Supplementary material

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Plasmid constructions – The YciM expression vectors were constructed as follows: the region encoding YciM was amplified from the chromosome of MC1000 strain using primers *yciM_NcoI* and *yciM_HindIII_pBAD* and cloned into a pBAD-HisB plasmid (Invitrogen), yielding pVN2. A C-terminal His-tagged version of YciM was constructed by amplifying the *yciM* gene from the chromosome of MC1000 strain using primers *yciM_NcoI* and *yciM_BglII_pQE60* and cloned into a pQE60 plasmid (Qiagen), yielding pCB25. The sequence coding for *yciM* fused to that coding for the C-terminal His-tag was then cut with *Nco*I and *Hin*dIII from pCB25 and inserted into a pBAD-HisB vector, yielding plasmid pHE43. The sequence encoding a truncated form of YciM (YciM*) lacking the first 22 amino acids was amplified and cloned into 2 different vectors: pBAD-HisB and pASK-IBA63a-plus. Two different versions of YciM* were produced by each vector: YciM* with a C-terminal tag or YciM* without any tag. In order to introduce the sequence encoding the C-terminal His-tagged version of YciM* into the pBAD-HisB vector, the coding sequence for the truncated form of YciM (YciM*) was amplified with primers *yciM***_NcoI* and *yciM_BglII_pBAD* using pHE43 as a template, cut with *Nco*I and *Bgl*II and then inserted into pHE43, generating plasmid pHE72. The sequence encoding the untagged YciM* version was cloned into the pBAD-HisB vector by amplifying *yciM** with primers *yciM***_Nco*I and *yciM_HindIII_pBAD* using pVN2 as a template. The PCR product was then cut with *Nco*I and *Hin*dIII and inserted into pVN2, generating plasmid pVN182. In order to clone *yciM** into the pASK-IBA63a-plus vector, *yciM** was amplified using pHE72 as a template with two different primers pairs: *yciM** *_NcoI* and *yciM_XhoI_pASK* or *yciM** *_NcoI* and

yciM_XhoI_STOP. The PCR products were then cut with *Nco*I and *Xho*I and inserted into a pASK-IBA63a-plus plasmid to generate pVN55 (coding for YciM* with a C-terminal Strep-tag) and pVN74 (coding for untagged YciM*), respectively.

Three mutant alleles expressing proteins YciM$^{*}_{SS/CCCC\text{-}Strep}$, YciM$^{*}_{SS/CCCC\text{-}His}$ and YciM$_{CC/SSSS}$were constructed by site-directed mutagenesis using the QuickChange Site-Directed Mutagenesis protocol (Stratagene) and plasmids pVN55, pHE72 and pVN2 as template, respectively. The expression plasmids for YciM$^{*}_{SS/CCCC\text{-}Strep}$ and YciM$^{*}_{SS/CCCC\text{-}His}$ were generated by sequentially replacing the codons for cysteines 184 and 256 by serine codons using the following mutagenic primers: *yciM_C1S1_Fw* and *yciM_C1S1_Rv* for cysteine C184, *yciM_C2S2_Fw* and *yciM_C2S2_Rv* for cysteine C256 (yielding plasmid pVN67 and pVN147, respectively). To produce the YciM$_{CC/SSSS}$ mutant protein, the codons for cysteines 357, 360, 371 and 374 were replaced by serine codons using primers *yciM_S1XXS2_Fw* and *yciM_S1XXS2_Rv* for C357 and C360 and *yciM_S3XXS4_Fw* and *yciM_S3XXS4_Rv* for C371 and C374 (yielding plasmid pVN181).

The gene encoding mCherry was PCR-amplified with primers *mCherry_Fw* and *mCherry_Rv* from the plasmid pRSET-B*mCherry* and ligated in frame at the 3' end of the *yciM* gene, replacing the sequence coding for the His-tag of the pHE43 or pHE72 plasmids. The resulting plasmids, designated as pHE64 and pVN68, encode the fusion proteins YciM$_{mCherry}$ and YciM$^{*}_{mCherry}$, respectively.

All plasmids were verified by sequencing.

45 **Table S1. Strains and plasmids used in this study**

Strains or plasmids	Relevant genotype or features	Source or Ref.
Strains		
MC1000	F$^-$ λ^- *araD*139 Δ*(ara-leu)*7697 Δ*(lacIY)*74 *galU galK rpsL*	(1)
BL21	F$^-$ *dcm ompT hsdS*($r_{B-}m_{B-}$) *gal* λ(DE3)	(2)
XL1-Blue	r*ecA*1 *endA*1 *gyrA*96 *thi*$^-$1 *hsdR*17 *supE*44 *relA*1 *lac* [F *proAB lacI qZ*ΔM15 *Tn10* (*Tetr*)]	Stratagene
BW25113	*rrnB*3 Δ*lacZ*4787 *hsdR*514 Δ(*araBAD*)567 Δ(*rhaBAD*)568 *rph-1*	(3)
CB23	MC1000 Δ*yciM::kan*	This study
JW1272	BW25113 Δ*yciM::kan*	(4)
JW2479	BW25113 Δ*yfgC::kan*	(4)
JW3119	BW25113 Δ*yraP::kan*	(4)
JW5105	BW25113 Δ*ybiX::kan*	(4)
NR1681	MC1000 Δ*yciS::kan*	This study
NR1647	MC1000 Δ*yciM*	This study
HE43	BL21 carrying pHE43	This study
HE64	CB23 carrying pHE64	This study
HE89	CB23 carrying pHE89	This study
VN15	MC1000 ara^R	This study
VN20	VN15 carrying empty pBAD-HisB	This study
VN26	MC1000 Δ*pyrF::kan*	This study
VN50	VN15 Δ*yciM::kan*	This study
VN57	BL21 carrying pVN55	This study
VN63	BL21 carrying pHE72	This study
VN64	NR1647Δ*rfaI::kan*	This study
VN69	BL21 carrying pVN67	This study
VN75	BL21 carrying pVN74	This study
VN90	NR1647Δ*rfaQ::kan*	This study
VN94	NR1647Δ*asmA::kan*	This study
VN110	NR1647Δ*rfaP::kan*	This study
VN148	BL21 carrying PVN147	This study
VN179	VN50 carrying empty pBAD-HisB	This study
VN187	VN50 carrying pVN2	This study
VN196	VN50 carrying pVN182	This study
VN198	BL21 carrying pVN182	This study
VN213	VN50 carrying pVN173	This study
VN237	CB23 carrying pVN68	This study
VN241	BL21 carrying pVN2	This study
VN250	MC1000 Δ*envC::kan*	This study
VN255	NR1647 Δ*prc::kan*	This study

VN262	NR1647 Δ*mrcA::kan*	This study
VN264	NR1647 Δ*ponB::kan*	This study
VN266	NR1647 Δ*pbpc::kan*	This study
VN268	NR1647 Δ*nlpD::kan*	This study
VN343	BL21 carrying pVN173	This study
NR2793	MC1000 *zib563*::Tn*10*	This study
NR2794	MC1000 *zib563*::Tn*10* Δ*envC::kan*	This study
NR2795	NR1647 *zib563*::Tn*10*	This study
NR2796	NR1647 *zib563*::Tn*10* Δ*envC::kan*	This study

Plasmids

pBAD-HisB	L-arabinose inducible expression vector, N-terminal His_6-tag	Invitrogen
pASK-IBA63a-plus	P_{Tet} dependent expression vector, C-terminal Strep-tag	IBA
pRSET-B-*mCherry*	P_{T7} dependent expression vector, C-terminal mCherry protein	Received from Xavier De Bolle, University of Namur
pQE60	P_{T5} dependent expression vector, C-terminal His_6 tag	Qiagen
pCB25	pQE60 with $YciM_{His}$, C-terminal His_6-tag	This study
pHE43	pBAD-HisB with $YciM_{His}$, C-terminal His_6-tag	This study
pHE72	pBAD-HisB with $YciM^{*}_{His}$, truncated protein, C-terminal His_6-tag	This study
pHE64	pBAD-HisB with $YciM_{mCherry}$, C-terminal mCherry	This study
pHE89	pFPV25 with *fruB*-GFP, constitutively active GFP in the cytoplasm	Received from A . Aersten, Katholieke Universiteit Leuven
pVN2	pBAD-HisB with YciM, full-length protein	This study
pVN55	pASK-IBA63a-plus with $YciM^{*}_{Strep}$, truncated protein, C-terminal Strep-tag	This study
pVN67	pASK-IBA63a-plus with $YciM^{*}_{SS/CCCC\text{-}Strep}$, truncated protein, C_{184} and C_{256} mutated, C-terminal Strep-tag	This study
pVN68	pBAD-HisB with $YciM^{*}_{mCherry}$, truncated protein, C-terminal mCherry	This study
pVN74	pASK-IBA63a-plus with $YciM^{*}$, truncated protein	This study
pVN147	pBAD-HisB with $YciM^{*}_{SS/CCCC\text{-}His}$, truncated protein, C_{184} and C_{256} mutated, C-terminal His_6-tag	
pVN173	pBAD-HisB with $YciM_{CC/SSSS}$, C_{357}, C_{360}, C_{371} and C_{374} mutated	This study

pVN182	pBAD-HisB with YciM*, truncated protein	This study	

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Table S2. Primers used in this study

Primer	Sequence (5'-3')
yciM_NcoI	TTACCATGGTGGAGTTGTTGTTTCTGCTTTTGCC
yciM_BglII_pQE60	TTAAGATCTACAGGCCATCAAGACCGCGAATCGG
yciM_HindIII_pBAD	TTAAAGCTTACAGGCCATCAAGACCGCGAATCGG
yciM**_NcoI*	TTACCATGGCGCAACAAAACAAGCAAGATGAAGC
yciM_BglII_pBAD	TTAAGATCTCAGGCCATCAAGACCGCG
yciM_XhoI_pASK	AATTACTCGAGCAGGCCATCAAGACCGCGAATCGGTTTAAT
yciM_XhoI_STOP	TTACTCGAGTTACAGGCCATCAAGACCGCG
yciM_C1S1_Fw	CGCGTCGAAATTGCCCATTTCTACAGTGAGTTAGCCCTGCAG
yciM_C1S1_Rv	CTGCAGGGCTAACTCACTGTAGAAATGGGCAATTTCGACGCG
yciM_C2S2_Fw	ACGCTGGAAATGTTGCAAACCAGCTATCAGCAGTTGGGTAAAACTGCC
yciM_C2S2_Rv	GGCAGTTTTACCCAACTGCTGATAGCTGGTTTGCAACATTTCCAGCGT
yciM_S1XXS2_Fw	CTCGTTATCGTAGCCAGAAAAGTGGTTTTA
yciM_S1XXS2_Rv	CGGTAAAACCACTTTTCTGGCTACGATAACGAG
yciM_S3XXS4_Fw	CTACTGGCATAGTCCGTCTAGTCGGGCCTGG
yciM_S3XXS4_Rv	CCAGGCCCGACTAGACGGACTATGCCAGTAG
mCherry_Fw	CACAGATCTGTGAGCAAGGGCGAG
mCherry_Rv	GTTAAGCTTACTTGTACAGCTCGTCC

The underlined nucleotides correspond to serine codons replacing the cysteine codons

Table S3. Mutants from the Keio collection sensitive to both rifampin and SDS-EDTA

Gene	SDS-EDTA	Rifampin	Gene	SDS-EDTA	Rifampin
bamB			*rfaP*		
bamE			*rimM*		
cmk			*rplI*		
dedD			*rpsF*		
dnaK			*rseA*		
fabH			*surA*		
fur			*tatB*		
galU			*tatC*		
gmhB			*tdK*		
ipp			*thyA*		
lipA			*tolB*		
lpcA			*tolC*		
pal			*tolQ*		
rbfA			*tolR*		
rfaC			*ubiE*		
rfaD			*ubiF*		
rfaE			*ubiH*		
rfaF			*ybiX*		
rfaG			*yciM*		
rfaH			*yfgC*		
rfaI			*yraP*		

The sensitivity to hydrophobic antibiotics and detergent of the mutants from the Keio collection was determined. The mutants were streaked on LB plates containing either rifampin (25 μg) or SDS-EDTA (0.05 %, 1.25 mM). Only the mutants that are sensitive to both conditions are listed in this table. Black: no growth, Grey: poor growth

Table S4. Sensitivity of *yciM*, *yraP*, *ybiX* and *yfgC* strains to various hydrophobic antibiotics

	Inhibition zone (mm)		
	Rifampin (25 µg)	Bacitracin (10 µg)	Erythromycin (15 µg)
WT	12 ± 0,5	<6	<6
yciM	21 ± 1	11 ± 2.9	13 ± 3.8
yraP	17.3 ± 0.5	13.7 ± 0.5	10.3 ± 1.3
ybiX	14.6 ± 0.5	<6	10.3 ± 1.9
yfgC	19 ± 1.4	<6	10.6 ± 0.9

The sensitivity of JW1272 (Δ*yciM::kan*), JW3119 (Δ*yraP::kan*), JW5104 (Δ*ybiX::kan*) and JW2479 (Δ*yfgC::kan*) strains to four hydrophobic antibiotics was determined by measuring the diameter of the inhibition zone using BBL Sensi-Discs Antimicrobial Susceptibility Test Discs containing rifampin (25 µg), bacitracin (10 µg) or erythromycin (15 µg). Discs have a diameter of 6 mm. Strains JW1272, JW3119, JW5104 and JW2479 are from the Keio collection (4).

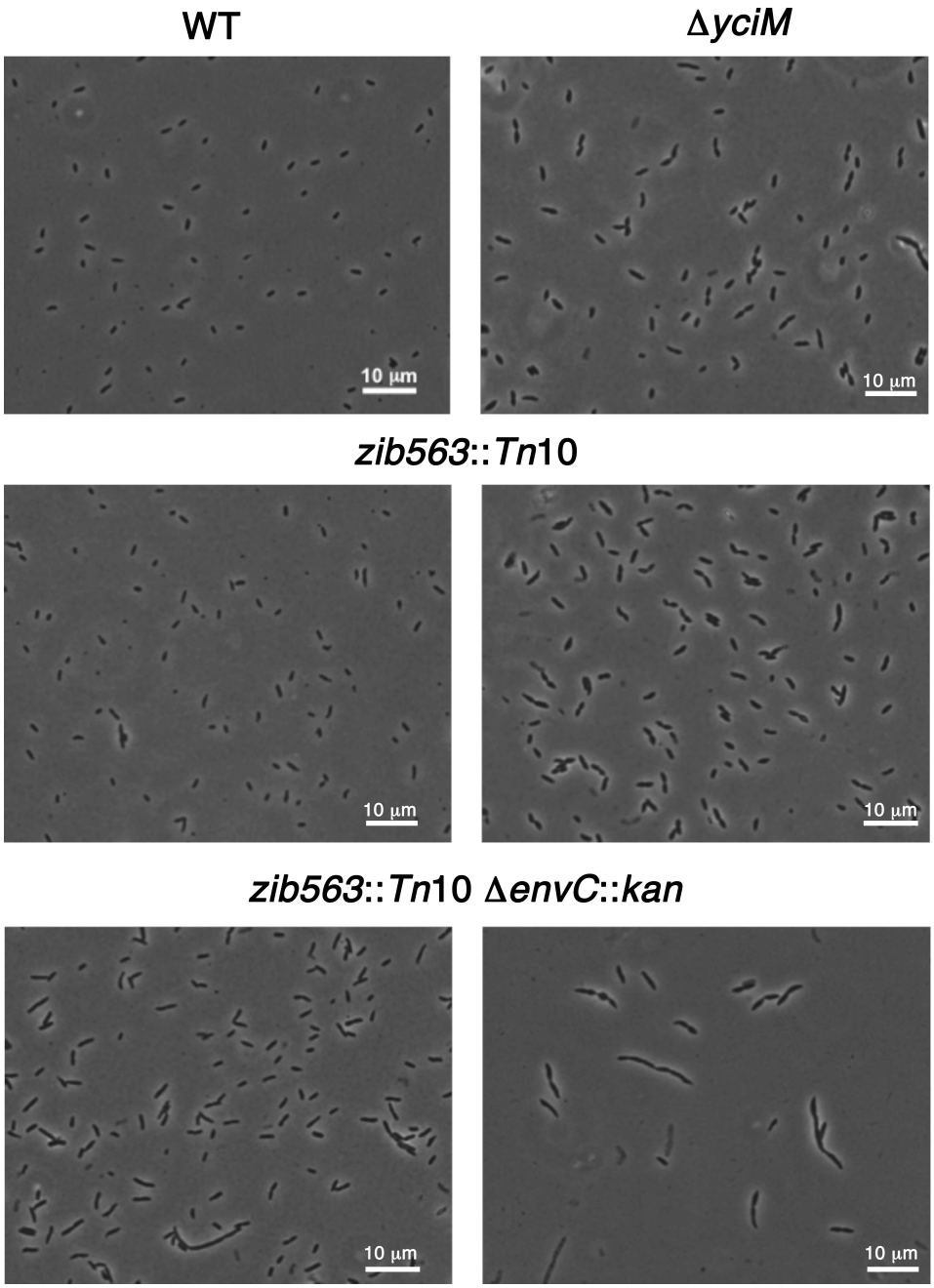

Figure S1. ***yciM envC* null alleles exhibit a synthetic phenotype**. Phase-contrast images of *yciM* and *envC* single and double mutant strains grown overnight on LB agar at 37°C show that cells lacking both YciM and EnvC exhibit severe morphological defects and increased lysis with respect to wild-type and single-mutant strains. The *zib563*::Tn*10* allele was used to co-transduce the Δ*envC::kan* allele as described in Materials and Methods.

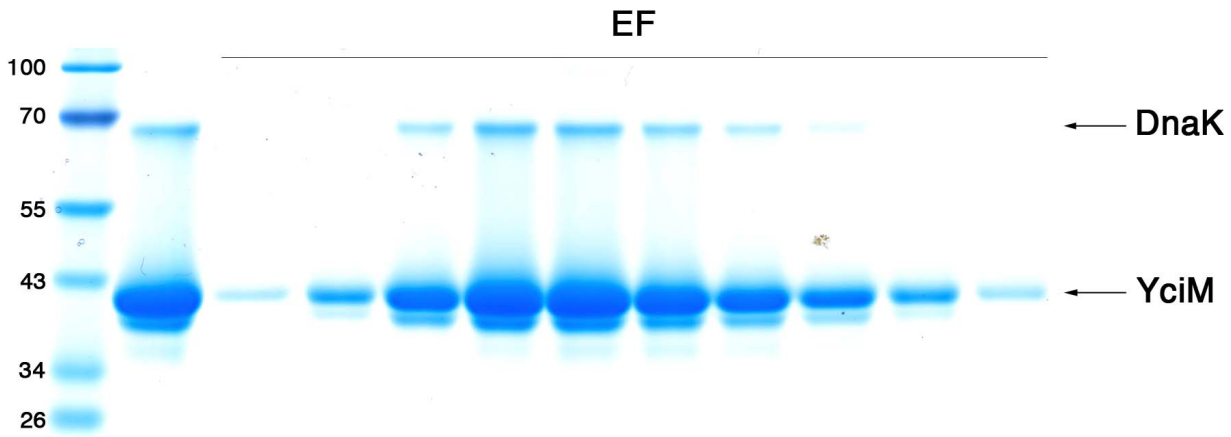

Figure S2. YciM* co-purifies with DnaK. The truncated version of YciM was purified by three steps of purification (affinity chromatography, anion exchange chromatography and gel filtration). The fractions that eluted from a Superdex S200 gel filtration column (EF) were loaded onto a SDS-PAGE and stained using the PageBlue Protein Saining Solution (Fermentas).

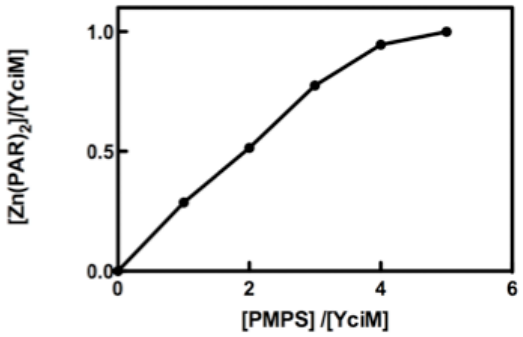

Figure S3. YciM binds zinc via 4 cysteine residues. The purified $YciM^{*}_{His}$ protein was incubated with PAR and A_{500} was monitored to follow the formation of the $Zn(PAR)_2$ complex. Stepwise additions of stoichiometric amount of PMPS induced zinc release. The [PMPS]/[YciM] ratio corresponds to the number of cysteine residues that have to be titrated to allow zinc release. The concentration of the $Zn(PAR)_2$ complex ($[Zn(PAR)_2]$) corresponds to the concentration of zinc released from YciM.

Figure S4. Aerobically-purified YciM$^{*}_{Strep}$ displays a pink color. YciM$^{*}_{Strep}$ was purified by affinity chromatography using a Strep-Tactin column, followed by an anion exchange chromatography and a gel filtration. The fractions that eluted from the gel filtration column were pooled and concentrated. The purified protein displays a bright pink color owing to its ability to bind iron.

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