Supplementary Tables

Supplementary Table S1. Characteristics of sporadic BC patients

Characteristics	BC patients (n,%)
TNM stage	
stage 0	1(0.4%)
stage I	69(30.1%)
stage II	72(31.4%)
stage III	15(6.6%)
stage IV	4(1.7%)
neoadj.*	50(21.8%)
unknown	18(7.9%)
Type of BC	
Ductal	179(78.2%)
Lobular	13(5.7%)
Ductal-Lobular	3(1.3%)
DCIS	4(1.7%)
Others	10(4.4%)
unknown	24(10.5%)
ER status[a]	
negative	21(9.2%)
positive	160(69.9%)
unknown	48(21.0%)
PR status[a]	
negative	36(15.7%)
positive	145(63.3%)
unknown	48(21.0%)
HER2 status[b]	
negative	165(72.1%)
positive	16(7.0%)
unknown	48(21.0%)

a Immunoreactive score (IRS), ER/PR negative: IRS 0–2; ER/PR positive: IRS 3–12
b HER2 negative: 0–1; HER2 positive: IHC-score 3. If IHC-score = 2, FISH/CISH was further analyzed and recognized positive if HER2 was amplified
*patients were treated with neoadjuvant chemotherapy, so no stage is given here

Supplementary Table S2. Bisulfite-specific primers for the target amplicons

Amplicon	Primer	Sequence	Amplicon size	No. of CpG	No. of Analyzed CpG
LINE1	sense	aggaagagagTTATTAGGGAGTGTTAGATAGTGGG	250	18	11
	antisense	cagtaatacgactcactatagggagaaggctAAAACCCTCTAAACC AAATATAAAAT			
Alu	sense	aggaagagagGTTTAGGTTGGAGTGTAGTGG	240	17	11
	antisense	cagtaatacgactcactatagggagaaggctCCTATAATCCCAACAC TTTAAAAAA			

Supplementary Table S3. Sequences of the target amplicons

Amplicon	Sequence
LINE1	TCACTAGGGAGTGCCAGACAGTGGGCGCAGGCCAGTGTGTGTGCGCACCGTGCGCGAGCCGA AGCAGGGCGAGGCATTGCCTCACCTGGGAAGCGCAAGGGGTCAGGGAGTTCCCTTTCCGAGT CAAAGAAAGGGGTGACGGACGCACCTGGAAAATCGGGTCACTCCCACCCGAATATTGCGCTTT TCAGACCGGCTTAAGAAACGGCGCACCACGAGACTATATCCCACACCTGGCTCAGAGGGTCCT
Alu	GCCCAGGCTGGAGTGCAGTGGCGCGATCTCGGCTCACTGCAACCTCCGCCTCCCGGGTTCAAG CGATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGCGCGCGCCACCACGCCCGGCTA ATTTTTGTATTTTTAGTAGAGACGGGGTTTCACCATGTTGGCCAGGCTGGTCTCGAACTCCTGA CCTCAGGTGATCCGCCCGCCTCGGCCTCCCAAAGTGCTGGGATTACAGG

Supplementary Table S4. Associations between quartile of Alu_CpG_14 methylation and the risk of breast cancer

Quartile	Alu_CpG_14 methylation	case, n=229	control, n=147	OR (95% CI)*	*P** value
1	0.43 - 0.49	98	45	2.23(1.21-4.13)	***0.01***
2	0.50 - 0.54	62	41	1.62(0.86-3.07)	*0.14*
3	0.55 - 0.56	35	27	1.49(0.73-3.06)	*0.27*
4	0.57 - 0.62	34	34	1.00(reference)	
P* for trend					*0.08*

* p value and p for trend were calculated by logistic regression and adjusted for age. Significant p values are in bold. α= 0.05

Supplementary Table S5. Combination analysis of LINE1 and Alu

CpG site	BC Cases median (IQR),n=220	Controls median (IQR)n=145	OR (95% CI)	P value*
Alu_CpG_1,2	0.21(0.19-0.23)	0.21(0.20-0.23)	0.18(0.05-0.61)	**0.006**
Alu_CpG_3	0.63(0.62-0.64)	0.63(0.62-0.64)	1.82(0.23-14.76)	0.574
Alu_CpG_11,12	0.53(0.52-0.55)	0.53(0.52-0.55)	13.39(2.17-82.84)	**0.005**
Alu_CpG_13	0.63(0.62-0.65)	0.65(0.62-0.67)	0.60(0.13-2.71)	0.503
Alu_CpG_14	0.50(0.48-0.55)	0.54(0.49-0.56)	5.13(1.69-15.55)	**0.004**
LINE1_CpG_1	0.76(0.74-0.79)	0.80(0.76-0.82)	0.05(0.01-0.21)	**5.76E-05**
LINE1_CpG_2	0.92(0.91-0.94)	0.93(0.91-0.94)	5.04(0.85-30.03)	0.076
LINE1_CpG_3,4,5	0.88(0.86-0.89)	0.88(0.87-0.90)	1.27(0.23-6.96)	0.783
LINE1_CpG_9	0.87(0.86-0.88)	0.88(0.87-0.90)	0.98(0.14-6.70)	0.984
LINE1_CpG_12	0.71(0.69-0.74)	0.74(0.71-0.77)	0.84(0.35-2.02)	0.696
LINE1_CpG_14	0.90(0.89-0.91)	0.91(0.90-0.92)	0.01(0.001-0.22)	**0.002**
LINE1_CpG_15	0.94(0.78-0.98)	0.90(0.80-0.98)	1.03(0.80-1.34)	0.807
LINE1_CpG_16,17	0.88(0.90-0.91)	0.88(0.90-0.91)	1.52(0.91-2.53)	0.114

*p values are calculated by multiple logistic regression and adjusted for age. Significant p values are in bold.
Abbreviations: IQR: interquartile range OR: odds ratio

Supplementary Table S6. Combination analysis of LINE1_CpG_1 and Alu_CpG_13

CpG site	BC Cases median (IQR), n=220	Controls median (IQR), n=147	OR (95% CI)	P value*
LINE1_CpG_1	0.76(0.74-0.79)	0.80(0.76-0.82)	0.09(0.04-0.20)	**2.30E-08**
Alu_CpG_13	0.63(0.62-0.65)	0.65(0.62-0.67)	1.76(0.64-4.86)	0.274

*p values are calculated by multiple logistic regression and adjusted for age. Significant p values are in bold.
Abbreviations: IQR: interquartile range OR: odds ratio

Supplementary Table S7. The LINE1 methylation in sporadic BC patients with different clinical characteristics

Clinical characteristics (N)	Group (N)	Median of Age	Median of methylation levels								
			CpG_1	CpG_2	CpG_3,4,5	CpG_9	CpG_12	CpG_14	CpG_15	CpG_16,17	MEAN
TNM stage (211)	stage 0 (1)	46	0.74	0.91	0.87	0.87	0.68	0.88	0.92	0.89	0.85
	stage I(69)	48	0.77	0.92	0.88	0.87	0.71	0.90	0.90	0.88	0.85
	stage II (72)	49	0.76	0.92	0.87	0.87	0.71	0.90	0.94	0.88	0.84
	stage III (15)	50	0.76	0.92	0.87	0.87	0.71	0.89	0.95	0.88	0.86
	stage IV (4)	48.5	0.77	0.91	0.87	0.87	0.73	0.89	0.95	0.86	0.86
	neoadj.* (50)	48.5	0.76	0.92	0.87	0.87	0.71	0.90	0.94	0.88	0.85
	P value (Kruskal- Wallis Test)	0.684	0.527	0.825	0.545	0.728	0.886	0.268	0.914	0.595	0.628
Type of BC (205)	Ductal (179)	49	0.76	0.92	0.88	0.87	0.71	0.90	0.93	0.88	0.85
	Lobular (13)	48	0.75	0.93	0.87	0.87	0.72	0.89	0.94	0.81	0.84
	Ductal-Lobular (3)	46	0.74	0.92	0.88	0.87	0.70	0.90	0.78	0.85	0.84
	DCIS (4)	46	0.74	0.91	0.87	0.87	0.69	0.88	0.96	0.89	0.85
	Others(10)	49.5	0.75	0.92	0.87	0.87	0.70	0.89	0.97	0.85	0.84
	P value (Kruskal- Wallis Test)	0.776	0.237	0.470	0.720	0.851	0.719	**0.022**	0.260	0.234	0.778
ER status (181)	ER negative (21)	48	0.76	0.92	0.87	0.87	0.70	0.89	0.95	0.88	0.85
	ER positive (160)	48	0.76	0.92	0.88	0.87	0.71	0.90	0.94	0.88	0.85
	P value (Mann-Whitney U)	0	0.445	0.902	0.750	0.812	0.337	0.669	0.599	0.924	0.919
PR status (181)	PR negative (36)	47	0.76	0.93	0.88	0.87	0.70	0.90	0.95	0.88	0.85
	PR positive (145)	49	0.76	0.92	0.87	0.87	0.71	0.90	0.92	0.88	0.85
	P value (Mann-Whitney U)	0	0.278	0.631	0.622	0.672	0.381	0.643	0.585	0.530	0.520

Supplementary Table S7.Continued

Clinical characteristics (N)	Group (N)	Median of Age	Median of methylation levels								
			CpG_1	CpG_2	CpG_3,4,5	CpG_9	CpG_12	CpG_14	CpG_15	CpG_16,17	MEAN
Her-2 status (181)	Her-2 negative (165)	48	0.76	0.92	0.87	0.87	0.71	0.90	0.93	0.88	0.85
	Her-2 positive (16)	46	0.75	0.92	0.88	0.86	0.70	0.89	0.96	0.88	0.84
	P value (Mann-Whitney U)	0	0.402	0.645	0.608	0.203	0.325	0.211	0.359	0.506	0.564
triple-negative BC (181)	non- triple neg. BC (161)	48	0.76	0.92	0.87	0.87	0.71	0.90	0.93	0.88	0.85
	triple neg. BC (20)	48	0.76	0.93	0.88	0.87	0.70	0.89	0.95	0.89	0.85
	P value (Mann-Whitney U)	0	0.288	0.871	0.815	0.705	0.206	0.604	0.376	0.978	0.968
Tumor Nr. (221)	1 tumor(217)	48	0.76	0.92	0.87	0.87	0.71	0.90	0.94	0.88	0.85
	2 tumor(4)	51	0.78	0.94	0.90	0.89	0.73	0.91	0.88	0.89	0.87
	P value (Mann-Whitney U)	0	0.780	0.940	0.900	0.890	0.730	0.905	0.875	0.890	0.867

* patients underwent neoadjuvant chemotherapy

Supplementary Table S8. The Alu methylation in sporadic BC patients with different clinical characteristics

Clinical characteristics (N)	Group (N)	Median of Age	Median of methylation levels							
			CpG_1,2	CpG_3	CpG_7	CpG_11,12	CpG_13	CpG_14	CpG_15,16,17	MEAN
TNM stage (211)	stage 0 (1)	46	0.21	0.61	0.47	0.52	0.61	0.48	0.64	0.51
	stage I(69)	48	0.21	0.63	0.46	0.54	0.64	0.51	0.65	0.51
	stage II (72)	49	0.21	0.63	0.46	0.53	0.63	0.50	0.65	0.51
	stage III (15)	50	0.21	0.62	0.46	0.54	0.62	0.49	0.62	0.51
	stage IV (4)	48.5	0.20	0.62	0.44	0.54	0.64	0.50	0.65	0.51
	neoadj.* (50)	48.5	0.21	0.63	0.46	0.54	0.63	0.50	0.64	0.51
	P value (Kruskal- Wallis Test)	0.684	0.695	0.670	0.543	0.261	0.471	0.398	0.382	0.926
Type of BC (205)	Ductal (179)	49	0.21	0.63	0.46	0.53	0.63	0.50	0.65	0.51
	Lobular (13)	48	0.21	0.63	0.47	0.54	0.63	0.50	0.64	0.51
	Ductal-Lobular (3)	46	0.17	0.63	0.46	0.53	0.62	0.48	0.62	0.49
	DCIS (4)	46	0.20	0.62	0.47	0.53	0.63	0.48	0.65	0.52
	Others(10)	49.5	0.22	0.63	0.47	0.53	0.62	0.50	0.64	0.51
	P value (Kruskal- Wallis Test)	0.776	0.756	0.847	**0.028**	0.327	0.199	0.347	0.524	0.350
ER status (181)	ER negative (21)	48	0.19	0.62	0.46	0.53	0.62	0.50	0.66	0.51
	ER positive (160)	48	0.21	0.63	0.46	0.53	0.63	0.50	0.65	0.51
	P value (Mann-Whitney U)	0.254	0.029	0.153	0.810	0.525	0.722	0.878	0.732	0.466
PR status (181)	PR negative (36)	47	0.20	0.62	0.46	0.54	0.63	0.50	0.65	0.51
	PR positive (145)	49	0.21	0.63	0.46	0.53	0.63	0.50	0.65	0.51
	P value (Mann-Whitney U)	0.060	0.515	0.909	0.567	0.302	0.947	0.636	0.644	0.752

Supplementary Table S8.Continued

Clinical characteristics (N)	Group (N)	Median of Age	Median of methylation levels							
			CpG_1,2	**CpG_3**	**CpG_7**	**CpG_11,12**	**CpG_13**	**CpG_14**	**CpG_15,16,17**	**MEAN**
Her-2 status (181)	Her-2 negative (165)	48	0.21	0.63	0.46	0.53	0.63	0.50	0.65	0.51
	Her-2 positive (16)	46	0.22	0.63	0.47	0.53	0.64	0.50	0.65	0.51
	P value (Mann-Whitney U)	0	0.312	0.831	0.649	0.351	0.914	0.295	0.350	0.489
triple-negative BC (181)	non- triple neg. BC (161)	48	0.21	0.63	0.46	0.53	0.63	0.50	0.65	0.51
	triple neg. BC (20)	48	0.19	0.62	0.46	0.53	0.62	0.50	0.66	0.51
	P value (Mann-Whitney U)	0	**0.043**	0.182	0.961	0.375	0.571	0.980	0.829	0.373
Tumor Nr. (221)	1 tumor(217)	48	0.21	0.63	0.46	0.53	0.63	0.50	0.65	0.51
	2 tumor(4)	51	0.23	0.64	0.47	0.55	0.64	0.53	0.66	0.54
	P value (Mann-Whitney U)	0.484	**0.042**	0.221	0.317	**0.047**	0.219	0.242	0.568	0.126

* patients underwent neoadjuvant chemotherapy