Supplementary Information

Rhs NADase effectors and their immunity proteins are exchangeable mediators of inter-bacterial competition in *Serratia*

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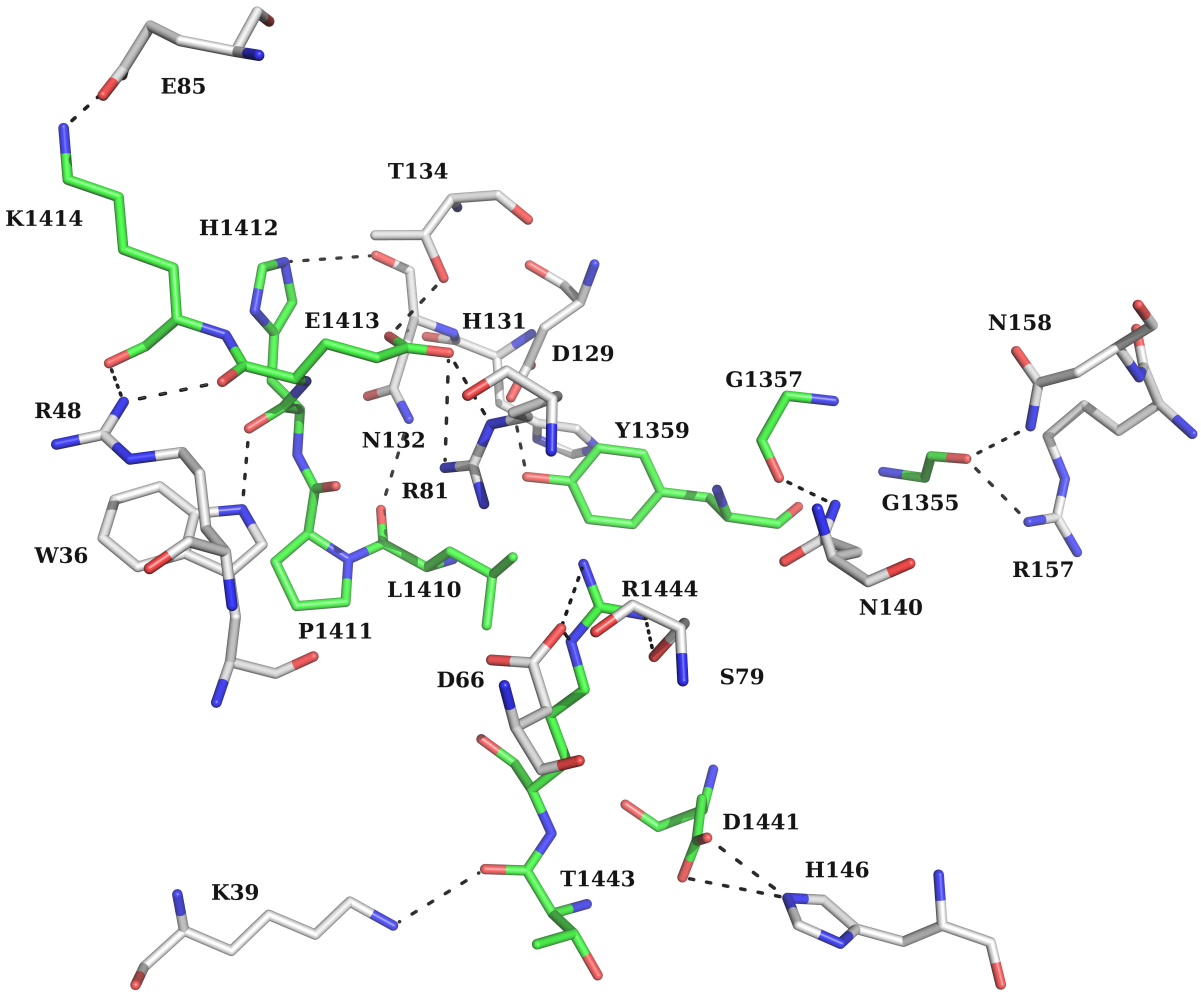
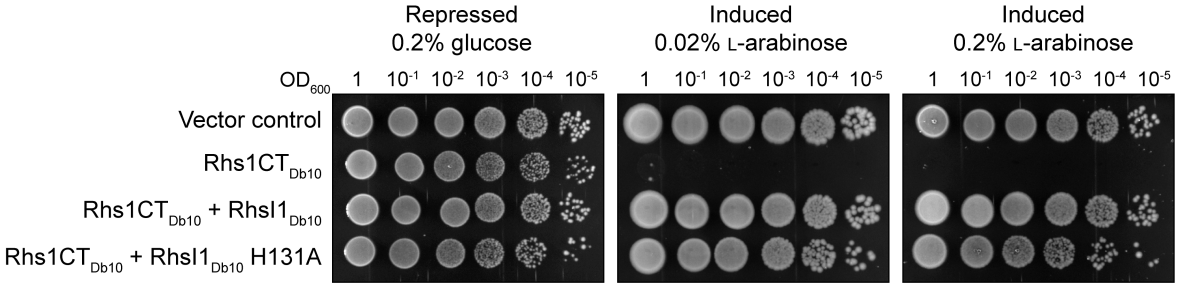
Supplementary Figure 1.



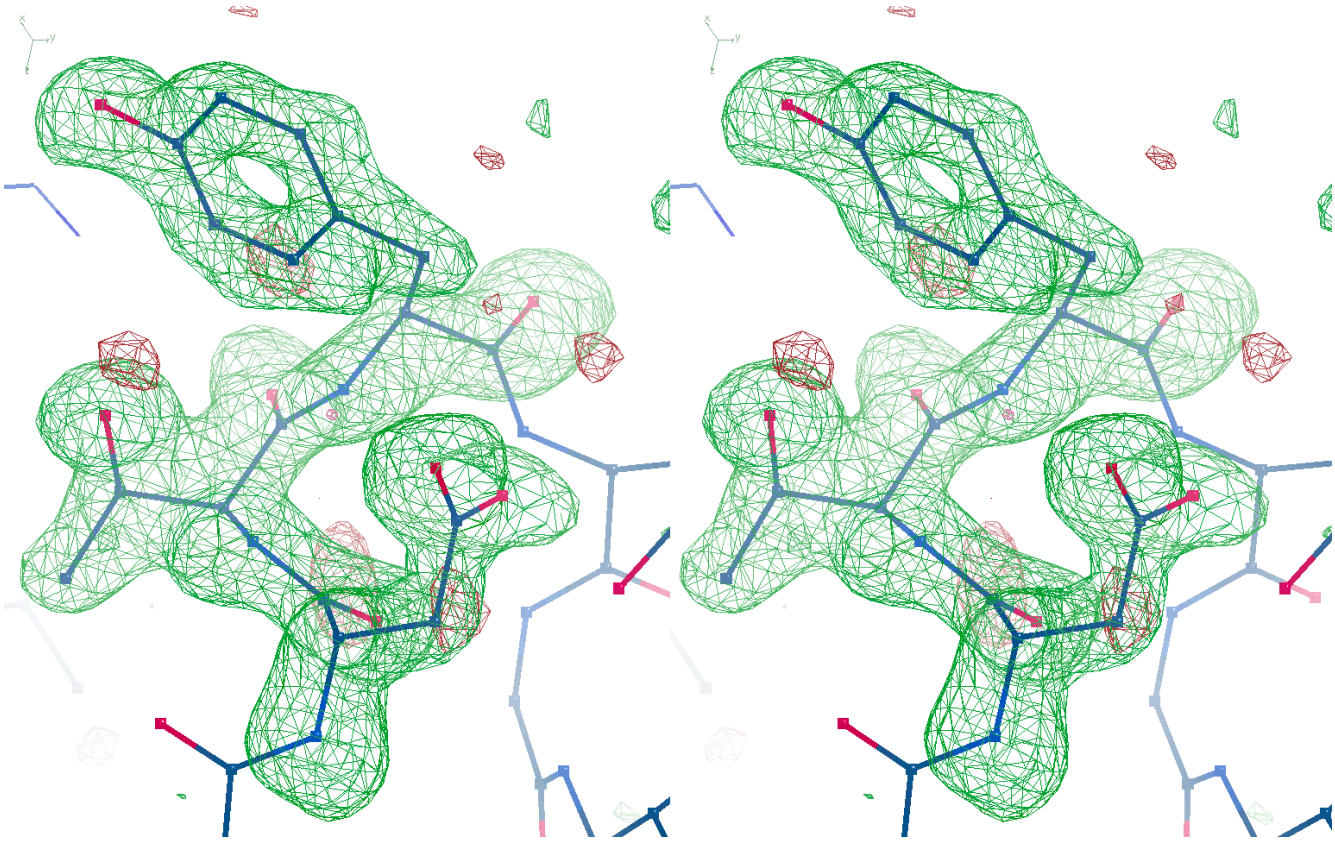
Supplementary Figure 1. Residues involved in the formation of the protein-protein interface between Rhs1CT$_{Db10}$ and RhsI1$_{Db10}$. Different coloring schemes were applied to the carbon atoms of Rhs1CT$_{Db10}$ (green) and RhsI1$_{Db10}$ (grey). Hydrogen bonds (within 2.5 Å–3.5 Å distance) are shown as dashed lines.

Supplementary Figure 2.



Supplementary Figure 2. RhsI1$_{Db10}$ H131A retains the ability to neutralise the toxicity of Rhs1CT$_{Db10}$ when co-expressed in *E. coli*. Growth of *E. coli* MG1655 carrying empty vector control plasmid (pBAD18-Kn) or plasmids directing the expression of wild type Rhs1CT$_{Db10}$ with N-terminal 3xFLAG tag alone (Rhs1CT$_{Db10}$), or Rhs1CT$_{Db10}$ together with wild type RhsI1$_{Db10}$ (+ RhsI1$_{Db10}$) or RhsI1$_{Db10}$ carrying a H131A substitution (+ RhsI1$_{Db10}$ H131A). Growth was on solid LB media with 0.2% glucose or 0.02% or 0.2% L-arabinose to repress or induce, respectively, gene expression. The data shown are representative of two independent experiments.

Supplementary Figure 3.



Supplementary Figure 3. A side-by-side stereo view of a representative portion of the electron density map for the Rhs1CT$_{Db10}$-RhsI1$_{Db10}$ crystal structure (PDB 6XTD). A fragment of the Fo-Fc omit map for RhsI1$_{Db10}$ with residues Asp4, Thr5 and Tyr6 contoured at 3σ level.

Supplementary Table 1. Bacterial strains and plasmids used in this study.

Name	Description/genotype	Source / Reference
Strains		
Serratia marcescens		
Db10	Wild type model strain	[1]
SJC1036	Wild type clinical isolate	[2]
SJC11	Db10 Δ*tssE* (*SMDB11_2271*)	[3]
FRC29	Db10 Δ*rhs2*Δ*vgrG1* (*SMBD11_1610, SMBD11_2244*)	[4]
RGM32	Db10 Rhs1_no CTI, Δ$rhs1CT_{Db10}$-*SMDB11_2281*. Lacks the region containing $rhs1CT_{Db10}$ (*SMDB11_2278* nt 3997-4422), *rhsI1 (SMDB11_2278A), SMDB11_2280* and *SMDB11_2281*.	This study
RGM33	Db10 Rhs1_CTI_{1036}, $rhs1CT_{1036}$- $rhsI1_{1036}$ from *S. marcescens* SJC1036 replacing $rhs1CT_{Db10}$-*SMDB11_2281*. This generates a fusion of amino acids 1-1329 of $Rhs1_{Db10}$ with amino acids 1330-1486 of $Rhs1_{1036}$ (the exchanged region is the CT plus the final three amino acids of the core domain immediately prior to the cleavage site).	This study
RGM35	Db10 Δ*tssE* Rhs1_CTI_{1036}, derived from SJC11	This study
RGM36	Db10 Δ*rhs2*Δ*vgrG1* Rhs1_CTI_{1036}, derived from FRC29	This study
KT72	Db10 rendered Sm^R using φIF3	[5]
JAD04	Db10 Δ2280_{Db10}, in-frame deletion of *SMDB11_2280*	This study
JAD10	JAD04 rendered Sm^R using φIF3	This study
Escherichia coli		
MG1655	Wild type (model K-12 strain)	[6]
BL21(DE3) pLysS	Protein overexpression strain. Chromosomal λDE3 encodes IPTG-inducible T7 RNA polymerase. pLysS directs expression of T7 lysozyme.	Novagen
CC118λ*pir*	Cloning host and donor strain for pKNG101-derived allelic exchange plasmids (λ*pir*)	[7]
HH26 pNJ5000	Mobilizing strain for conjugal transfer	[8]
Plasmids		
pRSFDuet-1	Protein overexpression vector for the co-expression of two target genes. Each multiple cloning site (MCS) is preceded by a T7 promoter and the first site allows for fusion of an N-terminal His_6 tag (Kn^R)	Novagen
pET15b-TEV	Vector for protein overexpression under the control of the T7 promoter. Permits fusion of an His_6 tag followed by a TEV protease cleavage site to the N-terminus of the overexpressed protein (Ap^R)	[9]
pBAD18-Kn	Arabinose-inducible expression vector; gene of interest is cloned downstream of the P_{ara} promoter (Kn^R)	[10]
pKNG101	Suicide vector for allelic exchange (Sm^R, *sacBR, mobRK2, oriR6K*).	[11]
pSC962	Coding sequence for His_6-$Rhs1CT_{Db10}$ (SMDB11_2278, amino acids 1333-1473) in MCS1 and untagged $RhsI1_{Db10}$ (SMDB11_2278A) in MCS2 of pRSFDuet-1.	This study
pSC981	Coding sequence for His_6-$Rhs1CT_{1036}$ (SJC1036_02424, amino acids 1333-1486) in MCS1 and untagged $RhsI1_{1036}$ (SJC1036_02425) in MCS2 of pRSFDuet-1.	This study
pSC3020	Coding sequence for $Rhs1CT_{1036}$ (SJC1036_02424, amino acids 1333-1486) in pET15b-TEV. The native, untagged protein is expressed.	This study
pSC3019	Coding sequences for $Rhs1CT_{1036}$ (SJC1036_02424, amino acids 1333-1486) and $RhsI1_{1036}$ (SJC1036_02425) in pET15b-TEV. The native, untagged proteins are expressed.	This study

pSC987	Coding sequences for $Rhs1CT_{1036}$ (SJC1036_02424, amino acids 1333-1486) and 2280_{Db10} (SMDB11_2280) in pET15b-TEV. The native, untagged proteins are expressed.	This study
pSC990	Coding sequence for 3xFLAG-$Rhs1CT_{Db10}$ (SMDB11_2278; amino acids 1333-1473) in pBAD18-Kn	This study
pSC992	Coding sequence for 3xFLAG-$Rhs1CT_{Db10}$ S1384A in pBAD18-Kn	This study
pSC993	Coding sequence for 3xFLAG-$Rhs1CT_{Db10}$ S1399A in pBAD18-Kn	This study
pSC994	Coding sequence for 3xFLAG-$Rhs1CT_{Db10}$ H1412A in pBAD18-Kn	This study
pSC999	Coding sequence for 3xFLAG-$Rhs1CT_{Db10}$ F1386A in pBAD18-Kn	This study
pSC3500	Coding sequence for 3xFLAG-$Rhs1CT_{Db10}$ R1418A in pBAD18-Kn	This study
pSC3501	Coding sequence for 3xFLAG-$Rhs1CT_{Db10}$ Q1452A in pBAD18-Kn	This study
pSC4115	Coding sequence for $RhsI1_{Db10}$ (SMDB11_2278A) in pSC990	This study
pSC4116	Coding sequence for $RhsI1_{Db10}$ (SMDB11_2278A) in pSC992	This study
pSC4117	Coding sequence for $RhsI1_{Db10}$ (SMDB11_2278A) in pSC993	This study
pSC4118	Coding sequence for $RhsI1_{Db10}$ (SMDB11_2278A) in pSC994	This study
pSC4119	Coding sequence for $RhsI1_{Db10}$ (SMDB11_2278A) in pSC999	This study
pSC4120	Coding sequence for $RhsI1_{Db10}$ (SMDB11_2278A) in pSC3500	This study
pSC4121	Coding sequence for $RhsI1_{Db10}$ (SMDB11_2278A) in pSC3501	This study
pSC4112	Coding sequence for $RhsI1_{Db10}$-HA (SMDB11_2278A) in pSC990	This study
pSC4113	Coding sequence for $RhsI1_{Db10}$-HA H131A in pSC990	This study
pSC620	pKNG101-derived allelic exchange plasmid for generation of an in-frame deletion of *SMDB11_2280*.	This study
pSC2776	pKNG101-derived allelic exchange plasmid for deletion of the Db10 chromosomal region $rhs1CT_{Db10}$ - *SMDB11_2281*.	This study
pSC2777	pKNG101-derived allelic exchange plasmid for replacement of $rhs1CT_{Db10}$ - *SMDB11_2281* on the chromosome of Db10 with $rhs1CT_{1036}$-$rhsI1_{1036}$ from *S. marcescens* SJC1036	This study

Supplementary Table 2. Oligonucleotide primers and synthetic gene fragments used for plasmid construction

Plasmid	Sequence of relevant primers (5'-3')[a]	Description
pSC962	TATAGGATCCGAAGCCACGATGCGCAGCAAC	Forward primer to clone SMDB11_2278 aa 1333-1473 in MCS1 of pRSFDuet-1 (*Bam*HI)
	TATA GAATTC TTACAATCCAATTTCAAAAGGC	Reverse primer to clone SMDB11_2278 aa 1333-1473 in MCS1 of pRSFDuet-1 (*Eco*RI)
	TATA CATATG ATGCAACTAGATACTTATGACG	Forward primer to clone SMDB11_2278A in MCS2 of pRSFDuet-1 (*Nde*I)
	TGTGCTCGAGTTAATAAATTATGTTATTCCTTTTGCAA TATTTTCG	Reverse primer to clone SMDB11_2278A in MCS2 of pRSFDuet-1 (*Xho*I)
pSC3020	ATGCTCTAGACGAACACAGTGAACGATAGCCATGAGT TGCGGCAATTCCTGG	Forward primer to clone SJC1036_02424 aa 1333-1486 in pET15b-TEV (*Xba*I)
	ATGCGGATCCTCAAATTGGCCGGATTCG	Reverse primer to clone SJC1036_02424 aa 1333-1486 in pET15b-TEV (*Bam*HI)
pSC3019	ATGCTCTAGACGAACACAGTGAACGATAGCCATGAGT TGCGGCAATTCCTGG	Forward primer to clone SJC1036_02424 aa 1333-1486 and SJC1036_02425 in pET15b-TEV (*Xba*I)
	ATGCGGATCCCTAGTGAAAAATGAAACCCAGC	Reverse primer to clone SJC1036_02424 aa 1333-1486 and SJC1036_02425 in pET15b-TEV (*Bam*HI)
pSC620	TATAACTAGTGCGACAACCCGAGAAATGC	Forward primer to clone upstream region of SMDB11_2280 in pKNG101 (*Spe*I)
	GCGCAAGCTTCTCTTTGTTCATAATATTTAAATAGATC TAGTG	Reverse primer to clone upstream region of SMDB11_2280 in pKNG101 (*Hin*dIII)
	TGTGAAGCTTTTCATTTTTCACTAGCGCTCTCTTAGAG	Forward primer to clone downstream region of SMDB11_2280 in pKNG101 (*Hin*dIII)
	TATAGGGCCCTTGCTCGTCCATGGCATC	Reverse primer to clone downstream region of SMDB11_2280 in pKNG101 (*Apa*I)
pSC4115-pSC4121	TATAGGTACCAAGAGGATTGTAAAATGCAAC	Forward primer to clone SMDB11_2278A into pSC990 and related plasmids (*Kpn*I)
	TATAGCATGCTTAATAAATTATGTTATTCC	Reverse primer to clone SMDB11_2278A into pSC990 and related plasmids (*Sph*I)

Plasmid	Details of synthetic insert
pSC981	Synthetic insert including coding sequences for His_6-$Rhs1CT_{1036}$ and untagged $RhsI1_{1036}$, produced by GeneArt (ThermoFisher): GGATCCAAGTTGCGGCAATTCCTGGAATAATTTTCAATCGAAGTCGAAAGGTGTTTTTGCATCCAGAAGCCAGGCATCTAA AGCTTATAATCTGTGGAAAAAACAAGATTGGGCGGCCTTAGAAAAATTCATGGGGCATGGCTCATGGCCTCCCAATAGAG GTTTTGTACAAGCAACGCCTACTACTCTTATGCCTGGAGCAAAAATTGATAGATACGGTGGCTGGACAAATAATGGCGTAT TCAACGACCGAGGTACCTTTGTCTCTCCTGCTGGAGCCTCTTTTGGCAGCCGGGCATTACCGTTAGATACACTGGAAAAACC GTATCGAGTATATGAGGTGGTTAAACCAATCCAAGCAGATATGGGGCCTGCTATACCGTGGTTTAACCAAGCTGGTGGAGG AACACAATTTGAACTGTCCAAATCCATCAGTCAACTTCTTGCAGAAGGCCGAATCCGGCCAATTTGAGTCGACAAGCTTGC GGCCGCATAATGCTTAAGTCGAACAGAAAGTAATCGTATTGTACACGGCCGCATAATCGAAATTAATACGACTCACTATAG GGGAATTGTGAGCGGATAACAATTCCCCATCTTAGTATATTAGTTAAGTATAAGAAGGAGATATACATATGAGCGAAAAAT ACGTTAGCGTGAAGAAAGATAAGCAACTGTTTGATGGTAACGAAGAGCAGTTTCACTTATTTAAAAAAGAAGTATCCATCC CTAACTCTAACGAAAAAGTACTTTATGATAACAGCTCTGCGATACCCATATGGGATATAGATGAAAACATCAGGAAGGCG ATCGCAGAAAAAATAAGCGGAATAAAAGTCATAGAGTATCCATATGACCCAATACCTTCATATAAAAATGAAAATCTGGA TATCTATGTTCAACCAGTAGAGTCGGACATGCACAAAAAAAGAATAACAATAATCACGGCGACGGGTGAATCACAACCAG CAAGATATAAGATAAAGTTGCTGGGTTTCATTTTTCACTAGCTCGAG
pSC987	Synthetic insert containing coding sequences for the 3' end of $Rhs1CT_{1036}$ and 2280_{Db10}, used to replace $RhsI1_{1036}$ in pSC3019 with $Db10_{2280}$ in exactly the same context, produced by GeneArt (ThermoFisher): GGTACCTTTGTCTCTCCTGCTGGAGCCTCTTTTGGCAGCCGGGCATTACCGTTAGATACACTGGAAAAACCGTATCGAGTAT ATGAGGTGGTTAAACCAATCCAAGCAGATATGGGGCCTGCTATACCGTGGTTTAACCAAGCTGGTGGAGGAACACAATTTG AACTGTCCAAATCCATCAGTCAACTTCTTGCAGAAGGCCGAATCCGGCCAATTTGAGGAATTATGAACAAAGAGAAAATTG TCATTACCCCATCAAACAGGCAACTATTTGATGGTAACGAAGAGCAGTTTCGTTTATTTAAAAAAGAAGTATCCATCCCTA ACTCTAACGAAAAAGTACTTTATGATAACAGCTCTGCGATACCCATATGGGATATAGATGAAAACATCAGGAAGGCGATC GCAGAAAAAATAAGCGGGATAAAAGTCATAGAGTACCCATATGACCCAATACCTTCATATAAAAATGAAAATCTAGATAT CTACATTCAGAATGTAGAGTCGGAAATGCACAAAAACAGAATAACAATAATCACGGCGACGGGTGAATCACAACCAGCAA GATATAAGATAAAGTTGCTGGGTTTCATTTTTCACTAGGGATCC
pSC990	Synthetic insert containing the coding sequence for parental $Rhs1CT_{Db10}$ (SMDB11_2278, amino acids 1333-1473) with an N-terminal 3xFLAG tag and a ribosome binding site for cloning into pBAD18-Kn, produced by GeneArt (ThermoFisher):

	GAGCTCAAGAGGAATACATATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACG ATGACAAAAAGCCACGATGCGCAGCAACCAAAGCCAACGATCATAATCAGGCAGCTTTTGGTCGACAATGGCAAGGTCGA GGTATCTACAAAGGACGAGACTCTTGGTCAAATATCATGTTGAAAGAAGGTGACATTGTTTATGGCGGCGCTCCAGGGCAA TCTGGTTTTTACTTCAACAAGGCGACGCTAGATGCTGCAGGTGGCAGTAGAGCCAAGCTATGGGAAAGTTTGCAGGTGCTT CCACATGAAAAATTTGGTTATAGATCTAAAATACAAGCATATAGAGTAAAAAGAGAAACGATTGCAGGAACAGGTAAAGC GATATCTCAAGACCCAACGAGATTCGGCGAAGGCGGAGGAACTCAATTTTTCCTTTCTAACTATAAAACTGTTCTTGAGCC AATTGATAAGCCTTTTGAAATTGGATTGTAATCTAGAGGTACCGCATGC
pSC992	Synthetic insert same as for pSC990 except for the incorporation of a S1384A mutation (tct→gct)
pSC993	Synthetic insert same as for pSC990 except for the incorporation of a S1399A mutation (agt→gct)
pSC994	Synthetic insert same as for pSC990 except for the incorporation of a H1412A mutation (cat→gcg)
pSC999	Synthetic insert same as for pSC990 except for the incorporation of a F1386A mutation (ttt→gcg)
pSC3500	Synthetic insert same as for pSC990 except for the incorporation of a R1418A mutation (aga→gct)
pSC3501	Synthetic insert same as for pSC990 except for the incorporation of a Q1452A mutation (caa→gcg)
pSC4112	Synthetic insert containing the coding sequence for parental $RhsI1_{Db10}$ (SMDB11_2278A) with a C-terminal HA tag and a ribosome binding site for cloning into pSC990, produced by GenScript: GGTACCAAGAGGATTGTAAAATGCAACTAGATACTTATGACGGAACACTAGAACTCGCGGGAATAACGCTAGGTACAGCG ACAACCCGAGAAATGCTAATAAAAGGCTCCAGATTGTGGGAGGGTTGGCCAGAAAAGTCAGATGGTAGGACTACATCATA CAGAACAATAATCAGTACAAAAAAAGAAAAAGCCGGCGATATATATATAATTGCCGACTTCTCCGGGGCCTTCATTACAG ATGCAGTGCTTTGCAGTTGGCGTTTTGCGCCTGAAAAACTCATGATGGGAATACAAAAAAAAGTAGAAGGTGCTATTACCA AAAATCTTCGAACATGGTTTTATGAAAAAACTCATATACAACTTCCAGTTAGTGGTTCATGGGGGCATATTGACGCTGCTTA TGACCCACATAATTTAACGGGAACAATAGTGTGCAACTATCGCAGTGCATTTCATACTGAGGATGAATGGCGAAAATATTG CAAAAGGAATAACATAATTTATGCCTATCCTTATGATGTTCCTGATTATGCATAAGCATGC
pSC4113	Synthetic insert same as for pSC4112 except for the incorporation of a H131A mutation (cat→gcc)
pSC2776	Synthetic insert for the deletion of *rhs1CT*$_{Db10}$ (*SMDB11_2278* nt 3997-4422) to *SMDB11_2281* (and the mutation of the final 10 nt of the core Rhs1CT to the corresponding bases in SJC1036, ttggggatgg→gttggggttg), flanked by 550 bp upstream and downstream sequence to facilitate allelic exchange by homologous recombination: TCTAGAAAGCCCAGCAGCATGTGCATTTCCACTATGATCCGCTCGGGCGGCGCACGCAAAAGCGGGTGTGGCAGCAAAGT CAGGATCTGCGCCAACCGGCCGGCAAATGCCAAACCACCACGTTTTTGTGGGAAGGTTTCCGGCTGCTGCAGGAAACTCGC GACGGCATGCCCCTCACCTATGTCTATGCCGATCAGGGCAGCTATGAACCTTTGGCGCGCATCGACGGCCACGCGCCGGCC CAAGTATTTTACTTCCACACGGCGCCGAACGGCGAACCGGAAAGCCTGACCGACAGCGACGGAACGCTGCGCTGGCAGAG CCACAGCAGCGCCTGGGGCCGCATAAAGTATGAGGAAAATCAGCAAGATCTGGATTACTCCCAAAACCTGCGCCTGCAGG GGCAATACCTGGATCGGGAAACGGGATTGCACTACAATTTGTTCCGCTATTACGATCCGGATATCGGCAGATTTACCCAGC ACGATCCGATAGGTTTGGCCGGCGGGATCAACCTGTACCAATACGCGCCGAATCCGCTGGGTTGGGTGGATCCGTTGGGGT TGTGAGAATTCACATGGAATAATTATCTTATGACTGTTCCTCTTTTAAATGAATCTTTAAATATCCCATCAAAAGGCTCCCA AAGAGCCTTTTGATACGCTACCGCGCCGTCTCACCACTTCTTCACCGTCTGGTAAATCTCGAGGTCGAAATAGCCGTCGGCC TCGCAGTCGTTGAGATAGTGCTCATAGCTCACCCCCTCAACCGGCTGGTAGCCGCTGGCCGGCAGCAGCTTCTGATAGAAC TCGCCCCACACCTGCTCGAAATCGCCATCGCTGATGCGCACGTGGTAGACCGCATACTGCCCGGCGGGCAGCGTCTGCACG GTCACGCCCTCGCTGCCCGCCGGCAGCGCGAAATCGTCCGCCACGCTTATCACTACGTCGGCACGCAGCTTTTCCGGCGCC ACTTCCGCCGGATCGTCCCAATACAGCACCAGCCATTTGCCAAACGGCACGCCGTGGCGTTTCCGCCATGCCAGCAGTTGC TGCGAGCCCTGCGGGATGGTCTGCGGATACGGCCCCACGACTCTCACTCCCACCACCTTCTCCGCCGCTTTATGGGCCC
pSC2777	Synthetic insert for the replacement of *rhs1CT*$_{Db10}$ - *SMDB11_2281* with *rhs1CT*$_{1036}$-*rhsI1*$_{1036}$ (and the mutation of the final 10 nt of the core Rhs1CT to the corresponding bases in SJC1036, ttggggatgg→gttggggttg), flanked by 550 bp upstream and downstream sequence to facilitate allelic exchange by homologous recombination: TCTAGAAAGCCCAGCAGCATGTGCATTTCCACTATGATCCGCTCGGGCGGCGCACGCAAAAGCGGGTGTGGCAGCAAAGT CAGGATCTGCGCCAACCGGCCGGCAAATGCCAAACCACCACGTTTTTGTGGGAAGGTTTCCGGCTGCTGCAGGAAACTCGC GACGGCATGCCCCTCACCTATGTCTATGCCGATCAGGGCAGCTATGAACCTTTGGCGCGCATCGACGGCCACGCGCCGGCC CAAGTATTTTACTTCCACACGGCGCCGAACGGCGAACCGGAAAGCCTGACCGACAGCGACGGAACGCTGCGCTGGCAGAG CCACAGCAGCGCCTGGGGCCGCATAAAGTATGAGGAAAATCAGCAAGATCTGGATTACTCCCAAAACCTGCGCCTGCAGG GGCAATACCTGGATCGGGAAACGGGATTGCACTACAATTTGTTCCGCTATTACGATCCGGATATCGGCAGATTTACCCAGC ACGATCCGATAGGTTTGGCCGGCGGGATCAACCTGTACCAATACGCGCCGAATCCGCTGGGTTGGGTGGATCCGTTGGGGT TGAGTTGCGGCAATTCCTGGAATAATTTTCAATCGAAGTCGAAAGGTGTTTTTGCATCCAGAAGCCAGGCATCTAAAGCTT ATAATCTGTGGAAAAAACAAGATTGGGCGGCCTTAGAAAAATTCATGGGGCATGGCTCATGGCCTCCCAATAGAGGTTTTG TACAAGCAACGCCTACTACTCTTATGCCTGGAGCAAAAATTGATAGATACGGTGGCTGGACAAATAATGGCGTATTCAACG ACCGAGGTACCTTTGTCTCTCCTGCTGGAGCCTCTTTTGGCAGCCGGGCATTACCGTTAGATACACTGGAAAAACCGTATCG AGTATATGAGGTGGTTAAACCAATCCAAGCAGATATGGGGCCTGCTATACCGTGGTTTAACCAAGCTGGTGGAGGAACAC AATTTGAACTGTCCAAATCCATCAGTCAACTTCTTGCAGAAGGCCGAATCCGGCCAATTTGAGGAATTATGAGCGAAAAAT ACGTTAGCGTGAAGAAAGATAAGCAACTGTTTGATGGTAACGAAGAGCAGTTTCACTTATTTAAAAAAGAAGTATCCATCC CTAACTCTAACGAAAAAGTACTTTATGATAACAGCTCTGCGATACCCATATGGGATATAGATGAAAACATCAGGAAGGCG ATCGCAGAAAAAATAAGCGGAATAAAAGTCATAGAGTATCCATATGACCCAATACCTTCATATAAAAATGAAAATCTGGA TATCTATGTTCAACCAGTAGAGTCGGACATGCACAAAAAAAGAATAACAATAATCACGGCGACGGGTGAATCACAACCAG CAAGATATAAGATAAAGTTGCTGGGTTTCATTTTTCACTAGACATGGAATAATTATCTTATGACTGTTCCTCTTTTAAATGA ATCTTTAAATATCCCATCAAAAGGCTCCCAAAGAGCCTTTTGATACGCTACCGCGCCGTCTCACCACTTCTTCACCGTCTGG TAAATCTCGAGGTCGAAATAGCCGTCGGCCTCGCAGTCGTTGAGATAGTGCTCATAGCTCACCCCCTCAACCGGCTGGTAG CCGCTGGCCGGCAGCAGCTTCTGATAGAACTCGCCCCACACCTGCTCGAAATCGCCATCGCTGATGCGCACGTGGTAGACC GCATACTGCCCGGCGGGCAGCGTCTGCACGGTCACGCCCTCGCTGCCCGCCGGCAGCGCGAAATCGTCCGCCACGCTTATC

	ACTACGTCGGCACGCAGCTTTTCCGGCGCCACTTCCGCCGGATCGTCCCAATACAGCACCAGCCATTTGCCAAACGGCACG CCGTGGCGTTTCCGCCATGCCAGCAGTTGCTGCGAGCCCTGCGGGATGGTCTGCGGATACGGCCCCACGACTCTCACTCCC ACCACCTTCTCCGCCGCTTTATGGGCCC

[a]Incorporated restriction sites for cloning into the respective vector are underlined.

Supplementary Table 3. Crystallographic statistics for the Rhs1CT$_{Db10}$-RhsI1$_{Db10}$ complex

Unit cell dimensions ***a*, *b*, *c* [Å]** **α, β, γ [°]**	39.66, 44.11, 46.81, 101.20, 96.13, 114.15
Space group	*P*1
X-ray source	Diamond Light Source, beamline I03
Wavelength [Å]	0.9150
Asymmetric unit	heterodimer
Resolution range	35.39-1.30 [1.32-1.30]
Total number reflections	668801 [33385]
Unique reflections	66880 [3188]
Redundancy	10.2 [10.5]
R_{merge}[a]	0.06 [1.2]
Wilson *B* [Å^2]	14.3
Completeness [%]	96.1 [93.9]
<I/σ[I]>	15.7 [2.1]
CC[1/2]	1.00 [0.800]
R_{pim}[b]	0.02 [0.40]
R_{work}/R_{free}[c] **[%]**	12.2/16.7
Number reflections for R_{work}/R_{free}	63553/3327
Protein residues	295
Ligands	6 bromides, 1 Bis-Tris
Water molecules	392
R.M.S.D. Bonds [Å]/Angles [°]	0.013/1.608
Ramachandran plot	
Residues in favored regions [%]	98
Residues in allowed regions [%]	2
Residues in outlier regions [%]	0
Average *B*-factors **Protein atoms [Å^2]**	18.3 Rhs1CT$_{Db10}$ 20.1 RhsI1$_{Db10}$
Water molecules [Å^2]	33.2
Ligands of interest [Å^2]	19.2

*Numbers in parenthesis are for the highest resolution shell. Values in parentheses correspond to the highest resolution shell. [a] $R_{merge} = \sum h \sum i |I[h,i] - \langle I[h] \rangle| / \sum h \sum i\, I[h,i]$; where I[*h*,*i*] is the intensity of the *i*th measurement of reflection *h* and <I[*h*]> is the mean value of I[*h*,*i*] for all *i* measurements.[b] $R_{pim} = \sum h\, [1/[/nh-1]]^{1/2} \sum i|\langle Ih \rangle - Ih,i|/\sum h \sum i\, Ih,i$ [c] $R_{work} = \sum hkl||F_o|-|F_c||/\sum|F_o|$, where F_o is the observed structure factor amplitude and the F_c is the structure-factor amplitude calculated from the model. R_{free} is calculated with a subset of data that are excluded from refinement calculations (5 %) using the same method as for R_{merge}.

Supplementary References

1. Flyg C, Kenne K, Boman HG. Insect pathogenic properties of *Serratia marcescens*: phage-resistant mutants with a decreased resistance to *Cecropia* immunity and a decreased virulence to *Drosophila*. *Microbiology* **120**, 173-181 (1980).

2. Williams DJ*, et al.* The genus *Serratia* revisited by genomics. *Nature Communications* **13**, 5195 (2022).

3. Murdoch SL, Trunk K, English G, Fritsch MJ, Pourkarimi E, Coulthurst SJ. The opportunistic pathogen *Serratia marcescens* utilizes type VI secretion to target bacterial competitors. *Journal of Bacteriology* **193**, 6057-6069 (2011).

4. Cianfanelli FR, Alcoforado Diniz J, Guo M, De Cesare V, Trost M, Coulthurst SJ. VgrG and PAAR Proteins Define Distinct Versions of a Functional Type VI Secretion System. *PLoS Pathogens* **12**, e1005735 (2016).

5. English G, Trunk K, Rao VA, Srikannathasan V, Hunter WN, Coulthurst SJ. New secreted toxins and immunity proteins encoded within the Type VI secretion system gene cluster of *Serratia marcescens*. *Molecular Microbiology* **86**, 921-936 (2012).

6. Blattner FR*, et al.* The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453-1462 (1997).

7. Herrero M, de Lorenzo V, Timmis KN. Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram-negative bacteria. *Journal of Bacteriology* **172**, 6557-6567 (1990).

8. Grinter NJ. A broad-host-range cloning vector transposable to various replicons. *Gene* **21**, 133-143 (1983).

9. Rao VA, Shepherd SM, English G, Coulthurst SJ, Hunter WN. The structure of *Serratia marcescens* Lip, a membrane-bound component of the type VI secretion system. *Acta Crystallogr D Biol Crystallogr* **67**, 1065-1072 (2011).

10. Guzman L-M, Belin D, Carson MJ, Beckwith J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *Journal of Bacteriology* **177**, 4121-4130 (1995).

11. Kaniga K, Delor I, Cornelis GR. A wide-host-range suicide vector for improving reverse genetics in gram-negative bacteria: inactivation of the *blaA* gene of *Yersinia enterocolitica*. *Gene* **109**, 137-141 (1991).