

LINE-1 hypomethylation in familial and sporadic cancer

Walter Pavicic, Emmi I. Joensuu, Taina Nieminen, and Päivi Peltomäki
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Supplementary Table S1

Average methylation dosage ratios and standard deviations for tumor and normal tissues shown in Fig. 3.

Tissue category	No.	LINE-1 Methylation	Average no. of hypermethylated TSG out of 24 [§]
Sporadic MSS-CRC	55	0.85 (0.06) [‡]	3.4
Normal tissue	55	0.93 (0.02)	0.2
P-value *		<0.001	<0.001
Sporadic MSI-CRC	52	0.87 (0.05)	7.3
Normal tissue	52	0.91 (0.04)	0.2
P-value		<0.001	<0.001
LS-CRC	43	0.84 (0.06)	3.8
Normal tissue	43	0.90 (0.05)	1.2
P-value		<0.001	0.001
FCCX	18	0.80 (0.08)	3.9
Normal tissue	18	0.84 (0.06)	2.1
P-value		0.03	0.004
Sporadic MSS-GC	34	0.79 (0.12)	2
Normal tissue	34	0.90 (0.05)	2.6
P-value		<0.001	<i>ns</i>
Sporadic MSI-GC	11	0.88 (0.04)	6.1
Normal tissue	11	0.90 (0.04)	2.3
P-value		<i>ns</i>	0.001
LS-GC	13	0.86 (0.05)	4.1
Normal tissue	13	0.90 (0.05)	1.2
P-value		0.04	<0.001
LS-EC	50	0.88 (0.07)	3
Normal tissue	50	0.90 (0.07)	1.3
P-value		<i>ns</i>	<0.001

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[‡] Number in parenthesis, standard deviation.

* Statistical significance for tumor vs. normal comparisons was determined with T-test or Wilcoxon test and significant values are in bold.

ns, non-significant

[§] Data from Gylling et al. [18], Joensuu et al. [19], and Nieminen et al. [20].

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Supplementary Table S2

Average methylation dosage ratios and standard deviations for tumor and normal tissues shown in Fig. 4.

Tissue category	No.	LINE-1 Methylation
LS-CRC	27	0.84 (0.06) [‡]
<u>N</u> ormal mucosa	27	0.91 (0.06)
<u>B</u> lood	27	0.95 (0.04)
	T vs. N	<0.001
P-value*	T vs. B	<0.001
	N vs. B	<0.01
FCCX	10	0.80 (0.10)
<u>N</u> ormal mucosa	10	0.85 (0.07)
<u>B</u> lood	10	0.89 (0.11)
	T vs. N	<i>ns</i>
P-value	T vs. B	<0.01
	N vs. B	<i>ns</i>
LS-EC	19	0.91 (0.06)
<u>N</u> ormal endometrium	19	0.93 (0.06)
<u>B</u> lood	19	0.95 (0.03)
	T vs. N	<i>ns</i>
P-value	T vs. B	<0.01
	N vs. B	<i>ns</i>

[‡] Number in parenthesis, standard deviation.

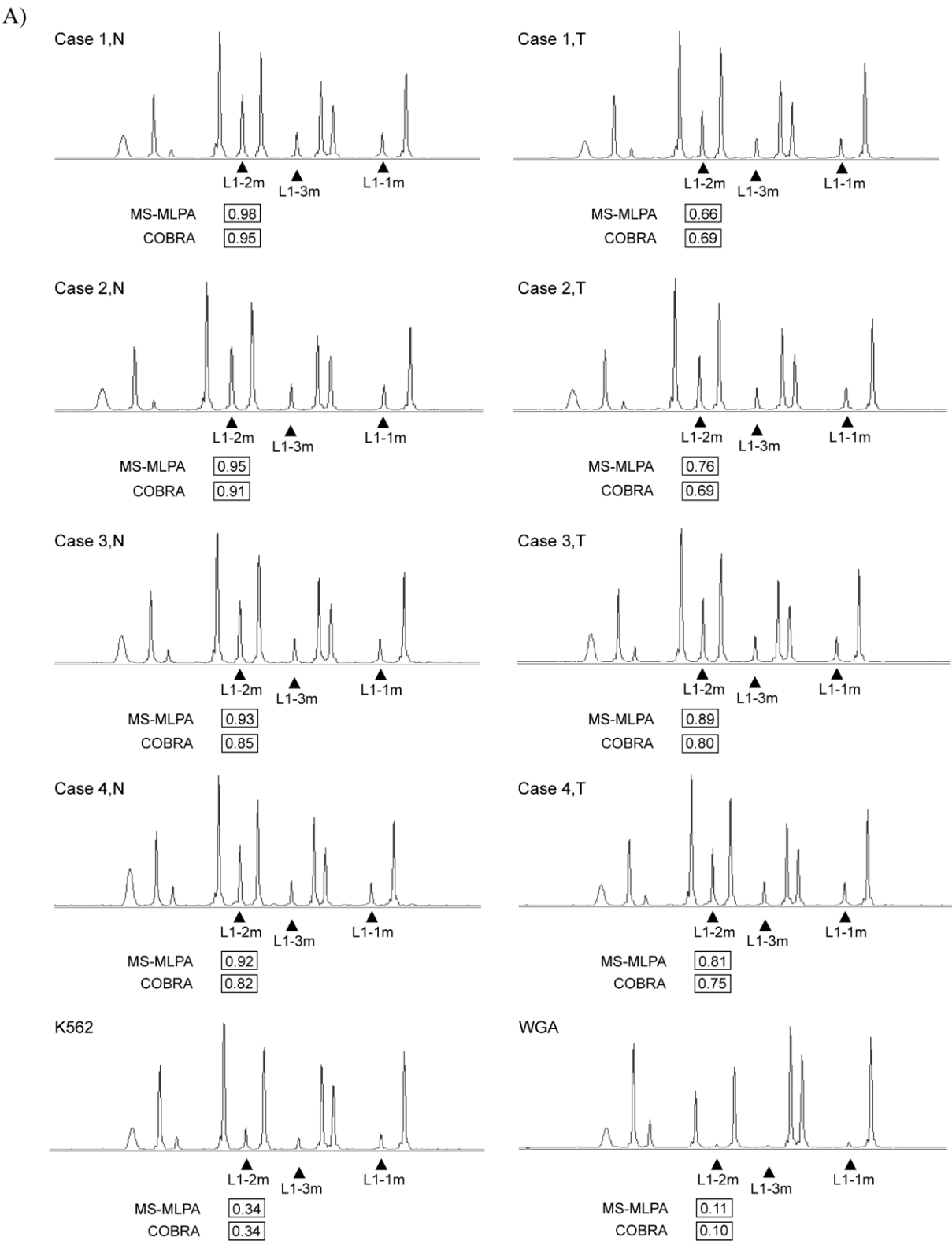
* Statistical significance for tumor vs. normal comparisons was determined with T-test or Wilcoxon test and significant values are in bold.

ns, non-significant.

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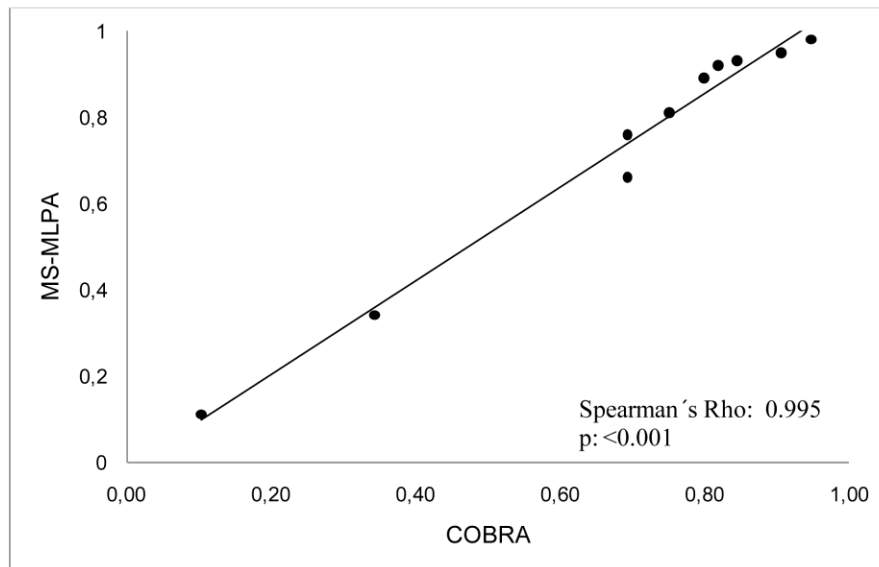
Supplementary Figure 1



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B)



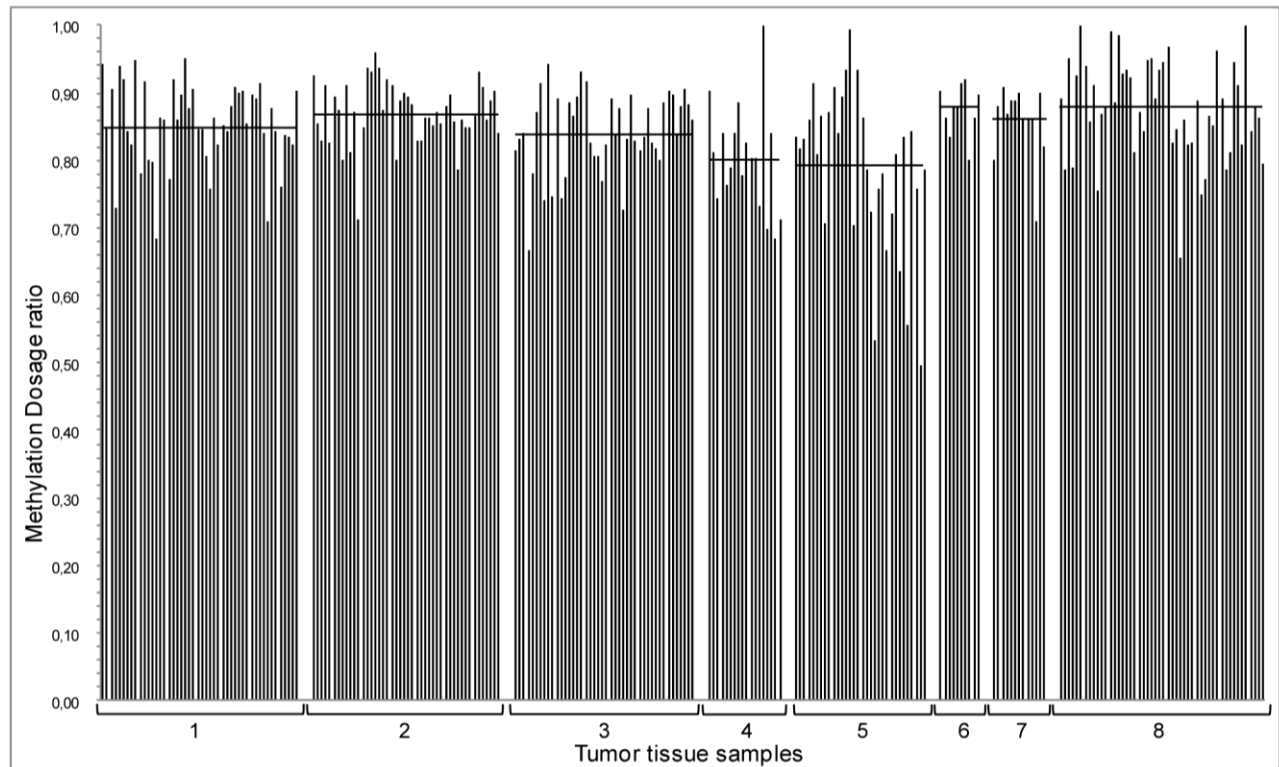
Supplementary Figure 1. Methylation analysis of LINE-1 by MS-MLPA vs. COBRA. A, four normal and tumor pairs from the present series (with cases 1 and 2 representing sporadic MSS-CRC and cases 3 and 4 sporadic MSI-CRC), the cell line K562, and a whole-genome amplified (WGA) sample are shown. Boxes indicate methylation values for LINE-1 region 2m which is monitored by both methods. B, Correlation analysis of the methylation values obtained by MS-MLPA (Dm, Y-axis) and COBRA (fraction of methylated LINE-1 sequences, X-axis).

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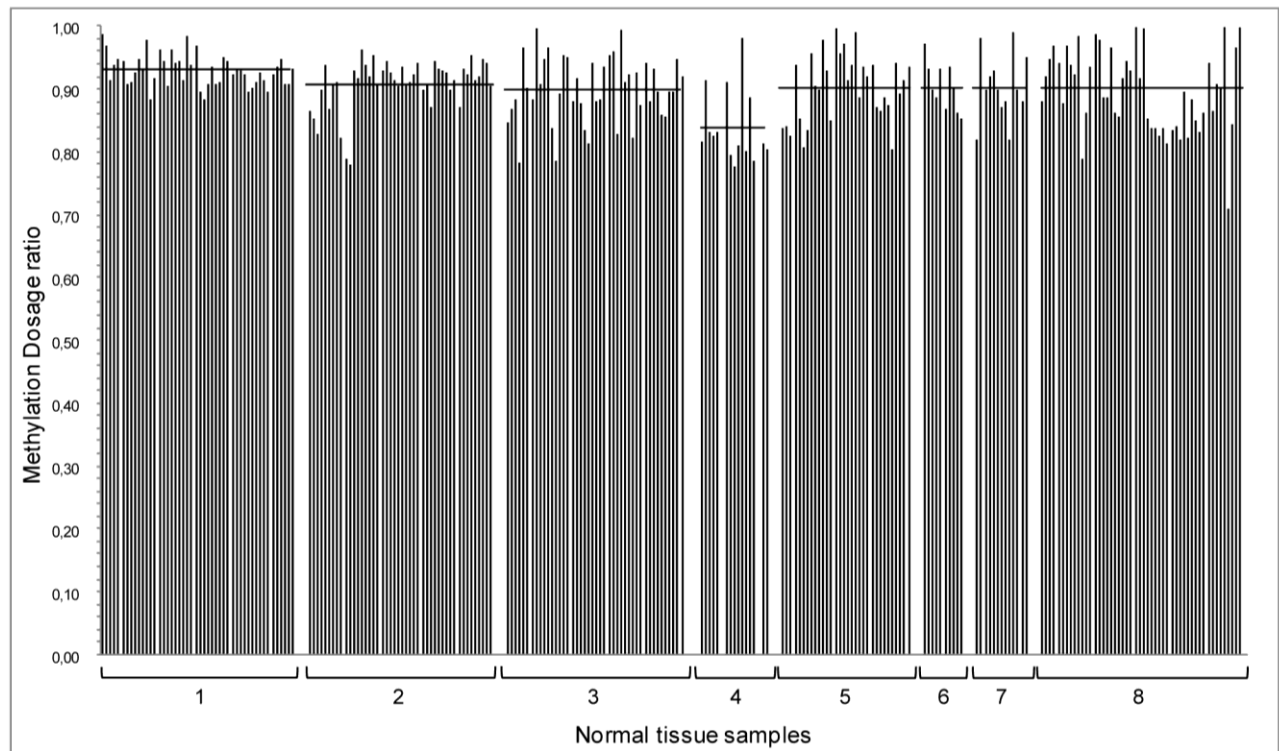
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Supplementary Figure 2

A) Tumor tissues



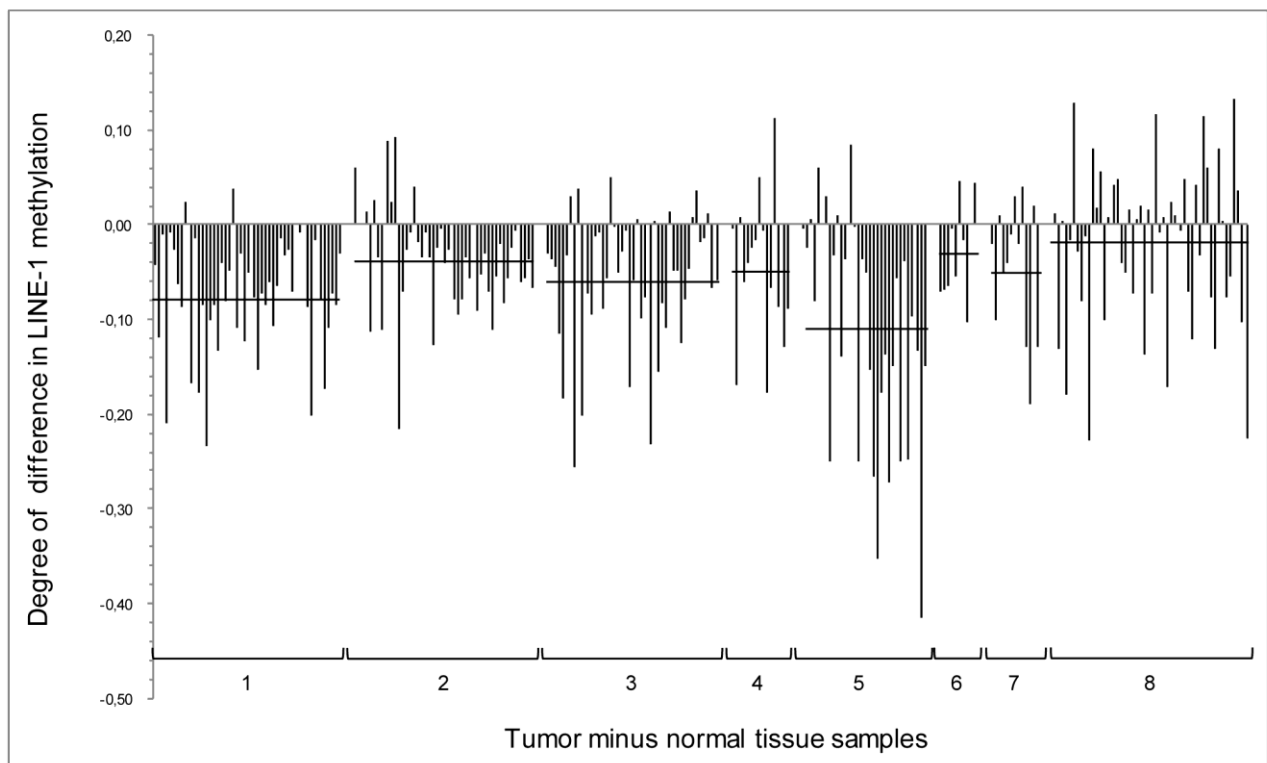
B) Normal tissues



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C) Tumor minus normal tissue difference



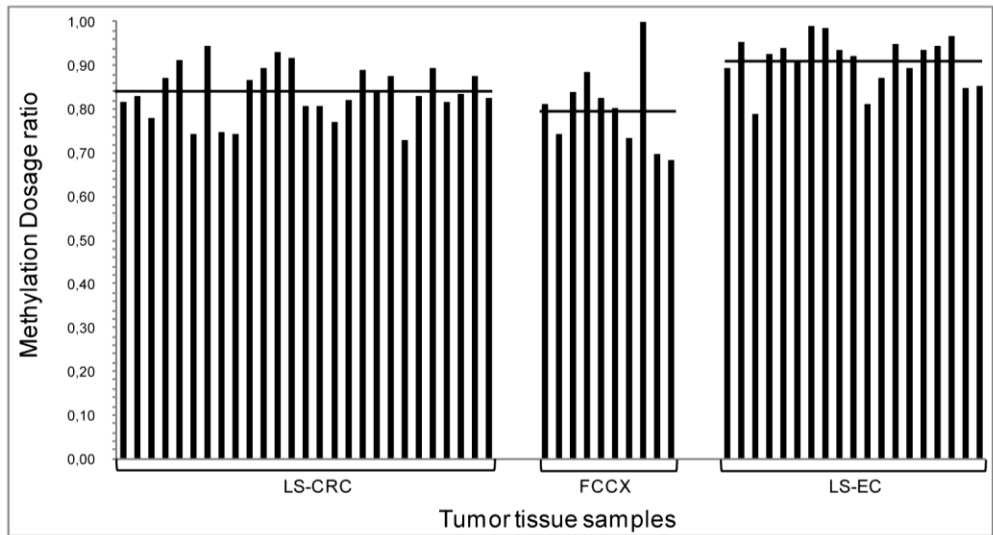
Supplementary Figure 2. Distribution of LINE-1 methylation (expressed as Dm values) in tumor (A) and paired normal DNA (B) from individual patients from groups 1 – 8 as specified in Fig. 3. The difference (Dm in tumor DNA minus Dm in normal DNA) is also given (C). Horizontal line indicates the average for each group.

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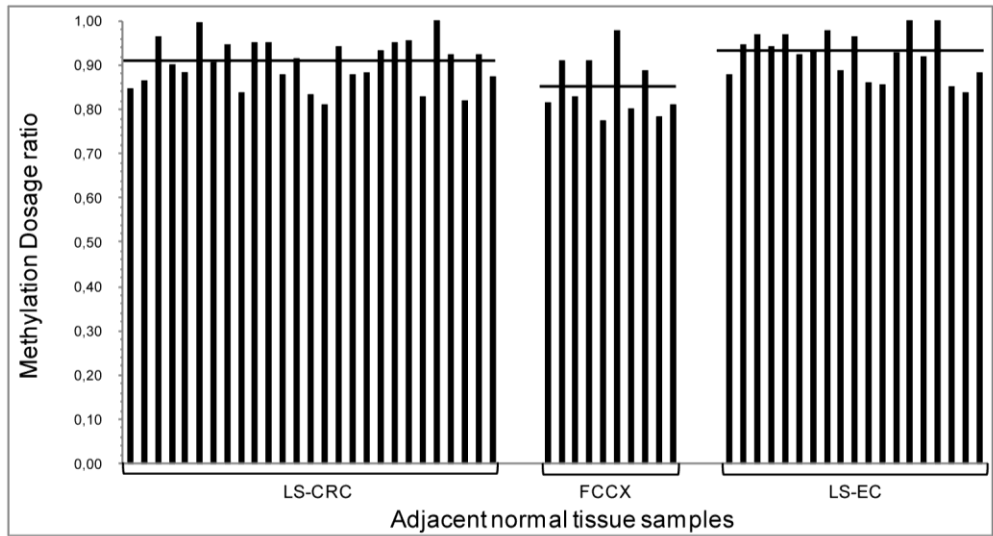
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Supplementary Figure 3

A) Tumor tissues



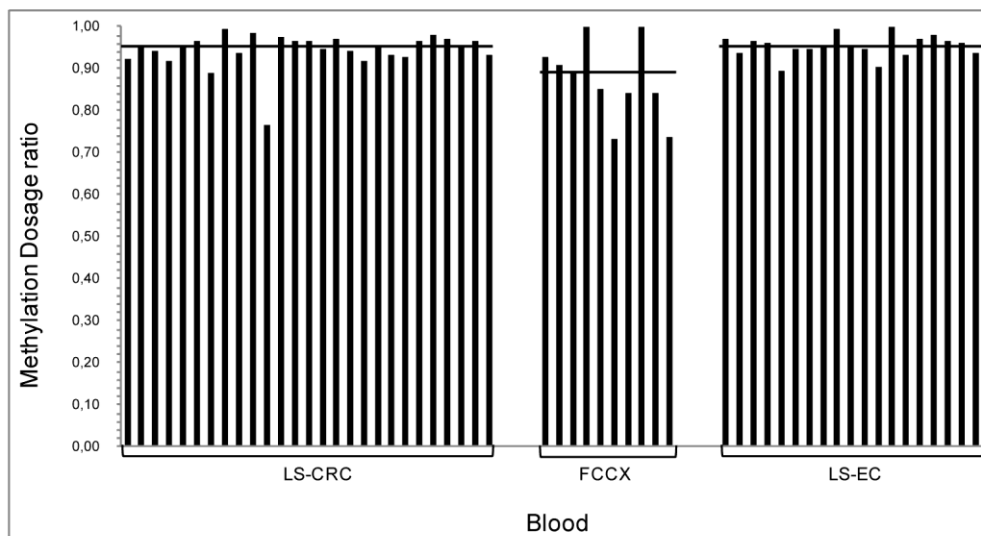
B) Adjacent normal tissues



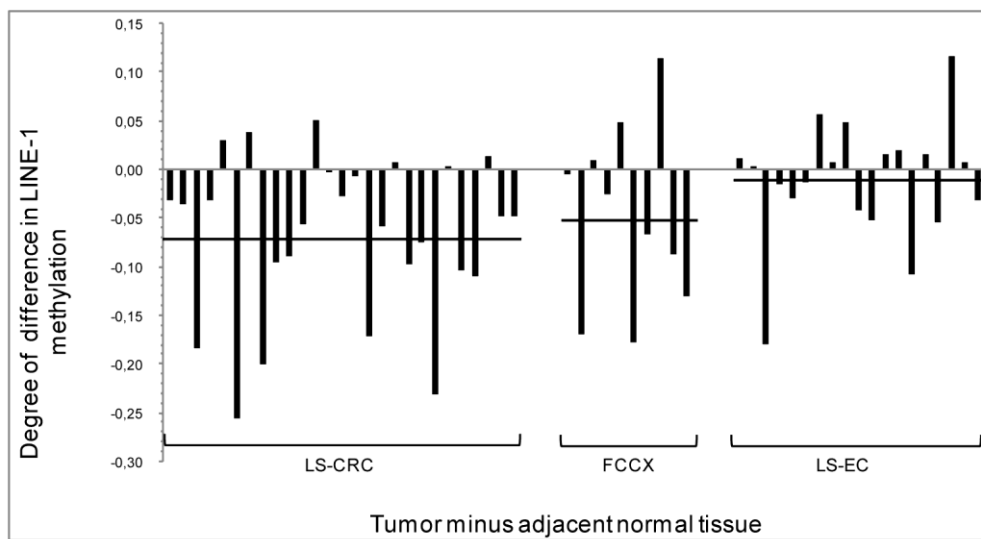
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C) Blood



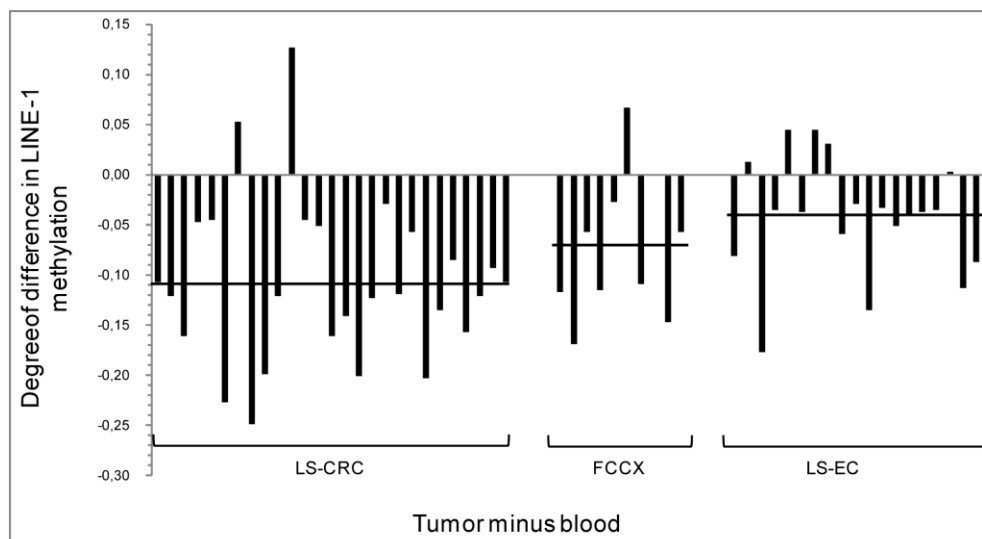
D) Tumor minus adjacent normal tissue difference



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E) Tumor minus blood



Supplementary Figure 3. Distribution of LINE-1 methylation (Dm) in tumor (A), adjacent normal mucosa or endometrium (B), and blood DNA (C) from individual patients with LS-CRC, FCCX, and LS-EC as specified in Fig. 4. The differences between tumor and normal DNA (D) and tumor and blood DNA (E) are also given. Horizontal line indicates the average for each group.