Supporting Information

This Supporting Information includes:

Supporting Experimental Section

Materials and General Methods. $HD5_{ox}$ was purified following overexpression of His_6-Met-HD5 in *E. coli* by a published procedure that affords the native 32-residue peptide with the correct disulfide bond linkages.[1]

AMA buffer and AMA media were prepared using Milli-Q water (18.2 $M\Omega cm^{-1}$) that was filtered through a 0.2-μm filter before use. LB, LB-Lennox, and trypticase soy broth (TSB) were prepared using de-ionized water. LB-Lennox broth was from Novagen. TSB, casamino acids, peptone and yeast extract for the preparation of LB broth were from Becton Dickinson. LPS ultrapure from *E. coli* K-12 was purchased from InVivogen. Colistin, vancomycin, and thiamine-HCl were purchased from Sigma. Folic acid and pantothenic acid were from Spectrum Chemicals. Nicotinamide, pyridoxal-HCl, and biotin were from Alfa Aesar. Riboflavin and chloramphenicol were from Calbiochem. Glucose and kanamycin were from VWR. Magnesium sulfate was from EMD.

The 2x AMA media employed for antimicrobial activity assays contained 10 mM potassium phosphate pH 7.4, 0.4 mM $MgSO_4$, 2% casamino acids, 2x vitamin mix (0.025% each of folic acid, pantothenic acid, nicotinamide, pyridoxal-HCl, thiamine-HCl and riboflavin; 0.05% biotin), 0.4% glucose.

The 1x AMA buffer employed in select antimicrobial activity assays contained 10 mM potassium phosphate pH 7.4 and 1% TSB.

The Keio Collection of strains and ASKA(-) strains were from the National Bio Resource Project at the National Institute of Genetics, Japan. The *imp4213* mutant strain and the corresponding WT strain (*E. coli* MC4100) were a gift from the laboratory of Prof. Daniel Kahne (Department of Chemistry and Chemical Biology, Harvard University). Primers for DNA sequencing were obtained from Integrated DNA Technologies and used as received (standard desalting protocol).

Acid-washed glass beads (425-600 μm) for cell lysis were from Sigma. The 96-metal-prong replicator was from Boekel Scientific. All OD_{600} and OD_{405} values were measured on a BioTek Synergy HT plate reader and processed with Gen 3.0 software. Microsoft Excel was used for data evaluation.

Antimicrobial Activity Assay Employed for Keio Screening. Strains were inoculated from agar plates or glycerol stocks in 150 μL of LB-Lennox (kanamycin 25 μg/mL) in a flat-bottom 96-well plate (Greiner Bio-One) using a 96-metal-prong replicator and incubated at 37 °C overnight. The next morning, an aliquot (1-2 μL) of overnight culture was transferred to 150 μL of low-salt LB (NaCl 50 mg/mL, kanamycin 25 μg/mL) in a flat-bottom 96-well plate (Greiner Bio-One) using the replicator. Low-salt LB was used because we observed that high salt concentrations inactivate $HD5_{ox}$, and replicator handling made it difficult to introduce a washing step to remove the salt-containing media. After incubation for 2 h at 37 °C, an aliquot (1-2 μL) of the culture was transferred with the replicator to 20 μL of sterile water in a 96-well U-bottom plate (Greiner Bio-One Cellstar), and immediately passed on to 50 μL of assay mixture in a sterile flat-bottom 96-well plate (polystyrene, Corning) using the replicator. The assay mixture consisted of 10 mM potassium phosphate pH 7.4 and 1% TSB with or without 8 μM $HD5_{ox}$. This concentration of $HD5_{ox}$ was selected after performing activity assays with four to eight randomly picked Keio mutants. At this concentration, the growth of all of the mutants was affected by $HD5_{ox}$ treatment. The plates were incubated at 37 °C for 1 h in a shaking incubator (150 rpm). After that time, a 50-μL aliquot of 2x AMA media (See General Materials and Methods) was added and the plates were sealed with parafilm. The plates were incubated first at 37 °C for 2-3 h, then at 30 °C overnight in a shaking incubator (150 rpm). The OD_{600} was measured 20 h after addition of the AMA media on a plate reader after stirring the cultures with the replicator.

Because the mutants exhibited varying fitness, different mutants reached different cell densities after the incubation time used in the AMA. Moreover, we observed that variations in cell density affect the antibacterial activity of $HD5_{ox}$. Because the screening was performed

using a 96-pin replicator, the amount of liquid and therefore the amount of cells for the actual AMA could not be adapted to the fitness of the mutant. To correct for variations in cell densities, screenings of large mutant libraries in liquid culture reported in the literature use the ratio of growth of the mutant treated with a certain compound to the growth of the untreated mutant.[2] As a result of the manual handling of the plates, we observed a day-to-day and plate-to-plate variation that resulted in different absolute OD_{600} values. This variation affects the calculated ratios and makes it difficult to define a definitive threshold for selecting sensitive strains. We therefore introduced a normalized fitness ratio, termed ϕ, which we define as the ratio of fitness of a mutant treated with $HD5_{ox}$ to the fitness of the untreated mutant. The fitness of a certain mutant is expressed relative to the mean OD_{600} measured for a plate (see Equations 1-3; t (treated, u (untreated)). This procedure normalizes the ratios and makes them independent of the absolute OD_{600} value, resulting in better consistency and higher reproducibility of the screen.

$$\text{fitness (t)} = \frac{OD_{600}\ \text{mutant (t)}}{OD_{600}\ \text{mean (t)}} \qquad \text{Equation 1}$$

$$\text{fitness (u)} = \frac{OD_{600}\ \text{mutant (u)}}{OD_{600}\ \text{mean (u)}} \qquad \text{Equation 2}$$

$$\phi\ \text{(mean)} = \frac{\text{fitness (t)}}{\text{fitness (u)}} = \frac{OD_{600}\ \text{mutant (t)}}{OD_{600}\ \text{mutant (u)}} \times \frac{OD_{600}\ \text{mean (u)}}{OD_{600}\ \text{mean (t)}} \qquad \text{Equation 3}$$

An example for a screening result and calculation of ϕ values of one plate of the Collection can be found in Figure S1 (Plate 1 of the Keio Collection).

The screening was first performed employing the AMA described above for Plates 1, 3, 7, and 9 of the Keio Collection (arbitrarily selected) to determine a reasonable threshold of ϕ value for selecting mutants for further testing. Subsequently, plates 1-9 were each screened

twice without eliminating any strains and the ϕ values were calculated for each strain using Equation 3.

Strains with low ϕ values were re-tested one or two times using an AMA adapted for the different growth of the mutants by introducing a pipetting step (described below, "AMA with normalized cell count"). From these preliminary experiments, strains with $\phi < 0.7$ repeatedly showed hypersensitivity. Therefore, the threshold for selecting a mutant as hypersensitive was set to $\phi \leq 0.7$ (Table S1). .

After completing the test screening, the entire Keio Collection was screened using the procedure described above. The screening was carried out in three iterative rounds, while decreasing the number of strains after each round by eliminating strains that exhibited ϕ values above the threshold of 0.7. For the first screening round, the set threshold of 0.7 was not regarded as strictly, and mutants with a ϕ-value above 0.7 but below 0.8 were also selected for further testing. Some strains could not be re-grown to log-phase from the overnight culture in the timeframe of 2 h (due to growth defects). For these problematic strains, the screening was repeated with an extended incubation time from 2 h to 2 h and 30 min to ensure that slow growing strains reached log-phase growth following dilution of the overnight culture (*vide supra*).

In total, out of 3985 strains screened, 367 mutants showed hypersensitivity towards $HD5_{ox}$ after the first screening round and 188 mutants could not be re-grown. The screening procedure was repeated for those mutants employing the longer incubation time. After the second round, 166 mutants showed a mean ϕ value of the two rounds ≤ 0.7. Out of those 166 mutants, 159 mutants were subjected to a third screening round (seven hypersensitive mutants were identified from plates 1, 3, 5, 7 and 9 and tested separately (see Table S1)). After the third screening round, ninety mutants were identified as hypersensitive to $HD5_{ox}$ based on the mean ϕ value of all three screening rounds (Table S2) to give a total of ninety-seven hypersensitive mutants.

Antimicrobial Activity Assay with Normalized Cell Count. As a first confirmation of the ninety-seven mutants obtained as hypersensitive hits from Keio screening, two more rounds

of screening without elimination were carried out. At this stage, as the number of strains to be handled was small enough to allow for correction of the growth of each individual mutant, an adapted AMA was employed. In this AMA, a pipetting step was introduced to normalize the cell count of different strains in a way that approximately the same number of cells was exposed to $HD5_{ox}$.

Strains were inoculated in 150 μL of LB Lennox (kanamycin 25 μg/mL) in 96-well flat-bottom plates (Greiner Bio-One). The cultures were grown overnight at 37 °C without shaking. The next morning, a 2-μL portion of each culture was added to 200 μL of LB (NaCl 50 mg/L, kanamycin 25 μg/mL) in a 96-well flat-bottom plate (Greiner Bio-One) using a pipette. The diluted cultures were incubated at 37 °C on a shaker (120 rpm). After 2 h and 10 min, the OD_{600} was measured on the plate reader (OD_{600} ~ 0.1 to 0.2). The log-phase cultures were immediately diluted 100 times in 2x AMA buffer (20 mM K_3PO_4 pH7 / 2% TSB) in two steps (20 μL mixed with 180 μL of 2 x AMA buffer). The equivalent of 50 μL of culture with an OD_{600} of 0.1 was diluted well-by-well with a pipette according to the measured OD_{600} to 100 μL with 2x AMA buffer (in total 200x dilution). A 25-μL portion of each of the normalized diluted cultures was added to 25 μL of 16 μM $HD5_{ox}$ or 25 μL of water in a 96-well flat-bottom plate (polystyrene, Costar). The plates were incubated at 37 °C (120 rpm). After 1 h, a 50-μL aliquot of 2x AMA medium was added to each well. The plates were again incubated on the shaker at 37 °C for 2.5 h, and were then incubated at 30 °C overnight. The OD_{600} was measured the next day after stirring the cultures with the replicator.

ϕ values were calculated using Equations 1-3. After the two additional AMA rounds, thirty-one out of the ninety-seven tested mutants consistently showed hypersensitivity to $HD5_{ox}$ (Table S2).

Data Analysis Using the EcoCyc Web Resource. The thirty-one genes identified in the screen were uploaded to the ecocyc.org webpage.[3] The genes were analyzed regarding enrichments (pathways and all gene ontology terms) using Fisher's Exact test without

correction. The obtained enriched groups and p-values can be found in Table S3 (the fifty groups with the lowest p-values are shown).

CFU Measurements to Confirm Hits from the Screen and Test Additional Strains of Interest. Mutants of interest were evaluated using an AMA with adapted cell density to eliminate false positives from the screen (e.g. slow growing mutants). As the number of mutants was small, the parent strain for the Keio mutants (*E. coli* BW25113, Thermo Fisher Scientific) was included as a reference, and ϕ values were calculated using the WT as reference instead of the mean value according to Equations 4-6.

$$\text{fitness (t)} = \frac{\text{CFU mutant (t)}}{\text{CFU WT (t)}} \qquad \text{Equation 4}$$

$$\text{fitness (u)} = \frac{\text{CFU mutant (u)}}{\text{CFU WT (u)}} \qquad \text{Equation 5}$$

$$\phi\ \text{(parent)} = \frac{\text{fitness (t)}}{\text{fitness (u)}} = \frac{\text{CFU mutant (t)}}{\text{CFU mutant (u)}} \times \frac{\text{CFU WT (u)}}{\text{CFU WT (t)}} \qquad \text{Equation 6}$$

E.coli K-12 Keio wild-type and mutants were grown overnight while shaking in 5 mL of TSB-dextrose with 25 μg/mL kanamycin (kanamycin was not used for the WT strain) in sterile 14-mL polypropylene culture tubes (VWR). The overnight culture was diluted 1:100 in 6 mL of fresh TSB-dextrose in sterile 14-mL polypropylene culture tubes (VWR), and grown while shaking until OD_{600} 0.5 was reached (ca. 2 h). Cultures were then centrifuged (3500 rpm for 10 min at 4 °C) to pellet the bacterial cells. The supernatant was discarded and the cell pellet was resuspended in 5 mL of 2x AMA buffer (20 mM sodium phosphate buffer supplemented with 2 % TSB, pH 7.4). The cell suspension was centrifuged again (3500 rpm for 10 min at 4 °C) and the supernatant discarded. The pellet was resuspended in 2x AMA buffer to an OD_{600} of 0.5.

The wild-type strain was further diluted 1:250 and the mutant strains diluted 1:200 in 2x AMA buffer to approximately 1.3 x 10^6 colony forming units (CFU)/mL.

Antimicrobial assays were performed in sterile polystyrene 96-well plates (Costar). Each well contained 25 μL of 2x (final concentrations of 16 μM, 8 μM, 4 μM, 2 μM, 1 μM, and 0 μM) sterile-filtered peptide stock (i.e. $HD5_{ox}$, colistin, or vancomycin) or a no-peptide control. Next, a 25-μL portion of each diluted bacterial culture was added to each well (6.25 x 10^5 CFU/mL final bacterial concentration). The plate was sealed with parafilm and incubated for 1 h (37°C, 150 rpm). Immediately after incubation, a 50-μL aliquot of 2x AMA media was added to each well. The plate was sealed with parafilm and incubated at 37 °C for 2 h (150 rpm). Following the 2-h incubation, the plate was incubated at 30 °C (150 rpm) overnight. Following the overnight incubation, wells were mixed by pipetting, and OD_{600} readings were taken using a plate reader. After OD_{600} measurements, a 20-μL aliquot was taken from each well and diluted with 180 μL of AMA buffer (10^{-1} dilution) in a sterile polystyrene 96-well plate (Costar). The solution was further diluted serially from 10^{-2}-10^{-6}. A 5-μL aliquot of each dilution and an undiluted sample were spotted onto prepared, pre-warmed trypticase soy agar (TSA) minus dextrose (Becton Dickinson) plates (VWR). The spots were allowed to dry, agar side up, for at least 30 min prior to being inverted and incubated at 37 °C for 13-15 h. The number of CFU obtained for each mutant and peptide treatment was determined by colony counting. Mean ϕ values of three independent measurements are shown in Figures S2 and S3.

Antimicrobial Activity Assay in Rich Media. To exclude the possibility that the observed hypersensitivity was due to reduced fitness of the mutants in minimal media, AMA assays with the WT and the two most sensitive mutants were performed in 50% Mueller-Hinton-Broth (MHB).

Strains were inoculated in 150 μL of LB Lennox (kanamycin 25 μg/mL) in 96-well flat-bottom plates (Greiner Bio-One). The cultures were grown overnight at 37 °C without shaking. The next morning, a 2-μL portion of each culture was added to 200 μL of LB (NaCl 50 mg/L, kanamycin 25 μg/mL) in a 96-well flat-bottom plate (Greiner Bio-One) using a pipette. The

diluted cultures were incubated at 37 °C on a shaker (120 rpm). After 2 h, the OD_{600} was measured on the plate reader (OD_{600} ~ 0.1 to 0.2). The log-phase cultures were immediately diluted 1000 times in MHB. The equivalent of 100 μL of culture with an OD_{600} of 0.1 was diluted well-by-well with a pipette according to the measured OD_{600} to 200 μL with MHB (in total 2000x dilution). A 25-μL portion of each of the normalized diluted cultures was added to 25 μL of 0, 8, 16, 32, and 64 μM $HD5_{ox}$ in water in a 96-well flat-bottom plate (polystyrene, Costar). The plates were incubated at 37 °C (120 rpm). After 1 h, a 5-μL aliquot was taken from each well and diluted with 45 μL of AMA buffer (10^{-1} dilution) in a sterile polystyrene 96-well plate (Costar). A 10-μL aliquot of the solution was further diluted serially with 90 μL of AMA buffer from 10^{-2}-10^{-4}. A 5-μL aliquot of each dilution and an undiluted sample were spotted onto prepared, pre-warmed trypticase soy agar (TSA) minus dextrose (Becton Dickinson) plates (VWR). The spots were allowed to dry, agar side up, for at least 30 min prior to being inverted and incubated at 37 °C for 13-15 h. The number of CFU obtained for each mutant and peptide treatment was determined by colony counting. Mean CFU of three independent measurements are shown in Figure S4.

Antimicrobial Activity Assay with the *imp4213* Mutant. Bacteria (*E. coli imp4213* mutant and WT MC4100) were grown overnight while shaking in 5 mL of media in sterile 14-mL polypropylene culture tubes (VWR). The overnight culture was diluted 1:100 in 6 mL of fresh media in sterile 14-mL polypropylene culture tubes (VWR), and grown while shaking until OD_{600} of 0.5 was reached. Cultures were then centrifuged (3500 rpm for 10 min at 4 °C) to pellet the bacterial cells. The supernatant was discarded and the cell pellet was resuspended in 5 mL of 1.1x AMA buffer (11 mM sodium phosphate buffer supplemented with 1.1% TSB, pH 7.4). The cell suspension was centrifuged again (3500 rpm for 10 min at 4 °C) and the supernatant discarded. The pellet was resuspended in 4 mL of 1.1x AMA buffer and further diluted with 1.1x AMA buffer to obtain an OD_{600} of 0.5. The bacterial suspension were further diluted in 1.1x AMA buffer 1:200 and used immediately.

Antimicrobial assays were performed in sterile polystyrene 96-well plates (Costar). Each well contained 10 μL of 10x sterile-filtered $HD5_{ox}$ or a no-peptide control. A 90-μL portion of diluted bacterial culture was added to each well. The plate was sealed with parafilm and incubated for 1 h (37 °C, 150 rpm). Immediately after incubation, a 20-μL aliquot was taken from each well and diluted with 180 μL of 1x AMA buffer (10^{-1} dilution) in another sterile polystyrene 96-well plate (Costar). The solution was further diluted serially from 10^{-2}-10^{-4}. A 5-μL portion of each dilution and an undiluted sample were spotted onto prepared, pre-warmed TSA minus dextrose (Becton Dickinson) plates (VWR). The spots were allowed to dry, agar side up, for at least 30 min prior to being inverted and incubated at 37 °C for 13-15 h. The number of colony forming units (CFU) was determined by colony counting. All assays were plated in triplicate in three independent trials.

Complementation of Keio Strains with ASKA(-) Clones. Electrocompetent Keio strains were first prepared. The Keio strains were grown overnight in 170 μL of TSB (kanamycin 25 μg/mL). The overnight cultures were diluted 1:100 in 10 mL of LB-Lennox (kanamycin 25 μg/mL) and grown to log-phase (2 h 15 min at 37 °C). The cultures were cooled on ice for 5 min. All subsequent centrifugation steps were performed at 3600 rpm and 4 °C (unless noted otherwise), and the cultures were kept on ice, and supernatants were removed by decanting. First, the cultures were washed once with 10 mL of ice-cold Milli-Q water, then twice with ice-cold10% (v/v) glycerol in Milli-Q water. The cell pellets were gently resuspended in 1 mL of 10% glycerol and transferred to microcentrifuge tubes. After centrifugation (4000 rpm, 5 min, 4 °C), the pellets were suspended in 200 μL of 10% glycerol, and two 100-μL aliquots of each strain were frozen on dry ice and stored at -80 °C.

For electroporation, one aliquot of the electrocompetent cells was allowed to thaw on ice. A 3-μL portion of the isolated plasmid harboring the corresponding ASKA(-) clone was added to the cells and the mixture was incubated for 15 min on ice. After incubation, the cells were carefully transferred to a chilled 2-mm electroporation cuvette (VWR). Electroporation was performed with the *E. coli* settings of the BioRad Electroporator (2.5 kV). Immediately after the

pulse, a 700-μL aliquot of SOC-LB media (warmed to 37 °C on a heating block) was added to the cuvette. The cells were transferred to a microcentrifuge tube, and were incubated at 37 °C for 45 min. After that incubation time, a 100-μL portion was spread on selective LB Lennox plates (kanamycin and chloramphenicol, 25 μg/mL each). The plates were dried and incubated overnight at 37 °C.

To confirm correct complementation, one colony from each electroporpation was picked from a transformation plate (see above) and inoculated in 2 mL of LB-Lennox (kanamycin and chloramphenicol, 25 μg/mL each). Plasmids were isolated from the overnight cultures using the Omega E.Z.N.A. DNA Kit, and the plasmids were eluted by using 50 μL of Milli-Q water. Plasmid identities were confirmed by restriction digest (*Sfi*I, New England Biolabs) and DNA sequencing (MIT Biopolymers Facility). The sequencing primer, 5'-CCT TTC GTC TTC ACC TCG AGA AAT-3', was designed for the pCA24N plasmid using Geneious Primer3

The AMA with the complemented strains and the corresponding mutants as comparison was performed as described above (AMA with Normalized Cell Count). ϕ values were calculated using the WT as the reference according to Equations 7-9.

$$\text{fitness (t)} = \frac{OD_{600}\ \text{mutant (t)}}{OD_{600}\ \text{WT (t)}} \qquad \text{Equation 7}$$

$$\text{fitness (u)} = \frac{OD_{600}\ \text{mutant (u)}}{OD_{600}\ \text{WT (u)}} \qquad \text{Equation 8}$$

$$\phi\ \text{(parent)} = \frac{\text{fitness (t)}}{\text{fitness (u)}} = \frac{OD_{600}\ \text{mutant (t)}}{OD_{600}\ \text{mutant (u)}} \times \frac{OD_{600}\ \text{WT (u)}}{OD_{600}\ \text{WT (t)}} \qquad \text{Equation 9}$$

Antimicrobial Activity Assay with Addition of LPS. An overnight culture of the Keio WT (*E. coli* BW25113) in LB-Lennox was diluted 100x in low-salt LB (NaCl 50 mg/L), and incubated for 2 h at 37 °C. The log-phase culture was diluted 200x in 1x AMA buffer (10 mM potassium phosphate pH 7.4 containing 1 % TSB). A dilution series of high-purity LPS (0 to 150 μg/mL) was incubated at 37 °C for 10 min with increasing concentrations of either $HD5_{ox}$ or vancomycin from 0 to 8 μM, or colistin from 0 to 9.2 μg/mL in 1x AMA buffer prior to addition of 25 μL of diluted cells. The mixture of cells, antibiotic, and LPS was incubated at 37 °C for 2 h on a shaker (150 rpm). After that time, 10 μL of each well of the LPS dilutions for 8 μM $HD5_{ox}$, 2.3 μg/ml of colistin, and 4 μM vancomycin were used for colony forming units (CFU) measurements (the cells were diluted in steps of 1:10 until 10^{-6}). The plates were incubated at 37 °C overnight, and the colonies were counted the next morning.

LAL Assay. The Thermo Pierce LAL Endotoxin Quantitation kit was used. LPS from *E. coli* was provided with the kit. The assay was performed following the instructions detailed in the manual provided with the kit with the following modifications: Colistin at 0, 2, 4, 8, 16 μg/mL (final concentrations, 25 μL) and vancomycin/ $HD5_{ox}$ at 0, 8, 16, 32, 64 μM (final concentrations, 25 μL) in 1x reaction buffer (10 mM Tris-HCl pH 7.5) were mixed with 25 μL of LPS (1.0 EU/mLof LPS final) in 1x reaction buffer in a 96-well plate. Standards and samples were incubated at 37 °C at 150 rpm for 30 min. After that time, a 25-μL portion of LAL was added, and the mixture was incubated for 10 min at 37 °C without shaking. A 50-μL portion of chromogenic substrate was added per well, and the plate was incubated for 6 min at 37 °C without shaking. The reaction was stopped by addition of 25 μL of 25% (v/v) acetic acid, and the absorbance at 405 nm was measured on a plate reader. Free LPS was calculated using an LPS standard curve and converted to bound LPS (bound LPS = 1.0 EU/mL – free LPS).[4]

Phase-contrast Microscopy. For cellular morphology studies, overnight cultures of *E. coli* BW25113 were grown in LB and the mutant strains were grown in LB supplemented with kanamycin (25 μg/mL) at 37 °C. The strains were diluted 1:100 in the respective media and

grown to a final OD_{600} of 0.6. The cells were centrifuged and resuspended twice in 1x AMA buffer (10 mM sodium phosphate buffer pH 7.4 containing 1% TSB). The cells were then diluted to 10^8 CFU/mL in 1x AMA buffer. The diluted bacterial cultures (90 μL) were treated with either a 10-μL portion of 400 μM of $HD5_{ox}$ or Milli-Q water for 1 h at 37 °C with shaking at 150 rpm. For imaging, a 5-μL aliquot of each resulting culture was gently placed on a 1% agarose pad and topped with a glass coverslip. The samples were imaged immediately using a Zeiss AxioPlan2 up-right microscope equipped with Hamamatsu Orca-ER camera outfitted with an 100x oil-immersion objective (1.3 NA PH3) housed in the W. M. Keck Microscopy Facility (Whitehead Institute) . The MetaMorph software package was employed for image acquisition, and the images were subsequently processed using Image J (Figure S6).

Supporting Tables.

Table S1. Screening of Plates 1, 3, 5, 7, and 9 of the Keio Collection.

Gene	ϕ1[a]	ϕ2[a]	ϕ3[b]	ϕ4[b]	Mean (ϕ3,ϕ4)	Mean (ϕ1-ϕ4)
acnB	0.47	0.71	0.72	1.05	0.89	0.74
atoS	0.82	0.87				
cpxA	0.54	0.5	0.81	0.74	0.78	0.65
cusB	0.61	0.67	0.76	0.84	0.80	0.72
eutT	0.53	0.94				
fbp	0.57	1.03				
fimD	0.64	0.44	0.99	0.97	0.98	0.76
glnG	0.86	0.7	0.87	1.14	1.01	0.89
htpX	0.45	0.89				
ihfA	0.33	0.74	1.14			
nuoI	0.33	1.11				
recA	0.6	0.91				
rpe	0.34	1.08				
rssB	0.68	0.52	0.53	0.54	0.54	0.57
speE	0.54	0.62	1.03	0.6	0.82	0.70
sucA	0.25	0.75				
sucB	0.54	1.01				
sucC	0.6	1.06				
tpiA	0.67	0.63	0.71	0.9	0.81	0.73

[a] ϕ1 and ϕ2 were calculated using Equations 1-3. [b] ϕ3, ϕ4 AMA with normalized cell count, calculated using Equations 7-9.

Table S2. Screening of the Keio Collection of Strains.

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
abgT	0.72	0.67	0.70	0.77	0.72				
argO	0.69	0.72	0.71						
aroB	0.63	0.47	0.55	2.36	1.15				
aroC	0.58	1.13	0.86						
aroE	0.36	1.47	0.92						
aroH	0.52	0.74	0.63	1.17	0.81				
aroK	0.72	0.84	0.78						

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
aspA	0.67	0.91	0.79						
atpB	0.67	0.49	0.58	0.62	0.59	1.22	1.39	1.31	0.88
atpC	0.61	0.39	0.50	0.65	0.55	0.69	0.73	0.71	0.61
atpE	0.46	0.41	0.44	0.61	0.49	1.47	1.43	1.45	0.88
atpF	0.73	0.92	0.83						
azoR	0.59	0.77	0.68	0.88	0.75				
bacA	0.79	1.12	0.96						
bcsC	0.65	1.3	0.98						
betT	0.7	1.09	0.90						
bipA	0.68	0.94	0.81						
cheZ	0.46	0.87	0.67	0.82	0.72				
chpB	0.6	0.83	0.72						
citG	0.55	1	0.78						
cmk	0.44	0.32	0.38	1.24	0.67	1.08	1.13	1.11	0.84
cobU	0.35	1.44	0.90						
cpsG	0.22	1.09	0.66	1.09	0.80				
csgC	0.69	0.66	0.68	0.78	0.71				
cspA	0.71	1.01	0.86						
cyaA	0.39	1.13	0.76						
cybB	0.74	1.1	0.92						
cydD	0.69	1.09	0.89						
dam	0.41	0.48	0.45	0.6	0.50	1.04	1.3	1.17	0.77
dcd	0.4	0.82	0.61	1.02	0.75				
dcuC	0.56	0.72	0.64	0.52	0.60	0.94	1.05	1.00	0.76
dcuC	0.71	0.82	0.77						
ddpX	0.44	0.36	0.40	0.96	0.59	0.94	1.08	1.01	0.76
deaD	0.51	0.93	0.72						
dgkA	0.56	0.97	0.77						
dipZ	0.57	1.04	0.81						
dksA	0.64	0.73	0.69	1.23	0.87				
dnaK	0.45	0.98	0.72						
dppB	0.62	1.06	0.84						
dsbB	0.6	0.76	0.68	0.81	0.72				
efp	0.54	0.36	0.45	1.07	0.66	1.12	1.23	1.18	0.86
entB	0.69	0.32	0.51	M	0.51	0.52	1.03	0.78	0.64
entF	0.38	0.79	0.59	1.36	0.84				
envC	0.76	0.58	0.67	1.11	0.82				
eutH	0.64	1.05	0.85						
fabH	0.53	0.36	0.45	0.84	0.58	0.8	0.7	0.75	0.65
fcl	0.37	1.21	0.79						
fdx	0.18	0.24	0.21	0.59	0.34	0.84	0.96	0.90	0.56

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
fepA	0.67	0.9	0.79						
fepB	0.4	1.29	0.85						
fepG	0.73	1.39	1.06						
fes	0.69	0.75	0.72						
fieF	0.62	0.74	0.68	1.28	0.88				
fimA	0.17	0.41	0.29	0.73	0.44	1.29	1.37	1.33	0.79
fimC	0.39	0.43	0.41	0.85	0.56	1.27	0.99	1.13	0.79
fimF	0.43	0.85	0.64	1.05	0.78				
fimH	0.29	0.95	0.62	0.99	0.74				
fimZ	0.77	1.31	1.04						
flgA	0.6	0.86	0.73						
flgB	0.58	1.19	0.89						
flgC	0.4	0.9	0.65	0.51	0.60	1.11	1.02	1.07	0.79
flgE	0.57	0.83	0.70	0.52	0.64	1.09	0.98	1.04	0.80
flgF	0.38	1.08	0.73						
flgG	0.55	0.95	0.75						
flgI	0.7	1.09	0.90						
flgJ	0.41	0.73	0.57	0.51	0.55	1.29	0.88	1.09	0.76
flgK	0.51	0.77	0.64	1.39	0.89				
flgL	0.73	0.69	0.71						
flhC	0.3	0.97	0.64	0.46	0.58	1.05	1.01	1.03	0.76
flhD	0.45	0.79	0.62	0.48	0.57	1.05	1.02	1.04	0.76
flhE	0.62	0.57	0.60	0.93	0.71				
flic	0.6	0.87	0.74						
fliE	0.39	1.27	0.83						
fliG	0.22	0.78	0.50	0.87	0.62	0.97	0.96	0.97	0.76
fliH	0.58	0.24	0.41	0.69	0.50	1.04	0.94	0.99	0.70
fliJ	0.31	0.94	0.63	0.73	0.66	0.97	0.84	0.91	0.76
fliM	0.55	0.74	0.65	0.6	0.63	1.16	0.95	1.06	0.80
fliN	0.61	0.9	0.76						
fliO	0.39	0.95	0.67	1.05	0.80				
fliR	0.46	0.73	0.60	0.75	0.65	0.99	0.91	0.95	0.77
frlA	0.68	0.92	0.80						
fur	0.52	1.17	0.85						
galE	0.43	0.98	0.71						
galU	0.24	1.24	0.74						
garD	0.56	0.93	0.75						
gcvR	0.57	0.52	0.55	0.45	0.51	0.77	0.81	0.79	0.62
glyA	0.28	0.43	0.36	2.05	0.92				
gmhB	0.54	0.6	0.57	0.8	0.65	0.34	0.5	0.42	0.56
gnsB	0.68	0.87	0.78						

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
gntK	0.73	1.47	1.10						
hda	0.74	1.21	0.98						
hipB	0.6	0.6	0.60	1.14	0.78				
hlpA	0.23	0.43	0.33	0.76	0.47	0.54	0.42	0.48	0.48
holC	0.75	1.05	0.90						
holD	0.44	0.81	0.63	0.7	0.65	0.82	1.01	0.92	0.76
hscA	0.53	0.87	0.70	0.86	0.75				
hscB	0.68	1.34	1.01						
htrC	0.67	0.69	0.68	0.72	0.69	1.15	1.05	1.10	0.86
hybF	0.67	0.96	0.82						
idnD	0.59	1.63	1.11						
iscS	0.46	1.16	0.81						
ivy	0.64	1.18	0.91						
kdgT	0.72	1.49	1.11						
ksgA	0.49	1.09	0.79						
lipA	0.28	0.44	0.36	1.78	0.83				
lipB	0.65	1.39	1.02						
lit	0.79	1.24	1.02						
lpcA	0.24	0.24	0.24	0.74	0.41	0.29	0.3	0.30	0.36
lpxL	0.67	1.05	0.86						
malE	0.69	1.2	0.95						
marB	0.64	0.76	0.70	0.84	0.75				
mdtF	0.79	0.78	0.79						
menD	0.53	1.2	0.87						
mglB	0.29	0.23	0.26	0.87	0.46	1.01	1.09	1.05	0.70
mhpB	0.63	0.6	0.62	0.95	0.73				
miaA	0.59	1.1	0.85						
mog	0.24	0.83	0.54	1.21	0.76				
molR	0.52	1.06	0.79						
mraW	0.54	0.95	0.75						
mutL	0.6	1.36	0.98						
nagZ	0.54	1.51	1.03						
narW	0.53	0.96	0.75						
ndk	0.49	0.78	0.64	0.92	0.73				
nohA	0.65	0.67	0.66	1.07	0.80				
paaY	0.76	0.99	0.88						
pdxB	0.44	0.77	0.61	0.71	0.64	0.96	0.91	0.94	0.76
pdxH	0.71	0.42	0.57	0.62	0.58	0.81	0.65	0.73	0.64
pfkB	0.56	1.18	0.87						
potB	0.66	0.82	0.74						
pphA	0.46	1.3	0.88						

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
ppiB	0.44	0.32	0.38	0.86	0.54	1.11	0.81	0.96	0.71
proQ	0.63	1.35	0.99						
proW	0.35	0.91	0.63	0.72	0.66	0.76	0.74	0.75	0.70
pspA	0.68	0.97	0.83						
pstS	0.66	0.86	0.76						
purN	0.51	0.71	0.61	1.42	0.88				
putP	0.72	0.8	0.76						
rbsA	0.68	0.49	0.59	0.9	0.69	1.14	1.47	1.31	0.94
rbsC	0.67	0.73	0.70	1.02	0.81				
rcsF	0.68	1.17	0.93						
recB	0.38	0.32	0.35	0.97	0.56	0.77	0.55	0.66	0.60
recC	0.56	0.63	0.60	0.91	0.70	0.71	0.52	0.62	0.67
recF	0.55	0.57	0.56	0.87	0.66	0.95	1.23	1.09	0.83
recO	0.63	0.65	0.64	1.47	0.92				
rem	0.63	0.6	0.62	1.62	0.95				
rep	0.3	0.7	0.50	0.95	0.65	0.97	1.09	1.03	0.80
rfaC	0.39	1.15	0.77						
rfaD	0.22	0.33	0.28	0.81	0.45	0.32	0.28	0.30	0.39
rfaE	0.2	0.21	0.21	0.58	0.33	0.25	0.31	0.28	0.31
rfaF	0.2	0.31	0.26	0.67	0.39	0.3	0.29	0.30	0.35
rfaG	0.31	0.72	0.52	0.81	0.61	1.16	0.87	1.02	0.77
rfe	0.56	0.84	0.70	0.93	0.78				
rffA	0.23	0.47	0.35	0.58	0.43	0.77	0.64	0.71	0.54
rffC	0.29	0.73	0.51	0.69	0.57	1.07	1.14	1.11	0.78
rffD	0.35	0.52	0.44	0.64	0.50	1.48	1.41	1.45	0.88
rffH	0.48	0.4	0.44	0.81	0.56	1.15	0.84	1.00	0.74
rffM	0.22	0.41	0.32	0.58	0.40	0.82	1.05	0.94	0.62
rffT	0.39	0.7	0.55	1.34	0.81				
rhsB	0.56	0.88	0.72						
rimJ	0.6	0.82	0.71						
rimK	0.61	1.26	0.94						
rluA	0.72	1.26	0.99						
rluD	0.66	0.8	0.73						
rnr	0.51	0.76	0.64	0.94	0.74				
rpmF	0.6	1.26	0.93						
rpmJ	0.61	0.54	0.58	1.22	0.79				
rseA	0.57	0.67	0.62	0.63	0.62	1.25	1.06	1.16	0.84
rspA	0.62	1.58	1.10						
ruvA	0.61	1.07	0.84						
rzpD	0.71	1.66	1.19						
sapB	0.68	1.06	0.87						

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
secB	0.36	0.73	0.55	0.67	0.59	0.53	0.37	0.45	0.53
sfmD	0.42	1.09	0.76						
sfmF	0.77	0.84	0.81						
sgcC	0.63	1.09	0.86						
sgcQ	0.59	0.78	0.69	0.67	0.68	1.04	0.94	0.99	0.80
sgrR	0.46	1.05	0.76						
slyA	0.7	0.66	0.68	1.55	0.97				
smpA	0.38	0.67	0.53	0.5	0.52	0.78	0.65	0.72	0.60
speA	0.66	M	0.66	1.3	0.98				
speB	0.75	0.91	0.83						
spr	0.61	0.76	0.69	0.73	0.70	1.03	0.71	0.87	0.77
spy	0.56	1.52	1.04						
srlD	0.66	0.82	0.74						
stfR	0.67	0.59	0.63	0.68	0.65	0.94	1.18	1.06	0.81
sufB	0.61	0.57	0.59	0.65	0.61	1.31	1	1.16	0.83
sufD	0.58	0.94	0.76						
surA	0.38	0.48	0.43	0.87	0.58	0.29	0.37	0.33	0.48
tatB	0.65	1.09	0.87						
tauD	0.3	1.31	0.81						
tgt	0.67	1.01	0.84						
thrL	0.67	0.61	0.64	1.34	0.87				
tnaB	0.56	0.74	0.65	0.61	0.64	0.96	0.95	0.96	0.76
tolA	0.54	1.61	1.08						
tolB	0.41	0.5	0.46	M	0.46	0.36	0.79	0.58	0.52
tolQ	0.25	M	0.25	0.93	0.59	0.29	0.7	0.50	0.54
trkH	0.72	1.12	0.92						
trmC	0.56	0.77	0.67	0.58	0.64	0.71	0.84	0.78	0.69
trpA	0.35	0.97	0.66	1.64	0.99				
trpB	0.52	0.83	0.68	1.73	1.03				
trpD	0.67	1.23	0.95						
tufA	0.75	0.9	0.83						
tynA	0.62	0.95	0.79						
ubiE	0.48	2.12	1.30						
ubiX	0.46	1.14	0.80						
ulaE	0.62	0.79	0.71						
ulaF	0.48	0.62	0.55	0.74	0.61	0.89	0.91	0.90	0.73
usg	0.6	0.66	0.63	0.82	0.69	0.9	1.07	0.99	0.81
uvrD	0.14	1.4	0.77						
wbbK	0.63	0.94	0.79						
wbbL	0.49	0.85	0.67	0.71	0.68	0.9	1.07	0.99	0.80
wcaF	0.37	0.4	0.39	1.2	0.66	0.94	1.48	1.21	0.88

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
wcaI	0.6	1.45	1.03						
wzxE	0.33	1.27	0.80						
xerC	0.47	1.89	1.18						
xerD	0.53	0.72	0.63	0.96	0.74				
yadN	0.59	1.43	1.01						
yafX	0.65	0.61	0.63	0.95	0.74				
yafY	0.73	1.25	0.99						
yafZ	0.54	1.23	0.89						
yagF	0.54	1.07	0.81						
yahD	0.74	1	0.87						
yahM	0.76	1.02	0.89						
yajC	0.64	0.35	0.50	0.42	0.47	0.74	0.75	0.75	0.58
ybaK	0.26	1.1	0.68	0.76	0.71				
ybcD	0.55	0.83	0.69	0.82	0.73				
ybdZ	0.73	1.46	1.10						
ybeB	0.6	0.81	0.71						
ybeQ	0.7	1.33	1.02						
ybeT	0.53	0.53	0.53	0.64	0.57	0.75	0.88	0.82	0.67
ybeU	0.5	1.02	0.76						
ybfF	0.53	1.04	0.79						
ybgC	0.25	1	0.63	0.98	0.74				
ybgH	0.29	0.84	0.57	1.32	0.82				
ybhR	0.6	0.95	0.78						
ybiC	0.55	1.2	0.88						
ybiJ	0.28	1.59	0.94						
ybiS	0.55	0.93	0.74						
ybiX	0.73	1.57	1.15						
ybjD	0.65	0.94	0.80						
ycaI	0.74	1.38	1.06						
ycaM	0.48	0.55	0.52	0.55	0.53	1.03	1.04	1.04	0.73
ycbJ	0.64	1.04	0.84						
yccC	0.67	1.62	1.15						
ycdB	0.25	0.87	0.56	1.06	0.73				
ycdG	0.68	0.55	0.62	0.82	0.68	1.23	0.95	1.09	0.85
ycdM	0.73	0.87	0.80						
yceD	0.25	1.07	0.66	1.08	0.80				
yceI	0.51	1.29	0.90						
ycfD	0.56	1.43	1.00						
ycfK	0.56	1.18	0.87						
ycfM	0.63	0.54	0.59	1.91	1.03				
ycfQ	0.72	0.84	0.78						

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
ycfS	0.7	1.05	0.88						
ycgM	0.6	1.63	1.12						
ycgV	0.52	1.01	0.77						
ychN	0.33	1.52	0.93						
yciA	0.68	1.21	0.95						
yciB	0.5	1.31	0.91						
yciI	0.21	1.17	0.69	0.94	0.77				
yciM	0.31	M	0.31	M					
yciT	0.42	0.86	0.64	1.37	0.88				
ycjU	0.58	1.57	1.08						
ydbJ	0.71	1.01	0.86						
ydcA	0.51	0.8	0.66	1.11	0.81				
ydcD	0.64	0.86	0.75						
ydcF	0.66	1.36	1.01						
ydcK	0.64	0.64	0.64	1.13	0.80				
ydcL	0.76	1.17	0.97						
ydcN	0.36	1.35	0.86						
ydcQ	0.66	1.45	1.06						
ydcY	0.65	1.17	0.91						
ydcZ	0.6	1.13	0.87						
yddB	0.69	0.97	0.83						
yddE	0.24	1.39	0.82						
yddK	0.5	0.43	0.47	0.83	0.59	0.94	1.09	1.02	0.76
ydeH	0.53	0.93	0.73						
ydeJ	0.48	1.73	1.11						
ydfC	0.68	0.78	0.73						
ydfH	0.58	1.08	0.83						
ydfO	0.67	0.96	0.82						
ydfW	0.57	0.7	0.64	0.91	0.73				
ydhW	0.69	0.73	0.71						
ydiK	0.61	0.75	0.68	0.74	0.70	0.85	1.03	0.94	0.80
ydiN	0.59	0.52	0.56	0.56	0.56	0.95	1	0.98	0.72
ydiP	0.56	1.1	0.83						
ydjJ	0.48	1.27	0.88						
yebG	0.51	0.7	0.61	1.12	0.78				
yebT	0.54	1.52	1.03						
yebU	0.46	0.69	0.58	1.03	0.73				
yecF	0.52	0.81	0.67	0.7	0.68				
yecH	0.69	0.67	0.68	1.11	0.82				
yecJ	0.69	0.85	0.77						
yecS	0.69	0.94	0.82						

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
yedD	0.65	1.08	0.87						
yedE	0.62	0.75	0.69	1.46	0.94				
yedK	0.61	0.72	0.67	0.76	0.70	1.13	1.2	1.17	0.88
yedN	0.39	0.56	0.48	1.4	0.78				
yedS	0.6	1.66	1.13						
yeeN	0.5	1.53	1.02						
yehD	0.65	1	0.83						
yehL	0.4	0.57	0.49	1.09	0.69	1.01	0.97	0.99	0.81
yeiP	0.49	0.43	0.46	0.88	0.60	0.7	0.88	0.79	0.68
yeiS	0.55	0.32	0.44	0.84	0.57	1.22	1.2	1.21	0.83
yeiT	0.66	1.26	0.96						
yfbG	0.61	1.02	0.82						
yfdG	0.33	0.35	0.34	0.57	0.42	0.79	0.79	0.79	0.57
yfeS	0.72	0.92	0.82						
yfhD	0.47	0.82	0.65	0.78	0.69	0.95	1.29	1.12	0.86
yfiC	0.63	0.93	0.78						
yfjU	0.28	1.22	0.75						
yfjX	0.72	0.59	0.66	0.89	0.73				
ygaY	0.66	0.8	0.73						
ygbF	0.47	0.53	0.50	0.81	0.60	0.75	0.78	0.77	0.67
ygcF	0.29	1.6	0.95						
ygcL	0.55	0.61	0.58	0.71	0.62	1.21	1.21	1.21	0.86
ygcO	0.37	0.82	0.60	0.95	0.71				
ygcP	0.23	1.49	0.86						
ygeN	0.69	0.78	0.74						
ygfU	0.45	1.67	1.06						
yggM	0.58	0.58	0.58	0.51	0.56	0.81	0.98	0.90	0.69
ygiE	0.23	0.78	0.51	0.94	0.65	1.17	1.24	1.21	0.87
ygiH	0.64	0.69	0.67	0.86	0.73				
ygiP	0.63	0.91	0.77						
yhbS	0.29	0.7	0.50	1.28	0.76				
yheL	0.79	0.65	0.72						
yhhF	0.76	0.46	0.61	0.84	0.69	1.04	1.04	1.04	0.83
yhiR	0.75	0.51	0.63	0.66	0.64	0.96	1.11	1.04	0.80
yhjK	0.4	1.14	0.77						
yicH	0.78	1.39	1.09						
yieK	0.43	1.19	0.81						
yihN	0.66	0.9	0.78						
yjbA	0.45	M	0.45	0.9	0.68				
yjbR	0.67	0.89	0.78						
yjeH	0.67	0.85	0.76						

Gene	ϕ1[a]	ϕ2[a]	Mean (ϕ1,ϕ2)	ϕ3[a]	Mean (ϕ1,ϕ2,ϕ3)	ϕ4[b]	ϕ5[b]	Mean (ϕ4,ϕ5)	Mean (ϕ1-ϕ5)
yjfJ	0.59	1.26	0.93						
yjfK	0.64	1.14	0.89						
yjfL	0.64	1.12	0.88						
yjfO	0.67	1.26	0.97						
yjgF	0.5	0.6	0.55	0.78	0.63	1.05	0.71	0.88	0.73
yjiQ	0.65	1.22	0.94						
yjjN	0.73	0.95	0.84						
yjjU	0.66	0.9	0.78						
ykgB	0.64	0.7	0.67	0.72	0.69	0.88	0.94	0.91	0.78
ykgG	0.71	0.78	0.75						
ylaB	0.7	1.19	0.95						
ylaC	0.76	0.82	0.79						
ylcG	0.35	0.67	0.51	0.76	0.59	0.67	0.6	0.64	0.61
ymgD	0.74	1.14	0.94						
ymgE	0.52	0.87	0.70	0.62	0.67	0.98	1.16	1.07	0.83
yncE	0.64	1.62	1.13						
yneF	0.39	0.74	0.57	0.79	0.64	1.19	1.14	1.17	0.85
ynfA	0.28	0.32	0.30	0.86	0.49	0.56	0.76	0.66	0.56
ynfC	0.69	0.57	0.63	0.55	0.60	1.11	1.05	1.08	0.79
ynfO	0.75	0.94	0.85						
ynjE	0.43	0.98	0.71						
yobD	0.51	1.08	0.80						
yoeB	0.7	0.7	0.70	1.02	0.81				
yojL	0.71	0.36	0.54	1.26	0.78				
ypfE	0.73	0.85	0.79						
yqeJ	0.66	0.75	0.71						
yqhC	0.7	0.98	0.84						
yrbE	0.75	0.88	0.82						
yrbF	0.6	0.89	0.75						
yrhB	0.78	0.57	0.68	0.82	0.72				
ytfH	0.68	1.15	0.92						

[a] ϕ1, ϕ2 and ϕ3 were obtained from the three individual screening rounds and calculated using Equations 1-3. [b] ϕ4 and ϕ5 result from the AMA with normalized cell count and were calculated using Equations 1-3. Mutants are listed in alphabetical order.

Table S3. Analysis of Hypersensitive Mutants for Enrichments Using the EcoCyc Web Resource.

Pathways Gene-Ontology-Terms All-Genes	p-values	Matches
ADP-L-glycero-beta-manno-heptose biosynthesis	1.84E-09	lpcA // "rfaD" // "gmhB" // "rfaE"
ADP-sugar Biosynthesis	1.84E-09	lpcA // "rfaD" // "gmhB" // "rfaE"
ADP-L-glycero-beta-D-manno-heptose biosynthetic process	2.97E-07	gmhB // "rfaD" // "rfaE"
ADP-L-glycero-beta-D-manno-heptose metabolic process	2.97E-07	gmhB // "rfaD" // "rfaE"
lipopolysaccharide core region biosynthetic process	5.27E-07	rfaD // "waaF" // "lpcA" // "gmhB" // "rfaE"
lipopolysaccharide core region metabolic process	5.27E-07	rfaD // "waaF" // "lpcA" // "gmhB" // "rfaE"
polysaccharide biosynthetic process	6.04E-07	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "rffM" // "yfdG"
polysaccharide metabolic process	1.23E-06	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "rffM" // "yfdG"
Sugar Nucleotides Biosynthesis	1.93E-06	lpcA // "rfaD" // "gmhB" // "rfaE" // "rffA"
carbohydrate biosynthetic process	4.01E-06	rfaD // "waaF" // "lpcA" // "gmhB" // "rfaE" // "rffA" // "rffM" // "yfdG"
cellular polysaccharide biosynthetic process	7.13E-06	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "rffM"
cellular carbohydrate biosynthetic process	9.03E-06	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "rffM"
cellular polysaccharide metabolic process	1.07E-05	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "rffM"
lipopolysaccharide biosynthetic process	1.32E-05	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD"
oligosaccharide biosynthetic process	1.37E-05	rfaD // "waaF" // "lpcA" // "gmhB" // "rfaE"
lipopolysaccharide metabolic process	1.42E-05	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD"
membrane assembly	3.45E-05	hlpA // "surA" // "bamE"
Gram-negative-bacterium-type cell outer membrane assembly	3.45E-05	hlpA // "surA" // "bamE"
Sugars Biosynthesis	3.57E-05	lpcA // "rfaD" // "gmhB" // "rfaE" // "rffA"
Carbohydrates Biosynthesis	4.59E-05	lpcA // "rfaD" // "gmhB" // "rfaE" // "rffA"
lipid biosynthetic process	4.72E-05	fabH // "rffA" // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD"
single-organism membrane organization	4.72E-05	hlpA // "surA" // "bamE"
oligosaccharide metabolic process	4.97E-05	rfaD // "waaF" // "lpcA" // "gmhB" // "rfaE"
Biosynthesis	7.05E-05	speE // "pdxH" // "fdx" // "atpC" // "lpcA" // "rfaD" // "gmhB" // "rfaE" // "entB" // "fabH" // "waaF" // "rffA" // "rffM"
membrane organization	8.11E-05	hlpA // "surA" // "bamE"
chaperone mediated protein folding requiring cofactor	1.38E-04	hlpA // "surA"
exodeoxyribonuclease V complex	1.38E-04	recC // "recB"
'de novo' posttranslational protein folding	1.38E-04	hlpA // "surA"
exodeoxyribonuclease V activity	1.38E-04	recB // "recC"
cellular lipid metabolic process	2.23E-04	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "fabH"
carbohydrate derivative biosynthetic process	2.49E-04	gmhB // "rfaD" // "rfaE" // "atpC" // "rffA" // "waaF" // "lpcA" // "rffM"
lipid metabolic process	2.71E-04	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "fabH"
protein import	2.75E-04	tolB // "tolQ"

Pathways Gene-Ontology-Terms All-Genes	p-values	Matches
'de novo' protein folding	2.75E-04	hlpA // "surA"
macromolecule metabolic process	2.83E-04	recC // "recB" // "rssB" // "tolB" // "yfdG" // "rffA" // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "rffM" // "yeiP" // "gcvR" // "hlpA" // "surA" // "cpxA" // "secB"
cellular carbohydrate metabolic process	2.98E-04	rffA // "rfaE" // "waaF" // "lpcA" // "gmhB" // "rfaD" // "rffM"
protein binding	2.99E-04	fabH // "speE" // "bamE" // "surA" // "hlpA" // "secB" // "recC" // "fdx" // "rfaD" // "tolB" // "recB" "entB"
protein transport	3.04E-04	secB // "hlpA" // "tolB" // "tolQ" // "surA"
establishment of protein localization	3.04E-04	secB // "hlpA" // "tolB" // "tolQ" // "surA"
unfolded protein binding	3.13E-04	surA // "hlpA" // "secB"
nucleotide-sugar biosynthetic process	4.19E-04	gmhB // "rfaD" // "rfaE"
double-strand break repair	4.56E-04	recC // "recB"
protein stabilization	4.56E-04	hlpA // "surA"
regulation of protein stability	4.56E-04	hlpA // "surA"
single-organism carbohydrate metabolic process	6.08E-04	rfaD // "waaF" // "lpcA" // "gmhB" // "rfaE" // "rffA" // "rffM" // "yfdG"
biosynthetic process	6.55E-04	rfaD // "waaF" // "lpcA" // "gmhB" // "rfaE" // "fabH" // "entB" // "speE" // "pdxH" // "yfdG" // "yeiP" // "atpC" // "rffA" // "rffM" // "rssB" // "gcvR" // "fdx"
nucleotide-sugar metabolic process	6.94E-04	gmhB // "rfaD" // "rfaE"
organophosphate biosynthetic process	8.23E-04	gmhB // "rfaD" // "rfaE" // "fabH" // "atpC" // "pdxH" // "lpcA"
cellular component assembly	8.44E-04	hlpA // "surA" // "bamE" // "fdx" // "secB"
chaperone-mediated protein folding	9.50E-04	hlpA // "surA"

Supporting Figures.

OD_{600} no $HD5_{ox}$	1	2	3	4	5	6	7	8	9	10	11	12
A	0.29	0.26	0.35	0.23	0.33	0.33	0.27	0.32	0.32	0.25	0.34	0.39
B	0.16	0.27	0.19	0.25	0.40	0.23	0.37	0.34	0.30	0.23	0.34	0.18
C	0.15	0.23	0.22	0.28	0.22	0.33	0.20	0.24	0.35	0.29	0.25	0.31
D	0.27	0.23	0.27	0.43	0.37	0.30	0.38	0.22	0.25	0.38	0.23	0.27
E	0.24	0.34	0.35	0.26	0.25	0.35	0.28	0.28	0.26	0.38	0.30	0.30
F	0.28	0.29	0.26	0.25	0.30	0.28	0.26	0.27	0.31	0.33	0.24	0.20
G	0.30	0.37	0.42	0.43	0.35	0.32	0.32	0.35	0.41	0.26	0.37	0.27
H	0.37	0.38	0.29	0.37	0.33	0.22	0.32	0.21	0.33	0.19	0.33	0.35
mean	0.29											

fitness untreated	1	2	3	4	5	6	7	8	9	10	11	12
A	1.00	0.88	1.17	0.78	1.11	1.11	0.92	1.08	1.08	0.85	1.14	1.31
B	0.54	0.92	0.66	0.84	1.36	0.78	1.25	1.14	1.02	0.77	1.16	0.62
C	0.50	0.77	0.73	0.95	0.73	1.12	0.68	0.83	1.18	1.00	0.83	1.05
D	0.92	0.79	0.91	1.45	1.24	1.01	1.30	0.74	0.85	1.29	0.79	0.92
E	0.81	1.17	1.18	0.89	0.84	1.20	0.96	0.95	0.87	1.28	1.02	1.02
F	0.95	0.97	0.87	0.86	1.01	0.94	0.87	0.93	1.04	1.12	0.83	0.68
G	1.02	1.24	1.43	1.45	1.18	1.08	1.10	1.20	1.40	0.89	1.24	0.91
H	1.27	1.30	0.99	1.27	1.13	0.73	1.10	0.70	1.11	0.65	1.11	1.18

OD_{600} $HD5_{ox}$	1	2	3	4	5	6	7	8	9	10	11	12
A	0.18	0.28	0.22	0.21	0.22	0.23	0.20	0.20	0.19	0.16	0.20	0.16
B	0.20	0.16	0.20	0.18	0.24	0.19	0.17	0.18	0.17	0.17	0.10	0.16
C	0.18	0.20	0.22	0.19	0.20	0.20	0.19	0.22	0.27	0.17	0.26	0.17
D	0.23	0.18	0.21	0.14	0.23	0.20	0.20	0.22	0.22	0.08	0.16	0.12
E	0.20	0.17	0.19	0.19	0.18	0.19	0.18	0.17	0.16	0.19	0.16	0.05
F	0.12	0.14	0.18	0.17	0.16	0.17	0.19	0.18	0.17	0.19	0.16	0.07
G	0.19	0.18	0.18	0.21	0.19	0.17	0.15	0.23	0.17	0.17	0.19	0.10
H	0.22	0.22	0.21	0.24	0.19	0.19	0.18	0.20	0.19	0.19	0.21	0.19
mean	0.18											

fitness treated	1	2	3	4	5	6	7	8	9	10	11	12
A	0.95	1.51	1.17	1.14	1.20	1.26	1.07	1.07	1.01	0.89	1.07	0.87
B	1.07	0.89	1.08	0.99	1.27	1.01	0.92	1.00	0.94	0.89	0.54	0.87
C	0.99	1.09	1.18	1.03	1.06	1.09	1.01	1.18	1.48	0.90	1.38	0.93
D	1.24	0.96	1.13	0.75	1.27	1.07	1.09	1.19	1.17	0.42	0.84	0.66
E	1.09	0.92	1.03	1.05	1.00	1.01	0.96	0.94	0.88	1.04	0.85	0.26
F	0.67	0.77	1.00	0.94	0.89	0.93	1.05	0.99	0.94	1.03	0.89	0.37
G	1.02	1.00	0.98	1.16	1.04	0.92	0.80	1.24	0.93	0.92	1.00	0.55
H	1.21	1.18	1.16	1.28	1.03	1.00	0.96	1.07	1.03	1.00	1.14	1.03

ϕ	1	2	3	4	5	6	7	8	9	10	11	12
A	0.96	1.72	1.00	1.46	1.09	1.14	1.17	0.99	0.94	1.05	0.94	0.66
B	1.99	0.97	1.65	1.19	0.94	1.29	0.73	0.87	0.93	1.15	0.47	1.41
C	1.99	1.43	1.60	1.09	1.45	0.98	1.50	1.42	1.26	0.90	1.66	0.89
D	1.34	1.21	1.24	0.52	1.02	1.06	0.84	1.61	1.37	0.32	1.06	0.72
E	1.35	0.79	0.88	1.17	1.18	0.84	1.00	0.99	1.01	0.81	0.83	0.26
F	0.70	0.80	1.15	1.09	0.88	0.99	1.20	1.07	0.90	0.92	1.08	0.55
G	1.00	0.80	0.68	0.80	0.88	0.86	0.73	1.03	0.67	1.03	0.81	0.60
H	0.96	0.90	1.17	1.01	0.91	1.37	0.88	1.53	0.93	1.54	1.03	0.87

Figure S1. Screening of Plate 1 of the Keio Collection. OD_{600} values for the treated and untreated strains were used to calculate a fitness for each strain, which was then converted to a ϕ value (Equations 1-3).

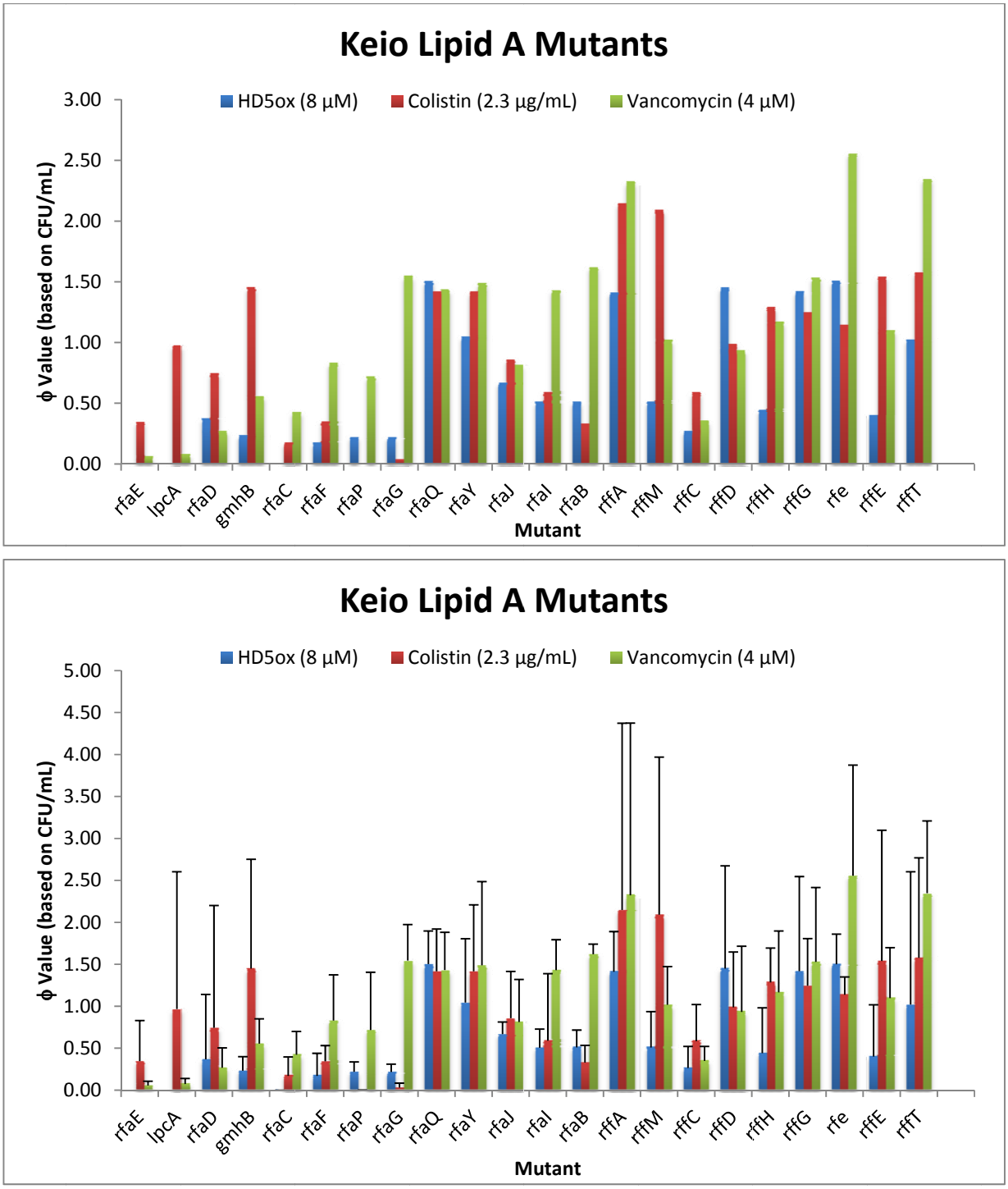

Figure S2. Results of antimicrobial activity assays performed by colony counting for strains related to LPS biosynthesis. Hits from the Keio screen and additional strains of interest were subjected to an AMA using either 8 μM HD5, 2.3 μg/mL colistin or 4 μM vancomycin. ϕ values were calculated from CFU/mL using Equations 4-6. Mean values of three independent measurements shown. Bottom: Error bars indicate standard deviations of the mean.

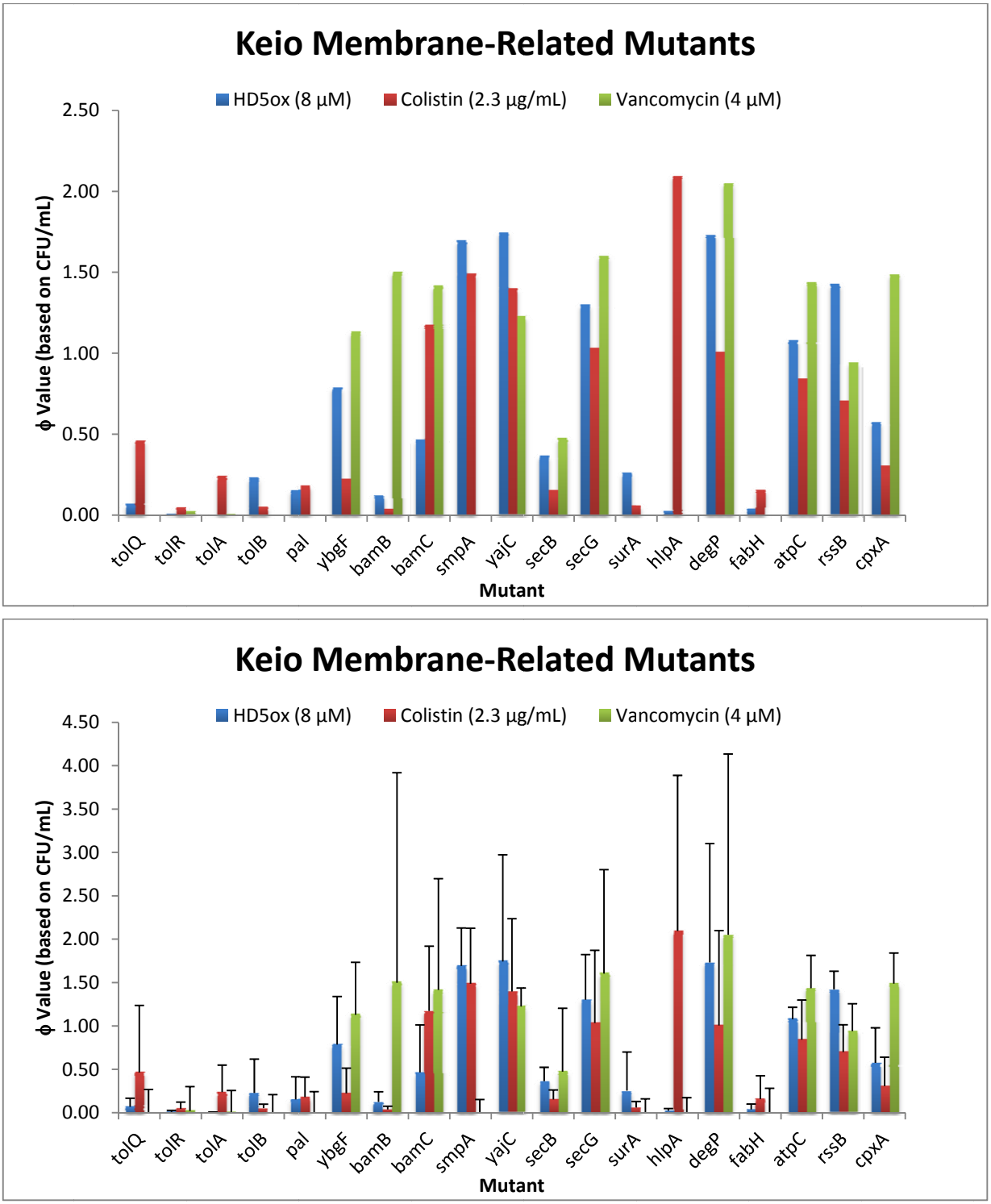

Figure S3. Results of antimicrobial activity assays performed by colony counting for strains related membrane integrity. Hits from the Keio screen and additional strains of interest were subjected to an AMA using either 8 μM HD5, 2.3 μg/mL colistin or 4 μM vancomycin. ϕ values were calculated from CFU/mL using Equations 4-6. Mean values of three independent measurements shown. Bottom: Error bars indicate standard deviations of the mean.

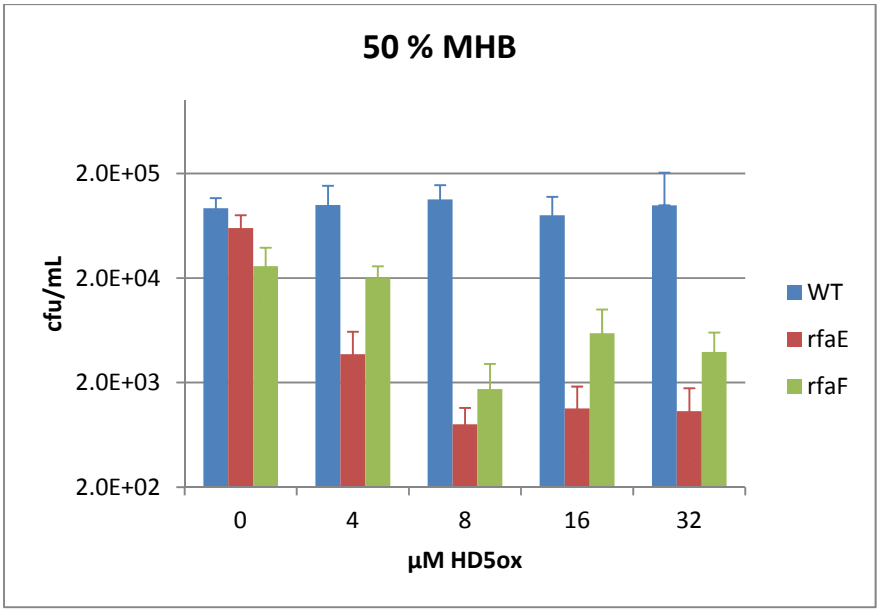

Figure S4. Results of antimicrobial activity assays in rich media (50% MHB) performed by colony counting for the WT and the two most sensitive mutants rfaE and rfaF. Mean values of three independent measurements shown. Error bars indicate standard deviations of the mean.

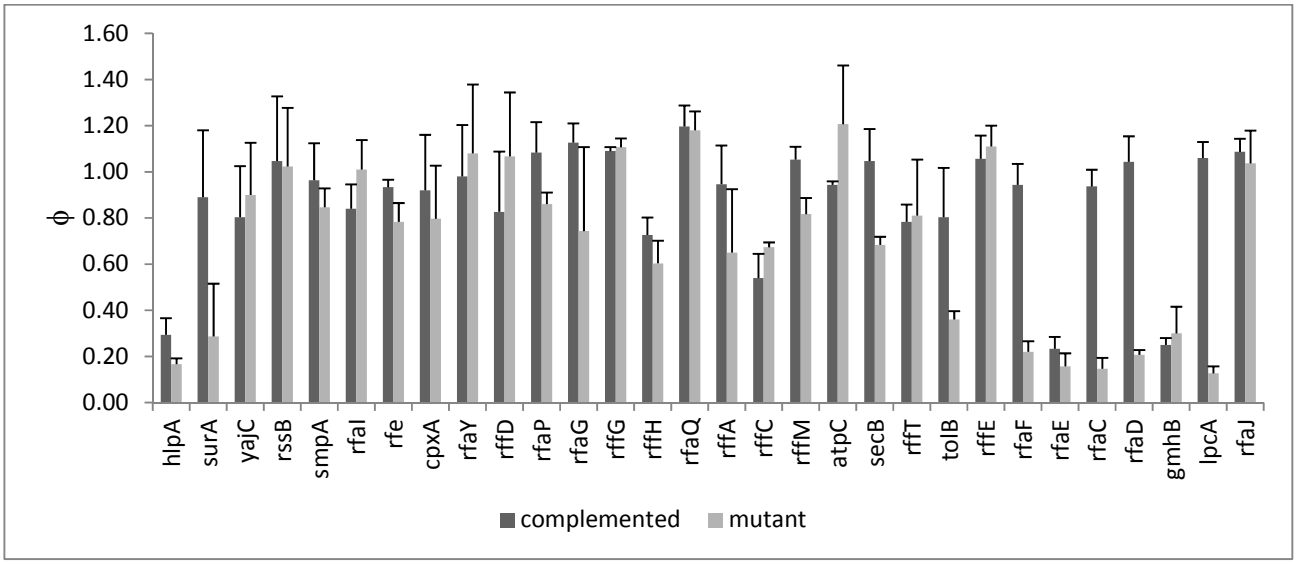

Figure S5. Results of the complementation antimicrobial activity assay. Mutants were transformed with plasmids expressing the genes that were knocked out in the mutant as indicated. Mutants and complemented strains were subjected to the AMA, and ϕ values were calculated using Equations 7-9. Mean of three independent replicates shown, error bars represent standard deviations.

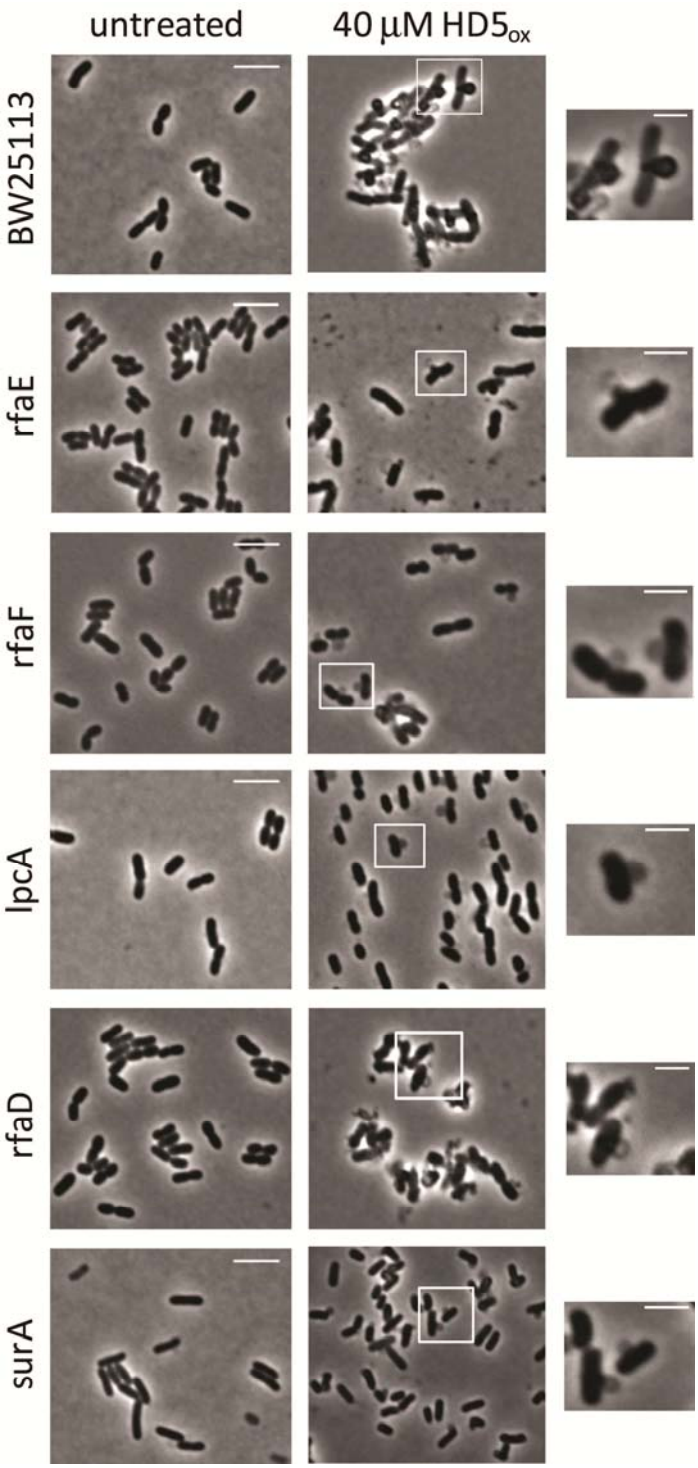

Figure S6. Phase-contrast images of the WT and the five most hypersensitive mutants. Left column, without $HD5_{ox}$. Middle column, cells were treated with 40 mM $HD5_{ox}$. All five hypersensitive mutants exhibit similar morphological changes upon $HD5_{ox}$ treatment compared to WT (formation of blebs, cell elongation). Scale bar represents 5 μm. Smaller images in the right columns show enlarged sections of treated cells (white frame) to highlight the observed phenotype. Scale bar represents 2 μm.

Supporting References.

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