

Localization and density of tertiary lymphoid structures associate with molecular subtype and clinical outcome in colorectal cancer liver metastases

Chong Zhang^{1,†}, Xiang-Yu Wang^{2,†}, Jie-Liang Zuo^{3,†}, Xue-Fu Wang^{4,5,†}, Xiao-Wen Feng^{4,5}, Bo Zhang², Yi-Tong Li², Chen-He Yi², Peng Zhang², Xiao-Chen Ma², Zhen-Mei Chen², Yue Ma², Jia-Hao Han², Bao-Rui Tao², Rui Zhang², Tian-Qi Wang¹, Li Tong¹, Wang Gu¹, Si-Yu Wang¹, Xiao-Fei Zheng¹, Wen-Kang Yuan¹, Zi-Jie Kan¹, Jie Fan⁶, Xiang-Yang Hu⁷, Jun Li^{3,*}, Chao Zhang^{1,*}, Jin-Hong Chen^{2,*}

¹Department of General Surgery, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, China

²Department of General Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China

³Department of General Surgery, Shanghai 10th People's Hospital, Tongji University, Shanghai 200072, China

⁴School of Pharmacy, Anhui Medical University, Hefei 230032, China

⁵Inflammation and Immune Mediated Diseases Laboratory of Anhui Province, Anhui Medical University, Hefei 230032, China

⁶Department of Pathology, Huashan Hospital, Fudan University, Shanghai, 200040 China

⁷Department of Pathology, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, China

[†]These authors contributed equally to this work.

*Correspondence:

Jin-Hong Chen, Department of General Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China. Email: jinhongch@hotmail.com.

Chao Zhang, Department of General Surgery, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, China. Email: 13965053990@163.com.

Jun Li, Department of General Surgery, Shanghai 10th People's Hospital, Tongji University, Shanghai 200072, China. Email: lijundfgd1@163.com.

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Supplementary methods

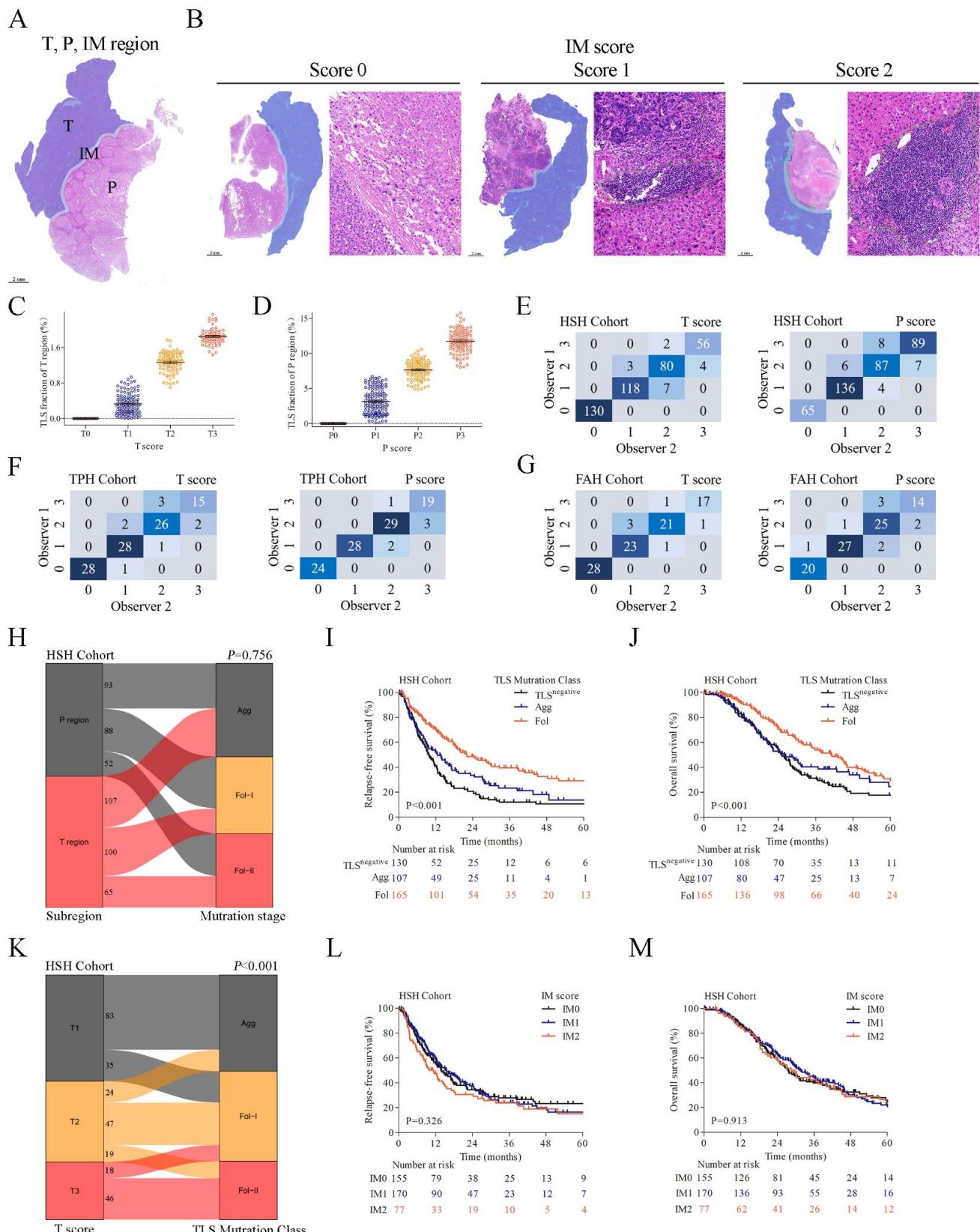
Evaluation of TLS abundance in the IM region

The subregions of WSI were shown in Supplementary Fig. 1A. TLS abundance in the IM region (IM score) was divided into three groups: (1) score 0: without TLS in the IM region; (2) score 1: the area of TLSs in the IM region is less than 50% and (3) score 2: the area of TLSs in the IM region is more than 50% (Supplementary Fig. 1B).

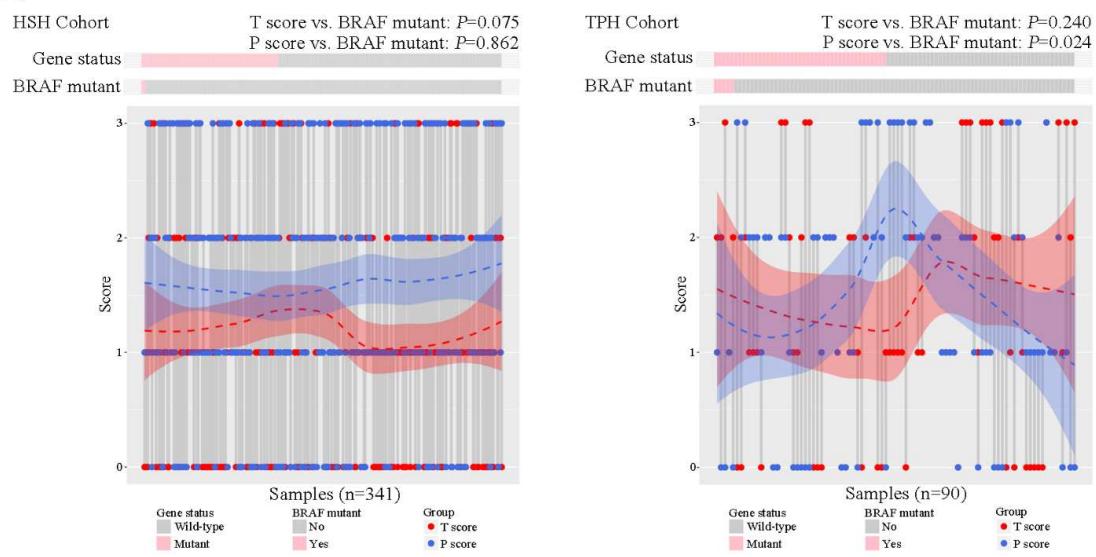
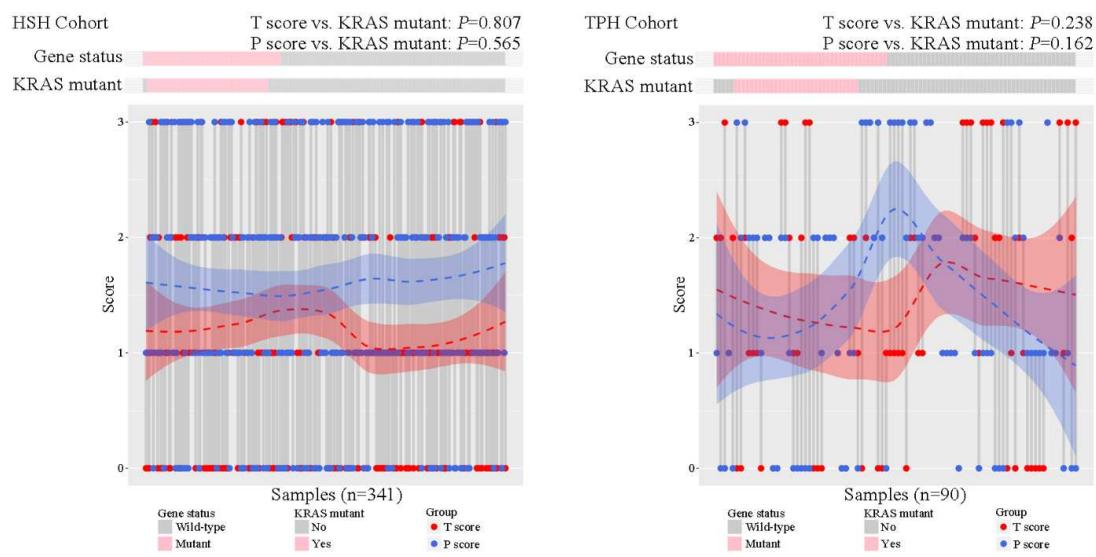
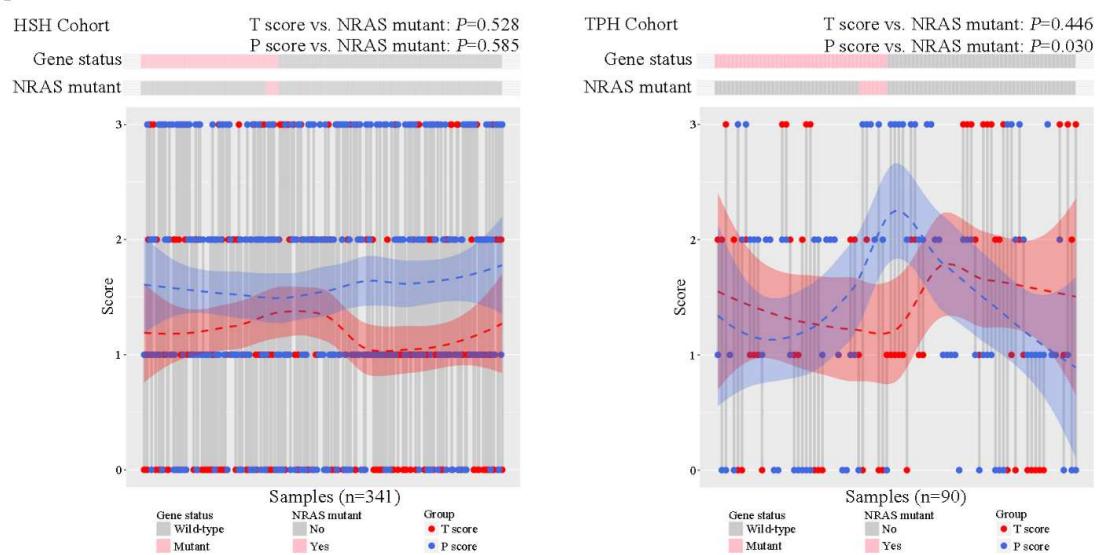
Verification of the TLS scoring system

The subregions of TLSs in Cohort D were assessed by pathologists and the TLS fraction in the T region and P region (area of TLSs/area of the scoring subregion) was calculated by computer. As shown in Supplementary Fig. 1C and D, T score 0, 1, 2 and 3 corresponded to TLS fractions 0%, 0%-0.8%, 0.8%-1.6% and 1.6%-100%, respectively, while P score 0, 1, 2 and 3 corresponded to TLS fractions 0%, 0%-5%, 5%-10% and 10%-100%, respectively. These findings further demonstrated that this TLS scoring system could represent the abundance of TLSs.

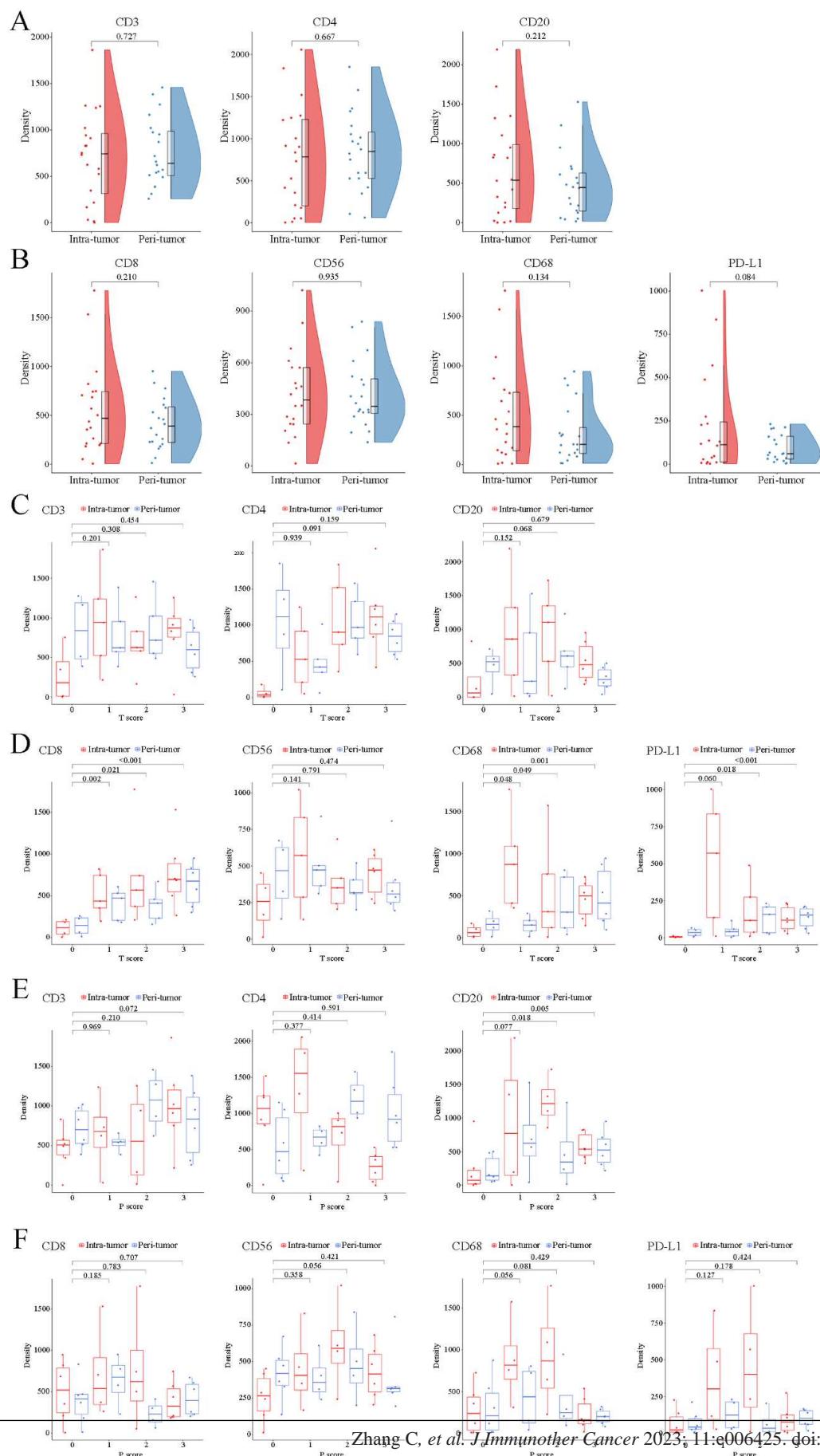
Supplementary figures



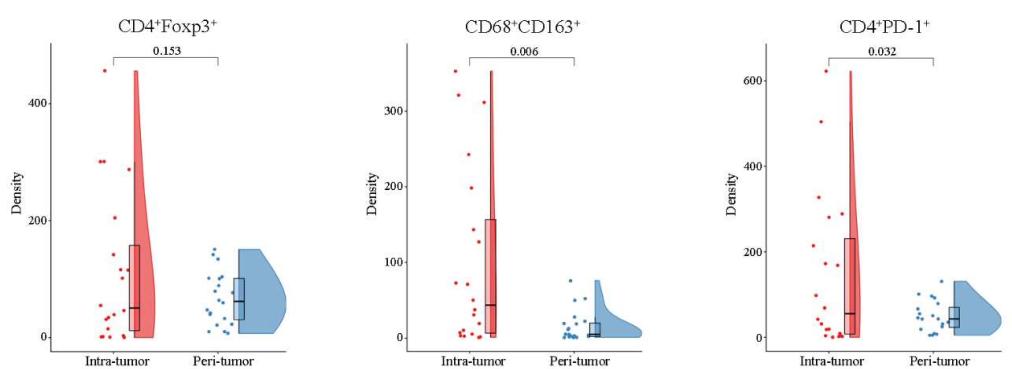
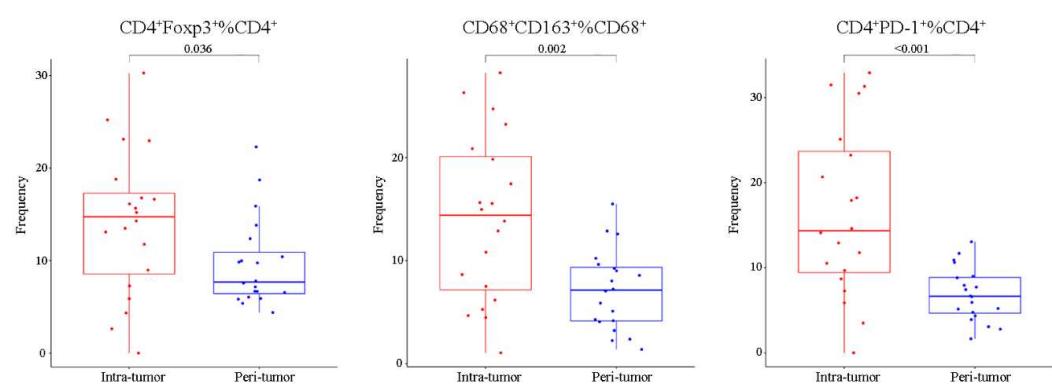
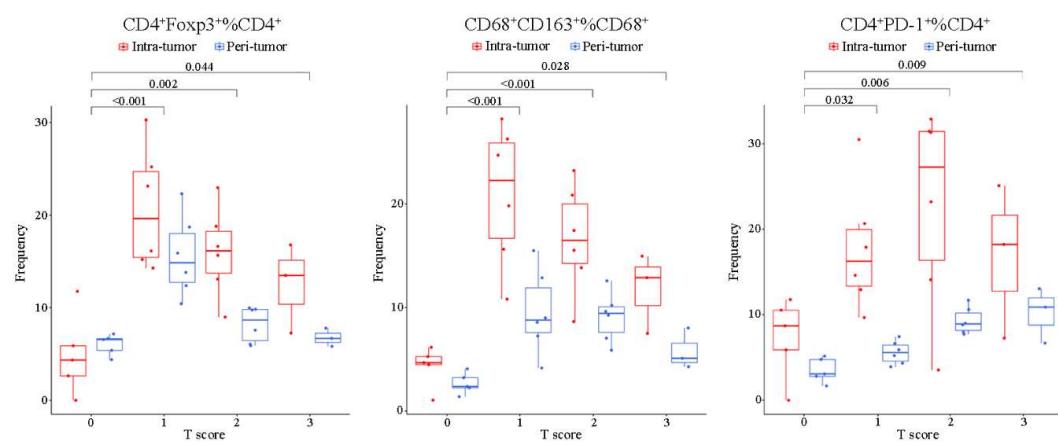
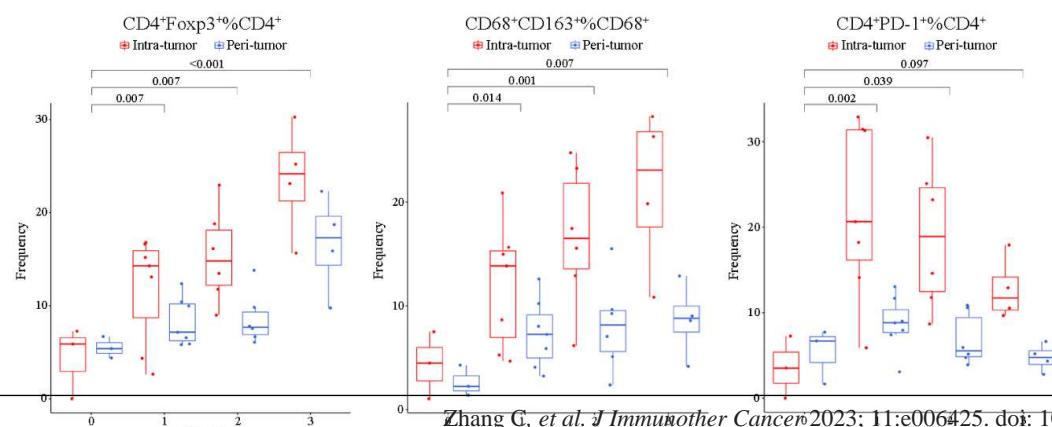
Supplementary Fig. 1. Additional information of the TLS scoring system. (A) Representative schematic diagram of intra-tumor (T), peri-tumor (P) and invasive margin (IM) in whole slide image (WSI). Peri-tumor region was highlighted in deep blue and invasive margin was highlighted in light blue. (B) Representative WSIs of H&E staining for TLS scoring system (IM score). (C, D) The distribution of TLS fraction with different T score (C) and P score (D) in the HSH Cohort. TLS fraction: area of TLSs/area of the scoring subregion. (E-G) Detailed T score, P score and IM score in the HSH Cohort (E), TPH Cohort (F) and FAH Cohort (G), respectively. (H) Correlation between T region, P region and maturation stages of TLSs in the HSH Cohort. (I, J) Kaplan-Meier curves showing relapse-free survival (I) and overall survival (J) of CRCLM patients stratified by TLSs Maturation Class in the HSH Cohort. (K) Correlation between T score and TLSs Maturation Class in the HSH Cohort. (L, M) Kaplan-Meier curves showing relapse-free survival (L) and overall survival (M) of CRCLM patients stratified by IM score in the HSH Cohort.

A**B****C**

Supplementary Fig. 2. Correlation between gene mutation and T score and P score. (A) Relevance of T score and P score to BRAF mutation in the HSH and TPH Cohorts. (B) Relevance of T score and P score to KRAS mutation in the HSH and TPH Cohorts. (C) Relevance of T score and P score to NRAS mutation in the HSH and TPH Cohorts. The pick represents a mutation of gene, while the gray represents a wild-type of gene at the top. The connected red and blue circles on each column represent a patient's T score and P score, respectively. The red and blue curves represent the overall distribution of T score and P score of patients, respectively.



Supplementary Fig. 3. The immune composition of TLSs of CRCLM patients in the HSH Cohort. (A, B) Quantification of the density of CD3, CD4, CD20, CD8, CD56, CD56, CD68 and PD-L1 in intra-tumor and peri-tumor TLSs. (C-F) Quantification of the density of CD3, CD4, CD20, CD8, CD56, CD56, CD68 and PD-L1 in intra-tumor and peri-tumor TLSs with different T score (C, D) and P score (E, F), respectively.

A**B****C****D**

Supplementary Fig. 4. The immune composition of TLSs of CRCLM patients in the TPH Cohort. (A, B) Quantification of the density (A) and frequency (B) of CD4⁺Foxp3⁺ Treg cells, CD4⁺PD-1⁺ Tfh cells and CD68⁺CD163⁺ M2 macrophages in intra-tumor and peri-tumor TLSs. (C, D) Quantification of the frequency of CD4⁺PD-1⁺ Tfh cells and CD4⁺Foxp3⁺ Treg cells within CD4⁺ T cells and CD68⁺CD163⁺ M2 macrophages within CD68⁺ macrophages in intra-tumor and peri-tumor TLSs with different T score (C) and P score (D).

Supplementary tables

Supplementary Table 1. Information of the antibodies used in our research.

Antibodies	Source	Catalogue
CD3	Bioss	bs-10498R
CD4	Abcam	ab133616
CD20	Dako	IR604
Foxp3	Abcam	ab20034
PD-1	CST	86163S
CD8	Abcam	ab178089
CD56	Abcam	ab75813
CD68	Abcam	ab213363
CD163	Abcam	ab182422
PD-L1	CST	13684S

Supplementary Table 2. Univariate and multivariate analysis of relapse-free survival of CRCLM patients in HSH Cohort

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Sex (male vs. female)	1.054 (0.822-1.352)	0.678		
Age, years (60< vs. ≥60)	0.869 (0.687-1.099)	0.242		
Occurrence of metastases (metachronous vs. synchronous)	1.187 (0.921-1.530)	0.186		
Primary tumor location (left vs. right)	0.836 (0.642-1.090)	0.185		
CEA (ng/ml) (5< vs. ≥5)	1.562 (1.185-2.060)	0.002	1.386 (1.030-1.864)	0.031
CA19-9 (U/ml) (40< vs. ≥40)	1.323 (1.041-1.682)	0.022	1.020 (0.783-1.331)	0.881
CA125 (U/ml) (35< vs. ≥35)	1.162 (0.902-1.498)	0.245		
Size, cm (5< vs. ≥5)	1.325 (1.008-1.742)	0.043	1.048 (0.746-1.473)	0.785
Tumor number (single vs. multiple)	1.675 (1.315-2.132)	<0.001	1.392 (1.053-1.902)	0.029
Liver fibrosis (yes vs. no)	1.058 (0.707-1.584)	0.784		
Tumor grade (G1-2 vs. G3)	1.236 (0.948-1.613)	0.118		
T stage (Tis-2 vs. T3-4)	1.350 (1.029-1.770)	0.030	0.861 (0.611-1.213)	0.392
Lymph node metastasis (yes vs. no)	1.345 (1.121-1.670)	0.035	1.172 (1.011-1.383)	0.042
Differentiation		0.621		
Poor	reference			
Moderate	1.124 (0.806-1.569)	0.491		
Well	1.072 (0.734-1.486)	0.841		
Gene status (wild-type vs. mutant)	1.123 (0.865-1.458)	0.383		
Neoadjuvant chemotherapy (yes vs. no)	1.372 (1.080-1.742)	0.010	1.254 (1.022-1.637)	0.035
TLS Maturation Class		<0.001		
TLS ^{negative}	reference			
Agg	1.805 (1.157-2.818)	0.009		
Fol-I	2.396 (1.550-3.705)	<0.001		
Fol-II	3.181 (2.098-4.822)	<0.001		
T score		<0.001		<0.001
0	reference		reference	
1	1.414 (1.089-2.046)	0.014	1.424 (1.097-1.983)	0.025
2	2.288 (1.485-3.502)	<0.001	1.718 (1.371-2.032)	<0.001
3	2.945 (1.944-4.461)	<0.001	2.204 (1.631-2.815)	<0.001
P score		<0.001		<0.001
0	reference		reference	
1	0.716 (0.403-0.904)	0.017	0.647 (0.311-1.015)	0.058
2	0.516 (0.386-0.689)	<0.001	0.323 (0.198-0.503)	<0.001
3	0.385 (0.212-0.408)	<0.001	0.211 (0.117-0.327)	<0.001

Abbreviations: CRCLM, colorectal cancer liver metastases; HR, hazard ratio; CI, confidential interval; CEA, carcinoembryonic antigen.

Supplementary Table 3. Univariate and multivariate analysis of overall survival of CRCLM patients in HSH Cohort

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Sex (male vs. female)	1.135 (0.877-1.469)	0.335		
Age, years (60< vs. ≥60)	0.901 (0.707-1.149)	0.401		
Occurrence of metastases (metachronous vs. synchronous)	1.073 (0.827-1.392)	0.598		
Primary tumor location (left vs. right)	1.054 (0.796-1.396)	0.712		
CEA (ng/ml) (5< vs. ≥5)	1.372 (1.032-1.825)	0.030	1.310 (1.002-1.714)	0.041
CA19-9 (U/ml) (40< vs. ≥40)	1.211 (0.946-1.551)	0.128		
CA125 (U/ml) (35< vs. ≥35)	0.992 (0.764-1.288)	0.952		
Size, cm (5< vs. ≥5)	1.166 (0.881-1.543)	0.283		
Tumor number (single vs. multiple)	1.743 (1.357-2.239)	<0.001	1.323 (1.013-1.729)	0.040
Liver fibrosis (yes vs. no)	1.390 (0.939-2.059)	0.100		
Tumor grade (G1-2 vs. G3)	1.500 (1.144-1.967)	0.003	1.171 (0.874-1.569)	0.290
T stage (Tis-2 vs. T3-4)	1.386 (1.050-1.829)	0.021	0.956 (0.702-1.302)	0.775
Lymph node metastasis (yes vs. no)	1.465 (1.099-1.953)	0.009	1.326 (1.056-1.787)	0.049
Differentiation		0.114		
Poor	reference			
Moderate	1.385 (0.979-1.960)	0.065		
Well	1.040 (0.775-1.397)	0.793		
Gene status (wild-type vs. mutant)	1.117 (0.851-1.467)	0.424		
Neoadjuvant chemotherapy (yes vs. no)	1.609 (1.253-2.065)	<0.001	1.572 (1.216-2.033)	0.001
TLS Maturation Class		<0.001		
TLS ^{negative}	reference			
Agg	3.785 (2.341-6.122)	<0.001		
Fol-I	3.224 (1.943-5.351)	<0.001		
Fol-II	3.110 (1.892-5.112)	<0.001		
T score		<0.001		<0.001
0	reference		reference	
1	1.692 (1.231-2.775)	0.037	1.318 (1.041-1.768)	0.046
2	2.749 (1.730-4.369)	<0.001	2.024 (1.486-2.327)	0.011
3	2.888 (1.836-4.542)	<0.001	2.233 (1.695-3.283)	<0.001
P score		<0.001		<0.001
0	reference		reference	
1	0.693 (0.511-0.941)	0.019	0.701 (0.515-1.003)	0.038
2	0.445 (0.330-0.599)	<0.001	0.473 (0.334-0.602)	<0.001
3	0.177 (0.102-0.309)	<0.001	0.209 (0.115-0.383)	<0.001

Abbreviations: CRCLM, colorectal cancer liver metastases; HR, hazard ratio; CI, confidential interval; CEA, carcinoembryonic

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Supplementary Table 4. Correlation between TLS scores and clinicopathological characteristics of CRCLM patients

Variables	HSH Cohort (n=402)										TPH Cohort (n=106)									
	T score				P value	P score				P value	T score				P value	P score				P value
	0	1	2	3		0	1	2	3		0	1	2	3		0	1	2	3	
Sex (%)					0.635					0.124					0.265					0.289
Male	90 (34.1)	76 (28.8)	55 (20.8)	43 (16.3)		38 (14.4)	90 (34.1)	59 (22.3)	77 (29.2)		21 (31.8)	16 (24.2)	19 (28.8)	10 (15.2)		18 (27.3)	18 (27.3)	19 (28.8)	11 (16.6)	
Female	40 (29.0)	42 (30.4)	35 (25.4)	21 (15.2)		27 (19.6)	46 (33.3)	38 (27.5)	27 (19.6)		7 (17.5)	13 (32.5)	10 (25.0)	10 (25.0)		6 (15.0)	10 (25.0)	12 (30.0)	12 (30.0)	
Age (years) (%)					0.129					0.102					0.920					0.548
<60	66 (32.7)	50 (24.8)	53 (26.2)	33 (16.3)		31 (15.4)	58 (28.7)	54 (26.7)	59 (29.2)		14 (24.6)	15 (26.3)	17 (29.8)	11 (19.3)		13 (22.8)	12 (21.1)	19 (33.3)	13 (22.8)	
≥60	64 (32.0)	68 (34.0)	37 (18.5)	31 (15.5)		34 (17.0)	78 (39.0)	43 (21.5)	45 (22.5)		14 (28.6)	14 (28.6)	12 (24.5)	9 (18.3)		11 (22.4)	16 (32.7)	12 (24.5)	10 (20.4)	
Occurrence of metastases (%)					0.107					0.512					0.313					0.089
Metachronous	40 (30.3)	31 (23.5)	34 (25.8)	27 (20.4)		26 (19.7)	40 (30.3)	31 (23.5)	35 (26.5)		8 (19.1)	14 (33.3)	10 (23.8)	10 (23.8)		14 (33.3)	11 (26.2)	9 (21.5)	13 (31.0)	
Synchronous	90 (33.3)	87 (32.2)	56 (20.8)	37 (13.7)		39 (14.4)	96 (35.6)	66 (24.4)	69 (25.6)		20 (31.3)	15 (23.4)	19 (29.7)	10 (15.6)		10 (15.6)	17 (26.5)	22 (34.3)	10 (15.6)	
Primary tumor location (%)					0.942					0.179					0.553					0.617
Left-sided	97 (32.3)	86 (28.7)	69 (23.0)	48 (16.0)		53 (17.7)	93 (31.0)	75 (25.0)	79 (26.3)		21 (28.8)	17 (23.3)	21 (28.8)	14 (19.1)		15 (20.5)	18 (24.7)	24 (32.9)	16 (21.9)	
Right-sided	33 (32.3)	32 (31.4)	21 (20.6)	16 (15.7)		12 (11.8)	43 (42.2)	22 (21.5)	25 (24.5)		7 (21.2)	12 (36.4)	8 (24.2)	6 (18.2)		9 (27.3)	10 (30.3)	7 (21.2)		0.048
CEA (ng/ml)					0.037					0.023					0.036					0.029
<5	34 (29.0)	27 (23.1)	29 (24.8)	27 (23.1)		28 (23.9)	42 (35.9)	23 (19.7)	24 (20.5)		6 (16.2)	7 (18.9)	13 (35.1)	11 (29.7)		4 (10.8)	15 (13.5)	10 (27.0)	8 (21.6)	
≥5	96 (33.7)	91 (31.9)	61 (21.4)	37 (13.0)		37 (13.0)	94 (33.0)	74 (26.0)	80 (28.0)		22 (31.9)	22 (31.9)	16 (23.2)	9 (13.0)		20 (29.0)	13 (18.9)	21 (30.4)	15 (21.7)	
CA19-9 (U/ml)					0.003					0.021					0.917					0.285
<40	69 (26.5)	80 (30.8)	61 (23.5)	50 (19.2)		53 (20.4)	83 (31.9)	61 (23.5)	63 (24.2)		9 (15.3)	20 (33.9)	18 (30.5)	12 (20.3)		14 (23.8)	17 (28.8)	16 (27.1)	12 (20.3)	
≥40	61 (43.0)	38 (26.8)	29 (20.4)	14 (9.8)		12 (8.5)	53 (37.3)	36 (25.3)	41 (28.9)		19 (40.4)	9 (19.2)	11 (23.4)	8 (17.0)						
CA125 (U/ml)					0.144					0.970										
<35	87 (30.1)	87 (30.1)	72 (24.9)	43 (14.9)		53 (18.3)	101 (35.0)	61 (21.1)	74 (25.6)		16 (24.2)	18 (27.3)	19 (28.8)	13 (19.7)		12 (18.2)	21 (31.8)	18 (27.3)	15 (22.7)	
≥35	43 (38.1)	31 (27.4)	18 (15.9)	21 (18.6)		12 (10.6)	35 (31.0)	36 (31.9)	30 (26.5)		12 (30.0)	11 (27.5)	10 (25.0)	7 (17.5)		12 (30.0)	7 (17.5)	13 (32.5)	8 (20.0)	
Size (cm) (%)					<0.001					0.944					0.046					0.341
<5	81 (25.6)	99 (31.2)	80 (25.2)	57 (18.0)		52 (16.4)	109 (34.4)	75 (23.6)	81 (25.6)		14 (18.9)	21 (28.4)	22 (29.7)	17 (23.0)		19 (25.7)	21 (28.4)	21 (28.4)	13 (17.5)	
≥5	49 (57.6)	19 (22.4)	10 (11.8)	7 (8.2)		13 (15.3)	27 (31.8)	22 (25.9)	23 (27.0)		14 (43.8)	8 (25.0)	7 (21.9)	3 (9.3)		5 (15.7)	7 (21.9)	10 (31.2)	10 (31.2)	
Tumor number (%)					<0.001					0.036					0.038					0.180
Single	48 (27.1)	45 (25.4)	39 (22.1)	45 (25.4)		38 (21.5)	60 (33.9)	42 (23.7)	37 (20.9)		9 (20.5)	10 (22.7)	11 (25.0)	14 (31.8)		11 (25.0)	14 (31.8)	14 (31.8)	5 (11.4)	
Multiple	82 (36.5)	73 (32.4)	51 (22.7)	19 (8.4)		27 (12.0)	76 (33.8)	55 (24.4)	67 (29.8)		19 (30.6)	19 (30.6)	18 (29.0)	6 (9.8)		13 (21.0)	14 (22.6)	17 (27.4)	18 (29.0)	
Liver fibrosis (%)					0.101					0.782					0.312					0.143
Yes	19 (47.5)	12 (30.0)	6 (15.0)	3 (7.5)		5 (12.5)	16 (40.0)	10 (25.0)	9 (22.5)		8 (38.1)	4 (19.1)	7 (33.3)	2 (9.5)		3 (14.3)	9 (42.9)	7 (33.3)	2 (9.5)	
No	111 (30.7)	106 (29.3)	84 (23.2)	61 (16.8)		60 (16.6)	120 (33.1)	87 (24.0)	95 (26.3)		20 (23.5)	25 (29.4)	22 (25.9)	18 (21.2)		21 (24.7)	19 (22.4)	24 (28.2)	21 (24.7)	
Tumor grade (%)					<0.001					0.076					0.047					0.030
G1-2	85 (28.1)	83 (27.5)	80 (26.5)	54 (17.9)		54 (17.9)	105 (34.8)	74 (24.5)	69 (22.8)		22 (30.6)	20 (27.8)	14 (19.4)	16 (22.2)		18 (25.0)	24 (33.3)	16 (22.2)	14 (19.5)	
G3	45 (45.0)	35 (35.0)	10 (10.0)	10 (10.0)		11 (11.0)	31 (31.0)	23 (23.0)	35 (35.0)		6 (17.6)	9 (26.5)	15 (44.1)	4 (11.8)		6 (17.6)	4 (11.8)	15 (44.1)	9 (26.5)	
T stage (%)					<0.001					0.629					0.029					0.090
Tis-2	78 (25.1)	99 (31.8)	75 (24.1)	59 (19.0)		51 (16.4)	109 (35.1)	75 (24.1)	76 (24.4)		7 (13.7)	16 (31.4)	15 (29.4)	13 (25.5)		17 (33.3)	12 (23.5)	13 (25.5)	9 (17.7)	
T3-4	52 (57.1)	19 (20.9)	15 (16.5)	5 (5.5)		14 (15.4)	27 (29.7)	22 (24.2)	28 (30.7)		21 (38.2)	13 (23.6)	14 (25.5)	7 (12.7)		7 (12.7)	16 (29.1)	18 (32.7)	14 (25.5)	
Lymph node metastasis (%)					<0.001					0.763					0.036					0.177
Yes	106 (36.3)	89 (30.5)	65 (22.3)	32 (10.9)		44 (15.1)	100 (34.2)	70 (24.0)	78 (26.7)		22 (34.4)	19 (29.7)	15 (23.4)	8 (12.5)		11 (17.2)	15 (23.4)	22 (34.4)	16 (25.0)	
No	24 (21.8)	29 (26.4)	25 (22.7)	32 (29.1)		21 (19.1)	36 (32.7)	27 (24.6)	26 (23.6)		6 (14.3)	10 (23.8)	14 (33.3)	12 (28.6)		13 (31.0)	13 (31.0)	9 (21.4)	7 (16.6)	
CRS (%)					<0.001					0.002					0.014					0.358
Low (0-2)	60 (23.7)	69 (27.3)	67 (26.5)	57 (22.5)		54 (21.3)	86 (34.0)	54 (21.3)	59 (23.4)		12 (17.1)	20 (28.6)	21 (30.0)	17 (24.3)		18 (25.7)	20 (28.6)	20 (28.6)	12 (17.1)	
High (3-5)	70 (47.0)	49 (32.9)	23 (15.4)	7 (4.7)		11 (7.3)	50 (33.6)	43 (28.9)	45 (30.2)		16 (44.4)	9 (25.0)	8 (22.2)	3 (8.4)		6 (16.6)	8 (22.2)	11 (30.6)	11 (30.6)	
Differentiation (%)					0.067					0.393					0.275					0.798
Poor	37 (38.1)	23 (23.7)	22 (22.7)	15 (15.5)		18 (18.6)	27 (27.8)	24 (24.7)	28 (28.9)		8 (36.4)	5 (22.7)	6 (27.2)	3 (13.7)		4 (18.2)	6 (27.2)	8 (36.4)	4 (18.2)	
Moderate	69 (35.0)	62 (31.5)	42 (21.3)	24 (12.2)		26 (13.2)	68 (34.5)	53 (26.9)	50 (25.4)		11 (18.6)	19 (32.2)	19 (32.2)	10 (17.0)		15 (25.4)	13 (22.1)	16 (27.1)	15 (25.4)	
Well	24 (22.2)	33 (30.6)	26 (24.1)	25 (23.1)		21 (19.4)	41 (38.0)	20 (18.5)	26 (24.1)		9 (36.0)	5 (20.0)	4 (16.0)	7 (28.0)		5 (20.0)	9 (36.0)	7 (28.0)	4 (16.0)	
Gene status (%)					0.683					0.555					0.254					0.004
Wild-type	67 (31.8)	66 (31.3)	45 (21.3)	33 (15.6)		34 (16.1)	71 (33.6)	50 (23.7)	56 (26.6)		9 (19.1)	16 (34.1)	9 (19.1)	13 (27.7)		8 (17.0)	16 (34.1)	11 (23.4)	12 (25.5)	
KRAS mutant	38 (33.3)	32 (28.1)	26 (22.8)	18 (15.8)		19 (16.7)	40 (35.1)	29 (25.4)	26 (22.8)		10 (32.3)	7 (22.5)	10 (32.3)	4 (12.9)		10 (32.3)	6 (19.4)	13 (41.9)	2 (6.4)	
NRAS mutant	5 (41.7)	4 (33.3)	1 (8.3)	2 (16.7)		2 (16.7)	2 (16.7)	5 (41.7)	3 (24.9)		3 (42.8)	1 (14.3)	2 (28.6)	1 (14.3)		1 (14.3)	0 (0)	2 (28.6)	4 (57.1)	
BRAF mutant	3 (75.0)	1 (25.0)	0 (0)	0 (0)		0 (0)	3 (75.0)	0 (0)	1 (25.0)		0 (0)	1 (20.0)	3 (60.0)	1 (20.0)		3 (60.0)	2 (40.0)	0 (0)	0 (0)	
Unknown	17 (27.9)	15 (24.6)	18 (29.5)	11 (18.0)		10 (16.4)	20 (32.8)	13 (21.3)	18 (29.5)		6 (37.5)	4 (25.0)	5 (31.3)	1 (6.2)		2 (12.4)	4 (25.0)	5 (31.3)	5 (31.3)	
Neoadjuvant chemotherapy (%)					0.146					0.294					0.482					0.778
Yes	81 (35.7)	70 (30.8)	44 (19.4)	32 (14.1)		38 (16.7)	68 (30.0)	57 (25.1)	64 (28.2)		18 (31.1)	17 (29.3)	14 (24.1)	9 (15.5)						

Supplementary Table 5. Correlation between the presence of TLS and the gene mutation of KRAS, NRAS and TP53 in GSE159216.

Variables	KRAS		P value	NRAS		P value	TP53		P value
	WT	Mut		WT	Mut		WT	Mut	
TLS (%)			0.232			0.214			0.900
TLS+	70 (40.9)	58 (33.9)		124 (72.5)	4 (2.3)		37 (21.7)	91 (53.2)	
TLS-	28 (16.4)	15 (8.8)		39 (22.8)	4 (2.3)		12 (7.0)	31 (18.1)	

Abbreviations: WT, wild-type; Mut, mutation.

Supplementary Table 6. Clinical characteristics and TLS scores of CRCLM patients in HSH and TPH Cohorts for multiplex immunohistochemistry.

No.	T score	P score	Occurrence of metastases	Primary tumor location	CEA, ng/ml	CA19-9, U/ml	CA125, U/ml	Size, cm	Tumor number	Liver fibrosis	Tumor grade	T stage	Lymph node metastasis	Differentiation	Gene status	Neoadjuvant chemotherapy	Immune Class
HSH Cohort																	
1	0	0	Synchronous	Left	1.9	10.0	22.4	4.0	Multiple	No	G2	T4	Yes	Moderate	Wild-type	Yes	Class III
2	0	3	Metachronous	Left	50.0	34.4	32.1	3.5	Multiple	No	G2	T4	Yes	Poor	Mutant	Yes	Class I
3	0	3	Synchronous	Left	3.6	31.8	6.4	1.5	Multiple	No	G2	T2	Yes	Moderate	Wild-type	Yes	Class I
4	0	3	Synchronous	Left	20.3	1540.0	25.4	2.0	Multiple	No	G2	T2	Yes	Poor	Mutant	No	Class I
5	1	0	Metachronous	Left	7.2	37.5	18.0	2.5	Single	No	G3	T2	No	Well	Wild-type	No	Class IV
6	1	1	Synchronous	Right	1438.0	0.6	12.6	13.0	Single	No	G3	T2	No	Moderate	Wild-type	No	Class III
7	1	2	Synchronous	Right	11.4	76.0	58.9	2.0	Single	No	G2	T3	Yes	Moderate	Wild-type	No	Class II
8	1	2	Metachronous	Left	81.1	25.7	89.4	2.8	Multiple	Yes	G2	T2	Yes	Poor	Wild-type	Yes	Class II
9	1	3	Synchronous	Left	5.9	58.2	61.1	5.0	Multiple	No	G3	T2	No	Well	Wild-type	Yes	Class I
10	2	0	Metachronous	Left	4.4	8.1	11.6	5.0	Multiple	No	G2	T3	Yes	Poor	Wild-type	Yes	Class IV
11	2	0	Synchronous	Left	30.5	975.4	7.1	6.0	Single	No	G3	T2	No	Moderate	Wild-type	No	Class IV
12	2	1	Synchronous	Left	12.1	12.7	20.1	2.0	Single	No	G3	T2	Yes	Poor	Mutant	Yes	Class III
13	2	2	Synchronous	Left	57.8	0.6	8.8	3.8	Multiple	No	G2	T2	Yes	Moderate	Wild-type	Yes	Class II
14	2	3	Synchronous	Right	18.4	0.9	17.5	3.0	Multiple	No	G1	T3	Yes	Moderate	Mutant	No	Class I
15	3	0	Synchronous	Right	8.4	86.1	5.3	1.4	Single	No	G2	T2	Yes	Well	Mutant	No	Class IV
16	3	0	Metachronous	Left	4.9	11.2	11.2	3.0	Single	Yes	G2	T2	No	Poor	Wild-type	No	Class IV
17	3	1	Metachronous	Left	17.7	70.2	28.4	2.6	Single	No	G2	T2	No	Moderate	Wild-type	Yes	Class IV
18	3	1	Synchronous	Left	101.0	20.8	12.0	6.5	Multiple	No	G3	T4	Yes	Moderate	Wild-type	No	Class IV
19	3	2	Synchronous	Left	42.2	118.5	98.4	2.7	Single	No	G2	T2	No	Moderate	Wild-type	No	Class III
20	3	3	Synchronous	Right	0.9	435.0	46.6	3.0	Multiple	No	G3	T2	Yes	Poor	Wild-type	Yes	Class II
TPH Cohort																	
1	0	0	Synchronous	Left	3.7	19.5	22.2	4.5	Multiple	No	G2	T2	Yes	Moderate	Mutant	Yes	Class III
2	0	1	Synchronous	Left	46.5	71.4	8.2	3.0	Multiple	Yes	G2	T3	Yes	Moderate	Wild-type	Yes	Class II
3	0	2	Metachronous	Left	81.2	182.7	69.6	8.0	Multiple	Yes	G2	T4	Yes	Poor	Mutant	No	Class I
4	0	2	Synchronous	Left	329.7	192.6	90.5	4.5	Multiple	Yes	G2	T3	Yes	Moderate	Wild-type	Yes	Class I
5	0	3	Metachronous	Left	2.5	50.1	37.3	5.0	Multiple	No	G2	T2	Yes	Well	Wild-type	Yes	Class I
6	1	1	Metachronous	Right	4.2	11.9	13.2	3.0	Single	No	G2	T3	No	Moderate	Wild-type	No	Class III
7	1	1	Metachronous	Left	21.6	61.0	12.8	2.5	Multiple	No	G2	T3	Yes	Moderate	Mutant	No	Class III
8	1	2	Synchronous	Right	2.7	8.0	11.4	3.5	Single	No	G3	T2	Yes	Well	Wild-type	No	Class II
9	1	2	Synchronous	Left	90.9	200.4	311.3	7.0	Multiple	No	G3	T4	Yes	Moderate	Mutant	No	Class II
10	1	3	Metachronous	Right	2.5	27.0	39.4	1.5	Single	No	G2	T2	Yes	Well	Wild-type	Yes	Class I
11	1	3	Synchronous	Left	1957.0	156.7	8.2	8.5	Multiple	No	G3	T4	Yes	Moderate	Wild-type	Yes	Class I
12	2	0	Metachronous	Left	4.9	38.4	40.1	2.5	Single	Yes	G2	T2	No	Moderate	Wild-type	No	Class IV
13	2	1	Metachronous	Right	53.5	32.4	9.1	6.0	Single	Yes	G3	T3	No	Poor	Mutant	Yes	Class III
14	2	1	Synchronous	Right	4.5	8.5	14.4	2.5	Multiple	No	G2	T2	No	Moderate	Wild-type	Yes	Class III
15	2	2	Metachronous	Left	1.52	30.0	40.3	3.0	Multiple	No	G2	T2	No	Moderate	Mutant	No	Class II
16	2	2	Synchronous	Left	328.5	784.0	618.3	7.5	Multiple	Yes	G3	T4	Yes	Poor	Wild-type	No	Class II
17	2	3	Metachronous	Right	142.8	67.6	40.1	6.5	Single	No	G3	T3	Yes	Poor	Wild-type	No	Class I
18	3	0	Metachronous	Left	6.3	31.2	9.5	4.0	Single	No	G2	T3	Yes	Moderate	Mutant	No	Class IV
19	3	0	Metachronous	Left	46.6	39.8	40.6	3.5	Multiple	No	G2	T3	No	Moderate	Wild-type	Yes	Class IV
20	3	1	Metachronous	Left	4.6	8.5	11.6	1.5	Single	No	G2	T2	No	Well	Wild-type	Yes	Class IV

Abbreviations: CRCLM, colorectal cancer liver metastases; CEA, carcinoembryonic antigen.

Supplementary Table 7. Corresponding relationships among T score, P score, Immune score, and Immune Class.

T score	P score	Immune score (RFS)	Immune score (OS)	Immune Class
0	3	-2.31	-2.7	Class I
1	3	-1.79	-2.03	
0	2	-1.54	-1.8	
2	3	-1.27	-1.36	
1	2	-1.02	-1.13	Class II
0	1	-0.77	-0.9	
3	3	-0.75	-0.69	
2	2	-0.5	-0.46	
1	1	-0.25	-0.23	Class III
0	0	0	0	
3	2	0.02	0.21	
2	1	0.27	0.44	
1	0	0.52	0.67	Class IV
3	1	0.79	1.11	
2	0	1.04	1.34	
3	0	1.56	2.01	

Abbreviations: RFS, recurrence-free survival; OS, overall survival.

Immune score (RFS) = $(0.52 \times \text{T score}) + (-0.77 \times \text{P score})$

Immune score (OS) = $(0.67 \times \text{T score}) + (-0.90 \times \text{P score})$