Supplementary Information

Title: SaeRS is responsive to the cellular respiratory status and regulates fermentative biofilm formation in *Staphylococcus aureus*.

Authors:

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Figure S1.

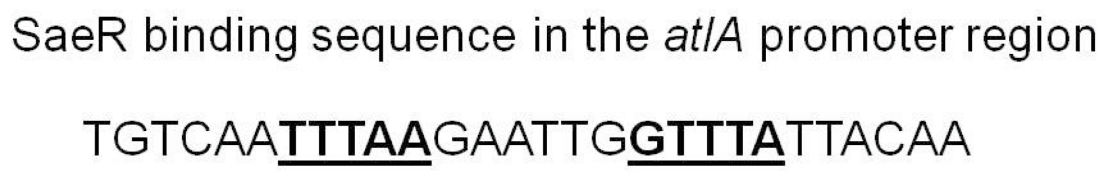

Figure S1. Putative SaeR binding site in the promoter region of *atlA*. Depiction of the putative SaeR binding sequence in the *atlA* promoter (underlined) (consensus sequence= GTTAAN_6GTTAA). The putative SaeR binding site is located ~180 base-pairs upstream of the translational start site on the negative strand. The binding site differed from the consensus sequence by two base pairs: the dispensable base pair guanine and one adenine. Two-base pair variations in the SaeR binding sequence and/or the replacement of an adenine base-pair have been observed in the promoter region for alternate SaeR regulon members such *coa* (GTTAA N_6 GTT**T**A), *nuc* (**A**TTAA N_6 **A**TTAA), and *tst* (**A**TTAA N_6 **T**TTAA).

Figure S2

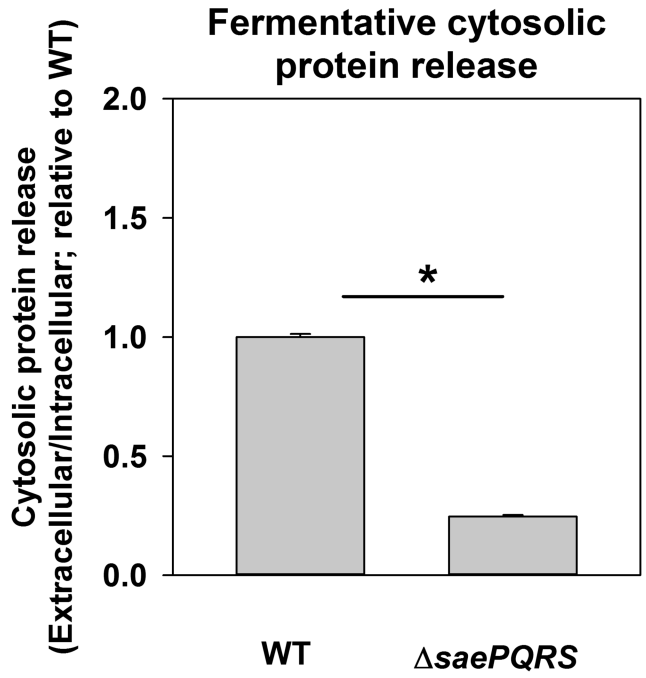

Figure S2. Cytosolic protein release is decreased in a Δ*saePQRS* strain during fermentative growth. Biofilms of the WT (JMB 1100) and Δ*saePQRS* (JMB 1335) were cultured fermentatively and the activity of the cytosolic protein catalase (Kat) was measured in the spent media supernatant. Extracellular Kat activity was normalized to intracellular Kat activity and thereafter to levels in the WT. The data represent the average value of triplicates. Error bars represent standard deviations. Statistical significance was calculated using a two-tail Student's t-test and * indicates p-value of <0.05.

Figure S3

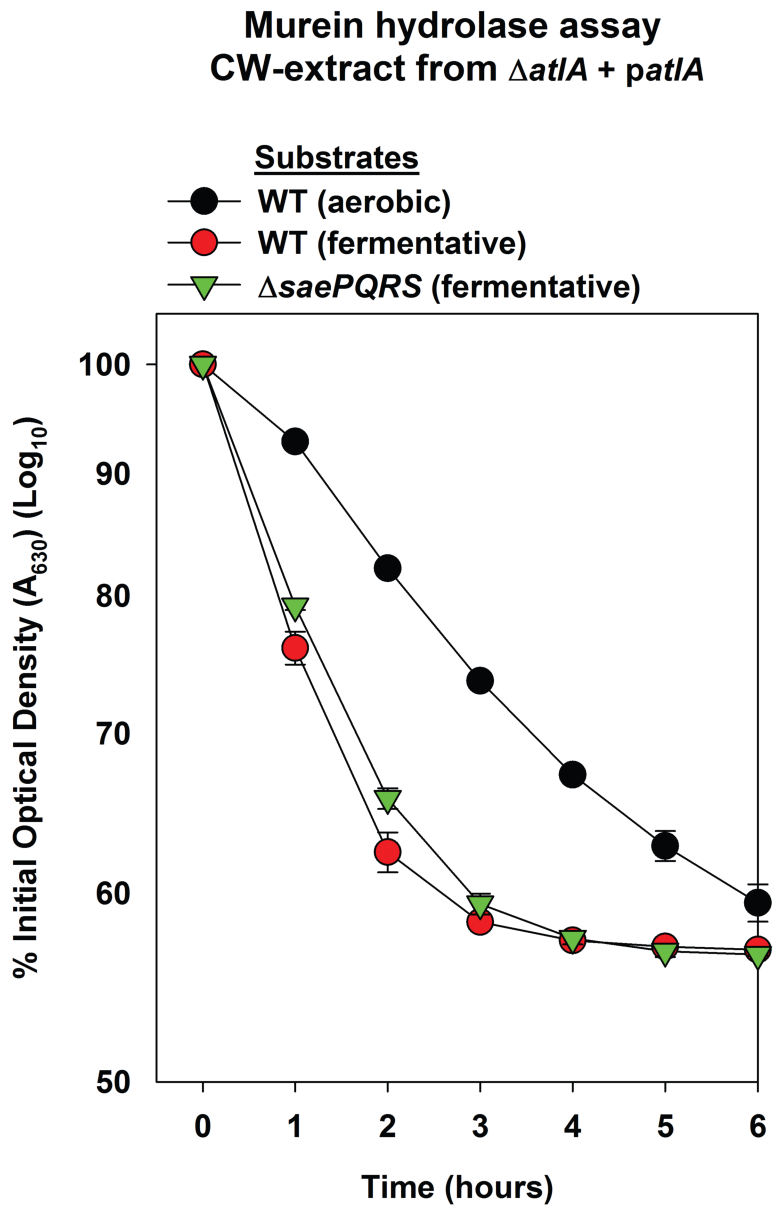

Figure S3. Heat-killed fermentatively cultured Δ*saePQRS* cells are cleaved at the same rate as the WT. Murein-hydrolase activity was determined using cell-wall (CW) extracts detached from a Δ*atlA* strain (KB 5000) carrying a plasmid encoding for full-length AtlA (p*atlA*). The substrates were heat-killed cells of the WT cultured aerobically

or fermentatively and the Δ*saePQRS* strain cultured fermentatively (pH of 7.5). The data represent the average value of technical duplicates from one set of substrate preparations. Heat-killed substrates were prepared and assayed on least two separate occasions and similar results were obtained. Error bars represent standard deviations. Error bars are displayed for all data, but might be too small to see on occasion.

Figure S4

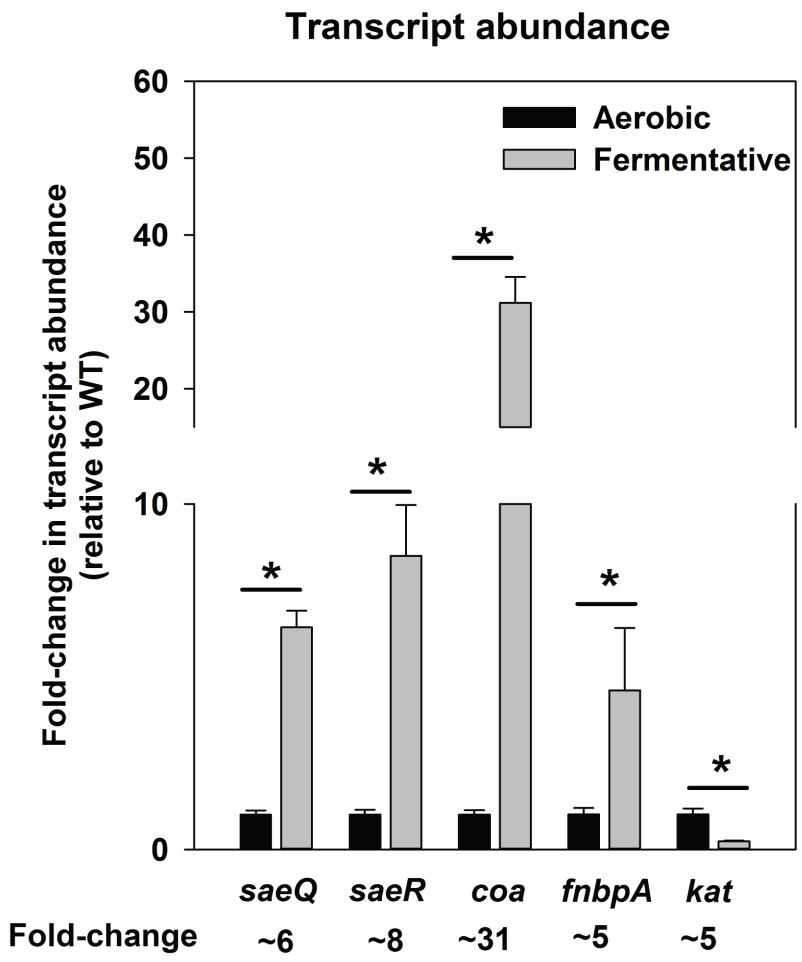

Figure S4. Transcript levels corresponding to Class 1 Sae regulon members are increased upon fermentative growth. Biofilms of the WT (JMB 1100) strain were cultured fermentatively and aerobically, mRNA was extracted, and the abundances of the *saeQ, saeR, coa, fnbpA* and *kat* transcripts were quantified. Data were normalized to 16S rRNA levels, and thereafter to levels observed in the WT cultured aerobically. Data represent the average value of biological triplicates and error bars represent standard deviations. Statistical significance was calculated using a two-tail Student's t-test and * indicates p-value of <0.05.

Figure S5

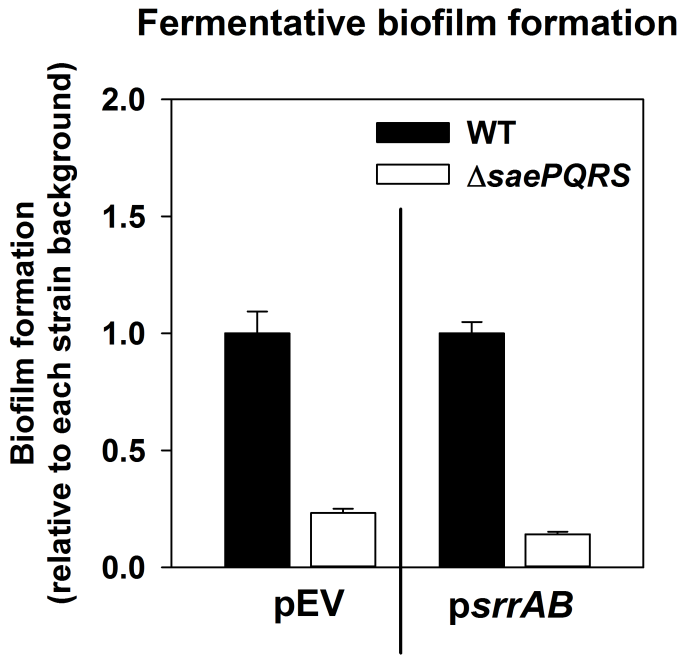

Figure S5. The presence of *srrAB* in multi-copy does not rescue the biofilm formation defect of the Δ*saePQRS* strain. Biofilm formation of the WT and Δ*saePQRS* (JMB 1335) strains carrying either pCM28 (pEV) or pCM28_*srrAB* (p*srrAB*) is displayed following fermentative growth. The data represent the average of eight wells and error bars represent standard deviations. Error bars are displayed for all data, but might be too small to see on occasion.

Figure S6

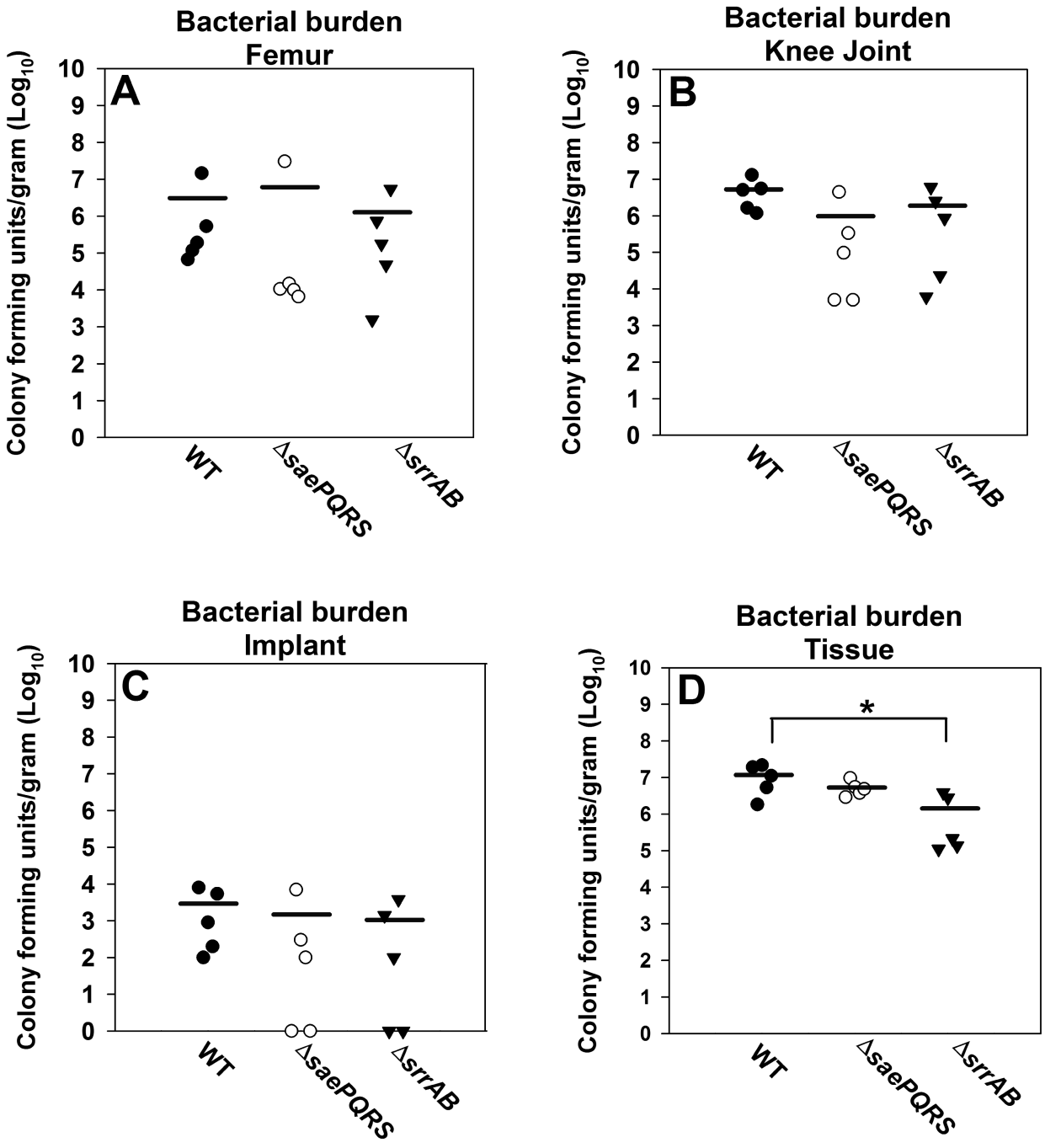

Figure S6. SaeRS and SrrAB influence biofilm persistence in a murine model of orthopedic implant biofilm infection. Panels A-D; Male C57BL/6 mice were infected with the WT (JMB 1100), Δ*saePQRS* (JMB 1335) and the Δ*srrAB* (JMB 1467) strains

(n=5/group). Animals were sacrificed at seven days following infection, whereupon the implant was sonicated and host tissues surrounding the infected orthopedic implant site were homogenized to quantitate bacterial burdens. Results are expressed as CFU per milliliter for the implant and CFU per gram of tissue (for the soft tissue surrounding the knee, knee joint containing ligament and tendon structures, and femur) to normalize for differences in sampling size. Significant differences in bacterial burdens between mice are denoted by asterisks (*, $p < 0.05$). Statistics were conducted using a one-way analysis of variance (ANOVA), followed by Bonferroni's multiple-comparison test.

Supplementary table 1: Oligonucleotides used in this study

RT- PCR primers

atlA For	GGTGCAGTCGGTAACCCTAGAT
altA Rev	TGAACGTGCAAATGAAGCATAGT
fnbpA For	CAGTAACAGAACAACCGTCAAACG
fnbpA Rev	TTGCTGGTTGTGCAGTTTGTG
saeQ For	TTATCATTCGTGTGGGTTCAGGTA
saeQ Rev	TCCGCCCGTTAATTTTTCG
saeR For	AAGAACATGATACCATTTACGCCTTA
saeR Rev	CCCTTGGACTAAATGGTTTTTTGA
coa For	TCGTTCAAGGTCCCGATTTT
coa Rev	CGGTGGGTTTGTATAATTATTGCTT

Cloning primers

saeSpCMdwn	TGATTACGAATTCATGATCGAATGCTAGCAGGCGGCATACAGTTAATTTCAATTGGTGT
saePYCC5	TAATATAGCGTAACTATAACGGTCCTAACTTGTTGTTTTTATTGTGGCAAAAGGTTTAT
saePYCC3	ATAAACCTTTTGCCACAATAAAAACAACAAGTTAGGACCGTTATAGTTACGCTATATTA