Supplementary Online Material

Supplementary tables

Table S1. Patient characteristics

	Study cohort (n=147)	Validation cohort (n = 418)
Age, mean (SD)	66.9 (11.2)	67.7 (12.4)
Sex		
Male	79 (53.7%)	200 (47.8%)
Female	68 (46.3%)	218 (52.2%)
Preoperative radiotherapy or chemoradiotherapy		
No	116 (78.9%)	418 (100%)
Yes	31 (21.1%)	0 (0%)
Tumor location		
Proximal colon	49 (33.3%)	130 (31.1%)
Distal colon	27 (18.4%)	116 (27.8%)
Rectum	71 (48.3%)	172 (41.1%)
WHO grade		
G1 (well)	20 (13.6%)	101 (24.2%)
G2 (moderately)	108 (73.5%)	246 (58.9%)
G3 (poorly)	19 (12.9%)	71 (17.0%)
TNM Stage		
Stage I	26 (17.8%)	90 (21.5%)
Stage II	55 (37.7%)	180 (43.1%)
Stage III	46 (31.5%)	98 (23.4%)
Stage IV	19 (13.0%)	50 (12.0%)
Mismatch repair (MMR) screening status		
MMR Deficient	11 (7.5%)	36 (9.0%)
MMR Proficient	136 (92.5%)	362 (91.0%)
Serrated histology		
No	114 (77.6%)	372 (89.0%)
Yes	34 (22.4%)	46 (11.0%)
Infiltrative growth pattern		
No	105 (71.4%)	341 (81.6%)
Yes	42 (28.6%)	77 (18.4%)
Necrosis percentage, median (IQR)	10.0 (5.0-25.0)	8.0 (5.0-15.0)

Table S2. Antibodies and protocols used in immunohistochemistry

Antigen	Antigen retrieval	Antibody type	Manufacturer	Clone	Code	Dilution	Incubation	Antibody visualization
MLH1	Tris-EDTA pH9*	mouse monoclonal	BD-Pharmingen	G168-15	551091	1:200	overnight	NovoLink
MSH2	Tris-EDTA pH9*	mouse monoclonal	BD-Pharmingen	G219-1129	556349	1:150	1 hr	EnVision
Ki-67	Tris-EDTA pH9*	mouse monoclonal	DAKO	MIB-1	M7240	1:300	30 min	EnVision
CD31	Tris-EDTA pH9*	mouse monoclonal	DAKO	JC70A	M0823	1:300	45 min	EnVision
CD105	Tris-EDTA pH9*	mouse monoclonal	DAKO	SN6h	M3527	1:50	1 hr	EnVision
von Willebrand factor (Factor VIII related antigen)	Tris-EDTA pH9*	rabbit polyclonal	DAKO	-	A0082	1:5000	30 min	EnVision

*In a microwave oven at 800W for 2 min and at 150W for 15 min.

Table S3. Correlations between tumor necrosis and clinicopathological characteristics in the study cohort (n=147)

	Tumor necrosis percentage	
Variable	Median (IQR)	P value
Age		
<65	10.5 (5.0-30.0)	0.724
≥65	9.0 (5.0-21.0)	
Sex		
Male	10.0 (5.0-25.0)	0.847
Female	10.0 (5.0-24.5)	
Location of tumor		
Proximal colon	10.0 (4.0-19.0)	0.656
Distal colon	11.0 (5.0-28.0)	
Rectum	9.0 (5.0-25.0)	
Preoperative RT/CRT		
No	9.5 (5.0-19.8)	0.188
Yes	11.0 (6.0-35.0)	
WHO grade		
G1 (well)	6.0 (3.5-20.8)	0.712
G2 (moderately)	10.0 (5.0-25.0)	
G3 (poorly)	8.0 (3.0-40.0)	
TNM Stage		
Stage I	5.0 (2.8-8.5)	8.5E-4
Stage II	10.0 (5.0-24.0)	
Stage III	10.5 (5.0-25.6)	
Stage IV	25.0 (10.0-45.0)	
Primary tumor		
pT1	8.0 (5.0-11.5)	4.0E-6
pT2	5.0 (2.0-6.8)	
pT3	11.0 (5.0-25.0)	
pT4	50.0 (15.0-70.0)	
Lymph nodes		
pN0 (0 nodes)	7.0 (4.5-16.5)	4.0E-3
pN1 (1-3 nodes)	8.5 (5.0-19.5)	
pN2 (≥4 nodes)	25.0 (9.5-42.5)	
Distant Metastasis		
No	8.0 (5.0-20.5)	5.0E-3
Yes	25.0 (10.0-45.0)	
Lymphatic invasion		
No	7.0 (4.0-18.0)	1.2E-3
Yes	14.0 (6.50-35.0)	
Blood vessel invasion		
No	7.0 (5.0-16.5)	1.3E-4
Yes	22.0 (13.0-50.0)	
Infiltrative growth pattern		
No	10.0 (5.0-24.5)	0.263

Yes	11.5 (5.0-27.5)	
Serrated histology		
No	11.0 (5.0-25.0)	0.014
Yes	5.0 (2.5-15.0)	
KRAS and BRAF mutation status		
KRAS	7.5 (3.8-14.3)	0.384
BRAF V600E	7.0 (2.8-28.8)	
wt KRAS and BRAF V600E	10.0 (5.0-28.0)	
MMR screening status		
MMR deficient	7.0 (2.0-30.0)	0.447
MMR proficient	10.0 (5.0-24.8)	

Abbreviations: MMR: mismatch repair. P values are for Mann-Whitney *U* test or Kruskal-Wallis test.

Table S4. Correlations between preoperative radiotherapy (RT) or chemoradioterapy (CRT) and microvascular density (MVD) and proliferation rate.

	Preoperative RT/CRT		
Variable	No (n=116)	Yes (n=31)	p value
CD31 MVD (1/mm^2)	44.8 (30.6-62.5)	37.1 (23.0-56.4)	0.085
vWF MVD (1/mm^2)	22.7 (16.3-31.9)	22.0 (10.5-31.6)	0.231
CD105 MVD (1/mm^2)	8.44 (4.53-15.0)	14.9 (7.15-21.6)	0.021
Proliferation rate, Ki-67 (%)	26.9 (12.3-52.0)	31.4 (2.8-62.1)	0.952

Table S5. Survival analysis follow-up and endpoint data

	DFS	CSS	OS
Study cohort (n=147)			
Included patients	124 (84.5%)	147 (100.0%)	147 (100.0%)
Excluded patients	23 (15.6%)[A]	0 (0.0%)	0 (0.0%)
Events	25 (20.2%)	32 (21.8%)	44 (29.9%)
Follow-up, median (IQR)	51.0 (19.2-60.0)	59.2 (36.0-60.0)	59.2 (36.0-60.0)
Follow-up in patients with events, median (IQR)	12.0 (7.1-20.1)	22.4 (15.4-31.5)	21.4 (13.1-31.8)
Follow-up in patients without events, median (IQR)	60.0 (26.4-60.0)	60.0 (52.1-60.0)	60.0 (57.8-60.0)
Validation cohort (n=418)			
Included patients	303 (72.5%)	352 (84.2%)	352 (84.2%)
Excluded patients	105 (25.1%)[B]	66 (15.8%)[C]	66 (15.8%)[C]
Events	92 (30.4%)	129 (36.7%)	175 (49.7%)
Follow-up, median (IQR)	25.0 (12.0-60.0)	31.0 (14.0-60.0)	31.0 (14.0-60.0)
Follow-up in patients with events, median (IQR)	12.0 (8.0-19.0)	20.0 (8.0-36.5)	16.0 (6.0-35.0)
Follow-up in patients without events, median (IQR)	41.0 (17.0-60.0)	60.0 (27.0-60.0)	60.0 (27.0-60.0)

[A]Palliative operation in 23/23 (100%) of the excluded cases.
[B]No follow-up data for 66/105 (62.9%) of the excluded patients. Palliative operation in 39/105 (37.1%) of the excluded cases.
[C]No follow-up data for 66/66 (100%) of the excluded patients.

Table S6. Receiver operating characteristics (ROC) analysis for determining cut-off points in detecting disease-free survival

	AUC	95%CI	Cut-off point
Tumor necrosis	0.628	0.513-0.743	10%
CD31 MVD	0.532	0.414-0.651	45/mm^2
vWF MVD	0.591	0.478-0.704	20/mm^2
CD105 MVD	0.604	0.476-0.732	10/mm^2
Ki-67	0.562	0.436-0.689	30%

Table S7. Univariate analysis of disease-free survival (DFS), cancer-specific survival (CSS), and overall survival (OS) according to tumor necrosis, microvascular density, proliferation rate, serrated growth pattern and infiltrative tumor border in the study cohort.

	DFS			CSS			OS		
	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value
Tumor necrosis (<10% vs. ≥10%)	2.34	1.03-5.30	0.041	2.74	1.27-5.93	0.010	2.08	1.11-3.88	0.022
CD31 MVD (<45/mm^2 vs. ≥45/mm^2)	1.02	0.46-2.23	0.966	0.56	0.27-1.17	0.124	1.79	0.96-3.33	0.068
vWF MVD (<20/mm^2 vs. ≥20/mm^2)	1.08	0.48-2.45	0.850	0.50	0.25-1.00	0.050	1.87	1.03-3.39	0.039
CD105 MVD (<10/mm^2 vs. ≥10/mm^2)	2.26	1.00-5.11	0.051	0.86	0.43-1.74	0.676	1.04	0.57-1.89	0.892
Ki-67 (≤30% vs. >30%)	0.56	0.25-1.26	0.162	0.71	0.35-1.45	0.349	1.12	0.62-2.03	0.716
Serrated growth pattern (No vs. Yes)	1.34	0.56-3.21	0.512	1.12	0.51-2.50	0.775	0.79	0.41-1.53	0.484
Infiltrative tumor border (No vs. Yes)	3.89	1.77-8.53	7.0E-4	2.71	1.35-5.44	4.9E-3	0.47	0.26-0.86	0.014

Abbreviations: MVD: microvascular density.

Table S8. Correlations between tumor necrosis and clinicopathological characteristics in the validation cohort (n=418)

	Necrosis percentage	
Variable	Median (IQR)	P value
Age		
<65	7.0 (5.0-15.0)	0.921
≥65	8.0 (5.0-15.0)	
Sex		
Male	8.0 (5.0-16.0)	0.807
Female	8.0 (5.0-15.0)	
Location of tumor		
Proximal colon	8.0 (4.9-15.5)	0.872
Distal colon	7.0 (4.0-17.5)	
Rectum	8.0 (4.5-15.0)	
WHO grade		
G1 (well)	5.0 (3.0-9.5)	2.6E-8
G2 (moderately)	9.0 (5.0-17.3)	
G3 (poorly)	10.0 (5.0-20.0)	
TNM Stage		
Stage I	5.0 (3.0-7.0)	1.5E-10
Stage II	9.0 (5.0-17.5)	
Stage III	10.0 (5.0-20.0)	
Stage IV	11.0 (5.0-21.25)	
Primary tumor		
pT1	3.0 (2.3-5.0)	1.3E-10
pT2	5.0 (3.0-8.0)	
pT3	10.0 (5.0-20.0)	
pT4	8.0 (5.0-22.5)	
Lymph nodes		
pN0 (0 nodes)	7.0 (4.0-15.0)	1.2E-3
pN1 (1-3 nodes)	10.0 (5.0-16.0)	
pN2 (≥4 nodes)	13.0 (5.0-20.0)	
Distant Metastasis		
No	8.0 (5.0-15.0)	0.029
Yes	11.0 (5.0-21.25)	
Lymphatic invasion		
No	7.0 (4.0-15.0)	0.065
Yes	9.0 (5.0-16.0)	
Blood vessel invasion		
No	7.0 (5.0-15.0)	0.012
Yes	12.0 (5.0-20.0)	
Infiltrative growth pattern		
No	8.0 (5.0-15.5)	0.869
Yes	7.0 (5.0-15.0)	
Serrated type growth pattern		
No	8.0 (5.0-17.0)	3.1E-6

Yes	5.0 (2.0-8.5)	
MMR screening status		
MMR deficient	7.0 (3.0-16.5)	0.208
MMR proficient	8.0 (5.0-15.0)	

Abbreviations: MMR: mismatch repair. P values are for Mann-Whitney *U* test or Kruskal-Wallis test.

Table S9. Cox regression model for the independent prognostic significance of tumor necrosis in the subgroup of TNM stage II patients in the validation cohort.

	DFS			CSS			OS		
	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value
WHO Grade (1-2 vs. 3)	1.69	0.73-3.93	0.216	1.64	0.66-4.06	0.285	1.93	0.97-3.83	0.060
Tumor location (Colon vs. Rectum)	1.75	0.92-3.32	0.087	1.76	0.89-3.50	0.105	1.49	0.86-2.59	0.154
Lymphatic or blood vessel invasion (No vs. Yes)	1.61	0.77-3.33	0.203	1.93	0.89-4.19	0.097	2.33	1.25-4.32	7.5E-3
Infiltrative tumor border (No vs. Yes)	4.70	2.19-10.1	7.3E-5	3.78	1.71-8.35	1.0E-3	2.51	1.30-4.87	6.4E-3
Tumor necrosis (<10% vs. ≥10%)	1.96	1.02-3.76	0.043	2.18	1.09-4.34	0.027	1.75	1.01-3.02	0.046

Supplementary figures

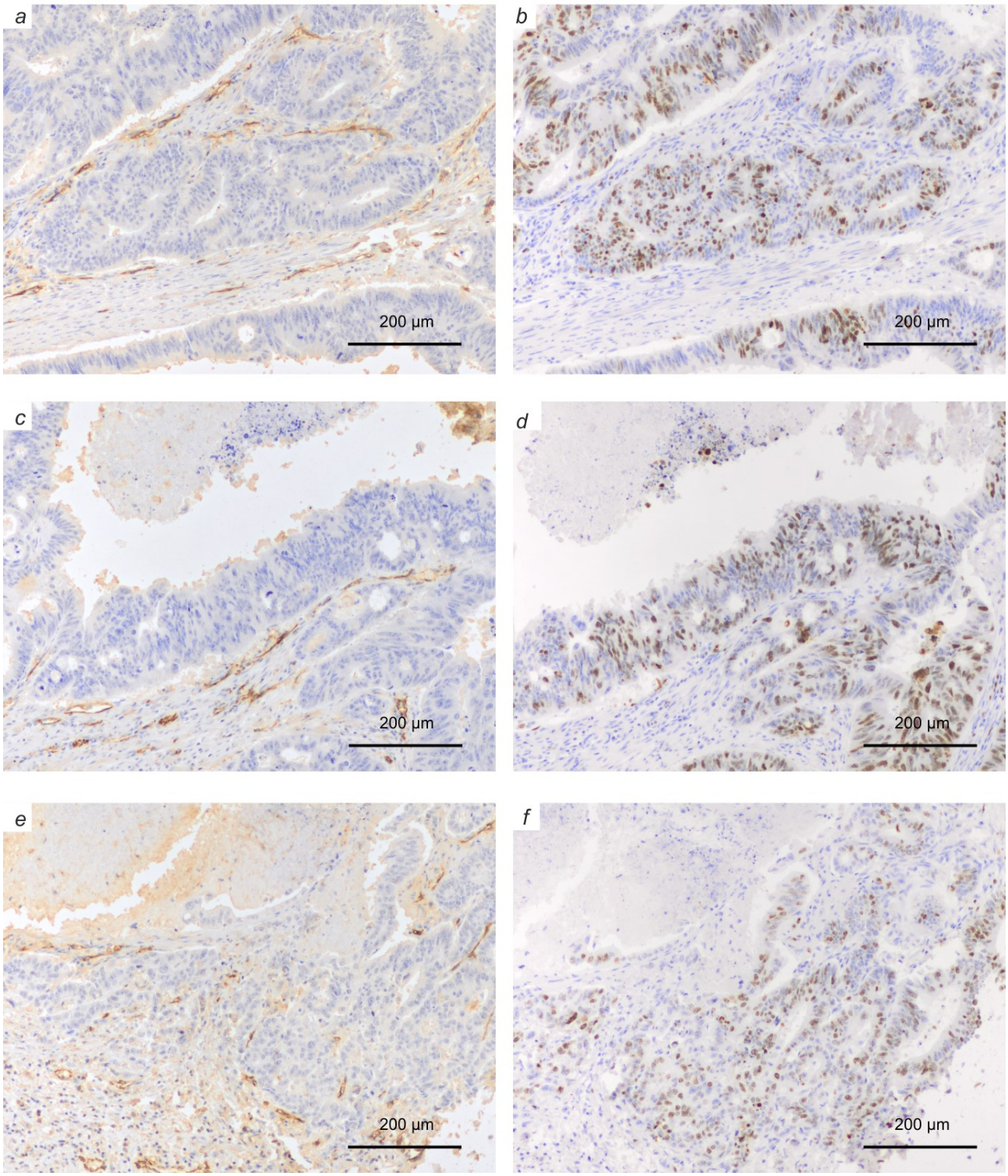

Fig S1. Example of CD31 MVD (a, c, e) and Ki-67 index (b, d, f) in different tumor areas and adjacent and distant to necrosis in one colorectal cancer case. Center of the tumor, no necrosis (a, b). Center of the tumor,

necrosis (c, d). Invasive front, necrosis (e, f). CD31 MVD in different tumor locations, as well as the proliferation rate in different tumor locations, had high correlation.