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FacZ is a GpsB-interacting protein that prevents aberrant division-site placement in *Staphylococcus aureus*

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Title: FacZ is a GpsB-interacting protein that prevents aberrant division-site placement in *Staphylococcus aureus*

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Supplemental Table Legends

Table S1. Tn-seq meta-analysis.

Table summarizes the relevant metrics from Tn-Seq analysis of the initial transposon library, END and CSD enrichments, and the ungated control sort. The numbers 2 and 3 refer to the sorting round (e.g., CSD 2 has been subjected to two rounds of sorting increased light scattering). The total number of unique TA sites and the percentage of total TA sites in the genome with transposon insertions are given, as well as median and mean number of insertions per gene in the library following enrichments. The number of genes in each library hit at least once, twice, or five times is shown at right, along with the percent of annotated genes bearing at least that many hits. Also, the number of genes meeting or exceeding these thresholds is compared to the number of mutants in the NTML ordered transposon library[2], which is commonly used in *S. aureus* research.

Table S2. Tn-seq data for the END and CSD enrichments.

Tn-seq results for the second and third rounds of sorting of the CSD and END enrichments. Relative enrichments compare the results of a given round of either CSD or END sorting to the ungated control. P-values are from Mann-Whitney U test. Genes are sorted by SAOUHSC locus number.

Table S3. Relative enrichment of transposon insertions in each gene following END and CSD enrichments versus the ungated control.

Tn-seq data showing the relative enrichments of the END and CSD sorts compared to an ungated control. Genes are sorted by SAOUHSC locus number.

Table S4. PCA-adjusted Tn-Seq results.

Following 2D-PCA rotation of the CSD and END sorted data, each gene's enrichment was assigned a new (X,Y) location, with X representing a genes relative enrichment along PC2, and Y representing the relative enrichment along PC1. These data were used to plot the PCA panel in **Fig. S1**. Genes are sorted by SAOUHSC locus number.

Table S5. PCA-adjusted hit list.

The top 50 hits from the PCA analysis based on maximum relative enrichment along PC1. "Δ" indicates the change in PC1 or PC2 relative enrichment between rounds of sorting. "Mean fold increase" and "% increasing in enrichment" are metrics to assess whether hits identified by PCA in sort 2 increase in PC1 enrichment from additional rounds of CSD and END sorting. In general, hits identified by PC1 enrichment enrich further from additional sorts, where those from PC2 do not, consistent with PC1-enrichment serving as a proxy for mutual CSD and END enrichment. Genes are displayed by decreasing relative enrichment along PC1.

Table S6. Δ*facZ* synthetic lethal Tn-seq hits

Tn-seq analysis of transposon libraries constructed in WT *S. aureus* [aTB015] versus one constructed in a Δ*facZ* [aTB259] strain. P-values are from Mann-Whitney U test. Genes are sorted by increasing Δ*facZ:WT* relative enrichment ratio.

Table S7. Annotated Δ*facZ* synthetic lethal Tn-seq hits

Tn-seq analysis of transposon libraries constructed in WT *S. aureus* [aTB015] versus one constructed in a Δ*facZ* [aTB259] strain. P-values are from Mann-Whitney U test. Genes are sorted by increasing Δ*facZ:WT* relative enrichment ratio. Only the top 50-most de-enriched loci from the Δ*facZ* synthetic lethal analysis are displayed; see **Table S6** for a complete list of unannotated loci. Loci were identified with gene names and brief descriptions from AureoWiki. Genes previously described as having a role in cell wall biosynthesis, morphogenesis, cell division, and cell separation are highlighted in yellow.

84 **Table S8: *S. aureus* strains used in this study**

***S. aureus* strain**	**Background**	**Relevant genotype**	**Source**	**Construction notes**
aTB001	USA300	WT	Pang, T. et al.[3]	-
aTB003	HG003	WT	Pang, T. et al.[3]	-
aTB004	RN4220	WT	Pang, T. et al.[3]	-
aTB015	RN4220	*ΔattB(f11)::Orf5, pTM378*	Wang, H. et al.[4]	-
aTB016	RN4220	*ΔattB(f11)::Orf5, pTM381*	Wang, H. et al.[4]	-
aTB033	RN4220	pTP044	Pang, T. et al.[3]	-
aTB111	USA300	*Tn5::USA300_0932 (SAOUHSC_00965)*	Fey, P. et al.[2]	-
aTB112	USA300	*Tn5::USA300_1792 (SAOUHSC_01975)*	Fey, P. et al.[2]	-
aTB113	USA300	*Tn5::USA300_2094 (SAOUHSC_02383)*	Fey, P. et al.[2]	-
aTB209	HG003	*Δspa::kan*	Pang, T. et al.[3]	-
aTB219	HG004	*pLOW-ftsZ-GFP*	This study	pLOW-ftsZ-GFP transduced into aTB003
aTB243	RN4220	*ΔfacZ::specR*	This study	Homologous recombination of pMR91-*ΔfacZ::specR* into aTB004
aTB251	HG003	*ΔfacZ::specR*	This study	Φ85 lysate of aTB243 was used to transduce *ΔfacZ::specR* into aTB003
aTB259	RN4220	*ΔfacZ::specR ΔattB(f11)::Orf5, pTM378*	This study	Φ85 lysate of aTB243 was used to transduce *ΔfacZ::specR* into aTB015
aTB261	RN4220	*ΔfacZ::specR ΔattB(f11)::Orf5, pTM381*	This study	Φ85 lysate of aTB243 was used to transduce *ΔfacZ::specR* into aTB016
aTB263	RN4220	*pGL485*	This study	pGL485 was transformed into aTB004
aTB287	HG003	*ΔsagB (lytD)*	This study	Provided by Walker lab
aTB315	RN4220	*pGL485 pLOW-facZ*	This study	*pLOW-facZ* was transformed into aTB263
aTB316	RN4220	*pGL485 pLOW-facZ-mCherry*	This study	*pLOW-facZ-mCherry* was transformed into aTB263
aTB317	RN4220	*pGL485 pLOW-facZ-6xHis*	This study	*pLOW-facZ-6xHis* was transformed into aTB263
aTB339	RN4220	*pTP044 pTP63d-facZ*	This study	*pTP63d-facZ* was transformed into aTB044
aTB341	HG003	*pTP63d-facZ*	This study	Φ85 lysate of aTB339 was used to transduce *pTP63-facZ* into aTB003
aTB347	HG003	*pLOW-facZ*	This study	Φ85 lysate of aTB315 was used to transduce *pLOW-facZ* into aTB003
aTB348	HG003	*pLOW-facZ-mCherry*	This study	Φ85 lysate of aTB316 was used to transduce *pTP63-facZ-mCherry* into aTB003
aTB349	HG003	*pLOW-facZ-6xHis*	This study	Φ85 lysate of aTB317 was used to transduce *pTP63-6xHis* into aTB003
aTB356	HG003	*ΔfacZ::specR pLOW-facZ*	This study	Φ85 lysate of aTB243 was used to transduce *ΔfacZ::specR* into aTB347
aTB358	HG003	*ΔfacZ::specR pLOW-facZ-mCherry*	This study	Φ85 lysate of aTB243 was used to transduce *ΔfacZ::specR* into aTB348
aTB372	HG003	*ΔfacZ::specR pTP63d-facZ*	This study	Φ85 lysate of aTB243 was used to transduce *ΔfacZ::specR* into aTB341
aTB374	RN4220	*pTP044 pTP63d-facZ-mCherry*	This study	*pTP63d-facZ-mCherry* was transformed into aTB033
aTB376	RN4220	*pTP044 pTP63-ftsZ-gfp*	This study	*pTP63-ftsZ-gfp* was transformed into aTB033
aTB378	HG003	*ΔezrA pTP63b-ezrA ΔfacZ::specR*	This study	Φ85 lysate of aTB243 was used to transduce *ΔfacZ::specR* into aTB481
aTB390	HG003	*ΔfacZ::specR pTP63d-facZ pLOW-FtsZ-GFP*	This study	Φ85 lysate of *RN4220 pLOW-ftsZ-GFP* was transduced into aTP372
aTB391	HG003	*ΔezrA pTP63d-ezrA pLOW-FtsZ-GFP*	This study	Φ85 lysate of *RN4220 pLOW-ftsZ-GFP* was transduced into aTP481
aTB392	HG003	*pTP63d-facZ-mCherry*	This study	Φ85 lysate of aTB374 used to transduce *pTP63-facZ-mCherry* into aTB003

aTB394	HG003	*pTP63d-ftsZ-GFP*	This study	ϕ85 lysate of aTB376 used to transduce *pTP63-ftsZ-GFP* into aTB003
aTB411	HG003	*Δspa::kan pLOW-01855-6xHis*	This study	Transformed *pLOW-01855-6xHis* from RN4220 into aTB209
aTB453	HG003	*ΔfacZ::specR gpsB (T)6→5 (truncation 187)*	This study	Spontaneous suppressor of aTB251 sensitivity to PC190723
aTB476	HG003	*ΔfacZ::specR gpsB(Y26*)*	This study	Spontaneous suppressor of aTB251 sensitivity to PC190723
aTB478	HG003	*ΔfacZ::specR gpsB (T)6-5 (trunc. 128)*	This study	Spontaneous suppressor of aTB251 sensitivity to PC190723
aTB492	HG003	*gpsB::Tn5*	This study	ϕ85 lysate of NTML strain *gpsB::Tn5(erm)* transduced into aTB003
aTB497	HG003	*gpsB::Tn5 ΔfacZ::specR*	This study	ϕ85 lysate of aTB243 was used to transduce Δ*facZ::specR* into aTB492
aTB513	HG003	*Δspa::kan pLOW-01855 pTP63-gpsB-FLAG*	This study	Transformed *pLOW-01855* from RN4220 into aTB209
aTB514	HG003	*Δspa::kan pLOW-01855-6xHis pTP63-gpsB-FLAG*	This study	Transformed *pLOW-01855-6xHis* from RN4220 into aTB209
aTB515	HG003	*pTP63-gpsB-mNeon*	This study	ϕ85 lysate of AGS063 was used to transduce *pTP63-gpsB-mNeon* into aTB003
aTB517	HG003	*pTP63-gpsB-mNeon pLOW-01855-mCherry*	This study	ϕ85 lysate of aTB316 was used to transduce *pLOW-facZ-mCherry* into aTB515
aTB519	HG003	*pTP63-gpsB-mNeon ΔfacZ::specR*	This study	ϕ85 lysate of aTB243 was used to transduce Δ*facZ::specR* into aTB515
aTB521	HG003	*pKK30_RFP*	This study	ϕ85 lysate of RN4220 *pKK30_RFP* was used to transduce pKK30_RFP into aTB003
aTB525	HG003	*ΔgpsB::Kan*	This study	ϕ85 lysate of TAS201 was used to transduce Δ*gpsB::Kan* into aTB003
aTB527	HG003	*ΔfacZ::specR pKK30_RFP*	This study	ϕ85 lysate of aTB521 was used to transduce pKK30_RFP into aTB251
aTB529	HG003	*ΔgpsB::kanR pKK30_RFP*	This study	ϕ85 lysate of aTB521 was used to transduce pKK30_RFP into TAS201
aTB540	HG003	*ΔfacZ::specR pTP63-facZ ΔgpsB::kanR*	This study	ϕ85 lysate of TAS201 was used to transduce Δ*gpsB::kanR* into aTB372
aTB542	HG003	*ΔfacZ::specR ΔgpsB::kanR pKK30-RFP*	This study	ϕ85 lysate of aTB243 was used to transduce Δ*facZ::specR* into aTB529
aTB549	HG003	*ΔfacZ::specR pTP63-facZ pKK30-RFP*	This study	ϕ85 lysate of aTB521 was used to transduce *pKK30_RFP* into aTB372
aTB565	RN4220	*pLOW-facZ(3R-3D)-6xHis ΔfacZ*	This study	Plasmid transformed into aTB243
aTB568	RN4220	*pLOW-facZ(R135D)-6xHis ΔfacZ*	This study	Plasmid transformed into aTB243
aTB570	RN4220	*pLOW-facZ(R138D)-6xHis ΔfacZ*	This study	Plasmid transformed into aTB243
aTB572	RN4220	*pLOW-facZ(R139D)-6xHis ΔfacZ*	This study	Plasmid transformed into aTB243
aTB574	RN4220	*pLOW-facZ(R160D)-6xHis ΔfacZ*	This study	Plasmid transformed into aTB243
aTB632	HG003	*pTP63-gpsB-FLAG*	This study	Provided by Walker lab
aTB643	RN4220	*pLOW-ftsW-GFP*	This study	*pLOW-ftsW-GFP* transformed into aTB004
aTB645	RN4220	*pLOW-GFP-pbpA*	This study	*pLOW-GFP-pbpA* transformed into aTB004
aTB649	HG003	*pTP63-gpsB-FLAG ΔfacZ::specR*	This study	ϕ85 lysate of aTB243 was used to transduce Δ*facZ::specR* into aTB632
aTB651	HG003	*pLOW-facZ(3R-3D)-6xHis*	This study	ϕ85 lysate of aTB565 transduced into aTB251
aTB663	HG003	*gpsB::Tn5 ΔezrA::kanR pTP63-ezrA*	This study	ϕ85 lysate of NTML strain *gpsB::Tn5(erm)* transduced into aTB264
aTB665	HG003	*pLOW-GFP-pbpA*	This study	ϕ85 lysate of aTB645 was used to transduce *pLOW-GFP-pbpA* into aTB251
aTB666	HG003	*pLOW-ftsW-GFP*	This study	ϕ85 lysate of aTB643 was used to transduce *pLOW-ftsW-GFP* into aTB251
aTB673	HG003	*ΔfacZ pLOW-GFP-pbpA*	This study	*pLOW-GFP-pbpA* purified from aTB645 and transformed into aTB251
aTB675	HG003	*ΔfacZ pLOW-ftsW-GFP*	This study	*pLOW-ftsW-GFP* purified from aTB643 and transformed into aTB251
aTB679	HG003	gpsB::Tn5 ΔezrA::kanR pTP63-ezrA ΔfacZ	This study	ϕ85 lysate of aTB243 was used to transduce Δ*facZ::specR* into aTB663
aTP071	RN4220	pTP10 integrant	This study	pTP10 transformed and integrated into RN4220; cloning intermediate for Δ*atl*

aTP103	HG003	Δ*atl*	This study	ϕ85 lysate of aTP071 was used to transduce Δ*atl::kanR* into HG003
aTP436	RN4220	pTP044 pTP63-ezrA	This study	pTP63-ezrA transformed into RN4220 pTP044
aTP455	RN4220	pTP89 integrant	This study	pTP10 transformed and integrated into RN4220; cloning intermediate for Δ*ezrA*
aTP481	HG003	Δ*ezrA pTP63-ezrA*	This study	Unpublished (Rudner collection)
TAS201	RN4220	Δ*gpsB::Kan*	This study	Provided by Walker lab
TAS079	RN4220	*pLOW-gpsB-FLAG*	This study	Provided by Walker lab
AGS063	RN4220	*pTP63-gpsB-mNeon*	This study	Provided by Walker lab
SB100	RN4220	*pKK30-RFP*	This study	Provided by Walker lab

All *S. aureus* strains used in the course of this study are noted in this table. See Methods section for a description of how strains were made.

Table S9: *B. subtilis* strains used in this study

***B. subtilis* strain**	**Relevant Genotype**	**Source**	**Construction Notes**
bDR11	*WT*	Youngman, P. et al.[5]	-
bDR2229	*amyE::P_{spac}-ftsZ-gfp*	Ben-Yehuda, S. & Losick, R.[6]	-
bDR2637	*sacA::Pveg-mCherry(phleo)*	Roney, I. & Rudner, D.[7]	-
bDR2660	*sacA::Pveg-BFP(phleo)*	Roney, I. & Rudner, D.[7]	-
bDR2789	*sacA::Pveg-GFP(phleo)*	Roney, I. & Rudner, D.[7]	-
bTB013	Δ*facZ::ermR*	This study	Marker crossed from deletion library into bDR11
bTB018	*amyE::Pspac-ftsZ-gfp* Δ*facZ::ermR*	This study	Marker crossed from deletion library into bDR2229
bTB039	*sacA::Pveg-GFP(phleo)* Δ*facZ::erm*	This study	Marker crossed from deletion library into bDR2789
bTB040	*sacA::Pveg-BFP(phleo)* Δ*facZ::erm*	This study	Marker crossed from deletion library into bDR2660
bTB041	*sacA::Pveg-BFP(phleo)* Δ*facZ::erm* Δ*gpsB::spec*	This study	Marker crossed from deletion library into bTB040
bTB044	*sacA::Pveg-GFP(phleo)* Δ*gpsB::spec*	This study	Marker crossed from deletion library into bDR2789

All *B. subtilis* strains used in the course of this study are noted in this table. See Methods section for a description of how strains were made.

91 **Table S10: Plasmids used in this study**

Plasmid	Relevant genotype & description	Marker	Source	Cut Sites/ ITA	Oligos
pTM378	ts origin, HMAR1 C9 transposase	Kan	Wang, H. et al.[4]	-	-
pTM381	ts origin, HMAR1 C9 transposase (truncated)	Kan	Wang, H. et al.[4]	-	-
pTP044	L54a integrase-bearing plasmid	Tet	Pang, T. et al.[3]	-	-
pLOW-ftsZ-GFP	*ftsZ-gfp* under P_{spac} promoter	Erm	Liew, A. et al.[8]	-	-
pLOW-GFP-ftsW	*gfp-ftsW* under P_{spac} promoter	Erm	This study	SalI/BamHI	oTB689-692
pLOW-pbp1-GFP	*pbp1-gfp* under P_{spac} promoter	Erm	This study	SalI/BamHI	oTB699-702
pGL485	High-copy plasmid constitutively expressing LacI	Cm	Liew, A. et al.[8]	-	-
pKK30	dsRed, constitutively expressed red fluorescence	Tmp	Rodriguez, M. et al. [9]	-	-
pLOW-facZ	*facZ* under P_{spac} promoter	Erm	Synthesized	-	-
pLOW-facZ-mCherry	*facZ-mCherry* under P_{spac} promoter	Erm	Synthesized	-	-
pLOW-facZ-6xHis	*facZ-6xHis* under P_{spac} promoter	Erm	Synthesized	-	-
pLOW-facZ(R135D)-6xHis	*facZ(R135D)-6xHis* under P_{spac} promoter	Erm	Synthesized	-	-
pLOW-facZ(R138D)-6xHis	*facZ(R138D)-6xHis* under P_{spac} promoter	Erm	Synthesized	-	-
pLOW-facZ(R139D)-6xHis	*facZ(R139D)-6xHis* under P_{spac} promoter	Erm	Synthesized	-	-
pLOW-facZ(R160D)-6xHis	*facZ(R160D)-6xHis* under P_{spac} promoter	Erm	Synthesized	-	-
pLOW-facZ(3R-3D)-6xHis	*facZ(R135D, R138D, R160D)-6xHis* under P_{spac} promoter	Erm	Synthesized	-	-
pLOW-gpsB-GFP	*gpsB-mNeon* under P_{spac} promoter	Erm	Walker lab	-	-
pTP63-lacZ	*lacZ* under P_{tet} promoter	Cm	Pang, T. et al.[3]	-	-
pTP63-ezrA	*ezrA* under P_{tet} promoter	Cm	Rudner lab, unpublished	-	-
pTP63-facZ	*facZ* under P_{tet} promoter	Cm	This study	KpnI/EcoRI	oTB561, 562
pTP63-facZ-mCherry	*facZ-mCherry* under P_{tet} promoter	Cm	This study	KpnI/EcoRI	oTB563, 564
pTP63-gpsB-mNeon	*gpsB-mNeon-FLAG* under P_{tet} promoter	Cm	This study	ITA	AGS059-064
pTP63-ftsZ-GFP	*ftsZ-GFP* under P_{tet} promoter	Cm	This study	KpnI/EcoRI	oTB566, 568
pMR91-ΔfacZ::specR	1.5 kb flanking sequence of *facZ* interrupted with *specR* marker, constitutive mScarlett, ts origin	Erm, Spec	This study	ITA	oTB490, 491, 492, 493
pLOW-gpsB-FLAG	*gpsB-FLAG* under P_{spac} promoter	Erm	This study	SalI/BamHI	oTS079, 80
pSUMO-FacZ 3x(127-146)	Purification plasmid for His-SUMO-tagged $FacZ_{(127-146)}$	Kan	This study	ITA	oRWB137, oRWB138

pSUMO-GpsB (1-75)	Purification plasmid for His-SUMO-tagged GpsB $FacZ_{(1-75)}$	Kan	This study	ITA	oRWB123, oRWB124

All plasmids produced in the course of this study were made by isothermal assembly (ITA) or restriction digest and ligation using pairs of restriction enzymes, as noted in this table. Plasmids were isolated from and are stored in either DH5α or BL21(DE3) *E. coli* strains, and are available upon request.

Table S11: Oligos used in this study **Oligo**	**Name**	**Sequence**	**Purpose**
oTB045	SpecR Seq F3	TGATTCCACGGTACCATTTCTTGC	Outward-facing sequencing primer for downstream homology arm of Δ*facZ::specR*
oTB065	SpecR Seq R4	AGTGCTCCCTGatGTCgacc	Outward-facing sequencing primer for upstream homology arm of Δ*facZ::specR*
oTB169	pMR091 MCS seq F	GACTTTACGAAACACGGAAACCG	Inward-facing sequencing primer for upstream homology arm of Δ*facZ::specR*
oTB170	pMR091 MCS seq R	atcagttcattgctcacgatatgtg	Inward-facing sequencing primer for downstream homology arm of Δ*facZ::specR*
oTB192	EzrA cPCR F	CATCTTCAATAAGGCTTGCTGC	Colony PCR for *ezrA* deletion
oTB193	EzrA cPCR R	CATCAGTCCAATTTGACAGAGTGC	Colony PCR for *ezrA* deletion
oTB410	pTP63_cPCR_F	CTCATTAAGCAGCTCTAATGCGC	Colony PCR for *pTP63* insert
oTB411	pTP63_cPCR_R	CCAGCGTTTCTGGGTGAGC	Colony PCR for *pTP63* insert
oTB416	del_Atl_cPCR_F1	GGCGAAGTCGGCAAATACTTCG	Colony PCR for *atl* deletion
oTB417	del_Atl_cPCR_R1	CGACGCATATCGTTGTAACACG	Colony PCR for *atl* deletion

oTB490	pMR091 01855 UpF	CGCTCGCGTATCGGTGATGGATCCCGATCAATTAGAGCAACTCGGTTATGTTTCG	Generate upstream flanking region of *facZ* for ITA
oTB491	pMR091 01855 UpR	cccggaaaaagagttgactaaatcaaTAAAAACGCCTCCTAATTAACATGTAATAATGTC	Generate upstream flanking region of *facZ* for ITA
oTB492	pMR091 01855 DnF	ccggtctatgttcatttagtcctccactaTTAATAATTAAACAAATGCACTTAAATGAGGTTGTTAC	Generate downstream flanking region of *facZ* for ITA
oTB493	pMR091 01855 DnR	gctctatataaaatatactcaaaatattatCCATGGtatGAATTCGCATTTGTTGTTTTTGAATTCTTATTACTAGTTTG	Generate downstream flanking region of *facZ* for ITA
oTB494	01855 up out F	GATGATGTTTGTGCATTTATGGTTAATGAAGG	Δ*facZ* sequencing primer
oTB495	01855 up in F	GATAATGCTGTTATTTTATTTATGGGTGCAGG	Δ*facZ* sequencing primer
oTB496	01855 dn in R	GTTGCTGCGTCATTTGTATATCCTCC	Δ*facZ* sequencing primer
oTB497	01855 dn out R	CATAGCTTACTGCTATGATTGATTATTCAACG	Δ*facZ* sequencing primer
oTB498	Spec Out Seq R	CAATAAACCCTTGCATAGGGATAACTTCG	Δ*facZ* sequencing primer
oTB499	Spec Out Seq F	CGTTACGTTATTAGTTATAGTTATTATAACATGTATTCACG	Δ*facZ* sequencing primer
oTB500	Spec Out Seq R2	GCAGTTCGTAGTTATCTTGGAGAGAATATTGAATG	Δ*facZ* sequencing primer
oTB501	Spec Out Seq F2	GTGTAAACCTATTCATTGTTTTAAAAATATCTCTTGCC	Δ*facZ* sequencing primer
oTB513	01855 outside f	GTTGATTTTATCAAACAACAAAGAGAACCGG	Colony PCR for *facZ* deletion
oTB514	01855 outside r	GGTAAGCACCTGAATGCCTACC	Colony PCR for *facZ* deletion
oTB538	pLOW MCS seq f1	GACTTTATCTACAAGGTGTGGC	Colony PCR *for pLOW* insert
oTB539	pLOW MCS seq f2	atcctctagagtcaattgtgagcgc	Colony PCR for *pLOW* insert
oTB535	PolyG-1st-1 primer	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGGGGGGGGGGGGG	Tn-seq primer (first PCR)
oTB536	Mariner PCR1	GCCATCTATGTGTCTAGAGAC	Tn-seq primer (first PCR)

oTB537	Rnd2_Staph_IL	AATGATACGGCGACCACCGAGATCTACACTCTTTCGGGGACTTATCAGCCAACCTG	Tn-seq primer (second PCR)
oTB538	pLOW MCS seq f1	GACTTTATCTACAAGGTGTGGC	Colony PCR/sequencing primer for *pLOW* constructs
oTB540	pLOW MCS seq r1	TTCAGGCTGCGCAACTGTTG	Colony PCR/sequencing primer for *pLOW* constructs
oTB545	pGL485 fwd	taatgtATCGATaataatggtttcttagacg	Colony PCR for *pGL485*
oTB546	pGL485 rev	tattatGTCGACagtcggcattatctc	Colony PCR for *pGL485*
oTB561	pTP063d-01855 f	acaTAAGGAGGaGGTACCatgGATTGGATTTTACCAATTGCTGG	Subclone *facZ* into pTP63 vector; KpnI cut site
oTB562	pTP063d-01855 r	CCACCTGGAATTCttaTTTATCTACTCTAGAAGTATAGCTATGATTTGCATCAGTTGC	Subclone *facZ* into pTP63 vector; EcoRI cut site
oTB563	pTP063d-01855-mCh f	AGGAGGaGGTACCatgGATTGGATTTTACCAATTGCTGG	Subclone *facZ-mCherry* into pTP63 vector; KpnI cut site
oTB564	pTP063d-01855-mCh r	CACCTGGAATTCCttaGGATCCGCCAGCACCTTTG	Subclone *facZ-mCherry* into pTP63 vector; EcoRI cut site
oTB566	FtsZ-GFP KpnI F	GGAGGaGGTACCATGTTAGAATTTGAACAAGGATTTAATCATTTAGCG	Subclone *ftsZ-GFP* into pTP63 vector; KpnI cut site
oTB568	FtsZ-GFP EcoRI R	GAACGTCTTTCTTCTCTATTTCTAATGAAGC	Subclone *ftsZ-GFP* into pTP63 vector; EcoRI cut site
oTB595	GpsB cPCR F	GTTCTTCAAGCAGATGTTAGTTGATTTTATGG	Colony PCR for inactivation of *gpsB*
oTB596	GpsB cPCR R	GGACTTTCCTCTATATAATATAGCGATTACCC	Colony PCR for inactivation of *gpsB*
oTB597	GpsB Seq F	GTGATAAATTAAAAAATGTAGGAGGCGTCC	Colony PCR for inactivation of *gpsB*
oTB598	GpsB Seq R	CTTTCTTAGTTATCGCCTGACAATCTGGC	Colony PCR for inactivation of *gpsB*

oTB599	GpsB cPCR F	GGATAAAACAAACTATACTTGTGATATTGTG	Colony PCR for inactivation of *gpsB*
oTB600	GpsB cPCR R	CAATCATCTCAGACTGTGTGAGC	Colony PCR for inactivation of *gpsB*
oTB689	pLOW GFP Nterm ITA F	cctgcaggcatgcctgcagGTCGACacaTAAGGAGGaGGTACCATGAGTAAAGGAGAAGAACTTTTCACTGG	Generate GFP fragment for ITA of GFP-Pbp1
oTB690	pLOW GFP Nterm ITA R	CAGACCAGCCGGACCCTCGAGTTTGTATAGTTCATCCATGCCATGTGTAATCC	Generate GFP fragment for ITA of GFP-Pbp1
oTB691	Linker-PBP1 ITA F	CTCGAGGGTCCGGCTGGTCTGATGGCGAAGCAAAAAATTAAAATTAAAAAAAATAAAATAGGGGC	Generate Pbp1 fragment for ITA of GFP-Pbp1
oTB692	PBP1-pLOW ITA R	GAATTCgagctcgcccggGGATCCTTAGTCCGACTTATCCTTGTCAGTTTTACTGTCAG	Generate Pbp1 fragment for ITA of GFP-Pbp1
oTB699	GFP pLOW Cterm ITA F	ggtggtagtggtggtagtggtggtATGAGTAAAGGAGAAGAACTTTTCACTGG	Generate GFP fragment for ITA of FtsW-GFP
oTB700	GFP pLOW Cterm ITA R	gccagtGAATTCgagctcgcccggGGATCCTTATTTGTATAGTTCATCCATGCCATGTGTAATCCC	Generate GFP fragment for ITA of FtsW-GFP
oTB701	pLOW-ftsW ITA F	cctgcaggcatgcctgcagGTCGACacaTAAGGAGGaGGTACCatgAAGAATTTTAGAAGTATTTTACGGTATATTGGTAAAACC	Generate FtsW fragment for ITA of FtsW-GFP
oTB702	ftsW-linker ITA R	CTCATaccaccactaccaccactaccaccATTAAATTGTCTTCTTATATCAACTTTTTGTTGTTGTTTCTTTCG	Generate FtsW fragment for ITA of FtsW-GFP
AGS59	pTP63 backbone F	GAATTCCAGGTGGCACTTTTCG	Amplification of pTP63
AGS60	pTP63 backbone R	GGTACCATCATACTCTATCAATGA	Amplification of pTP63
AGS61	GpsB F	GAGTATGATGGTACCgtttctaatgaggtggaaaaaATGTCAGATGTTTCATTGAAAT	Generate GpsB fragment for ITA of GpsB-mNeon
AGS62	GpsB R	AGCTCCACCAGCGCTACCACCACCTTTACCAAATACAGCTTTTTCT	Generate GpsB fragment for ITA of GpsB-mNeon
AGS63	mNeonGreen F	AGCGCTGGTGGAGCTGTGAGTAAAGGTGAGGAGGA	Generate mNeon fragment for ITA of GpsB-mNeon

AGS64	mNeonGreen R	TGCCACCTGGAATTCTTActtgtcgtcatcgtctttgtagtcTTTATACAACTCATCCATTCCCAT	Generate mNeon fragment for ITA of GpsB-mNeon
oTS079	rbs_{rpoB}-GpsB F	GCAGGTCGACCATAATTTTTGAGGGGTGAATCTGTATGTCAGATGTTTCATTGAAATTATCA	Generate GpsB-FLAG insert
oTS080	GpsB-FLAG	CCCGGGGATCCTTACTTGTCGTCATCGTCTTTGTAG	Generate GpsB-FLAG insert
AMo67	gpsB_Wanner_KO_30H_U_o67	AAGATCAAAGTTTCTAATGAGGTGGAAAAA CATAAAAACAACTCGTAGCTTATCAAAG	Generate fragment for amplifying KanR marker (for deleting *gpsB*)
AMo68	gpsB_Wanner_KO_30H_D_o68	GTAAGACAGTTAAACTTTTGTATTTAGTAA CAATGACCTAAGAGGTGTGG	Generate fragment for amplifying KanR marker (for deleting *gpsB*)
oRWB123	GpsB SUMO Fwd	TGAACAGATTGGCGGCATGTCAG	Insert for pSUMO-FacZ 3x(127-146)
oRWB124	GpsB 75 SUMO Rev	catggatccttattacgtagcaacacgaaggcgaag	Insert for pSUMO-FacZ 3x(127-146)
oRWB125	Sumo-FacZ 127 Fwd	TgaacagattggcggcGAAATTGCAGATAAGTGGCAA	Insert for pSUMO-GpsB(1-75)
oRWB126	Sumo-FacZ 145 Rev	ccatggatccttattaTGCCTTGTAGTTTGCAGATCC	Insert for pSUMO-GpsB(1-75)

All oligos used in the course of this study are noted in this table.

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