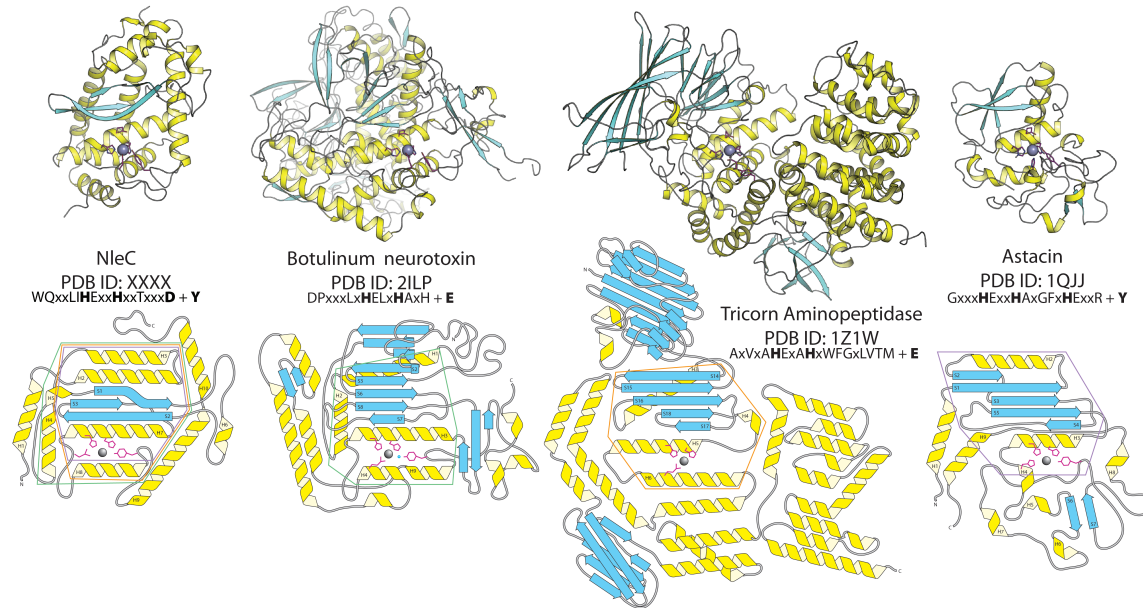
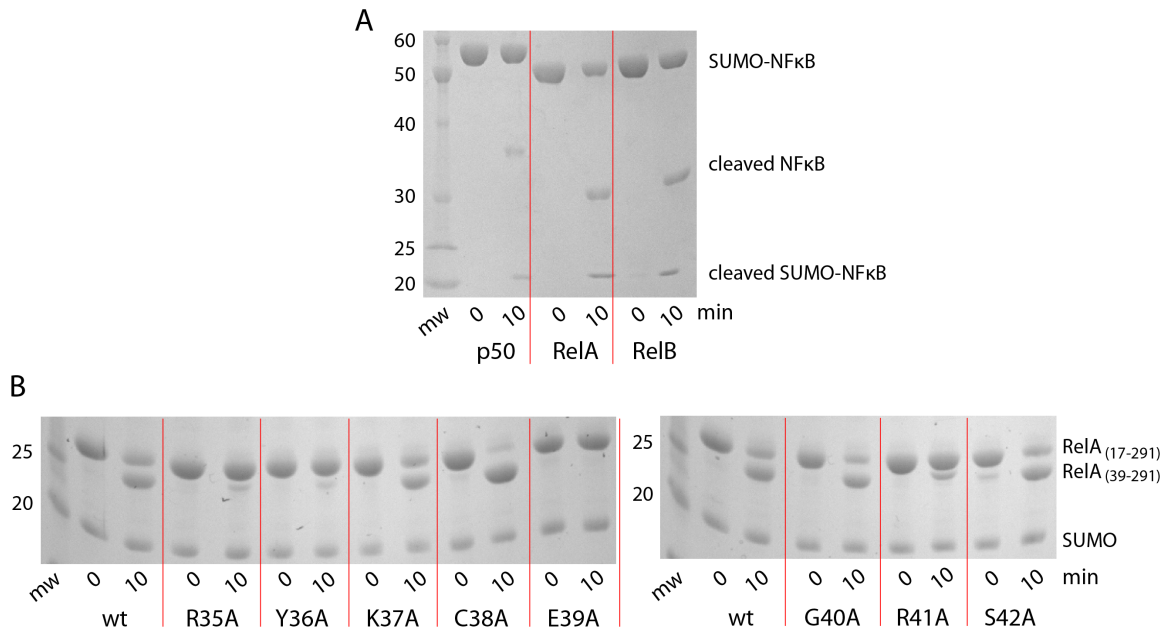


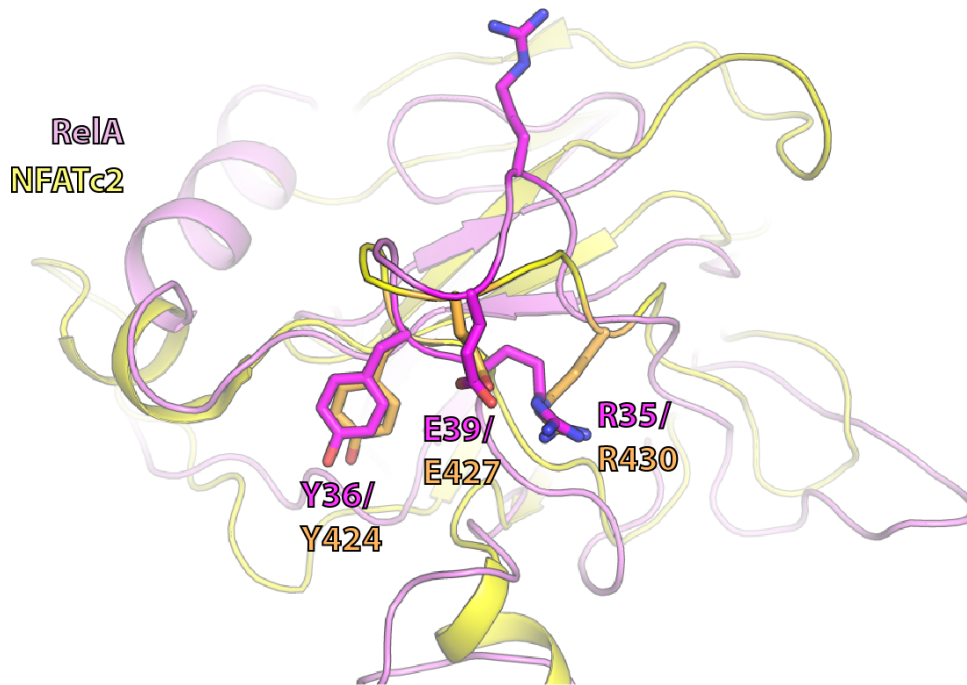
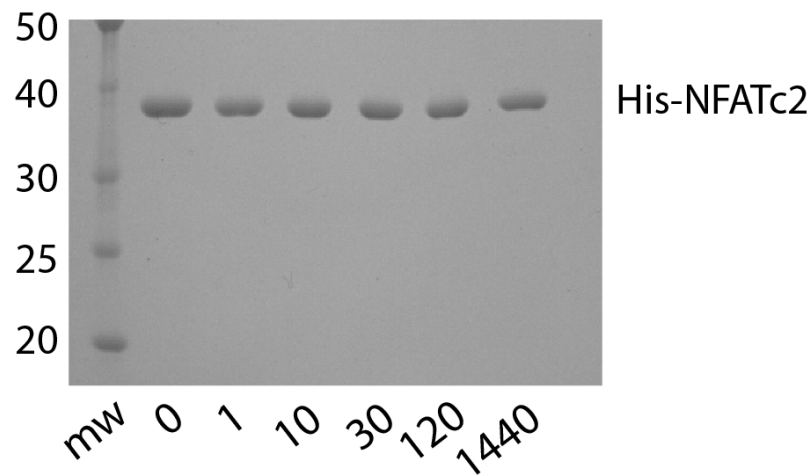
Supplementary Materials



Supplementary Figure S1. Structural and topological comparison of NleC with other Zincins. Representations of proteins have α -helices in yellow, β -strands in blue, and loops in grey. The active site Zn^{2+} is shown as a grey sphere, while the coordinating residues and active site glutamate are depicted in magenta stick format. The topology diagrams highlight the similarity of the NleC structure to the other Zincins. The similar structures are highlighted in the NleC structure, with green for botulinum neurotoxin, orange for tricorn aminopeptidase, and purple for astacin. The conserved active site motif which contains the coordinating residues in bold is also shown for each Zincin.

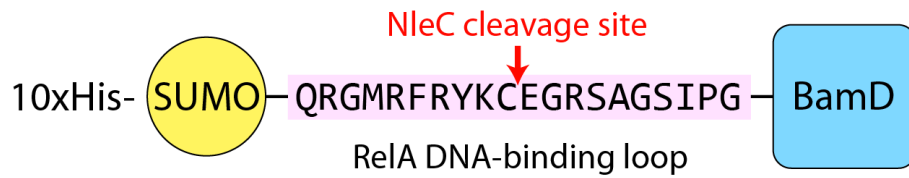


Supplementary Figure S2. SDS-PAGE results for NleC cleavage of NF κ B subunits. A) Cleavage of NF κ B subunits by NleC. 20 μ M RelA, RelB or p50 was incubated for 10 minutes with 20 nM NleC before quenching the reaction by addition of running buffer and boiling for 2 minutes. This sample was run on an SDS-PAGE with a zero timepoint control, before the addition of NleC. B) Cleavage of alanine mutants of RelA by NleC. 20 μ M of each RelA alanine mutant was incubated with 20 nM NleC for 10 minutes. Samples were quenched by addition of running buffer and boiling for 2 minutes. Samples are shown before the addition of NleC and after 10-minute incubation. The SUMO tag was cleaved before the activity assay.

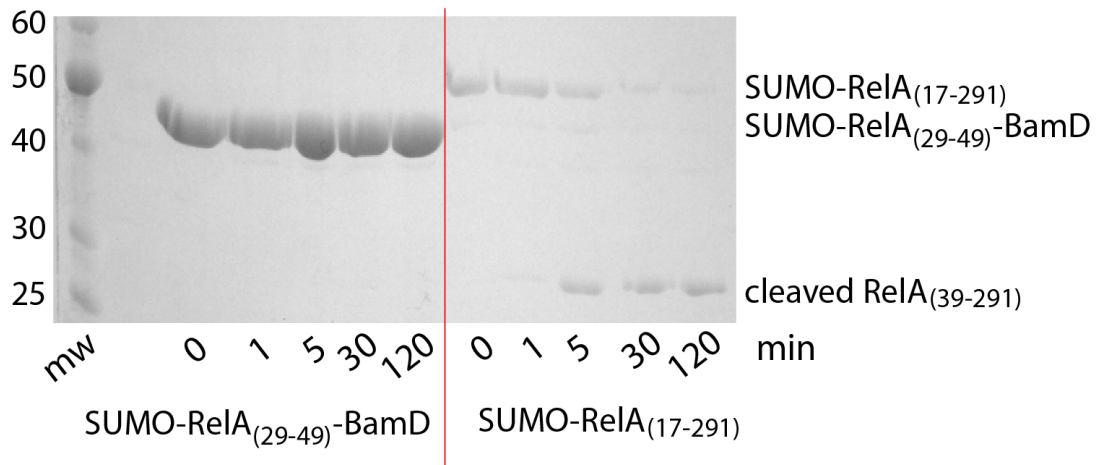
A**B**

Supplementary Figure S3. NFATc2 comparison to RelA. A) Overlay the DNA-binding domains of RelA and NFATc2. NFATc2 is in yellow and RelA in pink. The residues in RelA that were found to be most important for efficient cleavage are shown in stick form. Residues in NFATc2 that overlay with these are also shown. PDB ID: 2AS5, chain N. B) Cleavage assay for NFATc2. 10 μ M NFATc2 was incubated with 20 nM NleC and timepoints taken over 24 hours. The timepoints were quenched with the addition of running buffer and boiling for 2 minutes. No cleavage is apparent.

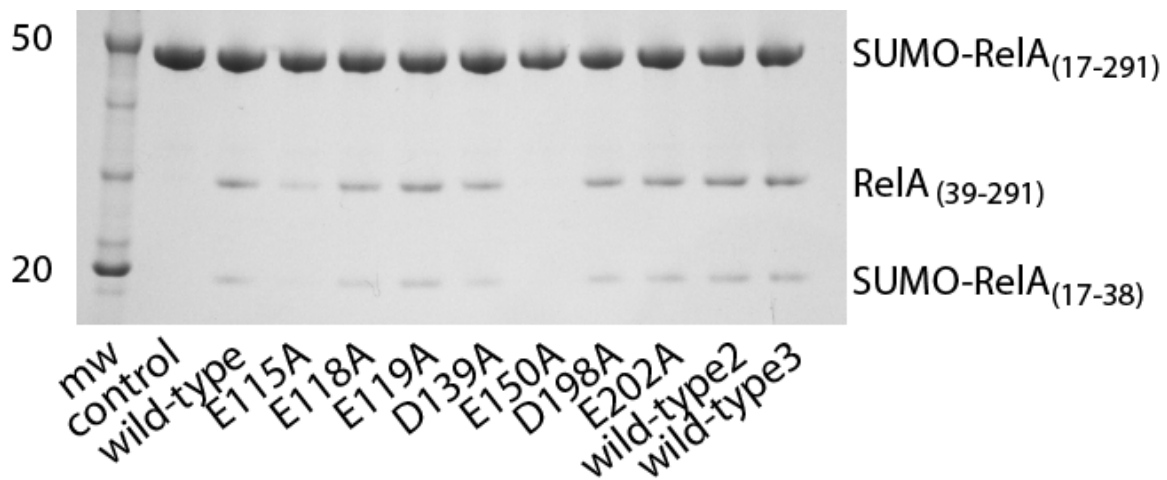
A



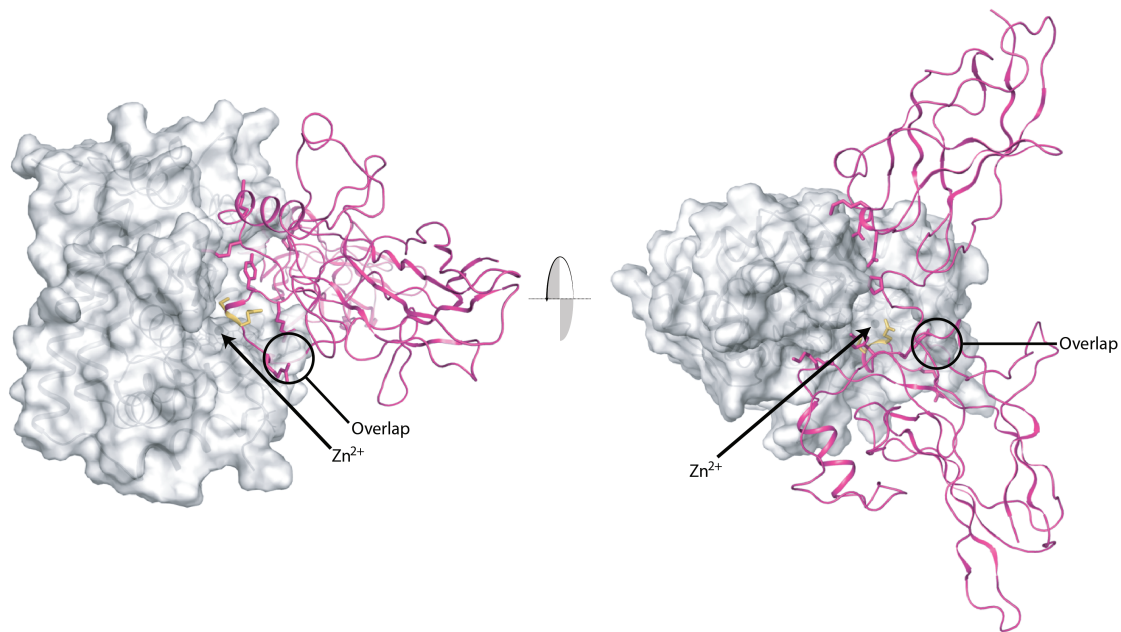
B



Supplementary Figure S4. Fusion protein containing NleC cleavage site in RelA. A) Schematic of 10x-His-SUMO-RelA (residues 29-49)-BamD fusion protein. In between the SUMO and BamD proteins, twenty total residues of RelA are centered around the NleC cleavage site. B) Cleavage assay for RelA fusion protein compared to RelA Rel-homology domain. 20 μ M of RelA(29-49) fusion protein and 5 μ M of RelA (17-291) were incubated with 20 nM NleC and timepoints taken. The reactions were quenched by addition of SDS running buffer and boiling for 2 minutes. No cleavage of the RelA (29-49) fusion protein is apparent.



Supplementary Figure S5. Representative SDS-PAGE result for NleC mutant cleavage of RelA. Seven NleC mutants were tested for their ability to cleave His-SUMO-RelA (residues 17-291). 20nM NleC was incubated with 20 μ M RelA for 10 minutes and the reaction quenched by addition of SDS running buffer and boiling for 2 minutes. The control lane contains no NleC.



Supplementary Figure S6. DNA mimicry-motivated model of NleC and RelA interaction. After the active site cleft of NleC is overlaid with the major groove of DNA and the negative charges aligned, the structure of RelA bound to DNA shows good shape complementarity with NleC and no significant clashes. NleC is shown as a transparent gray surface representation, with RelA in pink. The scissile bond of RelA is depicted in yellow. The active site zinc is depicted as a dark grey sphere. The minor clashes between the two structures are highlighted in a circle, and map to two dynamic loops in NleC and RelA. (Rel A is PDB ID: 2RAM, chain B.)

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
Arsenophonus nasoniae/1-515
Arsenophonus nasoniae/1-507
Vibrio nigripulchritudo/1-887
Arsenophonus nasoniae/1-579

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
Arsenophonus nasoniae/1-515
Arsenophonus nasoniae/1-507
Vibrio nigripulchritudo/1-887
Arsenophonus nasoniae/1-579

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
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Arsenophonus nasoniae/1-579

EHEC/1-330

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E. coli O111:H-/1-328
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E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
Arsenophonus nasoniae/1-515
Arsenophonus nasoniae/1-507
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Arsenophonus nasoniae/1-579

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
Arsenophonus nasoniae/1-515
Arsenophonus nasoniae/1-507
Vibrio nigripulchritudo/1-887
Arsenophonus nasoniae/1-579

Diagram showing protein domain structure with beta2, TT, eta3, alpha5, TT, beta3, beta4, TT, beta5 domains. Amino acid sequence alignment for various EHEC strains across these domains.

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
Arsenophonus nasoniae/1-515
Arsenophonus nasoniae/1-507
Vibrio nigripulchritudo/1-887
Arsenophonus nasoniae/1-579

Diagram showing protein domain structure with TT, alpha6, alpha7 domains. Amino acid sequence alignment for various EHEC strains across these domains.

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
Arsenophonus nasoniae/1-515
Arsenophonus nasoniae/1-507
Vibrio nigripulchritudo/1-887
Arsenophonus nasoniae/1-579

Diagram showing protein domain structure with alpha8, eta4, alpha9 domains. Amino acid sequence alignment for various EHEC strains across these domains.

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
Arsenophonus nasoniae/1-515
Arsenophonus nasoniae/1-507
Vibrio nigripulchritudo/1-887
Arsenophonus nasoniae/1-579

Diagram showing protein domain structure with TT, eta5 domains. Amino acid sequence alignment for various EHEC strains across these domains.

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
Arsenophonus nasoniae/1-515
Arsenophonus nasoniae/1-507
Vibrio nigrripulchritudo/1-887
Arsenophonus nasoniae/1-579

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
E. coli/1-229
Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
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Arsenophonus nasoniae/1-507
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Arsenophonus nasoniae/1-579

EHEC/1-330

EHEC/1-330
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Yersinia aldovae/1-339
E. coli/1-351
S. typhimurium/1-330
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Vibrio tasmaniensis/1-518
Vibrio sp./1-518
Vibrio azureus/1-518
Photobacterium damsela/1-512
Vibrio caribbenthicus/1-518
Arsenophonus nasoniae/1-486
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Arsenophonus nasoniae/1-507
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Arsenophonus nasoniae/1-579

EHEC/1-330

EHEC/1-330
E. coli/1-328
Citrobacter rodentium/1-330
E. coli O111:H-/1-328
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Vibrio nigrripulchritudo/1-887
Arsenophonus nasoniae/1-579

Supplementary Figure S7. Alignment of NleC family members. Other NleC proteins were identified by Psi-BLAST and redundant proteins (with over 98% identity) removed for analysis. Residues in the active site of the protein are completely conserved. Some NleC family members contain insertions, but these occur in loops and not in regular secondary structure elements, making it likely that these proteins retain a fold similar to Sakai strain NleC. Some of the NleC family members have additional domains, either at the amino or carboxy terminus.

Plasmid	Gene	Residues	Organism	Gene Source	Tag	Background	Primers	Resistance
pMS692	NleC	1-330	EHEC str. Sakai	EHEC str. Sakai DNA	C-term Intein-CBD	pTYB2	ggaattccatgaaaattccctcattacagtcc tccccgggttgctgattgtgtttgtccac	Amp
pMS846	NleC	19-330	EHEC str. Sakai	EHEC str. Sakai DNA	C-term Intein-CBD	pTYB2	ggaattccatgattctgtctccattgctc tccccgggttgctgattgtgtttgtccac	Amp
pMS1120*	NleC E115A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	CAGAAACGAATACGCGCTTAACGAAGAATCTTC GAAGATTCTCGTTAAGCGGTATTGTTTCTCG	Amp
pMS1121*	NleC E118A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	CGAATACGAGCTTAACGCGAATCTTCTGTC GACAGAAGATTCTGCGTTAAGCTCGTATTCTG	Amp
pMS1122*	NleC E119A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	CGAATACGAGCTTAACGAAGCATCTTCTGTCAAATTGATG CATCAATTTTGACAGAAGATGCTTTCGTTAAGCTCGTATTCTG	Amp
pMS1123*	NleC D139A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	CGAATTATGAAATATGCTGTGCGGCAAGAGC GCTCTTGCCCGACAGCATATTCATATAATTCTG	Amp
pMS1124*	NleC E150A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	GCCAAATTTCCCATTTGTGACAGCAGGAGAAAACG CGTTTTCTCCTGCTGCAAAATGGGAAAATTGGC	Amp
pMS1125*	NleC D198A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	GCGATCCATCTGGAGCTAGTAATATAGAGTTAGG CCTAACTCTATATTACTAGCTCCAGATGGATCGC	Amp
pMS1134*	NleC E202A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	GGAGATAGTAATATAGCGTTAGGACCCACCG CGGTGGGTCTAAAGCTATATTACTATCTCC	Amp
pMS1013*	RelA	17-291	Human	J Goodrich, Univ of CO	10x-His-SUMO	pET24d	GGATCTGGCCCTATGTGGAGATC CGGCTCAACTGGCAGGTAAGGAAATTC GTACCGGCTCAACTGGCAGGTAAGGAAATTC	Kan
pMS845	RelA	1-210	Human	J Goodrich, Univ of CO	6x-His	pET24d	ATcatgCCATGGacgaactgttccccct CATCATgtcgcTAAatccccaccgaggcag	Kan
pMS1020*	RelA	29-49	Human	J Goodrich, Univ of CO	10x-His-SUMO-RelA-BamD	pET24d	GGATCACCATGGCAGCGGTATGCGCTTCCGC CTTATGTATTGCTGCTGTTTGGCGGATG AGCTCTTATGATTGCTGCTGTTTGGCGGATG	Kan
pMS1032	RelA R35A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CATGCGCTTgCgCTACAAGTGCAGGGGCGCT GCATTGTAGgCgGAAGCGCATGCCCGCTGCT	Kan
pMS1033	RelA Y36A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	GCGCTTCCGcGcCAAGTGCAGGGGCGCTCCG CTCGCACTTgCgCGGAAGCGCATGCCCGCT	Kan
pMS1034	RelA K37A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CTTCCGCTAcgGTGCGAGGGGCGCTCCGCGG CCCTCGCACgCgTAGCGGAAGCGCATGCCCC	Kan
pMS1035	RelA C38A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CCGCTACAAGgCgCGAGGGGCGCTCCGCGGCA GCGCCCTCGgCCTGTAGCGGAAGCGCATGC	Kan
pMS1036	RelA E39A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	TACAAGTGCgCGGGGCGCTCCGCGGCGAGCAT CGGAGCGCCCGcGCGCACTTGTAGCGGAAGCGC	Kan
pMS1037	RelA G40A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	AAGTGCgAGGcGCGCTCCGCGGCGAGCATCC CGCGGAGCGcGcCTCGCACTTGTAGCGGAAG	Kan
pMS1038	RelA R41A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	GTGCGAGGGGgCCTCCGCGGCGAGCATCCAG GCCCGCGAGgCgCCCCTCGCACTTGTAGCGGA	Kan
pMS1039	RelA S42A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CGAGGGCGcGCGCGGCGAGCATCCAGCGC CTGCCCGCGcGCGCCCTCGCACTTGTAGC	Kan
pMS1114	RelB	124-413	Human	Functional Genomics Facility - Univ of CO	10x-His-SUMO	pET24d	ggagcgCAGCCGACCTGGTCATC gtacctcaCGTTTCCGCTTCTTGTC ctcaCGTTTCCGCTTCTTGTC	Kan
pMS1115	p50	39-363	Human	J Goodrich, Univ of CO	10x-His-SUMO	pET24d	ggagcgACAGCTGGGCCATACCTTC gtacctcaACGTTTCTCTGCaCTTCTTC ctcaACGTTTCTCTGCaCTTCTTC	Kan

Supplementary table ST1.