Serum levels of ITGBL1 as an early diagnostic biomarker for hepatocellular carcinoma with hepatitis B virus infection

SUPPLEMENTARY MATERIALS

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1 Definitions of Liver cirrhosis

(1) Histopathology is consistent with the diagnosis of liver cirrhosis;

(2) Without liver biopsy results, the patient should meet 1 of the following criteria:

− Ascites

− Hepatic encephalopathy

− Upper gastro-intestinal (esophageal and/or gastroduodenal) bleeding

− Hepatorenal syndrome

But non-cirrhotic portal hypertension should be excluded.

(3) If none of the criteria above are met, the patient should meet any 2 of the following

criteria:

− Liver imaging (including B ultrasound, LSM or CT) showing characteristics of cirrhosis: nodular liver and/or splenomegaly

− Platelet count <150,000/mm^3 in the absence of other explanation

− Liver stiffness measurement (by Fibroscan) >12 kPa

− Gastro-esophageal varices as visualized by upper endoscop

Supplementary Table 1: Patients clinicopathological characteristics in training and validation cohort.

Baseline clinicopathological characteristics.

Variable	Training cohort(n=299)	Validation cohort(n=417)	p-Value
Age, mean±SD(years)	52±12	52±12	P=0.7221
Gender(male), n(%)	238(79.60)	321(76.99)	P=0.412
Platelet count(×109/L)	147(88.5-200.5)	164(91.5-209)	P=0.1774
White blood cells（×109/L）	5.2(4.05-6.50)	5.41(4.10-6.63)	P=0.3515
ALT, (IU/L)	34(22-61)	31(20-51)	P=0.0234
AST, (IU/L)	36(24-67)	30(23-48)	P=0.0010
GGT, (IU/L)	43(24-118)	36(21-70)	P=0.0024
ITGBL1	55.42(33.68-77.65)	54.03(34.33-78.57)	P=0.7194
AFP, (ng/mL)	5.89(2.72-38.51)	3.575(2.40-16.23)	P=0.0008
≥400 ng/ml, n (%)	30(10.03)	40(9.59)	P=0.899
≥20 ng/ml, n (%)	88(29.43)	92(22.06)	P=0.429
<20 ng/ml, n (%)	211(70.57)	257(61.63)	
Albumin(μmol/L)	40.65(34.0-44.53)	42(36-46)	P=0.0030
Total bilirubin(μmol/L)	17.5(13.5-29.2)	17.8(12.85-27.5)	P=0.5580
HBeAg-Positive, n (%)	72(24.08)	111(26.62)	P=0.487
Cirrhosis, n (%)			P=0.096
Yes	158(52.84)	194(46.52)	
No	141(47.15)	223(53.47)	
Child class, n (%)			P=0.035
A	241(80.60)	332(79.61)	
B	47(15.71)	62(14.8)	
C	10(3.34)	9(2.15)	
Missing	1(0.33)	14(3.35)	
BCLC stage, n (%)			P=0.383
0+A	51(17.05)	65(15.58)	
B	21(7.02)	27(6.47)	
C-D	33(11.03)	35(8.39)	
Missing	1(0.33)	6(1.43)	

Abbreviations: ITGBL1, Integrin beta-like 1; AFP, alpha‐fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpetidase; HBeAg, hepatitis B e antigen; BCLC, Barcelona Clinic Liver Cancer staging system

Supplementary Table 2: The value of AFP and ITGBL1 alone or in combination in the diagnosis of HBV-related HCC

	AUC (95%CI)	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Positive LR	Negative LR
HCC vs At-risk							
Training							
ITGBL1	0.773 (0.716-0.823)	66.98	71.62	62.8	75.2	2.36	0.46
AFP20	0.705 (0.644-0.760)	58.49	82.43	70.5	73.5	3.33	0.5
AFP400	0.617 (0.554-0.677)	25.47	97.97	90.0	64.7	12.57	0.76
ITGBL1+AFP20	0.840 (0.789-0.883)	70.75	82.43	74.3	79.7	4.03	0.35
ITGBL1+AFP400	0.820 (0.767-0.865)	69.81	77.7	69.2	78.2	3.13	0.39
Validation							
ITGBL1	0.732 (0.682-0.778)	62.41	74.07	59.7	76.2	2.41	0.51
AFP20	0.773 (0.725-0.816)	60.15	94.44	87.0	79.4	10.83	0.42
AFP400	0.650 (0.598-0.700)	30.08	100	100	69.9		0.7
ITGBL1+AFP20	0.868 (0.828-0.902)	69.92	92.13	84.5	83.3	8.88	0.33
ITGBL1+AFP400	0.822 (0.778-0.861)	75.94	74.07	64.3	83.3	2.93	0.32
HCC vs LC							
Training							
ITGBL1	0.721 (0.648-0.787)	58.49	77.27	80.5	53.7	2.57	0.54
AFP20	0.679 (0.603-0.748)	58.49	77.27	80.5	53.7	2.57	0.54
AFP400	0.627 (0.550-0.700)	25.47	100	100	45.5		0.75
ITGBL1+AFP20	0.794 (0.726-0.852)	78.3	68.18	79.8	66.2	2.46	0.32
ITGBL1+AFP400	0.793 (0.725-0.851)	69.81	77.27	83.1	61.4	3.07	0.39
Validation							
ITGBL1	0.717 (0.654-0.773)	62.41	73.27	75.5	59.7	2.33	0.51
AFP20	0.756 (0.696-0.810)	60.15	91.09	89.9	63.4	6.75	0.44
AFP400	0.650 (0.586-0.711)	30.08	100	100	52.1		0.7
ITGBL1+AFP20	0.847 (0.794-0.891)	64.66	92.08	91.5	66.4	8.16	0.38
ITGBL1+AFP400	0.811 (0.755-0.859)	75.94	73.27	78.9	69.8	2.84	0.33

Abbreviations: ITGBL1, Integrin beta-like 1; AFP, α-fetoprotein; AUC, area under curve; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; HCC, hepatocellular carcinoma; LC, liver cirrhosis; at-risk controls, patients with chronic hepatitis B and HBV-related cirrhosis;ITGBL1, Integrin beta-like 1; AFP, alpha fetoprotein; AFP20, 20 ng/mL of alpha fetoprotein as a cutoff value; AFP400, 400 ng/mL of alpha fetoprotein as a cutoff value.; AUC, area under curve; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Supplementary Table 3: Results for measurement of serum ITGBL1, AFP, or both in the detection of early-stage HBV-related HCC by the different staging systems.

	Training								Validation							
	AUC (95%CI)	Sensitivity (%)	Specificity (%)	PPV(%)	NPV(%)	Positive LR	Negative LR	p value	AUC (95%CI)	Sensitivity y(%)	Specificity y(%)	PPV(%)	NPV(%)	Positive LR	Negative LR	p value
Early-stage HCC (BCLC 0+A HCC) vs HBV-LC and CHB																
ITGBL1	0.787(0.723-0.842)	62.75	79.73	51.6	86.1	3.10	0.47		0.764(0.710-0.813)	67.69	74.07	44.0	88.4	2.61	0.44	
AFP20	0.638(0.567-0.704)	45.10	82.43	46.9	81.3	2.57	0.67	P=0.0049	0.718(0.662-0.770)	49.23	94.44	72.7	86.1	8.86	0.54	P=0.3041
AFP400	0.539(0.467-0.610)	9.80	97.97	62.5	75.9	4.84	0.92	P<0.0001	0.608(0.548-0.665)	21.54	100	100	80.9		0.78	P=0.0002
ITGBL1+AFP20	0.824(0.764-0.875)	82.35	68.24	47.2	91.8	2.59	0.26	P=0.0784	0.851(0.804-0.891)	83.08	71.30	46.6	93.3	2.89	0.24	P=0.0011
ITGBL1+AFP400	0.800(0.737-0.853)	68.63	77.70	51.5	87.8	3.08	0.40	P=0.3701	0.818(0.767-0.861)	76.92	74.07	47.2	91.4	2.97	0.31	P=0.0114
Early-stage HCC (Milan criteria HCC) vs HBV-LC and CHB																
ITGBL1	0.786(0.719-0.843)	66.67	75.68	40.0	90.3	2.74	0.44		0.728(0.672-0.779)	62.12	74.07	42.3	86.5	2.40	0.51	
AFP20	0.620(0.546-0.691)	41.67	82.43	36.6	85.3	2.37	0.71	P=0.0104	0.722(0.666-0.774)	50.00	94.44	73.3	86.1	9.00	0.53	P=0.9043
AFP400	0.518(0.443-0.592)	5.56	97.97	40.0	81.0	2.74	0.96	P<0.0001	0.598(0.539-0.656)	19.70	100	100	80.3		0.8	P=0.0034
ITGBL1+AFP20	0.819(0.756-0.872)	83.33	68.24	39.0	94.4	2.62	0.24	P=0.1659	0.836(0.788-0.878)	62.12	92.13	70.7	88.8	7.89	0.41	P=0.0002
ITGBL1+AFP400	0.790(0.724-0.847)	69.44	75.00	40.3	91.0	2.78	0.41	P=0.6784	0.790(0.738-0.836)	72.73	74.07	46.2	89.9	2.81	0.37	P=0.0069
Early-stage HCC (TNM stgeing HCC) vs HBV-LC and CHB																
ITGBL1	0.758(0.688-0.818)	100	37.16	26.2	100	1.59	0		0.765(0.711-0.814)	72.13	74.07	44.0	90.4	2.78	0.38	
AFP20	0.609(0.534-0.681)	39.39	82.43	33.3	85.9	2.24	0.74	P=0.0159	0.726(0.670-0.778)	50.82	94.44	72.1	87.2	9.15	0.52	P=0.4347
AFP400	0.520(0.445-0.595)	6.06	97.97	40.0	82.4	2.99	0.96	P<0.0001	0.598(0.538-0.657)	19.67	100	100	81.5		0.8	P=0.0004
ITGBL1+AFP20	0.777(0.709-0.835)	72.73	67.57	33.3	91.7	2.24	0.40	P=0.3834	0.863(0.817-0.902)	88.52	71.30	46.6	95.7	3.08	0.16	P=0.0012
ITGBL1+AFP400	0.762(0.693-0.822)	63.64	74.32	34.5	89.4	2.48	0.49	P=0.7282	0.827(0.777-0.869)	81.97	74.07	47.2	93.6	3.16	0.24	P=0.0120

Abbreviations: ITGBL1, Integrin beta-like 1; AFP, α-fetoprotein; AUC, area under curve; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; HCC, hepatocellular carcinoma; CHB, chronic hepatitis B virus infection; LC, liver cirrhosis, BCLC, Barcelona Clinic Liver Cancer staging system.
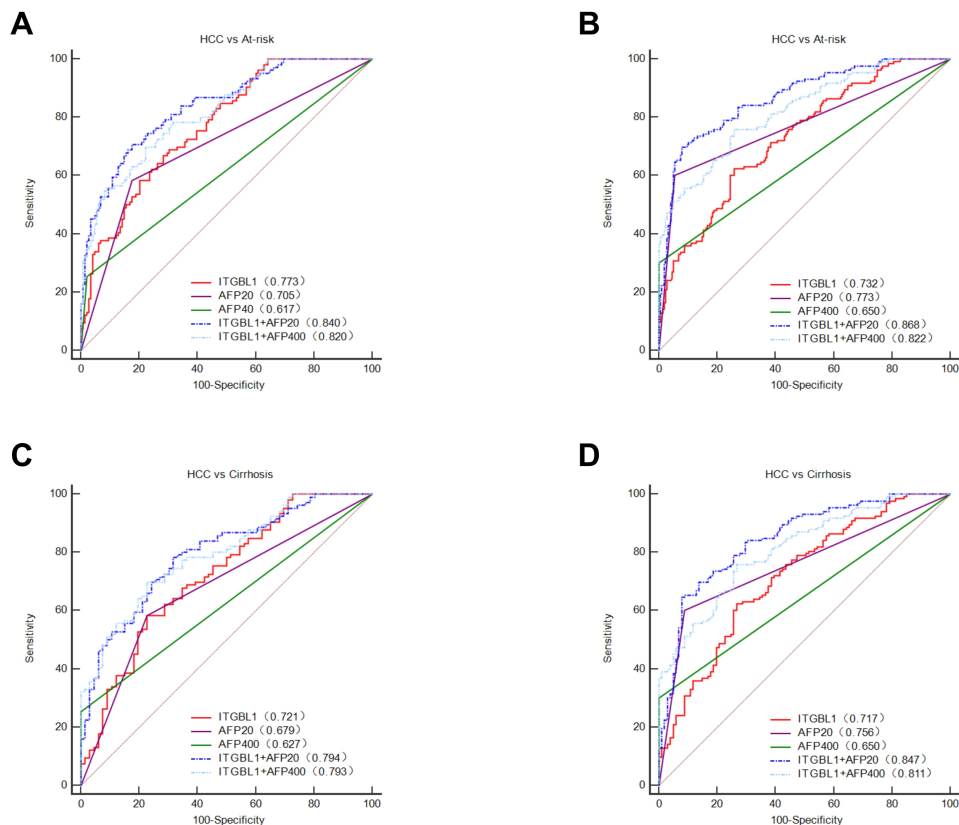
Supplementary Table 4: Results for measurement of ITGBL1 in serum in the diagnosis of AFP<20ng/mL and AFP<400ng/mL patients with HBV-related HCC and early-stage HBV-related HCC.

		AUC (95%CI)	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Positive LR	Negative LR
AFP(<400ng/ml)								
HCC vs At-risk								
	Training	0.772(0.712-0.825)	70.89	69.66	56.0	81.5	2.34	0.42
	Validation	0.745(0.693-0.793)	65.59	74.07	52.1	83.3	2.53	0.46
HCC vs Cirrhosis								
	Training	0.723(0.642-0.794)	59.49	77.27	75.8	61.4	2.62	0.52
	Validation	0.730(0.662-0.791)	65.59	73.27	69.3	69.8	2.45	0.47
Early-stage HCC vs At-risk								
	Training	0.788(0.723-0.844)	69.57	75.17	47.1	88.6	2.8	0.4
	Validation	0.768(0.712-0.817)	70.59	74.07	39.1	91.4	2.72	0.4
Early-stage HCC vs Cirrhosis								
	Training	0.740(0.649-0.819)	65.22	77.27	66.7	76.1	2.87	0.45
	Validation	0.752(0.676-0.819)	70.59	73.27	57.1	83.1	2.64	0.4
AFP(<20ng/ml)								
HCC vs At-risk								
	Training	0.756(0.683-0.819)	68.18	68.85	44.1	85.7	2.19	0.46
	Validation	0.744(0.686-0.796)	81.13	55.88	32.3	91.9	1.84	0.34
HCC vs Cirrhosis								
	Training	0.698(0.595-0.788)	61.36	70.59	64.3	67.9	2.09	0.55
	Validation	0.737(0.658-0.807)	79.25	59.78	53.2	83.3	1.97	0.35
Early-stage HCC vs At-risk								
	Training	0.786(0.712-0.849)	75	68.85	35.6	92.3	2.41	0.36
	Validation	0.767(0.708-0.819)	66.67	75.49	30.6	93.3	2.72	0.44
Early-stage HCC vs Cirrhosis								
	Training	0.733(0.622-0.826)	67.86	70.59	55.9	80.0	2.31	0.46
	Validation	0.764(0.680-0.835)	87.88	55.43	41.4	92.7	1.97	0.22

Abbreviations: ITGBL1, Integrin beta-like 1; AFP, α-fetoprotein; AUC, area under curve; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; HCC, hepatocellular carcinoma; CHB, chronic hepatitis B virus infection; LC, liver cirrhosis; at-risk controls, patients with chronic hepatitis B and HBV-related cirrhosis.
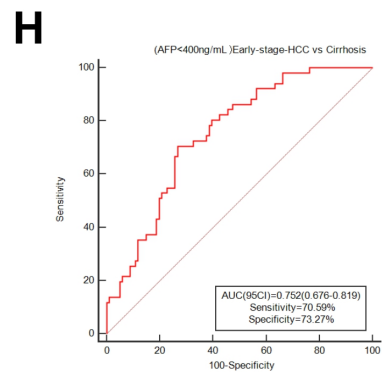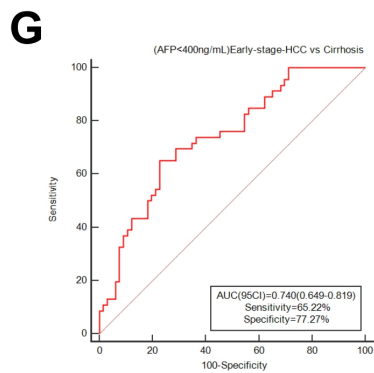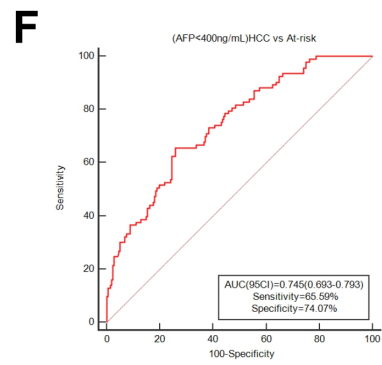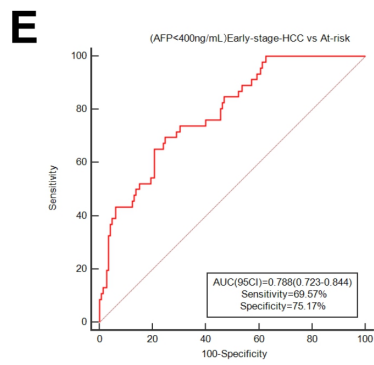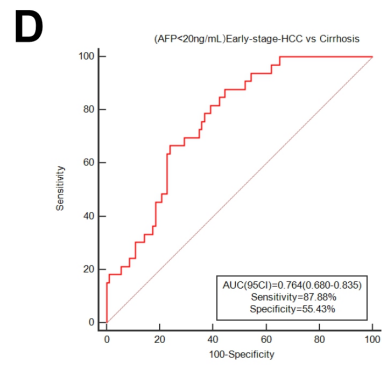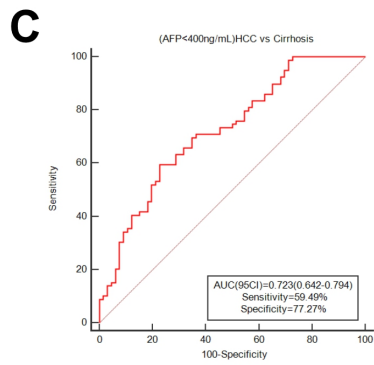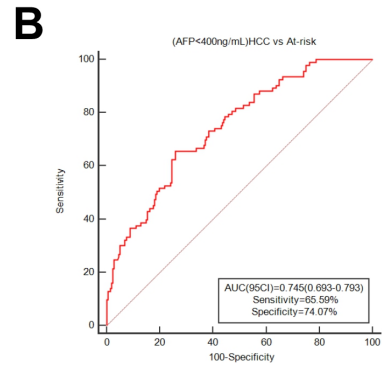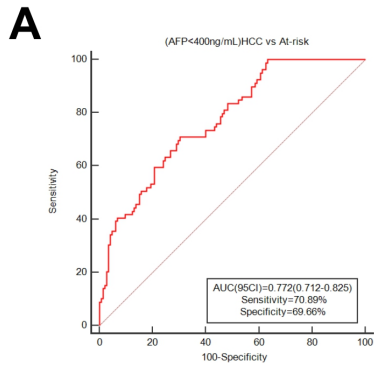
Supplementary Figure 1: Diagnostic performance for serum ITGBL1 for HBV-HCC patients.

ROC curve for ITGBL1, AFP, or both, for patients with all HBV-HCC versus controls at risk of HBV-HCC in the training cohort (A). ROC curve of ITGBL1, AFP, or both, for patients with all HBV-HCC versus controls at risk of HBV-HCC in the validation cohort (B). ROC curve for ITGBL1, AFP, or both, for patients with all HBV-HCC versus controls at HBV-LC in the training cohort (C). ROC curve of ITGBL1, AFP, or both, for patients with all HBV-HCC versus controls at HBV-LC in the validation cohort (D). Abbreviations: ROC, receiver operating characteristics; HCC, hepatocellular carcinoma; At-risk controls, patients with chronic hepatitis B and HBV-related cirrhosis; HBV, hepatitis B virus; LC, liver cirrhosis; ITGBL1, Integrin beta-like 1; AFP, alpha fetoprotein; AFP20, 20 ng/mL of alpha fetoprotein as a cutoff value; AFP400, 400 ng/mL of alpha fetoprotein as a cutoff value.
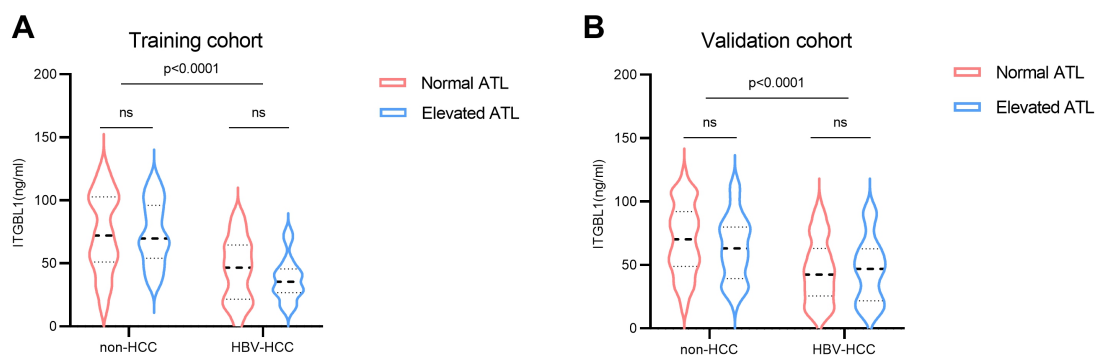
Supplementary Figure 2: Diagnostic performance for serum ITGBL1 in AFP <400 ng/mL HBV-HCC patients and early-stage HBV-HCC

ROC curve for ITGBL1 distinguishing individuals with AFP <400 ng/mL HBV-HCC from at-risk groups in the training cohort(A). ROC curve for ITGBL1 distinguishing individuals with AFP <400 ng/mL HBV-HCC from at-risk groups in the validation cohort(B). ROC curve for ITGBL1 distinguishing individuals with AFP <400 ng/mL HBV-HCC from HBV-LC patientsin the training cohort (C). ROC curve for ITGBL1 distinguishing individuals with AFP<400 ng/mL HBV-HCC from HBV-LC patientsin the validation cohort (D). ROC curve of ITGBL1 distinguishing individuals with AFP <400 ng/mL early-stage HBV-HCC from at-risk groups the training cohort (E). ROC curve of ITGBL1 distinguishing individuals with AFP <400 ng/mL early-stage HBV-HCC from at-risk groups the validation cohort (F). ROC curve of ITGBL1 distinguishing individuals with AFP <400 ng/mL HBV-HCC from HBV-HCC patients the training cohort(G). ROC curve of ITGBL1 distinguishing individuals with AFP<400 ng/mL HBV-HCC from HBV-HCC patients the validation cohort(G). Abbreviations: ROC, receiver operating characteristics; HCC, hepatocellular carcinoma; At-risk groups, patients with chronic hepatitis B and HBV-related cirrhosis; HBV, hepatitis B virus; LC, liver cirrhosis; ITGBL1, Integrin beta-like 1; AFP, alpha fetoprotein;AUC, area under the ROC curve; CI, confidence interval.
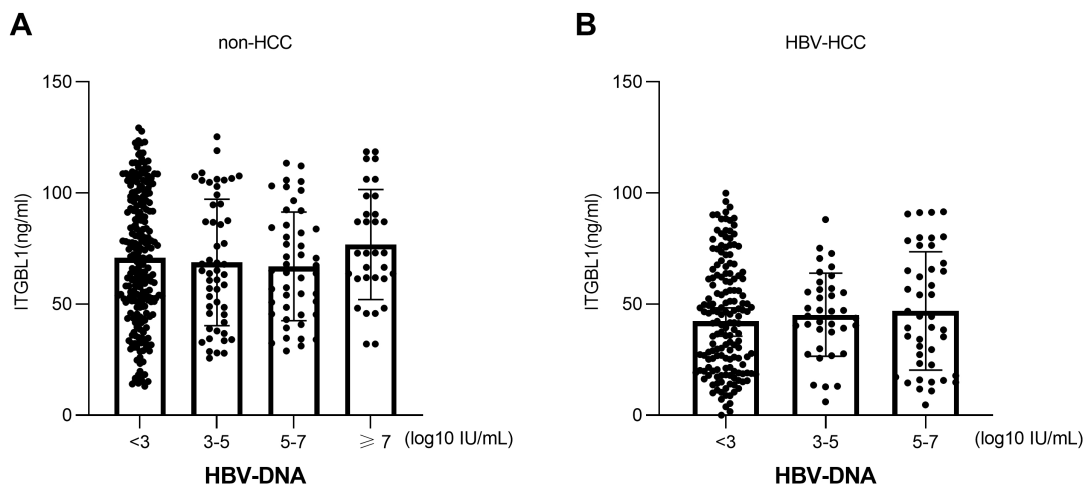
A — (AFP<400ng/mL)HCC vs At-risk

Sensitivity / 100-Specificity

AUC(95CI)=0.772(0.712-0.825)
Sensitivity=70.89%
Specificity=69.66%

B — (AFP<400ng/mL)HCC vs At-risk

AUC(95CI)=0.745(0.693-0.793)
Sensitivity=65.59%
Specificity=74.07%

C — (AFP<400ng/mL)HCC vs Cirrhosis

AUC(95CI)=0.723(0.642-0.794)
Sensitivity=59.49%
Specificity=77.27%

D — (AFP<20ng/mL)Early-stage-HCC vs Cirrhosis

AUC(95CI)=0.764(0.680-0.835)
Sensitivity=87.88%
Specificity=55.43%

E — (AFP<400ng/mL)Early-stage-HCC vs At-risk

AUC(95CI)=0.788(0.723-0.844)
Sensitivity=69.57%
Specificity=75.17%

F — (AFP<400ng/mL)HCC vs At-risk

AUC(95CI)=0.745(0.693-0.793)
Sensitivity=65.59%
Specificity=74.07%

G — (AFP<400ng/mL)Early-stage-HCC vs Cirrhosis

AUC(95CI)=0.740(0.649-0.819)
Sensitivity=65.22%
Specificity=77.27%

H — (AFP<400ng/mL)Early-stage-HCC vs Cirrhosis

AUC(95CI)=0.752(0.676-0.819)
Sensitivity=70.59%
Specificity=73.27%

Supplementary Figure 3: Comparison of ITGBL1 levels in serum of non-HCC and HBV-HCC patients with normal and elevated ALT.

The level of ITGBL1 in serum of the non-HCC and HBV-HCC patients with normal and elevated ALT in training cohort (A). The level of ITGBL1 in serum of the non-HCC and HBV-HCC patients with normal and elevated ALT in validation cohort (B). Black horizontal lines are median and quartiles. Abbreviations: ITGBL1, Integrin beta-like 1; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; non-HCC groups, patients with chronic hepatitis B and HBV-related cirrhosis; ALT, alanine aminotransferase; NS, not significant.

Supplementary Figure 4: The level of ITGBL1 in serum of non-HCC and HBV-HCC patients with different HBV DNA levels.

The level of ITGBL1 in serum of the non-HCC patients with different HBV DNA level (A). The level of ITGBL1 in serum of the HBV-HCC patients with different HBV DNA level (B). Black horizontal lines are median and quartiles. Abbreviations: ITGBL1, Integrin beta-like 1; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; non-HCC groups, patients with chronic hepatitis B and HBV-related cirrhosis;NS, not significant.

Supplementary Figure 5: Comparison of ITGBL1 levels in serum of non-HCC and HBV-HCC patients with antiviral therapy or not.

The level of ITGBL1 in serum of the non-HCC and HBV-HCC patients with antiviral therapy and no treatment in training cohort (A). The level of ITGBL1 in serum of the non-HCC and HBV-HCC patients with antiviral therapy and no treatment in validation cohort (B). Black horizontal lines are median and quartiles. Abbreviations: ITGBL1, Integrin beta-like 1; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; non-HCC groups, patients with chronic hepatitis B and HBV-related cirrhosis;NS, not significant.