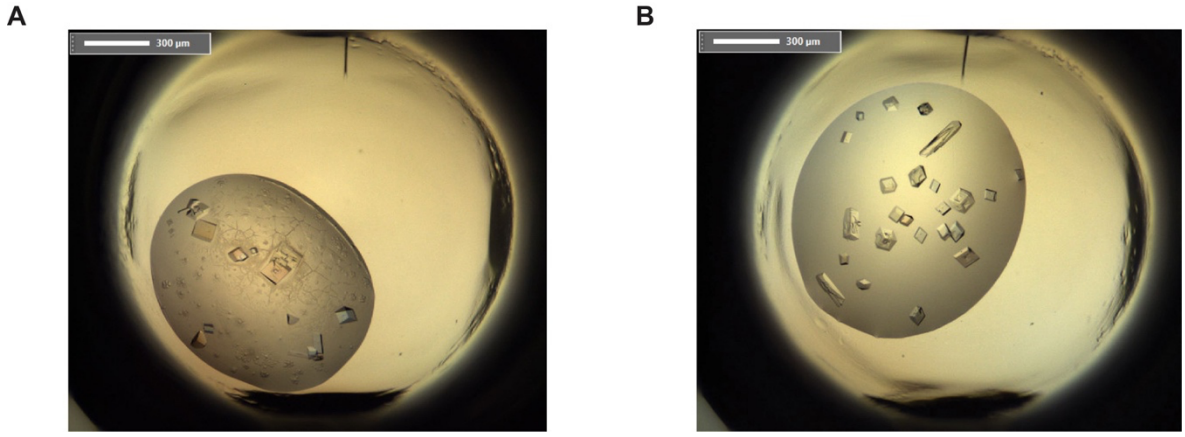
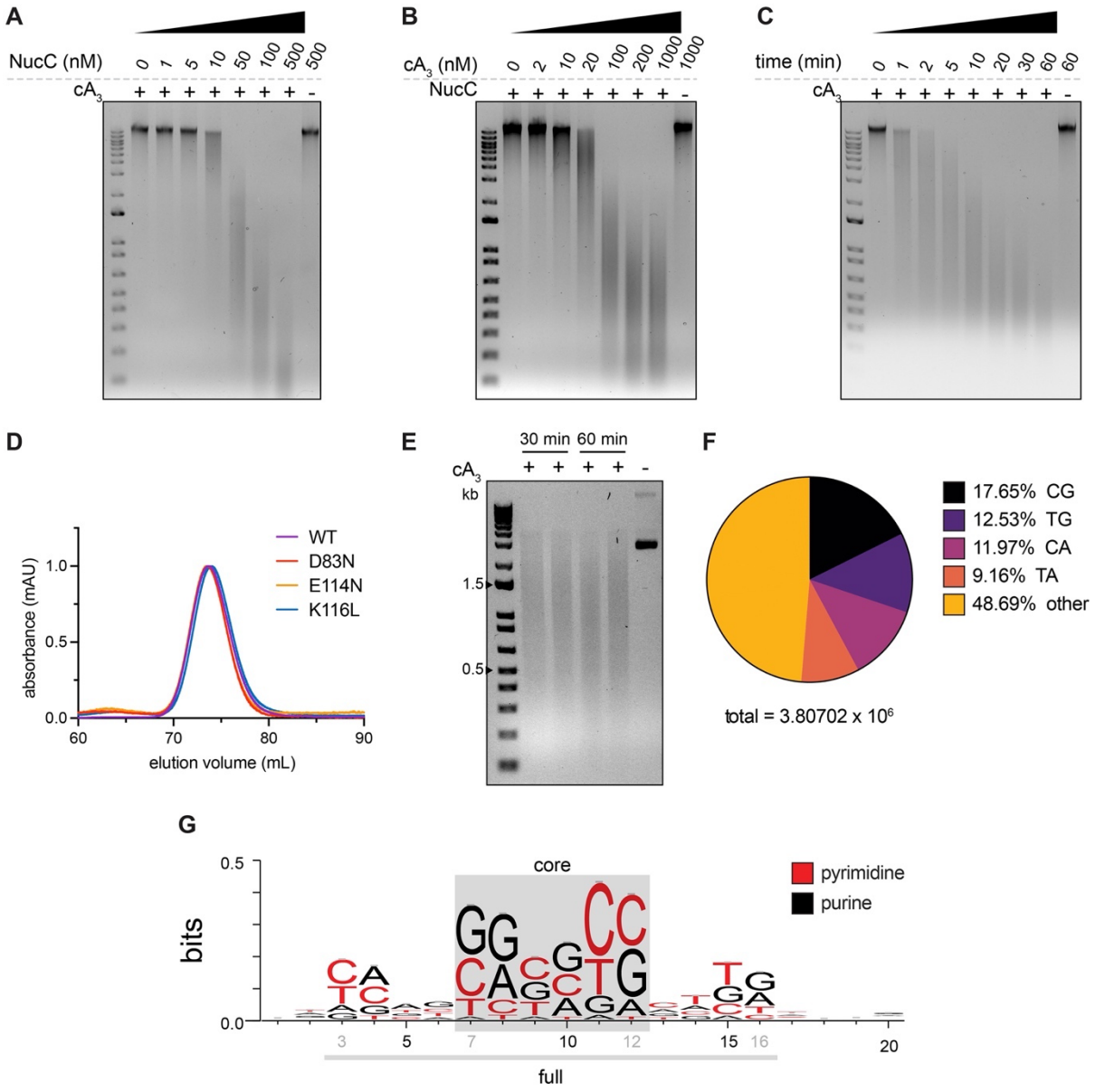
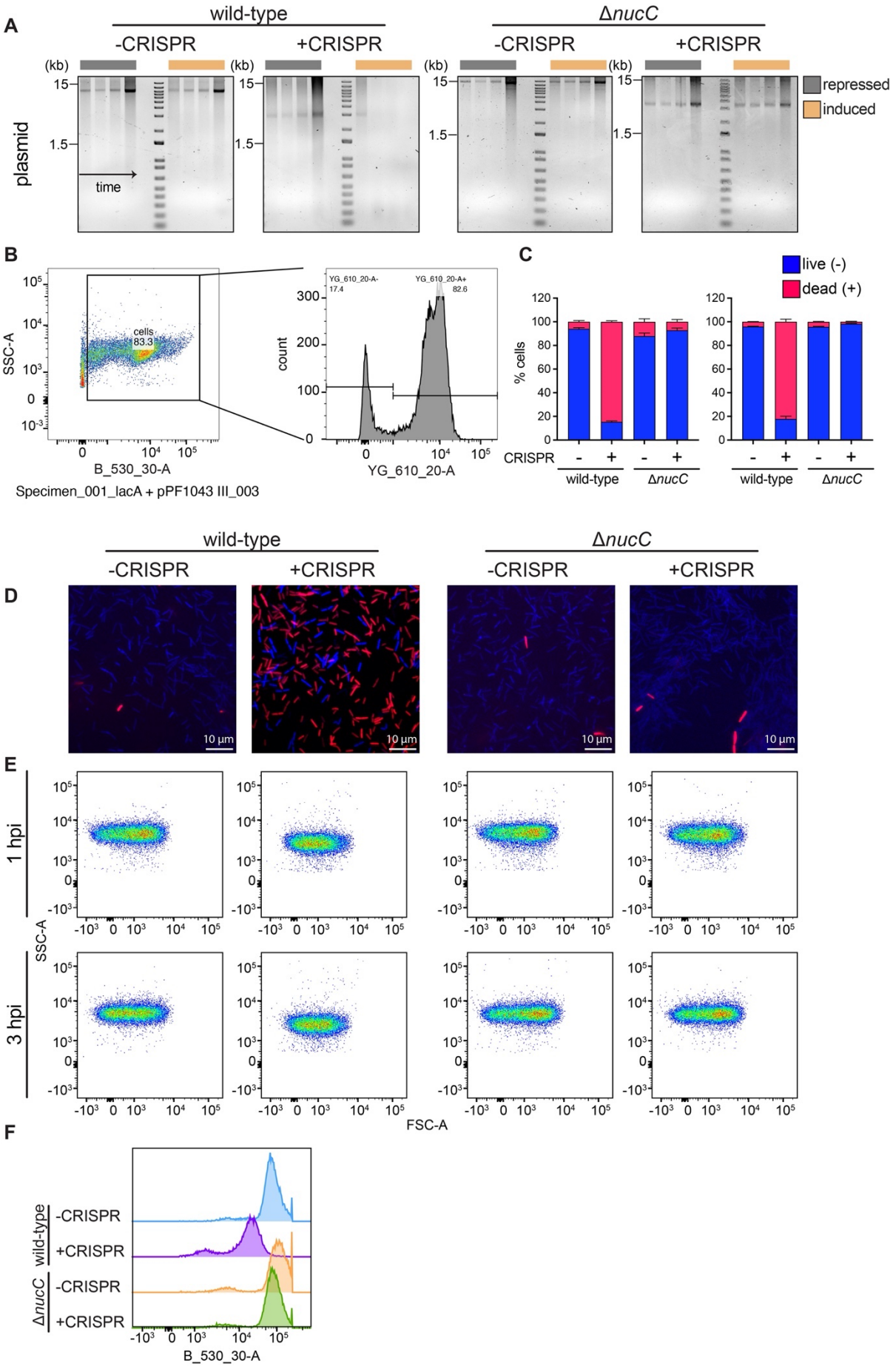
SUPPLEMENTARY MATERIAL



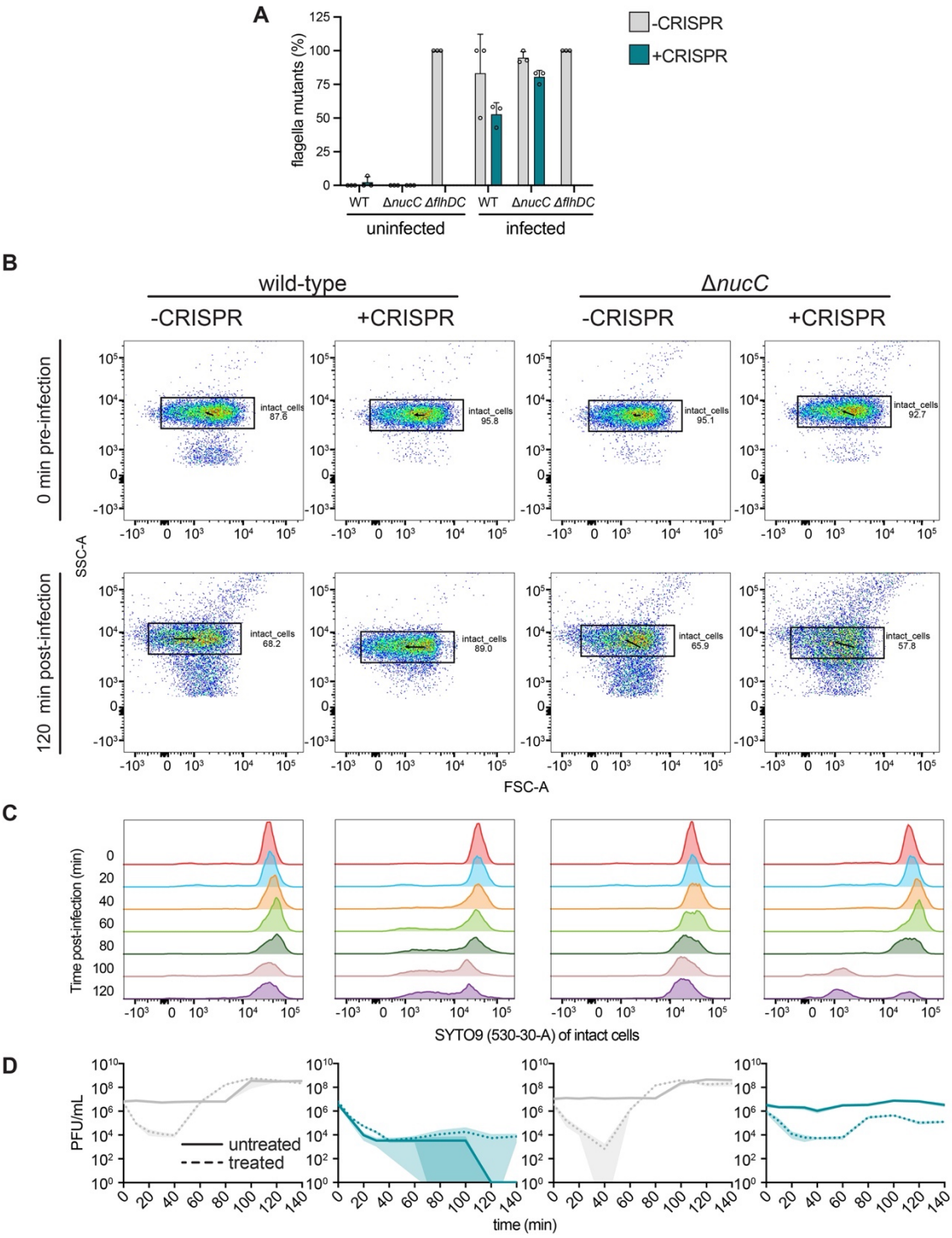
Supplementary Figure S1. NucC crystallization. (**A**) Crystallization of *Serratia* NucC apo form at 1.83 Å; NucC = 56 mg/mL, 0.1 M Na acetate pH 5.5, 10% PEG 8K, 10% PEG 1K, 0.8 M Na formate. (**B**) Co-crystallization of *Serratia* NucC bound to cA_3 at 1.48 Å; NucC = 24.5 mg/mL (+1.2x cA_3/trimer), 0.1 M Tris pH 7.5, 0.2 Ca acetate, 25% PEG MME 2K. Crystallographic information is reported in **Supplementary Table S1**.



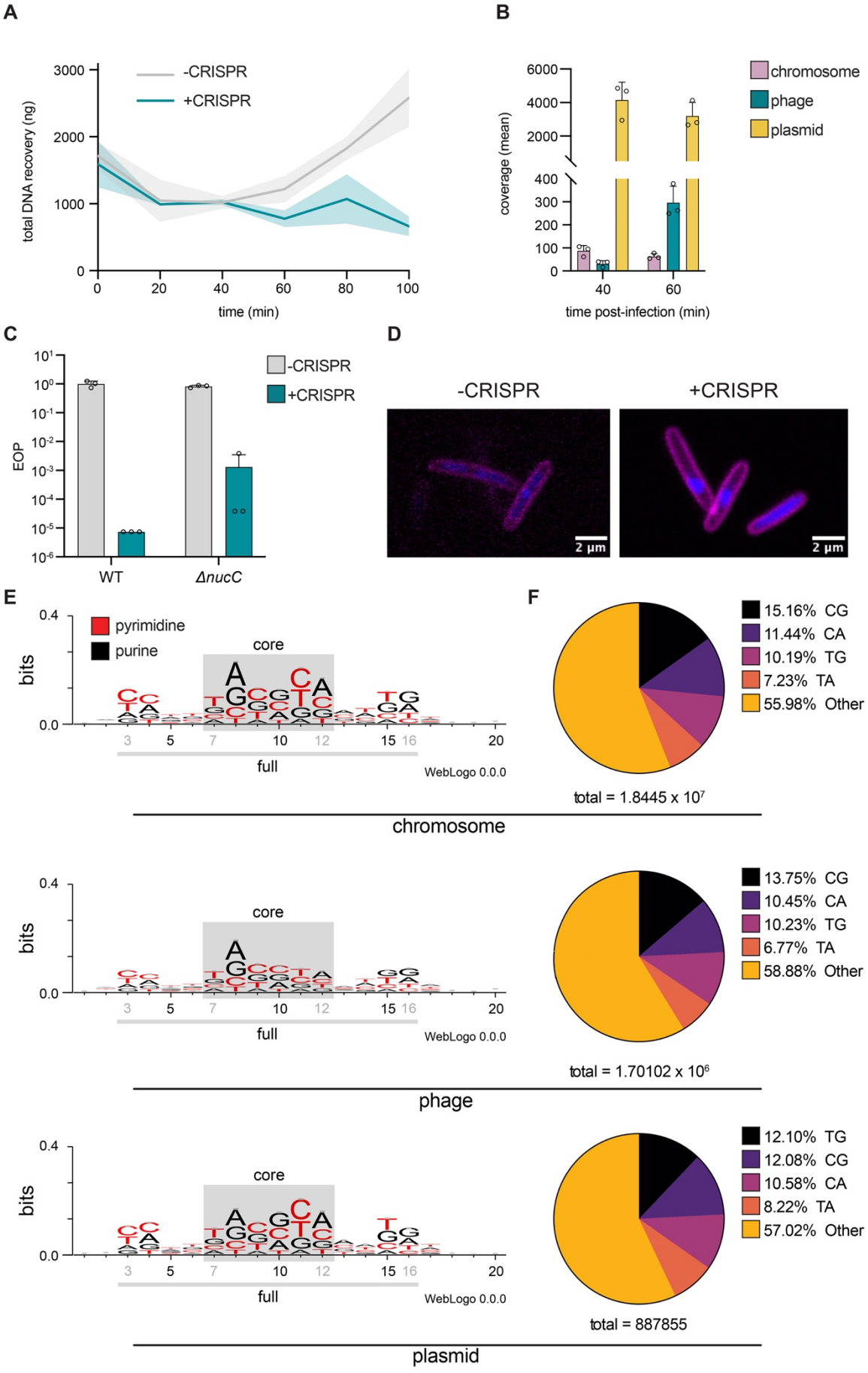
Supplementary Figure S2. *Serratia* NucC is activated by cA_3 and degrades double-stranded DNA *in vitro*. (**A**) *Serratia* gDNA cleavage assay with increasing concentrations of wild-type NucC in the presence (+, 1000 nM) or absence (-) of cA_3. (**B**) *Serratia* gDNA cleavage assay with increasing concentrations of the activator cA_3 in the presence (+, 100 nM) or absence (-) of wild-type NucC. (**C**) *Serratia* gDNA cleavage assay with wild-type NucC in the presence of cA_3 and increasing incubation times. (**D**) Size exclusion chromatogram of wild-type NucC (purple, pPF2513) and nuclease active site NucC mutants D83N (red, pPF2669), E114N (orange, pPF2671) and K116L (blue, pPF2673). (**E**) NucC cleavage of plasmid for deep sequencing of degradation products. Plasmid pPF1043 was incubated with NucC in the presence of cA_3 for 30 min or 60 min. (**F**) Nucleotide preferences at outermost motif positions (3 and 16). Four pyrimidine:purine combinations (C:G, T:G, C:A, T:A) at positions 3 and 16 of the predicted *in vitro* motif account for 51.31% of all sequences. (**G**) Cleavage site preference of NucC, represented as a WebLogo coloured according to pyrimidine (red) or purine (black) nitrogenous base nature. Mapping, per-base coverage and WebLogo summaries are reported in **Supplementary Tables S2**, **S3** and **S4**, respectively.


A
wild-type
ΔnucC
-CRISPR
+CRISPR
-CRISPR
+CRISPR
(kb)
15
1.5
plasmid
time
repressed
induced
B
SSC-A
cells
83.3
B_530_30-A
Specimen_001_lacA + pPF1043 III_003
YG_610_20-A-
17.4
YG_610_20-A+
82.6
count
YG_610_20-A
C
live (-)
dead (+)
% cells
CRISPR
wild-type
ΔnucC
D
wild-type
ΔnucC
-CRISPR
+CRISPR
10 µm
E
1 hpi
3 hpi
SSC-A
FSC-A
F
wild-type
ΔnucC
-CRISPR
+CRISPR
B_530_30-A

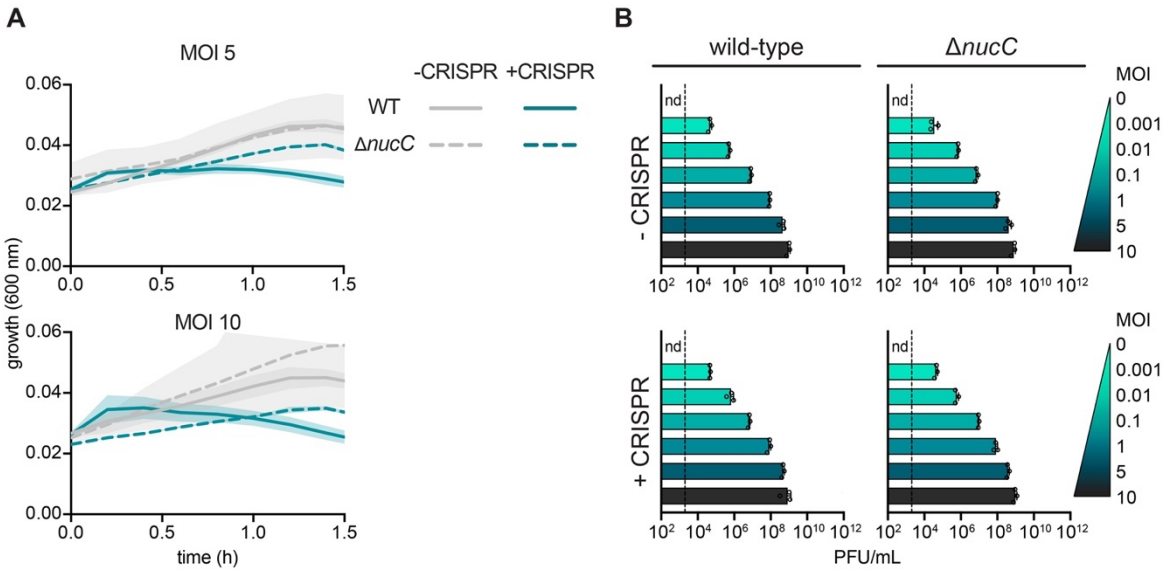
Supplementary Figure S3. Type III immunity triggers cell death by destruction of the bacterial genome via NucC. (**A**) Plasmid DNA was purified from plasmid targeting cultures at 0 h, 0.5 h, 1 h and 3 h post-induction/repression and analysed via gel electrophoresis. (**B**) Flow cytometry gating strategy adopted for cell viability assay during plasmid targeting assay. (**C**) Percentage of live (blue, negative stain) and dead cells (red, positive stain) observed for cell viability assay during plasmid targeting at 1 h and 3 h post-induction (hpi). Cell counts for cell viability assay at 1 hpi and 3 hpi are reported in **Supplementary Table S5**. (**D**) Cell membrane integrity was analysed via confocal microscopy at 3 hpi by staining with propidium iodide which discriminates living cells (blue, negative stain) from dead cells (red, positive stain). (**E**) Forward versus side scatter (FSC vs SSC) of representative samples from cell viability assay during plasmid targeting at 1 hpi and 3 hpi. FSC is indicative of cell size, and SSC relates to the complexity or granularity of the cell. (**F**) SYTO9 fluorescence distributions of representative samples from cell viability assay during plasmid targeting at 1 hpi. The left-skewed distributions of wild-type +CRISPR sample is consistent with DNA degradation.



Supplementary Figure S4. Type III immunity against the jumbo phage results in cell death of the infected individual and prevents jumbo phage reproduction. (**A**) Percentage of flagella mutants identified after the cell survival assay. A swimming assay was performed from colonies from cell survival assay. (**B**) Scattering profiles of wild-type and *nucC* mutants before and 120 min post-infection. One representative plot of n=3 shown. (**C**) SYTO9 fluorescence distributions of representative samples from (B) and **Figure 4E**. The left-skewed distributions of wild-type anti-phage samples are consistent with DNA degradation. Data for (**B**) and (**C**) shown in **Supplementary Table S6**. (**D**) One-step growth curves performed during jumbo-phage infections and targeting.


A
-CRISPR
+CRISPR
total DNA recovery (ng)
time (min)
B
chromosome
phage
plasmid
coverage (mean)
time post-infection (min)
C
-CRISPR
+CRISPR
EOP
WT
ΔnucC
D
-CRISPR
+CRISPR
2 µm
E
pyrimidine
purine
core
bits
full
WebLogo 0.0.0
F
15.16% CG
11.44% CA
10.19% TG
7.23% TA
55.98% Other
total = 1.8445 x 10^7
chromosome
13.75% CG
10.45% CA
10.23% TG
6.77% TA
58.88% Other
total = 1.70102 x 10^6
phage
12.10% TG
12.08% CG
10.58% CA
8.22% TA
57.02% Other
total = 887855
plasmid

Supplementary Figure S5. The jumbo phage DNA-containing protein shell excludes NucC. (**A**) Quantification of total DNA extracted throughout a single round of jumbo phage infection from **Figure 5A.** (**B**) Average coverage (per-base) of *Serratia* (chromosome), PCH45 jumbo phage genome (phage) and pPF1467 (plasmid) at 40 and 60 min post-infection. Coverage was calculated using the mosdepth command line tool. The data shown represents biological triplicates plotted as the mean ± standard deviation. (**C**) EOP assay with wild-type (WT) and Δ*nucC* cells expressing mEGFP-NucC with a plasmid with no spacer (-CRISPR) or a spacer targeting PCH45 capsid mRNA (+CRISPR). The data shown represents biological triplicates plotted as the mean ± standard deviation. (**D**) Confocal microscopy of -CRISPR and +CRISPR *Serratia* upon jumbo phage (PCH45) infection. Membranes (magenta) and DNA (blue) were stained with FM4-64 and DAPI, respectively. (**E**) NucC cleaves at partial palindromes in plasmid, phage and chromosomal sequences. Weblogos from deep sequencing of DNA fragments from *in vivo* cleavage by NucC, coloured according to pyrimidine (red) or purine (black) nitrogenous base nature. Cleavage occurs between motif nt positions 10 and 11. (**F**) Nucleotide preferences at outermost motif positions (3 and 16). Four pyrimidine:purine combinations (C:G, T:G, C:A, T:A) at positions 3 and 16 of the predicted *in vivo* motifs account for between 42-51% of all sequences. Mapping, per-base coverage and WebLogo summaries are reported in **Supplementary Tables S2**, **S3** and **S4**, respectively.



Supplementary Figure S6. NucC provides jumbo phage immunity by protecting the population at low phage doses. (**A**) Zoom in first 1.5 h of infection time courses for MOI 5 and MOI 10 from **Figure 6**. (**B**) Initial (0 hpi) phage titres of infection time courses presented in **Figure 6** were measured. Data are biological triplicates plotted as the mean ± standard deviation. Phage infection resulting in no countable plaques were labelled non-determined (nd). Limit of detection of phage titres indicated with a dashed line.

Supplementary Table S1. Crystallographic information (**Figure 1**).

	Apo-NucC	**NucC bound to cA_3**
deposition ID	D_1292122076	D_1292121303
space group	P21	H32
wavelength	1.00003	1.00003
cell parameters	79.468 95.492 94.536	114.265 114.265 96.069
	90.000 92.052 90.000	90.0 90.0 120.0
resolution	42.613-1.827 (1.858-1.827)	43.213-1.480 (1.506-1.480)
number of observations	848140 (41495)	798761 (38328)
number unique	124878 (6266)	40117 (1987)
Rmerge	0.049 (0.731)	0.117 (1.517)
Rpim	0.020 (0.304)	0.027 (0.354)
mean(I)/sd(I)	23.7 (2.2)	17.6 (2.2)
completeness	100 (99.9)	100 (100)
multiplicity	6.8 (6.6)	19.9 (19.3)
CC(1/2)	1.0 (0.832)	0.999 (0.773)
refinement		
reflections used for refinement	124208 (12390)	40111 (3970)
reflections used for R-free	6303 (630)	1962 (192)
R-work	0.1670 (0.2332)	0.1578 (0.2233)
R-free	0.2076 (0.2848)	0.1811 (0.2430)
RMS(bonds)	0.01	0.011
RMS(angles)	1.01	1.13
ramachandran favored (%)	97.72	96.72
ramachandran outliers (%)	0.22	0
rotamer outliers (%)	0.83	0

Statistics for the highest-resolution shell are shown in parentheses.

Supplementary Table S2. Mapping summaries resulting from deep sequencing of *in vitro* (**Figure 2**) and *in vivo* (**Figure 5**) degradation products.

sample	paired-end reads	total reads	reads aligned to pPF1043	reads aligned to PCH45	reads aligned to WT	reads aligned to pPF1467	total reads aligned	overall alignment rate (%)	unaligned reads
In_vitro (PE mapping)*	3884552	7769104	7292544	NA	NA	NA	7292544	93.87	476560
In_vitro (SE mapping)**	NA	3884552	3808751	NA	NA	NA	3808751	98.05	75801
WT_40_min I	4660657	9321314	NA	137370	8667662	398276	9203308	98.73	118006
WT_40_min II	4174729	8349458	NA	131134	7740966	380078	8252178	98.83	97280
WT_40_min III	2668582	5337164	NA	54100	4973460	237412	5264972	98.65	72192
WT_60_min I	2847136	5694272	NA	912266	4469758	221772	5603796	98.41	90476
WT_60_min II	4041545	8083090	NA	1308200	6304376	340350	7952926	98.39	130164
WT_60_min III	2958763	5917526	NA	859610	4734120	231548	5825278	98.44	92248

* this is paired end mapping of full length reads (R1 and R2), used to estimate sequencing depth (coverage) of plasmid

** this is mapping of only R1 (truncated to 15 nt), used for the motif search

Supplementary Table S3. Per-base coverage summaries resulting from deep sequencing of *in vitro* (**Figure 2**) and *in vivo* (**Figure 5**) degradation products.

	pPF1043			PCH45			WT			pPF1467		
sample	**mean coverage**	**min coverage**	**max coverage**	**mean coverage**	**min coverage**	**max coverage**	**mean coverage**	**min coverage**	**max coverage**	**mean coverage**	**min coverage**	**max coverage**
In_vitro (PE mapping)	122297.1	0	343905	NA	NA	NA	NA	NA	NA	NA	NA	NA
WT_40_min I	NA	NA	NA	39.55	0	88	105.73	0	680	4853.76	0	9864
WT_40_min II	NA	NA	NA	38.33	0	78	95.57	0	715	4679.12	0	9836
WT_40_min III	NA	NA	NA	15.79	0	45	61.67	0	416	2941.05	0	6342
WT_60_min I	NA	NA	NA	262.77	0	846	54.59	0	1165	2667.01	0	9228
WT_60_min II	NA	NA	NA	378.26	0	1246	77.47	0	1570	4131.47	0	16051
WT_60_min III	NA	NA	NA	249.19	0	748	58.21	0	1214	2805.58	0	10788

Supplementary Table S4. Sequence numbers used to build Weblogo motifs from deep sequencing of *in vitro* (**Figure 2**) and *in vivo* (**Figure 5**) degradation products.

name	total R1 from BAM files	total extracted fasta from BED annotation file	total extracted fasta >20nt	extracted fasta >20nt pPF1043	extracted fasta >20nt PCH45	extracted fasta >20nt pPF1467	extracted fasta >20nt WT
In_vitro (R1 mapped)	3808751	3808742	3807021	3807021	NA	NA	NA
WT_40_min I	4601654	4601454	4598153	NA	68671	195679	4333803
WT_40_min II	4126089	4125898	4122903	NA	65553	186891	3870459
WT_40_min III	2632486	2632371	2630657	NA	27047	116906	2486704
WT_60_min I	2801898	2801670	2799256	NA	456043	108357	2234856
WT_60_min II	3976463	3976207	3972988	NA	653994	166830	3152164
WT_60_min III	2912639	2912428	2909945	NA	429717	113192	2367036

	total 20nt sequences for building WebLogos			
	pPF1043	PCH45	pPF1467	WT
In_vitro (R1 mapped)	3807021	NA	NA	NA
WT_concatenation	NA	1701025	887855	18445022

Supplementary Table S5. Flow cytometry viability data during plasmid targeting at 1 hpi and 3 hpi (**Figure 3**).

1 hour post-induction								
sample	**cells | count**	**cells/PI | count**	**% dead**	**mean**	**sd**	**% alive**	**mean**	**sd**
WT -CRISPR (#1)	18152	1177	6.48	**5.96**	**1.04**	93.52	**94.04**	**1.04**
WT -CRISPR (#2)	18747	892	4.76			95.24		
WT -CRISPR (#3)	18282	1214	6.64			93.36		
WT +CRISPR (#1)	15548	13033	83.82	**84.65**	**0.83**	16.18	**15.35**	**0.83**
WT +CRISPR (#2)	16118	13641	84.63			15.37		
WT +CRISPR (#3)	16094	13759	85.49			14.51		
Δ*nucC* -CRISPR (#1)	17601	2300	13.07	**12.03**	**2.59**	86.93	**87.97**	**2.59**
Δ*nucC* -CRISPR (#2)	18317	1665	9.09			90.91		
Δ*nucC* -CRISPR (#3)	17384	2424	13.94			86.06		
Δ*nucC* +CRISPR (#1)	18341	918	5.01	**7.14**	**1.93**	94.99	**92.86**	**1.93**
Δ*nucC* +CRISPR (#2)	18300	1404	7.67			92.33		
Δ*nucC* +CRISPR (#3)	17984	1574	8.75			91.25		
3 hour post-induction								
sample	**cells | count**	**cells/PI | count**	**% dead**	**mean**	**sd**	**% alive**	**mean**	**sd**
WT -CRISPR (#1)	17623	664	3.77	**4.01**	**0.22**	96.23	**95.99**	**0.22**
WT -CRISPR (#2)	18424	752	4.08			95.92		
WT -CRISPR (#3)	19014	795	4.18			95.82		
WT +CRISPR (#1)	9215	7329	79.53	**82.15**	**2.27**	20.47	**17.85**	**2.27**
WT +CRISPR (#2)	15970	13330	83.47			16.53		
WT +CRISPR (#3)	15357	12815	83.45			16.55		
Δ*nucC* -CRISPR (#1)	19611	753	3.84	**4.27**	**0.37**	96.16	**95.73**	**0.37**
Δ*nucC* -CRISPR (#2)	18740	837	4.47			95.53		
Δ*nucC* -CRISPR (#3)	19131	862	4.51			95.49		
Δ*nucC* +CRISPR (#1)	19549	249	1.27	**1.70**	**0.43**	98.73	**98.30**	**0.43**
Δ*nucC* +CRISPR (#2)	19172	409	2.13			97.87		
Δ*nucC* +CRISPR (#3)	19293	325	1.68			98.32		

Supplementary Table S6. Flow cytometry data during phage infection (**Figure 4**)

strain	time post-infection (min)	intact_cells | Count	intact_cells | Median (B_530_30-A)	intact_cells | Freq. of Total (10,000)	debris | Count	debris | Freq. of Total (10,000)
WT -CRISPR (#1)	0	9085	38444	90.8	915	9.2
WT -CRISPR (#2)	0	8115	38352	81.2	1885	18.8
WT -CRISPR (#3)	0	8761	37984	87.6	1239	12.4
WT -CRISPR (#1)	20	9285	40736	92.8	715	7.2
WT -CRISPR (#2)	20	9236	40054	92.4	764	7.6
WT -CRISPR (#3)	20	9271	40638	92.7	729	7.3
WT -CRISPR (#1)	40	8837	50164	88.4	1163	11.6
WT -CRISPR (#2)	40	8734	56643	87.3	1266	12.7
WT -CRISPR (#3)	40	8938	49921	89.4	1062	10.6
WT -CRISPR (#1)	60	8329	56643	83.3	1671	16.7
WT -CRISPR (#2)	60	7933	60050	79.3	2067	20.7
WT -CRISPR (#3)	60	7731	63510	77.3	2269	22.7
WT -CRISPR (#1)	80	6439	51272	64.4	3561	35.6
WT -CRISPR (#2)	80	6855	52278	68.5	3145	31.5
WT -CRISPR (#3)	80	6862	44226	68.6	3138	31.4
WT -CRISPR (#1)	100	6083	32486	60.8	3917	39.2
WT -CRISPR (#2)	100	3067	27085	30.7	6933	69.3
WT -CRISPR (#3)	100	5805	30232	58.1	4195	41.9
WT -CRISPR (#1)	120	6710	33516	67.1	3290	32.9
WT -CRISPR (#2)	120	7084	29517	70.8	2916	29.2
WT -CRISPR (#3)	120	6846	30016	68.5	3154	31.5
WT +CRISPR (#1)	0	9293	34914	92.9	707	7.1
WT +CRISPR (#2)	0	9176	38259	91.8	824	8.2
WT +CRISPR (#3)	0	9577	33597	95.8	423	4.2
WT +CRISPR (#1)	20	9314	33759	93.1	686	6.9
WT +CRISPR (#2)	20	9265	39193	92.7	735	7.3
WT +CRISPR (#3)	20	9443	33840	94.4	557	5.6
WT +CRISPR (#1)	40	9213	31263	92.1	787	7.9
WT +CRISPR (#2)	40	9108	29873	91.1	892	8.9
WT +CRISPR (#3)	40	9262	32253	92.6	738	7.4
WT +CRISPR (#1)	60	8968	25038	89.7	1032	10.3
WT +CRISPR (#2)	60	9054	25458	90.5	946	9.5
WT +CRISPR (#3)	60	9089	22079	90.9	911	9.1
WT +CRISPR (#1)	80	8831	20472	88.3	1169	11.7
WT +CRISPR (#2)	80	8885	19438	88.8	1115	11.2
WT +CRISPR (#3)	80	8956	20472	89.6	1044	10.4
WT +CRISPR (#1)	100	9083	10650	90.8	917	9.2
WT +CRISPR (#2)	100	8913	6502	89.1	1087	10.9
WT +CRISPR (#3)	100	9019	9884	90.2	981	9.8
WT +CRISPR (#1)	120	8695	8281	87	1305	13.0
WT +CRISPR (#2)	120	8486	6253	84.9	1514	15.1

WT +CRISPR (#3)	120	8898	10554	89	1102	11.0
Δ*nucC* -CRISPR (#1)	0	9588	30670	95.9	412	4.1
Δ*nucC* -CRISPR (#2)	0	9535	34580	95.3	465	4.7
Δ*nucC* -CRISPR (#3)	0	9512	33037	95.1	488	4.9
Δ*nucC* -CRISPR (#1)	20	9250	31869	92.5	750	7.5
Δ*nucC* -CRISPR (#2)	20	9315	37258	93.2	685	6.8
Δ*nucC* -CRISPR (#3)	20	9265	36372	92.7	735	7.3
Δ*nucC* -CRISPR (#1)	40	8811	36812	88.1	1189	11.9
Δ*nucC* -CRISPR (#2)	40	8662	46420	86.6	1338	13.4
Δ*nucC* -CRISPR (#3)	40	8771	45972	87.7	1229	12.3
Δ*nucC* -CRISPR (#1)	60	8352	34085	83.5	1648	16.5
Δ*nucC* -CRISPR (#2)	60	8025	46985	80.2	1975	19.8
Δ*nucC* -CRISPR (#3)	60	8633	41531	86.3	1367	13.7
Δ*nucC* -CRISPR (#1)	80	8011	24450	80.1	1989	19.9
Δ*nucC* -CRISPR (#2)	80	7360	33921	73.6	2640	26.4
Δ*nucC* -CRISPR (#3)	80	8106	29944	81.1	1894	18.9
Δ*nucC* -CRISPR (#1)	100	8160	20617	81.6	1840	18.4
Δ*nucC* -CRISPR (#2)	100	6837	26764	68.4	3163	31.6
Δ*nucC* -CRISPR (#3)	100	7770	24218	77.7	2230	22.3
Δ*nucC* -CRISPR (#1)	120	8368	19995	82.7	1632	17.3
Δ*nucC* -CRISPR (#2)	120	7315	25458	73.2	2685	26.8
Δ*nucC* -CRISPR (#3)	120	6588	25702	65.9	3412	34.1
Δ*nucC* +CRISPR (#1)	0	9343	38352	93.4	657	6.6
Δ*nucC* +CRISPR (#2)	0	9460	34085	94.6	540	5.4
Δ*nucC* +CRISPR (#3)	0	9265	38352	92.7	735	7.3
Δ*nucC* +CRISPR (#1)	20	9178	41732	91.8	822	8.2
Δ*nucC* +CRISPR (#2)	20	9176	38630	91.8	824	8.2
Δ*nucC* +CRISPR (#3)	20	9185	41132	91.8	815	8.2
Δ*nucC* +CRISPR (#1)	40	8665	58463	86.7	1335	13.3
Δ*nucC* +CRISPR (#2)	40	8795	52405	87.9	1205	12.1
Δ*nucC* +CRISPR (#3)	40	8611	61982	86.1	1389	13.9
Δ*nucC* +CRISPR (#1)	60	8002	61530	80	1998	20.0
Δ*nucC* +CRISPR (#2)	60	8497	50900	85	1503	15.0
Δ*nucC* +CRISPR (#3)	60	8027	59179	80.3	1973	19.7
Δ*nucC* +CRISPR (#1)	80	7387	34331	73.9	2613	26.1
Δ*nucC* +CRISPR (#2)	80	5624	43694	56.2	4376	43.8
Δ*nucC* +CRISPR (#3)	80	5547	44119	55.5	4453	44.5
Δ*nucC* +CRISPR (#1)	100	3664	1407	36.6	6336	63.4
Δ*nucC* +CRISPR (#2)	100	5809	21820	58.1	4191	41.9
Δ*nucC* +CRISPR (#3)	100	4910	1034	49.1	5090	50.9
Δ*nucC* +CRISPR (#1)	120	5637	1804	56.4	4363	43.6
Δ*nucC* +CRISPR (#2)	120	6276	21975	62.8	3724	37.2
Δ*nucC* +CRISPR (#3)	120	5781	16847	57.8	4219	42.2

Supplementary Table S7. Strains used in this study.

name	genotype/phenotype	reference
***Serratia* sp ATCC 39006**		
LacA	Lac- EMS mutant ("wild-type" parental strain for this study)	(Thomson et al., 2000)
PCF686	LacA-derivative, Δ*nucC*	(Malone et al., 2020)
PCF879	LacA-derivative, Δ*flhDC*::Cm	(Hampton et al., 2016)
Escherichia coli		
DH5α	cloning strain. F^- , φ80Δd*lac*ZM15, Δ(*lacZYA*–arg*F*)U169, *endA1*, *recA1*, *hsdR*17 (rK^- mK^+), *deoR*, *thi-1*, *sup*E44, λ^-, *gyr*A96, *rel*A1	(Taylor et al., 1993)
ST18	auxotrophic donor for biparental conjugation. S17-1 λ*pir* Δ*hemA*	(Jackson et al., 2020; Thoma and Schobert, 2009)
BL21(DE3)	protein expression strain. Str., B, $F^–$, *ompT*, *gal*, *dcm*, *lon*, $hsdS_B(r_B^– m_B^–)$, λ(DE3, [*lacI*, *lacUV5-T7p07*, *ind1*, *sam7*, *nin5*]), $[malB^+]_{K-12}(\lambda^S)$	(Studier and Moffatt, 1986)
Bacteriophages		
PCH45	lytic jumbo phage, family *Myoviridae;* infects *Serratia* sp. ATCC 39006	(Malone *et al.*, 2020)
JS26	lytic non-jumbo phage, family *Siphoviridae*; infects Serratia sp. ATCC 39006	(Malone et al., 2022)

Supplementary Table S8. Oligonucleotides used in this study.

name	sequence (5′-3′)	description
cloning		
pPF2513-F	TACTTCCAATCCAATGCAATGACTAATCAGGCAAAAAA	F primer for cloning *Serratia nucC* into expression vector, generating pPF2513
pPF2513-R	TTATCCACTTCCAATGTTATTATTCCAGACTATCTATAT	R primer for cloning *Serratia nucC* into expression vector, generating pPF2513
PF2767	AGACTCAGGTCGTCTTTGTGCGCTTTGTAGAAAGCAGCG	F primer complement of PF2768 to generate spacer targeting phage JS26 in pPF1477
PF2768	AGGACGCTGCTTTCTACAAAGCGCACAAAGACGACCTGA	R primer complement of PF2767 to generate spacer targeting phage JS26 in pPF1477
PF3896	CAAAGAGGAGAAATTAACTATGACTAATCAGGCAAAAAAGTTATCTAG	F1 primer for Gibson assembly of *Serratia nucC* into pQE-80L-stuffer, generating pPF2007
PF3897	TCATACTAGGATCCGCATGCAAAGAGGAGAAATTAACTATGACTAATCAGG	F2 primer for Gibson assembly of *Serratia nucC* into pQE-80L-stuffer, generating pPF2007
PF3898	TGGCTGCAGGTCGACCCGGGTTATTCCAGACTATCTATATACACCCGC	R primer for Gibson assembly of *Serratia nucC* into pQE-80L-stuffer, generating pPF2007
PF4688	GCGAATTCGAGCTCGGTACCAAAGAGGAGAAATTAACTATGGTGAG	F primer for amplification of gBlock PF3809, overlap with pBAD30 for Gibson assembly (KpnI*);* pPF2290 cloning
PF4689	CTTTTTTGCCTGATTAGTCATGGATCCGCCTCCACCG	R primer for amplification of gBlock PF3809, overlap with PF4690; pPF2290 cloning
PF4690	AGGGCGGTGGAGGCGGATCCATGACTAATCAGGCAAAAAAGTTATC	F primer for amplification of *Serratia nucC* + linker (Glyx5-Ser), overlap with PF4689; pPF2290 cloning
PF4691	CAAAAGGTCATCCACTGCAGTTATTCCAGACTATCTATATACACCC	R primer for amplification of *Serratia nucC*, overlap with pBAD30 for Gibson assembly (PstI*)*; pPF2290 cloning
PF3809	TCGTCTTCACCTCGAGAAATCAAAGAGGAGAAATTAACTATGGTGAG CAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGA GCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGA GGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCAT CTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGAC CACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCA CATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTAC GTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCA TCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGG GGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCAT GGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGC CACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC CACTACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGA AGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGA TCACTCTCGGCATGGACGAGCTGTACAAGGGCGGTGGAGGCGGAT CCCCTGTTGATAGATCCAGTAATGAC	gBlock template for amplification of RBS-mEGFP(no STOP codon)-linker(Gly5x-Ser); pPF2290 cloning
PF5145	TATAGAATTCAAAGAGGAGAAATTAACTATGACTAATCAGGCAAAAAAGT	F primer for cloning *Serratia nucC* + artificial RBS into pPF1618, generating pPF2503 (EcoRI)
PF5146	TATAAAGCTTTTATTCCAGACTATCTATATACACCCGCC	R primer for cloning *Serratia nucC* + artificial RBS into pPF1618, generating pPF2503 (HindIII)

name	sequence (5′-3′)	description
PF5149	TATAGAATTCAAAGAGGAGAAATTAACTATGGAGGACCTTGATGCG	F primer for cloning *Thermus thermophilus csm6* + artificial RBS into pPF1618, generating pPF2505 (EcoRI)
PF5150	TATAAAGCTTTTAGAACCCCAAGGGTACGGGT	R primer for cloning *Thermus thermophilus csm6* + artificial RBS into pPF1618, generating pPF2505 (HindIII)
PF5539	CCAGATAAATGCAGTGATTTTTG	F primer for site-directed mutagenesis of *Serratia nucC* active site (D83N) in pPF2513, generating pPF2669
PF5540	TGCATTTATCTGGTCGCTG	R primer for site-directed mutagenesis of Serratia *nucC* active site (D83N) in pPF2513, generating pPF2669
PF5541	GTACTGAATGTTAAACCAACCAT	F primer for site-directed mutagenesis of Serratia *nucC* active site (E114N) in pPF2513, generating pPF2671
PF5542	TTAACATTCAGTACCGCGTAC	R primer for site-directed mutagenesis of Serratia *nucC* active site (E114N) in pPF2513, generating pPF2671
PF5543	GGTTCTTCCAACCATTAATAAAACC	F primer for site-directed mutagenesis of Serratia *nucC* active site (K116L) in pPF2513, generating pPF2673
PF5544	GTTGGAAGAACCTCCAGTA	R primer for site-directed mutagenesis of Serratia *nucC* active site (K116L) in pPF2513, generating pPF2673
NucC cleavage assays (Figure 2G-H)		
PF73	GACTCTAGACACGTGGAGAAACCAAAGCC	F primer for amplification of a *Serratia* chromosomic region, generating a 1419 bp product for cleavage assays. Binds XRE family transcriptional regulator CDS
PF807	GATCCCGGGTCAGTTCCTTGCCGTAGC	R primer for amplification of a *Serratia* chromosomic region, generating a 1419 bp product for cleavage assays. Binds DUF165 domain-containing protein CDS
PF6283	CCCTACGCTCCCTCCAGCGCTGTCGGGGATATAGTCACTCGGAGTT AGAGAGTTTTAGGATTGATTACTGAACTCTAGTATGGTAAACTGTGA AAACTCATAAAGCTGACGAAGTAAAAGAATCAAACTAATAACTCAAT CCAGTCTAAAGAGTAGAAAGTTGGTGAAAGATTGTGAGTCAGTCACT TAATGGTCTTAGA	no motif negative control gBlock
PF6284	CCCTACGCTCCCTCCAGCGCTGTCGGGGATATAGTCACTCGGCAAG GGCGCCCTTGAGGATTGATTACTGAACTCTAGTATGGTAAACTGTGA AAACTCATAAAGCTGACGAAGTAAAAGAATCAAACTAATAACTCAAT CCAGTCTAAAGAGTAGAAAGTTGGTGAAAGATTGTGAGTCAGTCACT TAATGGTCTTAGA	full motif gBlock
PF6285	CCCTACGCTCCCTCCAGCGCTGTCGGGGATATAGTCACTCGGAGTT GGCGCCTTTTAGGATTGATTACTGAACTCTAGTATGGTAAACTGTGA AAACTCATAAAGCTGACGAAGTAAAAGAATCAAACTAATAACTCAAT CCAGTCTAAAGAGTAGAAAGTTGGTGAAAGATTGTGAGTCAGTCACT TAATGGTCTTAGA	core motif gBlock
screening		
PF2202	TATTGCATGCGGCTGACGATCTGGCGTC	chromosomal *Serratia nucC*, F primer
PF2199	TCTTGGATCCGCTAGCGGCCTGCCGGAGAAC	chromosomal *Serratia nucC*, R primer
PF138	CACACTTTGCTATGCCATAG	pPF781-derived plasmids, F primer
PF1702	CGAAGACGAAAGGGCCTCGTGATACGCAAGCTTTATGGCTTGTAAA CCGTTTTGTG	pPF781-derived plasmids, R primer

name	sequence (5′-3′)	description
PF4181	AAAGAAATCATAAAAAATTTATTTGCTTTGTGAGCGGAT	pPF976-derived plasmids, F primer
PF3737	TTTATGCATCTTCAGTCAGGGAGCGTC	pPF976-derived plasmids, R primer
PF2231	TTTTACTAGTAGACGTTCAACAACGTCATG	PCH45 capsid gene, F primer
PF2232	TTTTGGTACCGAAGTTATATTCGCGCGGTG	PCH45 capsid gene, R primer
PF138	CACACTTTGCTATGCCATAG	pPF1618-derived plasmids, F primer
PF210	GTCATTACTGGATCTATCAACAGG	pPF1618-derived plasmids, R primer

Supplementary Table S9. Plasmids used in this study.

name	description	features	construction	reference
protein expression and purification (Figs. 1 and 2)				
pPF2007	template for *nucC* cloning into expression vector	pBR322/ori, RP4/oriT, ApR, lacI/T5	Gibson assembly using PF3896, PF3897 + PF3898 and SphI and SmaI to Gibson assemble *nucC* into pQE-80L-oriT stuffer	This study
pPF2513	6xHis-TEV-NucC expression vector	ColE1/ori, f1/ori, KmR, lacI/T7	pPF2513-F + pPF2513-R paired in a PCR to amplify *nucC* from pPF2007 and clone it into an expression vector through Ligation Independent Cloning.	This study
pPF2669	6xHis-TEV-NucC D83N mutant expression vector	ColE1/ori, f1/ori, KmR, lacI/T7	PF5539 + PF5540 paired in a PCR to introduce NucC D83N mutation in pPF2513	This study
pPF2671	6xHis-TEV-NucC E114N mutant expression vector	ColE1/ori, f1/ori, KmR, lacI/T7	PF5541 + PF5542 paired in a PCR to introduce NucC E114N mutation in pPF2513	This study
pPF2673	6xHis-TEV-NucC K116L mutant expression vector	ColE1/ori, f1/ori, KmR, lacI/T7	PF5543 + PF5544 paired in a PCR to introduce NucC K116L mutation in pPF2513	This study
plasmid targeting (Fig. 3)				
pPF781	untargeted control for the type III-A system	p15A/ori, RP4/oriT, CmR, pBAD/araC	pBAD30 derivative	(Patterson et al., 2016)
pPF1043	targeted type III-A with protospacer complementary to *Serratia* CRISPR3 spacer 1	p15A/ori, RP4/oriT, CmR, pBAD/araC	pPF781 derivative	(Patterson *et al.*, 2016)
phage targeting (Figs. 4, 5, 6 and 7)				
pPF976	type III-A repeat-BsaI-repeat construct for artificial crRNA	pBR322/ori, RP4/oriT, KmR, lacI/T5	pPF260 derivative	(Malone *et al.*, 2020)
pPF1467	anti-PCH45 III-A spacer overexpression. III-A_PCH45_PS4 (capsid protein)	pBR322/ori, RP4/oriT, KmR, lacI/T5	pPF976 derivative	(Malone *et al.*, 2020)
pPF1477	anti-JS26 III-A spacer overexpression. III-A_JS26_PS8 (capsid protein)	pBR322/ori, RP4/oriT, KmR, lacI/T5	PF2767 and PF2768 were annealed, digested with BsaI and ligated into pPF976 cut with BsaI	This study
NucC localization microscopy (Fig. 5)				
pPF2290	mEGFP-NucC expression vector	p15A/ori, RP4/oriT, GmR, pBAD/araC	Gibson assembly PF4688/PF4689 (gblockPF3809) + PF4690/PF4691 (LacA)	This study
accessory nuclease swap (Fig. 7)				
pPF1618	empty vector control for expression	RK2/ori, OriT, ApR, pBAD/araC	pSEVA1810 (pBAD) and pSEVA121 (ApR, RK2) were digested with PacI and EcoRI, and ligated into one vector.	This study
pPF2503	*Serratia* NucC expression vector	RK2/ori, OriT, ApR, pBAD/araC	PF5145 + PF5146 paired in a PCR to amplify *nucC* from *Serratia* LacA and cloned it into pPF1618 (EcoRI/HindIII)	This study
pC0075	*Thermus thermophilus* Csm6 template		TtCsm6 His6-TwinStrep-SUMO-BsaI (Addgene #115270)	(Gootenberg et al., 2018)
pPF2505	*T. thermophilus* Csm6 expression vector	RK2/ori, OriT, ApR, pBAD/araC	PF5149 + PF5150 paired in a PCR to amplify *csm6* from pAddgene_115270 and clone it into pPF1618 (EcoRI/HindIII)	This study

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