Supplemental Table 1. Clinical and molecular characteristics of colon cancer according to LINE-1 methylation level*

Characteristic	All patients	N (%) LINE-1 methylation level				P
		≥75%	60–75%	45–60%	<45%	
Total N	643	40	334	238	31	
Sex (cohort)						.10
Male (Health Professionals Follow-up Study)	282 (44)	25 (63)	145 (43)	100 (42)	12 (39)	
Female (Nurses' Health Study)	361 (56)	15 (37%)	189 (57)	138 (58)	19 (61)	
Mean age ± SD, y	66.6 ± 8.3	68.2 ± 7.6	66.9 ± 8.3	66.0 ± 7.9	66.0 ± 7.9	.11
Year of diagnosis						.07
Before 1990	99 (15)	3 (7.5)	49 (15)	37 (16)	10 (32)	
1990–1999	468 (73)	30 (75)	242 (72)	176 (74)	20 (65)	
2000–2002	76 (12)	7 (18)	43 (13)	25 (11)	1 (3.2)	
Tumor location†						.10
Proximal	369 (58)	24 (62)	206 (62)	124 (53)	15 (50)	
Distal	265 (42)	15 (38)	124 (38)	111 (47)	15 (50)	
Tumor stage						.05
I	131 (20)	7 (18)	63 (19)	57 (24)	4 (13)	
II	221 (34)	15 (38)	134 (40)	62 (26)	10 (32)	
III	164 (26)	10 (25)	75 (22)	70 (29)	9 (29)	
IV	84 (13)	6 (15)	38 (11)	32 (13)	8 (26)	
Unknown	43 (6.7)	2 (5.0)	24 (7.2)	17 (7.1)	0	
Tumor grade						.36
Low	570 (89)	32 (80)	297 (89)	216 (91)	25 (81)	
High	71 (11)	8 (20)	36 (11)	21 (8.9)	6 (19)	
CIMP						<.001
CIMP-0 (no methylated promoters)	268 (42)	12 (30)	121 (36)	119 (50)	16 (52)	
CIMP-low (1/8–5/8 methylated promoters)	252 (39)	13 (33)	137 (41)	89 (37)	13 (42)	
CIMP-high (≥6/8 methylated promoters)	123 (19)	15 (38)	76 (23)	30 (13)	2 (6.5)	

MSI						<.001
MSS (no unstable marker)	460 (73)	22 (58)	227 (70)	186 (78)	25 (83)	
MSI-low (1–30% unstable markers)	52 (8.3)	2 (5.3)	21 (6.5)	27 (11)	2 (6.7)	
MSI-high (≥30% unstable markers)	118 (19)	14 (37)	76 (23)	25 (11)	3 (10)	
Chromosomal instability (CIN)‡						.01
Negative	118 (27)	11 (48)	66 (30)	36 (21)	5 (28)	
1+	211 (49)	9 (39)	102 (47)	94 (54)	6 (33)	
2+	91 (21)	3 (13)	45 (21)	39 (22)	4 (22)	
3–4+	14 (3.2)	0	6 (2.7)	5 (2.9)	3 (17)	
p53 expression						.08
Negative	388 (61)	31 (78)	205 (62)	133 (56)	19 (61)	
Positive	249 (39)	9 (22)	125 (38)	103 (44)	12 (39)	
BRAF mutation						.004
Negative	519 (84)	23 (62)	264 (82)	206 (89)	26 (87)	
Positive	100 (16)	14 (38)	57 (18)	25 (11)	4 (13)	
KRAS mutation						.73
Negative	399 (63)	21 (55)	207 (63)	150 (63)	21 (68)	
Positive	234 (37)	17 (45)	120 (37)	87 (37)	10 (32)	

*CIMP = CpG island methylator phenotype; CIN = chromosomal instability; LINE-1 = long interspersed nucleotide element-1; MSI = microsatellite instability; MSS = microsatellite stable; SD = standard deviation. Tumor stage according to American Joint Commission on Cancer (AJCC) criteria. Low and high tumor grades are defined as the presence of ≥50% glandular areas and the presence of <50% glandular areas, respectively. *P* values (two-sided) were calculated using the chi-square test.

†Proximal colon includes cecum to transverse colon, and distal colon includes splenic flexure to sigmoid colon.

‡The number of chromosome segments positive for loss of homozygosity (among 18q, 2p, 5q and 17q).

Supplementary figure legends.

Supplemental Figure 1. Distribution of age and LINE-1 methylation level in colon cancer. Tumor samples taken from participants in the Health Professionals Follow-up Study (n=282) and the Nurses' Health study (n=361) were assayed for LINE-1 methylation by pyrosequencing. P (two-sided) was calculated using the chi square test, and correlation coefficient r was calculated using linear regression analysis**.**

Supplemental Figure 2. Receiver operator characteristics (ROC) curve for LINE-1 methylation as a diagnostic test to predict 5-year colon cancer–specific survival (dead vs alive). **Solid line**, ROC curve; **hatched line**, diagonal line for an imaginary test with no predictive value**.**

Supplemental Figure 3. Stratified analysis of colon cancer–specific mortality. Log_e(adjusted hazard ratio [HR]) with 95% confidence intervals (CIs) for a 30% decline in LINE-1 methylation are shown. $P_{interaction}$ (two-sided) values were calculated using the likelihood ratio test. CIMP = CpG island methylator phenotype; CIN = chromosomal instability; HPFS = Health Professionals Follow-up Study; MSI = microsatellite instability; MSS = microsatellite stable; NHS = Nurses' Health Study. * p53 status was determined by immunohistochemistry.