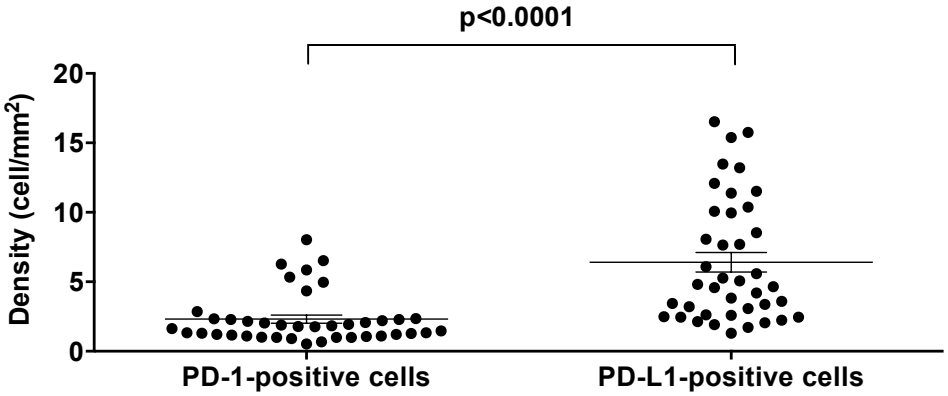
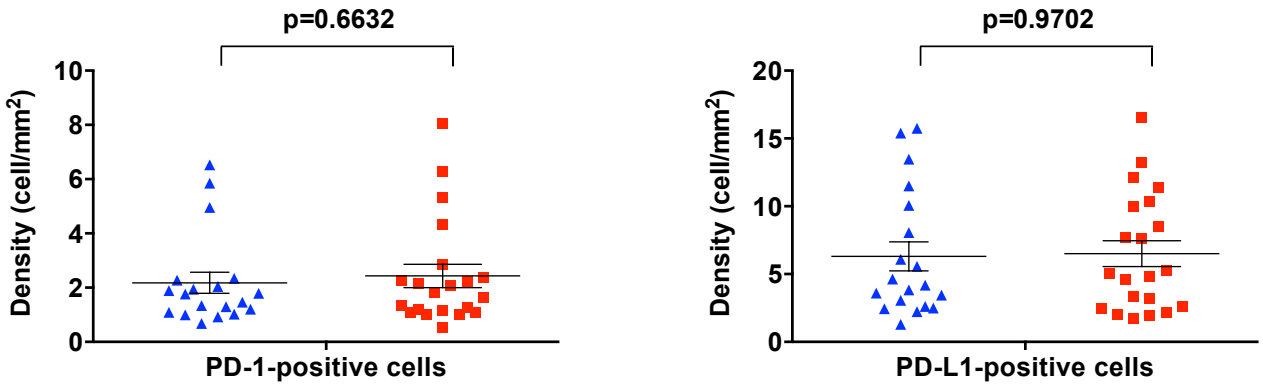
Figure S1

A



	PD-1-positive cells	PD-L1-positive cells
No. of patients	40	40
Mean±SEM	2.31±0.28	6.40±0.70
Median (range)	1.78 (0.52-8.04)	4.72 (1.29-16.52)

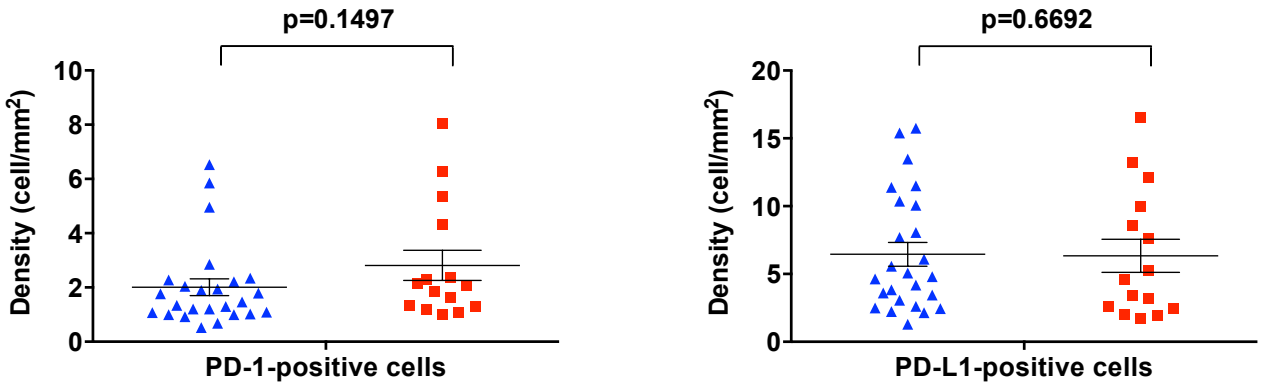
B



	PD-1-positive cells		PD-L1-positive cells	
No. of patients	19	21	19	21
Mean±SEM	2.18±0.38	2.43±0.42	6.30±1.07	6.50±0.95
Median (range)	1.77 (0.68-6.53)	1.83 (0.52-8.04)	4.19 (1.29-15.75)	5.07 (1.71-16.52)

▲ Without deletions spanning the pre-S2 gene segment
■ With deletions spanning the pre-S2 gene segment

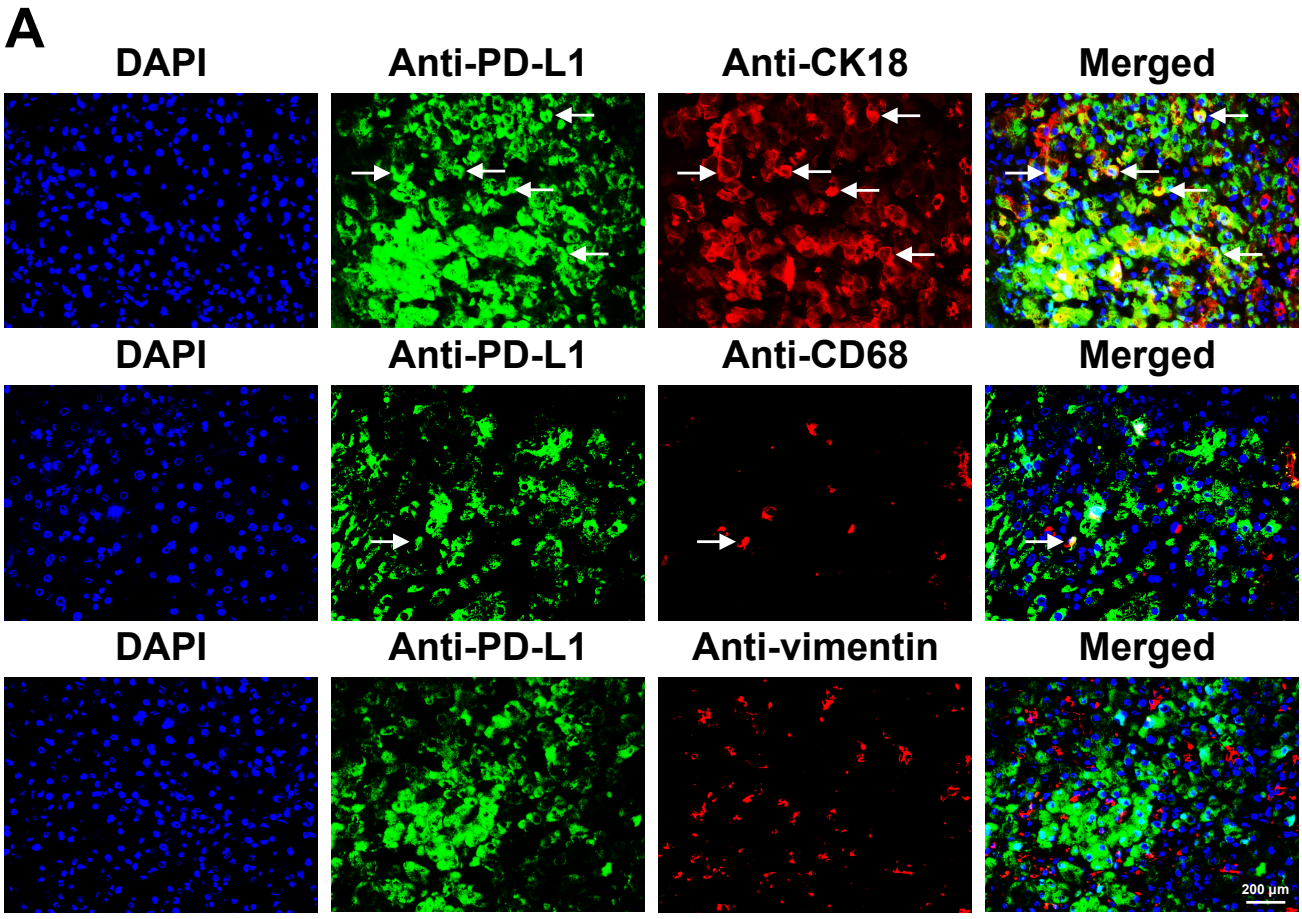
C

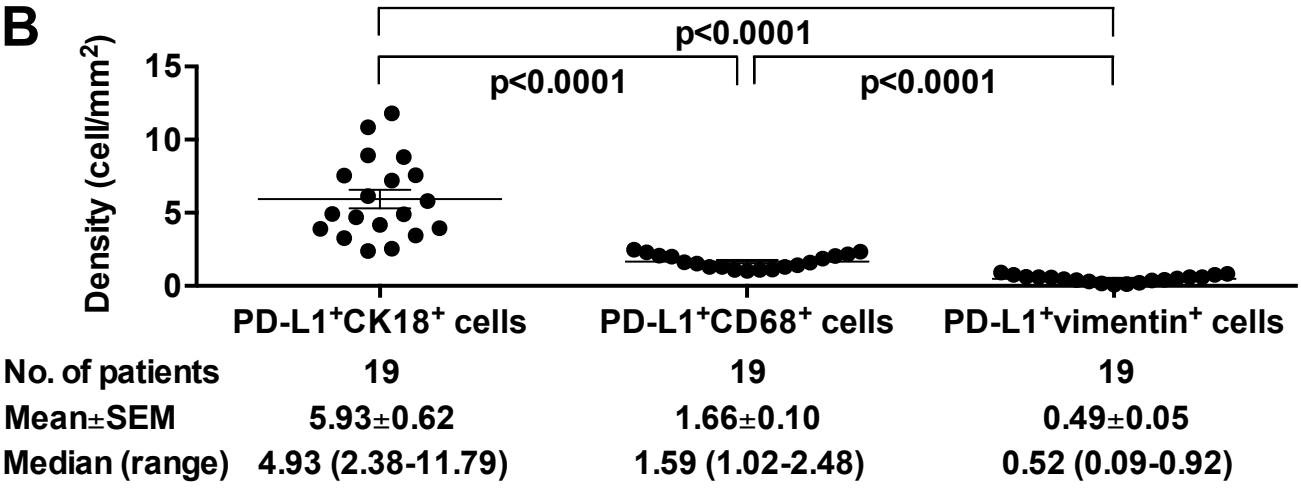


	PD-1-positive cells		PD-L1-positive cells	
No. of patients	25	15	25	15
Mean±SEM	2.01±0.30	2.81±0.55	6.45±0.87	6.34±1.22
Median (range)	1.47 (0.52-6.53)	2.07 (1.00-8.04)	4.81 (1.29-15.75)	4.58 (1.71-16.52)

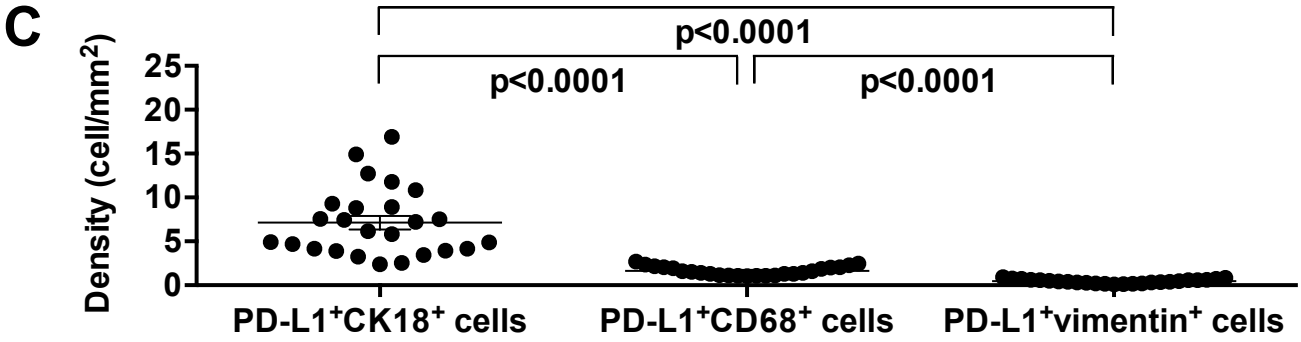
▲ Low percentage of pre-S2 plus pre-S1+pre-S2 deletions
■ High percentage of pre-S2 plus pre-S1+ pre-S2 deletions

Figure S2





	PD-L1$^+$CK18$^+$ cells	PD-L1$^+$CD68$^+$ cells	PD-L1$^+$vimentin$^+$ cells
No. of patients	19	19	19
Mean±SEM	5.93±0.62	1.66±0.10	0.49±0.05
Median (range)	4.93 (2.38-11.79)	1.59 (1.02-2.48)	0.52 (0.09-0.92)



	PD-L1$^+$CK18$^+$ cells	PD-L1$^+$CD68$^+$ cells	PD-L1$^+$vimentin$^+$ cells
No. of patients	25	25	25
Mean±SEM	7.13±0.77	1.64±0.10	0.45±0.04
Median (range)	6.15 (2.38-16.93)	1.53 (1.02-2.71)	0.42 (0.09-0.92)

Supplementary Figure Legends

Figure S1. Comparative analysis of the density of PD-1/PD-L1-positive cells in non-HCC tissues between the pre-S2 mutant-positive and -negative patients. (A) Density of PD-1- and PD-L1-positive cells in non-HCC tissues in total patients. (B) Density of PD-1-positive cells (left graph) and PD-L1-positive cells (right graph) in non-HCC tissues between patients with and without deletion spanning pre-S2 gene segment. (C) Density of PD-1-positive cells (left graph) and PD-L1-positive cells (right graph) in non-HCC tissues between patients with high and low percentage of pre-S2 plus pre-S1+pre-S2 deletion. Horizontal lines represented the mean values of the distribution. A P value < 0.05 was considered to indicate statistical significance.

Figure S2. Expression profiles of PD-L1 in different cell populations in HCC tissues of pre-S2 mutant-negative/low patients. (A) The expression of PD-L1 on tumor cells, Kupffer cells, and LSECs in HCC tissues of pre-S2 mutant-negative/low patients was detected by fluorescent IHC staining of PD-L1 (green in color) together with CK18 (red in color), CD68 (red in color), and vimentin (red in color), respectively. The HCC tumor cells and Kupffer cells with PD-L1 expression appeared

yellow and were indicated by white arrows in the single-color and merged images. Nuclei were counterstained with DAPI (blue in color). Shown were representative results. Original magnification, ×40. Scale bar, 200 μm. (B) Density of PD-L1/CK18 double-positive (PD-L1^{+}CK18^{+}), PD-L1/CD68 double-positive (PD-L1^{+}CD68^{+}), and PD-L1/vimentin double-positive (PD-L1^{+}vimentin^{+}) cells in HCC tissues of patients without deletion spanning pre-S2 gene segment. (C) Density of PD-L1^{+}CK18^{+}, PD-L1^{+}CD68^{+}, and PD-L1^{+}vimentin^{+} cells in HCC tissues of patients with low percentage of pre-S2 plus pre-S1+pre-S2 deletion. Horizontal lines represented the mean values of the distribution. A P value < 0.05 was considered to indicate statistical significance.

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients

Patient No.	Total reads	Pre-S deletion type (%)[a]	Deletions spanning the pre-S2 gene segment	Percentage of pre-S2 plus pre-S1+pre-S2 deletions[c]
1	346,100	1. **wild-type (99.278)**[b] 2. pre-S1 del (0.660) 3. pre-S2 del (0.053) 4. pre-S1+pre-S2 del (0.009)	negative	low
2	333,826	1. **wild-type (96.592)** 2. pre-S1 del (2.906) 3. pre-S2 del (0.469) 4. pre-S1+pre-S2 del (0.033)	negative	low
3	327,640	1. **wild-type (99.000)** 2. pre-S1 del (0.927) 3. pre-S2 del (0.062) 4. pre-S1+pre-S2 del (0.012)	negative	low
4	343,499	1. **wild-type (97.851)** 2. pre-S1 del (2.037) 3. pre-S2 del (0.108) 4. pre-S1+pre-S2 del (0.005)	negative	low
5	332,222	1. **wild-type (98.964)** 2. pre-S1 del (0.943) 3. pre-S2 del (0.084) 4. pre-S1+pre-S2 del (0.009)	negative	low

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients (continued)

Patient No.	Total reads	Pre-S deletion type (%)[a]	Deletions spanning the pre-S2 gene segment	Percentage of pre-S2 plus pre-S1+pre-S2 deletions[c]
6	342,663	1. **wild-type (97.781)** 2. pre-S1 del (2.099) 3. pre-S2 del (0.112) 4. pre-S1+pre-S2 del (0.008)	negative	low
7	341,941	1. **wild-type (96.830)** 2. pre-S1 del (1.728) 3. pre-S2 del (1.341) 4. pre-S1+pre-S2 del (0.102)	negative	low
8	324,302	1. **wild-type (98.571)** 2. pre-S1 del (1.173) 3. pre-S2 del (0.178) 4. pre-S1+pre-S2 del (0.078)	negative	low
9	323,225	1. **wild-type (96.915)** 2. pre-S1 del (2.701) 3. pre-S2 del (0.364) 4. pre-S1+pre-S2 del (0.020)	negative	low
10	325,092	1. **wild-type (98.069)** 2. pre-S1 del (1.263) 3. pre-S2 del (0.609) 4. pre-S1+pre-S2 del (0.059)	negative	low

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients (continued)

Patient No.	Total reads	Pre-S deletion type (%)[a]	Deletions spanning the pre-S2 gene segment	Percentage of pre-S2 plus pre-S1+pre-S2 deletions[c]
11	353,564	1. **pre-S1 del (92.118)** 2. **wild-type (7.278)** 3. pre-S2 del (0.372) 4. pre-S1+pre-S2 del (0.231)	negative	low
12	322,716	1. **pre-S1 del (75.241)** 2. **wild-type (22.338)** 3. pre-S2 del (1.891) 4. pre-S1+pre-S2 del (0.530)	negative	low
13	336,064	1. **wild-type (71.590)** 2. **pre-S1 del (28.270)** 3. pre-S2 del (0.129) 4. pre-S1+pre-S2 del (0.011)	negative	low
14	325,894	1. **pre-S1 del (75.109)** 2. **wild-type (23.879)** 3. pre-S1+pre-S2 del (0.687) 4. pre-S2 del (0.325)	negative	low
15	365,835	1. **pre-S1 del (86.404)** 2. **wild-type (12.695)** 3. pre-S2 del (0.737) 4. pre-S1+pre-S2 del (0.163)	negative	low

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients (continued)

Patient No.	Total reads	Pre-S deletion type (%)[a]	Deletions spanning the pre-S2 gene segment	Percentage of pre-S2 plus pre-S1+pre-S2 deletions[c]
16	321,533	1. **wild-type (82.957)** 2. **pre-S1 del (10.988)** 3. pre-S2 del (4.178) 4. pre-S1+pre-S2 del (1.877)	negative	low
17	349,212	1. **wild-type (93.746)** 2. **pre-S1 del (5.846)** 3. pre-S2 del (0.398) 4. pre-S1+pre-S2 del (0.018)	negative	low
18	344,222	1. **pre-S1 del (76.151)** 2. **wild-type (19.034)** 3. pre-S2 del (4.624) 4. pre-S1+pre-S2 del (0.192)	negative	low
19	314,062	1. **wild-type (94.701)** 2. **pre-S1 del (5.086)** 3. pre-S2 del (0.137) 4. pre-S1+pre-S2 del (0.077)	negative	low
20	326,601	1. **wild-type (58.461)** 2. **pre-S2 del (37.194)** 3. pre-S1 del (2.939) 4. pre-S1+pre-S2 del (1.407)	positive	high

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients (continued)

Patient No.	Total reads	Pre-S deletion type (%)[a]	Deletions spanning the pre-S2 gene segment	Percentage of pre-S2 plus pre-S1+pre-S2 deletions[c]
21	339,776	1. **wild-type (59.263)** 2. **pre-S2 del (37.934)** 3. pre-S1 del (1.940) 4. pre-S1+pre-S2 del (0.862)	positive	high
22	352,523	1. **pre-S2 del (56.155)** 2. **wild-type (42.610)** 3. pre-S1+pre-S2 del (0.718) 4. pre-S1 del (0.516)	positive	high
23	343,602	1. **pre-S2 del (94.816)** 2. **wild-type (5.049)** 3. pre-S1+pre-S2 del (0.070) 4. pre-S1 del (0.066)	positive	high
24	325,089	1. **wild-type (65.077)** 2. **pre-S2 del (28.468)** 3. pre-S1 del (4.516) 4. pre-S1+pre-S2 del (1.940)	positive	high
25	312,304	1. **wild-type (91.356)** 2. **pre-S2 del (8.015)** 3. pre-S1 del (0.379) 4. pre-S1+pre-S2 del (0.251)	positive	low

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients (continued)

Patient No.	Total reads	Pre-S deletion type (%)[a]	Deletions spanning the pre-S2 gene segment	Percentage of pre-S2 plus pre-S1+pre-S2 deletions[c]
26	348,782	1. **wild-type (46.914)** 2. **pre-S1 del (45.517)** 3. **pre-S2 del (6.834)** 4. pre-S1+pre-S2 del (0.735)	positive	low
27	349,650	1. **pre-S1 del (76.228)** 2. **wild-type (12.583)** 3. **pre-S2 del (10.622)** 4. pre-S1+pre-S2 del (0.567)	positive	low
28	338,492	1. **pre-S1+pre-S2 del (46.237)** 2. **pre-S2 del (26.927)** 3. **pre-S1 del (14.368)** 4. **wild-type (12.467)**	positive	high
29	322,281	1. **wild-type (69.001)** 2. **pre-S1 del (20.530)** 3. **pre-S2 del (9.463)** 4. pre-S1+pre-S2 del (1.006)	positive	low
30	367,627	1. **wild-type (50.938)** 2. **pre-S1 del (19.760)** 3. **pre-S1+pre-S2 del (15.021)** 4. **pre-S2 del (14.280)**	positive	high

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients (continued)

Patient No.	Total reads	Pre-S deletion type (%)[a]	Deletions spanning the pre-S2 gene segment	Percentage of pre-S2 plus pre-S1+pre-S2 deletions[c]
31	314,247	1. **wild-type (75.230)** 2. **pre-S1+pre-S2 del (13.878)** 3. **pre-S1 del (10.730)** 4. pre-S2 del (0.162)	positive	low
32	323,119	1. **pre-S2 del (41.477)** 2. **pre-S1+pre-S2 del (39.126)** 3. **wild-type (12.348)** 4. **pre-S1 del (7.048)**	positive	high
33	345,498	1. **pre-S1+pre-S2 del (45.703)** 2. **pre-S2 del (29.458)** 3. **wild-type (14.926)** 4. **pre-S1 del (9.913)**	positive	high
34	345,927	1. **wild-type (30.944)** 2. **pre-S2 del (30.409)** 3. **pre-S1+pre-S2 del (29.105)** 4. **pre-S1 (9.542)**	positive	high
35	330,111	1. **pre-S1 del (40.086)** 2. **wild-type (34.919)** 3. **pre-S2 del (16.065)** 4. **pre-S1+pre-S2 del (8.930)**	positive	high

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients (continued)

Patient No.	Total reads	Pre-S deletion type (%)[a]	Deletions spanning the pre-S2 gene segment	Percentage of pre-S2 plus pre-S1+pre-S2 deletions[c]
36	337,893	1. **pre-S1 del (29.181)** 2. **wild-type (23.836)** 3. **pre-S2 del (23.645)** 4. **pre-S1+pre-S2 del (23.338)**	positive	high
37	319,570	1. **wild-type (30.973)** 2. **pre-S1+pre-S2 del (27.774)** 3. **pre-S1 del (27.161)** 4. **pre-S2 del (14.091)**	positive	high
38	317,285	1. **pre-S1 del (43.130)** 2. **wild-type (41.965)** 3. **pre-S2 del (9.508)** 4. **pre-S1+pre-S2 del (5.397)**	positive	low
39	337,345	1. **wild-type (36.868)** 2. **pre-S1 del (35.238)** 3. **pre-S2 del (21.130)** 4. **pre-S1+pre-S2 del (6.763)**	positive	high
40	326,346	1. **wild-type (58.984)** 2. **pre-S2 del (34.533)** 3. pre-S1+pre-S2 del (4.497) 4. pre-S1 (1.986)	positive	high

Table S1. List of the pre-S genotyping result by NGS-based analysis in 40 HBV-related HCC patients (continued)

[a]The total percentage of pre-S gene DNA in each type of pre-S deletion was shown in descending order.

[b]The pre-S deletion type above the cut-off percentage (5.049) was shown in bold.

[c]The percentage of pre-S2 plus pre-S1+pre-S2 deletions above 24.995 was defined as high percentage.

Abbreviations: NGS, next-generation sequencing; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; del, deletion.