Supplementary information for

Identification and characterization of a 25-lncRNA prognostic signature for early recurrence in hepatocellular carcinoma

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SUPPLEMENTARY FIGURES and FIGURE LEGENDS

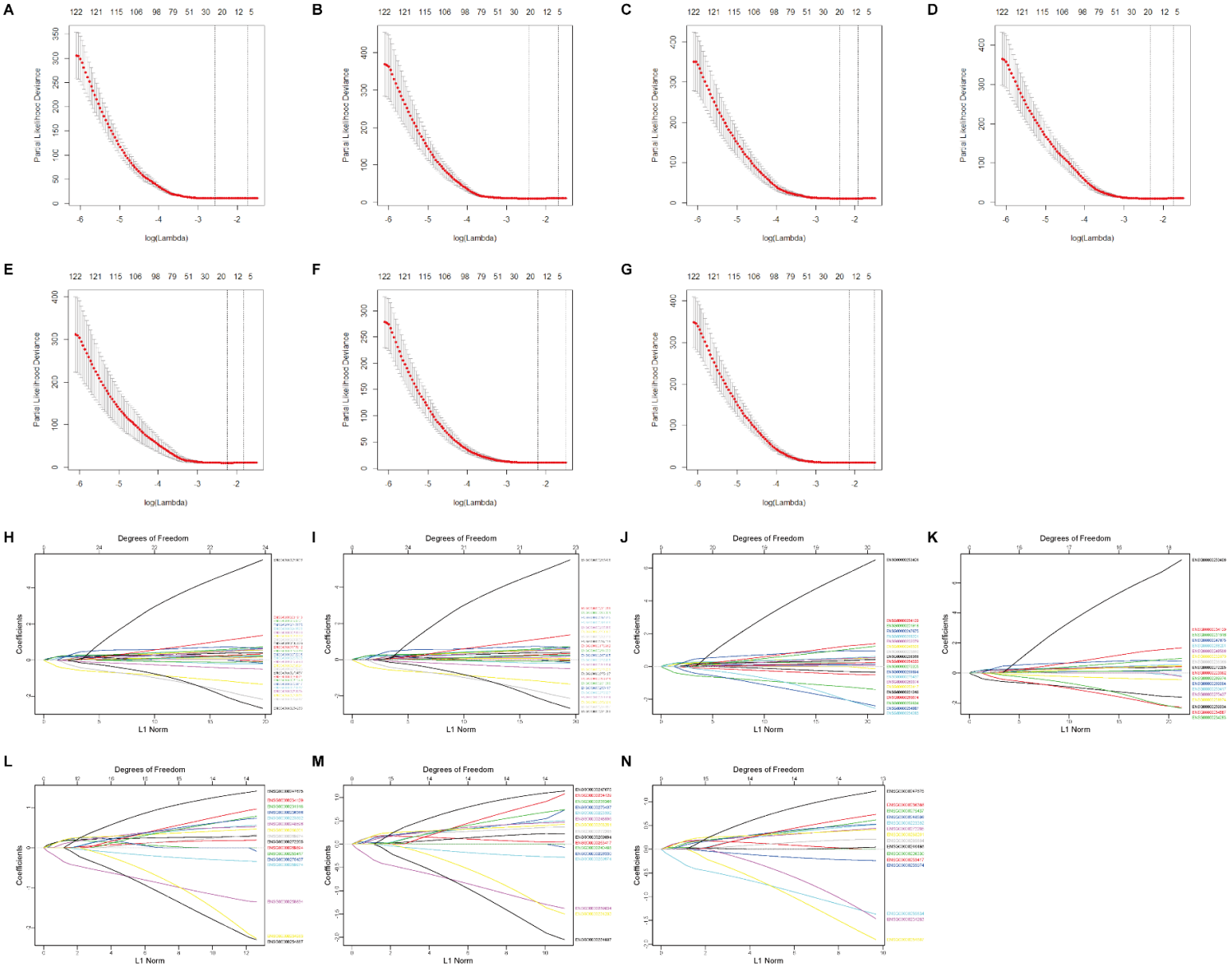

Figure S1 LASSO regression results of 7 constructed LncRNA-based risk signature

A-G) Ten-fold cross-validation for tuning parameter selection in the LASSO model. The dotted vertical lines are drawn at the optimal values by minimum criteria (lambda.min, left vertical dotted line) and 1-SE criteria (lambda.1se, right vertical dotted line); lambda.min is 0.07681466, 0.0883182, 0.09252353, 0.09692911, 0.1063796, 0.1114449 and 0.1167515 in A)-G) respectively; H-N) Partial LASSO coefficient profiles of the 358 lncRNAs. The dotted vertical line was plotted at the value selected using 10-fold cross-validation in A)-G), for which the optimal λ (lambda.min) resulted in H) 25 non-zero coefficients for A); I) 24 non-zero coefficients for B); J) 20 non-zero coefficients for C); K) 18 non-zero coefficients for D); L) 16 non-zero coefficients for E); M) 16 non-zero coefficients for F); N) 15 non-zero coefficients for G)

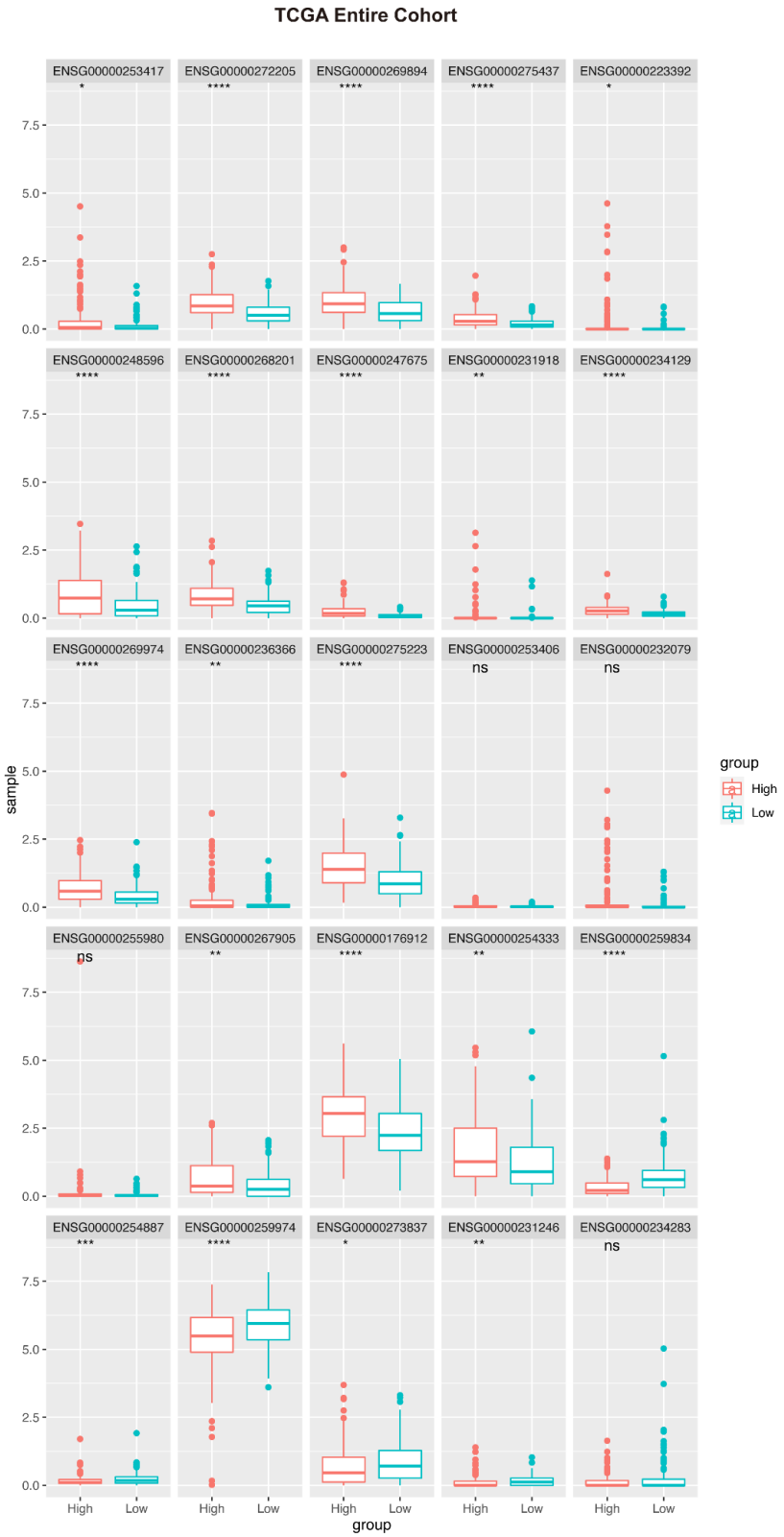

Figure S2 The expression comparison of lncRNAs in the low- and high-risk groups

Non-pair Wilcoxon test was used to compared the expression of lncRNAs between low- and high groups (ns: non-specific; *: P<0.05; **: P<0.01; ***: P<0.001, ****: P<0.0001).

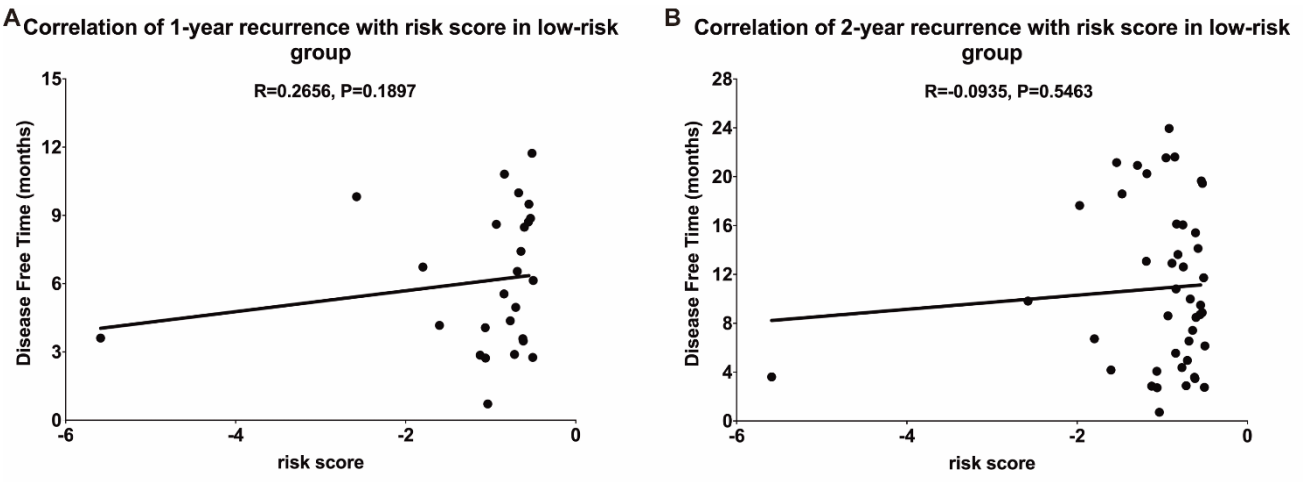

Figure S3 Correlation analysis of recurrence with risk score in the low-risk group

A-B) Correlation between risk score and 1-year DFS (R=0.2656, P=0.1897) (A) and 2-year DFS (R=-0.0935, P=0.5463) (B).

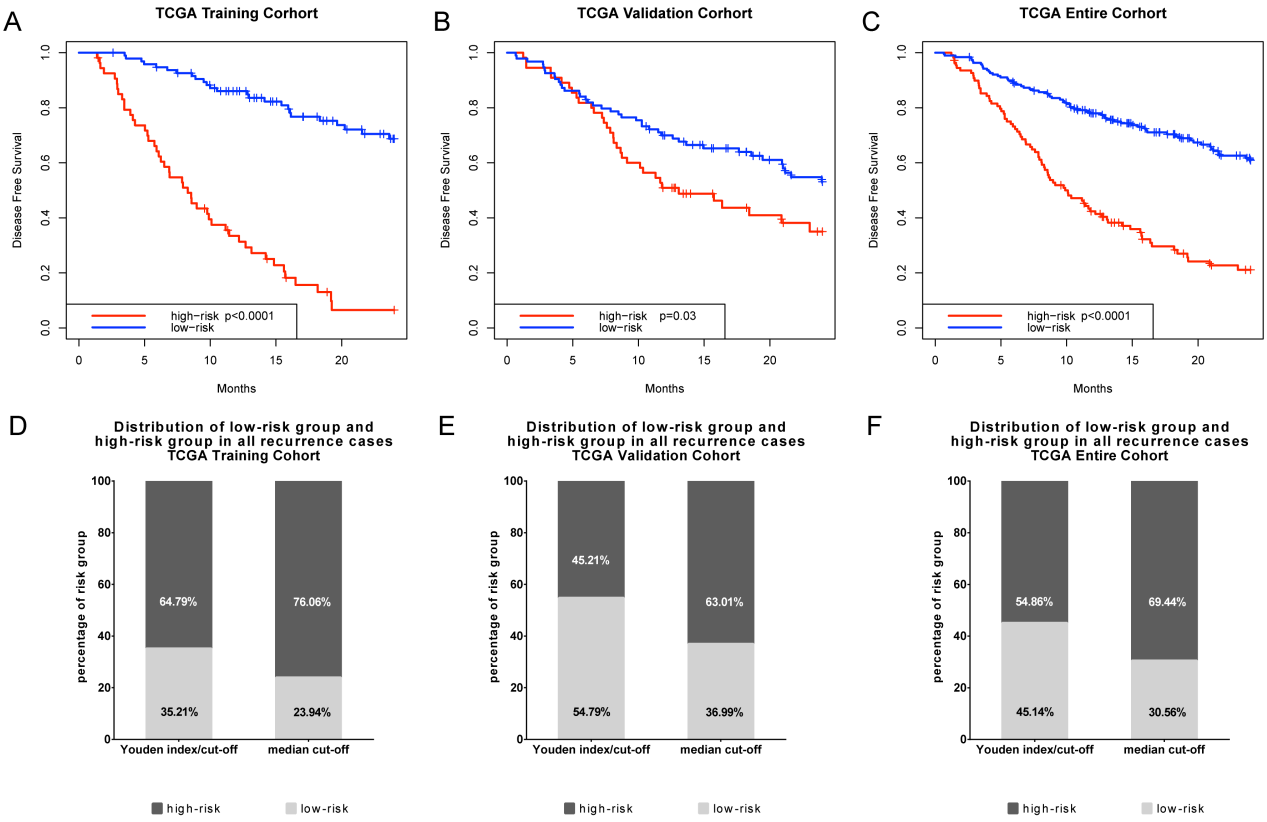

Figure S4 Comparison of different cut-off threshold

A)-C) KM plot of the 25-lncRNA risk signature with 2-year DFS was analyzed in the training cohort (N=150, P<0.0001) (A), validation cohort (N=149, P=0.03) (B), and the entire TCGA cohort (N=299, P<0.0001) (C). The statistical significance was determined by the log-rank test. The patients in each cohort were stratified into the high-risk and low risk groups based on the cut-off risk score set to -0.2 in the training cohort. D) 64.79% (cut-off=-0.2) and 76.06% (cut-off=median) HCC patients with recurrence were classified in the high-risk group based on different cut-off, and 35.21% (cut-off=-0.2) and 23.94% (cut-off=median) HCC patients with recurrence were assigned in the low-risk group; E) 45.21% (cut-off=-0.2) and 63.01% (cut-off=median) HCC patients with recurrence were classified in the high-risk group based on different cut-off, and 54.79% (cut-off=-0.2) and 36.99% (cut-off=median) HCC patients with recurrence were assigned in the low-risk group; F) 54.86% (cut-off=-0.2) and 69.44% (cut-off=median) HCC patients with recurrence were classified in the high-risk group based on different cut-off, and 45.14% (cut-off=-0.2) and 30.56% (cut-off=median) HCC patients with recurrence were assigned in the low-risk group.

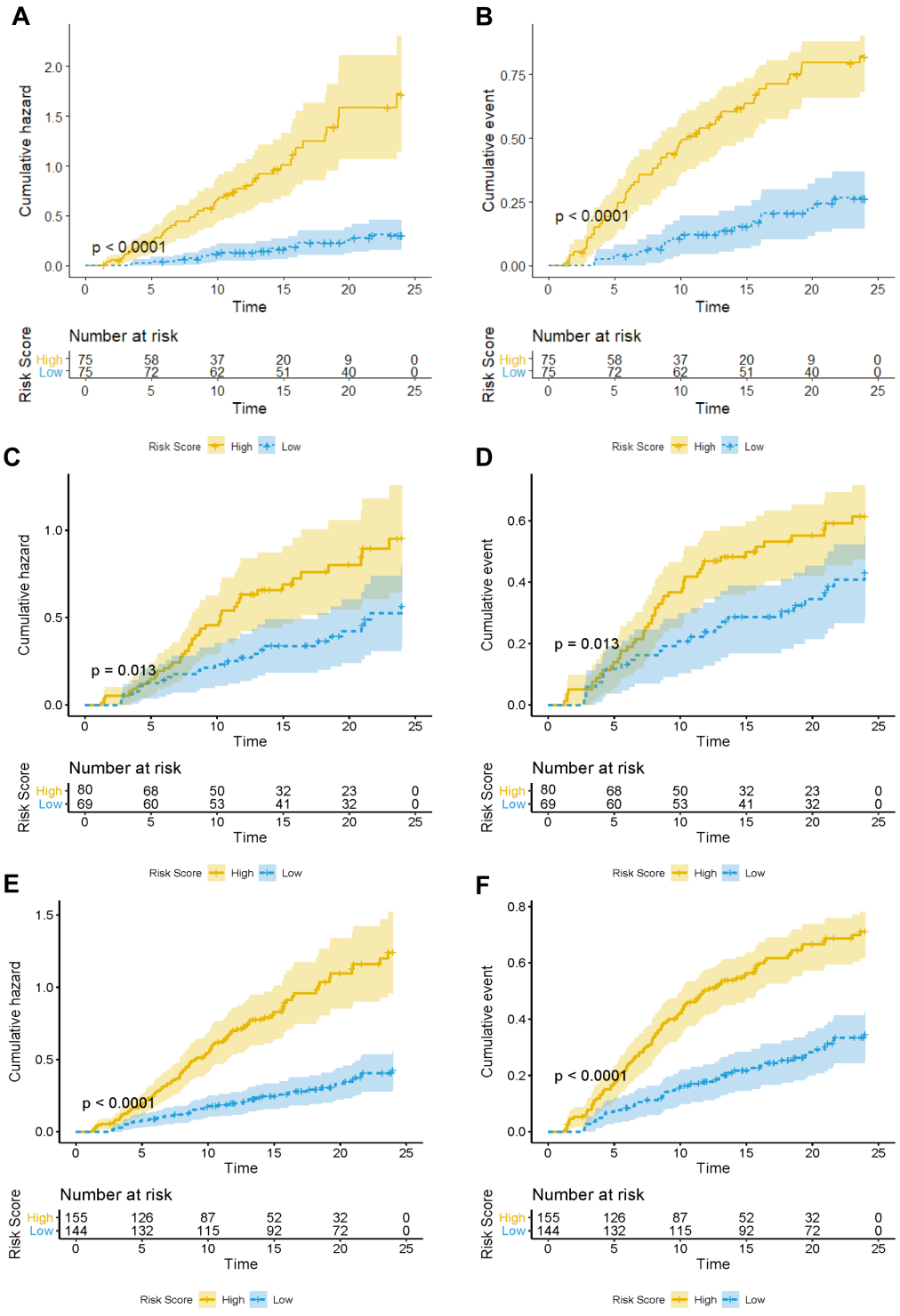

Figure S5 Recurrence analysis of HCC patients

A, C, E) Cumulative hazard curves showed higher recurrence hazard in the high-risk group of the training cohort (N=150) (P<0.0001) (A), validation cohort (N=149) (P=0.015) (C) and entire TCGA cohort (N=299) (P<0.0001) (E); B, D, F) Cumulative event curves showed higher recurrence events in the high-risk group of the training cohort (N=150) (P<0.0001) (B), validation cohort (N=149) (P=0.015) (D) and entire TCGA cohort (N=299) (P<0.0001) (F).

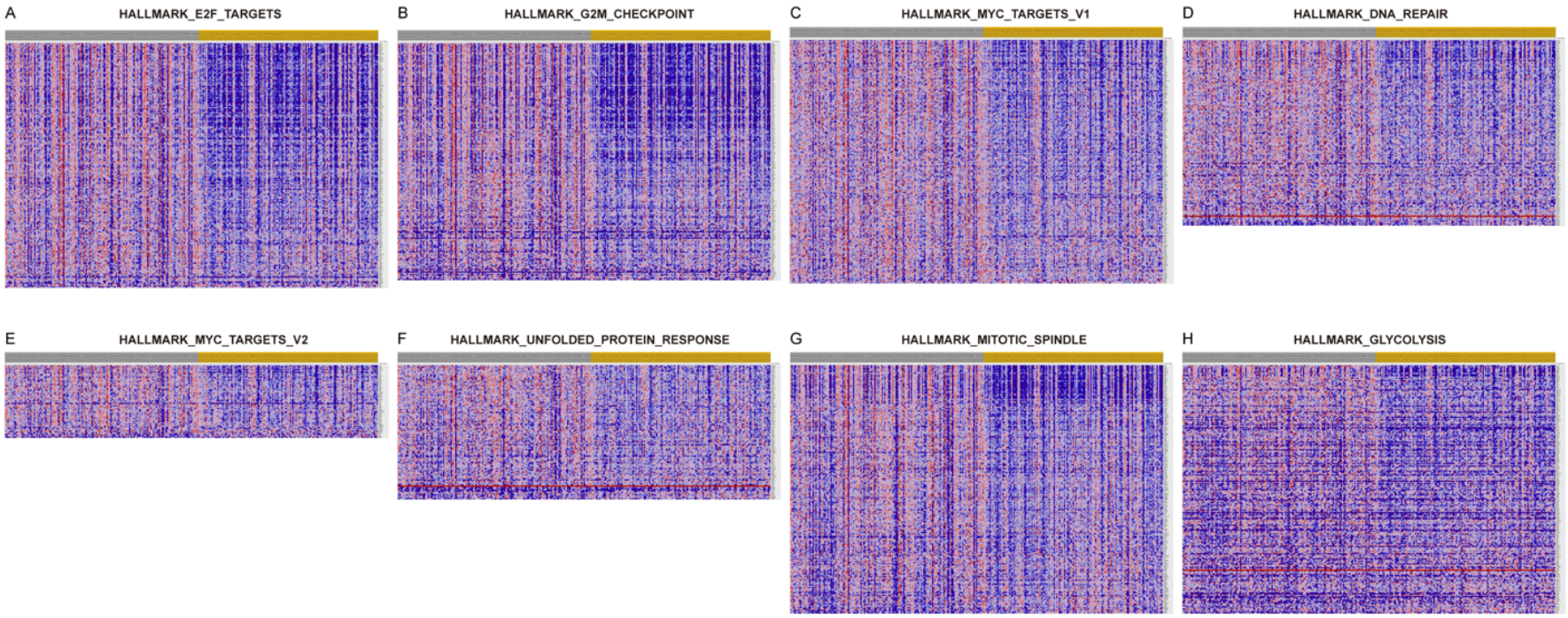

Figure S6 Heatmap for the genes of the significant pathways

Heatmap of genes in the low-risk group (brown) and the high-risk group (grey) of A) HALLMARK_E2F_TARGETS; B) HALLMARK_G2M_CHECKPOINT; C) HALLMARK_MYC_TARGETS_V1; D) HALLMARK_DNA_REPAIR; E) HALLMARK_MYC_TARGETS_V2; F) HALLMARK_UNFOLDED_PROTEIN_RESPONSE; G) HALLMARK_MITOTIC_SPINDLE; H) HALLMARK_GLYCOLYSIS.

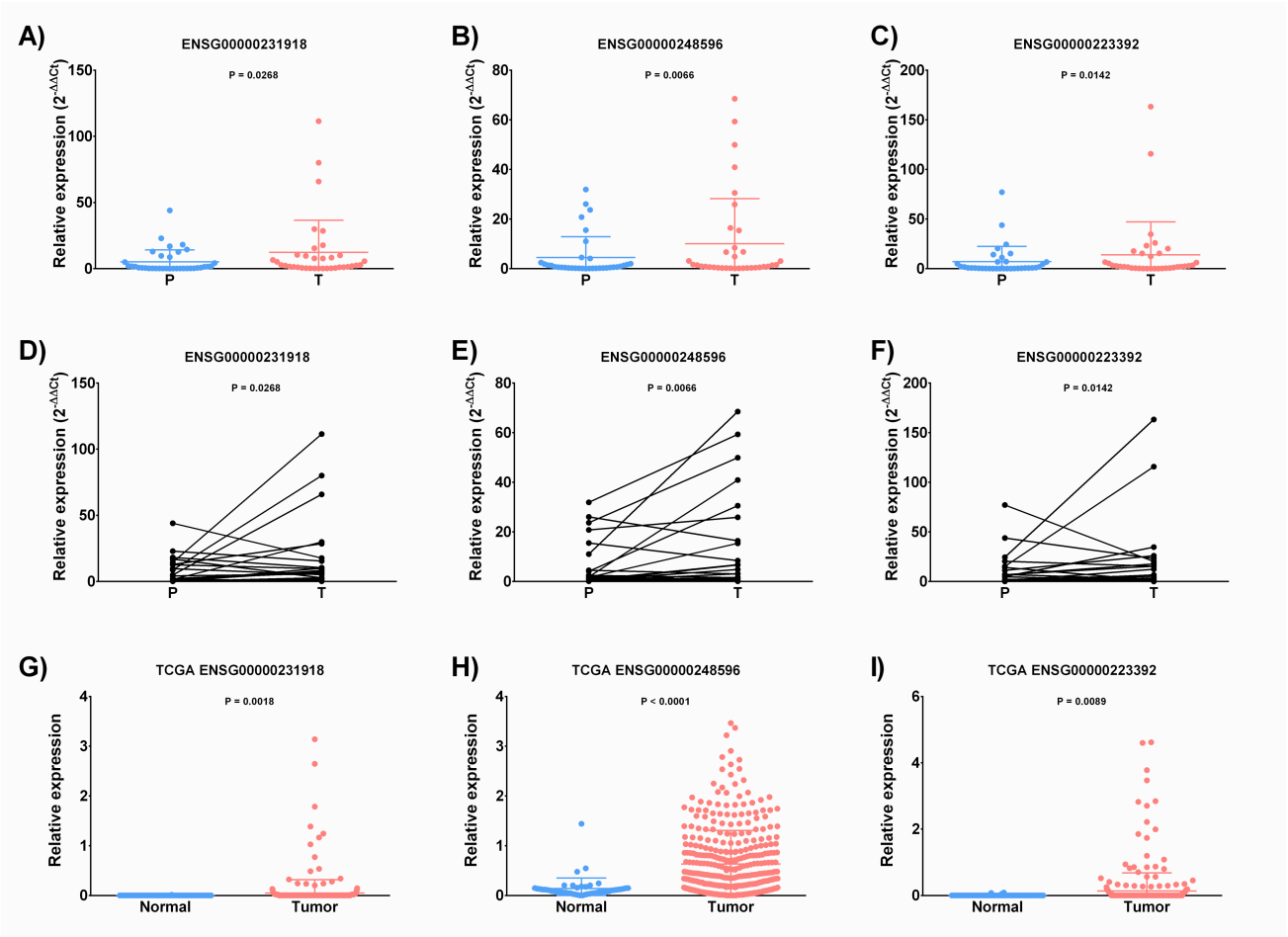

Figure S7 LncRNA expression in clinical samples and TCGA database

A)-C) Relative expressions of lncRNAs were higher in clinical HCC tissues than those in paracancerous tissues (n=36) for A) ENSG00000231918 (P = 0.0268), B) ENSG00000248596 (P = 0.0066), and C) ENSG00000223392 (P = 0.0142); D)-E) 36 Paired HCC tissues and paracancerous tissues ;G)-I) Relative expressions of lncRNAs were higher in TCGA HCC samples (N=373) than those in normal samples (N=50) for G) ENSG00000231918 (P = 0.0018), H) ENSG00000248596 (P < 0.0001), and I) ENSG00000223392 (P = 0.0089).

SUPPLEMENTARY TABLES

Table S1 Clinical characteristics of 299 HCC patients involved in the study

Characteristics	Number of Cases
Disease Free Survival (299/299)	
Disease Free	125(41.81%)
Recurred/Progressed	174(58.19%)
Overall Survival (299/299)	
Alive	220(73.58%)
Dead	79(26.42%)
TNM Stage (281/299)	
Stage I	145(51.6%)
Stage II	67(23.84%)
Stage III+IV	69(24.56%)
Fibrosis (181/299)	
Established Cirrhosis	62(34.25%)
Fibrous Speta	22(12.15%)
No Fibrosis	60(33.15%)
Nodular Formation and Incomplete Cirrhosis	7(3.87%)
Portal Fibrosis	30(16.57%)
Vascular Invasion (251/299)	
Macro	13(5.18%)
Micro	73(29.08%)
None	165(65.73%)
AFP Range (236/299)	
≤ 20 ng/ml	133(56.36%)
>20 ng/ml	103(43.64%)
Race (292/299)	
AMERICAN INDIAN OR ALASKA NATIVE	1(0.34%)
ASIAN	133(45.55%)
BLACK OR AFRICAN AMERICAN	11(3.77%)
WHITE	147(50.34%)
Sex (299/299)	
Female	90(30.1%)
Male	209(69.9%)
Age Range (299/299)	
≤ 50	60(20.07%)
>50	239(79.93%)

Table S2 7 group of lncRNAs sequence summarized from LASSO regression analysis

Model	NO. of LncRNAs	LncRNAs
1	25	ENSG00000253417, ENSG00000272205, ENSG00000269894, ENSG00000275437, ENSG00000223392, ENSG00000248596, ENSG00000268201, ENSG00000247675, ENSG00000231918, ENSG00000259834, ENSG00000234129, ENSG00000269974, ENSG00000254887, ENSG00000259974, ENSG00000236366, ENSG00000275223, ENSG00000253406, ENSG00000232079, ENSG00000273837, ENSG00000255980, ENSG00000267905, ENSG00000176912, ENSG00000254333, ENSG00000231246, ENSG00000234283
2	24	ENSG00000253417, ENSG00000272205, ENSG00000269894, ENSG00000275437, ENSG00000223392, ENSG00000248596, ENSG00000268201, ENSG00000247675, ENSG00000231918, ENSG00000259834, ENSG00000234129, ENSG00000269974, ENSG00000254887, ENSG00000259974, ENSG00000236366, ENSG00000275223, ENSG00000253406, ENSG00000232079, ENSG00000273837, ENSG00000267905, ENSG00000176912, ENSG00000254333, ENSG00000231246, ENSG00000234283
3	20	ENSG00000253417, ENSG00000272205, ENSG00000269894, ENSG00000275437, ENSG00000223392, ENSG00000248596, ENSG00000268201, ENSG00000247675, ENSG00000231918, ENSG00000259834, ENSG00000234129, ENSG00000269974, ENSG00000254887, ENSG00000259974, ENSG00000236366, ENSG00000253406, ENSG00000232079, ENSG00000254333, ENSG00000231246, ENSG00000234283
4	18	ENSG00000253417, ENSG00000272205, ENSG00000269894, ENSG00000275437, ENSG00000223392, ENSG00000248596, ENSG00000268201, ENSG00000247675, ENSG00000231918, ENSG00000259834, ENSG00000234129, ENSG00000269974, ENSG00000254887, ENSG00000259974, ENSG00000236366, ENSG00000253406, ENSG00000232079, ENSG00000234283
5	16	ENSG00000253417, ENSG00000272205, ENSG00000269894, ENSG00000226330, ENSG00000240498, ENSG00000275437, ENSG00000223392, ENSG00000248596, ENSG00000268201, ENSG00000247675, ENSG00000259834, ENSG00000234129, ENSG00000254887, ENSG00000259974, ENSG00000236366, ENSG00000234283
6	16	ENSG00000253417, ENSG00000272205, ENSG00000269894, ENSG00000275437, ENSG00000223392, ENSG00000248596, ENSG00000268201, ENSG00000247675, ENSG00000231918, ENSG00000259834, ENSG00000234129, ENSG00000269974, ENSG00000254887, ENSG00000259974, ENSG00000236366, ENSG00000234283
7	15	ENSG00000253417, ENSG00000272205, ENSG00000269894, ENSG00000226330, ENSG00000240498, ENSG00000275437, ENSG00000223392, ENSG00000248596, ENSG00000268201, ENSG00000247675, ENSG00000259834, ENSG00000254887, ENSG00000259974, ENSG00000236366, ENSG00000234283

Table S3 AUC and C-index of predication models

	1-year DFS			2-year DFS		
	AUC	**95% CI**	**C-index**	**AUC**	**95% CI**	**C-index**
RS+TNM+VI+AFP	78.8%	72.02%-85.56%	0.75	76.8%	70.3%-83.35%	0.74
RS	73.9%	66.49%-81.22%	0.71	72.0%	64.95%-79.02%	0.70
TNM	65.0%	57.63%-72.36%	0.63	67.2%	60.52%-73.83%	0.63
VI	63.5%	56.4%-70.55%	0.62	60.3%	53.9%-66.76%	0.60
AFP	64.6%	57.56%-71.6%	0.63	61.4%	54.73%-68.06%	0.61

Table S4 Clinical information of 36 HCC patients

Patient NO	Gender	Age	HBsAg	HCVAb	Cirrhosis	AFP	ALT	Tumor Size	Tumor Number	TNM	Recurrence
1	Female	50	+	-	+	1028	55	12	1	III	Not Available
2	Female	71	-	-	-	18.4	12	8	1	I	Not Available
3	Male	61	-	+	-	3.02	19	1.8	1	I	Not Available
4	Female	64	+	-	+	8.67	61	5.5	>1	III	Not Available
5	Male	64	+	-	+	4.75	14	6	1	I	Not Available
6	Male	76	-	-	-	1.21	17	4	1	I	Not Available
7	Female	35	+	-	-	1185	20	15	1	III	Not Available
8	Female	64	-	-	-	3.65	24	4.5	1	I	Not Available
9	Male	65	+	-	-	8256	47	9	1	I	Not Available
10	Male	67	+	-	-	4.84	18	4.5	1	II	Not Available
11	Male	60	+	-	+	2.46	22	2	1	I	Not Available
12	Male	62	+	-	-	81.7	32	2	1	III	Recurrence
13	Female	55	+	-	-	3.08	27	9.2	1	III	Not Available
14	Male	60	+	-	-	2.38	42	4.5	1	I	Not Available
15	Male	56	+	-	+	1438	32	1.5	1	I	Not Available
16	Male	75	-	-	-	8.35	28	8	1	I	Not Available
17	Male	47	+	-	-	39241	26	4	>1	III	Not Available
18	Male	59	+	-	+	2.29	12	1.5	>1	II	Not Available
19	Male	69	+	-	+	2884	26	3	>1	III	Recurrence

20	Male	55	+	-	-	14.7	23	17	>1	III	Recurrence
21	Male	54	+	-	-	40.6	36	4.5	1	I	Not Available
22	Male	64	+	-	-	2.47	17	3	>1	II	Not Available
23	Female	66	+	-	+	423	56	9	>1	III	Recurrence
24	Female	71	-	-	-	611	8	6	1	III	Not Available
25	Male	47	+	-	+	>60500	62	20	1	IV	Recurrence
27	Male	43	+	-	+	2.27	27	1.3	>1	II	Not Available
28	Female	66	+	-	+	5.1	20	3	1	I	Not Available
29	Male	67	-	-	+	5832	33	2.8	>1	IV	Recurrence
30	Male	60	+	-	+	550.1	33	6	1	IV	Recurrence
33	Female	53	-	+	+	2.63	35	3.7	1	I	Not Available
34	Female	47	+	-	-	2.01	26	16	>1	III	Not Available
35	Male	29	+	-	+	24.7	83	9	>1	III	Recurrence
36	Male	62	+	-	-	205	21	5.5	1	I	Not Available
37	Female	50	+	-	+	11.3	72	3	>1	II	Not Available
38	Male	41	+	-	+	2072	31	12	>1	III	Recurrence
40	Male	54	+	-	-	1241	80	14	>1	III	Not Available

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