Section A: Materials and Methods

A1. Materials and Instruments

A1.1. Oligonucleotides and Materials.

The sequences of all DNA and RNA oligonucleotides are provided in Table S1. The DNA oligonucleotides were purchased from Sangon Biotech (Shanghai, China), and purified by high-performance liquid chromatography (HPLC). The RNA oligonucleotides were purchased from General Biol (Anhui, China), and purified by high-performance liquid chromatograph (HPLC). T7 RNA polymerase was purchased from New England Biolabs. The Cas13a protein was obtained as gifts from the laboratory of Professor Yongbin Xu (Department of Bioengineering, College of Life Science, Dalian Nationalities University). Taq II DNA Polymerase, M-MuLV Reverse Transcriptase, NTP mix, dNTP mix, and Liquid RNase Inhibitor were purchased from Sangon Biotech (Shanghai, China). Agarose and DNA markers were purchased from Thermo Fisher Scientific. All other chemicals were purchased from Sigma-Aldrich and used without further purification. Nuclease-free water was purchased from Solarbio Life Sciences (Beijing, China). The genomic DNA extraction kit was purchased from Tiangen Biotech (Beijing, China). All human blood samples and the genomic RNA of throat swab samples were acquired from Dalian Municipal Central Hospital.

A1.2. Instruments.

The fluorescence images were obtained using a Typhoon 5 variable mode imager (GE Healthcare) and analyzed using Image Quant software (Molecular Dynamics). Fluorescence measurements were performed using a Tecan Spark microplate reader (Switzerland) with excitation at 485 nm and emission at 520 nm. Polymerase chain reactions were conducted on a thermal cycler (BioRad-T100).

A1.3. Buffers used in this work.

(1) 1 × Cas13a cleavage buffer (1 × CCB): 20 mM HEPES-Na, pH 6.8, 50 mM KCl, 5 mM $MgCl_2$, 10 μg/mL bovine serum albumin, 10 μg/mL yeast tRNA, 0.01% CA-630, 5% glycerol.

(2) 1 × 10-23 DNAzyme cleavage buffer (1 × DCB): 50 mM Tris-HCl, PH 7.5, 100 mM NaCl 10 mM $MgCl_2$.

(3) 1 × Reverse Transcription buffer (1 × RTB): 50 mM Tris-HCl, pH 8.3, 50 mM KCl, 4 mM $MgCl_2$, 10 mM DTT.

(4) 1 × VPCR buffer (1 × VPB): 10 mM Tris-HCl, PH 8.9, 50 mM KCl 1.5 mM $MgCl_2$.

A2. Methods

A2.1. 10-23 DNAzyme-induced cleavage of RNA substrate (Figure 2c).

For studying the cleavage of RNA substrate (RS) by 10-23 DNAzyme (Dz), 5' FAM-labeled RNA substrate was used. In a typical experiment for 10-23 DNAzme cleavage, 2 μL of 1 μM FAM labeled RNA substrate (FAM-RS-GU/GC/GA/GG) was mixed with 1 μL of 100 μM 10-23DZ1 in 1× DCB, and heated at 90 °C for 2 min before being cooled to RT. This was followed by the addition of 2 μL of 100 mM $MgCl_2$ and 0.5 μL of RNase inhibitor (40 U/μL) to a total volume of 20 μL. The reaction mixture was then incubated at 37 °C for 20 min. Then, 10 μL of the above cleavage mixture was mixed with 10 μL of 2× RNA loading buffer (95% (v/v) formamide, 0.02% (w/v) SDS, 0.02% (w/v) bromophenol blue, 0.01% (w/v) xylene cyanol) containing 1 mM EDTA. The mixture was then run on a 10% dPAGE gel. The gel was scanned and analyzed by Image Quant software.

A2.2. Procedure of DOES-CRISPR reaction (Figure 2d)

Cas13a (4 pmol) was pre-assembled with 2 pmol crRNA-CY1* (Y1* = G, U, C) at 37 °C for 10 min in 100 μL of 1× CCB containing 1 U/μL RNase inhibitor. 2 μL of 1 μM 10-23DZ1, 2 μL of 10 nM RS-GU or RS-GY1 (Y1 = C, A, or G), and 1.5 μL of 10 μM FQ-ssRNA reporter were added to the mixture, followed by a measurement of fluorescence intensity using a microplate reader for 30 min at 37 °C (λ_{ex}: 485 nm; λ_{em}: 520 nm).

A2.3. Procedure of Cas13a reaction (Figure 2e)

For the Cas13a-only reaction, the procedure was carried out according to the protocol described in A2.2. except that: 2 μL of 1 μM 10-23DZ1 was not used.

A2.4. DOES-CRISPR reaction for miRNA detection (Figure 3)

dPAGE analysis of miRNA cleavage product. The cleavage of miRNA was carried out in 1× DCB according to the protocol described in A2.1. except that: 5' FAM-labeled miR-17 and miR-20X (X = a, b), or let-7e and let-7Z (Z = a, b, c, or d) were used as the substrate of 10-23DZ-miR (or 10-23DZ-let).

DOES-CRISPR reaction. The procedure was carried out according to the protocol described in A2.1. except that: crRNA-miR-17 (or crRNA-let-7e), 10-23DZ-miR (or 10-23DZ-let), miR-17 (or let-7e), and miR-20X (or let-7Z) were used.

Cas13a-based detection. The Cas13a-based detection was performed in the same way as the above description except that: 2 μL of 1 μM 10-23DZ-miR (or 10-23DZ-let) was not used.

A2.5. DOES-CRISPR reaction for CYP2C19*17 gene detection (Figure 4c)

*dPAGE analysis of ssRNA-CYP2C19*17 cleavage product.* The cleavage of the corresponding RNA of the CYP2C19*17 gene was carried out in 1×

DCB according to the protocol described in A2.1. except that: 5' FAM-RS-CYP-WT and 5' FAM-RS-CYP-Mut were used as the substrate of 10-23DZ-CYP-Mut.

VPCR Reaction. VPCR was performed in 50 μL of a solution containing 1 × VPB, 50 μM dNTPs, 0.1 U/μL of Taq II DNA Polymerase, 1 μM forward primer-CYP (FP-CYP), 1 μM reverse primer-CYP (RP-CYP), and 10^5 copies/μL synthetic standard target ssDNA template (RS-CYP-WT/Mut). The VPCR amplification was carried out on a thermal cycler with a thermal profile beginning at 95 °C for 1 min, followed by 40 cycles at 95 °C for 1 s and 50 °C for 1 s with the ramp rate of 1.6 °C/s, 1 cycle at 72 °C for 5 min, and ending at 12 °C. The products of VPCR were analyzed by 2% agarose gel electrophoresis.

T7 transcription-DOES-CRISPR reaction. Cas13a (4 pmol) was pre-assembled with 2 pmol crRNA-CYP-WT at 37 °C for 10 min in 100 μL of 1× CCB containing 1 U/μL RNase inhibitor. Then, 2 μL of 100 μM 10-23DZ-CYP-Mut, 1 μL of the above VPCR dsDNA product, 2 μL of 25 mM NTP, 2 μL of 50 U/μL T7 RNA polymerase, and 1.5 μL of 10 μM FQ-ssRNA reporter were added to the mixture. After incubation at 37 °C for 10 min, the fluorescence intensity was recorded by a microplate reader (λ_{ex}: 485 nm; λ_{em}: 520 nm).

Cas13a-based detection. The Cas13a-based detection was performed in the same way as the above description except that: 2 μL of 100 μM 10-23DZ-CYP-Mut was not used.

A2.6. Analysis of CYP2C19*17 gene in human blood samples (Figure 4d)

Genomic DNA extraction (step I): In a typical experiment, 200 μL of the blood sample was purified to obtain gDNA using a gDNA extraction kit. The obtained gDNA was dissolved in 100 μL of nuclease-free water and analyzed by 1% agarose gel electrophoresis.

VPCR amplification (step II): The VPCR mixture containing 5 μL of extracted gDNA, 50 nM dNTPs, 0.1 U/μL of Taq II DNA Polymerase, 1 μM FP-CYP, and 1 μM RP-CYP was mixed in 50 μL of 1× VPB. The VPCR was carried out using the following procedure: 95 °C for 2 min, followed by 40 cycles of 95 °C for 1 s and 50 °C for 1 s with the ramp rate of 1.6 °C/s, 1 cycle of 72 °C for 5 min, and ending at 12 °C.

T7 transcription-DOES-CRISPR assay (step III): Then, 1 μL of the dsDNA product was added to the T7 transcription-DOES-CRISPR system and incubated at 37 °C for 10 min.

A2.7. DOES-CRISPR reaction for SARS-CoV-2 detection (Figure 5d)

dPAGE analysis of wild-type SARS-CoV-2 RNA cleavage product. The cleavage of wild-type SARS-CoV-2 RNA was carried out in 1× DCB according to the protocol described in A2.1. except that: 5' FAM-labeled RS-S253P-

WT/Mut, RS-I82T-WT/Mut, RS-S373P-WT/Mut, RS-Y4665H-WT/Mut, or RS-D5225-WT/Mut were used as the substrate of 10-23DZ-S253P-WT, 10-23DZ-I82T-WT, 10-23DZ-S373P-WT, 10-23DZ-Y4665H-WT, or 10-23DZ-D5225-WT.

DOES-CRISPR reaction. The procedure was carried out according to the protocol described in A2.1. except that: crRNA-S253P (crRNA-I82T, crRNA-S373P, crRNA-Y4665H, or crRNA-D5225), 10-23DZ-S253P-WT (10-23DZ-I82T-WT, 10-23DZ-S373P-WT, 10-23DZ-Y4665H-WT, or 10-23DZ-D5225-WT) and RS-S253P-WT/Mut (RS-I82T-WT/Mut, RS-S373P-WT/Mut, RS-Y4665H-WT/Mut, or RS-D5225-WT/Mut) were used.

Cas13a-based detection. The Cas13a-based detection was performed in the same way as the above description except that: 5 μL of 1 μM 10-23DZ-S253P-WT, 10-23DZ-I82T-WT, 10-23DZ-S373P-WT, 10-23DZ-Y4665H-WT, or 10-23DZ-D5225-WT was not used.

A2.8. Analysis of the SARS-CoV-2 gene in human throat swabs (Figure 6)

The genomic RNA (gRNA) was obtained from Dalian Municipal Central Hospital and analyzed according to the following steps.

Reverse transcription (step I): 10 μL of the obtained gRNA, 9 μL of nuclease-free water, 1.5 μL of 10mM dNTP, and 1.5 μL of 5 μM reverse primer (RP-S253P or RP-D5225) were mixed and heated at 65 for 5 min before being chilling on ice. To this mixture, 200 U (units) of M-Mulv Reverse Transcriptase and 30 U (units) of RNase inhibitor were added to a total volume of 30 μL and incubated at 42 °C for 20 min.

VPCR amplification (step II): VPCR: 5 μL of obtained cDNA (cDNA-Omicron or cDNA-XBB.1), 50 nM dNTPs, 0.1 U/μL of Taq II DNA Polymerase, 1 μM forward primer (FP-S253P or FP-D5225), and 1 μM reverse primer (RP-S253P or RP-D5225) were mixed in 50 μL of 1× VPB. The VPCR was carried out using the following procedure: 95 °C for 2 min, followed by 40 cycles of 95 °C for 1 s and 50 °C for 1 s with the ramp rate of 1.6 °C/s, 1 cycle of 72 °C for 5 min, and ending at 12 °C.

T7 transcription-DOES-CRISPR assay (step III): 1 μL of the dsDNA product was added to the T7 transcription-DOES-CRISPR reaction system and incubated at 37 °C for 30 min.

Section B: Supporting Tables and Figures.

B1. Supplementary Tables

Table S1. Sequences of DNA/RNA oligonucleotides used in this work.

Name of DNA/RNA oligonucleotide	Sequence (5′-3′)
RNA Substrate	
(FAM)-RS-AA	(FAM) ACU CAC UAU AGG GAG AGG CUG GGA UAA CUU UGU GGA
(FAM)-RS-AU	(FAM) ACU CAC UAU AGG GAG AGG CUG GGA UAU CUU UGU GGA
(FAM)-RS-AG	(FAM) ACU CAC UAU AGG GAG AGG CUG GGA UAG CUU UGU GGA
(FAM)-RS-AC	(FAM) ACU CAC UAU AGG GAG AGG CUG GGA UAC CUU UGU GGA
(FAM)-RS-GA	(FAM) ACU CAC UAU AGG GAG AGG CUG GGA UGA CUU UGU GGA
(FAM)-RS-GU	(FAM) ACU CAC UAU AGG GAG AGG CUG GGA UGU CUU UGU GGA
(FAM)-RS-GG	(FAM) ACU CAC UAU AGG GAG AGG CUG GGA UGG CUU UGU GGA
(FAM)-RS-GC	(FAM) ACU CAC UAU AGG GAG AGG CUG GGA UGC CUU UGU GGA
(FAM)-miR-17	(FAM) CAA AGU GCU UAC AGU GCA GGU AG
(FAM)-miR-20a	(FAM) UAA AGU GCU UAU AGU GCA GGU AG
(FAM)-miR-20b	(FAM) CAA AGU GCU CAU AGU GCA GGU AG
(FAM)-let-7a	(FAM) UGA GGU AGU AGG UUG UAU AGU U
(FAM)-let-7b	(FAM) UGA GGU AGU AGG UUG UGU GGU U
(FAM)-let-7c	(FAM) UGA GGU AGU AGG UUG UAU GGU U
(FAM)-let-7d	(FAM) AGA GGU AGU AGG UUG CAU AGU U
(FAM)-let-7e	(FAM) UGA GGU AGG AGG UUG UAU AGU U
(FAM)-RS-CYP-WT	(FAM) UUU GUG UCU UCU GUU CUC AAA GCA UCU CUG AUG UAA GAG A
(FAM)-RS-CYP-Mut	(FAM) UUU GUG UCU UCU GUU CUC AAA GUA UCU CUG AUG UAA GAG A
(FAM)-RS-Y4665H-WT	(FAM) AGU GGG AUU UGU UAA AAU AUG ACU UCA CGG AAG AGA GGU U
(FAM)-RS-Y4665H-Mut	(FAM) AGU GGG AUU UGU UAA AAC AUG ACU UCA CGG AAG AGA GGU U
(FAM)-RS-D5225-WT	(FAM) UGU ACC UUC CUU ACC CAG AUC CAU CAA GAA UCC UAG GGG C
(FAM)-RS-D5225-Mut	(FAM) UGU ACC UUC CUU ACC CAG ACC CAU CAA GAA UCC UAG GGG C
(FAM)-RS-S373P-WT	(FAM) UGU CCU AUA UAA UUC CGC AUC AUU UUC CAC UUU UAA GUG U
(FAM)-RS-S373P-Mut	(FAM) UGU CCU AUA UAA UUC CGC ACC AUU UUC CAC UUU UAA GUG U
(FAM)-RS-S253P-WT	(FAM) UCA CAC AAU CGA CGG UUC AUC CGG AGU UGU UAA UCC AGU A
(FAM)-RS-S253P-Mut	(FAM) UCA CAC AAU CGA CGG UUC ACC CGG AGU UGU UAA UCC AGU A
(FAM)-RS-I82T-WT	(FAM) CAC CGG UGG AAU UGC UAU CGC AAU GGC UUG UCU UGU AGG C
(FAM)-RS-I82T-Mut	(FAM) CAC CGG UGG AAU UGC UAC CGC AAU GGC UUG UCU UGU AGG C
DNASubstrate	
DS-CYP-WT	CTA AAA CAA AGT TTT AGC AAA CGA TTT TTT TTT TCA AAT TTG TGT CTT CTG TTC TCA AAG CAT CTC TGA TGT AAG AGA TAA TGC GCC ACG ATG GGC ATC AGA AGA CCT CA
DS-CYP-Mut	CTA AAA CAA AGT TTT AGC AAA CGA TTT TTT TTT TCA AAT TTG TGT CTT CTG TTC TCA AAG TAT CTC TGA TGT AAG AGA TAA TGC GCC ACG ATG GGC ATC AGA AGA CCT CA
10-23 DNAzyme	
10-23DZ1	CCA CAA AGA GGC TAG CTA CAA CGA ATC CCA GCC
10-23DZ2	CCA CAA AGG GGC TAG CTA CAA CGA ATC CCA GCC
10-23DZ-miR	CCT GCA CTA GGC TAG CTA CAA CGA AAG CAC TTT
10-23DZ-let	ATA CAA CCT AGG CTA GCT ACA ACG ATA CCT CA
10-23DZ-CYP-Mut	TCA GAG ATA GGC TAG CTA CAA CGA TTT GAG AAC
10-23DZ-Y4665H-WT	GAA GTC ATA GGC TAG CTA CAA CGA TTT AAC AAA
10-23DZ-D5225-WT	CTT GAT GGA GGC TAG CTA CAA CGA CTG GGT AAG
10-23DZ-S373P-WT	GGA AAA TGA GGC TAG CTA CAA CGA GCG GAA TTA
10-23DZ-S253P-WT	AAC TCC GGA GGC TAG CTA CAA CGA GAA CCG TCG
10-23DZ-I82T-WT	CCA TTG CGA GGC TAG CTA CAA CGA AGC AAT TCC
crRNA	
crRNA-CY1* (Y1 = G, U, C)	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AAC AAA GY_1*C AUC CCA GCC UCU
crRNA-UY2* (Y2 = G, U, C)	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AAC AAA GY_2*U AUC CCA GCC UCU
crRNA-CY3* (Y3 = A, U, C)	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AAC AAA GY_3*C AUC CCA GCC UCU
crRNA-miR-17	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC ACU ACC UGC ACU GUA AGC AC

crRNA-let-7e	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AAA CUA UAC AAC CUC CUA CCU
crRNA-CYP-WT	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AUU ACA UCA GAG AUG CUU UGA
crRNA-Y4665H	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AUC CGU GAA GUC AUG UUU UAA
crRNA-D5225	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AGG AUU CUU GAU GGG UCU GGG
crRNA-S373P	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AAA AUG GUG CGG AAU UAU AUA
crRNA-S253P	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AUU AAC AAC UC C GGG UGA ACC
crRNA-I82T	GAC CAC CCC AAA AAU GAA GGG GAC UAA AAC AUU GCG GUA GCA AUU CCA CCG
PCR Primer	
Forward Primer-CYP	GAA TTC TAA TAC GAC TCA CTA TAG GGT TTG TGT CTT CTG TTC TC
Reverse Primer-CYP	GCG CAT TAT CTC TTA CAT
Forward Primer-S373P	GAA TTC TAA TAC GAC TCA CTA TAG GGC TGA TTA TTC TGT CCT ATAT
Reverse Primer-S373P	
Forward Primer-D5225	TAA AAG TGG AAA ATG ATG C
Reverse Primer-D5225	GAA TTC TAA TAC GAC TCA CTA TAG GGG TAC CTT CCT TAC CCA GAT
FQ-ssRNA Reporter	TAT CAT CTA CAA AAC AGC C UUUUU (5’ 6-FAM, 3’ Dabcyl)

Table S2. Comparison of the performance of DOES-CRISPR with CRISPR-based nucleic acid detection.

Targets	Sensitivity	Assay time	Ref.
RNA	90 fM	4 h	[1]
RNA	2.8 fM	3 h	[2]
DNA	40 copies/μL	3-4 h	[3]
DNA	100 copies/μL	45 min	[4]
RNA	5 pM	15 min	This work
DNA	10 copies/μL	55 min	This work

B2. Supplementary Figures

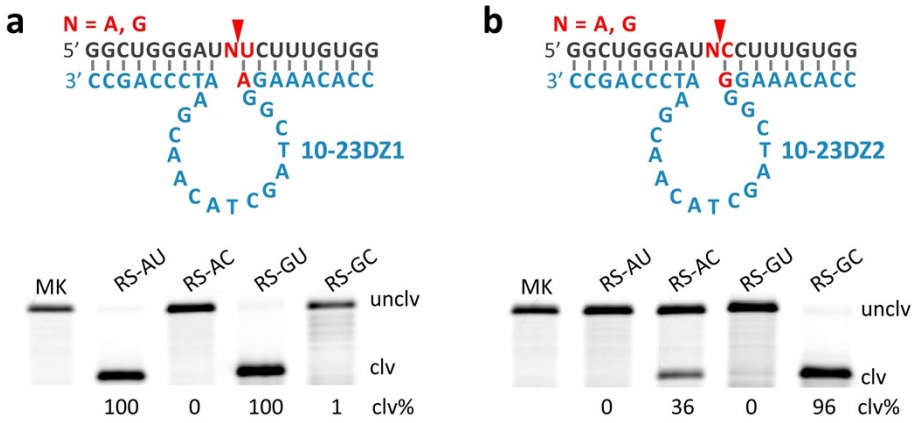

Fig. S1. 10% dPAGE analysis of the cleavage of 5' FAM-labeled a) RS-NU or b) RS-NC substrate (N = A or G) by 10-23DZs. MK = marker, unclv = uncleaved, clv = cleaved.

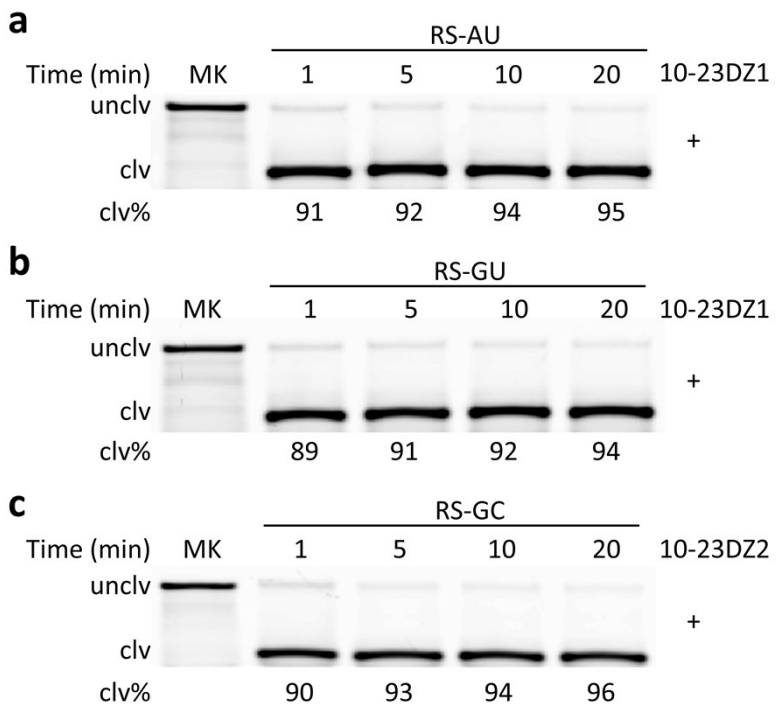

Fig. S2. 10% dPAGE analysis of the kinetic responses of 10-23DZ1 to (a) RS-GU, (b) RS-AU, and 10-23DZ2 to (c) RS-GC substrate. MK = marker, unclv = uncleaved, clv = cleaved.

Experimental details: The protocol is similar to the 10-23 DNAzyme-induced cleavage of RNA substrate in **A2.1.** except for the reaction mixtures were incubated at 37 °C for specified time points (1, 5, 10, and 20 min) and stopped by the addition of 10 µL of 2 × urea PAGE loading buffer.

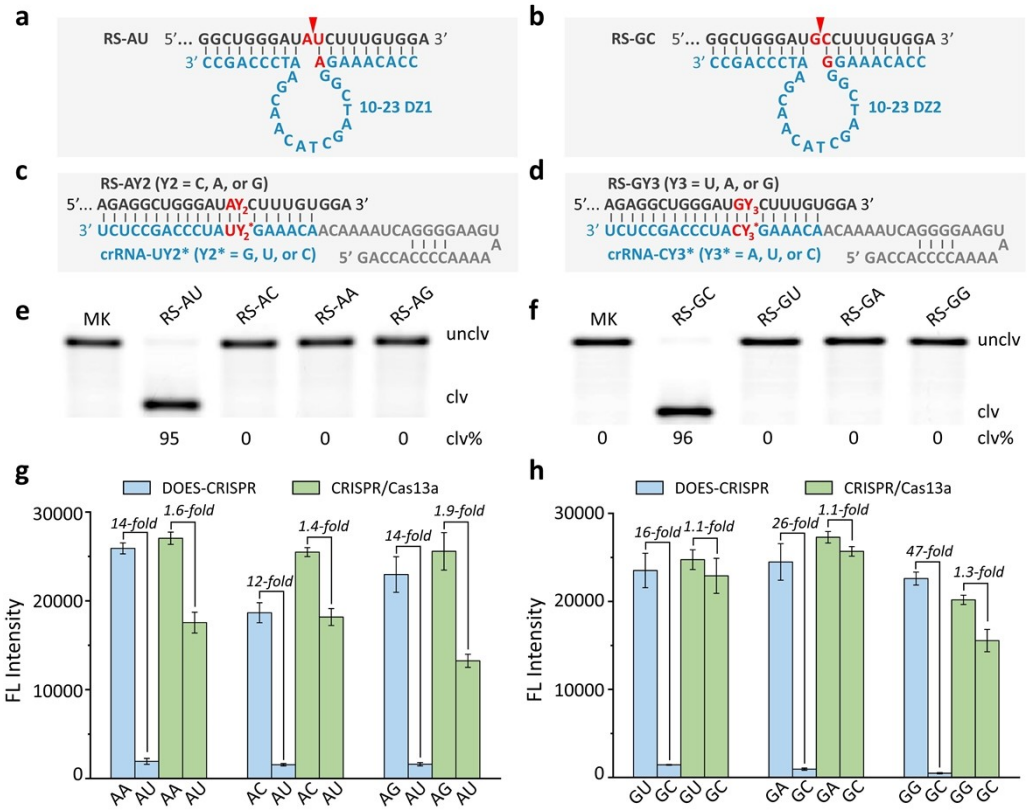

Fig. S3. Sequences of (a) RS-AU and 10-23DZ1, (b) RS-GC and 10-23DZ2, (c) RS-AY2 (Y2 = C, A, or G) and crRNA-UY2* (Y2* = G, U, or C), (d) RS-GY3 (Y3 = U, A, or G) and crRNA-CY3* (Y3* = A, U, or C). Cleavage occurs at the position indicated by the arrow. 10% denaturing (8 M urea) polyacrylamide gel electrophoresis (dPAGE) analysis of (e) the cleavage of 5' FAM-labeled RS-AU and RS-AY2 by 10-23DZ1, and (f) the cleavage of 5' FAM-labeled RS-GC and RS-GY3 by 10-23DZ2. MK = marker, unclv = uncleaved, clv = cleaved. Fluorescence responses of DOES-CRISPR and CRISPR/Cas13a toward (g) RS-AY2 when using RS-AU as the off-target RNA, and (h) RS-GY3 when using RS-GC as the off-target RNA. The error bars represent standard deviations of three independent experiments.

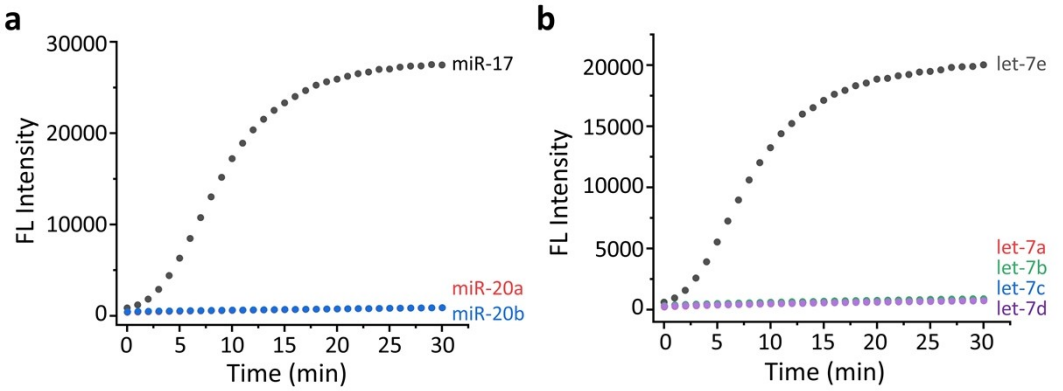

Fig. S4. Real-time fluorescence monitoring of FQ-ssRNA cleavage by DOES-CRISPR-based (a) miR-17 detection in the presence of miR17 or miR-20X (X = a or b), and (b) let-7e detection in the presence of let-7e or let-7Z (Z = a, b, c, or d).

Experimental details: For the time-course analysis of FQ-ssRNA cleavage, the protocol is similar to the DOES-CRISPR reaction for miRNA detection in **A2.4.** except for the fluorescence intensity was measured for 30 min at 37 °C using a microplate reader with fluorescence measurements taken every minute.

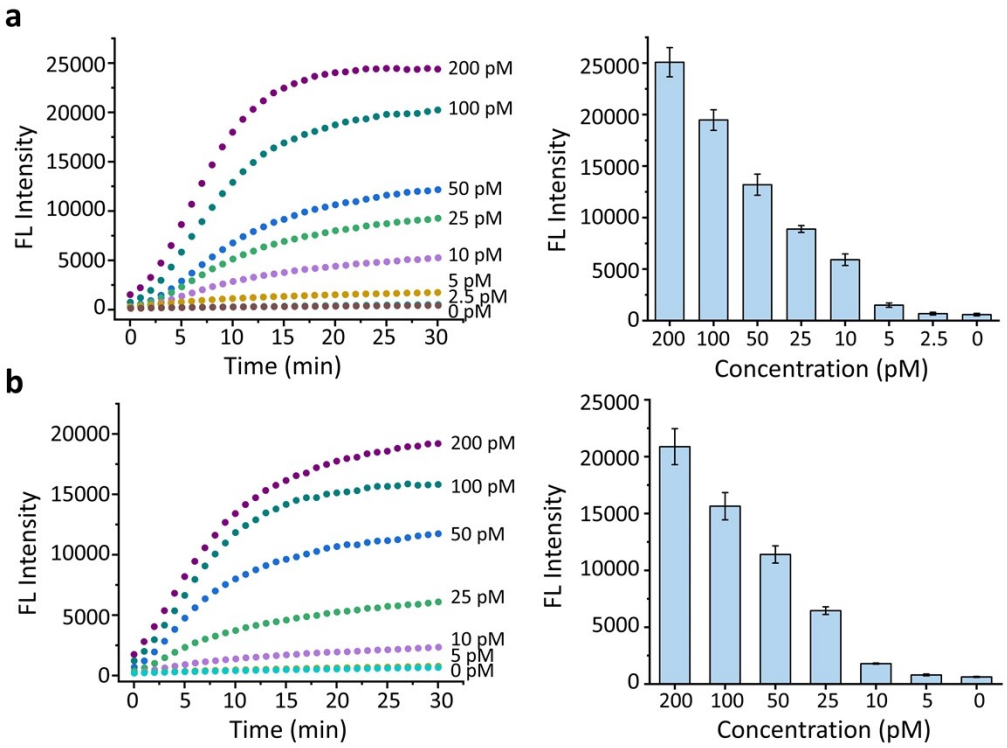

Fig. S5. Real-time fluorescence monitoring and fluorescence intensity measurement of FQ-ssRNA cleavage by DOES-CRISPR at various concentrations (pM) of (a) miR-17 and (b) let-7e. All experiments and assays were repeated at least three times. The data were expressed as mean ± s.d.

Experimental details: For the sensitivity analysis, the protocol is similar to the DOES-CRISPR reaction for miRNA detection in **A2.4.** except for 0, 2.5, 5, 10, 25, 50, 100, and 200 pM miR-17 (or let-7e) were used.

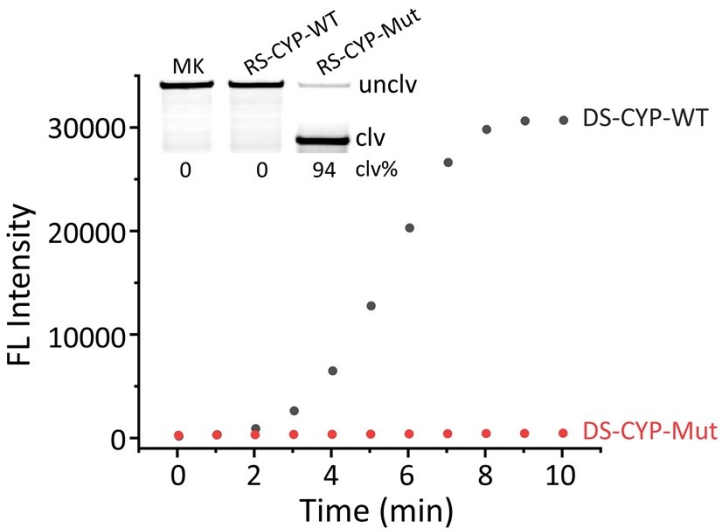

Fig. S6. Real-time fluorescence monitoring of FQ-ssRNA cleavage by VPCR-DOES-CRISPR-based wide-type CYP2C19*17 gene detection in the presence of DS-CYP-WT or DS-CYP-Mut. The inset shows the cleavage of fluorophore-labeled RS-CYP-WT and RS-CYP-Mut. MK = marker, unclv = uncleaved, clv = cleaved.

Experimental details: For the time-course analysis of FQ-ssDNA cleavage, the protocol is similar to the one described in **A2.5.** except for the fluorescence intensity was measured for 10 min at 37 °C using a microplate reader with fluorescence measurements taken every minute.

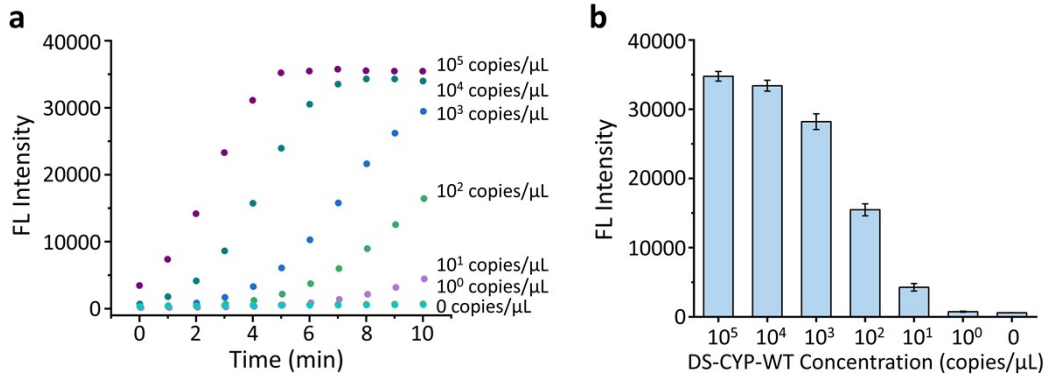

Fig. S7. (a) Real-time fluorescence monitoring and (b) fluorescence intensity measurement of FQ-ssRNA cleavage by VPCR-DOES-CRISPR at various DS-CYP-WT concentrations (copies/μL).

Experimental details: For the time-course analysis of FQ-ssDNA cleavage and sensitivity analysis, the protocol is similar to the one described in **A2.5.** except that: the fluorescence intensity was measured for 10 min at 37 °C using a microplate reader with fluorescence measurements taken every minute, and 0, 10^0, 10^1, 10^2, 10^3, 10^4, and 10^5 copies/μL DS-CYP-WT were used.

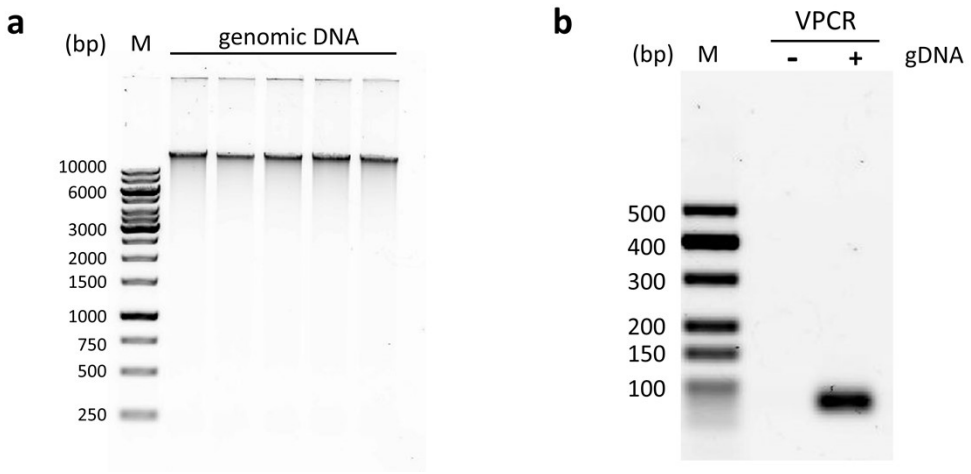

Fig. S8. (a) 1% Agarose gel electrophoresis analysis of the extracted genomic DNA from clinical blood samples. (b) 2% Agarose gel electrophoresis analysis of the VPCR-amplified products of gDNA extracted from clinical blood samples.

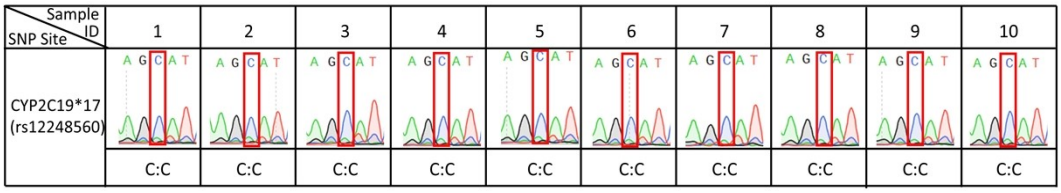

Fig. S9. Sanger sequencing analysis of C806T mutation in the gDNA from clinical whole blood samples.

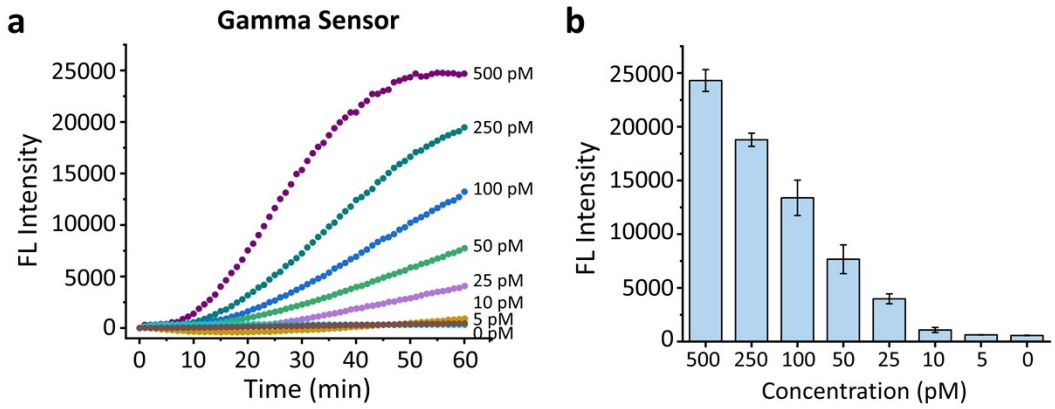

Fig. S10. (a) Kinetics and (b) quantification of fluorescence intensity in response to different concentrations of synthetic standard RS-S253P-Mut. All experiments and assays were repeated at least three times. The data were expressed as mean ± s.d.

Experimental details: For the time-course analysis of FQ-ssRNA cleavage and sensitivity analysis, the protocol is similar to the one described in **A2.7.** except for: the fluorescence intensity was measured for 60 min at 37 °C using a microplate reader with fluorescence measurements taken every minute, and 0, 5, 10, 25, 50, 100, 250, and 500 pM RS-S253P-Mut were used.

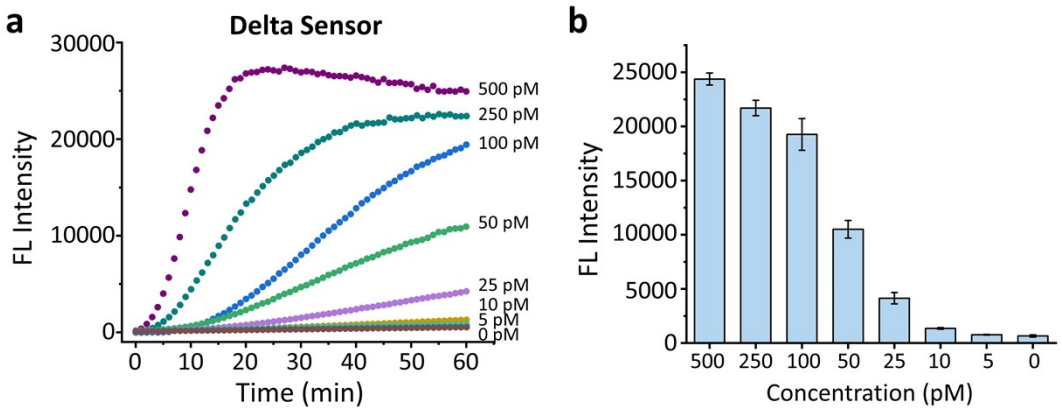

Fig. S11. (a) Kinetics and (b) quantification of fluorescence intensity in response to different concentrations of synthetic standard RS-I82T-Mut. All experiments and assays were repeated at least three times. The data were expressed as mean ± s.d.

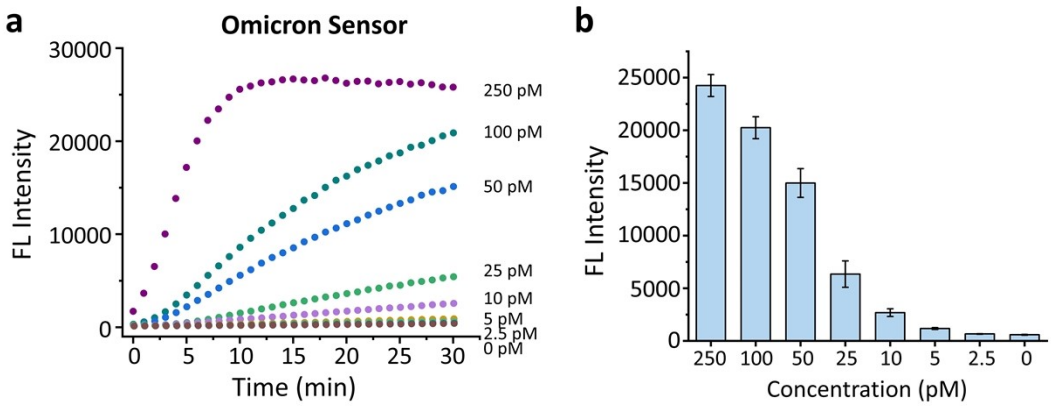

Fig. S12. (a) Kinetics and (b) quantification of fluorescence intensity in response to different concentrations of synthetic standard RS-S373P-Mut. All experiments and assays were repeated at least three times. The data were expressed as mean ± s.d.

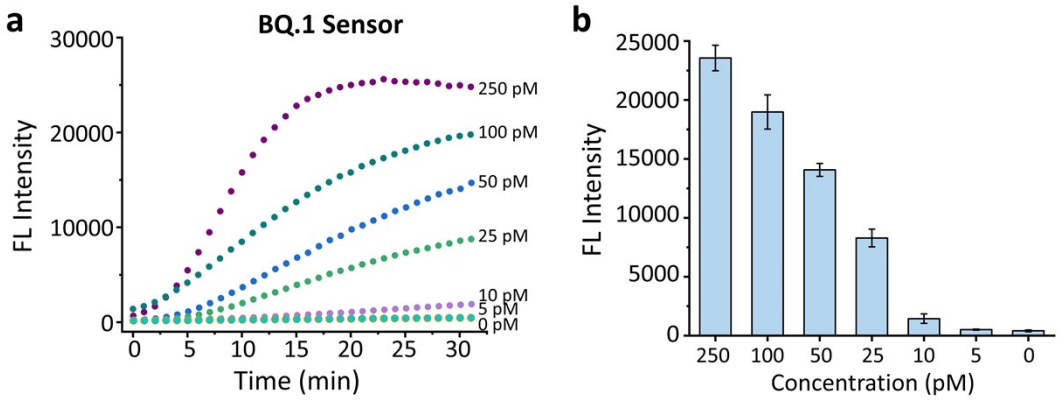

Fig. S13. (a) Kinetics and (b) quantification of fluorescence intensity in response to different concentrations of synthetic standard RS-Y4665H-Mut. All experiments and assays were repeated at least three times. The data were expressed as mean ± s.d.

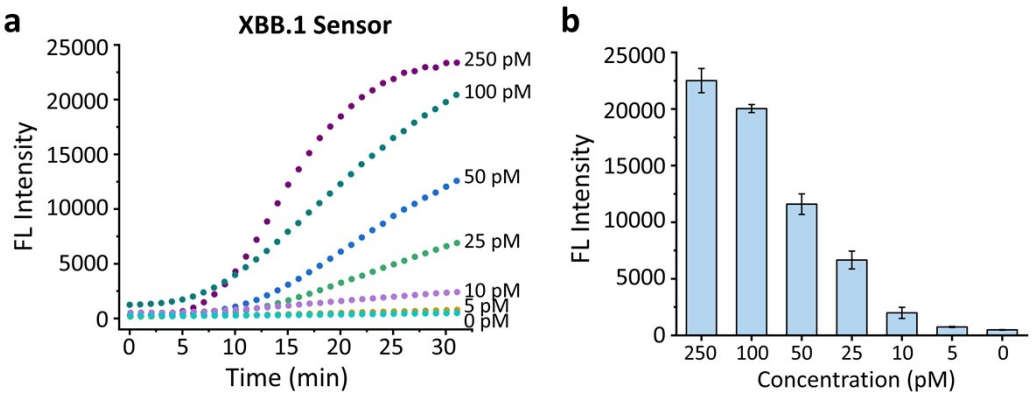

Fig. S14. (a) Kinetics and (b) quantification of fluorescence intensity in response to different concentrations of synthetic standard RS-D5225-Mut. All experiments and assays were repeated at least three times. The data were expressed as mean ± s.d.

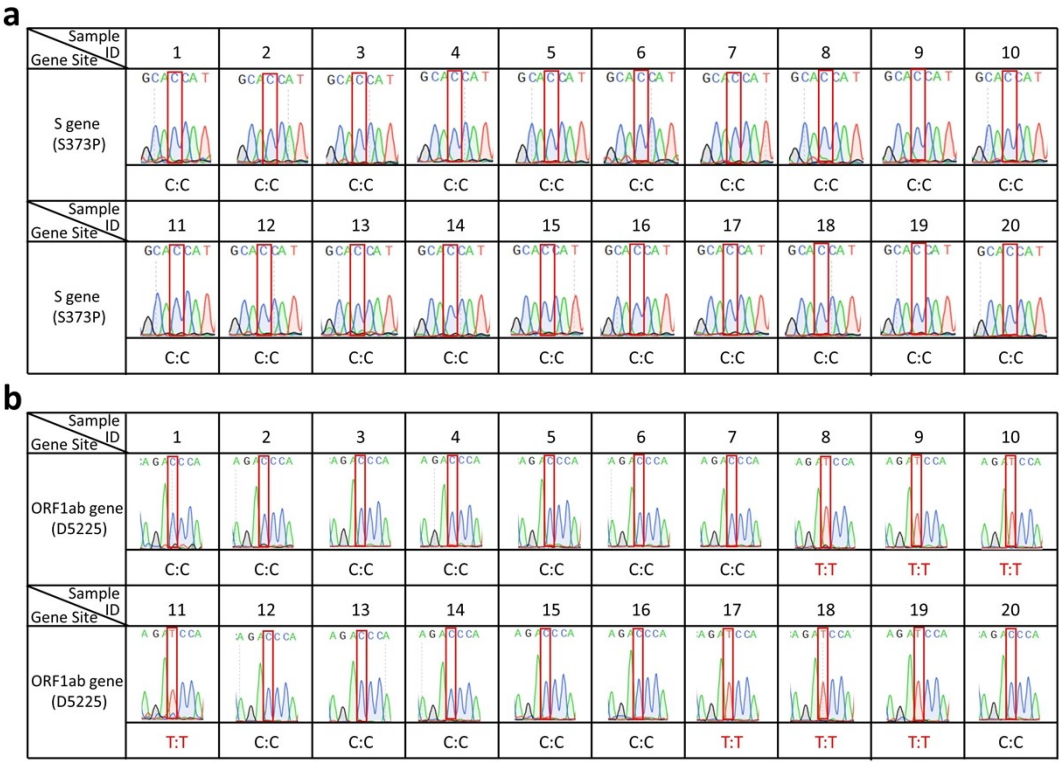

Fig. S15. Sanger sequencing analysis of (a) the S373P site in S gene and (b) the D5225 site in ORF1ab gene from clinical throat swabs.

References

[1] R. Wang, X. Zhao, X. Chen, X. Qiu, G. Qing, H. Zhang, L. Zhang, X. Hu, Z. He, D. Zhong, Y. Wang and Y. Luo, *Anal. Chem.*, 2020, **92**, 2176-2185.
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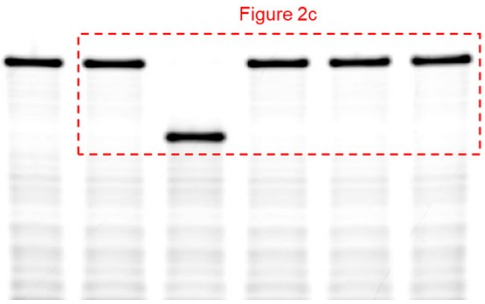
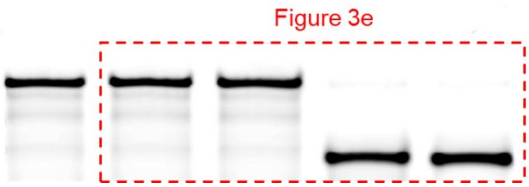
[3] Q. Wang, B. Zhang, X. Xu, F. Long and J. Wang, *Sci. Rep.*, 2018, **8**, 1-13.
[4] Y. Wu, Y. Liu, Y. Chang and M. Liu, *Anal. Chem.*, 2023, **95**, 10127-10135.

Uncropped gel images



Uncropped PAGE gel for Figure 2c. The red box indicates the region presented in Figure 2c.



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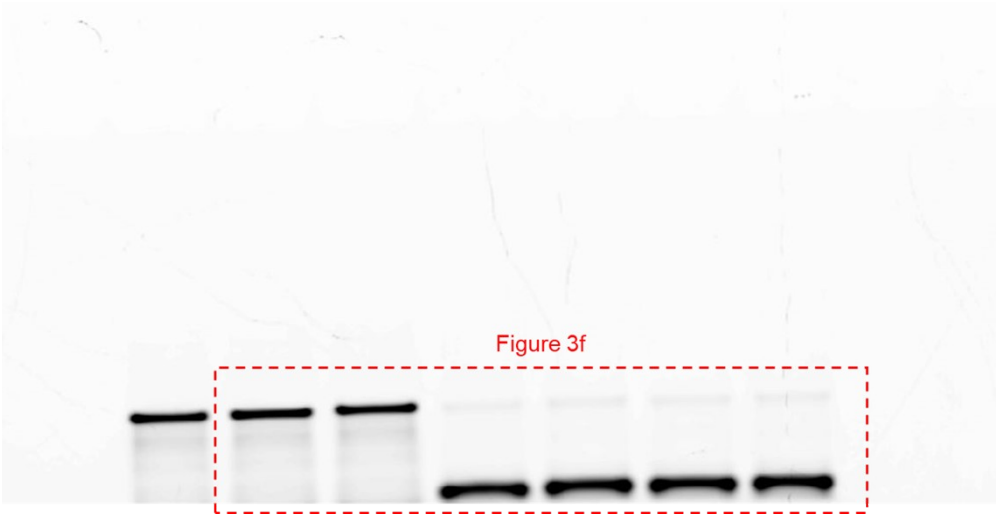
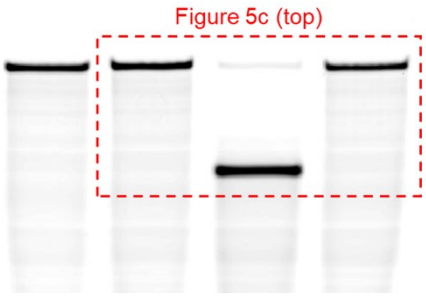
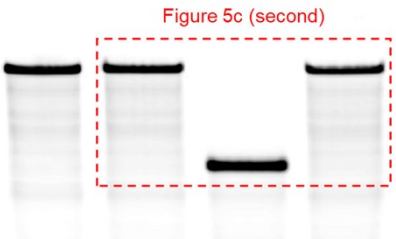


Uncropped PAGE gel for Figure 3f. The red box indicates the region presented in Figure 3f.



Uncropped PAGE gel for Figure 5c (top). The red box indicates the region presented in Figure 5c (top).



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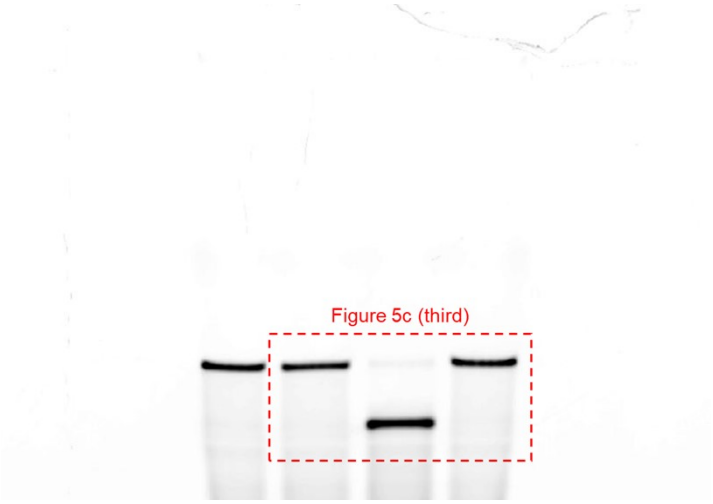
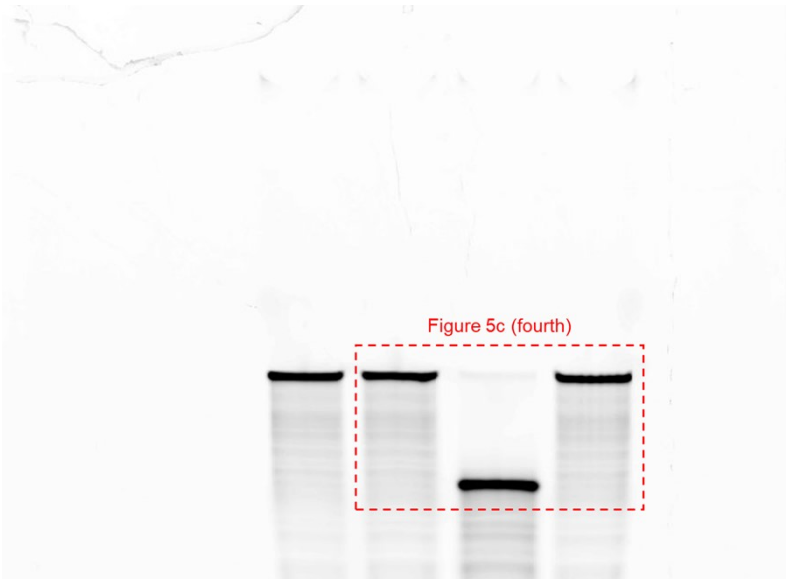


Uncropped PAGE gel for Figure 5c (third). The red box indicates the region presented in Figure 5c (third).



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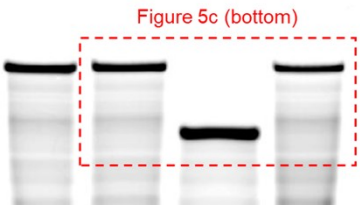
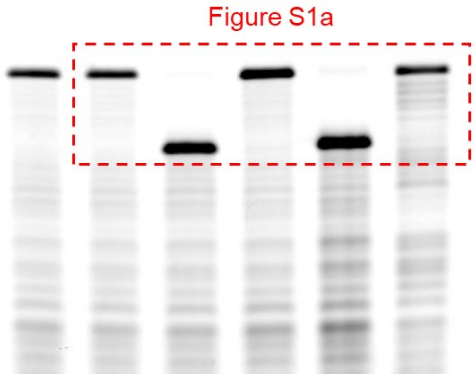
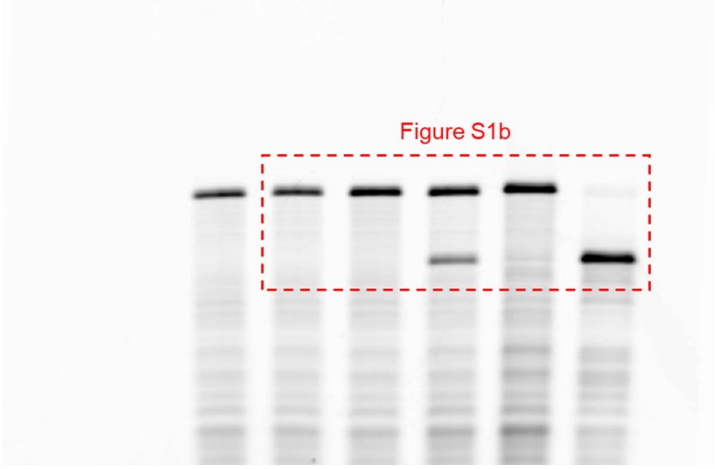


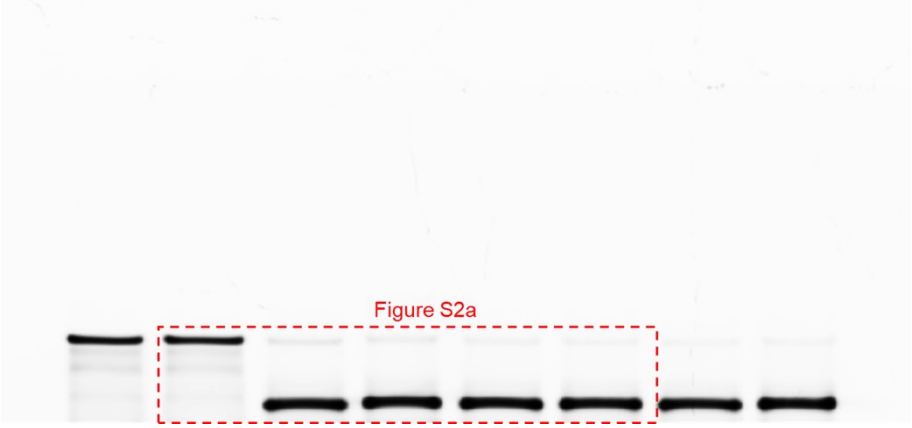
Uncropped PAGE gel for Figure 5c (bottom). The red box indicates the region presented in Figure 5c (bottom).



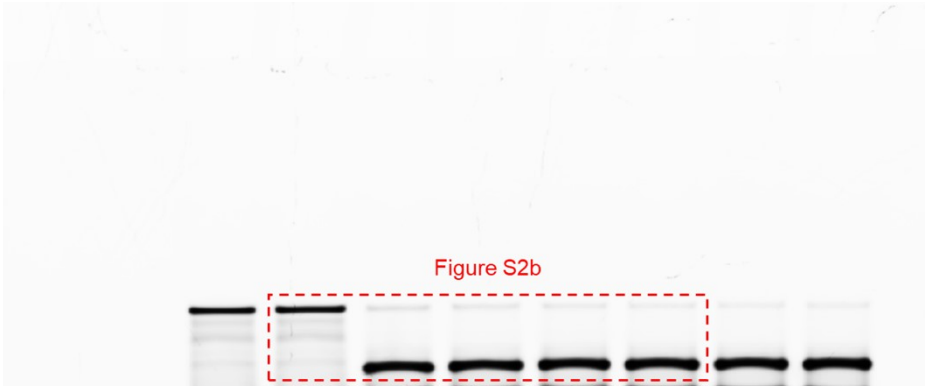
Uncropped PAGE gel for Figure S1a. The red box indicates the region presented in Figure S1a.



Uncropped PAGE gel for Figure S1b. The red box indicates the region presented in Figure S1b.

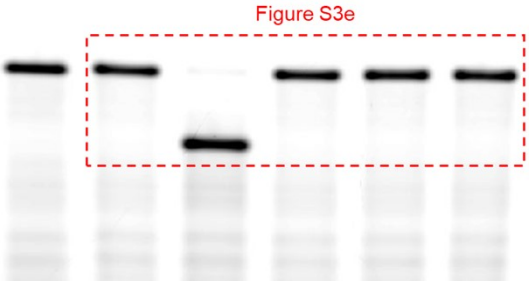


Uncropped PAGE gel for Figure S2a. The red box indicates the region presented in Figure S2a.

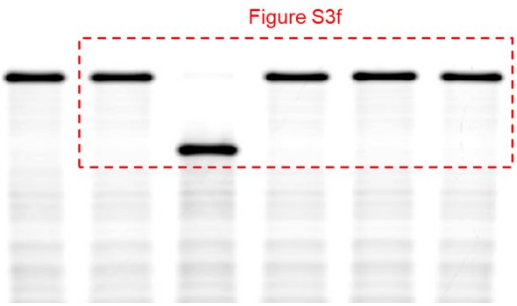


Uncropped PAGE gel for Figure S2b. The red box indicates the region presented in Figure S2b.

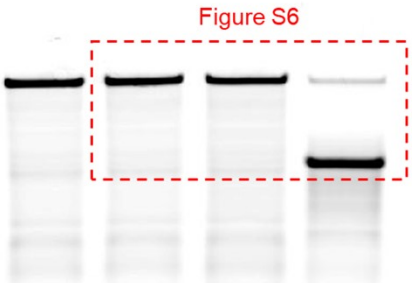
Uncropped PAGE gel for Figure S2c. The red box indicates the region presented in Figure S2c.



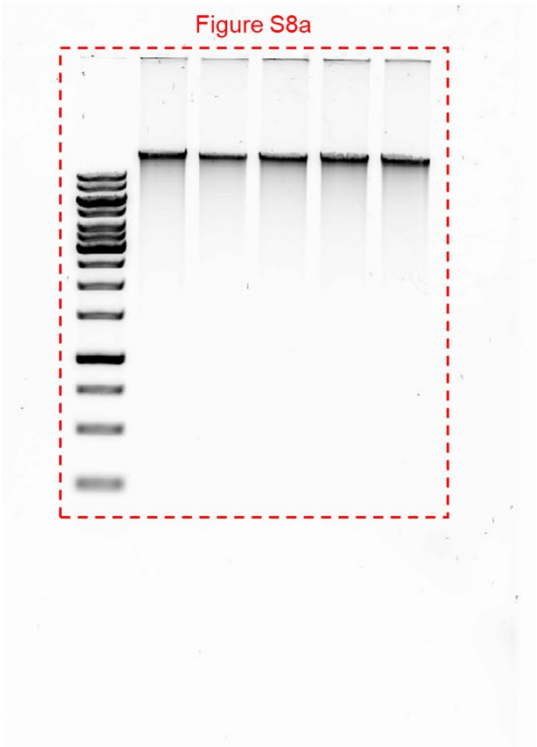
Uncropped PAGE gel for Figure S3e. The red box indicates the region presented in Figure S3e.



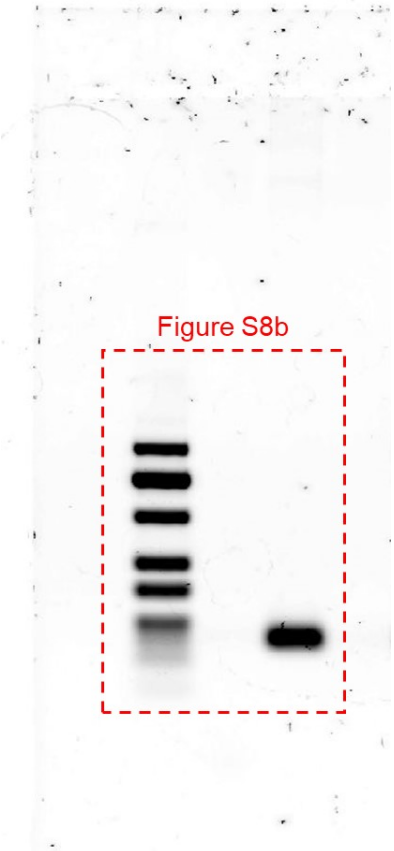
Uncropped PAGE gel for Figure S3f. The red box indicates the region presented in Figure S3f.



Uncropped PAGE gel for Figure S6. The red box indicates the region presented in Figure S6.



Uncropped PAGE gel for Figure S8a. The red box indicates the region presented in Figure S8a.



Uncropped PAGE gel for Figure S8b. The red box indicates the region presented in Figure S8b.