Table S1. Oligonucleotides used in this study

Transposon primer1: TACGGCGAAGGATCACTCATGG
Transposon primer2: TATATATTTCTGCTTCGCTAGG
AA502: CGCCCGGGTTGCTCTTTATTTGCTTTATTGTC
AA505: AGCCCGGGCGCATGTTTGAAGAAGCAGGTTTCATAAAT
AA508: GGGGACAAGTTTGTACAAAAAAGCAGGCTGGAAACAACTGTTAGCAAATTGGAAAG
AA509: GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAAACTTAAAAGTAGTGATTTCGGATGT
CAW430: GACACGGTCGCGAATATTTTAAACGGGTACTACGGAAGC
CAW431: GCTTCCGTAGTACCCGTTTAAAATATTCGCGACCGTGTC
CAW437: GGGGACAAGTTTGTACAAAAAAGCAGGCTGTGCTCGGGTCTAGTGACTG
CAW438: GGGGACCACTTTGTACAAGAAAGCTGGGTGAGGTAGAGGCATCGCGAC
DLS365: GGGGACAAGTTTGTACAAAAAAGCAGGCTGATTGGATTTCGAGTATACAGC
DLS366: CCCCGGATCCTCAGGGCGATTTATTGTTACC
DLS367: CCCCGGATCCTTATTACACAACTGATGAAGCG
DLS368: GGGGACCACTTTGTACAAGAAAGCTGGGTGGTCTGAAGTAAGTGAGCAAGACC

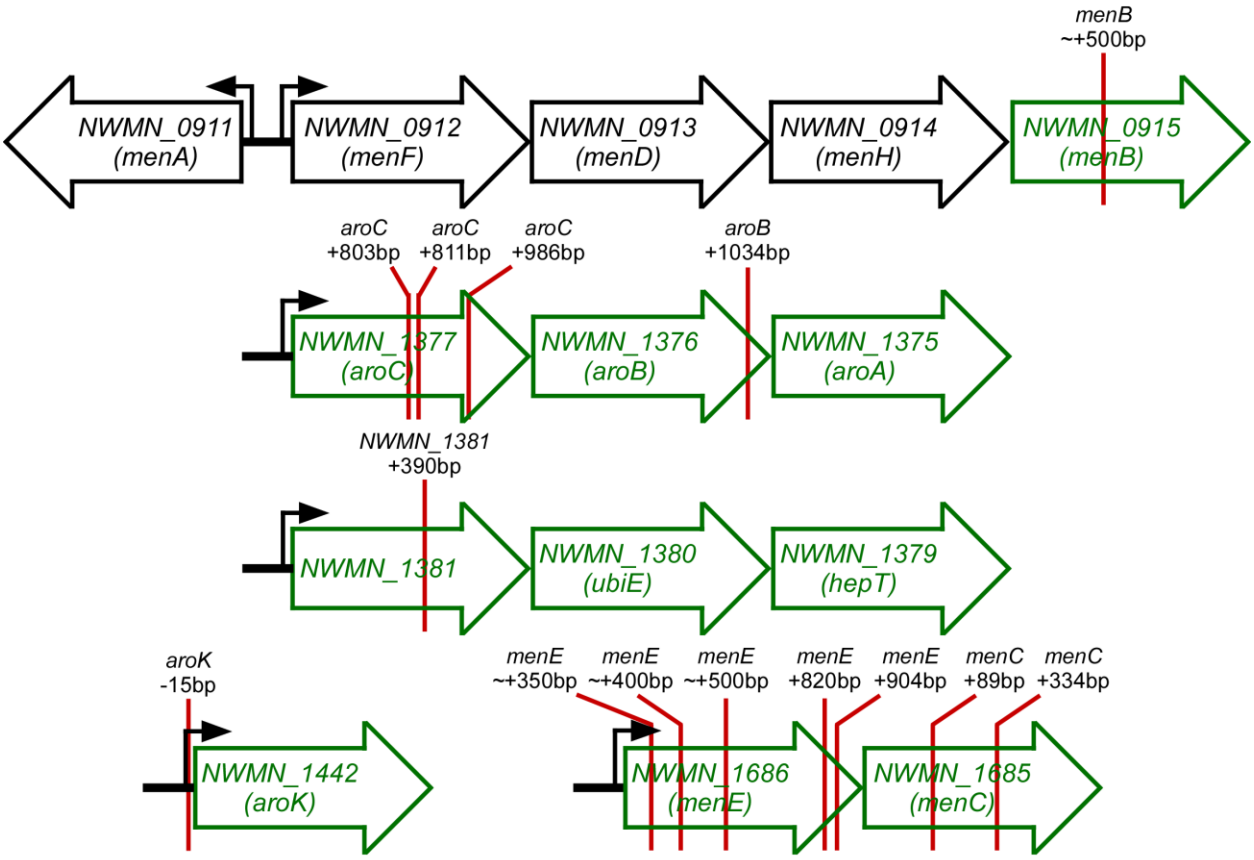

Fig. S1. Operonic arrangement of the menaquinone (MK) biosynthesis genes disrupted in the tranposon mutagenesis of *S. aureus* Δ*hrtA*. Transposon lesions are depicted in red, and the exact site of each lesion is denoted as base pairs relative to the translational start site of the gene containing the insertion. Genes that are disrupted by at least one of the identified transposon insertions are highlighted in green. Because the transposon insertion creates a polar deletion, all genes downstream of the lesion and located within the same operon are predicted to be disrupted by the transposon. The promoter elements and direction of transcription are depicted by the small black arrows.

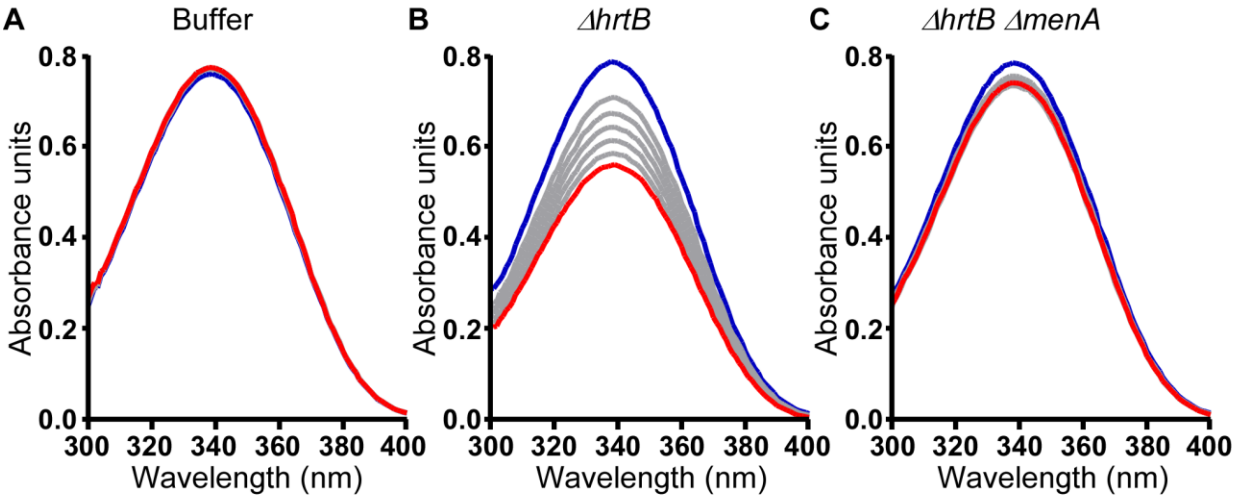

Fig. S2. MK-deficient *S. aureus* strains do not respire and, therefore, do not oxidize NADH. Inverted membranes were prepared from *S. aureus* strains by sonicating protoplasts suspended in 20 mM phosphate buffer pH 7.6. Samples were diluted to a final protein concentration of 0.25 μg/μL and incubated in the presence of 150 μM NADH. Spectral scans were taken of each sample at 5 minute intervals over the course of 30 minutes. NADH oxidation is measured by decrease in absorbance at 340 nm. Blue lines represent the initial spectral reading and red lines represent the 30 minute spectral reading. **A.** NADH does not degrade over the course of 30 minutes in buffer alone. **B.** Inverted membranes prepared from Δ*hrtB* are capable of oxidizing significant levels of NADH. **C.** Inverted membranes prepared from Δ*hrtB* Δ*menA* do not significantly oxidize NADH over the course of 30 minutes. Data shown are representative of biological triplicates.

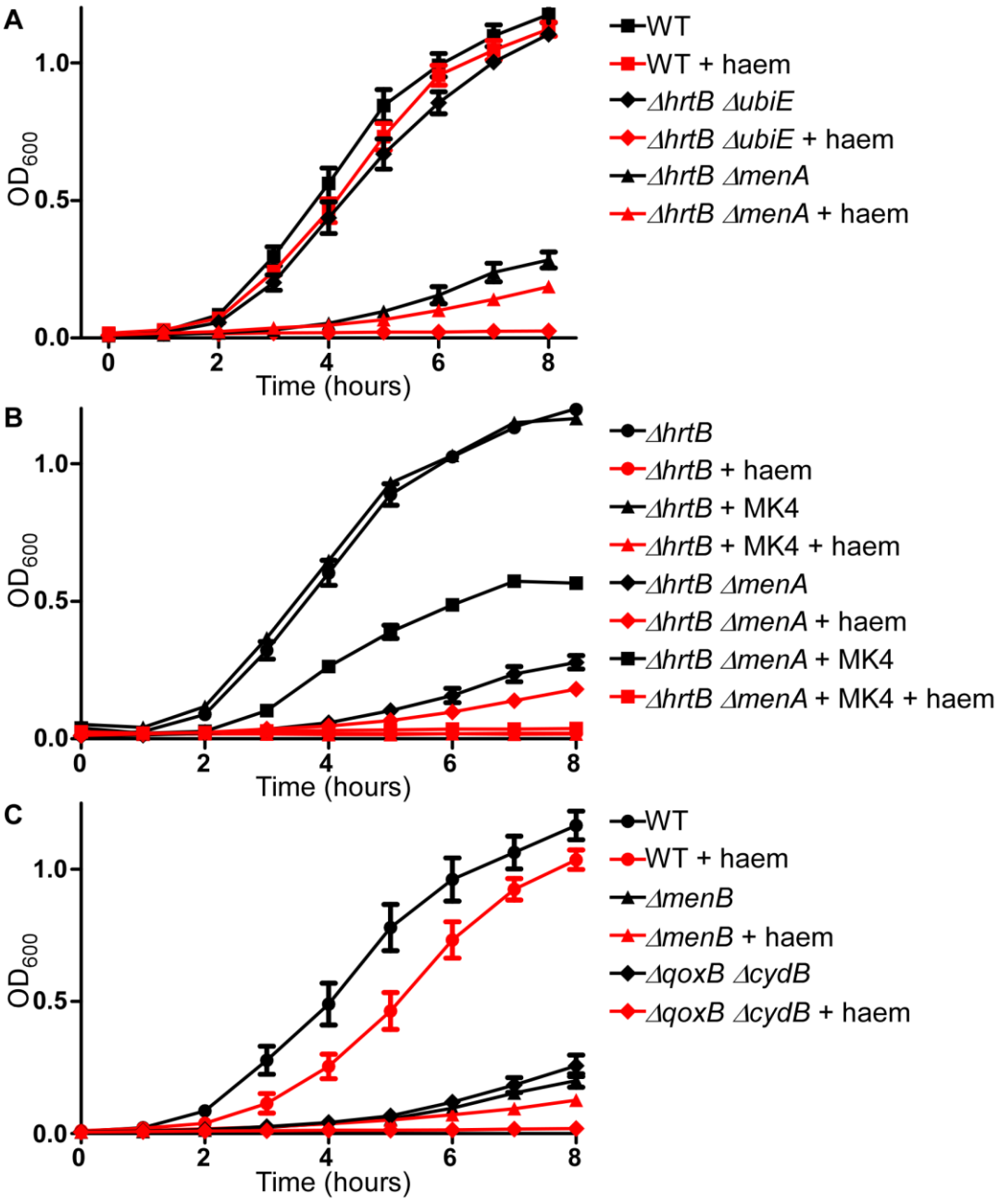

Fig. S3. *S. aureus* growth curves. A. The growth of *S. aureus* strains was monitored hourly by measuring optical density at 600 nm of cells cultured in TSB in the presence or absence of 6 μM haem. **B.** The growth of *S. aureus* strains was monitored hourly for cells cultured in the presence or absence of 6 μM haem and 12.5 μM MK4. **C.** The growth of *S. aureus* strains was monitored hourly for cells cultured in the presence or absence of 10 μM haem. Growth assays were performed at least three times on three separate days. Additionally, technical triplicates were performed on each day. Error bars represent SEM of the biological replicates.