**Table S3. Strains used in this study.**

| Strain | Genotype[a] | Source/Reference[b] |
|---|---|---|
| DH5α | *F– hsdR17 deoR recA1 endA1 phoA supE44 thi-1 gyrA96 relA1 Δ(lacZYA-argF)U169 ϕ80dlacZΔM15* | Gibco BRL |
| TB10 | MG1655 *λΔcro-bio nad::Tn10* | [1] |
| MG1655 | *rph-1 ilvG rfb-50* | [2] |
| TB28 | *MG1655 ΔlacIZYA::frt* | [3] |
| GL38 | MG1655 Δ*rcsB::frt* | This study[a] |
| GL44 | MG1655 Δ*relA::frt* | This study[a] |
| GL74 | MG1655 Δ*rfaP::Kan*$^R$ | This study[a] |
| GL76 | MG1655 Δ*rfaH:: Kan*$^R$ | This study[a] |
| GL82 | MG1655 Δ*prc:: Kan*$^R$ | This study[a] |
| GL112 | MG1655 Δ*rnt:: Kan*$^R$ | This study[a] |
| GL120 | MG1655 Δ*efp::Kan*$^R$ | This study[a] |
| GL122 | MG1655 Δ*sspA::Kan*$^R$ | This study[a] |
| AG04 | MG1655 Δ*lpp::Kan*$^R$ | This study[a] |
| HC408 | MG1655 Δ*slt::Kan*$^R$ | This study[a] |
| GL101 | MG1655 Δ*arcA::Kan*$^R$ | This study[a] |
| GL90 | MG1655 Δ*gcvR::Kan*$^R$ | This study[a] |
| GL92 | MG1655 Δ*crr::Kan*$^R$ | This study[a] |
| GL70 | MG1655 Δ*lepA::Kan*$^R$ | This study[a] |
| GL72 | MG1655 Δ*trxB::Kan*$^R$ | This study[a] |
| GL124 | MG1655 Δ*cysE::Kan*$^R$ | This study[a] |
| GL78 | MG1655 Δ*pgm::Kan*$^R$ | This study[a] |
| GL84 | MG1655 Δ*nlpI::Kan*$^R$ | This study[a] |
| GL80 | MG1655 Δ*opgH::Kan*$^R$ | This study[a] |
| GL206 | MG1655 Δ*nlpD::Kan*$^R$ | This study[a] |

| | | |
|---|---|---|
| GL66 | MG1655 Δ*tufA::Kan*$^R$ | This study[a] |
| GL204 | MG1655 Δ*rcsB::frt* Δ*rfaP::Kan*$^R$ | This study[a] |
| GL205 | MG1655 Δ*rcsB::frt* Δ*nlpD::Kan*$^R$ | This study[a] |
| GL203 | MG1655 Δ*rcsB::frt* Δ*lpp::Kan*$^R$ | This study[a] |
| GL207 | MG1655 Δ*relA::frt* Δ*tufA::Kan*$^R$ | This study[a] |
| GL243 | MG1655 Δ*relA::frt* Δ*efp::Kan*$^R$ | This study[a] |
| GL62 | MG1655 Δ*rcsB::frt* Δ*slt::Kan*$^R$ | This study[a] |
| GL180 | MG1655 Δ*relA::frt* Δ*slt::Kan*$^R$ | This study[a] |
| GL68 | MG1655 Δ*mepS::frt* | This study[a] |
| GL67 | MG1655 Δ*ponB::frt* | This study[a] |
| TB153 | TB28 *ΔmepS::Kan*$^R$ | This study[a] |
| DL84 | TB28 *Δprc::frt* | This study[a] |
| HC604 | TB28 *Δprc::frt ΔmepS::Kan*$^R$ | This study[a] |
| TU278 | TB28 Δ*lysA::frt* Δ*ampD::frt* | [4] |
| HC533 | MG1655 Δ*lysA::frt* Δ*ampD::frt* Δ*ponA::frt* Δ*pbpC::frt* Δ*mtgA::frt* $^{MS}$*ponB* | [5] |

[a] The indicated strains were generated by P1 transduction by moving the indicated gene replacement alleles from the Keio collection [26] to the desired genetic background. In cases were the allele was unavailable or incorrect in our copy of the collection, equivalent mutants were regenerated by recombineering using strain TB10 and then transduced from this background. Kan$^R$ cassettes are flanked by *frt* sites for removal by FLP recombinase. An *frt* scar remains following removal of the cassette using FLP expressed from pCP20.


[b]
1. Johnson JE, Lackner LL, Hale CA, de Boer PAJ. ZipA is required for targeting of DMinC/DicB, but not DMinC/MinD, complexes to septal ring assemblies in Escherichia coli. J Bacteriol. 2004;186: 2418–2429.
2. Guyer MS, Reed RR, Steitz JA, Low KB. Identification of a sex-factor-affinity site in E. coli as gamma delta. Cold Spring Harb Symp Quant Biol. 1981;45 Pt 1: 135–140.
3. Bernhardt TG, de Boer PAJ. Screening for synthetic lethal mutants in Escherichia coli and identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase activity. Molecular Microbiology. 2004;52: 1255–1269. doi:10.1111/j.1365-2958.2004.04063.x
4. Cho H, Uehara T, Bernhardt TG. Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. Cell. 2014;159: 1300–1311. doi:10.1016/j.cell.2014.11.017
5. Cho H, Wivagg CN, Kapoor M, Barry Z, Rohs PDA, Suh H, et al. Bacterial cell wall biogenesis is mediated by SEDS and PBP polymerase families functioning semi-autonomously. Nat Microbiol. 2016;1: 16172. doi:10.1038/nmicrobiol.2016.172