Supporting Information

Proximity-Activated Guide RNA of CRISPR-Cas12 for Programmable Diagnostic Detection and Gene Regulation

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Experimental Materials

Purified AsCas12a nuclease (AsCpf1) was purchased from GenScript (Nanjing, China). The lysogeny broth ingredients (tryptone, yeast extract), 30% polyacrylamide gel electrophoresis (PAGE), *N,N,N',N'*-Tetramethylethylenediamine (TEMED), agarose dry powder, Goldview stain solution, GelRed stain solution, 10X TBE buffer, 50X TAE buffer, DNA marker and loading buffer were gotten from Beijing BioDee Biotech. Co., Ltd. All inorganic and organic reagents, such as $MgCl_2$, rare earth metal chlorides, N, N-dimethylforamide (DMF) and theophylline were purchased from Aladdin Co,.Ltd. (Shanghai, China). Dynabeads™ M-280 Streptavidin and separation magnets (DynaMag-2) were commercially available from Invitrogen Co. (Shanghai, China). The Proteinase K (10 mg/mL) was gotten from Biorigin Biotech. Co. Ltd. (Beijing, China). DNase/RNase-Free Deionized Water was purchased from and Thermo Fisher (USA). The 2× Hieff Canace® AdvanceFast PCR mix (With Dye), Hifair® Ⅲ One Step RT-qPCR SYBR Green Kit, MolPure® Bacterial RNA Kit, MolPure® Cell RNA Kit and Hieff Trans™ Liposomal Transfection Reagent were purchased from Yeasen Biotechnology Co., Ltd. (Shanghai, China). The commercial plasmids were obtained from Addgene (USA) or synthesized by GenScript (Nanjing, China). The *MG1655* and *DH5α* E coli bacteria were purchased from Zomanbio Co., Ltd. (Beijing, China). The solution of KanRmycin (***KanR***, 100mg/ml), ampicillin (***Amp***, 50mg/ml) and chloramphenicol (***Cmt***, 25mg/ml, alcohol) were made from Biorigin Biotech. Co. Ltd. (Beijing, China). The T4 DNA Ligase (5 U/μL) and 10X buffer solution were obtained from Thermo Fisher Scientific (USA). pEASY®-Basic Seamless Cloning and Assembly Kit was purchased from TransGen Biotech Co., Ltd. The ATP (100 mM), UTP (100 mM), CTP (100 mM), GTP (100 mM), dNTPs mixture (100 mM) and restriction endonucleases for plasmids clone and constructions were obtained from New England Biolabs inc. (Beijing). Plasmid extraction kit (endotoxin-free or with endotoxin) were purchased from TianGen Biotech Co., LTD. (Beijing, China). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), Dulbecco's Phosphate Buffered Saline (DPBS), 100 IU/mL penicillin-streptomycin and 0.25% Trypsin were purchased from Sigma Aldrich (Shanghai, China) and Corning Cellgro (NY, USA). The Opti-MEM medium and SYBR Gold Nucleic Acid Gel Stain were obtained from Thermo Fisher Scientific (USA). HEK293T cell line were obtained from Cell Resource Center, Peking Union Medical College (which is the headquarter of National Infrastructure of Cell Line Resource, NSTI). GenCRISPR™ LbuCas13a Nuclease and 10X buffer were purchased from GenScript (Nanjing, China).

Fig. S1.

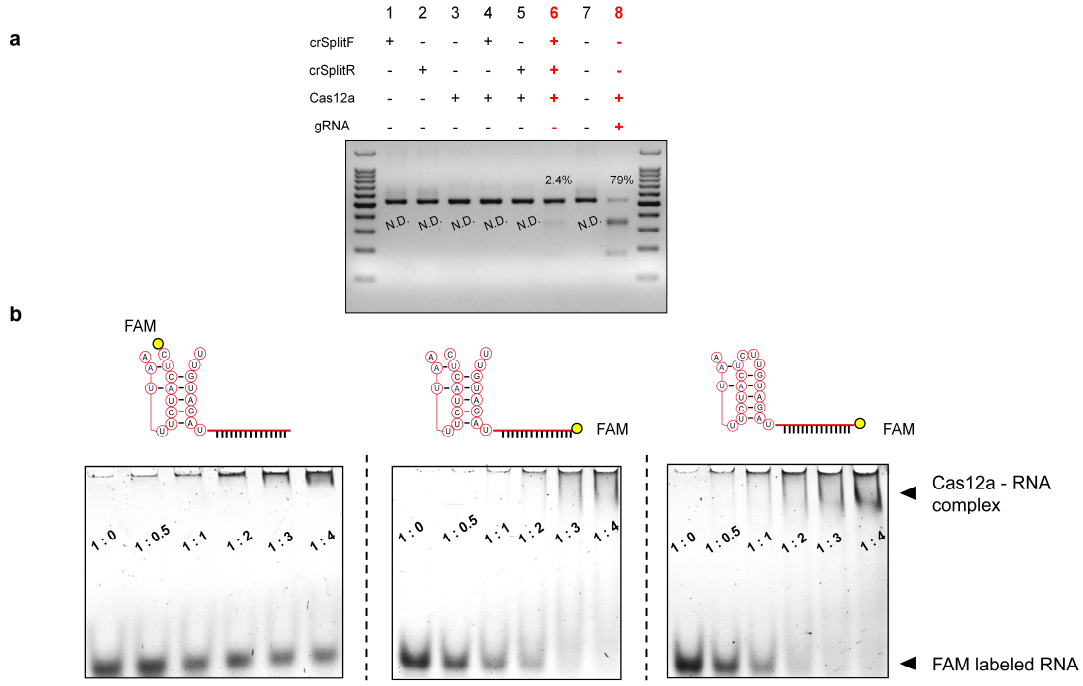


Figure S1 The activity of spliting the gRNAs of AsCas12a

(a) The *cis*-cleavage activity of CRISPR-Cas12a after splitting gRNA at the "UCUU" region. The results of gel electrophoresis were analyzed using ***ImageJ*** software, with the black markings indicating the dsDNA cleavage efficiency of CRISPR-Cas12a. N.D. represents that no products were detected. From top to bottom of DNA ladder: 1500 bp, 1000 bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp.

(b) Electrophoretic mobility shift assay (EMSA) image (native 5% PAGE, ice incubated). The varying concentrations of Cas12a were incubated with FAM labeled crSplitF/crSplitR complex and gRNA (100 nM), respectively. The ratios listed represent the concentrations of RNA: the concentrations to Cas12a protein.

According to the figure, the binding ability of Cas12a to crSplitR is similar to that of normal gRNA. However, the binding ability to crSplitF is significantly reduced.

Fig. S2.



Figure S2 The sequence of the dsDNA activator. The yellow region is the spacer sequence of the gRNA (PAM: TTTC).

Fig. S3.

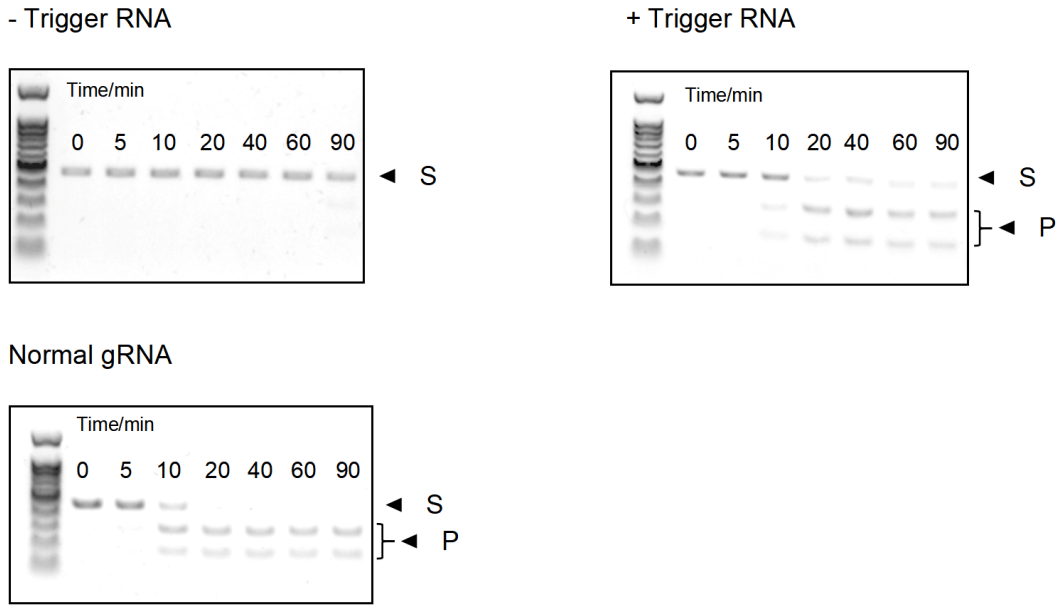


Figure S3 Characterization of the kinetics of RNA-inducible PARC-Cas12a cleavage. Trigger RNA: 50 nM, dsDNA: 100 ng, Cas12a: 20 μg, normal gRNA: 50 nM, crRNA-F and crRNA-R complexes: 50 nM. From top to bottom of DNA ladder: 1500 bp, 1000 bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp. "S" represents the substrate dsDNA, and "P" represents the cleavage products.

Fig. S4.

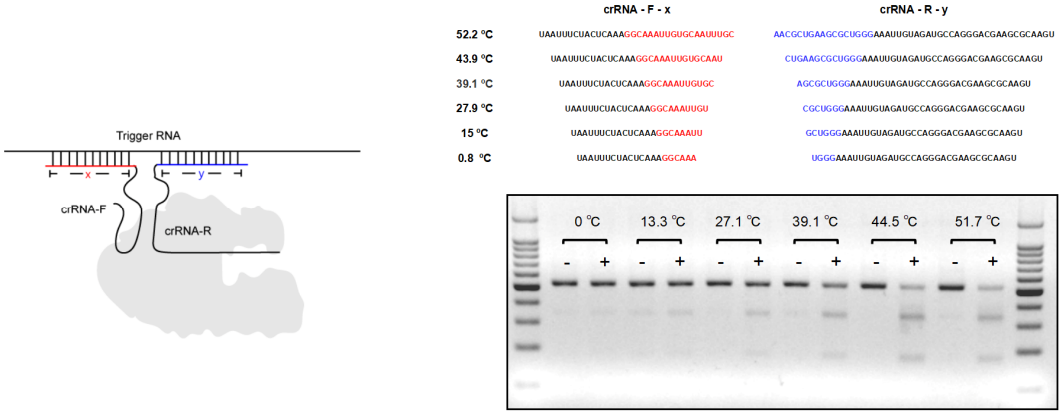


Figure S4 The impact of the RNA-recognition arm lengths of crRNA-F/crRNA-R on CRISPR activity. The dsDNA activator and cleavage products were characterized by agarose gel electrophoresis (1%, 120 V, 35 min). "x" represents the sequence marked in **red** in the table. "y" represents the sequence marked in **blue** in the table. The melting temperatures (Tm) of "x" and "y" were estimated using the IDT tool (*sg.idtdna.com*) with Na^+: 10 mM, Mg^{2+}: 10 mM, RNA concentration: 50 nM. From top to bottom of the DNA ladder: 1500 bp, 1000 bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp.

According to the figure, the Tm values of the two recognition arms need to exceed 37°C to effectively activate the CRISPR-Cas12a system.

Fig. S5.

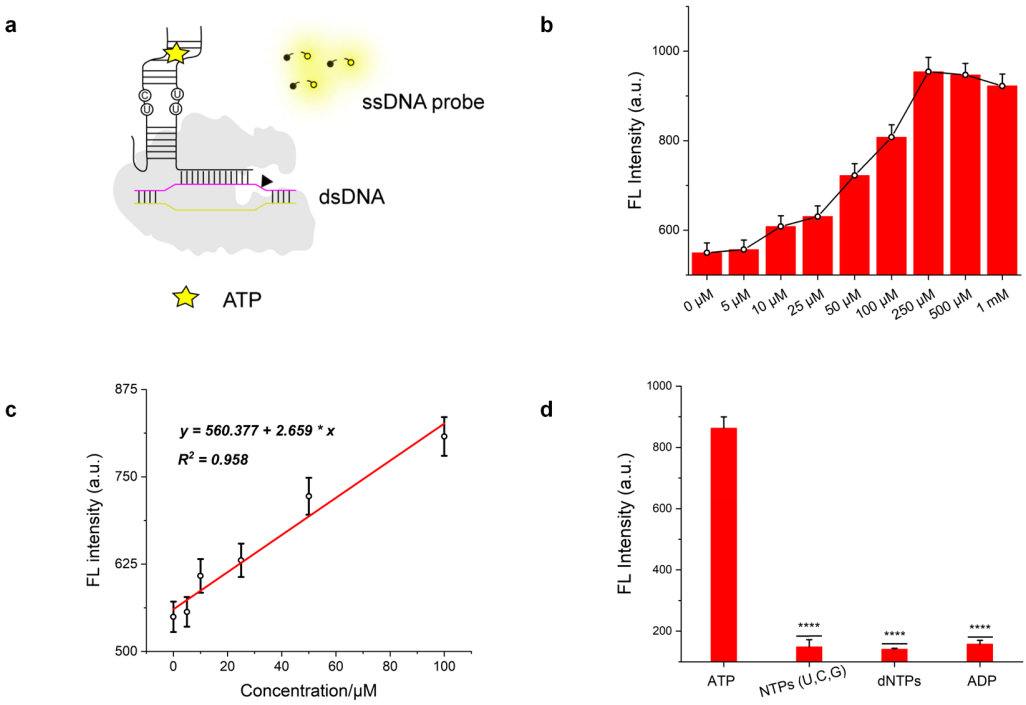


Figure S5 ATP-regulated *trans*-cleavage activity of PARC-Cas12a.

(a) The ssDNA probe (500 nM, 5′ FAM, 3′ BHQ-1) was cleaved by the PARC-Cas12a system in the presence of ATP, and the fluorescence was recorded by a microplate reader (excitation: 488 nm, emission: 525 nm).

(b)- (c) ATP concentration-dependent (1000, 500, 200, 100, 75, 50 μM) *trans*-cleavage activity of PARC-Cas12a.

(d) The selectivity of ATP detection. Both ATP and interferences were 1 mM.

Error bars represent the standard deviation of three measurements. ****$P < 0.001$, calculated by *Student's t-test.*

Fig. S6.

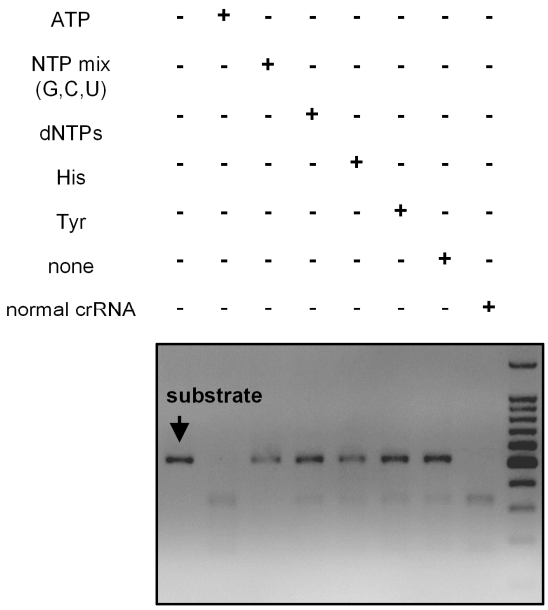


Figure S6 The selectivity of ATP-inducible PARC-Cas12a. Each interference at a concentration of 1 mM was incubated with Cas12a (20 μg), crATP-F/crATP-R (50 nM), and dsDNA (100 ng) at 37°C for 2 hours. The results were characterized by 1% agarose gel. From top to bottom of the DNA ladder: 1500 bp, 1000 bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp. ATP: adenosine triphosphate; NTP mix: auanosine triphosphate (G), cytidine triphosphate (C), and uridine triphosphate (U); His: histidine; Tyr: tyrosine; none: ddH_2O.

Fig. S7.

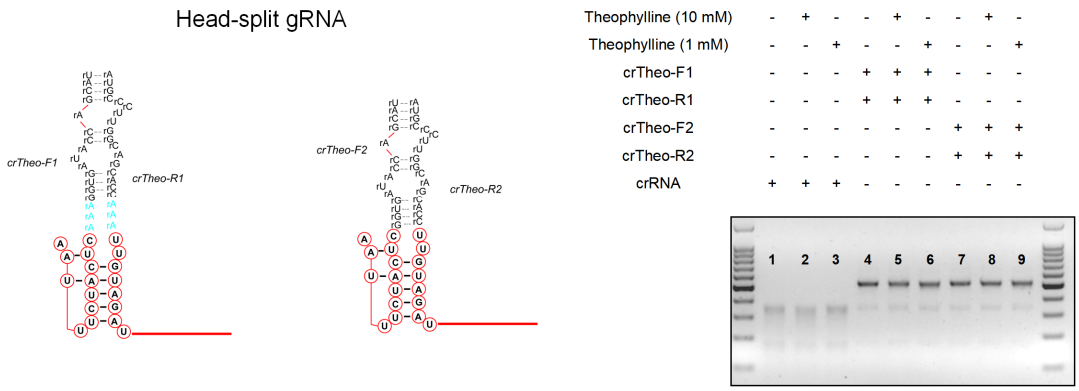


Figure S7 Feasibility of split gRNA for the theophylline response. The theophylline RNA aptamer sequence was split into two fragments as described in the literature(1). We designed two forms of split gRNA. In one form (left), three adenine bases (rA) were added at the junction of the theophylline aptamer and the gRNA sequence to prevent the aptamer sequence from interfering with the binding of the gRNA to Cas12a. In the other form (right), the theophylline aptamer was directly integrated with the gRNA. The cleavage reaction was characterized by 1% agarose gel. Both engineered forms of the gRNA failed to activate CRISPR-Cas12a system by theophylline. DNA markers from top to bottom: 1500 bp, 1000 bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp.

Fig. S8.

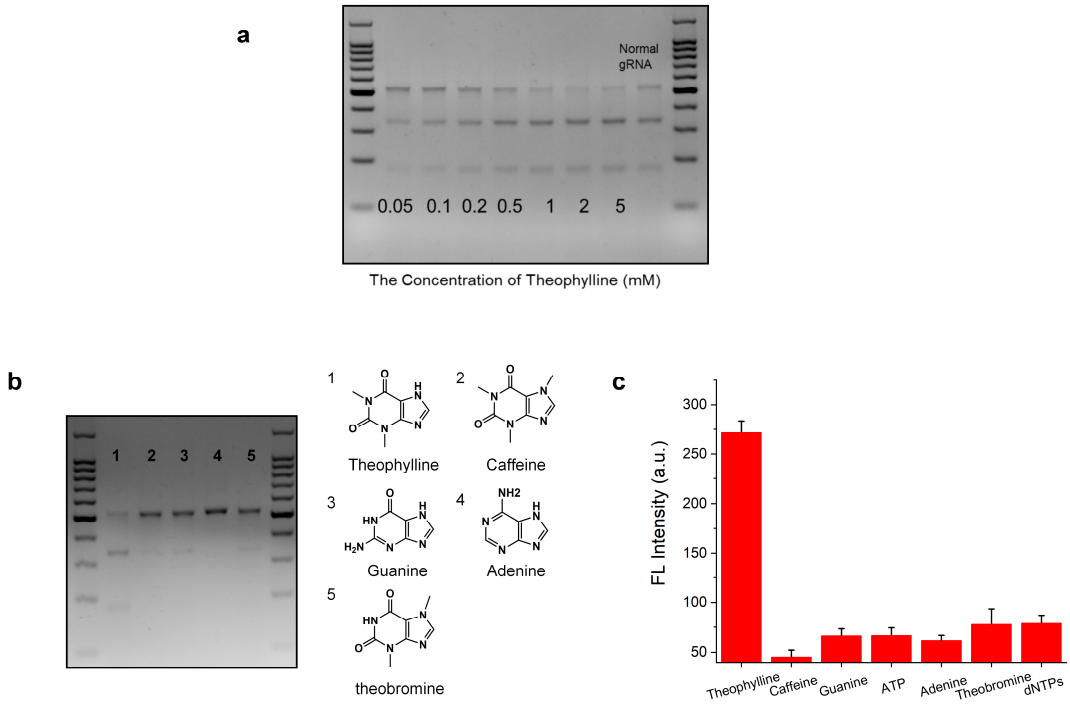


Figure S8 Theophylline-regulated cleavage activity of PARC-Cas12a.

(a) Agarose gel (1%) analysis revealed the theophylline concentration-dependent (5 mM, 2 mM, 1 mM, 0.5 mM, 0.2 mM, 0.1 mM, 0.05 mM) *cis*-cleavage activity of Cas12a.

(b) The selectivity of *cis*-cleavage by theophylline-inducible PARC-Cas12a. Line 1: target theophylline 10 mM; line 2: caffeine 10 mM; line 3: guanine 10 mM; line 4: adenine 10 mM; line 5: theobromine 10 mM. Different small molecules were incubated with crTheo (50 nM), AsCas12a (20 μg), and the dsDNA activator (100 ng), and the products were analyzed by agarose gel electrophoresis (1%).

(c) The selectivity of *trans*-cleavage activity by the theophylline-inducible PARC-Cas12a was evaluated. Interference concentrations were set at 1 mM, and fluorescence measurements were taken at 37℃ after 1 hour. Error bars represent the standard deviation of three measurements.

From top to bottom of the DNA ladder: 1500 bp, 1000 bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp.

Fig. S9.

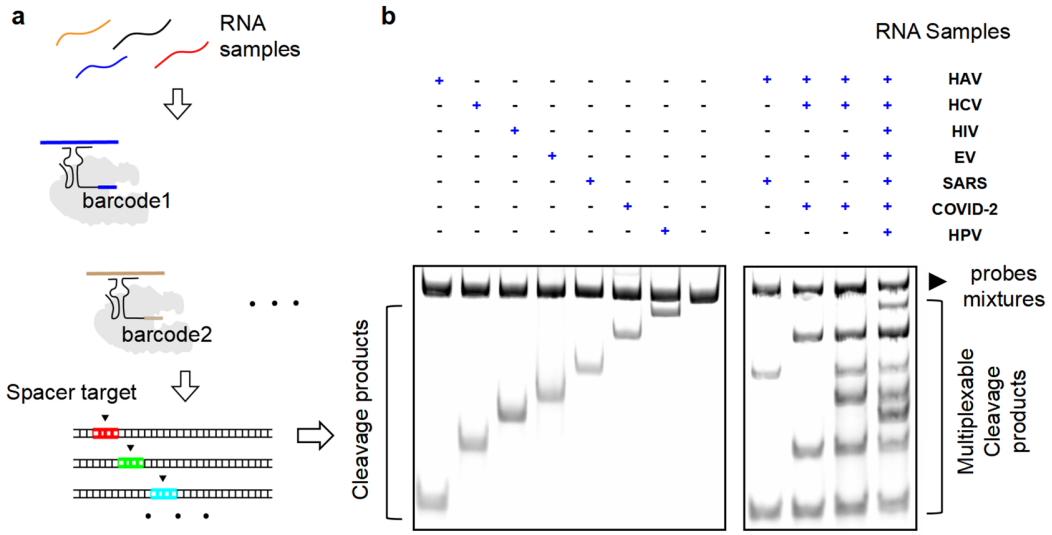


Figure S9 Multiplexable RNA detection. (a) Programmable multiplex RNA detection was achieved by connecting the RNA recognition arms of crRNA-F/R to the spacer sequence. (b) Identification of seven different viral mRNA fragments. Seven 5′ FAM-labeled dsDNA mixtures (sequences listed in Table S4) were incubated with PARC-Cas12a and RNA samples. Left graph: different RNA samples corresponded to dsDNA cleavage products of varying lengths; right graph: multiple RNA sequences were detected simultaneously.

Fig. S10.

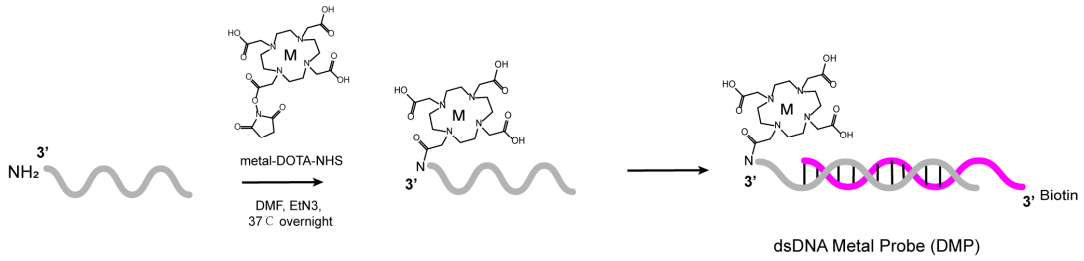


Figure S10 The scheme for constructing the dsDNA metal probe (DMP). This process referred to the literatures [2, 3]. In a solution of 100 μM NH_2-modified ssDNA, a 40-fold excess of the metal-chelating DOTA-NHS compound was added, and the reaction was allowed to proceed overnight at 37°C. After HPLC purification, the metal-modified ssDNA was annealed with the biotin-modified complementary strand to form the dsDNA metal probe.

Fig. S11.

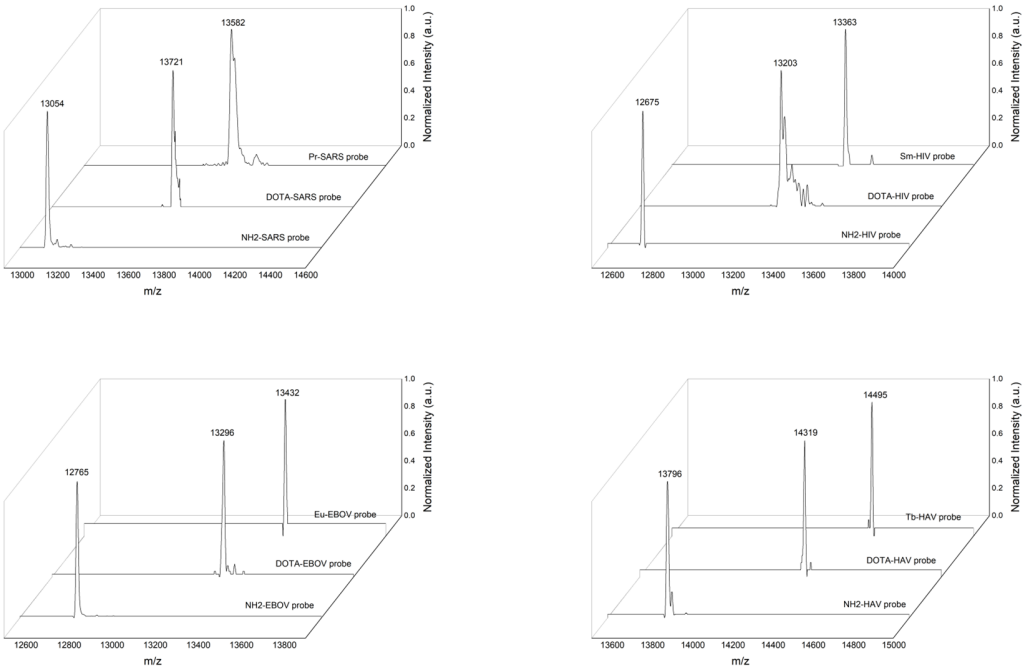


Figure S11 MALDI-TOF-MS analysis of metal-labeled DNA probes. The SARS probe was labeled with the Pr element. The HIV probe was labeled with the Sm element. The HIV probe was labeled with the Eu element. The HAV probe was labeled with the Tb element. The sequences of these metal-labeled DNA probes are listed in Table S5. The slight discrepancy between the measured and theoretical molecular weights was due to the mass measurement error in the MALDI-TOF-MS used. For the analysis, 3-Hydroxypicolinic acid (3-HPA) was used as the matrix. In the process, 1 μL of the DNA target was mixed with 1 μL of 3-HPA (10 μM). After drying at room temperature, the mass was determined using MALDI-TOF-MS.

Fig. S12.

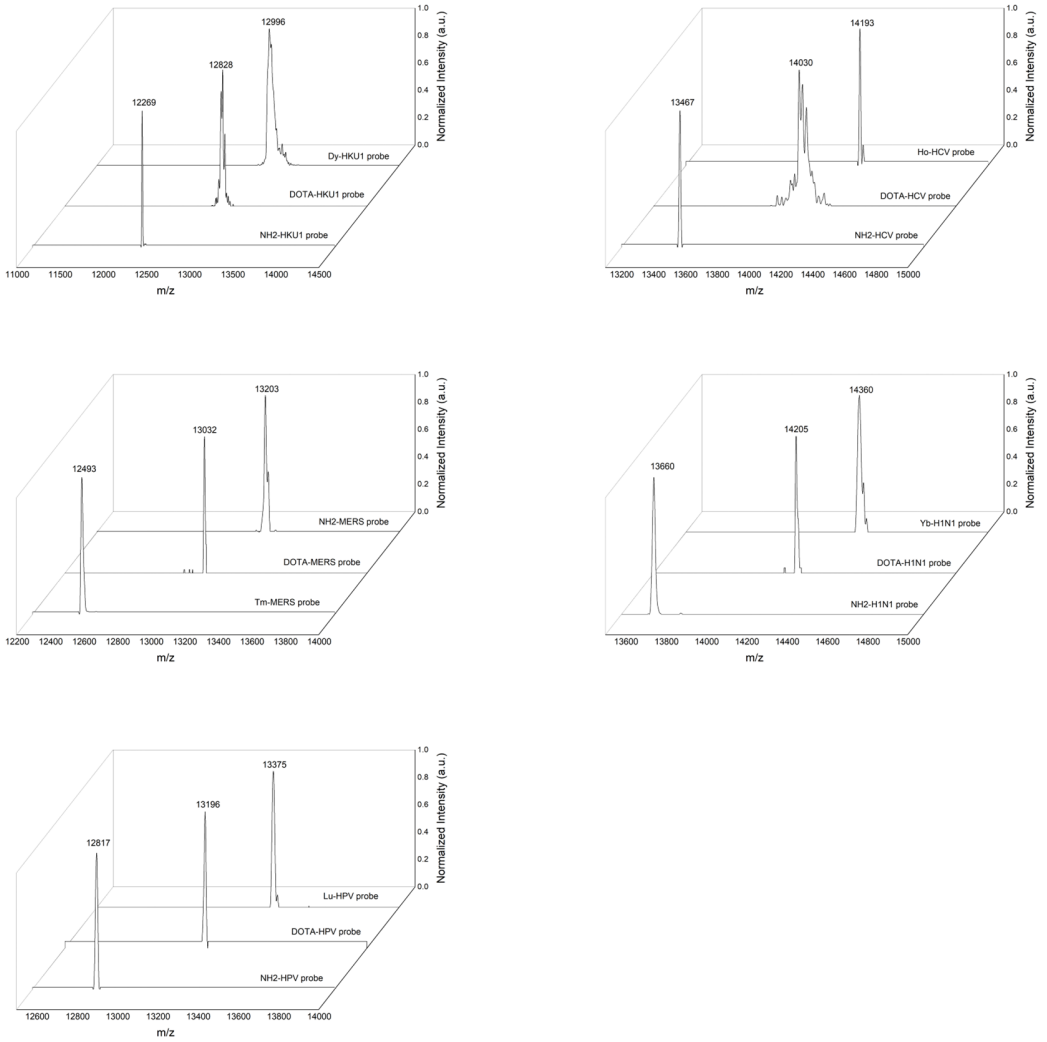


Figure S12 MALDI-TOF-MS analysis of DMPs. The HKU1 probe was labeled with the Dy element. The HCV probe was labeled with the Ho element. The MERS probe was labeled with the Tm element. The H1N1 probe was labeled with the Yb element. The slight discrepancy between the measured and theoretical molecular weights was due to the mass measurement error in the MALDI-TOF-MS used. The sequences of these metal-labeled DNA probes are listed in Table S5. For the analysis, 3-Hydroxypicolinic acid (3-HPA) was used as the matrix. 1 μL of the DNA target (20 μM) was mixed with 1 μL of 3-HPA (10 μM). After drying at room temperature, the mass was determined by MALDI-TOF-MS.

Fig. S13.

Element	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Isotope/ abundance	89 m/z 100%	138 m/z 0.09%	136 m/z 0.19%	**141 m/z 100%**	142 m/z 27.20%	144 m/z 3.07%	151 m/z 47.81%	152 m/z 0.20%	**159 m/z 100%**	156 m/z 0.06%	**165 m/z 100%**	162 m/z 0.14%	**169 m/z 100%**	168 m/z 0.13%	**175 m/z 97.41%**
		139 m/z 99.91%	138 m/z 0.25%		143 m/z 12.20%	**147 m/z 14.99%**	**153 m/z 52.19%**	154 m/z 2.18%		158 m/z 0.10%		164 m/z 1.61%		170 m/z 3.04%	176 m/z 2.59%
			140 m/z 88.45%		144 m/z 23.80%	148 m/z 11.24%		155 m/z 14.8%		160 m/z 2.34%		166 m/z 33.61%		171 m/z 14.28%	
			142 m/z 11.11%		145 m/z 8.30%	149 m/z 13.82%		156 m/z 20.47%		161 m/z 18.91%		167 m/z 22.93%		**172 m/z 21.83%**	
					146 m/z 17.20%	150 m/z 7.38%		157 m/z 15.65%		162 m/z 25.51%		168 m/z 26.78%		173 m/z 16.13%	
					148 m/z 5.70%	152 m/z 26.75%		158 m/z 24.84%		**163 m/z 24.90%**		170 m/z 14.93%		174 m/z 31.83%	
					150 m/z 5.60%	154 m/z 22.75%		160 m/z 21.86%		164 m/z 28.18%				176 m/z 12.76%	

Figure S13 The diagram illustrates the isotopes and their abundances for 15 different rare earth metal elements. The elements marked in blue were selected as detection targets for ICP-MS analysis. In this assay, the selected isotopes did not exhibit mass spectral overlap.

Fig. S14.

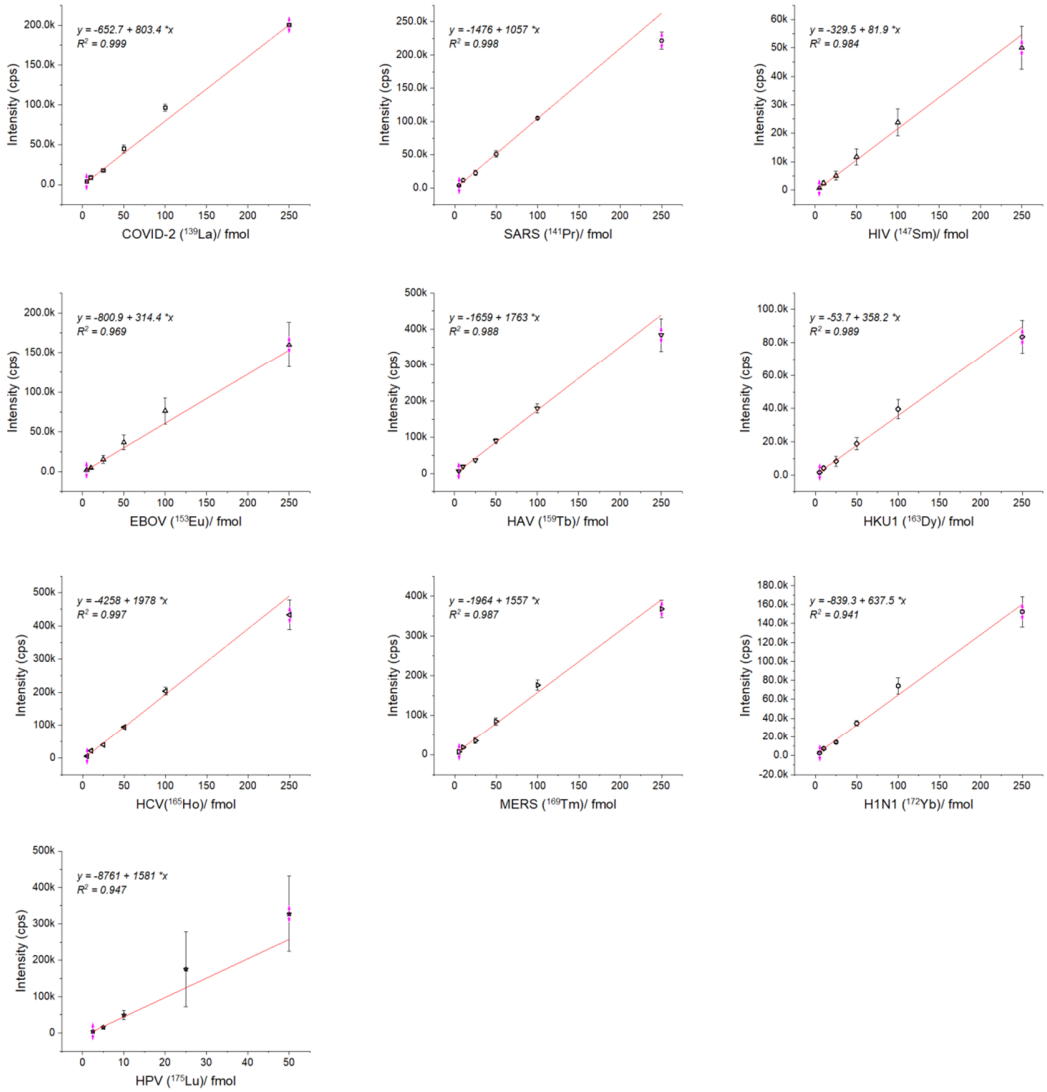


Figure S14 Calibration curves for multiplexed RNA detection. Simultaneous quantitative analysis of specific RNA fragments from ten different viruses (sequences listed in Table S2) was performed using the SMB-DMPs strategy. Error bars represent the standard deviation of three measurements.

Fig. S15.

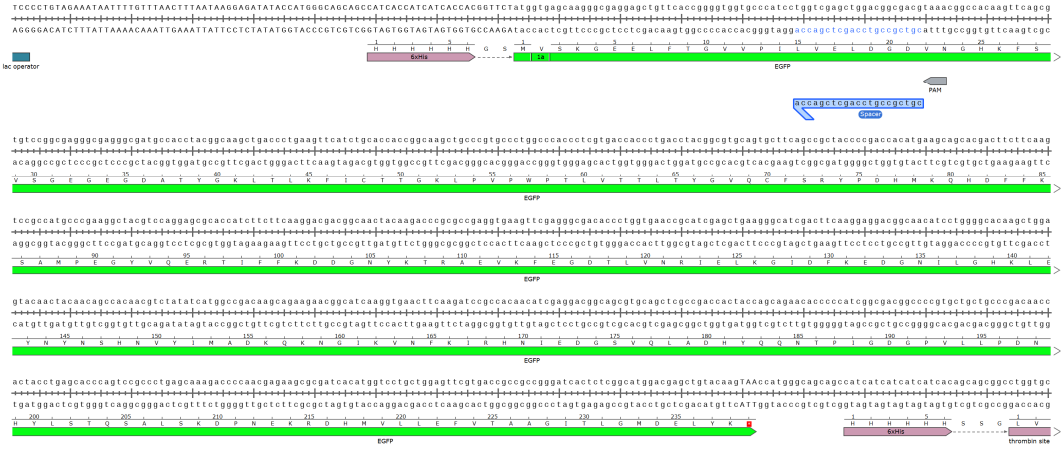


Figure S15 The GFP gene was employed as a substrate for the PARC-Cas12a cleavage experiment in *E.coli*. The spacer sequence was marked in **blue** and listed in Table S8. The PET-GFP plasmid is described in Table S7.

Fig. S16.

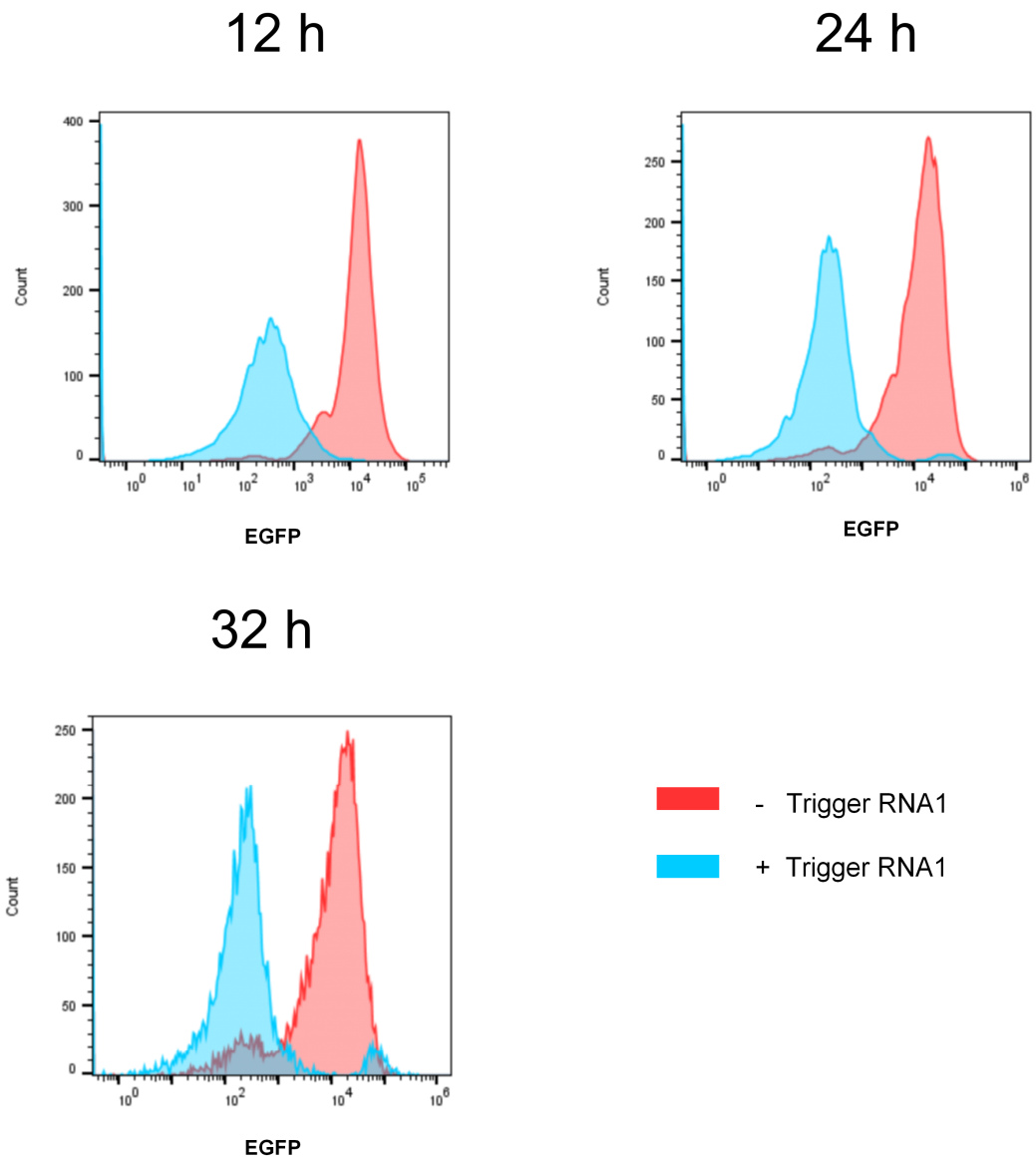


Figure S16 Changes in GFP fluorescence in MG1655-GFP cells transfected with PACYC-Trigger 1 or PACYC-no Trigger 1 were measured at 12, 24, and 32 hours. These cells were transfected with PACYC-Trigger 1 (+) and PACYC-no Trigger 1 (-), respectively, and GFP fluorescence was recorded at different time points by flow cytometry. After 24 hours of Trigger RNA and PARC-Cas12a expression, the signal-to-noise ratio was maximized, indicating clear cleavage of the GFP gene within the bacteria. Conversely, the GFP fluorescence intensity remained high in the no Trigger RNA group. Error bars represent the standard deviation of three measurements.

Fig. S17.

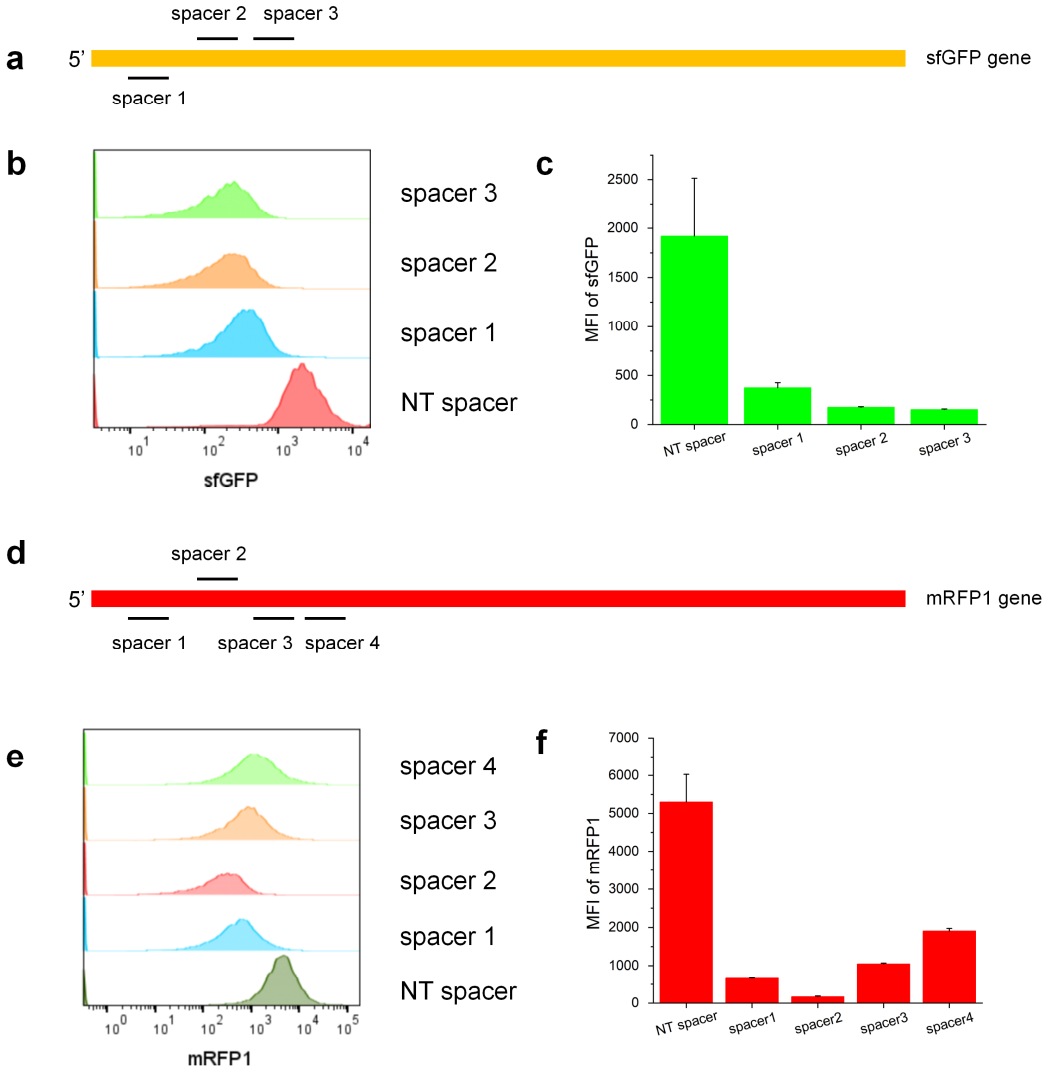


Figure S17 Flow cytometry screening of CRISPRi spacer sequences targeting sfGFP and mRFP1 genes in *E.coli*. (a) and (d) show the positions of spacers on the sfGFP and mRFP1 genes. The details of gene sequences are listed in Table S7 and Table S9. (b) and (e) show flow cytometry histograms for sfGFP and mRFP1 expression with each of the spacers compared with a non-targeting (NT) spacer control. (c) and (f) display the mean fluorescence intensity (MFI) of sfGFP and mRFP1 following CRISPRi with different spacers. Error bars represent data from three independent parallel experiments. These results highlight the specific positions and effectiveness of the spacer sequences in targeting and downregulating the expression of sfGFP and mRFP1 genes via CRISPRi.

Fig. S18.

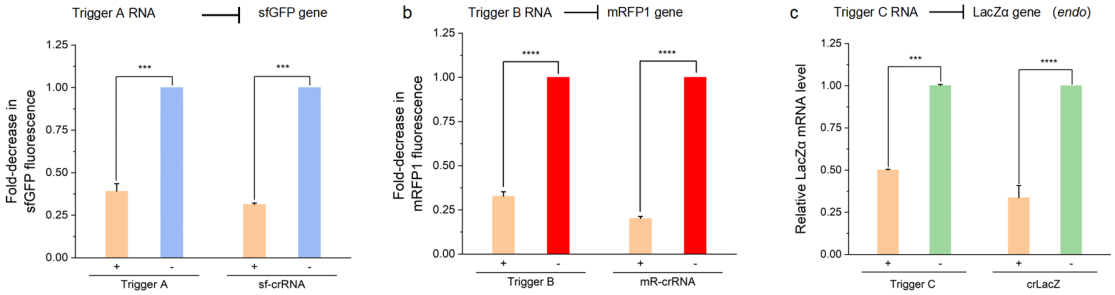


Figure S18 RNA-inducible CRISPRi in *E. coli*.

(a)Artificial Trigger-A RNA effectively suppressed exogenous sfGFP expression, with an inhibition efficiency of approximately 60.9%. sf-crRNA represents the normal gRNA (Figure S19, spacer 3) for CRISPRi targeting the sfGFP gene.

(b) Artificial Trigger B RNA effectively suppressed exogenous mRFP1 expression with an inhibition efficiency of approximately 67.2% using the PARC-CRISPR12a system. mR-crRNA represents the normal gRNA (Figure S19, spacer 2) for CRISPRi targeting the mRFP1 gene.

(c) The relative expression levels of *lacZα* mRNA were detected by real-time qPCR. Artificial Trigger C RNA effectively suppressed endogenous *lacZα* mRNA expression with an inhibition efficiency of approximately 49.9% using the PARC-CRISPR12a system. crLacZ represents the normal gRNA for CRISPRi targeting the *lacZ* gene.

The symbols "+" and "-" indicate the presence or absence of the corresponding overexpressed RNA sequence in the bacteria. Reporter fold change is defined as the sfGFP fluorescence observed with induction of the indicated trigger on the x-axis over the sfGFP fluorescence observed without induction of the trigger or without gRNA. Error bars represent data from three independent parallel experiments. ***P < 0.001 and ****P < 0.0001 were calculated by *Student's t-test*.

Fig. S19.

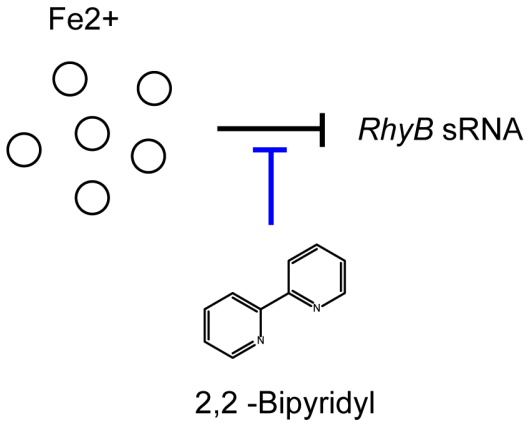


Figure S19 According to the literature[4, 5], the mechanism of PARC-Cas12a activation involves the induction of RyhB under conditions of iron starvation.

Fig. S20.

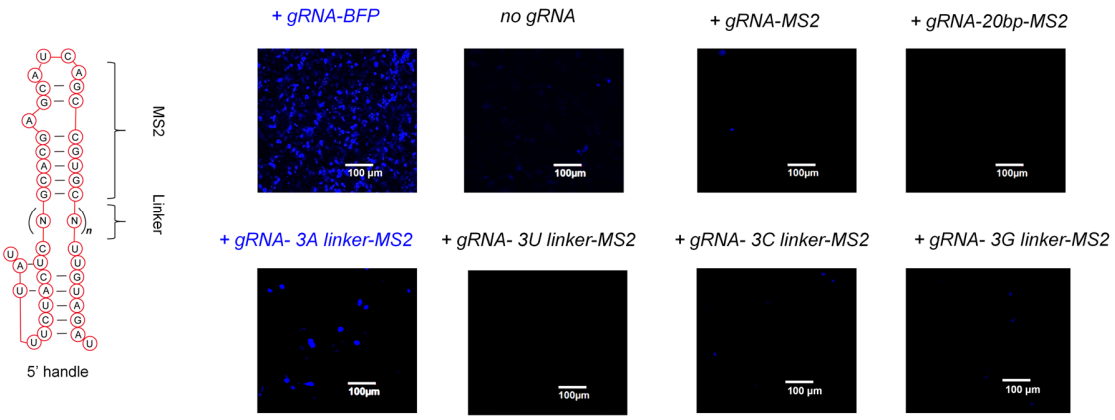


Figure S20 Laser confocal imaging analysis of embedding gRNA with the MS2 element at the "UCUU" stem-loop position and its effect on CRISPR-dCas12a activity. Normal gRNA-BFP significantly activated the dCas12a-VPR, which in turn activated tagBFP gene, resulting in bright fluorescence. In contrast, only the MS2-3A-gRNA design resulted in some cells displaying bright blue fluorescence, while other designs showed almost no cells with efficient blue fluorescence expression.

Fig. S21.

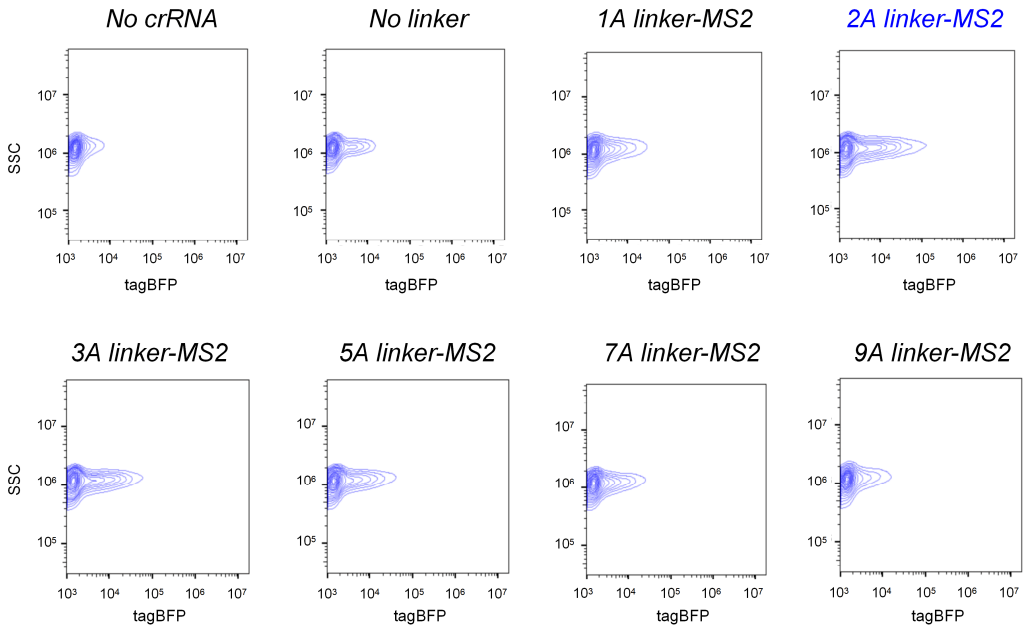


Figure S21 Impact of the number of A bases at the linker position in the MS2-gRNA chimera on CRISPR-Cas12a activation. The results show that when the number of A bases reaches 2, the MS2-gRNA performs with the highest efficiency. As the number of A bases increases beyond 2, the efficiency of CRISPRa significantly decreases, resulting in reduced blue fluorescence in cells.

Fig. S22.

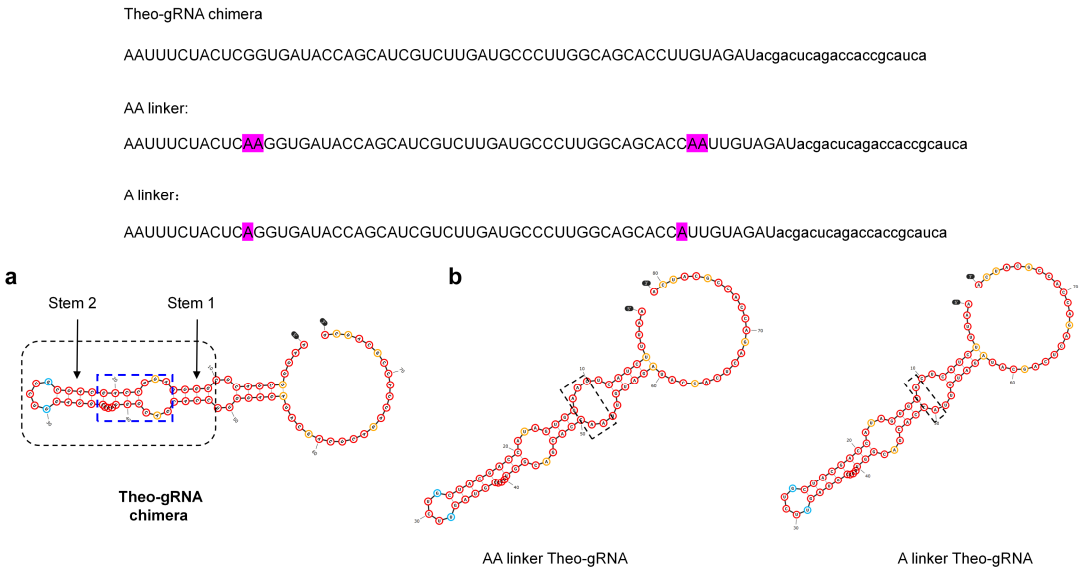


Figure S22 The sequences and structures of theophylline aptamer-embedded gRNA and its variants with single-A and double-A linkers (sequences in Table S10). (a) The design of the theophylline aptamer and gRNA chimera (Theo-gRNA chimera). The theophylline aptamer is highlighted within the **black dashed box**, which includes the theophylline recognition sequence in the ***blue*** box(6). ***Stem1*** is tightly connected to the "UCUU" region of the gRNA, while ***Stem2*** enhances the binding affinity of the theophylline aptamer. (b) The addition of one or two adenine nucleotides between the theophylline aptamer and the gRNA sequence does not cause significant structural changes.

Fig. S23.

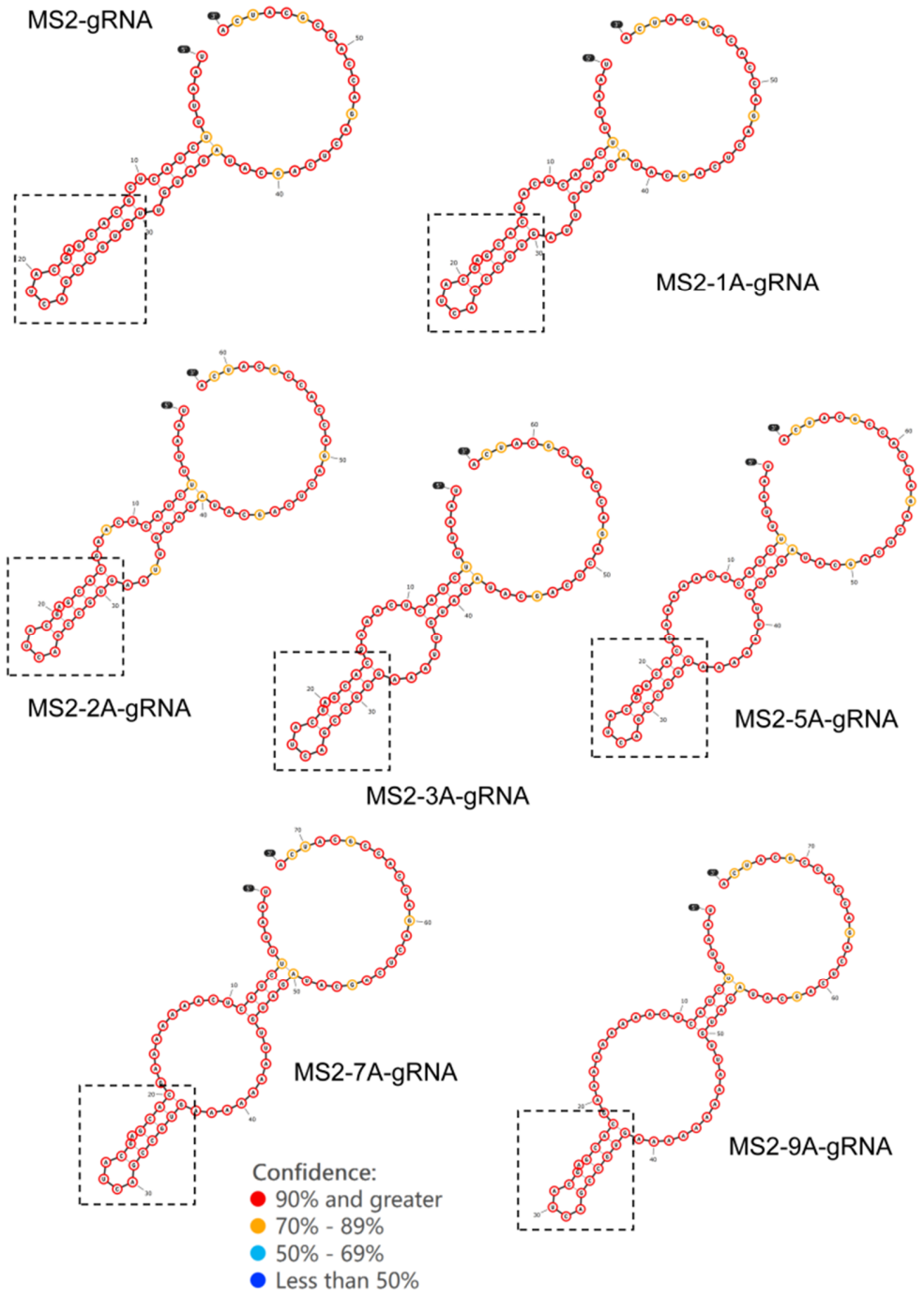


Figure S23 Predicted structures of the MS2 hairpin aptamer and gRNA chimera designs by *SnapGene* software. The structures were predicted using Snapgene software, and the section enclosed by the dashed line represents the MS2 RNA element sequence.

Table S1 RNA and DNA sequences of RNA-inducible PARC-Cas12a and small molecules -inducible PARC-Cas12a in vitro

Name	Sequence	5′	3′
gRNA	UAAUUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		
gRNA-FAM	UAAUUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU	FAM	
crRNA-F	UAAUUUCUACUCAAAGGCAAAUUGUGCAAU		
crRNA-R	CUGAAGCGCUGGGAAAUUGUAGAUGCCAGGGACGAAGCGCAAGU		
crSplitF	UAAUUUCUACUC		
crSplitF-FAM	UAAUUUCUACUC		FAM
crSplitR	UUGUAGAUGCCAGGGACGAAGCGCAAGU		
crSplitR-FAM	UUGUAGAUGCCAGGGACGAAGCGCAAGU	FAM	
crATP-F	UAAUUUCUACUCAAAA**dAdCdCdTdGdGdGdGdGdAdGdTdAdT**		
crATP-R	**dTdGdCdGdGdAdGdGdAdAdGdGdT**AAAAUUGUAGAUGCCAGGGACGAAGCGCAAGU		
crTheo-F1	AAUUUCUACUCAAA**GGUGAUACCAGCAU**		
crTheo-R1	**AUGCCCUUGGCAGCACC**AAAUUGUAGAUGCCAGGGACGAAGCGCAAGU		
crTheo-F2	AAUUUCUACUC**GGUGAUACCAGCAU**		
crTheo-R2	**AUGCCCUUGGCAGCACC**UUGUAGAUGCCAGGGACGAAGCGCAAGU		
Theo-gRNA	AAUUUCUACUCAAA**GGGAUACCAGCAUCGUCUUGAUGCCCUUGGCAGCCC**AAAUUGUAGAUGCCAGGGACGAAGCGCAAGU		
Trigger RNA	GGCCGCAAAUUGCACAAUUUGCCCCCAGCGCUUCAGCGUUCUUCGGAA		

crRNA (LbuCas 13a)	GACCACCCCAAAAAUGAAGGGGACUAAAACGAAGCGCU GGGGGCAAAUUG		
crCOVID 2-F	UAAUUUCUACUCAAAGGCAAAUUGUGCAAU		
crCOVID 2-R	CUGAAGCGCUGGGAAAUUGUAGAUGCCAGGGACGAAGC GCAAGU		
ssDNA activator	tctttcgccagggacgaagcgcaagtgacgg		
dsDNA activator	tgggtaatctgccctgcactctgggataagccttggaaacggggtctaatacc ggatatcacaatctctcgcatgggggttgttgaaagttctggcggtgcagg atgaacccgcggcctatcagcttgttggtggggtagtggcctaccaaggcga cgacgggtagccggcctgagagggtgaccggccacactgggactgagac acggcccagactcctacgggaggcagcagtggggaatattgcacaatggg cgcaagcctgatgcagcgacgccgcgtgagggatgacggccttcgggttgt aaacctctttcgccagggacgaagcgcaagtgacggtacctggataagaag caccggctaactacgtgccagcagccgcggtaatacgtagggtgcgagcgt tgtccggatttattgggcgtaaagagctcgtaggcggtttgtcgcgtcggcc gtgaaatctccatgcttaacgtggagcgtgcggtcgatacgggcagacttga gttcggt		
FQ probe	TTTTTT	FAM	BHQ1

Note:

dN: DNA modification

Table S2 RNA sequences of Viruses in this paper

Name	Sequence	Full name
HAV	AGUACCUGAGACAUUUCCUGAAUUGAAGCCUGGAGAGUCC	Hepatovirus A
HCV	AGCGGUCACAACCUCGUGGAAGACGUCAGCCUAUCCCCAA	Hepatitis C virus
HIV	GUUGCAACUCACGGUCUGGGGCAUUAAGCAGCUCCAGGC	Human immunodeficiency virus
EBOV	CCACUGAGGCUGAGAAGCAACUCCAACAAUAUGCAGAGUC	Ebolavirus
SARS	AAAUUGGCUACUACCGAAGAGCUACCCGACGAGUUCGUG	Severe acute respiratory syndrome-related coronavirus
COVID-2	GCAAAUUGCACAAUUUGCCCCCAGCGCUUCAGCGUUCU	Severe acute respiratory syndrome coronavirus 2 (COVID-19)
HPV	UACUGCAAGCAACAGUUACUGCGACGUGAGGUAUAUGACU	Human papillomavirus
MERS	GGGTCAGGATATGCAGCCGACCTGAAGAGCACACAGAATGCCAT	Middle East respiratory syndrome coronavirus
HKU1	GCTAATCACCAAGCTGACACTTCTACTCCCTCCGATGTTTCGTCAAGG	Human coronavirus HKU1
H1N1	TGTGCAGAGTATCCGGCTGTTTATGAGAACTGGATCATGGTGGTCATTCA	Influenza A virus subtype H1N1

Table S3 split gRNA sequences of Multiplex Detection Platform

name	Sequence	5′	3′
crHAV-F	UAAUUUCUACUCAAUCAGGAAUGUCUCA		
crHAV-R	cuccaggcuucaauAAUUGUAGAUcuacgguauccaccagauuuc		
crHCV-F	UAAUUUCUACUCAAuuccacgagguugu		
crHCV-R	ggauaggcugacgucAAUUGUAGAUcaaaggcagccuccggcuuuc		
crHIV-F	UAAUUUCUACUCAAccagaccgugaguug		
crHIV-R	gagcugcuuaaugccAAUUGUAGAUuaaagacaccggcuggcauuua		
crEBOV-F	UAAUUUCUACUCAAugcuucucagccuc		
crEBOV-R	gcauauuguuggagAAUUGUAGAUgaaaugaccuauggugauuuc		
crSARS-F	UAAUUUCUACUCAAucuucgguaguagc		
crSARS-R	acucgucggguagcAAUUGUAGAUgaaacaccacgggcgacuuua		
crCOVID2-F	UAAUUUCUACUCAAggcaaauugugcaau		
crCOVID2-R	cugaagcgcugggAAUUGUAGAUcaaauccugcgguguccuuug		
crHPV-F	UAAUUUCUACUCAAaguaacuguugcuug		
crHPV-R	auauaccucacgucgAAUUGUAGAUuaaagccggauaccuccggac		
crMERS-F	UAAUUUCUACUCAAcataaacagccggat		
crMERS-R	caccatgatccagttcAAUUGUAGAUcgucgccguccagcucgacca		
crHKU1-F	UAAUUUCUACUCAAgaagtgtcagcttgg		
crHKU1-R	aacatcggagggagtAAUUGUAGAUacgacucagaccaccgcauca		
crH1N1-F	UAAUUUCUACUCAAcggctgcatatcct		
crH1N1-R	tgtgtgctcttcaggAAUUGUAGAUcaaauuacggcguccacuuug		

Table S4 dsDNA sequences of Multiplex Detection Platform

Name	Sequence	5′	3′
HAV-dsDNA	atgcgtccggcgtagaggatcgagatctgtcgactttc**CTACGGTATCCACCAGATTTC**tacgttgtaccgaggtggaaatgtagttaatccgtataggctggtccaaggcggcacaatatcgtcaaaaacgtaggagtagtttcaagggttcgcgagttcatggtgtcaaatcagttattccgcacgacaaccactacctgagcacccccgttagaccgatacgttcaaacgtacgtgctagcgaattcattaaagaggagaaaaggtaccatggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagttcgag	FAM	
HCV-dsDNA	atgcgtccggcgtagaggatcgagatctgtcgactttcacgtcggagtatgtacgtttc**CAAAGGCAGCCTCCGGCTTTC**gtggatataagcagatcatcatccgttgttttgttcttcaggcccactagcgccccagtacgacatggattccatgcttctaaaattagccagtagtcccgcaaaggtgtaactaggaatccgggagcccgttagaccgatacgttcaaacgtacgtgctagcgaattcattaaagaggagaaaggtaccatggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagttcgag	FAM	
HIV-dsDNA	atgcgtccggcgtagaggatcgagatctgtcgactttcacgtcggagtatgtacgtttccggcgcgatagcatacgtttc**TAAAGACACCGGCTGGCATTTA**gtaactccgagctaaaatcgaacctgtccggcttcgaccagccaaacaggtctgaattagtttcgtgttctcatcccgtactttcttttcgtgaatcagatatttcccgttagaccgatacgttcaaacgtacgtgctagcgaattcattaaagaggagaaaggtaccatggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtccggcgaggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagttcgag	FAM	
EV-dsDNA	atgcgtccggcgtagaggatcgagatctgtcgactttcacgtcggagtatgtacgtttccggcgcgatagcatacgtttccgattgccaggccccatatttta**GAATGACCTATGGTGATTTC**gcacctgcgtataggtacctccattcgccatgcgcgcatgacaagttggcttagactcgccccagttattgctgaacagctttgcccgt	FAM	

	tagaccgatacgttcaaacgtacgtgctagcgaattcattaaagaggagaaag gtaccatggtgagcaagaggcgaggagctgttcaccggggtggtgcccatcc tggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtccggcga gggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcacc accggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacgg cgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaa gtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacg acggcaactacaagacccgcgccgaggtgaagttcgag		
SARS-dsDNA	atgcgtccggcgtagaggatcgagatctgtcgactttcacgtcggagtatgtac gtttccggcgcgatagcatacgtttccgattgccaggccccatatttactagagt gcacaacctgtttc**GAAACACCACGGGCGACTTTA**tcactcctgtta gcccttgggactgcgaaagtcgtgggaacttcatgataataaaggattaatccc gttagaccgatacgttcaaacgtacgtgctagcgaattcattaaagaggagaa aggtaccatggtgagcaagggcgaggagctgttcaccggggtggtgcccat cctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtccggc gagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgca ccaccggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctac ggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttc aagtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaagga cgacggcaactacaagacccgcgccgaggtgaagttcgag	FAM	
COVID2-dsDNA	atgcgtccggcgtagaggatcgagatctgtcgactttcacgtcggagtatgtac gtttccggcgcgatagcatacgtttccgattgccaggccccatatttactagagt gcacaacctgtttctgtagacgtttggaggatttatcactcctgttagcccttggg actgcgaaagtcgtgggaacttcatgataataaaggactttta**CAAATCCTG CGGTGTCCTTTG**gggattgtgctagcgaattcattaaagaggagaaag gtaccatggtgagcaagaggcgaggagctgttcaccggggtggtgcccatcc tggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtccggcga gggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcacc accggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacgg cgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttcaa gtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacg acggcaactacaagacccgcgccgaggtgaagttcgag	FAM	
HPV-dsDNA	atgcgtccggcgtagaggatcgagatctgtcgactttcacgtcggagtatgtac gtttccggcgcgatagcatacgtttccgattgccaggccccatatttactagagt gcacaacctgtttctgtagacgtttggaggatttatcactcctgttagcccttggg actgcgaaagtcgtgggaacttcatgataataaaggactttaccgcaagaggc ccggcagtacgggattgtgctagcgaattcattaaagaggagaaaggtaccat ggtgagcaaggagcgaggagctgttcaccggggtggtgcccatcctggtcg agctggacggcgactttg**TAAAGCCGGATACCTCCGGAC**ccggcg agggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcac caccggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacg gcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgacttcttca	FAM	

	agtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggac gacggcaactacaagacccgcgccgaggtgaagttcgag		

Table S5 dsDNA Metal Probe (DMP) sequences of Multiplex Detection Platform

Name	Sequence	5′	3′
HAV-Tb	GTCGACTTTTCCTACGGTATCCACCAGATTTCTACGTTGTAC		Tb-DOTA
HCV-Ho	TGTACGTTTCCAAAGGCAGCCTCCGGCTTTCGTGGATATAA		Ho-DOTA
HIV-Sm	CATACGTTTCTAAAGACACCGGCTGGCATTTAGTAACTCCGA		Sm-DOTA
EV-Eu	CCATATTTAGAAATGACCTATGGTGATTTCGCACCTGCGTA		Eu-DOTA
SARS-Pr	AACCTGTTTCGAAACACCACGGGCGACTTTATCACTCCTGT		Pr-DOTA
COVID2 -La	AAGGACTTTACAAATCCTGCGGTGTCCTTTGGGGATTGTGCTA		La-DOTA
HPV-Lu	GCGACTTTGTAAAGCCGGATACCTCCGGACCCGGCGAGGGCGAG		Lu-DOTA
MERS-Tm	TGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCA		Tm-DOTA
HKU1-Dy	CTCGACGTTTGACGACTCAGACCACCGCATCACTCTCCCT		Dy-DOTA
H1N1-Yb	ACCATGGGCAGCTTTGCAAATTACGGCGTCCACTTTGAGCCATC		Yb-DOTA

Table S6 Covid-2 N-gene sequence[7]

Name	Sequence	5′	3′
COVID2-N-gene	CTTCCATGCCAATGCGCGACATTCCGAAGAACGCTGAAGCGCTGGGGGGCAAATTGTGCAATTTGCGGCCAATGTTTGTAATCAGTTCCTTGTCTGATTAGTTCCTGGTCCCCAAAATTTCCTTGGGTTTGTTCTGGACCACGTCTGCCGAAAGCTTGTGTTACATTGTATGCTTTAGTGGCAGTACGTTTTTGCCGAGGCTTCTTAGAAGCCTCAGCAGCAGATTTCTTAGTGACAGTTTGGCCTTGTTGTTGTTGGCCTTTACCAGACATTTTGCTCTCAAGCTGGTTCAATCTGTCAAGCAGCAGCAAAGCAAGAGCAGCATCACCGCCATTGCCAGCCATTCTAGCAGGAGAAGTTCCCCTACCTGCTGCCTGGAGTTGAATTTCTTGAACTGTTGCGACTACGTGATGAGGAACGAGAAGAGGCTTGACTGCCGCCTCTGCTCCCTTCTGCGTAGAAGCCTTTTGGCAATGTTGTTCCTTGAGGAAGTTGTAGCACGATTGCAGCATTGTTAGCAGGATTGCGGGTGCCAATGTGATCTTTTGGTGTATTCAAGGCTCCCTCAGTTGCAACCCATATGATGCCGTCTTTGTTAGCACCATAGGGAAGTCCAGCTTCTGGCCCAGTTCCTAGGTAGTAGAAATACCATCTTGGACTGAGATCTTTCATTTTACCGTCACCACCACGAATTCGTCTGGTAGCTCTTCGGTAGTAGCCAATTTGGTCATCTGGACTGCTATTGGTGTTAATTGGAACGCCTTGTCCTCGAGGGAATTTAAGGTCTTCCTTGCCATGTTGAGTGAGAGCGGTGAACCAAGACGCAGTATTATTGGGTAAACCTTGGGGCCGACGTTGTTTTGATCGCGCCCCACTGCGTTCTCCATTCTGGTTACTGCCAGTTGAATCTGAGGGTCCACCAAACGTAATGCGGGGTGCATTTCGCTGATTTTGGGGTCCATTATCAGACCTATAGTGAGTCGTATTA		

Table S7 The sequences of Plasmids

Name/	The detailed information of the plasmid	antibiotic resistance genes
PACYC-dAsCpf1(E993A)	https://benchling.com/s/seq-Np1cgBewa5cvHEkckJKF?m=slm-qjxOLLQuLGD7vwsZJd2Q	Cmt
PACYC-AsCpf1	https://benchling.com/s/seq-emkdiQRNDGz8AUX9iqmx?m=slm-lhYcSj4BrN3pBzqBBeyf	Cmt
PET-GFP-crRNA	https://benchling.com/s/seq-K45atCEx1W7qEAlwvSxO?m=slm-Hy0xMwGMW4R4izaEYX8D	Amp
PET-GFP	https://benchling.com/s/seq-DSV7zM4F3cTx2iutp4M3?m=slm-lvcEor3OGQ6rVAZqcxdd	Amp
PACYC-Trigger 1	https://benchling.com/s/seq-BLUxEPq82jFQR1JMGFXY?m=slm-24Rh4DC42ep2k2nmSdt2	Cmt
PACYC-no Trigger 1	https://benchling.com/s/seq-2krrU8fnE25PSBaU9t84?m=slm-qn7zepyfl0bVLGiM2ujG	Cmt
PSC101-sfGFP	https://benchling.com/s/seq-Ru7PFfXe6vDBsaHd8xV6?m=slm-aulGgIaDXS9a40GBBvni	KanR
PSC101-mRFP1	https://benchling.com/s/seq-A2QjosP8KoXkofSwLN96?m=slm-nI3fOomZ467YGpGcyVDF	KanR
PSC101-sfGFP-mRFP1	https://benchling.com/s/seq-BwvCVujVqWqOOJLRnIDB?m=slm-dwHLbXasJQHvahQkaDQP	KanR
PACYC-sf-crRNA	https://benchling.com/s/seq-Z5e9kxcfDvDuNyEA3aKy?m=slm-mjg8cE2xgx3dWwLNXMF0	Cmt
PACYC-crLacZα	https://benchling.com/s/seq-qCN8amndN2jhyJAaVEA3?m=slm-lZfYCY8UsZQCEbYuUljX	Cmt

PACYC-Trigger A	https://benchling.com/s/seq-7sF2PIuhtBvfkYK4oHOT?m=slm-3eije59bhFKBAmdIXllV	Cmt
PACYC-no Trigger A	https://benchling.com/s/seq-FQAaZhVlqmCcxogayD14?m=slm-9owZRPmMmx1ltFoLPcHM	Cmt
dAsCas12a (D908A)	https://benchling.com/s/seq-mQ0TNinbje6xUs13TmoG?m=slm-6C2ft8hUeGRzBkoXUB2N	Amp
dAsCas12a-VPR	https://benchling.com/s/seq-f02CpRfGxXUNlqJ1rXzr?m=slm-iLcqArE93MA7Giiqvhc0	Amp
dAsCas12a-VPR-gRNA	https://benchling.com/s/seq-O5vpLhjzrVRFHXw5A42H?m=slm-XVnCyZscf4McHuORp2Jf	Amp
TRE-miniCMV-tagBFP	https://benchling.com/s/seq-jnhOP2kO7H7s55uZHw1h?m=slm-1z7gQZOxZLhfKjy0Zbjq	Amp
U6-gRNA-TRE-miniCMV-tagBFP	https://benchling.com/s/seq-Szz2LEVmiNy30V2kWAbd?m=slm-s1O7GoNvFJI67rm5sWSI	Amp
U6-MS2/2A-TRE-miniCMV-tagBFP	https://benchling.com/s/seq-AQEqT3TeT301JKGYpq66?m=slm-ljX3z1xly2sM1cfvvYRx	Amp
U6-PP7/1A-TRE-miniCMV-tagBFP	https://benchling.com/s/seq-46EhM1CrOQssydyfzjd7?m=slm-VoTcwQhjJwVE4jGtHjZh	Amp
U6-crSplitF-U6-crSplitR-TRE-miniCMV-tagBFP	https://benchling.com/s/seq-oQOCIfyoAt5OhxmY81uz?m=slm-AcLYtSchidRAOhkM3FF7	Amp
U6-Theo/2A-TRE-miniCMV-tagBFP	https://benchling.com/s/seq-2yHqkMe4lRkeeGNyzpsO?m=slm-68cIm25n7r4kLIFC2Kh0	Amp
MCP-Suntag 10X	https://benchling.com/s/seq-HD0Yo7OgD8iDGB6oLSGi?m=slm-HUTmnkpfXreyDn5d5NyX	Amp

MCP-Suntag 24X	https://benchling.com/s/seq-0muaAiru8MXkS0ldELIg?m=slm-ulefGPfNznQ2ngwEZnJL	Amp
scFv-sfGFP-VP64	https://benchling.com/s/seq-IV7BLuBJBAbtAJCsal97?m=slm-hkZBJlTRqkTwWGIctNJZ	Amp
U6-sgRNA/MS2(Cas9)-TRE-miniCMV-tagBFP	https://benchling.com/s/seq-5eu6zbL6wdHIJBeMzIWN?m=slm-Sj4BYpHWUMUqESgDjyNA	Amp
dCas9	https://benchling.com/s/seq-6PBLYM40rLMfkB69OQTK?m=slm-shLm4rqBSAFm5ImzJldl	Amp

Table S8 Sequences for Cas12a gene-cleavage experiment in bacteria. The spacers were marked in blue and the arms of the split crRNA binding to the Trigger RNA were labeled green. "TTTTTTTTT" represents the terminator of J23119 promoter.

Name	Sequence
J23119 promoter	ttgacagctagctcagtcctaggtataatactagt
GFP-gRNA (spacer in blue)	taatttctactcttgtagatCGTCGCCGTCCAGCTCGACCATTTTTTTTT
Trigger 1	ggccgcaaattgcacaatttgcccccagcgcttcagcgttcttcggaaTTTTTTTTT
crRNA-F1	taatttctactcaaggcaaattgtgcaatTTTTTTTTT
crRNA-R1	ctgaagcgctgggaattgtagatcgtcgccgtccagctcgaccaTTTTTTTTT
Trigger 2	catggagacgcagaagcccttcagcggccagtagcatctgactttgagcctcagggtctgagtgTTTTTTTT
crRNA-F2	taatttctactcaactgaagggcttctgcTTTTTTTTT
crRNA-R2	agatgctactggccgaattgtagatcgtcgccgtccagctcgaccaTTTTTTTTT
crRNA-F_3	tAAtttCtACtCAAGGCTTTTTTTTT
crRNA-F_6	tAAtttCtACtCAAGGCAAATTTTTTTTT
crRNA-F_9	tAAtttCtACtCAAGGCAAAttGTTTTTTTTT
crRNA-F_{12}	tAAtttCtACtCAAGGCAAAttGtGCTTTTTTTTT
crRNA-F_{15}	tAAtttCtACtCAAGGCAAAttGtGCAAtTTTTTTTTT

Table S9 Sequences for CRISPRi experiment in bacteria. The spacers were marked in blue and the arms of the split crRNA binding to the Trigger RNA were labeled green. "TTTTTTTTT" represents the terminator of J23119 promoter.

Name	Sequence
sf-spacer1	TAATTTCTACTCTTGTAGATCTAACCATAGAACCGTGGTGATGTTTTTTTTT
sf-spacer2	TAATTTCTACTCTTGTAGATCCGGCGTTGTGCCGATTCTGGTGTTTTTTTTT
sf-spacer3/ sf-crRNA	TAATTTCTACTCTTGTAGATGCGTTCGTGGCGAAGGCGAAGGTTTTTTTTTT
mR-spacer1	TAATTTCTACTCTTGTAGATataacgtcttcgctactcgccatTTTTTTTTT
mR-spacer2 (mR-crRNA)	TAATTTCTACTCTTGTAGATaaagttcgtatggaaggttccgtTTTTTTTTT
mR-spacer3	TAATTTCTACTCTTGTAGATgaactcgtgaccgttaacggaacTTTTTTTTT
mR-spacer4	TAATTTCTACTCTTGTAGATgcggtctgggtaccttcgtacggTTTTTTTTT
NT spacer/ NT crRNA/ NT gRNA	TAATTTCTACTCTTGTAGATactattgagaaccagcaattgTTTTTTTTT
crLacZ	TAATTTCTACTCTTGTAGATcaacgtcgtgactgggaaaacccTTTTTTTTT
RyhB sRNA	GCGAUCAGGAAGACCCUCGCGGAGAACCUGAAAGCACGACAUUGCUCAC AUUGCUUCCAGUAUUACUUAGCCAGCCGGGUGCUGGC
Trigger A	AAACAAATTGTACCTGTAACCGTCGCTTCTGAATGTCGTTCTTCCATTTTTTTT T
Trigger B	AAATCAATACGACAGCGGTCACACAAGTACTTCTCAGCACAATTTTTTTTT
Trigger C	GGCCGCAAATTGCACAATTTGCCCCCAGCGCTTCAGCGTTCTTCGGAATTTT TTTTT
crA-F	TAATTTCTACTCAAGGTTACAGGTACAATTTTTTTTTTT
crA-R	ACATTCAGAAGCGACAATTGTAGATGCGTTCGTGGCGAAGGCGAAGGTTTT TTTTT
crB-F	TAATTTCTACTCAAGACCGCTGTCGTATTTTTTTTTT

crB-R	CTGAGAAGTACTTGTGAATTGTAGATAAAGTTCGTATGGAAGGTTCCGTTTTTTTTTT
crC-F	TAATTTCTACTCAAGGCAAATTGTGCAATTTTTTTTTT
crC-R	CTGAAGCGCTGGGAATTGTAGATCAACGTCGTGACTGGGAAAACCCTTTTTTTTT
crRyhB-F	TAATTTCTACTCAATGAGCAATGTCGTGTTTTTTTTT
crRyhB -R	GTAATACTGGAAGCAATAATTGTAGATGCGTTCGTGGCGAAGGCGAAGGTTTTTTTTT

Table S10 Sequences for PARC-Cas12a system in eukaryotic cell. The spacers were marked in blue. The MS2 and PP7 RNA element were marked in red. The linker sequences between RNA element and gRNA were **bold**. The hybrid sequences of crRNA-F and crRNA-R are denoted in *italic underline*. The aptamer of theophylline was marked in green. "TTTTTTTTT" represents the terminator of U6 promoter.

Name	Sequence
U6 promoter	gagggcctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattgga attaatttgactgtaaacacaaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgg gtagtttgcagttttaaaattatgttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcg atttcttggctttatatatcttgtggaaaggacgaaacacc
gRNA-BFP	TAATTTCTACTCTTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
MS2-gRNA	TAATTTCTACTCGCACGAGCATCAGCCGTGTTGTAGATACGACTCAGACCAC CGCATCATTTTTTTTT
MS2-1A-gRNA	TAATTTCTACTC**A**GCACGAGCATCAGCCGTGC**A**TTGTAGATACGACTCAGA CCACCGCATCATTTTTTTTT
MS2-2A-gRNA	TAATTTCTACTC**AA**GCACGAGCATCAGCCGTG**AA**TTGTAGATACGACTCAG ACCACCGCATCATTTTTTTTT
MS2-3A-gRNA	TAATTTCTACTC**AAA**GCACGAGCATCAGCCGTG**AAA**TTGTAGATACGACTC AGACCACCGCATCATTTTTTTTT
MS2-5A-gRNA	TAATTTCTACTC**AAAAA**GCACGAGCATCAGCCGTG**AAAAA**TTGTAGATAC GACTCAGACCACCGCATCATTTTTTTTT
MS2-7A-gRNA	TAATTTCTACTC**AAAAAAA**GCACGAGCATCAGCCGTG**AAAAAAA**TTGTAG ATACGACTCAGACCACCGCATCATTTTTTTTT
MS2-9A-gRNA	TAATTTCTACTC**AAAAAAAAA**GCACGAGCATCAGCCGTG**AAAAAAAAA**T TGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
MS2-2A-gRNA-3 bp	TAATTTCTACTC**AAGGA**GCACGAGCATCAGCCGTG**TCCAA**TTGTAGATACG ACTCAGACCACCGCATCATTTTTTTTT
MS2-2A-gRNA -6 bp	TAATTTCTACTC**AAGTGGGA**GCACGAGCATCAGCCGTG**TCCCACAA**TTGTA GATACGACTCAGACCACCGCATCATTTTTTTTT
MS2-2A-gRNA -12 bp	TAATTTCTACTC**AAGTGGGACGCGTG**GCACGAGCATCAGCCGTG**CACGCG TCCCAC**AATTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT

MS2-2A-gRNA -24 bp	TAATTTCTACTC**AAGTGGGACGCGTGCAAGCAGGTTGA**GCACGAGCATC AGCCGTG**CTCAACCTGCTTGCACGCGTCCCACAA**TTGTAGATACGACTCA GACCACCGCATCATTTTTTTTT
PP7-gRNA	TAATTTCTACTCCCAGCAGAGCATATGGGCTCGCTGGTTGTAGATACGACTC AGACCACCGCATCATTTTTTTTT
PP7-1A-gRNA	TAATTTCTACTC**A**CCAGCAGAGCATATGGGCTCGCTGG**A**TTGTAGATACGA CTCAGACCACCGCATCATTTTTTTTT
PP7-2A-gRNA	TAATTTCTACTC**AA**CCAGCAGAGCATATGGGCTCGCTGG**AA**TTGTAGATAC GACTCAGACCACCGCATCATTTTTTTT
PP7-3A-gRNA	TAATTTCTACTC**AAA**CCAGCAGAGCATATGGGCTCGCTGG**AAA**TTGTAGAT ACGACTCAGACCACCGCATCATTTTTTTT
PP7-5A-gRNA	TAATTTCTACTC**AAAAA**CCAGCAGAGCATATGGGCTCGCTGG**AAAAA**TTGT AGATACGACTCAGACCACCGCATCATTTTTTTT
PP7-7A-gRNA	TAATTTCTACTC**AAAAAAA**CCAGCAGAGCATATGGGCTCGCTGG**AAAAAA A**TTGTAGATACGACTCAGACCACCGCATCATTTTTTTT
sgRNA-MS2 (Cas9)	ACGACTCAGACCACCGCATCGTTTTAGAGCTAGGCCAGCACGAGCATCAGC CGTGCCTGCAGGGCCTAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT
crSplit-F-3 bp	TAATTTCTACTC*GGA*TTTTTTTTT
crSplit-R-3 bp	*TCC*TTGTAGATACGACTCAGACCACCGCATCATTTTTTTT
crSplit-F-8 bp	TAATTTCTACTCAA*GGAGCACG*TTTTTTTT
crSplit-R-8 bp	*CGTGCTCC*AATTGTAGATACGACTCAGACCACCGCATCATTTTTTTT
crSplit-F-12 bp	TAATTTCTACTCAA*GTGGGACCTCGG*TTTTTTTT
crSplit-R-12 bp	*CCGAGGTCCCAC*AATTGTAGATACGACTCAGACCACCGCATCATTTTTTTT
crSplit-F-20 bp	TAATTTCTACTCAA*GTGGGACGCGTGCCTCGGCC*TTTTTTTT
crSplit-R-20 bp	*GGCCGAGGCACGCGTCCCAC*AATTGTAGATACGACTCAGACCACCGCATC ATTTTTTTT

crSplit-F-32 bp	TAATTTCTACTCAA*GTGGGACGCGTGCAAGCAGGTTGACCTCGGCC*TTTTTTTTT
crSplit-R-32 bp	*GGCCGAGGTCAACCTGCTTGCACGCGTCCCAC*AATTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
crSplit-F-50 bp	TAATTTCTACTCAA*ACTGTGGTTCATCGCCGTGTGGGACGCGTGCAAGCAGGTTGACCTCGGCC*TTTTTTTTT
crSplit-R-50 bp	*GGCCGAGGTCAACCTGCTTGCACGCGTCCCACACGGCGATGAACCACAGT*AATTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
Theo-gRNA chimera	TAATTTCTACTCGGTGATACCAGCATCGCGTCTTGCGATGCCCTTGGCAGCACCTTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
Theo-gRNA-2A/ Theo-gRNA	TAATTTCTACTC**AA**GGTGATACCAGCATCGCGTCTTGCGATGCCCTTGGCAGCACC**AA**TTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
Theo-gRNA-1A	TAATTTCTACTC**A**GGTGATACCAGCATCGCGTCTTGCGATGCCCTTGGCAGCACC**A**TTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
NT Theo-gRNA	TAATTTCTACTC**AA**GGTGATACCAGCATCGCGTCTTGCGATGCCCTTGGCAGCACC**AA**TTGTAGATACTATTGAGAACCAGCAATTGTTTTTTTTT
TS-Theo-DNMT1	TAATTTCTACTC**AA**GGTGATACCAGCATCGCGTCTTGCGATGCCCTTGGCAGCACC**AA**TTGTAGAT CTCAAGGGCTCTCACAAACCCTTTTTTTTTT
NTS-Theo-DNMT1	TAATTTCTACTC**AA**GGTGATACCAGCATCGCGTCTTGCGATGCCCTTGGCAGCACC**AA**TTGTAGAT CTGGCTATACGACCTTAGGTGGGTTTTTTTTT
TS-Theo-IL1RN	TAATTTCTACTC**AA**GGTGATACCAGCATCGCGTCTTGCGATGCCCTTGGCAGCACC**AA**TTGTAGAT TGCTAGCCTGAGTCACCCTCCTGTTTTTTTTT
NTS-Theo-IL1RN	TAATTTCTACTC**AA**GGTGATACCAGCATCGCGTCTTGCGATGCCCTTGGCAGCACC**AA**TTGTAGAT CAGGAGGGTGACTCAGGCTAGCA TTTTTTTTT
Theo-gRNA-4 bp	TAATTTCTACTC**AA**GGTGATACCAG**CATC**GTCTT**GATG**CCCTTGGCAGCACC**AA**TTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
Theo-gRNA-6 bp	TAATTTCTACTC**AA**GGTGATACCAG**CATCGC**GTCTT**GCGATG**CCCTTGGCAGCACC**AA**TTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT
Theo-gRNA-9 bp	TAATTTCTACTC**AA**GGTGATACCAG**CATCGCACG**GTCTT**CGTGCGATG**CCCTTGGCAGCACC**AA**TTGTAGATACGACTCAGACCACCGCATCATTTTTTTTT

Theo-gRNA-14 bp	TAATTTCTACTC**AA**GGTGATACCAG**CATCGCACGCAGAT**GTCTT**ATCTGCG TGCGATG**CCCTTGGCAGCACC**AA**TTGTAGATACGACTCAGACCACCGCATC ATTTTTTTTT

Table S11 qRT-PCR primer sequences

Name	Sequence	5′	3′
F-LacZ(8)	GACGTCTCGTTGCTGCATAAAC		
R-LacZ(8)	TCGCCGCACATCTGAACTTCA		
F-16S(9)	TAATACGGAGGGTGCAAGCG		
R-16S(9)	CTTCGCCACCGGTATTCCTC		
F-EGFP	ATGGTGAGCAAGGGCGAGGA		
R-EGFP	TCACGAACTCCAGCAGGACCAT		
F-tagBFP	GACGGCTGCCTCATCTAC		
R-tagBFP	TCTTAGCGGGTTTCTTGG		
F-*DNMT1*	GAGGAGGGCTACCTGGCTAA		
R-*DNMT1*	GCTTAGCCTCTCCATCGGAC		
F-*IL1RN*	GGAATCCATGGAGGGAAGAT		
R-*IL1RN*	TGTTCTCGCTCAGGTCAGTG		
F- *GAPDH*	CAATGACCCCTTCATTGACC		
R- *GAPDH*	TTGATTTTGGAGGGATCTCG		

Table S12 ICP-MS operation parameters

Parameter	Value
cool gas flow (L/min)	13
auxiliary gas flow (L/min)	0.7
nebulizer gas flow (L/min)	0.96
sample uptake (s)	45
dwell time (ms)	0.02
channel	3
number of repeats per sample	3
PC detector voltage (V)	1265
RF power (W)	1578.61
analogue detector voltage (V)	-1960

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