SUPPORTING INFORMATION

Negative regulation of MurZ and MurA underlies the essentiality of GpsB- and StkP-mediated protein phosphorylation in *Streptococcus pneumoniae* D39

Ho-Ching Tiffany Tsui[1†*], Merrin Joseph[1†], Jiaqi J. Zheng[1], Amilcar J. Perez[1], Irfan Manzoor[1], Britta E. Rued[1], John D. Richardson[1], Pavel Branny[2], Linda Doubravová[2], Orietta Massidda[3], and Malcolm E. Winkler[1*]

[1]Department of Biology, Indiana University Bloomington, Bloomington, IN, USA
[2]Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic
[3]Department of Cellular, Computational, and Integrative Biology (CIBIO), University of Trento, Italy

[†]Contributed equally to this work.

*Corresponding authors:
Malcolm E. Winkler
Department of Biology
Indiana University Bloomington (IUB)
1001 E 3rd St
Bloomington, IN 47405 USA
Phone: 812-856-1318
E-mail: winklerm@indiana.edu

*Ho-Ching Tiffany Tsui
Department of Biology
Indiana University Bloomington (IUB)
1001 E 3rd St
Bloomington, IN 47405 USA
Phone: 812-856-1781
E-mail: tttsui@indiana.edu

Contents:

Figure S15. Characterization of mutant strains with catalytic site mutations *murZ*(C116S) or *murA*(C120S).

Figure S16. Δ*murZ* or *murZ*(C116S) mutants cultured in C+Y media, pH 6.9 do not show growth defects, but form significantly enlarged cells compared to WT.

Figure. S17. Ectopic overexpression of *murZ*(D280Y) decreases cell size and inhibits growth similar to overexpression of *murZ.*

Figure. S18. Suppression of Δ*gpsB* and Δ*stkP* lethality by *murZ*(I265V).

Figure S19. Cellular amounts of MurA, or N-terminal-tagged MurZ or MurA fusion proteins are unchanged in Δ*clpP* mutants.

Figure S20. Complementation of Δ*stkP* growth and morphological defects by ectopic overexpression of *stkP*.

Figure S21. Suppression of growth and morphological Δ*stkP* phenotypes in BHI broth by *murZ*(D280Y) or overexpression of *murZ* or *murA*.

Table S1. *Streptococcus pneumoniae* strains and oligonucleotide primers used in this study

Strains used in this study			
Strain number	Genotype (description)[a]	Antibiotic resistance[b]	Reference or source
EL59	R6	None	(Hoskins *et al.*, 2001)
IU1690	D39 *cps*$^+$ (D39W)	None	(Lanie *et al.*, 2007, Slager *et al.*, 2018)
IU1781	D39 *cps*$^+$ *rpsL1*	Str^R	(Lanie *et al.*, 2007)
IU1824[c]	D39 *rpsL1* Δ*cps2A'-cps2H'* = D39 *rpsL1* Δ*cps*	Str^R	(Lanie *et al.*, 2007)
IU1945	D39 Δ*cps2A'-cps2H'*= D39 Δ*cps*	None	(Lanie *et al.*, 2007)
E193	D39 Δ*cps* Δ*pbp1b*::P$_c$-*erm*	Erm^R	(Land *et al.*, 2013)
E655	D39 Δ*cps* Δ*rodZ*::P$_c$-*erm*	Erm^R	(Tsui *et al.*, 2016)
K180	D39 Δ*cps* Δ*pbp1b*::P$_c$-[*kan-rpsL*$^+$]	Kan^R	(Tsui *et al.*, 2014)
E740	D39 Δ*cps* Δ[*phpP-stkP*]::P$_c$-*erm sup2* (IU1945 transformed with fusion Δ[*phpP-stkP*]::P$_c$-*erm* amplicon)	Erm^R	This Study
E765	D39 Δ*cps* Δ*murA*::P$_c$-*erm* (IU1945 X fusion Δ*murA*::P$_c$-*erm*)	Erm^R	This Study
E767	D39 Δ*cps* Δ*murZ*::P$_c$-*erm* (IU1945 X fusion Δ*murZ*::P$_c$-*erm*)	Erm^R	This Study
E780	D39 Δ*cps* Δ*clpC*::P$_c$-*erm* (IU1945 X fusion Δ*clpC*::P$_c$-*erm*)	Erm^R	This Study
K761	D39 Δ*cps* Δ*khpB*::P$_c$-[*kan-rpsL*$^+$]	Kan^R	(Zheng *et al.*, 2017)
K765	D39 Δ*cps* Δ*murA*::P$_c$-[*kan-rpsL*$^+$] (IU1945 X fusion Δ*murA*::P$_c$-[*kan-rpsL*$^+$])	Kan^R	This study
K767	D39 Δ*cps* Δ*murZ*::P$_c$-[*kan-rpsL*$^+$] (IU1945 X fusion Δ*murZ*::P$_c$-[*kan-rpsL*$^+$])	Kan^R	This Study
K779	D39 Δ*cps* Δ*clpC*::P$_c$-[*kan-rpsL*$^+$] (IU1945 X fusion Δ*clpC*::P$_c$-[*kan-rpsL*$^+$])	Kan^R	This Study
K787	D39 Δ*cps* Δ*ireB*::P$_c$-[*kan-rpsL*$^+$] (IU1945 X fusion Δ*ireB*::P$_c$-[*kan-rpsL*$^+$])	Kan^R	This Study
IU4355	*D39 rpsL1* Δ*cps* Δ*bgaA*::*kan*-t1t2-P$_{fcsk}$-*secA*-L-FLAG^3	Str^R Kan^R	(Tsui *et al.*, 2011)
IU4888	D39 Δ*cps* Δ*gpsB*<>*aad9*//Δ*bgaA*::*kan*-t1t2-P$_{fcsK}$-*gpsB*$^+$	Kan^R Spc^R	(Land *et al.*, 2013)
IU4970	D39 Δ*cps* *mreC*-L-FLAG^3-P$_c$-*erm*	Erm^R	(Land & Winkler, 2011)

IU5845	D39 Δ*cps* Δ*gpsB*<>*aad9 sup2*	Spc^R	(Rued *et al.*, 2017)
IU6441	D39 Δ*cps* Δ*gpsB*<>*aad9 sup3*	Spc^R	(Rued *et al.*, 2017)
IU6442	D39 Δ*cps* Δ*gpsB*<>*aad9 sup1*	Spc^R	(Rued *et al.*, 2017)
IU6444	D39 Δ*cps* Δ*gpsB*<>*aad9 sup5*	Spc^R	(Rued *et al.*, 2017)
IU7397	D39 Δ*cps* Δ*pbp2b*<>*aad9* //Δ*bga*::*kan*-t1t2-P_{fcsk}-*pbp2b*$^+$	Spc^R Kan^R	(Tsui *et al.*, 2014)
IU7673	D39 Δ*cps rpsL1 phpP*$^+$-P_c-[*kan-rpsL*$^+$]-*stkP*$^+$	Kan^R	(Rued *et al.*, 2017)
IU7735	D39 Δ*cps rpsL1* Δ*gpsB*<>*aad9 sup27* with spontaneous *ireB*(Q84(STOP)) mutation (IU1824 X Δ*gpsB*<>*aad9* amplicon from IU4888). Original Δ*gpsB* suppressor strain in IU1824 background.	Str^R Spc^R	This study
IU7736	D39 Δ*cps rpsL1* Δ*gpsB*<>*aad9 sup6*	Str^R Spc^R	(Rued *et al.*, 2017)
IU7824	D39 Δ*cps* Δ[*spd_1031-1037*]::P_c-*erm*	Erm^R	(Rued *et al.*, 2017)
IU7923	D39 Δ*cps* Δ*stkP*::P_c-*erm*	Erm^R	(Rued *et al.*, 2017)
IU8108	D39 Δ*cps* Δ[*spd_1029-1030*]::P_c-*erm* (IU1945 X fusion Δ[*spd_1029-1030*]::P_c-*erm*)	Erm^R	This study
IU8122	D39 Δcps Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*ftsZ*$^+$	Tet^R	(Zheng *et al.*, 2017)
IU8224	R6 Δ*gpsB*<>*aad9*	Spc^R	(Rued *et al.*, 2017)
IU8271	D39 Δ*cps* Δ[*spd_1029-1037*]::P_c-[*kan-rpsL*$^+$]	Kan^R	(Rued *et al.*, 2017)
IU8742	D39 Δ*cps* Δ*gpsB*<>*aad9 phpP*(G229D) Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*phpP*$^+$ (IU6442 X fusion Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*phpP*$^+$ amplicon)	Spc^R Tet^R	This Study
IU8791	D39 Δ*cps* Δ*mltG*::P_c-*aad9*//Δ*bgaA*::*kan*-t1t2-P_{fcsK}-*mltG*$^+$	Kan^R Spc^R	(Tsui *et al.*, 2016)
IU8872	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{mltG}–*mltG*$^+$	Tet^R	(Tsui *et al.*, 2016)
IU9036	D39 Δ*cps rpsL1* Δ*khpA*	Str^R	(Zheng *et al.*, 2017)
IU9262	Rx1 Δ*gpsB*<>*aad9 sup4*	Spc^R	(Rued *et al.*, 2017)
IU9600	D39 Δ*khpA*::P_c-*erm*	Erm^R	(Zheng *et al.*, 2017)
IU9613	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*rodZ*$^+$ (IU1824 X fusion Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*rodZ*$^+$)	Str^R Tet^R	This Study
IU9765	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*rodZ*$^+$	Tet^R	(Tsui *et al.*, 2016)

IU9805	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*sepF*$^{+}$ (IU1945 X fusion Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*sepF*$^{+}$)	Kan^{R}	This Study
IU9931	D39 Δ*cps* Δ*rodZ*<>*aad9*//Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*rodZ*$^{+}$	Spc^{R} Tet^{R}	(Tsui *et al.*, 2016)
IU9990	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*pbp2b*$^{+}$	Tet^{R}	(Zheng *et al.*, 2017)
IU9992	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*pbp1b*$^{+}$ (IU1945 X fusion Δ*bgaA*::tet-P_{Zn}-RBS^{ftsA}-*pbp1b*$^{+}$)	Tet^{R}	This Study
IU10063	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*pbp2x*$^{+}$	Tet^{R}	(Perez *et al.*, 2019)
IU10220	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*mreC*$^{+}$ (IU1824 X fusion Δ*bgaA*::tet-P_{Zn}-RBS^{ftsA}-*mreC*$^{+}$)	Str^{R} Tet^{R}	This Study
IU10592	D39 Δ*cps rpsL1* Δ*khpB*	Str^{R}	(Zheng *et al.*, 2017)
IU10596	D39 Δ*cps rpsL1* Δ*khpA* Δ*khpB*	Str^{R}	(Zheng *et al.*, 2017)
IU10659	D39 Δ*cps rpsL1* Δ*khpA* Δ*rodZ*<>*aad9*	Str^{R} Spc^{R}	(Zheng *et al.*, 2017)
IU10922	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*rodA*$^{+}$	Tet^{R}	(Tsui *et al.*, 2016)
IU11049	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murG*$^{+}$ (IU1945 X fusion Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murG*$^{+}$)	Kan^{R}	This Study
IU11077	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murZ*$^{+}$ (IU1945 X fusion Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murZ*$^{+}$)	Kan^{R}	This Study
IU11079	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murA*$^{+}$ (IU1945 X fusion Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murA*$^{+}$)	Kan^{R}	This Study
IU11083	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{Zn}-*mraY*$^{+}$ (IU1945 X fusion Δ*bgaA*::*kan*-t1t2-P_{Zn}-*mraY*$^{+}$)	Kan^{R}	This Study
IU11094	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{Zn}-*uppS*$^{+}$ (IU1945 X fusion Δ*bgaA*::*kan*-t1t2-P_{Zn}-*uppS*$^{+}$)	Kan^{R}	This Study
IU11286	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*gpsB*$^{+}$	Tet^{R}	(Cleverley *et al.*, 2019)
IU11456	D39 Δ*stkP*::P_{c}-*erm sup4*	Erm^{R}	(Rued *et al.*, 2017)
IU11628	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{Zn}-*mapZ*$^{+}$ (IU1945 X fusion Δ*bgaA*::*kan*-t1t2-P_{Zn}-*mapZ*$^{+}$)	Kan^{R}	This Study
IU11846	D39 Δ*cps* Δ*gpsB*<>*aad9 sup9*	Spc^{R}	This Study
IU11912	D39 Δ*cps* Δ*stkP*::P_{c}-*cat sup3* (IU1945 X fusion Δ*stkP*::P_{c}-*cat*)	Cm^{R}	This Study
IU11914	D39 Δ*cps* Δ*gpsB*<>*aad9 sup11*	Spc^{R}	This Study
IU11918	D39 Δ*cps* Δ*gpsB*<>*aad9 sup10*	Spc^{R}	This Study
IU11954	D39 Δ*cps* Δ*gpsB*<>*aad9 sup8*	Spc^{R}	This Study
IU11955	D39 Δ*cps* Δ*gpsB*<>*aad9 sup7*	Spc^{R}	(Rued *et al.*, 2017)
IU12192	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*ftsW*$^{+}$ (IU1824 X fusion Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*ftsW*$^{+}$)	Str^{R} Tet^{R}	This Study
IU12286	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*ftsZ*$^{+}$	Str^{R} Tet^{R}	(Zheng *et al.*, 2017)

IU12307	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*ftsA*$^+$	Str^R Tet^R	(Mura *et al.*, 2017)
IU12310	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*ftsA*$^+$	Str^R Tet^R	(Mura *et al.*, 2017)
IU12428	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*murA*$^+$ Δ*gpsB*<>*aad9* (IU11079 X Δ*gpsB*<>*aad9* amplicon from IU4888)	Kan^R Spec^R	This Study
IU12462	D39 Δ*cps rpsL1* Δ*clpC::*P_c-*kan-rpsL*$^+$ (IU1824 X Δ*clpC::*P_c-*kan-rpsL*$^+$ amplicon from K779)	Kan^R	This Study
IU12678	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*cozE*$^+$ (IU1945 X fusion Δ*bgaA*::tet-P_{Zn}-RBS^{ftsA}-*cozE*$^+$)	Tet^R	This Study
IU12704	D39 Δ*cps* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*ftsA*$^+$ Δ*pbp2b*<>*aad9* (IU12307 X Δ*pbp2b*<>*aad9* from IU7397)	Tet^R Spec^R	This Study
IU12707	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*ftsA*$^+$ Δ*pbp2b*<>*aad9*	Tet^R Spec^R	(Zheng *et al.*, 2017)
IU12712	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P_{ftsA}-RBS^{ftsA}-*ftsA*$^+$ (IU1945 X fusion Δ*bgaA*::*kan*- t1t2-P_{ftsA}-RBS^{ftsA}-*ftsA*)	Kan^R	This study
IU12719	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P_{ftsA}-RBS^{ftsA}-*ftsA* (IU1824 X fusion Δ*bgaA*::*kan*- t1t2-P_{ftsA}-RBS^{ftsA}-*ftsA*)	Str^R Kan^R	This study
IU12744	D39 Δ*cps rpsL1 khpB*(T89A)	Str^R	(Zheng *et al.*, 2017)
IU12883	D39 Δ*cps rpsL1* Δ*khpA* Δ*gpsB*<>*aad9*	Str^R Spc^R	(Zheng *et al.*, 2017)
IU12977	D39 Δ*cps rpsL1* Δ*khpB* Δ*gpsB*<>*aad9*	Str^R Spc^R	(Zheng *et al.*, 2017)
IU13249	D39 Δ*cps rpsL1 murZ*-L-FLAG^3-P_c-*erm* (IU1824 X fusion *murZ*-L-FLAG^3-P_c-*erm*)	Erm^R Str^R	This Study
IU13251	D39 Δ*cps rpsL1 murA*-L-FLAG^3-P_c-*erm* (IU1824 X fusion *murA*-L-FLAG^3-P_c-*erm*)	Erm^R Str^R	This study
IU13283	D39 Δ*cps rpsL1* Δ*khpA murZ*-L-FLAG^3-P_c-*erm* (IU9036 X *murZ*-L-FLAG^3-P_c-*erm* from IU13249)	Erm^R Str^R	This Study
IU13285	D39 Δ*cps rpsL1* Δ*khpA murA*-L-FLAG^3-P_c-*erm* (IU9036 X *murA*-L-FLAG^3-P_c-*erm* from IU13251)	Erm^R Str^R	This Study
IU13327	D39 Δ*cps rpsL1* CEP::P_{Zn}-*ezrA*$^+$ Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*ezrA*$^+$	Kan^R Str^R	(Perez *et al.*, 2021)
IU13393	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*murZ*$^+$ (IU1824 X Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murZ*$^+$ amplicon from IU11077)	Kan^R Str^R	This Study
IU13395	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA} -*murA*$^+$ (IU1824 X Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murA*$^+$ amplicon from IU11079)	Kan^R Str^R	This Study
IU13396	D39 Δ*cps rpsL1* Δ*murZ*::P_c-[*kan-rpsL*$^+$] (IU1824 X Δ*murZ*::P_c-[*kan-rpsL*$^+$] amplicon from K767)	Kan^R	This Study
IU13438 IU13439	D39 Δ*cps rpsL1 murZ*(D280Y) (IU13396 X *murZ*(D280Y) amplicon from IU11914)	Str^R	This Study

IU13485	D39 Δ*cps rpsL1 murZ*(D280Y) Δ*gpsB*<>*aad9* (IU13438 X Δ*gpsB*<>*aad9* amplicon from IU4888)	Str^R Spc^R	This Study
IU13491	D39 Δ*cps rpsL1* Δ*murA*::P$_c$-[*kan-rpsL*$^+$] (IU1824 X Δ*murA*::P$_c$-[*kan-rpsL*$^+$] amplicon from K765)	Kan^R	This Study
IU13493	D39 Δ*cps rpsL1* Δ*khpA* Δ*murZ*::P$_c$-[*kan-rpsL*$^+$] (IU9036 X Δ*murZ*::P$_c$-[*kan-rpsL*$^+$] amplicon from K767)	Kan^R	This Study
IU13495	D39 Δ*cps rpsL1* Δ*khpA* Δ*murA*::P$_c$-[*kan-rpsL*$^+$] (IU9036 X Δ*murA*::P$_c$-[*kan-rpsL*$^+$] amplicon from K765)	Kan^R	This Study
IU13502	D39 Δ*cps rpsL1 murZ*-L-FLAG^3 (IU13396 X fusion *murZ*-L-FLAG^3)	Str^R	This Study
IU13505	D39 Δ*cps rpsL1 murZ*(D280Y) Δ*gpsB*<>*aad9* (IU13438 X Δ*gpsB*<>*aad9* amplicon from IU4888)	Str^R Spc^R	This Study
IU13509	D39 Δ*cps rpsL1 murZ*(D280Y) Δ*gpsB*<>*aad9* (IU13438 X Δ*gpsB*<>*aad9* amplicon from IU4888)	Str^R Spc^R	This Study
IU13536	D39 Δ*cps rpsL1* Δ*murZ* (IU13396 X fusion Δ*murZ*)	Str^R	This Study
IU13538	D39 Δ*cps rpsL1* Δ*murA* (IU13491 X fusion Δ*murA*)	Str^R	This Study
IU13542	D39 Δ*cps rpsL1* Δ*khpA* Δ*murZ* (IU13493 X fusion Δ*murZ*)	Str^R	This Study
IU13545	D39 Δ*cps rpsL1* Δ*khpA murZ*-L-FLAG^3 (IU13493 X fusion *murZ*-L-FLAG^3)	Str^R	This Study
IU13546	D39 Δ*cps rpsL1* Δ*khpA* Δ*murA* (IU13495 X fusion Δ*murA*)	Str^R	This Study
IU13590	D39 Δ*cps rpsL1* Δ*ireB*::P$_c$-[*kan-rpsL*$^+$] (IU1824 X Δ*ireB*::P$_c$-[*kan-rpsL*$^+$] amplicon from K787)	Kan^R	This Study
IU13600	D39 Δ*cps rpsL1 murZ*(D280Y)-L-FLAG^3 (IU13396 X fusion *murZ*(D280Y)-L-FLAG^3)	Str^R	This Study
IU13604	D39 Δ*cps rpsL1* Δ*ireB* markerless (IU13590 X fusion Δ*ireB* markerless amplicon)	Str^R	This Study
IU13606	D39 Δ*cps rpsL1 ireB*(Q84(STOP)) (IU13590 X *ireB*(Q84(STOP)) amplicon from IU7735)	Str^R	This Study
IU13680	D39 Δ*cps* Δ*pbp1b*::P$_c$-*aad9* (IU1945 X fusion Δ*pbp1b*::P$_c$-*aad9*)	Spc^R	This Study
IU13756	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murZ*$^+$ Δ*gpsB*<>*aad9* (IU11077 X Δ*gpsB*<>*aad9* amplicon from IU4888)	Kan^R Spc^R	This Study
IU13757	D39 Δ*cps* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murA*$^+$ Δ*gpsB*<>*aad9* (IU11079 X Δ*gpsB*<>*aad9* amplicon from IU4888)	Kan^R Spc^R	This Study
IU13772	D39 Δ*cps rpsL1 murZ*-L-FLAG^3 Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murZ*-L-FLAG^3 (IU13502 X fusion Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murZ*-L-FLAG^3)	Str^R Kan^R	This Study

IU13794	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*divIVA*$^+$ (R6 annotation) (IU1824 X fusion Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}–*divIVA*$^+$)	Tet^R Str^R	This Study
IU13881	D39 Δ*cps rpsL1 khpB*(T89D)	Str^R	(Zheng *et al.*, 2017)
IU13883	D39 Δ*cps rpsL1 khpB*(T89E)	Str^R	(Zheng *et al.*, 2017)
IU13987	D39 Δ*cps rpsL1* Δ*khpB*::P_c-[*kan-rpsL*$^+$] *murZ*-L-$FLAG^3$ (IU13502 X Δ*khpB*::P_c-[*kan-rpsL*$^+$] from K761)	Kan^R	This Study
IU13989	D39 Δ*cps rpsL1* Δ*khpA* Δ*khpB*::P_c-[*kan-rpsL*$^+$] *murZ*-L-$FLAG^3$ (IU13545 X Δ*khpB*::P_c-[*kan-rpsL*$^+$] from K761)	Kan^R	This Study
IU14014	D39 Δ*cps rpsL1* Δ*khpB murZ*-L-$FLAG^3$ (IU13987 X Δ*khpB* from IU10592)	Str^R	This Study
IU14016	D39 Δ*cps rpsL1* Δ*khpA* Δ*khpB murZ*-L-$FLAG^3$ (IU13989 X Δ*khpB* from IU10592)	Str^R	This Study
IU14028	D39 Δ*cps rpsL1 murA*-L-$FLAG^3$ (IU13491 X fusion *murA*-L-$FLAG^3$)	Str^R	This Study
IU14030	D39 Δ*cps rpsL1* Δ*khpA murA*-L-$FLAG^3$ (IU13495 X fusion *murA*-L-$FLAG^3$)	Str^R	This Study
IU14082	D39 Δ*cps rpsL1* Δ*clpC*::P_c-*erm murZ*-L-$FLAG^3$ (IU13502 X Δ*clpC*::P_c-*erm* from E780)	Str^R Erm^R	This Study
IU14084	D39 Δ*cps rpsL1* Δ*murA*::P_c-*erm murZ*-L-$FLAG^3$ (IU13502 X Δ*murA*::P_c-*erm* from E765)	Str^R Erm^R	This Study
IU14086	D39 Δ*cps rpsL1* Δ*clpC*::P_c-*erm murA*-L-$FLAG^3$ (IU14028 X Δ*clpC*::P_c-*erm* from E780)	Str^R Erm^R	This Study
IU14088	D39 Δ*cps rpsL1* Δ*murZ*::P_c-*erm murA*-L-$FLAG^3$ (IU14028 X Δ*murZ*::P_c-*erm* from E767)	Str^R Erm^R	This Study
IU14210	D39 Δ*cps rpsL1 murZ*(I265V) (IU13396 X *murZ*(I265V) amplicon from EL59)	Str^R	This Study
IU14234	D39 Δ*cps rpsL1 murZ*(I265V) Δ*gpsB<>aad9* (IU14210 X Δ*gpsB<>aad9* amplicon from IU4888)	Str^R Spc^R	This Study
IU14270	D39 Δ*cps* Δ*mraY<>aad9*//Δ*bgaA*::*kan*-t1t2-P_{Zn}-*mraY*$^+$ (IU11083 X fusion Δ*mraY<>aad9*)	Spc^R Kan^R	This Study
IU14272	D39 Δ*cps* Δ*uppS<>aad9*// Δ*bgaA*::*kan*-t1t2-P_{Zn}-*uppS*$^+$ (IU11094 X fusion Δ*mraY<>aad9*)	Spc^R Kan^R	This Study
IU14274	D39 Δ*cps* Δ*murG<>aad9*//Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*murG*$^+$ (IU11049 X fusion Δ*murG<>aad9*)	Spc^R Kan^R	This Study
IU14312	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*- P_{Zn}-RBS^{ftsA}-*pbp1a*$^+$ (IU1824 X fusion Δ*bgaA*::*tet*-P_{Zn}- RBS^{ftsA}-*pbp1a*$^+$)	Str^R Tet^R	This Study
IU14318	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*pbp2a*$^+$ (IU1824 X fusion Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*pbp2a*$^+$)	Str^R Tet^R	(Cleverley *et al.*, 2019)
IU14738	D39 Δ*cps rpsL1 iht*-L_6-*mapZ* markerless	Str^R	(Perez *et al.*, 2019)
IU14974	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*stkP*$^+$ (IU1824 X fusion Δ*bgaA*::*kan*-t1t2-P_{Zn}-*stkP*$^+$)	Str^R Kan^R	This Study

IU15124	D39 Δ*cps rpsL1 murZ*(I265V) Δ*gpsB<>aad9* (IU14210 X Δ*gpsB<>aad9* amplicon from IU4888)	Str^R Spc^R	This Study
IU15143	D39 Δ*cps rpsL1 murA*(D281Y) (IU13491 X fusion amplicon *murA*(D281Y))	Str^R	This Study
IU15145	D39 Δ*cps rpsL1 murA*(E282Y) (IU13491 X fusion amplicon *murA*(E282Y))	Str^R	This Study
IU15355	D39 Δ*cps rpsL1* Δ*rodZ<>aad9*//Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*rodZ*$^+$ (IU9613 X Δ*rodZ<>aad9* from IU9931)	Str^R Tet^R Spc^R	This Study
IU15357	D39 Δ*cps rpsL1* Δ*rodZ*::P$_c$-*aad9*//Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*rodZ*$^+$ (IU9613 X Δ*rodZ*::P$_c$-*aad9* from IU6987)	Str^R Tet^R Spc^R	This Study
IU15361	D39 Δ*cps rpsL1* Δ*rodZ*::P$_c$-*aad9*//Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*ftsA*$^+$ (IU12310 X Δ*rodZ*::P$_c$-*aad9* from IU6987)	Str^R Tet^R Spc^R	This Study
IU15371	D39 Δ*cps* Δ*rodZ*::P$_c$-*aad9*//Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*rodZ*$^+$ (IU9765 X Δ*rodZ*::P$_c$-*aad9* from IU6987)	Tet^R Spc^R	This Study
IU15386	D39 Δ*cps rpsL1* Δ*rodZ*::P$_c$-*aad9* Δ*bgaA*::*tet*-P$_{ftsA}$-*ftsA* (IU12719 X Δ*rodZ*::P$_c$-*aad9* from IU6987)	Str^R Tet^R Spc^R	This Study
IU15531	D39 Δ*cps rpsL1* Δ*khpA* Δ*rodZ*::P$_c$-*erm* (IU9036 X Δ*rodZ*::P$_c$-*erm* from E655)	Str^R Erm^R	This Study
IU15636	D39 Δ*cps rpsL1* Δ*rodZ*::P$_c$-*erm*//Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*rodZ*$^+$ (IU9613 X Δ*rodZ*::P$_c$-*erm* from E655)	Str^R Tet^R Erm^R	This Study
IU15641	D39 Δ*cps* Δ*rodZ*::P$_c$-*erm*//Δ*bgaA*::*tet*-P$_{ftsA}$-*ftsA* (IU12712 X Δ*rodZ*::P$_c$-*erm* from E655)	Str^R Tet^R Erm^R	This Study
IU15860	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA} -*murZ*$^+$ Δ*gpsB<>aad9* (IU13393 X Δ*gpsB<>aad9* amplicon from IU4888)	Kan^R Str^R Spc^R	This Study
IU15862	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA} -*murA*$^+$ Δ*gpsB<>aad9* (IU13395 X Δ*gpsB<>aad9* amplicon from IU4888)	Kan^R Str^R Spc^R	This Study
IU15873	D39 Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*gpsB*$^+$ (IU1690 X Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*gpsB*$^+$ from IU11286)	Tet^R	This Study
IU15875	D39 *rpsL1* Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*gpsB*$^+$ (IU1781 X Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*gpsB*$^+$ from IU11286)	Str^R Tet^R	This Study
IU15877	D39 Δ*cps rpsL* Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*gpsB*$^+$ (IU1824 X Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*gpsB*$^+$ from IU11286)	Str^R Tet^R	This Study
IU15879	D39 Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murZ*$^+$ (IU1690 X Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murZ*$^+$ amplicon from IU11077)	Kan^R	This Study
IU15880	D39 Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murA*$^+$ (IU1690 X Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murA*$^+$ amplicon from IU11079)	Kan^R	This Study
IU15882	D39 *rpsL1* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murZ*$^+$ (IU1781 X Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murZ*$^+$ amplicon from IU11077)	Str^R Kan^R	This Study

IU15884	D39 *rpsL1* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murA*$^+$ (IU1781 X Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murA*$^+$ amplicon from IU11079)	Str^R Kan^R	This Study
IU15889	D39 Δ*cps rpsL1* Δ*clpC*::P$_c$-*erm* (IU1824 X Δ*clpC*::P$_c$-*erm* from E780)	Str^R Erm^R	This Study
IU15899	D39 *rpsL1* Δ*murZ*::P$_c$-[*kan-rpsL*$^+$] (IU1781 X Δ*murZ*::P$_c$-[*kan-rpsL*$^+$] from K767)	Kan^R	This Study
IU15917	D39 *rpsL1 murZ*(D280Y) (IU15899 X *murZ*(D280Y) from IU13438)	Str^R	This Study
IU15939	D39 Δ*cps rpsL1 murZ*(C116S) (IU13396 X fusion *murZ*(C116S))	Str^R	This Study
IU15941	D39 Δ*cps rpsL1 murZ*(C116S)-L-FLAG^3 (IU13396 X fusion *murZ*(C116S)-L-FLAG^3)	Str^R	This Study
IU15943	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$- RBS^{ftsA}-*murZ*(C116S) (IU1824 X fusion Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murZ*(C116S))	Str^R Kan^R	This Study
IU15949	D39 Δ*cps rpsL1 murA*(C120S) (IU13491 X fusion *murA*(C120S))	Str^R	This Study
IU15951	D39 Δ*cps rpsL1 murA*(C120S)-L-FLAG^3 (IU13491 X fusion *murA*(C120S)-L-FLAG^3)	Str^R	This Study
IU15954	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA} -*murA*(C120S) (IU1824 X fusion Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murA*(C120S))	Str^R Kan^R	This Study
IU15955	D39 Δ*cps rpsL1* Δ*bgaA*::tet-Pzn-*phpP*$^+$ (IU1824 X *bgaA*::tet-Pzn-*phpP*$^+$ amplicon from IU8742)	Tet^R	This Study
IU15983	D39 Δ*cps rpsL1 murA*-L-FLAG^3// Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murA*$^+$-L-FLAG^3 (IU14028 X fusion Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murA*$^+$-L-FLAG^3)	Str^R Kan^R	This Study
IU16176	D39 Δ*murZ*::P$_c$-*erm* (IU1690 X Δ*murZ*::P$_c$-*erm* amplicon from E767)	Erm^R	This Study
IU16178	D39 Δ*murA*::P$_c$-*erm* (IU1690 X Δ*murA*::P$_c$-*erm* amplicon from E765)	Erm^R	This Study
IU16196	D39 Δ*cps rpsL1* Δ*khpA* Δ*gpsB*<>*aad9* (IU9036 X Δ*gpsB*<>*aad9* from IU4888)	Str^R Spc^R	This Study
IU16259	D39 Δ*cps rpsL1* Δ*murZ*// Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murZ*$^+$ (IU13536 X Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murZ*$^+$ amplicon from IU11077)	Str^R Kan^R	This Study
IU16262	D39 Δ*cps rpsL1* Δ*murZ* // Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murA*$^+$ (IU13536 X Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murA*$^+$ amplicon from IU11079)	Str^R Kan^R	This Study
IU16265	R6 Δ*murZ*::P$_c$-*erm* (EL59 X Δ*murZ*::P$_c$-*erm* amplicon from E767)	Erm^R	This Study
IU16267	R6 Δ*murA*::P$_c$-*erm* (EL59 X Δ*murZ*::P$_c$-*erm* amplicon from E765)	Erm^R	This Study
IU16295	D39 Δ*cps rpsL1* Δ[*spd_1029-1030*]::P$_c$-*erm* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*murZ*$^+$ (IU13393 X Δ[*spd_1029-1030*]::P$_c$-*erm* amplicon from IU8108)	Kan^R Erm^R	This Study

IU16298	D39 Δ*cps rpsL1* Δ[*spd_1031-1037*]::P_c-*erm* Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*murZ*$^+$ (IU13393 X Δ[*spd_1031-1037*]::P_c-*erm* amplicon from IU7824)	Kan^R Erm^R	This Study
IU16330	D39 Δ*cps rpsL1* Δ*murZ*// Δ*bgaA::kan*- t1t2-P_{Zn}-RBS^{ftsA} -*murZ* Δ*murA*::P_c-*erm* (IU16259 X Δ*murA*::P_c-*erm* from E765)	Erm^R Kan^R	This Study
IU16332	D39 Δ*cps rpsL1* Δ*murZ* Δ*murA*::P_c-*erm*// Δ*bgaA::kan*- t1t2-P_{Zn}-RBS^{ftsA} -*murA* (IU16262 X Δ*murA*::P_c-*erm* from E765)	Erm^R Kan^R	This Study
IU16334	D39 Δ*cps rpsL1* Δ*bgaA::kan*- t1t2-P_{Zn}-RBS^{ftsA} -*murZ*(D280Y) (IU1824 X fusion Δ*bgaA::kan*- t1t2-P_{Zn}-RBS^{ftsA} -*murZ*(D280Y))	Str^R Kan^R	This Study
IU16336	D39 Δ*cps rpsL1 murZ*(D280Y)// Δ*bgaA::kan*- t1t2-P_{Zn}-RBS^{ftsA} -*murZ*(D280Y) (IU13438 X fusion Δ*bgaA::kan*- t1t2-P_{Zn}-RBS^{ftsA} -*murZ*(D280Y))	Str^R Kan^R	This Study
IU16370	D39 Δ*cps rpsL1* Δ*gpsB*<>*aad9*//Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*gpsB*$^+$ (IU15877 X Δ*gpsB*<>*aad9* amplicon from IU4888)	Spc^R Tet^R	This Study
IU16883	D39 Δ*cps rpsL1* Δ*stkP*::P_c-*erm sup1* (IU1824 X Δ*stkP*::P_c-*erm* from IU7923)	Erm^R	This Study
IU16885, IU16895	D39 Δ*cps rpsL1 murZ*(D280Y) Δ*stkP*::P_c-*erm* (IU13438 X Δ*stkP*::P_c-*erm* amplicon from IU7923)	Str^R Erm^R	This Study
IU16897	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*murZ*$^+$ Δ*stkP*::P_c-*erm* (IU13393 X Δ*stkP*::P_c-*erm* amplicon from IU7923)	Erm^R Kan^R	This Study
IU16910	D39 Δ*cps rpsL1* Δ*khpA* Δ*stkP*::P_c-*erm* (IU9036 X Δ*stkP*::P_c-*erm* amplicon from IU7923)	Str^R Erm^R	This Study
IU16912	D39 Δ*cps rpsL1* Δ*khpB* Δ*stkP*::P_c-*erm* (IU10592 X Δ*stkP*::P_c-*erm* amplicon from IU7923)	Str^R Erm^R	This Study
IU16915	D39 Δ*cps rpsL1* Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*murA*$^+$ Δ*stkP*::P_c-*erm* (IU13395 X Δ*stkP*::P_c-*erm* amplicon from IU7923)	Erm^R Kan^R	This Study
IU16933, IU16934	D39 Δ*cps rpsL1* Δ*stkP*::P_c-*erm*//Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}-*stkP*$^+$ (IU14974 X Δ*stkP*::P_c-*erm* amplicon from IU7923)	Erm^R Kan^R	This Study
IU17134	D39 Δ*cps rpsL1* Δ*clpE*::P_c-[*kan-rpsL*$^+$] (IU1824 X fusion Δ*clpE*::P_c-[*kan-rpsL*$^+$)	Kan^R	This Study
IU17136	D39 Δ*cps rpsL1* Δ*clpL*::P_c-[*kan-rpsL*$^+$] (IU1824 X fusion Δ*clpL*::P_c-[*kan-rpsL*$^+$])	Kan^R	This Study
IU17138	D39 Δ*cps rpsL1* Δ*clpP*::P_c-[*kan-rpsL*$^+$] (IU1824 X fusion Δ*clpP*::P_c-[*kan-rpsL*$^+$])	Kan^R	This Study
IU17146	D39 Δ*cps rpsL1* Δ*clpP*::P_c-*erm* (IU1824 X fusion Δ*clpP*::P_c-*erm*)	Str^R Erm^R	This Study
IU17150	D39 Δ*cps rpsL1* Δ*clpE*::P_c-*erm murZ*-L-$FLAG^3$ (IU13502 X fusion Δ*clpE*::P_c-*erm*)	Str^R Erm^R	This Study
IU17152	D39 Δ*cps rpsL1* Δ*clpL*::P_c-*erm murZ*-L-$FLAG^3$ (IU13502 X fusion Δ*clpL*::P_c-*erm*)	Str^R Erm^R	This Study
IU17154	D39 Δ*cps rpsL1* Δ*clpP*::P_c-*erm murZ*-L-$FLAG^3$ (IU13502 X fusion Δ*clpP*::P_c-*erm*)	Str^R Erm^R	This Study

IU17158	D39 Δ*cps rpsL1* Δ*clpE*::P$_c$-*erm murA*-L-FLAG^3 (IU14028 X fusion Δ*clpE*::P$_c$-*erm*)	Str^R Erm^R	This Study
IU17160	D39 Δ*cps rpsL1* Δ*clpL*::P$_c$-*erm murA*-L-FLAG^3 (IU14028 X fusion Δ*clpL*::P$_c$-*erm*)	Str^R Erm^R	This Study
IU17162	D39 Δ*cps rpsL1* Δ*clpP*::P$_c$-*erm murA*-L-FLAG^3 (IU14028 X fusion Δ*clpP*::P$_c$-*erm*)	Str^R Erm^R	This Study
IU17170	D39 Δ*cps rpsL1 murZ*-HA (IU13396 X fusion *murZ*-HA)	Str^R	This Study
IU17469, IU17475	D39 Δ*cps rpsL1 murZ*(I265V) Δ*stkP*::P$_c$-*erm* (IU14210 X Δ*stkP*::P$_c$-*erm* amplicon from IU7923)	Str^R Erm^R	This Study
IU17603	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P$_{ftsA}$-*ftsA* Δ*pbp2b<>aad9* (IU12719 X Δ*pbp2b<>aad9* from IU7397)	Str^R Tet^R Spc^R	This Study
IU17605	D39 Δ*cps* Δ*bgaA*::*tet*-P$_{ftsA}$-*ftsA* Δ*rodZ*::P$_c$-*aad9* (IU12712 X Δ*rodZ*::P$_c$-*aad9* from IU6987)	Str^R Tet^R Spc^R	This Study
IU17607	D39 Δ*cps* Δ*bgaA*::*tet*-P$_{ftsA}$-*ftsA* Δ*pbp2b<>aad9* (IU12712 X Δ*pbp2b<>aad9* from IU7397)	Str^R Tet^R Spc^R	This Study
IU17609	D39 Δ*cps* Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*ftsA*$^+$ Δ*pbp2b<>aad9* (IU12307 X Δ*rodZ*::P$_c$-*aad9* from IU6987)	Str^R Tet^R Spc^R	This Study
IU17619	D39 Δ*cps rpsL1 murZ*(E190A E192A) (IU13396 X fusion *murZ*(E192A))	Str^R	This Study
IU17622	D39 Δ*cps rpsL1 murZ*(E192A) (IU13396 X fusion *murZ*(E192A))	Str^R	This Study
IU17623	D39 Δ*cps rpsL1 murZ*(D195A) (IU13396 X fusion *murZ*(D195A))	Str^R	This Study
IU17627	D39 Δ*cps rpsL1 murZ*(E259A) (IU13396 X fusion *murZ*(E259A))	Str^R	This Study
IU17764	D39 Δ*cps rpsL1* F-*murZ* (IU13396 X fusion F-*murZ*)	Str^R	This Study
IU17766	D39 Δ*cps rpsL1* HA-*murZ* (IU13396 X fusion HA-*murZ*)	Str^R	This Study
IU17768	D39 Δ*cps rpsL1* F-*murA* (IU13491 X fusion F-*murA*)	Str^R	This Study
IU17770	D39 Δ*cps rpsL1* HA-*murA* (IU13491 X fusion HA-*murA*)	Str^R	This Study
IU17838	D39 Δ*cps rpsL1 iht*-L$_6$-*murZ* with spontaneous L88F mutation in *iht* (IU13396 X fusion *iht*-L$_6$-*murZ*)	Str^R	This Study
IU17840	D39 Δ*cps rpsL1 iht*-L$_6$-*murZ* with spontaneous Z21F mutation in *iht* (IU13396 X fusion *iht*-L$_6$-*murZ*)	Str^R	This Study
IU17841	D39 Δ*cps rpsL1 iht*-L$_6$-*murA* (IU13491 X fusion *iht*-L$_6$-*murA*)	Str^R	This Study
IU17865	D39 Δ*cps rpsL1* Δ*clpP*::P$_c$-*erm iht*-L$_6$-*murZ* with spontaneous L88F mutation in *iht* (IU17838 X Δ*clpP*::P$_c$-*erm* from IU17154)	Str^R Erm^R	This Study
IU17867	D39 Δ*cps rpsL1* Δ*clpP*::P$_c$-*erm iht*-L$_6$-*murZ* with spontaneous Z21F mutation in *iht* (IU17840 X Δ*clpP*::P$_c$-*erm* from IU17154)	Str^R Erm^R	This Study

IU17869	D39 Δ*cps rpsL1 iht*-L$_6$-*murA* Δ*clpP*::P$_c$-*erm* (IU17841 X Δ*clpP*::P$_c$-*erm* from IU17154)	Str^R Erm^R	This Study
IU17957	D39 Δ*cps rpsL1 murZ*-L-FLAG^3-P$_c$-*erm* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$ (IU14974 X *murZ*-L-FLAG^3-P$_c$-*erm* from IU13249)	Erm^R Kan^R	This Study
IU17959	D39 Δ*cps rpsL1 murA*-L-FLAG^3-P$_c$-*erm* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$ (IU14974 X *murA*-L-FLAG^3-P$_c$-*erm* from IU13251)	Erm^R Kan^R	This Study
IU17961	D39 Δ*cps rpsL1 murZ*-L-FLAG^3-P$_c$-*erm* Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*phpP*$^+$ (IU15955 X *murZ*-L-FLAG^3-P$_c$-*erm* from IU13249)	Erm^R Tet^R	This Study
IU17963	D39 Δ*cps rpsL1 murA*-L-FLAG^3-P$_c$-*erm* Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*phpP*$^+$ (IU15955 X *murA*-L-FLAG^3-P$_c$-*erm* from IU13251)	Erm^R Tet^R	This Study
IU18555	D39 Δ*cps rpsL1* Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$ (IU1824 X fusion Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$)	Tet^R	This Study
IU18643	D39 Δ*cps rpsL1 phpP*$^+$-P$_c$-[*kan-rpsL*$^+$]-*stkP*$^+$ //Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$ (IU18555 X *phpP*$^+$-P$_c$-[*kan-rpsL*$^+$]-*stkP*$^+$ from IU7673)	Kan^R Tet^R	This Study
IU18663	D39 Δ*cps rpsL1* Δ*clpP* markerless (IU17138 X fusion Δ*clpP* markerless)	Str^R	This Study
IU18665	D39 Δ*cps rpsL1* Δ*stkP* markerless//Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$ (IU18643 X fusion Δ*stkP* markerless)	Str^R Tet^R	This Study
IU19079	D39 Δ*cps rpsL1 murZ*(D280Y)-L-FLAG^3-P$_c$-*erm* Δ*stkP* markerless//Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$ (IU18665 X fusion *murZ*(D280Y)-L-FLAG^3-P$_c$-*erm*)	Erm^R Str^R Tet^R	This Study
IU19081	D39 Δ*cps rpsL1 murZ*-L-FLAG^3-P$_c$-*erm* Δ*stkP* markerless//Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$ (IU18665 X *murZ*-L-FLAG^3-P$_c$-*erm* from IU13249)	Erm^R Str^R Tet^R	This Study
IU19201	D39 Δ*cps rpsL1* Δ*clpP*::P$_c$-*erm* Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA} -*murA*$^+$ (IU13395 X Δ*clpP*::P$_c$-*erm* amplicon from IU17146)	Kan^R Str^R Erm^R	This Study
IU19821	D39 Δ*cps rpsL1* Δ*spd_0567*::P$_c$-[*sacB-kan-rpsL*$^+$] (IU1824 X fusion Δ*spd_0567*::P$_c$-[*sacB-kan-rpsL*$^+$])	Kan^R	This Study
IU19835	D39 Δ*cps rpsL1 spd_0567*$^+$ (IU19821 X *spd_0567*$^+$ from IU1690)	Str^R	This Study

Primers used to construct strains and plasmids and for assays			
Primer	Sequence (5' to 3')	Template[c]	Amplicon Product
For construction of E740 (Δ[*phpP-stkP*]::P$_c$-*erm*)			
P1485	CCAAGCCTTGTTGGAGGCGAATAATTCCCT	D39	5' fragment with 60 bp of 5' *phpP*
P1486	CATTATCCATTAAAAATCAAACGGATCCTAGACATA GTCTTGGTTATTTGTTCGTTTCTG		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	Pc-*erm* cassette[d]	Pc-*erm*

Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1497	CAAAAGCATAAGGAAAGGGGCCCAATAAGAC TAG AGTCAAGATTTCAATCTACAAACCTA	D39	3' fragment with 60 bp of 3' *stkP*
P1496	CAATACCAAGGCGACAGAAGTTCCTGCCCC		
For construction of E765 (Δ*murA*::P_c-*erm*)			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	5' fragment with 60 bp of 5' *murA*
P1560	CATTATCCATTAAAAATCAAACGGATCCTACT CGATCGTCACGCTTCCTACCAGACGATT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[d]	P_c-*erm*
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1561	AAACGTCCAAAAGCATAAGGAAAGGGGCCCA AGTTGGCGCAGCTAGGTGCTAAGATTCAG	D39	3' fragment with 60 bp of 3' *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of E767 (Δ*murZ*::P_c-*erm*)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	5' fragment with 60 bp of 5' *murZ*
P1556	CATTATCCATTAAAAATCAAACGGATCCTAAC CACTAATAGTGATTTCACCTTGCAGTGG		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[d]	P_c-*erm*
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1557	AAACGTCCAAAAGCATAAGGAAAGGGGCCCT CTGATATTATCGAAAAATTACGTAATTTA	D39	3' fragment with 60 bp of 3' *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of E780 (Δ*clpC*::P_c-*erm*)			
P1663	GACTAGAGCACGTCAGTTATGCCTATGGTC	D39	5' fragment with 60 bp of 5' *clpC*
P1665	CATTATCCATTAAAAATCAAACGGATCCTAAT GTCCAGCAACCATGTAGGCACTTTCGAT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[d]	P_c-*erm*
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1666	AAACGTCCAAAAGCATAAGGAAAGGGGCCCG CAGGCAGCATACTTAAGATTGGTGTCAAA	D39	3' fragment with 60 bp of 3' *clpC*
P1664	AAATCCACTGTTACATCCTGATATCGCCAA		
For construction of K765 (Δ*murA*::P_c-[*kan-rpsL*$^+$])			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	5' fragment with 60 bp of 5' *murA*
P1560	CATTATCCATTAAAAATCAAACGGATCCTACT CGATCGTCACGCTTCCTACCAGACGATT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-[*kan-rpsL*$^+$] cassette[d]	P_c-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1561	AAACGTCCAAAAGCATAAGGAAAGGGGCCCA AGTTGGCGCAGCTAGGTGCTAAGATTCAG	D39	3' fragment with 60 bp of 3' *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		

For construction of K767 (∆*murZ*::P$_c$-[*kan-rpsL*$^+$])			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	5' fragment with 60 bp of 5' *murZ*
P1556	CATTATCCATTAAAAATCAAACGGATCCTAAC CACTAATAGTGATTTCACCTTGCAGTGG		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P$_c$-[*kan-rpsL*$^+$] cassette[d]	P$_c$-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1557	AAACGTCCAAAAGCATAAGGAAAGGGGCCCT CTGATATTATCGAAAAATTACGTAATTTA	D39	3' fragment with 60 bp of 3' *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of K779 (∆*clpC*::P$_c$-[*kan-rpsL*$^+$])			
P1663	GACTAGAGCACGTCAGTTATGCCTATGGTC	D39	5' fragment with 60 bp of 5' *clpC*
P1665	CATTATCCATTAAAAATCAAACGGATCCTAAT GTCCAGCAACCATGTAGGCACTTTCGAT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P$_c$-[*kan-rpsL*$^+$] cassette[d]	P$_c$-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1666	AAACGTCCAAAAGCATAAGGAAAGGGGCCCG CAGGCAGCATACTTAAGATTGGTGTCAAA	D39	3' fragment with 60 bp of 3' *clpC*
P1664	AAATCCACTGTTACATCCTGATATCGCCAA		
For construction of K787 (∆*ireB*(*spd_0180*)::P$_c$-[*kan-rpsL*$^+$])			
P1711	GAGTGTCAATGAAGTTCTCAATCTGATTATGG AAACACC	D39	5' upstream of *ireB* + 30 bp of 5' *ireB*
P1713	CATTATCCATTAAAAATCAAACGGATCCTAAA AACGTACTGTTTCTTCAGTAAATCCCAT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P$_c$-[*kan-rpsL*$^+$] cassette[f]	P$_c$-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1714	AACGTCCAAAAGCATAAGGAAAGGGGCCCTA TCTCAAAGGACAAGGAGTCGATCTATAAC	D39	30 bp of 3' *ireB* and downstream of *ireB*
P1712	CCACTGGACGTTCCAACTCTTCCCCATTTC		
For construction of IU8108 (∆[*spd_1029-1030*]::P$_c$-*erm*)			
P1514	GCTGGTCAAATCTGGGAGCCTTTTACTGAT	D39	5' fragment with 60 bp of 5' *spd_1030*
P1513	CATTATCCATTAAAAATCAAACGGATCCTAAC AAACTTGATCCAAACCAGACTTGG		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P$_c$-*erm* cassette[d]	P$_c$-*erm*
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1512	CAAAAGCATAAGGAAAGGGGCCCCGTTGGC GTTTAACTGTGATTATGAA	D39	3' fragment with 60 bp of 3' *spd_1029*
P1510	ACCATTGCCACTGCGAACATGGTCTACAGC		
For construction of IU8742 (∆*bgaA::tet-P$_{Zn}$-RBS^{ftsA}-phpP$^+$*)			
TT657	CGCCCCAAGTTCATCACCAATGACATCAAC	IU8122	*bgaA'*

BR01	TTCCTTCCTAATCCGATATCTTGTAATAGATTT ATGAACACCTTGTTCATTATCATTATC		*tet*-P_{Zn}-*RBS*^{ftsA}
BR02	AATGAACAAGGTGTTCATAAATCTATTACAAG ATATCGGATTAGGAAGGAACTGACAC	D39	*phpP*$^{+}$
BR03	CAACTGGTTTATGAGAAAGTAAGTTCTTTCAT TCTGCATCCTCCTCGTTCA		
BR04	ACGAGGAGGATGCAGAATGAAAGAACTTACT TTCTCATAAACCAGTTGCTG	D39	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU9613 (Δ*bgaA*::*tet*-P_{Zn}-*RBS*^{ftsA}-*rodZ*$^{+}$)			
TT657	CGCCCCAAGTTCATCACCAATGACATCAAC	IU8122	*bgaA'* *tet*-P_{Zn}-*RBS*^{ftsA}
TT769	CCTCTCCAATTGTTTTTTTTCTCATTACATCGC TTCCTCTCTATCTTCCTTGT		
TT770	GGAAGATAGAGAGGAAGCGATGTAATGAGAA AAAAAACAATTGGAGAGGTTTTAC	D39	*rodZ*$^{+}$
TT771	ACTGGTTTATGAGAAAGTAAGTTCTTTTAATTT TTAGTAAAGGTTACAGTGATTTGTCCA		
TT772	AAATCACTGTAACCTTTACTAAAAATTAAAAG AACTTACTTTCTCATAAACCAGTTGCTG	D39	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU9805 (Δ*bgaA*::*kan*-t1t2-P_{Zn}-*sepF*$^{+}$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9689	5' Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS_{ftsA-}
AJP32	ACATCGCTTCCTCTCTATCTTCCTTGTTATAAT AGATTTATGAACACCTTGTTCATTATC		
AJP107	GGAAGATAGAGAGGAAGCGATGTAATGTCTT TAAAAGATAGATTCGATAGATTTATAGAT	D39	*sepF*$^{+}$
AJP108	CAACTGGTTTATGAGAAAGTAAGTTCTTTTAT CGTACTCTATTTCGCTTCATATCAAAA		
AJP109	GATATGAAGCGAAATAGAGTACGATAAAAGA ACTTACTTTCTCATAAACCAGTTGCTG	D39	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU9992 (Δ*bgaA*::tet-P_{Zn}-RBS^{ftsA}–*pbp1b*$^{+}$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU8122	5' Δ*bgaA*::tet-t1t2-P_{Zn}-RBS^{ftsA}
BR74	TGATTTTGCATGGATTTCCTCACTACATCGCT TCCTCTCTATCTTCCTTGTTATA		
BR73	AGGAAGATAGAGAGGAAGCGATGTAGTGAG GAAATCCATGCAAAATCAATTAA	D39	*pbp1b*$^{+}$
BR76	CAACTGGTTTATGAGAAAGTAAGTTCTTTTAT CGTCTCGCCCTTGAAGAAGAG		
BR75	TCTTCAAGGGCGAGACGATAAAAGAACTTAC TTTCTCATAAACCAGTTGCTGC	IU8122	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU10220 (Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}-*mreC*$^{+}$)			
TT657	CGCCCCAAGTTCATCACCAATGACATCAAC	IU9613	*bgaA'* *tet*-P_{Zn}-*RBS*^{ftsA}
TT865	GACATATTTTGATTTTTTAAAACGGTTCATTA CATCGCTTCCTCTCTATCTTCCTTGTTA		

TT866	ACAAGGAAGATAGAGAGGAAGCGATGTAAT GAACCGTTTTAAAAAATCAAAATATGTCAT	D39	*mreC*+
TT867	AACTGGTTTATGAGAAAGTAAGTTCTTTTATG AATTCCCCACTAATTCTATCACATCTAC		
TT868	ATGTGATAGAATTAGTGGGGAATTCATAAAA GAACTTACTTTCTCATAAACCAGTTGCTG		*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU11049 (Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murG*+)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9805	5' containing Δ*bgaA*::*kan*-t1t2-P$_{Zn}$
JQ199	CCCCCCACCTGTAAAGACAATTTTTTTCATTA CATCGCTTCCTCTCTATCTTCCTTGTTA		
JQ200	TAACAAGGAAGATAGAGAGGAAGCGATGTAA TGAAAAAAATTGTCTTTACAGGTGGGGGG	D39	*murG*+
JQ201	AGCAACTGGTTTATGAGAAAGTAAGTTCTTTT ATGATAAATCTTTTTTCAACAATTGATA		
JQ202	TATCAATTGTTGAAAAAAGATTTATCATAAAAG AACTTACTTTCTCATAAACCAGTTGCT	IU9805	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU11077 (Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murZ*+)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9805	5' containing Δ*bgaA*::*kan*-t1t2-P$_{Zn}$
JQ222	TAATCCACCATTGATAACAATTTTTCTCATTAC ATCGCTTCCTCTCTATCTTCCTTGTTA		
JQ223	TAACAAGGAAGATAGAGAGGAAGCGATGTAA TGAGAAAAATTGTTATCAATGGTGGATTA	D39	*murZ*+
JQ224	AGCAACTGGTTTATGAGAAAGTAAGTTCTTTT AATCCTCAACAAGTCTAATATCCGCTCC		
JQ225	GGAGCGGATATTAGACTTGTTGAGGATTAAA AGAACTTACTTTCTCATAAACCAGTTGCT	IU9805	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU11079 (Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*murA*+)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9805	5' containing Δ*bgaA*::*kan*-t1t2-P$_{Zn}$
JQ226	ATCGCCACCTTGAACCACAATTTTATCCATTA CATCGCTTCCTCTCTATCTTCCTTGTTA		
JQ227	TAACAAGGAAGATAGAGAGGAAGCGATGTAA TGGATAAAATTGTGGTTCAAGGTGGCGAT	D39	*murA*+
JQ228	AGCAACTGGTTTATGAGAAAGTAAGTTCTTTT ATTCATCTTCATCATTTGCCTCAATCCG		
JQ229	CGGATTGAGGCAAATGATGAAGATGAATAAA AGAACTTACTTTCTCATAAACCAGTTGCT	IU9805	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU11083 (Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-*mraY*+)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9805	5' containing Δ*bgaA*::*kan*-t1t2-P$_{Zn}$
JQ195	CACAATTCCAGCACTGATGGAAATAAACATTA CATCGCTTCCTCTCTATCTTCCTTGTTA		
JQ196	TAACAAGGAAGATAGAGAGGAAGCGATGTAA TGTTTATTTCCATCAGTGCTGGAATTGTG	D39	*mraY*+
JQ197	AGCAACTGGTTTATGAGAAAGTAAGTTCTTTT ACATCAAATACAAAATTGCGAGGGTCAG		

JQ198	CTGACCCTCGCAATTTTGTATTTGATGTAAAA GAACTTACTTTCTCATAAACCAGTTGCT	IU9805	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU11094 (Δ*bgaA*::*kan*-t1t2-P_{Zn}-*uppS*$^+$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9805	5' containing Δ*bgaA*::*kan*-t1t2-P_{Zn}
JQ191	AGCCTTATCTTTCTTAAAAAATCCAAACATTAC ATCGCTTCCTCTCTATCTTCCTTGTTA		
JQ192	TAACAAGGAAGATAGAGAGGAAGCGATGTAA TGTTTGGATTTTTTAAGAAAGATAAGGCT	D39	*uppS*$^+$
JQ193	AGCAACTGGTTTATGAGAAAGTAAGTTCTTCT AAACTCCTCCAAATCGGCGATGACGACG		
JQ194	CGTCGTCATCGCCGATTTGGAGGAGTTTAGA AGAACTTACTTTCTCATAAACCAGTTGCT	IU9805	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU11628 (Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA} -*mapZ*$^+$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9805	Δ*bgaA*::*kan*-t1t2-P_{Zn}-RBS^{ftsA}
AJP32	ACATCGCTTCCTCTCTATCTTCCTTGTTATAAT AGATTTATGAACACCTTGTTCATTATC		
AJP223	AAGGAAGATAGAGAGGAAGCGATGTAATGAG TAAAAAAAGACGAAATCGTCATAAA	D39	*mapZ*$^+$
AJP224	CAACTGGTTTATGAGAAAGTAAGTTCTTTTAG TAGTCCAAGTCATCCGCATGAC		
AJP225	ATGCGGATGACTTGGACTACTAAAAGAACTTA CTTTCTCATAAACCAGTTGCTG	D39	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU11912 (Δ*stkP*::P_c-*cat*)			
TT546	AGAGAGTCATCCCGAGTTCGAGCAGGTAAA	D39	5' fragment with 60 bp of 5' *stkP*
TT654	CATTATCCATTAAAAATCAAACGGATCCTATC GACCAATCTGTTTGACAATCCG		
kanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	IU11119[e]	P_c-*cat*
kanrpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1497	CAAAAGCATAAGGAAAGGGGCCCAATAAGAC TAGAGTCAAGATTTCAATCTACAAACCTA	D39	3' fragment with 60 bp of 3' *stkP*
P1496	CAATACCAAGGCGACAGAAGTTCCTGCCCC		
For construction of IU12192 (Δ*bgaA*::tet- P_{Zn}- RBS^{ftsA}-*ftsW*$^+$)			
TT657	CGCCCCAAGTTCATCACCAATGACATCAAC	IU8122	Δ*bgaA*::tet- P_{Zn}-RBS^{ftsA}
YT50	ATAATTTAATAAGTGCCTCTTACTAATCTTCAT TACATCGCTTCCTCTCTATCTTCCTTG		
YT51	GGAAGATAGAGAGGAAGCGATGTAATGAAGA TTAGTAAGAGGCACTTATTAAATTATTCC	D39	*ftsW*$^+$
YT52	CAGCAACTGGTTTATGAGAAAGTAAGTTCTTC TACTTCAACAGAAGGTTCATTGGTTGAT		
YT53	ATCAACCAATGAACCTTCTGTTGAAGTAGAAG AACTTACTTTCTCATAAACCAGTTGCTG	IU8122	*bgaA'* to downstream

CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU12678 (Δ*bgaA*::tet- P_{Zn}- RBS^{ftsA}-*cozE*$^+$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU8122	Δ*bgaA*::tet- P_{Zn}-RBS^{ftsA}
TT968	CAAAAAAATAATTTATTTCTACGAAACATTACATCGCTTCCTCTCTATCTTCCTTGTTAT		
TT969	AAGGAAGATAGAGAGGAAGCGATGTAATGTTTCGTAGAAATAAATTATTTTTTTGGACCA	D39	*cozE*$^+$
TT970	CTGGTTTATGAGAAAGTAAGTTCTTTTACTTAGCTAATTCTCTTTCTCGTTCTTTCATTA		
TT971	AAGAACGAGAAAGAGAATTAGCTAAGTAAAAGAACTTACTTTCTCATAAACCAGTTGCTG	IU8122	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU12712 and IU12719 (Δ*bgaA*::*kan*-t1t2-P_{ftsA}-RBS^{ftsA}-*ftsA*)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9621	5' *bgaA*'-Kan-T1T2
SC484	GAGCAAAAAAGAAAGCTCTGTGGTAGAAACGCAAAAAGGCCATCCGTCAGG		
SC483	GACGGATGGCCTTTTTGCGTTTCTACCACAGAGCTTTCTTTTTTGCTCTTAGAGAG	D39	P_{ftsA}-*ftsA*$^+$
AJP49	CAACTGGTTTATGAGAAAGTAAGTTCTTTTATTCGTCAAACATGCTTCCGATC		
AJP50	CGGAAGCATGTTTGACGAATAAAAGAACTTACTTTCTCATAAACCAGTTGC	D39	*bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU13249 (*murZ*-L-FLAG^3-P_c-*erm*)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murZ* + *murZ*
JQ315	GCCAGAACCAGCAGCGGAGCCAGCGGAACCATCCTCAACAAGTCTAATATCCGCTCCTAA		
JQ179	GGTTCCGCTGGCTCCGCTGCTGGTTCTGGC	IU4970	L-FLAG^3-P_c-*erm*
JQ184	TTATTTCCTCCCGTTAAATAATAGATAACTAT		
JQ316	ATAGTTATCTATTATTTAACGGGAGGAAATAAACCGTAGAGGTGTTTATGAATATTTGGA	D39	Downstream *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU13251 (*murA*-L-FLAG^3-P_c-*erm*)			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream of *murA* + *murA*
JQ317	GCCAGAACCAGCAGCGGAGCCAGCGGAACCTTCATCTTCATCATTTGCCTCAATCCGCTG		
JQ179	GGTTCCGCTGGCTCCGCTGCTGGTTCTGGC	IU4970	L-FLAG^3-P_c-*erm*
JQ184	TTATTTCCTCCCGTTAAATAATAGATAACTAT		
JQ318	ATAGTTATCTATTATTTAACGGGAGGAAATAAGAAATCAAGCTACGTAGTCAAGCGTTTA	D39	Downstream *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU13502, IU13545 (*murZ*-L-FLAG^3)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	IU13249	*murZ*-L-FLAG^3

JQ338	GGTCCAAATATTCATAAACACCTCTACGGTTT ATTTATCATCATCATCTTTATAATCTTT		
JQ339	AAAGATTATAAAGATGATGATGATAAATAAAC CGTAGAGGTGTTTATGAATATTTGGACC	D39	Downstream *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU13536, IU13542 (Δ*murZ*)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murZ* + 60 bp 5' *murZ*
JQ344	TAAATTACGTAATTTTTCGATAATATCAGAACC ACTAATAGTGATTTCACCTTGCAGTGG		
JQ345	CCACTGCAAGGTGAAATCACTATTAGTGGTT CTGATATTATCGAAAAATTACGTAATTTA		60 bp 3' of *murZ* + downstream *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU13538, IU13546 (Δ*murA*)			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream of *murA* + 60 bp 5' *murA*
JQ346	CTGAATCTTAGCACCTAGCTGCGCCAACTTC TCGATCGTCACGCTTCCTACCAGACGATT		
JQ347	AATCGTCTGGTAGGAAGCGTGACGATCGAGA AGTTGGCGCAGCTAGGTGCTAAGATTCAG		60 bp 3' of *murA* + downstream *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU13600 (*murZ*(D280Y)-L-FLAG^3)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	IU13438	Upstream of *murZ* + *murZ*(D280Y)
JQ315	GCCAGAACCAGCAGCGGAGCCAGCGGAACC ATCCTCAACAAGTCTAATATCCGCTCCTAA		
JQ179	GGTTCCGCTGGCTCCGCTGCTGGTTCTGGC	IU13502	L-FLAG^3 + downstream of *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU13604 (Δ*ireB* markerless)			
P1711	GAGTGTCAATGAAGTTCTCAATCTGATTATGG AAACACC	D39	5' upstream of *ireB* + 15 bp of 5' *ireB*
TT1030	CATTATCCATTAAAAATCAAACGGATCCTAAA AACGTACTGTTTCTTCAGTAAATCCCAT		
TT1031	AACGTCCAAAAGCATAAGGAAAGGGGCCCTA TCTCAAAGGACAAGGAGTCGATCTATAAC	D39	18 bp of 3' *ireB* and downstream of *ireB*
P1712	CCACTGGACGTTCCAACTCTTCCCCATTTC		
For construction of IU13680 (Δ*pbp1b*::P$_c$-*aad9*)			
P222	CGTTCGTGTGGCGCTGCTTCAAATTGTT	D39	Upstream of *pbp1b* and 100 bp of 5' *pbp1b*
P456	CATTATCCATTAAAAATCAAACGGATCCTATT GAACCTTTCTTGCCAGGTCTAGCTGATT		
KanrpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	IU8791	P$_c$-*aad9*
KanrpsL reverse	CAAAAGCATAAGGAAAGGGGCCC		
P225	CAAAAGCATAAGGAAAGGGGCCCTCTAGCGA TAGCAGTAACTCAAGTACTACACGACCTT	D39	60 bp of 3' *pbp1b* and

P522	AACGGCAACCACCAAAGGAGAAACCAAGGA		downstream of *pbp1b*
For construction of IU13772 (Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murZ*-L-FLAG^{3})			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU11077	Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murZ*
JQ315	GCCAGAACCAGCAGCGGAGCCAGCGGAACC ATCCTCAACAAGTCTAATATCCGCTCCTAA		
JQ179	GGTTCCGCTGGCTCCGCTGCTGGTTCTGGC	IU4355	L-FLAG^{3}-*bgaA*’
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU13794 (Δ*bgaA*::*tet*-P_{Zn}-RBS^{ftsA}–*divIVA*$^{+}$ (R6))			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU8122	Δ*bgaA*::*tet*-t1t2-P_{Zn}-RBS^{ftsA}
YT72	TAATGATGTAATTGGCATTCTATTCCTCACTA CATCGCTTCCTCTCTATCTTCCTTGTTA		
YT73	TAACAAGGAAGATAGAGAGGAAGCGATGTAG TGAGGAATAGAATGCCAATTACATCATTA	D39	*divIVA*$^{+}$ (R6 annotation)
YT62	AGCAACTGGTTTATGAGAAAGTAAGTTCTTCT ACTTCTGGTTCTTCATACATTGGGCCAA		
YT63	GGCCCAATGTATGAAGAACCAGAAGTAGAAG AACTTACTTTCTCATAAACCAGTTGCTGC	IU8122	*bgaA’* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU14028, IU14030 (*murA*-L-FLAG^{3})			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	IU13251	Upstream of *murA* + *murA*-L-FLAG^{3}
JQ340	CTGAATCTTAGCACCTAGCTGCGCCAACTTTT ATTTATCATCATCATCTTTATAATCTTT		
JQ341	AAAGATTATAAAGATGATGATGATAAATAAAA GTTGGCGCAGCTAGGTGCTAAGATTCAG	D39	Downstream of *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU14270 (Δ*mraY*<>*aad9*)			
TT345	GTGACCCAGACGCAAATGATTCGTGCCTTT	D39	upstream of *mraY*
TT1076	TATTCAAATATATCCTCCTCATATTAGTCTCCT AAAGTTAATGTAATTTTTTTAATGTCC		
TT1077	AAATTACATTAACTTTAGGAGACTAATATGAG GAGGATATATTTGAATACATACGAACAA	IU4888	*aad9* ORF
TT1078	ATCAGGGTGCCATTCTTATAATTTTTTTAATCT GTTATTTAAATAGTTTATAGTTAAATT		
TT1079	TATAAACTATTTAAATAACAGATTAAAAAAATT ATAAGAATGGCACCCTGATGTTTCAGG	D39	downstream of *mraY*
TT1080	CTGCTGTCAAGTTTCGACCCAGTTTAGCAAG G		
For construction of IU14272 (Δ*uppS*<>*aad9*)			
TT1070	GCCATTCTGACGATCATCCGAGACCTTGGT	D39	upstream of *uppS*
TT1071	GTATGTATTCAAATATATCCTCCTCATGATCTT ATTCCTATTCAAAAATCTATCGTTTCA		
TT1072	CGATAGATTTTTGAATAGGAATAAGATCATGA GGAGGATATATTTGAATACATACGAACA	IU4888	*aad9* ORF

TT1073	GGGTCATATTTCCTCTTATAATTTTTTTAATCT GTTATTTAAATAGTTTATAGTTAAATT		
TT1074	ACTATTTAAATAACAGATTAAAAAAATTATAAG AGGAAATATGACCCAGGATTTACAGAA	D39	downstream of *uppS*
TT1075	GGTTAAAATTCCGAGCATAGCGTTTCCTCCG TC		
For construction of IU14274 (Δ*murG*<>*aad9*)			
TT1064	CCAACCTCATGCCAACTCATATCGACTACCAT G	D39	upstream of *murG*
TT1065	CGTATGTATTCAAATATATCCTCCTCATATTTT ATTCTTTTAACTCCGCTACTGTGTCG		
TT1066	ACAGTAGCGGAGTTAAAAGAATAAAATATGA GGAGGATATATTTGAATACATACGAACA	IU4888	*aad9* ORF
TT1067	TTGACATTTACTTTCCTTATAATTTTTTTAATCT GTTATTTAAATAGTTTATAGTTAAAT		
TT1068	TTAAATAACAGATTAAAAAAATTATAAGGAAA GTAAATGTCAAAAGATAAGAAAAATGAG	D39	downstream of *murG*
TT1069	GCCGCCTTGAGTTCTGGGCTAATTTGAGCA		
For construction of IU14312 (Δ*bgaA*::tet- P$_{Zn}$- RBS^{ftsA}-*pbp1a*$^{+}$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU8122	Δ*bgaA*::tet- P$_{Zn}$- RBS^{ftsA}
BR62	CGCAGAATCGTTGGTTTGTTCATTACATCGCT TCCTCTCTATCTTCCTTGTTATAATA		
BR61	ACAAGGAAGATAGAGAGGAAGCGATGTAATG AACAAACCAACGATTCTGCGC	D39	*pbp1a*$^{+}$
BR64	CAGCAACTGGTTTATGAGAAAGTAAGTTCTTT TATGGTTGTGCTGGTTGAGGATTCTG		
BR63	GAATCCTCAACCAGCACAACCATAAAAGAAC TTACTTTCTCATAAACCAGTTGCTGC	D39	*bgaA'* to downstream
CS121	GCACCAAGTGAATTGCCTCAAGAAAGC		
For construction of IU14974 (Δ*bgaA*::*kan*-t1t2-P$_{Zn}$-RBS^{ftsA}-*stkP*$^{+}$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU9805	Δ*bgaA*::*kan*-t1t2-P$_{Zn}$
BR12	AAATCTTGCCGATTTGGATCATTACATCGCTT CCTCTCTATCTTCCTTGT		
BR13	GGAAGATAGAGAGGAAGCGATGTAATGATCC AAATCGGCAAGATTTTTG	D39	*stkP*$^{+}$
BR14	GCAGCAACTGGTTTATGAGAAAGTAAGTTCTT TTAAGGAGTAGCTGAAGTTGTTTTAGGT		
BR15	CAATCTACAAACCTAAAACAACTTCAGCTACT CCTTAAAAGAACTTACTTTCTCATAAAC	IU9805	*bgaA'* to downstream
CS121	GCACCAAGTGAATTGCCTCAAGAAAGC		
For construction of IU15143 (*murA*(D281Y))			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream *murA* + 5' *murA*(D281Y)
TT1145	ACGAACACGAATTCCTTCGTATTCTTCAATTA CTTCAACA		

TT1146	TGTTGAAGTAATTGAAGAATACGAAGGAATTC GTGTTCGT	D39	3’ m*urA*(D281Y) + downstream *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU15145 (murA(E282Y))			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream *murA* + 5’ *murA*(E282Y)
TT1147	GAGAACGAACACGAATTCCGTAGTCTTCTTC AATTACTTCA		
TT1148	TGAAGTAATTGAAGAAGACTACGGAATTCGT GTTCGTTCTC	D39	3’ m*urA*(E282Y) + downstream *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU15939 (*murZ*(C116S))			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream *murZ* + 5’ *murZ*(C116S)
TT1203	CGGACGAGGACCAAGATCAGATCCTCCCGG TAGACCAA		
TT1204	TTGGTCTACCGGGAGGATCTGATCTTGGTCC TCGTCCG	D39	3’ *murZ*(C116S) + downstream *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU15941 (*murZ*(C116S)-L-FLAG^3)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	5’ *murZ*(C116S)
TT1203	CGGACGAGGACCAAGATCAGATCCTCCCGG TAGACCAA		
TT1204	TTGGTCTACCGGGAGGATCTGATCTTGGTCC TCGTCCG	IU13502	3’ *murZ*(C116S) -L-FLAG^3
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU15943 (Δ*bgaA*::*kan*-t1t2-P$_{Zn}$- RBS^{ftsA} -*murZ*(C116S))			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU13393	Δ*bgaA::kan*-t1t2-P$_{Zn}$-*murZ*(C116S)
TT1203	CGGACGAGGACCAAGATCAGATCCTCCCGG TAGACCAA		
TT1204	TTGGTCTACCGGGAGGATCTGATCTTGGTCC TCGTCCG	IU13393	*murZ*(C116S) and 3’ bgaA
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU15949 (*murA*(C120S))			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream *murA* + 5’ *murA*(C120S)
TT1205	AGGACGGCTACCAATCGTAGAACCACCTGGC ATGGATA		
TT1206	TATCCATGCCAGGTGGTTCTACGATTGGTAG CCGTCCT	D39	3’ m*urA*(C120S) + downstream *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU15951 (*murA*(C120S)-L-FLAG^3)			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream *murA* + 5’ *murA*(C120S)
TT1205	AGGACGGCTACCAATCGTAGAACCACCTGGC ATGGATA		
TT1206	TATCCATGCCAGGTGGTTCTACGATTGGTAG CCGTCCT	IU14028	3’ *murA*(C120S) -L-FLAG^3 +

P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		downstream *murA*
For construction of IU15954 (Δ*bgaA*::*kan*-t1t2-P_{Zn}- RBS^{ftsA} -*murA*(C120S))			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU13395	Δ*bgaA*::*kan*-t1t2-P_{Zn}-5' *murA*(C120S)
TT1205	AGGACGGCTACCAATCGTAGAACCACCTGGCATGGATA		
TT1206	TATCCATGCCAGGTGGTTCTACGATTGGTAGCCGTCCT	IU13395	*3' murA*(C120S)-3' *bgaA'*
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU15983 (Δ*bgaA*::*kan*-t1t2-P_{Zn}- RBS^{ftsA} -*murA*-L-$FLAG^3$)			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU13395	Δ*bgaA*::*kan*-t1t2-P_{Zn}-*murA*
JQ317	GCCAGAACCAGCAGCGGAGCCAGCGGAACCTTCATCTTCATCATTTGCCTCAATCCGCTG		
JQ179	GGTTCCGCTGGCTCCGCTGCTGGTTCTGGC	IU4355	L-$FLAG^3$-*bgaA*'
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU16334, IU16336 (Δ*bgaA*::*kan*-t1t2-P_{Zn}- RBS^{ftsA} -*murZ*(D280Y))			
P146	TGGCCATTCATCGCTGGTCGTGCTGAAAT	IU13393	Δ*bgaA*::*kan*-t1t2-P_{Zn}-5' *murZ*(D280Y)
TT1230	TTCCTCGACAAAAATGCTGTATTCAGATACAGTCATTCTCA		
TT1231	TGAGAATGACTGTATCTGAATACAGCATTTTTGTCGAGGAA	IU13393	*3' murZ*(D280Y)-*bgaA*'
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU17134 (△*clpE*::P_c-[*kan-rpsL*$^+$])			
P1730	ACGAACAATCTCCGAAACATAAGCACCACT	D39	Upstream of *clpE* + 60 bp of 5' *clpE*
P1727	CATTATCCATTAAAAATCAAACGGATCCTAATTGAGATTGGTGTAAAGATGAATTGTTGA		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-[*kan-rpsL*$^+$] cassette[d]	P_c-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1728	AAACGTCCAAAAGCATAAGGAAAGGGGCCCAACATTCAGATTAAATCTGCCAAAAAAGCT	D39	60 bp of 3' *clpE* + downstream
P1729	TTCTTATGGCATATTCAATAGATTTTCGTA		
For construction of IU17136 (△*clpL*::P_c-[*kan-rpsL*$^+$])			
P1726	ATTAGTTTGTTTGCCTATGGAGTTATTGCC	D39	Upstream of *clpL* + 60 bp of 5' *clpL*
P1723	CATTATCCATTAAAAATCAAACGGATCCTAACCCATCAATTGGTTAAATAAATCATCCAT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-[*kan-rpsL*$^+$] cassette[d]	P_c-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1724	CAAAAGCATAAGGAAAGGGGCCCGCTAAACATCTGGAAGCAGATATGGAAGAT	D39	60 bp of 3' *clpL* + downstream
P1725	TTCGTAAACTGGGTATCAACGTAACCTTTG		
For construction of IU17138 (△*clpP*::P_c-[*kan-rpsL*$^+$])			
P1722	CGAATGGACGACTACGCCCAATACCTTTAT	D39	

P1719	CATTATCCATTAAAAATCAAACGGATCCTAC AGCATAATGATGCGGTCTTTGAGAAGACG		Upstream of *clpP* + 90 bp of 5' *clpP*
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-[*kan-rpsL*$^+$] cassette[d]	P_c-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1720	AAACGTCCAAAAGCATAAGGAAAGGGGCCC CAGGAAACACTTGAATATGGCTTTATTGAT	D39	60 bp of 3' *clpP* + downstream
P1721	GTGTAAAGAACAACTTTCTTAGCATTTAAT		
For construction of IU17150, IU17158 (∆*clpE*::P_c-*erm*)			
P1730	ACGAACAATCTCCGAAACATAAGCACCACT	D39	Upstream of *clpE* + 60 bp of 5' *clpE*
P1727	CATTATCCATTAAAAATCAAACGGATCCTAAT TGAGATTGGTGTAAAGATGAATTGTTGA		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[d]	P_c-*erm*
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1728	AAACGTCCAAAAGCATAAGGAAAGGGGCCC AACATTCAGATTAAATCTGCCAAAAAAGCT	D39	60 bp of 3' *clpE* + downstream
P1729	TTCTTATGGCATATTCAATAGATTTTCGTA		
For construction of IU17152, IU17160 (∆*clpL*::P_c-*erm*)			
P1726	ATTAGTTTGTTTGCCTATGGAGTTATTGCC	D39	Upstream of *clpL* + 60 bp of 5' *clpL*
P1723	CATTATCCATTAAAAATCAAACGGATCCTAA CCCATCAATTGGTTAAATAAATCATCCAT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[d]	P_c-*erm*
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1724	CAAAAGCATAAGGAAAGGGGCCCGCTAAAC ATCTGGAAGCAGATATGGAAGAT	D39	60 bp of 3' *clpL* + downstream
P1725	TTCGTAAACTGGGTATCAACGTAACCTTTG		
For construction of IU17146, IU17154, IU17162 (∆*clpP*::P_c-*erm*)			
P1722	CGAATGGACGACTACGCCCAATACCTTTAT	D39	Upstream of *clpP* + 90 bp of 5' *clpP*
P1719	CATTATCCATTAAAAATCAAACGGATCCTAC AGCATAATGATGCGGTCTTTGAGAAGACG		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-*erm* cassette[d]	P_c-*erm*
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1720	AAACGTCCAAAAGCATAAGGAAAGGGGCCC CAGGAAACACTTGAATATGGCTTTATTGAT	D39	60 bp of 3' *clpP* + downstream
P1721	GTGTAAAGAACAACTTTCTTAGCATTTAAT		
For construction of IU17170 (*murZ*-HA)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murZ* + *murZ*-HA
MJ062	GCATAATCTGGAACATCATATGGATAATCCT CAACAAGTCTAATATCCGCTCCTAA		
MJ063	GGATTATCCATATGATGTTCCAGATTATGCT TAAACCGTAGAGGTGTTTATGAATATTTG	D39	

P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		HA + downstream of *murZ*
For construction of IU17619 (*murZ*(E190A E192A))			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murA* + *murA*(E190A E192A)
TT1360	AGCTACATCAATAATCGCAGGTGCACGGGCTG CATTTTCA		
TT1361	TGAAAATGCAGCCCGTGCACCTGCGATTATTG ATGTAGCT	D39	*murA*(E190A E192A) + downstream of *murA*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU17622 (*murZ*(E192A))			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murA* + *murA*(E192A)
TT1358	GTAGCTACATCAATAATCGCAGGTTCACGGGC TGCATTT		
TT1359	AAATGCAGCCCGTGAACCTGCGATTATTGATG TAGCTAC	D39	*murA*(E192A) + downstream of *murA*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU17623 (*murZ*(D195A))			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murA* + *murA*(D195A)
TT1356	TATTCAAGAGAGTAGCTACAGCAATAATCTCAG GTTCACGGG		
TT1357	CCCGTGAACCTGAGATTATTGCTGTAGCTACTC TCTTGAATA	D39	*murA*(E195A) + downstream of *murA*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU17627 (*murZ*(E259A))			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murA* + *murA*(E259A)
TT1362	GCAATAAACCCTTCCAGGTGTGCGTAAAGAAC ATTATTTA		
TT1363	TAAATAATGTTCTTTACGCACACCTGGAAGGGT TTATTGC	D39	*murA*(E259A) + downstream of *murA*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU17764 (F-*murZ*)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murZ* + F
TT1366	CTTTTATCATCATCATCTTTATAATCCATTCT AAGTTTTCAATACTCTTTCAAGATTTCT		
TT1367	TAGAATGGATTATAAAGATGATGATGATAAA AGAAAAATTGTTATCAATGGTGGATTACC	D39	F-*murZ* + downstream of *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU17766 (HA-*murZ*)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murZ* + HA
TT1368	AGCATAATCTGGAACATCATATGGATACATT CTAAGTTTTCAATACTCTTTCAAGATTTC		
TT1369	AATGTATCCATATGATGTTCCAGATTATGCTA GAAAAATTGTTATCAATGGTGGATTACC	D39	HA-*murZ* + downstream of *murZ*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		

For construction of IU17768 (F-*murA*)			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream of *murA* + F
TT1370	ATCTTTATCATCATCATCTTTATAATCCATACTCGTTTCCTTTACTCTTGATTTCATAAT		
TT1371	AAACGAGTATGGATTATAAAGATGATGATGATAAAGATAAAATTGTGGTTCAAGGTGGCG	D39	F-*murA* + downstream of *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU17770 (HA-*murA*)			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream of *murA* + HA
TT1372	AGCATAATCTGGAACATCATATGGATACATACTCGTTTCCTTTACTCTTGATTTCATAAT		
TT1373	CGAGTATGTATCCATATGATGTTCCAGATTATGCTGATAAAATTGTGGTTCAAGGTGGCG	D39	HA-*murA* + downstream of *murA*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU17838, IU17840 (*iht*-L$_6$-*murZ*)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	D39	Upstream of *murZ*
TT1392	AAAAATTTCCAAACCTTTTTTATCCATTCTAAGTTTTCAATACTCTTTCAAGATTTCTAA		
TT1393	AATCTTGAAAGAGTATTGAAAACTTAGAATGGATAAAAAAGGTTTGGAAATTTTTTTGGC	IU14738	*iht*-L$_6$
TT1394	TTGCAGTGGTAATCCACCATTGATAACAATTTTTCTACCAGAACCTTGACCAGATCCTGG		
TT1395	CAAGGACCAGGATCTGGTCAAGGTTCTGGTAGAAAAATTGTTATCAATGGTGGATTACCA	D39	*murZ* + downstream
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU17841 (*iht*-L$_6$-*murA*)			
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	D39	Upstream of *murA*
TT1396	CAAAAAAATTTCCAAACCTTTTTTATCCATACTCGTTTCCTTTACTCTTGATTTCATAAT		
TT1397	TATGAAATCAAGAGTAAAGGAAACGAGTATGGATAAAAAAGGTTTGGAAATTTTTTTGGC	IU14738	*iht*-L$_6$
TT1398	CAGACGATTATCGCCACCTTGAACCACAATTTTATCACCAGAACCTTGACCAGATCCTGG		
TT1399	CAGGACAAGGACCAGGATCTGGTCAAGGTTCTGGTGATAAAATTGTGGTTCAAGGTGGCG	D39	*murA* + downstream
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
For construction of IU18555 (Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}-*stkP*$^+$)			
TT657	CGCCCCAAGTTCATCACCAATGACATCAAC	IU9990	Δ*bgaA*::*tet*-P$_{Zn}$-RBS^{ftsA}
TT1435	AAATCTTGCCGATTTGGATCATTACATCGCTTCCTCTCTATCTTCCTTGTTATAATAGAT		
TT1436	ATAACAAGGAAGATAGAGAGGAAGCGATGTAATGATCCAAATCGGCAAGATTTTTGCCGG	IU14974	*stkP*$^+$- *bgaA'* to downstream
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU18663 (Δ*clpP* markerless)			
TT1374	CACCCACTGATTCAACACAAATTGTCAATCTTGC	D39	Upstream *stkP* + 90 bp 5' *clpP*

TT1455	ATCAATAAAGCCATATTCAAGTGTTTCCTGCAGCATAATGATGCGGTCTTTGAGAAGACG		
TT1456	CGTCTTCTCAAAGACCGCATCATTATGCTGCAGGAAACACTTGAATATGGCTTTATTGAT	D39	60 bp of 3' *clpP* + downstream
TT1377	ACCTGCTTTTGTAGCGTTCGCTACCGCAG		
For construction of IU18665 (Δ*stkP* markerless)			
TT546	AGAGAGTCATCCCGAGTTCGAGCAGGTAAA	D39	Upstream *stkP* + 60 bp 5' *stkP*
TT1315	TGTAGATTGAAATCTTGACTCTAGTCTTATTTCGACCAATCTGTTTGACAATCCG		
TT1316	GGATTGTCAAACAGATTGGTCGAAATAAGACTAGAGTCAAGATTTCAATCTACAAACC	D39	60 bp of 3' *stkP* + downstream
P1496	CAATACCAAGGCGACAGAAGTTCCTGCCCC		
For construction of IU19079 (*murZ*(D280Y)-L-FLAG^3-P_c-*erm*)			
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	IU13438	*murZ*(D280Y)
JQ315	GCCAGAACCAGCAGCGGAGCCAGCGGAACCATCCTCAACAAGTCTAATATCCGCTCCTAA		
JQ179	GGTTCCGCTGGCTCCGCTGCTGGTTCTGGC	IU13249	L-FLAG^3-P_c-*erm* + downstream
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
For construction of IU19821 (Δ*spd-0567*::P_c-[*sacB-kan-rpsL*$^+$])			
TT1522	GTCCCTATTGATGCGGAATTTGACTGTCCC	D39	5' fragment with 90 bp of 5' *spd_0567*
TT1527	CATTATCCATTAAAAATCAAACGGATCCTATCCCATAGTCGCATCCACTACGACATCCTC		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-[*kan-rpsL*$^+$] cassette[d]	P_c-[*sacB-kan-rpsL*$^+$][f]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
TT1528	CAAAAGCATAAGGAAAGGGGCCCGTCAACAACCCGCCGTTTTTAGTGATGATT	D39	3' fragment with 60 bp of 3' *spd_0567*
TT1520	CCAGAAGCATCATTCAAGAGTCCTTCGCCC		

Templates and primers used to generate amplicons for transformation assays			
TT196	GCCAAGCCCTGAGACAAATAGTAGTCGTTGGT	IU4888	Δ*gpsB*<>*aad9*
TT197	TTTGATACGATCTGCTGCCCGAAGCCAAAGGT		
P1554	GATTTTGTGGTACGACGGGCATGTATAGCG	E767	Δ*murZ*::P_c-*erm*
P1555	TGAACCTGAAATCCCCCTGTAACCAGAACT		
P1558	TCAGGAGACTACAGGTGGTTCTTCCGATGT	E765	Δ*murA*::P_c-*erm*
P1559	CTTAGTACCTGTTCTAGCCCTGCTTAAACT		
TT571	GAGCGAGTGCTTGATGCCTGTGCGGCTCCA	IU7923	Δ*stkP*::P_c-*erm*
P1496	CAATACCAAGGCGACAGAAGTTCCTGCCCC		
TT329	CAACTGATATAGTTGGAAGTGAGGAGTCCATTTCCC	IU9931	Δ*rodZ* <>*aad9*
P1385	ACAACACCTGCAATGGCCACACGTTGCTTT		
TT329	CAACTGATATAGTTGGAAGTGAGGAGTCCATTTCCC	IU6987	Δ*rodZ* ::P_c-*aad9*
P1385	ACAACACCTGCAATGGCCACACGTTGCTTT		

TT329	CAACTGATATAGTTGGAAGTGAGGAGTCCATT TCCC	E655	Δ*rodZ* ::P_c-*erm*
P1385	ACAACACCTGCAATGGCCACACGTTGCTTT		
TT452	GGAGGGTTGGCTGTGGGTGGCTACAAGAAC	IU7397	Δ*pbp2b* <>*aad9*
TT352	TGAAGGACTGGAAAGACCACTGCACCTTCT		
P104	AATGAGACGTGTTGCCATTGCAGG	IU1751	Δ*mreCD* <>*aad9*
P107	TGTCGCTTTCTCAGCAGCAAGACT		
P222	CGTTCGTGTGGCGCTGCTTCAAATTGTT	E193	Δ*pbp1b*::P_c*erm*
P522	AACGGCAACCACCAAAGGAGAAACCAAGGA		
P222	CGTTCGTGTGGCGCTGCTTCAAATTGTT	IU13680	Δ*pbp1b* ::P_c-*aad9*
P522	AACGGCAACCACCAAAGGAGAAACCAAGGA		
P222	CGTTCGTGTGGCGCTGCTTCAAATTGTT	K180	Δ*pbp1b*::P_c-[*kan-rpsL*$^+$]
P522	AACGGCAACCACCAAAGGAGAAACCAAGGA		

Primers used to confirm deletion junction in Δ*gpsB sup3*		
Primer name	Sequence (5’ to 3’)	
P1, P1510	ACCATTGCCACTGCGAACATGGTCTACAGC	
P2, TT1345	GCACCAAGGTTCCCAGCATCAAGGTCAGC	
P3, TT1346	TGGCAAACGTGACTCAGTCAATGTCGCTGC	
P4, TT1347	CTAGTCTTTACAAGTATCTAACCGAGGAGGTTGAAA ACGATCAG	
Primers used for detection of *spd_1033 to spd_1035* in Δ*gpsB* suppressor strains		
Primer name	Sequence (5’ to 3’)	Product
P1481	TTATGTAGGAGGAACCGAGGGCGGAGGAAT	*3’ spd_1036 to 5’ spd_1032*
P1482	AGACGAGTGTTCCATAGCCGACTCCTTCATTT	
Primers used for qRT-PCR		
Primer name	Sequence (5’ to 3’)	Gene name
JQ342	GGAGCTACTGTTAAGCGTTATG	*murZ*
JQ343	CGCCTTAAGGTGTAAGTCAATC	
KK489	AAAGGTCGTGGTGGTAAGGGAATG	*gyrA*
KK490	GCATCTTGATCCAGGCGCATTACT	
Primers used for MurA(*Spn*) plasmid construction		
Primer name	Sequence (5’ to 3’)	
AJP431	CGAAGCATAAACATCTGTCAATTCTTCGCTAATTTCT TTTTTATT	
AJP432	AATAAAAAAGAAATTAGCGAAGAATTGACAGATGTTT ATGCTTCG	

AJP435	ACGACCGAAAACCTGTATTTTCAGGGCATGGATAAA ATTGTGGTTCAAGGTGGCGAT	
AJP436	GATCTCAGTGGTGGTGGTGGTGGTTTATTCATCTTC ATCATTTGCCTCAATCCG	

[a]FLAG-tag fusions ((C)-L-FLAG^3) were made to the carboxyl-ends (C) of reading frames. The amino acid sequence of the FLAG epitope is DYKDDDDK (Ramos-Montanez *et al.*, 2008, Wayne *et al.*, 2010). The FLAG-tag used in this study contained a linker sequence (L; GSAGSAAGSG) followed by three tandem copies of the FLAG epitope (FLAG^3).

[b]Antibiotic resistance markers: Erm^R, erythromycin; Kan^R, kanamycin; Spc^R, spectinomycin; Str^R, streptomycin; Cm^R, chloramphenicol; Tet^R, tetracycline.

[c]Genomic DNA of indicated *S. pneumoniae* strains was used as templates for PCR reactions, except for P_c-[*kan*-*rpsL*$^+$] and P_c-*erm* cassettes.

[d]P_c-*erm* and P_c-[*kan*-*rpsL*$^+$] cassettes are described in (Tsui *et al.*, 2011).

[e]Genotype of IU11119 is *ezrA*-L_0-*sfgfp*-P_c-*cat*, as described in (Perez *et al.*, 2019).

[f]P_c-[*sacB*-*kan*-*rpsL*$^+$] is described in (Li *et al.*, 2014).

Table S2. Blastn results using *phtD* as query sequence against *S. pneumoniae* D39 database

spd #	% identity	alignment length	Mis matches	gap opens	q. start	q. end	s. start	s. end	evalue	bit score
spd_0889, phtD	100	2562	0	0	899901	902462	899901	902462	0	4732
spd_1037, phtB	100	1324	0	0	900760	902083	1063403	1062080	0	2446
spd_1037, phtB	78.7	700	125	14	899901	900585	1064304	1063614	2.6E-125	446
spd_1038, phtA	77.3	699	137	12	899901	900585	1066912	1066222	1.2E-108	390
spd_1038, phtA	89.1	258	28	0	900760	901017	1066011	1065754	4.6E-88	322

Table S3. Blastn results using *spd_0966* as query sequence against *S. pneumoniae* D39 database[a]

spd #	% identity	alignment length	Mis matches	gap opens	q. start	q. end	s. start	s. end	evalue	bit score
spd_0966	100	1492	0	0	978724	980215	978724	980215	0	2691
spd_0758	93	1518	84	4	978724	980215	768573	770089	0	2244
spd_1641	91	1479	109	3	978724	980185	1656439	1654961	0	2102
spd_0986	91	1477	119	3	978724	980183	998197	999673	0	2053
spd_1666	88	1489	127	5	978724	980187	1679982	1678514	0	1929
spd_0048	86	1486	132	4	978724	980183	40190	41625	0	1793
spd_1708	86	831	98	6	979359	980188	1709287	1708478	0	951
spd_1708	89	595	42	1	978724	979294	1709869	1709275	0	793
spd_0034	87	404	51	2	979781	980183	29738	30140	1.43E-136	483
spd_1681	98	284	5	1	979905	980187	1697825	1697542	1.43E-136	482
spd_1681	92	319	24	1	978724	979041	1698142	1697824	1.63E-129	460
spd_0022	84	386	61	1	979463	979848	21517	21901	6.08E-116	414

[a]The reading frame of *spd_0966* was assigned to be from 978757 to 980059 on the complementary strand of D39 genome. The blastn analysis was performed with sequence 978724 to 980215, corresponding to 156 bp upstream to 33 bp downstream of *spd_0966*.

Table S4. Blastn results using *spd_1690* to *spd_1703* (*rRNA*) as query sequence against *S. pneumoniae* D39 database[a]

spd #	% identity	alignment length	Mis matches	gap opens	q. start	q. end	s. start	s. end	evalue	bit score
(*rRNA-2*) *spd_1690* to *spd_1703*	100	5998	0	0	1699037	1705034	1699037	1705034	0	10817
(*rRNA-3*) *spd_1804* to *spd_1817*	99.9	5998	5	0	1699037	1705034	1796588	1802585	0	10795
(*rRNA-4*) *spd_1889* to *spd_1894*	100.0	5289	1	0	1699746	1705034	1859222	1864510	0	9534
(*rRNA-1*) *spd_0015* to *spd_0019*	99.9	5216	6	0	1699819	1705034	20042	14827	0	9380

[a]The blastn analysis was performed with sequence 1699037 to 1705034, corresponding to *spd_1690* to *spd_1703*. The genes from *spd_1690* to *spd_1703* are tRNA-pro (*spd_1690*); tRNA-arg (*spd_1691*); tRNA-leu (*spd_1692*); tRNA-gly (*spd_1693*); tRNA-thr (*spd_1694*); tRNA-leu (*spd_1695*); tRNA-lys (*spd_1696*); tRNA-asp (*spd_1697*); tRNA-val (*spd_1698*); *rrfB* (5S rRNA, *spd_1699*); *rrlB* (23S rRNA, *spd_1700*), tRNA-ala (*spd_1701*); *rrsB* (16S rRNA, *spd_1702*) and tRNA-glu (*spd_1703*). Alignment of *spd_1804* to *spd_1817* covers all sequence from *spd_1690* to *spd_1703.* Alignment of *spd_1889* to *spd_1894* covers tRNA-val to tRNA-glu, and alignment of *spd_0015* to *spd_0019* covers from 5S rRNA to tRNA-glu.

Table S5. Suppression of Δ*gpsB* lethality in *S. pneumoniae* D39 or R6 strains[a]

A. Transformation with Δ*gpsB*<>*aad9* amplicon

Genetic background	Recipient strains	Number of colonies 22 h after transformation
D39 Δ*cps rpsL1*	1. WT (IU1824)	0
	2. WT + Zn[b,c]	0
	3. *gpsB*$^+$//P_{Zn}-*gpsB*$^+$(IU15877)	0
	4. *gpsB*$^+$//P_{Zn}-*gpsB*$^+$ + Zn[c]	>500
	5. *murZ*(D280Y) (IU13438)	>500 small
	6. *murZ*(I265V, R6 allele) (IU14210)	>500 small
	7. *murZ*(E259A) (IU17627)	>500 small
	8. *murZ*(E190A E192A) (IU17619)	0
	9. *murZ*(E192A) (IU17622)	0
	10. *murZ*(E195A) (IU17623)	0
	11. *murA*(D281Y) (IU15143)	0
	12. *murA*(E282Y) (IU15145)	0
	13. *murZ*$^+$//P_{Zn}-*murZ*$^+$(IU13393)	0
	14. *murZ*$^+$//P_{Zn}-*murZ*$^+$ + Zn[b]	>500 small
	15. *murZ*$^+$//P_{Zn}-*murZ*(C116S)(IU15943)	0
	16. *murZ*$^+$//P_{Zn}-*murZ*(C116S) + Zn[b]	0
	17. *murA*$^+$//P_{Zn}-*murA*$^+$(IU13395)	0
	18. *murA*$^+$//P_{Zn}-*murA*$^+$ + Zn[c]	>500
	19. *murA*$^+$//P_{Zn}-*murA*(C120S)(IU15954)	0
	20. *murA*$^+$//P_{Zn}-*murA*(C120S) + Zn[c]	0
	21. Δ*murZ* (IU13536)	0
	22. Δ*murA* (IU13538)	0
	23. Δ*clpC*::P_c-[*kan-rpsL*$^+$] (IU12462)	0
	24. Δ*clpP*::P_c-[*kan-rpsL*$^+$] (IU17138)	0
	25. Δ*clpE*::P_c-[*kan-rpsL*$^+$] (IU17134)	0
	26. Δ*clpL*::P_c-[*kan-rpsL*$^+$] (IU17136)	0
	27. *murZ*-L-$FLAG^3$ (IU13502)	0
	28. *murA*-L-$FLAG^3$ (IU14028)	0
	29. *murZ*(D280Y)-L-$FLAG^3$ (IU13600)	>500 small
	30. Δ*khpA* (IU9036)	>500, small
	31. Δ*khpB* (IU10592)	>500, small
	32. *khpB*(T89A)(IU12744)	0
	33. *khpB*(T89D)(IU13881)	0
	34. *khpB*(T89E)(IU13883)	0
	35. Δ*khpA* Δ*murZ* (IU13542)	0
	36. Δ*khpA* Δ*murA* (IU13546)	>500, very small
D39 Δ*cps*	37. WT (IU1945)	0
	38. *murZ*$^+$//P_{Zn}-*murZ*$^+$(IU11077)	0
	39. *murZ*$^+$//P_{Zn}-*murZ*$^+$ + Zn[b]	>500 small
	40. *murA*$^+$//P_{Zn}-*murA*$^+$(IU11079)	0
	41. *murA*$^+$//P_{Zn}-*murA*$^+$ + Zn[c]	>500
R6[d]	42. WT, EL59 (*murZ*(I265V))	>500 small
	43. Δ*murZ*::P_c-*erm* (IU16265)	0
	44. Δ*murA*::P_c-*erm* (IU16267)	>500 small

B. Transformation with Δ*murZ*::P_c-*erm* amplicon

Genetic background	Recipient strains	Number of colonies 22 h after transformation
D39 Δ*cps rpsL1*	1. WT (IU1824)	>500
	2. Δ*murA* (IU13538)	0
	3. *murA*(C120S)(IU15949)	0
	4. *murA*-L-FLAG^3 (IU14028)	>500
	5. Δ*khpA* Δ*gpsB*<>*aad9* (IU12883, IU16196)	0
R6[d]	6. WT, EL59 (*murZ*(I265V))	>500
	7. Δ*gpsB*<>*aad9* (IU8224)	0

C. Transformation with Δ*murA*::P_c-*erm* amplicon

Genetic background	Recipient strains	Number of colonies 22 h after transformation
D39 Δ*cps rpsL1*	1. WT (IU1824)	>500
	2. Δ*murZ* (IU13536)	0
	3. *murZ*(C116S)(IU15939)	0
	4. *murZ*(D280Y)(IU13438)	>500
	5. *murZ*-L-FLAG^3 (IU13502)	>500
	6. Δ*khpA* Δ*gpsB*<>*aad9* (IU12883, IU16196)	~ 25 to 50, small[e]
R6[d]	7. WT, EL59, (*murZ*(I265V))	>500
	8. Δ*gpsB*<>*aad9* (IU8224)	>500 small

[a]Transformations and visualization of colonies were performed as described in Experimental procedures and footnote to Table 3. Colony sizes are relative to colonies transformed with positive control Δ*pbp1b* amplicons containing the same antibiotic selection marker.

[b]0.2 mM $ZnCl_2$ + 0.02 mM $MnSO_4$ were added to transformation mixes and in subsequent steps to induce expression of *murZ* under control of the P_{Zn} zinc-inducible promoter in the ectopic *bgaA* site.

[c]0.4 mM $ZnCl_2$ + 0.04 mM $MnSO_4$ were added to transformation mixes and in subsequent steps to induce ectopic expression of *gpsB* or *murA.*

[d]R6 strain contains a spontaneous *murZ*(I265V) mutation compared to D39 strain (Lanie *et al.*, 2007).

[e]Both IU12883 and IU16196, two independent Δ*khpA* Δ*gpsB*<>*aad9* isolates obtained from independent transformations, have very low transformation efficiency. Transformation of these

strains with a positive control Δ*pbp1b* amplicon also yielded the same low number (25 to 50) of transformants.

Table S6. Overexpression strains in D39 Δ*cps* backgrounds that did not suppress Δ*gpsB* essentiality[a]

In IU1824 (D39 Δ*cps rpsL1*) background

Recipient strain genotype[a]	Strain[b]
P_{Zn}-*stkP*$^{+}$	IU14974
P_{Zn}-*pbp1a*$^{+}$	IU14312
P_{Zn}-*pbp2a*$^{+}$	IU14318
P_{Zn}-*mreC*$^{+}$	IU10220
P_{Zn}-*rodZ*$^{+}$	IU9613
CEP- P_{Zn}-*ezrA*$^{+}$ Δ*bgaA*:: P_{Zn}-*ezrA*$^{+}$	IU13327
P_{Zn}-*divIVA*$^{+}$ (R6 annotation)	IU13794
P_{Zn}-*ftsA*$^{+}$	IU12310
P_{Zn}-*ftsZ*$^{+}$	IU12286
P_{Zn}-*ftsW*$^{+}$	IU12192

In IU1945 (D39 Δ*cps*) background

Recipient strain genotype[a]	Strain
P_{Zn}-*pbp2x*$^{+}$	IU10063
P_{Zn}-*pbp2b*$^{+}$	IU9990
P_{Zn}-*pbp1b*$^{+}$	IU9992
P_{Zn}-*mltG*$^{+}$	IU8872
P_{Zn}-*rodA*$^{+}$	IU10922
P_{Zn}-*mraY*$^{+}$	IU11083
P_{Zn}-*uppS*$^{+}$	IU10094
P_{Zn}-*murG*$^{+}$	IU11049
P_{Zn}-*cozE*$^{+}$	IU12678
P_{Zn}-*mapZ*$^{+}$	IU11628
P_{Zn}-*sepF*$^{+}$	IU9805

[a]Recipient strains and Δ*gpsB*<>*aad9* were obtained as described in Table S1. Transformations with 1 mL of transformation mixture were performed as described in *Experimental procedures*. Final concentrations of 0.4 mM $ZnCl_2$ + 0.04 mM $MnSO_4$ were present in the transformation mixes and in subsequent steps to induce gene expression mediated by the P_{Zn} zinc-inducible promoter in the ectopic *bgaA* site for all strains except for IU10063 (P_{Zn}-*pbp2x*), which was transformed in the presence of 0.2 mM $ZnCl_2$ + 0.02 mM $MnSO_4$. IU14974 (P_{Zn}-*stkP*) was tested with 0.1, 0.2 and 0.4 mM $ZnCl_2$ and 1/10 concentration of $MnSO_4$. No colonies were obtained with these overexpression strains when transformed with a Δ*gpsB* amplicon in the

presence of $ZnCl_2$, while more than 500 colonies were obtained with strains that overexpressed *gpsB*, *murZ*, or *murA* (see Table 1).

[b]The Zn-induced expression of the ectopic genes in these strains have been shown to complement the respective deletions in the native site, except for IU9992 (P_{Zn}-*pbp1b*$^+$) because of the lack of overt phenotypes caused by Δ*pbp1b*.

SUPPLEMENTAL REFERENCES

Cleverley, R.M., Rutter, Z.J., Rismondo, J., Corona, F., Tsui, H.T., Alatawi, F.A., Daniel, R.A., Halbedel, S., Massidda, O., Winkler, M.E., and Lewis, R.J. (2019) The cell cycle regulator GpsB functions as cytosolic adaptor for multiple cell wall enzymes. *Nature communications* **10**: 261.

Hoskins, J., Alborn, W.E., Jr., Arnold, J., Blaszczak, L.C., Burgett, S., DeHoff, B.S., Estrem, S.T., Fritz, L., Fu, D.J., Fuller, W., Geringer, C., Gilmour, R., Glass, J.S., Khoja, H., Kraft, A.R., Lagace, R.E., LeBlanc, D.J., Lee, L.N., Lefkowitz, E.J., Lu, J., Matsushima, P., McAhren, S.M., McHenney, M., McLeaster, K., Mundy, C.W., Nicas, T.I., Norris, F.H., O'Gara, M., Peery, R.B., Robertson, G.T., Rockey, P., Sun, P.M., Winkler, M.E., Yang, Y., Young-Bellido, M., Zhao, G., Zook, C.A., Baltz, R.H., Jaskunas, S.R., Rosteck, P.R., Jr., Skatrud, P.L., and Glass, J.I. (2001) Genome of the bacterium Streptococcus pneumoniae strain R6. *Journal of bacteriology* **183**: 5709-5717.

Land, A.D., Tsui, H.C., Kocaoglu, O., Vella, S.A., Shaw, S.L., Keen, S.K., Sham, L.T., Carlson, E.E., and Winkler, M.E. (2013) Requirement of essential Pbp2x and GpsB for septal ring closure in Streptococcus pneumoniae D39. *Molecular microbiology* **90**: 939-955.

Land, A.D., and Winkler, M.E. (2011) The requirement for pneumococcal MreC and MreD is relieved by inactivation of the gene encoding PBP1a. *Journal of bacteriology* **193**: 4166-4179.

Lanie, J.A., Ng, W.L., Kazmierczak, K.M., Andrzejewski, T.M., Davidsen, T.M., Wayne, K.J., Tettelin, H., Glass, J.I., and Winkler, M.E. (2007) Genome sequence of Avery's virulent serotype 2 strain D39 of Streptococcus pneumoniae and comparison with that of unencapsulated laboratory strain R6. *Journal of bacteriology* **189**: 38-51.

Li, Y., Thompson, C.M., and Lipsitch, M. (2014) A modified Janus cassette (Sweet Janus) to improve allelic replacement efficiency by high-stringency negative selection in Streptococcus pneumoniae. *PloS one* **9**: e100510.

Mura, A., Fadda, D., Perez, A.J., Danforth, M.L., Musu, D., Rico, A.I., Krupka, M., Denapaite, D., Tsui, H.T., Winkler, M.E., Branny, P., Vicente, M., Margolin, W., and

Massidda, O. (2017) Roles of the Essential Protein FtsA in Cell Growth and Division in Streptococcus pneumoniae. *Journal of bacteriology* **199**.

Perez, A.J., Cesbron, Y., Shaw, S.L., Bazan Villicana, J., Tsui, H.T., Boersma, M.J., Ye, Z.A., Tovpeko, Y., Dekker, C., Holden, S., and Winkler, M.E. (2019) Movement dynamics of divisome proteins and PBP2x:FtsW in cells of Streptococcus pneumoniae. *Proceedings of the National Academy of Sciences of the United States of America* **116**: 3211-3220.

Ramos-Montanez, S., Tsui, H.C., Wayne, K.J., Morris, J.L., Peters, L.E., Zhang, F., Kazmierczak, K.M., Sham, L.T., and Winkler, M.E. (2008) Polymorphism and regulation of the spxB (pyruvate oxidase) virulence factor gene by a CBS-HotDog domain protein (SpxR) in serotype 2 Streptococcus pneumoniae. *Molecular microbiology* **67**: 729-746.

Rued, B.E., Zheng, J.J., Mura, A., Tsui, H.T., Boersma, M.J., Mazny, J.L., Corona, F., Perez, A.J., Fadda, D., Doubravova, L., Buriankova, K., Branny, P., Massidda, O., and Winkler, M.E. (2017) Suppression and synthetic-lethal genetic relationships of DeltagpsB mutations indicate that GpsB mediates protein phosphorylation and penicillin-binding protein interactions in Streptococcus pneumoniae D39. *Molecular microbiology* **103**: 931-957.

Slager, J., Aprianto, R., and Veening, J.W. (2018) Deep genome annotation of the opportunistic human pathogen Streptococcus pneumoniae D39. *Nucleic acids research* **46**: 9971-9989.

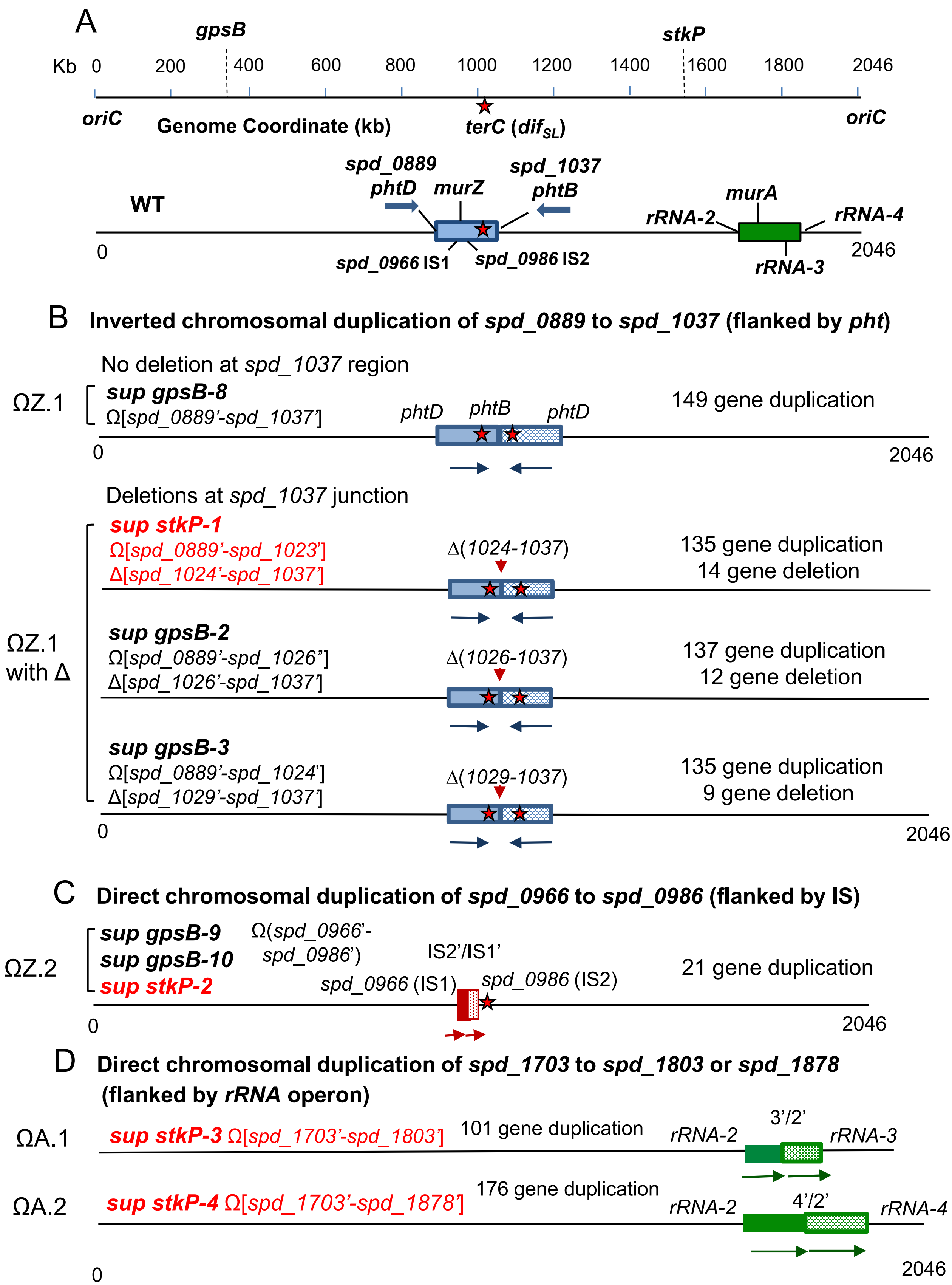
Tsui, H.C., Keen, S.K., Sham, L.T., Wayne, K.J., and Winkler, M.E. (2011) Dynamic distribution of the SecA and SecY translocase subunits and septal localization of the HtrA surface chaperone/protease during Streptococcus pneumoniae D39 cell division. *mBio* **2**: e00202-00211.

Tsui, H.C., Zheng, J.J., Magallon, A.N., Ryan, J.D., Yunck, R., Rued, B.E., Bernhardt, T.G., and Winkler, M.E. (2016) Suppression of a deletion mutation in the gene encoding essential PBP2b reveals a new lytic transglycosylase involved in peripheral peptidoglycan synthesis in Streptococcus pneumoniae D39. *Molecular microbiology* **100**: 1039-1065.

Tsui, H.T., Boersma, M.J., Vella, S.A., Kocaoglu, O., Kuru, E., Peceny, J.K., Carlson, E.E., VanNieuwenhze, M.S., Brun, Y.V., Shaw, S.L., and Winkler, M.E. (2014) Pbp2x localizes separately from Pbp2b and other peptidoglycan synthesis proteins during later stages of cell division of Streptococcus pneumoniae D39. *Molecular microbiology* **94**: 21-40.

Wayne, K.J., Sham, L.T., Tsui, H.C., Gutu, A.D., Barendt, S.M., Keen, S.K., and Winkler, M.E. (2010) Localization and cellular amounts of the WalRKJ (VicRKX) two-component regulatory system proteins in serotype 2 Streptococcus pneumoniae. *Journal of bacteriology* **192**: 4388-4394.

Zheng, J.J., Perez, A.J., Tsui, H.T., Massidda, O., and Winkler, M.E. (2017) Absence of the KhpA and KhpB (JAG/EloR) RNA-binding proteins suppresses the requirement for PBP2b by overproduction of FtsA in Streptococcus pneumoniae D39. *Molecular microbiology* **106**: 793-814.



A
gpsB
stkP
Kb 0 200 400 600 800 1000 1200 1400 1600 1800 2046
oriC
Genome Coordinate (kb)
terC (difSL)
oriC
WT
spd_0889
phtD
murZ
spd_1037
phtB
murA
rRNA-2
rRNA-4
rRNA-3
spd_0966 IS1
spd_0986 IS2
0
2046
B Inverted chromosomal duplication of spd_0889 to spd_1037 (flanked by pht)
No deletion at spd_1037 region
ΩZ.1
sup gpsB-8
Ω[spd_0889'-spd_1037']
phtD phtB phtD
149 gene duplication
0
2046
Deletions at spd_1037 junction
sup stkP-1
Ω[spd_0889'-spd_1023']
Δ[spd_1024'-spd_1037']
Δ(1024-1037)
135 gene duplication
14 gene deletion
ΩZ.1
with Δ
sup gpsB-2
Ω[spd_0889'-spd_1026"]
Δ[spd_1026'-spd_1037']
Δ(1026-1037)
137 gene duplication
12 gene deletion
sup gpsB-3
Ω[spd_0889'-spd_1024']
Δ[spd_1029'-spd_1037']
Δ(1029-1037)
135 gene duplication
9 gene deletion
0
2046
C Direct chromosomal duplication of spd_0966 to spd_0986 (flanked by IS)
ΩZ.2
sup gpsB-9
sup gpsB-10
sup stkP-2
Ω(spd_0966'-spd_0986')
IS2'/IS1'
spd_0966 (IS1)
spd_0986 (IS2)
21 gene duplication
0
2046
D Direct chromosomal duplication of spd_1703 to spd_1803 or spd_1878 (flanked by rRNA operon)
ΩA.1
sup stkP-3 Ω[spd_1703'-spd_1803']
101 gene duplication
rRNA-2
3'/2'
rRNA-3
ΩA.2
sup stkP-4 Ω[spd_1703'-spd_1878']
176 gene duplication
rRNA-2
4'/2'
rRNA-4
0
2046

Fig. S1

Figure S1. Duplication and duplication/deletion regions in Δ*gpsB* and Δ*stkP* suppressor strains. A. Chromosome coordinates representation in a linear scheme in kb. *terC* (dif_{SL} sequence, red star) is located at 83 to 53 bp upstream of *xerS* (*spd_1023*) as described by (Le Bourgeois *et al.*, 2007). (B) The duplication patterns are grouped into ΩZ.1 or ΩZ.2 (duplication of *murZ* region) or ΩA.1 or ΩA.2 (duplication of *murA* region). ΩZ.1 duplications are flanked by *phtD* and *phtB*, while ΩZ.2 duplications are bordered by degenerate IS elements *spd_0966* and *spd_0986.* ΩA.1 or ΩA.2 are bordered by tRNA/rRNA gene clusters. In ΩZ.1, represented by *sup gpsB-8,* large inverted duplications (shaded region, >135 genes) are flanked by *phtB* (*spd_1037*), and *phtD* (*spd_0889*), two oppositely transcribed genes with identical 1324 nt sequence at the 3' end. No flanking deletion is found in this strain. In group ΩZ.1 with Δ, duplication of the *phtD* to *phtB* region is accompanied by gene deletions in the *spd_1037 region.* In *sup stkP-1,* the regions from ≈50 bp upstream of *spd_1024* to *spd_1037* at both duplications are deleted, leading to the resulting genotype of Ω[*spd_0889*'-*spd_1023*'] Δ[*spd_1024*-*spd_1037*']. In *sup gpsB-2*, the deletion junction is within *spd_1026,* leading to the resulting genotype of Ω[*spd_0889*'-*spd_1026*'] Δ[*spd_1026*'-*spd_1037*']. In *sup gpsB-3*, the deletion occurs between *spd_1029* and *spd_1037* in one segment and *spd_1037* to *spd_1024* in the other segment, leading to Ω[*spd_0889*'-*spd_1024*'] Δ[*spd_1029*'-*spd_1037*'] (see Figure 3 for detail). Two copies of *terC* (red star) are present in ΩZ.1 and 'ΩZ.1 with Δ' classes. (C) ΩZ.2 suppressors contain 21-gene duplication flanked by *spd_0966* to *spd_0986,* two degenerate transposase IS1167 genes. *Sup gpsB-9* and *sup stkP-2* contain tandem duplications, while *sup gpsB-10* contains a higher level amplification. (D) In ΩA.1 and ΩA.2 suppressors, the duplications are flanked by tRNA/rRNA gene clusters, *rRNA-2, rRNA-3*, or *rRNA-4.* Duplicated fragments are represented by shaded segments. Arrows represent the sequence directions.

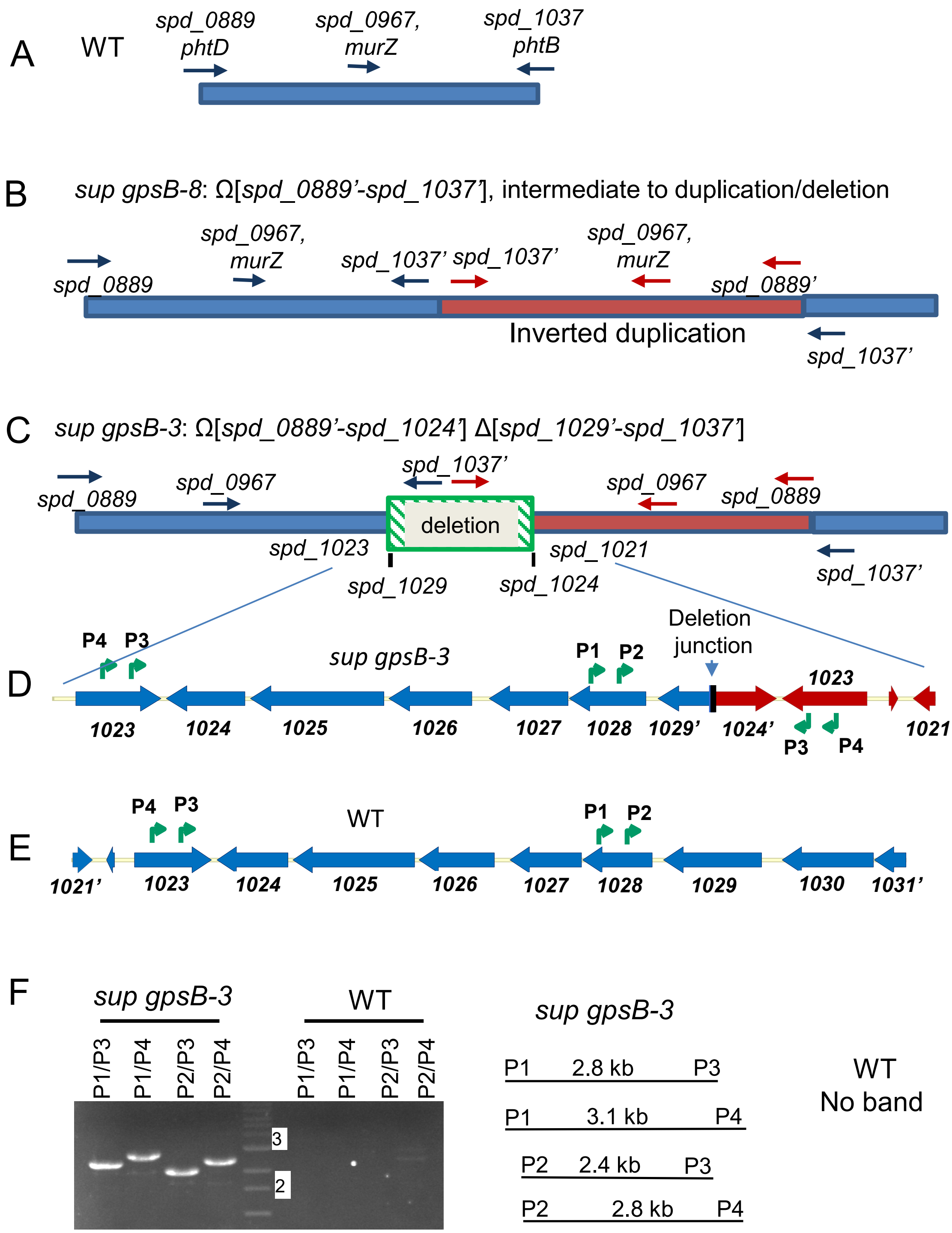

A
WT
spd_0889
phtD
spd_0967,
murZ
spd_1037
phtB
B
sup gpsB-8: Ω[spd_0889'-spd_1037'], intermediate to duplication/deletion
spd_0889
spd_0967,
murZ
spd_1037'
spd_1037'
spd_0967,
murZ
spd_0889'
Inverted duplication
spd_1037'
C
sup gpsB-3: Ω[spd_0889'-spd_1024'] Δ[spd_1029'-spd_1037']
spd_0889
spd_0967
spd_1037'
deletion
spd_0967
spd_0889
spd_1023
spd_1029
spd_1021
spd_1024
spd_1037'
D
P4
P3
sup gpsB-3
P1
P2
Deletion
junction
1023
1023
1024
1025
1026
1027
1028
1029'
1024'
P3
P4
1021'
E
P4
P3
WT
P1
P2
1021'
1023
1024
1025
1026
1027
1028
1029
1030
1031'
F
sup gpsB-3
WT
P1/P3
P1/P4
P2/P3
P2/P4
P1/P3
P1/P4
P2/P3
P2/P4
3
2
sup gpsB-3
P1 2.8 kb P3
P1 3.1 kb P4
P2 2.4 kb P3
P2 2.8 kb P4
WT
No band

Fig. S2

Figure S2. Model for formation of the chromosomal *sup gpsB-3* duplication/deletion (ΩZ.1 with Δ) that suppresses Δ*gpsB* by large inverted duplication followed by small deletion of the duplication junction. (A) Arrangement of *spd_0889* to *spd_1037* in a WT strain. (B) Chromosomal arrangement of *sup gpsB-8* which contains an inverted duplication (orange segment) from *spd_0889* (*phtD*) to *spd_1037* (*phtB*). (C) In *sup gpsB-3*, inverted duplication was followed by the deletion between *spd_1029* and *spd_1037* in one segment and *spd_1037* to *spd_1024* in the other segment, leading to Ω[*spd_0889*'-*spd_1024'*] ∆[*spd_1029*'-*spd_1037'*]. (D) Enlargement of the deletion junction in *sup gpsB-3* between *spd_1023* of one segment and *spd_1021* of the other segment. Primers P1, P2, P3, and P4 are used to confirm the rearrangement. (E) Location of primers P1 to P4 in the WT strain. (F) PCR analysis to confirm the chromosomal arrangement shown in C. Bands of expected sizes were obtained with four sets of primers using *sup gpsB-3* strain as DNA template, while no bands was obtained using DNA obtained from the WT parent (IU1945).

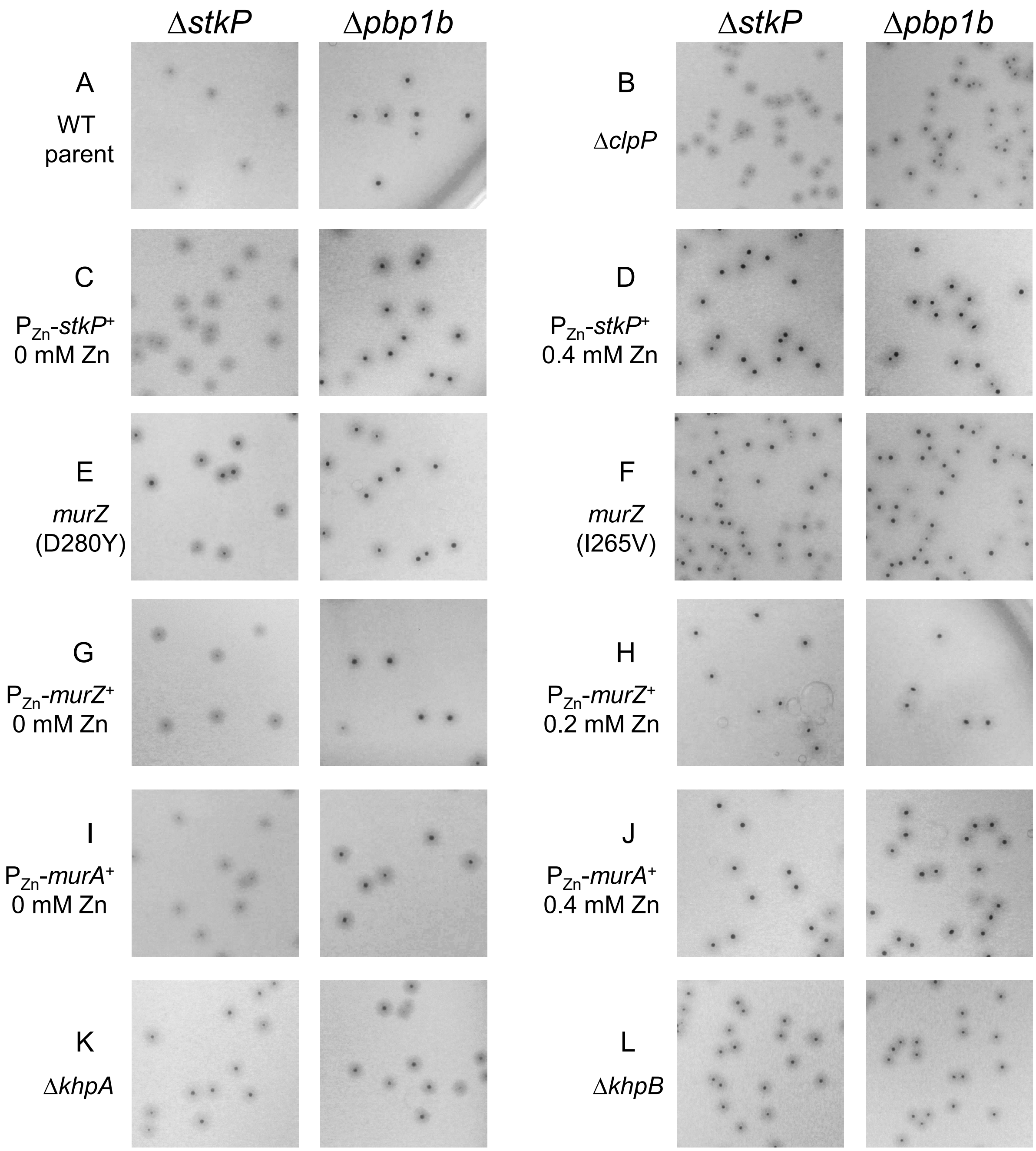


Figure S3. *murZ*(D280Y), *murZ*(I265V)*, ΔkhpA/B* mutations, and overexpression of *murZ* or *murA* suppress the faint colony phenotype of Δ*stkP* on TSAII-BA plates. (A) Parent D39 Δ*cps rpsL1* strain (IU1824), (B) Δ*clpP* (IU17138), (C and D) *stkP*$^+$//P_{Zn}-*stkP*$^+$ (IU14974), (E) *murZ*(D280Y (IU13438), (F) *murZ*(I265V (IU14210), (G and H) *murZ*$^+$//P_{Zn}-*murZ*$^+$ (IU13393), (I and J) *murA*$^+$//P_{Zn}-*murA*$^+$ (IU13395), (K) Δ*khpA* (IU9036)*,* and (L) Δ*khpB* (IU10592) were transformed with a Δ*stkP*::P_c-*erm* or a positive control amplicon Δ*pbp1b*::P_c-*erm* as described in *Experimental procedures.* Images of colonies on the TSAII-BA transformation plates were taken with a light source under the plates after 20h incubation in a 37°C 5% CO_2 incubator.

Fig. S3

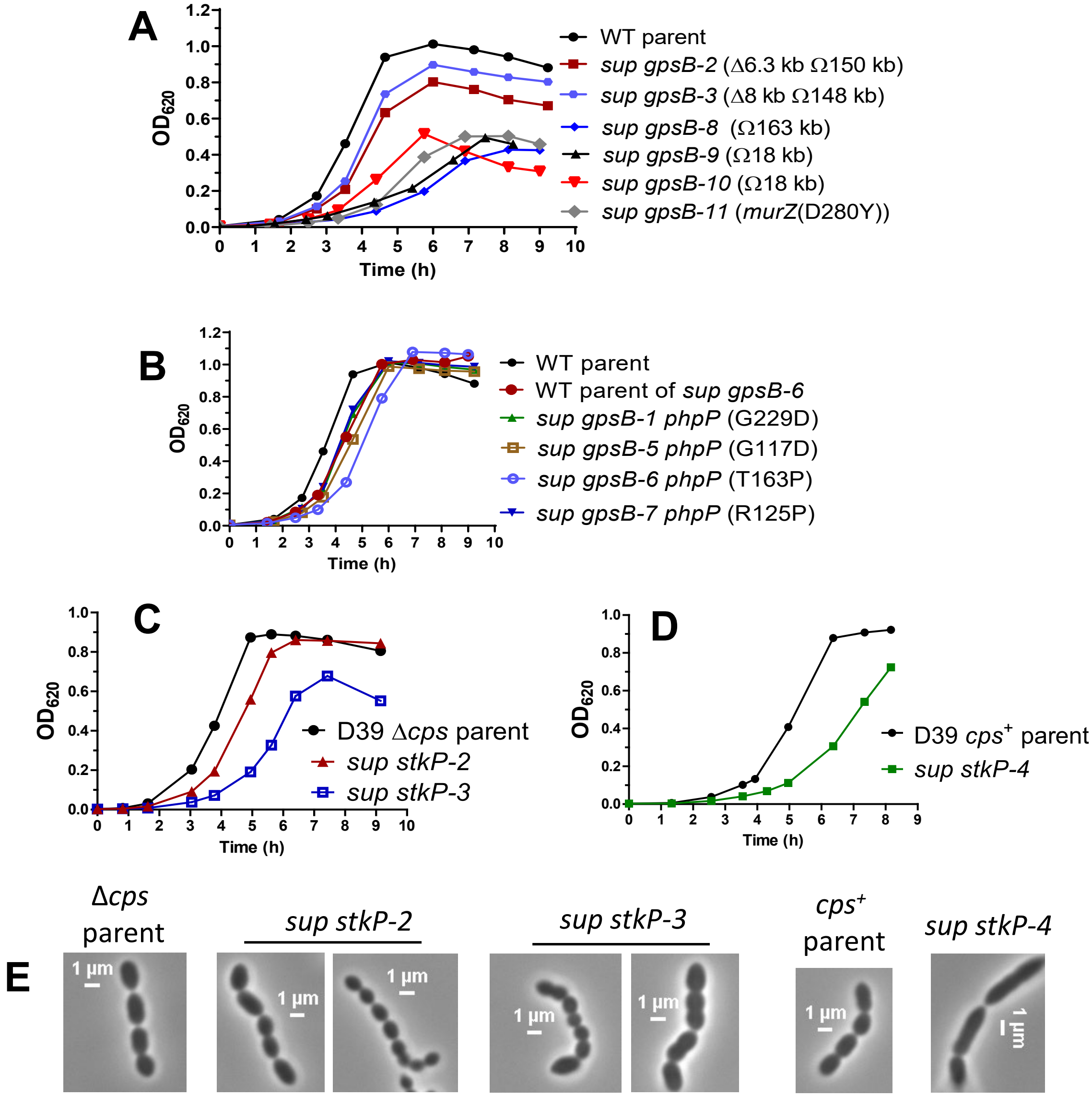
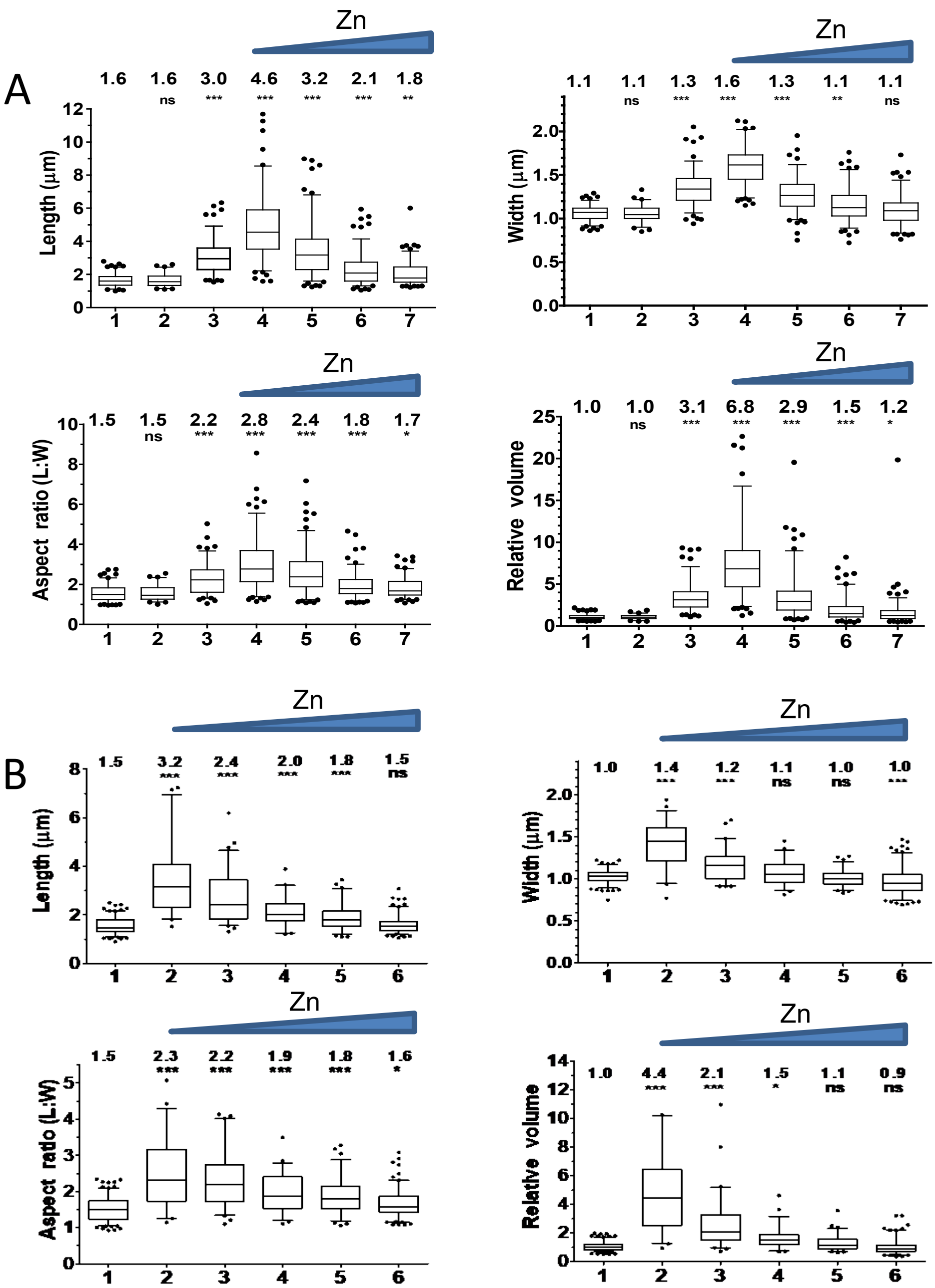


Figure S4. Growth profiles of Δ*gpsB* and Δ*stkP* suppressor strains. (A) and (B), Δ*gpsB* suppressor strains with mutations in *phpP* exhibit better growth profile compared to Δ*gpsB* suppressor strains with large chromosomal deletion. (A) Growth curves of D39 Δ*cps* WT parent (IU1945) and Δ*gpsB* suppressor strains with large chromosomal duplication and deletion (*sup gpsB-2 and -3*), strains with large duplications (*sup gpsB -8* to *-10)* and *sup gpsB -11* which contains a *murZ*(D280Y) mutation. (B) Growth curves of WT parents D39 Δ*cps* (IU1945), and D39 Δ*cps rpsL1* (IU1824), and Δ*gpsB* suppressor strains containing mutations in *phpP*. (C) Growth curves of D39 Δ*cps* WT parent (IU1945) and *sup stkP-2* and *-3* strains. (D) Growth curves of D39 WT parent (IU1690) and *sup stkP-4* strain. (E) Phase contrast images of parents, and *sup stkP-2 to -4* strains. Growth curve and micrographs of *sup stkP-1* are shown in Fig. S20. Detailed summaries of genotypes, growth rates and growth yields of various Δ*gpsB* and Δ*stkP sup* strains are listed in Table 1 and Table 3, respectively.

Fig. S4



	Genotype	Zn (mM)
1	WT	0
2		0.4
3	Δ*gpsB* *murZ* (D280Y)	0
4	Δ*gpsB* P_{Zn}-*murZ*$^{+}$	0
5		0.1
6		0.2
7		0.3

	Genotype	Zn (mM)
1	WT	0
2	Δ*gpsB* P_{Zn}-*murA*$^{+}$	0
3		0.2
4		0.3
5		0.4
6		0.5

Figure S5. Box-and-whisker plots of cell dimensions of *murZ*(D280Y) and overexpression strains of *murZ* and *murA* in a Δ*gpsB* background. (A) Box-and-whisker plots (whiskers, 5 and 95 percentile) of cell lengths, widths, aspect ratios (cell length to width) and relative cell volumes of strains grown without or with indicated (Zn^{2+}/(1/10)Mn^{2+}) shown in Fig. 4. For both (A) and (B), P values were obtained by one-way ANOVA analysis (GraphPad Prism, Kruskal-Wallis test). *, **, *** and ns denote p<0.05, p<0.01, p<0.001, not significant, respectively when compared to WT.

Fig. S5

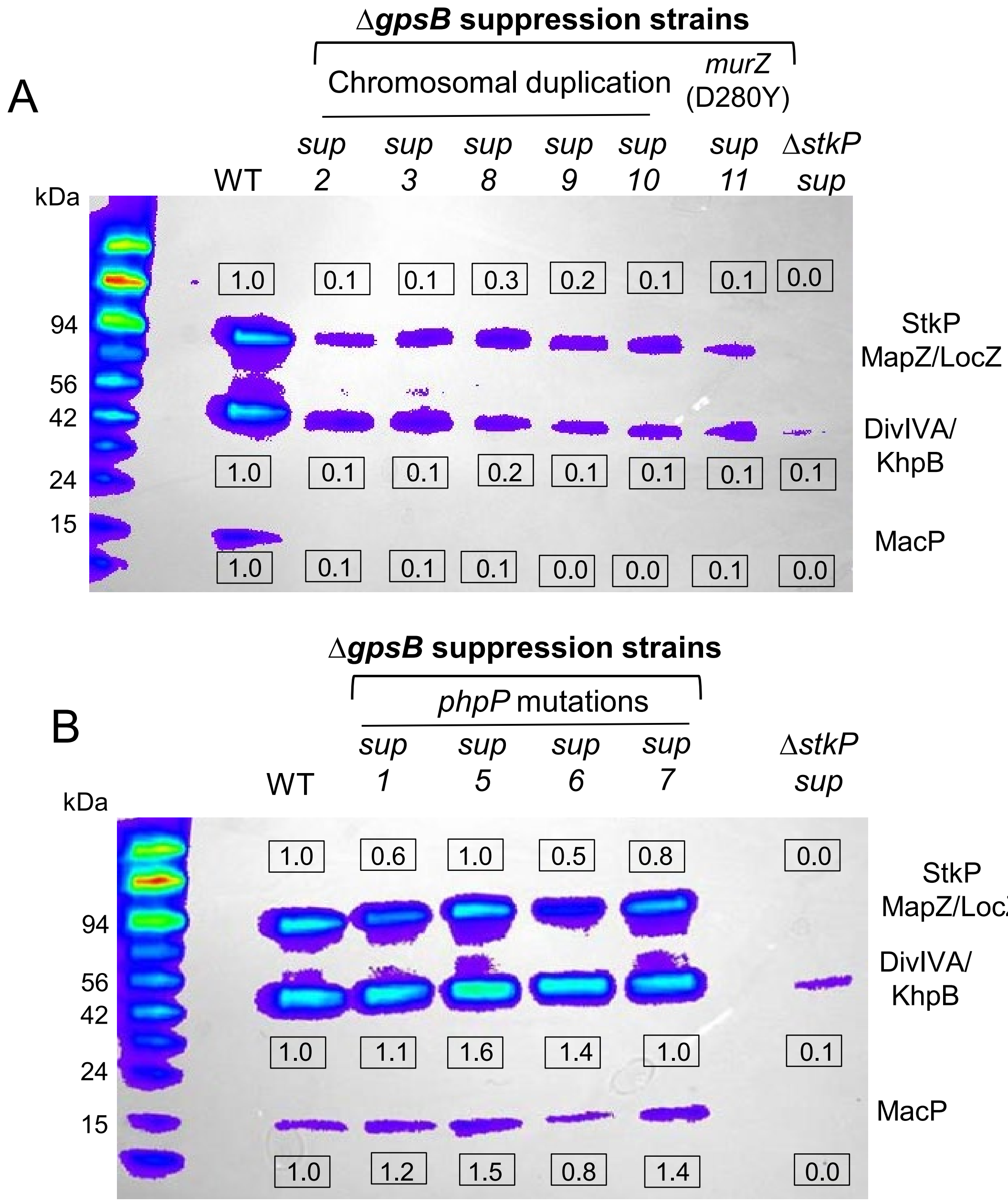


Figure S6. Protein phosphorylation profiles in Δ*gpsB* suppression strains. Western blot with α-pThr antibody to detect protein phosphorylation on Thr resides for WT D39 Δ*cps* parent strain (IU1945), Δ*gpsB* suppressor strains listed in Table 1, and a Δ*stkP* strain that contains uncharacterized suppressor mutation(s) (IU7923). Mean relative values of band intensities (±SEM) compared to the wild-type (WT) strain indicated for the phosphorylated MapZ/StkP, DivIVA/KhpB or MacP bands are shown in boxes above or below the blots. (A) Western blot for Δ*gpsB* suppressor strains containing chromosomal duplications and deletions (*sup2* and *sup3*), chromosomal duplications (*sup8*, *sup9* and *sup10*), or *murZ*(D280Y) mutation (*sup11*). (B) Western blot for Δ*gpsB* suppressor strains containing mutations in *phpP*.

Fig. S6

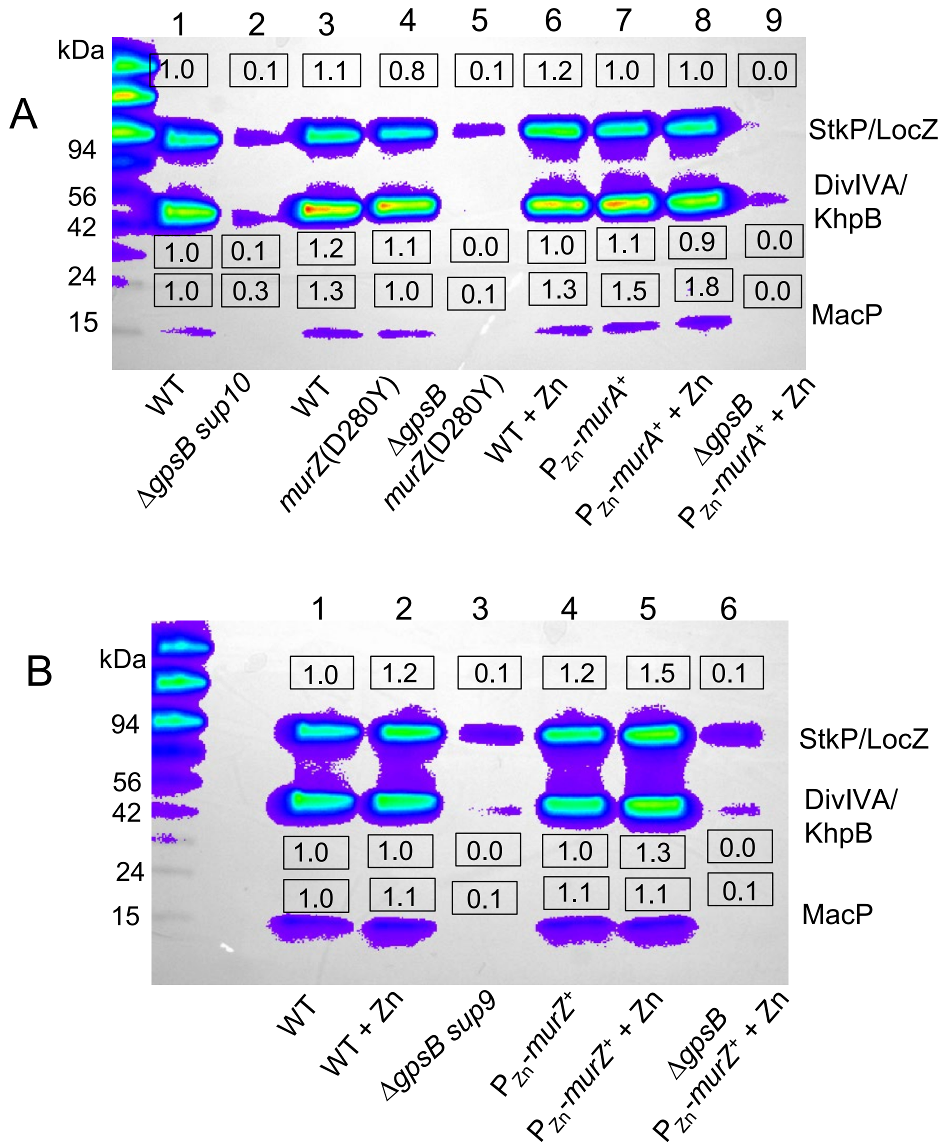


Figure S7. Overexpression of *murZ* or *murA* or the presence of *murZ*(D280Y) suppresses Δ*gpsB* lethality by a protein-phosphorylation independent mechanism. (A). Western blot with α-pThr antibody to detect protein phosphorylation on Thr resides for Δ*gpsB* strains containing *murZ*(D280Y) mutation, or *murA* overexpression. Mean relative values of band intensities (±SEM) compared to the wild-type (WT) strain indicated for the phosphorylated MapZ/StkP, DivIVA or MacP bands are shown in boxes above or below the blots. Strains 1 to 9 used for A are 1, IU1945 (WT D39 Δ*cps*); 2, IU11918; 3, IU1824 (WT D39 Δ*cps rpsL1*); 4, IU13439; 5, IU13485; 6, IU1945 + 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}) ; 7, IU11079; 8, IU11079 + 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}); 9, IU13757 + 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}). (B) Western blot for Δ*gpsB* suppressor strains overexpressing *murZ*. Strains are listed as follows: 1, IU1945; 2, IU1945 + 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}); 3, IU11846; 4, IU11077; 5, IU11077 + 0.2 mM (Zn^{2+}/(1/10)Mn^{2+}); 6, IU13756 + 0.2 mM (Zn^{2+}/(1/10)Mn^{2+}).

Fig. S7

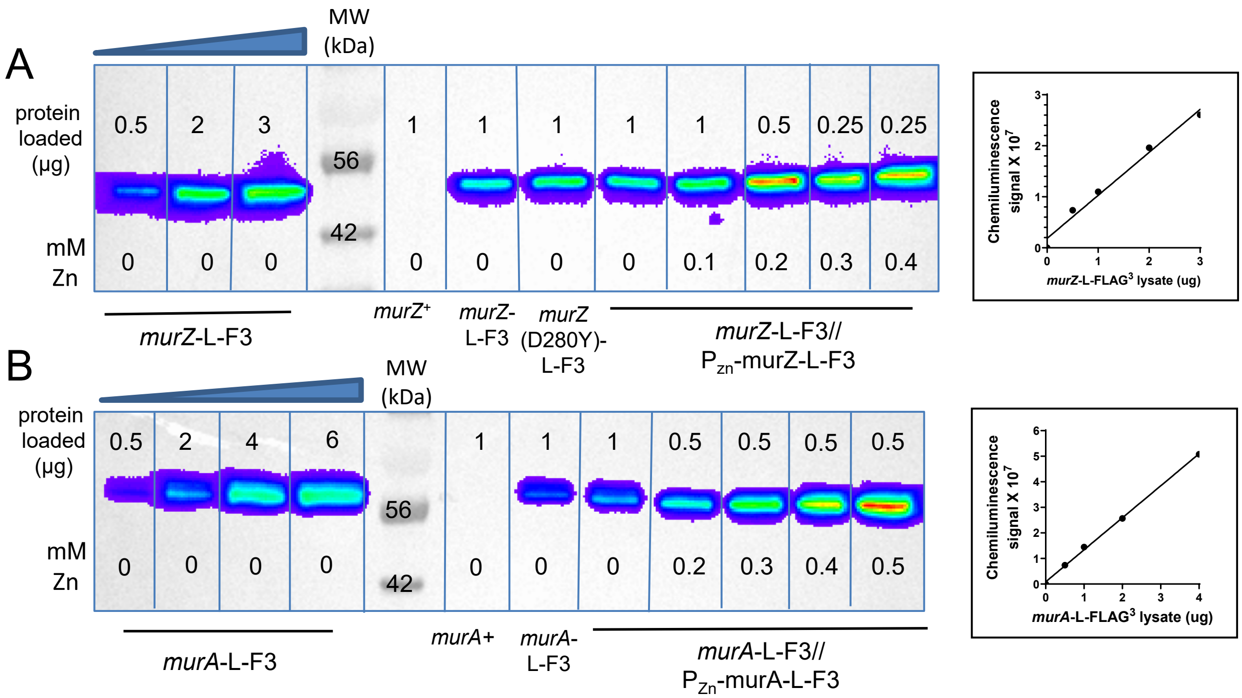


Figure S8. **Quantitation of relative MurZ-L-F3 (A) and MurA-L-F3 (B) cellular amounts by western blot using anti-FLAG antibody.** Strains and growth conditions are listed in legend to Fig. 5. The µg amounts of total protein loaded for each strain, and (Zn^{2+}/(1/10)Mn^{2+}) concentrations present in the BHI growth media are shown above and below the bands, respectively. The amounts of proteins loaded per lane for each sample were adjusted so that the intensity values are within the linear range obtained with the standard curve using various µg amounts of IU13502 or IU14028 samples. Plot of µg of lysate obtained from IU13502 or IU14028 loaded vs chemiluminescence signal intensities are shown to the right of the blots.

Fig. S8

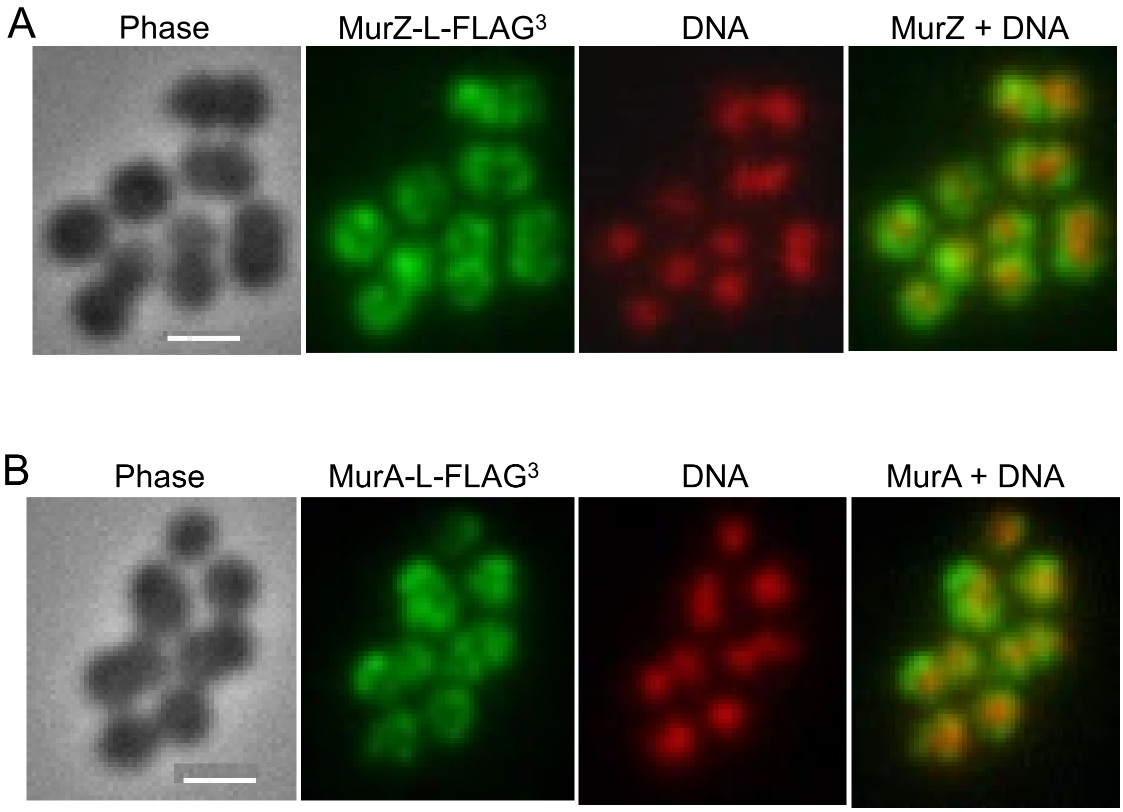
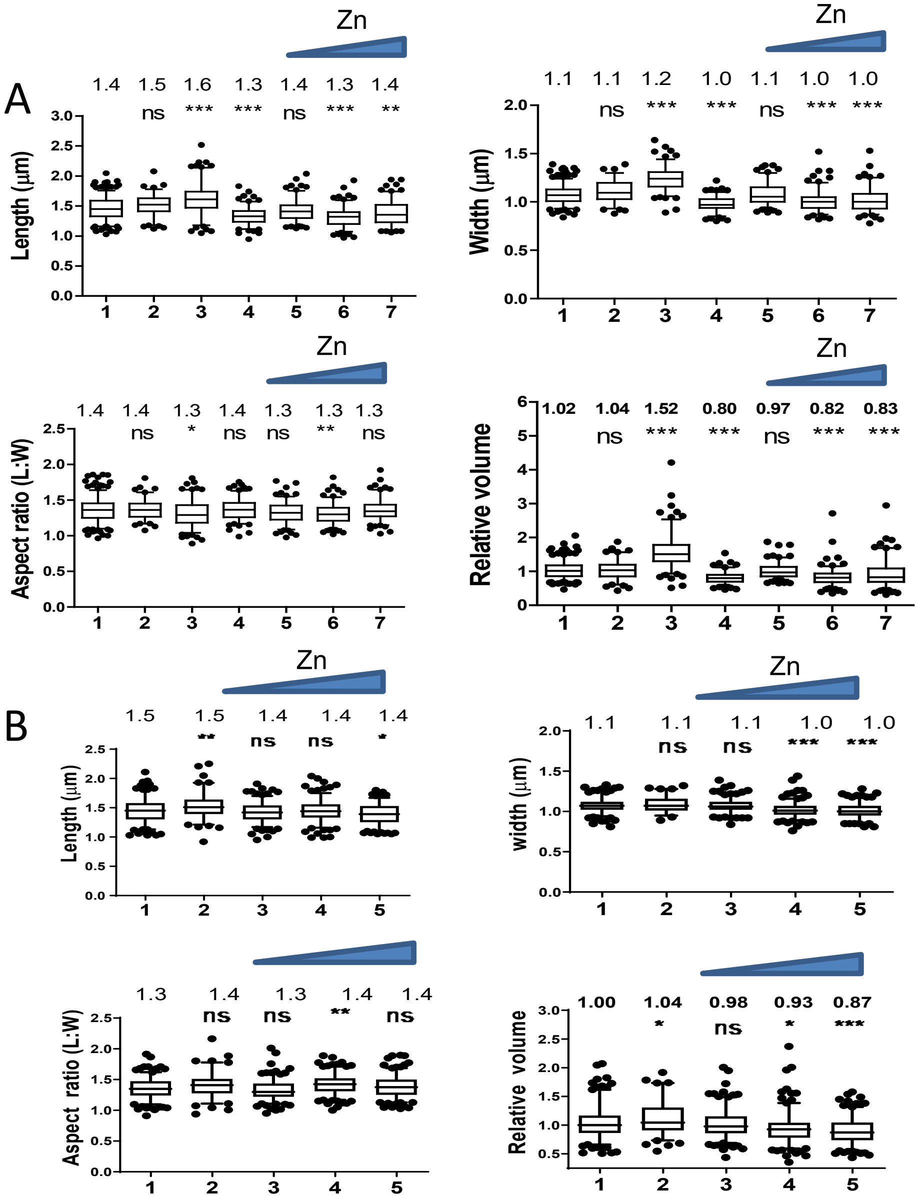


Figure S9. MurZ and MurA show general cytoplasmic distribution. Immunofluorescence microscopy was performed as described in *Experimental procedures* using (A) IU13502 (*murZ*-L-FLAG^3) or (B) IU14028 (*murA*-L-FLAG^3). Nucleoid DNA was labeled with a mounting media SlowFade gold antifade reagent containing DNA staining reagent DAPI. Scale bar: 1 μm.

Fig. S9



	Genotype	Zn (mM)
1	WT	0
2		0.4
3	Δ*murZ*	0
4	*murZ* (D280Y)	0
5	P_{Zn}-*murZ*+	0
6		0.2
7		0.4

	Genotype	Zn (mM)
1	WT	0
2	Δ*murA*	0
3	P_{Zn}-*murA*+	0
4		0.3
5		0.5

Figure S10. Box-and-whisker plots of cell dimensions of *murZ*(D280Y) and overexpression strains of *murZ* and *murA*. (A) Box-and-whisker plots (whiskers, 5 and 95 percentile) of cell lengths, widths, aspect ratios (cell length to width) and relative cell volumes of strains grown with or without (Zn^{2+}/(1/10)Mn^{2+}) of strains shown in Fig. 6. 1, WT (IU1824); 2, WT + 0.4 mM (Zn^{2+}/(1/10)Mn^{2+}); 3, Δ*murZ* (IU13536); 4, *murZ*(D280Y) (IU13438); 5, 6 and 7, *murZ*+//P_{Zn}-*murZ*+ (IU13393) grown in 0, 0.2, or 0.4 mM (Zn^{2+}/(1/10)Mn^{2+}), respectively. (B) 1, WT (IU1824); 2, Δ*murA* (IU13538); 3, 4 and 5, *murA*+//P_{Zn}-*murA*+ (IU13395) grown in 0, 0.3, or 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}), respectively. P values were obtained by one-way ANOVA analysis (GraphPad Prism, Kruskal-Wallis test). *, **, *** and ns denote p<0.05, p<0.01, p<0.001, not significant, respectively when compared to WT.

Fig. S10

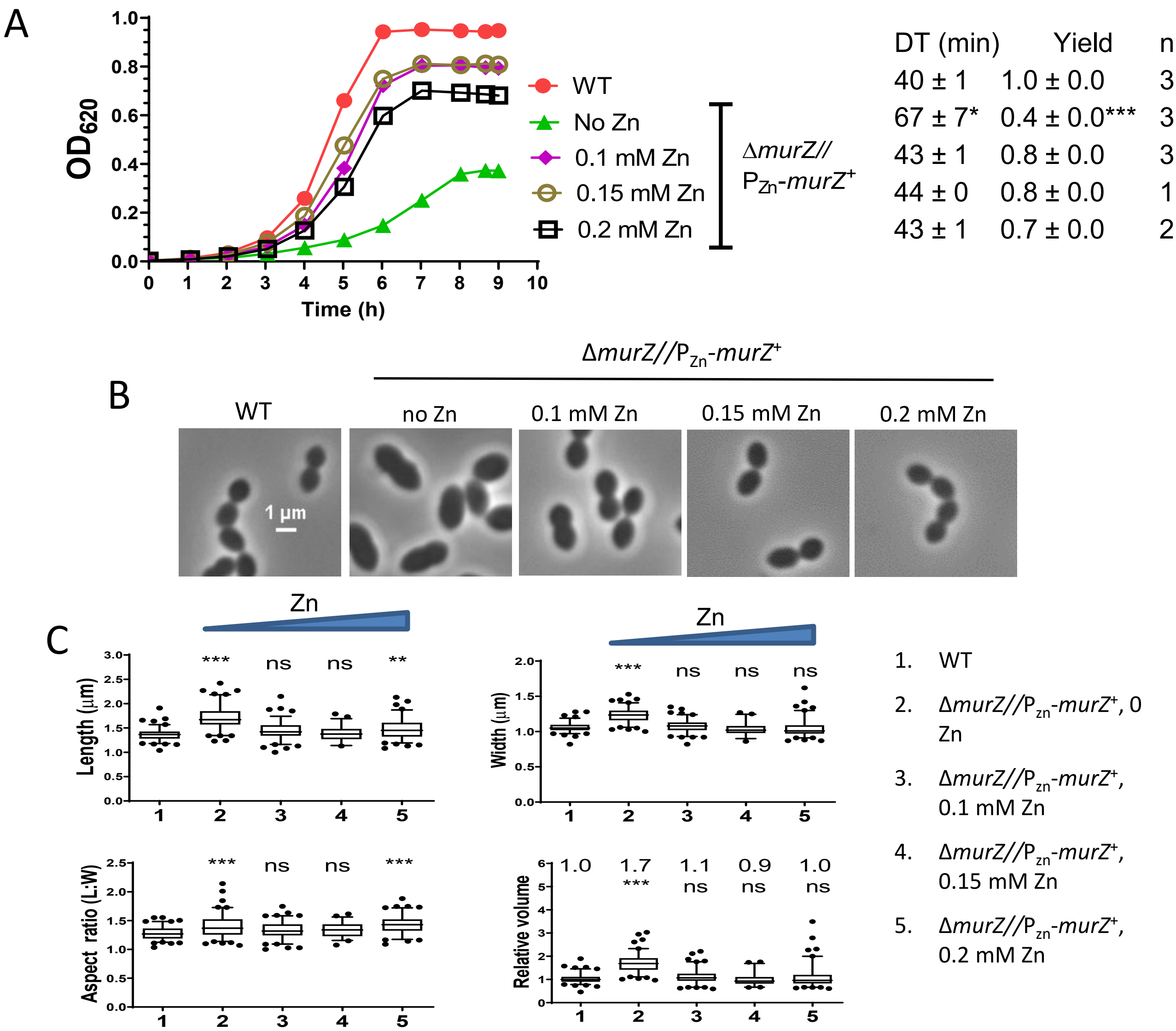


Figure S11. Complementation of Δ*murZ* growth and morphological defects by ectopic overexpression of *murZ*. Parent D39 Δ*cps rpsL1* strain (IU1824) was grown overnight in BHI broth with no additional (Zn^{2+}/(1/10)Mn^{2}+), and Δ*murZ*//P_{Zn}-*murZ*+ (IU16259) strains was grown overnight in BHI supplemented with 0, 0.1, 0.15 or 0.2 mM (Zn^{2+}/(1/10)Mn^{2+}), and diluted with fresh BHI containing the same concentrations of Zn^{2+}/(1/10)Mn^{2+} as the overnight cultures. (A) Representative growth curves, averages and SEMs of doubling times (DT) and maximal growth yields (OD_{620}) during 9 hours of growth. (B) Representative phase-contrast images taken between 3 and 4.5 h of growth for all strains and conditions. (C) Box-and-whisker plots of cell dimensions of strains grown with or without (Zn^{2+}/(1/10)Mn^{2+}). P values were obtained by one-way ANOVA analysis (GraphPad Prism, Kruskal-Wallis test). *, **, *** and ns denote p<0.05, p<0.01, p<0.001, not significant, respectively when compared to WT.

Fig. S11

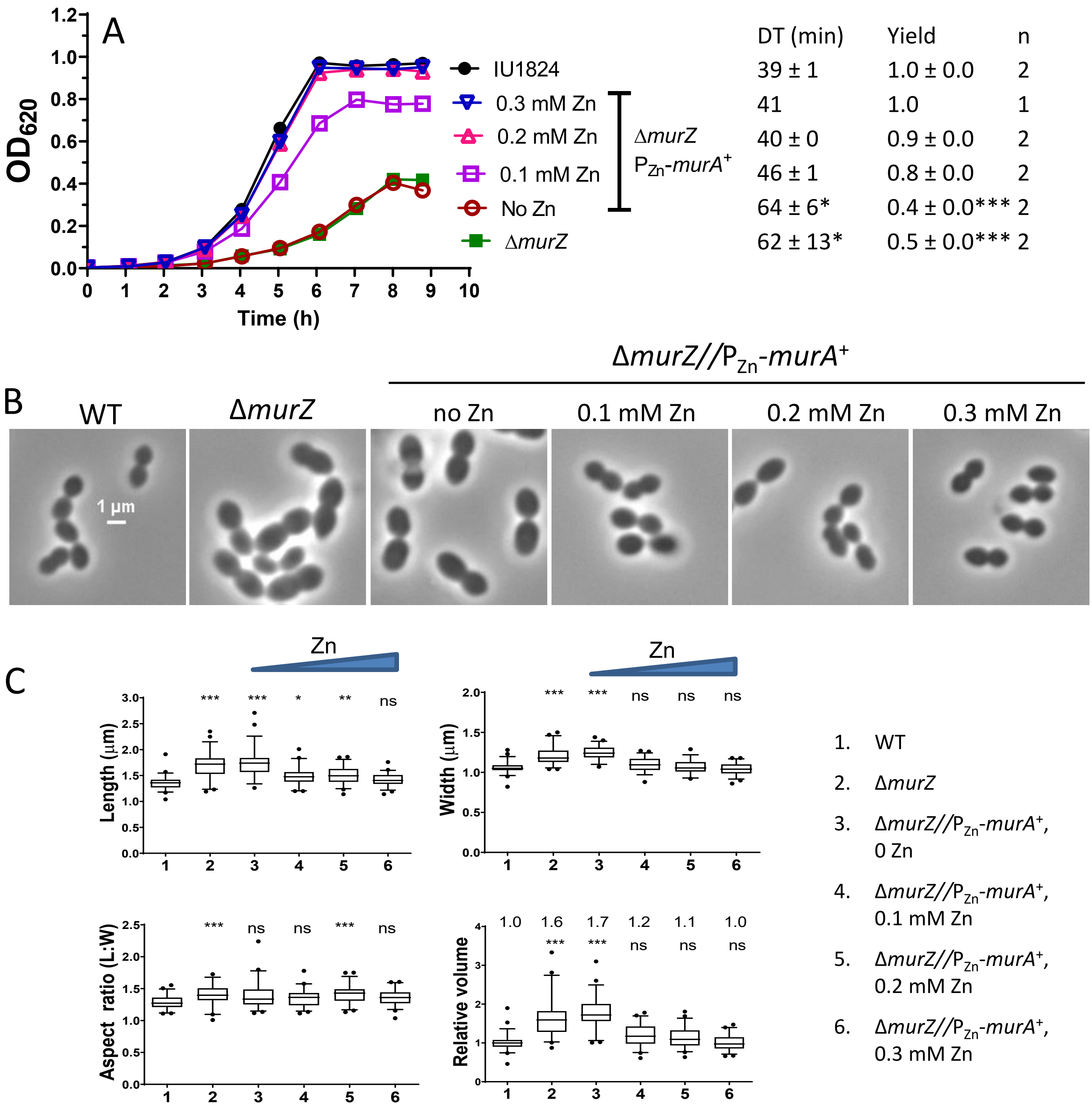
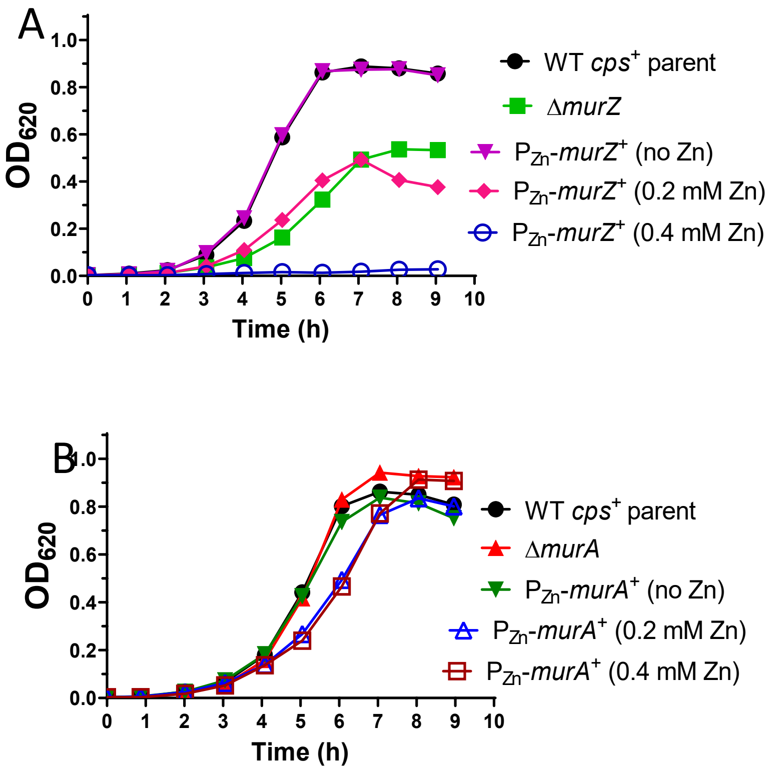


Figure S12. Complementation of Δ*murZ* growth and morphological defects by ectopic overexpression of *murA*. Parent D39 Δ*cps rpsL1* strain (IU1824), Δ*murZ* (IU13536), and Δ*murZ murA*//P_{zn}-*murA* (IU16262) strains were grown overnight and during the day in BHI broth with no additional or indicated concentrations of (Zn^{2+}/(1/10)Mn^{2+}) as described in legend to Fig. S11.

Fig. S12

D39 *cps*⁺ genetic background



(A)

Strain	DT (min)	Yield	n
WT *cps*⁺ parent	45 ± 3	0.9 ± 0.0	3
Δ*murZ*	54 ± 1	0.5 ± 0.0 ***	2
P_{Zn}-*murZ*⁺ (no Zn)	47 ± 4	0.9 ± 0.0	3
P_{Zn}-*murZ*⁺ (0.2 mM Zn)	55 ± 4	0.5 ± 0.0 ***	3
P_{Zn}-*murZ*⁺ (0.4 mM Zn)	>100 ***	0.08 ± 0.05 ***	3

(B)

Strain	DT (min)	Yield	n
WT *cps*⁺ parent	45 ± 3	0.9 ± 0.0	3
Δ*murA*	41 ± 1	0.9 ± 0.0	2
P_{Zn}-*murA*⁺ (no Zn)	49 ± 5	0.8 ± 0.0	3
P_{Zn}-*murA*⁺ (0.2 mM Zn)	55 ± 4	0.9 ± 0.0	3
P_{Zn}-*murA*⁺ (0.4 mM Zn)	55 ± 5	0.9 ± 0.0	3

Figure S13. Growth phenotypes of deletion and overexpression of *murZ* or *murA* in D39 encapsulated strains are similar to those in unencapsulated Δ*cps* D39 strains. (A) Growth in BHI broth of wild-type D39 *cps*⁺ parent (IU1690), isogenic Δ*murZ*::P_c-*erm* (IU16176), and *murZ*//P_{Zn}-*murZ*⁺ (IU15879). (B) Deletion or overexpression of *murA* in an encapsulated derivative of strain D39 did not result in growth defects when cultured in BHI broth. Strains tested are D39 *cps*⁺ parent (IU1690), isogenic Δ*murA* (IU16178), and *murA*⁺//P_{Zn}-*murA*⁺ (IU15880).

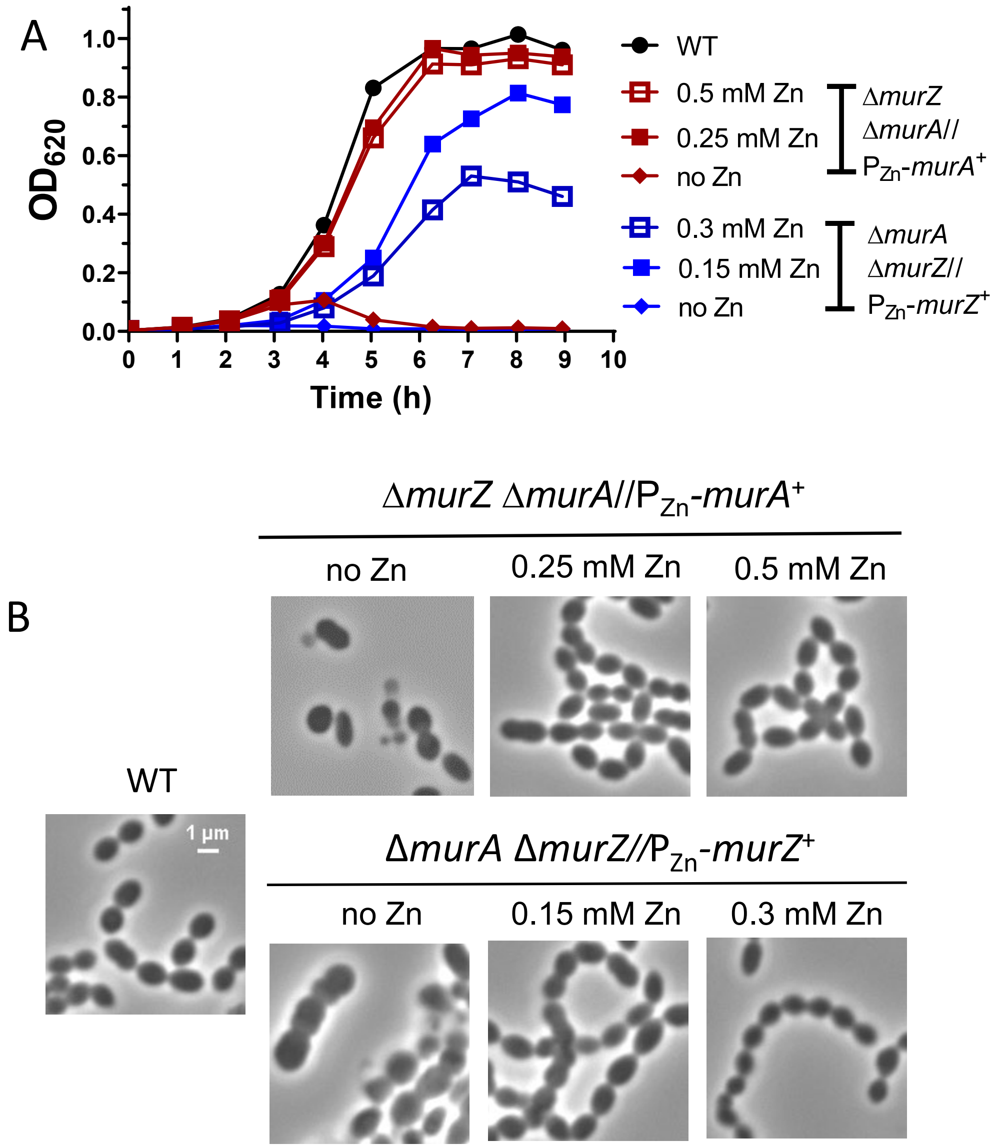
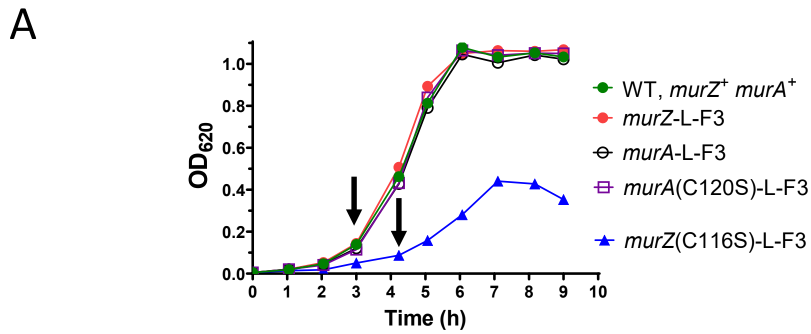
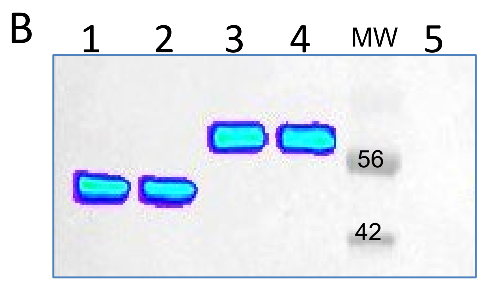


Figure S14. Depletion of MurA in a Δ*murZ* strain, or depletion of MurZ in a Δ*murA* strain results in cell lysis, but not cell elongation. Parent D39 Δ*cps rpsL1* strain (IU1824), Δ*murZ* Δ*murA*//P_{Zn}-*murA*$^+$ (IU16332), Δ*murA* Δ*murZ*//P_{Zn}-*murZ*$^+$ (IU16330) strains were grown overnight in BHI containing 0.15 or 0.25 mM Zn^{2+} (Zn^{2+}/(1/10)Mn^{2+}) for IU16332 and IU16330, respectively, and diluted into BHI broth containing the indicated (Zn^{2+}/(1/10)Mn^{2+}) concentration. (A) Growth curve and (B) microscopic images taken between 3 to 4 h of growth.





Lane	Genotype
1	*murZ*-L-FLAG³
2	*murZ*(C116S)-L-FLAG³
3	*murA*-L-FLAG³)
4	*murA*(C120S)-L-FLAG³
5	*murZ*⁺ *murA*⁺ WT

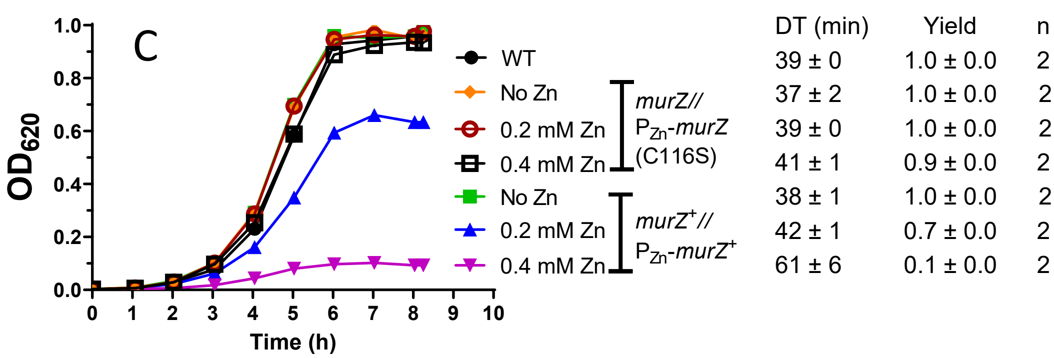


Figure S15. Characterization of mutant strains with catalytic site mutations *murZ*(C116S) or *murA*(C120S). (A) Growth curves in BHI broth of non-FLAG-tagged *murZ*⁺ WT (IU1824), *murZ*-L-FLAG³ (IU13502), *murZ*(C116S)-L-FLAG³ (IU15941), *murA*-L-FLAG³ (IU14028), and *murA*(C120S)-L-FLAG³ (IU15951). *murZ*(C116S)-L-FLAG³ strain showed a defective growth profile in BHI, similar to Δ*murZ* strains (see Fig. 6A). Arrows indicate when samples were withdrawn for protein preparation. (B) Western blot using an anti-FLAG antibody of protein samples prepared from above strains. C) Overexpression of MurZ in a *murZ*⁺//P_{Zn}-*murZ*⁺ strain (IU13393) resulted in growth inhibition in BHI broth, while overexpression of catalytically inactive *murZ*(C116S) in a *murZ*⁺//P_{Zn}-*murZ*(C116S) strain had no effect on growth.

Fig. S15

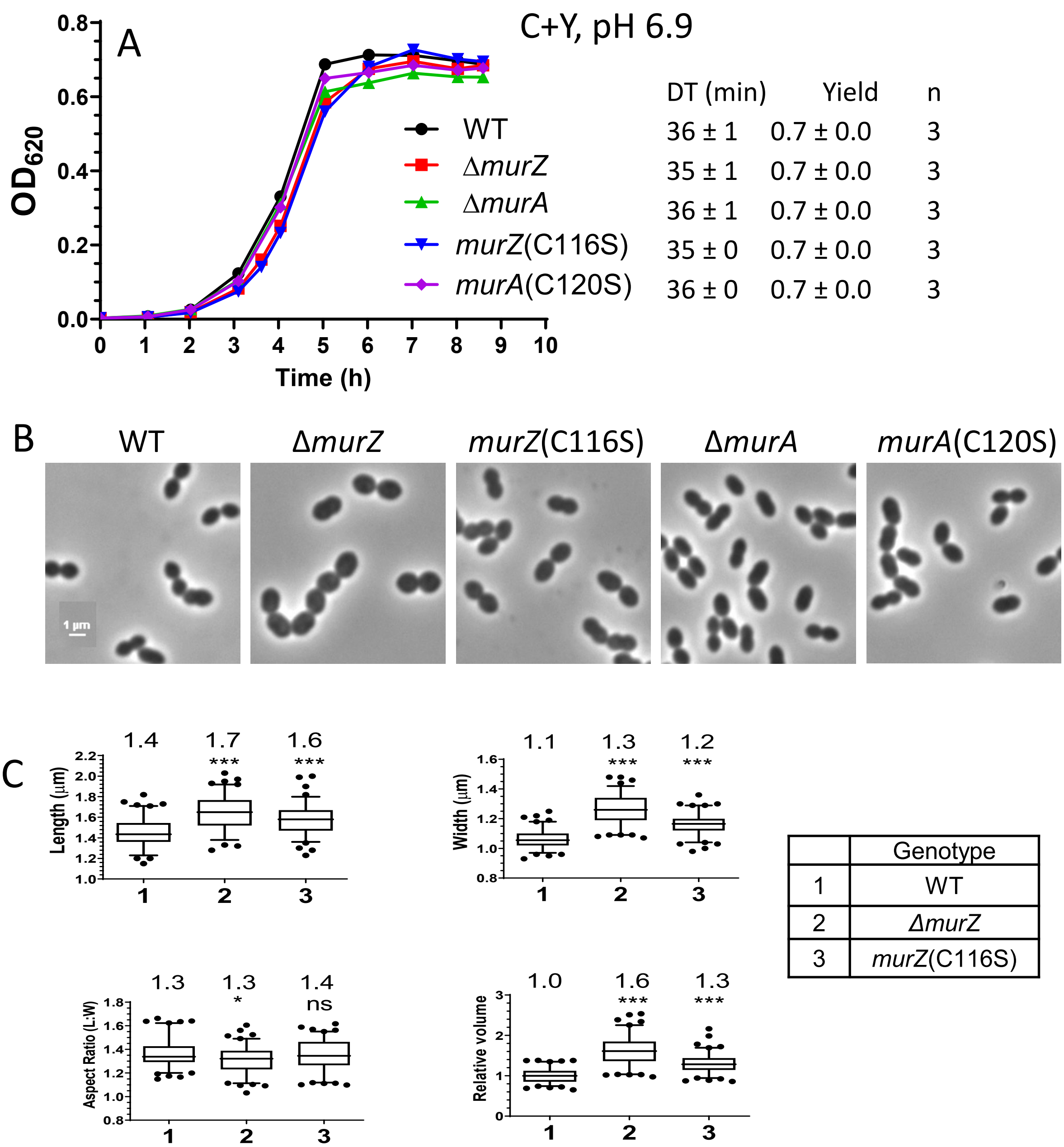


Figure S16. Δ*murZ* or *murZ*(C116S) mutants cultured in C+Y media, pH 6.9 do not show growth defects, but form significantly enlarged cells compared to WT. (A) Growth curves of WT (IU1824), Δ*murA* (IU13538), *murA*(C120S) (IU15949), Δ*murZ* (IU13536), and *murZ*(C116S) (IU15939). Strains were grown overnight in BHI broth, centrifuged to remove BHI, and resuspended in C+Y, pH 6.9 medium to OD_{620} ≈ of 0.003 for growth curves. (B) Cells were imaged OD_{620} ≈ of 0.1 to 0.15. Scale bar = 1 µm. (C) Box-and-whisker plots (whiskers, 5 and 95 percentile) of cell lengths, widths, aspect ratios (cell length to width) and relative cell volumes of 100 cells for each strain from two experiments. P values were obtained by one-way ANOVA analysis (GraphPad Prism, Kruskal-Wallis test). *, **, *** and ns denote p<0.05, p<0.01, p<0.001, not significant, respectively when compared to WT.

Fig. S16

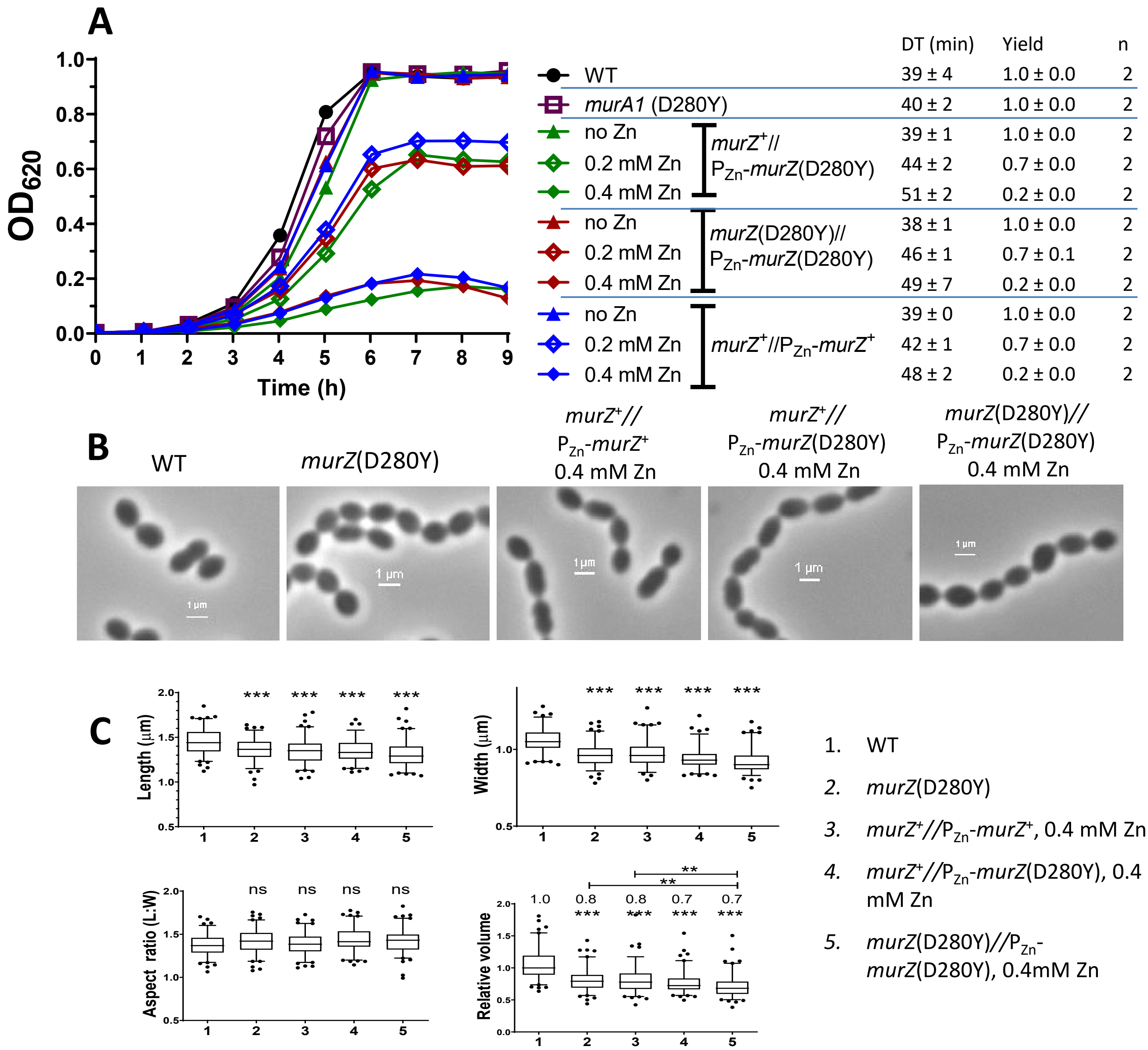


Figure. S17. Ectopic overexpression of *murZ*(D280Y) decreases cell size and inhibits growth similar to overexpression of *murZ*. Parent D39 Δ*cps rpsL1* strain (IU1824), *murZ*(D280Y) (IU13438), *murZ*$^+$//P$_{Zn}$-*murZ*$^+$ (IU13393), *murZ*$^+$//P$_{Zn}$-*murZ*(D280Y) (IU16334), and *murZ*(D280Y)//P$_{Zn}$-*murZ*(D280Y) (IU16336) strains were grown overnight in BHI broth with no additional (Zn^{2+}/(1/10)Mn^{2}+), diluted to OD_{620} ≈ 0.003 in the morning with no additional, 0.2 mM, or 0.4 mM (Zn^{2+}/(1/10)Mn^{2+}). (A) Representative growth curves, averages and SEMs of doubling times and maximal growth yields (OD_{620}) during 9 hours of growth. n denotes number of independent growths. (B) Representative phase-contrast images. (C) Box-and-whisker plots of cell dimension. P values were obtained by one-way ANOVA analysis (GraphPad Prism, Kruskal-Wallis test). *, **, *** and ns denote $p<0.05$, $p<0.01$, $p<0.001$, not significant, respectively when compared to WT.

Fig. S17

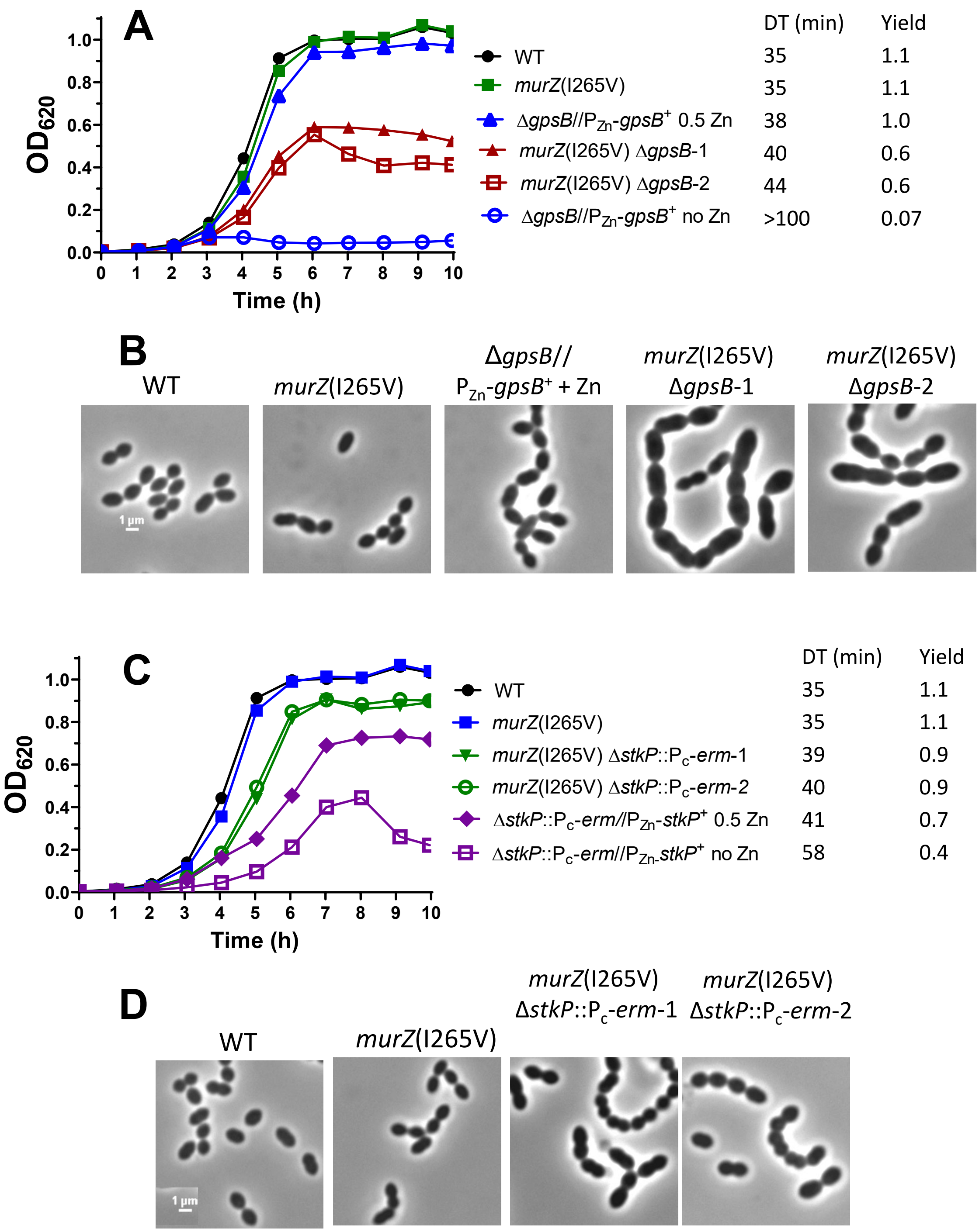
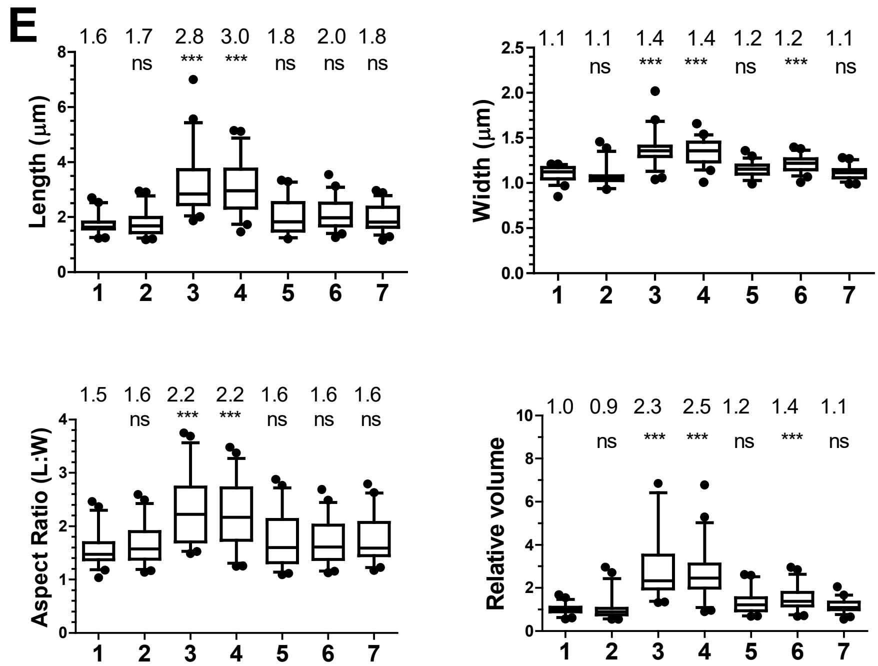


Fig. S18A to S18D



	Genotype
1	WT
2	*murZ*(I265V)
3	*murZ*(I265V) Δ*gpsB-1*
4	*murZ*(I265V) Δ*gpsB-2*
5	*murZ*(I265V) Δ*stkP*::P_c-*erm*-1
6	*murZ*(I265V) Δ*stkP*::P_c-*erm*-2
7	Δ*gpsB*//P_{Zn}-*gpsB*$^+$, 0.5 mM Zn

Figure. S18. Suppression of Δ*gpsB* and Δ*stkP* lethality by *murZ*(I265V). (A) Growth curves of WT (IU1824), *murZ*(I265V) (IU14210), two independent isolates of *murZ*(I265V) Δ*gpsB* (IU14234, IU15124), and Δ*gpsB*//P_{Zn}-*gpsB*$^+$ (IU16370) strains. Strains were grown overnight and diluted for growth during the day in BHI broth as described in *Experimental procedures*. IU16370 was grown overnight in BHI broth with 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}), and diluted to OD_{620} ≈ 0.003 in the morning with fresh BHI not supplemented with (Zn^{2+}/(1/10)Mn^{2+}) or containing 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}). (B) Microscopic images of cells in (A) grown to OD_{620} ≈0.15. (C) Growth curves of WT (IU1824), *murZ*(I265V) (IU14210), two independent isolates of *murZ*(I265V) Δ*stkP*::P_c-*erm* (IU17469, IU17475), and Δ*stkP*::P_c-*erm*//P_{Zn}-*stkP*$^+$ (IU16933) grown similar to IU16370. (D) Microscopic images of cells in (C) grown to OD ≈ 0.15. Scale bar = 1 μm. (E) Box-and-whisker plots (whiskers, 5 and 95 percentile) of cell lengths, widths, aspect ratios (cell length to width) and relative cell volumes of strains shown in (B) and (D). P values were obtained by one-way ANOVA analysis (GraphPad Prism, Kruskal-Wallis test). *, **, *** and ns denote p<0.05, p<0.01, p<0.001, not significant, respectively when compared to WT.

Fig. S18E

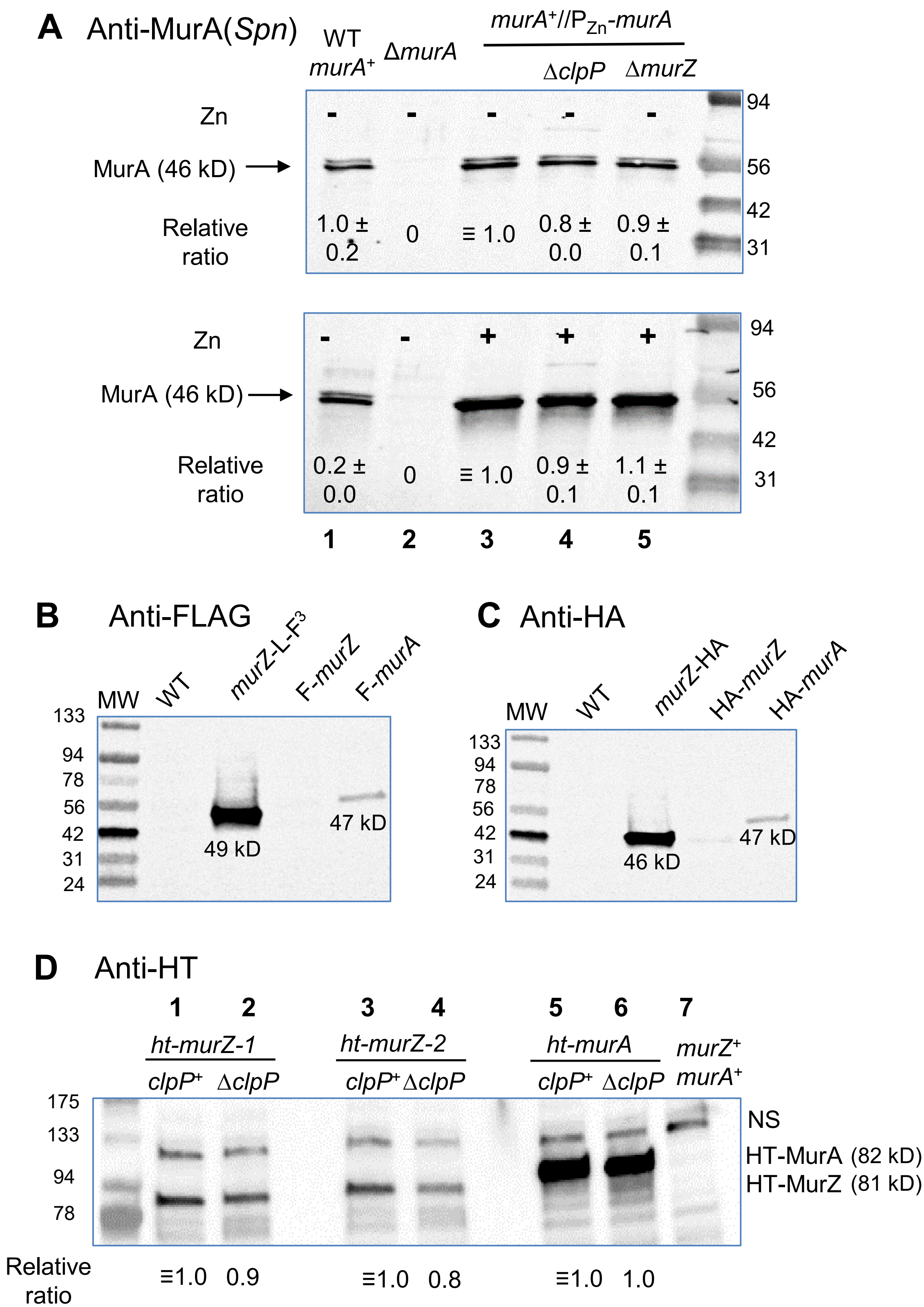
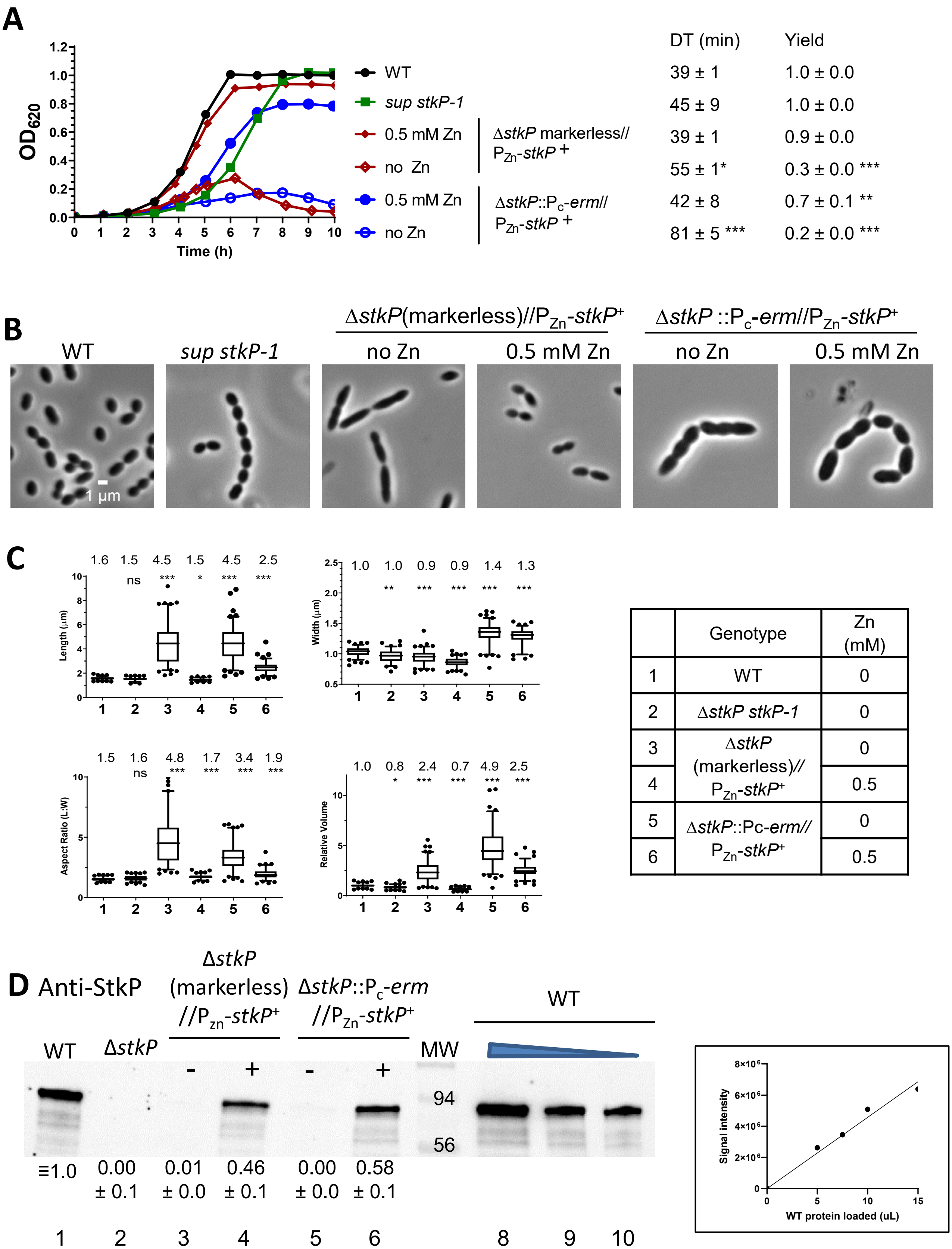

A Anti-MurA(Spn)
WT murA+
ΔmurA
murA+//PZn-murA
ΔclpP ΔmurZ
Zn
MurA (46 kD)
Relative ratio
1.0 ± 0.2
0
≡ 1.0
0.8 ± 0.0
0.9 ± 0.1
94
56
42
31
0.2 ± 0.0
1.1 ± 0.1
0.9 ± 0.1
1 2 3 4 5
B Anti-FLAG
MW WT murZ-L-F3 F-murZ F-murA
133 94 78 56 42 31 24
49 kD
47 kD
C Anti-HA
MW WT murZ-HA HA-murZ HA-murA
46 kD
47 kD
D Anti-HT
1 2 3 4 5 6 7
ht-murZ-1
ht-murZ-2
ht-murA
clpP+ ΔclpP
murZ+ murA+
175 133 94 78
NS
HT-MurA (82 kD)
HT-MurZ (81 kD)
Relative ratio
≡1.0 0.9
≡1.0 0.8
≡1.0 1.0

Fig. S19

Figure S19. Cellular amounts of MurA, or N-terminal-tagged MurZ or MurA fusion proteins are unchanged in Δ*clpP* mutants. (A) Representative western blot using anti-MurA (*Spn*) antibody (1:7000 dilution) of lysates collected after 3.5 h of growth, where – or + indicates the absence or presence of 0.4 mM (Zn^{2+}/(1/10)Mn^{2+}) in the BHI broth. Western blotting was performed as described in *Experimental procedures* using Licor IR Dye800 CW secondary antibody detected with Azure Biosystem 600. 10 μL (≈4 μg) of protein samples were loaded in each lane. Lane 1, wild-type (IU1824); lane 2, Δ*murA* (IU13538); lane 3, P_{Zn}-*murA* (IU13395); lane 4, P_{Zn}-*murA* Δ*clpP* (IU19201); lane 5, P_{Zn}-*murA* Δ*murZ* (IU16262). A standard curve was generated by loading 2.5, 5, 10, 15 or 20 μL of IU13395 (P_{Zn}-*murA*) samples (lanes not shown). Calculated protein amounts (mean ± SEM) relative to P_{Zn}-*murA* (IU13395) are based on two independent experiments. Signals obtained with anti-MurA antibody were normalized with total protein stain in each lane using Totalstain Q-NC (Azure Scientific). (B and C) Stable expression of C-terminal tagged MurZ (MurZ-L-F^3) and MurZ (MurZ-HA) compared to N-terminal tagged MurZ (F-MurZ, HA-MurZ) and MurA (F-MurA, HA-MurA). (B) Western blot results with lysates obtained from strains IU1824 (WT), IU13502 (*murZ*-L-F^3), IU17764 (F-*murZ*), and IU17768 (F-*murA*). (C) Western blot results of lysates obtained from strains IU1824 (WT), IU17170 (*murZ*-HA), IU17766 (HA-*murZ*), and IU17770 (HA-*murA*). (D) Western blot showing that HT-MurZ and HT-MurA cellular amounts are similar in *clpP*⁺ and ∆*clpP* strains. Lysates were obtained from strains IU17838 (*ht-murZ* isolate 1*,* lane 1), IU17865 (*ht-murZ* ∆*clpP* isolate 1*,* lane 2*)*, IU17840 (*ht-murZ* isolate 2*,* lane 3), IU17869 (*ht-murZ* ∆*clpP,* isolate 2 lane 4*),* IU17841 (*ht-murA,* lane 5), IU17869 (*ht-murZ* ∆*clpP,* lane 6) and WT (lane 7). The band below 133 kD is a nonspecific (NS) band since it is also present in a non-HT-tagged strain (lane 7). Expected molecular weights of MurZ-L-F^3, F-MurZ, F-MurA, MurZ-HA, HA-MurZ, HA-MurA, HT-MurA and HT-MurZ are 49, 46, 47, 46, 46, 47, 82 and 81 kDa respectively. All fusion MurA constructs migrate at higher positions than expected. Antibodies used for detection are described in *Experimental procedures* and signals were detected with an Azure Biosystem 600. 9 μg of crude lysate was loaded on each lane for B-D.



	Genotype	Zn (mM)
1	WT	0
2	Δ*stkP stkP-1*	0
3	Δ*stkP* (markerless)// P_{Zn}-*stkP*+	0
4		0.5
5	Δ*stkP*::Pc-*erm*// P_{Zn}-*stkP*+	0
6		0.5

Fig. S20

Figure S20. Complementation of Δ*stkP* growth and morphological defects by ectopic overexpression of *stkP*. Parent D39 Δ*cps rpsL1* strain (IU1824), *sup stkP-1* (IU16883), Δ*stkP*(*markerless)//*P_{Zn}-*stkP*$^+$ (IU18665) and Δ*stkP*::P_c-*erm//*P_{Zn}-*stkP*$^+$ (IU16933) strains were grown overnight in BHI broth with no additional (Zn^{2+}/(1/10)Mn^{2+}) (IU1824 and IU16883) or with 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}) (IU18665 and IU16933) as described in *Experimental procedures*. Strains were diluted to OD_{620} ≈ 0.003 in the morning with fresh BHI containing no (Zn^{2+}/(1/10)Mn^{2+}) or indicated concentrations of (Zn^{2+}/(1/10)Mn^{2+}). (A) Growth curves, doubling times, and maximal growth yields (OD_{620}) during 9 h of growth. (B) Representative phase-contrast images taken between 3.5 to 4 h of growth. Scale bar = 1 µm. Growth curves and microscopy were performed in two independent experiments. (C) Box-and-whisker plots (whiskers, 5 and 95 percentile) of cell lengths, widths, aspect ratios, and relative cell volumes. P values were obtained by one-way ANOVA analysis (GraphPad Prism, Kruskal-Wallis test). *, **, *** and ns denote $p<0.05$, $p<0.01$, $p<0.001$, not significant, respectively when compared to WT. (D) Quantitative Western blot with anti-StkP antibody showing relative StkP levels inducted by an ectopic Zn-controlled promoter. Lane 1, wild-type (WT, IU1824), 2, *sup stkP-1* (IU16883), 3-4, Δ*stkP*(markerless)//P_{Zn}-*stkP*$^+$ (IU18665) with 0 and 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}), respectively, and 5-6 Δ*stkP*::P_c-*erm//*P_{Zn}-*stkP*$^+$ (IU16933) with 0 and 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}), respectively. Samples were normalized based on culture OD_{620} before addition of lysis buffer, and 10 µL (≈3 µg) of lysate were loaded in lanes 1-6. Lane 7, molecular weight standard. Lanes 8-10, 15, 7.5 and 5 µL of WT lysates, respectively, were used to generate the standard curve to the right. SDS-PAGE and western blotting were carried out as described in *Experimental procedures* using Licor IR Dye800 CW secondary antibody detected with Azure Biosystem 600. – or + indicates the absence of presence of (Zn^{2+}/(1/10)Mn^{2+}) in the BHI broth. Signals obtained with anti-StkP antibody were normalized with total protein stain in each lane using Totalstain Q-NC (Azure Scientific).

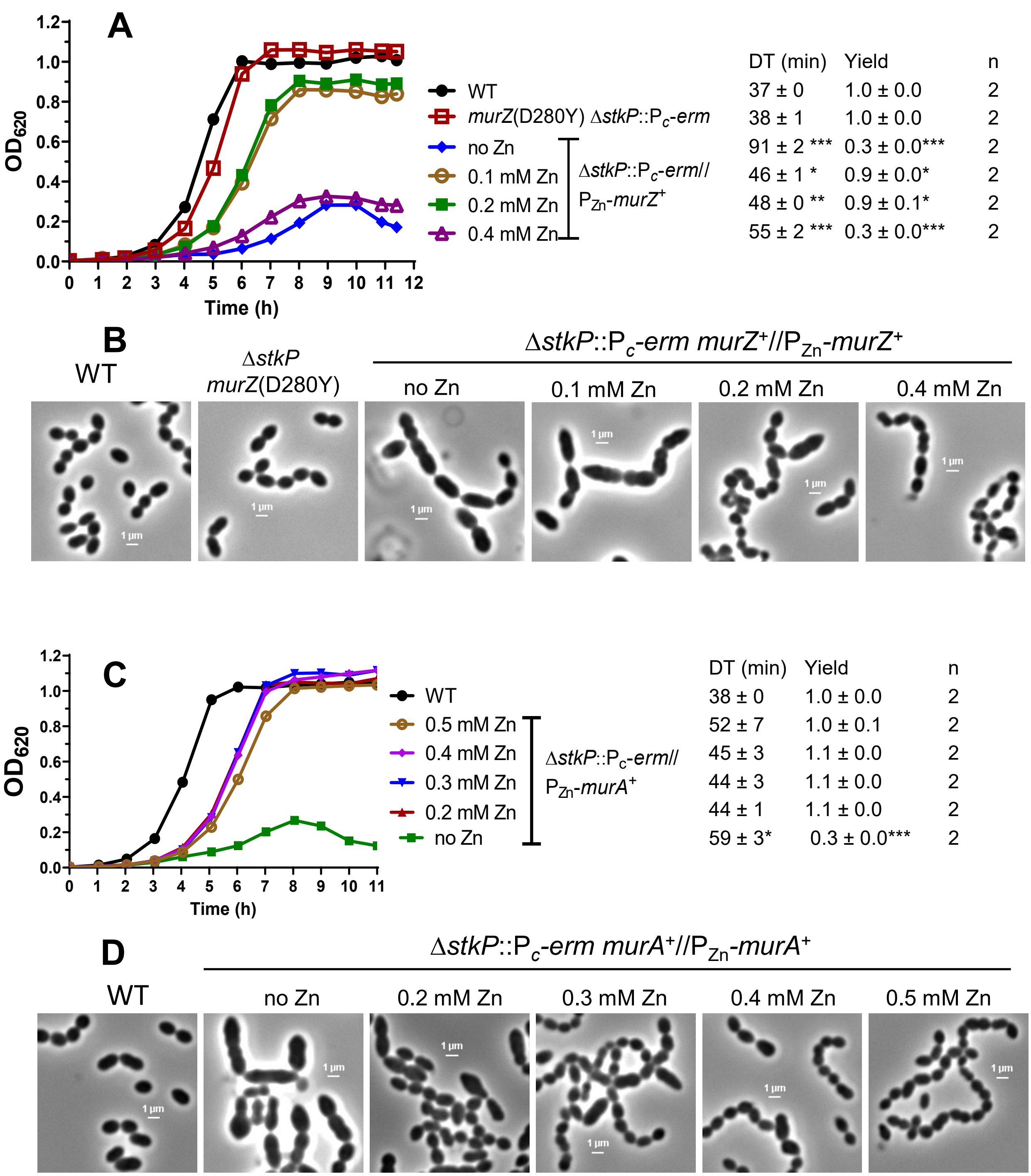
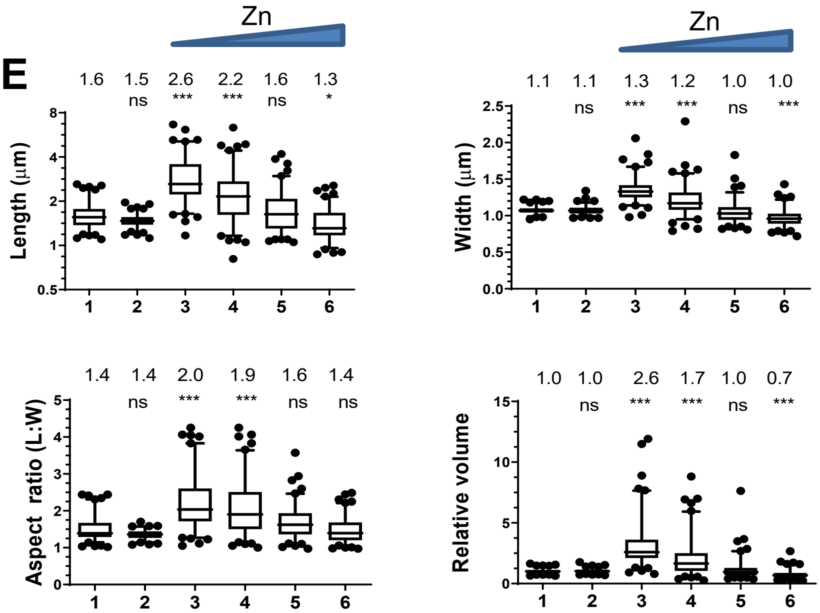


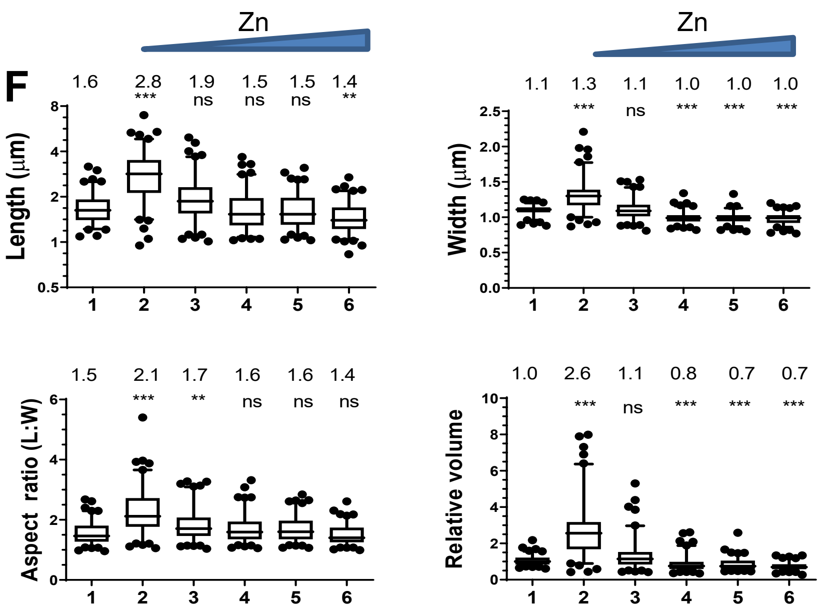
Fig. S21A to S21D

E



	Genotype	Zn (mM)
1	WT	0
2	*murZ* (D280Y) Δ*stkP*::P$_c$-*erm*	0
3	Δ*stkP*::P$_c$-*erm*// P$_{Zn}$-*murZ*$^+$	0
4		0.1
5		0.2
6		0.4

F



	Genotype	Zn (mM)
1	WT	0
2	Δ*stkP*::P$_c$-*erm*// P$_{Zn}$-*murA*$^+$	0
3		0.2
4		0.3
5		0.4
6		0.5

Fig. S21E and S21F

Figure S21. Suppression of growth and morphological Δ*stkP* phenotypes in BHI broth by *murZ*(D280Y) or overexpression of *murZ* or *murA*. (A and B) Parent D39 Δ*cps rpsL1* strain (IU1824), *murZ*(D280Y) Δ*stkP*::Pc-*erm* (IU16885), and Δ*stkP*::Pc-*erm murZ*+//P_{Zn}-*murZ*+ (IU16897) were grown overnight in BHI broth without (IU1824 and IU16885) or with 0.2 mM (Zn^{2+}/(1/10)Mn^{2+}) . Overnight cultures were diluted in the morning in BHI for IU1824 and IU16885, and in BHI supplemented with 0 to 0.4 mM (Zn^{2+}/(1/10)Mn^{2+}) for IU16897. (A) Representative growth curves, averages, and SEMs of doubling times (DT) and maximal growth yields (OD_{620}). (B) Representative phase-contrast images taken between at 3.5 h for IU1824 and IU16885, and between 5 to 5.8 h for IU16897. Similar growth curves and morphology results were obtained with an independent Δ*stkP murZ*(D280Y) isolate, IU16895. (C) Parent D39 Δ*cps rpsL1* strain (IU1824) and a Δ*stkP*::P_c-*erm murA*+//P_{Zn}-*murA*+ (IU16915) strain were grown overnight in BHI broth with no or 0.4 mM (Zn^{2+}/(1/10)Mn^{2+}), respectively. Overnight cultures were diluted to $OD_{620} \approx 0.003$ in the morning in BHI for IU1824, and in BHI supplemented with 0 to 0.5 mM (Zn^{2+}/(1/10)Mn^{2+}) for IU16915. (D) Representative phase-contrast images taken at 3 h for IU1824, and between 4 to 5 h for IU16915. (E) and (F) Box-and-whisker plots (whiskers, 5 and 95 percentile) of cell lengths, widths, aspect ratios, and relative cell volumes of above strains grown with or without (Zn^{2+}/(1/10)Mn^{2}+). P values were obtained by one-way ANOVA analysis (GraphPad Prism, Kruskal-Wallis test). *, **, *** and ns denote $p<0.05$, $p<0.01$, $p<0.001$, not significant, respectively when compared to WT.