Supporting Information

Direct quantification of damaged nucleotides in oligonucleotides using a single molecule detection interface

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Materials and Methods

Reagents

Trypsin-EDTA, Na_3PO_4, Tris(hydroxymethyl)aminomethane, HCl, KCl and Decane (anhydrous, ≥99%) were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO). 1, 2-Diphytanoyl-*sn*-glycero-3-phosphocholine (powder, ≥99%) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). The reagents and materials we used are all of the analytical grade. DNA samples were purchased from Sangon Biotech Co., Ltd. (Shanghai). The solutions in the experiments were preparing with ultrapure water (18.2 MΩ cm at 25 °C) from a Milli-Q system (Billerica, MA). The tris-KCl buffer solution used in nanopore experiments contains 1.0 M KCl, 10 mM TRIS and 1.0 mM EDTA at pH=8.0. The K238Q mutant aerolysin nanopore was expressed, purified and activated in our laboratory.

Purification of K238Q mutant aerolysin nanopore

The forward and reverse primers of K238Q were synthesized by Shanghai Personal Biotechnology. The sequence of forward primer contains mutant amino acid (CAA): 5'-CAAGTCACCACCAAAAACAAATTC-3'; The sequence of reverse primer: 5'-CTCGCTCAGACCGTAGGTATTGG-3'. The mutant expression vectors were constructed by the overlap extension PCR method. Wild-type plasmids and primers were added in high-fidelity KOD-Plus PCR system, which operated in a Thermal Cycler (Bio-Rad) with the following conditions: 94 °C for 2 min; 98 °C for 15 s; 68 °C for 7 min 30 s for 15 cycles; and 72 °C for 10 min. The mutant plasmids were transformed into E. coli DH5α cells after digestion and cyclization. The K238Q plasmids were extracted by TIANprep Mini Plasmid Kit. The mutant plasmids were transformed into E. coli BL21 (DE3) pLysS cells to express. A 0.5 mM final concentration of isopropyl β-D-Thiogalactoside was added to the bacterial culture to induce protein expression for 6 h at 16 °C. Bacteria lysate was loaded onto a Ni-NTA affinity resin column and washed with gradient Imidazole to remove the unbound proteins (20 mM Na_3PO_4, 0.5 M NaCl, pH 7.4, and 50 mM Imidazole; 20 mM Na_3PO_4, 0.5 M NaCl, pH 7.4, and 100 mM Imidazole). K238Q proaerolysin proteins were washed and collect with elution buffer containing 20 mM Na_3PO_4, 0.5 M NaCl, pH 7.4, and 300 mM Imidazole. The molecular weight of the collected protein was confirmed by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

Single channel recording

The K238Q aerolysin nanopore was constructed on a lipid bilayer in a 50 μm orifice of the Delrin bilayer cup (Warner Instruments, Hamden, CT). Both *cis* and *trans* compartments of the recording chamber contained 1.0 mL tris-KCl buffer solution prepared above. The potential is applied by using a pair of Ag/AgCl electrodes. After the a single K238Q nanopore was formed, the DNA samples were added the *cis* chamber to the final concentration of 2.0 μM for each sample. The experiments in our work were conducted at 22 ± 2 °C.

Data acquisition and analysis

The current traces from single channel recording were measured with a patch clamp amplifier (Axon 200B equipped with a Digidata 1440A A/D converter, Molecular Devices, USA) with the *cis* chamber connected to ground. The amplified signal was low-pass filtered at 5 kHz and sampled at 100 kHz by running the Clampex 10.4 software (Molecular Devices, USA). The data were analysed by using Mosaic 1.3 software and Origin-Lab 9.0.

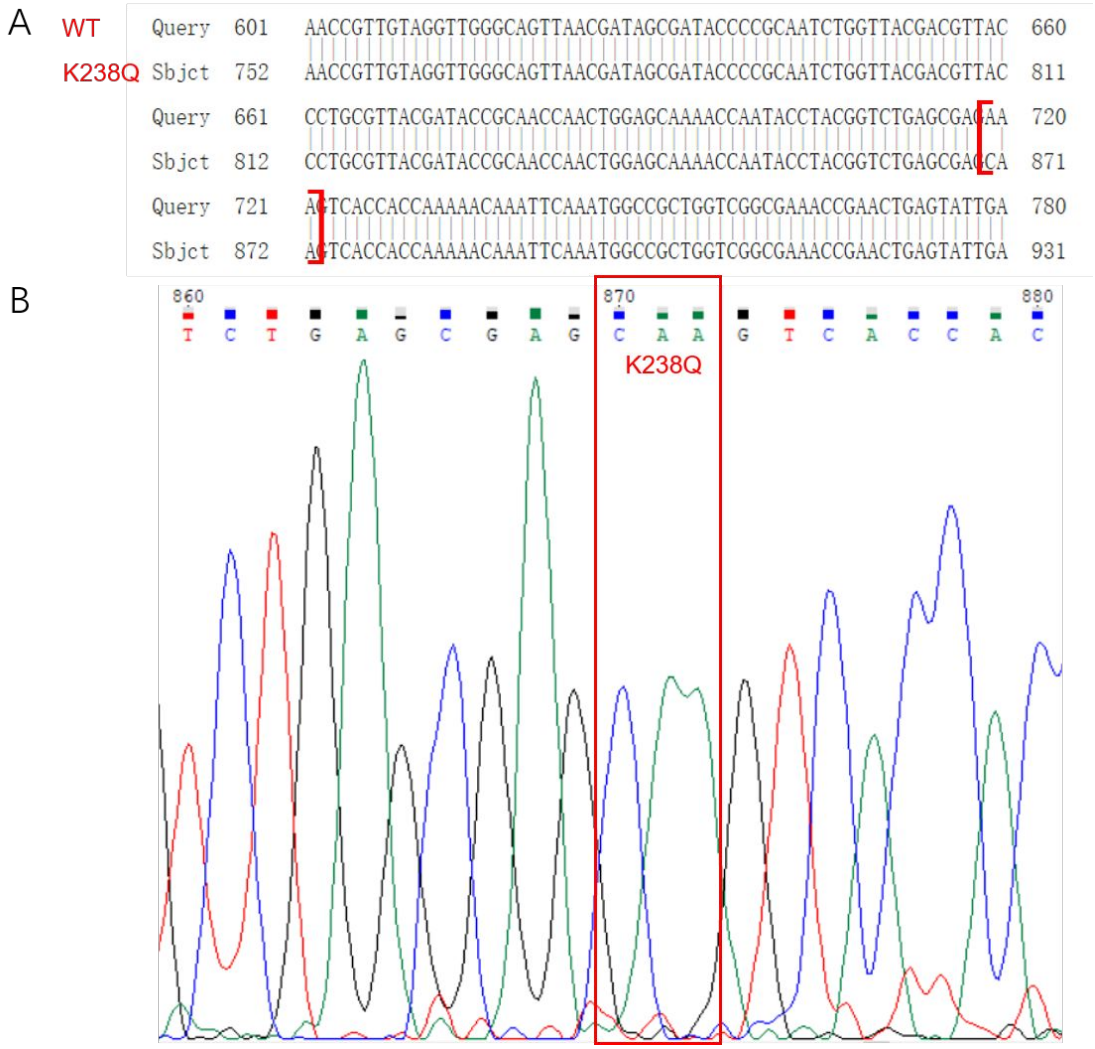

Fig. S1 Sequencing spectra of plasmid of K238Q. The mutant codon is shown in the red rectangle.

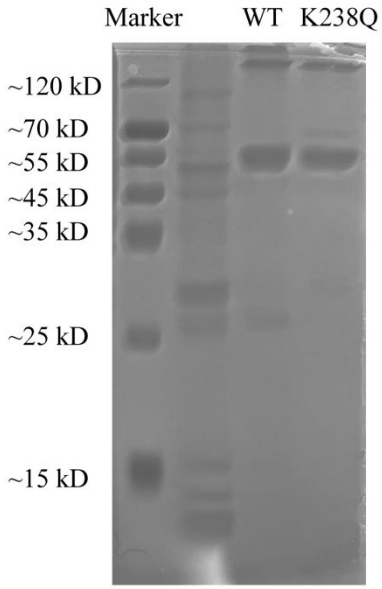

Fig. S2 SDS-PAGE analysis of WT and K238Q mutant proaerolysin.

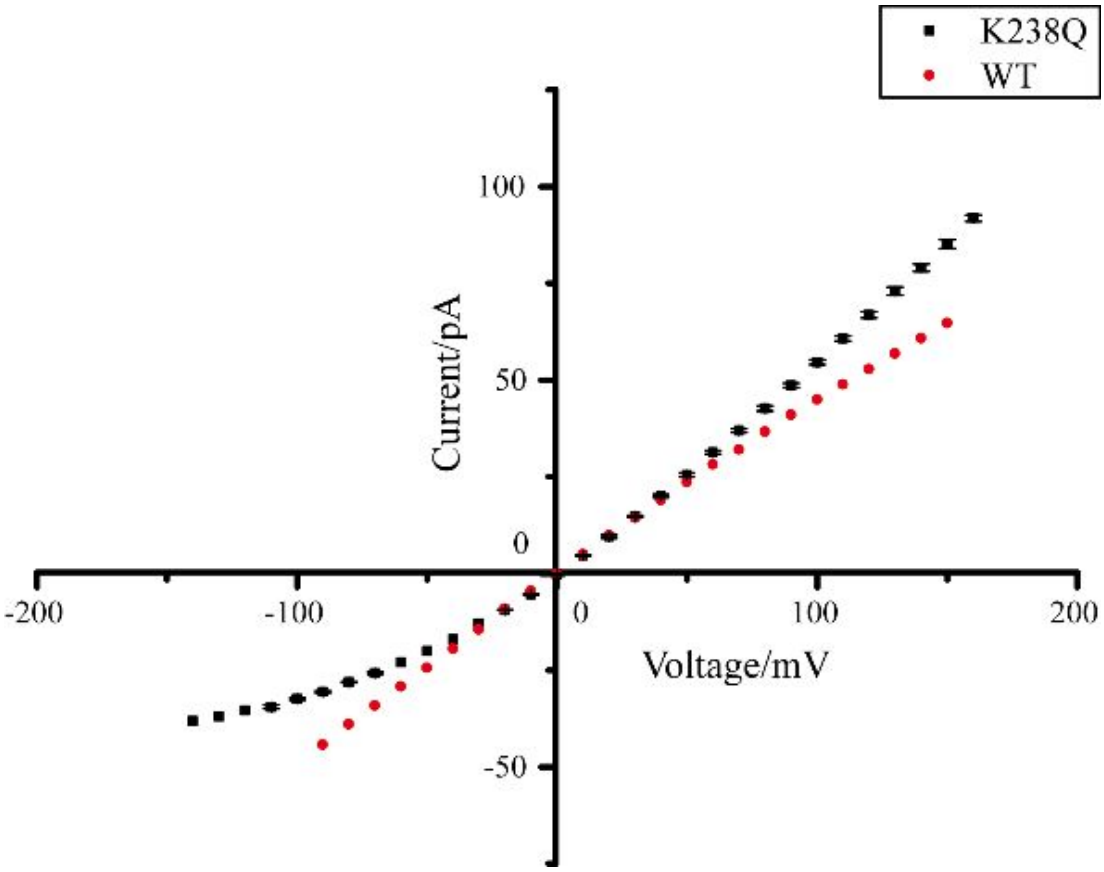

Fig. S3 The conductance of WT Aerolysin nanopore and K238Q Aerolysin nanopore.

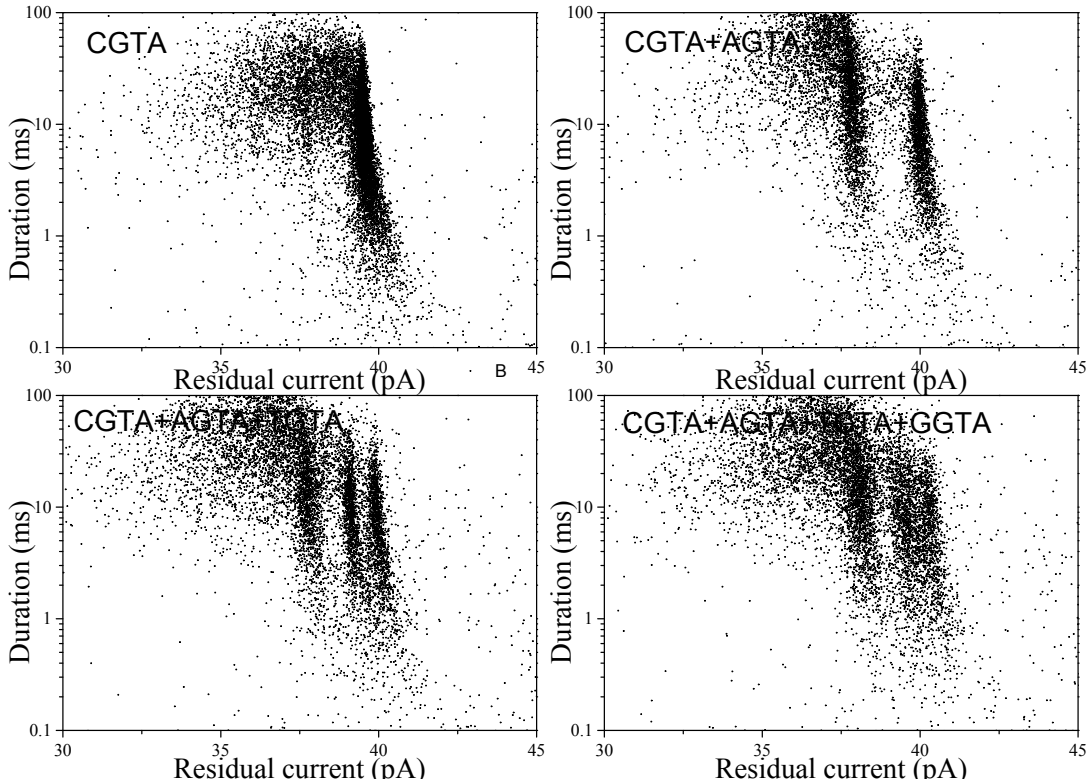

Fig S4 Discrimination of 1 μM XGTA (X = A, G, T, C) (ground side addition) by single K238Q nanopore. Asymmetric KCl was used (*cis*/*trans*=0.5M/1M).