# Supporting Information

## Meeske et al. 10.1073/pnas.1504967112

### SI Experimental Procedures

**Strain Construction.** For gene deletion mutants that are derived from the *Bacillus subtilis* knockout collection, each deletion was confirmed by PCR using oKO000 (within the erythromycin resistance cassette) and an upstream gene-specific primer: $murJ_{Bs}$ (oAM73), *yabM* (oAM65), *ykvU* (oAM77), *spoVB* (oAM81), *dinF* (oKO3), *yoeA* (oKO5), *yisQ* (oKO7), *norM* (oKO9), *epsK* (oKO11), *sigM* (oKO82), *amj* (oKO83), *minJ* (oKO84), or *divIVA* (oKO85).

All other deletion mutants were generated by isothermal assembly (1) with three PCR products: an antibiotic resistance cassette and upstream and downstream fragments that flank the locus to be deleted. Spectinomycin and tetracycline resistance cassettes, flanked by lox66 and lox71 sites, were amplified by PCR with oJM28 and oJM29 from pWX466 and pWX469, respectively. For the *murG* deletion, an erythromycin resistance cassette lacking a transcriptional terminator (amplified using oligonucleotide primers oDR1049 and oDR1050 from pDR242) was used. Upstream and downstream PCR products were amplified from *B. subtilis* 168 genomic DNA using the following oligonucleotide primers: $murJ_{Bs}$ (oAM73, oAM74, oAM75, or oAM76), *tuaB* (oKO001, oAM295, oAM296, or oKO002), *murG* (oAM316, oAM317, oAM318, or oAM319), and *tua* operon (oAM320, oAM321, oAM324, or oAM325).

Strain CS4 (λred *bla-araC*-$P_{BAD}$::*murJ*) was constructed as described by Ruiz (2). A PCR product harboring *bla*, *araC*, and the arabinose promoter was generated using primers CS50 and CS51, and plasmid pKD46 (3) as a DNA template. The resulting product contained 30-bp flanking regions of the murJ promoter. After PCR purification, the fragment was electroporated into strain TB10, selecting for ampicillin resistance in the presence of arabinose. A P1 lysate was prepared from strain CS4 and used to construct strain CS7 (TB28 *bla-araC*-$P_{BAD}$::*murJ*) by transduction into strain TB28.

The *E. coli* $murJ_{Ec}$(A29C) allele was constructed essentially as described by Tsang and Bernhardt (4). Briefly, strain CS34 [TB28 $murJ_{Ec}$(A29C)/pTD29] was constructed by allelic exchange using a suicide plasmid harboring *sacB* (5). Overnight culture of the donor strain SM10/pCS89 [$murJ_{Ec}$(A29C) *sacB cat*] was mixed in equal volume with 100 μL of overnight culture of recipient strain TB28/pTD29, followed by the addition of 200 μL of LB. The mixture was incubated statically at 37 °C for 6 h, serially diluted, and plated on LB supplemented with 50 μg/mL spectinomycin and 25 μg/mL chloramphenicol. One colony was picked and dispersed in 50 μL of 10 mM $MgSO_4$. Excision of the pCS89 plasmid from the resulting strain was done by plating serial dilutions of the colony suspension on M9 medium supplemented with 0.2% (wt/vol) maltose and 6% (wt/vol) sucrose. Colonies on the sucrose plate were screened for chloramphenicol sensitivity, spectinomycin, and sucrose resistance. Isolates were then tested for MTSES sensitivity. The presence of $murJ_{Ec}$(A29C) was further confirmed by PCR and sequencing. *lysA*::*kan* was introduced into strain CS34/pTD29 by P1 transduction to generate strain CAM274 [$murJ_{Ec}$(*A29C*) *lysA*::*kan*]/pTD29. Plasmid pTD29 was then cured by propagation without selection to produce strain CAM274. Finally, plasmids pTD29 (*Plac-gfp spec*), pCS81 (*Plac-*$murJ_{Bs}$ *spec*), pCS83 (*Plac-amj spec*), and pAM137 (*Plac-*$murJ_{Ec}$ *spec*) were transformed into CAM274.

**Antibiotic Cassette Loop-Out.** Strains with loxP-flanked antibiotic cassettes were transformed with pWX496 (temperature-sensitive plasmid with constitutively expressed Cre recombinase marked with a *kan* resistance gene). Transformants were selected on LB supplemented with 10 μg/mL kanamycin at 30 °C, a permissive temperature for pWX496 replication. Transformants were then streaked on LB and incubated at 42 °C, a nonpermissive temperature for plasmid replication. Single colonies were then restreaked on LB, LB(kan), and LB[macrolide-lincosamide-streptogramin B (MLS)], and incubated 37 °C. Strains that grew on LB, but not LB(kan) or LB(MLS), had lost pWX496 and the erythromycin resistance cassette. Markerless deletions were confirmed by PCR with oligonucleotide primers flanking the deletion.

**Plasmid Construction.** pAM126 (*ycgO::*$P_{spank}$*-murG cat*) was generated by ligation with a HindIII-NheI *murG* PCR product (amplified with oligonucleotide primers oAM314 and oAM315 from 168 genomic DNA) and pAM012 (*ycgO::Pspank* cat*). This plasmid contains the weak IPTG-inducible promoter *Pspank**, also called *Pspank* (*T-7A*) (6).

pAM131 (*ycgO::*$P_{spank}$*-amj cat*) was generated by ligation with a HindIII-NheI *amj* PCR product (amplified with oligonucleotide primers oAM367 and oAM368 from 168 genomic DNA) and pAM011 (*ycgO::Pspank cat*). This plasmid contains the medium-strength IPTG-inducible promoter *Pspank*.

pAM132 (*ycgO::*$P_{spank}$**-amj cat*) was generated by ligation with a HindIII-NheI *amj* PCR product (amplified with oligonucleotide primers oAM367 and oAM368 from 168 genomic DNA) and pAM012 (*ycgO::Pspank* cat*). This plasmid contains the weak IPTG-inducible promoter *Pspank**, also called *Pspank* (*T-7A*) (6).

pCS81 ($P_{lac}$-$murJ_{Bs}$ *spec*) was generated by isothermal assembly of *ytgP* (amplified with oligonucleotide primers CS152 and CS153) and pTD29 ($P_{lac}$-*gfp spec lacI* pSC101 *ori*) (amplified with oligonucleotide primers CS142 and CS143).

pCS82 ($P_{lac}$-*ykvU spec*) was generated by isothermal assembly of *ykvU* (amplified with oligonucleotide primers CS150 and CS151) and pTD29 (amplified with oligonucleotide primers CS142 and CS143).

pCS83 ($P_{lac}$-*amj spec*) was generated by isothermal assembly of *amj* (amplified with oligonucleotide primers CS148 and CS149) and pTD29 (amplified with oligonucleotide primers CS142 and CS143).

pCS84 ($P_{lac}$-*yabM spec*) was generated by isothermal assembly of *yabM* (amplified with oligonucleotide primers CS146 and CS147) and pTD29 (amplified with oligonucleotide primers CS142 and CS143).

pCS85 ($P_{lac}$-*spoVB spec*) was generated by isothermal assembly of *spoVB* (amplified with oligonucleotide primers CS144 and CS145) and pTD29 (amplified with oligonucleotide primers CS142 and CS143).

pCS89 [*yceM-*$murJ_{Ec}$*(A29C)-flgN sacB cat*] was generated by ligation with a SacI-XbaI PCR product (amplified first with oligonucleotide primers CS171 and CS168 and CS172 and CS169, and then with CS171 and CS172) with pMT17 cut with SacI and XbaI.

pAM157 (*Plac-*$murJ_{Ec}$ *spec*) was generated by isothermal assembly of $murJ_{Ec}$ (amplified with oligonucleotide primers oAM412 and oAM413) and pTD29 (amplified with oligonucleotide primers CS142 and CS143).

pAM147 (*amyE::*$P_{amj}$*-lacZ cat*) was generated by ligation with an EcoRI-BamHI *Pamj* PCR product (amplified with oligonucleotide primers oAM399 and oAM400 from 168 genomic DNA) and pDG1661 (*amyE::lacZ cat*) (7).

pAM149 (*amyE::$P_{sigM}$-lacZ cat*) was generated by ligation with an EcoRI-BamHI *PsigM* PCR product (amplified with oligonucleotide primers oAM403 and oAM404 from 168 genomic DNA) and pDG1661 (*amyE::lacZ cat*).

pAM155 (*amyE::$P_{amj}$-yfp cat*) was generated by ligation with an EcoRI-HindIII *Pamj* PCR product (amplified with oligonucleotide primers oAM399 and oAM414 from 168 genomic DNA) and pNS008 (*amyE::PspoIIQ-yfp cat*) (8).

pWX496 [*$P_{PA}$-cre-ori*(*ts*) *kan*] was generated by a three-way ligation with an EcoR-SpeI fragment from pDR244, an EcoRI-BamHI fragment from pDR243, and a BamHI-SpeI PCR product containing the *kan* gene (amplified with oligonucleotide primers oWX292 and oWX293 from pKM373). pDR244 [*$P_{PA}$-cre-ori*(*ts*) *spec*] was generated by ligation with a SpeI-EcoRI PCR product containing *$P_{PA}$-cre* (amplified with oligonucleotide primers oDR946 and oDR947 from *$P_{PA}$-cre* plasmid DNA) (9) and pDR243 cut with SpeI and EcoRI. pDR243 [*ori*(*ts*) *spec*] was generated by ligation with an EagI-BamHI PCR product containing a temperature-sensitive origin (amplified with oligonucleotide primers oDR944 and oDR945 from pE194 plasmid DNA) and pWX297 (a pACYC-based plasmid containing *spec*).

**Tn-Seq.** Tn-seq was performed as described previously (10, 11). Briefly, Magellan6 transposon library DNA was transformed into competent *B. subtilis* cells. Approximately 500,000 transformants were pooled, and genomic DNA was isolated. Samples were digested with MmeI, followed by adapter ligation. Transposon–chromosome junctions were amplified in 16 PCR cycles. PCR products were gel-purified and sequenced on the Illumina HiSeq platform using TruSeq reagents (Tufts University Core Facility Genomics).

**Fluorescence Microscopy.** Exponentially growing cells were concentrated by centrifugation at 6,800 × *g* for 1 min and immobilized on 2% (wt/vol) agarose pads containing growth medium. Fluorescence microscopy was performed using an Olympus BX61 microscope equipped with an UplanF1 100× phase-contrast objective lens and a CoolSnapHQ digital camera (Photometrics) or a Nikon TE2000 inverted microscope with a Nikon CFI Plan Apo VC 100× objective lens. Images were acquired using Metamorph or Nikon Elements software. Membranes were stained with 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate (TMA-DPH) (50 μM; Molecular Probes). Exposure times were 400 ms for TMA-DPH and mCherry. Image analysis and processing was performed in Fiji.

**Colicin M Susceptibility Assay.** Lipid II flippase activity in *E. coli* was measured as described previously (12). Briefly, cells were grown in M9 medium (supplemented with maltose, Met, Thr, and Lys) at 37 °C to an $OD_{600}$ of 0.3. [$^3$H]-*m*-DAP (American Radiolabeled Chemicals) was added at a final concentration of 1.5 μCi/mL to label PG precursors. Cells were treated 15 min later with 50 μM MTSES and/or 50 ng/mL purified colicin M. After 10 min, cells were pelleted and resuspended in boiling water for 30 min to extract soluble colicin M cleavage products. Samples were centrifuged at 100,000 × *g* for 20 min at 4 °C, and the supernatant was collected, lyophilized, and analyzed by RP-HPLC. Colicin M cleavage products were measured using an inline radioflow detector and quantified using Chromeleon software (Dionex). To measure lipid-linked precursor levels, the hot-water–insoluble pellet was washed, resuspended in 100 μL of 10 mM Tris·HCl (pH 7.4), to which an equal volume of 6 M pyridinium acetate/1-butanol (1:2 vol/vol) was added. This mixture was vortexed and centrifuged at 3,000 × *g* for 30 s at room temperature. The organic layer was transferred to a new tube and washed once with 100 μL of butanol-saturated water, the extraction was repeated three times, and organic fractions were pooled. One hundred microliters of extract was added to 10 mL of Ecolite (MP Biomedicals) scintillation fluid for scintillation counting.


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8. Sullivan NL, Marquis KA, Rudner DZ (2009) Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. *Cell* 137(4): 697–707.
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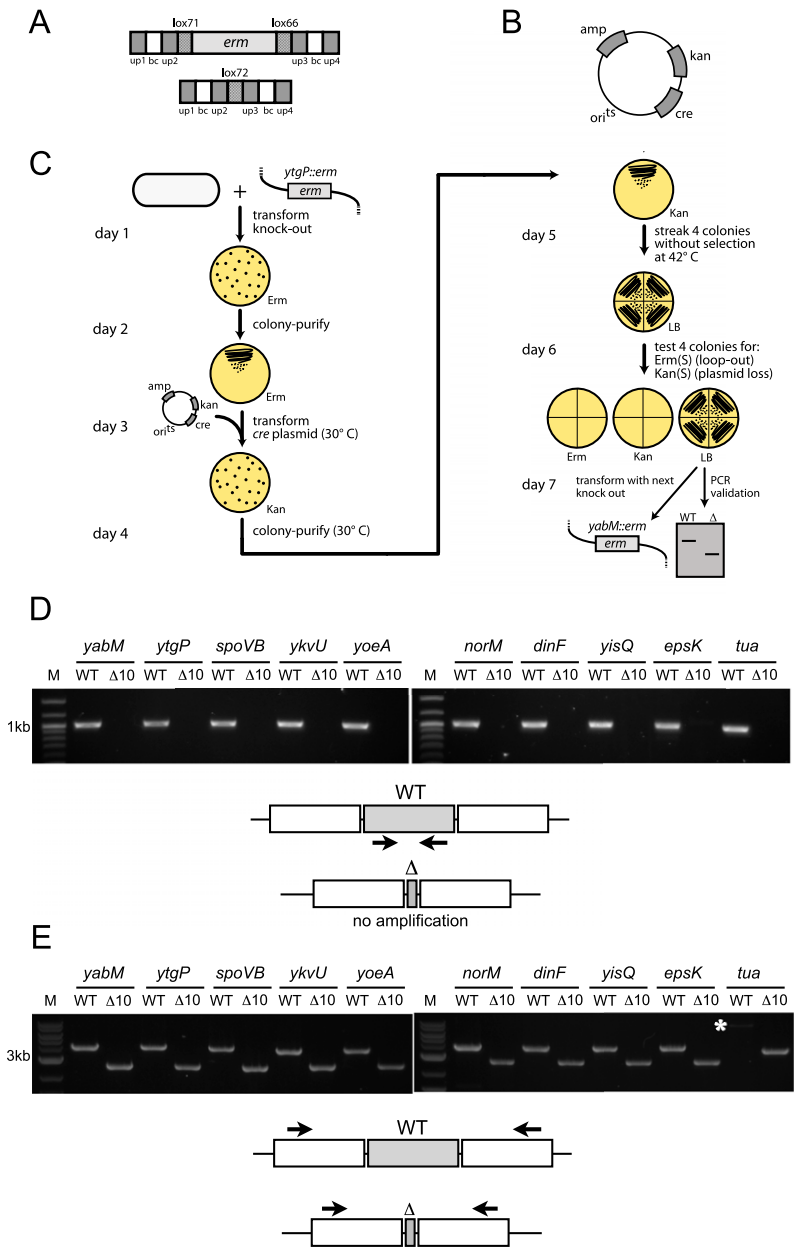


**Fig. S1.** Flow diagram for generating the decuple (Δ10) deletion mutant and confirmation of deletions by PCR. (*A*) Schematic diagram of the deletion cassette before and after removal of the antibiotic resistance gene. The erythromycin resistance gene (*erm*) is flanked by lox71 and lox66 sites recognized by the P1 Cre recombinase and by a unique barcode (bc), each flanked by universal primer (up) sites. Cre-mediated recombination generates a lox72 site in place of the lox-flanked *erm* gene. The lox72 site is inefficiently recognized by Cre (1). (*B*) Schematic diagram of the Cre-bearing plasmid (2) used to generate unmarked deletions. A temperature-sensitive origin for replication in *B. subtilis* (ori$^{ts}$) and kanamycin (kan) and ampicillin (amp) resistance genes for selection in *B. subtilis* and *E. coli* are indicated. (*C*) Flow diagram for building the strain lacking 10 MOP family members. Competent *B. subtilis* cells were transformed with genomic DNA harboring a deletion mutant (*ytgP*::*erm*) selecting for erythromycin resistance. Transformants were streak-purified and transformed with the Cre plasmid shown in *B*, selecting for kanamycin resistance at 30 °C to maintain the ts plasmid. Transformants were streak-purified at 30 °C, and four colonies were then streaked at 42 °C without selection to cure the plasmid. One colony from each isolate was screened for erythromycin sensitivity (to test for Cre-mediated loop-out of the *erm* cassette) and kanamycin sensitivity (to test for loss of the Cre plasmid) and was streak-purified without selection. Isolates that were erm-sensitive and kan-sensitive were analyzed by PCR to confirm that the cassette had excised and were transformed with genomic DNA of the next *erm*-marked deletion (*yabM*::*erm*). (*D* and *E*) Genomic DNAs from WT (BDR11) and the strain (BAM485) lacking all 10 MOP family members (Δ10) were used as templates for 10 separate PCR reactions. The oligonucleotide primers used in *D* anneal to sequences within the indicated genes. If the gene is present anywhere in the genome, a 1-kb product should be amplified. If the gene is absent, no product will be generated. The oligonucleotide primers used in *E* anneal to regions flanking the indicated genes (or *tua* operon). If the gene is present at its native locus, a product of ~4 kb (or 9 kb in the case of *tua*) should be amplified. If the deletion is present, a smaller product is generated. If chromosomal rearrangements between lox72 scars have occurred, no product would be generated. The large PCR product from the *tua* operon is indicated (asterisk) in *E*.

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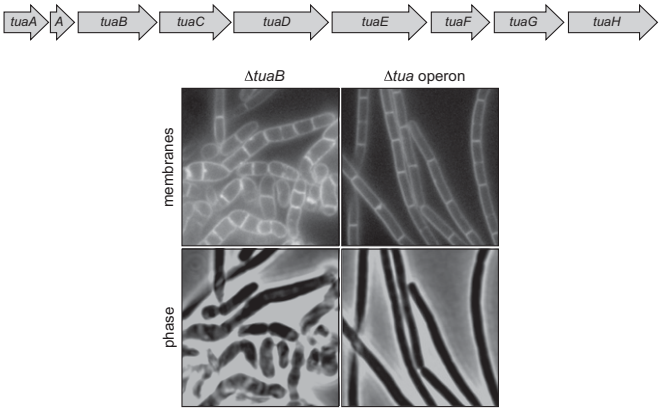


**Fig. S2.** Cells lacking *tuaB* have morphological defects that are suppressed by deletion of the entire *tua* operon. A schematic diagram of the *tua* operon encoding the techuronic biosynthetic pathway is shown. *tuaA* is broken into two disrupted pseudogenes in *B. subtilis* 168. The indicated strains (BAM440 and BAM456) were grown to midexponential phase, stained with the membrane dye TMA-DPH, and examined by fluorescence microscopy. (Scale bar: 1 μm.) Only the deletion of *tuaB* was found to have cell shape defects.

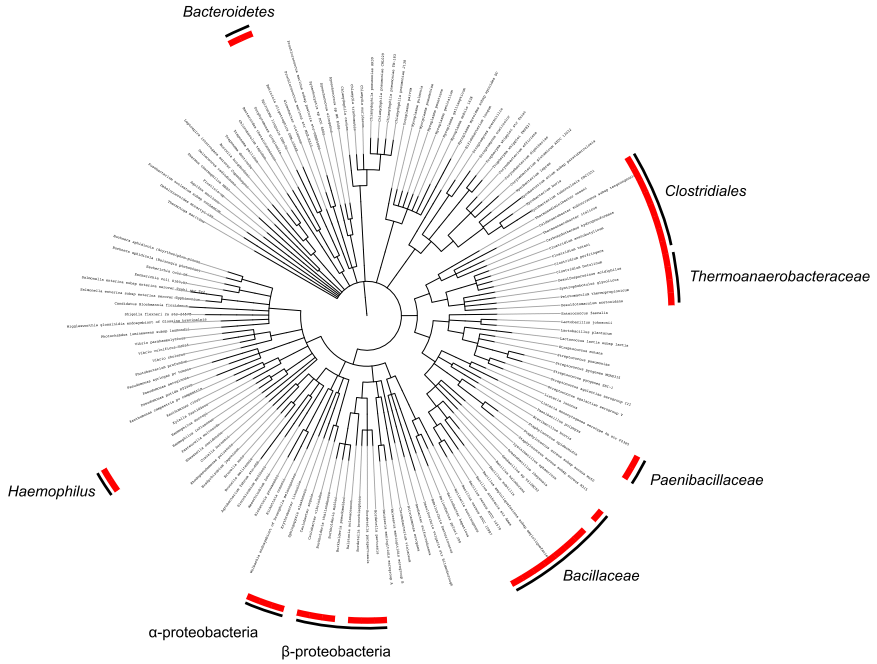


**Fig. S3.** Phylogenetic distribution of Amj. Amj orthologs (red curves) were identified in the genomes of a subset of gram-positive and gram-negative bacteria, including *B. pertussis*, *B. mallei*, and *C. botulinum*. The phylum with the largest number of sequenced genomes containing Amj orthologs is the Firmicutes. The tree was generated using phyloT (phylot.biobyte.de) and visualized in iTOL (1), and it was manually pruned to remove Eukarya and Archaea.

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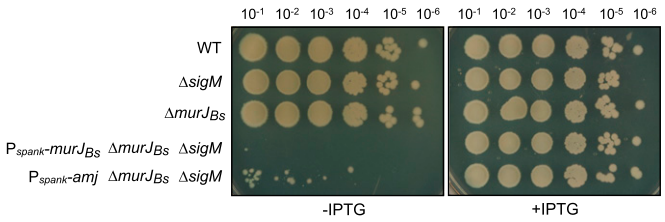



**Fig. S4.** Expression of *amj* suppresses the Δ*sigM* Δ$murJ_{Bs}$ synthetic lethality. Spot dilutions of the indicated strains in the presence and absence of inducer are shown. The five strains were grown in the presence of IPTG (500 μM) to an optical density of ∼1. The cultures were washed twice without inducer, resuspended at an $OD_{600}$ of 1.25, and serially diluted. Five microliters of each dilution was spotted onto LB agar plates with and without inducer.

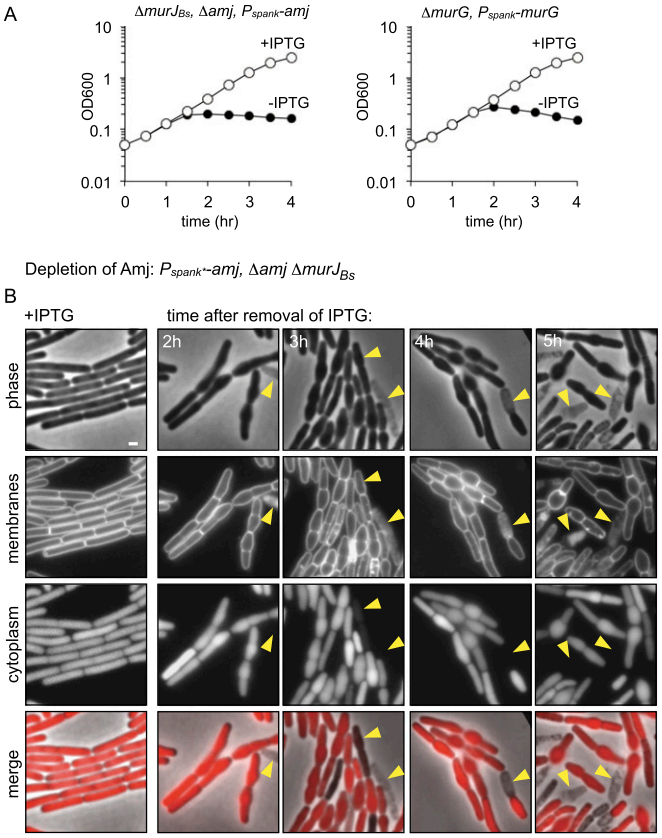



**Fig. S5.** (Continued)

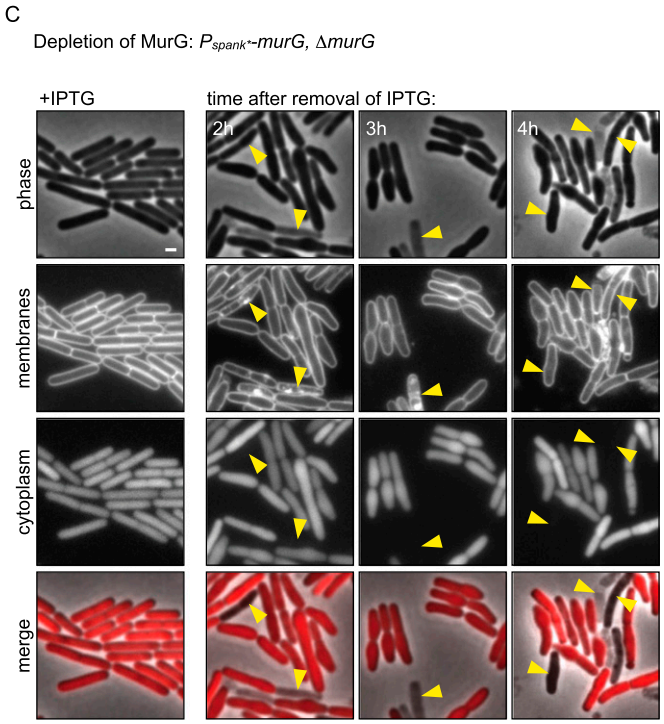
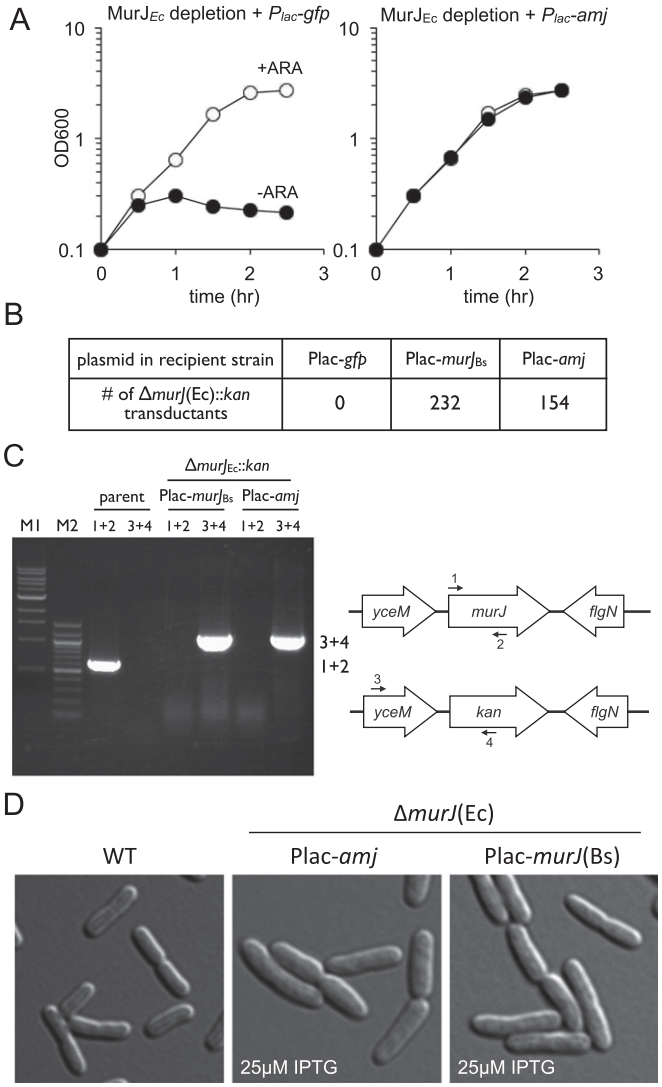



**Fig. S5.** Cytological analysis of *B. subtilis* cells during depletion of Amj in the absence of $MurJ_{Bs}$ and depletion of MurG. (*A*) Cessation of growth following depletion of Amj in the absence of $MurJ_{Bs}$. Depletion of the lipid II synthase MurG is shown for comparison. The indicated strains (BAM555 and BAM566) were grown to exponential phase in the presence of IPTG (500 μM), washed twice with media lacking inducer, and back-diluted to an $OD_{600}$ of 0.05 in casein hydrolysate (CH) medium with or without inducer. Growth was monitored over time. (*B*) Representative images at indicated times during Amj depletion in a strain (BAM585) lacking $MurJ_{Bs}$. Cells were grown to exponential phase in the presence of IPTG (500 μM), washed twice with CH medium lacking inducer, and diluted to an $OD_{600}$ of 0.05 in CH medium with or without inducer. The cells were then monitored by fluorescence microscopy at hours 2–5. (Scale bar: 1 μm.) (*C*) Same as in *B*, but depletion of MurG in strain BAM587. Both strains contain mCherry. An overlay of cytoplasmic fluorescence and phase contrast (merge) is shown. Examples of cell lysis (yellow carets) are indicated. (Scale bar: 1 μm.)




| plasmid in recipient strain | Plac-*gfp* | Plac-$murJ_{Bs}$ | Plac-*amj* |
|---|---|---|---|
| # of Δ*murJ*(Ec)::*kan* transductants | 0 | 232 | 154 |

**Fig. S6.** *amj* and $murJ_{Bs}$ complement a $murJ_{Ec}$-null mutant in *E. coli*. (*A*) Amj expression suppresses the loss of viability following depletion of $MurJ_{Ec}$. Strains CAM252 ($MurJ_{Ec}$ depletion + pGFP) and CAM249 ($MurJ_{Ec}$ depletion + pAmj) were grown to stationary phase in the presence of arabinose, washed twice in LB lacking inducer, and back-diluted to an $OD_{600}$ of 0.01 in LB containing arabinose (+ARA) or IPTG (−ARA). The cultures were grown for seven generations and back-diluted to an $OD_{600}$ of 0.1, maintaining arabinose or IPTG. Growth curves are from the second back-dilution. (*B*) *E. coli* strains harboring $P_{lac}$-*amj*, $P_{lac}$-$murJ_{Bs}$, or $P_{lac}$-*gfp* were separately transduced with lysates from a Δ$murJ_{Ec}$::*kan* strain harboring a complementing allele at an ectopic location. The table shows the number of transductants obtained for each strain. (*C*) PCR confirmation that the $P_{lac}$-*amj* and $P_{lac}$-$murJ_{Bs}$ strains contain Δ$murJ_{Ec}$::*kan* at the endogenous $murJ_{Ec}$ locus and lack a WT copy of $murJ_{Ec}$. (*D*) Cytological analysis of *E. coli* strains lacking $MurJ_{Ec}$ complemented with $P_{lac}$-*amj* or $P_{lac}$-$murJ_{Bs}$ but grown with 25 μM IPTG instead of 1 mM IPTG. (Scale bar: 1 μm.)

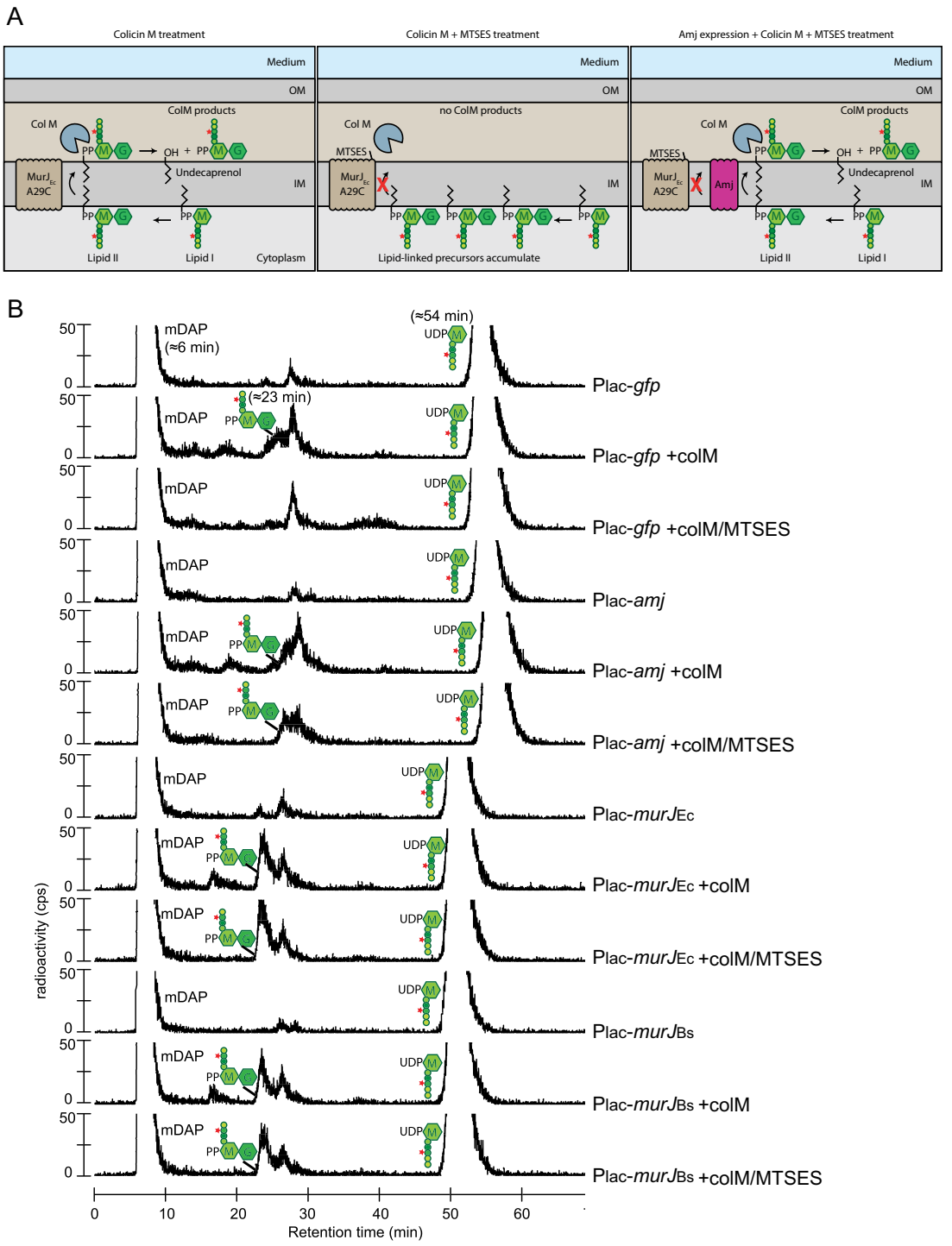



**Fig. S7.** Colicin M flippase assay and HPLC traces. (*A*) Schematic diagram depicting the in vivo lipid II flippase activity assay. PG precursors are labeled with [$^3$H]-*m*-DAP. Exogenously added colicin M (ColM) cleaves flipped lipid II, and soluble products are analyzed by HPLC. In this experiment, all strains harbor the functional $murJ_{Ec}$(A29C) allele, which can be inactivated by addition of MTSES. Upon MTSES addition, ColM-dependent cleavage products are reduced and lipid-linked precursors accumulate. Lipid-linked precursors are monitored by scintillation counting. By contrast, addition of MTSES to cells expressing Amj (or $MurJ_{Ec}$ or $MurJ_{Bs}$) generates ColM-dependent cleavage products and does not accumulate lipid-linked precursors. IM, inner membrane; OM, outer membrane. (*B*) Shown are representative chromatograms from the RP-HPLC analysis. *E. coli* $murJ_{Ec}$(A29C) Δ*lysA* strains harboring $P_{lac}$-$murJ_{Ec}$, $P_{lac}$-*amj*, $P_{lac}$-$murJ_{Bs}$, or $P_{lac}$-*gfp* were grown in M9 medium to an $OD_{600}$ of ∼0.2. PG precursors were labeled by adding [$^3$H]-*m*-DAP. After 15 min, ColM and/or MTSES was added as indicated to the right of the chromatograms. Cells were harvested 10 min later and extracted with hot water as described in *Experimental Methods*. Lyophilized hot-water extract was dissolved in 50 mM triethylammonium formate (pH 4.6) and 6% (vol/vol) methanol, and separated by RP-HPLC using isocratic elution in the same buffer, followed by detection with an in-line radioflow detector. The ColM cleavage product in *A* is further processed by carboxypeptidases to generate the tetrapeptide product shown. Retention times of the ColM cleavage product and UDP-N-acetylmuramic acid-pentapeptide vary between runs and are indicated in parentheses. The ColM cleavage product coelutes with a separate product generating partially overlapping peaks. For Fig. 5*C*, the amount of ColM cleavage product was determined by measuring the area under both peaks and comparing between samples.

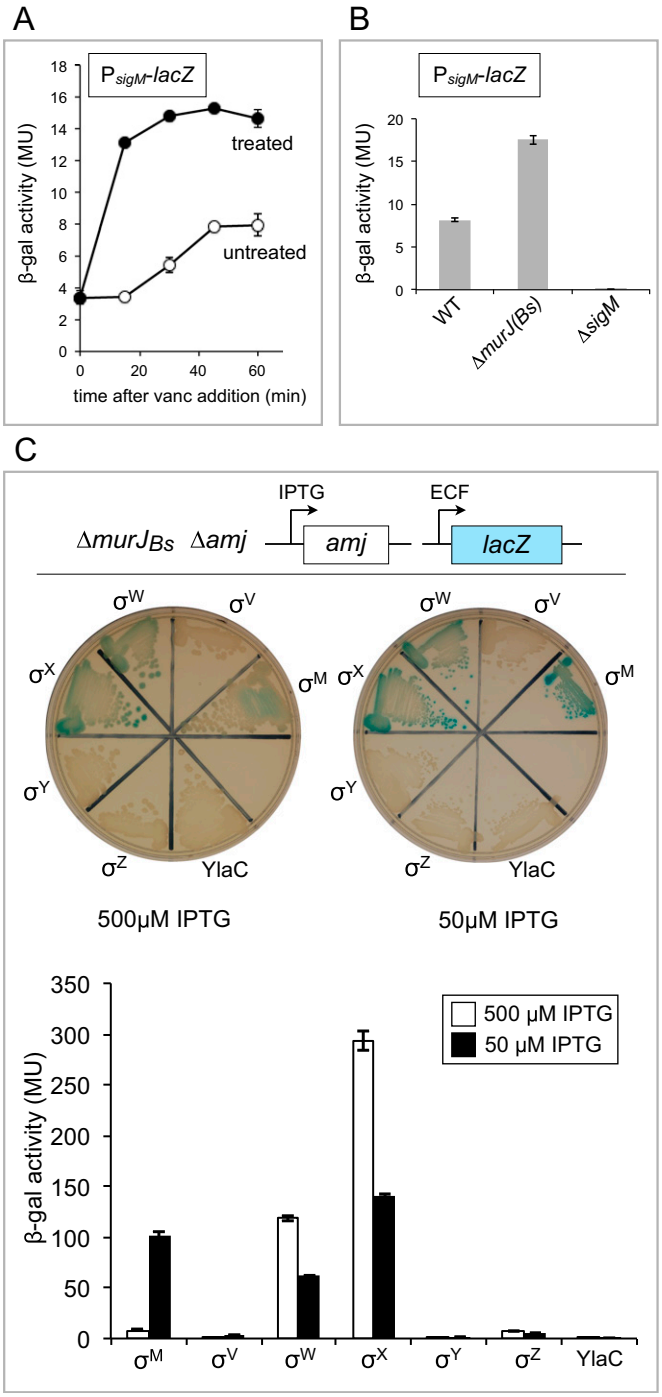



**Fig. S8.** $\sigma^M$ activity increases in MurJ$_{Bs}$ mutant cells expressing low levels of *amj* under inducible control. (*A*) Increase in expression from the P$_{sigM}$ promoter upon vancomycin (vanc) treatment. Exponentially growing cultures harboring a P$_{sigM}$ fusion to *lacZ* were treated with 2 μg/mL vancomycin and assayed at the indicated times for β-gal activity. Error bars represent SEM from two independent experiments. (*B*) Indicated strains harboring a P$_{sigM}$ promoter fusion to *lacZ* were harvested during exponential growth and assayed for β-gal activity. (*C*) $\sigma^M$ is the only extracytoplasmic function (ECF) sigma factor whose activity increases in cells lacking MurJ$_{Bs}$ but expressing low levels of *amj*. (*Top*) Δ*murJ*$_{Bs}$ Δ*amj* double mutant complemented by an IPTG-inducible *amj* fusion containing *lacZ* reporters to the promoters of the indicated sigma factors (1) were streaked on LB agar plates containing X-gal and the indicated amount of IPTG. (*Bottom*) Bar graph showing β-gal activity of the same strains grown in LB with the indicated amount of IPTG. Error bars represent SEM from two experiments. MU, Miller units.

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**Table S1. Strains, plasmids, and oligonucleotides**

| Construct | Genotype/description/sequence |
|---|---|
| *B. subtilis* strain | |
| BDR11 | WT (PY79) |
| BDR2413 | WT, *trpC2* (168) |
| BAM433 | *tuaB::tet* |
| BAM456 | (*tuaABCDEFGH*)*::tet* = *tua::tet* |
| BAM485 | Δ*spoVB::lox72*, Δ$murJ_{Bs}$*::lox72*, Δ*yabM::lox72*, Δ*ykvU::lox72*, Δ*yoeA::lox72*, Δ*norM::lox72*, Δ*dinF::lox72*, Δ*yisQ::lox72*, Δ*epsK::lox72*, Δ*tua::tet* |
| BAM486 | Δ*spoVB::lox72*, Δ$murJ_{Bs}$*::lox72*, Δ*yabM::lox72*, Δ*ykvU::lox72*, Δ*yoeA::lox72*, Δ*norM::lox72*, Δ*dinF::lox72*, Δ*yisQ::lox72*, Δ*epsK::lox72*, Δ*tua::tet*, *sacA::*$P_{veg}$*-mcherry* (*spec*) |
| BDR2648 | *sacA::*$P_{veg}$*-mcherry* (*spec*) |
| BAM490 | Δ*ykvU::lox72*, Δ*spoVB::lox72*, Δ$murJ_{Bs}$*::lox72*, Δ*yabM::lox72*, *trpC2* |
| BAM495 | *divIVA::erm*, *trpC2* |
| BAM496 | *minJ::erm*, *trpC2* |
| BAM501 | $murJ_{Bs}$*::spec*, *trpC2* |
| BAM503 | *sigM::erm*, *trpC2* |
| BAM504 | *amj::erm*, *trpC2* |
| BAM505 | *ycgO::*$P_{spank}$*-amj* (*cat*), *trpC2* |
| BAM506 | *ycgO::*$P_{spank}$**-amj* (*cat*), *trpC2* |
| BAM555 | *ycgO::*$P_{spank}$**-amj* (*cat*), $murJ_{Bs}$*::spec*, *amj::erm*, *trpC2* |
| BAM558 | *ycgO::*$P_{spank}$*-amj* (*cat*), $murJ_{Bs}$*::spec*, *sigM::erm*, *trpC2* |
| BAM564 | *ycgO::*$P_{spank}$**-murG* (*cat*), *trpC2* |
| BAM566 | *ycgO::*$P_{spank}$**-murG* (*cat*), *murG::erm*, *trpC2* |
| AM585 | *ycgO::*$P_{spank}$**-amj* (*cat*), $murJ_{Bs}$*::spec*, *amj::erm*, *sacA::*$P_{veg}$*-mcherry* (*tet*), *trpC2* |
| BAM587 | *ycgO::*$P_{spank}$**-murG* (*cat*), *murG::erm*, *sacA::*$P_{veg}$*-mcherry* (*tet*), *trpC2* |
| BAM754 | *amyE::*$P_{amj}$*-lacZ* (*cat*), *trpC2* |
| BAM755 | *amyE::*$P_{amj}$*-lacZ* (*cat*), $murJ_{Bs}$*::spec*, *trpC2* |
| BAM756 | *amyE::*$P_{amj}$*-lacZ* (*cat*), *sigM::erm*, *trpC2* |
| BAM762 | *amyE::*$P_{sigM}$*-lacZ* (*cat*), *trpC2* |
| BAM763 | *amyE::*$P_{sigM}$*-lacZ* (*cat*), $murJ_{Bs}$*::spec*, *trpC2* |
| BAM764 | *amyE::*$P_{sigM}$*-lacZ* (*cat*), *sigM::erm*, *trpC2* |
| BAM815 | *ycgO::*$P_{spank}$**-amj*, $murJ_{Bs}$*::spec*, *amj::erm*, *amyE::*$P_{amj}$*-lacZ* (*cat*), *trpC2* |
| BAM819 | *amyE::*$P_{amj}$*-yfp* (*cat*), *trpC2* |
| BAM820 | *amyE::*$P_{amj}$*-yfp (cat)*, $murJ_{Bs}$*::spec*, *trpC2* |
| BAM821 | *amyE::*$P_{amj}$*-yfp* (*cat*), *sigM::erm*, *trpC2* |
| *E. coli* strain | |
| TB10 | *rph1 ilvG rfb-50* λΔ*cro-bio nad::Tn10* |
| TB28 | MG1655 Δ*lacZYA* |
| CS4 | *TB10 bla-araC-*$P_{BAD}$*-*$murJ_{Ec}$ |
| CS7 | TB28(Δ*lacZYA*) *bla-araC-*$P_{BAD}$*-*$murJ_{Ec}$ |
| CAM252 | TB28(Δ*lacZYA*) *bla-araC-*$P_{BAD}$*-*$murJ_{Ec}$ pTD29 ($P_{lac}$*-gfp*) |
| CAM247 | TB28(Δ*lacZYA*) *bla-araC-*$P_{BAD}$*-*$murJ_{Ec}$ pCS81 ($P_{lac}$*-*$murJ_{Bs}$) |
| CAM248 | TB28(Δ*lacZYA*) *bla-araC-*$P_{BAD}$*-*$murJ_{Ec}$ pCS82 ($P_{lac}$*-ykvU*) |
| CAM249 | TB28(Δ*lacZYA*) *bla-araC-*$P_{BAD}$*-*$murJ_{Ec}$ pCS83 ($P_{lac}$*-amj*) |
| CAM250 | TB28(Δ*lacZYA*) *bla-araC-*$P_{BAD}$*-*$murJ_{Ec}$ pCS84 ($P_{lac}$*-yabM*) |
| CAM251 | TB28(Δ*lacZYA) bla-araC-*$P_{BAD}$*-*$murJ_{Ec}$ pCS85 ($P_{lac}$*-spoVB*) |
| CAM291 | $murJ_{Ec}$*(A29C), lysA::kan, pTD29* ($P_{lac}$*-gfp*) |
| CAM292 | $murJ_{Ec}$*(A29C), lysA::kan, pAM157* ($P_{lac}$*-*$murJ_{Ec}$) |
| CAM293 | $murJ_{Ec}$*(A29C), lysA::kan, pCS81* ($P_{lac}$*-*$murJ_{Bs}$) |
| CAM294 | $murJ_{Ec}$*(A29C), lysA::kan, pCS83* ($P_{lac}$*-amj*) |
| CAM296 | $murJ_{Ec}$*::kan, pAM157* ($P_{lac}$*-*$murJ_{Ec}$) |
| CAM297 | $murJ_{Ec}$*::kan, pCS81* ($P_{lac}$*-*$murJ_{Bs}$) |
| CAM298 | $murJ_{Ec}$*::kan, pCS83* ($P_{lac}$*-amj*) |
| Plasmid | |
| pAM126 | *ycgO::*$P_{spank*}$*-murG lacI* (*cat*) (*amp*) |
| pAM131 | *ycgO::*$P_{spank}$*-amj lacI* (*cat*) (*amp*) |
| pAM132 | *ycgO::*$P_{spank*}$*-amj lacI* (*cat*) (*amp*) |
| pTD29 | $P_{lac}$*-gfp* (*spec*) *lacI pSC101 ori* |
| pCS81 | $P_{lac}$*-*$murJ_{Bs}$ (*spec*) |
| pCS82 | $P_{lac}$*-ykvU* (*spec*) |
| pCS83 | $P_{lac}$*-amj* (*spec*) |
| pCS84 | $P_{lac}$*-yabM* (*spec*) |
| pCS85 | $P_{lac}$*-spoVB* (*spec*) |

**Table S1. Cont.**

| Construct | Genotype/description/sequence |
|---|---|
| pCS89 | *yceM-murJ*$_{Ec}$*(A29C)-flgN sacB cat* |
| pWX496 | $P_{PA}$*-cre* (*kan*), *ori*(*ts*) (*kan*) (*amp*) |
| pAM147 | *amyE::*$P_{amj}$*-lacZ* (*cat*) (*amp*) |
| pAM149 | *amyE::*$P_{sigM}$*-lacZ* (*cat*) (*amp*) |
| pAM155 | *amyE::*$P_{amj}$*-yfp* (*cat*) (*amp*) |
| pAM157 | $P_{lac}$*-murJ*$_{Ec}$ (*spec*) |
| Primer | |
| oAM65 | GCTTCAAGATGAAATGATCGACCGC |
| oAM73 | CTGAAAGGATCCTTCGGCAG |
| oAM74 | CTGAGCGAGGGAGCAGAACTTGACATGTTTCTTCATCAC |
| oAM75 | GTTGACCAGTGCTCCCTGCATGCAGGCTGATGAACTAG |
| oAM76 | CGTACGCAGTTGATAGTCGTG |
| oAM77 | GCTTCAAGATGAAATGATCGACCGC |
| oAM81 | GAATTGCGCGCCTACTGATG |
| oAM295 | CTGAGCGAGGGAGCAGAAGGCATCATATTCCCTACTTC |
| oAM296 | GTTGACCAGTGCTCCCTGCTAAGAAAAGGACGTTTGTCAT |
| oAM314 | GCGAAGCTTAGACTGGGGGAAAAAAGAAATG |
| oAM315 | CGCGCTAGCCGCATTCCATTTGCTTTTCTAC |
| oAM316 | GATTGCTTTGACTACTAAAAAAAC |
| oAM317 | TCCTGCCTTTCCTCCCTCTCGCATTTCTTTTTTCCCCCA |
| oAM318 | GAGAGCACAGATACGGCGAAAAAATAGTAGAAAAGCAAATGGA |
| oAM319 | CAGAGATTGTTGATACTTTACTG |
| oAM320 | GGCTTCTTATATGCAGACGC |
| oAM321 | CTGAGCGAGGGAGCAGAACTTTTCTCTGCACTCACTGAC |
| oAM324 | GTTGACCAGTGCTCCCTGCCTATAAATTGGGATGCGCG |
| oAM325 | CAGTACAACGAATTCAACAAC |
| oAM332 | GGCATAGGTCATCATAACATC |
| oKO001 | CATGCAAATGCTAATGATAGC |
| oKO002 | GATCTTCCAATTTATAATGG |
| oKO003 | GTTGAACCGTTTCAGGAAGC |
| oKO004 | GCGTTACTTCATATTGCATG |
| oKO005 | CACTCCGGAAAACGTTTGGC |
| oKO006 | CCGAAGCAACGACGAAAAG |
| oKO007 | CACGAGAGCGATCATATTC |
| oKO008 | TATTCTCTACTCCGCCAAG |
| oKO009 | GATTTCAGCTCCTTCATCAC |
| oKO010 | CATTTTATCCCGGACACGG |
| oKO011 | GCTATCAATGGCTTAAAGACATC |
| oKO012 | CCGGTATGGAACGGATCAC |
| oKO082 | GATATCTTTCTGATCTTCACTG |
| oKO083 | CAGTCTGTTAAAGCGTGAATGG |
| oKO084 | CTTAAAGTGGACATGAACAATG |
| oKO085 | CGATTTGTGAACCATATCTCG |
| oAM345 | CCTTTCTTGGTATTGCGGTC |
| oAM346 | CGCAGGAAAAACTGTGTATC |
| oAM347 | GATCATGTCGTTTATGTCATCC |
| oAM348 | CTTATACGAATAGCCTGCATG |
| oAM349 | CTACATTAACTCAATTCGGTC |
| oAM350 | CAGCTCCTGCCAAATTAAG |
| oAM351 | CTGAATGCCTTGAATTTGTCG |
| oAM352 | CACAACAGCTGCAAGTAATAC |
| oAM353 | GCAATGTGCTGCAATCAG |
| oAM354 | GTAAAGCGTCTCTTGCTC |
| oAM355 | CCGATATTCATTACCCAAGC |
| oAM356 | ACATCGGGGTCAGAAGTG |
| oAM357 | GGAAACGCATTGCAGGTTTC |
| oAM358 | CGATCCACTTCAAATATTGTTC |
| oAM359 | CGATACACTGATGCTCAGC |
| oAM360 | CTGGTTCAAGGATAATGGTC |
| oAM361 | GCCTCTGTTTTGATTTCCTTG |
| oAM362 | GATACGTAAAGCGGCGTAAAG |
| oAM363 | CACAACGGTTACAGTTTTCG |
| oAM364 | CTGTACAGCTGCAAACAGTG |

**Table S1. Cont.**

| Construct | Genotype/description/sequence |
|---|---|
| oAM367 | GCGAAGCTTGAAATTGGAGGAAGAATAACG |
| oAM368 | GCGGCTAGCCGGATTAAGGAACTATAAATAAG |
| oAM374 | GCGCTCGAGATGGTCAGCAAGGGAGAG |
| oAM375 | CGCGGATCCTTATTTGTATAATTCGTCCATTCCAC |
| oAM376 | CGCGAATTCCGATTACGATTACGTCATATTG |
| oAM377 | GCGCTCGAGGCCTGCATGTCTGCCTTTC |
| AD-11a | GTTCAGAGTTCTACAGTCCGACGATCACTTGANN |
| AD-11b | /5Phos/TCAAGTGATCGTCGGACTGTAGAACTCTGAACCTGTC |
| AD-9a | GTTCAGAGTTCTACAGTCCGACGATCTTAGGCNN |
| AD-9b | /5Phos/GCCTAAGATCGTCGGACTGTAGAACTCTGAACCTGTC |
| CS142 | ATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTC |
| CS143 | TAAGCTTAGTAAAGCCCTCGCTAGATT |
| CS144 | AATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCGAAACAGACGTTTTTAAAG |
| CS145 | AATCTAGCGAGGGCTTTACTAAGCTTATCGGATGATCAACCGTCCGATAATG |
| CS146 | GAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGACGATTCAATAGGCGT |
| CS147 | AATCTAGCGAGGGCTTTACTAAGCTTACCCGCCATTTTGTTCTCTCC |
| CS148 | ATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCATGTCATTACAACACAAGTA |
| CS149 | AATCTAGCGAGGGCTTTACTAAGCTTAAAACCACTTTGTCAGCCACG |
| CS150 | AATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGAATCGATTTGTGAAAGGGATC |
| CS151 | AATCTAGCGAGGGCTTTACTAAGCTTATCCTTCAATTCGCGGCG |
| CS152 | AATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGTCAAGCAAACTGTTAAGAGGC |
| CS153 | AATCTAGCGAGGGCTTTACTAAGCTTAGCCTGCATGTCTGCCTTTC |
| oJM28 | TTCTGCTCCCTCGCTCAG |
| oJM29 | CAGGGAGCACTGGTCAAC |
| oDR1049 | GAGGGAGGAAAGGCAGGA |
| oDR1050 | CGCCGTATCTGTGCTCTC |
| oAM399 | CGCGAATTCGTTTACTTTCCAGAATGATTCTTC |
| oAM400 | CGCGGATCCATTTCATCCTAGAGATAAGACTG |
| oAM403 | CGCGAATTCTAGTAATGGTGTTTGATTAGG |
| oAM404 | CGCGGATCCCTATGTTATACACGCATAAGAAAG |
| oAM412 | AATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGAATTTATTAAAATCGCTGGC |
| oAM413 | AATCTAGCGAGGGCTTTACTAAGCTTACACCGTCCGGCGGGC |
| oAM414 | CGCAAGCTTATTTCATCCTAGAGATAAGAC |
| oWX292 | TATCCCGGGGGATCCAAATTCCTCGTAGGCGCTCG |
| oWX293 | GCGACTAGTATGCTAGCGAGAATTGGACCTTTACAG |
| oDR944 | CGCGGATCCGGACGCAGCGCATCACACG |
| oDR945 | GCGCGGCCGTCTTTTGCGCAGTCGGCTT |
| oDR946 | CGCGAATTCGTAATGAGGTTCGGATTCATCC |
| oDR947 | GCCACTAGTCTAATCGCCATCTTCCAGCAG |
| CS168 | CCATCCCTGCGCCAAAGATTCTGCAGACAATTGCGTCTCGTGCGAAGCC |
| CS169 | GGCTTCGCACGAGACGCAATTGTCTGCAGAATCTTTGGCGCAGGGATGG |
| CS171 | GGGAGAGCTCATTGCGCAAAAAGCGTGGTTACCGG |
| CS172 | ATCCTCTAGAAATTACTGTGAAAACGCAGCAACTACGCC |