Supporting Information

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SI Materials and Methods

Bacterial Strains and Materials. Bacterial strains, plasmids and primers in this study are listed in Table S3. [1-^{14}C]Oleic acid (56.3 mCi/mmol), [^{3}H]oleic acid (54.6 Ci/mmmol), [^{3}H]myristic acid (43.5 Ci/mmol, [γ-^{32}P] ATP (3,000 Ci/mmol) were purchased from Perkin-Elmer. [1-^{14}C]myristic acid (55 mCi/mmoll) was purchased from American Radiolabeled Chemicals. Fatty acids (FA) were purchased from Sigma-Aldrich and Larodan Fine Chemicals. Lipids were obtained from Avanti-Polar Lipids, Ni-NTA agarose from Qiagen and streptavidin magnetic beads from Thermo Scientific.

Strain Construction. All strains used in this study were derivatives of strain AH1263, a USA300 isolate (1). To minimize polar effects on neighboring genes, we constructed an in-frame deletion mutant of SAUSA300_1318 (*fakB2*). Approximately 700 bp upstream of *fakB2* was amplified by PCR from the AH1263 chromosome using primers JB68 and JB69, which added EcoRI and NheI recognition sites to the 5′ and 3′ ends, respectively. Similarly, ∼750 bp downstream of *fakB2* was amplified using primers JB70 and JB71 with added NheI and SalI sites to the 5′ and 3′ ends, respectively. Each PCR product was digested with the appropriate restriction endonucleases and ligated in a single step into pJB38 digested with EcoRI and SalI, generating pJLB14. Doing so generated an in-frame deletion plasmid, such that the start and stop codons of *fakB2* were on either side of the NheI recognition site. pJLB14 was transferred to AH1263 and allelic exchange performed as previously described (1) to generate the Δ*fakB2* strain JLB30. To make the Δ*fakB1* single and Δ*fakB1* Δ*fakB2* double-mutants, the SAUSA300_1318::ΦNΣ from NE1540 (2) was transferred via phage transduction to AH1263 and JLB30, resulting in JLB27 (*fakB1*::ΦNΣ) and JLB31 (*fakB1*:ΦNΣ Δ*fakB2*), respectively. All mutations were confirmed using PCR.

The *fakA* gene was amplified using primers JB41 and JB42 before digestion and ligation into the NdeI and XhoI sites of pET28a. The SAUSA300_0733 (*fakB1*) and *SAUSA300_1318* (*fakB2*) genes were synthesized by Invitrogen Gene Art Strings with NdeI and BamHI restriction sites and ligated into pET15b to produce pCS106 (pET15b-*fakB1*) and pCS107 (pET15b-*fakB2*). The *fakB1* gene from USA300 was amplified using primers fakB1-F and fakB1-R primers. The forward primer anneals 143 bp upstream of *fakB1* gene in the USA300_FPR3757 genome and the reverse primer anneals at the end of the gene. The amplified product was cloned into the BamHI and PstI sites of pCM28. The plasmid sequence of pCS105 was confirmed and it was transformed into USA300 JLB31 (Δ*fakB1* Δ*fakB2* double-knockout) by phage transduction. Because *fakB2* appears to be cotranscribed with *folA* (SAUSA300_1319), we chose to place *fakB2* under control of the constitutive *sarA* promoter in pCM29 for complementation studies. *fakB2* and its native ribosome binding site were amplified by PCR from the AH1263 chromosome using primers JB75 and JB76. The PCR product was digested with EcoRI and ligated into pCM29 digested sequentially with SmaI and EcoRI, generating pJLB16.

Protein Purification. Plasmids containing *fakA*, *fakB1*, and *fakB2* genes were transformed into *Escherichia coli* strain BL21 (DE3). Next, 1 L of LB was inoculated to A_{600} = 0.05 and grown with vigorous shaking until A_{600} = 0.8–1.0, when protein expression was induced with the addition of 1 mM Isopropyl β-D-1-thiogalactopyranoside. Cultures were grown with shaking for an additional 3 h before the cells were harvested by centrifugation at 4,000 × *g* for 15 min. The pellets were resuspended in 20 mL binding buffer (20 mM Tris·HCl, pH 8.0, 0.5 M NaCl) and lysed using a microfluidizer. Debris was removed by centrifugation at 36,000 × *g* and the soluble fraction applied to a 5 mL Ni-NTA column equilibrated with binding buffer. The column was washed with 3 × 50 mL binding buffer with increasing concentrations of imidazole (0 mM, 25 mM, 50 mM) and the proteins were eluted using binding buffer containing 400 mM imidazole. After analysis by SDS/PAGE, the proteins were purified by size-exclusion on a Superdex 200 16/600 column (GE Healthcare) using 20 mM Tris·HCl, pH 7.5, 0.2 M NaCl as the buffer.

Metabolic Labeling. *Staphylococcus aureus* strains were cultured in LB media containing 10 mg/mL FA- free BSA until A_{600} = 0.5. Next, 25 mL of culture was then transferred to a flask containing either 20 μM [^{14}C]oleate and 180 μM oleate or 20 μM [^{14}C]myristic acid and 180 μM myristic acid from which the ethanol was allowed to evaporate for 1 h before addition of the culture. The cultures were then incubated at 37 °C while shaking. Next, 5 mL of culture was removed every 10 min for 30 min. The cells were harvested by centrifugation, washed once with 11 mL of LB with 10 mg/mL BSA and once with water. The cell pellet was resuspended in 100 μL water and the lipids were extracted with 360 μL chloroform/methanol/HCl [1/2/0.02 (vol/vol/vol)]. Phases were separated after addition of 120 μL chloroform and 120 μL 2 M KCl. Radiolabeled lipids were quantified by a combination of scintillation counting and TLC analysis.

FA Kinase Assay. The reaction mix for FA kinase (Fak) assay contained 0.1 M Tris·HCl (pH 7.5), 20 mM $MgCl_2$, 10 mM ATP, 1% Triton X-100, 20 μM [1-^{14}C]oleic acid or myristic acid, 0.2 μM FakA, and 0.2 μM FakB1 or 0.2 μM FakB2 in a 60-μL volume. The reaction was initiated with the addition of the Fak enzymes and incubated at 37 °C for 20 min. Next 40 μL of the reaction was spotted on DE81 disks and washed three times with ethanol containing 1% acetic acid to remove the unreacted FA. The disks were dried and counted using a Beckman scintillation counter. Alternatively, the reaction was analyzed by TLC using Silica Gel G layers plate in chloroform/methanol/acetic acid (90/10/10) (vol/vol/vol) solvent system. The plate was dried and analyzed using a Bioscan (AR-2000).

Liposome Preparation for Fak Assays. Fak assays were performed either in the presence of 1% Triton X-100 or [^{14}C]oleate containing liposomes. The liposomes were prepared as described previously (3) [^{14}C]oleic acid was incorporated into 100-nm unilamellar dioleoylphosphotidylglycerol vesciles composed of dioleoylphosphatidyl glycerol/oleic acid (90/10 mol%). The vesicles were prepared by drying phosphatidylglycerol and oleic acid and rehydrating them in 0.1 M Tris·HCl (pH 7.5) by repeated vortexing and incubating at room temperature for 30 min, followed by sonication at 200 W for 2 min and passage through a micro extruder equipped with a 100-nm polycarbonate filter 20–30 times. Liposomes equivalent to 20 μM oleic acid were added to a Fak assay.

Mass Spectrometry of Acyl-PO_4. A 2-mL Fak reaction containing 14.8 μM FakA, 1.5 μM FakB1, and 37.5 μM 18:1 (Δ9) FA was incubated at 37 °C for 2 h. The acyl-phosphate product was extracted using 7.2 mL of chloroform/methanol/HCl (1:2:0.02) and 2.4 mL of chloroform and 2.4 mL of 2 M KCl. The organic phase was removed and dried. The sample was dissolved in 100 μL of

chloroform, and injected onto Waters C-18 column using an auto sampler on a Waters Acquity UPLC system paired with single quadrupole mass spectrometer. The acyl-PO_4 was separated using a gradient of water + 0.1% formic acid:acetonitrile + 0.1% formic acid [95%: 5–5%: 95% (vol/vol)] over a period of 4.5 min and analyzed in a ES− mode. Ion source parameters were set as dynamic as follows: capillary voltage 2.73 kV, cone voltage 11.48 V, extractor voltage 0.24 V, source temperature 146 °C, desolvation temperature 350 °C, cone gas flow 50 L/h, desolvation gas flow 800 L/h, ion energy 0.57, gain 1.0, multiplier −698.43, and scan range 110–1,000 Da. The data were analyzed using MassLynx 4.1 software.

Analytical Ultracentrifugation. Experiments were carried out in a ProteomeLab XL-I analytical ultracentrifuge with an eight-hole rotor (Beckman An-50Ti) and cells containing sapphire or quartz windows and charcoal-filled Epon double-sector center pieces (Beckman Coulter). Analytical ultracentrifugation were performed in 20 mM Tris, pH 7.5, 0.2 M NaCl. The density and viscosity of the ultracentrifugation buffer at 20 °C were measured with a DMA 5000M density meter and an AMVn viscometer (Anton Paar). The partial specific volume at 20 °C and the molecular weight of the protein were calculated based on its amino acid composition (4) using the software SEDFIT (https://sedfitsedphat.nibib.nih.gov/software/default.aspx). Buffer from the size-exclusion column used during protein purification was used as the ultracentrifugation buffer and optical reference. For the sedimentation velocity experiment, the loading volume of 400 μL was identical for the reference and sample chambers of the double-sector centerpiece. Fringe displacement data at time intervals of 1.0 min were collected with the Rayleigh interference system for 12 h at a rotor speed of 50,000 rpm and analyzed with SEDFIT software (https://sedfitsedphat.nibib.nih.gov/software/default.aspx) using the model for continuous sedimentation coefficient distribution $c(s)$ with deconvolution of diffusional effects (5, 6). The sedimentation coefficient distribution $c(s)$ was calculated with maximum entropy regularization at a confidence level of $P = 0.68$ and at a resolution of sedimentation coefficients of $n = 100$. The positions of the meniscus and bottom, as well as time-invariant and radial noises, were fitted. Sedimentation equilibrium was attained at a rotor temperature of 4 °C at increasing speeds of 10,000 (for 45 h), 15,000 (for 30 h), and 25,000 rpm (for 18 h). Protein at concentrations of between 25 and 35 μM (130 μL) were loaded into double-sector centerpieces and absorbance distributions recorded at 280 in 0.001-cm radial intervals with 20 replicates for each point. Global least-squares modeling were performed at multiple rotor speeds with the software SEDPHAT (https://sedfitsedphat.nibib.nih.gov/software/default.aspx) using the following models: single species, two and three discrete species, reversible monomer-dimer self-association, and the reversible two-site heterogeneous association models (6).

Acyl-[^{32}P]phosphate Purification. To generate ^{32}P-labeled acyl-PO_4, a 100-μL Fak reaction assay containing 14.8 μM FakA, 1.5 μM FakB2, 20 μM oleic acid, and 2 μL of [γ- ^{32}P]ATP (3,000 Ci/mmol) was incubated at 37 °C for 2 h. The acyl phosphate product was extracted using 360 μL of chloroform:methanol:HCl (1:2:0.02) and 120 μL of chloroform and 120 μL of 2 M KCl. The organic phase was purified using an aminopropyl column (DSC-NH_2). The column was prewashed with hexane and the FA was eluted with ether containing 2% acetic acid, the acyl-PO_4 was eluted with a mixture containing hexane/propanol/ethanol/0.1 M ammonium acetate/formic acid/phosphoric acid (21/17.5/5/2.5/0.025/2.7) (vol/vol/vol/vol/vol/vol). The acyl-PO_4 fraction was dried under N_2 and resuspended in chloroform.

FA and Acyl-PO_4 Binding to FakB. FA binding to FakB2 was evaluated by combining 26 μM FakB2. and 0.92 μM [^{3}H]oleic acid in Fak buffer (20 mM Tris·HCl, pH 7.5, 0.2 M NaCl) for 20 min at room temperature. The mixture was subsequently separated on a Superdex S200 10/300 GL (GE Healthcare) equilibrated in Fak buffer. The inclusion volume was separated into 0.2-mL fractions and analyzed by scintillation counting. [^{32}P]oleoyl-phosphate generated using a 200 μL Fak reaction assay containing 14.8 μM FakA, 1.5 μM FakB2, 20 μM oleic acid and 2 μL of [γ-^{32}P] ATP. The acyl phosphate product was extracted using 360 μL of chloroform/methanol/HCl (1:2:0.02) and 120 μL of chloroform and 120 μL of 2M KCl. The organic phase was removed and dried under nitrogen. The dried acyl-PO_4 was dissolved in 20 μL ethanol (10,000 cpm/μL) and 10 μL added to 483 μM FakB2 in a volume of 190 μL. The reaction was allowed to incubate for 20 min before analyzed by gel filtration, as described above.

FakB Ligand Binding Assays. Substrate specificity of FakB1 and FakB2 was examined by incubating FakB1 and FakB2 with different radiolabeled FA. The reaction (200 μL) consisted of 42 μM of either FakB1 or FakB2 and 9 μM [^{14}C]fatty acid in PBS, pH 7.4. Reactions were incubated at room temperature for 20 min before the addition of 200 μL PBS and removal of unbound fatty acid using a 2 mL zeba spin desalting column (Thermo Scientific). The elution fractions were analyzed by scintillation counting.

The affinity of FakB1 and FakB2 for different FA was tested using a competition assay (7, 8) with some modifications. [^{3}H]Myristic acid (FakB1) or [^{3}H]oleic acid (FakB2) binding was assessed by mixing 8 μM FakB with 1 μM [^{3}H]fatty acid and 10 μM competing FA in 200 μL PBS. The concentration of ethanol and DMSO were kept at 1% and 0.5% respectively. Binding was initiated by the addition of FakB and duplicate reactions were incubated at room temperature for 30 min. Subsequently, 200 μL of PBS was added and unbound fatty acid removed using a 2-mL Zeba spin desalting column. The elution fractions were analyzed by scintillation counting.

FA Composition of Recombinant FakBs. The FA bound to FakB1 and FakB2 proteins purified from *E. coli* were identified using GC/MS. A lipid extraction was performed on 1 mL of purified FakB1 (14 mg/mL) and FakB2 (28 mg/mL) by the addition of 3.6 mL (chloroform/methanol/HCl 1/2/0.02) (vol/vol/vol). The samples were vortexed before the addition of 1.2 mL chloroform and 1.2 mL 2 M KCl to achieve phase separation. The organic phase was removed, dried under nitrogen and dissolved in 1 mL anhydrous methanol with two drops of acetyl-chloride. Methyl esters were prepared and analyzed using a Hewlett Packard 5890 Gas Chromatograph (9).

Liposome Transfer Assays. Liposomes (PtdGro/[^{14}C]oleic acid/biotin-phosphatidylethanolamine, 80/10/10) were produced and used to test the ability of FakB to pick up oleic acid incorporated into liposomes. Reaction mixtures contained 10 μL of liposomes (CONC), varying amounts of FakB2 and PBS up to 200 μL. Reactions were incubated at room temperature for 5 min before the addition of 50 μL streptavidin magnetic beads (Pierce) and 200 μL PBS. Samples were incubated at room temperature on a rotating drum for 15 min before the liposomes were removed with the streptavidin magnetic beads by centrifugation. The supernatant containing the FakB2 with bound [^{14}C]oleic acid was analyzed by scintillation counting. The transfer of FakB2-associated FA to liposomes was tested using PtdGro liposomes and PtdGro/oleic acid liposomes (90/10). Binding of [^{14}C]oleic acid to FakB2 was accomplished by incubating 200 μg of FakB2 with 1.4 μCi [^{14}C]oleic acid in 700 μL of PBS. After incubating at room temperature for 30 min, unbound FA was removed using a 2-mL Zeba Spin desalting column equilibrated in PBS. Then,

100 μL of the desalted FakB2 was incubated with 100 μL of either PtdGro liposomes or PtdGro/oleic acid liposomes and diluted to 1 mL with PBS. After incubation for 15 min at room temperature, 100 μL Ni-NTA agarose beads were added to each reaction and incubated on a rotating drum for 15 min. Ni-NTA bound FakB2 was removed from solution by centrifugation and the liposome associated [^{14}C]oleic acid detected by liquid scintillation counting.

Affymetrix Array Analysis. The effect of a *fakA* deletion on global gene expression was analyzed using the *S. aureus* Affymetrix array technology as described previously (10). Briefly, triplicate cultures were grown to A_{600} = 0.45 in LB and harvested by centrifugation. RNA was immediately extracted using Ambion RNAqueous kit (Applied Biosystems) according to manufacturer's instructions but with the inclusion of a lysostaphin treatment to lyse cells. RNA quality was checked and Affymetrix protocol used to synthesize, fragment and terminally label cDNA. Data were collected and analyzed with the manufacturer's software and Spotfire DecisionSite 9.1.1. Changes reported if they were greater than twofold with P value of <0.05 using Student t test to compare values.

Measurement of mRNA Levels. Quantative RT-PCR (qRT-PCR) was used to determine the levels of transcript mRNA. Bacterial strains were grown in LB to A_{600} = 3.0, where the cells were harvested by centrifugation and washed with 1 mL RNAlater solution (Ambion). Total RNA was then isolated and analyzed as described previously (10). Briefly, RNA was isolated immediately after cell harvesting with Ambion RNAqueous kit (Applied Biosystems) per the manufacturer's instructions but with the inclusion of a lysostaphin treatment to lyse the cells. Turbo DNA-free kit (Ambion) was used to remove genomic DNA and cDNA synthesized with SuperScript II reverse transcriptase (Invitrogen). qRT-PCR was performed in triplicate using the ABI Prism 7700 Sequence Detection System using distilled water and RNA samples prepared without the cDNA synthesis step as negative controls. The *gmk* gene was selected as the calibrator to compare mRNA levels between strains. The primers used were described previously (10). The values compared using the threshold cycle (C_T) method (11) and the amount of cDNA present ($2^{-\Delta CT}$) was reported to the *gmk* calibrator.

Hemolysis Assays and Immunoblotting. The hemolysis assay was conducted on LB agar plates supplemented with 3% defibrinated sheep blood. *S. aureus* strains were grown to A_{600} = 6.0 and 1 μL spotted onto agar plates. Plates were incubated at 37 °C for 24 h before image recorded. For immunoblotting, strains AH1263, Δ*fakA*, Δ*fakB1*, Δ*fakB2*, and Δ*fakB1* Δ*fakB2* were grown to A_{600} = 5.0 in LB medium. The cells were harvested by centrifugation, the culture medium removed and sterilized by filtration (0.2 μm). The medium was desalted using a 10-mL Zeba Spin desalting column and 40 μL of each sample separated by SDS/PAGE. The proteins were transferred to a polyvinylidene fluoride membrane and probed with rabbit alpha-toxin antisera (Sigma-Aldrich) at a 1:500 dilution. After 1 h, membrane was washed three times with Tris-buffered saline with 0.1% triton. The secondary antibody used was alkaline phosphatase conjugated goat anti-rabbit (GE Healthcare) used at 1:5,000 dilution, incubated for 1 h and visualized by electrochemical fluorescence using a Typhoon FLA 9500.

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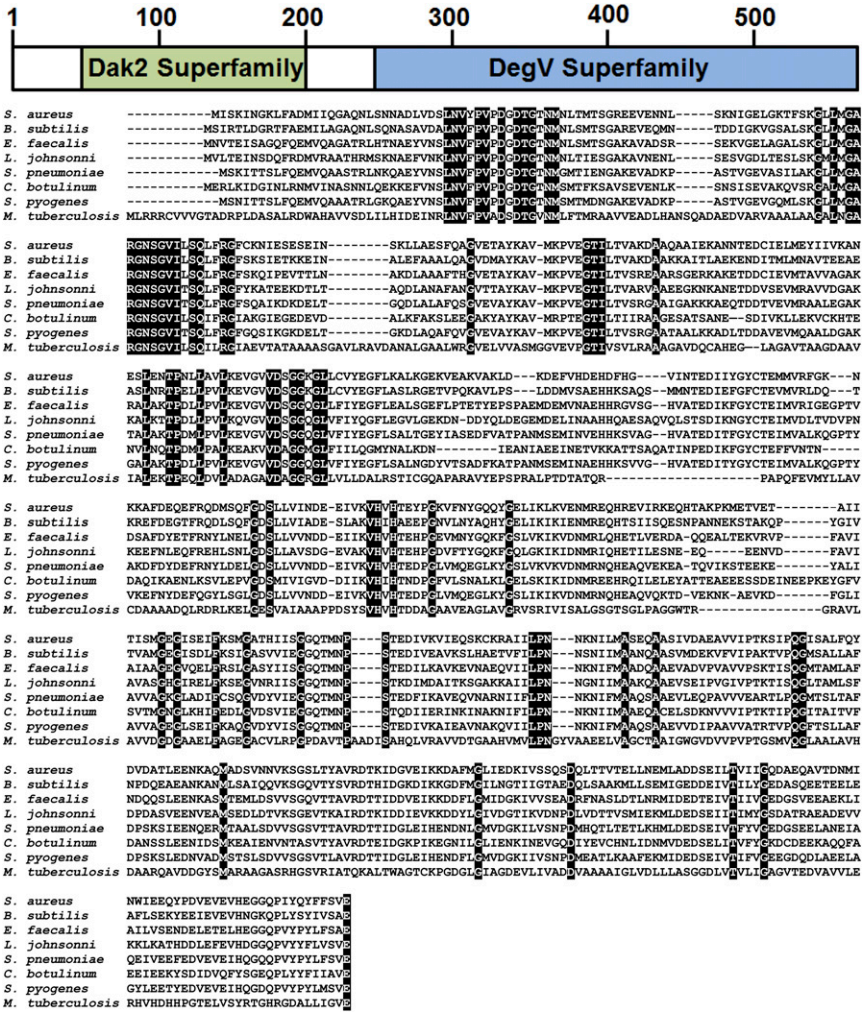

Fig. S1. Overall domain structure of FakA. The amino-terminal Dak2 domain of FakA extends from the LNVPVPDGDTGTNM motif through the VDSGGKGL motif, and the carboxyl-terminal domain is predicted to have a DegV-like fold. Alignment of FakA homologs from different bacteria. The percent identity to *S. aureus* (100%) is indicated in parentheses. *Bacillus subtilis* (56%), *Enterococcus faecalis* (53%), *Lactobacillus johnsonni* (48%), *Streptococcus pneumoniae* (48%), *Clostridium botulinum* (43%), *Streptococcus pyogenes* (48%), and *Mycobacterium tuberculosis* (25%). Residues that are identical in all FakA proteins are indicated in black.

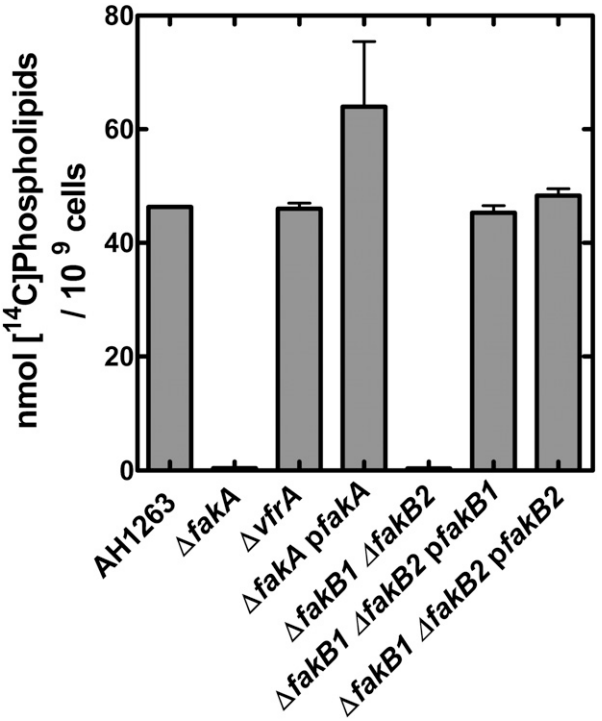

Fig. S2. FA incorporation into mutant *S. aureus* and its complemented derivatives. Cells were labeled with [^{14}C]oleate for 20 min; lipids were extracted and incorporation into phospholipids analyzed by TLC as described in *Materials and Methods*.

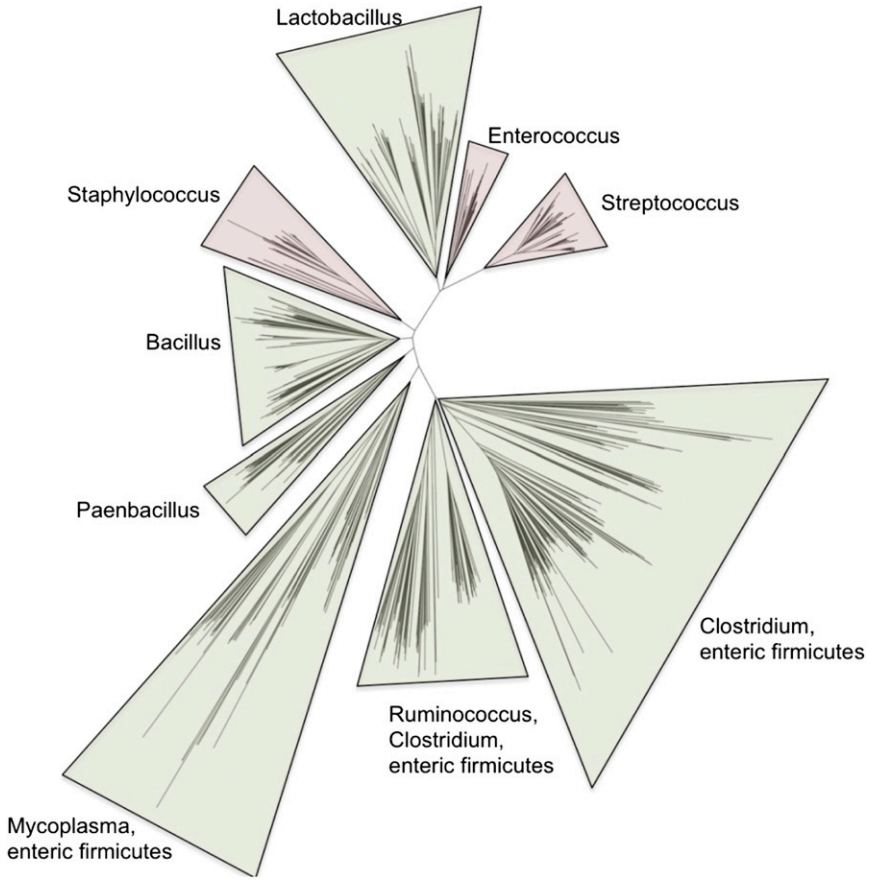

Fig. S3. Phylogenetic distribution of *fakA* gene in the Firmicute family.

Table S1. Equilibrium ultracentrifugation of *S. aureus* FakA, FakB1, and FakB2

Protein	mg/mL*	s_{20} (Svedberg)†	Mw (kDa)‡	$(f/f_0)_w$§
FakA	0.93	5.46 (91%)	131 (60.496)	1.64
FakB1	0.90	2.82 (83%)	33.8 (32.065)	1.22
FakB2	0.92	2.57 (98%)	34.6 (30.645)	1.33

*Concentration of peak in mg/mL.

†Sedimentation coefficient taken from the ordinate maximum of each peak in the best-fit *c*(*s*) distribution in 20 mM Tris pH 7.5, 0.2 M NaCl buffer at 20 °C with percentage protein amount in parenthesis. Sedimentation coefficient (*s*-value) is a measure of the size and shape of a protein in a solution with a specific density and viscosity at a specific temperature. The percent of the total protein in the peak is shown in parenthesis.

‡Molar mass values taken from the *c*(*s*) distribution that was transformed to the *c*(*M*) distribution. The theoretical monomer mass is given in parenthesis.

§Best-fit weight-average frictional ratio values $(f/f_0)_w$ taken from the *c*(*s*) distribution.

Table S2. Gene-expression changes in *S. aureus* strain AH1263 compared with its Δ*fakA* derivative based on triplicate Affymetrix arrays

Gene locus	Name	Δ*fakA*/AH1263*	*P* value	Function
SA2439	*sasF*	3.87	<0.001	Putative surface anchored protein
SA0290	Hypothetical protein	3.08	<0.001	Hypothetical protein
SA0739	Hypothetical protein	3.00	0.0011	Hypothetical protein
SA0291	Hypothetical protein	2.93	<0.001	Hypothetical protein
SA0738	Hypothetical protein	2.86	<0.001	Hypothetical protein
SA1318	Hypothetical protein	2.85	<0.001	Hypothetical protein
SA0279	Hypothetical protein	2.60	<0.001	Hypothetical protein
SA0280	Hypothetical protein	2.59	<0.001	Hypothetical protein
SA0277	*esaC*	2.57	<0.001	ESAT-6 protein cluster
SA0278	*esxB*	2.52	<0.001	ESAT-6 protein cluster
SA1320	Hypothetical protein	2.49	<0.001	Hypothetical protein
SA0520	*sdrA*	2.34	<0.001	Virulenece factor
SA0275	*essB*	2.29	<0.001	ESAT-6 protein cluster
SA0273	*essA*	2.25	<0.001	ESAT-6 protein cluster
SA2133	Hypothetical protein	2.09	<0.001	Hypothetical protein
SA1056	Hypothetical protein	2.09	<0.001	Hypothetical protein
SA0272	*esaA*	2.08	<0.001	ESAT-6 protein cluster
SA2167	*scrA*	2.04	<0.001	PTS system, sucrose-specific IIBC components, putative
SA1142	*glpD*	2.02	<0.001	Glycerol-3-phosphate dehydrogenase
SA0211	Hypothetical protein	2.11	<0.001	Oxidoreductase
SA1004	Hypothetical protein	2.18	0.0014	Fibrinogen binding protein precursor
SA1754	Hypothetical protein	2.18	<0.001	Complement inhibitor
SA0213	Hypothetical protein	2.19	<0.001	Hypothetical protein
SA0212	Hypothetical protein	2.23	<0.001	Putative sugar:phosphate epimerase
SA0309	*geh*	2.25	<0.001	Glycerol ester hydrolase
SA0821	*argH*	2.27	<0.001	Argininosuccinate lyase
SA0822	*argG*	2.60	<0.001	Argininosuccinate synthase
SA2303	*smpC*	2.80	<0.001	Hypothetical protein
SA1007	*hla*	3.08	<0.001	alpha-toxin
SA2206	*sbi*	3.23	<0.001	IgG-binding protein SBI
SA0663	*saeP*	3.38	<0.001	Hypothetical protein
SA1003	*efb*	3.56	<0.001	Fibrinogen binding protein
SA0662	*saeQ*	3.95	<0.001	Hypothetical protein
SA1000	*ehp*	4.82	<0.001	Fibrinogen binding protein
SA1069	*fakA*	67.45	<0.001	Fatty acid kinase

S. aureus genes listed had significantly ($P < 0.05$) different expression levels (± 2.0 fold). The data were abstracted from the complete dataset deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE57340. ESAT-6, early secreted antigenic target 6 kDa protein. Green, up-regulated; red, down-regulated.

*Ratio of expression levels in Δ*fakA* compared with AH1263.

Table S3. Bacterial strains, plasmids, and primers used in this study

Bacterial strains, plasmids, and primers	Description	Source
Bacterial strains		
RN4220	Restriction negative strain	(1)
AH1263	USA300 CA-MRSA strain LAC without p03	(1)
JLB1	AH1263 Δ*vfrA*	(2)
JLB2	AH1263 Δ*fakA* (Δ*vfrB*)	(2)
JLB27	AH1263 Δ*fakB1::ΦNΣ*	Present study
JLB30	AH1263 Δ*fakB2*	Present study
JLB31	AH1263 *fakB1::ΦNΣ* Δ*fakB2*	Present study
Plasmids		
pET28a	Km^R	Novagen
pET15b	Amp^R	Novagen
pCM28	*E. coli–S. aureus* shuttle vector with P_{sarA}	(3)
pCM29	*E. coli–S. aureus* shuttle vector	(4)
pJLB165	pCM28 containing *fakA* and promoter	(2)
pJB38	Allelic exchange plasmid	(5)
pCS106	pET15b-*fakB1*	Present study
pCS107	pET15b-*fakB2*	Present study
pCS105	pCM28 w/*fakB1* and promoter	Present study
pJLB16	pCM29 containing *fakB2* gene	Present study
pJLB11	pET28a containing *fakA* gene	Present study
Primers		
fakB1-F	ATATGGATCCATAATAACTCCTTAATTCATAAGC	Present study
fakB1-R	ATTACTGCAGTTATGTTAATCTTATTTTTCTGCC	Present study
JB41	GGCATATGATTAGCAAAATTAATGGTAAATTATTTGC	Present study
JB42	GGCTCGAGTTATTCTACTGAAAAGAAATATTGATAAATTG	Present study
JB68	CCGAATTCGTCATTGCATAGTTAGCTAACCATATAGAC	Present study
JB69	CCGCTAGCCATGGTCGTTTTCCCCCTTATTTTTTACG	Present study
JB70	CCGCTAGCTAAATTTAATCTTTTCAGTGTTAATTACTTC	Present study
JB71	CCGTCGACCTTTAGCAAAATGATTCCAATATTCATTC	Present study
JB75	CCCGTAAAAAATAAGGGGGAAAACGACCATGAC	Present study
JB76	CCGAATTCAATTTACTTCTTAAGGACTACGAGGCCAATC	Present study

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