Construction of strains and plasmids. Oligonucleotide primers used for genetic constructions are indicated in Table S3. All genetic constructs and chromosomal integrations were validated by DNA sequencing. Chromosomal integrations of *mVENUS* fusions to *dgcZ* wild-type or mutant variants were generated by standard λRED-mediated recombineering (Datsenko and Wanner, 2000). The wild-type *dgcZ* gene was first replaced with a construct carrying the kanamycin resistance cassette fused to a counter-selectable marker (the L-rhamnose inducible *ccdB* toxin gene) and then it was substituted with PCR-generated fusions of *dgcZ* wild-type or mutant variants to *mVENUS*. Selection was performed on minimal medium agar plates containing 0.2 % L-rhamnose. *mCHERRY* fusions to *frdA* and *frdB* were generated using the same approach. The *dgcZ*(+) and *frdA*(+) strains were obtained by replacing the *PRham-ccdB-kan* element with the *dgcZ* and *frdA* genes amplified from *E. coli* MG1655. The Δ*frdA*^{Frt} strain was obtained by replacing the *PRham-ccdB-kan* element with *frdA::Frt* obtained by amplification from Δ*frdA* strain of the Keio collection, after removal of the kanamycin resistance cassette. All integrations generated by λRED-mediated recombineering were tested by DNA sequencing. To generate the *dgcZ-mVENUS* fusion, *mVENUS* was amplified from the plasmid template pDL-mVENUS (D. Cavalcanti De Lucena, unpublished) with the primers P3_dgcZ_mVenus_optim and P4_mVenus_rev, and *dgcZ* with SacI_NcoI_dgcZ_FWd and P2 _dgcZ_mVenus. The *dgcZ* fragment was then digested with SacI and EcoRI, and *mVENUS* with EcoRI and HindIII. Digested *dgcZ* and *mVENUS* fragments were inserted into the pBAD18 plasmid using the SacI and HindIII restriction sites. The resulting plasmid pBAD-DgcZ-mVENUS was used as template for PCR of the *dgcZ-mVENUS* construct applied for recombineering. As result of this cloning strategy, a DNA sequence of 93 nucleotides coding for a flexible linker of 31 amino acids (sequence provided by D. Cavalcanti De Lucena and indicated in Table S3) was inserted between *dgcZ* and the *mVENUS* coding region.

The plasmid carrying *yfbR-mCHERRY* was obtained by cloning *yfbR* and *mCHERRY* into pCJ30 (Bibikov et al., 1997). *yfbR* was amplified using yfbR_BamHI_fwd and yfbR_KpnI_rev primers and subsequently cutted with BamHI and KpnI. *mCHERRY* was amplified from pWBP20911mCherry (Charoenpanich et al., 2015) using mCHERRY_KpnI_fwd and P4_mVENUS_rev and cutted with KpnI and HindIII. Digested *yfbR* and *mCHERRY* fragments were inserted into the pCJ30 vector, cutted with KpnI and HindIII.

For bacterial two-hybrid (BACTH) assays, bait and prey sequences were inserted into vectors pKT25, pKNT25, pUT18, pUT18C (Battesti and Bouveret, 2012) using the restriction sites for BamHI and KpnI present on the plasmids.

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Charoenpanich P, Soto MJ, Becker A, McIntosh M. 2015. Quorum sensing restrains growth and is rapidly inactivated during domestication of *Sinorhizobium meliloti*. Environ Microbiol Rep **7:**373-382

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MS-based protein identification. Samples were digested in gel by the addition of Sequencing Grade Modified Trypsin (Promega) and incubation at 37 °C overnight, according to the manufacturers protocols. Peptides were eluted by using 200 µL MeCN for 30 min in a sonic-bath. Subsequently, the supernatant was transferred to a fresh tube and after evaporation of the solvent in a vacuum concentrator dry samples were dissolved in 25 µL 10 % acetonitrile/0.1 % TFA.

The mass spectrometric analysis of the samples was performed using an Orbitrap Velos Pro mass spectrometer (ThermoScientific). An Ultimate nanoRSLC-HPLC system (ThermoScientific), equipped with a home-made nano 1.8 µm C18 RP column was connected online to the mass spectrometer through a nanospray ion source. Depending on sample concentration, 1-15 µL of the tryptic digest were usually injected onto a C18 pre-concentration column. Automated trapping and desalting of the sample was performed at a flowrate of 6 µL/min using water/0.05 % formic acid as solvent.

Separation of the tryptic peptides was achieved with the following gradient of water/0.045 % formic acid (solvent A) and 80 % acetonitrile/0.05 % formic acid (solvent B) at a flow rate of 300 nL/min: holding 4 % B for five minutes, followed by a linear gradient to 45 % B within 30 minutes and linear increase to 95 % solvent B in additional 5 minutes. The column was connected to a stainless steel nanoemitter (ThermoScientific) and the eluent sprayed directly towards the heated capillary of the mass spectrometer using a potential of 2300 V. A survey scan with a resolution of 60000 within the Orbitrap mass analyzer was combined with at least three data-dependent MS/MS scans with dynamic exclusion for 30 s either using CID with the linear ion-trap or using HCD and orbitrap detection at a resolution of 7500.

Data analysis was performed using Proteome Discoverer (ThermoScientific) with SEQUEST and MASCOT (version 2.2; Matrix science) search engines using a user defined database containing the sequence of the protein of interest.

TABLE S1 Bacterial strains

Strain designation	Genotype	Reference
MG1655	Wt	(Blattner et al., 1997)
Tr1-5	*csrA::Tn5*(kan)	(Romeo et al., 1993)
AB400	*csrA::Tn5*(kan)	(Boehm et al., 2009)
GL60	*ibpA–mCherry::Frt-kan-Frt*	(Li and Young, 2012)
AB1299	*csrA::Tn5*(kan), *dgcZ*(E208Q)	(Zähringer et al., 2013)
AB1484	*csrA::Tn5*(kan), *dgcZ*(E208Q), *pgaD-3xFlag*	(Zähringer et al., 2013)
BTH101		(Battesti and Bouveret, 2012)
AB3348	*dgcZ::PRha_ccdB-kan*	This work
AB3320	*csrA::Tn5*(kan), *dgcZ*(+)	This work
AB3367	*csrA::Tn5*(kan), *dgcZ-mVENUS*	This work
AB3401	*csrA::Tn5*(kan), *dgcZ*(+), *pgaD-3xFlag*	This work
AB3402	*csrA::Tn5*(kan), *dgcZ-mVENUS*, *pgaD-3xFlag*	This work
AB3321	*csrA::Tn5*(kan), Δ*dgcZ::Frt*	This work
AB3403	*csrA::Tn5*(kan), *dgcZ-mVENUS, ibpA-mCHERRY*	This work
AB3411	*csrA::Tn5*(kan), *dgcZ-mVENUS*, *frdA-mCHERRY*	This work
AB3405	*csrA::Tn5*(kan), *dgcZ-mVENUS, frdB-mCHERRY*	This work
AB629	Δ*dgcZ*	This work
AB3369	Δ*cpxR*	This work
AB3372	Δ*dgcZ*, Δ*cpxR*	This work
AB3408	*csrA::Tn5*(kan), *dgcZ(H79L,H83L)-mVENUS*	This work
AB3409	*csrA::Tn5*(kan), *dgcZ*(H79L,H83L,E208Q)-*mVENUS*	This work
AB3412	*csrA::Tn5*(kan), *dgcZ*(H79L,H83L), *pgaD-3xFlag*	This work
AB3389	Δ*frdA*, *csrA::Tn5*(kan)	This work
AB3391	Δ*dgcZ*, Δ*frdA*, *csrA::Tn5*(kan)	This work
AB3390	Δ*frdB*, *csrA::Tn5*(kan)	This work
AB3413	*frdA::ccdB-kan*	This work
AB3414	*frdA::ccdB-kan, csrA::Frt*	This work
AB3415	*frdA*(+), *csrA::Tn5*	This work
AB3416	Δ*frdA*^{Frt}, *csrA::Tn5*	This work
AB1718 (Δ7)	Δ*pdeH*, Δ*pdeL*, Δ*pdeA*, Δ*pdeR*, Δ*pdeN*, Δ*pdeC*, Δ*pdeF*	This work
AB1872 (Δ9)	Δ*pdeH*, Δ*pdeL*, Δ*pdeA*, Δ*pdeR*, Δ*pdeN*, Δ*pdeC*, Δ*pdeF*, Δ*pdeB*, Δ*pdeI*	This work
AB3383	Δ*pdeH*, Δ*pdeL*, Δ*pdeA*, Δ*pdeR*, Δ*pdeN*, Δ*pdeC*, Δ*pdeF*, *pgaD-3xFlag csrA::Tn5*(kan)	This work

AB3384	Δ*pdeH,* Δ*pdeL,* Δ*pdeA,* Δ*pdeR,* Δ*pdeN,* Δ*pdeC,* Δ*pdeF,* Δ*pdeB,* Δ*pdeI, pgaD-3xFlag, csrA::Tn5*(kan)	This work
AB614	Δ*pdeI*	This work
AB613	Δ*pdeB*	This work
AB3420(Δ2)	Δ*pdeI,* Δ*pdeB*	This work
AB3424	Δ*pdeI, csrA::Tn5*(kan)*, pgaD-3xFlag*	This work
AB3425	Δ*pdeB, csrA::Tn5*(kan)*, pgaD-3xFlag*	This work
AB3426	Δ*pdeI,* Δ*pdeB, csrA::Tn5*(kan)*, pgaD-3xFlag*	This work
AB3427	Δ*pdeH,* Δ*pdeL,* Δ*pdeA,* Δ*pdeR,* Δ*pdeN,* Δ*pdeC,* Δ*pdeF,* Δ*pdeB,* Δ*pdeI, pgaD-3xFlag,* Δ*dgcZ*	This work
AB3428	Δ*pdeH,* Δ*pdeL,* Δ*pdeA,* Δ*pdeR,* Δ*pdeN,* Δ*pdeC,* Δ*pdeF,* Δ*pdeB,* Δ*pdeI, pgaD-3xFlag,* Δ*dgcZ, csrA::Tn5*(kan)	This work

TABLE S1 References

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TABLE S2 Plasmids

Plasmid name	Feature	Reference
pBAD18	Expression vector	(Guzman et al., 1995)
pDL-mVENUS	Vector used for amplification of mVENUS template	*
pWBP20911	Vector used for amplification of mCHERRY template	(Charoenpanich et al., 2015)
pDgcZ	Expression vector (pBAD18) for *dgcZ*	This work
pDgcZ-mVENUS	Expression vector (pBAD18) for *mVENUS* fusion to *dgcZ*	This work
pCJ30	Expression vector	(Bibikov et al., 1997)
pCJ30-YfbR-mCHERRY	Expression vector for *mCHERRY* fusion to *yfbR*	This work
pNlpE	Expression vector (pCJ30) for *nlpE*	This work
pKT25	Standard plasmid for bacterial two-hybrid	(Battesti and Bouveret, 2012)
pKNT25	Standard plasmid for bacterial two-hybrid	(Battesti and Bouveret, 2012)
pUT18	Standard plasmid for bacterial two-hybrid	(Battesti and Bouveret, 2012)
pUT18C	Standard plasmid for bacterial two-hybrid	(Battesti and Bouveret, 2012)
pUT18-zip	Standard plasmid for bacterial two-hybrid	(Battesti and Bouveret, 2012)
pKT25-zip	Standard plasmid for bacterial two-hybrid	(Battesti and Bouveret, 2012)
pKT25-DgcZ	Plasmid for bacterial two-hybrid	This work
pKT25-CZB	Plasmid for bacterial two-hybrid	This work
pKT25-GGDEF	Plasmid for bacterial two-hybrid	This work
pKT25-FrdA	Plasmid for bacterial two-hybrid	This work
pKT25-FrdB	Plasmid for bacterial two-hybrid	This work
pKNT25-DgcZ	Plasmid for bacterial two-hybrid	This work
pKNT25-FrdA	Plasmid for bacterial two-hybrid	This work
pKNT25-FrdB	Plasmid for bacterial two-hybrid	This work
pUT18-DgcZ	Plasmid for bacterial two-hybrid	This work
pUT18-FrdA	Plasmid for bacterial two-hybrid	This work

pUT18-FrdB	Plasmid for bacterial two-hybrid	This work
pUT18C-DgcZ	Plasmid for bacterial two-hybrid	This work
pUT18C-FrdA	Plasmid for bacterial two-hybrid	This work
pUT18C-FrdB	Plasmid for bacterial two-hybrid	This work

TABLE S2 References

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* Gifted from Daniella Cavalcanti de Lucena (unpublished)

TABLE S3 Primers

Primer name	Sequence	Use
dgcZ-pKD45_rev_new	GAATCGTTGACACAGTAGCA TCAGTTTTCTCAATGAATGT TAAACGGAGCacccggatat tatcgtgaggatgcG	Generation of *dgcZ::PRha-ccdb-kan* strain
dgcZ_KO_pKD45_fwd	Gatcaagaagacaacggaaa ttgatgccatcttgttaaat ctcaatTCATTTCGAACCCC AGAGTCCCGC	Generation of *dgcZ::PRha-ccdb-kan* strain
1651-dgcZ_test_fwd	Gccggaccagatgatcaaca ttagtgg	Generation of *dgcZ*(+) strain
1652-dgcZ_test_rev	tgactaatgaacggagataatccctcacc	Generation of *dgcZ*(+) strain
SacI_NcoI_dgcZ_FWd	tatagagctcGGAGTGCCAT GGatgATCAAGAAGACAACG GAAATTGATG	Generation of *dgcZ-mVENUS* strain and cloning in pBAD18
P2_dgcZ_mVenus	GGGGGAATTCGGTACCCATC CTAGGAATCTCCTTTCTAGA atgAACTCGGTTAATCACAT TTTGTTCGTCA	Generation of *dgcZ-mVENUS* strain and cloning in pBAD18
P3_dgcZ_mVenus_optim	Taccgaattccccctcgata tcgggcccggcctatctggc ctgggagctggacttccttc actgATGGTGAGCAAGGGCG AGGAG	Generation of *dgcZ-mVENUS* strain and cloning in pBAD18
P4_mVenus_rev	tataAAGCTTTTACTTGTAC AGCTCGTCCATGCCG	Generation of *dgcZ-mVENUS* strain and cloning in pBAD18
P0_dgcZ_fwd_recomb	ACTGTGAAAAAGGAGTGGCA ATGATCAAGAAGACAACGGA AATTGATG	Recombination of *dgcZ-mVENUS* strain and mutants
P5_mVenus_recomb	GAATCGTTGACACAGTAGCA TCAGTTTTCTCAATGAATGT TAAACGGAGCtataAAGCTT TTACTTGTACAGCTCGTCCA	Recombination of *dgcZ-mVENUS* strain and mutants
dgcZ_E208Q_fwd	CGCTACGGGGGCCAAGAATT TATC	Generation of *dgcZ*(H79L,H83L,E208Q)-*mVENUS* fusion
dgcZ_E208Q_rev	GATAAATTCTTGGCCCCCGT AGCG	Generation of *dgcZ(H79L,H83L,E208Q)-mVENUS* fusion
dgcZ_H83L_fwd	caacatatgCTTaactgtgg tcggg	Generation of

		dgcZ(H79L,H83L)-*mVENUS* fusion
dgcZ_H83L_rev	cccgaccacagttAAGcata tgttg	Generation of *dgcZ*(H79L,H83L)-*mVENUS* fusion
frdA_pkd45_fwd	CTTACCCTGAAGTACGTGGC TGTGGGATAAAAACAATCTG GAGGAATGTCtcatttcgaa ccccagagtcccgc	Generation of *frdA::PRha-ccdb-kan* strain
frdA_pkd45_rev(2)	CCACCTCAATTTTCAGGTTT TTCATCtcaGCcatTCGCCT TCTCCTTCTTacccggatat tatcgtgaggatgcG	Generation of *frdA::PRha-ccdb-kan* strain
frdA_mCHERRY_fwd	CTTACCCTGAAGTACGTGGC TGTGGGATAAAAACAATCTG GAGGAATGTCgtgCAAACCT TTCAAGCCGATCTTG	Generation of *frdA-mCHERRY* strain
frdA_mCHERRY_rev2	CCACCTCAATTTTCAGGTTT TTCATCtcaGCcatTCGCCT TCTCCTTCTttacttgtaca gctcgtccatgcc	Generation of *frdA-mCHERRY* strain
FrdA_fwd	CAGGTACTTACCCTGAAGTACGTGGCT	Generation of *frdB::Frt* and *frdB*(+) strain
frdA_rev_recom	GTCATAAGGCACTTCATAGAATGCGCT	Generation of *frdB::Frt* and *frdB*(+) strain
frdB_pkd45_fwd	AGCGGATGCAGCCGATAAGG CGGAAGCAGCCAATAAGAAG GAGAAGGCGAtcatttcgaa ccccagagtcccgc	Generation of *frdB::PRha-ccdb-kan* strain
frdB_pkd45_rev	ACGTCATTGGCCGTACATAC GGTTTACGTTTAGTCGTCAT GTTGCACTCCacccggatat tatcgtgaggatgcG	Generation of *frdB::PRha-ccdb-kan* strain
frdB_mCHERRY_fwd	AGCGGATGCAGCCGATAAGG CGGAAGCAGCCAATAAGAAG GAGAAGGCGAatgGCTGAGA TGAAAAACCTGAAAAT	Generation of *frdB-mCHERRY* strain
frdB_mCHERRY_rev	ACGTCATTGGCCGTACATAC GGTTTACGTTTAGTCGTCAT GTTGCACTCCttacttgtac agctcgtccatgcc	Generation of *frdB-mCHERRY* strain
dgcZ_HindIII_rev	atatAAGCTTttaAACTCGGTTAATCACATTTTGTTCGTC	Cloning of *dgcZ* in pBAD18
SacI_NcoI_dgcZ_FWd	tatagagctcGGAGTGCCATGGatgATCAAGAAGACAACGG AAATTGATG	Cloning of *dgcZ* in pBAD18
nlpE_pcj30_BamHI_fwd	GCTGCAGGATCCGGTGAAAA AAGCGATAGTGACAGCGA	Cloning of *nlpE* in pCJ30
nlpE_pcj30_HindIII_rev	CCAAGCTTGttaCTGCCCCA AACTACTGCAATCC	Cloning of *nlpE* in pCJ30

yfbR_BamHI_fwd	CTAGAGGATCCCAAACAGAG CCATTTCTTTGCCCATCT	Cloning of *yfbR-mCHERRY* in pCJ30. In addition, primer P4_mVENUS_rev was used
yfbR_KpnI_rev	CTTAGGTACCcgAGCGGTGA ATCCTGGCTAATCTCAT	Cloning of *yfbR-mCHERRY* in pCJ30. In addition, primer P4_mVENUS_rev was used
mCHERRY_KpnI_fwd	cgGGTACCGGTGAGCAAGGG CGAGGAGCTG	Cloning of *yfbR-mCHERRY* in pCJ30. In addition, primer P4_mVENUS_rev was used
dgcZ_BamHI_fwd	tataGGATCCAatgATCAAG AAGACAACGGAAATTGATG	Cloning of *dgcZ* in BTH plasmids
dgcZ_KpnI_rev	CTTAGGTACCcgAACTCGGT TAATCACATTTTGTTCGTCA	Cloning of *dgcZ* in BTH plasmids
CZB_KpnI_rev	CTTAGGTACCcgGCTACGGA TCGTCAGCAAATAAATTT	Cloning of CZB domain of *dgcZ* in BTH plasmids. dgcZ_BamHI_fwd was used in addition
GGDEF_BamHI_fwd	CTAGAGGATCCCGGAGTGCC TGTGatgAATATGGATGTTT TGACGGGATTGC	Cloning of GGDEF domain of *dgcZ* in BTH plasmids. DgcZ_KpnI_rev was used in addition
frdA_BamHI_fwd	CTAGAGGATCCCCAAACCTT TCAAGCCGATCTTGC	Cloning of *frdA* in BTH plasmids.
frdA_KpnI_rev	CTTAGGTACCcgGCCATTCG CCTTCTCCTTCTTATTG	Cloning of *frdA* in BTH plasmids.
frdB_BamHI_fwd	CTAGAGGATCCCGCTGAGAT GAAAAACCTGAAAATTGAGG T	Cloning of *frdB* in BTH plasmids.
frdB_KpnI_rev	CTTAGGTACCcgGCCATTCG CCTTCTCCTTCTTATTG	Cloning of *frdB* in BTH plasmids.
Linker used for the *dgcZ-mVENUS* construct	catTCTAGAAAGGAGATTCCTAGGATGGGTACCgaattccc cctcgatatcgggcccggcctatctggcctgggagctggacttccttcactg	

Table S4 (accompanying Excel file) Putative DgcZ-3xFLAG protein interaction partners identified by Co-immunoprecipitation (CoIP). The file contains two sheets listing results from CoIPs in stationary and exponential phase. Each sheet contains two lists: i) candidates identified at least by two unique peptides and three peptides and ii) candidates identified by less unique peptides / peptides. The lists are sorted according to the parameter “Area” which gives a measure of the relative abundances of the proteins in the sample. None of the candidates was identified in any of the negative control CoIPs. Column ΣCoverage indicates the percentages of the protein sequence covered by identified peptides. Column Σ#Proteins lists the number of proteins that contains the identified peptides, i.e. 1 indicates that the identified peptides can be unequivocally assigned to one specific protein. Column Σ# Unique Peptides indicates the number of distinct unique peptides identified for each protein. Column Σ# Peptides indicates the number of peptide sequences identified. Column Σ# PSMs shows the total number of identified peptide sequences (PSM = peptide spectrum matches) for the protein, including those redundantly identified. Column #AAs lists the number of amino acids of each identified protein.

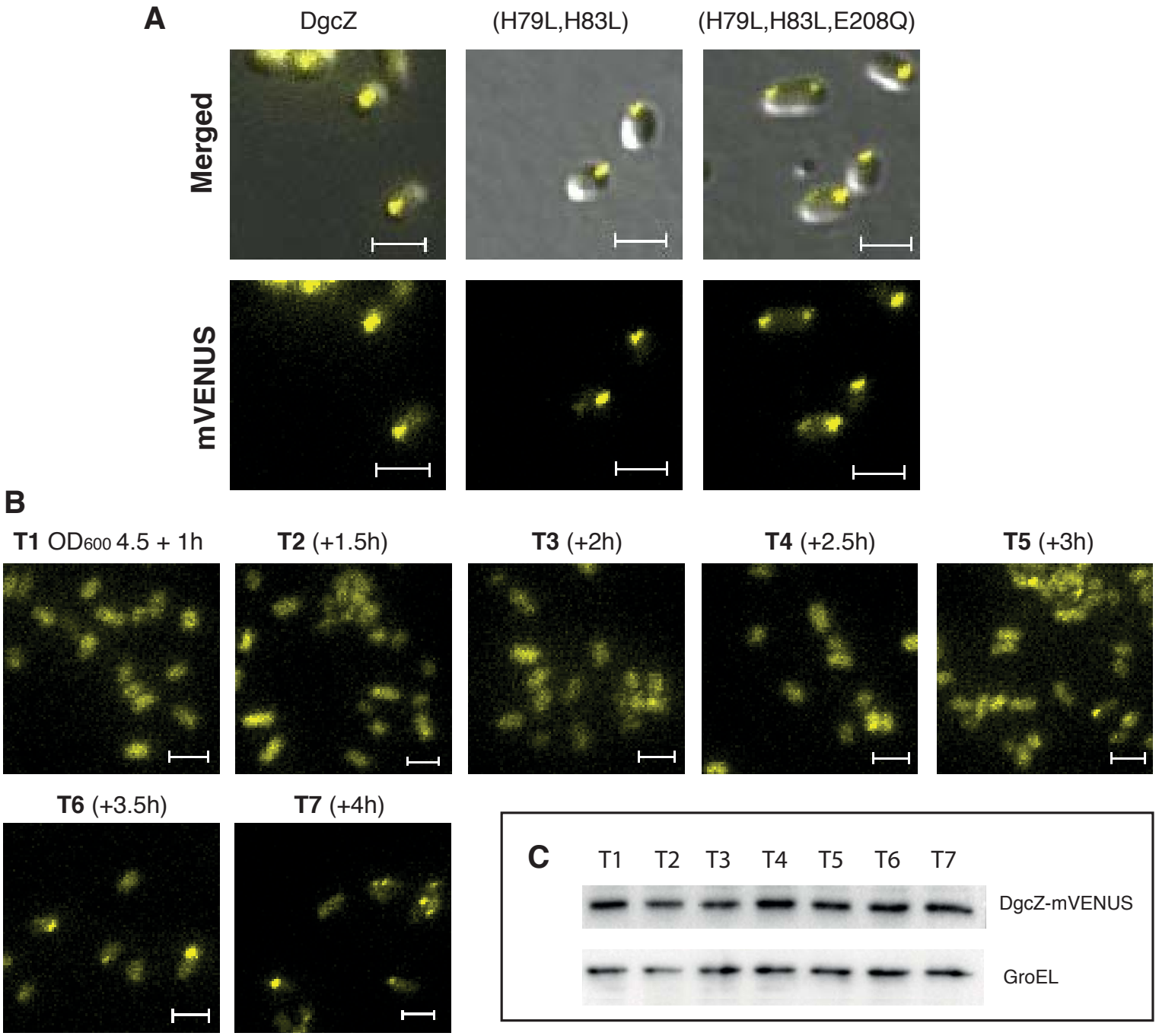


FIG S1 Polar localization of DgcZ is not controlled by protein activity or concentration. (A) Localization of wild-type, hyperactive (H79L, H83L) and inactive (H79L, H83L, E208Q) DgcZ protein fused to mVENUS. The constructs encoding the mVENUS fusions were chromosomally integrated at the native *dgcZ* locus. All strains carry *csrA::Tn5*. Size bars are 2 µm and are identical in each picture. (B) DgcZ-mVENUS localization in the *csrA::Tn5/dgcZ-mVENUS* strain grown in LB medium at 37 °C. Pictures and protein aliquots were taken every 30 minutes starting 1 hour after the bacterial culture reached an OD_{600} of 4.5. Size bars as (A). (C) Western blot detection of DgcZ and GroEL protein extracted during the experiment shown in panel B.

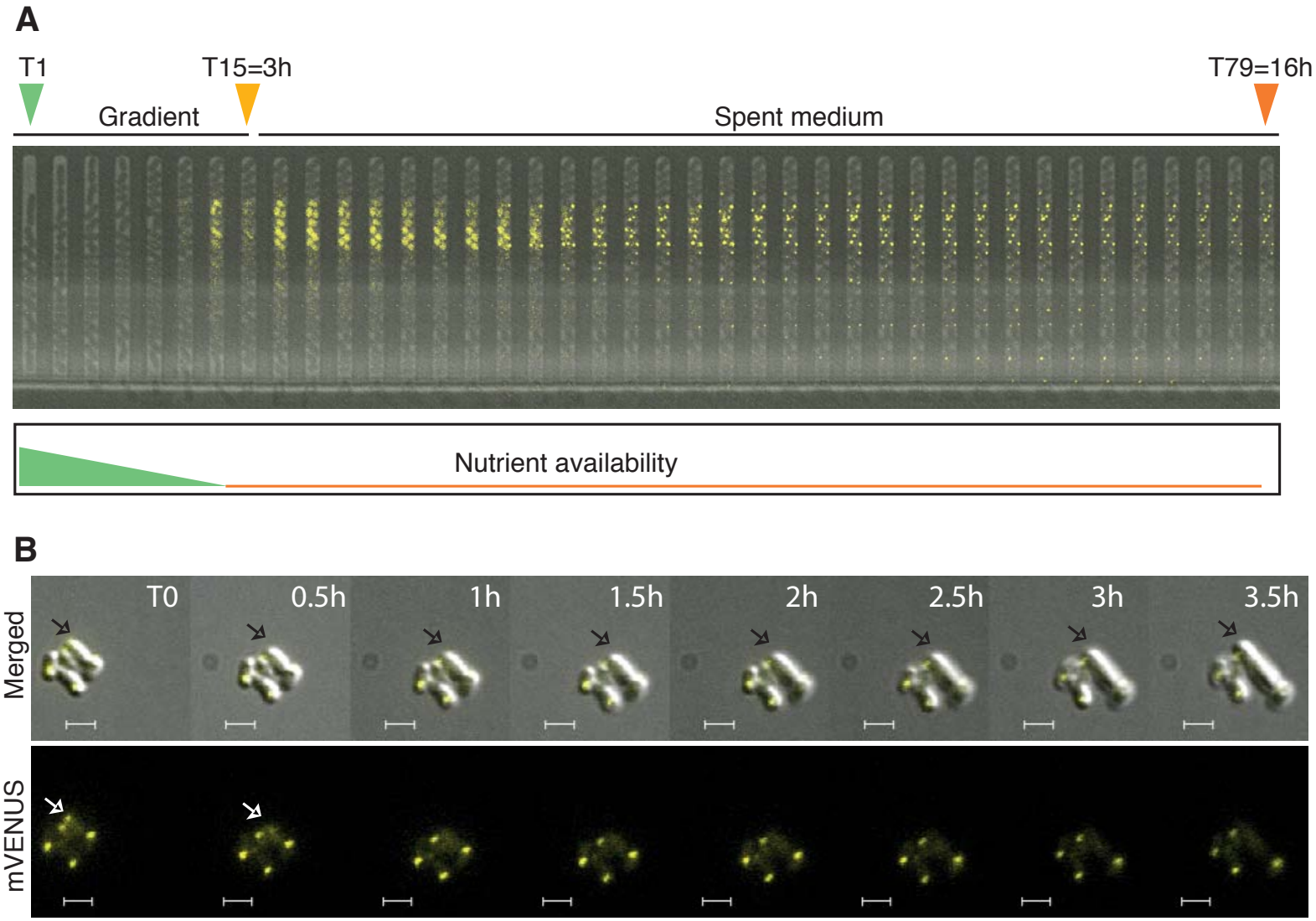


FIG S2 DgcZ polar foci are observed in non-dividing bacteria and vanish upon nutrient addition before growth resumes. (A) Fluorescence microscopy of the *dgcZ-mVENUS/ibpA-mCHERRY/csrA::Tn5* strain in microfluidic chambers. An excerpt of the experiment (described in Materials and Methods) representing the 3 hours phase of the gradient from fresh to spent medium followed by 13 hours of spent medium is shown. The image represents a montage of pictures taken every 24 minutes. (B) Fluorescence microscopy of the *dgcZ-mVENUS/csrA::Tn5* strain. Bacteria from stationary phase (48 h) were spotted onto a microscopy slide (LB, 1 % agarose) and images were taken every 30 minutes. White arrows indicate a DgcZ focus that disappeared when cells resumed growth. The respective bacterial cell is indicated by black arrows in the merged channel (DIC + mVENUS). Size bars are 2 µm and are identical in each picture.

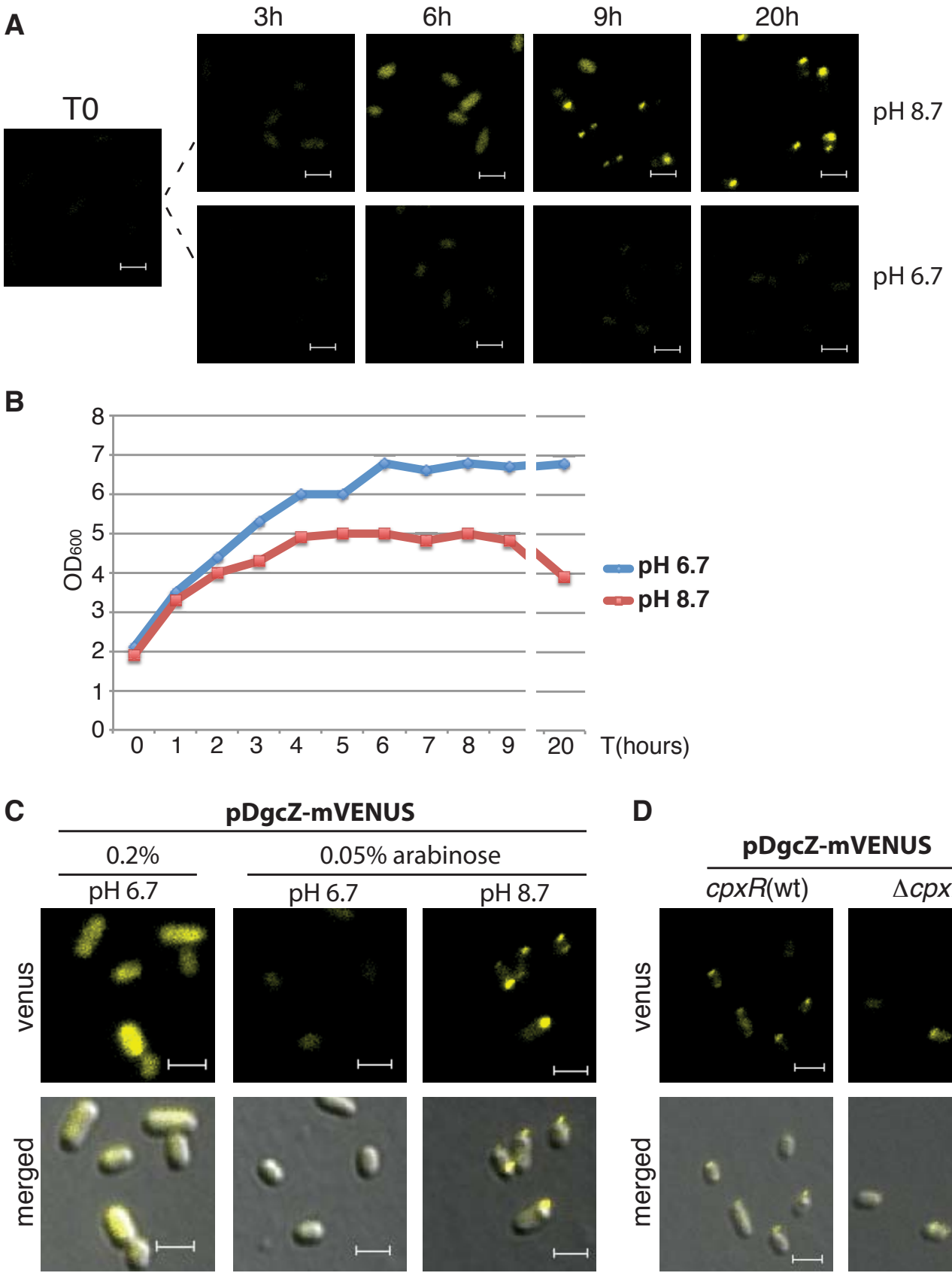
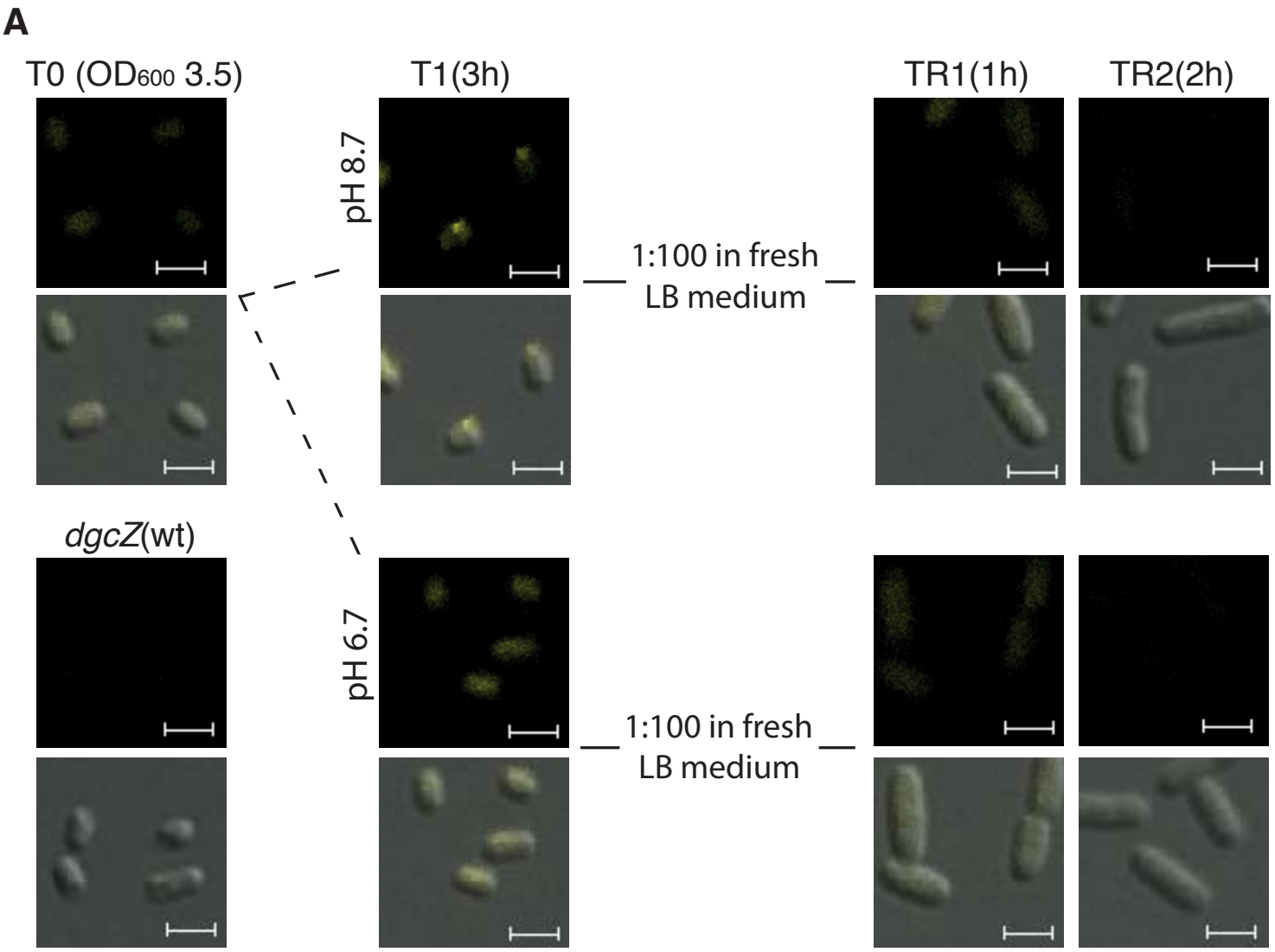


FIG S3 Localization of the DgcZ-mVENUS protein at different pH. (A) *E. coli dgcZ-mVENUS / csrA::Tn5* was grown in LB medium until OD_{600} of 2.5 and then diluted (1:100) in buffered LB medium at pH 6.7 or 8.7 and grown at 37 °C under shaking conditions. Fluorescence microscopy images were taken after 3, 6, 9 and 20 hours. (B) Growth curves from the experiment shown in panel A. (C) Microscopy pictures of *E. coli* MG1655 transformed with pDgcZ-mVENUS and grown in buffered LB medium at pH 6.7 or 8.7 at 37 °C for 24 hours until the cultures reached stationary phase. The medium was supplemented with 100 µg/ml ampicillin and 0.2% or 0.05 % arabinose was added to induce expression of *dgcZ-mVENUS*. (D) Fluorescence microscopy images of *E.coli* MG1655 *cpxR*(wt) and Δ*cpxR* transformed with pDgcZ-mVENUS and grown in LB medium at 37 °C for 24 hours. LB was supplemented with 0.02 % arabinose and ampicillin. Size bars are 2 µm and identical in each picture.



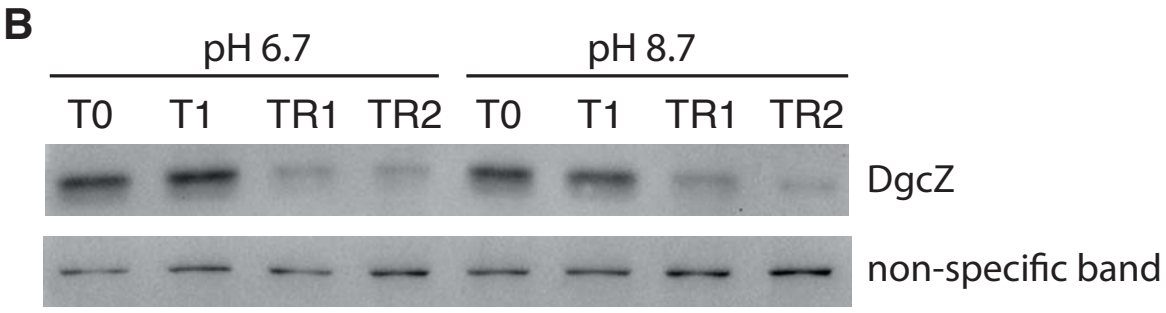


FIG S4 Decrease in DgcZ-mVENUS levels upon dilution of bacterial cultures in fresh LB medium. (A) *E. coli dgcZ-mVENUS/csrA::Tn5* was grown in 20 ml LB medium until OD_{600} of 3.5, then transferred (washed and resuspended in the same volume of medium) to buffered (K_2HPO_4 50 mM, KH_2PO_4 15 mM) spent LB medium at pH 6.7 or 8.7 and cultivated under shaking conditions at 37 °C. After 3 hours both samples were diluted (1:100) in fresh (unbuffered) LB medium and cultivated for two hours. Microscopy pictures were taken at the time points indicated in the figure. Size bars are 2 µm and are identical in each picture. (B) Western blot detection of DgcZ in protein samples extracted in the experiment described in (A). As a negative control, a non-specific band detected by the anti-DgcZ antibody is shown.

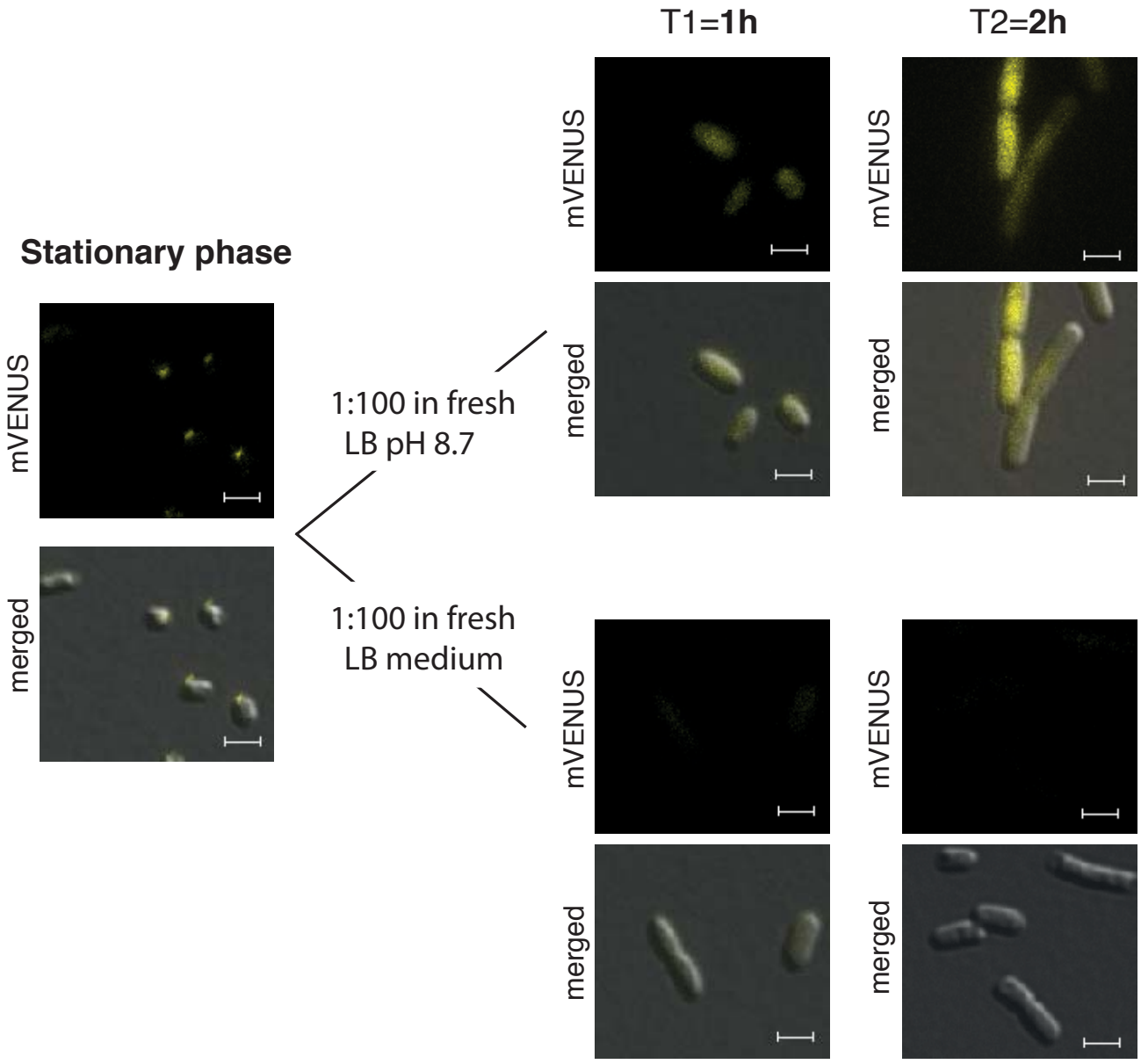


FIG S5 Localization and signal intensity of DgcZ-mVENUS upon dilution of bacterial cultures in fresh LB medium. (A) The d*gcZ-mVENUS/csrA::Tn5* strain was grown in 3 ml LB medium at 37 °C for 24 hours until stationary phase and then resuspended (1:100 ratio) in buffered (K2HPO4 50 mM, KH2PO4 15 mM) LB medium at pH 8.7 or in (unbuffered) LB medium. Tubes were then placed back to 37 °C under shaking conditions for 2 hours. After 1 and 2 hours microscopy pictures were taken. Size bars are 2 µm and are identical in each picture.

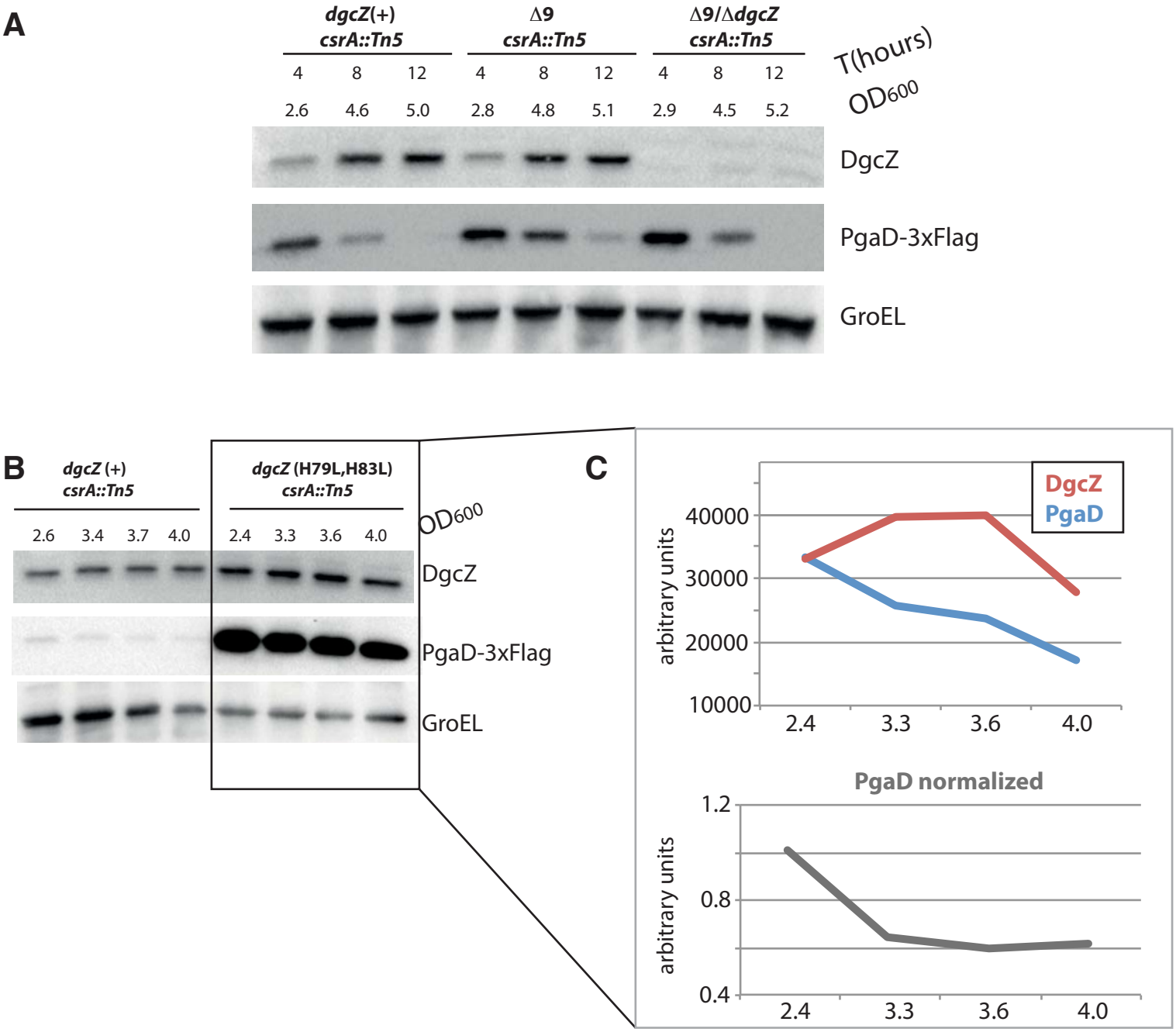
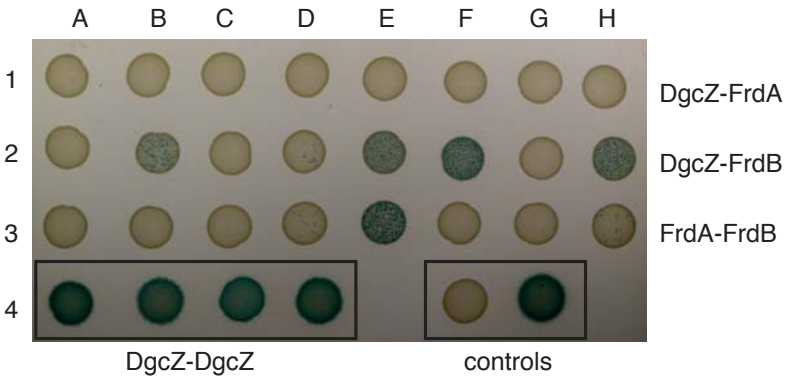


FIG S6 Detection of PgaD levels in *dgcZ*(+), *dgcZ*(H79L,H83L), Δ9 and Δ9/Δ*dgcZ* strains. (A) Western blot detection of DgcZ, PgaD-3xFlag and GroEL proteins in the *dgcZ*(+), Δ9 and Δ9/Δ*dgcZ* strains in the *pgaD-3xFlag/csrA::Tn5* background. Strains were grown under shaking conditions in LB medium at 37 °C. (B) Western blot detection of DgcZ, PgaD-3xFlag and GroEL protein extracted from the *pgaD-3xFlag/csrA::Tn5* strains carrying either *dgcZ*(wt) or the *dgcZ*(H79L,H83L) allele. Strains were grown as described in (A). (C) Western blot quantification. Intensities of DgcZ and PgaD bands are shown in the upper chart. The chart at the bottom shows PgaD-normalized values obtained dividing PgaD by DgcZ signals. Intensities of DgcZ bands were quantified using ImageJ.

A



B

Code	Combination	pUTs (High-copy)		pK(N)Ts (Low-copy)	
		C-term	N-term	N-term	C-term
1A	DgcZ-FrdA	DgcZ		FrdA	
1B	DgcZ-FrdA	DgcZ			FrdA
1C	DgcZ-FrdA		DgcZ	FrdA	
1D	DgcZ-FrdA		DgcZ		FrdA
1E	FrdA-DgcZ	FrdA		DgcZ	
1F	FrdA-DgcZ	FrdA			DgcZ
1G	FrdA-DgcZ		FrdA	DgcZ	
1H	FrdA-DgcZ		FrdA		DgcZ
2A	DgcZ-FrdB	DgcZ		FrdB	
2B	DgcZ-FrdB	DgcZ			FrdB
2C	DgcZ-FrdB		DgcZ	FrdB	
2D	DgcZ-FrdB		DgcZ		FrdB
2E	FrdB-DgcZ	FrdB		DgcZ	
2F	FrdB-DgcZ	FrdB			DgcZ
2G	FrdB-DgcZ		FrdB	DgcZ	
2H	FrdB-DgcZ		FrdB		DgcZ
3A	FrdA-FrdB	FrdA		FrdB	
3B	FrdA-FrdB	FrdA			FrdB
3C	FrdA-FrdB		FrdA	FrdB	
3D	FrdA-FrdB		FrdA		FrdB
3E	FrdB-FrdA	FrdB		FrdA	
3F	FrdB-FrdA	FrdB			FrdA
3G	FrdB-FrdA		FrdB	FrdA	
3H	FrdB-FrdA		FrdB		FrdA
4A	DgcZ-DgcZ	DgcZ		DgcZ	
4B	DgcZ-DgcZ	DgcZ			DgcZ
4C	DgcZ-DgcZ		DgcZ	DgcZ	
4D	DgcZ-DgcZ		DgcZ		DgcZ
4F	Negative contr.	pUT18		pKT25	
4G	Positive contr.	zip		zip	

C

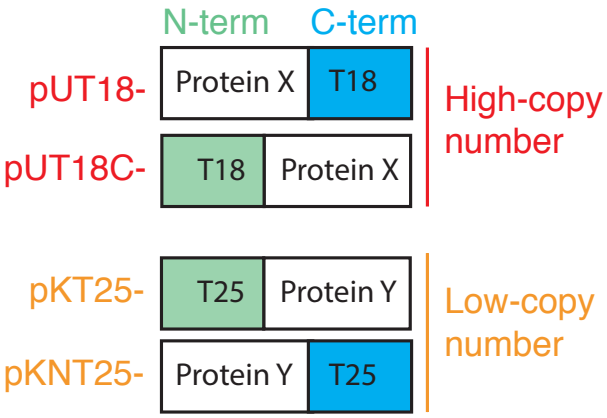


FIG S7 Bacterial two-hybrid assay. (A) Validation of interactions between DgcZ and FrdA-B. N- (N-term) and C- terminal (C-term) fusions of the proteins were constructed and all combinations were tested. Growth conditions are indicated in Materials and Methods. (B) Combinations used in panel A. (C) Schematic representation of plasmid features.

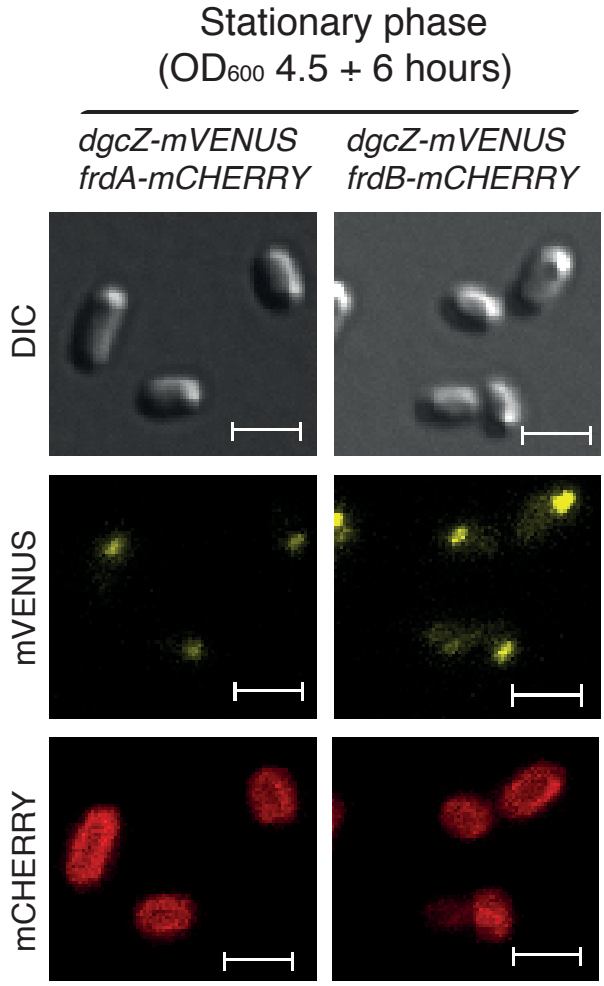


FIG S8 Fluorescence microscopy of strains expressing *dgcZ-mVENUS* and *frdA-B-mCHERRY*. *E. coli csrA::Tn5* cells carrying chromosomally integrated *dgcZ-mVENUS* and either *frdA-mCHERRY* or *frdB-mCHERRY* were grown in LB medium at 37 °C and microscopic images were taken in the stationary growth phase. Size bars are 2µm.

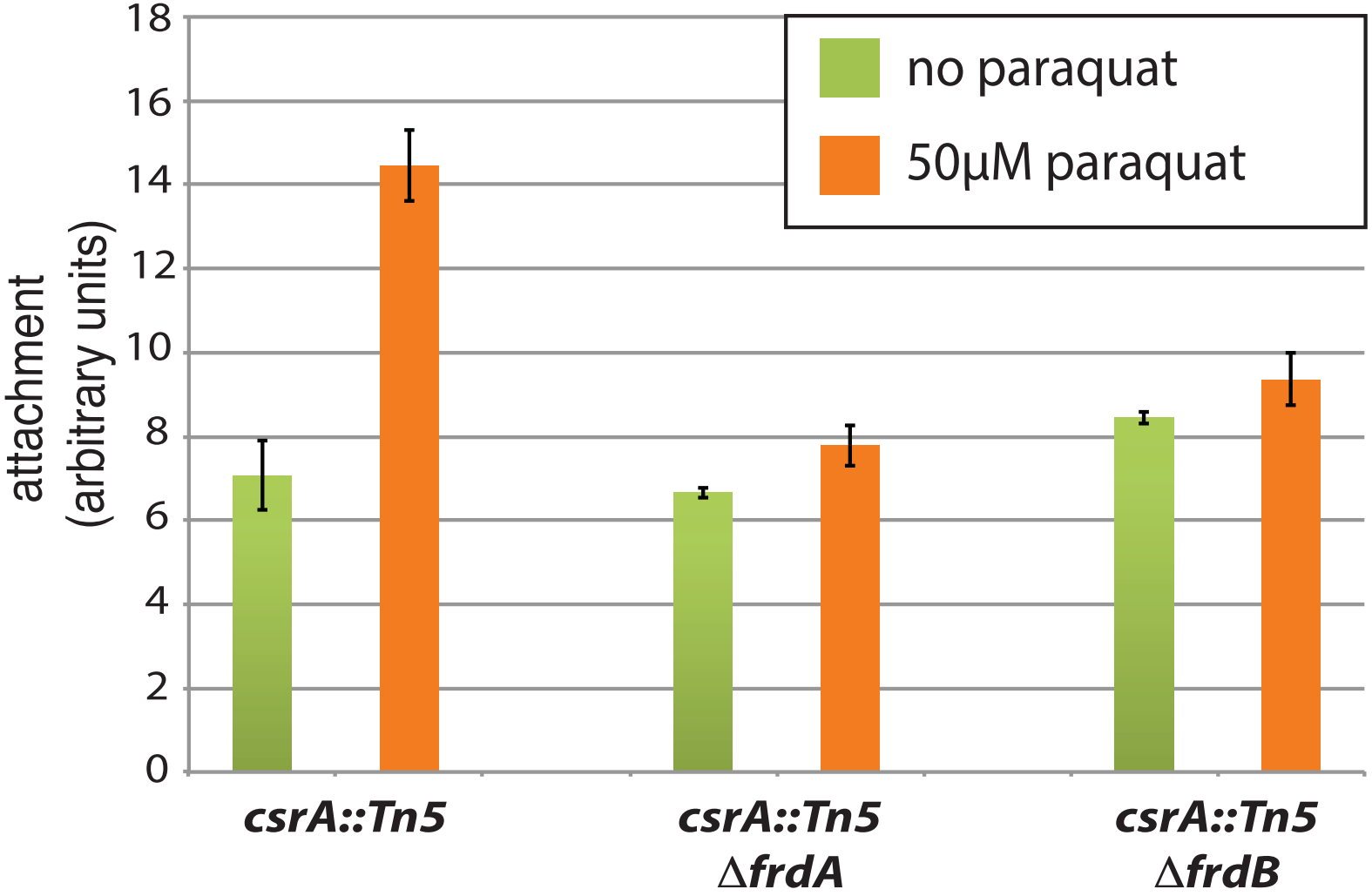


FIG S9 Effects of paraquat on biofilm formation of the *csrA::Tn5*, *csrA::Tn5/ΔfrdA* and *csrA::Tn5/ΔfrdB* strains. Attachment of the *csrA::Tn5/ΔfrdB* strain is compared with attachment of the *csrA::Tn5* and of the *csrA::Tn5/ΔfrdA* strains, shown in figure 7A. LB medium was supplemented with 0 or 50 μM paraquat. All strains were grown on the same 96-well plate. Results are averages of 5 wells inoculated with single colonies. Error bars are SEM.

VIDEO S1 (accompanying video file) Time-lapse microscopy for localization of DgcZ-mVENUS protein in bacteria grown in microfluidic chambers. The video shows the DgcZ localization in the *dgcZ-mVENUS/ibpA-mCHERRY/csrA::*Tn*5* strain. Each frame shows a merged image (phase contrast and mVENUS channel). Images were taken every 12 minutes. Bacteria were flushed into the microfluidic chamber and LB medium was applied for 2 hours (not shown here). A gradient from fresh to nutrient-depleted (spent) medium was applied (3 h, frame 0 - 15), followed by 16 h of growth in spent medium (frame 16 - 95), 1 h of gradient from spent to fresh medium (frame 95 - 100) and 20 h of fresh medium (frame 101 - 200).