

## Supplemental material

Westbye *et al.* *The protease ClpXP and the PAS-domain protein DivL regulate CtrA and gene transfer agent production in Rhodobacter capsulatus.*

### Supplemental Figure Legends

#### Figure S1.

**A.** Effect of CtrA phosphorylation on the *ghsAB* head spike promoter.  $\beta$ -galactosidase activity of cells containing the chromosomally integrated *ghsAB* promoter-*lacZ* reporter (pABW848). WT strain SB1003, SB1003  $\Delta ctrA$  or mutant encoding CtrA(D51A) or CtrA(D51E) *in trans* on pD51A or pD51E, respectively. **B.** Effect of CtrA phosphorylation on lysis in cells lacking the histidine kinase CckA. Membrane-bound LH2 pigment in culture supernatant of strain SBpG (“WT”), the SBpG  $\Delta 280$  (RcGTA overproducer) and SBpG  $\Delta 280 \Delta cckA$  double mutant, and double mutant with plasmid encoding phosphomimetic CtrA(D51E) or non-phosphorylatable CtrA(D51A). Representative curves shown.

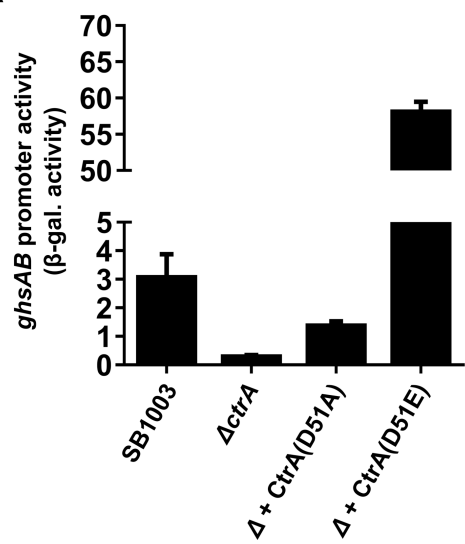
#### Figure S2.

**A.** Multiple sequence alignment showing conservation of the C-terminal part of the CtrA protein from selected Alphaproteobacteria. Box highlights the poorly conserved C-terminal tail. Alignment performed using entire CtrA sequence, residues 121 to end shown. **B.** Genetic organization of *clpP* and *clpX* in selected members of the Alphaproteobacteria (*Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Ruegeria pomeroyi*, *Brucella abortus*, *Sinorhizobium meliloti*, *Caulobacter crescentus*) and the gammaproteobacterium *Escherichia coli*. Gene name or apparent function of ORFs surrounding *clpP-clpX* based on annotations in NCBI are indicated: NDH (NADH dehydrogenase), RidA-F (encoding a RidA-Family protein), GlxA (GlxA-family transcription factor), HflX (GTPase HflX), Cls (lipid biosynthesis-related enzyme), *lon* (Lon protease), *tig* (trigger factor), *cicA* (haloacid dehalogenase-like hydrolase, essential for growth of *C. crescentus*). NCBI nucleotide accession number indicated. Drawn

approximately to scale. **C.** Transduction frequencies of cell-free culture supernatant. SB1003 or SB1003 *ΔctrA* cells encoding WT-CtrA (CtrA), the CtrA Asp-Asp mutant (CtrA-DD) or the CtrA lacking the three C-terminal residues (CtrA $\Delta$ 3) *in trans* on pLK754, pLK755 or pLK756, respectively. ND indicates not detected. Bars show frequencies from one transduction experiment (n=1).

## Supplemental figures

### A



### B

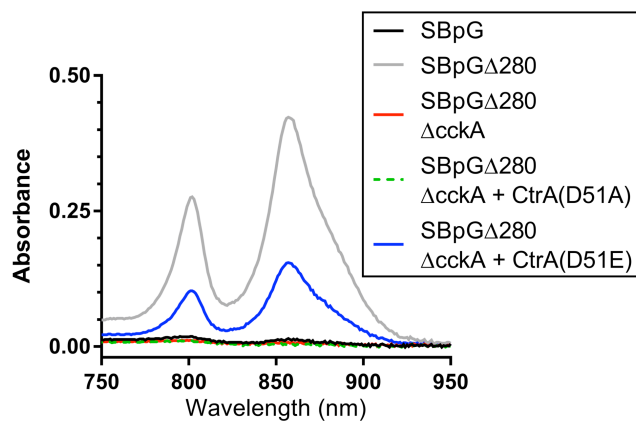


Figure S1.

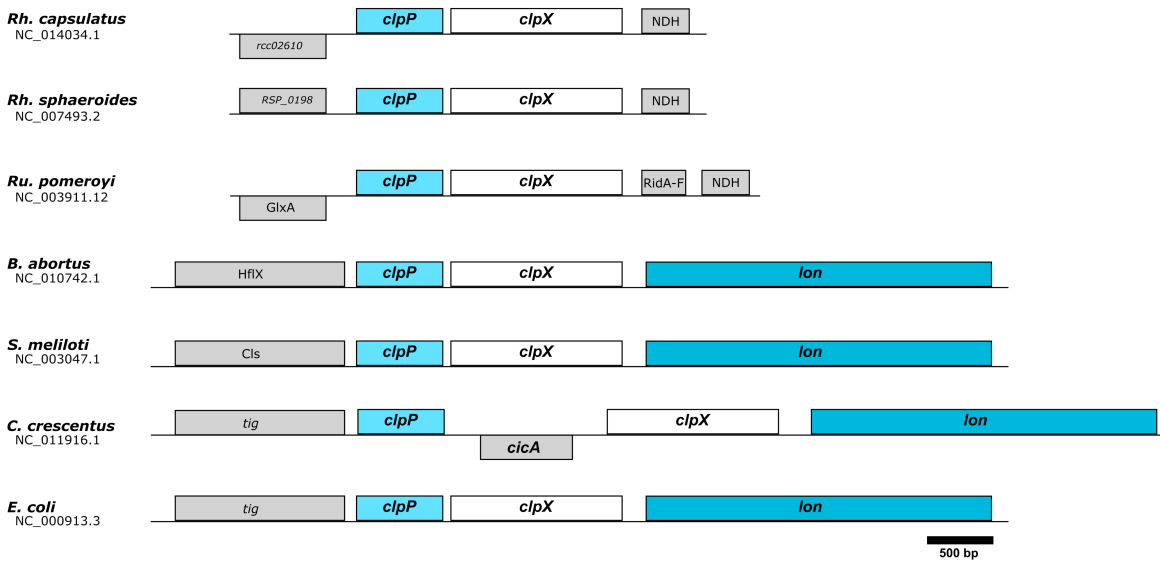
**A**

<i>Ro. nubinhibens</i> (WP_040618121.1)	121	GHSQSVITGKILVNLDAKTVEVEGKPVHLTGKEYQMLELLSLRKGTTLTKEMFLNHLYG
<i>Ru. pomeroyi</i> (WP_011047414.1)	121	GHSQSVITGKILVNLDAKTVEVEGKPVHLTGKEYQMLELLSLRKGTTLTKEMFLNHLYG
<i>Rh. capsulatus</i> (WP_013067387.1)	121	GHSQSVITGKILVNLDAKTVEVEGKPVHLTGKEYQMLELLSLRKGTTLTKEMFLNHLYG
<i>Rh. sphaeroides</i> (YP_352679.1)	121	GHAQSVITGKILVNLDAKTVEVEGKPVHLTGKEYQMLELLSLRKGTTLTKEMFLNHLYG
<i>C. crescentus</i> (YP_002518503.1)	121	GHAQSVITGKILVNLDAKTVEVEGKPVHLTGKEYQMLELLSLRKGTTLTKEMFLNHLYG
<i>S. meliloti</i> (NP_386824.1)	121	GHAQSVITGKILVNLDAKTVEVEGKPVHLTGKEYQMLELLSLRKGTTLTKEMFLNHLYG
<i>B. abortus</i> (WP_002964699.1)	121	GHAQSVITGKILVNLDAKTVEVEGKPVHLTGKEYQMLELLSLRKGTTLTKEMFLNHLYG
	121	** ** *.** . *****.* * *****

<i>Ro. nubinhibens</i> (WP_040618121.1)	181	GMDEPELKIIDVFICKLRKLSQAIGGDNHLETVWGRGYVLRDEESTQNDRDGLAIGA
<i>Ru. pomeroyi</i> (WP_011047414.1)	181	GMDEPELKIIDVFICKLRKLSNAIGGENYLETVWGRGYVLRDEQADGLPDPRIAVNA
<i>Rh. capsulatus</i> (WP_013067387.1)	181	GMDEPELKIIDVFICKLRKLSAEVIGGENYLETVWGRGYVLRDEQDGLDRR-MVVGGA
<i>Rh. sphaeroides</i> (YP_352679.1)	181	GMDEPELKIIDVFICKLRKLSAETGGESVLETVWGRGYVLRDEVTETDRR-FAIGA
<i>C. crescentus</i> (YP_002518503.1)	181	GMDEPELKIIDVFICKLRKLSAASAHCKHHLETVWGRGYVLRDENEQVNAA-----
<i>S. meliloti</i> (NP_386824.1)	181	GMDEPELKIIDVFICKLRKLSANAAAGANYLETVWGRGYVLRDEEGSDYLETA-----
<i>B. abortus</i> (WP_002964699.1)	181	GMDEPELKIIDVFICKLRKLSDAVSGNQSYLETVWGRGYVLRDEDAEMRESA-----
	181	***** .. *****.*

**B**



**C**

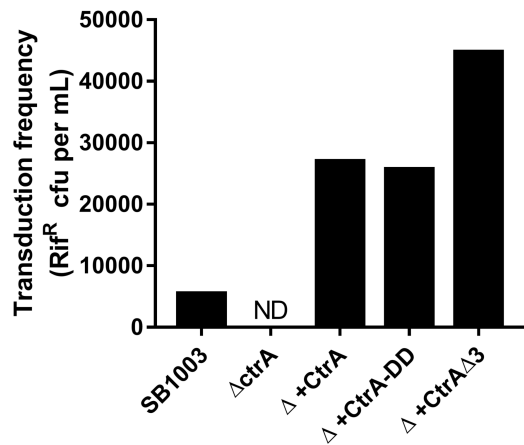


Figure S2.