**SUPPLEMENTARY TABLES**

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| **Supplementary file 1a; Table S1. Muropeptide composition of the peptidoglycan of strains BW25113**$Δ$**6*ldt*, M1.5, and BW25113 “wild type”** |
| **Strain** | **Muropeptide (%) a** |
|  **β-lactam b**  | **Tri** | **Tetra** | **Penta** | **Tri**$\rightarrow $**Tri** | **Tri**$\rightarrow $**Tetra** | **Tri**$\rightarrow $**Penta** | **Tetra**$\rightarrow $**Tri** | **Tetra**$\rightarrow $**Tetra** |
| BW25113Δ6*ldt* |  |  |  |  |  |  |
|  | None (9) c | ND | 74 $\pm $ 2 | ND | ND | ND | ND | ND | 26 $\pm $ 2 |
|  | AZT (5) c | ND | 77 $\pm $ 1 | ND | ND | ND | ND | ND | 23 $\pm $ 1 |
|  | MEL (5) c | ND | 70 $\pm $ 2 | ND | ND | ND | ND | ND | 30 $\pm $ 2 |
| M1.5 |  |  |  |  |  |  |  |  |  |
|  | None (12) c | 31 $\pm $ 3 | 36 $\pm $ 4 | ND | 7 $\pm $ 1 | 8 $\pm $ 1 | ND | 7 $\pm $ 1 | 12 $\pm $ 1 |
|  | AMP (10) c | 29 $\pm $ 2 | 21 $\pm $ 3 | 6 $\pm $ 2 | 13 $\pm $ 2 | 24 $\pm $ 1 | 6 $\pm $ 0.4 | 1 $\pm $ 0.3 | ND |
| BW25113 "Wild type" |  |  |  |  |  |  |
|  | None (5) c | 24 ± 4 | 48 ± 6 | ND | ND | 2 ± 1 | ND | 2 ± 0.2 | 23 ± 3 |
|  | AZT (5) c | 14 ± 5 | 61 ± 3 | ND | ND | 3 ± 0.3 | ND | 3 ± 0.3 | 19 ± 3 |
|  | MEL (5) c | 28 ± 8 | 46 ± 9 | ND | ND | 3 ± 0.3 | ND | 3 ± 1 | 20 ± 1 |

a Data are the mean ± standard deviation from independent experiments (see note c).

b β-lactam added to the growth medium. AZT, aztreonam at 12 µg/mL; MEC, mecillinam at 2.5 µg/mL; AMP, ampicillin at 16 µg/mL.

c The number of independent peptidoglycan analyses is indicated in parenthesis.

**Abbreviations:** ND, not detected; Tri, tripeptide monomer; Tetra, tetrapeptide monomer; Penta, pentapeptide monomer; Tri→Tri, Tri→Tetra, and Tri→Penta, 3→3 cross-linked dimers; Tetra→Tri and Tetra→Tetra, 4→3 cross-linked dimers. The inter-peptide cross-link direction is indicated using the donor→acceptor conventional notation.

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| **Supplementary file 1b; Table S2. Generation time for growth of BW25113 derivatives in M1 minimal medium**  |
| **Strain** | **Generation time (min)** |
|  | **β-lactam a** | **Experiment 1** | **Experiment 2** |
| M1.5 |  |  |
|  | None  | 90 ± 2 | 85 ± 3 |
|  | AMP  | 163 ± 9 | 165 ± 13 |
| BW25113Δ6*ldt* |  |  |
|  | None  | 67 ± 1 | 66 ± 1 |
|  | AZT **b** | 64 ± 1 | 63 ± 1 |
|  | MEL **b**  | 63 ± 1 | 65 ± 1 |
| BW25113 "W ild type" |  |  |
|  | None  | 63 ± 8 | 64 ± 8 |
|  | AZT **b** | 74 ± 5 | 63 ± 8 |
|  | MEL **b**  | 55 ± 2 | 58 ± 6 |

a β-lactam added to the growth medium. AZT, aztreonam at 12 µg/mL; MEC, mecillinam at 2.5 µg/mL; AMP, ampicillin at 16 µg/mL.

b For growth in the presence of aztreonam and mecillinam, the values were deduced from the variation in OD600 during 90 min.

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| **Supplementary file 1c; Table S3. Susceptibility of *E. coli* strains to β-lactams** |
|  | MIC (µg/mL)  |
| Strain | Aztreonam  | Mecillinam  |
| BW25113 | 0.0625 | 0.125 |
| BW25113Δ6*ldt* | 0.0625 | 0.125 |
| Data are the medians from six experiments. |