MiR-494-3p promotes PI3K/AKT pathway hyperactivation and human hepatocellular carcinoma progression by targeting PTEN

Hui Lin[1#], Zhi-Ping Huang[4#], Jiao Liu[5#], Yun Qiu[2#], Yuan-ping Tao[4], Meng-chao Wang[4], Hui Yao[2], Ke-zhu Hou[1*], Fang-ming Gu[4*], Xuan-fu Xu[3*]

Supplementary Table 1. Univariable analysis for RFS and OS

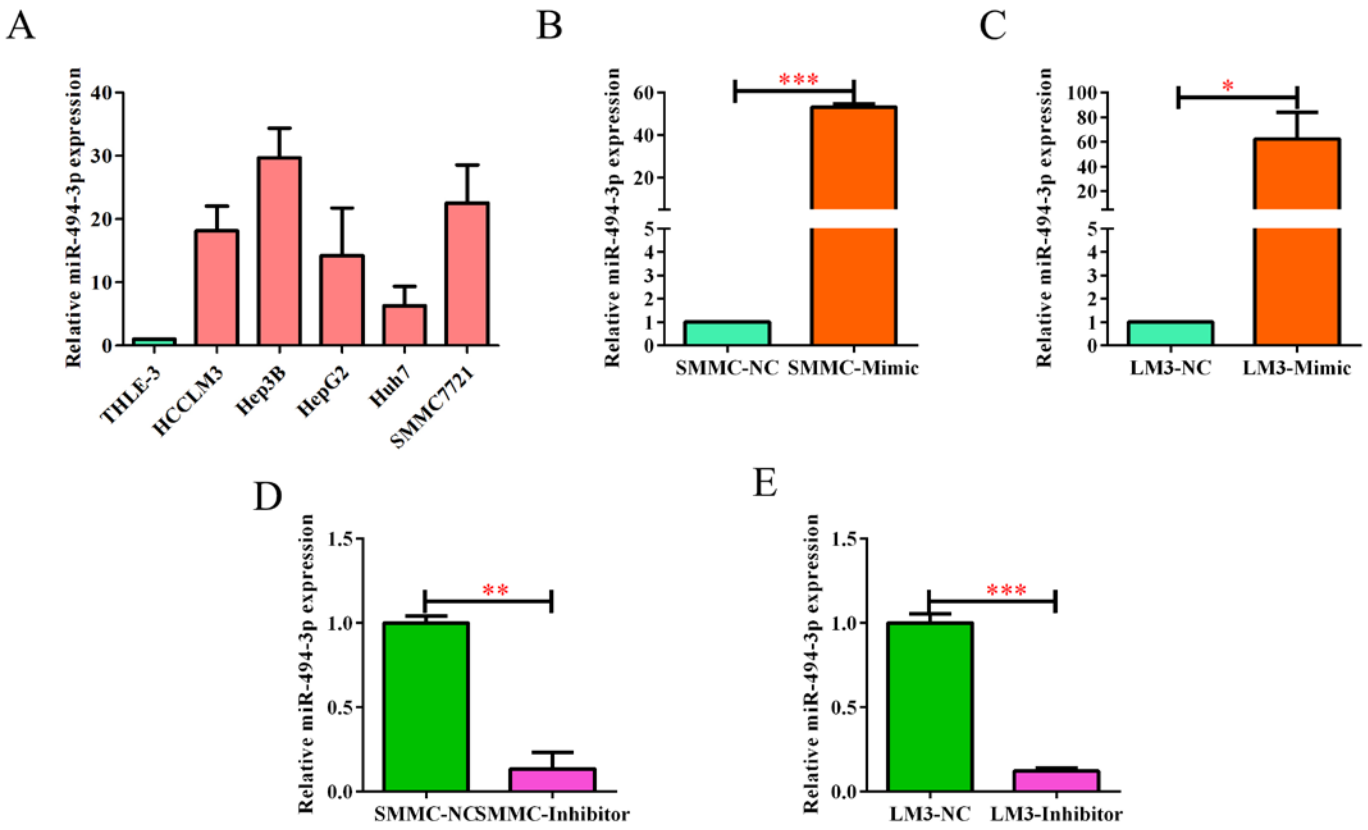
Variable	RFS			OS		
	P	HR	95% CI	P	HR	95% CI
Age, years, ≥55 vs. <55	0.700	1.060	0.787 1.428	0.236	1.233	0.872 1.745
Sex, male vs. female	0.178	1.378	0.864 2.197	0.471	1.212	0.718 2.045
AFP, μg/L, ≥20 vs <20	0.382	1.140	0.850 1.528	0.031	1.451	1.035 2.034
Cirrhosis absent vs. present	0.219	0.835	0.626 1.113	0.573	0.907	0.646 1.273
Tumor diameter, cm, ≥5 vs. <5	0.001	1.725	1.237 2.406	0.048	1.477	1.003 2.177
Tumor number, multiple vs. solitary	0.047	1.501	1.005 2.240	0.047	1.566	1.006 2.439
Capsule, absent vs. present	0.752	0.953	0.706 1.286	0.588	0.908	0.641 1.286
Vascular invasion, present vs. absent	0.000	1.967	1.439 2.689	0.000	2.458	1.722 3.507
TNM stage, III and IV vs. I and II	0.081	1.407	0.958 2.067	0.003	1.886	1.235 2.879
BCLC stage, C and D vs. A and B	0.081	1.454	0.955 2.213	0.034	1.651	1.040 2.623
miR-494-3p expression, high vs. low	0.027	1.366	1.037 1.801	0.001	1.757	1.272 2.428

Abbreviation: AFP, alpha fetoprotein; BCLC stage, Barcelona Clinic Liver Cancer stage.

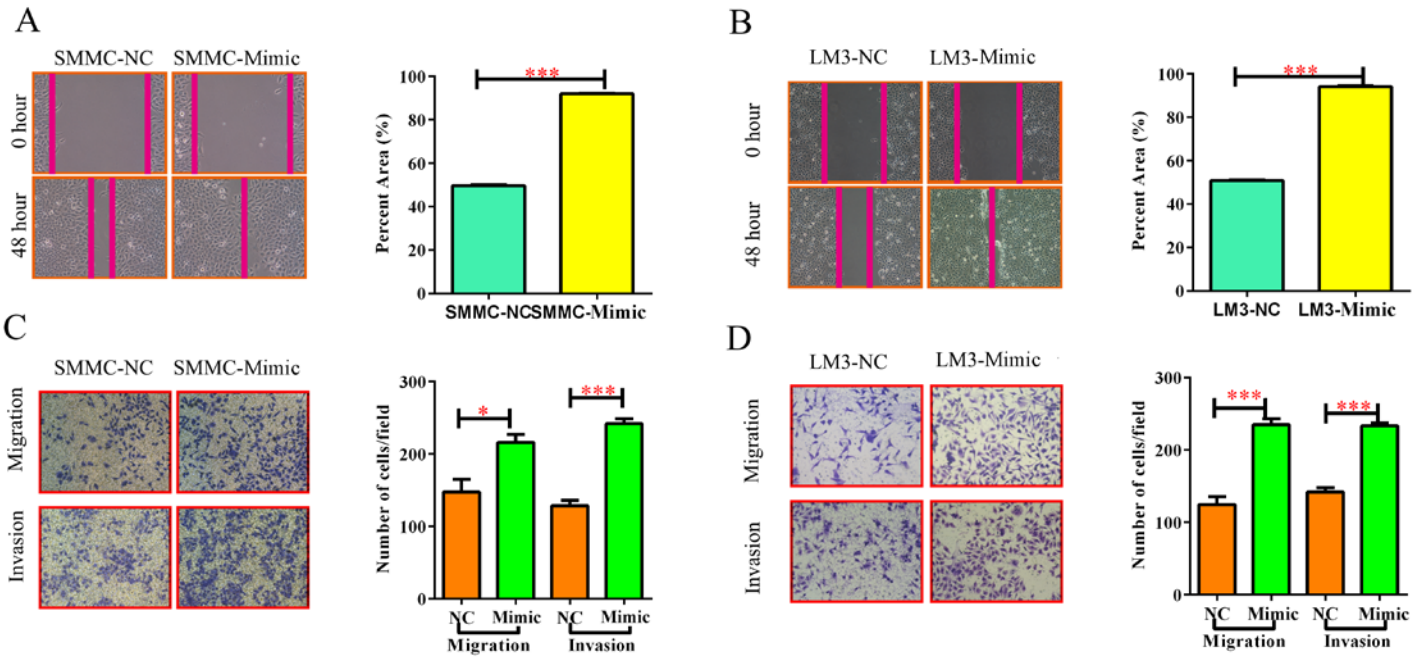
Supplementary Table 2 RT-qPCR primers:

Gene	Forward	Reverse
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
PTEN	TGGATTCGACTTAGACTTGACC	AGGATATTGTGCAACTCTGCAA
ACTIN	CACTCTTCCAGCCTTCCTT	CTCGTCATACTCCTGCTTGCT

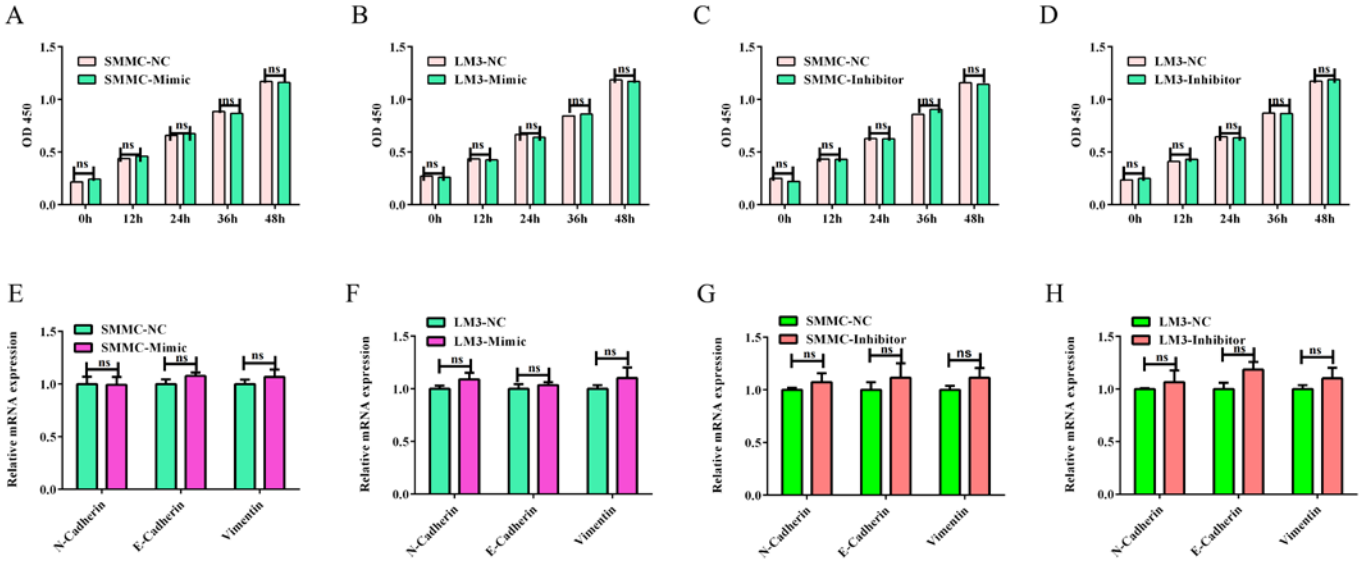
Supplementary figure 1



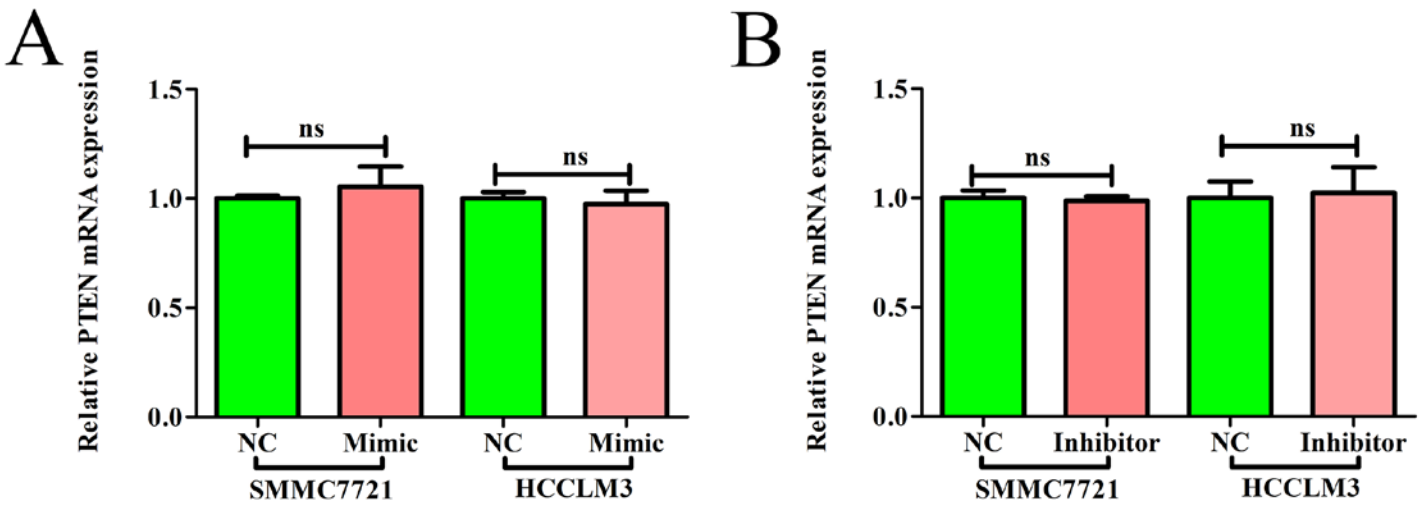
Supplementary figure 2



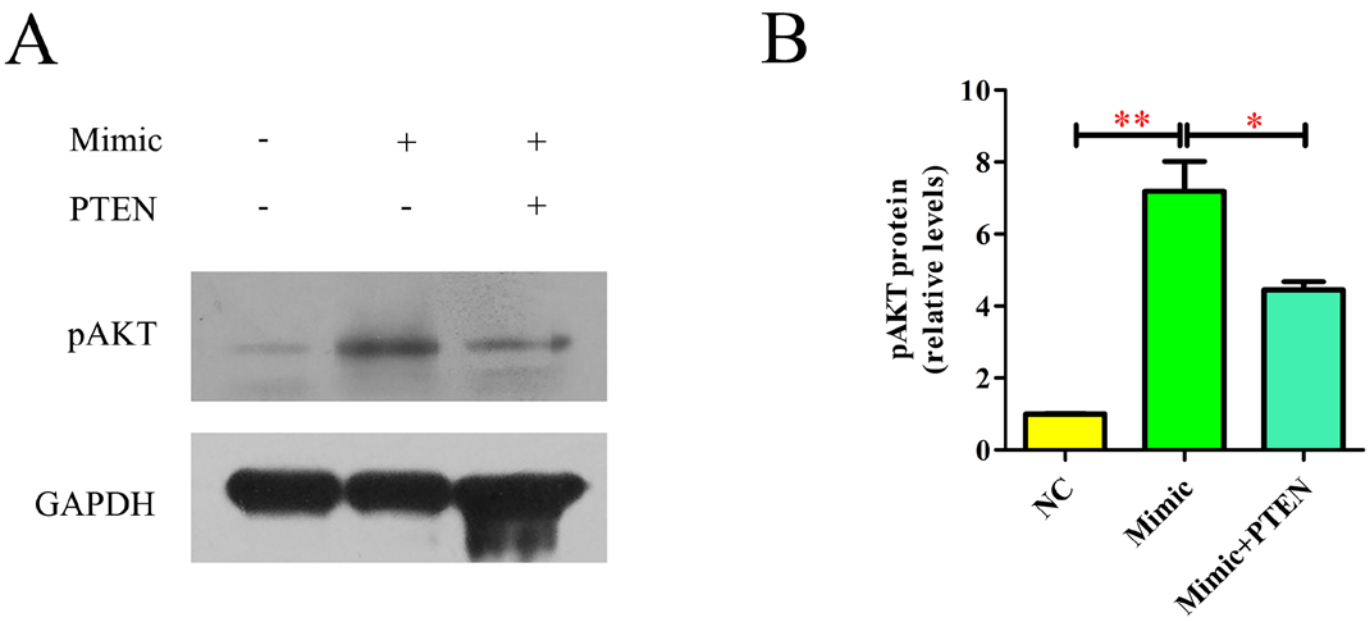
Supplementary figure 3



Supplementary figure 4



Supplementary figure 5



Supplementary legends

Supplement Figure1

(**A**) miR-494-3p expression levels in various human HCC cell lines and human hepatocyte THLE-3 cells. (**B**) miR-494-3p expression levels were significantly increased in SMMC-mimic cells compared with those in SMMC-NC cells. (**C**) miR-494-3p expression levels were significantly inhibited in SMMC-inhibitor cells compared with those in SMMC -NC cells. (**D**) miR-494-3p expression levels were significantly increased in LM3-mimic cells compared with those in LM3-NC cells. (**E**) miR-494-3p expression levels were significantly inhibited in LM3-inhibitor cells compared with those in LM3-NC cells. (*p<0.05, **p<0.01, ***p<0.001)

Supplement Figure2

(**A-B**) Wound-healing (**C-D**) Transwell migration and Matrigel invasion assays in each HCC cells. Cells were counted in 3 randomized fields at a magnification of 100×. The error bar represents the mean±SD of triplicate assays (*p<0.05, ** p<0.01, and *** p<0.001).

Supplement Figure3

(A-D) The growth curves of the SMMC-Mimic, LM3-Mimic, SMMC-Inhibitor, LM3-Inhibitor and their controls, respectively, in CCK8 assay. **(E-H)** Caspase-3, Caspase-7, and Caspase-9 mRNA levels were compared by RT-PCR, (*p<0.05, **p<0.01, ***p<0.001)

Supplement Figure4

(**A**) PTEN mRNA expression in response to miR-494-3p Inhibitor was determined by qRT-PCR in SMMC7721 and HCCLM3. (**B**) PTEN mRNA expression in response to miR-494-3p inhibitor was determined by qRT-PCR in SMMC7721 and HCCLM3 , (*p<0.05, **p<0.01, ***p<0.001)

Supplement Figure5

(**A**) Western blot of pAKT expression (representative of three experiments) 48 h after transfection with GFP-PTEN or empty vector. (**B**) Densitometric quantification. GAPDH was used as a loading control in. Values represent mean ± SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001