

Fig. S1. Comparison of growth of wild-type, $\Delta msrA1B$ and complemented $\Delta msrA1B$.

The growth of *S. aureus* wild-type, $\Delta msrA1B$ and complemented $\Delta msrA1B$ in tryptic soy broth (TSB) supplemented with human serum albumin (HSA) was monitored by measuring the OD at 550 nm. The OD at the mid-log phase of growth for each is plotted, n = 3 experiments.

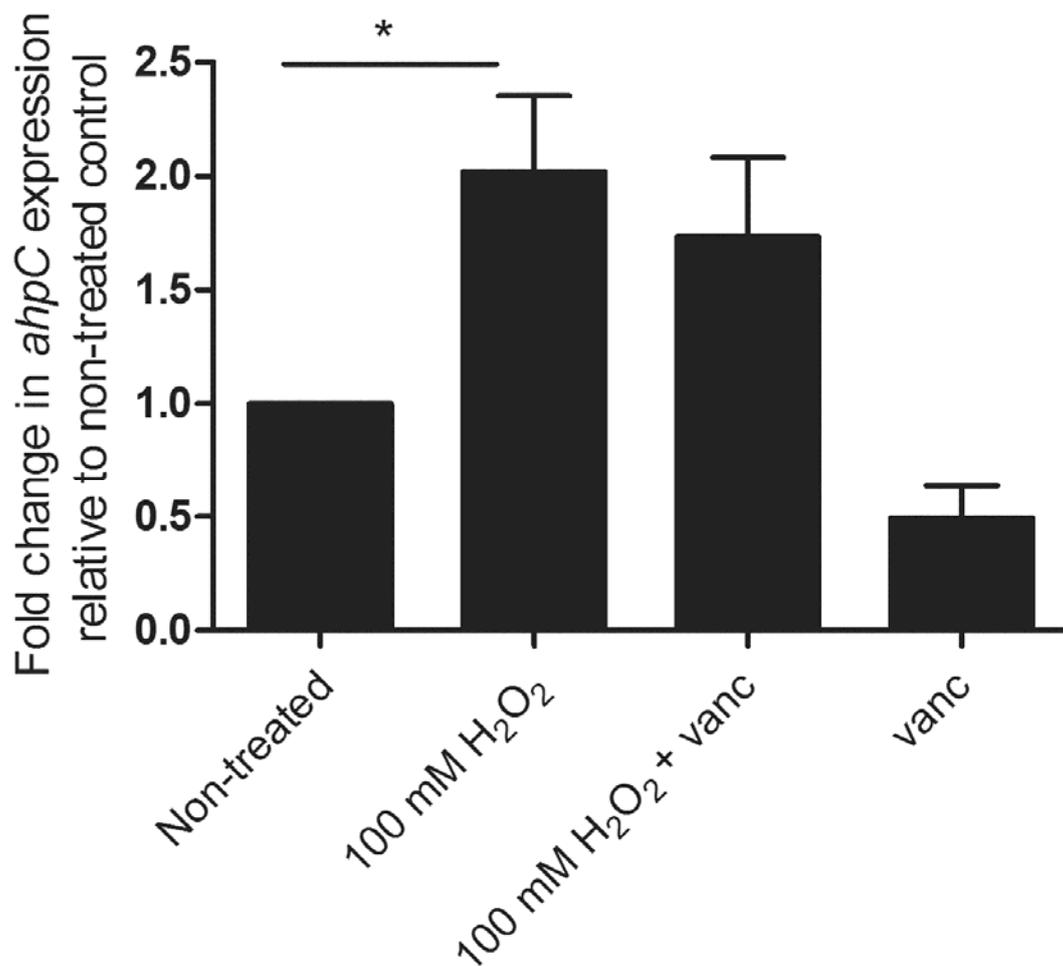


Fig. S2. Expression of *ahpC* in *S. aureus* treated with oxidants in the presence and absence of vancomycin.

Wild-type *S. aureus* cultured in minimal media was left untreated or treated with H₂O₂ in the presence or absence of vancomycin or with vancomycin alone for one hour at 37°C.

Bacteria were then assessed for *ahpC* expression by real-time PCR (forward primer 5'-TCAACGTGGTACATTTCATTATCGA-3' reverse primer '5' TGTACTAGCGTCACGGCCAAT 3') and the data were normalized to *gapdh* expression. The data represent the fold change in *ahpC* expression relative to the non-treated control, mean ± SEM, n = 6. A paired t-test was used to compare sample conditions, * p < 0.05.

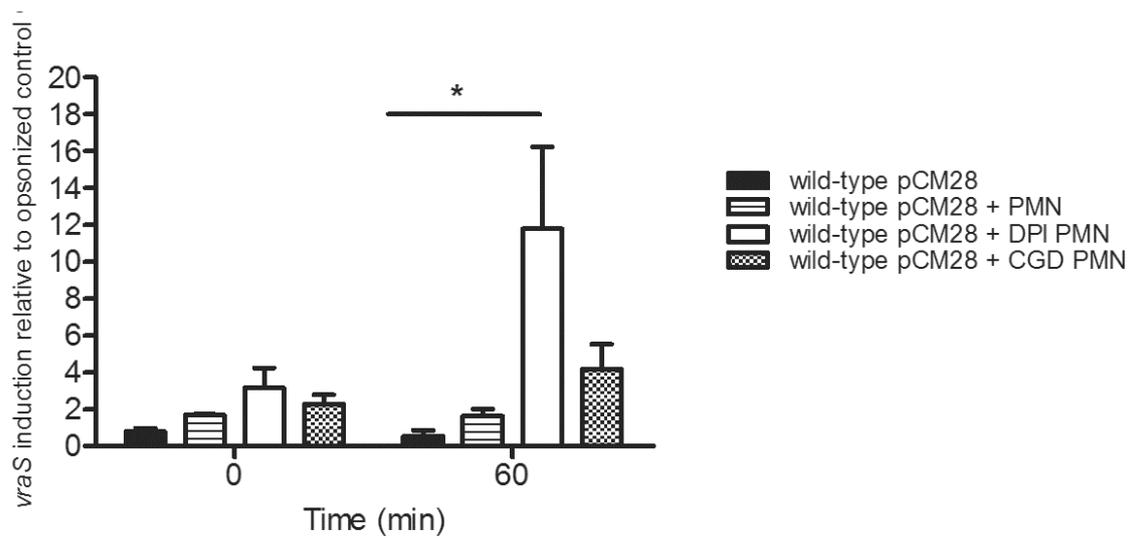


Fig. S3. Expression of *vraS* in *S. aureus* ingested by PMN.

The expression of *vraS* was measured by real-time PCR in the wild-type strain ingested by normal, DPI-treated or CGD PMN. Samples were prepared as stated in the legend of Figure 3C in the main article. Data at time = 60 minutes are analysed by 1-way ANOVA followed with a Tukey's post-test, * $p < 0.05$.

**Supplementary Table 1. Oligonucleotide sequences relevant to TCS
mutant construction.**

Oligo	Sequence 5' to 3'
SAUSA300_0217-18 up5EcoRI	GGGGAATCCAACAACGACAGGCTATCAACATATGCGTGC
SAUSA300_0217-18 up3fuse	ACGCGTGGTACCGCTAGCGCTAGCGATGTCTATAAGGTTTGTATGCCGTCATTG
SAUSA300_0217-18 dwn5fuse	GCTAGCGGTACCACGCGTACGCGTCTAATGATTATGGCGCCTCCATTTTGTCTATG
SAUSA300_0217-18 dwn3BamHI	CCCGGATCCGGACAGATGTTTTTCATCGTCTTCTATGTTGC
SAUSA300_254-5up5EcoRI	GGGGAATCCAGCGTCTCAACGACGTTGAACAAACG
SAUSA300_254-5up3fuse	ACGCGTGGTACCGCTAGCGCGATAGCACGATAGCACCTCAGTGAATT
SAUSA300_254-5dwn5fuse	GCTAGCGGTACCACGCGTGCATAGGATTACTTTAACAGTAATCC
SAUSA300_254-5dwn3Sall	GGGGTCGACCACCTAATGTGTGACCACTTGTTCCAAGTGC
SAUSA300_645-6upEcoRI	GGGGAATTCTGCCAAAATAACACAATCATCAGCAAGAG
SAUSA300_645-6up3fuse	ACGCGTGGTACCGCTAGCCTAGTAGTATTTGCATCCATATCACCCAATATCATTTAG
SAUSA300_645-6dwn5fuse	GCTAGCGGTACCACGCGTGGGACAACGTGTCAGATTGATTTTCCCATTA
SAUSA300_645-6dwn3Sall	CCCGTCGACCCCTTGGTATAATTCAGTGAAAATTTGACC
SAUSA300_1219-20 up5EcoRI	GGGGAATTCGGCGCCAAAGATTAATGGTAACACCATTTAC
SAUSA300_1219-20 Up3fuse	ACGCGTGGTACCGCTAGCGCTAGCCCGGCAATAGGAAAATCAGATAAACTATAGACG
SAUSA300_1219-20 dwn5fuse	GCTAGCGGTACCACGCGTACGCGTGGAAAGTGAGTCAATAATCAAATCTTACTCAATA
SAUSA300_1219-20 dwn3Sall	CCCGTCGACGTAATACACCTACAATAATGATGGCTACCAC
SAUSA300_1441-2up5EcoRI	CCCGAATTCGGACTAGTTGAGGCTAAAGTGGTAAATGAACAGCG
SAUSA300_1441-2up3fuse	ACGCGTGGTACCGCTAGCGTATTTGTTTCGACATACAGGTCATACCTCCC
SAUSA300_1441-2dwn5fuse	GCTAGCGGTACCACGCGTGGGAAAGGCACAACATTTATTATTAATACTACC
SAUSA300_1441-2dwn3Sall	CCCGTCGACCTGTGCACCCCATATTCTATTGGACG
SAUSA300_1638-9up5EcoRI	GGGGAATTCGGCTCATTTAAGCAGTGGGGTTACGA
SAUSA300_1638-9up3fuse	ACGCGTGGTACCGCTAGCCCCACTACCTATGCATGAGTTTTACTC
SAUSA300_1638-9dwn5fuse	GCTAGCGGTACCACGCGTGGTAACAGCGCGTGTATTTTTCACG
SAUSA300_1638-9dwn3Sall	CCCGTCGACGCCATACTAATGAGGCCATATGAAAGCC
SAUSA300_1798-99 up5EcoRI	GGGGAATCCCTACTGTTTCTGGTCCAGTCGATGAATTGGTTCGAC
SAUSA300_1798-99 up3fuse	ACGCGTGGTACCGCTAGCGCTAGCCGCTCCAATTCATTTTTACGTGTATTAATACTATTAT
SAUSA300_1798-99 dwn5fuse	GCTAGCGGTACCACGCGTACGCGTGCATCGTACACAAGCAGCAATTTATGC
SAUSA300_1865-66up5EcoRI	GGGGAATCCGGCGAAACATGACACACAAATATATATCAACGC
SAUSA300_1865-66 up3fuse	ACGCGTGGTACCGCTAGCGCAGCTAGCATGCTATATACTAAGATGAGCATTG
SAUSA300_1865-66dwn5fuse	GCTAGCGGTACCACGCGTGCCTAATCACAATATAACATCAAATAGACACC
SAUSA300_1865-66dwn3Sall	CCCGTCGACGCCGTAATGGAATCGTATTAATACTACTAAGTG
SAUSA300_2035-36 Up5EcoRI	GGGGAATCCCCCATAAATCCATTAATGCCAGAAAATGTTTGAC
SAUSA300_2035-36 up3fuse	ACGCGTGGTACCGCTAGCGCTAGCGGATTCAGTGTGACATAACCTTCACCTCG

SAUSA300_2035-36 dwn5fuse	GCTAGCGGTACCACGCGTACGCGTGGCTATGTTAATAAGACTGAAATGCCTAGTTTAAG
SAUSA300_2035-36 dwn3Sall	CCCGTCGACCGGTAAACCAAGTGGTTCTCGTAACAGAAATAGT'
SAUSA300_2308-9up5EcoRI	GGGGAATCCGCACTCAATAAATGTCTTGTCTTAATACC
SAUSA300_2308-9up3fuse	ACGCGTGGTACCGCTAGCGGGAGAAGTGAATCGATATGAAATTAGCG
SAUSA300_2308-9dwn5fuse	GCTAGCGGTACCACGCGTGGAGGTATCTTTTATATTGATTAGCGCTTTCTAGCC
SAUSA300_2308-9dwn3sall	CCCGTCGACGCGCTCCTTTATAGATTTTAAATAACGTCTAGAAG
SAUSA300_2338-9 up5EcoRI	GGGGAATTCGAGGTGAAAGGATTTGACACCAGAAGC
SAUSA300_2338-9 up3fuse	ACGCGTGGTACCGCTAGCGCTAGCGACTTACACCCTAATTCATCTCATTATTTATC
SAUSA300_2338-9 dwn5fuse	GCTAGCGGTACCACGCGTACGCGTGC GCGAGAGGAAATGATTATCTTCTGGCGC
SAUSA300_2338-9dwn3BamHI	CCCGGATCCCATATGTCTTTGAGTCATTATCCAATCCAAG
SAUSA300_2558-9up5EcoRI	GGGGAATCCAGAACTAAAAACCGTTGACACGATAATAAGG
SAUSA300_2558-9 up3fuse	ACGCGTGGTACCGCTAGCGCTAGCCCTGCTGCAATGACGGCTCTTGATTTG
SAUSA300_2558-9 down5fuse	GCTAGCGGTACCACGCGTACGCGTGGGTACAACATTTACGATTCAATTTCCAGATGAAT
SAUSA300_2558-9 dwn3BamHI	CCCGCTAGCCTATAATCTTCTTCTTCAATTGAATTATCCTTTGTTATC

Supplementary methods

Construction of the $\Delta msrA1B$ and $\Delta vraSR$ strains.

Approximately 500 base pairs upstream and downstream of the *msrA1B* genes (SAUSA300_1316-7) were amplified using with polymerase chain reaction (PCR) with wild-type chromosomal DNA as a template and the following primer pairs; *msrA1_5EcoRI* and *msrA1_up3NheI*; *msrB_dwn5mluI* and *msrB_dwn3BamHI*. The amplicon was used as a template for a fusion PCR reaction using the *msrA1_5EcoRI* and *msrB_dwn3BamHI* primer pair. The fusion PCR product was digested with *EcoRI* and *BamHI*. The digested product was ligated into similarly digested pJB38, transformed into *E. coli* DH5 α cells and transformants were selected with ampicillin (150 μ g/ml). Colonies containing pJMB176 were grown overnight after which plasmids were isolated and transformed into RN4220. Transformants were selected on tryptic soy agar (TSA) containing chloramphenicol (Cm 30 μ g/ml) at 30°C. The pJMB176 plasmid was isolated from RN4220 and transformed into wild-type via electroporation and cells were allowed to recover at 30°C before selection on TSA-Cm at 30°C. Single colonies were used to inoculate TSB containing Cm and cultures were grown overnight at 42°C to select for single recombinants. Cultures were plated on TSA-Cm and incubated overnight at 42°C. Single colonies were used to inoculate TSB and cultures were grown overnight. Cultures were diluted before plating 100 μ l on TSA-anhydrotetracycline (150 ng/ml). Colonies were screened for the double recombination event using PCR and the primers *msrA_veri5* and *msrA_veri3*.

The *vraSR* mutant was created using the same protocol as outlined above with the following exceptions: 1. The upstream and downstream

regions of *vraSR* (SAUSA300_1865-66) was amplified using the following primer pairs: 1865up5EcoRI and 1865up3fuse; 1865down5fuse and 1865down3Sall; 2. The 1865up5EcoRI and 1865down3Sall primer pair was used for the fusion PCR reaction; 3. Colonies containing cells that underwent the double recombination event were verified using the 1865veri5 and 1865veri3 primer pair.

Construction of complementing plasmids and strains. PCR was used to amplify the *msrA1* and *msrB* gene (SAUSA300_1317-8) and their native promoter using wild-type chromosomal DNA as a template and the primers *msrA1B_comp5EcoRI* and *msrA1B_comp3BamHI*. The PCR product was digested with EcoRI and BamHI. The digested fragment was gel ligated into similarly digested pCM28, and transformed into *E. coli* DH5 α cells. Cells containing the plasmid were selected for using LB-Amp150 plates, The resulting plasmid was isolated and transformed into RN4220, selected on TSA-Cm30 and after verification by sequencing was subsequently transduced into the Δ *msrA1B* strain using bacteriophage 80 α . The *vraSR* genes were cloned using the same protocol outlined above with the following exceptions: 1. The pEPSA5 plasmid was used instead of pCM28; 2. The primers 1865comp3Sall and 1865compBamHI were used to amplify *vraSR*; 3. The superoxide dismutase (*sodM*) ribosomal binding site was engineered upstream of the ATG start codon. For oligonucleotide sequences refer to Table 2.

Construction of TCS mutants

S. aureus chromosomal mutations were created using homologous recombination of a knock-out vector introduced by transduction mediated by bacteriophage 80 α . Approximately, 500-1000 bp regions upstream and downstream of the genes encoding the TCS response regulator and histidine kinase were generated by PCR. These fragments were fused using extension overlap PCR to generate a 1.0-2.0 kb fragment which was restriction enzyme digested and cloned into the poly-linker of similarly digested pJB38. The generated vectors were transformed into *S. aureus* RN4220 and lysates were made with bacteriophage 80 α . Following transduction into the target *S. aureus* strains at 30°C, clones exhibiting chloramphenicol resistance were selected on TSA containing chloramphenicol at 45°C. Clones able to grow at the elevated temperature were isolated a second time at 45°C, and bacterial clones were grown in TSB at 30°C. These bacterial cultures were sub-cultured 1:1000 daily for 3 days, and the cultures were plated and bacterial isolates were screened for chloramphenicol sensitivity [1]. Antibiotic sensitive isolates were screened for the chromosomal deletion by PCR. For oligonucleotide sequences relevant to TCS mutant construction refer to Supplementary Table 1.

Msr Activity Assay

15ml of cell suspension was pelleted at 1900 g for 10 minutes at 4°C. The cells were washed once with 10 ml of assay buffer (50 mM TRIS-HCl pH7.5, 145 mM NaCl), pelleted again and then re-suspended into 200 μ l of assay buffer. 25 μ g of lysostaphin was added to each sample and bacteria were lysed at 37°C for 10 minutes. The lysates were sonicated on ice and then 70

µl of each sample was incubated with 2 mM Dabsyl-methionine sulfoxide and 20 mM DTT in assay buffer to a final volume of 100 µl. After 1 hour at 37°C, reactions were quenched with 50 µl of acetonitrile. Samples were cleared by centrifugation at 20,000 x g for 5 minutes at 4°C and then analyzed using reverse phase HPLC. The mobile phases were as follows, solvent A (25 mM KH₂PO₄ pH 7.0) and solvent B (70% acetonitrile, 30% methanol). A LC-Dabs column (Supelco) was equilibrated with 20% A at a flow rate of 1.5 ml / minute. 10 µl of the sample was injected onto the column and the dabsyl methionine sulfoxide and dabsyl-methionine were separated using the following time program: (i) 0.5, 25 %B, duration 1 minute (ii) 3 minutes, 32 %B, duration 1 minute (iii) 10 minutes, 40% B duration 7 minutes (iv) 17 minutes, 60 %B, duration 9 minutes (v) 26 minutes, 75%B, duration 1 minute. The protein concentration of the lysates was measured and the conversion of methionine sulfoxide to methionine was determined using the area under the chromatographic peak for each species.

References

1. Bae T, Schneewind O: Allelic replacement in *Staphylococcus aureus* with inducible counter-selection. *Plasmid* 2006;55:58-63.