Supporting information

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Materials and methods

DNA preparation:

DNA was purchased from Takara Biotechnology (Dalian) containing either cytosine, 5-methylcytosine, 5-hydroymethylcytosine or formylcytosine.

The preparation of G-ladder:

1 μl DNA (100 mM), 10 μl Tris-EDTA buffer (100 mM, pH 7.8) and 89 μl distilled water were added to the G reaction tube and 40 μl distilled water, 20μl CH_3COONa-CH_3COOH buffer (1 M, pH 5.0), and 40 μl ethanethiol were added to the other tube to prepare DMS stop buffer. Then added 2 μl DMS to the G reaction tube and immediately closed the tubes and mixed. The tube was incubated at room temperature for eight minutes, followed by adding the DMS stop buffer plus 100% ethanol having been prechilled to −20°C to the G reaction tube and mixing. The mixture was freezed at −80°C for 2h, and centrifuged for 20 minutes. After the piperidine treatment, the DNA was precipitated as the step mentioned above.

DNA oxidations:

ssDNA oxidation: DNA was made up to 21 μL with NaOH (50mM final concentration) at 4℃, then 4 μL of a $KRuO_4$(Alpha Aeser) solution (6 mM in 50 mM NaOH) was added and the reaction was held at 4℃ for 1h. the reaction was purified by pore film.

dsDNA oxidation: DNA was made up to 21 μL with NaOH (50mM final concentration), then was denatured at 37℃ for 30 min. The reaction was snap cooled at 4 ℃ for 20 min, then 4 μL of a $KRuO_4$(Alpha Aeser) solution (6 mM in 50 mM NaOH) was added immediately. After the reaction at 4℃ for 1 h, the DNA was purified, and then denatured in NaOH again. The reaction was snap cooled again before the second time oxidization. Finally the DNA was purified by pore film.

The DNA sequencing analysis by polyacrylamide gel electrophoresis:

Following the steps of piperidine cleavage and freeze drying, the DNA samples were loaded into a 20% polyacrylamide gel for electrophoresis at 20 V/cm; this electrophoresis occurred at room temperature in 1×TBE buffer. The gel was then analysed using the Pharos FX Molecular Imager (Bio-Rad, USA).

PAGE analysis

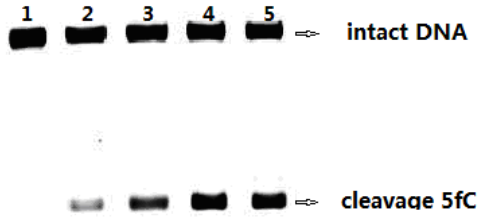


Figure S1. Polyacrylamide gel electrophoresis analysis of DNA1(X, Z=C,Y=5fC). Treatment of the DNA **1** (20pmol) with piperidine at 90 ℃for 30 mins. The volume fractions of piperidine in lanes 1-5 were 0%, 5%, 10%, 15% and 20%, respectively.

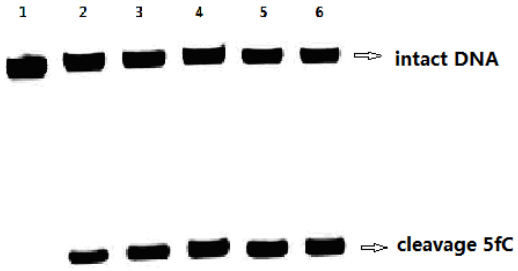
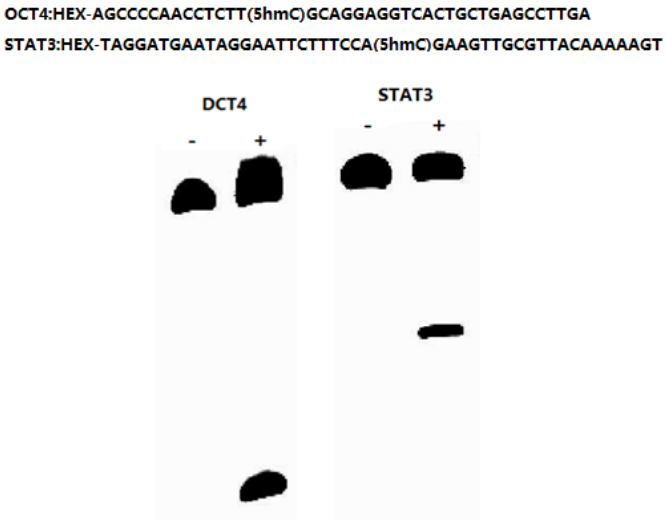


Figure S2. Polyacrylamide gel electrophoresis analysis of DNA **1**(X, Z=C, Y=5fC). Treatment of the DNA **1** (20pmol)with 15% piperidine at 90℃. The reacting time in lanes 1-6 was 0h, 0.5h, 1h, 1.5h, 2h, and 2.5h, respectively.



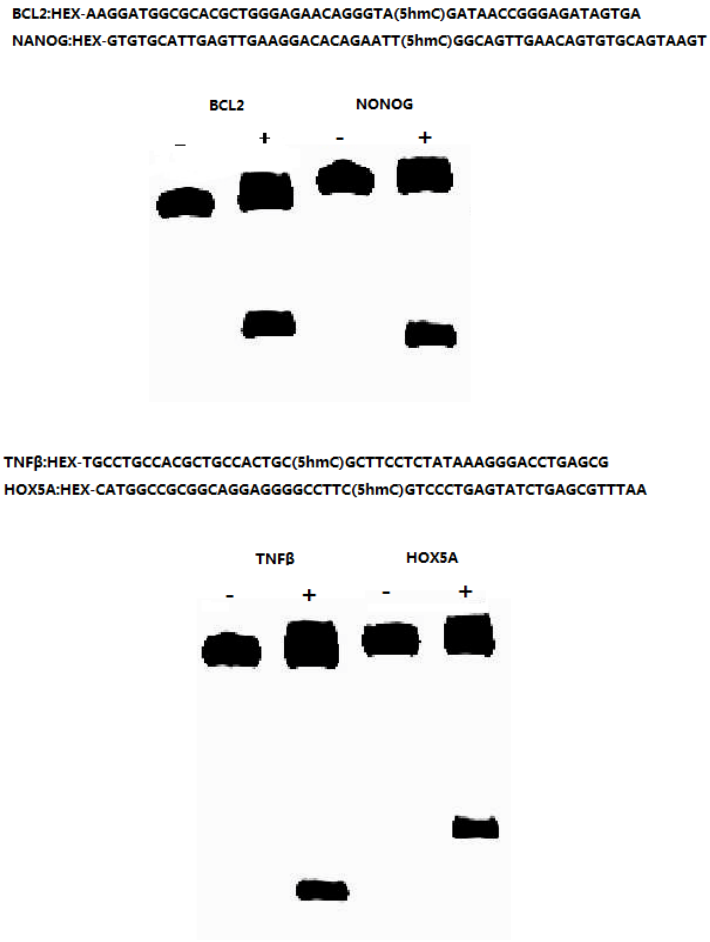


Figure S3. Polyacrylamide gel electrophoresis analysis of the DNA fragments containing 5hmC first oxidized by $KRuO_4$ and then treated with piperidine in the promoter regions of human STAT3, BCL2, OCT4, NANOG, HOX5A and TNF- β genes. Lane '-', the DNA fragments were treated without oxidation and reacted with piperidine directly; Lane'+', the DNA fragments were treated with oxidation and then piperidine.