Supplementary information for

Molecular sandwich-based DNAzyme catalytic reaction towards transducing efficient nanopore electrical detection for antigen proteins

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Supplementary Methods:

1. Preparation of sandwich complex

(1) *Carboxyl activated Magnetic beads (MBs)*: 100 µL of MBs were taken and washed three times with 200 µL of MES (2-(N-morpholino) ethanesulfonic acid, ≥ 99.5 %, 25 mM, pH 5.0) solution, followed dispersed in 200 µL MES solution containing 50 µL EDC (1-(3-Dimethylaminopropyl)-3 ethylcarbodiimide hydrochloride, ≥ 98 %, 50 mg/mL) and 50 µL NHS (N-hydroxysuccinimide, ≥ 97 %, 50 mg/mL) and incubated at room temperature for 30 minutes (vortexed every 5 minutes to ensure sufficient reaction). MBs were then isolated from the supernatant and washed three times with 500 µL MES.

(2) *Antibody-functionalized MBs*: Carboxyl-activated MBs were mixed with anti-HIV-1 p24 antibody (capture antibody) in 100 µL of MES solution and incubated for 30 min at room temperature, then isolated and incubated with 100 µL of Tris-HCI (50 mM, pH 7.4) for 20 mins to quench the excess activated carboxylic acid groups, then blocked by 100 µL 0.05% BSA solution for 10 min and resuspended in PBS for further use.

(3) *Entrapment of antigen proteins using capture antibody-antigen-detection antibody sandwich:* Recombinant HIV-1 p24 protein was mixed with 20 µL capture antibody-modified MBs (washed three times with 500 µL assay buffer before use) and human serum (1000 folds diluted) at final concentration of 10 pM, and incubated for 30 mins at room temperature to make standard samples. Next, biotin-modified anti-HIV-1 p24 antibody (detection antibody) was mixed well with standard samples and incubated at room temperature for 30 mins. After the sandwich structures were formed, MBs carrying sandwich complex were washed five times with 500 µL washing buffer.

(4) *Attachment of streptavidin in MBs:* streptavidin (≥13 units/mg protein, S4762) was added and incubated with MBs carrying sandwich complex for 30 mins at room temperature. After incubation, MBs were resuspended and washed five times with 200 µL PBS solution, and finally stored in 100 µL PBS solution for further use.

(5) *DNAzyme linkage*: streptavidin attached MBs were incubated with 30 µL biotin-modified DNAzyme (seq:

GAGGTCTTGACCTCATGAGGCTAGCTACAACGAGGTTAGGCCATTTGGCC T, 10 μM) for 30 mins at room temperature to form sandwich complexes. Finally, they were washed 5 times with 100 μL of PBS solution and stored in 30 μL of PBS solution. Assay buffer consists of 0.1 M NaCl, 0.025% Tween 20, 0.1% BSA, and 10 mM Na_2HPO_4 (pH 7.2). Washing buffer consists of 0.15 M NaCl and 10 mM Na_2HPO_4.PBS buffer consists of 136.56 nM NaCl, 2.67 mM KCl, 8.1 mM Na_2HPO_4 and 1.76 mM KH_2PO_4 (pH=7.4).

2. Gel electrophoresis

Electrophoresis experiment as follows: Agarose gels for DNA electrophoresis (1×TBE, 110 mV, instrument PowerPac Basic, Shanghai) were prepared with 3.75 mL of 40% preformed gel, 3.75 mL of 1×TBE, 50 μL of 10% APS, and 3 μL of TEMED, with 5 μL of standard sample (5 μM) in each lane. After electrophoresis, 5 μL of staining agent and 50 mL of 1×TBE were mixed well, and then the agarose gel was shaken with the mixed solution on an Orbital shaker (Kylin-Bell TS-100) for 15 mins at low frequency away from light to complete the staining of the gel. Finally, the agar gel was photographed and recorded under a UV-visible projection reflectometer (Shanghai Jingke Industrial, WFH-201BJ).

Supplementary Figures:

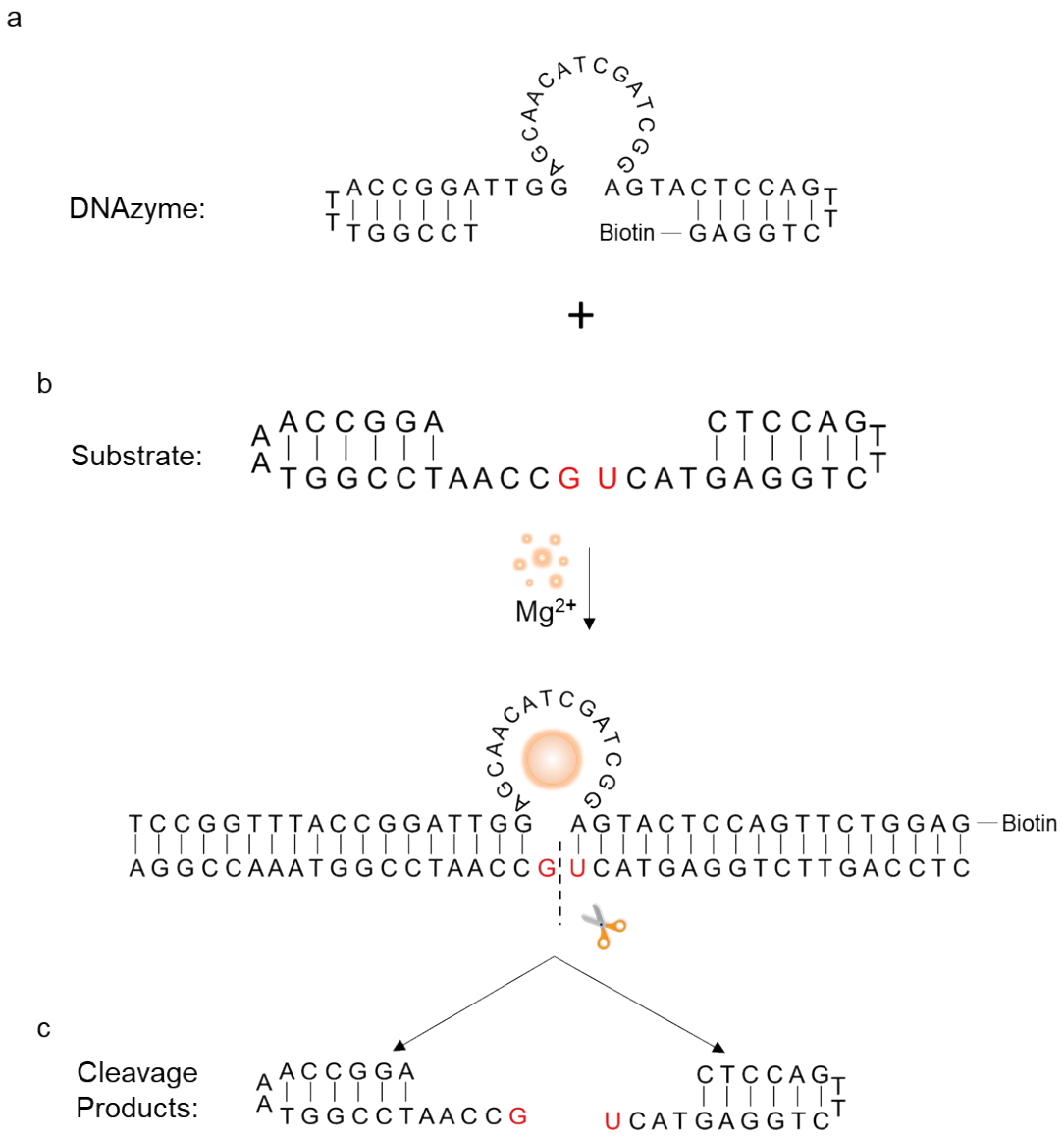

Fig. S1 The sequences of DNAzyme, substrate and cleavage fragments. (a) DNAzyme; (b) Substrate and (c) Cleavage fragments.

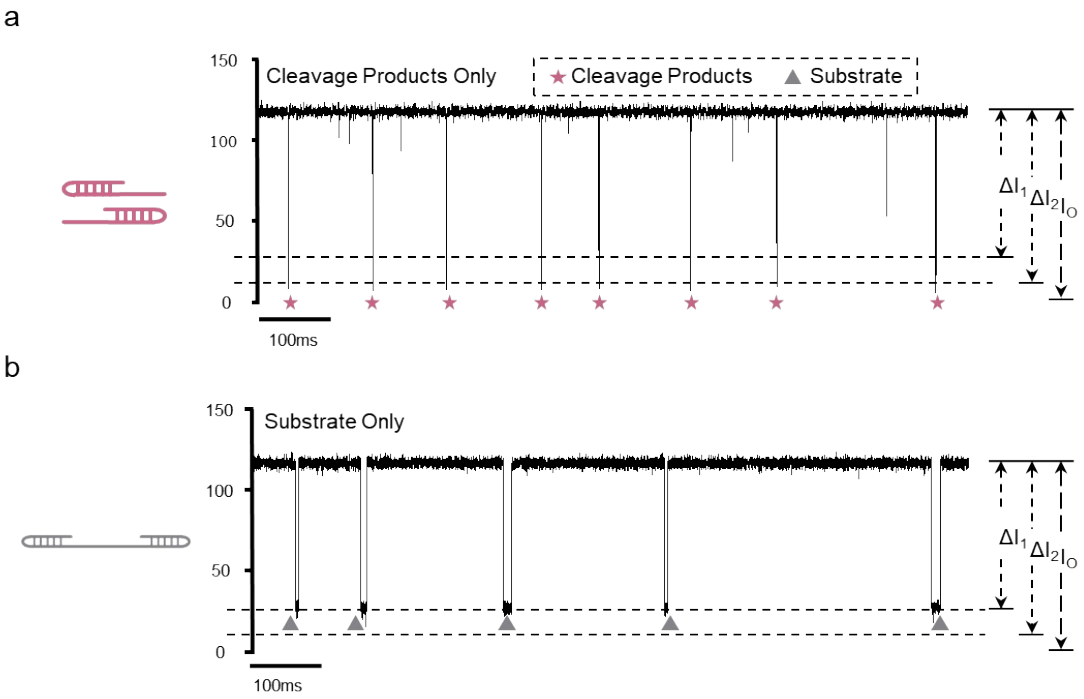

Fig. S2 Nanopore detection of nucleic acids as control experiments. Representative current traces display individual events caused by (a) Synthesized cleavage fragments and (b) Nucleic acids substrate.

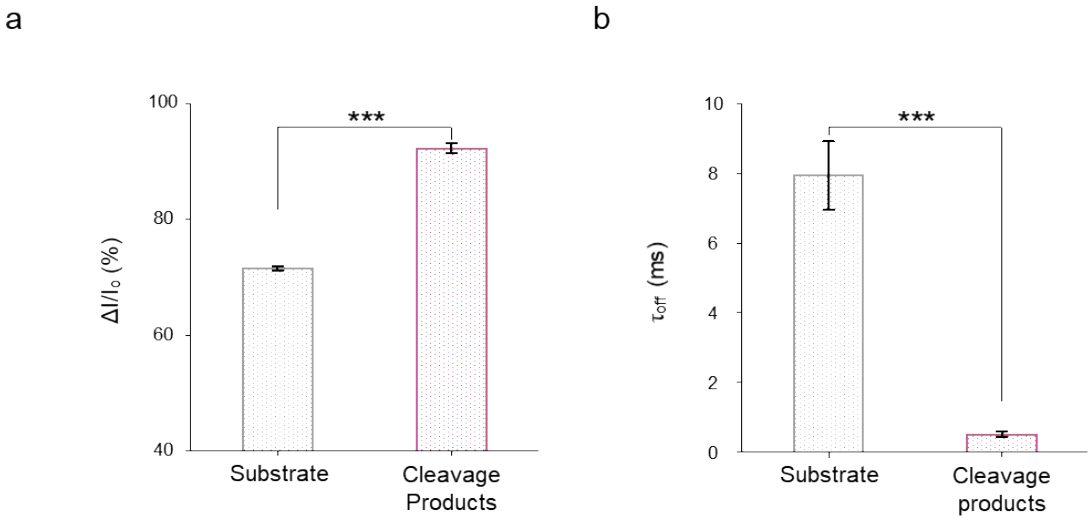

Fig. S3 Nanopore events signatures of the substrate and cleavage fragments. (a) $\Delta I/I_O$ and (b) τ_{off} ($P \leq 0.001$).

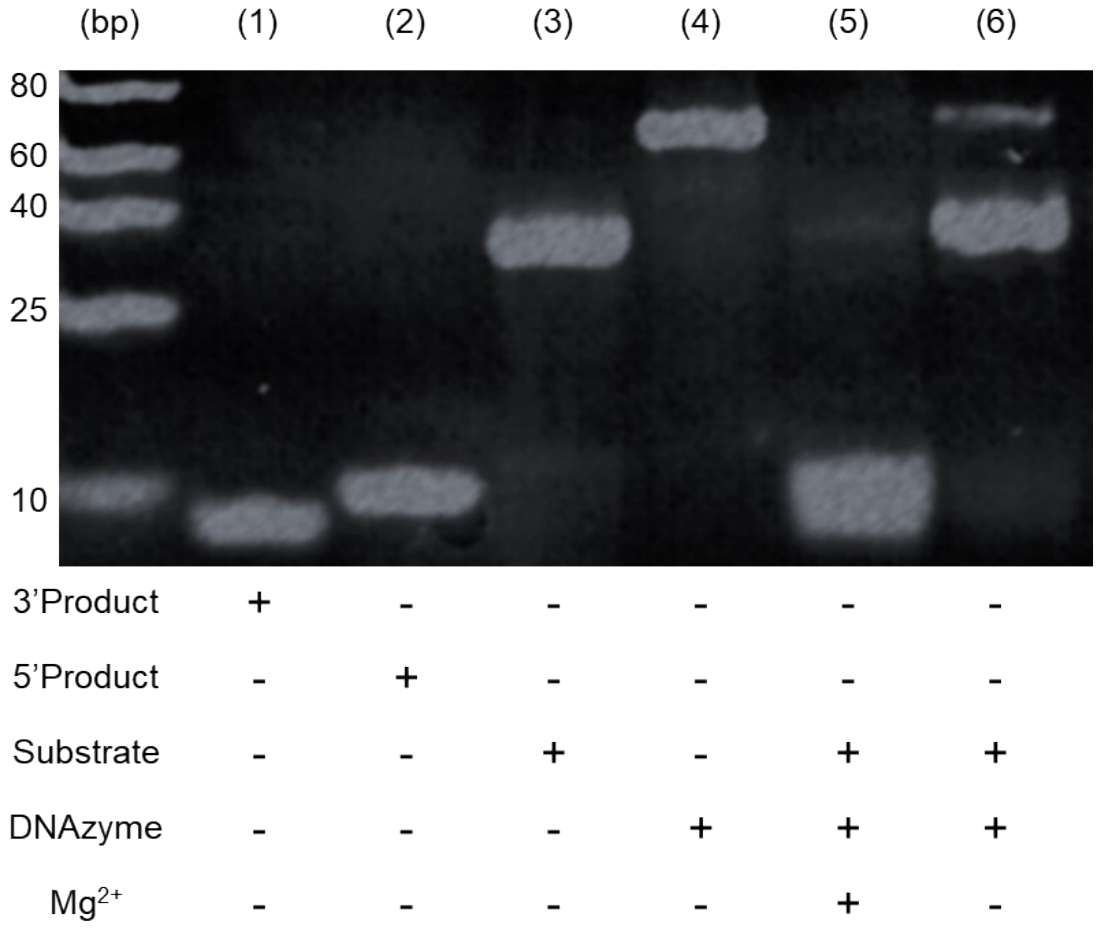

Fig. S4 Electrophoresis of DNAzyme catalytical reaction in the presence of Mg^{2+}.

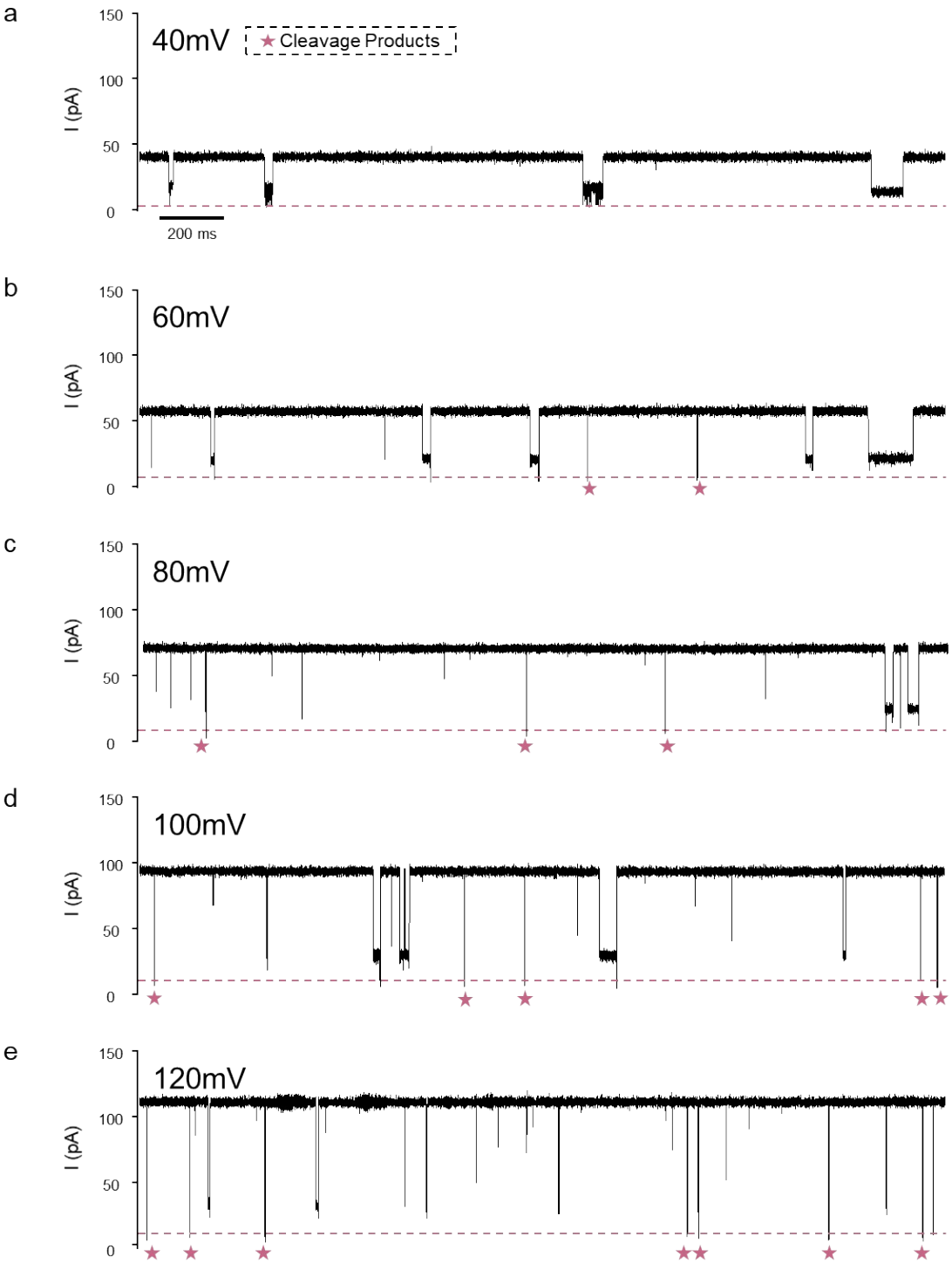

Fig. S5 Voltage-dependent detection for DNAzyme catalytical reaction. Representative current traces produced under (a) 40mV. (b) 60 mV. (c) 80 mV. (d) 100 mV and (e) 120 mV. Red stars represent the signals of cleavage fragments obtained by the DNAzyme catalytical reaction.

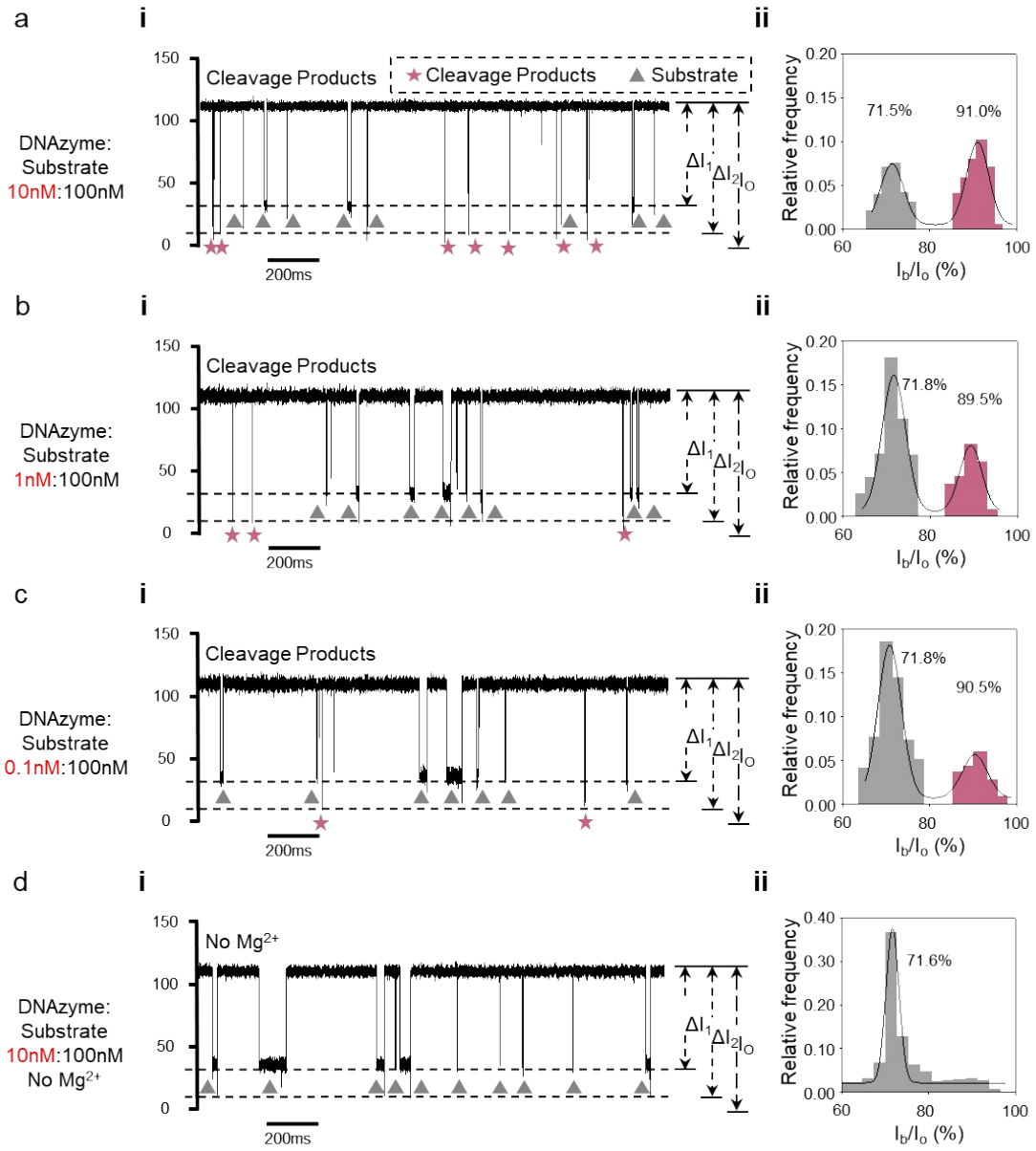

Fig. S6 DNAzyme concentration-dependent determination. (a) 10 nM. (b) 1 nM. (c) 0.1 nM. (d) 10 nM without Mg^{2+} as control. Substrate = 100 nM. (i) Representative current traces of DNAzyme catalytical reaction. (ii) Corresponding statistical analyses of $\Delta I/I_O$.

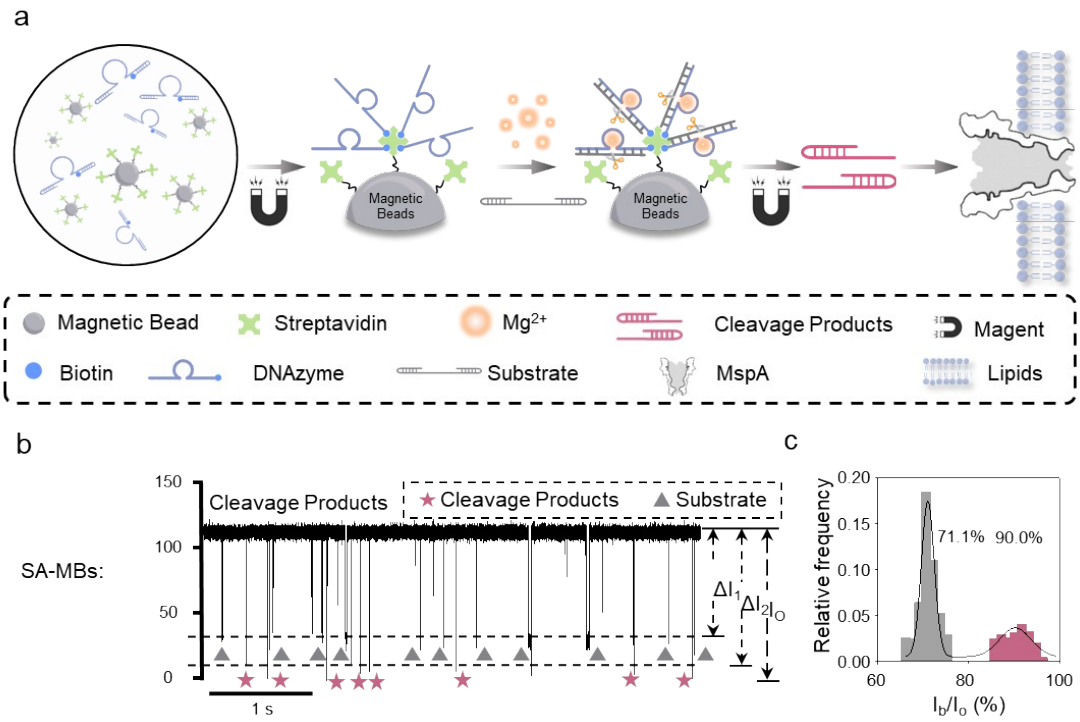

Fig. S7 Magnetic beads immobilized DNAzyme cleavage in nanopore detection. (a) Streptavidin-modified magnetic beads (SA-MBs) were incubated with biotin-modified DNAzyme to form complex structure for nanopore DNAzyme cleavage detection. (b) Representative current traces of DNAzyme cleavage detection. (c) Corresponding statistical analyses of $\Delta I/I_O$ of signatured events. All experiments were performed in *cis*:1 M [BMIM]Cl *trans*:1.5 M [BMIM]Cl solution containing 10 mM Tris at pH 7.4. The number of experiments n = 3.

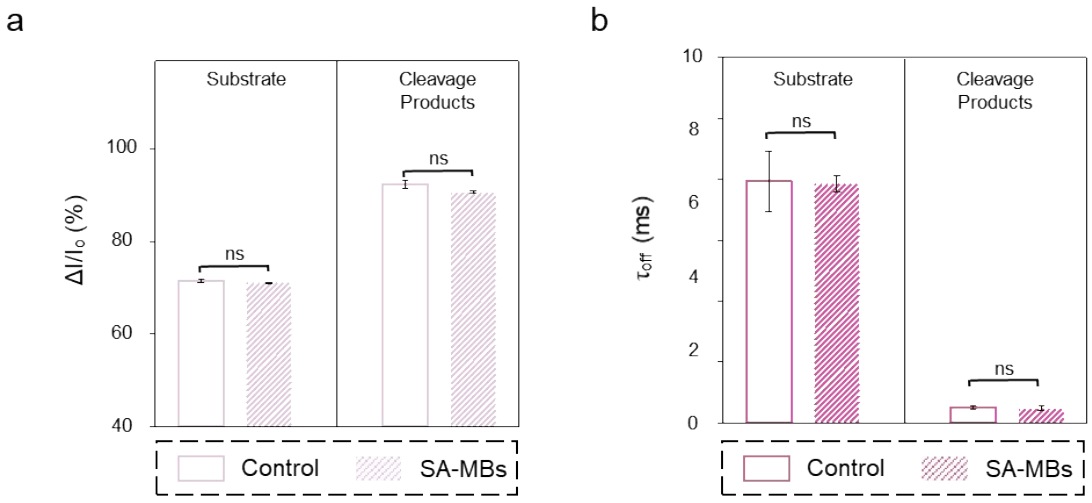

Fig. S8 Comparison of detected cleavage events produced by magnetic beads immobilized DNAzyme and free DNAzyme. Corresponding statistical analysis of the (a) $\Delta I/I_O$ and (b) τ_{off} in nanopore DNAzyme cleavage detection. SA-MBs represents substrate and cleavage fragments detected by magnetic beads immobilized DNAzyme cleavage. Control represents substrate and cleavage fragments released by free DNAzyme (all $P > 0.05$). There is no significant difference between Control and SA-MBs.

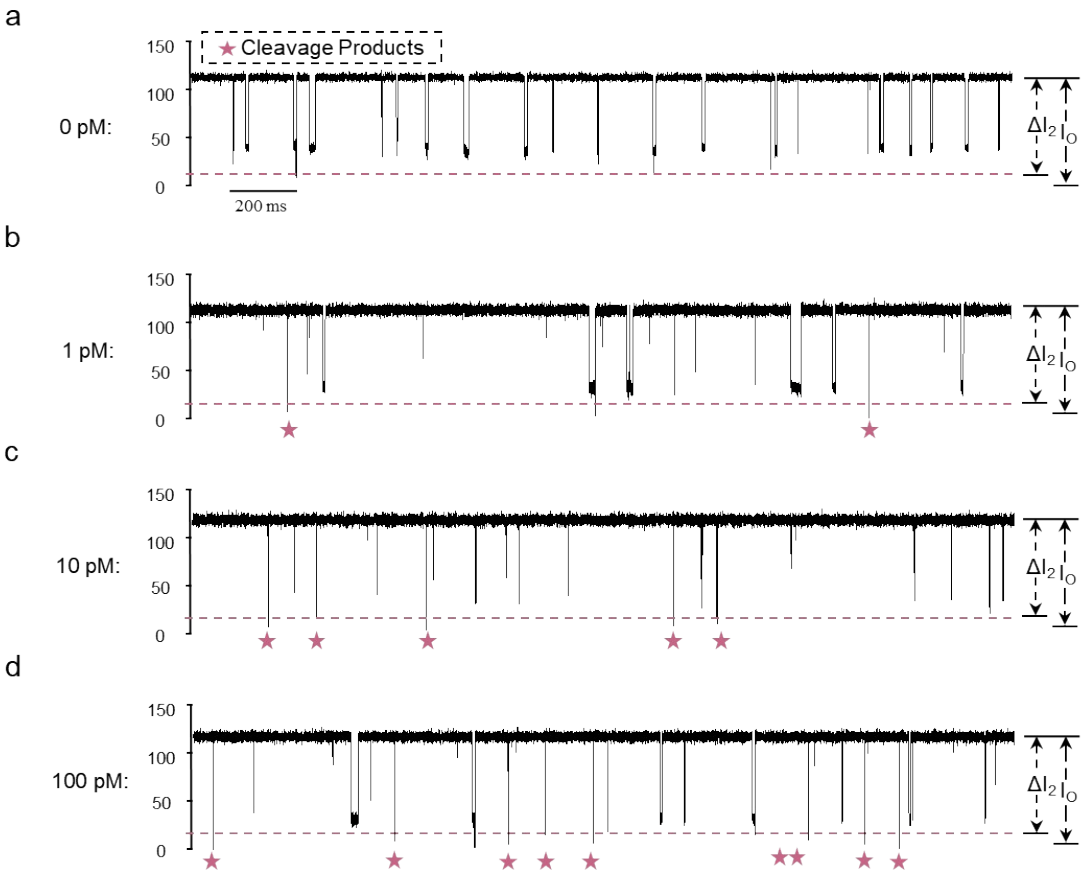

Fig. S9 p24 antigen concentration-dependent detection. Representative current traces of molecular sandwich-based DNAzyme catalytic reaction towards transducing efficient nanopore electrical detection for antigen proteins (a) 0 pM p24; (b) 1 pM p24; (c) 10 pM p24 and (d) 100 pM p24.

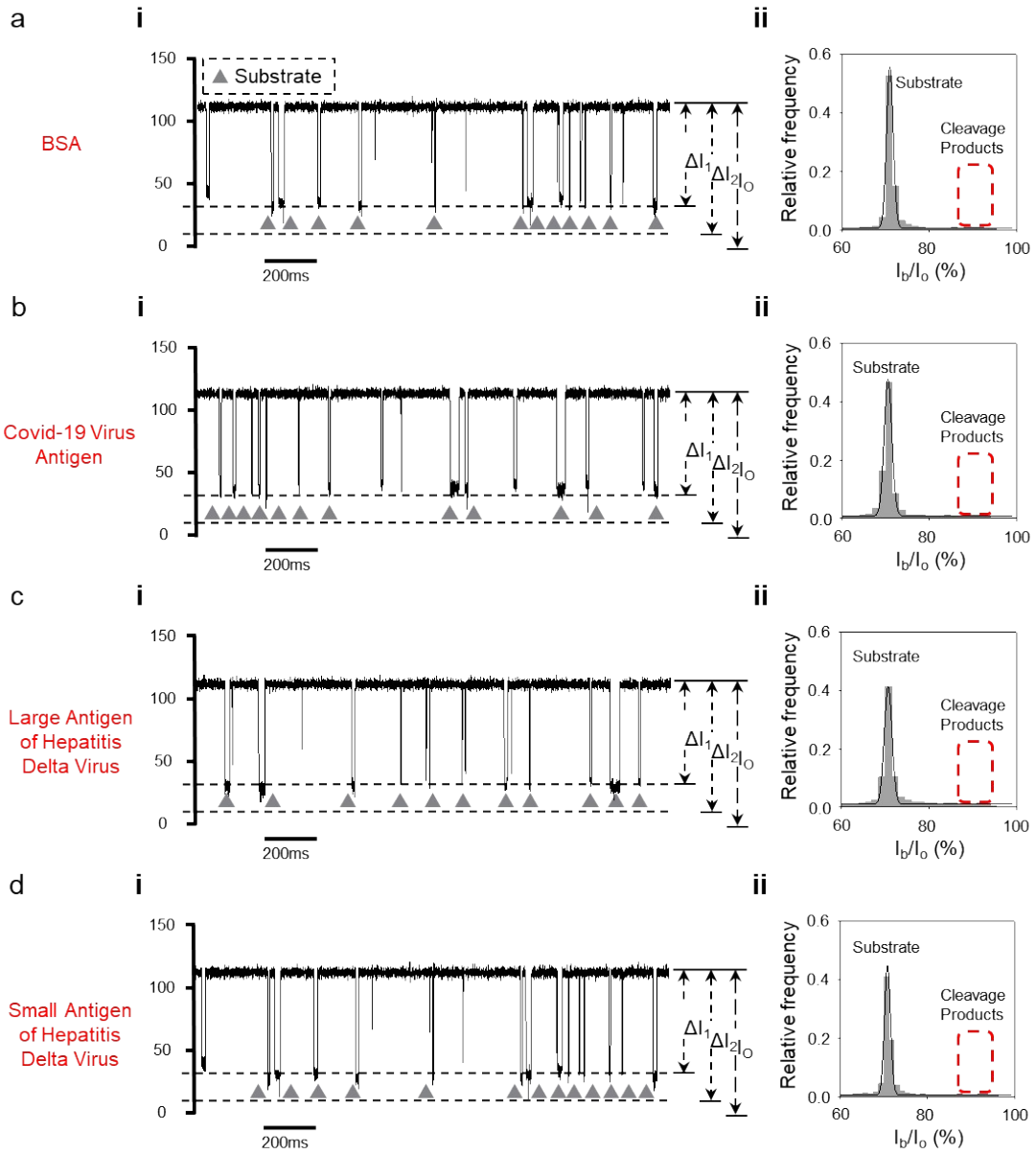

Fig. S10 Molecular sandwich-based DNAzyme catalytic reaction towards selective nanopore electrical detection for p24 antigen proteins. (a) BSA; (b) Covid-19 Virus Antigen; (c) Large Antigen of Hepatitis Delta Virus; (d) Small Antigen of Hepatitis Delta Virus. (i) Representative current traces of molecular sandwich-based DNAzyme catalytic reaction towards selective nanopore electrical detection for antigen proteins (ii) Corresponding statistical analyses of $\Delta I/I_O$ of current events by nanopore electrical detection for antigen proteins.