Supporting information

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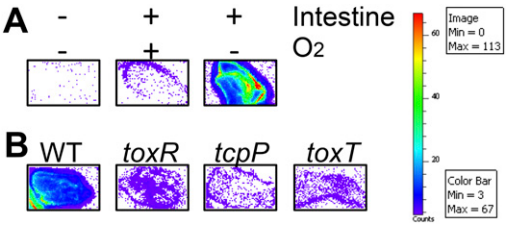
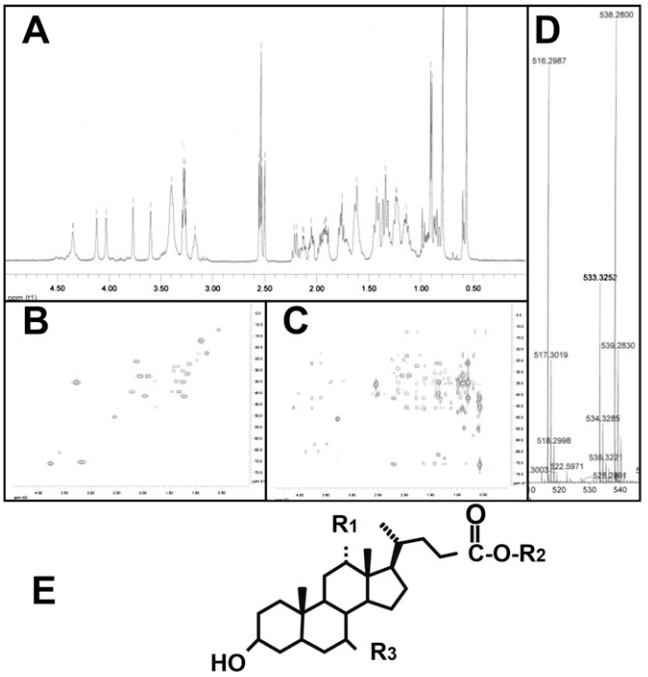


Fig. S1. Virulence gene induction in an ex vivo model. (*A*) Fragments of mouse small intestines were mounted in dishes covered with LB medium and placed at 37 °C in an anaerobic chamber (O_2^-) or ambient incubator (O_2^+). *Vibrio cholerae* cells containing a P_{tcpA}-*lux* reporter were then loaded on the top of opened intestinal tissues. After 3 h, dishes were photographed in the dark using a LAS4010 ImageQuant and analyzed using Living Image 3.2. (*B*) Mouse intestinal tissues were incubated in an anaerobic chamber the same as in *A*. WT and in-frame deletion mutants containing a P_{tcpA}-*lux* reporter were used.



Bile acid tested	R1	R2	R3
Taurocholic (TC)	OH	NH-CH_2-CH_2SO_3H	OH
Glycocholic (GC)	OH	NH-CH_2-COOH	OH
Cholic (CA)	OH	H	OH
Deoxycholic (DC)	OH	H	H
Chenodeoxycholic (CDC)	H	H	OH

Fig. S2. Virulence activation factor-1 (VAF-1) is identified as taurocholate. (*A*–*D*) Characterization data for VAF-1, including ^1H (*A*), ^1H-^{13}C heteronuclear single quantum coherence (HSQC) (*B*), and ^1H-^{13}C heteronuclear multiple bond correlation (HMBC) (*C*) NMR spectra (600 MHz, DMSO-d_6) as well as mass spectral data [high-resolution electrospray ionization-time of flight-mass (ESI-TOF-MS)] (*D*). The proposed structure was verified by high-resolution mass spectrometry [*m/z* (MH+) calculated for C26H46NO7S 516.2995, found 516.2987]. (*E*) Chemical structures of different bile salts.

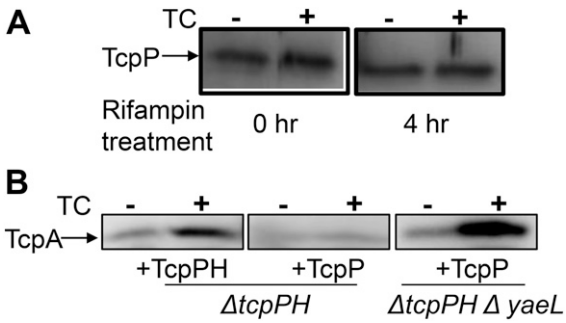


Fig. S3. Bile salts do not affect TcpP stability. (*A*) TcpP protein stability. *Vibrio cholerae* containing P_{BAD}-*tcpPH* plasmids were grown in LB containing 0.01% arabinose in the absence or in the presence of 100 μM taurocholate (TC) until OD_{600} ~0.2, at which point 0.2 mg/mL rifampin was added to terminate transcription. Cultures were further incubated for 4 h. One-milligram cell lysates were subjected to SDS/PAGE and Western blot using anti-TcpP antiserum. (*B*) TcpA production in *tcpPH* mutant and *tcpPH/YaeL* double mutant containing P_{BAD}-*tcpP* or P_{BAD}-*tcpPH* plasmids. Strains were grown in LB containing 0.01% arabinose in the absence or in the presence of 100 μM TC until OD_{600} ~0.2. One-milligram cell lysates were subjected to SDS/PAGE and Western blot using anti-TcpA antiserum.

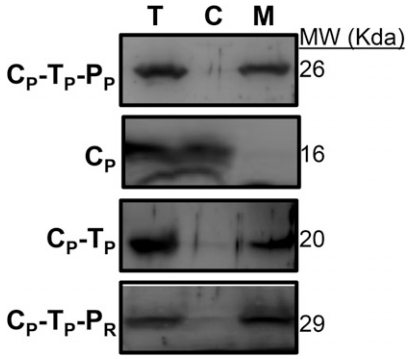


Fig. S4. Cellular localization of TcpP truncation and chimeric mutants. *Escherichia coli* containing indicated P_{BAD}-protein chimeras were grown in LB containing 0.01% arabinose until $OD_{600} \approx 0.2$. Cells were then collected and lysed by sonication. One milligram of total protein (T), cytoplasmic fraction (C), and membrane fraction (M) were subjected to SDS/PAGE and Western blot using anti-TcpP antibody. C, cytoplasmic domain; P, periplasmic domain; $_P$, TcpP; $_R$, ToxR; T, transmembrane domain.

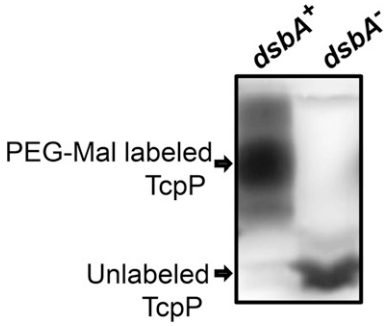


Fig. S5. Differential thiol trapping of TcpP in *Vibrio cholerae*. *V. cholerae tcpPH* (pBAD-*tcpH*) or *tcpPH dsbA* (pBAD-*tcpH*) containing a plasmid expressing TcpP125-221-FLAG-tag fusion (cytoplasmic domain of TcpP was removed to avoid interference of cysteine residues in cytoplasmic domain) using the P_{BAD} promoter were grown in LB containing 0.01% arabinose until OD_{600} ~0.2. The cultures were treated with 50 mM iodoacetamide before isolating the membrane so that any free thiol groups were irreversibly blocked. After trichloroacetic acid precipitation and extensive washing, oxidized thiol groups were reduced by addition of 50 mM DTT in denaturing buffer. These reduced cysteine residues were then alkylated by addition of 10 mM PEG-maleimide (PEG-Mal). Samples were mixed with nonreducing SDS-sample buffer and 30 μg of membrane protein were loaded onto 15% SDS-polyacrylamide gels. TcpP was detected by Western blot analysis by using monoclonal anti-FLAG tag HRP-conjugated antibody.

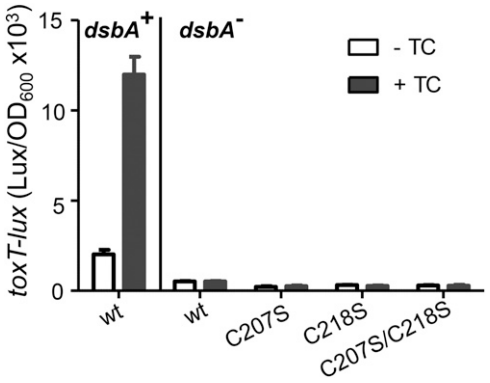


Fig. S6. Bile salts do not affect TcpP activity in *dsbA* mutants. *Vibrio cholerae tcpPH* (pBAD-*tcpH*) and *tcpPH* (pBAD-*tcpH*)Δ*dsbA* mutants containing P_{toxT}-*lux* reporter and WT or cysteine mutant *tcpP* under the control of the P_{BAD} promoter on plasmids were grown in LB containing 0.01% arabinose in the absence or in the presence of 100 μM taurocholate (TC) until OD_{600} ~0.2. Luminescence was then measured and reported as light units/OD_{600}. Data are mean and SD of three independent experiments.

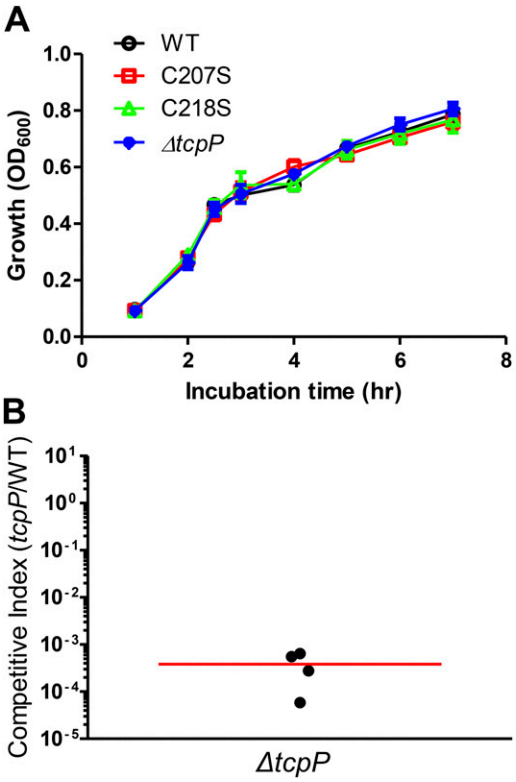


Fig. S7. In vitro growth and colonization of *tcpP* mutants. (*A*) WT and *tcpP* C207S, C218S, and Δ*tcpP* were grown in LB at 37 °C. OD_{600} was measured at the time points indicated. Data are mean and SD of three independent experiments. (*B*) Infant mouse colonization. Six-day-old CD-1 infant mice were inoculated with the mixture of *tcpP* null mutants and WT at a 1:1 ratio. After a 6-h period of colonization, intestinal homogenates were collected, and the ratio of mutant-to-WT bacteria was determined and normalized against input ratios.

Table S1. VAF activity from various sources

Sample	Tissue*	VAF activity[†]
Conventional adult mouse[c]	Stomach	—
	Small intestine	+++
	Large intestine	+
Germ-free adult mouse[‡]	Small intestine	+++
Infant mouse[¶]	Small intestine	+++
Infant mouse	Small intestine/dialysis[§]	—

*Tissues were homogenized in 5 mL PBS buffer.

[†]Ten percent cleared homogenates were added in LB containing C6706 (p*PtcpA-luxCDABE*) reporter cells and incubated anaerobically at 37 °C for 4 h. Compared with that of PBS control, below 10-fold induction of tcpA was recorded as —, 10–50-fold as +, and over 50-fold as +++.

[‡]Five-week-old BALB/c mice were obtained from The Jackson Laboratory (conventional) or maintained in plastic isolator units and fed autoclaved feed and water (germ-free).

[¶]Five-day-old infant CD-1 mice were used.

[§]The homogenate was dialyzed using a 1-K_d cutoff membrane.