Antibacterial photosensitization through activation of coproporphyrinogen oxidase


*Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232; †Department of Chemistry, Vanderbilt University, Nashville, TN 37232; ‡Vanderbilt Institute for Chemical Biology, Nashville, TN 37232; §Department of Biomedical Engineering, Vanderbilt University, Nashville, TN 37232; †Vanderbilt Vaccine Center, Vanderbilt University Medical Center, Nashville, TN 37232; ‡Biomedical Informatics, Vanderbilt University School of Medicine, Nashville, TN 37203; ‡Department of Biochemistry, Vanderbilt University, Nashville, TN 37232; §Department of Electrical Engineering and Computer Science, Vanderbilt University, Nashville, TN 37232; ‡Biomedical and Health Sciences Institute, University of Georgia, Athens, GA 30602; ‡Department of Microbiology, University of Georgia, Athens, GA 30602; and ‡Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602.

Edited by Ferric C. Fang, University of Washington School of Medicine, Seattle, WA, and accepted by Editorial Board Member Carl F. Nathan June 26, 2017 (received for review January 10, 2017)

Gram-positive bacteria cause the majority of skin and soft tissue infections (SS TIs), resulting in the most common reason for clinic visits in the United States. Recently, it was discovered that Gram-positive pathogens use a unique heme biosynthesis pathway, which implicates this pathway as a target for development of antibacterial therapies. We report here the identification of a small-molecule activator of coproporphyrinogen oxidase (CgoX) from Gram-positive bacteria, an enzyme essential for heme biosynthesis. Activation of CgoX induces accumulation of coproporphyrin III and leads to photosensitization of Gram-positive pathogens. In combination with light, CgoX activation reduces bacterial burden in murine models of SSTI. Thus, small-molecule activation of CgoX represents an effective strategy for the development of light-based antimicrobial therapies.

Significance

Skin and soft tissue infections (SSTIs) account for a majority of visits to hospitals and clinics in the United States and are typically caused by Gram-positive pathogens. Recently, it was discovered that Gram-positive bacteria use a unique pathway to synthesize the critical cellular cofactor heme. The divergence of the heme biosynthesis pathways between humans and Gram-positive bacteria provides a unique opportunity for the development of new antibiotics targeting this pathway. We report here the identification of a small-molecule activator of coproporphyrinogen oxidase (CgoX) from Gram-positive bacteria that induces accumulation of coproporphyrin III and leads to photosensitization of Gram-positive pathogens. In combination with light, CgoX activation reduces bacterial burden in murine models of SSTI.

Small-molecule VU0038882 (‘882) was previously identified in a screen for activators of the S. aureus heme-sensing system two-component system (HssRS) (11–13) (Fig. 1B). Upon activation, HssRS induces the expression of the heme-regulated transporter (HrTAb) to alleviate heme toxicity (11–13). HssRS activation is triggered by massive accumulation of heme in ‘882-exposed bacteria (11). In addition to activating HssRS, treatment with ‘882 induces toxicity to bacteria undergoing fermentation by impacting iron–sulfur cluster biogenesis (11, 14). Through a medicinal chemistry approach, the HssRS-activating properties of ‘882 were decoupled from its toxicity, suggesting two distinct cellular targets for this small molecule (11, 14, 15). Before this investigation, the mechanism by which ‘882 activates heme biosynthesis to trigger HssRS had not been uncovered.

We report here the identification of the cellular target of ‘882 responsible for inducing heme biosynthesis through the use of a Psor-driven suicide strain. ‘882 activates CgoX from Gram-positive bacteria, an enzyme essential for heme biosynthesis. Activation of CgoX induces accumulation of the product of the

E6652–E6659  |  PNAS  |  Published online July 24, 2017

www.pnas.org/cgi/doi/10.1073/pnas.1700469114


Conflict of interest statement: M.C.S., B.F.D., G.A.S., and E.P.S. have filed a patent for the small-molecule activity reported in this paper.

This article is a PNAS Direct Submission. F.C.F. is a guest editor invited by the Editorial Board.

Freely available online through the PNAS open access option.

1To whom correspondence should be addressed. Email: eric.skaar@vanderbilt.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1700469114/-/DCSupplemental.
reaction, CPIII, a photoreactive molecule of demonstrated utility in treating bacterial infections (6, 16, 17). Photodynamic therapy (PDT) uses a photosensitizing molecule activated by specific wavelengths of light to produce reactive oxygen species that lead to cell death (6). Most US Food and Drug Administration-approved photosensitizers are aminolevulinic acid (ALA) derivatives, which serve as prodrugs through their conversion to porphyrins in the heme biosynthetic pathway (6). Through ‘882-dependent activation of CgoX, CPIII accumulates in a similar manner, inducing photosensitization specifically in Gram-positive bacteria, and ‘882-PDT reduces bacterial burden in murine models of SSTI (Fig. S1). Thus, small-molecule activation of CgoX represents a promising strategy for the development of light-based antimicrobial therapies.

Results

Construction of an ‘882-Responsive Suicide Strain. To identify the target of ‘882, a suicide strain was created enabling selection of S. aureus strains that are unresponsive to ‘882. Because ‘882 is not toxic to wild-type (WT) bacteria, a genetic approach was used to engineer toxicity to S. aureus upon treatment with ‘882. S. aureus hrtAB was replaced with two copies of the E. coli gene encoding the RNA interferase toxin RelE. Upon heme or ‘882 stimulation, toxicity is induced in hrtAB::relE. (E) Strain CgoX.T183K exhibits a normal growth phenotype on tryptic soy agar (TSA), suggesting the CgoX T183K does not restrict CgoX function. (F) Strain CgoX.T183K is unresponsive to ‘882, but retains the ability to respond to heme as measured by P₈882-PDT. *P < 0.001 compared with vehicle treated for each strain. (G) HPLC analysis of WT, CgoX.T183K, and ΔCgoX ± ‘882. ‘882 increases CPIII production in WT cells but not in CgoX.T183K. (Inset) ‘882-induced CPIII accumulation induces fluorescence in treated cells.

Selection of ‘882-Resistant Suicide Strains. Isolates of hrtAB::relE exhibiting spontaneous resistance to ‘882 were identified at a frequency of 0.0079%, and the stability of resistance was ensured through serial passage. Genomic DNA was isolated and the hssRS/hrtAB locus was sequenced. Whole-genome sequencing was performed on isolates lacking mutations in hssRS/Pht to identify mutations conferring resistance to ‘882 in the hrtAB::relE strain background. This analysis revealed a T183K mutation in CgoX, an enzyme required for heme biosynthesis. The CgoX T183K mutation was reconstructed in WT S. aureus (CgoX.T183K), and this strain exhibits normal growth, suggesting the CgoX T183K mutation prevents the response of S. aureus to ‘882 without restricting heme biosynthesis, as seen in a strain lacking CgoX (ΔCgoX) (18) (Fig. 1E). In addition, the T183K mutation abolished ‘882 sensing to the same level as ΔhssRS, whereas heme sensing remained intact (Fig. 1F). Taken together, these results demonstrate that the CgoX T183K mutation prevents ‘882-induced activation of hrtAB in S. aureus.

‘882 Induces CPIII Accumulation. To determine the impact of ‘882 on the heme biosynthesis pathway, intermediates of heme biosynthesis were quantified following ‘882 exposure. Porphobilinogen (PBG), an early heme biosynthetic precursor, is unaffected by ‘882 (Fig. 1A and Fig. S2). In contrast, CPIII, the product of CgoX, is greatly increased following ‘882 treatment (Fig. 1A and G). Notably, CPIII accumulation is not observed in CgoX.T183K or ΔCgoX upon ‘882 treatment (Fig. 1G). CPIII is the only fluorescent molecule in the heme biosynthesis pathway, and ‘882 exposure triggers dramatic fluorescence in S. aureus (Fig. 1G, Inset). The HPLC fraction corresponding to the elution time of the CPIII standard was analyzed by LC-MS/MS and confirmed to be CPIII (Fig. S3). These results demonstrate that ‘882 exposure leads to accumulation of CPIII in S. aureus and implicate CgoX as a candidate target of the molecule.

‘882 Activates CgoX from Gram-Positive Bacteria in Vitro. To determine whether ‘882 directly activates S. aureus CgoX, recombinant S. aureus WT CgoX and CgoX.T183K were purified. Importantly,
these two enzymes display similar $K_m$ and $V_{\text{max}}$ values, demonstrating the T183K mutation does not affect baseline CgoX activity (Fig. 2G and Fig. S4A). Upon treatment with '882, WT CgoX displayed 205% activity with an $AC_{150}$ of 92 nM, whereas the T183K mutant did not respond to '882 at concentrations up to 10 μM (Fig. 3A and G). These data establish '882 as a small-molecule activator of CgoX.

To determine whether '882 retains activity against CgoX from other Gram-positive organisms, recombinant Bacillus subtilis and P. acnes CgoX were purified (7). $K_m$ and $V_{\text{max}}$ values were determined to characterize baseline activity of the enzymes, and each was tested for '882-induced activation (Fig. 2B and G and Fig. S4B). '882 activates CgoX from B. subtilis and P. acnes with $AC_{150}$ values of 42 and 16 nM, respectively (Fig. 2B and G). Importantly, human HemY, a protoporphyrinogen oxidase as opposed to a coproporphyrinogen oxidase, did not respond to '882 treatment (Fig. 2C and Fig. S4C). These results establish '882 as a small-molecule activator of CgoX from Gram-positive bacteria.

**Structure–Activity Relationship Studies of '882.** Recently, a series of structural analogs of '882 were prepared and screened in a phenotypic HssRS activation assay using a XylE reporter gene to monitor transcription of hrtAB as a response to heme accumulation

---

![Figure 2](https://www.pnas.org/cgi/doi/10.1073/pnas.1700469114) Surdel et al.

**Fig. 2.** '882 activates CgoX from Gram-positive bacteria and identification of a region important for regulation of CgoX. (A, B, and F) CgoX activity was assayed with increasing '882 concentrations and 5 μM CPGIII. (C) HemY activity was assayed with increasing '882 concentrations and 5 μM PPGIX. (D) The residue of B. subtilis CgoX homologous to S. aureus T183 (blue) is located in a region distinct from the active site (Y366, yellow) (19, 51). B. subtilis CgoX shares 46% identity with S. aureus CgoX. A residue homologous to S. aureus N186 (red) faces a cleft leading into the active site. (E) Magnified view of residues homologous to S. aureus T183 and N186 residues (carbon in light blue, nitrogen in dark blue, and oxygen in red). (G) $K_m$, $V_{\text{max}}$, $AC_{150}$, and activation (in percentage) for each enzyme were determined.
Both mutations inhibited the activation of CgoX by 882, suggesting that these compounds do not have improved bioavailability over 882 in staphylococcal cells. In addition, these 882 derivatives are also inactive against the T183K mutant, suggesting all four diarylpyrazoles share a common binding site. The remaining eight diarylpyrazoles were equally inactive when assayed against CgoX or the T183K mutant. Collectively, these structure–activity relationship studies support activation of CgoX by a common allosteric binding site.

**Structural Analysis of the 882–CgoX Interaction.** To gain insight into the potential binding site of 882 within CgoX, the previously solved crystal structure of *B. subtilis* CgoX was interrogated (19) (Fig. 2D). Based on the location of *S. aureus* T183 within CgoX, a nearby amino acid, N186, was implicated as potentially important due to its proximity on a short helix facing a cleft leading into the active site (Fig. 2D and E). The N186 residue was mutated to tyrosine, phenylalanine, or alanine and the activity of the mutant enzyme was examined in the presence or absence of 882 (Fig. 2F and G and Fig. S4D). CgoX N186Y and CgoX N186F exhibit increased baseline activity relative to WT CgoX, suggesting this region is important for positive enzyme regulation (Fig. 2G and Fig. S4D). Consistent with this, all three mutations abolish the ability of CgoX to respond to 882 treatment (Fig. 2F and G). Importantly, the helix containing these residues is in a distinct region from the active site of the molecule (Fig. 2D). Taken together, these data support a model whereby 882 binds to the region of CgoX containing residues 183–186 and acts as an allosteric modulator of enzyme activity. Mutations in this portion of the enzyme may mimic 882-induced changes in tertiary structure leading to enzyme activation.

**In Silico Docking Identifies a Functional Domain Important for 882 Activation.** Despite significant efforts, we were unsuccessful in our attempts to solve the complete crystal structure of 882 in complex with CgoX from various Gram-positive organisms. A flexible loop encompasses residues 183–186 that were identified in our initial mutational analysis. Importantly, previously published structures of CgoX are also incomplete in this region (19, 20). Therefore, to further our understanding of the functional domain required for 882 activity in the absence of a crystal structure, the 882–CgoX interaction was modeled by in silico docking to identify additional residues that may be required for 882-induced activity of CgoX. Based upon this analysis, six more residues within CgoX were selected for mutational analysis to interrogate their importance for 882-dependent activation (Fig. 3A). Mutations were made in *S. aureus* CgoX, creating the enzyme variants V146M, M167F, Y171A, F184A, F187W, and D450Y. Upon induction, V146M, Y171A, F184A, and D450Y led to unstable enzymes that could not be purified (Fig. S5). CgoX M167F and F187W expressed equivalently to WT (Fig. S5), were purified, and interrogated for 882-induced activation. Both mutations inhibited the activation of CgoX by 882 (Fig. 3B). Taken together, these data begin to define a pocket within
CgoX that appears to be required for ‘882-dependent activation of the enzyme (Fig. 3C).

**CgoX Activation Induces Photosensitization of Gram-Positive Bacteria.**

PDT is frequently used to treat bacterial skin infections and involves the use of a photosensitizer and a light source to destroy cells through the production of reactive oxygen species (6). Porphyrin intermediates of the heme biosynthesis pathway are the most common photosensitizers used in clinics. The production of porphyrin intermediates is often up-regulated through the addition of ALA, the first committed precursor in the heme biosynthetic pathway (Fig. 1A). A major limitation of ALA-PDT for the treatment of infectious diseases is the lack of specificity of this therapy, which induces photosensitivity in both bacterial and human cells. Due to the specificity of ‘882 for CgoX from Gram-positive bacteria, this molecule should selectively sensitize bacteria to light while avoiding host toxicity. To test this hypothesis, various strains of *S. aureus* were grown in the presence of ALA, ‘882, or both and exposed to 395-nm light, the absorbance maximum for CPIII as determined experimentally (data not shown). Both ALA and ‘882 treatment led to significant growth inhibition of *S. aureus* following exposure to 68 J/cm² light (Fig. 4A). An additive effect was seen when ‘882 and ALA were in combination, likely due to ALA increasing precursor availability for CgoX. Notably, ALA and ‘882 combined decreased *S. aureus* viability by six logs compared with untreated cells (Fig. 4A). The toxicity of ‘882-PDT is conserved across some of the most important causes of human skin infections, including *S. epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *B. anthracis*, and *P. acnes* (Fig. 4B–D). These results establish the utility of ‘882-PDT as a potential therapeutic for the treatment of skin infections caused by Gram-positive bacteria.

**‘882-PDT Decreases Bacterial Burdens in Vivo.** To determine the in vivo efficacy of ‘882-PDT, a murine model of superficial *S. aureus* skin infection was used, which leads to considerable skin ulceration (21). Mice infected with *S. aureus* USA300 LAC and treated with ‘882-PDT or ‘882/ALA-PDT exhibited significantly lower bacterial burden per wound following infection compared with untreated animals (Fig. 4G). Histologically, all groups showed varying degrees of inflammation, with neutrophils being the most abundant cells present; however, bacterial burden was noticeably reduced in ‘882-PDT- and ‘882/ALA-PDT-treated samples (Fig. 4E and F). Topical administration of 2% mupirocin ointment, as a positive control antibiotic, also significantly reduced bacterial burden compared with untreated mice. (Fig. 4G).

To evaluate the broad utility of ‘882-PDT as a therapy for common skin infections, the efficacy of ‘882-PDT in a murine

Table 1. Structural analogs of ‘882 activate HemY

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound designation</th>
<th>Compound abbreviation</th>
<th>In vivo activity: % activity of ‘882 at 50 μM (SEM)</th>
<th>In vitro activity: % activity of WT HemY at 1 μM (SEM)</th>
<th>In vitro activity: % activity of T183K HemY at 1 μM (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Structure 1]</td>
<td>VU038882</td>
<td>a</td>
<td>100 (9)*</td>
<td>192 (21)</td>
<td>69.3 (8.8)</td>
</tr>
<tr>
<td>![Structure 2]</td>
<td>VU0812197</td>
<td>b</td>
<td>0.335 (0.276)*</td>
<td>300 (42)</td>
<td>72.3 (11.5)</td>
</tr>
<tr>
<td>![Structure 3]</td>
<td>VU0420372</td>
<td>c</td>
<td>1.67 (0.55)*</td>
<td>230 (40)</td>
<td>72.1 (7.5)</td>
</tr>
<tr>
<td>![Structure 4]</td>
<td>VU0812187</td>
<td>d</td>
<td>0.206 (0.113)*</td>
<td>153 (15)</td>
<td>35.7 (4.7)</td>
</tr>
<tr>
<td>![Structure 5]</td>
<td>VU0420382</td>
<td>e</td>
<td>0.812 (0.297)*</td>
<td>128 (12)</td>
<td>78.3 (7.9)</td>
</tr>
<tr>
<td>![Structure 6]</td>
<td>VU0125897</td>
<td>f</td>
<td>28.2 (0.8)*</td>
<td>112 (16)</td>
<td>110 (19)</td>
</tr>
<tr>
<td>![Structure 7]</td>
<td>VU0476720</td>
<td>g</td>
<td>0.392 (0.146)*</td>
<td>111 (13)</td>
<td>95.6 (9.7)</td>
</tr>
<tr>
<td>![Structure 8]</td>
<td>VU0476725</td>
<td>h</td>
<td>0.299 (0.188)*</td>
<td>109 (17)</td>
<td>137 (12)</td>
</tr>
<tr>
<td>![Structure 9]</td>
<td>VU0366053</td>
<td>i</td>
<td>0.715 (0.093)*</td>
<td>89.6 (11.4)</td>
<td>106 (12)</td>
</tr>
<tr>
<td>![Structure 10]</td>
<td>VU0404345</td>
<td>j</td>
<td>1.19 (0.31)*</td>
<td>81.9 (24.4)</td>
<td>75.8 (10.0)</td>
</tr>
<tr>
<td>![Structure 11]</td>
<td>VU0476722</td>
<td>k</td>
<td>0.286 (0.136)*</td>
<td>79.4 (9.0)</td>
<td>95.2 (15.2)</td>
</tr>
<tr>
<td>![Structure 12]</td>
<td>VU0812191</td>
<td>l</td>
<td>0.245 (0.036)*</td>
<td>77.8 (14.9)</td>
<td>72.1 (9.5)</td>
</tr>
</tbody>
</table>

*Previously published data (15).
\( \text{model of } P. \text{ acnes } \text{infection was determined. Both '882-PDT and '882/ALA-PDT led to a significant decrease in bacterial burden compared with untreated mice, highlighting the utility of '882 as a possible compound for the treatment of acne (Fig. 4H). Taken together, these findings establish PDT coupled with small-molecule activation of CgoX as an effective therapeutic strategy to specifically target Gram-positive pathogens.} \)

\section*{Discussion}

These results define the small-molecule '882 as an activator of Gram-positive CgoX. Treatment with '882 leads to massive heme accumulation resulting in HssRS activation in \( S. \text{ aureus} \). Residues required for '882-induced CgoX activation have been identified, thereby revealing a functional domain involved in the activation of CgoX. In addition, treatment with '882 leads to photosensitization of Gram-positive bacteria, reducing bacterial burdens in vivo. Taken together, these results establish '882 as an activator of Gram-positive CgoX and provide proof-of-principle for small-molecule activation of CgoX as a potential therapeutic strategy for the treatment of bacterial infections.

Synthetic small-molecule activators are rare, with only a handful identified to date (22). Identifying the targets of small molecules is a major obstacle in biomedical research (23–25). Phenotypic high-throughput screens using small-molecule libraries often fail to result in the identification of numerous molecules with unknown targets. Several approaches have been successful in identifying intracellular targets of small molecules (26–32). Here, we report a genetic selection strategy based on the creation of a suicide strain that enables the identification of spontaneous resistant mutants to an activating compound that is typically nontoxic. This strategy can be adapted to a variety of systems where a small molecule activates a specific gene expression program, and may enable the identification of targets for numerous small-molecule activators.

The mechanisms by which heme biosynthesis is regulated in Gram-positive bacteria are largely unknown (9). The identification of '882 as an activator of CgoX establishes this molecule as a valuable tool for interrogating the heme biosynthetic pathway in Gram-positive bacteria to further understand the synthesis of this essential cofactor. Interestingly, it has previously been demonstrated that CgoX activity is modulated in vitro to a similar extent by addition of ChdC (previously known as HemQ), the terminal enzyme in the Gram-positive heme biosynthesis pathway (33). This suggests that the interaction of CgoX with '882 may not be a random occurrence but may represent an inappropriate hijacking of a normal in vivo regulatory mechanism. The '882-dependent activation of CgoX with '882 may not be a random occurrence but may represent an inappropriate hijacking of a normal in vivo regulatory mechanism. The '882-dependent activation of CgoX establishes this molecule as a valuable tool for studying the regulation of heme biosynthesis in Gram-positive bacteria.

Notably, enzyme activators have numerous properties that make them ideal therapeutics (22). Whereas inhibitors often require the ability to inhibit the enzyme by 90%, activators can induce phenotypes with small increases in enzyme activity, indicating that derivatives of '882 with slight increases in activity could lead to dramatic increases in CPIII accumulation and therefore antibacterial properties (22). Specifically, this has been seen with small-molecule activators of glucokinase, where a 1.5-fold increase in enzymatic activity has shown significant effects in vivo. These molecules are now used as therapeutics for diabetes (22, 34–36).

In addition, inhibitors often bind active sites of enzymes, which are typically well conserved across homologous enzymes. In contrast, activators often bind allosteric sites, which are less well conserved across enzymes, thus increasing specificity and limiting off-target effects (22, 34, 37). Structural analysis of the '882–CgoX interaction has identified a functional domain of CgoX that suggests an allosteric mechanism of action for '882-induced activation (Figs. 2 and 3). In addition, we have identified specific
residues that when mutated increase activity, further supporting that this portion of the enzyme is important in positive regulation (Fig. 2). Therefore, that this portion of the enzyme is important in positive regulation residues that when mutated increase activity, further supporting that the use of PDT has begun to expand beyond SSTIs. Gastrointestinal endoscopes have been developed that emit wavelengths that activate porphyrins in patients to detect cancer, and similar strategies have been interrogated for their ability to treat gastrointestinal infections (38, 39). Osteomyelitis and contamination of orthopedic devices are some of the most common invasive bacterial infections, and PDT strategies to combat these infections are being developed (40–46). Finally, PDT-based strategies are in development for a variety of other diseases, including parasitic, dental, and sinus infections (47, 48). One of the key limitations for PDT approaches involving photosensitizers that need to be activated with light at shorter wavelengths (below 500 nm) is the lack of deep-tissue penetration of light at those wavelengths. Therefore, the development of strategies to increase the penetration of light may considerably improve the therapeutic potential of porphyrin-based PDT. As the utility of PDT-based therapies expands, so too will the potential clinical utility of small-molecule activators of CgoX.

The ability of 582 to specifically photosensitize Gram-positive bacteria circumvents the nonspecific nature of ALA-PDT, which has limited the use of ALA-PDT for the treatment of infectious diseases (6, 49, 50). Furthermore, this provides proof-of-concept that activation of bacterial porphyrin production through specific activation of CgoX is a viable therapeutic strategy that could be adapted to Gram-negative bacteria and other infectious diseases (6, 16, 47). Therefore, the development of 582-PDT has the potential to significantly expand the value of light-based therapies for the treatment of the most common causes of skin infections.

Materials and Methods

Descriptions of growth conditions, strain construction, suicide strain selection experiments, genomic analysis, heme precursor quantification, promoter activity assays, CgoX activity assays, in silico docking studies, photosensitization assays, superficial skin infection studies, and chemical synthesis can be found in SI Materials and Methods. Bacterial strains, expression constructs, plasmids, and primers used in this study can be found in Tables S1–S4. All research involving animals described in this paper was reviewed and approved by the Vanderbilt University Institutional Animal Care and Use Committee.

ACKNOWLEDGMENTS.

We thank members of the E.P.S. laboratory for critical reading of the manuscript. Core services performed through Vanderbilt University Medical Center’s Digestive Disease Research Center were supported by NIH Grant P30DK058404 Core Scholarship. The following reagents were provided by the Network on Antimicrobial Resistance in Staphylococcus aureus for distribution by BEI Resources, National Institute of Allergy and Infectious Diseases, NIH: N590F (ATCC 25923) and Nebraska Transmutant Memory Screening Array (NR-48501). This work was supported by Public Health Service Award T32 GM07347 from the National Institute of General Medical Studies for the Vanderbilt Medical School-Microbiology Program (to M.C.S. and P.L.T.), Grant R01 AI069233 (to M.C.S., L.I.L., and E.P.S.), Grant R01 AI073843 (to M.C.S. and E.P.S.), Grant T32 GM008554-18 (to L.I.L.), Grant T32 GM065086 (to B.F.D.), Grant R01 LM010685 (to P.L.T.), Grant T32 ES007028 (to M.A.), Vanderbilt Vaccine Center startup funds (to R.N. and L.S.G.), Vanderbilt University Medical Center Faculty Research Scholars award (to R.N. and L.S.G.), and Walter Reed Army Institute of Research Grant W81XWH-17-2-0003.


52. Fey PD, et al. (2013) A genetic resource for rapid and comprehensive phenotype screening of nonessential Staphylococcus aureus genes. MBio 4:e00537–e12.


Supporting Information

SI Materials and Methods

Bacterial Strains and Growth Conditions. Cloning was performed in Escherichia coli DH5α (Invitrogen). Strains, plasmids, and primers used are described in Tables S1–S4. All proteins were expressed using E. coli strain BL21(DE3) pREL (11). All S. aureus and S. epidermidis strains were grown in tryptic soy broth (TSB) or agar (TSA). E. coli and B. anthracis were grown in lysogeny broth (LB) or lysogeny broth agar (LBA), and P. acnes in brain heart infusion (BHI) broth or agar, unless otherwise stated.

Strain Construction. The suicide strain was constructed by allelic replacement as previously described (52). PCR was performed with Phusion Polymerase (Thermo Scientific), unless stated otherwise. The genomic context upstream of htrAB was amplified using primers MS0001b and MS019, and the downstream fragment was amplified using primers MS020 and MS006b. The fragments were fused by PCR SOEing to create an NdeI site between the upstream and downstream fragments (53). The 3′-adenosine overhangs were added by incubation with ExTaq (TaKaRa) for 20 min at 72 °C. The resulting product was ligated into PCR2.1 according to manufacturer’s instructions (Life Technologies). reLe was amplified from E. coli DH5α using primers MS023 and MS024. The plasmid and PCR products were digested with NdeI (New England Biolabs) and ligated with T4 DNA Ligase (New England Biolabs) to insert the tox gene between upstream and downstream fragments. The plasmid DNA was isolated from transformants, and a strain harboring two copies of reLe in the correct orientation was selected for downstream applications. Using this plasmid as a template, primers MS001 and MS006 were used to amplify the suicide construct. This was inserted into pKORI, and allelic exchange was performed as previously described (52).

S. aureus strain CgoX::ermC was described previously and the CgoX::ermC allele was transduced into strain Newman using the Ph-85 bacteriophage (54, 55). To create an S. aureus strain harboring the T183K mutation in CgoX, cgoX was amplified from the point mutant isolated in the suicide selection using primers pKORI_CgoX_FattB and pKORI_CgoX_RattB. This construct was moved into pKORI and allelic replacement was used as previously described (52).

Suicide Strain Selection. Overnight bacterial cultures of suicide strain or ΔhtrB were subcultured 1:100 into 5 mL of TSB and grown for 8 h. One hundred microliters of a 1:40,000 dilution were plated on media containing ‘882 (5–15 μM). The resistant colonies were passaged twice on TSA to ensure resistance was genetically stable, and rechallenged by plating on heme or ‘882. The colonies that retained resistance to ‘882 but were sensitive to heme were used for downstream analysis.

Genome Sequencing and Analysis. Genomic DNA was isolated from mutant strains using the Wizard Genomic Kit (Promega) and sequenced to identify mutations in htrAB::relE. Strains containing mutations in this locus were eliminated from subsequent analyses. The genomes from strains lacking mutation in this locus were sequenced by Perkin-Elmer on the MiSeq Platform. Whole-genome sequencing analysis was automated with a tool written in the Python programming language. The input files were the Newman genome (.fas) and the mutations file (.csv). The program iterates through each mutation determining the mutation type and resultant amino acid change, if applicable. The input .csv is preprocessed to combine proximal mutations in the same strain as both must be considered due to their combined codon effect. The script also includes options to specify the organism ID, the base pair radius around the mutation to use for genome matching, an e-value threshold for allowable search results, and a customizable delimeter character for visualization. The program takes into account both strand directions when searching to ensure complete coverage as well as potential missed search matches at the edges of the genome. All mutations are classified based on their effect—silent, substitution, frameshift, truncation, or deletion.

The Bacterial, Archaeal, and Plant Plastid Code (transl_table = 11) was used as our translation table; more information can be found at https://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi.

Mutations in noncoding regions or that produced silent mutations in the amino acid sequence were removed. Mutations were compared with a control suicide strain not subjected to selection to eliminate mutations present in the parent strain. The genes identified in this analysis were resequenced in the relevant strain to confirm the presence of the mutation.

Heme Precursor Quantification. Cells were grown in TSB overnight in the presence or absence of 40 μM ‘882. The cells were pelleted and lysed as previously described (11). PBG was quantified from lysate as previously described (56). Briefly, modified Erlich’s reagent was prepared by dissolving 1 g of p-dimethylaminobenzaldehyde (Sigma) in 16 mL of 70% perchloric acid and bringing the final volume to 50 mL with glacial acetic acid. The lysate was mixed with fresh Erlich’s reagent and incubated at room temperature for 10 min. The absorbance at 555 nm was determined and compared with a standard curve using fresh PBG (Frontier Biosciences) to determine the concentration. For HPLC analysis, protoplast lysates were incubated with Dianon HP20 beads for 1 h at 4 °C. Hydrophobic molecules were eluted from the beads with 3 mL of acetone. The samples were protected from light. The hydrophobic molecules were concentrated in vacuo for 24 h at room temperature. The samples were resuspended in 200 μL of 1:1 water/acetonitrile with 0.1% trifluoroacetic acid. HPLC was performed as previously described (57). Absorbance was measured at a wavelength of 400 nm to identify heme precursor molecule peaks. The fractions corresponding to the retention time of CPIII and heme standards were collected and lyophilized. The lyophylized fractions were resuspended in 100 μL of 1:1 water/acetonitrile and analyzed by LC-MS/MS as previously described (56).

Promoter Activity Assay. Promoter activity assay was performed as previously described (13). Briefly, phtrAB::XylE was electroporated into the strain CgoX.T183K. The other strains used have been previously described (11). Overnight cultures were diluted 1:100 into TSB supplemented with 10 μg/mL chloramphenicol under the following conditions: vehicle (DMSO), 1 μM heme, or 10 μM ‘882. Cells were grown for 6 h and assayed as previously described (12). The experiment was performed in triplicate on three separate days (n = 9). Data are presented as mean ± SEM. Student’s t test was performed to determine significance.

S. aureus CgoX Expression Construct. S. aureus CgoX was amplified from either Newman or the suicide strain resistant to ‘882 containing the T183K mutation using primers pET15_CgoXF1 and pET15_CgoXR and inserted into pET15b using the Gibson Assembly Cloning Kit (New England Biolabs). The point mutations in...
CgoX were created using Pfu mutagenesis with primers described in Table S4 (58).

**Enzyme Expression and Purification.** The plasmids described in Table S2 were transformed into BL21(DE3) pREL. An overnight of a strain harboring each CgoX expression construct was diluted 1:100 into Terrific Broth (Fisher Scientific) supplemented with the appropriate antibiotics and 10 μg/mL riboflavin. Cells containing Homo sapiens HemY were grown at 37 °C overnight. The cells containing S. aureus and P. acnes CgoXwere grown at 37 °C until they reached an OD_{600} of 0.7, induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and grown at 30 °C overnight. The cells containing B. subtilis CgoX were grown at 37 °C until they reached an OD_{600} of 0.7, induced with 0.1 mM IPTG, and grown for 5 h 37 °C. The cells were washed with PBS and stored at −80 °C until protein was harvested. The cells were resuspended in lysis buffer [50 mM Tris-Mops, pH 8.0, 0.1 M potassium chloride, and 1% sodium cholate hydrate supplemented with 1 mg/mL lysozyme and one Pierce Protease Inhibitor Tablet (Thermo Scientific), homogenized using a Dounce homogenizer, and passed through an EmulsiFlex (Avestin) four times. Lysate was centrifuged at 40,000 × g for 1 h and filtered with a 0.22-μM filter.

S. aureus and P. acnes CgoX and H. sapiens HemY were purified using HisPur Cobalt Superflow Agarose (Thermo Scientific). The lysate was mixed with agarose and incubated at 4 °C with rotating for 30 min. The lysate and beads were poured into a gravity column followed by washing with 10 column volumes of 5 mM imidazole in the lysis buffer. Proteins were eluted with 250 mM imidazole in lysis buffer in 5 column volumes. B. subtilis protein was purified on an AKTA FPLC (GE Healthcare Scientific) with a linear gradient from 0 to 500 mM imidazole in lysis buffer. Glycerol was added to 10% total volume, and protein was aliquoted and stored at −80 °C. A fresh aliquot was used each day for assays.

All purified enzymes were analyzed by SDS/PAGE and tested for activity to verify purity (Fig. S5).

**CgoX Activity Assay.** Purified CgoX was assayed as previously described (59). The reactions were performed in 300-μL volume and incubated at 37 °C for 1 h with an exposure time of 0.7, induced with 0.1 mM IPTG, and grown for 5 h 37 °C. The cells were washed with PBS and stored at −80 °C until protein was harvested. The cells were resuspended in lysis buffer [50 mM Tris-Mops, pH 8.0, 0.1 M potassium chloride, and 1% sodium cholate hydrate supplemented with 1 mg/mL lysozyme and one Pierce Protease Inhibitor Tablet (Thermo Scientific), homogenized using a Dounce homogenizer, and passed through an EmulsiFlex (Avestin) four times. Lysate was centrifuged at 40,000 × g for 1 h and filtered with a 0.22-μM filter.

S. aureus and P. acnes CgoX and H. sapiens HemY were purified using HisPur Cobalt Superflow Agarose (Thermo Scientific). The lysate was mixed with agarose and incubated at 4 °C with rotating for 30 min. The lysate and beads were poured into a gravity column followed by washing with 10 column volumes of 5 mM imidazole in the lysis buffer. Proteins were eluted with 250 mM imidazole in lysis buffer in 5 column volumes. B. subtilis protein was purified on an AKTA FPLC (GE Healthcare Scientific) with a linear gradient from 0 to 500 mM imidazole in lysis buffer. Glycerol was added to 10% total volume, and protein was aliquoted and stored at −80 °C. A fresh aliquot was used each day for assays.

All purified enzymes were analyzed by SDS/PAGE and tested for activity to verify purity (Fig. S5).

**Identification of Additional Mutations That Affect ‘882 Activity.** Protoporphyrinogen oxidase structure (PDB ID code 3I6D, chain A) from Bacillus subtilis was used for the structural analysis. Molecular docking simulations with ligand ‘882 were performed using the AutoDock 4.2 and AutoDock tools (60). The protein was prepared for docking by assigning polar hydrogens, solvation parameters, and Kollman united atom charges, whereas Gasteiger charges were assigned to the ligand. Water molecules were removed from the input structure. The ligand was modeled as flexible around rotatable bonds. The grid box was centered around residues T189 and Q192 (S. aureus T183 and N186). Flexible docking was performed by modeling as flexible these two residues, as well as the nearby Y177, F190, and F193. The default grid box size was adjusted to allow a free rotation of the ligand, and set at 70 × 70 × 70, with a grid spacing of 0.375 Å. Grid maps were generated using the AutoGrid program. The Lamarckian genetic algorithm (LGA) was used for the conformer search, with default parameters, including selection window (10 generations), population size (150), and maximum number of energy evaluations (2,500,000) (61). A total of 8,000 docked conformations was generated, and the 100 lowest-energy models were selected for further analysis. Potential mutations at residues, for which at least one ligand conformation (from the top 100) was within 4 Å, were modeled structurally, for selection of mutations to destabilize the interactions between the protein and the ‘882 ligand. Structural models were visualized using the PyMOL software. Two types of mutations were selected for experimental validation: (i) larger amino acid side chains that could sterically hinder ligand binding but that could be accommodated within the protein binding pocket, or (ii) Ala mutations at residues that were found to have substantial ligand interactions in the docking models.

**Light Source and Photosensitivity Assays.** A 395-nm wavelength LED (M395L4; Thorlabs) was mounted 4.5 cm above the sample and collimated using a collimation lens (ACF2520-A; Thorlabs). The diode was powered by a T-cube LED driver (LED1DB M00325270; Thorlabs) producing 179 mW at the sample with a circular spot size 1 cm in diameter covering an area of 0.785 cm² with an irradiance of 227.9 mW/cm² with an exposure time of 5–6 min. All light-killing experiments were performed on 3 separate days and averaged.

Overnight cultures of S. aureus, S. epidermidis, S. lugdunensis, or B. anthracis were subcultured 1:50 into TS and either 50 μM ‘882, 4 mM δ-aminolevulinic acid hydrochloride (ALA) (Frontier Biosciences), or a combination of the two. Cultures were grown for 3 h to reach exponential phase of growth. Cell pellets were washed once with ice-cold PBS at two times the original culture volume, centrifuged, and resuspended in the original culture volume with ice-cold PBS. Cells were diluted 1:10 into ice-cold PBS. Twenty-five microliters were transferred into a black 96-well plate with flat, clear bottom and exposed to light to 0 or 68 J/cm². The bacteria were serially diluted, plated, and CFUs were counted after 20 h of growth on LB agar.

A 5-d anaerobic culture of P. acnes in BHI was removed from the anaerobic chamber (Coy) and subcultured 1:100 into BHI with or without (TS) 50 μM ‘882 or 4 mM ALA. Cultures were subcultured 1:50 into TSB and either 50 μM ‘882 or 4 mM ALA. Cultures were grown at 30 °C overnight. The cells containing P. acnes were grown measured with an exposure time of 6 min delivering a total energy dose of ~82 J/cm². Control mice were either left untreated or were administered a dose of mupirocin ointment 2% (TARO Pharmaceuticals) with a sterile-cotton tipped applicator. Eight hours postinfection, groups of mice were treated with 20 μL of
1 mM '882 in 2% Tween 80 in PBS, 20% ALA and 1 mM '882 in
2% Tween 80 PBS, 2% Tween 80 PBS vehicle alone, or a second
dose of mupirocin ointment. Two to 3 h after application of
compounds, groups of mice were challenged with a second dose
of light or left untreated for a total dose of 164 J/cm². Upon
completion of the second dose of light treatment, skin samples
containing the wound were homogenized using a Bullet Blender
(Next Advanced) according to the manufacturer’s instructions.
The homogenate was serially diluted and plated. Data are repre-
sented as mean ± SEM. A one-way ANOVA with Dunn’s
multiple-comparisons test was performed to determine signifi-
cance (GraphPad).

Histological Examinations. Sections of skin were excised after the
mice were killed, and then placed in 10% Neutral Buffered
Formalin. Fixed tissues were then processed routinely by de-
hydration and embedded in paraffin. Five-micrometer sections
were trimmed and placed on charged slides for staining with
H&E. Skin sections were evaluated for inflammation, presence
of neutrophils, mononuclear leukocytes, and bacteria using a
previously described semiquantitative scoring system (21).

SI Chemical Synthesis

General Procedures. All nonaqueous reactions were performed in
flame-dried flasks under an atmosphere of argon. Stainless-steel
syringes were used to transfer air- and moisture-sensitive liquids.
Reaction temperatures were controlled using a thermocouple
thermometer and analog hotplate stirrer. Reactions were con-
ducted at room temperature (∼23 °C), unless otherwise noted.
Flash column chromatography was conducted using silica gel
230–400 mesh. Analytical TLC was performed on E. Merck silica
gel 60 F254 plates and visualized using UV and iodine stain.

Materials. All solvents and chemicals were purchased from Sigma-
Aldrich, unless otherwise noted. Dichloromethane (DCM) and
tetrahydrofuran (THF) were used as received in a bottle with a
SureSeal. Triethylamine was distilled from calcium hydride and
stored over KOH. Deuterated solvents were purchased from
Cambridge Isotope Laboratories. Trimethylsilylacetylene was
purchased from Oakwood Chemicals. S1 and S3 were prepared
using previously described procedures (62, 63). Biotin azide
(PEG4 carboxamide-6-azidohexanyl biotin) was prepared by the
Vanderbilt Institute of Chemical Biology chemical synthesis
core. The preparation and characterization of ‘882 derivatives
presented in Table 1 have been previously described (15).

Instrumentation. 1H NMR spectra were recorded on Bruker 400-
or 600-MHz spectrometers and are reported relative to deuter-
ated solvent signals. Data for 1H NMR spectra are reported as
follows: chemical shift (δ ppm), multiplicity (s = singlet, d =
doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br =
broad, app = apparent), coupling constants (in hertz), and in-
tegration. 13C NMR spectra were recorded on Bruker 100- or
150-MHz spectrometers and are reported relative to deuterated
solvent signals. 19F NMR were recorded on a Bruker 376-MHz
spectrometer. Low-resolution mass spectrometry (LRMS) was con-
ducted and recorded on an Agilent Technologies 6130 Quadrupole
instrument.

SI Experimental Procedures

1-(2-Hydroxy-4-(trimethylsilyl)ethynyl)phenyl)
ethanone (S2). To a stirred solution of 4-bromo-2-hydroxy-
acetophenone (S1) (220 mg, 1.02 mmol) in tetrahydrofuran (4 mL)
was added triethylamine (284 μL, 2.04 mmol), bis(triphenylphos-
phine)palladium dichloride (36.0 mg, 0.051 mmol), copper(I) io-
dide (9.7 mg, 0.051 mmol), and trimethylsilylacetylene (216 μL,
1.53 mmol). The mixture was stirred overnight at room tempera-
ture under an atmosphere of argon until judged complete by
TLC. The reaction was filtered through Celite, concentrated, and
purified by flash chromatography to provide 208 mg (88%) of S2
as white crystals. 1H NMR (400 MHz, CDCl3) δ 7.64 (d, J = 8.24 Hz,
1H), 7.85 (d, J = 1.40 Hz, 1H), 6.96 (dd, J = 8.24 Hz, J = 1.52 Hz,
1H), 2.61 (s, 3H), 0.23 (s, 9H); 13C NMR (100 MHz, CDCl3)
δ 204.0, 162.1, 131.2, 130.6, 122.5, 121.7, 119.5, 103.7, 98.8,
26.8, –0.1; LRMS calculated for C13H17O2Si+ (M+H)+ m/z:
233.1, measured 233.2.
Gram-positive bacteria cause the majority of skin and soft tissue infections (SSTIs), resulting in the most common reason for clinic visits in the United States. Recently, it was discovered that Gram-positive pathogens use a unique heme biosynthesis pathway, which implicates this pathway as a target for development of antibacterial therapies. We report here the identification of a small-molecule activator of coproporphyrinogen oxidase (CgoX) from Gram-positive bacteria, an enzyme essential for heme biosynthesis. Activation of CgoX induces accumulation of coproporphyrin III and leads to photosensitization of Gram-positive pathogens. In combination with light, CgoX activation reduces bacterial burden in murine models of SSTI. Thus, small-molecule activation of CgoX represents an effective strategy for the development of light-based antimicrobial therapies.

Fig. S2. 882 does not affect early heme biosynthesis. Porphobilinogen (PBG) was quantified to determine 882-induced effects on early heme biosynthesis intermediates. No effect on PBG was seen.
Fig. S3. ‘882 induces accumulation of CPIII. HPLC fractions containing either the CPIII standard (A) or the corresponding fraction from ‘882-treated WT cells (B) were analyzed by LC-MS/MS. MS/MS fragmentation of the peak at m/z 654.340, the mass of CPIII, is shown. The MS/MS fragmentation of this peak in both samples is nearly identical and consistent with expected major fragments, positively identifying the peak within these fractions as CPIII.
Fig. S4. Characterization of CgoX activity from Gram-positive bacteria and HemY from H. sapiens. (A–D) Activities of recombinant CgoX from S. aureus (WT, T183K, N186Y, N186F), B. subtilis, P. acnes, and H. sapiens HemY with varying concentrations of coproporphyrinogen III (CPGIII) for Gram-positive CgoX and protoporphyrinogen IX (PPGIX) for H. sapiens HemY to determine $K_m$ and $V_{max}$.

Fig. S5. Purity of CgoX or HemY used in biochemical assays. After purification, each protein was analyzed by SDS/PAGE to verify purity.
### Table S1. Bacterial strains

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>S. aureus strain Newman</td>
<td>Ref. 64</td>
</tr>
<tr>
<td>Wild type</td>
<td>S. aureus strain USA300</td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>S. aureus strain USA500</td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>S. aureus strain RN6390</td>
<td></td>
</tr>
<tr>
<td>ΔhrtB</td>
<td>In-frame deletion of hrtB in Newman background</td>
<td>Ref. 65</td>
</tr>
<tr>
<td>RN4220</td>
<td>S. aureus cloning intermediate</td>
<td>Ref. 66</td>
</tr>
<tr>
<td>hrtAB::reiE</td>
<td>S. aureus strain Newman expressing two copies of reiE at the hrtAB locus</td>
<td>This study</td>
</tr>
<tr>
<td>CgoX,T183K</td>
<td>S. aureus strain Newman harboring a T183K mutation in CgoX</td>
<td>This study</td>
</tr>
<tr>
<td>ΔCgoX</td>
<td>S. aureus strain Newman harboring a transposon interrupting CgoX expression</td>
<td>This study</td>
</tr>
<tr>
<td>ΔhssRS</td>
<td>In-frame deletion of hssRS in Newman background</td>
<td>Ref. 67</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>Strain NRS6</td>
<td>BEI</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>HIP05979 (NRS9)</td>
<td>BEI</td>
</tr>
<tr>
<td>S. lugdunensis</td>
<td>Timothy Foster</td>
<td></td>
</tr>
<tr>
<td>B. anthracis</td>
<td>Strain Sterne</td>
<td>Ref. 68</td>
</tr>
<tr>
<td>P. acnes</td>
<td>ATCC 6919</td>
<td>ATCC</td>
</tr>
</tbody>
</table>

### Table S2. Expression constructs

<table>
<thead>
<tr>
<th>Protein</th>
<th>Expression vector</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>T183K S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>N186F S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>N186Y S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>Y171A S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>D450Y S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>F187W S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>F184A S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>M167F S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>V146M S. aureus CgoX</td>
<td>pET15b</td>
<td>This study</td>
</tr>
<tr>
<td>B. subtilis CgoX</td>
<td>pTrcHisA</td>
<td>Ref. 33</td>
</tr>
<tr>
<td>H. sapiens HemY</td>
<td>pTrcHisB</td>
<td>Ref. 2</td>
</tr>
<tr>
<td>P. acnes CgoX</td>
<td>pTrcHisA</td>
<td>Ref. 69</td>
</tr>
</tbody>
</table>

### Table S3. Plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>phrt.XylE</td>
<td>12</td>
</tr>
<tr>
<td>pKORI</td>
<td>52</td>
</tr>
<tr>
<td>Primer name</td>
<td>Sequence</td>
</tr>
<tr>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td>SA_cgoX_T183K_T</td>
<td>AGTTTGATGAGTACGTTTCCTAATTTTAAAG</td>
</tr>
<tr>
<td>SA_cgoX_T183K_B</td>
<td>CTTTTAAATTTAGAAGGAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>N186Y_T_NWMN1723</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>N186Y_B_NWMN1723</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>N186F_T_NWMN1723</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>N186F_B_NWMN1723</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>SA_Y171A_T</td>
<td>CTTTTAAATTTAGAAGGAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>SA_Y171A_B</td>
<td>CAAATTCGTAACCCACATACACCCCAATTTAAGG</td>
</tr>
<tr>
<td>SA_D450Y_T</td>
<td>GCGGGTGGAGACTCCTTATTGTATTACGCAAGG</td>
</tr>
<tr>
<td>SA_D450Y_B</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>SA_F187W_T</td>
<td>GAGTTTGATGAGTACGTTTCCTAATTTTAAAG</td>
</tr>
<tr>
<td>SA_F187W_B</td>
<td>CTTTTAAATTTAGAAGGAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>SA_F184A_T</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>SA_F184A_B</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>SA_M167F_T</td>
<td>GAGTTTGATGAGTACGTTTCCTAATTTTAAAG</td>
</tr>
<tr>
<td>SA_M167F_B</td>
<td>CTTTTAAATTTAGAAGGAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>SA_V146M_T</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>SA_V146M_B</td>
<td>GCTCTTCTTTTCTTTTTCTTTAAAAATAGAAAAACGCTACCTCATAAACCT</td>
</tr>
<tr>
<td>MS001</td>
<td>GGGGACAGTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>MS001</td>
<td>GGGGACAGTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>MS006</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>MS006</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>MS023</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>MS024</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>MS019</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>MS020</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>pet15_cgoXF1</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>pet15_cgoXF2</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>pKOR1_cgoX_FattB</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
<tr>
<td>pKOR1_cgoX_RattB</td>
<td>GGGGACCACTTTTGTACAAAAAAGCAGGCTGATTTGCTTAGGACGTGCTGC</td>
</tr>
</tbody>
</table>