

**Supplementary Table 1. Univariate and Multivariate Analysis of Factors Correlated with Tumor-free Survival.**

	Variable	Cases number	HR (95% CI)	P value	
<b>Univariate analysis</b>	Age (year), >50: ≤50	71:81	0.919 (0.591-1.427)	0.705	
	Gender, male: female	123:29	0.515 (0.265-1)	0.05	
	AFP (μg/L), >20: ≤20	97:54	1.661 (1.023-2.697)	0.04*	
	Serum bilirubin (μmol/L), >17: ≤17	38:114	1.446 (0.89-2.349)	0.136	
	Serum albumin (g/L), >40: ≤40	114:38	1.321 (0.773-2.257)	0.309	
	ALT (U/L), >40: ≤40	63:89	1.758 (1.133-2.727)	0.012*	
	HBs antigen, positive: negative	118:34	1.516 (0.852-2.7)	0.157	
	HBe antigen, positive: negative	46:106	1.006 (0.623-1.623)	0.981	
	Tumor size (cm), >5: ≤5	75:77	2.785 (1.738-4.464)	<0.001*	
	Tumor number, single: multiple	107:45	2.184 (1.39-3.43)	0.001*	
	Encapsulation, complete: no	78:74	0.578 (0.37-0.903)	0.016*	
	Liver cirrhosis, with: without	114:38	1.618 (0.935-2.8)	0.086	
	Differentiation, I+II: III+IV	52:100	2.277 (1.345-3.853)	0.002*	
	Microvascular invasion, yes: no	34:118	3.638 (2.275-5.815)	<0.001*	
	TNM stage, I: II: III	60:42:50	1.716 (1.309-2.249)	<0.001*	
	BCLC stage, A:B:C	52:78:22	2.18 (1.55-1.55)	<0.001*	
	miR-99a, low: high	111:41	0.435 (0.24-0.788)	0.006*	
	<b>Multivariate analysis</b>	AFP (μg/L), >20: ≤20	97:54	0.975 (0.56-1.697)	0.928
		ALT (U/L), >40: ≤40	63:89	1.267 (0.777-2.068)	0.343
Tumor size (cm), >5: ≤5		75:77	2.182 (1.245-3.823)	0.006*	
Tumor number, single: multiple		107:45	1.181 (0.688-2.029)	0.546	
Encapsulation, complete: no		78:74	0.837 (0.498-1.405)	0.5	
Differentiation, I+II: III+IV		52:100	1.273 (0.695-2.33)	0.435	
Microvascular invasion, yes: no		34:118	2.458 (1.34-4.51)	0.004*	
TNM stage, I: II: III		60:42:50	1.094 (0.723-1.656)	0.671	
BCLC stage, A:B:C		52:78:22	1.177 (0.7-1.979)	0.539	
miR-99a, low: high		111:41	0.443 (0.233-0.842)	0.013*	

**Supplementary Table 2. Univariate and Multivariate Analysis of Factors Correlated with Over-all Survival.**

	Variable	Cases number	HR (95% CI)	P value	
<b>Univariate analysis</b>	Age (year), >50: ≤50	71:81	0.874 (0.536-1.426)	0.59	
	Gender, male: female	123:29	0.522 (0.249-1.095)	0.085	
	AFP (μg/L), >20: ≤20	97:54	1.801 (1.035-3.134)	0.037*	
	Serum bilirubin (μmol/L), >17: ≤17	38:114	1.713 (1.018-2.885)	0.043*	
	Serum albumin(g/L), >40: ≤40	114:38	1.141 (0.641-2.033)	0.653	
	ALT (U/L), >40: ≤40	63:89	1.642 (1.009-2.672)	0.046*	
	HBs antigen, positive: negative	118:34	1.556 (0.813-2.975)	0.182	
	HBe antigen, positive: negative	46:106	1.211 (0.724-2.025)	0.465	
	Tumor size (cm), >5: ≤5	75:77	2.881 (1.538-5.395)	0.001*	
	Tumor number, single: multiple	107:45	2.484 (1.52-4.061)	<0.001*	
	Encapsulation, complete: no	78:74	0.504 (0.304-0.834)	0.008*	
	Liver cirrhosis, with: without	114:38	2.251 (1.147-4.419)	0.018*	
	Differentiation, I+II: III+IV	52:100	2.881 (1.538-5.395)	0.001*	
	Microvascular invasion, yes: no	34:118	4.719 (2.868-7.763)	<0.001*	
	TNM stage, I: II: III	60:42:50	1.795 (1.33-2.424)	<0.001*	
	BCLC stage, A:B:C	52:78:22	2.368 (1.625-3.452)	<0.001*	
	miR-99a, low: high	111:41	0.405 (0.206-0.794)	0.009*	
	<b>Multivariate analysis</b>	AFP (μg/L), >20: ≤20	97:54	0.912 (0.485-1.715)	0.776
		Serum bilirubin (μmol/L), >17: ≤17	38:114	1.302 (0.711-2.384)	0.393
ALT (U/L), >40: ≤40		63:89	0.97 (0.516-1.823)	0.924	
Tumor size (cm), >5: ≤5		75:77	2.071 (1.067-4.02)	0.032*	
Tumor number, single: multiple		107:45	1.119 (0.603-2.076)	0.721	
Encapsulation, complete: no		78:74	0.824 (0.452-1.504)	0.528	
Liver cirrhosis, with: without		114:38	2.224 (1.037-4.772)	0.04*	
Differentiation, I+II: III+IV		52:100	1.302 (0.711-2.384)	0.393	
Microvascular invasion, yes: no		34:118	2.669 (1.351-5.276)	0.005*	
TNM stage, I: II: III		60:42:50	1.237 (0.778-1.967)	0.37	
BCLC stage, A:B:C	52:78:22	1.198 (0.671-2.139)	0.541		
miR-99a, low: high	111:41	0.433 (0.209-0.897)	0.024*		

Analysis was conducted on 152 HCC patients. Hazard ratios (95% confidence interval) and *p* values were calculated using univariate or multivariate Cox proportional hazards regression in SPSS 18.0. \* *P* < 0.05 by Cox proportional hazards regression.

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; HR, hazard ratio; HBV, hepatitis B virus; TNM, tumor-node-metastasis.

**Supplementary Table 3. Sequence of primers used.**

Gene		Primer Sequence (5'-3')
miR-99a	Sense primer	GCAACCCGTAGATCCGAT
miR-21	Sense primer	GAGTGCTAGCTTATCAGACTGA
let-7a	Sense primer	CATCGTGAGGTAGTAGGTTGT
<i>Ago2</i>	Sense primer	TCCACCTAGACCCGACTTT
	Anti-sense primer	GTTCCACGATTTCCCTGTT
<i>Pten</i>	Sense primer	CGGCAGCATCAAATGTTTCAG
	Anti-sense primer	AACTGGCAGGTAGAAGGCAACTC
<i>U6</i>	Sense primer	GTGGACCGCACAAGCTCGCT
	Anti-sense primer	TTGTTGAACGGCACTGTGTATAGCA
<i>Gapdh</i>	Sense primer	GGTGAAGGTCGGAGTCAACG
	Anti-sense primer	CAAAGTTGTCATGGATGHACC

**Supplementary Table 4. RNAi candidate target sequences for AGO2.**

<b>Sequence name</b>	<b>Sequence (5'-3')</b>
NS-siRNA	TTCTCCGAACGTGTCACGT
<i>Ago2</i> -Si1	CGTCCGTGAATTTGGAATCAT
<i>Ago2</i> -Si2	CGGCAAGAAGAGATTAGCAAA
<i>Ago2</i> -Si3	GCACAGCCAGTAATCGAGTTT
<i>Ago2</i> -Si4	CAATCAAATTACAGGCCAATT
<i>Ago2</i> -Si5	CCAGATTTCAAACCTGGATTT

## Supplementary Figure Legend:

**Figure S1.** MiR-99a expression in HCC cell lines detected by qRT-PCR.

**Figure S2.** Restoration of miR-99a in HCC by the recombinant adenovirus expressing miR-99a *in vitro* (a) and *in vivo* (b). Cells transduced by Ad5-miR-99a or Ad-blank (empty adenovirus). Ctrl, untreated HCC cells. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

**Figure S3.** Necrotic areas detected by H&E staining in xenograft tissues.

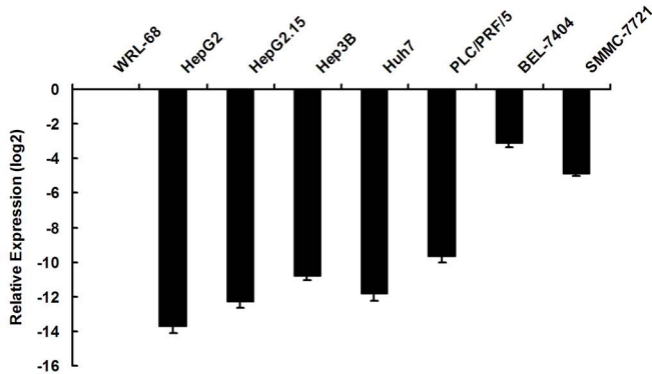
**Figure S4.** *Ago2* expression in HCC cell lines detected by qRT-PCR (a) and Western blot. (b).

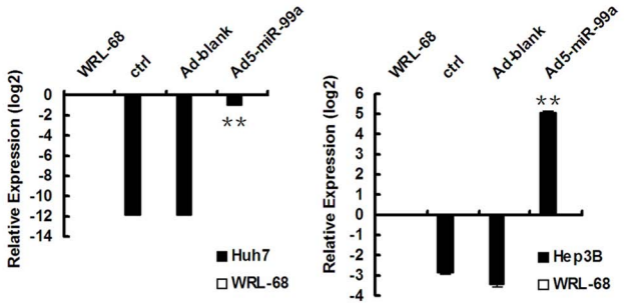
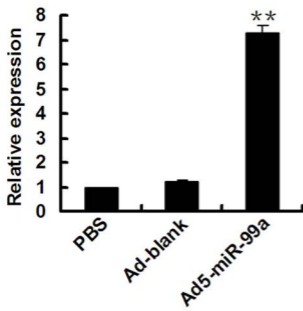
**Figure S5.** Knocked-down of *Ago2* by lentivirus-mediated RNAi. The interference efficiency of five candidate *Ago2*-siRNAs was examined in HEK-293T cells cotransfected with *Ago2* over-expressing vector. NC, cells treated by transfection reagent; Ctrl, cells cotransfected with *Ago2* over-expression vector and control vector expressing nonsilencing-siRNA; Si1-Si5, cells cotransfected with *Ago2* over-expression vector and control vector expressing *Ago2*-specific siRNA; Group 1, 0.6  $\mu$ g over-expression plasmid and 0.3  $\mu$ g siRNA plasmid was co-transfected; Group 2, 0.6  $\mu$ g over-expression plasmid and 0.6  $\mu$ g siRNA plasmid

**Figure S6.** Expression of miR-21a and let-7a in miR-99a over-expressing or *Ago2*-knockdown Huh7 cells (a) and Hep3B (b) cells as determined by qRT-PCR. Ad-blank and Ad5-miR-99a, HCC cells transduced by Ad-blank and Ad5-miR-99a, respectively. Ctrl and AGO2i, HCC cells stably expressing control siRNA and *Ago2*-specific siRNA, respectively. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

**Figure S7.** Expression of cancer-associated proteins in *Ago2*-knockdown Hep3B cells. GAPDH served as loading control. Ctrl and AGO2i indicated Hep3B cells stably expressing control siRNA and *Ago2*-specific siRNA, respectively. Ad5-miR-99a, the recombinant adenovirus expressing miR-99a; Ad-blank, the control adenovirus without transgene.

**Figure S8.** Expression of let-7a (a) and miR-21a (b) in HCC cell lines detected by qRT-PCR.

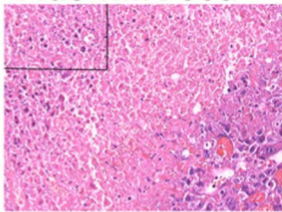
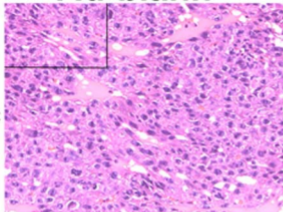


**a****b**

**Ad-blank**

**Ad5-miR-99a**

200x



400x

