SUPPLEMENT

Cyclic di-GMP regulates multiple cellular functions in the symbiotic α-proteobacterium *Sinorhizobium meliloti*

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Media and antibiotic concentrations

TY medium (5 g/L tryptone, 3 g/L yeast extract, 0.4 g $CaCl_2*2H_2O$). LB medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl). YM medium (0.5 g/L K_2HPO_4, 0.5 g/L KH_2PO_4, 0.2 g/L $MgSO_4*7H_2O$, 0.1 g/L NaCl, 1 g/L yeast extract, 10 g/L mannitol, pH 7.2). MOPS-buffered minimal medium (MM) (10 g/L MOPS, 10 g/L mannitol, 3.55 g/L sodium glutamate, 0.246 g/L $MgSO_4*7H_2O$, 0.25 mM $CaCl_2$, 2 mM K_2HPO_4, 10 mg/L $FeCl_3*6H_2O$, 1 mg/L biotin, 3 mg/L H_3BO_3, 2.23 mg/L $MnSO_4*H_2O$, 0.287 mg/L $ZnSO_4*7H_2O$, 0.125 mg/L $CuSO_4*5H_2O$, 0.065 mg/L $CoCl_2*6H_2O$, 0.12 mg/L $NaMoO_4*2H_2O$, pH 7.2). MGM medium (11 g/L $Na_2HPO_4*2H_2O$, 3 g/L KH_2PO_4, 0.5 g/L NaCl, 1 g/L sodium glutamate, 10 g/L mannitol, 0.2 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mg/L biotin).

Plant medium (147 mg/L $CaCl_2*2H_2O$, 68 mg/L KH_2PO_4, 3.35 mg/L Fe citrate, 61.7 mg/L $MgSO_4*7H_2O$, 43.5 mg/L K_2SO_4, 0.169 mg/L $MnSO_4*H_2O$, 123.5 mg/L H_3BO_4, 0.144 mg/L $ZnSO_4*7H_2O$, 0.05 mg/L $CuSO_4*5H_2O$, 0.028 mg/L $CoSO_4*7H_2O$, 0.024 mg/L $Na_2MoO_2*2H_2O$)

Antibiotics were added to *S. meliloti* or *A. tumefaciens* cultures at the following concentrations: streptomycin, 600 mg/L, gentamicin, 40 mg/L, kanamycin, 200 mg/L tetracyclin, 10 mg/L. For liquid cultures of *S. meliloti* antibiotic concentrations were reduced to the half. For *E. coli,* 100 mg/L ampicillin, 10 mg/L gentamicin, 50 mg/L kanamycin and 5 mg/L tetracyclin were used.

TABLE S1 Strains and plasmids used in this study.

Strain or plasmid	Properties	Reference
S. meliloti		
Rm2011	Wild type, Str^r	Casse *et al*. (1979)
Rm101	Rm2011 *mucR*::Spec^r	Becker *et al.* (1997)
Sm2B5001	Rm2011 Δ*rem*	Bahlawane *et al.* (2008)
Rm2011mTn5STM.1.08.H02	Rm2011 mTn5 insertion into *rem*	Pobigaylo *et al.* (2006)
Rm2011mTn5STM.1.08.B07	Rm2011 mTn5 insertion into *flgH*	Pobigaylo *et al.* (2006)
Rm2011mTn5STM.2.11.F09	Rm2011 mTn5 insertion into *SMa0453-SMa0461* intergenic locus	Pobigaylo *et al.* (2006)
Sm2B3001	Rm2011 restored *expR*	Bahlawane *et al.* (2008)
Rm2011 *expR*$^+$ *exoY wgeB*	Sm2B3001 Δ*exoY wgeB*::pK18mob2-mCh	Charoenpanich *et al.* (2015)
Rm2011 *expR*$^+$ *sinI*	Sm2B3001 Δ*sinI*	McIntosh *et al.* (2009)
Rm2011 *exoY*	Rm2011 Δ*exoY*	This work
Rm2011 *exoY wgeB*	Rm2011 Δ*exoY wgeB*::pK18mob2-mCh	This work
mucR exoY wgeB	Rm101 Δ*exoY wgeB*::pK18mob2-mCh	This work
mucR SMc01792	Rm101 *SMc01792*::pK19mob2ΩHMB	This work
Rm2011 *expR*$^+$ *SMc01792*	Sm2B3001 *SMc01792*::pK19mob2ΩHMB	This work
Rm2011 *expR*$^+$ *sinI SMc01792*	Sm2B3001 Δ*sinI SMc01792*::pK19mob2ΩHMB	This work
Rm2011 *pilA1*	Rm2011 Δ*pilA1*	This work
Rm2011 *pleD*	Rm2011 *pleD*::pK19mob2ΩHMB	This work
Rm2011 *SMa0137*	Rm2011 *SMa0137*::pK19mob2ΩHMB	This work
Rm2011 *SMa0369*	Rm2011 Δ*SMa0369*	This work
Rm2011 *SMa1548*	Rm2011 *SMa1548*::pK19mob2ΩHMB	This work
Rm2011 *SMa2301*	Rm2011 *SMa2301*::pK19mob2ΩHMB	This work
Rm2011 *SMb20389*	Rm2011 *SMb20389*::pK19mob2ΩHMB	This work
Rm2011 *SMb20447*	Rm2011 *SMb20447*::pK19mob2ΩHMB	This work
Rm2011 *SMb20523*	Rm2011 *SMb20523*::pK19mob2ΩHMB	This work
Rm2011 *SMb20900*	Rm2011 *SMb20900*::pK19mob2ΩHMB	This work
Rm2011 *SMb21517*	Rm2011 *SMb21517*::pK19mob2ΩHMB	This work
Rm2011 *SMc00033*	Rm2011 *SMc00033*::pK19mob2ΩHMB	This work
Rm2011 *SMc00038*	Rm2011 *SMc00038*::pK19mob2ΩHMB	This work
Rm2011 *SMc00507*	Rm2011 Δ*SMc00507* (Δ*mcrA*)	This work
Rm2011 *SMc00507 SMc00999*	*SMc00507 SMc00999*::pK19mob2ΩHMB	This work
Rm2011 *SMc00887*	Rm2011 *SMc00887*::pK19mob2ΩHMB	This work
Rm2011 *SMc00992*	Rm2011 *SMc00992*::pK19mob2ΩHMB	This work
Rm2011 *SMc00999*	Rm2011 *SMc00999*::pK19mob2ΩHMB	This work
Rm2011 *SMc01464*	Rm2011 *SMc01464*::pK19mob2ΩHMB	This work
Rm2011 *SMc01792*	Rm2011 *SMc01792*::pK19mob2ΩHMB	This work
Rm2011 *SMc03141*	Rm2011 *SMc03141*::pK19mob2ΩHMB	This work
Rm2011 *SMc03178*	Rm2011 *SMc03178*::pK19mob2ΩHMB	This work
Rm2011 *SMc03942*	Rm2011 *SMc03942*::pK19mob2ΩHMB	This work
Rm2011 *SMc04015*	Rm2011 *SMc04015*::pK19mob2ΩHMB	This work
Rm2011 ΔI	Rm2011 Δ*pleD*	This work
Rm2011 ΔII	Rm2011 ΔI Δ*SMc04015*	This work
Rm2011 ΔIII	Rm2011 ΔII Δ*SMb20523*	This work
Rm2011 ΔIV	Rm2011 ΔIII Δ*SMc01464*	This work
Rm2011 ΔV	Rm2011 ΔIV Δ*SMa2301*	This work
Rm2011 ΔVI	Rm2011 ΔV Δ*SMb20389*	This work
Rm2011 ΔVII	Rm2011 ΔVI Δ*SMb20447*	This work
Rm2011 ΔVIII	Rm2011 ΔVII Δ*SMb20900*	This work
Rm2011 ΔIX	Rm2011 ΔVIII Δ*SMc00038*	This work
Rm2011 ΔX	Rm2011 ΔIX Δ*SMa1548*	This work
Rm2011 ΔXI	Rm2011 ΔX Δ*SMc03178*	This work
Rm2011 ΔXII	Rm2011 ΔXI Δ*SMa0137*	This work
Rm2011 ΔXIII	Rm2011 ΔXII Δ*SMc00992*	This work
Rm2011 ΔXIV	Rm2011 ΔXIII Δ*SMc03942*	This work
Rm2011 ΔXV	Rm2011 ΔXIV Δ*SMc00887*	This work
Rm2011 ΔXVI	Rm2011 ΔXV Δ*SMc00033*	This work
Rm2011 *expR*$^+$ ΔI	Sm2B3001 Δ*pleD*	This work
Rm2011 *expR*$^+$ ΔII	Rm2011 *expR*$^+$ ΔI Δ*SMc04015*	This work
Rm2011 *expR*$^+$ ΔIII	Rm2011 *expR*$^+$ ΔII Δ*SMb20523*	This work
Rm2011 *expR*$^+$ ΔIV	Rm2011 *expR*$^+$ ΔIII Δ*SMc01464*	This work
Rm2011 *expR*$^+$ ΔV	Rm2011 *expR*$^+$ ΔIV Δ*SMa2301*	This work
Rm2011 *expR*$^+$ ΔVI	Rm2011 *expR*$^+$ ΔV Δ*SMb20389*	This work
Rm2011 *expR*$^+$ ΔVII	Rm2011 *expR*$^+$ ΔVI Δ*SMb20447*	This work
Rm2011 *expR*$^+$ ΔVIII	Rm2011 *expR*$^+$ ΔVII Δ*SMb20900*	This work
Rm2011 *expR*$^+$ ΔIX	Rm2011 *expR*$^+$ ΔVIII Δ*SMc00038*	This work
Rm2011 *expR*$^+$ ΔX	Rm2011 *expR*$^+$ ΔIX Δ*SMa1548*	This work
Rm2011 *expR*$^+$ ΔXI	Rm2011 *expR*$^+$ ΔX Δ*SMc03178*	This work
Rm2011 *expR*$^+$ ΔXII	Rm2011 *expR*$^+$ ΔXI Δ*SMa0137*	This work
Rm2011 *expR*$^+$ ΔXIII	Rm2011 *expR*$^+$ ΔXII Δ*SMc00992*	This work

Rm2011 *expR*$^+$ ΔXIV	Rm2011 *expR*$^+$ ΔXIII *SMc03942*	This work
Rm2011 *expR*$^+$ ΔXV	Rm2011 *expR*$^+$ ΔXIV *SMc00887*	This work
Rm2011 *expR*$^+$ ΔXVI	Rm2011 *expR*$^+$ ΔXV *SMc00033*	This work
Rm2011 Gm^R	Rm2011 pSM10 integrated into the chromosome	This work
Rm2011 ΔXVI Gm^R	Rm2011 ΔXVI pSM10 integrated into the chromosome	This work
Rm2011 *expR*$^+$ Gm^R	Sm2B3001 pSM10 integrated into the chromosome	This work
Rm2011 *expR*$^+$ ΔXVI Gm^R	Rm2011 *expR*$^+$ ΔXVI pSM10 integrated into the chromosome	This work
PleD-EGFP	Rm2011 *pleD*::pK18mob2-*pleD-egfp*	This work
SMb20447-EGFP	Rm2011 *SMb20447*::pK18mob2-*SMb20447*-EGFP	This work
SMb20523-EGFP	Rm2011 *SMb20523*::pK18mob2-*SMb20523*-EGFP	This work
SMb21517-EGFP	Rm2011 *SMb21517*::pK18mob2-*SMb21517*-EGFP	This work
SMc01464-EGFP	Rm2011 *SMc01464*::pK18mob2-*SMc01464*-EGFP	This work
SMc03178-EGFP	Rm2011 *SMc03178*::pK18mob2-*SMc03178*-EGFP	This work
A. tumefaciens		
NTL4 (pZLR4)	Derivative of NT1 with an internal deletion of the *tetC58* locus, *traG-lacZ,* Gm^r	Shaw *et al.* (1997)
E. coli		
S17-1	*E. coli* 294 Thi RP4-2-Tc::Mu-Km::Tn7 integrated into the chromosome	Simon *et al.* (1983)
DH5α	F$^-$ *endA1 supE44 thi-1l-recA1 gyrA96 relA1 deoRD*(*lacZYA-argF*)U169	Grant *et al*. (1990)
M15pREP4	Nx^s Str^s Rif^s Thi$^-$ Lac$^-$ Ara$^+$ Gal$^+$ Mtl$^-$ F$^-$ RecA$^+$ Uvr$^+$ Lon$^+$	Qiagen, Hilden, Germany
Plasmid		
pDJS31	pET24b(+); *dgcA*WT, Km^r	Skotnicka *et al.* (2015)
pK18mob2-EGFP	pK18mob2::EGFP, *lacZ*, *mob,* Km^r	N. Meier
pK18mobsacB	*lacZ*, *mob*, *sacB,* Km^r	Schäfer *et al.* (1994)
pK19mob2ΩHMB	integrative plasmid containing a transcription-termination sequence, Km^r	Luo *et al.* (2005)
pPHU231-EGFP	broad-host-range low copy expression vector containing *egfp*, used for generating promoter-EGFP fusions, Tc^r	M. McIntosh
pMMB67EH	broad-host-range expression vector containing *tac* promoter, Gm^r	Fürste *et al.* (1986)
pSM10	*S. meliloti* integrative vector containing partial *recA* and *alaS* sequences, Gm^r	Selbitschka *et al.* (1995)
pSRKKm	pBBR1MCS-2-derived broad-host-range expression vector containing *lac* promoter and *lacI*^{q}, lacZα$^+$, Km^r	Khan *et al.* (2008)
pWBT	pSRKGm containing T5 promoter, Gm^r	M. McIntosh
pWH844	expression vector containing His$_6$-tag sequence and T5 promoter, Amp^r	Schirmer *et al.* (1997)
pSRKKm-EGFP	pSRKKm containing EGFP, Km^r	This work
pSRKGm-*parB-mcherry*	pSRKGm carrying *S. meliloti parB* translationally fused to mCherry, Gm^r	This work
Integrative plasmids for gene mutation		
pK19mob2ΩHMB-*SMb20389*	pK19mob2ΩHMB carrying internal fragment of *SMb20389*	Becker *et al.* (2009)
pK18mob2-*wgeB*-mCh	pK18mob2-mCh carrying internal fragment of *wgeB*	P. Charoenpanich
pK19mob2ΩHMB-*pleD*	pK19mob2ΩHMB carrying internal fragment of *pleD*	This work
pK19mob2ΩHMB-*SMa2301*	pK19mob2ΩHMB carrying internal fragment of *SMa2301*	This work
pK19mob2ΩHMB-*SMb20523*	pK19mob2ΩHMB carrying internal fragment of *SMb20523*	This work
pK19mob2ΩHMB-*SMc01464*	pK19mob2ΩHMB carrying internal fragment of *SMc01464*	This work
pK19mob2ΩHMB-*SMc04015*	pK19mob2ΩHMB carrying internal fragment of *SMc04015*	This work
pK19mob2ΩHMB-*SMa0137*	pK19mob2ΩHMB carrying internal fragment of *SMa0137*	This work
pK19mob2ΩHMB-*SMa1548*	pK19mob2ΩHMB carrying internal fragment of *SMa1548*	This work
pK19mob2ΩHMB-*SMb20447*	pK19mob2ΩHMB carrying internal fragment of *SMb20447*	This work
pK19mob2ΩHMB-*SMb20900*	pK19mob2ΩHMB carrying internal fragment of *SMb20900*	This work
pK19mob2ΩHMB-*SMc00033*	pK19mob2ΩHMB carrying internal fragment of *SMc00033*	This work
pK19mob2ΩHMB-*SMc00038*	pK19mob2ΩHMB carrying internal fragment of *SMc00038*	This work
pK19mob2ΩHMB-*SMc00074*	pK19mob2ΩHMB carrying internal fragment of *SMc00074*	This work
pK19mob2ΩHMB-*SMc00887*	pK19mob2ΩHMB carrying internal fragment of *SMc00887*	This work
pK19mob2ΩHMB-*SMc00992*	pK19mob2ΩHMB carrying internal fragment of *SMc00992*	This work
pK19mob2ΩHMB-*SMc03141*	pK19mob2ΩHMB carrying internal fragment of *SMc03141*	This work
pK19mob2ΩHMB-*SMc03178*	pK19mob2ΩHMB carrying internal fragment of *SMc03178*	This work
pK19mob2ΩHMB-*SMc03942*	pK19mob2ΩHMB carrying internal fragment of *SMc03942*	This work
pK19mob2ΩHMB-*SMb21517*	pK19mob2ΩHMB carrying internal fragment of *SMb21517*	This work
pK19mob2ΩHMB-*SMc00999*	pK19mob2ΩHMB carrying internal fragment of *SMc00999*	This work
pK19mob2ΩHMB-*SMc01792*	pK19mob2ΩHMB carrying internal fragment of *SMc01792*	This work
Integrative plasmids for C-terminal EGFP tagging		
pK18mob2-*pleD*-EGFP	pK18mob2-EGFP carrying C-terminal portion of *pleD*	This work
pK18mob2-*SMb20523*-EGFP	pK18mob2-EGFP carrying C-terminal portion of *SMb20523*	This work
pK18mob2-*SMc01464*-EGFP	pK18mob2-EGFP carrying C-terminal portion of *SMc01464*	This work
pK18mob2-*SMb20447*-EGFP	pK18mob2-EGFP carrying C-terminal portion of *SMb20447*	This work
pK18mob2-*SMc03178*-EGFP	pK18mob2-EGFP carrying C-terminal portion of *SMc03178*	This work

pK18mob2-*SMb21517*-EGFP	pK18mob2-EGFP carrying C-terminal portion of *SMb21517*	This work
Deletion constructs		
pK18mobsacB-*exoY*	pK18mobsacB carrying flanking regions of *exoY*	P. Charoenpanich
pK18mobsacB-*pleD*	pK18mobsacB carrying flanking regions of *pleD*	This work
pK18mobsacB-*SMa2301*	pK18mobsacB carrying flanking regions of *SMa2301*	This work
pK18mobsacB-*SMb20389*	pK18mobsacB carrying flanking regions of *SMb20389*	This work
pK18mobsacB-*SMb20523*	pK18mobsacB carrying flanking regions of *SMb20523*	This work
pK18mobsacB-*SMc01464*	pK18mobsacB carrying flanking regions of *SMc01464*	This work
pK18mobsacB-*SMc04015*	pK18mobsacB carrying flanking regions of *SMc04015*	This work
pK18mobsacB-*SMa0137*	pK18mobsacB carrying flanking regions of *SMa0137*	This work
pK18mobsacB-*SMa1548*	pK18mobsacB carrying flanking regions of *SMa1548*	This work
pK18mobsacB-*SMb20447*	pK18mobsacB carrying flanking regions of *SMb20447*	This work
pK18mobsacB-*SMb20900*	pK18mobsacB carrying flanking regions of *SMb20900*	This work
pK18mobsacB-*SMc00033*	pK18mobsacB carrying flanking regions of *SMc00033*	This work
pK18mobsacB-*SMc00038*	pK18mobsacB carrying flanking regions of *SMc00038*	This work
pK18mobsacB-*SMc00887*	pK18mobsacB carrying flanking regions of *SMc00887*	This work
pK18mobsacB-*SMc00992*	pK18mobsacB carrying flanking regions of *SMc00992*	This work
pK18mobsacB-*SMc03178*	pK18mobsacB carrying flanking regions of *SMc03178*	This work
pK18mobsacB-*SMc03942*	pK18mobsacB carrying flanking regions of *SMc03942*	This work
pK18mobsacB-*SMa0369*	pK18mobsacB carrying flanking regions of *SMa0369*	This work
pK18mobsacB-*SMc00507*	pK18mobsacB carrying flanking regions of *SMc00507* (*mcrA*)	This work
pK18mobsacB-*pilA1*	pK18mobsacB carrying flanking regions of *pilA1*	This work
Overexpression constructs		
pWBT-*SMb20447*	pWBT carrying *SMb20447* coding sequence	P. Charoenpanich
pWBT-*pleD*	pWBT carrying *pleD* coding sequence	This work
pWBT-*pleD*$_{\mathrm{GGAAF}}$	pWBT carrying *pleD*$_{\mathrm{GGAAF}}$ coding sequence	This work
pWBT-*SMa2301*	pWBT carrying *SMa2301* coding sequence	This work
pWBT-*SMb20389*	pWBT carrying *SMb20389* coding sequence	This work
pWBT-*SMb20523*	pWBT carrying *SMb20523* coding sequence	This work
pWBT-*SMc01464*	pWBT carrying *SMc01464* coding sequence	This work
pWBT-*SMc04015*	pWBT carrying *SMc04015* coding sequence	This work
pWBT-*SMa0137*	pWBT carrying *SMa0137* coding sequence	This work
pWBT-*SMa1548*	pWBT carrying *SMa1548* coding sequence	This work
pWBT-*SMb20900*	pWBT carrying *SMb20900* coding sequence	This work
pWBT-*SMc00033*	pWBT carrying *SMc00033* coding sequence	This work
pWBT-*SMc00038*	pWBT carrying *SMc00038* coding sequence	This work
pWBT-*SMc00074*	pWBT carrying *SMc00074* coding sequence	This work
pWBT-*SMc00887*	pWBT carrying *SMc00887* coding sequence	This work
pWBT-*SMc00992*	pWBT carrying *SMc00992* coding sequence	This work
pWBT-*SMc03178*	pWBT carrying *SMc03178* coding sequence	This work
pWBT-*SMc03942*	pWBT carrying *SMc03942* coding sequence	This work
pWBT-*SMb21517*	pWBT carrying *SMb21517* coding sequence	This work
pWBT-*SMc00507*	pWBT carrying *SMc00507* (*mcrA*) coding sequence	This work
pWBT-*SMc00999*	pWBT carrying *SMc00999* coding sequence	This work
pWBT-*pleD-SMc00507*	pWBT carrying *pleD and SMc00507* (*mcrA*) coding sequences	This work
pWBT-*pleD SMc00507*$_{\mathrm{AxxxA}}$	pWBT carrying *pleD and SMc00507*$_{\mathrm{AxxxA}}$ (*mcrA*$_{\mathrm{AxxxA}}$) coding sequences	This work
pWBT-*dgcA*	pWBT carrying *C. crescentus dgcA* coding sequence	This work
pWBT-*yhjH*	pWBT carrying *E. coli yhjH* coding sequence	This work
pSRKKm-PT5-*pleD*	NdeI/HindIII fragment from pWBT-*pleD* cloned into pSRKKm	This work
pMMB67EH-*pleD*	EcoRI/HindIII fragment from pWBT-*pleD* cloned into pMMB67EH	This work
pWH844-McrA	pWH844 carrying *SMc00507* (*mcrA*) coding sequence	This work
pWH844-McrA$_{\mathrm{AxxxA}}$	pWH844 carrying *SMc00507*$_{\mathrm{AxxxA}}$ (*mcrA*$_{\mathrm{AxxxA}}$) coding sequence	This work
pWH844-McrA$_{\mathrm{D35A/S37A/G40A}}$	pWH844 carrying *SMc00507*$_{\mathrm{D35A/S37A/G40A}}$ (*mcrA*$_{\mathrm{D35A/S37A/G40A}}$) coding sequence	This work
pWH844-*SMc00074*$_{390\text{-}970}$	pWH844 carrying *SMc00074* (aa 390-970) coding sequence	This work
FRET-based biosensor constructs		
pMMB67EH-Spy	pMMB67EH containing synthetic *ycgR* fused to CYPet and YPet	Christen *et al.* (2010)
pMMB67EH-CYPet-*12aa*-YPet	pMMB67EH containing a 12 aa linker fused to CYPet and YPet	This work
pMMB67EH-CYPet-*mcrA*-YPet	pMMB67EH containing *SMc00507* (*mcrA*) fused to CYPet and YPet	This work
Promoter-EGFP fusion plasmids		
pLK115	pPHU231-EGFP containing *wgeA* promoter	Charoenpanich *et al.* (2013)
pLK64	pPHU231-EGFP containing *sinI* promoter	McIntosh *et al.* (2008)
pPHU231-P*exoY*-EGFP	pPHU231-EGFP containing *exoY* promoter	This work
pPHU231-P*SMc00507*$_{\mathrm{ATG}}$-EGFP	pPHU231-EGFP containing *SMc00507*$_{\mathrm{ATG}}$ promoter	This work
pPHU231-P*SMc00507*$_{\mathrm{TTG}}$-EGFP	pPHU231-EGFP containing *SMc00507*$_{\mathrm{TTG}}$ promoter	This work
pSRKKm-P*SMc01794*-EGFP	pSRKKm-EGFP containing *SMc01794* promoter	This work

TABLE S2 Primers used in this study.

Primer	Sequence	Purpose
SMc03142-stop-f	GTTTCGGCTGGATGCCGAGA	*SMc03142* stop codon verification
SMc03142-stop-r	ATCGACTCCTTCAGTGCATTG	
pleD-int-f	ATATAAGCTTACCAGCCAATGTGAAGCTCCT	*pleD* internal fragment
pleD-int-r	ATATCTGCAGTGCAGATCGTTCACGGGC	
SMa2301-int-f	ATATAAGCTTCCCTAGATGGGCAAGGATG	*SMa2301* internal fragment
SMa2301-int-r	ATATCTGCAGTGAAGGCGGTGGCCAGAG	
SMb20523-int-f	ATATAAGCTTCTTCGTGGTTGCGTTTCTGA	*SMb20523* internal fragment
SMb20523-int-r	ATATCTGCAGTGGATCAGGAAGAACGGCAT	
SMc01464-int-f	ATATAAGCTTAATGAGCTTTCTCTCCGCCATA	*SMc01464* internal fragment
SMc01464-int-r	ATATCTGCAGAAGGACGAGAAGTAGAGCTGCG	
SMc04015-int-f	ATATAAGCTTTATGATCCGCATGATCGACTG	*SMc04015* internal fragment
SMc04015-int-r	ATATCTGCAGTACGTCCGTAATGTCAACGGC	
SMa0137-int-f	ATATAAGCTTATCGCAATGGCCTTGATG	*SMa0137* internal fragment
SMa0137-int-r	ATATCTGCAGAGCTTCCTGCTCGTTGGTCT	
SMa1548-int-f	ATATAAGCTTTCTCTTCCAGCGGCTTATGG	*SMa1548* internal fragment
SMa1548-int-r	ATATCTGCAGTGATATCCACGATCGTTACGAC	
SMb20447-int-f	ATATAAGCTTCGCCCATTCCGACGAACTT	*SMb20447* internal fragment
SMb20447-int-r	ATATCTGCAGGGGTCGCCTGATGTAGGAGAA	
SMb20900-int-f	ATATAAGCTTGAGCTTTTCCATCGCGGT	*SMb20900* internal fragment
SMb20900-int-r	ATATCTGCAGGGTCGGGGATCAGGACGA	
SMc00033-int-f	ATATAAGCTTCCAAATCGTGACGCTTGC	*SMc00033* internal fragment
SMc00033-int-r	ATATCTGCAGAGCAAAGCGAGGAAGGAGC	
SMc00038-int-f	ATATAAGCTTTGAACGCGGACGACAATG	*SMc00038* internal fragment
SMc00038-int-r	ATATCTGCAGAAGATCGGTTCGCCGGTTT	
SMc00074-int-f	ATATAAGCTTGTTTCTGGTCGCACTCGTCGT	*SMc00074* internal fragment
SMc00074-int-r	ATATCTGCAGAGAGTATGCGCTGCGAGC	
SMc00887-int-f	ATATAAGCTTCGTTCGTCGATCCATTGACC	*SMc00887* internal fragment
SMc00887-int-r	ATATCTGCAGGCACTTCCTTGAGCATCTGG	
SMc00992-int-f	ATATAAGCTTTGCAGGAGAGCCTGACGT	*SMc00992* internal fragment
SMc00992-int-r	ATATCTGCAGTGCAGCCTGAGAAAGCGGT	
SMc03141-int-f	ATATAAGCTTCGGCACAGTGTCGAGACG	*SMc03141* internal fragment
SMc03141-int-r	ATATCTGCAGACGTGCCATCCAGCATCTT	
SMc03178-int-f	ATATAAGCTTCTACCTTCCGGCCGTCAT	*SMc03178* internal fragment
SMc03178-int-r	ATATCTGCAGGGTAATCGAGGCCGATCGC	
SMc03942-int-f	ATATAAGCTTCGTAAGCTCGCTCTACCAGAAT	*SMc03942* internal fragment
SMc03942-int-r	ATATCTGCAGGTGAGATCATCGAGGCAAGC	
SMb21517-int-f	ATATAAGCTTTGCAGCAGGTCAATGCGGT	*SMb21517* internal fragment
SMb21517-int-r	ATATCTGCAGCAAGGCGCGAGGCCATTT	
SMc00999-int-f	ATATAAGCTTAGAACAGCGCCCCGAAAC	*SMc00999* internal fragment
SMc00999-int-r	ATATCTGCAGAGCTTCTCGCGCTTGCGT	
SMc01792-int-f	ATATAAGCTTAAGGACGGGACAAGGGGCAAT	*SMc01792* internal fragment
SMc01792-int-r	ATATCTGCAGCGATTTGAGAAAGAAGAGGCCGA	
pleD-C-f	ATATGTCGACCCCGCTTCATTCCGATCCT	*pleD* 3’-end for protein localization
pleD-C-r	ATATTCTAGAGGCAGCGGCAGCGACGAC	
SMb20523-C-f	ATATGTCGACATCTATGCCGCAGTTGCG	*SMb20523* 3’ end for protein localization
SMb20523-C-r	ATATTCTAGACGTCGGTCGATACTTTCCAGA	

SMc01464-C-f	ATATGTCGACTTCGAGCCTGTTCGATTTCCT	*SMc01464* 3’-end for protein localization
SMc01464-C-r	ATATTCTAGACGCCGAGTGGCGCAGTTC	
SMb20447-C-f	ATATGTCGACCGGTCGTCGGTTTCGAAG	*SMb20447* 3’-end for protein localization
SMb20447-C-r	ATATTCTAGATGTTCGCTGAAGACCTGCC	
SMc03178-C-f	ATATGTCGACAGTTCTGTCCGAGCTCGCT	*SMc03178* 3’-end for protein localization
SMc03178-C-r	ATATTCTAGAGGCGGCGCGCCACCGCCGCTCGAAGA	
SMb21517-C-f	ATATAAGCTTTCGATACATGGTCGGTCTCG	*SMb21517* 3’-end for protein localization
SMb21517-C-r	ATATTCTAGAGGAATGCAGCTGCCGCGAT	
pleD-l-f	ATATGAATTCAACGCAACGAGTCCGCCCAT	*pleD* left flanking region
pleD-l-r	ATATTCTAGAATCGATCTTTCCGGCAGTCA	
pleD-r-f	ATATTCTAGATCCGCCGCACATGCTGAG	*pleD* right flanking region
pleD-r-r	ATATAAGCTTCAATCGGTTGTCCACGGCTTT	
SMa2301-l-f	ATATGAATTCTTTGGCTGGAAGACGACAC	*SMa2301* left flanking region
SMa2301-l-r	ATATGGTACCTTCATATGGCAAATCGTGGC	
SMa2301-r-f	ATATGGTACCCGGCGCGACGAGAGCGGGATTA	*SMa2301* right flanking region
SMa2301-r-r	ATATGGATCCTTGGCGAGCAAGGTTTCG	
SMb20389-l-f	ATATGAATTCTTCGATGATCGGTGCGCA	*SMb20389* left flanking region
SMb20389-l-r	ATATGGTACCTTCTATCTCCCTCGTTCGTGA	
SMb20389-r-f	ATATGGTACCGTCATCCTCGACGCCTGA	*SMb20389* right flanking region
SMb20389-r-r	ATATAAGCTTAGCATCGCGACATTCGTG	
SMb20523-l-f	ATATGAATTCCATTCGGCGGGCAATCAC	*SMb20523* left flanking region
SMb20523-l-r	ATATTCTAGATGGCAGAATCCAATATACGCA	
SMb20523-r-f	ATATTCTAGAGGCCAAGCGGGCAATTGAT	*SMb20523* right flanking region
SMb20523-r-r	ATATAAGCTTTATGCTGTGATGAGTTTCGGC	
SMc01464-l-f	ATATGGATCCACGAGCCGGTCCACGTCGT	*SMc01464* left flanking region
SMc01464-l-r	ATATTCTAGATATGCGAGGCCATTGGTAA	
SMc01464-r-f	ATATTCTAGACTTCTCGCACGATGGATCCT	*SMc01464* right flanking region
SMc01464-r-r	ATATAAGCTTATGCGGCAGACCTTAGTCTC	
SMc04015-l-f	ATATGGATCCAGGTGTCGACCGAGAGATTGA	*SMc04015* left flanking region
SMc04015-l-r	ATATTCTAGACAGGGCCTTGAGGTGATCA	
SMc04015-r-f	ATATTCTAGACGCGTCAAGCGTCATACG	*SMc04015* right flanking region
SMc04015-r-r	ATATAAGCTTAATGCCTGTTGAAGGCCC	
SMa0137-l-f	ATATGGATCCGCGCCCTGACGAATACCCA	*SMa0137* left flanking region
SMa0137-l-r	ATATTCTAGACCGCACTCCTCCTGATAGG	
SMa0137-r-f	ATATTCTAGATTTTGGCCCGAGCGTCCCTAA	*SMa0137* right flanking region
SMa0137-r-r	ATATAAGCTTGCTGCTTGACGATACTGGCA	
SMa1548-l-f	ATATGGATCCACGGGTAATGGCAGCGAT	*SMa1548* left flanking region
SMa1548-l-r	ATATTCTAGAGACACGACACTTAGACCGGTGA	
SMa1548-r-f	ATATTCTAGACTCGATTGGCGCAGTTGC	*SMa1548* right flanking region
SMa1548-r-r	ATATAAGCTTCGAGAAGCCCTGTACAAGGA	
SMb20447-l-f	ATATGAATTCGCGCATTCTGTCGAACGC	*SMb20447* left flanking region
SMb20447-l-r	ATATTCTAGATCTCGCCTTTCCCTTCCGTT	
SMb20447-r-f	ATATTCTAGAAGTGGGTCAATGCCGGGAAATTA	*SMb20447* right flanking region
SMb20447-r-r	ATATAAGCTTCACCTCTTCGATGGCGCCTTTA	
SMb20900-l-f	ATATGAATTCAGGTGCCCTCCGACACGATA	*SMb20900* left flanking region
SMb20900-l-r	ATATGGTACCCGCGATGAAATGCGTCGA	
SMb20900-r-f	ATATGGTACCCTCGTCATGAGACAAAAAGGC	*SMb20900* right flanking region
SMb20900-r-r	ATATGGATCCGATGGTTGGCTTCAACCG	
SMc00033-l-f	ATATGAATTCAATCAGCGGGAGGCGTCCAT	*SMc00033* left flanking region

SMc00033-l-r	ATATTCTAGAGCCGGGCTCCAGGGATAGGACA	
SMc00033-r-f	ATATTCTAGAGCAGCGATGCAAGGCGACCTTT	*SMc00033* right flanking region
SMc00033-r-r	ATATGGTACCGCAGCACGACAGCCTTGAAAT	
SMc00038-l-f	ATATGGATCCTTCGAGTACGGCAAAGGCTTT	*SMc00038* left flanking region
SMc00038-l-r	ATATTCTAGAGCGAAACCTCCGAAGGAGC	
SMc00038-r-f	ATATTCTAGATGTTCGCGAGCGGCGGTAA	*SMc00038* right flanking region
SMc00038-r-r	ATATAAGCTTATCATTTCGTTCGGCGGC	
SMc00887-l-f	ATATGGATCCAATGGCGAGGAGCTCGAC	*SMc00887* left flanking region
SMc00887-l-r	ATATTCTAGATGTCATTGCGATCCGCTCCG	
SMc00887-r-f	ATATTCTAGAGAGCATTATCGGGATACGGC	*SMc00887* right flanking region
SMc00887-r-r	ATATAAGCTTTTCGCCTCCATGTGGATG	
SMc00992-l-f	ATATGGTACCACTATGCGCCGGAATGGC	*SMc00992* left flanking region
SMc00992-l-r	ATATTCTAGAGGTCACTGGAAGTTCGCC	
SMc00992-r-f	ATATTCTAGACGTCACTTGCGAGAGAAGGA	*SMc00992* right flanking region
SMc00992-r-r	ATATGAATTCTGCCGTCGCAAAGGCAGAA	
SMc03178-l-f	ATATGGATCCTCGCCGCTCGTCGGCTTTT	*SMc03178* left flanking region
SMc03178-l-r	ATATTCTAGAGGTGCGGCTTCAAAAGAGCAAT	
SMc03178-r-f	ATATTCTAGACCGGTATCCGGCCCTCGCCTTCA	*SMc03178* right flanking region
SMc03178-r-r	ATATAAGCTTGTCGCGGGCGTGTGCGTTATGG	
SMc03942-l-f	ATATGGATCCTTCACGACCACATCCTGGC	*SMc03942* left flanking region
SMc03942-l-r	ATATTCTAGAGAACCTAAGCTCCGGTTTGC	
SMc03942-r-f	ATATTCTAGACGCCTTTCGGCGCAATGT	*SMc03942* right flanking region
SMc03942-r-r	ATATAAGCTTTTGTCCTCCAGGCGACAGCATT	
SMa0369-l-f	ATATGAATTCCTGGAGCCTCGGTCGGGA	*SMa0369* left flanking region
SMa0369-l-r	ATATTCTAGATCTGAGCCTCAACGGCCGC	
SMa0369-r-f	ATATGAATTCCTGGAGCCTCGGTCGGGA	*SMa0369* right flanking region
SMa0369-r-r	ATATTCTAGATCTGAGCCTCAACGGCCGC	
SMc00507-l-f	ATATGAATTCAATATTTCTCTCCGGGCCAGAA	*SMc00507* left flanking region
SMc00507-l-r	ATATTCTAGATCTTCACATGGAGCACCTAAAG	
SMc00507-r-f	ATATTCTAGACCTTGAGTGAAGGACCGGAT	*SMc00507* right flanking region
SMc00507-r-r	ATATAAGCTTTGTGCCTCATATATCCAATCGC	
pilA1-l-f	ATATGAATTCTTAGGGTGAGGGGCAGGG	*pilA1* left flanking region
pilA1-l-r	ATATTCTAGAGACTATTCTCCTCAAACTTCACTTGT	
pilA1-r-f	ATATTCTAGACGGCCGACTGATTACCTAACA	*pilA1* right flanking region
pilA1-r-r	ATATAAGCTTAGGTTGATCAGGAATTGGTGTAA	
pleD-f	ATATTCTAGAATGACTGCGCGCATCCTC	*pleD* ORF cloning
pleD-r	ATATAAGCTTTCAGGCAGCGGCAGCGAC	
pleD-GGAAF-f	gccgccTTCGTGGTCGTCATGCCGGATA	*pleD* GGEEF mutation to GGAAF
pleD-GGAAF-r	TGACGACCACGAAggcggcCCCGCCGAAACGGCAGGCA	
SMa2301-f	ATATTCTAGAATGCAACTCGCGAGTTCAT	*SMa2301* ORF cloning
SMa2301-r	ATATGTCGACCTATACGGTCGGCAGATCG	
SMb20389-f	ATATAAGCTTATTAAAGAGGAGAAATCTAGAATGGGAC AAGCCGTACGAATC	*SMb20389* ORF cloning, usage of XbaI recognition site
SMb20389-r	ATATGGTACCTCAGGCGTCGAGGATGACGAA	
SMb20523-f	ATATGTCGACATTAAAGAGGAGAAATCTAGAATGGGC GGTGCGATCTCACTT	*SMb20523* ORF cloning, usage of XbaI recognition site
SMb20523-r	ATATGGTACCTTACGTCGGTCGATACTTTCCA	
SMc01464-f	ATATTCTAGAATGGCTGAAACGCAAGCCG	*SMc01464* ORF cloning
SMc01464-r	ATATAAGCTTTCACGCCGAGTGGCGCAGTT	
SMc04015-f	ATATTCTAGAATGGGCCTGCAAGCCGCA	*SMc04015* ORF cloning
SMc04015-r	ATATGTCGACCTAAGGGGTCTGATCCGGG	

SMa0137-f	ATATTCTAGAATGAGGTTTGTGGCTGGCAAA	*SMa0137* ORF cloning
SMa0137-r	ATATGGTACCTCATGAGCGTGAGCTAGAAGAG	
SMa1548-f	ATATTCTAGAATGAACGCACCGACGCCC	*SMa1548* ORF cloning
SMa1548-r	ATATGGTACCTTAGAGGGAACCGGCCGC	
SMb20900-f	ATATTCTAGAATGCTCGCCTATGACGGGGGA	*SMb20900* ORF cloning
SMb20900-r	ATATAAGCTTTTAAGCCCCTACCCGATGC	
SMc00033-f	ATATAAGCTTATTAAAGAGGAGAAATCTAGAATGTCGGCCGCCCCCGCAGAAAT	*SMc00033* ORF cloning, usage of XbaI recognition site
SMc00033-r	ATATGGTACCTCAGGCGCGGTCGAGGGGCT	
SMc00038-f	ATATTCTAGAATGGCAAGAAAAACGTCCC	*SMc00038* ORF cloning
SMc00038-r	ATATAAGCTTTCACCGCCGCTCAGAGGAAA	
SMc00074-f	ATATTCTAGAATGCCCCTGACCCGTAAG	*SMc00074* ORF cloning
SMc00074-r	ATATGGATCCTCAAGCCCGCTTCATCAG	
SMc00887-f	ATATTCTAGAATGACATTGCAGGAACTCGGTA	*SMc00887* ORF cloning
SMc00887-r	ATATAAGCTTTCAGCGCACCGCCGTATC	
SMc00992-f	ATATTCTAGAATGACCCTCGGCAAGCGT	*SMc00992* ORF cloning
SMc00992-r	ATATAAGCTTTCAGCGCCGGCTTGCAGC	
SMc03178-f	ATATTCTAGAATGTCCCCTCTGTCGCGCTTTCTC	*SMc03178* ORF cloning
SMc03178-r	ATATAAGCTTTCAGGCGGCGCGCCACCG	
SMc03942-f	ATATTCTAGAATGACCCGCAATGAACGGG	*SMc03942* ORF cloning
SMc03942-r	ATATGGTACCTCAGGCGGTCCGCTCCGC	
SMb21517-f	ATATAAGCTTATTAAAGAGGAGAAATCTAGAATGGAACATCTGAGAAGATTCGA	*SMb21517* ORF cloning, usage of XbaI recognition site
SMb21517-r	ATATGGTACCCTAGGAATGCAGCTGCCG	
SMc00507-f	ATATAAGCTTATTAAAGAGGAGAAATCTAGAATGGTTTACAAGGACAGTGTTCAG	*SMc00507* ORF cloning, usage of XbaI recognition site; usage of HindIII recognition site for combined overexpression with *pleD*
SMc00507-r	ATATGGTACCTCAGCGAAAGAACTTGTAATAGGA	
SMc00999-f	ATATTCTAGAATGTTCTCCTTCCAGCATGCG	*SMc00999* ORF cloning
SMc00999-r	ATATGGTACCTTACAGAAACTCGGTCAGTGCCT	
SMc00507-RxxxR-f	gccgcctcttcggccGAAGAGACCAAGATAACCGGAA	*SMc00507* mutation to R9A/R13A
SMc00507-RxxxR-r	TCTTGGTCTCTTCggccgaagaggcggcctgaacactgtccttgtaaaccat	
SMc00507-DxSxxG-f	gcccttgccgacgaagccATCTGCTTCAGGCTGCTCTTC	*SMc00507* mutation to D35A/S37A/G40A
SMc00507-DxSxxG-r	GCCTGAAGCAGATggcttcgtcggcaagggcCACCACAATCCCGTTGGTC	
dgcA-f	ATATTCTAGAATGAAAATCTCAGGCGCCC	*dgcA* ORF cloning
dgcA-r	atatAAGCTTtcaAGCGCTCCTGCGCTTG	
yjhH-f	ATATTCTAGAATGATAAGGCAGGTTATCCAGC	*yjhH* ORF cloning
yjhH-r	ATATAAGCTTTTATAGCGCCAGAACCGCCGTATT	
12aa-oligo1	ATATACTAGTGGCTCGCCGGGCCTCCAGGAGTTCGGTACCTATA	12 aa linker for CYPet and YPet
12aa-oligo2	TATAGGTACCGAACTCCTGGAGGCCCGGCGAGCCACTAGTATAT	
SMc00507-FRET-f	ATATACTAGTCAGCTGTCTCAATCAGGTTTCGA	*SMc00507* as a linker for CYPet and YPet
SMc00507-FRET-r	ATATGGTACCGCGAAAGAACTTGTAATAGGACG	
His6-*SMc00507*-f	ATATGGATCCGTTTACAAGGACAGTGTTCAGCGT	*SMc00507* ORF cloning for N-terminal His6-tagging
His6-*SMc00507*-r	ATATAAGCTTTCAGCGAAAGAACTTGTAATAGGA	
His6-*SMc00074*-f	ATATGGATCCACCGTCATGCAGCACGCCTTTT	*SMc00074*$^{390-970}$ ORF cloning for N-terminal His6-tagging
His6-*SMc00074*-r	ATATGTCGACTCAAGCCCGCTTCATCAG	
P*exoY*-f	gttcaagcttTGCCTTGGGTGCTACCTCTTG	*exoY* upstream intergenic region
P*exoY*-r	tgctctagaCTTCATAGAGGTGACTCCAT	
P*SMc00507*-f	ATATAAGCTTCTACTGCATGTTTCCTTTAATCG	*SMc00507* upstream intergenic regions
P*SMc00507*-TTG-r	ATATTCTAGACAGCTGCAATCTTCACATGGA	
P*SMc00507*-ATG-r	ATATTCTAGAGTAAACCATGGTTTCCTCGAA	

P*SMc01794*-f	ATATCTGCAGCGAACATCCGGTCGGAGC	*SMc01794* upstream intergenic region
P*SMc01794*-r	ATATTCTAGAGGTCGACATGCGGTTAGG	
egfp-f	ggaggagctcttaagcttgtatctagactgcagATGGTGAGCAAGGGCGAGG	*egfp* ORF cloning
egfp-r	gtacggtaccTTACTTGTACAGCTCGTCCATG	
pleD-valid	AAACTGCTTTCGGCGGCCT	validation of integration site for *pleD* mutation, combined with PCR1
SMa2301-valid	AGATCAGGTAAAGGCCGAGC	validation of integration site for *SMa2301* mutation, combined with PCR1
SMb20389-valid	CGCATTCTGTAGTTGGTTATGG	validation of integration site for *SMb20389* mutation, combined with PCR1
SMb20523-valid	AGATAATCCGCCGCCGTG	validation of integration site for *SMb20523* mutation, combined with PCR1
SMc01464-valid	AAATCGAACAGGCTCGAAGC	validation of integration site for *SMc01464* mutation, combined with PCR1
SMc04015-valid	TAGTCGATGTCGATGATCGC	validation of integration site for *SMc04015* mutation, combined with PCR1
SMa0137-valid	ACCAGATCGCTCTCGCCG	validation of integration site for *SMa0137* mutation, combined with PCR1
SMa1548-valid	AACTTGCGGGTGAAGGTCGA	validation of integration site for *SMa1548* mutation, combined with PCR1
SMb20447-valid	CCCAATGTGTCGTTGATCG	validation of integration site for *SMb20447* mutation, combined with PCR1
SMb20900-valid	GGCGAAGGATCACCATGC	validation of integration site for *SMb20900* mutation, combined with PCR1
SMc00033-valid	CGCAGAGCAGTTTCAAGCC	validation of integration site for *SMc00033* mutation, combined with PCR1
SMc00038-valid	GCTTCGATCAGCAGCACCA	validation of integration site for *SMc00038* mutation, combined with PCR1
SMc00074-valid	AAGGCGTTGACGGTATCCTT	validation of integration site for *SMc00074* mutation, combined with PCR1
SMc00887-valid	CTCAAGCTGCGTCGCGTG	validation of integration site for *SMc00887* mutation, combined with PCR1
SMc00992-valid	TTTCTGACGCAGCATCTGG	validation of integration site for *SMc00992* mutation, combined with PCR1
SMc03141-valid	GAAGCCTGGCGATCTGAGCT	validation of integration site for *SMc03141* mutation, combined with PCR1
SMc03178-valid	TTCCGGACCGGTGGTTAC	validation of integration site for *SMc03178* mutation, combined with PCR1
SMc03942-valid	ATCATCATCGGCAGGCAG	validation of integration site for *SMc03942* mutation, combined with PCR1
SMb21517-valid	GAAACTGTCCAAGGCGATTCT	validation of integration site for *SMb21517* mutation, combined with PCR1
SMc00999-valid	CCCTTCATGTTACCCAGCCT	validation of integration site for *SMc00999* mutation, combined with PCR1
SMc01792-valid	ATCGAATAGGCGATGAATGC	validation of integration site for *SMc01792* mutation, combined with PCR1
PCR1	CGGGCCTCTTCGCTATT	Standard sequencing primer 1
PCR2	TTAGCTCACTCATTAGG	Standard sequencing primer 2
SMc03942-seq	TGGCGCTCGACCCGGAAAGAA	*SMc03942* sequencing primer
SMa0137-seq	GAAACGATAGGATATTTCTCC	*SMa0137* sequencing primer
SMa1548-seq1	CGCAAAGCGTGACGCGGATGG	*SMa1548* sequencing primer 1
SMa1548-seq2	ACCATCACGGAAAGCCGTTCCGAAT	*SMa1548* sequencing primer 2
SMa1548-seq3	TACAACCTCTGACGGAGTCAC	*SMa1548* sequencing primer 3
SMb20900-seq	AAGCGAGGGAGAGCTCCTTC	*SMb20900* sequencing primer
SMc00033-seq	TGCGATCTGATGCAGGGCTA	*SMc00033* sequencing primer
SMc00038-seq	TGCGCTGCCATCCATGGCCTCG	*SMc00038* sequencing primer
SMc00992-seq	CAGATGCTGCGTCAGAAAGTACT	*SMc00992* sequencing primer
SMc03178-seq1	TCTTCGGCGACCCGGCAATCT	*SMc03178* sequencing primer 1
SMc03178-seq2	CGCTTCCTGGACCAGATTCT	*SMc03178* sequencing primer 2
SMc00074-seq1	ACGCCTACAAGGATACCGTCA	*SMc00074* sequencing primer 1
SMc00074-seq2	ATCGTGCTCCTGATCGGC	*SMc00074* sequencing primer 2
SMc00074-seq3	ACAATGTCCTCATCGCGCT	*SMc00074* sequencing primer 3

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Supplemental figures

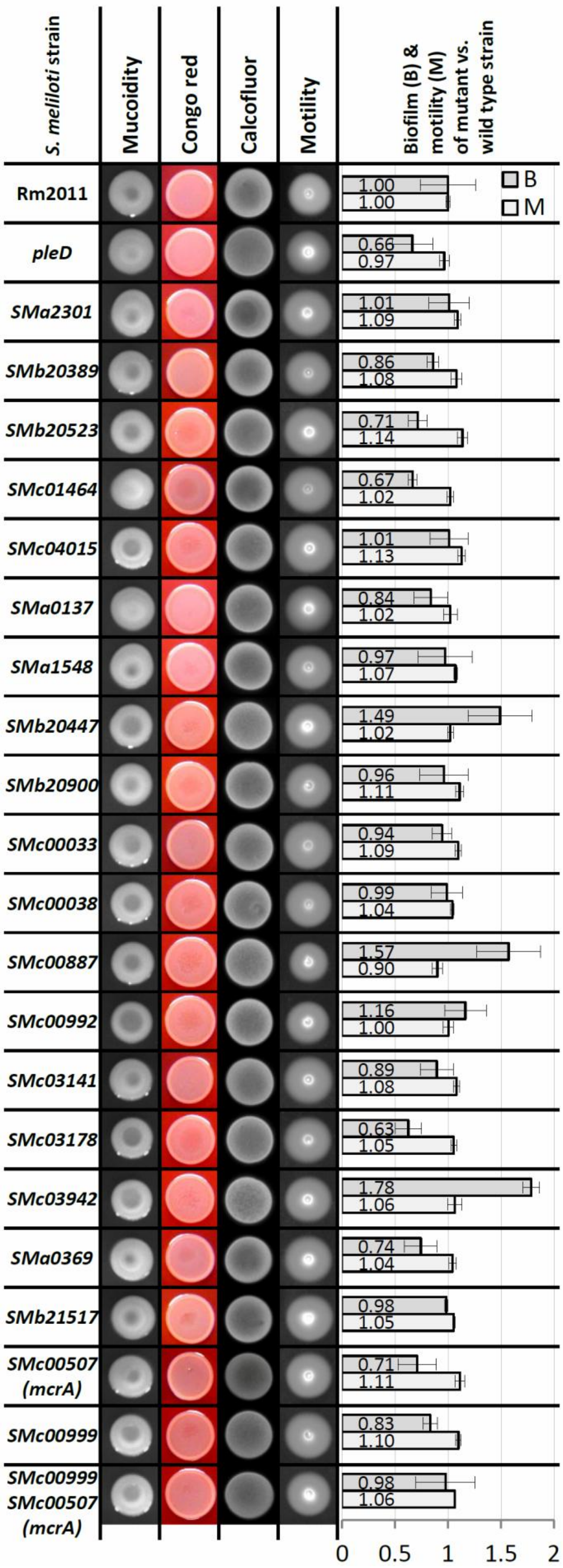


FIG S1 Phenotypic analysis of gene knock-outs in Rm2011. Error bars indicate standard deviations of three biological replicates. The A_{570}/OD_{600} mean value for Rm2011 was 0.131±0.034 (set to 1).

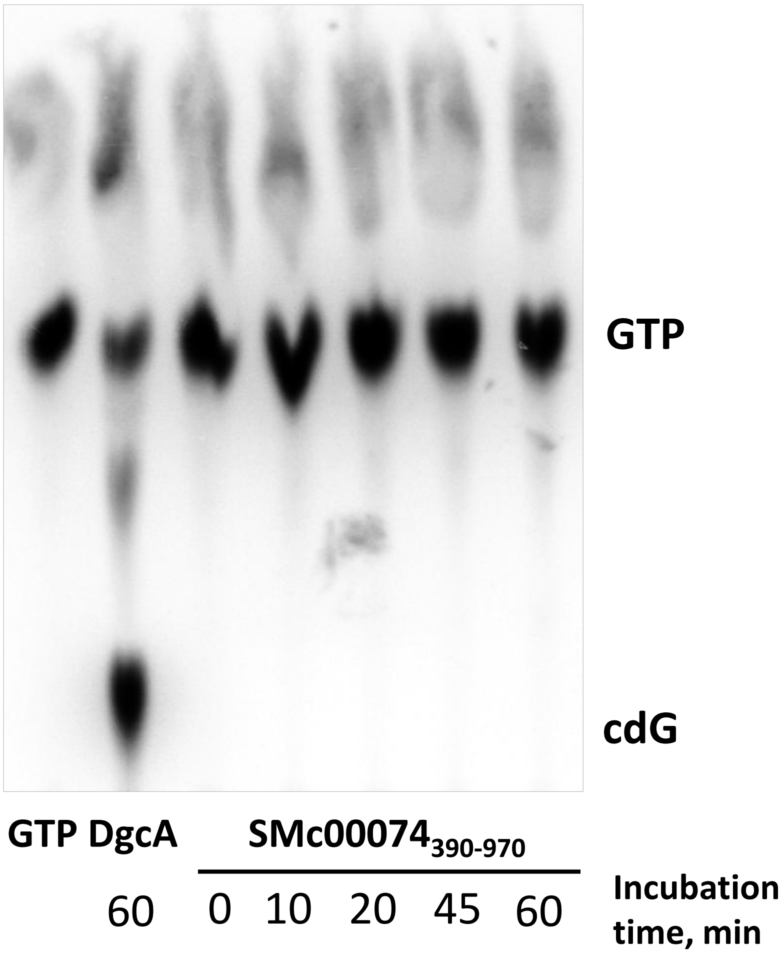


FIG S2 Assay for DGC activity of the C-terminal portion of SMc00074 containing the predicted DGC and PDE domains. Thin-layer chromatography of reaction mixtures containing radiolabelled GTP and purified protein. Absence of a cdG signal and unchanged intensity of the GTP band indicate that SMc00074$_{390-970}$ does not have DGC activity.

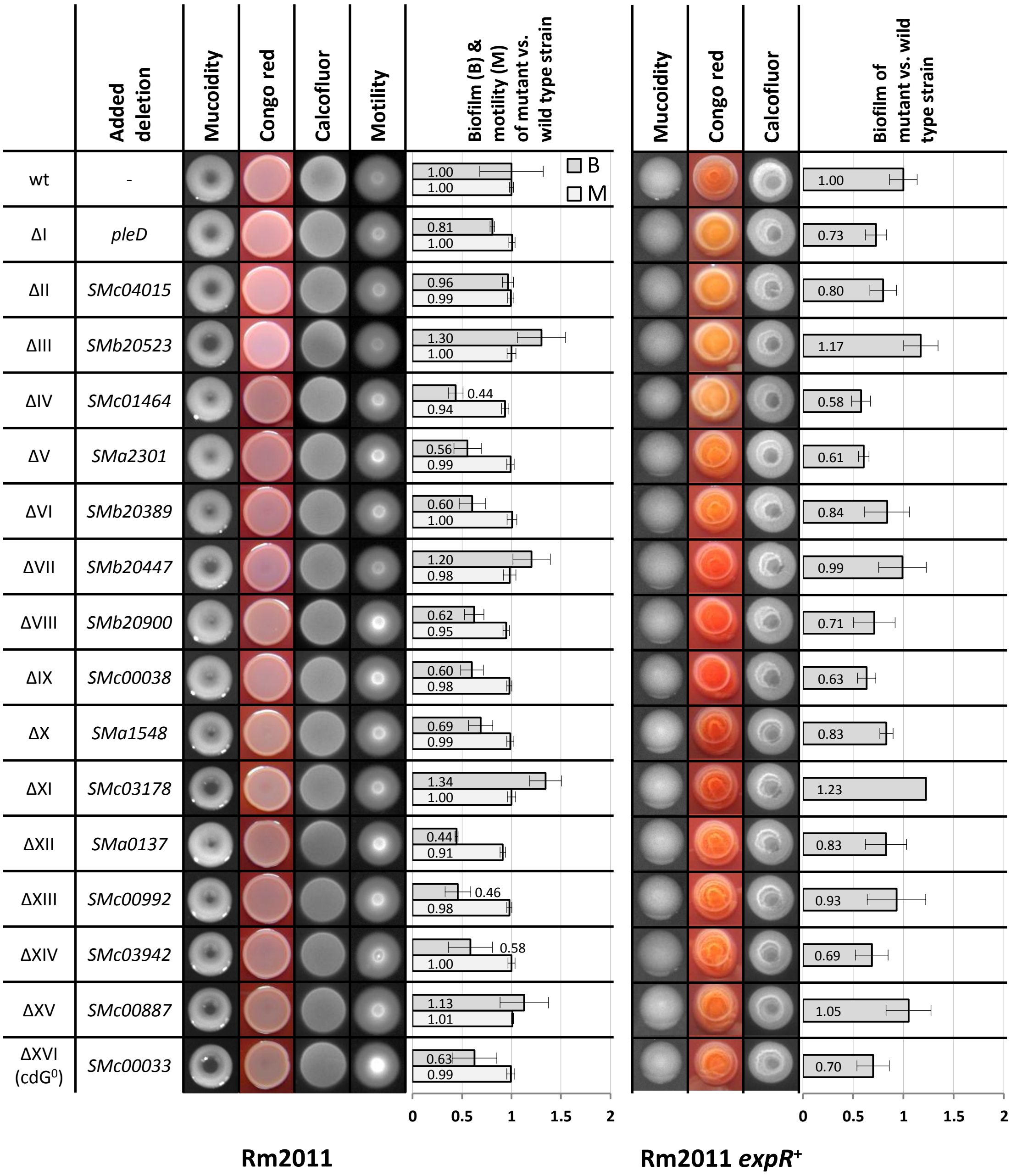


FIG S3 Phenotypic analysis of Rm2011 and Rm2011 *expR*$^{+}$ carrying multiple markerless deletions of DGC/PDE genes. Error bars indicate standard deviations of three biological replicates. The A_{570}/OD_{600} mean values for Rm2011 and Rm2011 *expR*$^{+}$ were 0.110±0.035 and 0.343±0.047, respectively (set to 1).

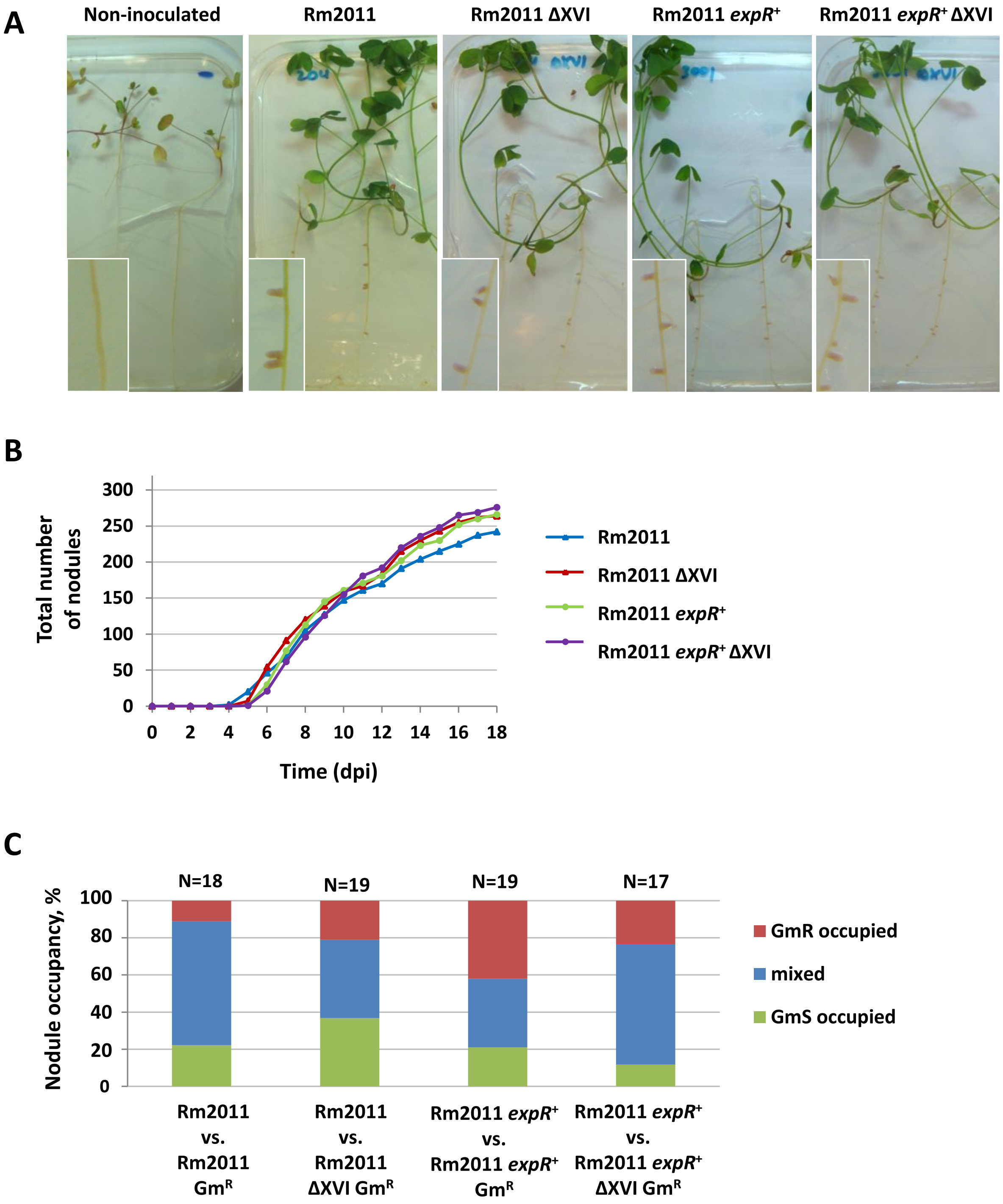


FIG S4 Symbiotic phenotype of cdG^0 strains. (A) Macroscopic appearance of *M. sativa* plants with root nodules formed by cdG^0 strains or corresponding parental strains 24 days post inoculation. Pink nitrogen-fixing nodules indicate efficient symbiosis. (B) Nodulation kinetics, determined for 32 plants per strain. (C) Competitive nodulation assay. Before inoculation, strains were mixed 1:1. N, number of analyzed nodules.

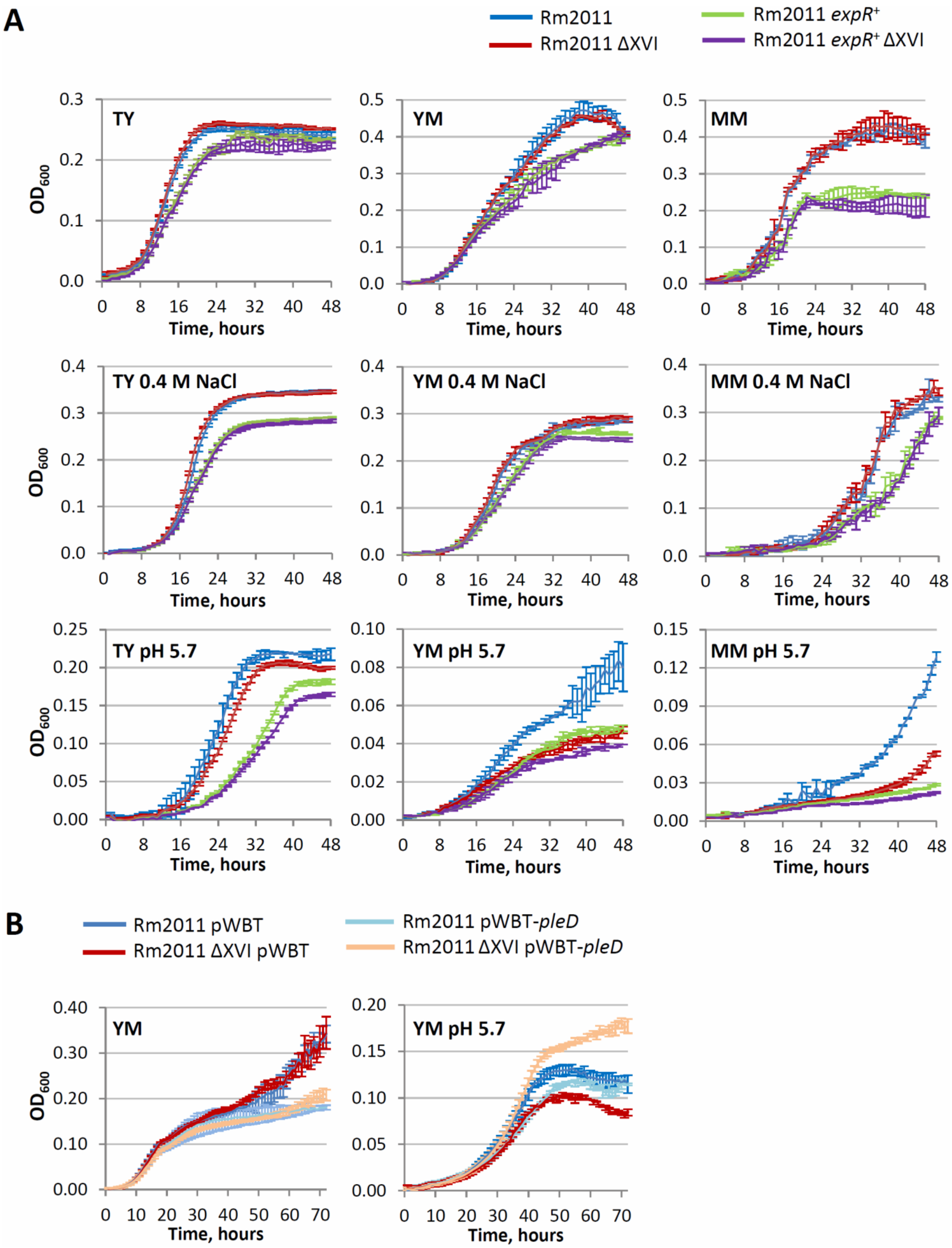


FIG S5 Growth of cdG^0 strains in different media. (A) Growth under normal, high-salt and acidic conditions. (B) Complementation of the growth defect of the Rm2011 cdG^0 strain at pH 5.7 by pWBT-*pleD* in presence of 100 µM IPTG. Error bars indicate standard deviations of three biological replicates.

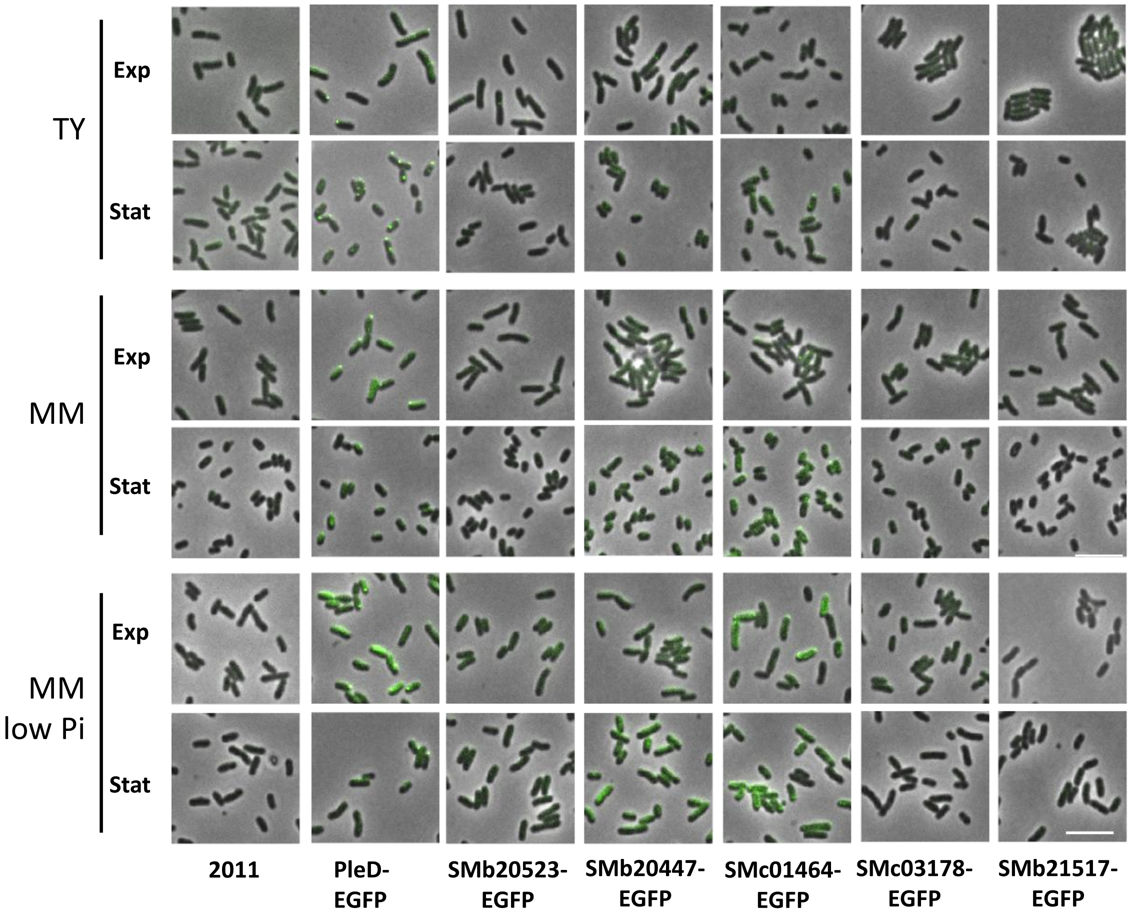


FIG S6 Detection of EGFP-tagged putative DGC/PDEs in Rm2011 cells grown on TY, MM or phosphate-limiting MM. Exp, exponential growth phase (OD_{600} of 0.6 to 0.8); Stat, stationary growth phase (after 40 h of growth). Scale bar, 5 µm. All images were taken using identical settings.

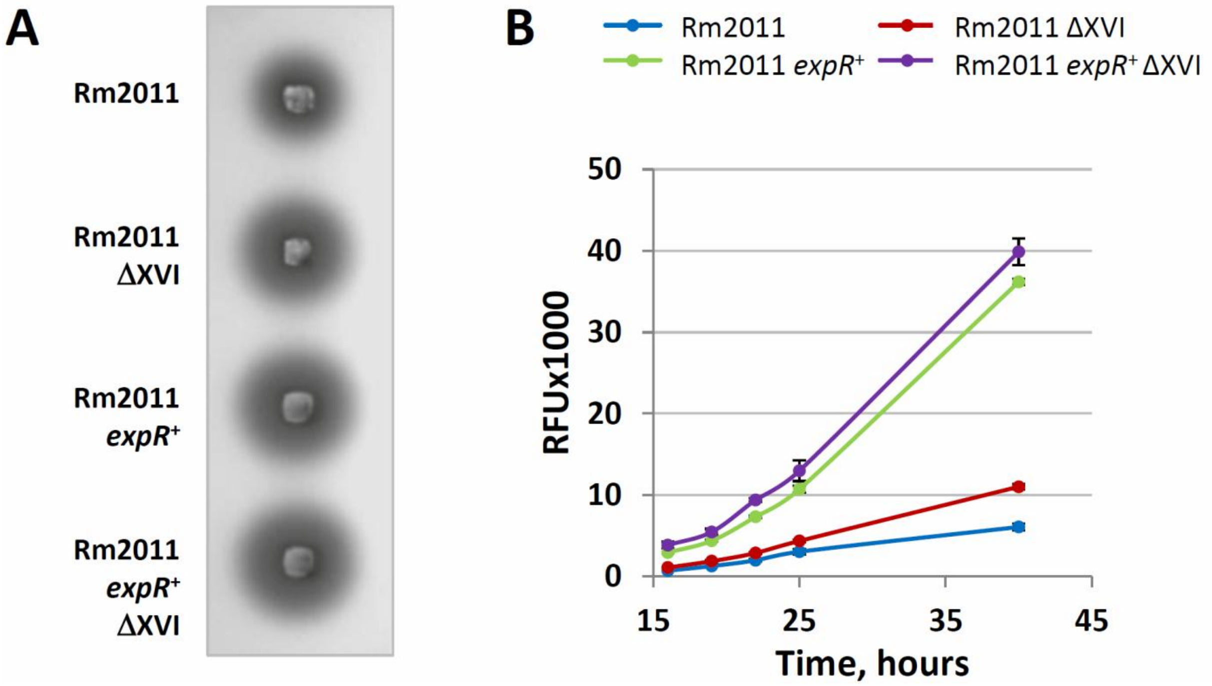
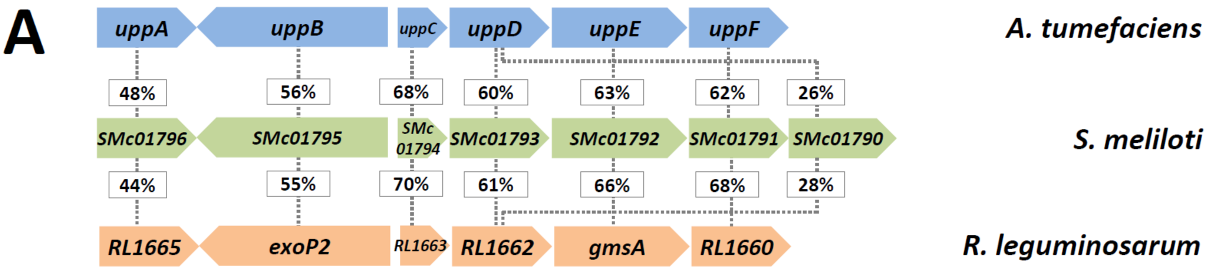


FIG S7 cdG negatively affects AHL production at the level of *sinI* transcription. (A) Semiquantitative detection of AHLs in supernatants of *S. meliloti* stationary phase cultures by *A. tumefaciens* NTL4 (pZLR4). (B) *sinI* promoter activity determined using a P_{sinI}-*egfp* fusion. RFU, relative EGFP fluorescence units. Error bars indicate standard deviations of three biological replicates.



SMc01790: Putative glycosyltransferase
SMc01791: Conserved hypothetical transmembrane protein
SMc01792: Putative surface polysaccharide biosynthesis glycosyltransferase
SMc01793: Putative glycosyltransferase
SMc01794: Putative polysaccharide export system periplasmic transmembrane protein
SMc01795: Surface polysaccharide transport protein
SMc01796: Conserved hypothetical protein

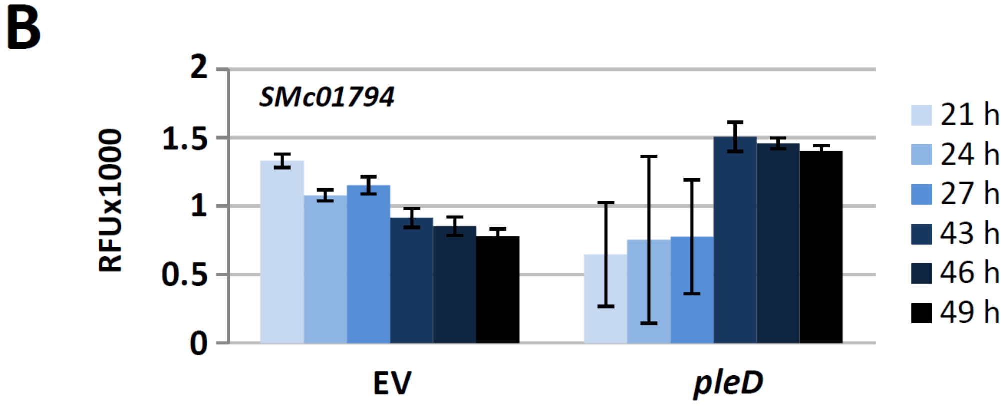


FIG S8 *S. meliloti SMc01790-SMc01796* gene cluster: architecture and expression upon overproduction of PleD. (A) Homology of the *SMc01790-SMc01796* gene cluster to the *A. tumefaciens upp* and *R. leguminosarum RL1660-RL1665* gene clusters. Percent numbers indicate amino acid sequence identities. (B) *SMc01794* promoter activity during growth in 30 % MM with or without *pleD* overexpression determined using a promoter-*egfp* fusion. EV, empty vector. RFU, relative EGFP fluorescence units. Error bars indicate standard deviations of three biological replicates.

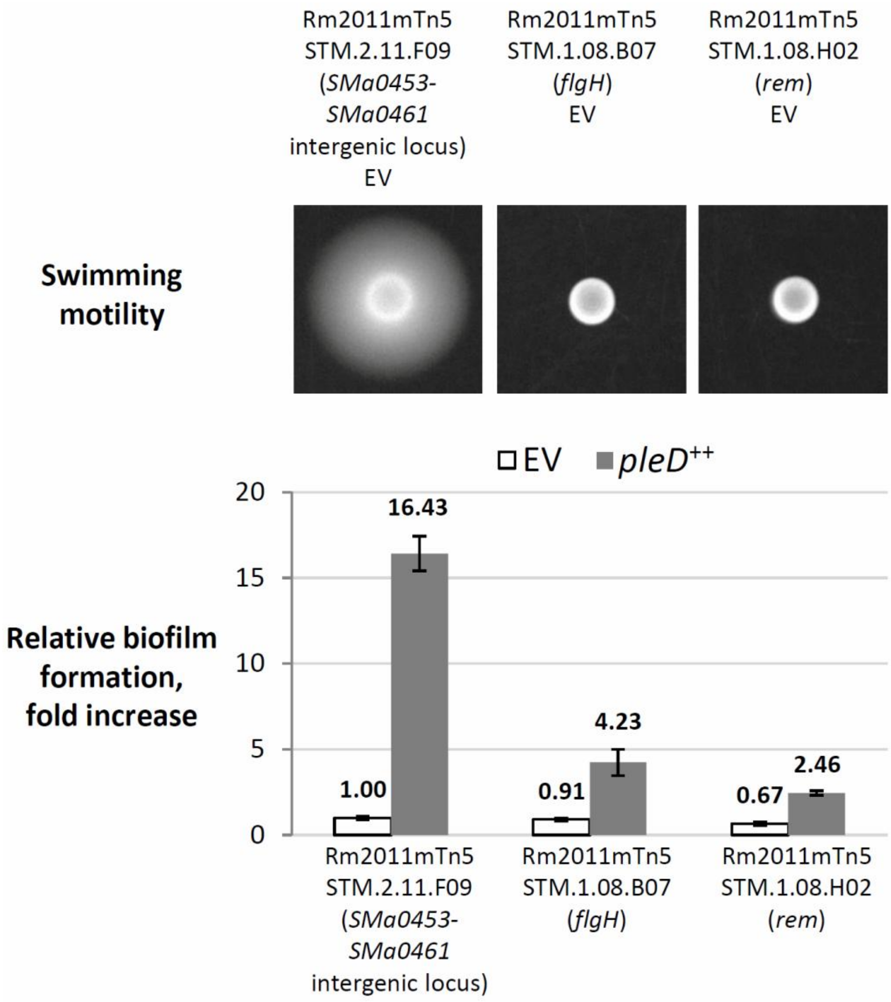
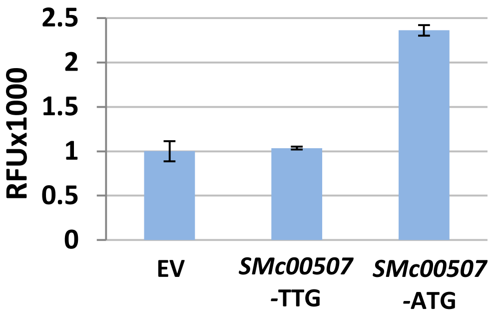


FIG S9 Biofilm formation of motility-deficient strains upon *pleD* overexpression. EV, empty pWBT vector. The A_{570}/OD_{600} mean value for Rm2011mTn5STM.2.11.F09 pWBT was 0.338±0.038 (set to 1). This control strain carrying an intergenic mTn5STM insertion is not affected in any phenotype analyzed in this study. Error bars represent standard deviations of three biological replicates.

A



***S. meliloti* chromosome 1905425-1905482**

```
TGTGAAGATTTGCAGCTGTCTCAATCAGGTTTCGAGGAAACCATGGTTTACAAGGACAG
```

Annotated start

Experimentally verified start

B

```
         10         20         30         40         50
MVYKDSVQRA SSREETKITG TVTCKTGSTN GIVVDLSDEG ICFRLLFDIG

         60         70         80         90        100
ARTGQEVTIR SEELGFLSGM VRWARGDRIG IKLNLSSNTA AQISSYYKFF

101
R
```

FIG S10 Experimental validation of the SM*c00507 (mcrA)* start codon. (A) *mcrA* promoter activity determined using promoter fusions to *egfp*, which were generated either using the annotated start codon TTG or the alternative start codon ATG. EV, empty vector. RFU, relative EGFP fluorescence units. Error bars indicate standard deviations of three biological replicates. (B) SMc00507 amino acid sequence with the ATG as translation start. RxxxR and DxSxxG motifs are indicated.

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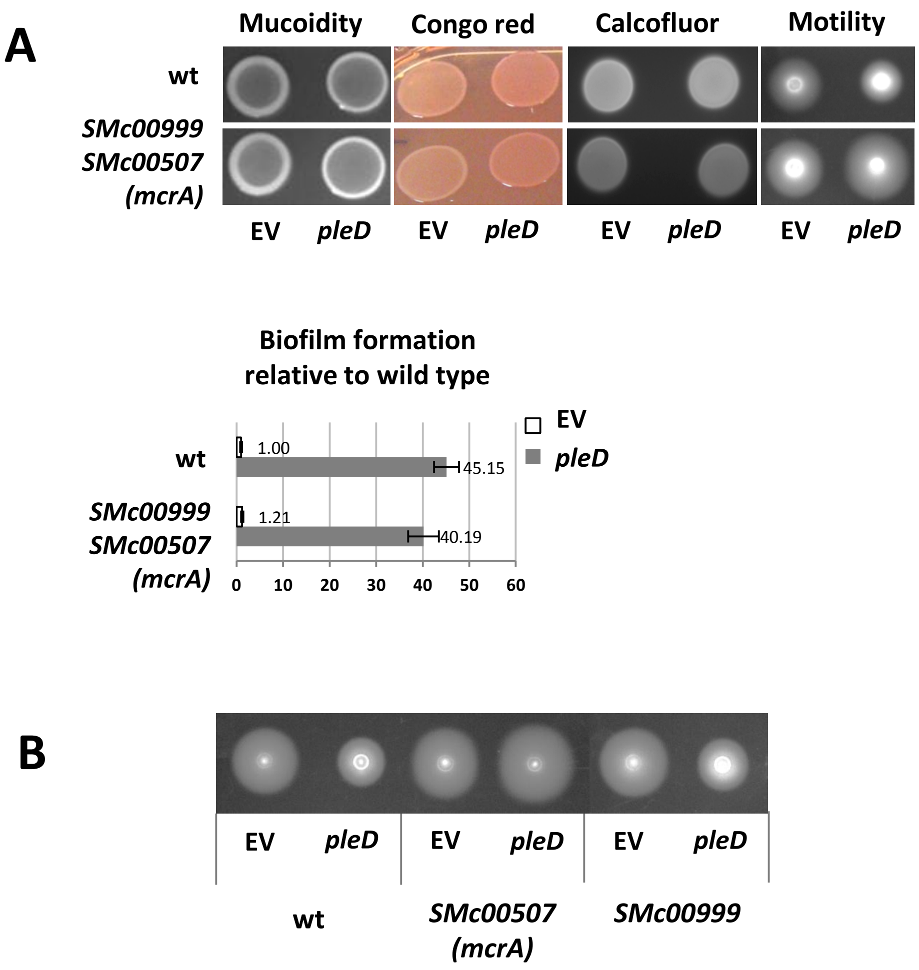

FIG S11 Identification of McrA (SMc00507) as a PilZ domain cdG receptor mediating repression of swimming motility upon *pleD* overexpression. (A) Phenotypic analysis of a double mutant in the PilZ domain protein-encoding genes *SMc00507* and *SMc00999* with or without *pleD* overexpression. Error bars indicate standard deviations of three biological replicates. The A_{570}/OD_{600} mean value for Rm2011 pWBT was 0.087±0.020 (set to 1). (B) Effect of *pleD* overexpression on motility of single mutants in *SMc00507* and *SMc00999*. EV, empty pWBT vector.