Supporting Information for:

Translating Daily COVID-19 Screening into a Simple Glucose Test: A Proof of Concept Study

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Section A: Materials and reagents

All oligonucleotides used in this study were synthesized by Shanghai Sangon Biological Engineering Co., Ltd. (Shanghai, China) and purified by HPLC. The detailed sequences are shown in Table S1. For stock solution preparation, all DNA sequences were dissolved and diluted with Millipore water to a final concentration of 10 μM. After annealing at 90 °C for 5 min, the resulting solutions were cooled down to room temperature and stored at 4 °C until use.

T4 DNA ligase (M0202S), phi29 DNA Polymerase (M0269L), EcoRI (R0101S), Engen® Lba Cas12a (Cpf1) (M0653T), 10X T4 DNA ligase buffer and Low MW DNA ladder (N3233) were purchased from New England Biolabs (Beijing, China) Ltd. SARS-CoV-2 Nucleocapsid Protein was provided by Cellregen LifeScience Co. (China). SARS-CoV-2 Nucleoprotein antibodies (Rabbit monoclonal antibody, 40143-R040 and 40143-R004) and SARS-CoV-2 Spike antibodies (Rabbit monoclonal antibody, 40150-D001 and 40150-D003) were purchased from Sino Biological Inc. (China). Tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP, A600974-0001) and streptavidin immunomagnetic beads (D110557) were provided by Shanghai Sangon Biological Engineering Co., Ltd. (Shanghai, China). SARS-CoV-2 Spike pseudo-viruses was obtained from Genomeditech Co., Ltd. (Shanghai, China). The commercial glucose meter and blood glucose test strips were purchased from Abbott Diabetess Care Ltd.(UK). A prototype of portable glucose meter (PGM) capable of measuring glucose down to 26.0 μM of glucose using commercially available Abbott Precision Xtra® glucose test strip was provided by GlucoSentient, Inc (USA). The prototype was developed using a Cypress PSoC Prototyping Kit and glucose measurement was achieved using chronoamperometry. Other reagents (analytical grade) were purchased from Sinopharm Chemical Reagent Co.(Shanghai, China). All solutions were prepared with Millipore water (18.25 $M\Omega \cdot cm^{-1}$).

Buffers used in this work:

PBS buffer: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4.

PBST buffer: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.05% Tween-20, pH 7.4.

Reporter solution: 0.1 M KOH, 2 M sucrose, pH 4.2.

0.5X TBE buffer: 4.5 mM Tris, 4.5 mM boric acid, 0.1 mM EDTA, pH 8.2.

1 M PBS buffer: 1370 mM NaCl, 27 mM KCl, 100 mM Na_2HPO_4, 20 mM KH_2PO_4, pH 5.0.

10X NE buffer 2.1: 500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 1 mg/ml BSA, pH 7.9

Buffer A: 0.1 M NaCl, 0.1 M sodium phosphate buffer, pH 7.3.

Section B: Table S1. Sequences of DNA oligonucleotides used in this work[a]

Name	Sequence (5'-3')
Padlock Probe N (PPN)	P-GCTGGGGGTTTTGAATTCCCTTTTTTTGGGAATTCTTTTCCTACTACCTCTTGTACCTACTGAACGCTGAAGC
phi29 Primer	GTAGGTACAAGAGGTAGTAG
Biotin-modified DNA (Biotin-DNA)	Biotin-TTATTTTATTTTATTTTATTGTAATAGGCTCATGGTAG
Padlock Probe 2 (PP2)	P-CCTATTACTTCCTTTTTTTTTTTTTTTTAATAATTTTTAATAATTTTTTTTTTCTACCATGAG
Connector	GGAAGTAATAGGCTCATGGTAG
Thiol-modified DNA (DNA-Thiol)	SH-TTTTTTTTTTCTACCATGAGCCTATTAC
crRNA for N gene	UAAUUUCUACUAAGUGUAGAUGAACGCUGAAGCGCUGGGGG
N gene-DNA	AATTTGCCCCCAGCGCTTCAGCGTTCTTCGGAA
N gene-RNA	AAUUUGCCCCCAGCGCUUCAGCGUUCUUCGGAA
Mismatched target DNA 1 (N-MT1)	AATTTGCCCCAAGCGCTTCAGCGTTCTTCGGAA
Mismatched target DNA 2 (N-MT2)	AATTTGCCCCAAGCGGTTCAGCGTTCTTCGGAA
Mismatched target DNA 3(N-MT3)	AATTTGCCCCAAGCGGTTCATCGTTCTTCGGAA
E gene-1	CTTGCTTTCGTGGTATTCTTGCTAGTTACACTA
E gene-2	ATTCTTCTAGAGTTCCTGATCTTCTGGTCTAAA
Random Target Analogue (RTA)	ATTACCAAAGCTTTGGTTAAACAAAGCTTAATG
Proximity Probe A (PPA)	SH-AAAAAAAAAAAAAAAAGACCACATTAGTTAAGACGCTT
Proximity Probe B (PPB)	SH-AAAAAAAAAAAAAAAATATGACAGAACTAGACACTCTT
Connector-L	P-CTAATGTGGTCAAAAAAAGAACGCTGAAGCGCTGGGGGAAAAAAGAATTCCCAAAAAAAGGGAATTCAAAA AAAAAAAAGAGTGTCTA
Connector-S	P-GTTCTGTCATATTTAAGCGTCTTAA
crRNA for N and Spike protein	UAAUUUCUACUAAGUGUAGAUGAACGCUGAAGCGCUGGGGG
F-Q Reporter	FAM-TTATT-BHQ1

[a]The bases in red indicate the mutated bases in target N gene.

Section C: Experimental details

C1. Procedure for SARS-CoV-2 N gene detection

C1.1 Preparation and characterization of the circular Padlock Probe 2 (cPP2) templates for RCA reaction on magnetic nanoparticles (MNPs)

For RCA reaction on MNPs-Primers, the circular DNA template (cPP2) was prepared and purified in advance according to the previous protocol.[1] Briefly, cyclization reaction solution was prepared via mixing 2 μL of Padlock Probe 2 (PP2, 10 μM), 2 μL of connector (10 μM), 2 μL of 10X T4 DNA ligase buffer and 13 μL of Millipore water. After annealing at 90 °C for 5 min, the resulting solutions were cooled down to room temperature. Afterwards, 1 μL of 400 units/μL T4 DNA ligase was added, and the ligation was allowed to proceed at 16 °C for 30 min. After separation and purification by denatured polyacrylamide gel electrophoresis (denatured PAGE, 10 %), the cyclized DNA padlock was dissolved in Millipore water and ready to serve as RCA template for further experiments.

The cyclization reaction of PP2 were characterized using 10 % denatured polyacrylamide gel electrophoresis (denatured PAGE) with the following experimental conditions: current (30 mA), 0.5X TBE buffer, 40 min.

The RCA reaction of cPP2 in solution were characterized using 10% native polyacrylamide gel electrophoresis (native PAGE) with the following experimental conditions: voltage (80 V), 0.5X TBE buffer, 90 min. Low MW DNA ladder was used as the marker.

C1.2 Preparation and characterization of the DNA-Invertase conjugates

The DNA-Invertase conjugates were synthesized by the maleimide-thiol reaction using a heterobifunctional linker sulfo-SMCC.[2] Briefly, 200 μL of 60 μM thiol-modified DNA (DNA-Thiol) was first mixed with 10 μL of 1 M PBS buffer (pH 5.0) and 5 μL of 30 mM TCEP. After vortexing for 30 s, the

solution was placed on a shaker for 60 min at 37 °C. After that, the TECP-treated DNA was purified by Amicon-3K using Buffer A for 8 times, and dispersed in 200 μL of Buffer A. In parallel, 200 uL of 20 mg/mL invertase in Buffer A was mixed with 0.4 mg of sulfo-SMCC, and incubated at room temperature for 60 min. To remove the excess sulfo-SMCC, the invertase solution was then purified by Amicon-100K using Buffer A for 8 times and dispersed in 200 μL of Buffer A. To prepare the DNA-Invertase conjugate, the above two solutions were further mixed and incubated at 4 °C for 2 hours. To remove un-conjugated DNA-Thiol, the solution of DNA-Invertase was purified by Amicon-100K for 8 times using Buffer A. The concentration of purified DNA-Invertase conjugate was quantified using a NanoDrop Onec (Thermo Fisher), and the final stock solution of DNA-Invertase was 10 μM.

The DNA-Invertase conjugate was characterized using 10% native PAGE with the following experimental conditions: voltage (80 V), 0.5X TBE buffer, 90 min. Low MW DNA ladder was used as the marker.

C1.3 Preparation and characterization of the poly-invertase-immobilized magnetic nanoparticles (MNPs-Poly-Invertase)

The poly-invertase-immobilized magnetic nanoparticles (MNPs-Poly-Invertase) were prepared by performing a RCA reaction on MNPs as follow: (1) First, 10 μL of streptavidin immunomagnetic beads (5 mg/mL) were washed using PBST buffer for 5 times, and then mixed with 5 μL of Biotin-DNA (2 μM) and 995 μL of PBS. The resulting solution was placed on a roller for 60 min at roon temperature to form the MNPs-Primers. To remove un-reacted Biotin-DNA, the MNPs-Primers were purified by magnetic separation for 5 times using a PBST buffer and dispersed in 100 μL of Millipore water. (2) RCA reaction was prepared via mixing 10 μL of circular Padlock Probe 2 (cPP2, 500 nM), 2 μL of dNTPs (10 mM), 0.5 μL of phi29 DNA polymerase (10 units/μL) and 20 μL of 10X phi29 DNA polymerase buffer with 67.5 μL

of Millipore water. The resulting solution (200 μL) was incubated at 30 °C for 30 min to perform the RCA reaction. After magnetic separation 5 times using a PBST buffer, the precipitate was dissolved in PBS (100 μL). The resulting solution was denoted as MNPs-Primers covered with RCA products (MNPs-RP). (3) Then, 5 μL of DNA-Invertase (10 μM) and 95 μL of PBS were added to 100 μL of MNPs-RP (0.5 mg/mL) and mixed thoroughly. The resulting solution was kept at roon temperature for 30 min to form the MNPs-Poly-Invertase. To remove un-reacted DNA-Invertase, the MNPs-Poly-Invertase were purified by magnetic separation for 5 times using a PBST buffer, dispersed in 100 μL of PBS, and stored at 4 °C until use.

The MNPs-Poly-Invertase was characterized by testing its catalytic activity of the conversion of sucrose to glucose using a PGM. Briefly, 10 μL of the MNPs-Poly-Invertase (0.5 mg/mL) was mixed with 40 μL of sucrose reporter solution, and incubated at 37 °C for 30 min. After that, 5 μL of the resulting solution was dropped on a glucose strip, and then tested using a PGM. 10 μL of PBS buffer was used as blank sample.

C1.4 Particle size distribution and zeta potential analysis

For hydrodynamic diameter and zeta potential measurement, 10 μL of MNPs, MNPs-Primers, MNPs-RP or MNPs-Poly-Invertase (with the same concentration as MNPs of 0.5 mg/mL) was added to 990 μL of PBS. After mixing thoroughly, the dynamic light scattering (DLS) measurement and zeta potential were measured on Malvern Instruments Zetasizer HS III (Malvern, UK) and 90Plus Dynamic Light Scattering Instrument (Brookhaven Instruments Corporation, USA) at room temperature, respectively.

C1.5 Design and characterization of CRISPR-Cas12a sensor for N gene detection based on rolling circle amplification and portable glucose meter (CRISPR-PGM)

The typical CRISPR-PGM procedure for N gene detection mainly consists of three steps: (1) the generation of Cas12a activator through N gene triggered RCA reaction; (2) the release of poly-invertase-DNA conjugate from MNPs-Poly-Invertase through the collateral cleavage by active Cas12a; (3) the production of PGM-detectable glucose signal through invertase-catalyzed hydrolysis of sucrose to glucose.

The following reagent mix was prepared in advance, and used according to the typical CRISPR-PGM procedure: (1) Reagent A contains 1 μL of phi 29-primer (1 μM), 1 μL of dNTPs (10 mM), 0.5 μL of phi 29 DNA Polymerase (phi29 DP), 2 μL of Millipore water and 0.5 μL of 10X phi 29 buffer. (2) Reagent B contains 1 μL of EcoRI (20 units/μL), 3.5 μL of Millipore water, and 0.5 μL of 10X EcoRI buffer. (3) Reagent C contains the pre-assembled Cas12a/crRNA ribonucleoprotein by mixing 7 μL of Millipore water, 1 μL of 10X NE buffer 2.1, 1 μL of Engen® Lba Cas12a (10 μM in 1X NE buffer 2.1) with 1 μL of crRNA (10 μM in DEPC-treated water) at 37 °C for 20 min.

The following is the detailed experimental condition for each steps:

Step 1: The hybridization and ligation of N gene-DNA with padlock probe was performed by slowly annealing 1 μL of N gene-DNA target with 1 μL of 10 μM padlock probe N (PPN) in 1X T4 DNA Ligase buffer, followed by the addition of 1 μL of T4 DNA Ligase (400 units/μL) and incubated for 20 min at 16 °C. Then, the RCA and cleavage reaction was triggered and accomplished by the stepwise addition of 5 μL of Reagent A and B, and incubated for 30 min at 30 °C and 37 °C, respectively. The amplified Cas12a activator was then obtained after thermal termination of the reaction.

Step 2: To release the poly-invertase-DNA conjugate, 10 μL of MNPs-Poly-Invertase (5 mg/mL) was used as the Cas12a substrate, and mixed with 90 μL of 1X NE buffer 2.1 containing 20 μL of amplified Cas12a activator (Step 1), and 5 μL of Reagent C, followed by incubating at 37 °C for 30 min.

Step 3: After magnetic separation, 10 μL of the supernatant solution (step 2) was collected, and incubated with 40 μL of sucrose reporter solution at 37 °C for 30 min. The generated glucose was then quantified using a PGM.

The products of ligation reaction were characterized using 10 % denatured PAGE with the following experimental conditions: current (30 mA), 0.5X TBE buffer, 40 min.

The products of RCA and cleavage reaction were characterized using 10% native PAGE with the following experimental conditions: voltage (80 V), 0.5X TBE buffer, 90 min. Low MW DNA ladder was used as the marker.

C1.6 Comparison of PGM signals for N gene-DNA and N gene-RNA using CRISPR-PGM system

1 μL of different concentrations (1 pM and 50 pM) of N gene-DNA or N gene-RNA was prepared in Millipore water, and tested according to the typical CRISPR-PGM procedure, respectively (Section C1.5).

C1.7 Fluorescent monitoring of the activation of CRISPR-RCA system using N gene-DNA

To evaluate whether the Cas12a/crRNA ribonucleoprotein can be activated by RCA products of N gene, a fluorecent cleavage assay was designed using a ssDNA fluorescence reporter (F-Q reporter). Briefly, 10 μL of 1 μM of pre-assembled Cas12a/crRNA ribonucleoprotein was mixed with 20 μL of RCA products obtained in the presence of different concentrations of N gene-DNA (step 1 in Section C1.5), 2.5 μL of F-Q reporter (10 μM), 1 μL of 10X NE buffer 2.1 and 6.5 μL of Millipore water at 37 °C for 30 min. After that, the fluorescent signal of the resulting solution was measured using a F-320 (TIANJIN GANGDONG SCI.&TECH. CO., Ltd. China). The excitation wavelength was 488 nm, and the fluorescence spectra were collected from 500 nm to 650 nm. For the real-time fluorescence monitoring, the excitation wavelength was set as 488 nm, and the emission wavelength was 520 nm. Control

experiments were performed using the same concentration of N gene-DNA by traditional CRISPR-Cas12 assay without RCA reaction.

C1.8 Sensitivity and selectivity test for N gene detection using CRISPR-PGM system

For sensitivity analysis, 1 μL of different concentrations of N gene-DNA was prepared in Millipore water, and tested according to the typical CRISPR-PGM procedure (Section C1.5).

For selectivity analysis, 1 μL of 50 pM of N gene-DNA, one mismatched base-contained target (N-MT1), two mismatched bases-contained target (N-MT2), three mismatched bases-contained target (N-MT3), two fragments of the E gene (E gene-1 and E gene-2) and random target analogue (RTA) were prepared in Millipore water, and tested according to the typical CRISPR-PGM procedure, respectively.(Section C1.5).

C1.9 Detection of N gene in saliva using CRISPR-PGM system

For N gene detection in saliva, 1.0 μL of different concentrations of N gene was spiked in saliva and tested according to the typical CRISPR-PGM experiment (Section C1.5).

C1.10 Detection of SARS-CoV-2 viral RNA in human throat swab samples from patients and healthy people

Human throat swab samples were kindly provided by our collaborators (Dr. Hu), and handled in a local hospital (Hefei, China), following the safety requirements of COVID-19 tests. SARS-CoV-2 viral RNA was extracted using a commercial viral RNA extraction kit (SDK60104, Jiangsu Bioperfectus Technologies Co., Ltd.) by an automatic rapid nucleic acid extractor (SSNP-3000A, Jiangsu Bioperfectus Technologies Co., Ltd.). For the detection of viral RNA from COVID-19 positive and negative people, 1.0 μL of RNA extract solution was used as the input, and tested according to the typical CRISPR-PGM experiment (Section C1.5).

C1.11 Live subject statement

The authors state that all experiments were performed in compliance with the relevant laws and institutional guidelines. The institutional committee of the Binhu Hospital of Hefei City approved the experiment. The authors also state that the informed consent was obtained for any experimentation with human subjects and the RNA extraction specimens of human throat swab used in this study didn't have any identifying information about all the participants. The Binhu Hospital of Hefei City is committed to the protection and safety of human subjects involved in the research. All experimental procedures were completed under biosafety level II conditions.

C2. Procedure for the detection of SARS-CoV-2 N protein using a proximity ligation assisted CRISPR-PGM system (PLA-CRISPR-PGM)

C2.1 Preparation and characterization of the antibody-DNA conjugates

The antibody-DNA conjugates were synthesized by the maleimide-thiol reaction using heterobifunctional linker sulfo-SMCC.[2] Briefly, 200 μL of 0.15 mg/mL SARS-CoV-2 Nucleoprotein antibody A (MAbA) or SARS-CoV-2 Nucleoprotein antibody B (MAbB) in PBS buffer was mixed with 200 μL of 1.5 mg/mL sulfo-SMCC. After vortexing for 30 s, the solution was placed on a shaker for 30 min at room temperature. The mixture was then purified by Amicon-100K using PBS buffer for 8 times, and dispersed in 200 μL of PBS buffer. In parallel, a certain amount of thiol-DNA (PPA or PPB, molar ratio: 10 fold to corresponding protein) in 200 μL Millipore was mixed with 10 μL of 1 M PBS buffer (pH 5), and 10 μL of 30 mM TCEP, and then incubated at 37 °C for 1 hour. The thiol-DNA was then purified by Amicon-3K using PBS buffer for 8 times, dispersed in 200 μL of PBS, and then mixed with the above solution of sulfo-SMCC-activated Nucleoprotein antibodies, respectively. The resulting solution was kept at 4 °C for 2 hours.

To remove unconjugated thiol-DNA, the solution for MAbA-PPA or MAbB-PPB was purified by Amicon-100K using PBS buffer for 8 times. The concentrations of purified antibody-DNA conjugates were quantified using a NanoDrop Onec (Thermo Fisher), and the final stock solution of MAbA-PPA and MAbB-PPB was 200 nM, respectively.

The antibody-DNA conjugates were characterized using 12% native PAGE with the following experimental conditions: voltage (80 V), 0.5X TBE buffer, 90 min. Low MW DNA ladder was used as the marker.

C2.2 Detection of N protein using PLA-CRISPR-PGM system

The typical PLA-CRISPR-PGM procedure for N protein detection mainly consists of three steps: (1) the generation of Cas12a activator through N protein triggered PLA-RCA reaction; (2) the release of poly-invertase-DNA conjugate from MNPs-Poly-Invertase through the collateral cleavage by active Cas12a; (3) the production of PGM-detectable glucose signal through invertase-catalyzed hydrolysis of sucrose to glucose.

The following reagent mix was prepared in advance, and used according to the typical PLA-CRISPR-PGM procedure: (1) Reagent B and C are the same as described in Section C1.5; (2) Reagent D contains 2 μL of MAbA-PPA (2 nM), 2 μL of MAbB-PPB (2 nM), 2 μL of connector-S (200 nM), 2 μL of connector-L (200 nM), 4 μL of T4 DNA ligase (2 units/μL), 4 μL of 10X T4 DNA ligase buffer, and 22 μL of Millipore water; (3) Reagent E contains 0.5 μL of dNTPs (25 mM), 0.5 μL of phi29 DNA polymerase (10 units/μL), 0.5 μL of 10X phi29 DNA polymerase buffer, and 3.5 μL of Millipore water.

The following is the detailed experimental condition for each steps:

Step 1: The PLA-RCA reaction was triggered and accomplished by the stepwise addition of 2 μL of N protein with Reagent D (38 μL), Reagent E (5 μL) and Reagent B (5 μL), and incubated for 30 min at 37

°C, 30 °C, and 37 °C, respectively. The amplified Cas12a activator was then obtained after thermal termination of the reaction.

Step 2: To release the poly-invertase-DNA conjugate, 10 μL of MNPs-Poly-Invertase (5 mg/mL) was used as the Cas12a substrate, and mixed with 90 μL of 1X NE buffer 2.1 containing 20 μL of amplified Cas12a activator (Step 1), and 5 μL of Reagent C, followed by incubating at 37 °C for 30 min.

Step 3: After magnetic separation, 10 μL of the supernatant solution (step 2) was collected, and incubated with 40 μL of sucrose reporter solution at 37 °C for 30 min. The generated glucose was then quantified using a PGM.

C2.3 Fluorescent monitoring of the activation of CRISPR-RCA system using N protein

To evaluate whether the Cas12a/crRNA ribonucleoprotein can be activated by N protein through the proximity ligation assisted RCA reaction, a fluorecent cleavage assay was designed using a ssDNA fluorescence reporter (F-Q reporter). Briefly, 5 μL of pre-assembled Cas12a/crRNA ribonucleoprotein, 2.5 μL of F-Q reporter (10 μM), 5 μL of 10X NE buffer 2.1 and 37.5 μL of Millipore water were mixed with 50 μL of PLA-RCA products obtained in the presence of different concentrations of N protein (step 1 in Section C2.2). After incubating at 37 °C for 30 min, the fluorescent signal of the resulting solution was measured using a F-320 (TIANJIN GANGDONG SCI.&TECH. CO,. Ltd. China). The excitation wavelength was 488 nm, and the fluorescence spectra were collected from 500 nm to 650 nm.

C2.4 Selectivity test for N protein detection using PLA-CRISPR-PGM system

For selectivity analysis, 2 μL of 200 pM N protein and other competing proteins were prepared in PBS, and tested according to the typical PLA-CRISPR-PGM procedure (Section C2.2).

C2.5 Detection of N protein in saliva using CRISPR-PGM system

For N protein detection in saliva, 2.0 μL of different concentrations of N protein was spiked in saliva and tested according to the typical PLA-CRISPR-PGM experiment (Section C2.2).

C3. Procedure for the detection of SARS-CoV-2 Spike pseudo-viruses using the PLA-CRISPR-PGM system

C3.1 Preparation and characterization of the Spike protein antibody-DNA conjugates

The antibody-DNA conjugates of Spike protein were synthesized by the maleimide-thiol reaction using heterobifunctional linker sulfo-SMCC.[2] The resulting antibody-DNA conjugates were named SMAbA-PPA and SMAbB-PPB, respectively. The detailed experimental procedure is described in “Section C2.1”

C3.2 Detection of Spike protein of SARS-CoV-2 pseudo-viruses using PLA-CRISPR-PGM system.

The typical PLA-CRISPR-PGM procedure for SARS-CoV-2 pseudo-viruses detection mainly consists of three steps: (1) SARS-CoV-2 pseudo-viruses lysis and protein extraction (2) the generation of Cas12a activator through Spike protein triggered PLA-RCA reaction; (3) the release of poly-invertase-DNA conjugate from MNPs-Poly-Invertase through the collateral cleavage by active Cas12a; (4) the production of PGM-detectable glucose signal through invertase-catalyzed hydrolysis of sucrose to glucose.

The following reagent mix was prepared in advance, and used according to the typical PLA-CRISPR-PGM procedure: (1) Reagent B, C and E are the same as described in Section C1.5 and C2.2; (2) Reagent F contains 2 μL of SMAbA-PPA (2 nM), 2 μL of SMAbB-PPB (2 nM), 2 μL of connector-S (200 nM), 2 μL of connector-L (200 nM), 4 μL of T4 DNA ligase (2 units/μL), 4 μL of 10X T4 DNA ligase buffer, and 22 μL of Millipore water;

The following is the detailed experimental condition for each steps:

Step 1: For the detection of Spike protein of SARS-CoV-2 pseudo-viruses using PLA-CRISPR-PGM system, the NE-PER extraction reagents (Thermo Fisher Scientific, USA) was used to extract the whole protein from the pseudo-viruses.

Step 2: The PLA-RCA reaction was triggered and accomplished by the stepwise addition of 2 μL of different concentrations of SARS-CoV-2 pseudo-viral extractive with Reagent D (38 μL), Reagent F (5 μL) and Reagent B (5 μL), and incubated for 30 min, 60 min and 30 min, respectively. The amplified Cas12a activator was then obtained after thermal termination of the reaction.

Step 3: To release the poly-invertase-DNA conjugate, 10 μL of MNPs-Poly-Invertase (5 mg/mL) was used as the Cas12a substrate, and mixed with 90 μL of 1X NE buffer 2.1 containing 20 μL of amplified Cas12a activator (Step 1), and 5 μL of Reagent C, followed by incubating at 37 °C for 30 min.

Step 4: After magnetic separation, 10 μL of the supernatant solution (step 2) was collected, and incubated with 40 μL of sucrose reporter solution at 37 °C for 60 min. The generated glucose was then quantified using a PGM.

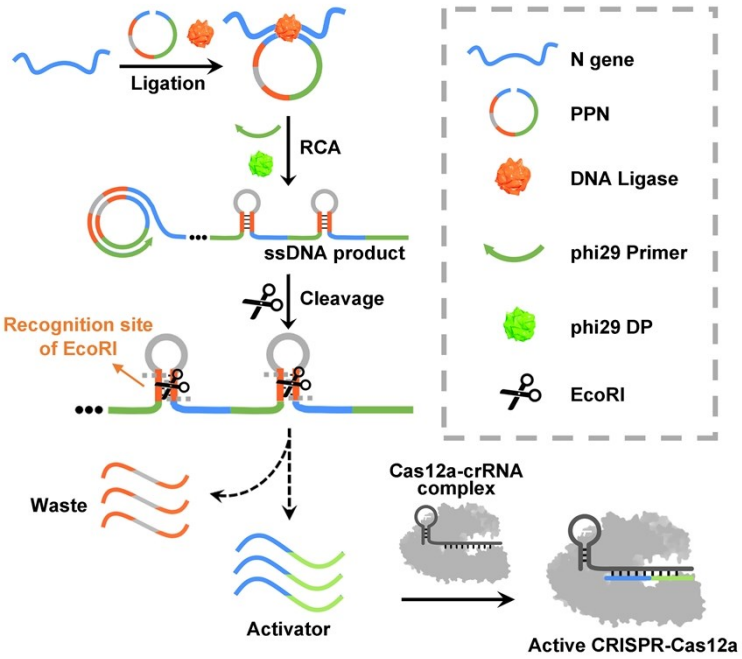

Fig. S1. Schematic illustration of the padlock probe-based rolling circle amplification (RCA) reaction coupled with the restriction endonuclease EcoRI-based amplification, generating large amounts of small DNA fragments for CRISPR-Cas12a activation.

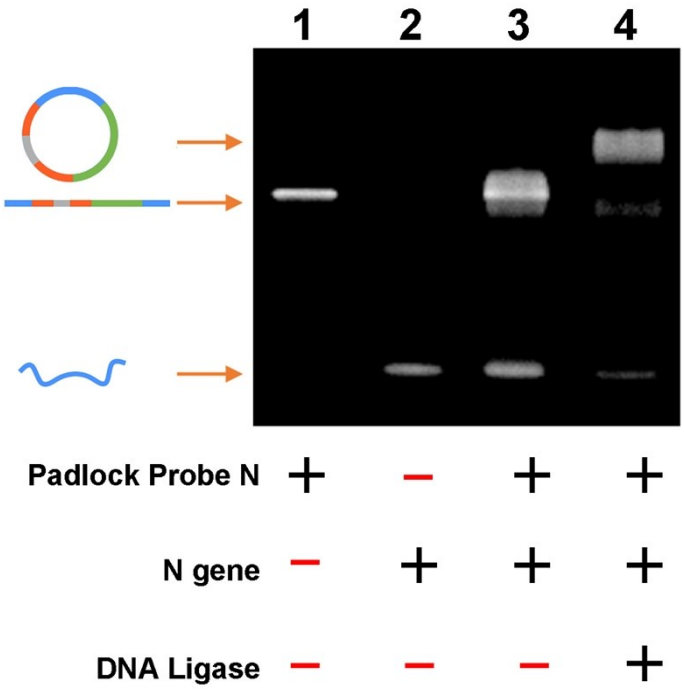

Fig. S2 Analysis of ligation reaction by denatured PAGE (10 %). Lane 1: Padlock Probe N (PPN); Lane 2: N gene-DNA; Lane 3: N gene-DNA + PPN; Lane 4: N gene-DNA + PPN + DNA ligase. The hybridization and ligation of N gene-DNA with padlock probe was performed by slowly annealing 1 µL of 10 µM N gene-DNA target with 1 µL of 10 µM padlock probe N (PPN) in 20 µL of 1X T4 DNA Ligase buffer, followed by the addition of 1 µL of T4 DNA Ligase (400 units/µL) and incubated for 20 min at 16 °C. The symbols “+” and “-”, indicate the presence and absence of the reagent, respectively.(Section C1.5, Step 1)

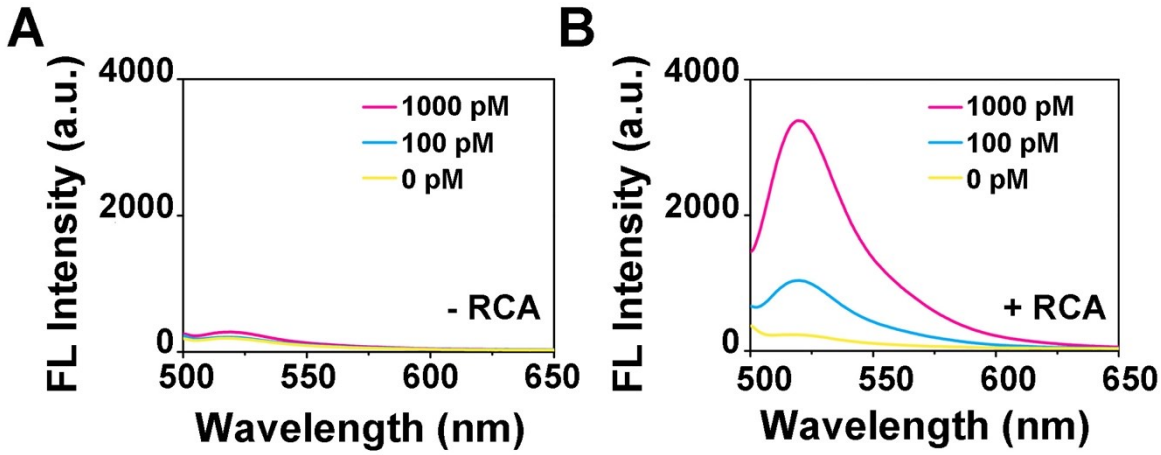

Fig. S3 Fluorescence responses of CRISPR-Cas12a assay for different concentrations of N gene target without RCA reaction (A) and with RCA reaction (B).

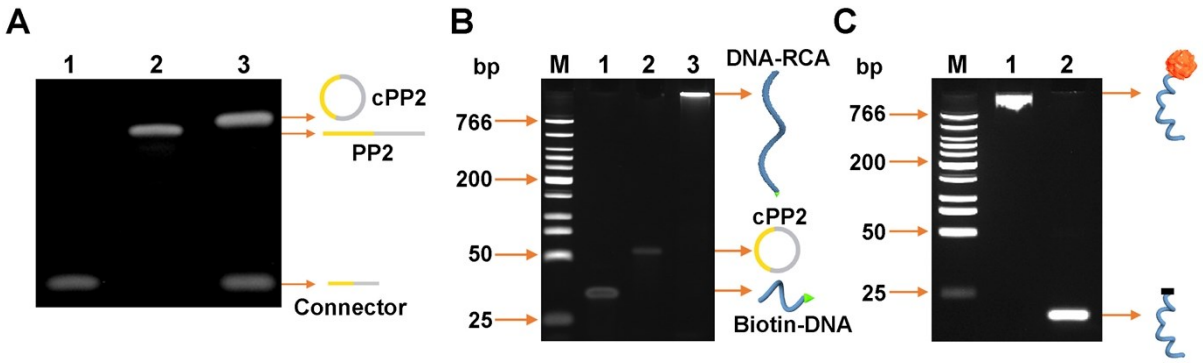

Fig. S4 (A) Denatured PAGE (10%) analysis of the cyclization reaction of the PP2. Lane 1: connector; Lane 2: PP2; Lane 3: connector + PP2 + DNA ligase. (B) Native PAGE (10%) analysis of the RCA reaction of cPP2. Lane 1: Biotin-DNA; Lane 2: cPP2; Lane 3: Biotin-DNA + cPP2 + dNTPs + phi29 DP. (C) Native PAGE (10%) analysis of the DNA-Invertase conjugates. Lane 1: DNA-Invertase conjugates; Lane 2: DNA-Thiol; M: Low molecular weight DNA ladder was used as the marker.

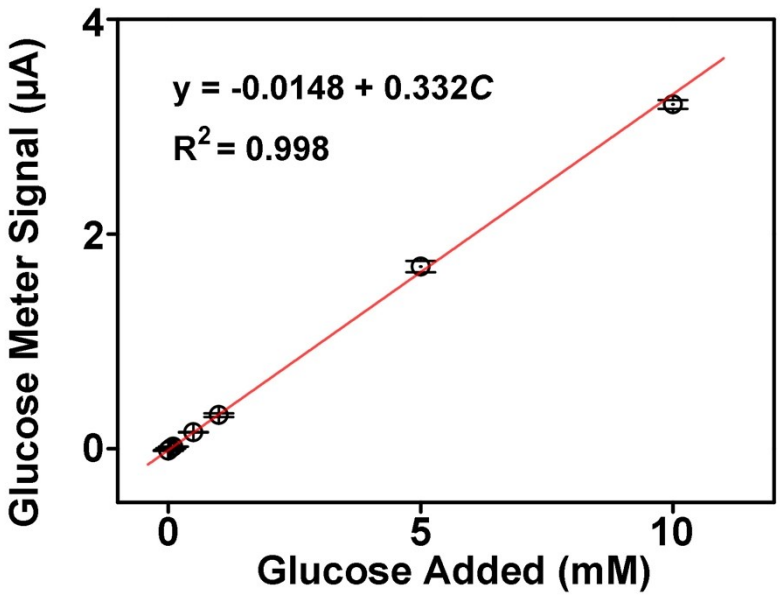

Fig. S5 The carlibration curve for glucose detection with GSI portable glucose meter (GSI-PGM) using the commercial glucose strips. Different concentrations of glucose were added in the reporter solution, and tested using the GSI-PGM. Error bars represent the standard deviations of three independent measurements.

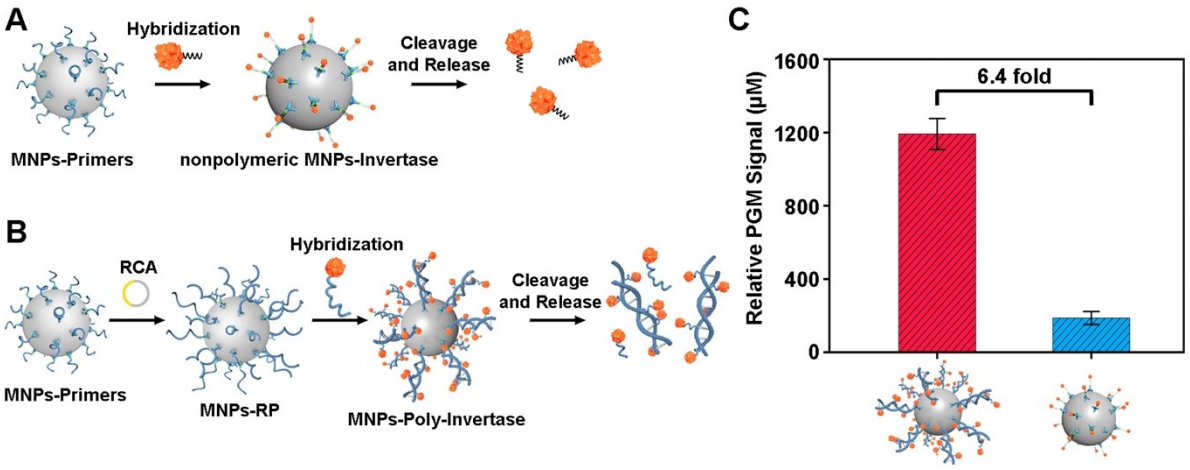

Fig. S6 Graphic illustration of the detection of N gene by CRISPR-RCA system with a nonpolymeric invertase immobilized magnetic beads (MNPs-Invertase) reporter (A) and MNPs-Poly-Invertase reporter (B). (C) Comparison of PGM signals for 10 pM of N gene using MNPs-Poly-Invertase (red) and nonpolymeric MNPs-Invertase (blue), measured by CRISPR-PGM system. Error bars represent the standard deviations of three independent measurements.

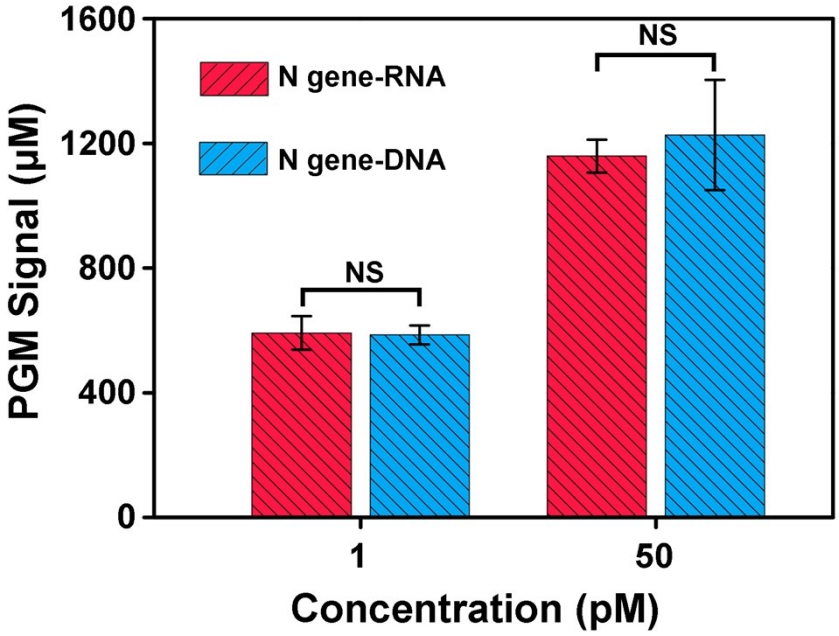

Fig. S7 Comparision of PGM signals of the CRISPR-PGM system for the detection of N gene-DNA and N gene-RNA. Error bars represent the standard deviations of three independent measurements, and NS indicated no significant difference.

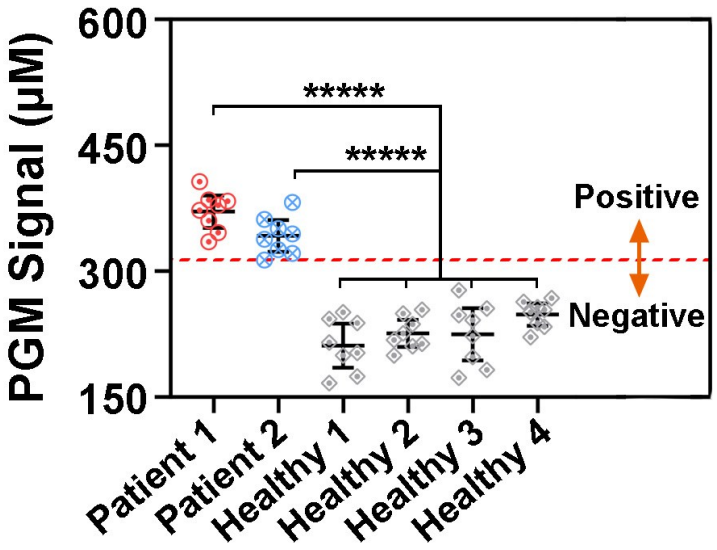

Fig. S8 Performance of the CRISPR-PGM system for N gene detection in healthy people and confirmed patients. The COVID-19 positive and negative samples were confirmed by RT-qPCR. ***** indicated P < 0.00001.

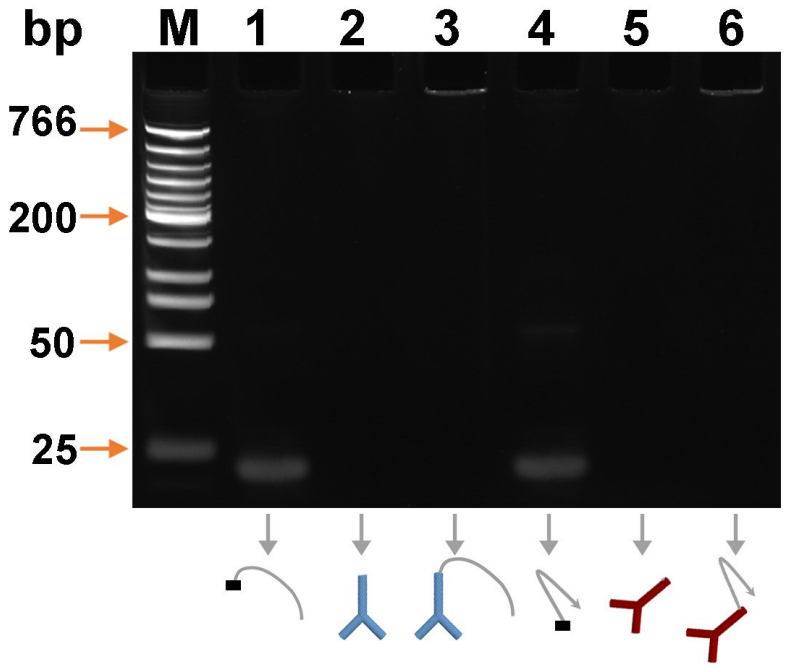

Fig. S9 Native PAGE (10%) analysis of the MAbA-PPA and MAbB-PPB conjugates. Lane 1: PPA; Lane 2: MAbA; Lane 3: MAbA-PPA conjugate; Lane 4: PPB; Lane 5: MAbB; Lane 6: MAbB-PPB conjugate; M: Low molecular weight DNA ladder was used as the marker. PPA: Proximity Probe A; MAbA: SARS-CoV-2 Nucleoprotein antibody A; PPB: Proximity Probe B; MAbB: SARS-CoV-2 Nucleoprotein antibody B.

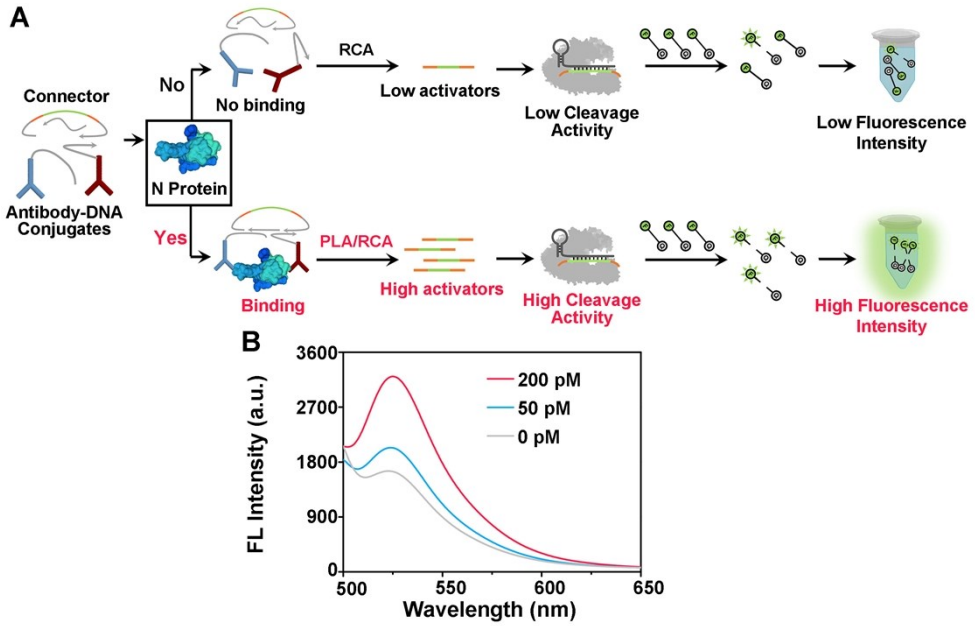

Fig. S10 Graphic illustration (A) and fluorescence spectra measurement (B) of N protein by the proximity ligation assisted CRISPR-RCA system with a single-stranded DNA fluorescence reporter.

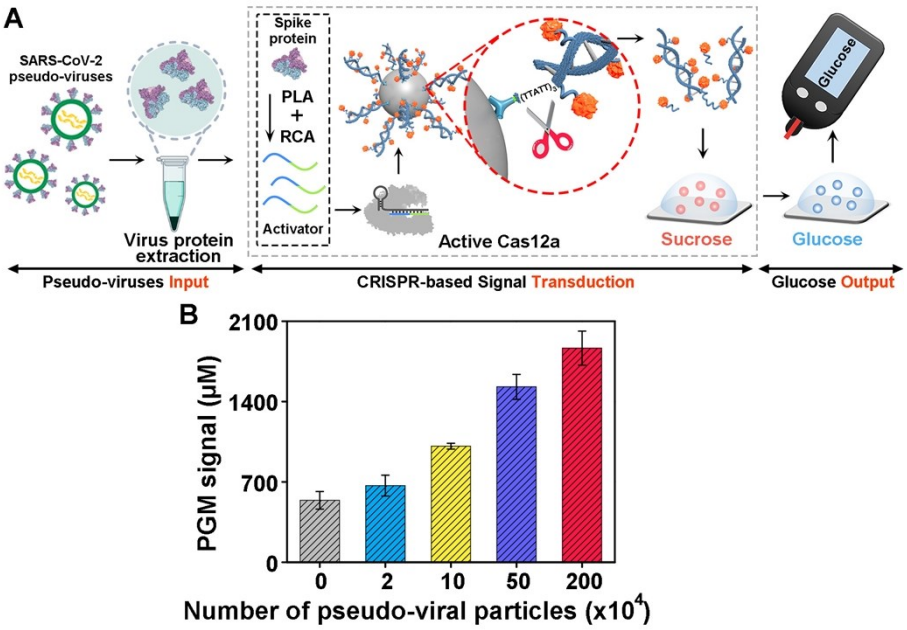

Fig. S11 Design and performance of PLA-CRISPR-PGM system for SARS-CoV-2 Spike pseudo-viruses detection. (A) Working principle and (B) feasibility test of PLA-CRISPR-PGM system for the detection of SARS-CoV-2 Spike pseudo-viruses.

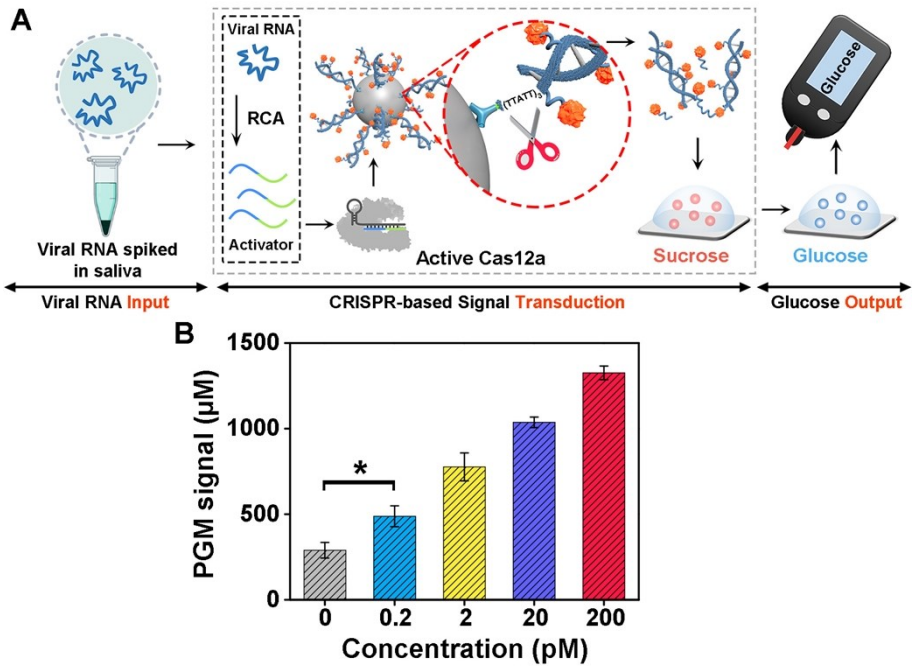

Fig. S12 Design and performance of CRISPR-PGM system for SARS-CoV-2 N gene detection in saliva. (A) Working principle for the detection of N gene spiked in saliva by the CRISPR-PGM method. (B) PGM signal increase of the sensor in saliva over different concentrations of N gene.

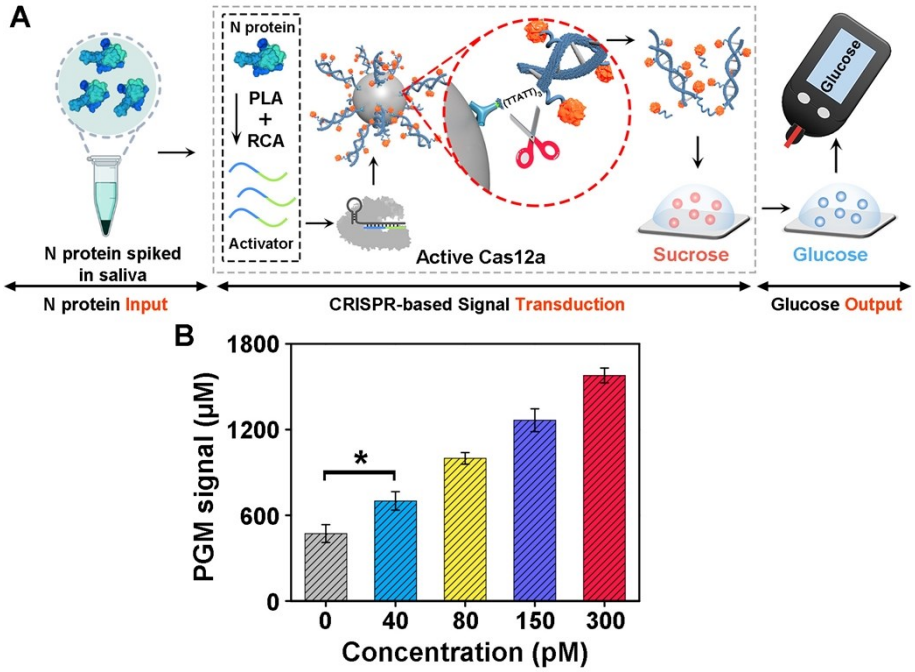

Fig. S13 Design and performance of CRISPR-PGM system for SARS-CoV-2 N protein detection in saliva. (A) Working principle for the detection of N protein spiked in saliva by the CRISPR-PGM method. (B) PGM signal increase of the sensor in saliva over different concentrations of N protein.

References:

1. S. X. Zhang, C. Chen, C. Xue, D. R. Chang, H. Xu, B. J. Salena, Y. F. Li and Z. S. Wu, *Angew. Chem., Int. Ed.*, 2020, **59**, 14584-14592.
2. J. J. Zhang and Y. Lu, *Angew. Chem., Int. Ed.*, 2018, **57**, 9702-9706.