Supplementary Table 1. Multivariate analysis results of clinicopathological characteristics and preoperative soluble PD-L1.

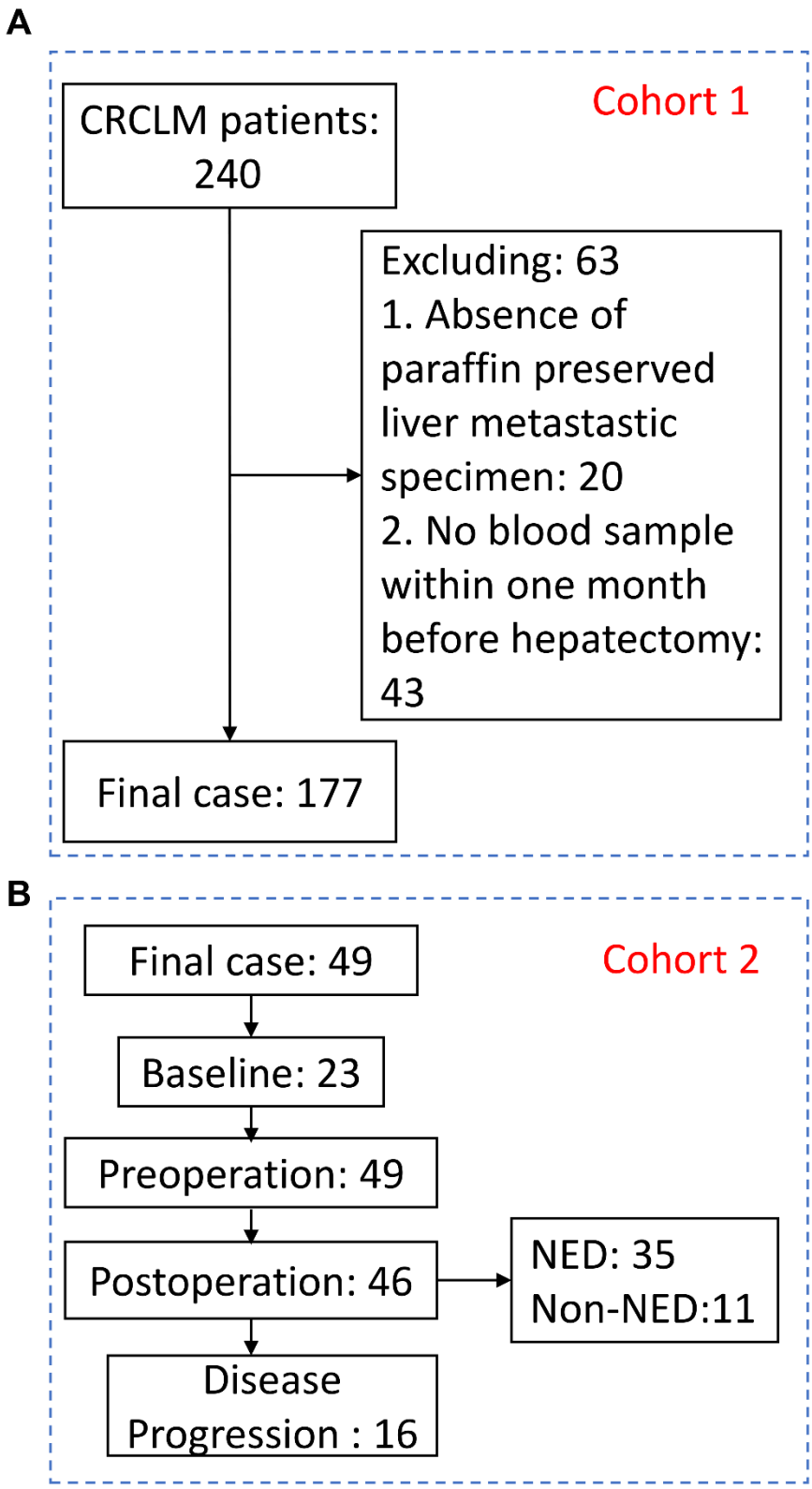
Variables	RFS		OS	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (> 65 vs. ⩽ 65)				
Gender (Female vs. male)				
Primary tumor site (Right-sided vs. Left-sided)				
Tumor grade (G3 vs. G1–2)	1.949(1.23-3.087)	**0.004**		
Pathological type (Mucinous vs. Non-mucinous)				
T-stage (T4 vs. Tis-3)			1.682(1.002-2.824)	**0.049**
N-stage (N1–2 vs. N0)	1.575(1.000-2.478)	**0.050**		
Preoperative CEA (> 200 vs. ⩽ 200 ng/ml)				
Interval from primary tumor resection to liver metastases (> 12 vs. ⩽ 12 months)				
Number of metastases (> 1 vs. ⩽ 1)				
Size of the largest metastasis (> 5cm vs. ⩽ 5cm)	2.396(1.355-4.237)	**0.003**	3.849(1.852-7.997)	**<0.001**
Preoperative chemotherapy (Yes vs. no)	2.472(1.609-3.798)	**<0.001**		
Postoperative chemotherapy (Yes vs. no)				
Ablation (Yes vs. no)				
Soluble PD-L1 (High vs. low)	1.555(1.033-2.341)	**0.034**	2.219(1.197-4.112)	**0.011**

Supplementary Table 2. Basic clinicopathological characteristics of patients in cohort 2.

Variables	NO. of patients	Percent
Age		
>=65	6	12.24%
<65	43	87.76%
Gender		
Male	38	77.55%
Female	11	22.45%
Pathological grade		
G1–2	45	91.84%
G3	4	8.16%
Histological subtype		
Non-mucinous	42	85.71%
Mucinous	0	0.00%
Missing	7	14.29%
Primary tumor T stage		
Tis-3	43	87.76%
T4	5	10.20%
Missing	1	2.04%
Primary tumor N stage		
N0	16	32.65%
N1-2	33	67.35%
Primary tumor site		
Right-sided	12	24.49%
Left-sided	37	75.51%
Primary Tumor size		
≤ 5 cm	23	46.94%
> 5 cm	5	10.20%
Missing	21	42.86%
Preoperative CEA		
≤ 200 ng/ml	48	97.96 %
> 200 ng/ml	1	2.04%
Interval from primary tumor resection to liver metastases		
≤ 12 months	41	83.67%
> 12 months	8	16.33%
Number of metastases per patient		
≤ 1	16	32.65%
> 1	33	67.35%
Size of the largest metastases		
≤ 5 cm	39	79.59%
> 5 cm	10	20.41%

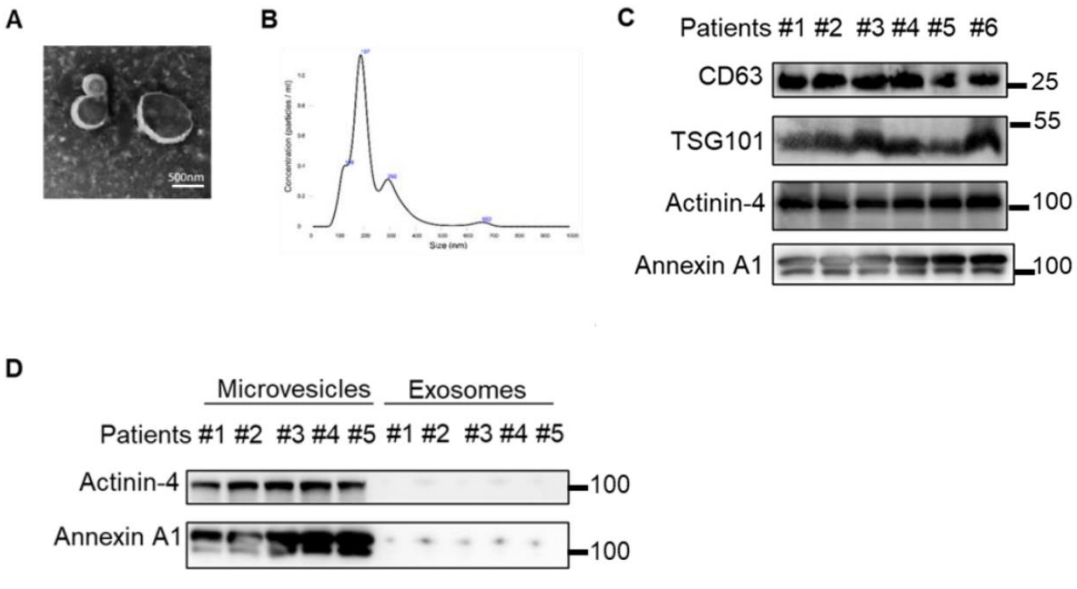
Resection type of liver metastasis and primary tumor		
Simultaneous resection	22	44.9%
Staged resection	27	55.1%
Preoperative chemotherapy		
No	9	18.37%
Yes	40	81.63%
Chemotherapy	23	46.94%
Cetuximab-based	11	22.45%
Bevacizumab-based	6	12.24%
Postoperative chemotherapy		
No	4	8.16%
Yes	45	91.84%
Chemotherapy	34	69.39%
Cetuximab-based	9	18.37%
Bevacizumab-based	2	4.08%
CRS		
0–2	45	91.84%
3–5	4	8.16%

Supplementary Figures

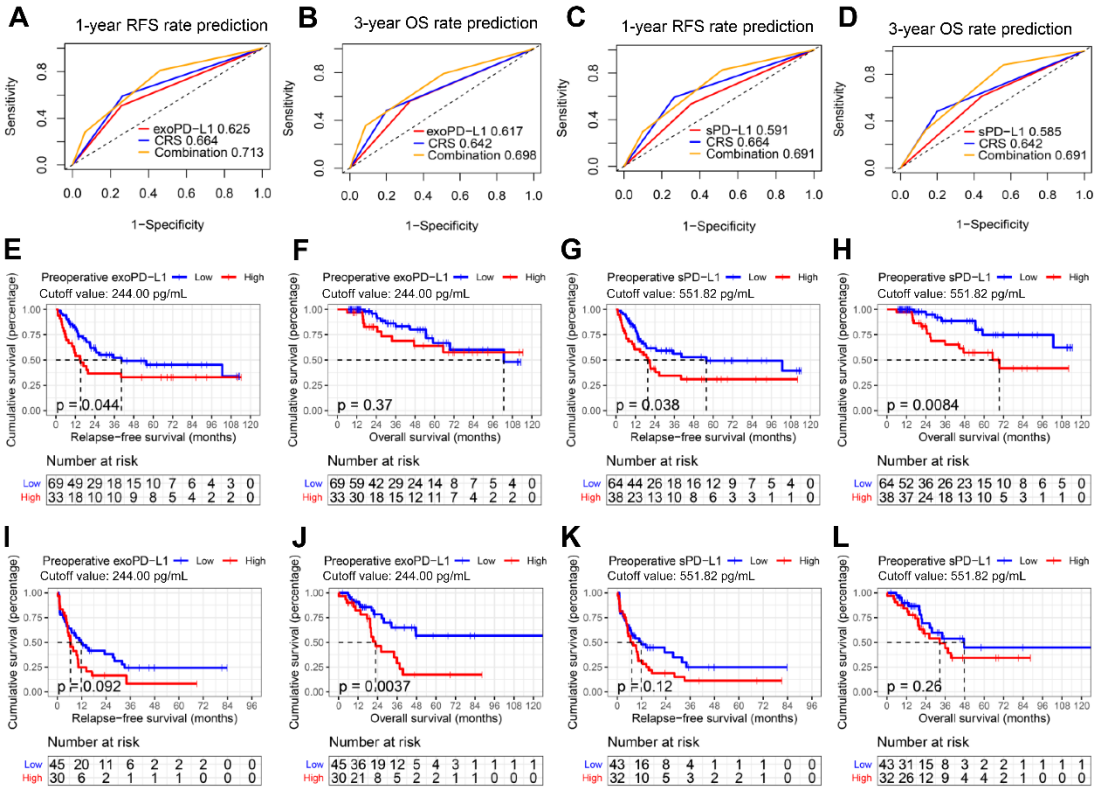


Supplementary Figure 1. Flow chart of the enrollment process in cohort

1 (A) and cohort 2 (B).

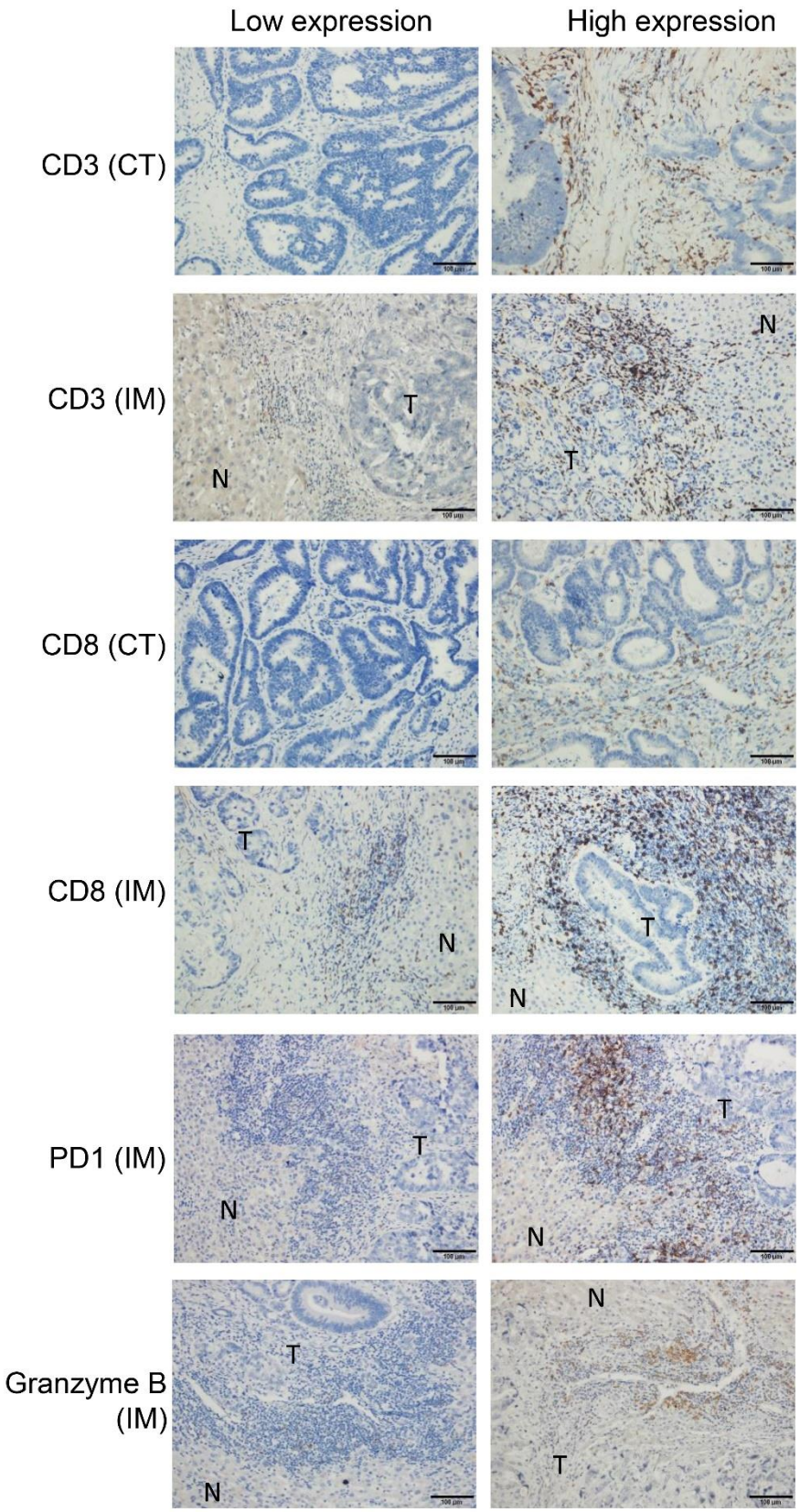


Supplementary Figure 2. Characterization of isolated microvesicles. (A) A representative TEM image of purified microvesicles from patients' plasma. Scale bar, 500 nm.; (B) Characterization of isolated microvesicles using nanoparticle tracking; (C) Representative immunoblots showing expression of CD63, TSG101, Actinin-4 and Annexin A1 in plasma microvesicles derived from six patients; (D) Representative immunoblots showing expression of Actinin-4 and Annexin A1 in plasma microvesicles and exosomes derived from five patients.



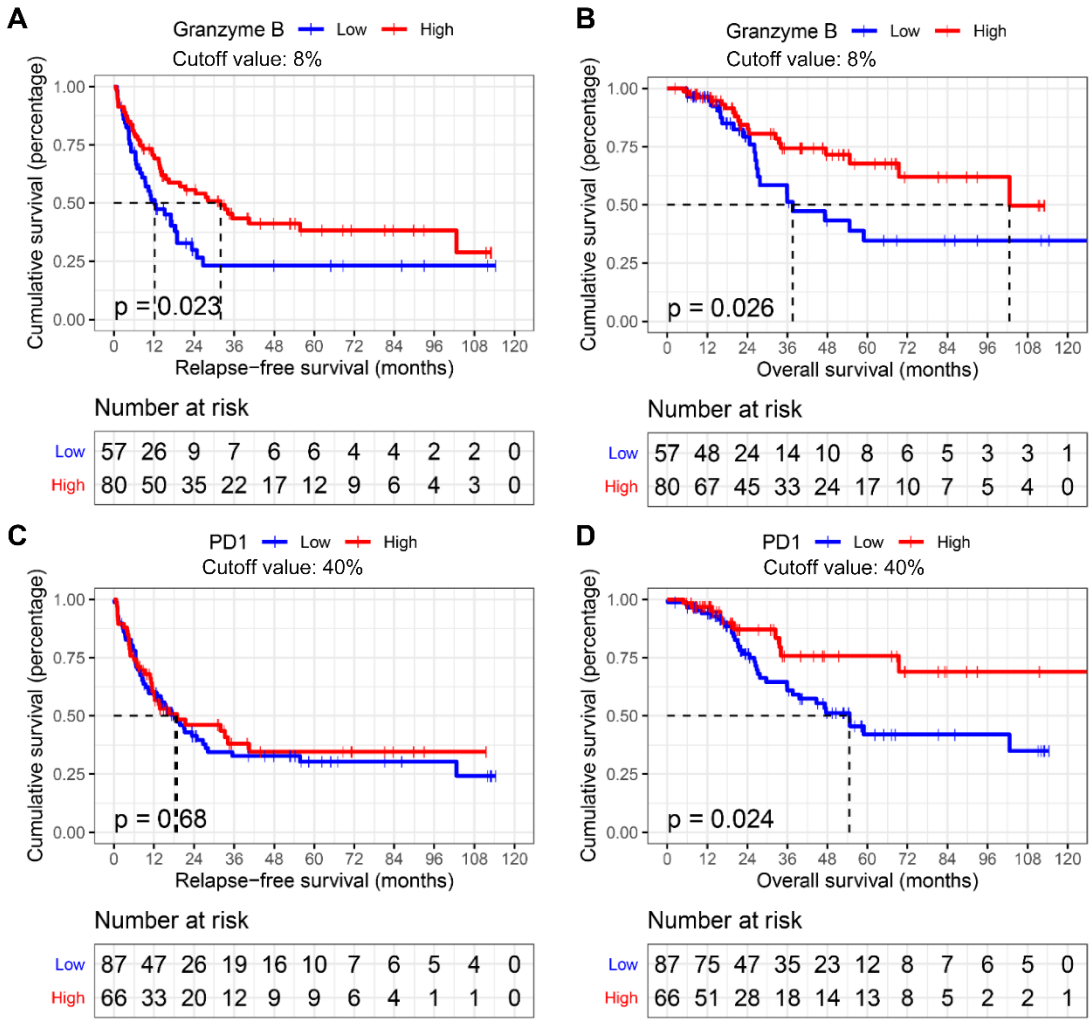
Supplementary Fig. 3. Preoperative exoPD-L1 and sPD-L1 improve survival prediction when combine with CRS and can predict the prognosis of patients with same CRS (0-2). ROC curve analysis of preoperative exoPD-L1 or CRS or combination group at (A) 1-year RFS rate prediction and (B) 3-year OS rate prediction; ROC curve analysis of preoperative sPD-L1 or CRS or combination group at (C) 1-year RFS rate prediction and (D) 3-year OS rate prediction; Kaplan–Meier estimates of (E) relapse-free survival (p=0.044) and (F) overall survival (p=0.37) in patients according to preoperative exoPD-L1 in patients with low CRS (0-2); Kaplan–Meier estimation of (G) relapse-free survival (p=0.038) and (H) overall survival (p=0.0084) in patients according to preoperative sPD-L1 in patients with low CRS (0-2); Kaplan–Meier estimates of (I) relapse-free

survival (p=0.092) and (J) overall survival (p=0.0037) in patients according to preoperative exoPD-L1 in patients with high CRS (3-5); Kaplan–Meier estimation of (K) relapse-free survival (p=0.12) and (L) overall survival (p=0.26) in patients according to preoperative sPD-L1 in patients with high CRS (3-5).



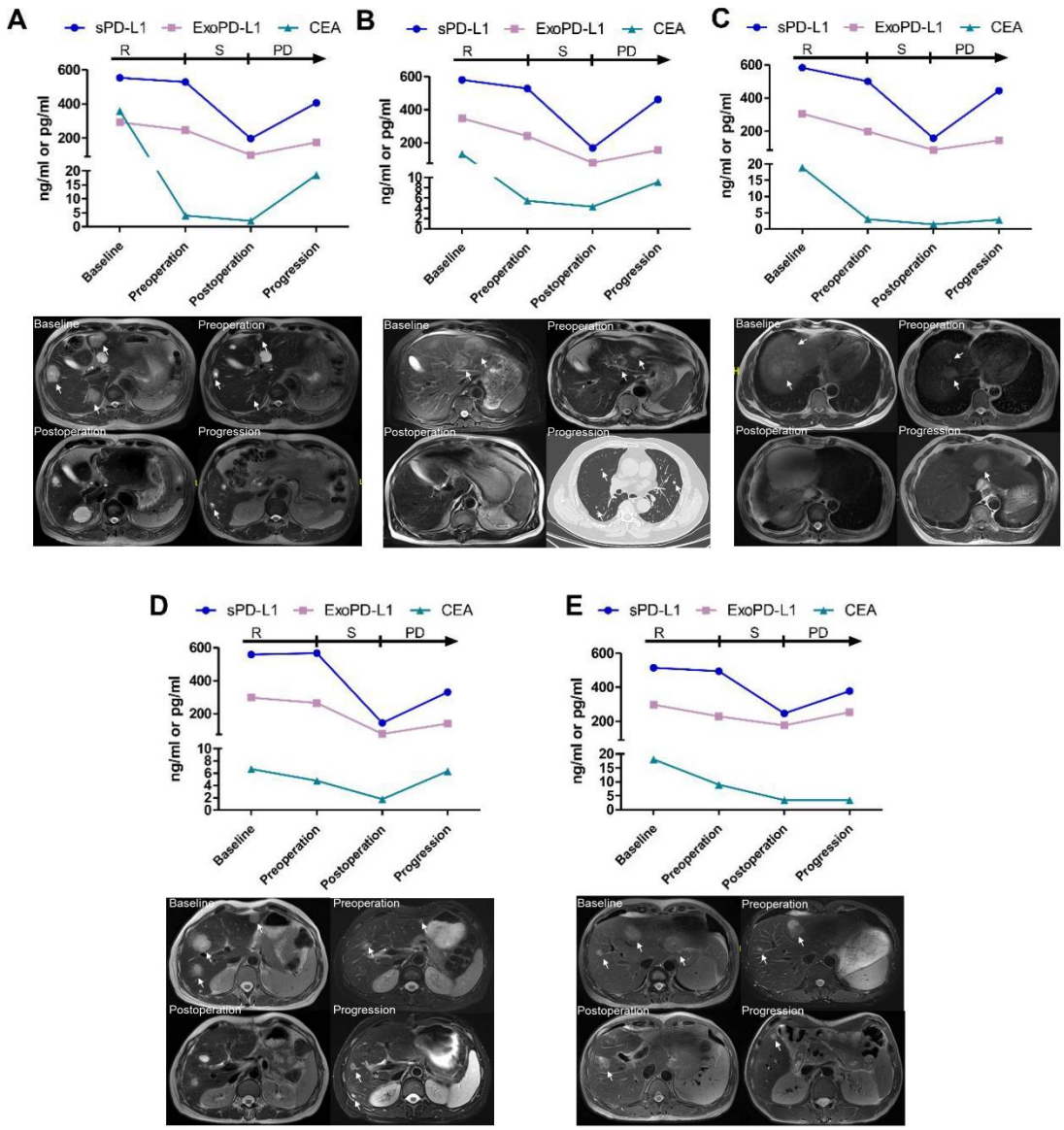
Supplementary Fig. 4. Immunohistochemical detection of CD3, CD8, PD1 and granzyme B expression at the tumor center (CT) and/or the

invasive margin (IM). Scale bars indicated 100 µm.



Supplementary Fig. 5. Both GB and PD1 expression at the invasive margin of colorectal liver metastases can be used as biomarkers of survival. Kaplan–Meier estimates of (A) relapse-free survival (p=0.023) and (B) overall survival (p=0.026) in patients according to GB expression at the invasive margin in colorectal liver metastasis; Kaplan–Meier estimates of (C) relapse-free survival (p=0.68) and (D) overall survival (p=0.024) in patients according to PD1 according to the expression of PD1 at the

invasive margin of colorectal liver metastases.



Supplementary Fig. 6. ExoPD-L1 can be used as follow-up markers in CRLM patients. (A-C) Concomitant imaging and circulating PD-L1 sampling as well as CEA results in three patients experiencing response after preoperative chemotherapy, but disease progression (> 6 months) after postoperative chemotherapy as observed in liver metastasis on MRI or CT scan; (D-E) Concomitant imaging and circulating PD-L1 sampling

as well as CEA in two patients experiencing response after preoperative chemotherapy, but early recurrence after postoperative chemotherapy as observed in liver metastasis on MRI or CT scan. Tumor metastases in the scans are indicated by arrows. R: Response to preoperative chemotherapy; S: surgery of hepatectomy; PD: progression of disease.