Figure S1. Heatmap displaying the hierarchical clustering analysis of tumor-infiltrating immune cells and six co-signaling molecules or the immunomodulatory factor IDO1 using immunohistochemical staining in the primary cohort. Each colored square in the figure indicates the Spearman correlation r-value between the two markers. Red color denotes a strong positive correlation (r=0.9, P<0.00001); yellow, no correlation (r=0); and green, negative correlation (r=-0.9). P, parenchyma; M, mesenchyme; TC, tumor cell; IC, immune cell; Foxp3, forkhead box p3; PD1, programmed cell death 1; PD-L1, PD1 ligand 1; TIM3, T-cell immunoglobulin mucin family member 3; LAG3, lymphocyte-activating 3; OX40, tumor necrosis factor receptor superfamily, member 4; ICOS, inducible T-cell costimulator; IDO1, indoleamine 2,3-dioxygenase 1.

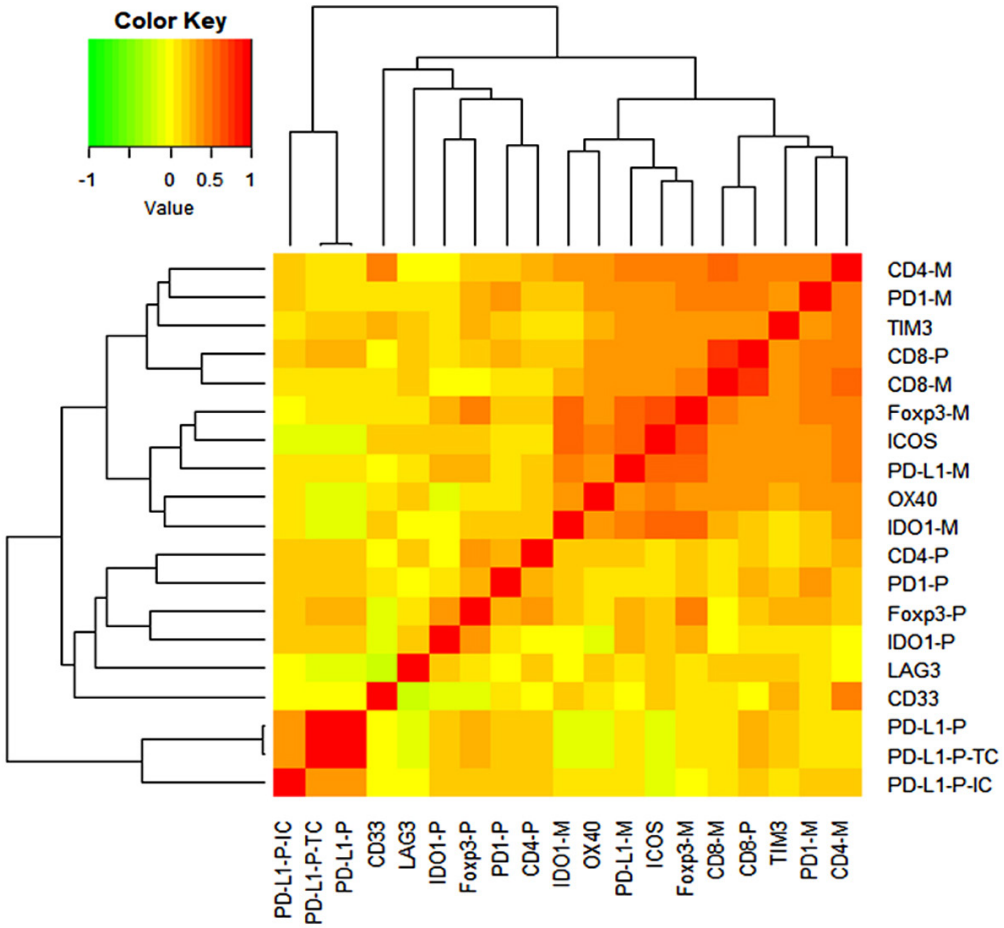

Figure S2. Overall survival of patients stratified by different CD8$^+$TIL infiltration status in both tumor regions was analyzed in combination. CD8$^+$TIL lowlow represents ≤1% CD8$^+$T cells infiltrating in the parenchyma with ≤5% CD8$^+$T cells infiltrating in the mesenchyme. CD8$^+$TIL highhigh indicates >1% CD8$^+$T cells infiltrating in parenchyma with >5% CD8$^+$T cells infiltrating in mesenchyme. CD8$^+$TIL heterogeneous is another combination (lowhigh/highlow) of CD8$^+$T cell infiltration status in both tumor regions. TIL, tumor-infiltrating lymphocytes.

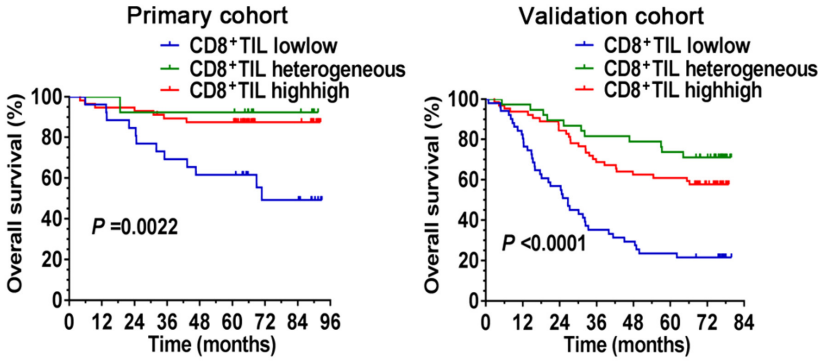

Figure S3. Survival analysis of immune markers in the primary cohort. Survival curves comparing overall survival of patients stratified by different (A) $CD4^+$TIL infiltration status and $Foxp3^+$T-regulatory cell infiltration status, (B) $PD1^+$TIL infiltrating status, as well as (C) PD-L1/LAG3/TIM3/OX40/ICOS/IDO1 expression status. TIL, tumor-infiltrating lymphocytes; Foxp3, forkhead box p3; PD1, programmed cell death 1; PD-L1, PD1 ligand 1; TIM3, T-cell immunoglobulin mucin family member 3; LAG3, lymphocyte-activating 3; OX40, tumor necrosis factor receptor superfamily, member 4; ICOS, inducible T-cell costimulator; IDO1, indoleamine 2,3-dioxygenase 1.

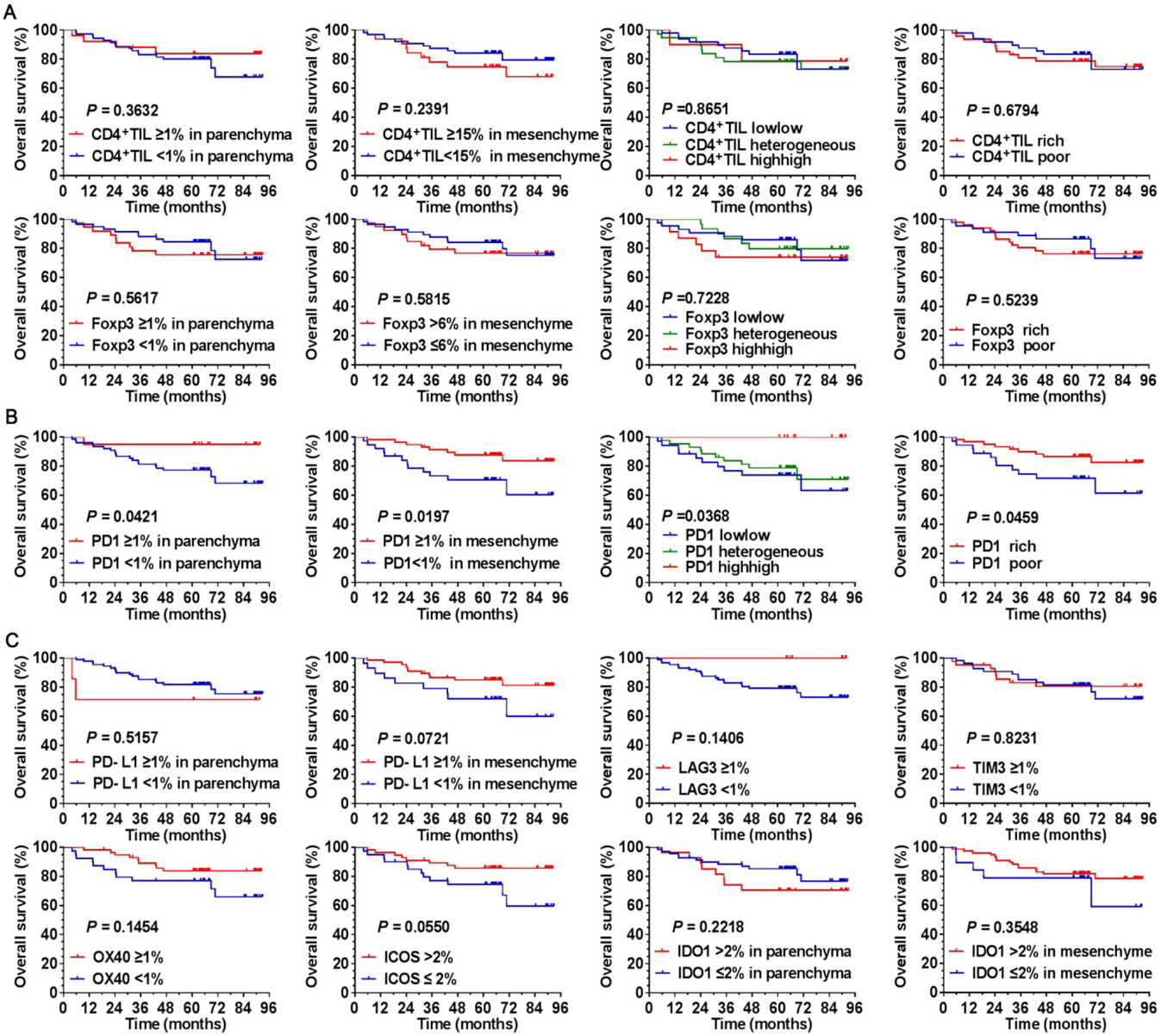

Figure S4. Survival curves comparing overall survival of patients stratified by different (A) TNM stage, (B) T stage, (C) N stage in the primary cohort. (D) Survival curves grouped by different immunoprofile in all patients with colorectal cancer (n=249).

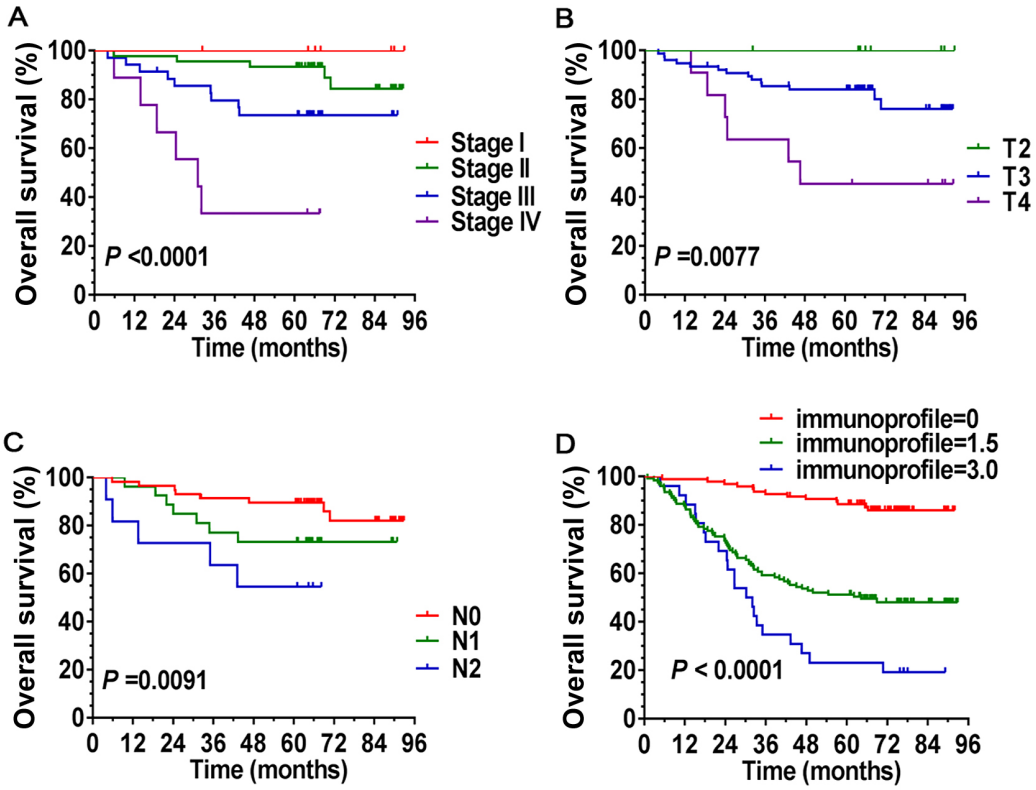

Table SI. Primary antibodies used for IHC/multiplex IHC.

Species	Antigen	Source	Clone	Supplier	Dilution (IHC/mIHC)
Human	CD4	Rabbit	EPR6855	Abcam	1:1,200
Human	CD8	Rabbit	SP16	ZSBio	1:50/1:400
Human	Foxp3	Mouse	236A/E7	Abcam	1:300
Human	CD33	Rabbit	SP266	Abcam	1:500/1:500
Human	PD-L1	Rabbit	EIL3N	Cell Signaling Technology	1:200
Human	PD1	Mouse	EH33	Cell Signaling Technology	1:200
Human	LAG3	Rabbit	D2G4O	Cell Signaling Technology	1:200
Human	TIM3	Rabbit	D5D5R	Cell Signaling Technology	1:500
Human	OX40	Rabbit	D1S6L	Cell Signaling Technology	1:100
Human	ICOS	Rabbit	D1K2T	Cell Signaling Technology	1:200
Human	IDO1	Rabbit	D5J4E	Cell Signaling Technology	1:800
Human	CK	Mouse	PAN-CK	Abcam	1:2,000

mIHC, multiplex immunohistochemistry.

Table SII. Kaplan-Meier survival analyses for the primary cohort (n=96) according to the minimum P-value cutoffs.

Biomarker	Optimal cutoff value (%)	Stratification	No. of patients	95% Confidence interval		Log-rank P-value
				Lower	Upper	
CD4-Parenchyma	<1	Low	71	69.407	83.639	0.363
	≥1	High	25	70.087	91.453	
CD4-Mesenchyme	<15	Low	64	74.297	87.445	0.239
	≥15	High	32	63.239	84.314	
CD8-Parenchyma	≤1	Low	33	56.849	78.829	0.003
	>1	High	63	78.792	90.113	
CD8-Mesenchyme	≤5	Low	32	58.689	80.466	0.011
	>5	High	64	77.506	89.345	
Foxp3-Parenchyma	<1	Low	59	72.318	86.420	0.562
	≥1	High	37	65.942	85.649	
Foxp3-Mesenchyme	≤6	Low	57	72.957	86.922	0.581
	>6	High	39	67.037	85.714	
CD33	<1	Low	50	80.771	92.501	0.002
	≥1	High	46	60.513	78.940	
PD-L1-Parenchyma-TC	<1	Low	89	73.672	84.981	0.516
	≥1	High	7	37.742	95.744	
PD-L1-Parenchyma-IC	<1	Low	95	NS	NS	0.594
	≥1	High	1	NS	NS	
PD-L1-Parenchyma	<1	Low	89	73.672	84.981	0.516
	≥1	High	7	37.742	95.744	
PD-L1-Mesenchyme	<1	Low	29	57.659	82.367	0.072
	≥1	High	67	76.015	87.935	
PD1-Parenchyma	<1	Low	76	68.465	82.252	0.042
	≥1	High	20	79.705	95.408	
PD1-Mesenchyme	<1	Low	38	58.270	80.192	0.020
	≥1	High	58	78.438	89.870	
LAG3	<1	Low	88	NS	NS	0.141
	≥1	High	8	NS	NS	

TIM3	<1	Low	54	70.672	85.633	0.823
	≥1	High	42	70.188	87.386	
OX40	<1	Low	39	62.199	82.938	0.145
	≥1	High	57	76.707	88.590	
ICOS	≤2	Low	40	61.561	81.656	0.055
	>2	High	56	76.491	89.100	
IDO1-Parenchyma	≤2	Low	69	73.750	86.587	0.222
	>2	High	27	61.833	84.922	
IDO1-mesenchyme	≤2	Low	19	54.708	86.684	0.355
	>2	High	77	74.378	86.078	

NS, no statistics were computed because all cases are censored.