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Supplementary Table 1. The DNAs used in this study

D1	palindromic 12-bp dsDNA	topstrand: 5'-ACCACXGGTGGT-3' X=5mC, 5hmC or 5fC
D2	palindromic FAM-18-bp dsDNA	topstrand: 5'-FAM-CAGCACACXGGTGTGCTG-3', X=C, 5mC, 5hmC , 5fC or 5caC
D3	biotinylated 26-bp dsDNAs	topstrand:5'-biotin-CAGTAGTCTGGACACACXGGTCATGA-3', bottom strand: 5'-TCATGACXGGTGTGTCCAGACTACTG-3', X=5mC, 5hmC or 5fC
D4	Biotin-free 26-bp dsDNAs	topstrand:5'-CAGTAGTCTGGACACACXGGTCATGA-3', bottom strand: 5'-TCATGACXGGTGTGTCCAGACTACTG-3', X=5C,5mC, 5hmC , 5fC or 5caC
D5	palindromic 58-bpDNA dsDNA	topstrand:5'-ACGATCAGATCCTAAGGCATCAGCACACXGGT GTGCTGATGCCTTAGGATCTGATCGT-3', X=5mC, 5hmC or 5fC
D6	palindromic 58-bpDNA AT-rich dsDNA	topstrand:5'-ACCAGCAGATGGCCAGGCATCAGATATAXGTA TATCTGATGCCTGGCCATCTGCTGGT-3' , X=5mC, 5hmC or 5fC
D7	58-bpDNA CG-rich dsDNA	topstrand:5'-ACTCAACAGACTACACAGTAGTGCCCCCXGCC CAGATGCTATTCAGTAACTGACACTG-3'; bottom strand: 5'-CAGTGTCAGTTACTGAATAGCATCTGGGX GGGGGGCACTACTGTGTAGTCTGTTGAGT -3' X=5mC, 5hmC or 5fC
D8	palindromic 100-bpDNA dsDNA	topstrand:5'-GCTTGGAGGTCCAAGCTAGCTACGATCAGATC CTAAGGCATCAGCACACXGGTGTGCTGATGCCTTAGGATC TGATCGTAGCTAGCTTGGACCTCCAAGC-3', X=5mC, 5hmC or 5fC.
D9	deuterium labeled biotinylated 20-bp dsDNA	topstrand:5'- Biotin-CTTGGACACACXGGTCATGA-3'; bottom strand: 5'-TCATGACCmGGTGTGTCCAAG -3' X=$[^2H]_3$-5mC
D10	biotinylated 20-bp dsDNA	topstrand:5'- Biotin-CTTGGACACACXGGTCATGA -3'; bottom strand: 5'- TCATGACCmGGTGTGTCCAAG -3' X=5mC

The DNAs were synthesized and annealed for the measurements of enzymatic activities and DNA-binding affinities of TET proteins.

Supplementary Table 2. TET activities on 5mC/5hm/5fC-DNA substrate

	12.5 μM TET1+58-bpDNA dsDNA			
	5mC (μM)	5hmC (μM)	5fC (μM)	5caC (μM)
5mC	1.400	ND	ND	ND
5mC+TET1(12.5 μM)	0.130±0.023	0.550±0.035	0.218±0.014	0.272±0.004
5hmC	ND	1.400	ND	ND
5hmC+TET1(12.5 μM)	ND	0.806±0.031	0.135±0.028	0.139±0.003
5fC	ND	ND	1.400	ND
5fC+TET1(12.5 μM)	ND	ND	0.937±0.085	0.199±0.035
	5 μM TET2+58-bpDNA dsDNA			
	5mC (μM)	5hmC (μM)	5fC (μM)	5caC (μM)
5mC	1.400	ND	ND	ND
5mC+TET2(5 μM)	0.035±0.008	0.224±0.039	0.305±0.033	0.529±0.010
5hmC	ND	1.400	ND	ND
5hmC+TET2(5 μM)		0.591±0.119	0.347±0.033	0.293±0.045
5fC	ND	ND	1.400	ND
5fC+TET2(5 μM)	ND	ND	0.868±0.063	0.426±0.011
	1 μM TET2+58-bpDNA dsDNA			
	5mC (μM)	5hmC (μM)	5fC (μM)	5caC (μM)
5mC	1.400	ND	ND	ND
5mC+TET2(1 μM)	0.124±0.008	1.019±0.106	0.409±0.069	0.069±0.016
5hmC	ND	1.400	ND	ND
5hmC+TET2(1 μM)	ND	1.253±2.56	0.172±0.047	0.046±0.046
5fC	ND	ND	1.400	ND
5fC+TET2(1 μM)	ND	ND	1.248±0.277	ND

58-bp DNA substrate containing one 5mCpG/5hmCpG/5fCpG site was incubated with TET1 or TET2 for reaction. ND indicates Non-Detectable. Quantification was calculated from three independent assays and the error bars represent ± SD for triplicate experiments. Note that 5 μM TET2 shows stronger enzymatic activity than 12.5 μM TET1. The results were reproducibly obtained under our experimental conditions. One possible explanation is that the difference does exist between TET1 and TET2 although we could not rule out the possibility that the difference is resulted from different quality/stability of the purified recombinant TET1 and TET2.

Supplementary Table 3. TET activities on 5mC/5hm/5fC-DNA substrate at different time points

Substrate	Time (min)	5mC (μM)	5hmC (μM)	5fC (μM)	5caC (μM)
5mC-DNA	0	1.400	ND	ND	ND
	5	0.363±0.052	0.920±0.62	0.039±0.030	ND
	10	0.246±0.021	1.047±0.057	0.238±0.016	ND
	20	0.155±0.006	1.003±0.081	0.346±0.043	0.021±0.021
	30	0.132±0.005	1.048±0.109	0.375±0.033	0.053±0.012
	40	0.124±0.008	1.019±0.106	0.409±0.069	0.069±0.016
5hmC-DNA	0		1.400	ND	ND
	5		1.426±0.163	0.038±0.038	ND
	10		1.423±0.127	0.134±0.049	ND
	20		1.128±0.179	0.122±0.028	0.026±0.026
	30		1.167±0.210	0.149±0.029	0.030±0.030
	40		1.253±0.256	0.172±0.047	0.046±0.046
5fC-DNA	0			1.400	ND
	5			1.288±0.293	ND
	10			1.316±0.266	ND
	20			1.299±0.252	ND
	30			1.329±0.337	ND
	40			1.249±0.277	ND

58-bp DNA substrate containing one 5mCpG /5hmCpG/5fCpG site was incubated with 1 μM TET2 for reaction. ND indicates Non-Detectable. Quantification was calculated from three independent assays and the error bars represent ± SD for triplicate experiments.

Supplementary Table 4. Steady-state kinetic analyses of TET2-mediated oxidation

0.5 μM TET2+58-bp 5mC dsDNA		2 μM TET2+58-bp 5hmC dsDNA		3 μM TET2+58-bp 5fC dsDNA	
5mC Concentration (μM)	5hmC generation (nM/s)	5hmC Concentration (μM)	5fC generation (nM/s)	5hmC Concentration (μM)	5caC generation (nM/s)
0.070	ND	0.140	ND	0.140	ND
0.140	0.319±0.073	0.350	0.402±0.042	0.350	0.331±0.001
0.350	0.399±0.071	0.700	0.596±0.088	0.700	0.451±0.029
0.700	0.707±0.030	1.050	0.644±0.056	1.400	0.795±0.030
1.05	0.728±0.026	1.4.00	0.837±0.32	2.800	0.925±0.039
1.40	0.745±0.039	2.800	0.917±0.032	5.600	1.112±0.073

58-bp DNA substrate containing one 5mCpG /5hmCpG/5fCpG site was incubated with 0.5 μM, 2 μM, and 3 μM TET2, respectively. ND indicates Non-Detectable. Quantification was calculated from two independent assays and the error bars represent ± SD for duplicate experiments.

Supplementary Table 5. TET2 activities on substrates with different lengths and sequences

Reaction	5mC (μM)	5hmC (μM)	5fC (μM)	5caC (μM)
26-bp dsDNA				
5mC	1.400	ND	ND	ND
5mC+TET2(1 μM)	0.107±0.008	0.586±0.046	0.403±0.033	0.297±0.044
5hmC	ND	1.400	ND	ND
5hmC+TET2(1 μM)	ND	0.838±0.226	0.499±0.071	0.239±0.057
5fC	ND	ND	1.400	ND
5fC+TET2(1 μM)	ND	ND	0.808±0.042	0.560±0.012
100-bp dsDNA				
5mC	1.400	ND	ND	ND
5mC+TET2(1 μM)	0.572±0.102	0.864±0.102	0.046±0.008	0.021±0.002
5hmC	ND	1.400	ND	ND
5hmC+TET2(1 μM)	ND	1.091±0.055	0.148±0.034	0.044±0.010
5fC	ND	ND	1.400	ND
5fC+TET2(1 μM)	ND	ND	1.311±0.064	0.08±0.007
58-bp dsDNA				
5mC	1.400	ND	ND	ND
5mC+TET2(1 μM)	0.368±0.47	0.671±0.114	0.262±0.046	0.071±0.030
5hmC	ND	1.400	ND	ND
5hmC+TET2(1 μM)	ND	1.025±0.044	0.378±0.021	0.194±0.006
5fC	ND	ND	1.4	ND
5fC+TET2(1 μM)	ND	ND	1.361±0.073	0.087±0.010
58-bp AT-rich dsDNA				
5mC	1.400	ND	ND	ND
5mC+TET2(1 μM)	0.284±0.049	0.838±0.057	0.180±0.072	0.031±0.007
5hmC	ND	1.400	ND	ND
5hmC+TET2(1 μM)	ND	0.693±0.075	0.411±0.078	0.196±0.006
5fC	ND	ND	1.400	ND
5fC+TET2(1μM)	ND	ND	0.893±0.018	0.574±0.08
58-bp CG-rich dsDNA				
5mC	1.400	ND	ND	ND
5mC+TET2(1 μM)	0.270±0.051	1.006±0.067	0.167±0.035	0.081±0.014
5hmC	ND	1.400	ND	ND
5hmC+TET2(1 μM)	ND	1.217±0.042	0.166±0.043	0.018±0.005
5fC	ND	ND	1.400	ND
5fC+TET2(1 μM)	ND	ND	1.391±0.103	0.068±0.012

Various DNA substrates were incubated with wild-type 1 μM TET2 protein for reaction. ND indicates Non-Detectable. Quantification was calculated from three independent assays and the error bars represent ± SD for triplicate experiments.

Supplementary Table 6. Effects of substrate and product on activities of TET2

Reaction	5mC (μM)	5hmC (μM)	5fC (μM)	5caC (μM)
1 μM TET2 + 26-bp biotinylated 5mC-DNA In the presence of 26-bp 5C/5hmC-DNA				
Biotin-5mC (no TET2)	1.400	ND	ND	ND
Biotin-5mC	0.174±0.038	0.925±0.123	0.431±0.083	0.293±0.219
Biotin-5mC +1.4 μM 5C	0.205±0.059	0.786±0.010	0.303±0.059	0.129±0.098
Biotin-5mC+0.7 μM 5hmC	0.182±0.039	0.946±0.041	0.343±0.099	0.188±0.137
Biotin-5mC +1.4 μM 5hmC	0.213±0.044	1.168±0.035	0.341±0.135	0.094±0.048
1 μM TET2 + 26-bp biotinylated 5hmC-DNA In the presence of 26-bp 5C/5fC-DNA				
Biotin-5hmC (no TET2)		1.400	ND	ND
Biotin-5hmC		0.663±0.87	0.423±0.017	0.378±0.143
Biotin-5hmC +1.4 μM 5C		0.772±0.058	0.351±0.035	0.137±0.090
Biotin-5hmC+0.7 μM 5fC		0.706±0.076	0.388±0.027	0.246±0.168
Biotin-5hmC +1.4 μM 5fC		0.751±0.096	0.457±0.076	0.168±0.119
1 μM TET2 + 26-bp biotinylated 5fC-DNA In the presence of 26-bp 5C/5caC-DNA				
Biotin-5fC (no TET2)			1.400	ND
Biotin-5fC			1.157±0.085	0.282±0.116
Biotin-5fC +1.4 μM 5C			1.096±0.039	0.164±0.079
Biotin-5fC+0.7 μM 5caC			0.989±0.038	0.428±0.085
Biotin-5fC +1.4 μM 5caC			0.986±0.075	0.430±0.063

Various DNA substrates were incubated with wild-type 1 μM TET2 protein for reaction. 26-bp biotinylated DNA was purified with strepavidin beads and applied for LC-MS/MS detection. ND indicates Non-Detectable. Quantification was calculated from three independent assays and the error bars represent ± SD for triplicate experiments.

Supplementary Table 7. Decreased enzymatic activities of TET2 by substrate deuteration

0.25μM TET2 + 20-bp biotinylated 5mC-DNA				
	5mC (μM)	5hmC (μM)	5fC (μM)	5caC (μM)
Biotin-5mC (no TET2)	1.400	ND	ND	ND
Biotin-5mC (with TET2)	1.140±0.196	0.117±0.063	ND	ND
0.5 μM TET2 + 20-bp biotinylated 5mC-DNA				
Biotin-5mC (no TET2)	1.400	ND	ND	ND
Biotin-5mC (with TET2)	1.415±0.124	0.758±0.051	ND	ND
0.25 μM TET2 + 20-bp biotinylated $[^2H]_3$-5mC-DNA				
	$[^2H]_3$-5mC (μM)	$[^2H]_2$-5hmC (μM)	$[^2H]_1$-5fC (μM)	5caC (μM)
Biotin-$[^2H]_3$-5mC (no TET2)	1.400	ND	ND	ND
Biotin-$[^2H]_3$-5mC (with TET2)	1.110±0.137	ND	ND	ND
0.5 μM TET2 + 20-bp biotinylated $[^2H]_3$-5mC-DNA				
Biotin-$[^2H]_3$-5mC (no TET2)	1.400	ND	ND	ND
Biotin-$[^2H]_3$-5mC (with TET2)	1.375±0.204	0.158±0.025	ND	ND

20-bp DNA substrate containing one 5mCpG/$[^2H]_3$-5mCpG site was incubated with 0.25 μM, 0.5 μM TET2 at 37℃ for 10 min followed by heat at 65℃for 10 min, respectively. 20-bp biotinylated DNA was purified with streptavidin beads and applied for LC-MS/MS detection. ND indicates Non-Detectable. Quantification was calculated from three independent assays and the error bars represent ± SD for triplicate experiments.