SUPPLEMENTARY DATA

A common variant in *MTHFR* influences response to chemoradiotherapy and recurrence of rectal cancer

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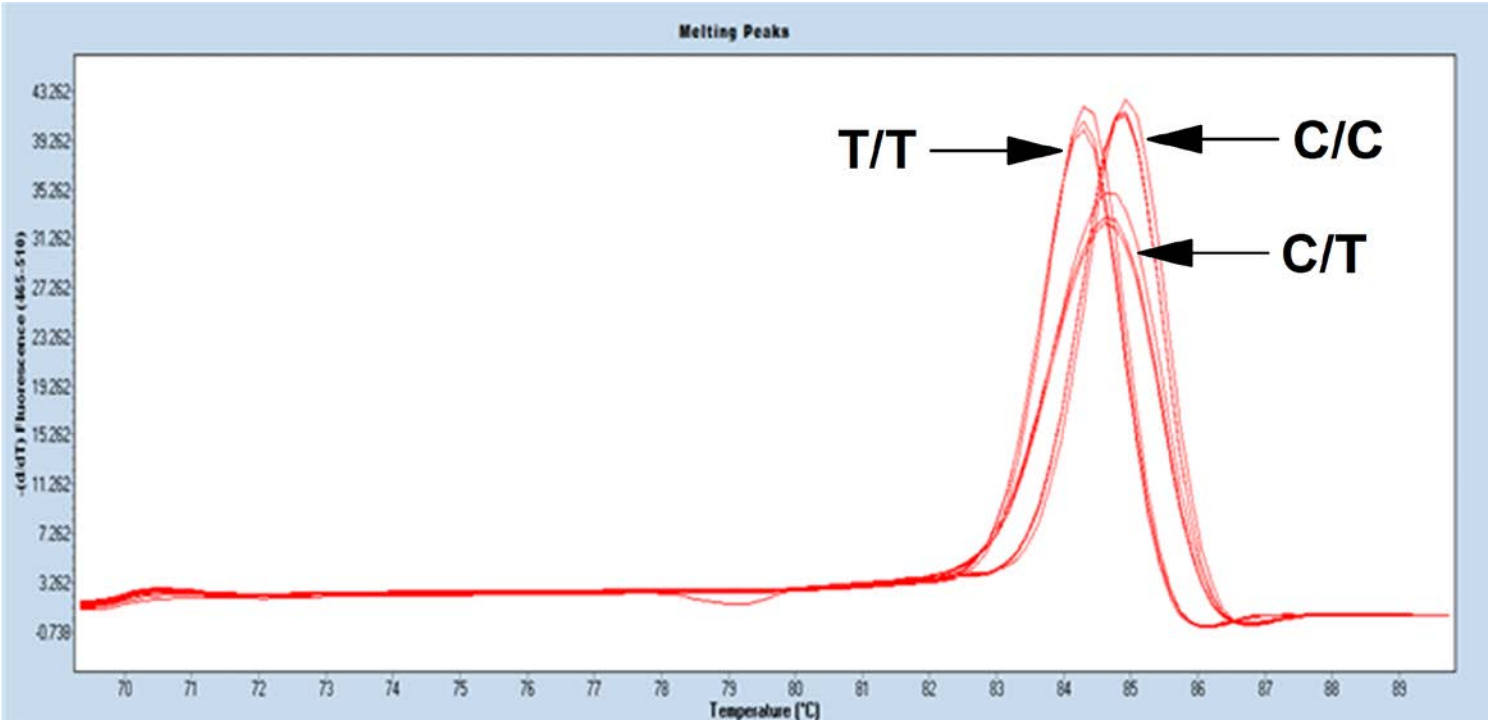
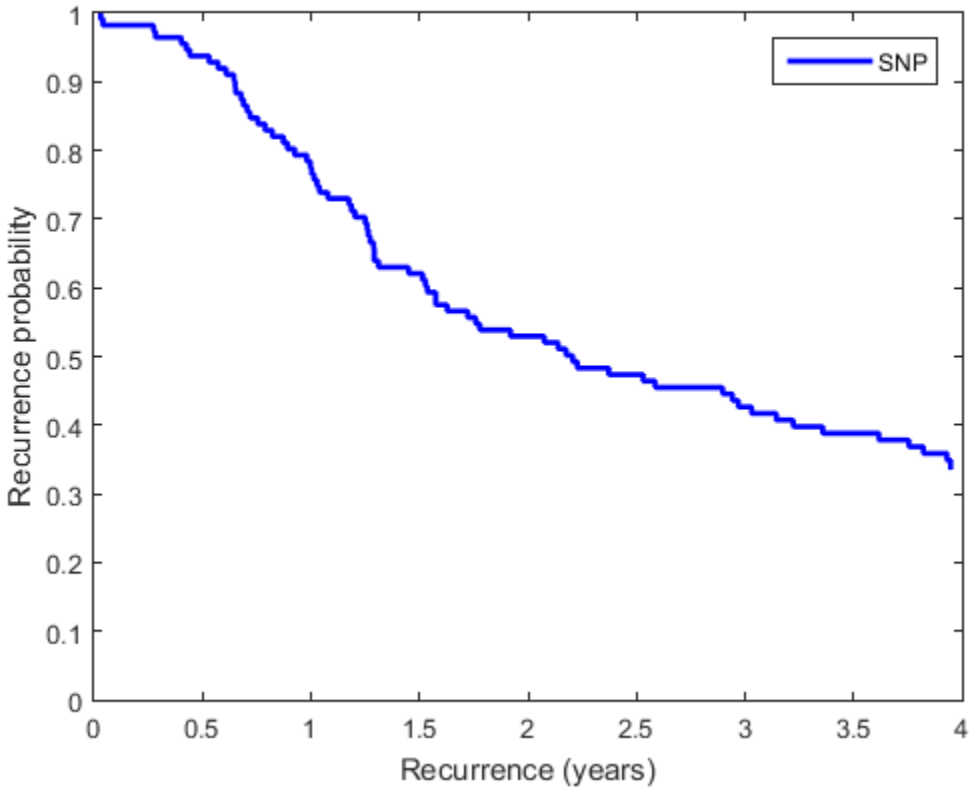
Supplementary Figures 1-2 and Supplementary Tables 1-4



Supplementary Figure 1. Melting peak profiles and sequence reactions for the three genotypes in a C>T transition. Based on nearest-neighbor thermodynamics, the homozygous for the mutation (T/T) melting peak appears at a lower temperature than (to the left of) the one of the homozygous for the wild type (C/C); whereas the heterozygous (C/T) melting peak appears at a temperature between those of the previous two and is of a smaller magnitude. Triplicate samples were used for each curve, demonstrating thus the reproducibility of the high-resolution melting method.

a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Recurrence probability
Recurrence (years)
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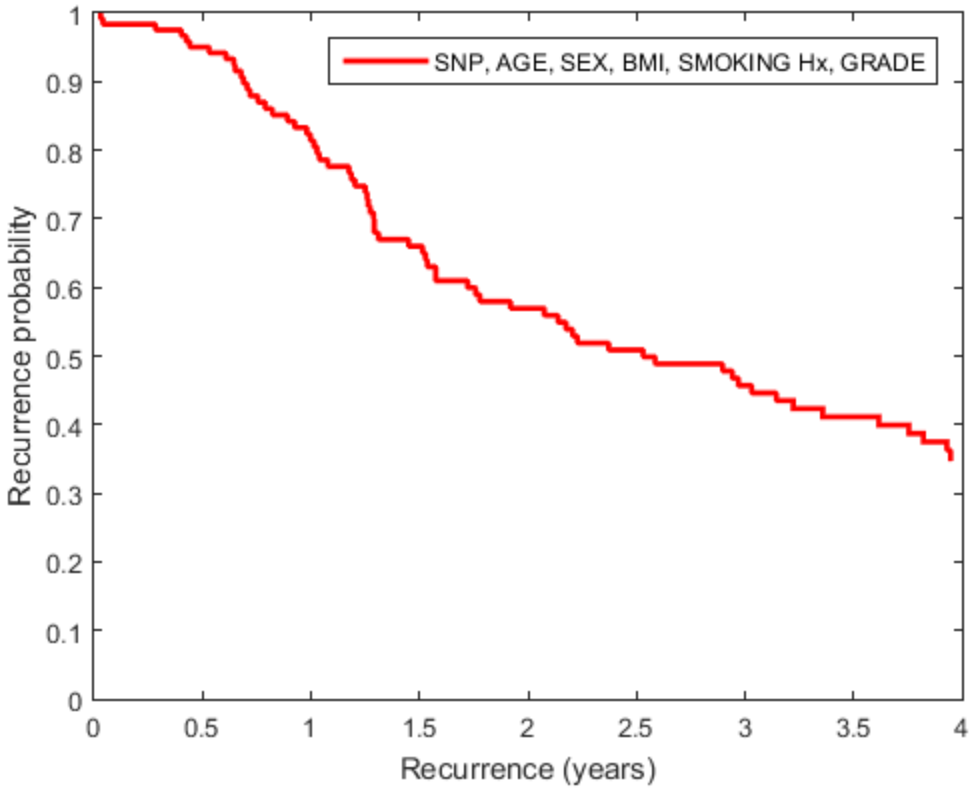
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.5
1
1.5
2
2.5
3
3.5
4

b


SNP, AGE, SEX, BMI, SMOKING Hx, GRADE
Recurrence probability
Recurrence (years)
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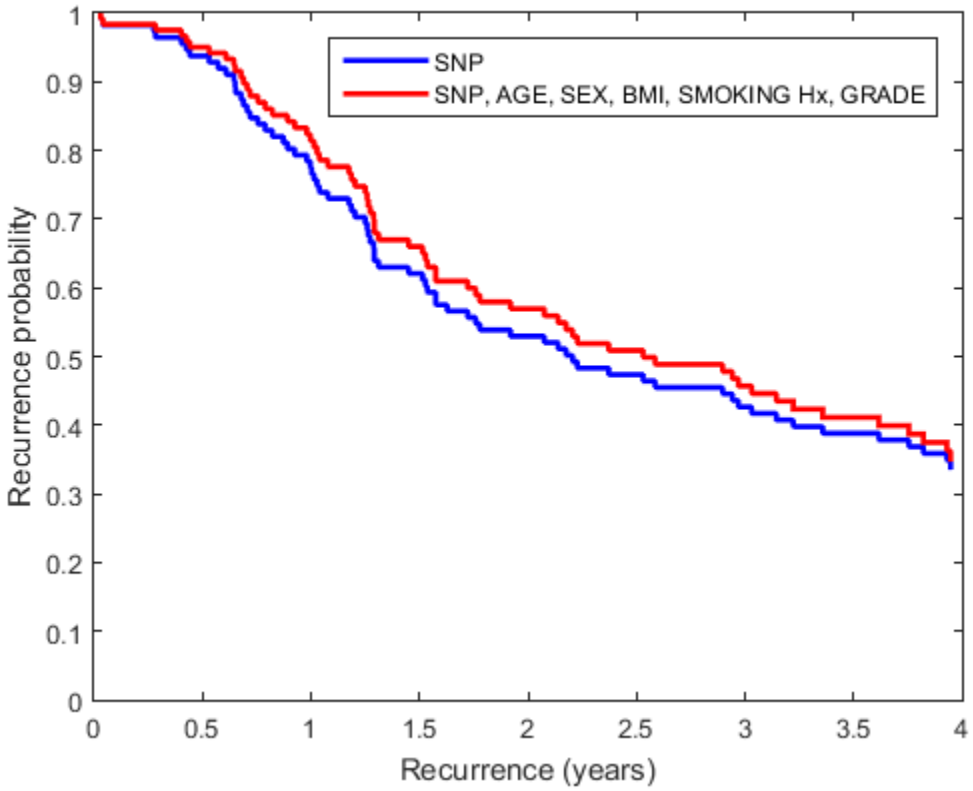
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.5
1
1.5
2
2.5
3
3.5
4

c



Supplementary Figure 2. Cox proportional hazards regression analysis in connection with the recurrence of rectal cancer study. (**a**) The graph (recurrence probability vs. recurrence) of the *MTHFR* SNP (single nucleotide polymorphism) variable by itself (Model A) (**Supplementary Table 4**) is shown. (**b**) The graph (recurrence probability vs. recurrence) of the *MTHFR* SNP variable and the covariates age, sex, BMI (body mass index), smoking history, and tumor grade (Model B) (**Supplementary Table 4**) is shown. (**c**) The overlaid graph of Model A (blue solid line) and Model B (red solid line) is shown for comparison purposes. The log-likelihood of the Model A was -302.5808, whereas the log-likelihood of the Model B was -265.9203 ($P = 2.09 \times 10^{-14}$), indicating that the Model B constitutes a statistically significant improvement over the Model A.

Supplementary Table 1. Demographical, clinical, and genotyping information about all subjects used in the study on the response to neoadjuvant chemoradiotherapy (CRT).

Subject No	Subject ID	Response	TRG	Sex	Age (yrs)	BMI	Grade	Smoking Hx	Genotype
1	10004552	RS	0	M	65.2	26.54	3	N	A
2	10004558	RS	0	F	71.4	37.55	3	N	A
3	10004575	RS	0	M	59.6	28.50	3	F	A
4	10004584	RS	0	F	69.8	21.37	3	N	A
5	10004593	RS	0	F	35.7	38.01	3	F	H
6	10004610	RS	0	M	62.5	27.28	3	N	H
7	10004612	RS	0	F	65.6	38.46	3	F	H
8	10004617	RS	0	F	56.3	23.81	3	N	A
9	10004630	RS	0	F	61.8	53.11	2	N	A
10	10004633	RS	0	M	60.3	29.76	2	F	A
11	10004637	RS	0	M	63.9	35.53	3	F	A
12	10004657	RS	0	M	43.4	23.94	3	N	A
13	10004662	RS	0	M	52.7	28.53	3	N	A
14	10004665	RS	0	M	55.5	32.13	3	N	A
15	10004666	RS	0	F	54.4	27.35	3	F	A
16	10004672	RS	0	F	68.8	20.43	2	F	A
17	10004677	RS	0	M	69.5	26.94	3	F	A
18	10004682	RS	0	M	52.7	28.53	3	N	H
19	10004695	RS	0	M	71.0	32.32	3	F	A
20	10004701	RS	0	M	63.5	28.01	2	N	H
21	10004709	RS	0	F	50.8	20.82	3	F	A
22	10004715	RS	0	M	45.9	43.92	3	N	A
23	10004727	RS	0	F	50.0	18.54	2	C	H
24	10004729	RS	0	M	77.4	33.63	2	F	H
25	10004734	RS	0	F	72.1	27.47	3	F	A
26	10004740	RS	0	M	48.7	21.39	3	C	A
27	10004751	RS	0	F	51.3	28.34	3	F	A
28	10004758	RS	0	M	53.0	50.61	3	N	M
29	10004764	RS	0	F	68.6	24.57	3	C	H
30	10004765	RS	0	M	37.3	34.07	2	N	A
31	10004768	RS	0	M	47.4	25.68	3	N	A
32	10004774	RS	0	M	47.9	25.77	2	F	A
33	10004816	RS	0	F	46.8	21.88	2	N	A
34	10004826	RS	0	F	58.6	33.06	4	N	A
35	10004834	RS	0	M	63.5	37.45	2	F	A
36	10004838	RS	0	M	56.8	27.51	2	unknown	H

37	10004554	NRS	3	M	63.7	29.32	3	C	H
38	10004562	NRS	3	M	54.8	53.45	3	N	H
39	10004568	NRS	3	M	70.1	27.95	3	F	A
40	10004573	NRS	3	M	58.7	24.77	4	N	M
41	10004582	NRS	3	M	50.3	34.15	3	F	A
42	10004586	NRS	3	M	54.8	53.45	3	N	H
43	10004587	NRS	3	M	66.0	34.29	3	F	M
44	10004599	NRS	3	M	68.4	26.17	3	C	H
45	10004600	NRS	3	M	44.4	19.52	3	C	H
46	10004606	NRS	3	M	59.2	21.08	3	C	A
47	10004618	NRS	3	M	69.8	33.63	3	F	H
48	10004620	NRS	3	M	68.0	25.25	4	C	H
49	10004623	NRS	3	M	71.3	26.58	3	N	A
50	10004627	NRS	3	M	76.3	26.42	3	F	H
51	10004631	NRS	3	M	61.7	26.42	3	N	A
52	10004632	NRS	3	M	55.3	30.28	3	C	A
53	10004638	NRS	3	M	60.5	29.99	3	F	A
54	10004640	NRS	3	F	71.1	27.22	3	N	A
55	10004641	NRS	3	M	45.1	23.76	3	F	A
56	10004642	NRS	3	M	57.8	32.15	3	F	A
57	10004649	NRS	3	M	74.1	35.63	4	F	M
58	10004652	NRS	3	M	64.7	26.73	3	N	A
59	10004653	NRS	3	M	59.3	24.19	3	F	H
60	10004654	NRS	3	M	65.5	31.08	3	N	A
61	10004659	NRS	3	M	67.4	30.33	3	F	A
62	10004660	NRS	3	M	75.4	39.39	4	N	A
63	10004670	NRS	3	M	48.3	31.92	3	F	M
64	10004678	NRS	3	M	53.7	28.20	3	N	A
65	10004679	NRS	3	M	73.0	25.00	2	F	H
66	10004686	NRS	3	M	66.1	25.73	3	N	A
67	10004689	NRS	3	M	71.7	26.67	3	N	M
68	10004692	NRS	3	M	63.9	24.65	3	C	A
69	10004697	NRS	3	M	55.7	25.38	3	F	A
70	10004700	NRS	3	M	31.8	22.20	3	N	A
71	10004703	NRS	3	M	54.9	31.51	3	F	M
72	10004716	NRS	3	F	55.2	23.27	3	F	A
73	10004717	NRS	3	M	71.5	23.75	3	N	A
74	10004721	NRS	3	M	58.4	28.76	3	N	M
75	10004723	NRS	3	M	65.4	31.62	3	N	A
76	10004725	NRS	3	F	65.1	23.19	3	F	A
77	10004730	NRS	3	M	44.9	27.19	3	F	M

78	10004741	NRS	3	M	59.7	26.85	3	N	A
79	10004742	NRS	3	M	77.4	22.08	4	C	A
80	10004749	NRS	3	F	59.5	43.28	3	N	A
81	10004750	NRS	3	F	64.4	23.70	3	F	H
82	10004754	NRS	3	M	56.2	38.31	3	N	A
83	10004790	NRS	3	M	59.0	34.57	3	C	H
84	10004792	NRS	3	F	34.3	21.57	2	N	M
85	10004798	NRS	3	M	39.6	33.02	3	N	H
86	10004800	NRS	3	M	46.8	31.07	3	N	H
87	10004802	NRS	3	F	43.2	13.26	3	C	M
88	10004803	NRS	3	M	38.5	25.98	3	C	H
89	10004805	NRS	3	F	42.0	21.12	3	F	H
90	10004808	NRS	3	F	60.8	20.29	3	C	M
91	10004819	NRS	3	M	47.0	28.64	3	N	H
92	10004822	NRS	3	M	50.0	22.42	3	C	A
93	10004823	NRS	3	M	47.2	26.26	3	C	A
94	10004825	NRS	3	F	34.8	22.26	3	N	H
95	10004830	NRS	3	F	44.7	23.81	3	C	H
96	10004831	NRS	3	M	50.8	34.75	3	N	H
97	10004835	NRS	3	M	57.2	26.90	3	F	A
98	10005037	NRS	3	M	58.0	26.15	2	F	A
99	10005044	NRS	3	M	73.4	22.27	3	N	H
100	10005065	NRS	3	F	61.7	25.82	3	N	H
101	10005069	NRS	3	M	59.6	29.40	3	F	H
102	10005070	NRS	3	M	62.4	38.63	3	F	H
103	10005081	NRS	3	F	48.4	39.91	3	N	H
104	10005087	NRS	3	M	53.3	27.29	3	N	A
105	10005094	NRS	3	M	46.6	30.35	3	C	H
106	10005096	NRS	3	M	60.1	31.24	3	N	A
107	10005098	NRS	3	M	44.4	20.18	3	N	H
108	10005109	NRS	3	M	60.8	23.21	unknown	F	A

The response to neoadjuvant CRT [response (RS) vs. no-response (NRS)], the tumor regression grading (TRG), the sex [(F): female; (M): male], the age in years at the time of surgery, the BMI (body mass index), the tumor grade, the smoking history [(C): current smoker; (F): former smoker; (N): never smoked], and the genotype [(A=C/C): homozygous ancestral (wild type); (H=C/T): heterozygous; (M=T/T): homozygous for the mutation] of all subjects are shown. In this study, there were 36 subjects (# 1-36) who responded to neoadjuvant CRT and experienced pathological complete recovery (RS) (TRG=0), and 72 subjects (# 37-108) who experienced no response or very poor response (NRS) (TRG=3).

Supplementary Table 2. Demographical, clinical, and genotyping information about all subjects used in the study on the recurrence of rectal cancer.

Subject No	Subject ID	Recurrence	Recurrence Time (yrs)	Sex	Age (yrs)	BMI	Grade	Smoking Hx	Genotype
1	10004552	NRC	n/a	M	65.2	26.54	3	N	A
2	10004558	NRC	n/a	F	71.4	37.55	3	N	A
3	10004575	NRC	n/a	M	59.6	28.50	3	F	A
4	10004584	NRC	n/a	F	69.8	21.37	3	N	A
5	10004593	NRC	n/a	F	35.7	38.01	3	F	H
6	10004610	NRC	n/a	M	62.5	27.28	3	N	H
7	10004612	NRC	n/a	F	65.6	38.46	3	F	H
8	10004617	NRC	n/a	F	56.3	23.81	3	N	A
9	10004630	NRC	n/a	F	61.8	53.11	2	N	A
10	10004633	NRC	n/a	M	60.3	29.76	2	F	A
11	10004637	NRC	n/a	M	63.9	35.53	3	F	A
12	10004657	NRC	n/a	M	43.4	23.94	3	N	A
13	10004662	NRC	n/a	M	52.7	28.53	3	N	A
14	10004665	NRC	n/a	M	55.5	32.13	3	N	A
15	10004666	NRC	n/a	F	54.4	27.35	3	F	A
16	10004672	NRC	n/a	F	68.8	20.43	2	F	A
17	10004677	NRC	n/a	M	69.5	26.94	3	F	A
18	10004682	NRC	n/a	M	52.7	28.53	3	N	H
19	10004695	NRC	n/a	M	71.0	32.32	3	F	A
20	10004701	NRC	n/a	M	63.5	28.01	2	N	H
21	10004709	NRC	n/a	F	50.8	20.82	3	F	A
22	10004715	NRC	n/a	M	45.9	43.92	3	N	A
23	10004727	NRC	n/a	F	50.0	18.54	2	C	H
24	10004729	NRC	n/a	M	77.4	33.63	2	F	H
25	10004734	NRC	n/a	F	72.1	27.47	3	F	A
26	10004740	NRC	n/a	M	48.7	21.39	3	C	A
27	10004751	NRC	n/a	F	51.3	28.34	3	F	A
28	10004758	NRC	n/a	M	53.0	50.61	3	N	M
29	10004764	NRC	n/a	F	68.6	24.57	3	C	H
30	10004765	NRC	n/a	M	37.3	34.07	2	N	A
31	10004768	NRC	n/a	M	47.4	25.68	3	N	A
32	10004774	NRC	n/a	M	47.9	25.77	2	F	A
33	10004816	NRC	n/a	F	46.8	21.88	2	N	A
34	10004826	NRC	n/a	F	58.6	33.06	4	N	A
35	10004834	NRC	n/a	M	63.5	37.45	2	F	A
36	10004838	NRC	n/a	M	56.8	27.51	2	unknown	H

37	10004544	RC	2.08	F	72.0	32.89	3	F	M
38	10004549	RC	1.18	M	65.3	25.21	2	F	H
39	10004564	RC	1.20	M	63.5	25.98	3	F	H
40	10004568	RC	1.72	M	70.1	27.95	3	F	A
41	10004569	RC	2.18	M	83.8	26.78	3	N	H
42	10004578	RC	3.22	F	38.4	25.29	3	C	H
43	10004587	RC	0.43	M	66.0	34.29	3	F	M
44	10004597	RC	3.36	F	61.4	32.83	3	N	H
45	10004598	RC	1.92	M	44.5	29.07	3	N	H
46	10004599	RC	2.94	M	68.4	26.17	3	C	H
47	10004606	RC	2.59	M	59.2	21.08	3	C	A
48	10004616	RC	0.68	M	47.6	28.60	3	F	A
49	10004634	RC	0.03	F	71.1	23.25	3	C	H
50	10004638	RC	1.26	M	60.5	29.99	3	F	A
51	10004641	RC	1.01	M	45.1	23.76	3	F	A
52	10004645	RC	0.82	F	64.8	24.82	3	F	A
53	10004656	RC	1.29	M	54.7	30.42	3	F	H
54	10004674	RC	1.29	M	54.7	30.42	3	F	A
55	10004681	RC	3.82	F	67.9	31.19	3	N	H
56	10004690	RC	2.37	F	76.2	29.07	2	N	A
57	10004692	RC	0.93	M	63.9	24.65	3	C	A
58	10004703	RC	3.03	M	54.9	31.51	3	F	M
59	10004712	RC	0.71	M	39.9	25.93	3	N	A
60	10004723	RC	1.76	M	65.4	31.62	3	N	A
61	10004731	RC	2.21	M	49.7	36.91	3	F	A
62	10004736	RC	1.45	M	60.7	22.96	3	F	A
63	10004739	RC	1.53	M	52.7	35.83	4	F	H
64	10004750	RC	2.23	F	64.4	23.70	3	F	H
65	10004782	RC	2.97	M	41.9	25.77	4	N	A
66	10004787	RC	2.53	M	47.4	22.17	3	N	H
67	10004788	RC	1.27	F	59.3	24.94	3	N	A
68	10004793	RC	2.14	F	49.4	27.09	3	N	H
69	10004797	RC	0.61	M	24.9	24.24	3	N	A
70	10004798	RC	0.98	M	39.6	33.02	3	N	H
71	10004804	RC	0.53	M	21.8	23.54	3	F	H
72	10004805	RC	3.93	F	42.0	21.12	3	F	H
73	10004811	RC	0.64	M	41.4	31.39	3	F	A
74	10004813	RC	1.54	F	40.8	18.12	3	N	H
75	10004817	RC	1.19	M	60.8	27.47	3	N	A
76	10004818	RC	1.31	M	43.3	29.89	2	F	A
77	10004829	RC	0.44	F	46.5	42.66	2	N	A

78	10004831	RC	1.04	M	50.8	34.75	3	N	H
79	10004833	RC	1.26	F	56.1	22.62	3	F	M
80	10004835	RC	0.72	M	57.2	26.90	3	F	A
81	10004836	RC	0.87	M	55.8	28.21	unknown	F	H
82	10004837	RC	3.76	M	67.4	26.58	2	N	H
83	10004840	RC	0.04	F	50.3	21.79	3	F	M
84	10004841	RC	3.95	M	50.1	36.95	2	C	A
85	10005039	RC	0.76	M	64.0	25.07	3	F	M
86	10005043	RC	0.28	M	60.8	26.77	2	N	H
87	10005049	RC	1.63	M	43.0	27.14	unknown	C	M
88	10005050	RC	1.25	M	55.1	35.10	3	N	H
89	10005051	RC	2.90	M	56.3	32.23	3	F	M
90	10005052	RC	1.51	M	55.1	20.23	3	F	A
91	10005053	RC	0.65	F	74.3	25.00	unknown	N	A
92	10005054	RC	1.78	F	52.6	38.53	3	N	H
93	10005056	RC	0.69	M	53.6	25.25	3	C	H
94	10005062	RC	1.29	F	30.5	23.01	2	F	M
95	10005065	RC	0.79	F	61.7	25.82	3	N	H
96	10005072	RC	1.00	M	45.0	26.79	3	F	A
97	10005074	RC	1.03	M	63.4	31.05	2	F	H
98	10005075	RC	3.14	M	54.2	23.57	4	F	H
99	10005076	RC	0.89	M	40.6	31.11	3	C	A
100	10005086	RC	0.57	F	47.0	16.98	unknown	F	H
101	10005093	RC	1.08	F	41.1	18.14	3	N	A
102	10005094	RC	0.40	M	46.6	30.35	3	C	H
103	10005098	RC	0.65	M	44.4	20.18	3	N	H
104	10005101	RC	1.00	F	49.5	24.71	unknown	F	A
105	10005102	RC	0.28	F	57.1	18.06	unknown	F	A
106	10005105	RC	1.58	F	39.3	22.04	3	N	A
107	10005112	RC	1.58	F	51.8	28.07	3	F	A
108	10005113	RC	3.62	F	23.0	23.73	3	N	A

The recurrence of rectal cancer [no-recurrence (NRC) vs. recurrence (RC)], the time of recurrence (measured in years from the date of surgery), the sex [(F): female; (M): male], the age in years at the time of surgery, the BMI (body mass index), the tumor grade, the smoking history [(C): current smoker; (F): former smoker; (N): never smoked], and the genotype [(A=C/C): homozygous ancestral (wild type); (H=C/T): heterozygous; (M=T/T): homozygous for the mutation] of all subjects are shown. In this study, there were 36 subjects (# 1-36) who did not experience recurrence of rectal cancer (NRC) within four years following surgery, and 72 subjects (# 37-108) who did experience recurrence of the disease (RC) within four years following surgery. (n/a): not applicable.

Supplementary Table 3. Logistic regression analysis results in connection with the study on the response to neoadjuvant chemoradiotherapy (CRT).

Variables	β coefficient	SE of β	P	OR	OR 95% CI (LL)	OR 95% CI (UL)	Notes
Y-intercept (β_0)	3.354650	2.538627	0.18635	28.6356	0.1977	4147.9039	NSS
***MTHFR* SNP**	**-1.590711**	**0.562650**	**0.00470**	**0.2038**	**0.0676**	**0.6139**	**SS**
AGE	0.003969	0.024009	0.86871	1.0040	0.9578	1.0524	NSS
SEX	**1.644917**	**0.567131**	**0.00373**	**5.1806**	**1.7046**	**15.7445**	**SS**
BMI	0.070987	0.038125	0.06261	1.0736	0.9963	1.1569	NSS
SMOKING Hx	-0.113737	0.370031	0.75856	0.8925	0.4321	1.8432	NSS
GRADE	**-2.120554**	**0.687796**	**0.00205**	**0.1200**	**0.0312**	**0.4619**	**SS**

The variables, including the y-intercept (β_0), the logistic regression coefficient (β), the standard error of the β coefficient, the probability of significance (P), the odds ratio (OR), and the 95% confidence interval [(LL): lower limit and (UL): upper limit] of the logistic regression analysis are shown. The analysis corroborated the statistical significance of the *MTHFR* SNP (single nucleotide polymorphism) variable when it was considered together with the covariates of age, sex, BMI (body mass index), smoking history, and tumor grade (P=0.00470). Furthermore, the analysis revealed that the covariates sex and tumor grade also had a statistically significant effect (P=0.00373 and P=0.00205, respectively). According to the analysis, patients with either the heterozygous (C/T) or the homozygous for the mutation (T/T) genotype were 0.2038 times less likely to respond to CRT than patients with the wild type (C/C) genotype; female patients were 5.1806 times more likely to respond to CRT [(15/28) = 53.57% of female patients were RS] than male patients [(21/80) = 26.25% of male patients were RS]; and patients with either tumor grade 3 [(24/87)=27.59% of grade 3 patients were RS] or tumor grade 4 [(1/6)=16.67% of grade 4 patients were RS] were 0.1200 times less likely to respond to CRT than patients with tumor grade 2 [(11/14)=78.57% of grade 2 patients were RS]. The covariates age, BMI, and smoking history had no statistically significant effect.

Supplementary Table 4. Cox proportional hazards regression analysis in connection with the study on the recurrence of rectal cancer.

Model	Variables	β coefficient	SE of β	P	HR	HR 95% CI (LL)	HR 95% CI (UL)	Notes
A	*MTHFR* SNP	0.547099	0.238120	0.02159	1.7282	1.0837	2.7561	SS
B	*MTHFR* SNP	0.721978	0.254390	0.00454	2.0585	1.2503	3.3892	SS
	AGE	-0.024709	0.011393	0.03010	0.9756	0.9541	0.9976	SS
	SEX	-0.589028	0.278827	0.03464	0.5549	0.3213	0.9584	SS
	BMI	-0.037424	0.021760	0.08546	0.9633	0.9230	1.0052	NSS
	SMOKING Hx	0.165081	0.178093	0.35396	1.1795	0.8320	1.6722	NSS
	GRADE	0.261660	0.253899	0.30274	1.2991	0.7898	2.1368	NSS

The particular model and its constituent variables, the Cox regression coefficient (β), the standard error of the β coefficient, the probability of significance (P), the hazard ratio (HR), and the 95% confidence interval [(LL): lower limit and (UL): upper limit] are shown. The Cox proportional hazards regression analysis corroborated the statistical significance of the *MTHFR* SNP (single nucleotide polymorphism) both in the case where it was the only variable (Model A, P=0.02159) and in the case where it was considered together with the covariates of age, sex, BMI (body mass index), smoking history, and tumor grade (Model B, P=0.00454). Furthermore, the Cox proportional hazards regression analysis revealed that two covariates, namely, age and sex also had a statistically significant – albeit much smaller – effect (P=0.03010 and P=0.03464, respectively). According to the combined model (Model B), patients with either the heterozygous (C/T) or the homozygous for the mutation (T/T) genotype were 2.0585 times more likely to experience recurrence of rectal cancer than patients with the wild type (C/C) genotype; older patients were 0.9756 times less likely to experience recurrence of the disease (mean age of the NRC group was 57.7 yrs) than younger patients (mean age of the RC group was 53.4 yrs); and female patients were 0.5549 times less likely to experience recurrence of the disease [(26/41) = 63.41% of female patients were RC] than male patients [(46/67) = 68.66% of male patients were RC]. The covariates BMI, smoking history, and tumor grade had no statistically significant effect. The log-likelihood of the Model A was -302.5808, whereas the log-likelihood of the Model B was -265.9203 ($P=2.09 \times 10^{-14}$) (**Supplementary Figure 2**). (SS): statistically significant. (NSS): not statistically significant.