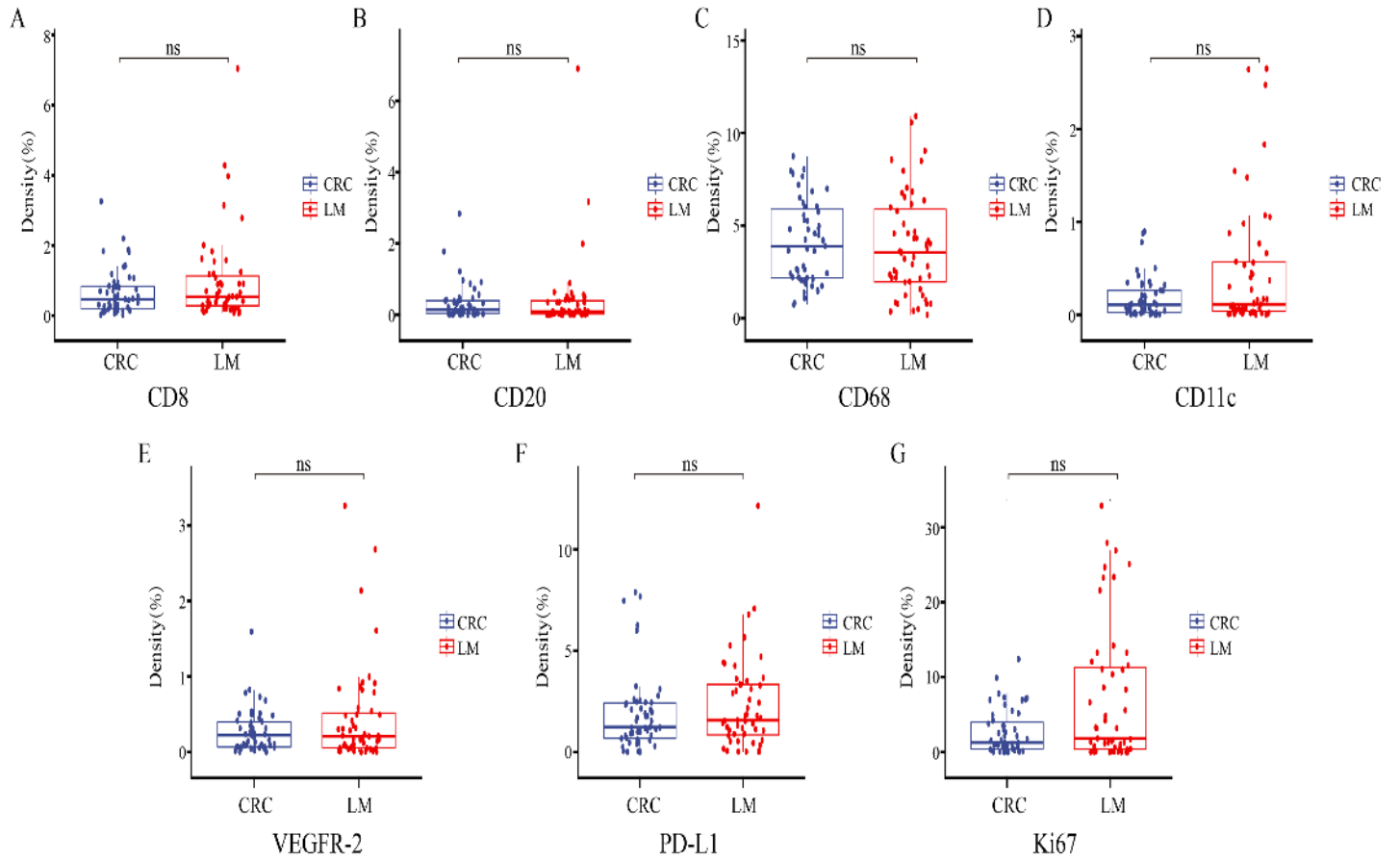


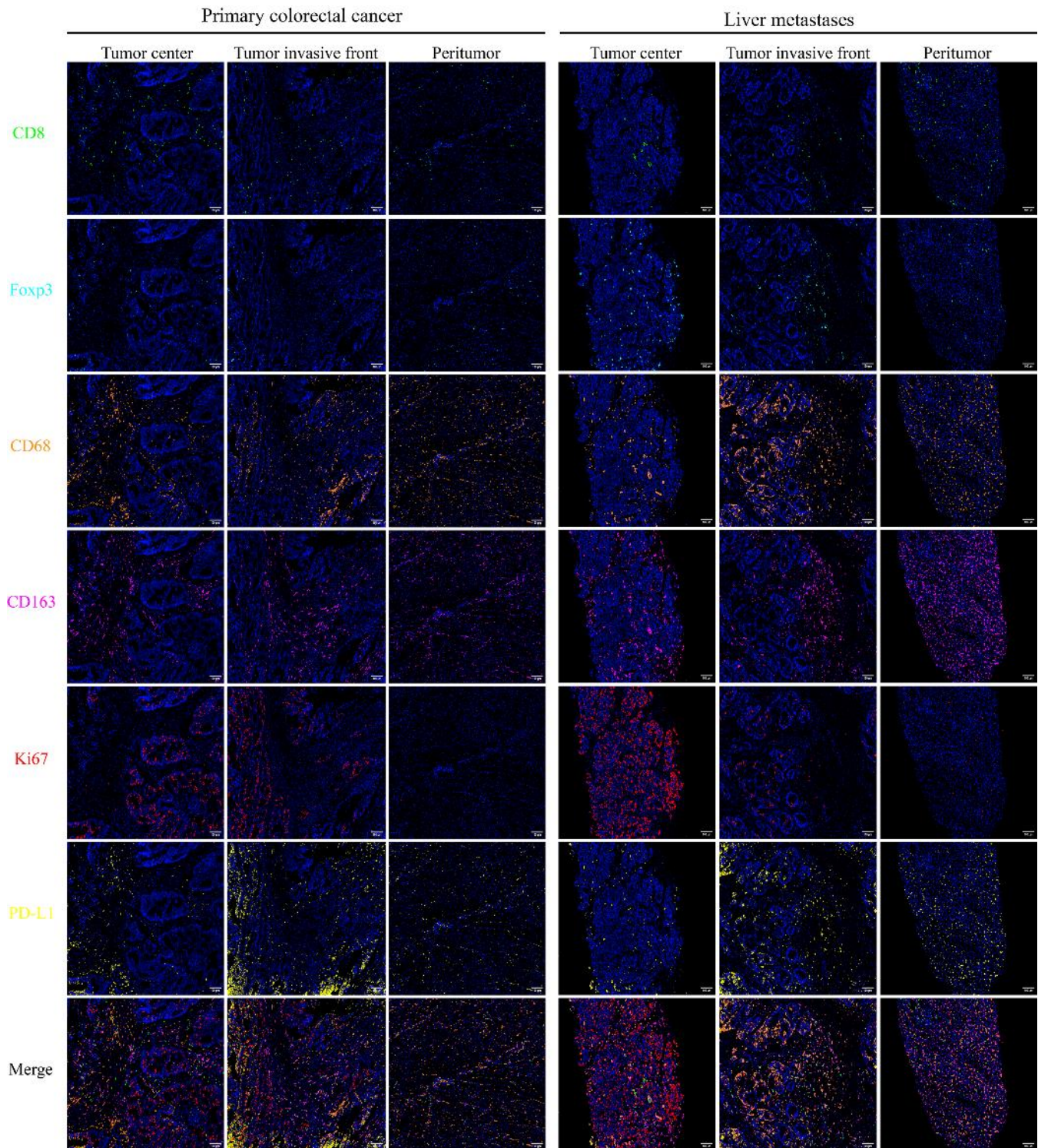
Supplementary Material

1 Supplementary Figures and Tables

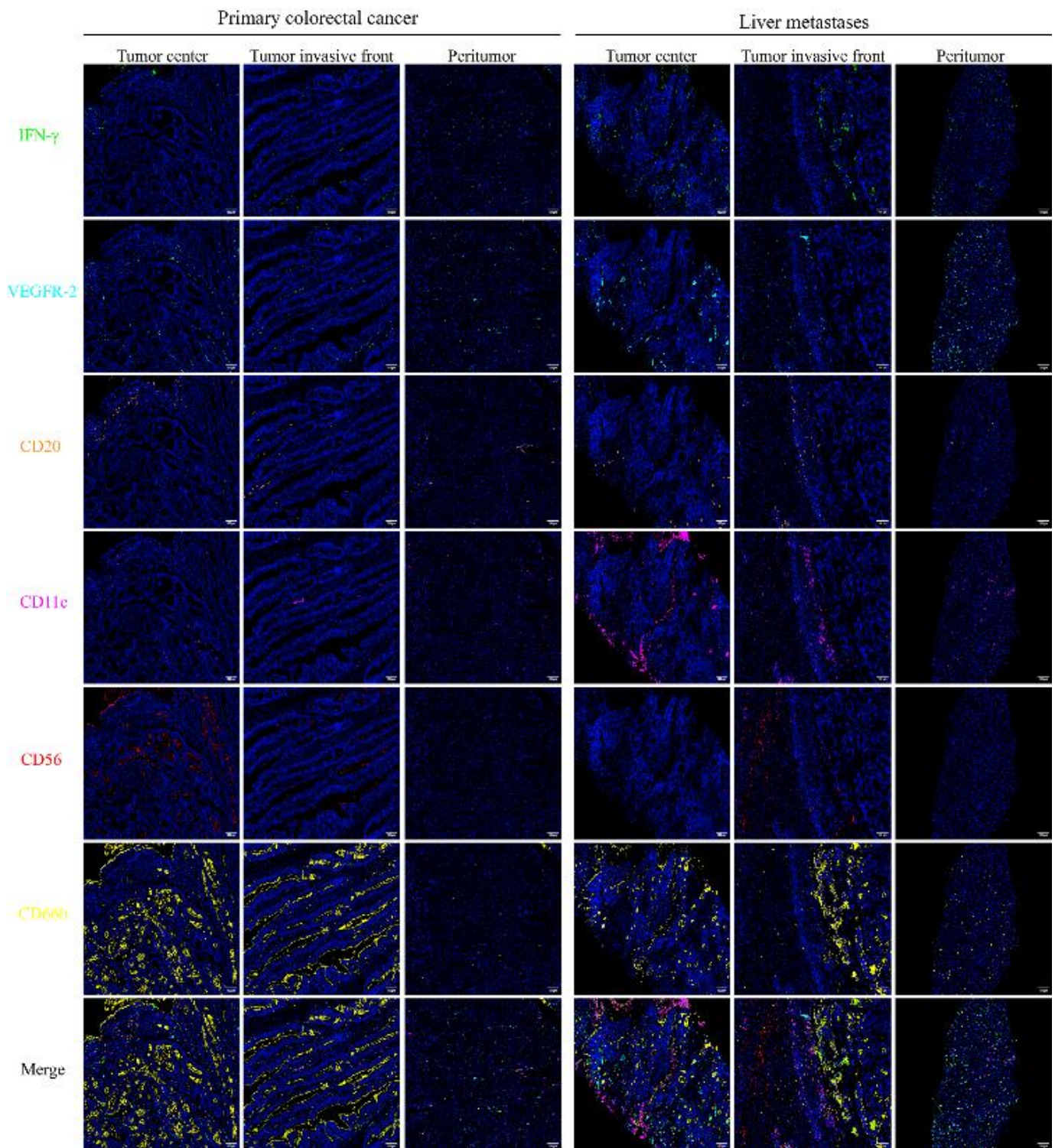
1.1 Supplementary Figures



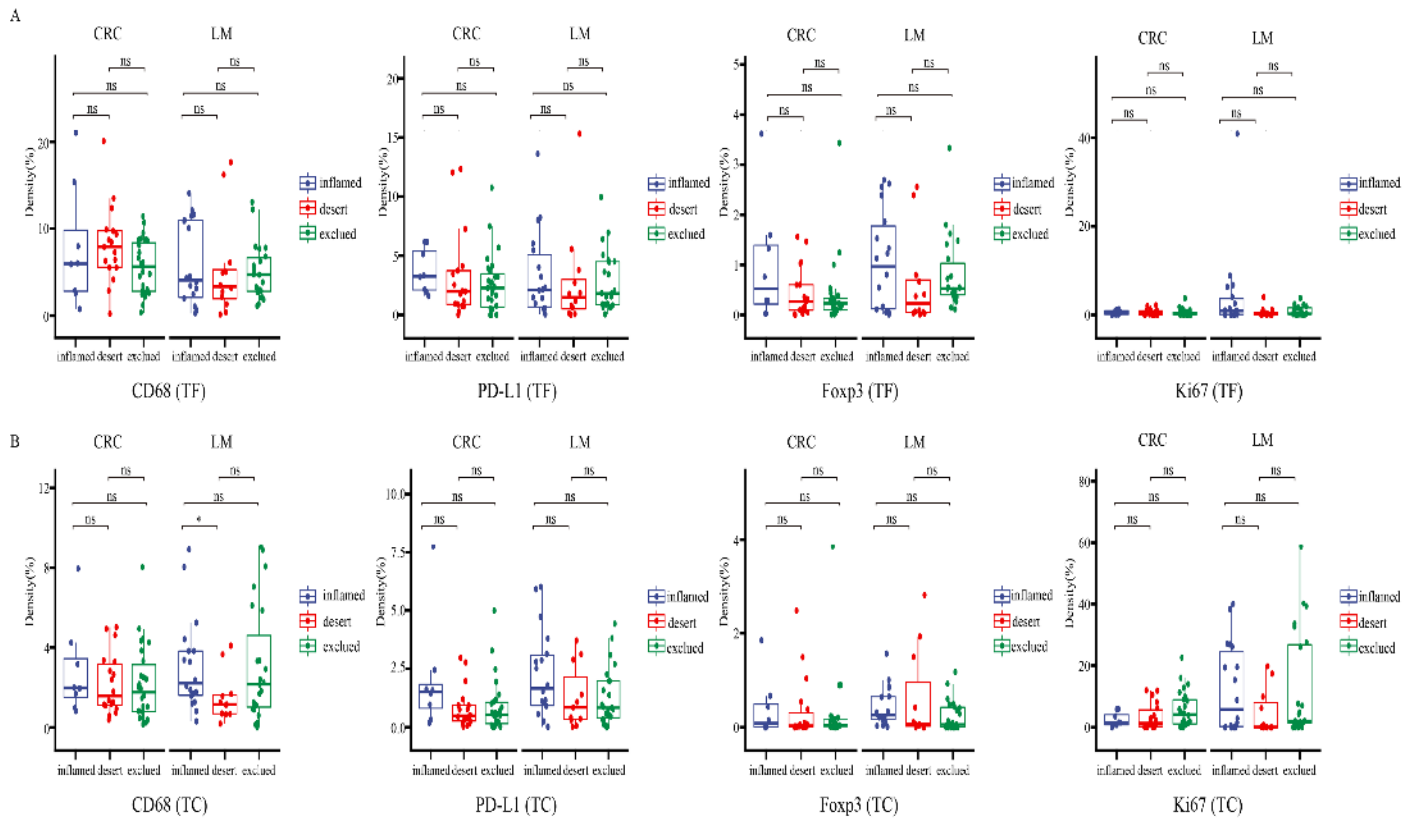
Additional file 1: Figure S1 General comparison of immune markers between primary tumors and liver metastases (A: CD8, B: CD20, C: CD68, D: CD11c, E: VEGFR-2, F: PD-L1, G: Ki67) ($p \geq 0.05$, not significant)



Additional file 3: Figure S2 Representative multiplex immunofluorescence images of 6 cell markers from panel 1 in the tumor center (TC), tumor invasive front (TF), and peritumoral (PT) regions of primary tumors and liver metastases



Additional file 4: Figure S3 Representative multiplex immunofluorescence images of 6 cell markers from panel 2 in the tumor center (TC), tumor invasive front (TF), and peritumoral (PT) regions of primary tumors and liver metastases



Additional file 7: Figure S4 Comparison of the three immunotypes according to the degree of CD8 infiltration. A: Comparison of the three immunotypes at the front of the tumor (CD68, Foxp3, PD-L1 and Ki67). C: Comparison of the three immunotypes at the center of the tumor (CD68, Foxp3, PD-L1 and Ki67). (* $p < 0.05$, $p \geq 0.05$, not significant)

1.2 Supplementary Tables

Additional file 2: Table S1 General comparison of immune markers between primary tumors and liver metastases

	Primary tumor			Liver metastases			<i>p</i> value
	Mean±SD	Median	25%, 75%	Mean±SD	Median	25%, 75%	
CD8	0.66±0.65	0.47	0.19 0.85	0.99±1.27	0.54	0.28 1.18	0.210
CD68	4.24±2.57	3.89	2.17 6.03	3.91±2.76	3.54	1.94 5.99	0.416
PD-L1	1.92±1.93	1.24	0.67 2.44	2.35±2.26	1.58	0.82 3.35	0.286
Ki67	2.67±2.96	1.28	0.41 4.19	7.18±9.21	1.81	0.36 11.56	0.136
Foxp3	0.38±0.56	0.15	0.07 0.36	0.58±0.52	0.47	0.18 0.73	< 0.001
CD163	1.72±1.02	1.56	0.90 2.28	2.72±2.41	2.06	1.11 3.53	0.025
INF-γ	0.18±0.39	0.02	0.00 0.11	0.34±0.61	0.07	0.01 0.40	0.030
CD20	0.33±0.50	0.15	0.04 0.40	0.41±1.07	0.09	0.03 0.42	0.292
CD66b	4.16±2.77	3.79	1.38 6.39	2.68±2.17	1.66	1.30 3.94	0.008
CD56	2.06±1.43	1.83	0.77 3.25	0.90±1.25	0.46	0.19 1.03	< 0.001
VEGFR-2	0.27±0.29	0.22	0.07 0.40	0.45±0.67	0.21	0.05 0.54	0.647
CD11c	0.18±0.22	0.11	0.02 0.27	0.46±0.69	0.11	0.04 0.57	0.159

p values were obtained from Wilcoxon's signed rank test

The bold values indicates significance at $p < 0.05$

Additional file 5: Table S2 Comparison of the immune markers of the TC, TF and PT regions

	Tumor Center			Tumor Front			Peritumor			<i>p</i> value	
	Mean±SD	Median	25%, 75%	Mean±SD	Median	25%, 75%	Mean±SD	Median	25%, 75%	TC:TF	TF:PT
Primary tumor											
CD8	0.25±0.38	0.09	0.03 0.31	1.36±1.95	0.81	0.34 1.53	0.19±0.31	0.06	0.02 0.26	< 0.001	< 0.001
CD68	2.29±1.83	1.87	0.86 3.31	6.74±4.39	6.06	2.92 8.73	2.17±1.88	1.66	0.82 3.09	< 0.001	< 0.001
PD-L1	1.05±1.38	0.58	0.20 1.35	3.01±2.88	2.22	0.91 3.90	0.76±0.93	0.36	0.05 1.19	< 0.001	< 0.001
Ki67	4.37±5.05	2.04	0.62 6.59	0.57±0.71	0.29	0.07 0.90	0.08±0.19	0.01	0.00 0.06	< 0.001	< 0.001
Foxp3	0.32±0.71	0.04	0.01 0.22	0.51±0.75	0.25	0.10 0.58	0.15±0.21	0.06	0.02 0.18	< 0.001	< 0.001
CD163	0.56±0.69	0.32	0.10 0.70	3.38±2.01	2.94	2.08 4.40	1.82±1.46	1.63	0.49 2.81	< 0.001	< 0.001
INF-γ	0.07±0.13	0.01	0.00 0.10	0.25±0.59	0.00	0.00 0.06	0.04±0.13	0.00	0.00 0.00	0.488	0.003
CD20	0.07±0.14	0.01	0.00 0.10	0.69±0.93	0.33	0.07 0.75	0.29±0.47	0.12	0.02 0.26	< 0.001	0.002
CD66b	6.83±5.34	6.00	1.75 11.11	1.60±1.88	1.00	0.24 2.10	0.10±1.76	0.10	0.03 1.11	< 0.001	< 0.001
CD56	2.91±2.61	2.50	0.83 4.15	1.00±1.28	0.44	0.17 1.32	0.32±0.91	0.06	0.00 0.23	< 0.001	< 0.001
VEGFR-2	0.08±0.13	0.04	0.00 0.09	0.54±0.58	0.33	0.11 0.74	0.15±0.28	0.04	0.00 0.22	< 0.001	< 0.001
CD11c	0.05±0.11	0.00	0.00 0.07	0.32±0.34	0.23	0.05 0.44	0.15±0.26	0.01	0.00 0.14	< 0.001	< 0.001
Liver metastases											
CD8	0.67±0.97	0.27	0.05 1.01	1.47±1.67	0.87	0.38 1.84	0.47±0.42	0.44	0.08 0.60	< 0.001	< 0.001
CD68	2.77±2.49	1.80	1.13 3.78	5.43±4.40	4.07	2.52 7.88	2.87±2.22	2.32	1.32 4.94	< 0.001	0.002
PD-L1	1.62±1.56	1.06	0.37 2.77	3.04±3.34	1.82	0.82 4.59	2.76±2.36	2.20	1.16 5.10	0.021	0.716
Ki67	11.05±14.80	2.02	0.08 19.70	1.99±5.82	0.36	0.07 1.86	1.44±6.89	0.13	0.04 0.45	0.006	0.008
Foxp3	0.39±0.57	0.17	0.03 0.49	0.86±0.87	0.53	0.12 1.46	1.03±1.28	0.40	0.14 1.73	< 0.001	0.682
CD163	1.25±1.80	0.65	0.31 1.45	4.70±4.31	3.24	1.84 5.72	6.02±3.49	6.29	2.99 8.02	< 0.001	0.010
INF-γ	0.48±0.92	0.02	0.00 0.64	0.22±0.43	0.05	0.02 0.30	0.40±0.84	0.03	0.00 0.44	0.795	0.605
CD20	0.22±1.21	0.00	0.00 0.06	0.56±1.07	0.18	0.04 0.74	0.61±0.98	0.26	0.08 0.80	< 0.001	0.370
CD66b	3.93±2.94	3.76	1.74 5.77	1.41±2.21	0.65	0.35 1.72	0.25±0.34	0.11	0.07 0.36	< 0.001	< 0.001
CD56	1.40±1.85	0.67	0.03 2.02	0.52±0.70	0.30	0.16 0.69	0.27±0.61	0.05	0.01 0.33	0.072	< 0.001
VEGFR-2	0.44±1.64	0.02	0.00 0.09	0.60±0.70	0.38	0.12 0.98	0.81±1.28	0.22	0.17 1.13	< 0.001	0.917
CD11c	0.15±0.40	0.04	0.00 0.12	0.79±1.02	0.24	0.05 1.53	0.18±0.30	0.05	0.01 0.26	< 0.001	< 0.001

p values were obtained from Wilcoxon's signed rank test

The bold values indicates significance at *p*<0.05

Additional file 6: Table S3 Comparison of primary tumors and liver metastases in different regions

	Primary tumor				Liver metastases				p value
	Mean±SD	Median	25%, 75%		Mean±SD	Median	25%, 75%		
Tumor center									
CD8	0.25±0.38	0.09	0.03	0.31	0.67±0.97	0.27	0.05	1.01	0.020
CD68	2.29±1.83	1.87	0.86	3.31	2.77±2.49	1.80	1.13	3.78	0.554
PD-L1	1.05±1.38	0.58	0.20	1.35	1.62±1.56	1.06	0.37	2.77	0.037
Ki67	4.37±5.05	2.04	0.62	6.59	11.05±14.80	2.02	0.08	19.70	0.520
Foxp3	0.32±0.71	0.04	0.01	0.22	0.39±0.57	0.17	0.03	0.49	0.134
CD163	0.56±0.69	0.32	0.10	0.70	1.25±1.80	0.65	0.31	1.45	0.005
INF-γ	0.07±0.13	0.01	0.00	0.10	0.48±0.92	0.02	0.00	0.64	0.120
CD20	0.07±0.14	0.01	0.00	0.10	0.22±1.21	0.00	0.00	0.06	0.188
CD66b	6.83±5.34	6.00	1.75	11.11	3.93±2.94	3.76	1.74	5.77	0.009
CD56	2.91±2.61	2.50	0.83	4.15	1.40±1.85	0.67	0.03	2.02	<0.001
VEGFR-2	0.08±0.13	0.04	0.00	0.09	0.44±1.64	0.02	0.00	0.09	0.517
CD11c	0.05±0.11	0.00	0.00	0.07	0.15±0.40	0.04	0.00	0.12	0.010
Tumor invasive front									
CD8	1.36±1.95	0.81	0.34	1.53	1.47±1.67	0.87	0.38	1.84	0.475
CD68	6.74±4.39	6.06	2.92	8.73	5.43±4.40	4.07	2.52	7.88	0.054
PD-L1	3.01±2.88	2.22	0.91	3.90	3.04±3.34	1.82	0.82	4.59	0.658
Ki67	0.57±0.71	0.29	0.07	0.90	1.99±5.82	0.36	0.07	1.86	0.147
Foxp3	0.51±0.75	0.25	0.10	0.58	0.86±0.87	0.53	0.12	1.46	0.009
CD163	3.38±2.01	2.94	2.08	4.40	4.70±4.31	3.24	1.84	5.72	0.298
INF-γ	0.25±0.59	0.00	0.00	0.06	0.22±0.43	0.05	0.02	0.30	0.008
CD20	0.69±0.93	0.33	0.07	0.75	0.56±1.07	0.18	0.04	0.74	0.170
CD66b	1.60±1.88	1.00	0.24	2.10	1.41±2.21	0.65	0.35	1.72	0.594
CD56	1.00±1.28	0.44	0.17	1.32	0.52±0.70	0.30	0.16	0.69	0.064
VEGFR-2	0.54±0.58	0.33	0.11	0.74	0.60±0.70	0.38	0.12	0.98	0.930
CD11c	0.32±0.34	0.23	0.05	0.44	0.79±1.02	0.24	0.05	1.53	0.206
Peritumor									
CD8	0.19±0.31	0.06	0.02	0.26	0.47±0.42	0.44	0.08	0.60	<0.001
CD68	2.17±1.88	1.66	0.82	3.09	2.87±2.22	2.32	1.32	4.94	0.103
PD-L1	0.76±0.93	0.36	0.05	1.19	2.76±2.36	2.20	1.16	5.10	<0.001
Ki67	0.08±0.19	0.01	0.00	0.06	1.44±6.89	0.13	0.04	0.45	<0.001
Foxp3	0.15±0.21	0.06	0.02	0.18	1.03±1.28	0.40	0.14	1.73	<0.001
CD163	1.82±1.46	1.63	0.49	2.81	6.02±3.49	6.29	2.99	8.02	<0.001
INF-γ	0.04±0.13	0.00	0.00	0.00	0.40±0.84	0.03	0.00	0.44	<0.001
CD20	0.29±0.47	0.12	0.02	0.26	0.61±0.98	0.26	0.08	0.80	0.011
CD66b	0.10±1.76	0.10	0.03	1.11	0.25±0.34	0.11	0.07	0.36	0.661
CD56	0.32±0.91	0.06	0.00	0.23	0.27±0.61	0.05	0.01	0.33	0.469
VEGFR-2	0.15±0.28	0.04	0.00	0.22	0.81±1.28	0.22	0.17	1.13	<0.001
CD11c	0.15±0.26	0.01	0.00	0.14	0.18±0.30	0.05	0.01	0.26	0.022

p values were obtained from Wilcoxon's signed rank test

The bold values indicates significance at $p < 0.05$