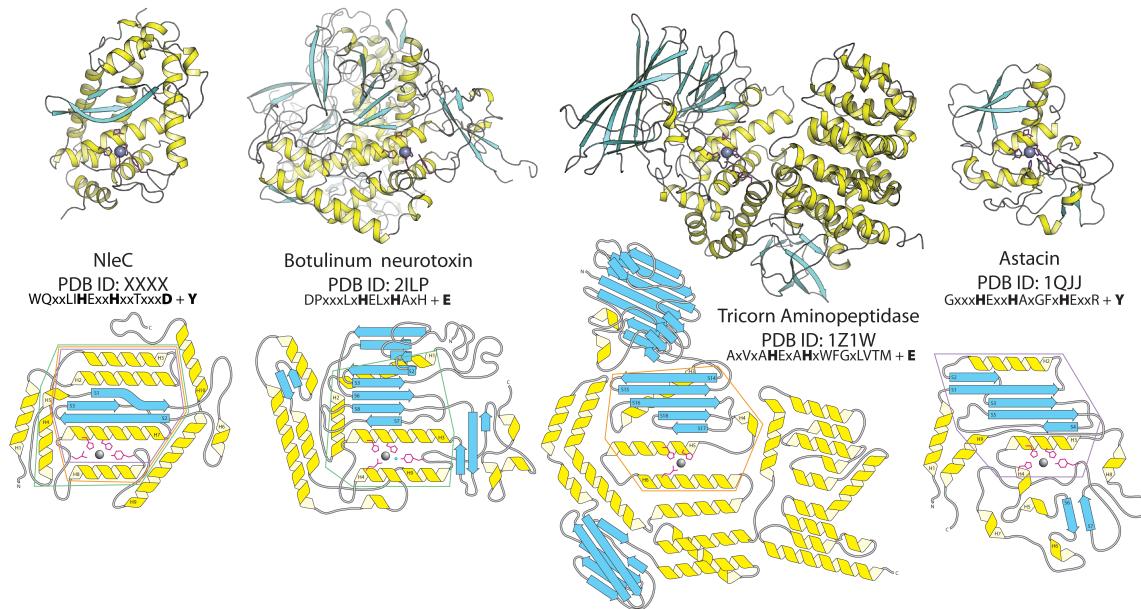
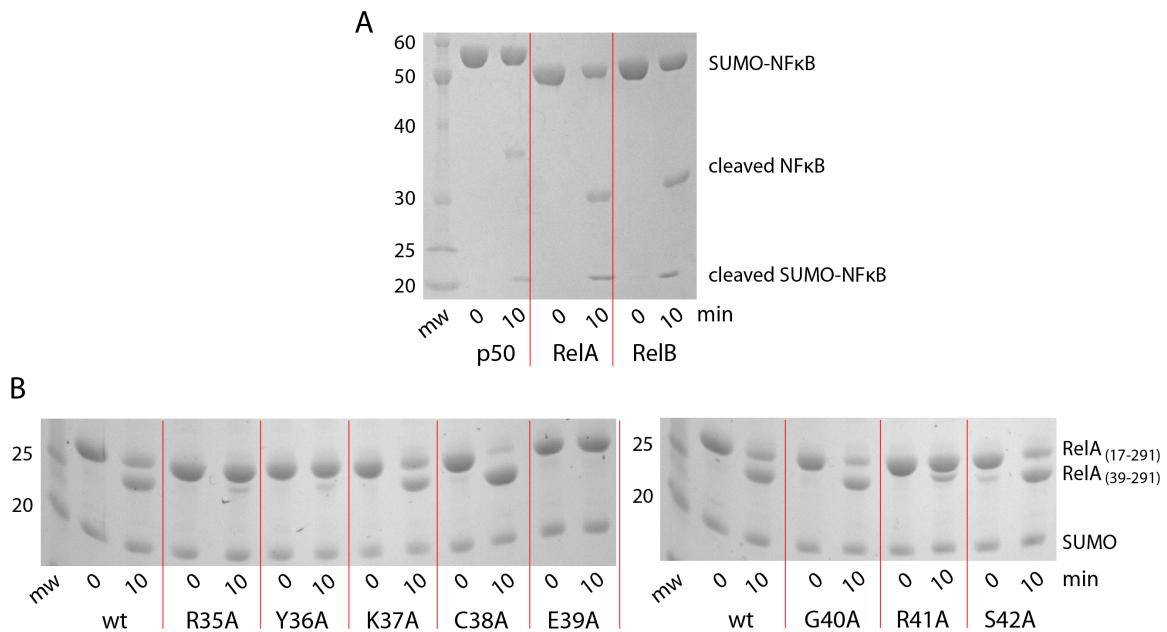


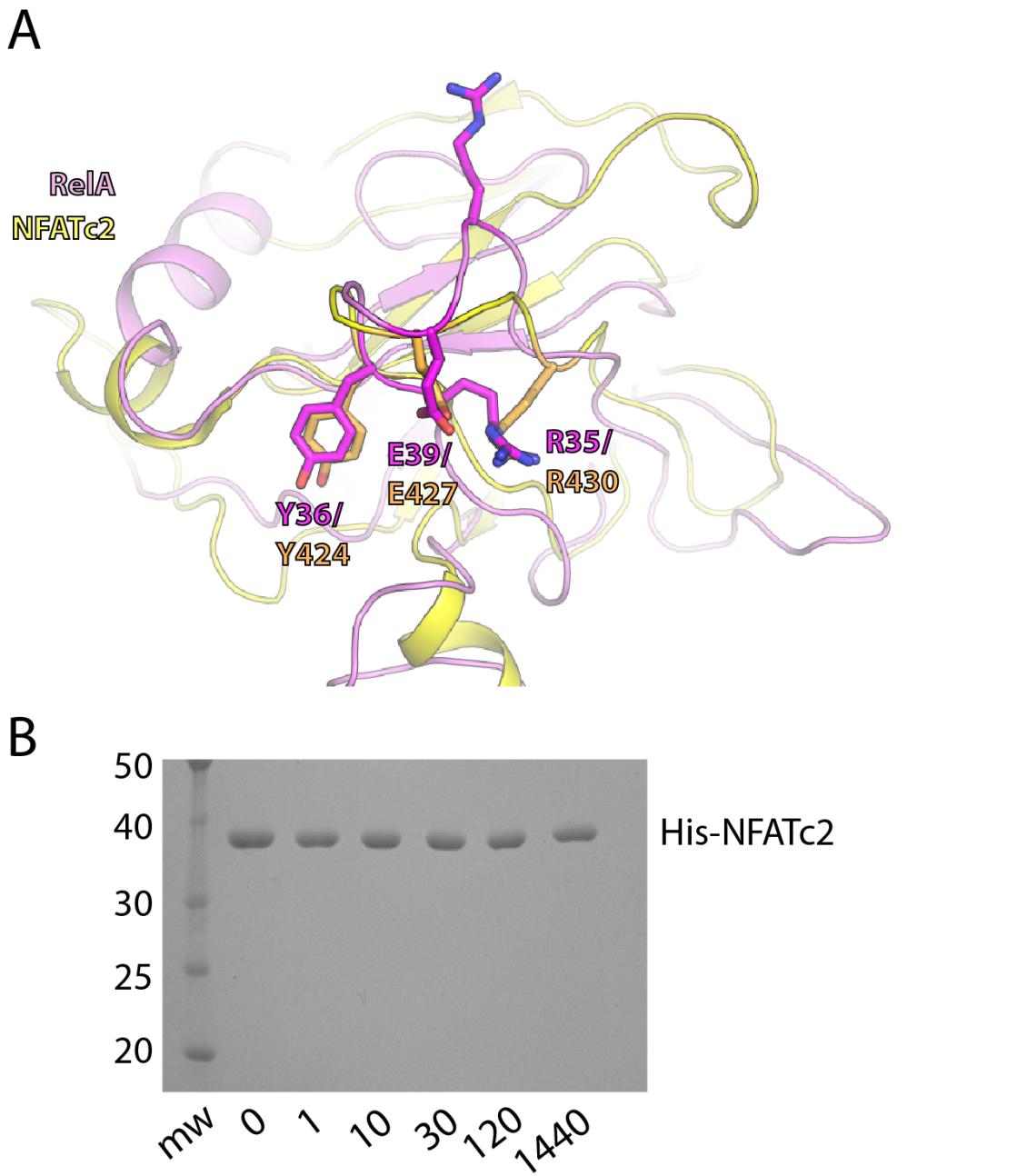
Supplementary Materials



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 NFκB subunits. A) Cleavage of NFκB subunits by NleC. 20 μM 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 μM 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.

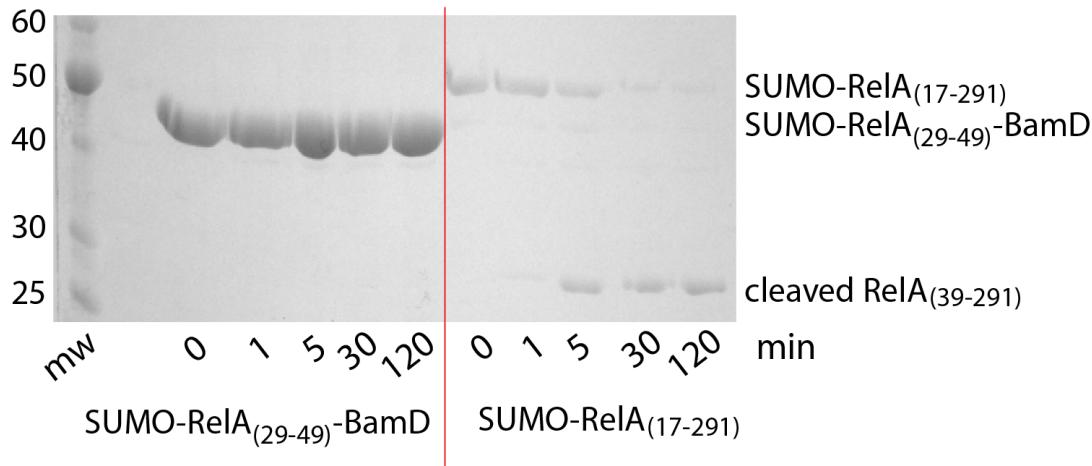


Supplementary Figure S3. NFATc2 comparison to RelA. A) Overlay the DNA-binding domains of RelA and NFATc2. NFATc2 is in yellow and RelA in pink. The residues in RelA that were found to be most important for efficient cleavage are shown in stick form. Residues in NFATc2 that overlap with these are also 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.

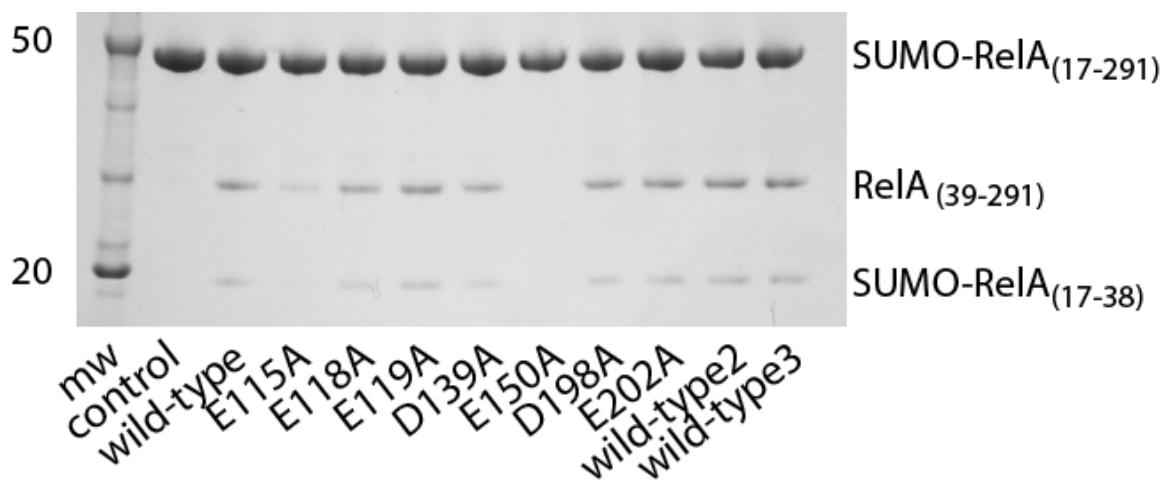
A



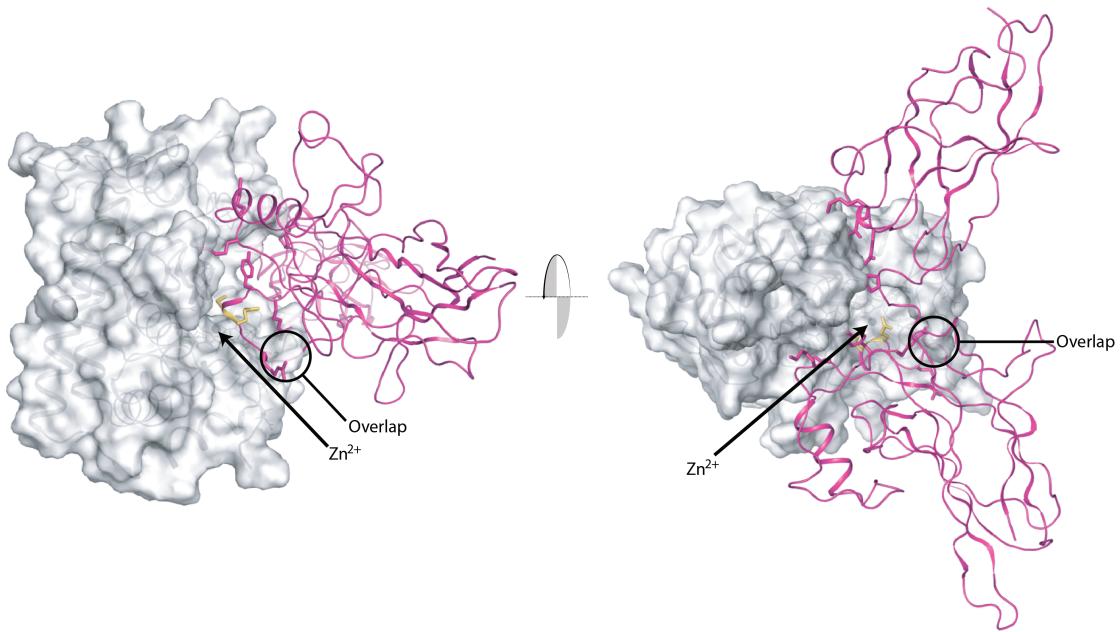
B



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 20μM 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. (Real A is PDB ID: 2RAM, chain B.)

<i>EHEC</i> /1-330		.
<i>EHEC</i> /1-330	1	.
<i>E. coli</i> /1-328	1	.
<i>Citrobacter rodentium</i> /1-330	1	.
<i>E. coli</i> _O111:H-/1-328	1	.
<i>Yersinia alvdovae</i> /1-339	1	.
<i>E. coli</i> /1-351	1	.
<i>S. typhimurium</i> /1-330	1	.
<i>E. coli</i> /1-229		.
<i>Vibrio tasmaniensis</i> /1-518	4	YPVLLLATL..
<i>Vibrio</i> _sp./1-518	4	KSLSVAIL..
<i>Vibrio_azureus</i> /1-518	4	HRY.FAVL..
<i>Photobacterium damseliae</i> /1-512	1MTAI..
<i>Vibrio_carribenthicus</i> /1-518	4	KVLVIS..
<i>Arsenophonus nasoniae</i> /1-486		.
<i>Arsenophonus nasoniae</i> /1-515	4	KN.....
<i>Arsenophonus nasoniae</i> /1-507		.
<i>Vibrio nigripulchritudo</i> /1-887	541	KLTTPPEANIEPNGEYARFDGVRHKFVRVMHQGEVFIGPAREISVAGRPKLEMNNNPGYEF
<i>Arsenophonus nasoniae</i> /1-579	4	NQPEVKQLQANQLEIIRNLISQGIIYYPYPQFEEHRPVYPIQGLDQLLEGQLDA...I

<i>EHEC/1-330</i>		<i>TT</i>	<i>α1</i>		<i>α2</i>
<i>EHEC/1-330</i>		
<i>E. coli/1-328</i>	4	PSLQ	SNFNFSAPAGYSAPIAPNRAENA	YADYVLIDIGKRI..	PLSAADLSNVYESVIRAVH
<i>Citrobacter rodentium/1-330</i>	4	PVLQ	PGFNNFAPAGYSAAVAPNRAENA	YADYVLIDIGKRI..	PLSAADLSNVYESVIRAVH
<i>E. coli_0111:H-/1-328</i>	4	PSLQ	PSFNFFAPAGYSAAVAPNRSNA	YADYVLIDIGKRI..	PLSAADLGNYENYESVIRAVH
<i>Yersinia alvdovae/1-339</i>	4	PVLQ	PSFNFFAPAGYSAAVAPNRAENA	YADYVLIDIGKQT..	PLSAADLSNVYENVIRAVH
<i>E. coli/I-351</i>	13	TSQ	PSFNFSQAEGYSAAVAPNHSNA	YADYVLIDIGKRI..	PTSTADLGNYENVYENVIRAH
<i>S. typhimurium/1-330</i>	4	PSLQ	SNFNFSAPAGYSAPIAPNRAENA	YADYVLIDIGKRI..	PLSAADLSNVYESVIRAVH
<i>E. coli/I-229</i>	4	SSST	PCLNFAPQKEYSAAVVPHPSKNA	YADYVLETGKRI..	PTPSAADLSNLYQSIVIAYH
<i>Vibrio tasmaniensis/1-518</i>	14	TVVT	P.RDAFAESERP....	ERDSDDLYANVVNTGIQQ..	PLTRTQRAAISSSATQSVV
<i>Vibrio_sp./1-518</i>	14	LAMN	T.KFVLANNDRP....	DQHSDEYADYVVNTGADQ..	QLTYQSQLVASSAISRSVV
<i>Vibrio_azureus/1-518</i>	13	LSSL	TITPVMANNEKP....	HYGSDEYADYVLSKGNEH..	RLTATQQLSEISSAISRSVV
<i>Photobacterium damselaiae/1-512</i>	7	LAIN	S.NFVLVLANNDKP....	DASDDKYADYVVRLGSEH..	PLNHTQIEISSAISRSVV
<i>Vibrio_carribenthicus/1-518</i>	12	LAMN	T.KFVLANNNGAP....	DRDSDDEYADYVVNRGANQ..	QLTYAQAMVAISSSAISRSVV
<i>Arsenophonus_nasoniae/1-486</i>	1	MKNRNEDAYASVYCGNDHEF..	SLSDDVLTIRTEIRTAVS
<i>Arsenophonus_nasoniae/1-515</i>	6	FRRSDGYAAHVITIGERN..	RLSSEAQLYDIVVRVRSQIR
<i>Arsenophonus_nasoniae/1-507</i>	1	..MQ	NNQDQVNQ.NEI....	FRKMDDYAEYVVNTGARA..	ELSSRQEIEEIMIIDVRRFTIN
<i>Vibrio_nigripulchriflido/1-887</i>	601	FPLD	IEGEFYGITRHGLKGMMNKLDS	YVEHVTAMGSD..	ALDPSRQVYEDNWSLGR
<i>Arsenophonus_nasoniae/1-579</i>	61	RALN	S.....L..	FAQSNOYAEFVCTEGNODIQ	DDPNFLISNYMIQJRAHJD

<i>EHEC</i> /1-330		β_2	TT	η_3	aaaa	α_5	TT	β_3	TT	β_4	TT	β_5
<i>EHEC</i> /1-330	.			eee		ELYYEY		DVGQEP	FFP..	.IC..		EAGENNDN..EEPYVSFSVA
<i>E. coli</i> /1-328				eee		ELYYEY		DVGQEP	PILP..	.IC..		EAGENNDN..EEPYVSFSVA
<i>Citrobacter rodentium</i> /1-330				eee		ELYYEY		DVGQEP	PILP..	.IC..		EAGENNDN..EEPYVSFSVA
<i>E. coli</i> _O111:H-/1-328	114	YELN	EESSVKIDD	IQS	LTCN	ELYYEY	DVGQEP	FFP..	.IC..		EAGENNDN..EEPYVSFSVA	
<i>Versinia alvodaei</i> /1-339	114	YERN	EEESPVKVDD	IQS	LTC	ELYYEY	DVGQEP	PILP..	.IC..		EAGENNDN..EEPYVSFSVA	
<i>E. coli</i> /1-351	114	YELN	GESPVKVDD	IQS	LTC	ELYYEY	DVGQEP	PILP..	.IC..		EAGENNDN..EEPYVSFSVA	
<i>S. typhimurium</i> /1-330	123	YERN	EEESPVKVDD	IQS	LTC	ELYYEY	DVGQEP	PVF..	.IC..		EAGENNDH..EEPYVSFSVA	
<i>E. coli</i> /1-229	114	YELN	GESPVEVD	IQS	LTC	ELYYEY	DVGQEP	PVF..	.IC..		EAGENNDH..EEPYVSFSVA	
<i>Vibrio tasmaniensis</i> /1-518	114	YEIN	EDSPVGVDs	IHL	LTHS	ELYYEY	EAGQEP	PILP..	.IC..		EARKDEH..EEAYILSFSAA	
<i>Vibrio</i> sp./1-518	15	TSLT	KNLLVKIDD	IQS	LTC	ELYYEY	DVGQEP	FFP..	.IC..		EAGENEN..EEPYVSFSVA	
<i>Vibrio</i> _azureus/1-518	118	YEVN	EEREGDFSD	VDE	VI	EDIEDS.	DAEQVP	PIIA..	.SH	EARENDY..GTPVIVNGVA		
<i>Photobacterium damselae</i> /1-512	118	YEIS	EEREGDFSD	VVD	V	IIDDIAYS.	DADQVP	PLIA..	.SH	EAREDYY..GNPVIWVIGAA		
<i>Vibrio carribenthicus</i> /1-518	118	YEIN	PDQDM	FED	I	IEAWIEEES.	DAATNS	PLIC..	.IN	EAKEDDE..GNVIVNMCIA		
<i>Arsenophonus nasoniae</i> /1-486	111	YEIN	NNREDEFSL	VSE	VSY	ADDIKNS.	NAQQV	PLIA..	.FN	EAREDRA..GTPIVVNMGVA		
<i>Arsenophonus nasoniae</i> /1-515	116	YEIN	EEREGDFSD	VVD	V	DDIDIEDS.	DADQVP	PIFA..	.NH	EAKEDNN..GTPIVVNMGVA		
<i>Vibrio nigripulchritudo</i> /1-887	92	YEIS	ENSEDR..	R	LRS	ISIQEIE	NAENMP	III..	.HG	EAGEDAN..EH..EEPYVNSPAA		
<i>Arsenophonus nasoniae</i> /1-579	117	YVMR	QYTDR..RR	LDQL	LSLT	EILTA.	TAETV	PIVPEEILTFEDAR	PDL..		FVSISI	
<i>Vibrio nigrifrons</i> /1-507	111	YEIL	..DR.HRRFQD	PIR	EE	ITRS.	TVD	TVPII..	..ISSN	NLTDPNII..QD..II	ITISVVA	
<i>Arsenophonus nasoniae</i> /1-579	711	YEYL	TAAEDTFSGs	LRN	VTAEH	ILGVN	..QFAPTE	DTALs..	NLNSPP	PVLS..	..NIYFSLPEGNINHPYIAIPVV	
<i>Vibrio nigripulchritudo</i> /1-887	166	YLRN	PNSNVRLEN	LAAY	DL	DTALS.	NLNSPP	PVLS..				

<i>EHEC/1-330</i>		<i>a6</i>		<i>a7</i>
<i>E. coli/1-330</i>				
<i>E. coli/1-328</i>				
<i>Citrobacter rodentium/1-330</i>				
<i>E. coli_O111:H-/1-328</i>	167	P DTD SY EMP S WQE EGLI HEII IHHV TGGS SDP . SGDS NIEL GPT E IL AR RVAQ EL GWS . VPD .		
<i>Yersinia alvdovae/1-339</i>	167	P DTD SY EMP S WQE EGLI HEII IHHV TGGS SDP . SGDS NIEL GPT E IL AR RVAQ EL GWS . VPD .		
<i>E. coli/1-351</i>	167	P DTD SY EMP S WQE EGLI HEII IHHV TGGS SDP . SGDS NIEL GPT E IL AR RVAQ EL GWS . VPD .		
<i>S. typhimurium/1-330</i>	167	P DTD SY EMP S WQE EGLI HEII IHHV TGGS SDP . SRDS NIEL GPT E IL AR RVAQ EL GWP . IPD .		
<i>E. coli/1-229</i>	167	P DTD SY EMP S WQE EGLI HEII IHHV TGGS SDP . SGDS NIEL GPT E IL AR RVAQ EL GWS . VPD .		
<i>Vibrio tasmaniensis/1-518</i>	68	P DTD SY EMP S WQE EGLI HEII IHHV TGGS SDP . SGDS NIEL GPT E IL AR RVAQ EL GWS . IPE .		
<i>Vibrio_sp./1-518</i>	171	P SI HSS D YPW WQEA GLI HEVI HHIT GS SD ADS LNRH . . GPT E IL AR RVAQ EL GWS . VPD .		
<i>Vibrio_azureus/1-518</i>	171	P RID S SE YSW WQEA ALI HEVI HHIT GS SDT D DEV DRH . . GPT E IL AR QIAN EL HWP . IPI .		
<i>Photobacterium damseliae/1-512</i>	170	P STH S E YSW WQEA ALI HE MI HHIT GS SDP D SE VRH . . GPT E IL AR QVARE NNW . VPS .		
<i>Vibrio_carribenthicus/1-518</i>	164	P SFL S GRY SW WQEA ALI HEV I HHV TGGS SDT D THEEN KQ . . GPT E IL AR QMVA EL HWT . IPT .		
<i>Arsenophonus_nasoniae/1-486</i>	169	P HINT S E YFW WQEA SLI HEVI HQ IT AS QDT D ELD DRH . . GPT E IL AR QIAN EL NW P . IPT .		
<i>Arsenophonus_nasoniae/1-515</i>	143	P SY T S RDYN H WQSS SLI HE LI HLT GS SDP . . ES NED RLL GPT E IL T Q RVA I D LN W H . IPQ .		
<i>Arsenophonus_nasoniae/1-507</i>	170	P NHN S VHYP FW QT GLI HEII I HA IT DAG DGP PENE Q TARL GPT E IL AN QTA RE IS WN . IPV .		
<i>Vibrio_nigripulchritudo/1-887</i>	162	P NYL S EHYP I WQY T LI HEII I HAI T NAG DGP PEGEE . . RIGPT D JL AY RIASE M DLR . IQR .		
<i>Arsenophonus_nasoniae/1-579</i>	765	P ADP T PRYE H WQGLI HE IM HVL TD AKD P . . SSASE N RL GPT E IL AR KVMA EM GKNN IFD .		
	220	P NPES Y FYP L WQAC LI HEII I HAI T NMAD PSL DGD PR . . LGPT E IL T Q VVIE MNVV . IRYP .		

			α8		η4		α9
EHEC/1-330		
EHEC/1-330	224	F K G Y A E P E R E A H L R L R N L N A L R Q A A M R H E E N E R A F F E R L G T I S				
E._coli/1-328	224	F K G Y A E P E R E A H L R L R N L N A L R Q A A M R H E E N E R A F F E R L G M I S				
Citrobacter rodentium/1-330	224	F I G Y A E P D P R E A H L R G R N L N A L R Q A A M R H E D N E R T F E R L G M I S				
E._coli_O111:H-/1-328	224	F K G Y A E P E R E A H L R L R N L N A L R Q A A M R H E K N E R A F F E R L G T I S				
Yersinia alvdovae/1-339	233	F I G Y A E P D P R E A H L R A R N L N A L R Q A A M R H E S N E G A F F E R L D M I S				
E._coli/1-351	224	F K G Y A E P E R E A H L R L R N L N A L R Q A A M R H E E N E R A F F E R L G T I S				
S._typhimurium/1-330	224	F T G Y A S P D P R V A H L R T R N L N A L R Q T A T R H E D N E E A F F E R L D V I S				
E._coli/1-229	125	F K G Y A E P E R E A H L R L R N L N A L R Q A A M R H E E N E R A F F E R L G T I S				
Vibrio_tasmaniensis/1-518	226	F S G Y G D P S R I Q A L K E R N F S A L I Q T I N R H P S E A S A L L O R L I S I I A				
Vibrio_sp./1-518	226	F K G Y A D P E R T Q A L K E R N F Q A L L H T I H R H P Y E A E A L L N R L A T I S				
Vibrio_azureus/1-518	225	F R G Y S D P E R T E G I Q R R N F N A L I D T I N R H P A E A S E L M S R L I T A I A				
Photobacterium_damselae/1-512	219	F K G Y S D P A R V E A I Q R E D F H S L L E M F O R G S E L G F L F T R L I T A I A				
Vibrio_carribenthicus/1-518	224	F K G Y A D P E R T R A I A E R G F R A L L Q T I D R H P S E T V A L L E R L I T A I S				
Arsenophonus_nasoniae/1-486	199	F R S Y N S P E R L Q A I R A R N F R S L L E S I D R H P R R E N E L L E R L I S I S				
Arsenophonus_nasoniae/1-515	228	F T A Y D S V D R N E F I S E Y E F O S L R O G I Y R H H O R G Y E L L E R L C D I N				
Arsenophonus_nasoniae/1-507	218	F N N Y Y S L E R I R A I Y E H D F A C L C E T I Y R H H E H P T E V I N R L F A I N				
Vibrio_nigripulchriflido/1-887	823	F A N Y Q P Q P E R E A W I K Q R D H L A V D L I E R V P V D R A L M K R L D K V A K Q R G T A K Q L I Q T				
Arsenophonus_nasoniae/1-579	277	OSRVM F N S Y D P D P R I R A A O Q E W V A L L H C L F R . S E N L S E R V N R L E I G					

<i>EHEC</i> /1-330		T.T	75	
<i>EHEC</i> /1-330
<i>E. coli</i> /1-328	267	DRYEASP.DFTEYSAVSNIGYGFQIQQHDFPGLAINDNLQDA.	.NQIQLYH	GAP.YIFTFG
<i>Citrobacter rodentium</i> /1-330	267	DRYEASP.DFTEYSAVPNIGYGFQIQQHDFPGLAINDNLQDA.	.NQIQLYH	GAP.YIFTFG
<i>E. coli</i> _O111:H-/1-328	267	DRYEASP.DFTEYSAVSNIGYGFQIQQHDFPGLAINDNLQDA.	.NQIQLYH	GAP.YIFTFG
<i>Versinia aldobae</i> /1-339	276	DKYEVSP.DFTEYAVVSNIEYGLQIQQHDFPGLAIDDNLQDA.	.NQIQLYH	GAP.YIFTFG
<i>E. coli</i> /I-351	267	DRYEASP.DFTEYSAVSNIGYGFQIQQHDFPGLAIDDNLQDA.	.NQIQLYH	GAP.YIFTFG
<i>S. typhimurium</i> /1-330	267	EGYEASA.DFTEYPVMSDMVKELNKPQDFPGLVINDNTMADPDQIQLYH	QP.YIFTFV	
<i>E. coli</i> /1-229	168	DRYEASP.DFTEYSAVSNIGYGFQIQQHDFPGLAINDNLQDA.	.NQIQLYH	GAP.YIFTFG
<i>Vibrio tasmaniensis</i> /1-518	269	RGSKASTSFLLTSFCSSM....NVLDPL....DRPNFD...DFSMCAA.	FPTG
<i>Vibrio</i> _sp./1-518	269	SGLKASP.RFSLLTSFCSSG....IDLP.....KLPDFDDD....DFSMCAA.	FPTG
<i>Vibrio</i> _azureus/I-518	268	SGLRASP.QFTLLTSSYCSS....QIDLPL....PLPDFDDD....HFNMAA.	FPTG
<i>Photobacterium</i> _damseliae/I-512	262	GKKKASP.DFGTLTSFCSSSEG....IRSFPP....KYPDHD...DFSGGA.	FPLP
<i>Vibrio</i> _carribenthicus/1-518	267	RGLKASA.SFSLLTSSFCSSG....IEELP....KLPDFDDD....DFSMCAA.	FPTG
<i>Arsenophonus</i> _nasoniae/1-486	242	ENVGASS.DFSSLSESSNDS....SVSTAACRFEHGDSDDD....FTST.	FFRG
<i>Arsenophonus</i> _nasoniae/1-515	271	HLLTSSP.NAGIATLSDEC....LIPT....HSSDEDT....PAHWGAF.	LLSLG
<i>Arsenophonus</i> _nasoniae/1-507	261	FFVENRPEDLNHPPQYFRY....LLPD....HLFCDDN....DVQICSA.	FFMG
<i>Vibrio</i> _nigripulchritudo/1-887	877	DQYPPAP.LLQP....	
<i>Arsenophonus</i> _nasoniae/1-579	324	DRMVAFIT....AAQPYAHFP....NAELLSTIC....PFDDD....QEHLPFP.	FPSG

EHEC/1-330

EHEC/1-330	323	.	DVDKHNQQ.
E._coli/1-328	323	.	DVDRHN.
Citrobacter_rodontium/1-330	323	.	DVDKHNQR.
E._coli_O111:H-/1-328	323	.	DVDRHN.
Yersinia_albovare/1-339	332	.	GADRHNR.
E._coli/I-351	324	CGQTQSAMIRLCSDIGLLLIDINGVDDG.	.
S._typhimurium/1-330	325	.	DKHNQR.
E._coli/1-229	224	.	DVDRHN.
Vibrio_tasmaniensis/1-518	312	ASASNS . . . GO.	CSFDVKDRAKPISDSIHFE . GGQFLIKERDLINLNLSVAKLA
Vibrio_sp./1-518	312	ASASSS . . . GS.	CSLDAAGRVDPVSSSITFE . GGQLLIQORDLKLNLNLLVAELA
Vibrio_azureus/1-518	311	ASASQS . . . VGR.	CSIDNALRVQPVTEDIRFA . AGYPLIKRDFKNLNLLVAKQA
Photobacterium_damselae/1-512	304	SASADS . . . SVE.	CTFDVLNRIEPVDDSIKFE . GGNLLIKNDFKNLNLRLVAQLS
Vibrio_caribbeanicus/1-518	310	ASASNL . . . GE.	CSLDAAGRVEPVSSSITFE . GGQLLIQORDLKLNLNLLVAKLA
Arsenophonus_nasoniae/1-486	287	AKAANY . . . LC.	HKKV . . . IIFE . NDLSLEYWHLKKYNNLIAELA
Arsenophonus_nasoniae/1-515	312	AHATHQ . . . R.	KDVFATKYKN . . . IVFNKEELPLTQWHELEKYGGLVIAEKA
Arsenophonus_nasoniae/1-507	303	ASASHC . . . K.	NNLFAAKYEN . . . IIFS . ENLPLTQFHLEKYNLVIAAMA
Vibrio_nigripulchritudo/1-887	.	.	.
Arsenophonus_nasoniae/1-579	364	AHGISV . . . A.	KSFFCINHSD . . . IIFN . DGLKLSNLHLIKYDAAATVA

EHEC/1-330

EHEC/1-330	.	.	.
E._coli/1-328	.	.	.
Citrobacter_rodontium/1-330	.	.	.
E._coli_O111:H-/1-328	.	.	.
Yersinia_albovare/1-339	.	.	.
E._coli/I-351	.	.	.
S._typhimurium/1-330	.	.	.
E._coli/1-229	.	.	.
Vibrio_tasmaniensis/1-518	360	FLRAKNGGGFYNKNWASWKSWYKASAW . KHLFGFGLYGYGQEQAQGNDIYNPYGIRFNDG	
Vibrio_sp./1-518	360	FMRAKNGGFYSKKSKSWKHWYKDSAW . KHLFGYGIYGYGLEEANGNSIYDPYGLTFNDG	
Vibrio_azureus/1-518	360	FLRAKNSGGFYAKNWWKSWYQASAW . KHAFGYGLYGYGLEQAMGNDLYNPYGLVFNDG	
Photobacterium_damselae/1-512	353	FLNAKKGSGFYRKWNDSWKSQASSW . KNGLNSGLYGYGHDESEGNDIYSPYGLTFNDG	
Vibrio_caribbeanicus/1-518	358	FLRAKNGGGFYSKNWESWKHWYKDSGW . KNLFGFYIYGYGLDEAEGNDIYSPYGLTFNDG	
Arsenophonus_nasoniae/1-486	325	SRVENGSGFYNNKKYKSWKEWYHSSAW . KTLGTYDGLSEIGKNASSKPYGEIFEDG	
Arsenophonus_nasoniae/1-515	355	VNRVEHGSFYKKKKYKSWKEWYQSLAW . KHVFGYGINGYQGAAAEGYFIYSPYGEIFEDC	
Arsenophonus_nasoniae/1-507	345	VNRAEQGSGFYKEECESWKEWYKSKERWKYLFGKGKIFEHGKYEAKGSFIYGPYGLFDDG	
Vibrio_nigripulchritudo/1-887	.	.	.
Arsenophonus_nasoniae/1-579	406	VNLVKNGSGFNFNKEYLLWDWYHDKEW . RGIFKSGIYDYGK . . . NLFNNECGLVFKDG	

EHEC/1-330

EHEC/1-330	.	.	.
E._coli/1-328	.	.	.
Citrobacter_rodontium/1-330	.	.	.
E._coli_O111:H-/1-328	.	.	.
Yersinia_albovare/1-339	.	.	.
E._coli/I-351	.	.	.
S._typhimurium/1-330	.	.	.
E._coli/1-229	.	.	.
Vibrio_tasmaniensis/1-518	419	SFSIGVVG . RDINNSTRS . . . DNFTTLAGTNWHTIRYAGQMFFDLNGRPVALVITDPLT	
Vibrio_sp./1-518	419	SFSIGVVG . QDVNKNTIS . . . DNFTTLAGTNWHTIKYAGQMFFDKNGRPVALVITDALT	
Vibrio_azureus/1-518	419	SFSVGVVG . KDVTSSTKS . . . DNFTTLAGTNWHTIKYAGQMFFDRNGRPVALVITDMMT	
Photobacterium_damselae/1-512	412	SFSIGFSSRKHINDNTKD . . . DNFVKLNNANWSSFYAGQMFFDKNKRPVALVITEPLN	
Vibrio_caribbeanicus/1-518	417	SFSIGVVG . QDVTKSTS . . . DNFTKLSGTNWHTIKYAGQMFFDMNGRPVALVITDVLT	
Arsenophonus_nasoniae/1-486	384	SFSVGVTS . DDAKKYGYN . . . DTWTNYFKDDKETI . YAGQMFFDKNRPVALLTITNKIS	
Arsenophonus_nasoniae/1-515	414	SFAVGITG . TDIKKWGN . . . DNWTNLAGENWSSAS . AGQMYFDKNGRPVAIVMTNLIT	
Arsenophonus_nasoniae/1-507	405	SFAVGVKG . KDIIEYGYN . . . DNWTTLSGKNWYPLEYAGQIYFDKNRPPIAVMTNPPI	
Vibrio_nigripulchritudo/1-887	.	.	.
Arsenophonus_nasoniae/1-579	460	SFLVGISG . MMLNSLKKNFGHEDKWVK . ENKNLINNSSFVGMFFDKNGRPVAVVFTVADK	

EHEC/1-330

EHEC/1-330	.	.	.
E._coli/1-328	.	.	.
Citrobacter_rodontium/1-330	.	.	.
E._coli_O111:H-/1-328	.	.	.
Yersinia_albovare/1-339	.	.	.
E._coli/I-351	.	.	.
S._typhimurium/1-330	.	.	.
E._coli/1-229	.	.	.
Vibrio_tasmaniensis/1-518	474	GGLGSGWSFVYTNGQWQYESQDDWDERLLNHS . VLSMDSDAPQFSL	
Vibrio_sp./1-518	474	GGFGSGWSFIYNNNGKQWYESKDDWDRFFKGS . TLSLDPHAPKFKL	
Vibrio_azureus/1-518	474	GGVGSWSFIYSEGKWLYPEPHDDWERYFANS . ELSLDAHAPQFIR	
Photobacterium_damselae/1-512	468	AAFGAGWSIYIKDGKWHYEAQDDWDQRLFKDS . TLSLDPHAPQFIN	
Vibrio_caribbeanicus/1-518	472	GGFGSGWSFVYNNNGKWEYESKDDWDRFFQGSSTLSDSNAPKFSTH	
Arsenophonus_nasoniae/1-486	438	GYLGKGSSLIYIKGGWTWNKDTWDIHNFKD . YESLDRYAPRFLIKNI	
Arsenophonus_nasoniae/1-515	468	GFFGAGWSFIYNEGKWEYESKDDWDRFAGR . TESLDPYAPRFLIKNI	
Arsenophonus_nasoniae/1-507	460	GLLGDGWSFIYNKGKWEYESKDDWDRFAGR . TESLDPYAPRFLIKNI	
Vibrio_nigripulchritudo/1-887	.	.	.
Arsenophonus_nasoniae/1-579	518	GFFGSGWSFIYNNKKWEYVLDENWDMNKFFGN . TKSLDSHAPRFLINKYIVKSIDSEHES	

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	ggaaatccatatgaaaattcccttattacagtcc tccccccgggtgcgtattgtgtttgtccac	Amp
pMS846	NleC	19-330	EHEC str. Sakai	EHEC str. Sakai DNA	C-term Intein-CBD	pTYB2	ggaaatccatatgtgcgtccattgc tccccccgggtgcgtattgtgtttgtccac	Amp
pMS1120*	NleC E115A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	CAGAAACGAATAACGCGCTTAACGAAGAACATTC GAAGATTCTCGTTAAGCGGTATTGTTCTG	Amp
pMS1121*	NleC E118A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	CGAATACGAGCTTAACGCAATCTCTGTC GACAGAAGATTCTGCCTTAAGCTCGTATTG	Amp
pMS1122*	NleC E119A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	CGAATACGAGCTTAACGAAAGCATCTCTGTC CATCAATTGACAGAAAGATGCTCGTTAAGCTCGTATTG	Amp
pMS1123*	NleC D139A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	CGAATTATATGAATATGCTGTCGGCAAGAGC GCTCTGCCGACAGCATATTATATAATTG	Amp
pMS1124*	NleC E150A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	GCCAATTTCCTCCATTGTCAGCAGGAGAAACG CGTTTCTCTGCTGCAAAATGGGAAATTG	Amp
pMS1125*	NleC D198A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	GCGATCCATCTGGAGCTAGTAATATAGAGTTAG CTTAACCTCTATATTACTAGCTCCAGATGGATCG	Amp
pMS1134*	NleC E202A	1-330	EHEC str. Sakai	pMS692	C-term Intein-CBD	pTYB2	GGAGATAGTAATATAGCGTTAGGACCCACCG CGGTGGGTCTAACGCTATTAATTCTATCTC	Amp
pMS1013*	RelA	17-291	Human	J Goodrich, Univ of CO	10x-His-SUMO	pET24d	GGATCTGGCCCTATGTGGAGATC CGGCTCAATCTGGCAGGTACTGGAAATTCC GTACCGGCTCAATCTGGCAGGTACTGGAAATTCC	Kan
pMS845	RelA	1-210	Human	J Goodrich, Univ of CO	6x-His	pET24d	ATcatgCCATGGacgactgtcccc CATCATgtcgacTTAatccccaccggaggcag	Kan
pMS1020*	RelA	29-49	Human	J Goodrich, Univ of CO	10x-His-SUMO-RelA-BamD	pET24d	GGATCACCATGGCAGCGCGTATGCGCTTCCGC CTTATGTATTGCTGCTGTTGCGGCGATG AGCTCTTATGTATTGCTGCTGTTGCGGCGATG	Kan
pMS1032	RelA R35A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CATCGCTTGCgcCTACAAGTGCAGGGGGCGCT GCACTTGTAGgcGAAGCGCATGCCCGCTGCT	Kan
pMS1033	RelA Y36A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	GCGCTTCGCgcCAAGTGCAGGGGGCGCTCCG CTCGCACTTgcGCAGGAGCGCATGCCCGCT	Kan
pMS1034	RelA K37A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CTTCCGCTACgcGTGCGAGGGGCGCTCCGCG CCCCTCGCACgcGTAGCGGAAGCGCATGCCCG CCGCTACAAGgcCGAGGGGCGCTCCGCGGGCA	Kan
pMS1035	RelA C38A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CGGCCCTCGgcCTTGAGCGGAAGCGCATGC TACAAGTGCAGGGCGCTCCGCGGGCAGCATCC	Kan
pMS1036	RelA E39A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CGGAGCGCCCCgcCGCACTTGTAGCGGAAGCG AAGTGCAGGGCgcCGCACTTGTAGCGGAAGCG CGCGGAGCGCgcCTCGCACTTGTAGCGGAAG	Kan
pMS1037	RelA G40A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	GTGCGAGGGGgcCTCCGCGGGCAGCATCCCAG GCCCGCGGAGgcCCCCCTCGCACTTGTAGCGGA CGAGGGGGCGcCGGGGGCAGCATCCCAGGC	Kan
pMS1038	RelA R41A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	CTGCCCGCGGcGCGCCCTCGCACTTGTAGCG ggagcgCAGCCGCACCTGGTCATC gtacctcaCGTTCGCTTCTGTCC	Kan
pMS1039	RelA S42A	17-291	Human	pMS1013	10x-His-SUMO	pET24d	ctcaCCGTTCCGCTTCTGTAGC ggagcgACAGCTGGGCCATACCTTC gtacctcaACGTTCCCTGCaCTTCTTC ctcaACGTTCTCTGCaCTTCTTC	Kan
pMS1114	RelB	124-413	Human	Functional Genomics Facility - Univ of CO	10x-His-SUMO	pET24d	ggagcgCAGCCGCACCTGGTCATC gtacctcaCGTTCGCTTCTGTCC	Kan
pMS1115	p50	39-363	Human	J Goodrich, Univ of CO	10x-His-SUMO	pET24d	ggagcgACAGCTGGGCCATACCTTC gtacctcaACGTTCCCTGCaCTTCTTC ctcaACGTTCTCTGCaCTTCTTC	Kan

Supplementary table ST1.