Supporting Information

Colorimetric Detection of SARS-CoV-2 and Drug-Resistant pH1N1 using CRISPR/dCas9

Jeong Moon,[a,e] *Hyung-Jun Kwon,*[b] *Dongeun Yong,*[c] *In-Chul Lee,*[b] *Hongki Kim,*[a] *Hyunju Kang,*[a] *Eun-Kyung Lim,*[a,d] *Kyu-Sun Lee,*[a] *Juyeon Jung,*[a,d,*] *Hyun Gyu Park,*[e,*] *and Taejoon Kang*[a,*]

[a]Bionanotechnology Research Center and [b]Functional Biomaterial Research Center, KRIBB, 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

[c]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea

[d]Department of Nanobiotechnology, KRIBB School of Biotechnology, UST, 217 Gajeong-ro, Yuseong-gu, Daejeon 34113, Republic of Korea

[e]Department of Chemical and Biomolecular Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

Corresponding Authors

*E-mail: kangtaejoon@kribb.re.kr (T.K.).

*E-mail: hgpark1@kaist.ac.kr (H.G.P.).

*E-mail: jjung@kribb.re.kr (J.J.).

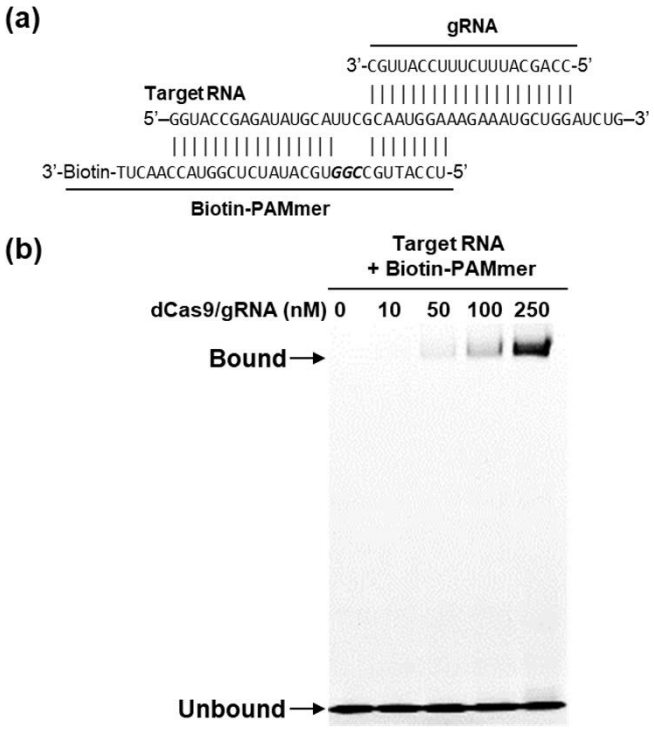

Figure S1. (a) Sequence of gRNA, target RNA, and biotin-PAMmer. (b) Electrophoretic mobility shift assay for binding test of RNA and biotin-PAMmer with dCas9/gRNA complex.

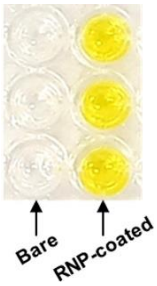

Figure S2. Confirmation of dCas9/gRNA RNP immobilization on microplate.

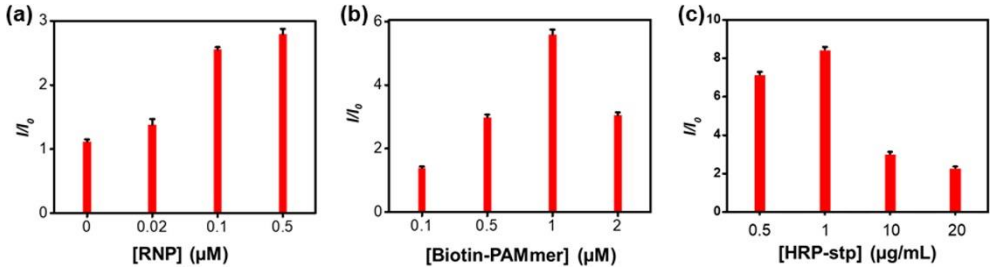

Figure S3. (a) Optimization of dCas9/gRNA RNP concentration. [Biotin-PAMmer] = 0.5 μM, [HRP-streptavidin] = 10 μg/mL, and [Target RNA] = 100 nM. (b) Optimization of biotin-PAMmer concentration. [RNP] = 0.1 μM, [HRP-streptavidin] = 10 μg/mL, and [Target RNA] = 100 nM. (c) Optimization of HRP-streptavidin concentration. [RNP] = 0.1 μM, [Biotin-PAMmer] = 0.5 μM, and [Target RNA] = 100 nM. I_0 and I represent OD_{450nm} values of control and target.

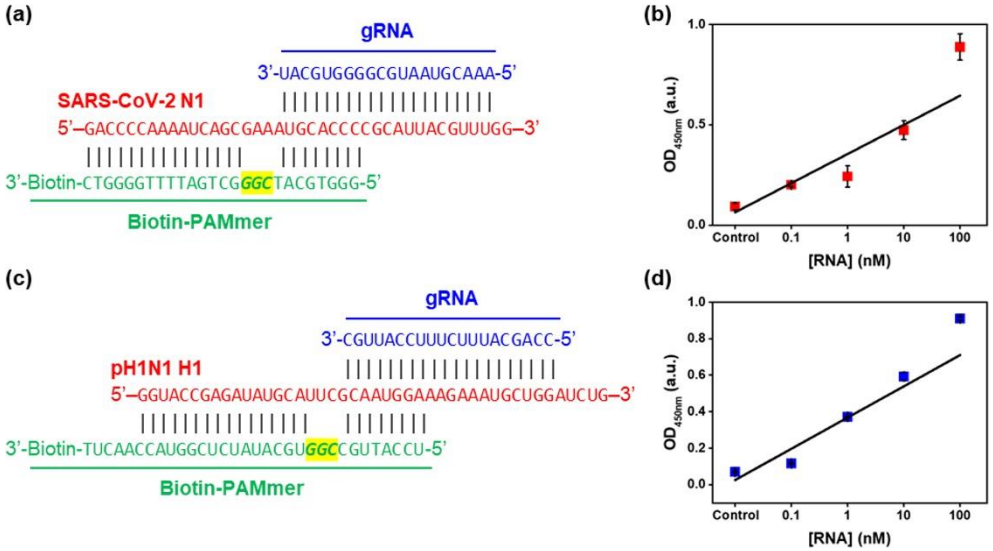

Figure S4. (a) Sequence of gRNA, SARS-CoV-2 N1, and biotin-PAMmer. (b) Linearly fitted line of Figure 2a. (c) Sequence of gRNA, pH1N1 H1, and biotin-PAMmer. (d) Linearly fitted line of Figure 2b.

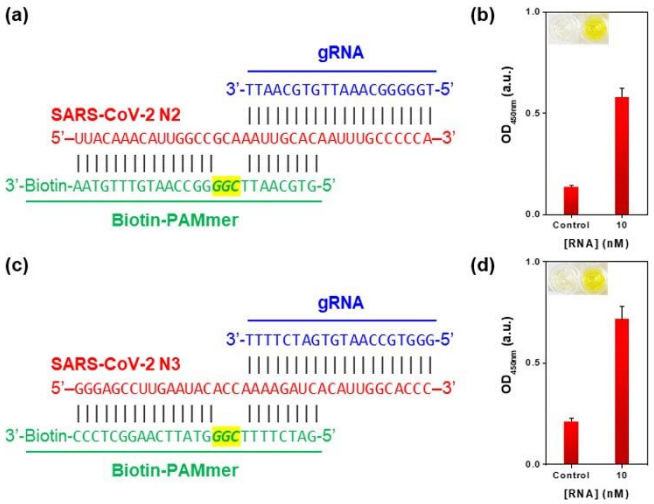

Figure S5. (a) Sequence of gRNA, SARS-CoV-2 N2, and biotin-PAMmer. (b) Plot of OD_{450nm} versus the SARS-CoV-2 N2 RNA (10 nM) and control samples. Inset is the photograph of microplate after the detection of SARS-CoV-2 N2 RNA using CRISPR/dCas9. (c) Sequence of gRNA, SARS-CoV-2 N3, and biotin-PAMmer. (d) Plot of OD_{450nm} versus the SARS-CoV-2 N3 RNA (10 nM) and control sample. Inset is the photograph of microplate after the detection of SARS-CoV-2 N3 RNA using CRISPR/dCas9.

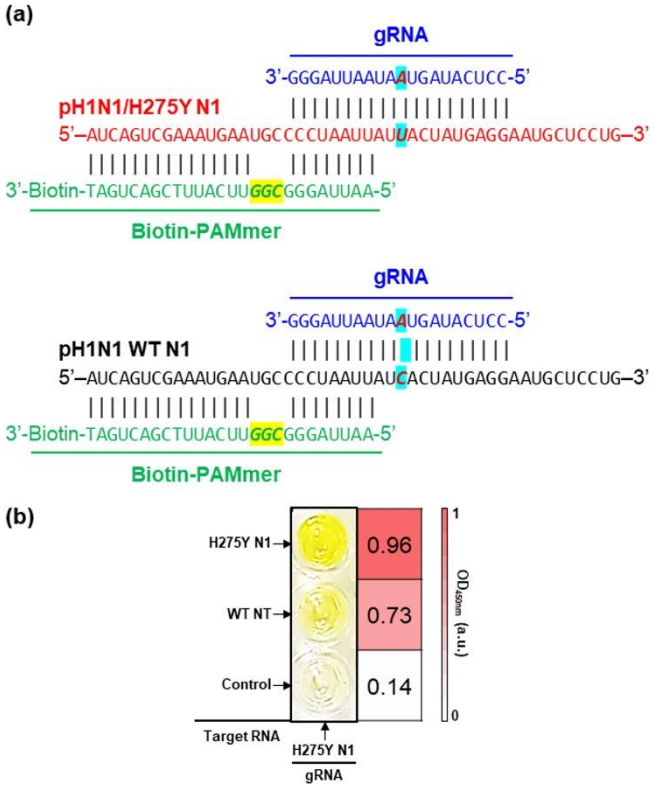

Figure S6. (a) Sequence of gRNA without additional mismatched point, pH1N1/H275Y N1, pH1N1 WT N1, and biotin-PAMmer. (b) Photograph of microplate and corresponding heat map after detection of various RNAs using CRISPR/dCas9. gRNA and target RNA are written in the bottom and left side of the microplate, respectively. The concentration of target RNAs is 100 nM.

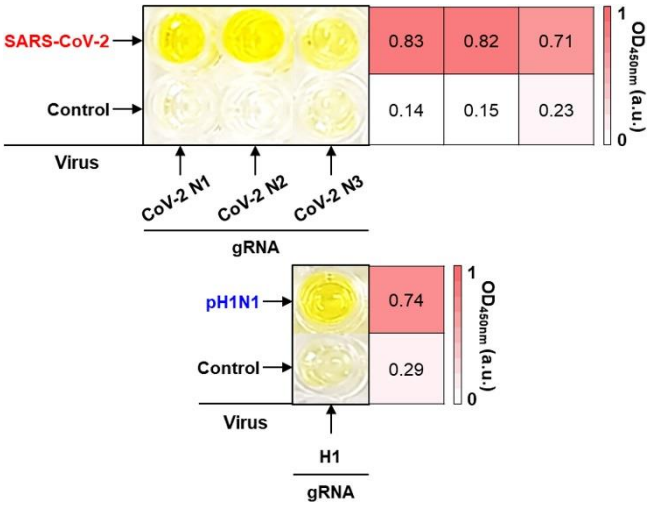

Figure S7. Photographs of microplates and corresponding heat maps after detection of SARS-CoV-2 (top) and pH1N1 (bottom) using CRISPR/dCas9. gRNA and target virus are written in the bottom and left side of the microplates, respectively. Only in the presence of SARS-CoV-2 and pH1N1, the color of dCas9/gRNA-attached wells turns yellow. The concentration of virus was 10 PFU/mL.

Table S1. Sequences used in this experiment.

gRNA	Sequence (5' → 3')
SARS-CoV-2 N1	mA*mA*mA* CGU AAU GCG GGG UGC AUG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC mU*mU*mU* U
SARS-CoV-2 N2	mU*mG*mG* GGG CAA AUU GUG CAA UUG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC mU*mU*mU* U
SARS-CoV-2 N3	mG*mG*mG* UGC CAA UGU GAU CUU UUG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC mU*mU*mU* U
pH1N1 H1	mC*mC*mA* GCA UUU CUU UCC AUU GCG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC mU*mU*mU* U
pH1N1 WT N1	mC*mC*mU* CUU AGU GAU AAU UAG GGG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC mU*mU*mU* U
pH1N1/H275Y N1	mC*mC*mU* CUU AGU AAU AAU UAG GGG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC mU*mU*mU* U
IFV H3	mC*mU*mU* CCA UUU GGA GUG AUG CAG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC mU*mU*mU* U
IFV H5	mC*mA*mA* CCA UCU ACC AUU CCC UGG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC mU*mU*mU* U
Target	**Sequence (5' → 3')**
SARS-CoV-2 N1	GAC CCC AAA AUC AGC GAA AUG CAC CCC GCA UUA CGU UUG G
SARS-CoV-2 N2	UUA CAA ACA UUG GCC GCA AAU UGC ACA AUU UGC CCC CA
SARS-CoV-2 N3	GGG AGC CUU GAA UAC ACC AAA AGA UCA CAU UGG CAC CC
pH1N1 H1	GGU ACC GAG AUA UGC AUU CGC AAU GGA AAG AAA UGC UGG AUC UG
pH1N1 WT N1	AUC AGU CGA AAU GAA UGC CCC UAA UUA UCA CUA UGA GGA AUG CUC CUG
pH1N1/H275Y N1	AUC AGU CGA AAU GAA UGC CCC UAA UUA UUA CUA UGA GGA AUG CUC CUG
IFV H3	UUG GCA AGU GCA AGU CUG AAU GCA UCA CUC CAA AUG GAA GCA UU
IFV H5	GGU UUU AUA GAG GGA GGA UGG CAG GGA AUG GUA GAU GGU UGG UAU G
SARS-CoV	AAC AUG CUU AGG AUA AUG GCC UCU CUU GUU CUU GCU CGC A
Biotin-PAMmer	**Sequence (5' → 3')**
SARS-CoV-2 N1	GGG TGC ATC GGG CTG ATT TTG GGG TC – Biotin
SARS-CoV-2 N2	GTG CAA TTC GGG GCC AAT GTT TGT AA – Biotin
SARS-CoV-2 N3	GAT CTT TTC GGG TAT TCA AGG CTC CC – Biotin
pH1N1 H1	rUrCrC rATrU GrCC rGGrU GrCA rUArU CrUC rGGrU ArCC rArAC rUT – Biotin
pH1N1 WT N1	rAArU rUArG GrGC GrGrU rUCA rUrUT CGA CrUG AT – Biotin
pH1N1/H275Y N1	rAArU rUArG GrGC GrGrU rUCA rUrUT CGA CrUG AT – Biotin
IFV H3	rGrUG rATrG CrArC GrGrA GrAC TrUrG rCAC rUTG rCrCA – Biotin
IFV H5	rArUT rCCrC TrGrC GrGrU CrCT CrCrC rUCT rATA rArAA – Biotin

Table S2. Diagnostic result of COVID-19 patients using PCR.

Number	Sample	Ct Value
1	Nasopharyngeal aspirate	24.80
2	Nasopharyngeal aspirate	31.91
3	Sputum	26.36
4	Sputum	20.37
5	Nasopharyngeal aspirate	31.53

Table S3. Comparison to other CRISPR/Cas system-based virus detection methods.

System name	Effector	Pre-amplification	Multi-plex	Time	Readout	Target type	LOD	Refs
NASBACC	Cas9	NASBA	N	~ 3 h	Colorimetric	RNA	fM	(Pardee et al. 2016)
SHERLOCK	Cas13a	RPA	N	2-5 h	Fluorescent	DNA/RNA	aM	(Gootenberg et al. 2017)
SHERLOCKv2	Cas13b	RPA	Y	0.5-3 h	Fluorescent; Colorimetric	DNA/RNA	zM	(Gootenberg et al. 2018)
HUDSON +SHERLOCK	Cas13a	RPA	N	~ 2 h	Fluorescent; Colorimetric	DNA/RNA	aM	(Myhrvold et Al. 2018)
HOLMES	Cas12a	PCR; RT-PCR	N	~ 1 h	Fluorescent	DNA/RNA	aM	(Li et al. 2018)
DETECTR	Cas12a	RPA	N	~ 2 h	Fluorescent	DNA	aM	(Chen et al. 2018)
HOLMESv2	Cas12b	LAMP; RT-LAMP; Asymmetric PCR	N	~ 1 h	Fluorescent	DNA/RNA	aM	(Li et al. 2019)
CASLFA	Cas9	PCR; RPA	N	~ 1 h	Colorimetric	DNA	hundred copies	(Wang et al. 2020b)
SHERLOCK	Cas13a	RPA	N	~ 1 h	Fluorescent; Colorimetric	RNA (SARS-CoV-2)	aM	(Zhang et al. 2020)
DETECTR	Cas12a	RT-LAMP	N	~ 45 min	Fluorescent; Colorimetric	RNA (SARS-CoV-2)	10 copies	(Broughton et al. 2020)
CRISPR-FDS	Cas12a	RT-PCR; RPA	N	~ 50 min	Fluorescent	RNA (SARS-CoV-2)	2 copies	(Huang et al. 2020)
This work	dCas9	-	Y	~ 1.5 h	Colorimetric	RNA (SARS-CoV-2)	pM	

REFERENCES

(1) Pardee, K.; Green, A. A.; Takahashi, M. K.; Braff, D.; Lambert, G.; Lee, J. W.; Ferrante, T.; Ma, D.; Donghia, N.; Fan, M.; Daringer, N. M.; Bosch, I.; Dudley, D. M.; O'Connor, D. H.; Gehrke, L.; Collins, J. J. Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. *Cell* **2016,** *165* (5), 1255-1266.

(2) Gootenberg, J. S.; Abudayyeh, O. O.; Lee, J. W.; Essletzbichler, P.; Dy, A. J.; Joung, J.; Verdine, V.; Donghia, N.; Daringer, N. M.; Freije, C. A.; Myhrvold, C.; Bhattacharyya, R. P.; Livny, J.; Regev, A.; Koonin, E. V.; Hung, D. T.; Sabeti, P. C.; Collins, J. J.; Zhang, F. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* **2017,** *356* (6336), 438-442.

(3) Gootenberg, J. S.; Abudayyeh, O. O.; Kellner, M. J.; Joung, J.; Collins, J. J.; Zhang, F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science* **2018,** *360* (6387), 439-444.

(4) Myhrvold, C.; Freije, C. A.; Gootenberg, J. S.; Abudayyeh, O. O.; Metsky, H. C.; Durbin, A. F.; Kellner, M. J.; Tan, A. L.; Paul, L. M.; Parham, L. A.; Garcia, K. F.; Barnes, K. G.; Chak, B.; Mondini, A.; Nogueira, M. L.; Isern, S.; Michael, S. F.; Lorenzana, I.; Yozwiak, N. L.; MacInnis, B. L.; Bosch, I.; Gehrke, L.; Zhang, F.; Sabeti, P. C. Field-deployable viral diagnostics using CRISPR-Cas13. *Science* **2018,** *360* (6387), 444-448.

(5) Li, S. Y.; Cheng, Q. X.; Wang, J. M.; Li, X. Y.; Zhang, Z. L.; Gao, S.; Cao, R. B.; Zhao, G. P.; Wang, J. CRISPR-Cas12a-assisted nucleic acid detection. *Cell Discov* **2018,** *4*, 20.

(6) Chen, J. S.; Ma, E.; Harrington, L. B.; Da Costa, M.; Tian, X.; Palefsky, J. M.; Doudna, J. A. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* **2018,** *360* (6387), 436-439.

(7) Li, L.; Li, S.; Wu, N.; Wu, J.; Wang, G.; Zhao, G.; Wang, J. HOLMESv2: A CRISPR-Cas12b-Assisted Platform for Nucleic Acid Detection and DNA Methylation Quantitation.

ACS Synth Biol **2019,** *8* (10), 2228-2237.

(8) Wang, X.; Xiong, E.; Tian, T.; Cheng, M.; Lin, W.; Wang, H.; Zhang, G.; Sun, J.; Zhou, X. Clustered Regularly Interspaced Short Palindromic Repeats/Cas9-Mediated Lateral Flow Nucleic Acid Assay. *ACS Nano* **2020,** *14* (2), 2497-2508.

(9) Zhang, F.; Abudayyeh, O. O.; Gootenberg, J. S. A protocol for detection of COVID-19 using CRISPR diagnostics. https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf.

(10) Broughton, J. P.; Deng, X.; Yu, G.; Fasching, C. L.; Servellita, V.; Singh, J.; Miao, X.; Streithorst, J. A.; Granados, A.; Sotomayor-Gonzalez, A.; Zorn, K.; Gopez, A.; Hsu, E.; Gu, W.; Miller, S.; Pan, C. Y.; Guevara, H.; Wadford, D. A.; Chen, J. S.; Chiu, C. Y. CRISPR-Cas12-based detection of SARS-CoV-2. *Nat Biotechnol* **2020,** *38* (7), 870-874.

(11) Huang, Z.; Tian, D.; Liu, Y.; Lin, Z.; Lyon, C. J.; Lai, W.; Fusco, D.; Drouin, A.; Yin, X.; Hu, T.; Ning, B. Ultra-sensitive and high-throughput CRISPR-p owered COVID-19 diagnosis. *Biosens Bioelectron* **2020,** *164*, 112316.