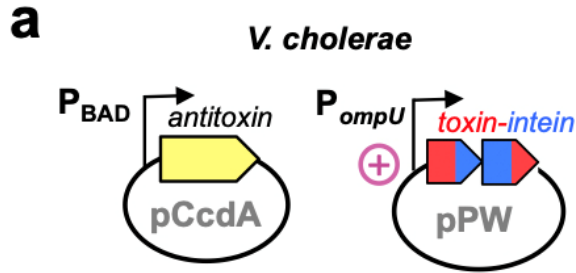
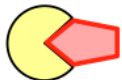



Supplementary Figure 1

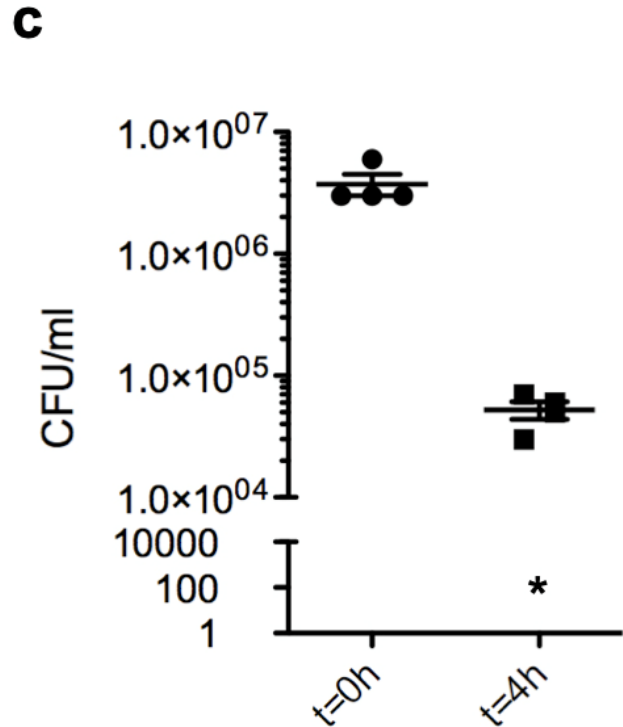
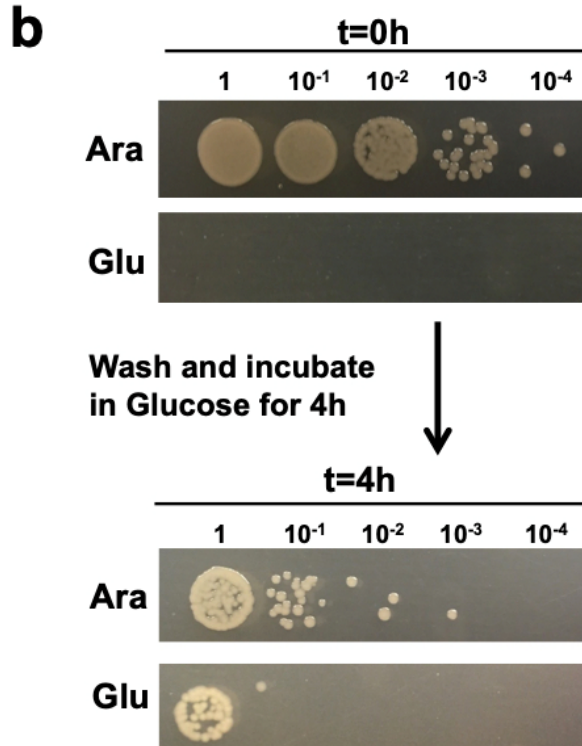
Toxicity comparison of single CDS toxin gene versus synthetic split toxin-intein fusion.

(a) Characteristics of plasmids pTox, pToxInt and pN. All constructions are derivative of pBAD43 a low copy number plasmid conferring spectinomycin (Sp) resistance. The pTox plasmid contains the *ccdB* toxin gene (red arrow) cloned under P_{BAD} promoter, while in pToxInt *ccdB* is disrupted by the *dnaE* split intein (blue rectangles), forming an operon (N and C terminal genes fusion). As a control we used the non-toxic CcdB-DnaE-N fusion plasmid, the pN plasmid. Numbers below plasmids show the transformation rates into *E. coli* in repressible condition (glucose) for toxin or toxin-intein expression. (b) Growth curve in liquid media containing Sp and glucose, of 18 transformants clones from pTox (red), pToxInt (blue) or pN (grey) plasmids. No differences were found between growth rate of bacteria containing the non-toxic pN plasmid and pToxInt plasmid. However, bacteria containing pTox plasmid were not able to growth in repressible conditions for toxin expression (n=18 mean \pm s.d.).



Arabinose conditions: 
 Toxin-antitoxin = bacteria growth

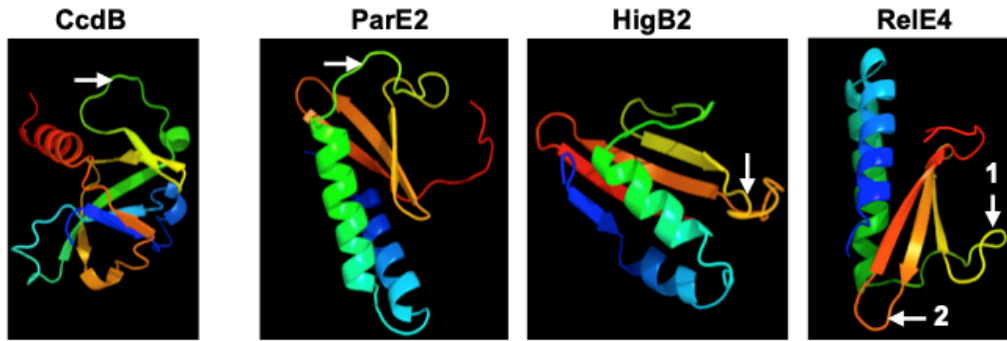
Glucose conditions: 
 Toxin = bacteria growth arrest



Supplementary Figure 2

Analysis of the killing effect of CcdB toxin in *V. cholerae*.

(a) Schematic representation of antitoxin-*ccdA* (pCcdA) and *ccdB* (pFW) expressing plasmids (left). When both plasmids are together in a bacterium, toxin-antitoxin complex allow bacteria to survive. Upon switch off of antitoxin expression (+ Glucose), the toxin is able to carry out its bactericidal or bacteriostatic effect. (b) *V. cholerae* O139 containing pCcdA and pPW plasmids grown in the presence of arabinose (allowing the antitoxin to be expressed) was diluted at OD=0,5 (time 0h), arabinose was removed and glucose was added in order to switch off antitoxin expression for 4h at 37°C. Viability at t=0 and t=4h was assessed by serial dilutions plated in drops on selective media + arabinose or + glucose. (c) Total bacteria (CFU/ml) able to grow on MH media + arabinose 0.2% at time 0h and 4h. After 4h upon exposure with the toxin, an important proportion of *V. cholerae* cells are not detected when the antitoxin is switched on (arabinose). * represents the proportion of mutants with CcdB inactivated, as they are able to grow in glucose conditions. This result indicates the bactericidal effect of CcdB toxin. Data numbers were calculated from four independent experiments (n=4, mean ± s.d.).

a**b****CcdB** (*Vibrio fischeri* MJ11)

MSQFTLYKNKDKSSAKTYPYFVDVQSDLLDNLNTRLVIPLTPIIELLDKKAPSHLCPTIHIDEGDFIM
 LTQOMTSVPVKILSEPVNELSTFRNEIIAAIDFLITGI

Split site

**ParE2** (*Vibrio cholerae* N16961)

MKPFNLTVAAKADLRDIALFTQRRWGKEQRNVYLKQFDDSFWLLAENPDIGKSCDEIREGYRKFPQG
 SHVIFYQQTGSQQIRVIRILHKSMDVNPIFGA

Split site

**HigB2** (*Vibrio cholerae* N16961)

MKSVFVESTIFEKYRDEYLSDEEYRLFQAEMLNPKLGDVIQGTGGLRKIRVASKGKGKRGGSRIIY
YFLDEKRRFYLLTIYGKNEMSDLNANQRKQLMAFMEAWRNEQS

Split site

**RelE4** (*Vibrio cholerae* N16961)

MIFWEEASLNDREKIFEFLYDFNPAAAKKTDELIEAKVENLLEQPLIGVQRDGIRGRLLIIPEISMI
VSYWVDGSKIRIMRVLHQKQKFPND

Split site 1

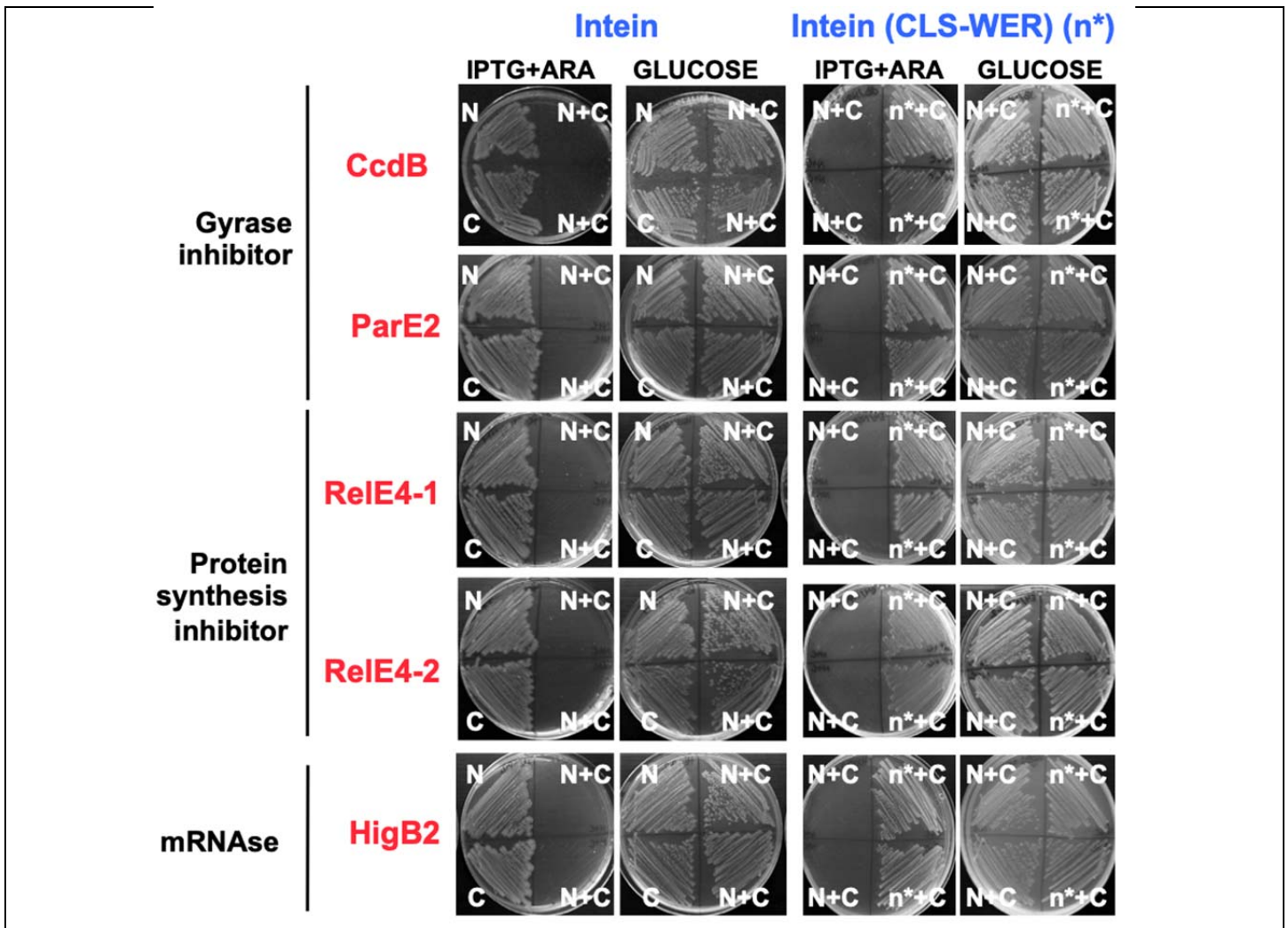


Split site 2

**Supplementary Figure 3**

Location of chosen split sites in the different toxins.

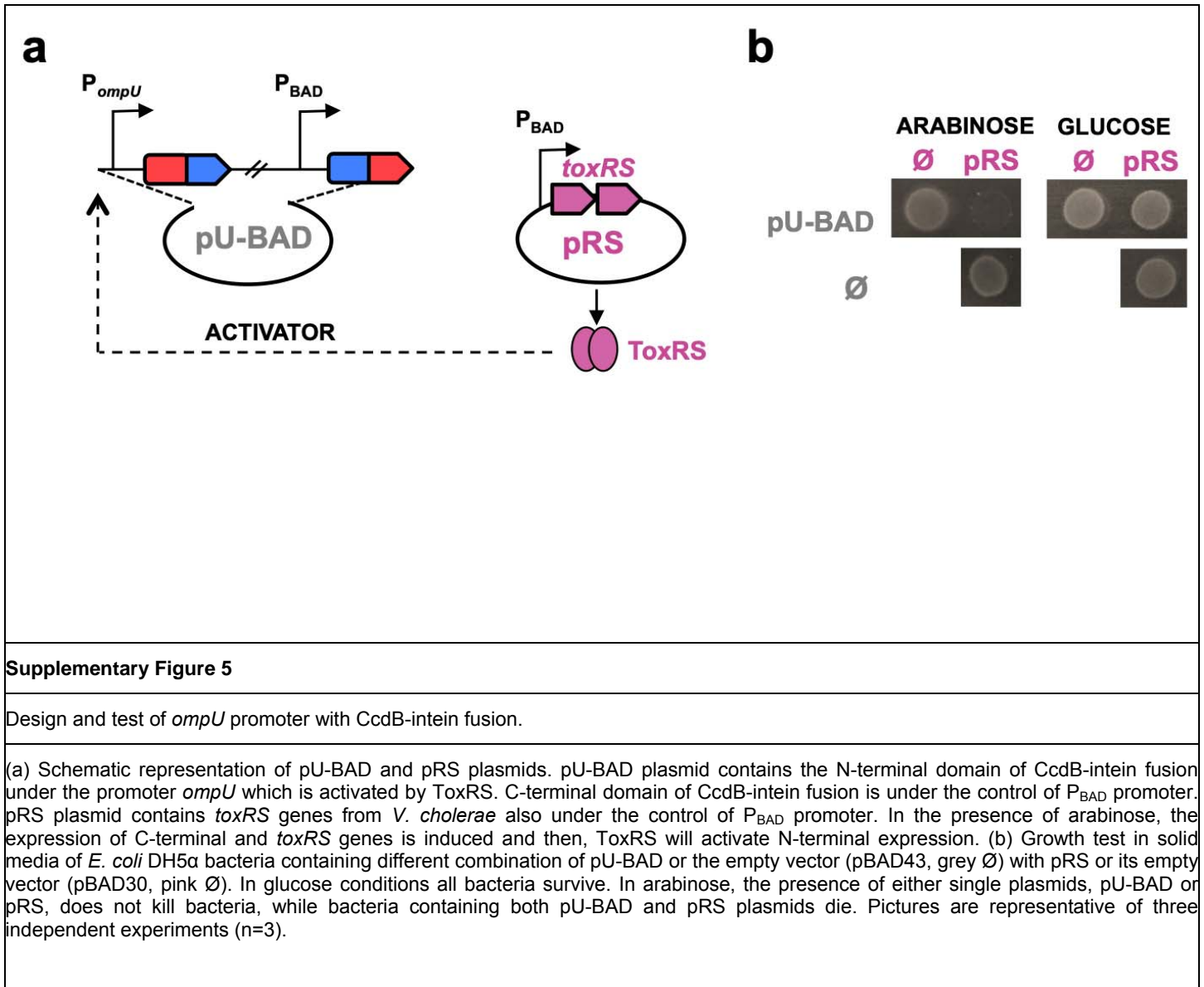
(a) 3D-structure model from Phyre2 of each toxin. The protein sequence follows rainbow colors from blue (N-terminal) to red (C-terminal). Split sites are indicated by white arrows. (b) Amino acid sequences of the four toxins we split. These toxins are: CcdB from *V. fischeri*, and ParE2, HigB2 and RelE4 from *V. cholerae*. Structural properties in their amino acid sequences are highlighted as follows: blue represents beta sheet and green represents alpha helix. These properties are predicted using Phyre2. Split sites are indicated by a red arrow. For RelE4, we tested two sites (1 and 2) which were both functional.

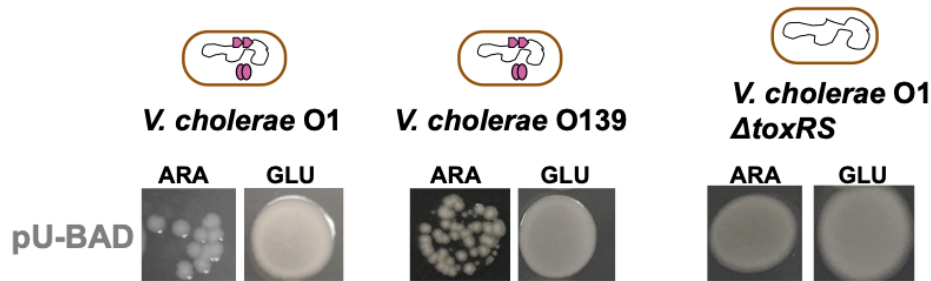
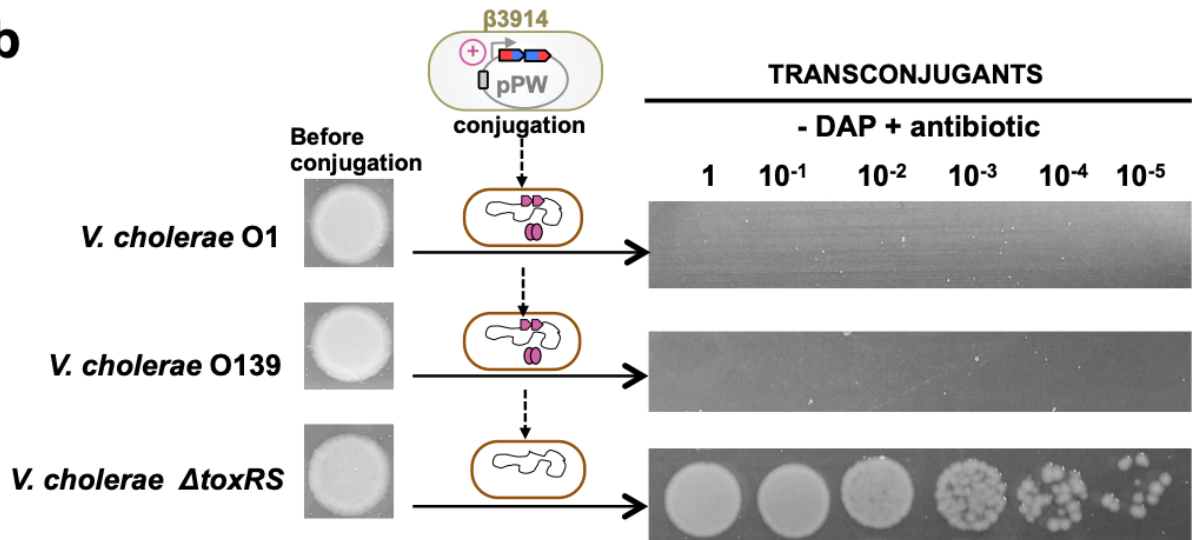


Supplementary Figure 4

Toxins from different families are reconstituted after intein-mediated splicing.

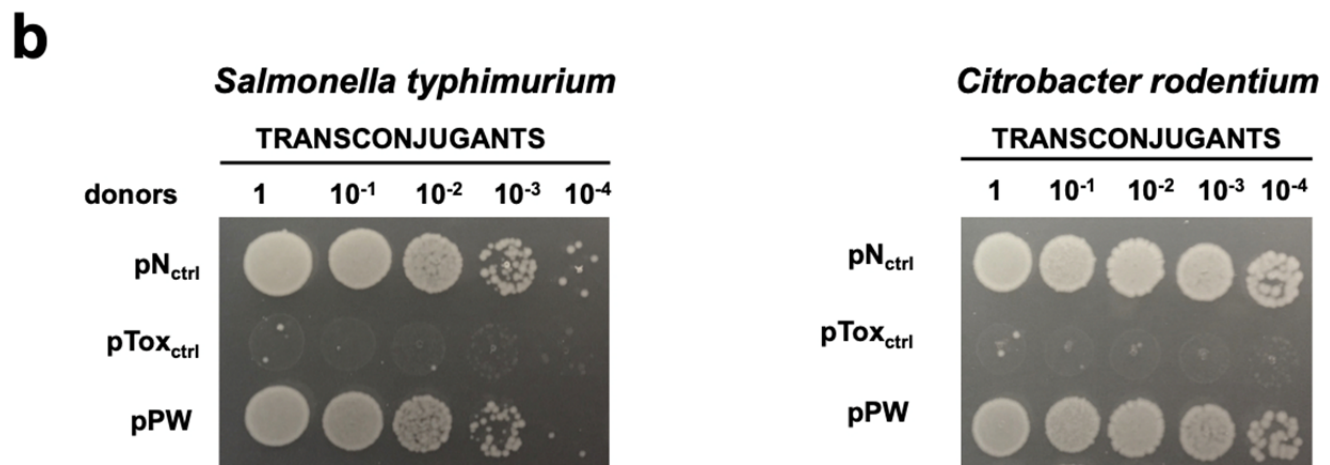
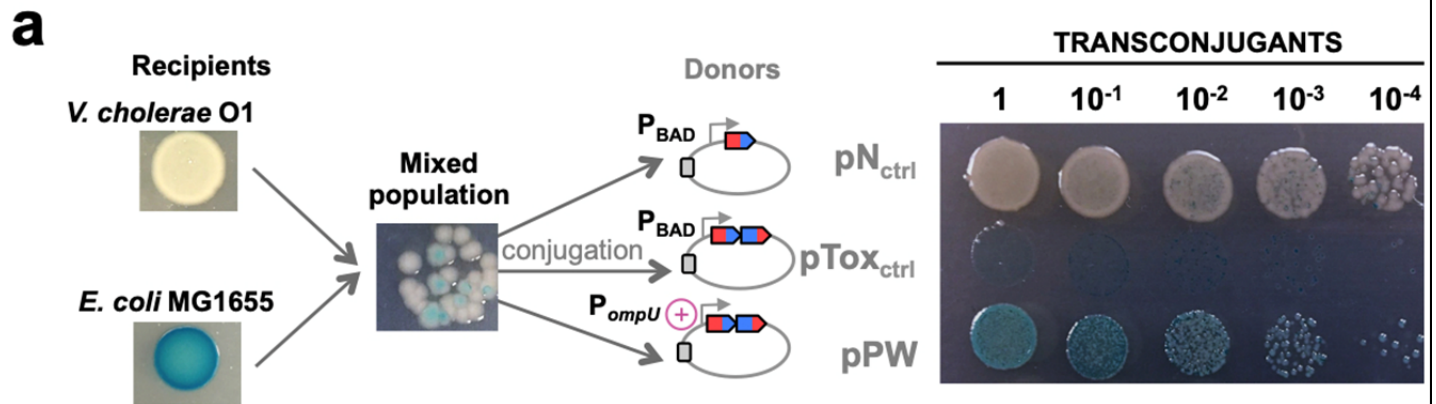
Viability of *E. coli* DH5 α bacteria containing different combination of plasmids with different toxins-intein fusions. Toxins and their corresponding families are indicated on the left. The split intein used is DNAE from *Nostoc punctiforme*. The left panel presents the viability assays using the native intein that carry out normal protein splicing; the right panel presents the viability assays using a N-terminal intein mutant (n*), in which residues CLS, which correspond to the catalytic Cys and the two following aminoacids from N-terminal domain were replaced by WER and splicing process does not take place (Cell. Mol. Life Sci., Volkmann, Mootz, 2013). Glucose represses the expression of the fusions and all strains grow. In presence of IPTG and arabinose (IPTG+Ara) bacteria containing both fusion plasmids (N+C) die. However bacteria containing only one half of the toxin-intein fusion (N or C) or the mutated version of N plasmid (n*) survive. Constructions and experimental set-up are described in the online methods. Experiments were repeated independently 3 times for the intact intein and twice for the inactivated version, with identical results.



a**b****Supplementary Figure 6**

Test of pU-BAD and of pPW, the genetic pathogenic-weapon, in *V. cholerae* serogroups O1 and O139.

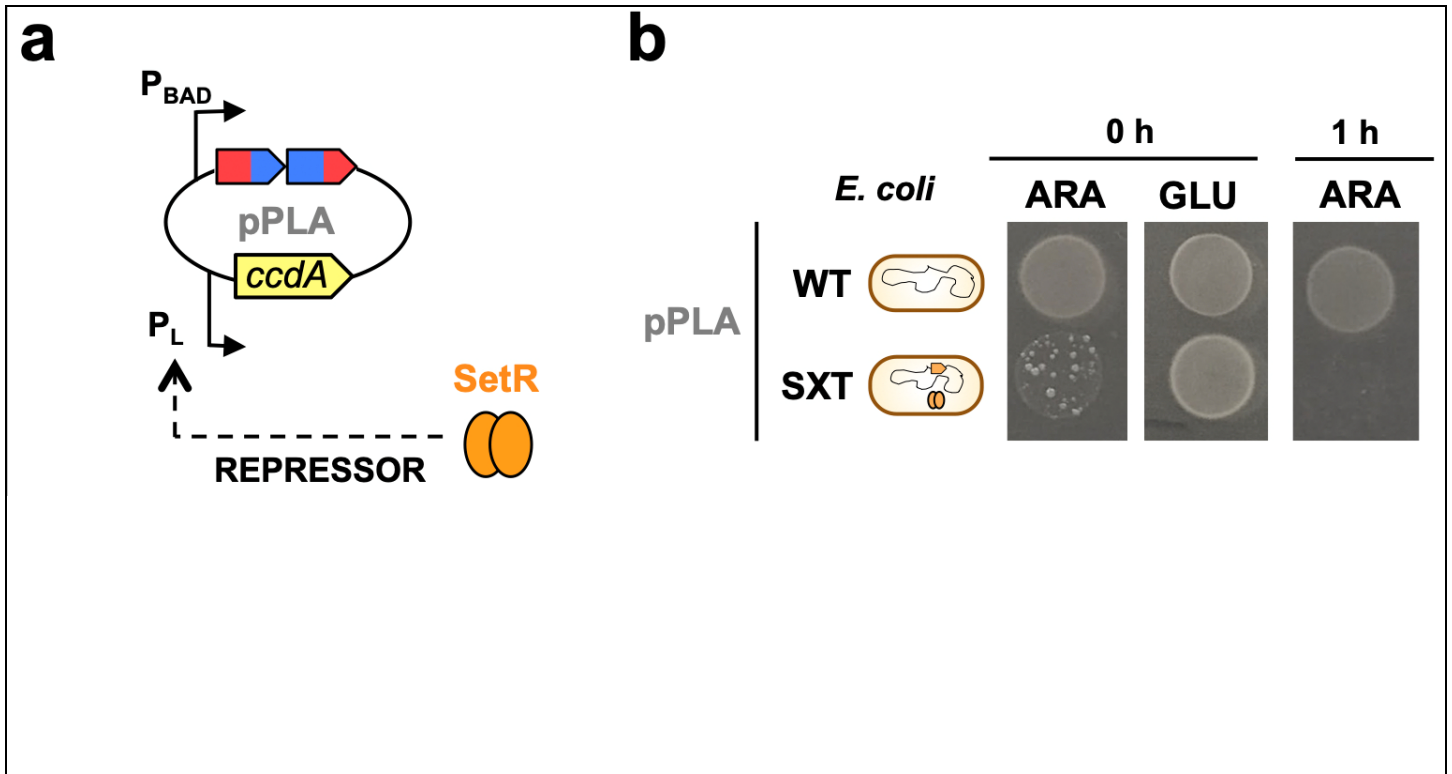
(a) Growth test on solid media of *V. cholerae* serogroups O1 and O139, and of the O1 mutant lacking *toxRS* ($\Delta toxRS$) carrying pU-BAD plasmid. In presence of glucose all bacteria survive. In arabinose, only wild type serogroups of *V. cholerae* that contain *toxRS* die, but not the $\Delta toxRS$ mutant. The colonies in arabinose for the two wild type *V. cholerae* strains illustrate the instability of pU-BAD plasmid in the presence of ToxRS. (b) Conjugation of the genetic weapon pPW into *V. cholerae* O1, O139 or the $\Delta toxRS$ -mutant as recipients from the *E. coli* donor b3914. The *ompU* promoter activated specifically by ToxRS from *V. cholerae* is represented by a circled + pink-symbol. Selection of transconjugants was done on Sp containing media. Upon conjugation of pPW plasmid, *V. cholerae* O1 and O139 die, while the transconjugants of the $\Delta toxRS$ mutant are fully viable. Pictures are representative of three independent experiments (n=3).



Supplementary Figure 7

Tunability of the system for specific killing.

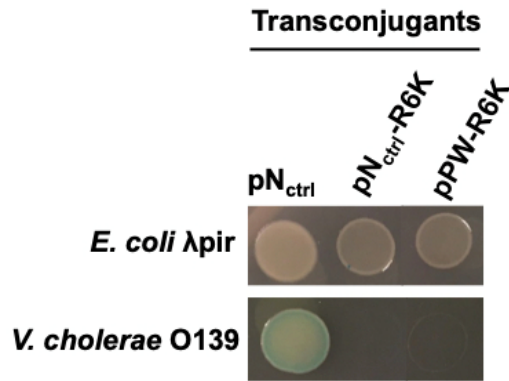
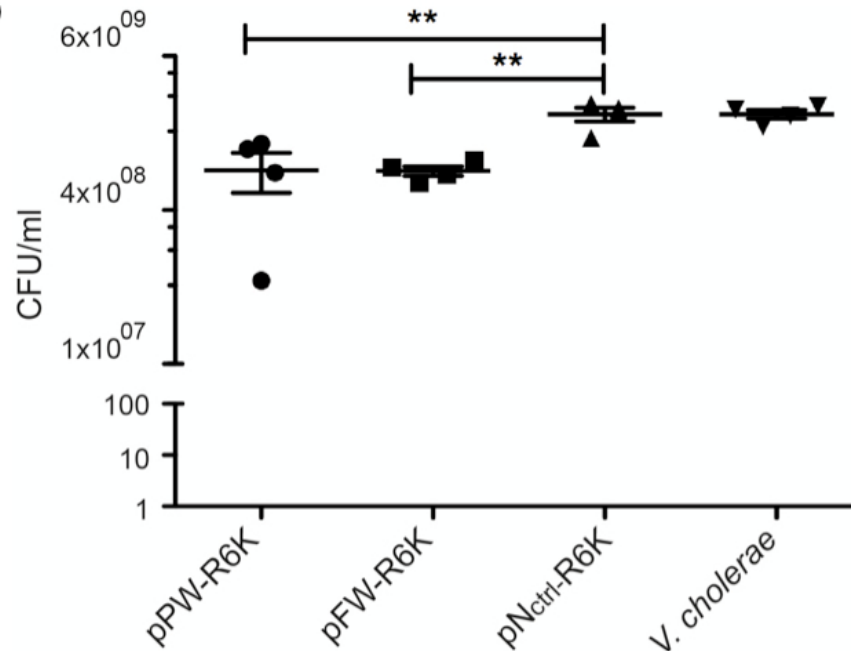
(a) Conjugation of pN_{ctrl} , $pTox_{ctrl}$ or pPW plasmids into 1:1 mixed population of *V. cholerae* O1 (white) and *E. coli* MG1655 (blue) as recipients. Transconjugants were selected on appropriate antibiotic media containing arabinose and X-gal. The non-toxic pN_{ctrl} plasmid conjugates the mixed population, and $pTox_{ctrl}$ plasmid kills both sub-populations. Upon conjugation of pPW plasmid only pathogenic *V. cholerae* O1 (white) die while *E. coli* MG1655 transconjugants are unaffected (blue). The *ompU* promoter activated specifically by ToxRS from *V. cholerae* is represented by a (+) pink-symbol. (b) pPW do not kill *Salmonella typhimurium* and *Citrobacter rodentium*. Transconjugants from pN_{ctrl} , $pTox_{ctrl}$ or pPW plasmids conjugation were selected on Sp containing media. Transconjugants from control plasmids pN_{ctrl} and $pTox_{ctrl}$ show that both bacterial strains are able to be conjugated in these conditions, and killed by the toxin-intein fusion. pPW transconjugants in these bacteria are fully viable, confirming that our system kills specifically only in the presence of ToxRS. Pictures are representative from three independent experiments.



Supplementary Figure 8

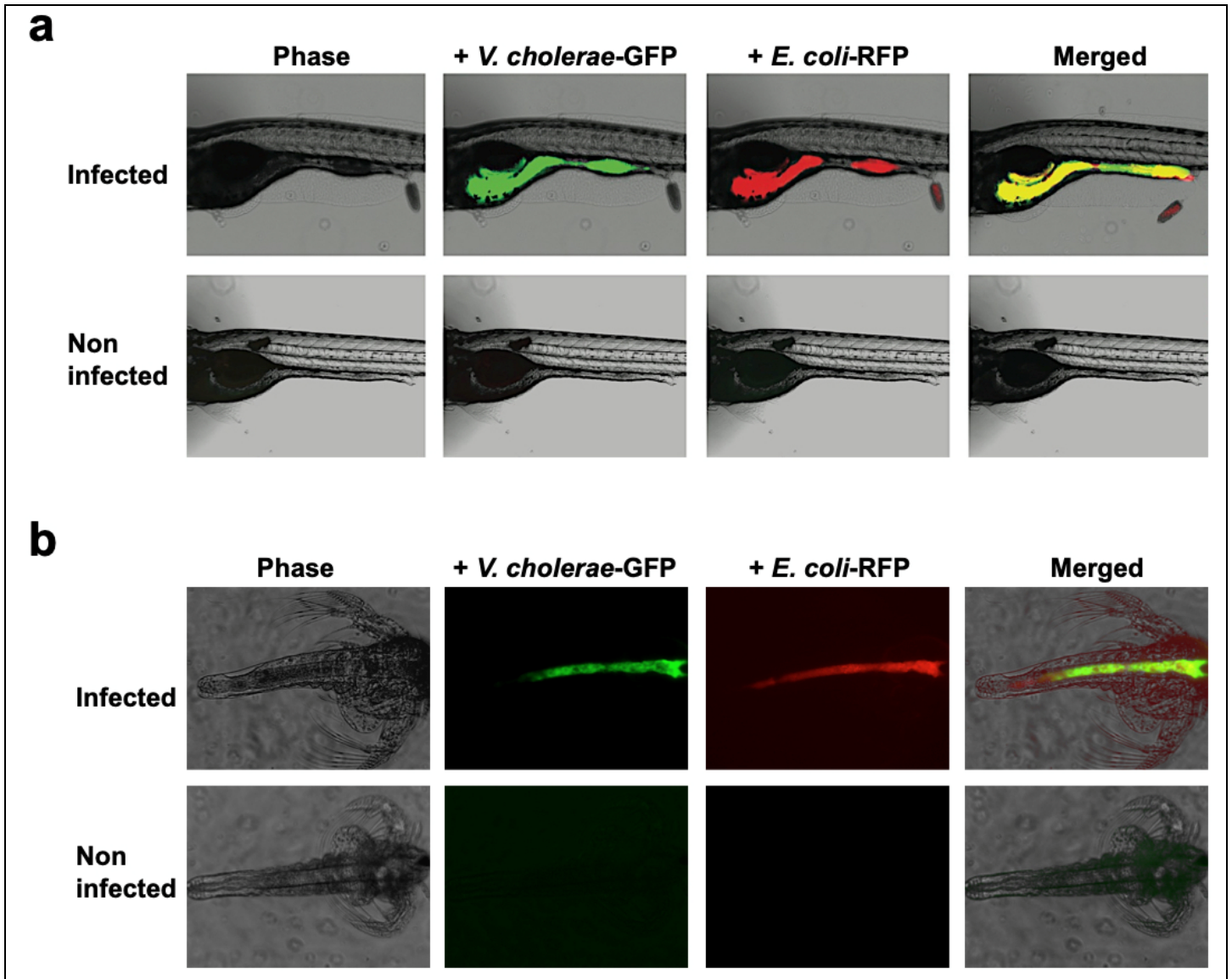
Design and test of P_L promoter to control the *ccdA* antitoxin expression to prevent the CcdB killing activity.

(a) Schematic representation of pPLA plasmid which is the pToxInt plasmid (Supplementary Fig. 1) containing *ccdA* antitoxin under the control of the SXT P_L promoter. (b) Growth test in solid media of *E. coli* lacking or containing SXT element chromosomally integrated (WT and SXT, respectively) transformed by pPLA. These strains were grown in the presence of glucose, then washed and re-suspended at OD=0.5 in MH media containing glucose or arabinose, then spotted directly on glucose or arabinose containing media (0h) or after 1h incubation at 37°C with arabinose (1h). Pictures are representative from three independent experiments.

a**b****Supplementary Figure 9**

Construction and test of a non-replicative and conjugative weapon.

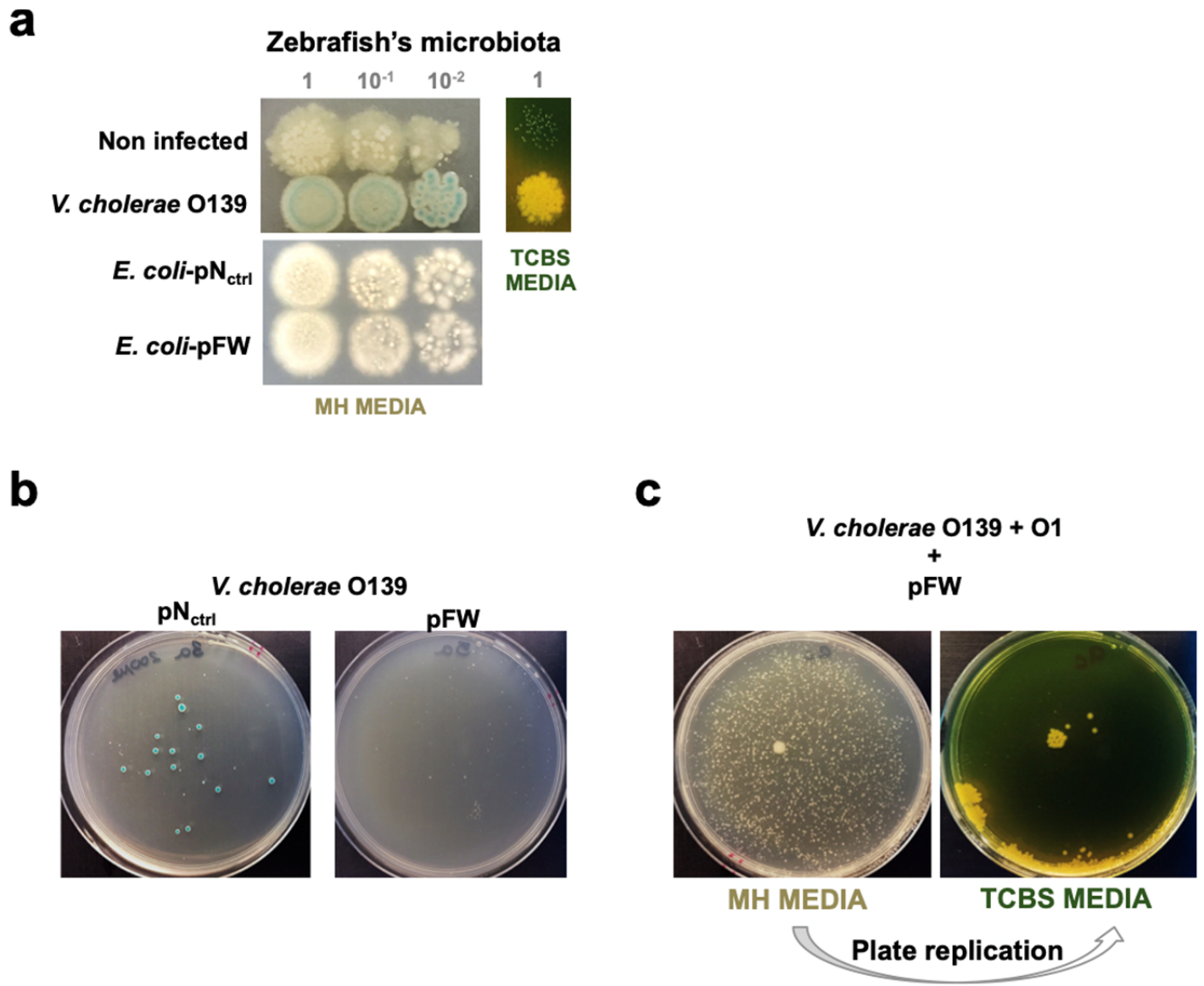
(a) Transconjugants selected in MH media with spectinomycin and X-gal. pN_{ctrl} plasmid containing pSC101 was used as control of conjugation. pN_{ctrl} and pPW plasmids containing R6K replication origin were able to replicate after conjugation only when *E. coli* λ pir was used as recipient but not into *V. cholerae* O139. (b) Killing assay after conjugation in *V. cholerae* O139 of pPW-R6K, pFW-R6K and pN_{ctrl}-R6K non-replicative plasmids. As one cannot select for transconjugants, we measure the number of viable *V. cholerae* after conjugation with the 3 plasmids. Conjugation of the pN_{ctrl}-R6K non-replicative plasmid show that conjugation by itself do not affect *V. cholerae* viability, i.e. similar CFU/ml than for the not conjugated bacteria (*V. cholerae*). When conjugated with either pPW-R6K or pFW-R6K non-replicative weapons, the *V. cholerae* O139 population decrease by 2-3 fold indicating that even if not replicating both plasmids efficiently kill *V. cholerae* O139. One-way ANOVA with Dunnett's Multiple Comparison Test was performed. pN_{ctrl}-R6K vs pPW-R6K, Mean Diff. = $2.383e+008$, $q = 4.183$, $**P < 0.05$, 95% CI of diff = ($8.937e+007$ to $3.871e+008$). pN_{ctrl}-R6K vs pFW-R6K, Mean Diff. = $2.308e+008$, $q = 4.227$, $**P < 0.05$, 95% CI of diff = ($9.187e+007$ to $3.896e+008$). Data numbers were calculated from four independent experiments ($n=4$, mean \pm s.d).



Supplementary Figure 10

Localization of *V. cholerae*-GFP and *E. coli*-RFP in the two *in vivo* models, zebrafish (*Danio rerio*) and *Artemia salina*.

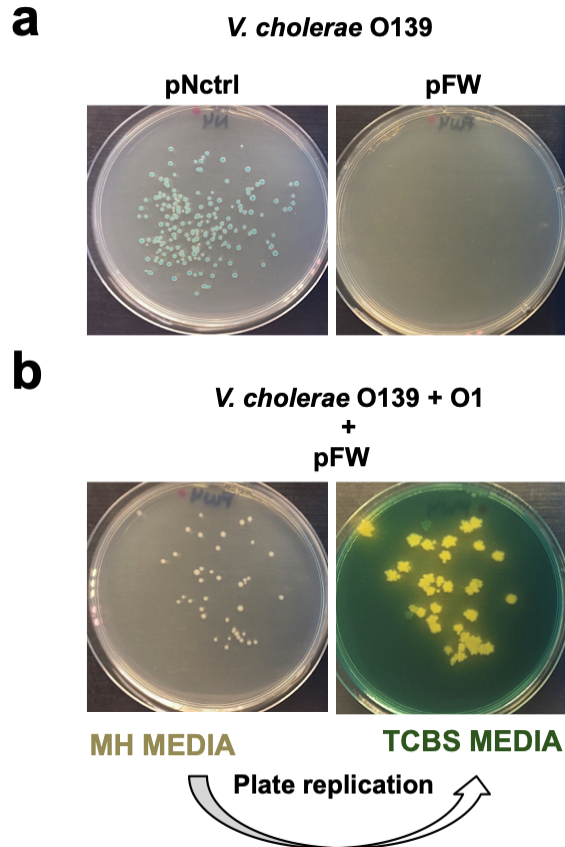
(a) Four-day-postfertilization zebrafish larvae were exposed to water containing *V. cholerae*-GFP and *E. coli*-RFP as described in methods. Infected and non-infected larvae were visualized by fluorescence microscopy using appropriate wavelength conditions enabling or not the visualization of GFP and RFP. Fluorescence was only detected in infected larvae and more precisely in the gut where both bacteria are co-localized. (b) *Artemia salina* stage nauplii were exposed to sterile PBS containing *V. cholerae*-GFP and *E. coli*-RFP as described in methods. Infected and non-infected nauplii were visualized by fluorescence microscopy using appropriate wavelength conditions enabling or not the visualization of GFP and RFP. Fluorescence was only detected in infected nauplii and more precisely in the gut where both bacteria are co-localized. Pictures are representative from three independent experiments.



Supplementary Figure 11

Analysis of zebrafish larvae microbiota and validation of the weapon *in vivo*.

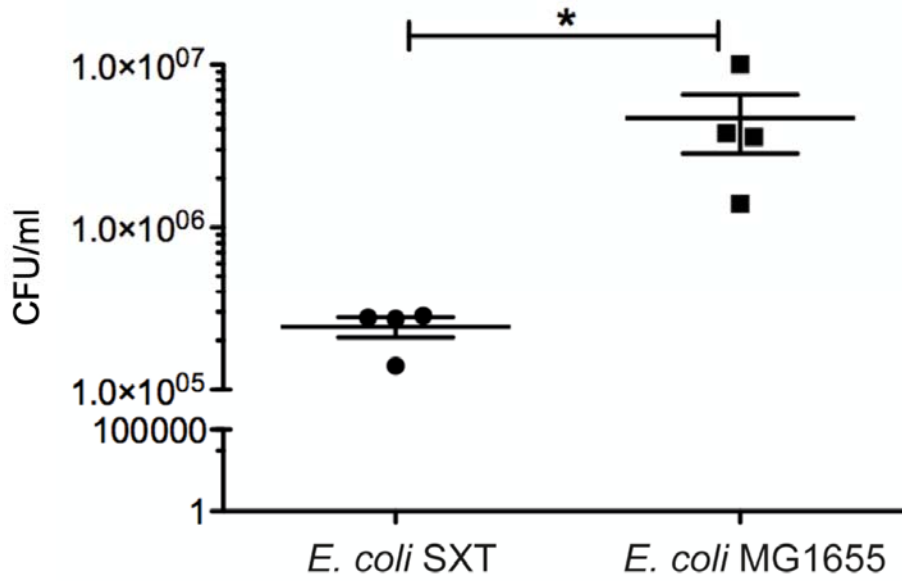
(a) Analysis of the non-infected and infected with *V. cholerae* O139 zebrafish larvae's microbiota. As a control, four-day post-fertilization zebrafish larvae were exposed to water containing 10⁸ of β3914 bacteria carrying pN_{ctrl} or pFW plasmid for 24 hours at 27°C, and then microbiota from five larvae were plated in MH media. Blue bacteria on MH X-gal correspond to *V. cholerae* O139 as attested by their yellow color on TCBS *Vibrio* specific media. (b) Transconjugants selected from five larvae of zebrafish larvae after pN_{ctrl} or pFW conjugation treatment on MH media with Sp and X-gal. Blue colonies were only detected upon conjugation of pN_{ctrl} plasmid but not after pFW conjugation due to the specific killing of *V. cholerae* by this plasmid. Pictures are representative from the experiment with only *V. cholerae* O139 shown in Fig. 4a. (c) Transconjugants selected from the infection with pFW of 1:1 mixed population of *V. cholerae* O1 and O139, on MH media with Sp and X-gal and then replicated on TCBS plates to follow yellow development specific of *Vibrio* growth. Pictures are representative of the experiments using mix of *Vibrio* in Fig. 4a. Pictures from (a) and (c) are representative from two independent experiments (10 zebrafish larvae) and from (b) are representative from three independent experiments (15 zebrafish larvae).



Supplementary Figure 12

Analysis of *Artemia salina* stage nauplii microbiota and validation of the weapon efficacy in this model.

(a) Transconjugants selected from 225 ± 15 *Artemia* after infection with *V. cholerae* O139 and after treatment with β 3914 bacteria carrying pN_{ctrl} or pFW conjugative plasmids, on MH media with Sp and X-gal. Blue colonies (*V. cholerae* O139) were only detected after conjugation with pN_{ctrl} plasmid but not after pFW conjugation, illustrating the specific killing of *V. cholerae* by pFW. Pictures are representative from the experiment with only *V. cholerae* O139 shown in Fig. 4b. (b) Transconjugants selected after infection with a mixed population of 1:1 *V. cholerae* O1 and O139, and treatment with β 3914 bacteria carrying the pFW conjugative plasmid. Selection was done into MH media with Sp and X-gal and then, replicated on TCBS plates to detect the yellow color specific of *Vibrio* presence. As expected pFW transconjugants were only detected in *V. cholerae* O1 (white). Pictures are representative from the experiment with mix of *Vibrios* in Fig. 4b. Pictures are representative from four independent experiments (900 ± 15 nauplii of *Artemia*).



Supplementary Figure 13

E. coli SXT killing efficiency when using donor: recipient ratio of 10:1.

After conjugation of pABRW, in either *E. coli* SXT (its specific target) or *E. coli* MG1655 (not targeted) the number of surviving recipient bacteria (CFU/ml) was determined by plating on MH media without antibiotic and with arabinose (0,2%). One-sided t-test Mann Withney was performed. *E. coli* SXT vs *E. coli* MG1655. P value=0.0143. *P < 0.05. Data numbers were calculated from four independent experiments (n=4, mean ± s.d.).