Supporting data

Staphylococcus aureus adaptation to aerobic low redox potential environments: implications for an intracellular lifestyle

Benjamin A. F. Christmas, Matthew D. Rolfe, Matthew Rose and Jeffrey Green

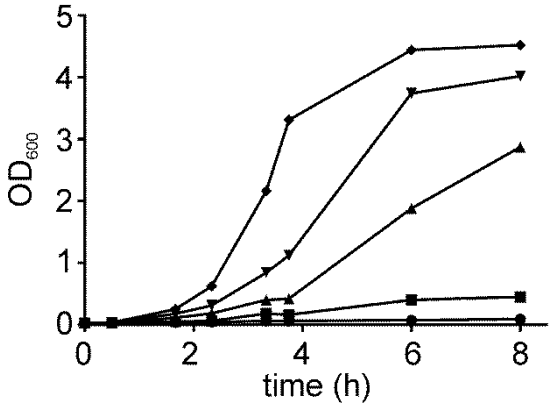

Fig. S1. The reducing agent dithiothreitol inhibits aerobic growth of *S. aureus* USA300 JE2. Growth (optical density at 600 nm, OD_{600}) of aerobic batch cultures of *S. aureus* in chemically defined medium supplemented with 0 (diamonds), 4.85 (inverted triangles), 9.7 (triangles), 19.4 (squares) or 38.8 (circles) mM dithiothreitol (DTT). Data are representative of three independent experiments.

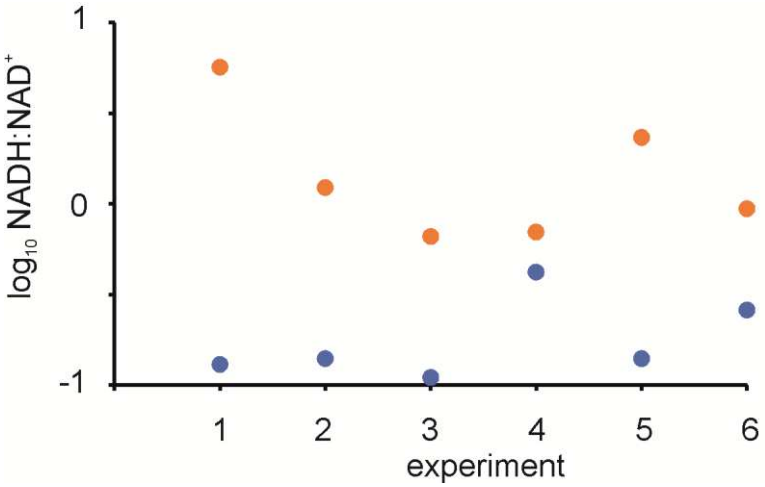

Fig. S2. Ratio of NADH:NAD^+ increases following exposure of *S. aureus* USA300 JE2 to a low redox potential environment. Aerobic batch cultures of *S. aureus* were grown to mid-exponential phase (OD_{600} ~ 0.9-1.1) and samples were removed to measure the intracellular NADH:NAD^+ ratios (blue circles). The cultures were perturbed by addition of reduced glutathione (10 mM) and after 1 h further samples were taken and the NADH;NAD^+ ratios were measured (orange circles). Data for six independent experiments are shown.

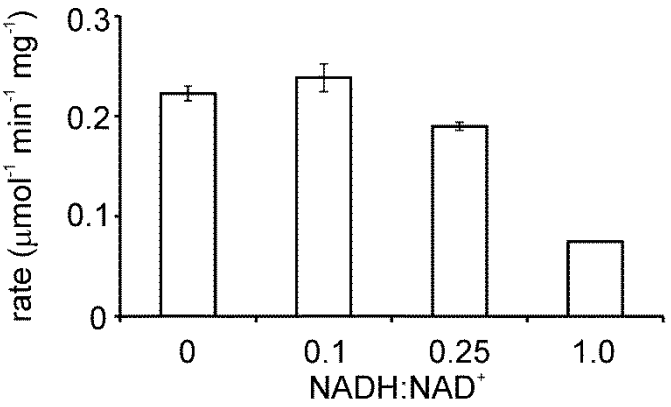

Fig. S3. *Staphylococcus aureus* USA300 JE2 pyruvate dehydrogenase complex is inhibited by NADH. Pyruvate dehydrogenase complex activity of *S. aureus* crude cell-free extracts was measured in the presence of different concentrations of NADH by measuring the production of CoASH from acetyl-CoA. Data shown are the mean values (n=3); error bars show the standard deviations.

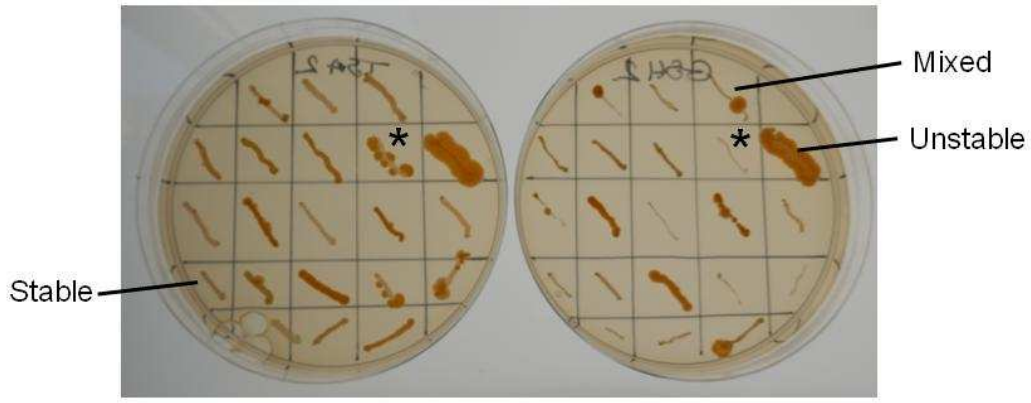

Fig. S4. Glutathione promotes maintenance of the SCV phenotype. SCVs selected by growth on Typtic Soy agar containing 3 μg ml^{-1} gentamicin were sequentially transferred to Tryptic Soy agar plates containing GSH (10 mM) and Tryptic Soy agar only. After incubation for 3 days the patches were scored as: Unstable, reversion to wild-type phenotype; Mixed, partial reversion; or Stable, SCV phenotype retained; examples are indicated above. The asterisks (*) indicate a SCV that exhibited a Mixed phenotype in the absence of GSH but retained the SCV phenotype in the presence of GSH.

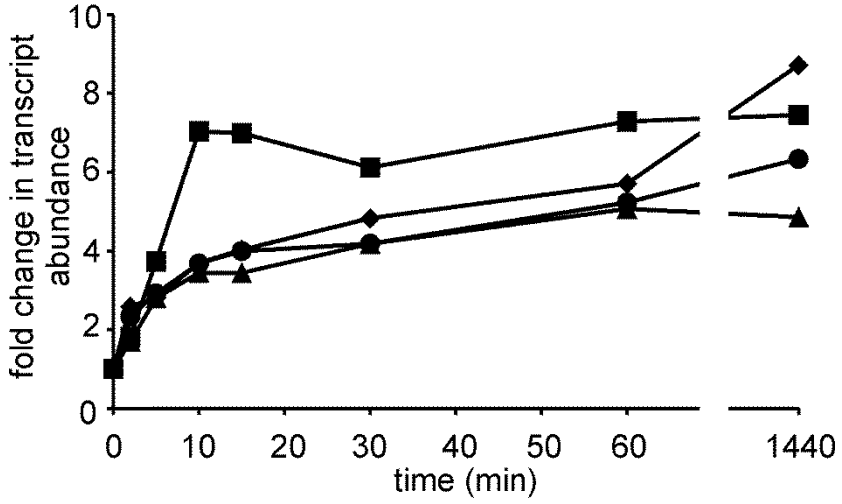

Fig. S5. Changes in abundance of transcripts associated with aerobic respiration after perturbation of aerobic steady-state chemostat cultures of *S. aureus* USA300 JE2 by addition of DTT. Changes in transcript abundances (fold changes of the indicated transcripts in the gene expression data relative to the initial steady-state levels, t = 0) for: *cyd* operon (*SAUSA_0986-0987*, squares); *qox* operon (*SAUSA_0960-0963*, diamonds); *ctaA* (*SAUSA_1015*, triangles) and *cyoE* (*SAUSA_1016*, circles). *Staphylococcus aureus* USA300 Je2 possesses a heme-copper oxidase (Qox) and a cytochrome *bd*-type oxidase (Cyd). Transcripts coding for the Cyd oxidase were maximally induced within 10 min of perturbation. Transcripts coding for Qox, the cytochrome oxidase assembly protein, CtaA, and the heme biosynthesis protein, CyoE, were also significantly up-regulated after exposure to DTT. These responses are interpreted as attempts to restore aerobic respiratory capacity.