Supplementary Material

Supplementary figure legends (Fig S1-S5)
Table S1 - Whole-genome sequencing of YodL-resistant suppressors
Table S2 - Strains
Table S3 - Plasmids
Table S4 - Oligos
Descriptions of strain and plasmid constructions

Supplementary Figure Legends

Fig S1. Growth curves in LB following misexpression of YodL and/or YisK. 2X P_{hy}-*yodL* (BAS191), 2X P_{hy}-*yisK* (BYD074) and 2X P_{hy}-*yodL*, 2X P_{hy}-*yisK* (BYD281) were grown in LB media at 37°C to mid-exponential diluted to an OD_{600} of <0.02. At time 0, 1 mM IPTG or 1 mM IPTG and the indicated concentration of $MgCl_2$ was added.

Fig S2. Misexpression of YodL and YisK on PAB media. (A) Cells were streaked on PAB solid media supplemented with 100 µg/ml spectinomycin and, when indicated, 1 mM IPTG and the denoted concentration of $MgCl_2$. Plates were incubated for ~16 hr at 37°C before image capture. (B) Cells were grown in PAB liquid media at 37°C to mid-exponential and back-diluted to an OD_{600} of <0.02. When indicated, 1 mM IPTG and the denoted concentration of $MgCl_2$ was added. Cells were then grown for 1.5 hrs at 37°C before image capture. Membranes are stained with TMA-DPH (white). All images are shown at the same magnification.

Fig S3. A strain harboring a GFP reporter without a promoter during a sporulation timecourse. BAS205 (P_{empty}-*gfp*) was induced to sporulate via resuspension, and membranes are stained with TMA (white). Signal from GFP was scaled identically for all images and pseudocolored green. All images are shown at the same magnification.

Fig S4. Strains lacking *yodL* and/or *yisK* appear morphologically similar to wildtype during a sporulation timecourse. *B. subtilis* 168 (wt), BYD276 (*ΔyodL*), BYD278 (*ΔyisK*) and BYD279 (*ΔyodL ΔyisK*) were grown induced to sporulate via resuspension, and cells were grown for the indicated amount of time at 37°C before image capture. Membranes are stained with TMA-DPH (white). All images are shown at the same magnification.

Fig S5. Location of MreB residues conferring resistance to YodL. The co-crystal structure of RodZ-MreB (2WUS)(1) was extracted from the Protein Data Bank. MreB is labeled in brown and RodZ is labeled in grey. The identity and locations of the amino acid substitutions obtained from the YodL spontaneous suppressor selections are indicated on the structure, marked by a black asterisk above the relevant amino acid on the sequence alignment. Substitutions that confer resistance to YodL over YisK are shown in bold. Residues previously implicated in the MreB-RodZ interaction interface (1) are indicated by red asterisks. The filled circles indicate the location of the substitutions in Mbl conferring resistance to YodL misexpression. $MreB_{R117G}$ (underlined) was identified in a suppressor selections conferring resistance to YodL as well as in suppressor selections conferring resistance to YisK.

Fig S6. Location of Mbl residues conferring resistance to YisK. The structure of *B. subtilis* Mbl, as predicted by I-TASSER (2), threaded to *T. maritima* MreB (1JCG)(3). The structure on the right is a surface prediction model. The identity and locations of the amino acid substitutions obtained from the YisK spontaneous suppressor selections are indicated on the structure, with substitutions conferring resistance to YisK over YodL in bold. The sequence alignment is of MreB from *T. maritima*, *B. subtilis* 168, *C. crescentus* NA1000, *E. coli* MG1655, and *V. cholera* N16961. The location of amino acid substitutions conferring YisK resistance are indicated by black asterisks. Residues also previously shown to confer resistance to A22 in *C. crescentus* NA1000 (4, 5) and *V. cholera* N16961 (6) are indicated by red and blue asterisks, respectively. The filled triangle corresponds to a residue shown by in vivo crosslinking to be important for the formation of antiparallel MreB protofilaments (7). The filled circle denotes the location of $MreB_{R117G}$, which was identified in spontaneous suppressor selections conferring resistance to both YodL and YisK. Mbl_{T317I} (underlined) was only identified in a spontaneous suppressor selection conferring resistance to YodL, although it exhibits cross-resistance to YisK (see Fig 3).

Table S1. Whole-genome sequencing of YodL-resistant suppressors

Suppressors of YodL	GENE	COORDINATE	REFERENCE	SAMPLE	DISTRIBUTION	VARIANT
SYL#1	*lmrB*	289144	C	A	50% C, 50% A	G317C
	yjgA	1284721	C	G	61% C, 39% G	G17R
	mreB	2860903	T	A	100% A	R282S
	biol	3089273	G	T	50% G, 50% T	R381I
	yyaC-parB	4205543	A	T	47% A, 53% T	intergenic
SYL#3	*mreB*	2861400	G	C	100% C	R117G
SYL#7	*mbl*	3747508	C	T	97% T	E250K
SYL#8	*mreB*	2861287	G	T	98% T	S154R
SYL#10	*mreB*	2861309	G	C	99% C	P147R
SYL#14	*mreB*	2860781	C	T	99% T	G323E
	folC	2866565	C	A	100% A	G14W

Table S1. Whole-genome sequencing analysis of genomic DNA from six YodL-resistant suppressors. BYD048 (three copies of P_{hy}-*yodL*) was used for suppressor selection. Candidates were analyzed by whole-genome sequencing as described in the materials and methods.

Table S2. Strains

Strain	Description	Reference
Parental		
B. subtilis 168 (1A866)	*Bacillus subtilis* laboratory strain 168 *trpC2*	BGSC*
DH5α	*F⁻, endA1, glnV44, thi-1, recA1, relA1, gyrA96, deoR, nupG, Φ80dlacZΔM15, Δ(lacZYA-argF)U169, hsdR17(r_K^- m_K^+), λ–*	
B. subtilis 3610	*spo0H::cat (sigH::cat)*	(8)

B. subtilis PY79	*spo0A::erm (RL891)*	(8)
B. subtilis 168		
BAS040	*amyE::*P_{hy}*-yodL (spec)*	This study
BAS041	*amyE::*P_{hy}*-yisK (spec)*	This study
BAS146	*ponA::erm, kanΩΔmreB*	This study
BAS147	*ponA::erm, kanΩΔmbl*	This study
BAS170	*amyE::*P_{yodL}*-lacZ (spec)*	This study
BAS171	*amyE::*P_{yodL}*-gfp (spec)*	This study
BAS191	*amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo)*	This study
BAS192	*amyE::*P_{yisK}*-lacZ (spec)*	This study
BAS193	*amyE::*P_{yisK}*-gfp (spec)*	This study
BAS205	*amyE::*P_{empty}*-gfp (spec)*	This study
BAS248	*ponA::erm, kanΩΔmbl*, *catΩΔmreBH*, *amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo)*	This study
BAS249	*ponA::erm, kanΩΔmbl*, *catΩΔmreBH*, *amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo)*	This study
BAS265	*spo0A::erm*	This study
BAS266	*amyE::*P_{yodL}*-gfp (spec), spo0A::erm*	This study
BAS267	*amyE::*P_{yisK}*-gfp (spec), spo0A::erm*	This study
BAS282	*sigH::cat*	This study
BAS301	*amyE::*P_{yodL}*-lacZ (spec), spo0A::erm*	This study
BAS302	*amyE::*P_{yisK}*-lacZ (spec), spo0A::erm*	This study
BAS303	*amyE::*P_{yodL}*-lacZ (spec), sigH::cat*	This study
BAS304	*amyE::*P_{yisK}*-lacZ (spec), sigH::cat*	This study
BAS305	*amyE::*P_{yodL}*-lacZ (spec), spo0A::erm, sigH::cat*	This study
BAS306	*amyE::*P_{yisK}*-lacZ (spec), spo0A::erm, sigH::cat*	This study
BDR992	*amyE::*P_{hy}*-lacZ (spec)*	David Z. Rudner
BKE10750	*yisK::erm*	BGSC*
BKE19640	*yodL::erm*	BGSC*
BKE22320	*ponA::erm*	BGSC*
BYD048	*amyE::*P_{hy}*-yodL (spec), ycgO::*P_{hy}*-yodL (tet), yhdG::*P_{hy}*-yodL (phleo), sacA::*P_{hy}*-lacZ (erm)*	This study
BYD074	*amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo)*	This study
BYD076	*amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat), sacA::*P_{hy}*-lacZ (erm)*	This study
BYD175	*ponA::erm, amyE::* P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo)*	This study
BYD176	*ponA::erm, amyE::* P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo)*	This study
BYD177	*kanΩmreB*$_{G323E}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD178	*kanΩmreB*$_{P147R}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD179	*kanΩmreB*$_{R282S}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD180	*kanΩmreB*$_{G143A}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD184	*kanΩmreB*$_{R117G}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD258	*ponA::erm, kanΩΔmbl, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo)*	This study
BYD259	*ponA::erm, kanΩΔmbl, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo)*	This study
BYD262	*ponA::erm, kanΩΔmreB, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo)*	This study
BYD263	*ponA::erm, kanΩΔmreB, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo)*	This study
BYD276	*ΔyodL*	This study
BYD278	*ΔyisK*	This study

BYD279	*ΔyodL, ΔyisK*	This study
BYD281	*amyE::*P_{hy}*-yisK (spec), ycgO::*P_{hy}*-yisK (tet), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD327	*kanΩmreB*$_{G323E}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD328	*kanΩmreB*$_{R117G}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD329	*kanΩmreB*$_{N145D}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD330	*kanΩmreB*$_{P147R}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD332	*kanΩmreB*$_{S154R,R230C}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD333	*kanΩmreB*$_{G143A}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD334	*kanΩmbl*$_{E250K}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD335	*kanΩmbl*$_{T317I}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD336	*kanΩmbl*$_{T158M}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD337	*kanΩmbl*$_{\Delta S251}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD338	*kanΩmbl*$_{P309L}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD339	*kanΩmbl*$_{G156D}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD340	*kanΩmbl*$_{T158A}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD341	*kanΩmbl*$_{D153N}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD342	*kanΩmbl*$_{R63C}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD343	*kanΩmbl*$_{M51I}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD344	*kanΩmbl*$_{A314T}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD345	*kanΩmbl*$_{E204G}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD346	*kanΩmbl*$_{E250K}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD348	*kanΩmbl*$_{T158A}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD349	*kanΩmbl*$_{G156D}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD351	*kanΩmbl*$_{D153N}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD352	*kanΩmbl*$_{M51I}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD353	*kanΩmbl*$_{A314T}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD354	*kanΩmbl*$_{E204G}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD361	*amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yodL (phleo)*	This study
BYD363	*kanΩmreB*$_{S154R,R230C}$*, amyE::*P_{hy}*-yisK (spec), yhdG::*P_{hy}*-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD365	*kanΩmreB*$_{R282S}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD404	*kanΩmreB*$_{N145D}$*, amyE::*P_{hy}*-yisK (spec), yhdG::Phy-yisK (phleo), yycR::*P_{hy}*-yisK (cat)*	This study
BYD405	*kanΩmbl*$_{R63C}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD406	*kanΩmbl*$_{\Delta S251}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD407	*kanΩmbl*$_{P309L}$*, amyE::*P_{hy}*-yodL (spec), yhdG::*P_{hy}*-yodL (phleo), yycR::*P_{hy}*-yodL (cat)*	This study
BYD510	*ΔyodL, ΔyisK, amyE::*P_{yisK}*-yisK (spec)*	This study

*Bacillus Genetic Stock Center

Table S3. Plasmids

Plasmid	Description	Reference
pAS015	*yhdG::*P_{hy}*-yisK (amp)*	This study
pAS040	*amyE::*P_{yodL}*-lacZ (amp)*	This study
pAS041	*amyE::*P_{yodL}*-gfp (amp)*	This study
pAS044	*amyE::*P_{yisK}*-lacZ (amp)*	This study
pAS045	*amyE::*P_{yisK}*-gfp (amp)*	This study
pAS047	*amyE::gfp (amp)*	This study
pAS067	*amyE::*P_{yisK}*-yisK (amp)*	This study
pDR111	*amyE::*P_{hy} *(amp)*	David Z. Rudner
pDR244	Temperature sensitive Cre recombinase plasmid (*amp*)(*spec*)	David Z.

		Rudner
pJH036	*sacA*::P_{hy}*-lacZ (amp)*	This study
pJW004	*yhdG*::P_{hy} *(amp)*	This study
pJW006	*amyE*::P_{hy}*-sirA-gfp (amp)*	(9)
pJW033	*ycgO*::P_{hy} *(amp)*	This study
pJW034	*yycR*::P_{hy} *(amp)(cat)*	This study
pKM062	*sacA::erm (amp)*	David Z. Rudner
pWX114	*yrvN*::P_{hy} *(amp)(kan)*	David Z. Rudner
pYD073	*yhdG*::P_{hy}*-yodL (amp)*	This study
pYD155	*yycR*::P_{hy}*-yodL (amp)*	This study
pYD156	*ycgO*::P_{hy}*-yisK (amp)*	This study

Table S4. Oligos

Oligo	**Sequence 5' to 3'**
OAM001	AGAAGCGTTAGCGGCAGCAAGTGAT
OAM002	CCATGTCTGCCCGTATTTCGCGTAAGGAAATCCATTATGTACTATTTCGATCAGACCAG
OAM009	GAAAACAATAAACCCTTGCATAGGGGGATCGGGCAAGGCTAGACGGGACTTACC
OAM010	ATGGACACAACAACAGCAAAACAGGC
OAM011	TAATGGATTTCCTTACGCGAAATA
OAM013	AGTAGTTCCTCCTTATGTAAGC
OAS064	TCCTCCTTTTCAAAAGAAAAAAAC
OAS067	TGTTACATATTGCTGCTTTTTGGT
OAS078	GGATCCCAGCGAACCATTTGA
OAS079	GTCGACAAATTCCTCGTAGGC
OAS080	CCTATCACCTCAAATGGTTCGCTGGGATCCAAAGCAAAAATACCCTAAAGGGAA
OAS081	GTCCCGAGCGCCTACGAGGAATTTGTCGACACACTTTTTTTTTCGTCGAATTAAG
OAS086	CGAATACATACGATCCTACAGC
OAS087	CCTATCACCTCAAATGGTTCGCTGGGATCCAAAAAGTTGGAAGCACAATAAGTT
OAS088	GTCCCGAGCGCCTACGAGGAATTTGTCGACATCACCTGGCATTGCCTTCTT
OAS089	ATTAATGGTGATATTCTTCATTGA
OAS091	AGATGGATGTGCTCCAGTGCTCCAAGATCTATACCAAGGTCT
OAS092	AGACCTTGGTATAGATCTTGGAGCACTGGAGCACATCCATCT
OAS095	GGAAGCTTGTCCATATTATCAAGATTTGCAGTACCGAGGTCAATA
OAS096	TATTGACCTCGGTACTGCAAATCTTGATAATATGGACAAGCTTCC
OAS114	TCTAAGGAATTCCTGTTTTAGTCGGCATAAGCAG
OAS116	GTAATCTTACGTCAGTAACTTCCACCAAGATCCCCTCCCTTTTATTT
OAS117	AAGAAATAAAAGGGAGGGGATCTTGGTGGAAGTTACTGACGTAAGAT
OAS118	ACTTAGGGATCCTTATTTTTGACACCAGACCAACT
OAS119	TGAAAAGTTCTTCTCCTTTACTCATCAAGATCCCCTCCCTTTTATTT
OAS120	AAGAAATAAAAGGGAGGGGATCTTGATGAGTAAAGGAGAAGAACTTTTC
OAS121	ACTTAGGGATCCTTATTTGTATAGTTCATCCATGCCAT
OAS134	TCTAAGGAATTCTCCTTTTCAGCTGCTCCCGAT
OAS135	GTAATCTTACGTCAGTAACTTCCACGTTATTCCTCCATCATCTTTTAAA
OAS136	ATTTAAAAGATGATGGAGGAATAACGTGGAAGTTACTGACGTAAGAT
OAS137	TGAAAAGTTCTTCTCCTTTACTCATGTTATTCCTCCATCATCTTTTAAA
OAS138	ATTTAAAAGATGATGGAGGAATAACATGAGTAAAGGAGAAGAACTTTTC
OAS148	TCTAAGGAATTCATGAGTAAAGGAGAAGAACTTTTC
OAS149	ACTTAGGGATCCTTATTTGTATAGTTCATCCATGCC
OAS274	TCTAAGGAATTCTCCTTTTCAGCTGCTCCCGA
OAS275	ACTTAGGGATCCTCAGCCAATTTGGTTTGACAG
OEA035	GGATAACAATTAAGCTTACATAAGGAGGAACTACTATGAAATTTGCGACAGGGGAACTT
OEA036	TTCCACCGAATTAGCTTGCATGCGGCTAGCCCAGTTTTATTCAGCCAATTTGGT

OEA275	GGATAACAATTAAGCTTACATAAGGAGGAACTACTATGATGTTATCCGTGTTTAAAAAG
OEA276	TTCCACCGAATTAGCTTGCATGCGGCTAGCTTTCTTTTCATTATGTCGTTTGTA
OJH159	CTGCAGGAATTCGACTCTCTA
OJH160	TAGCTTGCATGCGGCTAGC
OJH185	CAGGAATTCGACTCTCTAGC
OJH186	CTCAGCTAGCTAACTCACATTAATTGCGTTGC

Strain Construction

The Gibson enzymatic assembly method has been described in detail (10).

Except where otherwise indicated, PCR amplifications are performed using *B. subtilis* 168 genomic DNA as template.

To generate unmarked derivatives the BGSC strains (BKE22320, BKE19640, and BKE10750) each strain was transformed with pDR244, selecting for spectinomycin resistance at 30°C. Isolated colonies were restreaked on LB media and placed at 42°C. Isolated colonies from these plates were streaked again on spectinomycin, MLS, and LB plates. Clones that were sensitive to both spectinomycin and MLS were used to generate freezer stocks. Loss of the erm resistance cassette was confirmed by PCR.

Moving suppressor mutations in *mbl* into clean genetic backgrounds

To move the mutant *mbl* alleles obtained in the suppressor selections, we generated linear Gibson assembly products corresponding to a region of ~1,500 bp upstream of *mbl* ("UP"), a kanamycin resistance cassette ("KAN"), and each mutant *mbl* allele plus ~1,500 bp downstream of *mbl* ("DOWN"). This linear double stranded DNA product was then transformed directly into *B. subtilis*. Double cross-over recombination was selected for by plating on LB plates supplemented with 10 µg/ml kanamycin. To confirm the presence of the relevant mutations, and the absence of others, the entire *mbl* ORF was sequenced. The "UP" fragment comprised a region of homology to the *B. subtilis* chromosome upstream of *mbl* (including a region downstream of *spoIIID),* and was amplified from genomic DNA using oAS86 and oAS87. The "KAN" fragment was made by amplifying a kanamycin cassette from pWX114a using oAS78 and oAS79. The "DOWN" fragment corresponded to a region including the *mbl* promoter, *mbl* and 1500bp downstream of *mbl* and was amplified from genomic DNA of each corresponding suppressor strain using oAS88 and oAS89. For each allele of interest, the three products were combined in single Gibson assembly reaction and transformed directly into *B. subtilis 168*, selecting for kanamycin resistance. To confirm the presence of the relevant mutation(s) and the absence of others, the entire *mbl* ORF was sequenced.

Moving suppressor mutations in *mreB* into clean genetic backgrounds

To move the mutant *mreB* alleles obtained in the suppressor selections, we generated linear Gibson assembly products corresponding to a region of ~1,500 bp upstream of *mreB* ("UP"), a kanamycin resistance cassette ("KAN"), and each mutant *mreB* allele plus ~1,500 bp downstream of *mreB* ("DOWN"). This linear double stranded DNA product was then transformed directly into *B. subtilis*. Double cross-over recombination was selected for by plating on LB plates supplemented with 10 µg/ml kanamycin. To confirm the presence of the relevant mutations, and the absence of others, the entire *mreB* ORF was sequenced. The "UP" fragment comprised a region of homology to the *B. subtilis* chromosome upstream of *mreB* (including a region downstream of *radC),* and was amplified from genomic DNA using oAS64 and oAS80. The "KAN" fragment was made by amplifying a kanamycin cassette from pWX114a using oAS78 and oAS79. The "DOWN" fragment corresponded to a region including the *mreB* promoter, *mreB* and 1500bp downstream of *mreB* and was amplified from genomic DNA of each corresponding suppressor strain using oAS81 and oAS67. For each allele of interest, the three products were combined in single Gibson assembly reaction and transformed

directly into B. subtilis 168, selecting for kanamycin resistance. To confirm the presence of the relevant mutation(s) and the absence of others, the entire *mreB* ORF was sequenced.

BAS040 was generated by transforming a linear Gibson assembly product encoding a region upstream of *amyE*, a spectinomycin cassette, the P_{hy} promoter, an optimized ribosome binding site, *yodL*, *lacI*, and a region downstream of *amyE* into *B. subtilis* 168, and selecting for spectinomycin resistance. The final strain was confirmed by PCR and sequencing. The region of upstream of *amyE* was PCR amplified from *B. subtilis* 168 genomic DNA using OAM009 and OAM010. The spectinomycin resistance cassette and the P_{hy} promoter were PCR amplified from pDR111 using primers OAM10 and OAM13, and included a ribosome binding site (TAAGGAGG). The *yodL* ORF was PCR amplified using OEA275 and OEA276. *lacI* was PCR amplified from pDR111 using OAM011 and OAM012. The region downstream of *amyE* was PCR amplified from *B. subtilis* 168 genomic DNA using OAM001 and OAM002.

BAS041 was generated by transforming a linear Gibson assembly product encoding a region upstream of *amyE*, a spectinomycin cassette, the P_{hy} promoter, an optimized ribosome binding site, *yisK*, *lacI*, and a region downstream of *amyE* into *B. subtilis* 168, and selecting for spectinomycin resistance. The final strain was confirmed by PCR and sequencing. The region of upstream of *amyE* was PCR amplified from *B. subtilis* 168 genomic DNA using OAM009 and OAM010. The spectinomycin resistance cassette and the P_{hy} promoter were PCR amplified from pDR111 using primers OAM10 and OAM13, and included a ribosome binding site (TAAGGAGG). The *yisK* ORF was PCR amplified using OEA035 and OEA036. *lacI* was PCR amplified from pDR111 using OAM011 and OAM012. The region downstream of *amyE* was PCR amplified from *B. subtilis* 168 genomic DNA using OAM001 and OAM002.

BAS147 was generated by directly transforming a Gibson assembly of a region upstream of *mbl*, a kanamycin resistance cassette, and an *mbl* knockout fragment (see below) directly into BKE22320, selecting for kanamycin resistance in the presence of 10 mM $MgCl_2$. The region upstream of *mbl* was amplified using OAS86 and OAS87 (*B. subtilis* 168 genomic DNA template). The kanamycin resistance cassette was amplified from pWX114a using OAS78 and OAS79. The *mbl* knockout fragment was made by amplifying a DNA product that included the first 42 bp of *mbl* and its upstream region using oAS86 and oAS95 (*B. subtilis* 168 genomic DNA template), amplifying a DNA product that included the last 42 bp *mbl* and its downstream region using oAS96 and oAS89 (*B. subtilis* 168 genomic DNA template), and splicing the two fragments together using overlap extension PCR with OAS088 and OAS089. The kanamycin-linked *mbl* knockout was confirmed by PCR amplification.

BAS170 was made by transforming *B. subtilis* 168 with pAS040 linearized with ScaI and selecting for spectinomycin resistance. Integrations were confirmed by starch test.

BAS171 was made by transforming *B. subtilis* 168 with pAS041 linearized with ScaI and selecting for spectinomycin resistance. Integrations were confirmed by starch test.

BAS192 was made by transforming *B. subtilis* 168 with pAS044 linearized with ScaI and selecting for spectinomycin resistance. Integrations were confirmed by starch test and sequencing.

BAS193 was made by transforming *B. subtilis* 168 with pAS045 linearized with ScaI and selecting for spectinomycin resistance. Integrations were confirmed by starch test and sequencing.

BAS265 was generated by transforming *B. subtilis* 168 with genomic DNA from RL891 and selecting for erythromycin resistance (erm). Recommend growth on 0.5 µg/ml erythromycin (erm) and 12.5 µg/ml lincomycin (0.5X normal antibiotic concentration).

BAS282 was generated by transforming *B. subtilis* 168 with genomic DNA from BJH154 and selecting on chloramphenicol.

BYD258 was generated by directly transforming a Gibson assembly of a region upstream of *mbl*, a kanamycin resistance cassette, and an *mbl* knockout fragment (see below) directly into BYD175, selecting for kanamycin resistance in the presence of 10 mM $MgCl_2$. The region upstream of *mbl* was amplified using OAS86 and OAS87 (*B. subtilis* 168 genomic DNA template). The kanamycin resistance cassette was amplified from pWX114a using OAS78 and OAS79. The *mbl* knockout fragment was made by amplifying a DNA product that included the first 42 bp of *mbl* and its upstream region using oAS86 and oAS95 (*B. subtilis* 168 genomic DNA template), amplifying a DNA product that included the last 42 bp *mbl* and its downstream region using oAS96 and oAS89 (*B. subtilis* 168 genomic DNA template), and splicing the two fragments together using overlap extension PCR with OAS088 and OAS089. The kanamycin-linked *mbl* knockout was confirmed by PCR amplification.

BYD259 was generated as described for BYD258, but transforming the final Gibson assembly into BYD176. The kanamycin-linked *mbl* knockout was confirmed by PCR amplification.

BYD262 was generated by directly transforming a Gibson assembly of a region upstream of *mreB*, a kanamycin resistance cassette, and an *mreB* knockout fragment (see below) directly into BYD175, selecting for kanamycin resistance in the presence of 10 mM $MgCl_2$. The region upstream of *mreB* was amplified using OAS64 and OAS80 (*B. subtilis* 168 genomic DNA template) and was designed to include the putative *radC* terminator/*mreB* promoter region to avoid the chances of disrupting the terminator. The kanamycin resistance cassette was amplified from pWX114a using OAS78 and OAS79. The *mreB* knockout fragment was made by amplifying the *mreBCD* promoter region and the first 42bp of *mreB* using oAS64 and oAS91 (*B. subtilis* 168 genomic DNA template), amplifying the last 42bp of *mreB* along with region downstream of *mreB* using OAS92 and OAS67 (*B. subtilis* 168 genomic DNA template), and splicing the two fragments together using overlap extension PCR with oAS81 and oAS67. The kanamycin-linked *mreB* knockout was confirmed by PCR amplification. The final integration product encodes identical sequences before and after the kanamycin cassette, so loss of the kanamycin resistance cassette is possible in the absence of selective pressure.

BYD263 was generated as described for BYD262, but transforming the final Gibson assembly into BYD176. The kanamycin-linked *mreB* knockout was confirmed by PCR amplification.

Plasmid construction

pAS015 was generated by cloning PCR product from OJH159 and OJH160 amplification of genomic DNA from BAS041 into pJW004 (EcoRI-NheI).

pAS040 was generated with overlap extension PCR. The “UP” product encoding the *yodL* promoter region was amplified from *B. subtilis* 168 genomic DNA with primer pair OAS114/OAS116. The “DOWN” product encoding *lacZ* was amplified from genomic DNA from SYL#8 (Table S1) using primer pair OAS117/OAS118. The two PCR products were used as template for overlap extension PCR using primer pair OAS114/OAS118. The amplified fragment was cut with EcoRI and BamHI and cloned into pDR111 cut with the same enzymes.

pAS041 was generated with overlap extension PCR. The “UP” product was amplified from *B. subtilis 168* genomic DNA with primer pair OAS114/OAS119. The “DOWN” product was amplified from pJW006 with primer pair OAS120/ OAS121. The two PCR products were used as template for overlap extension PCR with primer pair OAS114/OAS121. The amplified fragment was cut with EcoRI and BamHI and cloned into pDR111 cut with the same enzymes.

pAS044 was generated with overlap extension PCR. The "UP" product encoding the *yisK* promoter region was amplified from *B. subtilis* 168 genomic DNA template using primer pair OAS134/OAS135. The "DOWN" product encoding the *lacZ* gene was amplified from genomic DNA of SYL#8 (Table S1) with primer pair OAS136/OAS118. The two PCR products were used as template for overlap extension PCR using primer pair OAS134/OAS118. The amplified fragment was cut with EcoRI and BamHI and cloned into pDR111 cut with the same enzymes.

pAS045 was generated with overlap extension PCR. The "UP" product was amplified from *B. subtilis* 168 genomic DNA template using primer pair OAS134/OAS137. The "DOWN" product was amplified from pJW006 with primer pair OAS138/ OAS121. The two PCR products were used as template for overlap extension PCR with primer pair OAS134/OAS121. The amplified fragment was cut with EcoRI and BamHI and cloned into pDR111 cut with the same enzymes.

pAS047 was generated by cloning PCR product from an OAS148 and OAS149 amplification of pJW006 into pDR111 (EcoRI-BamHI).

pAS067 was generated by cloning the PCR product obtained from an OAS274 and OAS275 amplification of *B. subtilis* 168 genomic DNA template (encoding 200bp upstream of *yisK* and *yisK*) into pDR111 (EcoRI-BamHI).

pJH036 was generated by cloning the PCR product obtained from an OJH185 and OJH186 amplification of genomic DNA from BDR992 (encoding P_{hy}-*lacZ)* into pKM062 (EcoRI-NheI).

pJW004 was generated by cloning the ~1770 bp BamHI-EcoRI fragment from pDR111 (encoding the P_{hy} promotor and *lacI*) into pBB280, an allelic exchange vector that integrates at the *yhdG* locus and confers phleomycin resistance.

pJW033 was generated by cloning the ~1770 bp BamHI-EcoRI fragment from pDR111 (encoding the P_{hy} promotor and *lacI*) into pKM086, an allelic integration vector that integrates at the *ycgO* locus and confers tetracycline resistance.

pJW034 was was generated by cloning the ~1770 bp BamHI-EcoRI fragment from pDR111 (encoding the P_{hy} promotor and *lacI*) into pNS037, an allelic exchange vector that integrates at the *yycR* locus and confers chloramphenicol resistance.

pYD073 was generated by cloning PCR product from OJH159 and OJH160 amplification using genomic DNA from BAS040 (amyE::P_{hy}-*yodL*) and cloning into the into the EcoRI-NheI sites of pJW004.

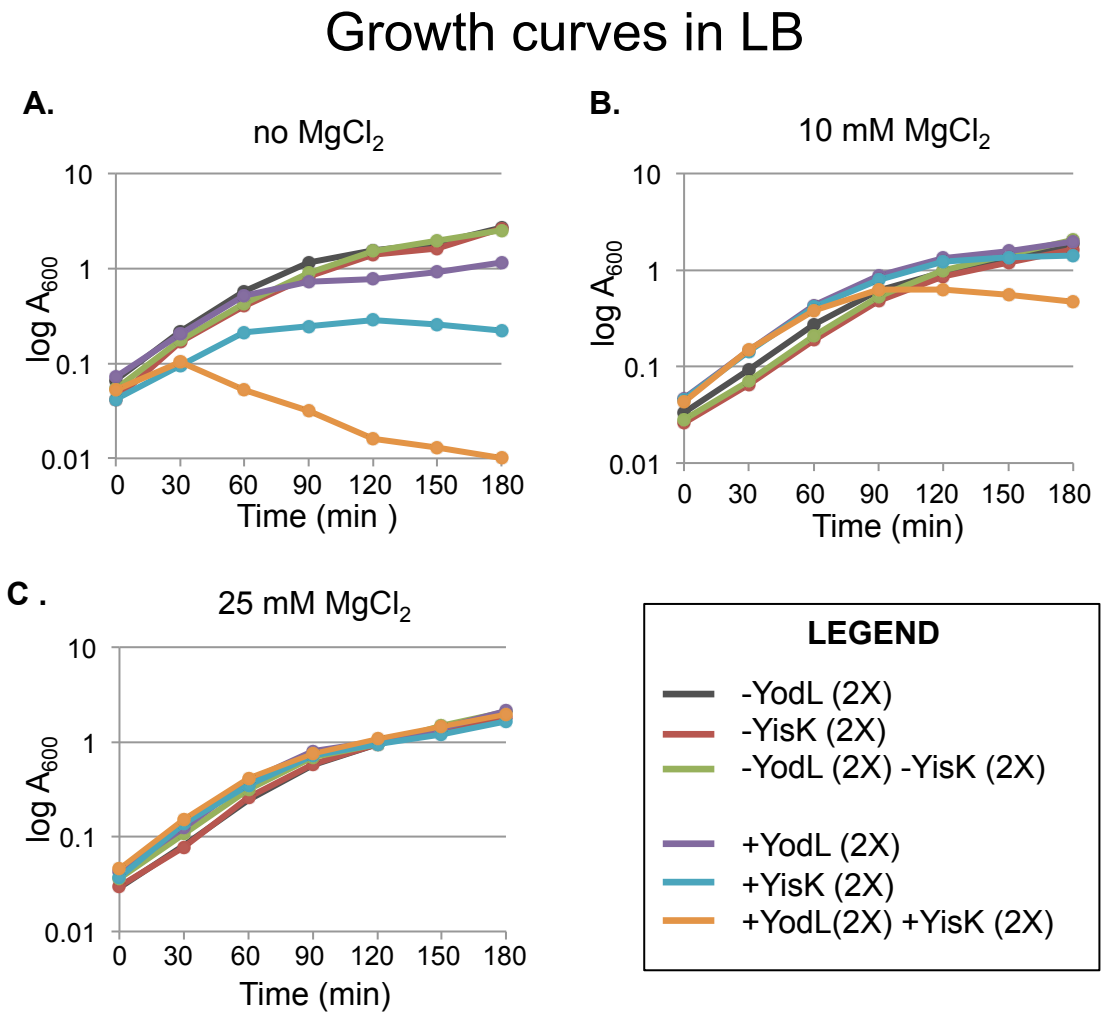
pYD155 was generated by cloning PCR product from OJH159 and OJH160 amplification using genomic DNA from BAS040 and cloning into the into the EcoRI-NheI sites of pJW034.

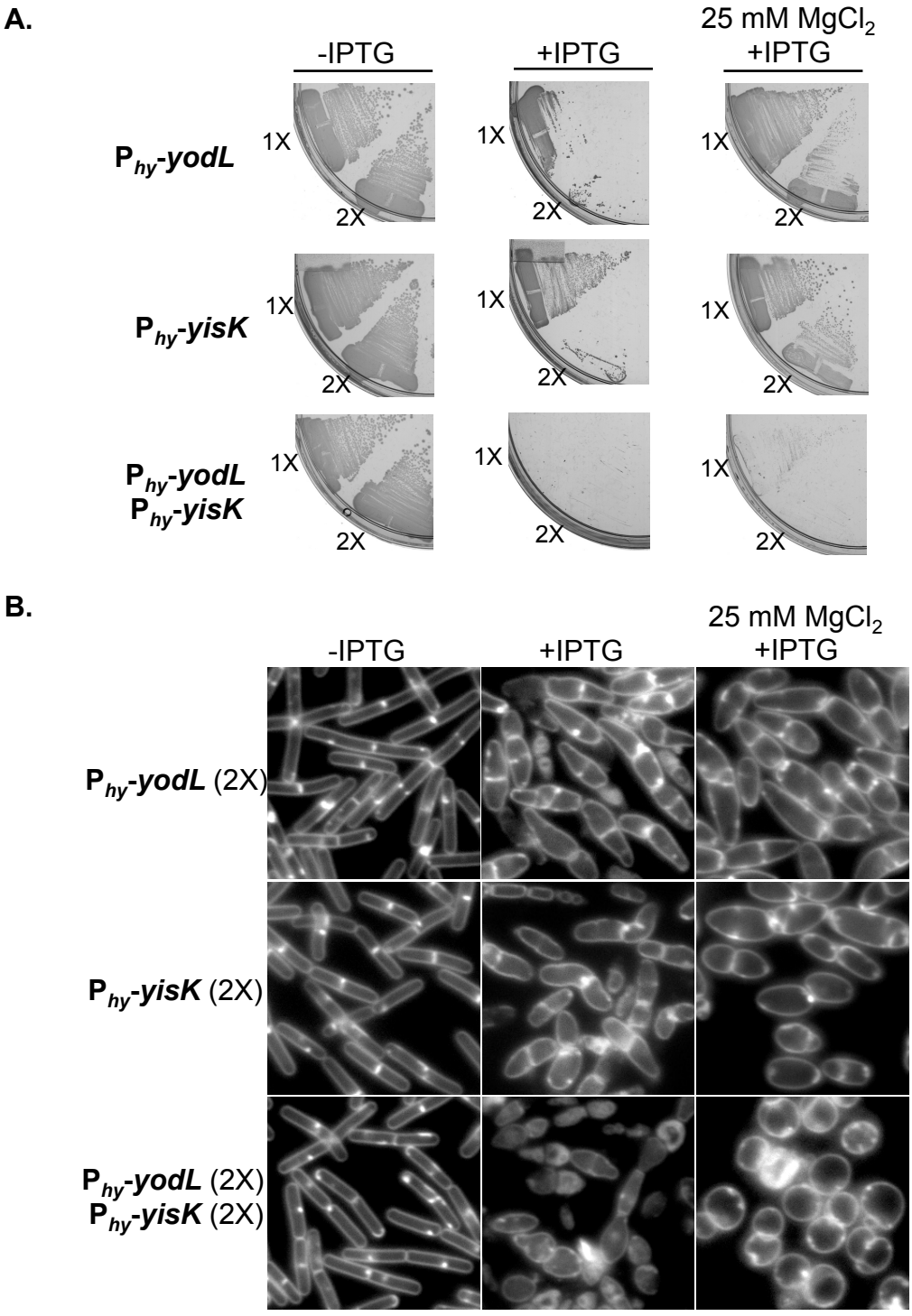
pYD156 was generated by cloning PCR product from OJH159 and OJH160 amplification using genomic DNA from BAS040 and cloning into the into the EcoRI-NheI sites of pJW033.

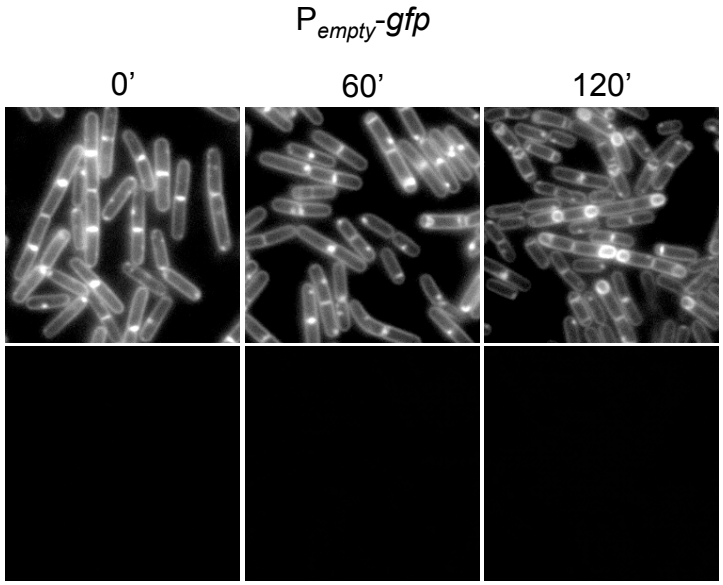
REFERENCES

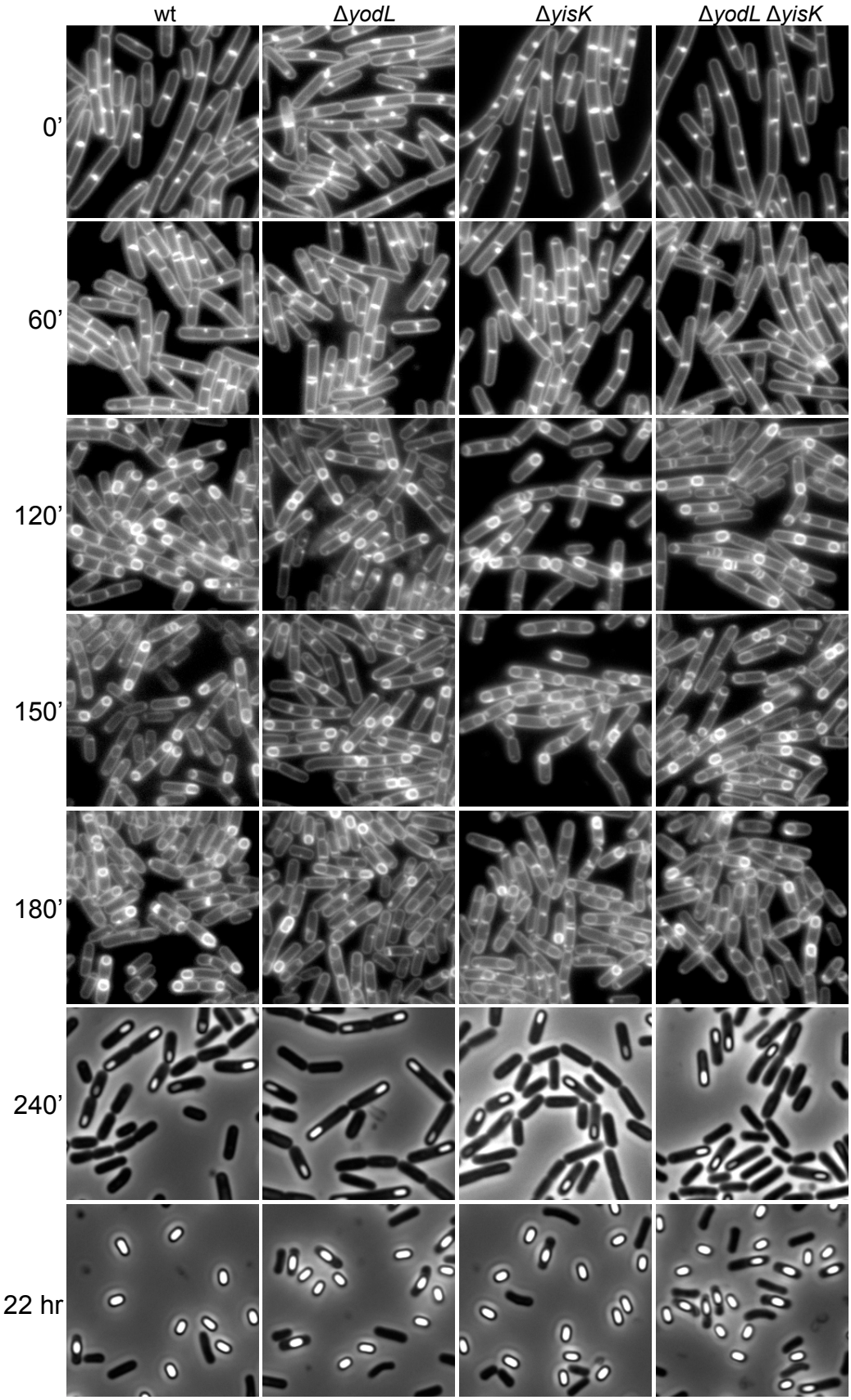
1. **van den Ent F, Johnson CM, Persons L, de Boer P, Lowe J.** 2010. Bacterial actin MreB assembles in complex with cell shape protein RodZ. EMBO J **29:**1081-1090.

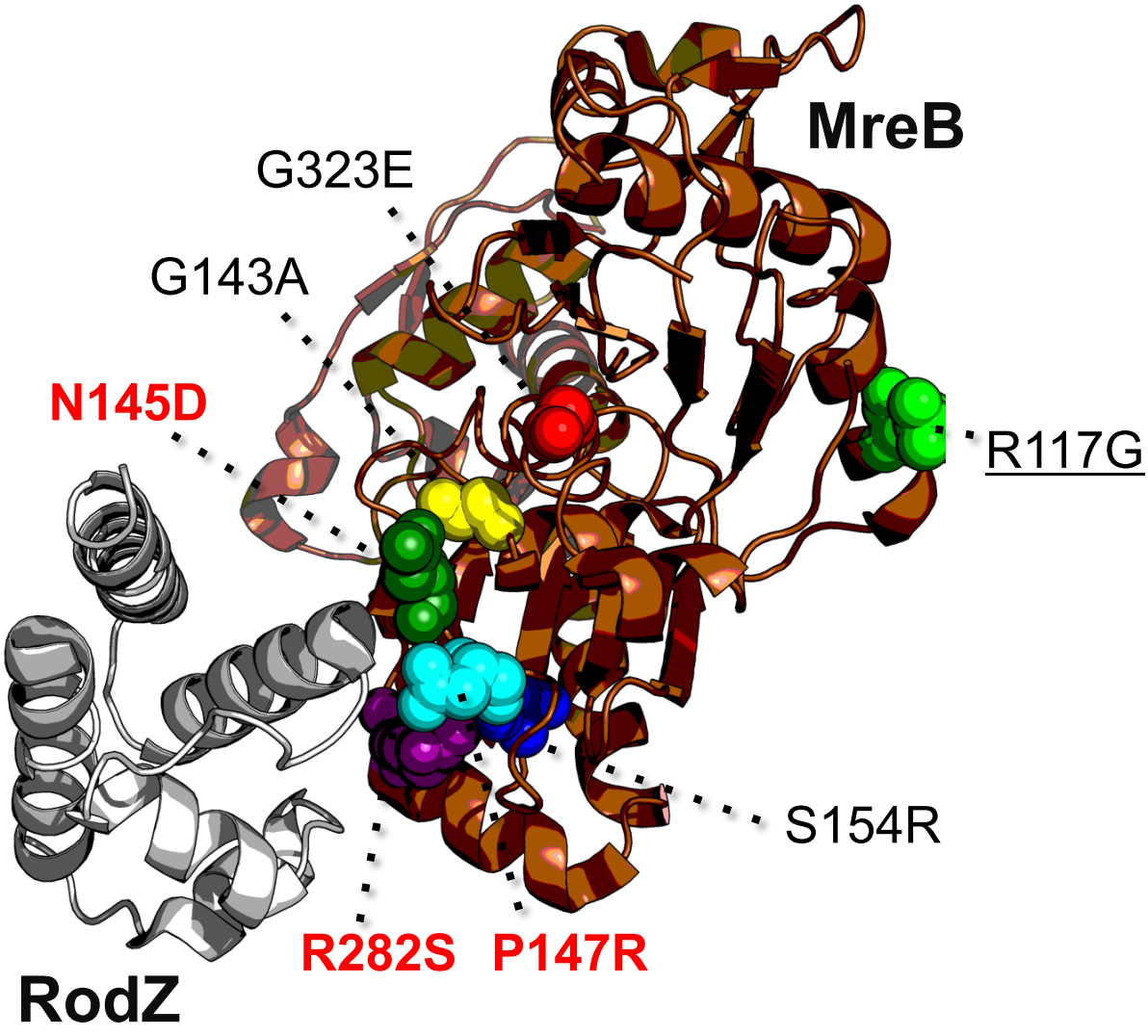
2. **Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y.** 2015. The I-TASSER Suite: protein structure and function prediction. Nat Methods **12:**7-8.
3. **van den Ent F, Amos LA, Lowe J.** 2001. Prokaryotic origin of the actin cytoskeleton. Nature **413:**39-44.
4. **Dye NA, Pincus Z, Fisher IC, Shapiro L, Theriot JA.** 2011. Mutations in the nucleotide binding pocket of MreB can alter cell curvature and polar morphology in *Caulobacter*. Mol Microbiol **81:**368-394.
5. **Gitai Z, Dye NA, Reisenauer A, Wachi M, Shapiro L.** 2005. MreB actin-mediated segregation of a specific region of a bacterial chromosome. Cell **120:**329-341.
6. **Srivastava P, Demarre G, Karpova TS, McNally J, Chattoraj DK.** 2007. Changes in nucleoid morphology and origin localization upon inhibition or alteration of the actin homolog, MreB, of *Vibrio cholerae*. J Bacteriol **189:**7450-7463.
7. **van den Ent F, Izore T, Bharat TA, Johnson CM, Lowe J.** 2014. Bacterial actin MreB forms antiparallel double filaments. Elife **3:**e02634.
8. **Branda SS, Gonzalez-Pastor JE, Ben-Yehuda S, Losick R, Kolter R.** 2001. Fruiting body formation by Bacillus subtilis. Proc Natl Acad Sci U S A **98:**11621-11626.
9. **Wagner JK, Marquis KA, Rudner DZ.** 2009. SirA enforces diploidy by inhibiting the replication initiator DnaA during spore formation in *Bacillus subtilis*. Mol Microbiol **73:**963-974.
10. **Gibson DG, Young L, Chuang RY, Venter JC, Hutchison CA, 3rd, Smith HO.** 2009. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods **6:**343-345.


Growth curves in LB
A.
no MgCl2
log A600
10
1
0.1
0.01
0 30 60 90 120 150 180
Time (min)
B.
10 mM MgCl2
log A600
10
1
0.1
0.01
0 30 60 90 120 150 180
Time (min)
C .
25 mM MgCl2
log A600
10
1
0.1
0.01
0 30 60 90 120 150 180
Time (min)
LEGEND
-YodL (2X)
-YisK (2X)
-YodL (2X) -YisK (2X)
+YodL (2X)
+YisK (2X)
+YodL(2X) +YisK (2X)


A.
-IPTG
+IPTG
25 mM MgCl2
+IPTG
P hy-yodL
P hy-yisK
P hy-yodL
P hy-yisK
1X
2X
B.
-IPTG
+IPTG
25 mM MgCl2
+IPTG
P hy-yodL (2X)
P hy-yisK (2X)
P hy-yodL (2X)
P hy-yisK (2X)


P empty -gfp
0’
60’
120’

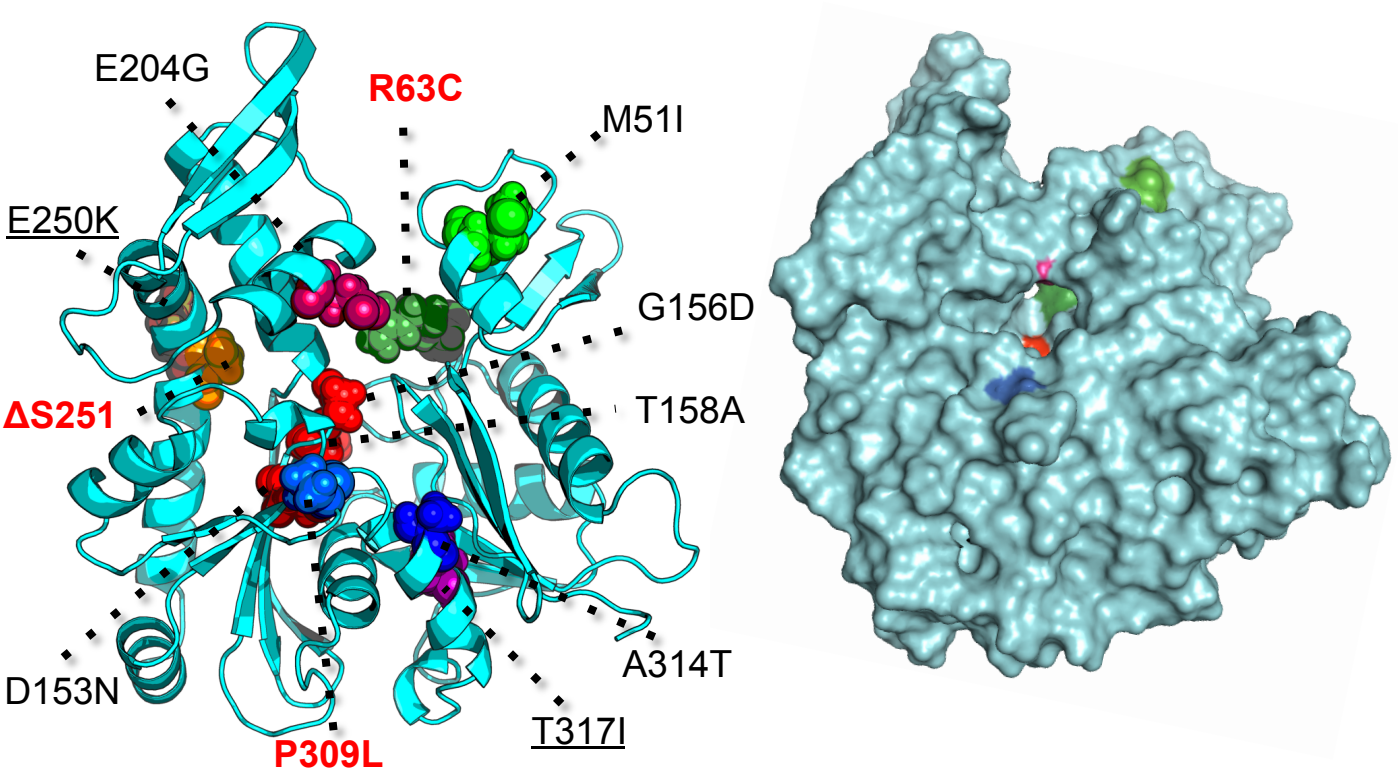

wt
ΔyodL
ΔyisK
ΔyodL ΔyisK
0’
60’
120’
150’
180’
240’
22 hr




```
T. maritima MreB     1 ---MLRKDIGIDLGTANTLVFLRGKGIVVNEPSVIAIDSTTGEILKVGLEAKNMIGKTPA
B. subtilis MreB     1 MFGIGARDLGIDLGTANTLVFVKGKGIVVREPSVVALQTDTKSIVAVGNDAKNMIGRTPG
B. subtilis Mbl      1 ---MFARDIGIDLGTANVLIHVKGKGIVLNEPSVVALDKNSGKVLAVGEEAARRMVGRTPG

T. maritima MreB    58 TIKAIRPMRDGVIADYTVALVMLRYFINKAKG--GMNLFKPRVVIGVPIGITDVERRAIL
B. subtilis MreB    61 NVVALRPMKDGVIADYETTATMMKYYINQAIKNKGMFARKPYVMVCVPSGITAVEERAVI
B. subtilis Mbl     58 NIVAIRPLKDGVIADFEVTEAMLKHFINKLN--VKGLFSKPRMLICCPTNITSVEQKAIK

T. maritima MreB   116 DAGLEAGASKVFLIEEPMAAAIGSNLNVEEPSGNMVVDIGGGTTEVAVISLGSIVTWESI
B. subtilis MreB    121 DATRQAGARDAYPIEEPFAAAIGANLPVWEPTGSMVVDIGGGTTEVAIISLGGIVTSQSI
B. subtilis Mbl     116 EAAEKSGGKHVYLEEEPKVAAIGAGMEIFQPSGNMVVDIGGGTTDIAVISMGDIVTSSSI

T. maritima MreB   176 RIAGDEMDEAIVQYVRETYRVAIGERTAERVKIEIGNVFPSKENDELETTVSGIDLSTGL
B. subtilis MreB    181 RVAGDEMDDAIINYIRKTYNLMIGDRTAEAIKMEIGSAEAPEESDNMEIRGR--DLLTGL
B. subtilis Mbl     176 KMAGDKFDMEILNYIKREYKLLIGERTAEDIKIKVATVFPDARHEEISIRGR--DMVSGL

T. maritima MreB   236 PRKLTLKGGEVREALRSVVVAIVESVRTTLEKTPPELVSDIIERGIFLTGGGSLLRGLDT
B. subtilis MreB    239 PKTIEITGKEISNALRDTVSTIVEAVKSTLEKTPPELAADIMDRGIVLTGGGALLRNLDK
B. subtilis Mbl     234 PRTITVNSKEVEEALRESVAVIVQAAKQVLERTPPELSADIIDRGVIITGGGALLNGLDQ

T. maritima MreB   296 LLQKETGISVIRSEEPLTAVAKGAGMVLDKVNILKKLQGAG
B. subtilis MreB    299 VISEETKMPVLIAEDPLDCVAIGTGKALEHIHLFKGKTR--
B. subtilis Mbl     294 LLAEELKVPVLVAENPMDCVAIGTGVMLDNMDKLPKRKLS-
```

B. subtilis Mbl

Threaded to *T. maritima* MreB (PDB:1JCG)




```
T. maritima MreB      1 -------MLRKDIGIDLGTANTLVFLRGKGIVVNEPSVIAIDSTTG----EILKVGLEAK
B. subtilis MreB      1 ----MFGIGARDLGIDLGTANTLVFVKGKGIVVREPSVVALQTDT----KSIVAVGNDAK
B. subtilis Mbl        1 -------MFARDIGIDLGTANVLIHVKGKGIVLNEPSVVALDKNSG----KVLAVGEEAR
C. crescentus MreB     1 MFSSLFGVISNDIAIDLGTANTLIYQKGKGIVLNEPSVVALRN--VGGRKVVHAVGIEAK
E. coli MreB           1 MLKKFRGMFSNDLSIDLGTANTLIYVKGQGIVLNEPSVVAIRQDRAGSPKSVAAVGHDAK
V. cholerae MreB       1 MFKKLRGMFSNDLSIDLGTANTLIYVKGQGIVLDEPSVVAIRQDKGRGGKTVAAVGHAAK

T. maritima MreB     50 NMIGKTPATIKAIRPMRDGVIADYTVALVMLRYFINKAKGG--MNLFKPRVVIGVPIGIT
B. subtilis MreB      53 NMIGRTPGNVVALRPMKDGVIADYETTATMMKYYINQAIKNKGMFARKPVVMVCVPSGIT
B. subtilis Mbl        50 RMVGRTPGNIVAIRPLKDGVIADFEVTEAMLKHFINKLNVKG--LFSKPRMLICCPTNIT
C. crescentus MreB     59 QMLGRTPGHMEAIRPMRDGVIADFEVAEEMIKYFIRKVFNRK-GFVN-PKVIVCVPSGAT
E. coli MreB           61 QMLGRTPGNIAAIRPMKDGVIADFFVTEKMLQHFIKQVHSNS-FMRPSPRVLVCVPVGAT
V. cholerae MreB       61 QMLGRTPGNISAIRPMKDGVIADFYVTEKMLQHFIRQVHDNS-VLKPSPRVLVCVPCGST

T. maritima MreB    108 DVERRAILDAGLEAGASKVFLIEEPMAAAIGSNLNVEEPSGNMVVDIGGGTTEVAVISLG
B. subtilis MreB      113 AVEERAVIDATRQAGARDAYPIEEPFAAAIGANLPVWEPTGSMVVDIGGGTTEVAIISLG
B. subtilis Mbl        108 SVEQKAIKEAAEKSGGKHVYLEEEPKVAAIGAGMEIFQPSGNMVVDIGGGTTDIAVISMG
C. crescentus MreB     117 AVERRAINDSCLNAGARRVGLIDEPMAAAIGAGLPIHEPTGSMVVDIGGGTTEVAVLSLS
E. coli MreB           120 QVERRAIRESAQGAGAREVFLIEEPMAAAIGAGLPVSEATGSMVVDIGGGTTEVAVISLN
V. cholerae MreB       120 QVERRAIRESALGAGAREVYLIDEPMAAAIGAGLRVSEPTGSMVIDIGGGTTEVAVISLN

T. maritima MreB    168 SIVTWESIRIAGDEMDEAIVQYVRETYRVAIGERTAERVKIEIGNVFPSKENDELETTVS
B. subtilis MreB      173 GIVTSQSIRVAGDEMDDAIINYIRKTYNLMIGDRTAEAIKMEIGSAEAP--EESDNMEIR
B. subtilis Mbl        168 DIVTSSSIKMAGDKFDMEILNYIKREYKLLIGERTAEDIKIKVATVFP--DARHEEISIR
C. crescentus MreB     177 GIVYSRSVRVGGDKMDEAIISYMRRHHNLLIGETTAERIKKEIGTARAPADGEGLSIDVK
E. coli MreB           180 GVVYSSSVRIGGDRFDEAIINYVRRNYGSLIGEATAERIKHEIGSAYP--GDEVREIEVR
V. cholerae MreB       180 GVVYSSSVRIGGDRFDEAIINYVRRNYGSLIGEATAEKIKHEIGSAYP--GDDVQEIEVR

T. maritima MreB    228 GIDLSTGLPRKLTLKGGEVREALRSVVVAIVESVRTTLEKTPPELVSDIIERGIPLTGGG
B. subtilis MreB      231 GRDLLTGLPKTIEITGKEISNALRDTVSTIVEAVKSTLEKTPPELAADIMDRGIVLTGGG
B. subtilis Mbl        226 GRDMVSGLPRTITVNSKEVEEALRESVAVIVQAAKQVLERTPPELSADIIDRGVIITGGG
C. crescentus MreB     237 GRDLMQGVPREVRISEKQAADALAEPVGQIVEAVKVALEATPPELASDIADKGIMLTGGG
E. coli MreB           238 GRNLAEGVPRGFTLNSNEILEALQEPLTGIVSAVMVALEQCPPELASDISERGMVLTGGG
V. cholerae MreB       238 GRNLAEGVPRSFTLNSNEILEALQEPLTGIVSAVMVALEQCPPELASDISENGMVLTGGG

T. maritima MreB    288 SLLRGLDTLLQKETGISVIRSEEPLTAVAKGAGMVLDKVNILKKLQGAG--
B. subtilis MreB      291 ALLRNLDKVISEETKMPVLIAEDPLDCVAIGTGKALEHIHLFKGKTR----
B. subtilis Mbl        286 ALLNGLDQLLAEELKVPVLVAENPMDCVAIGTGVMLDNMDKLPKRKLS----
C. crescentus MreB     297 ALLRGLDAEIRDHTGLPVTVADDPLSCVALGCGKVLEHPKWMKGVLESTLA
E. coli MreB           298 ALLRNLDRLLMEETGIPVVVAEDPLTCVARGGGKALEMIDMHGGDLFSEE-
V. cholerae MreB       298 ALLKDLDRLLMEETGIPVVIADDPLTCVARGGGKALEMIDMHGGDLFSEE-
```