Supporting Information

Discovery of the Rnase activity of CRISPR-Cas12a and its distinguishing cleavage efficiency on various substrates

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Table S1

Oligonucleotides	Sequence (5'->3')
TS-D	**GCTTATCAGACTGATGGTTGA**
DNA-1	**CY3-TTATT-BHQ2**
DNA-2	**CY3- CGTATGTTGACTTCACGTTCATACG -CY5**
DNA-3	**CY3-GGGGTTGGTTGTGTTGGGTGTTGTGTGGGTAGGGCGGGTTGGGCCAACCCC-CY5**
TS-R	**GCUUAUCAGACUGAUGGUUGA**
crRNA	**UAAUUUCUACUAAGUGUAGAUUCAACAUCAUCUGAUAAGC**
RNA-1	**CY3-UUAUU-BHQ2**
RNA-2	**CY3-CAGUCGUUACGCUACUCGACUG-CY5**
R11	**CY3-UUAUUUUAUUU-BHQ2**
R14	**CY3-UUAUUUUAUUUUAU-BHQ2**
R17	**CY3-UUAUUUUAUUUUAUUUU-BHQ2**
R20	**CY3-UUAUUUUAUUUUAUUUUAUU-BHQ2**
R25	**UUAUUUUAUUUUAUUUUAUUUUAUU**
R30	**UUAUUUUAUUUUAUUUUAUUUUAUUUUAUU**
R35	**UUAUUUUAUUUUAUUUUAUUUUAUUUUAUUUUAUU**
R40	**UUAUUUUAUUUUAUUUUAUUUUAUUUUAUUUUAUUUUAUU**
miR-122	**UGGAGUGUGACAAUGGUGUUUG**
miR-1a	**UGGAAUGUAAAGAAGUAUGUAU**

Experiment

Materials and instruments

All the oligonucleotides and Acryl/Bis 30% Solution (29:1) were bought from Sangon (Shanghai, China). DNA and RNA strands were dissolved in DEPC (diethyl pyrocarbonate) treated water as stock solutions. LbCas12a were purchased from New England Biolab. And fluorometric assay was performed on F-2700 (HITACHI).

Methods

We first investigated the cleavage activities of LbaCas12a toward linear ssRNA substrates,10 μL reactions consisting of 50 nM LbCas12a/crRNA,50 nM activator (TS-D or TS-R), 1 μM RNA substrate (R11/R14/R17/R20) and 1ß NEBuffer 2.1 were performed in 37 ℃ for 0/30/60/120 min, then analyzed by polyacrylamide gel electrophoresis (PAGE) with a concentration of 20% for 30min at 120 V. Then RNA substrates labeled with fluorophore were used fluorometric assay in 37℃ for 2 hours.

For exploring the trans-cleavage activity to RNA-hairpin, RNA-1 served as substrate. 10 μL reactions consisting of 50 nM LbCas12a/crRNA, 50 nM activator (TS-D or TS-R), 1 μM RNA-1 substrate and 1ß NEBuffer 2.1 were incubating in 37℃ for 0/30/60/120 min, then analyzed by PAGE with a concentration of 20% for 30min at 120 V. Then 20 μL reactions with same composition and incubating time as electrophoretic assay were taken fluorometric assay after diluted to 100 μL.

In the same time, DNA-hairpin (DNA-2) also was analyzed by means of electrophoretic and fluorometric assays. 10 μL reactions consisting of 50 nM LbCas12a/crRNA, 50 nM activator (TS-D or TS-R), 1 μM DNA-2 substrate and 1ß NEBuffer 2.1 were incubated in 37 ℃ for 0/5/10/15/20 min, then analyzed by PAGE with a concentration of 20% for 30min at 120 V. Then Solution was incubated in fluorescence plate directly with a volume of 100 μL composed of 50 nM LbCas12a/crRNA, 50 nM activator (TS-D or TS-R), 1 μM DNA-2 substrate and 1ß NEBuffer 2.1 , and taken measurement every 5 min.

To calculate initial velocity to DNA-2 with ssDNA/ssRNA activator, a 100 μL reaction consisting of 1 nM LbCas12a/crRNA, 0.1 nM activator (TS-D or TS-R),1 μM/500 nM/200 nM/100 nM/50 nM/20 nM/10 nM DNA-2 and 1ß NEBuffer 2.1 was performed in fluorescence plate every 2s for 3600s.

Then we investigated the cleavage activities of LbaCas12a toward short ssDNA and ssRNA substrates. 20 μL reactions consisting of 50 nM LbCas12a/crRNA,50 nM activator (TS-D or TS-R), 1 μM short ssDNA and ssRNA substrate and 1× NEBuffer 2.1 were performed in 37 ℃ for 60 min, terminating reactions in 85 ℃ for 5min. Then reaction solution was taken fluorometric assay after diluted to 100 μL.

To comparing trans-cleavage activity to different DNA substrate, a 100 μL reaction consisting of 50 nM LbCas12a/crRNA,50 nM DNA activator (TS-D),1 μM DNA substrate (DNA-1/DNA-2/DNA-3) and 1× NEBuffer 2.1 was performed in fluorescence plate every 2s for 7200s.

The healthy serum samples were obtained from The Fourth Hospital of Changsha. Approved was obtained from the local agency review board and informed consent was obtained from subject for serum sampling. The serum sample was centrifuged at 10,000 × g for 10 min at 4 ℃. Then 50 μL of supernatant was mixed with 50 μL of DEPC treated water, and the solution was heated to 65 ℃ for 10 min to inactivate deoxyribonuclease. Finally, the serum of healthy donor was spiked with different concentrations of miR-21 (0 20 50 100 200 300 500 nM)and miR-21 mimic (0 2 5 10 20 50 100 nM), and 1 μL of serum samples was added into LbCas12a-crRNA-hairpin DNA reporter system. The reaction and fluorescent detection were performed with the above mentioned procedures.

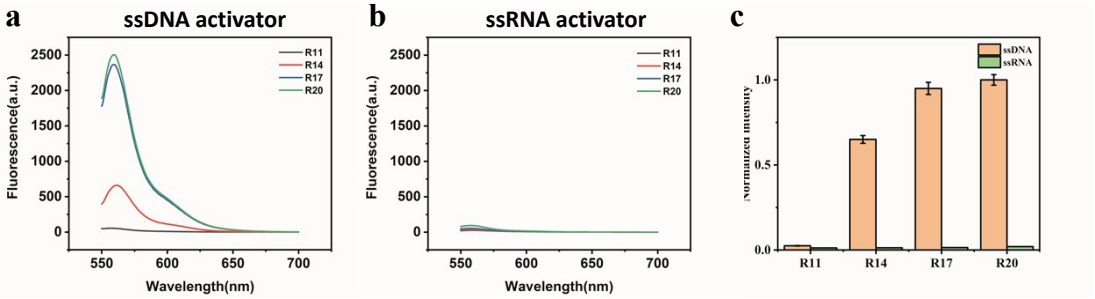

Fig. S1 Fluorescence comparison to different length of liner RNA substrates. a. Activator is ssDNA. b. Activator is ssRNA. c. Normalized intensity of fluorescence in a and b. Concentration of substrate is 1 μM and reaction time is 2 h. LbCas12a-crRNA 50 nM, activator 50 nM, RNA substrate 1 μM**.**

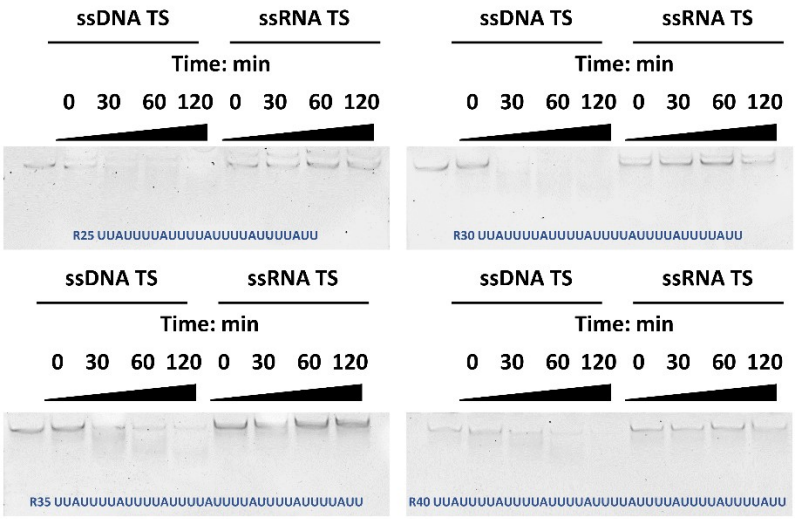

Fig. S2 Activity comparison of LbCas12a-crRNA-targeted ssDNA/ssRNA complex on linear ssRNA with 25-40 bases in length. LbCas12a-crRNA 50 nM, targeted ssDNA/ssRNA 50 nM, linear ssRNA substrate 1 μM.

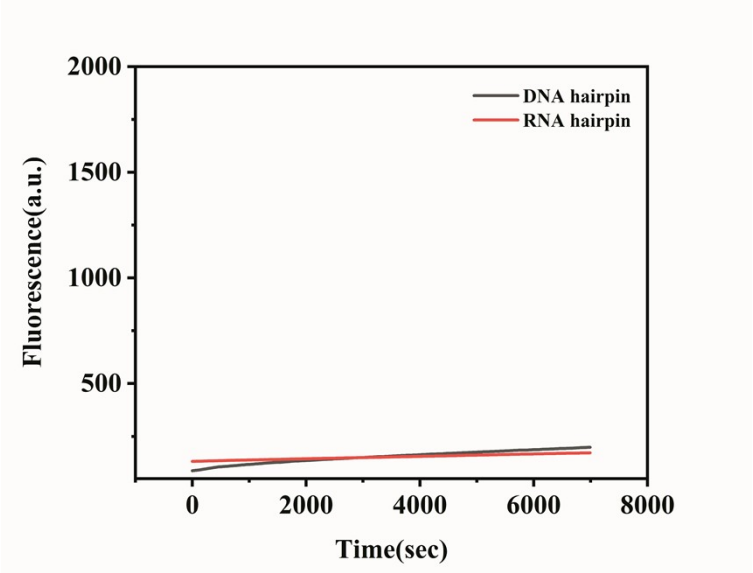

Fig. S3 The fluorescence changes of DNA or RNA hairpin when incubated with LbCas12a/crRNA only. LbCas12a/crRNA 50 nM, hairpin DNA/RNA 1 μM.

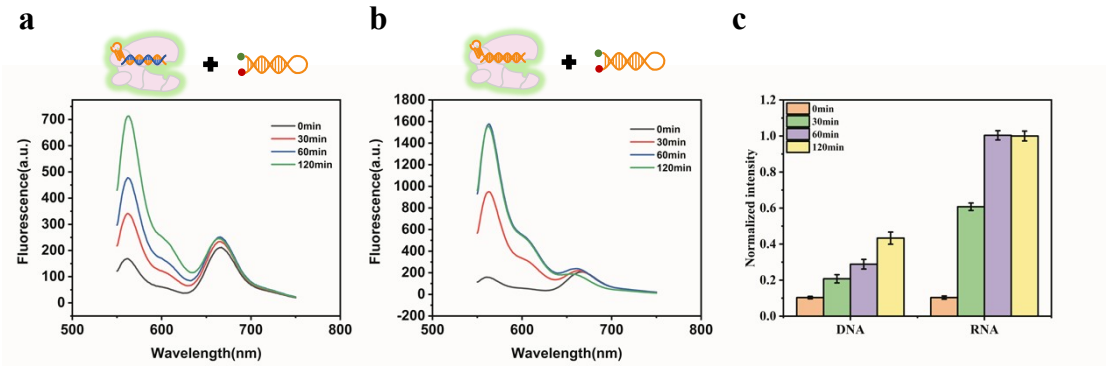

Fig. S4 The trans-cleavage comparison of LbCas12a activated by ssDNA/ssRNA to RNA hairpin with different time. a. Activator is ssDNA; b. Activator is ssRNA; c. Normalized intensity comparison of fluorescence in 560 nm between a and b. LbCas12a-crRNA 50 nM, activator 50 nM, hairpin RNA substrate 1 μM**.**

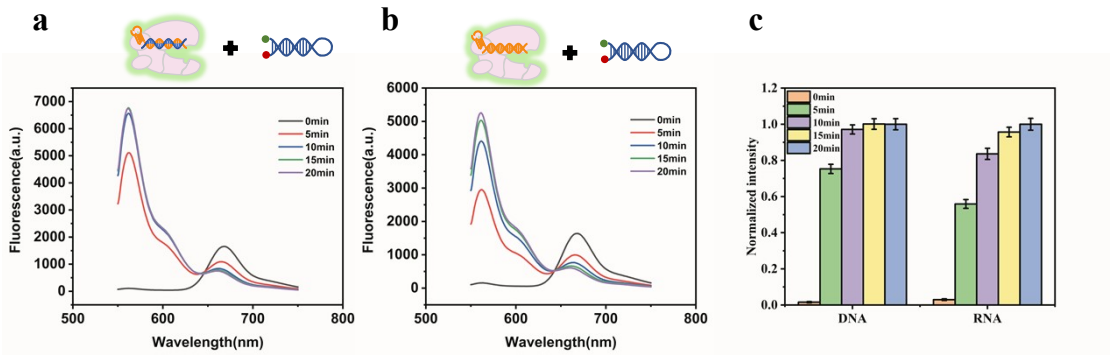

Fig. S5 The trans-cleavage comparison of LbCas12a activated by ssDNA/ssRNA to DNA-2 hairpin with different time. a. Activator is ssDNA; b. Activator is ssRNA; c. Normalized intensity comparison of fluorescence in 560 nm between a and b. LbCas12a-crRNA 50 nM, activator 50 nM, hairpin DNA substrate 1 μM**.**

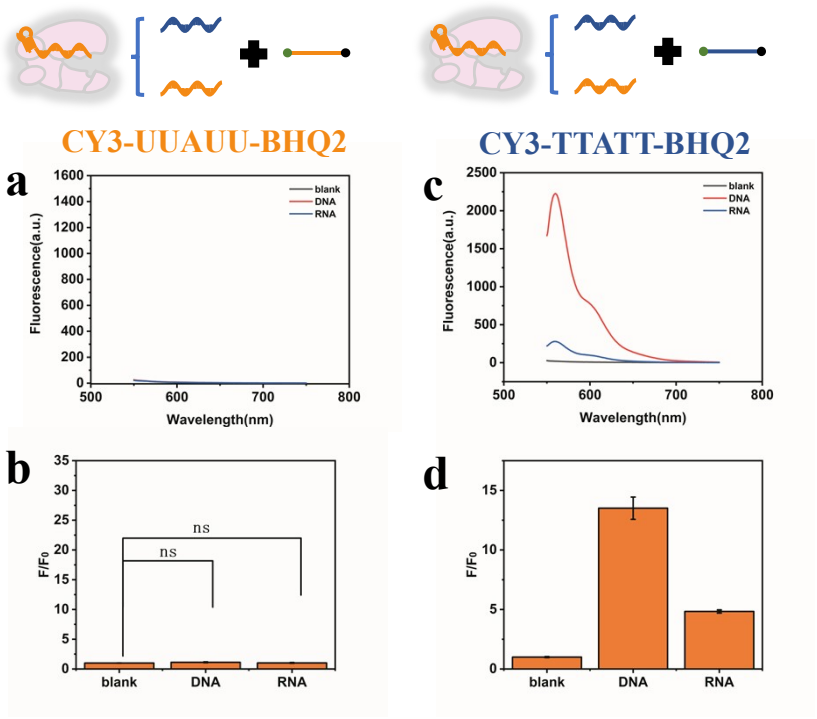

Fig. S6 Only short strand substrate is DNA, it can be trans-cleaved. a. Fluorescence comparison with ssDNA/ssRNA activator to short RNA substrate; b. F/F_0 comparison of fluorescence in 560 nm of a; c. Fluorescence comparison with ssDNA/ssRNA activator to short DNA substrate; d. F/F_0 comparison of fluorescence in 560 nm of c. Error bars indicated the standard deviations of three experiments. ns means no significant difference, Student's t-test. LbCas12a-crRNA 50 nM, activator 50 nM, ssDNA or ssRNA substrate 1 μM**.**

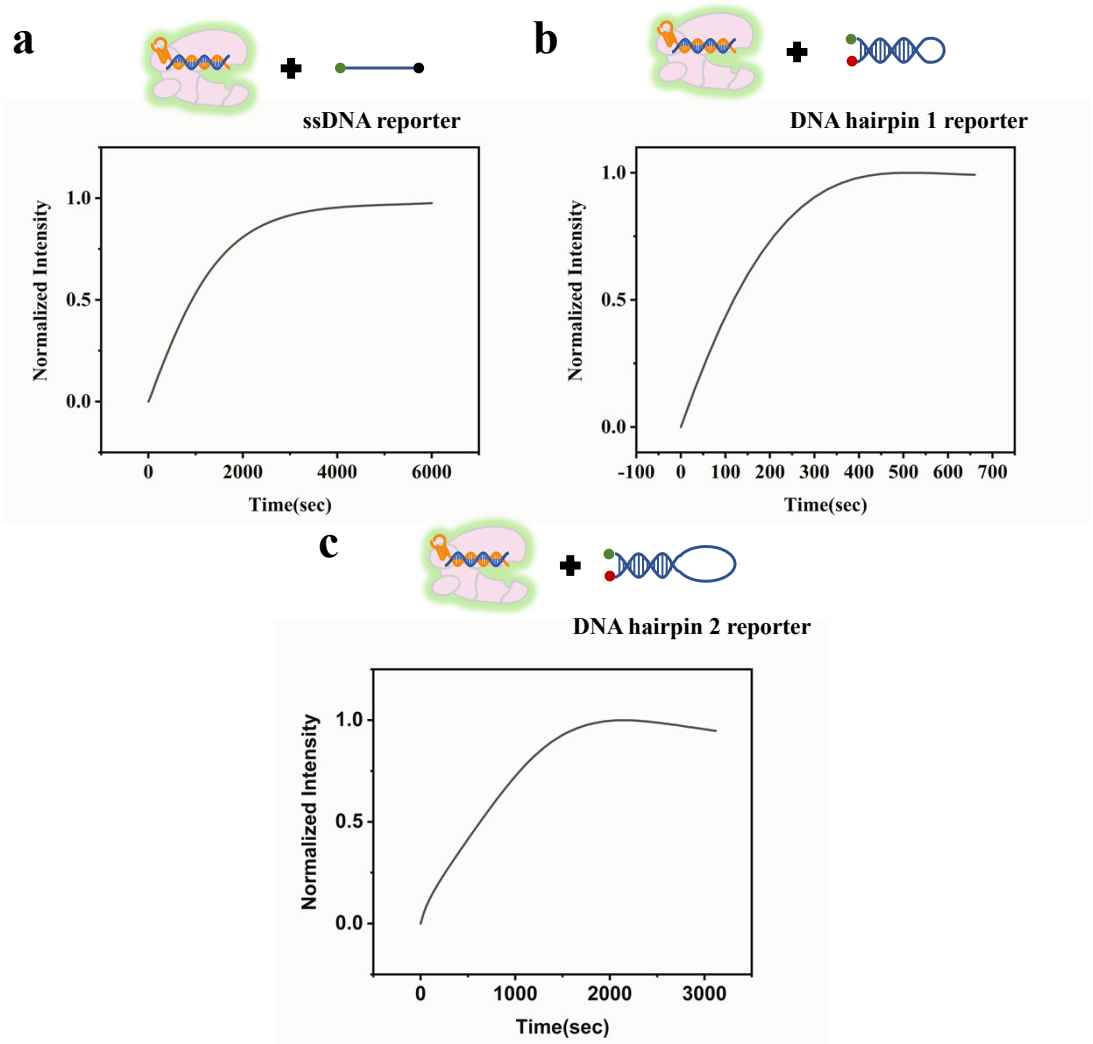

Fig. S7 Comparison of trans-cleavage activity to different DNA reporters. a. Reporter is short DNA(DNA-1); b. Reporter is DNA hairpin 1(DNA-2); c. Reporter is DNA hairpin 2 (DNA-3). LbCas12a-crRNA 50 nM, activator 50 nM, DNA substrate 1 μM**.**

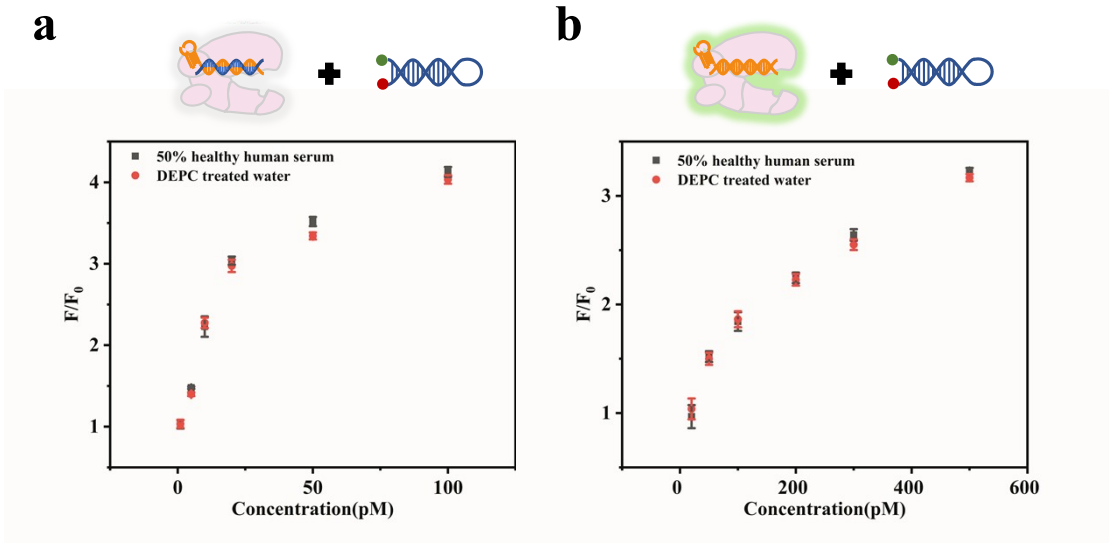

Fig. S8 a. F/F_0 to miR-21 mimic spiked in 50% healthy human serum and DEPC treated water at different concentrations with LbCas12a-crRNA-hairpin DNA reporter. b. F/F_0 to miR-21 spiked in 50% healthy human serum and DEPC treated water at different concentrations with LbCas12a-crRNA-hairpin DNA reporter. LbCas12a/crRNA 50 nM, hairpin DNA reporter 1 μM, reaction time 1 h. The data are presented as mean ± s.d. of three replicate measurements.

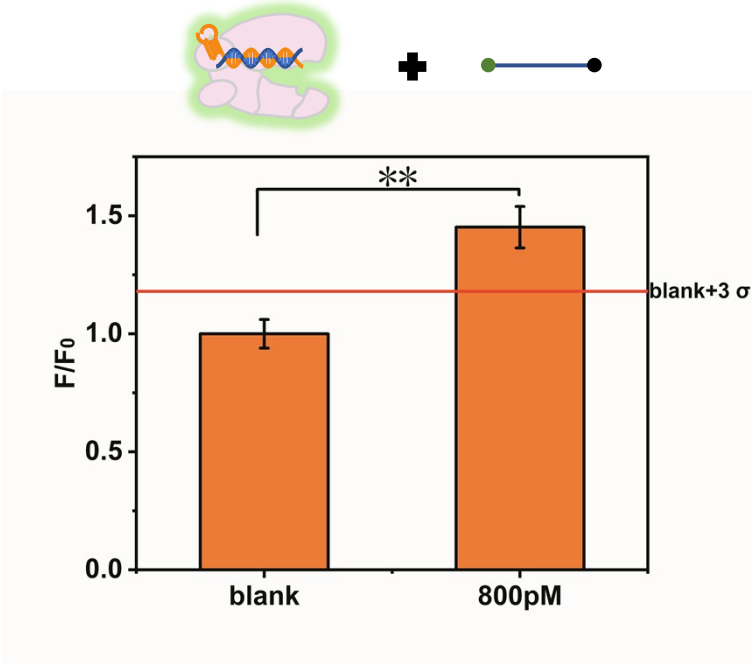

Fig. S9 Limit of detection to ssDNA with short DNA as reporter. According to 3σ standard**,** the limit of detection to ssDNA is 800 pM. Error bars indicated the standard deviations of three experiments. ** means p<0.01, Student's t-test. LbCas12a-crRNA 50 nM, activator 50 nM, short ssDNA substrate 1 μM**.**

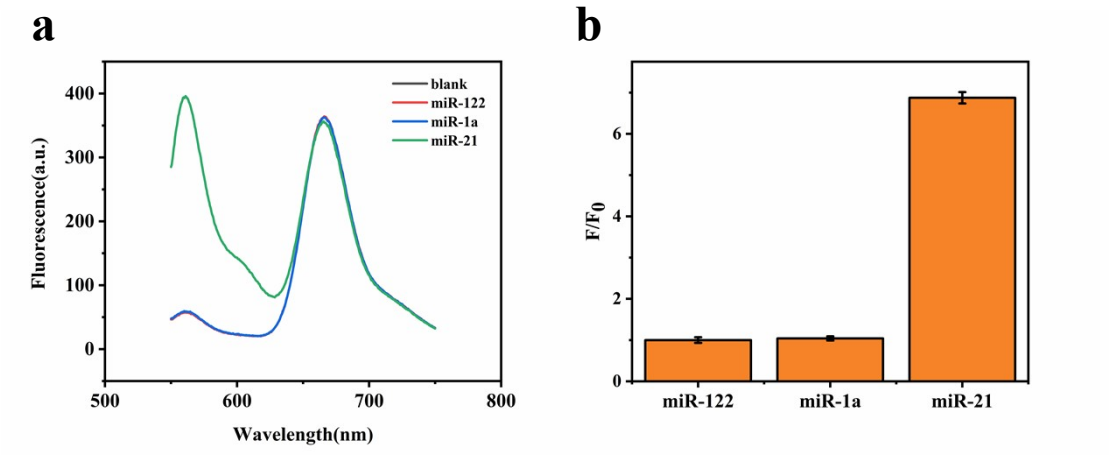

Fig. S10 a. Fluorescence responses of LbCas12a-crRNA-hairpin DNA reporter toward different microRNA. b. F/F_0 of fluorescence in 560nm of a. microRNA 1 nM, reaction time 1 h. The data are presented as mean ± s.d. of three replicate measurements.