

Supplementary Tables

Supplementary Table 1. Characteristics of HCC Patients Used for Survival Analysis

P. No.	Age	Sex	HBV						HCV Ab	Liver Cirrhosis	HCC			
			HB sAg	HB sAb	HB eAg	HB eAb	HB cAb	Source			TNM Staging	Histological Grade	AFP	
1	36	M	—	+	—	+	—	—	+	primary HCC	T1 N0 M0	II	++	
2	35	M	+	—	—	+	+	—	+	primary HCC	T1 N0 M0	II	++++	
3	33	F	+	—	—	+	+	—	—	primary HCC	T1 N0 M0	II	++++	
4	32	M	+	—	—	+	+	—	+	primary HCC	T1 N0 M0	III	+	
5	51	M	+	—	—	+	+	—	+	primary HCC	T1 N0 M0	III	++	
6	67	M	+	—	—	+	+	—	—	primary HCC	T1 N0 M0	I	—	
7	43	M	+	—	—	+	+	—	+	primary HCC	T2 N0 M0	II	+++	
8	64	M	+	—	—	+	+	—	+	primary HCC	T1 N0 M0	II	—	
9	57	M	+	—	—	+	+	—	+	primary HCC	T1 N0 M0	II	—	
10	60	M	—	—	—	—	—	—	—	primary HCC	T1 N0 M0	II	—	
11	55	F	+	—	—	+	+	—	—	primary HCC	T3 N1 M0	II	+	
12	36	M	+	—	—	+	+	—	—	primary HCC	T3 N2 M0	II	—	
13	37	M	+	—	+	—	+	—	+	primary HCC	T1 N0 M0	III	++++	
14	36	M	+	—	—	+	+	—	+	primary HCC	T1 N0 M0	II	—	
15	53	M	+	—	—	+	+	—	+	primary HCC	T1 N0 M0	I	—	
16	71	M	+	—	+	—	+	—	+	primary HCC	T1 N0 M0	I	—	
17	30	M	+	—	+	—	+	—	+	primary HCC	T1 N0 M0	II	—	

18	71	M	+	-	-	+	+	-	+	primary HCC	T3 N2 M0	I	-
19	42	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	III	+++
20	58	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	-
21	49	M	+	-	-	-	-	-	+	primary HCC	T3 N1 M0	III	+
22	40	M	+	-	-	+	+	-	-	primary HCC	T3 N1 M0	III	+++
23	52	F	+	-	+	-	+	-	+	primary HCC	T3 N3 M0	II	++++
24	59	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	II	-
25	55	M	-	-	-	-	-	+	+	primary HCC	T3 N2 M0	IV	+
26	27	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	-
27	34	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	-
28	51	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	III	++++
29	54	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	II	++++
30	52	F	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++++
31	42	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	-
32	57	M	+	-	-	+	+	-	+	primary HCC	T3 N2 M0	III	+++
33	40	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	III	-
34	31	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++++
35	52	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++++
36	70	F	-	-	-	-	-	-	+	primary HCC	T1 N0 M0	III	++
37	32	F	+	-	-	+	+	-	-	primary HCC	T3 N1 M0	III	-
38	45	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++
39	51	M	-	-	-	-	-	-	+	primary HCC	T3 N1 M0	III	++++

40	42	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	++++
41	71	F	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	-
42	55	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	++++
43	48	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++++
44	50	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	III	+
45	41	M	+	-	-	+	+	-	+	primary HCC	T3 N2 M0	III	++++
46	65	M	+	+	-	+	-	-	+	primary HCC	T1 N0 M0	III	++++
47	52	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	-
48	35	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	I	+
49	55	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++
50	20	M	+	-	-	+	+	-	-	primary HCC	T3 N3 M0	III	++++
51	48	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++
52	40	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	II	++++
53	58	M	-	-	-	+	+	-	+	primary HCC	T3 N3 M0	II	++
54	59	M	+	-	+	+	-	-	+	primary HCC	T1 N0 M0	II	++++
55	31	F	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	II	-
56	31	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++
57	70	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	-
58	45	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	II	+
59	60	F	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	+++
60	60	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	II	+
61	54	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	II	+

62	60	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++++
63	45	M	+	-	-	+	+	-	+	primary HCC	T3 N2 M0	II	-
64	52	M	-	+	-	-	+	-	+	primary HCC	T3 N1 M0	III	+++
65	46	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	I	++
66	53	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	II	++++
67	60	M	-	+	-	-	+	-	+	primary HCC	T4 N0 M0	II	-
68	58	F	+	-	-	+	+	-	-	primary HCC	T3 N1 M0	II	-
69	40	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	III	-
70	40	M	+	-	-	+	+	-	-	primary HCC	T4 N0 M0	II	++++
71	56	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	II	++++
72	46	M	+	-	-	+	+	-	-	primary HCC	T2 N0 M0	III	++++
73	46	M	+	-	-	+	+	-	-	primary HCC	T2 N0 M0	III	++++
74	35	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	-
75	51	M	+	-	+	-	+	-	-	primary HCC	T2 N0 M0	III	-
76	44	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++++
77	57	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	III	+
78	39	M	+	-	+	-	+	-	+	primary HCC	T2 N0 M0	III	++++
79	68	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++
80	57	M	+	-	-	+	+	-	-	primary HCC	T2 N0 M0	III	-
81	40	M	+	-	+	-	+	-	-	primary HCC	T2 N0 M0	III	+
82	50	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++
83	37	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	-

84	63	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++
85	39	M	+	-	-	+	+	-	+	primary HCC	T3 N0 M0	III	-
86	46	M	+	-	-	+	+	-	-	primary HCC	T2 N0 M0	III	-
87	44	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	-
88	62	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	II	++
89	35	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	-
90	53	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	-
91	57	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	-
92	37	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	+++
93	53	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	+
94	51	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	+
95	72	M	-	+	-	-	+	-	-	primary HCC	T1 N0 M0	III	-
96	63	F	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	-
97	52	M	-	-	-	+	+	-	-	primary HCC	T3 N0 M0	III	-
98	43	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++
99	44	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++
100	36	M	+	-	-	+	+	-	-	primary HCC	T2 N0 M0	III	++++
101	49	M	+	-	-	+	+	-	+	primary HCC	T3 N0 M0	III	-
102	40	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	III	++++
103	32	M	+	-	-	+	+	-	-	primary HCC	T2 N0 M0	III	++++
104	58	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++
105	57	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++++

106	39	M	+	-	+	-	+	-	-	primary HCC	T3 N0 M0	III	++++
107	59	M	+	-	-	+	+	-	+	primary HCC	T2 N0 M0	III	++
108	55	M	+	-	-	-	+	-	-	primary HCC	T2 N0 M0	III	+
109	50	M	+	-	+	-	+	-	-	primary HCC	T3 N1 M0	III	++++
110	39	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	I	++
111	58	M	-	-	-	-	-	-	-	primary HCC	T3 N1 M0	III	-
112	30	M	+	-	+	-	+	-	-	primary HCC	T1 N0 M0	II	-
113	61	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	I	-
114	54	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	+
115	28	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	++
116	42	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	II	-
117	49	M	+	-	+	-	+	-	+	primary HCC	T2 N0 M0	III	+
118	45	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	++++
119	47	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	I	++++
120	48	M	+	-	+	-	+	-	+	primary HCC	T1 N0 M0	III	-
121	35	M	-	-	-	-	-	-	+	primary HCC	T1 N0 M0	II	++++
122	63	F	-	-	-	-	-	-	+	primary HCC	T3 N1 M0	III	-
123	36	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	II	++++
124	35	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	II	-
125	37	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	++
126	44	F	+	-	-	+	+	-	+	primary HCC	T3 N3 M0	III	++++
127	67	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	-

128	46	M	+	-	-	+	+	-	+	primary HCC	T3 N3 M0	II	++++
129	46	M	+	-	+	-	+	-	-	primary HCC	T2 N0 M0	III	++
130	47	M	+	-	-	+	+	-	+	primary HCC	T3 N1 M0	III	++++
131	41	M	+	-	+	-	+	-	+	primary HCC	T2 N0 M0	II	-
132	42	M	+	-	+	-	+	-	-	primary HCC	T3 N0 M0	I	-
133	38	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	++++
134	55	M	+	-	+	-	+	-	-	primary HCC	T1 N0 M0	II	++
135	68	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	-
136	36	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	II	-
137	78	F	-	-	-	-	-	-	+	primary HCC	T1 N0 M0	II	-
138	48	M	+	-	-	+	+	-	+	primary HCC	T1 N0 M0	III	+
139	43	F	-	-	-	-	-	-	-	primary HCC	T1 N0 M0	II	-
140	50	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	II	-
141	58	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	II	-
142	37	M	+	-	-	+	+	-	-	primary HCC	T1 N0 M0	II	-

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; P, patient; HBsAg, hepatitis B surface antigen; HBsAb, antibody against HBsAg; HBeAg, hepatitis B e antigen; HBeAb, antibody against HBeAg; HBcAb, antibody against hepatitis B core antigen; TNM, tumor-node-metastasis; AFP, alpha-fetoprotein; M, male; F, female.

Supplementary Table 2. Sequences of RNA Oligonucleotides and Primers Used

Name	Primer Sequence (5'-3')
miRNA Duplexes	
miR-99a sense strand	AACCCGUAGAUCCGAUCUUGUG
miR-99a antisense strand	CAAGAUCGGAUCUACGGGUUUU
NC sense strand	UUCUCCGAACGUGUCACGUTT
NC antisense strand	ACGUGACACGUUCGGAGAATT
Primers for 3'UTR Cloning	
mTOR 3'UTR F	CGACGCGTGGAGGCCAGATGTGCCCATC
mTOR 3'UTR R	GGGTTTAAACATGTTTAAAATTCTGATGTCATTTATTGG
IGF-1R 3'UTR F	CGACGCGTGGATCCTGGCACAGAGAAGAGTTAC
IGF-1R 3'UTR R	GGGTTTAAACGTGCCATGCACTGCTGGGCTCC
FGFR3 3'UTR F	CGACGCGTCCCAACAATGTGAGGGGTCCCTAGC
FGFR3 3'UTR R	AGCTTTGTTTAAACCGGAAAGTTCGTCGCTGGGTTAACAA
FZD5 3'UTR F	CGACGCGTCCTGTTTCCATCCATATACTTACTTCCCCCA
FZD5 3'UTR R	AGCTTTGTTTAAACCTCATCAAGCTGCTCTGGGTTTCCTTTTG
FZD8 3'UTR F	GGACTAGTCGAGGAGAGCCAAGTGCAGCGAAG
FZD8 3'UTR R	AGCTTTGTTTAAACCAGGGTTTTGAAACCTTTAGTGATACCATGCA
Primers for RT-PCR	
U6 F	GTGGACCGCACAAGCTCGCT
U6 R	TTGTTGAACGGCACTGTGTATAGCA
β -actin F	CTCGCTTCGGCAGCACA
β -actin R	AACGCTTCACGAATTTGCGT
pri-miR-99a F	AGTGTGACGTTGACATCCGT
pri-miR-99a R	GCAGCTCAGTAACAGTCCGC
C21orf34 F	TTTGCTTCAAACGACAACAAGAG
C21orf34 R	TTCGAGCATGGCCGGATTG

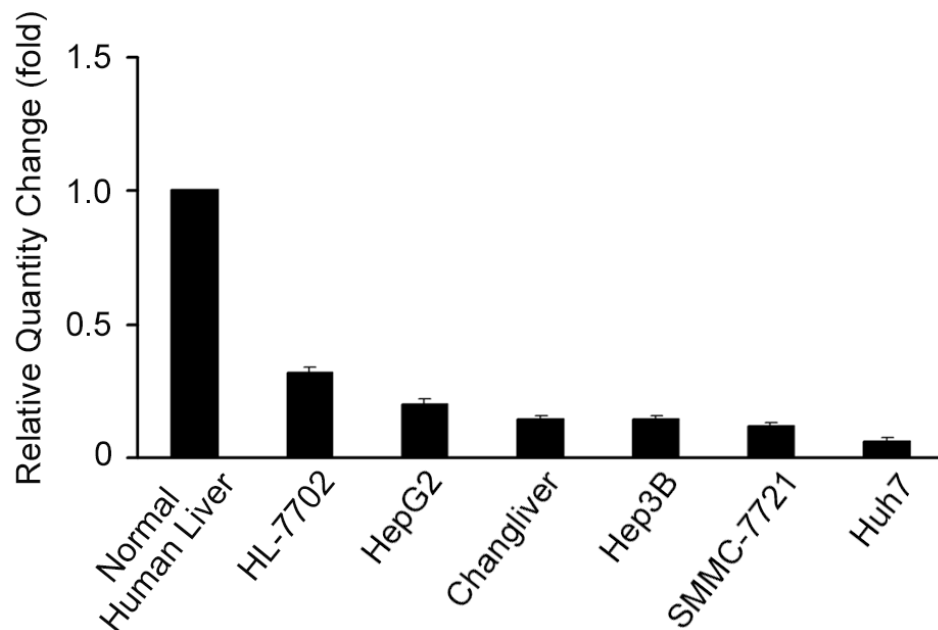
Abbreviations: NC, negative control; RT, reverse-transcription; F, forward primer; R, reverse primer.

Supplementary Table 3. Enrichment Analysis of Predicted miR-99a Targets in KEGG Cell Signaling Pathway Database.

Pathway	Count	<i>P</i> value
Pathways in cancer	9	0.015187
Metabolic pathways	9	0.913787
MAPK signaling pathway	7	0.042937
Chemokine signaling pathway	6	0.027574
Leukocyte transendothelial migration	5	0.014499
Jak-STAT signaling pathway	5	0.036139
Cytokine-cytokine receptor interaction	5	0.081776
Focal adhesion	5	0.093158
Long-term potentiation	4	0.011716
Colorectal cancer	4	0.017167
Progesterone-mediated oocyte maturation	4	0.040815
Cell adhesion molecules (CAMs)	4	0.073579
Wnt signaling pathway	4	0.104772

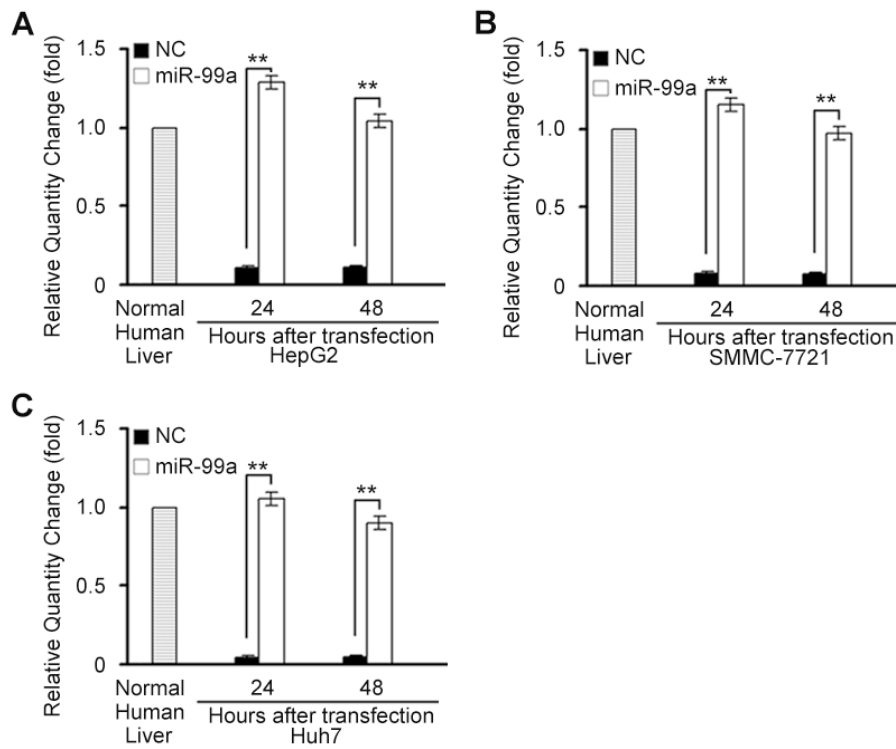
Only the top 5 enriched cell signaling pathways of predicted miR-99a targets in KEGG database were shown. A hypergeometric test was used to validate the significance with indicated *P* value.

Supplementary Figure 1



Supplementary Figure 1. Expression of miR-99a in human liver tissue and cell lines. The mature miR-99a expression was analyzed by real-time qRT-PCR in normal human liver tissue and the indicated human liver cell line (HL-7702) or HCC cell lines (HepG2, Changliver, Hep3B, SMMC-7721, Huh7). The normalized miR-99a expression in normal human liver tissue in each experiment was set as relative 1. Data are shown as mean \pm s.d. (n = 4) of one representative experiment. Similar results were obtained in at least three independent experiments.

Supplementary Figure 2



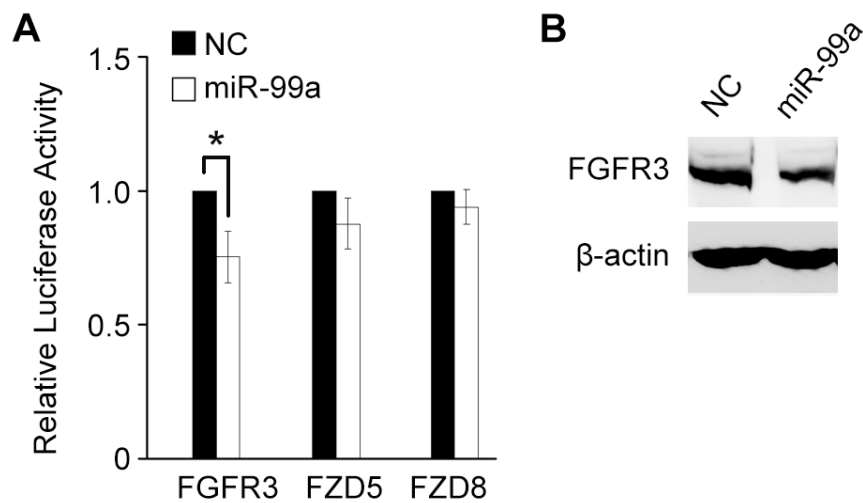
Supplementary Figure 2. Restoration efficiency of miR-99a in HCC cell lines. HepG2, SMMC-7721 and Huh7 cells were transfected with miR-99a or NC mimics. miR-99a expression was analyzed by real-time qRT-PCR 24 or 48 hours after transfection. The normalized miR-99a expression in normal human liver tissue in each experiment was set as relative 1. Data are shown as mean \pm s.d. ($n = 4$) of one representative experiment. Similar results were obtained in at least three independent experiments. **, $P < 0.01$.

Supplementary Figure 3

		294	300
mTOR 3'UTR	5'	CCAUAACUUUAGAAAUACGGGUU	3'
miR-99a	3'	GUGUUCUAGCCUAGAUGCCCAA	5'
Mutant 3'UTR	5'	CCAUAACUUUAGAAAUGCCCAU	3'
		5597	5603
IGF-1R 3'UTR	5'	UUAAGUCCAGUAGAUUACGGGUA	3'
miR-99a	3'	GUGUUCUAGCCUAGAUGCCCAA	5'
Mutant 3'UTR	5'	UUAAGUCCAGUAGAUUAGCCCAA	3'
		537	543
FGFR3 3'UTR	5'	CUCAGAGACUGAAAUACGGGUA	3'
miR-99a	3'	GUGUUCUAGCCUAGAUGCCCAA	5'
		3830	3836
FZD5 3'UTR	5'	UUUGGUGACUUUUGAUACGGGUU	3'
miR-99a	3'	GUGUUCUAGCCUAGAUGCCCAA	5'
		543	549
FZD8 3'UTR	5'	UACACCCCACGUAAAUACGGGUU	3'
miR-99a	3'	GUGUUCUAGCCUAGAUGCCCAA	5'

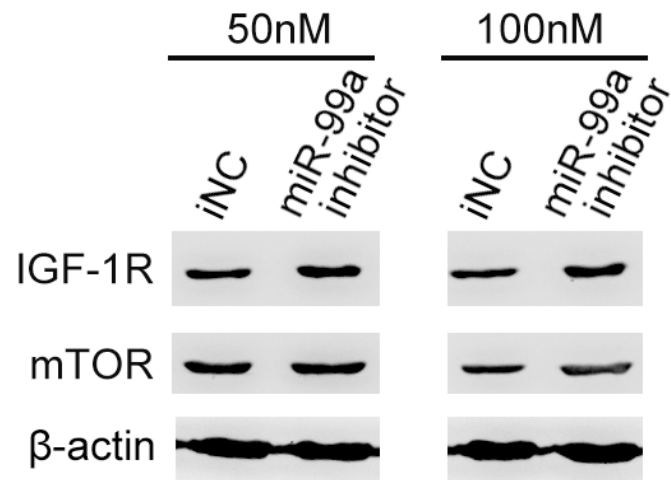
Supplementary Figure 3. TargetScan prediction of miR-99a pairing site and mutated sequence in the complementary site for the seed region.

Supplementary Figure 4



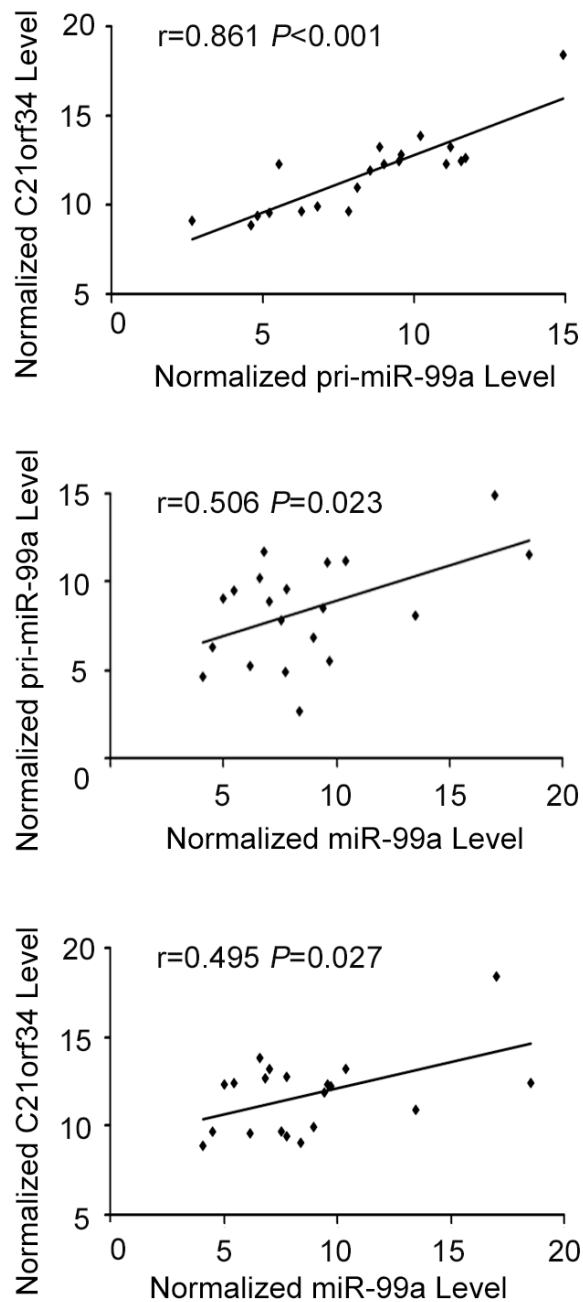
Supplementary Figure 4. Putative targets of miR-99a. (A) Analysis of luciferase activity. HepG2 cells were co-transfected with indicated RNA duplex, pRL-TK and pMIR-Report firefly luciferase reporter plasmid containing wild-type 3'-UTR of putative target gene. After 24 hours, firefly luciferase activity was measured and normalized by Renilla luciferase activity. The normalized luciferase activity of NC-transfectant in each experiment was set as relative luciferase activity 1. Data are shown as mean \pm s.d. (n = 4) of one representative experiment. Similar results were obtained in at least three independent experiments. *, P < 0.05. (B) Restoration of miR-99a reduces protein level of FGFR3. FGFR3 expression in HepG2 cells was analyzed by Western blotting 60 hours after transfection with miR-99a or NC mimics.

Supplementary Figure 5



Supplementary Figure 5. Effects of knocking down miR-99a in HL-7702 cells. HL-7702 cells were transfected with miR-99a inhibitor or inhibitor NC (iNC) at the indicated concentration. Expression of IGF-1R and mTOR were analyzed by Western blotting 60 hours after transfection.

Supplementary Figure 6



Supplementary Figure 6. Positive correlation between expression of mature miR-99a, primary miR-99a and C21orf34 in HCC and matching liver tissues. RNA levels of mature miR-99a, primary miR-99a (pri-miR-99a) and the host gene C21orf34 in HCC and matching liver tissues (n=20) were detected by qRT-PCR. The mature miR-99a level was normalized to U6 expression, while C21orf34 and pri-miR-99a were normalized to β -actin expression. Statistical analysis was performed using Pearson's correlation coefficient. Each data was expressed with a log₂ scale.