

Global Methylome Scores Correlate with Histological Subtypes of Colorectal Carcinoma and Show Different Associations with Common Clinical and Molecular Features

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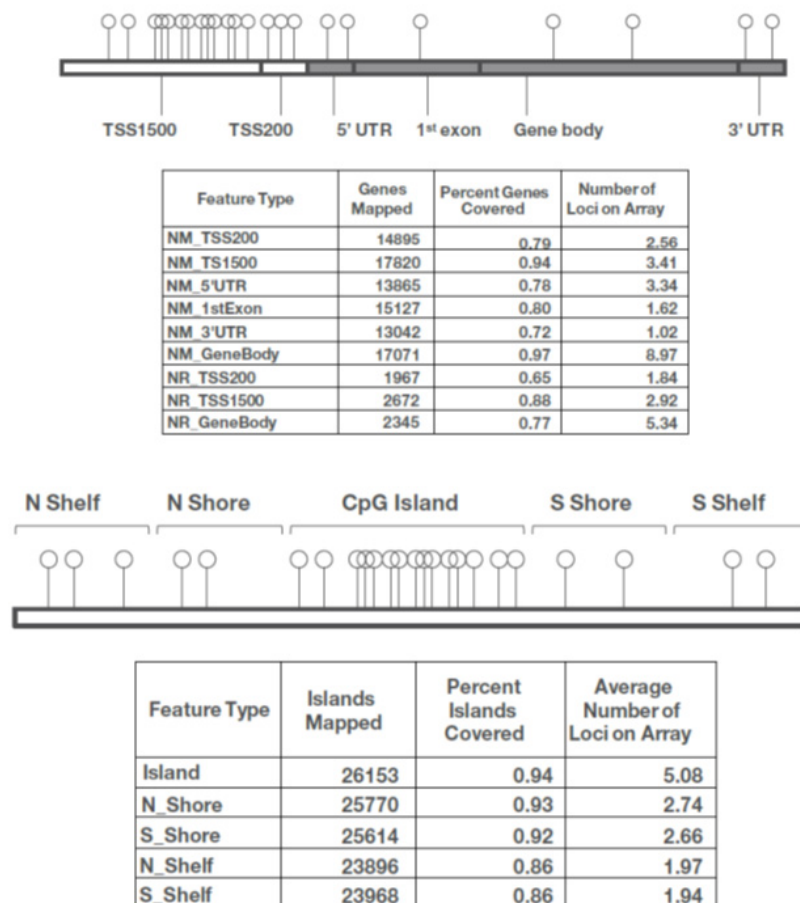


Figure S1. These regions were defined based on the manifest and annotation data from the R-packages "IlluminaHumanMethylation450kmanifest" [25] and "IlluminaHumanMethylation450kanno.ilmn12.hg19" [36].

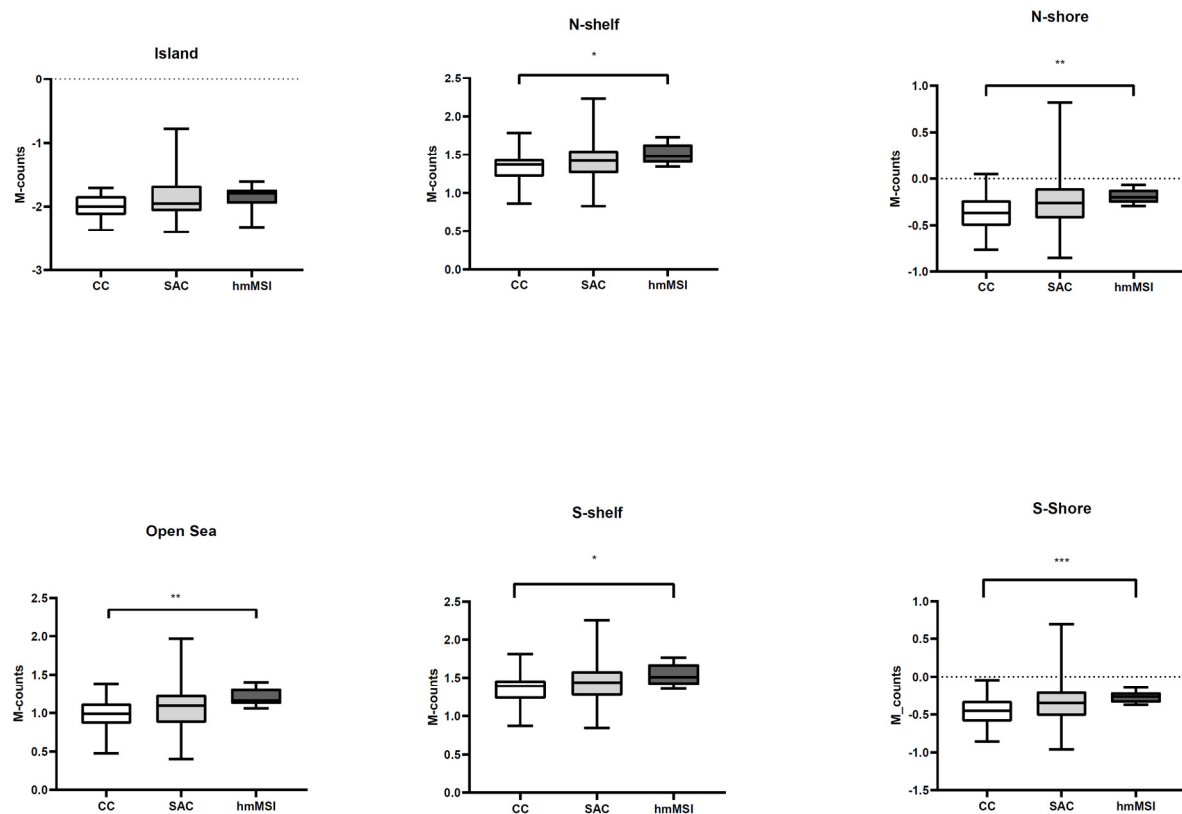


Figure S2. Boxplot graphs showing the methylation differences in the regions referred to CpG islands amongst the different histological CRC subtypes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

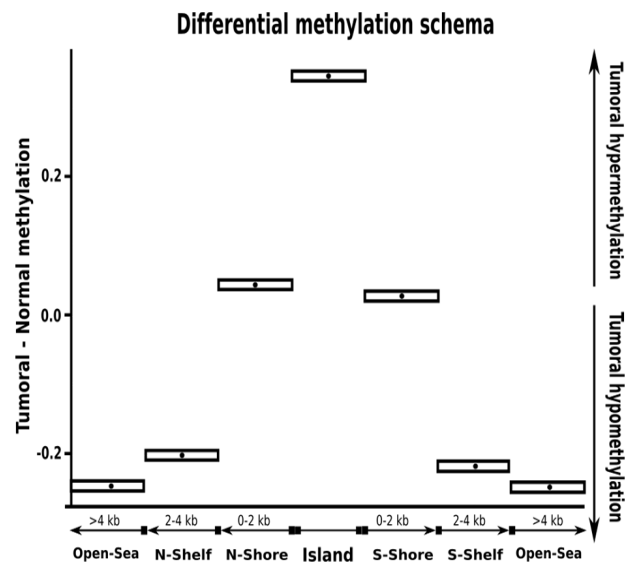


Figure S3. Differential methylation schema (tumoral CRC M-value–normal adjacent colorectal mucosa M-value) showing the tumor-associated hyper- and hypomethylation according to the genome locations with respect to the CpG island.

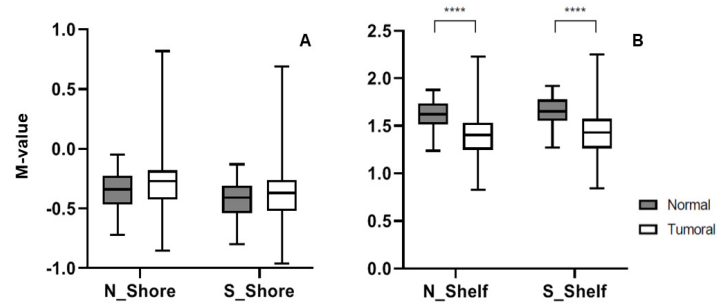


Figure S4. Comparison between normal and tumoral methylation in North and South directions of Shores (A) and Shelves (B) areas with respect to CpG island. **** $p < 0.0001$.

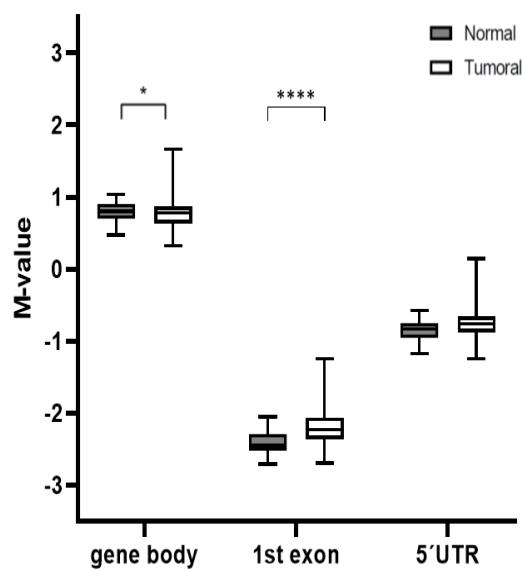


Figure S5. Comparison between normal and tumoral methylation in different gene-related locations; i.e. 5'Untranslated Region (5'UTR), First exon and gene body * $p > 0.05$, **** $p < 0.0001$.

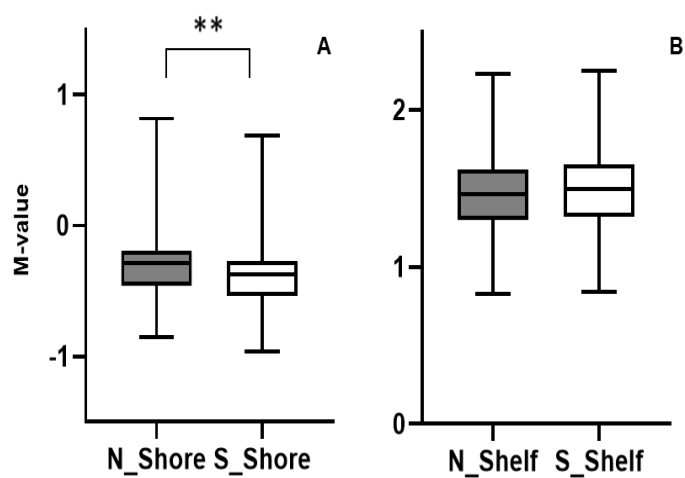


Figure S6. Comparison between normal and tumoral methylation in different locations; North and South directions of Shores (A) and Shelves (B) areas with respect to CpG island. ** $p < 0.01$

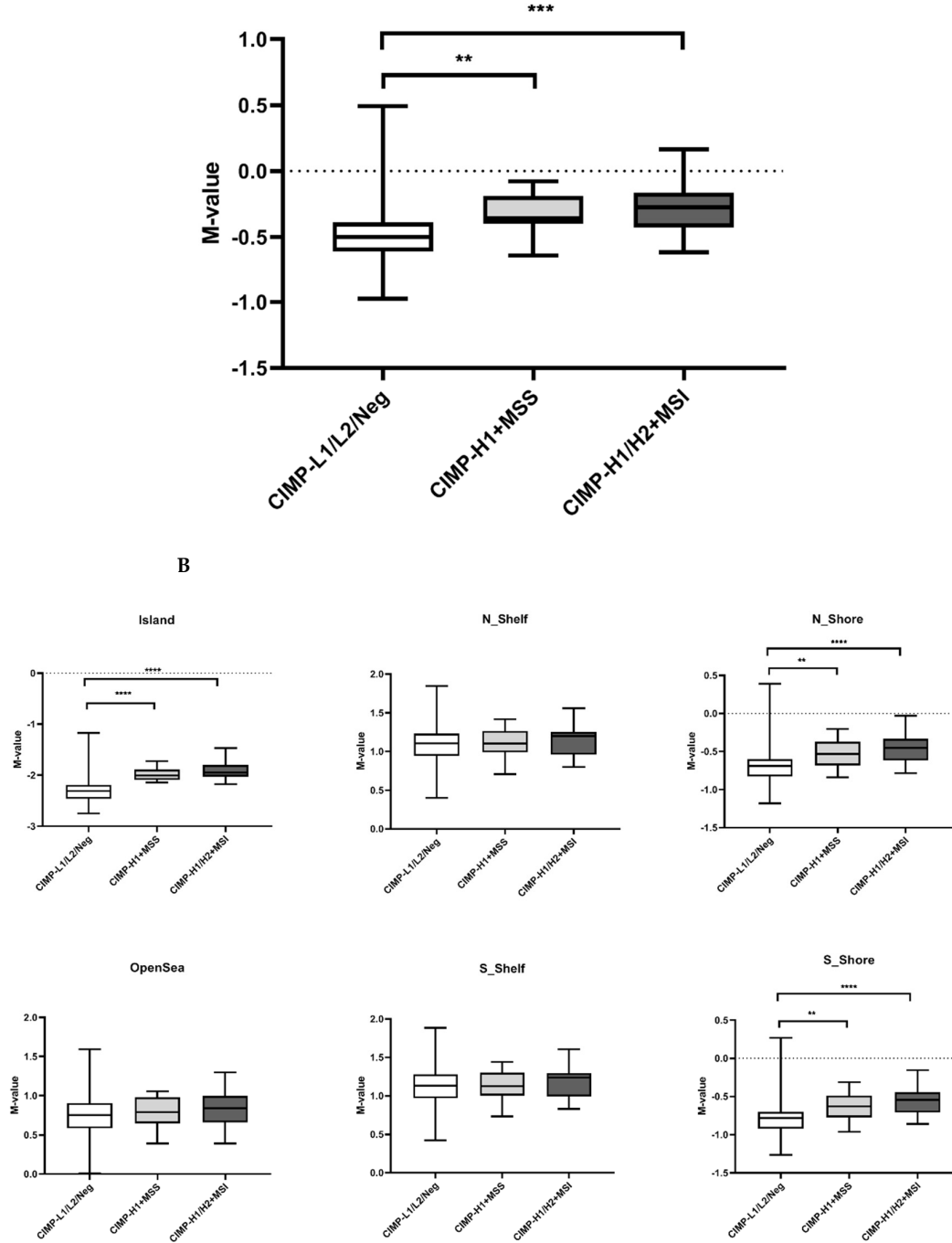


Figure S7. Validation of global methylation patterns in an independent series. (A), boxplot graph showing the global methylation level amongst the groups enriched in CC (CIMP-L1-L2-Neg/CIMP-H2/MSS+), SAC (CIMP-H1/MSS+), and hmMSI-H (CIMP-H1-H2/MSI+) histological subtypes. Kruskal Wallis test **** $p < 0.0001$. Mann Whitney Test (graphs) ** $p < 0.01$, *** $p < 0.001$ (B), boxplot graphs showing the methylation differences in the regions referred to CpG islands amongst the groups enriched in CC (CIMP-L1-L2-Neg/CIMP-H2/MSS+), SAC (CIMP-H1/MSS+), and hmMSI-H (CIMP-H1-H2/MSI+) histological subtypes ** $p < 0.01$, **** $p < 0.0001$.

Table S1. Association of global methylation scores of adjacent normal mucosa specimens with histopathological features.

			250 bp			1 kb			2 kb		
		<i>n</i>	Median	IQR	<i>p</i>	Median	IQR	<i>p</i>	Median	IQR	<i>p</i>
Status	normal	35	0.2660	0.0034	0.0100 *	0.4224	0.0083	<0.0001 *	0.6345	0.0157	<0.0001 *
	tumoral	82	0.2698	0.0106		0.4106	0.0211		0.5943	0.0309	
Type	CC										
	normal	14	0.2664	0.0033	0.8110	0.4232	0.0054	<0.0001 *	0.6358	0.0077	<0.0001 *
	tumoral	32	0.2653	0.0092		0.4051	0.0198		0.5871	0.0271	
	SAC										
	normal	15	0.2662	0.0035	0.0160	0.4222	0.0113	0.0010 *	0.6360	0.0274	<0.0001 *
	tumoral	40	0.2705	0.0104		0.4121	0.0205		0.5933	0.0341	
	MSI										
	normal	6	0.2646	0.0073	0.0050 *	0.4208	0.0102	0.5620	0.6272	0.0222	0.0930
	tumoral	10	0.2764	0.0095		0.4237	0.0142		0.6132	0.0347	

Note: *p*-values for Mann-Whitney's *U* test. Chi-squared tests applied for testing the bivariate associations of each variable with type of tumor. The Bonferroni correction for multiple comparisons was applied. Associations with type of tumor are considered significant (marked with *) if $p < 0.05/4 = 0.0125$. CC: Conventional Carcinoma, SAC: Serrated adenocarcinoma, hmMSI-H: Colorectal cancer showing histological and molecular features of high level of microsatellite instability.

Table S2. Associations between global methylation scores with the methylation status of the genes included in the CIMP panel.

			250 bp			1 kb			2 kb		
		<i>n</i>	Median	IQR	<i>p</i>	Median	IQR	<i>p</i>	Median	IQR	<i>p</i>
CAGNA1G	Unmet	55	0.2681	0.0105	0.0530	0.4079	0.0210	0.2430	0.5902	0.0316	0.3640
	Met	25	0.2721	0.0115		0.4152	0.0219		0.5974	0.0301	
CDKN2A	Unmet	39	0.2665	0.0084	0.0110	0.4066	0.0219	0.5220	0.5900	0.0276	0.7250
	Met	41	0.2721	0.0109		0.4152	0.0236		0.5970	0.0334	
CRABP1	Unmet	39	0.2676	0.0098	0.1270	0.4095	0.0225	0.9190	0.5939	0.0334	0.6000
	Met	41	0.2709	0.0128		0.4115	0.0220		0.5939	0.0301	
IGF2	Unmet	14	0.2656	0.0089	0.1840	0.4093	0.0245	0.9600	0.5981	0.0408	0.5020
	Met	66	0.2702	0.0115		0.4114	0.0210		0.5939	0.0281	
MLH1	Unmet	59	0.2676	0.0101	0.0260	0.4079	0.0214	0.2110	0.5900	0.0313	0.4600
	Met	21	0.2731	0.0104		0.4152	0.0197		0.5974	0.0281	
NEUROG1	Unmet	22	0.2667	0.0096	0.1060	0.4093	0.0238	0.8800	0.5952	0.0327	0.6130
	Met	58	0.2702	0.0114		0.4122	0.0210		0.5933	0.0309	
RUNX3	Unmet	48	0.2686	0.0100	0.1300	0.4085	0.0222	0.8440	0.5939	0.0319	0.6510
	Met	32	0.2721	0.0143		0.4135	0.0211		0.5941	0.0311	
SOCS1	Unmet	78	0.2698	0.0106	0.4110	0.4106	0.0215	0.7970	0.5943	0.0309	0.2410
	Met	2	0.2747			0.4073			0.5747		

Note: *p*-values for Mann-Whitney's *U* test. The Bonferroni correction for multiple comparisons was applied for each site; *p* values $< 0.05/8 = 0.0063$ were considered statistically significant.