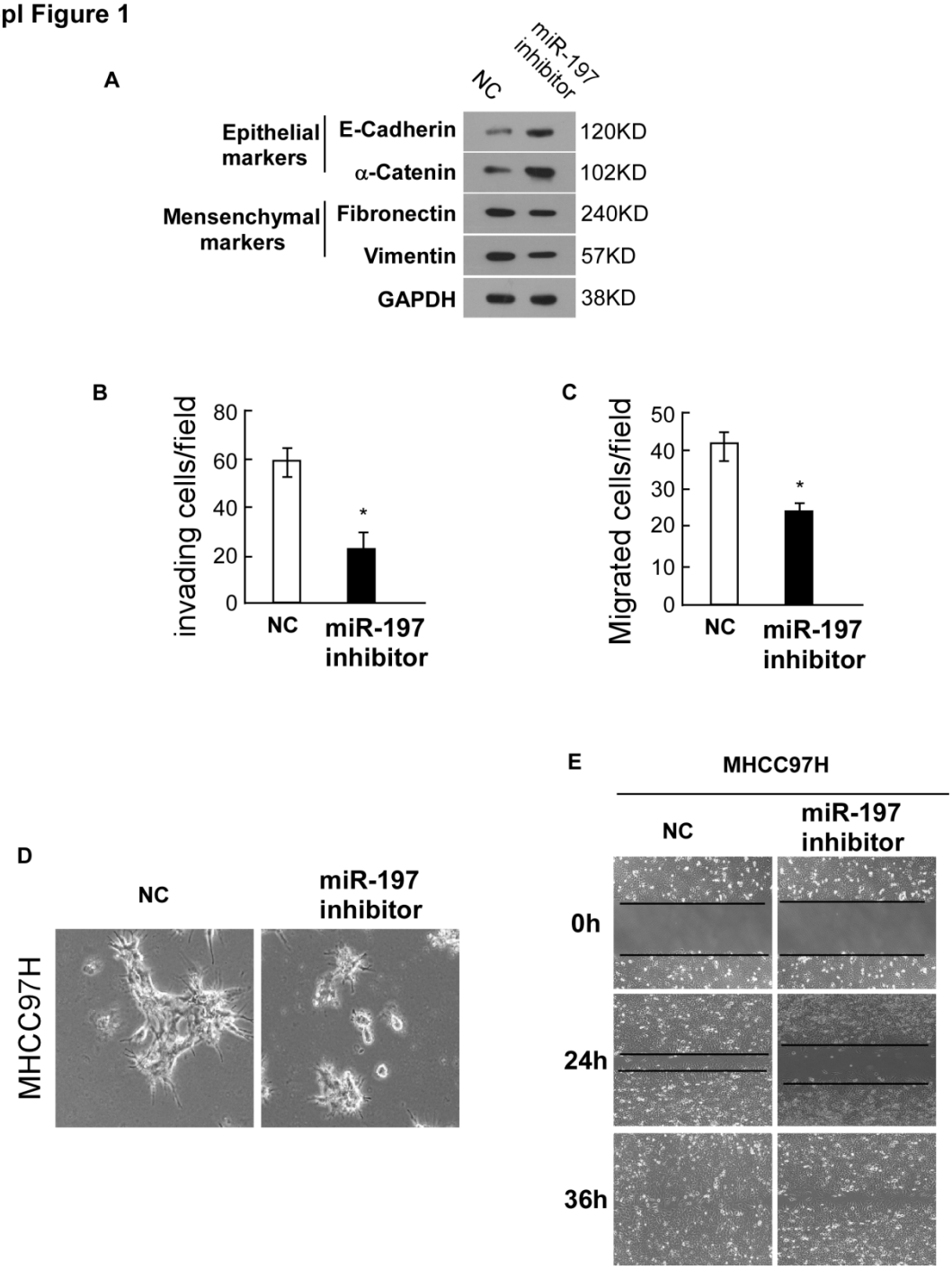
Supplemental figures

Suppl Figure 1

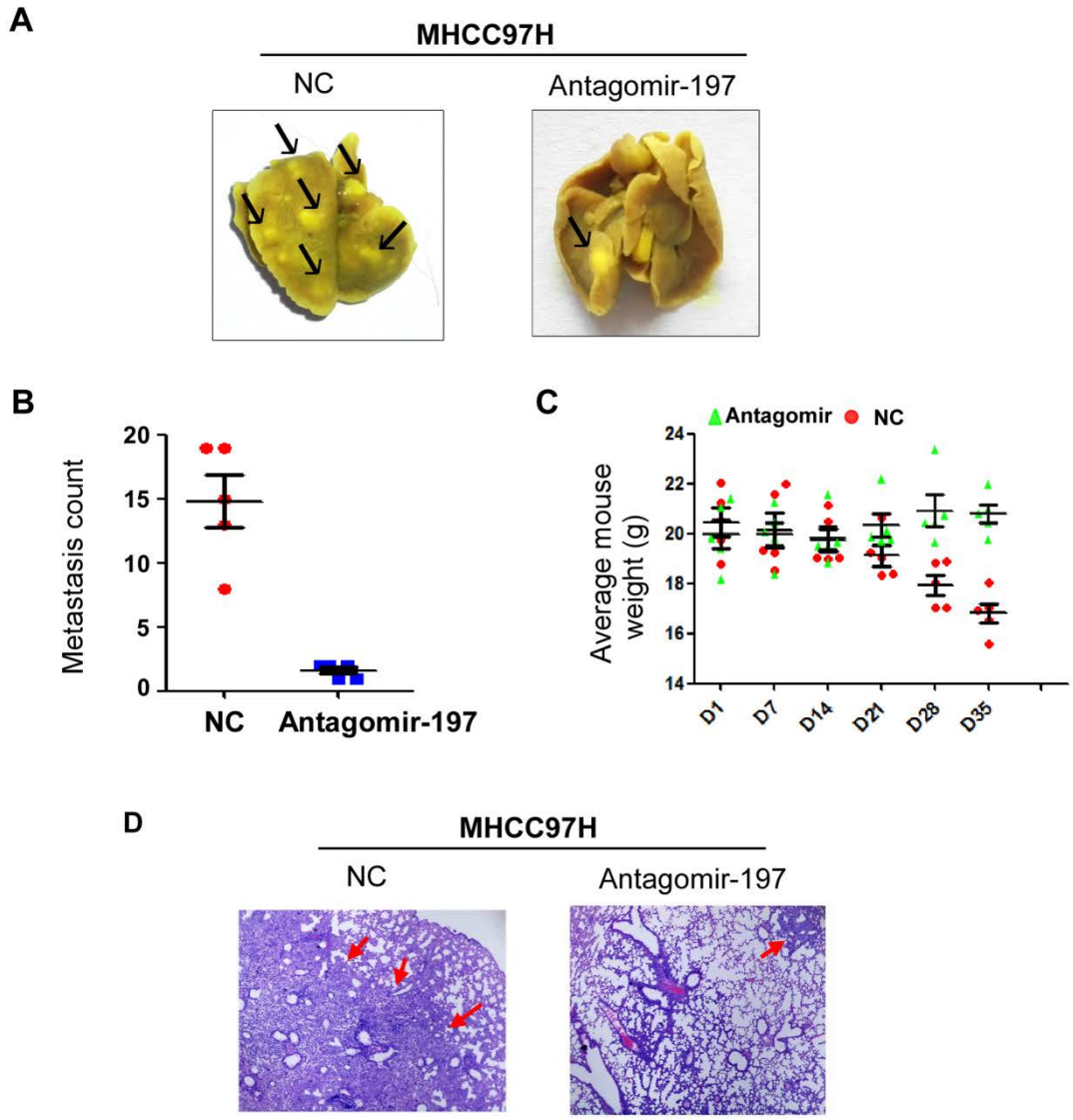


Supplemental Figure 1. EMT is attenuated by down-regulation of miR-197 in HCC cell lines in vitro.

(A) Expression of epithelial cell markers (E-cadherin, a-catenin) and mesenchymal cell markers (vimentin and fibronectin) in MHCC97H cells were examined. GAPDH

was used as a loading control. Representative micrographs of indicated invading (B) or migrating (C) MHCC97H cells in 5 random fields analyzed by Matrigel-coated or -non-coated Transwell assays, respectively. (D) Representative micrographs of indicated cells grown on Matrigel for 10 days in 3D spheroid invasion assay.(E) Representative micrographs of wound healing assay of MHCC97H cells. Wound closures were photographed at 0 and 24 hours after wounding.

Suppl Figure 2

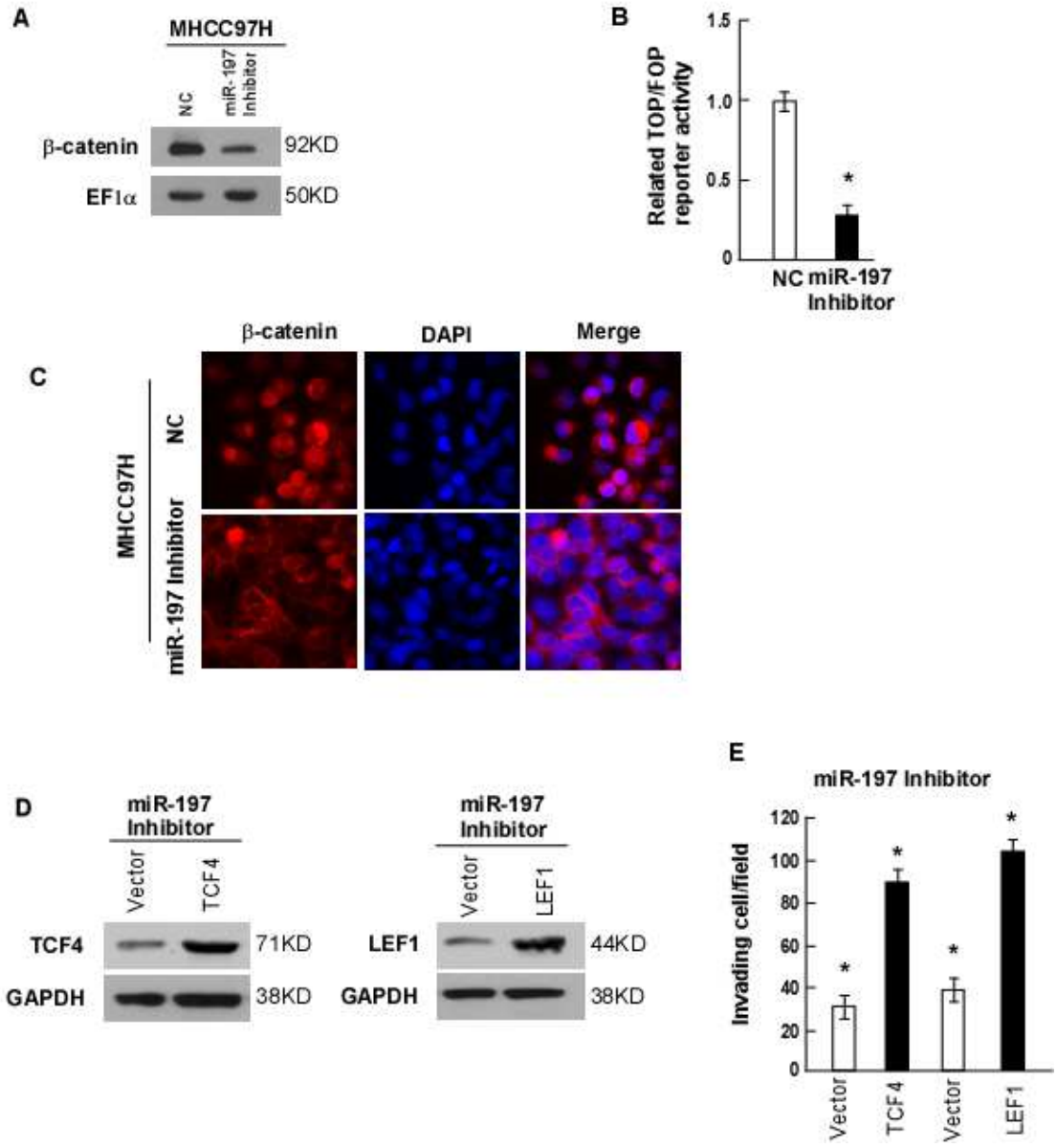


Supplemental Figure 2. Inhibition of miR-197 reduced metastasis of HCC cell lines *in vivo*.

(A) Representative bright-field imaging of the lungs. On days 30, mice receiving transplants of MHCC-97H cells were anesthetized and the lungs were collected. Arrows indicate surface metastatic nodules. (B) Number of visible surface metastatic lesions in mice (n=5 per group) receiving tail vein injection of MHCC97H cells. (C) Weights of BALB/c mice that received transplants of MHCC97H cells with different

treatment. (D) Lung metastases in the mice in which MHCC97H cells were implanted were confirmed by H&E staining.

Suppl Figure 3

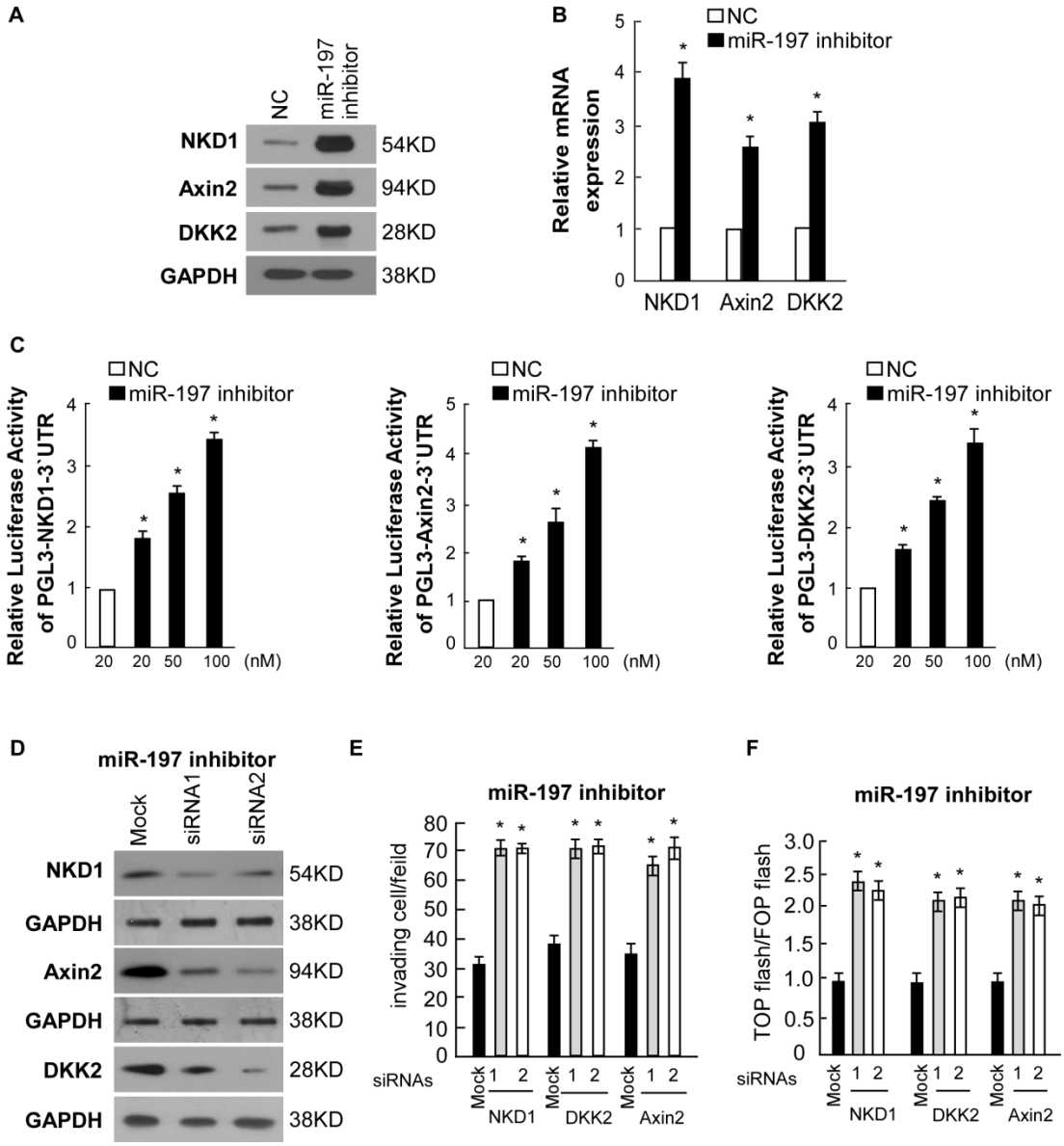


Supplemental Figure 3. Downregulation of miR-197 in MHCC97H cells inhibited Wnt/β-catenin signaling.

(A) Altered nuclear translocation of β-catenin in response to deregulated miR-197 expression. Nuclear fractions of indicated cells were analyzed by WB analysis. EF-1αwas used as a loading control. (B) Indicated cells transfected with TOP flash or FOP flash and RenillapRL-TK plasmids were subjected to dual-luciferase assays 48 hours after transfection. Reporter activity detected was normalized by Renilla

luciferase activity. *P< 0.05. (C) Subcellular β-catenin localization in indicated cells was assessed by immunofluorescence staining. (D) Overexpression of TCF4 or LEF1 in miR-197-down-regulated MHCC97H cells confirmed by WB analysis. (E) Quantification of invading cells impacted by overexpression of TCF4 or LEF1 in Transwell assay.

Suppl Figure 4



Supplemental Figure 4. Antagonism of miR-197 inhibited EMT of MHCC97H cells.

(A) WB analysis of the protein levels of NKD1, Axin2, and DKK2 in response to deregulated miR-197 expression of MHCC97H cells. (B) Real-time RT-PCR analysis of mRNA levels of NKD1, Axin2, and DKK2 in indicated treatment. (C) Luciferase assay of pGL3- NKD1-3′-UTR, pGL3- Axin2-3′-UTR, and pGL3- DKK2-3′-UTR reporters cotransfected with increasing amounts (20, 50 and 100 nM) of miR-197 inhibitor oligonucleotides in MHCC-97H cells. (D) WB analysis confirmed the

transfection of specific siRNAs of NKD1, Axin2, and DKK2 in miR-197-silenced cells. (E) Quantification of indicated invading cells with specific siRNA of NKD1, Axin2 and DKK2 transfection. (F) Luciferase assay of TCF/LEF transcriptional activity in indicated MHCC97H cells transfected with specific siRNA of NKD1, Axin2 and DKK2.

Supplemental Table S1. Clinicopathological characteristics of clinical samples and expression of miR-197 in liver cancer

Characteristics		No. Patients	%
Age (years)	≤50	65	61.9
	>50	40	38.1
Gender	Male	96	91.4
	Female	9	8.6
TNM classification	I	10	9.5
	II	70	66.7
	III	25	23.8
HBsAg	Positive	100	95.2
	Negative	5	4.8
AFP	≥400 ng/ml	54	51.4
	<400ng/ml	51	48.6
Tumor size	>3 cm	85	81.0
	≤3 cm	20	19.0
Tumor number	>1	29	27.6
	= 1	76	72.4
Metastasis status (at follow-up)	No metastasis	68	64.8
	Metastasis	37	35.2
Expression of miR-197	High expression	40	38.1
	Low expression	65	61.9