SUPPLEMENTARY MATERIALS AND METHODS

Ethics statement

Female BALB/c mice aged 7 weeks or male C57BL/6J mice aged 7 weeks were purchased from Jackson Laboratories (Bar Harbor, ME) and housed in specific pathogen-free facilities at the University of Colorado Anschutz Medical Center Animal Facility (Aurora, CO). Mice were allowed to acclimate for 1 week before experimentation. At experimental end points, mice were killed through carbon dioxide inhalation, followed by cervical dislocation. All animal work was approved by and performed in accordance with the University of Colorado Anschutz Medical Campus Institutional Animal Care and Use Program under the protocol numbers 00486 and 00941.

Growth conditions and reagents

All staphylococcal strains were grown in tryptic soy broth (TSB) at 37 °C with shaking at 220 r.p.m. For strains with pDB59, chloramphenicol was added to a final concentration of 10 μ g/ml. For strains with pCM40, erythromycin was added to a final concentration of 10 μ g/ml. *Staphyloccocus warneri* synthetic autoinducing peptides (AIPs) were custom synthesized by AnaSpec (Fremont, CA).

Conditioned media inhibition experiments

For conditioned media assays, *S. warneri* strains and methicillin-resistant *S. aureus* reporters were grown overnight for 20 hours as described. A total of 1 ml of *S. warneri* culture was pelleted, and conditioned media was filtered through a Costar (MilliporeSigma, Burlington, MA) Spin-X centrifuge tube (0.22 μ m, cellulose acetate filter). Reporters were prepared by subculturing 1:500 in fresh TSB with chloramphenicol to maintain the pDB59 accessory gene regulator reporter plasmid. Conditioned media was added at 20% (vol/vol) to a 96-well black culture plate (Corning, Corning, NY) and two-fold serially diluted to 0.15% (vol/vol). A total of 100 μl reporter was added to a final volume of 200 μl per well. Cultures were grown in a Stuart (Cole-Parmer, Staffordshire, United Kingdom) humidified incubator at 37 °C with shaking at 1,000 r.p.m. At hourly time points up to 24 hours, plates were measured on a Tecan Group (Männedorf, Switzerland) Infinite Pro plate reader to quantify growth (optical density of 600 nm) and YPF signal (excitation of 480 nm, emission of 515 nm). For synthetic AIP experiments, the peptide was resuspended in neat DMSO, or a DMSO control was added from stocks of 20 μ M to indicated concentrations. *S. epidermidis* reporter assays were conducted in the same manner as methicillin-resistant *Staphylococcus aureus* reporter assays.

Liquid chromatography-mass spectrometry AIP identification

AIP identification was accomplished using a previously published method (Todd et al., 2016). Liquid chromatography-mass spectrometry data were obtained on a Q Exactive Plus quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) with an electrospray ionization source coupled to an Aquity UPLC system (Waters, Milford, MA). Spent media samples were injected onto an Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm, Waters) at a flow rate of 300 μl/min and injection volume of 5 μl. A binary solvent gradient of water with 0.1% formic acid and acetonitrile (CH_3CN) with 0.1% formic acid was used. The gradient began with an 80:20 (water:CH_3CN) hold for 1 minute 30 seconds, followed by a linear increase to 60% CH_3CN over 5 minutes. The gradient was held at 40:60 (water:CH_3CN) for 30 seconds, increased to 100% CH_3CN over 1 minute, and held at 100% CH_3CN for 1 minute before returning to 80:20 for a total run time of 10 minutes. The first 1 minute 30 seconds of eluent was diverted to waste. Positive ionization mode was used with a scan range of m/z 450−2,000, with a resolving power of 35,000. The instrument parameters were set as follows: spray voltage of 3.5 kV, capillary temperature of 256 °C, sheath gas of 48, auxiliary gas of 12, spare gas of 2, probe heater temperature of 412.50 °C, and S-lens radio frequency level of 50. Confirmation of AIP was done using tandem mass spectrometry. The ion of the predicted AIP, calculated at m/z 960.39256, was isolated and subjected to high-energy collision dissociation fragmentation using a normalized collision energy of 30. Tandem mass spectrometry spectra were collected at a resolving power of 17,500.

Epicutaneous infection

To prepare bacteria for AIP experiments, AH1263 (strain LAC) was grown overnight in TSB, then subcultured at 1:50 in fresh TSB and allowed to grow to an OD_{600} (optical density of a sample measured at a wavelength of 600 nm) of 1. Bacterial cells were washed and pelleted in PBS and resuspended in sterile saline to achieve an inoculum of 1×10^8 colony-forming units (CFUs) in 100 μl. LAC and synthetic AIP-I (50 μ g) or vehicle (DMSO) were combined immediately before application on gauze. For competition experiments, AH1263 (strain LAC) and *S. warneri* (AH5628) were grown overnight in TSB, then subcultured at 1:50 in fresh TSB and allowed to grow to an OD_{600} of 1. Bacterial cells were washed and pelleted in PBS and resuspended in sterile saline to achieve an inoculum of 1×10^8 CFU in 50 μl for competition or 1×10^8 CFU in 100 μl L for a single challenge. The cochallenge inoculum was combined in a 1:1 ratio immediately before application on gauze. Inoculum concentration was verified by colony counting after 24 hours of incubation at 37 °C.

C57BL/6J mice were anesthetized with 2% isoflurane, backs were shaved, and residual hair was removed with a 1-minute application of Nair (Church & Dwight, Ewing Township, NJ). Mice were allowed to recover for 24 hours after hair removal. Bacteria were applied to the back skin for 72 hours on a 2-cm^2 piece of sterile gauze affixed with Tegaderm and covered in a Band-Aid. A Tewameter TM300 (Courage + Khazaka electronic GmbH, Köln, Germany) was used to determine changes to epithelial barrier integrity 72 hours after infection. Two sites per lesion were measured. To enumerate bacterial CFUs on the skin after the challenge with AIP, the full-thickness 2-cm^2 atopic lesion was excised with sterile scissors, added to 0.5 ml PBS with 1 mm zirconia/silica homogenization beads (BioSpec, Bartlesville, OK), and homogenized for three intervals of 1 minute each. The suspension was serially diluted and plated on nonselective (tryptic soy agar) and selective (mannitol salt agar, and mannitol salt agar supplemented with 5.2 μ g/ml cefoxitin) media. Plates were incubated overnight before colony counting.

Dermonecrosis infection

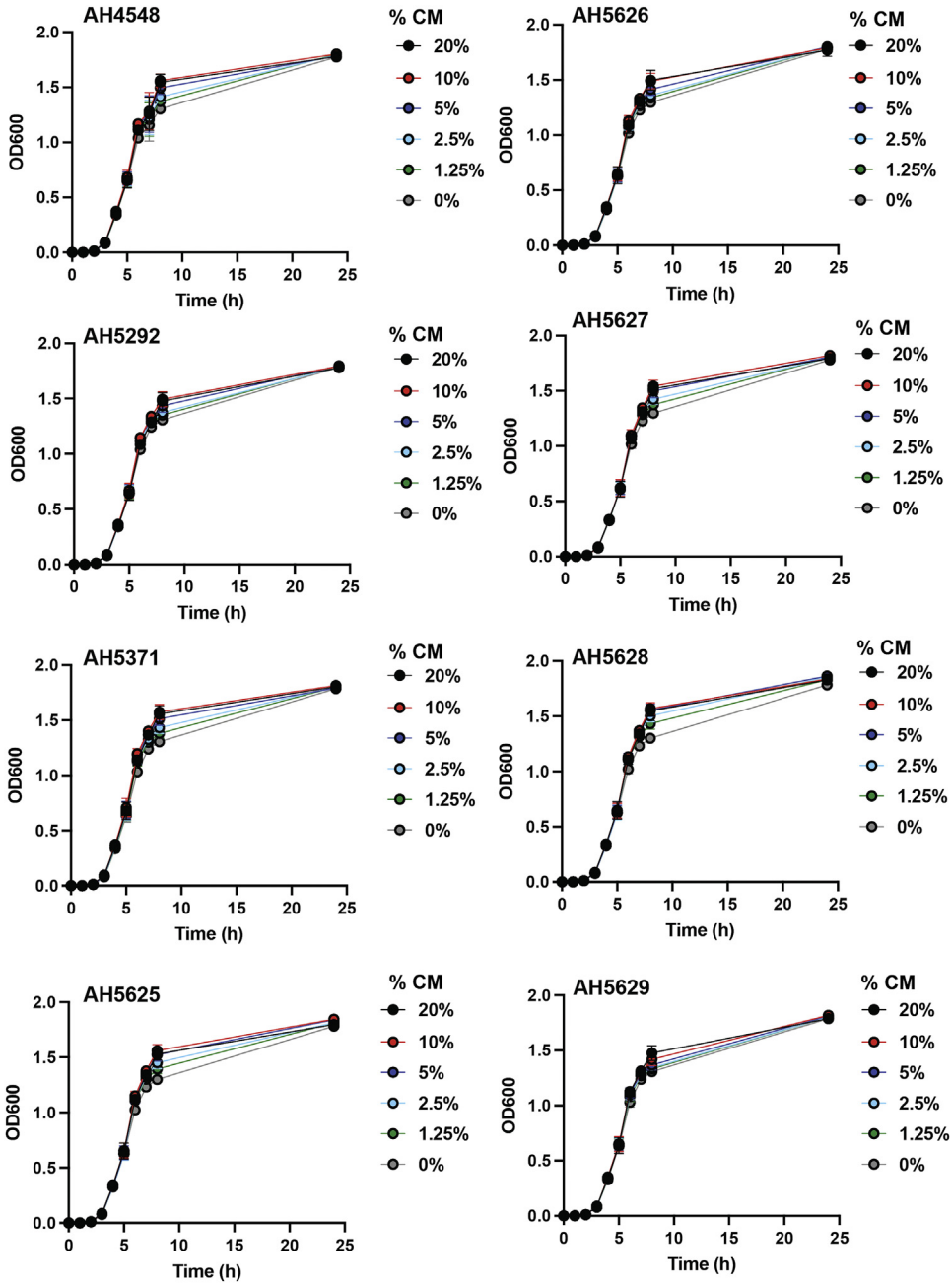
To prepare bacteria for the dermonecrosis model, *S. aureus* AH1758 (RN4850) was grown overnight in TSB, then subcultured at 1:100 in fresh TSB, and allowed to grow to early exponential phase (OD_{600} of 0.5–0.7). Bacterial cells were washed and pelleted in PBS and resuspended in sterile saline to achieve an inoculum of 1×10^8 CFU in 50 µl. Inoculum concentration was verified by colony counting after 24 hours of incubation at 37 °C. One day before the challenge, BALB/c mouse abdomens were shaved, and residual hair was removed with a 30-second application of Nair (Church & Dwight). Immediately before injection, abdomens were sanitized with alcohol wipes. A total of 50 µl inoculum suspensions containing 1×10^8 CFU *S. aureus* and *S. warneri* AIP-II (50 µ g in neat DMSO) or DMSO alone were injected intradermally. *S. aureus* and AIP were mixed immediately before injection. Body weights were measured before infection and every day thereafter for 7 days. To determine lesion size, digital images were taken using a Canon Powershot ELPH180 (Canon Europe, Hayes, United Kingdom) camera and analyzed with ImageJ (National Institutes of Health, Bethesda, MD) software.

Statistical Analysis

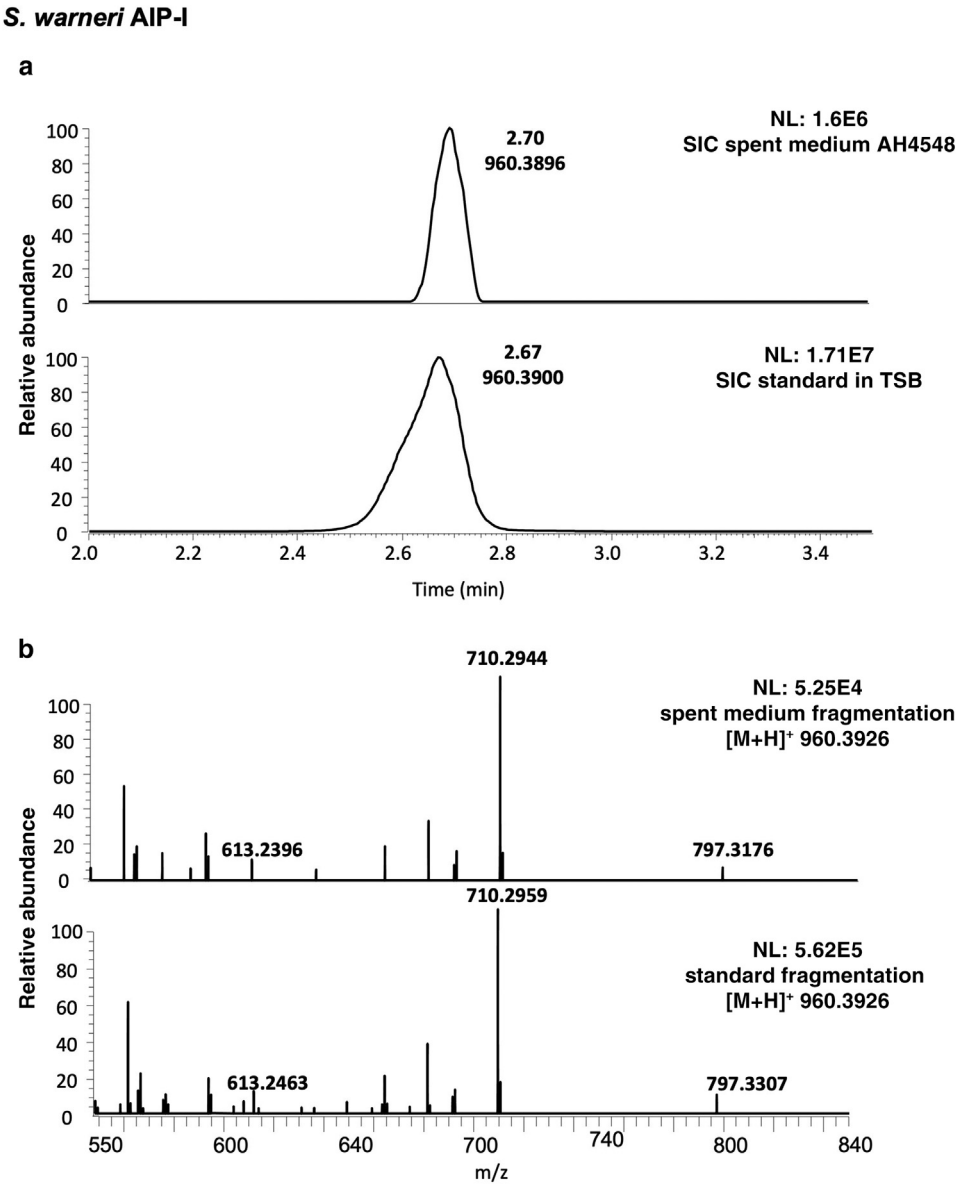
All analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA) software. In vitro data are presented as the mean and SD, and in vivo data are presented as the mean and SEM; a $P < 0.05$ was considered significant. Test choices are indicated in the figure legend.

SUPPLEMENTARY REFERENCES

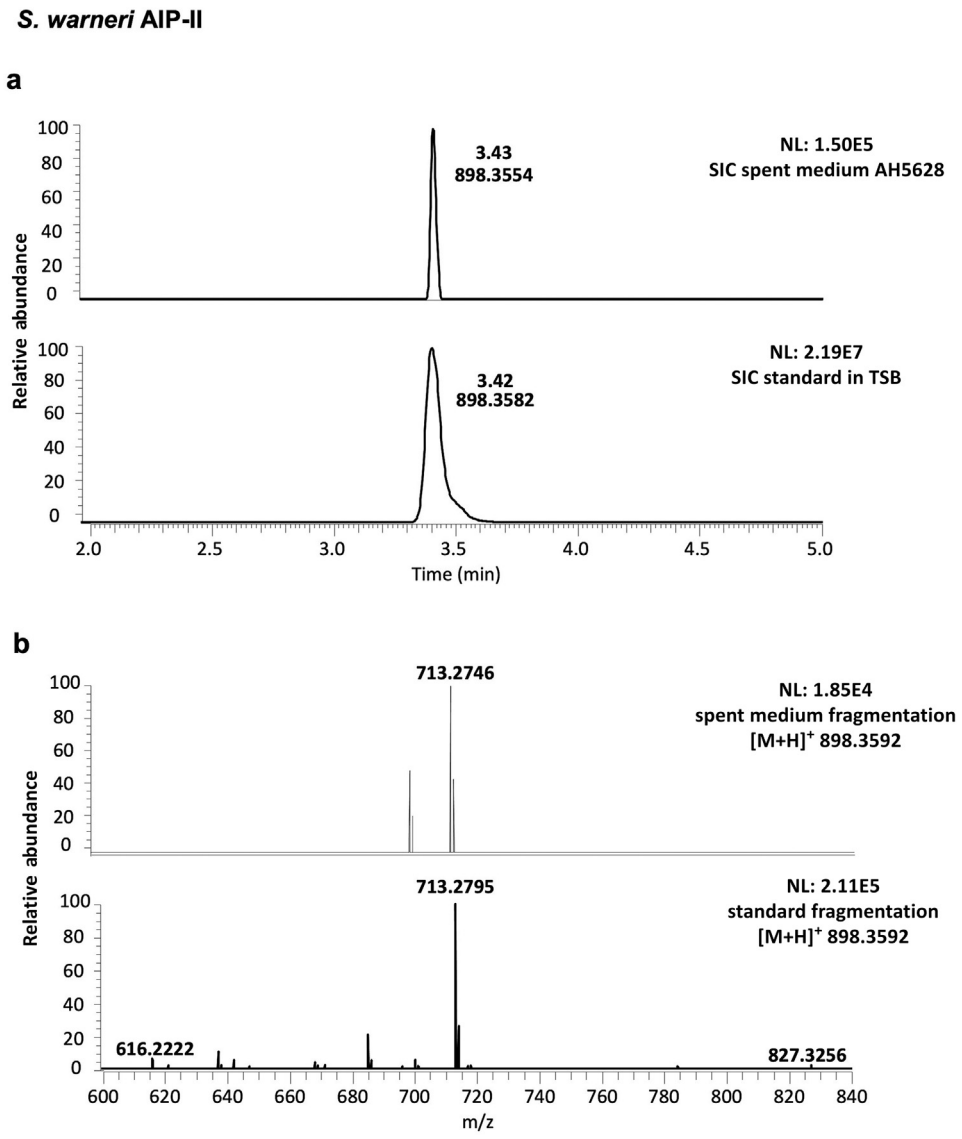
Todd DA, Zich DB, Ettefagh KA, Kavanaugh JS, Horswill AR, Cech NB. Hybrid Quadrupole-Orbitrap mass spectrometry for quantitative measurement of quorum sensing inhibition. J Microbiol Methods 2016; 127:89–94.



Supplementary Figure S1. *S. warneri* conditioned media does not inhibit MRSA *agr*-I growth. The MRSA *agr*-I P3::YFP reporter strain was incubated with various indicated concentrations (v/v) of conditioned media from overnight cultures of *S. warneri* strains (identified by number AHXXXX). The OD (600 nM) for 1–8 and 24 hours is shown. Data are pooled from three independent experiments, and mean ± SD is shown. *agr*, accessory gene regulator; CM, conditioned medium; h, hour; MRSA, methicillin-resistant *Staphylococcus aureus*; OD, optical density.



Supplementary Figure S2. Synthetic AIP-I validation. (**a**) SIC for the calculated AIP-I mass (m/z = 960.3926) from strain AH4548 for both the spent medium (top) and the synthetic AIP standard in TSB (bottom). (**b**) MS/MS spectra for identified AIP-I ion in the spent medium (top) and synthetic AIP in TSB (bottom). AIP, autoinducing peptide; min, minute; MS/MS, tandem mass spectrometry; SIC, selected-ion chromatograms; TBS, tryptic soy broth.



Supplementary Figure S3. Synthetic AIP-II validation. (**a**) SIC for the calculated AIP-II mass (m/z = 898.3592) from strain AH5628 for the spent medium (top) and the synthetic AIP in TSB (bottom). (**b**) MS/MS spectra for identified AIP-II ion in both the spent medium (top) and the synthetic AIP in TSB (bottom). AIP, autoinducing peptide; MS/MS, tandem mass spectrometry; SIC, selected-ion chromatograms; TBS, tryptic soy broth.