Electronic Supplementary Information

Combination of gallium(III) with acetate for combating antibiotic resistant *Pseudomonas aeruginosa*

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Supplementary Methods

Growth of *P. aeruginosa*. *Pseudomonas aeruginosa* PAO1 was cultured in M9 minimal medium (Sigma), supplemented with 0.2% casein hydrolysate (Sigma) unless otherwise stated. Cells were grown at 37 °C on a culture shaker at 250 rpm to mid-log phase, then diluted with fresh culture medium to OD_{600} of 0.1-0.2 and subjected to metal or metabolite treatment. Cells were harvested by centrifugation at 5,000 *g* for 15 mins at 4 °C for further experiments.

Identification of Ga-binding proteins in *P. aeruginosa*. Two different approaches were applied for proteome-wide identification of Ga-binding proteins in *P. aeruginosa*. The fluorescent probe Ga(III)-*TRACER* was prepared by reacting equimolar amounts of $Ga(NO_3)_3$ with the fluorescent ligand NTA-AC in Tris buffer (20 mM Tris, 150 mM NaCl, pH 7.2), and the formation of Ga(III)-*TRACER* was confirmed by ESI-MS. To label Ga-binding proteins with the fluorescent probe Ga(III)-*TRACER*, *P. aeruginosa* liquid cultures grown to mid-log phase were incubated with 50 μM Ga(III)-*TRACER* at 37 °C in the dark for 30 mins. Cells were irradiated by UV light at 365 nm for 20 mins, and then centrifuged to obtain the cell pellets. An aliquot of *P. aeruginosa* cells resuspended in PBS (OD_{600} = 0.3) was subjected to confocal imaging. Fluorescent and phase contrast images were captured by a Carl Zeiss LSM700 Inverted Confocal Microscope. The remaining cells were resuspended in Tris buffer and lysed by sonication. The soluble proteins were separated by SDS-PAGE. The silver-stained protein bands corresponding to the fluorescent spots were excised from the gel, subjected to trypsin digestion and analyzed by Orbitrap-LC-MS.

Ga-binding proteins in *P. aeruginosa* were also identified by Ga-IMAC experiments modified from previous study.[1] *P. aeruginosa* cells were lysed in ice-cold Buffer A (20 mM Tris, 300 mM NaCl, 1 mM TCEP, pH 7.6) and centrifuged (20,000 *g*, 20 mins) to remove cell debris. The bacterial protein extracts were diluted to *ca*. 2 mg/mL. A final concentration of 100 μM gallium nitrate solution was added to half of the protein extracts and was incubated at 4 °C for 4-5 hrs to pre-saturate the protein Ga-binding sites. The Ga-NTA column with a bed volume of 2 mL was washed thoroughly and equilibrated with Buffer A and then loaded with 10 mg of the Ga-treated and untreated protein extracts. After incubation at 4 °C overnight, on-column protein digestion was performed with the addition of trypsin (1:25 w/w, Sequencing Grade, Promega) in Buffer A and incubated at 37 °C for 1 hr. The unbound peptide fragments and excessive proteolytic enzymes were washed off with 10 C.V. of Buffer A, and the metal-binding peptides were finally eluted with

5 C.V. of Buffer A supplemented with 50 mM EDTA. After DTT and IAA treatment, the eluted peptides were desalted using C-18 reverse phase chromatography (Waters), which was performed immediately after IMAC separation to minimize peptide oxidation and modification. The desalted peptides eluted with 60% ACN, 0.1% TFA were freeze-dried overnight and re-dissolved in 5% ACN, 0.1% FA prior to Orbitrap-LC-MS analysis. The MS data were analyzed with MaxQuant for label-free quantification and proteins were searched against the UniProtKB/SwissPort database. Data were plotted with the fold change and significance of the LFQ intensity values generated from MaxQuant analysis.

Expression and purification of *Pa*RpoB/C. The *rpoB* and *rpoC* genes were amplified by PCR from *P. aeruginosa* PAO1 with primer pairs RpoB(*Bam*HI)-for (5'-TAATGGATCCGATGGCTTACTCATACACTGAGAAAAA-3'), RpoB(*Hind*III)-rev (5'-ATTAAAGCTTTTATTCGGTTTCCAGTTCGATGTC-3'), RpoC(*Eco*RI)-for (5'-TAATGAATTCTTTGAAAGACTTGCTTAATCTGTTGAA-3') and RpoC(*Hind*III)-rev (5'-ATTAAAGCTTTTAGTTACCGCTCGAGTTCAGC-3') (the restriction sites are underlined). The digested DNA fragments were purified and ligated into the same digestion sites of pET-47b(+) vector. The constructed plasmids were sequenced to confirm that no mutation occurred during the manipulation. *Escherichia coli* BL21 (DE3) cells harboring the expression vector were grown in LB medium supplemented with kanamycin (30 μg/mL) at 37 °C to an OD_{600} of 0.6-0.8, followed by the addition of 0.2 mM isopropyl β-D-thiogalactoside (IPTG) to induce protein overexpression. After further culture at 22 °C for 12 hrs, the cells were harvested by centrifugation (5,000 *g*, 20 min) and resuspended in Tris buffer B (20 mM Tris, 300 mM NaCl, pH 7.2). The cells were lysed by homogenization and sonication, and centrifuged (20,000 *g*, 30 mins) to remove cell debris. The cell lysates were then subjected to Ni-NTA resin (Thermo) equilibrated with Tris buffer B. The bound proteins were eluted by 500 mM imidazole-containing Tris buffer B, followed by Prescission Protease cleavage at 4 °C overnight. The proteins were further purified by HiTrap Heparin HP column (GE Healthcare).

Characterization of Ga-binding to *Pa*RpoB/C. Isothermal titration calorimetry (ITC) experiments were carried out to characterize Ga(III) binding to purified *Pa*RpoB/C in vitro. The EDTA-treated RpoB and RpoC proteins were desalted and prepared in Tris buffer C (20 mM Tris, 300 mM NaCl, 5 mM NaH_2PO_4, 2 mM sodium citrate, pH 7.6) with a final concentration of 20

μM. The Ga titrant solution was prepared by diluting $Ga(NO_3)_3$ in the same Tris buffer C with final concentration of 1 mM. ITC experiments were performed on Malvern MicroCal ITC200 at 25 °C. The signals of Ga(III) titrated into Tris buffer C were recorded as background control. The ITC data were analyzed using the Origin software and fitted by one-set-of-sites binding model.

Cellular thermal shift assay (CETSA) was performed to evaluate Ga(III) binding to *Pa*RpoB/C in intact bacterial cells. Control and Ga-treated *P. aeruginosa* cells were harvested and washed with chilled PBS for three times. Equal amounts of cell suspensions (*ca*. 10^9 cells) were aliquoted into PCR tubes and heated at different temperatures for 3 mins, followed by immediate cooling at room temperature for 3 mins, and the heat-cool cycles were repeated for three times. The cells were then lysed by three freeze-thaw cycles using liquid nitrogen, and were subsequently centrifuged at 20,000 *g* for 20 mins at 4 °C to pellet the precipitated proteins. The supernatants were separated by SDS-PAGE followed by western blot analysis using *Pa*RpoB and *Pa*RpoC polyclonal antibodies.

Analysis of nascent mRNA transcripts in *P. aeruginosa*. EU labeling experiments were performed according to the protocol of Click-iT Nascent RNA Capture Kit (Thermo). Briefly, bacterial cells at early log phase were pulsed with 0.5 mM 5-ethynyl uridine (EU) for 4 hrs in the presence of different metals or metabolites. Total RNA was isolated using SV Total RNA Isolation System (Promega) and used in a copper catalyzed click reaction with azide-modified biotin, and then binds to streptavidin magnetic beads provided by the kit. cDNA synthesis was performed with the nascent mRNA transcripts directly captured on the beads using VILO cDNA synthesis kit (Thermo) followed by analysis with RT-PCR. The primers used are listed in Table S2.

GC-MS acquisition and data processing. Bacterial metabolite extraction was performed as previously described[2] with some modifications. Bacterial cells after 4 hrs treatment were harvested by the same amount according to the measured OD_{600}. After washing with cold 0.9% NaCl, cell pellets were resuspended in 1 mL cold methanol, with the addition of 5 μg ribitol (Sigma) as an internal standard. Cells were then lysed on a high-throughput Tissuelyser, and the supernatant was dried on a SpeedVac concentrator system (Thermo Fisher) for 5-6 hrs at room temperature.

The dried samples were then subjected to derivatization. Briefly, 80 μL of 20 mg/mL methoxylamine (Sigma) in pyridine was added and incubated for 90 mins at 37 °C, followed by

the addition of 80 µL of N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA, Sigma) and incubated at 37 °C for another 30 mins. After centrifugation, the clear derivated samples were transferred to the GC-MS autosampler vial. Analysis was carried out on Agilent 7890AGC coupled to mass spectrometry 5975C equipped with a HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent). Helium was employed as carrier gas with a constant flow rate of 1 mL/min. Electron impact ionization (EI) energy was 70 eV and the mass scan range was 33 - 550 m/z at a rate of 2 scan/s. 1 µL of derivatized sample was injected to the injector at 280 °C. The initial column temperature was held for 3 mins at 85 °C and then ramped at 20 °C/min to 310 °C and held for 7 mins. For each tested group, six independent samples were analyzed.

The computational tool, eRah written in R language,[3] was employed to analyze the GC-MS data for spectral deconvolution, alignment, and metabolite quantification and identification. Metabolites were identified according to the reference spectra in Golm Metabolome Database (GMD), and National Institute of Standards and Technology (NIST) MS library. The resulting data matrix was normalized to the integrated peak area of ribitol in each sample. Principal component analysis (PCA) was applied to reduce the high dimension of the data set. One-way ANOVA followed by LSD post-hoc multiple comparisons test in SPSS statistics (version 24) was used to test the significance of metabolites. MetaboAnalyst 3.0 was employed to analyze the statistically enriched pathways over the identified metabolites.

Exogenous metabolite/inhibitor addition assay. Growth curves of *P. aeruginosa* were measured on Cytation3 multi-mode reader using the dynamics model. Bacteria grown to mid-log phase were diluted to OD_{600} of 0.1 in 24-well plate, with the supplementation of appropriate doses of gallium nitrate and exogenous metabolites or inhibitors of TCA cycle (malonate) and ETC (sodium azide). A serial concentrations of metabolites were supplemented including amino acids (isoleucine, leucine, valine, proline, glutamic acid and phenylalanine) and different central carbon metabolites (fumarate, succinate, α-ketoglutarate, citrate, malate, oxaloacetate, glyoxylate, pyruvate, acetate, gluconate, glucose, mannitol, fructose, arabinose and ribose) with a total carbon concentration of 60 mM. Plate was incubated at 37 °C in the plate reader with orbital shaking at 220 rpm. Bacterial growth was monitored for 12 hrs *via* OD_{600} measurement at 30 mins intervals.

Measurement of Ga uptake. Bacterial cells were harvested at different time points by the same

amount according to the measured OD_{600}. After washing with cold PBS containing 5 mM EDTA, cell pellets were digested with 70% HNO_3 (trace metal basis, Sigma) at 60 °C overnight. Samples were diluted to a final concentration of *ca.* 5% HNO_3 and analyzed by ICP-MS (iCAP RQ, Thermo Fisher).

Measurement of NADH and NAD^+. The experiment was performed according to established protocols.[4] Briefly, to the harvested 10^9 bacterial cells, 200 μL of either 0.2 M HCl (for NAD^+ extraction) or 0.2 M NaOH (for NADH extraction) were added. After incubation at 55 °C for 10 mins, the samples were cooled on ice, and neutralized with 0.1 M NaOH or 0.1 M HCl. After centrifugation, 5 μL of clear supernatant from each sample were added to 90 μL of the reagent mix in a 96-well plate. The reagent mix contained 2× bicine buffer (1.0 M, pH 8.0), 1× water, 1× 40 mM EDTA, 1× 100% ethanol, 1× 4.2 mM thiazolyl blue, and 2× 16.6 mM phenazine ethosulphate. The colorimetric assay was initiated by the addition of 5 μL solution containing 1 mg/mL alcohol dehydrogenase II in 0.1 M bicine buffer (pH 8.0). The reaction was performed at 30 °C by recording the absorbance of reduced thiazolyl blue at 570 nm for 30 mins. The rate of increase in absorbance over time was used as the measure of NAD(H) concentration. A standard curve derived from known concentrations of NADH was assayed simultaneously.

Measurement of ATP. Cellular ATP contents of *P. aeruginosa* were measured with BacTiter-Glo Microbial Cell Viability Assay (Promega). Bacterial cells after 4 hrs treatment were harvested, washed with PBS and diluted to 10^8 cells/mL based on OD_{600}. To an aliquot of 100 μL of bacterial suspension, 100 μL of BacTiter-Glo reagent were added. The mixture was incubated at room temperature for 15 mins, and luminescence was measured on Cytation 3 multi-mode reader (BioTek). A standard curve was prepared using serial dilutions of ATP in the range of 1 μM to 10 pM and measured simultaneously.

Bacterial respiration. Bacterial oxygen consumption rate (OCR) was quantified on an XF^e96 Extracellular Flux Analyzer (Agilent). Before the XF assay, sensors were submerged into XF calibrant and kept in a non-CO_2 37 °C incubator overnight for hydration. XF cell culture microplates were precoated with 2 μg poly-D-lysine (PDL) and dried overnight. *P. aeruginosa* cultures (in M9 medium) diluted to OD_{600} of ~0.01 were added to the assay plate, and then centrifuged for 10 mins at 1,400 *g* for cell attachment. A volume of 6 μL gallium nitrate solution

with different concentrations was loaded into the injection ports. To assure uniform cellular seeding, basal OCR was measured for two cycles (2 mins mix, 2 mins wait, 3 mins measurement) before injection of the compound. After injection, 15 measuring cycles were performed. Each sample was tested in five replicate wells. Data were analyzed by Wave Desktop 2.2 software (Agilent).

Persister assay. Persister cells were prepared according to the previous studies.[5, 6] Briefly, *P. aeruginosa* overnight culture was diluted in LB broth and grown to OD_{600} of 0.2~0.3. Cells were then diluted 1:1000 in 50 mL LB and grown for 16 hrs at 37 °C with 250 rpm shaking to obtain stationary phase cells. Cells were then treated with 10 μg/mL ciprofloxacin or 10 μg/mL ofloxacin for 4 hrs, and the remaining persister cells were washed with PBS and resuspended in M9 medium to OD_{600} of 0.2. Growth of the persister cells exposed to $Ga(NO_3)_3$ or metabolites in 24-well plate was monitored for 24 hrs on Cytation 3 multi-mode reader.

Bacterial infection assay. A549 cells (10^5 cells per well) were seeded in a 24-well plate in F-12 medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). Cells were incubated at 37 °C in humidified 5% CO_2 atmosphere for 24 hrs to reach 80% confluence. Bacteria were added to cell monolayers at a multiplicity of infection (MOI) of 10 per cell in antibiotics-free cell culture medium, and the plates were centrifuged for 10 mins at 1,000 *g*. After 1 hr of infection at 37 °C, extracellular bacteria were removed by incubating the cells in 50 μg/mL gentamicin-containing F-12 medium for 1 hr, followed by extensive washing with PBS. The infected cells were then cultured for 24 hrs in the presence of $Ga(NO_3)_3$, $Fe(NO_3)_3$ or sodium acetate (ACE), or the combination of metal with acetate. To examine the cell-associated bacteria, cells were lysed by sterilized 1% Triton X-100 in PBS, and the bacterial colonies in serial diluted cell lysates were enumerated by agar plating.

Bacterial cytotoxicity assay was performed according to the previous study.[7] A549 cells were pretreated with $Ga(NO_3)_3$ or ACE, or their combination for 4 hrs. *P. aeruginosa* cells grown to mid-log phase in LB broth were diluted 1:10 in F-12 medium and induced at 37 °C for 1 hr. Infection was initiated by adding bacteria to A549 cells at MOI of 10. Morphology of the cells was captured by the inverted brightfield microscopy on Cytation 3 multi-mode reader. The percentage of live cells were determined by MTT assay. 1 mL of FBS-free F-12 medium containing 1% PS and 100 μL MTT solution (5 mg/mL) was added to each well, and the plates

were incubated for an additional 4 hrs. After careful removal of the medium, 1 mL DMSO was added to each well and the plate was shaken for 10 mins. The absorbance at 490 nm was measured.

Murine wound infection model. All experiments were performed in accordance with the guidelines approved by committee on the use of Live Animals in Teaching and Research (CULATR), the University of Hong Kong. The animals used were six-to-eight-week-old, female BALB/c mice (18-22g) purchased from Charles River Laboratories, Inc. The animals could freely access to food and water, and were randomized to cages for each experiment.

In the wound infection model, static overnight cultures of *P. aeruginosa* PAO1 were harvested and washed with PBS for three times before further use. An excisional wound (with an area of *ca.* 4 mm^2) was created on the flank of each mouse. A number of 5×10^8 CFU of bacteria in an aliquot of 10 μL were dropped to the wound. Groups (n=5 for each group) of mice received monotherapy of $Ga(NO_3)_3$ (2 mg/kg), ACE (1 g/kg), and the combination therapy of $Ga(NO_3)_3$ and ACE. All the compounds were mixed in cream and smeared on the infection site twice daily post infection. Mice administrated with vehicle were set as the control. All the mice were sacrificed 48 hrs post infection and the infection sites were collected in an aliquot of 0.5 mL PBS and homogenized on Qiagen Tissue Lyser II at a frequency of 30 Hz for 5 mins. The bacterial loads per tissue were numerated by agar plating.

Hemolysin assay. Hemolysin levels in *P. aeruginosa* culture supernatants were determined referring to a previous protocol.[8] Briefly, rabbit blood cell was prepared by washing the defibrinated rabbit blood with BSA buffer (20 mM KH_2PO_4, 150 mM NaCl, 1 mM $MgCl_2$, 1 mg/ml BSA) twice, and then centrifuged at 2,000 *g* for 2 mins to remove the precipitates. *P. aeruginosa* cells grown at 37 °C for 20 hrs in the absence or presence of studied compounds were collected and centrifuged at 12,000 *g* at 4 °C for 5 mins. An aliquot of 0.4 mL bacterial supernatant was diluted to 1 mL with BSA buffer and mixed with 20 μl of freshly defibrinated rabbit blood cell. After incubation at 37 °C for 25 mins, intact blood cells were removed by centrifugation at 5,500 *g* for 1 min at room temperature and the optical densities of the supernatants were measured at 543 nm to determine the extracted hemolysin levels.

Transcriptome sequencing and data processing. *P. aeruginosa* cells after 4 hrs treatment with 80 μM $Ga(NO_3)_3$ or 30 mM ACE, or their combination were harvested for transcriptome

sequencing. After extraction of the total RNA, bacterial rRNAs were removed by RiboMinus™ transcriptome isolation kit (Thermo Fisher). Libraries were prepared with VAHTS™ total RNA-seq (H/M/R) library prep kit (Vazyme). RNA (3 μg) was fragmented and subjected to first- and second-strand synthesis, 3'-end adenylation, adaptor ligation and PCR amplification. After purification of the PCR products, library quality was checked on an Agilent 2100 Bioanalyzer.

For gene expression arrays, quality control and quantification of gene expression levels were performed with HTSeq v0.6.1. The raw data were firstly processed through in-house perl scripts to remove adapter-containing, poly-N-containing and low quality reads, and the clean reads were mapped to the reference genome of *P. aeruginosa* PAO1. After counting the reads numbers mapped to each gene, FPKM (Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced) of each gene was calculated based on the length of the gene and the reads count mapped to this gene. Differential expression analysis between two groups was performed using the DESeq R package (1.18.0), which determines differential gene expressions using a model based on the negative binomial distribution. The resulting *p*-values were adjusted using the Benjamini and Hochberg's method for false discovery rate (FDR) control. Differentially expressed genes were selected with an adjusted *p*-value < 0.05.

Isotope tracer experiment. To track the metabolic flux of acetate in the absence and presence of Ga(III), nontargeted isotope fate detection (NTFD) analysis was performed as previously described.[9, 10] *P. aeruginosa* cells grown to log phase were diluted to OD_{600} of 0.3 with M9 medium in the absence or presence of 80 μM $Ga(NO_3)_3$, with the supplementation of 30 mM unlabeled ACE or 1:1 mixture of unlabeled and [U-$^{13}C_2$] labeled ACE (total 30 mM). Bacterial cells were harvested after 4 hrs incubation at 37 °C and the metabolites were extracted and analyzed according to the methods described in GC-MS acquisition. Three biological replicates were performed for each growth condition.

The detection of stable isotope labeled metabolites was executed using the NTFD software which provides the mass isotopomer distributions (MID) data of all labeled compounds detected in the GC-MS data.[11] Effectiveness of each tracer was identified from the MID data. The identification of labeled metabolites was carried out by the chemstation of Agilent combined with the NIST database. Both the spectrum obtained from the labeled chromatogram and the spectrum obtained from the unlabeled chromatogram was normalized by their total signals.

Growth of *P. aeruginosa* PAO1 transposon mutants. Selected strains with transposon insertions on studied genes were obtained from the *P. aeruginosa* PAO1 transposon mutant library (University of Washington). After confirming the mutants by PCR using flanking primers and transposon specific primers, strains were stocked in LB medium containing 5% DMSO (v/v). Growth curves of the mutant strains in the presence of $Ga(NO_3)_3$ (20 μM) or ACE (30 mM) or their combinations were measured on Cytation3 multi-mode reader. Strains were cultured in M9 medium at 37 ºC in 24-well plate without the supplementation of antibiotics.

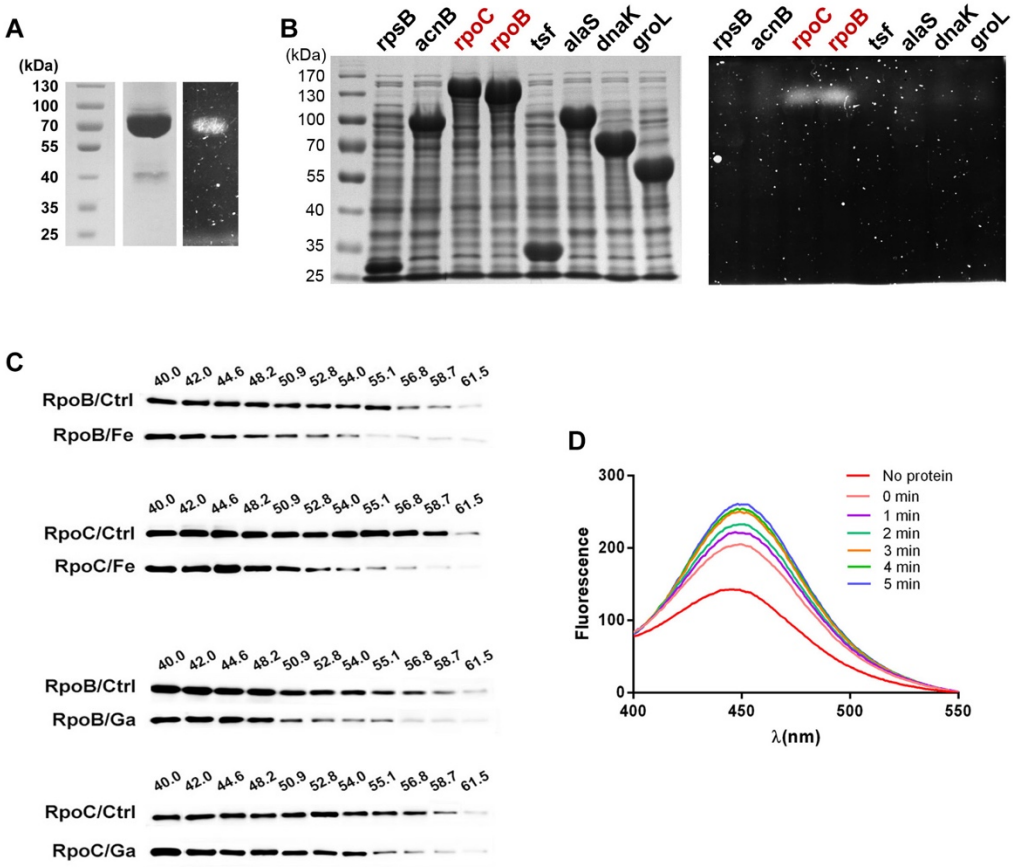

Fig. S1 (A) Fluorescent labelling of apo-lactoferrin by Ga(III)-*TRACER* on a SDS-PAGE gel. After incubating the probe with apo-hLF in a 1:1 molar ratio, samples were subjected to UV irradiation at 365 nm for 10 mins to allow the formation of covalent bond between the probe and protein prior to SDS-PAGE analysis. (B) Fluorescent labeling of Ga-binding proteins by Ga(III)-*TRACER* on a SDS-PAGE gel. The proteins enriched by Ga-IMAC with high scores were overexpressed in *E. coli* BL21. *Pa*RpoB and *Pa*RpoC could be fluorescent labeled by Ga(III)-*TRACER*, indicating binding of Ga(III) to the two proteins in cells. (C) Representative western blot results of Ga(III)-targeting proteins evaluated by CETSA. (D) Time-dependent fluorescence spectra of Ga(III)-*TRACER* (1 μM) after the addition of *Pa*RpoB (10 μM). The binding of Ga(III)-*TRACER* to *Pa*RpoB led to *ca*. 2-fold fluorescence enhancement within 5 mins.

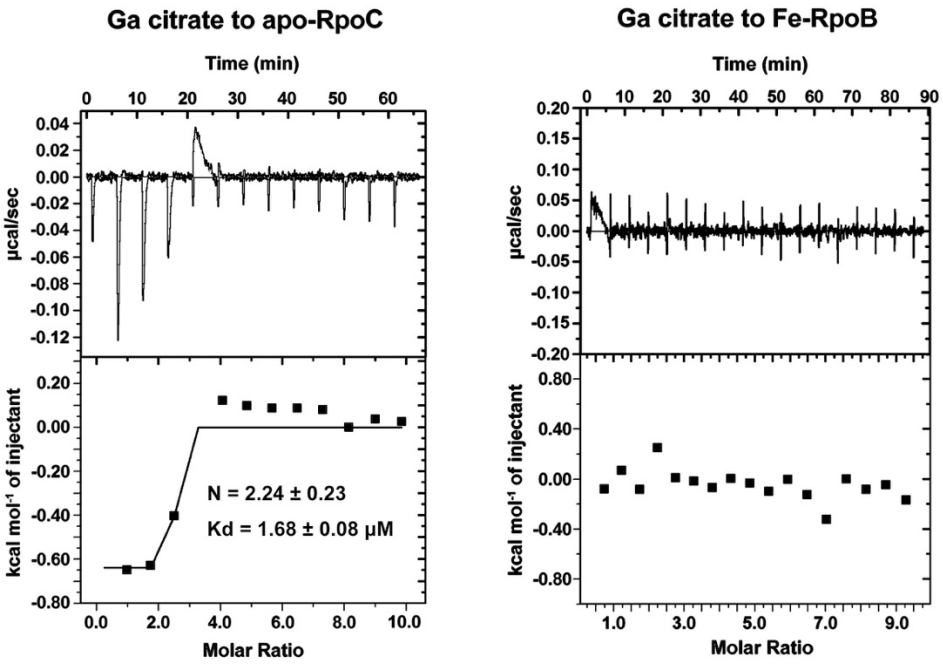

Fig. S2 Calorimetric titration of gallium citrate (1 mM) to apo-RpoC (*Left*) and Fe-RpoB (*Right*). Fe(III) bound to RpoB was in a molar ratio of 2:1. The protein concentration of 20 µM was used in the experiments. The titration curve of Ga(III) to apo-RpoC was fitted to a one-set-of-sites binding model using Origin software.

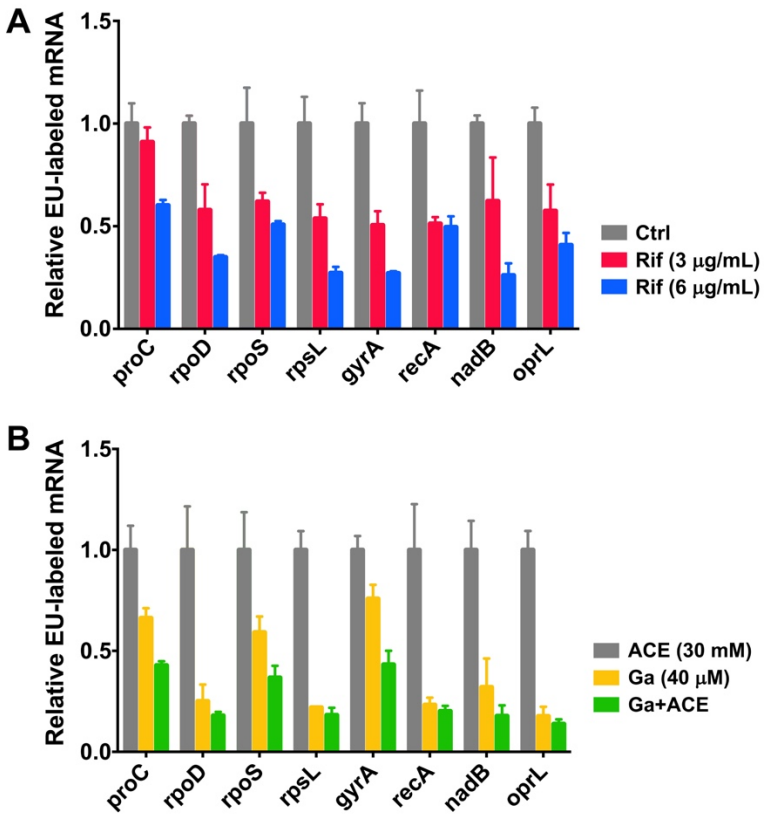

Fig. S3 Analysis of EU-labeled mRNA levels from *P. aeruginosa* treated with different concentrations of rifamycin (A) or $Ga(NO_3)_3$ and sodium acetate (B). Rifamycin and Ga(III) treatment lead to obvious decrease of nascent mRNA levels of the selected house-keeping genes, indicative of inhibited bacterial transcription.

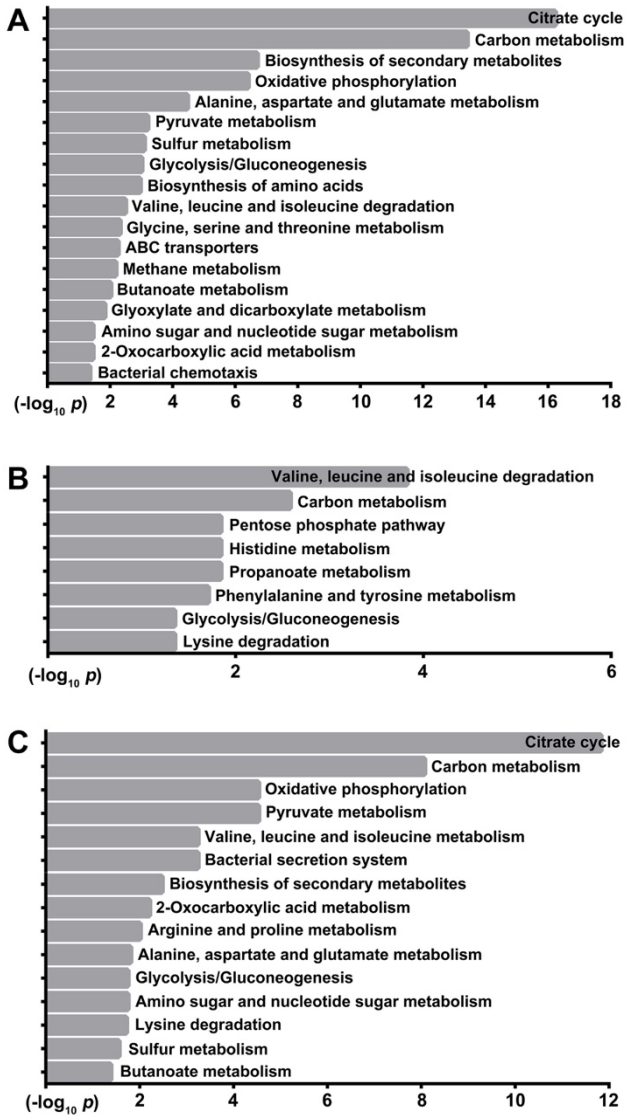

Fig. S4 Pathway enrichment analysis of differentially regulated gene transcriptions in *P. aeruginosa* upon treatment with 80 μM $Ga(NO_3)_3$ (A) or 30 mM ACE (B) or combination (C).

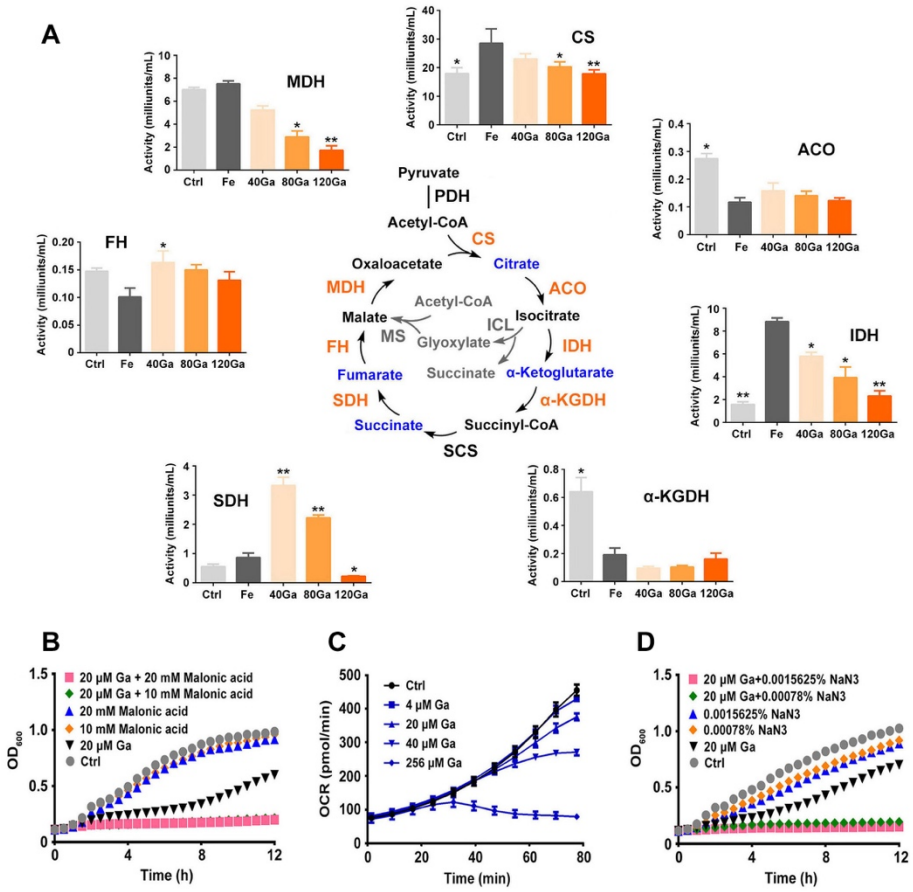

Fig. S5 Ga(III) treatment inhibits *P. aeruginosa* TCA cycle activity and attenuates bacterial respiration. (A) Effect of Ga(III) on enzyme activities in *P. aeruginosa* TCA cycle. Dose-dependent decrease in the enzyme activities were generally detected. The experimental groups are corresponding to non-treated control (Ctrl), 10 μM Fe(III) (Fe), 10 μM Fe(III) and 40 μM Ga(III) (40Ga), 10 μM Fe(III) and 80 μM Ga(III) (80Ga), 10 μM Fe(III) and 120 μM Ga(III) (120Ga) treatment conditions. The asterisks indicate significant difference from the 10 μM Fe(III) treatment group (*, $0.01 < p < 0.05$; **, $p < 0.01$) (B) TCA cycle inhibition by malonate on the inhibitory effect of Ga(III). (C) Real time changes in oxygen consumption rate (OCR) of *P. aeruginosa* in response to Ga(III) treatment. (D) ETC inhibition by NaN_3 on the inhibitory effect of Ga(III).

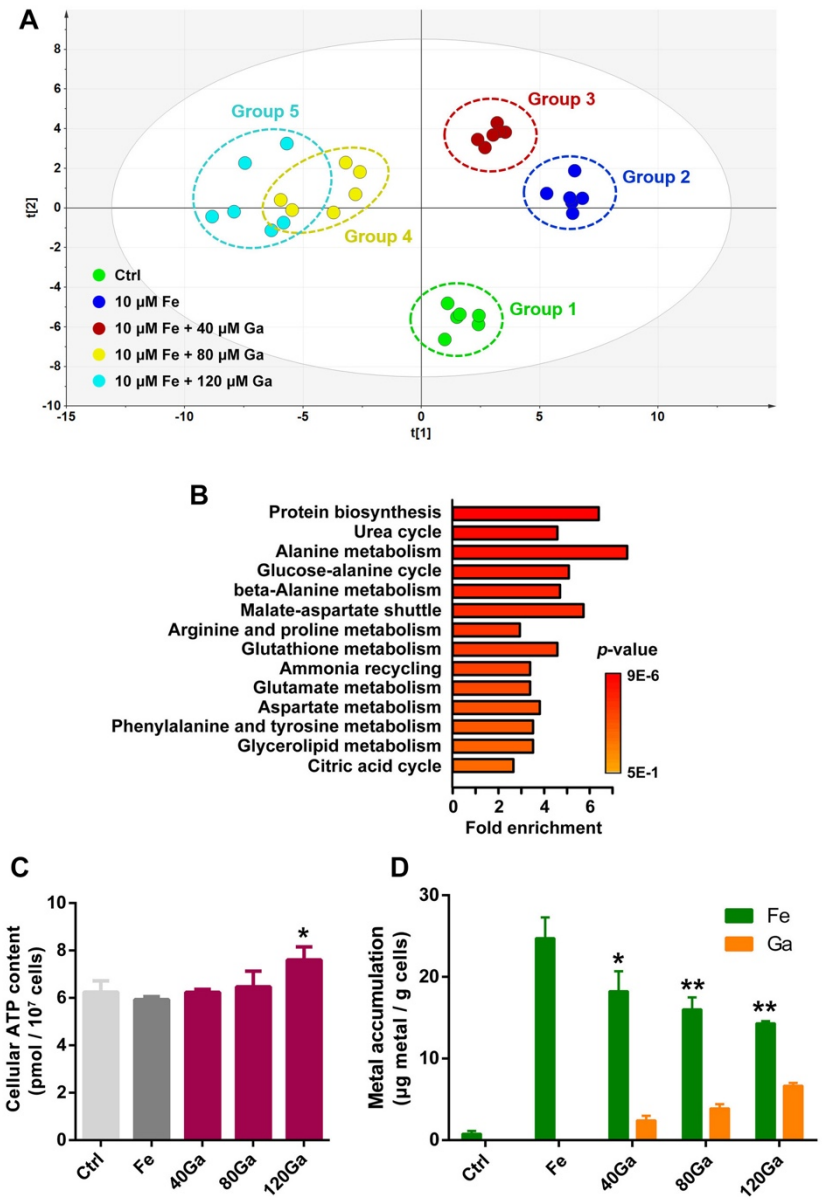

Fig. S6 (A) PCA analysis of GC-MS profiles obtained from control, Fe- and Ga-treated *P. aeruginosa* metabolite extracts. Each dot represents the metabolome profiling data from one individual sample. The metabolite profiles of *P. aeruginosa* under different Fe- and Ga-treatment conditions could be clearly separated. (B) Pathway enrichment analysis based on differentially regulated metabolites. (C) ATP content and (D) metal accumulation of *P. aeruginosa* under different Fe(III) and Ga(III) treatment conditions. The asterisks indicate significant difference from the 10 μM Fe(III) treatment group (*, $0.01 < p < 0.05$; **, $p < 0.01$). The experimental groups are corresponding to non-treated control (Ctrl), 10 μM Fe(III) (Fe), 10 μM Fe(III) and 40 μM Ga(III) (40Ga), 10 μM Fe(III) and 80 μM Ga(III) (80Ga), 10 μM Fe(III) and 120 μM Ga(III) (120Ga) treatment conditions.

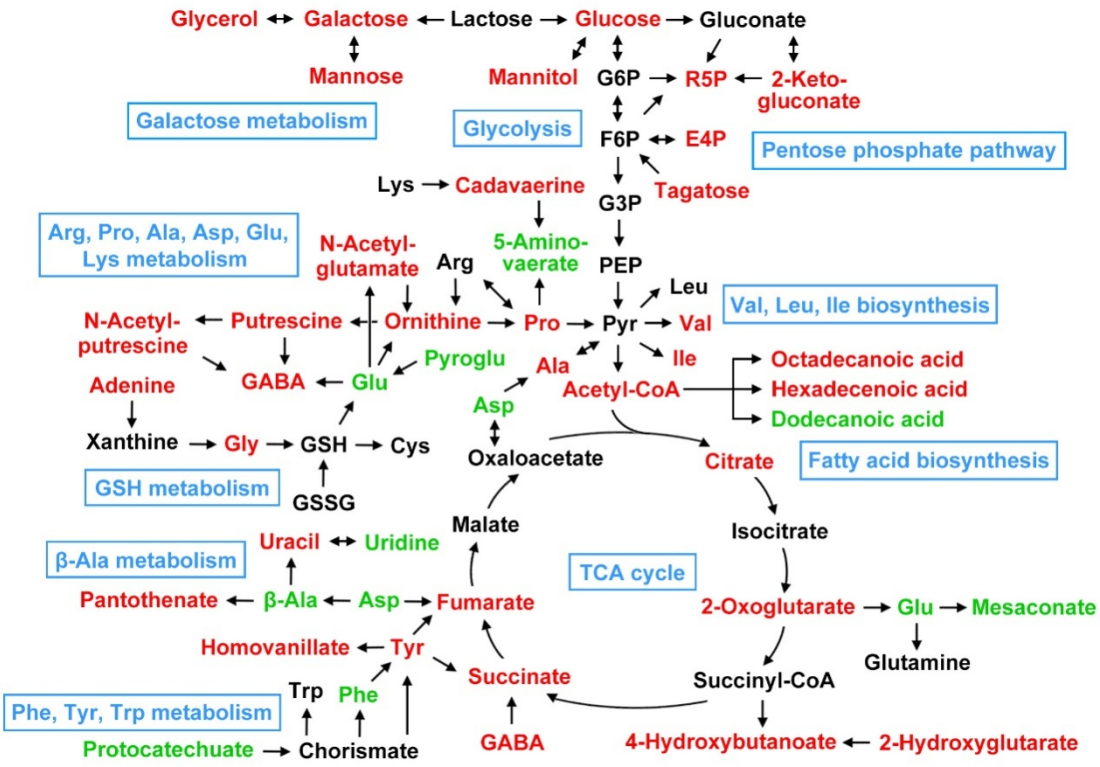

Fig. S7 Summary of metabolic pathways affected by Ga(III) in *P. aeruginosa*. Metabolites colored in red or green represent a higher or lower level in 120 μM Ga(III) treatment *P. aeruginosa* extracts compared to the 10 μM Fe(III) treatment group. The affected metabolic pathways are indicated in the figure.

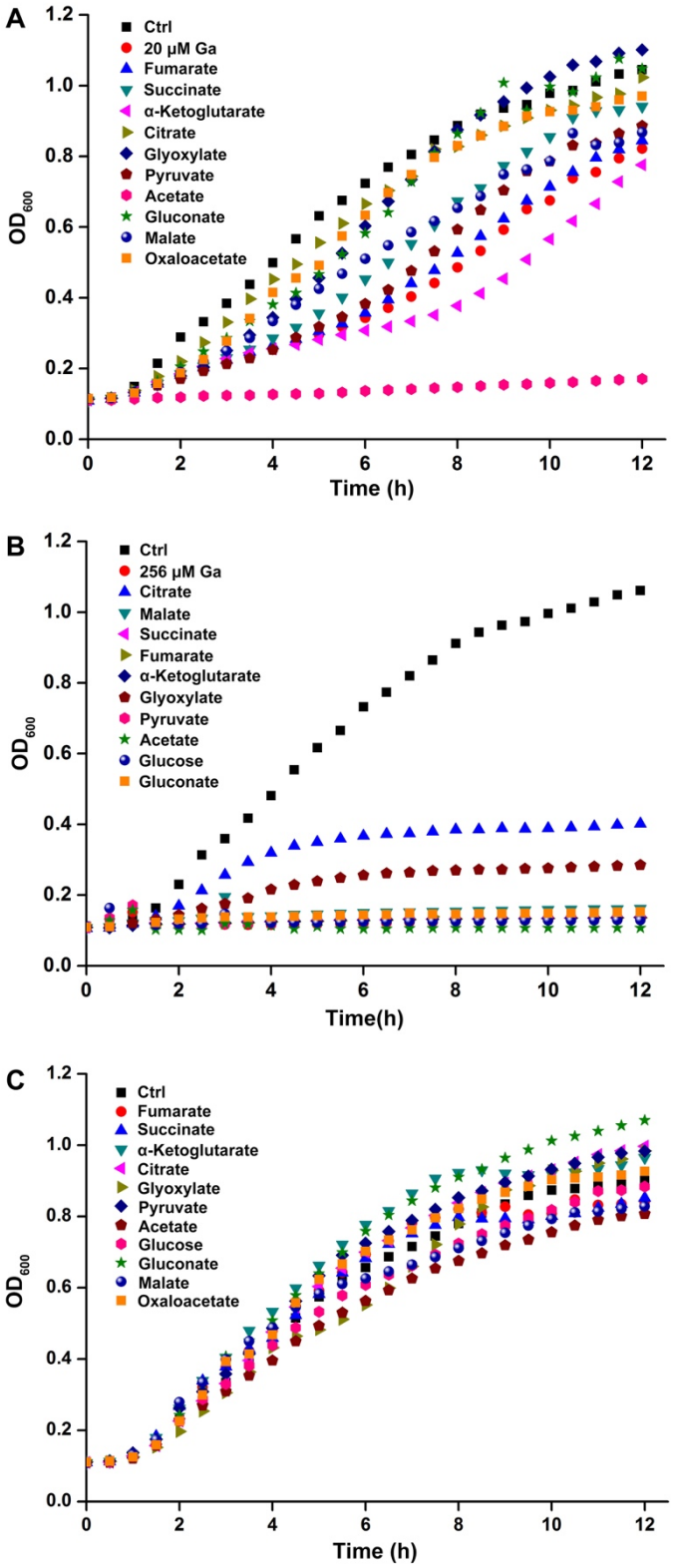

Fig. S8 Growth curves of *P. aeruginosa* in the presence of various central carbon metabolites with the supplementation of low (A) or high (B) concentrations of $Ga(NO_3)_3$, or metabolites alone (C). The supplemented metabolites are with a total carbon concentration of 60 mM. Data show one representative result of three independent experiments.

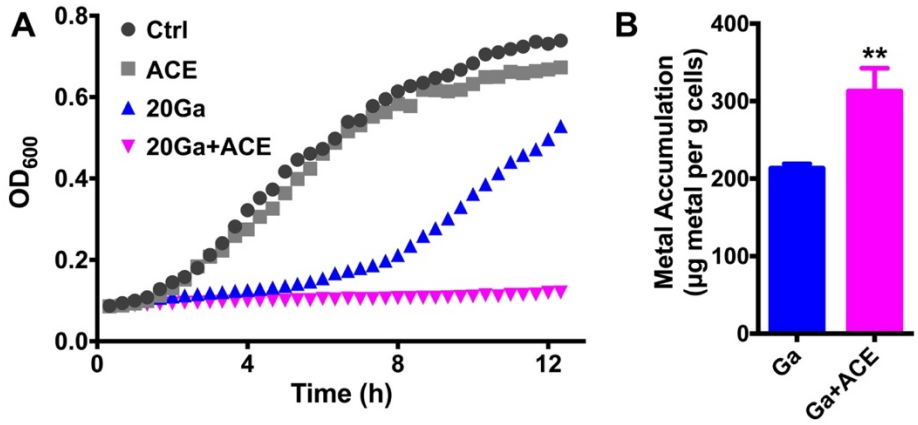

Fig. S9 Acetate enhances the antimicrobial activity of Ga(III) on a clinical isolated *P. aeruginosa* strain through increased Ga uptake. (A) Growth curves of *P. aeruginosa* clinical isolated strain in the presence of Ga(III) (20 μM) or the combination with acetate (30 mM). (B) Bacterial Ga accumulation. Clinical *P. aeruginosa* strain grown to OD = 0.2 were subjected to Ga(III) (80 μM) or the combined treatment for 4 h, and *ca.* 1×10^{10} cells were collected for the determination of Ga content.

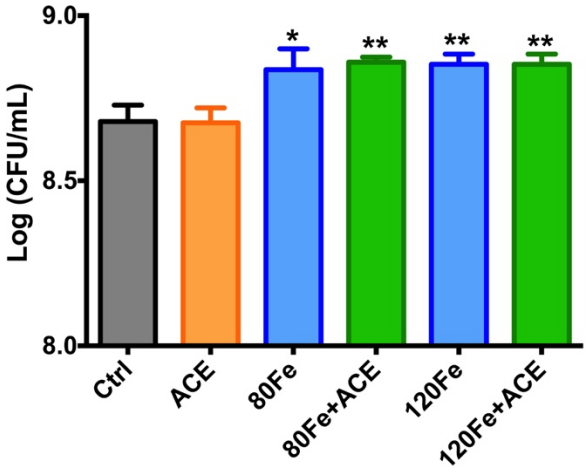

Fig. S10 A549 cell-associated bacterial colony counts in the *P. aeruginosa* cell infection model. The infected cells were cultured in the presence of $Fe(NO_3)_3$ (80 μM or 120 μM) or sodium acetate (ACE, 30 mM), or their combinations. The asterisks indicate significant difference from the control group (*, $0.01 < p < 0.05$; **, $p < 0.01$)

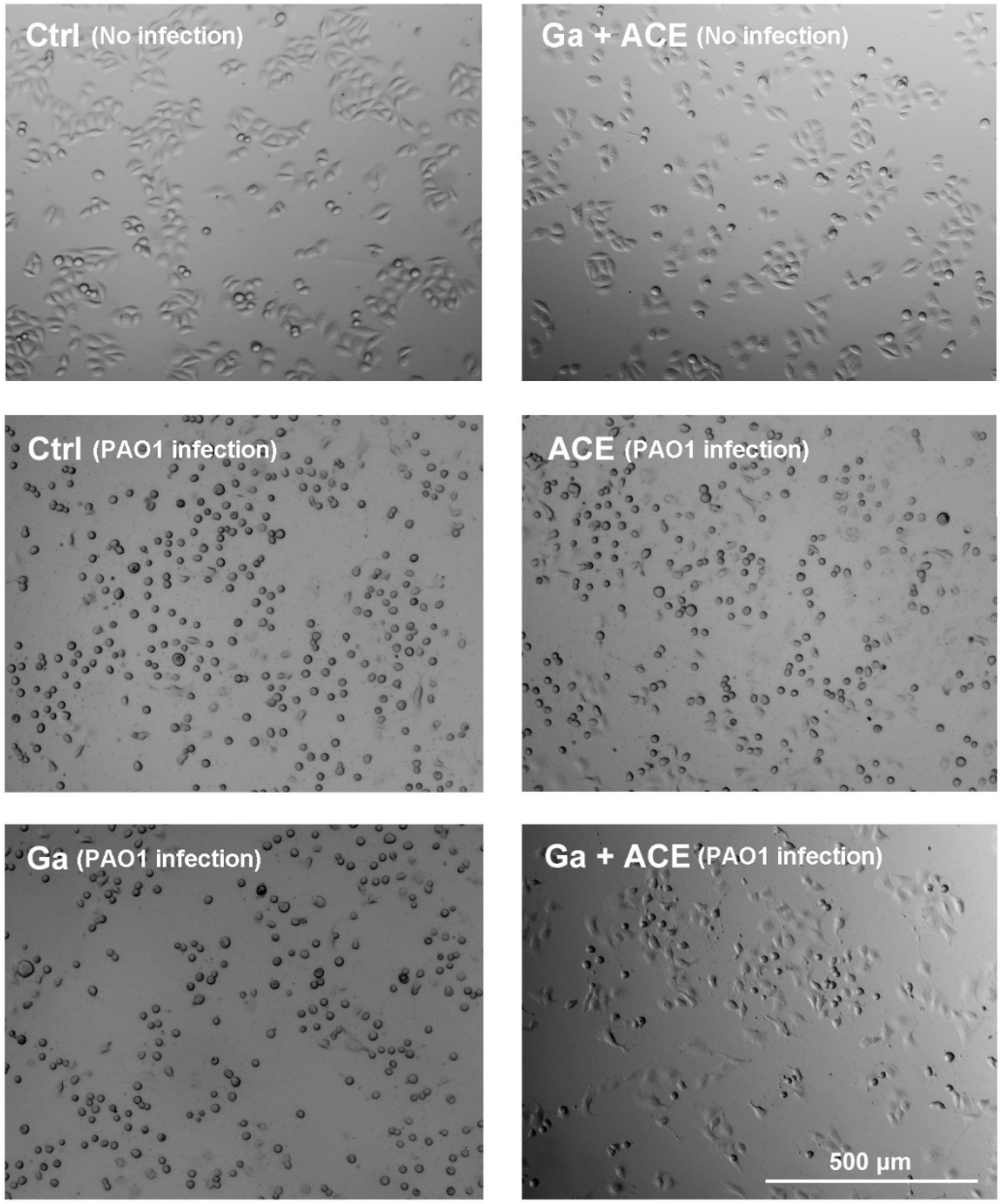

Fig. S11 Combined use of Ga(III) and acetate reduced the cytotoxic effect of *P. aeruginosa* in A549 cells. Images were captured using the 4 × magnification objective of the microscopy on Cytation 3 reader. The four *P. aeruginosa* infection groups (4 h infection) are corresponding to untreated control (Ctrl), 30 mM acetate (ACE), 80 µM Ga(III) (Ga), and 80 µM Ga(III) and 30 mM acetate (Ga+ACE). Upon *P. aeruginosa* infection, reduced cell death was observed in the presence of both Ga(III) and acetate.

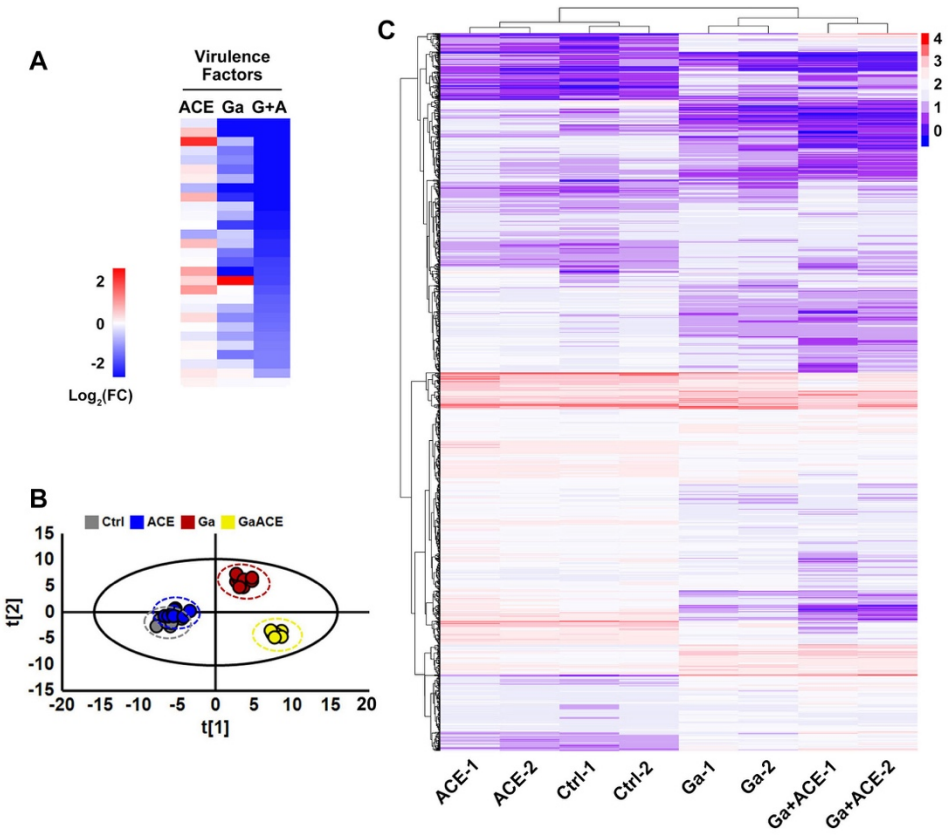

Fig. S12 Transcriptomic and metabolomic features of *P. aeruginosa* in response to gallium or acetate or their combinations. (A) Transcription profiles of genes encoding virulence factors of *P. aeruginosa* upon treatment with $Ga(NO_3)_3$ (80 μM) or sodium acetate (30 mM) or their combinations for 4 hrs. Values are reported as $\log_2$ fold changes over untreated control (corresponding to data in Supplementary Table 4). (B) Principal component analysis (PCA) score plot of control and treatment groups. (C) Cluster analysis of differentially regulated gene transcriptions in control and treatment groups of *P. aeruginosa*. The cluster map was created based on the normalized $\log_{10}$(FPKM) values.

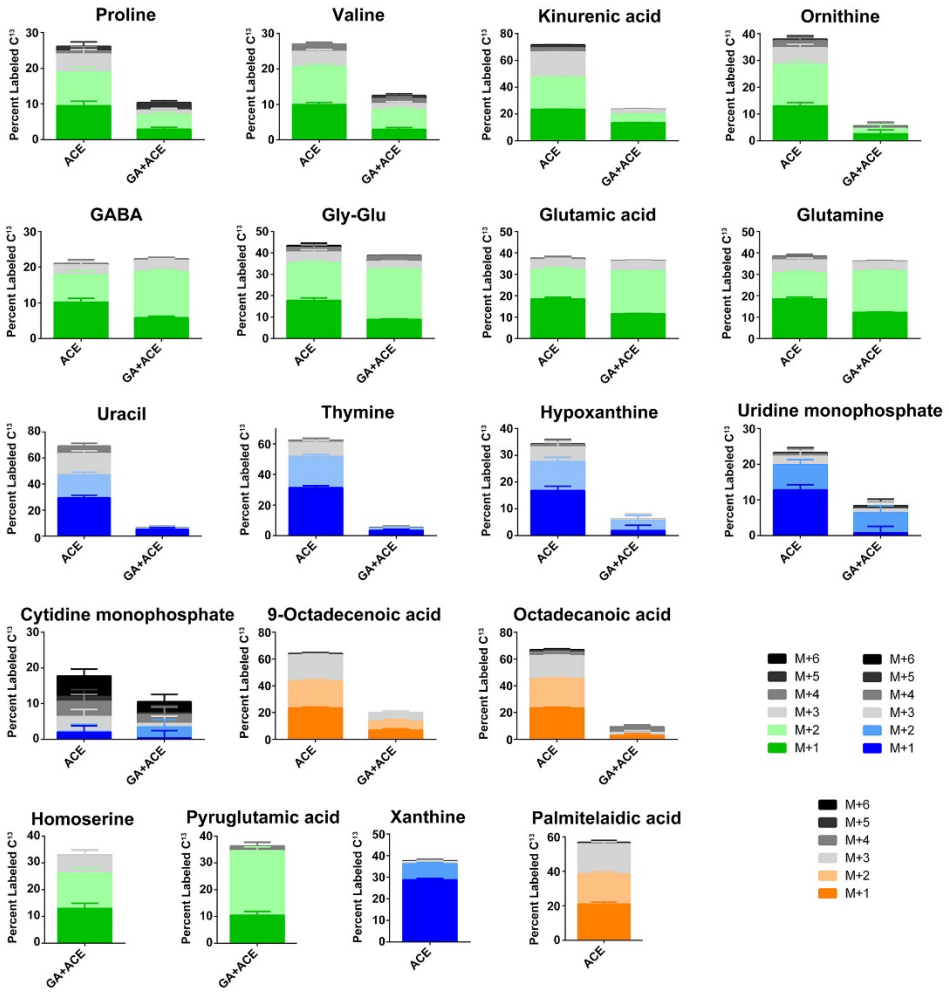

Fig. S13 Mass isotopomer distributions of $^{13}C_2$-acetate detected in various metabolites upon treatment with or without $Ga(NO_3)_3$ (80 μM). The metabolites colored in green belong to amino acids, blue to nucleotides and orange to fatty acids.

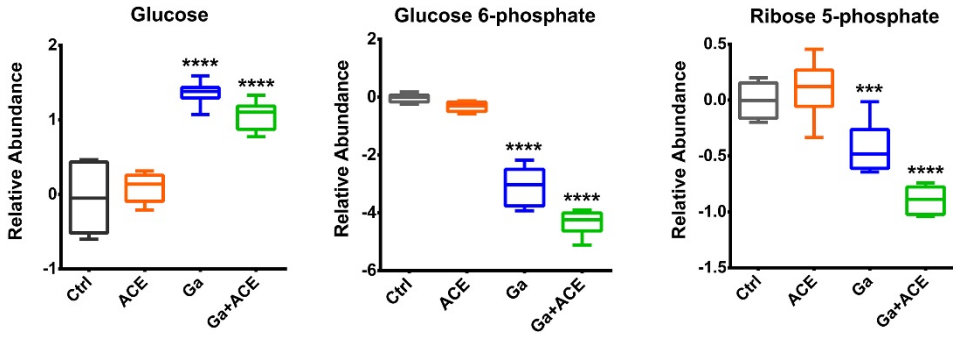

Fig. S14 Relative abundance of key metabolites in glycolysis and pentose phosphate pathway upon treatment with $Ga(NO_3)_3$ (80 μM) or ACE (30 mM) or their combinations. The asterisks indicate significant difference from the control group (***, $0.0001 < p < 0.001$; ****, $p < 0.0001$).

Table S1. Peptide mass fingerprints of *Pa*RpoB and *Pa*RpoC analyzed by Orbitrap LC-MS

Uniprot ID	Gene Name	Protein Name	Protein MW (kDa)	Peptide Sequence	Charge	Peptide Mass (Da)
Q9HWC9	*rpoC*	DNA-directed RNA polymerase subunit beta'	154.3	HLNVFEGEQVNR	3	1441.72
				LGIQAFEPVLIEGK	2	1513.86
				FATSDLNDLYR	2	1314.63
				YIVNEIQDVYR	2	1411.72
				IGLASPEMIR	2	1086.60
				SVITVGPTLR	2	1042.63
Q51561	*rpoB*	DNA-directed RNA polymerase subunit beta	150.7	STGSYSLVTQQPLGGK	2	1622.84
				APEDVTLMDVSPK	2	1401.69
				LQQGDDLAPGVLK	2	1353.74
				IVEGATFER	2	1021.53
				YDLSAVGR	2	880.45

Table S2. Primers used for RT-PCR analysis

Gene ID	Gene		Sequence (5'-3')
PA0393	*proC*	For	CAGGCCGGGCAGTTGCTGTC
		Rev	GGTCAGGCGCGAGGCTGTCT
PA0576	*rpoD*	For	GGGCGAAGAAGGAAATGGTC
		Rev	CAGGTGGCGTAGGTGGAGAA
PA3622	*rpoS*	For	CTCCCCGGGCAACTCCAAAAG
		Rev	CGATCATCCGCTTCCGACCAG
PA4268	*rpsL*	For	GCAAGCGCATGGTCGACAAGA
		Rev	CGCTGTGCTCTTGCAGGTTGTGA
PA3168	*gyrA*	For	TGTGCTTTATGCCATGAGCGA
		Rev	TCCACCGAACCGAAGTTGC
PA3617	*recA*	For	AGATCATCGATCTGGGCGTG
		Rev	TGGTCGCGAATGGTCTTCTC
PA0761	*nadB*	For	CGATGTACTGGTCATCGGCA
		Rev	GGTGGTATTCGAAGCTGCCT
PA0973	*oprL*	For	CGTGCGATCACCACCTTCTA
		Rev	CTCGCCCAGAGCCATATTGT

Table S3. Metabolites identified by GC-MS in *P. aeruginosa* PAO1

Identified Compound	PubChem CID	*p*-value *	Average Integrated Peak Area				
			Ctrl	10 Fe	40 Ga	80 Ga	120 Ga
Pyridine, 2-hydroxy- (1TMS)	8871	0.511	682246	1037531	983616	887125	905251
Lactic acid (2TMS)	107689	0.000	3656989	988792	1732014	5783190	7722360
L-Alanine (3TMS)	5950	0.000	4538491	1900318	9062271	5597338	6141267
Glycolic acid (2TMS)	757	0.359	4516184	3194322	3391943	3002132	3414117
Phosphoric acid monomethyl ester (2TMS)	13130	0.016	1604714	910143	1740276	1629000	1276147
Valine (2TMS)	6287	0.000	447716	60417	1062645	8352568	8375696
Butanoic acid, 4-hydroxy- (2TMS)	10413	0.000	3445	12401	38651	72406	91519
Norvaline, DL- (2TMS)	824	0.588	1021266	777397	1098288	798010	943445
Ethanolamine (3TMS)	700	0.002	1235399	5790962	13029588	12138870	15302595
Propane-1,2-diol (2TMS)	1030	0.000	3555	2027	1127021	1578303	531293
Isoleucine (2TMS)	6306	0.000	393	348	490	822542	3128453
Proline (2TMS)	145742	0.000	624	727	877	657008	1420780
Glycine (3TMS)	750	0.000	5890060	6457828	11766319	16479837	14967721
Heneicosanoic acid (1TMS)	16898	0.661	960976	1003295	1313036	922016	993518
Succinic acid (2TMS)	1110	0.000	800479	599249	1143453	2093834	2399872

Fumaric acid, 2-methyl- (2TMS)	638129	0.000	1306331	3545107	3695692	1293421	1506272
Uracil (2TMS)	1174	0.000	1002949	184327	1071890	1923566	2607799
Fumaric acid (2TMS)	444972	0.000	709956	472893	288900	697500	904782
Benzoic acid, 3,4-dihydroxy- (3TMS)	72	0.000	205696	458866	440899	230670	221772
Homovanillic acid (2TMS)	1738	0.003	638454	353981	474226	495251	653409
Glutaric acid (2TMS)	743	0.000	2499414	1357734	946745	458346	263198
Alanine, beta- (3TMS)	239	0.000	479490	1071464	1933628	981626	750017
Aspartic acid (2TMS)	5960	0.000	345423	3691717	4491349	539090	172868
Glycerol (3TMS)	753	0.000	2989	4929	27787	56284	152444
Pyroglutamic acid (1TMS)	7405	0.000	40749853	50016030	109095242	82265157	77907984
Butanoic acid, 4-amino- (3TMS)	119	0.006	4815536	16410938	32433703	22673307	22398740
Glutamic acid (2TMS)	33032	0.000	4342446	13063157	13634291	3038576	3195621
Phenylalanine (1TMS)	6140	0.000	3471604	11577552	7458293	916239	499763
Glutaric acid, 2-hydroxy- (3TMS)	43	0.000	24892	17287	31343	118741	72802
Glutaric acid, 2-oxo- (2TMS)	51	0.000	2095	23873	91507	54060	64188
Valeric acid, 5-amino- (3TMS)	138	0.000	59315256	46642888	27636990	7400527	3337646
Putrescine, N-acetyl- (2TMS)	122356	0.000	586105	853807	3432947	2222735	2536749

Glyceric acid-2,3-diphosphate (5TMS)	61	0.018	280979	268967	528840	377454	252331
Dodecanoic acid (1TMS)	3893	0.003	190843	237440	181019	91555	125251
Pyrophosphate (4TMS)	644102	0.000	26762	826	2582	113555	169483
Adenine (2TMS)	190	0.000	577161	456937	455737	641773	607581
Arabinose (4TMS)	66308	0.028	193804	433765	804506	491256	491164
Ribitol (5TMS)	6912	-	8513522	8721038	10651634	8368441	8534133
Glycerol-2-phosphate (4TMS)	2526	0.000	3314370	1204074	3764293	4713054	5250977
Putrescine (4TMS)	1045	0.000	8393094	2492419	18681265	25517020	25519803
Ornithine (3TMS)	6262	0.000	407	293	47797	1162686	432736
Glycerol-3-phosphate (4TMS)	754	0.021	13088025	11380015	19492783	18501298	17202024
Ethanolaminephosphate (4TMS)	1015	0.000	14689107	7688300	12771541	16812160	14008568
Glutamic acid, N-acetyl- (2TMS)	185	0.000	397390	74199	306296	179031	301800
Citric acid (4TMS)	311	0.000	420714	594322	1788555	1405172	1622701
Cadaverine (4TMS)	273	0.000	1323577	291697	1748473	1642258	620031
Tetradecanoic acid (1TMS)	11005	0.040	105785	172966	338271	223445	202978
N-α-Acetyl-L-Lysine (3TMS)	192590	0.000	2075	5336	7676	2262459	1187207
Tagatose (5TMS)	92092	0.000	216546	110432	106793	177658	622211
Tyrosine (2TMS)	6057	0.000	1207997	395267	1315820	1451477	1646766

Gluconic acid, 2-oxo- (5TMS)	3035456	0.000	525892	297676	403646	360416	1846591
Glucose (5TMS)	5793	0.000	1759678	1174804	2893401	3511934	4146328
Mannose (5TMS)	18950	0.000	266543	221864	528996	431618	692419
Mannitol (6TMS)	6251	0.000	2117057	1099573	2305694	2947892	2586524
Erythrose-4-phosphate (4TMS)	122357	0.000	3498	1155	5377	130910	586506
Ribulose-5-phosphate (5TMS)	53477706	0.000	7316	5198	9506	246705	1386223
Pantothenic acid, D- (3TMS)	6613	0.000	1033	1025	6253	63285	42498
Hexadecenoic acid, 9-(Z)- (1TMS)	5282743	0.000	141508	1296361	5071257	1814586	1387204
Hexadecanoic acid (1TMS)	985	0.013	6921737	16319838	31587729	21533357	24323339
Galactose (1MEOX) (5TMS)	439357	0.000	250123	52654	120408	425678	403157
Heptadecanoic acid (1TMS)	10465	0.575	21631	30090	39849	24118	24058
Octadecanoic acid (1TMS)	5281	0.000	3718796	4788161	7319700	9296432	12231961

* One-way ANOVA was used for the significance analysis among different Fe- and Ga-treatment groups. The value of 0.000 represents p-value < 0.001.

Table S4. Regulations of genes coding for *P. aeruginosa* virulence factors

Gene Name	ACE/Ctrl*	Ga/Ctrl*	(Ga+ACE)/Ctrl*	Gene Description
algC	-0.1192	-0.6013	-1.2668	Phosphomannomutase/phosphoglucomutase
aprA	-0.0488	-0.0266	-1.3037	Alkaline metalloproteinase
estA	-0.6046	-2.2565	-2.2492	Esterase
exoS	0.0950	-0.0571	-0.0440	Exoenzyme S
exoT	-0.2084	-0.7695	-1.0850	Exoenzyme T
exoY	-0.7161	-0.4077	-1.8191	Adenylate cyclase
lasA	0.6166	-1.7160	-2.1766	Protease LasA
lasB	-0.1910	-50.79	-7.6126	Elastase
phnA	-0.2437	-1.0969	-2.8696	Anthranilate synthase component 1, pyocyanine specific
phnB	-0.3937	-0.9703	-2.8338	Anthranilate synthase component 2, pyocyanine specific
phzS	1.6957	-0.5544	-3.1673	5-methylphenazine-1-carboxylate 1-monooxygenase
plcB	0.7514	-2.4203	-1.5395	Phospholipase C
pldA	0.0076	-1.5699	-1.8963	Phospholipase D
pqsE	0.1459	-0.6515	-2.4149	Thioesterase PqsE
pslA	0.0331	-1.1019	-1.0312	Biofilm formation protein PslA
pslE	0.0145	-0.4848	-1.1650	Biofilm formation protein PslE
pslF	0.8308	-0.0152	-1.4060	Biofilm formation protein PslF
pslG	0.2870	-0.9455	-1.2112	Biofilm formation protein PslG

pslH	0.5417	-0.4595	-1.7266	Biofilm formation protein PslH
pvdA	0.1932	0.0780	-0.7533	L-ornithine N5-oxygenase
pvdE	-0.0546	-1.0867	-1.7221	Pyoverdine biosynthesis protein PvdE
pvdF	-0.0559	-0.6396	-1.9008	Pyoverdine synthetase F
pvdH	0.2229	-1.1965	-2.4316	Diaminobutyrate--2-oxoglutarate aminotransferase
pvdL	0.0294	-1.3385	-1.5700	Peptide synthase
pvdS	0.3218	2.1103	-1.5057	Pyoverdine biosynthesis and secreted toxins
rhlA	-0.1007	-0.4057	-2.1187	3-(3-hydroxydecanoyloxy)decanoate synthase
rhlI	-0.1879	-0.2580	-1.0262	Acyl-homoserine-lactone synthase
rhlR	0.0714	-0.3066	-1.0411	Regulatory protein RhlR
toxA	0.4642	-3.6282	-3.6614	Exotoxin A

* Values displayed as $\log_2$ fold changes over untreated control group.

Table S5. Significantly regulated gene transcripts in *P. aeruginosa* upon acetate treatment

Gene Name	ACE/Ctrl*	Regulation	Gene Description
PA2010	5.5126	Up	Transcriptional regulator
hpd	5.6272	Up	4-hydroxyphenylpyruvate dioxygenase
maiA	5.0877	Up	Maleylacetoacetate isomerase
phhA	4.7925	Up	Phenylalanine 4-monooxygenase
PA5106	4.5154	Up	N-formimino-L-glutamate deiminase
hmgA	4.1557	Up	Homogentisate 1,2-dioxygenase
PA1137	3.9316	Up	Oxidoreductase
phhC	3.0067	Up	Aromatic amino acid aminotransferase
PA0534	2.8890	Up	Hypothetical protein
hutH	2.6638	Up	Histidine ammonia-lyase
hutC	2.6119	Up	Histidine utilization repressor HutC
PA3860	2.4457	Up	Acyl-CoA synthetase
aroP2	2.3159	Up	Aromatic amino acid transporter AroP
PA0530	2.3036	Up	Class III pyridoxal phosphate-dependent aminotransferase
phzB1	2.2069	Up	Phenazine biosynthesis protein
dhcB	2.1538	Up	Dehydrocarnitine CoA transferase subunit B
PA0187	2.1026	Up	Hypothetical protein
PA0529	2.0974	Up	Hypothetical protein

atoB	2.0790	Up	Acetyl-CoA acetyltransferase
hutU	2.0003	Up	Urocanate hydratase
PA4130	-2.1208	Down	Sulfite/nitrite reductase
mdcA	-2.1258	Down	Malonate decarboxylase subunit alpha
mdcE	-2.2215	Down	Malonate decarboxylase subunit gamma
PA0215	-2.2592	Down	Malonate transporter MadL
metE	-2.4178	Down	5-methyltetrahydropteroyltriglutamate
PA0216	-2.6761	Down	Malonate transporter MadM
PA3568	-3.0708	Down	Propionyl-CoA synthetase
PA3710	-3.2033	Down	GMC-type oxidoreductase
mmsA	-3.2550	Down	Methylmalonate-semialdehyde dehydrogenase
mmsB	-3.4503	Down	3-hydroxyisobutyrate dehydrogenase
PA3709	-3.8141	Down	Major facilitator superfamily transporter
PA4133	-4.0199	Down	Cbb3-type cytochrome C oxidase subunit I

* Values displayed as $\log_2$ fold change. Only gene transcriptions with fold change > 4 and adjusted p-value < 0.05 are listed.

Table S6. NTFD analysis of metabolites labeled by ^{13}C in the presence of [U-$^{13}C_2$] acetate.

Metabolite	RT Unlabeled	RT Labeled	Frag	M+0 (%)	M+1 (%)	M+2 (%)	M+3 (%)	M+4 (%)	M+5 (%)	M+6 (%)
Lactic acid	6.0127	5.9888	219	94.76	3.32	2.29	0.25			
Alanine	6.9749	6.9511	116	90.98	7.59	1.47	-0.05			
			218	93.51	3.69	2.92	0.31			
Valine	9.7613	9.7492	156	75.36	9.96	10.73	4.22	1.95	0.08	
Proline	11.8629	11.8626	142	74.91	9.51	9.43	5.05	0.83	1.33	
Succinic acid	12.3647	12.3644	172	65.75	16.43	14.21	3.69	0.12		
			247	65.50	17.90	12.31	3.74	0.73		
Uracil	12.9136	12.9075	255	39.81	29.51	17.55	16.37	5.83		
Fumaric acid	13.3328	13.3326	245	72.54	14.57	9.17	3.26	0.61		
Serine	13.5571	13.5569	204	94.42	5.66	-0.02	-0.05			
Threonine	14.2006	14.2003	57	92.50	5.63	5.89	-2.82	-2.71		
			117	92.01	4.64	3.60	-0.08	-0.17		
			291	90.53	5.03	4.87	-0.90	0.62		
Thymine	14.5194	14.5073	197	41.40	32.51	21.30	6.63	-1.60	-0.64	
			270	37.85	31.34	20.46	9.15	1.34	0.07	
beta-Alanine	15.2454	15.2452	160	91.13	5.28	1.20	2.86			
GABA	17.7485	17.7423	246	79.28	10.19	7.78	2.64	0.42		
			304	78.32	11.13	7.58	2.39	0.59		
Glycyl-L-Glutamic acid	17.8311	17.8250	84	60.15	22.61	13.16	3.84	0.76	0.01	0.01
			186	56.51	18.78	17.51	5.62	1.94	0.37	0.02
			276	56.85	17.77	17.61	4.92	2.17	0.01	0.90
2-Hydroxyglutaric acid	18.8878	18.8876	129	65.03	25.72	8.85	-0.13	5.82	-3.49	
Glutamic acid	19.9563	19.9502	84	63.47	19.18	15.47	3.92	-0.63	0.13	

			156	62.89	18.52	13.89	4.53	1.58	-0.07	
			230	62.96	18.72	13.61	4.08	0.75	-0.03	
			246	64.52	17.23	13.70	4.07	0.59	-0.11	
			320	62.55	18.52	13.89	4.30	0.90	-0.06	
Putrescine	22.4475	22.4709	200	94.54	2.12	2.15	1.06	0.22		
			214	94.56	2.05	2.15	1.01	0.28		
Glycerol-2-phosphate	23.0555	23.0612	129	91.05	4.55	5.50	0.28			
			256	94.61	5.25	0.06	0.06			
			357	92.39	4.09	3.60	-0.04			
Glutamine	23.2740	23.2737	245	61.72	18.47	12.32	5.87	1.57	0.17	
			347	56.24	18.20	13.14	8.96	3.94	-0.05	
Ethanolamine phosphate	23.4215	23.4272	100	94.09	6.92	1.48				
Cytidine monophosphate	23.6577	23.6633	111	84.96	1.94	0.28	4.17	4.24	1.36	5.78
Hypoxanthine	23.8407	23.8404	193	67.71	16.86	10.77	5.49	0.97	0.16	
Ornithine	24.1831	24.1769	420	62.34	13.03	15.51	6.21	2.61	0.60	
Citric acid	24.2362	24.2419	273	60.70	21.46	14.27	3.52	0.45	-0.05	0.02
Cadaverine	24.5254	24.5370	174	93.43	5.42	1.60	-0.37	-0.08	-0.01	
Tyrosine	26.6093	26.6091	192	93.41	4.64	1.38	-0.16	0.32	-0.15	0.02
Xanthine	28.1147	28.1085	353	65.67	26.70	4.55	0.50	1.57	-0.09	
			368	62.34	28.82	7.38	1.02	0.01	0.34	
Uridine-5'-monophosphate	28.2032	28.2030	169	78.51	11.45	6.61	2.76	0.47	0.95	0.01
			243	79.76	12.03	5.66	2.23	0.42	0.04	0.01
			258	77.23	12.81	7.04	2.38	0.62	0.14	0.22
Palmitelaidic acid	28.3213	28.3151	129	52.40	21.02	17.44	17.49	-2.14	0.87	-3.91
Hexadecanoic acid	28.7463	28.7461	299	92.67	0.27	-0.03	0.14	2.11	1.02	2.24
Ribofuranose-5-phosphate	28.9825	28.9940	217	84.74	12.04	3.18	-0.10	0.38	0.27	

D-Galactose	30.3756	30.3577	160	94.92	4.23	0.73	0.42	-0.33	-0.02	0.01
(E)-9-Octadecenoic acid	31.9223	31.9220	129	46.29	23.35	20.25	19.51	-4.02	0.98	0.01
			145	47.94	24.62	26.74	11.27	-2.88	-2.39	-2.26
Octadecanoic acid	32.2293	32.2290	231	33.08	23.28	22.04	17.06	2.74	1.35	0.56
			319	35.57	23.55	23.42	15.93	2.50	0.68	-0.23
Kinurenic acid	33.2446	33.2503	230	29.08	23.42	24.16	18.70	3.06	1.48	0.76
			304	29.68	41.32	20.29	5.71	2.64	1.03	0.02
D-Fructose-phosphate	33.3214	33.3211	129	94.44	4.38	2.48	-0.67	4.97	-4.78	0.01
			449	88.45	11.51	1.91	-1.07	-0.40	0.17	-0.94
Glucose-6-phosphate	33.5162	33.5160	247	93.77	3.46	1.14	-0.84	-3.16	1.47	3.32
Hexadecanoic acid	37.8374	37.8313	371	76.76	-2.12	3.61	0.96	5.97	2.52	9.77

Table S7. NTFD analysis of metabolites labeled by ^{13}C in the presence of [U-$^{13}C_2$] acetate and gallium.

Metabolite	RT Unlabeled	RT Labeled	Frag	M+0 (%)	M+1 (%)	M+2 (%)	M+3 (%)	M+4 (%)	M+5 (%)	M+6 (%)
Lactic acid	5.9890	6.0302	219	94.97	2.93	1.85	0.24			
Alanine	6.9513	6.9866	116	94.49	5.05	0.48	0.02			
			218	92.01	6.48	0.93	-0.50			
Valine	9.7494	9.7670	156	90.16	2.96	5.61	1.67	1.38	0.89	
Proline	11.8569	11.8627	142	90.60	2.90	4.17	1.05	0.26	2.00	
Succinic acid	12.3646	12.3645	172	69.08	9.99	**17.79**	4.67	-0.74		
			247	68.47	9.56	**18.00**	3.81	0.32		
Uracil	12.9077	12.9135	255	95.00	5.21	0.55	0.41	0.38		
Fumaric acid	13.3327	13.3326	245	80.81	6.46	**10.56**	2.08	0.28		
Serine	13.5571	13.5569	204	94.71	5.15	0.19	-0.02			
Threonine	14.2005	14.2004	57	89.80	6.70	9.20	-3.79	-3.81		
			117	90.52	4.94	4.71	-0.10	-0.06		
			291	91.54	0.85	8.06	-0.93	0.76		
Thymine	14.5134	14.5192	197	92.19	9.92	-2.59	0.02	0.02	0.01	
			270	94.81	3.49	1.45	0.05	0.01	0.27	
beta-Alanine	15.2395	15.2453	160	90.01	2.96	2.69	3.84			
Homoserine	15.8239	15.8238	218	71.99	**16.54**	9.42	3.28	-0.95		
			230	68.53	**12.99**	**13.24**	6.50	0.10		
Pyroglutamic acid	17.5772	17.5771	230	64.77	**11.27**	**21.12**	6.36	-2.20	-2.27	
			258	65.66	8.29	**25.87**	-2.80	2.43	0.43	
			273	63.66	**10.44**	**24.14**	0.01	1.74	-0.12	
GABA	17.7543	17.7542	246	77.66	5.84	**12.99**	3.09	0.42		
			304	75.22	7.67	**13.98**	2.80	0.34		
Glycyl-L-Glutamic	17.8311	17.8309	84	63.65	**13.77**	**18.98**	3.89	0.29	-0.03	0.01

acid			186	62.42	9.05	**22.73**	3.87	2.18	0.08	-0.23
			276	61.37	8.92	**23.63**	3.44	2.54	0.10	0.08
2-Hydroxyglutaric acid	18.8937	18.8935	129	64.32	**29.98**	8.22	-0.96	4.63	-4.96	
Glutamic acid	19.9563	19.9502	84	64.98	**12.96**	**21.12**	4.00	-1.54	0.02	
			156	64.24	**11.98**	**19.84**	4.56	0.69	-0.29	
			230	63.97	**12.58**	**19.47**	4.08	0.08	-0.09	
			246	64.49	**10.57**	**20.87**	4.06	0.03	-0.02	
			320	63.60	**11.57**	**20.06**	4.48	0.31	0.04	
Glutamine	23.2739	23.2797	245	63.96	**12.19**	**19.41**	4.17	0.38	0.01	
			347	61.76	8.23	**23.71**	3.35	2.72	0.39	
Ethanolamine phosphate	23.4215	23.4213	100	92.19	1.04	0.06				
Cytidine monophosphate	23.6635	23.6634	111	91.91	0.36	3.07	1.01	2.53	0.45	3.13
Hypoxanthine	23.8406	23.8405	193	94.85	1.89	3.72	0.54	0.07	0.01	
Ornithine	24.1771	24.1770	420	94.77	2.59	1.95	0.09	0.73	0.13	
Citric acid	24.2420	24.2419	273	63.38	**19.13**	**13.76**	3.67	0.99	0.03	-0.84
Uridine-5'-monophosphate	28.2032	28.2030	204	93.47	0.75	5.71	0.91	0.23	0.78	0.01
Hexadecanoic acid	28.7463	28.7461	299	94.68	1.99	-0.04	0.04	0.62	0.76	1.35
Ribofuranose-5-phosphate	28.9706	28.9764	215	92.52	1.58	-6.04	-3.21	-1.18	7.27	
D-Galactose	30.3697	30.3695	160	93.53	1.31	2.86	0.11	0.21	2.31	1.56
(E)-9-Octadecenoic acid	31.9222	31.9221	129	84.65	6.75	6.81	6.33	-2.79	-0.78	-1.83
			145	84.98	8.52	6.99	3.13	-0.88	-1.23	-1.10
Octadecanoic acid	32.2942	32.2940	171	94.95	4.76	-0.27	1.83	-1.17	-0.54	-0.08
			285	94.01	3.04	-0.67	2.04	4.05	0.08	-0.01
Kinurenic acid	33.2387	33.2385	230	77.18	**13.34**	6.49	3.39	0.02	-0.56	0.20

			304	78.23	**17.96**	3.62	0.18	-0.08	0.12	0.01
D-Fructose-phosphate	33.3154	33.3153	129	91.25	6.15	6.88	-1.42	3.69	-5.03	0.02
			449	89.05	4.64	4.81	2.14	0.64	-1.64	0.33
Glucose-6-phosphate	33.5102	33.5101	174	90.07	-2.44	3.47	2.33	-2.35	9.47	-1.98
Hexadecanoic acid	37.8314	37.8313	371	83.11	0.01	2.43	0.38	3.84	0.73	3.96

References

1. Y. Wang, C. N. Tsang, F. Xu, P. W. Kong, L. Hu, J. Wang, I. K. Chu, H. Li and H. Sun, *Chem. Commun.*, 2015, **51**, 16479-16482.
2. B. Peng, Y. B. Su, H. Li, Y. Han, C. Guo, Y. M. Tian and X. X. Peng, *Cell Metab.*, 2015, **21**, 249-261.
3. X. Domingo-Almenara, J. Brezmes, M. Vinaixa, S. Samino, N. Ramirez, M. Ramon-Krauel, C. Lerin, M. Diaz, L. Ibanez, X. Correig, A. Perera-Lluna and O. Yanes, *Anal. Chem.*, 2016, **88**, 9821-9829.
4. N. R. Glasser, S. E. Kern and D. K. Newman, *Mol. Microbiol.*, 2014, **92**, 399-412.
5. K. R. Allison, M. P. Brynildsen and J. J. Collins, *Nature*, 2011, **473**, 216-220.
6. S. Meylan, C. B. Porter, J. H. Yang, P. Belenky, A. Gutierrez, M. A. Lobritz, J. Park, S. H. Kim, S. M. Moskowitz and J. J. Collins, *Cell Chem. Biol.*, 2017, **24**, 195-206.
7. P. Uusitalo, U. Hagglund, E. Rhoos, H. Scherman Norberg, M. Elofsson and C. Sundin, *J Antibiot.*, 2017, **70**, 937-943.
8. X. Liao, F. Yang, R. Wang, X. He, H. Li, R. Y. T. Kao, W. Xia and H. Sun, *Chem. Sci.*, 2017, **8**, 8061-8066.
9. K. Hiller, C. M. Metallo, J. K. Kelleher and G. Stephanopoulos, *Anal. Chem.*, 2010, **82**, 6621-6628.
10. Y. B. Su, B. Peng, H. Li, Z. X. Cheng, T. T. Zhang, J. X. Zhu, D. Li, M. Y. Li, J. Z. Ye, C. C. Du, S. Zhang, X. L. Zhao, M. J. Yang and X. X. Peng, *Proc. Natl. Acad. Sci. USA*, 2018, **115**, E1578-E1587.
11. K. Hiller, A. Wegner, D. Weindl, T. Cordes, C. M. Metallo, J. K. Kelleher and G. Stephanopoulos, *Bioinformatics*, 2013, **29**, 1226-1228.