Electronic supplementary information

A dual-amplification fluorescent sensing platform for ultrasensitive assay of nuclease and ATP based on rolling circle replication and exonuclease III-aided recycling

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This file contains:

1. Experimental Section

1.1 Gel Electrophoresis.

A 5% agarose gel electrophoresis analysis of the interaction between S1 nuclease and sDNA was carried out in 0.5× TBE (Tris-borate running buffer (pH 9.2) containing 2 mM EDTA) with ethidium bromide and goldview as the fluorescence indicator. Electrophoresis was carried out at a constant potential of 110 V for 2 h with loading of 20 μL of each sample into the lanes. A 0.7% agarose gel electrophoresis analysis of RCA products was carried out in 0.5× TBE (Tris-borate running buffer (pH 9.2) containing 2 mM EDTA) with goldview as the fluorescence indicator. Electrophoresis was carried out at a constant potential of 100 V for 1h with loading of 20 μL of each sample into the lanes. The images were acquired with a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company).

1.2 Cell culture and S1 nuclease extraction

HeLa cells were prepared as described in our recent paper.[1] Briefly, HeLa cells were cultured in RPMI 1640 cell culture medium supplemented with 10% fetal calf serum (FBS) and 100 IU per mL of penicillin-streptomycin, and the cells were maintained at 37 °C in a humidified atmosphere (95% air and 5% CO_2) and were kept in a logarithmic growth phase by routine passage every 2-3 days. Then, cells were collected in EP tube, washed twice with 10 mM PBS (10 mM PB, 0.1 M NaCl, pH 7.4) by centrifuging at 2000 rpm for 3 min at 4 °C. The cells were suspended in 200 mL of ice cold lysis buffer provided by Sangon Biotechnology Co., Ltd. (Shanghai, China) (the product number: BSP022) by swaying at least three times at a concentration of 1.0×10^7 cells per mL, kept on ice for 30 min and then centrifuged at 12000 rpm for 20 min at 4 °C. Last, the supernatant was carefully moved to the sterilized tube, and was frozen at -80 °C for further experiments.

2. Figures S1-S8 with Legends

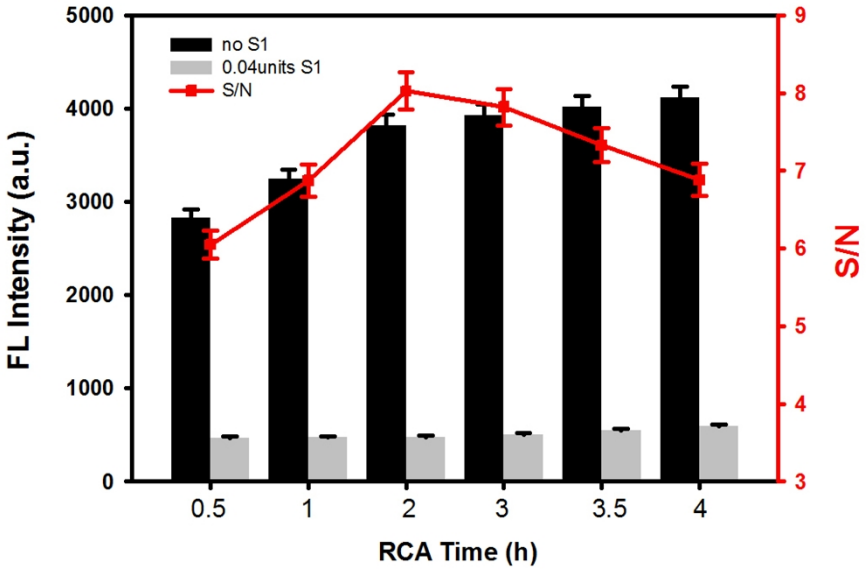

Fig. S1 Effect of RCA reaction time on fluorescence response in S1 nuclease assay. Error bars represented standard deviations from three repeated experiments.

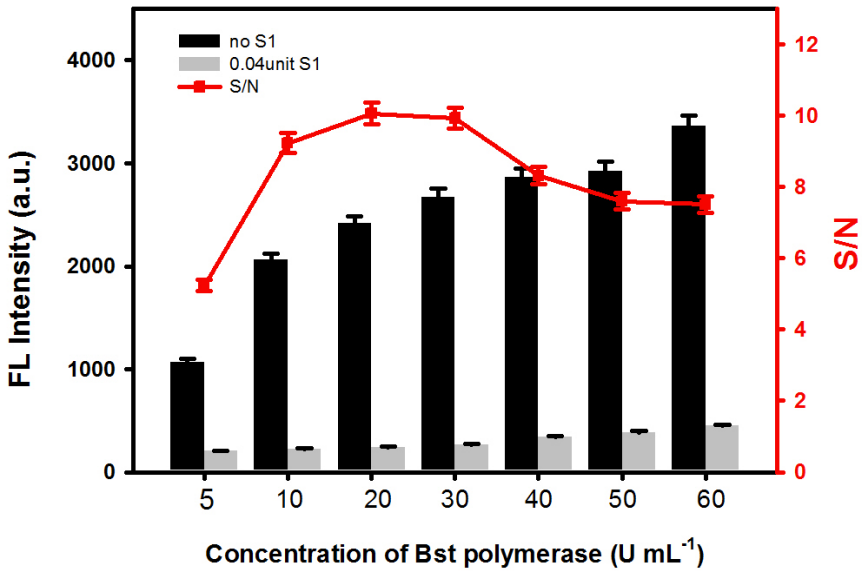

Fig. S2 Effect of polymerase concentration on fluorescence response in S1 nuclease assay. Error bars represented standard deviations from three repeated experiments.

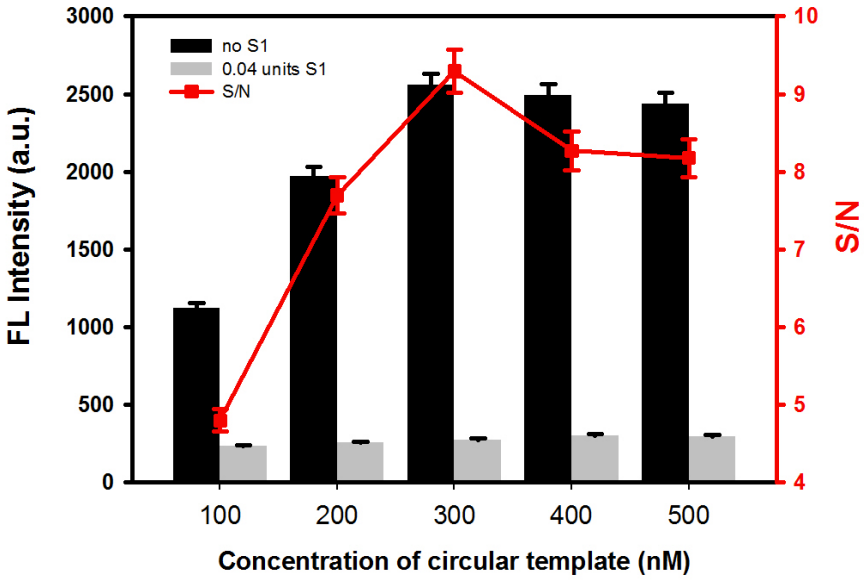

Fig. S3 Effect of circular DNA template (CT) concentration on fluorescence response in S1 nuclease assay. Error bars represented standard deviations from three repeated experiments.

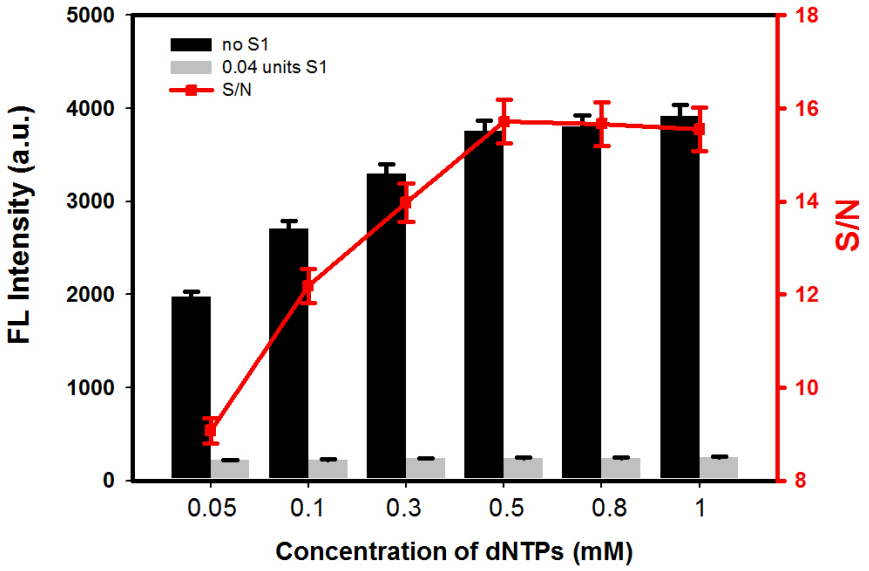

Fig. S4 Effect of dNTPs concentration on fluorescence response in S1 nuclease assay. Error bars represented standard deviations from three repeated experiments.

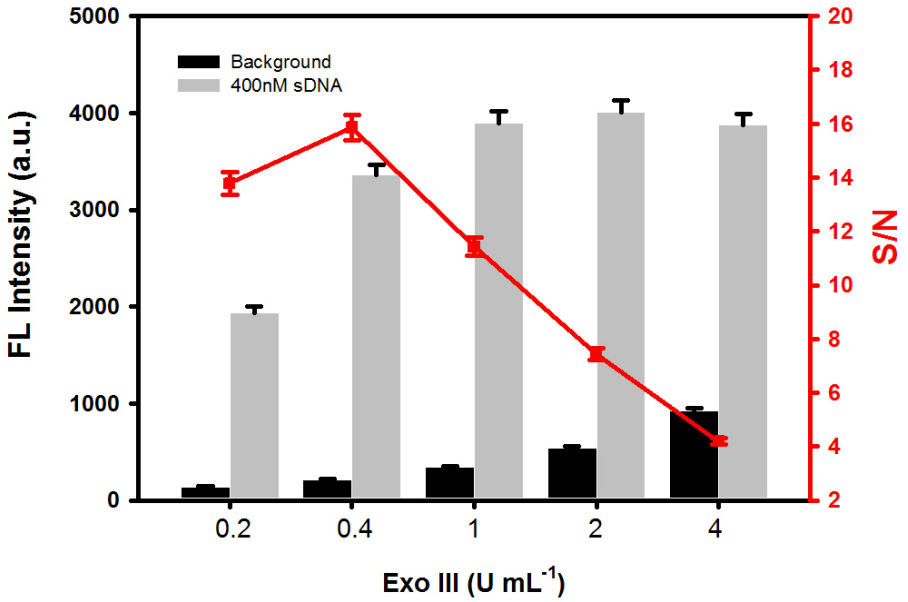

Fig. S5 Influence of the Exo III concentration on the S1 nuclease assay. Error bars represented standard deviations from three repeated experiments.

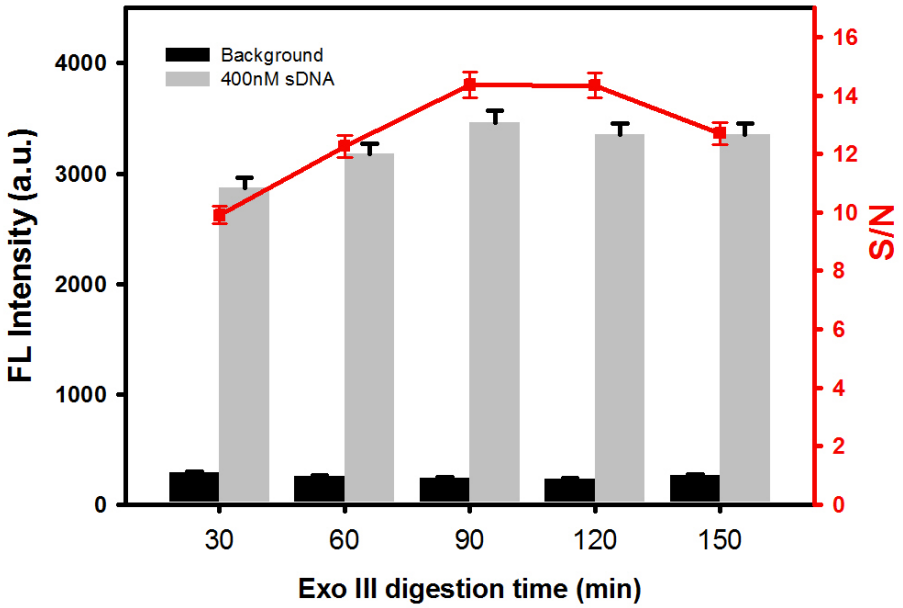

Fig. S6 Influence of the Exo III-catalyzed digestion time on the S1 nuclease assay. Error bars represented standard deviations from three repeated experiments.

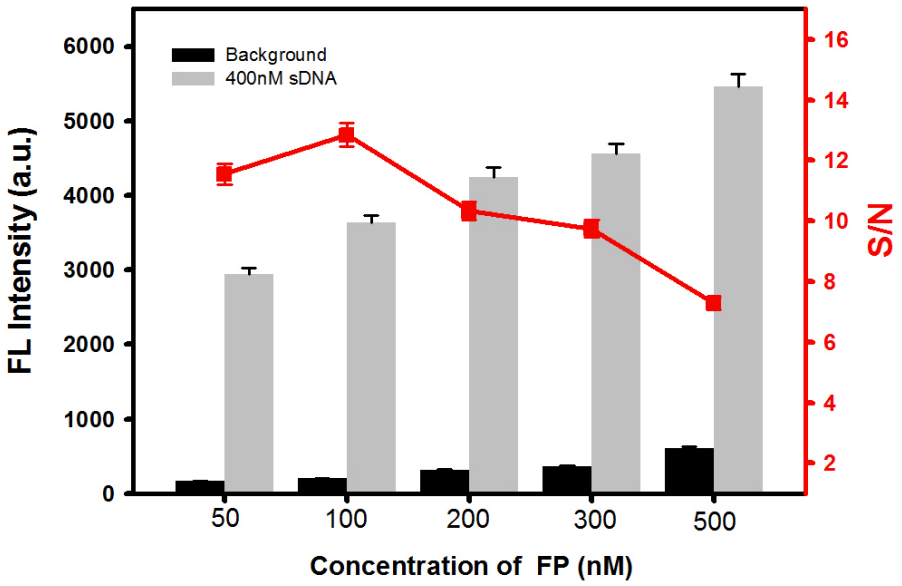

Fig. S7 Influence of the TaqMan probe (FP) concentration on the S1 nuclease assay. Error bars represented standard deviations from three repeated experiments.

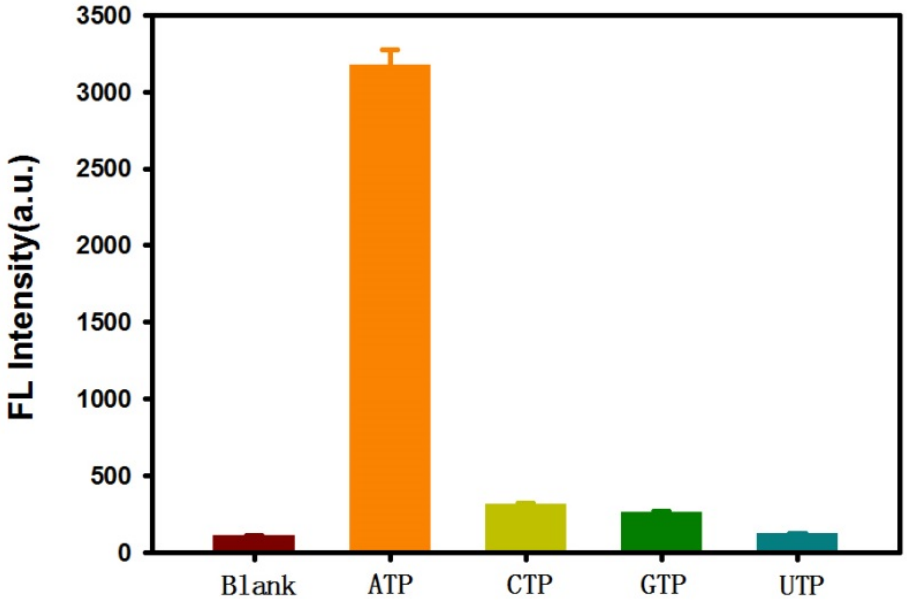

Fig. S8 Selectivity of the developed sensing platform for ATP compared to ATP analogs (UTP, CTP and GTP). The concentration of ATP was 2.0 mM and ATP analogs were 20.0 mM.

3. Supporting Tables 1

Table S1 Names and sequences (5'-3') used in this work.

Name	Sequences
sDNA	GTA GGT TGT AGG AGG AGA ATT GGG
Padlock probe	PO_3-CCT ACA ACC TAC AAA CCT CGA CTG CAA GCT CCGA CCT ACC CAC CAC CCA ATT CTC CT
TaqMan probe (FP)	CGA CCT(TAMRA) ACC C-FAM

4. References

1. H. B. Wang, S. Wu, X. Chu and R. Q. Yu, *Chem. Commun.*, 2012, **48**, 5916.