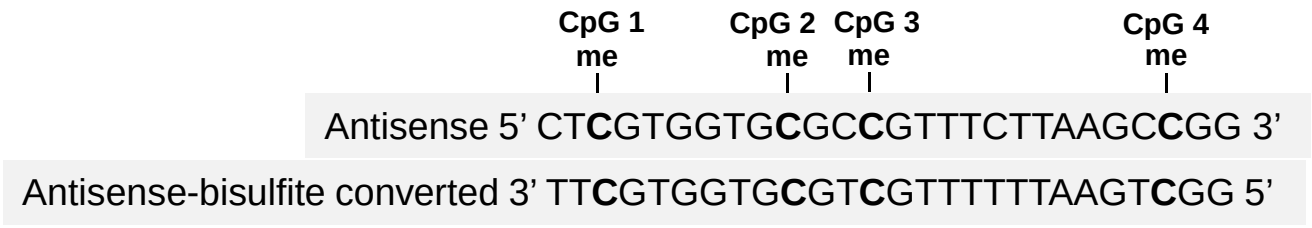
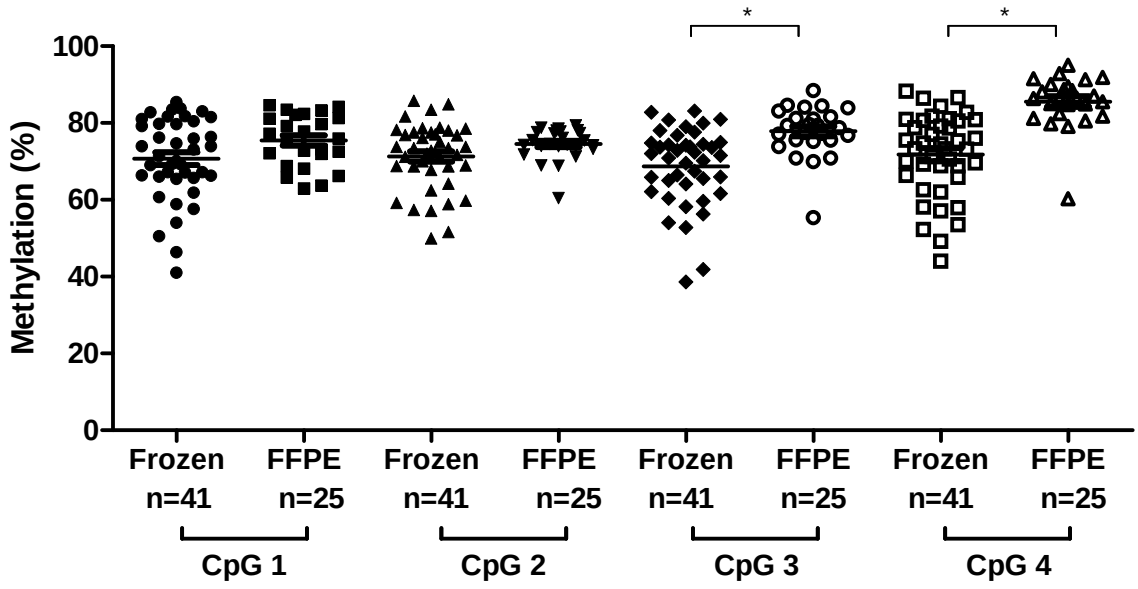
Supplementary figure 1.
Four CpG sites of LINE-1 analyzed in bisulfite-converted DNA



Supplementary figure 2.
The differences of LINE-1 methylation level (%) between frozen and FFPE tissues were observed at CpG3 and CpG4 ($p < 0.001$, $p < 0.001$, respectively).



Supplementary figure 3.

The average LINE-1 methylation levels at CpG1 and CpG4 together were significantly hypomethylated in the presence of venous invasion in frozen tissues, FFPE tissues and combined (p = 0.018, p = 0.038, p = 0.002, respectively).

*p < 0.05 (unpaired t test).

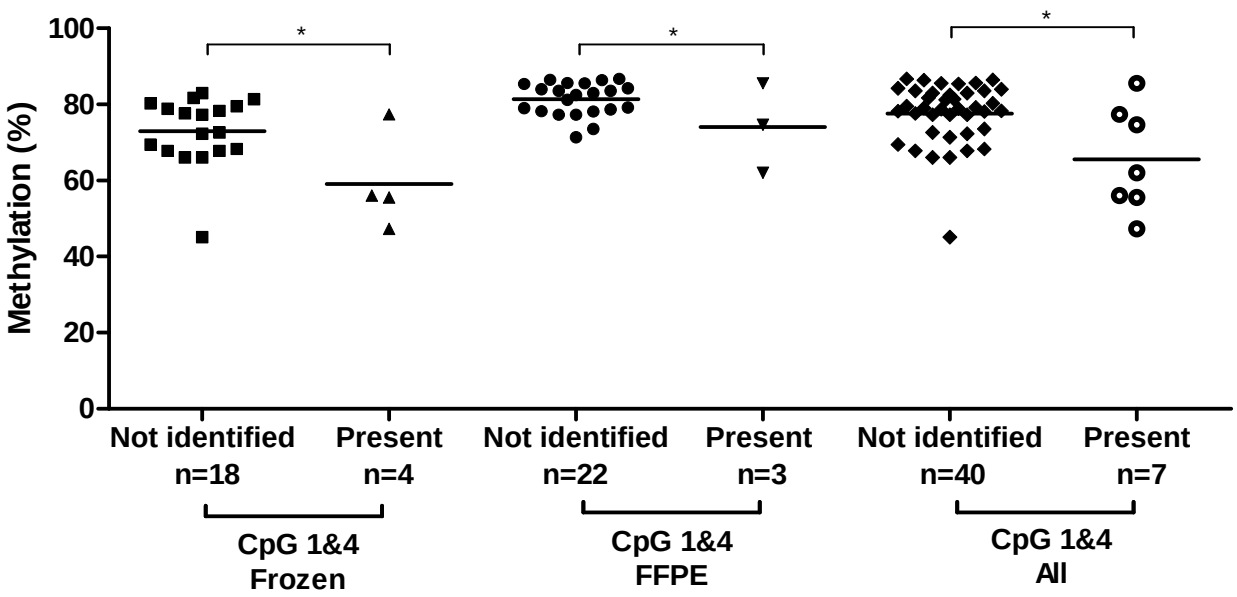


Table S1. Clinicopathological characteristics of patients with GC in frozen tissue set in this study

Characteristic	Gastric cancer (n=41)	Percentage
Gender		
Male	30	73.17%
Female	11	26.83%
Age, years		
≤ 61	18	43.90%
> 61	23	56.10%
T classification		
T1	0	0.00%
T2	14	34.15%
T3	20	48.78%
T4	7	17.07%
N classification		
N0	15	36.59%
N1	7	17.07%
N2	8	19.51%
N3	11	26.83%
Distance metastasis		
Absent	39	95.12%
Present	2	4.88%
TNM stage		
I	0	0.00%
II	26	63.41%
III	13	31.71%
IV	2	4.88%
Lauren Classification		
Intestinal	20	48.78%
Diffuse	12	29.27%
Mixed	1	2.44%
Unknown	8	19.51%
WHO classification		
Differentiated	18	43.90%
Undifferentiated	20	48.78%
Unknown	3	7.32%
Tumor location		
Upper	11	26.83%
Middle	8	19.51%
Lower	21	51.22%
Entire	1	2.44%

Lymphatic invasion		
Identified	11	26.83%
Present, mural, peritumoral	11	26.83%
Unknown	19	46.34%
Venous invasion		
Identified	18	43.90%
Present, mural, peritumoral	4	9.76%
Unknown	19	46.34%
Perineural invasion		
Identified	16	39.02%
Present, mural, peritumoral	6	14.63%
Unknown	19	46.34%

Table S2. Clinicopathological characteristics of patients with GC in FFPE tissue set in this study

Characteristic	Gastric cancer (n=25)	Percentage
Gender		
Male	12	48.00%
Female	13	52.00%
Age, years		
≤ 57	14	56.00%
> 57	11	44.00%
T classification		
T1	12	48.00%
T2	0	0.00%
T3	1	4.00%
T4	12	48.00%
N classification		
N0	13	52.00%
N1	0	0.00%
N2	0	0.00%
N3	12	48.00%
Distance metastasis		
Absent	23	92.00%
Present	2	8.00%
TNM stage		
I	0	0.00%
II	23	92.00%
III	0	0.00%
IV	2	8.00%
Lauren Classification		
Intestinal	8	32.00%
Diffuse	15	60.00%
Mixed	1	4.00%
Unknown	1	4.00%
WHO classification		
Differentiated	6	24.00%
Undifferentiated	15	60.00%
Unknown	4	16.00%
Tumor location		
Upper	5	20.00%
Middle	5	20.00%
Lower	15	60.00%
Entire	0	0.00%

Lymphatic invasion		
Identified	11	44.00%
Present, mural, peritumoral	14	56.00%
Unknown	0	0.00%
Venous invasion		
Identified	22	88.00%
Present, mural, peritumoral	3	12.00%
Unknown	0	0.00%
Perineural invasion		
Identified	12	48.00%
Present, mural, peritumoral	13	52.00%
Unknown	0	0.00%

Table S3. Primers for LINE-1 methylation analysis

LINE-1 methylation analysis	**Forward primer (5' → 3')**
Forward primer for PCR (5′→3′)	TTTTGAGTTAGGTGTGGGATATA
Reverse primer for PCR (5′→3′)	Biotin-AAAATCAAAAAATTCCCTTTC
Sequencing primer (5′→3′)	AGTTAGGTGTGGGATATAGT