Fig. S1. Cheng et al.

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CLUSTAL O(1.2.4) multiple sequence alignment


PgtA      MHWPLIWKKVQWLFWSYSKMKSTVLLIDDDQDVLDSYLHLMSIAGLTAKAVIDPTQAMSH  60
VC1522    ------MSPVLDLNQTHLYQAFSVLLVDDEVGMQLVLKKALSKWFSRVDLASSIEEAEVL  54
FlrC      ---------------MKMAQSKVLIVEDDEGLREALIDTLALAGYEWLEADCAEDALLK  44
LuxO      ------------------------MVEDTASVAALYRSYLTPLDIDINIVGTGRDAIES  35
NtrC      ---------------MSMSRGYVWVVDDDSSIRWVMEKTLSSAHIKCETFADAESVLLA  44
DctD-1    -------------------MCDVFLIDDESDIRIALAQSFELADLNAQFFASVEEALLA  40
DctD-2    ------------------MESQRIALIEDDDIVRQATGQWLQLAGFDVAMFADGASALAA  42
                           ::::*    :              :                 ..

                       Predicted phosphorylation site
PgtA      IQPD-WNGVVLLDMYMPQLHGMELLKQIKAVDDKIPVIVITGHGDIPMAVEAVKMGACDF 119
VC1522    RG-EHHYDLLILDINLPGRSGIEWEEAFTDPDHRADVIFMTGFADLETAISALKLGASDF 113
FlrC      LKSHS-VDIVVSDVQMAGMGGLALLRSIKQHWPNLPVLLMTAYANIQDAVSAMKDGAIDY 103
LuxO      IGRRE-PDLILLDLRLPDMTGMDVLYAVKEKSPDVPIVFMTAHGSIDTAVEAMRHGAQDF  94
NtrC      LERET-PDVLVSDIRMPGMDGIALLNQVHQRTPELPVIIMTAHSDLDAAVNAYQQGAFEY 103
DctD-1    IKAQGLPLVIVSDICLPGLSGQNLLSSVLHQDSELPVILITGHGDISMAVKAMHDGAYDF 100
DctD-2    IEQQSFA-AVVSDVRLPDTDGLSLLVAKNVQSLMPVILITGHGDVDMAVKALHQGAFDF 101
                   :: *:.:      *        .    ::.:*.....:  *:.:*  :  ** ::

PgtA      LEKPINPPQLLTLIKQHLELRRSFIEQ-----KLLLSRSVKRELVGKSAQMEQIRSHVAQ 174
VC1522    ILKPFNLEQMLQAVQRCMNKRLNERLQYALQR--DFQRHCTTQIIGSSVQTELLKQRIVQ 171
FlrC      MAKPFAPEVLLNMVSRYAPVKSD---------------DNGDAVVADTKSLK-LLALADK 147
LuxO      LIKPCEADRLRVTVNNAIRKASK------LKNDVDNKNQNYQGFIGSSQTMQAVYRTIDS 148
NtrC      LPKPFDVDETLTLVERAIAHGQEQRKTS----HRPSENYSAPEIIGEAPAMQEVFRAIGR 159
DctD-1    IEKPFAPERLIDTVHRAIEKRRLTLENRQLKRSLKVSQTLGPRIIGETAAIQTLRDTIAQ 160
DctD-2    IEKPFQPERLANTVAQAVSQYQNALSSQSRAHYLASAKGLEQILIGQCKSIQLLREQIAK 161
          : **         :        .:                    .:..      :  :

PgtA      YALLNRHVVIVGESGCGRHTIAYLIHQLTTNNGHLPWVELVA--------------SAKT 220
VC1522    FAPSRASVLIEGESGTGKELVARGIHEASGR--QGPFVPINCGAIAPELLESELFGHTSG 229
FlrC      VAKTDANVMILGPSGSGKEVMSRYIHNASPR-KEGPFIAINCAAIPDNMLEATLFGYEKG 206
LuxO      AASSKASIFITGESGTGKEVCAEAIHAASKR-GDKPFIAINCAAIPKDLIESELFGHVKG 207
NtrC      LSRSSISVLINGESGTGKEVAHALHRHSPR-AQKPFIALNMAAIPKDLIESELFGHEKG 218
DctD-1    VADTQADILLFGETGTGKELVARSLHEQSSR-REQNFVAINCGAVPENLIESELYGHEKG 219
DctD-2    VAAMDTNVIIYGETGCGKELVAQCLHQASER-QRGQFVAINCGAIPENLFESELFGHEAG 220
                   :     :.:   *  :*  *:.       :  :*   .       :: :

                 GAFTGA domain
PgtA      EW----STVEPKVQESAGGTLLIDHIELLSEEVQRNVVQSLLN-------QERLNRPRTR 269
VC1522    AFTGAKKSREGLFRVANGGTLFLDEIGEMPLTMQASLLRVLEQRAVRPVGSEKEVNVDVR 289
FlrC      AFTGAVQACPGKFEQAQGGTILLDEISEMDLNLQAKLLRVLQEREVERLGSRKSIKLDVR 266
LuxO      AFTGAATERQGAAEAADGGTLFLDELCEMDLDLQTKLLRFIQTGTFQKVGSSKMKSVDVR 267
NtrC      AFTGANTVRQGRFEQANGGTLFLDEIGDMPLDIQTRLLRVLADGQFYRVGGHVAIKVDVR 278
DctD-1    AFTGADSRRVGKFEHAQGGTLFLDEIESMPMQAQIRLLRVLQERVIERIGSNELIPLDIR 279
DctD-2    AFTGAVKRRIGKLELADKGTLFLDEIESMPLAMQVKVLRVLQDHVVERVGSNQPITIDLR 280
          :       . :  **:::*.: :      *    :::  :               *

PgtA      VIAVIDQPPEQLIAQNQLLPELYYLLNQGQIDVPLLRQRPDDIAALFHYFLKQSCYKLGK 329
VC1522    VVAATNRNLQQEVENGRFRGDLYYRLNVLKIEVSPLRERKQDLHELLPFFTKMLCAELGM 349
FlrC      VLATSNRDLKQYVQAGHFREDLYYRLNVFPLTWPALCERKDDIEPLANHLIERHCKKLGL 326
LuxO      FVCATNRDPWKEVQEGRFREDLYYRLYVIPLHLPPLRARGDDVIEIAYSLLGFMSKEEGK 327
NtrC      IVAATHQNLEKLVHQGKFREDLFHRLNVIRIHIPSLRERRQDIEKLTKHFLALAAKELGV 338
DctD-1    VIAATKIDLKQAAAEGKFRQDLYYRLNIVTLTIPPLRERREDIPALFHHFLLVAAARYGK 339
DctD-2    VIAAAKEELRQ---HAEFRQDLFYRLNVAQLYLPPLRERGDDILLLFEHFCVQVKPTWR- 336
          .:..   :      .:    :*:: *       *   * :*:              *

PgtA      PMPNVEPHYLALLRAHQWPGNVRELRNVAELYAIGIVKLAG-------KE---------- 372
VC1522    PMPKWAYEDISSMQDYDWPGNIRELKNLIERCLLLGKPPA----------HYWRELNGLP 399
FlrC      PVPSIAPNAITKLLNYPWPGNVRELDNVVQRALILSENGHIQSEHILLEGVDWHDASSLQ 386
LuxO      DFVRLSAEVVERFRQYEWPGNVRQLQNVLRNVVVLNEGREITLDMLPPPL---------- 377
NtrC      EMKTLNPRTVDILTKLDWPGNVRQLENMCRWLTVMASGSEV-LPSDLPSE---------- 387
DctD-1    AATALTASDVQSLLSHDWPGNVRELRNAAERYVLLGKLAQF-------AE---------- 382
DctD-2    ---SLSEADRLALLTYRWPGNVRELRNVALRYALDDSISVGEILASCPGM---------- 383
                       :  ****:*:* *                   :

PgtA      ------------------------------------RLYNQEEMLSPLDELVDDYEKQVIE 397
VC1522    IYAEPEVTMS--------QSNNSEI-------SLGDNVTQPSLGYPNDWPLSAVEKAHIQ 444
FlrC      QAVAGE-SMAAPQ--IKPVAEPE---VFKPL-VQGGGQNSSSQSSGLGGELRDQEFAIIL 439
LuxO      ---N---QMSAPINRALPLAHENKVSVH-----------------EIFPLWMTEKQAIE 413
NtrC      --LL---SER------KASHFDNDISWQKQLETWAKSALASGETELLAYALPEFERILLE 436
DctD-1    ----------------------------------SHEPKSKLLGLNEQVAEFEKSLLE 406
DctD-2    ----------------------------------TEEEASAGLPLAIQVHNFERKVIA 407
                                                       :    *     :

PgtA      DALFLFSGRVADAANYLQVPRKKLYLRMKKHGLEKDAFKARG- 439
VC1522    QIVALHEGNKSAAARDLGVARKTLERKYKEWESEDLSHES--- 484
FlrC      DTLAECQGRRKEMAEKLGISPRTLRYKLAKMRDAGIDIPG--- 479
LuxO      QAIEACDGNIPRAATYLDVSPSTIYRKLQTWNEKVKEKEKER- 455
NtrC      AALHHTNGHKQEAAKVLGWGRNTLTRKLKELY----------- 468
DctD-1    QTLIECGGSIKATMERLHLPRKTLYDKMQKYQLDKESYKP--- 446
DctD-2    QALHRHQGNISEVMKELDLPRRTLNQKMQKFGLLRGDFTAQEE 450
             :      *         *        .:     :
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Fig. S2. Cheng et al.

Fig. S3. Cheng et al.

CLUSTAL O(1.2.4) multiple sequence alignment

```
NtrC        MSMSRGYVWVVDDDSSIRWVMEKTLSSAHIKCETFADAESVLLALERETPDVLVSDIRMP 60
NtrC_Ec     --MQRGIVWVVDDDSSIRWVLERALAGAGLTCTTFENGAEVLEALASKTPDVLLSDIRMP 58
NtrC_Sm     --MQRGIVWVVDDDSSIRWVLERALAGAGLTCTTFENGNEVLAALASKTPDVLLSDIRMP 58
              *.** *************:*::*:.* :.* ** :. .** **  :*****:******

NtrC        GMDGIALLNQVHQRTPELPVIIMTAHSDLDAAVNAYQQGAFEYLPKPFDVDETLTLVERA 120
NtrC_Ec     GMDGLALLKQIKQRHPMLPVIIMTAHSDLDAAVSAYQQGAFDYLPKPFDIDEAVALVERA 118
NtrC_Sm     GMDGLALLKQIKQRHPMLPVIIMTAHSDLDAAVSAYQQGAFDYLPKPFDIDEAVALVERA 118
            ****:***:*::** * ****************.******:*******:**::.:*****

NtrC        IAHGQEQRKTSHRPSENYSAPEIIGEAPAMQEVFRAIGRLSRSSISVLINGESGTGKELV 180
NtrC_Ec     ISHYQEQQQPRNV-QLNGPTTDIIGEAPAMQDVFRIIGRLSRSSISVLINGESGTGKELV 177
NtrC_Sm     ISHYQEQQQPRNI-EVNGPTTDMIGEAPAMQDVFRIIGRLSRSSISVLINGESGTGKELV 177
            *:* ***::    : . * : ::*********:*** **********************

NtrC        AHALHRHSPRAQKPFIALNMAAIPKDLIESELFGHEKGAFTGANTVRQGRFEQANGGTLF 240
NtrC_Ec     AHALHRHSPRAKAPFIALNMAAIPKDLIESELFGHEKGAFTGANTIRQGRFEQADGGTLF 237
NtrC_Sm     AHALHRHSPRAKAPFIALNMAAIPKDLIESELFGHEKGAFTGANTIRQGRFEQADGGTLF 237
            ***********: ********************************:*******:*****

NtrC        LDEIGDMPLDIQTRLLRVLADGQFYRVGGHVAIKVDVRIVAATHQNLEKLVHQGKFREDL 300
NtrC_Ec     LDEIGDMPLDVQTRLLRVLADGQFYRVGGYAPVKVDVRIIAATHQNLEQRVQEGKFREDL 297
NtrC_Sm     LDEIGDMPLDVQTRLLRVLADGQFYRVGGYAPVKVDVRIIAATHQNLERRVQEGKFREDL 297
            **********:*****************:. :******:********: *::*******

NtrC        FHRLNVIRIHIPSLRERRQDIEKLTKHFLALAAKELGVEMKTLNPRTVDILTKLDWPGNV 360
NtrC_Ec     FHRLNVIRVHLPPLRERREDIPRLARHFLQVAARELGVEAKLLHPETEAALTRLAWPGNV 357
NtrC_Sm     FHRLNVIRIHLPPLRERREDIPRLARHFLQVAARELGVEAKLLHPETETALTRLAWPGNV 357
            ********:*:* *****:** :*::*** :**:***** * *:*.*    **:* *****

NtrC        RQLENMCRWLTVMASGSEVLPSDLPSELLSERKASH--FDNDISWQKQLETWAKSALASG 418
NtrC_Ec     RQLENTCRWLTVMAAGQEVLIQDLPGELFESTVAESTSQMQPDSWATLLAQWADRALRSG 417
NtrC_Sm     RQLENTCRWLTVMAAGQEVLIQDLPGELFEASTPDSPSHLPPDSWATLLAQWADRALRSG 417
            ***** *******:*.*** .***.**:.     .        ** . *  **. ** **

NtrC        ETELLAYALPEFERILLEAALHHTNGHKQEAAKVLGWGRNTLTRKLKELY-- 468
NtrC_Ec     HQNLLSEAQPELERTLLTTALRHTQGHKQEAARLLGWGRNTLTRKLKELGME 469
NtrC_Sm     HQNLLSEAQPELERTLLTTALRHTQGHKQEAARLLGWGRNTLTRKLKELGME 469
            . :**: * **:** ** :**:**:*******::***************
```

```
#  Percent Identity  Matrix - created by Clustal2.1
#
#

    1: NtrC        100.00   73.12   72.90
    2: NtrC_Ec      73.12  100.00   96.16
    3: NtrC_Sm      72.90   96.16  100.00
```
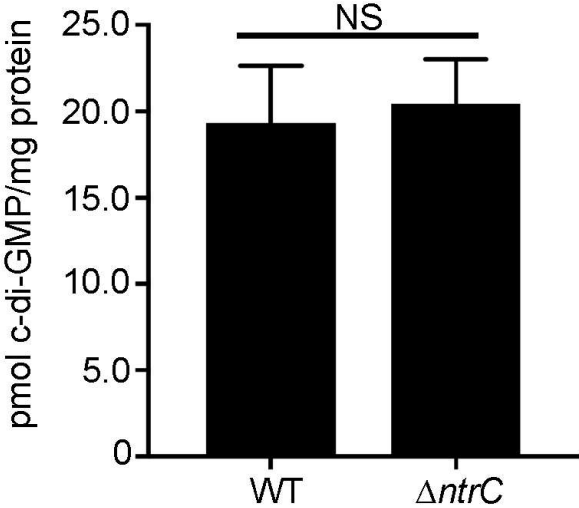
Figure S1. Multiple sequence alignment of *V. cholerae* NtrC family. Amino acid sequence alignment of *V. cholerae* NtrC family RR using Clustal Omega. The aspartate residue that is predicted to be the site of phosphorylation, and the GAFTGA region that is required for RpoN interaction are shown inside an empty box. The asterisk denotes identity, semicolon and colon denote conservative and semiconservative variations respectively.

Figure S2. Absence of NtrC has no significant impact on the c-di-GMP pool. C-di-GMP quantification was performed using Liquid Chromatography and Mass Spectrometry. Nucleotides were extracted from WT and Δ*ntrC* cultures grown to exponential phase. The graph represents the average and standard deviation of pmols of c-di-GMP/mg of total protein obtained from six independent biological samples. A two-tailed Mann Whitney test was used to compare the expression between WT and Δ*ntrC*. NS, not significant.

Figure S3. Multiple sequence alignment of NtrC from *V. cholerae*, *E. coli* and *S. typhimurium*. Amino acid sequence alignment of *V. cholerae* NtrC, *E. coli* NtrC (NtrC_Ec), and *S. typhimurium* NtrC (NtrC_Sm). The asterisk denotes identity, semicolon and colon denote conservative and semiconservative variations respectively. The percent identity matrix created with Clustal Omega is shown.