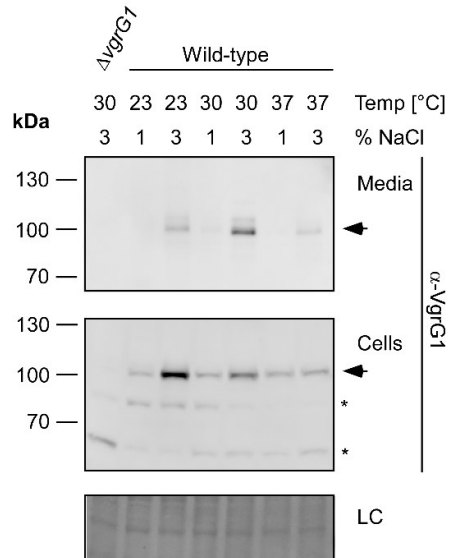


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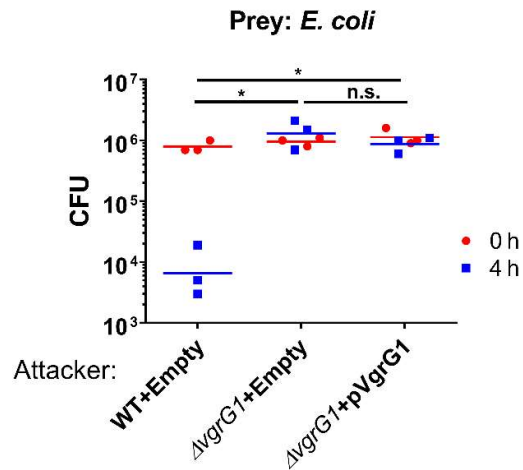
Type VI secretion system MIX-effectors are targeted at both bacterial and eukaryotic neighbors

Table of Contents

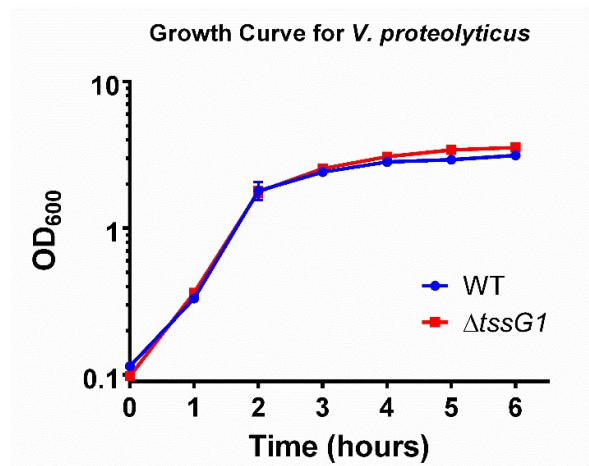
Appendix Figure S1. T6SS1 is most active under warm marine-like conditions.	2
Appendix Figure S2. Exogenous complementation of VgrG1 does not rescue loss of bactericidal activity.	3
Appendix Figure S3. Deletion of tssG1 does not affect Vpr growth.	4
Appendix Figure S4. Schematic representation of the gene neighborhoods of new Vpr T6SS1 effectors identified by comparative proteomics.	5
Appendix Figure S5. Vpr01570 contains a C-terminal CNF toxin domain.	6
Appendix Figure S6. Vpr01s_11_00400 is homologous to C-terminus of insecticidal toxin Txp40.	7
Appendix Figure S7. Deletion of vprh does not impair Vpr bactericidal activity.	8
Appendix Figure S8. Deletion of vpr01570 does not affect T6SS1 activity or bacterial growth.	9



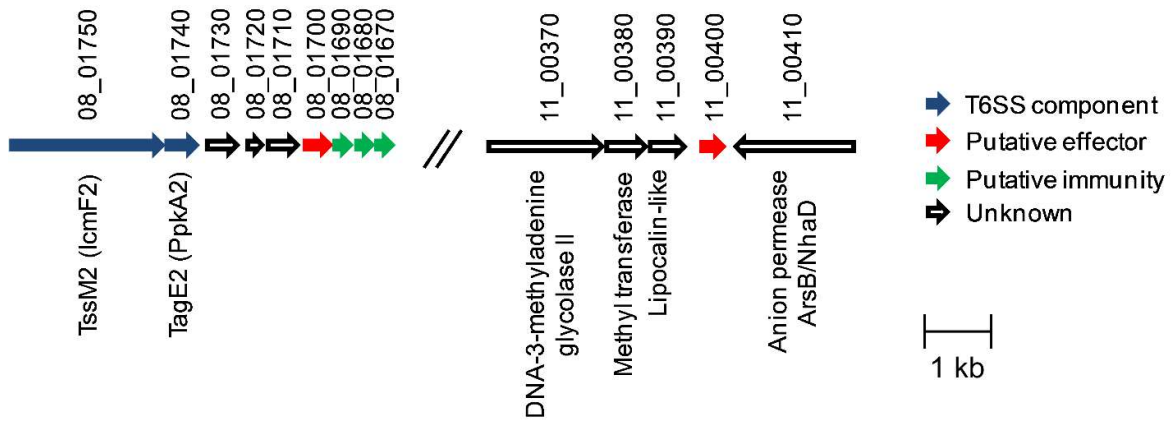
Appendix Figure S1. T6SS1 is most active under warm marine-like conditions. Expression (Cells) and secretion (Media) of *Vpr VgrG1* after 5 h incubation under the indicated temperature and salinity conditions. $\Delta vgrG1$ deletion strain was used as negative control. VgrG1 was detected by immunoblot using specific antibodies against VgrG1. Arrow marks VgrG1 signal; asterisks mark non-specific bands. Loading control (LC) is shown for total protein lysate. Experiment was performed twice with similar results. A representative experiment is shown.



Appendix Figure S2. Exogenous complementation of VgrG1 does not rescue loss of bactericidal activity. Viability counts of *E. coli* prey before (0 h) and after (4 h) co-culture with indicated *Vpr* strains on media containing 3% NaCl at 30°C. Attackers contain either an empty expression vector (Empty), or vector for arabinose-inducible expression of VgrG1. Asterisks mark statistical significance between samples at 4 h timepoint ($P < 0.05$) determined using unpaired, two-tailed Student *t* test; n.s.=not significant; WT=wild-type.



Appendix Figure S3. Deletion of *tssG1* does not affect *Vpr* growth. Growth of *Vpr* strains in MLB (3% NaCl) at 30°C measured as absorbance optical density at 600 nm (OD₆₀₀). Data are means \pm standard deviation (n=3). WT=wild-type.



Appendix Figure S4. Schematic representation of the gene neighborhoods of new *Vpr* T6SS1 effectors identified by comparative proteomics. Genes are represented by arrows indicating direction of translation. Locus tags (*vpr01s_xx_xxxxx*) shown above. Encoded proteins and known domains shown below.

```

Vpr01570 313 MVGEWDGEGYSTGEQVSMTQLTGRGPFYLYKTPRAPRAKFWFSRIQRAEFSAEKASERLS
VopC      88 NVKKADAKSITALKSGDLHLKKGKGIIGMKG-----GDNKLPFKCTIVNDD--
CNF1     720 SIESTSKSNFQKLSRGNIDVLKGRGSISSSTRQRAIYPYFEAANADEQQPLFFYIKKDRFD
CNFy     720 NVAEISSINFRRLLNSGNIINVLLKGRGVFSSRRLREIYLRFDAANADELRLPGDVYVKKTKFD

Vpr01570 373 DSDQRDVLECSDGTMQTSFNKSMNAWV-----THFWDDNPLPEELDVLVL
VopC     134 -KNG-AHLSQGTNLA-TNG---IKSMAGDSVRAAQLIPGTPLGQFYNSAPLDDSFNVVHL
CNF1     780 -NHGYDQYFYDNTVG-LNGIPTLNTYTGEI PSDSSSLG----STYWKKYNLTNETS IIRV
CNFy     780 -SMGYDSHFYNEGIG-INGAPT LNTYTGEYVADSSSQG----ATYWLKYNLTNETS IIKV

Vpr01570 418 HNGYTGAAGVRIDLDLDPGQQILVTG*TL*SGCTMVTGVKGRQFYALHAGT-AI PNEWK
VopC     188 PNGQRGVNLKIPLSEFYSEKKFLFSNGALSGCMTCTAIDKNNLYIFHVKGKDGNDTSPWK
CNF1     834 SNSARGANGIKIALEEVQEGKPVIIITS*NL*SGCTTIVARKEGYIKVHTGT-TK A-GF
CNFy     834 SNSARGANGIKIALEEVLEENKPVVIT*SL*LGCTVVFARKGEYFMAVHTGN-SESLI-GF

Vpr01570 477 TGQHGVRDNYALLKLMMPENDLTAPPEWDNDGMPALL-DCFSQATMAYSG---KPEAKMQ
VopC     248 TNVDGSSLIQKNMKMLLGQNSDSLNNGIQ--GLIDYCSKNFDKAIIQYCGHGEQYSG---
CNF1     892 TSTTGKVKAVEVLELLTKEPIPRVEGIMSNDFLVDYLSENFEDSLITYSSSEKKPDSQIT
CNFy     892 TSTSGVAKAIEVLSLISELEVPALPDVINNNTLVEYLSDNEDSALISYSSSSSLKPNSMIN

Vpr01570 532 --RADARCF
VopC     303 --RKNIHLEF
CNF1     952 IIRDNVSVF
CNFy     952 ISRENVSTF

```

Appendix Figure S5. Vpr01570 contains a C-terminal CNF toxin domain. Multiple sequence alignment of Vpr01570 MIX-effector C-terminal toxin domain and other CNF toxin domains. Residue numbers shown left of the alignment. Conserved residues marked in yellow (similar) and red (identical). Conserved catalytic residues marked by asterisk. Alignments shown for: Vpr01570 (GAD68163), *V. parahaemolyticus* VopC (VPA1321), *E. coli* CNF1 (UTI89_C4921), and *Yersinia pseudotuberculosis* CNFy (YPK_2615).

```

VPR01S_11_00400      1 M-----
V. mimicus           1 M-----
Txp40_Xenorhab.     1 MVENTVVENTMVIKPVTTTPSVIQLTPDDRGEYQPVEKQIAGDIIRVLEFKQTNESHTGLY
Txp40_Photorhab.    1 M-----VIQLTPDDRSGYPPVEKQIAGDIVRILNFKQTDGHTASY

VPR01S_11_00400      2 -----
V. mimicus           2 -----
Txp40_Xenorhab.     61 GIAYRAKKVIIAYALAVSGIHNVSQLPEDYYKKNKDNTGRIYQEYMSNLSSALLGENGDQI
Txp40_Photorhab.    42 GIEYRAKKIILAYALAVSGIHNVSKLPDDYYKKNKETAERIYQEYMSNLSSALLGENGDQI

VPR01S_11_00400      2 -----
V. mimicus           2 -----
Txp40_Xenorhab.     121 SKDMANDFTQNELEFGGQRLKNTWDIPDLENKLLLEDYSDEDKLLALYFFASQELPMEANQ
Txp40_Photorhab.    102 SKDMANGFYKNELDFEGQYQNIWNVPELENKPLSAYSDDDKLLALYFFSVQEI PLEENQ

VPR01S_11_00400      2 -----
V. mimicus           2 -----
Txp40_Xenorhab.     181 QSNAANFFKVIDFLLILSAVTSLGKRIFSKNFYNGLETKSLENYIERKKLSKPFRRPPQK
Txp40_Photorhab.    162 QSNAARFFKLIIDFLLTSAVTSLGRRIFSKNFYNGLESKLENYIERKKFPKPFRRPPQR

VPR01S_11_00400      2 -----SH-----WRINKSAVRW-----SRPTSSAAQR
V. mimicus           2 -----PG-----WRLDKSRVNW-----NRTITSAAQD
Txp40_Xenorhab.     241 LPDGRTGYLAGPPKAPKLPTTSSSTATTSTAASSNWRVSLQKLRDNPSRNAFMQMDDAAKR
Txp40_Photorhab.    222 LPDGRIGYLAGPTEAPK-----WRVSFKELKNNKSRNGFSNMEGAAKQ

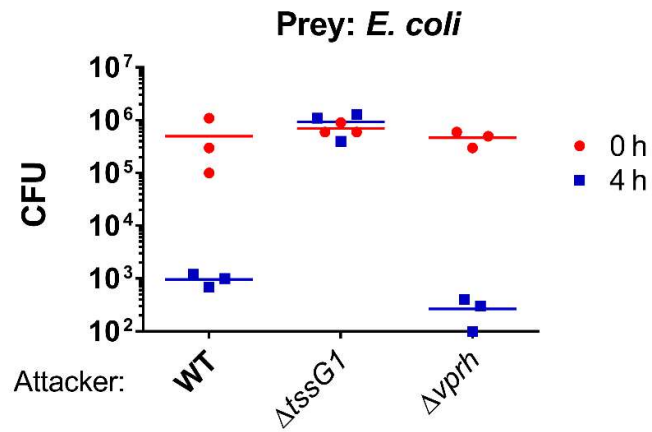
VPR01S_11_00400      24 NFDLQNEIFHNGTDP RHAADEFDS----HVECLDKGNVWVSIIRLSQRDRVVRLDETCH
V. mimicus           24 KLDMEQNEIFNKGTDPRIAAKQFDS----HCECLDRTNNIWSIIRLSQSDRVVFRINDIDH
Txp40_Xenorhab.     301 KYSSFIKEV-QKGNPRAAATSIGTKSGSNFEKL-QGRDLYSIRLSQEHRTVTSINNTDQ
Txp40_Photorhab.    265 KYSSFIKEV-QKGNAPQTAAKSIGTASGSNLEKL--PNNLYSVRLSQKDRVTFTQNDTDN

VPR01S_11_00400      80 VCIIEGVGGHY---
V. mimicus           80 ICVIEAVGGHY---
Txp40_Xenorhab.     359 IMEIQSVGTHYQNI
Txp40_Photorhab.    322 TMTVHHSVGTHTYKNI

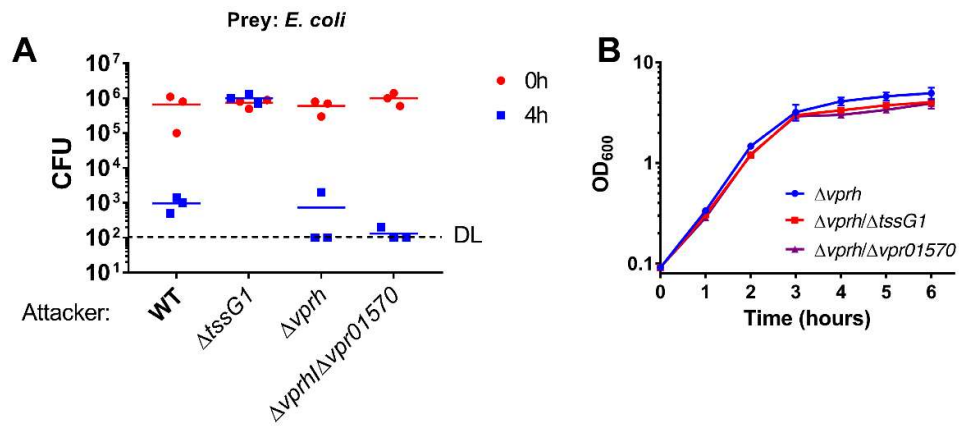
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Appendix Figure S6. Vpr01s_11_00400 is homologous to C-terminus of insecticidal toxin Txp40.

Multiple sequence alignment of Vpr01s_11_00400 and Txp40 toxins. Residue numbers shown left of the alignment. Conserved residues marked in yellow (similar) and red (identical). Alignments shown for: Vpr01s_11_00400 (GAD68047), AL543_05135 from *Vibrio mimicus* (AMG02395), Txp40 from *Xenorhabdus nematophila* AN6/1 (CEK22105), and Txp40 from *Photorhabdus luminescens* subsp. *laumondii* (KTL63163).



Appendix Figure S7. Deletion of *vprh* does not impair *Vpr* bactericidal activity. Viability counts of *E. coli* prey before (0 h) and after (4 h) co-culture with indicated *Vpr* strains on media containing 3% NaCl at 30°C. WT=wild-type.



Appendix Figure S8. Deletion of *vpr01570* does not affect T6SS1 activity or bacterial growth. (A) Viability counts of *E. coli* prey before (0 h) and after (4 h) co-culture with indicated *Vpr* strains on media containing 3% NaCl at 30°C. WT=wild-type; DL=detection limit. **(B)** Growth of *Vpr* strains in MLB (3% NaCl) at 30°C measured as absorbance optical density at 600 nm (OD₆₀₀). Data are means ± standard deviation (n=3). WT=wild-type.