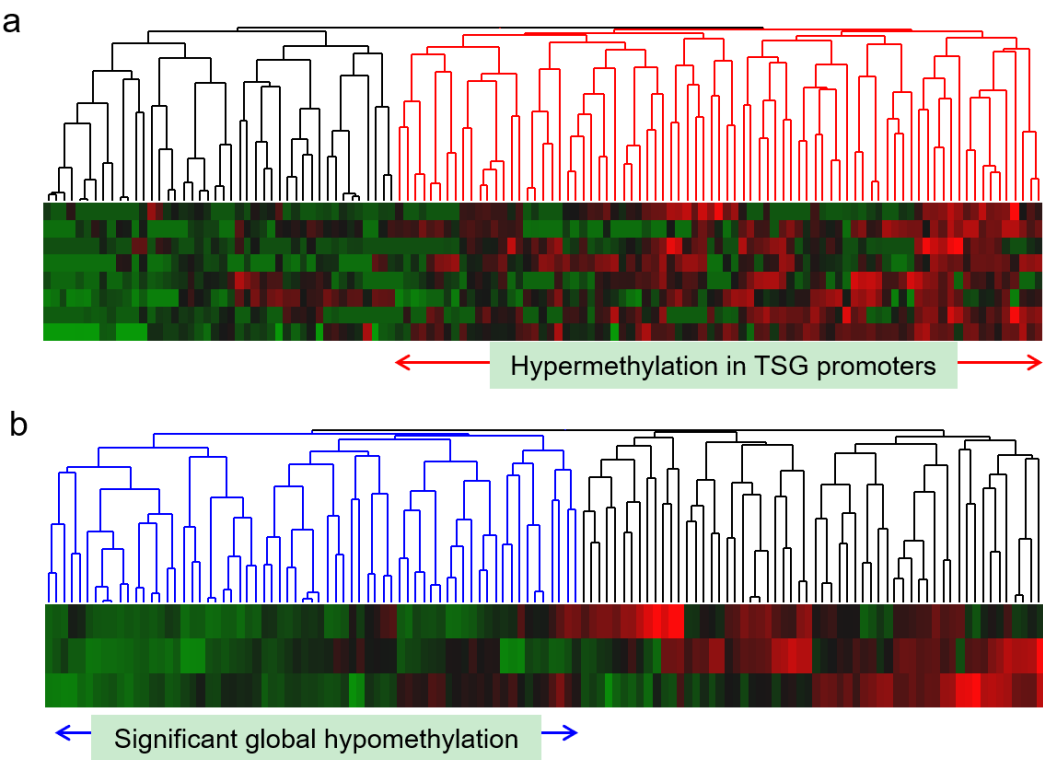


# Supplementary Materials: Molecular Scoring of Hepatocellular Carcinoma for Predicting Metastatic Recurrence and Requirements of Systemic Chemotherapy

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**Figure S1.** Classifications of HCCs based on methylation status of 8 tumor suppressor gene (TSG) promoters and 3 kind of repetitive DNA sequences (rDNAs). **(a)** HCCs with hypermethylation in the promoters of TSGs were determined using hierarchical clustering analysis. **(b)** Hypomethylation at rDNAs, which were the representative of global hypomethylation, was also determined as carrying significant hypomethylation.

**Table S1.** Mutations detected in HCCs from hepatic resection.

Sample ID	CNTTB1	TP53	TERT Promoter
1T	none	none	(-146) G→A
2T	none	none	(-124) G→A
3T	none	none	(-124) G→A
4T	none	none	none
5T	none	none	(-124) G→A
6T	codon 45 TCT→CCT	codon 151 CCC→GCC	(-124) G→A
7T	none	none	(-124) G→A
8T	codon 32 GAC→TAC	none	(-124) G→A
9T	codon 45 TCT→CCT	none	(-124) G→A
10T	codon 36 CAT→CGT	none	none
11T	none	none	none
12T	none	intron 7 AGgt→AGgg	(-124) G→A
13T	codon 33 TCT→TGT	none	none

14T	none	none	(-124) G→A
15T	codon 36 CAT→CCT	none	(-124) G→A
16T	none	none	none
17T	none	codon 285 GAG→TAG	(-124) G→A
18T	none	none	(-124) G→A
19T	none	none	(-124) G→A
20T	codon 41 ACC→GCC	codon 141 TGC→TAC	(-124) G→A
21T	none	codon 130 CTC→TTC	none
22T	codon 41 ACC→GCC	none	(-124) G→A
23T	codon 45 TCT→TTT	none	(-124) G→A
24T	none	none	(-124) G→A
25T	none	none	(-124) G→A
26T	none	intron 6 AGgt→AGgg	(-124) G→A
27T	none	codon 208 GAC→GCC	(-124) G→A
28T	none	none	(-124) G→A
29T	none	none	(-124) G→A
30T	none	codon 247 CGG→CAG	none
31T	none	codon 278 CCT→TCT	(-124) G→A
32T	none	none	(-124) G→A
33T	none	none	(-124) G→A
34T	none	none	none
35T	none	none	(-124) G→A
36T	codon 45 TCT→CCT	none	(-124) G→A
37T	none	codon 147 del (1bp)	none
38T	none	none	none
39T	codon33 TCT→CCT	none	(-124) G→A
40T	none	none	(-124) G→A
41T	none	none	(-124) G→A
42T	codon37 TCT→TGT	codon 193 CAT→TAT	(-124) G→A
43T	none	none	(-124) G→A
44T	none	none	(-124) G→A
45T	none	none	none
46T	none	codon 161 GCC→TCC	(-146) G→A
47T	codon33 TCT→TTT	none	(-124) G→A
48T	none	none	none
49T	none	none	none
50T	codon44 CCT→GCT	codon 177 CCC→CGC	(-124) G→A
51T	none	codon 132 AAG→AAT	(-124) G→A
52T	none	none	(-124) G→A
53T	none	none	(-124) G→A
54T	codon32 GAC→AAC	none	(-124) G→A
55T	none	intron 5 agGT→atGT	(-124) G→A
56T	none	none	(-124) G→A
57T	none	none	none
58T	codon 36 CAT→CCT	codon 215 AGT→AAT	(-124) G→A
59T	none	codon 139 AAG→ACG	none
60T	none	codon 157 GTC→GCC	(-124) G→A
61T	none	none	none
62T	none	intron 4 tcctacagTA→ccctacagTA	(-124) G→A
63T	none	codon 244 GGC→TGC	(-124) G→A
64T	none	codon 156 CGC→CAC	none
65T	none	codon 240 AGT→CGT	(-124) G→A
66T	none	none	none
67T	none	none	(-124) G→A
68T	codon34 GGA→AGA	none	(-127) G→A
69T	none	none	(-124) G→A
70T	none	none	none
71T	none	none	none
72T	none	none	none
73T	none	codon 258 GAA→AAA	none
74T	codon34 GGA→GTA	none	none
75T	none	none	none
76T	codon45 TCT→TTT	codon 285 GAG→AAG	none
77T	none	codon 236 TAC→TGC	(-124) G→A
78T	none	none	(-124) G→A

79T	none	none	(-124) G→A
80T	none	none	(-124) G→A
81T	none	none	none
82T	none	none	none
83T	none	none	(-124) G→A
84T	codon 44 to 55 del (33bp)	none	(-124) G→A (-68) G→A
85T	codon 37 TCT→TTT	none	(-124) G→A
86T	codon 45 TCT→CCT	none	(-124) G→A
87T	none	none	(-124) G→A
88T	none	none	(-124) G→A
89T	codon 41 ACC→GCC	none	(-124) G→A
90T	codon 41 ACC→GCC	none	(-124) G→A
91T	none	none	(-124) G→A
92T	codon 45 TCT→CCT	none	none
93T	none	none	none
94T	none	none	(-124) G→A
95T	codon 41 ACC→GCC	codon 239 AAC→GAC	(-124) G→A
96T	codon 41 ACC→GCC	none	(-124) G→A
97T	none	none	(-124) G→A
98T	none	none	none
99T	codon36 CAT→CCT	none	(-124) G→A
100T	none	none	none
101T	none	none	none
102T	none	none	(-124) G→A
103T	none	none	(-124) G→A
104T	none	none	none
105T	none	none	(-124) G→A
106T	none	none	(-124) G→A
107T	none	none	(-124) G→A
108T	none	none	(-124) G→A
109T	codon 32 GAC→AAC	none	(-124) G→A
110T	codon 45 TCT→CCT	none	none
111T	none	none	(-124) G→A
112T	none	none	none
113T	codon 41 ACC→GCC	none	(-124) G→A
114T	none	none	(-124) G→A
115T	none	none	(-124) G→A
116T	none	none	(-124) G→A
117T	none	codon 270 TTT→GTT	none
118T	none	none	(-124) G→A
119T	none	none	none
120T	none	none	(-124) G→A
121T	none	none	(-124) G→A
122T	none	none	none
123T	none	none	none
124T	none	none	(-124) G→A
125T	none	none	none

**Table S2.** The details of the clinical background of 25 patients who underwent liver transplantation.

Clinical Factors	Median (25th–75th Percentile) or Number of Cases
Age (years old)	59 (54–62.5)
Sex	
Male/female	19/6
Virus	
HBV/HCV/HBV& HCV/negative	10/13/1/1
Serum AFP <sup>1)</sup> (ng/mL)	41 (9.4–222)
Serum DCP <sup>2)</sup> (mAU/mL)	54 (16.8–151.5)
Maximum tumor size (cm)	2.6 (1.8–3.2)
Number of tumor	
Single/multiple/missing	3/22
Tumor differentiation	
Well/moderately/poorly/missing	3/17/5

<sup>1)</sup> AFP;  $\alpha$ -fetoprotein <sup>2)</sup> Des-gamma carboxyprothrombin.

**Table S3.** Mutations detected in HCCs from liver transplantation.

Sample ID	CNTTB1	TP53	TERT promoter
T03H	none	none	(-124) G→A
T04H	none	none	none
T05H	codon 41 ACC→GCC	codon 183 TCA→CCA	none
T10H	none	codon 181 CGC→TGC	(-124) G→A
T15H12	none	none	(-124) G→A
T21H1	none	codon 237 ATG→ATA	(-124) G→A
T22H	codon 45 TCT→CCT	none	(-124) G→A
T26H1	none	codon 286 GAA→TAA	none
T29H	none	codon 234 TAC→TAA	(-124) G→A
T32H	none	none	(-124) G→A
T35H1	none	none	(-124) G→A
T38H1	none	none	none
T42H	none	none	(-124) G→A
T43H	none	none	none
T47H	codon 45 to 47 del (9 bp)	none	(-124) G→A
T51H	codon 32 GAC→GGC	none	(-124) G→A
T52H2	none	none	none
T53H	none	none	(-124) G→A
T55H	none	codon 239 AAC→GAC	(-124) G→A
T57H1	codon 45 TCT→TTT	codon 248 CGG→TGG	(-124) G→A
T61H4	codon 32 GAC→GGC	none	(-124) G→A
T77H1	none	none	(-124) G→A
T78H1	none	codon 173 GTG→ATG	none
T86H1	none	intron 5 TGgt→TGgc	(-124) G→A
T95H2	codon 41 ACC→GCC	none	(-124) G→A

**Table S4.** Factors associated with disease-free survival of HCC patients who underwent liver transplantation.

Clinicopathological backgrounds	No. of Cases		Univariate	Multivariate	
	Total	With Event	<i>p</i> value	<i>p</i> value	HR (95% CI)
AFP					
≥200 ng/mL	6	5	<0.0001	0.0969	14.8 (0.64–1413)
<200 ng/mL	18	1			1
PIVKA-II					
≥400 AU/mL	3	3	0.0003	0.5050	2.01 (0.27–22.6)
<400 AU/mL	21	3			1
Size					
≥3.0 cm	9	5	0.0170	0.9617	0.93 (0.04–31.0)
<3.0 cm	16	1			
Differentiation					
Mod-poor	22	6	0.3325		
Well	3	0			
Number of tumor					
Multiple	22	6	0.3325		
Single	3	0			
Milan criteria					
Out	17	6	0.1782		
In	8	0			
Molecular pattern					
Aggressive	10	5	0.0090	0.8200	1.42 (0.07–44.0)
Mild	14	1			

supplementary S5 in an individual.

**Table S6.** The details of the clinical background of 125 patients who underwent hepatectomy.

Clinical Factors	Median (25th–75th Percentile) or Number of Cases
Age (years old)	63 (56–69)
Sex (male/female)	90/35
Virus	
HBV/HCV/HBV& HCV/negative	27/75/2/21
Serum AFP <sup>1)</sup> (ng/mL)	55 (7.3–487.5)
Maximum tumor size (cm)	3.6 (2.7–6)
Vascular invasion	
Presence/absence/missing	58/65/2
Number of tumor	
Single/multiple/missing	55/58/12
Tumor differentiation	
Well/moderately/poorly/missing	36/64/21/4

<sup>1)</sup> AFP;  $\alpha$  fetoprotein.

**Table S7.** The details of PCR primers and conditions for mutational analyses.

Target genes	Primers	T <sub>m</sub>
<i>CNTTB1</i> exon 3	Forward: 5'-(ATGGAACCAGACAGAAAAG)-3' Reverse: 5'-(TACAGGACTTGGGAGGTATC)-3'	58 °C
<i>TP53</i> exon 5-1	Forward: 5'-(TTATCTGTTCACCTGTGCCC)-3' Reverse: 5'-(TCATGTGCTGTGACTGCTTG)-3'	60 °C
<i>TP53</i> exon 5-2	Forward: 5'-(TTCCACACCCCGCCCGGCA)-3' Reverse: 5'-(ACCCTGGGCAACCAGCCCTG)-3'	65 °C
<i>TP53</i> exon 6	Forward: 5'-(ACGACAGGGCTGGTTGCCCA)-3' Reverse: 5'-(CTCCCAGAGACCCAGTTGC)-3'	65 °C
<i>TP53</i> exon 7	Forward: 5'-(GGCCTCATCTTGGGCCTGTG)-3' Reverse: 5'-(CAGTGTGCAGGGTGGCAAGT)-3'	64 °C
<i>TP53</i> exon 8	Forward: 5'-(CTGCCTCTTGCTTCTCTTT)-3' Reverse: 5'-(TCTCCTCCACCGCTTCTTGT)-3'	60 °C
<i>TERT</i> promoter	Forward: 5'-(CAGCGCTGCCTGAAACTC)-3' Reverse: 5'-(GTCCTGCCCCCTTCACCTT)-3'	62 °C



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