The conserved DNA-binding protein WhiA is involved in cell division in *Bacillus subtilis*

Supplemental Material

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Content:

Table S1. Bacterial strains and plasmids used in this study.

B. subtilis	**Relevant genotype**	**Source & comment**
168	*trpC2* (0168 ED)	wild-type for this study
1283	*trpC2* Δ*noc*::*spc*	(1)
1356	*trpC2* Δ*zapA-yshB*::*tet*	(2)
1801	*trpC2* chr:: pJSIZDpble (P_{spac}-*ftsZ ble*)	(3)
2020	*trpC2 amyE*::(P_{xyl}-*gfpmut1-ftsZ spc*)	J. Sievers (unpublished)
3309	*trpC2 minCD*::*kan*	Laboratory stock
4701	*trpC2 amyE*::P_{xyl}-*noc-yfpmut1*	(4)
BFA2863	*trpC2* Δ*sepF*::*ery(pMutin4YlmF)*	(5)
KS6	*trpC2* Δ*zapA-yshB*::*tet*	168::chr. DNA 1356
KS44	*trpC2* Δ*ezrA*::*cat*	D. Claessen, unpublished
KS50	*trpC2* Δ*lacA*::*cat* Δ*zapA-yshB*::*tet* pLOSS-zapA	This work
KS162	*trpC2* Δ*zapA-yshB*::*tet* P_{spac}-*ftsZ* ble	KS6::chr. DNA 1801
KS207	*trpC2* Δ*yvcL*::pMutin4YvcL(*ery)*	This work
KS267	*trpC2* Δ*yvcL*::*TnYLB-1(kan)*	This work, transposon disruption
KS268	*trpC2* P_{spac}-*ftsZ ble*	168::chr. DNA 1801
KS338	*trpC2* Δ*minCD*::*kan*	168::chr. DNA 3309
KS341	*trpC2* Δ*yvcL*::*TnYLB-1(kan)* Δ*sepF*::*ery*	KS267::chr. DNA BFA2863
KS344	*trpC2* Δ*yvcL*::*TnYLB-1(kan)* Δ*ezrA*::*cat*	KS267::chr. DNA KS44
KS345	*trpC2* Δ*noc*::*spc*	168::1283
KS354	*trpC2* Δ*yvcL*:: *TnYLB-1(kan)* Δ*noc::spc*	KS267::chr. DNA KS345
KS356	*trpC2* Δ*yvcL*::*ery* Δ*minCD*::*kan*	KS207::chr. DNA KS338
KS396	*trpC2 amyE*::P_{xyl}-*yvcL spc*	This work; 168:: pSG1729YvcLΔGFP
KS400	*trpC2* Δ*yvcL*::*kan*	This work
KS438	*trpC2 yvcL*::P_{spac}-*yvcL ery*	This work
KS696	*trpC2 yvcL*	This work
KS742	*trpC2* Δ*lacA*::*cat* Δ*zapA-yshB*::*tet* Δ*yvcL* pLOSS-YvcL	This work; Δ*yvcL* originated from KS696
KS748	*trpC2 yvcL* P_{spac}-*ftsZ* ble	KS696::chr. DNA 1801
KS754	*trpC2 yvcL*::P_{spac}-*yvcL ery* Δ*zapA-yshB*::*tet amyE*::P_{xyl}-*gfp-ftsZ*(*spc*) pMAP65(*lacI*,*kan*)	This work, the P_{xyl}-*gfp-ftsZ* fusion originates from 2020
KS859	*trpC2 yvcL*::P_{spac}-*yvcL ery* Δ*zapA-yshB*::*tet aprE*::*lacI spc*	KS891::chr. DNA KS6
KS891	*trpC2 yvcL*::P_{spac}-*yvcL ery aprE*::*lacI spc*	KS438::pAPNC213
KS902	*trpC2 gtaB*:: TnYLB-1 *kan* Δ*yvcL*::*ery*	This work
KS903	*trpC2 pgcA*:: TnYLB-1 *kan* Δ*yvcL*::*ery*	This work
KS1015	*trpC2* Δ*yvcL*::*ery* Δ*ugtP*::neo	KS207::chr. DNA PG237, this work
PG8	*trpC2 amyE*::P_{xyl}-*ftsZ cat*	168::chr. DNA YK059
PG237	*trpC2* Δ*ugtP*::*neo*	Pamela Gamba, unpublished
PG725	*trpC2 amyE*::P_{xyl}-*noc-yfpmut1*	168::4701
PG727	*trpC2 amyE*::P_{xyl}-*noc-yfpmut1 ΔyvcL::kan*	KS400::4701
PG732	*trpC2 amyE*::P_{xyl}-*mgfpmut1-yvcL spc*	168::pSG1729YvcL(mGFP),this work
PG735	*trpC2 amyE*::P_{xyl}-*ftsZ cat* Δ*zapA-yshB*::*tet*	PG8::chr. DNA KS6
PG736	*trpC2* Δ*yvcL*:: *kan amyE*::P_{xyl}-*mgfpmut1-yvcL spc*	KS400::chr. DNA PG732
PG737	*trpC2 amyE*::P_{xyl}-*ftsZ cat* Δ*yvcL*::*kan*	PG8::chr. DNA KS400
PG738	*trpC2 amyE*::P_{xyl}-*ftsZ cat* Δ*zap -yshB*::*tet* Δ*yvcL*::*kan*	PG735::chr. DNA KS400
PG739	*trpC2* Δ*zapA-yshB*::*tet* Δ*noc*::*spc*	KS6::KS345
PG740	*trpC2* Δ*zapA-yshB*::*tet* Δ*minCD*::*kan*	KS6::KS338
YK059	CRK6000 *amyE*::P_{xyl}-*ftsZ cat*	(6)
E. coli		
XL1Blue	*recA1 endA1 gyrA96 thi1 hsdR17 supE44 relA1 lac* [F´ proA+B+*lacI*qZΔM15 Tn10 (TetR)]	Stratagene
KS432	XL1Blue::pQE60EYvcL	This work

Plasmid	**Relevant genotype/comment**	**Source & comment**
pAPNC213	*bla aprE5' spc lacI* P_{spac}-mcs *aprE3'*	(7)
pBEST501	bearing the kanamycin cassette	(8)
pQE60E	derived from pQE60 (Qiagen), expression vector for C-terminal His-6 fusions	L. Hamoen, unpublished
pQE60EYvcL	pQE60E containing *yvcL-his6*	This work
pLOSS*	*bla spc* P_{spac}-mcs P_{divIVA}-*lacZ lacI* rep_{pLS20} (GA→CC)	(9)
pLOSS-zapA	pLOSS* containing *zapA-yshB*	This work
pLOSS-YvcL	pLOSS* containing *yvcL*	This work
pMAP65	pUB110 P_{pen}-*lacI*	(10)
pMarB	*bla erm* P_{ctc}Himar1 *kan* (TnYLB-1)	(11)

pMutin4	*bla erm lacZ lacI*	(12)
pMutin4YvcL	integrates into *yvcL* gene	This work
pMutin4YvcLKO	pMutin4 containing 3' fragment of *yvcK* and 5' fragment of *yvcL*	This work
pMut4YKO	pMutin4YvcLKO introducing a stop codon and *Eco*RI site into *yvcL*	This work.
pMutiYvcL	used for construction of an *yvcL-crh-yvcN* conditional mutant	This work
pSG1729	*bla amy*E3' *spc* P_{xyl}-*gfpmut1*' *amy*E5'	(13)
pSG1729YvcL	*bla amy*E3' *spc* P_{xyl}-*gfpmut1-yvcL amy*E5'	This work
pSG1729YvcL(mGFP)	*bla amy*E3' *spc* P_{xyl}-*mgfpmut1-yvcL amy*E5'	This work
pSG1729YvcLΔGFP	*bla amy*E3' *spc* P_{xyl}-*yvcL amy*E5'	This work

Table S2. Oligonucleotides used in this study.

Name	Restriction site	Sequence (5'-3')	Reference/ used for
HS410		CCTGTCCACACAATCTAAACTTTCGAAAGATCCC	A206K mutation in GFP coding sequence
HS411		GGGATCTTTCGAAAGTTTAGATTGTGTGGACAGG	A206K mutation in GFP coding sequence
km3	*Bam*HI	GGGGGATCCAAGACGAAGAGGATGAAG	*kan*^{R} amplification
km4	*Eco*RI	CCCGAATTCAGAGTATGGACAGTTGCG	*kan*^{R} amplification
KS80	-	GTACAGGTCTTTCTGTATTG	KS400 construction
KS83	-	GGCGCTCTGACATGACCATC	KS400 construction
KS84	*Eco*RI	CGGACAGAATTCACCGTCACTTTAAAATAAC	KS400 construction
KS89	*Bam*HI	TGAGGTGGATCCATGTCATTTGCATCAGAAA	pQE60E construction
KS94	*Hind*III	GCTAAGCTTGCATTCTTCCCAATTTGCTC	pMutiYvcL construction
KS95	*Bam*HI	GATGGATCCCTCATTTCAAGGCTTCATTC	pMutiYvcL, KS400 construction
KS97	*Bam*HI	GATGGATCCTTACGGTTATTTTAAAGTG	pMutiCrh, pLOSS-YvcL construction
KS98	*Xho*I	GATCCTCGAGTAAAGTGACGGTTTGCCCTG	pSG1729YvcLΔGFP construction
KS99	*Bgl*II	GATCAGATCTTAAAGTGACGGTTTGCCCTG	pQE60E construction
KS120		AATTCTGATAACTGGAAGTGAAGGACTG	pMut4YKO construction
KS121		CCAGTTATCAGAATTCTTTTTTTGTTTCTGATG	pMut4YKO construction
KS128	*Noc*I	ACTGAAAGCGGCCGCCCTTGAAATGAGGTGGC	pLOSS-YvcL construction
KS185		AATGGAGACTGGATTGCTGTAG	*xynD* qPCR
KS186		TTACCGTCTGCACTGTCGAG	*xynD* qPCR
KS187		ATACGCGCAGCATCTTAACG	*ynfF* qPRC
KS188		GTATTTCTTGCGGCGTCCAC	*ynfF* qPCR
KS198		TCTGAGCCAGAACCTGATCC	*spoVID* qPCR
KS199		AGTCTCCGCTGGAGAGTCTG	*spoVID* qPCR
KS200		GATACTAAATCCGCCGGAAAC	*acoC* qPCR
KS201		TCAAATGTCAGGCTGAGTGG	*acoC* qPCR
OIPCR1		GCTTGTAAATTCTATCATAATTG	(11)
OIPCR2		AGGGAATCATTTGAAGGTTGG	(11)
OIPCR3		GCATTTAATACTAGCGACGCC	(11)
STG101		ATACCGCGGAGATACACGAG	*scpB* Fw qPCR
STG102		TTTGACGGCACTTCAATCAG	*scpB* Rw qPCR
STG410		TTGACGACAAGCGTGAAAAG	*rplC* Fw qPCR
STG411		TTcatACGcatCcatTTCCA	*rplC* Rw qPCR
yshA-F	*Noc*I	GATGCGGCCGCCACTTTTCGCTGTATATACC	pLOSS-ZapA construction
yshB-R	*Bam*HI	GATGGATCCGACGTTCACATATGTTTCCATC	pLOSS-ZapA construction
yvcL-C5	*Kpn*I	GCCTTGGGTACCGGTGGCTATATGTCATTTG	pSG1729YvcLΔGFP construction
yvcL-F1	*Hind*III	GCTAAGCTTAAACCGCCATCTCGTACTC	pMutin4YvcL construction
yvcL-R1	*Bam*HI	GATGGATCCAATTGACGAGGCGGTTGACC	pMutin4YvcL construction
yvcL-N5	*Hind*III	GCCTTGAAGCTTGGTGGCTATATGTCATTTG	pSG1729YvcL construction
yvcL-N3	*Eco*RI	GAACTTGAATTCTCTGTTGAACCATAAGATC	pSG1729YvcL construction

Table S3. Sporulation efficiency.

Strain	**Genotype**	**Total cell count**	**# Phase bright spores**	**% Sporulation**
168	wild type	2997	1988	66.3
KS207	Δ*yvcL::ery*	2536	1233	48.6
KS696	Δ*yvcL*	1933	808	41.8

The sporulation efficiencies are calculated from the number of phase bright spores relative to the total number of cells. Cultures were grown in DSM medium for 24 hours at 37°C (two biological and two technical replicates). Spore and cell numbers were determined by light microscopy.

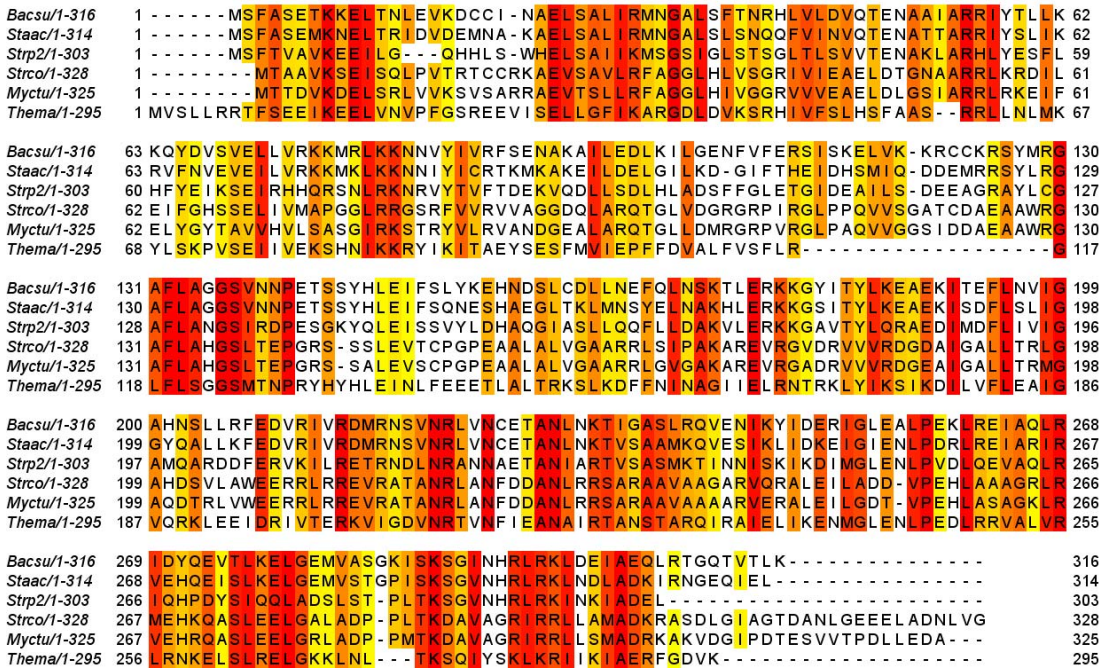

Fig. S1. Sequence alignment of WhiA-like proteins. The protein sequences were aligned using ClustalW and the alignment was coloured using JalView programme (14). WhiA-like proteins from the following organisms were aligned: *(Bacsu) Bacillus subtilis, (Staac) Staphylococcus aureus, (Strp2) Streptococcus pneumoniae; (Strco) Streptomyces coelicolor; (Myctu) Mycobacterium tuberculosis,* and *(Thema) Thermotoga maritima*. Conserved residues are coloured, with the least variable conserved residues in red background.

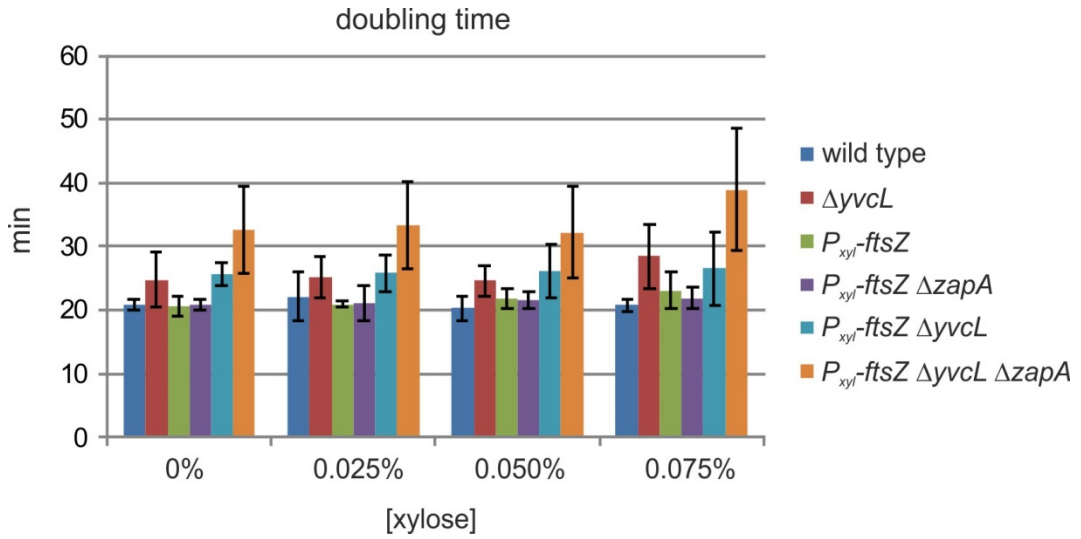

Fig. S2. Growth rates of *yvcL* mutants. Cells of strains: 168 (wild type), KS400 (Δ*yvcL*), PG8 (P_{xyl}-*ftsZ*), PG735 (P_{xyl}-*ftsZ* Δ*zapA*), PG737 (P_{xyl}-*ftsZ* Δ*yvcL*), and PG738 (P_{xyl}-*ftsZ* Δ*zapA* Δ*yvcL*) were initially grown in LB medium with 0.05% xylose, then diluted and grown in LB medium supplemented with increasing concentrations of xylose at 37°C in a plate reader. The doubling times and standard deviations were calculated from three independent experiments.

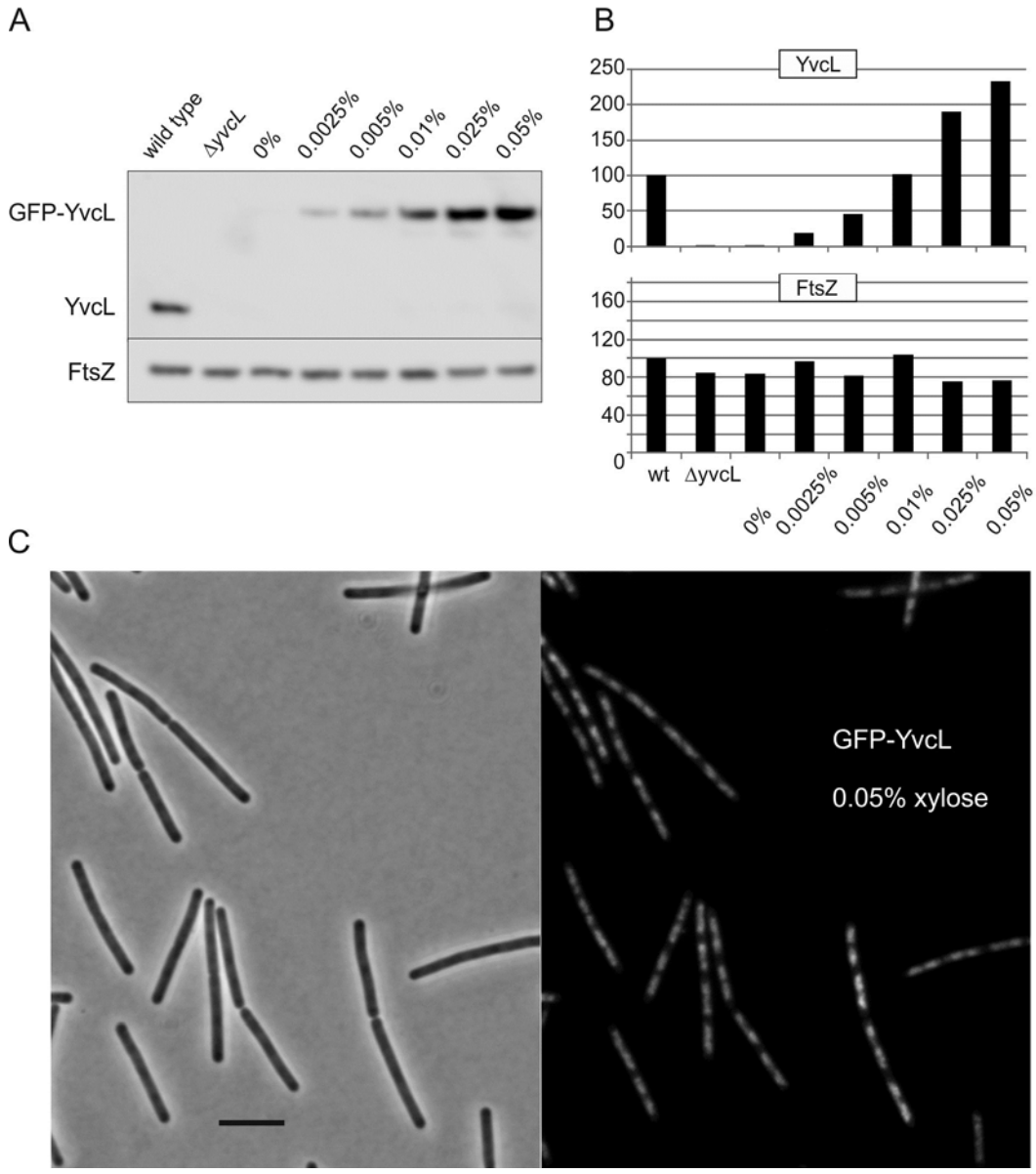

Fig. S3. GFP-YvcL expression levels and localization. (A) Western blot analysis using YvcL antibodies to determine cellular YvcL and GFP-YvcL levels. Strains 168 (wild type), Δ*yvcL*, and PG736 (P_{xyl}-*mgfp-yvcL*) were grown in LB medium at 30°C. In case of PG736, different concentrations of xylose were added (0% up to 0.05%). FtsZ immunodetection was used as internal control. (B) Band intensities (arbitrary units) of the Western blot in (A). (C) Fluorescence microscopy image of strain PG736 grown in LB medium supplemented with 0.05% xylose at 30°C. Scale bar 5 µm.

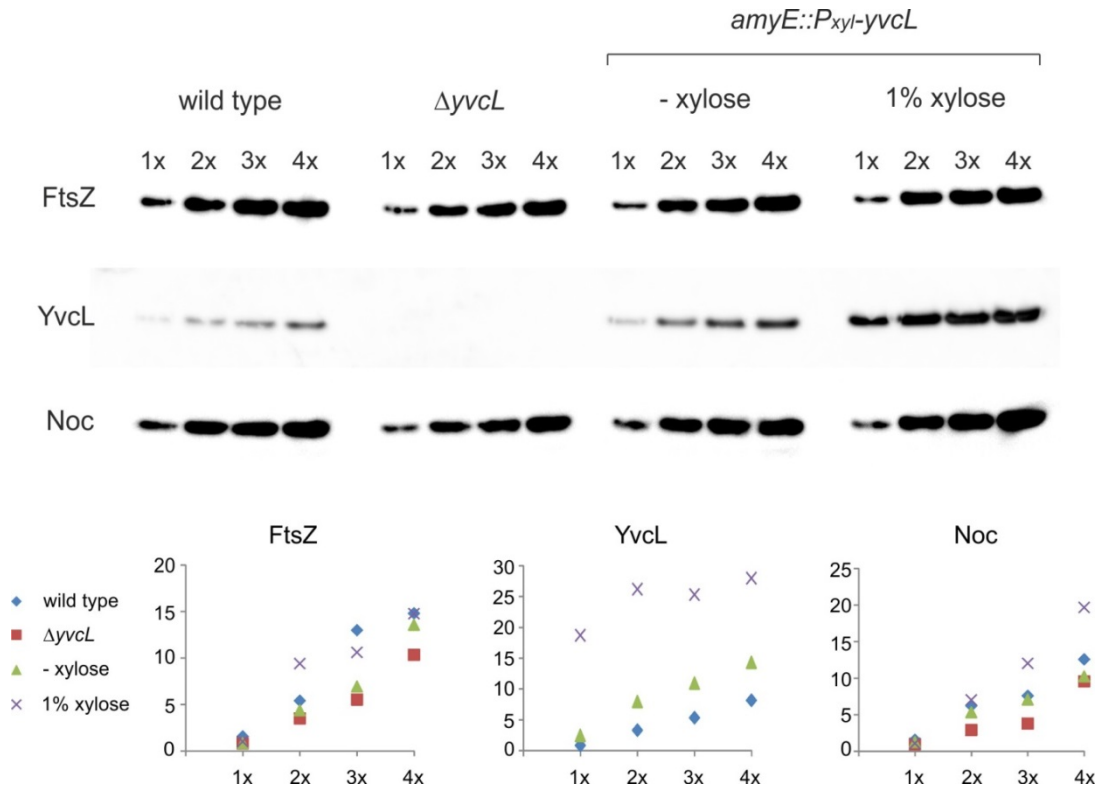

Fig. S4. FtsZ levels in ΔyvcL and YvcL-overproduction strains. Western blots indicating FtsZ, YvcL and Noc levels in wild type, *yvcL* mutant strain, and a strain overexpressing YvcL are shown. Samples were diluted (1 to 4 fold) before loading onto the protein gel to facilitate quantification. Graphs presenting the intensities (arbitrary units) of the protein bands are shown below. Strains 168 (wild type), KS400 (Δ*yvcL*), and KS396 (P_{xyl}-*yvcL*) were grown to OD_{600}~0.4 in LB medium at 37°C. Protein concentrations of cell extracts were measured by Bradford protein assay, and immunodetection of Noc was used as internal control.

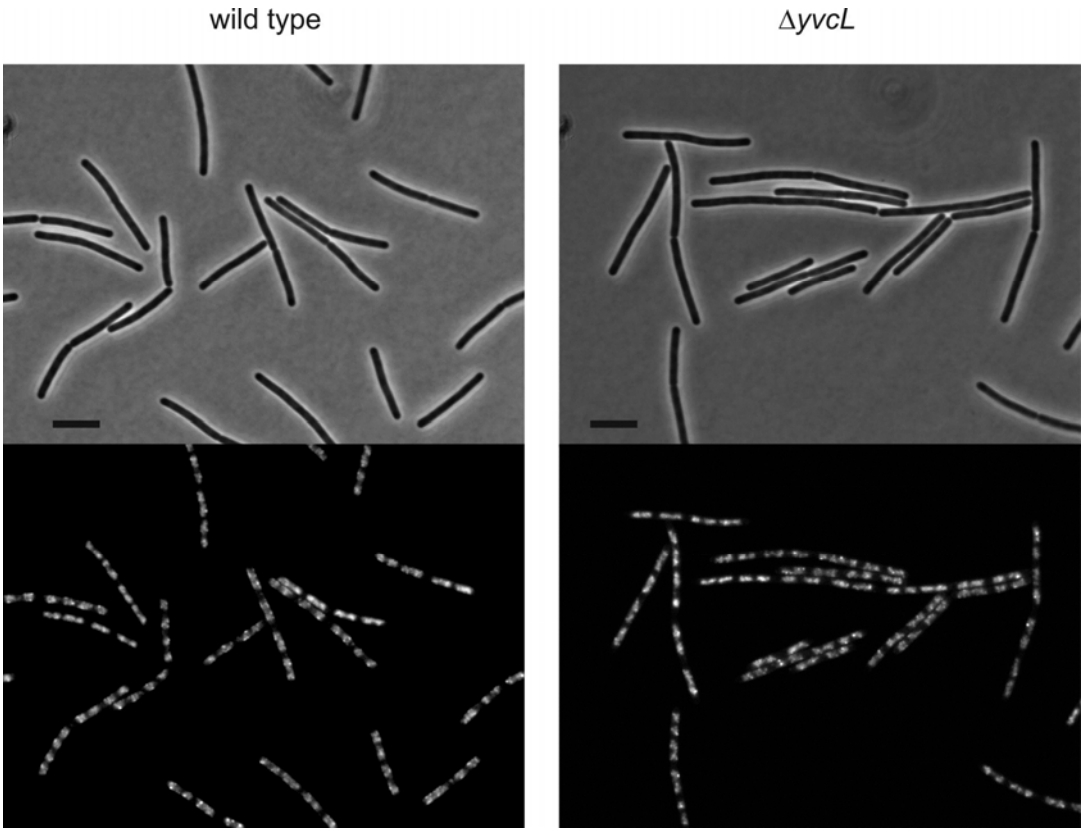

Fig. S5. Localization of Noc-YFP in *yvcL* mutant cells. For fluorescence microscopy, strains PG725 (P_{xyl}-*noc-yfp*) and PG727 (P_{xyl}-*noc-yfp* Δ*yvcL*) were grown in LB medium at 30°C, supplemented with 0.5% xylose to express Noc-YFP. Scale bar 5 µm.

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