SUPPLEMENTAL INFORMATION

(p)ppGpp imposes graded transcriptional changes to impair motility and promote antibiotic tolerance in biofilms

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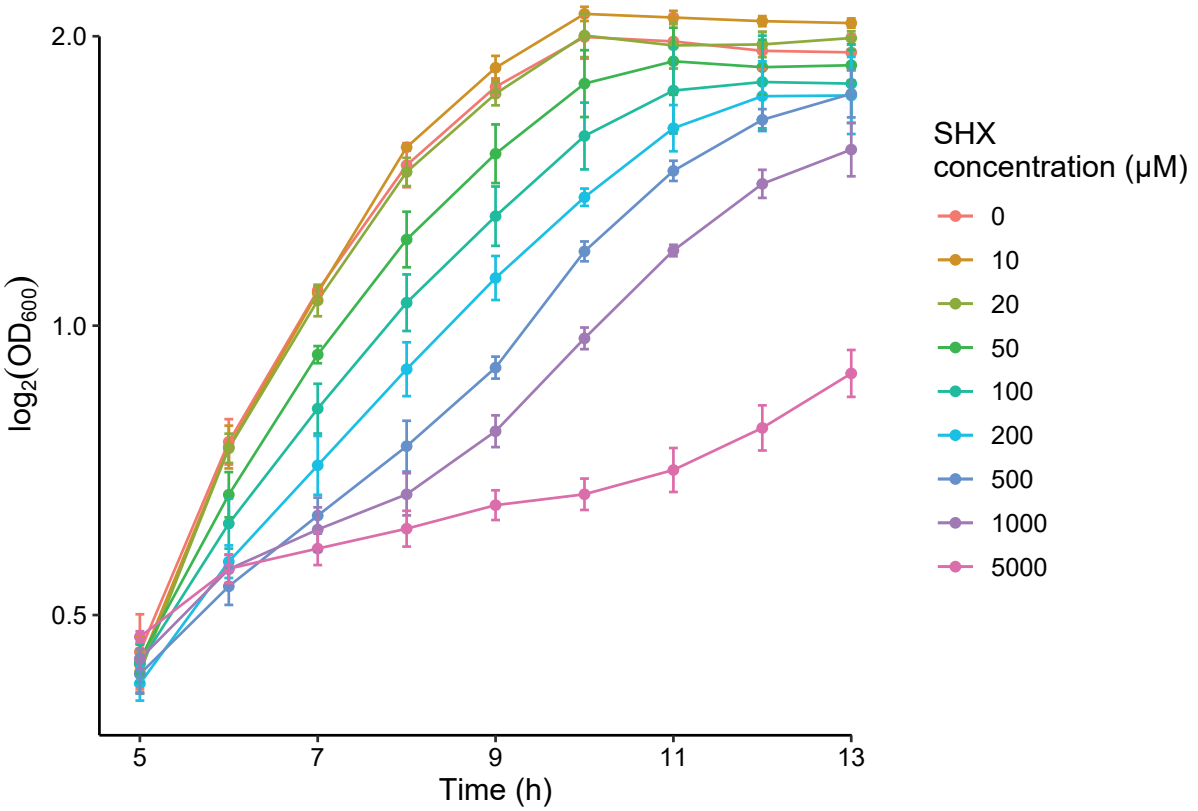
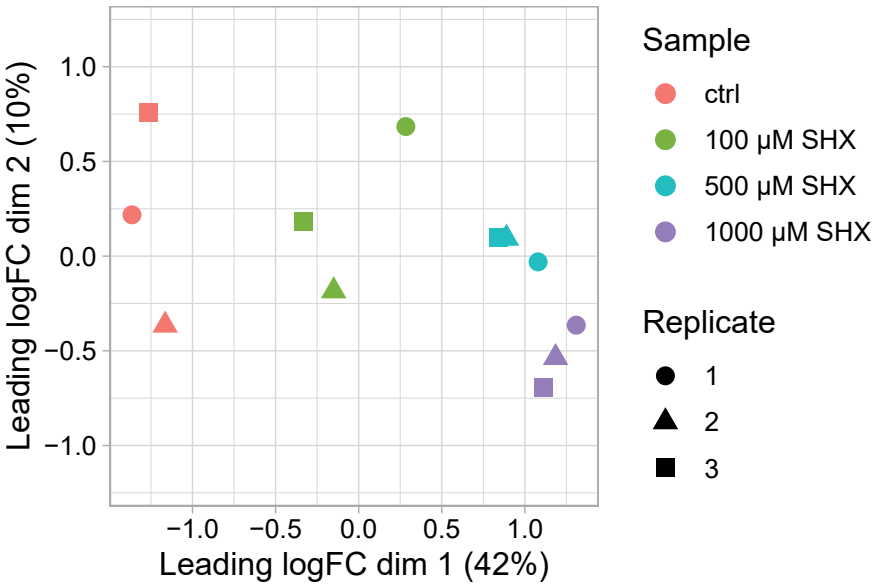
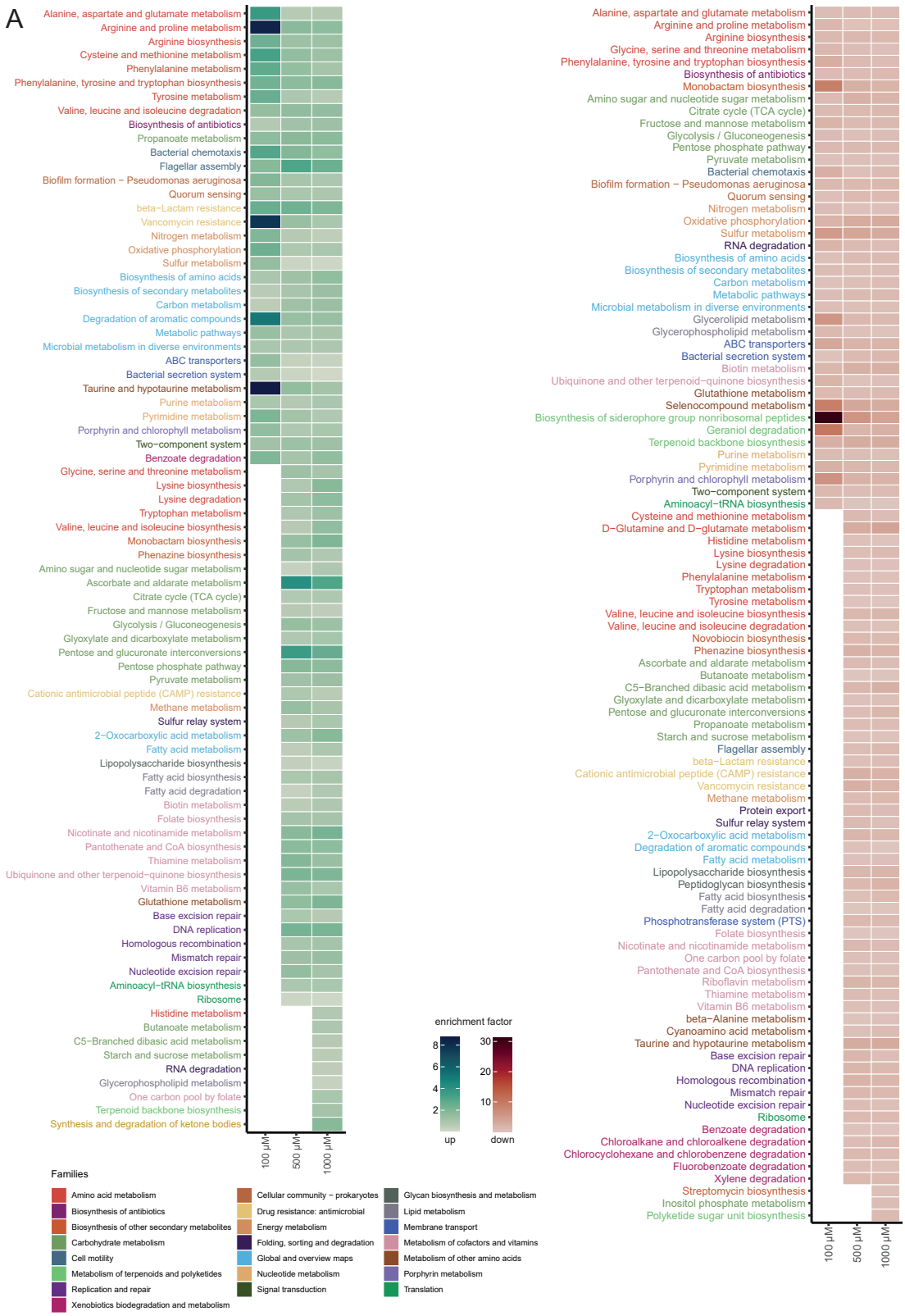


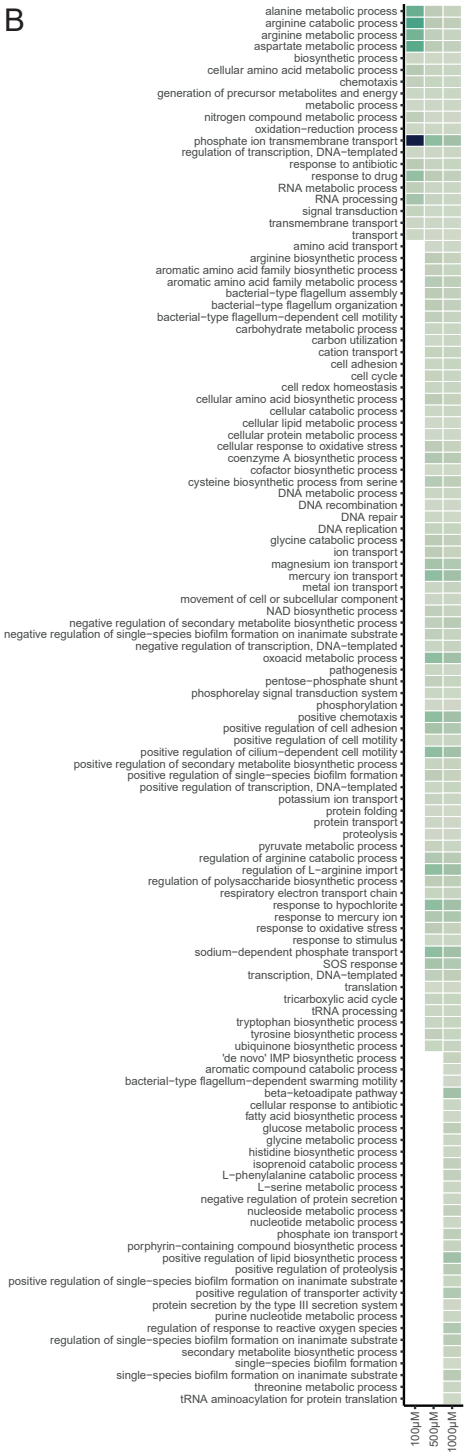
Supplementary Figure 1 (linked to Figure 1A). Effect of increasing concentrations of serine hydroxamate (SHX) on the growth of *P. aeruginosa* PA14.
The growth of exponentially *P. aeruginosa* PA14 was measured following treatment with increasing concentration of SHX. The experiment was performed in triplicates. Data points are shown as mean ± standard deviation.

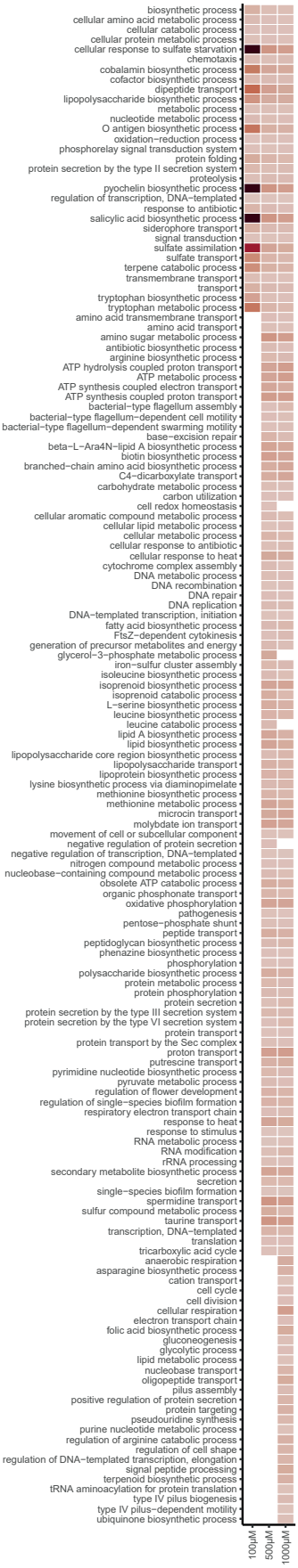


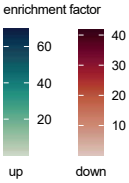
Supplementary Figure 2 (linked to Figure 2). Principal Component Analysis (PCA) of transcriptomic data from *P. aeruginosa* PA14 exponentially grown and treated with increasing concentrations of serine hydroxamate (SHX).
The analysis was performed on three biological replicates for each treatment condition. The first two principal components (dim 1 and dim2) are plotted, with each point representing a replicate sample. The plot shows the clustering of replicates from untreated and each SHX treatment group, highlighting the variation in gene expression profiles in response to different SHX concentrations. The distance between the clusters indicates the degree of transcriptional variation across treatments.


A
Alanine, aspartate and glutamate metabolism
Arginine and proline metabolism
Arginine biosynthesis
Cysteine and methionine metabolism
Phenylalanine metabolism
Phenylalanine, tyrosine and tryptophan biosynthesis
Tyrosine metabolism
Valine, leucine and isoleucine degradation
Biosynthesis of antibiotics
Propanoate metabolism
Bacterial chemotaxis
Flagellar assembly
Biofilm formation − Pseudomonas aeruginosa
Quorum sensing
beta−Lactam resistance
Vancomycin resistance
Nitrogen metabolism
Oxidative phosphorylation
Sulfur metabolism
Biosynthesis of amino acids
Biosynthesis of secondary metabolites
Carbon metabolism
Degradation of aromatic compounds
Metabolic pathways
Microbial metabolism in diverse environments
ABC transporters
Bacterial secretion system
Taurine and hypotaurine metabolism
Purine metabolism
Pyrimidine metabolism
Porphyrin and chlorophyll metabolism
Two−component system
Benzoate degradation
Glycine, serine and threonine metabolism
Lysine biosynthesis
Lysine degradation
Tryptophan metabolism
Valine, leucine and isoleucine biosynthesis
Monobactam biosynthesis
Phenazine biosynthesis
Amino sugar and nucleotide sugar metabolism
Ascorbate and aldarate metabolism
Citrate cycle (TCA cycle)
Fructose and mannose metabolism
Glycolysis / Gluconeogenesis
Glyoxylate and dicarboxylate metabolism
Pentose and glucuronate interconversions
Pentose phosphate pathway
Pyruvate metabolism
Cationic antimicrobial peptide (CAMP) resistance
Methane metabolism
Sulfur relay system
2−Oxocarboxylic acid metabolism
Fatty acid metabolism
Lipopolysaccharide biosynthesis
Fatty acid biosynthesis
Fatty acid degradation
Biotin metabolism
Folate biosynthesis
Nicotinate and nicotinamide metabolism
Pantothenate and CoA biosynthesis
Thiamine metabolism
Ubiquinone and other terpenoid−quinone biosynthesis
Vitamin B6 metabolism
Glutathione metabolism
Base excision repair
DNA replication
Homologous recombination
Mismatch repair
Nucleotide excision repair
Aminoacyl−tRNA biosynthesis
Ribosome
Histidine metabolism
Butanoate metabolism
C5−Branched dibasic acid metabolism
Starch and sucrose metabolism
RNA degradation
Glycerophospholipid metabolism
One carbon pool by folate
Terpenoid backbone biosynthesis
Synthesis and degradation of ketone bodies
100 μM
500 μM
1000 μM
enrichment factor
8
6
4
2
30
20
10
up
down
Families
Amino acid metabolism
Biosynthesis of antibiotics
Biosynthesis of other secondary metabolites
Carbohydrate metabolism
Cell motility
Metabolism of terpenoids and polyketides
Replication and repair
Xenobiotics biodegradation and metabolism
Cellular community − prokaryotes
Drug resistance: antimicrobial
Energy metabolism
Folding, sorting and degradation
Global and overview maps
Nucleotide metabolism
Signal transduction
Glycan biosynthesis and metabolism
Lipid metabolism
Membrane transport
Metabolism of cofactors and vitamins
Metabolism of other amino acids
Porphyrin metabolism
Translation
Alanine, aspartate and glutamate metabolism
Arginine and proline metabolism
Arginine biosynthesis
Glycine, serine and threonine metabolism
Phenylalanine, tyrosine and tryptophan biosynthesis
Biosynthesis of antibiotics
Monobactam biosynthesis
Amino sugar and nucleotide sugar metabolism
Citrate cycle (TCA cycle)
Fructose and mannose metabolism
Glycolysis / Gluconeogenesis
Pentose phosphate pathway
Pyruvate metabolism
Bacterial chemotaxis
Biofilm formation − Pseudomonas aeruginosa
Quorum sensing
Nitrogen metabolism
Oxidative phosphorylation
Sulfur metabolism
RNA degradation
Biosynthesis of amino acids
Biosynthesis of secondary metabolites
Carbon metabolism
Metabolic pathways
Microbial metabolism in diverse environments
Glycerolipid metabolism
Glycerophospholipid metabolism
ABC transporters
Bacterial secretion system
Biotin metabolism
Ubiquinone and other terpenoid−quinone biosynthesis
Glutathione metabolism
Selenocompound metabolism
Biosynthesis of siderophore group nonribosomal peptides
Geraniol degradation
Terpenoid backbone biosynthesis
Purine metabolism
Pyrimidine metabolism
Porphyrin and chlorophyll metabolism
Two−component system
Aminoacyl−tRNA biosynthesis
Cysteine and methionine metabolism
D−Glutamine and D−glutamate metabolism
Histidine metabolism
Lysine biosynthesis
Lysine degradation
Phenylalanine metabolism
Tryptophan metabolism
Tyrosine metabolism
Valine, leucine and isoleucine biosynthesis
Valine, leucine and isoleucine degradation
Novobiocin biosynthesis
Phenazine biosynthesis
Ascorbate and aldarate metabolism
Butanoate metabolism
C5−Branched dibasic acid metabolism
Glyoxylate and dicarboxylate metabolism
Pentose and glucuronate interconversions
Propanoate metabolism
Starch and sucrose metabolism
Flagellar assembly
beta−Lactam resistance
Cationic antimicrobial peptide (CAMP) resistance
Vancomycin resistance
Methane metabolism
Protein export
Sulfur relay system
2−Oxocarboxylic acid metabolism
Degradation of aromatic compounds
Fatty acid metabolism
Lipopolysaccharide biosynthesis
Peptidoglycan biosynthesis
Fatty acid biosynthesis
Fatty acid degradation
Phosphotransferase system (PTS)
Folate biosynthesis
Nicotinate and nicotinamide metabolism
One carbon pool by folate
Pantothenate and CoA biosynthesis
Riboflavin metabolism
Thiamine metabolism
Vitamin B6 metabolism
beta−Alanine metabolism
Cyanoamino acid metabolism
Taurine and hypotaurine metabolism
Base excision repair
DNA replication
Homologous recombination
Mismatch repair
Nucleotide excision repair
Ribosome
Benzoate degradation
Chloroalkane and chloroalkene degradation
Chlorocyclohexane and chlorobenzene degradation
Fluorobenzoate degradation
Xylene degradation
Streptomycin biosynthesis
Inositol phosphate metabolism
Polyketide sugar unit biosynthesis
100 μM
500 μM
1000 μM

B

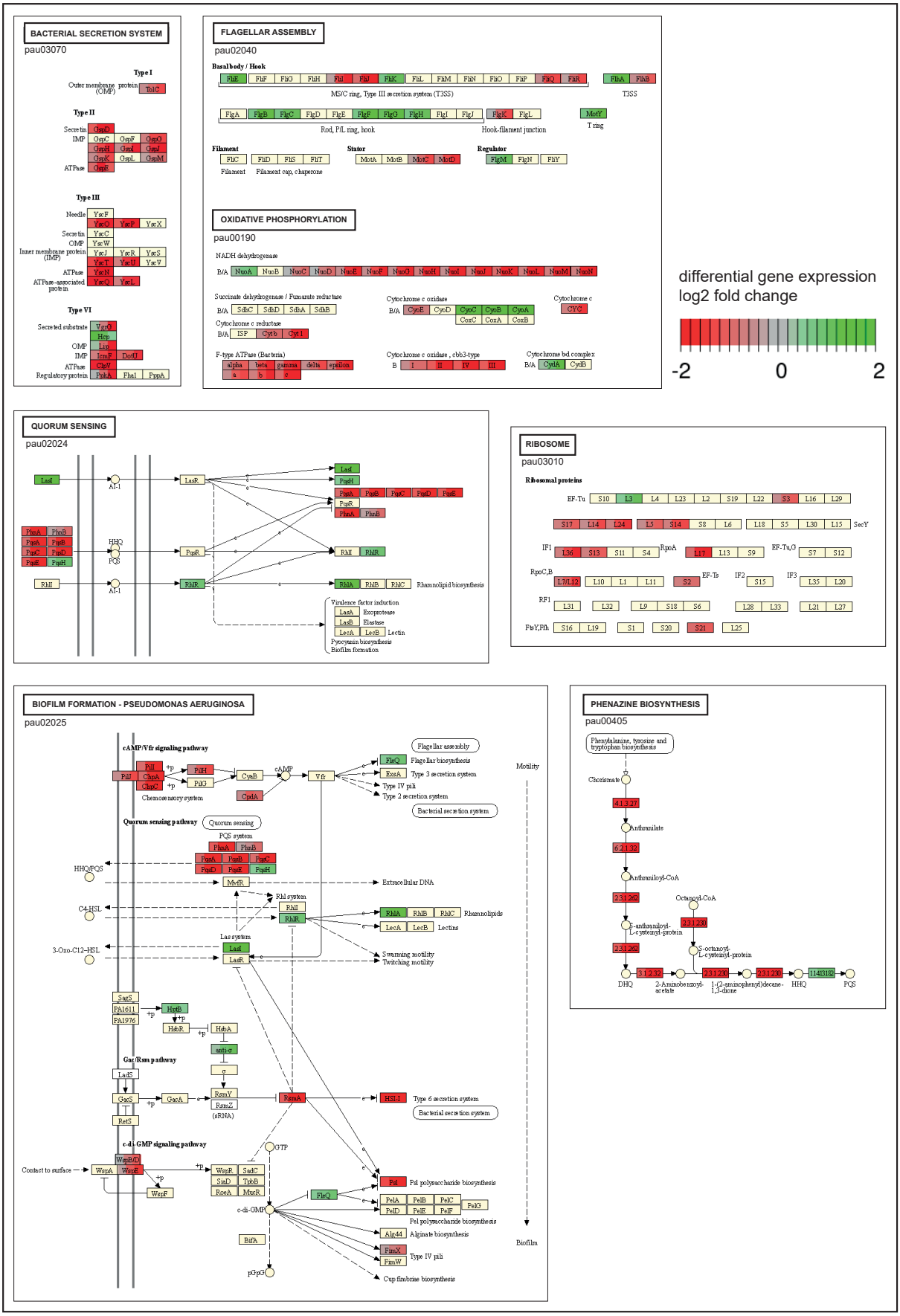

alanine metabolic process
arginine catabolic process
arginine metabolic process
aspartate metabolic process
biosynthetic process
cellular amino acid metabolic process
chemotaxis
generation of precursor metabolites and energy
metabolic process
nitrogen compound metabolic process
oxidation−reduction process
phosphate ion transmembrane transport
regulation of transcription, DNA−templated
response to antibiotic
response to drug
RNA metabolic process
RNA processing
signal transduction
transmembrane transport
transport
amino acid transport
arginine biosynthetic process
aromatic amino acid family biosynthetic process
aromatic amino acid family metabolic process
bacterial−type flagellum assembly
bacterial−type flagellum organization
bacterial−type flagellum−dependent cell motility
carbohydrate metabolic process
carbon utilization
cation transport
cell adhesion
cell cycle
cell redox homeostasis
cellular amino acid biosynthetic process
cellular catabolic process
cellular lipid metabolic process
cellular protein metabolic process
cellular response to oxidative stress
coenzyme A biosynthetic process
cofactor biosynthetic process
cysteine biosynthetic process from serine
DNA metabolic process
DNA recombination
DNA repair
DNA replication
glycine catabolic process
ion transport
magnesium ion transport
mercury ion transport
metal ion transport
movement of cell or subcellular component
NAD biosynthetic process
negative regulation of secondary metabolite biosynthetic process
negative regulation of single−species biofilm formation on inanimate substrate
negative regulation of transcription, DNA−templated
oxoacid metabolic process
pathogenesis
pentose−phosphate shunt
phosphorelay signal transduction system
phosphorylation
positive chemotaxis
positive regulation of cell adhesion
positive regulation of cell motility
positive regulation of cilium−dependent cell motility
positive regulation of secondary metabolite biosynthetic process
positive regulation of single−species biofilm formation
positive regulation of transcription, DNA−templated
potassium ion transport
protein folding
protein transport
proteolysis
pyruvate metabolic process
regulation of arginine catabolic process
regulation of L−arginine import
regulation of polysaccharide biosynthetic process
respiratory electron transport chain
response to hypochlorite
response to mercury ion
response to oxidative stress
response to stimulus
sodium−dependent phosphate transport
SOS response
transcription, DNA−templated
translation
tricarboxylic acid cycle
tRNA processing
tryptophan biosynthetic process
tyrosine biosynthetic process
ubiquinone biosynthetic process
'de novo' IMP biosynthetic process
aromatic compound catabolic process
bacterial−type flagellum−dependent swarming motility
beta−ketoadipate pathway
cellular response to antibiotic
fatty acid biosynthetic process
glucose metabolic process
glycine metabolic process
histidine biosynthetic process
isoprenoid catabolic process
L−phenylalanine catabolic process
L−serine metabolic process
negative regulation of protein secretion
nucleoside metabolic process
nucleotide metabolic process
phosphate ion transport
porphyrin−containing compound biosynthetic process
positive regulation of lipid biosynthetic process
positive regulation of proteolysis
positive regulation of single−species biofilm formation on inanimate substrate
positive regulation of transporter activity
protein secretion by the type III secretion system
purine nucleotide metabolic process
regulation of response to reactive oxygen species
regulation of single−species biofilm formation on inanimate substrate
secondary metabolite biosynthetic process
single−species biofilm formation
single−species biofilm formation on inanimate substrate
threonine metabolic process
tRNA aminoacylation for protein translation
100μM
500μM
1000μM


biosynthetic process
cellular amino acid metabolic process
cellular catabolic process
cellular protein metabolic process
cellular response to sulfate starvation
chemotaxis
cobalamin biosynthetic process
cofactor biosynthetic process
dipeptide transport
lipopolysaccharide biosynthetic process
metabolic process
nucleotide metabolic process
O antigen biosynthetic process
oxidation−reduction process
phosphorelay signal transduction system
protein folding
protein secretion by the type II secretion system
proteolysis
pyochelin biosynthetic process
regulation of transcription, DNA−templated
response to antibiotic
salicylic acid biosynthetic process
siderophore transport
signal transduction
sulfate assimilation
sulfate transport
terpene catabolic process
transmembrane transport
transport
tryptophan biosynthetic process
tryptophan metabolic process
amino acid transmembrane transport
amino acid transport
amino sugar metabolic process
antibiotic biosynthetic process
arginine biosynthetic process
ATP hydrolysis coupled proton transport
ATP metabolic process
ATP synthesis coupled electron transport
ATP synthesis coupled proton transport
bacterial−type flagellum assembly
bacterial−type flagellum−dependent cell motility
bacterial−type flagellum−dependent swarming motility
base−excision repair
beta−L−Ara4N−lipid A biosynthetic process
biotin biosynthetic process
branched−chain amino acid biosynthetic process
C4−dicarboxylate transport
carbohydrate metabolic process
carbon utilization
cell redox homeostasis
cellular aromatic compound metabolic process
cellular lipid metabolic process
cellular metabolic process
cellular response to antibiotic
cellular response to heat
cytochrome complex assembly
DNA metabolic process
DNA recombination
DNA repair
DNA replication
DNA−templated transcription, initiation
fatty acid biosynthetic process
FtsZ−dependent cytokinesis
generation of precursor metabolites and energy
glycerol−3−phosphate metabolic process
iron−sulfur cluster assembly
isoleucine biosynthetic process
isoprenoid biosynthetic process
isoprenoid catabolic process
L−serine biosynthetic process
leucine biosynthetic process
leucine catabolic process
lipid A biosynthetic process
lipid biosynthetic process
lipopolysaccharide core region biosynthetic process
lipopolysaccharide transport
lipoprotein biosynthetic process
lysine biosynthetic process via diaminopimelate
methionine biosynthetic process
methionine metabolic process
microcin transport
molybdate ion transport
movement of cell or subcellular component
negative regulation of protein secretion
negative regulation of transcription, DNA−templated
nitrogen compound metabolic process
nucleobase−containing compound metabolic process
obsolete ATP catabolic process
organic phosphonate transport
oxidative phosphorylation
pathogenesis
pentose−phosphate shunt
peptide transport
peptidoglycan biosynthetic process
phenazine biosynthetic process
phosphorylation
polysaccharide biosynthetic process
protein metabolic process
protein phosphorylation
protein secretion
protein secretion by the type III secretion system
protein secretion by the type VI secretion system
protein transport
protein transport by the Sec complex
proton transport
putrescine transport
pyrimidine nucleotide biosynthetic process
pyruvate metabolic process
regulation of flower development
regulation of single−species biofilm formation
respiratory electron transport chain
response to heat
response to stimulus
RNA metabolic process
RNA modification
rRNA processing
secondary metabolite biosynthetic process
secretion
single−species biofilm formation
spermidine transport
sulfur compound metabolic process
taurine transport
transcription, DNA−templated
translation
tricarboxylic acid cycle
anaerobic respiration
asparagine biosynthetic process
cation transport
cell cycle
cell division
cellular respiration
electron transport chain
folic acid biosynthetic process
gluconeogenesis
glycolytic process
lipid metabolic process
nucleobase transport
oligopeptide transport
pilus assembly
positive regulation of protein secretion
protein targeting
pseudouridine synthesis
purine nucleotide metabolic process
regulation of arginine catabolic process
regulation of cell shape
regulation of DNA−templated transcription, elongation
signal peptide processing
terpenoid biosynthetic process
tRNA aminoacylation for protein translation
type IV pilus biogenesis
type IV pilus−dependent motility
ubiquinone biosynthetic process
100μM
500μM
1000μM


enrichment factor
60
40
20
40
30
20
10
up
down

Supplementary Figure 3 (linked to Figure 2). Pathway analysis of gene expression changes in *P. aeruginosa* PA14 treated with increasing concentrations of serine hydroxamate (SHX).

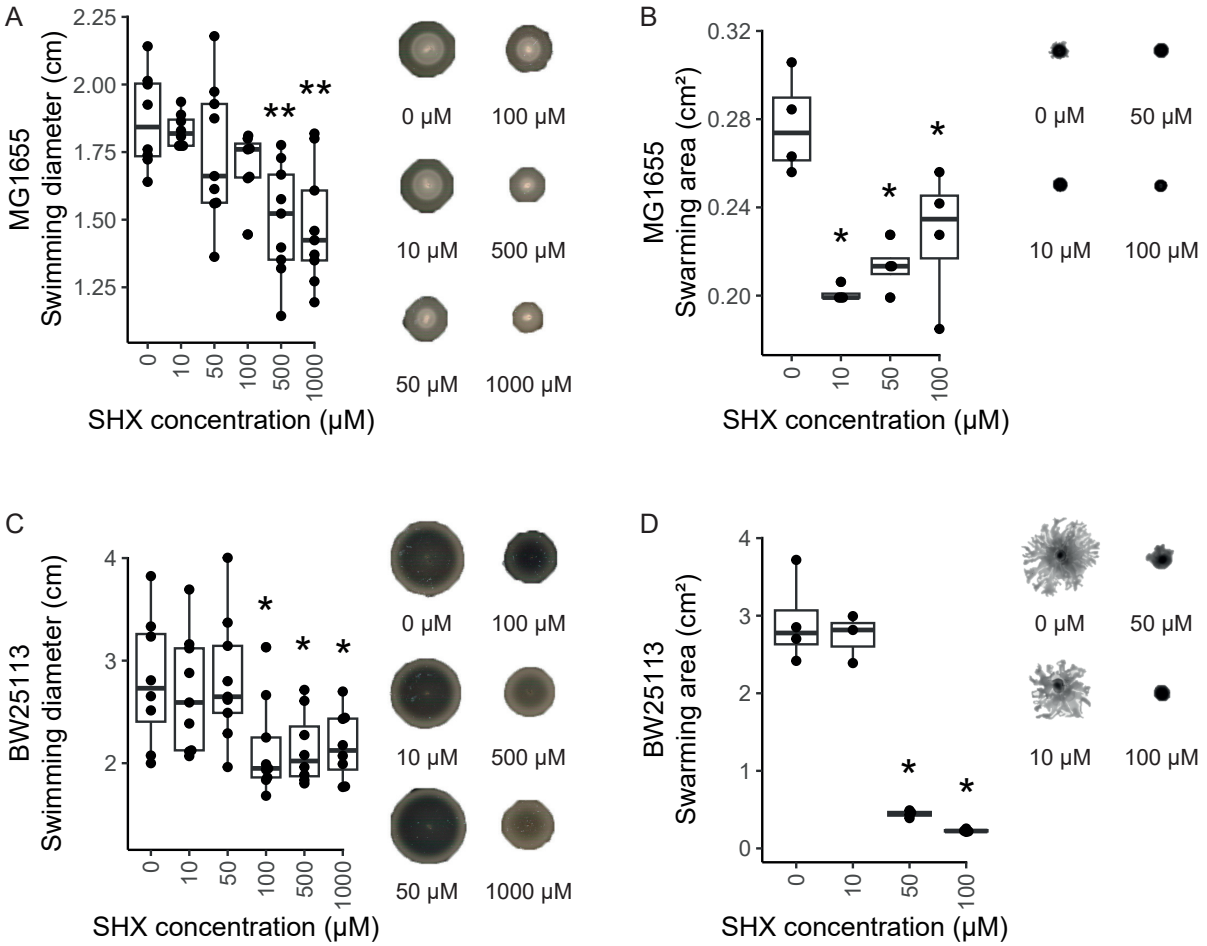
(A) KEGG and (B) Gene Ontology (GO) pathway enrichment analysis showing cellular pathways whose expression is dependent on increased (p)ppGpp levels. The pathways that were upregulated are shaded in green, and those that were downregulated are shaded in red, with the intensity of the shading representing the enrichment factor (i.e., the significance of the pathway's differential regulation). The analysis was performed with SHX treatments at 100 μM, 500 μM, and 1000 μM, with changes relative to the exponentially grown, untreated control sample.


BACTERIAL SECRETION SYSTEM
pau03070
Type I
Outer membrane protein (OMP)
TolC
Type II
Secretin
IMP
ATPase
Type III
Needle
Secretin
OMP
Inner membrane protein (IMP)
ATPase
ATPase-associated protein
Type VI
Secreted substrate
OMP
IMP
ATPase
Regulatory protein
FLAGELLAR ASSEMBLY
pau02040
Basal body / Hook
MS/C ring, Type III secretion system (T3SS)
T3SS
Rod, P/L ring, hook
Hook-filament junction
T ring
Filament
Filament
Filament cap, chaperone
Stator
Regulator
OXIDATIVE PHOSPHORYLATION
pau00190
NADH dehydrogenase
Succinate dehydrogenase / Fumarate reductase
Cytochrome c reductase
Cytochrome c oxidase
Cytochrome c
F-type ATPase (Bacteria)
Cytochrome c oxidase , cbb3-type
Cytochrome bd complex
differential gene expression
log2 fold change
-2
0
2
QUORUM SENSING
pau02024
AI-1
HHQ
PQS
AI-1
Rhamnolipid biosynthesis
Virulence factor induction
Exoprotease
Elastase
Lectin
Pyocyanin biosynthesis
Biofilm formation
RIBOSOME
pau03010
Ribosomal proteins
EF-Tu
RpoA
EF-Tu,G
RpoC,B
EF-Ts
IF2
IF3
RF1
FtsY,Ffh
SecY
BIOFILM FORMATION - PSEUDOMONAS AERUGINOSA
pau02025
cAMP/Vfr signaling pathway
Chemosensory system
cAMP
Vfr
Flagellar assembly
Flagellar biosynthesis
Type 3 secretion system
Type IV pili
Type 2 secretion system
Bacterial secretion system
Motility
Quorum sensing pathway
Quorum sensing
PQS system
HHQ/PQS
Extracellular DNA
MvfR
Rhl system
C4-HSL
Rhamnolipids
Lectins
Las system
3-Oxo-C12-HSL
Swarming motility
Twitching motility
Gac/Rsm pathway
(sRNA)
Type 6 secretion system
Bacterial secretion system
c-di-GMP signaling pathway
GTP
Contact to surface
Pel polysaccharide biosynthesis
c-di-GMP
Alginate biosynthesis
Type IV pili
Cup fimbriae biosynthesis
Biofilm
PHENAZINE BIOSYNTHESIS
pau00405
Phenylalanine, tyrosine and tryptophan biosynthesis
Chorismate
Anthranilate
Anthraniloyl-CoA
Octanoyl-CoA
DHQ
2-Aminobenzoyl-acetate
HHQ
PQS

62
63

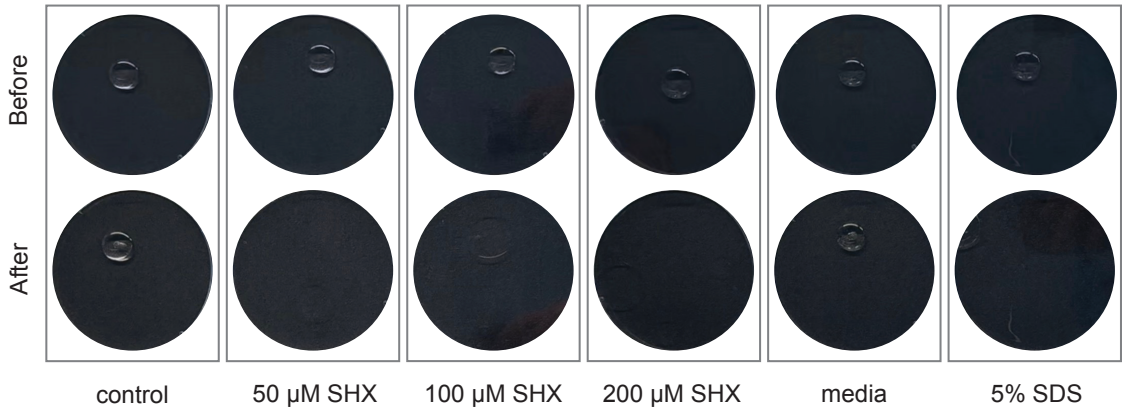
Supplementary Figure 4: Visualisation of a selection of cellular pathways affected upon (p)ppGpp induction.

This figure visualises the transcriptional effects of (p)ppGpp on genes from selected cellular pathways derived from KEGG enrichment analysis. Each gene is color-coded to reflect the observed effects at increasing SHX treatments (100 µM, 500 µM, and 1000 µM) relative to the exponentially grown untreated control sample. The colours red and green indicate $\log_2$ decreases or increases in gene expression, respectively, while genes in yellow are unaffected by (p)ppGpp. These pathways are selected to illustrate the graded effects of (p)ppGpp induction on cellular processes, but do not represent all regulated pathways or genes.



Supplementary Figure 5 (linked to Figure 3). (p)ppGpp induction negatively affects motility.

A-D) The ability of *E. coli* MG1655 and BW25113 to swim (A and C, respectively) or swarm (B and D, respectively) was determined in the presence of increasing serine hydroxamate (SHX) concentrations. Increase in the SHX concentration abolished both motility phenotypes. The experiments were repeated at least four times and representative images of swimming and swarming motility agar plates are shown on the right side of each panel. Significance was tested by using the Wilcoxon test (*P≤0.05, **P≤0.005).



Supplementary Figure 6. (p)ppGpp induction promotes biosurfactant production. *P. aeruginosa* PA14 cultures treated with increasing concentrations of serine hydroxamate (SHX) (50 µM, 100 µM, and 200 µM) were added to mineral oil on the surface of distilled water (A). A 5% SDS solution was also added as a positive control. This resulted in oil displacement by SHX-treated cultures and SDS (B). Untreated cultures and media showed no effect on oil displacement, confirming the biosurfactant production induced by (p)ppGpp. These are representative images from at least three independent experiments.

Supplementary Table 1: List of genes differentially regulated upon activation of the stringent response. Genes with unknown function are italicised and in bold.

Supplementary Table 2: Minimal inhibitory concentrations (MIC) of ciprofloxacin or tobramycin for *Pseudomonas aeruginosa* PA14 or *Escherichia coli* MG1655 in the presence of increasing concentrations of serine hydroxamate (SHX).

SHX (μM)	Ciprofloxacin (μg/mL)		Tobramycin (μg/mL)	
	PA14	MG1655	PA14	MG1655
0	0.125	0.016	2	0.5
50	0.125	0.016	2	0.5
100	0.063	0.016	1	0.5
200	0.063	0.016	1	0.5
500	0.063	0.016	1	0.25
1000	0.031	0.008	0.5	0.25

Supplementary Table 3: List of bacterial strains, chemicals, reagents, kits, devices, and informatics tools used in this study.

Reagent or Resource	Source	Identifier
Bacterial Strains		
Pseudomonas aeruginosa UCBPP-PA14	Laboratory stock	N/A
Pseudomonas aeruginosa PA01	Laboratory stock	N/A
Pseudomonas aeruginosa CH7480	Laboratory stock	N/A
Escherichia coli MG1655	Laboratory stock	N/A
Escherichia coli BW25113	Laboratory stock	N/A
Chemicals		
DL-serine hydroxamate	Merck	Cat#S4503
RNAprotect Bacteria Reagent	Qiagen	Cat#76506
FastAP buffer	ThermoFisher Scientific	Cat#EF0651
TurboDNAse	Invitrogen	Cat#AM1907
T4 RNA Ligase 1	New England Biolabs	Cat#M0437
SMARTScribe Reverse Transcriptase	Takara	Cat#639536
Exonuclease I	New England Biolabs	Cat#M0293
AccuPrime Taq DNA Polymerase	Invitrogen	Cat#12346-086
CAS Amino Acids	ThermoFisher Scientific	Cat#228820
Tobramycin	Merck	Cat#T1783

Ciprofloxacin	Merck	Cat#17850
Congo red	Merck	Cat#C6767
Coomassie Brilliant blue	Merck	Cat#112553
Phosphorus-32 Radionuclide	Perkin Elmer	Cat#NEX053H001MC
Yeast extract	Carl Roth	Cat#2363.2
Sodium chloride (NaCl)	Carl Roth	Cat#3957.1
Tryptone	Carl Roth	Cat#8952.2
Magnesium sulphate (MgSO4)	Carl Roth	Cat#1A99.2
Calcium chloride (CaCl2)	Carl Roth	Cat#A119.1
Ammonium chloride (NH4Cl)	Carl Roth	Cat#5470.1
Potassium dihydrogen phosphate (KH2PO4)	Carl Roth	Cat#P018.1
di-Sodium hydrogen phosphate heptahydrate (Na2HPO4 · 7H2O)	Carl Roth	Cat#X987.2
Iron(II) sulphate heptahydrate (FeSO4 · 7H2O)	Carl Roth	Cat#3722.1
D(+)-Glucose monohydrate	Carl Roth	Cat#6887.1
TE Buffer	ThermoFisher Scientific	Cat#12090015
Lysozym	Carl Roth	Cat#8259.2
2-Mercaptoethanol	Carl Roth	Cat#4227.3
BD Bacto Agar	ThermoFisher Scientific	Cat#10455513
Casamino acids	MP Biomedicals	Cat#113060012-CF
Mineral oil	Merck	Cat#330760
Other		
Fragment Analyzer	Agilent	https://www.agilent.com
Qubit 2.0	ThermoFisher Scientific	https://www.thermofisher.com
QIAshredder column	Qiagen	Cat#79656
NovaSeq 6000	Illumina	https://www.illumina.com/
RNeasy Mini Kit	Qiagen	Cat#74106
DNA-free DNA Removal kit	Qiagen	Cat#AM1906
RNAClean XP magnetic beads	Beckman Coulter	Cat#A63987
RNA Clean & Concentrator Kit	Zymo Reasearch	Cat#R1017
RiboZero	Illumina	Cat#RZMB12324
Half-area 96-well µClear microtiter plate	Greiner Bio-One	Cat#675096
BREATHseal cover foil	Greiner Bio-One	Cat#676050
LIVE/DEAD® BacLight® Bacterial Viability Kit	Invitrogen	Cat#L7012

Confocal laser scanning microscope SP8	Leica Microsystems	https://www.leica-microsystems.com/
PEI (Polyethylenimine) Cellulose Plates	Merck	Cat#Z122882
Typhoon FLA 9500	GE Healthcare	N/A
Software and Algorithms		
FastQC	https://github.com/s-andrews/FastQC	v0.11.9
bowtie2	Langmead & Salzberg, 2012	v1.3.1
featureCounts	Liao et al., 2014	v2.0.1
edgeR	Chen et al., 2024	N/A
KOBAS-i	https://bioinfo.org/kobas/genelist/	v3.0
pathview	Luo & Brouwer, 2013	N/A
eulerr	DOI: 10.32614/CRAN.package.eulerr	7.0.2
Developer XD 64	Definiens	N/A
Imaris	Bitplane	v7.6
FIJI	Schindelin et al., 2012; DOI 10.1038/nmeth.2019	v1.54f

Supplementary Table 4: List of the barcoded adapter sequences used in the preparation of the RNA-seq library.

Barcode ID	Barcode Sequence 5' --> 3'; 5' phosphorylated
L01	ACCAAGTCGAGATCGGAAGAGCGTCGTGTA
L02	AGCAGCCACAGATCGGAAGAGCGTCGTGTA
L03	AGTAACTGCAGATCGGAAGAGCGTCGTGTA
L04	ATCATCGTGAGATCGGAAGAGCGTCGTGTA
L05	ATTACCACGAGATCGGAAGAGCGTCGTGTA
L06	AAGAATTATAGATCGGAAGAGCGTCGTGTA
L07	AGGCCCAAGAGATCGGAAGAGCGTCGTGTA
L08	ATACAACATAGATCGGAAGAGCGTCGTGTA
L09	ATGAACCAGAGATCGGAAGAGCGTCGTGTA
L10	AATATGGACAGATCGGAAGAGCGTCGTGTA
L11	ACAACTCGCAGATCGGAAGAGCGTCGTGTA
L12	ACGGAGGGCAGATCGGAAGAGCGTCGTGTA
L13	AACATTATTAGATCGGAAGAGCGTCGTGTA
L14	AATCACTTGAGATCGGAAGAGCGTCGTGTA
L15	ACCCGTCTTAGATCGGAAGAGCGTCGTGTA
L16	ACTCGGTACAGATCGGAAGAGCGTCGTGTA
L17	AGTCTGGCGAGATCGGAAGAGCGTCGTGTA
L18	ATCCCGCGGAGATCGGAAGAGCGTCGTGTA

L19	ACGGCACTTAGATCGGAAGAGCGTCGTGTA
L20	AGAGATTGTAGATCGGAAGAGCGTCGTGTA
L21	ATACAGATGAGATCGGAAGAGCGTCGTGTA
L22	ATGGGAGACAGATCGGAAGAGCGTCGTGTA
L23	ACCCTACAGAGATCGGAAGAGCGTCGTGTA
L24	ACTCTAACTAGATCGGAAGAGCGTCGTGTA
L25	AAAGTGTTGAGATCGGAAGAGCGTCGTGTA
L26	AGAGCCATCAGATCGGAAGAGCGTCGTGTA
L27	AGGTCCTCTAGATCGGAAGAGCGTCGTGTA
L28	ATACCGGCCAGATCGGAAGAGCGTCGTGTA
L29	ACCCTCGGCAGATCGGAAGAGCGTCGTGTA
L30	ACTGGATCGAGATCGGAAGAGCGTCGTGTA
L31	ATTTCTAACAGATCGGAAGAGCGTCGTGTA
L32	ACCGGTACCAGATCGGAAGAGCGTCGTGTA

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