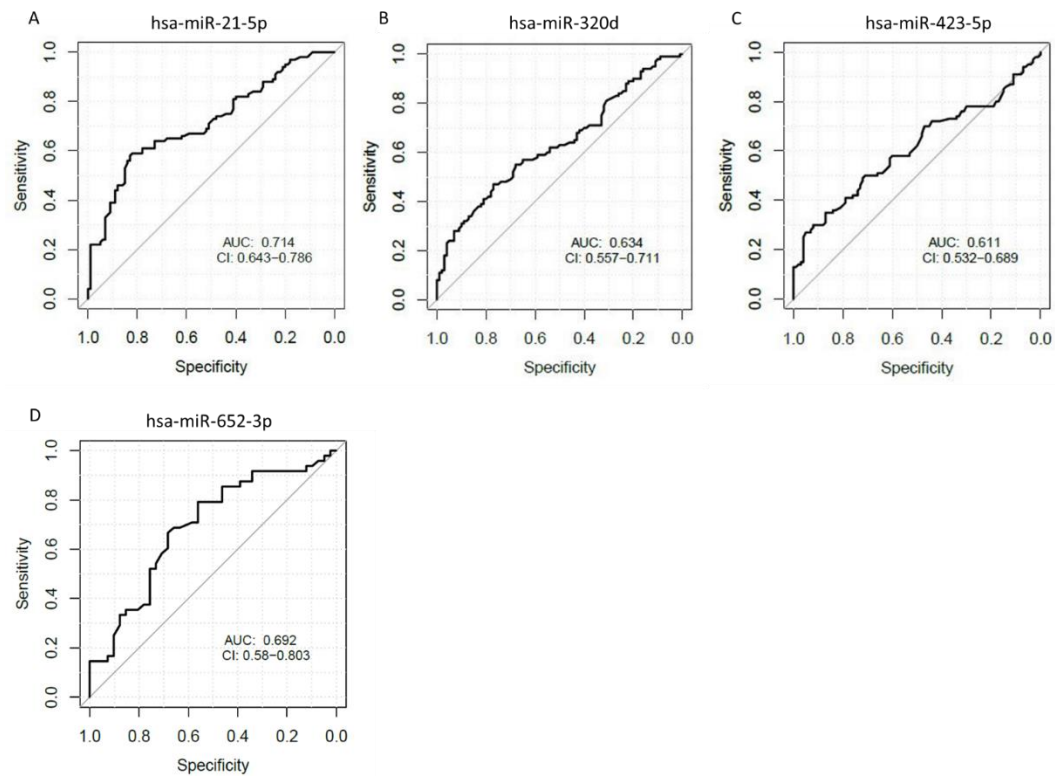
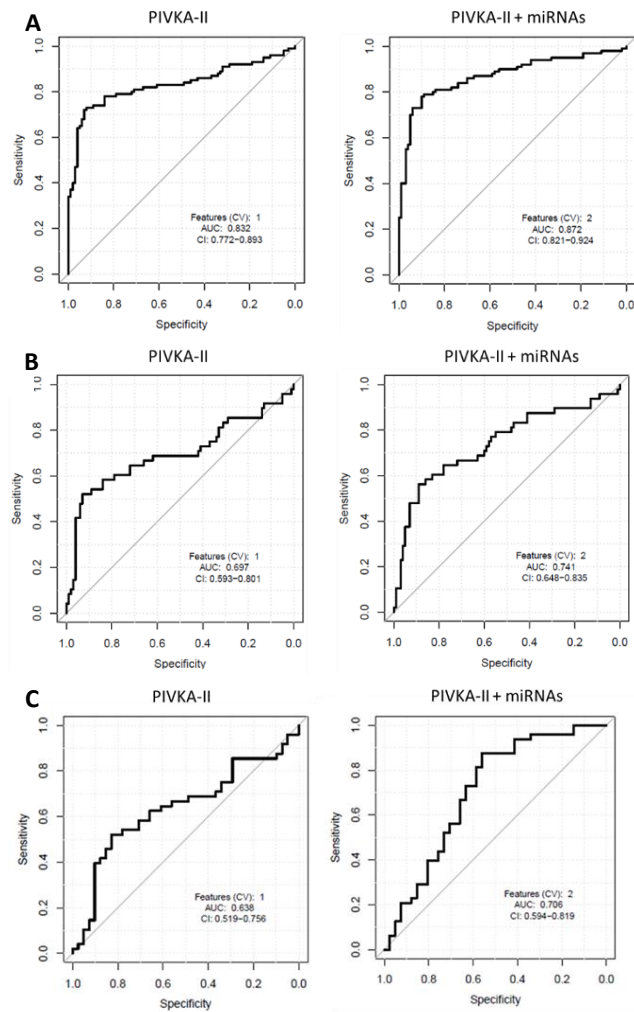
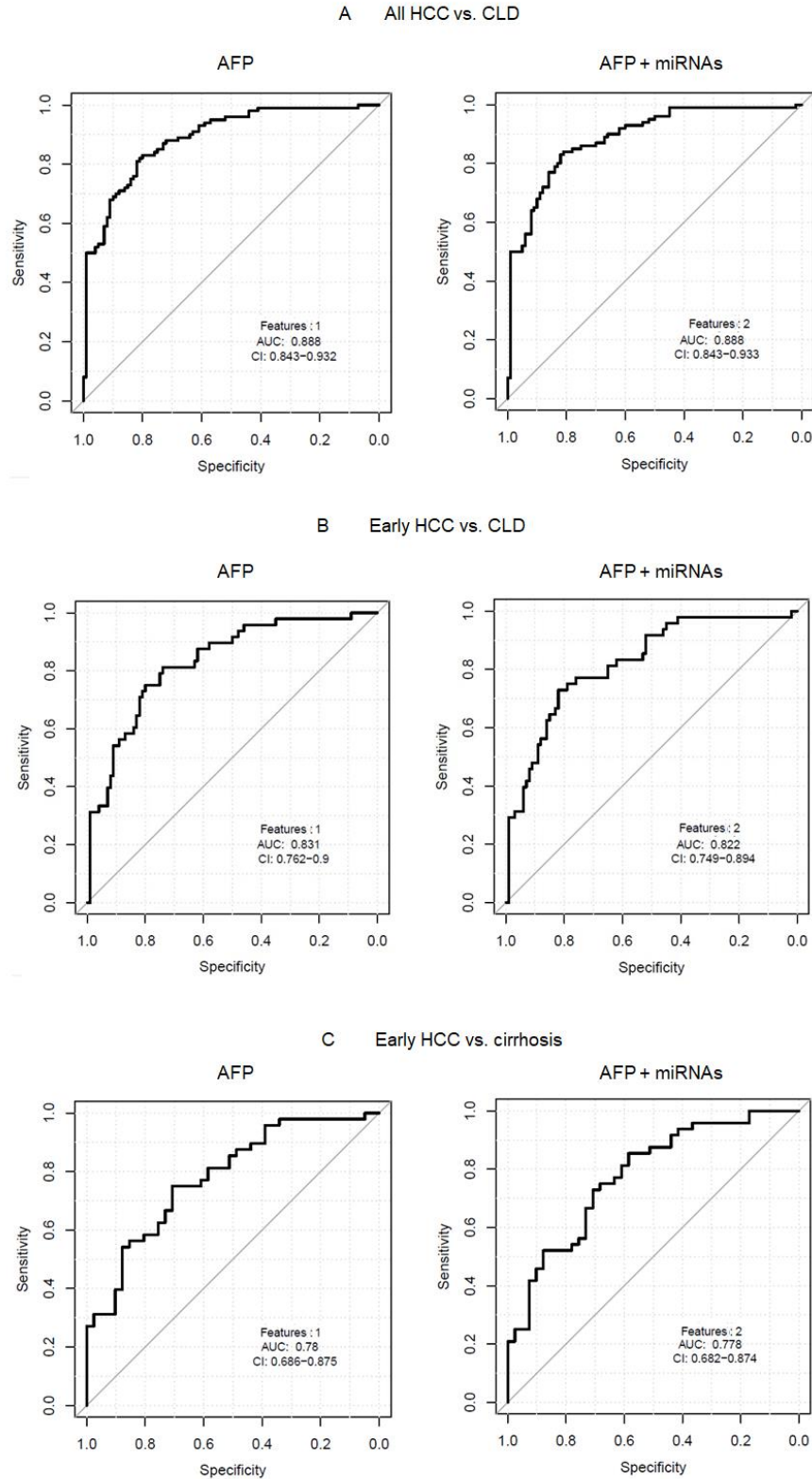




Supplementary Figure 1 Receiver operator characteristics curves and area under the curve for univariate analysis of RT-qPCR results for training cohort. A-C: All HCC *vs* CLD; D: Early HCC *vs* cirrhosis. AUC: Area under the curve; CI: Confidence interval; CLD: chronic liver disease; HCC: hepatocellular carcinoma.

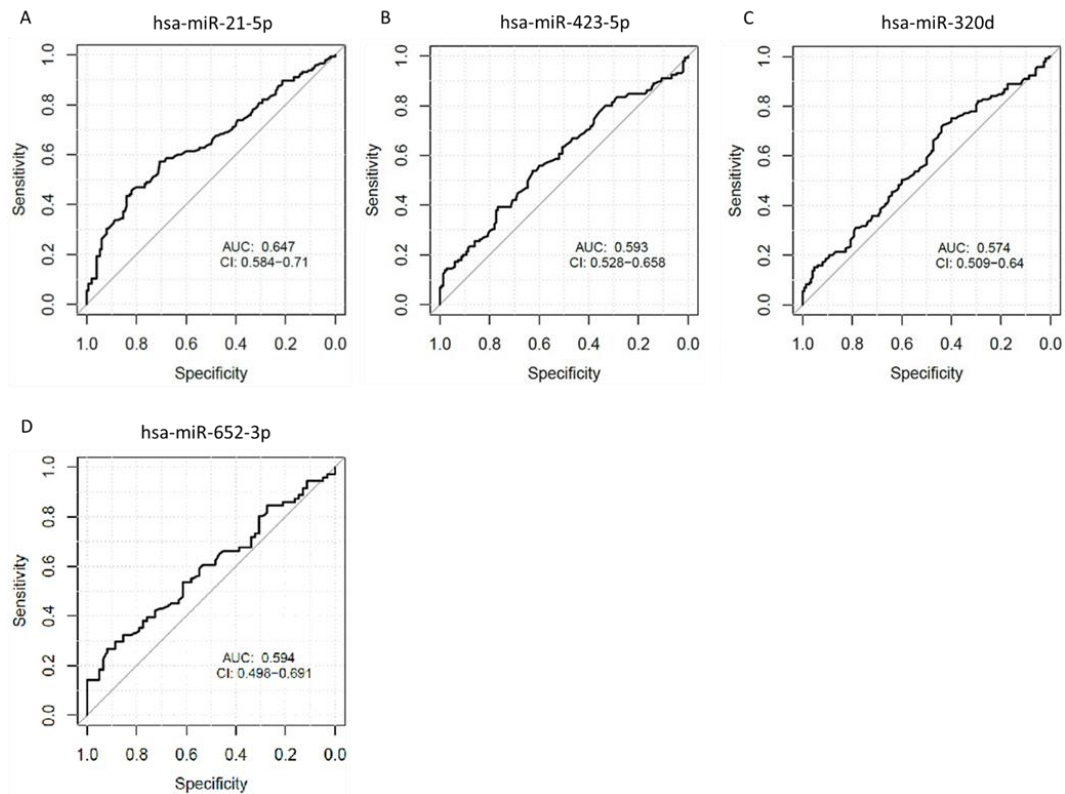


Supplementary Figure 2 Receiver operator characteristics curves and area under the curve for multivariate analysis of RT-qPCR results in training cohort (cross-validation procedure) for comparison of all HCC *vs* CLD (A), early HCC *vs* CLD (B), and early HCC *vs* cirrhosis (C). Left-hand panels show PIVKA-II alone. Right-hand panels show PIVKA-II in combination with the best of the 26 selected miRNAs in each cross-validation run, resulting from the logistic regression model. AUC: Area under the curve; CI: Confidence interval; CLD: Chronic liver disease; CV: Cross-validation; HCC: Hepatocellular carcinoma; PIVKA-II: Protein induced by vitamin K absence/antagonist-II.



Supplementary Figure 3 Receiver operator characteristics curves and area under the curve for multivariate analysis of RT-qPCR for comparison of all HCC *vs* CLD (A), early HCC *vs* CLD (B), and early HCC *vs* cirrhosis (C), in training cohort (cross-validation procedure); for AFP alone and in combination with the best of the 26

selected miRNAs in each cross-validation run, resulting from the logistic regression model. AUC: Area under the curve; CI: Confidence interval; CLD: Chronic liver disease; HCC: Hepatocellular carcinoma.



Supplementary Figure 4 Receiver operator characteristics curves and area under the curve for univariate analysis of RT-qPCR results in the validation cohort. A-C: All HCC *vs* CLD; D: Early HCC *vs* cirrhosis. (A and B) Wilcoxon $Q < 0.05$, (C and D) Wilcoxon $Q > 0.05$. AUC: Area under the curve; CI: Confidence interval; CLD: Chronic liver disease; HCC: Hepatocellular carcinoma.

Supplementary Table 1 Characteristics of study participants in the screening, training, and validation panels

				Ethnicity, <i>n</i> (%)			BCLC stage, <i>n</i> (%)				
	Age, mean $\pm$ SD	Male, <i>n</i> (%)	Female, <i>n</i> , (%)			Non-White/-Asian					
Patients				White	Asian		0	A	B	C	D
Screening panel RT-PCR											
(n=60)											
HBV(n=16)	53.6 $\pm$ 12.3	11 (69%)	5 (31%)	0 (0%)	16 (100%)	0 (0%)	-	-	-	-	-
HCV (n=8)	54.4 $\pm$ 8.0	4 (50%)	4 (50%)	0 (0%)	8 (100%)	0 (0%)	-	-	-	-	-
Cirrhosis											
(HBV, HCV)											
(n=19)	56.6 $\pm$ 6.1	9 (47%)	10 (53%)	0 (0%)	18 (95%)	1 (5%)	-	-	-	-	-
Early HCC											
(n=10)	56.4 $\pm$ 5.1	8 (80%)	2 (20%)	1 (10%)	9 (90%)	0 (0%)	2 (20%)	8 (80%)	-	-	-
Late HCC											
(n=7)	56.0 $\pm$ 5.9	6 (86%)	1 (14%)	0 (0%)	7 (100%)	0 (0%)	-	-	3 (43%)	3 (43%)	1 (14%)
Screening panel NGS (n=100)											
HBV (n=21)	53.3 $\pm$ 9.4	14 (67%)	7 (33%)	0 (0%)	21 (100%)	0 (0%)	-	-	-	-	-
HCV (n=17)	56.6 $\pm$ 6.5	11 (65%)	6 (35%)	0 (0%)	17 (100%)	0 (0%)	-	-	-	-	-

Cirrhosis											
(HBV, HCV)											
(n=22)	61.1 ± 13.3	12 (55%)	10 (45%)	0 (0%)	22 (100%)	0 (0%)	-	-	-	-	-
Early HCC									14		
(n=18)	56.0 ± 6.8	13 (72%)	5 (28%)	0 (0%)	18 (100%)	0 (0%)	4 (22%)	(78%)	-	-	-
Late HCC										14	
(n=22)	57.6 ± 6.4	22 (96%)	1 (4%)	0 (0%)	22 (96%)	1 (4%)	-	-	6 (27%)	(64%)	2 (9%)
Training panel (n=200)											
HBV (n=40)	51.2 ± 9.8	21 (52%)	19 (48%)	0 (0%)	38 (95%)	2 (5%)	-	-	-	-	-
HCV (n=19)	55.4 ± 10.3	9 (47%)	10 (53%)	0 (0%)	19 (100%)	0 (0%)	-	-	-	-	-
Cirrhosis											
(HBV, HCV)											
(n=41)	56.5 ± 10.9	21 (51%)	20 (49%)	8 (20%)	33 (80%)	0 (0%)	-	-	-	-	-
Early HCC									45		
(n=48)	61.2 ± 10.0	36 (75%)	12 (25%)	8 (17%)	40 (83%)	0 (0%)	3 (6%)	(94%)	-	-	-
Late HCC									23	25	
(n=52)	61.5 ± 9.5	44 (85%)	8 (15%)	9 (17%)	42 (81%)	1 (2%)	-	-	(44%)	(48%)	4 (8%)
Validation panel (n=300)											
HBV (n=59)	52.6 ± 9.3	34 (58%)	25 (42%)	0 (0%)	59 (100%)	0 (0%)	-	-	-	-	-
HCV (n=28)	51.9 ± 8.6	16 (57%)	12 (43%)	0 (0%)	28 (100%)	0 (0%)	-	-	-	-	-

Cirrhosis

(HBV, HCV)

(n=63)	59.9 ± 9.3	30 (48%)	33 (52%)	10 (16%)	52 (82%)	1 (2%)	-	-	-	-	-
Early HCC								45			
(n=71)	59.8 ± 9.5	50 (70%)	21 (30%)	9 (13%)	60 (84%)	2 (3%)	6 (8%)	(92%)	-	-	-
Late HCC									29	42	
(n=79)	60.0 ± 10.4	69 (87%)	10 (13%)	16 (21%)	62 (78%)	1 (1%)	-	-	(36%)	(54%)	8 (10%)

BCLC: Barcelona-Clínic Liver Cancer; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; NGS: Next-generation sequencing; RT-qPCR: Real-time quantitative polymerase chain reaction.

Supplementary Table 2 Details on the put-miRs and their corresponding chromosome location, chromosome strand, start and stop position, sequence and primer design IDs from Exiqon

put-miR	Chromosome	Chromosome strand	Start position	Stop position	Sequence	Primer design IDs	
put-miR-4	X	+	34234106	34234131	TAACAGGTCTGTGATGCTCTTAGATG	n/a	
put-miR-6	14	+	106318490	106318516	GTGACGGTGGTACTGTAGAAGAGGCTC	705658-1, 705658-3	705658-2,
put-miR-9	8	+	30145594	30145620	CCTTATGGCCAGGGCTGTGTGACTCTC	705662-1, 705662-3	705662-2,
put-miR-25	9	+	139906806	139906832	TAGCCCTTGTTGTTGTAGAAAACCTGC	715532-1,715532-2, 715532-3	
put-miR-27	17	+	5535752	5535778	TTGCTGGTCTCTGGACTGTAGCAAAGC	705666-1, 705666-3	705666-2,
put-miR-40	20	+	5666168	5666194	TAAGGGAACCTGGGAATTCTGAGTGGAC	n/a	
put-miR-43	17	+	61706875	61706897	GAGATAACTGGCTTGTGGCAGCC	n/a	
put-miR-44	7	+	31037734	31037760	CCTTGGTAAATCAGGATGTTGGGACTC	n/a	
put-miR-46	5	+	157964600	157964627	GCACTCTGGGACTGCTGGATTCTGCTGA	705670-1, 705670-3	705670-2,
put-miR-49	1	+	180916565	180916591	TGGTGCCTGGACTGGGAACGGCTGTGG	n/a	
put-miR-52	20	+	20839917	20839943	TTGGGATTGTTGGGAGAATACTCTGACTC	n/a	

put-miR-56	3	+	164059152	164059175	ATATCTCGCTGGGGCCTCCAAAAT	715536-1, 715536-3	715536-2,
put-miR-58	6	+	57441807	57441824	ATGAGCTGTGGATAGGGA	n/a	
put-miR-62	10	+	11359731	11359747	TTGAGGGCCTCTGGAGA	n/a	
put-miR-66	3	+	58560289	58560315	CTCCTCTGTAGAATTGTGATGCTGTGA	705674-1, 705674-3	705674-2,
put-miR-67	1	+	215247347	215247373	CATGACTCAGTGTGGAAAATGGACTGG	n/a	
put-miR-79	16	+	226786	226811	AGTATGGTGCGGAGGCCCTGGAGAGG	715540-1, 715540-3	715540-2,
put-miR-82	10	+	121201806	121201828	CTGAGCTCTCTGCACTCCCAGGC	n/a	
put-miR-83	11	+	5512687	5512713	AGAGGAGAATGATCAGAGGGGTGGAAC	705678-1, 705678-3	705678-2,
put-miR-86	1	+	163173054	163173080	GATTCTTAGGACTGTAAGGGCTCCTGG	705682-1, 705682-3	705682-2,
put-miR-91	4	+	6752141	6752166	CACACTGCTGGTCGGTGGCAGAACTG	705686-1, 705686-3	705686-2,
put-miR-94	18	+	11755658	11755684	ATATCTGTAGAATTTCTAAAATGGAGG	n/a	
put-miR-99	1	+	10782719	10782735	TTCGGACTGGCCCAGGG	715544-1, 715544-3	715544-2,

put-miR-100	22	+	23631896	23631922	TTTGATGGGACTAGTGGACTTTGGTTC	705690-1, 705690-3	705690-2,
put-miR-109	1	+	49216789	49216806	GATAGGGGTGAAAGGCTG	n/a	
put-miR-117	13	+	102651846	102651862	GATAGGGGTGAAAGGCT	n/a	
put-miR-118	22	+	50629605	50629629	TCCCGGCTGTCCAAGAAGAGGGCAG	715548-1, 715548-3	715548-2,
put-miR-125	16	+	78398673	78398699	CCAGGGAACAGTGTAGCATCTGATTAC	n/a	
put-miR-128	21	+	37609464	37609490	CCAGTATGTCGGGGCTGGGACTGACTG	705694-1, 705694-3	705694-2,
put-miR-129	5	+	141152733	141152759	GACCCTGTAGAACTGTCTGTCTGGTCC	n/a	
put-miR-136	17	+	80798700	80798726	TGGTGTCGGTTTGAGGACTGGGAAAGG	n/a	
put-miR-137	10	+	8349794	8349820	GTTTTGGACTCACATTGAACCTCTCCT	n/a	
put-miR-139	19	+	49469042	49469061	CGCGATGATGTGGCTCTGGA	n/a	

N/A: Not applicable; put-miR: Putative microRNA; RT-qPCR: Real-time quantitative polymerase chain reaction.

Supplementary Table 3 Results of the NGS screening

A

miRNA	Average TMM All HCC	Average TMM CLD	Fold change All HCC/CLD	AUC	P (Wilcoxon)	value
hsa-miR-185-5p	1259.97	627.47	2.01	0.76	0.00009	
hsa-miR-320a¹	25946.21	17100.15	1.52	0.74	0.00033	
hsa-miR-423-5p	103492.33	126264.88	-1.22	0.69	0.00412	
hsa-miR-664a-5p	165.28	119.62	1.38	0.69	0.00569	
hsa-miR-21-3p	15.62	9.12	1.71	0.68	0.00791	
hsa-miR-203a	42.26	115.24	-2.73	0.67	0.01167	
hsa-miR-421¹	31.56	22.03	1.43	0.67	0.01338	
hsa-miR-431-3p	2.49	1.53	1.63	0.67	0.00397	
hsa-miR-6734-5p	8.05	13.85	-1.72	0.67	0.00129	
hsa-miR-4433-3p	6.49	10.44	-1.61	0.67	0.01385	
hsa-miR-200a-5p	3.39	2.85	1.19	0.66	0.00280	
hsa-let-7b-5p	23783.46	33534.68	-1.41	0.66	0.01552	
hsa-miR-320d	349.67	136.47	2.56	0.66	0.01715	
hsa-miR-193b-3p	0.50	0.35	1.41	0.66	0.00394	
hsa-miR-873-3p	2.17	1.21	1.80	0.66	0.00733	
hsa-miR-21-5p¹	2.64	1.97	1.34	0.66	0.02048	
hsa-miR-6852-3p	0.96	0.65	1.49	0.65	0.00900	
hsa-miR-3151-5p	0.72	0.88	-1.23	0.65	0.00110	
hsa-let-7i-3p	3.02	1.85	1.63	0.65	0.00549	
hsa-miR-1183	3.56	4.53	-1.27	0.65	0.00232	

B

miRNA	Average TMM Early HCC	Average TMM CLD	Fold change Early HCC/CLD	AUC	P (Wilcoxon)	value
hsa-miR-30a-3p	114.47	183.33	-1.60	0.72	0.00814	
hsa-miR-25-3p	12300.18	7037.94	1.74	0.71	0.01019	

hsa-miR-7706	139.18	83.29	1.67	0.69	0.02002
hsa-miR-186-5p	819.35	487.46	1.68	0.68	0.02364
hsa-miR-200c-3p	48.18	30.46	1.58	0.67	0.04263
hsa-miR-25-5p	50.82	32.60	1.56	0.66	0.04561
hsa-miR-6852-3p	2.29	1.34	1.71	0.64	0.00561
hsa-miR-6514-5p	5.71	3.75	1.52	0.64	0.01496
hsa-miR-550a-5p	30.56	40.65	-1.33	0.63	0.01923
hsa-miR-185-5p	1026.94	717.54	1.43	0.63	0.00765
hsa-miR-2110	396.76	313.83	1.26	0.63	0.02003
hsa-miR-3135a	3.18	2.06	-1.63	0.62	0.00958
hsa-miR-873-3p	6.47	4.37	1.48	0.61	0.01712
hsa-miR-1226-5p	2.59	1.77	1.46	0.61	0.01266
hsa-miR-16-5p	236.06	159.23	1.48	0.60	0.02363
hsa-miR-552-3p	2.29	1.53	1.50	0.59	0.01805
hsa-miR-4747-5p	2.35	1.71	1.37	0.58	0.01668
hsa-miR-23b-3p	17.80	25.81	-1.45	0.57	0.01715
hsa-miR-7155-3p	2.18	2.77	-1.27	0.57	0.01869
hsa-miR-106b-3p	937.23	768.23	1.22	0.56	0.02407

C

miRNA	Average TMM all HCC	Average TMM CLD	Fold change all HCC/CLD	AUC	P value (Wilcoxon)
put-miR-86	5491.18	1452.64	3.78	0.74	0.00007
put-miR-100	5970.08	1323.74	4.51	0.73	0.00119
put-miR-25	18417.77	7275.76	2.53	0.73	0.00447
put-miR-67	4592.23	2701.31	1.70	0.72	0.01178
put-miR-27	9227.85	4545.74	2.03	0.71	0.00003
put-miR-9	3311.85	838.71	3.95	0.71	0.00130
put-miR-52	4857.69	3154.35	1.54	0.71	0.00783
put-miR-91	6681.38	2005.00	3.33	0.70	0.00115
put-miR-46	6645.74	2144.79	3.10	0.70	0.00347

put-miR-83	8571.97	3283.97	2.61	0.69	0.00031
put-miR-128	5580.26	1287.24	4.34	0.69	0.00035
put-miR-137	4761.95	2710.82	1.76	0.67	0.00812
put-miR-6	3913.00	1890.34	2.07	0.66	0.00196
put-miR-139	4376.87	2291.56	1.91	0.66	0.01197
put-miR-125	4872.49	2578.04	1.89	0.66	0.00351
put-miR-40	6261.56	3818.03	1.64	0.65	0.00485
put-miR-66	22941.64	9335.47	2.46	0.64	0.00034
put-miR-49	3525.00	2303.92	1.53	0.63	0.00651
put-miR-44	4932.64	2429.87	2.03	0.62	0.00259
put-miR-94	4621.10	2924.75	1.58	0.62	0.00651

D

miRNA	Average TMM Early HCC	Average TMM CLD	Fold Early /CLD	change HCC AUC	P (Wilcoxon)	value
put-miR-118	38488.41	76920.60	-2.00	0.73	0.09334	
put-miR-79	17266.35	8407.37	2.05	0.72	0.07279	
put-miR-56	1723.47	3737.67	-2.17	0.71	0.07041	
put-miR-82	2224.46	3425.67	-1.54	0.70	0.09804	
put-miR-99	81596.81	35171.04	2.32	0.70	0.18802	
put-miR-58	1174.89	2373.27	-2.02	0.69	0.06682	
put-miR-86	2980.06	1576.75	1.89	0.68	0.00651	
put-miR-83	7163.06	3271.08	2.19	0.68	0.02123	
put-miR-6	2198.41	1675.15	1.31	0.68	0.07975	
put-miR-4	1356.76	2806.87	-2.07	0.67	0.03842	
put-miR-136	3029.29	1771.52	1.71	0.66	0.05503	
put-miR-117	1242.71	2522.69	-2.03	0.66	0.07998	
put-miR-109	1401.12	2902.80	-2.07	0.65	0.09749	
put-miR-43	1817.44	3598.54	-1.98	0.63	0.08731	
put-miR-62	13807.06	9937.81	1.39	0.62	0.07804	

put-miR-44	3099.06	2093.96	1.48	0.61	0.10573
put-miR-66	15964.71	9037.35	1.77	0.59	0.05524
put-miR-129	2448.00	2841.17	-1.16	0.59	0.07771
put-miR-100	4957.65	3699.74	1.34	0.58	0.02475
put-miR-125	2934.82	2223.35	1.32	0.55	0.05837

Top 20 lists of known and unknown (put-miR) miRNAs, sorted by descending AUC. Fold change and *P* value (Wilcoxon) are shown. A: Known miRNAs for comparison of all HCC *vs* CLD; B: Known miRNAs for early HCC *vs* CLD; C: Unknown miRNAs for all HCC *vs* CLD; D: Unknown miRNAs for early HCC *vs* CLD. Emboldened miRNAs were selected for clinical validation. ¹miRNAs were detected in both screenings, RT-qPCR and NGS. TMM = trimmed mean of M-values (a method for normalization in the EdgeR Statistics software package). The TMM normalization compensates for the sample-specific effects caused by the variation of library size/sequencing depth between samples. AUC: Area under the curve; CLD: Chronic liver disease; HCC: Hepatocellular carcinoma; NGS: Next-generation sequencing; RT-qPCR: Real-time quantitative polymerase chain reaction; TMM: Trimmed mean of M-values.

Supplementary Table 4 Results of RT-qPCR screening

A

miRNA	Fold change HCC/CLD	all	AUC	P (Wilcoxon)	value
hsa-miR-421¹	1.49		0.83	0.0001	
hsa-miR-28-5p	-1.65		0.80	0.0004	
hsa-miR-21-5p¹	1.59		0.79	0.0006	
hsa-miR-103a-3p	-1.65		0.78	0.012	
hsa-miR-301a-3p	-1.50		0.78	0.013	
hsa-miR-15b-5p	1.33		0.77	0.0017	
hsa-miR-140-5p	-1.37		0.76	0.0014	
hsa-miR-30b-5p	-1.63		0.75	0.0033	
hsa-miR-10b-5p	1.37		0.75	0.0041	
hsa-miR-495-3p	-1.50		0.73	0.0068	
hsa-miR-345-5p	-1.36		0.73	0.0088	
hsa-miR-320a¹	1.37		0.72	0.0093	
hsa-miR-324-3p	-1.36		0.72	0.01	
hsa-miR-18a-5p	-1.33		0.72	0.0093	
hsa-let-7g-5p	-1.30		0.71	0.0146	
hsa-miR-126-5p	1.42		0.71	0.0173	
hsa-miR-30a-5p	1.46		0.71	0.0146	
hsa-let-7d-5p	-1.34		0.71	0.0139	
hsa-miR-92a-3p	1.43		0.70	0.0205	
hsa-let-7f-5p	1.35		0.69	0.0283	

B

miRNA	Fold change early HCC/CLD	AUC	<i>P</i> (Wilcoxon)	value
hsa-miR-15b-5p	4.21	0.97	<0.0001	
hsa-miR-339-3p	-2.73	0.87	0.0006	
hsa-let-7b-5p	1.96	0.86	0.0009	
hsa-miR-18a-5p	-1.93	0.86	0.0009	
hsa-miR-10b-5p	2.81	0.86	0.0007	
hsa-miR-301a-3p	-1.78	0.86	0.0008	
hsa-miR-151a-5p	-2.25	0.85	0.0011	
hsa-miR-652-3p	-2.22	0.85	0.001	
hsa-miR-324-3p	-1.67	0.85	0.0013	
hsa-miR-103a-3p	-1.95	0.85	0.0011	
hsa-miR-32-5p	2.03	0.85	0.001	
hsa-miR-92a-3p	1.85	0.83	0.0023	
hsa-miR-140-5p	-1.70	0.83	0.0021	
hsa-miR-221-3p	-2.96	0.83	0.0021	
hsa-miR-486-5p	2.43	0.82	0.0034	
hsa-miR-15a-5p	2.19	0.82	0.0029	
hsa-let-7f-5p	1.73	0.82	0.003	
hsa-let-7g-5p	-1.45	0.81	0.0042	
hsa-miR-142-5p	-2.15	0.81	0.0043	
hsa-miR-26b-5p	2.01	0.81	0.0042	

Top 20 lists of miRNAs, sorted by descending AUC. Fold change and *P* value (Wilcoxon) are shown. miRNAs for comparison of all HCC *vs* CLD (A), and early HCC *vs* CLD (B). Emboldened miRNAs were selected for clinical validation. ¹miRNAs were detected both in RT-qPCR and NGS screening. AUC: Area under the curve; CLD: Chronic liver disease; HCC: Hepatocellular carcinoma; NGS: Next-generation sequencing; RT-qPCR: Real-time quantitative polymerase chain reaction.