

Supplementary Material

Exosomal miR-451a Functions as a Tumor Suppressor in Hepatocellular Carcinoma by Targeting LPIN1

Shaorong Zhao^a Jianjun Li^a Guomin Zhang^a Qiong Wang^a Chao Wu^a
Quansheng Zhang^b Hang Wang^a Peiqing Sun^c Rong Xiang^a
Shuang Yang^a

^aTianjin Key Laboratory of Tumor Microenvironment and Neurovascular Regulation, Medical College of Nankai University, Tianjin, China, ^bTianjin Key Laboratory of Organ Transplantation, Tianjin First Center Hospital, Tianjin, China, ^cDepartment of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC, USA

Supplementary materials

Quantitative PCR primers	
miR-451a	RT: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACTGGAT ACGACAACTCA-3'
miR-451a	Forward: 5'-CGAAACCGTTACCATTAC-3'
	Reversed: 5'-GTGCAGGGTCCGAGGT-3'
U6	RT: 5'-AAAATATGGAACGCTTCACGAATTTG-3'
U6	Forward: 5'-CTCGCTTCGGCAGCATATACT-3'
	Reversed: 5'-ACGCTTCACGAATTTGCGTGTC-3'
LPIN1	Forward: 5'-CCAGCCCAATGGAAACCTCC-3'
	Reversed: 5'-AGGTGCATAGGGATAACTTCCTG-3'
Construction of full-length LPIN1 expression vector	
LPIN1	Forward: 5'- TCCCCCGGGGGAGCCACCATGAATTACGTGGGGCAGTTAG-3'
	Reversed: 5'-CGACGCGTCGTTACGCTGAGGCAGAATGAAT-3'
Construction of shRNA vectors	
sh185	5'- AAAAGGAATCTGTGGATTTGCATTTGGATCCAAATGCAAATCCACAGATTCC-3'
sh1031	5'- AAAAGCGAATCTTCAGACACTTTATGGATCCATAAAGTGTCTGAAGATTCGC-3'

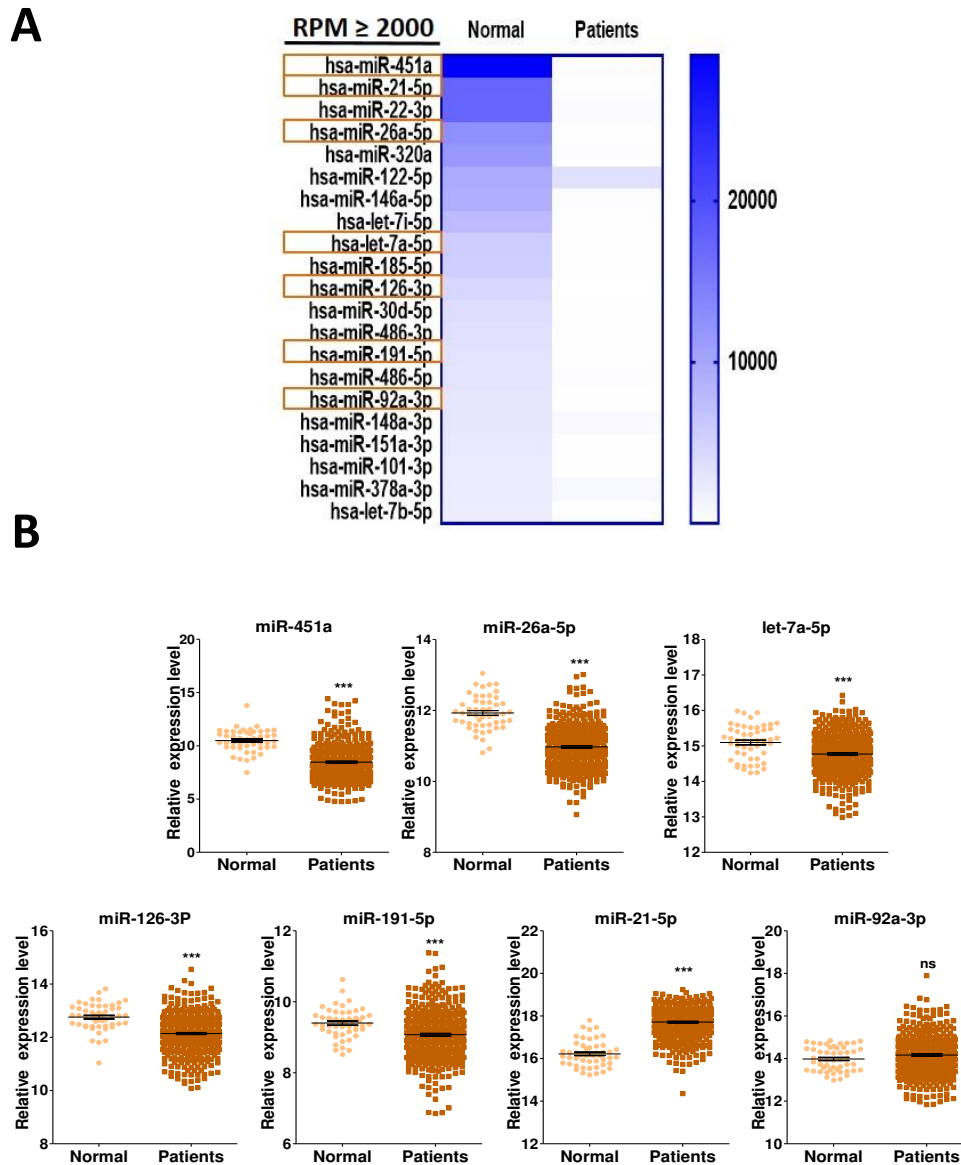


Figure S1 miRNome analysis according to the number of reads per million (RPM) and TCGA database. A, There were 21 downregulated miRNAs with an RPM value greater than 2000 in normal subjects. B, Five miRNAs (miR-451a, miR-26-5p, let-7a-5p, miR-126-3p, and miR-191-5p) were significantly downregulated in hepatocellular carcinoma (HCC) patients in the TCGA database. *** $P < 0.001$.

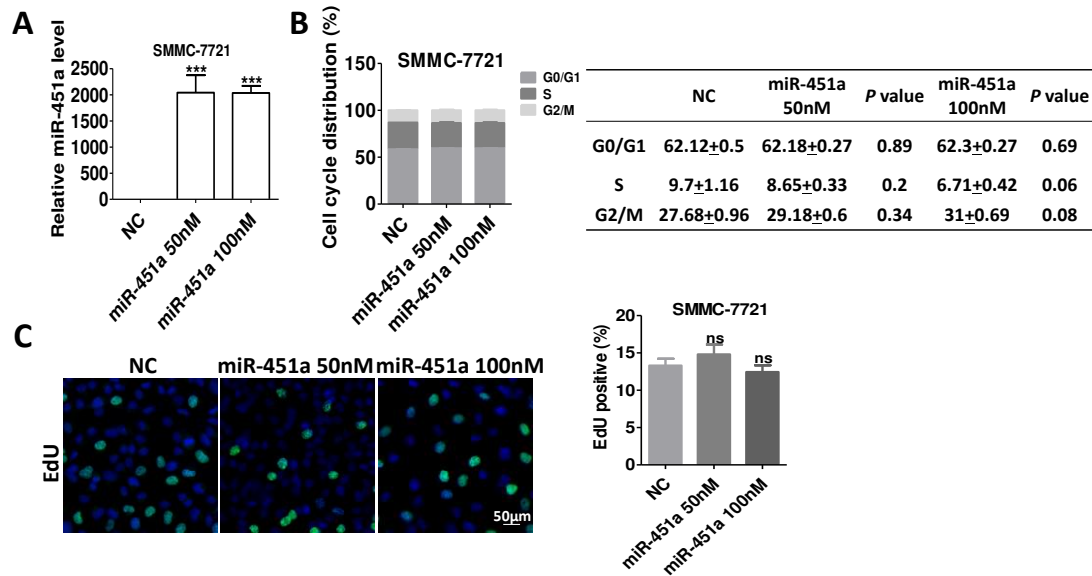


Figure S2 miR-451a does not affect hepatocellular carcinoma (HCC) cell cycle distribution. A, Different concentrations of miR-451a mimics were transfected into SMMC-7721 cells for 48 h. The expression of miR-451a was examined by quantitative PCR. Data were normalized to the level of U6. B and C, Different concentrations of miR-451a mimics were transfected into SMMC-7721 cells. Cell cycle distribution was measured by propidium iodide (PI) (B) and EdU (C) staining assays. NC, negative control; *** $P < 0.001$

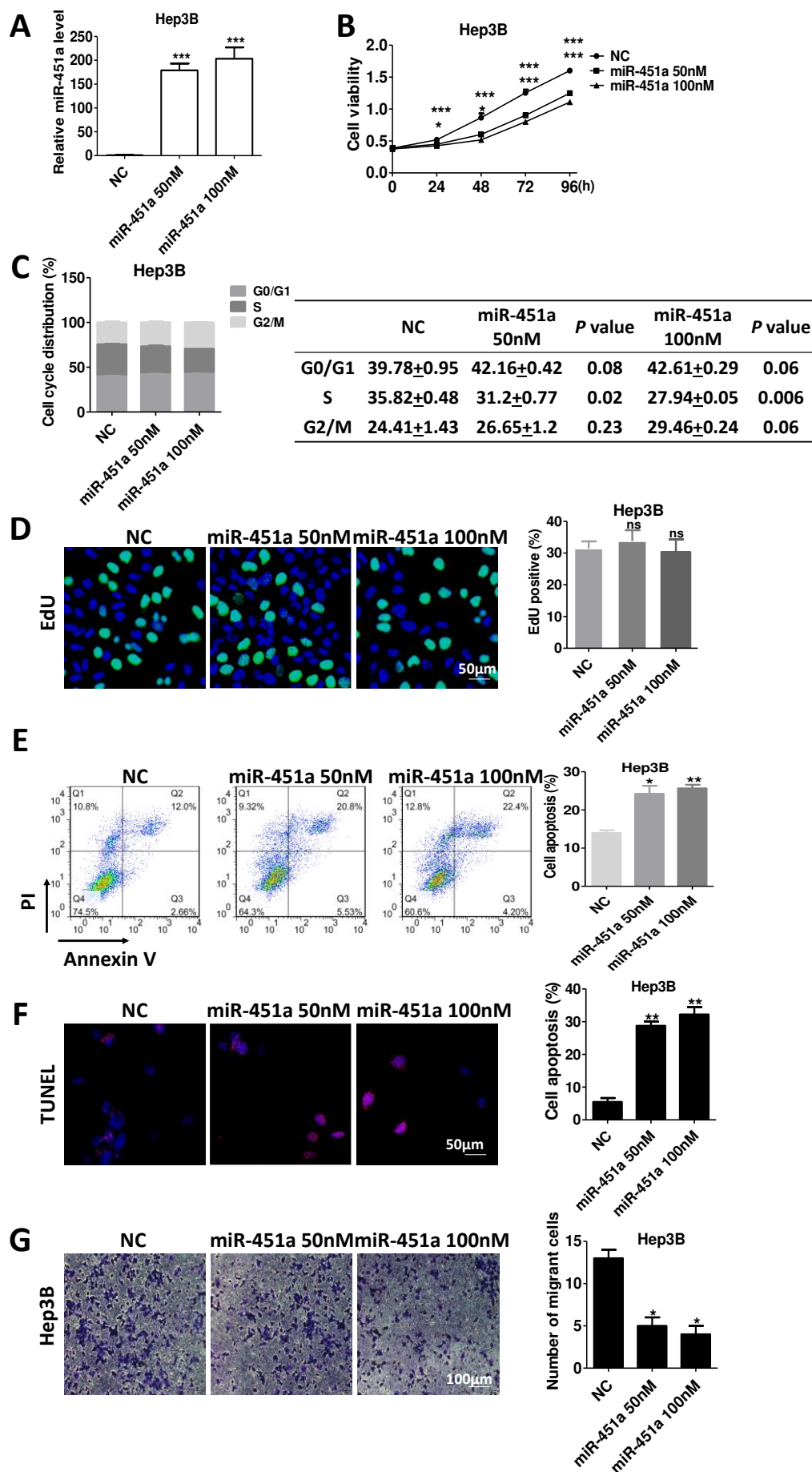


Figure S3 miR-451a regulates hepatocellular carcinoma (HCC) cell apoptosis and migration. A–G, Different concentrations of miR-451a mimics were transfected into Hep3B cells. The expression of miR-451a was examined by quantitative PCR (A). Cell viability was measured by CCK8 assays (B). Cell cycle distribution was measured by propidium iodide (PI) (C) and EdU (D) staining assays. Cell apoptosis was measured by Annexin V (E) and TUNEL (F) assays. Cell migration was determined by transwell assays (G). NC, negative control; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

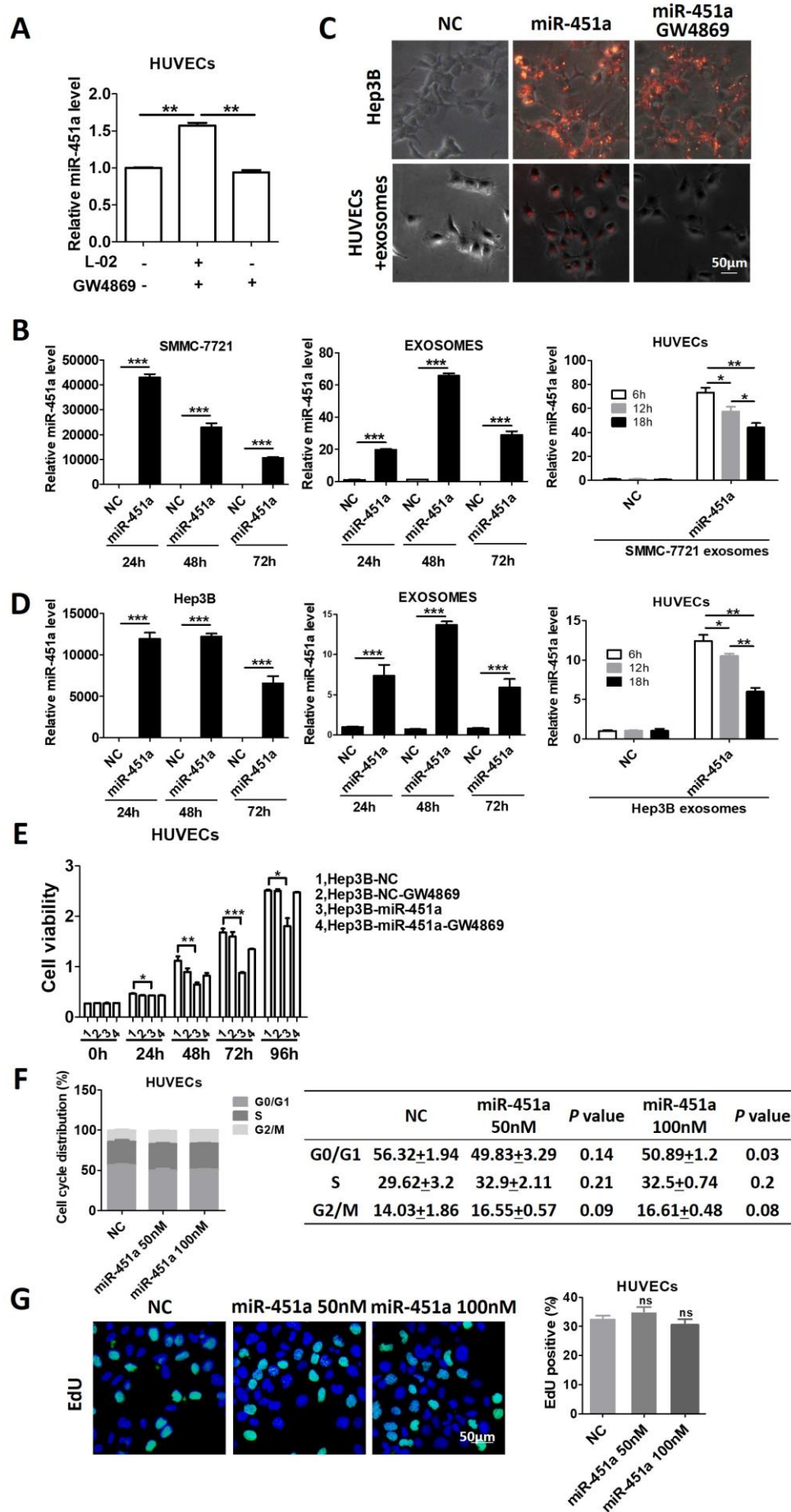


Figure S4 Exosomal miR-451a was delivered from hepatocellular carcinoma (HCC) cell lines to human umbilical vein endothelial cells (HUVECs). A, The expression of miR-451a in HUVECs was examined by quantitative PCR. HUVECs cocultured with exosomes from L-02 cell lines. GW4869 is an inhibitor of exosome secretion. B, Cellular and exosomal RNA were extracted at the indicated time points and analyzed for miR-451a levels. Left: SMMC-7721 cells transfected with 50 nM miR-451a mimics. Middle: SMMC-7721-derived supernatant exosomes. Right: HUVECs co-cultured with SMMC-7721-derived exosomes (48 h). C, Exosomal miR-451a was delivered from Hep3B cells to HUVECs. Top: Hep3B cells transfected with miR-451a-Cy3 mimics, and cultured in the presence or absence of exosome secretion inhibitor GW4869. Bottom: HUVECs incubated with Hep3B-derived exosomes. D, Cellular and exosomal RNA were extracted at the indicated time points and analyzed for miR-451a levels using U6 as an internal control. Left: Hep3B cells transfected with 50 nM of miR-451a mimics. Middle: Hep3B-derived supernatant exosomes. Right: HUVECs co-cultured with Hep3B-derived exosomes (48 h). E, Exosomes were derived from Hep3B cells transfected with miR-451a mimics in the presence or absence of GW4869, and were co-cultured with HUVECs. Cell viability was examined by CCK8 assays. F and G, Different concentrations of miR-451a mimics were transfected into HUVECs. Cell cycle distribution was measured by propidium iodide (PI) (F) and EdU (G) staining assays. NC, negative control; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

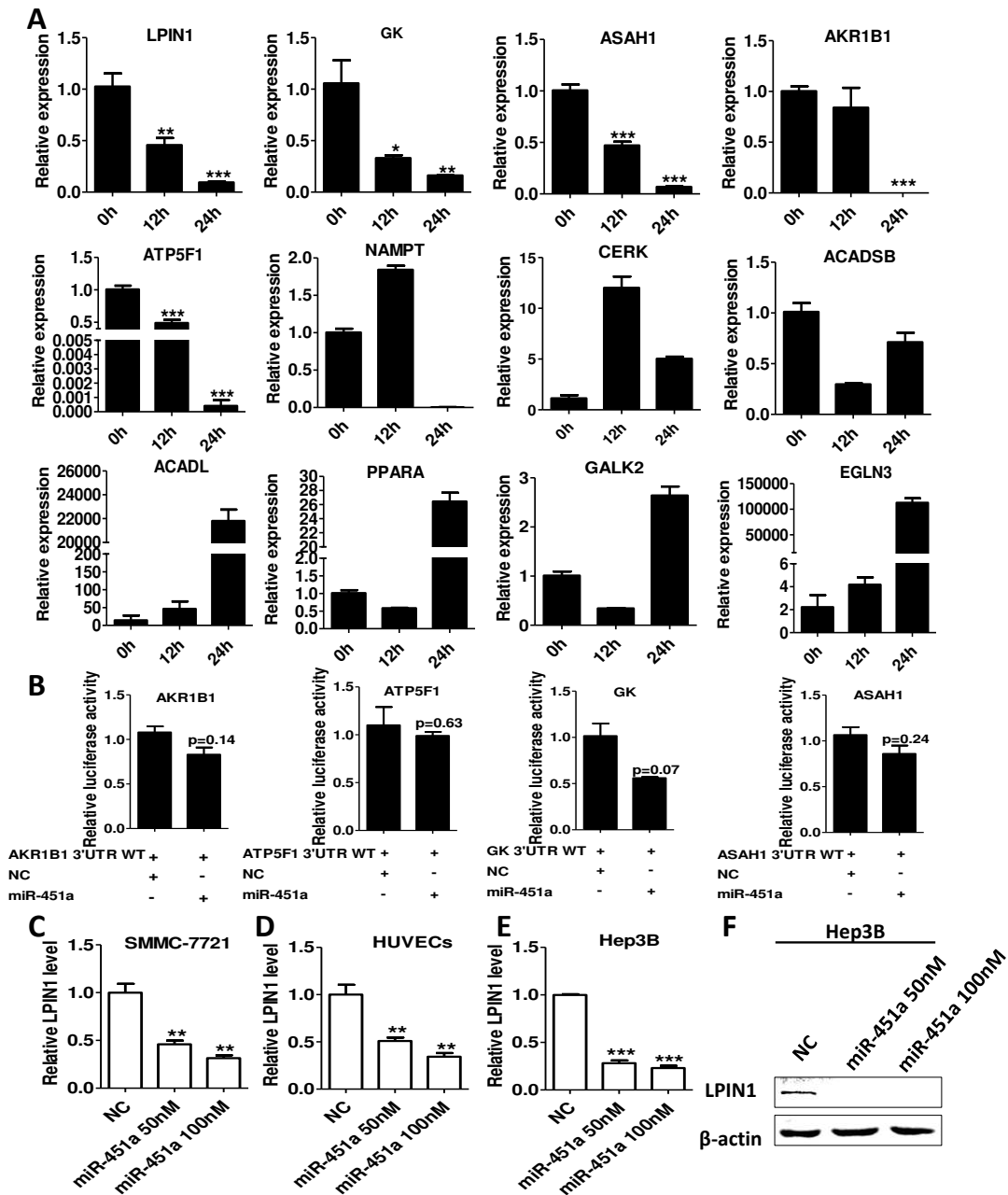


Figure S5 LPIN1 is a target of miR-451a. A, miR-451a mimics were transfected into SMMC-7721 cells. The expression of 12 candidate targets was measured by quantitative PCR at the indicated time points. B, 293T cells were co-transfected with miR-451a mimics and different wild-type promoter luciferase reporter constructs of *AKR1B1*, *ATP5F1*, *GK* and *ASAH1*. Luciferase activities were determined 36 h after transfection. Luciferase values were normalized to *Renilla* activities. C–F, Different concentrations of miR-451a mimics were transfected into SMMC-7721 cells (C), human umbilical vein endothelial cells (HUVECs) (D) and Hep3B cells (E). The

expression of LPIN1 was detected by quantitative PCR. F, Different concentrations of miR-451a mimics were transfected into Hep3B cells. The expression of LPIN1 was detected by western blotting. NC, negative control; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

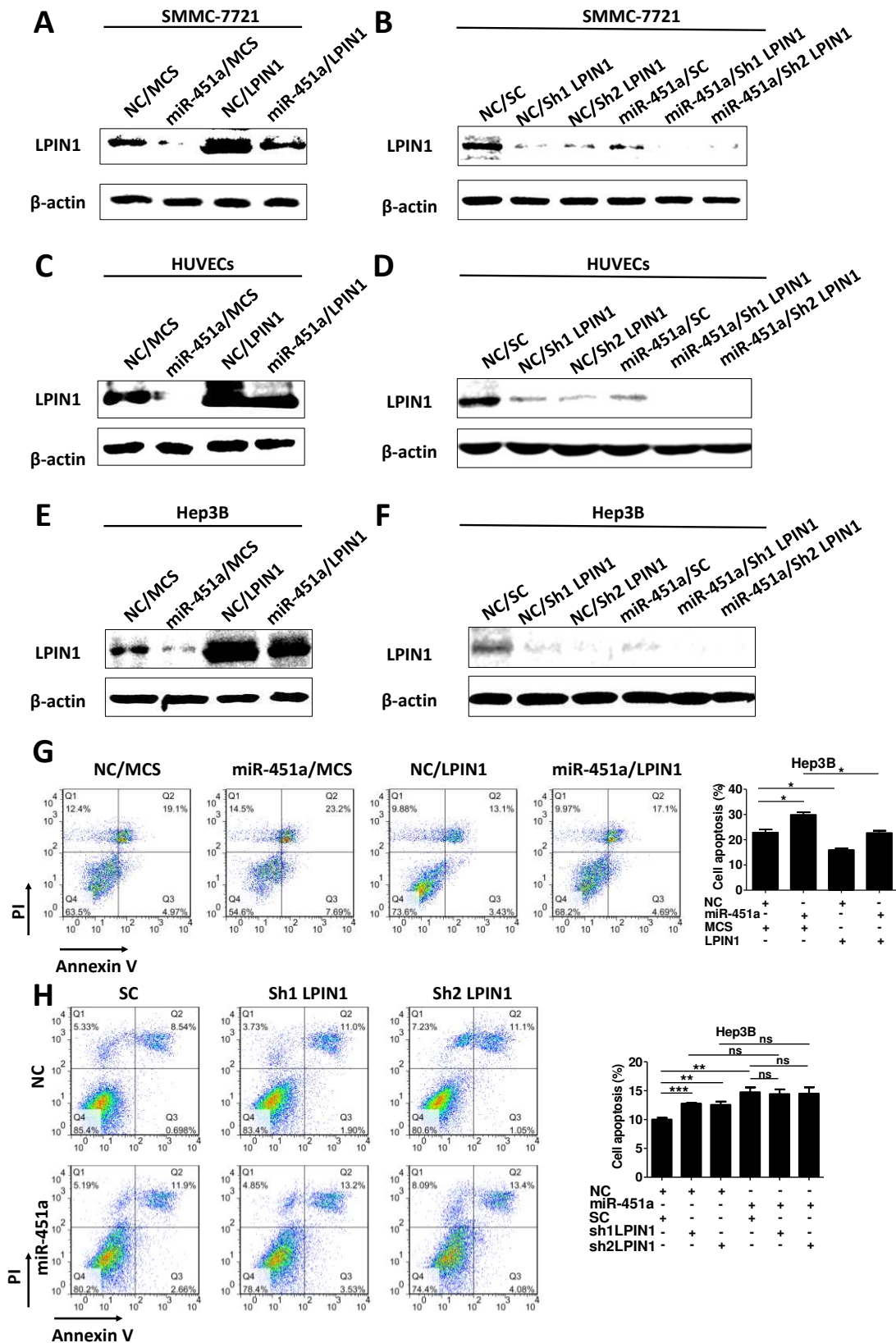


Figure S6 LPIN1 is a critical target during miR-451a-induced hepatocellular carcinoma (HCC) cell apoptosis. A, C, and E, SMMC-7721 cells (A), human umbilical vein

endothelial cells (HUVECs) (C) and Hep3B cells (E) were transfected with an LPIN1 expression plasmid in the presence or absence of miR-451a mimics. The expression of LPIN1 was determined by western blotting. B, D, and F, SMMC-7721 cells (B), HUVECs (D), and Hep3B cells (F) were transfected with specific LPIN1-targeting shRNAs in the presence or absence of miR-451a mimics. The expression of LPIN1 was determined by western blotting. G, Hep3B cells were transfected with an LPIN1 expression plasmid in the presence or absence of miR-451a mimics. Apoptosis was determined by Annexin V assays. H, Hep3B were transfected with specific LPIN1-targeting shRNAs in the presence or absence of miR-451a mimics. Apoptosis was determined by Annexin V assays. PI, propidium iodide; NC, negative control; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table S1 All relative miR-451a-associated gene sets based on GSEA.

NAME	NES	FDR q-val
GO_SMALL_MOLECULE_CATABOLIC_PROCESS	2.301848	0.003673
GO_ORGANONITROGEN_COMPOUND_CATABOLIC_PROCESS	2.298454	0.001837
GO_MONOCARBOXYLIC_ACID_METABOLIC_PROCESS	2.248486	0.002851
GO_LIPID_CATABOLIC_PROCESS	2.215521	0.00436
GO_ALPHA_AMINO_ACID_METABOLIC_PROCESS	2.215225	0.003884
GO_ORGANIC_ACID_CATABOLIC_PROCESS	2.215046	0.003398
GO_CELLULAR_LIPID_CATABOLIC_PROCESS	2.192454	0.003611
GO_SULFUR_AMINO_ACID_METABOLIC_PROCESS	2.177759	0.004838
GO_LIPID_HOMEOSTASIS	2.169536	0.005424
GO_CELLULAR_AMINO_ACID_CATABOLIC_PROCESS	2.168884	0.005062
GO_ORGANIC_HYDROXY_COMPOUND_CATABOLIC_PROCESS	2.164413	0.005168
GO_REGULATION_OF_FATTY_ACID_METABOLIC_PROCESS	2.161405	0.004998
GO_FATTY_ACID_METABOLIC_PROCESS	2.146384	0.006609
GO_ORGANIC_HYDROXY_COMPOUND_METABOLIC_PROCESS	2.142804	0.006496
GO_BLOOD_MICROPARTICLE	2.205424	0.002912
GO_OXYGEN_BINDING	2.204853	0.002647
GO_VESICLE_LUMEN	2.250178	0.003801
GO_IRON_ION_BINDING	2.224138	0.004729
GO_SECRETORY_GRANULE_LUMEN	2.206314	0.003235
GO_COFACTOR_BINDING	2.168502	0.00481

Table S2 Targets of miR-451a as predicted by Targetscan.

Target gene	3P-seq tags + 5	Cumulative weighted context++ score	Total context++ score	Metabolic process
PSMB8	18	-0.55	-1.09	
CDKN2B	373	-0.85	-1.05	
CMTM6	169	-1.00	-1.03	
MTRNR2L13	5	-0.97	-0.97	
OSR1	15	-0.93	-0.93	
ATF2	123	-0.6	-0.91	
OLSP16	444	-0.3	-0.3	
CXCL16	385	-0.78	-0.78	
Ckorf21	5	-0.78	-0.78	
TBC1D98	5042	-0.75	-0.75	
BATF	5	-0.73	-0.73	
ATP5F1	575	-0.42	-0.69	ATP synthesis
GRSF1	4414	-0.38	-0.69	
RFC25	97	-0.67	-0.68	
NEDD9	1164	-0.63	-0.63	
EVL	291	-0.6	-0.6	
HELLS	329	-0.60	-0.6	
MIF	1623	-0.57	-0.6	
FAM9C	5	-0.59	-0.59	
CBLN4	5	-0.59	-0.59	
SEC23IP	765	-0.57	-0.57	
AP000350.10	1627	-0.52	-0.55	
TMEM170B	489	-0.47	-0.54	
WIF24	84	-0.51	-0.51	
POU3F2	274	-0.51	-0.51	
C11orf91	998	-0.44	-0.51	
CY8561D1	105	-0.49	-0.49	
CTDSP1	1299	-0.49	-0.49	
ASAH	5	-0.48	-0.48	Sphingolipid metabolism
FAM91A1	731	-0.48	-0.48	
MSC	27	-0.48	-0.48	
TSC1	838	-0.45	-0.47	
WDFY2	85	-0.39	-0.47	
CTNBP1	493	-0.33	-0.47	
C11orf90	230	-0.37	-0.46	
CPHE3	756	-0.43	-0.44	
ACP4	19	-0.33	-0.44	
CRIP2	3446	-0.43	-0.43	
MCCE2	1590	-0.43	-0.43	
KIAA1217	320	-0.41	-0.43	
NKSA2	121	-0.35	-0.42	
FNIA	7	-0.41	-0.41	
CERK	123	-0.39	-0.41	Sphingolipid metabolism
SSU72	262	-0.36	-0.41	
PSSCR2	5	-0.40	-0.40	
S1PR2	10	-0.40	-0.40	
GDH1	21	-0.40	-0.40	
C12orf5	520	-0.39	-0.39	
PTTG3IP	1170	-0.32	-0.39	
SAMD4B	46	-0.39	-0.39	
NHSL1	393	-0.38	-0.38	
GALK2	626	-0.38	-0.38	Galactose metabolism
NAMPT	375	-0.35	-0.38	Nicotinamide mononucleotide synthesis
PPARA	82	-0.32	-0.38	Cholesterol and lipid metabolism
AEBP2	1228	-0.37	-0.37	
ACAD5B	239	-0.37	-0.37	Fatty acid metabolism
UNC93A	5	-0.37	-0.37	
GK	38	-0.37	-0.37	
KCTD10	147	-0.37	-0.37	
VAPA	2926	-0.36	-0.36	
TBCL	9	-0.33	-0.36	
UPY3A	44	-0.35	-0.35	
MECK2	5	-0.35	-0.35	
ZNTF66	2	-0.35	-0.35	
C22orf23	6	-0.35	-0.35	
EGLN3	71	-0.35	-0.35	Oxidoreductase activity
BAK1	15	-0.35	-0.35	
SGK1	269	-0.34	-0.35	
KMAU2	5234	-0.34	-0.35	
ADAM10	385	-0.32	-0.35	
LETM2	12	-0.34	-0.34	
CACNA1B	5	-0.34	-0.34	
DKFZP779J2370	20	-0.34	-0.34	
UBCL3	1318	-0.34	-0.34	
CDKN2D	186	-0.34	-0.34	
LPIN1	357	-0.33	-0.34	Lipid metabolism
NFATC1	104	-0.32	-0.34	
AKR1B1	41	-0.32	-0.34	Glucose metabolism
RAD18	211	-0.31	-0.34	
ACADL	5	-0.33	-0.33	Fatty acid metabolism
IL6R	13	-0.3	-0.33	
TMEM143	160	-0.32	-0.32	
PNMAGC	5	-0.32	-0.32	
CYR1	5	-0.32	-0.32	
MYOG	5	-0.32	-0.32	
MPPED2	42	-0.32	-0.32	
C4orf46	128	-0.32	-0.32	
PNMAGA	5	-0.32	-0.32	
TNFRSF25	8	-0.32	-0.32	
CAND2	92	-0.32	-0.32	
APBA1	12	-0.31	-0.31	
EREG	5	-0.31	-0.31	
NPTN	2918	-0.31	-0.31	
AC137932.1	5	-0.31	-0.31	
PRICKLE2	20	-0.31	-0.31	
SLC7A6OS	751	-0.31	-0.31	
CHLD2	4044	-0.31	-0.31	
AKTIP	46	-0.30	-0.30	
TRAPPC3L	5	-0.30	-0.30	
THAP4	744	-0.30	-0.30	
TSC1	1	-0.47	0.2	
BTBD9	1	-0.40	0.2	
S1PR2	1	-0.40	0.21	
C11orf90	1	-0.46	0.21	
AEBP2	1	-0.37	0.21	
DKFZP779J2370	1	-0.34	0.21	
CDKN2D	1	-0.34	0.21	
TBCL	1	-0.36	0.21	
PRICKLE2	1	-0.31	0.21	
SAMD4B	1	-0.39	0.21	
YWHAZ	1	-0.23	0.21	
PNMAG	1	-0.26	0.21	
CAB39	1	-0.31	0.21	
TRIM66	1	-0.19	0.21	
PRR12	1	-0.28	0.21	
SZRD1	1	-0.30	0.21	
PSMD11	1	-0.31	0.21	
OSR1	1	-0.93	< 0.10	
ATF2	1	-0.91	< 0.10	
PSMB8	1	-1.09	< 0.10	
GK	1	-0.37	< 0.10	Glycerol metabolism
VAPA	1	-0.36	< 0.10	
MEF2D	1	-0.26	< 0.10	
FBXO33	1	-0.19	< 0.10	
GATA2B	1	-0.10	< 0.10	
VPS18	1	-0.20	< 0.10	
MIF	1	-0.60	ORF	
DBNL	1	-0.07	ORF	