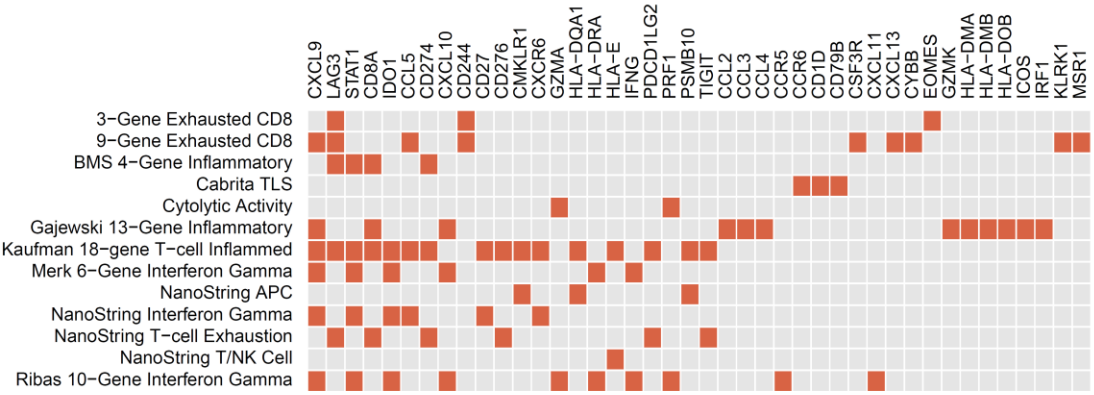
Supplementary Methods and Materials

Published gene signatures that may be associated with response to immune checkpoint inhibitor (ICI) therapy

The following gene signatures were used in the present study to evaluate their predictive and prognostic values for HCC patients who receive ICI therapy:



The TIDE score was calculated using the online tool (http://tide.dfci.harvard.edu/) where the parameter of cancer type was set as 'other' (1). The IMPRES score were calculated as previously described (2). IMPRES encompasses 15 pairwise transcriptomics relations between immune checkpoint genes. Because CD137L and VISTA were not profiled by the NanoString PanCancer Immune Profiling panel, only 13 pairwise genes were used for scoring IMPRES in our ICI therapy cohort. The prediction value of PD-L1 was evaluated in our study using the PD-L1(CD274) gene expression. The prediction values of other signatures published in the literature were the average values among all members (3-8).

β-catenin analysis

DNA was extracted using the truXTRAC® FFPE total NA Kits (Covaris). Exon 3 of CTNNB1 was amplified using the following primers: 5'-CAATGGGTCATATCACAGATTCTT-3' and 5'-TCTCTTTTCTTCACCACAACATTT-3'. Direct sequencing was performed to detect mutations by using an ABI PRISM 3730xl DNA Analyzer. The immunohistochemistry staining of β-catenin and glutamine synthetase was performed as previous described (9)

Immune subtypes

The immune class of the ICI treatment cohort was determined as previous described (10). In accordance with the manufacturer's instructions, CIBERSORT analysis of mRNA expression data of the ICI treatment cohort was performed in the Absolute mode with LM22 signature matrix on the web (https://cibersort.stanford.edu/) (11)

Supplementary Tables

Supplementary Table 1. Antibodies used to measure CD8 T cell exhaustion by multiplex immunofluorescence staining.

Antibody Target	Clone	Dilution	Fluorophore
anti-CD8	Cat# 790-4460, clone SP57, Roche	1:1	Opal 570
anti-PD1	Cat# 760-4895, clone NAT105, Roche	1:1	Opal 690
anti-LAG3	Cat# AC-0294, clone EP294, Cell marque	1:100	Opal 520

Supplementary Table 2 Clinico-pathological characteristics of the ICI therapy cohort

Characteristics	All patients (%)	Patients with adequate tumor sample for biomarker analysis		*p* value*
		Discovery (%)	Validation (%)	
No. of patients	61	24	18	
Age				0.35
≦ 60 years	29 (47.5)	12 (50)	6 (33.3)	
> 60 years	32 (52.5)	12 (50)	12 (66.7)	
Sex				1.00
Men	53 (86.9)	22 (91.7)	16 (88.9)	
Women	8 (12.9)	2 (8.3)	2 (11.1)	
Etiology				1.00
HBV	48 (78.7)	19 (79.2)	13 (72.2)	
HCV	8 (13.1)	3 (12.5)	3 (16.7)	
Other	5 (8.2)	2 (8.3)	2 (11.1)	
ALBI grade				0.51
1	42 (68.9)	16 (66.7)	14 (77.8)	
2	19 (31.1)	8 (33.3)	4 (22.2)	
NLR				0.75
≦ 3	33 (54.1)	15 (62.5)	10 (55.6)	
> 3	28 (45.9)	9 (31.5)	8 (44.4)	
AFP (ng/ml)				0.54
≦ 100	32 (52.5)	12 (50.0)	11 (61.1)	
> 100	29 (47.5)	12 (50.0)	7 (38.9)	
Macrovascular invasion				**0.04**
No	42 (68.9)	14 (58.3)	16 (88.9)	
Yes	19 (31.1)	10 (41.7)	2 (11.1)	
Extra-hepatic spread				0.57
No	7 (11.5)	1 (4.2)	2 (11.1)	
Yes	54 (88.5)	23 (95.8)	16 (88.9)	
Liver tumor volume# > 50%				1.00
No	56 (91.8)	23 (95.8)	17 (94.4)	
Yes	5 (8.2)	1 (4.2)	1 (5.6)	
Prior systemic therapy				**< 0.0001**
None	19 (31.1)	2 (8.3)	10 (55.6)	
1st-line	27 (44.3)	10 (41.7)	8 (44.4)	
2nd-line	15 (24.6)	12 (50.0)	0 (0.0)	
Response to prior systemic therapy				**0.004**
No treatment	19 (31.1)	2 (8.4)	10 (55.6)	
SD	21 (34.4)	11 (45.8)	4 (21.1)	
PD	21 (34.4)	11 (45.8)	4 (21.4)	
Therapeutic regimen				1.00
Single-agent anti-PD1/ anti-PD-L1	28 (45.9)	13 (54.2)	9 (50.0)	
Anti-PD1/ anti-PD-L1-based	33 (54.1)	11 (45.8)	9 (40.0)	

combination				
Objective response				0.12
PD/SD	18/19 (60.7)	8/10 (75.0)	4/5 (50.0)	
PR/CR	22/1 (37.7)	6/0 (25.0)	8/1 (50.0)	
Not evaluable	1[@] (1.6)	0 (0.0)	0 (0.0)	

ALBI grade: albumin-bilirubin grade

NLR: neutrophil-lymphocyte ratio

AFP: alpha-fetoprotein level

* Fisher's exact test was used to assess the difference between the discovery and validation cohorts. The result was in bold, if $p < 0.05$

#: Estimated percentage of liver volume

[@]: The patient died after 1 cycle of ICI-based combination therapy without formal evaluation of response by imaging.

Supplementary Table 3. The regimen of immune checkpoint inhibitor (ICI)-based regimens used for subjects in the discovery and validation cohorts.

Discovery cohort			**Validation cohort**		
Subject no.	**ICI-based regimen**	**Time from tumor sample collection (year)**	**Subject no.**	**ICI-based regimen**	**Time from tumor sample collection (year)**
S01	Nivolumab+Ipilimumab	0.1	V02	Codrituzumab+Atezolizumab	0.7
S02	Nivolumab+Ipilimumab	2.7	V04	Durvalumab	4.4
S03	Nivolumab+Ipilimumab	1.2	V05	Durvalumab	0.2
S06	Nivolumab+Ipilimumab	1.5	V06	Atezolizumab+Bevacizumab	4.3
S08	Nivolumab+Ipilimumab	1.5	V07	Atezolizumab+Bevacizumab	1.6
S10	Nivolumab+Ipilimumab	2.1	V10	Atezolizumab+Bevacizumab	0
S11	Nivolumab+Ipilimumab	0.4	V12	Atezolizumab+Bevacizumab	3.1
S12	Nivolumab+Ipilimumab	0	V13	Atezolizumab	0.9
S14	Nivolumab+Ipilimumab	2	V14	Atezolizumab	0.5
S16	PDR001+MBG453	0	V16	Atezolizumab	0.3
S17	PDR001+MBG453	2.8	V17	Atezolizumab	0.2
S18	Nivolumab	5.6	V18	INC280+PDR001	1.2
S19	Nivolumab	1	V19	INC280+PDR001	0.5
S20	Nivolumab	6.2	V22	BGB-A317	1.3
S21	Nivolumab	2.2	V23	BGB-A317	0.9
S22	Nivolumab	1.4	V25	GT90001+Nivolumab	0.3
S23	Nivolumab	2.1	V27	GT90001+Nivolumab	0.7
S24	Nivolumab	3.7	V29	Nivolumab	0.1
S25	Nivolumab	2.9			
S26	Nivolumab	0.1			
S27	Nivolumab	0.1			
S29	Nivolumab	0.3			
S30	Nivolumab	12			
S31	Nivolumab	0.4			

Supplementary Table 4. Odd ratios of various clinical-pathologic characteristics for objective response to ICI therapy

Characteristics	Objective response to ICI therapy	
N = 61	OR (95% IC)	*p* value*
Age (≦60 vs. > 60 years)	1.03 (0.32-3.33)	1.00
Sex (women vs. men)	0.96 (0.13-5.58)	1.00
Etiology (other etiologies vs. HBV)	0.55 (0.12-2.42)	0.51
ALBI grade (1 vs. 2)	0.52 (0.12-1.92)	0.39
NLR (≦3 vs. > 3)	1.20 (0.37-3.97)	0.79
AFP (≦100 vs. > 100 ng/ml)	2.51 (0.77-8.57)	0.11
Macrovascular invasion (No vs. Yes)	0.91 (0.24-3.18)	1.00
Extra-hepatic spread (No vs. Yes)	0.43 (0.06-2.81)	0.41
Liver tumor volume# (≦50% vs.> 50%)	1.65 (0.11-24.4)	0.63
Prior systemic therapy (No vs. 1 or more prior therapy)	0.70 (0.20-2.52)	0.57

* the p-values were derived from Fisher's exact test

estimated percentage of liver volume

Supplementary Table 5. Univariate Cox regression analysis in the ICI therapy cohort

Characteristics (*n*)	Progression-free survival		Overall survival	
	HR (95% IC)	*p* value*	HR (95% IC)	*p* value*
N = 61	Events = 44		Events = 39	
Age				
≦ 60 years (29)	Ref		Ref	
> 60 years (32)	0.81 (0.45-1.47)	0.49	1.24 (0.65-2.35)	0.52
Sex				
Men (53)	Ref		Ref	
Women (8)	1.65 (0.73-3.72)	0.23	1.28 (0.53-3.06)	0.58
Etiology				
HBV (48)	Ref		Ref	
HCV (8)	0.28 (0.07-1.15)	0.08	1.18 (0.46-3.06)	0.73
Other (5)	1.45 (0.51-4.09)	0.48	1.50 (0.52-4.30)	0.44
ALBI grade				
1 (42)	Ref		Ref	
2 (19)	1.33 (0.70-2.53)	0.37	1.92 (1.01-3.66)	**0.047**
NLR				
≦ 3 (33)	Ref		Ref	
> 3 (28)	1.03 (0.56-1.86)	0.94	1.28 (0.68-2.40)	0.44
AFP (ng/ml)				
≦ 100 (32)	Ref		Ref	
> 100 (29)	1.00 (0.55-1.81)	1.00	1.41 (0.75-2.66)	0.29
Macrovascular invasion				
No (42)	Ref		Ref	
Yes (19)	1.12 (0.60-2.11)	0.73	1.40 (0.72-2.70)	0.32
Extra-hepatic spread				
No (7)	Ref		Ref	
Yes (54)	2.48 (0.76-8.02)	0.13	1.91 (0.59-6.22)	0.28
Liver tumor volume > 50%				
No (56)	Ref		Ref	
Yes (5)	0.61 (0.15-2.52)	0.49	1.54 (0.47-5.03)	0.48
Prior systemic therapy				
None (19)	Ref		Ref	
1 or more lines (42)	1.58 (0.81-3.09)	0.18	1.41 (0.65-3.04)	0.38

***** The result was in bold, if $p < 0.05$

Supplementary Table 6. Bivariate Cox regression analysis in the ICI therapy discovery cohort to evaluate the predictive value of the 9-gene exhausted CD8 T cell signature in different clinico-pathological sub-groups.

Characteristics	Progression-free survival		Overall survival	
	HR (95% IC)	*p* value*	HR (95% IC)	*p* value*
N = 24	Event = 21		Event = 21	
9-gene Exhausted CD8	0.44 (0.24-0.84)	**0.012**	0.39 (0.19-0.81)	**0.012**
Age				
≦ 60 years	Ref		Ref	
> 60 years	1.17 (0.48-2.86)	0.73	1.76 (0.72-4.29)	
9-gene Exhausted CD8	0.47 (0.24-0.89)	**0.019**	0.43 (0.20-0.90)	**0.025**
Sex				
Men	Ref		Ref	
Women	3.86 (0.69-21.5)	0.12	2.02(0.41-9.99)	0.39
9-gene Exhausted CD8	0.42 (0.20-0.87)	**0.019**	0.39 (0.18-0.83)	**0.014**
Etiology				
HBV	Ref		Ref	
HCV	1.48 (0.28-7.83)	0.64	1.32 (0.28-6.21)	0.73
Other	2.12 (0.46-9.81)	0.34	0.65 (0.14-2.89)	0.57
9-gene Exhausted CD8	0.44 (0.23-0.83)	**0.012**	0.41 (0.20-.85)	**0.017**
ALBI grade				
1	Ref		Ref	
2	0.78 (0.31-1.97)	0.59	0.92 (0.37-2.31)	0.86
9-gene Exhausted CD8	0.47 (0.25-0.91)	**0.025**	0.34 (0.15-0.79)	**0.012**
NLR				
≦ 3	Ref		Ref	
> 3	0.74 (0.28-1.93)	0.54	1.68 (0.63-4.46)	0.30
9-gene Exhausted CD8	0.41 (0.21-0.78)	**0.007**	0.34 (0.17-0.75)	**0.008**
AFP (ng/ml)				
≦ 100	Ref		Ref	
> 100	2.01 (0.79-5.11)	0.14	2.49 (0.99-6.31)	0.054
9-gene Exhausted CD8	0.45 (0.24-0.85)	**0.014**	0.41 (0.20-0.86)	**0.018**
Macrovascular invasion				
No	Ref		Ref	
Yes	1.02 (0.40-2.59)	0.96	1.08 (0.44-2.65)	0.88
9-gene Exhausted CD8	0.52 (0.27-0.99)	**0.046**	0.48 (0.22-1.01)	0.054
Extra-hepatic spread				
No	Ref		Ref	
Yes	1.2e8 (0-Inf)	0.99	3.4e7 (0-Inf)	0.99
9-gene Exhausted CD8	0.46 (0.24-0.87)	**0.017**	0.42 (0.20-0.88)	**0.02**
Liver tumor volume > 50%				
No	Ref		Ref	
Yes	9.87 (0.87-111.9)	0.06	7.64 (0.69-84.9)	0.10
9-gene Exhausted CD8	0.40 (0.21-0.76)	**0.0054**	0.35 (0.16-0.75)	**0.007**
Prior systemic therapy				
None	Ref		Ref	
1 or more lines	0.24 (0.05-1.24)	0.088	0.24 (0.05-1.25)	0.090

Supplementary Table 7. Clinico-pathological features of patients in the ICI therapy cohort who received single-agent ICI and combination ICI therapy

Characteristics	Single-agent (%)	Combination (%)	*p* value*
N	22	20	
Age			0.58
≦ 60 years	8 (36.4)	10 (50)	
> 60 years	14 (63.6)	10 (50)	
Sex			1.00
Men	20 (90.9)	18 (90.0)	
Women	2 (9.1)	2 (10.0)	
Etiology			0.87
HBV	16 (72.7)	16 (80.0)	
HCV	4 (18.2)	2 (10.0)	
Other	2 (9.1)	2 (10.0)	
ALBI grade			0.74
1	15 (68.2)	15 (75.0)	
2	7 (31.8)	5 (25.0)	
NLR			0.76
≦ 3	14 (60.9)	11 (55.0)	
> 3	9 (39.1)	9 (45.0)	
AFP (ng/ml)			0.23
≦ 100	10 (45.5)	13 (65.0)	
> 100	12 (54.5)	7 (35.0)	
Macrovascular invasion			0.50
No	17 (77.3)	13 (65.0)	
Yes	5 (22.7)	7 (35.0)	
Extra-hepatic spread			0.60
No	1 (4.5)	2 (10.0)	
Yes	21 (95.5)	18 (90.0)	
Liver tumor volume > 50%			1.00
No	21 (95.5)	19 (95.0)	
Yes	1 (4.5)	1 (5.0)	
Prior systemic therapy			0.31
None	8 (36.4)	4 (20.0)	
1 or more lines	14 (63.6)	16 (80.0)	
Objective response			0.53
PD/SD	6/7 (59.1)	6/8 (70.0)	
PR/CR	8/1 (40.9)	6/0 (30.0)	

* Fisher's exact test.

Supplementary Table 8. Bivariate Cox regression analysis in the samples with single-agent regimen.

Characteristics	Progression-free survival		Overall survival	
	HR (95% IC)	*p* value*	HR (95% IC)	*p* value*
N = 22	Event = 18		Event = 17	
9-gene Exhausted CD8	0.45 (0.25-0.81)	**0.008**	0.62 (0.35-1.12)	0.11
Age				
≦ 60	Ref		Ref	
> 60	1.29 (0.49-3.39)	0.60	1.19 (0.43-3.34)	0.73
9-gene Exhausted CD8	0.50 (0.27-0.93)	**0.028**	0.71 (0.37-1.34)	0.28
Gender				
Male	Ref		Ref	
Female	6.09 (0.80-46.4)	0.081	3.63 (0.58-22.8)	0.17
9-gene Exhausted CD8	0.55 (0.30-1.02)	**0.057**	0.56 (0.29-1.09)	0.09
Etiology				
HBV	Ref		Ref	
HCV	0.21 (0.03-1.75)	0.15	2.03 (0.37-11.1)	0.42
Other	0.51 (0.06-4.22)	0.53	1.65 (0.19-14.1)	0.65
9-gene Exhausted CD8	0.40 (0.22-0.75)	**0.004**	0.52 (0.28-0.99)	0.04
ALBI grade				
1	Ref		Ref	
2	0.52 (0.17-1.61)	0.26	0.47 (0.14-1.58)	0.22
9-gene Exhausted CD8	0.47 (0.26-0.85)	**0.013**	0.68 (0.38-1.22)	0.19
NLR				
≦ 3	Ref		Ref	
> 3	0.65 (0.24-1.80)	0.41	0.49 (0.15-1.62)	0.24
9-gene Exhausted CD8	0.47 (0.26-0.85)	**0.012**	0.68 (0.39-1.19)	0.17
AFP (ng/ml)				
≦ 100	Ref		Ref	
> 100	0.73 (0.28-1.93)	0.52	0.33 (0.10-1.12)	0.08
9-gene Exhausted CD8	0.48 (0.26-0.87)	**0.016**	0.65 (0.36-1.18)	0.16
Macrovascular invasion				
No	Ref		Ref	
Yes	1.62 (0.55-4.85)	0.38	1.83 (0.60-5.55)	0.29
9-gene Exhausted CD8	0.49 (0.27-0.88)	**0.017**	0.66 (0.36-1.18)	0.16
Extra-hepatic spread				
No	Ref		Ref	
Yes	1.8e7 (0-Inf)	1.00	5.7e7 (0-Inf)	1.00
9-gene Exhausted CD8	0.48 (0.26-0.88)	**0.017**	0.67 (0.36-1.23)	0.20
Liver tumor > 50%				
No	Ref		Ref	
Yes	1.1e10 (0-Inf)	1.00	13.5 (0.80-226.6)	0.07
9-gene Exhausted CD8	0.49 (0.24-0.98)	**0.044**	0.58 (0.27-1.23)	0.15
Prior systemic therapy				
None	Ref		Ref	
1 or more lines	1.25 (0.34-4.54)	0.74	0.78 (0.17-3.58)	0.75

***** The result was in bold, if $p < 0.05$

Supplementary Table 9. Bivariate Cox regression analysis in the samples with combination regimen.

Characteristics	Progression-free survival		Overall survival	
	HR (95% IC)	*p* value*	HR (95% IC)	*p* value*
N = 20	Event = 15		Event = 10	
9-gene Exhausted CD8	0.49 (0.27-0.87)	**0.015**	0.24 (0.09-0.64)	**0.0045**
Age				
≦ 60	Ref		Ref	
> 60	0.93 (0.32-2.65)	0.88	1.69 (0.40-7.13)	0.48
9-gene Exhausted CD8	0.49 (0.28-0.88)	**0.017**	0.29 (0.11-0.77)	**0.013**
Gender				
Male	Ref		Ref	
Female	0.79 (0.09-6.61)	0.83	2.5e-8 (0-Inf)	0.99
9-gene Exhausted CD8	0.49 (0.28-0.87)	**0.015**	0.26 (0.09-0.71)	**0.0083**
Etiology				
HBV	Ref		Ref	
HCV	0.64 (0.08-5.19)	0.67	0.79 (0.09-6.77)	0.83
Other	2.07 (0.41-10.4)	0.38	1.21 (0.24-6.13)	0.82
9-gene Exhausted CD8	0.49 (0.28-0.86)	**0.014**	0.16 (0.04-0.59)	**0.006**
ALBI grade				
1	Ref		Ref	
2	1.21 (0.37-3.94)	0.76	8.41 (1.44-48.9)	**0.018**
9-gene Exhausted CD8	0.48 (0.27-0.85)	**0.013**	0.19 (0.06-0.60)	**0.0045**
NLR				
≦ 3	Ref		Ref	
> 3	1.32 (0.46-3.75)	0.61	3.02 (0.68-13.3)	0.14
9-gene Exhausted CD8	0.48 (0.27-0.84)	**0.011**	0.24 (0.09-0.64)	**0.0042**
AFP (ng/ml)				
≦ 100	Ref		Ref	
> 100	1.47 (0.47-4.56)	0.51	6.09 (1.30-28.5)	**0.022**
9-gene Exhausted CD8	0.44 (0.23-0.92)	**0.010**	0.24 (0.08-0.68)	**0.0072**
Macrovascular invasion				
No	Ref		Ref	
Yes	0.51 (0.16-1.66)	0.26	0.64 (0.16-2.62)	0.53
9-gene Exhausted CD8	0.51 (0.29-.91)	**0.022**	0.30 (0.11-0.81)	**0.018**
Extra-hepatic spread				
No	Ref		Ref	
Yes	2.07 (0.25-16.9)	0.50	8.9e7 (0-Inf)	1.00
9-gene Exhausted CD8	0.52 (0.30-0.91)	**0.021**	0.27 (0.10-0.73)	**0.010**
Liver tumor > 50%				
No	Ref		Ref	
Yes	1.5e-8 (0-Inf)	1.00	2.9e-8 (0-Inf)	1.00
9-gene Exhausted CD8	0.38 (0.19-0.78)	**0.009**	0.33 (0.12-0.88)	**0.027**
Prior systemic therapy				
None	Ref		Ref	
1 or more lines	0.37 (0.08-1.69)	0.20	9.1e7 (0-Inf)	1.00

***** The result was in bold, if $p < 0.05$

Supplementary Table 10. Multivariate Cox regression analysis in the samples with single-agent regimen.

Characteristics	Progression-free survival		Overall survival	
	HR (95% IC)	*p* value*	HR (95% IC)	*p* value*
N = 22	Event = 18		Event = 17	
9-gene Exhausted CD8	0.44 (0.22-0.88)	**0.020**	0.71 (0.36-1.38)	0.31
Age				
≦ 60	Ref		Ref	
> 60	0.83 (0.23-3.42)	0.86	0.78 (0.23-2.60)	0.69
ALBI grade				
1	Ref		Ref	
2	0.59 (0.17-2.03)	0.40	0.75 (0.19-2.89)	0.67
NLR				
≦ 3	Ref		Ref	
> 3	0.65 (0.15-2.73)	0.55	0.49 (0.13-1.89)	0.30
AFP (ng/ml)				
≦ 100	Ref		Ref	
> 100	0.89 (0.28-2.84)	0.85	0.37 (0.09-1.45)	0.15

***** The result was in bold, if $p < 0.05$

Supplementary Table 11. Multivariate Cox regression analysis in the samples with combination regiment.

Characteristics	Progression-free survival		Overall survival	
	HR (95% IC)	*p* value*	HR (95% IC)	*p* value*
N = 20	Event = 15		Event = 10	
9-gene Exhausted CD8	0.47 (0.26-0.87)	**0.015**	0.16 (0.05-0.59)	**0.0054**
Age				
≦ 60	Ref		Ref	
> 60	0.86 (0.26-2.90)	0.81	2.53 (0.41-15.7)	0.32
ALBI grade				
1	Ref		Ref	
2	0.92 (0.23-3.71)	0.91	2.90 (0.43-19.8)	0.28
NLR				
≦ 3	Ref		Ref	
> 3	1.52 (0.46-5.05)	0.49	1.37 (0.25-7.37)	0.71
AFP (ng/ml)				
≦ 100	Ref		Ref	
> 100	1.56 (0.41-5.95)	0.51	5.73 (0.64-51.4)	0.12

***** The result was in bold, if $p < 0.05$

Supplementary Table 12. Clinical-pathologic characteristics of the primary-metastatic HCC cohort

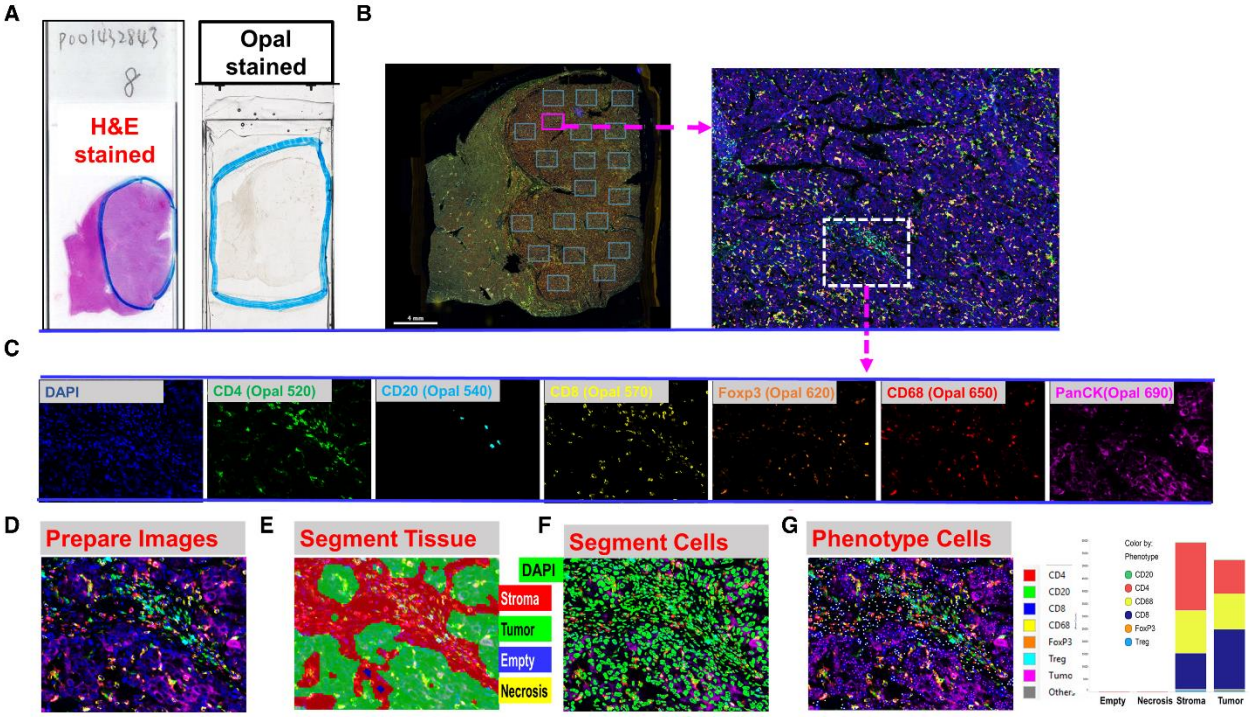
Characteristics	No. (%)
N	31
Age (years)	25-78 (median 48)
Sex	
Men	27 (87.1)
Women	4 (12.9)
Largest tumor diameter	
<= 5 cm	7 (22.5)
> 5 cm & <= 10 cm	10 (32.3)
> 10 cm	14 (45.2)
Tumor numbers	
1	26 (83.9)
2	3 (9.7)
>= 3	2 (6.4)
Etiology	
HBV	26 (83.9)
HCV	3* (9.7)
Other	2 (6.4)
Macrovascular invasion	
No	30 (96.9)
Yes	1 (3.1)
Cirrhosis	
No	10 (32.3)
Yes	21 (67.7)
Pathology stages (AJCC)	
I	10 (32.3)
II	10 (32.3)
III	10 (32.3)
IV	1 (3.1)

* One patient had both HBV and HCV infection.

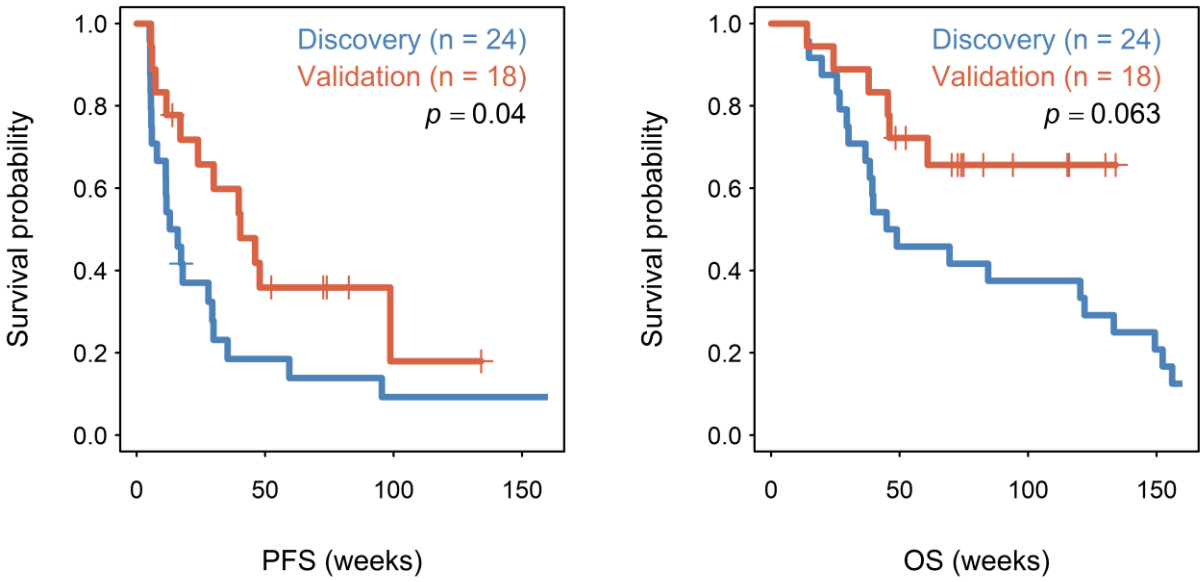
Supplementary Figures

Supplementary Figure 1. Analysis flowchart of immune cell composition by using multiplex immunofluorescence staining

(A) Identification of the tumor parts of each specimen by the pathologist.
(B) Acquisition of multispectral images to cover the whole area of the specimens and normalization for exposure by using the Phenochart and the inForm software.
(C) Unmixing of the multispectral images to their component images by using the spectral libraries of each fluorophore.
(D) Selection of representative tumor images to establish the training algorithm
(E) Manual tissue segmentation (tumor, stroma, necrosis, and blank areas)
(F) Cell segmentation based on the nuclear DAPI staining.
(G) Cell phenotyping and measurement of proportions of different immune cells.



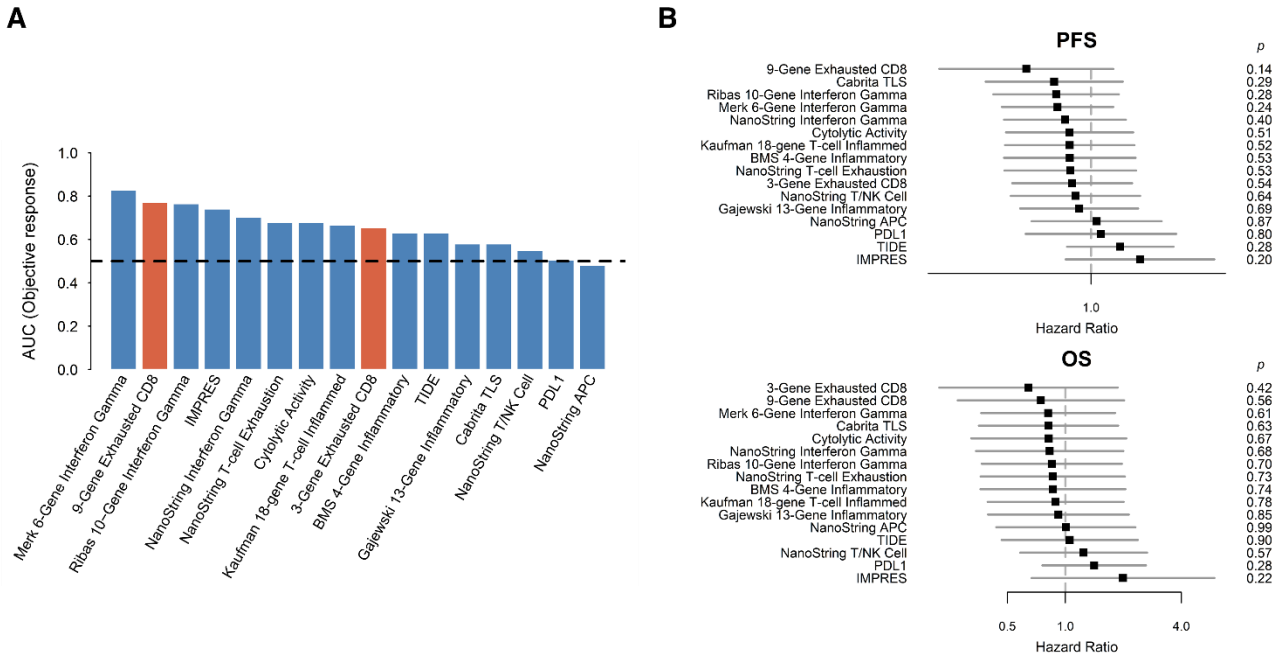
Supplementary Figure 2. PFS and OS curves of the ICI therapy discovery and validation cohorts



Supplementary Figure 3. Predictive ability of T-cell signatures in the validation cohort

(A) Bar plot depicting area under the receiver-operating characteristics (ROC) curve (AUC) for prediction of objective response by different immune cells in the ICI therapy validation cohort (n = 18). The performance of a random predictor (AUC = 0.5) was denoted by the dashed line.

(B) Forest plots showing hazard ratios of various immune cells for progress-free survival (PFS, left) and overall survival (OS, right) in the ICI therapy validation cohort (n = 18). Horizontal bars represented the 95% CIs of HR

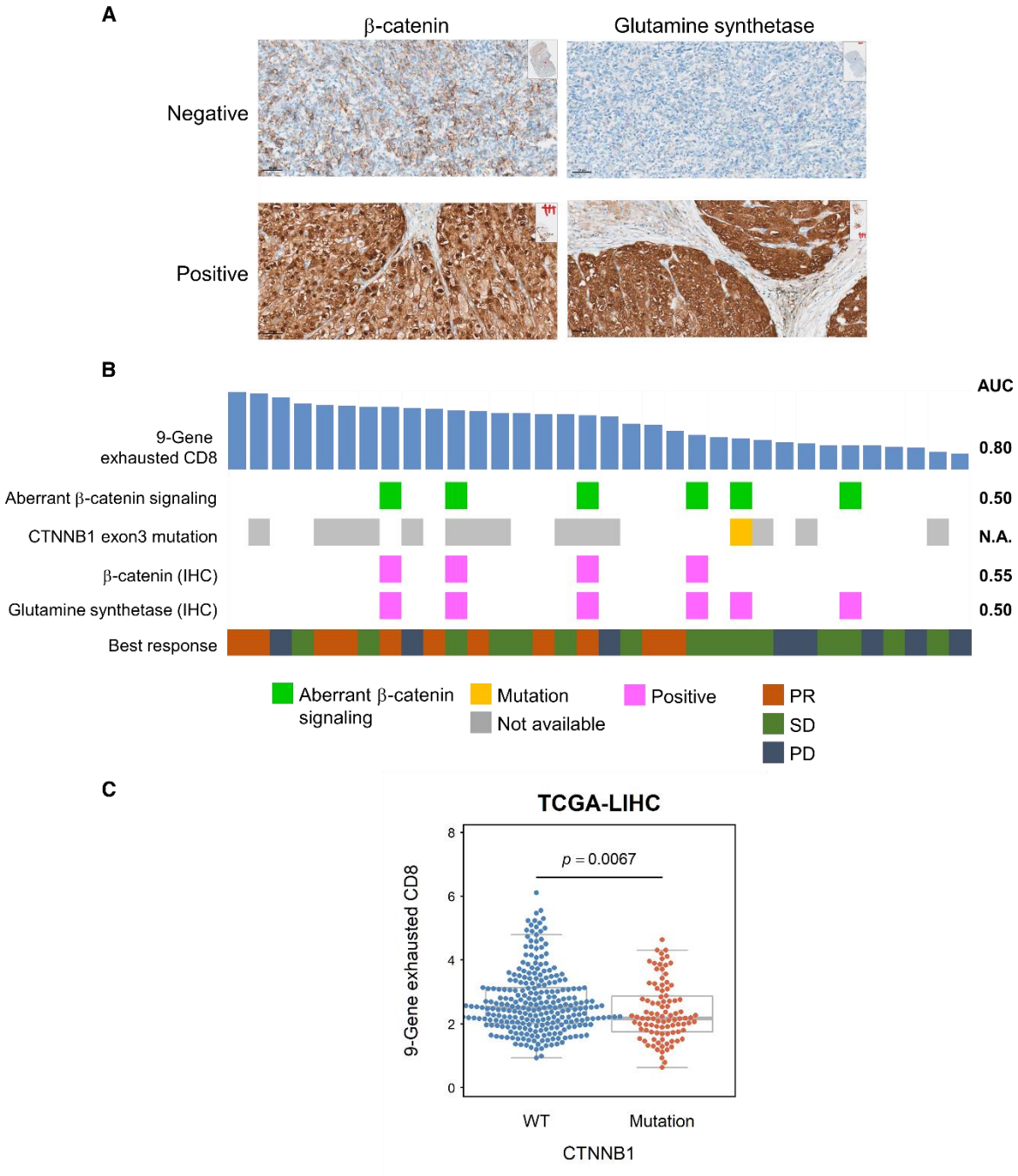


Supplementary Figure 4. Association between 9-gene exhausted CD8 signature and β-catenin signaling

(A) Representative images from the tumors with low (top) and high (bottom) nuclear β-catenin and cytoplasmic glutamine synthetase expression.

(B) The status of β-catenin in the ICI treatment cohort evaluated by Sanger sequencing and immunostaining. The aberrant β-catenin signaling is defined as one of positive of CTNNB1 mutation, expression of nuclear β-catenin and cytoplasmic glutamine synthetase.

(C) 9-gene exhausted CD8 signatures between samples without/with CTNNB1 mutations in TCGA-LHIC.



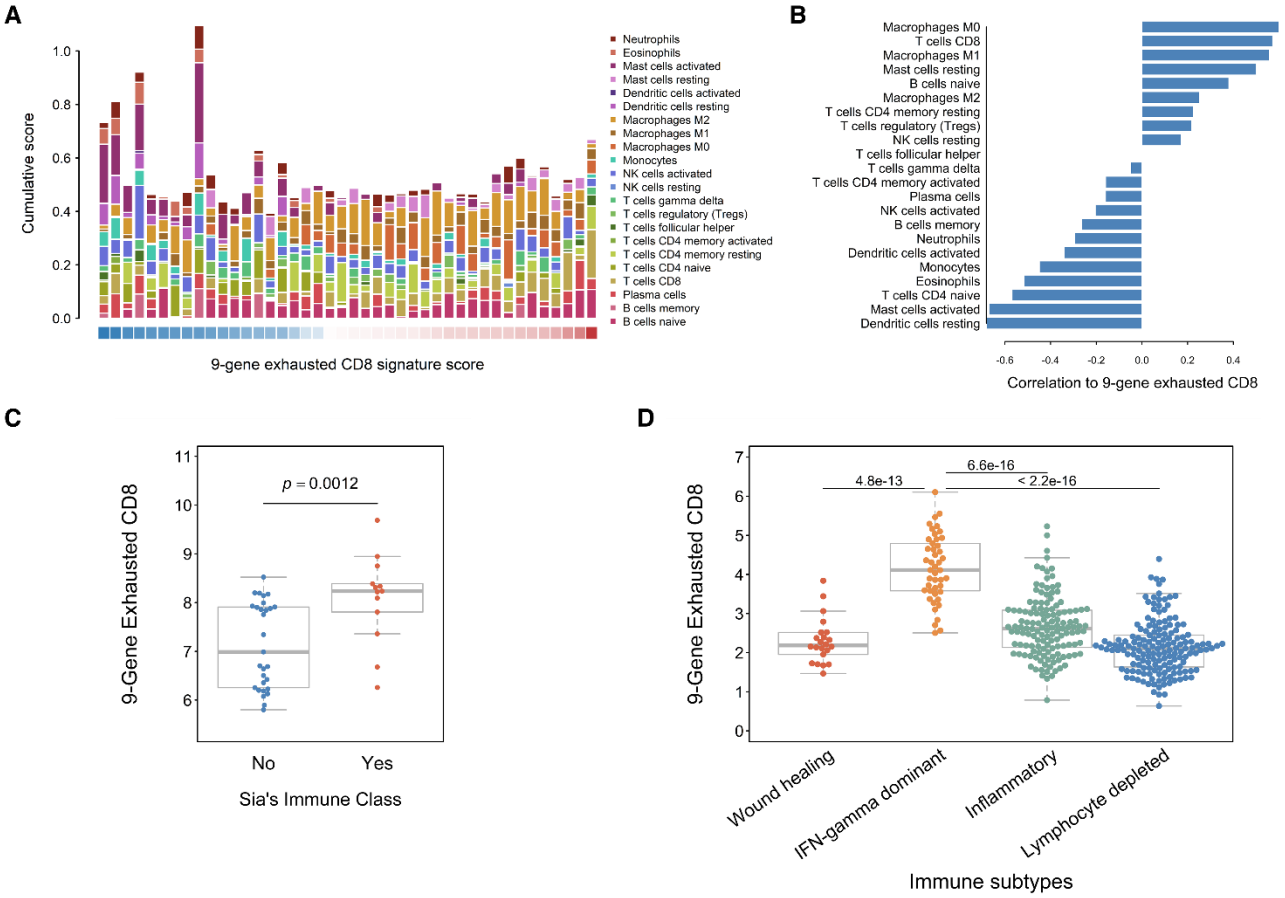
Supplementary Figure 5. Association between 9-gene exhausted CD8 signature and other immune-based subtypes in HCC

(A) Cumulative CIBERSORT score for various types of immune cells in each sample. The order of samples was based on the 9-gene exhausted signature.

(B) The correlation of CIBERSORT score for immune cells and 9-gene exhausted CD8 signature.

(C) Distribution of 9-gene exhausted CD8 signature between samples in and not in the immune class defined by Sia and the colleagues (10).

(D) Distribution of 9-gene exhausted CD8 signature among different immune subtypes defined by Thorsson and the colleagues (12).



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