Supplemental Methods

SSD0Fe medium recipe. A 25X Tris-salts mixture was made with the following: Tris-HCl (0.75 M, pH 8.8); NaCl (0.198 M); KH_2PO_4 (0.05 M); NH_4Cl (0.43 M); glucose (0.5 M). One liter of SSD0Fe was made by combining 40 mL of the 25X stock, 6 g BD Bacto Casamino Acids, 3.9 mL of a 0.01 M L-tryptophan stock solution, 0.6 mL of a 0.01 M Thiamine-HCl stock solution, 3.2 mL of a 0.01 M nicotinic acid stock solution, and an appropriate amount of filtered water (MilliQ Advantage Q-10; Millipore). 10 g Chelex (200-400 mesh; Biorad) was added and the mixture was stirred 1 h at room temperature (RT). The resulting solution was sterile filtered (0.2 µm, 90 mm filter; Nalgene) and mixed with 50 µL of a sterile, 1 M $MgCl_2*6H_2O$ solution. SSD0Fe was left at RT for at least 14 d before use in assays.

Isolation and putative speciation of primary nostril strains of *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*. After rubbing a swab (BBL CultureSwab) around the surface of the nasal vestibule (inside of a nostril), about 1/8 of each swab was used to inoculate two sets of sterile BHIT agar and Columbia CNA 5% sheep's blood agar prior to incubation at 37°C, one set each aerobically and anaerobically. (After inoculation, nostril swabs were immediately stored at -80°C.) Morphologically distinct colonies were picked and colony purified using the growth conditions of initial isolation prior to stocking in medium with 20% glycerol (v/v) at -80°C. Strains KPL1845 and KPL1815 were designated as putative *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively, based on a combination of phenotypic assays (e.g., CHROMagar *S. aureus* for KPL1845 and API Staph V4.1 for both) and sequencing of the V1-3 region of 16S rRNA genes from each isolate. A combination of both physical

assays and molecular biology was also used to assign KPL1844 as a putative *Propionibacterium granulosum* and KPL1849 as a putative *Propionibacterium acnes*. Per Downes and Wade (1), growth in 20% bile salts, hydrolysis of esculin, and production of indole were used to compare known isolates of *Propionibacterium acnes*, *avidum*, and *granulosum* with KPL isolates (**Table S1**). Furthermore, 16S rRNA gene and *rpoB* gene sequences from KPL isolates were used as bait for a blastn search. These loci were sequenced as previously described (1) and deposited in GenBank (KF906602-KF906611).

Because KPL1855 had a lipid requirement for growth like many *Corynebacterium* spp., we modified an existing MLST-like approach to putatively speciate KPL1855 as *Corynebacterium accolens*. We utilized 16S rRNA gene and *rpoB* gene sequences from the genome sequence of KPL1855 completed as part of the Human Microbiome U54 initiative, Broad Institute. These gene sequences were compared to similar sequences from 21 previously deposited *Corynebacterium* spp. and one outgroup taxon (*Rhodococcus equi*) (2). These sequences were aligned with the ClustalW algorithm using MEGA5 (3). Trimmed, aligned 16S DNA (1283-1297bp) and *rpoB* (3144-3153bp) sequences were concatenated and subject to phylogenetic reconstruction using a general time-reversible model with a gamma shape parameter and a proportion of invariable sites (GTR+Γ+$\mathbf{I}$) via Bayesian inference (Ba). The GTR+Γ+$\mathbf{I}$ evolutionary model was chosen by evaluating likelihood scores of 56 potential evolutionary models using the Akaike information criterion as implemented by Modeltest 3.7 (4). Ba was done with nst “6” and rate “invgamma” settings (GTR+Γ+$\mathbf{I}$) with heated chains set to a temperature value of 0.25 (to ensure an appropriate amount of chain swapping) using

the software package MrBayes 3.1.2 (5). For Ba, majority-rule consensus tree construction and statistical analysis of clade membership/presence was assessed by sampling an appropriately stationary posterior probability distribution of 800,000 total generations every 100 generations. For our purposes, an appropriately stationary distribution was defined, as recommended by Ronquist and colleagues (6), as an average standard deviation of split frequencies of less than 0.01 for ~70 to 90% of samples between two, independent Metropolis-coupled Markov Chain Monte Carlo runs. Majority-rule consensus trees drawn from the stationary sample distribution were used for the assessment of the posterior probabilities of all clades (**Fig. S7**). Statistical analysis of clade membership/presence was also assessed by both maximum parsimony and maximum likelihood bootstrapping. ML bootstrapping was performed by treating gaps as missing, searching heuristically using simple addition, subtree pruning and regrafting for swaps, and swapping on “best only” with 200 pseudoreplicates as implemented by PAUP*4.0b10 (7). MP bootstrapping was performed by treating gaps as missing, searching heuristically using simple addition, tree bisection reconnection for swaps, and swapping on “best only” with 200 bootstrap pseudoreplicates as implemented by PAUP*4.0b10 (7).

Preparation of *Propionibacterium* spp. conditioned medium (CM) crude extract and HPLC fractionation. From solid medium, *Propionibacterium* spp. were inoculated into 2 L of BHI at pH 6.1, either with 1 mM 5–aminolevulinic acid in DMSO (*P. granulosum* KPL1844) or without (*P. acnes* KPA171202). Each flask was incubated in an anaerobic, light-protected environment at 37°C for 90 h. The CM was collected by pelleting the cells by centrifugation (3250 x g, 15 min, 4°C) and was extracted with 150

mL (volume measured dry in a beaker before equilibration) of Diaion HP-20 (Sigma-Aldrich) beads by stirring for 4 h in a light-protected environment. Beads were washed 5X with distilled H_2O (dH_2O), then extracted 3X with 150 mL MeOH. Extractions were combined and dried down to produce dry crude extract of CM.

Dry crude extract was dissolved in 10 mL MeOH; this solution was filtered and 1 mL injected into a preparative HPLC (1200 Series, Agilent Technologies) on a semi-preparative C18 column (Phenomenex Luna 5 µM C18, 250 x 10 mm) with detection at λ=280 and 400 nm. Mobile phase A was 0.1% trifluoroacetic acid (TFA) in dH_2O; mobile phase B was 0.1% TFA in acetonitrile. Separation was obtained at a flow rate of 7 mL/min with a gradient program that started at 10% B followed by a 20-min step that raised eluent B to 100%. Washing at 100% B was performed for 2 min (yielding a total analysis time of 22 min). Fractions were collected at 2 min intervals and immediately dried down for storage at -80°C in a light-protected environment.

Analytical HPLC porphyrin quantitation from cell-free conditioned medium. For each strain, 5 mL cultures were grown at 37°C to stationary phase under the following conditions: *S. aureus* and *S. epidermidis* – TSB, 18 h, aerobic; *C. accolens* – BHIT, 30 h, aerobic; *P. granulosum* and *P. acnes* – BHI, 50 h, anaerobic. Cells were pelleted as above. Each CM was filtered (0.22 µm) to remove cells and extracted with an equal volume of ethyl acetate:acetic acid (3:1, v/v). The organic phase (containing extracellular porphyrins) was collected and all solvent evaporated. Dried residue was resuspended in 250 µL acetone:MeOH (1:1, v/v) and filtered to remove insoluble material (= 20X concentration compared to original CM). Cell pellets were washed once with 5 mL dH_2O to remove residual extracellular porphyrin then lyophilized to determine

dry cell mass for standardization of the production results. For determination of intracellular porphyrin levels, the three pellets of each strain were combined and homogenized in 5 mL ethyl acetate:acetic acid (3:1, v/v). The solvent was evaporated after filtration and the dried residue resuspended in 250 µL acetone:MeOH (1:1, v/v).

Fifty µl of each acetone:MeOH resuspension was injected into an analytical HPLC (1200 Series, Agilent Technologies) on a C18 column (Phenomenex Luna 5 µM C18, 100 x 4.6 mm) with detection at λ=400 nm. Mobile phase A was 0.1% TFA in dH_2O; mobile phase B was 0.1% TFA in acetonitrile. Separation was obtained at a flow rate of 1 mL/min with a gradient program that started at 10% B, changing to 25% B in 3 min followed by a slow increase to 45% B over 40 min After this, the solvent composition was increased to 100% B over an additional 7 min followed by a 5 min wash at 100% B (yielding a total analysis time of 55 min). Intracellular porphyrin levels were below the detection limit, which is not surprising given the small amounts of cell pellets combined from the three 5 mL cultures of each bacterial strain.

A standard curve was generated with 100 µM stock solutions made with uroporphyrin III (UIII) (Frontier Scientific, Logan, UT), coproporphyrin I (CI) & III (CIII) (Santa Cruz Biotechnology, Dallas, TX), and protoporphyrin IX (PIX) (Sigma-Aldrich). Stock solutions were combined in equimolar quantities and a dilution series was analyzed over 0.025-2.5 µM. For each porphyrin, the peak area at λ=400 nm was plotted versus concentration. A linear trendline with the intercept set to zero was fitted for these data. For quantification of an individual sample's porphyrin concentration, the peak areas were converted to concentrations using appropriate trendline equations from the above standards. The average of three biological replicates was calculated and corrected by

subtracting the porphyrin concentration in the medium. To allow comparison, production levels were standardized by determining the amount of porphyrin produced (pmol) per dry weight of lyophilized cells (mg).

Live-dead staining of *S. aureus.* We chose staining to study cell death, rather than culture-based colony-forming unit (cfu) counting, as the difference in the prevalence of aggregates impacted enumeration of cfus. After a 4 h exposure to either 50 μM CIII in DMF or DMF alone in the aggregation assay described above, 100 μL *S. aureus* cells were mixed with 1 μL of a 1.67 mM Syto 9 and 10 mM propidium iodide solution (Bac Light, Invitrogen/Molecular Probes) and incubated at RT in the dark for 15 min. Syto 9-stained (fluorescent green – "alive") and propidium iodide-stained (fluorescent red – "dead") cells were visualized with a Nikon Eclipse 80i epifluorescence microscope equipped with a Intensilight C-HGFI lamp and a D5-Qi1Mc digital camera using 1000X total magnification to define a "field of view" (FOV). For each FOV, the percentage of red and green cells was calculated (~150-300 total cells per FOV) and these data were averaged for 3-5 FOV for each biological replicate. The average percentage of red cells for each of five biological replicates was averaged together for final statistical analyses.

References for Supplemental Methods

1. **Downes J, Wade WG.** 2009. *Propionibacterium acidifaciens* sp. nov., isolated from the human mouth. Int J Syst Evol Microbiol **59:**2778-2781.
2. **Khamis A, Raoult D, La Scola B.** 2004. *rpoB* gene sequencing for identification of *Corynebacterium* species. J Clin Microbiol **42:**3925-3931.
3. **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S.** 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol **28:**2731-2739.
4. **Posada D, Crandall KA.** 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics **14:**817-818.
5. **Huelsenbeck JP, Ronquist F.** 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics **17:**754-755.

6. **Ronquist F, Huelsenbeck JP.** 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:**1572-1574.
7. **Swofford DL.** 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.