Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

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Results

Here we describe in more detail the rationale and results that contributed to the development of STOPCovid. We first simplified the viral RNA extraction method. Most clinical labs currently perform an RNA extraction step using commercial RNA extraction kits, which requires multiple fluid handling steps and relies on reagents that have been difficult to obtain steady supplies of. To eliminate this step, we tested various viral lysis buffers for extraction of lentiviral RNA and added the lysate directly into RT-qPCR reactions (Fig. S1A and S1B). We found that addition of DNA QuickExtract to COVID-19 positive patient nasopharyngeal (NP) swab viral transport media (VTM) followed by heating at 95 ℃ for 5 mins was comparable to the standard QIAmp Viral RNA Miniprep extraction (Fig. S1C).

Integrating the isothermal amplification step with the CRISPR-mediated detection step required developing an optimal common reaction chemistry capable of supporting both steps (Fig. S1D). To amplify the viral RNA, we chose reverse transcription followed by Loop-mediated isothermal amplification (RT-LAMP) [16]. While we have focused on Recombinase polymerase amplification [22] in the past [1,2,5], LAMP reagents are readily available from multiple commercial suppliers, are easily multiplexed [23], and rely on defined buffers that are amenable to optimization with Cas enzymes. We designed 29 sets of LAMP primers targeting different regions of the SARS-CoV-2 genome and identified 6 primer sets targeting genes ORF1ab, S, and N that were capable of SARS-CoV-2 RNA detection (Fig. S1E).

LAMP operates at 55-65 ℃, requiring a thermostable Cas enzyme for a one-pot chemistry, such as Cas12b from *Alicyclobacillus acidiphilus* (AapCas12b) [17]. The AapCas12b locus does not contain an identifiable CRISPR array, but it can function up to 65 ℃ with a single guide RNA (sgRNA) based on the direct repeat from *Alicyclobacillus macrosporangiidus* Cas12b (AmCas12b) [17](Fig. S2A). Replacing the AmCas12b sgRNA with an sgRNA from *Alicyclobacillus acidoterrestris* Cas12b (AacCas12b) (Shmakov et al., 2015), which is highly similar to AapCas12b, produced more robust and specific nuclease activity, possibly due to the stronger hairpin formed near the 3' end of the sgRNA (Fig. S2B and S2C).

We next systematically evaluated all possible spacers targeting our top LAMP amplicons to identify the ideal combination of primers and guide sequence (Fig. S2D). For the top 4 combinations of LAMP primer set and guide, we titrated the concentration of magnesium in a one-pot reaction to find the best combination of buffer, primer set, and guide, yielding an assay targeting gene N (Fig. S2E). For this assay, we found that all 6 LAMP primers and spacer targeting regions were perfectly conserved in at least 99.5% of >31,000 sequenced human SARS-CoV-2 genomes [24].

Using these conditions, we tested both AapCas12b and AacCas12b in a one-pot reaction. We found that AapCas12b generated faster (34 min to half maximum signal compared to 39 min) and stronger collateral activity (1.6 times maximum signal) than AacCas12b in the one-pot reaction (Fig. S3A). We note that although AacCas12b has been reported to work with LAMP in one-pot assays at 55℃ [25], it did not provide adequate activity in our assay at 60 ℃, a temperature more optimally suited for LAMP, possibly because it is unstable at higher temperatures. We further optimized the AapCas12b one-pot reaction by screening 94 additives to improve thermal stability (Fig. S3B), finding that addition of taurine improved reaction kinetics (Fig. S3C and S3D). We termed this optimized one-pot assay STOP and its application for SARS-CoV-2 detection STOPCovid. As expected, STOPCovid detection produces signal only when the target is present, whereas LAMP alone (either as a stand-alone reaction or in the STOPCovid mastermix) can produce nonspecific signal (Fig. S3E), highlighting the additional specificity provided by CRISPR detection.

We used STOPCovid.v1 with a lateral flow readout and SARS-CoV-2 genome standards spiked into pooled healthy saliva or NP swab VTM to determine the sensitivity, robustness, ideal incubation temperature, and readout time of the assay. We found that the STOPCovid.v1 chemistry can detect 100 copies of SARS-CoV-2 per reaction and reproducibly detect 200 copies over 30 replicates (Fig. S4A and S4B). STOPCovid.v1 detection was stochastic below 100 copies per reaction and produced variable signal levels between replicates, potentially resulting from variability in the timing of LAMP (Fig. S4A and S4B). The ideal incubation parameters were 60 ℃ for at least 50 minutes for lateral flow, though longer incubation times do not affect the results (Fig. S4C and S4D). The reaction components could be formulated as a mastermix, which maintained functionality after 6 freeze-thaw cycles (Fig. S4E). The assay exhibited no cross-reactivity with the SARS-CoV or MERS-CoV genomes (Fig. S4F) and could be performed using either a standard heat block or via a water bath maintained by a commercially-available low-cost (under $40USD) sous-vide cooker (Fig. S4G and S4H).

In addition to lateral flow readout, STOPCovid.v1 is also compatible with fluorescence readout, which allows for simultaneous detection of an internal control using orthogonal fluorescent dyes. By introducing LAMP primer sets targeting an abundant control gene into the STOPCovid reaction, LAMP amplification signal detected by SYTO 9 nucleic acid stain before 28 minutes indicates specific amplification of human sample, while Cas12b collateral cleavage of a HEX reporter probe indicates presence of SARS-CoV-2. We screened 114 LAMP primers sets targeting *ACTB* and *GAPDH* to identify 19 LAMP primer sets that could be multiplexed with the N LAMP primer set at 50% or 20% of the total primer set concentration (Fig. S5). The best control LAMP primer set, *ACTB* Set 1, could be multiplexed with the N gene LAMP primer set at 20% of the total primer amount (Fig. S5). Using SARS-CoV-2 genome standards spiked into pooled healthy saliva or NP swabs, we determined the multiplex reaction is sensitive down to 200 copies of SARS-CoV-2 genome per reaction (Fig. S6A through S6D). Our assay detected

30/30 replicates at 300 genomic copies and 27/30 replicates at 200 genomic copies (Fig. S6E and S6F). Similar to lateral flow, the fluorescent multiplexed assay was most robust at 60℃ (Fig. S6G and S6H).

We then evaluated STOPCovid.v1 with lateral flow readout on 43 SARS-CoV-2 RNA positive (Ct values ranging from 17 to 38) and 15 negative patient NP swab VTM samples. STOPCovid.v1 had a sensitivity of 84% and specificity of 100% for detecting 2 out of 3 replicates for the 43 positive samples (Fig. S7A through S7C). Samples that tested false negative with STOPCovid.v1 had RT-qPCR Ct values above 32 (Fig. S7A). Parallel work from the Virology Lab at the University of Washington on 19 positive and 7 negative patient samples showed that STOPCovid.v1 had a sensitivity of 84% and specificity of 100% (Fig. S7D). Patient sample dilution series revealed that STOPCovid.v1 sensitivity comparable to RT-qPCR Ct value of 30.8 (Fig. S7E). In addition, we found that lysis at a lower temperature (60℃ or 22℃) is a viable alternative for streamlining the STOPCovid.v1 assay workflow, albeit with a slight decrease in sensitivity (increased Ct value of 0.8/0.2 at 60 ℃ and 1.1/0.9 for 22 ℃ for CDC N1/N2 probe sets) (Fig. S8A through S8C). Together, these results demonstrate the feasibility of detecting SARS-CoV-2 without RNA extraction in a one-pot reaction, but based on the distribution of Ct values for 2,105 SARS-CoV-2 positive patients (Fig. S8D), STOPCovid.v1 would only capture 60.5 % of RT-qPCR-positive patients.

We therefore sought to boost sensitivity by increasing sample input via sample concentration. In our initial attempt to simplify viral RNA extraction, we only used 2.5 µL of the 3 mL NP swab VTM (0.083 % of the total NP swab sample). We reasoned that capturing all of the viral RNA from an NP or anterior nasal (AN) swab would increase sample input by 1,200-fold and dramatically increase sensitivity. We tested sample concentration using two magnetic bead formulations, laboratory-developed [26] and commercial (Beckman SPRIselect), prior to STOPCovid detection. We found that both formulations could efficiently capture viral RNA (SARS-CoV-2 genomic standards spiked into DNA QuickExtract lysis buffer with human background RNA) into one 50 µL STOPCovid reaction using the standard magnetic bead purification method (Fig. S9A). In addition, we determined we could elute the sample from the beads by directly adding STOPCovid mastermix to the magnetic beads without a separate water elution step (Fig. S9A). As the laboratory-developed magnetic beads are significantly cheaper ($0.20USD/reaction compared to $6.13USD/reaction) and easier to modify than the commercial version, we moved forward with the laboratory-developed magnetic beads. We found that introducing the bead concentration step increased sensitivity, enabling detection of as little as 100 viral copies per sample compared to 5,000 copies per sample without this step (Fig. S9B).

To streamline the magnetic bead concentration workflow, we eliminated the ethanol wash step and combined the lysis and magnetic bead binding steps by optimizing the salt concentration of the one-pot reaction and testing alternative QuickExtract formulations (Fig. S9C through S9E).

Using SARS-CoV-2 virus-like particles (SARS-CoV-2 RNA targets in a noninfectious viral coat), we found that Plant QuickExtract combined with magnetic bead binding in a KCl buffer produced the best results in lieu of any washing steps, further simplifying extraction to a total of 15 minutes with minimal hands on time (Fig. S9E). We refer to this streamlined workflow as STOPCovid.v2 (Fig. 1A).

We further optimized the STOPCovid.v2 chemistry by titrating Cas12b and sgRNA concentrations. While characterizing STOPCovid.v2, we noticed that STOPCovid.v2 occasionally produced false positive results. We hypothesized that this was due to partial overlap between the sgRNA and LAMP BIP primer that contributed to sporadic Cas12b collateral activity. We therefore titrated Cas12b and sgRNA concentrations and found that the optimal concentration of Cas12b and sgRNA for reducing the proportion of false positive results while retaining true positive signal was 31.3 nM (Fig. S10A). We verified that this concentration of Cas12b and sgRNA maintained the same sensitivity (Fig. S10B).

We compared STOPCovid.v2 to the standard CDC workflow that uses QIAmp Viral RNA Miniprep extraction followed by RT-qPCR (Fig. S10C). Starting with different concentrations of SARS-CoV-2 virus-like particles, we performed a direct comparison of the two assay workflows. We confirmed that the CDC SARS-CoV-2 RT-qPCR protocol could reliably detect down to 1,000 copies per mL of VTM, the reported limit of detection (LOD) of the assay (https://www.fda.gov/media/134922/download). By contrast, STOPCovid.v2 was able to reliably detect viral loads as low as 100 copies per sample (equivalent to 33 copies per mL VTM) for the fluorescence readout and 250 copies (equivalent to 83 copies per mL VTM) for the lateral flow readout. We typically detect positive signal in less than 45 minutes by fluorescence read out and 80 minutes by lateral flow. Introduction of the magnetic bead concentration step in STOPCovid.v2 allowed for detecting viral RNA from the entire swab sample, or 600-fold higher input, than the CDC RT-qPCR workflow. As a result, STOPCovid.v2 could reliably detect 30-fold lower viral load than the CDC RT-qPCR workflow (100 copies per sample, or 33 copies per mL, compared to 1,000 copies per mL). These results suggest that though the STOPCovid.v2 reaction is 20 times less sensitive than the RT-qPCR reaction itself (detects 100 copies per reaction compared to 5 copies per reaction), adding an initial sample concentration in the STOPCovid.v2 workflow resulted in higher overall sensitivity.

We evaluated the performance of the optimized STOPCovid.v2 chemistry on SARS-CoV-2 positive patient samples. Patient NP swab sample dilution series revealed that STOPCovid.v2 had a limit of detection comparable to an RT-qPCR Ct value of 40.3, which could in theory capture 99.7% of SARS-CoV-2 positive patients (Fig. S8D, S10D, and S10E). Blinded testing by an external laboratory at the University of Washington on 202 SARS-CoV-2 positive and 200 negative patient NP swab VTM samples showed that the sensitivity and specificity of STOPCovid.v2 were 93.1% and 98.5% respectively (Fig. 1B and 1C, Fig. S11A, and Table 1).

STOPCovid.v2 false negative samples had RT-qPCR Ct values that were greater than 37. Positive samples were detected in 15-45 mins. Finally, we validated STOPCovid.v2 using dry AN swabs. We confirmed that STOPCovid.v2 could detect SARS-CoV-2 virus-like particles spiked into SARS-CoV-2 negative dry AN swabs (Fig. S11B and S11C). STOPCovid.v2 correctly identified 5 SARS-CoV-2 positive patient AN swabs (Ct values ranging from 19 to 36) (Fig. S11D and S11E).

To aid users of STOPCovid, we have developed a mobile phone application to help the user interpret lateral flow results (Fig. S10C and S12). We note that lateral flow testing requires opening post-amplification tubes, and users should consider separate pre- and post-amplification workspaces to reduce chances of cross contamination between samples and false positives.

Methods

RT-qPCR reactions

RT-qPCR reactions were performed using the TaqPath 1-Step RT-qPCR Master Mix (ThermoFisher A15300) using TaqMan probes and primer sets (Table S1) and measured on a Roche LightCycler 480. RT-qPCR quantification was performed using the second derivative maximum method from the LightCycler software.

Viral nucleic acid extraction

To compare crude lysis methods with standard RNA extraction methods, viral samples were purified using the QIAamp Viral RNA Mini Kit (Qiagen 52906), using 100 µL sample input and 100 µL elution volume.

Lentiviral lysis and quantification

To evaluate crude lysis methods, red fluorescent protein sequence packaged into lentivirus as described previously [29] was directly used for downstream quantification or diluted 1:1 in candidate lysis buffers and boiled at 95 ℃ for 5 minutes. Lysis efficiency was determined using 2 µL of crude sample as input into a 20 µL RT-qPCR reaction, performed as described above.

Mock clinical sample preparation

Nasopharyngeal (NP) swabs from healthy donors (Lee Biosolutions 991-31-NC) were dipped in 2 mL E-MEM (VWR 10128-214) to dislodge material from the swab. NP swabs from 5 patients were pooled to simulate SARS-CoV-2-negative NP swab matrix. Saliva from 5 healthy donors (Lee Bisolution 991-05-S) was pooled together to simulate SARS-CoV-2 negative saliva matrix. Clinical matrices were heated at 95 ℃ for 5 minutes with an equal volume of QuickExtract DNA Extraction Solution (Lucigen QE09050), followed by the addition of SARS-CoV-2 genomic standards (Twist Biosciences 102019) at indicated concentrations to create mock clinical samples for downstream detection.

Loop-mediated Isothermal Amplification (LAMP)

LAMP primer sets (Table S2) were designed using LAMP Designer 1.16 (Premier Biosoft) or GLAPD [30]. LAMP reactions were performed using final concentrations of 1.6 uM FIP/BIP primers, 0.2 uM F3/B3 primers, and 0.4 uM LoopF/B primers. LAMP primer sets were screened using the WarmStart Lamp Kit (New England Biolabs E1700) with 1X fluorescent LAMP dye and 25 ng of background human RNA in 20 µL reactions. RNA targets for LAMP primer screening (Table S2) were transcribed using HiScribe T7 Quick High Yield RNA Synthesis Kit (New England Biolabs E2050). Optimizations of LAMP primer sets selected for further evaluation were performed using 1X Isothermal Amplification Buffer (20 mM Tris-HCl, 50 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Tween 20, pH 8.8) (New England Biolabs B0537), 1.4 mM dNTPs (New England Biolabs N0447), 6.4 units of Bst2.0 WarmStart DNA

Polymerase (New England Biolabs M0538), 6 units of WarmStart RTx Reverse Transcriptase (New England Biolabs M0380), variable concentrations of added $MgSO_4$ (New England Biolabs B1003) and 1X fluorescent LAMP dye in 20 µL reactions containing 10% mock NP clinical sample with SARS-CoV-2 genomic standards. LAMP reactions were performed at 60 ℃ (unless otherwise indicated) for 1-2 hrs on a qPCR machine (Roche LightCycler 480 or BioRad CFX) with fluorescent measurements every 2 minutes.

Cas12b Protein Purification

AacCas12b and AapCas12b orthologs were expressed and purified with a modified protocol [31]. Briefly, AacCas12b (human codon optimized) bacterial expression vector was transformed into BL21-CodonPlus (DE3)-RIPL Competent Cells (Agilent 230280), and AapCas12b bacterial expression vector was transformed into BL21(DE3) Competent E. coli (NEB C2527). Protein sequences for AacCas12b and AapCas12b are listed in Table S3. A 12.5 mL starter culture was grown in Terrific Broth media (TB) supplemented with 100 µg/ml ampicillin for 12 h, which was used to inoculate 12 L of TB for growth at 37°C and 150 rpm until an OD600 of 0.4. After cells were cooled down to 16°C, protein expression was induced by supplementation with IPTG (Goldbio I2481C) to a final concentration of 0.5 mM. The cells were incubated at 16°C for 16 h for protein expression, and then harvested by centrifugation for 20 min at 4°C at 4000 rpm (Beckman Coulter Avanti J-E, rotor JLA9.100). Cell pellet was stored at -80°C for later purification. All subsequent steps were performed at 4°C. Cell pellet was resuspended in 600 mL of lysis buffer (20 mM Tris-HCl, 500 mM NaCl, 1 mM DTT, pH 8.0) supplemented with cOmplete ULTRA Tablets (Millipore sigma 6538282001), 1 mg/mL lysozyme (Sigma L6876), and 250 units/L benzonase (Sigma E1014). Cells were disrupted by the LM20 Microfluidizer system at 18,000 PSI. Lysate was cleared by centrifugation for 30 min at 4°C at 9500 rpm (Beckman Coulter Avanti J-E, rotor JLA-10.500). The cleared lysate was applied to 5 mL of packed Strep-Tactin Sepharose (IBA 2-1201-010) and incubated with rotation for 1 h, followed by washing of the protein-bound resin in 150 mL of lysis buffer. The resin was resuspended in 15 mL of lysis buffer supplemented with 1.2 mg of Ulp1 SUMO protease, and incubated at 4°C for 16 h with rotation. The suspension was applied to a column for elution and separation from resin by gravity flow. The resin was washed with 15 mL of lysis buffer, and this additional eluate was combined with the first 15 mL of eluate. The combined 30 mL of eluate was diluted with 30 mL of cation exchange buffer A (20 mM Tris-HCl, 5% glycerol, 1 mM DTT, pH 7.5) to lower the salt concentration for cation exchange chromatography. The resulting 60 mL protein solution was loaded onto a 5 mL HiTrap SP HP cation exchange column (GE Healthcare) via FPLC (AKTA PURE, GE Healthcare) and eluted over a 250 mM to 2 M NaCl salt gradient made by cation exchange buffer A and B (20 mM Tris-HCl, 2 M NaCl, 5% glycerol, 1 mM DTT, pH 7.5). The resulting fractions were tested for presence of the protein by NuPAGE (Invitrogen) and eStain L1 Protein Staining System (GenScript). The fractions containing the protein were pooled and concentrated by an Amicon Ultra-15 Centrifugal Filter Units (50KDa NMWL, Millipore UFC905024) to 1 mL. The concentrated protein was loaded onto a gel filtration column

(Superdex 200 Increase 10/300 GL, GE Healthcare) equilibrated with Cas12 protein storage buffer (50 mM Tris-HCl, 600 mM NaCl, 2.5% glycerol, 2 mM DTT, pH 7.5) via FPLC. The resulting fractions from gel filtration were analyzed and the fractions containing the protein were pooled, snap frozen as 2 mg/mL aliquots, and stored at -80°C.

Cas12b collateral detection

Cas12b detection reactions were performed using 1X Isothermal Amplification Buffer, 8mM $MgSO_4$, 250 nM Cas12b protein, 250 nM sgRNA, 250 nM fluorescent reporter (/5HEX/TTTTT/3IABkFQ/) and 1 µL of completed LAMP reaction in 10 µL reactions. Cas12b detection reactions were performed at 60 ℃ (unless otherwise indicated) for 1-2 hrs on a qPCR machine (Roche LightCycler 480 or BioRad) with fluorescent measurements every 2 minutes.

One-pot LAMP-Cas12b fluorescent reactions

One-pot LAMP-Cas12b reactions were performed using 1X Isothermal Amplification Buffer, 1.4 mM dNTPs, 6.4 units of Bst2.0 WarmStart DNA Polymerase, 6 units of WarmStart RTx Reverse Transcriptase, 250 nM Cas12b protein, 250 nM sgRNA, 200 nM fluorescent reporter, 1.6 uM FIP/BIP primers, 0.2 uM F3/B3 primers, 0.4 uM LoopF/B primers and variable concentrations of $MgSO_4$ in 20 µL reactions. Where indicated, mock clinical sample with SARS-CoV-2 genomic standard was added at 10% of the final reaction volume to simulate crude input from sample lysis. One-pot reactions were performed at 60 ℃ (unless otherwise indicated) for 1-2 hrs on a qPCR machine (BioRad) with fluorescent measurements every 2 minutes.

Conservation analysis of STOPCovid targeting sites

Human SARS-CoV-2 nucleotide sequences were downloaded from Gisaid database [24] on May 30th 2020. Completed genomes (sequence length >29,000 nt and less than 5% of ambiguous nucleotides) were aligned using mafft [32] to obtain a global alignment of 31,614 sequences. For each target, we extracted a sub alignment embedding the region of the target from the genome alignment and removed sequences with ambiguous nucleotides. Then, we determined the number of sequences that either perfectly matched the target or had substitutions and indels.

Additive Screening

Additives from the Hampton Research Solubility and Stability Screen (Hampton Research HR2-072) were added at 20% of the final reaction volume of one-pot LAMP-Cas12b fluorescent detection reactions unless otherwise indicated.

STOPCovid Reactions

STOPCovid reactions were one-pot LAMP Cas12b reactions described above, using N Set 2 LAMP primers, N set 2 AacCas12b sgRNA 11, and 50 mM taurine. When performing lateral flow reactions, the fluorescent reporter was substituted for a lateral flow collateral reporter (/56-FAM/TTTTTTT/3Bio/). Unless otherwise indicated, STOPCovid lateral flow reactions were

incubated at 60 ℃ for one hour. After incubation, a HybriDetect Dipstick (Milenia Biotec GmbH MGHD 1) was added to the reaction and the liquid was allowed to flow to the top of the strip for subsequent detection. The final concentration of the lateral flow collateral reporter was titrated (125-200 nM) on each lot of HybriDetect Dipsticks to achieve maximal signal-to-noise ratio. Sous-Vide cooker (Amazon B07H9N9PMQ) was used where indicated. To maximize sensitivity and specificity, the final Aap Cas12b and sgRNA concentration was titrated to 31.3 nM. This concentration was used for validating STOPCovid.v2 on patient NP and anterior nasal (AN) samples.

STOPCovid multiplexed control reactions

Multiplexed reactions were performed as described for fluorescent STOPCovid reactions, with indicated ratios of N set 2 LAMP primers to control LAMP primers and the addition of 250 nM SYTO-9 Fluorescent Nucleic Acid Stain (ThermoFisher S34854). Total LAMP Primer concentration was consistent between single target and multiplexed STOPCovid reactions.

SARS-CoV-2 clinical sample collection

Nasopharyngeal swabs were collected in PBS, viral transport medium (ThermoFisher Scientific), or universal transport media (Becton Dickinson) and submitted to the Virology Laboratory at the University of Washington. SARS-CoV-2 was detected by a laboratory-developed RT-qPCR test using CDC distributed N1 and N2 gene primer/probe sets or tests from Hologic (Panther Fusion) and Roche (cobas). The Panther Fusion SARS-CoV-2 assay (Hologic) amplifies and detects two regions of ORF1ab. The cobas SARS-CoV-2 assay on the 6800 platform (Roche Diagnostics) qualitatively detects two viral targets: ORF1ab and E-gene. The averages of the observed Ct values in each test were used to determine the overall Ct value of the SARS-CoV-2 positive samples. Previous studies have shown that the average Ct values between each assay are comparable [33]. After analysis, samples were stored at -80°C until further analysis. Samples from the University of Washington were shipped to the Broad Institute for evaluating STOPCovid.v1 performance.

Estimation of LOD using patient samples

Clinical samples that tested positive for SARS-CoV-2 from the cobas SARS-CoV-2 assay were stored at 4 °C and used within 12 h to determine the LOD. A five-fold serial dilution of SARS-CoV-2 positive patient NP swab samples in UTM (Becton Dickinson) were generated. For STOPCovid.v1, samples were lysed with an equal volume of DNA QuickExtract for 5 mins at 95 °C. 5 µL of lysates were used for the STOPCovid.v1 lateral flow reaction and incubated at 60 °C for one hour, following the signal readout using a HybridDetect Dipstick (Milenia). Ct 3.3 was added between the 5-fold dilutions of samples. To estimate the LOD of the STOPCovid.v2 fluorescent reaction of the total NP swab sample, three replicates of five-fold serial dilution of SARS-CoV-2 positive patient NP swab samples in UTM were generated. 50 µL of diluents were transferred to the extraction buffer (260 µL of laboratory-developed KCl magnetic beads, 40 µL

of 10X QuickExtract Plant DNA Extraction Solution, and 50 µL water) using foam swabs (Puritan 25-1506). Nucleic acids bound to magnetic beads were subjected to a STOPCovid.v2 reaction at 60 °C on an ABI 7500 qPCR machine (Thermo Fisher) with measurements every 1 min for 45 min. Since 50 µL of the NP swab sample in UTM, out of 3 mL total, were used, Ct 5.9 was added to the observed average Ct value of the original sample, and Ct 3.3 was added between the 5-fold dilutions of samples. To project the proportion of SARS-CoV-2 positive patients that an assay with the estimated LOD could capture, the average Ct values obtained from the dilution series were compared to the larger 2,105 patient Ct values obtained from the cobas RT-qPCR assay.

To evaluate the performance of STOPCovid.v2, a blinded test of 202 SARS-CoV-2 positive samples and 200 negative samples was performed. Similar to the dilution series, as only 50 µL of the NP swab sample in 3mL of UTM was used instead of the entire swab, Ct 5.9 was added to the observed Ct value of the original sample, and only samples with Ct values lower than 34 were used. 50 µL of randomized and blinded NP swab samples in UTM were transferred to 50 µL of water and 300 µL of extraction buffer. STOPCovid.v2 reaction was performed as described above and fluorescent measurements were taken every 2 min during a 44 min period in an ABI 7500 qPCR machine (Thermo Fisher). Fluorescence threshold for classifying samples as positive or negative was set to approximately 10% of the average steady state fluorescence signal, or 100,000 RFU on the ABI 7500 qPCR machine.

Laboratory-developed magnetic bead preparation

Laboratory-developed magnetic beads for RNA concentration were prepared as previously described [26]. Briefly, 10 mL of Sera-Mag SpeedBeads Carboxyl Magnetic Beads Hydrophobic (GE Healthcare 65152105050250) were washed with ddH_20 and resuspended in 500 mL of bead binding buffer (10 mM Tris-HCl pH 8.0, 1M NaCl, 18 % PEG-8000, and 1mM EDTA). Where indicated, 1M NaCl was replaced with 1M KCl.

Bead Extraction and Concentration

400 µL of laboratory-developed or commercial (SPRISelect, Beckman Coulter B23317) magnetic beads were mixed with 200 µL of heat-inactivated (95 ℃ for 5 minutes). QuickExtract DNA Extraction Solution spiked with indicated quantity of SARS-CoV-2 genomic standard and 1.25 µg of background human RNA. This mixture was allowed to bind for 5 minutes at room temperature, before transfer onto a magnetic plate (Alpaqua A001219). Beads were washed with 1 mL of 70% ethanol, and allowed to dry for 5 minutes. 50 µL of STOPCovid mastermix was directly added to the beads and the reaction mixture was incubated at 60 ℃ for 1-2 hrs. Where indicated, the washing and drying steps outlined above were skipped and a modified STOPCovid mastermix was used. Specifically, a modified 1X Isothermal Amplification Buffer containing no KCl (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Tween 20, pH 8.8) was used in place of the buffer described above.

Combination of lysis and magnetic bead binding

To evaluate our ability to perform efficient viral lysis and nucleic acid binding in parallel, 100 µL of mock SARS-CoV-2 virus (SARS-CoV-2 RNA targets in a noninfectious viral coat) from AccuPlex SARS-CoV-2 Verification Panel (SeraCare 0505-0132) with 125 ng/µL background human RNA were either added to 100 µL of lysis buffer alone or 100 µL of lysis buffer combined with 400 µL of laboratory-developed KCl magnetic beads. Concentrations of lysis buffers, QuickExtract DNA Extraction Solution and QuickExtract Plant DNA Extraction Solution (Lucigen QEP70750), indicated the final working concentration. Samples containing no magnetic beads were incubated at the indicated temperature for 5 minutes, followed by the addition of 400 µL of laboratory-developed KCl magnetic beads. Samples containing combined bead/lysis mixtures were incubated at room temperature for 10 minutes. All samples were subsequently treated with the bead extraction and concentration steps described above with no wash step.

Comparison of STOPCovid.v2 to CDC RT-qPCR assays

To create mock samples simulating the addition of swabs, different amounts of mock SARS-CoV-2 virus from the AccuPlex SARS-CoV-2 Verification Panel were diluted in PBS containing 25ng/µL background human RNA. 50 µL of mock sample was either added to extraction buffer (400 µL of laboratory-developed KCl magnetic beads, 60 µL of 10X QuickExtract Plant DNA Extraction Solution, and 90 µL of PBS) for detection with STOPCovid.v2 or to 1 mL E-MEM media for subsequent nucleic acid extraction and RT-qPCR. STOPCovid.v2 fluorescent reactions were incubated at 60 ℃ on a qPCR machine with measurement every 2 minutes. STOPCovid.v2 lateral flow reactions were allowed to proceed at 60 ℃ for 80 minutes before addition of a detection strip. RT-qPCR reactions were performed according to CDC recommendations (https://www.fda.gov/media/134922/download), using the N1 primer and probe set.

Evaluation of STOPCovid.v2 using dry anterior nasal swabs

SARS-CoV-2 negative patient dry AN swabs (Lee Biosolutions 991-31-NC) were dipped into extraction buffer (260 µL of laboratory-developed KCl magnetic beads, 40 µL of 10X QuickExtract Plant DNA Extraction Solution, and 100 µL water) for detection with STOPCovid.v2. For simulating SARS-CoV-2 positive AN swabs, water in the extraction buffer was replaced with mock SARS-CoV-2 virus from the AccuPlex SARS-CoV-2 Verification Panel. SARS-CoV-2 positive patient dry AN swabs were collected from SARS-CoV-2 positive patients at Brigham and Women's Hospital. SARS-CoV-2 positive patients were identified using NP swabs and the Panther Fusion (Hologic) assay. Dry AN swabs were collected within 48 hours of the NP swab RT-qPCR test. To collect the AN swabs, a flocked AN swab (Miraclean Technology 93050) was inserted approximately 1 inch into the left nostril of the patient. Once in place, the swab was rotated 3 times and then kept in place for 15 seconds to absorb nasal secretions. The same procedure was repeated with the right nostril using the same swab. Dry AN

swabs were stored at 4 ℃ and tested using STOPCovid.v2 within 24 hours as described above for negative AN swabs. Fluorescence threshold for classifying samples as positive or negative was set to approximately 10% of the average steady state fluorescence signal, or 500 RFU on the Biorad CFX96 qPCR machine.

Lateral Flow Band Quantification

Acquired images were converted to 8-bit grayscale using photoshop and then imported into ImageLab software (BioRad Image Lab Software 6.0.1). Images were inverted and lanes were manually adjusted to fit the lateral flow strips. Bands were picked automatically and the background was adjusted manually to allow band comparison. Width of bands and background adjustment was kept constant between all bands in the same image. The band intensity ratio is calculated as the intensity of the top (test) band divided by the bottom (control) band.

Mobile Phone Application

For classifying STOPCovid lateral flow strip results, the mobile application used >50 images containing >500 lateral flow strips that have been manually annotated as positive or negative to set the appropriate threshold for the band intensity ratio. As the lateral flow strip typically has a faint background for the top (test) band, this threshold was determined to be 0.14. To use the STOPCovid mobile phone application for interpreting lateral flow trip results, download the Expo application for iOS (https://apple.co/2c6HMtp) or Android (https://bit.ly/2bZq5ew). Launch the Expo application, tap "Sign in to your account", and sign in with the STOPCovid team's Expo credentials. Please contact the STOPCovid team through the STOPCovid website (https://www.stopcovid.science/) for credentials. Open STOPCovid from the Expo application.

STOPCovid.v2 Reagents and Protocol

Sequences

LAMP Primers (IDT):

F3: 5′– GCTGCTGAGGCTTCTAAG –3′
B3: 5′– GCGTCAATATGCTTATTCAGC –3′
FIP: 5′– GCGGCCAATGTTTGTAATCAGTAGACGTGGTCCAGAACAA –3′
BIP: 5′– TCAGCGTTCTTCGGAATGTCGCTGTGTAGGTCAACCACG –3′
LoopF: 5′– CCTTGTCTGATTAGTTCCTGGT –3′
LoopB: 5′– TGGCATGGAAGTCACACC –3′

AapCas12b Guide RNA (Synthego):

5′– GUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCCAGGUGGCAA
AGCCCGUUGAGCUUCUCAAAUCUGAGAAGUGGCAC**CGAAGAACGCUGAAGCGCUG** –3′
(The spacer targeting N gene is underlined.)

AapCas12b Protein Sequence:

MAVKSMKVKLRLDNMPEIRAGLWKLHTEVNAGVRYYTEWLSLLRQENLYRRSPNGDGE
QECYKTAEECKAELLERLRARQVENGHCGPAGSDDELLQLARQLYELLVPQAIGAKGD
AQQIARKFLSPLADKDAVGGLGIAKAGNKPRWVRMREAGEPGWEEEKAKAEARKSTDR
TADVLRALADFGLKPLMRVYTDSDMSSVQWKPLRKGQAVRTWDRDMFQQAIERMMSWE
SWNQRVGEAYAKLVEQKSRFEQKNFVGQEHLVQLVNQLQQDMKEASHGLESKEQTAHY
LTGRALRGSDKVFEKWEKLDPDAPFDLYDTEIKNVQRRNTRRFGSHDLFAKLAEPKYQ
ALWREDASFLTRYAVYNSIVRKLNHAKMFATFTLPDATAHPIWTRFDKLGGNLHQYTF
LFNEFGEGRHAIRFQKLLTVEDGVAKEVDDVTVPISMSAQLDDLLPRDPHELVALYFQ
DYGAEQHLAGEFGGAKIQYRRDQLNHLHARRGARDVYLNLSVRVQSQSEARGERRPPY
AAVFRLVGDNHRAFVHFDKLSDYLAEHPDDGKLGSEGLLSGLRVMSVDLGLRTSASIS
VFRVARKDELKPNSEGRVPFCFPIEGNENLVAVHERSQLLKLPGETESKDLRAIREER
QRTLRQLRTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPMDANQMTPDWREAFEDELQ
KLKSLYGICGDREWTEAVYESVRRVWRHMGKQVRDWRKDVRSGERPKIRGYQKDVVGG
NSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRFAITLREHIDHAKEDRLKKLA
DRIIMEALGYVYALDDERGKGKWVAKYPPCQLILLEELSEYQFNNDRPPSENNQLMQW
SHRGVFQELLNQAQVHDLLVGTMYAAFSSRFDARTGAPGIRCRRVPARCAREQNPEPF
PWWLNKFVAEHKLDGCPLRADDLIPTGEGEFFVSPFSAEEGDFHQIHADLNAAQNLQR
RLWSDFDISQIRLRCDWGEVDGEPVLIPRTTGKRTADSYGNKVFYTKTGVTYYERERG
KKRRKVFAQEELSEEEAELLVEADEAREKSVVLMRDPSGIINRGDWTRQKEFWSMVNQ
RIEGYLVKQIRSRVRLQESACENTGDI*

Reporter DNA (IDT):

Fluorescence: 5′– /5HEX/TTTTT/3IABkFQ/ –3′
Lateral flow: 5′– /56-FAM/TTTTTTT/3Bio/ –3′

Patient samples

Patient nasopharyngeal (NP) and anterior nasal (AN) swab samples should be collected and processed according to the appropriate biosafety procedure. Please reference the 2020 CDC COVID-19 test protocol for details on specimen collection. Swabs that do not soak up excessive volumes (>80 µL) of extraction buffer, such as flocked swabs, should be used.

Reagents

Extraction Buffer:

- Sera-Mag SpeedBeads Carboxyl Magnetic Beads (VWR 65152105050250)
- Poly(ethylene glycol) 8,000 (Millipore Sigma 89510)
- 3 M Potassium chloride solution (Millipore Sigma 60135)
- 1 M Tris-HCl, pH 8.0 (Thermo Fisher Scientific AM9855G)
- UltraPure 0.5 M EDTA, pH 8.0 (Thermo Fisher Scientific 15575020)
- UltraPure DNAse/RNAse-Free Distilled Water (Thermo Fisher Scientific 10977015)
- 10× concentrated QuickExtract Plant DNA Extraction Solution (Lucigen, custom order). Once thawed, aliquot and store at –20 °C to avoid >3 freeze-thaw cycles.

STOPCovid.v2 Reaction:

- 1 M Tris-HCl, pH 8.0 (Thermo Fisher Scientific AM9855G)
- 2 M Ammonium sulfate solution (Millipore Sigma 76399)
- 1 M Magnesium sulfate solution (Millipore Sigma M3409)
- Tween 20 (Millipore Sigma P9416)
- Bst 2.0 WarmStart DNA Polymerase (New England BioLabs M0538L)
- WarmStart RTx Reverse Transcriptase (New England BioLabs M0380L)
- 100 mM Magnesium sulfate, supplied with M0538L and M0380L
- AapCas12b protein (New England BioLabs, custom order) or purified as described previously[1]
- 10 mM Deoxynucleotide (dNTP) Solution Mix (New England BioLabs N0447L)
- Taurine (Millipore Sigma 86329)
- Proteinase K Inhibitor (Millipore Sigma 539470)

- Dimethyl sulfoxide (Millipore Sigma D2650)
- UltraPure DNAse/RNAse-Free Distilled Water (Thermo Fisher Scientific 10977015)
- (Optional) AccuPlex SARS-CoV-2 Reference Material Kit v2 (SeraCare 0505-0133)
- (Optional) HybriDetect Dipstick (Milenia Biotec GmbH MGHD 1)

Equipment

- 60 °C heat block, water bath, or sous-vide immersion cooker
- Magnetic separator
- (Optional) qPCR machine for fluorescence readout

Reagent Setup

Laboratory-developed magnetic beads:

1. Vortex the Sera-Mag SpeedBeads Carboxyl Magnetic Beads for 1 min. Add 10 mL to a 50 mL conical tube.
2. Using a 50 mL magnetic separator, pellet the magnetic beads.
3. Aspirate the supernatant and discard.
4. Wash beads twice with 10 mL of ddH_2O, resuspending the pellet each time by vortexing for 30 s.
5. Pellet the beads for the last time.
6. Prepare the bead buffer by mixing the following in a 500 mL reagent bottle.

Component	Amount
3 M KCl	167 mL
1 M Tris-HCl	5 mL
0.5 M EDTA	1 mL
PEG-8000	90 g

7. Fill with ddH_2O to 500 mL and invert 10 times to mix.
8. Filter through a vacuum filtration unit according to the manufacturer's instructions.
9. Remove the final wash fluid on the beads and add 10 mL of bead buffer.
10. Vortex for 30 s to resuspend the beads and transfer to the 500 mL bottle containing the bead buffer.
11. Invert to homogenize the beads with the buffer.
12. Beads can be aliquoted and stored at 4 °C for 1 month or –20 °C for up to 1 year.

Extraction Buffer:

1. Prepare the extraction buffer by mixing the following in a 50 mL conical tube.

Component	Amount (mL)
Magnetic beads	26
10× QuickExtract Plant	4
ddH_2O	10
Total	40

2. Aliquot for storage at –20 °C for up to 1 year. Avoid more than 1 additional freeze-thaw cycle.

10× LAMP Primer Pool:

1. Resuspend LAMP primers with ddH_2O to 100 µM.
2. Prepare the 10× LAMP primer pool by mixing the following in a 1.5 mL tube.

Component	Amount (µL)
F3 primer	20
B3 primer	20
FIP primer	160
BIP primer	160
LoopF primer	40
LoopB primer	40
ddH_2O	560
Total	1000

3. Aliquot for storage at –20 °C for up to 1 year.

10× STOPCovid.v2 reaction buffer:

1. Prepare the 10× STOPCovid.v2 reaction buffer by mixing the following in a 15 mL conical tube.

Component	Initial concentration	Final concentration	Amount (mL)
Tris-HCl	1 M	0.2 M	2
$(NH_4)_2SO_4$	2 M	0.1 M	0.5
Mg_2SO_4	1 M	0.02 M	0.2
Tween 20	100 %	1 %	0.1
ddH_2O			7.2
Total			10

2. Adjust the pH of the solution to 8.8. The pH should be very close to 8.8 before adjusting.
3. Aliquot for storage at –20 °C for up to 1 year.

500 mM Taurine:

1. Resuspend 6.258 g of Taurine in 100 mL of ddH_2O. Heating at 37 °C may be required for resuspension.
2. Aliquot for storage at room temperature for up to 1 year.

10× Proteinase K Inhibitor:

1. Resuspend 10 mg of Proteinase K Inhibitor with 150 µL of DMSO to make the stock solution.
2. Dilute stock solution 1:100 with ddH_2O to make working aliquots. Store both stock and working solutions at –20 °C for up to 1 year.

STOPCovid.v2 reaction:

1. Prepare the STOPCovid.v2 reaction by mixing the following for one reaction. Scale up volume as needed for additional reactions.

Component	Initial concentration	Final concentration	Amount (µL)
STOPCovid.v2 reaction buffer	10×	1×	5
dNTPs	10 mM	1.4 mM	7
Mg_2SO_4	100 mM	8 mM	4
Bst 2.0 DNA Polymerase	8,000 units/mL	320 units/mL	2
RTx Reverse Transcriptase	15,000 units/mL	300 units/ml	1
LAMP primer pool	10×	1×	5
AapCas12b protein	2 mg/mL or 15.4 µM	31.25 nM	0.102
AapCas12b sgRNA	100 µM	31.25 nM	0.0156
Reporter DNA (fluorescence)*	100 µM	250 nM	0.125
Taurine	500 mM	50 mM	5
Proteinase K Inhibitor	10×	1×	5
ddH_2O			15.757
Total			50

*If performing lateral flow readout, use the lateral flow reporter instead of the fluorescence reporter, at a final concentration of 125-500 nM titrated for best signal-to-

noise on each lot of Milenia HybriDetect Dipstick.

2. Aliquot and store at –20 °C for up to 1 year.

Protocol

1. Thaw extraction buffer and STOPCovid.v2 reaction at room temperature. Keep STOPCovid.v2 reaction on ice after thawing. Allow extraction buffer to equilibrate to room temperature before proceeding.
2. Vortex extraction buffer for 15s.
3. Dip dry NP or AN swab in 400 µL of extraction buffer in a 1.5 mL tube. Swirl swab against the side of the tube to dislodge material.
4. Vortex or pipette to mix. Incubate at room temperature for 10 mins.
5. Place sample and extraction buffer mix on magnet. Wait 5 mins or until solution is clear.
6. Aspirate the supernatant. Make sure to remove all of the supernatant.
7. Add 50 µL of STOPCovid reaction. Remove tube from magnet and spin down beads using a microcentrifuge.
8. Vortex or pipette to mix.
9. Pellet the beads at 5,000×g for 1 min. Lower speeds or a plate spinner may work as well.
10. Depending on the readout method:
    Fluorescence readout:
    a. Run the reaction at 60 °C for 45 mins
    b. Read out the fluorescence using the VIC/HEX channel on a qPCR machine.
    Lateral flow readout:
    a. Run the reaction at 60 °C for 80 mins.
    b. To prevent sample contamination, transfer sample to a different work area. Place a HybriDetect Dipstick into each reaction and wait for the reaction to flow through the dipstick. Generally, 2 mins is enough time to develop a positive band at the limit of detection.

Supplementary Figures

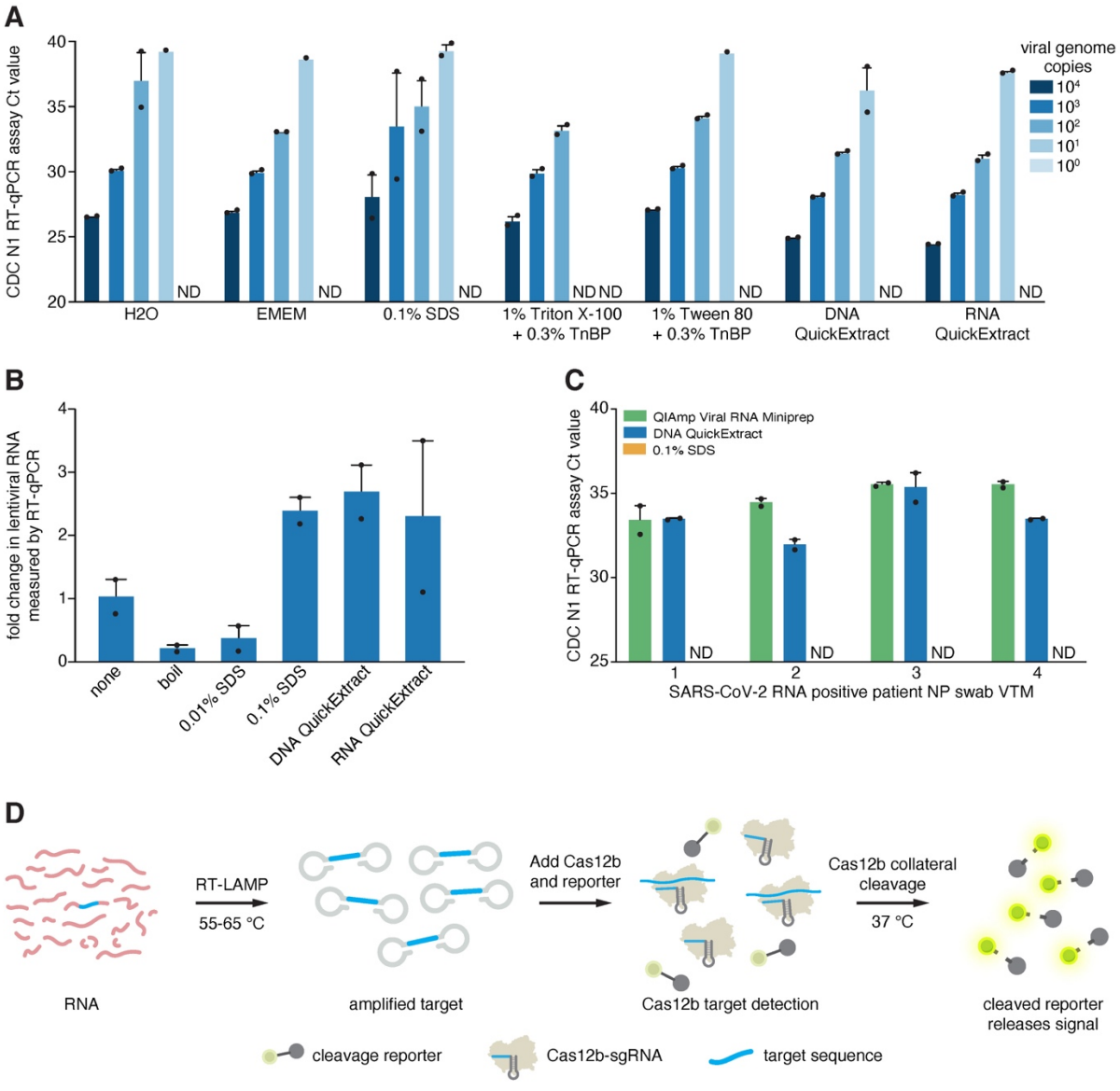


Fig. S1 | Sample extraction using DNA QuickExtract and top LAMP primer sets targeting SARS-CoV-2. A, Effects of different lysis buffers on RT-qPCR. Different amounts of SARS-CoV-2 genomic standards were spiked into lysis buffers and used as input for the CDC N1 RT-qPCR assay. **B,** Comparison of lysis buffers for extracting lentiviral RNA measured by RT-qPCR targeting the RFP RNA. **C,** Comparison of lysis buffers to the standard QIAmp Viral RNA Miniprep extraction on SARS-CoV-2 RNA positive patient nasopharyngeal (NP) swab viral transport media (VTM). The 0.1 % SDS condition did not produce any detectable Ct values. ND, not detected. **D,** Schematic of reverse transcription followed by Loop-mediated Isothermal Amplification (RT-LAMP) and CRISPR-mediated detection. Viral RNA is reverse transcribed and amplified using RT-LAMP. Cas12b detection of the LAMP product triggers collateral cleavage of a reporter to produce signal for detection. **E,** Comparison of LAMP primer sets targeting different regions of the SARS-CoV-2 genome to two established LAMP primer sets measured by real-time fluorescence across a range of concentrations of SARS-CoV-2 RNA amplicons at 65 ℃. NTC, no template control.

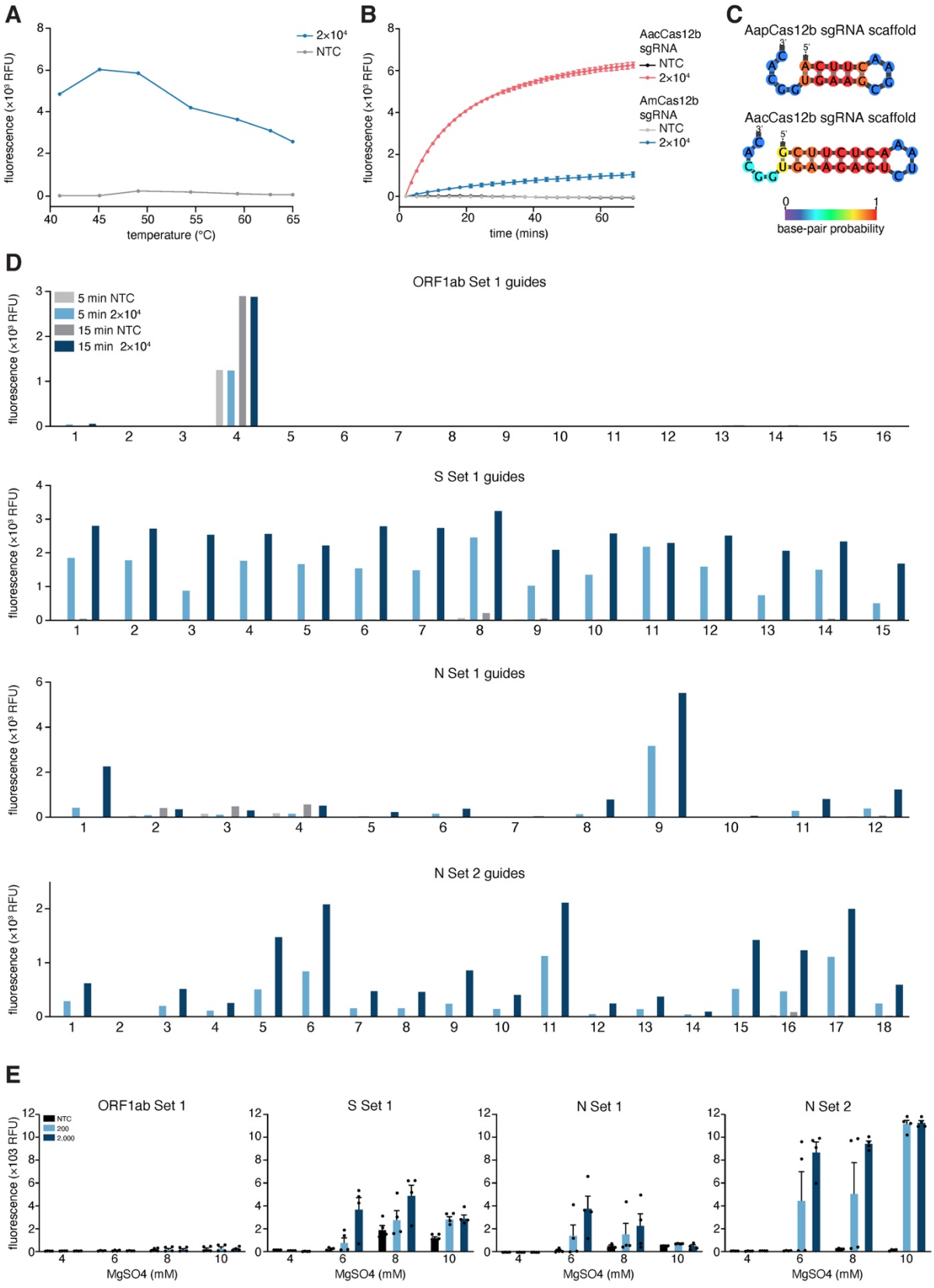

A
fluorescence (x10³ RFU)
temperature (°C)
2×10⁴
NTC
B
fluorescence (x10³ RFU)
time (mins)
AacCas12b sgRNA
NTC
2×10⁴
AmCas12b sgRNA
NTC
2×10⁴
C
AapCas12b sgRNA scaffold
AacCas12b sgRNA scaffold
base-pair probability
D
ORF1ab Set 1 guides
5 min NTC
5 min 2×10⁴
15 min NTC
15 min 2×10⁴
fluorescence (x10³ RFU)
S Set 1 guides
N Set 1 guides
N Set 2 guides
E
ORF1ab Set 1
S Set 1
N Set 1
N Set 2
fluorescence (x103 RFU)
NTC
200
2,000
MgSO4 (mM)

Fig. S2 | Optimization of Aap sgRNAs targeting SARS-CoV-2 LAMP amplicons for one-pot reactions. A, Temperature dependence of AapCas12b collateral activity when incubated with RT-LAMP amplified SARS-CoV-2 genomic standards. Collateral activity was measured as end point fluorescence after incubation. **B,** Comparison of AapCas12b collateral activity with either a previously published AmCas12b sgRNA scaffold [18] or an AacCas12b-based scaffold when incubated with RT-LAMP amplified SARS-CoV-2 genomic standards. **C,** Comparison of 3' end of Aap and Aac Cas12b sgRNA scaffolds. Color indicates base-pairing probability determined by RNAfold. **D,** Collateral activity of AapCas12b using different guides (Table S4) when incubated with RT-LAMP amplified SARS-CoV-2 genomic standards or no template control, NTC. Assay was performed at 60 ℃ and collateral activity was measured by fluorescence after 5 and 15 mins. **E,** Comparison of the best Cas12b sgRNA targeting each of the 4 top LAMP sets at different Magnesium concentrations in the reaction. Different amounts of SARS-CoV-2 genomic standards spiked into NP swab were used as input. NTC, no template control.

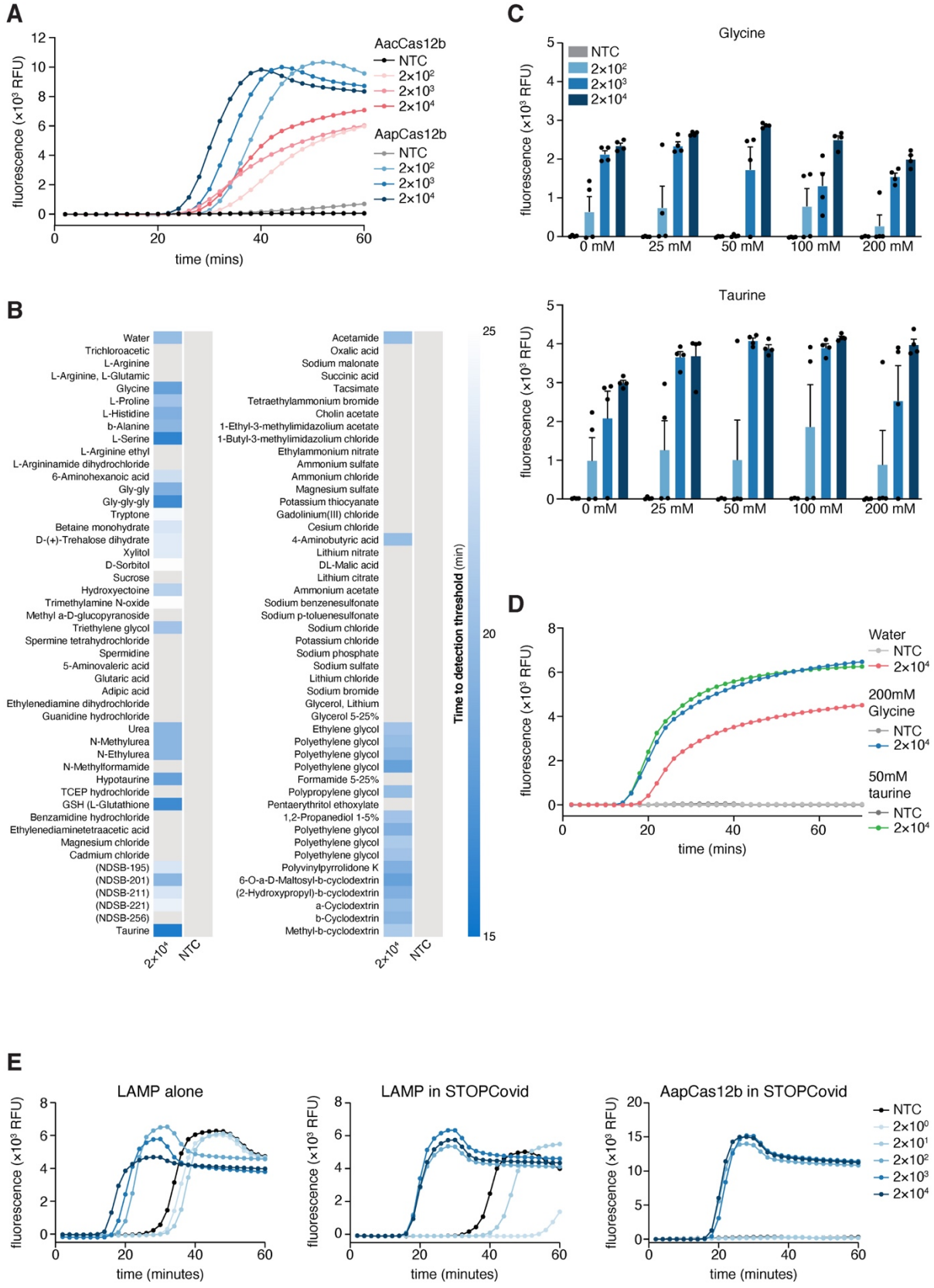

A
fluorescence (×10³ RFU)
time (mins)
AacCas12b
NTC
2×10²
2×10³
2×10⁴
AapCas12b
NTC
2×10²
2×10³
2×10⁴
B
Water
Trichloroacetic
L-Arginine
L-Arginine, L-Glutamic
Glycine
L-Proline
L-Histidine
b-Alanine
L-Serine
L-Arginine ethyl
L-Argininamide dihydrochloride
6-Aminohexanoic acid
Gly-gly
Gly-gly-gly
Tryptone
Betaine monohydrate
D-(+)-Trehalose dihydrate
Xylitol
D-Sorbitol
Sucrose
Hydroxyectoine
Trimethylamine N-oxide
Methyl a-D-glucopyranoside
Triethylene glycol
Spermine tetrahydrochloride
Spermidine
5-Aminovaleric acid
Glutaric acid
Adipic acid
Ethylenediamine dihydrochloride
Guanidine hydrochloride
Urea
N-Methylurea
N-Ethylurea
N-Methylformamide
Hypotaurine
TCEP hydrochloride
GSH (L-Glutathione
Benzamidine hydrochloride
Ethylenediaminetetraacetic acid
Magnesium chloride
Cadmium chloride
(NDSB-195)
(NDSB-201)
(NDSB-211)
(NDSB-221)
(NDSB-256)
Taurine
2×10⁴
NTC
Acetamide
Oxalic acid
Sodium malonate
Succinic acid
Tacsimate
Tetraethylammonium bromide
Cholin acetate
1-Ethyl-3-methylimidazolium acetate
1-Butyl-3-methylimidazolium chloride
Ethylammonium nitrate
Ammonium sulfate
Ammonium chloride
Magnesium sulfate
Potassium thiocyanate
Gadolinium(III) chloride
Cesium chloride
4-Aminobutyric acid
Lithium nitrate
DL-Malic acid
Lithium citrate
Ammonium acetate
Sodium benzenesulfonate
Sodium p-toluenesulfonate
Sodium chloride
Potassium chloride
Sodium phosphate
Sodium sulfate
Lithium chloride
Sodium bromide
Glycerol, Lithium
Glycerol 5-25%
Ethylene glycol
Polyethylene glycol
Polyethylene glycol
Polyethylene glycol
Formamide 5-25%
Polypropylene glycol
Pentaerythritol ethoxylate
1,2-Propanediol 1-5%
Polyethylene glycol
Polyethylene glycol
Polyethylene glycol
Polyvinylpyrrolidone K
6-O-a-D-Maltosyl-b-cyclodextrin
(2-Hydroxypropyl)-b-cyclodextrin
a-Cyclodextrin
b-Cyclodextrin
Methyl-b-cyclodextrin
2×10⁴
NTC
Time to detection threshold (min)
25
20
15
C
Glycine
fluorescence (×10³ RFU)
NTC
2×10²
2×10³
2×10⁴
0 mM
25 mM
50 mM
100 mM
200 mM
Taurine
fluorescence (×10³ RFU)
0 mM
25 mM
50 mM
100 mM
200 mM
D
fluorescence (×10³ RFU)
time (mins)
Water
NTC
2×10⁴
200mM
Glycine
NTC
2×10⁴
50mM
taurine
NTC
2×10⁴
E
LAMP alone
fluorescence (×10³ RFU)
time (minutes)
LAMP in STOPCovid
fluorescence (×10³ RFU)
time (minutes)
AapCas12b in STOPCovid
fluorescence (×10³ RFU)
time (minutes)
NTC
2×10⁰
2×10¹
2×10²
2×10³
2×10⁴

Fig. S3 | Optimization of STOPCovid with additive reagents. A, Comparison of STOPCovid performance (monitored by real-time fluorescence) when using either AacCas12b or AapCas12b and varying amounts of SARS-CoV-2 genomic standards. AacCas12b sgRNA was used for both enzymes. **B,** Heat map comparing a panel of chemical additives for effects on the performance of STOPCovid for detection of 2×10^4 copies of SARS-CoV-2 genomic standards spiked into NP swab. **C,** Titration of Glycine and Taurine additive concentrations for effects on performance of STOPCovid detection of varying amounts of SARS-CoV-2 genomic standards spiked into NP swab. **D,** STOPCovid fluorescence signal over time with either glycine or taurine additives. **E,** Comparison of fluorescence signal generated by LAMP alone (left), LAMP in STOPCovid (middle), and AapCas12b in STOPCovid (right) for varying amounts of SARS-CoV-2 genomic standards. LAMP signal was measured using SYTO9 nucleic acid stain and AapCas12b signal was measured using collateral cleavage of a HEX reporter. NTC, no template control.

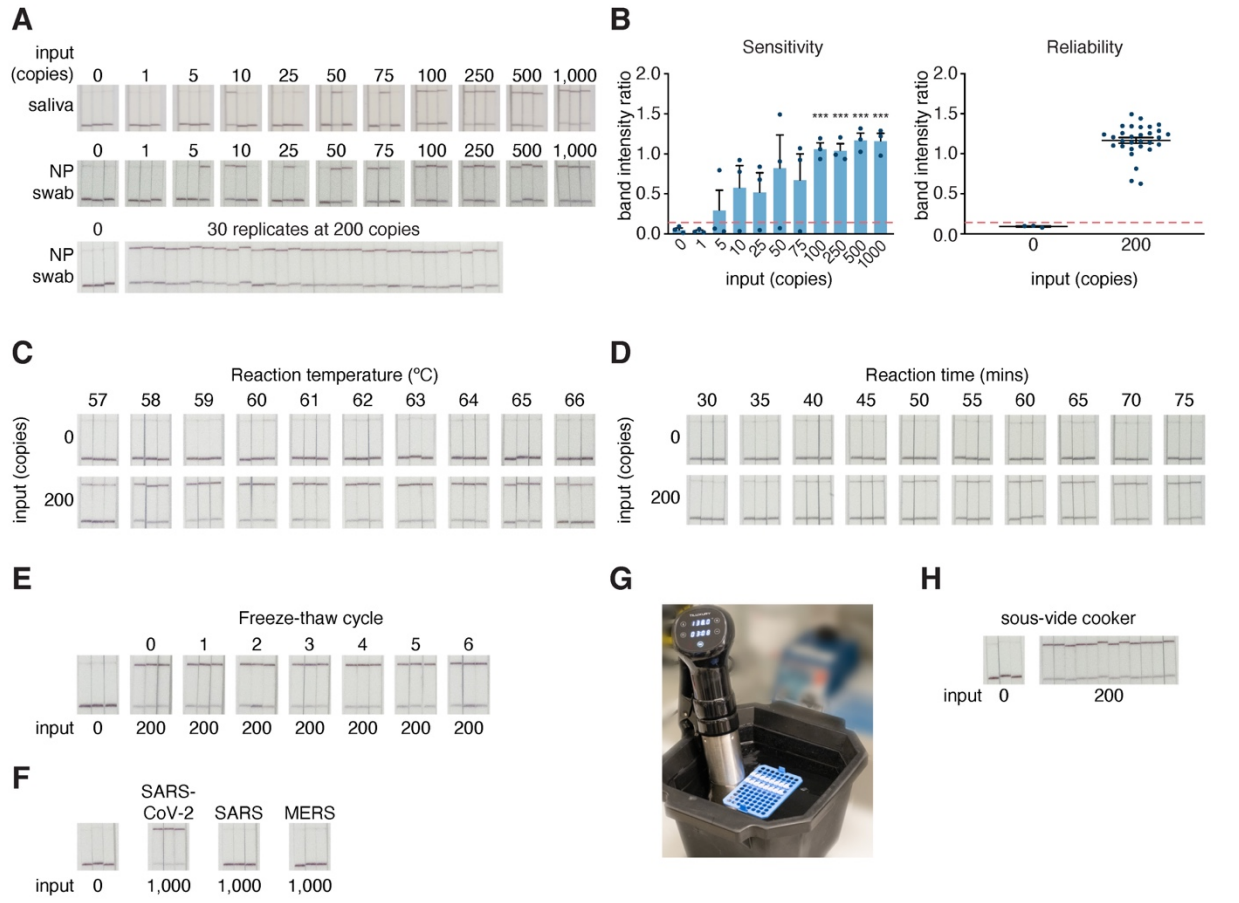


Fig. S4 | Performance of STOPCovid.v1 with lateral flow readout. A, Determination of the sensitivity for STOPCovid.v1 with lateral flow readout at 60 minutes using three replicates per condition. Different amounts of SARS-CoV-2 genomic standards spiked into saliva (top) or nasopharyngeal (NP) swab (middle) were lysed by adding an equal volume of DNA QuickExtract and heating for 5 mins at 95 °C. At 200 genome copies per reaction in NP swab, STOPCovid.v1 yielded positive results for all 30 replicates (bottom). **B,** Quantitation of the band intensity ratio (top band/bottom band) for the NP swab sensitivity (left) and reproducibility (right) from panel (**A**). ***, $P < 0.001$. Red dashed line indicates threshold for classification. **C,** Effect of reaction temperature on STOPCovid.v1 lateral flow detection for 200 SARS-CoV-2 copies per reaction. **D,** Effect of reaction incubation time on STOPCovid.v1 lateral flow detection for 200 SARS-CoV-2 copies per reaction. **E,** Effect of mastermix freeze-thaw cycles on STOPCovid.v1 lateral flow detection for 200 SARS-CoV-2 copies per reaction. **F,** Evaluation of cross-reactivity for COVID-19 STOPCovid.v1 lateral flow test for gene N from SARS and MERS. All inputs were at 1,000 copies per reaction. **G,** Example STOPCovid.v1 setup using a 60°C water bath powered by a simple commercial sous-vide device. **H,** STOPCovid.v1 lateral flow detection of SARS-CoV-2 at 200 copies per reaction using a 60°C sous-vide water bath.

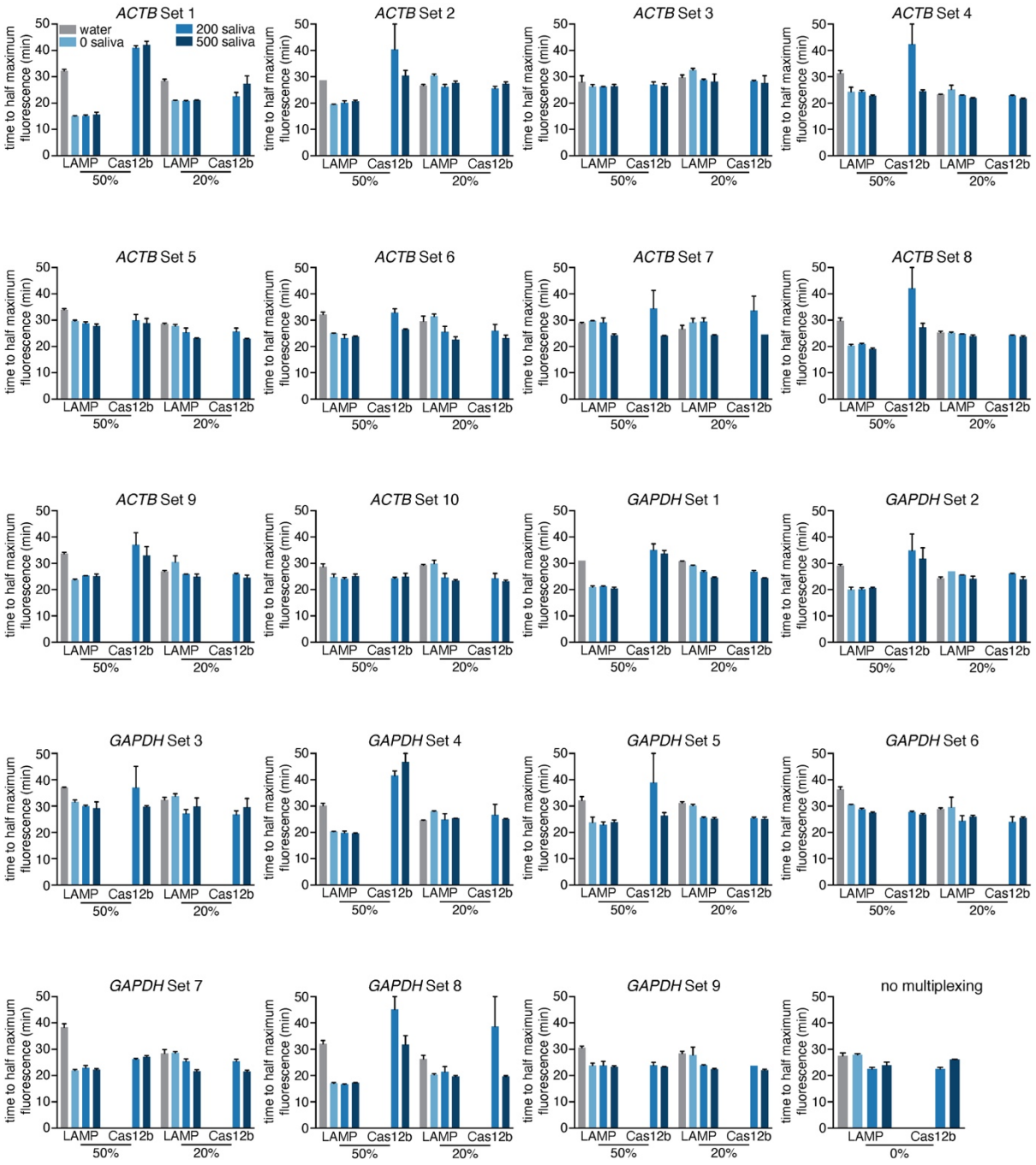


Fig. S5 | Multiplexing internal control LAMP primer sets in STOPCovid.v1. Comparison of LAMP primer sets targeting *ACTB* and *GAPDH* genes to control for sample extraction and isothermal amplification on different amounts of SARS-CoV-2 genomic standards spiked into saliva or water control. Control primer sets were mixed with the STOPCovid.v1 LAMP primer set targeting the N gene at 50% or 20% of the total primer concentration. LAMP signal was detected using the SYTO9 nucleic acid stain and Cas12b signal was detected via collateral cleavage of a HEX reporter probe. Values indicate time to reach the half maximum fluorescence for each reaction.

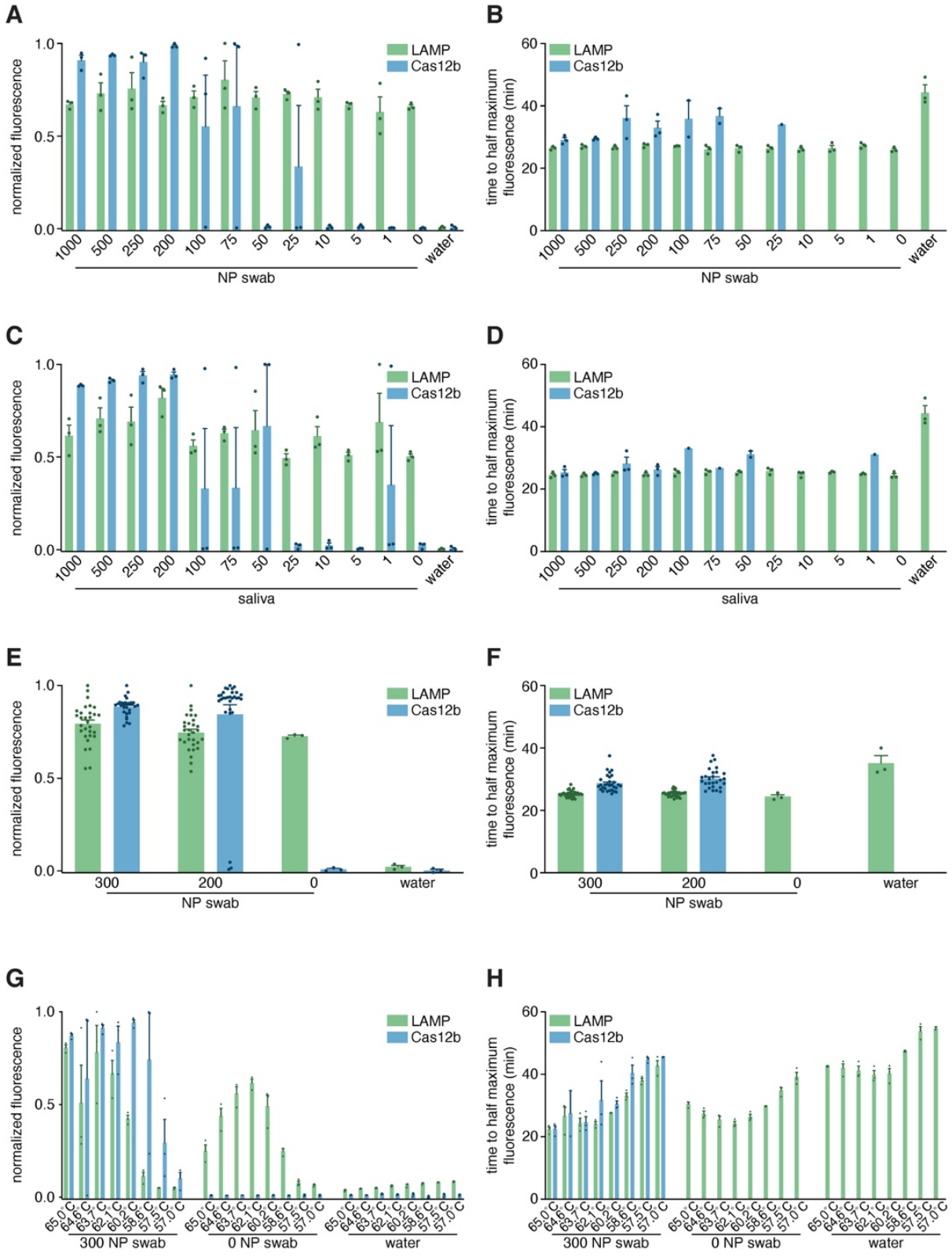
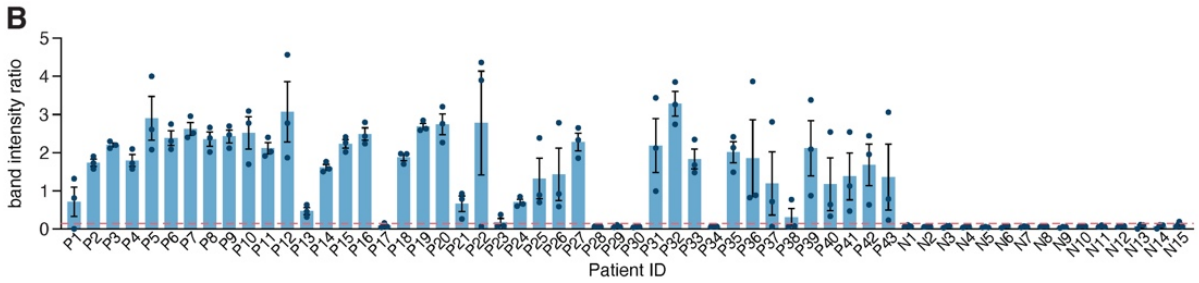

A
normalized fluorescence
1.0
0.5
0.0
LAMP
Cas12b
1000
500
250
200
100
75
50
25
10
5
1
0
water
NP swab
B
time to half maximum fluorescence (min)
60
40
20
0
C
saliva
D
E
300
200
0
F
G
65.0°C
64.6°C
63.7°C
62.1°C
60.2°C
58.6°C
57.5°C
57.0°C
300 NP swab
0 NP swab
H

Fig. S6 | Performance of multiplexed STOPCovid.v1 fluorescence readout. LAMP primer sets targeting *ACTB* or the SARS-CoV-2 gene N were pooled at 20% and 80% of the total primer concentration respectively. LAMP signal was detected using the SYTO9 nucleic acid stain and Cas12b signal was detected via collateral cleavage of a HEX reporter probe. Endpoint normalized fluorescence signal was determined at 28 mins for LAMP and at 45 mins for Cas12b. **A,** Determination of the sensitivity for multiplexed STOPCovid.v1 with fluorescence readout using three replicates per condition. Different amounts of SARS-CoV-2 genomic standards spiked into nasopharyngeal (NP) swab was used for input. Values represent endpoint normalized fluorescence. **B,** Same as (**A**) with values indicating time to reach the half maximum fluorescence. **C,** Determination of sensitivity for multiplexed STOPCovid.v1 using SARS-CoV-2 genomic standards spiked into saliva as input. Values represent endpoint fluorescence signals. **D,** Same as (**C**) with values indicating time to reach the half maximum fluorescence. **E,** Reliability assessment of multiplexed STOPCovid.v1 using 30 replicates at 300 copies and 200 copies of SARS-CoV-2 genomic standards spiked into NP swab. **F,** Same as (**E**) with values indicating time to reach the half maximum fluorescence. **G,** Effect of reaction temperature on multiplexed STOPCovid.v1 detection for 300 SARS-CoV-2 copies spiked into NP swab per reaction. Data represents three replicates per condition. **H,** Same as (**G**) with values indicating time to reach the half maximum fluorescence.



C

Broad Institute
SAR-CoV-2 infection status

STOPCovid.v1 (2/3 replicates)	Pos.	Neg.	Total	Predictive value
Pos.	**36** (True pos.)	**0** (False pos.)	**36**	PPV=100%
Neg.	**7** (False neg.)	**15** (True neg.)	**22**	NPV=68%
Total	**43**	**15**		
	Sensitivity 84%	**Specificity 100%**		

D

UW Virology
SAR-CoV-2 infection status

STOPCovid.v1 (2/3 replicates)	Pos.	Neg.	Total	Predictive value
Pos.	**16** (True pos.)	**0** (False pos.)	**16**	PPV=100%
Neg.	**3** (False neg.)	**7** (True neg.)	**10**	NPV=70%
Total	**19**	**7**		
	Sensitivity 84%	**Specificity 100%**		

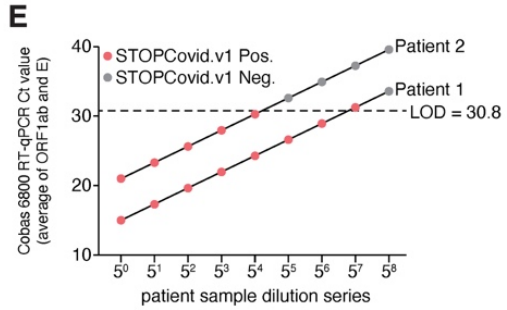


Fig. S7 | Evaluation of STOPCovid.v1 on SARS-CoV-2 patient nasopharyngeal swab samples. A, STOPCovid.v1 results for 43 unique SARS-CoV-2 positive and 15 negative patient nasopharyngeal (NP) swab samples in triplicate detected by lateral flow readout. Prior to STOPCovid.v1, NP swab viral transport media (VTM) was lysed by adding an equal volume of DNA QuickExtract and heating for 5 mins at 95 °C. **B,** Quantification of the band intensity ratios of lateral flow results from panel (**A**). Red dashed line indicates threshold for classification. **C,** Table summarizing the STOPCovid.v1 results on patient NP swab samples tested at Broad Institute. STOPCovid.v1 results were considered positive if 2 out of 3 replicates were positive. **D,** Table summarizing STOPCovid.v1 results on patient NP swab samples tested at the University of Washington Virology lab. **E,** Determination of the limit of detection using 5-fold serial dilutions of SARS-CoV-2 positive patient NP swab samples. Ct values represent the average of ORF1ab and E gene assays from the Cobas 6800 RT-qPCR assay.

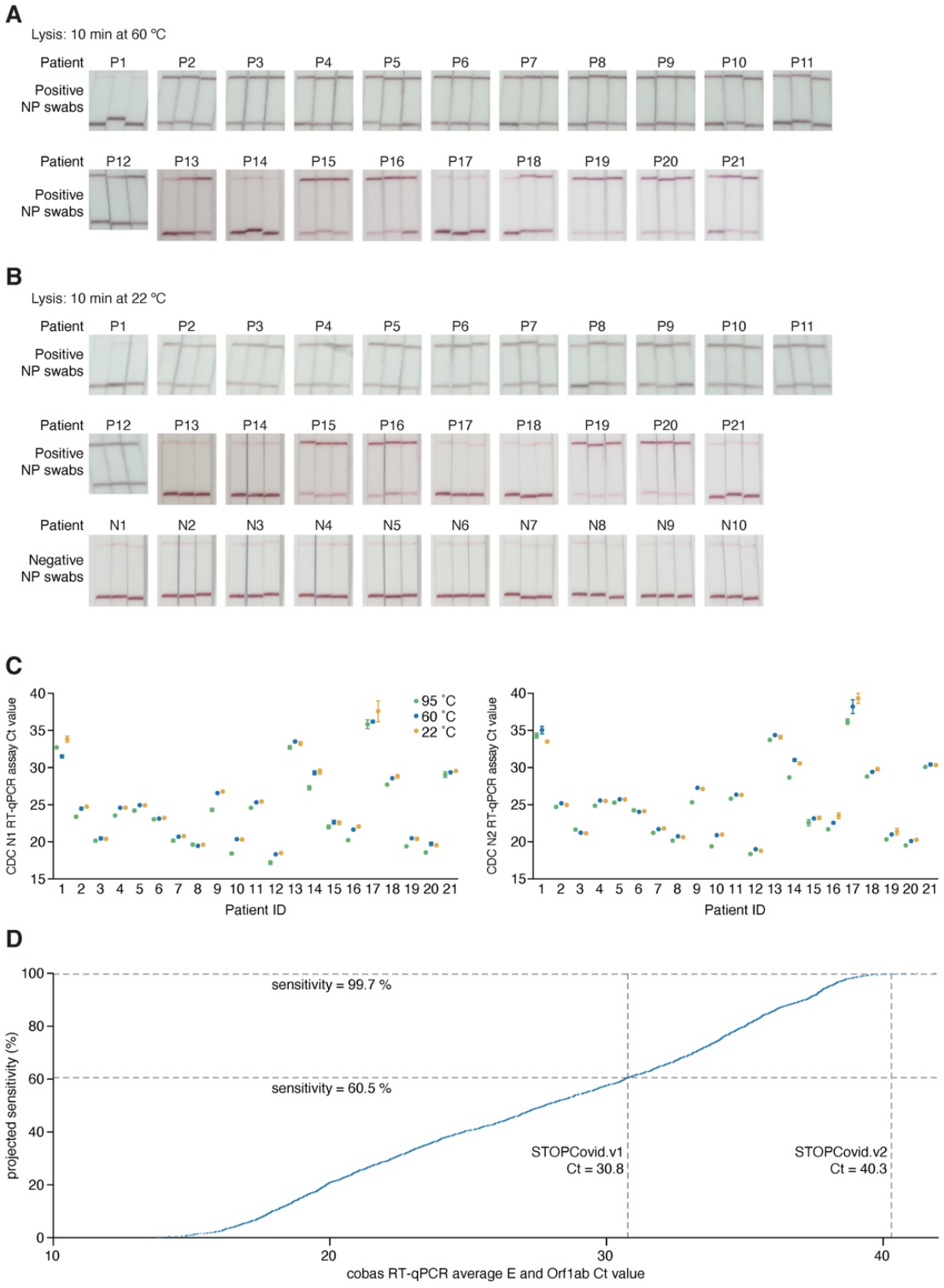

A
Lysis: 10 min at 60 °C
Patient P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11
Positive NP swabs
Patient P12 P13 P14 P15 P16 P17 P18 P19 P20 P21
Positive NP swabs
B
Lysis: 10 min at 22 °C
Patient P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11
Positive NP swabs
Patient P12 P13 P14 P15 P16 P17 P18 P19 P20 P21
Positive NP swabs
Patient N1 N2 N3 N4 N5 N6 N7 N8 N9 N10
Negative NP swabs
C
CDC N1 RT-qPCR assay Ct value
95 °C
60 °C
22 °C
Patient ID
CDC N2 RT-qPCR assay Ct value
Patient ID
D
projected sensitivity (%)
sensitivity = 99.7 %
sensitivity = 60.5 %
STOPCovid.v1
Ct = 30.8
STOPCovid.v2
Ct = 40.3
cobas RT-qPCR average E and Orf1ab Ct value

Fig. S8 | Lysis temperature optimization and Ct distribution of SARS-CoV-2 patient nasopharyngeal swab samples. A, STOPCovid.v1 results for 21 unique SARS-CoV-2 positive and 10 negative patient nasopharyngeal (NP) swab samples in triplicate detected by lateral flow readout. Prior to STOPCovid.v1, NP swab viral transport media (VTM) was lysed by adding an equal volume of DNA QuickExtract and heating for 10 mins at 60 °C. **B,** Same as (**A**) lysed for 10 mins at 22 °C. **C,** Comparison of Ct values for the same set of samples lysed at 95°C, 60°C and 22°C from panels (**A**) and (**B**) and Fig. S7A. For patients 9 and 10, due to the low volume of samples available, samples lysed at 60°C and 22°C were diluted 1:2 prior to STOPCovid.v1 and RT-qPCR. **D,** Scatterplot of 2,105 SARS-CoV-2 positive patient Ct values from the University of Washington Virology lab. Patient NP swab samples were tested using the cobas SARS-CoV-2 assay. The averages of ORF1ab and E-gene Ct values are shown. For samples with undetected ORF1ab or E-gene results, only the Ct value of the detected assay is shown.

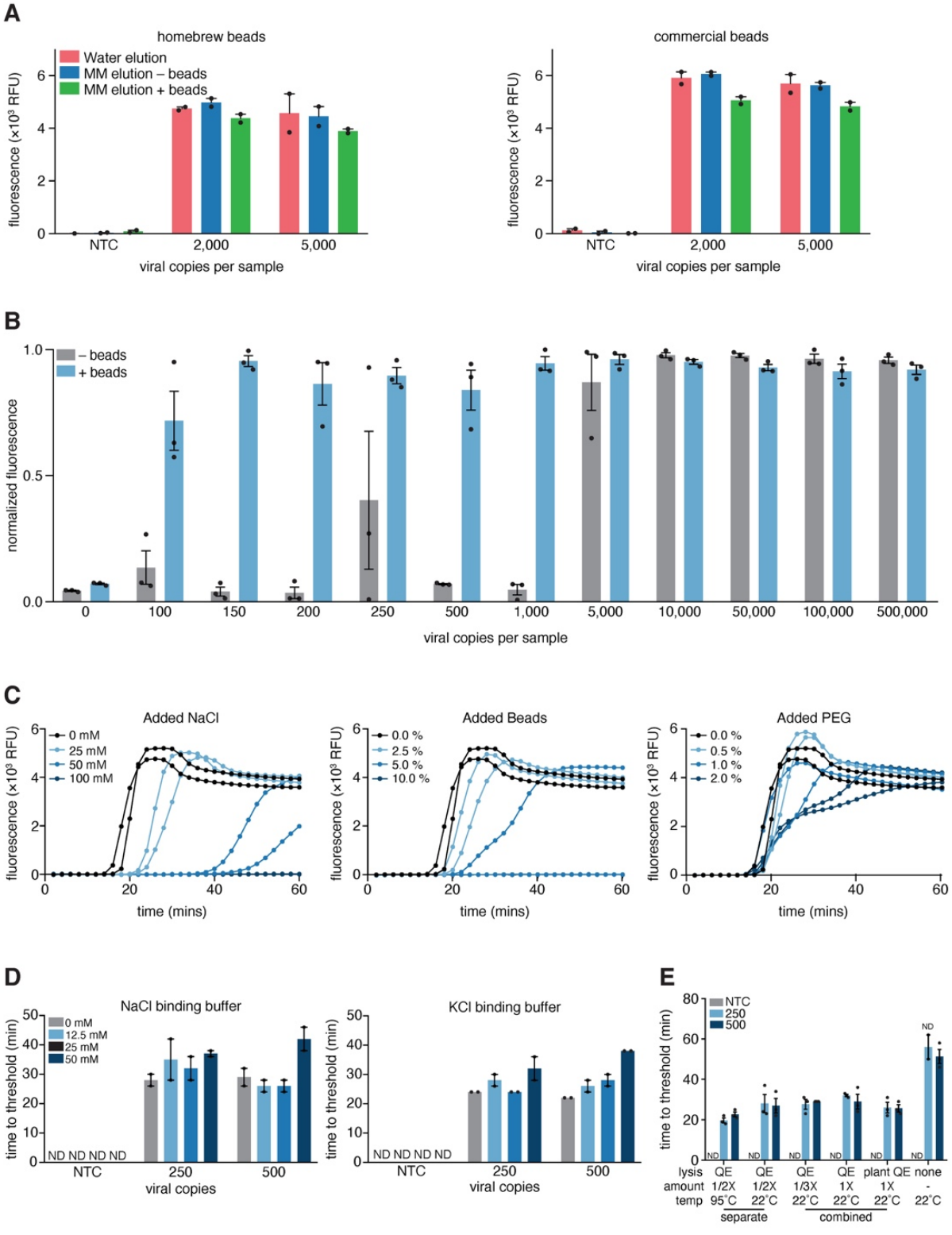

A
homebrew beads
Water elution
MM elution – beads
MM elution + beads
fluorescence (×10³ RFU)
NTC
2,000
5,000
viral copies per sample
commercial beads
B
– beads
+ beads
normalized fluorescence
0
100
150
200
250
500
1,000
5,000
10,000
50,000
100,000
500,000
viral copies per sample
C
Added NaCl
0 mM
25 mM
50 mM
100 mM
time (mins)
Added Beads
0.0 %
2.5 %
5.0 %
10.0 %
Added PEG
0.0 %
0.5 %
1.0 %
2.0 %
D
NaCl binding buffer
0 mM
12.5 mM
25 mM
50 mM
time to threshold (min)
ND ND ND ND
viral copies
KCl binding buffer
E
NTC
250
500
ND
lysis
amount
temp
QE
1/2X
95˚C
22˚C
1/3X
1X
plant
none
separate
combined

Fig. S9 | Optimization of STOPCovid.v2 by concentration sample input using magnetic bead concentration. A, Comparison of laboratory-developed [26] and commercial (Beckman SPRIselect) magnetic bead formulations for sample concentration prior to STOPCovid.v2. Samples were eluted in water, STOPCovid.v2 mastermix (MM), or STOPCovid.v2 mastermix retaining the magnetic beads. Values represent endpoint fluorescence signal at 45 mins. **B,** Determination of the sensitivity for purification with and without beads using fluorescence readout. Different amounts of SARS-CoV-2 genomic standards spiked into 200 µL of DNA QuickExtract with 1.25 µg of human RNA were used as the starting sample for both methods. **C,** Titration of different magnetic bead buffer components in STOPCovid.v2 reactions using 1,000 SARS-CoV-2 genomic standards spiked into 200 µL of DNA QuickExtract with 1.25 µg of human RNA. Fluorescence traces represent data from two biological replicates. **D,** Comparison of magnetic beads with either 1M NaCl (top) or KCl (bottom) in the buffer for sample concentration without ethanol wash prior to STOPCovid.v2. STOPCovid.v2 reactions contained varying concentrations of KCl. Different amounts of SARS-CoV-2 genomic standards spiked into 200 µL of DNA QuickExtract with 1.25 µg of human RNA were used as the starting sample. **E,** Comparison of different lysis approaches: buffer, amount of buffer, and temperature, either as a separate step or combined with magnetic bead binding. Mock SARS-CoV-2 virus (SARS-CoV-2 RNA targets in a noninfectious viral coat) were used to assess lysis efficacy. NTC, no template control. ND, not detected.

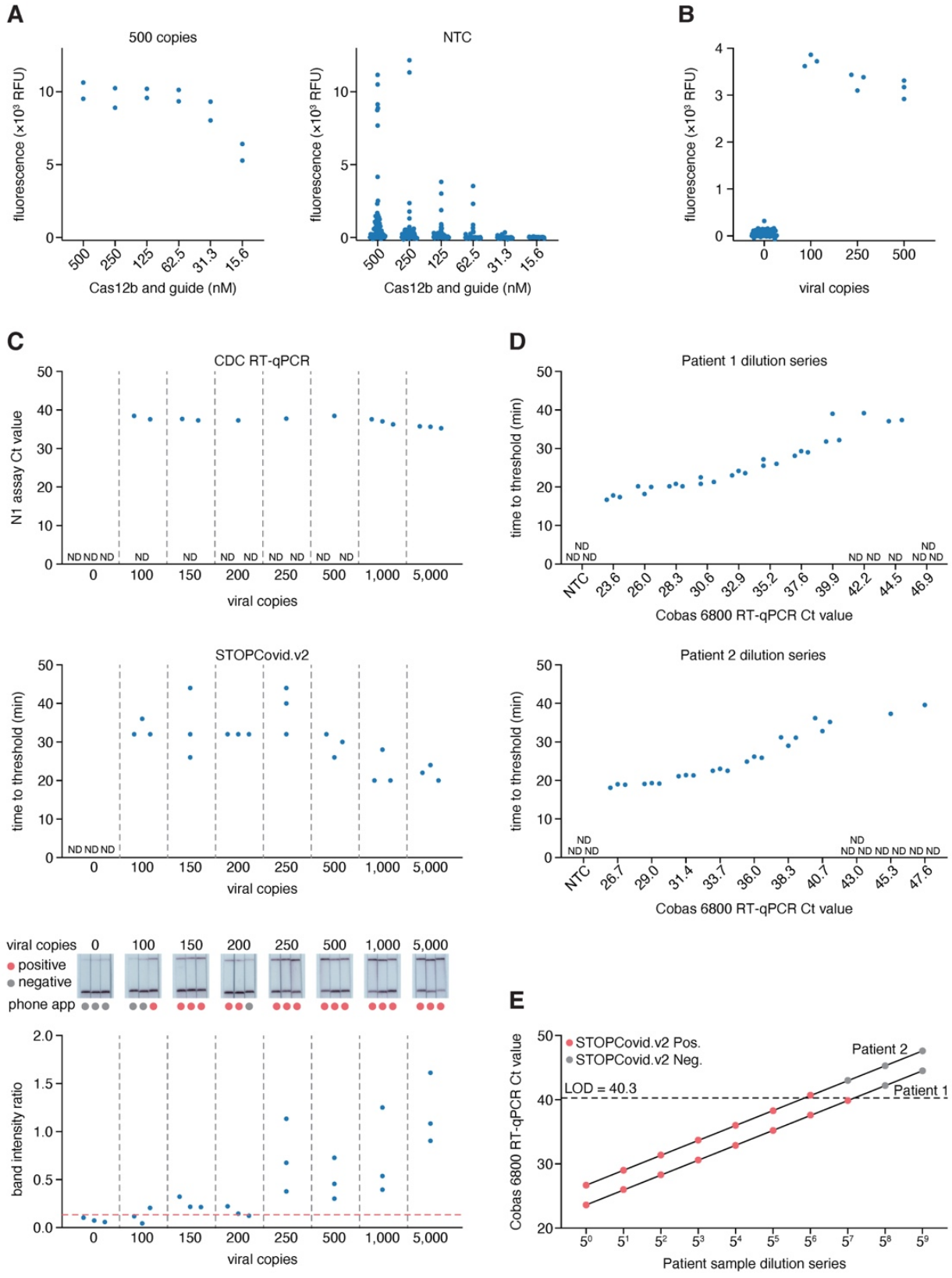

A
500 copies
NTC
fluorescence (×10³ RFU)
Cas12b and guide (nM)
500
250
125
62.5
31.3
15.6
B
viral copies
C
CDC RT-qPCR
N1 assay Ct value
ND
0
100
150
200
250
500
1,000
5,000
STOPCovid.v2
time to threshold (min)
viral copies
phone app
positive
negative
band intensity ratio
D
Patient 1 dilution series
Cobas 6800 RT-qPCR Ct value
NTC
23.6
26.0
28.3
30.6
32.9
35.2
37.6
39.9
42.2
44.5
46.9
Patient 2 dilution series
26.7
29.0
31.4
33.7
36.0
38.3
40.7
43.0
45.3
47.6
E
STOPCovid.v2 Pos.
STOPCovid.v2 Neg.
LOD = 40.3
Patient 2
Patient 1
Patient sample dilution series

Fig. S10 | Optimization of STOPCovid.v2 and limit of detection assessment. A, Titration of AapCas12b and sgRNA concentrations for effects on STOPCovid.v2 performance. 500 copies of SARS-CoV-2 genomic standards were directly added to STOPCovid.v2 reactions without magnetic bead concentration. Two biological replicates were used for 500 copies and for 62 biological replicates were used for NTC (no template control) to evaluate sensitivity and specificity. Values represent endpoint fluorescence at 45 mins. **B,** Confirmation that lowering the AapCas12b and sgRNA concentrations to 31.3 nM does not affect the sensitivity of STOPCovid.v2. Different amounts of mock SARS-CoV-2 virus (SARS-CoV-2 RNA targets in a noninfectious viral coat) were added to extraction buffer containing lysis buffer and magnetic beads. Three biological replicates were used for 100, 250, or 500 copies and 87 biological replicates were used for 0 copies to evaluate sensitivity and specificity. Values represent endpoint fluorescence at 45 mins. **C,** Comparison of the limit of detection (LOD) for the standard CDC workflow (top) to STOPCovid.v2 (middle and bottom). SARS- CoV-2 virus-like particles (SARS-CoV-2 RNA targets in a noninfectious viral coat) and 1.25 µg of human RNA were added to VTM or extraction buffer containing lysis buffer and magnetic beads. STOPCovid.v2 fluorescence readout (middle) was evaluated using time for the fluorescence signal to reach the classification threshold (see Supplementary Appendix Methods). STOPCovid.v2 lateral flow readout (bottom) was quantified and interpreted using a mobile phone application with the red dashed line indicating the threshold for classification. ND, not detected. **D,** Dilution series using two SARS-CoV-2 RNA positive patient nasopharyngeal (NP) swab viral transport medium (VTM) to estimate sensitivity of STOPCovid.v2. A swab was used to transfer 50 µL of the sample into the extraction buffer. Ct values represent projected values for each 5-fold dilution in VTM. NTC, no template control. **E,** Estimation of sensitivity by averaging the highest Ct values that were STOPCovid.v2 positive in all 3 replicates for each patient.

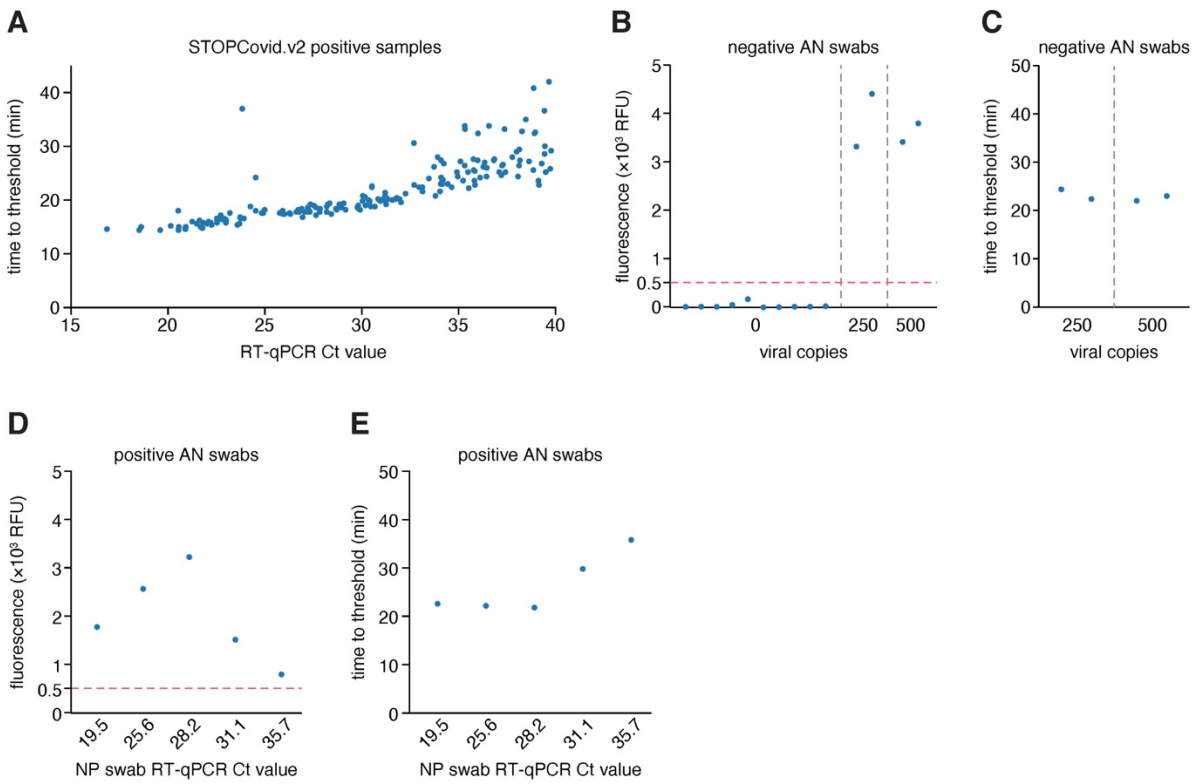
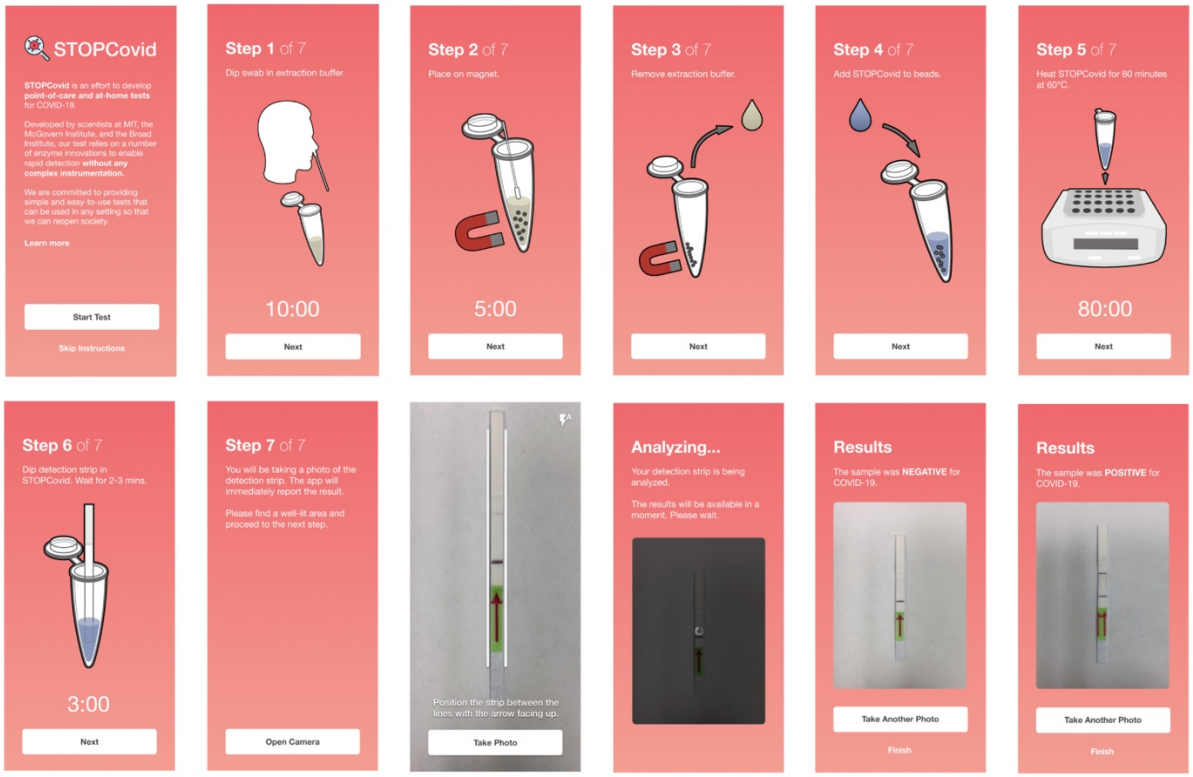


Fig. S11 | Performance evaluation of STOPCovid.v2 using SARS-CoV-2 patient samples. A, STOPCovid.v2 results for 202 SARS-CoV-2 positive patient nasopharyngeal (NP) swab viral transport medium (VTM) samples detected by fluorescence readout. A swab with 50 µL of the sample was dipped into the extraction buffer. Ct values were determined using standard RT-qPCR workflows. Time to reach the fluorescence threshold for 188 STOPCovid.v2 positive samples is shown. **B-C,** STOPCovid.v2 results for SARS-CoV-2 negative patient anterior nasal (AN) swabs. Dry AN swabs with mock SARS-CoV-2 virus were dipped in extraction buffer. Data represents endpoint fluorescence (**B**) or time to fluorescence threshold (**C**). **D-E,** STOPCovid.v2 results for 5 SARS-CoV-2 positive patient dry AN swabs. Data represents endpoint fluorescence (**D**) or time to fluorescence threshold (**E**). Ct values represent respective NP swab RT-qPCR results.

A


STOPCovid
STOPCovid is an effort to develop point-of-care and at-home tests for COVID-19.
Developed by scientists at MIT, the McGovern Institute, and the Broad Institute, our test relies on a number of enzyme innovations to enable rapid detection without any complex instrumentation.
We are committed to providing simple and easy-to-use tests that can be used in any setting so that we can reopen society.
Learn more
Start Test
Skip Instructions
Step 1 of 7
Dip swab in extraction buffer.
10:00
Next
Step 2 of 7
Place on magnet.
5:00
Next
Step 3 of 7
Remove extraction buffer.
Next
Step 4 of 7
Add STOPCovid to beads.
Next
Step 5 of 7
Heat STOPCovid for 80 minutes at 60°C.
80:00
Next
Step 6 of 7
Dip detection strip in STOPCovid. Wait for 2-3 mins.
3:00
Next
Step 7 of 7
You will be taking a photo of the detection strip. The app will immediately report the result.
Please find a well-lit area and proceed to the next step.
Open Camera
Position the strip between the lines with the arrow facing up.
Take Photo
Analyzing...
Your detection strip is being analyzed.
The results will be available in a moment. Please wait.
Results
The sample was NEGATIVE for COVID-19.
Take Another Photo
Finish
Results
The sample was POSITIVE for COVID-19.
Take Another Photo
Finish

B

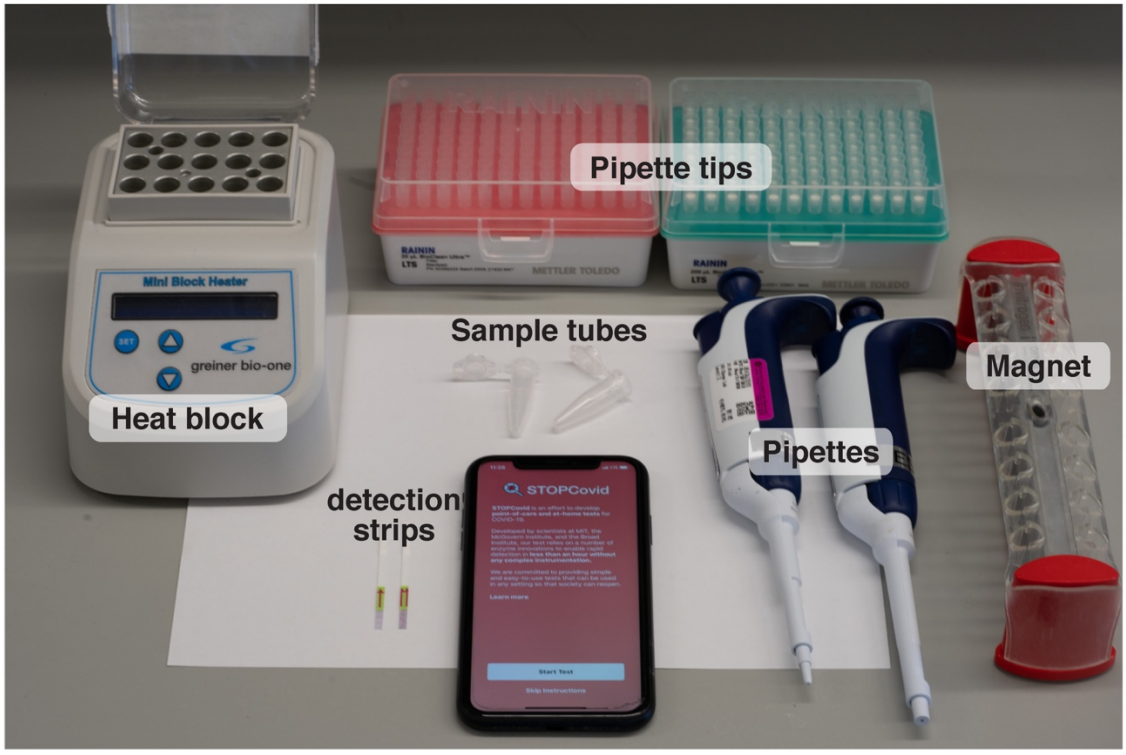

Pipette tips
Mini Block Heater
greiner bio-one
Heat block
Sample tubes
Magnet
Pipettes
detection strips
STOPCovid

Fig. S12 | Mobile phone application and equipment required for STOPCovid. A, Wireframe of mobile phone application for interpreting the STOPCovid.v2 lateral flow readout. Application starts with the STOPCovid.v2 workflow followed by camera capture of a lateral flow strip image and result interpretation. **B,** Equipment and consumables needed for running STOPCovid.v2.

Supplementary Tables

Table S1 | Primer and probe sequences

Name	Sequence
CDC N1 F	GACCCCAAAATCAGCGAAAT
CDC N1 R	TCTGGTTACTGCCAGTTGAATCTG
CDC N1 Probe	FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1
CDC N2 F	TTACAAACATTGGCCGCAAA
CDC N2 R	GCGCGACATTCCGAAGAA
CDC N2 Probe	FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1
RFP F	ACTTGATGTTGACGTTGTAGG
RFP R	AGTTCATCTACAAGGTGAAGCT
RFP Probe	FAM-CCCCGTAATGCAGAAGAAGA-BHQ1

Table S2 | LAMP target and primer set sequences

Name	Sequence
ORF1ab Set 1 target RNA	CGCAACATTAAACCAGTACCAGAGGTGAAAATACTCAATAA TTTGGGTGTGGACATTGCTGCTAATACTGTGATCTGGGACTA CAAAAGAGATGCTCCAGCACATATATCTACTATTGGTGTTTG TTCTATGACTGACATAGCCAAGAAACCAACTGAAACGATTTG TGCACCACTCACTGTCTTTTTTGATGGTAGAGTTGATGGTCA AGTAGACTTATTTAGAAATGCCCGTAATGGTGTTCTTATTAC
ORF1ab Set 2 target RNA	AATTACAGAATAATGAGCTTAGTCCTGTTGCACTACGACAGA TGTCTTGTGCTGCCGGTACTACACAAACTGCTTGCACTGATG ACAATGCGTTAGCTTACTACAACACAACAAAGGGAGGTAGG TTTGTACTTGCACTGTTATCCGATTTACAGGATTTGAAATGG GCTAGATTCCCTAAGAGTGATGGAACTGGTACTATCTATACA
S Set 1 target RNA; S Set 2 target RNA	AGATCCATCAAAACCAAGCAAGAGGTCATTTATTGAAGATC TACTTTTCAACAAAGTGACACTTGCAGATGCTGGCTTCATCA AACAATATGGTGATTGCCTTGGTGATATTGCTGCTAGAGACC TCATTTGTGCACAAAAGTTTAACGGCCTTACTGTTTTGCCAC CTTTGCTCACAGATGAAATGATTGCTCAATACACTTCTGCAC TGTTAGCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAG GTGCTGCATTACAAATACCATTTGCTATGCAAATGGCTTATA GGTTTAATGGTATTGGAGTTACACAGAATGTTCTCTATGAGA ACC
N Set 1 target RNA; Zhang et al target RNA	CCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTC CAGATGACCAAATTGGCTACTACCGAAGAGCTACCAGACGA ATTCGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAG ATGGTATTTCTACTACCTAGGAACTGGGCCAGAAGCTGGACT TCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTGCAAC TGAGGGAGCCTTGAATACACCAAAAGATCACATTGGCACCC GCAATCCTGCTAACAATGCTGCAATCGTGCTA
N Set 2 target RNA	TAAGAAATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAA AACGTACTGCCACTAAAGCATACAATGTAACACAAGCTTTCG GCAGACGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGAC CAGGAACTAATCAGACAAGGAACTGATTACAAACATTGGCC GCAAATTGCACAATTTGCCCCCAGCGCTTCAGCGTTCTTCGG AATGTCGCGCATTGGCATGGAAGTCACACCTTCGGGAACGT GGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAGAT CCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATT GACGCATACAAAACA

Broughten et al target RNA	TGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCA CTAAAGCATACAATGTAACACAAGCTTTCGGCAGACGTGGT CCAGAACAAACCCAAGGAAATTTTGGGGACCAGGAACTAAT CAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCAC AATTTGCCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCA TTGGCATGGAAGTCACACCTTCGGGAACGTGGTTGACCTACA CAGGTGCCATCAAATTGGATGACAAAGATCCAAATTTCAAA GATCAAGTCATTTTGCTGAATAAGCATATTGACGCATACAAA ACA
ORF1ab Set 1 F3	AACCAGTACCAGAGGTGAA
ORF1ab Set 1 B3	CACCATTACGGGCATTTCTA
ORF1ab Set 1 FIP	TGTGCTGGAGCATCTCTTTTGTTTTGGGTGTGGACATTGC
ORF1ab Set 1 BIP	AGCCAAGAAACCAACTGAAACGCTACTTGACCATCAACTCT ACC
ORF1ab Set 1 LoopF	GTCCCAGATCACAGTATTAGCA
ORF1ab Set 1 LoopB	TGTGCACCACTCACTGTC
ORF1ab Set 2 F3	TAATGAGCTTAGTCCTGTTGC
ORF1ab Set 2 B3	GTACCAGTTCCATCACTCTTAG
ORF1ab Set 2 FIP	AGCTAACGCATTGTCATCAGTGACGACAGATGTCTTGTGC
ORF1ab Set 2 BIP	AACACAACAAAGGGAGGTAGGTTCTAGCCCATTTCAAATCC TG
ORF1ab Set 2 LoopF	CAAGCAGTTTGTGTAGTACCG
ORF1ab Set 2 LoopB	TTGTACTTGCACTGTTATCCGA
S Set 1 F3	AAACCAAGCAAGAGGTCATT
S Set 1 B3	GTATTTGTAATGCAGCACCTG
S Set 1 FIP	TGCACAAATGAGGTCTCTAGCACAGATGCTGGCTTCATCAA
S Set 1 BIP	TTGCCACCTTTGCTCACAGATTCCAACCAGAAGTGATTGTAC
S Set 1 LoopF	CAATATCACCAAGGCAATCACC
S Set 1 LoopB	TGATTGCTCAATACACTTCTGC
S Set 2 F3	CAGATGCTGGCTTCATCAA
S Set 2 B3	GAGAACATTCTGTGTAACTCCA
S Set 2 FIP	TGGCAAAACAGTAAGGCCGTTAATGGTGATTGCCTTGGTG
S Set 2 BIP	TTCTGCACTGTTAGCGGGTACGTATTTGTAATGCAGCACCTG
S Set 2 LoopF	AAATGAGGTCTCTAGCAGCAAT
S Set 2 LoopB	AATCACTTCTGGTTGGACCTT
N Set 1 F3	CAAGGCGTTCCAATTAACAC
N Set 1 B3	GCAGCATTGTTAGCAGGA
N Set 1 FIP	ACCGTCACCACCACGAATTCCAATAGCAGTCCAGATGACC
N Set 1 BIP	CTAGGAACTGGGCCAGAAGCACCCATATGATGCCGTCT

N Set 1 LoopF	GCTCTTCGGTAGTAGCCAATT
N Set 1 LoopB	GACTTCCCTATGGTGCTAACAA
N Set 2 F3	GCTGCTGAGGCTTCTAAG
N Set 2 B3	GCGTCAATATGCTTATTCAGC
N Set 2 FIP	GCGGCCAATGTTTGTAATCAGTAGACGTGGTCCAGAACAA
N Set 2 BIP	TCAGCGTTCTTCGGAATGTCGCTGTGTAGGTCAACCACG
N Set 2 LoopF	CCTTGTCTGATTAGTTCCTGGT
N Set 2 LoopB	TGGCATGGAAGTCACACC
ACTB Set 1 F3	AAGATGAGATTGGCATGGC
ACTB Set 1 B3	GCAAGGGACTTCCTGTAAC
ACTB Set 1 FIP	CTCCAACCGACTGCTGTCTTTGGCTTGACTCAGGATTT
ACTB Set 1 BIP	CCCAAAGTTCACAATGTGGCCGCATCTCATATTTGGAATGAC
ACTB Set 1 LoopF	ACCTTCACCGTTCCAGTT
ACTB Set 1 LoopB	GGACTTTGATTGCACATTGTTG
ACTB Set 2 F3	ACTCTTCCAGCCTTCCTT
ACTB Set 2 B3	GATCCACACGGAGTACTTG
ACTB Set 2 FIP	TGTGTTGGCGTACAGGTCAACTACCTTCAACTCCATCATG
ACTB Set 2 BIP	CACCATGTACCCTGGCATTGATCTTGATCTTCATTGTGCTG
ACTB Set 2 LoopF	GGATGTCCACGTCACACT
ACTB Set 2 LoopB	GACAGGATGCAGAAGGAGA
ACTB Set 3 F3	GGCCGTCTTCCCCTCCAT
ACTB Set 3 B3	CGCAGCTCATTGTAGAAGGT
ACTB Set 3 FIP	TTGCTCTGGGCCTCGTCGCAGGCACCAGGGCGTGA
ACTB Set 3 BIP	TGAAGTACCCCATCGAGCACGGGTGGTGCCAGATTTTCTCCA
ACTB Set 3 LoopF	CCTTCTGACCCATGCCCACCA
ACTB Set 3 LoopB	CATCGTCACCAACTGGGACGAC
ACTB Set 4 F3	GCACCAGGGCGTGATG
ACTB Set 4 B3	TCAGCAGCACGGGGTG
ACTB Set 4 FIP	GAGGATGCCTCTCTTGCTCTGGGTGGGCATGGGTCAGAAG
ACTB Set 4 BIP	AGTACCCCATCGAGCACGGCACGCAGCTCATTGTAGAAGGT
ACTB Set 4 LoopF	CCTCGTCGCCCACATAGGAATC
ACTB Set 4 LoopB	TCGTCACCAACTGGGACGACA
ACTB Set 5 F3	TACGTTGCTATCCAGGCTGT
ACTB Set 5 B3	AGTCAGGTCCCGGCCA
ACTB Set 5 FIP	TGACCCCGTCACCGGAGTCCGCTATCCCTGTACGCCTCT
ACTB Set 5 BIP	CCCACACTGTGCCCATCTACGCAGGTCCAGACGCAGGAT
ACTB Set 5 LoopF	CGATGCCAGTGGTACGGCC
ACTB Set 5 LoopB	GGGGTATGCCCTCCCCCAT
ACTB Set 6 F3	GCTATCCCTGTACGCCTCT

ACTB Set 6 B3	CGGTGAGGATCTTCATGAGG
ACTB Set 6 FIP	AGATGGGCACAGTGTGGGTGGGCCGTACCACTGGCA
ACTB Set 6 BIP	GGGGTATGCCCTCCCCCATTCAGTCAGGTCCCGGC
ACTB Set 6 LoopF	CGTCACCGGAGTCCATCACGA
ACTB Set 6 LoopB	GCCATCCTGCGTCTGGACC
ACTB Set 7 F3	GGGAAATCGTGCGTGACA
ACTB Set 7 B3	GGCTGGAAGAGTGCCTCA
ACTB Set 7 FIP	GGAAGCAGCCGTGGCCATCAAGGAGAAGCTGTGCTACGT
ACTB Set 7 BIP	TCCTCCCTGGAGAAGAGCTACGCCGCTCATTGCCAATGGT
ACTB Set 7 LoopF	TTGCTCGAAGTCCAGGGCG
ACTB Set 7 LoopB	NULL
ACTB Set 8 F3	AAGGAGAAGCTGTGCTACGT
ACTB Set 8 B3	TGCCCAGGAAGGAAGGC
ACTB Set 8 FIP	CGTAGCTCTTCTCCAGGGAGGAGCCCTGGACTTCGAGCA
ACTB Set 8 BIP	CCTGACGGCCAGGTCATCACCTGGAAGAGTGCCTCAGGG
ACTB Set 8 LoopF	AGCAGCCGTGGCCATCTC
ACTB Set 8 LoopB	ATTGGCAATGAGCGGTTCCGC
ACTB Set 9 F3	GGCATCCACGAAACTACCTT
ACTB Set 9 B3	ACTTGCGCTCAGGAGGAG
ACTB Set 9 FIP	CCGCCAGACAGCACTGTGTTTCATGAAGTGTGACGTGGAC
ACTB Set 9 BIP	CACCACCATGTACCCTGGCATTTCATTGTGCTGGGTGCC
ACTB Set 9 LoopF	GGCGTACAGGTCTTTGCGGAT
ACTB Set 9 LoopB	TGCCGACAGGATGCAGAAGG
ACTB Set 10 F3	ATGCAGAAGGAGATCACTGC
ACTB Set 10 B3	TTTGCGGTGGACGATGGA
ACTB Set 10 FIP	CACACGGAGTACTTGCGCTCACTGGCACCCAGCACAATG
ACTB Set 10 BIP	CTCCATCCTGGCCTCGCTGTCGTCATACTCCTGCTTGCTG
ACTB Set 10 LoopF	NULL
ACTB Set 10 LoopB	CCACCTTCCAGCAGATGTGGAT
GAPDH Set 1 F3	AAAGGGTCATCATCTCTGC
GAPDH Set 1 B3	ATGATGTTCTGGAGAGCC
GAPDH Set 1 FIP	GCTAAGCAGTTGGTGGTGGAACCATGAGAAGTATGACAAC
GAPDH Set 1 BIP	GGTATCGTGGAAGGACTCATCCATCCACAGTCTTCTGG
GAPDH Set 1 LoopF	TTGCTGATGATCTTGAGGC
GAPDH Set 1 LoopB	CAGTCCATGCCATCACTG
GAPDH Set 2 F3	ATGAGAAGTATGACAACAGCC
GAPDH Set 2 B3	GATGATGTTCTGGAGAGCC
GAPDH Set 2 FIP	TGTCATGGATGACCTTGGCAGATCATCAGCAATGCCTC
GAPDH Set 2 BIP	GGTATCGTGGAAGGACTCATGGCCATCCACAGTCTTCTG

GAPDH Set 2 LoopF	GCTAAGCAGTTGGTGGTG
GAPDH Set 2 LoopB	ACAGTCCATGCCATCACT
GAPDH Set 3 F3	GTGAAGGTCGGAGTCAAC
GAPDH Set 3 B3	CTCCATGGTGGTGAAGAC
GAPDH Set 3 FIP	GGGTGGAATCATATTGGAACATAGTGGATATTGTTGCCATCA
GAPDH Set 3 BIP	CACCGTCAAGGCTGAGAAATCTCGCTCCTGGAAGAT
GAPDH Set 3 LoopF	ACCATGTAGTTGAGGTCAATG
GAPDH Set 3 LoopB	GGAAGCTTGTCATCAATGGA
GAPDH Set 4 F3	ACCATGAGAAGTATGACAACAG
GAPDH Set 4 B3	GATGATGTTCTGGAGAGCC
GAPDH Set 4 FIP	TGTCATGGATGACCTTGGCCTCAAGATCATCAGCAATGC
GAPDH Set 4 BIP	GGTATCGTGGAAGGACTCATGGCCATCCACAGTCTTCTG
GAPDH Set 4 LoopF	GCTAAGCAGTTGGTGGTG
GAPDH Set 4 LoopB	ACAGTCCATGCCATCACT
GAPDH Set 5 F3	GTGAAGGTCGGAGTCAAC
GAPDH Set 5 B3	GACTCCACGACGTACTCA
GAPDH Set 5 FIP	TGGGTGGAATCATATTGGAACAGGATATTGTTGCCATCAATG AC
GAPDH Set 5 BIP	ACCGTCAAGGCTGAGAACGATCTCGCTCCTGGAAGA
GAPDH Set 5 LoopF	AACCATGTAGTTGAGGTCAATG
GAPDH Set 5 LoopB	GGAAGCTTGTCATCAATGGAA
GAPDH Set 6 F3	TGGTATCGTGGAAGGACTCA
GAPDH Set 6 B3	CGTTCAGCTCAGGGATGAC
GAPDH Set 6 FIP	ACGCCACAGTTTCCCGGAGGTGACCACAGTCCATGCCA
GAPDH Set 6 BIP	CGCGGGGCTCTCCAGAACACTTGCCCACAGCCTTGG
GAPDH Set 6 LoopF	ACAGTCTTCTGGGTGGCAGTGA
GAPDH Set 6 LoopB	CATCCCTGCCTCTACTGGCGC
GAPDH Set 7 F3	TCGTGGAAGGACTCATGACC
GAPDH Set 7 B3	CGTTCAGCTCAGGGATGAC
GAPDH Set 7 FIP	ACGCCACAGTTTCCCGGAGGACAGTCCATGCCATCACTGC
GAPDH Set 7 BIP	CGCGGGGCTCTCCAGAACACTTGCCCACAGCCTTGG
GAPDH Set 7 LoopF	GCCATCCACAGTCTTCTGGGTG
GAPDH Set 7 LoopB	CATCCCTGCCTCTACTGGCGC
GAPDH Set 8 F3	GACCACAGTCCATGCCATC
GAPDH Set 8 B3	GGCCATGCCAGTGAGCT
GAPDH Set 8 FIP	TGTTCTGGAGAGCCCCGCGACTGCCACCCAGAAGACT
GAPDH Set 8 BIP	CATCCCTGCCTCTACTGGCGCTCCCGTTCAGCTCAGGGAT
GAPDH Set 8 LoopF	ACGCCACAGTTTCCCGGAGG
GAPDH Set 8 LoopB	TGCCAAGGCTGTGGGCAA

GAPDH Set 9 F3	GCGCTGCCAAGGCTGT
GAPDH Set 9 B3	CCCAGGATGCCCTTGAGG
GAPDH Set 9 FIP	GTGGGGACACGGAAGGCCAGGGCAAGGTCATCCCTGA
GAPDH Set 9 BIP	TGCCAACGTGTCAGTGGTGGACTGCTTCACCACCTTCTTGA
GAPDH Set 9 LoopF	CCAGTGAGCTTCCCGTTCAGC
GAPDH Set 9 LoopB	TGCCGTCTAGAAAAACCTGCCA

Table S3 | Cas12b Protein Sequences

Protein	Sequence
AacCas 12b	MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYR RSPNGDGEQECDKTAEECKAELLERLRARQVENGHRGPAGSDDELLQLAR QLYELLVPQAIGAKGDAQQIARKFLSPLADKDAVGGLGIAKAGNKPRWVR MREAGEPGWEEEKEKAETRKSADRTADVLRALADFGLKPLMRVYTDSEMS SVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQEYAKLVEQ KNRFEQKNFVGQEHLVHLVNQLQQDMKEASPGLESKEQTAHYVTGRALRG SDKVFEKWGKLAPDAPFDLYDAEIKNVQRRNTRRFGSHDLFAKLAEPEYQA LWREDASFLTRYAVYNSILRKLNHAKMFATFTLPDATAHPIWTRFDKLGGN LHQYTFLFNEFGERRHAIRFHKLLKVENGVAREVDDVTVPISMSEQLDNLLP RDPNEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRRGARDVYL NVSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSDYLAEHPDD GKLGSEGLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNSKGRVPFFFPIK GNDNLVAVHERSQLLKLPGETESKDLRAIREERQRTLRQLRTQLAYLRLLV RCGSEDVGRRERSWAKLIEQPVDAANHMTPDWREAFENELQKLKSLHGICS DKEWMDAVYESVRRVWRHMGKQVRDWRKDVRSGERPKIRGYAKDVVG GNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRFAITLREHIDHAKED RLKKLADRIIMEALGYVYALDERGKGKWVAKYPPCQLILLEELSEYQFNND RPPSENNQLMQWSHRGVFQELINQAQVHDLLVGTMYAAFSSRFDARTGAP GIRCRRVPARCTQEHNPEPFPWWLNKFVVEHTLDACPLRADDLIPTGEGEIF VSPFSAEEGDFHQIHADLNAAQNLQQRLWSDFDISQIRLRCDWGEVDGELV LIPRLTGKRTADSYSNKVFYTNTGVTYYERERGKKRRKVFAQEKLSEEEAE LLVEADEAREKSVVLMRDPSGIINRGNWTRQKEFWSMVNQRIEGYLVKQIR SRVPLQDSACENTGDI*
AapCas 12b	MAVKSMKVKLRLDNMPEIRAGLWKLHTEVNAGVRYYTEWLSLLRQENLY RRSPNGDGEQECYKTAEECKAELLERLRARQVENGHCGPAGSDDELLQLA RQLYELLVPQAIGAKGDAQQIARKFLSPLADKDAVGGLGIAKAGNKPRWV RMREAGEPGWEEEKAKAEARKSTDRTADVLRALADFGLKPLMRVYTDSD MSSVQWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGEAYAKLV EQKSRFEQKNFVGQEHLVQLVNQLQQDMKEASHGLESKEQTAHYLTGRAL RGSDKVFEKWEKLDPDAPFDLYDTEIKNVQRRNTRRFGSHDLFAKLAEPKY QALWREDASFLTRYAVYNSIVRKLNHAKMFATFTLPDATAHPIWTRFDKLG GNLHQYTFLFNEFGEGRHAIRFQKLLTVEDGVAKEVDDVTVPISMSAQLDD LLPRDPHELVALYFQDYGAEQHLAGEFGGAKIQYRRDQLNHLHARRGARD VYLNLSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSDYLAEHP DDGKLGSEGLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNSEGRVPFCF PIEGNENLVAVHERSQLLKLPGETESKDLRAIREERQRTLRQLRTQLAYLRL LVRCGSEDVGRRERSWAKLIEQPMDANQMTPDWREAFEDELQKLKSLYGI CGDREWTEAVYESVRRVWRHMGKQVRDWRKDVRSGERPKIRGYQKDVV GGNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRFAITLREHIDHAKE DRLKKLADRIIMEALGYVYALDDERGKGKWVAKYPPCQLILLEELSEYQFN NDRPPSENNQLMQWSHRGVFQELLNQAQVHDLLVGTMYAAFSSRFDARTG APGIRCRRVPARCAREQNPEPFPWWLNKFVAEHKLDGCPLRADDLIPTGEG EFFVSPFSAEEGDFHQIHADLNAAQNLQRRLWSDFDISQIRLRCDWGEVDGE

	PVLIPRTTGKRTADSYGNKVFYTKTGVTYYERERGKKRRKVFAQEELSEEE AELLVEADEAREKSVVLMRDPSGIINRGDWTRQKEFWSMVNQRIEGYLVK QIRSRVRLQESACENTGDI*AAALEARKEAELAAATAEQ*

Table S4 | Cas12b spacer sequences

Name	sgRNA Sequence
Orf1ab Set 1 Guide 1	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTA GTCCCAGATCACAGTATT
Orf1ab Set 1 Guide 2	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT AGTCCCAGATCACAGTAT
Orf1ab Set 1 Guide 3	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTG TAGTCCCAGATCACAGTA
Orf1ab Set 1 Guide 4	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCT GCTAATACTGTGATCTGG
Orf1ab Set 1 Guide 5	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGC TATGTCAGTCATAGAACA
Orf1ab Set 1 Guide 6	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTT GGCTATGTCAGTCATAGA
Orf1ab Set 1 Guide 7	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCT TGGCTATGTCAGTCATAG
Orf1ab Set 1 Guide 8	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT TTCTTGGCTATGTCAGTC
Orf1ab Set 1 Guide 9	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT GTTTGTTCTATGACTGAC
Orf1ab Set 1 Guide 10	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACAG TTGGTTTCTTGGCTATGT
Orf1ab Set 1 Guide 11	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCA GTTGGTTTCTTGGCTATG
Orf1ab Set 1 Guide 12	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT TCTATGACTGACATAGCC
Orf1ab Set 1 Guide 13	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTT CTATGACTGACATAGCCA
Orf1ab Set 1 Guide 14	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTA TGACTGACATAGCCAAGA

Orf1ab Set 1 Guide 15	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT GCACCACTCACTGTCTTT
Orf1ab Set 1 Guide 16	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTG CACCACTCACTGTCTTTT
S Set 1 Guide 1	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTG CACAAATGAGGTCTCTAG
S Set 1 Guide 2	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCC TTGGTGATATTGCTGCTA
S Set 1 Guide 3	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT GCACAAATGAGGTCTCTA
S Set 1 Guide 4	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTG TGCACAAATGAGGTCTCT
S Set 1 Guide 5	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACAA CTTTTGTGCACAAATGAG
S Set 1 Guide 6	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT GCACAAAAGTTTAACGGC
S Set 1 Guide 7	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTG CACAAAAGTTTAACGGCC
S Set 1 Guide 8	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACAA CGGCCTTACTGTTTTGCC
S Set 1 Guide 9	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACAC GGCCTTACTGTTTTGCCA
S Set 1 Guide 10	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCT GTTTTGCCACCTTTGCTC
S Set 1 Guide 11	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTG CCACCTTTGCTCACAGAT
S Set 1 Guide 12	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGC CACCTTTGCTCACAGATG
S Set 1 Guide 13	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCC ACCTTTGCTCACAGATGA

S Set 1 Guide 14	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGC TCACAGATGAAATGATTG
S Set 1 Guide 15	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCT CACAGATGAAATGATTGC
N Set 1 Guide 1	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT CTGGTAGCTCTTCGGTAG
N Set 1 Guide 2	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCC GTCACCACCACGAATTCG
N Set 1 Guide 3	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACAC CGTCACCACCACGAATTC
N Set 1 Guide 4	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTA CCGTCACCACCACGAATT
N Set 1 Guide 5	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACAT TTTACCGTCACCACCACG
N Set 1 Guide 6	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCA TTTTACCGTCACCACCAC
N Set 1 Guide 7	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGA CTGAGATCTTTCATTTTA
N Set 1 Guide 8	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT GGTGGTGACGGTAAAATG
N Set 1 Guide 9	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCT AGGTAGTAGAAATACCAT
N Set 1 Guide 10	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTG GCCCAGTTCCTAGGTAGT
N Set 1 Guide 11	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCT ACTACCTAGGAACTGGGC
N Set 1 Guide 12	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTA CTACCTAGGAACTGGGCC
N Set 2 Guide 1	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCT GGTCCCCAAAATTTCCTT

N Set 2 Guide 2	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT TCCTGGTCCCCAAAATTT
N Set 2 Guide 3	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTC TGATTAGTTCCTGGTCCC
N Set 2 Guide 4	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCT TGTCTGATTAGTTCCTGG
N Set 2 Guide 5	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTA ATCAGTTCCTTGTCTGAT
N Set 2 Guide 6	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGT AATCAGTTCCTTGTCTGA
N Set 2 Guide 7	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCG GCCAATGTTTGTAATCAG
N Set 2 Guide 8	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGC GGCCAATGTTTGTAATCA
N Set 2 Guide 9	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTG CAATTTGCGGCCAATGTT
N Set 2 Guide 10	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGC CGCAAATTGCACAATTTG
N Set 2 Guide 11	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCG AAGAACGCTGAAGCGCTG
N Set 2 Guide 12	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCA CAATTTGCCCCCAGCGCT
N Set 2 Guide 13	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGC CCCCAGCGCTTCAGCGTT
N Set 2 Guide 14	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACCC CCCAGCGCTTCAGCGTTC
N Set 2 Guide 15	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACAG CGTTCTTCGGAATGTCGC
N Set 2 Guide 16	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACTT CGGAATGTCGCGCATTGG

N Set 2 Guide 17	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGG AATGTCGCGCATTGGCAT
N Set 2 Guide 18	GTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCC AGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGC ATGGAAGTCACACCTTCG

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Author contributions

J.J., A.L., J.S.G., O.O.A., and F.Z. conceived the study and designed experiments. J.J., A.L., J.S.G., and O.O.A. performed most of the experiments and analyzed data. M. Saito and M. Segel purified protein and prepared reagents for collaborators. N.K. performed patient sample validation experiments and dilution series at the University of Washington under the supervision of A.L.G. and K.R.J.. A.E.W. and D.T.H. collected positive patient AN swabs. R.P.J.B. and A.R. performed image quantification and developed the mobile application. R.K.M. assisted with IRB application, sample procurement, collaboration coordination. G.F. performed the conservation analysis. E.I.I. and R.N.K. purified guide RNAs. N.K., R.B., M.W.H., X.G.Y., J.Z.L., B.D.W., A.L.G., and K.R.J. provided patient samples. J.J., A.L., J.S.G., O.O.A., and F.Z. wrote the manuscript with help from all authors.

Ethical Statement

This study was performed under the MIT IRB 2005000151, the University of Washington IRB STUDY00010205, and Partners Healthcare IRB 2020P000804.

Declaration of conflicts of interest

F.Z., O.O.A., J.S.G., J.J., and A.L. are inventors on patent applications related to this technology filed by the Broad Institute, with the specific aim of ensuring this technology can be made freely, widely, and rapidly available for research and deployment. O.O.A., J.S.G., and F.Z. are co-founders, scientific advisors, and hold equity interests in Sherlock Biosciences, Inc. F.Z. is also a co-founder of Editas Medicine, Beam Therapeutics, Pairwise Plants, and Arbor Biotechnologies.

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