

Six Cell Cycle-related Genes Are Prognostic Biomarkers and Correlated with Immune Infiltrates in Hepatocellular Carcinoma

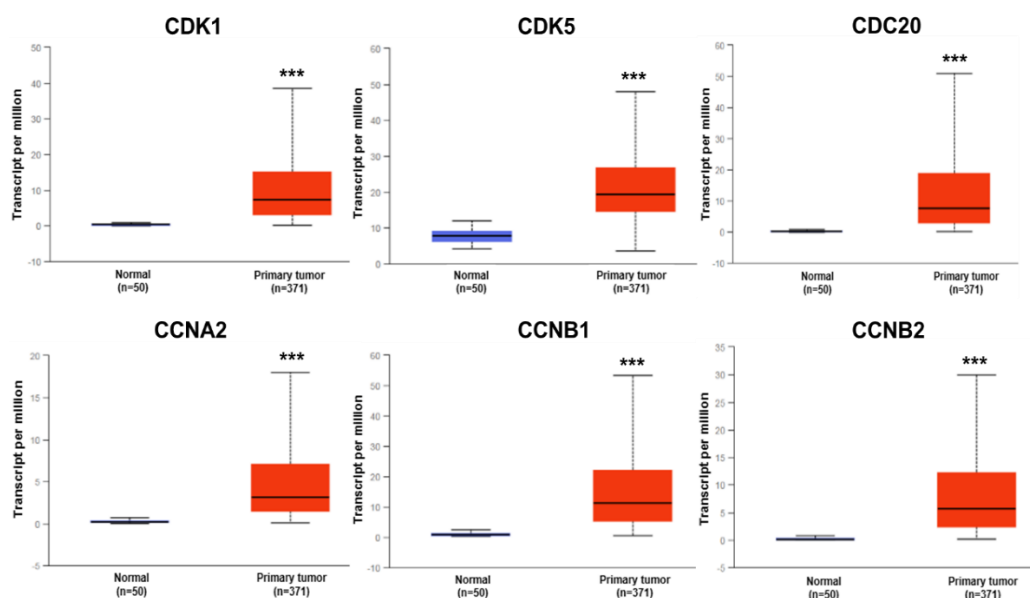
Ying Shi¹, Xiaopu Sang², Jiali Deng¹, Xiaoni Chen³, Fenfang Wu^{1,3*}, Anlong Xu^{1,2*}

¹State Key Laboratory of Biocontrol, Guangdong Province Key Laboratory of Pharmaceutical Functional Genes, College of Life Sciences, Sun Yat-Sen University, Guangzhou, China; ²School of Life Sciences, Beijing University of Chinese Medicine, Beijing, China; ³Department of Central Laboratory, Shenzhen Hospital, Beijing University of Chinese Medicine, Shenzhen, China.

*Correspondence: Anlong Xu (xuanlong@bucm.edu.cn); Fenfang Wu (wufenfang19@126.com)

The Expression Levels of Cell Cycle-related Genes and Correlation with Pathological Parameters in HCC

We also examined cell cycle-related genes expression between the tumor and normal tissues using UALCAN database (tumor sample: n = 371 vs. normal sample: n = 50) (**Supplementary Figure 1**). The result showed that *CDK1* ($P = 1E-12$), *CDK5* ($P = 1.62458935193399E-12$), *CDC20* ($P = 1.62436730732907E-12$), *CCNA2* ($P = 1.62447832963153E-12$), *CCNB1* ($P = 1E-12$), and *CCNB2* ($P = 1E-12$) expression was significantly increased compared with normal tissues.



SUPPLEMENTARY FIGURE 1 | *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* expression increased in HCC presented in the form of a boxplot (Ualcan database). (***) $P < 0.001$.

Expression of Cell Cycle-related Genes in HCC Based on *TP53* Mutation Status in UALCAN Database

We individually analyzed the expression of six hub cell cycle-related genes in HCC based on *TP53* mutation status. The results showed that the mRNA expression levels of *CDK1* ($P = 8.44949999967426E-07$), *CDK5* ($P = 2.18779999999752E-05$), *CDC20* ($P = 3.42399997244058E-09$), *CCNA2* ($P = 2.377900E-02$), *CCNB1* ($P = 1.00539999658977E-08$) and *CCNB2* ($P = 1.34450000022213E-08$) was significantly increased in *TP53*-mutant sample compared to *TP53*-

nonmutant (**Supplementary Table 1**).

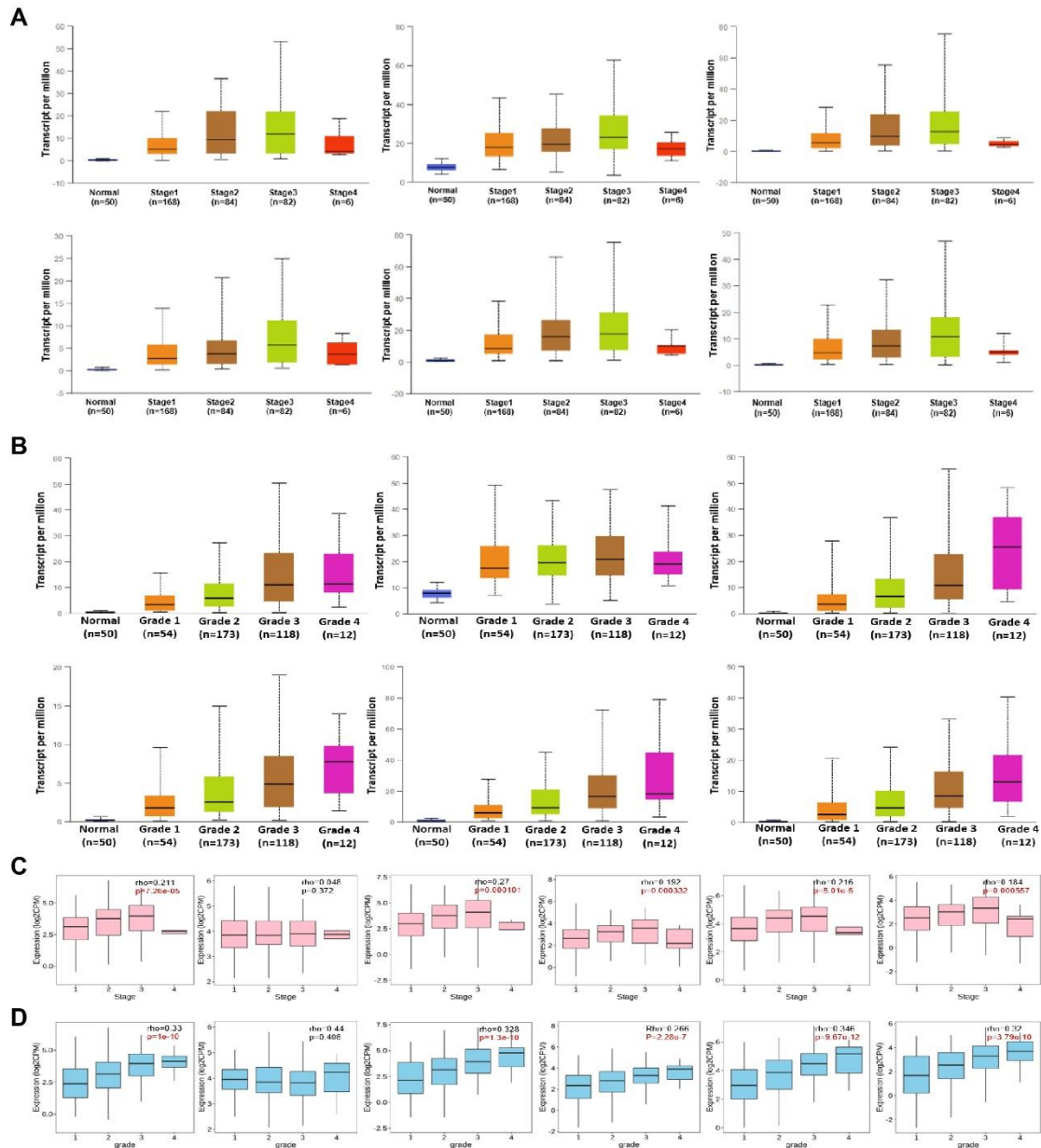
SUPPLEMENTARY TABLE 1 | Expression of Cell Cycle-related Genes in HCC Based on *TP53* Mutation Status in UALCAN Database.

Gene name	TPM (median)		<i>P</i> value
	<i>TP53</i> -Mutant (105)	<i>TP53</i> -NonMutant (255)	
CDK1	12.931	5.496	8.44949999967426E-07
CDK5	24.333	18.114	2.18779999999752E-05
CDC20	22.126	5.238	3.42399997244058E-09
CCNA2	5.838	2.513	2.377900E-02
CCNB1	21.55	8.566	1.00539999658977E-08
CCNB2	11.719	4.731	1.34450000022213E-08

TPM, Transcript per million. Bold values indicate statistically significant ($P < 0.05$).

Associations of *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* mRNA Expression with Clinico-pathological Variables in HCC Patients.

Additionally, the association of the expression of these genes with the HCC stage and grade was analyzed. UALCAN database showed that *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1* and *CCNB2* expression were significant differences in different stage and grade, respectively. (**Supplementary Figure 2**). Meanwhile, TISIDB database showed that *CDK1* ($P = 7.26E-05$), *CDC20* ($P = 0.000101$), *CCNA2* ($P = 0.000332$), *CCNB1* ($P = 5.01E-05$) and *CCNB2* ($P = 0.000557$) high expression in stage 2 and 3 compared with normal and stage 1. The results also showed that *CDK1* ($P = 1E-10$), *CDC20* ($P = 1.3E-10$), *CCNA2* ($P = 2.28E-7$), *CCNB1* ($P = 9.67E-12$) and *CCNB2* ($P = 3.79E-10$) high expression in grade 2, 3 and 4 compared with normal and stage 1.

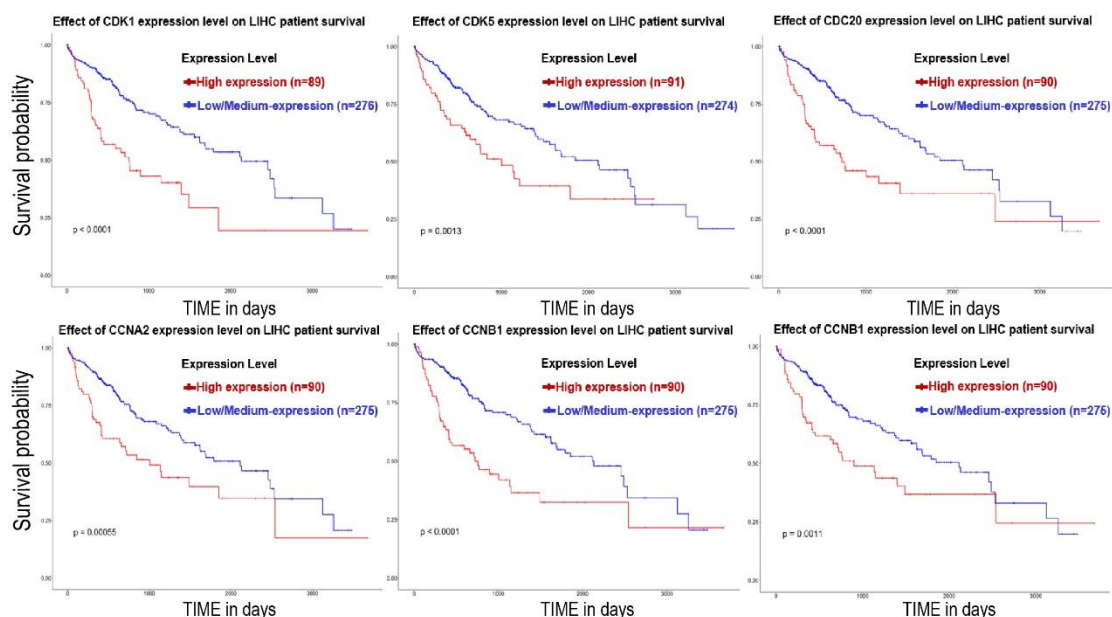


SUPPLEMENTARY FIGURE 2 | A. Correlation of the *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* expression with tumor stage among HCC cases (UALCAN database). **B.** Correlation of the *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* expression with tumor grade among HCC cases (UALCAN database). **C.** Correlation of the *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* expression with tumor stage among HCC cases (TISIDB database). **D.** Correlation of the *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* expression with tumor grade among HCC cases (TISIDB database).

Relationship between Elevated *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* mRNA Expression and Dismal Prognosis for HCC Cases.

The crucial cell cycle-related genes efficiency in HCC patient survival was also found. The UALCAN database was utilized to examine the relationship of mRNA expression of *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* with HCC patient survival based on the public datasets. Our

results suggested that increased *CDK1* ($P = 0.0001$), *CDK5* ($P = 0.0011$), *CDC20* ($P = 0.0001$), *CCNA2* ($P = 0.0013$), *CCNB1* ($P = 0.0001$), and *CCNB2* ($P = 0.00055$) showed a significant relationship with poor OS. (Supplement Figure 3).



SUPPLEMENTARY FIGURE 3 | Significance of the *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* mRNA expression in predicting the prognosis for HCC cases (UALCAN database).

***CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1*, and *CCNB2* Expression Is Correlated with Immune Infiltration Level in HCC.**

We also used TIMER database to analyze whether *CDK1*, *CDK5*, *CDC20*, *CCNA2*, *CCNB1* and *CCNB2* expression were correlated with immune infiltration levels in HCC. Our results showed that the levels of cell cycle-related genes expression had remarkable correlations with B cell, CD8+ T cells, CD4+ T cells, neutrophils, macrophages, and DCs in HCC (Figure 5). The P -value were listed in Supplementary Table 2.

SUPPLEMENTARY TABLE 2 | P -value of the cell cycle-related genes expression correlations with B cell, CD8+ T cells, CD4+ T cells, neutrophils, macrophages, and DCs in HCC.

Gene	B cell	CD8+ T cell	CD4+ T cell	Macrophage	Neutrophils	Dendritic cell
<i>CDK1</i>	2.97e-20	2.38e-09	2.72e-10	2.60e-18	4.98e-11	1.17e-17
<i>CDK5</i>	1.28e-02	0.305	0.0704	1.31e-02	6.89e-04	1.64e-02
<i>CDC20</i>	8.39e-19	2.01e-11	4.78e-07	6.63e-16	2.12e-09	3.54e-19
<i>CCNA2</i>	7.31e-21	1.63e-11	1.60e-09	1.21e-14	2.41e-12	8.02e-22
<i>CCNB1</i>	2.94e-20	1.12e-08	9.33e-08	5.42e-16	6.81e-11	1.15e-16
<i>CCNB2</i>	6.57e-22	2.78e-09	7.86e-09	6.27e-18	1.49e-09	6.57e-19

Bold values indicate statistically significant ($P < 0.05$).

SUPPLEMENTARY TABLE 3 | Correlation analysis between cell cycle-related genes and markers of immune cells in TIMER.

Description	Gene markers	CDK1		CDK5		CDC20	
		Cor	P	Cor	P	Cor	P
CD8+ T cell	CD8A	0.198	***	-0.04	0.438	0.218	***
	CD8B	0.184	***	0.009	0.857	0.256	***
T cell (general)	CD3D	0.274	***	0.06	0.25	0.364	***
	CD3E	0.202	***	0.124	**	0.217	***
	CD2	0.216	***	0.12	*	0.243	***
B cell	CD19	0.273	***	0.043	0.408	0.275	***
	CD79A	0.158	***	0.18	***	0.165	*
Monocyte	CD86	0.284	***	0.069	***	0.326	***
	CD115 (CSF1R)	0.131	*	0.088	0.0918	0.179	***
TAM	CCL2	0.039	0.459	0.02	0.701	0.038	0.464
	CD68	0.23	***	0.076	0.142	0.242	***
	IL10	0.219	***	0.012	0.822	0.23	***
M1 Macrophage	INOS (NOS2)	0.02	0.695	0.116	*	0.098	0.058
							8
	IRF5	0.394	***	0.367	***	0.325	***
M2 Macrophage	COX2(PTGS2)	0.101	0.0526	-0.109	*	0.034	0.514
	CD163	0.067	0.0197	0.043	0.406	0.046	0.38
	VSIG4	0.08	0.123	0.149	*	0.098	0.058
							8
Neutrophils	MS4A4A	0.089	0.0859	0.056	0.285	0.089	0.086
							9
	CD66b (CEACAM8)	0.123	*	0.031	0.558	0.092	0.077
							5
Natural killer cell	CD11b (ITGAM)	0.257	***	0.223	***	0.317	***
	CCR7	0.089	0.0873	0.195	***	0.035	0.499
	KIR2DL1	0.035	0.5	0.042	0.418	0.058	0.262
Dendritic cell	KIR2DL3	0.167	**	0.076	0.145	0.139	**
	KIR2DL4	0.195	***	0.134	***	0.228	***
	KIR3DL1	0.01	0.843	0.007	0.893	0.033	0.528
	KIR3DL2	0.085	0.102	-0.012	0.821	0.082	0.113
	KIR3DL3	0.064	0.219	-0.022	0.675	0.062	0.231
	KIR2DS4	0.075	0.148	0.02	0.708	0.018	0.73
Th1	HLA-DPB1	0.154	**	0.03	0.563	0.196	***
	HLA-DQB1	0.132	*	0.025	0.628	0.193	***
	HLA-DRA	0.17	***	0.027	0.601	0.18	***
	HLA-DPA1	0.141	**	0.041	0.426	0.151	*
	BDCA-1(CD1C)	0.121	*	-0.16	*	0.063	0.225
	BDCA-4(NRP1)	0.231	***	0.145	*	0.114	*
Th1	CD11c (ITGAX)	0.331	***	0.045	0.384	0.318	***
	T-bet (TBX21)	0.081	0.119	-0.095	0.0683	0.073	0.159
	STAT4	0.262	***	-0.121	0.0194	0.263	***

	STAT1	0.372	***	0.031	0.552	0.287	***
	IFN- γ (IFNG)	0.266	***	0.108	*	0.319	***
	TNF- α (TNF)	0.252	***	-0.022	***	0.265	***
Th2	GATA3	0.208	***	-0.111	*	0.203	***
	STAT6	0.114	*	0.108	*	-0.026	0.62
	STAT5A	0.265	***	0.208	***	0.26	***
	IL13	0.068	0.19	0.024	0.641	0.101	0.052
Tfh	BCL6	0.161	**	0.112	0.0306	0.075	0.15
	IL21	0.156	**	-0.002	0.962	0.136	0.087
							2
Th17	STAT3	0.114	*	0.108	*	0.025	0.627
	IL17A	0.135	**	-0.118	0.0232	0.043	0.411
Treg	FOXP3	0.163	**	0.065	0.211	0.097	0.061
							2
Treg	CCR8	0.392	***	0.011	0.828	0.31	***
	STAT5B	0.247	***	0.225	***	0.069	0.183
	TGF β (TGFB1)	0.278	***	0.013	0.807	0.27	***
T cell exhaustion	PD-1 (PDCD1)	0.33	***	-0.046	0.377	0.37	***
	CTLA4	0.357	***	-0.016	0.765	0.396	***
	LAG3	0.294	***	0.013	0.8	0.372	***
	TIM-3	0.296	***	0.074	0.154	0.352	***
	(HAVCR2)						
	GZMB	0.092	0.0779	0.061	0.244	0.121	*

Description	Gene markers	CCNA2		CCNB1		CCNB2	
		Cor	P	Cor	P	Cor	P
CD8+ T cell	CD8A	0.236	***	0.186	***	0.227	***
	CD8B	0.206	***	0.188	***	0.23	***
T cell (general)	CD3D	0.271	***	0.278	***	0.303	***
	CD3E	0.228	***	0.189	***	0.222	***
	CD2	0.225	***	0.205	***	0.238	***
B cell	CD19	0.268	***	0.232	***	0.272	***
	CD79A	0.185	***	0.137	**	0.177	***
Monocyte	CD86	0.322	***	0.316	***	0.318	***
	CD115 (CSF1R)	0.172	***	0.166	**	0.156	*
TAM	CCL2	0.076	0.144	0.034	0.512	0.065	0.211
	CD68	0.221	***	0.272	***	0.216	***
	IL10	0.247	***	0.249	***	0.235	***
M1 Macrophage	INOS (NOS2)	0.022	0.674	0.028	0.597	0.014	0.786
	IRF5	0.369	***	0.377	***	0.379	***
	COX2(PTGS2)	0.125	*	0.08	0.124	0.078	0.133
M2 Macrophage	CD163	0.125	**	0.082	0.114	0.059	0.253
	VSIG4	0.125	*	0.106	**	0.077	0.141
	MS4A4A	0.133	*	0.106	*	0.087	0.0945

Neutrophils	CD66b (CEACAM8)	0.091	0.0802	0.118	*	0.1	0.0546
	CD11b (ITGAM)	0.325	***	0.304	***	0.271	***
	CCR7	0.129	*	0.046	0.382	0.077	***
Natural killer cell	KIR2DL1	0.013	0.809	0.038	0.467	0.025	0.628
	KIR2DL3	0.179	***	0.152	**	0.16	**
	KIR2DL4	0.218	***	0.195	***	0.214	***
	KIR3DL1	0.043	0.404	-0.03	0.566	-0.001	0.985
	KIR3DL2	0.07	0.18	0.055	0.288	0.096	0.0642
	KIR3DL3	0.083	0.111	0.036	0.49	0.096	0.0642
	KIR2DS4	0.088	0.089	0.037	0.474	0.052	0.317
Dendritic cell	HLA-DPB1	0.2	***	0.185	***	0.194	***
	HLA-DQB1	0.168	**	0.161	**	0.184	***
	HLA-DRA	0.218	***	0.196	***	0.183	***
	HLA-DPA1	0.198	**	0.166	**	0.164	*
	BDCA-1(CD1C)	0.131	*	0.108	*	0.136	**
	BDCA-4(NRP1)	0.246	***	0.183	***	0.19	***
	CD11c (ITGAX)	0.354	***	0.334	***	0.315	***
Th1	T-bet (TBX21)	0.098	0.0599	0.05	0.34	0.086	0.0979
	STAT4	0.263	***	0.221	***	0.249	***
	STAT1	0.387	***	0.375	***	0.366	***
	IFN- γ (IFNG)	0.274	***	0.276	***	0.293	***
	TNF- α (TNF)	0.268	***	0.279	***	0.268	***
Th2	GATA3	0.227	***	0.188	***	0.222	***
	STAT6	0.099	0.0575	0.054	0.287	0.048	0.354
	STAT5A	0.276	***	0.232	***	0.254	***
	IL13	0.128	0.0133	0.101	0.0527	0.101	0.0526
Tfh	BCL6	0.161	*	0.1	0.0554	0.089	0.0861
	IL21	0.181	***	0.171	***	0.164	*
Th17	STAT3	0.175	***	0.096	0.0651	0.051	0.331
	IL17A	0.109	*	0.081	0.121	0.099	0.0576
Treg	FOXP3	0.213	***	0.139	0.0073	0.163	*
Treg	CCR8	0.429	***	0.375	***	0.363	***
	STAT5B	0.302	***	0.177	***	0.195	***
	TGF β (TGFB1)	0.275	***	0.303	***	0.295	***
	PD-1 (PDCD1)	0.324	***	0.323	***	0.356	***
T cell exhaustion	CTLA4	0.335	***	0.34	***	0.351	***
	LAG3	0.293	***	0.293	***	0.362	***
	TIM-3 (HAVCR2)	0.348	***	0.33	***	0.318	***
	GZMB	0.087	0.0944	0.078	0.136	0.114	*

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation. * P <0.01; ** P <0.001; *** P <0.0001.

