Supplementary information for

The DNA-Cu nanocluster and exonuclease I integrated label-free reporting system for CRISPR/Cas12a-based SARS-CoV-2 detection with minimized background signal

Siying Xie[a], Cheng Qin[b], Furong Zhao[a], Zhaoyang Shang[a], Pei Wang[a,c], Muhammad Sohail[a,c], Xing Zhang[a, *], Bingzhi Li[a, *]

[a] School of Food Science and Pharmaceutical Engineering, Nanjing Normal University, Nanjing 210023, China

[b] School of Life Sciences and Chemical Engineering, Jiangsu Second Normal University, Nanjing 210013, China

[c] School of Life Sciences, Nanjing Normal University, Nanjing 210023, China

Manuscript prepared for *Journal of Materials Chemistry B*.

* Corresponding Author:

Bingzhi Li (bingzhili@njnu.edu.cn)

Xing Zhang (zhangxing@njnu.edu.cn)

Table S1. Oligonucleotides used in this study

Names	Sequence (from 5' to 3')
ss-Target	GTTCAATCTGTCAAGCAGCAG
ds-Target-1	TTTGCTGCTGCTTGACAGATTGAAC
ds-Target-2	GTTCAATCTGTCAAGCAGCAGCAAA
cr-RNA	UAAUUUCUACUAAGUGUAGAUCUGCUGCUUGACAGAUUGAAC
T12	TTTTTTTTTTTT
T14	TTTTTTTTTTTTTT
T16	TTTTTTTTTTTTTTTT
T18	TTTTTTTTTTTTTTTTTT
T20	TTTTTTTTTTTTTTTTTTTT
T22	TTTTTTTTTTTTTTTTTTTTTT
T24	TTTTTTTTTTTTTTTTTTTTTTTT
T26	TTTTTTTTTTTTTTTTTTTTTTTTTT
T28	TTTTTTTTTTTTTTTTTTTTTTTTTTTT
T30	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
T30-6	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCCCCC
T30-8	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCCCCCCC
T30-10	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCCCCCCCCC
T30-15	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCTCGCTTCCCCTTC
T30-20	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCTCGCTTCCCCTTCTCC TT
T30-25	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCTCGCTTCCCCTTCTCC TTTCATC
p6	GGGGGG
p8	GGGGGGGG
p10	GGGGGGGGGG
p15	GAAGGGGAAGCGAGG
p20	CCTCGCTTCCCCTTCTCCTT

p25	GATGAAAGGAGAAGGGGAAGCGAGG
SM1	GATCAATCTGTCAAGCAGCAG
SM2	GTTCAATCAGTCAAGCAGCAG
DM1	GTTCAATCTGTCAAGCTGCTG
DM2	GATCATTCTGTCAAGCAGCAG
TM1	GATCAATATGTCAAGCTGCAG
SARS-CoV	GTTCAATCTGTCTAGCAGCAA
MERS-CoV	GCATCAGTGGCGCCATCTTCA
SM1-1	GTTCAATCTGTCAAGCAGCAGCAGA
SM1-2	TCTGCTGCTGCTTGACAGATTGAAC
SM2-1	GTTCAATCTGTCAAGCAGCAGCAAT
SM2-2	ATTGCTGCTGCTTGACAGATTGAAC
DM1-1	GTTCAATCTGTCAAGCAGCGCCAAA
DM1-2	TTTGGCGCTGCTTGACAGATTGAAC
DM2-1	GTTCAATCTGTCAAGCAGCAGCATT
DM2-2	AATGCTGCTGCTTGACAGATTGAAC
TM1-1	GTTCAATCTGTCAAGTCACAGCAAA
TM1-2	TTTGCTGTGACTTGACAGATTGAAC
SARS - CoV (1)	GCTATTGCTGCTAGACAGATTGAAC
SARS - CoV (2)	GTTCAATCTGTCTAGCAGCAATAGC
MERS - CoV (1)	TCCATGAAGATGGCGCCACTGATGC
MERS - CoV (2)	GCATCAGTGGCGCCATCTTCATGGA
PCR-F Primer	GGGGAACTTCTCCTGCTAGAAT
PCR-R Primer	CAGACATTTTGCTCTCAAGCTG
N gene	atgtctgataatggaccccaaaatcagcgaaatgcaccccgcattacgtttggtggaccctcagattcaact
	ggcagtaaccagaatggagaacgcagtggggcgcgatcaaaacaacgtcggccccaaggtttacccaat
	aatactgcgtcttggttcaccgctctcactcaacatggcaaggaagaccttaaattccctcgaggacaaggc
	gttccaattaacaccaatagcagtccagatgaccaaattggctactaccgaagagctaccagacgaattcgt
	ggtggtgacggtaaaatgaaagatctcagtccaagatggtatttctactacctaggaactgggccagaagct

	ggacttccctatggtgctaacaaagacggcatcatatgggttgcaactgagggagccttgaatacaccaaa
	agatcacattggcacccgcaatcctgctaacaatgctgcaatcgtgctacaacttcctcaaggaacaacatt
	gccaaaaggcttctacgcagaagggagcagaggcggcagtcaagcctcttctcgttcctcatcacgtagtc
	gcaacagttcaagaaattcaactccaggcagcagtaggggaacttctcctgctagaatggctggcaatggc
	ggtgatgctgctcttgctttgctgctgcttgacagattgaaccagcttgagagcaaaatgtctggtaaaggcc
	aacaacaacaaggccaaactgtcactaagaaatctgctgctgaggcttctaagaagcctcggcaaaaacg
	tactgccactaaagcatacaatgtaacacaagctttcggcagacgtggtccagaacaaacccaaggaaatt
	ttggggaccaggaactaatcagacaaggaactgattacaaacattggccgcaaattgcacaatttgccccc
	agcgcttcagcgttcttcggaatgtcgcgcattggcatggaagtcacaccttcgggaacgtggttgacctac
	acaggtgccatcaaattggatgacaaagatccaaatttcaaagatcaagtcattttgctgaataagcatattga
	cgcatacaaaacattcccaccaacagagcctaaaaaggacaaaaagaagaaggctgatgaaactcaagc
	cttaccgcagagacagaagaaacagcaaactgtgactcttcttcctgctgcagatttggatgatttctccaaa
	caattgcaacaatccatgagcagtgctgactcaactcaggcctaa
E gene	atgtactcattcgtttcggaagagacaggtacgttaatagttaatagcgtacttctttttcttgctttcgtggtattc
	ttgctagttacactagccatccttactgcgcttcgattgtgtgcgtactgctgcaatattgttaacgtgagtcttg
	taaaaccttctttttacgtttactctcgtgttaaaaatctgaattcttctagagttcctgatcttctggtctaa
ORF1ab	ggaagccaatatggatcaagaatcctttggtggtgcatcgtgttgtctgtactgccgttgccacatagatcatc
(GenBankNO.NC_0	caaatcctaaaggattttgtgacttaaaaggtaagtatgtacaaatacctacaacttgtgctaatgaccctgtg
45512,13201-	ggttttacacttaaaaacacagtctgtaccgtctgcggtatgtggaaaggttatggctgtagttgtgatcaact
15600)	ccgcgaacccatgcttcagtcagctgatgcacaatcgtttttaaacgggtttgcggtgtaagtgcagcccgt
	cttacaccgtgcggcacaggcactagtactgatgtcgtatacagggcttttgacatctacaatgataaagtag
	ctggttttgctaaattcctaaaaactaattgttgtcgcttccaagaaaaggacgaagatgacaatttaattgatt
	cttactttgtagttaagagacacactttctctaactaccaacatgaagaaacaatttataatttacttaaggattgt
	ccagctgttgctaaacatgacttctttaagtttagaatagacggtgacatggtaccacatatatcacgtcaacg
	tcttactaaatacacaatggcagacctcgtctatgctttaaggcattttgatgaaggtaattgtgacacattaaa
	agaaatacttgtcacatacaattgttgtgatgatgattatttcaataaaaaggactggtatgattttgtagaaaac
	ccagatatattacgcgtatacgccaacttaggtgaacgtgtacgccaagctttgttaaaaacagtacaattct
	gtgatgccatgcgaaatgctggtattgttggtgtactgacattagataatcaagatctcaatggtaactggtat
	gatttcggtgatttcatacaaaccacgccaggtagtggagttcctgttgtagattcttattattcattgttaatgcc

tatattaaccttgaccagggctttaactgcagagtcacatgttgacactgacttaacaaagccttacattaagt

gggatttgttaaaatatgacttcacggaagagaggttaaaactctttgaccgttattttaaatattgggatcaga

cataccacccaaattgtgttaactgtttggatgacagatgcattctgcattgtgcaaactttaatgttttattctct

acagtgttcccacctacaagttttggaccactagtgagaaaaatatttgttgatggtgttccatttgtagtttcaa

ctggataccacttcagagagctaggtgttgtacataatcaggatgtaaacttacatagctctagacttagtttta

aggaattacttgtgtatgctgctgaccctgctatgcacgctgcttctggtaatctattactagataaacgcacta

cgtgcttttcagtagctgcacttactaacaatgttgcttttcaaactgtcaaacccggtaatttttaacaaagactt

ctatgactttgctgtgtctaagggtttctttaaggaaggaagttctgttgaattaaaacacttcttctttgctcagg

atggtaatgctgctatcagcgattatgactactatcgttataatctaccaacaatgtgtgatatcagacaactac

tatttgtagttgaagttgttgataagtactttgattgttacgatggtggctgtattaatgctaaccaagtcatcgtc

aacaacctagacaaatcagctggttttccatttaataaatggggtaaggctagactttattatgattcaatgagt

tatgaggatcaagatgcacttttcgcatatacaaaacgtaatgtcatccctactataactcaaatgaatcttaag

tatgccattagtgcaaagaatagagctcgcaccgtagctggtgtctctatctgtagtactatgaccaatagac

agtttcatcaaaaattattgaaatcaatagccgccactagaggagctactgtagtaattggaacaagcaaatt

ctatggtggttggcacaacatgttaaaaactgtttatagtgatgtagaaaaccctcaccttatgggttgggatt

atcctaaatgtgatagagccatgcctaacatgcttagaattatggcctcacttgttcttgctcgcaaacataca

acgtgttgtagcttgtcacaccgtttctatagattagctaatgagtgtgctcaagtattgagtgaaatggtcatg

tgtggcggttcactatatgttaaaccaggtggaacctcatcaggagatgccacaactgcttatgctaatagtg

tttttaacatttgtcaagctgtacggccaatgttaatgcacttttatctactgatggtaacaaaattgccgataagt

atgtc

The blue and red bases correspond to crRNA and DNA template in Figure 1, respectively. The green bases represent the mutation in target sequence (SM: single-base mutation; DM: double-base mutation; TM: triple-base mutation). The yellow background highlights the target nucleic acid sequence in N gene of SARS-CoV-2.

Table S2. The cost analysis of this reporter

Component	Amount	Cost ($)	Number of uses	Cost/1000 reactions ($)
EXO I	20 KU	157	4000	39
T30+20 (DNA)	5 OD	5	500	10
$CuSO_4 \cdot 5H_2O$	500 g	7	3.15E5	0.000175
NaAA	25 g	7	4E7	0.023
			Total cost per 1000 reactions ($)	**49.02**

Table S3. Comparison of our reporter and dual-labeled fluorescence reporters

	Probes	Main components	Cost / 1000 reactions ($)	Ref.
1	FQ reporter	5-FAM, 3-BHQ1	220	*Biosens. Bioelectron*, 2021, **172**, 112766.
2	G-triplex reporter	5-FAM, 3-TAMRA	88.39	*Biosens. Bioelectron*, 2021, **187**, 113292.
3	dsDNA-reporter	5-FAM, 3-BHQ1	63	*ACS Synth. Biol.*, 2021, **10**, 1785-1791.
4	**Our reporter**	**EXO Ⅰ T30+20 (DNA) $CuSO_4 \cdot 5H_2O$ NaAA**	**49.02**	**This work**

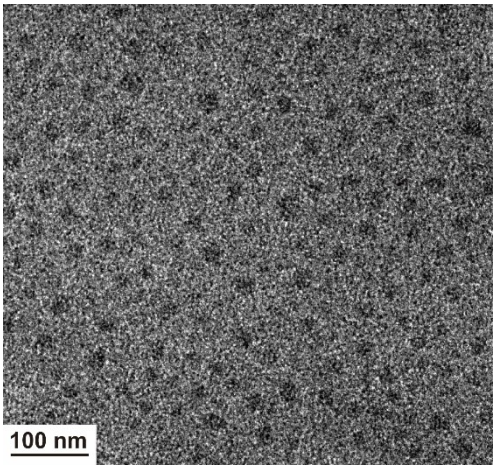

Figure S1. The TEM of synthetic DNA-CuNCs. 10 μM DNA template and 40 mM NaAA were mixed in dark for 2 min in the CRISPR reaction buffer, and then adding 0.4 mM Cu^{2+}, forming DNA-CuNCs.

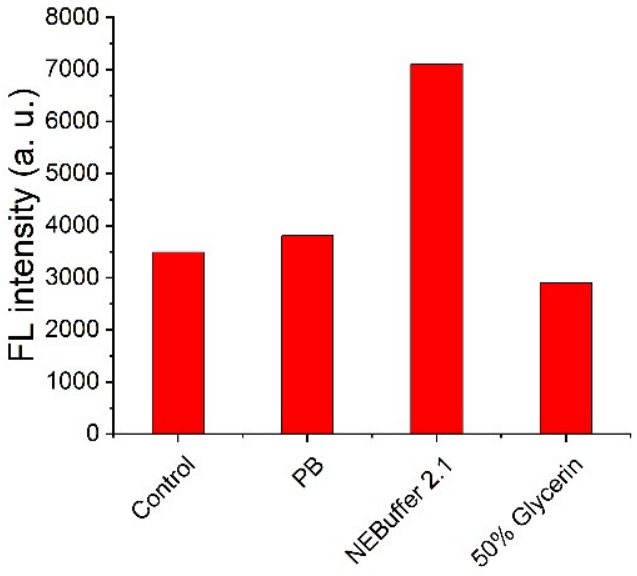

Figure S2. Fluorescence intensity of DNA-CuNCs synthesized in different buffer. Control refer to H_2O. The added volume of each buffer was consistent with CRISPR reaction.

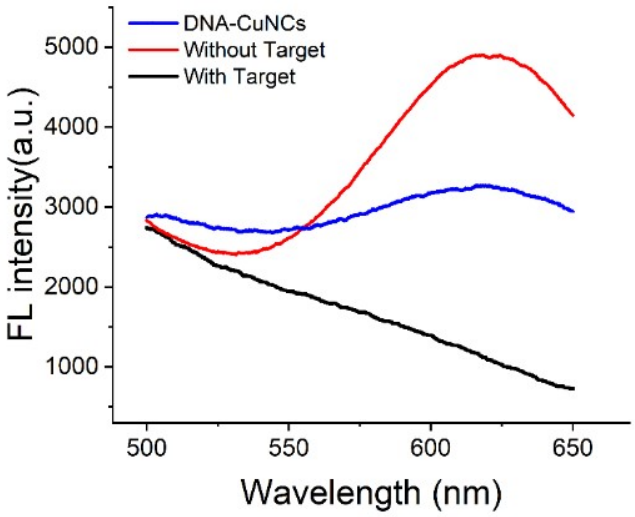

Figure S3. The verification feasibility of CRISPR-CNS via fluorescence spectra. Fluorescence spectra of the DNA-CuNCs (blue), CRISPR-CNS without target (red) and CRISPR-CNS with target (black).

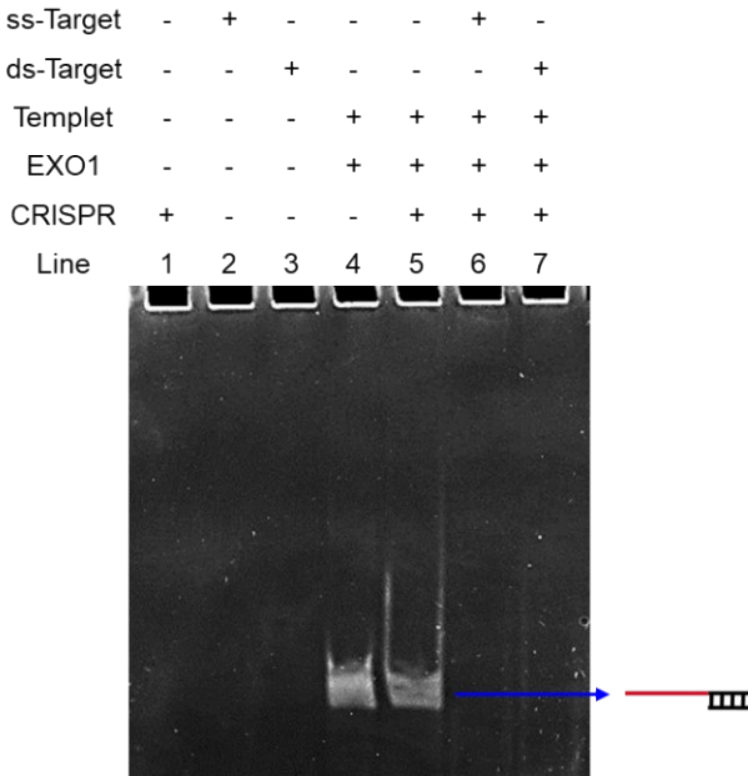

Figure S4. The suitability of ss-target and ds-target for CRISPR-CNS via 15% polyacrylamide gel electrophoresis.

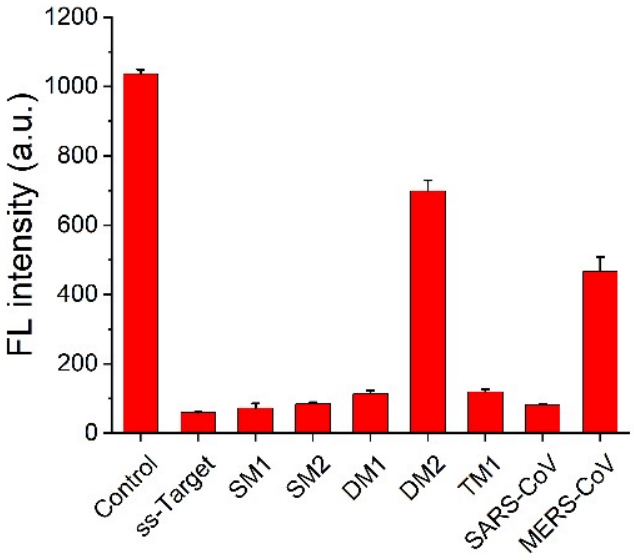

Figure S5. The selectivity of detection with ssDNA. The ss-Target concentration added for detection was 50 nM, while the concentration of non-target nucleic acids was 500 nM, which was 10-folds ss-Target concentration.