SI Appendix

Method S1

Engineering the genome of *Vibrio cholerae.* All the recombinant vectors were developed using restriction enzymes and T_4 DNA ligase (NEB, USA). Desired DNA fragments were PCR amplified by using high fidelity Taq DNA polymerase (NEB, USA). *E. coli* FCV14 was used as the cloning host. All gene deletions and replacements were performed via homologous recombination using the derivatives of suicide vectors pKAS32 or pDS132 (1, 2). *E. coli* β2163 was used as a donor strain and *V. cholerae* N16961 or its derivatives were used as a recipient strain. For all gene deletions, around 500 to 700 base pair (bp) homologous regions upstream and downstream of the respective open reading frame were PCR amplified using the specific primer combinations (*SI appendix,* **Table S3**).

For removing different GIs (genomic islands) from the *V. cholerae* genome, first a part of GI was PCR amplified and then cloned into a cloning vector pSW23T (*SI appendix,* **Table S1**). The recombinant vector was used as a template for inverse PCR for replacing a part of the cloned DNA fragment with the *sacB-cat* or *sacB-cat-lacZ* allele. Finally, the recombinant vector carrying *sacB-cat* or *sacB-cat-lacZ* allele flanked by the up stream and down stream regions of the interested GI was transferred to the recipient *V. cholerae* strain by conjugation. The vector integrated in the GI through homologous recombination. The first-cross over strain carrying recombinant vector in the GI became resistant to chloramphenicol but sensitive to streptomycin and sucrose. The second crossover strain carrying only *sacB-cat* or *sacB-cat-lacZ* in the GI without plasmid backbone was isolated by growing the strain on to LA plates supplemented with streptomycin (1 mg/ml) with or without X-gal. Finally, the *V. cholerae* strain without GI in the chromosome was isolated by growing cells on the LA plate supplemented with 15% sucrose. The GI excised strains were confirmed by sensitivity to chloramphenicol and further confirmed by PCR with specific primer sets (*SI appendix,* **Table S3**), one primer from the core genome and another from of the GI integration site region (**Fig. 3**).

Recombinant vectors construction: For *lexA* gene deletion, recombinant vector pAP17 was constructed from previously used vector pBS52 (3). The region carrying upstream and downstream of *lexA* along with *aph1* gene, in between, was PCR amplified from pBS52 using primers 878-879 **(***SI appendix,* **Table S3).** It was digested with Xba1 XbaI and ligated into similarly digested pDS132.

The suicide plasmid, pAP8 carrying upstream and downstream regions of *recA* of *V. cholerae* N16961 was constructed to delete *recA* gene from VCE232. *aph1* gene cassette was digested from pUC4K using PstI to introduce into similarly digested pDA2 and named as pAP8. Similarly, to delete *recA* from Classical O395, pAP16 was constructed. Upstream region of *recA* was PCR amplified from O395 strain using primers 729-754 **(***SI appendix,* **Table S3)** and introduced into pUC18 after digestion with SacI and KpnI to generate pAP13. *aph1* gene was amplified from pUC4K using primers 756-757 **(***SI appendix,* **Table S3),** was digested with KpnI and SphI and inserted within pAP13 and named as pAP14. Subsequently, the downstream region of *recA* was amplified using primers 755-730 **(***SI appendix,* **Table S3)** and digested with SphI and XbaI to introduce into similarly digested pDS132 to construct pAP15. The upstream region of *recA* along with *aph1* gene was digested from pAP14 using SacI and SphI and inserted into similarly digested pAP15, to finally generate pAP16.

To construct pAP7, RS2 region from the genome of CTXϕ of environmental strain VCE232 was PCR amplified using primers 500-501 **(***SI appendix,* **Table S3)**, further digested with EcoRI and NotI & cloned into similarly digested vector pSW23T. Similarly, RS2 region from the genome of CTXϕ of O139 strain SG24 was amplified by using primers 679-680 **(***SI appendix,* **Table S3)**, further digested with PstI and XbaI & cloned in pSW23T, to develop pAP9. The *cat* gene in pBD56 was replaced with AmpR obtained from pEM7 using PvuII and Nco1. The vector was named as pAP10.

To introduce point mutation [Tyrosine replaced with Phenylalanine (T1204A)] within *rstA* gene, inverse PCR of pBS66 was done by using primers 1106-1107 **(***SI appendix,* **Table S3)** followed by PNK treatment for subsequent ligation. The vector was designated as pAP21.

In order to delete the entire VSP-1, we first PCR amplified the *VC0177* - *VC0178* using 861 and 862 primers and cloned into the *Not*I and *Sac*I sites of pSW23T to make pSB29 which was then used as a template for inverse PCR with 863 and 864 primers. Simultaneously the *sacB-cat* region was PCR amplified from pDS132 with 869 and 870 primers. This *sacB-cat* region was digested with *Nru*I and *Nsi*I and ligated into the similarly digested inverse PCR product and the clone was named as pSB30. Then the *sacB-cat* region flanked by the *VC0177* and *VC0178* was sub-cloned

from pSB30 to pKAS32 at the *Not*I and *Sac*I sites and the final recombinant vector was named as pSB31.

To delete the entire VSP-2, *VC0500* was PCR amplified using 865 and 866 primers and cloned into the *Not*I and *Xba*I sites of pSW23T to make pBIP13, which was then used as a template for inverse PCR with 867 and 868 primers. The *sacB-cat* region was PCR amplified from pDS132 with 870 and 871 primers. The *sacB-cat* amplicon was digested with *Xho*I and *Nsi*I and ligated into the similarly digested inverse PCR product and the clone was named as pBIP15. Furthermore, the *sacB-cat* region flanked by the up stream and down stream region of *VC0500* was sub-cloned from pBIP17 to pKAS32 at the *Not*I and *Xba*I sites and the final recombinant vector was named as pBIP38.

In order to delete the entire VPI-1, we first PCR amplified the *VC0826* - *VC0829* using 947 and 948 primers and cloned into the *Not*I and *Sac*I sites of pSW23T to make pSB35 which was then used as a template for inverse PCR with 949 and 950 primers. Simultaneously the *sacB-cat* region was PCR amplified from pDS132 with 869 and 951 primers. This *sacB-cat* region was digested with *Nru*I and *Xho*I and ligated into the similarly digested inverse PCR product and the clone was named as pSB36. Furthermore, the *sacB-cat* region flanked by the *VC0827* and *VC0828* was sub-cloned from pSB36 to pKAS32 at the *Not*I and *Sac*I sites and the final recombinant vector was named as pSB37.

To delete the entire VPI-2, *VC1796-VC1797* was PCR amplified using 910 and 911 primers (*SI appendix,* **Table S3)** and cloned into the *Not*I and *Sac*I sites of pSW23T to make pSB32, which was then used as a template for inverse PCR with 912 and 913 primers (*SI appendix,* **Table S3)**. The *sacB-cat* region was PCR amplified from pDS132 with 869 and 870 primers. The *sacB-cat* amplicon was digested with *Nru*I and *Nsi*I and ligated into the similarly digested inverse PCR product and the clone was named as pSB33. Furthermore, the *sacB-cat* region flanked by the *VC1796* and *VC1797* was sub-cloned from pSB33 to pKAS32 at the *Not*I and *Sac*I sites and the final recombinant vector was named as pSB34.

For the deletion of entire SXT element, around 1kbp upstream and downstream sequences of *s053* region of SXT were PCR amplified using primers 1076-1108 and 1109-1110, respectively (*SI appendix,* **Table S3)** and cloned to generate pSM19. Inverse PCR of pSM19 was performed using primers 1220-1221 (*SI appendix,* **Table S3)**. The *aadA* gene was PCR amplified using primers 1222-1223 and introduced

within pSM19 by digesting XbaI and NdeI, to generate pJV36. PCR, restriction digestion, agarose gel shift assay and DNA sequencing confirmed the recombinant vectors.

Construction of VSP-1 deleted *V. cholerae* strain SB25. pSB31 was conjugally transferred into N16961 for developing VSP-1::*sacB-cat* reporter strain adopting sequential allelic exchange method. The reporter strain SB23 carrying VSP-1::*sacB-cat* allele was confirmed by sucrose sensitivity, chloramphenicol resistance and PCR with 890 and 891 primers (*SI appendix,* **Table S3**). Finally, the VSP-1 deleted strain SB25 was isolated by selecting the overnight grown SB23 onto LA plate supplemented with 15% sucrose. This SB25 was confirmed by chloramphenicol sensitivity and PCR assay using 908 and 909 primers (*SI appendix,* **Table S3**).

Construction of VSP-2 deleted *V. cholerae* strain DD2.

Recombinant vector pBIP38 was conjugally transferred into the N16961 for tagging VSP-2 with the *sacB-cat* allele by similar allelic exchange method. The N16961 derivative carrying *sacB-cat* tagged VSP-2 was named DD1. Sucrose sensitivity, chloramphenicol resistance and PCR with 927 and 928 primers (*SI appendix,* **Table S3**) confirmed DD1. Finally, the VSP-2 deleted strain DD2 was isolated by plating the over night grown DD1 onto LA plate supplemented with 15% sucrose. This DD2 was further confirmed by chloramphenicol sensitivity and PCR assay.

Construction of VPI-1 deleted *V. cholerae* strain SB31.

For developing VPI-1::*sacB-cat* reporter strain, the pSB37 was conjugally transferred into the N16961. The vector was integrated in the intergenic region of *VC0827* and *VC0828* by homologous recombination. Subsequent allelic exchange experiment was used to remove the vector backbone and develop SB30. We confirmed VPI-1::*sacB-cat* genotype by sucrose sensitivity, chloramphenicol resistance and PCR assay (**Fig. 3**). Finally, the VPI-1 deleted strain SB31 was isolated by selecting the overnight grown SB30 onto LA plate supplemented with 15% sucrose. Chloramphenicol sensitivity and PCR assay further confirmed SB31.

Construction of VPI-2 deleted *V. cholerae* strain SB27. To delete the VPI-2, pSB34 was conjugally transferred into the N16961 in which the VPI-2 was tagged with the *sacB-cat* region by replacing the intergenic region of *VC1796* and *VC1797* and this strain was named as SB26 which was confirmed by sucrose sensitivity, chloramphenicol resistance and PCR with 925 and 926 primers (*SI appendix,* **Table S3**). Finally the VPI-2 deleted strain SB27 was isolated by plating the over night

grown SB26 onto LA plate supplemented with 15% sucrose. This SB27 was further confirmed by chloramphenicol sensitivity and PCR assay (**Fig. 3**).

Construction of SXT deleted *V. cholerae* strain. For the deletion of SXT element in the host strain *V. cholerae O1 Ogawa* 41081 the construct pJV36 was used. The Stp^S host strain *V. cholerae O1 Ogawa* 41081 was made Stp^R by growing the strain in the presence of sub lethal concentration of streptomycin (5 ug/ml). The streptomycin resistant mutant strain was then used for the subsequent experiments. The final construct pJV36 (*SI appendix,* **Table S1**) was conjugally transferred to the host bacterium. The SXT was tagged with the *sacB-aadA* cassette by replacing the s053 region and this strain was named as JV3 (**Table 1**), which was confirmed by sucrose sensitivity, spectinomycin resistance and PCR with 1119 and 1120 primers (*SI appendix,* **Table S3**). Finally, the SXT deleted strain JV4 was isolated by plating the over night grown JV3 onto LA plate supplemented with 15% sucrose. This JV4 was further confirmed by spectinomycin sensitivity and PCR using primers 1046 and 1234 specific for the SXT integrase gene (*SI appendix,* **Table S3**).

Construction of VSP-1 and VSP-2 deleted N16961 derivative SB33. Recombinant strain SB33 was developed by sequentially deleting the VSP-1 and VSP-2 from N16961 as described above. First VSP-1 was deleted from N16961 and the strain was named as SB25, then VSP-2 of SB25 was flagged with *sacB-cat* by deleting *VC0500* and the strain was named as SB32. Finally, growing the SB32 onto LA plate supplemented with 15% sucrose isolated SB33, which was devoid of both VSP-1 and VSP-2.

Construction of other genetically modified *V. cholerae* strains. In order to flag the pathogenicity islands with *sacB-cat-lacZ,* firstly endogenous $lacZ_{Vc}$ were deleted from SB27, SB31 and SB33 by using pBD50 (*SI appendix,* **Table S1**) and the strains were named as SB42, SB43 and SB44, respectively (**Table 1**). $lacZ_{Ec}$ was cloned at the *Nru*I site of pSB37 and pSB34 to make pSB43 and pSB44, respectively (*SI appendix,* **Table S1**). pSB43 was used to develop SB45 and SB51, whereas pSB44 were used to develop SB46 and SB49 (**Table 1**). SB45, SB46, SB49 and SB51 were grown in LB and plated onto sucrose plate to isolate the SB47, SB48, SB50 and SB52, respectively (**Table 1**).

For the co-culture and growth competition assays streptomycin resistant *V. cholerae* strains N16961, SB25, DD2, SB33 and SB52 were tagged with a chloramphenicol resistance gene by using pBD60 (*SI appendix,* **Table S1**) and the strains were name as

SB56, SB58, SB59, SB60 and SB63 respectively (**Table 1**). Similarly, N16961 and O395 were also tagged with zeocin resistance by using pBD61 and pBD66 (4) and the strains were designated as SB57 and SB62, respectively (**Table 1**).

For the construction of Δ*lexA* mutant strain,the recombinant vector, pAP17 was introduced into N16961 and its derivatives by conjugation. and The transconjugants were selected in the presence of chloramphenicol. Exchange of the native allele with Δ*lexA:: aph1* allele present on plasmid backbone was confirmed by PCR and UV sensitivity. Recombinant vectors pAP8 and pAP16, the derivatives of suicide vector pDS132 carrying the *ΔrecA::aph1* allele, were used for the construction of the *ΔrecA* mutants AP8 and AP10 from environmental VCE232 and classical O395 *V. cholerae* strains, respectively, using allelic exchange strategy explained previously (3). Excision of the native allele was confirmed by chloramphenicol sensitivity, PCR and UV sensitivity assay. Similarly, the Δ*lexA* Δ*recA* double mutant strain was constructed by following allelic exchange method. Briefly, the recombinant vector pDA2 was introduced into Δ*lexA* strain (BS20) by conjugation. The first crossovers were selected on the Stp (50 μg/ml) and Cam (2 μg/ml) containing selection plates and were replica plated on $Stp_{50}Cam_2$ & $Sucrose_{15\%}$ containing plates. The colony showing the sucrose sensitive phenotype was selected for the second crossover. The second crossovers were selected on Stp_{50} $Suc_{15\%}$ plates. Replica plating, UV sensitivity assay and PCR confirmed deletion of the native *recA* gene with plasmid backbone. The Δ*lexA* Δ*recA* double mutant strain was named as JV5.

Supplementary Figures

Figure S1

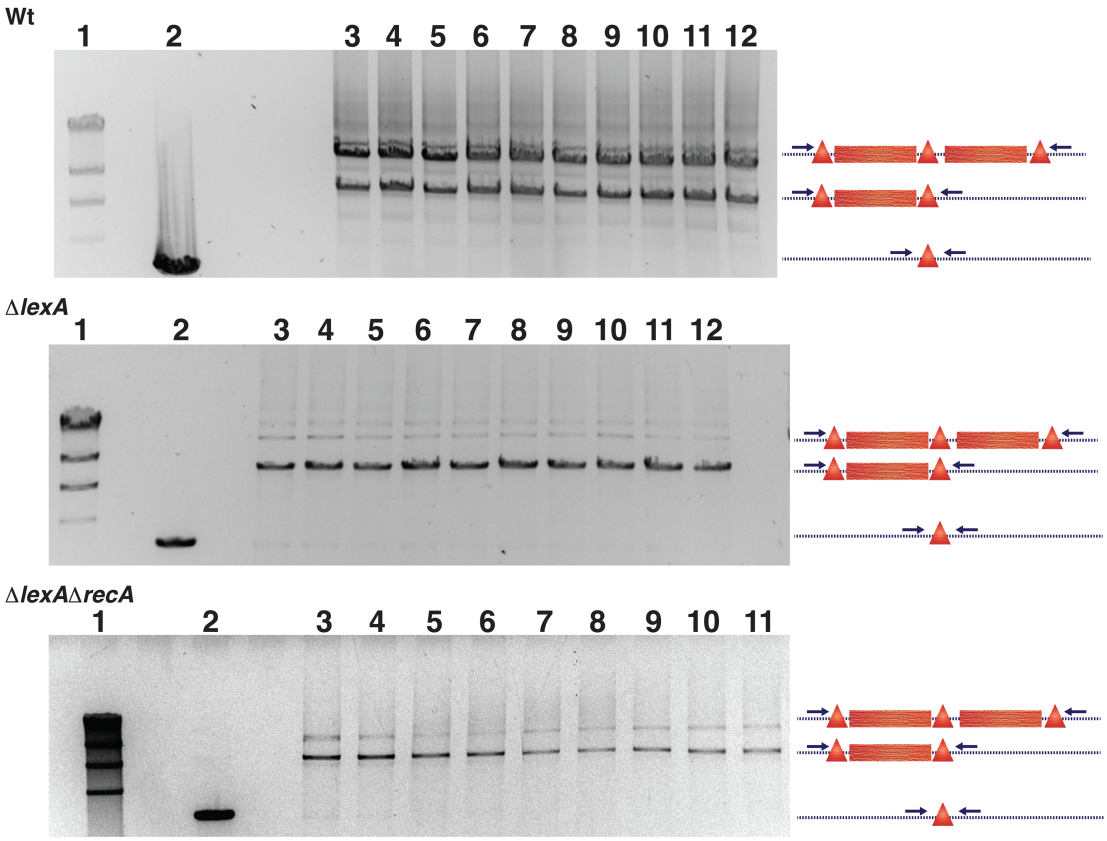

Figure S1: Single or multiple copies of integration of RS2 element (pBS66) in the *dif1* site of *lexA* positive **(a)** Δ*lexA* and **(b)** Δ*lexA*Δ*recA* *V. cholerae* strains. Integration copy numbers was measured by amplifying *dif1* loci using set of primers that bind to the flanking sequences of *dif1*. Single and double integrations produced ~8kb and ~12kb long DNA amplicons, respectively. Absence of integration produced ~3.5 kb long amplicon. λ genomic DNA digested with Hind III was used as size marker.

(a) Lane 1, λ HindIII; 2, BS1; 3-12, BS1::pBS66

(b) Lane 1, λ HindIII; 2, BS20; 3-12, BS20::pBS66

(c) Lane 1, λ HindIII; 2, JV5; 3-11, JV5::pBS66

Figure S2

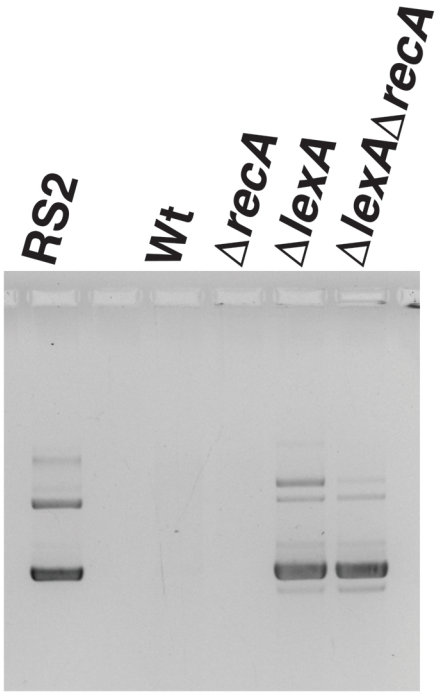

Figure S2: Stability of replicative and integrative module (RS2) of CTXΦ in the presence and absence of *lexA* gene. The RS2 module was introduced into *V. cholerae* strains by conjugation. Replicative genome of RS2 was isolated by alkaline lysis method. Relevant genotype of the *V. cholerae* strains were mentioned on the top of each wells. Replicative RS2 DNA isolated from *E. coli* cell was used as control.

Figure S3

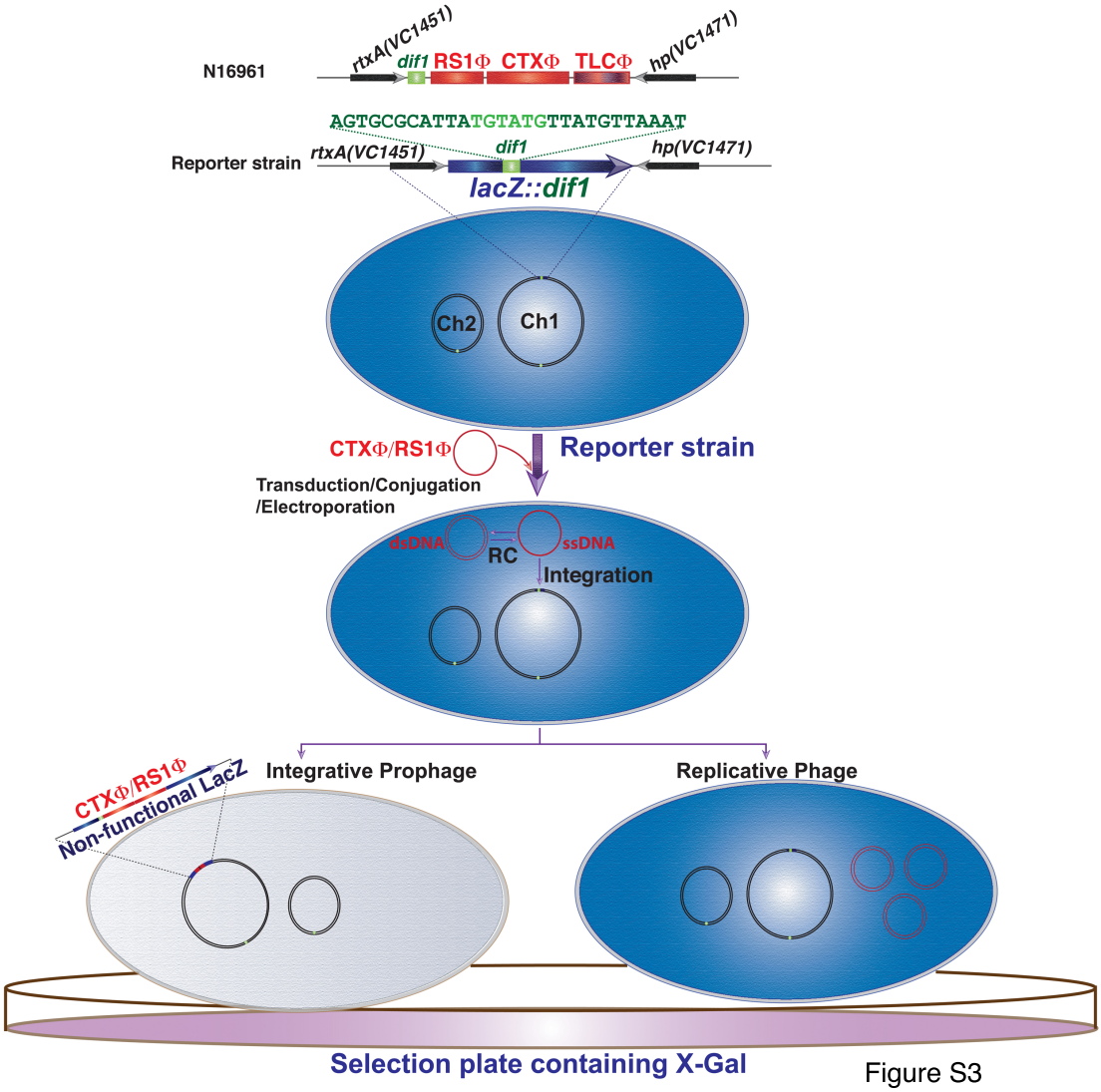

Figure S3: Schematic of CTXΦ integration in *Vibrio cholerae* reporter strain. The *dif1*-RS1-CTX-TLC prophage array has been replaced with a functional *lacZ-dif1* allele in an endogenous *lacZ* deleted *V. cholerae* strain adopting allelic exchange method. The CTXΦ or RS1Φ was introduced into the reporter strain by conjugation. Once inside the host cytoplasm, the (+)ssDNA genome of the phages either converted into double stranded replicative DNA using host replication machinery or integrated into *lacZ-dif1* site by site-specific recombination exploiting XerC-XerD recombinases. Integration of CTXΦ at the *dif1* site in the *lacZ-dif1* allele abolished the β-galactosidase activity of the reporter strain and the colony turned white on the selection plate supplemented with 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal). Arrows and their directions indicate open reading frames and their transcription direction, respectively. RC=Rolling circle replication.

Figure S4

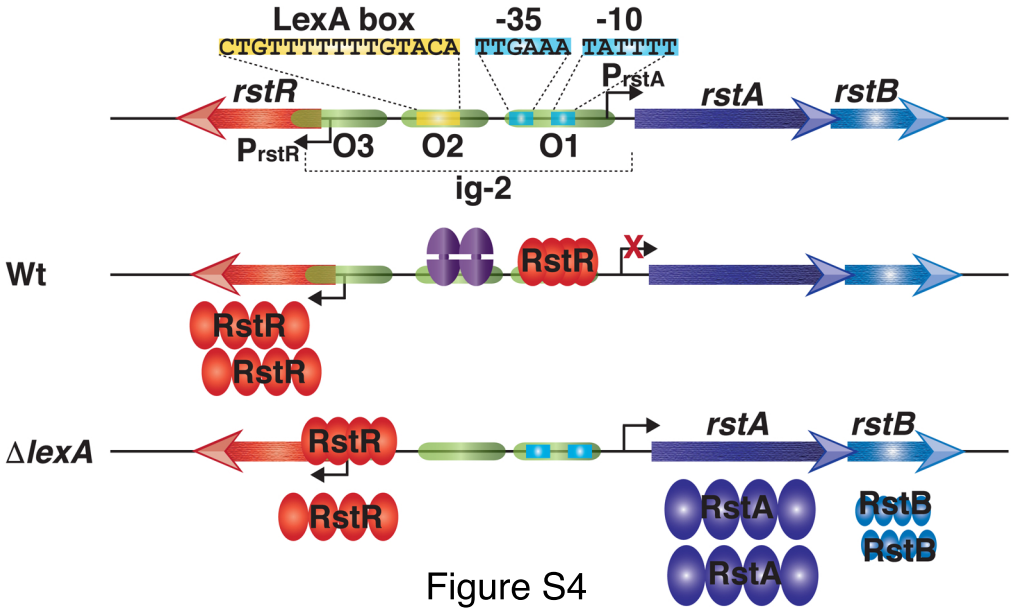

Figure S4: Overview of P_{rstA} promoter region in the CTXΦ genome and LexA regulation. In normal physiological condition, the CTXΦ encoded repressor RstR and SOS regulator LexA binds to the P_{rstA} promoter and repress the expression of RstA. Binding of LexA to the O2 operator site reduces the binding affinity of RstR on its own promoter (P_{rstR}) and allows *rstR* transcription. In the absence of LexA, RstR binds to the O2 and O3 operator sites and thereby repressed the transcription from P_{rstR} promoter. Reduced level of RstR derepressed transcription from P_{rstA} and produce high-level of RstA. Elevated level of RstA initiates rolling circle replication in the integrated CTXΦ.

Supplementary Tables

Table S1: Plasmids used in the present study

Plasmids	Genotype and Phenotype	References
pKAS32	*oriR6K mobRP4 rpsL bla;* conjugative vector, Amp^r	(1)
pSW23T	pSW23*::oriT* RP4 *oriV* R6K, Cam^r	(2)
pDS132	*oriR6K mobRP4 sacB cat;* conjugative vector, Cam^r	Laboratory collection
pCR2.1	pBR322 *ori*, cloning vector, Amp^r, Kan^r	Invitrogen
pEM7	pUC *ori bla sh ble*; Amp^r, Zeo^r	Invitrogen Life Technology, USA
pUC18	*ori*pMB *lacZα bla* Amp^r	Laboratory collection
pUC4K	ColE1 *lacZα bla aph;* Amp^r, Kan^r	Laboratory collection
pFX497	*repA, OriT-RP4, bla,* low copy no vector; Amp^r,	Laboratory collection
pBD50	pKAS32*::lacZvcUp-Dwn*	Laboratory collection
pBD56	pSW23T*::CTXϕ*^{ET}, Cam^r	(3)
pBD60	pSW23T*::xbs CTXϕ,* Cam^r	(4)
pBD61	pBD60 *Δcat::sh ble,* Zeo^r	(4)
pBD66	pBD62 *ΔattP*,* Zeo^r	(4)
pBS52	pKAS32*::ΔlexA::aph1*, Amp^r, Kan^r	(3)
pBS22	pSW23T*::RS2 (Classical),* Cam^r	(5)
pBS66	pSW23T*::RS2 (El Tor),* Cam^r	(5)
pMEV30	pSW23T*::RS1 (El Tor),* Cam^r	(5)
pBS73	pSW23T*::VGJϕ,* Cam^r	(6)
pBS90	pSW23T*::TLCϕ,* Cam^r	(7)
pDA2	pDS132:: *UprecA-DwnrecA (N16961),* Cam^r	(3)
pAP7	pSW23T*::RS2*^{Env} *(VCE232)*	This study
pAP8	pDA2*::aph,* Kan^r	This study
pAP9	pSW23T*::RS2*^{O139} *(SG24)*	This study
pAP10	pBD56*::Δcat:bla,* Amp^r	This study
pAP16	pDS132*::UprecA-aph-DwnrecA (O395),* Kan^r	This study
pAP17	pDS132*::UplexA-aph-DwnlexA,* Kan^r	This study
pAP18	pCR2.1*::RS2*^{ET}, Amp^r	This study
pAP21	pBS66*::rstA*,* Cam^r	This study
pAP24	pAP21*::Δcat:sh ble, Zeo*^{r}	This study

pSB29	pSW23T*::up-dw VC0177,* Cam^r	This study
pSB30	pSB29*::sacB-cat,* Cam^r, Suc^s	This study
pSB31	pKAS32*::VC0177-sacB-cat-VC0178,* Amp^r, Cam^r, Suc^s, Strp^s	This study
pSB32	pSW23T*::up-dw VC1796,* Cam^r	This study
pSB33	pSB32*::sacB-cat,* Cam^r, Suc^s	This study
pSB34	pKAS32*::VC01796-sacB-cat-VC01797,* Amp^r, Cam^r, Suc^s, Strp^s	This study
pSB35	pSW23T*:: up-dw VC0828,* Cam^r	This study
pSB36	pSB35*::sacB-cat,* Cam^r, Suc^s	This study
pSB37	pKAS32*::VC0827-sacB-cat-VC0828*, Amp^r, Cam^r, Suc^s, Strp^s	This study
pSB43	pSB37*::lacZEc,* Amp^r, Cam^r, Suc^s, Strp^s	This study
pSB44	pSB34*::lacZ$_{Ec}$*, Amp^r, Cam^r, Suc^s, Strp^s	This study
pBIP13	pSW23T*::VC0500,* Cam^r	This study
pBIP15	pSW23T*::UpVC0500-DwVC0500,* Cam^r	This study
pBIP17	pSW23T*::UpVC0500-sacB-cat-DwVC0500,* Cam^r, Suc^s	This study
pBIP38	*pKAS32::upVC0500-sacB-cat-dwVC0500*, Amp^r, Cam^r, Suc^s, Strp^s	This study
pMEV78	pDS132 Up*dif1-lacZ::dif1*-Dwn*dif1*; Cmr	(5)
pJV39.02	pSW23T: Integration module of VSP-I	This study
pJV40.02	pSW23T: Integration module of VSP-II	This study
pJV42	pSW23T: Integration module of VPI-2	This study
pJV45	pSW23T: Integration module of VPI-1	This study
pJV37	pSW23T: Integration module of SXT	This study
pSM19	pBD61ΔXBS::*sacB-cat,rpsL,*unique MCS, Zeo^r, Cam^r, Suc^s, Strp^s	This study
pJV36	pKAS32:*UpmosT-sacB:aadA-DwnmosT(MO10),* Zeo^r, Cam^s	This study

386 **Table S2.** Conjugation and replication efficiency of different CTXΦ and its
387 derivatives in O1 El Tor, classical and environmental *Vibrio cholerae* strains

Bacterial strain	**Phage or its derivative**	**Infection and replication efficiency (Mean ± SD)**
N16961	pBS66	$2.44x10^4$ ± 2.27E+04
N16961	pBS22	$3.32x10^5$ ± 1.82E+05
BS1	pBD56	$4.82x10^6$ ± 3.47E+06
BS1	pBS66	$6.39x10^5$ ± 6.34E+05
BS1	pBS22	$3.8x10^4$ ± 3.56E+04
BS1	pAP7	$1.53x10^6$ ± 2.22E+06
BS1	pMEV30	$3.14x10^6$ ± 2.05E+06
BS1	pAP10:pBD56	$3.6x10^4$ ± 1.65E+04
BS1	pAP10:pBS66	$1.58x10^5$ ± 2.18E+05
BS1	pAP10:pBS22	$6.19x10^4$ ± 6.26E+04
BS1	pAP10:pMEV30	$5.45x10^4$ ± 4.33E+04
BS11	pBD56	$6.68x10^4$ ± 4.37E+04
BS11	pBS66	$5.1x10^5$ ± 4.37E+05
BS11	pBS22	$1.22x10^5$ ± 2.42E+04
BS11	pAP7	1.037E+006 ±1.264E+006
AP7	pBD56	0.0 ± 0.0
AP7	pBS66	0.0 ± 0.0
AP7	pBS22	$4.64x10^5$ ± 1.29E+05
AP7	pAP7	$2.15x10^5$ ± 2.91E+05
BS11	pAP10:pBD56	0.0 ± 0.0
O395	pBS66	1.423E+006 ±1.253E+006
AP10	pBS66	$1.86x10^5$ ± 2.11E+05
O395	pBS22	$1.61x10^4$ ± 1.03E+04
AP10	pBS22	0.0 ± 0.0
O395	pAP7	$2.1x10^5$ ± 2.91E+05
AP10	pAP7	$1.08x10^5$ ± 7.41E+04
O395	pAP9	$3.18x10^4$ ± 2.19E+04
AP10	pAP9	$1.84x10^4$ ± 8.31E+03
VCE232	pBS66	$7.6x10^4$ ± 9.00E+04
AP8	pBS66	$5.53x10^3$ ± 8.72E+03
VCE232	pBS22	$2.56x10^4$ ± 1.03E+04
AP8	pBS22	$1.1x10^5$ ± 1.30E+05

VCE232	pAP7	$1.04x10^4$ ± 2.88E+03
AP8	pAP7	0.0 ± 0.0
VCE232	pAP9	$7.26x10^3$ ± 5.19E+03
AP8	pAP9	$2.73x10^4$ ± 4.16E+03

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393 **Table S3:** Primers used in the present study
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Primer Name	Sequences (5'-3')	RE site	Use
70	ATGACCATGATTACGGATTC	-	Amplification of *lacZ::dif1* region
71	TTTTTGACACCAGACCAAC	-	Amplification of *lacZ::dif1* region
861	CGG**GCGGCCGC**CGCTGATTCATCATCCTCAC	NotI	Amplification of *VC0177 – VC0178* (VSP-1)
862	GCG**GAGCTC**CGACCATCCCTATGAAACTC	SacI	Amplification of *VC0177 – VC0178* (VSP-1)
863	CCG**TCGCGA**CTCCAGGCAGACTAGCTCTG	NruI	Inverse PCR of pSB29
864	GCG**ATGCAT**ACCTCAAGCACATAACTAGC	NsiI	Inverse PCR of pSB29
865	CGG**GCGGCCGC**CGGCGGAAGCTGACTAATAC	NotI	Amplification of *VC0500* (VSP-2)
866	GG**TCTAGA**GTGCTTCGCAAGGCTTTATG	XbaI	Amplification of *VC0500* (VSP-2)
867	GCG**ATGCAT**GGACTCTCTGAACAACTCGC	NsiI	Inverse PCR of pBIP13
868	CCG**CTCGAG**TGTAGCACTAGGGTTACTAG	XhoI	Inverse PCR of pBIP13
869	CCG**TCGCGA**AGCCATGACCCGGGAATTAC	NruI	Amplification of *sacB cat* region from pDS132
870	GCG**ATGCAT**CACATATACCTGCCGTTCAC	NsiI	Amplification of *sacB cat* region from pDS132
871	CCG**CTCGAG**AGCCATGACCCGGGAATTAC	XhoI	Amplification of *sacB cat* region from pDS132
890	ATCGCTCTGGAGTGAATACC	-	VSP-1- *sacB cat* tagged strain conformation
891	GTTGCCATATCGCGAAAGAC	-	VSP-1- *sacB cat* tagged strain conformation
908	GTTTGATCGGCCCTTATAGC	-	VSP -1 deletion confirmation
909	AGTCTCCACTGCGTAGTTTC	-	VSP -1 deletion confirmation
910	CGG**GCGGCCGC**TTACGCTTAGCCGCGACTTG	NotI	Amplification of *VC1796-VC1797* (VPI-2)
911	GCG**GAGCTC**GCGCCACGTTCTGATCATTG	SacI	Amplification of *VC1796-VC1797* (VPI-2)

912	CCG**TCGCGA**ACTCGTGTTGCTAGCTCATG	NruI	Inverse PCR of pSB32
913	GCG**ATGCAT**GTGATGCGAGTATTCGCTTG	NsiI	Inverse PCR of pSB32
925	CGCACGTTCCACAAGTTCAG	-	VPI- 2 *sacB cat* tagged strain conformation
926	CCTAAGCGCAGTATGGAAGC	-	VPI- 2 *sacB cat* tagged strain conformation
927	TGGACGAGTTGAGTTCTAGG	-	VSP- II *sacB cat* tagged strain conformation
928	GAAGACGCGATAAGTGAGTG	-	VSP- II *sacB cat* tagged strain conformation
947	CGG**GCGGCCGC**AAAGCACTTTGGCCTCATGG	NotI	Amplification of *VC0826 – VC0829* (VPI-1)
948	GCG**GAGCTC**TAACATAACCCGCAGCATCG	SacI	Amplification of *VC0826 – VC0829* (VPI-1)
949	CCG**TCGCGA**GTGAATGTTGCACGTGTTTC	NruI	Inverse PCR of pSB35
950	CCG**CTCGAG**AGCGCCTACTCGTCACAGAC	XhoI	Inverse PCR of pSB35
951	CCG**CTCGAG**CACATATACCTGCCGTTCAC	XhoI	Amplification of *sacB cat* region from pDS132
1119	GCTGGTACTGGTGGGTATTGTG	-	SXT specific primers
1120	CGAAGCGCGGATGCTCTACTC	-	SXT specific primers
1046	CTCTATGGGCACTGTCCACATT	-	SXT specific primers
1076	**GCGGCCGC**GTAGGCAGTTCACTTAAGG	Not 1	*s053* upstream region amplification
1108	GC**ACTAGT**CTCTGGCACTGTGATTTG	Spe1	*s053* upstream region amplification
1109	**GCTAGC**CCGAGTCCACATAGGACAAC	Nhe1	*s053* downstream region amplification
1110	GC**CTCGAG**GGCCAGTCGTTGTTGAGAAG	Xho1	*s053* downstream region amplification
1220	GC**CATATG**CTGCAGGAATTCGATATCAAG	NdeI	Inverse PCR to amplify upstream and downstream region of *most*
1221	GC**TCTAGA**CTTCCTTAGCTCCTGCCCTATG	XbaI	Inverse PCR to amplify upstream and downstream region of *most*
1222	GC**TCTAGA**GCGTATGCGCTCACGCAACTG	XbaI	Amplification of Spectinomycin resistance cassette
1223	GC**CATATG**ATTATTTGCCGACTACCTTGG	NdeI	Amplification of Spectinomycin resistance cassette
1232	CACCTTATCTGAATGCTGCC	-	Amplification of integration module of SXT-outer
1233	GATTCCTCGCAACGAATGAG	-	Amplification of integration module of SXT-outer

1234	CG**CTGCAG**CTGATTATGAGAGTTGTCGTC	PstI	Amplification of integration module of SXT-inner
1235	GC**TCTAGA**TCAATGGCACGGCGGAGATG	Xba1	Amplification of integration module of SXT-inner
1247	GC**GCGGCCGC**GTATTGTCATATCTAGTCTG	NotI	Amplification of integration module of VPI-2
1248	GC**ACTAGT**CAGATTAGCTAACCTATTGG	SpeI	Amplification of integration module of VPI-2
1251	GC**GCGGCCGC**TCCTAAAGGGCACCTAGTTG	NotI	Amplification of integration module of VSP-II
1252	GC**ACTAGT**CCATAGAATGGAGTTCAGAG	SpeI	Amplification of integration module of VSP-II
1259	GC**ACTAGT**TCAATGGCACGGCGGAGATG	SpeI	Amplification of integration module of SXT
1260	GC**GCGGCCGC**TCTGCTCTTTGAGCGGAATG	NotI	Amplification of integration module of VPI-2 (inner)
1261	GC**ACTAGT**CAATAAGTATGAATCGATATC	SpeI	Amplification of integration module of VPI-2 (inner)
1289	GC**GAATTC**TCGCATACATCATCGACCAG	EcoRI	Amplification of integration module of VSP-I
1290	CC**GAGCTC**CAACATGAAGTAGGCGGCAC	SacI	Amplification of integration module of VSP-I
1304	ACTATGATTTGGTGGAGCTGGGGTAAGTTGA	-	Amplification of *attP* VPI-1
1305	TCAACTTACCCCAGCTCCACCAAATCATAGT	-	Amplification of *attP* VPI-1
1306	ACTAGTGATTGGTGGGCTGGGGGAGTTGA	-	Amplification of *attB* VPI-1
1307	TCAACTCCCCCAGCCCACCAATCACTAGT	-	Amplification of *attB* VPI-1

RE=Restriction Enzyme

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