Supplementary information

A novel miniature CRISPR-Cas13 system for SARS-CoV-2 diagnostics

Ahmed Mahas[1], Qiaochu Wang[1], Tin Marsic[1], and Magdy M. Mahfouz[1,] *

[1]*Laboratory for Genome Engineering and Synthetic Biology, Division of Biological Sciences, 4700 King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia.*

***Correspondence**: Magdy M. Mahfouz (magdy.mahfouz@kaust.edu.sa)

Keywords: Cas13, COVID-19, SARS-CoV-2, RT-LAMP, nucleic acid detection, biosensors, molecular diagnostics, Point-of-care diagnostics

This file includes:

- **Supplementary Figure 1:** Computational pipeline for the identification of the RNA-targeting mCas13 protein.
- **Supplementary Figure 2:** Protein purification of mCas13.
- **Supplementary Figure 3**: Evaluation of mCas13 activity.
- **Supplementary Figure 4:** Characterization of mCas13 sensitivity to single mismatch between crRNA and the target sequence.
- **Supplementary Figure 5:** Visual detection of SARS-CoV-2 RNA with RT-LAMP mCas13.
- **Supplementary Figure 6:** Validation of mCas13-based visual detection with SARS-CoV-2 clinical samples.

- **Supplementary table 1:** crRNA sequences used in this study.
- **Supplementary table 2:** RT-LAMP primers used in this study.
- **Supplementary table 3:** Primers to PCR amplify N gene regions for IVT.
- **Supplementary table 4:** RNA reporter designs and sequences used in this study.
- **Supplementary table 5:** mCas13 DNA sequence and tag sequences for protein **purification.**
- **Supplementary table 6:** mCas13 protein sequence and tag sequences for protein **purification.**
- **Supplementary table 7:** Clinical sample information.
- **Supplementary table 8:** Properties of different CRISPR-based assays for SARS-CoV-2 detection.

A

Obtain protein sequences of novel compact Cas13s from Xu *et al.*, 2020

↓

Identify similar sequences:
BLASTp protein sequences against all proteins (-nr database) using NCBI BLASTp tool

↓ Consider only hits with Query cover >90%

Identify putitive and previously uncharachtrized Cas13 protein (Hypothetical proteins) with high sequence similarites (>90%) to the novel compact Cas13 proteins

↓ 1 candidate *

Align the protein sequence of the putative Cas13 protein against all novel compact Cas13 protein sequences

↓

Confirm the presence of the conserved RxxxxH motifs of the HEPN ribonuclease domain

↓

Obtain the metagenomic contigs of the identified putative Cas13 protein Accession# DSVK01000191.1

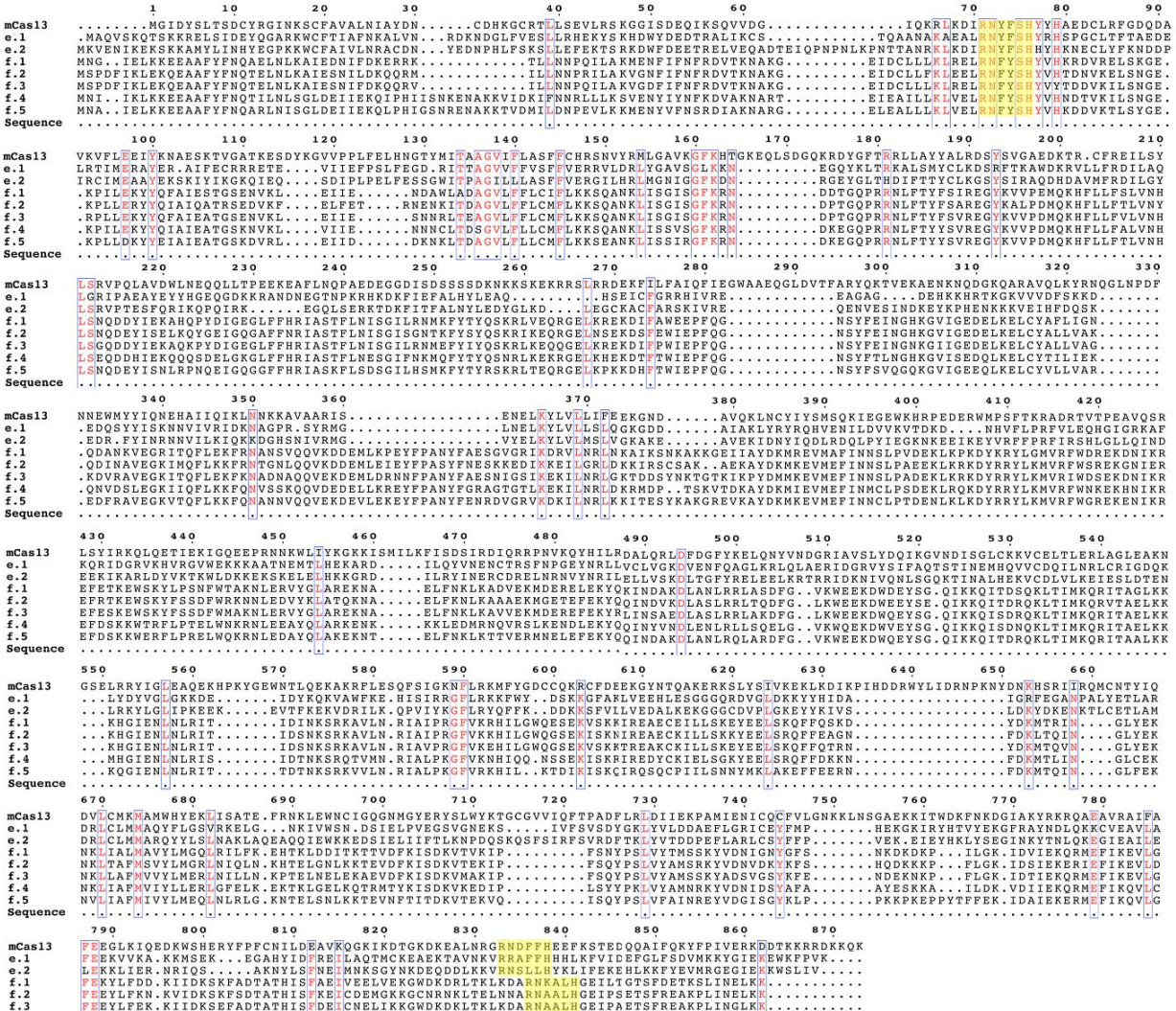
↓

Identify associated CRISPR arrays using CRISPRCasFinder

↓

Predict direct repeat (DR) orientation

B

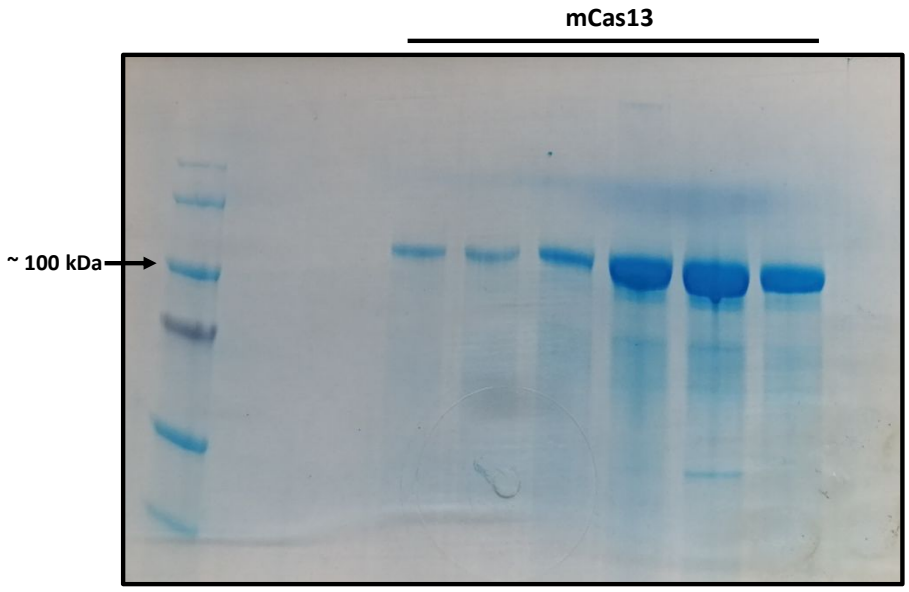


Supplementary Figure 1: Computational pipeline for the identification of the RNA-targeting mCas13 protein.

A- Schematic description of the sequence similarity searching strategies for the identification of novel miniature Cas13 effectors.

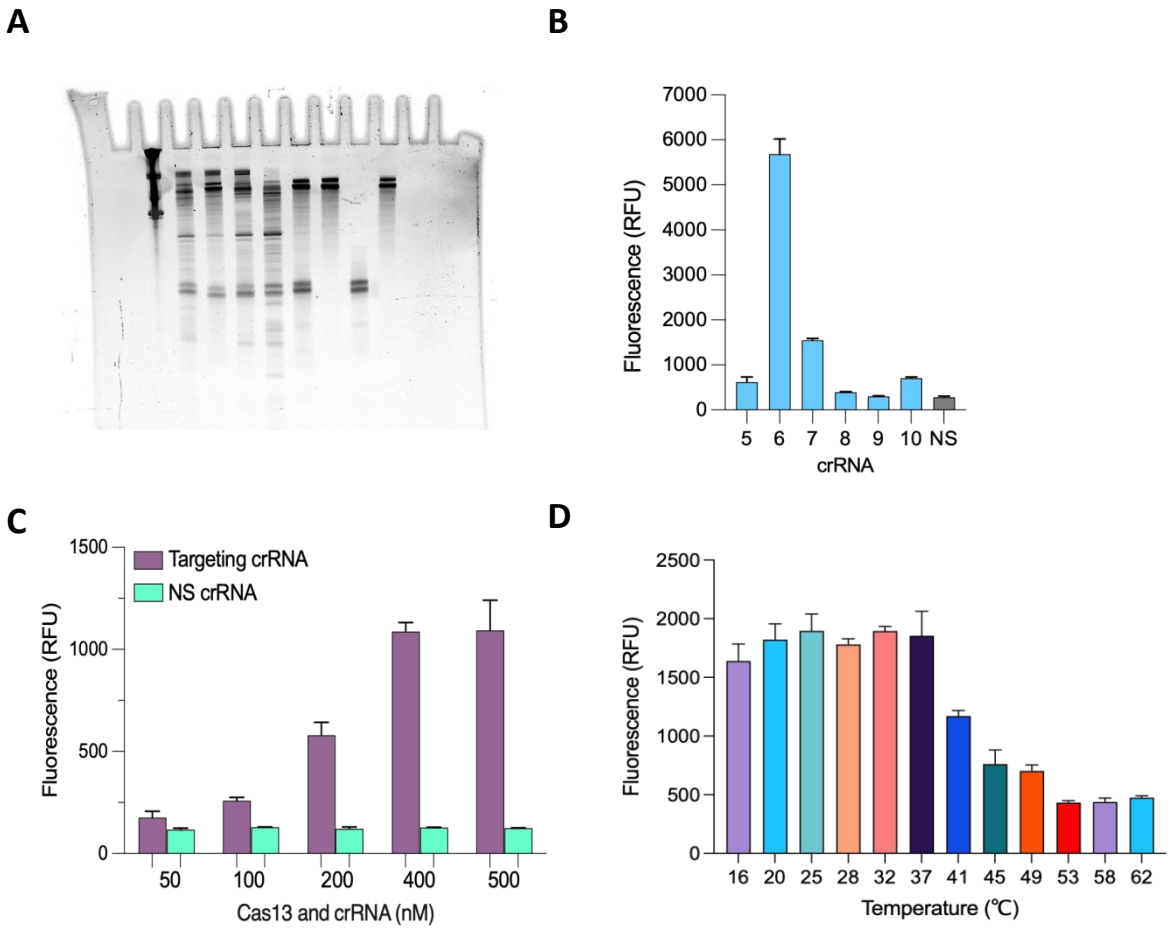
* BLASTp analysis was performed before January 2021.

B- Multiple sequence alignment of mCas13 and compact Cas13e and Cas13f orthologues. Protein sequences of mCas13, Cas13e.1, Cas13e.2, Cas13f.1, Cas13f.2, Cas13f.3, Cas13f.4 and Cas13f.5 were aligned using ClustalW in MEGAX and ESPript was used to generate the alignment visualization. Strictly conserved residues (%Strict) are shown in red text within blue rectangles. Conserved *RxxxxH* motifs of the HEPN domains are highlighted with yellow background.



Supplementary Figure 2: Protein purification of mCas13.

SDS-PAGE gel of purified mCas13 after size exclusion step. The size of mCas13 protein after removal of SUMO tag is ~ 102 kDa.



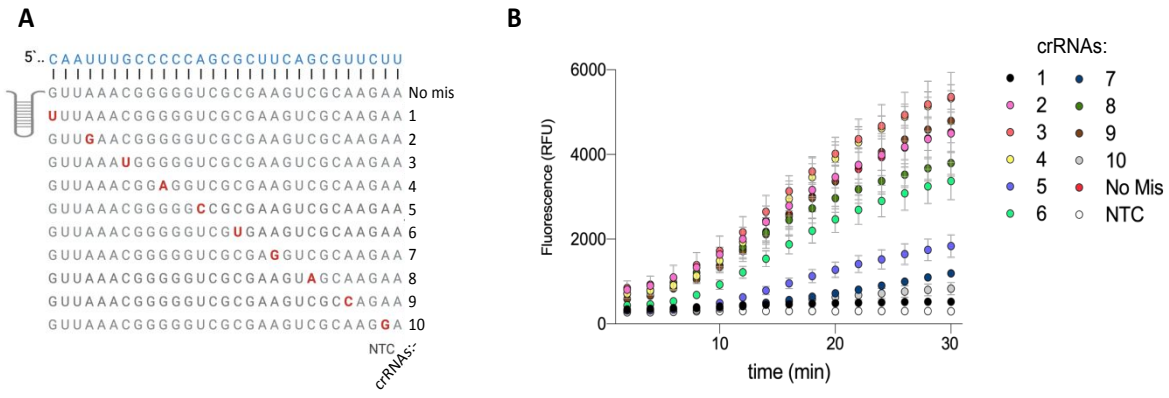
Supplementary Figure 3: Evaluation of mCas13 activity.

A- Uncropped image of the denaturing gel in figure 1B.

B- Activity assessment of mCas13 with different crRNA sequences (related to Figure 1B). Reactions containing mCas13 and one of the different crRNAs targeting the SARS-CoV-2 *N* gene were performed in the presence of the F-UUAUU-Q reporters. NS: non-specific (not SARS-CoV-2 targeting) crRNA. Values shown as mean ± SD (n = 3).

C- Titration of mCas13 and crRNA concentrations for optimal performance. 100 nM of *in vitro* transcribed RNA of SARS-CoV-2 N gene was used as the target sequence in mCas13 reactions. Values represent endpoint fluorescence at 30 mins. Values shown as mean ± SD (n = 3).

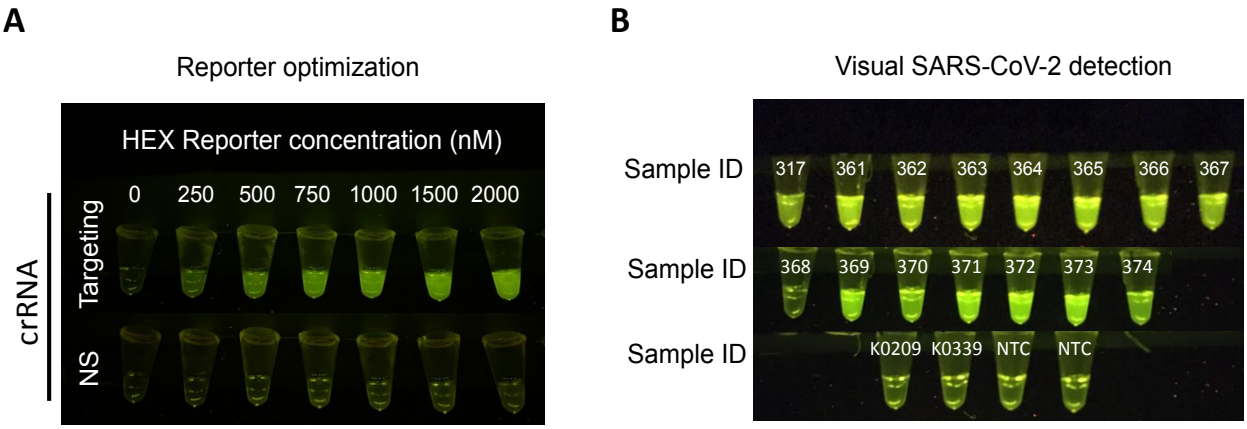
D- Temperature dependence of mCas13 collateral activity. Collateral activity was measured as end point fluorescence after incubation with 100 nM of *in vitro* transcribed RNA of SARS-CoV-2 *N* gene for 45 mins. Values shown as mean ± SD (n = 3).



Supplementary Figure 4: Characterization of mCas13 sensitivity to single mismatch between crRNA and the target sequence.

A- Sequences of crRNA 4 (Figure 1B) and the position of the synthetically introduced single mismatches.

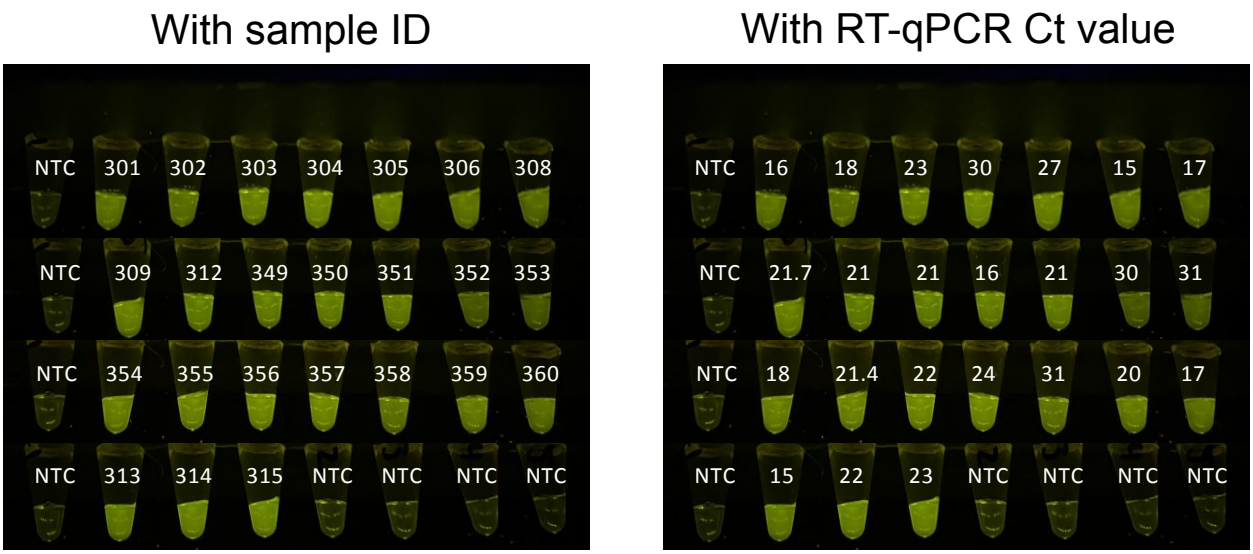
B- Real-time fluorescence measurements of the collateral cleavage activity of mCas13 with different crRNAs with different mismatches relative to no template control (NTC) and crRNA with no mismatch (No Mis) (related to Figure 1C). Values shown as mean ± SD (n = 3).



Supplementary Figure 5: Visual detection of SARS-CoV-2 RNA with RT-LAMP mCas13.

A- Optimization of fluorescent reporter (HEX reporter) concentration for clear visual detection of mCas13 collateral activity. mCas13 was incubated with crRNA 4 or non-specific (NS) crRNA and 100 nM of *in vitro* transcribed RNA of SARS-CoV-2 N gene using different concentrations of RNA reporter molecule with the sequence UUAUU that is conjugated to 5' HEX for 45 mins. Reactions were visualized with p51 fluorescence visualizer and photo was taken with a smart phone.

B- Different repeat of the experiment done in Figure 3D. Picture was taken with different smart phone used in the other figures. NTC: non-template control.



Supplementary Figure 6: Validation of mCas13-based visual detection with SARS-CoV-2 clinical samples.

RT-LAMP mCas13 visual detection reactions of 24 SARS-CoV-2 RT-qPCR positive samples and no template control (NTC) reactions used to generate the heatmap in figure 3E. Left, reaction tubes with fluorescence readouts are shown with the clinical sample ID. Right, reaction tubes with fluorescence readouts are shown with RT-qPCR Ct values indicated.

Supplementary table 1: crRNA sequences used in this study.

Name	Sequence	Note
crRNA 1	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCACAAGGAACTGATTACAAACATTGGCCGCACCCTATAGTGAGTCGTATTAATTTC	crRNAs for SARS-CoV-2 N gene Target 1 **Used in Figure 1B, C**
crRNA 2	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCACTGATTACAAACATTGGCCGCAAATTGCACCCTATAGTGAGTCGTATTAATTTC	
crRNA 3	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCGTTCTTCGGAATGTCGCGCATTGGCATGGCCCTATAGTGAGTCGTATTAATTTC	
crRNA 4*	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTGCCCCCAGCGCTTCAGCGTTCTTCCCTATAGTGAGTCGTATTAATTTC	
crRNA 5	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCAAGATCACATTGGCACCCGCAATCCTGCTACCCTATAGTGAGTCGTATTAATTTC	crRNAs for SARS-CoV-2 N gene Target 2 **Used in supplementary Figure 3B**
crRNA 6	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCACAATGCTGCAATCGTGCTACAACTTCCTCCCTATAGTGAGTCGTATTAATTTC	
crRNA 7	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCGTTCCTCATCACGTAGTCGCAACAGTTCACCCTATAGTGAGTCGTATTAATTTC	
crRNA 8	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCACAGTTCAAGAAATTCAACTCCAGGCAGCACCCTATAGTGAGTCGTATTAATTTC	
crRNA 9	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCGATGCTGCTCTTGCTTTGCTGCTGCTTGACCCTATAGTGAGTCGTATTAATTTC	
crRNA 10	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCAGGCCAAACTGTCACTAAGAAATCTGCTGCCCTATAGTGAGTCGTATTAATTTC	
NS	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCTTATGCCTCTTCCGACCATCAAGCATTTTACCCTATAGTGAGTCGTATTAATTTC	Non-specific targeting

crRNA 4-Mis-1	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCAAATTTGCCCC CAGCGCTTCAGCGTTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-2	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAACTTGCCCC CAGCGCTTCAGCGTTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-3	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTACCCC CAGCGCTTCAGCGTTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-4	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTGCCTC CAGCGCTTCAGCGTTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-5	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTGCCCC CGGCGCTTCAGCGTTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-6	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTGCCCC CAGCACTTCAGCGTTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-7	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTGCCCC CAGCGCTCCAGCGTTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-8	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTGCCCC CAGCGCTTCATCGTTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-9	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTGCCCC CAGCGCTTCAGCGGTCTTCCCTATAGTGAGTCGTATTAATTTC
crRNA 4-Mis-10	GCTGGAGCAGCCCTCGATTTGCAGGGTAATCACAGCCAATTTGCCCC CAGCGCTTCAGCGTTCCTCCCTATAGTGAGTCGTATTAATTTC

*crRNA 4 was used for all SARS-CoV-2 detection experiments

Supplementary table 2: RT-LAMP primers used in this study.

SC-T7-FIP	GCGGCCAATGTTTGTAATCAGTTAATACGACTCACTATAGGGAGACGTGGTCCAGAACAA	

Name	Sequence	Note
DE-F3	GAAATTTGGATCTTTGTCATCC	Original and modified DETECTR primers. Modified part (T7 promoter sequence) shown in red.
DE-B3	GAAATTTGGATCTTTGTCATCC	
DE-FIP	TGCGGCCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC	
DE-BIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG	
DE-LF	TTCCTTGTCTGATTAGTTC	
DE-LB	ACCTTCGGGAACGTGGTT	
DE-FIP-T7	TGCGGCCAATGTTTGTAATCAGTAATACGACTCACTATAGGCCAAGGAAATTTTGGGGAC	
DE-BIP-T7	CGCATTGGCATGGAAGTTAATACGACTCACTATAGGCACTTTGATGGCACCTGTGTAG	
SC-F3	GCTGCTGAGGCTTCTAAG	Original and modified STOPCovid primers. Modified part (T7 promoter sequence) shown in red.
SC-B3	GCGTCAATATGCTTATTCAGC	
SC-FIP	GCGGCCAATGTTTGTAATCAGTAGACGTGGTCCAGAACAA	
SC-BIP	TCAGCGTTCTTCGGAATGTCGCTGTGTAGGTCAACCACG	
SC-LF	CCTTGTCTGATTAGTTCCTGGT	
SC-LB	TGGCATGGAAGTCACACC	

SC-T7-BIP	TCAGCGTTCTTCGGAATGTCGCTAATACGACTCACTATAGGGTGTGTAGGTCAACCACG	

Supplementary table 3: Primers to PCR amplify N gene regions for IVT.

N gene-1-T7-F	GAAATTAATACGACTCACTATAGGGAGGCTTCTAAGAAGCCTCGGC
N gene-1-R_2	CTGTCTCTGCGGTAAGGCTTGAG
N gene-target 2-T7-F	GAAATTAATACGACTCACTATAGGGTTGCAACTGAGGGAGCCTTG
N gene-target-2-R	GCTTCTTAGAAGCCTCAGCAGCAG

Supplementary table 4: RNA reporter designs and sequences used in this study.

UA reporter-FAM	5'-/56-FAM/UUAUU/3IABKFQ/-3'
UA reporter-HEX	5'-/HEX/UUAUU/3IABKFQ/-3'
Poly U reporter-FAM	5'-/56-FAM/UUUUUU/3IABKFQ/-3'
Poly A reporter-FAM	5'-/56-FAM/AAAAAA/3IABKFQ/-3'
Mix reporter-FAM	5'-/56-FAM/UGACGU/3IABKFQ/-3'

Supplementary table 5: mCas13 DNA sequence and tag sequences for protein purification.

Name	Protein sequence
mCas13	atgggcagcagccatcatcatcatcatcacagcagcggcctggtgccgcgcggcagccatatggctagctggagccatccgcagtttgaaaaaggtggt ggtagcggtggtggttcaggtggtagtgcatggtcacaccctcagtttgagaaaatgtcggactcagaagtcaatcaagaagctaagccagaggtcaag ccagaagtcaagcctgagactcacatcaatttaaaggtgtccgatggatcttcagagatcttcttcaagatcaaaaagaccactcctttaagaaggctgat ggaagcgttcgctaaaagacagggtaaggaaatggactccttaagattcttgtacgacggtattagaatccaagctgatcagacccctgaagatttggac atggaggataacgatattattgaggctcacagagaacagattggtggatccATGGGAATAGACTATAGTCTAACATCAGATTGCTACCG TGGTATCAATAAATCTTGTTTCGCTGTGGCGCTGAACATTGCTTACGACAACTGCGATCATAAAGGCTGCCGTACCCT GCTGTCGGAGGTTTTGCGCTCTAAAGGCGGTATTTCGGACGAACAGATTAAGTCCCAAGTTGTCGATGGTATCCAGA AGCGCCTGAAGGATATCCGTAACTATTTCAGCCATTATTATCATGCAGAGGATTGCCTGCGCTTTGGTGACCAGGATG CGGTGAAAGTGTTTCTGGAGGAGATTTATAAGAATGCAGAGAGCAAAACGGTCGGTGCGACCAAAGAGTCTGACTA CAAGGGCGTTGTACCTCCGCTCTTTGAATTACATAATGGTACATACATGATTACCGCAGCGGGTGTCATCTTTCTCGCC AGCTTCTTCTGCCATCGTAGCAACGTTTATCGCATGCTGGGTGCCGTCAAAGGCTTCAAACACACCGGCAAAGAGCA GCTGTCCGATGGCCAGAAACGTGACTATGGCTTTACGCGTCGCCTGCTCGCTTACTATGCTCTGCGTGACTCCTACAG CGTTGGCGCAGAAGATAAAACGCGTTGCTTTCGTGAAATTCTGTCGTATCTGTCACGTGTTCCACAGTTGGCGGTGG ACTGGCTGAACGAACAACAATTGTTGACGCCGGAAGAAAAGGAGGCGTTTCTGAACCAGCCGGCAGAGGACGAGG GCGGTGACATCTCTGACAGCAGTAGCTCCGACAAAAACAAAAAGTCCAAAGAGAAAAGACGCAGCCTGCGCCGTGA TGAGAAGTTCATTTTGTTTGCCATCCAATTTATCGAGGGCTGGGCAGCAGAGCAGGGTCTGGATGTTACCTTCGCTCG TTACCAGAAGACCGTTGAAAAGGCTGAGAACAAAAACCAGGACGGTAAACAAGCGCGTGCAGTTCAGCTCAAGTAC CGCAACCAAGGCCTGAACCCGGATTTCAACAACGAGTGGATGTATTACATCCAGAACGAGCACGCGATTATTCAGAT TAAACTGAATAATAAGAAAGCGGTTGCAGCGCGTATTAGCGAAAATGAACTGAAGTATTTGGTCCTGTTGATCTTCG AGGAGAAGGGTAATGACGCGGTGCAGAAGTTGAATTGCTACATCTACAGCATGAGCCAGAAGATTGAGGGTGAAT GGAAACACCGTCCGGAAGATGAGCGTTGGATGCCGAGCTTCACCAAACGAGCGGACCGCACCGTGACCCCGGAAGC CGTCCAAAGCCGTCTGAGCTATATCCGCAAACAGCTGCAAGAAACCATTGAGAAGATTGGTCAAGAAGAACCGCGTA ACAACAAGTGGCTGATCTACAAGGGCAAAAAGATCTCTATGATTTTGAAGTTCATCAGTGATTCGATCCGTGATATTC AACGCCGTCCGAATGTTAAACAATATCACATTTTGCGTGACGCACTGCAACGTCTCGACTTCGATGGTTTTTATAAGG AACTTCAGAATTATGTTAATGACGGCCGCATTGCCGTAAGCCTGTATGACCAAATCAAGGGCGTGAATGACATCAGC GGTCTGTGTAAAAAAGTTTGTGAACTGACCTTGGAAAGGCTAGCGGGTCTGGAGGCTAAAAACGGCAGCGAACTGA GACGTTACATCGGCCTGGAAGCGCAGGAGAAGCACCCGAAGTACGGTGAATGGAATACCCTGCAAGAAAAAGCGA AACGCTTTCTGGAGTCTCAGTTCTCCATTGGTAAGAATTTTTTGCGTAAAATGTTTTACGGCGATTGTTGTCAAAAGCG CTGCTTCGATGAGGAGAAAGGCTACAACACCCAGGCGAAGGAGCGCAAAAGCCTGTATTCCATCGTTAAGGAGAAG CTGAAGGATATCAAGCCGATCCATGATGACCGTTGGTATCTGATTGATCGTAACCCGAAAAACTACGATAATAAGCA CAGCCGCATCATCCGTCAGATGTGCAACACGTACATCCAAGATGTGTTATGCATGAAGATGGCGATGTGGCACTACG AGAAATTAATCTCCGCGACTGAGTTCCGTAACAAGTTGGAGTGGAACTGCATTGGTCAGGGTAACATGGGTTATGAA CGTTACTCACTGTGGTACAAGACCGGTTGCGGTGTGGTGATCCAATTCACGCCAGCTGATTTCCTGCGGCTGGACATT ATTGAAAAACCGGCAATGATCGAGAACATCTGCCAGTGTTTCGTGCTGGGCAACAAAAAGCTAAACAGCGGCGCGG AAAAGAAAATCACTTGGGATAAATTCAACAAGGACGGCATAGCGAAATACCGTAAGCGCCAGGCCGAGGCGGTGC GTGCGATCTTCGCGTTTGAAGAGGGTCTCAAGATCCAAGAAGACAAATGGTCACACGAACGTTACTTCCCGTTTTGTA ATATCTTGGATGAAGCCGTGAAGCAGGGGAAAATCAAAGATACCGGTAAGGACAAAGAAGCCTTAAATCGTGGTCG TAACGACTTTTTTCATGAAGAATTTAAAAGCACCGAAGACCAGCAAGCGATTTTTCAAAAATACTTCCCGATTGTTGA ACGGAAGGACGACACCAAAAAACGTCGCGATAAGAAACAGAAGTAA

- 6x His affinity tag, Thrombin site, Strep-tag II, SUMO, mCas13 protein.

Supplementary table 6: mCas13 protein sequence and tag sequences for protein purification.

Name	Protein sequence
mCas13	MGSSHHHHHHSSGLVPRGSHMASWSHPQFEKGGGSGGGSGGSAWSHPQFEKMSDSEVNQEAKPEVKPE VKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAH REQIGGSMGIDYSLTSDCYRGINKSCFAVALNIAYDNCDHKGCRTLLSEVLRSKGGISDEQIKSQVVDGIQKRLK DIRNYFSHYYHAEDCLRFGDQDAVKVFLEEIYKNAESKTVGATKESDYKGVVPPLFELHNGTYMITAAGVIFLAS FFCHRSNVYRMLGAVKGFKHTGKEQLSDGQKRDYGFTRRLLAYYALRDSYSVGAEDKTRCFREILSYLSRVPQL AVDWLNEQQLLTPEEKEAFLNQPAEDEGGDISDSSSSDKNKKSKEKRRSLRRDEKFILFAIQFIEGWAAEQGLD VTFARYQKTVEKAENKNQDGKQARAVQLKYRNQGLNPDFNNEWMYYIQNEHAIIQIKLNNKKAVAARISENE LKYLVLLIFEEKGNDAVQKLNCYIYSMSQKIEGEWKHRPEDERWMPSFTKRADRTVTPEAVQSRLSYIRKQLQE TIEKIGQEEPRNNKWLIYKGKKISMILKFISDSIRDIQRRPNVKQYHILRDALQRLDFDGFYKELQNYVNDGRIAV SLYDQIKGVNDISGLCKKVCELTLERLAGLEAKNGSELRRYIGLEAQEKHPKYGEWNTLQEKAKRFLESQFSIGKN FLRKMFYGDCCQKRCFDEEKGYNTQAKERKSLYSIVKEKLKDIKPIHDDRWYLIDRNPKNYDNKHSRIIRQMCN TYIQDVLCMKMAMWHYEKLISATEFRNKLEWNCIGQGNMGYERYSLWYKTGCGVVIQFTPADFLRLDIIEKP AMIENICQCFVLGNKKLNSGAEKKITWDKFNKDGIAKYRKRQAEAVRAIFAFEEGLKIQEDKWSHERYFPFCNIL DEAVKQGKIKDTGKDKEALNRGRNDFFHEEFKSTEDQQAIFQKYFPIVERKDDTKKRRDKKQK*

- 6x His affinity tag, Thrombin site, Strep-tag II, SUMO, mCas13 protein. G-SUMO cleavage site (after this site).

Supplementary table 7: Clinical sample information.

Sample number	Sample ID	Ct value	Batch #
301	155369	16	4
302	155397	18	4
303	155398	23	4
304	155399	30	4
305	155402	27	4
306	155902	15	4
308	155908	17	4
309	155916	21.7	4
312	156852	21	4
313	156854	15	4
314	156855	22	4
315	156859	23	4
317	158512	19	4
349	161073	21	4

350	161080	16	4
351	161081	21	4
352	161082	30	4
353	161083	31	4
354	161087	18	4
355	161088	21.4	4
356	161089	22	4
357	161091	24	4
358	161093	31	4
359	161095	20	4
360	161096	17	4
361	161098	29	4
362	161100	20	4
363	161105	23	4
364	161115	16	4
365	161117	15	4

366	161122	19	4
367	161123	22	4
368	161127	34	4
369	161130	18	4
370	161144	23	4
371	161149	19	4
372	161152	16	4
373	161159	21	4
374	161162	18	4
K0209	**K0209**	N1:36.94 N2: 39.28	-
K0339	**K0339**	N1: 38.27 N2: 39.41	-

Supplementary table 8: Properties of different CRISPR-based assays for SARS-CoV-2 detection

Assay name	Amplification	CRISPR/Cas effector	One-pot	Assay time	Limit of detection	Validation using clinical samples	Visual detection	Reference
mCas13 detection	RT-LAMP	mCas13b	No	<60 min	4 coipes/µL of synthetic SARS-CoV-2 genome RNA	Yes	LED-based detection	This work
DETECTR	RT-LAMP	LbCas12a	No	30-45 min	10 coipes/µL of *in vitro* transcription SARS-CoV-2 RNA	Yes	Strip-based detection	Broughton *et al.* 2020[1]
SHERLOCK	RT-RPA	LwaCas13a	No	55-85 min	3 coipes/µL of extracted SARS-CoV-2 RNA (Ct 33.5 for *N* gene)	Yes	Strip-based detection	Patchsung *et al.* 2020[2]
STOPCovid	RT-LAMP	AacCas12b and AapCas12b	Yes (only for fluorescence detection)	40-70 min	2 coipes/µL of SARS-CoV-2 genome RNA	Yes	Strip-based detection	Joung *et al.* 2020[3]
CASdetec	RT-RAA	AaCas12b	No	40-60 min	5 coipes/µL of *in vitro* transcription SARS-CoV-2 RNA	No	LED-based detection	Guo *et al.* 2020[4]
AIOD-CRISPR	RT-RPA	LbaCas12a	Yes	20-40 min	~5 copies/µL of *in vitro* transcription SARS-CoV-2 RNA	Yes	LED or UV-based detection	Ding *et al.* 2020[5]
Direct CRISPR-Cas13a assay	No	LbuCas13a	Yes	5-30 min	~100 copies/µL of *in vitro* transcription SARS-CoV-2 RNA	Yes	Mobile phone-based fluorescence microscope and reaction chamber	Fozouni *et al.* 2021[6]
iSCAN	RT-LAMP	LbCas12a for two-pot; AacCas12b for one-pot	Yes (for one-pot iSCAN detection)	40-60 min	~10 copies/µL of synthetic SARS-CoV-2 genome RNA	Yes	Strip or UV-based detection	Ali *et al.* 2020[7]
CREST	PCR (with portable thermocycler)	LwaCas13a	No	<120 min	10 coipes/µL of *in vitro* transcription SARS-CoV-2 RNA	Yes	LED-based detection	Rauch *et al.* 2021[8]

References

[1] 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., and Chiu, C. Y. (2020) CRISPR-Cas12-based detection of SARS-CoV-2, *Nat Biotechnol 38*, 870-874.

[2] Patchsung, M., Jantarug, K., Pattama, A., Aphicho, K., Suraritdechachai, S., Meesawat, P., Sappakhaw, K., Leelahakorn, N., Ruenkam, T., Wongsatit, T., Athipanyasilp, N., Eiamthong, B., Lakkanasirorat, B., Phoodokmai, T., Niljianskul, N., Pakotiprapha, D., Chanarat, S., Homchan, A., Tinikul, R., Kamutira, P., Phiwkaow, K., Soithongcharoen, S., Kantiwiriyawanitch, C., Pongsupasa, V., Trisrivirat, D., Jaroensuk, J., Wongnate, T., Maenpuen, S., Chaiyen, P., Kamnerdnakta, S., Swangsri, J., Chuthapisith, S., Sirivatanauksorn, Y., Chaimayo, C., Sutthent, R., Kantakamalakul, W., Joung, J., Ladha, A., Jin, X., Gootenberg, J. S., Abudayyeh, O. O., Zhang, F., Horthongkham, N., and Uttamapinant, C. (2020) Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA, *Nat Biomed Eng 4*, 1140-1149.

[3] Joung, J., Ladha, A., Saito, M., Kim, N. G., Woolley, A. E., Segel, M., Barretto, R. P. J., Ranu, A., Macrae, R. K., Faure, G., Ioannidi, E. I., Krajeski, R. N., Bruneau, R., Huang, M. W., Yu, X. G., Li, J. Z., Walker, B. D., Hung, D. T., Greninger, A. L., Jerome, K. R., Gootenberg, J. S., Abudayyeh, O. O., and Zhang, F. (2020) Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing, *N Engl J Med 383*, 1492-1494.

[4] Guo, L., Sun, X., Wang, X., Liang, C., Jiang, H., Gao, Q., Dai, M., Qu, B., Fang, S., Mao, Y., Chen, Y., Feng, G., Gu, Q., Wang, R. R., Zhou, Q., and Li, W. (2020) SARS-CoV-2 detection with CRISPR diagnostics, *Cell Discov 6*, 34.

[5] Ding, X., Yin, K., Li, Z., Lalla, R. V., Ballesteros, E., Sfeir, M. M., and Liu, C. (2020) Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay, *Nat Commun 11*, 4711.

[6] Fozouni, P., Son, S., Diaz de Leon Derby, M., Knott, G. J., Gray, C. N., D'Ambrosio, M. V., Zhao, C., Switz, N. A., Kumar, G. R., Stephens, S. I., Boehm, D., Tsou, C. L., Shu, J., Bhuiya, A., Armstrong, M., Harris, A. R., Chen, P. Y., Osterloh, J. M., Meyer-Franke, A., Joehnk, B., Walcott, K., Sil, A., Langelier, C., Pollard, K. S., Crawford, E. D., Puschnik, A. S., Phelps, M., Kistler, A., DeRisi, J. L., Doudna, J. A., Fletcher, D. A., and Ott, M. (2021) Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy, *Cell 184*, 323-333 e329.

[7] Ali, Z., Aman, R., Mahas, A., Rao, G. S., Tehseen, M., Marsic, T., Salunke, R., Subudhi, A. K., Hala, S. M., Hamdan, S. M., Pain, A., Alofi, F. S., Alsomali, A., Hashem, A. M., Khogeer, A., Almontashiri, N. A. M., Abedalthagafi, M., Hassan, N., and Mahfouz, M. M. (2020) iSCAN: An RT-LAMP-coupled CRISPR-Cas12 module for rapid, sensitive detection of SARS-CoV-2, *Virus Res 288*, 198129.

[8] Rauch, J. N., Valois, E., Solley, S. C., Braig, F., Lach, R. S., Audouard, M., Ponce-Rojas, J. C., Costello, M. S., Baxter, N. J., Kosik, K. S., Arias, C., Acosta-Alvear, D., and Wilson, M. Z. (2021) A Scalable, Easy-to-Deploy Protocol for Cas13-Based Detection of SARS-CoV-2 Genetic Material, *J Clin Microbiol 59*.