

## **Web Appendix**

### **MATERIALS AND METHODS**

#### **Study population**

We utilized the data from two prospective cohort studies, the Nurses' Health Study (NHS, which started in 1976) and the Health Professionals Follow-up Study (HPFS, which started in 1986) (1, 2). Questionnaires were sent to participants every two years to update information on smoking status, diet, and other lifestyle factors, and to identify newly diagnosed diseases. In the present study, we excluded men and women with a baseline history of cancer (except non-melanoma skin cancer). We also excluded the participants who did not provide smoking information on the baseline questionnaire. A total of 88,397 women and 45,807 men were eligible for inclusion in the analysis. Based on the availability of adequate follow-up and tumor molecular data, 1,260 colorectal cancer cases, diagnosed from 1980 through 2008, were used as outcome data. Baseline characteristics of participants without available tumor molecular information were similar to those for cases with available data [age at diagnosis, 66.1 vs. 66.4 years old; never smoker, 40.6% vs. 39.9%; mean cumulative pack-years smoked, 14.8 vs. 14.9; mean pack-years smoked before age 30, 4.8 vs. 4.9; age at start of smoking 20 years old or younger, 31.7% vs. 31.3%; body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>, 10.8% vs. 10.8% (P>0.1 for all comparisons)].

Informed consent was obtained from all participants. This study was approved by the Human Subjects Committees at Harvard School of Public Health and Brigham and Women's Hospital.

### **Assessment of incident colorectal cancer**

Every two years, participants were asked whether they had received a new diagnosis colon or rectal cancer. In addition, the National Death Index was searched to ascertain deaths, and any diagnosis of colorectal cancer that contributed to death or that was a secondary diagnosis (3). For putative colorectal cancer cases, the medical records were reviewed, and cases with carcinoma-in-situ, non-epithelial tumors, and metastases from other body sites were excluded. We retrieved formalin-fixed paraffin-embedded colorectal cancer tissue blocks from hospitals throughout the U.S. where participants with colorectal cancer had undergone surgical resection (4). We collected diagnostic biopsy specimens for rectal cancer patients who received preoperative treatment, to minimize bias from treatment. Hematoxylin and eosin stained tissue sections from all colorectal cancer cases were reviewed by a pathologist (S.O.).

### **DNA extraction, Pyrosequencing of *BRAF*, and microsatellite instability (MSI) analysis**

DNA was extracted from paraffin embedded tissue. PCR and Pyrosequencing were performed for *BRAF* codon 600 (5). MSI analysis was performed using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487) (6). MSI-high was defined as instability in  $\geq 30\%$  of the markers, and microsatellite stability (MSS) was defined as instability in 0-29% of the markers (MSI-low cancers were incorporated into MSS cancers because both display similar features).

### **Methylation analysis for CpG islands**

Using validated bisulfite DNA treatment and real-time PCR (MethyLight), we quantified DNA methylation in eight CIMP-specific promoters [*CACNA1G*, *CDKN2A* (p16), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3* and *SOCS1*] (6-9). CIMP-high was defined as the presence of  $\geq 6/8$  methylated promoters and CIMP-low/negative was defined as 0/8-5/8 methylated promoters, according to previously established criteria (6-9).

### **Immunohistochemistry for DNMT3B**

DNMT3B results were limited to cases where tumor tissues were available for tissue microarray (TMA) construction. Immunohistochemistry for DNMT3B (HGNC ID: 2979) using TMA was performed as previously described (10), using a monoclonal primary antibody against DNMT3B (1:150 dilution; catalog No. IMG-184A; clone 52A1018; Imgenex, San Diego, CA). This antibody was generated using full-length mouse recombinant Dnmt3b, and has been demonstrated to recognize human DNMT3B ([www.imgenex.com/antibody\\_details.php?catalog=IMG-184A](http://www.imgenex.com/antibody_details.php?catalog=IMG-184A)). DNMT3B positivity (i.e., overexpression) was defined as  $\geq 30\%$  of tumor cells with at least weak nuclear staining. Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically-stained slides were interpreted by one of the investigators (K.N.) unaware of other data. A random selection of 141 cases was examined by a second observer (K.S.) unaware of other data; concordance between the two observers was 0.91 ( $\kappa=0.60$ ,  $P<0.001$ ), indicating substantial agreement (10).

## REFERENCES

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Web Table 1. Relations between tumor molecular features, tumor location, and sex in 1,260 colorectal cancer cases in the Nurses' Health Study (1980-2008) and the Health Professionals Follow-up Study (1986-2008)

		Within each of these subsets of cancer cases					
		CIMP-high (N=205)	MSI-high (N=188)	<i>BRAF</i> -mutated (N=178)	DNMT3B-positive (N=108)	Proximal colon cancer (N=591)	Women (N=718)
Prevalence of:	CIMP-high		76%	73%	32%	32%	21%
	MSI-high	68%		56%	27%	29%	19%
	<i>BRAF</i> -mutated	62%	52%		26%	25%	20%
	DNMT3B-positive	31%	29%	28%		21%	16%
	Proximal colon cancer	87%	87%	82%	60%		50%
	Women	70%	68%	76%	66%	60%	

Abbreviations: CIMP, CpG island methylator phenotype; DNMT3B, DNA methyltransferase 3B; MSI, microsatellite instability.

Web Table 2. Duration of Smoking Cessation and Incident Colorectal Cancer Risk by Molecular Subtypes<sup>a</sup> in the Nurses' Health Study (1980-2008) or the Health Professionals Follow-up Study (1986-2008)

	Current smoker		Cessation 1-4 years		Cessation 5-9 years		Cessation 10-19 years		Cessation 20-39 years		Cessation ≥40 years		$P_{\text{trend}}^b$	$P_{\text{heterogeneity}}^c$
	HR		HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI		
<b>Women</b>														
Person-years		381,181		125,752		119,058		238,551		338,742		62,676		
<b>All colorectal cancer</b>														
N		101		38		65		85		111		29		
Age-adjusted	1	Referent	0.98	0.67, 1.42	1.51	1.11, 2.07	1.02	0.76, 1.36	0.80	0.61, 1.05	0.79	0.52, 1.21	0.003	
Multivariate <sup>d</sup>	1	Referent	0.99	0.68, 1.43	1.52	1.11, 2.08	1.01	0.75, 1.36	0.79	0.60, 1.05	0.79	0.51, 1.21	0.004	
<b>CIMP status</b>														
CIMP-low/negative														
N		71		25		51		67		89		20		
Age-adjusted	1	Referent	0.93	0.59, 1.47	1.77	1.23, 2.55	1.21	0.86, 1.70	0.98	0.71, 1.34	0.91	0.55, 1.52	0.16	
Multivariate <sup>d</sup>	1	Referent	0.94	0.59, 1.48	1.79	1.24, 2.57	1.21	0.86, 1.70	0.97	0.70, 1.34	0.91	0.54, 1.52	0.19	0.01
CIMP-high														
N		25		12		12		15		18		7		
Age-adjusted	1	Referent	1.12	0.56, 2.23	0.90	0.45, 1.81	0.56	0.29, 1.06	0.39	0.21, 0.72	0.44	0.19, 1.02	0.0003	
Multivariate <sup>d</sup>	1	Referent	1.14	0.57, 2.27	0.90	0.45, 1.80	0.56	0.29, 1.07	0.39	0.21, 0.73	0.43	0.18, 1.02	0.001	
<b>MSI status</b>														
MSS														
N		76		24		52		65		91		20		
Age-adjusted	1	Referent	0.84	0.53, 1.32	1.67	1.17, 2.38	1.08	0.77, 1.51	0.91	0.67, 1.24	0.83	0.50, 1.38	0.09	
Multivariate <sup>d</sup>	1	Referent	0.84	0.53, 1.34	1.67	1.17, 2.39	1.08	0.77, 1.51	0.91	0.66, 1.24	0.83	0.50, 1.38	0.10	0.05
MSI-high														
N		21		12		10		14		14		8		
Age-adjusted	1	Referent	1.34	0.65, 2.72	0.92	0.43, 1.96	0.65	0.33, 1.28	0.38	0.19, 0.76	0.61	0.27, 1.41	0.002	
Multivariate <sup>d</sup>	1	Referent	1.35	0.66, 2.76	0.91	0.43, 1.94	0.65	0.33, 1.28	0.38	0.19, 0.76	0.61	0.26, 1.40	0.003	

**BRAF mutation status***BRAF*-wildtype

N		79	25	51	65	91	22							
Age-adjusted	1	Referent	0.84	0.53, 1.32	1.56	1.09, 2.22	1.01	0.73, 1.41	0.86	0.63, 1.17	0.84	0.51, 1.36	0.06	
Multivariate <sup>d</sup>	1	Referent	0.85	0.54, 1.33	1.56	1.09, 2.23	1.00	0.72, 1.40	0.85	0.62, 1.16	0.83	0.51, 1.36	0.07	0.11

*BRAF*-mutated

N		19	11	12	16	17	6							
Age-adjusted	1	Referent	1.34	0.63, 2.83	1.29	0.62, 2.68	0.92	0.47, 1.80	0.56	0.29, 1.08	0.62	0.24, 1.57	0.01	
Multivariate <sup>d</sup>	1	Referent	1.35	0.64, 2.85	1.29	0.62, 2.67	0.92	0.47, 1.82	0.56	0.29, 1.08	0.62	0.24, 1.58	0.01	

**DNMT3B expression status**

## DNMT3B-negative

N		61	22	30	44	59	6							
Age-adjusted	1	Referent	0.96	0.59, 1.56	1.24	0.80, 1.92	1.05	0.71, 1.55	0.89	0.62, 1.29	0.49	0.21, 1.16	0.06	
Multivariate <sup>d</sup>	1	Referent	0.97	0.59, 1.58	1.26	0.81, 1.96	1.07	0.72, 1.58	0.91	0.63, 1.32	0.50	0.21, 1.18	0.08	0.14

## DNMT3B-positive

N		12	3	7	2	8	1							
Age-adjusted	1	Referent	0.67	0.19, 2.38	1.31	0.51, 3.37	0.20	0.04, 0.90	0.49	0.20, 1.22	0.29	0.04, 2.30	0.03	
Multivariate <sup>d</sup>	1	Referent	0.69	0.19, 2.45	1.34	0.52, 3.43	0.21	0.05, 0.96	0.52	0.21, 1.29	0.29	0.04, 2.30	0.03	

**Men**

Person-years		58,327	36,153	36,662	74,205	172,684	64,012							
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**All colorectal cancer**

N		38	22	21	44	131	76							
Age-adjusted	1	Referent	0.93	0.55, 1.58	0.87	0.51, 1.49	0.81	0.52, 1.25	0.91	0.63, 1.31	0.92	0.61, 1.37	0.78	
Multivariate <sup>d</sup>	1	Referent	0.97	0.57, 1.65	0.89	0.52, 1.52	0.85	0.54, 1.32	0.97	0.67, 1.41	1.01	0.67, 1.52	0.83	

**CIMP status**

## CIMP-low/negative

N		32	17	15	38	105	52							
Age-adjusted	1	Referent	0.83	0.46, 1.50	0.72	0.39, 1.34	0.82	0.51, 1.33	0.87	0.58, 1.31	0.82	0.52, 1.30	0.59	
Multivariate <sup>d</sup>	1	Referent	0.86	0.47, 1.56	0.74	0.40, 1.38	0.87	0.54, 1.41	0.95	0.63, 1.42	0.91	0.57, 1.45	0.97	0.70

## CIMP-high

N		6	3	3	3	19	9							
Age-adjusted	1	Referent	0.99	0.24, 4.00	0.84	0.21, 3.40	0.38	0.09, 1.55	0.80	0.31, 2.05	0.57	0.19, 1.65	0.51	
Multivariate <sup>d</sup>	1	Referent	1.09	0.27, 4.44	0.90	0.22, 3.66	0.43	0.11, 1.77	0.88	0.34, 2.26	0.66	0.22, 1.95	0.66	

<b>MSI status</b>														
<b>MSS</b>														
N			32	16	16	36	110	66						
Age-adjusted	1	Referent	0.79	0.43, 1.44	0.78	0.43, 1.44	0.78	0.48, 1.27	0.90	0.60, 1.35	0.95	0.61, 1.48	0.88	
Multivariate <sup>d</sup>	1	Referent	0.82	0.45, 1.50	0.80	0.43, 1.46	0.81	0.50, 1.32	0.96	0.64, 1.44	1.04	0.66, 1.62	0.56	0.25
<b>MSI-high</b>														
N			6	4	4	6	16	9						
Age-adjusted	1	Referent	1.09	0.30, 3.91	1.13	0.31, 4.05	0.70	0.22, 2.19	0.66	0.25, 1.70	0.56	0.19, 1.63	0.21	
Multivariate <sup>d</sup>	1	Referent	1.13	0.32, 4.07	1.16	0.32, 4.17	0.74	0.23, 2.33	0.71	0.27, 1.86	0.63	0.22, 1.86	0.32	
<b>BRAF mutation status</b>														
<b>BRAF-wildtype</b>														
N			35	17	19	40	116	67						
Age-adjusted	1	Referent	0.75	0.42, 1.35	0.84	0.48, 1.47	0.79	0.50, 1.25	0.85	0.58, 1.25	0.85	0.56, 1.31	0.75	
Multivariate <sup>d</sup>	1	Referent	0.78	0.44, 1.41	0.86	0.49, 1.50	0.82	0.52, 1.30	0.91	0.61, 1.34	0.93	0.60, 1.43	0.88	0.98
<b>BRAF-mutated</b>														
N			3	3	1	3	13	7						
Age-adjusted	1	Referent	2.37	0.46, 12.0	0.61	0.06, 5.96	0.73	0.15, 3.69	1.37	0.38, 4.95	1.14	0.28, 4.67	0.93	
Multivariate <sup>d</sup>	1	Referent	2.39	0.47, 12.1	0.59	0.06, 5.74	0.76	0.15, 3.86	1.40	0.39, 5.06	1.22	0.30, 5.01	0.98	
<b>DNMT3B expression status</b>														
<b>DNMT3B-negative</b>														
N			12	13	8	28	64	31						
Age-adjusted	1	Referent	1.72	0.78, 3.81	1.03	0.42, 2.53	1.71	0.86, 3.39	1.51	0.81, 2.83	1.65	0.83, 3.29	0.40	
Multivariate <sup>d</sup>	1	Referent	1.76	0.80, 3.89	1.05	0.43, 2.59	1.79	0.90, 3.55	1.60	0.86, 3.00	1.82	0.91, 3.64	0.19	0.08
<b>DNMT3B-positive</b>														
N			5	2	1	3	8	4						
Age-adjusted	1	Referent	0.92	0.18, 4.83	0.34	0.04, 2.98	0.49	0.11, 2.10	0.43	0.14, 1.35	0.39	0.10, 1.53	0.15	
Multivariate <sup>d</sup>	1	Referent	0.94	0.18, 4.96	0.35	0.04, 3.03	0.50	0.12, 2.17	0.45	0.15, 1.41	0.40	0.10, 1.60	0.17	

Abbreviations: CI, confidence interval; CIMP, CpG island methylator phenotype; DNMT3B, DNA methyltransferase 3B; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable; N, No. of cases.

<sup>a</sup> All models were stratified with age.

<sup>b</sup> Based on the linear trend test across the median values in each category. To test whether duration of smoking cessation reduced the cancer risk compared with current smoking, trend test and heterogeneity test were performed on current and past smokers excluding never smokers.

<sup>c</sup> Tests for heterogeneity (for a multivariate HR linear trend) showed significance of differential association of cessation with colorectal cancer risk by molecular subtypes. (i.e., CIMP-low/negative vs. CIMP-high; MSS vs. MSI-high; *BRAF*-wildtype vs. *BRAF*-mutated; DNMT3B-negative vs. DNMT3B-positive).

<sup>d</sup> Models were adjusted for body mass index, family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level, alcohol consumption, total caloric intake and red meat intake.



Web Table 3. Duration of Smoking Cessation and Incident Colorectal Cancer Risk by Cancer Anatomical Subsites and Molecular Subtypes<sup>a</sup> in the Nurses' Health Study (1980-2008) and the Health Professionals Follow-up Study (1986-2008)

	Current smoker		Cessation 1-4 years		Cessation 5-9 years		Cessation 10-19 years		Cessation 20-39 years		Cessation ≥40 years		<i>P</i> <sub>trend</sub> <sup>b</sup>	<i>P</i> <sub>heterogeneity</sub> <sup>c</sup>
	HR		HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI		
Person-years		439,508		161,905		155,720		312,757		511,426		126,688		
<b>Proximal colon cancer</b>														
<b>CIMP status</b>														
CIMP-low/negative														
N		34		16		25		37		75		28		
Age-adjusted	1	Referent	1.08	0.59, 1.96	1.48	0.88, 2.49	1.07	0.67, 1.72	1.06	0.70, 1.61	0.95	0.56, 1.61	0.35	
Multivariate <sup>d</sup>	1	Referent	1.09	0.60, 1.98	1.51	0.89, 2.54	1.09	0.68, 1.76	1.07	0.70, 1.63	0.97	0.57, 1.65	0.49	0.06
CIMP-high														
N		26		15		13		18		30		15		
Age-adjusted	1	Referent	1.32	0.70, 2.50	0.94	0.48, 1.83	0.62	0.34, 1.14	0.52	0.30, 0.89	0.56	0.29, 1.09	0.003	
Multivariate <sup>d</sup>	1	Referent	1.36	0.72, 2.59	0.96	0.49, 1.87	0.64	0.35, 1.19	0.52	0.30, 0.89	0.58	0.30, 1.13	0.01	
<b>MSI status</b>														
MSS														
N		40		15		26		35		78		34		
Age-adjusted	1	Referent	0.85	0.47, 1.54	1.29	0.78, 2.12	0.84	0.53, 1.33	0.90	0.61, 1.33	0.84	0.52, 1.37	0.22	
Multivariate <sup>d</sup>	1	Referent	0.86	0.47, 1.56	1.31	0.79, 2.16	0.86	0.54, 1.36	0.90	0.61, 1.33	0.86	0.53, 1.40	0.31	0.17
MSI-high														
N		22		16		13		17		28		16		
Age-adjusted	1	Referent	1.62	0.85, 3.11	1.12	0.56, 2.24	0.69	0.37, 1.32	0.59	0.33, 1.04	0.71	0.36, 1.40	0.01	
Multivariate <sup>d</sup>	1	Referent	1.66	0.86, 3.17	1.13	0.56, 2.25	0.71	0.37, 1.34	0.59	0.33, 1.05	0.72	0.37, 1.43	0.02	
<b>BRAF mutation status</b>														
<i>BRAF</i> -wildtype														
N		45		18		26		37		86		38		
Age-adjusted	1	Referent	0.90	0.52, 1.56	1.12	0.69, 1.83	0.76	0.49, 1.18	0.85	0.59, 1.23	0.79	0.50, 1.26	0.24	
Multivariate <sup>d</sup>	1	Referent	0.91	0.53, 1.59	1.15	0.71, 1.88	0.78	0.50, 1.21	0.86	0.59, 1.25	0.82	0.51, 1.29	0.34	0.09
<i>BRAF</i> -mutated														
N		17		13		13		16		22		12		
Age-adjusted	1	Referent	1.73	0.84, 3.58	1.51	0.73, 3.12	0.92	0.46, 1.84	0.66	0.34, 1.25	0.80	0.37, 1.73	0.01	
Multivariate <sup>d</sup>	1	Referent	1.75	0.85, 3.63	1.50	0.72, 3.10	0.94	0.47, 1.88	0.66	0.34, 1.25	0.80	0.37, 1.74	0.01	

**DNMT3B expression status**

## DNMT3B-negative

N		29		16		13		24		47		14		
Age-adjusted	1	Referent	1.34	0.72, 2.48	0.95	0.49, 1.83	0.94	0.54, 1.63	0.96	0.60, 1.55	0.82	0.42, 1.61	0.42	
Multivariate <sup>d</sup>	1	Referent	1.32	0.71, 2.44	0.96	0.49, 1.85	0.97	0.56, 1.68	0.98	0.60, 1.57	0.85	0.43, 1.67	0.55	0.08

## DNMT3B-positive

N		9		5		4		2		11		3		
Age-adjusted	1	Referent	1.41	0.47, 4.23	0.86	0.26, 2.82	0.24	0.05, 1.10	0.59	0.24, 1.44	0.37	0.10, 1.45	0.02	
Multivariate <sup>d</sup>	1	Referent	1.45	0.48, 4.38	0.89	0.27, 2.92	0.25	0.05, 1.17	0.62	0.25, 1.52	0.38	0.10, 1.49	0.03	

**Distal colorectal cancer****CIMP status**

## CIMP-low/negative

N		69		26		40		68		115		42		
Age-adjusted	1	Referent	0.83	0.53, 1.31	1.29	0.87, 1.91	1.08	0.77, 1.51	0.90	0.66, 1.22	0.89	0.59, 1.35	0.18	
Multivariate <sup>d</sup>	1	Referent	0.84	0.53, 1.32	1.27	0.86, 1.89	1.06	0.75, 1.49	0.91	0.67, 1.24	0.91	0.60, 1.38	0.21	0.44

## CIMP-high

N		4		0		2		0		7		1		
Age-adjusted	1	Referent	-	-	0.82	0.15, 4.52	-	-	0.59	0.16, 2.10	0.19	0.02, 1.80	0.30	
Multivariate <sup>d</sup>	1	Referent	-	-	0.80	0.14, 4.45	-	-	0.60	0.17, 2.16	0.19	0.02, 1.84	0.30	

**MSI status**

## MSS

N		67		25		41		66		119		50		
Age-adjusted	1	Referent	0.83	0.52, 1.32	1.37	0.92, 2.02	1.07	0.76, 1.52	0.94	0.69, 1.29	1.05	0.71, 1.55	0.52	
Multivariate <sup>d</sup>	1	Referent	0.84	0.53, 1.33	1.35	0.91, 1.99	1.05	0.74, 1.49	0.95	0.70, 1.30	1.06	0.71, 1.58	0.55	0.02

## MSI-high

N		5		0		1		3		2		1		
Age-adjusted	1	Referent	-	-	0.34	0.04, 2.90	0.53	0.12, 2.28	0.14	0.03, 0.72	0.14	0.02, 1.33	0.02	
Multivariate <sup>d</sup>	1	Referent	-	-	0.32	0.04, 2.76	0.54	0.13, 2.30	0.14	0.03, 0.73	0.15	0.02, 1.40	0.02	

**BRAF mutation status***BRAF*-wildtype

N		69		24		43		68		117		50		
Age-adjusted	1	Referent	0.76	0.48, 1.22	1.37	0.93, 2.01	1.05	0.75, 1.48	0.87	0.64, 1.18	0.95	0.64, 1.40	0.22	
Multivariate <sup>d</sup>	1	Referent	0.76	0.48, 1.22	1.34	0.91, 1.97	1.03	0.73, 1.44	0.87	0.64, 1.18	0.96	0.65, 1.42	0.24	0.66

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N		4		1		0		3		8		0		
Age-adjusted	1	Referent	0.65	0.07, 5.95	-	-	0.92	0.20, 4.27	1.35	0.39, 4.65	-	-	0.80	
Multivariate <sup>d</sup>	1	Referent	0.67	0.07, 6.15	-	-	0.94	0.20, 4.41	1.37	0.39, 4.75	-	-	0.84	

**DNMT3B expression status**

## DNMT3B-negative

N		44		19		24		48		75		21		
Age-adjusted	1	Referent	0.96	0.56, 1.65	1.25	0.76, 2.06	1.33	0.88, 2.02	1.05	0.71, 1.54	1.00	0.58, 1.75	0.54	
Multivariate <sup>d</sup>	1	Referent	0.96	0.56, 1.66	1.25	0.75, 2.06	1.32	0.87, 2.01	1.07	0.72, 1.57	1.03	0.59, 1.80	0.67	0.27

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## DNMT3B-positive

N		8		0		4		3		5		2		
Age-adjusted	1	Referent	-	-	1.21	0.36, 4.06	0.42	0.11, 1.62	0.39	0.12, 1.21	0.61	0.12, 3.08	0.19	
Multivariate <sup>d</sup>	1	Referent	-	-	1.18	0.35, 4.01	0.43	0.11, 1.65	0.40	0.13, 1.25	0.62	0.12, 3.16	0.20	

Abbreviations: CI, confidence interval; CIMP, CpG island methylator phenotype; DNMT3B, DNA methyltransferase 3B; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable; N, No. of cases.

<sup>a</sup> All models were stratified with age and sex.

<sup>b</sup> Based on the linear trend test across the median values in each category. To test whether duration of smoking cessation reduced the cancer risk compared with current smoking, trend test and heterogeneity test were performed on current and past smokers excluding never smokers.

<sup>c</sup> Tests for heterogeneity (for a multivariate HR linear trend) showed significance of differential association of cessation with colorectal cancer risk by molecular subtypes. (i.e., CIMP-low/negative vs. CIMP-high; MSS vs. MSI-high; *BRAF*-wildtype vs. *BRAF*-mutated; DNMT3B-negative vs. DNMT3B-positive).

<sup>d</sup> Models were adjusted for body mass index, family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level, alcohol consumption, total caloric intake and red meat intake.

Web Table 4. Duration of Smoking Cessation and Incident Colorectal Cancer Risk by Molecular Subtypes<sup>a</sup> in the Nurses' Health Study (1980-2008) and the Health Professionals Follow-up Study (1986-2008)

	Never smoker		Cessation ≥40 years		Cessation 39-20 years		Cessation 19-10 years		Cessation 9-5 years		Cessation 4-1 years		Current smoker		$P_{\text{trend}}^b$ ( $P_{\text{heterogeneity}}^c$ )
	HR		HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	
Person-years		1,383,154		126,688		511,426		312,757		155,720		161,905		439,508	
<b>All colorectal cancer</b>															
N		490		105		242		129		86		60		139	
Age-adjusted	1	Referent	1.26	1.01, 1.56	1.13	0.97, 1.32	1.18	0.97, 1.44	1.61	1.28, 2.03	1.21	0.92, 1.58	1.23	1.02, 1.49	0.19
Multivariate <sup>d</sup>	1	Referent	1.23	0.99, 1.54	1.08	0.93, 1.27	1.13	0.93, 1.38	1.54	1.22, 1.94	1.16	0.88, 1.53	1.18	0.97, 1.43	0.29
<b>CIMP status</b>															
(0.02)															
CIMP-low/negative															
N		377		72		194		105		66		42		103	
Age-adjusted	1	Referent	1.10	0.84, 1.43	1.15	0.96, 1.37	1.26	1.01, 1.57	1.61	1.24, 2.10	1.07	0.78, 1.48	1.18	0.94, 1.47	0.17
Multivariate <sup>d</sup>	1	Referent	1.08	0.83, 1.41	1.11	0.93, 1.33	1.22	0.97, 1.52	1.56	1.19, 2.03	1.04	0.75, 1.44	1.13	0.90, 1.42	0.25
CIMP-high															
N		71		16		37		18		15		15		31	
Age-adjusted	1	Referent	1.07	0.61, 1.86	1.15	0.77, 1.72	1.15	0.68, 1.93	1.98	1.13, 3.47	2.41	1.37, 4.24	2.22	1.44, 3.41	0.001
Multivariate <sup>d</sup>	1	Referent	1.06	0.61, 1.85	1.11	0.74, 1.66	1.12	0.66, 1.88	1.90	1.08, 3.33	2.37	1.35, 4.17	2.12	1.37, 3.27	0.001
<b>MSI status</b>															
(0.02)															
MSS															
N		400		86		201		101		68		40		108	
Age-adjusted	1	Referent	1.14	0.89, 1.46	1.11	0.93, 1.32	1.15	0.92, 1.43	1.59	1.23, 2.06	0.98	0.71, 1.36	1.19	0.96, 1.48	0.26
Multivariate <sup>d</sup>	1	Referent	1.12	0.88, 1.44	1.08	0.90, 1.28	1.11	0.89, 1.38	1.53	1.18, 1.99	0.96	0.69, 1.33	1.15	0.92, 1.43	0.36
MSI-high															
N		63		17		30		20		14		16		27	
Age-adjusted	1	Referent	1.30	0.75, 2.27	1.09	0.70, 1.68	1.45	0.87, 2.41	2.13	1.18, 3.81	2.78	1.60, 4.84	2.19	1.38, 3.47	0.001
Multivariate <sup>d</sup>	1	Referent	1.29	0.74, 2.25	1.05	0.68, 1.63	1.40	0.84, 2.33	2.02	1.12, 3.62	2.71	1.55, 4.72	2.10	1.32, 3.33	0.002

<b><i>BRAF</i> mutation status</b>																	(0.10)
<i>BRAF</i> -wildtype																	
N			404		89		207		105		70		42		114		
Age-adjusted	1	Referent	1.14	0.89, 1.45	1.12	0.95, 1.33	1.19	0.96, 1.48	1.64	1.27, 2.12	1.04	0.76, 1.43	1.28	1.04, 1.58	0.12		
Multivariate <sup>d</sup>	1	Referent	1.12	0.88, 1.43	1.09	0.92, 1.29	1.14	0.92, 1.42	1.58	1.22, 2.05	1.01	0.73, 1.40	1.23	0.99, 1.53	0.18		
<i>BRAF</i> -mutated																	
N			67		13		30		19		13		14		22		
Age-adjusted	1	Referent	1.10	0.60, 2.03	1.05	0.68, 1.63	1.26	0.76, 2.11	1.72	0.94, 3.13	2.12	1.18, 3.80	1.45	0.88, 2.36	0.02		
Multivariate <sup>d</sup>	1	Referent	1.09	0.59, 2.01	1.02	0.66, 1.58	1.23	0.74, 2.06	1.64	0.90, 2.99	2.08	1.16, 3.72	1.40	0.86, 2.30	0.02		

<b>DNMT3B expression status</b>																	(0.03)
DNMT3B-negative																	
N			238		37		123		72		38		35		73		
Age-adjusted	1	Referent	1.12	0.78, 1.61	1.18	0.95, 1.48	1.36	1.04, 1.77	1.33	0.94, 1.88	1.28	0.89, 1.83	1.16	0.89, 1.52	0.40		
Multivariate <sup>d</sup>	1	Referent	1.12	0.77, 1.61	1.16	0.92, 1.45	1.31	1.00, 1.72	1.28	0.90, 1.81	1.23	0.86, 1.76	1.11	0.84, 1.46	0.61		
DNMT3B-positive																	
N			52		5		16		5		8		5		17		
Age-adjusted	1	Referent	0.57	0.22, 1.47	0.66	0.37, 1.16	0.42	0.17, 1.06	1.31	0.61, 2.78	1.00	0.40, 2.51	1.32	0.75, 2.30	0.01		
Multivariate <sup>d</sup>	1	Referent	0.54	0.21, 1.40	0.64	0.36, 1.14	0.40	0.16, 1.02	1.23	0.58, 2.62	0.96	0.38, 2.41	1.23	0.70, 2.16	0.01		

Abbreviations: CI, confidence interval; CIMP, CpG island methylator phenotype; DNMT3B, DNA methyltransferase 3B; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable; N, No. of cases.

<sup>a</sup> All models were stratified with age and sex.

<sup>b</sup> Based on the linear trend test across the median values in each category. To test whether duration of smoking cessation reduced the cancer risk compared with current smoking, trend test and heterogeneity test were performed on current and past smokers excluding never smokers.

<sup>c</sup> Tests for heterogeneity (for a multivariate HR liner trend) showed significance of differential association of cessation with colorectal cancer risk by molecular subtypes. (i.e., CIMP-low/negative vs. CIMP-high; MSS vs. MSI-high; *BRAF*-wildtype vs. *BRAF*-mutated; DNMT3B-negative vs. DNMT3B-positive).

<sup>d</sup> Models were adjusted for body mass index, family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level, alcohol consumption, total caloric intake and red meat intake.

Web Table 5. Duration of Smoking Cessation and Colorectal Cancer Risk by Molecular Subtypes in Strata of Cumulative Pack-years Smoked<sup>a</sup> in the Nurses' Health Study (1980-2008) and the Health Professionals Follow-up Study (1986-2008)

	Pack-years $\geq$ 20							Pack-years<20							
	Current smoker		Cessation <10 years		Cessation $\geq$ 10 years		$P_{\text{trend}}^b$ ( $P_{\text{heterogeneity}}^c$ )	Current smoker		Cessation <10 years		Cessation $\geq$ 10 years		$P_{\text{trend}}^b$ ( $P_{\text{heterogeneity}}^c$ )	
	HR		HR	95% CI	HR	95% CI		HR	95% CI	HR	95% CI	HR	95% CI		
Person-years		339,425		187,701		302,071			87,434		117,251		623,562		
<b>All colorectal cancer</b>															
N		113		107		214			18		29		250		
Age-adjusted	1	Referent	1.23	0.94, 1.61	1.10	0.87, 1.40	0.87	1	Referent	0.90	0.50, 1.63	0.89	0.54, 1.44	0.49	
Multivariate <sup>d</sup>	1	Referent	1.22	0.94, 1.60	1.09	0.86, 1.38	0.91	1	Referent	0.90	0.50, 1.63	0.88	0.54, 1.44	0.50	
<b>CIMP status</b>							(0.02)								(0.07)
CIMP-low/negative															
N		81		76		161			16		25		202		
Age-adjusted	1	Referent	1.25	0.91, 1.72	1.13	0.85, 1.49	0.67	1	Referent	0.90	0.48, 1.71	0.88	0.52, 1.48	0.38	
Multivariate <sup>d</sup>	1	Referent	1.24	0.90, 1.71	1.12	0.84, 1.49	0.69	1	Referent	0.90	0.47, 1.70	0.87	0.52, 1.47	0.35	
CIMP-high															
N		29		27		36			0		1		32		
Age-adjusted	1	Referent	1.09	0.64, 1.86	0.58	0.35, 0.97	0.02	1	Referent	-	-	-	-	0.21	
Multivariate <sup>d</sup>	1	Referent	1.09	0.64, 1.85	0.57	0.34, 0.96	0.02	1	Referent	-	-	-	-	0.20	
<b>MSI status</b>							(0.12)								(0.37)
MSS															
N		84		74		168			17		25		209		
Age-adjusted	1	Referent	1.17	0.85, 1.61	1.06	0.81, 1.40	0.88	1	Referent	0.81	0.43, 1.51	0.80	0.48, 1.33	0.24	
Multivariate <sup>d</sup>	1	Referent	1.16	0.84, 1.59	1.05	0.79, 1.39	0.95	1	Referent	0.81	0.43, 1.51	0.79	0.48, 1.32	0.20	
MSI-high															
N		25		27		35			1		2		31		
Age-adjusted	1	Referent	1.26	0.73, 2.19	0.69	0.41, 1.18	0.07	1	Referent	0.92	0.08, 10.3	1.35	0.18, 10.2	0.63	
Multivariate <sup>d</sup>	1	Referent	1.25	0.72, 2.17	0.69	0.40, 1.18	0.08	1	Referent	0.91	0.08, 10.3	1.35	0.18, 10.1	0.60	

<b><i>BRAF</i> mutation status</b>		(0.57)							(0.07)						
<i>BRAF</i> -wildtype															
N		89		78		176		18		25		215			
Age-adjusted	1	Referent	1.14	0.84, 1.56	0.99	0.76, 1.30	0.75	1	Referent	0.76	0.41, 1.40	0.76	0.46, 1.24	0.16	
Multivariate <sup>d</sup>	1	Referent	1.14	0.83, 1.55	0.98	0.75, 1.29	0.71	1	Referent	0.76	0.41, 1.41	0.75	0.46, 1.23	0.14	
<i>BRAF</i> -mutated															
N		21		24		31		0		2		29			
Age-adjusted	1	Referent	1.46	0.81, 2.65	0.96	0.54, 1.71	0.42	1	Referent	-	-	-	-	0.20	
Multivariate <sup>d</sup>	1	Referent	1.44	0.80, 2.60	0.94	0.53, 1.67	0.41	1	Referent	-	-	-	-	0.22	
<b>DNMT3B expression status</b>		(0.03)							(0.74)						
DNMT3B-negative															
N		59		50		97		13		17		129			
Age-adjusted	1	Referent	1.17	0.80, 1.71	1.16	0.83, 1.64	0.46	1	Referent	0.74	0.36, 1.55	0.83	0.46, 1.48	0.50	
Multivariate <sup>d</sup>	1	Referent	1.15	0.78, 1.68	1.17	0.83, 1.65	0.45	1	Referent	0.75	0.36, 1.56	0.82	0.46, 1.48	0.49	
DNMT3B-positive															
N		14		11		14		1		2		12			
Age-adjusted	1	Referent	1.09	0.49, 2.42	0.59	0.27, 1.28	0.04	1	Referent	1.05	0.09, 11.7	0.77	0.10, 6.05	0.59	
Multivariate <sup>d</sup>	1	Referent	1.09	0.49, 2.44	0.59	0.27, 1.27	0.04	1	Referent	1.02	0.09, 11.3	0.75	0.10, 5.89	0.56	

CI, confidence interval; CIMP, CpG island methylator phenotype; DNMT3B, DNA methyltransferase 3B; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable; N, No. of cases.

<sup>a</sup> All models were stratified with age and sex.

<sup>b</sup> Based on the liner trend test by using the median value of each category. To test whether duration of smoking cessation reduced the cancer risk compared with current smoking, trend test and heterogeneity test were performed on current and past smokers excluding never smokers.

<sup>c</sup> Tests for heterogeneity (for a multivariate HR linear trend) showed significance of association of cessation with colorectal cancer risk by molecular subtypes.

<sup>d</sup> Models were adjusted for body mass index, family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level, alcohol consumption, total calorie intake and red meat intake.

Web Table 6. Smoking Status, Cumulative Pack-years and Incident Colorectal Cancer Risk by Molecular Subtypes<sup>a</sup> in the Nurses' Health Study (1980-2008) or the Health Professionals Follow-up Study (1986-2008)

	Never	Smoking status						Cumulative pack-years						
		Former		Current		$P_{\text{trend}}^b$ ( $P_{\text{heterogeneity}}^c$ )	1-19		20-39		≥40		$P_{\text{trend}}^b$ ( $P_{\text{heterogeneity}}^c$ )	
		HR	95% CI	HR	95% CI		HR	95% CI	HR	95% CI	HR	95% CI		
<b>Women</b>														
Person-years	1,012,758		890,018		381,181			637,996		356,668		249,213		
<b>All colorectal cancer</b>														
N	285		332		101			171		119		129		
Age-adjusted	1	Referent	1.23	1.05, 1.44	1.26	1.00, 1.58	0.01	1.06	0.87, 1.28	1.27	1.03, 1.58	1.46	1.18, 1.80	0.0001
Multivariate <sup>d</sup>	1	Referent	1.23	1.04, 1.45	1.26	0.99, 1.59	0.02	1.06	0.87, 1.28	1.27	1.02, 1.59	1.46	1.17, 1.81	0.0002
<b>CIMP status</b>							(0.08)							(0.01)
CIMP-low/negative														
N	218		255		71			141		92		84		
Age-adjusted	1	Referent	1.25	1.04, 1.50	1.10	0.83, 1.44	0.13	1.13	0.91, 1.40	1.28	1.00, 1.63	1.26	0.98, 1.63	0.04
Multivariate <sup>d</sup>	1	Referent	1.25	1.04, 1.51	1.10	0.83, 1.45	0.14	1.14	0.92, 1.41	1.28	1.00, 1.64	1.26	0.97, 1.63	0.05
CIMP-high														
N	53		65		25			22		24		39		
Age-adjusted	1	Referent	1.22	0.85, 1.76	2.12	1.31, 3.43	0.01	0.75	0.46, 1.24	1.43	0.88, 2.32	2.21	1.46, 3.34	<0.0001
Multivariate <sup>d</sup>	1	Referent	1.23	0.85, 1.77	2.10	1.29, 3.42	0.01	0.76	0.46, 1.25	1.43	0.88, 2.33	2.18	1.44, 3.32	<0.0001
<b>MSI status</b>							(0.18)							(0.02)
MSS														
N	223		255		76			143		89		88		
Age-adjusted	1	Referent	1.22	1.01, 1.45	1.16	0.89, 1.51	0.08	1.12	0.91, 1.38	1.20	0.94, 1.54	1.29	1.00, 1.65	0.04
Multivariate <sup>d</sup>	1	Referent	1.22	1.01, 1.47	1.16	0.89, 1.52	0.09	1.13	0.91, 1.39	1.21	0.94, 1.55	1.28	0.99, 1.66	0.05
MSI-high														
N	48		59		21			20		23		34		
Age-adjusted	1	Referent	1.25	0.85, 1.82	1.91	1.13, 3.22	0.02	0.76	0.45, 1.29	1.53	0.93, 2.51	2.14	1.38, 3.33	<0.0001
Multivariate <sup>d</sup>	1	Referent	1.24	0.85, 1.83	1.90	1.13, 3.22	0.02	0.77	0.45, 1.29	1.52	0.92, 2.50	2.13	1.36, 3.33	0.0001



<b>BRAF mutation status</b>		(0.94)											(0.04)		
<i>BRAF</i> -wildtype															
N		220		258		79		146		90		89			
Age-adjusted	1	Referent	1.24	1.04, 1.49	1.25	0.96, 1.62	0.02	1.16	0.94, 1.43	1.23	0.96, 1.58	1.33	1.04, 1.71	0.02	
Multivariate <sup>d</sup>	1	Referent	1.24	1.03, 1.50	1.25	0.96, 1.63	0.03	1.16	0.94, 1.44	1.23	0.96, 1.58	1.33	1.03, 1.71	0.03	
<i>BRAF</i> -mutated															
N		55		62		19		20		23		36			
Age-adjusted	1	Referent	1.14	0.79, 1.64	1.37	0.81, 2.34	0.24	0.65	0.39, 1.09	1.31	0.81, 2.14	1.95	1.27, 2.98	0.0003	
Multivariate <sup>d</sup>	1	Referent	1.14	0.79, 1.65	1.36	0.80, 2.33	0.25	0.66	0.39, 1.10	1.31	0.80, 2.14	1.93	1.26, 2.97	0.0004	
<b>DNMT3B expression status</b>		(0.15)											(0.17)		
DNMT3B-negative															
N		143		164		61		92		58		68			
Age-adjusted	1	Referent	1.25	1.00, 1.57	1.28	0.94, 1.73	0.05	1.12	0.86, 1.46	1.18	0.87, 1.60	1.51	1.13, 2.02	0.01	
Multivariate <sup>d</sup>	1	Referent	1.26	1.00, 1.58	1.25	0.92, 1.71	0.07	1.13	0.87, 1.48	1.18	0.86, 1.61	1.48	1.09, 1.99	0.01	
DNMT3B-positive															
N		38		21		12		11		9		11			
Age-adjusted	1	Referent	0.57	0.33, 0.97	1.07	0.55, 2.06	0.49	0.50	0.26, 0.99	0.72	0.35, 1.49	0.88	0.45, 1.72	0.75	
Multivariate <sup>d</sup>	1	Referent	0.57	0.33, 0.98	1.02	0.53, 1.99	0.44	0.51	0.26, 1.01	0.71	0.34, 1.49	0.84	0.43, 1.67	0.67	
<b>Men</b>															
Person-years		370,397		388,351		58,327		206,897		154,604		89,204			
<b>All colorectal cancer</b>															
N		205		299		38		129		107		87			
Age-adjusted	1	Referent	1.18	0.98, 1.41	1.31	0.92, 1.87	0.04	1.12	0.90, 1.40	1.08	0.85, 1.37	1.19	0.92, 1.54	0.24	
Multivariate <sup>d</sup>	1	Referent	1.08	0.90, 1.31	1.13	0.79, 1.62	0.36	1.06	0.84, 1.33	0.98	0.77, 1.25	1.04	0.80, 1.36	0.95	
<b>CIMP status</b>		(0.24)											(0.07)		
CIMP-low/negative															
N		159		230		32		103		86		64			
Age-adjusted	1	Referent	1.17	0.95, 1.44	1.39	0.95, 2.04	0.05	1.17	0.91, 1.51	1.12	0.86, 1.46	1.13	0.84, 1.51	0.47	
Multivariate <sup>d</sup>	1	Referent	1.08	0.87, 1.34	1.19	0.80, 1.76	0.33	1.10	0.85, 1.42	1.03	0.78, 1.35	0.98	0.72, 1.33	0.81	
CIMP-high															
N		18		38		6		12		12		17			
Age-adjusted	1	Referent	1.64	0.93, 2.88	2.38	0.93, 6.09	0.04	1.23	0.59, 2.58	1.28	0.61, 2.67	2.31	1.18, 4.53	0.02	
Multivariate <sup>d</sup>	1	Referent	1.50	0.85, 2.64	1.93	0.74, 5.00	0.10	1.18	0.56, 2.46	1.11	0.53, 2.34	2.00	1.01, 3.95	0.06	

<b>MSI status</b>		(0.04)							(0.01)						
<b>MSS</b>															
N		177		249		32		111		86		71			
Age-adjusted	1	Referent	1.12	0.92, 1.37	1.28	0.87, 1.87	0.13	1.11	0.87, 1.41	1.00	0.77, 1.30	1.11	0.84, 1.47	0.66	
Multivariate <sup>d</sup>	1	Referent	1.04	0.85, 1.27	1.11	0.75, 1.64	0.57	1.05	0.82, 1.34	0.92	0.70, 1.20	0.98	0.73, 1.31	0.65	
<b>MSI-high</b>															
N		15		39		6		14		14		16			
Age-adjusted	1	Referent	2.12	1.16, 3.87	3.05	1.17, 7.97	0.01	1.69	0.81, 3.51	1.89	0.91, 3.95	3.06	1.49, 6.29	0.003	
Multivariate <sup>d</sup>	1	Referent	1.95	1.06, 3.56	2.55	0.97, 6.72	0.02	1.62	0.78, 3.38	1.68	0.80, 3.51	2.70	1.31, 5.58	0.01	
<b>BRAF mutation status</b>		(0.36)							(0.14)						
<b>BRAF-wildtype</b>															
N		184		264		35		115		97		75			
Age-adjusted	1	Referent	1.15	0.95, 1.39	1.37	0.95, 1.98	0.06	1.11	0.88, 1.40	1.08	0.84, 1.39	1.14	0.87, 1.50	0.39	
Multivariate <sup>d</sup>	1	Referent	1.07	0.87, 1.30	1.19	0.82, 1.74	0.34	1.05	0.83, 1.34	0.99	0.77, 1.28	1.01	0.76, 1.35	0.95	
<b>BRAF-mutated</b>															
N		12		27		3		11		5		12			
Age-adjusted	1	Referent	1.86	0.94, 3.71	1.59	0.44, 5.73	0.13	1.64	0.72, 3.76	0.88	0.31, 2.50	2.64	1.16, 5.99	0.07	
Multivariate <sup>d</sup>	1	Referent	1.73	0.87, 3.45	1.42	0.39, 5.13	0.21	1.58	0.69, 3.62	0.79	0.27, 2.26	2.37	1.04, 5.42	0.12	
<b>DNMT3B expression status</b>		(0.56)							(0.04)						
<b>DNMT3B-negative</b>															
N		95		145		12		68		46		35			
Age-adjusted	1	Referent	1.26	0.96, 1.63	0.81	0.44, 1.48	0.50	1.34	0.98, 1.84	1.02	0.71, 1.45	1.00	0.67, 1.48	0.69	
Multivariate <sup>d</sup>	1	Referent	1.18	0.90, 1.54	0.71	0.39, 1.32	0.96	1.29	0.93, 1.77	0.95	0.66, 1.37	0.89	0.59, 1.33	0.32	
<b>DNMT3B-positive</b>															
N		14		18		5		4		7		12			
Age-adjusted	1	Referent	0.99	0.49, 2.01	2.21	0.78, 6.23	0.32	0.51	0.17, 1.56	0.97	0.39, 2.43	2.23	1.01, 4.90	0.03	
Multivariate <sup>d</sup>	1	Referent	0.89	0.44, 1.81	1.91	0.67, 5.44	0.52	0.48	0.16, 1.46	0.86	0.34, 2.16	1.92	0.86, 4.28	0.07	

CI, confidence interval; CIMP, CpG island methylator phenotype; DNMT3B, DNA methyltransferase 3B; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable; N, No. of cases.

<sup>a</sup> All models were stratified with age.

<sup>b</sup> Based on the liner trend test by using the median value of each category.

<sup>c</sup> Tests for heterogeneity (for a multivariate HR linear trend) of the associations of smoking with one molecular subtype versus the other molecular subtype (i.e., CIMP-low/negative vs. CIMP-high; MSS vs. MSI-high; *BRAF*-wildtype vs. *BRAF*-mutated; DNMT3B-negative vs. DNMT3B-positive).

<sup>d</sup> Models were adjusted for body mass index, family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level, alcohol consumption, total caloric intake and red meat intake.

Web Table 7. Pack-years Smoked Before Age 30, Age at Start Smoking and Incident Colorectal Cancer Risk by Molecular Subtypes<sup>a</sup> in the Nurses' Health Study (1980-2008) or the Health Professionals Follow-up Study (1986-2008)

	Never	Pack-years smoked before age 30					Age at start of smoking					
		HR	1-9		≥10		$P_{\text{trend}}^b$ ( $P_{\text{heterogeneity}}^c$ )	≥20		<20		$P_{\text{trend}}^b$ ( $P_{\text{heterogeneity}}^c$ )
		HR	95% CI	HR	95% CI		HR	95% CI	HR	95% CI		
<b>Women</b>												
Person-years	1,012,758		929,803		282,366			532,947		737,349		
<b>All colorectal cancer</b>												
N	285		306		96			199		226		
Age-adjusted	1	Referent	1.19	1.01, 1.39	1.21	0.96, 1.53	0.05	1.22	1.02, 1.47	1.19	0.99, 1.41	0.04
Multivariate <sup>d</sup>	1	Referent	1.19	1.00, 1.40	1.20	0.95, 1.52	0.07	1.22	1.02, 1.47	1.18	0.99, 1.42	0.06
<b>CIMP status</b>												
						(0.58)					(0.52)	
CIMP-low/negative												
N	218		232		71			153		169		
Age-adjusted	1	Referent	1.17	0.98, 1.41	1.16	0.89, 1.52	0.16	1.25	1.01, 1.53	1.14	0.93, 1.40	0.15
Multivariate <sup>d</sup>	1	Referent	1.18	0.97, 1.42	1.15	0.88, 1.52	0.20	1.25	1.01, 1.54	1.14	0.93, 1.41	0.17
CIMP-high												
N	53		63		19			41		45		
Age-adjusted	1	Referent	1.32	0.92, 1.91	1.33	0.78, 2.24	0.18	1.30	0.86, 1.95	1.33	0.89, 1.99	0.15
Multivariate <sup>d</sup>	1	Referent	1.33	0.92, 1.92	1.32	0.78, 2.24	0.20	1.30	0.86, 1.96	1.33	0.89, 1.99	0.16
<b>MSI status</b>												
						(0.73)					(0.48)	
MSS												
N	223		234		72			157		168		
Age-adjusted	1	Referent	1.16	0.96, 1.39	1.15	0.88, 1.50	0.20	1.24	1.01, 1.53	1.11	0.91, 1.36	0.23
Multivariate <sup>d</sup>	1	Referent	1.16	0.96, 1.40	1.14	0.87, 1.49	0.26	1.25	1.01, 1.54	1.11	0.90, 1.37	0.27
MSI-high												
N	48		60		15			38		40		
Age-adjusted	1	Referent	1.41	0.96, 2.06	1.17	0.65, 2.10	0.33	1.34	0.87, 2.05	1.32	0.86, 2.01	0.18
Multivariate <sup>d</sup>	1	Referent	1.40	0.95, 2.05	1.17	0.65, 2.10	0.35	1.33	0.87, 2.05	1.32	0.86, 2.02	0.19

<b><i>BRAF</i> mutation status</b>							(0.80)					(0.47)
<i>BRAF</i> -wildtype												
N		220		236		74			153		177	
Age-adjusted	1	Referent	1.19	0.99, 1.43	1.20	0.92, 1.57	0.10	1.23	1.00, 1.51	1.20	0.98, 1.46	0.06
Multivariate <sup>d</sup>	1	Referent	1.18	0.98, 1.43	1.19	0.91, 1.56	0.14	1.23	0.99, 1.51	1.19	0.97, 1.46	0.08
<i>BRAF</i> -mutated												
N		55		61		16			44		36	
Age-adjusted	1	Referent	1.22	0.85, 1.76	1.06	0.60, 1.85	0.61	1.37	0.92, 2.03	0.98	0.64, 1.50	0.90
Multivariate <sup>d</sup>	1	Referent	1.22	0.84, 1.76	1.05	0.60, 1.84	0.64	1.37	0.92, 2.04	0.98	0.64, 1.50	0.93
<b>DNMT3B expression status</b>							(0.09)					(0.02)
DNMT3B-negative												
N		143		162		46			103		118	
Age-adjusted	1	Referent	1.22	0.98, 1.53	1.16	0.83, 1.62	0.22	1.23	0.95, 1.58	1.23	0.96, 1.57	0.09
Multivariate <sup>d</sup>	1	Referent	1.21	0.96, 1.53	1.13	0.81, 1.59	0.31	1.22	0.94, 1.58	1.21	0.94, 1.56	0.12
DNMT3B-positive												
N		38		22		8			17		15	
Age-adjusted	1	Referent	0.64	0.38, 1.09	0.70	0.32, 1.51	0.20	0.76	0.43, 1.36	0.58	0.32, 1.05	0.07
Multivariate <sup>d</sup>	1	Referent	0.64	0.37, 1.08	0.68	0.32, 1.48	0.18	0.77	0.43, 1.36	0.57	0.31, 1.03	0.06
<b>Men</b>												
Person-years		370,397		155,258		278,104			211,436		239,430	
<b>All colorectal cancer</b>												
N		205		108		204			148		176	
Age-adjusted	1	Referent	1.06	0.84, 1.34	1.15	0.95, 1.40	0.08	1.05	0.85, 1.30	1.20	0.98, 1.48	0.08
Multivariate <sup>d</sup>	1	Referent	0.99	0.78, 1.25	1.03	0.84, 1.26	0.50	0.97	0.78, 1.21	1.09	0.88, 1.34	0.46
<b>CIMP status</b>							(0.06)					(0.12)
CIMP-low/negative												
N		159		90		151			121		132	
Age-adjusted	1	Referent	1.14	0.88, 1.48	1.10	0.88, 1.38	0.30	1.11	0.87, 1.41	1.17	0.93, 1.48	0.18
Multivariate <sup>d</sup>	1	Referent	1.06	0.81, 1.38	0.99	0.78, 1.24	0.93	1.03	0.81, 1.32	1.05	0.83, 1.34	0.67
CIMP-high												
N		18		11		31			16		26	
Age-adjusted	1	Referent	1.27	0.60, 2.71	2.00	1.11, 3.59	0.02	1.22	0.62, 2.41	1.96	1.07, 3.60	0.03
Multivariate <sup>d</sup>	1	Referent	1.16	0.54, 2.48	1.77	0.98, 3.20	0.04	1.11	0.56, 2.19	1.75	0.95, 3.21	0.07

<b>MSI status</b>							(0.02)					(0.22)
<b>MSS</b>												
N		177		89		168			118		150	
Age-adjusted	1	Referent	1.00	0.77, 1.29	1.09	0.88, 1.35	0.28	0.96	0.76, 1.22	1.18	0.94, 1.47	0.17
Multivariate <sup>d</sup>	1	Referent	0.93	0.72, 1.21	0.99	0.80, 1.24	0.84	0.90	0.70, 1.14	1.07	0.85, 1.35	0.59
<b>MSI-high</b>												
N		15		15		30			25		20	
Age-adjusted	1	Referent	2.17	1.05, 4.47	2.41	1.29, 4.51	0.003	2.46	1.29, 4.70	1.86	0.95, 3.65	0.07
Multivariate <sup>d</sup>	1	Referent	2.03	0.98, 4.18	2.18	1.16, 4.10	0.01	2.29	1.20, 4.38	1.68	0.85, 3.31	0.13
<b>BRAF mutation status</b>							(0.09)					(0.05)
<b>BRAF-wildtype</b>												
N		184		98		179			136		151	
Age-adjusted	1	Referent	1.07	0.83, 1.37	1.13	0.92, 1.38	0.16	1.07	0.86, 1.34	1.14	0.92, 1.42	0.24
Multivariate <sup>d</sup>	1	Referent	1.00	0.78, 1.28	1.02	0.83, 1.27	0.63	1.00	0.80, 1.26	1.04	0.83, 1.30	0.73
<b>BRAF-mutated</b>												
N		12		7		21			9		20	
Age-adjusted	1	Referent	1.24	0.49, 3.14	1.92	0.95, 3.90	0.03	1.07	0.45, 2.56	2.36	1.15, 4.87	0.02
Multivariate <sup>d</sup>	1	Referent	1.18	0.47, 3.00	1.74	0.85, 3.55	0.06	1.01	0.42, 2.42	2.15	1.04, 4.45	0.03
<b>DNMT3B expression status</b>							(0.64)					(0.89)
<b>DNMT3B-negative</b>												
N		95		60		85			76		73	
Age-adjusted	1	Referent	1.26	0.91, 1.74	1.05	0.78, 1.40	0.65	1.19	0.88, 1.62	1.09	0.80, 1.49	0.53
Multivariate <sup>d</sup>	1	Referent	1.20	0.86, 1.67	0.96	0.71, 1.29	0.82	1.13	0.83, 1.54	1.00	0.73, 1.38	0.95
<b>DNMT3B-positive</b>												
N		14		5		16			10		13	
Age-adjusted	1	Referent	0.75	0.27, 2.08	1.20	0.59, 2.45	0.43	0.99	0.44, 2.25	1.24	0.58, 2.66	0.59
Multivariate <sup>d</sup>	1	Referent	0.68	0.24, 1.88	1.05	0.51, 2.16	0.69	0.92	0.40, 2.09	1.08	0.50, 2.33	0.86

CI, confidence interval; CIMP, CpG island methylator phenotype; DNMT3B, DNA methyltransferase 3B; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable; N, No. of cases.

<sup>a</sup> All models were stratified with age.

<sup>b</sup> Based on the liner trend test by using the median value of each category.

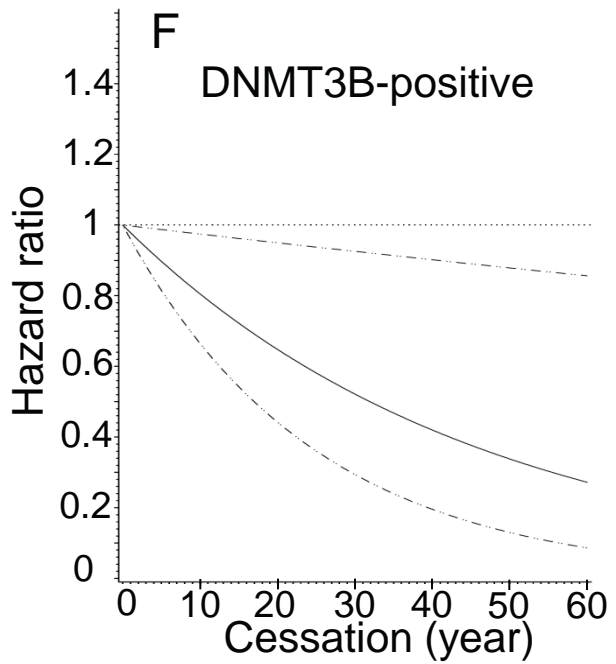
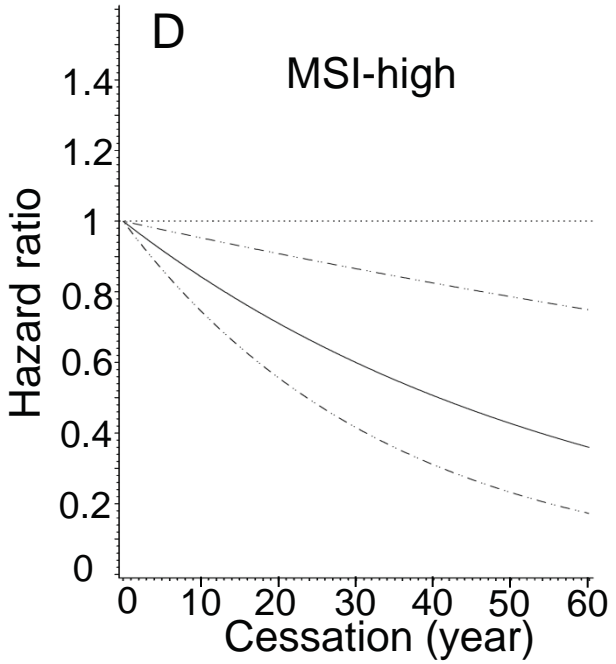
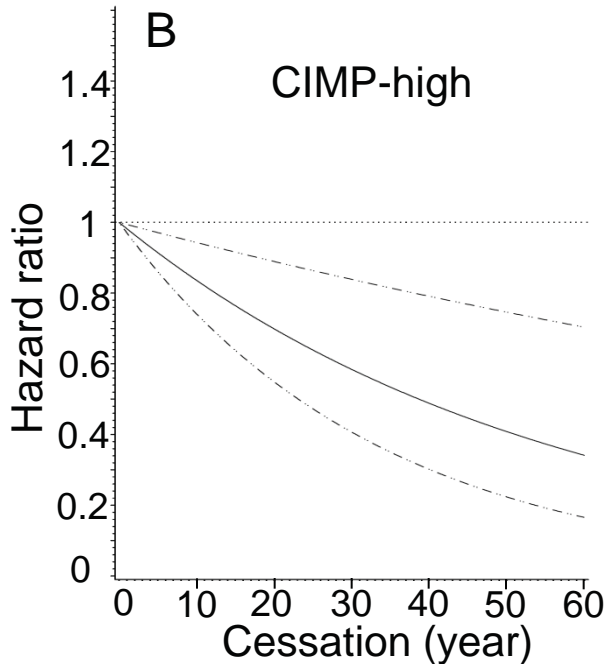
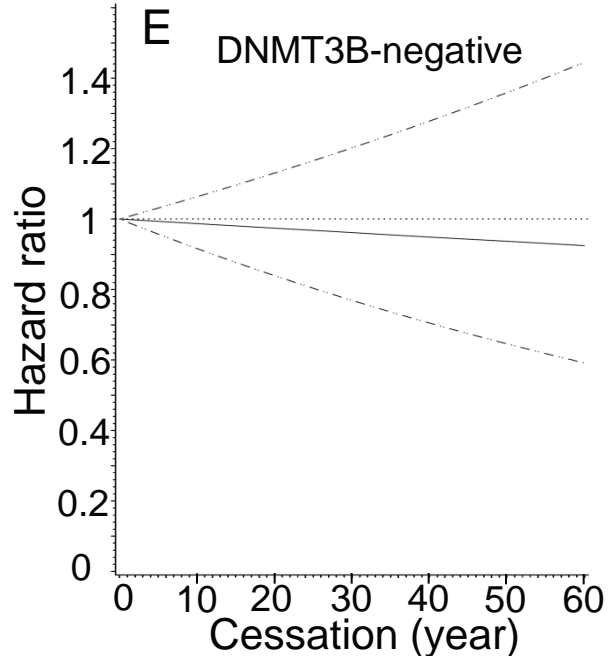
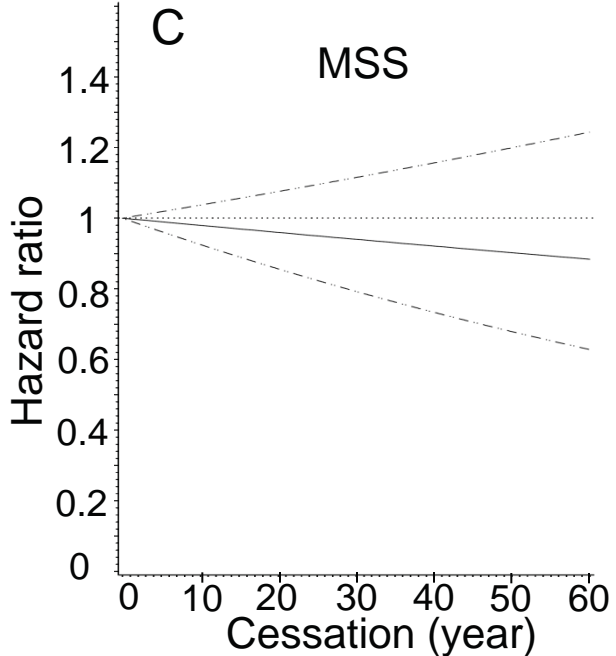
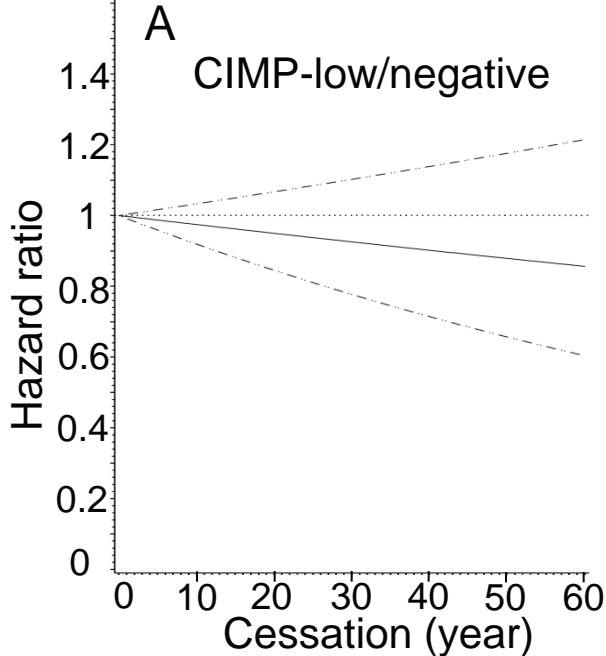
<sup>c</sup> Tests for heterogeneity (for a multivariate HR linear trend) of the associations of smoking with one molecular subtype versus the other molecular subtype (i.e., CIMP-low/negative vs. CIMP-high; MSS vs. MSI-high; *BRAF*-wildtype vs. *BRAF*-mutated; DNMT3B-negative vs. DNMT3B-positive).

<sup>d</sup> Models were adjusted for body mass index, family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level, alcohol consumption, total caloric intake and red meat intake.

## Web Figure legend

Web Figure 1. Restricted cubic splines to demonstrate the relationship between smoking cessation duration (year) and colorectal cancer risk by molecular subtypes in the Nurses' Health Study (1980-2008) and the Health Professionals Follow-up Study (1986-2008). Multivariate hazards ratio (the solid line) with 95% confidence interval (the dashed lines) is shown. (A) CIMP-low/negative colorectal cancer risk. (B) CIMP-high colorectal cancer risk. (C) MSS colorectal cancer risk. (D) MSI-high colorectal cancer risk. (E) DNMT3B-negative colorectal cancer risk. (F) DNMT3B-positive colorectal cancer risk.

CIMP, CpG island methylator phenotype; DNMT3B, DNA methyltransferase 3B; MSI, microsatellite instability; MSS, microsatellite stable.



**Web Figure 1**