Supplemental Materials for

Sensitive tracking of circulating viral RNA through all stages of SARS-CoV-2 infection

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Supplemental Table 1. Oligonucleotide sequences used in this study.

Oligonucleotide ID	Sequence	Target
ORF1ab-F	CCCTGTGGGTTTTACACTTAA	ORF1ab
ORF1ab-R	ACGATTGTGCATCAGCTGA	ORF1ab
gRNA -ORF1ab	UAAUUUCUACUCUUGUAGAUCACAUACCGCAGACGGUACAGAC	ORF1ab
RPP30-F	CTCGGATCCATCTCACTGCAA	RPP30
RPP30-R	TGCAACAACATCATAGAGCCG	RPP30
gRNA-RPP30	UAAUUUCUACUCUUGUAGAUAGAGCAACUUCUUCAAGGGCCC	RPP30
Probe	FAM-TTTTTTTTTTT-BHQ	

Underlined sequence indicates the region complementary to ORF1b or RPP30 target sequences

Supplemental Table 2. SnapGene (version 5.0.8) *in silico* analysis results for Orf1ab specificity of CRISPR-ABC primers and gRNA using the indicated genomic viruses and virus RNA entries for SARS-CoV-2 isolates from different countries, other coronaviruses, and common respiratory viruses.

Virus	Gene Bank No.	Isolation country	Primer specificity	gRNA specificity
SARS-CoV-2	MN908947.3	China	+	+
	LC529905.1	Japan	+	+
	MN985325.1	United States	+	+
	MT350282.1	Brazil	+	+
	MT233519.1	Spain	+	+
	MT470100.1	France	+	+
	MT007544.1	Australia	+	+
	MT324062.1	South Africa	+	+
SARS-CoV	NC_004718.3		-	-
MERS-CoV	NC_019843.3		-	-
HCoV-229E	NC_002645.1		-	-
HCoV-OC43	NC_006213.1		-	-
HCoV-HKU1	NC_006577.2		-	-
HCoV-NL63	NC_005831.2		-	-
RSV	NC_001803.1		-	-
Influenza A	NC_002023.1		-	-
Influenza B	NC_002204.1		-	-

Symbols indicate primers judge able (+) or not able (-) to amplify or bind sequence derived from the indicated viruses using following criteria: at least 10 matching bases separated by no mismatches, no more than 5 total mismatches, and a $T_m > 50$ °C.

Supplemental Table 3. Sources from viruses and viral RNA analyzed in Figure 2B.

No.	Organism	Source	Item.	Type
1	SARS-CoV	BEI Resources	NR-52346	Genomic RNA
2	MERS-CoV	BEI Resources	NR-45843	Genomic RNA
3	HCoV 229E	BEI Resources	NR-52726	Virus
4	HCoV OC43	BEI Resources	NR-52725	Virus
5	HCoV HKU1	ATCC	VR-3262SD	Synthetic RNA
6	HCoV NL63	BEI Resources	NR-470	Genomic RNA
7	RSV	BEI Resources	NR-43976	Genomic RNA
8	Influenza A	BEI Resources	NR-2760	Genomic RNA
9	Influenza B	BEI Resources	NR-10048	Genomic RNA

Supplemental Table 4. Gene targets and analytical sensitivity of reported SARS-CoV-2 nucleic assays.

Method	CRISPR-ABC	RT-qPCR	RT-qPCR	RT-LAMP	LAMP-CRISPR	RT-PRA-CRISPR	INSIGHT
LoD (copies/test) *	1	5, 16	15	100	20	50	10
LoD (copies/μL)	0.2	1, 3	1.5	20	10	10	10
Volume analyzed	5	5	10	5	2	5	1
Virus target gene(s)	ORF1ab	N1, N2	N	Nsp3	E, N	RdRP, ORF1ab	S
Sample type	Plasma	Nasal & throat swabs	Nasopharyngeal & nasal swabs	Spiked sample	Nasal swab	Spiked sample	Spiked sample
Read out	Fluorescence	Fluorescence	Fluorescence	Colorimetric	Colorimetric	Fluorescence	Fluorescence
Source	This work	IDT #	Zymo $	Journal (1)	Journal (2)	Journal (3)	Journal (4)

* LoD (copy/test) was directly reported or calculated using reported LoD concentrations and analyzed sample volumes.

Reported LoDs for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (IDT) with a QIAmp DSP Viral RNA Mini Kit or an EZ1 DSP Kit.

$ Reported LoD of the Zymo Quick SARS-CoV-2 RT-PCR Kit.

Supplemental Table 5. Demographic data of the NHP model populations.

Group	Animal ID	Sex	Age (years)	Species
1	NB86	Male	7.53	African Green Monkey
	NB78	Male	7.53	African Green Monkey
	NB76	Male	7.53	African Green Monkey
	NC06	Male	7.53	African Green Monkey
2	KN90	Male	7.15	Indian Rhesus Macaque
	JG28	Male	10.18	Indian Rhesus Macaque
	IR12	Male	10.85	Indian Rhesus Macaque
	IJ01	Male	11.15	Indian Rhesus Macaque

Supplemental Table 6. Demographics and clinical characteristics of COVID-19 cases analyzed in Figure 4 and Supplemental Figure 7.

Patient Characteristics*	**Hospitalized (n=25)**	**Non-Hospitalized (n=9)**	**P-value[#]**
Age, Median [quartile Q1, Q3]	68 [57, 74]	40 [36, 46]	<0.0001
Female, n (%)	13 (52.0%)	4 (44.4%)	0.6975
Days from symptom onset to sample collection, Median [Q1, Q3]	7 [5, 7]	5 [2, 5]	0.1823
COVID-19 related symptoms, n (%)			
Mild symptoms (e.g., fever, cough, fatigue, headache, sore throat, muscle or joint pain, chills or dizziness)	25 (100%)	9 (100%)	-
Severe symptoms (e.g., shortness of breath, loss of appetite, confusion, persistent chest pain or pressure)	19 (76.0%)	4 (44.4%)	-
Oxygen requirement at time of sample collection, n (%)			
Room air	4 (16%)	-	-
Ventilator support	18(72%)	-	-
COVID-19 resolution, n (%)			
Discharged	22 (88%)	-	-
Died	3 (12%)	-	-

*All COVID-19 cases were diagnosed by positive nasal/nasopharyngeal swab RT-PCR results using FDA EUA-approved assays. RT-PCR kit suppliers/designers and their targets were: RealStar (S gene and E gene); Cobas (ORF1 a/b and E gene); Panther Fusion (OFR1 a/b gene), Xpert Xpress (N2 and E), and the CDC (N1 and N2).

[#] P <0.05 by Mann Whitney U test (age and days from symptom onset) or Chi-square tests (sex).

Supplemental Table 7. Detection performance comparison of CRISPR-ABC and RT-qPCR in blood collected from 34 COVID-19 patients and 125 non-COVID-19 patients.

Blood assay results:	COVID-19 Cases	Non-COVID-19 Cases*
CRISPR-ABC Positive	31	1
CRISPR-ABC Negative	3	124
Sensitivity	91.2%	--
Specificity	--	99.2%
RT-qPCR Positive	15	0
RT-qPCR Negative	19	125
Sensitivity	44.2%	--
Specificity	--	100%

* Samples collected before COVID-19 outbreak.

[#] CDC RT-qPCR assay targeting the SARS-CoV-2 N1 gene region.

Supplemental Table 8. Demographics of children described in Figure 5A.

Case ID	Age (years)	Sex	Nasal swab assay*	First nasal swab test results	Days from first nasal swab to first serum sample	First CRISPR-ABC results	First IgG test results #
P1	14	Female	CDC	Negative	1	Negative	Negative
P2	0.5	Male	ID NOW	Negative	1	Negative	Negative
P3	12	Female	CDC	Negative	1	Negative	Negative
P4	14	Male	CDC	Negative	1	Negative	Negative
P5	14	Male	CDC	Negative	1	Negative	Negative
P6	3.6	Female	CDC	Negative	5	Negative	Negative
P7	11	Female	CDC	Negative	1	Negative	Negative
P8	14	Male	CDC	Negative	-1	Negative	Negative
P9	15	Female	CDC	Negative	0	Negative	Negative
P10	5	Male	ID NOW	Negative	2	Negative	Negative
P11	14	Male	ID NOW	Negative	4	Negative	Negative
P12	14	Female	ID NOW	Negative	0	Negative	Negative
P13	17	Male	ID NOW & Cobas	Negative	0	Negative	Negative
P14	10	Male	CDC	Negative	1	Negative	Negative
P15	17	Female	CDC	Negative	1	Negative	Negative
P16	15	Female	ID NOW	Negative	0	Negative	Negative
P17	15	Male	ID NOW	Negative	0	Negative	Negative
P18	17	Male	CDC	Negative	0	Negative	Negative
P19	13	Female	ID NOW	Negative	0	Negative	Negative
P20	14	Female	ID NOW	Negative	0	Negative	Negative
P21	4	Female	CDC	Negative	1	Negative	Negative

P22	2.8	Female	CDC	Negative	2	Negative	Negative
P23	13	Female	ID NOW	Negative	0	Negative	Negative
P24	14	Female	ID NOW	Negative	0	Negative	Negative
P25	4	Male	ID NOW	Negative	-3	Negative	Negative
P26	13	Female	ID NOW	Negative	1	Negative	Negative
P27	17	Female	CDC	Negative	1	Negative	Negative
P28	1.3	Male	ID NOW	Negative	6	Positive	Negative
P29	1.5	Male	ID NOW	Negative	1	Positive	Positive
P30	4	Male	CDC	Negative	-5	Positive	Positive
P31	6	Male	ID NOW	Positive	-9	Positive	Negative
P32	0.17	Female	ID NOW	Positive	5	Positive	Positive

* All these test assays are approved by FDA for EUA. CDC, indicated the CDC RT-qPCR assay that target N1 and N2 gene; ID NOW, indicated ID NOW COVID-19 assay that target RdRp gene, and the Cobas, indicated Cobas SARS-CoV-2 RT-PCR assay that target ORF1ab and E gene.

The IgG was tested as described in the Method.

Supplemental Table 9. Potential applications and benefits of CRISPR-ABC.

Application	Potential Benefit
COVID-19 diagnosis	CRISPR-ABC assays could serve as a secondary COVID-19 diagnostic, particularly for suspected cases with negative RT-qPCR nasal swab results, since detectable levels of SARS-CoV-2 RNA appears to persist longer in the circulation than in nasal tissue and may thus detect ongoing lower respiratory tract or extrapulmonary infections longer detectable by nasal swab RT-qPCR assays.
COVID-19 prognosis	RT-qPCR studies indicate that the detection and abundance of SARS-CoV-2 RNA in the circulation correlates with and predicts COVID-19 severity. RT-qPCR has poor sensitivity when applied to detect SARS-CoV-2 RNA in serum and plasma samples, limiting the clinical utility of this potential prognostic biomarker. The enhanced sensitivity of CRISPR-ABC assays, however, render such analyses practical.
COVID-19 evaluation	Treatment evaluation: SARS-CoV-2 RNAemia is expected to reflect virus and/or viral RNA shedding from infected pulmonary, and potentially extrapulmonary, tissue that serve as an indicator of disease severity. RNAemia decreases in response to treatment should thus serve as a direct measure of positive treatment responses. Disease clearance: SARS-CoV-2 RNAemia may also better reflect disease clearance than nasal swab results, given that viral RNA levels in nasal tissue can decrease well before those in the lower respiratory tract. CRISPR-ABC analysis of plasma or serum samples may thus provide a better means to evaluate disease clearance than RT-qPCR results for nasal swab samples.

Supplemental Figure 1.

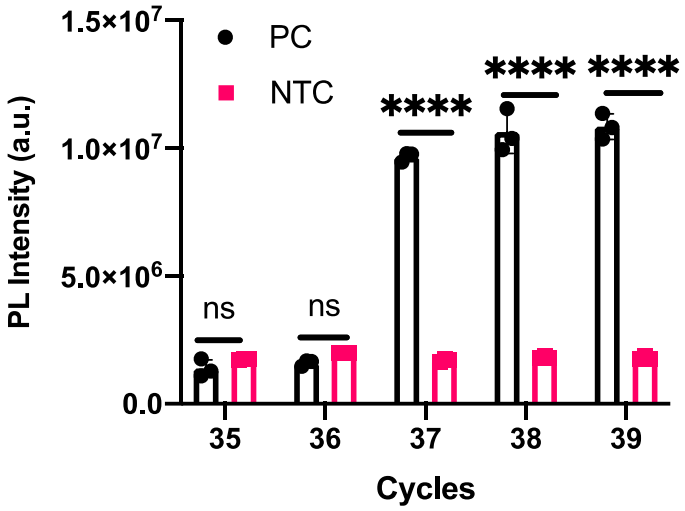

Fig. S1. PCR amplification cycles required for consistent detection a PC (positive control) at a concentration of 0.2 copy/μL (estimated as single copy target per test). RT-PCR reactions performed with a synthetic SARS-CoV-2 RNA sample diluted to contain a single target gene and nuclease-free water (NTC, no template control), using 35+ amplification cycles, and then directly added to CRISPR Cas12a reactions and analyzed for target-specific fluorescence. Since fluorescent signal was consistently detected only with ≥ 37 PCR cycles, all subsequent assays used 38 PCR cycles prior to CRISPR detection. Bar graphs represents the mean ± SD, of three technical replicates. (ns, $p > 0.05$; ****, $p < 0.0001$ by unpaired t-test comparisons between the PC and NTC samples at different cycles as corrected for multiple comparisons by the Holm-Sidak method).

Supplemental Figure 2.

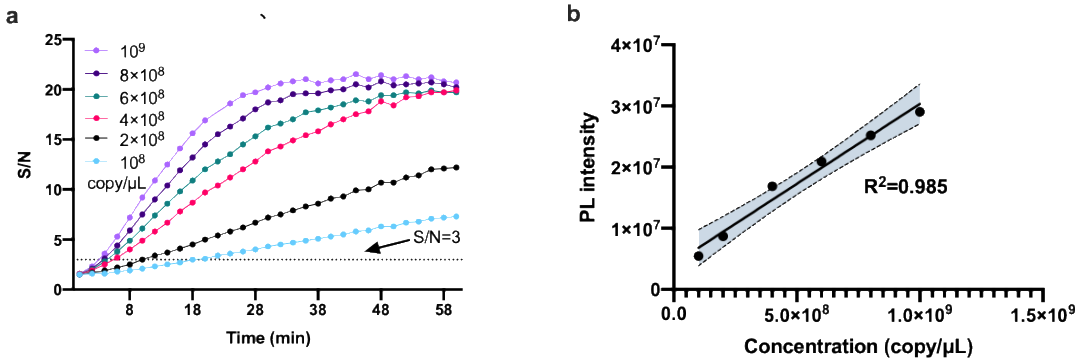

Fig. S2. CRISPR reaction time optimization across a range of input PCR amplicon concentrations. **a**, A known amount of the ORF1ab DNA amplicon was spiked into enzyme-free RT-PCR reactions and serially diluted to obtain RT-PCR sample-based concentration standards (0, 10^8, 2×10^8, 4×10^8, 6×10^8, 8×10^8, and 10^9 copies/μL). A 20μL aliquot of each standard was then mixed with 10 μL of CRISPR and analyzed every 2 min for 1 h in a plate reader that held samples at 37°C to evaluate CRISPR-mediated probe conversion in response to target concentration. CRISPR activity was expressed as signal-to-noise (S/N), using the blank (0 copies/μL) sample to evaluate the template-independent change in fluorescence background over time. The lowest concentration standard (10^8 copy/μL), representing amount of target predicted from a single target, reached the minimum S/N requirement (S/N=3) after 18 min, and the S/N of highest concentration standard reached a stable plateau at 34 min. **b**, CRISPR-mediated fluorescent signal exhibited a strong linear relationship with input template concentration when evaluated after 20 min, leading to this time point being chosen all further analyses. The shaded area indicates the 95% confidence interval of the fitted line..

Supplemental Figure 3.

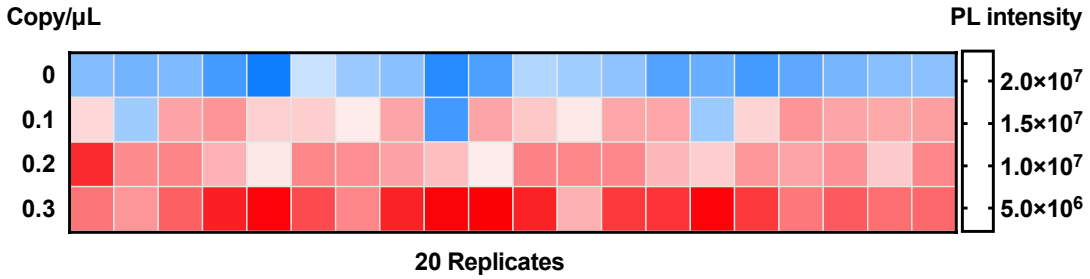

Fig. S3. Heat map of CRISPR-ABC reproducibility for SARS-COV-2 RNA detection in replicate samples of healthy human plasma spiked with 0 to 0.3 copies/μL of SARS-CoV-2 RNA. A positive detection rate >95% was not achieved in samples containing < 0.2 copies/μL, implying the LoD of CRISPR-ABC for SARS-CoV-2 RNA in plasma is approximately 0.2 copies/μL.

Supplemental Figure 4.

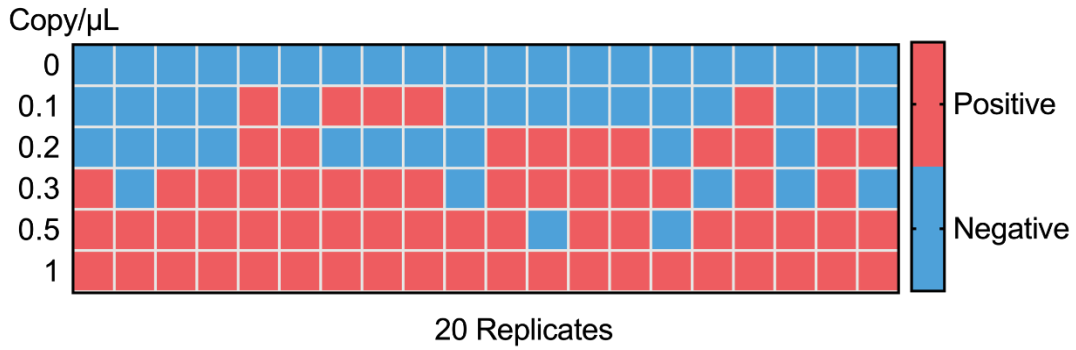

Fig. S4. Schematic of RT-qPCR reproducibility for SARS-COV-2 RNA detection in replicate samples of healthy human plasma spiked with 0 to 1 copies/µL of heat-inactivated SARS-CoV-2 virus. RT-qPCR analyses of plasma RNA extracts were performed using the CDC RT-qPCR kit specific for the N1 gene target region of SARS-CoV-2, and samples with Ct values less than 40 were considered SARS-CoV-2 positive. A positive detection rate >95% was not achieved in samples containing < 1 copy/µL, implying the limit of detection of RT-qPCR for SARS-CoV-2 RNA in plasma is approximately 1 copy/µL.

Supplemental Figure 5.

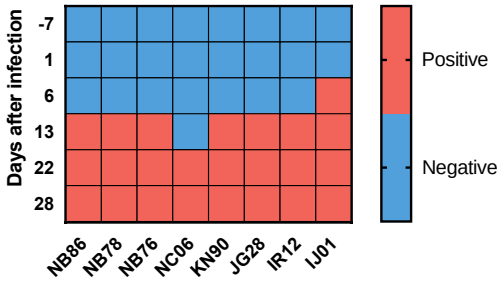

Fig. S5. Schematic of positive and negative detection of SARS-CoV-2 S protein specific IgM in NHP plasma samples collected at the indicated time relative to SARS-CoV-2 exposure.

Supplemental Figure 6.

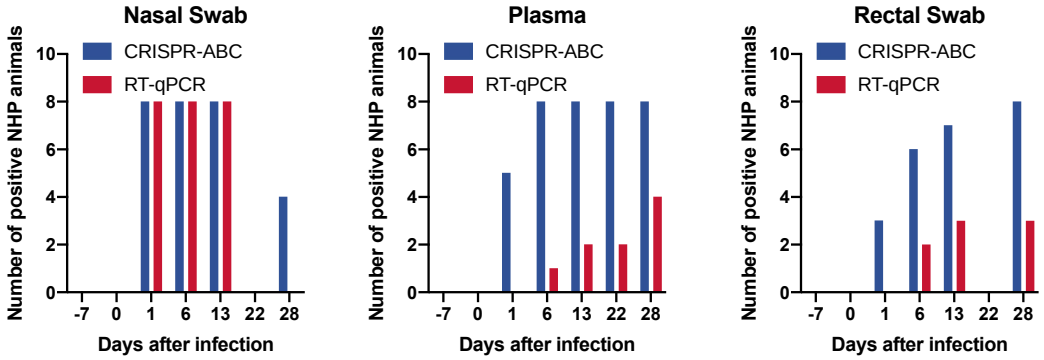

Fig. S6. Comparison of CRISPR-ABC and RT-qPCR positive rates in all NHP plasma and nasal and rectal swab samples shown in **Figure 2B** and **2C**. RT-qPCR analyses of plasma RNA extracts were performed using the CDC RT-qPCR kit specific for the N1 gene target region of SARS-CoV-2, and samples with Ct values less than 40 were considered SARS-CoV-2 positive.

Supplemental Figure 7.

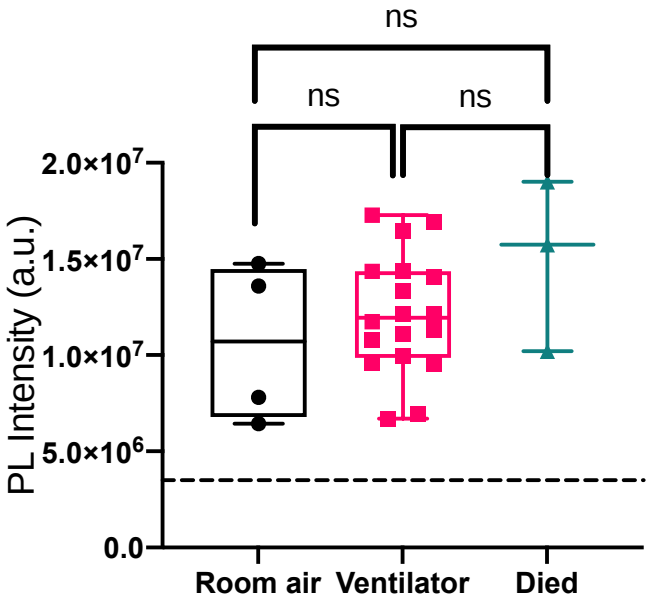

Fig. S7. CRISPR-ABC signal from residual blood samples of hospitalized COVID-19 patients after their categorization by disease severity. Patients were segregated by their disease severity according to their need for oxygen (room air; N=4 or ventilator; N=18) or failure to recover (died; N=3). Data are presented as box plots indicating the maximum, Q3, median, Q1, and minimum values of PL intensity for each group, and the mean of triplicate values for each individual. Dashed line indicates the limit of detection of the CRISPR-ABC assay; ns, p >0.05 by one-way ANOVA. CRISPR-ABC signal did not differ by sample type in this analysis (**Supplemental Figure 12**).

Supplemental Figure 8.

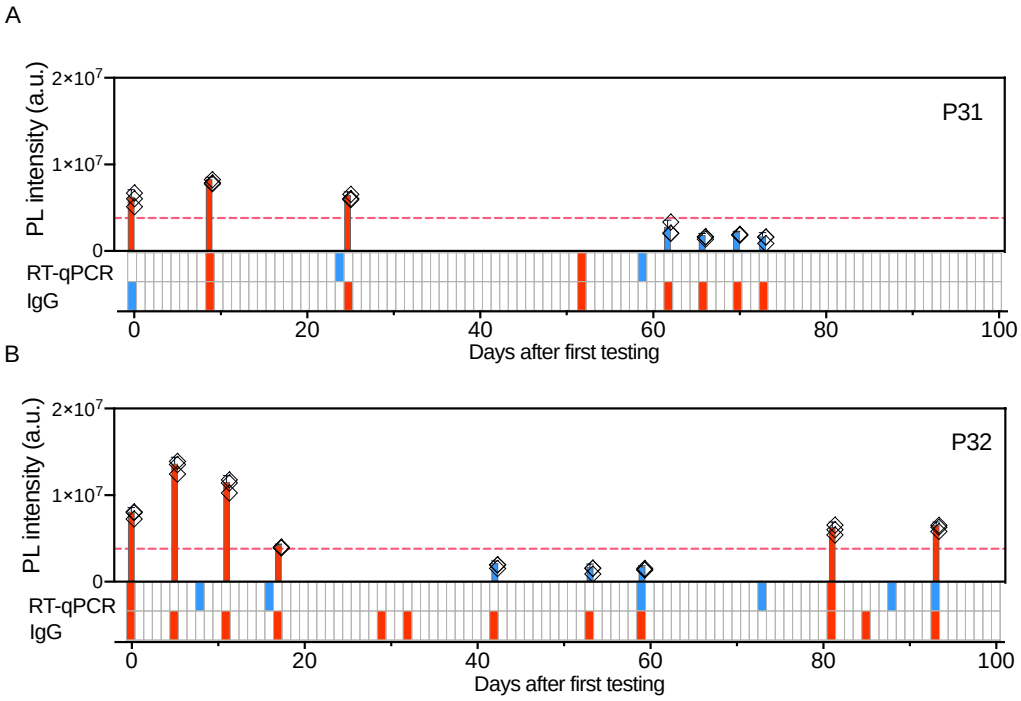

Fig. S8. Plasma CRISPR-ABC results of pediatric cases P31 and P32. Positive (red) and negative (blue) results for COVID-19 plasma CRISPR-ABC, nasal RT-qPCR, and serological results at the indicated time points after first evaluation. Data indicate the mean ± SD of three technical replicates, with CRISPR-ABC assay technical replicate values (diamond symbols) indicated for each analyzed sample.

Supplemental Figure 9.

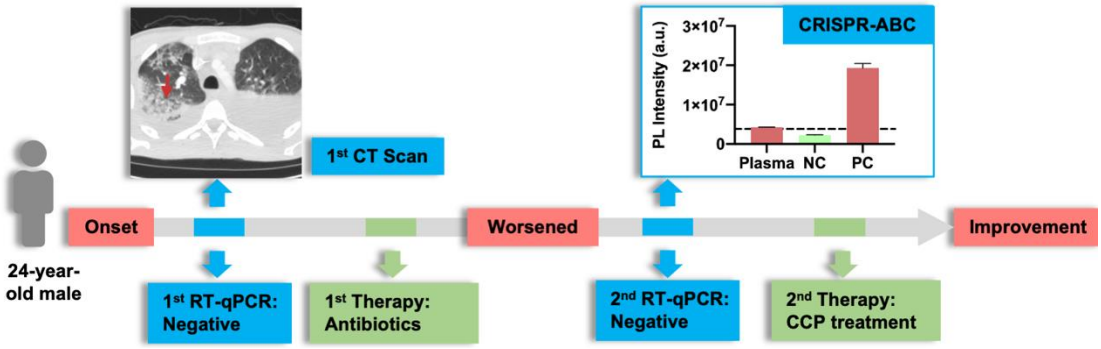

Fig. S9. Case history schematic (not to scale) for a 24-year-old male who presented with shortness of breath and fatigue, a 3 month history of flu-like symptoms, had a negative result from nasal RT-PCR test for COVID-19, and was diagnosed with acute myeloid leukemia (AML) and post-obstructive pneumonia, for which he was admitted to an outside hospital and started on broad spectrum antibiotics. A chest CT performed at transfer to Tulane revealed ground glass opacities (red arrow) but nasal and nasopharyngeal RT-PCR results were COVD-19 negative, and he was started on broad spectrum antibiotics for pneumonia. At hospital day 2, the patient was found to be tachycardiac and hypotensive with increased work of breathing, and was transferred to the ICU and started on a broader course of antibiotics. A second nasal RT PCR performed on hospital day 4 again COVID-19 negative, but a retrospective CRISPR-ABC result for a sample drawn on hospital day 4 was positive. The patient received treatment for AML from hospital days 3 to 10 post-admission and 1 unit of COVID-19 convalescent plasma (CCP) on hospital day 5, and revealed significantly improved vitals after initiation of chemotherapy and CCP. The CRISPR-ABC results present the mean ± SD of three technical replicates for each sample.

Supplemental Figure 10.

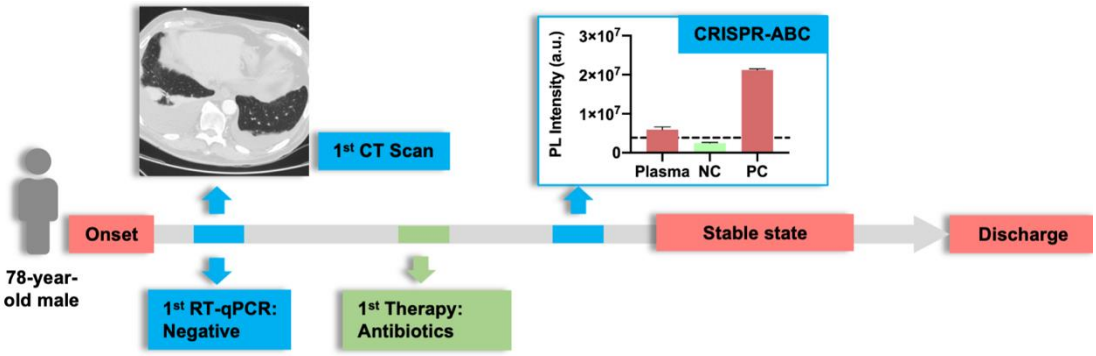

Fig. S10. Case history schematic (not to scale) for a 78-year-old male with history of T-cell prolymphocytic leukemia after autologous stem cell transplant, initially presented with fever and a 2-week history of fatigue. Chest x-ray was notable only for right pleural effusion, which was previously observed at initial diagnosis of T-cell prolymphocytic leukemia. Chest CT revealed only bibasilar atelectasis (lower lung collapse) with bilateral small pleural effusions (fluid buildup). Nasal RT-PCR (was negative for COVID-19, and the patient was given a dose of two antibiotics (vancomycin and cefepime) and continued on cefepime for an additional day due to a low absolute neutrophil count (1400/mm^3). A CRISPR-ABC assay retrospectively performed on a blood sample drawn on hospital day 2 was positive, but this patient demonstrated stable vital signs and did not require any COVID-19 specific treatment prior to discharge. The CRISPR-ABC results present the mean ± SD of three technical replicates for each sample.

Supplemental Figure 11.

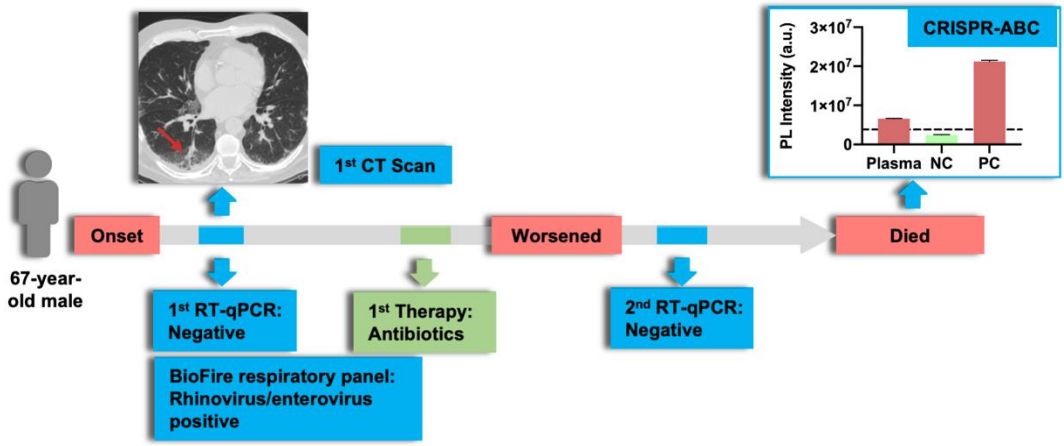

Fig. S11. Case history schematic (not to scale) for a 67-year-old male with a history of acute myeloid leukemia evolved from myelodysplastic syndrome following a hematopoietic stem cell transplant, presented with a two-day history of shortness of breath, cough, and a worsening skin rash. Initial evaluation was unremarkable except for tachycardia to 140 bpm. A chest CT revealed ill-defined peribronchovascular opacities in the left upper and lower lung fields (red arrow), raising concern for COVID-19, but he tested negative for COVID-19 by nasopharyngeal RT-PCR, and positive for rhinovirus/ enterovirus on the BioFire respiratory panel. On hospital day 11, his respiratory culture grew multi-drug resistant *Stenotrophomonas maltophilia*, and he was started on intravenous antibiotics. Despite aggressive antimicrobial therapy, his condition continued to worsen, requiring supplemental oxygen. On hospital day 28, he developed hypothermia and acute respiratory distress and was transferred to the ICU where he was intubated. Bronchoscopy was performed, but no definitive diagnosis was made. RT-PCR assays performed on a nasal swab and BAL specimen were COVID-19 negative. Despite aggressive antimicrobial therapy and supportive care, he developed multiorgan failure and died on hospital day 36. Shortly thereafter, CRISPR-ABC was performed on a blood sample collected on hospital day 36, which was positive. The CRISPR-ABC results present the mean ± SD of three technical replicates for each sample.

Supplemental Figure 12.

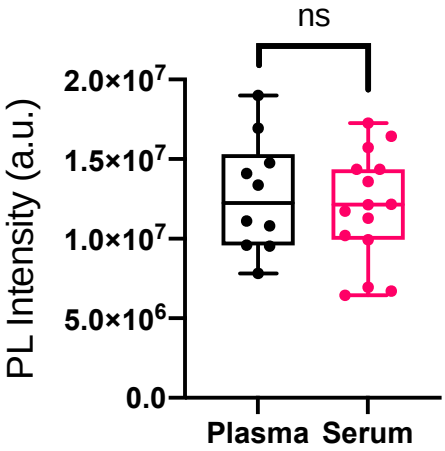

Fig. S12. CRISPR-ABC test results for 10 plasma and 15 serum samples collected from hospitalized cases. Data are presented as box plots indicating the maximum, Q3, median, Q1, and minimum values of PL intensity for each group, and also depict the mean of triplicate values for each individual. (ns, P>0.05 by Mann Whitney U test)

Supplemental Results for the five at-risk cases

Both CRISPR-positive patients who improved after receiving CCP presented with shortness of breath and had chest CT scans that revealed ground glass opacities suggestive of COVID-19, but had negative nasal swab RT-qPCR tests results. Each of these patients also failed to respond to antibiotics administered for pneumonia, but demonstrated positive responses following CCP therapy. The first case (**Figure 5A, Supplemental Data 2: Case 1**)(5), a 53-year-old female with pre-existing acute lymphoblastic leukemia (ALL), had multiple negative RT-qPCR results with respiratory samples obtained following chest radiography results suggestive of COVID-19, and was therefore started on antibiotic therapy for pneumonia and administered intravenous immunoglobulin for hypogammaglobulinemia. Over the following 48 hours her respiratory status worsened to require supplemental oxygen, at which time an investigational plasma CRISPR-ABC test returned a result positive for SARS-CoV-2. This patient was then transferred to the COVID-19 isolation ward on suspicion of an active SARS-CoV-2 infection, received a unit of CCP, and exhibited marked reductions in her shortness of breath, cough, fever, and supplemental oxygen requirement within the next 24 hours (**Figure 5A**). Due to continual lingering symptoms, this patient received an additional unit of CCP 1 week after initial infusion, after which she continued to improve and was discharged with resolution of all symptoms.

The second case (**Supplemental Figure 9, Supplemental Data 2: Case 2**), a 24-year-old male initially presented to an outside hospital with concern for a new diagnosis

of acute myeloid leukemia (AML), and was noted to have had intermittent flu-like symptoms for roughly 3 months prior to admission in June 2020, at which time he was found to have a moderate sized pericardial effusion and cardiac tamponade, and a pericardial window and drain were placed roughly 2 days prior to transfer to Tulane Medical Center to relieve tamponade physiology. This patient was also diagnosed with a post-obstructive pneumonia, for which he was started on broad spectrum antibiotics prior to transfer. Bilateral pleural effusion noted for this case after transfer was considered likely to have arisen from tamponade physiology, but right upper lobe ground glass opacities noted on the chest CT in this patient appeared consistent with COVID-19, and were judged by the attending infectious disease physicians to be less likely to represent atypical pneumonia. This patient had negative nasal RT-PCR test results at admission and upon transfer, and worsened following antibiotic treatment, becoming tachycardiac and hypotensive, demonstrated increased respiratory effort, and was transferred to the ICU where he was started on a broader course of antibiotics without major signs of improvement. Workups for other infectious etiologies all came back negative, and the patient again tested negative for COVID-19 by nasal swab RT-qPCR, but tested positive upon investigational CRISPR-ABC plasma assay analysis, and improved upon subsequent treatment with CCP and chemotherapy.

Both CRISPR-positive patients who did not receive CCP presented with shortness of breath or fatigue with CT scans providing evidence consistent with pneumonia or COVID-19. The first of these cases (**Supplemental Figure 10, Supplemental Data 2:**

Case 3), a 78-year-old male with a history of T-cell prolymphocytic leukemia, was noted to have fever, some dyspnea, and fatigue, lower lung collapse with small regions of bilateral fluid buildup, and to have had close contact with his wife, who had COVID-19 roughly 6 weeks before he presented with COVID-19-associated symptoms. Prior to this evaluation, this patient presented with a right lung pleural effusion that was positive for malignancy, and subsequently developed bilateral pulmonary effusions that were negative for malignancy and deemed likely to be due to fluid shift from apheresis and/or known heart failure. This patient lacked CT findings typical of COVID-19 and tested negative for COVID-19 by nasal swab RT-qPCR, and was therefore started on antibiotics. He exhibited stable vital signs during his hospitalization, and was discharged without receiving CCP therapy, but retrospective CRISPR-ABC analysis of a plasma sample drawn early after hospitalization tested positive for SARS-CoV-2, suggesting this patient had mild/moderate COVID-19 that resolved without direct therapeutic intervention.

The second case (**Supplemental Figure 11, Supplemental Data 2: Case 4**) had a history of AML and presented with shortness of breath, cough, a skin rash, tachycardia, and ill-defined peribronchovascular opacities, but tested negative for COVID-19 by RT-qPCR, with evidence for rhinovirus/enterovirus infection, and subsequently for a multi-drug resistant bacterial infection, for which he was started on intravenous antibiotics. However, this patient exhibited an extended gap between his rhinovirus diagnosis and his subsequent physiologic deterioration (~28 days) and demonstrated improvement

during this intervening period. This patient failed to respond to aggressive antimicrobial therapy, developed a need for supplemental oxygen that progressed to intubation, and ultimately died as a result of multi-organ failure. RT-qPCR results for nasal swab and bronchoalveolar lavage samples obtained during this period did not detect SAS-CoV-2, but a retrospective CRISPR-ABC assay performed on a plasma sample collected on the day the patient died was SARS-CoV-2 RNA positive (**Supplemental Figure 11**). Studies indicate that there is a relatively high rate of SARS-CoV-2 co-infection with other viruses, with one study reporting a general co-infection rate ≥20% (6) and another reporting a 6.9% co-infection rate with Enterovirus/Rhinovirus (7), thus it is plausible that this patient had both infections.

The single leukemia patient that did not have a positive CRISPR-ABC result, demonstrated findings consistent with bacterial infection upon evaluation of her clinical response. This patient, a 26-year-old female, had a history of AML (**Figure 5B, Supplemental Data 2: Case 5**), and presented with fever, tachycardia and hypotension, and a right upper lung lobe nodule, but tested negative for COVID-19 by nasal swab RT-qPCR. She was started on broad spectrum antibiotics and antifungals, but continued to spike fevers with tachycardia and hypoxia, and a second CT revealed bilateral diffuse ground glass opacity within the lower lung. However, a second nasal swab RT-qPCR test for COVID-19 performed on hospital day 6 was negative, as was a retrospective plasma CRISPR-ABC test. She was continued on broad spectrum antibiotics with an escalated antifungal treatment, after which she slowly improved,

278 and was discharged for treatment of her AML at another site. Due to the absence of
279 any CRISPR-ABC or RT-qPCR positive results and a positive response to antibiotics,
280 this patient was considered not to had COVID-19.

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