Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information for

Purely electrical SARS-CoV-2 sensing based on single-molecule counting

Xander F. van Kooten[#,1], Yana Rozevsky[#,1], Yulia Marom[1], Efrat Ben Sadeh[1] and Amit Meller[*,1]

[1]Department of Biomedical Engineering, Technion- IIT, Haifa 32000, Israel

# Equally contributing authors

* Corresponding author, e-mail: ameller@technion.ac.il

The supplementary information includes:

I. Supplementary Figures

Figure S1. Gel electrophoresis analysis of cDNA fragments obtained from synthetic SARS-CoV-2 RNA.

Figure S2. Positive control using synthetic SARS-CoV-2 and total human RNA extract.

Figure S3. Nanopore analysis of clinical SARS-CoV-2 samples.

Figure S4. Nanopore analysis of additional positive SARS-CoV-2 samples.

Figure S5. Nanopore repeats of the SARS-CoV-2 negative samples.

Figure S6. Nanopore analysis of SARS-CoV-2 negative samples.

Figure S7. Gel electrophoresis validation for on-chip and off-chip processes.

Figure S8. Additional nanopores SARS-CoV-2 positive samples analyses on-chip and off-chip.

Figure S9. Nanopore analysis results of a SARS-CoV-2 sample processed on-chip and off-chip.

Figure S10. Nanopore analysis results of pre-COVID-19 clinical sample.

Figure S11. Nanopore quantification of SARS-CoV-2 synthetic RNA and the human reference gene RPP30.

II. Supplementary Tables

Table S1: Gene-specific primers used in SARS-CoV-2 experiments.

Table S2: RT-qPCR clinical samples information.

I. Supplementary Figures

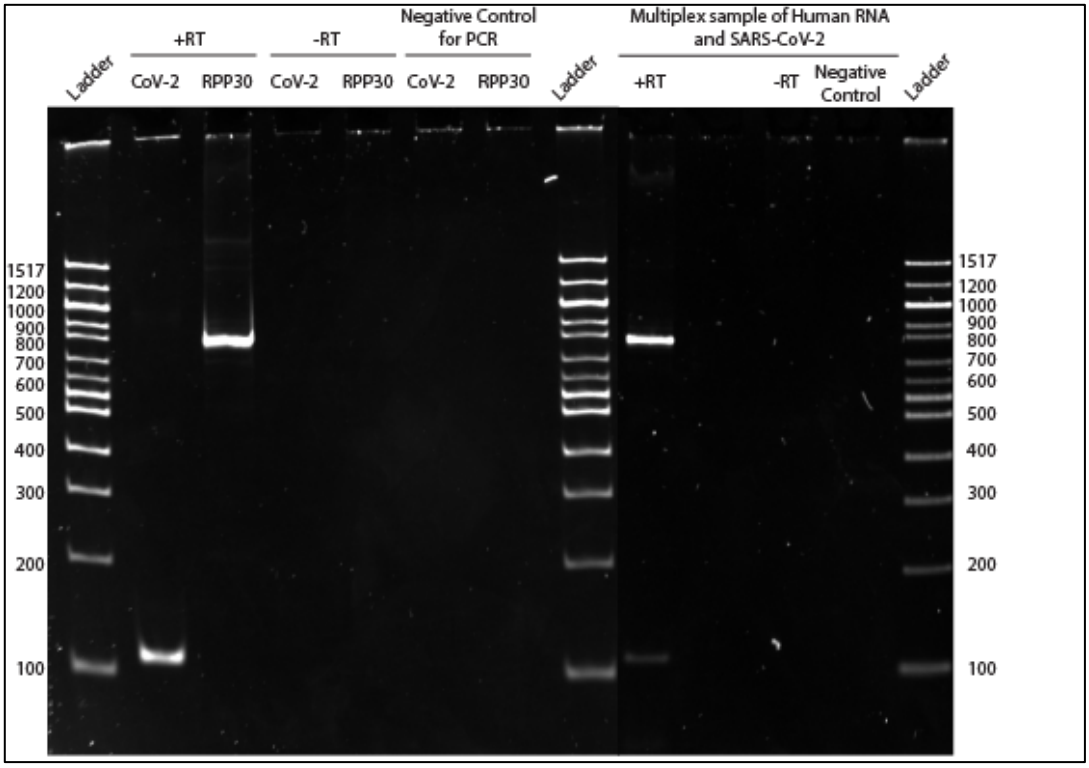

Figure S1. Gel electrophoresis analysis of cDNA fragments obtained from synthetic SARS-CoV-2 RNA. Amplified cDNA samples were prepared as described in the methods section, using only synthetic SARS-CoV-2 or only extracted RNA from HCT116 cells or combined multiplex sample from both. The '+RT' samples, show an expected band for the cDNA fragments, 107 bp for SARS-CoV-2 RdRP gene and 758 bp for human RPP30 were separated in 4% PAGE, stained with SYBR Gold, and imaged by GelDoc EZ (BioRad). In the combined multiplexed sample, the two fragments are obtained. Negative control samples ('-RT') were subjected to the same preparation procedure without the RT enzyme. Similarly, the 'negative control for PCR' has no template.

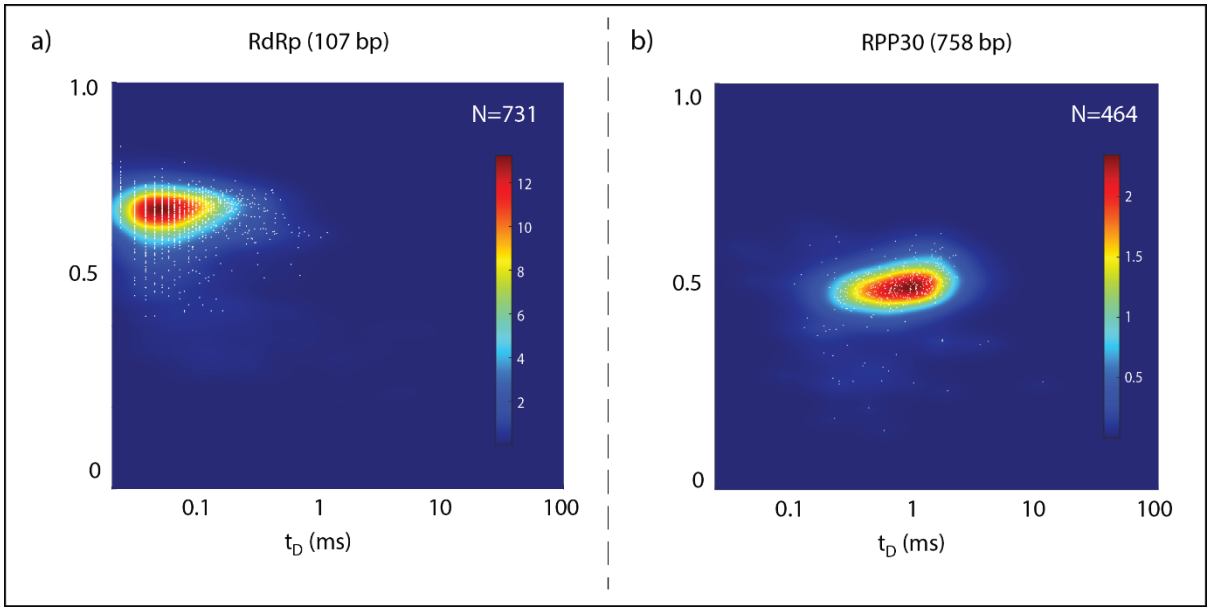

Figure S2. Positive control using synthetic SARS-CoV-2 and total human RNA extract. A step-by-step control experiment was conducted firstly by adding (**a**) RdRP gene fragments, washing thoughtfully and then inserting (**b**) RdRP gene fragments, using the same nanopore device (G = 9.33 nS and 300 mV bias). Both species gene fragments were generated using the sample processing described in section 3.1, which includes the following steps of transcription, second strand synthesis and digestion of all unwanted background. The 2D density scatter plots are presented here for (**a**) RdRP gene fragment (107 bp) and (**b**) RPP30 gene fragment (758 bp).

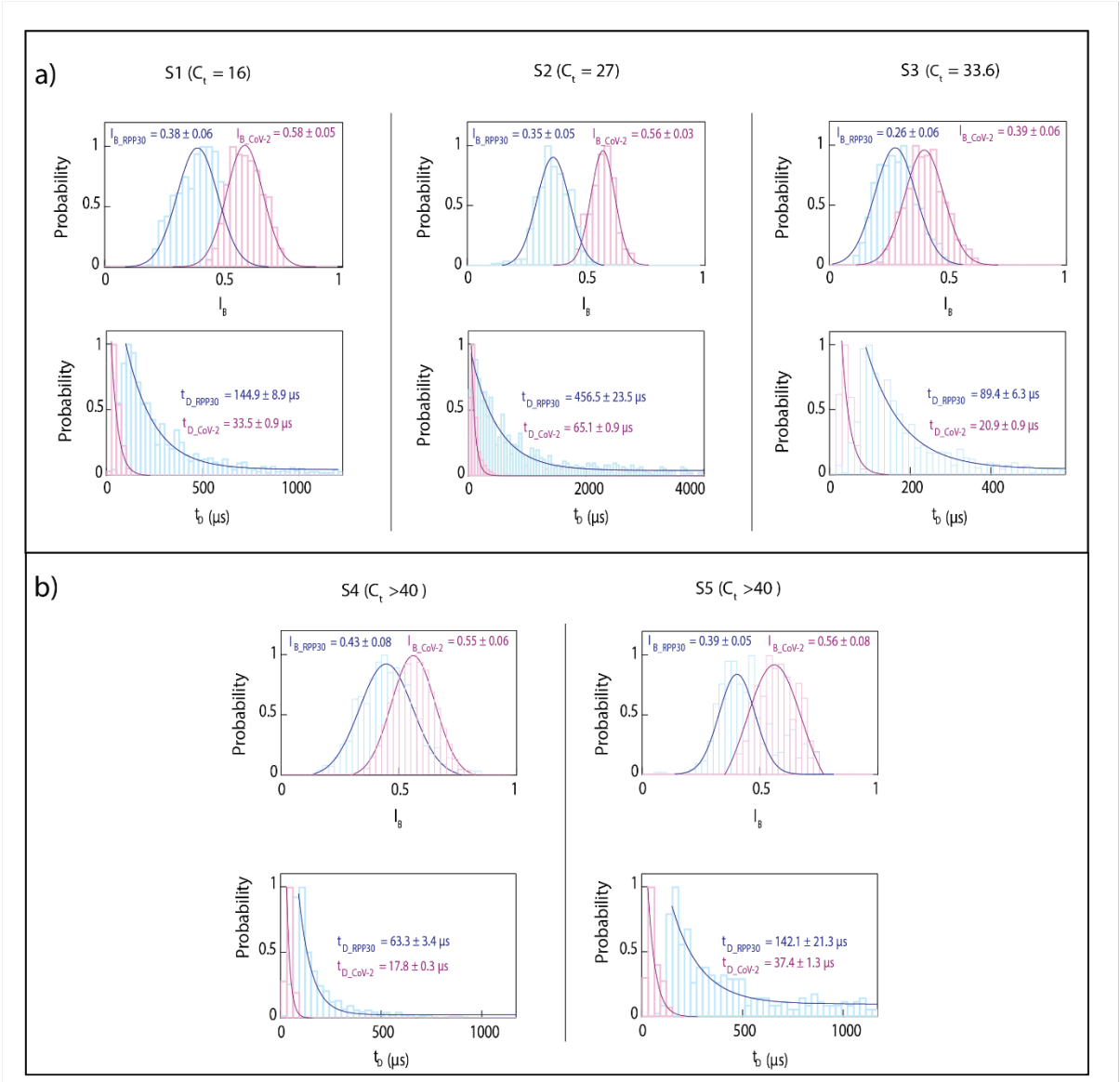

Figure S3. Nanopore analysis results of clinical SARS-CoV-2 samples. Clinical samples were categorized by RT-qPCR as (**a**) positive, patient samples S1-S3, or (**b**) negative/undetermined, patient samples S4-S5, according to their cycle thereshold (C_T). Each clinical sample was analyzed using separate nanopore device as described in the main text and in Fig. 2. In the upper panel, fractional blockage histograms with gaussian fit are shown. In the bottom panel, dwell time distributions with exponential tail fit are presented for each sample. In each case, the magenta color correspond to SARS-CoV-2 RdRP gene fragments while the cyan color correspond to the reference human gene RPP30.

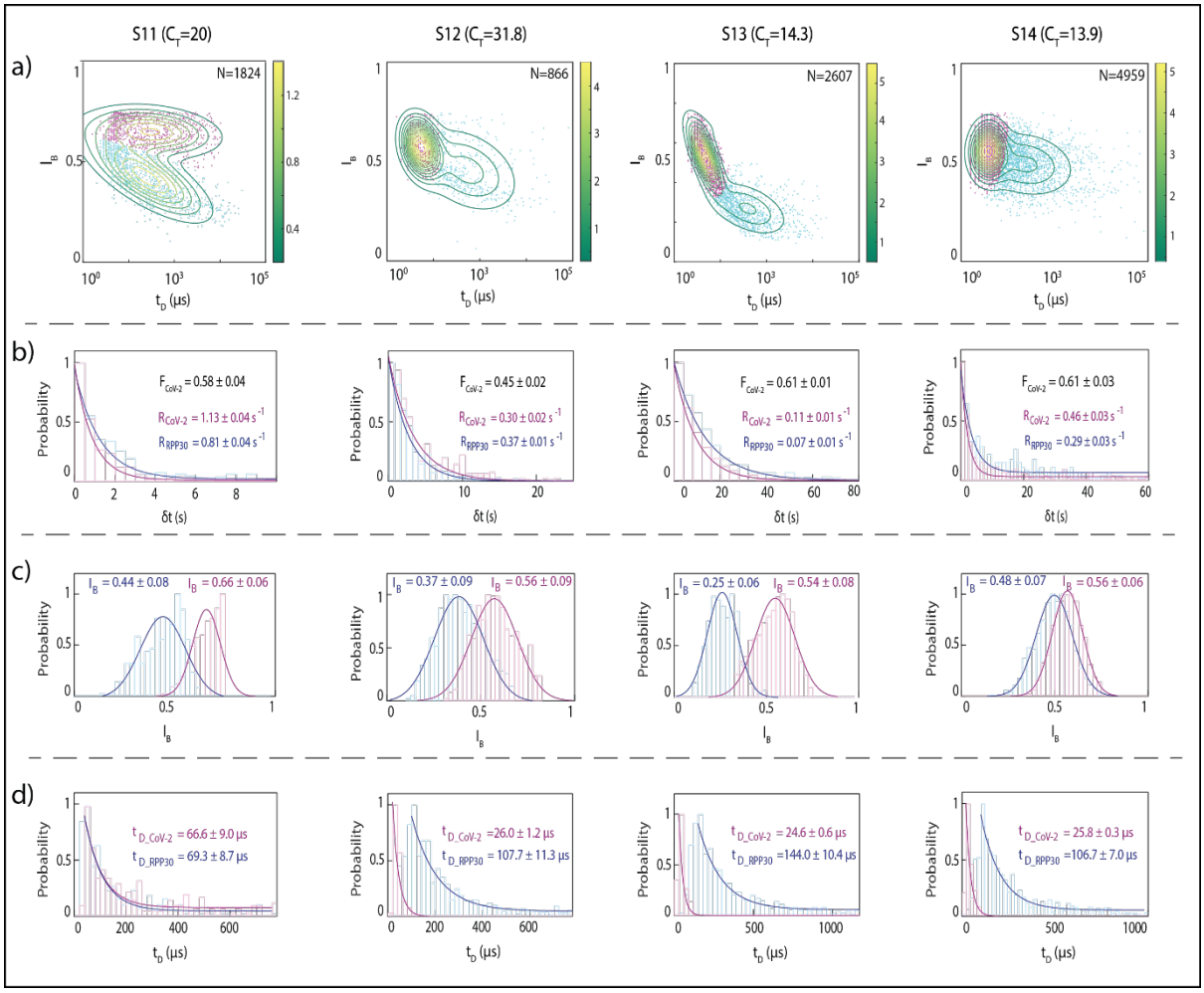

Figure S4. Nanopore analysis of additional SARS-CoV-2 positive samples. In (**a**) the GMM clustering event diagram is presented. In (**b**) the inter-event time histograms with exponential fit are presented. In (**c**) the fractional blockage histograms with gaussian fit are shown. In (**d**) the dwell time distributions with exponential tail fit are shown. In each case, the magenta color corresponds to SARS-CoV-2 RdRP gene fragments and the cyan color corresponds to the reference human gene RPP30.

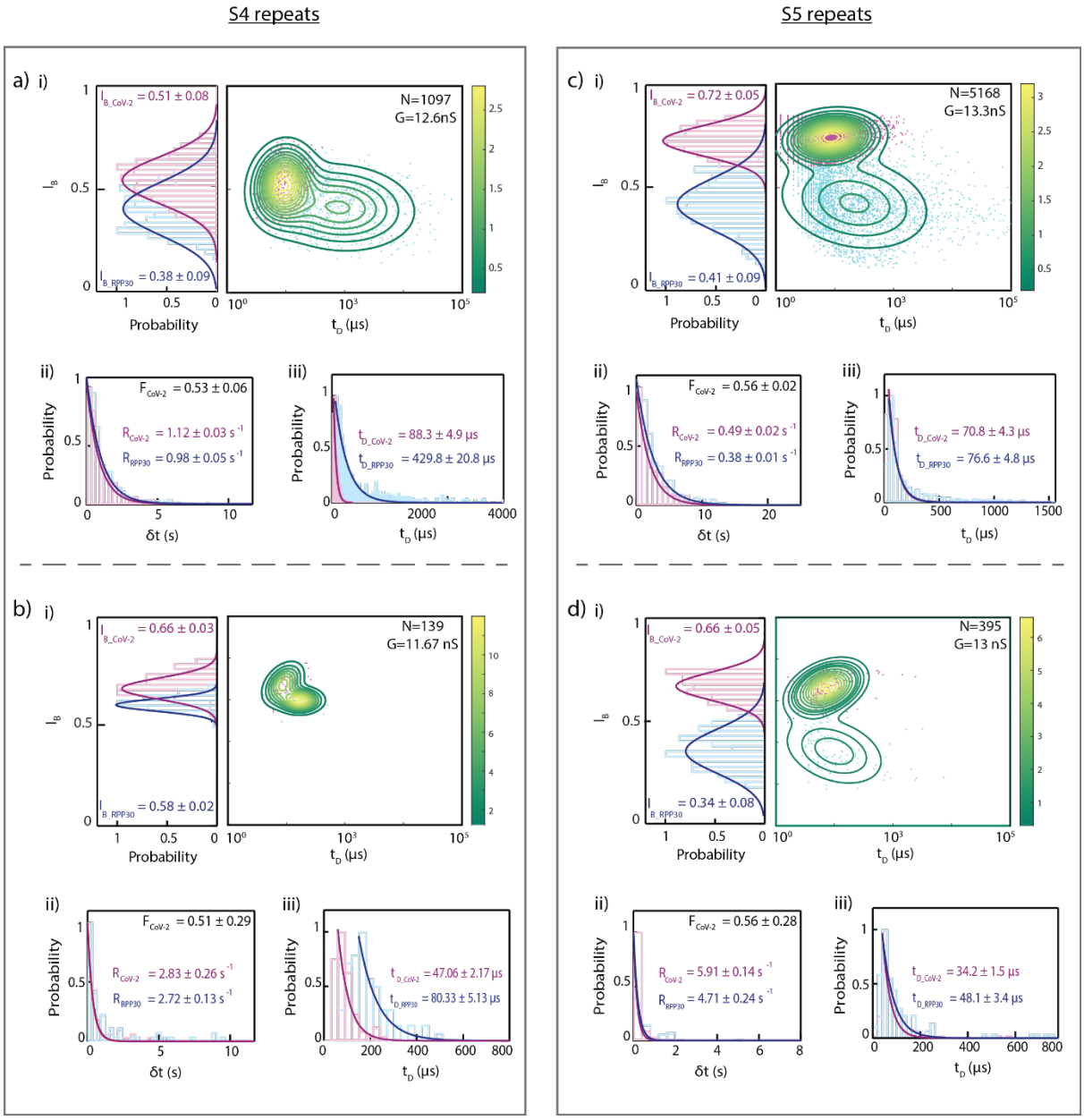

Figure S5. Nanopore repeats of the SARS-CoV-2 negative samples.

Additional four separate nanopore experiments were conducted using the suspected false negative samples, S4 (**a-b**) and S5 (**c-d**). This sample was determined to be negative by RT-qPCR (no signal detected at 40 PCR cycles). In (i), the GMM clustering separate into two populations. The fractional blockage histogram with gaussian fit are shown on the y-axis of each scatter plot. For each population, the mean inter-event histogram with exponential fit (ii) and the dwell time histogram with exponential tail fit (iii), are presented.

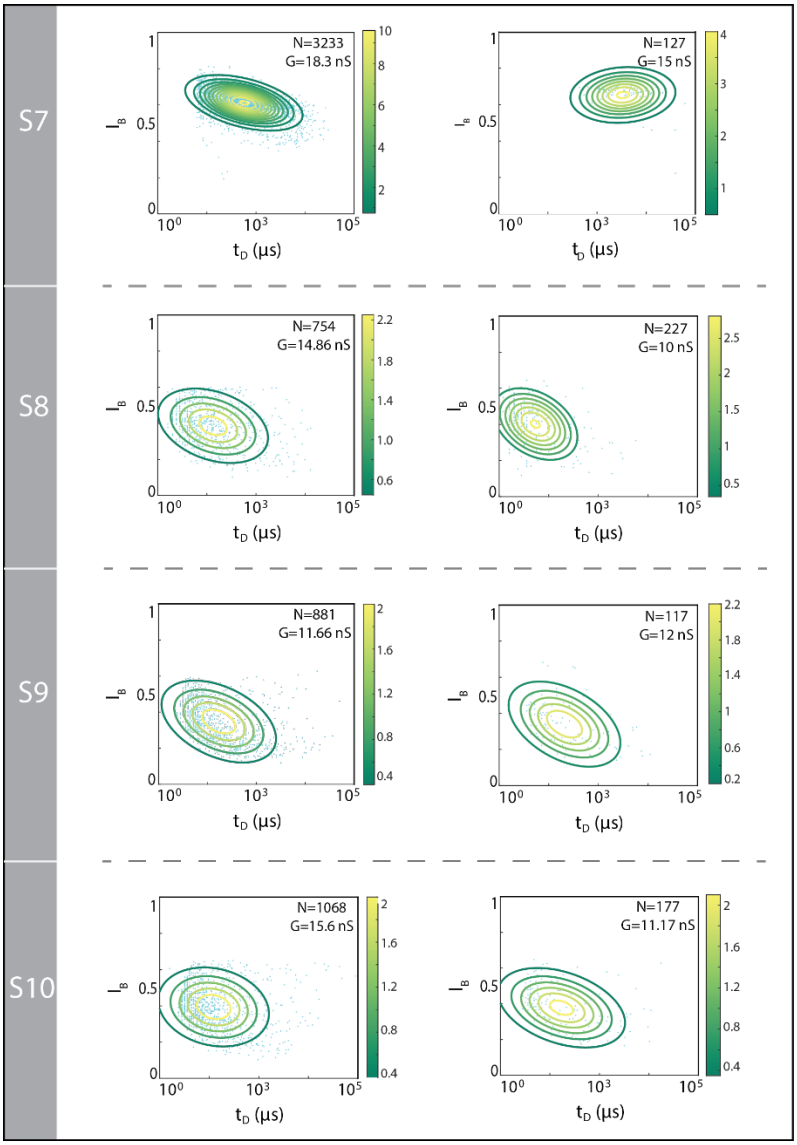

Figure S6. Nanopore analysis of the clinical SARS-CoV-2 negative samples.

Nanopore experiments were conducted using the negative samples determined by RT-qPCR with no signal detected for C_T up to 40 PCR cycles. The GMM clustering could recognize only a single population of events in all these cases, representing events obtained only from the human reference gene, RPP30.

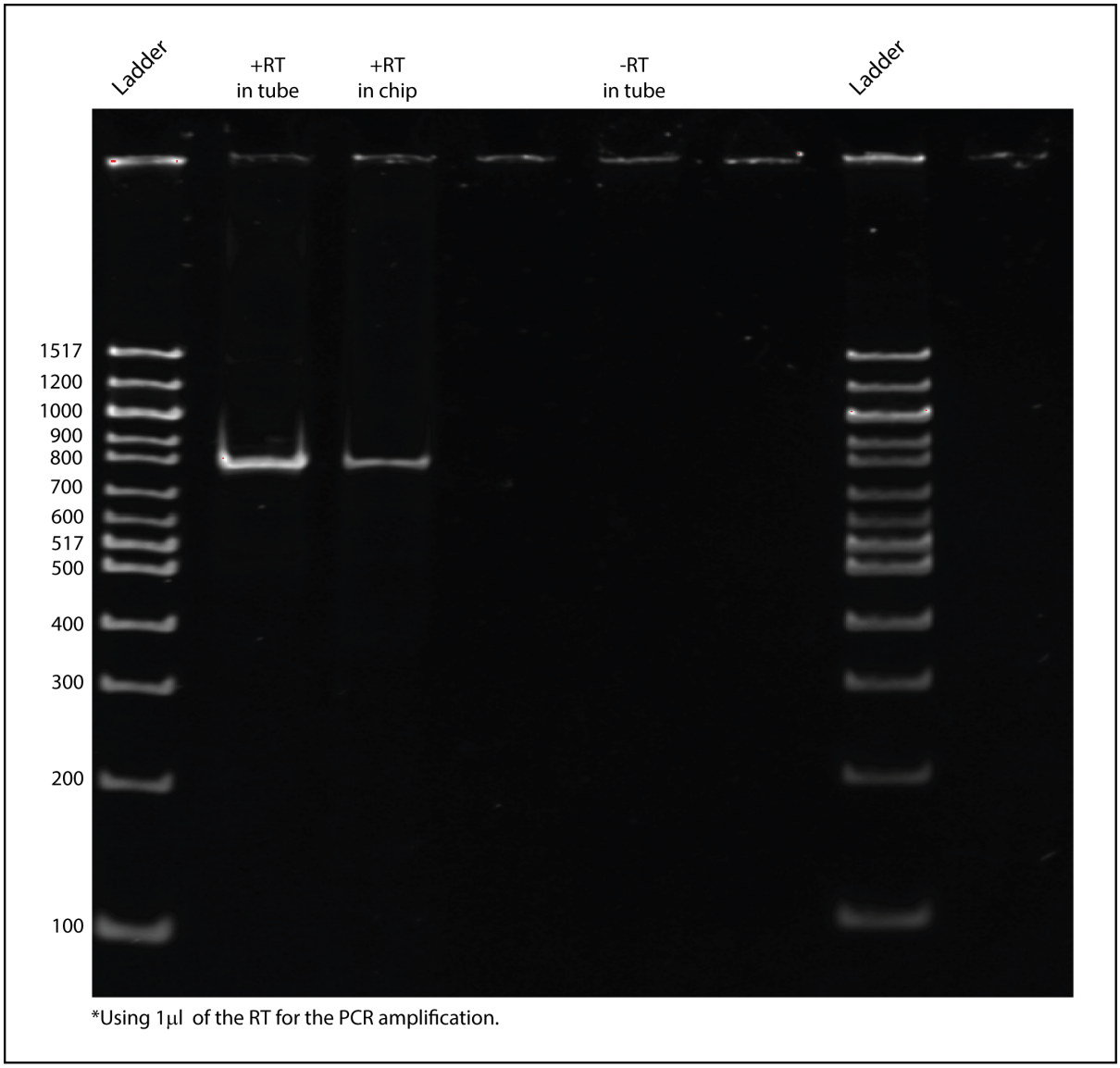

Figure S7. Gel electrophoresis validation for on-chip and off-chip processes. PCR amplified cDNA fragments of the human RPP30 are prepared as described in SI-1. 50 ng and 25 ng of total RNA extracted from HCT116 cells were processed in Eppendorf tubes ("in tube") or on-chip, processing respectively. All reactions were incubated at 60 °C for 30 min and imaged using gel electrophoresis. Both reactions produced ds-cDNA at the expected length, confirming successfully processing on-chip.

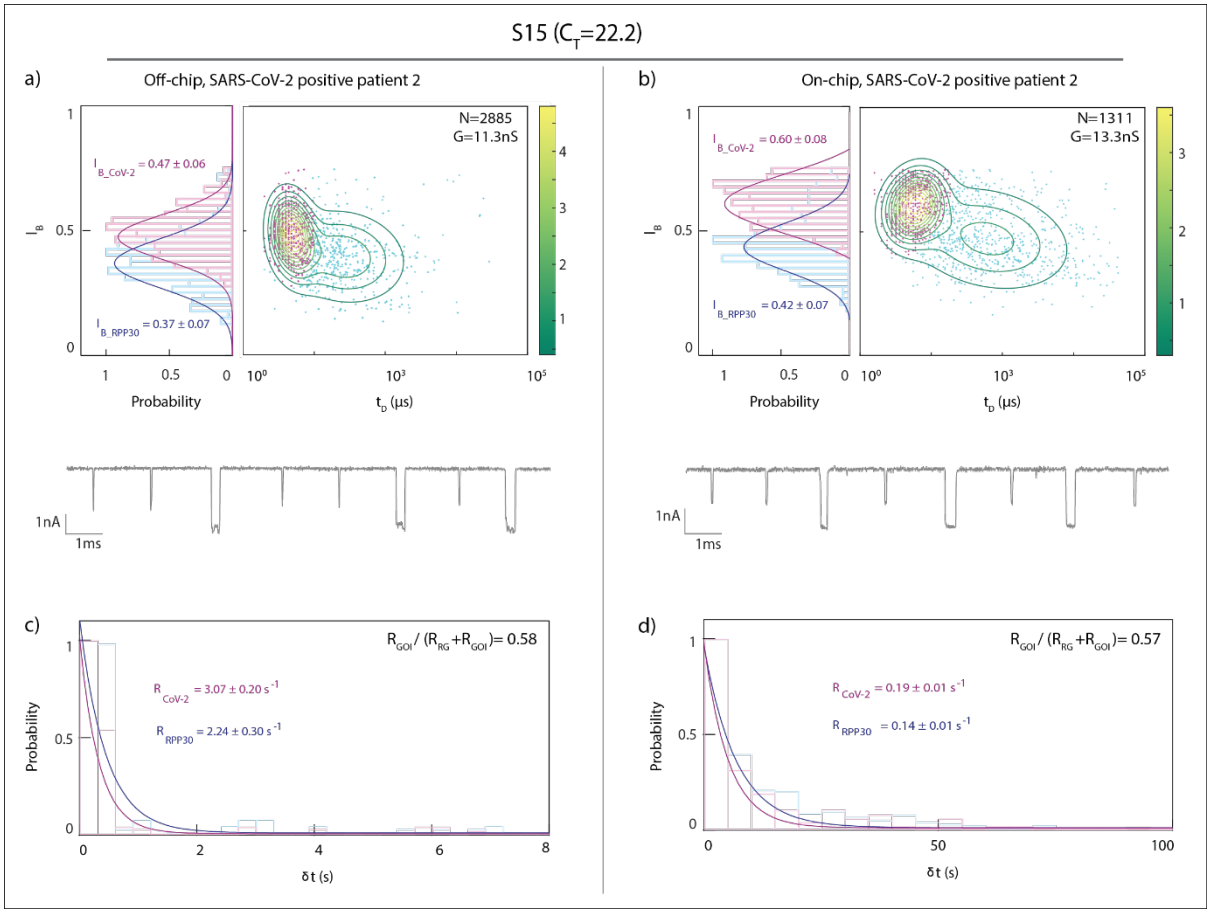

Figure S8. Additional nanopores SARS-CoV-2 positive samples analyses on-chip and off-chip. Additional SARS-CoV-2 positive sample, with C_T of 22.1 was run in two separate nanopores, with conductance of 11.3 nS and 13.3 nS after processing off-chip and on-chip, respectively. In (**a-b**), on top panel, the event diagram and the corresponding fractional blockage with gaussian fit on the y-axis are shown. In the bottom panel, representative events are shown. In (**c-d**), the corresponding inter-event time histograms with exponential fit are presented.

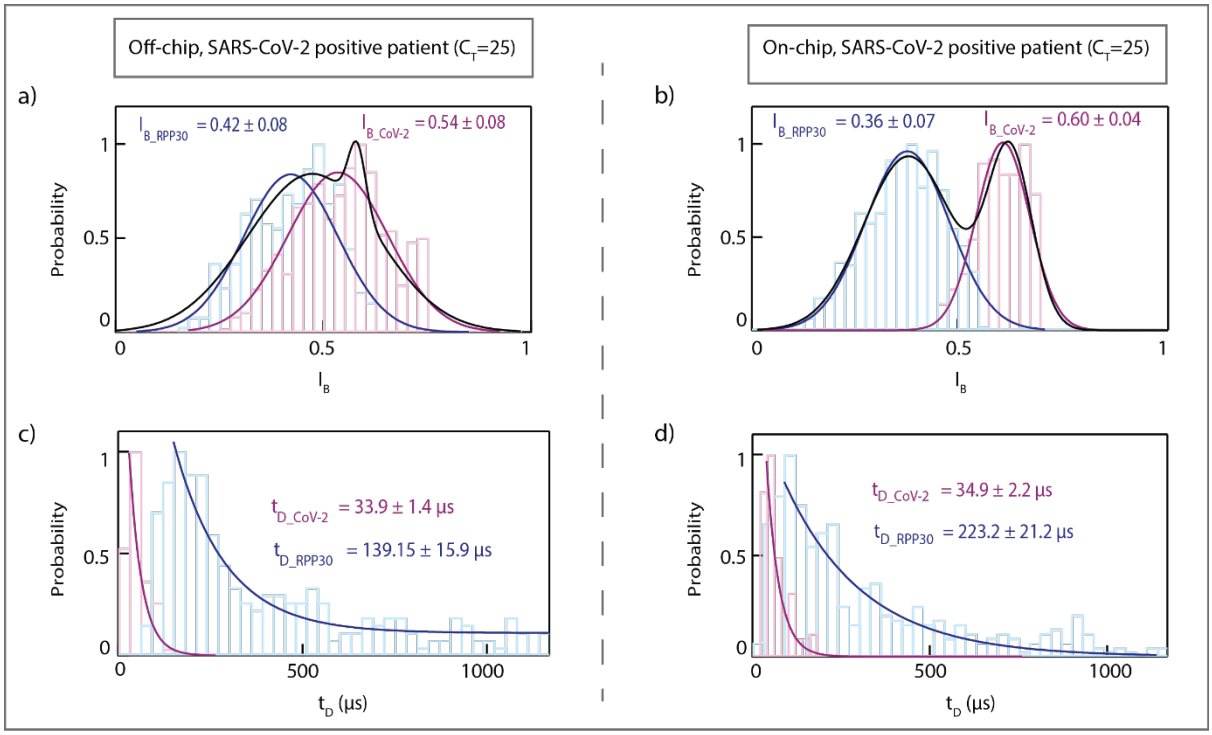

Figure S9. Nanopore analysis results of a SARS-CoV-2 sample processed on-chip and off-chip. Data analysis of the "on"- and "off"- chip experiments presented in the main text and Fig. 3. The fractional blockage distribution with gaussian fits are shown in (**a**) and (**b**) for "off"- and "on"- chip experiments respectively. In (**c**) and (**d**) the dwell-time distributions with exponential tail fit are presented for "off" and "on" chip experiments respectively.

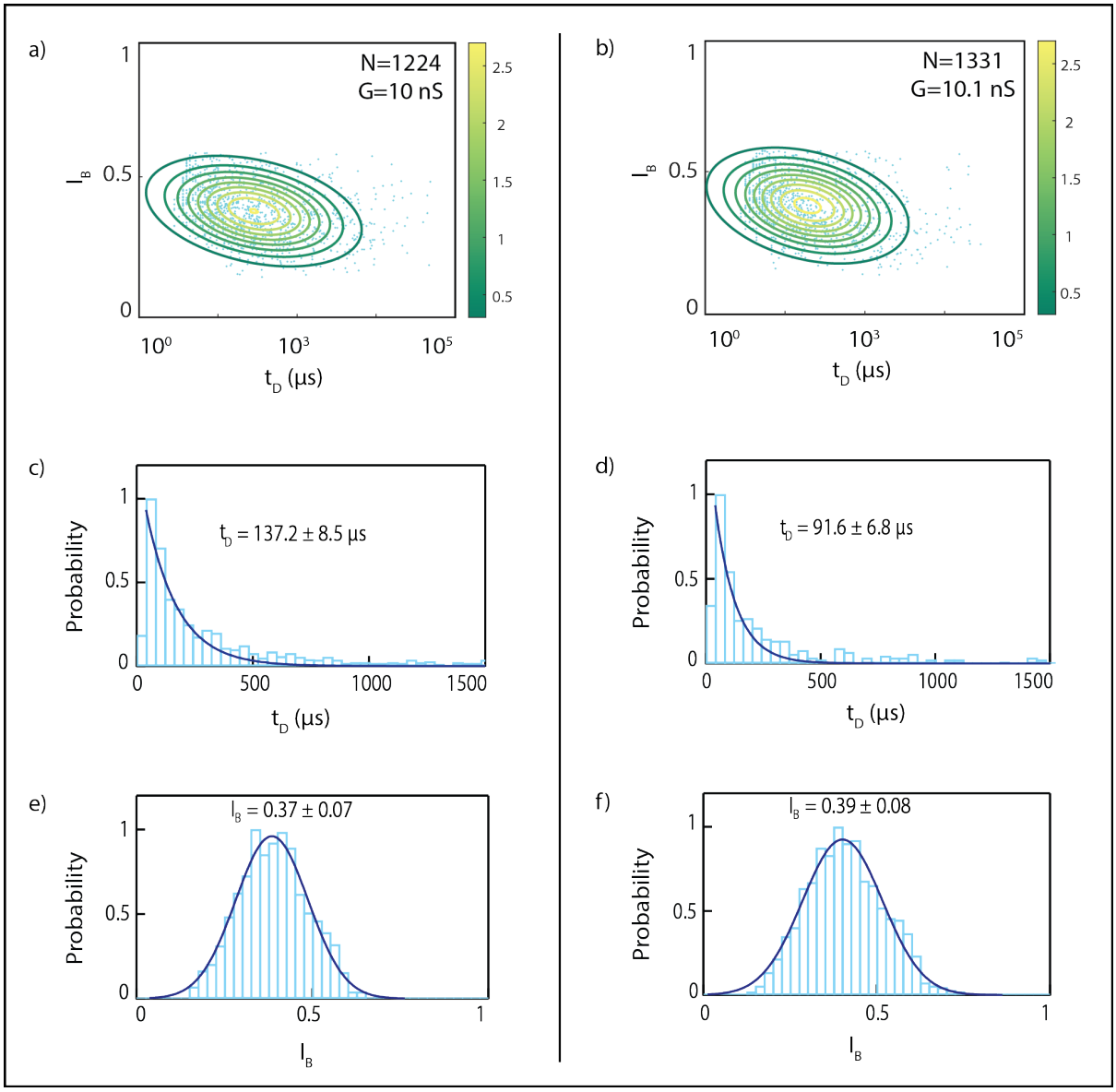

Figure S10. Nanopore analysis results of pre-COVID-19 clinical sample. The pre-COVID-19 clinical sample is used here as true negative control was run in two repeats. The GMM analysis is shown in (**a-b**). The corresponding normalized dwell time histograms are shown in (**c-d**). The normalized fractional blockage histograms are shown in (**e-f**).

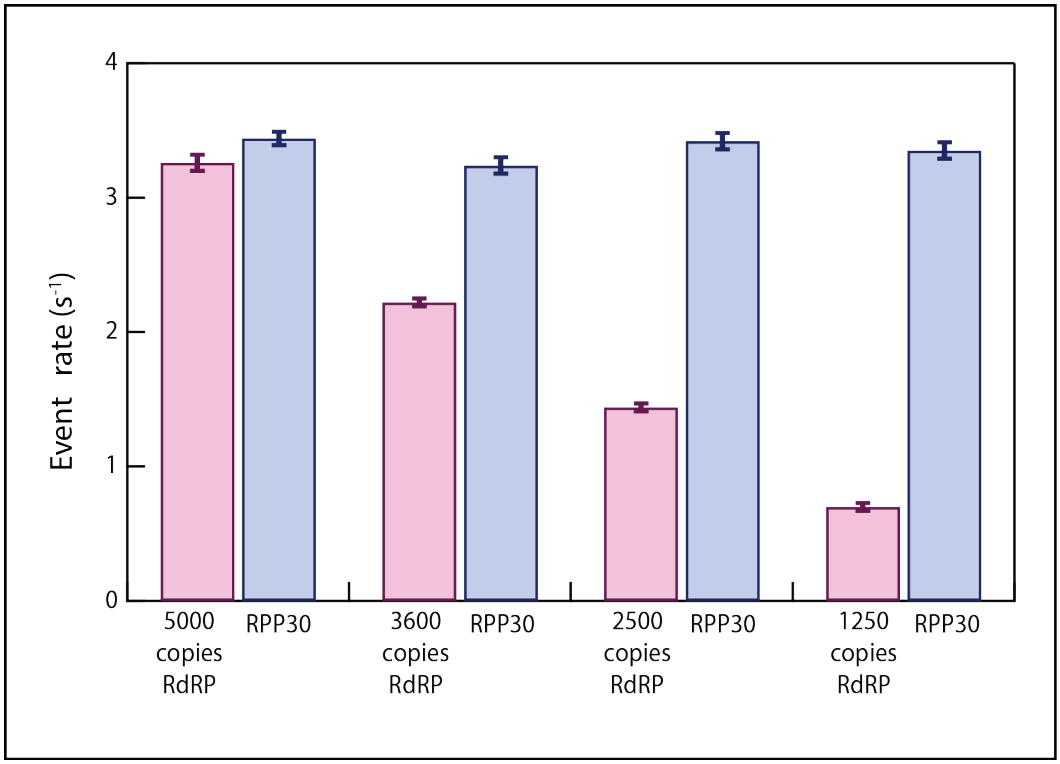

Figure S11. Nanopore quantification of SARS-CoV-2 RNA and the human reference gene RPP30. Different starting amounts of synthetic SARS-CoV-2 (in units of copy numbers) each mixed with a fixed amount of total RNA (0.25 ng) from HCT116 cell lines, underwent sample processing described in section 3.1. Here, we measure the event rate for starting amount of 1250, 2500, 3600 and 5000 copies of RdRP gene fragments. The RPP30 events' rate remains constant same relatively, as expected. Experiments were conducted with nanopores of ~14 nS.

II. Supplementary Tables

Table S1. Gene-specific primers used in SARS-CoV-2 experiments.

No	Gene	Oligo Name	Sequence (5'---3')
1	Human RPP30	hRPP30 For	AGATTTGGACCTGCGAGC
2		hRPP30 Rev	GACAATCTTCATCTCCTTCTGAT
3	Viral SARS-CoV-2 RdRP	vRdRP For1	GGTAACTGGTATGATTTCGGT
4		vRdRP Rev1	CTGGTCAAGGTTAATATAGGCATT
5	Viral SARS-CoV-2 RdRP	vRdRP For2	CTCATCAGGAGATGCCAC
6		vRdRP Rev2	GCAATTTTGTTACCATCAGTAGAT

Table S2. RT-qPCR clinical samples information.

Sample	C_T values		
	E gene	RdRP gene	N gene
S1	13.84	16.03	18
S2	26.63	27.22	28.53
S3	-	33.62	32.67
S4	-	-	-
S5	-	-	-
S6	24.04	24.86	26.6
S7	-	-	-
S8	-	-	-
S9	-	-	-
S10	-	-	-
S11	19.53	20.28	22.16
S12	30.53	31.77	32.19
S13	16.19	14.3	18.63
S14	12.27	13.89	16.71
S15	21.21	22.07	23.43