ONLINE METHODS

Strains and culture conditions.

Unless otherwise noted, bacterial cultures were grown at 37ºC with Luria-Bertani (LB) medium (Lennox) or Mueller-Hinton (MH) solid media supplemented when appropriate, with the following antibiotics: 50 µg/ml kanamycin (Kan), 50 µg/ml chloramphenicol (Cm), 100 µg/ml carbenicillin (Carb), 50 or 100 µg/ml spectinomycin (Sp) for *E. coli* and 100 µg/ml Sp for *Vibrio cholerae*. Selection of transconjugants was carried using 100 µg/ml Sp in all cases, except for *V. mimicus* and *V. vulnificus* where we used 50 µg/ml Sp. Bacterial strains used in this study are listed in Supplementary Table 1. Other molecules were added to the media with the following concentrations: 40 µg/ml 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (Xgal), 0.3 mM Diaminopimelic acid (DAP), 1% glucose and 0,2% arabinose.

Plasmid construction.

Plasmids are listed in Supplementary Table 1 and primers in Supplementary Table 6. All plasmid sequences were verified through sequencing.

To generate the N and C plasmids for each toxin-intein fusion, the N- and C-terminal toxin regions were amplified with primers F-toxin-EcoRI/R-toxin-intein and F-toxin-intein/R-toxin-XbaI, respectively. N-and C-terminal intein regions were amplified with primers F-intein-toxin/R-intein-XbaI and F-intein-EcoRI/R-intein-toxin, respectively. As DNA templates for toxins we used chromosomal DNA from *V. cholerae* in all cases and *V. fischeri* for *ccdB*. Intein amplification was done with chromosomal DNA from the cyanobacteria *Nostoc punctiforme*. PCR products of N- and C-terminal regions were fused by Gibson assembly[35]. Each toxin-intein fusion was then digested with EcoRI/XbaI (Thermo Fisher) and then cloned in EcoRI/XbaI digested pBAD43[36] and pSU38[37] (or pSU18) plasmids, respectively (Supplementary Table 1). To generate the

mutated version of N-terminal plasmid (n*) whole plasmids were amplified using primers F-Int-tox-mut/R-int-tox-mut.

To assemble the pU-BAD plasmid (Supplementary Fig. 5) we first cloned the C-terminal CcdB-Npu fusion into a pBAD18 plasmid (EcoRI-XbaI). An *ompU* promoter was inserted upstream the N-terminal ccdB/Npu fusion in N plasmid by PCR. The *ompU* promoter region was amplified using F-PompU-1/R-PompU-dB (size=352 bp). This promoter was chosen based on previous work[21] that showed its high induction in the presence of ToxR. A region containing the pSC101 origin was amplified using R-BAD43-BAD18/F-4126 primers and the N-terminal CcdB-Npu plasmid as template. A second region containing the N-terminal fusion was amplified using 4217/R-BAD43-BAD18 primers and N-terminal plasmids also as templates. Other regions containing the C-terminal fusion and Kan resistance gene were amplified using R-BAD18-BAD43/F-BAD18-BAD43 primers and the C-terminal CcdB-Npu pBAD18 plasmid as template. PCR products were then fused by Gibson assembly[35] producing the pU-BAD plasmid.

To generate the pRS plasmid, (Supplementary Fig. 5) the *toxRS* operon from *V. cholerae* O1 was amplified using F-toxR-SacI/R-toxS-XbaI primers, digested with SacI and XbaI and ligated with SacI-XbaI digested plasmid pBAD30. The native RBS sequence of *toxR* was kept.

To assemble the toxin-intein N and C-terminal fusions as an operon (pToxInt plasmid), N- and C-fusions were amplified using F-CcdB-EcoRI/R-Int-N-Int-C and F-Int-C-Int-N/R-Int-XbaI primers and then ligated by Gibson assembly[35], digested with EcoRI/XbaI and cloned into a pBAD43-EcoRI/XbaI digested plasmid. The fusion contains the following sequence: 5' TGATAAGGAGGTAACATATG 3' between the N and C genes. This sequence contains the RBS sequence necessary for translation of the C-terminal fusion. The pTox plasmid was created by amplification of the *ccdB* toxin gene from *V. fischeri* DNA with F-CcdB-EcoRI/R-CcdB-XbaI primers, EcoRI/XbaI digestion and ligation into a pBAD43-EcoRI/XbaI digested plasmid. *E. coli* XL2blue strain that

contains F' plasmid integrated in the chromosome (containing the *ccdB/ccdA* TA system and conferring resistance to CcdB), was used to transform with this ligation in order to obtain positive clones.

To assemble the pPW genetic weapon, the *ompU* promoter was amplified as previously described and ligated by Gibson assembly[35] with the product of pToxInt plasmid PCR using F-dB-PompU/R-BAD-PU1 primers.

The pPLA plasmid was constructed first by amplifying by PCR the PL promoter[38] using DNA from *V. cholerae* O139 and F-PL-plasmid/R-PL-ccdA as primers. Then, the *ccdA* antitoxin gene was amplified using the F-ccdA-PL/R-ccdA-plasmid primers and *V. fischeri* DNA. Finally, the pTox-Int plasmid was also amplified using F-plasmid-dA/R-plasmid-PL primers. Ligation by Gibson assembly[35] of the three PCR products resulted in the pPLA plasmid.

Mobilizable genetic weapons were created by amplifying the origin of transfer *oriT* RP4 using F-pSW23-BAD/R-oriT-BAD43 primers and the plasmid pSW23T[39] as template. Then, the *oriT* PCR product was ligated through Gibson assembly[35] with the amplified plasmid using F-BAD-pSW/R-BAD43-oriT primers and the weapon or control plasmids as template.

To assemble the Final Weapon we the plasmid pFW (Figure 3) as follow. The *ompU* promoter-1 was ligated into the pABRW plasmid as previously described for the pU-BAD construction. In order to fine-tune the RBS of *ompU* in this plasmid as well as the PL promoters,, PCRs were performed using F-ccdB-SD-OK/R-PU-SD-OK and F-PL-SD-T/R-PL-SD-T primer pairs, respectively. Finally, to generate the pFW plasmid, an operator O1 sequence (see [38]) was added into the PL promoter by PCR amplification of the pFW2 plasmid using F-PL-O1/R-PL-O1 primers.

To generate the pPW-R6K, pFW-R6K and pNctrl-R6K plasmids we first amplified the R6K replication origin using F-R6K-weapon/R-R6K-weapon primers and the pMP7[40] plasmid as template. Then, the pPW, pFW and pNctrl plasmids were amplified using F-

weapon-R6K/R-weapon-R6K primers. Finally, PCR fragments were ligated by Gibson assembly[35].

ΔtoxRS strain construction

DNA regions 500 bp upstream and downstream of the *toxRS* operon were amplified using F-toxRup-p7/R-toxRups and F-toxSdow/R-toxSdow-p7, respectively. The amplified fragments were ligated by Gibson assembly[35] and then cloned into an R6K γ-*ori*-based suicide vector, pSW7848[40] that encodes the *ccdB* toxin gene under the control of an arabinose-inducible promoter, P_{BAD}. For conjugal transfer of plasmids into *V. cholerae* strains, *E. coli* β3914 was used as the donor. Clones where integration of the entire plasmid in the chromosome by single crossover occurred were selected. Elimination of the plasmid backbone resulting from a second recombination step was selected as described ref 39.

Transformation assays

DH5α chimiocompetent cells (Invitrogen) were transformed with 150 ng of pTox, pToxInt or pN plasmids (Supplementary Fig. 1a). Transformants were then tested in Sp containing media with glucose or arabinose to analyze toxin integrity. 10 to 12% of pTox-transformed clones from were able to grow in the presence of arabinose. Four independent clones were analyzed by sequencing and they all carried an insertion sequence in the *ccdB* toxin gene. These clones were responsible for pTox transformation rate decrease in comparison with the pToxInt and pN plasmids.

DH5α cells (Invitrogen) were co-transformed with two plasmids simultaneously. Both plasmids were then simultaneously selected (Supplementary Fig.4).

Transformation of the donor strain β3914 was performed in the presence of DAP.

Growth tests

Eighteen independent clones from DH5α transformation were inoculated in p96 microplates containing LB media with Sp and glucose. The TECAN Infinite 200 microplate reader (TECAN, Männedorf, Germany) was used to determine growth curves, with absorbance (620nm) taken at 6-minute intervals for a period of 12 h. The obtained OD values were plotted as seen on Supplementary Fig. 1b.

In Supplementary Fig. 2 for analysis of bactericide effect of CcdB toxin: *V. cholerae* O139 was co-transformed with antitoxin-*ccdA (*pBAD24-*ccdA)* and pPW plasmids in the presence of arabinose allowing the antitoxin to be expressed. pPW plasmid contains the toxin-intein under the control of *ompU* promoter, which is always active in *V. cholerae*. Bacteria culture supplemented with antibiotics for maintaining both plasmids and arabinose, were diluted at OD=0.5 (time 0h). Then bacteria were washed three times with MH media with antibiotics and glucose, in order to switch off antitoxin expression, and incubated for 4h at 37ºC. Total bacteria were calculated by the CFU/ml at time 0h and 4h present in MH media with antibiotics and with glucose (1%) or arabinose (0,2%). Data numbers were calculated from four independent experiments (n=4).

Conjugation assays.

Overnight cultures of donor and recipient strains were diluted 1:100 in culture media with antibiotic and grown at 37ºC for 2-3 hours. Then, cultures were diluted to an OD_{600} = 0.5. The different conjugation experiments were performed by a filter mating procedure described previously[41] with a donor/recipient ratio of 1::1. When the recipients were composed of a mixed population the donor/mixed-recipient ratio was 1::0.5-0.5. Before mixing the different bacteria, cultures were washed three times with fresh media to remove antibiotics. In Supplementary Table 2 bacteria were mixed in different proportions (2:1 and 3:1) to test whether this would impact conjugation efficiency. Conjugation was performed during 4h at 37ºC on filter in MH plates

supplemented with DAP (and containing NaCl until 332mM final concentration in the case of *V. vulnificus*).

In vivo **conjugation in zebrafish larvae and** ***Artemia salina***

All animal experiments described in the present study were conducted at the Institut Pasteur according to European Union guidelines for handling of laboratory animals (http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm) and were approved by the Institut Pasteur Animal Care and Use Committee and the Direction Sanitaire et Veterinaire de Paris under permit #A-75-1061. Conjugative killing was assessed as follow. Four-day-postfertilization zebrafish larvae were exposed to water containing 10^4 CFU/ml of *V. cholerae* O139 for 2 hours at 27ºC (Figure 4a) or a 1::1 mixed population containing 10^5 CFU/ml *V. cholerae* O139 + *V. cholerae* O1 (Figure 5a, mix *Vibrio*) for 2 hours at 27ºC. Then, larvae were washed in sterile water three times and then placed into a well containing 10^7 or 10^6 CFU/ml (Figure 4a, *V. cholerae* O139 and mix *Vibrio*, respectively) of the *E. coli* β3914-*Δdap* donor strain containing either the pN_{ctrl} or pFW plasmid for 24 hours at 27ºC. In Supplementary Fig. 11b and 11c, infection dose for *Vibrio* was the same than for Fig. 4a. Larvae were transferred to bacteria-free wells, washed in sterile water three times and then placed into a well containing Tricaine (Sigma-Aldrich #E10521) at 200 mg/ml to euthanize them. Finally, they were transferred to a tube containing calibrated glass beads (acid washed, 425 um to 600 um, Sigma-Aldrich #G8722) and 500 µl of water. Five larvae were mashed using FastPrep® Cell Disrupter (BIO101/FP120 QBioGene) for 45 seconds at maximum speed (6,5 m/sec) to analyze their microbiota (Supplementary Fig. 11) in MH Media + X-gal or TCBS media for selection of *V. cholerae*. Blue bacteria corresponding to *V. cholerae* O139 were detected in MH media. Transconjugants selection was done into MH Media + X-gal and Sp and then, replication of these MH plates were done on TCBS media to specific identify *V. cholerae*. Strain identity was confirmed through

yellow color development in TCBS *Vibrio* specific media. The amoeba *Tetrahymena thermophila* (*T. thermophila*) was added to feed larvae during the experiment.

Groups of 225±15 larvaes of *Artemia salina* stage nauplii suspended in 1ml volume of seawater were washed using sterile cell strainer Nylon filters 100 µm pore size (Falcon) and three times with the same volume (3x1ml) of sterile PBS (D8537, Sigma). Nauplii were suspended in 1ml PBS and then infected with 10^7 *V. cholerae* O1 or a mix of 10^7 *V. cholerae* O1 and O139 for 2 hours in agitation at 27ºC. Then nauplii were washed as previously described and exposed to 10^7 of β3914 - *⊿dap* bacteria with pN_{ctrl} or pFW plasmid for 4 hours at 27ºC. These experiments were repeated four times independently. The microbiota from 1ml containing 225±15 nauplii were analyzed as previously described for zebrafish. In the case of *Artemia*, we have used M63B1 minimal media where *Artemia* feel asleep and then put them on ice, previous the use of fast-prep (FastPrep® Cell Disrupter (BIO101/FP120 QBioGene) for 45 seconds at maximum speed (6,5 m/sec)). Transconjugants were selected from 225±15 nauplii after pN_{ctrl} or pFW conjugation treatment into MH media with Sp and X-gal (Supplementary Fig. 12a,b). For the identification of *V. cholerae* in the mix of both serogroups (Fig. 4b and Supplementary Fig. 12b), replication of these MH plates were done into TCBS media to specificaly identify *V. cholerae*. Strain identity was confirmed through yellow color development in TCBS *Vibrio* specific media.

Co-localization of *E. coli* and *V. cholerae* in the zebrafish larvae and *A. salina* by Microscopy.

Co-localization of *E. coli* and *V. cholerae* in the zebrafish larvae was assessed as follow. Four-day-postfertilization zebrafish larvae were exposed to water containing 10^6 CFU/ml *V. cholerae O1*-GFP for 2 hours at 27ºC. Then washed in sterile water three times and then placed into a well containing 10^7 CFU/ml of *E. coli*-RFP for 24 hours at 27ºC. Larvae were removed from the well and then placed into a well containing

Tricaine for euthanize them. Infected and non-infected larvae were visualized by fluorescence microscopy (EVOS FL microscope-Life technologies) using appropriate wavelength conditions enabling or not the visualization of GFP and RFP. Fluorescence was only detected in infected larvae and more precisely into the gut where both bacteria are co-localized.

In the case of *A. salina* stage nauplii the microscopy experiment was done using 10^7 *V. cholerae*-GFP for 2 hours in agitation at 27ºC. Then nauplii were washed as previously described and exposed to 10^7 of *E. coli*-RFP strain for 2 hours. Microscopy conditions were performed as for zebrafish experiment.

Statistics

In Supplementary Fig. 9, one-way ANOVA with Dunnett's Multiple Comparison Test was performed. PNcontrol-R6K vs pPW-R6K, Mean Diff. = 2.383e+008, q = 4.183, **P<0.05, 95% CI of diff = (8.937e+007 to 3.871e+008). PNcontrol-R6K vs pFW-R6K, Mean Diff. = 2.308e+008, q = 4.227, **P<0.05, 95% CI of diff = (9.187e+007 to 3.896e+008).

In Supplementary Fig. 13, one-sided t-test Mann Withney was performed. *E. coli* SXT vs *E. coli* MG1655. P value = 0.0143. *P < 0.05.

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