

Supplementary Information

Active demethylation of non-CpG sites in DNA is initiated by TET2 5-methylcytosine dioxygenase

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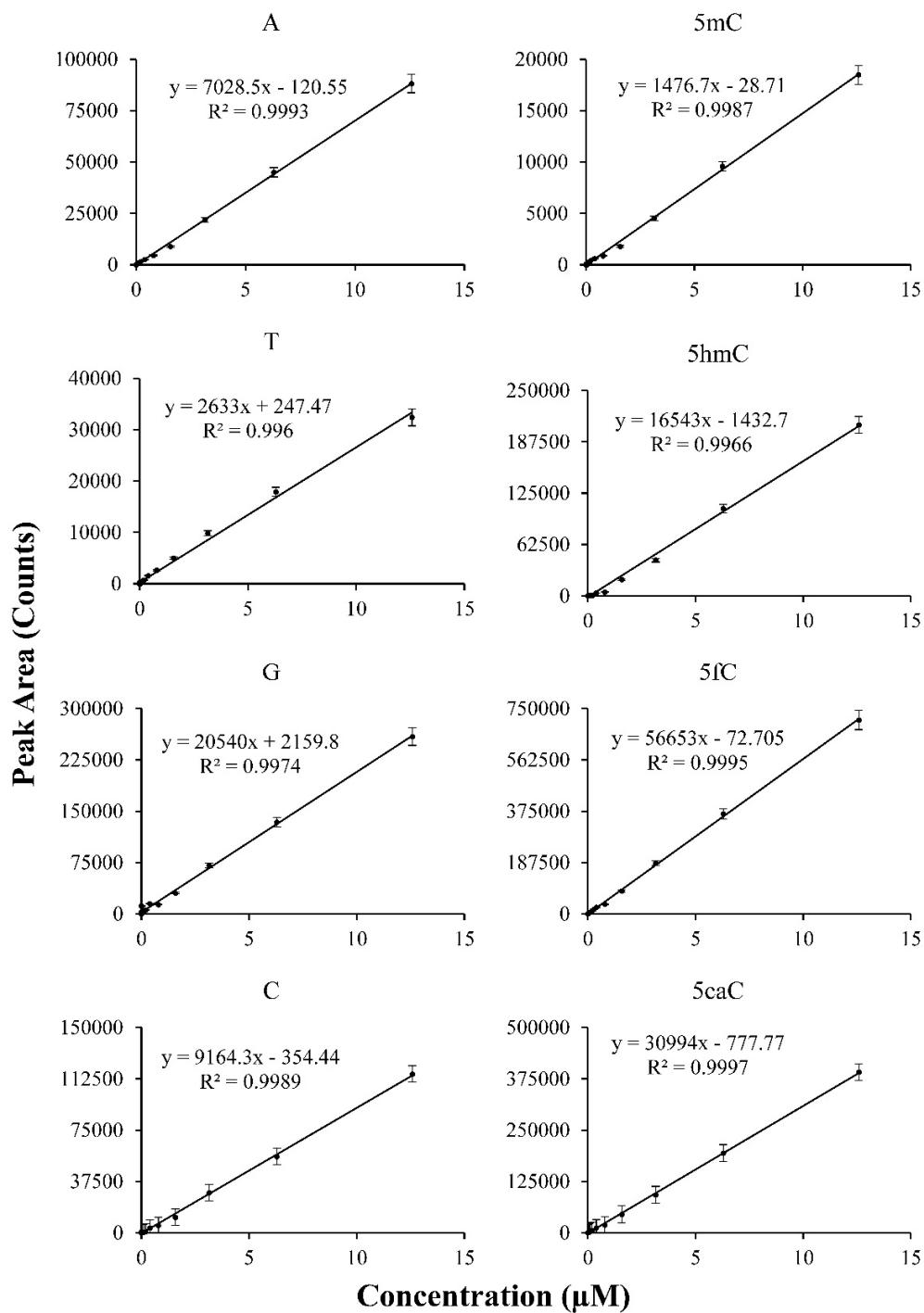


Figure S1. Standard curves of different nucleosides in negative mode.

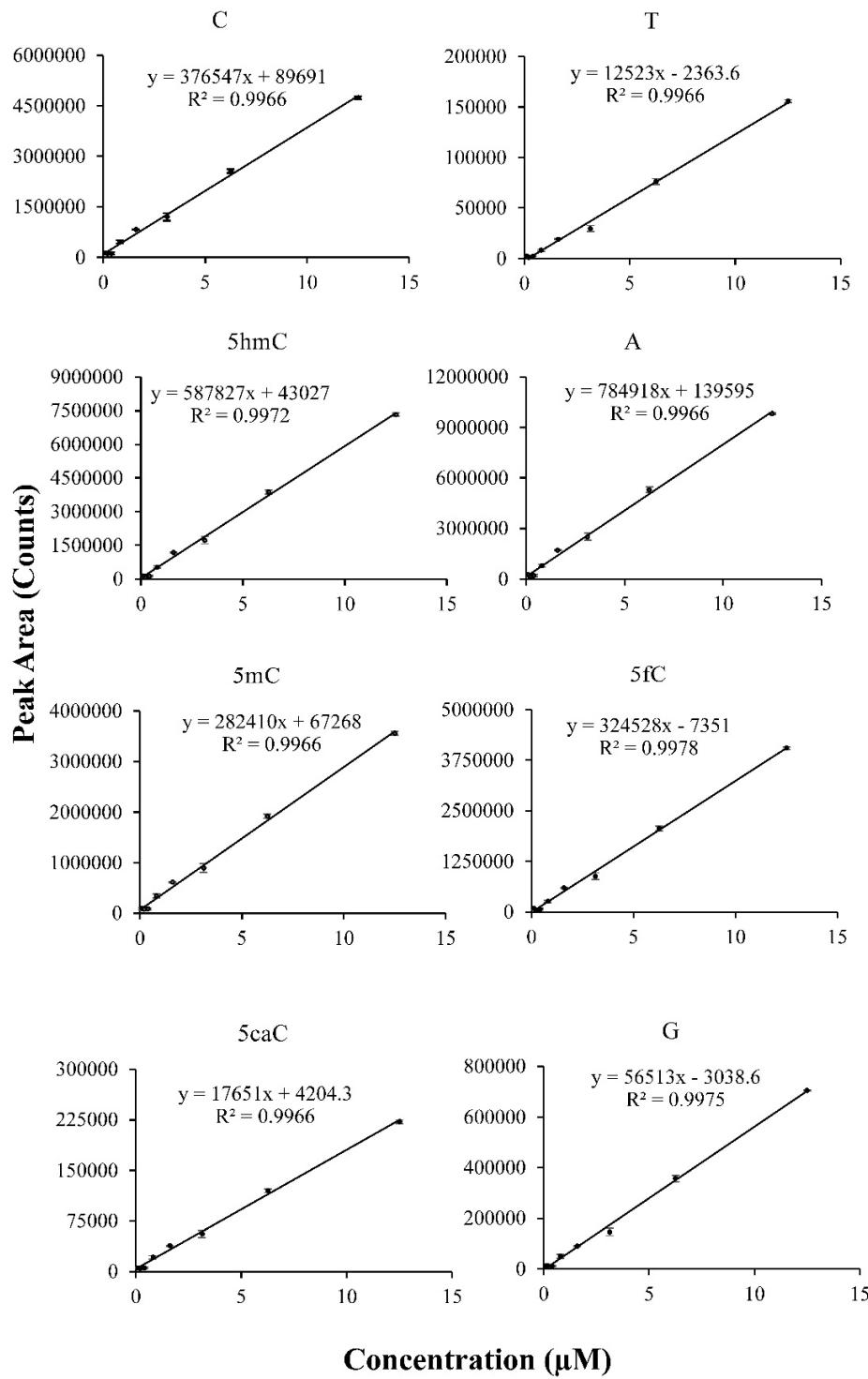


Figure S2. Standard curves of different nucleosides in positive mode.

Deoxyribonucleosides	Structure	Nucleoside Base	Structure
2-Deoxy-5methylcytidine Q1 242.2 (+) MODE Q1 240.1 (-) MODE		5-methylcytosine Q3 126.1 (+) MODE Q3 124.1 (-) MODE	
2-Deoxy-5-hydroxymethylcytidine Q1 258.2 (+) MODE Q1 256.2 (-) MODE		5-hydroxymethylcytosine Q3 142.1 (+) MODE Q3 140.1 (-) MODE	
2-Deoxy-5-formylcytidine Q1 256.2 (+) MODE Q1 254.2 (-) MODE		5-formylcytosine Q3 140.1 (+) MODE Q3 138.1 (-) MODE	
2-Deoxy-5-carboxyldcytidine Q1 272.2 (+) MODE Q1 270.2 (-) MODE		5-carboxyldcytosine Q3 156.1 (+) MODE Q3 154.1 (-) MODE	

Table S1. The chemical structure of four different modified deoxyribonucleosides, indicating the charge fragmentation position and the mass transition used for MRM detection.

Nucleosides	MRM Channels (Q1/Q3)	DP	EP	CEP	CE	CXP	LOD (μ M)	LLOQ (μ M)
C	226.1/110.1	-35	-9	-10	-24	-3	0.10	0.32
5hmC	256.2/140.1	-50	-9	-14	-22	-3	0.20	0.65
5mC	240.1/124.1	-40	-10.5	-10	-16	-3	0.20	0.65
5caC	270.2/154.1	-50	-4	-12	-24	-3	0.05	0.16
T	241.2/115.1	-40	-8	-14	-12	-3	0.20	0.35
A	250.2/134.1	-35	-10	-10	-22	-3	0.10	0.32
5fC	254.2/138.1	-50	-2	-10	-24	-3	0.01	0.02
G	266.2/150.1	-60	-7.5	-14	-26	-3	0.003	0.01

Global method parameters were: CUR (curtain gas) 50, temperature 500 °C, GS1 and GS2 (gas flows) 50 (arbitrary unit), collision associated dissociation (CAD) medium, ion spray voltage (IS) 4500 V for positive mode and -4500 V for negative mode

Table S2. Mass spectrometric parameters for the most intense MS/MS transitions of the eight nucleosides in negative mode detection.

Nucleosides	MRM Channels (Q1/Q3)	DP	EP	CEP	CE	CXP	LOD (μ M)	LLOQ (μ M)
C	228.1/112.1	21	7	14	15	4	0.36	1.09
5hmC	258.2/142.1	36	3.5	14	17	4	0.25	0.75
5mC	242.2/126.1	30	10.5	12	17	4	0.49	1.49
5caC	272.2/156.1	45	5	12	19	3	0.12	0.37
T	243.2/117.1	16	8	14	15	4	0.16	0.48
A	252.2/136.1	41	9	14	17	4	0.33	1.00
5fC	256.2/140.1	11	6	14	15	4	0.24	0.72
G	268.2/152.1	21	7	14	37	4	0.25	0.76

Global method parameters were: CUR (curtain gas) 50, temperature 500 °C, GS1 and GS2 (gas flows) 50 (arbitrary unit), collision associated dissociation (CAD) medium, ion spray voltage (IS) 4500 V for positive mode and -4500 V for negative mode

Table S3. Mass spectrometric parameters for the most intense MS/MS transitions of the eight nucleosides in positive mode detection.