Electronic Supplementary Information

Dual recognition element-controlled logic DNA circuit for COVID-19 detection based on exonuclease III and DNAzyme

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Experimental Section

Materials and reagents

All reagents were of analytical grade and obtained from Sigma-Aldrich (St. Louis, Mo). All HPLC-purified sequences were purchased from Sangon Biotech Co., Ltd (Shanghai, China) and listed in Table S1. Milli-Q purified water (18.2 MΩ/cm) was used to prepare the buffer solution.

Table S1 Sequences of the oligonucleotides used in the experiments.

Name	Sequence
Specific	5'-AGACAACTAC TATTCAAACA-3'
Homologous	5'-ATTCCCACCA ACAGAGCCTA-3'
H1	5'-GCTCCTACACCTACGTCTTTTTTTAGACGTAGGTGTAGGAGC TGTTTGAATA-3'
H2	5'-CATCATATTCCCTCCATATTTTTTTATGGAGGGAATATGATG GTAGTTGTCT-3'
H3	5'-AGACGTAGGGACTCCGAGCCGGACGAAGTTAATATGATGTTTTTTTGGCTCGGAG TC CCTACGTCTTAGGCTCTGT-3'
M_{S1}	5'-AGATAACTAC TATTCAAACA-3'
M_{S2}	5'-AGATATCTAC TATTCAAACA-3'
M_{S3}	5'-AGATATCTTC TATTCAAACA-3'
M_{SNC}	5'-TCTTGTGATG ATGGGTTTAG-3'
M_{H1}	5'-ATTCCCACCA ACAGAGGCTA-3'
M_{H2}	5'-ATTCCCACCA ACAGTGGCTA-3'
M_{H3}	5'-ATTCCCACCA ACTGTGGCTA-3'
M_{HNC}	5'-TCCGGGTTTGGGTTTTGTGG-3'
Substrate strand	5'-TATGGAGGG AACT(Dabcyl)rAGGT(FAM)C TGTAGGAGC TTTTTT-3'

Bioinformatics analysis

The complete genomes of COVID-19 and 25 similar sequences have been downloaded from GISAID (https://www.gisaid.org/) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Phylogenetic trees were reconstructed using MEGA 6.0 software with bootstrap values based on 1000 replications.

With the phylogenetic results, genome alignment between the complete genomes of COVID-19 and several mostly similar sequences were made using MEGA 6.0 software, then

obtaining the homologous sequence (Homologous) and the specific sequence (Specific) of COVID-19.

Analytical procedure

All of the DNA probes were dissolved into 20 mM Tris-HCl buffer (pH 8.0, 150 mM NaCl, 50 mM KCl, and 20 mM $MgCl_2$). All hairpins (H1, H2, and H3) were heated to 95 °C for 5min and allowed to cool to room, respectively. For the detection of COVID-19, 400 nM hairpins (H1, H2, and H3) were incubated with different concentrations of Specific and Homologous at 25 °C for 30 min in presence of 20U Exo III. Subsequently, 100 nM the substrate strand were introduced into the above solution and incubated at room temperature for 45 min. The fluorescence spectra were recorded from 500 to 650 nm (E_x = 488 nm, E_m = 520 nm). The fluorescence measurements were measured by a SpectraMax i3x (Molecular Devices).

To investigate the selectivity of this biosensor for the Specific detection, 10 nM Specific and 10 nM other mutations, including a single-base mismatched DNA (MS1), a two base mismatched DNA (MS2), a three base mismatched DNA (MS3), and the non-complementary DNA (MSNC) were performed in the presence of Homologous.

In order to evaluate the selectivity of the logic system for the Homologous detection, 10 nM other mutations (*e.g.*, MH1: a single-base mismatched DNA; MH2: a two base mismatched DNA; MH3: a three base mismatched DNA; MHNC: the non-complementary DNA) were tested in the same with the assistant of Specific.

Analysis of real samples

All real samples (*e.g.*, human serum, human urine, and mouth saliva) were filtered with 0.22 μm microfiltration membrane to remove the insoluble particles. Different concentrations of Specific and Homologous spiked in the real samples were quantified using our logic system, respectively.

Polyacrylamide gel electrophoresis (PAGE) experiment

1.0 mL 10 X TBE buffer, 3.8 mL H_2O, 5.0 mL Acr-bis (30%) ,100 μL APS (10%), and 4 μL TEMED

were mixed and polymerized to prepare 15 % PAGE gel for 3 h at room temperature. 3 μL reaction solution, 1 μL 6 X loading buffer, and 2 μL 100 X SYBR Green solution were mixed to prepare the PAGE samples. The electrophoresis analysis was carried out at 90 V for 60 min in 1 X TBE buffer. The gel was scanned with the gel image analysis scanner (Bio-Rad).

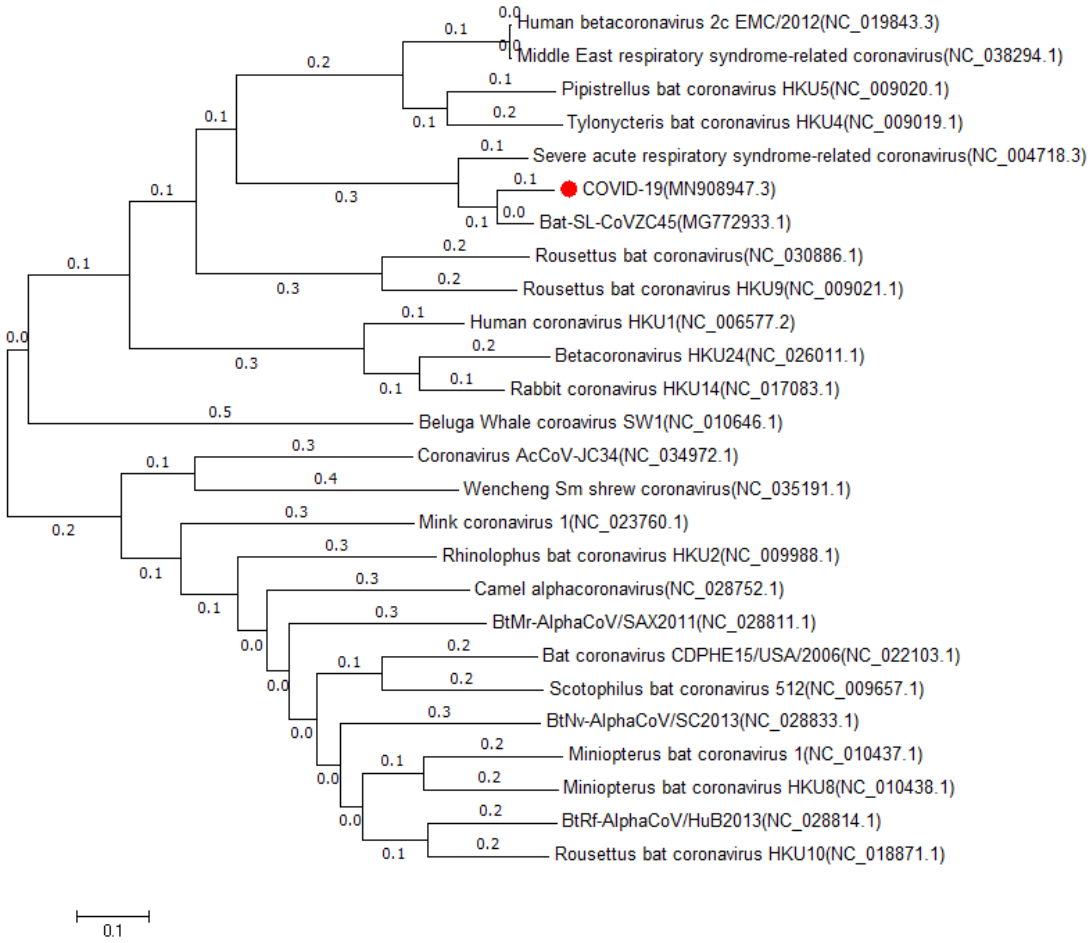

Fig. S1 Maximum likelihood phylogenetic trees of genome sequences of COVID-19. The trees were mid-point rooted for clarity only. The scale bar represents the number of substitutions per site.

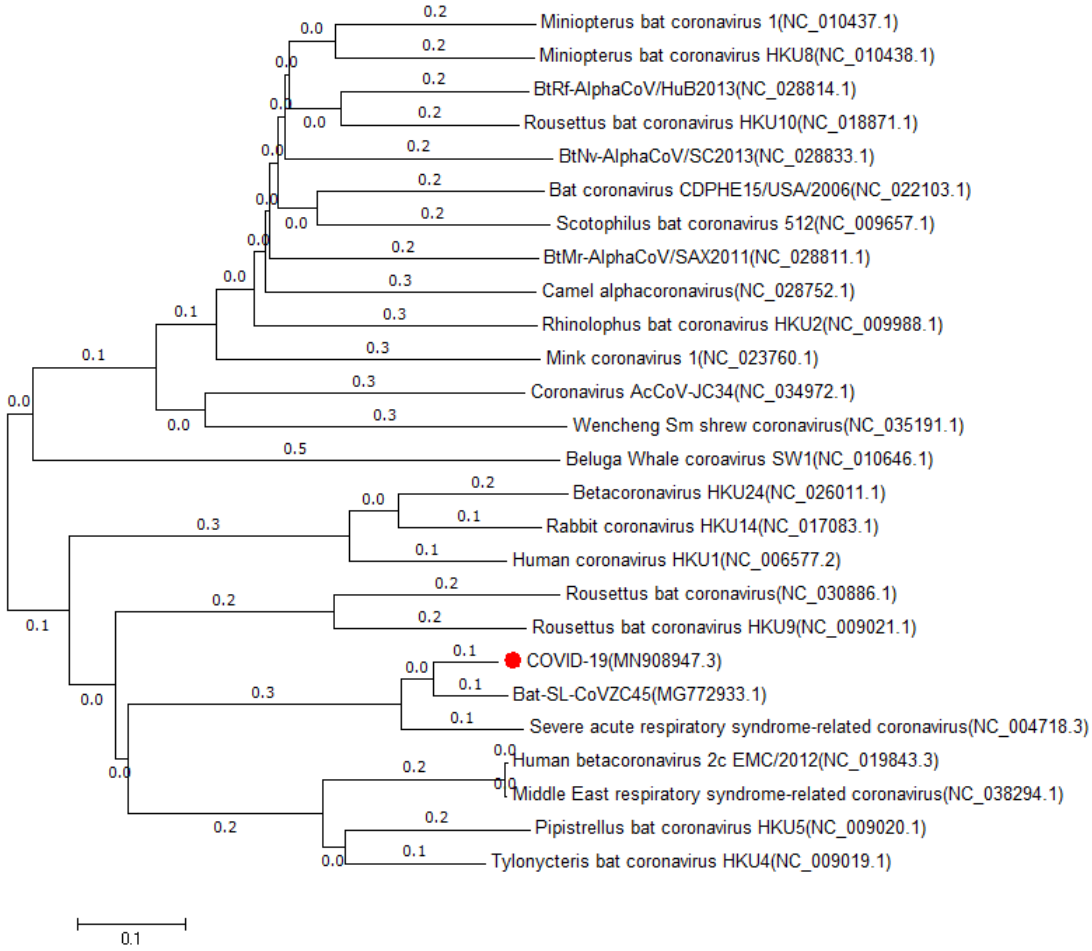

Fig. S2 Neighbor joining distance trees of genome sequences of COVID-19. The trees were mid-point rooted for clarity only. The scale bar represents the number of substitutions per site.

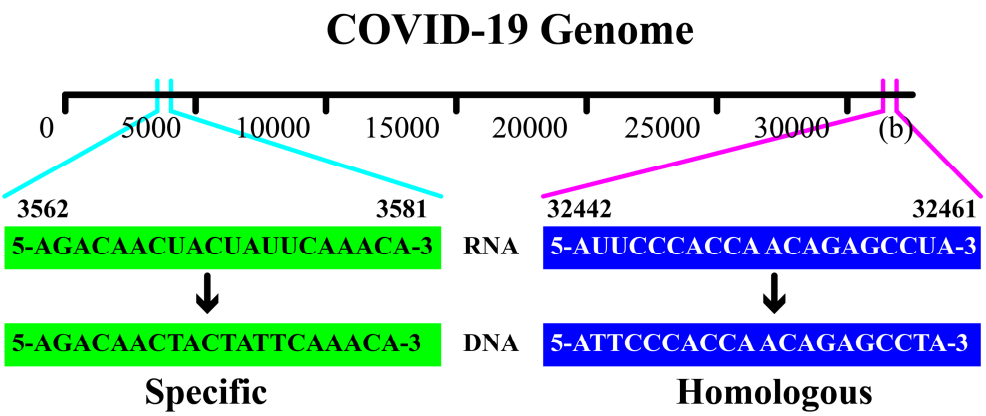

Fig. S3 The positions of Specific and Homologous in the COVID-19 viral RNA genome.

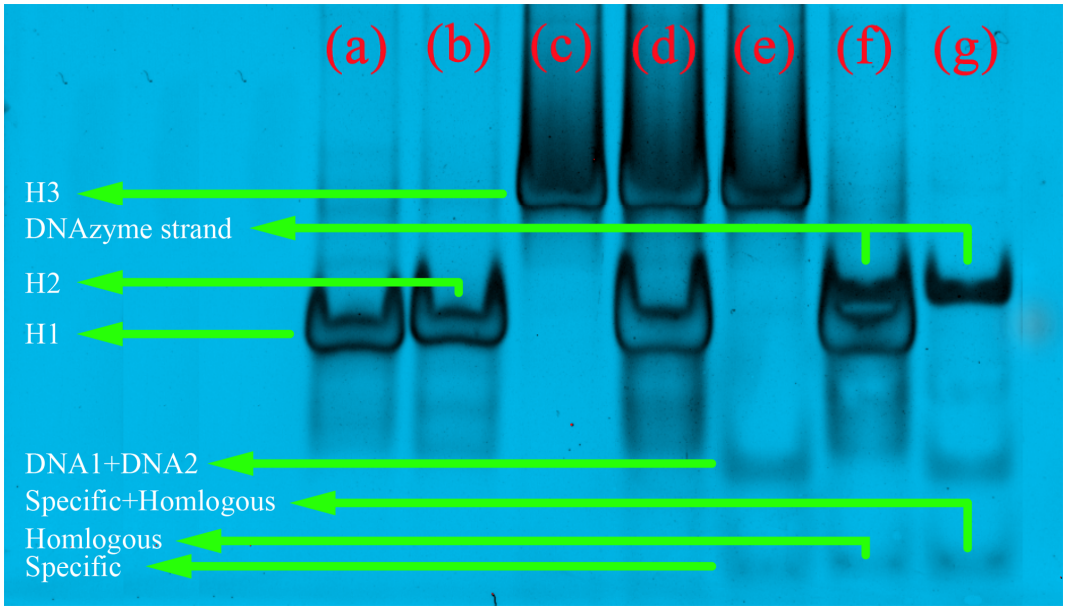

Fig. S4 The PAGE results under different conditions: (a) H1; (b) H2; (c) H3; (d) H1+H2+H3+Exo III; (e) Specific+H1+H2+H3+Exo III; (f) Homologous+H1+H2+H3+Exo III; (g) Specific+Homologous+H1+H2+H3+Exo III.

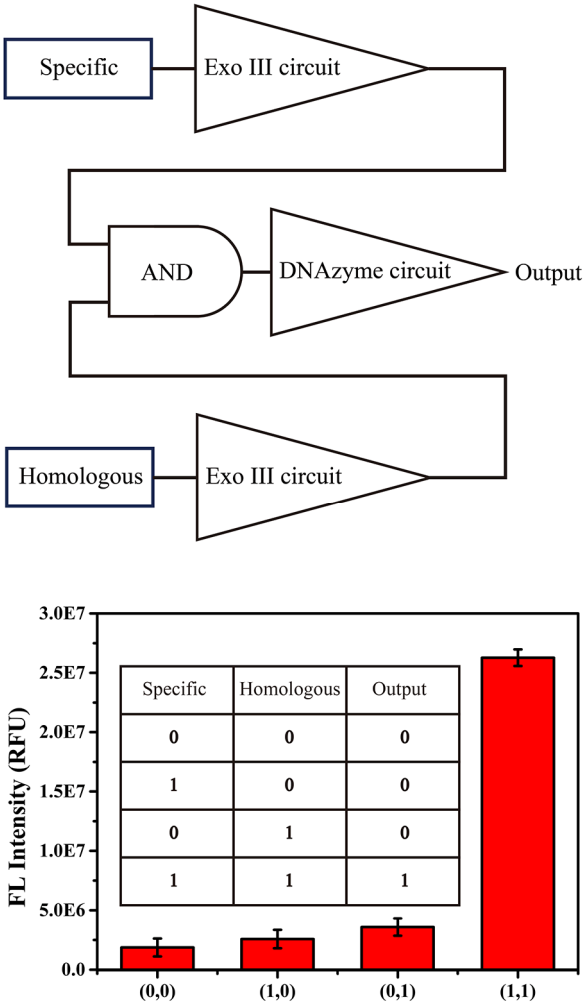

Fig. S5 (A) Schematic diagram of the AND logic circuit. (B) The histograms show the fluorescence intensity under different situations. Error bars represent the standard deviation from three independent measurements. The inset shows the truth table.

Optimization of the assay conditions

In order to obtain the best performance of dual recognition element-controlled logic DNA circuit, several key parameters, including the reaction temperature, the reaction time of Exo III, and Exo III dosage were investigated. In this work, the reaction temperature plays an important role in the sensing process. It not only effects the stability of all hairpins, but also the catalytic ability of Exo III. The effect of reaction temperature on the response of the DNA circuit was investigated by detecting 100 nM Specific at different temperatures (4 °C, 25 °C, 37 °C, and 45 °C). The blank sample (in the absence of the Specific) at each temperature was tested under the same conditions. As shown in Fig. S6, the fluorescence signal increased accordingly with the increase of the temperature from 4 to 37 °C. However, further increasing the temperature to 45°C, the response signal will decrease (cyan histogram in Fig. S6). This is probably because the enzyme activity of Exo III is inhabited over the optimal temperature (37 °C) and even becomes inactive at 45 °C. Also, the higher temperature may change the conformation of hairpins, resulting in the increase of the background (gray histogram in Fig. S6). In order to obtain the best signal-to-background ratio (S/N) (red line in Fig. S6), 25 °C was selected as the optimum reaction temperature. As shown in Fig. S7, the fluorescence signal of the DNA circuit containing 100 nM Homologous gradually reached its plateau when the reaction time was 30 min, which suggest that Exo III-assised signal amplification was completely achieved. Thus, 30 min was employed as the optimal reaction time. In addition, the effect of the enzyme dosage was also studied on the basis of the fluorescence response toward the detection of 100 nM Spcific. As depicted in Fig.S7, the response signal increased with the enhancement of Exo III dosage and reached a platform after 20U. Therefore, 20U was adopted as the optimal enzyme dosage.

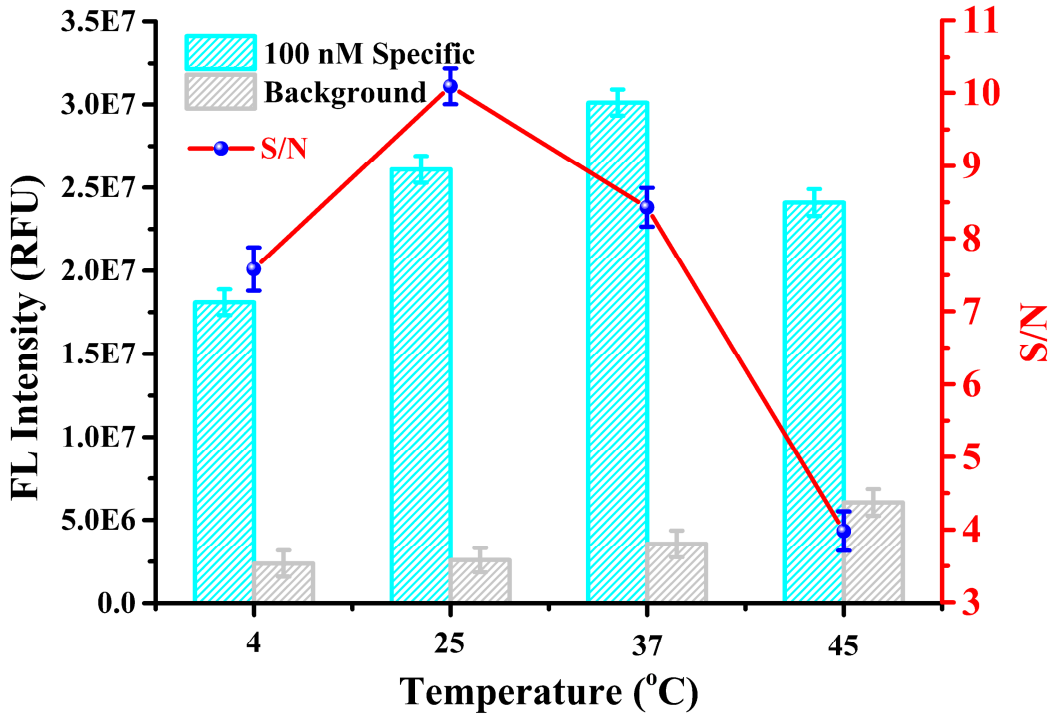

Fig. S6 Effect of the reaction temperature on the response of the sensing system. The histograms represent the fluorescence intensity of the solution in the presence of 100 nM Specific (cyan) and in the absence of Specific (gray), respectively. The red line represents the S/N ratio. The corresponding error bars represent the standard deviation of three independent measurements obtained at each reaction temperature. 100 nM Homologous, 400 nM hairpins (H1, H2, and H3), 100 nM Substrate strand, and 20U Exo III.

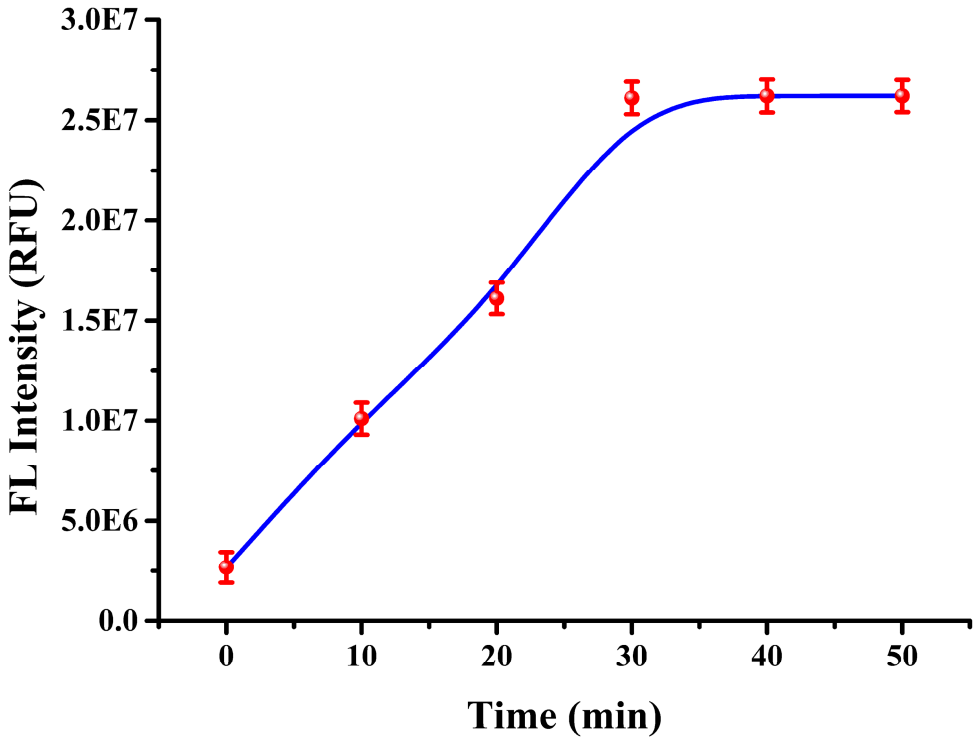

Fig. S7 Effect of the reaction time of Exo III-assised signal amplification on the fluorescence intensity of the proposed method for the detection of Specific (100 nM). Reactions were performed at room temperature. 100 nM Homologous, 400 nM hairpins (H1, H2, and H3), 100 nM substrate strand, and 20U Exo III.

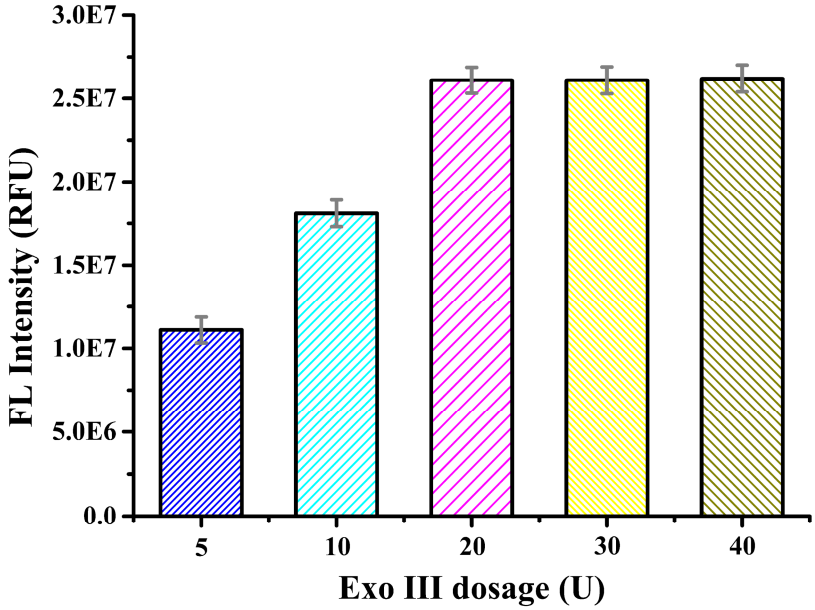

Fig. S8 The effect of the Exo III dosage on the aptasensor performance. The experiments were carried out at room temperature. 100 nM Homologous, 400 nM hairpins (H1, H2, and H3), and 100 nM substrate strand.

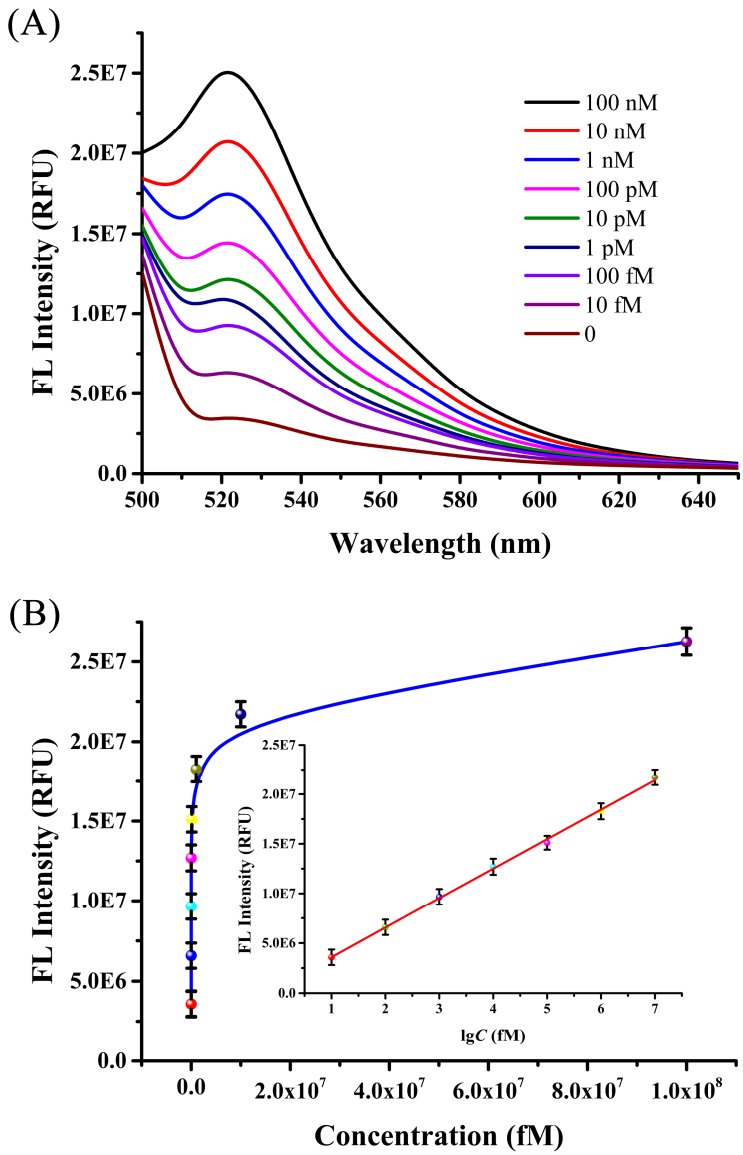

Fig. S9 (A) Fluorescence spectra of the sensing system in the presence of different Homologous concentrations. (B) Correlation of the fluorescence intensity at 520 nm with the Homologous concentrations. Inset depicts the linear relationship between the fluorescence peak intensity and the logarithm of Homologous concentrations. The error bars are standard derivation obtained from three independent experiments. 100 nM Specific; 400 nM hairpins (H1, H2, and H3); 100 nM Substrate strand; 20U Exo III.

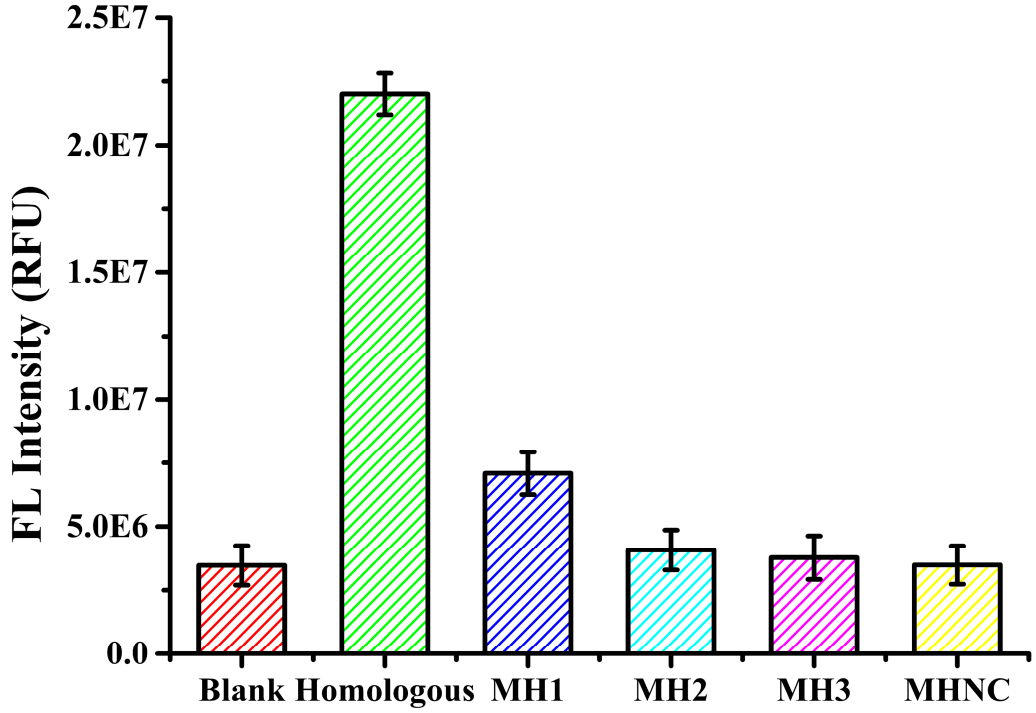

Fig. S10 Selectivity investigation of the logic system for the Homologous against other mutations. The concentration of each DNA is 10 nM. The error bars are standard derivation obtained from three independent experiments. 100 nM Specific; 400 nM hairpins (H1, H2, and H3); 100 nM Substrate strand; 20U Exo III.

Table S2 Comparison of our method and some previously sensors for COVID-19 detection.

Method	Strategy	LOD	Ref.
Colorimetric	thiol-modified antisense oligonucleotide-capped AuNPs	0.18 ng/μL	[1]
FET	SARS-CoV-2 spike antibody-conjugated grapheme sheet	2.42×10^2 copies/mL	[2]
Colorimetric	Nucleocapsid antigen	0.65 ng/μL	[3]
Photothermal	Complementary DNA receptors-functionalized two-dimensional gold nanoislands	0.22 pM	[4]
Colorimetric	DNA aptamers	20 pM	[5]
Fluorescent	CRISPR Cas12a/g RNAsystem	5 copies/test	[6]
Electrochemical	Secondary antibody-conjugated magnetic beads	8 ng/mL	[7]
Fluorescent	A DNA nanoscaffold hybrid chain reaction	0.96 pM	[8]
SPR	Gold nanospikes	0.5 pM	[9]
Fluorescent	Exo III and DNAzyme-mediated signal amplification	3.3 fM	[This work]

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Table S3 Analysis of real samples containing the Specific at different concentrations.

Sample	Spiked	Found[a]	Recovery	RSD
serum	10 nM	9.80 nM	98.0%	6.4%
serum	1 nM	0.94 nM	94.0%	5.9%
serum	100 pM	103.0 pM	103.0%	4.3%
serum	1 pM	0.97 pM	97.0%	4.8%
serum	100 fM	105.4 fM	105.4%	5.4%
urine	10 nM	9.83nM	98.3%	5.7%
urine	1 nM	0.95 nM	95.0%	4.6%
urine	100 pM	102.1 pM	102.1%	4.9%
urine	1 pM	0.99 pM	99.0%	5.5%
urine	100 fM	103.5 fM	103.5%	6.1%
saliva	10 nM	9.60 nM	96.0%	4.5%
saliva	1 nM	1.04 nM	104.0%	6.2%
saliva	100 pM	101.9 pM	101.9%	5.3%
saliva	1 pM	0.99 pM	99.0%	4.1%
saliva	100 fM	99.8 fM	99.8%	4.8%

[a]The data reported in the table represents the average of five measurements.

Table S4 Detection of Homologous in real samples by the proposed biosensor.

Sample	Added	Found[a]	Recovery	RSD
serum	10 nM	9.70 nM	97.0%	6.7%
serum	1 nM	1.02 nM	102.0%	6.0%
serum	100 pM	106.0 pM	106.0%	5.8%
serum	1 pM	0.93 pM	93.0%	4.0%
serum	100 fM	103.4 fM	103.4%	5.4%
urine	10 nM	9.45 nM	94.5%	6.5%
urine	1 nM	0.95 nM	95.0%	4.9%
urine	100 pM	102.1 pM	102.1%	5.3%
urine	1 pM	0.96 pM	96.0%	5.7%
urine	100 fM	101.7 fM	101.7%	4.4%
saliva	10 nM	9.49 nM	94.9%	6.6%
saliva	1 nM	1.04 nM	104.0%	4.8%
saliva	100 pM	99.3 pM	99.3%	4.2%
saliva	1 pM	0.94 pM	94.0%	6.3%
saliva	100 fM	97.8 fM	97.8%	5.9%

[a]The data reported in the table represents the average of five measurements.