Supplementary Information

Study of porphyrin modified liquid exfoliated graphene field effect transistor for evaluating DNA methylation degree

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Detailed discussion for Raman spectra. As shown in **Fig. 1A**, the typical bands of graphene material can be observed in the spectra LEG and TAPP decorated LEG layers, which are D band (~1347 cm^{-1}), G band (~1570 cm^{-1}) and 2D band (~2678 cm^{-1}). The D band can be due to the structural imperfections in the carbon basal plane or edge site, whereas the G band is the so-called characteristic peak of graphite, corresponding to the sp^2 carbon bond stretching of E2g mode.[24] The 2D band is the overtone of the D band and corresponds to elastic phonon scattering in the presence of inelastic phonon emission, it is typically used to indicate graphene structure quality.[21] In LEG's spectrum, the intensity ratio of D peak to G peak (I_D/I_G≈0.73) indicates there are structural defects around the nano-flakes or on the basal plane, which may be formed by the ultrasonic water bath[20] and exfoliation[22].

Meanwhile, the spectral properties of TAPP are evidenced,[23] which are: the peak in TAPP at 1600 cm^{-1} corresponds to the stretching vibration of C=C on benzene ring; $C_\beta C_\beta$ bond stretching vibration is located at 1547 and 1513 cm^{-1}; the peak at 1457 cm^{-1} represents the stretching vibration of $C_\alpha C_m$ bond; the peaks at 1386 and 1325 cm^{-1} signify the quarter-ring vibrations of protonated and non-protonated pyrrole rings, respectively; 1/2 ring vibrations of protonated and non-protonated pyrrole are identified by peaks at 1362 and 1289 cm^{-1}; respiratory vibrations of protonated and non-protonated pyrrole ring are identified by peaks at 1009 and 979cm^{-1}, respectively; it is found, there is deformed vibration of pyrrole ring at 880 and 827 cm^{-1}; the peak located at 1244 cm^{-1} corresponds to C_m-phenyl symmetric stretching vibration.

The TAPP coating on LEG film can be identified by the integrated spectral features in the spectrum of TAPP@LEG (the black curve in **Fig. 1A**) which has been explained in the manuscript. The underlying physical mechanism is

attributed to the electrostatic interaction in the long range and the π-π stacking interaction in the short range, according to the literature 18.

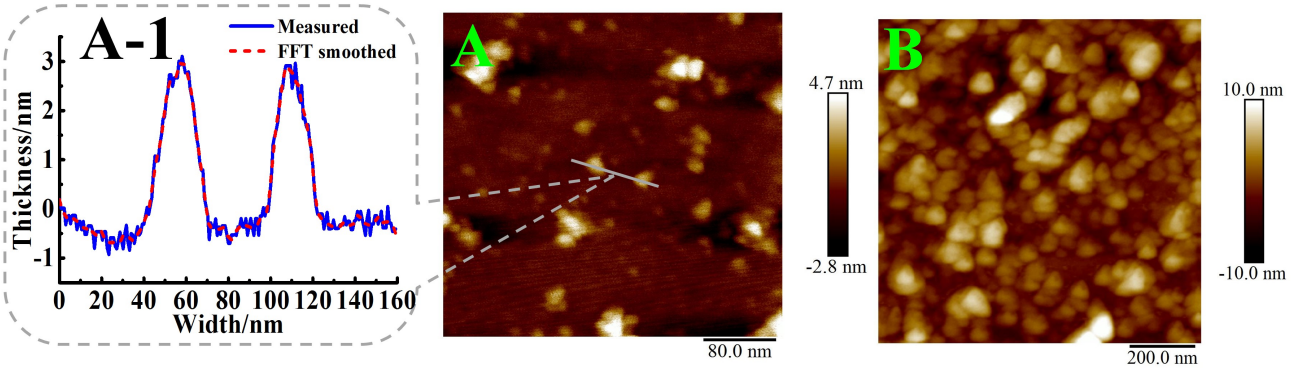

Fig. S1. AFM images of LEG (A) and TAPP-LEG (B). The 2D image of LEG shows the LEG flakes have inhomogeneous shapes. The height of two representative flakes are measured in A-1, the average thickness is about 3.5 nm, and the lateral size is about 40 nm. The as-prepared LEG is approximately four layers, according to the literature 6. In (B), TAPP layers are scattered on the few-layer LEG film.

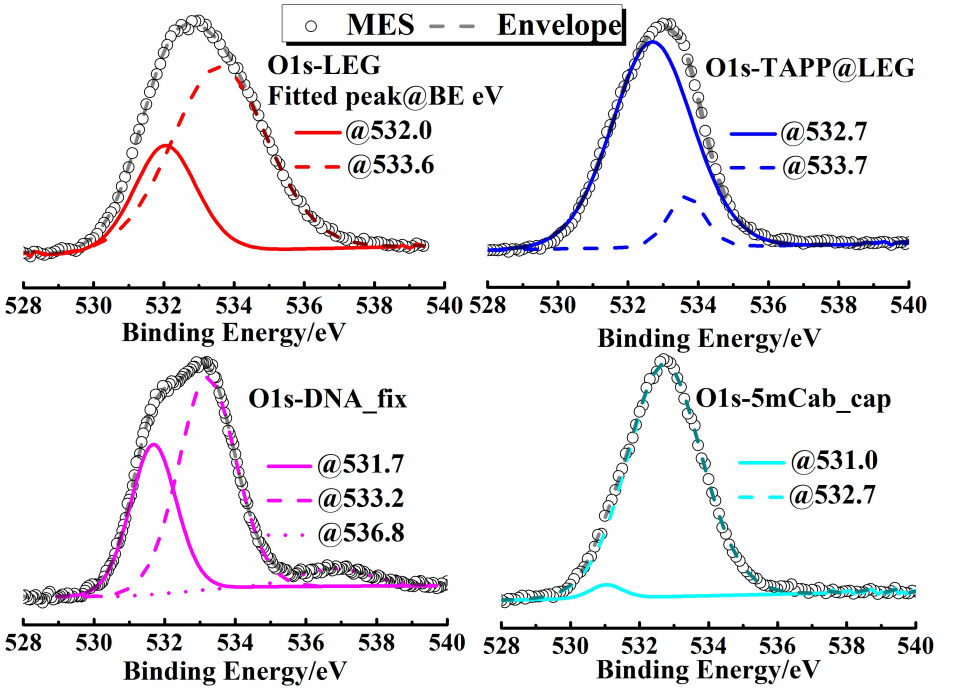

Fig. S2. O1s core spectra of LEG, TAPP@LEG, DNA_fix and 5mCab_cap. In LEG, the O1s fitted peaks at 532.0 eV and 533.6 eV are contributed by -OH and O=C-O groups in LEG, respectively. In TAPP@LEG, the peaks at 532.7 eV and 533.7 eV are contributed by -OH and O=C-O groups in LEG, respectively. In DNA_fix, the peak at 531.7 eV and 533.2 eV corresponds to C=O, P=O, P-O-…Na^+ in DNA molecules and C-O-P, C-O-C in DNA molecules[26], respectively, the peak at 536.8 eV belongs to the entrapped water in DNA molecules[31]. In 5mCab_cap, the main peak around 532.7 eV may be assigned to -CO-NH- in protein molecules.

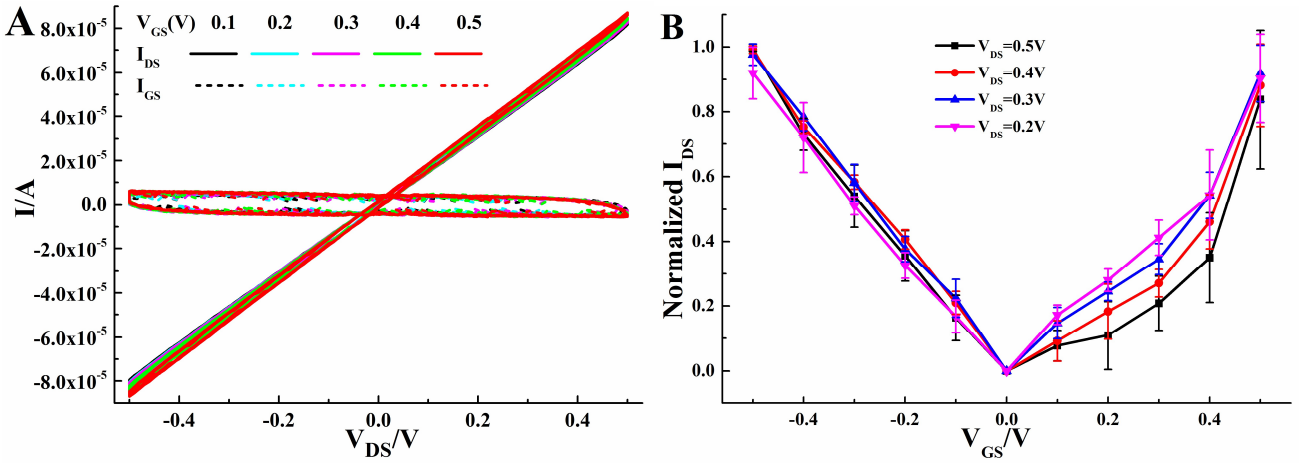

Fig. S3. Blank LEG-FET electronic features when . **A,** the output characteristic curves when V_{DS} is scanned from -0.5 V to 0.5 V for ten circles, at the controlled V_{GS} (0.5, 0.4, 0.3, 0.2, 0.1 V). **B** is the transferring curves, when V_{DS} is controlled at 0.5, 0.4, 0.3, 0.2 V. Error bars are standard deviations of the mean value of normalized I_{DS} (n=3).

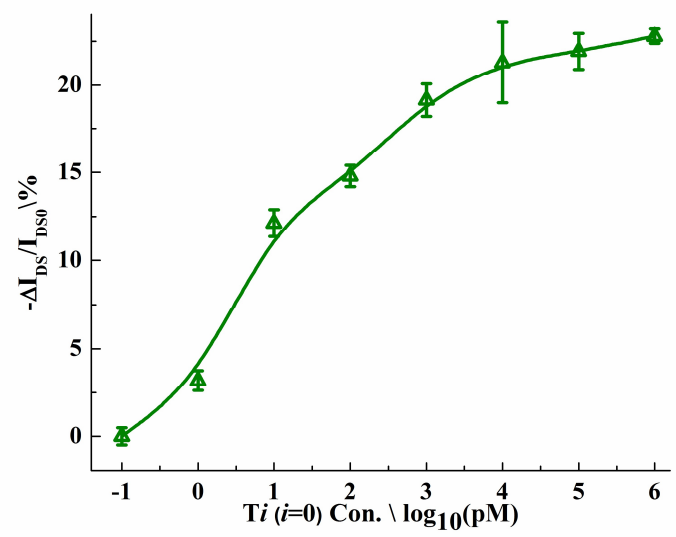

Fig. S4. Current responding of TAPP-LEG-FET to the concentration of testing chains (T*0* as an example), I_{DS0} is the measured I_{DS} after TAPP activation. I_{DS} is decreased by the immobilized nucleic acid strings, which induce an extra resistance on the gate surface [Anal. Chem. 90 (2018) 5153-5161]. The concentration of 100 nM is used as the optimized concentration because the curve of I_{DS} changing ratio tends to be saturated at 100 nM.

Table S1. Data of the expected N_{mC} (N_E) versus the observed one (N_O) for each of the tested sequences Ti (*i*=0 to 4) are presented. In which N_O is calculated by the measured Y data ($-\Delta I_{DS}/I_{DS}$) and the fitted line, in Fig. 1A when C_{5mCab} is 0.1 μg/ml. For example, for T*1,* the fitted line is *Y*=2.67*X*-0.14, the sensor's responding is Y=2.71, N_O=(2.71+0.14)/2.67≈1.1, its N_E is designed as 1, then N_E/N_O≈0.9. The average of N_E/N_O is calculated at about 1.05.

	T0	T1	T2	T3	T4
N_E	0	1	2	3	4
N_O	0.0	1.1	1.5	3.0	4.5
N_E/N_O	0	0.9	1.3	1.0	0.9
The averaged N_E/N_O			1.05		

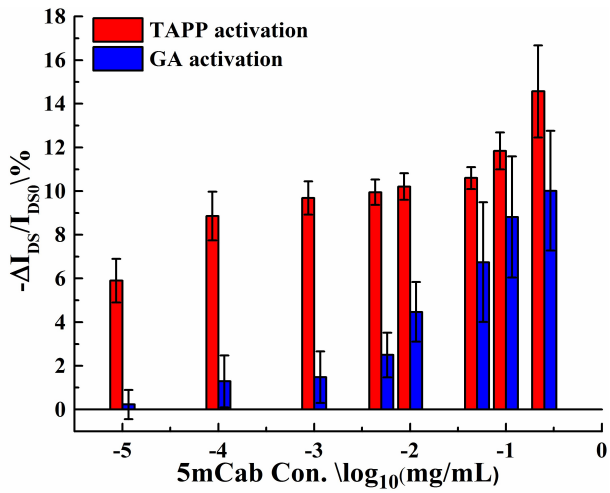

Fig. S5. The comparison of methylation detection results for T*4* by TAPP and GA activated LEG-FETs. Error bars are standard deviations of the average value of $-\Delta I_{DS}/I_{DS0}$ (n=3), I_{DS0} is the measured I_{DS} after BSA blocking.

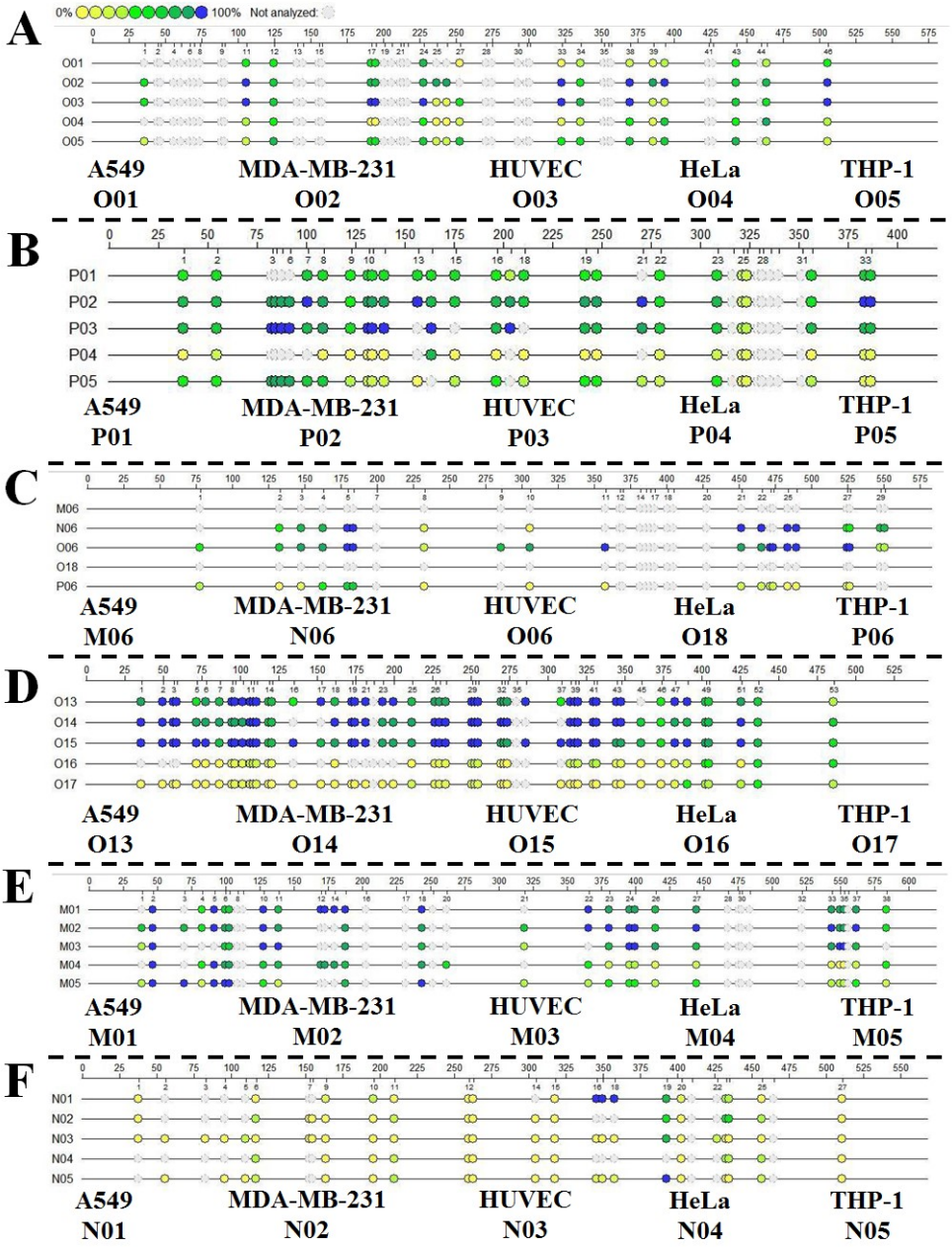

Fig. S6. MS measured methylation degree for the ssDNA samples which are extracted from the cells A549, MDA-MB-231, HUVEC, HeLa and THP-1. A to F are the results by using six different primers P1 to P6, respectively. The color points from yellow to blue correspond to the percentage (0~100%) of methylated at each of the sites. The raw data of **Fig. S6A~F** are listed in **Table S2~7**, respectively, the total amount of 5mC (N_{mC}) for each of ssDNA sample by using different Pi (i=1, 2, 3, 4, 5, 6) are calculated by summing each CpG's methylation level which are listed in **Table S2~7**.

Table S2. The raw data of **Fig. S6A** and the calculated N_{mC}. "NA" means "not analyzed".

CpG_serial number	Position in genomic sequence	A549	MDA-MB-231	HUVEC	HeLa	THP-1
CpG_1	35	NA	0.54	0.56	NA	0.23
CpG_2.3.4.5	44:46:55:60	NA	NA	NA	NA	NA
CpG_6.7.8.9.10	66:69:73:88:90	NA	NA	NA	NA	NA
CpG_11	105	0.41	1	1	0.34	0.25
CpG_12	124	0.44	0.67	0.68	0.43	0.77
CpG_13.14.15.16	140:144:155:157	NA	NA	NA	NA	NA
CpG_17.18	191:194	0.54	0.76	0.99	0.02	0.56
CpG_19.20.21.22.23	200:205:211:213:216	NA	NA	NA	NA	NA
CpG_24	227	0.72	0.7	1	0.44	0.64
CpG_25.26	236:243	NA	0.86	0.14	0.08	0.21
CpG_27	252	0	NA	0.54	0.29	0.66
CpG_28.29.30.31.32	270:274:292:298:300	NA	NA	NA	NA	NA
CpG_33	322	0.09	0.95	0.92	0.15	0.45
CpG_34	335	0.39	0.59	0.79	0.36	0.54
CpG_35.36.37	351:354:356	NA	NA	NA	NA	NA
CpG_38	369	0.21	1	1	0.42	0.66
CpG_39	385	0.15	0.81	0.22	0.17	0.21
CpG_40	393	0.34	0.97	0.26	0.63	0.63
CpG_41	423	NA	NA	NA	NA	NA
CpG_42	426	NA	NA	NA	NA	NA
CpG_43	442	0.44	0.67	0.68	0.43	0.77
CpG_44	459	NA	NA	NA	NA	NA
CpG_45	463	0.39	0.78	0.55	0.87	0.33
CpG_46	505	0.41	1	1	0.34	0.25
N_{mC}	**-**	**5.07**	**12.06**	**11.32**	**4.99**	**7.72**

Table S3. The raw data of **Fig. S6B** and the calculated N_{mC}. "NA" means "not analyzed".

CpG_serial number	Position in genomic sequence	A549	MDA-MB-231	HUVEC	HeLa	THP-1
CpG_1	37	0.45	0.73	0.73	0	0.48
CpG_2	54	0.58	0.73	0.69	0.23	0.41
CpG_3.4.5.6	82:84:87:91	NA	0.84	0.97	NA	0.85
CpG_7	100	0.61	0.99	0.77	NA	0.7
CpG_8	108	0.56	0.81	0.85	0.07	0.66
CpG_9	122	0.43	0.5	0.59	0.08	0.3
CpG_10.11.12	131:133:139	0.59	0.87	0.91	0.09	0.24
CpG_13	156	0.52	1	NA	NA	0.08
CpG_14	163	0.57	0.79	1	0.75	NA
CpG_15	175	0.56	0.63	NA	0	0.38
CpG_16	196	0.45	0.73	0.73	0	0.48
CpG_17	203	0.34	0.84	1	NA	NA
CpG_18	210	0.56	0.63	NA	0	0.38
CpG_19.20	241:247	0.44	0.81	0.77	0.07	0.46
CpG_21	270	NA	0.96	0.82	NA	0.36
CpG_22	279	0.45	0.56	0.61	0.12	0.23
CpG_23	308	0.58	0.73	0.69	0.23	0.41

CpG_24	316	NA	NA	NA	NA	NA
CpG_25.26	321:323	0.21	0.26	0.39	0.13	0.16
CpG_27.28.29.30.31	329:331:335:339:351	NA	NA	NA	NA	NA
CpG_32	356	0.55	0.58	0.73	0	0.35
CpG_33.34	383:386	0.62	1	0.8	0.15	0.18
N_{mC}	-	**11.52**	**21.32**	**19.74**	**2.45**	**10.94**

Table S4. The raw data of **Fig. S6C** and the calculated N_{mC}. "NA" means "not analyzed".

CpG_serial number	Position in genomic sequence	A549	MDA-MB-231	HUVEC	HeLa	THP-1
CpG_1	77	NA	NA	0.48	NA	0.37
CpG_2	132	NA	0.43	0.68	NA	0
CpG_3	147	NA	0.77	0.85	NA	0.18
CpG_4	162	NA	0.75	0.84	NA	0.5
CpG_5.6	179:183	NA	1	0.95	NA	0.61
CpG_7	199	NA	NA	NA	NA	NA
CpG_8	232	NA	0.14	0.28	NA	0
CpG_9	285	NA	NA	0.78	NA	NA
CpG_10	305	NA	0	0.88	NA	0.08
CpG_11	357	NA	NA	0.97	NA	0
CpG_12.13.14.15.16.17.18.19	367:369:381:385:388:391:400:404	NA	NA	NA	NA	NA
CpG_20	427	NA	NA	NA	NA	NA
CpG_21.22	451:465	NA	0.91	0.83	NA	0.23
CpG_23.24	471:473	NA	NA	1	NA	0.21
CpG_25.26	483:489	NA	1	0.94	NA	0.02
CpG_27.28	524:526	NA	0.47	0.95	NA	0.19
CpG_29.30	547:550	NA	0.73	0.37	NA	NA
N_{mC}	-	**0**	**10.31**	**15.84**	**0**	**3.65**

Table S5. The raw data of **Fig. S6D** and the calculated N_{mC}. "NA" means "not analyzed".

CpG_serial number	Position in genomic sequence	A549	MDA-MB-231	HUVEC	HeLa	THP-1
CpG_1	35	0.84	0.95	0.92	NA	0.01
CpG_2.3.4	49:56:58	1	1	0.97	NA	0.06
CpG_5	71	0.58	0.81	1	0	0
CpG_6	77	0.81	0.83	0.95	0	0
CpG_7	86	0.71	0.84	0.78	0	0.09
CpG_8.9.10	94:96:101	0.92	0.86	0.95	0.16	0.13
CpG_11.12.13	106:108:110	0.94	0.96	0.94	0.26	0.14
CpG_14.15	118:120	0.78	0.76	0.78	0.11	0
CpG_16	134	0.45	NA	1	NA	0
CpG_17	152	1	NA	0.8	NA	0
CpG_18	161	0.77	0.92	0.84	0.06	0.02
CpG_19.20.21	172:174:181	0.98	0.92	0.96	NA	0.07
CpG_22	186	NA	NA	NA	NA	NA
CpG_23.24	192:199	0.91	0.82	0.88	NA	0
CpG_25	211	0.74	0.81	0.84	0.01	0.21
CpG_26.27.28	226:229:233	0.9	1	0.95	0.14	0.13
CpG_29.30.31	250:252:254	0.94	0.96	0.94	0.26	0.14

CpG_32.33.34	269:271:273	0.83	0.96	0.87	0.02	0.06
CpG_35	279	NA	NA	NA	NA	NA
CpG_36	285	0.92	NA	1	NA	NA
CpG_37	308	0.45	NA	1	NA	0
CpG_38.39.40	314:317:319	0.99	1	0.97	0.2	0.02
CpG_41.42	329:331	0.91	0.93	0.97	0.36	0
CpG_43.44	344:347	0.92	0.92	0.9	0.1	0.05
CpG_45	360	NA	0.66	0.78	0	0
CpG_46	373	0.46	0.43	0.52	0.09	0
CpG_47	382	0.95	0.81	1	0.16	0.18
CpG_48	390	1	0.76	0.95	0.27	0.44
CpG_49.50	402:404	0.65	0.74	0.84	0.48	0.36
CpG_51	425	0.87	1	1	0.09	0.38
CpG_52	436	0.65	0.66	0.72	0.54	0.44
CpG_53	485	0.35	0.67	0.57	0.52	0.32
N_{mC}	-	**42.39**	**42.47**	**46.06**	**6.96**	**5.16**

Table S6. The raw data of **Fig. S6E** and the calculated N_{mC}. "NA" means "not analyzed".

CpG_serial number	Position in genomic sequence	A549	MDA-MB-231	HUVEC	HeLa	THP-1
CpG_1	38	NA	0.63	0.33	NA	0.34
CpG_2	46	0.93	0.97	1	0.91	0.92
CpG_3	69	NA	0.72	NA	NA	1
CpG_4	82	0.48	0.55	NA	0.42	0.22
CpG_5	91	1	1	NA	1	1
CpG_6.7	99:102	0.66	0.81	0.65	0.86	0.96
CpG_8	108	NA	NA	NA	NA	NA
CpG_9	112	NA	NA	NA	NA	NA
CpG_10	127	1	1	0.96	0.66	0.45
CpG_11	138	0.77	0.84	1	0.76	0.23
CpG_12.13.14	169:172:179	1	NA	NA	0.83	NA
CpG_15	187	0.9	0.87	0.88	0.76	0.74
CpG_16	202	NA	NA	NA	NA	NA
CpG_17	231	NA	NA	NA	NA	NA
CpG_18	243	0.99	0.87	0.73	0.89	1
CpG_19	251	NA	NA	NA	NA	NA
CpG_20	261	NA	NA	NA	0.52	NA
CpG_21	318	NA	0.63	0.33	NA	0.34
CpG_22	365	1	0.94	NA	0.43	0.3
CpG_23	380	0.85	0.9	0.76	0.26	0.44
CpG_24.25	395:399	0.88	0.97	0.95	0.33	0.49
CpG_26	414	0.79	0.69	0.88	0.18	0.35
CpG_27	444	0.85	0.9	0.76	0.26	0.44
CpG_28.29.30.31	467:475:477:483	NA	NA	NA	NA	NA
CpG_32	521	NA	NA	NA	NA	NA
CpG_33	543	0.89	0.97	0.86	0.12	0.33
CpG_34.35	549:552	0.89	0.86	0.96	0.06	0.31
CpG_36	555	NA	NA	NA	NA	NA
CpG_37	561	0.85	0.9	0.76	0.26	0.44

CpG_38	583	0.48	0.55	NA	0.42	0.22
N_{mC}	-	**19.64**	**19.21**	**14.37**	**12.84**	**12.28**

Table S7. The raw data of **Fig. S6F** and the calculated N_{mC}. "NA" means "not analyzed".

CpG_serial number	Position in genomic sequence	A549	MDA-MB-231	HUVEC	HeLa	THP-1
CpG_1	37	0	0.1	0.06	NA	NA
CpG_2	55	NA	NA	0.02	NA	0
CpG_3	82	NA	NA	0	NA	NA
CpG_4	95	NA	NA	0	NA	0
CpG_5	109	NA	NA	0.22	NA	0
CpG_6	116	0.01	0.31	0	0.34	0.28
CpG_7.8	152:154	NA	0	0	NA	NA
CpG_9	163	0.2	0	0	0	0
CpG_10	195	0.25	0	0.04	0.06	0.05
CpG_11	209	0.03	0.28	0.01	0.23	0.32
CpG_12.13	259:262	0	0.1	0.05	0.01	0.05
CpG_14	304	NA	0	0	0.07	0
CpG_15	317	0	0	0	0	0
CpG_16.17.18	345:349:357	0.93	NA	0.04	NA	0.12
CpG_19	392	0.75	0.59	0.71	NA	1
CpG_20	402	0.2	0	0	0	0
CpG_21	409	NA	NA	NA	NA	NA
CpG_22	426	NA	NA	0.28	NA	NA
CpG_23.24	432:434	0.31	0.51	0.07	0.39	0
CpG_25	456	0.01	0.31	0	0.34	0.28
CpG_26	464	NA	NA	NA	NA	NA
CpG_27	510	0	0	0	0	0
N_{mC}	-	**4.86**	**2.81**	**1.7**	**1.84**	**1.39**