

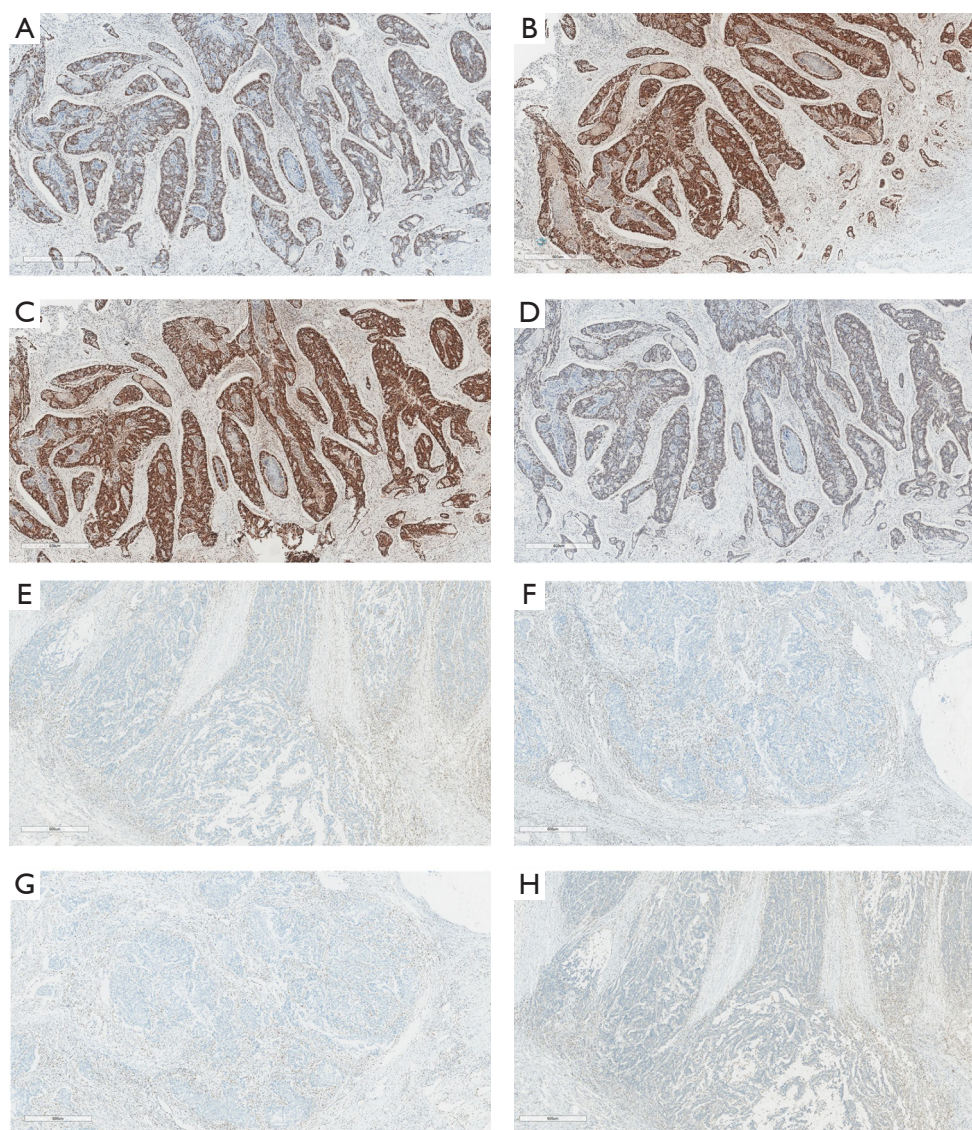


Figure S1 Representative images of MMR proteins. (A) Representative images of MLH1 being positive; (B) representative images of MSH2 being positive; (C) representative images of MSH6 being positive; (D) representative images of PMS2 being positive; (E) representative images of MLH1 being negative; (F) representative images of MSH2 being negative; (G) representative images of MSH6 being negative; (H) representative images of PMS2 being negative. Tumor tissues were stained with MLH1, MSH2, MSH6, PMS2 by IHC (600 μ m). MMR, mismatch repair; IHC, immunohistochemistry.

Table S1 Selected baseline characteristics before and after propensity score matching in chemotherapy group

Characteristics	Before matching				After matching (1:4)			
	pMMR, n=401, n (%)	dMMR, n=33, n (%)	P	Standardized difference	pMMR, n=132, n (%)	dMMR, n=33, n (%)	P	Standardized difference
Age, years			0.025	0.540			0.923	0.105
<65	301 (75.1)	31 (93.9)			127 (96.2)	31 (93.9)		
≥65	100 (24.9)	2 (6.1)			5 (3.8)	2 (6.1)		
Gender			0.974	0.042			0.930	0.017
Male	284 (70.8)	24 (72.7)			97 (73.5)	24 (72.7)		
Female	117 (29.2)	9 (27.3)			35 (26.5)	9 (27.3)		
Grade of differentiation			0.090	0.315			0.564	0.110
Well/moderate	352 (87.8)	25 (75.8)			106 (80.3)	25 (75.8)		
Poorly	49 (12.2)	8 (24.2)			26 (19.7)	8 (24.2)		
Mucinous adenocarcinoma			<0.001	0.495			0.278	0.201
No	381 (95.0)	26 (78.8)			114 (86.4)	26 (78.8)		
Yes	20 (5.0)	7 (21.2)			18 (13.6)	7 (21.2)		
Location from anal verge, cm			0.417	0.232			0.948	0.065
<5	172 (42.9)	11 (33.3)			44 (33.3)	11 (33.3)		
5–10	191 (47.6)	17 (51.5)			65 (49.2)	17 (51.5)		
>10	38 (9.5)	5 (15.2)			23 (17.4)	5 (15.2)		
Clinical T stage			0.232	0.222			0.928	0.080
T2	5 (1.2)	1 (3.0)			3 (2.3)	1 (3.0)		
T3	327 (81.5)	24 (72.7)			93 (70.5)	24 (72.7)		
T4	69 (17.2)	8 (24.2)			36 (27.3)	8 (24.2)		
Clinical N stage			1.000	0.025			0.797	0.050
N0	105 (26.2)	9 (27.3)			39 (29.5)	9 (27.3)		
N1-2	296 (73.8)	24 (72.7)			93 (70.5)	24 (72.7)		

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

Table S2 Selected baseline characteristics before and after propensity score matching in chemoradiation group

Characteristics	Before matching				After matching (1:4)			
	pMMR, n=390, n (%)	dMMR, n=30, n (%)	P	Standardized difference	pMMR, n=120, dMMR, n=30, n (%)	n (%)	P	Standardized difference
Age, years			0.891	0.073			0.954	0.069
<65	314 (80.5)	25 (83.3)			103 (85.8)	25 (83.3)		
≥65	76 (19.5)	5 (16.7)			17 (14.2)	5 (16.7)		
Gender			0.717	0.112			1.000	0.040
Male	280 (71.8)	23 (76.7)			94 (78.3)	23 (76.7)		
Female	110 (28.2)	7 (23.3)			26 (21.7)	7 (23.3)		
Grade of differentiation			1.000	0.049			1.000	<0.001
Well/moderate	359 (92.1)	28 (93.3)			112 (93.3)	28 (93.3)		
Poorly	31 (7.9)	2 (6.7)			8 (6.7)	2 (6.7)		
Mucinous adenocarcinoma			1.000	0.028			1.000	0.050
No	375 (96.2)	29 (96.7)			117 (97.5)	29 (96.7)		
Yes	15 (3.8)	1 (3.3)			3 (2.5)	1 (3.3)		
Location from anal verge, cm			0.162	0.298			0.849	0.114
<5	212 (54.4)	16 (53.3)			69 (57.5)	16 (53.3)		
5–10	158 (40.5)	10 (33.3)			39 (32.5)	10 (33.3)		
>10	20 (5.1)	4 (13.3)			12 (10.0)	4 (13.3)		
Clinical T stage			0.321	0.235			0.312	0.228
T2	8 (2.1)	1 (3.3)			1 (0.8)	1 (3.3)		
T3	289 (74.1)	19 (63.3)			86 (71.7)	19 (63.3)		
T4	93 (23.8)	10 (33.3)			33 (27.5)	10 (33.3)		
Clinical N stage			0.268	0.239			1.000	0.036
N0	77 (19.7)	9 (30.0)			38 (31.7)	9 (30.0)		
N1-2	313 (80.3)	21 (70.0)			82 (68.3)	21 (70.0)		

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

Table S3 Univariate and multivariate Cox proportional hazards regression models for LRFS and DFS

Variables	Local recurrence-free survival				Disease-free survival			
	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P	Univariate HR (95%CI)	P	Multivariate HR (95% CI)	P
Age, years								
<65	1	0.189	1	0.230	1	0.192	1	0.186
≥65	0.62 (0.31–1.26)		0.64 (0.32–1.32)		0.79 (0.55–1.13)		0.78 (0.54–1.13)	
Gender								
Male	1	0.024	1	0.054	1	0.739	1	0.562
Female	0.46 (0.23–0.90)		0.51 (0.26–1.01)		0.95 (0.70–1.29)		1.10 (0.80–1.50)	
Grade of differentiation								
Well/moderate	1	0.001	1	0.081	1	<0.001	1	0.408
Poorly	2.73 (1.50–4.96)		1.74 (0.93–3.23)		1.95 (1.35–2.80)		1.17 (0.80–1.71)	
Mucinous adenocarcinoma								
No	1	0.478			1	0.191		
Yes	1.44 (0.52–3.98)				1.44 (0.83–2.47)			
Location from anal verge, cm								
≥5	1	0.108			1	0.628		
<5	1.52 (0.91–2.53)				1.06 (0.85–1.32)			
Clinical T stage								
T2–3	1	0.006	1	0.012	1	0.082	1	0.407
T4	1.20 (1.06–1.37)		1.19 (1.04–1.37)		1.07 (0.99–1.16)		1.04 (0.95–1.12)	
Clinical N stage								
N0	1	0.026	1	0.161	1	0.023	1	0.351
N1–2	2.45 (1.11–5.38)		1.79 (0.79–4.01)		1.52 (1.06–2.18)		1.19 (0.82–1.73)	
Neoadjuvant therapy pattern								
Neoadjuvant chemotherapy alone	1	0.074	1	0.143	1	0.09	1	0.753
Fluorouracil based- radiotherapy	0.63 (0.37–1.05)		0.65 (0.37–1.16)		0.79 (0.60–1.04)		0.95 (0.70–1.29)	
Postoperative chemotherapy								
No	1	0.747	1	0.305	1	0.008	1	0.114
Yes	1.14 (0.52–2.50)		0.65 (0.28–1.48)		2.09 (1.22–3.60)		1.56 (0.90–2.72)	
ypT stage								
T0-2	1	<0.001	1	0.059	1	<0.001	1	<0.001
T3-4	3.52 (1.91–6.49)		2.08 (0.97–4.46)		3.67 (2.63–5.11)		2.78 (1.84–4.21)	
ypN stage								
N0	1	<0.001	1	0.009	1	<0.001	1	<0.001
N1-2	3.29 (1.99–5.45)		2.09 (1.20–3.62)		3.38 (2.56–4.47)		2.27 (1.67–3.07)	
pCR								
No	1	0.066	1	0.811	1	0.002	1	0.653
Yes	0.34 (0.11–1.07)		0.85 (0.22–3.22)		0.39 (0.22–0.70)		1.17 (0.59–2.31)	
TRG				0.907		<0.001		
0-1	1	0.022	1		1		1	0.660
2-3	1.38 (1.05–1.81)		1.02 (0.73–1.43)		1.34 (1.15–1.55)		1.04 (0.87–1.24)	
MMR status								
pMMR	1	0.746	1	0.686	1	0.022	1	0.013
dMMR	0.83 (0.26–2.63)		0.79 (0.24–2.54)		0.42 (0.20–0.88)		0.38 (0.18–0.81)	

LRFS, local recurrence-free survival; DFS, disease-free survival; HR, hazards ratio; CI, confidence interval; pCR, pathologic complete response; TRG, tumor regression grade; MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

Table S4 Univariate and multivariate Cox proportional hazards regression models for DFS in patients with ypStage II/III and patients with ypT0N0 and Stage I

Variables	ypStage II/III				ypT0N0 and Stage I			
	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P	Univariate HR (95%CI)	P	Multivariate HR (95% CI)	P
Age, years								
<65	1	0.211	1	0.348	1	0.259	1	0.306
≥65	0.78 (0.53–1.15)		0.83 (0.56–1.23)		0.55 (0.19–1.56)		0.58 (0.2–1.65)	
Gender								
Male	1	0.246	1	0.371	1	0.550	1	0.530
Female	1.22 (0.87–1.71)		1.17 (0.83–1.64)		0.80 (0.38–1.68)		0.79 (0.37–1.67)	
Grade of differentiation								
Well/moderate	1	0.069	1	0.351	1	0.834	1	0.961
Poorly	1.42 (0.97–2.07)		1.20 (0.82–1.77)		0.81 (0.11–5.92)		1.05 (0.14–8.09)	
Mucinous adenocarcinoma								
No	1	0.437			1	0.996		
Yes	1.24 (0.72–2.15)				0 (0-Inf)			
Location from anal verge, cm								
≥5	1	0.398			1	0.271		
<5	1.14 (0.84–1.54)				1.49 (0.73–3.03)			
Clinical T stage								
T2–3	1	0.271	1	0.156	1	0.177	1	0.168
T4	1.05 (0.96–1.14)		1.06 (0.98–1.16)		0.78 (0.55–1.12)		0.78 (0.54–1.11)	
Clinical N stage								
N0	1	0.010	1	0.050	1	0.240	1	0.046
N1–2	1.77 (1.15–2.72)		1.56 (1.00–2.42)		0.65 (0.32–1.33)		0.47 (0.22–0.99)	
Neoadjuvant therapy pattern								
Neoadjuvant chemotherapy alone	1	0.156	1	0.429	1	0.373	1	0.431
Fluorouracil based-radiotherapy	0.8 (0.59–1.09)		0.87 (0.62–1.22)		1.38 (0.68–2.81)		1.34 (0.64–2.81)	
Postoperative chemotherapy								
No	1	0.251	1	0.527	1	0.068	1	0.039
Yes	1.39 (0.79–2.45)		1.21 (0.68–2.15)		6.39(0.87–46.81)		8.31 (1.12–61.84)	
ypT stage								
T0–2	1	0.911	1	0.350				
T3–4	1.03 (0.57–1.86)		1.37 (0.71–2.65)					
ypN stage								
N0	1	<0.001	1	<0.001				
N1–2	1.99 (1.47–2.69)		1.92 (1.38–2.66)					
pCR								
No					1	0.396	1	0.542
Yes					1.36 (0.67–2.76)		1.28 (0.58–2.80)	
TRG								
0–1	1	0.169	1	0.211	1	0.066	1	0.187
2–3	1.14 (0.95–1.36)		1.14 (0.93–1.40)		0.66 (0.42–1.03)		0.72 (0.44–1.17)	
MMR status								
pMMR	1	0.037		0.020	1	0.322	1	0.294
dMMR	0.42 (0.19–0.95)		0.38 (0.17–0.86)		0.37 (0.05–2.68)		0.34 (0.05–2.55)	

DFS, disease-free survival; HR, hazards ratio; CI, confidence interval; pCR, pathologic complete response; TRG, tumor regression grade; MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

Table S5 Univariate and multivariate Cox proportional hazards regression models for DFS in chemotherapy and chemoradiation subgroup patients

Variables	Chemotherapy				Chemoradiation			
	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Age, years								
<65	1	0.996	1	0.998	1	0.574	1	0.789
≥65	0 (0–Inf)		0 (0–Inf)		1.29 (0.53–3.14)		1.15 (0.41–3.18)	
Gender								
Male	1	0.702	1	0.662	1	0.665	1	0.853
Female	0.87 (0.42–1.79)		0.85 (0.40–1.80)		0.83 (0.36–1.92)		0.92 (0.38–2.23)	
Grade of differentiation								
Well/moderate	1	0.128	1	0.464	1	0.083	1	0.298
Poorly	1.31 (0.92–1.86)		1.15 (0.70–1.67)		1.52 (0.95–2.45)		1.34 (0.77–2.31)	
Mucinous adenocarcinoma								
No	1	0.385			1	0.327		
Yes	1.44 (0.63–3.27)				2.05 (0.49–8.58)			
Location from anal verge, cm								
≥5	1	0.490			1	0.805		
<5	0.78 (0.39–1.58)				0.92 (0.46–1.82)			
Clinical T stage								
T2–3	1	0.217	1	0.324	1	0.290	1	0.981
T4	1.11 (0.94–1.32)		1.09 (0.91–1.31)		1.10 (0.92–1.31)		1.00 (0.80–1.24)	
Clinical N stage								
N0	1	0.555	1	0.824	1	0.037	1	0.199
N1–2	1.24 (0.60–2.56)		0.92 (0.42–1.99)		2.76 (1.06–7.14)		2.00 (0.70–5.73)	
Neoadjuvant therapy pattern								
Neoadjuvant chemotherapy alone								
Fluorouracil based-radiotherapy								
Postoperative chemotherapy								
No	1	0.182	1	0.410	1	0.057	1	0.133
Yes	3.87 (0.53–28.22)		2.34 (0.31–17.73)		4.02 (0.96–16.84)		3.15 (0.70–14.04)	
ypT stage								
T0–2	1	0.002	1	0.082	1	0.006	1	0.180
T3–4	1.45 (1.14–1.83)		1.3 (0.97–1.74)		1.28 (1.07–1.52)		1.18 (0.93–1.52)	
ypN stage								
N0	1	0.008	1	0.174	1	0.002	1	0.065
N1–2	1.54 (1.12–2.13)		1.28 (0.90–1.84)		1.77 (1.24–2.52)		1.47 (0.98–2.21)	
pCR								
No	1	0.356	1	0.795	1	0.059	1	0.424
Yes	0.39 (0.05–2.86)		1.35 (0.14–13.05)		0.32 (0.10–1.05)		0.57 (0.15–2.24)	
TRG								
0–1	1	0.200	1	0.634	1	0.825	1	0.317
2–3	1.31 (0.87–1.97)		1.12 (0.69–1.82)		1.04 (0.73–1.47)		0.80 (0.51–1.25)	
MMR status								
pMMR	1	0.997	1	0.997	1	0.987	1	0.498
dMMR	0 (0–Inf)		0 (0–Inf)		0.99 (0.43–2.29)		0.73 (0.29–1.83)	

DFS, disease-free survival; HR, hazards ratio; CI, confidence interval; pCR, pathologic complete response; TRG, tumor regression grade; MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.