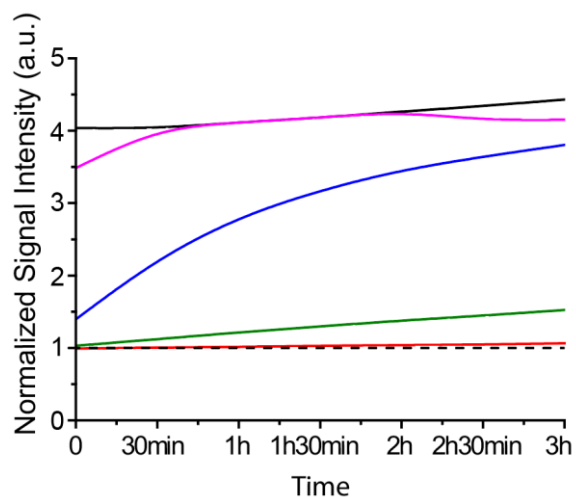


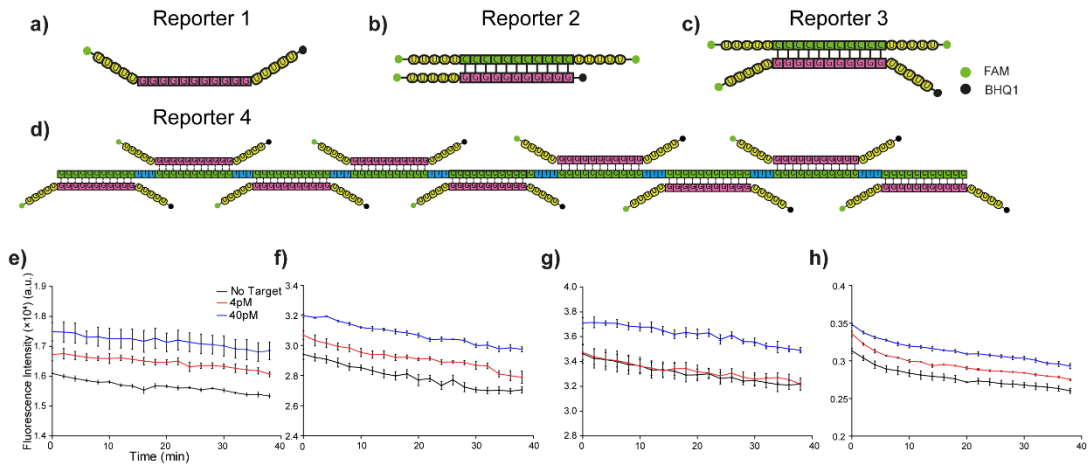
Supplementary for Amplification-free detection of viral RNA by super resolution imaging-based CRISPR/Cas13a System

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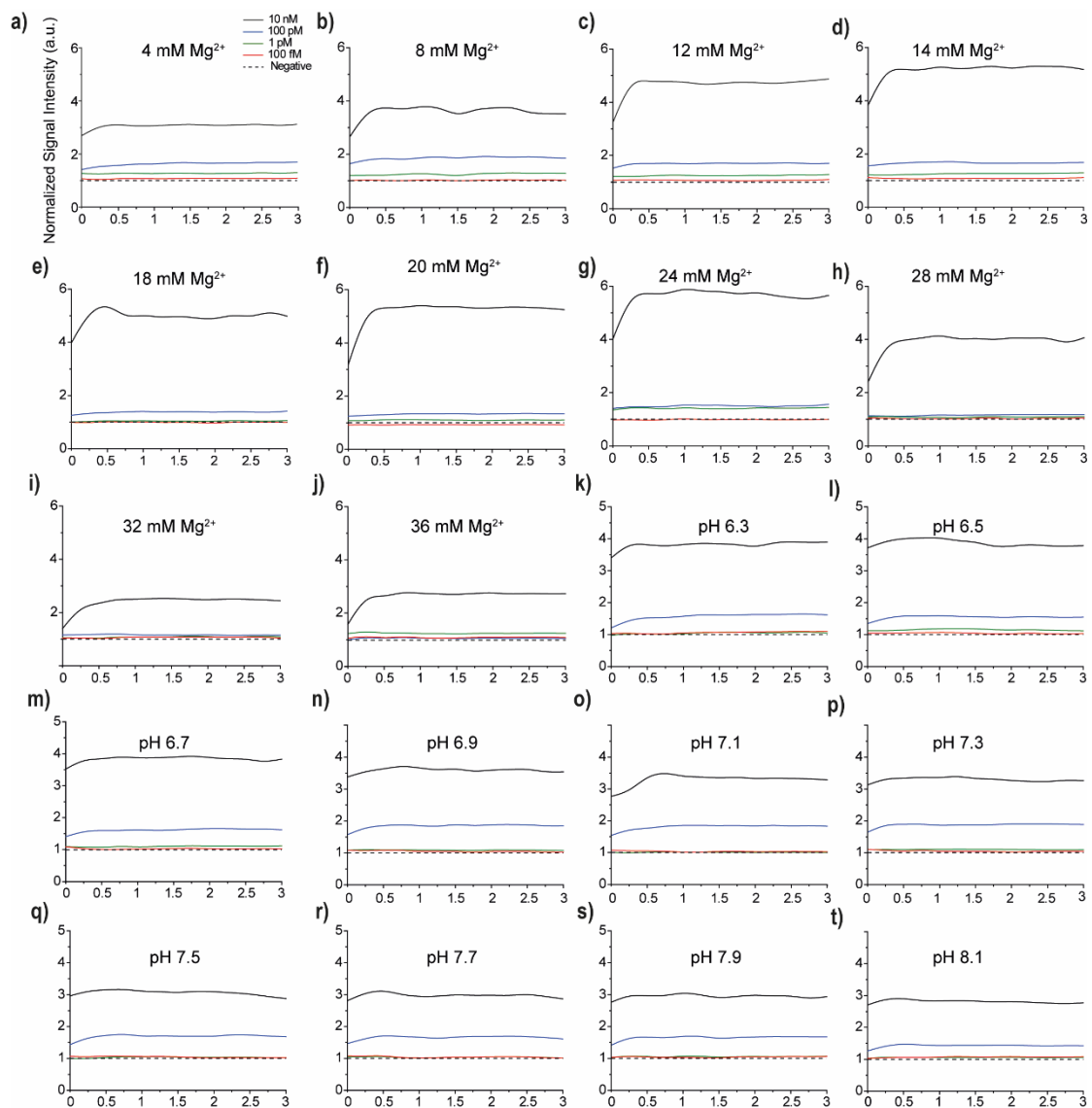
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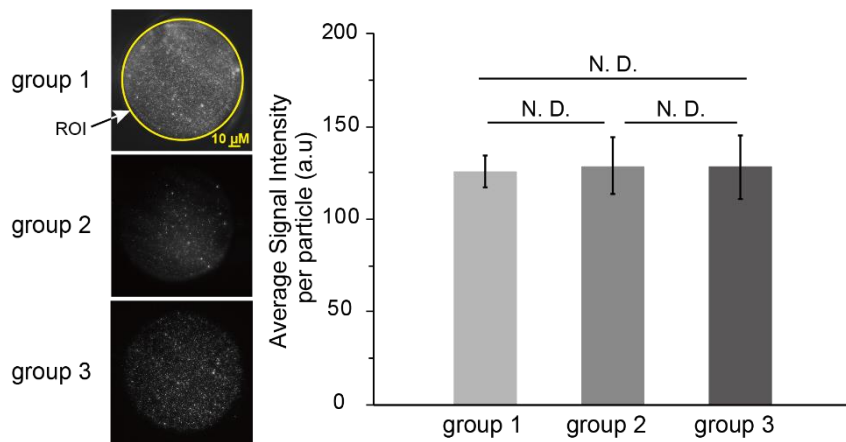
Supplementary Figure 1. Target RNA from 1 pM to 10 nM is detected using 5U reporter.



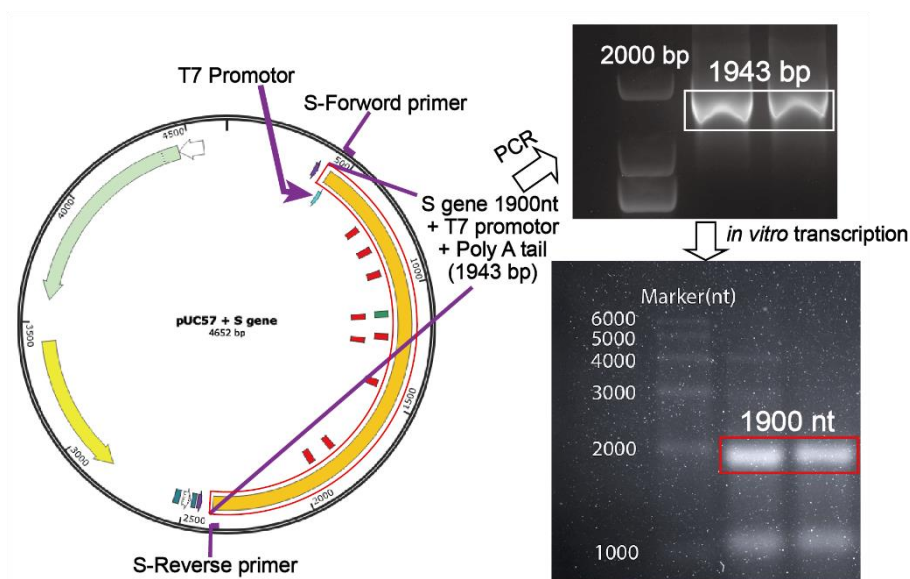
Supplementary Figure 2. **a)** Reporter 1 - BHQ1-5U-11G-5U-FAM. **b)** Reporter 2 - FAM-5U-11C-5U-FAM paired with BHQ1-5U-11G-FAM. **c)** Reporter 3 - FAM-5U-11C-5U-FAM paired with BHQ1-5U-11G-5U-FAM. **d)** Reporter 4 - nine 11C-3T repeats paired with BHQ1-5U-11G-U-FAM. **e - h)** The signal intensity of the CRISPR/Cas13a assays with 4 pM and 40 pM target RNA using the reporter 1 to 4 respectively.



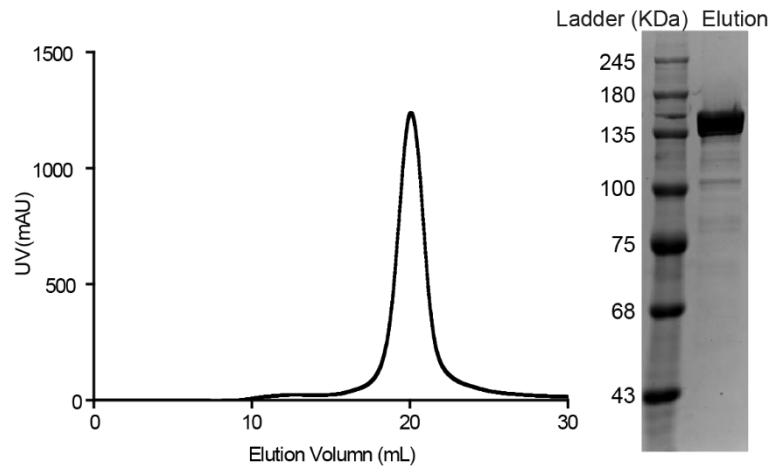
Supplementary Figure 3. Optimization of CRISPR/Cas13a assay with Mg²⁺ and pH. a-j) Mg²⁺ concentration from 4 mM to 36 mM. k-t) Different pH for off-coverslip assay from pH 6.3 to pH 8.1 by step 0.2.



Supplementary Figure 4. The average intensity per fluorescent particle from random TIRF images group 1 to group 3.



Supplementary Figure 5. The plasmid, PCR, and *in vitro* transcription of SARS-CoV-2 S gene. a) The pUC57 plasmid contains 1900 nt S gene template, T7 promotor, and polyA tail. b) The amplified target DNA fragment. The agarose gel shows the size of the amplified target DNA is about 1900 bp consistent with the theoretical size. c) RNA fragment of S gene. The transcription of 1900 nt S gene RNA is confirmed by denaturing formaldehyde agarose gel.



Supplementary Figure 6. FPLC chromatograms: a representative chromatogram of cation exchange (Mono S, GE Healthcare Life Sciences) *LwaCas13a*. The UV absorbance in milli-arbitrary units (mAU) is plotted against the elution volume in milliliters. Coomassie-stained SDS-PAGE gel is used to confirm the size of *LwaCas13a* after elution from cation exchange chromatography.

Supplementary Table 1: crRNA used in this study.

5U reporter	5' 6FAM-UUUUU-BHQ1 3'
8U reporter	5' 6FAM-UUUUUUUU-BHQ1 3'
14U reporter	5' 6FAM-UUUUUUUUUUUUUU-BHQ1 3'
18U reporter	5' 6FAM-UUUUUUUUUUUUUUUUUU-BHQ1 3'
22U reporter	5' 6FAM-UUUUUUUUUUUUUUUUUUUUUU-BHQ1 3'

Supplementary Table 2: Target sequence.

Target-1 (1900 nt)	<p>5'-AAUGUUUGUUUUUCUUGUUUUAUUGCCACUAGUCUCUAGUCAGUG UGUUAAUCUUACAACCAGAACUCAAUUACCCCCUGCAUACACUAAU CUUUCACACGUGGUGUUUAUACCCUGACAAAGUUUUCAGAUCCUCA GUUUUACAUUAACUCAGGACUUGUUCUUAACCUUUCUUUCCAAUGU UACUUGGUUCCAUGCUAUACAUGUCUCUGGGACCAUGGUACUAAGA GGUUUGAUAAACCUGUCCUACCAUUUAUGAUGGUGUUUAUUUUGCU UCCACUGAGAAGUCUAACAUAUAAGAGGCUGGAUUUUUGGUACUAC UUUAGAUUCGAAGACCCAGUCCCUACUUAUUGUAAUAACGCUACUA AUGUUGUUAUUAAAGUCUGUGAAUUUCAAUUUUGUAAUGAUCCAUUU UUGGGUGUUUAUUACCACAAAACAACAAAAGUUGGAUGGAAAGUGA GUUCAGAGUUUAUUCUAGUGCGAAUAAUUGCACUUUUGAAUAUGUCU CUCAGCCUUUCUUAUGGACCUUGAAGGAAAACAGGGUAAUUUCAA AAUCUUAGGGAAUUUGUGUUUAAGAAUAUUGAUGGUUAUUUUAAAA UAUUUCUAAGCACACGCCUAUUAAUUUAGUGCGUGAUCUCCUCAG GGUUUUUCGGCUUUAGAACCAUUGGUAGAUUUGCCAAUAGGUUUAA CAUCACUAGGUUCAAACUUUACUUGC UUACAUGAAGUUUUUGA CUCCUGGUGAUUCUUCUUCAGGUUGGACAGCUGGUGCUGCAGCUU UAUGUGGGUUAUCUUCAACCUAGGACUUUCUAUUAAAAUUAUAUGA AAAUGGAACCAUACAGAUGCUGUAGACUGUGCACUUGACCCUCUCU CAGAAACAAAGUGUACGUUGAAAUCUUCACUGUAGAAAAGGAAUC UAUCAAACUUCUAACUUUAGAGUCCAACCAACAGAAUCUAUUGU AUUUCUAAUUAUACAACUUGUGCCCUUUUGGUGAAGUUUUUAAACG CCACCAGAUUUGCAUCUGUUUAUGCUUGGAACAGGAAGAGAAUCAGC AACUGUGUUGCUGAUUAUUCUGUCCUAUAUAAUCCGCAUCAUUUUC CACUUUUAAGUGUUAUGGAGUGUCUCCUACUAAAUUAAAUGAUCUCU GCUUUACUAAUGUCUAUGCAGAUUCAUUUGUAAUUAGAGGUGAUGAA GUCAGACAAAUCGCUCAGGGCAAACUGGAAAGAUUGCUGAUUAUAA UUAUAAAUAACAGAUUAUUUACAGGCUGCGUUAUAGCUUGGAAU CUAACAAUCUUGAUUCUAAGGUUGGUGGUAUUUAUUAAUACCUGU AGAUUGUUUAGGAAGUCUAAUCUCAAACCUUUUGAGAGAGAUUUUC AACUGAAAUCUAUCAGGCCGGUAGCACACCUUGUAAUGGUGUUGAAG GUUUUAAUUGUACUUCUCCUUUACAUCUAUUGGUUCCAACCCACU</p>
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	AAUGGUGUUGGUUACCAACCAUACAGAGUAGUAGUACUUUCUUUUGA ACUUCUACAUGCACCAGCAACUGUUUGUGGACCUAAAAAGUCUACUA AUUUGGUUAAAAACAAAUGUGUCAAUUUCACUUCAAUGGUUUAACA GGCACAGGUGUUCUACUGAGUCUACAAAAAGUUUCUGCCUUCCA ACAAUUUGGCAGAGACAUUGCUGACACUACUGAUGCUGUCCGUGAUC CACAGACACUUGAGAUUCUUGACAUUACCAUGUUCUUUUGGUGGU GUCAGUGUUUAACACCAGGAACAAAUACUUCUAACCAGGUUGCUGU UCUUUAUCAGGAUGUUAACUGCACAGAAGUCCCUGUUGCUAUUCAUG CAGAUCAACUUA-3'
Target-2 (99 nt)	5'-AACUUUACUUGC UUACA UAGAAGUUAUUUGACUCCUGGUGAUUC UUCUUCAGGUUGGACAGCUGGUGCUGCAGCUUAUUUAUGUGGGUUAUC UUCAACCU-3'

Supplementary Table 3: crRNAs used in this study

crRNA-1	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac UAACAAUAGAUUCUGUUGGUUGGACUCUA-3'
crRNA-2	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac GUAGGGACUGGGUCUUCGAAUCUAAAGUA-3'
crRNA-3	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac GCCGAAAAACCCUGAGGGAGAUCACGCAC-3'
crRNA-4	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac AAACCCUGAGGGAGAUCACGCACUAAAUU-3'
crRNA-5	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac GAAGAAUCACCAGGAGUCAAAUAACUUCU-3'
crRNA-6	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac UAUCAAACCUUUAGUACCAUUGGUCC-3'
crRNA-7	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac AACUCACUUCCAUCUUAUUUGUUGUU-3'
crRNA-8	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac UUACCACCAACCUUAGAAUCAAGAUUGUU-3'
crRNA-9	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac AAACCUUCAACACCAUACAAGGUGUGCU-3'
crRNA-10	5'-gauuuagacuacccccaaaaacgaaggggacuaaaac UGCAGCACCAGCUGUCCAACCUGAAG-3'

*The lower cases represent the 36-nt repeat region for LwaCas13a and the capital cases represent the 28-nt guide region.