

TABLE S1 Free amino acids concentrations in CS-3, HS and MS

| Amino acid | CS-3 (μM) | HS (μM) | MS (μM) |
|---------------|------------------------|----------------------|----------------------|
| Alanine | 265 | 358 | 344 |
| Arginine | 72 | 79 | 27 |
| Asparagine | 8 | 15 | 3 |
| Aspartic acid | 8 | 32 | 34 |
| Cystine | 89 | 139 | 219 |
| Glutamine | 112 | 45 | 44 |
| Glutamic acid | 118 | 278 | 243 |
| Glycine | 199 | 257 | 261 |
| Histidine | 28 | 52 | 18 |
| Isoleucine | 41 | 52 | 38 |
| Leucine | 103 | 128 | 143 |
| Lysine | 59 | 136 | 146 |
| Methionine | 20 | 7 | 35 |
| Phenylalanine | 37 | 66 | 56 |
| Proline | 112 | 199 | 178 |
| Serine | 66 | 128 | 129 |
| Threonine | 41 | 94 | 100 |
| Tryptophan | 21 | 30 | 34 |
| Tyrosine | 34 | 52 | 56 |
| Valine | 111 | 154 | 140 |

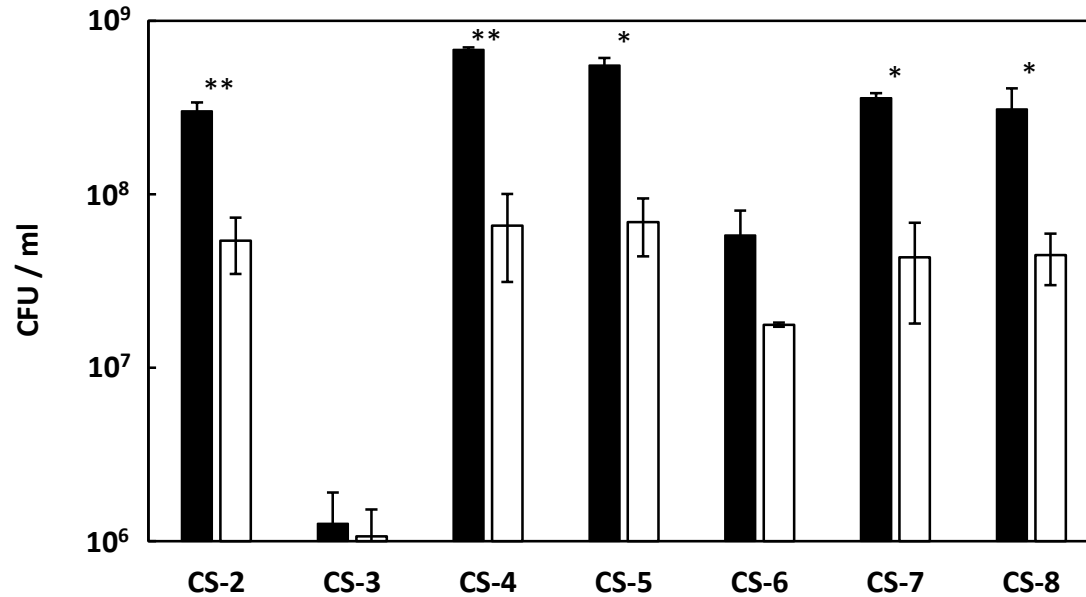


Fig. S1 Growth of the *asd*-inactivated mutant in 7 lots of CS. CFUs of MW2 WT (black bar) and Δasd (white bar) grown in 7 lots of CS for 24 h were measured by the method described in the Materials and Methods section. The data shown represent the mean \pm SD of measurements performed in triplicate. Statistical analysis was performed using Student's *t*-test (*, $P < 0.05$; **, $P < 0.01$).

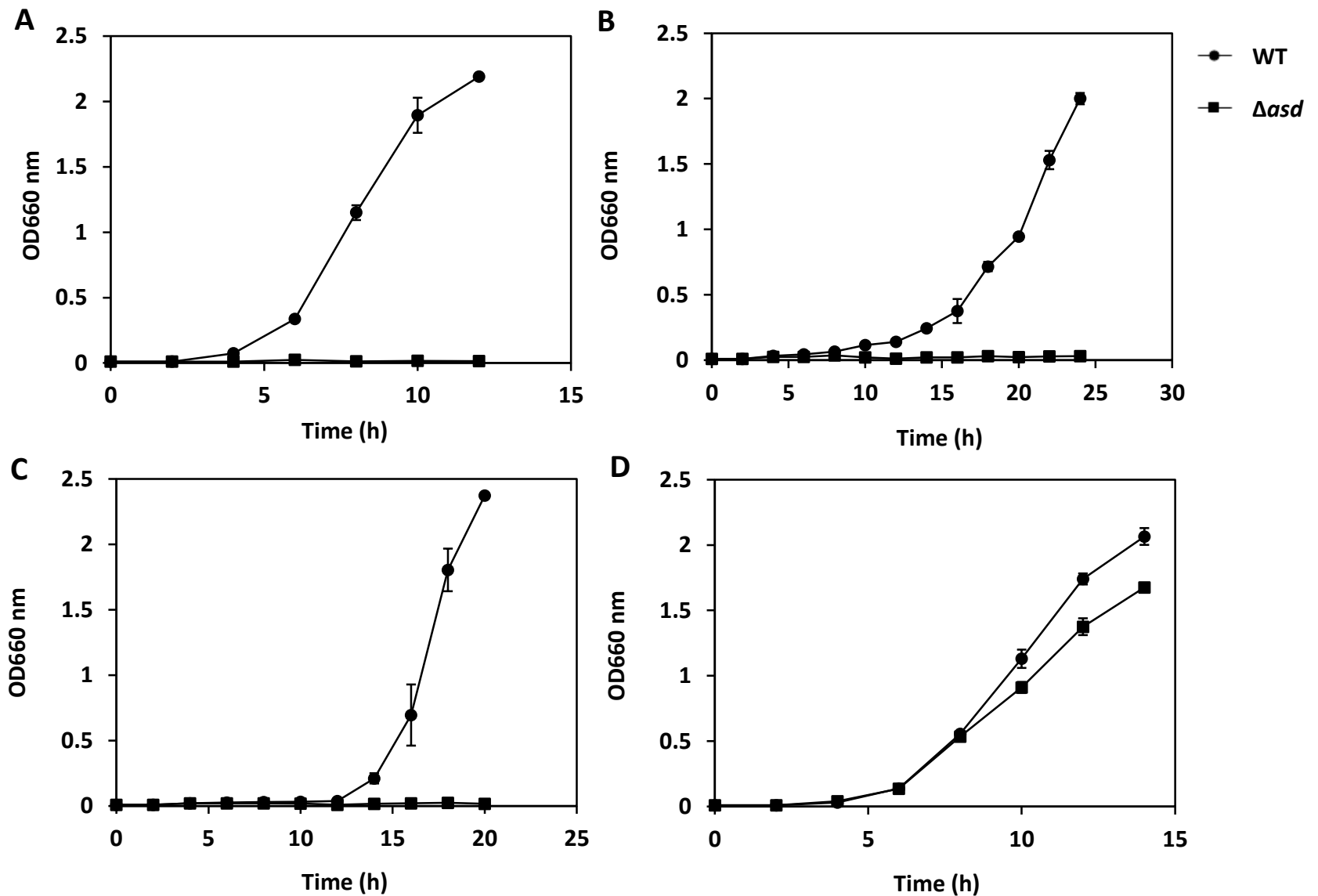


Fig. S2 Growth curve of the *asd*-inactivated mutant in CDM lacking lysine, methionine, threonine or isoleucine. Bacterial density of *S. aureus* MW2 WT and Δasd grown in CDM lacking lysine (A), methionine (B), threonine (C) or isoleucine (D) were measured using the method described in the Materials and Methods section. The data shown represent the mean \pm SD of measurements performed in triplicate.

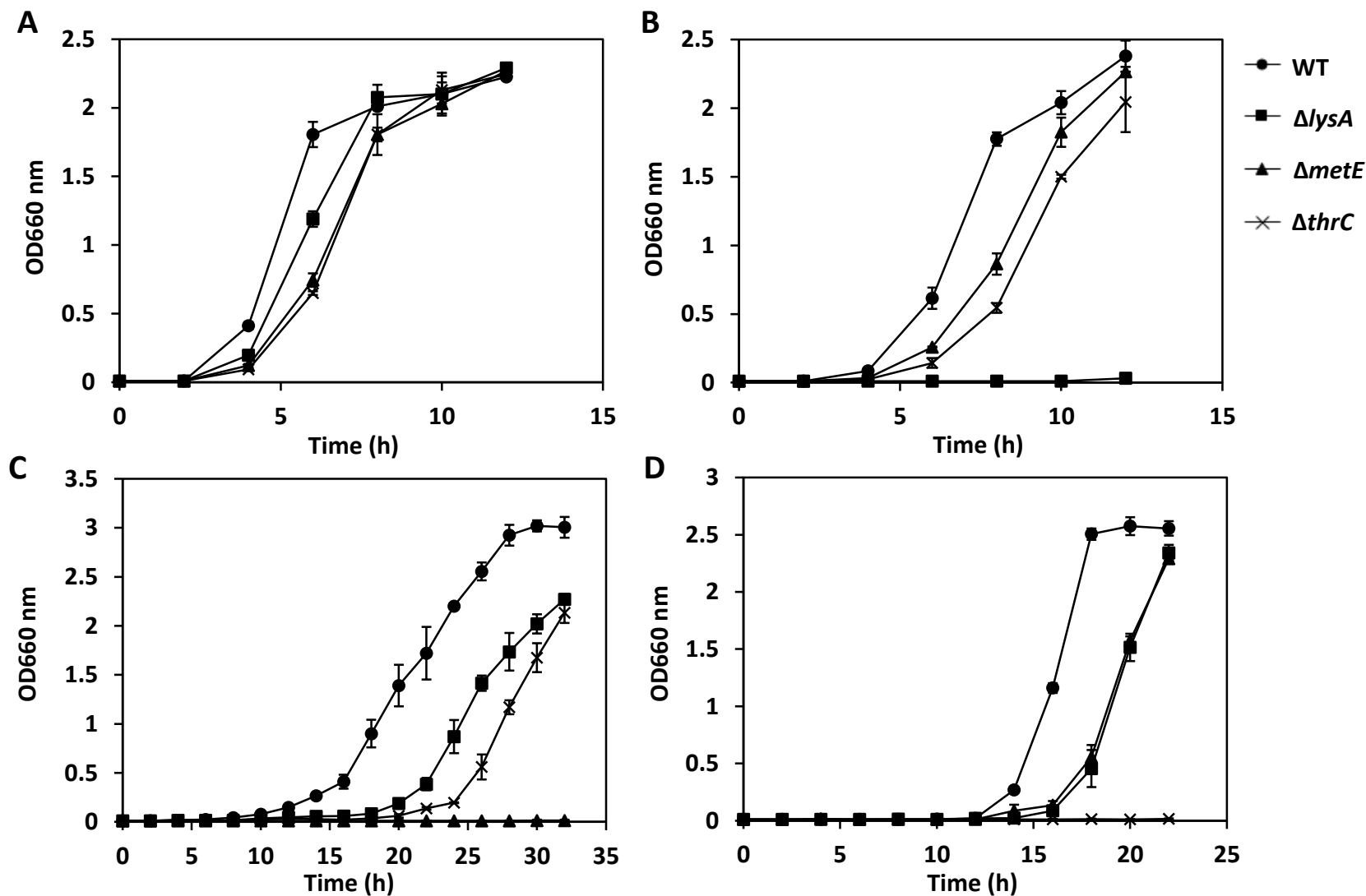


Fig. S3 Growth curve of the lysine, methionine or threonine biosynthesis mutants in CDM lacking lysine, methionine or threonine. Bacterial density of *S. aureus* MW2 WT, $\Delta lysA$, $\Delta metE$ and $\Delta thrC$ grown in CDM (A), CDM lacking lysine (B), methionine (C) and threonine (D) were measured using the method described in the Materials and Methods section. The data shown represent the mean \pm SD of measurements performed in triplicate.

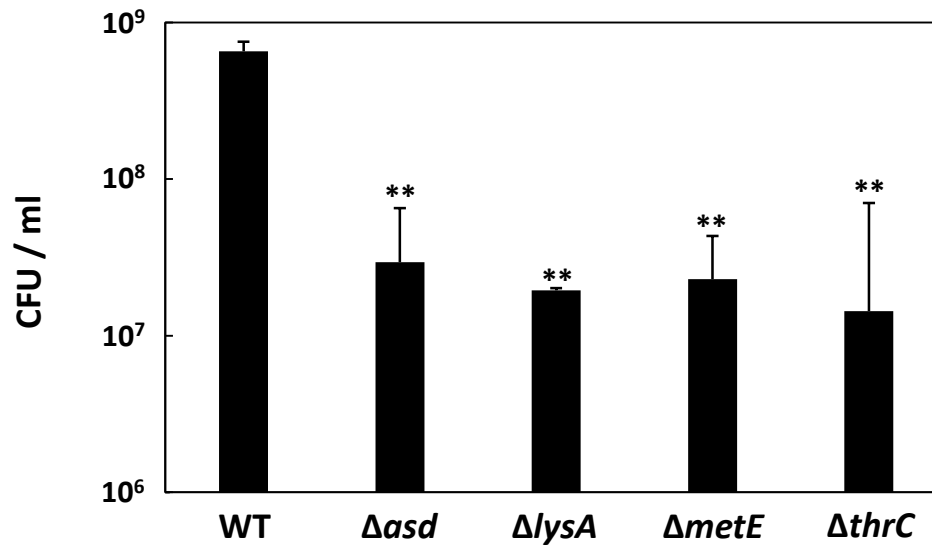


Fig. S4 Growth of the lysine, methionine and/or threonine biosynthesis mutants in MS. CFUs of MW2 WT, *asd*, *lysA*, *metE* and *thrC*-inactivated mutants grown in MS for 24 h were measured by the method described in the Materials and Methods section. The data shown represent the mean \pm SD of measurements performed in triplicate. Significant differences compared with WT were determined by Dunnett's test (**, $P < 0.01$).

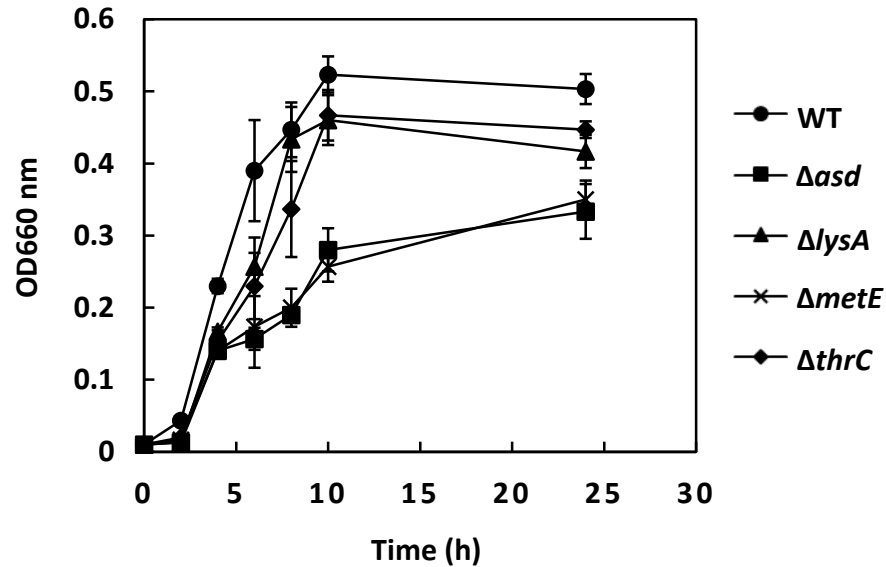


Fig. S5 Growth curve of the lysine, methionine and/or threonine biosynthesis mutants in CDM mimicking HS. Bacterial density of *S. aureus* MW2 WT, $\Delta lysA$, $\Delta metE$ and $\Delta thrC$ grown in CDM mimicking the concentrations of amino acids and glucose in HS were measured using the method described in the Methods section. The data shown represent the mean \pm SD of measurements performed in triplicate.