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Electronic Supplementary Information

An *in vitro* site-specific cleavage assay of CRISPR-Cas9 using a personal glucose meter

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Experimental details:

Reagents and Materials. DNA oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd (Shanghai, China). N-hydroxysuccinimide (NHS), 1,4-phenylene diisothiocyanate (PDITC), 1-(3-diaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Alfa Aesar (Tianjin, China). Cas9 nuclease and sgRNA in vitro one-step transcription kit was acquired from Novoprotein (Shanghai, China). Trisodium citrate, ethylene glycol and sodium acetate were obtained from China National Pharmaceutical Group Corp (Shanghai, China). Invertase was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Nuclease-free water was used in the Cas9 cleavage experiment. Buffer A: 0.1 M NaCl, 0.1 M sodium phosphate buffer, pH 7.3. Phosphate buffer saline (PBS, 10 mM, pH 7.4, 150 mM NaCl, 1 mM MgCl). PBST: PBS with 0.05% Tween 20. Other aqueous solutions used in experiments were prepared using deionized water (18.2 MΩ·cm).

Instruments. Transmission electron microscopy (TEM) was performed on HT7700 electron microscope (Hitachi, Japan). Zeta potential were measured on Malvern Zeta Sizer Nano (Malvern Instruments). The concentration and UV-vis absorbance of DNA were carried out on nanodrop one (Thermo Scientific, USA). Gel electrophoresis analysis was performed on gel electrophoresis apparatus (Bio-Rad, USA). Yuwell personal glucose meter (580) was used in this experiment.

EXPERIMENTAL SECTION

Synthesis of carboxyl-functionalized magnetic beads (MNPs). Trisodium citrate (0.2 g) and $FeCl_3$ (0.325 g) were added into ethylene glycol (20 mL). The mixture was sonicated for 5 min. Then NaAc (1.20 g) was added. After vigorous stir for 30 min, the mixture was sealed into a Teflon-lined stainless-steel autoclave. The autoclave was maintained at 200 ℃ for 10 h. The product was washed several times with ethanol and dried in vacuum.

Modification of H_2 on MNPs (MNPs-H_2). 100 μL synthesized MNPs (10 mg/mL) were washed with MES buffer (25 mM, pH=5.0) twice. The supernatant was separated using magnetic separation. The 50 μL EDC (50 mg/mL) and 50 μL NHS (50 mg/mL) were added and the mixture was shaken for 30 min to activate the carboxyl group. Afterwards, the MNPs were washed with MES buffer twice and dispersed in 100 μL MES buffer. 5 μL capture probe H_2 (100 μM) was added and the mixture was shaken at room temperature overnight. The product was washed several times with PBST buffer and dispersed in 100 μL PBS buffer containing 1% BSA. The mixture was shaken for 1 h. After that, the product was washed several times with PBS and then dispersed in 100 μL PBS for further use.

Quantification of H_2 on MNPs. The reacted mixture of H_2 and MNPs was separated by magnet. The H_2 in the supernatant was determined by nanodrop. Afterwards, the obtained MNPs-H_2 was added with 100 μL PBST. The mixture was also separated by magnet and the H_2 in the supernatant was measured by nanodrop again. Then the total H_2 without reacting with MNPs was measured. The H_2 modified on MNPs could be calculated by: (total H_2 mass-H_2 mass in the supernatant)/ MNPs mass.

Synthesis of invertase-H_1. 30 μL H_1 (100 μM) was mixed with 15 μL 0.1 M sodium borate buffer (pH 9.2). Then 0.5 mL DMF containing 2 mg PDITC was added. The mixture was stirred at room temperature in the dark for 2 h. Afterwards, 3 mL deionized water and 3 mL 1-butanol were added to the above mixture. The solution was centrifuged and the supernatant was discarded. 1-butanol was used to extract the aqueous phase three times. Then the solution was purified with buffer A by Amicon-3K five times to obtain the PDITC-activated amine-DNA solution. Finally, the PDITC-activated amine-DNA was mixed with 10 mg invertase in buffer A (2 mL) and the

mixture was stirred at room temperature for 48 h. The obtained invertase-H_1 solution was purified with buffer A by Amicon-30K and then diluted to 5 mg/mL with PBS for further use.

Generation of sgRNA. The sgRNA was prepared by method provided by manufacturer. Briefly, 10 μL 2* sgRNA reaction buffer, 2 μL sgRNA forward primer (10 μM), 2 μL enzyme mix and 6 μL RNase free water were added in a 1.5 mL centrifuge tube. The mixture was incubated at 37 ℃ for 2 h. Then, 1 μL DNase I was added and the mixture was incubated at 37 ℃ for 10 min to remove DNA template. After that, the mixture was incubated at 75 ℃ for 10 min to inactivate the DNase I.

Target DNA cleavage and combination with invertase-H_1. 0.3 μM (final concentration) Cas9 nuclease was firstly mixed with sgRNA and incubated at 25 ℃ for 10 min. 200 nM (final concentration) target DNA was added (final volume 20 μL) and the mixture was incubated at 37 ℃ for 1 h. Then the mixture was heat to 70 ℃ and maintained 10 min. 1 μL of the mixture was added to 20 μL invertase-H_1 solution (5 mg/mL). The mixture was firstly incubated for 10 min at 50 ℃ and then incubated for 10 min in ice water mixture.

DNA cleavage detection by PGM. 0.1 mg MNPs-H_2 was obtained from MNPs-H_2 solution by magnetic separation. The mixture of target DNA and invertase-H_1 was mixed with the obtained 0.1 mg MNPs-H_2. Afterwards, the mixture was first incubated for 10 min at 37 ℃ and then incubated for 30 min in ice water mixture. The MNPs remnants was obtained by magnetic separation and washed three times with PBS. Then 50 μL 0.5 M sucrose solution was added to the MNPs remnants. The mixture was incubated for 3 h at 45 ℃. The supernatant was measured by PGM.

Supplementary Figures:

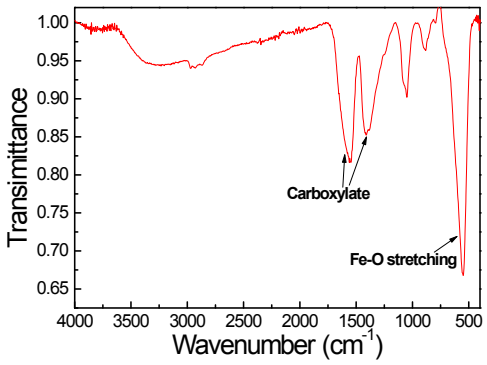


Fig. S1 FT-IR spectrum of MNPs.

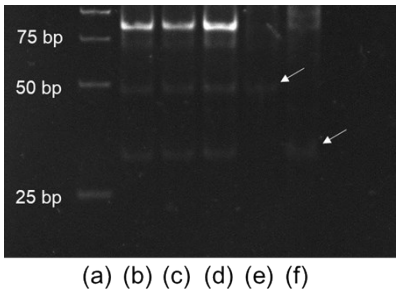


Fig. S2 Native polyacrylamide gel electrophoresis analysis of cleaved DNAs hybridized with MNPs, invertase, MNPs-H_2 and invertase-H_1. (a) marker; (b) control; (c) MNPs; (d) invertase; (e) MNPs-H_2; (f) invertase-H_1.

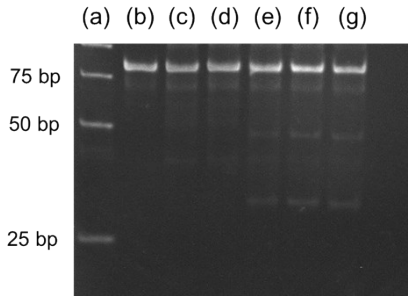


Fig. S3 Polyacrylamide gel electrophoresis analysis of target DNA cleavage with different concentrations of Cas9 nuclease. (a) marker; (b) DNA (0 nM); (c) 1.56 nM; (d) 3.9 nM; (e) 7.8 nM; (f) 15.6 nM; (g) 23.4 nM.

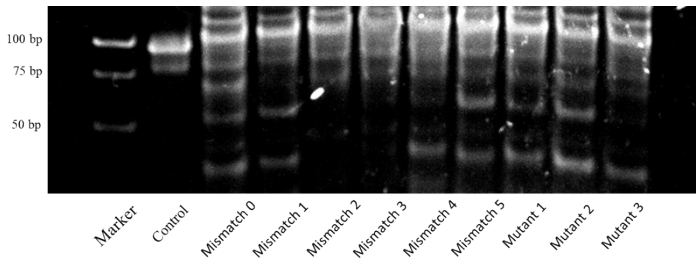
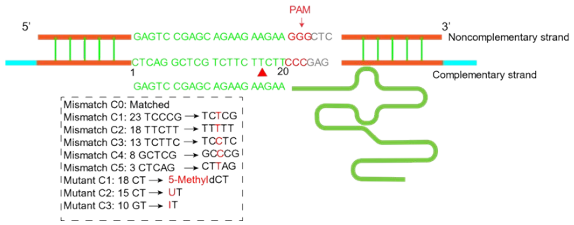


Fig. S4 Polyacrylamide gel electrophoresis analysis of target DNA cleavage with single-base change on noncomplementary strand.

(A)



(B)

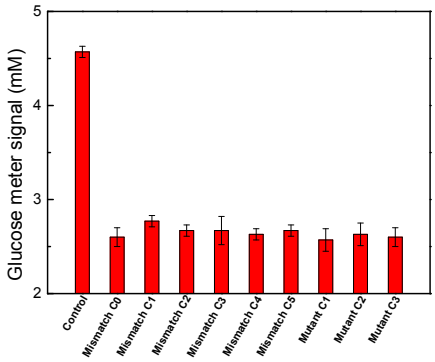
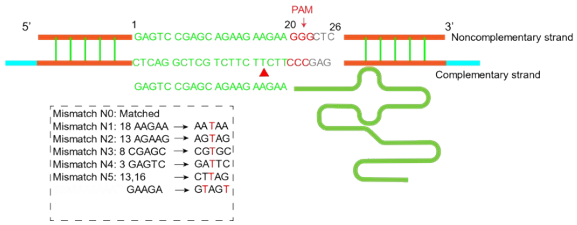


Fig. S5 (A) Single-base change on complementary strand of target DNA. (B) Glucose meter signal of cleaved DNA with single-base change on complementary strand.

(A)



(B)

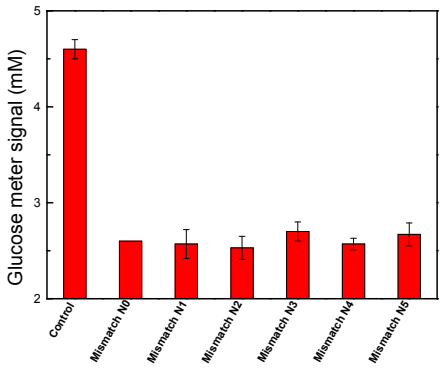


Fig. S6 (A) Base change on noncomplementary strand of target DNA. (B) Glucose meter signal of cleaved DNA with base change on noncomplementary strand.

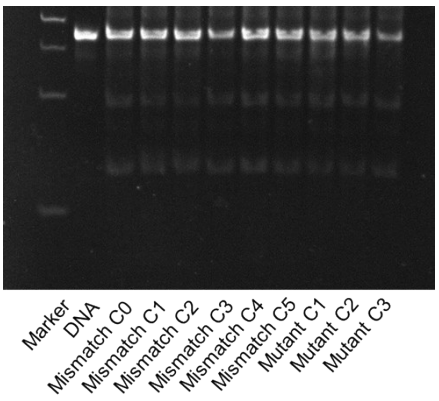


Fig. S7 Polyacrylamide gel electrophoresis analysis of cleaved DNA with single-base change on complementary strand.

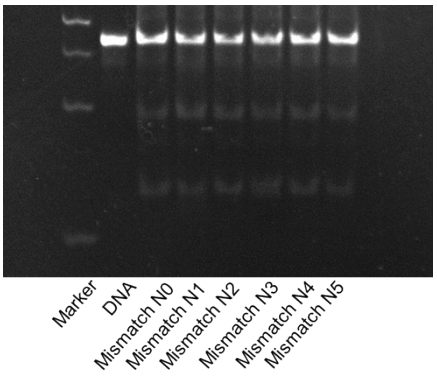


Fig. S8 Polyacrylamide gel electrophoresis analysis of cleaved DNA with base change on noncomplementary strand.

Table S1 Oligonucleotides sequence and base change sequences.

Noncomplementary DNA	5'-AGGAA GGGCC T GAGTC CGAGC AGAAG AAGAA GGG CTCCC ATCAC ATC- 3'
H_1	5'-H_2N-AAA AAA AAA AAA GTC TCC CGA GAT- 3'
Capture DNA (H_2)	5'-TCA CAG ATG AGT AAA AAA AAA AAA AAA AAA AAA AAA-NH_2- 3'
Complementary DNA	5'-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CCC TTCTT CTTCT GCTCG GACTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3'
sgRNA forward primer	5'-TTA ATA CGA CTC ACT ATA GG GAGTC CGAGC AGAAG AAGAA GTT TTA GAG CTA GAA ATA GCA- 3'
Mismatch 1	5'-AGGAA GGGCC T GAGTC CGAGC AGAAG AAGAA GGG TTCCC ATCAC ATC- 3'
Mismatch 2	5'-AGGAA GGGCC T GAGTC CGAGC AGAAG AAGAA GGT CTCCC ATCAC ATC- 3'
Mismatch 3	5'-AGGAA GGGCC T GAGTC CGAGC AGAAG AAGAA GTG CTCCC ATCAC ATC- 3'
Mismatch 4	5'-AGGAA GGGCC T GAGTC CGAGC AGAAG AAGAA TGG CTCCC ATCAC ATC- 3'
Mismatch 5	5'-AGGAA GGGCC T GAGTC CGAGC AGAAG AAGAT GGG CTCCC ATCAC ATC- 3'
Mutant 1	5'-AGGAA GGGCC T GAGTC UGAGC AGAAG AAGAA GGG CTCCC ATCAC ATC- 3'
Mutant 2	5'-AGGAA GGGCC T GAGTC CGAG（Metyl-dC） AGAAG AAGAA GGG CTCCC ATCAC ATC- 3'

Mutant 3	5’-AGGAA GGGCC T GAGTC CGAIC AGAAG AAGAA GGG CTCCC ATCAC ATC- 3’
Mismatch C1	5’-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CTC TTCTT CTTCT GCTCG GACTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3’
Mismatch C2	5’-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CCC TTTTT CTTCT GCTCG GACTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3’
Mismatch C3	5’-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CCC TTCTT CTCCT GCTCG GACTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3’
Mismatch C4	5’-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CCC TTCTT CTTCT GCCCG GACTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3’
Mismatch C5	5’-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CCC TTCTT CTTCT GCTCG GATTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3’
Mutant C1	5’-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CCC TT（Metyl-dC）TT CTTCT GCTCG GACTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3’
Mutant C2	5’-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CCC TTCTT UTTCT GCTCG GACTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3’
Mutant C3	5’-ACT CAT CTG TGA GCA CCT GAT GTGAT GGGAG CCC TTCTT CTTCT ICTCG GACTC A GGCCC TTCCT AGC TCG ATC TCG GGA GAC TTT TTT- 3’
Mismatch N1	5’-AGGAA GGGCC T GAGTC CGAGC AGAAG AATAA GGG CTCCC ATCAC ATC- 3’
Mismatch N2	5’-AGGAA GGGCC T GAGTC CGAGC AGTAG AAGAA GGG CTCCC ATCAC ATC- 3’
Mismatch N3	5’-AGGAA GGGCC T GAGTC CGTGC AGAAG AAGAA GGG CTCCC ATCAC ATC- 3’

Mismatch N4	5’-AGGAA GGGCC T GATTC CGAGC AGAAG AAGAA GGG CTCCC ATCAC ATC- 3’
Mismatch N5	5’-AGGAA GGGCC T GAGTC CGAGC AGTAG TAGAA GGG CTCCC ATCAC ATC- 3’