Supplemental data to
A type III CRISPR-Cas system mediates co-transcriptional DNA cleavage at the transcriptional bubbles in close proximity to active effectors

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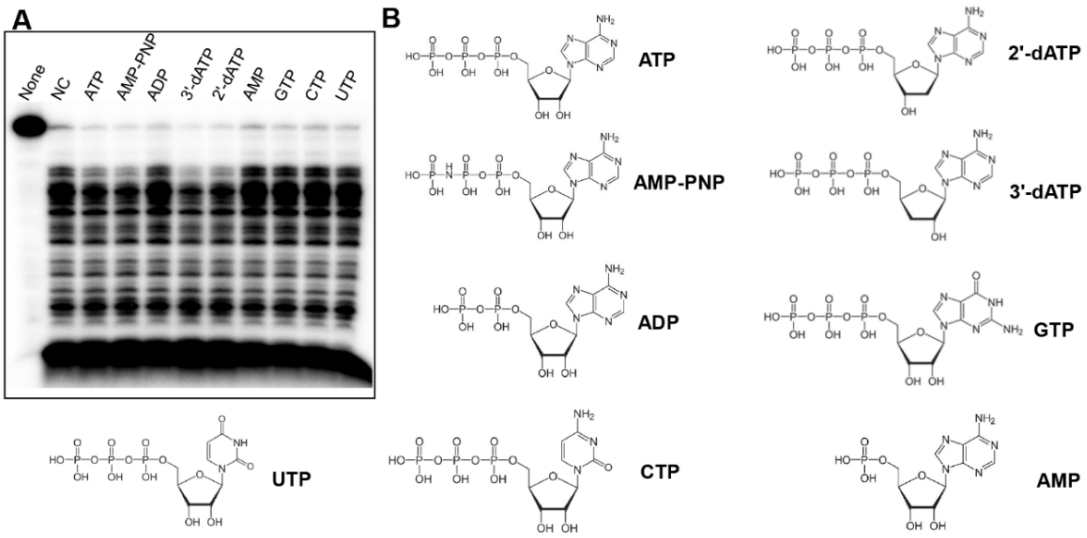


Figure S1 Effect of ATP and its analogues on the ssDNase Activity of LdCsm complex. (**A**) RNA-activated ssDNA cleavage of LdCsm in the presence of ATP or its analogues. NC: Negative control without ATP. (**B**) Structures of ATP and its analogues.

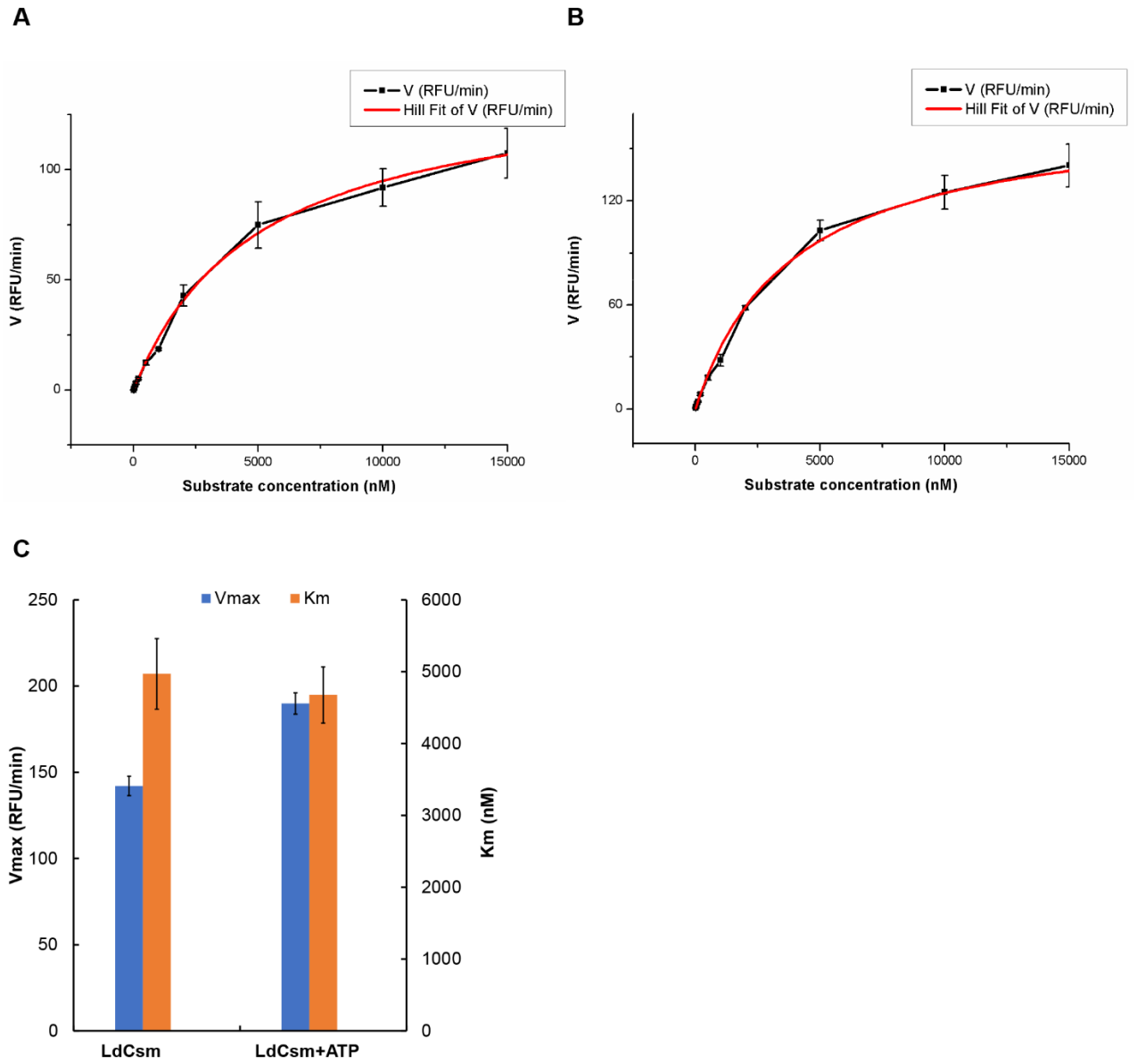


Figure S2 Michaelis-Menten modeling of ssDNA cleavage by LdCsm complex. (**A & B**) Michaelis-Menten modeling of LdCsm ssDNA cleavage in the absence (A) or presence (B) of ATP. The fluorescence production rates (V) were calculated for all tested substrate concentrations [S] and plotted against the substrate concentrations. The resulting curves were fitted with the Michaelis-Menten model. (**C**) The kinetic parameters of ssDNA degradation LdCsm with or without ATP. RFU: Relative Fluorescence Unit.

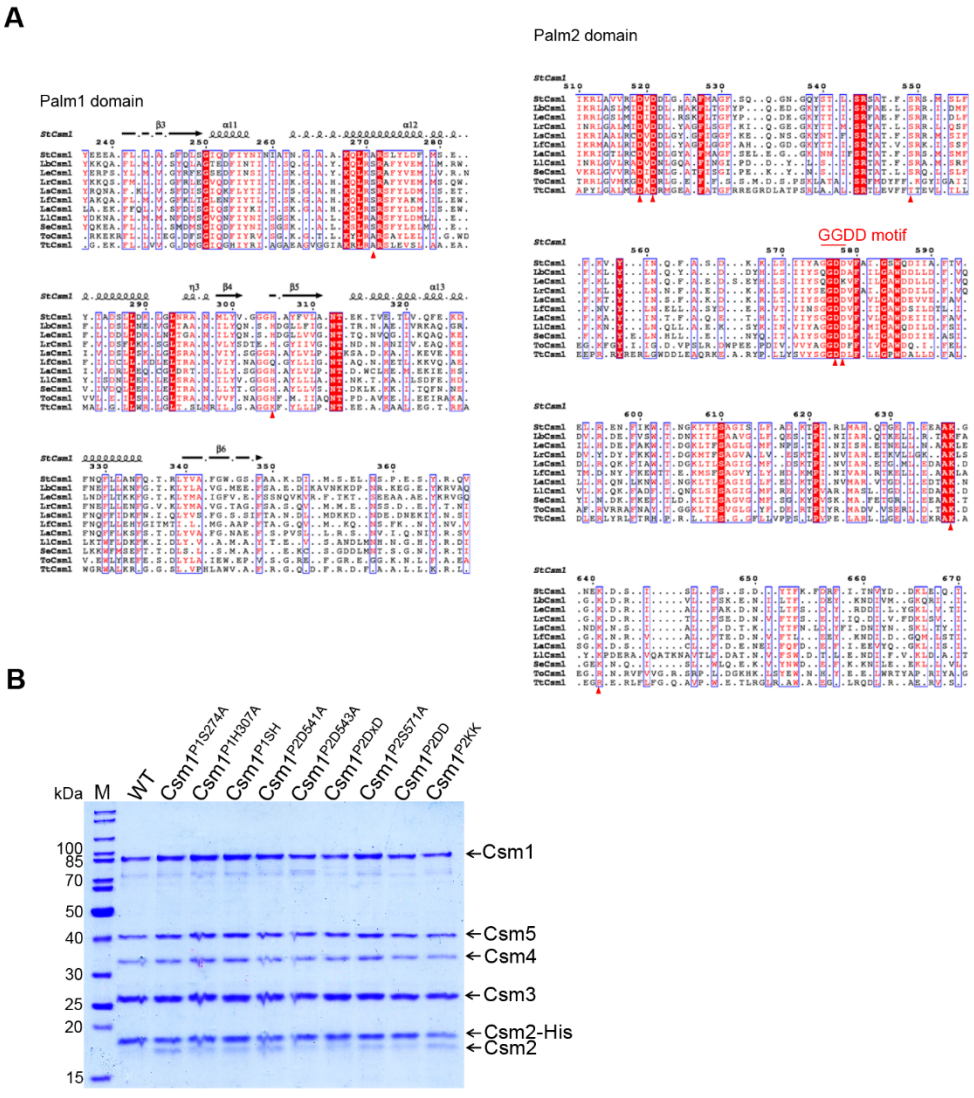
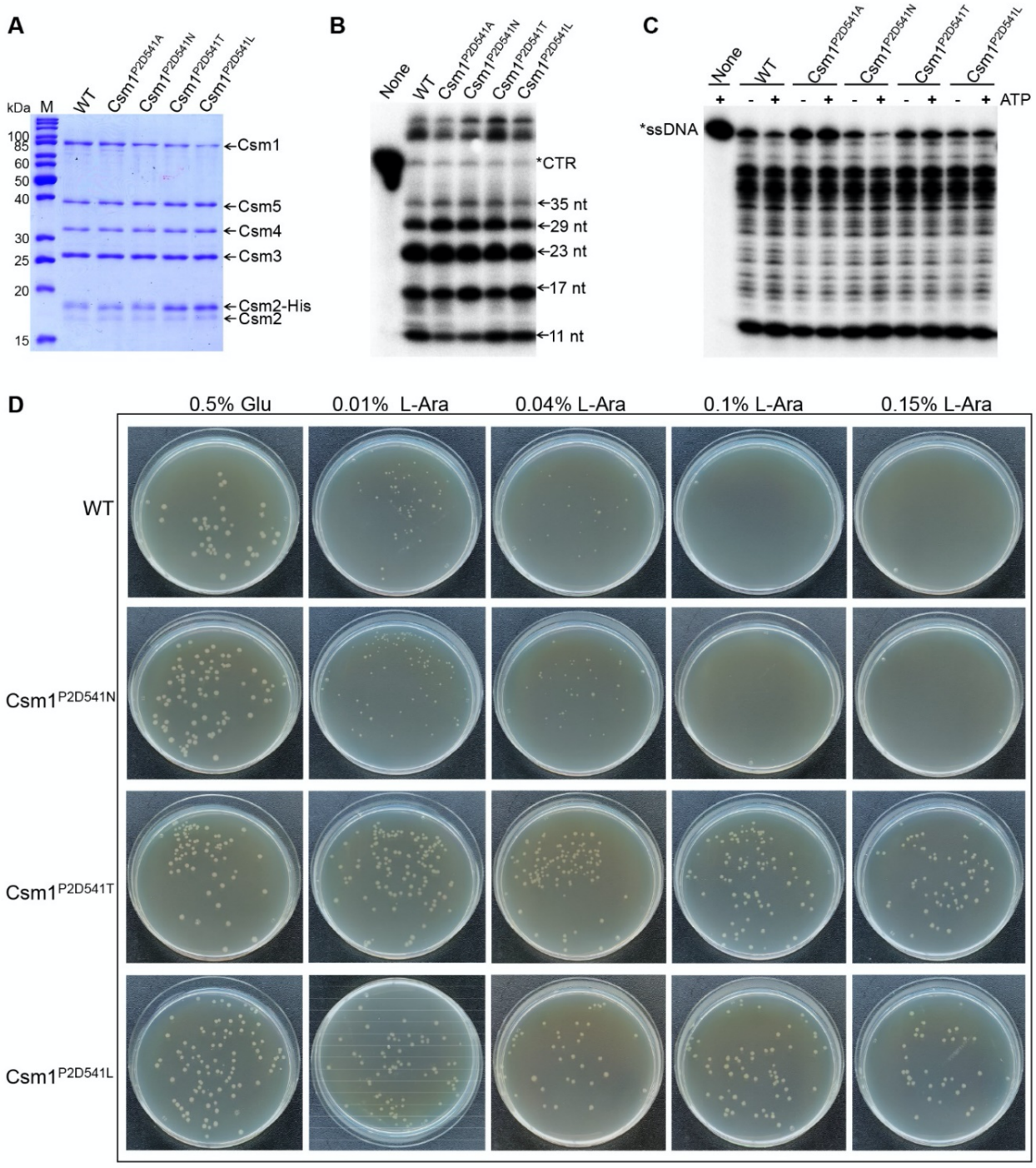


Figure S3 Purification of LdCsm1 mutations. (**A**) Conserved residues in LdCsm1. Ten Csm1 homologues: *Lactobacillus bulgaricus* (Lb), *Streptococcus thermophilus* (St), *Lactobacillus equicursoris* (Le), *Lactobacillus ruminis* (Lr), *Lactobacillus salivarius* (Ls), *Lactobacillus acidipiscis* (La), *Lactococcus lactis* subsp. *lactis* (Ll), *Staphylococcus epidermidis* (Se), *Thermococcus onnurineus* (To) and *Thermus thermophilus* (Tt) were selected and aligned using MEGA5 (1) and visualized using ESPript 3 (2). Some highly conserved residues marked with red arrows were chosen to produce the LdCsm1 variants. (**B**) Coomassie blue-stained SDS-PAGE gel of protein components of the wild-type and mutated LdCsm1 effector complexes. M: protein size marker.


A
kDa
M
WT
Csm1P2D541A
Csm1P2D541N
Csm1P2D541T
Csm1P2D541L
100
85
60
50
40
30
25
20
15
←Csm1
←Csm5
←Csm4
←Csm3
←Csm2-His
←Csm2
B
None
WT
Csm1P2D541A
Csm1P2D541N
Csm1P2D541T
Csm1P2D541L
*CTR
←35 nt
←29 nt
←23 nt
←17 nt
←11 nt
C
None
WT
Csm1P2D541A
Csm1P2D541N
Csm1P2D541T
Csm1P2D541L
+ - + - + - + - + - + ATP
*ssDNA
D
0.5% Glu
0.01% L-Ara
0.04% L-Ara
0.1% L-Ara
0.15% L-Ara
WT
Csm1P2D541N
Csm1P2D541T
Csm1P2D541L

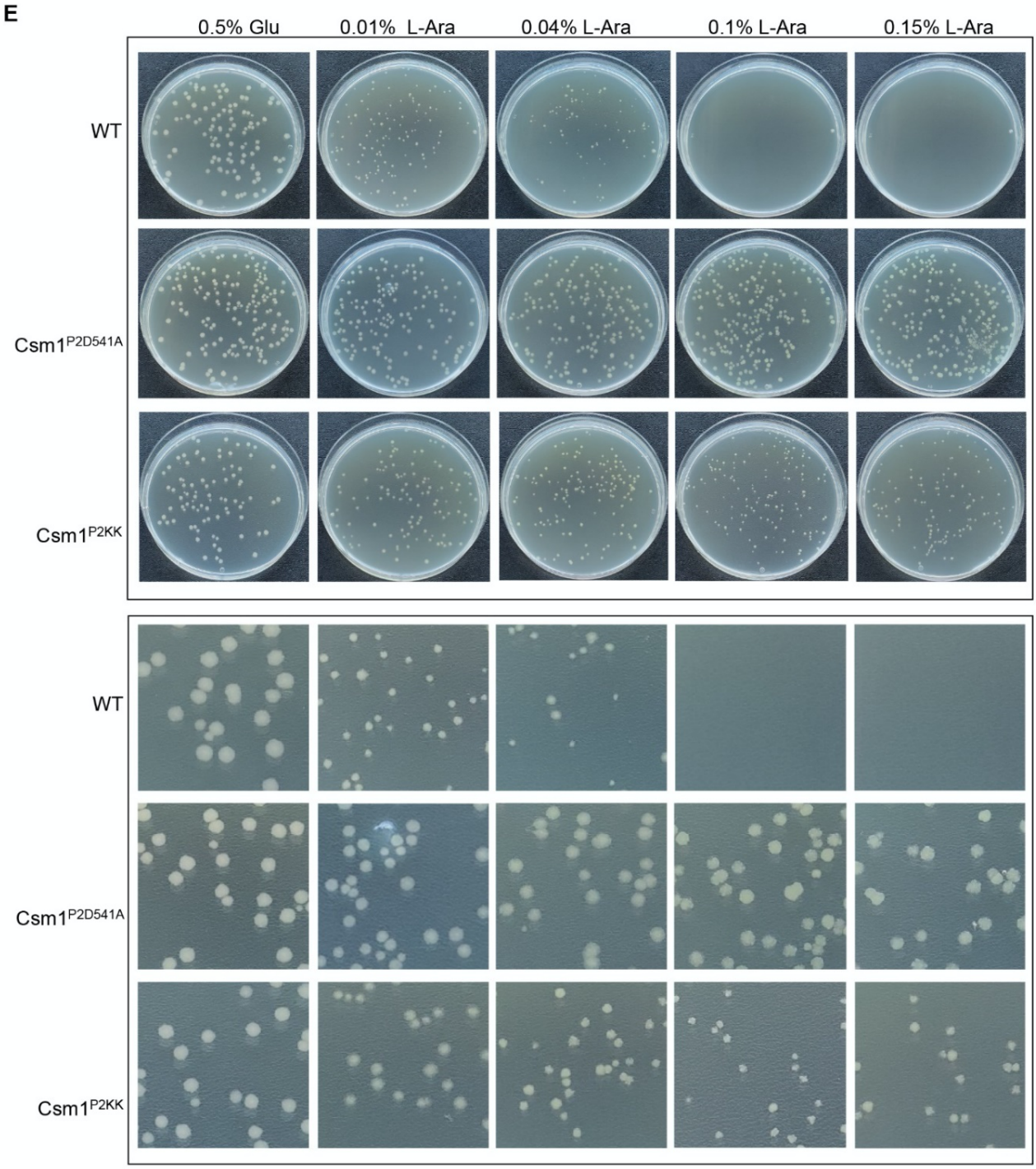


Figure S4 Effect of LdCsm1 D541 mutations on the target RNA cleavage, ssDNA cleavage and the *in vivo* anti-plasmid activity of the LdCsm system. (**A**) SDS-PAGE analysis of protein components of LdCsm effectors carrying LdCsm1 D541 mutations, M: protein size marker. (**B**) Effect of LdCsm1 D541 mutations on the target RNA cleavage. (**C**) Effect of LdCsm1 D541 mutations on the RNA-activated ssDNA cleavage in the presence of ATP. (**D**) Effect of LdCsm1 D541 mutations on the anti-plasmid activity. (**E**) Effect of other Palm 2 mutation of LdCsm1 on the anti-plasmid activity. LdCsm1 mutations tested: D541N ($Csm1^{P2D541N}$), D541L ($Csm1^{P2D541L}$), D541T ($Csm1^{P2D541T}$), D541A ($Csm1^{P2D541A}$) and K659/K663A ($Csm1^{P2KK}$).

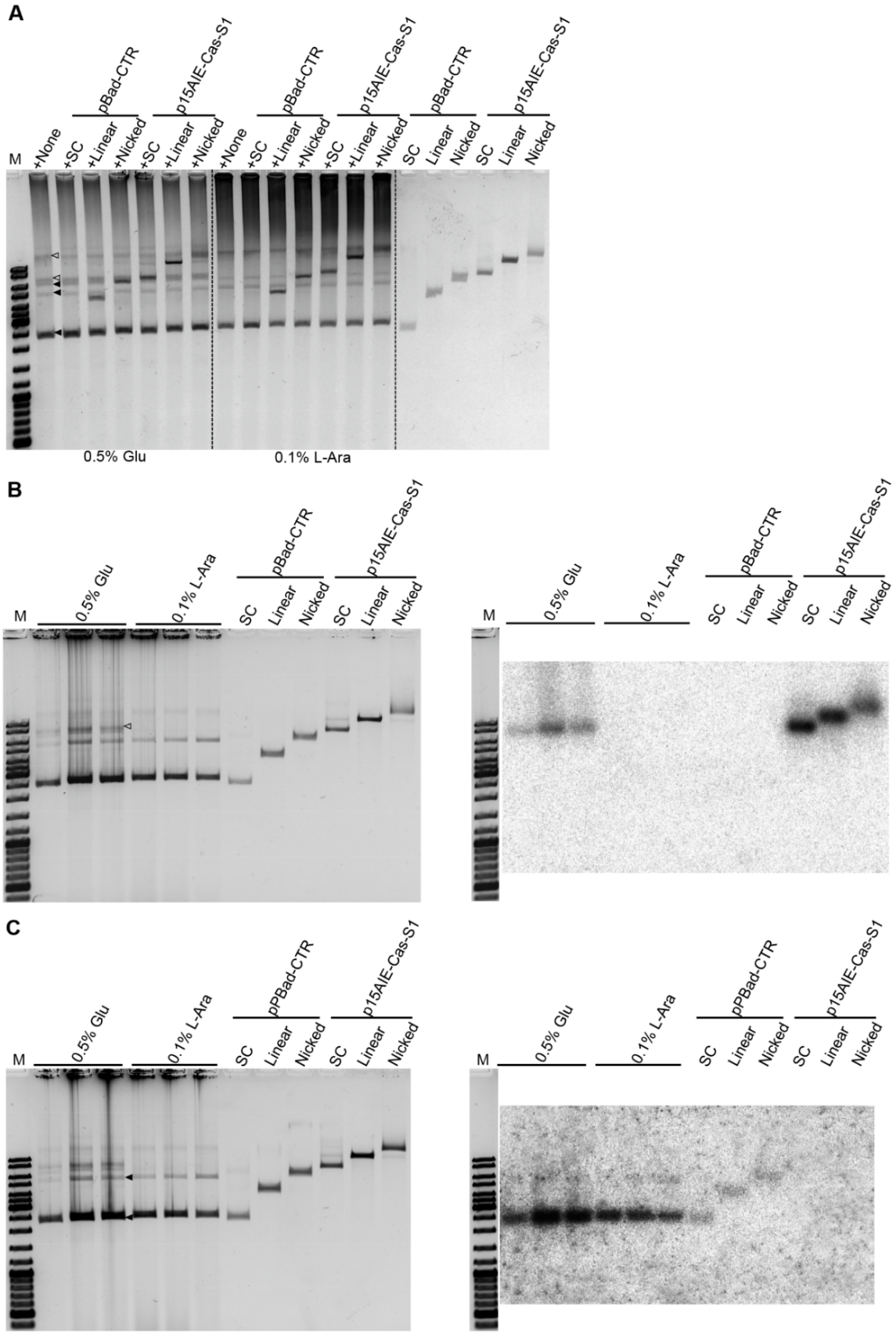


Figure S5 Detection of p15AIE-Cas-S1 and pBad-CTR in minipreparation assay. (**A**) Agarose gel analysis of plasmid DNAs mini prepared from *E. coli* colonies addition with different pattern of p15AIE-Cas-S1 and

pBad-CTR. The plasmid DNAs were prepared from 0.5% glucose and 0.1% L-arabinose colonies, respectively. SC, supercoiled. Empty triangles represent supercoiled (bottom) and nicked (top) of p15AIE-Cas-S1, filled triangles represent supercoiled (bottom) and nicked (top) of pBad-CTR, the middle triangle represent a unclassified band of pBad-CTR, which be indicated by southern blotting in (**C**). (**B & C**) Southern blotting analysis of p15AIE-Cas-S1 and pBad-CTR. **Left panel**, Gel-Red® staining before transfer. Empty triangle represents p15AIE-Cas-S1 band in (**B**), filled triangles represent pBad-CTR bands in (**C**). **Right panel**, southern blotting analysis of p15AIE-Cas-S1 and pBad-CTR, respectively. Empty triangles indicate the plasmid p15AIE-Cas-S1, filled triangles indicate the plasmid pBad-CTR. SC, supercoiled.

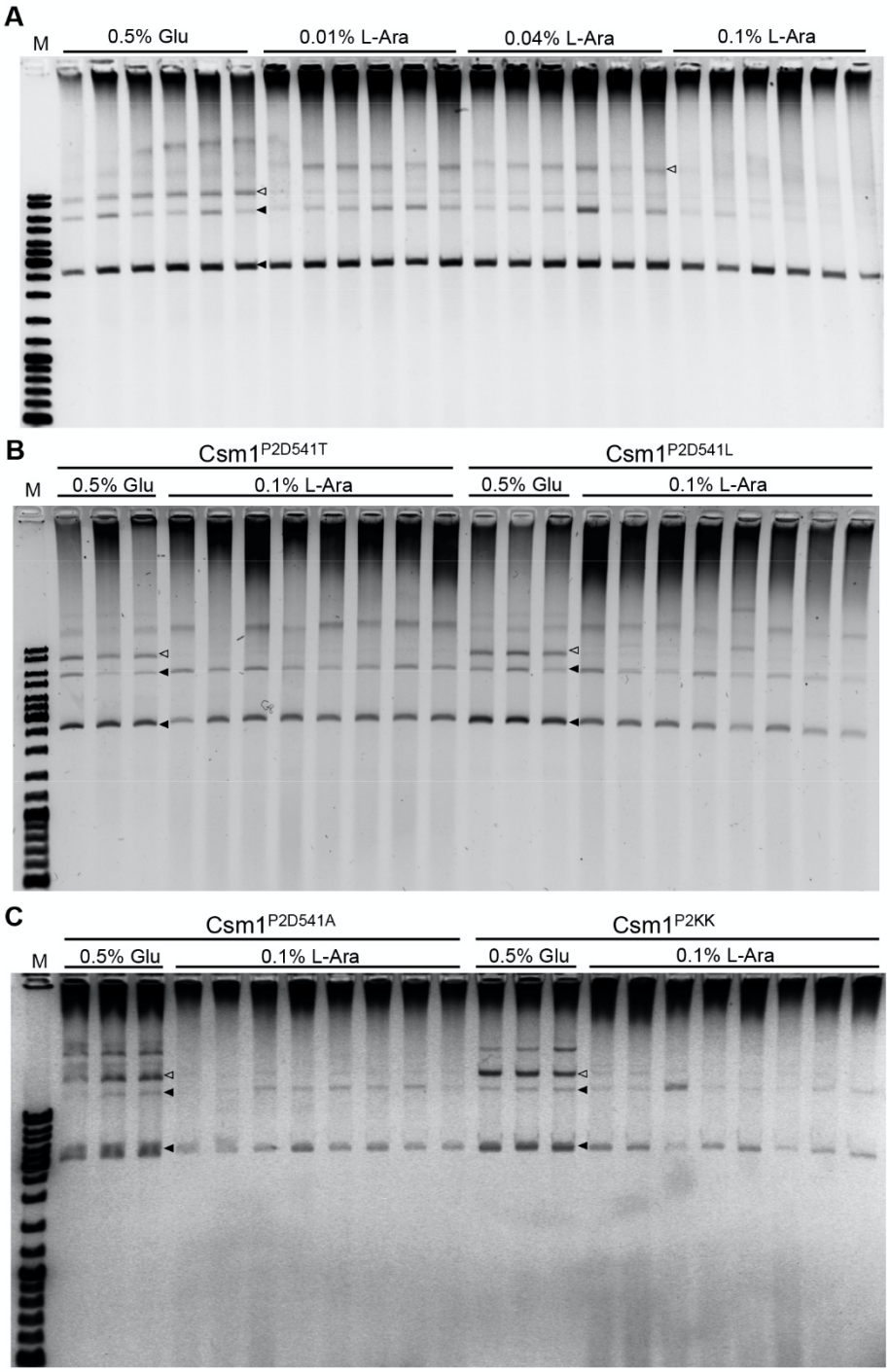


Figure S6 Anti-plasmid efficiency of the wild-type and LdCsm1-mutated LdCsm systems. The wild-type (**A**) and LdCsm1 mutated (**B & C**) LdCsm systems mediate efficient plasmid clearance preferably to nontarget plasmid in *E. coli*. The *E. coli* colonies of $Csm1^{P2D541T}$, $Csm1^{P2D541L}$, $Csm1^{P2D541A}$ and $Csm1^{P2KK}$ from Figure S4 were employed for plasmid minipreparation. Empty triangles denote p15AIE-Cas-S1, the nontarget plasmid; filled triangles indicate pBad-CTR, the target plasmid.

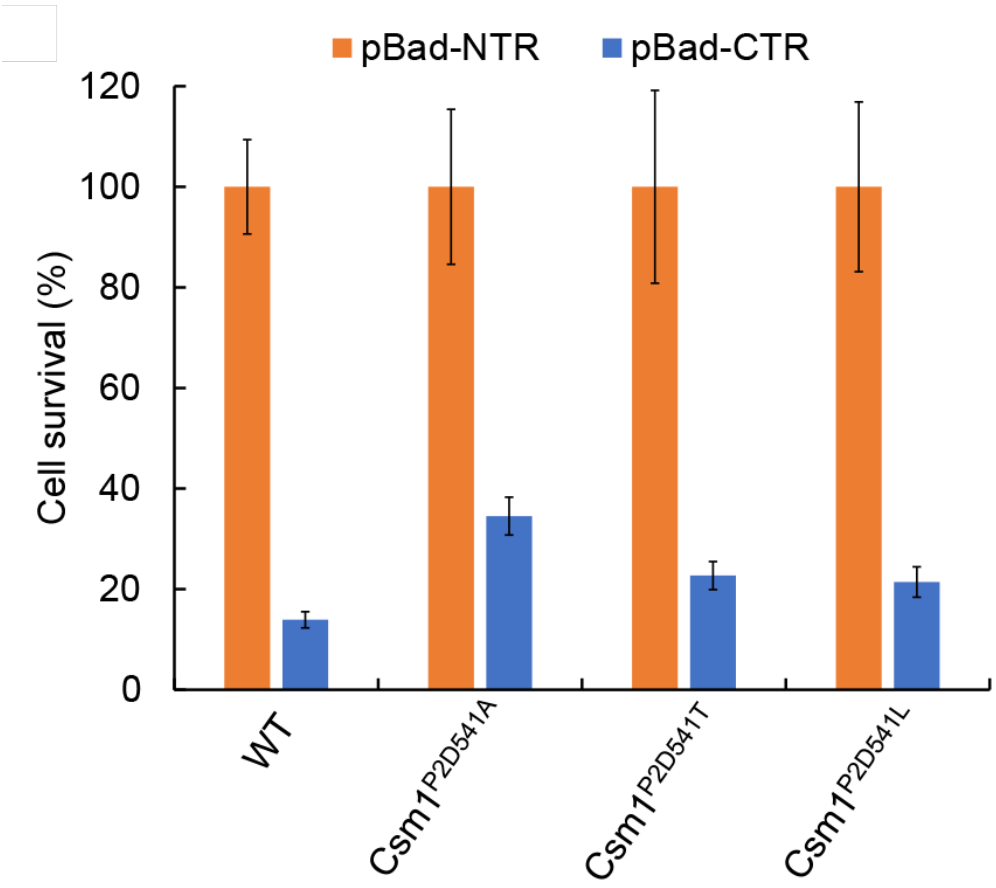


Figure S7 Survival rates of *E. coli* cells in the stepwise induction cultures Viable cells were determined for 120 min samples in **Figure 5A and 5B**. Viable cell counts with pBad-NTR were set up to 100% for each LdCsm effector, with which relative survival rates were calculated. Values shown are averages of three independent assays, bars representing the standard deviations.

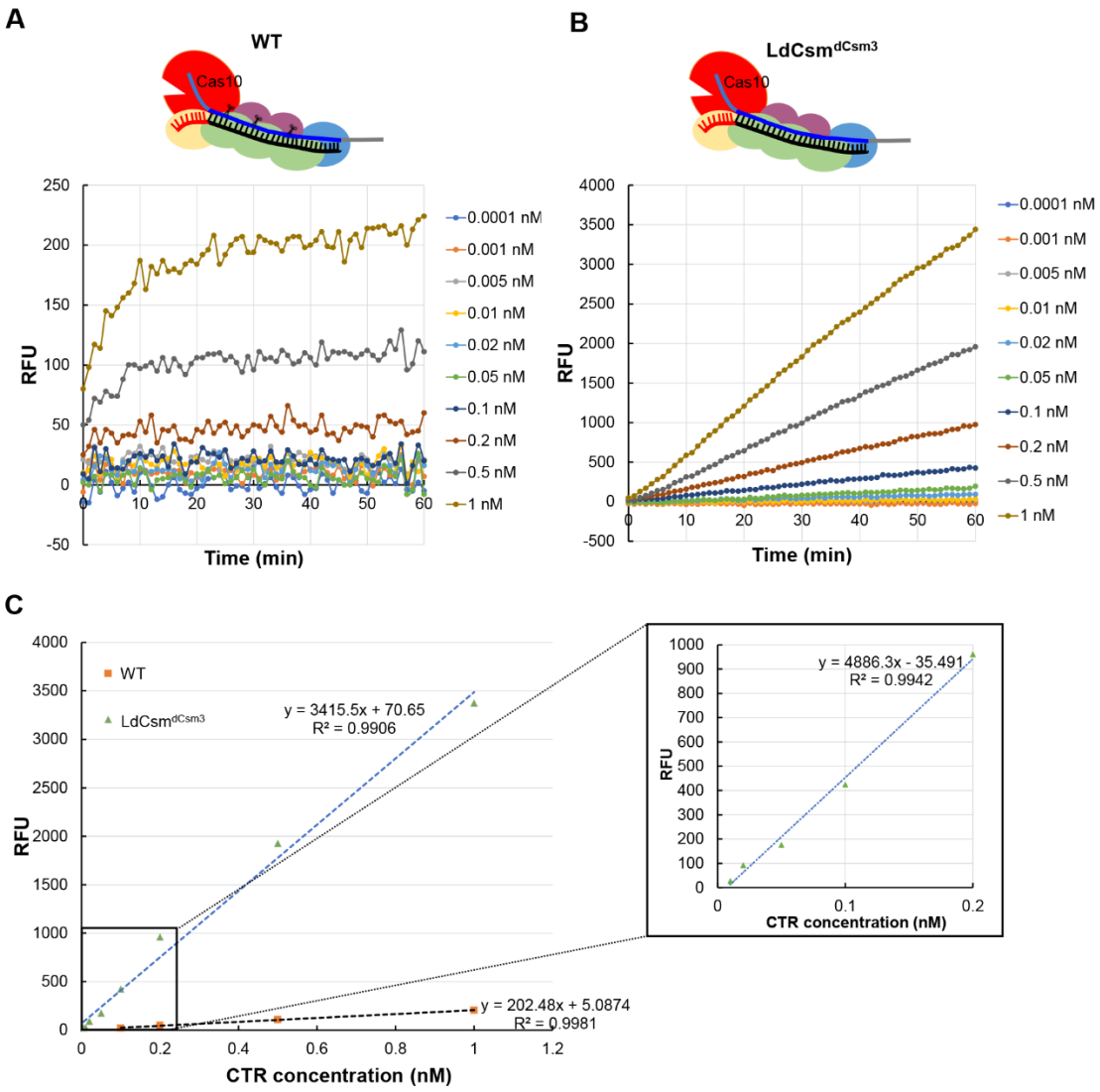


Figure. S8 LdCsm^{dCsm3} system may provide a potential platform for specific RNA quantification. Quantified time-course fluorescence signal generated by WT LdCsm (**A**) and LdCsm^{dCsm3} (**B**) effectors with varying concentrations of S1-46 RNA (CTR). (**C**) The fluorescence accumulation exhibits a positively linear relation to the increase of target RNA concentrations in a large range (0.01 nM to 1 nM for LdCsm^{dCsm3} effector).

Table S1 Nucleic acid oligo and substrates used in this study.

ssDNA and RNA	Sequence (5'-3')	Description
oligos		
Re-LPwt-F	GCTAACAACTTATCTCCGCTAGAAGGAGACGAGAACGCAGCACTGAAGGGCGGAAAC	For plasmid pUCE-LPwt construction
Re-LCwt-F	GCTAACAACTTATCTCCGCTAGAAGGAGACGAGAACGCGGCACTGAAGGGCGGAAAC	For plasmid pUCE-LCwt construction
Re-LPd-F	GCTAACAACTTATCTCCGCTAGAAGGAGACGAGAACGCACCACTGAAGGGCGGAAAC	For plasmid pUCE-LPd construction
Re-LCd-F	GCTAACAACTTATCTCCGCTAGAAGGAGACGAGAACGCGCCACTGAAGGGCGGAAAC	For plasmid pUCE-LCd construction
Re-L-R	GTTCTCGTCTCCTTCTAGCGGAGATAAGTTGTTAGCGAATGCTAAGT	For plasmids pUCE-LPwt, pUCE-LCwt, pUCE-LPd, pUCE-LCd construction
L-R1	TGTTAGCGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGG	For plasmids pUCE-LPwt, pUCE-LCwt, pUCE-LPd, pUCE-LCd construction
Csm1-S274A-F	AGCTGCGGGCCCGGGCATTTTACGTTG	For LdCsm1 S274A mutation
Csm1-S274A-R	ATGCCCGGGCCCGCAGCTGCTTGTAAGCTC	For LdCsm1 S274A mutation
Csm1N-H307A-F	CAGAACAGCGCTGATGGTCTGCTTTTTATTG	For LdCsm1 H307A mutation
Csm1N-H307A-R	GACCATCAGCGCTGTTCTGGTAAAGGATATTC	For LdCsm1 H307A mutation
Csm1N-D541A-F	TTGATGATCGCTATTGATGACCTCCATGCCAA	For LdCsm1 D541A mutation
Csm1N-D541A-R	CATCAATAGCGATCATCAAAGAAGCAAGC	For LdCsm1 D541A mutation
Csm1N-D543A-F	GATCGATATTGCTGACCTCCATGCCAAATTCTT	For LdCsm1 D543A mutation
Csm1N-D543A-R	GAGGTCAGCAATATCGATCATCAAAGAAG	For LdCsm1 D543A mutation
Csm1N-S571A-F	GGAACTTGCCAGAAGAATTGACATGTTC	For LdCsm1 S571A mutation
Csm1N-S571A-R	AATTCTTCTGGCAAGTTCCGCGAATCTGCTG	For LdCsm1 S571A mutation
Csm1N-K659/663A-F	CAGCATTTGCCGGCGCGGACCGGATTGTGTTGTTC	For LdCsm1 K659/663A mutation
Csm1N-K659/663A-R	CCGCGCCGGCAAATGCTGCTGTTCTTAACAGTTCC	For LdCsm1 S571A K659/663A mutation
Csm3-D34A-F	GGTGCGATCGCCAACCCGATAATTAAGGATCC	For LdCsm3 D34A mutation
Csm3-D34A-R	ATCGGGTTGGCGATCGCACCAATAGCCGCA	For LdCsm3 D34A mutation
SalI-Cas6-F	AGGGTCGACGTGCAGAAGATAAGTTTAGTTTG	For LdCsm1 Palm1 mutations
SacI-Csm1-R	CTCTGGAAGAGCTCAGCATAG	For LdCsm1 Palm1 mutations
SacI-Csm1-F	CTATGCTGAGCTCTTCCAGAG	For LdCsm1 Palm 2 mutations
StuI-Csm1-R	GTAAATGAAGGCCTTACCTCG	For LdCsm1 Palm 2 mutations
StuI-Csm1-F	CGAGGTAAGGCCTTCATTTAC	For LdCsm3 D34A mutation
KpnI-Csm4-R	GACAGTGGTACCGCGTATAAGC	For LdCsm3 D34A mutation
Repeat-probe	GTTCTCGTCTCCTTCTAGCGGAGATAAGTTGTTAGC	For p15AIE-Cas-S1 southern blotting
pBR322-probe	TGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATT	For pBad-CTR southern blotting
Kan-probe	GCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAG	For pBad-CTR southern blotting
Nucleic acid		

substrates		
S10 RNA	AUAGAAUGCCCCCAUUAUACAAUAUCUACGUUUUAGAUGAAAAAAA	Unspecific RNA
S1-40 (PTR)	UGUUAAGUCUGGUUUCCCUCCAGGGUAUCUAAGCUUUGAA	Target RNA Lacking any 3'-flanking sequence
S1-46 (CTR)	UGUUAAGUCUGGUUUCCCUCCAGGGUAUCUAAGCUUUGAAAAAAAA	Target RNA with noncomplementary 3'-flanking sequence with crRNA
S1-48 (NTR)	UGUUAAGUCUGGUUUCCCUCCAGGGUAUCUAAGCUUUGAAGUUCUCGU	Target RNA with complementary 3'-flanking sequence with crRNA
S10-60 ssDNA	ACTATAGGGAGAATAGAATGCCCCCATTATACAATATCTACGTTTTAGATGACCCCCCCC	Unspecific ssDNA substrate
FAM-poly-16T-BHQ1	FAM-TTTTTTTTTTTTTTTT-BHQ1	fluorophore quencher ssDNA reporter
Target dsDNA (non-template strand)	TAATACGACTCACTATAGGGAGATGTTAAGTCTGGTTTCCCTCCAGGGTATCTAAGCTTTGAACAAGAGCACCCGGGCACGCGTCTCCGG	Target dsDNA substrate in vitro
Nontarget dsDNA (non-template strand)	TCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGA	Nontarget dsDNA substrate in vitro

Mismatch RNAs	**Sequence (3'-5')**
Perfect target (PT)	**5'**-ACGAGAACUUCAAAGCUUAGAUACCCUGGAGG-**3' (crRNA)** |||||||||||||||||||||||||| AAAAAAAAGUUUCGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-1	AAAAAAUAGUUUCGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-2	AAAAAAAUGUUUCGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-3	AAAAAAAACUUUCGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-4	AAAAAAAAGAUUCGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-5	AAAAAAAAGUAUCGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-6	AAAAAAAAGUUACGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-7	AAAAAAAAGUUUGGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-8	AAAAAAAAGUUUCCAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-9	AAAAAAAAGUUUCGUAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-10	AAAAAAAAGUUUCGAUUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-1-2	AAAAAAUUGUUUCGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-3-4	AAAAAAAACAUUCGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-5-6	AAAAAAAAGUAACGAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-7-8	AAAAAAAAGUUUGCAAUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-9-10	AAAAAAAAGUUUCGUUUCUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-11-12	AAAAAAAAGUUUCGAAAGUAUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-13-14	AAAAAAAAGUUUCGAAUCAUUGGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-15-16	AAAAAAAAGUUUCGAAUCUAACGGACCUCCCUUUGGUCUGAAUUGUGG
SS1-17-18	AAAAAAAAGUUUCGAAUCUAUGCCACCUCCCUUUGGUCUGAAUUGUGG
SS1-19-20	AAAAAAAAGUUUCGAAUCUAUGGGUGCUCCCUUUGGUCUGAAUUGUGG
SS1-21-22	AAAAAAAAGUUUCGAAUCUAUGGGACGACCCUUUGGUCUGAAUUGUGG
SS1-23-24	AAAAAAAAGUUUCGAAUCUAUGGGACCUGGCUUUGGUCUGAAUUGUGG

Table S2 Strains and plasmids utilized in this work.

Strains	**Reference**	**Description**
Lactobacillus delbrueckii subsp. *bulgaricus* ND04	A donation from (LABCC, IMAU)	http://www.bio149.cn/
Escherichia coli JM109	Home-made	N/A
E. coli BL21 (DE3)	Home-made	N/A
Plasmids	**Reference**	**Description**
p15AIE-Cas	Lin et al. (3)	N/A
p15AIE-Cas(Csm1^{P1S274A})	This study	A LdCsm1 mutant carrying S274A substitution in Palm1 domain
p15AIE-Cas(Csm1^{P1H307A})	This study	A LdCsm1 mutant carrying H307A substitution in Palm1 domain
p15AIE-Cas(Csm1^{P1SH})	This study	A LdCsm1 mutant carrying S274A and H307A substitution in Palm1 domain
p15AIE-Cas(Csm1^{P2D541A})	This study	A LdCsm1 mutant carrying D541A substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2D543A})	This study	A LdCsm1 mutant carrying D543A substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2DxD})	Lin et al. (3)	A LdCsm1 mutant carrying D541A and D543A substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2D541N})	This study	A LdCsm1 mutant carrying D541N substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2D541T})	This study	A LdCsm1 mutant carrying D541T substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2D541L})	This study	A LdCsm1 mutant carrying D541L substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2S571A})	This study	A LdCsm1 mutant carrying S571A substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2DD})	Lin et al. (3)	A LdCsm1 mutant carrying D599A and D600A substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2KK})	This study	A LdCsm1 mutant carrying K659A and K663A substitution in Palm2 domain
p15AIE-Cas^{dCsm3}	This study	A LdCsm3 mutant carrying D34A substitution
p15AIE-Cas-S1	Lin et al. (3)	N/A
p15AIE-Cas(Csm1^{P2D541A})-S1	This study	A LdCsm1 mutant carrying D541A substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2D541N})-S1	This study	A LdCsm1 mutant carrying D541N substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2D541T})-S1	This study	A LdCsm1 mutant carrying D541T substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2D541L})-S1	This study	A LdCsm1 mutant carrying D541L

		substitution in Palm2 domain
p15AIE-Cas(Csm1^{P2KK})-S1	This study	A LdCsm1 mutant carrying K659A and K663A substitution in Palm2 domain
pUCE	Lin et al. (3)	N/A
pUCE-S1	Lin et al. (3)	N/A
pUCE-LPwt	This study	A plasmid carrying spacer for *L. plantarum* 16S rRNA detection using WT LdCsm
pUCE-LCwt	This study	A plasmid carrying spacer for *L. casei* 16S rRNA detection using WT LdCsm
pUCE-LPd	This study	A plasmid carrying spacer for *L. plantarum* 16S rRNA detection using with LdCsm3 dead mutant
pUCE-LCd	This study	A plasmid carrying spacer for *L. casei* 16S rRNA detection using with LdCsm3 dead mutant
pET30a-Csm2	Lin et al. (3)	N/A
pBad-G	Lin et al. (3)	N/A
pBad-CTR	Lin et al. (3)	N/A
pBad-NTR	Lin et al. (3)	N/A

REFERENCE

1. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*, **28**, 2731-2739.
2. Robert, X. and Gouet, P. (2014) Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res*, **42**, W320-324.
3. Lin, J., Feng, M., Zhang, H. and She, Q. (2020) Characterization of a novel type III CRISPR-Cas effector provides new insights into the allosteric activation and suppression of the Cas10 DNase. *Cell Discov*, **6**, 29.