SUPPLEMENTARY INFORMATION

Cell cycle-regulated release of a replication inhibition complex in *Vibrio cholerae*

DATA MATERIALS AVAILABILITY:

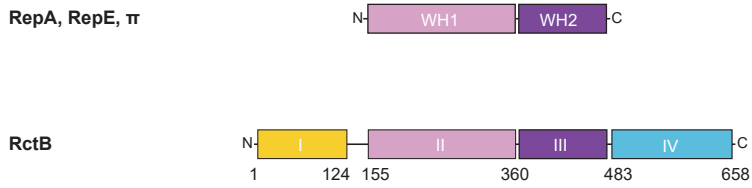
The sequencing data from main and supplementary figures are publicly available in The European Nucleotide Archive (ENA). For RctB ChIP-seq in wt context, the accession numbers are ERR12420290 (IP), ERR12420289 (INPUT) and ERR12420106 (no FLAG, negative control). For RctB ChIP-seq in stationary phase: ERR12420287 (IP) and ERR12420288 (INPUT). For RctB ChIP-seq in 29m C>A mutated context, ERR12420285 (IP) and ERR12420286 (INPUT). For ParB2 ChIP-seq, ERR12420283 (IP) and ERR12420284 (INPUT). For RctB-L651P ChIP-seq in wt context, ERR12420292 (IP) and ERR12420291 (INPUT). For RctB ChIP-seq in Δ*crtS* context, ERR12421767 (IP) and ERR12421766 (INPUT). For RctB-L651P ChIP-seq in Δ*crtS* context, ERR12492377 (IP) and ERR12492376 (INPUT). For RctB ChIP-seq in a context with *crtS* relocated to attTN7 site, ERR12421765 (IP) and ERR12421764 (INPUT). For RctB ChIP-seq in a context with two *crtS* sites inserted in attTN7 site, ERR12421763 (IP) and ERR12421762 (INPUT). For RctB ChIP-seq in Δ*dam* context ERR12493891 (IP), ERR12493890 (INPUT) and ERR12493887 (no FLAG, negative control). For RctB-L651P ChIP-seq in Δ*dam* context ERR12493889 (IP) and ERR12493888 (INPUT). For RctB ChIP-seq in synchronized population: at 0min after release ERR12421787 (IP) and ERR12421786 (INPUT), at 15min ERR12421789 (IP) and ERR12421788 (INPUT), at 30min ERR12421791 (IP) and ERR12421790 (INPUT), at 45min ERR12421793 (IP) and ERR12421792 (INPUT), at 60min ERR12421795 (IP) and ERR12421794 (INPUT). Finally, for MFA analysis: wt strain ERR12492335, mutant strain with *crtS* near *ori1* ERR12492336 and mutant with two *crtS* sites at wt and at *ori1* ERR12492337.

SUPPLEMENTARY FIGURES

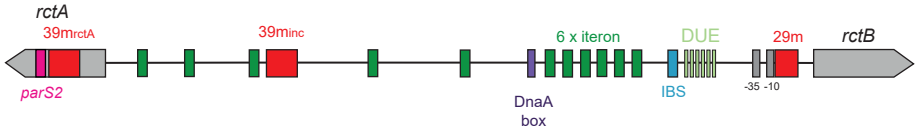
Supplementary Figure 1. Iteron-type initiators and *ori2* structure

a. Comparison of protein domain organization between Chr2 initiator (RctB) and iteron-type plasmid initiators (RepA, RepE, Pi). The different structural domains are indicated (WH = winged helix). **b.** Representation of Chr2 origin of replication (ori2) and nucleotide sequence of iterons, 29/39m and DNA unwinding element (DUE). RctB binding sites are indicated by dark green (iterons) and red (29/39m) rectangles. *Ori2* contains eleven iterons, six of which are essential for replication, while the others have a regulatory function. The three 29/39m sites act as strong negative regulators of *ori2* initiation. Single-stranded RctB binding sites (5'-ATCA) in the DUE are represented as light green bars. Other protein binding sites are indicated: IBS (IHF binding site), DnaA box, *parS2* (ParB2 binding site). Conserved bases in the sequence alignments are shown in bold. Dam-methylated GATC motifs within the iterons are shown in purple. Within the DUE, RctB binding sites to single-stranded DNA (5'-ATCA) are shown in green.

a



b



39mrctA	GC**GGAA**CG**AT**TAAACCGAGCCACT**A**AG**TTACGG**TGAA**TG**	**Array of 6 x iterons**
39minc	GC**GGAA**GC**AT**GTAAATTCATTATC**A**AT**TTACGG**TCGA**TG**	
29m	TT**GGAA**CT**AT**A----------GTG**A**TA**TTACGG**TAAG**TG**	

TT**GATC**A**T**GCTT
TT**GATC**A**T**TGAT
AT**GATC**A**T**GCTT
AT**GATC**A**T**GCTT
TT**GATC**A**T**GGTT
AT**GATC**A**T**GCTT

DUE (6 x ATCA) TCACAG**ATCA**TTAG**ATCA**CTCTA**ATCA**TATTTA**ATCA**TTTAA**ATCA**GAAAG**ATCA**GTTATT

Figure S1

Supplementary Figure 2: RctB ChIP Signal across all detected binding loci.

Y-axis: Normalized RctB ChIP Signal (IP Coverage/INPUT Coverage); X-axis: Genomic Coordinates on V. cholerae N16961 Reference Genome (CP028827.1 and CP028828.1). A 2,000-bp window is centered around the peak. ChIP signals are shown in blue, and the genetic context is depicted above the graph with CDS represented by grey arrows.

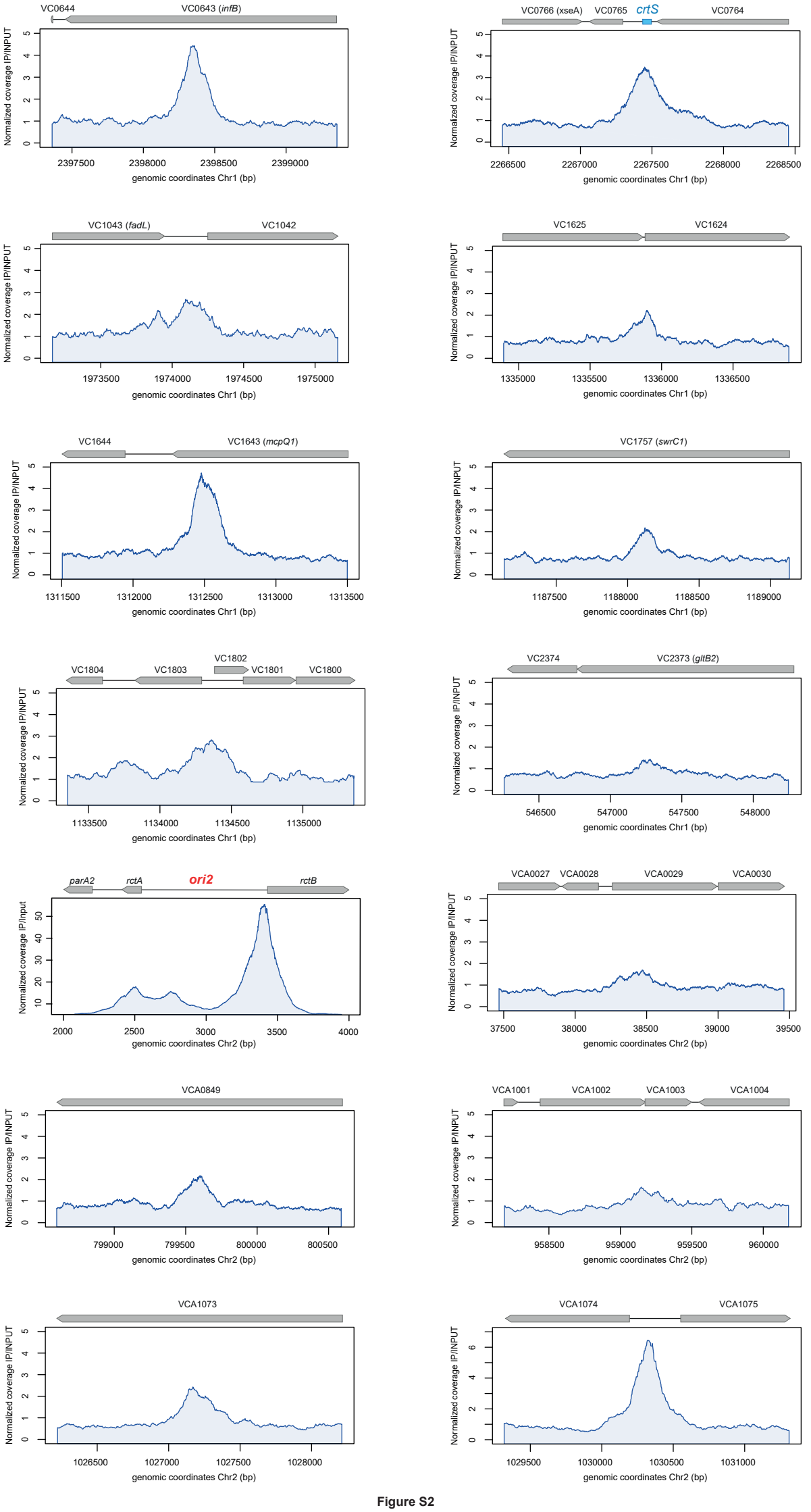

VC0644
VC0643 (infB)
Normalized coverage IP/INPUT
genomic coordinates Chr1 (bp)
VC0766 (xseA)
VC0765
crtS
VC0764
VC1043 (fadL)
VC1042
VC1625
VC1624
VC1644
VC1643 (mcpQ1)
VC1757 (swrC1)
VC1804
VC1803
VC1802
VC1801
VC1800
VC2374
VC2373 (gltB2)
parA2
rctA
ori2
rctB
Normalized coverage IP/Input
genomic coordinates Chr2 (bp)
VCA0027
VCA0028
VCA0029
VCA0030
VCA0849
VCA1001
VCA1002
VCA1003
VCA1004
VCA1073
VCA1074
VCA1075

Figure S2

Supplementary Figure 3: RctB ChIP profile remains similar between growing and not growing phases.

On y axis: RctB normalized ChIP signal (IP coverage/INPUT coverage), on x axis: genomic coordinates on *V. cholerae* N16961 reference genome (CP028827.1 and CP028828.1). A 2.000bp window is represented and centered around the peak. ChIP signals are represented in blue for exponential phase (EXP , OD_{600nm}=0.5) and in red for stationary phase (STAT , overnight culture). Genetic context (CDS in grey arrows) and known RctB binding sites are depicted on top of the graph: genes as grey arrow, 39mer site as red boxes, iterons as green rectangles, *crtS* as a cyan box.

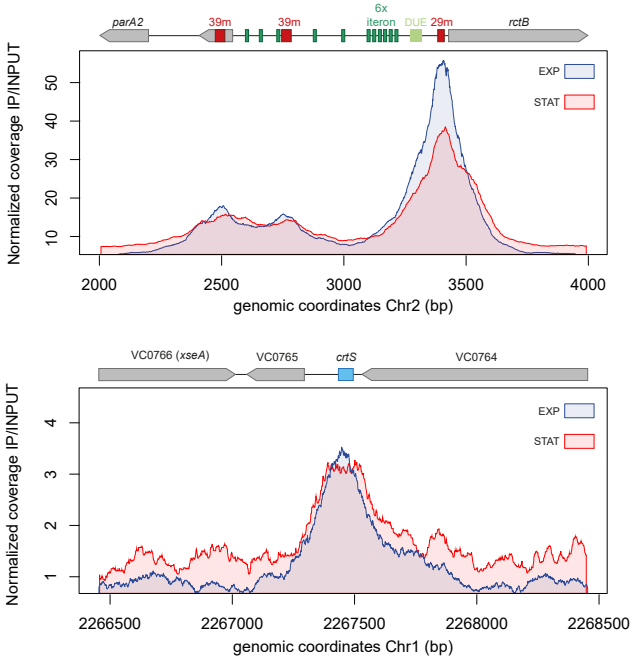
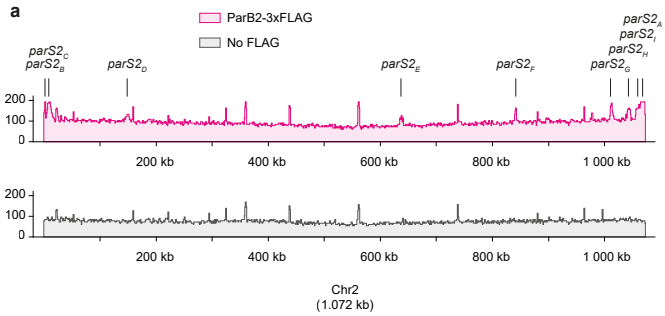

parA2
39m
39m
6x
iteron
DUE
29m
rctB
EXP
STAT
Normalized coverage IP/INPUT
10
20
30
40
50
2000
2500
3000
3500
4000
genomic coordinates Chr2 (bp)
VC0766 (xseA)
VC0765
crtS
VC0764
EXP
STAT
Normalized coverage IP/INPUT
1
2
3
4
2266500
2267000
2267500
2268000
2268500
genomic coordinates Chr1 (bp)

Figure S3

Supplementary Figure 4: ParB2 ChIP signal covers the *inc* region of *ori2*.

a. on y axis: coverage from the IP reads, on x axis: genomic coordinates on *Vibrio cholerae* N16961 reference Chr2 (CP028828.1). Raw coverage is represented in pink for ParB2-3xFLAG and in grey for the wt strain (negative control). Already identified *parS* sites [1] are represented on top of the graph. **b.** on left y axis: RctB normalized ChIP signal (IP coverage/INPUT coverage), on right y axis (different scale): ParB2 normalized ChIP signal, on x axis: genomic coordinates on *V. cholerae* N16961 reference Chr2 (CP028828.1). A 2.000bp window containing *ori2* is represented. ChIP signals are represented in blue for RctB and in pink for ParB2. Genetic context (CDS in grey arrows) and known RctB binding sites are depicted on top of the graph: genes as grey arrow, 39mer site as red boxes, iterons as green rectangles and *parS* site as pink rectangle.


a
ParB2-3xFLAG
No FLAG
parS2C
parS2B
parS2D
parS2E
parS2F
parS2G
parS2A
parS2I
parS2H
200
100
0
200 kb
400 kb
600 kb
800 kb
1 000 kb
Chr2
(1.072 kb)

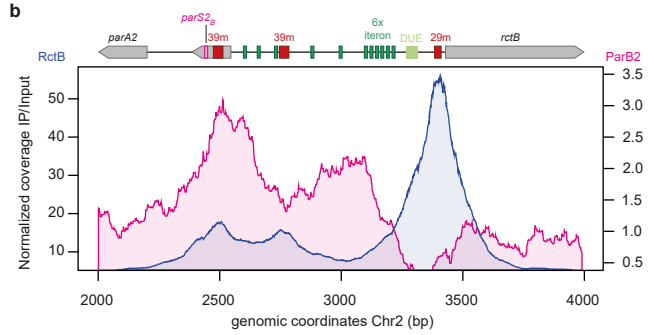
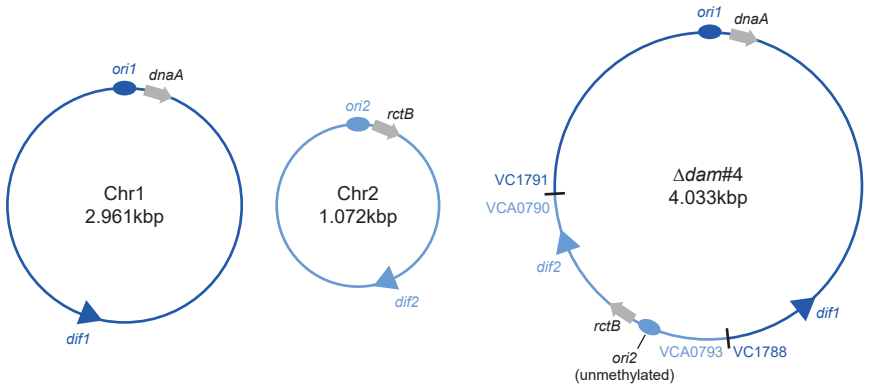

b
parS2B
parA2
39m
39m
6x
iteron
DUE
29m
rctB
RctB
ParB2
Normalized coverage IP/Input
50
40
30
20
10
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2000
2500
3000
3500
4000
genomic coordinates Chr2 (bp)

Figure S4

Supplementary Figure 5: RctB binding pattern on *ori2* is maintained in absence of Dam methylation.

a. On the left: *V. cholerae* N16961 wt genome organization, on the right: *Δdam* strain genome organization in which *ori2* is inactivated and Chr2 is inserted in Chr1 (mutant Δ*dam*#4 identified in [2]. **b**. on y axis: RctB normalized ChIP signal (IP coverage/INPUT coverage), on x axis: genomic coordinates on *Vibrio cholerae* N16961 reference Chr1 (CP028827.1). A 2.000bp window containing *ori2* is represented. RctB ChIP signal is represented in blue wt strain and in purple for *Δdam* strain. Genetic contexts are depicted on top of the graph as in Fig.2a.

a


ori1
dnaA
Chr1
2.961kbp
dif1
ori2
rctB
Chr2
1.072kbp
dif2
ori1
dnaA
VC1791
VCA0790
Δdam#4
4.033kbp
dif2
dif1
rctB
ori2
(unmethylated)
VCA0793
VC1788

b

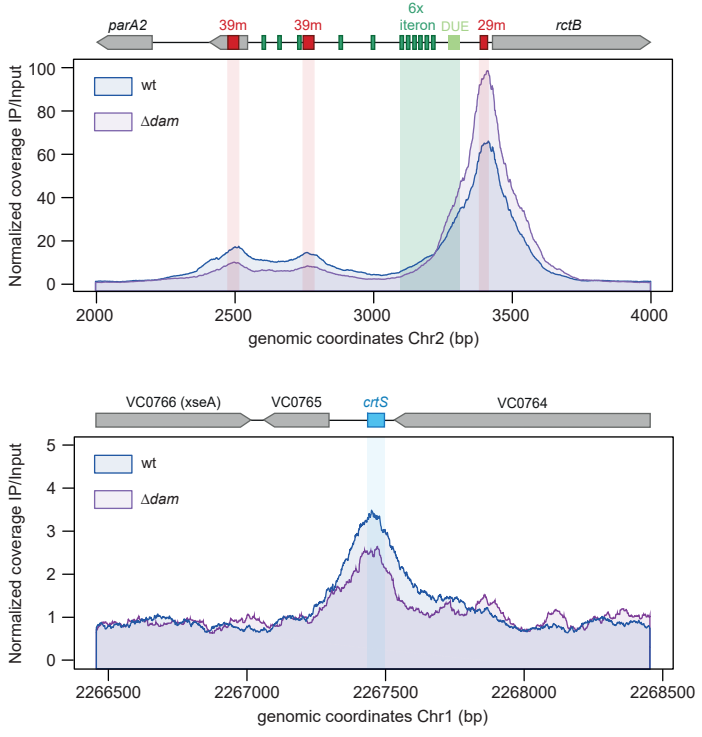

parA2
39m
39m
6x
iteron
DUE
29m
rctB
Normalized coverage IP/Input
wt
Δdam
genomic coordinates Chr2 (bp)
VC0766 (xseA)
VC0765
crtS
VC0764
Normalized coverage IP/Input
wt
Δdam
genomic coordinates Chr1 (bp)

Figure S5

Supplementary Figure 6: Size Exclusion Chromatography (SEC) of RctB Domain IV

The SEC profile of $RctB^{IV}$ was obtained on a Citiva Superdex 200 Increase 10/300. The chromatogram shows the signal intensity of $RctB^{IV}$ (blue curve), detected at a wavelength of 280 nm and measured in arbitrary units (a.u.) (left y-axis), plotted against the retention volume in mL (x-axis). The BioRad molecular weight standards (bovine thyroglobulin, 670 kDa, bovine γ-globuline, 158 kDa, chicken ovalbumin, 44 kDa, horse myoglobin, 17 kDa and vitamin B12, 1.35 kDa) were run under the same conditions, and their elution positions are indicated with black circles with the molecular weight in kDa, indicated on the right y-axis.

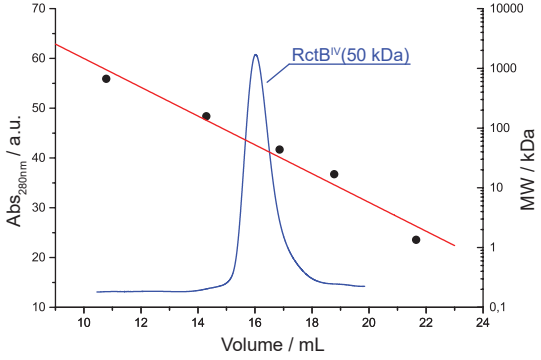
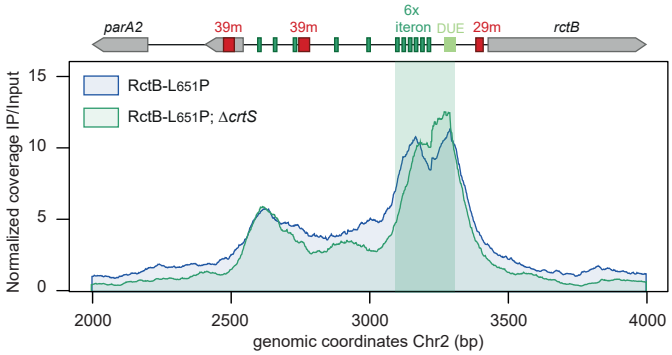

RctB^IV(50 kDa)
Abs280nm / a.u.
MW / kDa
Volume / mL
70
60
50
40
30
20
10
10000
1000
100
10
1
0,1
10
12
14
16
18
20
22
24

Figure S6

Supplementary Figure 7: Effect of the deletion of *crtS* and *dam* on the binding of RctB-L_{651}P. Same legend as in Supplementary Fig.2. **a, b.** RctB ChIP signal is represented in blue for L651P mutant, green in the Δ*crtS* and purple in the Δ*dam* genetic background.

a


parA2
39m
39m
6x
iteron
DUE
29m
rctB
RctB-L651P
RctB-L651P; ΔcrtS
Normalized coverage IP/Input
0
5
10
15
2000
2500
3000
3500
4000
genomic coordinates Chr2 (bp)

b

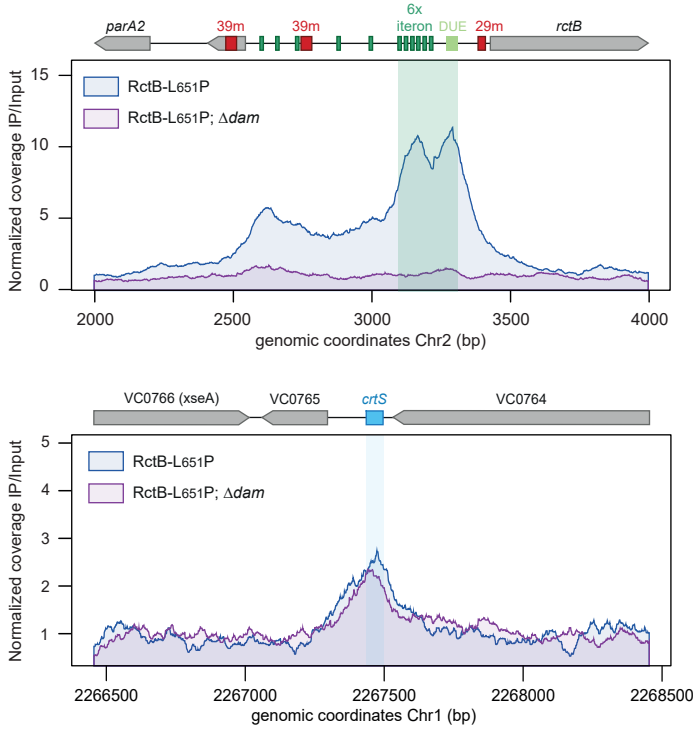

parA2
39m
39m
6x
iteron
DUE
29m
rctB
RctB-L651P
RctB-L651P; Δdam
Normalized coverage IP/Input
0
5
10
15
2000
2500
3000
3500
4000
genomic coordinates Chr2 (bp)
VC0766 (xseA)
VC0765
crtS
VC0764
RctB-L651P
RctB-L651P; Δdam
Normalized coverage IP/Input
1
2
3
4
5
2266500
2267000
2267500
2268000
2268500
genomic coordinates Chr1 (bp)

Figure S7

Supplementary Figure 8: The effect of *crtS* on RctB binding is restricted to *ori2*

Barplot representing the maximum height of RctB ChIP peaks along the genome (logarithmic scale). Peak maximal height was calculated from the average of two independent ChIP experiments, for *ori2* the peak height represented is the one on the 29m.

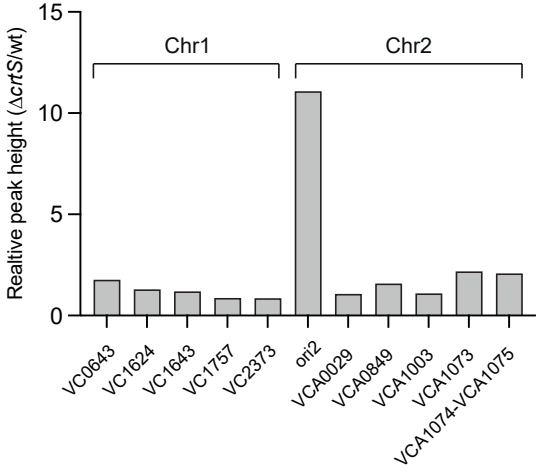

15
10
5
0
Realtive peak height (ΔcrtS/wt)
Chr1
Chr2
VC0643
VC1624
VC1643
VC1757
VC2373
ori2
VCA0029
VCA0849
VCA1003
VCA1073
VCA1074-VCA1075

Figure S8

Supplementary Figure 9. Observation of RctB binding in presence of *crtS* by TEM.

a. TEM observations of nucleoprotein complexes formed by RctB on a 944bp DNA fragment containing a *crtS* site located 1/3 of its length. RctB binding to *crtS* induces a kink in the DNA. **b.** TEM images of a mixture of unmethylated DNA substrates containing *crtS* and *ori2* (for better visibility, only 29/39m are shown, as RctB does not bind to unmethylated iterons). Sporadic intermolecular contacts, mediated by RctB bound to *crtS* and 29/39m, are observed. White arrows indicate nucleoprotein complexes.

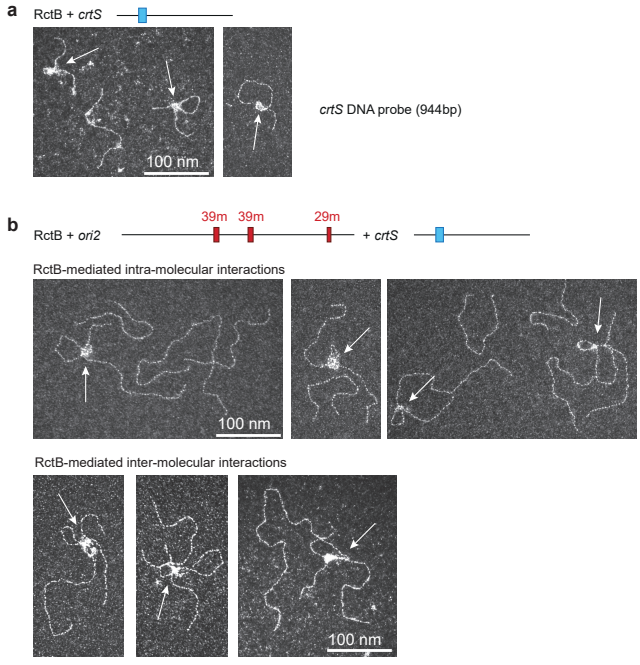

a
RctB + crtS
100 nm
crtS DNA probe (944bp)
b
RctB + ori2
39m
39m
29m
+ crtS
RctB-mediated intra-molecular interactions
100 nm
RctB-mediated inter-molecular interactions
100 nm

Figure S9

Supplementary Figure 10. Two chromosomal copies of *crtS* have no influence on RctB binding at *ori2*.

a. Relative Chr2 copy number to Chr1 (*ori2*/*ori1*) measured by dPCR on gDNA of non-replicating *V. cholerae* (stationary phase), in strains with one chromosomal copy of *crtS* (Δ*crtS*; *attTn7*::*crtS*) or two chromosomal copies of *crtS* (Δ*crtS*; *attTn7*::2x*crtS*). The *attTn7* site is located near gene VC0487 on Chr1. The endogenous *crtS* site is deleted. **b.** ChIP-seq of RctB in V. cholerae Δ*crtS* mutants carrying either one or two copies of *crtS* in the *attTn7* site of Chr1. Legend same as Fig. 2a.

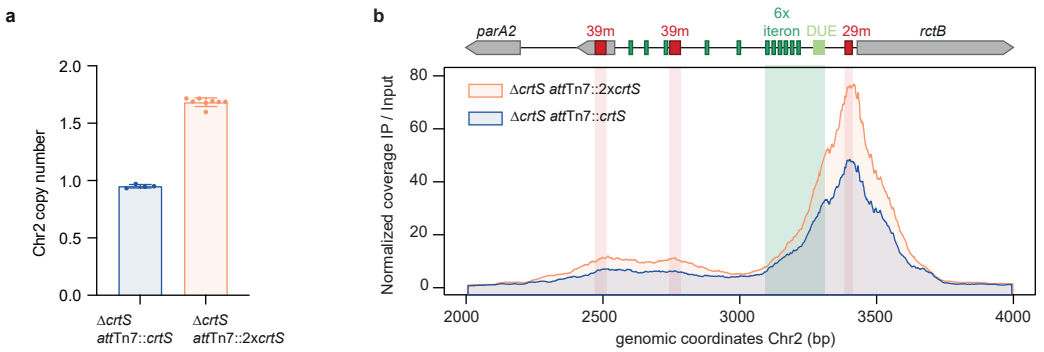

a
Chr2 copy number
2.0
1.5
1.0
0.5
0.0
ΔcrtS attTn7::crtS
ΔcrtS attTn7::2xcrtS
b
parA2
39m
39m
6x iteron
DUE
29m
rctB
Normalized coverage IP / Input
80
60
40
20
0
ΔcrtS attTn7::2xcrtS
ΔcrtS attTn7::crtS
2000
2500
3000
3500
4000
genomic coordinates Chr2 (bp)

Figure S10

Supplementary Figure 11: RctB binding pattern on *crtS* in synchronized population. ChIP-seq experiments on synchronized population at different timepoints after SHX removal (15min, 30min, 45min, 60min). On y axis: RctB normalized ChIP signal (IP coverage/INPUT coverage), on x axis: genomic coordinates on *V. cholerae* N16961 reference Chr1 (CP028827.1). A 2.000bp window is represented centered around *crtS*. Genetic is depicted on top of the graph (genes in grey arrows, *crtS* in light blue square).

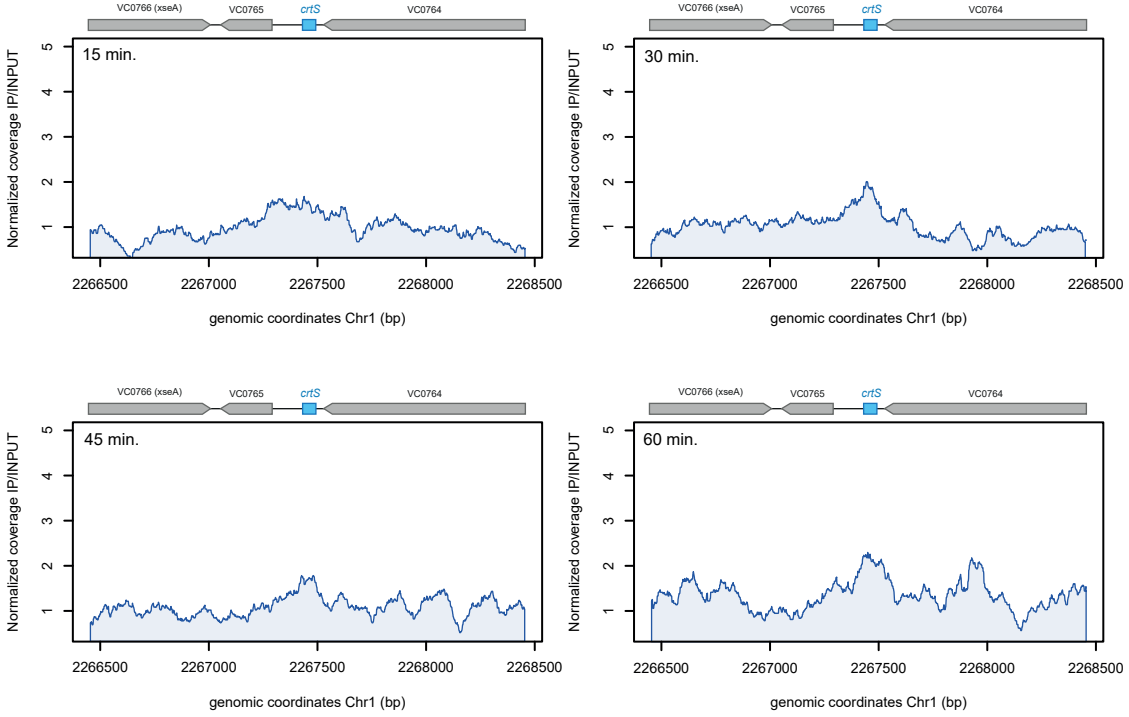

VC0766 (xseA)
VC0765
crtS
VC0764
15 min.
30 min.
45 min.
60 min.
Normalized coverage IP/INPUT
1
2
3
4
5
2266500
2267000
2267500
2268000
2268500
genomic coordinates Chr1 (bp)

Figure S11

Supplementary Figure 12

Marker frequency analysis on the input sample (not immunoprecipitated) from the ChIP experiment. Coverage is represented on y axis and genomic coordinates of both chromosomes (in kilo base pair) are present on the x axis. Coverage has been smoothed with a 10kb sliding window along the genome. Linear regression analysis was performed separately on each half of the chromosomes, and the best-fit lines were superimposed on the plot.

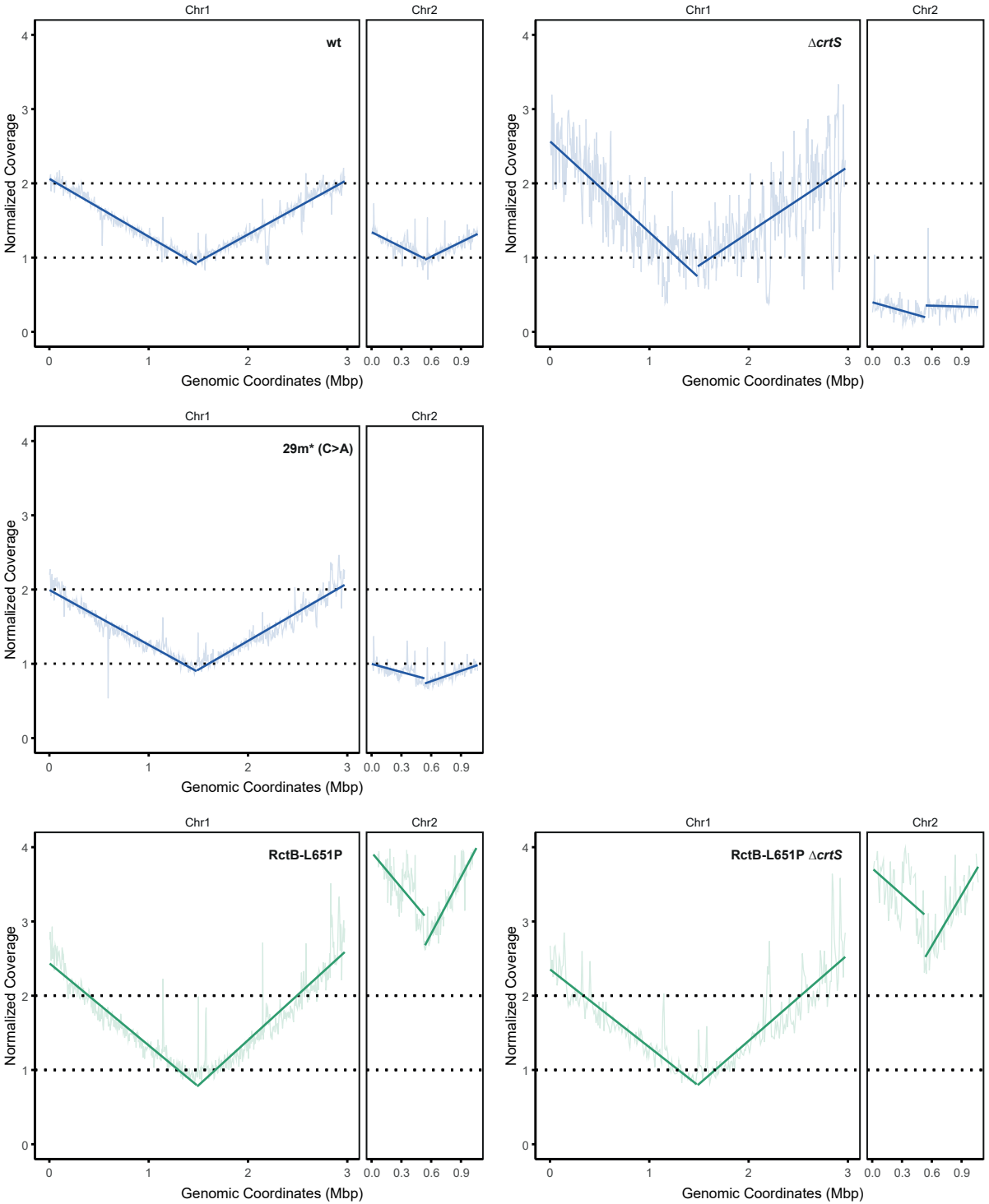

Chr1
Chr2
wt
Normalized Coverage
Genomic Coordinates (Mbp)
Chr1
Chr2
ΔcrtS
Normalized Coverage
Genomic Coordinates (Mbp)
Chr1
Chr2
29m* (C>A)
Normalized Coverage
Genomic Coordinates (Mbp)
Chr1
Chr2
RctB-L651P
Normalized Coverage
Genomic Coordinates (Mbp)
Chr1
Chr2
RctB-L651P ΔcrtS
Normalized Coverage
Genomic Coordinates (Mbp)

Figure S12

Supplementary Table 1: RctB binding sites identified from ChIP-seq experiment. ChIP reads data were aligned on *Vibrio cholerae* N16961 reference genome (CP028827.1 for Chr1 and CP028828.1 for Chr2). Peak summit, p-value and fold enrichment are calculated with MACS2.0 program by comparing RctB-FLAG versus wt (no FLAG) ChIP-seq data. ChIP peaks motif are found using the MEME suite, two sequences are significantly enriched and correspond to already known iterons and 39m sites.

					RctB ChIPseq enrichment factor					
Chromosome	**Peak summit position**	**Locus context**	**site type**	**Discovered in**	**wt**	**Δ*crtS***	**RctB-L651P**	**RctB-L651P, Δ*crtS***	**Δ*dam***	**RctB-L651P, Δdam**
Chr1	547255	VC2373, CDS	39m-like	this study	2,1	1,8	1,6	1,0	1,4	1,3
Chr1	1134355	VC1802-VC1803, intergenic	N / S	this study	3,2	20,6	5,9	6,2	2,8	8,5
Chr1	1188134	VC1757, CDS	39m-like	this study	3,1	2,7	1,7	1,4	2,6	1,5
Chr1	1312502	VC1643, CDS	39m-like	this study	6,9	8,2	2,2	1,4	2,3	1,6
Chr1	1335897	VC1624, CDS	39m-like	this study	3,0	3,9	1,5	1,1	2,1	1,5
Chr1	1974161	VC1042-VC1043, intergenic	N / S	this study	2,3	11,0	2,1	2,3	2,4	2,2
Chr1	2267454	VC0764-VC0765, intergenic	*crtS*	[1]	4,4	NA	2,7	NA	2,5	2,4
Chr1	2398355	VC0643, CDS	39m-like	this study	5,1	9,0	1,5	1,0	2,8	1,1
Chr2	3414	*ori2*	29/39m, iteron, ssDNA-ATCA	[2]	58,3	646,2	9,2	13,3	98,1	2,5
Chr2	38465	VCA0029, CDS	iteron	[1]	2,2	2,3	1,4	0,8	1,5	1,5
Chr2	799599	VCA0849, CDS	39m-like	this study	3,3	5,2	1,5	1,1	2,2	1,5
Chr2	959185	VCA1003, CDS	iteron	[1]	2,6	2,9	1,6	1,1	1,5	1,7
Chr2	1027217	VCA1073, CDS	iteron	[1]	3,3	7,1	2,5	2,9	1,7	1,9
Chr2	1030315	VCA1074-VCA1075, intergenic	39m	[1]	8,6	17,9	1,5	1,2	7,2	1,5

N / S : no similarity with known sequences

References

1 Baek, J. H. & Chattoraj, D. K. Chromosome I controls chromosome II replication in Vibrio cholerae. PLoS genetics 10, e1004184, doi:10.1371/journal.pgen.1004184 (2014).

2 Egan, E. S. & Waldor, M. K. Distinct replication requirements for the two Vibrio cholerae chromosomes. Cell 114, 521-530, doi:10.1016/S0092-8674(03)00611-1 (2003).

Supplementary Table 2. Plasmids

Experiment	Relevant figures	Description	Name / Reference
pORI2-derivatives for copy number monitoring	1c	pSW23T::*ori2*$_{V.cholerae}$, *oriT*$_{RP4}$, *ori*$_{R6K}$, Cm^r	pORI2 [3]
Expression vectors for protein purification	-	pET24b(+)	Novagen
	3a-c	pET24b::*rctBIV*	This study
	3d	pET24b::*rctB*	This study
Expression vectors for Bacterial two-hybrid	3e (pKT25 Empty)	pKT25 (P$_{lac}$-*cyaA*T25) *ori*$_{p15A}$, Kan^r	pKT25 [4]
	3e (pKT25 Zip)	pKT25-*zip* (Leucine zipper)	pKT25-zip [4]
	3e (pKT25 D314P)	pKT25::*rctB*$_{D314P}$	pFF102 [5]
	3e (pUT18C Empty)	pUT18C (P$_{lac}$-*cyaA*T18) *ori*$_{ColE1}$, Ap^r	pUT18C [4]
	3e (pUT18C Zip)	pUT18C-*zip* (Leucine zipper)	pUTC18-zip [4]
	3e (pUT18C D314P)	pUT18C:: *rctB*$_{D314P}$	pAT01 [5]
	3e (pUT18C D314P-L651P)	pUT18C:: *rctB*$_{D314P-L651P}$	pFF128 [5]
	3e (pUT18C D314P-I546G-I548G)	pUT18C::*rctB*$_{D314P-I546G-I548G}$	This study
	3e (pUT18C D314P-A565P)	pUT18C::*rctB*$_{D314P-A565P}$	This study
	3e (pUT18C D314P-Δ591-596)	pUT18C::*rctB*$_{D314P-Δ591-596}$	This study
	3e (pUT18C D314P-I625P)	pUT18C::*rctB*$_{D314P-I625P}$	This study
Vectors used for genetic engineering	Suicide plasmid for allele exchange	pMP7 (*oriV*$_{R6Kγ}$ *oriT*$_{RP4}$ *araC*-P$_{BAD}$-*ccdB ; cat*)	pMP7 (#pSW7848) [6]
	Supp 4	pMP7::parB2-3xFLAG (Cter)	This study
	1a, 2a, 5	pMP7::rctB-3xFLAG (Cter)	This study
	3f	pMP7-*rctB*$_{L651P-3xFLAG(Cter)}$	This study
	4a (*crtS* excision)	pMP7-[frt-*crtS-arr2*-frt]	pMP184 [3]
		ori$_{pSCS101}$ *repA* *oriT*$_{RP4}$ *araC* P$_{BAD}$-*flp* ; *bla*	pMP108 [3]
	Transposition in *attTn7* site	Tn7 helper – *ori*$_{pSCS101}$ *repA*$_{ts}$ oriT$_{RP4}$ *araC* P$_{BAD}$-*tnsABCD* ; *bla*	pMVM1 [3]
		Tn7 shuttle – *oriV*$_{R6Kγ}$ *oriT*$_{RP4}$:: [Tn7R-aadA7-MCS-Tn7L]; cat	pMP234 [5]
		pMP234 :: [Tn7R- aadA7-crtS-Tn7L]	This study
		pMP234:: [Tn7R- aadA7-2xcrtS-Tn7L]	This study

Supplementary Table 3. Bacterial strains

Experiment	Relevant figures	Description	Name (Reference)
Cloning strain	-	E. coli lacIq thi-1 supE44 endA1 recA1 hsdR17 gyrA462 zei-298::Tn10 (Tcr) Δ*thyA*::(*erm-pir*116)	π3813 [7]
Donor strain for conjugation	-	E. coli F- RP4-2-Tc::Mu ΔdapA::(erm-pir) *gyr*A462 *zei*-298::Tn10	β3914 [7]
Bacterial two-hybrid	3e	*E. coli* F−, *cya*-99, *ara*D139, *gal*E15, *gal*K16, *rps*L1, *hsd*R2, *mcr*A1, *mcr*B1	BTH101 [4]
Protein purification	3a-d	E. coli BL21	Thermo Scientific
Vibrio cholerae N16961	-	N16961 *hap*R+	N16961rep [8]
	Supp 5	N16961 Δ*dam* (fused chromosomes)	Δdam#4 [2]
	1e	N16961 Δ*rctB* (fused chromosomes)	MCH1 [6]
Chr2 copy number	1d	N16961 VC1042-aph-VC1043	This study
		N16961 VC0643 inactivated, TTACGCAGAGTG > TTGCGAAGGGTA	This study
		N16961 VC1643 inactivated, TTACGGCTAACG > TCACAGCCAACG	This study
ChIP-seq	1a, 2a, 5 Supp 2, 3, 11	N16961, rctB-3xFLAG (Cter)	This study
	2c	N16961, rctB::rctB-3xFLAG (Cter); 29m(C>A)	This study
	3f	N16961, rctB::rctB-L651P-3xFLAG (Cter)	This study
	Supp 4	N16961, parB2::parB2-3xFLAG (Cter)	This study
	Supp 5	Δdam#4, rctB::rctB-3xFLAG (Cter)	This study
	Supp 7	N16961, rctB::rctB-L651P-3xFLAG (Cter); ΔcrtS	This study
	Supp 7	Δdam#4 , rctB::rctB-L651P-3xFLAG (Cter)	This study
	4a Supp 8	N16961, rctB::rctB-3xFLAG (Cter); ΔcrtS	
	Supp 10	N16961, rctB::rctB-3xFLAG (Cter); ΔcrtS; attTn7::crtS	
	Supp 10	N16961, rctB::rctB-3xFLAG (Cter); ΔcrtS; attTn7::2xcrtS	
pORI2 copy number	1c	E. coli MG1655-*rpsL**, Strep^r	J665 [3]
		J665 lacZ::crtS	J666 [3]
		J665 lacZ::VC1042-VC1043	This study
		J665 lacZ::VC1803	This study
		J665 lacZ::VC0643	This study
		J665 lacZ::VC1624	This study
		J665 lacZ::VC1643	This study
		J665 lacZ::VC1757	This study
		J665 lacZ::VC2373	This study
Fluorescent microscopy	4c	N16961[parSP1@VC2759][parST1@VC0783][lacO@VCA1092][lacZ::(lacI-RFP-T,parBT1-yGFP)][attTn7::CFP-parBP1]	This study
	4d	N16961-VC0023-crtS-VC0024 [parSP1@VC2759][parST1@VC0783][lacO@VCA1092][lacZ::(lacI-RFP-T,parBT1-yGFP)][attTn7::CFP-parBP1]	This study
Marker Frequency Analysis	4e	$crtS_{wt}$ (N16961ChapRΔlacZ)	WT [9]
		$crtS_{ori1}$ (N16961ChapRΔlacZ ΔcrtS with crtS inserted between VC0023/VC0024)	$crtS_{VC23}$ [9]
		$crtS_{wt}$-$crtS_{ori1}$ (N16961ChapRΔlacZ with crtS inserted between VC0023/VC0024)	$crtS_{WT/VC23}$ [9]

Supplementary Table 4. Oligonucleotides

Primer and probe sequences used in dPCR and RT-dPCR reactions			
Target	Orientation	Primers (5 '→ 3')	Dual Labeled Probes (5 '→ 3')
ori1 *V. cholerae*	FWD	GCTGCTCGACAAATGGAAC	[FAM]-TCCGATGGAAATGTTGGTGAAACACATTCT-[BHQ1]
	REV	AAGATGCGGACTGACCAC	
ori2 *V. cholerae*	FWD	TGTCTCGTCGTCATACCG	[HEX]-ATCTGATCCGCGAACTTCGTCGTCTCT-[BHQ1]
	REV	CTTCACTCCCCTTCCCTTC	
oriC *E. coli*	FWD	CCACCGAGAAGAACATGGAG	[FAM]-ATTGTCCAGAAGGTGGCTGGGGGGTTTT-[BHQ1]
	REV	GCCGCAGGATTACATAGGAC	
pORI2	FWD	TTATGGTGAAAGTTGGAACCTC	[HEX]-GCCGATCAACGTCTCATTTTCGCCA-[BHQ1]
	REV	GCCGAATAAATACCTGTGACG	
VC1042	FWD	TCAAAATCCATCACCACTTCC	(FAM)-CCTTCACCCATCACATAACGCACACCA-(BHQ1)
	REV	TGCTTACCAAATCTTGCCTC	
VC1803	FWD	TCACTGCAAAGCTATGTGTC	(HEX)-AGCGTTGGTTACGCTTCATAAGACAC-(BHQ1)
	REV	TTTCTTCTCCAGTCAAAAGCC	
gyrA	FWD	AATGTGCTGGGCAACGACTG	[Cy5]-CACCCTCATGGTGACAGTGCGGTTT-[BHQ2]
	REV	GAGCCAAAGTTACCTTGGCC	
Primers used to generate TEM substrates			
crtS substrate	MV577	GCCAATATCGCACGAATTCC	
	MV485	TCGCCCATTCACTTGATCCG	
ori2 *substrate*	MV109	AGGCGTAATGAACCTTGTGG	
	MV226	CACGCAGTGAGATCAGATTC	

Supplementary Table 5. X-ray data collection and processing

The $CC_{1/2}$ criterion was used to determine the resolution range. Values for the outer shell are given in parentheses.

Sample	**RctB^{IV}**
Diffraction source	Soleil PX1
Wavelength (Å)	0.9786
Temperature (K)	100.0
Detector	Eiger-X 16M
Crystal-detector distance (mm)	332.4
Rotation range per image (°)	0.1
Exposure time per image (s)	0.01
Space group	$P2_12_12$
a, *b*, *c* (Å)	64.9 94.8 47.6
α, β, γ (°)	90.0 90.0 90.0
Mosaicity (°)	0.2
Resolution range (Å)	53.55 – 2.34
Total Nº. of reflections	145328 (7004)
Nº. of unique reflections	11993 (611)
Completeness (%)	93.8 (95.6)
Redundancy	12.1 (11.5)
$\langle I/\sigma(I)\rangle$	10.6 (2.8)
$CC_{1/2}$	0.99 (0.89)
R_{pim}	0.064 (0.282)
Overall *B* factor / Wilson plot (Å^2)	28.9
R-factor (%)	19.2
R_{free}-factor (%)	26.7
Ramachandran profile (%)	
Core	96.3
Allowed	3.7
Outliers	0.0
R.m.s. deviations	
Bond lengths (Å)	0.023
Bond angles (º)	2.02
Number of atoms	2180
Macromolecules	1917
Solvent	214
Other	49
B-factors (Å2)	
All atoms	46.3
Macromolecules	46.7
Solvent atoms	42.0
Other atoms	49.7
PDB ID	

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