Section A: Materials and Methods

A1. Materials

A1.1. DNA oligonucleotides.

The DNA oligonucleotides (Table S1) were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China), and purified by 10% denaturing (8 M urea) polyacrylamide gel electrophoresis (dPAGE) or high-performance liquid chromatograph (HPLC).

The DNA library (98-nt) contains two constant sequence elements at the 5' ends (16-nt) and 3' ends (12-nt) and a central random region of 70-nt (N70, where N is 25% A, 25% G, 25% C and 25% T). The RNA-containing DNA substrate (RDS, 28-nt) contains an adenosine ribonucleotide (R) as the cleavage site, flanked by a fluorescein-dT (F) and a dabcyl-dT (Q). The reverse primer (RP2) contains C3 spacer linker and poly-A (20-nt) fragment at the 5' end. The C3 spacer prevents the poly-A tail from being amplified.

A1.2. Enzymes and chemicals.

T4 DNA ligase, Thermus thermophilus DNA polymerase, ATP and dNTPs were purchased from Sangon Biotech. T4 polynucleotide kinase (PNK) was obtained from Thermo Fisher Scientific. All other chemicals were purchased from Sigma-Aldrich and used without further purification.

A1.3. Bacterial cells and culture conditions.

The *Burkholderia cocovenenans* (BC, CICC 10574), *Escherichia coli BL21* (EC, CICC 24719), *Bacillus subtilis* (BS, CICC 10002), and *Klebsiella pneumoniae* (KP, CICC 13883) were purchased from China Center of Industrial Culture Collection. The *Staphylococcus aureus* (SA, ATCC 6538), and *Pseudomonas aeruginosa* (PA, ATCC 9027) were purchased from Agricultural Culture Collection of China. BC cells were cultured in Luria Bertani (LB) medium at 30 °C. In addition to KP, other bacterial cells were cultured in LB medium at 37 °C. KP cells were cultured in Tryptic Soy Broth (TSB) medium at 37 °C.

A1.4. Instrumentation.

Fluorescence measurements were performed with a microplate reader (Tecan, Switzerland). The fluorescent images of gels were obtained using a Typhoon 5 variable mode imager (GE Healthcare) and analyzed using Image Quant software (Molecular Dynamics). DNAzymes-in-Droplets (DID) assay was carried out on the Naica digital PCR system (Stilla Technologies, Villejuif, France) with Sapphire chips (Stilla Technologies, Villejuif, France).

A2. Methods

A2.1. Preparation of fresh crude extracellular mixtures (CEMs) and crude intracellular mixtures (CIMs).

BC cells were cultured in 5 mL of Luria Bertani (LB) with continuous shaking at 30 °C. 1 mL of the bacterial culture was centrifuged at 11000 g for 5 min at 4 °C. The supernatant was taken as the fresh CEM-BC and stored at -20 °C. The cell pellet was re-suspended in 300 µL of 1× SB, sonicated for 30 s, put on the ice for 2 min. The cell suspension was centrifuged at 13,000 rpm for 10 min at 4 °C, and collected as the fresh CIM-BC. All the bacterial cells were cultured for a different period time until the OD_{600} (optical density at 600 nm) reached ~ 1.

A2.2. In vitro selection procedures.

Ligation of DL1 to RDS (Step I). 600 pmol of RDS was phosphorylated using 10 units (U) of T4 Polynucleotide Kinase (T4 PNK) in 1× PNK buffer with 2 mM ATP at 37 °C for 40 min in a 50 µL reaction volume. Then, 500 pmol of DL1, 600 pmol of DS1 and 10 µL of 10× T4 DNA ligase buffer were added to this solution and the volume was adjusted to 97 µL with ddH_2O. The mixture was heated at 90 °C for 3 min and cooled to room temperature (RT) for 15 min. 3 µL of 5 U/µL T4 DNA ligase (TDL) were added, followed by incubation at RT for 2.5 h.

Purification of DL1-RDS (Step II). The obtained products in the reaction mixture from step I were concentrated by standard ethanol precipitation and purified by 10% denatured polyacrylamide gel electrophoresis (10% dPAGE).

Negative selection (Step III). The purified DL1-RDS was dissolved in 20 µL of ddH_2O and quantified by NanoDrop. 50 µL of 2× selection buffer (2× SB) (100 mM HEPES, 300 mM NaCl, 30 mM $MgCl_2$, 0.02% Tween 20, pH 7.0) was added and adjusted the volume to 100 µL using ddH_2O. The mixture was heated at 90 °C for 2 min and cooled to RT for 15 min. Followed by incubation at RT for 5 h, the reaction was stopped by ethanol precipitation.

Purification of uncleaved DL1-RDS (Step IV). The uncleaved DL1-RDS molecules from step III were purified by 10% dPAGE, dissolved in 20 µL of ddH_2O and stored at -20 °C.

Positive selection (Step V). The purified DL1-RDS molecules (200 pmol) was mixed with 50 µL of 2 × SB. The mixture was heated at 90 °C for 2 min, and cooled to RT for 15 min. This was followed by the addition of 25 µL of fresh CEM-BC (final volume: 100 µL). Followed by incubation at RT for 2 h, the reaction was stopped by ethanol precipitation.

Purification of cleaved products (Step VI). The cleaved molecules from step V were purified by 10% dPAGE, dissolved in 20 µL of ddH_2O and stored at -20 °C until use. The percent of the cleaved products was quantified using

Image Quant software and calculated using: %clv = (clv/6)/[(clv/6) + unclv], clv = volume of cleaved band; unclv = volume of uncleaved band.

PCR1 (Step VII). The purified cleaved products were used as the DNA template for PCR1. PCR1 was typically conducted with the template, 1 μM each of FP and RP1, 50 μM each of dNTPs (dATP, dTTP, dGTP and dCTP), 1× PCR buffer (10 mM Tris-HCl, pH8.9, 50 mM KCl, 1.5 mM $MgCl_2$), 5 U of Thermus thermophilus DNA polymerase, and ddH_2O (final volume: 50 μL). The DNA was amplified using the following thermocycling steps: 94 ºC for 1 min; n cycles of 94 ºC for 30 s, 50 ºC for 45 s and 72 ºC for 45 s (n represents the numbers of amplification cycles, typically 12 ~ 15 cycles); 72 ºC for 1 min. The PCR products were assessed by 2% agarose gel electrophoresis.

PCR2 (Step VIII). PCR2 was conducted using PCR1 product as the template. 1 μL of the PCR1 product was diluted to 20 μL, and 1 μL of which was used in PCR2. FP and RP2 were used in PCR2 according to the protocol described above. The numbers of cycles between different rounds were typically between 11 and 13 cycles, to achieve full amplification (revealed by RT-PCR).

Purification of PCR2 products (Step IX). The PCR2 products were concentrated by ethanol precipitation and purified by 10% dPAGE. Because the C3 spacer in RP2 prevents the amplification of the A20 fragment, the non-coding strand was longer than the coding strand. The coding strand was then purified by 10% PAGE.

The coding DNA strand prepared above was then ligated to RDS, and used for the next round of selection. A total of 14 cycles was conducted. The DNA population from round 14 was subjected to deep sequencing using the MiSeq (Illumina) sequencing platform.

A2.3. DNAzyme activity assay (Figure 1c).

The top 5 sequences were chemically synthesized and ligated to RDS, named RFD-BC1, RFD-BC2, RFD-BC3, RFD-BC4 and RFD-BC5, respectively. Responses of top 5 RFDs to CEM-BC were carried out as follows: 2 μL of 5 μM each RFDs, 50 μL of 2× SB and 23 μL ddH_2O were mixed. To this mixture were added 25 μL of CEM-BC (10^5 CFU), and the resultant mixture was incubated at RT for 2 h before ethanol precipitation. The resultant products were analyzed by 10% dPAGE (8 M urea).

A2.4. Fluorescence assay (Figure 1e).

Real-time fluorescence response of RFD-BC1 was analyzed using Microplate reader. In a typical experiment, 25 μL of CEM-BC, 50 μL of 2× SB and 23 μL of ddH2O were mixed in 96-well plates. Following incubation at RT for 5 min, 2 μL of 5 μM RFD-BC1 was added to each well and incubated at

RT. Following this step, time-dependent fluorescence at λex/λem = 485 nm/520 nm was recorded every 5 min for 5 h.

A.2.5. pH dependency (Figure 2a).

The reaction buffer pH was adjusted using the following buffering reagents: citrate for pH 2.0-3.0, acetate for pH 4.0-5.0, MES for pH 6.0, and HEPES for pH 7.0-8.0 at 25 ºC (each used at 50 mM containing 150 mM NaCl, 15 mM $MgCl_2$, and 0.01% Tween 20). The protocol was similar to the one described in **A2.3.**

A.2.6. Kinetic analysis (Figure 2b).

Kinetic analysis of RFD-BC1 was carried out at pH 5.0, pH 6.0, and pH 7.0 in the presence of CEM-BC. The detailed protocol was similar to the one described in **A2.3.** excepted that the mixture was incubated for 1 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, and 4 h. Apparent rate constants were calculated by curve-fitting the percent cleavage of RFD-BC1 in the presence of CEM-BC versus reaction time using Origin 8.0 where $Y = Y_{max}\,[1\text{-}e^{-kt}]$, Y_{max} represents the maximal cleavage yield and k is the observed first-order rate constant (k_{obs}).

A.2.7. Divalent metal ion dependency (Figure 2c).

The divalent mental ion (M^{2+}) dependency analysis was carried out in 1× RB (50 mM acetate, 0.01% Tween 20, pH 5.0) containing 15 mM of different M^{2+} (Mg^{2+}, Mn^{2+}, Ca^{2+}, Pb^{2+}, Ba^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}). The protocol was similar to the one described in **A.2.3.**

A.2.8. Examination of the nature of the target (Figure 3a).

SDS treatment: 5 μL of 10% SDS was added to 45 μL of CEM-BC and the mixture was incubated at RT for 30 min; EDTA treatment: 5 μL of 100 mM EDTA was added to 45 μL of CEM-BC; RNase inhibitor (RL) treatment: 5 μL of RL (5 U/μL) was added to 25 μL of CEM-BC and the mixture was incubated at RT for 30 min; Proteinase K (PK) treatment: 5 μL of PK (2 mg/mL) was added to 45 μL of CEM-BC and the mixture was incubated at 37°C for 30 min; heating treatment: 50 μL of CEM-BC was heated at 90°C for 10 min, and cooled at RT for 15 min. All the cleavage test was carried out in 1× RB (50 mM acetate, 15 mM $MgCl_2$, 0.01% Tween 20, pH 5.0) and the detailed steps were similar to the one described in **A.2.3.**

A.2.9. Estimation of molecular weight of the target (Figure 3b).

300 μL of CEM-BC was passed through a molecular sizing centrifugal column with cut-off of 3 K (3,000 Daltons), 10 K, 30 K, 50 K and 100 K (GE Healthcare), respectively. The filtrate was collected and used in the cleavage test in 1× RB (50 mM acetate, 15 mM $MgCl_2$, 0.01% Tween 20, pH 5.0). The protocol was similar to the one described in **A.2.3.**

A.2.10. Specificity test (Figure 3c).

The specificity test was carried out in 1× RB according to the protocol described in **A.2.3.** except that: CEM-EC, CEM-KP, CEM-PA, CEM-SA, CEM-BS were used in the presence and absence of (5 U/μL) ribolock.

A.2.11. Kinetic analysis of truncated trans-acting DNAzyme (Figure 4b).

Kinetic analysis of RFD-BC1T1 was carried out as follows: 2 μL of 5 μM RFD-BC1T1, 2 μL of 5 μM RDS, 25 μL of CEM-BC (10^5 CFU), 50 μL of 2× RB (100 mM acetate, 30 mM $MgCl_2$, 0.02% Tween 20, pH 5.0) and 21 μL ddH_2O were mixed (final volume: 100 μL). Each mixture was incubated at RT for 1 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, and 4 h. Then the reaction was stopped by ethanol precipitation, and the resultant products were analyzed by 10% dPAGE (8 M urea).

A.2.12. Feasibility of DNAzymes-in-Droplets (DID) assay (Figure 6b).

In a typical experiment, BC cells were resuspended in 100 μL of 1× RB, sonicated for 1 min, put on the ice for 1 min, and sonicated for 1 more min. 1 μL of 10 μM RFD-BC1T1, 1 μL of 10 μM RDS, 5 μL of cell lysates, 12.5 μL of 2× RB, 4.5 μL of ddH_2O, and 1 μL of 25× Alexa Fluor 647 (AF647) were mixed in a 25 μL final volume as positive sample. The mixture was quickly pipetted into the Sapphire Chips and moved into the Naica Geode Thermocycler. Following incubation at 25 °C for 1 h, the chip was imaged using the Naica Prism3 reader.

A.2.13. Sensitivity of DID assay (Figures 6c).

The sensitivity test was carried out in droplets in a similar way as described in **A.2.12.** except that: different concentrations of BC cell lysates (final concentration: 1, 10, 10^2, 10^3, 10^4, and 10^5 CFU) were used.

A.2.14. Selectivity of DID assay (Figure 6e).

The specificity test was carried out in 1× RB according to the protocol described in **A.2.12**. except that: CEM-EC, CEM-KP, CEM-PA, CEM-SA, CEM-BS were used in the presence of (5 U/μL) ribolock.

Section B: Supporting Tables and Figures.

B1. Supplementary Tables

Table S1. Sequences of DNA oligonucleotides used in this work.

DNA oligonucleotide	Sequence (5' - 3')
DNA library (DL1, 98 nt)	CACGGATCCTGACAAG-N_{70}-CAGCTCCGTCCG
RNA-containing DNA substrate (RDS, 28 nt)	ACTCTTCCTA GCFRQGGTTC GATCAAGA (F = fluorescein-dT; Q = dabcyl-dT)
Forward primer (FP, 16 nt)	CACGGATCCTGACAAG
Reverse primer 1 (RP1, 12 nt)	CGGACGGAGCTG
Reverse primer 2 (RP2, 32 nt)	A_{20}/Spacer C3/CGGACGGAGCTG
DNA Splint (DS1, 23 nt)	CTAGGAAGAGTCGGACGGAGCTG
RFD-BC1 (126 nt)	CACGGATCCT GACAAG**GGAG CTCGCAGGTT CGACGGAAGG ATGCCGCATT GAGTCGCCAG TAAACTGGGG AGACGGCTGG AAGGGT**CAGC TCCGTCCGAC TCTTCCTAGC FRQGGTTCGA TCAAGA
RFD-BC2 (126 nt)	CACGGATCCT GACAAG**GGAG ATGTGCTGCG GCTCAATTAT GAATGTCTGG ATACGGCAAA GCCGCTGATG ACATTGCCAG**

RFD-BC3 (126 nt)	**GGGGGT**CAGC TCCGTCCGAC TCTTCCTAGC FRQGGTTCGA TCAAGA CACGGATCCT GACAAG**GTTG CTGAGCACCA ACGCGCGGTG TCGAGGAGAG TCCCGACGGG TCTACGTGAA TTTACGTGTA TTTGTA**CAGC TCCGTCCGAC TCTTCCTAGC FRQGGTTCGA TCAAGA
RFD-BC4 (126 nt)	CACGGATCCT GACAAG**GTTA AGGATCGGCA GATAATTGAG TCGCCGCGAT GACGCGGGGA GACGGCTTCG GAAGAGAGAG GGTTCT**CAGC TCCGTCCGAC TCTTCCTAGC FRQGGTTCGA TCAAGA
RFD-BC5 (126 nt)	CACGGATCCT GACAAG**ACAT GGGTGAAGCC AAGATCCACA CGGCAACGTA GGTTACCGTC GCTTGGGGAG ATGCGGATCG GCTATA**CAGC TCCGTCCGAC TCTTCCTAGC FRQGGTTCGA TCAAGA
RFD-BC1S1 (71 nt)	GCATTGAGTC GCCAGTAAAC TGGGGAGACG GCTGGAAGGG TCGACTCTTC CTAGCFRQGG TTCGATCAAG A
RFD-BC1T1 (43 nt)	GCATTGAGTC GCCAGTAAAC TGGGGAGACG GGCTAGGAAG AGT

B2. Supplementary Figures

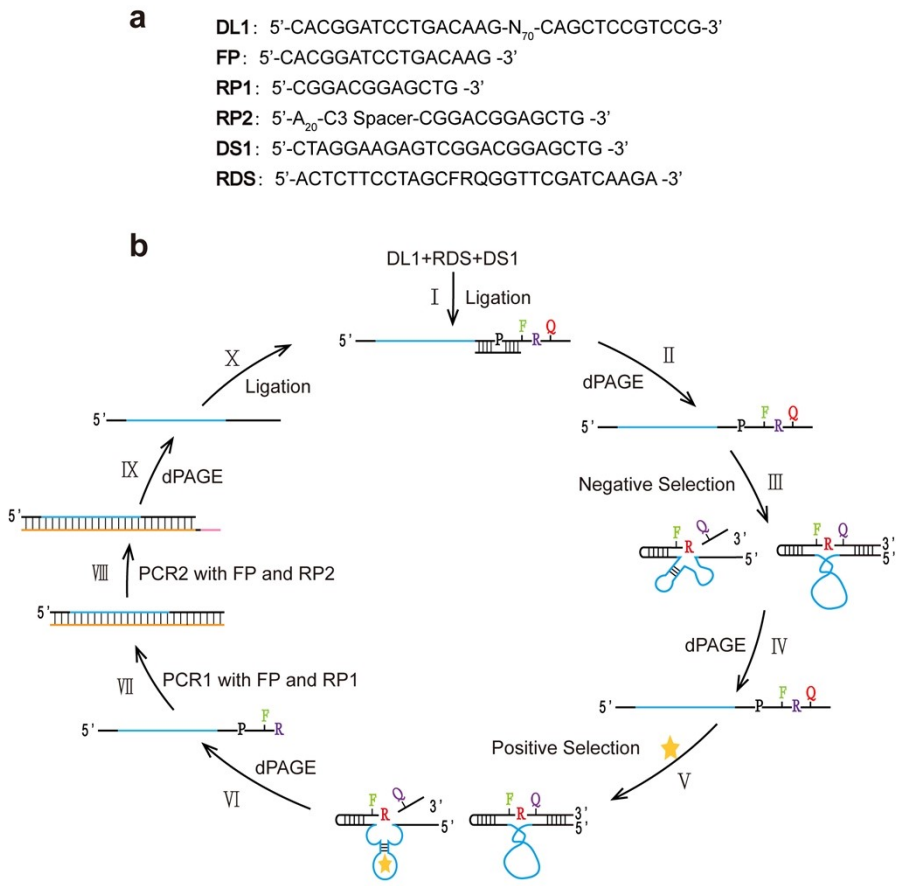


Fig. S1. a) The sequences of the DNA molecules used for in vitro selection experiment. b) In vitro selection scheme. I) Ligation of DL1 to RDS; II) purification of ligated DL1-RDS; III) negative selection with 1 × SB; IV) purification of uncleaved DL1-RDS by dPAGE; V) positive selection with CEM-BC; VI) purification of cleaved products by dPAGE; VII) PCR1 using FP and RP1 as primers; VIII) PCR2 with FP and RP2 as primers; IX) purification of the amplified DL1 strand by dPAGE; X) ligation of the amplified DL1 to RDS.

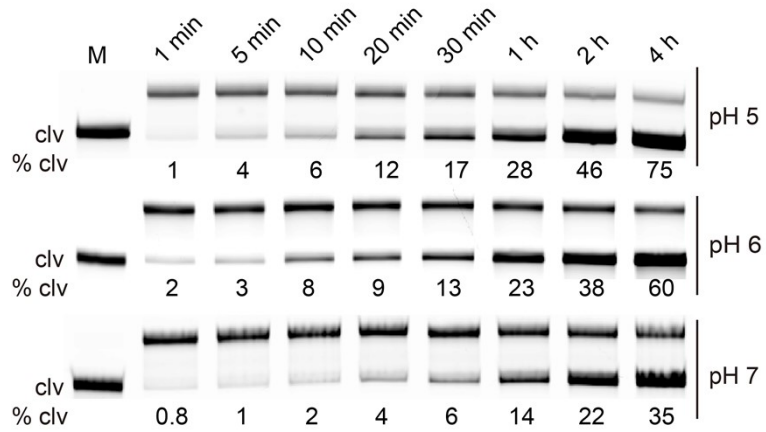


Fig. S2. 10% dPAGE analysis of the kinetic responses of RFD-BC1 to CEM-BC at pH values ranging from 5.0 to 7.0. M = DNA marker, clv = cleaved.

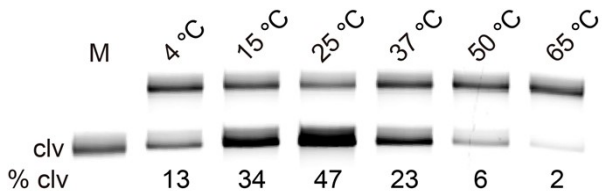


Fig. S3. 10% dPAGE analysis of the activity of RFD-BC1 to CEM-BC at various reaction temperatures (4 °C, 15 °C, 25 °C, 37 °C, 50 °C, and 65 °C). clv = cleaved.

Experimental details: The cleavage reactions were set up in 1× RB (50 mM acetate containing 15 mM $MgCl_2$, and 0.01% Tween 20, pH 5.0) in a similar way as described in **A.2.3.** above except that: the reaction was performed at different temperatures including 4 °C, 15 °C, 25 °C, 37 °C, 50 °C, and 65 °C.

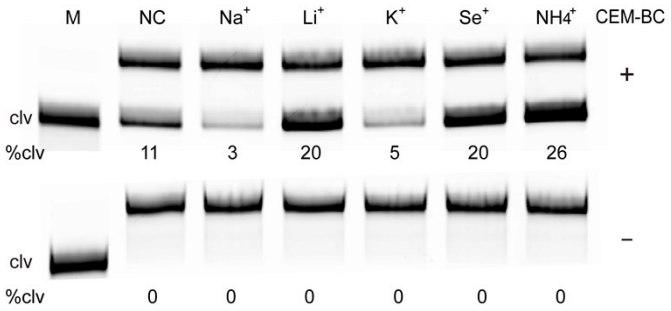


Fig. S4. 10% dPAGE analysis of the activity of RFD-BC1 in the presence of CEM-BC and various monovalent metal ions. NC: negative control, refers to the cleavage reaction carried out in 50 mM acetate (pH 5.0) containing 0.01% Tween 20. clv = cleaved.

Experimental details: The cleavage reactions were set up in 1× RB (50 mM acetate, 0.01% Tween 20, pH 5.0) containing 150 mM of different M^+ (Na^+, Li^+, K^+, Se^+, and NH_4^+). The protocol was similar to the one described in **A.2.3.**

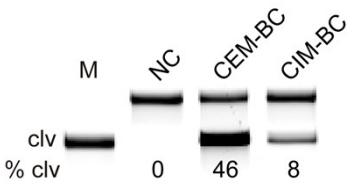


Fig. S5. 10% dPAGE analysis of the activity of RFD-BC1 in the presence of CEM-BC and CIM-BC. clv = cleaved.

Experimental details: The cleavage reactions were carried out in 1× RB (50 mM acetate, 15 mM $MgCl_2$, 0.01% Tween 20, pH 5.0). The protocol was similar to the one described in **A.2.3.**

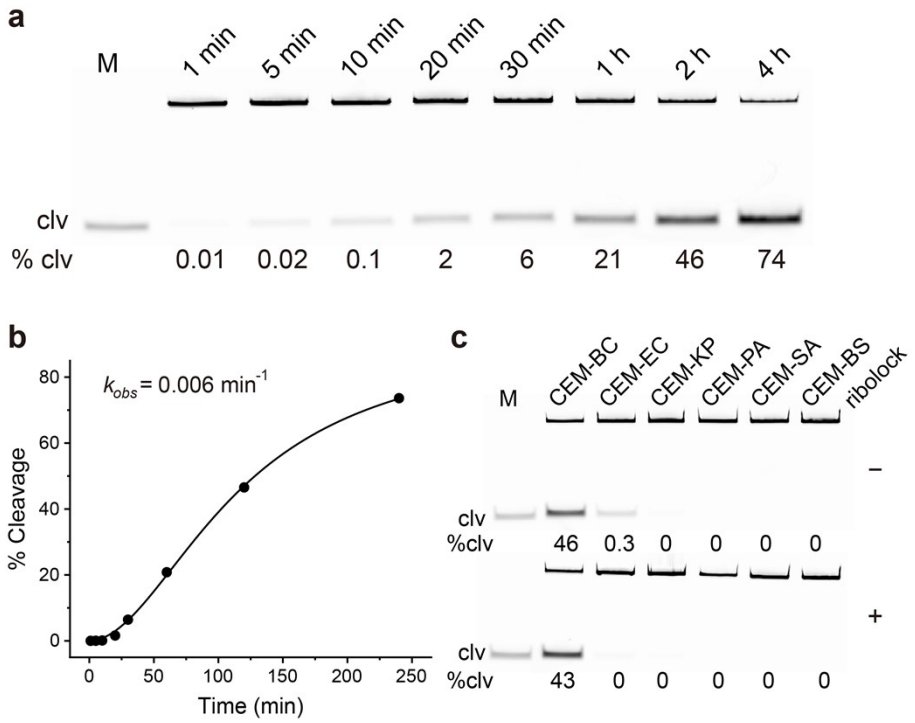


Fig. S6. (a) 10% dPAGE analysis of the kinetic responses of RFD-BC1 (without internal F and Q modifications) to CEM-BC. RFD-BC1 was labelled with a fluorescein (F) at the 3' end. %clv = cleavage percentage. M = DNA marker, clv = cleaved. (b) Kinetic analysis. (c) Responses of RFD-BC1 to the CEMs from various bacteria, including *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), *Pediococcus acidilactici* (PA), *Staphylococcus aureus* (SA), and *Bacillus subtilis* (BS) in the absence and presence of ribolock. The reaction time for all the cleavage reactions was 2 h.

Experimental details: The cleavage reactions were carried out in 1× RB (50 mM acetate, 15 mM $MgCl_2$, 0.01% Tween 20, pH 5.0). The protocol was similar to the one described in **A.2.3.** and **A.2.10.**

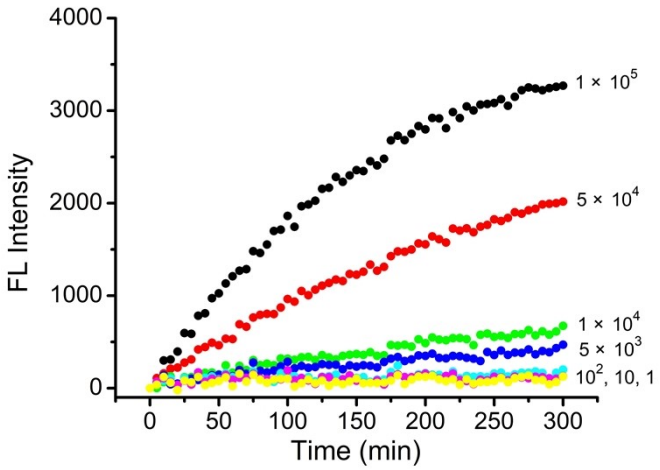


Fig. S7. Real-time fluorescence monitoring of RFD-BC1 in the presence of CEM-BC prepared from different numbers of BC cells.

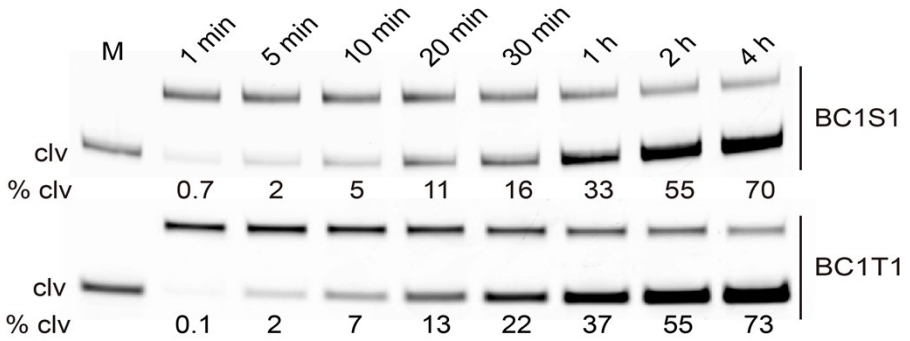


Fig. S8. 10% dPAGE analysis of the kinetic responses of RFD-BC1S1 and RFD-BC1T1 to CEM-BC. clv = cleaved. M = DNA marker.

Experimental details: The cleavage reactions were carried out in 1× RB (50 mM acetate, 15 mM $MgCl_2$, 0.01% Tween 20, pH 5.0). The protocol was similar to the one described in **A.2.3.**

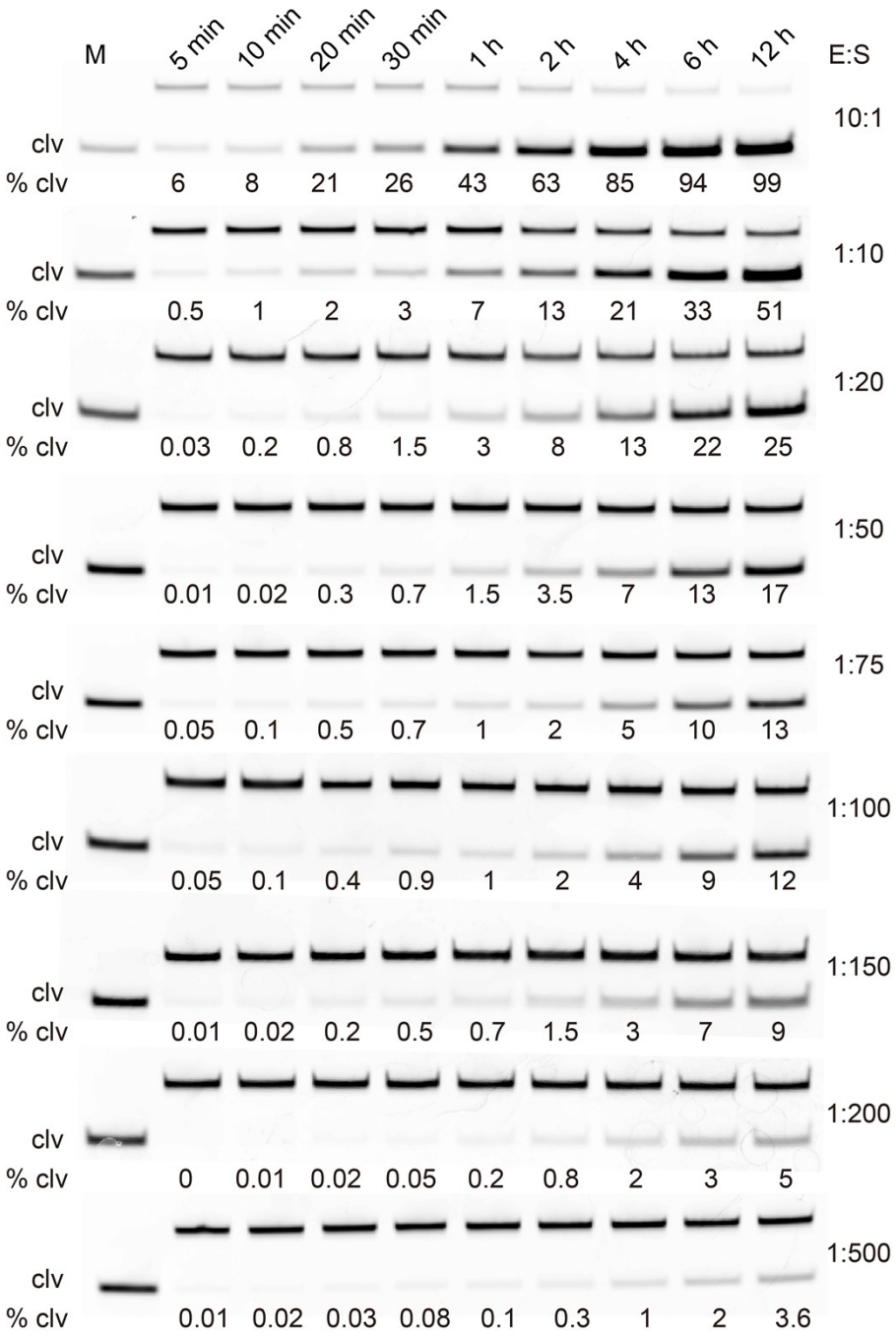


Fig. S9. 10% dPAGE analysis of the kinetic responses of single and multiple turnover assays with RFD-BC1T1 (E) and various concentrations of RDS (S) in the presence of CEM-BC. clv = cleaved. M = DNA marker.

Experimental details: The cleavage reactions were carried out in 1× RB (50 mM acetate, 15 mM $MgCl_2$, 0.01% Tween 20, pH 5.0). The protocol was similar to the one described in **A.2.3.**

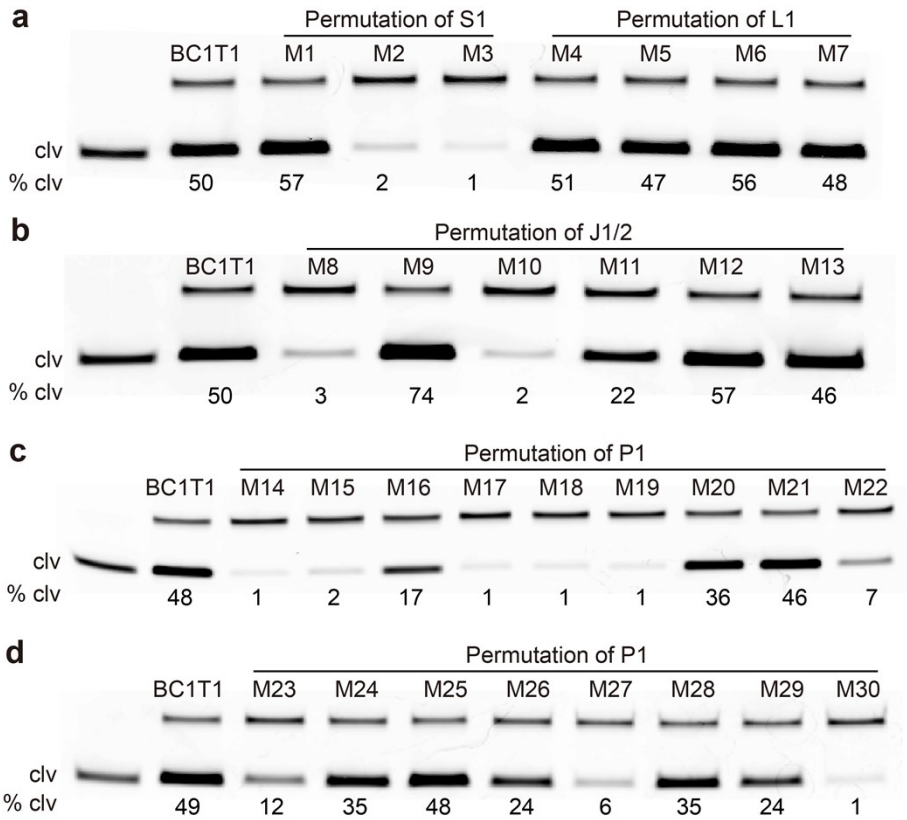


Fig. S10. 10% dPAGE analysis of RFD-BC1T1 with permutated S1, L1, J1/2, and P1 to CEM-BC. clv = cleaved. M = DNA marker.

Experimental details: The cleavage reactions were carried out in 1× RB (50 mM acetate, 15 mM $MgCl_2$, 0.01% Tween 20, pH 5.0). The protocol was similar to the one described in **A.2.3.**

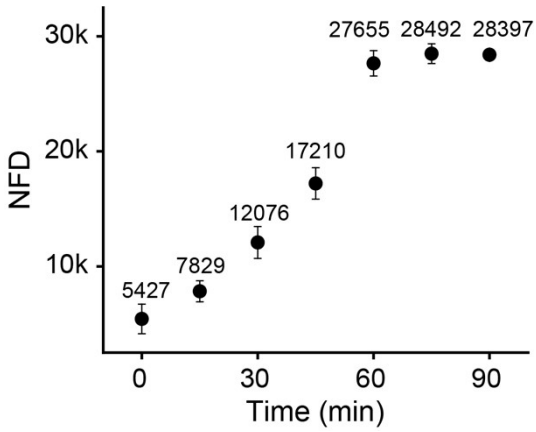


Fig. S11. Kinetic analysis of trans-acting RFD-BC1T1/RSD in droplets.

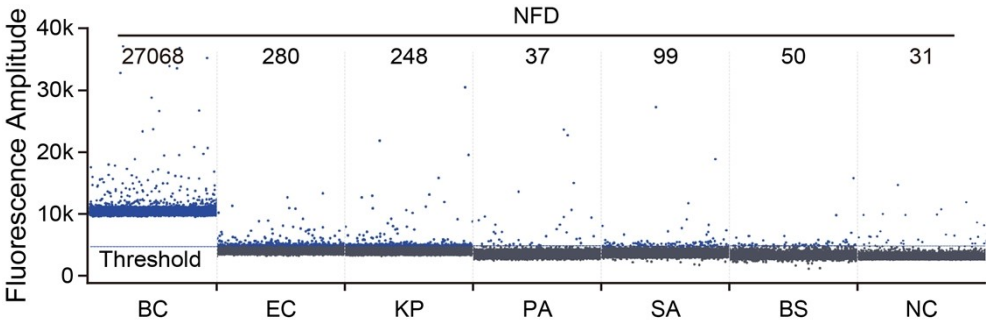
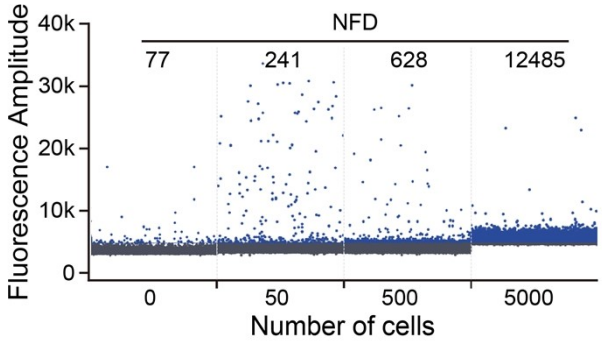


Fig. S12. Specificity of DID assay for various bacteria. EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, PA: *Pediococcus acidilactici*, SA: *Staphylococcus aureus*, and BS: *Bacillus subtilis*.

a



b

Samples	Added (CFU)	Recovery (%)	RSD (%)
Tremella	50	85.1-97.4	7.07
	500	94.2-107.6	7.27
	5000	76.7-92.6	9.54

Fig. S13. a) The fluorescence response of DID assay to various concentrations of BC cells spiked in tremella sample. b) Recovery (%) and relative standard deviation (RSD) of the DID assay.