Supplementary Figures

Figure S1 Loss-of-function gdhZ mutants display growth defects suppressed by carbon sources that do not require GdhZ to be catabolized in vivo

(A-D) Wild-type, ΔgdhZ and gdhZK837A strains were grown in complex peptone yeast extract (PYE) medium (A), supplemented with alanine (B) or xylose (C), or in (D) synthetic medium with glucose as the only carbon source (M2G). (E-F) Wild-type, ΔgdhZ, gdhZ-gfp and ΔgdhZ P_{xylX::venus-gdhZ} strains were grown in complex peptone yeast extract (PYE) medium (E), supplemented with 0.05% xylose (F). Note that the leakage of the P_{xylX} in PYE without xylose allows sufficient expression of venus-gdhZ to partially complement the growth of ΔgdhZ. The doubling time of each strain cultivated in each medium are indicated in brackets.
Figure S2 ΔgdhZ displays a delay in the G2 phase of the cell cycle

Flow cytometry and fluorescence microscopy were used to monitor respectively DNA content and cell cycle regulated proteins (StpX, MipZ, and TipN) throughout the Caulobacter cell cycle (Figure 1). Wild-type (A) and ΔgdhZ (B) cells were synchronized and samples were withdrawn every 30 min and prepared for FACS analyses as described in the Methods. The wild-type (A) and ΔgdhZ (B) strains expressing stpX-gfp (RH507, RH799), mipZ-cfp (RH522, RH836) and tipN-gfp (RH506, RH752) were synchronized and imaged every 30 minutes by fluorescence microscopy. StpX-GFP is a marker for the swarmer-to-stalked transition, since it is detected at the pole when the stalk synthesis is initiated (Hughes et al, 2010; Hughes et al, 2013). MipZ-CFP is a G1-to-S transition marker since the single MipZ-CFP focus observed in the G1/swarmer cells is duplicated upon DNA replication initiation (Thanbichler & Shapiro 2006). TipN-GFP is a late cell division protein used to measure the time spent in G2 phase (Huitema et al, 2006; Lam et al, 2006).

Both strains (wild-type and ΔgdhZ) have a similar pattern for all the cell cycle regulated proteins except for TipN-GFP, which remains much monger at the midcell in late predivisinal cells of ΔgdhZ than in wild-type cells. Indeed, the G1-to-S and swarmer-to-stalked transitions occur concomitantly in wild-type and ΔgdhZ cells (StpX-GFP polar accumulation, MipZ-CFP duplication). However, ΔgdhZ cells spend a much longer time in G2, delaying constriction (2n DNA content and midcell TipN localization). Scale bar, 5 μm.
**Figure S3** Inactivation of *gdhZ* leads to a severe growth defect that is not mitigated by constitutive expression of *ftsZ*

(A) Constitutive expression of *ftsZ-yfp* suppresses Δ*gdhZ* cell division defect. The wild-type and Δ*gdhZ* strains bearing an additional copy of *ftsZ* fused to *yfp* at the *vanA* locus (P*vanA::ftsZ-yfp*) were grown in PYE and *ftsZ-yfp* expression was induced by addition of 0.5 mM vanillate. Cell size distribution was plotted at different timepoints post-induction for (right) wild-type P*vanA::ftsZ-yfp* (RH53) and (left) Δ*gdhZ* P*vanA::ftsZ-yfp* (RH853).

(B) Growth curves of wild-type P*vanA::ftsZ-yfp* (RH53) and Δ*gdhZ* P*vanA::ftsZ-yfp* (RH853). Constitutive expression of *ftsZ-yfp* does not affect growth of wild-type and Δ*gdhZ* strains. Cells were grown in PYE supplemented with 0.5 mM of vanillate.
A

B

C

D

mcherry-fzlC  gdhZ-venus P_{XylX::ftsZ}

3 hrs xylose depletion

GdhZ-GFP

rel. fluo. intensity

rel. cell position

mcherry-fzlC merge
gdhZ-gfp
gdhZ-gfp mcherry-fzlC
**Figure S4** GdhZ midcell localization relies on the presence of FtsZ at the division site.

(A) Colocalization of GdhZ-GFP with mCherry-FzIC, determined by phase contrast and fluorescence microscopy of *gdhZ-gfp* P*xyIX::mcherry-fzIC* (RH1866). The expression of *mcherry-fzIC* was induced by adding 0.1 % xylose to the cultures 2 hrs before imaging. Scale bar 5 µm.

(B) Colocalization of GdhZ-Venus with mCherry-FzIC in cells depleted for FtsZ, determined by taking phase contrast and fluorescence micrographs of Δ*xylX gdhZ-venus* P*vanA::mcherry-fzIC* P*xyIX::ftsZ* (RH1897) cells. Cells were cultivated in PYE supplemented with 0.5 mM vanillate and 0.1% xylose to mid-exponential phase then washed and resuspended in PYE with 0.5 mM vanillate but without xylose.

(C) Immunoblots of FtsZ and MreB levels in *ftsZ* depletion strain. Δ*xylX gdhZ-venus* P*xyIX::ftsZ* (RH993) cells were grown in PYE supplemented with 0.1% xylose to mid-exponential phase, then washed and resuspended in PYE without xylose.

(D) Fluorescence intensity profile along cell length of *ftsZ* depletion strain. Δ*xylX gdhZ-venus* P*xyIX::ftsZ* (RH993) cells were grown in PYE supplemented with 0.1% xylose to mid-exponential phase, then washed and resuspended in PYE without xylose for 3 hours prior imaging and analysis. “0” and “1” correspond to the poles and “0.5” to the midcell position.
**Figure S5** Kinetics parameters for the oxidative deamination of glutamate catalysed by GdhZ

(A) Oxidative deamination activity of glutamate by GdhZ, determined as a function of NAD$^+$ concentration, displays a classical Michaelis-Menten behaviour. $K_m$ (38 µM) and $V_{max}$ (83 mM) values were obtained by a double-reciprocal plot of NAD$^+$ data (data not shown).

(B) Hill plot obtained by transforming the sigmoidal glutamate saturation curve. The $S_{0.5}$ (42.5 mM) and Hill coefficient (1.6) was calculated, the latter value indicating positive cooperativity in glutamate binding.
Figure S6 Active GdhZ stimulates GTPase activity and protofilaments disassembly of FtsZ and FtsZ_{E79K}

(A) Stimulation of FtsZ’s GTPase activity with GdhZ in the presence of only one of its substrates. Phosphate inorganic (P_i) release over time by FtsZ (0.5 μM) incubated with 1 mM GTP, 0.5 μM GdhZ in the absence or presence of 100 mM glutamate, or 5 mM NAD^+, or both 100 mM glutamate and or 5 mM NAD^+. The rates of GTPase (Pi molecules released per FtsZ per min) are indicated for each condition.
(B) GdhA from bovine liver does not modulate FtsZ’s GTPase activity. Phosphate inorganic \( (P_i) \) release over time by FtsZ (0.5 \( \mu \)M) incubated with 1 mM GTP, 5 mM \( \text{NAD}^+ \), 100 mM glutamate and 0, 0.5 or 1 \( \mu \)M GdhA (bovine liver glutamate dehydrogenase, Sigma) respectively, showing that GdhA does not activate the GTPase activity of FtsZ. The number of \( P_i \) molecules per FtsZ per minute for each condition is indicated in brackets.

(C) FtsZ\(_{E79K}\) is sensitive to active GdhZ. Phosphate inorganic \( (P_i) \) release over time by FtsZ\(_{E79K}\) (0.5 \( \mu \)M) incubated with 1 mM GTP, 5 mM \( \text{NAD}^+ \), 100 mM glutamate and 0, 0.5 or 1 \( \mu \)M GdhZ respectively, showing that FtsZ\(_{E79K}\) is sensitive to active GdhZ. The number of \( P_i \) molecules per FtsZ per minute for each condition is indicated in brackets.

(D) GdhZ\(_{K837A}\) does not modulate FtsZ’s GTPase activity. Phosphate inorganic \( (P_i) \) release over time by FtsZ (0.5 \( \mu \)M) incubated with 1 mM GTP, 5 mM \( \text{NAD}^+ \), 100 mM glutamate and 0, 0.5 or 1 \( \mu \)M GdhZ\(_{K837A}\) respectively, showing that GdhZ catalytic activity is required for stimulating the GTPase activity of FtsZ. The number of \( P_i \) molecules per FtsZ per minute for each condition is indicated in brackets.

(E) Neither GdhA from bovine liver, nor GdhZ\(_{K837A}\) does modulate FtsZ’s higher order structures. Negative stain electron microscopy of FtsZ (0.5 \( \mu \)M) incubated with 1 mM GTP, 5 mM \( \text{NAD}^+ \) and 100 mM glutamate without GdhZ\(_{K837A}/\text{GdhA (i)}\), or with 0.5 \( \mu \)M GdhA (ii) or 0.5 \( \mu \)M GdhZ\(_{K837A}\) (iii), showing that neither GdhZ\(_{K837A}\) nor GdhA can stimulate the GTPase activity of FtsZ. Scale bar, 200 nm.

(F) FtsZ\(_{E79K}\)’s higher order structures is disassembled by active GdhZ. Negative stain electron microscopy of FtsZ\(_{E79K}\) (0.5 \( \mu \)M) with 1 mM GTP, 5 mM \( \text{NAD}^+ \), and 100 mM glutamate without GdhZ (i, ii), or with 0.5 \( \mu \)M GdhZ (iii), showing that FtsZ\(_{E79K}\) is sensitive to active GdhZ. Note that \textit{in vitro}, FtsZ\(_{E79K}\) forms longer protofilaments and bundles than wild-type FtsZ. Scale bar, 200 nm for (i, ii) and 100 nm (iii).
A catalytically inactive mutant of GdhZ (GdhZ_{K837A}) phenocopies ΔgdhZ cell division defects

(A) GdhZ_{K837A} is a stable protein that does not display any catalytic activity. The activity of GdhZ (Blue line) and GdhZ_{K837A} (Red line) was monitored by measuring at 340 nm (oxydative deamination) the production of NADH + H^+ over time in a reaction mixture containing 100 mM HK_2PO_4/H_2NaPO_4 (pH 7.2), 5 mM NAD^+, 100 mM glutamate and 5 µg of enzymes. Experiments were started by the addition of NAD^+ to the mixture and were performed at 30 °C. Immunoblot of GdhZ and GdhZ_{K837A} steady state levels in wild-type, ΔgdhZ and gdhZ_{K837A} cell lysates.

(B) Cell size distribution of wild-type (RH50) and gdhZ_{K837A} (RH938) strains grown in complex PYE media. The cell length was measured by using microbeTracker software (Sliusarenko et al, 2011). The mean cell size ± standard deviation (in µm) of the different strains is indicated in brackets.

(C) Phase contrast imaging of wild-type (RH50), gdhZ_{K837A} (RH938), ftsZ_{E79K} (NR6102) and ftsZ_{E79K}gdhZ_{K837A} (RH957), showing that the combination of ftsZ_{E79K} with gdhZ_{K837A} leads to a conspicuous filamentation. Wild-type and ftsZ_{E79K} images were the same than the ones presented in Figure 2B. Scale bar, 5 µm.

(D) Localization of GFP-FzlC determined by phase contrast and fluorescence microscopy of wild-type (RH1733) and gdhZ_{K837A} (RH1906) cells expressing gfp-fzlC.
from $P_{xy/\lambda}$ at the $xylX$ locus. Expression of $gfp-fzIC$ was induced by adding 0.1% xylose to the cultures 1 hr before imaging. Scale bar, 5 $\mu$m.

(E) Co-immunoprecipitation (Co-IP) experiment performed on protein extracts of wild-type (RH50), 3FLAG-gdhZ (RH743) and 3FLAG-gdhZ$_{K837A}$ (RH940) strains showing that GdhZ can, independently of its catalytic activity, pull down FtsZ. GdhZ/GdhZ$_{K837A}$ and FtsZ were detected by western blot using respectively anti-GdhZ and anti-FtsZ antibodies before (IN) and after (IP) immunoprecipitation with anti-FLAG antibodies.
**Figure S8** Cell cycle dependent proteolysis of GdhZ by ClpXP

(A) Quantification of cellular 3FLAG-GdhZ, FtsZ and CtrA levels during the cell cycle of the wild-type strain, as determined by immunoblots shown in Figure 5a. Data were normalized to the maximal level (100%) of each protein and quantified using ImageJ software.

(B) Cell cycle abundance of 3FLAG-GdhZ\textsubscript{AA::DD} in a synchronized population of \textit{gdhZ\textsubscript{AA::DD}} (RH1122) strain, showing that the substitution of the last two alanine residues by two glutamate residues abolishes the cell cycle oscillation of the protein.

(C) Cell cycle abundance of 3FLAG-GdhZ in synchronized populations of \textit{ΔpopA} (RH828), \textit{ΔcpdR} (RH829) and \textit{ΔrcdA} (RH827) strains, showing that PopA, CpdR and RcdA are required for GdhZ cell cycle oscillation.
Figure S9 CtrA induces the transcription of P<sub>gdhZ</sub>

(A) The activity of the response regulator CtrA is controlled throughout the cell cycle by its temporal degradation and phosphorylation (reviewed in (Curtis & Brun 2010). Both regulations rely on another response regulator DivK, whose activity is mainly controlled by two antagonist proteins, the histidine kinase DivJ and the phosphatase PleC. Phosphorylation of DivK triggers a cascade leading to the inactivation of CtrA~P by dephosphorylation and ClpXP-dependent proteolytic degradation. On the contrary, dephosphorylation of DivK leads to stabilization of CtrA~P which, in turn, activates the transcription of >100 genes (Fumeaux <i>et al</i>, 2014).

(B) β–galactosidase assay using the P<sub>gdhZ</sub>–<i>lacZ</i> fusion to determine the activity of <i>gdhZ</i> promoter in various mutants affecting CtrA activity. Wild-type (RH1332), ∆<i>pleC</i> (RH1711), ∆<i>divJ</i> (RH1712) strains harbouring P<sub>gdhZ</sub>–<i>lacZ</i> fusion were grown at 30 °C in PYE to mid-exponential phase. Wild-type (RH1332) and div<sup>KCS</sup> (RH1713) harbouring P<sub>gdhZ</sub>–<i>lacZ</i> fusion were first grown at permissive temperature (30 °C) in PYE to mid-exponential phase, before being shifted for 3 hrs at the restrictive temperature (20 °C). The relative β–galactosidase activity was normalized to the activity of P<sub>gdhZ</sub>–<i>lacZ</i> fusion in the wild-type strain grown at 30 °C (100%). Error bars = SD, n = 3.
(C) Steady-state levels of GdhZ in $divK^{CS}$ (RH316) and $ctrA^{TS}$ (RH212) cells. Accordingly to the model summarized in Figure S8a, transcription of CtrA-activated genes increases in $divK^{CS}$ and decreases in $ctrA^{TS}$ cells, after a shift at the restrictive temperature (20 °C and 32 °C, respectively). MreB, which is not under the control of CtrA, was used as a loading control. Cryo-sensitive and thermo-sensitive strains were first grown at permissive temperature as indicated and shifted for 3 hours at restrictive temperature. The relative steady-state levels of GdhZ was normalized to wild-type strain grown at permissive temperature 32 °C for $divK^{CS}$ and 25 °C for $ctrA^{TS}$ (100%). The dotted line indicates that the bands depicted were not contiguous in the original blot.
Figure S10 GdhZ and KidO act in synergy to coordinate Z-ring disassembly

(A) Cell size distribution of ΔxylX ΔkidO gdhZ-gfp (RH1904) and ΔxylX ΔkidO gdhZ-gfp PxyL::kidOAA::DD (RH1905) strains showing that GdhZ and KidO work in synergy to block cell division. Note that the expression of kidOAA::DD was not induced with xylose, since the leakage of PxyL was sufficient to induce strong filamentation. Cell length was measured by using microbeTracker software (Slusarenko et al, 2011).

(B) Morphology of ΔxylX (RH984), ΔxylX gdhZ-gfp (RH962), ΔxylX ΔkidO PxyL::kidOAA::DD (RH1509) and ΔxylX ΔkidO gdhZ-gfp PxyL::kidOAA::DD (RH1905) cells, showing that GdhZ and KidO work in synergy to block cell division. For the reasons mentioned above, the expression of kidOAA::DD was not induced with xylose. Scale bar, 5 µm.

(C) Co-immunoprecipitation (Co-IP) experiments performed on protein extracts of ΔxylX ΔkidO PxyL::kidO-mCherry (RH1502) and ΔxylX ΔkidO PxyL::kidO-mCherry 3FLAG-gdhZ (RH1324) strains, showing that GdhZ pull down KidO. The expression of kidO-mCherry was induced with 0.05 % xylose for 1hr. GdhZ and KidO-mCherry were detected by western blotting using respectively anti-GdhZ and anti-mCherry antibodies before (IN) and after (IP) immunoprecipitation with anti-FLAG antibodies.
## Supplementary Tables

### Table S1 Oligonucleotides used in this study

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<td>pH650</td>
<td>pVCHYN-5-fzlC</td>
<td>This study</td>
</tr>
<tr>
<td>Name</td>
<td>Description and relevant genotype</td>
<td>Reference</td>
</tr>
<tr>
<td>------------</td>
<td>----------------------------------------------------------------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>RH10</td>
<td>S17-1 ((F- λ- endA thi pro recA hsdR2 (r-m+) RP4-2-Tet::Mu-Km::Tn7)</td>
<td>(Simon R., 1983)</td>
</tr>
<tr>
<td>RH319</td>
<td>MT607 (pro-B2 thi-I hsdR17 (r-m+) supE44 recA56)</td>
<td>(Casadaban &amp; Cohen 1980)</td>
</tr>
<tr>
<td>RH355</td>
<td>Y2HGold MATa trp1-901 leu2-3 112 ura3-52 his3-200 gal4Δ gal80Δ LYS2 : : GAL1UAS–Gal1TATA–HIS3</td>
<td>Clontech</td>
</tr>
<tr>
<td>RH356</td>
<td>Y2HGold MATa trp1-901 leu2-3 112 ura3-52 his3-200 gal4Δ gal80Δ LYS2 : : GAL1UAS–Gal1TATA–HIS3</td>
<td>Clontech</td>
</tr>
<tr>
<td>RH603</td>
<td>(F- ompT gal dcm lon hsdSB (r-m-) λ (DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])</td>
<td>Novagen</td>
</tr>
<tr>
<td>RH783</td>
<td>Top10 (φ80lacZΔM15 araD139 Δ(ara-leu)7697 galE15 galK16 Δ (lac)X74 rpsL(StrR) nupG recA1 endA1 mcrA Δ (mrr-hsdRMS-mcrBC)</td>
<td>Life Technologies</td>
</tr>
<tr>
<td>RH50</td>
<td>NA1000</td>
<td>(Evinger &amp; Agabian 1977)</td>
</tr>
<tr>
<td>RH53</td>
<td>NA1000 P_vanA :ftsZ-yfp</td>
<td>(Thanbichler &amp; Shapiro 2006)</td>
</tr>
<tr>
<td>RH203</td>
<td>NA1000 ΔdivJ</td>
<td>(Wheeler &amp; Shapiro 1999)</td>
</tr>
<tr>
<td>RH212</td>
<td>NA1000 ctrA401</td>
<td>(Quon et al, 1996)</td>
</tr>
<tr>
<td>RH217</td>
<td>NA1000 ΔpleC</td>
<td>(Aldridge et al, 2003)</td>
</tr>
<tr>
<td>RH315</td>
<td>NA1000 ΔpopA</td>
<td>(Duerig et al, 2009)</td>
</tr>
<tr>
<td>RH316</td>
<td>NA1000 divK341</td>
<td>(Hung &amp; Shapiro 2002)</td>
</tr>
<tr>
<td>RH323</td>
<td>NA1000 ΔrcdA::hyg</td>
<td>(McGrath et al, 2006)</td>
</tr>
<tr>
<td>RH339</td>
<td>NA1000 ΔacpdR</td>
<td>(Skerker et al, 2005)</td>
</tr>
<tr>
<td>RH506</td>
<td>NA1000 tipN::tipN-gfp</td>
<td>(Huitema et al, 2006)</td>
</tr>
<tr>
<td>RH507</td>
<td>NA1000 stpX::stpX-gfp</td>
<td>(Hughes et al, 2010)</td>
</tr>
<tr>
<td>RH521</td>
<td>NA1000 ΔtipN</td>
<td>(Lam et al, 2006)</td>
</tr>
<tr>
<td>RH534</td>
<td>NA1000 ΔgdhZ</td>
<td>This study</td>
</tr>
<tr>
<td>RH602</td>
<td>NA1000 pGFPC-2-gdhZ</td>
<td>This study</td>
</tr>
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<td>RH728</td>
<td>NA1000 pMCS-2-gdhZ-3FLAG</td>
<td>This study</td>
</tr>
<tr>
<td>RH740</td>
<td>NA1000 ΔgdhZ P_xyl::venus-gdhZ</td>
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</tr>
<tr>
<td>RH743</td>
<td>NA1000 3FLAG- gdhZ</td>
<td>This study</td>
</tr>
<tr>
<td>RH752</td>
<td>NA1000 ΔgdhZ tipN::tipN-gfp</td>
<td>This study</td>
</tr>
<tr>
<td>RH799</td>
<td>NA1000 ΔgdhZ stpX::stpX-gfp</td>
<td>This study</td>
</tr>
<tr>
<td>RH827</td>
<td>NA1000 ΔrcdA::hyg 3FLAG-gdhZ</td>
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<td>RH828</td>
<td>NA1000 ΔpopA 3FLAG-gdhZ</td>
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<td>RH829</td>
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<tr>
<td>RH830</td>
<td>NA1000 cpdR051A 3FLAG-gdhZ</td>
<td>This study</td>
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<tr>
<td>RH836</td>
<td>NA1000 ΔgdhZ mipZ-mcfp</td>
<td>This study</td>
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RH853  NA1000 ΔgdhZ P_{vanA}::ftsZ::yfp
RH903  NA1000 ΔkidO
RH904  NA1000 ΔgdhZ ΔkidO
RH938  NA1000 gdhZ_{K837A}
RH940  NA1000 3FLAG-gdhZ_{K837A}
RH957  NA1000 ftsZ_{E79K} ΔxylX gdhZ_{K837A}
RH958  NA1000 gdhZ_{K837A} ΔxylX
RH967  NA1000 ΔxylX pGFPC-2-gdhZpXCHYC-5-ftsZ
RH975  NA1000 ΔclpP::Ω P_{xylo}::clpP
RH984  NA1000 ΔxylX
RH991  NA1000 3FLAG-gdhZ P_{xylo}::clpP
RH992  NA1000 ΔxylX pVENC-1-gdhZ
RH993  NA1000 ΔxylX pVENC-1-CCNA_00086 P_{xylo}::ftsZ
RH1117  NA1000 ftsZ_{E79K} (NR6102)
RH1122  NA1000 3FLAG-gdhZAA::DD
RH1144  NA1000 ΔgdhZ P_{vanA}::kidO-mcherry
RH1256  NA1000 ΔxylX ΔgdhZ
RH1312  NA1000 ΔxylX pXGFPN2-mreB
RH1313  NA1000 ΔxylX ΔgdhZ pXGFPN2-mreB
RH1324  NA1000 3FLAG-gdhZ ΔxylX ΔkidO pXCHYC-5-kidO
RH1329  NA1000 ftsZ_{E79K} ΔgdhZ
RH1332  NA1000 P_{CCNA_00086}::lacZ
RH1494  NA1000 ftsZ_{E79K} ΔxylX
RH1495  NA1000 ΔkidO ΔxylX
RH1496  NA1000 ΔgdhZ ΔkidO ΔxylX
RH1503  NA1000 ΔgdhZ ΔkidO ΔxylX pXCHYC-5-kidO
RH1508  NA1000 ΔkidO ΔxylX pXC-5-kidO
RH1509  NA1000 ΔkidO ΔxylX pXC-5-kidOAA::DD
RH1510  NA1000 ΔgdhZ ΔkidO ΔxylX pXC-5-kidO
RH1511  NA1000 ΔgdhZ ΔkidO ΔxylX pXC-5-kidOAA::DD
RH1564  NA1000 3FLAG-gdhZ ΔxylX
RH1566  NA1000 3FLAG-gdhZ ΔxylX ΔkidO
RH1671  NA1000 ΔsocAB
RH1672  NA1000 3FLAG-gdhZ ΔsocAB
RH1675  NA1000 3FLAG-gdhZ ΔsocAB ΔclpP::Ω
| RH1711 | NA1000 P<sub(CCNA_00086-lacZ ΔpleC</sub> | This study |
| RH1712 | NA1000 P<sub(CCNA_00086-lacZ ΔdivJ</sub> | This study |
| RH1713 | NA1000 P<sub(CCNA_00086-lacZ ΔK<sub>341</sub></sub> | This study |
| RH1733 | NA1000 ΔxylX pXGFPN-2-ftsZ/C | This study |
| RH1734 | NA1000 ΔxylX ΔgdhZ pXGFPN2-ftsZ/C | This study |
| RH1834 | NA1000 ΔxylX pXCHYC-5-ftsZ | This study |
| RH1834 | NA1000 ΔxylX pXCHYC-5-ftsZ | This study |
| RH1868 | NA1000 ΔxylX ΔgdhZ pXCHYC-5-ftsZ | This study |
| RH1897 | NA1000 ΔxylX gdhZ-venus P<sub>vanA::mcherry-ftsZ/C</sub> P<sub>xylX::ftsZ</sub> | This study |
| RH1904 | NA1000 ΔkidO ΔxylX pGFPC-2-gdhZ | This study |
| RH1905 | NA1000 ΔxylX ΔkidO gdhZ-gfp P<sub>xylX::kidOAA::DD</sub> | This study |
| RH1906 | NA1000 ΔxylX gdhZ<sub>K837A</sub> pXGFPN2-ftsZ/C | This study |
Supplementary Material and Methods

Plasmids construction

**pHR275** (pXGFPN-2-*mreB*)

*C. crescentus mreB* was amplified from NA1000 gDNA by PCR with primers 64 and 65. The PCR product was then digested with *Asp* 718I and *Eco* RI and ligated into the pXGFPN-2 vector cut with the same restriction enzymes.

**pHR284** (pGBKT7-*ftsZ_{1-324}*)

*C. crescentus ftsZ_{1-324}* was amplified from NA1000 gDNA by PCR with primers 72 and 80. The PCR product was then digested with *Nco* I and *Eco* RI and ligated into the pGBKT7 cut with the same restriction enzymes.

**pHR312** (pNPTS138-Δ*gdhZ*)

Upstream and downstream regions of *C. crescentus CCNA_00086* were amplified from NA1000 gDNA by PCR respectively with primers 116/117 and 118/119. The PCR products were then digested respectively with *Hind* III/*Eco* RI and *Eco* RI/Bam HI; and ligated into the pNPTS138 vector cut with *Hind* III and Bam HI.

**pHR315** (pVENC-1-*gdhZ*)

The 3' end of *CCNA_00086* was amplified from NA1000 gDNA by PCR with primers 125 and 126. The PCR product was then digested with *Asp* 718I and *Eco* RI and ligated into the pVENC-1 vector cut with the same restriction enzymes.

**pHR328** (pGFPC-2-*gdhZ*)

The 3' end of *CCNA_00086* was amplified from NA1000 gDNA by PCR with primers 125 and 126. The PCR product was then digested with *Asp* 718I and *Eco* RI and ligated into the pGFPC-2 vector cut with the same restriction enzymes.

**pHR338** (pXVENN-1-*gdhZ*)

*gdhZ* was amplified from NA1000 gDNA by PCR with primers 188 and 189. The PCR product was then digested with *Asp* 718I and *Eco* RI and ligated into the pXVENN-1 vector cut with the same restriction enzymes.

**pHR348** (pNPTS138-*gdhZ_{A1606D,A1607D}* )

DNA fragment of *C. crescentus CCNA_00086* encompassing mutations A1606D and A1607D was synthesized as a gBlock (IDT), amplified by PCR with primers 473/474 and cloned into pNPTS138 cut with *Eco* RV.

**pHR354** (pET-21b-*gdhZ*)
CCNA_00086 was amplified from NA1000 gDNA by PCR with primers 186 and 189.
The PCR product was first cloned into TOPO-XL. TOPO-XL-gdhZ was then digested
with Nde I and Eco RI and ligated into the pET-21b vector cut with the same
restriction enzymes.

pHR371 (pNPTS138-3FLAG-gdhZ)
Upstream and 5’ regions of C. crescentus CCNA_00086 were amplified from NA1000
gDNA by PCR respectively with primers 266/267 and 270/269. The PCR products
were then digested respectively with Bam HI/Nde I and Nde I/Hind III; and ligated into
the pNPTS138 vector cut with Bam HI and Hind III.

pHR429 (pMCS-2-gdhZ-3FLAG)
The 3’ end of CCNA_00086 was amplified from NA1000 gDNA by PCR with primers
125 and 218. The PCR product was then digested with Asp 718I and Eco RI and
ligated into the pMCS-1 vector cut with the same restriction enzymes.

pHR501 (pMCS-5-lacZ)
lacZ was cut off from pSKoriTΩlacZ digested with Asp 718I/Bam HI and ligated into
pMCS-5 cut with Asp 718I/Bgl II.

pHR516 (pNPTS138-ΔxylX)
Upstream and downstream regions of C. crescentus CC0823 were amplified from
NA1000 gDNA by PCR respectively with primers 707/708 and 709/710 and cloned
into pSK. The pSK-707/708 and pSK-709/710 recombinant plasmids were then
digested respectively with Hind III/Eco RI and Eco RI/Bam HI; and ligated into the
pHR253 (pNPTS138) vector cut with Hind III and Bam HI.

pHR529 (pMCS-5-PgdhZ-lacZ)
PgdhZ was amplified from NA1000 gDNA by PCR with primers 732 and 733 and
cloned into pSK. The pSK-732/733 recombinant plasmid was then digested with Hind
III and Asp 718I and ligated into the pMCS-5-lacZ vector cut with the same restriction
enzymes.

pHR547 (pNPTS138-gdhZK837A)
DNA fragment of C. crescentus CCNA_00086 encompassing catalytic site mutation
K837A was synthesized as a gBlock (ITD), amplified by PCR with primers 746/747
and cloned into pNPTS138 cut with Eco RV.

pHR559 (pXCHYC-5-ftsZ)
C. crescentus ftsZ was amplified from NA1000 gDNA by PCR with primers 129 and 175 and cloned into pSK. The pSK-129/175 recombinant plasmid was then digested with Nde I and Eco RI and ligated into the pXCHYC-5 vector cut with the same restriction enzymes.

pHR561 (pXCHYC-5-kidO)
C. crescentus kidO was amplified from NA1000 gDNA by PCR with primers 773 and 391 and cloned into pSK. The pSK-129/175 recombinant plasmid was then digested with Nde I and Eco RI and ligated into the pXCHYC-5 vector cut with the same restriction enzymes.

pHR564 (pXC-5-kidOAA::DD)
C. crescentus kidOAA::DD was amplified from NA1000 gDNA by PCR with primers 773 and 775 and cloned into pSK. The pSK-773/775 recombinant plasmid was then digested with Nde I and Asp 718I and ligated into the pHR566 (pXC-5) vector cut with the same restriction enzymes.

pHR566 (pXC-5).
pXGFPC-5 cut with Eco RI/Nhe I (to cut out egfp), blunted with Klenow and self-ligated.

pHR569 (pXC-5-kidO)
C. crescentus kidO was amplified from NA1000 gDNA by PCR with primers 773 and 774 and cloned into pSK. The pSK-773/774 recombinant plasmid was then digested with Nde I and Asp 718I and ligated into the pHR566 (pXC-5) vector cut with the same restriction enzymes.

pHR573 (pET-21b-gdhZK837A)
gdhZK837A was amplified from RH938 (NA1000 gdhZK837A) gDNA by PCR with primers 186 and 189 and cloned into pSK. The pSK-186/189 recombinant plasmid was then digested with Nde I and Eco RI and ligated into the pET-21b vector cut with the same restriction enzymes.

pHR575 (pET-21b-ftsZE79K*)
ftsZE79K was amplified from NA1000 ftsZE79K gDNA by PCR with primers 129/69 and cloned into pSK. The pSK-129/69 was then digested respectively with Nde I and Eco RI, and ligated into the pET-21b vector cut with the same restriction enzymes.

pHR602 (pXGFPN-2-fzlC)
C. crescentus CC0100 was amplified from NA1000 gDNA by PCR with primers 843 and 844 and cloned into pSK. The pSK-843/844 recombinant plasmid was then digested with Asp718I and Eco RI and ligated into the pXGFPN-2 vector cut with the same restriction enzymes.

pHR606 (pNPTS138-ΔsocAB)
Upstream and downstream regions of C. crescentus CC3514-CC3515 were amplified from NA1000 gDNA by PCR respectively with primers 848/849 and 850/851 and cloned into pSK. The pSK-848/849 and pSK-850/851 recombinant plasmids were then digested respectively with Hind III/Eco RI and Eco RI/Bam HI; and ligated into the pNPTS138 vector cut with Hind III and Bam HI.

pHR624 (pET-21b-kidO*)
kidO was amplified from NA1000 gDNA by PCR with primers 773/855 and cloned into pSK. The pSK-773/855 was then digested respectively with Nde I and Eco RI, and ligated into the pET-21b vector cut with the same restriction enzymes.

Description of Strains

RH53 (NA1000 P\textsubscript{vanA}:ftsZ-yfp)
Electroporation of pHR212 (P\textsubscript{vanA}:ftsZ-yfp) into NA1000 was selected on PYE Kan.

RH534 (NA1000 ΔgdhZ)
Biparental mating between NA1000 and RH528 (S17-1-pNPTS138-ΔgdhZ) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\textsuperscript{R} candidates were streaked on PYE Kan and PYE. Kan\textsuperscript{S} colonies were screened by PCR with primers 139/140.

RH536 (NA1000 gdhZ-venus)
Biparental mating between NA1000 and RH530 (S17-1-pVENC-1-gdhZ) was selected on PYE Nal Spec/Strep.

RH602 (NA1000 gdhZ-gfp)
Biparental mating between NA1000 and RH573 (S17-1-pGFPC-2-gdhZ) was selected on PYE Nal Kan.

RH728 (NA1000 gdhZ-3FLAG)
Biparental mating between NA1000 and RH666 (S17-1-pMCS-2-gdhZ-3FLAG) was selected on PYE Nal Kan.
**RH740** (NA1000 *gdhZ*-venus)
Biparental mating between NA1000 and RH735 (S17-1-pXVENN-1-*gdhZ*) was selected on PYE Nal Spec/Strep.

**RH743** (NA1000 3FLAG-*gdhZ*)
Biparental mating between NA1000 and RH742 (S17-1-pNPTS138-3FLAG-*gdhZ*) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and PYE. Kan<sup>S</sup> colonies were screened by PCR with primers 139/170 followed by digestion with *Nde* I (619 bp + 550 bp for 3FLAG and 1103 bp for wt).

**RH752** (NA1000 Δ*gdhZ* tipN::tipN-egfp)
A CR30 lysate made on RH506 (NA1000 *tipN::tipN-egfp*) was transduced into RH534 (NA1000 Δ*gdhZ*). Transductants were selected on PYE Kan.

**RH799** (NA1000 Δ*gdhZ* stpX::stpX-egfp)
A CR30 lysate made on RH507 (NA1000 *stpX::stpX-egfp*) was transduced into RH534 (NA1000 Δ*gdhZ*). Transductants were selected on PYE Kan.

**RH827** (NA1000 ΔrcdA::hyg 3FLAG-*gdhZ*)
Biparental mating between RH323 (NA1000 ΔrcdA::hyg) and RH742 (S17-1-pNPTS138-3FLAG-*gdhZ*) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and PYE. Kan<sup>S</sup> colonies were screened by PCR with primers 139/170 followed by digestion with *Nde* I (619 bp + 550 bp for 3FLAG and 1103 bp for wt).

**RH828** (NA1000 ΔpopA 3FLAG-*gdhZ*)
Biparental mating between RH315 (NA1000 ΔpopA) and RH742 (S17-1-pNPTS138-3FLAG-*gdhZ*) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and PYE. Kan<sup>S</sup> colonies were screened by PCR with primers 139/396 followed by digestion with *NdeI* (619 bp + 915 bp for 3FLAG and 1534 bp for wt).

**RH829** (NA1000 ΔcpdR 3FLAG-*gdhZ*)
Biparental mating between RH339 (NA1000 ΔcpdR) and RH742 (S17-1-pNPTS138-3FLAG-*gdhZ*) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and
PYE. Kan\(^S\) colonies were screened by PCR with primers 139/396 followed by digestion with \(Nde\)I (619 bp + 915 bp for 3FLAG and 1534 bp for wt).

**RH830 (NA1000 \(cpdR_{DS1A}\) 3FLAG-gdhZ)**

Biparental mating between RH347 (NA1000 \(cpdR_{DS1A}\)) and RH742 (S17-1-pNPTS138-3FLAG-gdhZ) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\(^R\) candidates were streaked on PYE Kan and PYE. Kan\(^S\) colonies were screened by PCR with primers 139/396 followed by digestion with \(Nde\)I (619 bp + 915 bp for 3FLAG and 1534 bp for wt)

**RH836 (NA1000 \(ΔgdhZ\) \(mipZ\)::mcfp)**

Biparental mating between RH534 (NA1000 \(ΔgdhZ\)) and S17-1-pNPTS138-\(mipZ\)::mcfp was selected on PYE Nal Kan. Three independent clones were streaked on PYE Kan plates, grown overnight in PYE and plated on PYE 3% Suc. Colonies were picked and streaked on PYE Kan and PYE plates. Kan\(^S\) colonies were screened by fluorescence microscopy.

**RH853 (NA1000 \(ΔgdhZ\) \(P_{vanA}::ftsZ-yfp\))**

A CR30 lysate made on RH53 (NA1000 \(P_{vanA}::ftsZ-yfp\)) was transduced into RH534 (NA1000 \(ΔgdhZ\)). Transductants were selected on PYE Kan.

**RH854 (NA1000 \(ΔgdhZ\) \(mipZ\)::mcfp \(P_{vanA}::ftsZ-yfp\))**

A CR30 lysate made on RH53 (NA1000 \(P_{vanA}::ftsZ-yfp\)) was transduced into RH836 (NA1000 \(ΔgdhZ\) \(mipZ\)::mcfp). Transductants were selected on PYE Kan.

**RH903 (NA1000 \(ΔkidO\))**

Triparental mating between NA1000, RH319 (MT607-pRK600) and RH1119 (Top10-pNPTS138-\(ΔkidO\)) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\(^R\) candidates were streaked on PYE Kan and PYE. Kan\(^S\) colonies were screened by PCR with primers 448/449.

**RH904 (NA1000 \(ΔgdhZ\) \(ΔkidO\))**

Triparental mating between RH534 (NA1000 \(ΔgdhZ\)), RH319 (MT607-pRK600) and RH1119 (Top10-pNPTS138-\(ΔkidO\)) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\(^R\) candidates were streaked on PYE Kan and PYE. Kan\(^S\) colonies were screened by PCR with primers 448/449 (1318 bp for deletion and 2092 bp for wt).

**RH938 (NA1000 \(gdhZ_{K837A}\))**
Triparental mating between NA1000, RH319 (MT607-pRK600) and RH1420 (Top10-pNPTS138-gdhZ<sub>K837A</sub>) was selected on PYE 0.2% Xyl Nal Kan, cultivated o/n in PYE 0.2% Xyl and plated on PYE 0.2% Xyl 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE 0.2% Xyl Kan, PERAQ and PYE 0.2% Xyl. Kan<sup>S</sup> colonies unable to grow on PERAQ were screened by PCR with primers 467/469 and sequencing with primer 467.

**RH940** (NA1000 3FLAG-gdhZ<sub>K837A</sub>)

Biparental mating between RH938 (NA1000 CCNA_<sub>00086</sub>K837A) and RH742 (S17-1-pNPTS138-3FLAG-gdhZ) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and PYE. Kan<sup>S</sup> colonies were screened by PCR with primers 139/170 followed by digestion with Nde I (619 bp + 550 bp for 3FLAG and 1103 bp for wt).

**RH957** (NA1000 ftsZ<sub>E79K</sub> ΔxylX gdhZ<sub>K837A</sub>)

Triparental mating between RH1494 (NA1000 ftsZ<sub>E79K</sub> ΔxylX), RH319 (MT607-pRK600) and RH1420 (Top10-pNPTS138-gdhZ<sub>K837A</sub>) was selected on PYE 0.2% Ala Nal Kan, cultivated o/n in PYE 0.2% Ala and plated on PYE 0.2% Ala 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE 0.2% Ala Kan, PERAQ and PYE 0.2% Ala. Kan<sup>S</sup> colonies unable to grow on PERAQ were screened by PCR with primers 467/469 (1508 bp) and sequencing with primer 467.

**RH958** (NA1000 CCNA_<sub>00086</sub>K837A ΔxylX)

Triparental mating between RH938 (NA1000 CCNA_<sub>00086</sub>K837A), RH319 (MT607-pRK600) and RH1273 (Top10-pNPTS138-ΔxylX) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and PYE. Kan<sup>S</sup> colonies unable to grow on a M2X medium were selected.

**RH967** (NA1000 gdhZ-gfp ΔxylX P<sub>xylX</sub>::ftsZ-mcherry)

A CR30 lysate made on RH1837 (NA1000 ΔxylX P<sub>xylX</sub>::ftsZ-mcherry) was transduced into RH602 (NA1000 gdhZ-gfp). Transductants were selected on PYE Tet.

**RH991** (NA1000 3FLAG-gdhZ P<sub>xylX</sub>::clpP ΔclpP::Ω)

A CR30 lysate made on NA1000 P<sub>xylX</sub>::clpP ΔclpP::Ω was transduced into RH743 (NA1000 3FLAG-gdhZ) (Jenal & Fuchs 1998). Transductants were first selected on PYE Tet (for the integration of P<sub>xylX</sub>::clpP). LHR66 was transduced again into this
strain to get clpP depletion strain (NA1000 3FLAG-CCNA_00086 P\textsubscript{xyly}::clpP ΔclpP::Ω. Transductants were selected on PYE Spec/Strep (for the deletion of clpP).

**RH992** (NA1000 Δ\textit{xyly}X pVEN\-1-gdhZ)

Biparental mating between RH984 (NA1000 Δ\textit{xyly}X) and RH530 (S17-1-pVEN-1-gdhZ) was selected on PYE Nal Spec/Strep.

**RH993** (NA1000 Δ\textit{xyly}X pVEN-1-gdhZ P\textsubscript{xyly}::ftsZ)

Biparental mating between RH992 (NA1000 Δ\textit{xyly}X pVEN-1-gdhZ) and RH978 (S17-1-P\textit{xyly}::ftsZ\textsubscript{63ΔC}) was selected on PYE 0.01% Xyl Nal Kan. KanR candidates were streaked on 0.01% Xyl PYE Kan and PYE Kan. Colonies requiring xylose for growth were selected.

**RH1122** (NA1000 3FLAG-gdhZ\textsubscript{AA::DD})

Triparental mating between RH743 (NA1000 3FLAG-gdhZ), RH319 (MT607-pRK600) and RH664 (Top10-pNPTS138-gdhZ\textsubscript{AA::DD}) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\textsuperscript{R} candidates were streaked on PYE Kan and PYE. Kan\textsuperscript{S} colonies were screened by PCR with primers 499/497.

**RH1144** (NA1000 ΔgdhZ P\textsubscript{\textit{vanAn}}::kidO-mcherry)

Triparental mating between RH534 (NA1000 ΔgdhZ), RH319 (MT607-pRK600) and RH1120 (Top10- P\textsubscript{\textit{vanAn}}::kidO-mcherry) was selected on PYE Nal Kan.

**RH1256** (NA1000 Δ\textit{xyly}X ΔgdhZ)

Biparental mating between RH984 (NA1000 Δ\textit{xyly}X) and RH528 (S17-1-pNPTS138-ΔgdhZ) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\textsuperscript{R} candidates were streaked on PYE Kan and PYE. Kan\textsuperscript{S} colonies were screened by PCR with primers 139/140.

**RH1312** (NA1000 Δ\textit{xyly}X P\textsubscript{\textit{xyly}}::gfp-mreB)

Biparental mating between RH984 (NA1000 Δ\textit{xyly}X) and RH441 (S17-1-pXGFPN-2-mreB) was selected on PYE Nal Kan.

**RH1313** (NA1000 Δ\textit{xyly}X ΔgdhZ P\textsubscript{\textit{xyly}}::gfp-mreB)

Biparental mating between RH1256 (NA1000 Δ\textit{xyly}X ΔgdhZ) and RH441 (S17-1-pXGFPN-2-mreB) was selected on PYE Nal Kan.

**RH1324** (NA1000 3FLAG-gdhZ Δ\textit{xyly}X Δ\textit{kidO} P\textsubscript{\textit{xyly}}::kidO-mcherry)
Triparental mating between RH1566 (NA1000 3FLAG-gdhZ ΔxylX ΔkidO), RH319 (MT607-pRK600) and RH1481 (Top10-pXCHYC-5-kidO) was selected on PYE Nal Tet.

**RH1329 (NA1000 ftsZ\_E79K ΔgdhZ)**

Biparental mating between RH1117 (NA1000 ftsZ\_E79K) and RH528 (S17-1-pNPTS138-ΔgdhZ) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\(^R\) candidates were streaked on PYE Kan and PYE. Kan\(^S\) colonies were screened by PCR with primers 139/140.

**RH1332 (NA1000 P\_gdhZ-lacZ)**

Triparental mating between RH50 (NA1000), RH319 (MT607-pRK600) and RH1318 (Top10-pMCS-5-P\_gdhZ-lacZ) was selected on PYE Nal Tet. Used for monitoring gdhZ promoter activity in a wild-type background using β-galactosidase assay. A CR30 lysate was prepared on RH1332 and transduced into different genetic backgrounds: ΔpleC (RH1711), ΔdivJ (RH1712), divK341 (RH1713).

**RH1494 (NA1000 ftsZ\_E79K ΔxylX)**

Triparental mating between RH1117 (NA1000 ftsZ\_E79K), RH319 (MT607-pRK600) and RH1273 (Top10-pNPTS138-ΔxylX) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\(^R\) candidates were streaked on PYE Kan and PYE. Kan\(^S\) colonies unable to grow on a M2X medium were selected.

**RH1495 (NA1000 ΔkidO ΔxylX)**

Triparental mating between RH903 (NA1000 ΔkidO), RH319 (MT607-pRK600) and RH1273 (Top10-pNPTS138-ΔxylX) selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\(^R\) candidates streaked on PYE Kan and PYE. Kan\(^S\) colonies unable to grow on a M2X medium were selected.

**RH1496 (NA1000 ΔgdhZ ΔkidO ΔxylX)**

Triparental mating between RH904 (NA1000 ΔgdhZ ΔkidO), RH319 (MT607-pRK600) and RH1273 (Top10-pNPTS138-ΔxylX) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc\(^R\) candidates were streaked on PYE Kan and PYE. Kan\(^S\) colonies unable to grow on a M2X medium were selected.

**RH1508 (NA1000 ΔkidO ΔxylX P\_xylX::kidO)**
Triparental mating between RH1495 (NA1000 ΔkidO ΔxylX), RH319 (MT607-pRK600) and RH1512 (Top10-pXC-5-ΔkidO) was selected on PYE Nal Tet.

**RH1509** (NA1000 ΔkidO ΔxylX P,xylX::kidOAA::DD)

Triparental mating between RH1495 (NA1000 ΔkidO ΔxylX), RH319 (MT607-pRK600) and RH1484 (Top10-pXC-5-ΔkidO) was selected on PYE Nal Tet.

**RH1510** (NA1000 ΔgdhZ ΔkidO ΔxylX P,xylX::kidO)

Triparental mating between RH1496 (NA1000 ΔgdhZ ΔkidO ΔxylX), RH319 (MT607-pRK600) and RH1512 (Top10-pXC-5-ΔkidO) was selected on PYE Nal Tet.

**RH1511** (NA1000 ΔgdhZ ΔkidO ΔxylX P,xylX::kidOAA::DD)

Triparental mating between RH1496 (NA1000 ΔgdhZ ΔkidO ΔxylX), RH319 (MT607-pRK600) and RH1484 (Top10-pXC-5-ΔkidO) was selected on PYE Nal Tet.

**RH1564** (NA1000 3FLAG-gdhZ ΔxylX)

Triparental mating between RH743 (NA1000 3FLAG-gdhZ), RH319 (MT607-pRK600) and RH1273 (Top10-pNPTS138-ΔxylX) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and PYE. Kan<sup>S</sup> colonies unable to grow on a M2X medium were selected.

**RH1566** (NA1000 3FLAG-gdhZ ΔxylX ΔkidO)

Triparental mating between RH1564 (NA1000 3FLAG-gdhZ ΔxylX), RH319 (MT607-pRK600) and RH1119 (Top10-pNPTS138-ΔkidO) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and PYE. Kan<sup>S</sup> colonies were screened by PCR with primers 448/449.

**RH1671** (NA1000 ΔsocAB)

Triparental mating between NA1000, RH319 (MT607-pRK600) and RH1670 (Top10-pNPTS138-ΔsocAB) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were streaked on PYE Kan and PYE. Kan<sup>S</sup> colonies were screened by PCR with primers 848/851.

**RH1672** (NA1000 3FLAG-gdhZ ΔsocAB)

Triparental mating between RH743 (NA1000 3FLAG-gdhZ), RH319 (MT607-pRK600) and RH1670 (Top10-pNPTS138-ΔsocAB) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc<sup>R</sup> candidates were
streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 848/851.

RH1675 (NA1000 3FLAG-CCNA_00086 ΔsocAB ΔclpP::Ω)
A CR30 lysate made on RH975 (NA1000 PxyI::clpP ΔclpP::Ω) was transduced into RH1672 (NA1000 3FLAG-CCNA_00086 ΔsocAB). Transductants were selected on PYE Spec/Strep.

RH1733 (NA1000 ΔxylX P_xylX::gfp-fzlC)
Triparental mating between RH984 (NA1000 ΔxylX), RH319 (MT607-pRK600) and RH1731 (Top10-pXGFPN-2-fzlC) was selected on PYE Nal Kan.

RH1734 (NA1000 ΔxylX ΔgdhZ P_xylX::gfp-fzlC)
Triparental mating between RH1256 (NA1000 ΔxylX ΔgdhZ), RH319 (MT607-pRK600) and RH1731 (Top10-pXGFPN-2-fzlC) was selected on PYE Nal Kan.

RH1834 (NA1000 ΔxylX P_xylX::ftsZ-mcherry)
Triparental mating between RH984 (NA1000 ΔxylX), RH319 (MT607-pRK600) and RH1479 (Top10-pXCHYC-5-ftsZ) was selected on PYE Nal Tet.

RH1868 (NA1000 ΔxylX ΔgdhZ P_xylX::ftsZ-mcherry)
Triparental mating between RH1256 (NA1000 ΔxylX ΔgdhZ), RH319 (MT607-pRK600) and RH1479 (Top10-pXCHYC-5-ftsZ) was selected on PYE Nal Tet.

RH1897 (NA1000 ΔxylX gdhZ-venus P_vanA::mcherry-fzlC P_xylX::ftsZ)
Triparental mating between RH993 (NA1000 ΔxylX pVENC-1-gdhZ P_xylX::ftsZ), RH319 (MT607-pRK600) and RH1841 (Top10-pVCHYN-5-fzlC) was selected on PYE Nal Tet.

RH1904 (NA1000 ΔkidO ΔxylX pGFPC-2-gdhZ)
Biparental mating between RH1495 (NA1000 ΔkidO ΔxylX) and RH573 (S17-1-pGFPC-2-gdhZ) was selected on PYE Nal Kan.

RH1905 (NA1000 ΔxylX ΔkidO gdhZ-gfp P_xylX::kidOAA::DD)
Biparental mating between RH1509 (NA1000 ΔkidO ΔxylX P_xylX:: kidOAA::DD), RH573 (S17-1-pGFPC-2-gdhZ) was selected on PYE Nal Kan.

FACS analysis
DNA content was measured using Fluorescence-Activated Cell Sorting (FACS). Cells were fixed in ice-cold Ethanol. Fixed samples were then washed twice in FACS staining buffer (10 mM Tris pH 7.2, 1 mM EDTA, 50 mM NaCitrate, 0.01% Triton X-100) containing 0.1 mg/ml RNaseA and incubated at room temperature (RT) for 30 min. Cells were then harvested by centrifugation for 2 min at 8,000 x g, resuspended in 1 ml FACS staining buffer containing 0.5 μM Sytox Green Nucleic acid stain (Life Technologies), and incubated at RT in the dark for 5 min. Samples were analyzed in flow cytometer (FACS Calibur, BD Biosciences) at laser excitation of 488 nm. At least 1 x 10^4 cells were recorded in triplicate for each experiment.
References


