Table S3. Metabolites eliciting a ≥ 2-fold change in IC50 for each antibiotic; related to Figure 2.

Well	Biolog Metabolite	Ampicillin	Ciprofloxacin	Gentamicin
1B01	D-Serine	X		
1B05	D-GlucuronicAcid		X	
1D01	L-Asparagine		X	
1E01	L-Glutamine		X	
1F10	Glyoxylic Acid		X	X
1G04	L-Threonine		X	
1G05	L-Alanine		X	
1G07	Acetoacetic Acid		X	
1H08	Pyruvic Acid		X	
2E02	Caproic Acid		X	
3A10	L-AsparticAcid			X
3B02	Glycine			X
3B05	L-Leucine		X	
3B11	L-Threonine		X	
3B12	L-Tryptophan		X	
3C02	L-Valine	X		
3C08	D-Serine		X	
3D11	Putrescine			X
3D12	Agmatine			X
3E02	β-Phenylethyl-amine			X
3E03	Tyramine			X
3F02	Adenine		X	
3F12	Inosine		X	
3H06	Ala-Leu		X	
4A03	Pyrophosphate		X	X
4B09	Guanosine-3'-monophosphate		X	
4B11	Guanosine-2',3'-cyclicmonophosphate		X	

Table S4. Metabolic pathways enriched from metabolites that elicited ≥ 2-fold change in ciprofloxacin (CIP) IC50, identified by Metabolite Set Enrichment Analysis in Ecocyc; related to Figure 2.

Metabolic Pathways Enriched from Metabolite Screening "Hits" for CIP	p-value
Aminoacyl-tRNA charging	1.98E-06
tRNA charging	1.98E-06
Proteinogenic amino acids biosynthesis	2.50E-06
Amino acids biosynthesis	4.32E-06
Amino acids degradation	1.27E-05
L-Tryptophan biosynthesis	2.32E-05
L-Tryptophan biosynthesis	2.32E-05
Degradation / utilization / assimilation	5.27E-05
Metabolic clusters	1.03E-04
L-Asparagine biosynthesis	1.37E-04
L-Asparagine biosynthesis I	1.37E-04
Superpathway of L-asparagine biosynthesis	1.37E-04
Proteinogenic amino acids degradation	1.59E-04
Adenine and adenosine salvage III	2.47E-04
Nucleic acids processing	4.51E-04
Adenine and adenosine salvage	6.12E-04
L-Alanine biosynthesis II	7.24E-04
Superpathway of aromatic amino acid biosynthesis	7.66E-04

Table S7. Metabolic pathways predicted to participate in ampicillin (AMP), ciprofloxacin (CIP) or gentamicin (GENT) lethality; related to Figure 3.

Metabolic Pathways Identified by White-Box Machine Learning	p_{AMP}	p_{CIP}	p_{GENT}
Superpathway of sulfate assimilation and cysteine biosynthesis	1.32E-05	1.01E-04	4.80E-05
Superpathway of histidine, purine, and pyrimidine biosynthesis	1.23E-06	2.89E-03	4.80E-05
Superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass	1.32E-05	2.11E-02	6.91E-05
Superpathway of purine nucleotides de novo biosynthesis II	6.34E-06	2.97E-03	2.01E-03
Pentose phosphate pathway	4.22E-05	2.97E-03	2.01E-03
Superpathway of phenylethylamine degradation	6.29E-04	2.97E-03	1.79E-03
Inosine-5'-phosphate biosynthesis I	7.85E-04	2.97E-03	2.01E-03
Superpathway of 5-aminoimidazole ribonucleotide biosynthesis	7.85E-04	2.97E-03	2.07E-02
Superpathway of glycolysis and the Entner-Doudoroff pathway	4.27E-02	2.25E-02	1.20E-04
L-Arginine biosynthesis I (via L-ornithine)	2.79E-04	1.00E+00	8.10E-04
Salvage pathways of pyrimidine ribonucleotides	4.22E-05	1.18E-01	8.41E-02
TCA cycle I (prokaryotic)	7.85E-04	3.05E-01	6.32E-02
Superpathway of glyoxylate bypass and TCA	6.29E-04	5.37E-01	1.36E-01

Table S8. Measured intracellular metabolite concentrations under adenine or uracil supplementation. Data are reported as mean ± SD and computed from LC-MS/MS measurements from n = 3 biological replicates; related to Figure 6.

Metabolite	Units	Control	Adenine	Uracil
α-Ketoglutarate	µM	271.50 ± 53.84	192.98 ± 49.97	212.32 ± 23.95
2-Phosphoglycerate / 3-Phosphoglycerate	mM	1.56 ± 0.32	1.10 ± 0.23	1.26 ± 0.23
Acetyl-CoA	mM	7.46 ± 1.32	7.83 ± 1.16	4.69 ± 0.44
Aconitate	µM	253.55 ± 189.21	112.84 ± 61.92	147.47 ± 35.42
Adenine	mM	0.06 ± 0.07	3.45 ± 2.88	0.08 ± 0.09
Adenosine	mM	0.15 ± 0.03	1.37 ± 1.12	0.14 ± 0.04
ADP	mM	0.45 ± 0.13	0.75 ± 0.16	0.28 ± 0.017
AMP	µM	176.79 ± 83.42	650.14 ± 329.04	148.52 ± 46.23
ATP	mM	9.26 ± 2.78	9.41 ± 7.80	5.04 ± 1.20
Citrate	mM	0.53 ± 0.08	0.53 ± 0.27	0.89 ± 0.25
Fructose biphosphate	mM	6.00 ± 3.65	4.46 ± 1.74	4.85 ± 2.39
Fructose-6-phosphate	mM	1.38 ± 0.21	1.22 ± 0.14	1.37 ± 0.18
Fumarate	mM	1.96 ± 0.38	1.46 ± 0.35	1.56 ± 0.26
Glucose 6-phosphate	mM	4.70 ± 0.63	4.10 ± 0.66	4.60 ± 0.17
Glyceraldehyde 3-phosphate	a.u.	1.00 ± 0.51	0.83 ± 0.25	1.05 ± 0.38
Glyoxylate	µM	777.74 ± 447.46	935.03 ± 484.73	582.14 ± 158.61
Isocitrate	uM	11.16 ± 7.27	9.68 ± 4.37	13.93 ± 4.81
Malate	mM	26.65 ± .797	13.88 ± 3.27	14.35 ± 0.68
NAD+	a.u.	1.00 ± 0.18	1.09 ± 0.13	0.84 ± 0.04
NADH	a.u.	1.00 ± 0.20	0.91 ± 0.13	0.71 ± 0.08
NADP+	a.u.	1.00 ± 0.30	1.05 ± 0.27	0.79 ± 0.18
NADPH	a.u.	1.00 ± 0.50	0.49 ± 0.22	1.42 ± 0.62
Oxeloacetate	nM	21.93 ± 25.11	35.39 ± 33.42	11.72 ± 10.56
Phosphoenoylpyruvate	µM	140.29 ± 36.81	81.94 ± 21.17	125.74 ± 30.46
Pyruvate	mM	2.12 ± 1.55	2.71 ± 2.32	1.43 ± 0.41
Succinyl-CoA	a.u.	1.00 ± 1.00	0.90 ± 0.58	0.59 ± 0.28
Succinate	mM	1.50 ± 0.66	2.17 ± 0.43	1.19 ± 0.28
UDP	µM	69.04 ± 30.18	122.73 ± 108.85	267.81 ± 122.37
UMP	µM	6.66 ± 6.39	13.90 ± 10.30	30.38 ± 20.35
Uracil	mM	0.01 ± 0.00	0.04 ± 0.01	21.47 ± 8.92
Uridine	µM	19.00 ± 6.05	101.35 ± 62.72	49.58 ± 20.58
UTP	mM	1.66 ± 0.50	1.08 ± 0.60	3.17 ± 0.56

Table S9. Oligonucleotides used for verifying *E. coli* gene deletions; related to STAR Methods.

Oligonucleotides
glyA forward primer: AGAAATCCGTTTCCGGTTGC
glyA reverse primer: CCCAGAACGGTATCACCTGG
purD forward primer: TATTGCGATGCTCTTCACCGA
purD reverse primer: CGTTTGTGATCCTGGCTGGT
purE forward primer: GAGAGTTGTGCACCACAGGA
purE reverse primer: CAGCACTTCGTCGGTCTGG
purK forward primer: CAGTTACTTGCCGAACGCAG
purK reverse primer: TTTTGTGTCCAGTGACCGCT
purM forward primer: ATTGACGCGGGTAATGCTCT
purM reverse primer: CCGCGACATCGTAATCCTCA
pyrC forward primer: TGGTGCATGGTGAAGTGACA
pyrC reverse primer: CCAGCGTGTCATCAGTCAGT
pyrE forward primer: GGTGGATTCCGGCATTGAGT
pyrE reverse primer: TGCGGCACAGTTGCAGTAAT
Keio insert primer: GATGTTTCGCTTGGTGGTCG