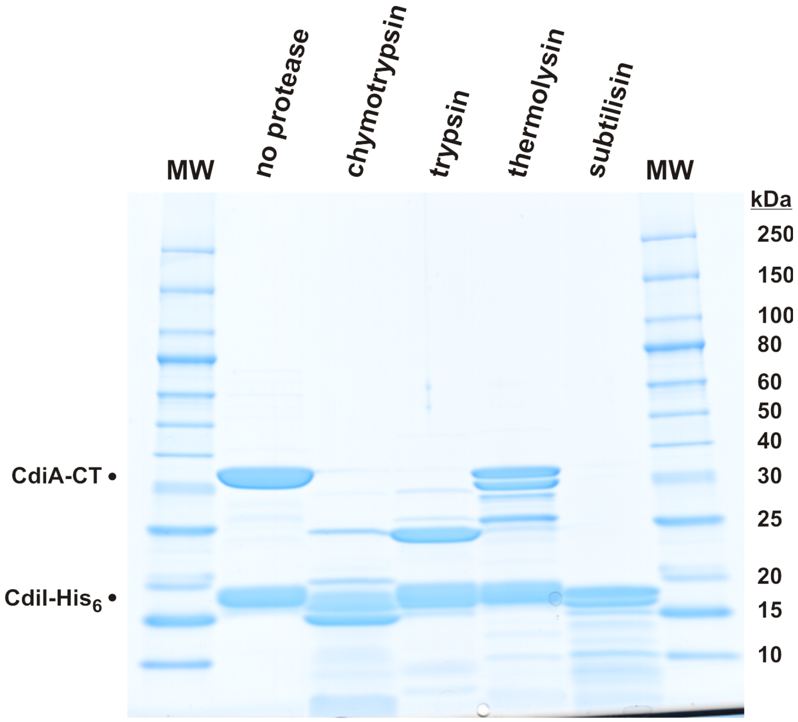

Figure S1. Limited proteolysis of the CdiA-CT/CdiI^{STECO31} complex. Affinity purified complex was treated with chymotrypsin, trypsin, thermolysin or subtilisin and analyzed by SDS-PAGE. The masses of the molecular weight (MW) standards are given to the right of the gel.

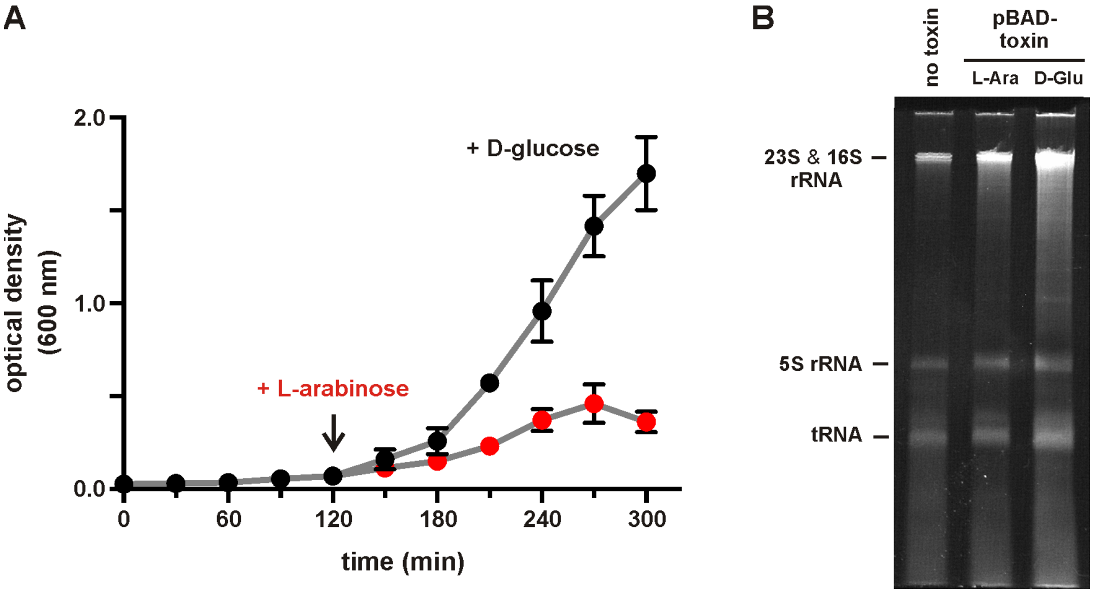

Figure S2. Activation of CdiA-CT$^{\text{STECO31}}$ in *E. coli* cells. A) *E. coli* X90 cells carrying pCH13308 were grown in LB media supplemented with 25 µg mL^{-1} tetracycline. After 2 h, cultures were split and one half supplemented with 0.4% D-glucose and the other with 0.2% L-arabinose. Cell growth was monitored by optical density at 600 nm (OD$_{600}$). The average OD$_{600}$ ± standard deviation is shown for two independent experiments. **B**) Total RNA was extracted from the cultures in panel A at 300 min, run on a denaturing polyacrylamide gel and stained with ethidium bromide. RNA from X90 cells that carry empty vector pCH450 was used as a no toxin control. The migration positions of transfer RNA (tRNA) and ribosomal RNAs (rRNA) are indicated.

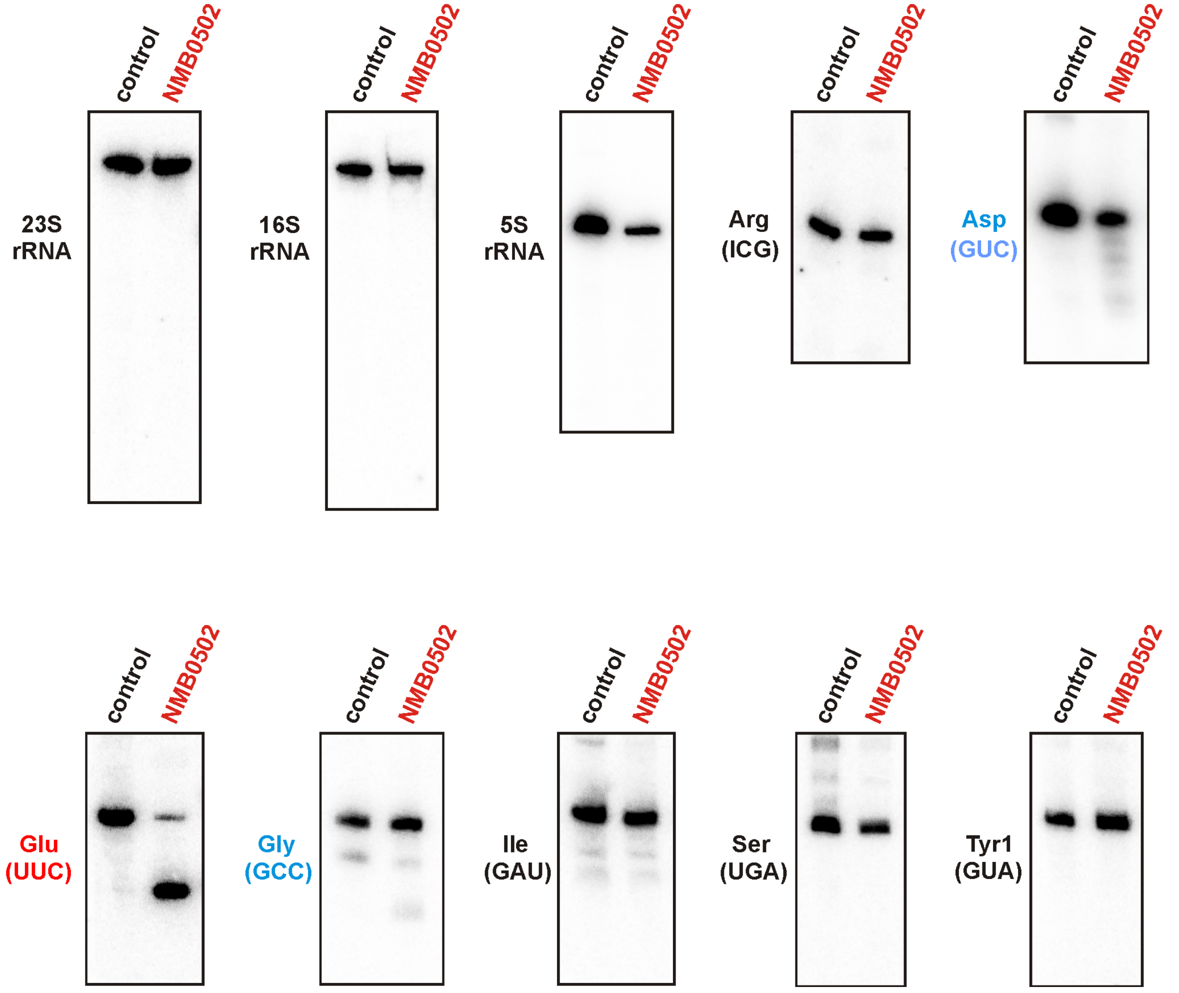

Figure S3. RNase activity of NMB0502. *E. coli* X90 cells carrying plasmid pCH13527 were grown in LB media supplemented with 25 μg mL^{-1} tetracycline. NMB0502 expression was induced with 0.4% L-arabinose for 2 h and then total RNA extracted for Northern blot analyses of the indicated rRNAs and tRNAs.

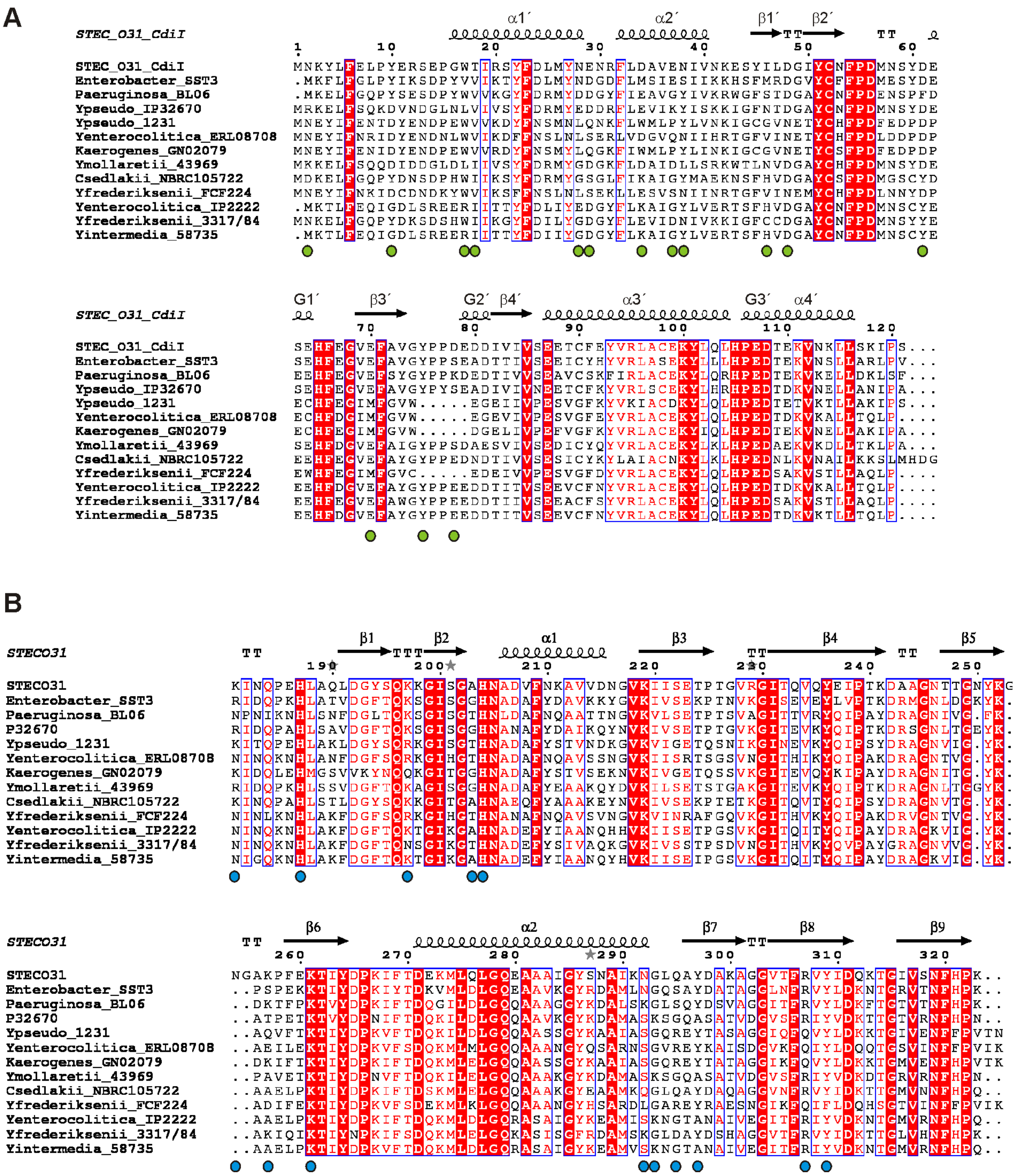

Figure S4. Close homologs of CdiI^{STECO31} and CdiA-CT^{STECO31}. A) CdiI^{STECO31} homologs were aligned using Clustal omega and rendered using the ESPript 3.0 server. Secondary structure elements for CdiI^{STECO31} are shown above the alignment. Sequences are from the following bacterial strains with NCBI protein identifiers given in parentheses: *E. coli* STEC_O31 (misannotated), *Enterobacter* sp. SST3 (WP_008500122.1), *P. aeruginosa* BL06 (unannotated), *Y. pseudotuberculosis* IP32670 (WP_032466733.1), *Y. pseudotuberculosis* 1231 (incorrect), *Y. enterocolitica* ERL08708 (misannotated), *K. aerogenes* GN02079 (misannotated), *Y. mollaretii* ATCC 43969 (WP_049611435.1), *C. sedlakii* NBRC 105722 (WP_042289363.1), *Y. frederiksenii* FCF224 (misannotated), *Y. enterocolitica* IP2222 (WP_019080485.1), *Y. frederiksenii* 3317/84 (WP_050129232.1), and *Y. intermedia* 58735 (WP_050075923.1). **B**) EndoU domains from close homologs of CdiA-CTSTECO31 were aligned as described in panel A. Secondary structure elements for CdiA-CT^{STECO31} are shown above the alignment. Sequences are from the following bacterial strains with NCBI protein identifiers given in parentheses: *E. coli* STEC_O31 (WP_001385946.1), *Enterobacter* sp. SST3 (WP_008500123.1), *P. aeruginosa* BL06 (ERY54707.1), *Y. pseudotuberculosis* IP32670 (WP_080987661.1), *Y. pseudotuberculosis* 1231 (WP_050090717.1), *Y. enterocolitica* ERL08708 (WP_050159162.1), *K. aerogenes* GN02079 (KLE56124.1), *Y. mollaretii* ATCC 43969 (WP_004876812.1), *C. sedlakii* NBRC 105722 (WP_052406297.1), *Y. frederiksenii* FCF224 (WP_050100436.1), *Y. enterocolitica* IP2222 (WP_019083962.1), *Y. frederiksenii* 3317/84 (WP_050129233.1), and *Y. intermedia* 58735 (WP_050075922.1).

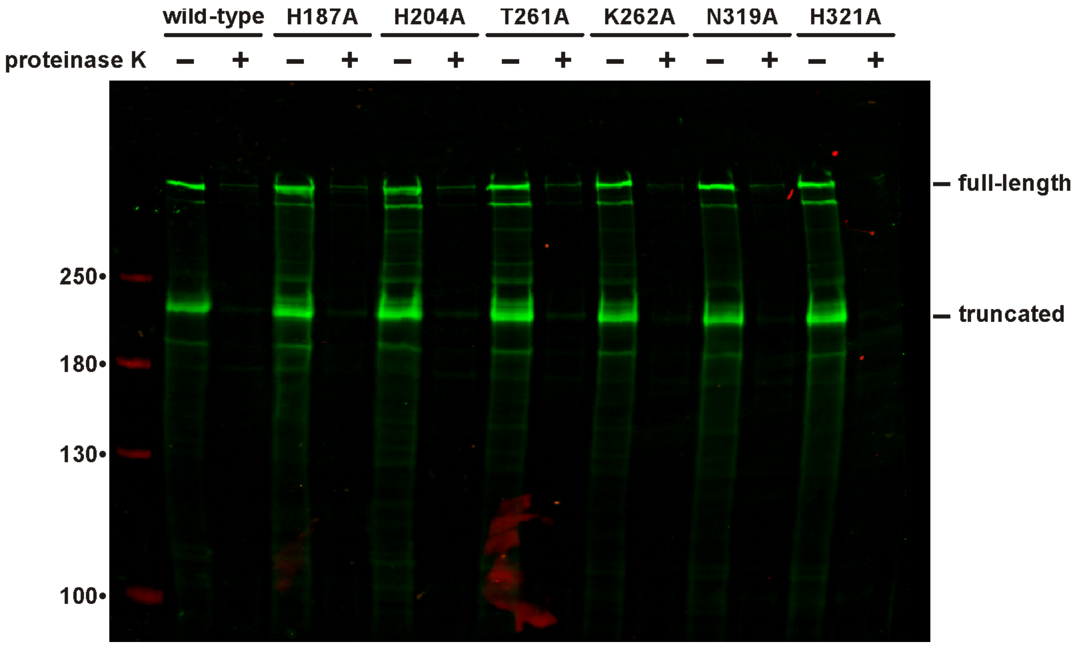

Figure S5. Immunoblot of chimeric CdiA proteins. Chimeric CdiA^{EC93} proteins that carry the indicated CdiA-CT^{STECO31} sequences were analyzed by immunoblotting using polyclonal antibodies to the N-terminal TPS domain of CdiA^{EC93}. Where indicated, extracellular protein was digested with proteinase K prior to extraction. Molecular mass standards (in kDa) appear in the red fluorescent channel. The migration positions of full-length and truncated CdiA are indicated on the right of the immunoblot.

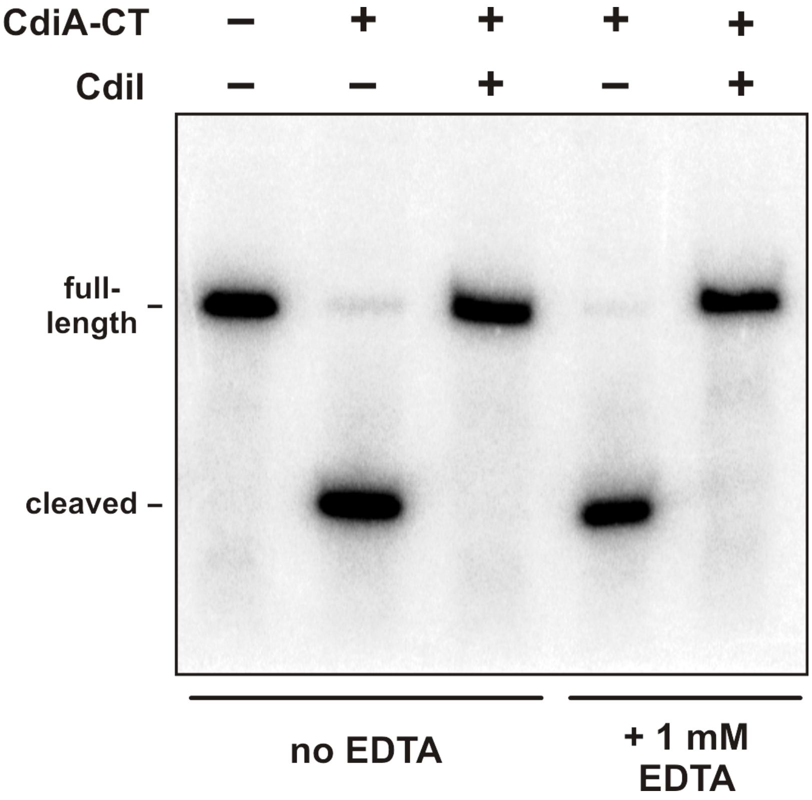

Figure S6. CdiA-CT^{STECO31} tRNase activity is metal-independent. Purified CdiA-CT^{STECO31} was incubated with *E. coli* total RNA for 30 min at 37 °C. Where indicated, reactions were supplemented with purified CdiI^{STECO31} and 1 mM EDTA. Reactions were analyzed by Northern blotting using an oligonucleotide probe to tRNA$_{UUC}^{Glu}$.

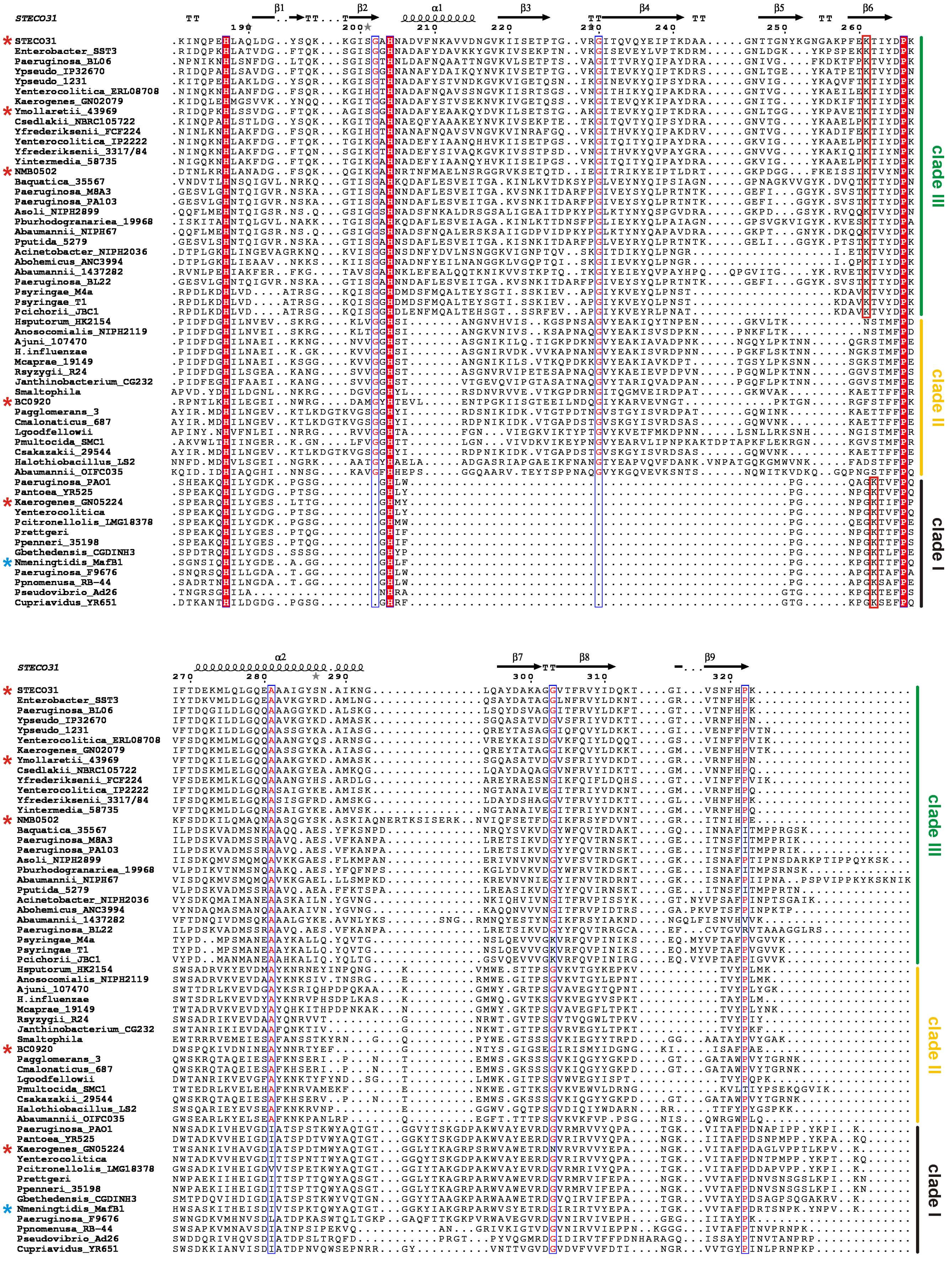
STECO31
TT
β1
β2
α1
β3
β4
β5
β6
190
200
210
220
230
240
250
260
STECO31
Enterobacter_SST3
Paeruginosa_BL06
Ypseudo_IP32670
Ypseudo_1231
Yenterocolitica_ERL08708
Kaerogenes_GN02079
Ymollaretii_43969
Csedlakii_NBRC105722
Yfrederiksenii_FCF224
Yenterocolitica_IP2222
Yfrederiksenii_3317/84
Yintermedia_58735
NMB0502
Baquatica_35567
Paeruginosa_M8A3
Paeruginosa_PA103
Asoli_NIPH2899
Pburhodogranariea_19968
Abaumannii_NIPH67
Pputida_5279
Acinetobacter_NIPH2036
Abohemicus_ANC3994
Abaumannii_1437282
Paeruginosa_BL22
Psyringae_M4a
Psyringae_T1
Pcichorii_JBC1
Hsputorum_HK2154
Anosocomialis_NIPH2119
Ajuni_107470
H.influenzae
Mcaprae_19149
Rsyzygii_R24
Janthinobacterium_CG232
Smaltophila
BC0920
Pagglomerans_3
Cmalonaticus_687
Lgoodfellowii
Pmultocida_SMC1
Csakazakii_29544
Halothiobacillus_LS2
Abaumannii_OIFC035
Paeruginosa_PAO1
Pantoea_YR525
Kaerogenes_GN05224
Yenterocolitica
Pcitronellolis_LMG18378
Prettgeri
Ppenneri_35198
Gbethedensis_CGDINH3
Nmeningtidis_MafB1
Paeruginosa_F9676
Ppnomenusa_RB-44
Pseudovibrio_Ad26
Cupriavidus_YR651
clade III
clade II
clade I
STECO31
α2
β7
β8
β9
270
280
290
300
310
320
STECO31
Enterobacter_SST3
Paeruginosa_BL06
Ypseudo_IP32670
Ypseudo_1231
Yenterocolitica_ERL08708
Kaerogenes_GN02079
Ymollaretii_43969
Csedlakii_NBRC105722
Yfrederiksenii_FCF224
Yenterocolitica_IP2222
Yfrederiksenii_3317/84
Yintermedia_58735
NMB0502
Baquatica_35567
Paeruginosa_M8A3
Paeruginosa_PA103
Asoli_NIPH2899
Pburhodogranariea_19968
Abaumannii_NIPH67
Pputida_5279
Acinetobacter_NIPH2036
Abohemicus_ANC3994
Abaumannii_1437282
Paeruginosa_BL22
Psyringae_M4a
Psyringae_T1
Pcichorii_JBC1
Hsputorum_HK2154
Anosocomialis_NIPH2119
Ajuni_107470
H.influenzae
Mcaprae_19149
Rsyzygii_R24
Janthinobacterium_CG232
Smaltophila
BC0920
Pagglomerans_3
Cmalonaticus_687
Lgoodfellowii
Pmultocida_SMC1
Csakazakii_29544
Halothiobacillus_LS2
Abaumannii_OIFC035
Paeruginosa_PAO1
Pantoea_YR525
Kaerogenes_GN05224
Yenterocolitica
Pcitronellolis_LMG18378
Prettgeri
Ppenneri_35198
Gbethedensis_CGDINH3
Nmeningtidis_MafB1
Paeruginosa_F9676
Ppnomenusa_RB-44
Pseudovibrio_Ad26
Cupriavidus_YR651
clade III
clade II
clade I

Figure S7. Clades of bacterial EndoU domains. EndoU domain sequences from predicted CdiA proteins (except BC0920 and MafB1) were aligned using Clustal omega and rendered using the ESPript 3.0 server. Secondary structure elements for CdiA-CT^{STECO31} are shown above the alignment. Sequences are from the following bacterial strains with NCBI protein identifiers given in parentheses: *E. coli* STEC_O31 (WP_001385946.1), *Enterobacter* sp. SST3 (WP_008500123.1), *P. aeruginosa* BL06 (ERY54707.1), *Y. pseudotuberculosis* IP32670 (WP_080987661.1), *Y. pseudotuberculosis* 1231 (WP_050090717.1), *Y. enterocolitica* ERL08708 (WP_050159162.1), *K. aerogenes* GN02079 (KLE56124.1), *Y. mollaretii* ATCC 43969 (WP_004876812.1), *C. sedlakii* NBRC 105722 (WP_052406297.1), *Y. frederiksenii* FCF224 (WP_050100436.1), *Y. enterocolitica* IP2222 (WP_019083962.1), *Y. frederiksenii* 3317/84 (WP_050129233.1), *Y. intermedia* 58735 (WP_050075922.1), *N. meningitidis* MC58 (NMB0502: WP_002225606.1), *B. aquatica* ATCC 35567 (WP_051323530.1), *P. aeruginosa* M8A.3 (ERX84232.1), *P. aeruginosa* PA103 (EYT98551.1), *A. soli* NIPH 2899 (ENV60496.1), *P. burhodogranariea* DSM 19968 (WP_008910291.1), *A. baumannii* NIPH 67 (ENW51352.1), *P. putida* MTCC 5279 (WP_052352499.1), *Acinetobacter* sp. NIPH 2036 (EPG38010.1), *A. bohemicus* ANC 3994 (WP_004649228.1), *A. baumannii* 1437282 (EXB27428.1), *P. aeruginosa* BL22 (ERY15522.1), *P. syringae* pv. maculicola M4a (KPB79949.1), *P. syringae* pv. tomato T1 (EEB58969.1), *P. cichorii* JBC1 (WP_025259970.1), *H. sputorum* HK 2154 (WP_007526435.1), *A. nosocomialis* NIPH 2119 (ENU48137.1), *A. junii* CIP 107470 (WP_004913022.1), *H. influenzae* (WP_049380285.1), *M. caprae* DSM 19149 (WP_029103641.1), *R. syzygii* R24 (CCA87764.1), *Janthinobacterium* sp. CG23_2 (WP_054267428.1), *S. maltophilia* (WP_049455930.1), *B. cereus* ATCC 14579 (BC0920: WP_000056051.1), *P. agglomerans* str. 3 (KYM73161.1), *C. malonaticus* str. 687 (WP_050497093.1), *L. goodfellowii* (WP_026738097.1), *P. multocida* SMC1 (WP_059245917.1), *C. sakazakii* ATCC 29544 (WP_052367197.1), *Halothiobacillus* sp. LS2 (WP_066099932.1), *A. baumanii* OIFC035 (WP_002132044.1), *P. aeruginosa* PA01 (WP_010895621.1), *Pantoea* sp. YR525 (SFU93835.1), *K. aerogenes* GN05224 (WP_080959078.1), *Y. enterocolitica* (CRX52240.1), *P. citronellolis* LMG 18378

(SFD80598.1), *P. rettgeri* (WP_052219300.1), *P. penneri* ATCC 35198 (EEG86312.1), *G. bethesdensis* CGDNIH3 (WP_025287064.1), *N. meningitidis* 8013 (MafB1: CAX49352.1), *P. aeruginosa* F9676 (AKQ15993.1), *P. pnomenusa* RB-44 (AHB76644.1), *Pseudovibrio* sp. Ad26 (WP_063312316.1), and *Cupriavidus* sp. YR651 (SDD11164.1). EndoU clades are indicated to the right of the alignment. Domains marked with asterisks have been characterized here (*) or elsewhere (*).

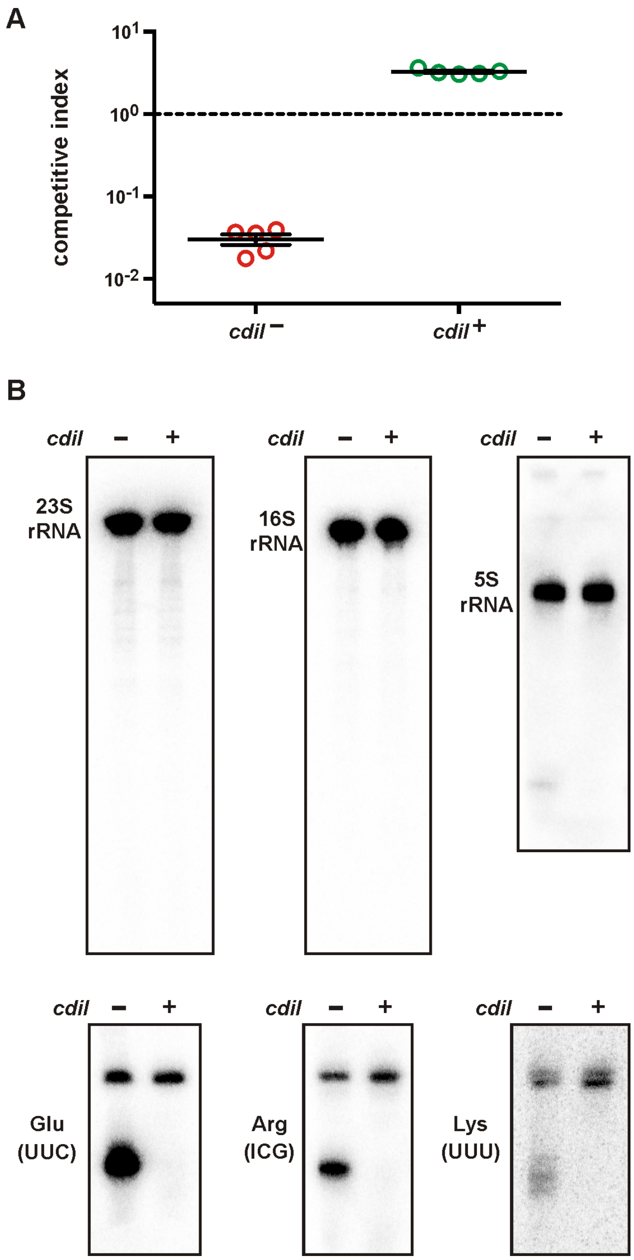

Figure S8. Growth inhibition and RNase activities of CdiA-CT$^{\text{GN05224}}$. A) *E. coli* inhibitors that deploy CdiA-CT$^{\text{GN05224}}$ were cultured at a 1:1 ratio with *E. coli* target cells that either lack (*cdiI*–) or carry (*cdiI*+) the *cdiI*$^{\text{GN05224}}$ immunity gene. Viable inhibitor and target bacteria were enumerated as colony forming units (cfu) at t = 0 and after 1 h of co-culture. Competitive indices $(\text{cfu}_{t\,=\,\text{final}}^{\text{targets}}/\text{cfu}_{t\,=\,\text{final}}^{\text{inhibitors}})/(\text{cfu}_{t\,=\,0}^{\text{targets}}/\text{cfu}_{t\,=\,0}^{\text{inhibitors}})$ for five independent experiments are reported together with mean ± standard error. **B**) RNA was extracted from the co-cultures in panel A for Northern blot analyses of the indicated rRNAs and tRNAs.

Table S1. Water-mediated hydrogen bonds at CdiA-CT/CdiI^{STECO31} interface.

water number	CdiA-CT atom	distance (Å)	CdiI atom	distance (Å)
HOH 20	Ser195 (OG)	3.06	Asp61 (OD2)	2.98
HOH 20	Ser195 (OG)	3.06	Ser63 (OG)	3.22
HOH 78	Lys197 (O)	2.58	Asn53 (OD1)	3.19
HOH 11	Lys198 (O)	3.04	Asp48 (OD1)	2.82
HOH 11	Lys198 (O)	3.04	Tyr51 (OH)	2.81
HOH 32	Gln235 (OE1)	2.84	Arg31 (NH1)	3.30
HOH 32	Gln235 (OE1)	2.84	Arg31 (NH2)	3.14
HOH 6	Tyr236 (OH)	2.67	Asp48 (OD1)	2.76
HOH 32	Pro258 (O)	2.98	Arg31 (NH1)	3.30
HOH 32	Pro258 (O)	2.98	Arg31 (NH2)	3.14
HOH 8	Glu260 (N)	2.82	Ile46 (O)	2.94
HOH 1	Glu260 (OE1)	2.89	Asp48 (N)	2.91
HOH 102	Glu260 (OE1)	3.02	Glu 29 (OE1)	3.11
HOH 1	Glu260 (O)	2.71	Asp48 (N)	2.91
HOH 6	Glu260 (O)	2.69	Asp48 (OD1)	2.76
HOH 5	Thr262 (N)	2.93	Asp48 (OD2)	2.78
HOH 13	Gln295 (N)	2.82	Ser21 (OG)	2.87
HOH 164	Gln295 (OE1)	2.83	Val73 (O)	2.72
HOH 164	Gln295 (OE1)	2.83	Asp81 (OD1)	3.20
HOH 2	Ala296 (O)	2.78	Tyr4 (OH)	2.65
HOH 102	Arg307 (NH2)	3.01	Glu29 (OE1)	3.11
HOH 29	Asn319 (ND2)	2.87	Tyr75 (N)	2.88
HOH 29	Ser318 (OG)	3.07	Tyr75 (N)	2.88

Table S2. Bacterial proteins that contain C-terminal EndoU domains*

N-terminal domain	internal domains	Bacterial species and strain	NCBI reference	protein length	EndoU clade	signal sequence	predicted immunity protein
DUF4280/PAAR-like (PF14107)		*Clostridium* sp. ASBs410	WP_025233650.1	467	III	no	WP_001188529.1
	S-type Pyocin (PF06958)	*Marinobacter excellens* HL-55	WP_052503287.1	524	II	no	WP_044388809.1
	S-type Pyocin (PF06958)	*Marinobacter excellens* LAMA 842	KXO10026.1	540	II	no	WP_061332007.1
PrsW (PF13367)		*Bacillus* sp. Leaf13	WP_082527539.1	687	III	no	WP_056524806.1
PrsW (PF13367)		*Bacillus butanolivorans* DSM 18926	WP_083450798.1	687	III	no	WP_053346692.1
PrsW (PF13367)		*Bacillus* sp. Soil768D1	WP_082612854.1	730	III	no	WP_057217521.1
PrsW (PF13367)		*Sporosarcina globispora* DSM 4	WP_082341174.1	721	III	no	WP_053435372.1
PrsW (PF13367)		*Bacillus novalis* NBRC 102450	WP_066086865.1	677	III	no	WP_066086866.1
PrsW (PF13367)		*Bacillus litoralis* C44	WP_066323900.1	739	III	no	WP_066325712.1
	phage Mu F (PF04233)	*Paeniclostridium sordellii* R31809	WP_057569752.1	548	II	no	WP_057569753.1
	phage Mu F (PF04233)	*Paeniclostridium sordellii* SSCC35109	WP_057567421.1	549	II	no	WP_057567422.1
	phage Mu F (PF04233)	*Clostridium baratii* 2789STDY5834907	WP_055225338.1	552	II	no	WP_054198869.1
	phage Mu F (PF04233)	*Clostridium paraputrificum* LH025	WP_099328373.1	554	II	no	WP_099328374.1
	phage Mu F (PF04233)	*Clostridium tetani* ATCC 453	WP_080744638.1	537	II	no	WP_035141751.1
	phage Mu F (PF04233)	*Clostridium* phage phiCT453A	YP_009216671.1	537	II	no	YP_009216672.1
	phage Mu F (PF04233)	*Clostridium tetani* ATCC 454	WP_080952904.1	537	II	no	WP_035141751.1
	phage Mu F (PF04233)	*Clostridium fallax* DSM 2631	SHE61975.1	536	II	no	SHE62005.1
	phage Mu F (PF04233)	*Paracoccus sanguinis* DSM 29303	WP_052176216.1	545	I	no	WP_036731562.1
SpvB (PF03534)	Rhs repeats; RHS core	*Vibrio harveyi* VHJR4	WP_049536940.1	2,277	I	yes	WP_017817642.1
SpvB (PF03534)	Rhs repeats; RHS core	*Vibrio harveyi* VH5	WP_049536940.1	2,277	I	yes	WP_017817642.1
SpvB (PF03534)	Rhs repeats; RHS core	*Vibrio* sp. L85	WP_079325389.1	2,277	I	yes	WP_017817642.1
SpvB (PF03534)	TcdB; Rhs repeats; RHS core	*Chitinimonas koreensis* DSM 14426	WP_084300605.1	2,189	I	no	unannotated
SpvB (PF03534)	VCBS; TcdB; Rhs repeats; RHS core	*Motiliproteus* sp. MSK22-1	WP_076715109.1	2,137	I	yes	WP_076715107.1
SpvB (PF03534)	Rhs repeats; PT-HINT (PF07591)	Actinobacteria bacterium OK074	KPI03717.1	2,301	I	yes	KPI03718.1
SpvB (PF03534)	Rhs repeats; RHS core; PT-HINT (PF07591)	*Streptomyces* sp. SolWspMP-sol7th	SCD57710.1	1,602	I	yes	WP_093853274.1
	Laminin_G_3; Rhs repeats; RHS core; PT-HINT (PF07591)	*Micromonospora echinospora* ATCC 15837	SCF34431.1	3,261	I	yes	WP_088984183.1
DNA_pol3_gamma3 (PF12169)	Rhs repeats; PT-HINT (PF07591)	*Streptomyces* sp. CC77	WP_071271283.1	2,321	I	yes	WP_071271286.1
	Rhs repeats; PT-HINT (PF07591)	*Streptomyces anulatus* ATCC 11523	WP_079044253.1	2,236	I	yes	WP_057665403.1
	PT-HINT (PF07591)	*Paenibacillus* sp. 1ZS3-15	WP_068667659.1	2,355	II	yes	WP_068667660.1
	Rhs repeats; PT-HINT (PF07591)	*Microlunatus soli* DSM 21800	WP_091527202.1	1,985	I	no	unannotated
	Rhs repeats	*Microbacterium testaceum* StLB037	WP_013586481.1	1,794	I	no	WP_013586482.1
	Rhs repeats	*Rathayibacter tritici* NCPPB 1953	WP_068252818.1	1,856	I	no	WP_068252815.1
	Rhs repeats	*Cellulomonas massiliensis* JC225	WP_019137609.1	1,396	I	yes	WP_019137608.1*
	Rhs repeats; PT-HINT (PF07591)	*Mycobacterium tuberculosis* 401416	CNE28938.1	2,321	I	yes	end of contig
Phage_GPD (PF05954)		*Paenibacillus glacialis* DSM 22343	WP_068533605.1	943	II	no	WP_068533603.1
Phage_GPD (PF05954)	phage_base_V (PF04717)	*Paenibacillus wulumuqiensis* Y24	WP_052723770.1	1,017	III	no	unannotated
Phage_GPD (PF05954)	VgrG superfamily	*Paenibacillus durus* ATCC 35681	WP_025698576.1	992	III	no	WP_025698578.1
Phage_GPD (PF05954)	VgrG superfamily	*Paenibacillus polymyxa* SC2	WP_013372466.1	965	II	no	WP_013372467.1
Phage_GPD (PF05954)	VgrG superfamily	*Paenibacillus polymyxa* KF-1	WP_058830052.1	965	II	no	WP_013372467.1
Phage_GPD (PF05954)		*Paenibacillus polymyxa* ND25	WP_064961954.1	965	II	no	WP_013372467.1
Phage_GPD (PF05954)		*Paenibacillus polymyxa* 1-43	WP_025676073.1	965	II	no	WP_013372467.1
Herpes_BLLF1 (PF05109)		*Nocardia asteroides* NBRC 15531	GAD85392.1	852	II	no	unannotated
Herpes_BLLF1 (PF05109)		*Pilimelia anulata* NRRL B-3741	WP_081723179.1	776	II	no	unannotated
Herpes_BLLF1 (PF05109)		*Nocardia asteroides* DSM 43373	SFL70581.1	886	II	no	unannotated
DNA_pol3_gamma3 (PF12169)	Spc7 (PF15402); Pneumovirinae glycoprotein G (PF05539); cADP-ribose hydrolase (PF02267); sialomucin (PF05283)	*Deinococcus aquatilis* DSM 23025	WP_020621305.1	1,686	III	no	WP_019008896.1
	DNA_pol3_gamma3 (PF12169); Nucleotidyl transferase AbiEii toxin (PF08843)	*Nocardia harenae* NBRC 108248	WP_067654967.1	2,768	I	no	none
	Nucleotidyl transferase AbiEii toxin (PF08843)	*Nocardia aobensis*	WP_036509317.1	1,851	I	no	none
	FlgD_Ig (PF13860); PT-HINT (PF07591)	*Brevibacillus* sp. Leaf182	WP_056491400.1	1,841	II	yes	WP_056491398.1
	FlgD_Ig (PF13860); PT-HINT (PF07591)	*Brevibacillus brevis* GZDF3.1	WP_064201482.1	1,841	II	yes	WP_064201483.1
	Isopren_C2-like (PF00432)	*Planomicrobium glaciei* UCD-HAM	WP_053167008.1	1,018	III	yes	WP_036809033.1
	Isopren_C2-like (PF00432)	*Planomicrobium glaciei* CGMCC 1.6846	WP_074509029.1	1,018	III	yes	WP_083335251.1
SMC_prok_A	YebA_transglutaminase (PF08379)	*Acetivibrio cellulolyticus* CD2	WP_010251285.1	1,382	III	yes	WP_010251287.1
TANFOR	fibronectin type 3	*Tenacibaculum ovolyticum* DSM 18103	WP_028888923.1	2,154	III	no	WP_028888924.1
Neuromodulin_N (PF10580)		*Paenibacillus* sp. FSL R5-0912	WP_052416555.1	1,359	I	no	WP_042237393.1
	Bacuni_01323_like (PF15283); Rhs repeats	Chitinophagaceae bacterium PMP191F	WP_054284430.1	1,506	I	no	WP_054284431.1
	PT-TG (PF14449)	*Fusobacterium* sp. HMSC064B11	WP_070766574.1	466	II	no	WP_060496433.1
	PT-TG (PF14449)	*Fusobacterium* sp. OBRC1	EUB34025.1	466	II	no	EUB34019.1
	peptidase_M23 (PF01551); PT-TG (PF14449)	*Streptomyces* sp. 769	WP_078876371.1	671	II	yes	WP_040108218.1*
		Providencia burhodogranariea DSM 19968	WP_008910513.1	1,394	I	no	WP_008910514.1
		Mycobacterium sp. UM_RHS	WP_024451231.1	523	I	no	WP_024451230.1
		Mycobacterium vulneris ACS3670	WP_065516086.1	524	I	no	WP_065516085.1
		Saccharopolyspora antimicrobica CPCC 201259	SFN01621.1	427	I	no	SFN01652.1
		Rhodococcus rhodnii LMG 5362	WP_010837416.1	451	I	no	WP_010837417.1
		Streptococcus parasanguinis ATCC 15912	AEH55766.1	416	II	no	AEH55765.1
		Clostridium sporogenes ATCC 19404	WP_061311230.1	561	II	no	WP_046340402.1
		Rugamonas rubra ATCC 43154	SFM63091.1	454	II	no	unannotated
		Nitriliruptor alkaliphilus DSM 45188	WP_052668235.1	700	II	no	WP_052668236.1
		Thermoactinomyces vulgaris NRRL F-5595	WP_054096214.1	490	III	no	WP_022737160.1
		Runella limosa DSM 17973	WP_051398261.1	545	III	no	WP_028526235.1

*Table does not include predicted MafB, T6SS-linked Rhs proteins, T7SS or T5SS/CdiA effector proteins.

Table S3. Predicted Actinobacterial proteins with internal EndoU domains.

Bacterial species and NCBI ID	**from**	**to**	**E-value**	**Conserved domains**	
***Saccharomonospora marina* XMU15**	5,273	5,446	6.61E-05	SMC_N superfamily	cl25732
WP_009151954.1	7,554	7,760	6.50E-07	Amidoligase_2 superfamily	cl13634
14,511 residues	9,686	9,778	5.08E-08	EndoU_bacteria	PF14436
	10,410	10,620	0.000932258	SMC_N superfamily	cl25732
	11,831	12,195	0.00143364	DNA_pol3_gamma3 superfamily	cl26386
***Amycolatopsis thermoflava* N1165**	580	797	3.71E-11	Rho superfamily	cl28310
WP_027931422.1	2,568	2,671	1.00E-18	EndoU_bacteria	PF14436
18,636 residues	2,772	2,923	0.000713927	Rho superfamily	cl28310
	7,776	8,003	0.00688798	DUF3084 superfamily	cl26472
	9,279	9,450	0.0010978	DNA_pol3_delta2 superfamily	cl26247
	16,935	17,178	2.08E-08	APH	PF01636
***Nocardia anaemiae* NBRC 100462**	422	968	8.44E-13	Atrophin-1 superfamily	cl26464
WP_062988149.1	801	1,181	7.76E-07	Atrophin-1 superfamily	cl26464
13,740 residues	1,211	1,671	5.12E-15	Atrophin-1 superfamily	cl26464
	1,502	1,987	3.14E-07	Herpes_ICP4_C superfamily	cl28033
	2,122	2,162	0.00431773	HTH_LacI	cd01392
	2,170	2,211	1.64E-06	HTH_LacI	cd01392
	2,170	2,257	0.000114812	PurR	COG1609
	2,687	2,742	5.11E-06	Peripla_BP_4 superfamily	cl26188
	2,922	2,993	0.000192999	EndoU_bacteria superfamily	PF14436
	3,386	3,446	5.78E-07	HTH superfamily	cl21459
	3,489	3,545	0.00879089	HTH superfamily	cl21459
	3,779	3,842	6.39E-05	HTH superfamily	cl21459
	5,981	6,273	3.44E-16	MFS_1	PF07690
	6,682	6,990	0.00435585	PRK10246 superfamily	cl25731
	7,349	7,769	0.000145206	Atrophin-1 superfamily	cl26464
	7,605	7,951	5.03E-07	Atrophin-1 superfamily	cl26464
	7,804	7,862	2.44E-10	HTH_LUXR	smart00421
	9,049	9,616	6.95E-107	D-aminoacylase	cd01297
	9,535	9,737	7.84E-06	Adenine_deam_C superfamily	cl26189
	9,859	9,914	0.0001258	HTH superfamily	cl21459
	10,517	10,875	1.46E-08	MFS	cd06174
	11,209	11,293	1.90E-06	Bac_transf superfamily	cl27431
	11,915	12,129	1.42E-12	APH	PF01636
***Amycolatopsis methanolica* 239**	617	784	1.77E-08	Rho superfamily	cl28310
WP_017981396.1	2,103	2,305	3.13E-08	Rho superfamily	cl28310
14,703 residues	2,630	2,733	3.00E-24	EndoU_bacteria	PF14436
	5,160	5,346	0.00540722	DNA_pol3_gamma3 superfamily	cl26386
	5,282	5,515	0.00247255	DNA_pol3_gamma3 superfamily	cl26386
	5,774	6,304	0.00257397	SMC_N superfamily	cl25732
	5,883	6,111	0.00247865	TolC	COG1538
	11,529	11,782	3.76E-05	APH superfamily	cl28124
	11,701	11,774	2.11E-05	PKc_like superfamily	cl21453
***Amycolatopsis* sp. ATCC 39116**	620	822	2.68E-09	Rho superfamily	cl28310
WP_020416785.1	775	928	0.00385785	PHA03169 superfamily	cl27451
17,637 residues	1,367	1,568	0.00181477	PRK09169 superfamily	cl28514
	2,625	2,726	3.00E-26	EndoU_bacteria	PF14436
	9,488	9,703	0.00488948	DNA_pol3_gamma3 superfamily	cl26386
	11,983	12,153	5.20E-05	FUSC superfamily	cl26163
	12,093	12,349	0.000129751	SMC_N superfamily	cl25732
	13,738	13,983	1.25E-10	APH	PF01636
	14,124	14,519	5.49E-06	DUF390 superfamily	cl25642
***Amycolatopsis taiwanensis* DSM 45107**	528	645	2.91E-06	Endomucin superfamily	cl25495
WP_043840807.1	531	789	2.55E-05	Herpes_BLLF1 superfamily	cl25496
27,216 residues	1,848	1,931	0.000120741	EndoU_bacteria superfamily	PF14436
	2,043	2,243	1.16E-05	Rho superfamily	cl28310
	2,546	3,127	3.32E-41	SecA_DEAD superfamily	cl26882
	3,486	3,806	0.00883741	DUF390 superfamily	cl25642
	7,470	7,702	0.00196047	APH superfamily	cl28124
	13,039	13,284	3.62E-06	APH	PF01636
	13,215	13,279	1.13E-06	PKc_like superfamily	cl21453
	17,042	17,430	1.67E-05	Atrophin-1 superfamily	cl26464
	25,109	25,199	0.00261893	Gln_amidase superfamily	cl21436

Table S4. Oligonucleotides

Identifier	Descriptive name	Sequence
204F40		5´-GTC TCT CCC ATG GTT GAT AAT AAC TAC CTG AGC GTG TCT G
204R49		5´ - TGG TGG TGC CCA GCG GAT GGG ATT TTA GAC AGT AAT TTG TTT ACT TTT T
204CdiIF		5´-GTC TCT CCC ATG AAT AAA TAT TTA TTT GAA TTG CCA TAT GAA CGT TCT G
CH367	Gly-GCC-probe	5´ - CTT GGC AAG GTC GTG CT
CH368	Pro-GGG-probe	5´ - CAC CCC ATG ACG GTG CG
CH374	Ala-UGC-probe	5´ - TCC TGC GTG CAA AGC AG
CH379	Arg-CCU-probe	5´ - CCT GCA ATT AGC CCT TAG G
CH380	Gly-UCC-probe	5´ - CCC GCA TCA TCA GCT TGG AAG GC
CH381	Leu-CAA-probe	5´ - CCC GCA CAG CGC GAA CGC CG
CH382	Phe-GAA-probe	5´ - TGC TCT ACC GAC TGA GCT A
CH383	Pro-CGG-probe	5´ - CTT CGT CCC GAA CGA AGT G
CH385	Thr-CGU-probe	5´ - CCT ACG ACC TTC GCA TT
CH406	5S probe	5´ - ATG CCT GGC AGT TCC CTA CTC TC
CH407	16S probe	5´ - TGC GCT TTA CGC CCA GTA ATT CC
CH408	23S probe	5´ - GTT TAG CCC CGT TAC ATC TTC CG
CH436	Arg-CCG-probe	5´ - CCT GAG ACC TCT GCC TCC GGA
CH451	Arg-UCU-probe	5´ - CCT GCG GCC CAC GAC TTA G
CH452	Arg-ICG-probe	5´ - CCT CCG ACC GCT CGG TTC G
CH487	Cys-GCA-probe	5´ - GGA CTA GAC GGA TTT GCA A
CH577	Ile-GAU-probe	5´ - ACC GAC CTC ACC CTT ATC AG
CH618	Trp-CCA-probe	5´ - CCC AAC ACC CGG TTT TGG
CH791	Gln-CUG-probe	5´ - TCG GAA TGC CGG AAT CAG A
CH798	Tyr1-GUA-probe	5´ - CTT CGA AGT CTG TGA CGG CAG
CH799	Tyr2-GUA-probe	5´ - CTT CGA AGT CGA TGA CGG CAG
CH800	Asp-QUC-probe	5´ - TCG AAC CCG CGA CCC CCT GCG
CH801	Asn-QUU-probe	5´ - CTC GAA CCA GTG ACA TAC GG
CH837	Ser-GCU-probe	5´ - CCC CGG ATG CAG CTT TTG ACC
CH1046	Leu-GAG-probe	5´ - CCC GTA AGC CCT ATT GGG CA

CH1047	Ser-UGA-probe	5´ - AAC CCT TTC GGG TCG CCG GTT TTC
CH1248	Val-UAG-probe	5´ - CGC CGA CCC CCT CCT TGT AAG
CH1249	Lys-UUU-probe	5´ - CCT GCG ACC AAT TGA TTA AA
CH1417	Glu-UUC-probe	5´ - CCC CTG TTA CCG CCG TG
CH1706	BC0921-Nhe-rev	5´ - CTC TAT GCT AGC TTT CAA TTC TTT ATT TTT TCC
CH2032	Gly-CCC-probe	5´ - CCC TCG TAT AGA GCT TGG GAA
CH2034	Leu-CAG-probe	5´ - CCC CCA CGT CCG TAA GGA CA
CH2035	Leu-UAG-probe	5´ - CAC CTT GCG GCG CCA GAA
CH2036	Leu-UAA-probe	5´ - CCC GCA CAG CGC GAA CGC CG
CH2037	fMet-CAU-probe	5´ - CGG GTT ATG AGC CCG ACG A
CH2038	Met-CAU-probe	5´ - CCT GTG ACC CCA TCA TTA TGA
CH2040	Ser-CGA-probe	5´ - GTA GAG TTG CCC CTA CTC CGG
CH2042	Thr-GGU-probe	5´ - CTG GGG ACC CCA CCC CT
CH2784	NMB0503-Kpn-for	5´ - GGT GGT ACC ATG AAA AAT AAT ATT TTT C
CH2785	NMB0503-Spe-rev	5´ - TTC ACT AGT GGT TTC ATG CAG GCT AC
CH2839	NMB0502-Nco-for	5´ - CTC CCA TGG TGA AAA ATA ATC AGC
CH3172	EC3006-CT(OE)-for	5´ - CAG GTA GGA ACT CGG TTG AGA ATA ATT ATC TTA GCG TGT CTG AAA AGA CAG AGC
CH3538	STECO31-CT-Nco-for	5´ - TTT CCA TGG TTG ATA ATA ACT ACC
CH3539	STECO31-cdiI-Spe-rev	5´ - TTT ACT AGT GGA TGG GAT TTT AGA C
CH3569	STECO31-mid-rev	5´ - GGT CTG GTG TCT AAC CTT TGG TTA GGA TGG GAT TTT AGA CAG TAA TT
CH3624	STECO31-cdiI-Xho-rev	5´ - TTT CTC GAG TTA GGA TGG GAT TTT AG
CH3747	Ymol-mid-for	5´ - CAG GTA GGA ACT CGG TTG AGA ATA ATA ACC TTA GTT TTG GCA AAG G
CH3748	Ymol-mid-rev	5´ - GGT CTG GTG TCT AAC CTT TGG GTT AAG CAG GTA ATT TAG TCA GTA AAT C
CH3854	STECO31-cdiI-Kpn-for	5´ - TTT GGT ACC ATG AAT AAA TAT TTA TTT GAA TTG C
CH3976	Ymol-cdiI-Kpn-for	5´ - TTT GGT ACC ATG AAA AAG GAA TTA TTT AGT CAA C
CH3977	Ymol-cdiI-Spe-rev	5´ - TTT ACT AGT AGC AGG TAA TTT AGT CAG
CH4016	STECO31-H187A-rev	5´ - CAA GTT GAG CTA AGG CTT CCG GCT G
CH4017	STECO31-H204A-rev	5´ - GAA TAC ATC AGC ATT AGC TGC ACC GCT AAT TCC

CH4018	STECO31-N319A-rev	5´ - CCT ATT TCG GGT GGA AGG CAC TTA CAA TTC C
CH4019	STECO31-H321A-rev	5´ - CCT ATT TCG GGG CGA AGT TAC TTA CAA TTC C
CH4100	EC93-cdiA-8220-for	5´ - GAA GCG ATG AAA GCA GCC AGG
CH4101	EC93-cdiA-8718-rev	5´ - CTC AAC CGA GTT CCT ACC TGC CTG
CH4102	EC93-cdiI-296-for	5´ - AAC CCA AAG GTT AGA CAC CAG ACC
CH4103	EC93-cdiI-+486-rev	5´ - AAG TAG GCA TTC TCG ACC CTG
CH4269	STECO31-K261A-rev	5´ - ATC ATA AAT AGT GGC CTC AAA AGG CTT
CH4270	STECO31-T262A-rev	5´ - AGG ATC ATA AAT GGC TTT CTC AAA AGG
CH4272	gltW-C33-marker	5´ - GAT TCG AAC CCC TGT TAC CGC CGT GAA A
CH4273	gltW-U36-marker	5´ - GGA TTC GAA CCC CTG TTA CCG CCG TG
CH4274	gltW-C39-marker	5´ - GGA TTC GAA CCC CTG TTA CCG CC
CH4275	gltW-RT-primer	5´ - GGA TTC GAA CCC CTG TTA CC
CH4398	BC0921-Kpn-for	5´ - ACA GGT ACC ATG AAA TAT CCA TAT AGC TTT GAA G
CH4649	tRNA-Glu 5´ probe	5´ - GAA AGG GCG GTG TCC TG
SK693	GN05224-CT-for	5´ - TTC TTG GGG CCG GAG AGC CGA GAG
SK694	GN05224-CT-rev	5´ - CCG GCG ATC TGC CGG TGT ACA AGC
SK733	GN05224-cdiI-Kpn-for	5´ - GAG CGG TAC CAT GAG TAT TAA TAA TAG
SK734	GN05224-cdiI-Xho-rev	5´ - GAG GCT CGA GTT ATC TCT TAT TTT CTG G
SK724	GN05224-CT-Nco-for	5´ - CAG ACC ATG GTG GAG AAT AAC TTC TTG GG
DL3986	GN05224-cdiI-Xho-rev	5´ - CCA GCT CGA GTC TCT TAT TTT CTG GAT TAA TAT ATA AAT ACC TAT TAT CTA TTT C