1. Extended Data

Figure #	**Figure title** **One sentence only**	**Filename** **This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.:** ***Smith_ED_Fig1.jpg***	**Figure Legend** **If you are citing a reference for the first time in these legends, please include all new references in the Online Methods References section, and carry on the numbering from the main References section of the paper.**
Extended Data Fig. 1	Further analyses of TseH killing, delivery, and structure	Hersch_ED_Fig1.tif	**a.** Relative survival (killing by *V. cholerae* V52 $TseH^{WT}$ / $TseH^{H64A}$) of *E. coli*, *A. hydrophila*, *Edwardsiella sp.*, *A. baylyi* ADP1 (ΔT6SS), or *V. parahaemolyticus* RIMD2210633 strains. Welch's one-way ANOVA with Dunnett's multiple comparisons test comparing each sample to *E. coli* MG1655; *, $p < 0.05$; **, $p < 0.01$; ns, not significant. **b.** Relative survival (killing by $TseH^{WT}$ strain relative to killing by $TseH^{H64A}$ strain) of *A. dhakensis* expressing TsiH or vector only control. Unpaired 2-tailed t-test; ***, $p < 0.001$. **c.** Survival of *A. dhakensis* after killing by $TseH^{H64A}$ strain expressing TseH or vector only. Vector control data resembles no-plasmid data shown in Fig. 1a and was only

			repeated in duplicate. Unpaired 2-tailed t-test with Welch's correction; **, $p < 0.01$. **d.** Extension of Figure 1b in the main text. Survival of *A. dhakensis* prey after killing by ΔPAAR2 strain expressing plasmid-borne TseH and full-length or C-terminal truncations of PAAR2. For PAAR2: V, vector; numbers, PAAR2 truncated to indicated amino acid length; Δin, codons 146-156 removed leaving downstream residues intact. One-way ANOVA with Dunnett's multiple comparisons test comparing to the sample expressing TseH and Vector control of PAAR2; ***, $p < 0.001$; ns, not significant. For all graphs, the mean and standard deviation are shown. Dots show individual replicates. **e.** Western blot showing whole cell lysate or eluted fraction after His-tag pull-down. BL21 DE3 *E. coli* expressed Flag-tagged TseH in combination with either His-tagged PAAR2 or Spy as a non-binding control. Number at left indicated ladder band size in kDa. Data is representative of two independent replicates. **f.** Circular permutation of catalytic residues

			causes swapping of N- and C-terminal lobes in the structure of TseH compared to Tse1 (PDB: 4EOB[18]), the other T6SS effector that belongs to NlpC/P60 family has reversed N- and C-lobe structure in comparison to TseH. **g.** Restricted access to the catalytic cysteine in certain peptidoglycan endopeptidases. The surface representations of SaCwlT and TseH show that the conformational changes in residues surrounding the active site are necessary for the substrate binding[21,22]. For all graphs, the mean and standard deviation are shown. Dots show individual replicates. $TseH^{WT}$, *V. cholerae* V52 with all anti-bacterial effectors inactivated except TseH; $TseH^{H64A}$, V52 with all anti-bacterial effectors inactivated including TseH.
Extended Data Fig. 2	Identified mutations that reduce TseH toxicity	Hersch_ED_Fig2.tif	
Extended Data Fig. 3	Additional *E. coli* mutants susceptible to T6SS-delivered TseH	Hersch_ED_Fig3.tif	**a.** Disk diffusion induction of plasmid-borne TAT-tagged TseH expression in bacterial lawns of *E. coli* MG1655. Wild-type (top) or H64A mutant (bottom) TseH are compared.

			Right disks contain the inducer of expression, arabinose (Ara), and left disks contain the repressor, glucose (Glu). Representative of three independent replicates. **b.** As in **a**, comparing induction of plasmid-borne TAT-tagged TseH^{WT} expression in *E. coli* MG1655 or *A. dhakensis* (ΔT6SS). Similar data for TseH^{WT} in *E. coli* has been shown previously and is shown again here for comparison[4]. **c.** Relative survival (killing by TseH^{WT} / TseH^{H64A}) of *E. coli* wild-type or knockout mutants from indicated categories. One-way ANOVA with Dunnett's multiple comparisons test comparing each sample to WT; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Data for WT (*E. coli*) is shown in Figure 2 and is shown again here for comparison. Aux., auxotrophs. **d.** RT-qPCR comparing expression of genes in wild-type *E. coli* prey after T6SS competition assay with TseH^{WT} or TseH^{H64A} killer strains. Value with TseH^{H64A} killer was set to 1 to show fold induction in response to TseH activity upon delivery by the T6SS. One-way ANOVA (with samples

			shown in Figure 2d) with Sidak's multiple comparisons test; ns, not significant. For both graphs, the mean and standard deviation are shown. Dots show individual replicates. TseH^{WT}, *V. cholerae* V52 with all anti-bacterial effectors inactivated except TseH; TseH^{H64A}, V52 with all anti-bacterial effectors inactivated including TseH.
Extended Data Fig. 4	Additional data for RcsA and BaeR regulon overexpression, and effect of Spy overexpression on permeability	Hersch_ED_Fig4.tif	**a.** Disk diffusion induction of plasmid-borne TAT-tagged TseH in bacterial lawns of *E. coli* BW25113 containing RcsA or vector only plasmids. IPTG was added throughout the lawn to induce RcsA. Disks contain arabinose to induce TAT-TseH expression. Representative of three independent replicates. **b.** Relative survival (killing by TseH^{WT} / TseH^{H64A}) of *E. coli* Δ*degP* mutant or *A. dhakensis* with vector (Vec) or overexpressing genes from the BaeR regulon. One-way ANOVA with Sidak's multiple comparisons test comparing samples to their respective empty vector controls; *, $p < 0.05$; **, $p < 0.01$; ns, not significant. Vector controls are shown in Figure 2 and are

			shown again here for comparison. Mean and standard deviations are shown. Dots show individual replicates. pSpy$^{\Delta Sec}$, plasmid expressing Spy with its Sec secretion tag deleted. **c.** Images of Δ*baeR E. coli* overexpressing plasmid-borne TAT-TseH and either Spy or vector-only controls. The phase-contrast and propidium iodide(PI) channels are merged. Scale bar shows 5 μm. Representative of three independent replicates. **d.** Quantification showing the percent of total cells showing PI+ fluorescence. N number at top indicates total cells counted. Dots show mean results from three independent experiments (used to calculate statistics) and error bars show one standard deviation. Unpaired 2-tailed t-test, p = 0.0513.
Extended Data Fig. 5	Additional data for Δ*wigR* strain T6SS susceptibility, immunity gene expression, VPS-mediated aggregation, and *Aeromonas* species do not encode RcsCDB, BaeSR, or WigKR	Hersch_ED_Fig5.tif	Relative survival (wild-type V52 / TseH^{H64A} killer strain) of *V. cholerae* V52 (T6SS^{+} background) prey (**a.**) or *V. cholerae* C6706 prey (**b.**) with intact ($^{+}$), deleted (Δ), or complemented (Δ,p) *wigR*. Stationary (Stat.) and log phase prey are shown. One-way ANOVA with Sidak's multiple

			comparisons test; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant. **c.** RT-qPCR measuring expression of T6SS immunity genes in Δ*wigR V. cholerae* V52 relative to in the *wigR+* strain (both in the ΔT6SS background). For all graphs, the mean and standard deviation are shown. Dots show individual replicates. **d.** Image showing settling of *V. cholerae* V52 grown at 37 °C or at 30 °C to induce VPS synthesis. Image after vortexing to disrupt aggregates is also shown for comparison (lower panel). Representative of three independent replicates. **e.** KEGG ortholog analysis across Gammaproteobacteria. KEGG Modules were compared for RcsCDB and BaeSR. WigK and WigR orthologs were assessed by sequence homology to VCA0565 and VCA0566 using an SW-score cutoff of 50% of maximum. Data is shown as the percentage of strains within each taxonomic group that contain an ortholog of all genes in the loci. Number in brackets indicates the number of strains in the taxonomic group in the KEGG database.

Extended Data Fig. 6	Table of data collection, phasing and refinement statistics for MAD (SeMet) structures	Hersch_ED_Fig6.tif	

2. Supplementary Information:

A. Flat Files

Item	**Present?**	**Filename** **This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf**	**A brief, numerical description of file contents.** **i.e.: *Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.***
Supplementary Information	Yes	Hersch_Supp_Info.pdf	Supplementary Information Table 1: Strains and plasmids
Reporting Summary	Yes	Hersch_Reporting_Summary.pdf	

B. Additional Supplementary Files

Type	**Number** **If there are multiple files of the same type this should be the numerical indicator. i.e. "1" for Video 1, "2" for Video 2, etc.**	**Filename** **This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: *Smith_ Supplementary_Video_1.mov***	**Legend or Descriptive Caption** **Describe the contents of the file**

Supplementary Video	Video 1	Hersch_Video1.mp4	To visualize the effect of TseH on the cell wall, video shows time-lapse fluorescence microscopy of *E. coli* cells expressing TseH (left), VgrG3 (middle) or TseL (right) effectors tagged to the periplasmic signal TAT. Cells were stained with 100 µg/mL of the PG specific dye WGA and spotted onto an agarose pad containing either 0.2% L-arabinose (TseH) or 1 mM IPTG (VgrG3 and TseL). Images were acquired every 20 sec during 25 min. Movie plays at a rate of 10 frames per second. Top row is a merge of phase and green

			(WGA) channels and bottom row shows green channel only. Scale bars, 5 μm. When expressing either TseH or the know cell-wall-targeting effector VgrG3, cells showed typical blebbing followed by PG sacculus deformation and cell lysis, whereas the effector TseL caused retraction and leaking of cytoplasmic content without obvious effect on PG. Video is representative of three independent replicates.

3. Source Data

Figure	**Filename** **This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: *Smith_SourceData_Fig1.xls,* or *Smith_ Unmodified_Gels_Fig1.pdf***	**Data description** **i.e.: Unprocessed Western Blots and/or gels, Statistical Source Data, etc.**
Source Data Fig. 1	Hersch_SourceData_Fig1,ED1d.xls	Data and statistical source data used for Figure 1 and ED Fig. 1d.
Source Data Extended Data Figure 1D	Hersch_SourceData_Fig1,ED1d copy.xls	Data and statistical source data used for Figure 1 and ED Fig. 1d. Please note that this is the same data as in Source Data for Fig 1.
Source Data Fig. 2	Hersch_SourceData_Fig2,ED3cd.xls	Data and statistical source data used for Figure 2 and ED Fig. 3c-d
Source Data Extended Data Figure 3	Hersch_SourceData_Fig2,ED3cd copy.xls	Data and statistical source data used for Figure 2 and ED Fig. 3c-d. Please note that this is the same data as in Source Data for Fig 2.
Source Data Fig. 3	Hersch_SourceData_Fig3.xls	Data and statistical source data used for Figure 3
Source Data Fig. 4	Hersch_SourceData_Fig4.xls	Data and statistical source data used for Figure 4
Source Data Fig. 5	Hersch_SourceData_Fig5.xls	Data and statistical source data used for Figure 5
Source Data Extended Data Fig. 1	Hersch_SourceData_ED1.xls	Data and statistical source data used for ED Fig. 1
Source Data Extended Data Fig. 1	Hersch_SourceBlots_ED1e.pdf	Uncropped western blots shown in ED1e
Source Data Extended Data Fig. 4	Hersch_SourceData_ED4.xls	Data and statistical source data used for ED Fig. 4
Source Data Extended Data Fig. 5	Hersch_SourceData_ED5.xls	Data and statistical source data used for ED Fig. 5