Supplementary online content

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Table S1. Baseline characteristics of the patient cohort according to tumor stages

Baseline Characteristics	Overall *n* (%)	Stage I *n* (%)	Stage II *n* (%)	Stage III *n* (%)	Stage IV *n* (%)	*P*#
Sex						
Women	910 (41.2)	151 (37.8)	315 (41.5)	321 (44.2)	118 (37.2)	0.209
Men	1296 (58.8)	249 (62.3)	444 (58.5)	405 (55.8)	191 (61.8)	
Age at diagnosis						
33 -< 65 years	717 (32.5)	122 (30.5)	215 (28.3)	260 (35.8)	116 (37.5)	0.015
65 -< 75 years	776 (35.2)	146 (36.5)	269 (35.4)	243 (33.5)	113 (36.6)	
75 -< 96 years	713 (32.3)	132 (33.0)	275 (36.2)	223 (30.7)	80 (25.9)	
BMI at diagnosis*						
< 25 kg/m^2	834 (38.0)	127 (31.9)	283 (37.5)	277 (38.2)	141 (45.9)	0.029
25-30 kg/m^2	932 (42.4)	180 (45.2)	322 (42.7)	308 (42.5)	118 (38.4)	
> 30 kg/m^2	430 (19.6)	91 (22.9)	150 (19.9)	140 (19.3)	48 (15.6)	
Smoking status*						
Never	907 (41.1)	145 (36.3)	313 (41.3)	319 (44.0)	125 (40.5)	< 0.001
Former	947 (43.0)	203 (50.8)	313 (41.3)	311 (42.9)	118 (38.2)	
Current	350 (15.9)	52 (13.0)	132 (17.4)	95 (13.1)	66 (21.4)	
Charlson comorbidity index						
0 (no comorbidity)	1281 (58.1)	233 (58.3)	402 (53.0)	439 (60.5)	200 (64.7)	0.026
1 (mild comorbidity)	479 (21.7)	80 (20.0)	182 (24.0)	155 (21.4)	59 (19.1)	
2+ (moderate comorbidity)	446 (20.2)	87 (21.8)	175 (23.1)	132 (18.2)	50 (16.2)	
Tumor sub-site*						
Distal colon †	738 (33.5)	123 (30.8)	277 (36.5)	223 (30.8)	114 (37.0)	< 0.001
Proximal colon ‡	796 (36.1)	127 (31.8)	314 (41.4)	254 (35.0)	101 (32.8)	
Rectum	669 (30.4)	150 (37.5)	167 (22.0)	248 (34.2)	93 (30.2)	

Note: * 12 missing values for tumor stage, 10 missing values for body mass index, 2 missing values for smoking status and 3 missing values for tumor subsite. Complete case analysis was applied when adjusting for the variables with missing values. † The distal colon includes the descending colon and the sigmoid colon. ‡ The proximal colon includes the cecum, the ascending colon, the right flexure, and the transverse colon and the left flexure. # *P* values were derived from Chi square test of difference in distributions of categorical variables between stages.

Table S2. Median and interquartile range of leukocyte proportion by characteristics of the study population at baseline[#]

Baseline Characteristics	CD4+ T cell	CD8+ T cell	B cell	NK cell	Monocyte	Neutrophil
Sex						
Women	**0.13 (0.09, 0.18)**	**0.07 (0.04, 0.10)**	**0.04 (0.03, 0.05)**	**0.05 (0.04, 0.08)**	**0.07 (0.06, 0.09)**	0.64 (0.54, 0.73)
Men	**0.11 (0.07, 0.16)**	**0.06 (0.04, 0.10)**	**0.03 (0.02, 0.04)**	**0.06 (0.04, 0.08)**	**0.08 (0.06, 0.10)**	0.65 (0.55, 0.74)
Age at diagnosis						
33 -< 65 year	**0.13 (0.09, 0.18)**	**0.07 (0.04, 0.10)**	**0.04 (0.03, 0.05)**	**0.06 (0.04, 0.08)**	**0.07 (0.06, 0.09)**	0.64 (0.54, 0.73)
65 -< 75 year	**0.12 (0.08, 0.17)**	**0.06 (0.04, 0.10)**	**0.04 (0.02, 0.05)**	**0.06 (0.04, 0.08)**	**0.07 (0.06, 0.09)**	0.64 (0.54, 0.73)
75 -< 96 year	**0.11 (0.07, 0.15)**	**0.06 (0.04, 0.10)**	**0.03 (0.02, 0.04)**	**0.06 (0.04, 0.08)**	**0.08 (0.06, 0.10)**	0.65 (0.56, 0.74)
Tumor stage						
I	**0.13 (0.09, 0.17)**	**0.07 (0.04, 0.10)**	**0.04 (0.03, 0.05)**	**0.06 (0.04, 0.10)**	**0.07 (0.06, 0.09)**	**0.62 (0.54, 0.71)**
II	**0.12 (0.08, 0.17)**	**0.06 (0.04, 0.10)**	**0.04 (0.03, 0.05)**	**0.06 (0.04, 0.08)**	**0.07 (0.06, 0.09)**	**0.64 (0.54, 0.73)**
III	**0.12 (0.07, 0.17)**	**0.06 (0.04, 0.10)**	**0.04 (0.02, 0.05)**	**0.06 (0.04, 0.08)**	**0.08 (0.06, 0.10)**	**0.64 (0.55, 0.74)**
IV	**0.11 (0.07, 0.15)**	**0.05 (0.03, 0.09)**	**0.03 (0.02, 0.04)**	**0.05 (0.04, 0.07)**	**0.07 (0.06, 0.09)**	**0.68 (0.56, 0.77)**
Tumor subsite						
Distal colon †	**0.13 (0.09, 0.18)**	**0.07 (0.04, 0.10)**	**0.04 (0.03, 0.05)**	**0.06 (0.04, 0.08)**	0.07 (0.06, 0.09)	**0.62 (0.53, 0.72)**
Proximal colon ‡	**0.13 (0.09, 0.17)**	**0.07 (0.04, 0.11)**	**0.04 (0.03, 0.05)**	**0.06 (0.04, 0.08)**	0.08 (0.06, 0.09)	**0.63 (0.53, 0.71)**
Rectum	**0.10 (0.06, 0.14)**	**0.05 (0.03, 0.09)**	**0.04 (0.02, 0.04)**	**0.05 (0.04, 0.08)**	0.07 (0.06, 0.09)	**0.69 (0.60, 0.77)**
Body mass index						
< 25 kg/m^2	0.12 (0.07, 0.17)	0.06 (0.04, 0.09)	**0.04 (0.02, 0.05)**	0.06 (0.04, 0.08)	0.08 (0.06, 0.10)	0.65 (0.55, 0.75)
25-30 kg/m^2	0.12 (0.08, 0.17)	0.07 (0.04, 0.10)	**0.03 (0.02, 0.05)**	0.06 (0.04, 0.08)	0.07 (0.06, 0.09)	0.64 (0.54, 0.73)
> 30 kg/m^2	0.12 (0.08, 0.17)	0.07 (0.04, 0.10)	**0.04 (0.03, 0.05)**	0.06 (0.04, 0.08)	0.07 (0.06, 0.09)	0.64 (0.54, 0.73)
Smoking status						
Never	0.12 (0.08, 0.17)	0.06 (0.04, 0.10)	**0.04 (0.03, 0.05)**	**0.06 (0.04, 0.08)**	**0.07 (0.06, 0.09)**	0.65 (0.55, 0.74)
Former	0.12 (0.08, 0.17)	0.06 (0.04, 0.10)	**0.03 (0.02, 0.05)**	**0.06 (0.04, 0.09)**	**0.08 (0.06, 0.10)**	0.64 (0.54, 0.73)
Current	0.12 (0.08, 0.18)	0.06 (0.04, 0.09)	**0.03 (0.02, 0.05)**	**0.05 (0.04, 0.07)**	**0.07 (0.06, 0.09)**	0.65 (0.55, 0.74)
Charlson Comorbidity Index						
0 (no comorbidity)	**0.13 (0.08, 0.17)**	**0.07 (0.04, 0.10)**	**0.04 (0.03, 0.05)**	0.06 (0.04, 0.08)	0.07 (0.06, 0.09)	**0.63 (0.54, 0.72)**
1 (mild comorbidity)	**0.12 (0.07, 0.16)**	**0.06 (0.04, 0.10)**	**0.04 (0.02, 0.05)**	0.06 (0.04, 0.08)	0.07 (0.06, 0.09)	**0.66 (0.56, 0.74)**
2+ (moderate comorbidity)	**0.10 (0.07, 0.15)**	**0.06 (0.03, 0.09)**	**0.03 (0.02, 0.04)**	0.06 (0.04, 0.08)	0.07 (0.06, 0.09)	**0.67 (0.56, 0.76)**

Note: # Wilcoxon-Mann-Whitney test was performed for comparing medians across groups of sex and tumor subsite. Jonckheere-Terpstra test was performed to determine the significance of the trend of ordinal variables. * 12 missing values for tumor stage, 10 missing values for body mass index, 2 missing values for smoking status and 3 missing values for tumor subsite. Complete case analysis was applied when adjusting for the variables with missing values. † The distal colon includes the descending colon and the sigmoid colon. ‡ The proximal colon includes the cecum, the ascending colon, the right flexure, and the transverse colon and the left flexure. Numbers printed in bold: Statistically significant difference or trend was observed ($P < 0.05$).

Table S3. Associations of leukocyte composition with all-cause and colorectal cancer (CRC)-specific mortality in total study population; Sensitivity analysis with additional adjustment for BMI, smoking status and CCI.

Leukocyte composition	Categories	All-cause mortality		CRC-specific mortality	
		n_{death}/n_{cases}	HR (95%CI) *	n_{death}/n_{cases}	HR (95%CI) *
CD4+T cell	Quartile 1	315/536	Ref.	190/532	Ref.
	Quartile 2	281/545	**0.86 (0.72, 1.02)**	144/538	**0.77 (0.61, 0.98)**
	Quartile 3	259/550	**0.76 (0.63, 0.92)**	143/550	**0.75 (0.59, 0.96)**
	Quartile 4	206/549	**0.63 (0.51, 0.77)**	113/546	**0.60 (0.46, 0.79)**
	Per SD increase	1061/2180	**0.84 (0.78, 0.91)**	590/2166	**0.83 (0.74, 0.92)**
CD8+T cell	Quartile 1	300/540	Ref.	173/534	Ref.
	Quartile 2	268/546	0.85 (0.71, 1.01)	140/543	0.77 (0.60, 0.98)
	Quartile 3	254/548	0.86 (0.71, 1.03)	148/545	0.90 (0.71, 1.14)
	Quartile 4	239/546	0.84 (0.69, 1.02)	129/544	0.87 (0.68, 1.12)
	Per SD increase	1061/2180	0.96 (0.90, 1.04)	590/2166	1.00 (0.91, 1.10)
B cell	Quartile 1	311/545	Ref.	183/543	Ref.
	Quartile 2	281/547	0.98 (0.82, 1.16)	150/543	0.95 (0.76, 1.20)
	Quartile 3	238/542	**0.69 (0.57, 0.84)**	128/539	**0.67 (0.52, 0.86)**
	Quartile 4	231/546	**0.70 (0.58, 0.85)**	129/541	**0.70 (0.55, 0.91)**
	Per SD increase	1061/2180	0.91 (0.82, 1.01)	590/2166	0.90 (0.78, 1.03)
NK cell	Quartile 1	278/545	Ref.	165/543	Ref.
	Quartile 2	277/545	1.05 (0.88, 1.25)	162/540	1.10 (0.87, 1.38)
	Quartile 3	247/544	0.93 (0.77, 1.13)	129/541	0.98 (0.76, 1.27)
	Quartile 4	259/546	0.92 (0.75, 1.12)	134/542	1.01 (0.77, 1.32)
	Per SD increase	1061/2180	0.94 (0.87, 1.01)	590/2166	0.95 (0.86, 1.05)
Monocyte	Quartile 1	260/545	Ref.	150/542	Ref.
	Quartile 2	249/548	0.88 (0.73, 1.07)	142/545	0.94 (0.74, 1.20)
	Quartile 3	277/545	1.15 (0.95, 1.38)	136/542	1.08 (0.84, 1.38)
	Quartile 4	275/542	1.01 (0.84, 1.23)	162/537	1.08 (0.84, 1.38)
	Per SD increase	1061/2180	1.03 (0.96, 1.10)	590/2166	1.02 (0.94, 1.11)
Neutrophil	Quartile 1	231/548	Ref.	127/546	Ref.
	Quartile 2	246/550	1.14 (0.94, 1.37)	124/548	1.06 (0.82, 1.38)
	Quartile 3	278/545	1.16 (0.94, 1.42)	163/540	1.09 (0.83, 1.43)
	Quartile 4	306/537	**1.44 (1.17, 1.78)**	176/532	1.31 (0.99, 1.74)
	Per SD increase	1061/2180	**1.14 (1.05, 1.23)**	590/2166	**1.14 (1.03, 1.26)**

Abbreviation: BMI, body mass index; CCI, Charlson comorbidity index; CI, confidence interval; HR, hazard ratio. Numbers printed in bold: statistically significantly different from 1 ($P < 0.05$). * HR was adjusted for age, sex, tumor stage, tumor subsite, timing of blood sampling, batch, body mass index, smoking status and Charlson comorbidity index. Complete case analysis was applied when adjusting for these variables with missing values.

Table S4. Associations of leukocyte composition with all-cause and colorectal cancer (CRC)-specific mortality among patients whose blood sample was taken prior to surgery, more than 1 month after surgery but not receiving chemo- or radiotherapy or more than 5 months after the first chemo- or radiotherapy

Leukocyte composition	Categories	All-cause mortality		CRC-specific mortality	
		n_{death}/n_{cases}	HR (95%CI)*	n_{death}/n_{cases}	HR (95%CI)*
CD4+T cell	Quartile 1	94/145	Ref.	53/142	Ref.
	Quartile 2	123/249	**0.66 (0.48, 0.90)**	55/247	**0.59 (0.39, 0.90)**
	Quartile 3	121/275	**0.56 (0.41, 0.78)**	54/275	**0.53 (0.34, 0.81)**
	Quartile 4	130/342	**0.49 (0.35, 0.68)**	63/342	**0.47 (0.30, 0.74)**
	Per SD increase	468/1011	**0.80 (0.71, 0.90)**	225/1006	**0.77 (0.66, 0.91)**
CD8+T cell	Quartile 1	89/149	Ref.	41/147	Ref.
	Quartile 2	98/201	0.75 (0.53, 1.06)	42/201	0.62 (0.37, 1.04)
	Quartile 3	132/292	0.85 (0.62, 1.16)	71/289	1.05 (0.67, 1.64)
	Quartile 4	149/369	**0.68 (0.50, 0.94)**	71/369	0.78 (0.49, 1.23)
	Per SD increase	468/1011	0.92 (0.83, 1.02)	225/1006	1.00 (0.86, 1.16)
B cell	Quartile 1	117/188	Ref.	61/187	Ref.
	Quartile 2	121/243	0.81 (0.61, 1.07)	54/242	0.90 (0.60, 1.35)
	Quartile 3	113/271	**0.54 (0.40, 0.73)**	53/269	**0.52 (0.34, 0.78)**
	Quartile 4	117/309	**0.52 (0.38, 0.71)**	57/308	**0.53 (0.36, 0.79)**
	Per SD increase	468/1011	0.78 (0.59, 1.04)	225/1006	0.86 (0.56, 1.31)
NK cell	Quartile 1	68/130	Ref.	38/127	Ref.
	Quartile 2	100/211	1.13 (0.79, 1.62)	49/211	1.27 (0.75, 2.15)
	Quartile 3	112/275	**0.67 (0.46, 0.99)**	51/275	0.87 (0.50, 1.51)
	Quartile 4	188/395	0.85 (0.59, 1.22)	87/393	1.17 (0.70, 1.96)
	Per SD increase	468/1011	0.89 (0.80, 0.98)	225/1006	0.95 (0.83, 1.08)
Monocyte	Quartile 1	91/208	Ref.	44/208	Ref.
	Quartile 2	80/218	0.81 (0.58, 1.12)	35/217	0.88 (0.53, 1.45)
	Quartile 3	144/291	1.24 (0.93, 1.64)	64/290	1.21 (0.79, 1.86)
	Quartile 4	153/294	1.18 (0.87, 1.60)	82/291	1.34 (0.85, 2.11)
	Per SD increase	468/1011	1.07 (0.97, 1.18)	225/1006	1.07 (0.93, 1.21)
Neutrophil	Quartile 1	152/382	Ref.	75/382	Ref.
	Quartile 2	142/318	**1.31 (1.04, 1.66)**	60/317	1.16 (0.81, 1.67)
	Quartile 3	105/197	**1.39 (1.04, 1.85)**	55/196	1.36 (0.91, 2.02)
	Quartile 4	69/114	**1.91 (1.30, 1.85)**	35/111	1.45 (0.85, 2.49)
	Per SD increase	468/1011	**1.26 (1.11, 1.43)**	225/1006	1.17 (0.97, 1.41)

Abbreviation: CI, confidence interval; HR, hazard ratio. Numbers printed in bold: statistically significantly different from 1 ($P < 0.05$). * HR was adjusted for age, sex, tumor stage, tumor subsite, timing of blood sampling, and batch. Complete case analysis was applied when adjusting for these variables with missing values.

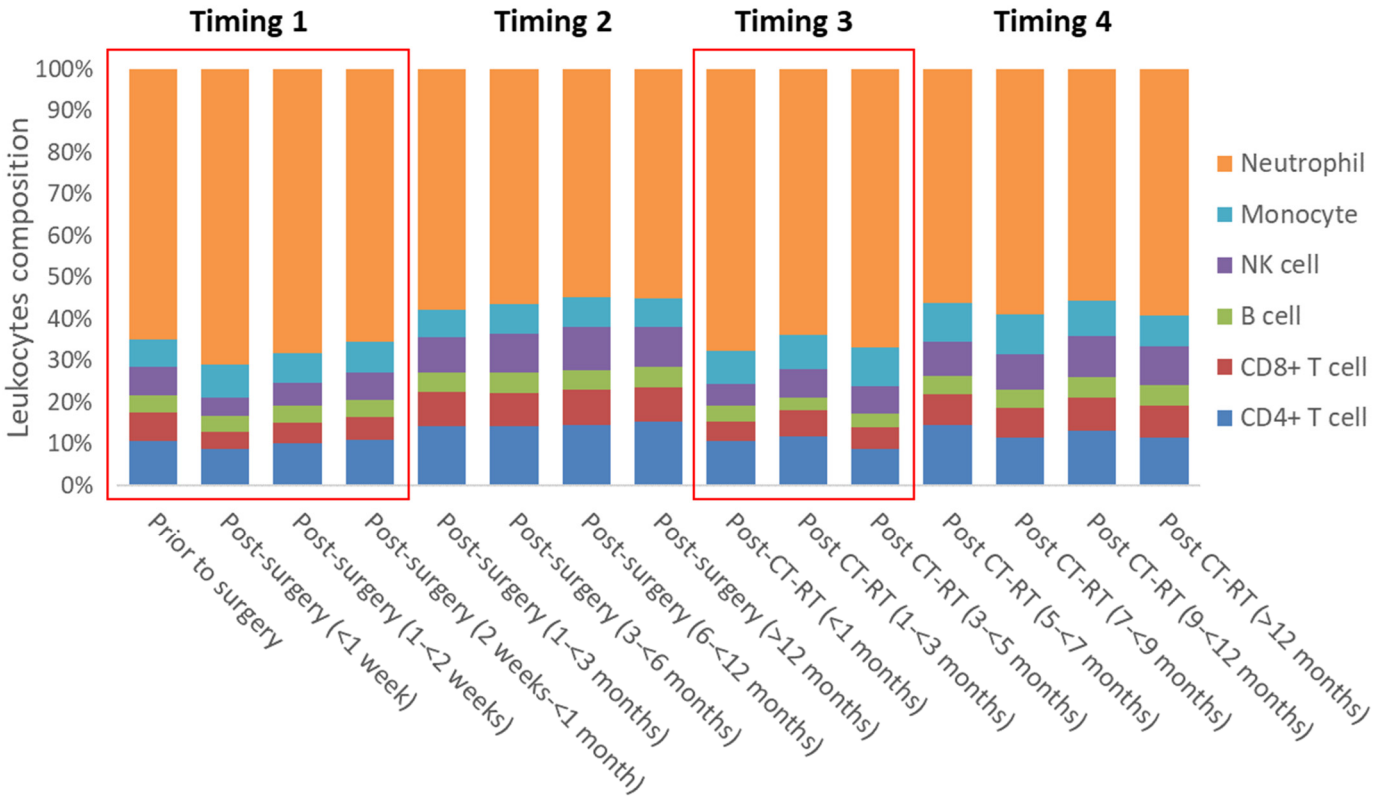

Figure S1. Leukocytes proportion according to the time of blood collection relative to treatments. Note: (a) CT-RT: chemotherapy or radiotherapy; The "post-surgery" categories include patients whose blood were collected after receiving surgery but before or not receiving chemotherapy or radiotherapy; The "post CT-RT" categories include patients whose blood samples were collected after receiving chemotherapy or radiotherapy. (b) The timing of blood sampling were grouped into four categories (1) prior to surgery and within 1 month after surgery (n = 1,006); (2) more than 1 month after surgery, but not receiving chemo- or radiotherapy (n = 569); (3) within 5 months after the first chemo- or radiotherapy (n = 278); (4) more than 5 months after the first chemo- or radiotherapy (n = 353).

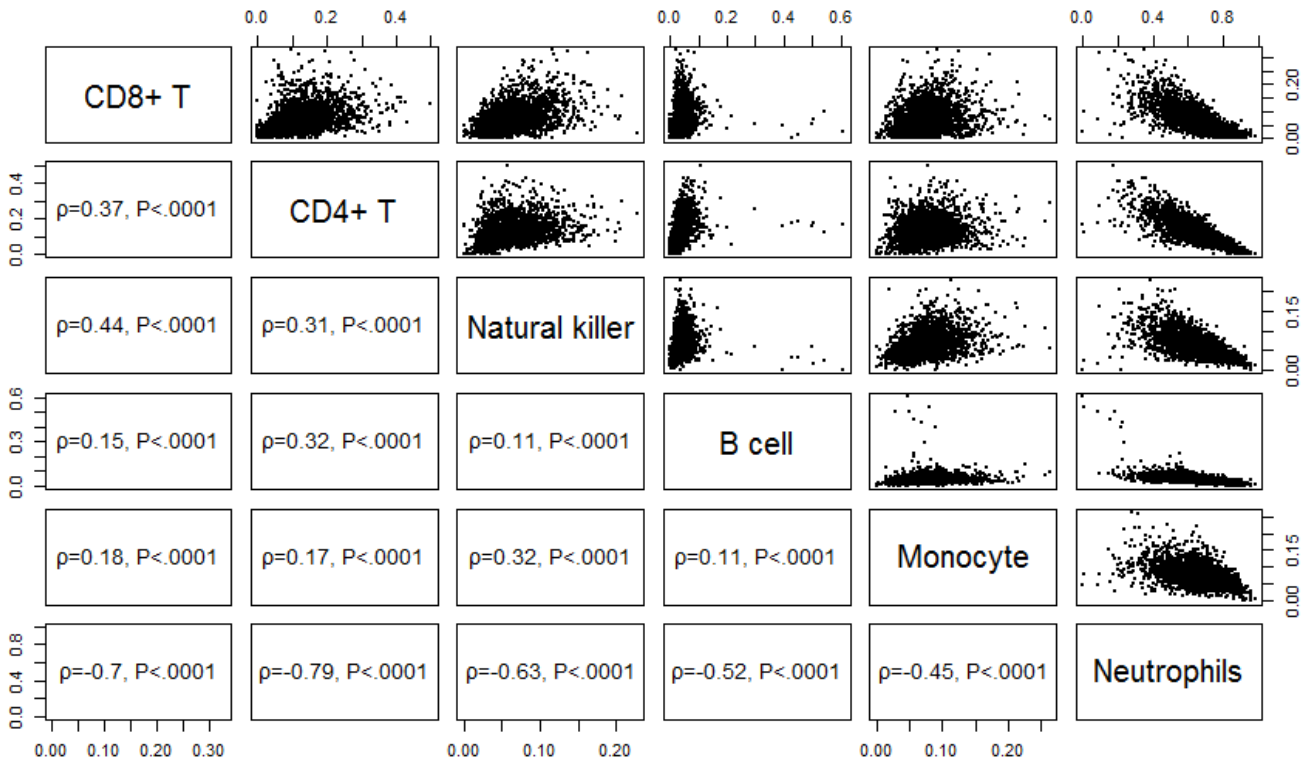

Figure S2. Correlations of leukocyte subtype proportions. ρ: Pearson correlation coefficient.

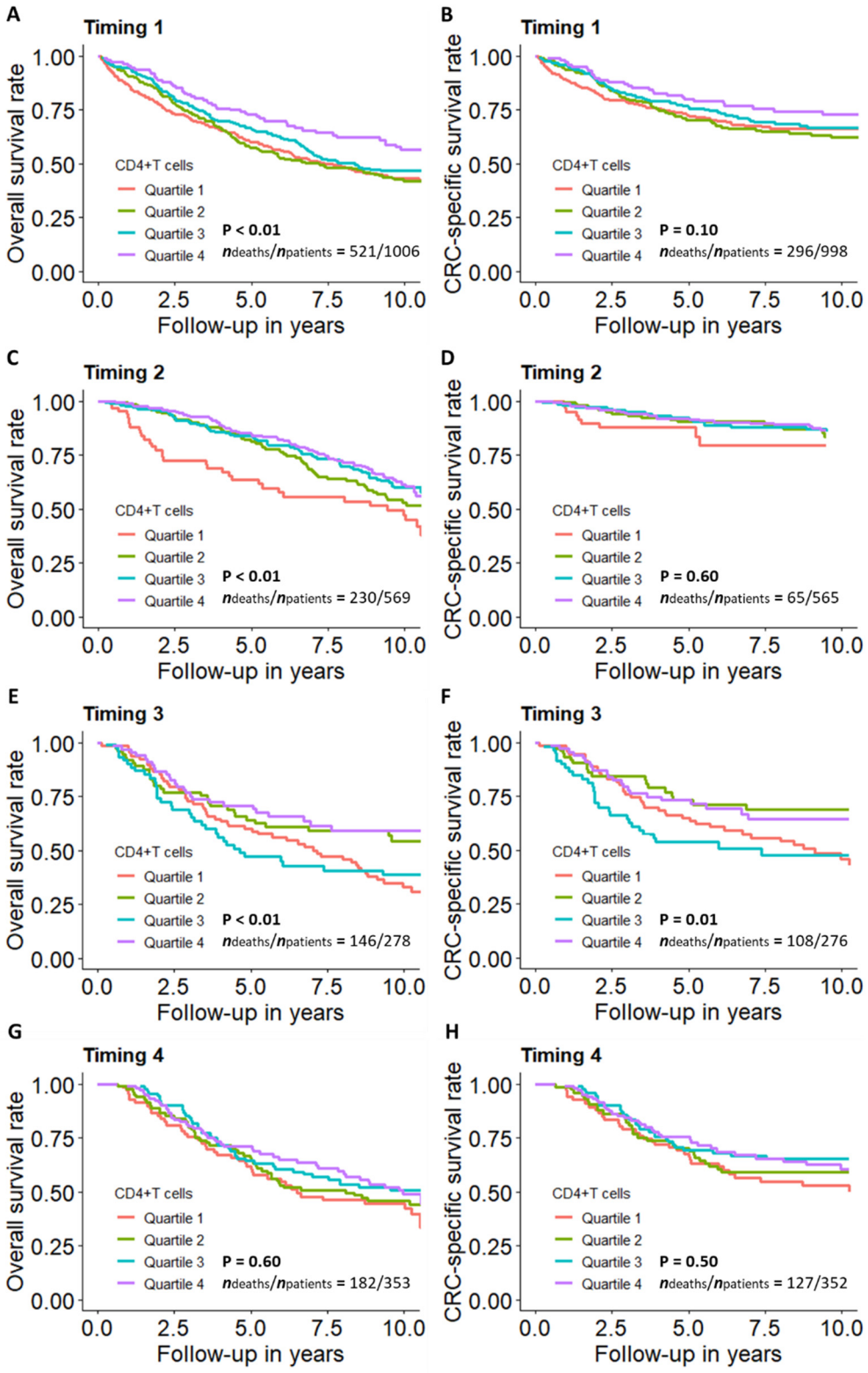

Figure S3. Survival curves for CRC patients across quartiles of CD4+ T cells proportion by the timing of blood sampling. Note: (A) overall and (B) CRC-specific survival curve among patients whose blood was sampled prior to surgery and within 1 month after surgery; (C) overall and (D) CRC-specific survival curve among patients whose blood was sampled more than 1 month after surgery, but not receiving chemo- or radiotherapy; (E) overall and (F) CRC-specific survival curve among patients whose blood was sampled within 5 months after chemo- or radiotherapy; (G) overall and (H) CRC-specific survival curve among patients whose blood was sampled more than 5 months after chemo- or radiotherapy. HR was adjusted for age, sex, tumor stage, subsite and batch. Quartile 1: lowest quartile; quartile 4: highest quartile.

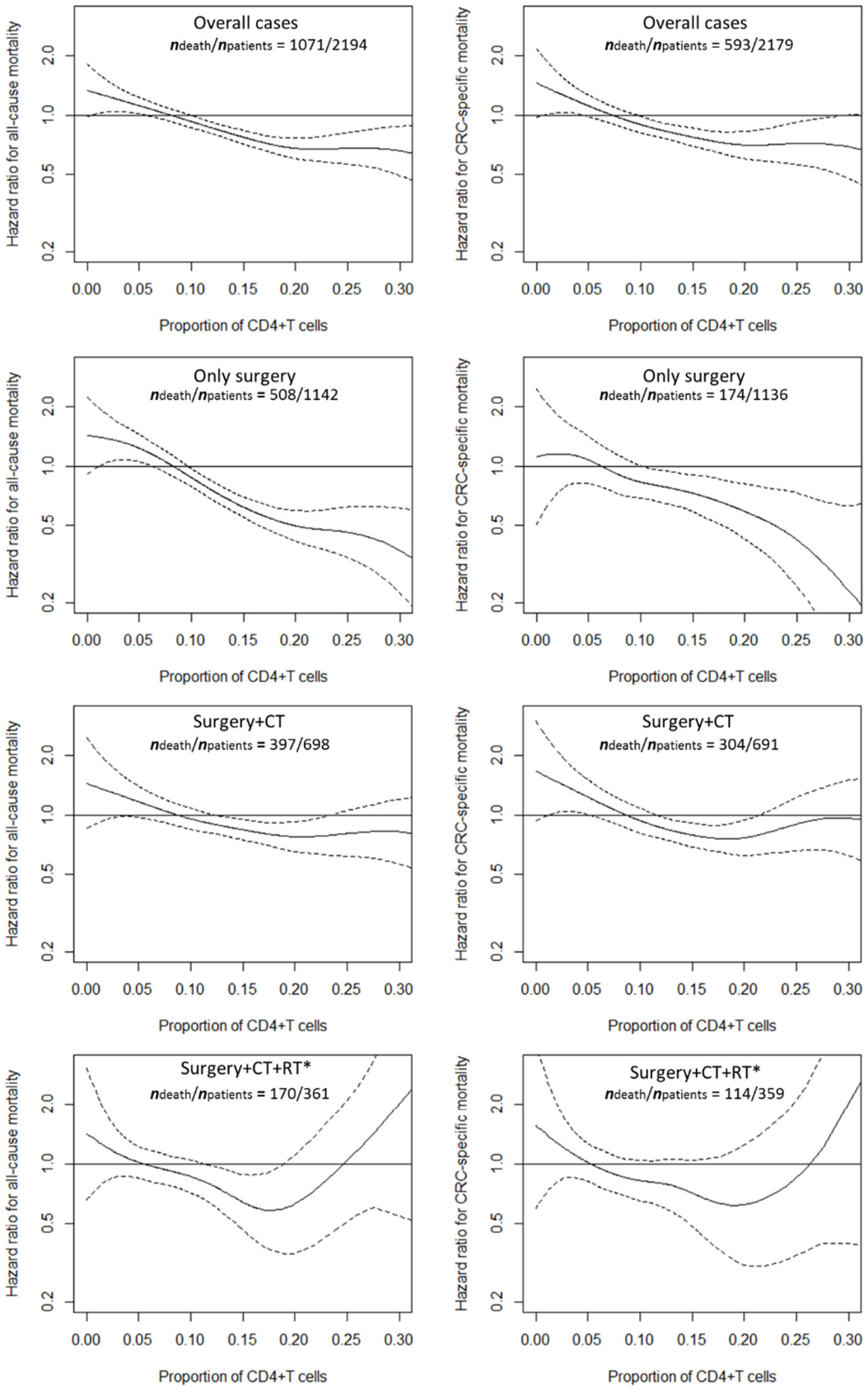

Figure S4. Adjusted restricted cubic splines for CRC patients across quartiles of CD4+ T cells proportion by therapies. Note: Hazard ratios were adjusted for age, sex, tumor stage, tumor subsite, timing of blood sampling and batch; 25th percentile of CD4+ T cells is set as the reference; In the group "surgery+CT+RT*", 42 patients only received surgery and radiotherapy; Abbreviations: CT, chemotherapy; RT, radiotherapy.