

Table S1. Primer sequences used in methylation-specific PCR

Primer	Sequence, 5'-3'	Annealing temperature
<i>p16</i> -MF	TTATTAGAGGGTGGGGCGGATCGC	60°C
<i>p16</i> -MR	GACCCCGAACCGCGACCGTAA	
<i>p16</i> -UF	TTATTAGAGGGTGGGGTGGATTGT	60°C
<i>p16</i> -UR	CAACCCCCAAACCACAACCATAA	
<i>DAPK</i> -MF	GGATAGTCGGATCGAGTTAACGTC	60°C
<i>DAPK</i> -MR	CCCTCCCAAACGCCGA	
<i>DAPK</i> -UF	GGAGGATAGTTGGATTGAGTTAATGTT	60°C
<i>DAPK</i> -UR	CAAATCCCTCCCAAACACCAA	
<i>ZIC1</i> -MF	GGATTTTTGTTCTGTAATC	56°C
<i>ZIC1</i> -MR	CCCGTTAACCAACGTTAAACG	
<i>ZIC1</i> -UF	GGGATTTTTGTTGTAAATT	56°C
<i>ZIC1</i> -UR	CCCATTAAACCACATTAAACA	
<i>Klotho</i> -MF	GTCGTCGTTGTAGTCGTTATC	62°C
<i>Klotho</i> -MR	CAACAAACGCCGATAATAACG	
<i>Klotho</i> -UF	TTGTTGTTGTAGTTGTTATT	62°C
<i>Klotho</i> -UR	CCAACAAACACCAATAATAAC	
<i>HOXD10</i> -MF	TTATT TATGG GGATT CGGGT TC	52°C
<i>HOXD10</i> -MR	AAAAAA AACCG AATCC AAAAC AAACG	
<i>HOXD10</i> -UF	ATTTA TTTAT GGGGA TTTGG GTTT	52°C
<i>HOXD10</i> -UR	AAAAAA AACCA AATCC AAAAC AAACA	
<i>RUNX3</i> -MF	TTACGAGGGGCGGTACGCCGG	62°C
<i>RUNX3</i> -MR	AAAACGACCGACGCGAACGCCTCC	
<i>RUNX3</i> -UF	TTATGAGGGGTGGTTGTATGTGGG	62°C
<i>RUNX3</i> -UR	AAAACAACCAACACAAACACCTCC	
<i>RASSF1A</i> -MF	GTGTTAACCGCGTTGCGTATC	60°C
<i>RASSF1A</i> -MR	AACCCCGCGAACTAAAAACGA	
<i>RASSF1A</i> -UF	TTTGGTTGGAGTGTGTTAATGTG	60°C
<i>RASSF1A</i> -UR	CAAACCCCCACAAACTAAAAACAA	

DAPK, Death-associated protein kinase; *ZIC1*, Zinc finger of the cerebellum 1; *HOXD10*, Homeobox D10; *RUNX3*, Runt-related transcription factor 3; *RASSF1A*, Ras association domain family 1A

Table S2. Correlation between histopathologic characteristics and methylation status of ZIC1, HOXD10 and RUNX3 in plasma from gastric cancer patients

Characteristics	N	<i>ZIC1</i>		<i>P value</i>	<i>HOXD10</i>		<i>P value</i>	<i>RUNX3</i>		<i>P value</i>
		M	U		M	U		M	U	
Location										
Antrum	85	65	20	0.056	39	46	0.778	33	52	0.119
Corpus	37	22	15		18	19		20	17	
Tumor size										
<5cm	70	51	19	0.230	31	39	0.987	32	38	0.546
≥5cm	45	28	17		20	25		18	27	
Differentiation										
Well/Moderately	41	28	13	0.924	20	21	0.559	14	27	0.178
Poorly	81	56	25		35	46		38	43	
Lymph node metastasis										
N0	56	38	18	0.672	22	34	0.208	24	32	1.000
N1-3	63	45	18		32	31		27	36	
Tumor invasion depth										
T1	40	27	13	0.704	12	28	0.023	18	22	0.737
T2-4	79	56	23		41	38		33	46	
Distant metastasis										
M0	113	78	35	0.433	51	62	1.000	49	64	0.138
M1	8	7	1		4	4		1	7	
TNM Stage										
I-II	63	46	17	0.488	25	38	0.184	28	35	0.467
III-IV	58	39	19		30	28		22	36	

ZIC1, Zinc finger of the cerebellum 1; *HOXD10*, Homeobox D10; *RUNX3*, runt-related transcription factor 3; M = Methylated; U = Unmethylated.