Supporting Information

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SI Materials and Methods

Bacterial Strains and Growth Conditions. *Escherichia coli* were grown in Luria–Bertani (LB) and *Staphylococcus aureus* were grown in tryptic soy broth (TSB) at 37 °C with shaking. For a complete list of strains, see Table S1. Antibiotic concentrations for maintenance of *E. coli* plasmids were 100 μg/mL for ampicillin (Amp) and 50 μg/mL for spectinomycin (Spec). *S. aureus* plasmids were maintained with 10 μg/mL chloramphenicol (Cam). Bacterial growth was monitored using a NanoDrop 2000c Spectrophotometer (Thermo Scientific) or a Tecan Infinite M200 plate reader (Tecan).

Recombinant DNA and Genetic Techniques. Restriction enzymes were obtained from New England Biolabs, and T4 DNA ligase was obtained from Promega Corporation. Primers used in this study are listed in Table S4 and were synthesized at Integrated DNA Technologies. DNA manipulations were performed in *E. coli* strain ER2566 or GM2163 (New England Biolabs). Plasmids from ER2566 were transformed into *S. aureus* strain RN4220 by electroporation as previously described (1). Plasmids from GM2163 were electroporated directly into LAC-derived strains as previously described (2). Chromosomal markers were moved by bacteriophage φ11 transduction (3). Plasmid constructs and *S. aureus* mutants were confirmed by DNA sequencing at the University of Iowa DNA Core Facility.

Plasmid and Strain Construction. *Δ**saePQRS**.* Two-step overlap PCR was used to create pJB38-Δ*saePQRS*, and 600-bp regions directly upstream and downstream of the operon were amplified from AH1263 genomic DNA with primer pairs JT240/MJT241 or CEF10/CEF11, respectively, where MJT241 and CEF 10 contained complementary overlap regions (Table S4). The upstream and downstream PCR products were purified by agarose gel electrophoresis and mixed in equal volumes. This mixture was used as template for the second round of PCR with primer pair MJT240/CEF11, which produced a single 1.2-kb piece of DNA. The PCR product was purified, digested with EcoRI and KpnI, and ligated into similarly digested pJB38 (4). The resulting plasmid was used to construct a markerless Δ*saePQRS* strain in the AH1263 background as previously described (4) and was named AH2216.
*Δ**saeS**.* Six hundred-base pair regions from the AH1263 genome directly upstream and downstream of *saeS* were PCR amplified using primer pairs CEF180/CEF181 and CEF182/CEF11, respectively (Table S4). The upstream and downstream PCR products were purified, mixed in equal volumes, and amplified with CEF180/CEF11. The resulting overlap PCR product was purified, digested with EcoRI and KpnI, and ligated into similarly digested pJB38. The resulting plasmid was used to construct a Δ*saeS* strain in the AH1263 background as previously described (4) and was named CFS99.

saeS Complementation Plasmid. To construct a C-terminally T7-tagged SaeS construct, *saeS* was amplified from AH1263 genomic DNA using primers MJT249 (5) and CEF67. The PCR product was purified by agarose gel electrophoresis, digested with EcoRI and XhoI, and ligated into pEPSA5 digested with the same enzymes (6).

hla Fluorescent Reporters. The promoter for the α-toxin gene (*hla*) was amplified by PCR using oligonucleotides CLM429 and CLM430 on AH1263 genomic DNA as template. The product was digested by HindIII and KpnI and cloned directly upstream of the superoxide dismutase (SOD) ribosome binding site (RBS) (7) and sGFP gene in pCM11 (8). The new plasmid was designated pCM27.

pCM27 was digested using HindIII and EcoRI, removing the *hla* promoter, SOD RBS, and sGFP gene. The digestion product was then ligated to similarly digested pLL29 (9). Plasmids were integrated into the chromosome of RN4220 as previously described (9). The integrated reporter was moved into LAC (AH1263) and Δ*saeS* (CFS99) using ϕ11.

Substituted Cysteine Accessibility Method. To construct a protein expression construct of SaeS with the SOD RBS and a C-terminal T7-tag, *saeS* was amplified from AH1263 genomic DNA using primer pair CEF66/CEF67. The PCR product was digested with EcoRI and XhoI and ligated to a similarly digested pEPSA5. Cysteine point mutations were constructed using two-step overlap PCR. N-terminal fragments were amplified using the WT SaeS expression plasmid as a template, an upstream vector primer (EPSA5for2), and reverse primer containing the cysteine substitution (Table S4). C-terminal *saeS-T7* fragments were amplified using the same template with forward primers containing cysteine substitutions and a downstream vector primer (EPSA5rev). PCR products were purified, and appropriate N- and C-terminal fragments were mixed in a 1:2 ratio. The mixtures were used as templates for the second round of PCR with primer pair EPSA5for2/EPSA5rev. Overlap PCR products were purified, digested with EcoRI and XhoI, and ligated to pEPSA5 digested with the same enzymes. Final constructs used for the substituted cysteine accessibility method (SCAM) contained only one native cysteine residue.

Cysteine residues in the SaeS SCAM constructs were labeled with MPB [N^{α}-(3-maleimidylpropionyl)biocytin; Invitrogen] and were detected by Western blotting with streptavidin HRP (Strep-HRP). Residues exposed to the extracellular milieu label under all conditions, residues in the cytoplasm were only accessible following permeablization of the cells with lysotaphin, and residues in the membrane were completely inaccessible during both conditions.

SCAM was performed in *S. aureus* strain AH2216 containing individual SaeS SCAM constructs according to previously published methods with minor modifications (5). In brief, overnight cultures were diluted 1:50 into TSB containing Cam and 0.1% xylose and grown in a 37 °C shaker until cultures reached an OD_{600} of 2.0. Cells were pelleted and resuspended to an OD_{600} of 25 in buffer A (100 mM Hepes, 250 mM sucrose, 25 mM $MgCl_2$, and 0.1 mM KCl, pH 7.5). One set of samples was pretreated with lysostaphin (AMBI Products) before labeling with MPB. The other set of samples were first treated with MPB, followed by incubation with lysostaphin. Both sample sets were solubilized by adding 400 μL T7 binding/wash buffer (4.29 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl, 1% Nonidet P-40 substitute, pH 7.3). Labeled SaeS constructs were immunoprecipitated using anti-T7 agarose, (Novagen) and eluted with T7 elution buffer (100 mM citric acid, pH 2.2, 0.5% SDS). Because boiling membrane proteins can cause aggregation, samples were heated at 60 °C for 10 min following addition of 2× SDS/PAGE loading buffer containing 5% (vol/vol) β-mercaptoethanol (BME). Later attempts to denature the proteins included adding additional BME, a range of dithiothreitol concentrations, and 9 M urea.

SaeS Extracellular Point Mutations. SaeS extracellular loop mutations were generated using two-step PCR with vector primers and

complementary mutagenesis primers. N-terminal fragments were amplified using the complementing plasmid pEPSA5-(WT)*saeS-T7* as a template, an upstream vector primer (EPSA5for2), and reverse primer containing the alanine substitution (Table S4). C-terminal *saeS-T7* fragments were amplified using the same template with forward primers containing alanine substitution and a downstream vector primer (EPSA5rev). PCR products were purified and mixed and used as the template for the second round of PCR with primer pair EPSA5for2/EPSA5rev. Overlap PCR products were purified, digested with EcoRI and XhoI, and ligated to pEPSA5 digested with the same enzymes. All mutants were confirmed as described above.

Immunoblotting. SaeS constructs and SrtA (loading control) were detected from whole cell lysates harvested during the fluorescent *hla* reporter assay. Cell pellets were resuspended in PBS, and cells were lysed using lysostaphin and DNaseI, followed by addition of 2× SDS loading buffer containing 5% (vol/vol) BME. Five microliters of each sample was electrophoresed on a 10% (vol/vol) polyacrylamide gel. Proteins were transferred to PVDF-P membranes (Millipore), and membranes were blocked overnight at 4 °C with 5% (wt/vol) milk in Tris-buffered saline (20 mM Tris·HCl, pH 7.0, with 137 mM NaCl) containing 0.05% Tween 20 (TBST).

For detection of T7-tagged SaeS proteins, anti-T7 antibody conjugated to HRP (Novagen) was diluted 1:15,000 in 0.05% TBST and incubated with PVDF-P membranes at room temperature for 1 h. Membranes were washed five times for 2 min each with 0.05% TBST, incubated with SuperSignal West Pico chemiluminescent substrate (Thermo Scientific) for 5 min, and exposed to X-ray film (Research Products International).

Concurrent detection of SrtA was performed by diluting anti-SrtA antibody (AbCam) 1:3,000 in 0.05% TBST. Antibody was incubated with membranes at room temperature for 1 h. Membranes were washed three times for 10 min with 0.05% TBST and then incubated with goat anti-rabbit HRP secondary antibody (1:20,000 in 0.05% TBST) for 1 h. Membranes were again washed three times for 10 min, incubated with SuperSignal West Pico chemiluminescent substrate, and exposed to X-ray film.

Statistics. Error bars are SEM. Statistical calculations were performed in Prism (version 6 for Mac; GraphPad Software) using Student *t* test or ANOVA with Tukey's posttest for multiple comparisons. Asterisks denote significance based on *P* values as follows: *0.01–0.05, **0.001–0.01, and ***<0.001.

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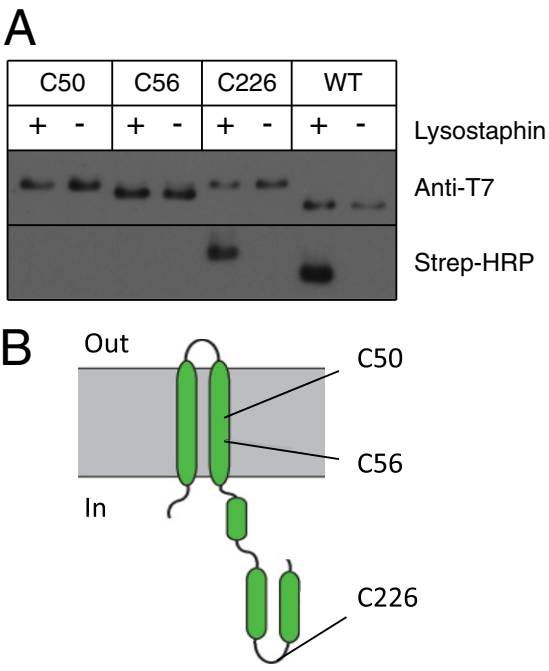

Fig. S1. SCAM results on native SaeS cysteine residues are consistent with topology prediction programs. (*A*) T7-tagged SaeS constructs containing only one of the three native cysteines were expressed in *S. aureus* USA300 strain LAC and labeled with MPB either with or without lysostaphin pretreatment. Protein expression was evaluated by T7 immunoblot while cysteine labeling was analyzed using Strep-HRP. Residues C50 and C56 did not label under either condition, suggesting they are inaccessible and buried in the membrane. Residue C226 only labeled following lysostaphin treatment, consistent with predictions that this residue is localized to the cytoplasm. Based on these findings, we would expect C226 to be the only accessible cysteine in the WT protein, which was run as a control and did not label without lysostaphin pretreatment. (*B*) Cartoon depiction of SCAM results and topology predictions.

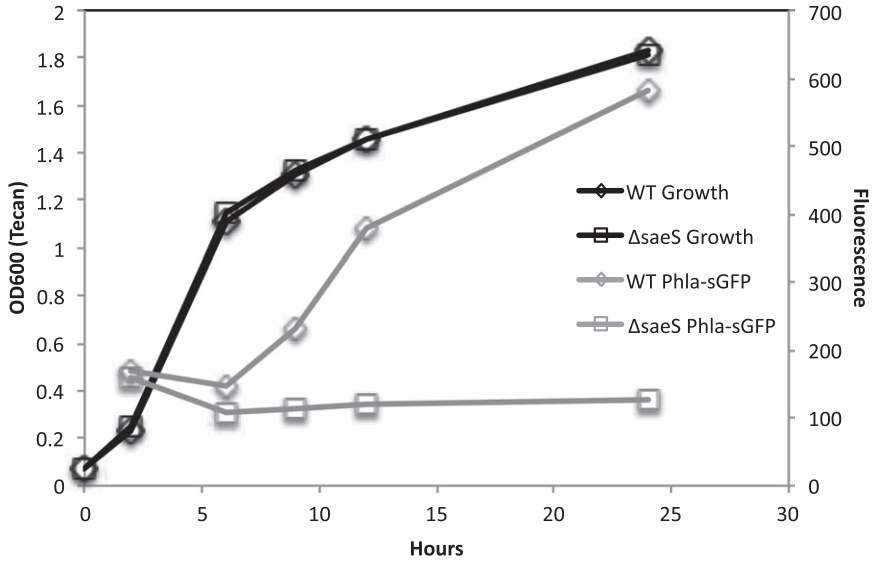

Fig. S2. Confirmation of *saeS* mutant using *hla* reporter. Growth and fluorescence from a fluorescent *hla* promoter fusion was monitored in *S. aureus* strain LAC and an isogenic Δ*saeS* mutant over the course of 24 h.

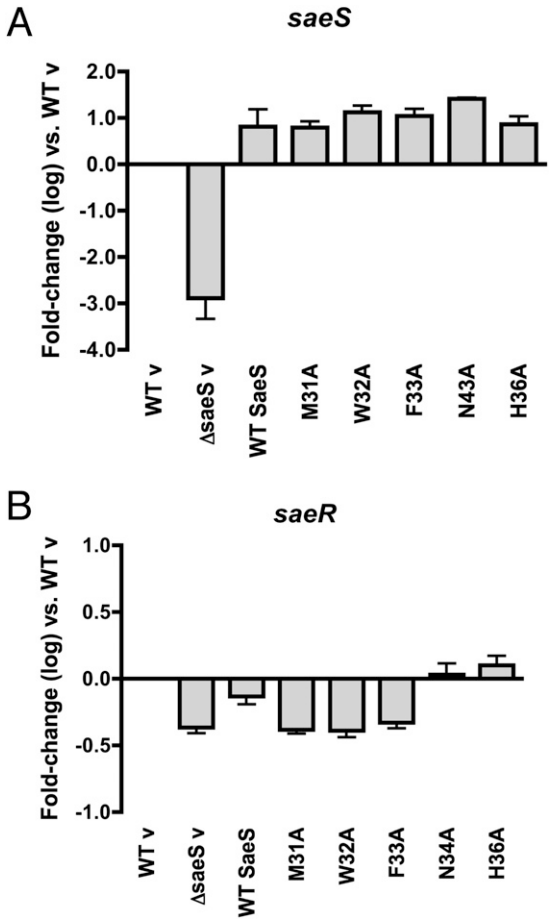

Fig. S3. In vitro basal transcription of *saeS* and *saeR* verified by qRT-PCR. RNA was extracted from bacteria harvested at midlog (OD_{600} = 1.5) and examined by TaqMan quantitative real-time PCR. Relative quantification of transcripts was determined by normalizing to *gyrB* and expressing mean values as $\log_{10}$ changes relative to the WT control. Two to four biological replicates of each experiment were analyzed in triplicate. (*A*) Expression of *saeS*. (*B*) Expression of *saeR*.

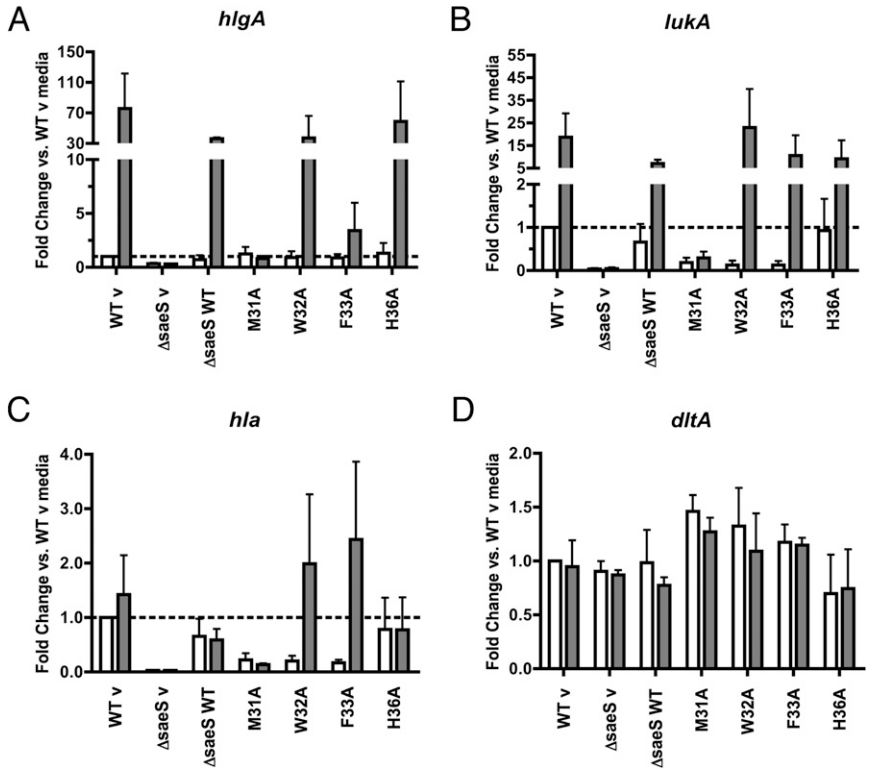

Fig. S4. SaeS mutant kinase strains have lower basal gene expression of *sae*-regulated toxins. QuantiGene 2.0 assays results from α-defensin 1 induced gene expression data in *S. aureus* strains. Data shown are normalized to *gyrB* calibrated to WT v without stimulus (media control) and displayed as mean fold change. Error bars are SEM of two biological replicates analyzed in triplicate. White bars, media control; gray bars, α-defensin. (*A*) *hlgA,* (*B*) *lukA,* (*C*) *hla,* and (*D*) *dltA* (control, gene without SaeR binding domain).

Table S1. Strains and plasmids used in this study

Strain or plasmid	Genotype/description	Resistance	Source
Strains			
Escherichia coli			
ER2566	Protein expression strain	None	New England Biolabs
GM2163	*dam- dcm-*	Cam	New England Biolabs
Staphylococcus aureus			
RN4220	Restriction modification deficient	None	(1)
AH1263	LAC (Erythromycin sensitive)		(2)
AH2216	AH1263 Δ*saePQRS*		This study
AH2270	AH1263::LL29-*hla*-sGFP	Tet	This study
CFS18	AH2216 - pEPSA5-SOD(WT)*saeS*-T7	Cam	This study
CFS41	AH2216 - pEPSA5-SOD(C50S/C56S)*saeS*-T7	Cam	This study
CFS42	AH2216 - pEPSA5-SOD(C50S/C226S)*saeS*-T7	Cam	This study
CFS43	AH2216 - pEPSA5-SOD(C56S/C226S)*saeS*-T7	Cam	This study
CFS99	AH1263 Δ*saeS*::LL29-*hla*-sGFP	Tet	This study
CFS111	AH2270 - pEPSA5	Tet, Cam	This study
CFS114	CFS99 - pEPSA5	Tet, Cam	This study
CFS121	CFS99 - pEPSA5-(WT)*saeS*-T7	Tet, Cam	This study
CFS174	CFS99 - pEPSA5-(M31A)*saeS*-T7	Tet, Cam	This study
CFS175	CFS99 - pEPSA5-(W32A)*saeS*-T7	Tet, Cam	This study
CFS176	CFS99 - pEPSA5-(F33A)*saeS*-T7	Tet, Cam	This study
CFS122	CFS99 - pEPSA5-(N34A)*saeS*-T7	Tet, Cam	This study
CFS177	CFS99 - pEPSA5-(G35A)*saeS*-T7	Tet, Cam	This study
CFS123	CFS99 - pEPSA5-(H36A)*saeS*-T7	Tet, Cam	This study
CFS178	CFS99 - pEPSA5-(M37A)*saeS*-T7	Tet, Cam	This study
CFS124	CFS99 - pEPSA5-(T38A)*saeS*-T7	Tet, Cam	This study
CFS179	CFS99 - pEPSA5-(L39A)*saeS*-T7	Tet, Cam	This study
Plasmids			
pJB38	Modified pKOR1 plasmid	Amp, Cam	(3)
pJB38-Δ*saePQRS*	Overlap of 600-bp flanking regions of *saePQRS*	Amp, Cam	This study
pJB38-Δ*saeS*	Overlap of 600-bp flanking regions of *saeS*	Amp, Cam	This study
pCM27	P*hla*-sGFP reporter in pCM11	Amp, Erm	This study
pLL29	φ11 attB integration vector	Spec, Tet	(4)
pLL29-hla-sGFP	P*hla*-sGFP reporter in φ11 integration vector	Spec, Tet	This study
pEPSA5	Xylose inducible shuttle vector	Amp, Cam	(5)
pEPSA5-SOD(WT)*saeS-T7*	WT SaeS, SOD RBS for protein expression	Amp, Cam	This study
pEPSA5-SOD(C50S/C56S)*saeS-T7*	C50S/C56S SaeS, SOD RBS for protein expression	Amp, Cam	This study
pEPSA5-SOD(C50S/C226S)*saeS- T7*	C50S/C226S SaeS, SOD RBS for protein expression	Amp, Cam	This study
pEPSA5-SOD(56S/C226S)*saeS-T7*	56S/C226S SaeS, SOD RBS for protein expression	Amp, Cam	This study
pEPSA5-(WT)*saeS-T7*	WT SaeS complementation	Amp, Cam	This study
pEPSA5-(M31A)*saeS-T7*	M31A SaeS complementation	Amp, Cam	This study
pEPSA5-(W32A)*saeS-T7*	W32A SaeS complementation	Amp, Cam	This study
pEPSA5-(F33A)*saeS-T7*	F33A SaeS complementation	Amp, Cam	This study
pEPSA5-(N34A)*saeS-T7*	N34A SaeS complementation	Amp, Cam	This study
pEPSA5-(G35A)*saeS-T7*	G35A SaeS complementation	Amp, Cam	This study
pEPSA5-(H36A)*saeS-T7*	H36A SaeS complementation	Amp, Cam	This study
pEPSA5-(M37A)*saeS-T7*	M37A SaeS complementation	Amp, Cam	This study
pEPSA5-(T38A)*saeS-T7*	T38A SaeS complementation	Amp, Cam	This study
pEPSA5-(L39A)*saeS-T7*	L39A SaeS complementation	Amp, Cam	This study

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Table S2. SaeS EC loop residues differentially regulate the *sae* regulon in response to human α-defensin 1 (values of data depicted in Fig. 5)

Gene	WT v α-def	saeS v α-def	saeS WT α-def	M31A α-def	W32A α-def	F33A α-def	H36A α-def
hlgA	75.56 ± 45.67	0.95 ± 0.20	74.05 ± 42.39	0.75 ± 0.20	34.54 ± 10.21	3.26 ± 1.73	32.65 ± 17.30
hlgB	19.17 ± 12.05	0.76 ± 0.38	20.64 ± 12.31	1.04 ± 0.11	295.24 ± 173.42	14.58 ± 10.04	11.53 ± 4.71
hlgC	17.16 ± 10.29	0.98 ± 0.15	11.13 ± 4.06	0.75 ± 0.17	16.91 ± 1.96	6.14 ± 3.24	9.25 ± 3.36
lukA	23.37 ± 10.50	1.08 ± 0.33	15.72 ± 7.70	1.71 ± 0.23	194.98 ± 17.56	65.67 ± 23.87	8.23 ± 2.18
sbi	5.98 ± 2.64	1.03 ± 0.22	5.13 ± 2.82	0.99 ± 0.20	89.13 ± 19.81	42.34 ± 30.24	3.27 ± 0.64
saeP	2.81 ± 1.09	1.06 ± 0.25	3.80 ± 1.16	1.15 ± 0.03	89.03 ± 21.70	44.83 ± 27.73	2.83 ± 0.18
saeR	5.66 ± 1.49	1.14 ± 0.16	4.61 ± 0.15	1.00 ± 0.21	43.90 ± 14.95	26.58 ± 13.42	3.87 ± 0.14
saeS	3.29 ± 0.83	—	0.94 ± 0.15	0.70 ± 0.18	0.77 ± 0.30	0.83 ± 0.04	0.78 ± 0.06
hla	1.42 ± 0.72	1.09 ± 0.30	0.97 ± 0.16	0.80 ± 0.35	8.96 ± 2.16	13.02 ± 4.36	0.94 ± 0.06
lukD	2.78 ± 0.89	0.78 ± 0.32	2.31 ± 1.09	0.87 ± 0.38	1.84 ± 0.17	1.46 ± 0.10	1.33 ± 0.09
lukF-PVl	6.53 ± 3.31	1.16 ± 0.34	4.58 ± 1.56	0.71 ± 0.26	8.86 ± 0.04	5.02 ± 1.90	3.27 ± 0.49
splA	2.99 ± 1.81	—	4.23 ± 2.74	1.12 ± 0.67	4.13 ± 3.05	2.73 ± 1.48	1.94 ± 0.02
ssl7	8.78 ± 4.30	1.30 ± 0.18	7.42 ± 4.01	0.71 ± 0.26	1.67 ± 0.16	0.99 ± 0.21	9.77 ± 4.42
agrA	1.25 ± 0.32	1.05 ± 0.15	1.01 ± 0.15	0.80 ± 0.15	0.88 ± 0.10	1.01 ± 0.10	1.12 ± 0.12
arlR	1.15 ± 0.41	0.92 ± 0.14	1.04 ± 0.40	0.59 ± 0.20	0.76 ± 0.00	0.89 ± 0.15	0.95 ± 0.01
rot	1.45 ± 0.66	0.84 ± 0.06	1.05 ± 0.49	0.58 ± 0.22	0.74 ± 0.04	0.81 ± 0.25	1.21 ± 0.05
sarA	1.50 ± 0.65	1.02 ± 0.15	0.95 ± 0.25	0.56 ± 0.27	0.68 ± 0.12	0.80 ± 0.20	0.96 ± 0.06
dltA	0.95 ± 0.24	0.98 ± 0.15	0.84 ± 0.19	0.71 ± 0.17	0.81 ± 0.05	1.01 ± 0.20	1.08 ± 0.20
mecA	1.24 ± 0.43	1.04 ± 0.21	0.98 ± 0.31	0.68 ± 0.20	0.76 ± 0.04	0.85 ± 1.90	1.08 ± 0.49

Relative gene expression in SaeS EC mutants was analyzed following a 30-min exposure to subinhibitory concentrations of human α-defensin 1 (0.48 μM) using the QuantiGene 2.0 assay. Transcript levels were normalized to *gyrB*, and each strain was calibrated to its matched sample grown in the absence of stimulus (media only). The ratio of gene expression in α-defensin treated vs. untreated *S. aureus* EC mutants is shown as an average of two separate experiments ± SEM. EC, extracellular; —, gene expression below limit of detection.

Table S3. SaeS EC loop methionine residue is essential for transcription of the *sae* regulon in response to human PMN phagocytosis (values of data depicted in Fig. 5)

Gene	WT v PMNs	saeS v PMNs	saeS WT PMNs	M31A PMNs	W32A PMNs	F33A PMNs	H36A PMNs
hlgA	224.85 ± 41.94	2.97 ± 0.46	210.59 ± 10.40	3.22 ± 0.68	429.63 ± 144.63	363.28 ± 115.03	134.72 ± 2.88
hlgB	195.88 ± 35.64	4.65 ± 1.77	210.24 ± 94.41	2.59 ± 1.54	667.97 ± 158.81	936.98 ± 305.86	68.34 ± 17.09
hlgC	73.26 ± 10.30	1.64 ± 0.38	81.14 ± 27.90	1.56 ± 0.67	118.08 ± 54.44	104.47 ± 42.61	27.04 ± 5.24
lukA	8.60 ± 0.46	0.52 ± 0.04	12.82 ± 3.64	0.65 ± 0.15	123.97 ± 22.22	106.00 ± 22.92	3.89 ± 0.76
sbi	3.32 ± 0.09	0.31 ± 0.03	5.73 ± 0.36	0.53 ± 0.08	144.90 ± 6.48	116.76 ± 5.02	2.66 ± 0.05
saeP	5.77 ± 0.20	0.91 ± 0.19	11.27 ± 3.40	1.08 ± 0.31	238.36 ± 58.19	197.54 ± 42.38	4.49 ± 1.09
saeR	3.17 ± 0.12	0.42 ± 0.04	3.82 ± 0.48	0.41 ± 0.08	9.13 ± 2.66	7.83 ± 1.51	1.93 ± 0.20
saeS	2.25 ± 0.08	0.62 ± 0.16	0.45 ± 0.03	1.27 ± 0.13	0.82 ± 0.26	0.57 ± 0.11	0.54 ± 0.03
hla	1.15 ± 0.15	0.65 ± 0.07	2.21 ± 0.60	0.74 ± 0.23	34.73 ± 10.13	46.07 ± 11.36	1.18 ± 0.28
lukD	1.03 ± 0.25	0.54 ± 0.01	1.42 ± 0.31	0.38 ± 0.05	1.26 ± 0.02	1.06 ± 0.06	0.80 ± 0.07
lukF-PVl	3.19 ± 0.06	0.97 ± 0.04	3.20 ± 0.27	0.84 ± 0.08	3.16 ± 0.34	3.22 ± 0.56	2.65 ± 0.75
splA	3.83 ± 0.98	0.48 ± 0.08	6.51 ± 3.00	—	14.07 ± 3.24	11.57 ± 3.33	2.42 ± 0.03
ssl7	3.67 ± 0.79	0.67 ± 0.14	2.78 ± 1.04	0.56 ± 0.12	0.92 ± 0.24	0.94 ± 0.26	2.90 ± 1.24
agrA	0.29 ± 0.07	0.39 ± 0.12	0.34 ± 0.06	0.32 ± 0.13	0.33 ± 0.11	0.34 ± 0.14	0.30 ± 0.09
arlR	0.60 ± 0.12	0.55 ± 0.12	0.67 ± 0.13	0.50 ± 0.15	0.77 ± 0.25	0.74 ± 0.19	0.52 ± 0.16
rot	0.60 ± 0.05	0.74 ± 0.02	0.72 ± 0.04	0.68 ± 0.00	0.79 ± 0.05	0.63 ± 0.04	0.64 ± 0.03
sarA	1.50 ± 0.23	2.04 ± 0.30	1.52 ± 0.30	2.00 ± 0.41	1.95 ± 0.59	1.52 ± 0.31	1.59 ± 0.38
dltA	0.44 ± 0.02	3 ± 0.0628	0.53 ± 0.03	0.36 ± 0.04	0.43 ± 0.03	0.40 ± 0.01	0.37 ± 0.04
mecA	0.52 ± 0.01	0.76 ± 0.08	0.53 ± 0.00	0.92 ± 0.17	0.75 ± 0.16	0.57 ± 0.07	0.58 ± 0.05

Relative gene expression in SaeS EC mutants was analyzed following a 30-min exposure to human PMNs (10:1 MOI) using the QuantiGene 2.0 assay. Transcript levels were normalized to *gyrB*, and each strain was calibrated to its matched sample grown in the absence of stimulus (media only). Data are shown as an average of two separate experiments ± SEM. PMN, polymorphonuclear leukocytes; —, gene expression below limit of detection.

Table S4. Oligonucleotides used in this study

Primer	Sequence	Description
CEF10	5′-GTTAGCAGATCTAGTACTAAACTTTATGGGTATCCTTCTC-3′	Downstream of *saeS* plus overlap
CEF11	5′-GTTGTTGGTACCGTTAGTTAATTGCTTTGTAGAAGAAG-3′	600-bp downstream of *saeS*, KpnI
CEF20	5′-GCATATATTTTAATGTGGTTTAACGGCGCTATGACACTAACTTTGACCTTAACG-3′	Mutating SaeS H36A, fwd
CEF21	5′-CGTTAAGGTCAAAGTTAGTGTCATAGCGCCGTTAAACCACATTAAAATATATGC-3′	Mutating SaeS H36A, rev
CEF22	5′-GCAATTGCATATATTTTAATGTGGTTTGCCGGCCATATGACACTAACTTTGACC-3′	Mutating SaeS N34A, fwd
CEF23	5′-GGTCAAAGTTAGTGTCATATGGCCGGCAAACCACATTAAAATATATGCAATTGC-3′	Mutating SaeS N34A, rev
CEF24	5′-TATTTTAATGTGGTTTAACGGCCATATGGCACTAACTTTGACCTTAACGACAATAATTAC-3′	Mutating SaeS T38A, fwd
CEF25	5′-GTAATTATTGTCGTTAAGGTCAAAGTTAGTGCCATATGGCCGTTAAACCACATTAAAATA-3′	Mutating SaeS T38A, rev
CEF39	5′-CGACAATAATTACAAGCTCTTTAACCTTATTAATAT-3′	Mutating SaeS C50S, fwd
CEF40	5′-ATATTAATAAGGTTAAAGAGCTTGTAATTATTGTCG-3′	Mutating SaeS C50S, rev
CEF41	5′-GCTGTTTAACCTTATTAATATCTAGTATTTTTATTAATCCAC-3′	Mutating SaeS C56S, fwd
CEF42	5′-GTGGATTAATAAAAATACTAGATATTAATAAGGTTAAACAGC- 3′	Mutating SaeS C56S, rev
CEF66	5′-GTTGTTGAATTCTTAGGAGGATGATTATTTATGGTGTTATCAATTAGAAGTCAAATCATT-3′	EcoRI, *saeS* forward with SOD RBS
CEF67	5′-GTTGTTCTCGAGTTAACCCATTTGCTGTCCACCAGTCATGCTAGCCATAGAGCCACC TGACGTAATGTCTAATTTGTGTAATG-3′	XhoI, downstream of *saeS*, C-term T7 tag with Gly-Gly-Ser linker, rev
CEF68	5′-CACATTAGAAGTGAATTTCTCTAACGAAATTGATGCATTTTA-3′	Mutating SaeS C226S, fwd
CEF69	5′-TAAAATGCATCAATTTCGTTAGAGAAATTCACTTCTAATGTG-3′	Mutating SaeS C226S, rev
CEF130	5′-GCATATATTTTAGCTTGGTTTAACGGC-3′	Mutating SaeS M31A, fwd
CEF131	5′-GCCGTTAAACCAAGCTAAAATATATGC-3′	Mutating SaeS M31A, rev
CEF132	5′-TATATTTTAATGGCTTTTAACGGCCAT-3′	Mutating SaeS W32A, fwd
CEF133	5′-ATGGCCGTTAAAAGCCATTAAAATATA-3′	Mutating SaeS W32A, rev
CEF134	5′-ATTTTAATGTGGGCTAACGGCCATATG-3′	Mutating SaeS F33A, fwd
CEF135	5′-CATATGGCCGTTAGCCCACATTAAAAT-3′	Mutating SaeS F33A, rev
CEF136	5′-ATGTGGTTTAACGCTCATATGACACTA-3′	Mutating SaeS G35A, fwd
CEF137	5′-TAGTGTCATATGAGCGTTAAACCACAT-3′	Mutating SaeS G35A, rev
CEF138	5′-TTTAACGGCCATGCTACACTAACTTTG-3′	Mutating SaeS M37A, fwd
CEF139	5′-CAAAGTTAGTGTAGCATGGCCGTTAAA-3′	Mutating SaeS M37A, rev
CEF140	5′-GGCCATATGACAGCTACTTTGACCTTA-3′	Mutating SaeS L39A, fwd
CEF141	5′-TAAGGTCAAAGTAGCTGTCATATGGCC-3′	Mutating SaeS L39A, rev
CEF180	5′-GTTGTTGAATTCGTAACAACGACAACTAGCGG-3′	EcoRI, 600 bp upstream of *saeS*
CEF181	5′-TAAATCGGAGTACTAGATCTATTATCGGCTCCTTTCAAATTTATATC-3′	Upstream of *saeS*, plus overlap
CEF182	5′-CGATAATAGATCTAGTACTCCGATTTATTTATAAAATAAAATGCAAAGAC-3′	Downstream of *saeS*, plus overlap
CLM429	5′-GTTGTTTAAGCTTAAGATCACCGGT-3′	1.6 kb upstream of *hla*, HindIII, fwd
CLM430	5′-GTTGTTGGTACCTTTCGTTCTAATAAC-3′	257 bp into *hla* ORF, KpnI, rev
MJT240	5′-GTTGTTGAATTCACCTGTATACATTACAGACC-3′	600 bp upstream of *saeP*, fwd
MJT241	5′-AAGTTTAGTACTAGATCTGCTAACACCACAAGCACCTAAAGCTAATGTTG-3′	Upstream of *saeP*, plus overlap, rev
EPSA5for2	5′-AGTTATAAAATAGATATCTCGGACCGTCAT-3′	100 bp upstream of MCS
EPSA5rev	5′-GGCAAATTCTGTTTTATCAGACCG-3′	100 bp downstream of MCS