Supporting Data

A Large-Scale Multicenter Study Validates AKR1B10 as a New Prevalent Serum Marker for Detection of Hepatocellular Carcinoma

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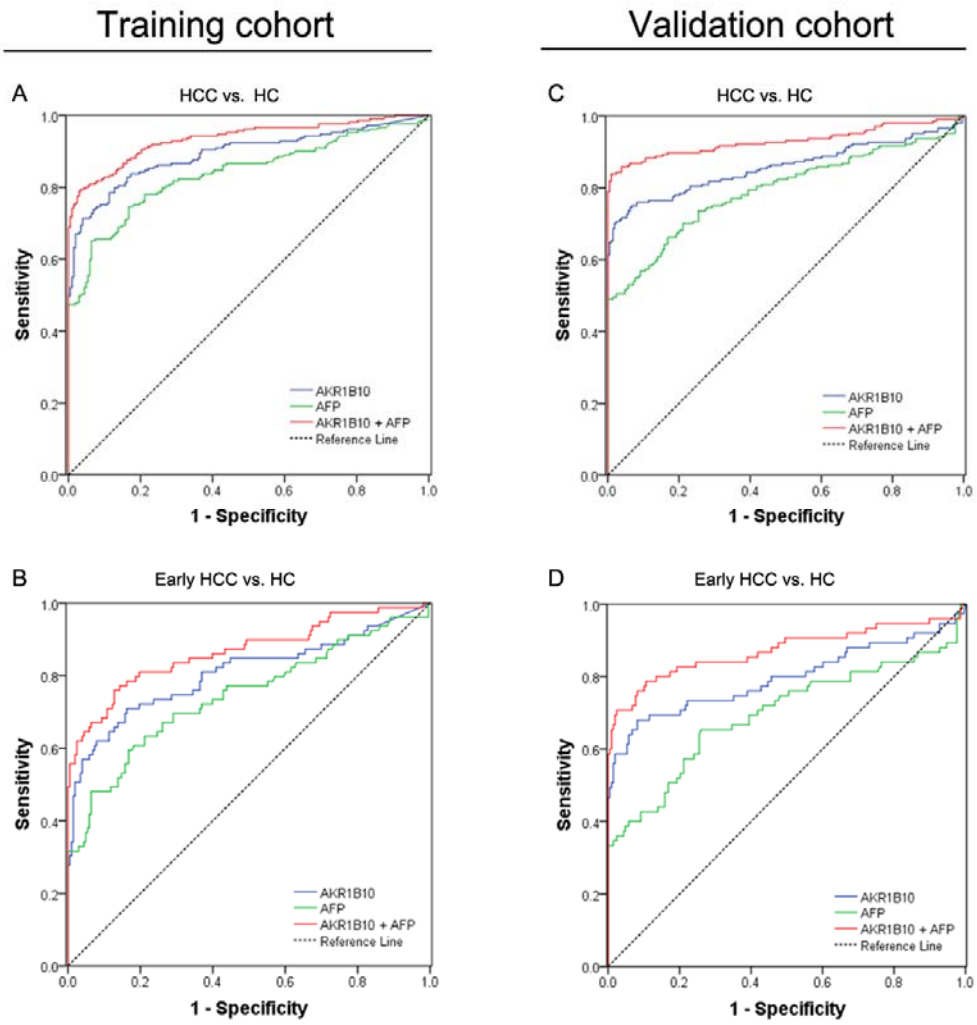
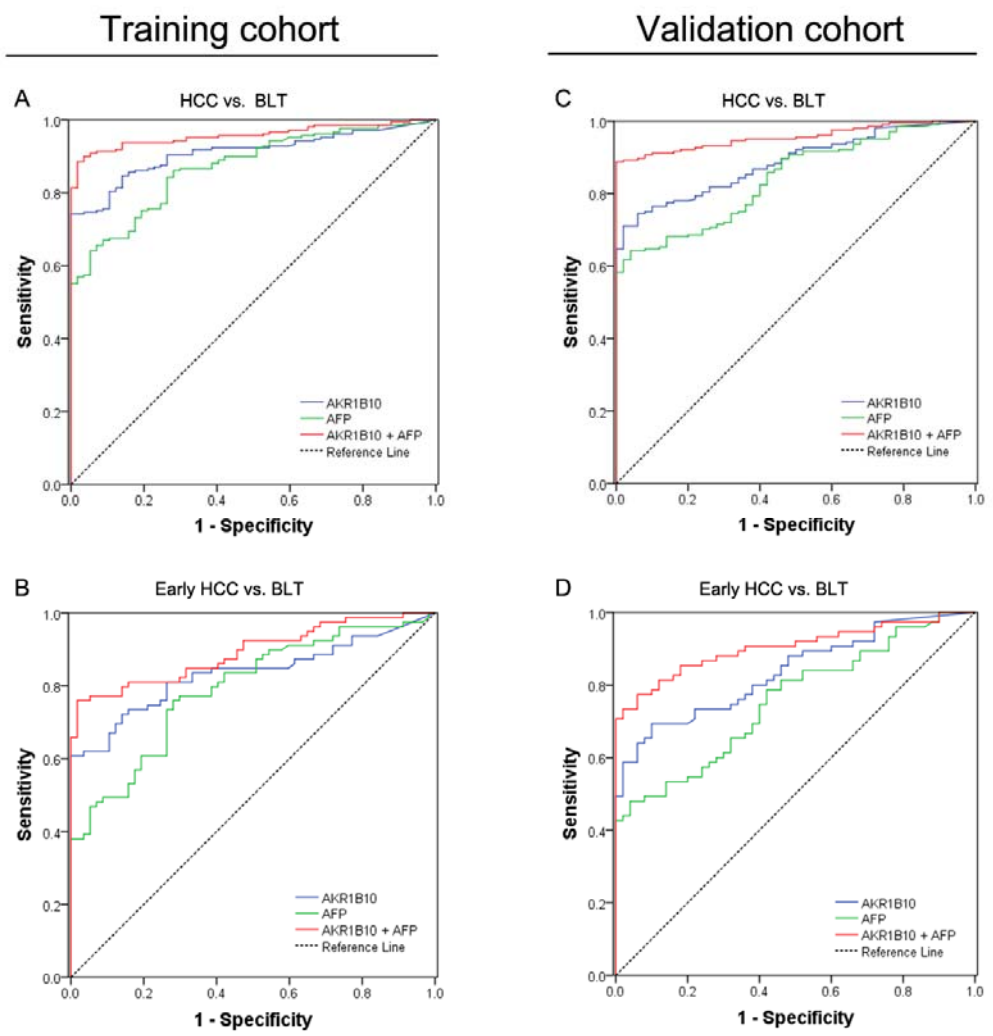
Supporting Figures



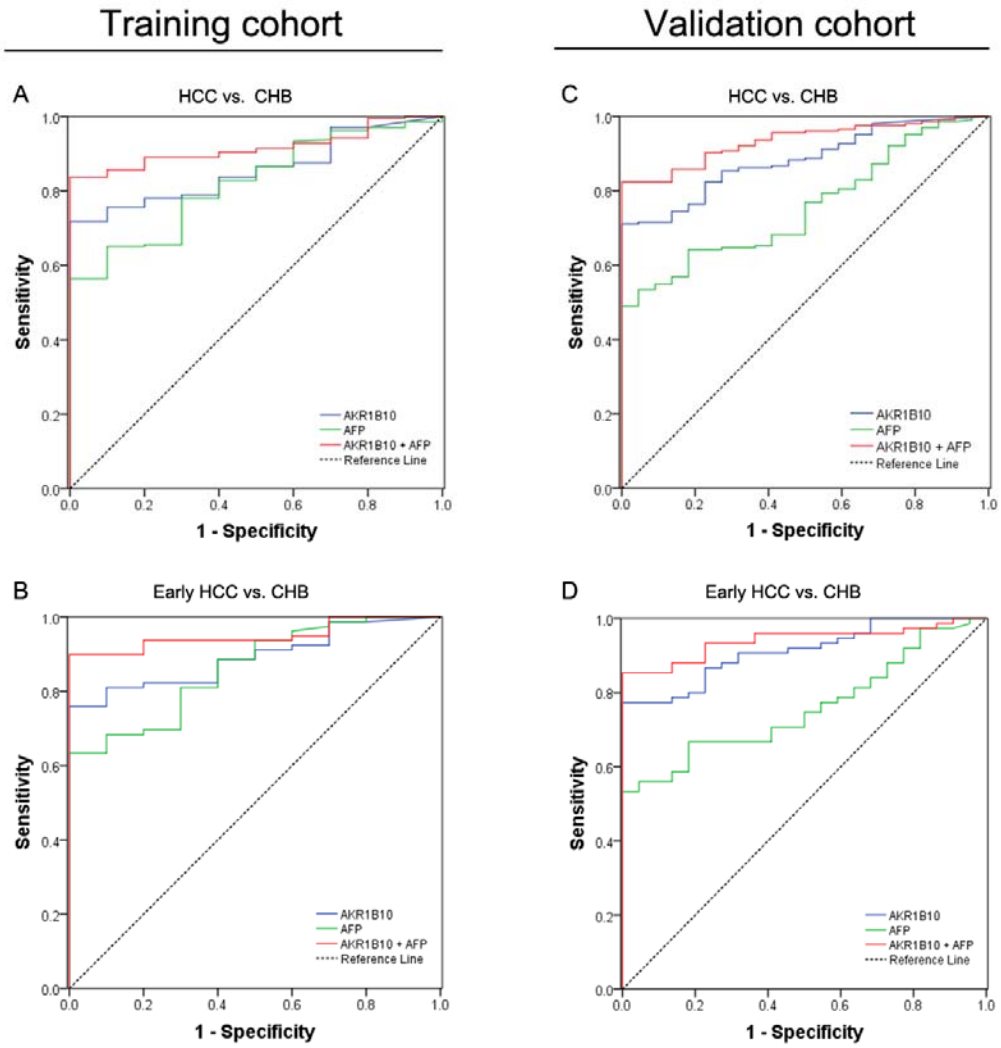
Supporting FIG. S1. Diagnostic accuracy of AKR1B10 in discrimination of HCC from HCs. (A and B) ROC curve analyses of AKR1B10, AFP, or both in training cohort for patients with HCC versus HCs and patients with early HCC versus HCs, respectively. (C and D) ROC curve analyses of AKR1B10, AFP, or both in validation cohort for patients with HCC versus HCs and patients with early HCC versus HCs, respectively.

Abbreviations: AFP, alpha-fetoprotein; AKR1B10, aldo-keto reductase family 1 member B10; HCC, hepatocellular carcinoma; HC, healthy control; ROC, receiver operating characteristic.



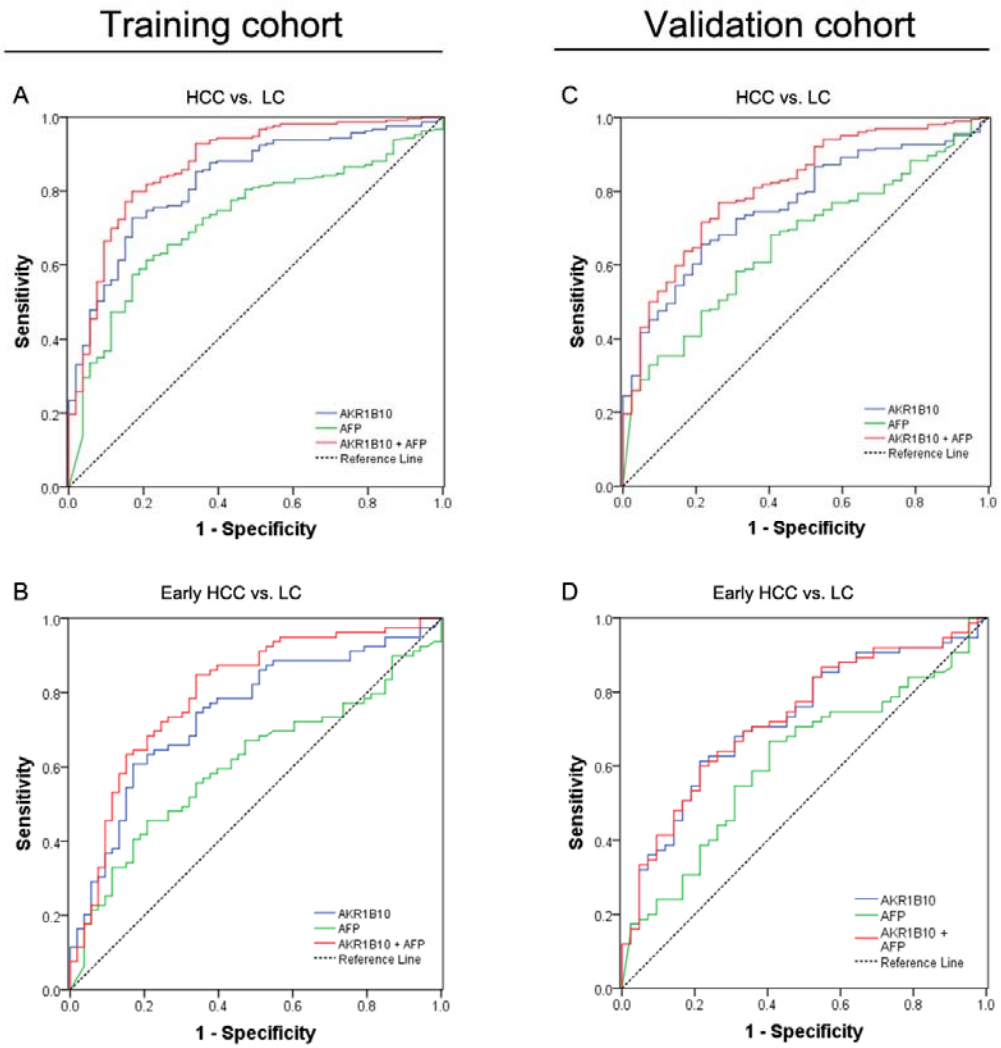
Supporting FIG. S2. Diagnostic accuracy of AKR1B10 in differentiating patients with HCC from patients with BLT. (A and B) ROC curve analyses of AKR1B10, AFP, or both in training cohort for patients with HCC versus patients with BLT and patients with early HCC versus patients with BLT, respectively. (C and D) ROC curve analyses of AKR1B10, AFP, or both in validation cohort for patients with HCC versus patients with BLT and patients with early HCC versus patients with BLT, respectively.

Abbreviations: AFP, alpha-fetoprotein; AKR1B10, aldo-keto reductase family 1 member B10; BLT, benign liver tumor; HCC, hepatocellular carcinoma; ROC, receiver operating characteristic.



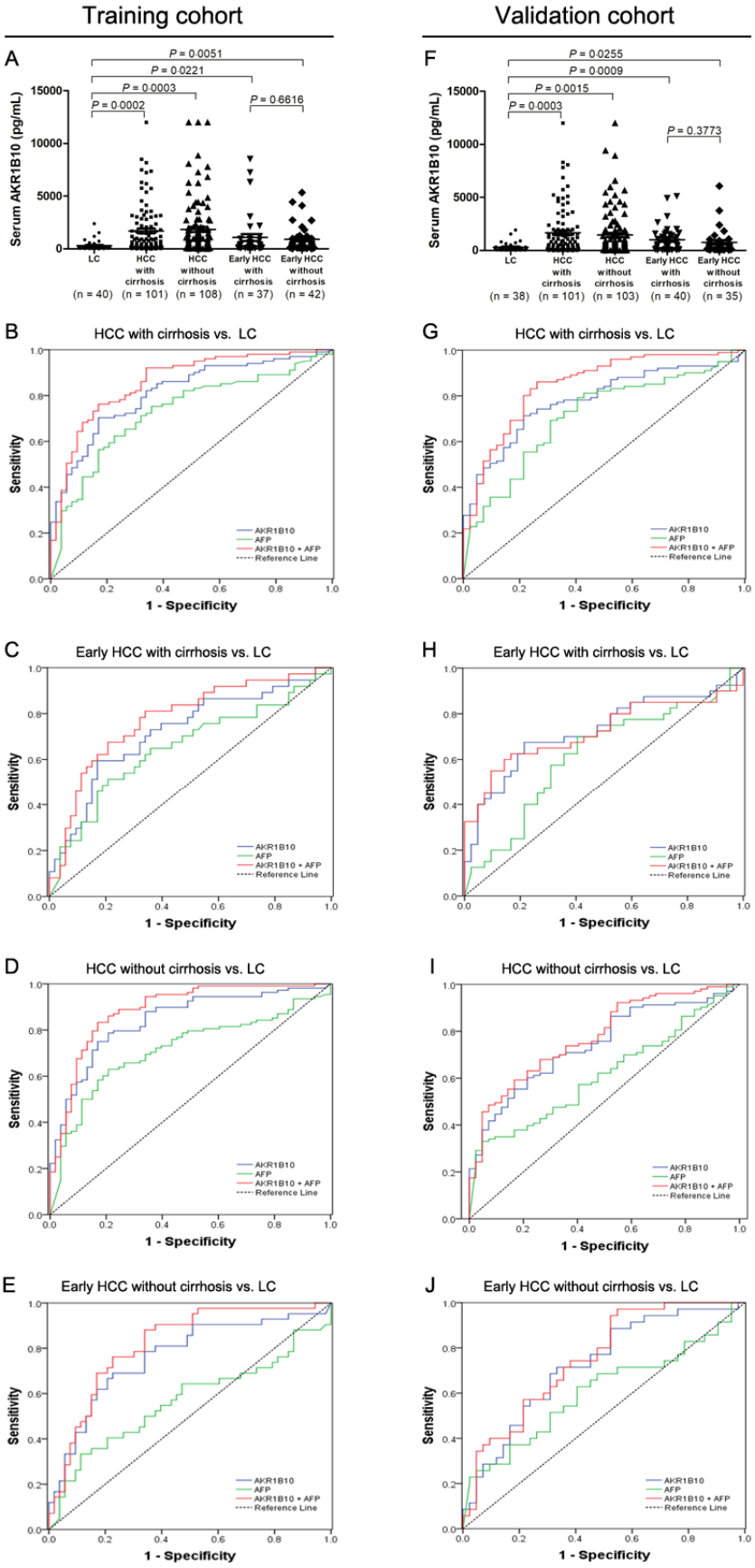
Supporting FIG. S3. Diagnostic accuracy of AKR1B10 in differentiating patients with HCC from patients with CHB. (A and B) ROC curve analyses of AKR1B10, AFP, or both in training cohort for patients with HCC versus patients with CHB and patients with early HCC versus patients with CHB, respectively. (C and D) ROC curve analyses of AKR1B10, AFP, or both in validation cohort for patients with HCC versus patients with CHB and patients with early HCC versus patients with CHB, respectively.

Abbreviations: AFP, alpha-fetoprotein; AKR1B10, aldo-keto reductase family 1 member B10; CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; ROC, receiver operating characteristic.

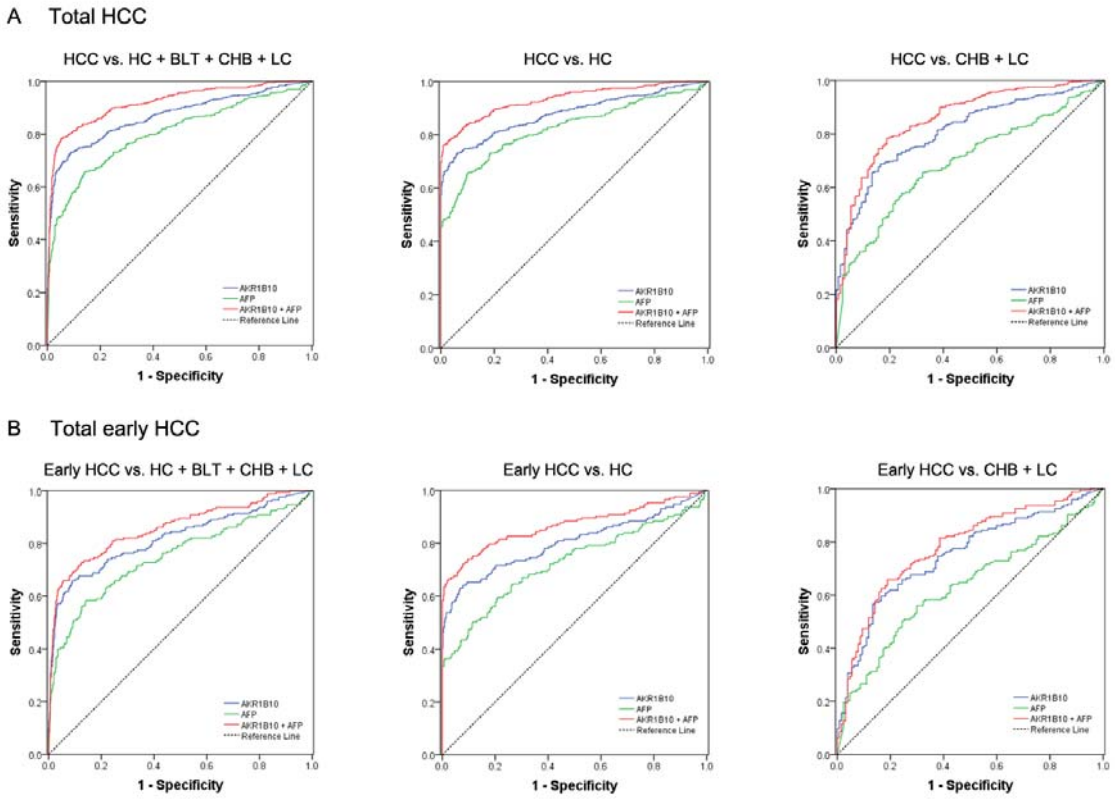


Supporting FIG. S4. Diagnostic accuracy of AKR1B10 in differentiating patients with HCC from patients with LC. (A and B) ROC curve analyses of AKR1B10, AFP, or both in training cohort for patients with HCC versus patients with LC and patients with early HCC versus patients with LC, respectively. (C and D) ROC curve analyses of AKR1B10, AFP, or both in validation cohort for patients with HCC versus patients with LC and patients with early HCC versus patients with LC, respectively.

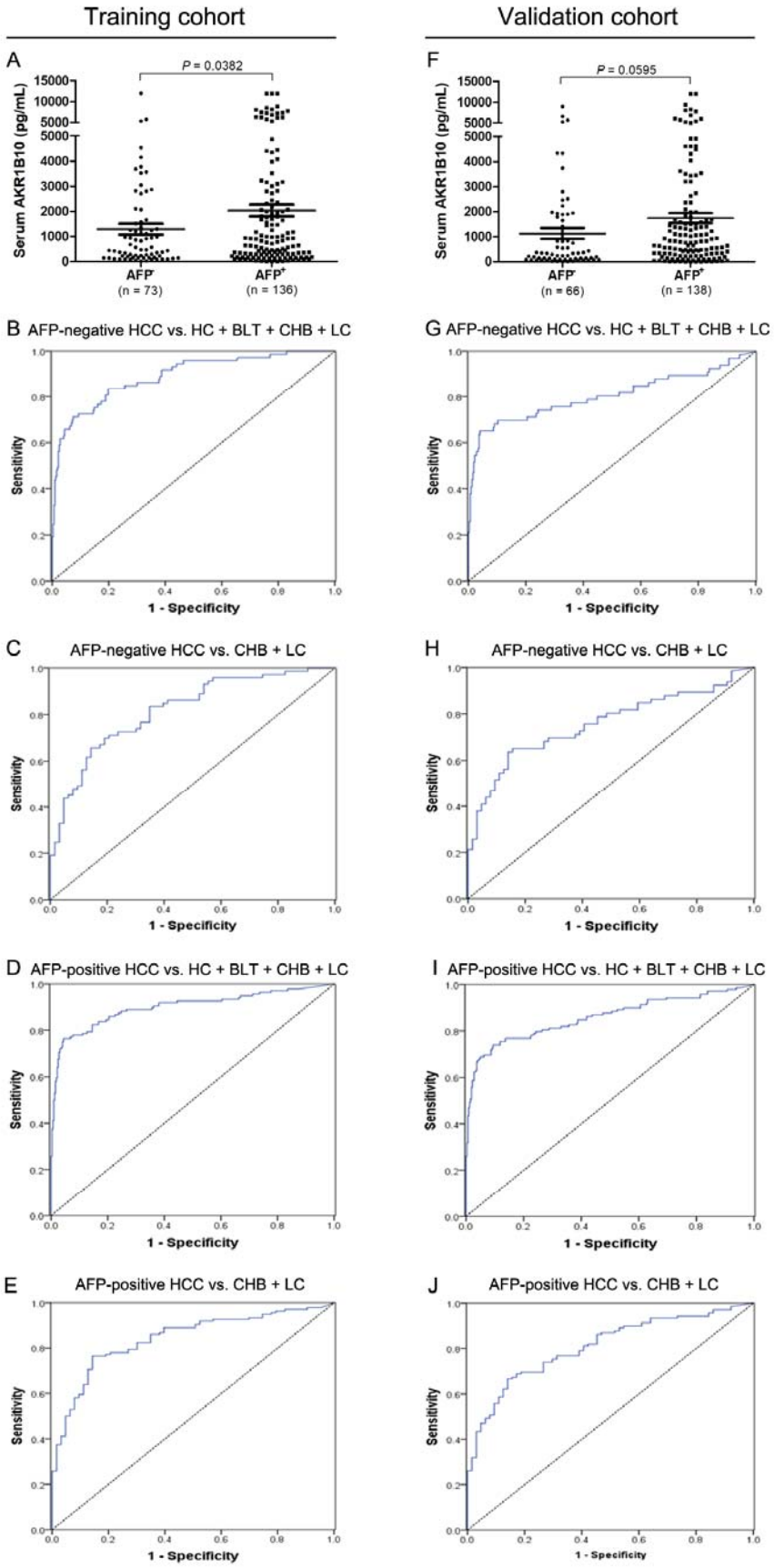
Abbreviations: AFP, alpha-fetoprotein; AKR1B10, aldo-keto reductase family 1 member B10; HCC, hepatocellular carcinoma; LC, liver cirrhosis; ROC, receiver operating characteristic.


Training cohort
Validation cohort
A
F
P = 0.0051
P = 0.0221
P = 0.0003
P = 0.0002
P = 0.6616
P = 0.0255
P = 0.0009
P = 0.0015
P = 0.0003
P = 0.3773
Serum AKR1B10 (pg/mL)
LC (n = 40)
HCC with cirrhosis (n = 101)
HCC without cirrhosis (n = 108)
Early HCC with cirrhosis (n = 37)
Early HCC without cirrhosis (n = 42)
LC (n = 38)
HCC with cirrhosis (n = 101)
HCC without cirrhosis (n = 103)
Early HCC with cirrhosis (n = 40)
Early HCC without cirrhosis (n = 35)
B
G
HCC with cirrhosis vs. LC
C
H
Early HCC with cirrhosis vs. LC
D
I
HCC without cirrhosis vs. LC
E
J
Early HCC without cirrhosis vs. LC
Sensitivity
1 - Specificity
AKR1B10
AFP
AKR1B10 + AFP
Reference Line

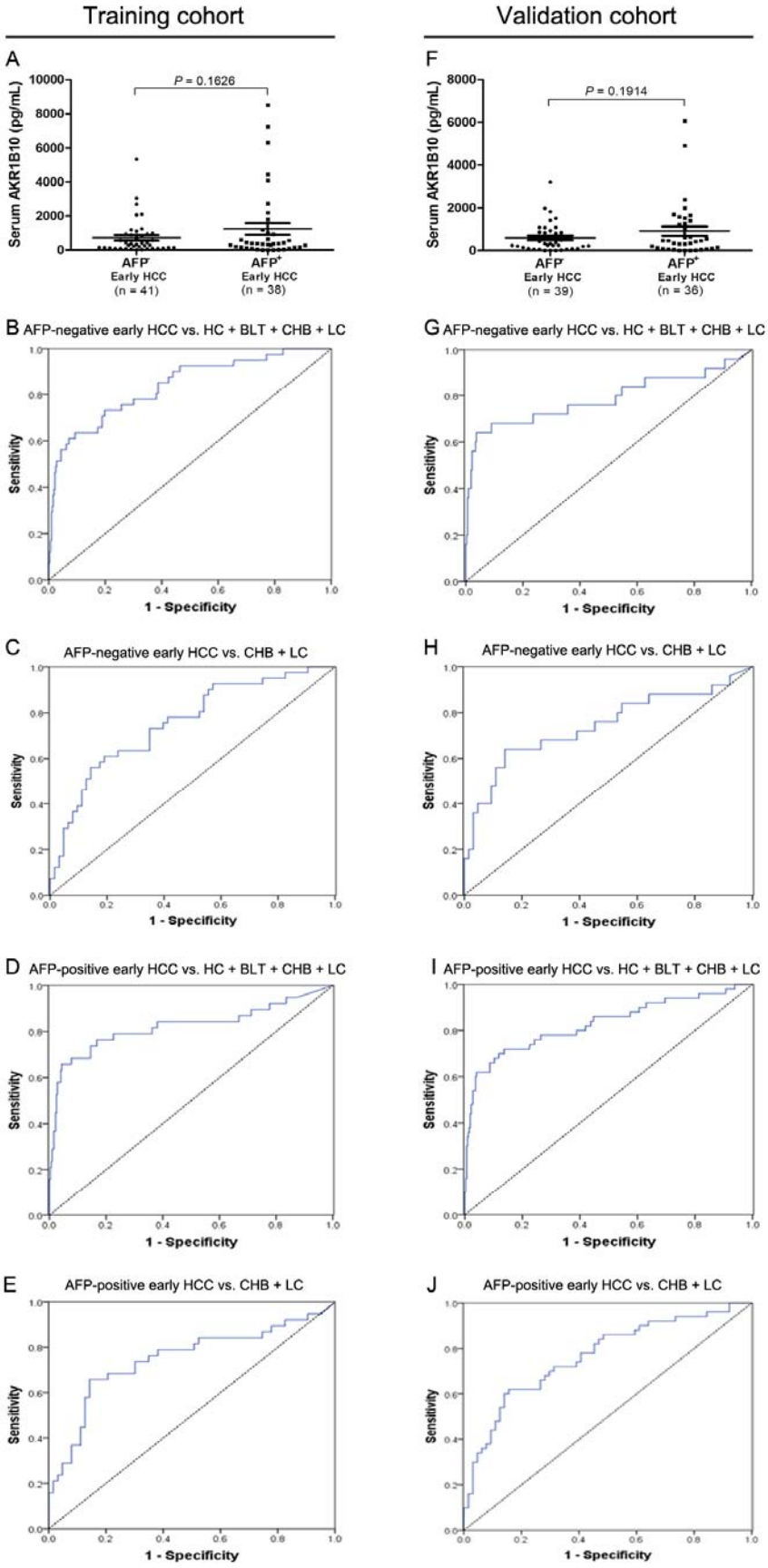
Supporting FIG. S5. Diagnostic accuracy of AKR1B10 in differentiating patients with HCC with or without cirrhosis from patients with LC. (A) Levels of AKR1B10 in patients with LC, patients with HCC with or without cirrhosis, and patients with early HCC with or without cirrhosis in training cohort. (B and C) ROC curve analyses of AKR1B10, AFP, or both in training cohort for patients with HCC with cirrhosis versus patients with LC and patients with early HCC with cirrhosis versus patients with LC, respectively. (D and E) ROC curve analyses of AKR1B10, AFP, or both in training cohort for patients with HCC without cirrhosis versus patients with LC and patients with early HCC without cirrhosis versus patients with LC. (F) Levels of AKR1B10 in patients with LC, patients with HCC with or without cirrhosis, and patients with early HCC with or without cirrhosis in validation cohort. (G and H) ROC curve analyses of AKR1B10, AFP, or both in validation cohort for patients with HCC with cirrhosis versus patients with LC and patients with early HCC with cirrhosis versus patients with LC, respectively. (I and J) ROC curve analyses of AKR1B10, AFP, or both in validation cohort for patients with HCC without cirrhosis versus patients with LC and patients with early HCC without cirrhosis versus patients with LC.



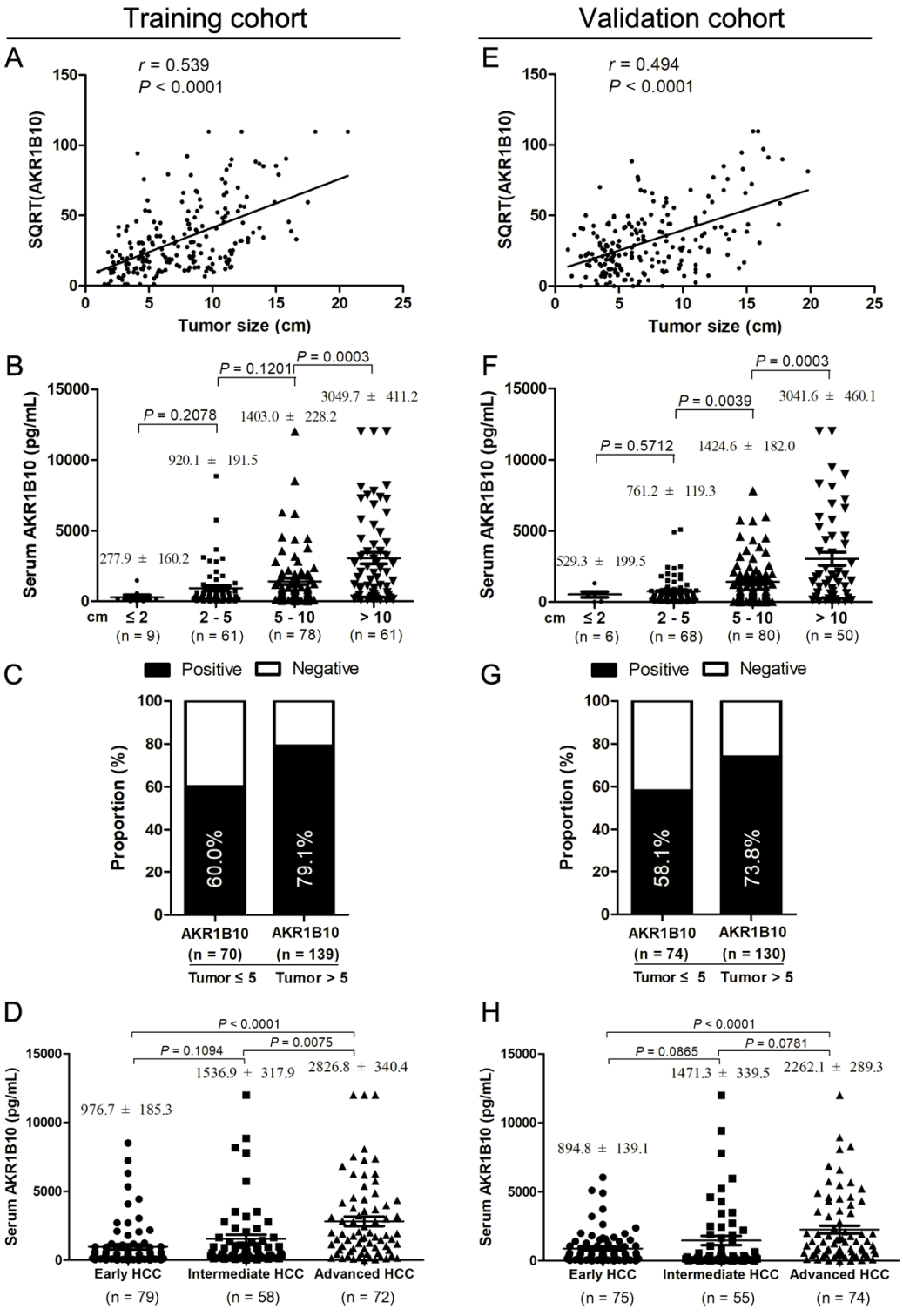
Supporting FIG. S6. Diagnostic accuracy of AKR1B10 in discrimination of patients with HCC from controls when participants in all three cohorts are combined. (A) ROC curves of AKR1B10, AFP, or both for total patients with HCC versus total controls, HCs, or high-risk controls (CHB + LC) from three cohorts. (B) ROC curves of AKR1B10, AFP, or both for total patients with early HCC versus total controls, HCs, or high-risk controls.


Training cohort
Validation cohort
A
P = 0.0382
Serum AKR1B10 (pg/mL)
AFP-
(n = 73)
AFP+
(n = 136)
F
P = 0.0595
AFP-
(n = 66)
AFP+
(n = 138)
B AFP-negative HCC vs. HC + BLT + CHB + LC
G AFP-negative HCC vs. HC + BLT + CHB + LC
C AFP-negative HCC vs. CHB + LC
H AFP-negative HCC vs. CHB + LC
D AFP-positive HCC vs. HC + BLT + CHB + LC
I AFP-positive HCC vs. HC + BLT + CHB + LC
E AFP-positive HCC vs. CHB + LC
J AFP-positive HCC vs. CHB + LC
Sensitivity
1 - Specificity

Supporting FIG. S7. Diagnostic performance of AKR1B10 in AFP-negative or AFP-positive HCC. (A) Levels of AKR1B10 in AFP-negative and AFP-positive patients with HCC in training cohort. (B and C) ROC curve analyses of AKR1B10 in AFP-negative patients with HCC versus all controls or versus high-risk controls (CHB + LC) in training cohort. (D and E) ROC curve analyses of AKR1B10 in AFP-positive patients with HCC versus all controls or versus high-risk controls in training cohort. (F) Levels of AKR1B10 in AFP-negative and AFP-positive patients with HCC in validation cohort. (G and H) ROC curve analyses of AKR1B10 in AFP-negative patients with HCC versus all controls or versus high-risk controls in validation cohort. (I and J) ROC curve analyses of AKR1B10 in AFP-positive patients with HCC versus all controls or versus high-risk controls in validation cohort.


Training cohort
Validation cohort
A
F
Serum AKR1B10 (pg/mL)
P = 0.1626
P = 0.1914
AFP- Early HCC (n = 41)
AFP+ Early HCC (n = 38)
AFP- Early HCC (n = 39)
AFP+ Early HCC (n = 36)
B AFP-negative early HCC vs. HC + BLT + CHB + LC
G AFP-negative early HCC vs. HC + BLT + CHB + LC
C AFP-negative early HCC vs. CHB + LC
H AFP-negative early HCC vs. CHB + LC
D AFP-positive early HCC vs. HC + BLT + CHB + LC
I AFP-positive early HCC vs. HC + BLT + CHB + LC
E AFP-positive early HCC vs. CHB + LC
J AFP-positive early HCC vs. CHB + LC
Sensitivity
1 - Specificity

Supporting FIG. S8. Diagnostic performance of AKR1B10 in AFP-negative or AFP-positive patients with early HCC. (A) Levels of AKR1B10 in AFP-negative and AFP-positive patients with early HCC in training cohort. (B and C) ROC curve analyses of AKR1B10 in AFP-negative patients with early HCC versus all controls or versus high-risk controls (CHB + LC) in training cohort. (D and E) ROC curve analyses of AKR1B10 in AFP-positive patients with early HCC versus all controls or versus high-risk controls in training cohort. (F) Levels of AKR1B10 in AFP-negative and AFP-positive patients with early HCC in validation cohort. (G and H) ROC curve analyses of AKR1B10 in AFP-negative patients with early HCC versus all controls or versus high-risk controls (CHB + LC) in validation cohort. (I and J) ROC curve analyses of AKR1B10 in AFP-positive patients with early HCC versus all controls or versus high-risk controls in validation cohort.


Training cohort
Validation cohort
A
r = 0.539
P < 0.0001
SQRT(AKR1B10)
Tumor size (cm)
E
r = 0.494
P < 0.0001
SQRT(AKR1B10)
Tumor size (cm)
B
P = 0.1201
P = 0.0003
P = 0.2078
3049.7 ± 411.2
1403.0 ± 228.2
920.1 ± 191.5
277.9 ± 160.2
Serum AKR1B10 (pg/mL)
cm ≤ 2 (n = 9)
2 - 5 (n = 61)
5 - 10 (n = 78)
> 10 (n = 61)
F
P = 0.0003
P = 0.0039
3041.6 ± 460.1
P = 0.5712
1424.6 ± 182.0
761.2 ± 119.3
529.3 ± 199.5
Serum AKR1B10 (pg/mL)
cm ≤ 2 (n = 6)
2 - 5 (n = 68)
5 - 10 (n = 80)
> 10 (n = 50)
C
Positive
Negative
Proportion (%)
60.0%
79.1%
AKR1B10 (n = 70)
AKR1B10 (n = 139)
Tumor ≤ 5
Tumor > 5
G
Positive
Negative
Proportion (%)
58.1%
73.8%
AKR1B10 (n = 74)
AKR1B10 (n = 130)
Tumor ≤ 5
Tumor > 5
D
P < 0.0001
P = 0.1094
P = 0.0075
1536.9 ± 317.9
2826.8 ± 340.4
976.7 ± 185.3
Serum AKR1B10 (pg/mL)
Early HCC (n = 79)
Intermediate HCC (n = 58)
Advanced HCC (n = 72)
H
P < 0.0001
P = 0.0865
P = 0.0781
1471.3 ± 339.5
2262.1 ± 289.3
894.8 ± 139.1
Serum AKR1B10 (pg/mL)
Early HCC (n = 75)
Intermediate HCC (n = 55)
Advanced HCC (n = 74)

Supporting FIG. S9. Stratified analysis of serum AKR1B10 levels with tumor size and stages. (A) Linear regression analyses of AKR1B10 levels and tumor size in training cohort. (B) Levels of AKR1B10 in patients with HCC with different tumor sizes in training cohort. (C) Positive rates of AKR1B10 in patients with HCC with tumor size ≤ 5 cm or > 5 cm in training cohort. (D) AKR1B10 levels in patients with HCC at different BCLC stages in training cohort. (E) Linear regression analyses of AKR1B10 levels and tumor size in validation cohort. (F) Levels of AKR1B10 in patients with HCC with different tumor sizes in validation cohort. (G) Positive rates of AKR1B10 in patients with HCC with tumor size ≤ 5 cm or > 5 cm in validation cohort. (H) AKR1B10 levels in patients with HCC at different BCLC stages in validation cohort.

Supporting Tables

Supporting TABLE S1. Eligibility criteria for selection of the participants.

Hepatocellular carcinoma

Inclusion criteria:

1. Age: ≥ 18 and ≤ 80 years, both male and female.
2. Diagnosed based on AFP serology and at least two imaging technologies (hepatic ultrasound, together with CT and/or MRI).
3. Confirmed histopathologically by two independent pathologists.
4. No preoperative chemotherapy, radiotherapy, transarterial chemoembolization, or ablation before collection of blood samples.
5. The subjects volunteer to sign the informed consent.

Exclusion criteria:

1. Patients with non-hepatocellular carcinoma (non-HCC).
2. Receiving any anticancer therapy before blood sample collection.
3. Pregnant or lactating women.
4. Those with clinical diagnosis of acute and chronic gastrointestinal diseases.
5. Those with fasting plasma glucose ≥ 7.0 mmol/L or casual plasma glucose ≥ 11.1 mmol/L.
6. Those with human immunodeficiency virus (HIV) infection or acquired immune deficiency syndrome (AIDS)-associated diseases.
7. Conditions that are considered not suitable for this study.

Healthy control

1. Age: ≥ 18 and ≤ 80 years, both male and female.
2. Serologically negative for hepatitis viruses (hepatitis B surface antigen [HBsAg], hepatitis B e antigen [HBeAg], anti-HBe, and anti-HBc).
3. No diabetes, no liver and gastrointestinal diseases, and no liver malignancies.
4. No history of other systematic malignancies.

Benign liver tumor

1. Age: ≥ 18 and ≤ 80 years, both male and female.
2. Hepatic hemangioma.
3. Focal nodular hyperplasia.
4. Hepatic adenoma.

Chronic hepatitis B

1. Age: ≥ 18 and ≤ 80 years, both male and female.
2. HBsAg-positive >6 months.
3. Serum HBV DNA > 105 copies/mL.
4. Persistent or intermittent elevation in AST or ALT levels.

Liver cirrhosis

1. Age: ≥ 18 and ≤ 80 years, both male and female.
2. With chronic hepatitis B infection history < 20 years.

3. Liver biopsy indicates cirrhosis and liver function detection indicates compensated phase of liver cirrhosis.

4. If no biopsy available, diagnosis must be supported by two imaging technologies.

Supporting TABLE 2. Characteristics of the subjects enrolled in each cohort.

Characteristics	n	Age (years)	Gender (M/F)	AFP (ng/mL)	HBsAg-Positive	Cirrhosis	AST (U/L)	ALT (U/L)	TBIL (μmol/L)	PT (s)	Albumin (g/L)	Tumor Number		Tumor Size (cm)			
												Single	Multiple	≤ 2	2-5	5-10	> 10
Discover cohort—ACHXSM CSU																	
HC	66	50.3 ± 13.1	45/21	9.7 ± 17.3	-	-	18.4 ± 6.3	19.5 ± 7.1	13.7 ± 6.0	-	46.3 ± 2.5	-	-	-	-	-	-
HCC	69	53.8 ± 11.1	58/11	436.7 ± 504.9	63	38	61.7 ± 49.9	44.3 ± 38.4	16.7 ± 7.9	13.0 ± 1.6	36.1 ± 4.0	28	41	3	11	35	20
Training cohort—ACHXSM CSU																	
HC	203	50.7 ± 11.0	128/75	9.1 ± 16.9	-	-	21.2 ± 7.1	20.4 ± 6.2	13.9 ± 5.6	-	47.6 ± 3.9	-	-	-	-	-	-
HCC	209	54.1 ± 12.0	186/23	400.2 ± 503.4	195	101	72.9 ± 66.5	49.9 ± 35.3	21.9 ± 20.1	13.7 ± 1.5	37.1 ± 5.1	111	98	9	61	78	61
BLT	57	47.7 ± 9.8	22/35	6.4 ± 8.1	26	6	29.0 ± 35.1	19.6 ± 20.1	12.7 ± 4.4	12.7 ± 1.1	46.3 ± 2.5	37	20	7	11	30	9
CHB	10	44.1 ± 12.9	5/5	53.9 ± 72.4	10	-	172.4 ± 214.3	270.1 ± 265.1	32.8 ± 31.3	11.9 ± 1.4	41.6 ± 5.3	-	-	-	-	-	-
LC	40	54.5 ± 9.8	32/17	123.4 ± 295.4	33	40	125.9 ± 162.4	111.4 ± 188.8	30.8 ± 20.2	13.7 ± 2.3	35.5 ± 6.3	-	-	-	-	-	-
Validation cohort																	
ACHXSM CSU																	
HC	75	49.6 ± 10.4	47/28	13.3 ± 24.7	-	-	20.1 ± 5.5	18.7 ± 7.4	14.2 ± 5.8	-	48.1 ± 3.4	-	-	-	-	-	-
HCC	71	52.8 ± 11.2	60/11	390.8 ± 501.9	59	38	56.7 ± 43.1	49.8 ± 36.5	22.4 ± 24.8	13.2 ± 1.5	39.3 ± 4.8	45	26	2	25	28	16
BLT	20	54.7 ± 12.8	11/9	11.5 ± 16.8	7	2	35.5 ± 44.6	24.2 ± 25.5	15.1 ± 6.6	12.7 ± 1.0	40.2 ± 4.7	6	14	0	7	11	2
LC	10	55.6 ± 13.6	7/3	115.9 ± 124.8	7	10	117.3 ± 120.5	94.2 ± 92.7	29.1 ± 17.0	12.7 ± 1.2	41.1 ± 4.9	-	-	-	-	-	-
HPPH																	
HC	63	51.2 ± 12.3	36/27	12.3 ± 21.4	-	-	22.3 ± 4.9	20.6 ± 6.1	13.7 ± 4.6	-	47.2 ± 3.1	-	-	-	-	-	-
HCC	64	54.4 ± 9.9	54/10	386.7 ± 493.7	60	30	65.1 ± 50.9	68.7 ± 52.1	28.7 ± 32.4	13.5 ± 1.7	37.2 ± 4.9	37	27	0	21	29	14
BLT	18	52.2 ± 11.7	7/11	10.7 ± 14.8	5	1	46.4 ± 49.1	39.7 ± 33.7	11.4 ± 3.4	12.3 ± 0.9	40.2 ± 2.6	5	13	0	4	9	5
CHB	9	40.3 ± 14.5	6/3	76.9 ± 182.1	6	-	251.6 ± 248.5	372.4 ± 179.3	31.8 ± 35.7	12.5 ± 1.2	43.2 ± 6.8	-	-	-	-	-	-
LC	13	54.9 ± 10.9	7/6	148.0 ± 263.8	8	13	129.0 ± 223.7	114.7 ± 247.9	22.5 ± 14.1	12.0 ± 1.6	37.8 ± 6.3	-	-	-	-	-	-
FAH NUSM																	

HC	70	52.9 ± 9.7	38/32	13.4 ± 22.5	-	-	19.1 ± 7.3	21.4 ± 5.2	12.3 ± 2.6	-	45.8 ± 4.9	-	-	-	-	-	-
HCC	69	53.3 ± 11.3	57/12	397.7 ± 514.5	63	33	75.6 ± 69.7	57.0 ± 43.5	22.1 ± 25.9	13.3 ± 2.2	36.8 ± 5.9	43	26	4	22	23	20
BLT	12	59.2 ± 17.7	5/7	11.8 ± 15.6	3	0	38.0 ± 29.5	36.9 ± 16.7	14.3 ± 5.3	13.6 ± 1.3	39.2 ± 7.0	3	9	0	8	3	1
CHB	13	41.0 ± 13.9	8/5	87.9 ± 224.9	9	-	232.7 ± 236.6	456.7 ± 466.5	35.5 ± 32.2	12.0 ± 1.6	41.8 ± 5.1	-	-	-	-	-	-
LC	15	55.7 ± 10.4	9/6	133.7 ± 315.1	10	15	123.2 ± 89.0	108.5 ± 126.9	36.1 ± 18.0	12.7 ± 2.3	36.8 ± 6.0	-	-	-	-	-	-

-, Not Available (NA).

Abbreviations: ACHXSM CSU, Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University; ALT, alanine aminotransferase; AST, aspartate transaminase; FAH NUSM, First Affiliated Hospital, Nanhua University School of Medicine; HPPH, Hunan Provincial People's Hospital; PT, prothrombin time; TBIL, total bilirubin. Data are presented as the mean ± SD.

Supporting TABLE 3. Information of the excluded patients in each center.

Characteristics	n	Age (years)	Gender (M/F)	AFP (ng/mL)	HBsAg-Positive	Cirrhosis	AST (U/L)	ALT (U/L)	TBIL (μmol/L)	PT (s)	Albumin (g/L)	Main Excluded Reasons
ACHXSM CSU	33	56.7 ± 10.7	19/14	127.5 ± 225.8	18	12	46.6 ± 44.7	52.1 ± 140.3	21.5 ± 37.7	13.6 ± 1.4	36.9 ± 5.2	ICC, other tumor history, anticancer therapy before blood collection, severe gastrointestinal diseases
HPPH	7	55.0 ± 13.6	3/4	123.0 ± 150.2	3	2	33.3 ± 15.1	30.6 ± 19.5	27.8 ± 37.1	12.8 ± 1.0	37.8 ± 3.2	ICC, other tumor history, severe gastrointestinal diseases
FAH NUSM	8	60.4 ± 12.6	6/2	153.7 ± 204.5	4	4	41.8 ± 22.8	35.5 ± 19.8	36.5 ± 58.7	13.2 ± 1.3	37.9 ± 2.9	ICC, other tumor history, anticancer therapy before blood collection

Abbreviations: ACHXSM CSU, Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAH NUSM, First Affiliated Hospital, Nanhua University School of Medicine; HPPH, Hunan Provincial People's Hospital; ICC, intrahepatic cholangiocarcinoma; PT, prothrombin time; TBIL, total bilirubin. Data are presented as the mean ± SD.

Supporting TABLE 4. Clinicopathological characteristics of patients with HCC in training and validation cohorts.

Variable		Training Cohort (n = 209)		Validation Cohort (n = 204)		*P* Value
		no.	Percentage (%)	no.	Percentage (%)	
Gender						
	Female	23	11.00	33	16.18	0.125
	Male	186	89.00	171	83.82	
Age (years)						
	≤ 55	108	51.67	117	57.35	0.247
	> 55	101	48.33	87	42.65	
HBsAg						
	Negative	14	6.70	22	10.78	0.141
	Positive	195	93.30	182	89.22	
AFP (ng/mL)						
	≤ 20	73	34.93	66	32.35	0.580
	> 20	136	65.07	138	67.65	
ALT (U/L)						
	≤ 40	102	48.80	114	55.88	0.150
	> 40	107	51.20	90	44.12	
AST (U/L)						
	≤ 40	80	38.28	96	47.06	0.071
	> 40	129	61.72	108	52.94	
Cirrhosis						
	No	108	51.67	103	50.49	0.810
	Yes	101	48.33	101	49.51	
Tumor size (range, cm)						
	≤ 5	71	33.49	71	34.80	0.859
	> 5	138	66.51	133	65.20	
Tumor number						
	Single	111	53.11	125	61.27	0.094
	Multiple	98	46.89	79	38.73	
Vascular invasion						
	No	144	68.90	128	62.75	0.743
	Yes	65	31.10	62	30.39	
	Missing			14	6.86	
Child-Pugh classification						
	A	161	77.03	160	78.43	0.733
	B + C	48	22.97	44	21.57	
Tumor differentiation						
	I-II	111	49.76	117	57.35	0.807
	III-IV	69	36.36	69	33.82	
	Missing	29	13.88	18	8.82	
BCLC stage						
	0 + A	79	37.80	75	36.76	0.828
	B + C + D	130	62.20	129	63.24	
TNM stage						
	I-II	86	41.15	92	45.10	0.144
	III-IV	123	58.85	98	48.04	
	Missing			14	6.86	

Supporting TABLE 5. Distribution of serum AKR1B10 concentrations of HCC and controls in training and validation cohorts.

Serum AKR1B10 (pg/mL)	Training Cohort					Validation Cohort				
	HC	HCC	BLT	CHB	LC	HC	HCC	BLT	CHB	LC
	(n = 203) (%)	(n = 209) (%)	(n = 57) (%)	(n = 10) (%)	(n = 40) (%)	(n = 208) (%)	(n = 204) (%)	(n = 50) (%)	(n = 22) (%)	(n = 38) (%)
0~268	198 (97.54)	57 (27.27)	57 (100)	10 (100)	31 (77.50)	201 (96.63)	65 (31.86)	48 (96.00)	19 (86.36)	27 (71.05)
269~500	5 (2.46)	35 (16.75)	0	0	3 (7.50)	7 (3.37)	28 (13.73)	2 (4.00)	3 (13.64)	4 (10.53)
501~750	0	12 (5.74)	0	0	2 (5.00)	0	14 (6.86)	0	0	3 (7.89)
751~1000	0	19 (9.09)	0	0	1 (2.50)	0	15 (7.35)	0	0	2 (5.26)
1001~1500	0	16 (7.66)	0	0	1 (2.50)	0	18 (8.82)	0	0	0
1501~2000	0	13 (6.22)	0	0	1 (2.50)	0	20 (9.80)	0	0	2 (5.26)
2001~3000	0	16 (7.66)	0	0	1 (2.50)	0	9 (4.41)	0	0	0
3001~6000	0	23 (11.00)	0	0	0	0	25 (12.25)	0	0	0
> 6000	0	18 (8.61)	0	0	0	0	10 (4.90)	0	0	0

Supporting TABLE 6. Association between AKR1B10 and clinicopathological data of HCC in training and validation cohorts. Serum AKR1B10 Cutoff: 267.9 pg/mL.

Variable		Training Cohort (n = 209)			Validation Cohort (n = 204)		
		Negative	Positive	*P* value	Negative	Positive	*P* value
		57	152		65	139	
Gender							
	Female	5	18	0.528	13	20	0.311
	Male	52	134		52	119	
Age (years)							
	≤ 55	35	73	0.085	39	78	0.601
	> 55	22	79		26	61	
HBsAg							
	Negative	2	12	0.359	11	11	0.053
	Positive	55	140		54	128	
AFP (ng/mL)							
	≤ 20	26	47	0.047	34	32	0.001
	> 20	31	105		31	107	
ALT (U/L)							
	≤ 40	36	66	0.011	45	69	0.009
	> 40	21	86		20	70	
AST (U/L)							
	≤ 40	36	44	0.001	41	55	0.002
	> 40	21	108		24	84	
Cirrhosis							
	No	27	81	0.446	39	64	0.063
	Yes	30	71		26	75	
Tumor size (range, cm)							
	≤ 5	28	42	0.003	31	43	0.020
	> 5	29	110		34	96	
Tumor number							
	Single	31	80	0.821	45	80	0.111
	Multiple	26	72		20	59	
Vascular invasion							
	No	38	106	0.669	44	84	0.098
	Yes	19	46		14	48	
	Missing				7	7	
Child-Pugh classification							
	A	41	120	0.283	53	107	0.461
	B + C	16	32		12	32	
Tumor differentiation							
	I-II	27	84	0.268	45	76	0.047
	III-IV	22	47		16	53	
	Missing	8	21		4	10	
BCLC stage							
	0 + A	31	48	0.002	28	47	0.201
	B + C + D	26	104		37	92	
TNM stage							
	I-II	22	64	0.646	25	67	0.206
	III-IV	35	88		35	63	
	Missing				5	9	

Supporting TABLE 7. Parameters of AKR1B10 in differentiating HCC from HC, BLT, CHB, or LC controls.

	Training Cohort							Validation Cohort						
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR	AUC (95%CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR	AUC (95%CI)
HCC vs. HC														
AKR1B10	71.3%	96.1%	94.9%	76.5%	18.29	0.30	0.892 (0.858-0.920)	72.5%	94.2%	92.5%	77.8%	12.58	0.29	0.850 (0.811-0.883)
AFP	65.1%	91.6%	91.3%	72.2%	7.76	0.37	0.838 (0.799-0.873)	73.5%	78.8%	77.3%	75.2%	3.48	0.34	0.814 (0.773-0.850)
AKR1B10 + AFP	79.0%	95.0%	96.5%	81.7%	15.71	0.22	0.935 (0.907-0.957)	74.0%	98.6%	98.1%	79.5%	51.32	0.26	0.910 (0.878-0.936)
Early-stage HCC vs. HC														
AKR1B10	70.9%	83.7%	62.9%	88.1%	4.36	0.35	0.806 (0.755-0.851)	68.0%	94.2%	81.0%	89.1%	11.79	0.34	0.826 (0.776-0.868)
AFP	59.5%	83.3%	58.0%	84.1%	3.55	0.49	0.744 (0.689-0.794)	65.3%	83.7%	59.0%	87.0%	4.00	0.41	0.757 (0.703-0.806)
AKR1B10 + AFP	76.0%	87.2%	69.8%	90.3%	5.93	0.28	0.866 (0.821-0.904)	70.7%	90.9%	73.6%	89.6%	7.74	0.32	0.838 (0.790-0.879)
HCC vs. BLT														
AKR1B10	68.0%	91.83	75.0%	88.8%	8.32	0.35	0.796 (0.744-0.841)	71.1%	98.0%	99.3%	45.4%	35.54	0.30	0.885 (0.839-0.922)
AFP	65.3%	74.04	47.6%	85.6%	2.52	0.47	0.697 (0.640-0.750)	64.2%	96.0%	98.5%	39.7%	16.05	0.37	0.841 (0.790-0.884)
AKR1B10 + AFP	70.7%	97.60	91.4%	90.2%	29.40	0.30	0.872 (0.827-0.908)	88.7%	98.0%	99.5%	68.1%	44.36	0.12	0.956 (0.922-0.977)
Early-stage HCC vs. BLT														
AKR1B10	60.7%	96.4%	96.0%	64.0%	17.32	0.41	0.833 (0.760-0.892)	69.3%	90.0%	91.2%	66.2%	6.93	0.34	0.836 (0.759-0.896)
AFP	75.9%	71.9%	78.9%	68.3%	2.71	0.33	0.79 1(0.713-0.856)	48.0%	96.0%	94.7%	55.2%	12.00	0.54	0.757 (0.672-0.829)
AKR1B10 + AFP	75.9%	98.3%	98.4%	74.7%	43.29	0.24	0.892 (0.827-0.939)	77.3%	94.0%	95.1%	73.4%	12.89	0.24	0.903 (0.838-0.949)
HCC vs. CHB														

AKR1B10	73.2%	90.0%	99.4%	13.8%	7.32	0.30	0.869 (0.817-0.911)	67.6%	95.5%	99.3%	24.1%	14.88	0.34	0.848 (0.795-0.892)
AFP	38.3%	90.0%	98.8%	6.5%	3.83	0.69	0.661 (0.595-0.724)	67.6%	77.3%	96.5%	20.5%	2.98	0.42	0.716 (0.652-0.774)
AKR1B10 + AFP	77.5%	90.0%	99.4%	16.1%	7.75	0.25	0.904 (0.857-0.940)	72.1%	90.9%	98.7%	26.0%	7.93	0.31	0.878 (0.828-0.918)
Early-stage HCC vs. CHB														
AKR1B10	62.0%	90.0%	98.0%	23.1%	6.20	0.42	0.792 (0.693-0.871)	62.7%	81.8%	92.2%	39.1%	3.45	0.46	0.824 (0.733-0.894)
AFP	26.6%	90.0%	95.5%	13.4%	2.66	0.82	0.542 (0.433-0.648)	66.7%	77.3%	90.9%	40.5%	2.93	0.43	0.679 (0.577-0.771)
AKR1B10 + AFP	62.0%	90.0%	98.0%	23.1%	6.20	0.42	0.828 (0.733-0.900)	64.0%	90.9%	96.0%	42.6%	7.04	0.40	0.822 (0.731-0.892)
HCC vs. LC														
AKR1B10	72.7%	83.0%	94.4%	43.6%	4.28	0.33	0.828 (0.776-0.871)	65.7%	78.6%	88.4%	32.0%	3.07	0.44	0.762 (0.704-0.814)
AFP	61.1%	79.3%	92.1%	34.1%	2.95	0.49	0.721 (0.663-0.775)	68.1%	59.5%	89.1%	27.8%	1.68	0.54	0.662 (0.599-0.721)
AKR1B10 + AFP	79.9%	83.0%	94.9%	51.2%	4.71	0.24	0.873 (0.826-0.911)	77.0%	73.8%	93.5%	39.7%	2.94	0.31	0.812 (0.758-0.859)
Early-stage HCC vs. LC														
AKR1B10	60.8%	83.0%	84.2%	58.7%	3.58	0.47	0.743 (0.660-0.816)	61.3%	78.6%	83.6%	53.2%	2.86	0.49	0.724 (0.633-0.802)
AFP	46.6%	79.2%	76.6%	49.4%	2.20	0.69	0.605 (0.516-0.689)	66.7%	59.5%	74.6%	50.0%	1.65	0.56	0.612 (0.517-0.700)
AKR1B10 + AFP	84.8%	66.0%	78.8%	74.5%	2.50	0.23	0.798 (0.719-0.863)	60.0%	78.6%	83.3%	52.4%	2.80	0.51	0.730 (0.640-0.807)

Abbreviations: AFP, alpha-fetoprotein; AKR1B10, aldo-keto reductase family 1 member B10; AUC, area under the curve; BLT, benign liver tumor; CHB, chronic hepatitis B virus; HCC, hepatocellular carcinoma; HC, healthy control; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

Supporting TABLE 8. Parameters of AKR1B10 in differentiating HCC with or without cirrhosis from LC.

	Training Cohort							Validation Cohort						
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR	AUC (95%CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR	AUC (95%CI)
HCC with cirrhosis vs. LC														
AKR1B10	70.3%	83.0%	88.7%	59.5%	4.14	0.36	0.813 (0.742-0.871)	71.3%	78.6%	88.9%	53.2%	3.33	0.37	0.782 (0.705-0.846)
AFP	62.4%	77.4%	84.0%	51.9%	2.75	0.49	0.729 (0.652-0.798)	79.2%	59.5%	82.5%	54.3%	1.96	0.35	0.711 (0.630-0.784)
AKR1B10 + AFP	76.2%	83.0%	89.5%	64.7%	4.49	0.29	0.859 (0.794-0.910)	86.1%	73.8%	88.8%	68.9%	3.29	0.19	0.848 (0.773-0.902)
Early-stage HCC with cirrhosis vs. LC														
AKR1B10	59.5%	83.0%	71.0%	74.6%	3.50	0.49	0.721 (0.616-0.810)	67.5%	78.6%	75.0%	71.7%	3.15	0.41	0.724 (0.614-0.817)
AFP	51.4%	79.2%	63.0%	70.0%	2.47	0.61	0.650 (0.542-0.748)	70.0%	59.5%	62.2%	67.6%	1.73	0.50	0.617 (0.503-0.722)
AKR1B10 + AFP	81.1%	66.0%	62.5%	83.3%	2.39	0.29	0.776 (0.676-0.857)	62.5%	83.3%	78.1%	70.0%	3.75	0.45	0.720 (0.610-0.813)
HCC without cirrhosis vs. LC														
AKR1B10	75.0%	83.0%	90.0%	62.0%	4.42	0.30	0.841 (0.776-0.894)	60.2%	78.6%	87.3%	44.6%	2.81	0.51	0.742 (0.663-0.811)
AFP	63.0%	79.2%	86.1%	51.2%	3.03	0.47	0.714 (0.638-0.782)	33.0%	95.2%	94.4%	36.7%	6.93	0.70	0.613 (0.529-0.693)
AKR1B10 + AFP	83.3%	83.0%	90.9%	71.0%	4.91	0.20	0.886 (0.826-0.930)	59.2%	83.3%	89.7%	45.5%	3.55	0.49	0.777 (0.701-0.842)
Early-stage HCC without cirrhosis vs. LC														
AKR1B10	69.0%	77.4%	70.7%	75.9%	3.50	0.40	0.764 (0.666-0.845)	71.4%	66.7%	64.1%	73.7%	2.14	0.43	0.723 (0.609-0.819)
AFP	33.3%	88.7%	70.0%	62.7%	2.94	0.75	0.566 (0.460-0.667)	62.9%	59.5%	56.4%	65.8%	1.55	0.62	0.606 (0.488-0.715)
AKR1B10 + AFP	88.1%	66.0%	67.3%	87.5%	2.59	0.18	0.817 (0.725-0.889)	71.4%	64.3%	62.5%	73.0%	2.00	0.44	0.751 (0.639-0.843)

Abbreviations: AFP, alpha-fetoprotein; AKR1B10, aldo-keto reductase family 1 member B10; AUC, area under the curve; HCC, hepatocellular carcinoma; HC, healthy control; LC, liver cirrhosis; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

Supporting TABLE 9. Differentiating parameters of AKR1B10 from pooled all patients with HCC and controls.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR	AUC (95%CI)
Total HCC vs. total HC + BLT + CHB + LC							
AKR1B10	73.2%	91.0%	84.7%	83.4%	8.14	0.29	0.869 (0.848-0.888)
AFP	65.8%	86.1%	76.1%	78.7%	4.71	0.40	0.803 (0.779-0.825)
AKR1B10 + AFP	78.4%	94.7%	90.9%	86.6%	14.67	0.23	0.920 (0.903-0.934)
Total early HCC vs. total HC + BLT + CHB + LC							
AKR1B10	65.9%	91.0%	64.0%	91.6%	7.32	0.37	0.819 (0.792-0.844)
AFP	58.4%	85.8%	50.0%	89.4%	4.11	0.49	0.749 (0.719-0.777)
AKR1B10 + AFP	71.7%	88.7%	60.8%	92.8%	6.37	0.32	0.857 (0.833-0.880)
Total HCC vs. total HC							
AKR1B10	72.8%	93.1%	91.4%	77.2%	10.53	0.29	0.876 (0.853-0.896)
AFP	65.6%	88.7%	85.4%	71.8%	5.79	0.39	0.817 (0.792-0.841)
AKR1B10 + AFP	79.3%	97.1%	96.0%	82.2%	23.63	0.21	0.930 (0.912-0.945)
Total early HCC vs. total HC							
AKR1B10	64.2%	92.2%	75.0%	87.6%	8.27	0.39	0.802 (0.769-0.832)
AFP	50.3%	88.5%	61.3%	83.1%	4.36	0.56	0.725 (0.689-0.759)
AKR1B10 + AFP	72.3%	92.2%	77.2%	90.2%	9.31	0.30	0.867 (0.839-0.892)
Total HCC vs. total CHB + LC							
AKR1B10	65.6%	86.6%	94.9%	39.9%	4.90	0.40	0.810 (0.760-0.840)
AFP	65.6%	67.7%	88.5%	34.1%	2.03	0.51	0.692 (0.654-0.729)
AKR1B10 + AFP	78.4%	81.1%	94.0%	49.8%	4.15	0.27	0.857 (0.828-0.884)
Total early HCC vs. total CHB + LC							
AKR1B10	56.6%	86.6%	85.2%	59.5%	4.23	0.50	0.747 (0.694-0.795)
AFP	56.1%	70.1%	71.9%	53.9%	1.87	0.63	0.629 (0.571-0.684)
AKR1B10 + AFP	65.9%	81.1%	82.6%	63.6%	3.49	0.42	0.779 (0.728-0.825)

Abbreviations: AFP, alpha-fetoprotein; AKR1B10, aldo-keto reductase family 1 member B10; AUC, area under the curve; BLT, benign liver tumor; CHB, chronic hepatitis B virus; HCC, hepatocellular carcinoma; HC, healthy control; LC, liver cirrhosis; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.