Supporting Information

"Repair and Fold" DNA Nanotweezers for Measuring DNA Alkylation Repair Mediated by ALKBH

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Reagents and Materials.

All oligonucleotides were synthesized by Sangon Biotech Co. Ltd., (Shanghai, China) and Accurate Biology (Changsha, China). Bovine Serum Albumin (BSA) and Trypsin were purchased from Genview (USA). Recombinant ALKBH2, ALKBH3 and FTO were acquired from Active Motif (Carlsbad, USA). Temozolomide (TMZ) and α-KG were bought from J&K Scientific. IOX-1 and HUHS015 were purchased from Shanghai Haoyuan Biomedical Technology Co., LTD. A549, MCF-7 and U87 cells were purchased from Procell Life Science &Technology Co., Ltd (Wuhan, China). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from CellMax. Penicillin-streptomycin (100x), trypsin, and BCA Protein Assay Kit were purchased from Beyotime (Shanghai, China).

Cell culture

All cells were cultured at 37°C under 5% CO_2 in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin.

Fluorescence assay

Generally, the fluorescence spectra were obtained with FS5 spectrofluorometer (Edinburgh, UK) with excitation wavelength of 530 nm. The relative FRET was calculated as follows:

$$\text{Relative FRET} = F_{Cy5} / F_{Cy3}$$

Where F_{Cy5} is the maximum fluorescence emission of Cy5 (λ_{em}= 670 nm) and F_{Cy3} is the maximum fluorescence emission of Cy3 (λ_{em}= 565 nm).

Optimization of methylation number of triplex

The oligonucleotides containing different number of 1MeA modification (S; 1*1MeA-S or 2*1MeA-S) were mixed with DNA stands (F, L) in HEPES buffer containing 10 mM $MgCl_2$ by using a 1:1:1 molar ratio. The mixture were heated to 95°C for 5 min, and then cooling to 25°C to form the 0-Me triplex, 1-Me triplex and 2-Me triplex construct. Then resulting triplex was diluted to final concentration of 100 nM with TAE-Mg^{2+} (40 mM Tris, 2 mM EDTA, 12.5 mM Mg^{2+}) buffer of different pHs (pH=5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0), respectively. Subsequently, the fluorescence signals were measured.

ALKBH2 mediated demethylation. The oligonucleotides containing two 1MeA modification (2*1MeA-S) were mixed with DNA stands (F, L) in HEPES buffer containing 10 mM $MgCl_2$ by using a 1:1:1 molar ratio. The mixture were heated to 95°C for 5 min, and then cooling to 25°C to form the triplex construct. The 1MeA-modified nanotweezer was reacted with 10 μM ALKBH2 in the reaction buffer (50 mM HEPES, 150 mM NaOH, 2 mM ascorbate, 75 μM $Fe(NH_4)_2(SO_4)_2$, 1 mM a-KG, 50 μg/ml BSA) for 5 h at 37°C. Then the reaction mixture was diluted into 100 μL with TAE-Mg^{2+} buffer to measure the fluorescence signal. The final concentration of the triplex was 100 nM.

Sensitivity assay of ALKBH2 mediated repair. The 1MeA-modified nanotweezer was incubated with different concentrations of ALKBH2 (0, 20, 40, 60, 80, 100, 150, 200, 300, 400 nM) in the reaction buffer for 5 h at 37°C. Then dilute with TAE-Mg^{2+} buffer before the fluorescence signal measurement.

Selectivity assay of ALKBH2 mediated repair. The 1MeA-modified nanotweezer was incubated with ALKBH2, ALKBH3, FTO, BSA and Trypsin in reaction buffer for 5 h at 37°C, respectively. The fluorescence signal measurement were performed as described above.

Evaluation of ALKBH2 inhibitor. Different concentrations of IOX1 (0.1, 1, 10, 20, 40, 60, 100, 150, 500 μM) were incubated with the 1MeA-modified nanotweezer in the reaction buffer at 37°C for 5 h, respectively. Subsequently, the fluorescence signals were measured and the IC50 value was determined.

Cellular extracts analysis. MCF-7, A549, U87 cells were collected and washed with 1× PBS buffer for three times. The nuclear proteins were obtained using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Finsher Scientific). Protein concentration was determined using Bicinchoninic Acid Assay (BCA) according to the manufacturer's instructions. The 1MeA-modified nanotweezer was incubated with 5 μg cellular extracts in the reaction buffer at 37°C for 5 h, respectively, and then the fluorescence signals were measured.
To evaluate the cellular response to drugs treatment, U87 cells were pretreated with 100 μM TMZ at 37°C for 24 h and the nuclear proteins were extracted. The remaining procedures followed the same protocol as described above.

ALKBH3 mediated demethylation. The oligonucleotides containing two 3MeC modification (2*3MeC-L) were mixed with DNA stands (F, S) in HEPES buffer containing 10 mM $MgCl_2$ by using a 1:1:1 molar ratio. The mixture were heated to 95°C for 5 min, and then cooling to 25°C to form the triplex construct. The 3MeC-modified nanotweezer was reacted with 10 μM ALKBH3 in the reaction buffer (50 mM HEPES, 150 mM NaOH, 2 mM ascorbate, 75 μM $Fe(NH_4)_2(SO_4)_2$, 1 mM a-KG, 50 μg/ml BSA) for 5 h at 37°C. Then the reaction mixture was diluted into 100 μL with TAE-Mg^{2+} (40 mM Tris, 2 mM EDTA, 12.5 mM Mg^{2+}) buffer to measure the fluorescence signal. The final concentration of the triplex was 100 nM.

Sensitivity assay of ALKBH3 mediated repair. The 3MeC-modified nanotweezer was incubated with different concentrations of ALKBH3 (0, 20, 40, 60, 80, 100, 150, 200, 300, 400 nM) in the reaction buffer for 5 h at 37°C. Then dilute with TAE-Mg^{2+} buffer before the fluorescence signal measurement.

Selectivity assay of ALKBH3 mediated repair. The 3MeC-modified nanotweezer was incubated with ALKBH2, ALKBH3, FTO, BSA and Trypsin in reaction buffer for 5 h at 37°C, respectively. The fluorescence signal measurement were performed as

described above.

Evaluation of ALKBH3 inhibitor. Different concentrations of HUHS015 (0.005, 0.01, 0.1, 0.5, 1, 5, 10, 20, 50 μM) were incubated with the 3MeC-modified nanotweezer in the reaction buffer at 37°C for 5 h, respectively. Subsequently, the fluorescence signals were measured and the IC50 value was determined.

RT-PCR analysis

A549, MCF-7 and U87 cells were washed with 1× PBS buffer for three times. Total RNA was extracted using Trizol. cDNA was obtained using Evo M-MLV RT mix kit with gDNA clean for qPCR (Accurate Biotechnology, China). The resulting cDNA was then utilized as the template for RT-PCR amplification using SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biotechnology, China) according to the manufacturer's protocol.

Table S1. Sequences of oligonucleotides.

Name	Sequence（5′-3′）
S	AAAGAAAGAAAGGGCGGG
1*1MeA -S	AAAGAA^{M1}AGAAAGGGCGGG
2*1MeA -S	AAAG^{M1}AAAG^{M1}AAAGGGCGGG
L	GGTTAGATGGTATGGTTTTTGTTTCTTTTTTTTTTTTTTTTTTTTTT CCCGCCCTTTCTTTCTTTCTGAGCGTAAGGTCTGG
2*3MeC-L	GGTTAGATGGTATGGTTTTTGTTTCTTTTTTTTTTTTTTTTTTTTTT CCCGCC^{M1}CTTT^{M1}CTTTCTTTCTGAGCGTAAGGTCTGG
F	Cy5-CCATACCATCTAACCTTTTTTTTTTCCAGACCTTACGC-Cy3

Table S2. Sequences of primers for RT-PCR.

Name	Sequence（5′-3′）
hALKBH2 F	GGTGACTGGACAGACCTTCAAC
hALKBH2 R	ACCGAAGGAGACAGAGGCAATG

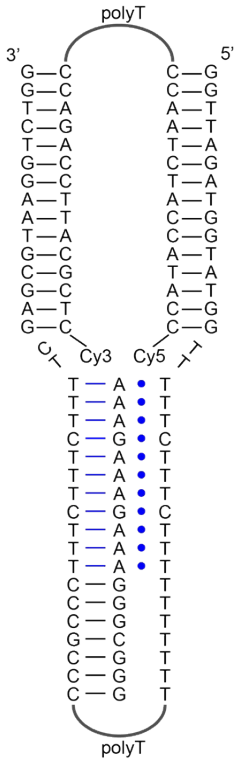


Figure S1. Schematic illustration of the DNA nanotweezer structure.

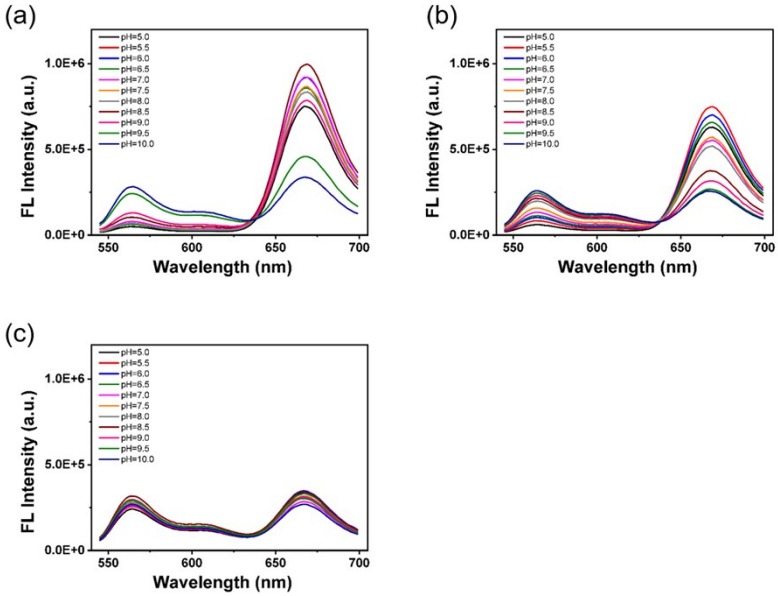


Figure S2. Fluorescence spectra of nanotweezer containing different number of 1MeA modification. (a) control nanotweezer; (b) 1-Me nanotweezer; (c) 2-Me nanotweezer.

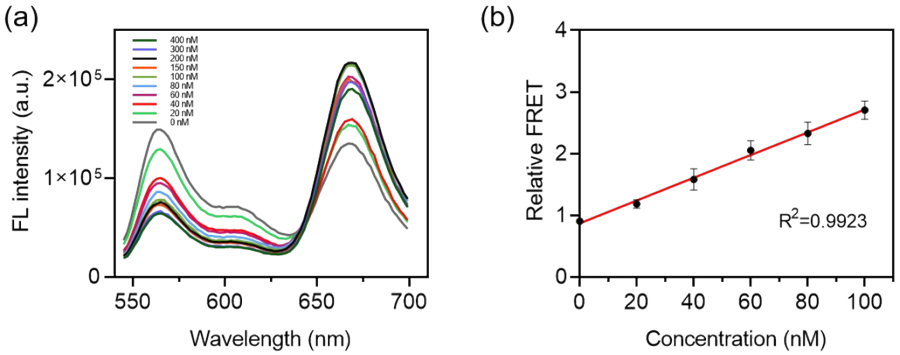
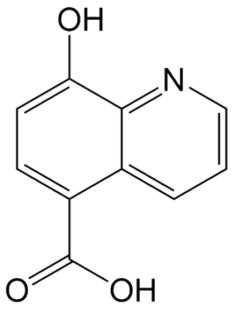


Figure S3. (a) Fluorescence responses of the 1MeA-modified DNA nanotweezer with various concentrations of ALKBH2. (b) The corresponding calibration plot with various concentrations of ALKBH2. Error bars represent three independent experiments.



IOX1

Figure S4. Chemical structure of IOX1.

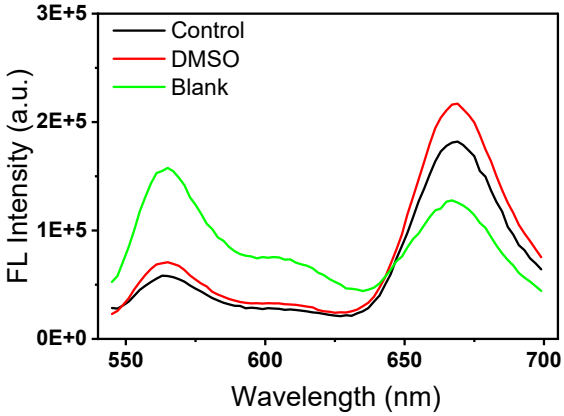


Figure S5. Fluorescence spectra of 1MeA-modified DNA nanotweezer with or without DMSO.

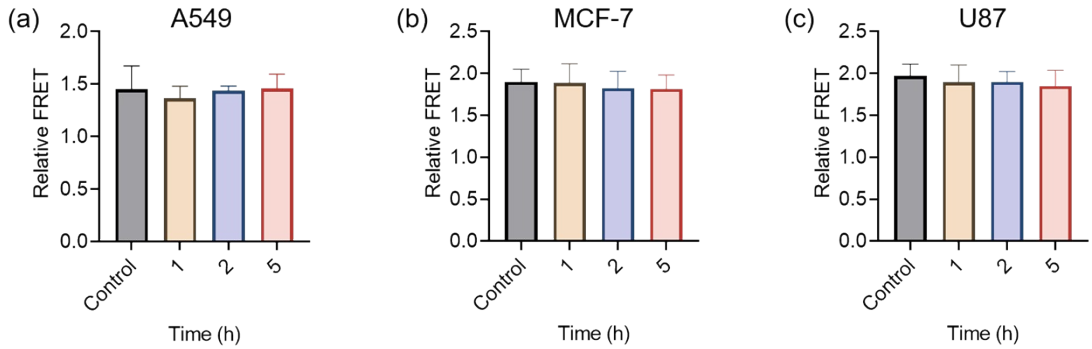


Figure S6. Evaluation of the stability of the nanotweezer in different cell lysate. Error bars represent S.D. from three independent experiments.

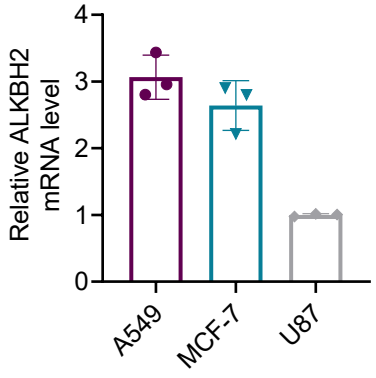


Figure S7. Evaluation of ALKBH2 expression levels in different cell lines by RT-PCR analysis.

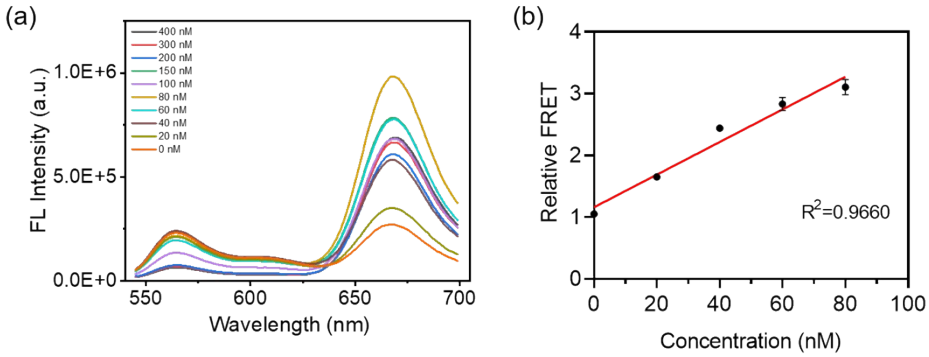
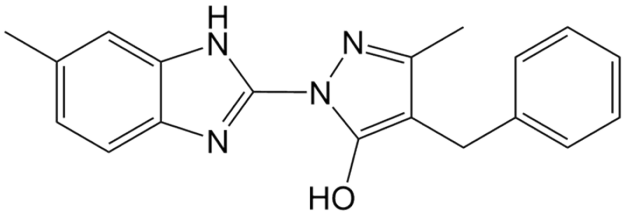


Figure S8. (a) Fluorescence responses of the 3MeC-modified DNA nanotweezer with various concentrations of ALKBH3. (b) The corresponding calibration plot with various concentrations of ALKBH3. Error bars represent three independent experiments.



HUHS015

Figure S9. Chemical structure of HUHS015.

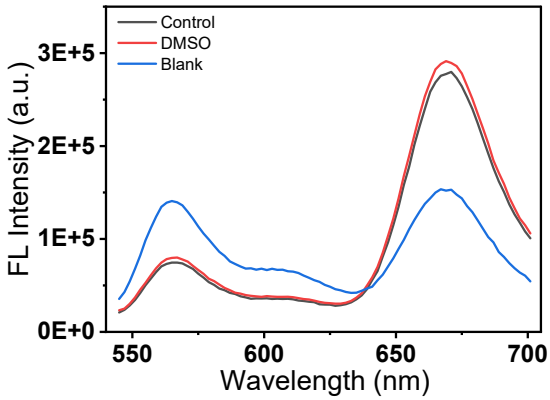


Figure S10. Fluorescence spectra of the 3MeC-modified DNA nanotweezer with or without DMSO.

Table S3. Comparison of our method with the reported methods.

Target	Method	LOD	Reference
ALKBH2	Fluorescence probe	Not provided	[5c]
ALKBH2	LC-MS	Not provided	[7]
ALKBH2	Melting temperature-based analysis	10 nM	[16]
ALKBH2	This work	2.4 nM	
ALKBH3	ELISA	0.1 ng/mL	/
ALKBH3	Fluorescence probe	Not provided	[5b]
ALKBH3	LC-MS	Not provided	[7]
ALKBH3	Electrochemical detection	0.04 ng/mL	[6e]
ALKBH3	This work	0.75 nM	