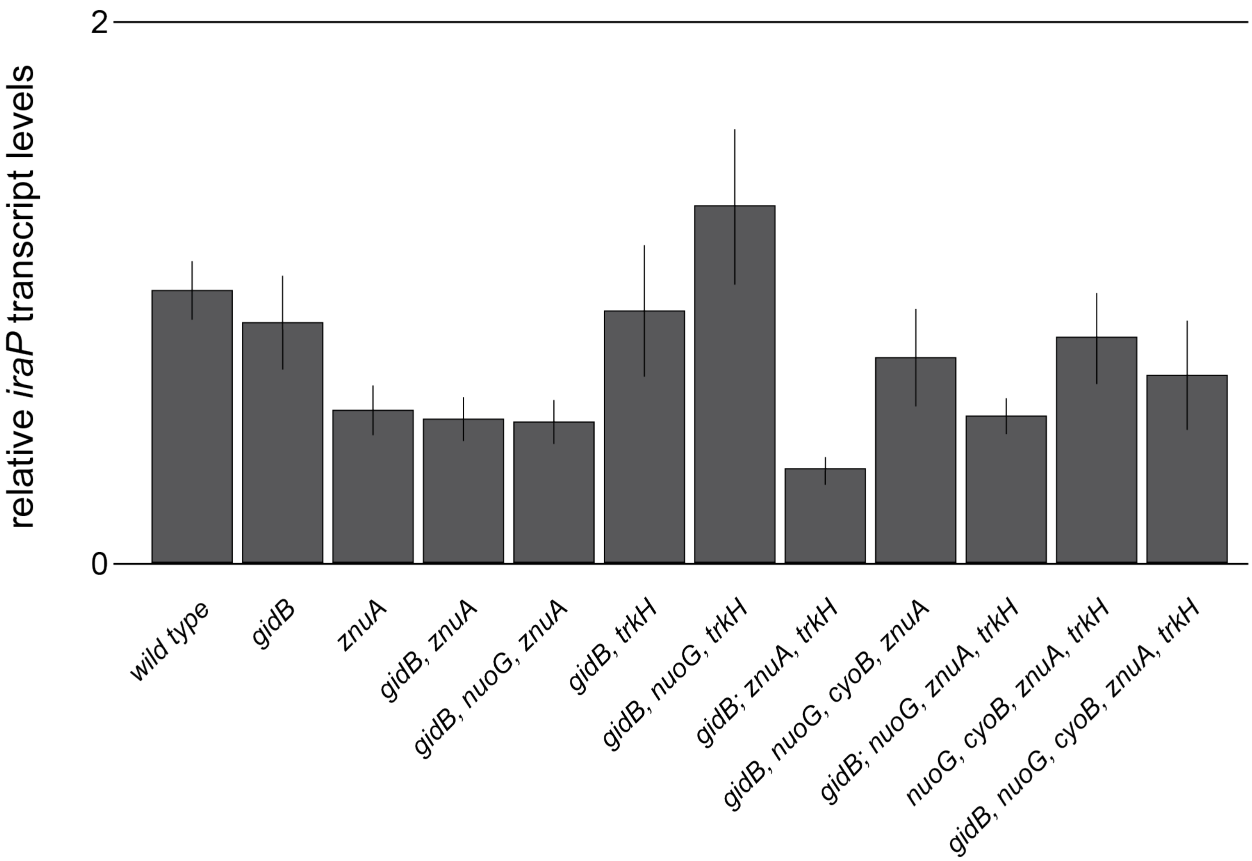
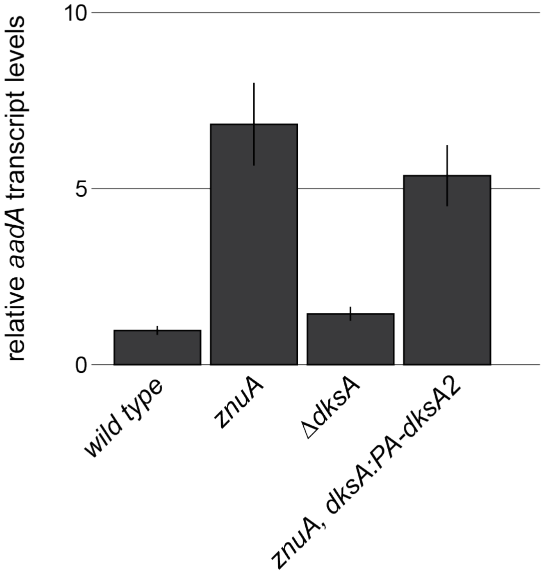
Supplementary Figures 1-2 and Supplementary Tables 1-8 for:

Evolution of high-level resistance during low-level antibiotic exposure

Wistrand-Yuen et al.



Supplementary Figure 1. Relative transcript levels of *iraP* in the wild type and selected mutants. No significant upregulation was observed in any of the samples. The values represent averages of 2 biological and 3 technical replicates. Error bars represent the standard deviation.



Supplementary Figure 2. Relative transcript levels of *aadA*. Expression of *aadA* was measured in wild type, reconstructed *znuA*-mutant, *dksA* knockout and a *znuA* mutant with a replacement of the native *dksA* gene with a zinc independent homologue from *Pseudomonas aeruginosa* (*PA-dksA2*). Error bars represent the standard deviation.

Supplementary Table 1. All mutations in the isolated mutants compared to wild type. Mutation types are single-nucleotide variants (SNVs), two or more SNVs in succession (MNVs), deletions and insertions. The symbol "*" represents a stop codon, while "fs" represents a frame shift mutation.

Strain	Position	Type	Annotation	Amino acid change
DA23868	85237	SNV	CDS: caiB	Val54Ala
DA23868	160039	SNV	CDS: secA	Pro451Ser
DA23868	225305	SNV	CDS: fhuA	Thr523Ala
DA23868	245972	Insertion		
DA23868	255731	SNV	CDS: tsf	Arg151Cys
DA23868	297812	Insertion	CDS: putative drug efflux protein	Val302fs
DA23868	302541	SNV	CDS: yafS	Pro130Leu
DA23868	302560	SNV	CDS: yafS	
DA23868	335622	SNV	CDS: putative RHS-like protein	Ala1024Val
DA23868	364624	Deletion	CDS: crl	Phe35fs
DA23868	370376	SNV		
DA23868	470999	Insertion		
DA23868	547591	SNV	CDS: hemH	
DA23868	680367	Deletion	CDS: rna	Asn145fs
DA23868	682157	SNV	CDS: citT	Tyr72Cys
DA23868	723009	SNV	CDS: hscC	Asp403Asn
DA23868	985689	SNV	CDS: hypothetical protein, CDS: hypothetical protein	His58Arg, Val150Ala
DA23868	1006741	SNV	CDS: putative nucleoside-diphosphate-sugar epimerase	Tyr264His
DA23868	1015778	Insertion		
DA23868	1133653	Insertion	CDS: host specificity protein J	Ala294fs
DA23868	1164914	SNV	CDS: yccS	Val307Ala
DA23868	1220293	SNV	CDS: putative inner membrane protein	Ala282Val
DA23868	1258842	Insertion	CDS: flgC	Val64fs
DA23868	1271172	SNV	CDS: rne	Tyr126His
DA23868	1343532	SNV	CDS: putative ABC-type transport system ATPase component	
DA23868	1346537	SNV		
DA23868	1357187	SNV	CDS: yeaL	Leu71Pro
DA23868	1363410	Deletion	Gene: yeaG	
DA23868	1375969	Deletion	CDS: selD	Arg126fs

DA23868	1527411	SNV	CDS: tppB	Trp179*
DA23868	1582873	SNV	CDS: rspA	His211Arg
DA23868	1593976	Deletion		
DA23868	1599457	SNV	CDS: ydeA	
DA23868	1610520	SNV	CDS: putative hydrogenase protein	Asp55Asn
DA23868	1618287	SNV	CDS: putative zinc-binding dehydrogenase	Trp18*
DA23868	1637147	SNV	CDS: putative glycosyl hydrolase	Val328Ala
DA23868	1639479	SNV	CDS: putative glycosyl hydrolase	
DA23868	1642462	Insertion	CDS: putative alpha amylase	Ala12fs
DA23868	1659045	SNV		
DA23868	1668866	SNV	CDS: nhoA	Glu117Gly
DA23868	1802376	SNV	CDS: yciM	Thr102Ala
DA23868	1845156	SNV	CDS: adhE	
DA23868	1862985	SNV	CDS: narK	Thr318Ala
DA23868	1870891	SNV	CDS: kdsA	
DA23868	1981602	SNV	CDS: yebK	Ala41Thr
DA23868	1984419	SNV	CDS: msbB	
DA23868	2064558	SNV	CDS: fliN	Asp9Asn
DA23868	2071971	SNV	CDS: yedI	Ala274Val
DA23868	2075768	SNV	RBS: RBS,	
DA23868	2092313	SNV	CDS: amn	
DA23868	2101006	SNV	CDS: cbiP	
DA23868	2149558	Insertion		
DA23868	2190052	Deletion	CDS: wcaI	Ile371fs
DA23868	2197408	SNV	CDS: wcaC	
DA23868	2436280	SNV	CDS: nuoE	Val69Ala
DA23868	2469205	SNV	CDS: putative cytoplasmic protein	Ala183Thr
DA23868	2690148	SNV	CDS: putative inner membrane protein	
DA23868	2712523	Insertion	CDS: yfhD	Val44fs
DA23868	2739118	Insertion	CDS: host specificity protein-J-like	Leu561fs
DA23868	2750091	SNV	CDS: minor capsid protein FII	
DA23868	2783659	SNV	CDS: srmB	
DA23868	2836458	SNV	CDS: STM2689	Val2959Ile
DA23868	2841809	SNV	CDS: putative ABC transporter transmembrane region	
DA23868	2847103	Insertion	CDS: phage tail-like protein	Val669fs
DA23868	2858360	SNV	CDS: phage tail-like protein	Gly52Ser
DA23868	2921898	SNV	CDS: iroD	

DA23868	2930399	Deletion	CDS: mig-14	Lys269fs
DA23868	2966261	SNV		
DA23868	2970562	SNV	tRNA: tRNA-Ser	
DA23868	2984651	SNV	CDS: ygaA	
DA23868	2996059	SNV	CDS: hycD	Ile164Thr
DA23868	3052487	SNV	CDS: mutS	Leu734Pro
DA23868	3060226	SNV	CDS: ygbK	Arg165Cys
DA23868	3083757	SNV	CDS: putative cytoplasmic protein	Phe94Leu
DA23868	3089591	SNV	CDS: cysI	Thr377Ala
DA23868	3101011	SNV	CDS: mazG	
DA23868	3111480	SNV	CDS: gudD	
DA23868	3129408	SNV	CDS: fucI	
DA23868	3132832	SNV	CDS: ygdE	
DA23868	3150051	SNV	CDS: ptr	Arg831Cys
DA23868	3161257	Insertion	CDS: ptsP	Gln303fs
DA23868	3186121	SNV	CDS: hypothetical protein	Arg133Cys
DA23868	3392258	Deletion		
DA23868	3397070	SNV	CDS: yqjD	Ala89Thr
DA23868	3418947	SNV	CDS: garD	Val91Ile
DA23868	3445076	SNV	CDS: mtr	Arg322Cys
DA23868	3532372	SNV	CDS: yhcQ	Thr181Met
DA23868	3539635	SNV	CDS: yhdP	
DA23868	3541535	SNV	CDS: maf	Thr125Ala
DA23868	3548156	Insertion	CDS: putative sulfite oxidase subunit YedY	Met20fs
DA23868	3563809	SNV	CDS: acrF	Gly510Asp
DA23868	3587650	SNV	CDS: rplO	
DA23868	3592121	SNV	CDS: rplP	Asp106Gly
DA23868	3676829	Insertion		
DA23868	3681105	SNV	CDS: rtcA	
DA23868	3681839	SNV	CDS: rtcB	Ala240Thr
DA23868	3711068	SNV	CDS: gntU	
DA23868	3808106	SNV		
DA23868	3945220	SNV	CDS: yicI	Tyr551Cys
DA23868	3997538	SNV	CDS: ilvB	
DA23868	4049508	SNV	CDS: trmE	Asp440Asn
DA23868	4063764	SNV	CDS: pstS	
DA23868	4064947	SNV	CDS: putative phosphotransferase system	Ala338Val

DA23868	4081716	SNV	CDS: gidB	Gln22*
DA23868	4086386	SNV	CDS: yieM	Ala450Val
DA23868	4131118	SNV	CDS: rffG	Gln222*
DA23868	4179218	SNV		
DA23868	4266460	SNV	CDS: yiiM	
DA23868	4290951	SNV	CDS: yiiR	Met112Val
DA23868	4320353	SNV	CDS: katG	
DA23868	4403441	SNV	CDS: aceA	Thr332Ala
DA23868	4441487	SNV	CDS: yjbF	Ala60Val
DA23868	4448384	SNV	CDS: malE	Phe305Ser
DA23868	4553316	SNV	CDS: putative anaerobic dimethylsulfoxide reductase subunit B	Pro72Leu
DA23868	4571807	SNV	CDS: fxsA	Pro103Ser
DA23868	4634053	SNV	CDS: cycA	Val143Ala
DA23868	4643962	Insertion		
DA23868	4696458	Insertion	Gene: treB	
DA23868	4720045	SNV	CDS: pepA	
DA23868	4739921	Insertion	CDS: putative cytoplasmic protein	Glu79fs
DA23868	4739981	SNV	CDS: putative cytoplasmic protein	Arg57His
DA23868	4784944	SNV	CDS: putative cytoplasmic protein	Tyr81His
DA23868	4842823	SNV	CDS: creA	Ala99Val
DA23869	391	SNV	CDS: thrA	Arg19Cys
DA23869	76853	SNV	CDS: carA	Leu325Pro
DA23869	81198	SNV	CDS: caiE	Val140Ala
DA23869	154309	Insertion	CDS: ftsQ	Glu262fs
DA23869	164202	SNV	CDS: guaC	
DA23869	185131	SNV	CDS: yacH	Ala113Val
DA23869	230358	Insertion		
DA23869	234939	SNV	CDS: stfE	Gly64Arg
DA23869	245174	SNV	CDS: htrA	Thr228Ala
DA23869	248205	SNV	CDS: putative inner membrane protein	Thr85Met
DA23869	252048	SNV	CDS: glnD	Ala257Thr
DA23869	258138	SNV	CDS: dxr	Met98Val
DA23869	302704	Insertion	CDS: yafS	Arg187fs
DA23869	316973	SNV	CDS: putative cytoplasmic protein	Val27Ala
DA23869	321982	SNV	CDS: hypothetical protein	Pro291Ser
DA23869	326320	SNV	CDS: putative inner membrane protein	Asn820Ser
DA23869	342584	SNV	CDS: safA	

DA23869	350623	SNV		
DA23869	352334	SNV	CDS: yafV	
DA23869	357677	SNV		
DA23869	368821	SNV	tRNA: thrW	
DA23869	443225	SNV	CDS: rdgC	Val206Ala
DA23869	496334	SNV	CDS: cyoB	Gly283Asp
DA23869	496381	SNV	CDS: cyoB	
DA23869	505578	SNV	CDS: lon	
DA23869	513676	Insertion	CDS: ybaE	Ser153fs
DA23869	546507	SNV	CDS: adk	Thr155Ile
DA23869	622700	SNV		
DA23869	774925	SNV	CDS: phrB	Pro49Ser
DA23869	806753	SNV	CDS: sucC	
DA23869	904789	SNV	CDS: ybiS	Ala11Val
DA23869	912823	SNV	CDS: pflF	Trp320*
DA23869	1110307	SNV	CDS: hypothetical protein	
DA23869	1110316	SNV	CDS: hypothetical protein	
DA23869	1110325	SNV	CDS: hypothetical protein	
DA23869	1162292	Insertion		
DA23869	1224740	Insertion	tRNA: serX	
DA23869	1232990	Deletion	RBS: RBS	
DA23869	1254229	SNV	CDS: mviM	
DA23869	1265494	SNV	CDS: flgK	Asn13Asp
DA23869	1363410	Deletion	Gene: yeaG	
DA23869	1405271	SNV	CDS: pfkB	
DA23869	1487338	Deletion	Misc. feature: SPI-2, CDS: sseE	Ala100fs
DA23869	1597469	SNV	CDS: marA	Met1?
DA23869	1598486	SNV	CDS: marC	
DA23869	1611940	SNV	CDS: putative Ni/Fe hydrogenase 1 b-type cytochrome subunit	Tyr34His
DA23869	1633128	SNV	CDS: putative transcriptional regulator	
DA23869	1650197	SNV	CDS: fdnH	Ser76Pro
DA23869	1653692	SNV		
DA23869	1781866	SNV	CDS: pspA	Ala182Val
DA23869	1788214	SNV	CDS: sapF	
DA23869	1885035	SNV	CDS: hydrogenase-1 small subunit	
DA23869	1941094	Deletion		
DA23869	1987304	Insertion	CDS: znuA	Ser19fs

DA23869	1992784	SNV	CDS: yebB	Ser54Gly
DA23869	1999723	SNV	CDS: yecO	
DA23869	2016562	SNV		
DA23869	2029377	SNV	RBS: RBS	
DA23869	2061641	SNV	CDS: fliJ	Gln115*
DA23869	2085229	Insertion		
DA23869	2095178	SNV	CDS: yeeO	Gln219*
DA23869	2178644	SNV		
DA23869	2295767	SNV	CDS: putative regulatory protein	Ala195Thr
DA23869	2433830	SNV	CDS: nuoG	Asp264Gly
DA23869	2446041	SNV	CDS: yfbT	
DA23869	2503789	SNV	CDS: vacJ	Glu244Lys
DA23869	2592702	SNV	CDS: narQ	Thr314Met
DA23869	2742856	SNV	CDS: phage tail component M-like protein	*110Trp
DA23869	2798241	Insertion	rRNA: rRNA	
DA23869	2816335	SNV	CDS: rimM	
DA23869	2821018	Insertion	CDS: putative cytoplasmic protein	Leu25fs
DA23869	2845190	SNV	CDS: late control-like protein	
DA23869	2912853	SNV	CDS: fljA	
DA23869	2940273	Insertion	CDS: gabD	Ala47fs
DA23869	3069492	SNV	CDS: truD	
DA23869	3079887	SNV	CDS: ygcH	Thr76Ala
DA23869	3079906	SNV	CDS: ygcH	
DA23869	3107032	SNV	CDS: barA	Val169Ala
DA23869	3143285	SNV	CDS: argA	Asp146Gly
DA23869	3147163	SNV	CDS: RecB	Val833Ala
DA23869	3169658	SNV	CDS: aas	
DA23869	3185444	SNV	CDS: putative cytoplasmic protein	Val192Ala
DA23869	3245214	SNV	CDS: putative mannitol dehydrogenase	Leu211Pro
DA23869	3257064	SNV	CDS: gshB	Met40Val
DA23869	3287613	SNV	CDS: putative oxidoreductase	
DA23869	3368021	SNV	CDS: ygiF	Gln97Arg
DA23869	3392258	Deletion		
DA23869	3415077	Insertion	Misc. RNA: rnpB	
DA23869	3482894	SNV	CDS: yrbK	Ala119Thr
DA23869	3552533	SNV	CDS: panF	Val87Ala
DA23869	3730830	SNV	CDS: livM	

DA23869	3794167	SNV	CDS: yhjL	Arg1083Cys
DA23869	3836547	SNV	CDS: cspA	
DA23869	3939879	SNV	CDS: gltS	Pro375Ser
DA23869	3965999	SNV	CDS: yicL	Thr111Ala
DA23869	3974022	SNV	CDS: putative transcriptional regulator	Thr435Ala
DA23869	3999035	SNV	CDS: integral membrane protein	Ala136Thr
DA23869	4003260	SNV	CDS: dsdX	
DA23869	4010699	SNV	CDS: yidQ	
DA23869	4028522	SNV	CDS: torS	Met698Ile
DA23869	4081275	SNV	CDS: gidB	Gln169*
DA23869	4096715	SNV	CDS: rbsR	Leu65Pro
DA23869	4158371	Insertion	CDS: yigG	Asp146fs
DA23869	4192743	SNV	CDS: trkH	Thr99Ala
DA23869	4300185	SNV	CDS: ftsN	Ala227Thr
DA23869	4302505	SNV	CDS: priA	Tyr611His
DA23869	4446742	SNV	CDS: malF	Gln412*
DA23869	4519496	SNV	CDS: nrfE	Thr296Ala
DA23869	4600831	Deletion	CDS: mutL	Trp121del
DA23869	4706682	SNV	CDS: ornithine carbamoyltransferase	Val124Ile
DA23869	4793345	SNV	CDS: putative transcriptional regulator	Glu833Gly
DA23869	4798813	Insertion	CDS: mdoB	Arg654fs
DA23869	4809418	Insertion	tRNA: leuV	
DA23869	4818547	SNV	CDS: yjjI	Arg469His
DA23872	50727	SNV	CDS: putative sodium galactoside symporter	
DA23872	84700	SNV	CDS: caiB	Tyr233Cys
DA23872	122305	SNV	CDS: araC	
DA23872	171432	SNV	CDS: putative cytoplasmic protein	Phe127Leu
DA23872	193565	SNV	CDS: putative transcriptional regulator	
DA23872	205743	SNV	CDS: stiC	Ser628Asn
DA23872	207172	SNV	CDS: stiC	Tyr152His
DA23872	233564	SNV	CDS: stfC	Ala754Val
DA23872	242024	SNV	CDS: btuF	Leu16Pro
DA23872	267043	SNV	CDS: lpxA	
DA23872	310051	Deletion	CDS: putative cytoplasmic protein	Thr261fs
DA23872	321801	Insertion	CDS: hypothetical protein	Ala232fs
DA23872	327522	SNV	CDS: putative inner membrane protein	Thr1221Ala
DA23872	347953	SNV		

DA23872	389590	SNV	CDS: putative inner membrane protein	Ser31Gly
DA23872	404378	SNV	CDS: mod	
DA23872	428383	SNV	CDS: yaiV	Gln66*
DA23872	446732	SNV	CDS: sbcC	Leu884Pro
DA23872	446962	SNV	CDS: sbcC	
DA23872	561004	SNV	CDS: ybbJ	Gly142Asp
DA23872	573832	SNV	CDS: ybbB	Thr318Ala
DA23872	625777	SNV	CDS: ybdG	Val370Ala
DA23872	673838	SNV	CDS: putative oxidoreductase protein	
DA23872	676897	SNV	CDS: putative hydrogenase protein	Met120Val
DA23872	681121	SNV	CDS: citT	
DA23872	685408	SNV	CDS: citE	Leu291Pro
DA23872	724743	Insertion	CDS: putative cytoplasmic protein	Leu263fs
DA23872	779010	SNV	CDS: ybgJ	
DA23872	796338	SNV	CDS: gltA	
DA23872	840871	SNV	CDS: galE	Val244Ile
DA23872	919408	Deletion	CDS: yliA	Asp437fs
DA23872	975401	SNV		
DA23872	1018676	Insertion	CDS: ybjY	Met24fs
DA23872	1070548	SNV	CDS: msbA	
DA23872	1099833	SNV		
DA23872	1215181	SNV		
DA23872	1224740	Insertion	tRNA: serX	
DA23872	1301502	SNV	CDS: mfd	Pro277Leu
DA23872	1556640	SNV	CDS: pntA	Arg318Cys
DA23872	1560683	SNV	CDS: ydgE	Gly31Ser
DA23872	1642275	SNV	CDS: putative alpha amylase	Val74Ala
DA23872	1732273	SNV	CDS: hrpA	Arg968Cys
DA23872	1788452	SNV	CDS: sapF	Ser100Pro
DA23872	1813959	SNV	CDS: sohB	
DA23872	1838108	SNV	CDS: oppC	Phe147Leu
DA23872	1862986	Insertion	CDS: narK	Thr318fs
DA23872	1879670	SNV	CDS: ychM	
DA23872	1988406	SNV	CDS: znuB	Trp73*
DA23872	2021974	SNV	CDS: flhD	Asp31Gly
DA23872	2026179	SNV	CDS: araH	Asp170Gly
DA23872	2210000	SNV	CDS: yegE	Val491Ala

DA23872	2444194	Deletion	CDS: yfbS	Val543fs
DA23872	2561611	SNV	CDS: hemF	Gly136Asp
DA23872	2615307	SNV	CDS: ppk	Arg124His
DA23872	2645744	SNV	CDS: ratA	Asp577Gly
DA23872	2646287	SNV	CDS: ratA	Asp396Gly
DA23872	2737238	SNV		
DA23872	2777392	SNV	CDS: rseC	Pro136Ser
DA23872	2823773	SNV	CDS: recN	Ser169Asn
DA23872	2824008	SNV	CDS: recN	
DA23872	2925343	SNV	CDS: iroN	
DA23872	2937830	SNV	RBS: RBS	
DA23872	3094368	SNV		
DA23872	3168903	SNV	CDS: aas	His379Arg
DA23872	3175670	Insertion	CDS: ygeA	Met10fs
DA23872	3263489	SNV	CDS: yggW	Ile123Thr
DA23872	3279213	SNV	CDS: putative transcriptional regulator	Thr122Ala
DA23872	3344168	SNV	CDS: parE	
DA23872	3359814	SNV	CDS: putative inner membrane protein	Ser9Asn
DA23872	3631572	Deletion	CDS: bigA	His802fs
DA23872	3678781	SNV	CDS: malT	Glu636*
DA23872	3683699	SNV	CDS: putative ribonucleoprotein related-protein	Asn319Ser
DA23872	3829179	SNV		
DA23872	3835753	Insertion		
DA23872	3909892	Insertion	CDS: rfaK	Gln265fs
DA23872	3978385	SNV	CDS: nepI	
DA23872	4068130	SNV	CDS: putative dipeptide/oligopeptide/nickel ABC-type transport system periplasmic component	
DA23872	4081556	SNV	CDS: gidB	Gly75Glu
DA23872	4244279	SNV	CDS: fdoG	Ala769Val
DA23872	4414847	SNV	CDS: yjbC	Tyr198Cys
DA23872	4504994	SNV	CDS: yjcD	Thr24Ala
DA23872	4601391	SNV	CDS: mutL	Lys307Arg
DA23872	4621196	SNV	CDS: yjfP	
DA23872	4642401	Deletion		
DA23872	4676758	SNV	RBS: RBS,	
DA23872	4713625	SNV	CDS: ytgA	
DA23872	4761505	SNV	CDS: trpS2	Tyr39Cys

DA23872	4767281	SNV		
DA23874	3060	Deletion	CDS: thrB	Asn89fs
DA23874	100449	SNV	CDS: kefC	Met558Val
DA23874	129259	SNV	CDS: leuD	Leu135Pro
DA23874	218255	Insertion	CDS: ligT	Ala10fs
DA23874	237104	Insertion	CDS: putative outer membrane protein	
DA23874	253245	SNV	CDS: map	
DA23874	272135	SNV	CDS: dnaE	Phe863Leu
DA23874	337446	Insertion	CDS: putative RHS-like protein	Leu120fs
DA23874	417817	Deletion		
DA23874	496416	SNV	CDS: cyoB	Asp256Asn
DA23874	518970	SNV	CDS: mdlB	
DA23874	661009	SNV	CDS: ybdB	Gln110*
DA23874	673098	SNV		
DA23874	793807	SNV	CDS: putative cytoplasmic protein	
DA23874	794629	SNV	CDS: nei	
DA23874	977144	Insertion	CDS: hypothetical protein	Gln113fs
DA23874	1048863	SNV	CDS: dmsC	Phe156Leu
DA23874	1117553	SNV	CDS: hypothetical protein	Lys232Glu
DA23874	1128378	Insertion	CDS: probable minor tail protein	Asn792fs
DA23874	1128798	SNV	CDS: probable minor tail protein	
DA23874	1198420	SNV		
DA23874	1232990	Deletion	RBS: RBS	
DA23874	1275289	SNV	CDS: yceD	
DA23874	1292479	SNV	CDS: thiK	Ile240Val
DA23874	1375139	Insertion	CDS: ydjA	Asp72fs
DA23874	1375969	Insertion	CDS: selD	Arg126fs
DA23874	1383031	SNV	CDS: argD	
DA23874	1412250	SNV	CDS: thrS	
DA23874	1593976	Deletion		
DA23874	1672348	SNV	CDS: putative outer membrane lipoprotein	Asn30Asp
DA23874	1689722	SNV	CDS: ydcR	Glu31Gly
DA23874	1696210	Deletion	CDS: ydcN	Met1?
DA23874	1739562	SNV	CDS: ydbH	Thr75Ala
DA23874	1945674	SNV	CDS: yebS	
DA23874	1948551	Insertion		
DA23874	1964193	Insertion	CDS: mig-3	Glu55fs

DA23874	2011348	Insertion	CDS: flhB	Arg29fs
DA23874	2021974	SNV	CDS: flhD	Asp31Gly
DA23874	2025489	SNV	CDS: otsB	
DA23874	2191482	Insertion	CDS: wcaH	Ala61fs
DA23874	2191634	SNV	CDS: wcaH, CDS: wcaG	*322Trp, Asp10Gly
DA23874	2237713	SNV	CDS: yegU	Phe59Ser
DA23874	2256471	SNV	CDS: yehV	
DA23874	2268330	SNV	CDS: yohF	Thr145Ala
DA23874	2272816	SNV	CDS: salicylate hydroxylase	Pro50Ser
DA23874	2337127	SNV	CDS: hypothetical protein	Ser20Gly
DA23874	2507250	SNV		
DA23874	2539831	SNV	CDS: ligA	Pro396Ser
DA23874	2585981	SNV	CDS: ypfG	
DA23874	2656147	SNV		
DA23874	2677887	SNV	CDS: fdx	Thr104Ala
DA23874	2696935	SNV	CDS: cadC	
DA23874	2787988	SNV	CDS: yfiF	Val156Ala
DA23874	2828043	Insertion	CDS: STM2689	Asp156fs
DA23874	2864334	SNV	CDS: terminase-like protein	Phe99Leu
DA23874	2923168	SNV	CDS: iroE	
DA23874	2949760	SNV	CDS: putative regulatory protein	
DA23874	2954321	SNV	CDS: nrdE	Arg612His
DA23874	3017731	SNV	Misc. feature: SPI-1, TTSS-1	
DA23874	3037974	SNV	Misc. feature: SPI-1, TTSS-1, CDS: invC	Thr3Ala
DA23874	3147016	SNV	CDS: RecB	Ala882Asp
DA23874	3160713	SNV	CDS: ptsP	Gln484*
DA23874	3164027	Deletion	CDS: mutH	Asn216fs
DA23874	3185632	SNV	CDS: putative cytoplasmic protein	
DA23874	3188195	SNV	CDS: stdC	Arg95Cys
DA23874	3206917	SNV	RBS: RBS,	
DA23874	3285551	SNV	CDS: putative amino acid transporter	Thr457Ala
DA23874	3315175	SNV	CDS: putative methyl-accepting chemotaxis protein	
DA23874	3326574	SNV	CDS: putative cation transporter	
DA23874	3376866	SNV	CDS: rpoD	Val153Ala
DA23874	3468761	SNV	CDS: dacB	Ala34Thr
DA23874	3501490	SNV	CDS: yhcG	Ala289Thr

DA23874	3587680	SNV	CDS: rplO	
DA23874	3666047	SNV	CDS: feoB	Ala634Thr
DA23874	3679441	SNV	CDS: malT	Ile856Val
DA23874	3796464	SNV	CDS: yhjL	Gln317Arg
DA23874	3824219	SNV	CDS: lpfC	Met801Lys
DA23874	3882244	SNV	CDS: putative inner membrane protein	Ala347Val
DA23874	3942757	SNV	CDS: yicH	Arg6Met
DA23874	3995090	SNV	CDS: putative regulatory protein	Val93Ala
DA23874	4081275	SNV	CDS: gidB	Gln169*
DA23874	4095010	SNV	CDS: rbsB	Ala149Val
DA23874	4116110	SNV	CDS: putative cytoplasmic protein	
DA23874	4387155	SNV		
DA23874	4409829	SNV	CDS: metH	
DA23874	4444719	SNV	CDS: yjbH	Thr670Met
DA23874	4532538	SNV	CDS: basR	Arg210His
DA23874	4647665	SNV	CDS: ytfN	Thr369Ala
DA23874	4671944	Deletion	Misc. feature: putative sugar kinase, ribokinase family, contains frameshift	
DA23874	4716168	SNV	CDS: valS	Gly828Ser
DA23874	4719833	SNV	CDS: pepA	Val303Ala
DA23874	4728461	SNV	CDS: idnK	His157Arg
DA23874	4739921	Insertion	CDS: putative cytoplasmic protein	Glu79fs
DA23874	4740249	SNV	CDS: putative cytoplasmic protein	
DA23874	4741935	SNV	CDS: putative ABC-type sugar/spermidine/putrescine transport system ATPase component	Gly37Arg
DA23874	4809426	SNV	tRNA: leuV	
DA23874	4809658	SNV	tRNA: leuQ	
DA23877	2021643	SNV	CDS: flhC	Ala24Ser
DA23877	3252058	SNV	CDS: metK	Gln120Leu
DA23877	3678860	SNV	CDS: malT	Leu662*
DA23877	4081563	SNV	CDS: gidB	Asp73Asn
DA23879	656069	MNV		
DA23879	1685668	Deletion	CDS: srfC	Asp536fs
DA23879	3677833	Deletion	CDS: malT	Tyr320fs
DA23879	4081582	Insertion	CDS: gidB	Gln67fs

Supplementary Table 2. Epistatic interaction terms based on background-averaged epistasis (ε) and on epistatic terms relative to the wild type sequence (λ). The different reconstructed variants are listed in binary order, and the phenotypic resistance values were linearized as $\log_2$(MIC) to fit the linear epistasis model.

Strain number	Binary genotype[1]	Interaction terms[1]	MIC streptomycin (mg L^{-1})	$\log_2$(MIC) y	Background-averaged epistasis ε	Epistatic term λ
DA6192	00000	*****	4	2	5.24	2
DA40124	00001	****1	3	1.58	0.05	-0.42
DA39336	00010	***1*	6	2.58	0.95	0.58
DA40128	00011	***11	4	2	0.11	-0.17
DA40118	00100	**1**	8	3	1.33	1
DA40482	00101	**1*1	12	3.58	0.16	1
DA40122	00110	**11*	12	3.58	0.05	0
DA40381	00111	**111	16	4	0.10	0
DA39332	01000	*1***	8	3	1.53	1
DA40126	01001	*1**1	6	2.58	0.05	0
DA40130	01010	*1*1*	16	4	0.45	0.42
DA40385	01011	*1*11	12	3.58	-0.10	0.17
DA40120	01100	*11**	16	4	-0.09	0
DA40378	01101	*11*1	16	4	-0.60	-0.58
DA40389	01110	*111*	32	5	1.19	0
DA40469	01111	*1111	32	5	1.21	0
DA40134	10000	1****	32	5	3.80	3
DA40473	10001	1***1	24	4.58	0.30	0
DA40142	10010	1**1*	64	6	0.41	0.42
DA40477	10011	1**11	96	6.58	0.40	1.17
DA40471	10100	1*1**	96	6.58	-0.05	0.58
DA40483	10101	1*1*1	96	6.58	-1.10	-0.58
DA40383	10110	1*11*	128	7	0.10	-0.58
DA40463	10111	1*111	128	7	0.21	-1
DA40138	11000	11***	96	6.58	0.84	0.58
DA40475	11001	11**1	192	7.58	0.52	1.42
DA40387	11010	11*1*	192	7.58	-0.10	-0.42
DA40465	11011	11*11	256	8	-0.55	-1.75
DA40379	11100	111**	256	8	0.40	-0.17
DA40461	11101	111*1	192	7.58	-0.04	-1.25
DA40467	11110	1111*	768	9.58	2.38	1.17
DA40479	11111	11111	1024	10	2.42	2.42

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[1] The five genes are *gidB, nuoG, cyoB, znuA* and *trkH*, corresponding to the bits in the binary genotype column in left-to-right order.

Supplementary Table 3. Relative growth rate and mutation rates of isolated mutants. The growth rate of the ancestral wild type strain DA6192 is set to 1.0. Fitness was measured as exponential growth rate in Mueller Hinton medium at 37°C. Mutation rates μ are the estimated rates per cell per division corrected by the plating efficiency, and they were calculated using the bz-rates web tool[1].

Strain	Relative growth rate	Relative growth rate SD	Mutation rate *μ*
DA6192	1	0.0262	2.1e-09
DA23868	0.96	0.0177	1.9e-07
DA23869	0.89	0.0260	2.9e-07
DA23872	0.92	0.0177	3.2e-07
DA23874	0.98	0.0168	3.9e-07

Supplementary Table 4. Populations evolved above MIC. Mutations in the 5 populations of *S. typhimurium* evolved in MH medium containing 200 mg L^{-1} streptomycin for 100 generations compared to wild type. Mutation types are single-nucleotide variants (SNVs), deletions and insertions, and "fs" represents a frame shift mutation. All mutations detected in >3 reads are listed.

Strain	Position	Type	Frequency	Annotation	Amino acid change
DA60384	3602590	SNV	100.00	CDS: rpsL	Lys43Arg
DA60384	2174308	Deletion	23.81	CDS: rfbI	Gly268fs
DA60385	3602590	SNV	100.00	CDS: rpsL	Lys43Arg
DA60385	2173959	SNV	36.36	CDS: rfbF	Tyr55Asp
DA60386	3602590	SNV	100.00	CDS: rpsL	Lys43Arg
DA60386	2171585	SNV	20.41	CDS: rfbH	Pro218Leu
DA60386	1853325	SNV	11.43		
DA60386	2173649	SNV	10.00	CDS: rfbF	Pro158Leu
DA60387	3602590	SNV	100.00	CDS: rpsL	Lys43Arg
DA60387	2168404	SNV	36.36	CDS: rfbV	Pro72Leu
DA60387	2852125	SNV	35.90	CDS: hypothetical protein	
DA60387	2852155	SNV	14.29	CDS: hypothetical protein	
DA60387	2854636	SNV	11.11	CDS: phage tail-like protein	
DA60388	3602590	SNV	100.00	CDS: rpsL	Lys43Arg
DA60388	2168088	Deletion	31.58	CDS: rfbV	Glu178fs
DA60388	3052876	SNV	14.29		

Supplementary Table 5. Isolated resistant mutants. Mutants of *S. typhimurium* clones isolated from serial passage in MH medium containing 1 mg L^{-1} streptomycin for 900 generations.

Strain	Genotype	Reference
DA23866	Mutant isolated by plating a serial passage population on 256 mg/L streptomycin, Lineage 10, Clone 1	This study
DA23867	Mutant isolated by plating a serial passage population on 256 mg/L streptomycin, Lineage 10, Clone 2	This study
DA23868	Mutant isolated by plating a serial passage population on 256 mg/L streptomycin, Lineage 10, Clone 3	This study
DA23869	Mutant isolated by plating a serial passage population on 256 mg/L streptomycin, Lineage 19, Clone 1	This study
DA23870	Mutant isolated by plating a serial passage population on 256 mg/L streptomycin, Lineage 19, Clone 2	This study
DA23871	Mutant isolated by plating a serial passage population on 256 mg/L streptomycin, Lineage 19, Clone 3	This study
DA23872	Mutant isolated by plating a serial passage population on 192 mg/L streptomycin, Lineage 16, Clone 1	This study
DA23873	Mutant isolated by plating a serial passage population on 192 mg/L streptomycin, Lineage 16, Clone 2	This study
DA23874	Mutant isolated by plating a serial passage population on 128 mg/L streptomycin, Lineage 7, Clone 1	This study
DA23875	Mutant isolated by plating a serial passage population on 128 mg/L streptomycin, Lineage 7, Clone 2	This study
DA23876	Mutant isolated by plating a serial passage population on 128 mg/L streptomycin, Lineage 7, Clone 3	This study
DA23877	Mutant isolated by plating a serial passage population on 96 mg/L streptomycin, Lineage 11, Clone 1	This study
DA23878	Mutant isolated by plating a serial passage population on 96 mg/L streptomycin, Lineage 11, Clone 2	This study
DA23879	Mutant isolated by plating a serial passage population on 96 mg/L streptomycin, Lineage 9, Clone 1	This study
DA23880	Mutant isolated by plating a serial passage population on 96 mg/L streptomycin, Lineage 9, Clone 2	This study
DA23881	Mutant isolated by plating a serial passage population on 96 mg/L streptomycin, Lineage 9, Clone 3	This study

Supplementary Table 6. Primers used for strain reconstruction. Primers with a name containing "dup" were used to amplify and insert a *cat-sacB* cassette near each gene using Lambda-Red recombineering, generating a marker-held tandem duplication, while primers with a name containing "scr" were used to screen and sequence the gene for mutations. Primer names containing ‘csb’ were used to amplify a *cat-sacB* cassette that was inserted into the target genes and subsequently replaced by single stranded oligos (ssLR) resulting in a gene knockout or by a *dksA* variant from *Pseudomonas aeruginosa* (*dksA2*).

Name	Sequence
gidB_dup_F	ATGCCCGATGCGCTTTCGCTTATCGGGCCTACAAATTTGTAGGCTGGAGCTGCTTC
gidB_dup_R	AAACGACGCGGCTTGTGTTAAAAATCGACAGGTATAACGCATATGAATATCCTCCTTAGTTCC
gidB_scr_F	CGATTCGATTTTTAGCAGAC
gidB_scr_R	GAAAAAACAAGGTATGCTGC
trkH_dup_F	AATACCATCATCGCTATTTTCCTGCTGATCTCCGGTTGTAGGCTGGAGCTGCTTC
trkH_dup_R	CCAGTAGTCCAACGATTCGGGTAATGGCGCGAAAATGCATATGAATATCCTCCTTAGTTCC
trkH_scr_F	CCGAATCGTTGGACTACT
trkH_scr_R	CGGAACATAGCAGCAAGAC
cyoB_dup_F	CGATGAGATTACTAAGGCAGGGCTGAAAAATGGCAACTGATGTAGGCTGGAGCTGCTTC
cyoB_dup_R	TAATTTTCCGAACATCTTATCTTCCTCAACCCTTGGAGTTTTATATCATAAATATCTGTGTCATATGAATATCCTCCTTAGTTCC
cyoB_scr_F	TTACCCCAGGTTTCGTTCA
cyoB_scr_R	GGTCTGATGAACCTGGTG
nuoG_dup_F	TGGCGCGCGTCTTGAGGATCTGCGGGAGGCGCAACAATGACTTGTAGGCTGGAGCTGCTTC
nuoG_dup_R	ATCGTAGCCATTAGCATGCTTCCAAATTAAAAATCGTTAATTATATCATAAATATCTGTGTCATATGAATATCCTCCTTAGTTCC
nuoG_scr_F	CCTCACCCAAGACCAGTA
nuoG_scr_R	ATACCAAAACGCGGAAGA
znuA_dup_F	GGGAAATGGTCACTCATGATTGAATTATTACTGCCCGGCTGTAGGCTGGAGCTGCTTC
znuA_dup_R	GGACTCACCCCGCTCGGTCACTTTACCGTGAACCCTGAGACATATGAATATCCTCCTTAGTTCC
znuA _scr_F	GTGCCTCAAAATCCTTCAG
znuA _scr_R	CGGAGAGTTTTTGCATGG
znuA_csb_P1	CGTCGGCAGCCTGGGTTGCACTCCCCCAAAGCGCGGCGGATGTAGGCTGGAGCTGCTTC
znuA_csb_P2	CATTATGTTACAGAAAAATACGCTTCTTTTCGCAGCATTACATATGAATATCCTCCTTAGTTCC
znuA_del_ssLR	TATCACATTTCACACATTCATTACGATGATTAGTCGCATTCGAGGAAGTGAATACGTGCAACAGATAGCCCGCTCTGTCG
screen_znuA_fw	TTATCAGAAGCGGCAAGT
screen_znuA_rv	ACCAAATGAGACCGAGAC
dksA_csb_P1	AAACTCCCGCCTGTCATAAATAGGGTAGAAACGAACGGGAGTGTAGGCTGGAGCTGCTTC
dksA_csb_P2	CGAACATGGGGATCGATAGTGCGTGTTAAGGAGAAGCAACCATATGAATATCCTCCTTAGTTCC
dksA_del_ssLR	CGAACATGGGGATCGATAGTGCGTGTTAAGGAGAAGCAACTCCCGTTCGTTTCTACCCTATTTATGACAGGCGGGAGTTT
PA_dksA2_LT2-rpl_fw	AAACTCCCGCCTGTCATAAATAGGGTAGAAACGAACGGGATCAGTTGTGCCGCACGTG
PA_dksA2_LT2-rpl_rv	CGAACATGGGGATCGATAGTGCGTGTTAAGGAGAAGCAACATGACCGAACAGGAACTGC

Supplementary Table 7. Unintended mutations in the reconstructed strains. The mutations in *yebB* and *lon* were co-transduced from the isolated mutants, while the mutations in *hemG* and *gyrA* appeared during the reconstruction process.

Strain	***yebB***	***hemG***	***lon***	***gyrA***
DA40124	wt	P81L	wt	wt
DA40120	wt	wt	P12syn	wt
DA40130	S54G	wt	wt	wt
DA40122	wt	wt	P12syn	wt
DA40473	wt	P81L	wt	A386T
DA40126	wt	P81L	wt	wt
DA40482	wt	P81L	P12syn	wt
DA40128	wt	P81L	wt	wt
DA40387	S54G	wt	wt	wt
DA40383	S54G	wt	P12syn	wt
DA40389	S54G	wt	wt	wt
DA40475	wt	P81L	wt	wt
DA40483	wt	P81L	wt	wt
DA40378	wt	P81L	wt	wt
DA40477	S54G	P81L	wt	wt
DA40385	S54G	P81L	wt	wt
DA40381	S54G	P81L	P12syn	wt
DA40467	S54G	wt	wt	wt
DA40461	wt	P81L	wt	wt
DA40465	S54G	P81L	wt	wt
DA40463	S54G	P81L	P12syn	wt
DA40469	S54G	P81L	wt	wt
DA40479	S54G	P81L	wt	wt

Supplementary Table 8. Primers used for RT-PCR

Name	**Sequence**
qPCR_aadA_fw	GGA GGT CAC TGT CGT GCT TT
qPCR_aadA_rv	ACC ATG TCC CAG TCC TGC T
qPCR_iraP_fw	GGG CAC TTG AAG GCG TAA
qPCR_iraP_rv	GCG CAG CAA TTC CGT ATC
qPCR_cysG_fw	CCA CAG GAG GAA ATC AAC CA
qPCR_cysG_rv	CGT ACC ACG CGT TTA CCT TT
qPCR_hcaT_fw	ACG ATT ACC AGG CGA TTC TG
qPCR_hcaT_rv	CGC TGG GAC GTA ACA ACA TAC

Supplementary Reference

1. Gillet-Markowska, A., Louvel, G. & Fischer, G. bz-rates: A Web Tool to Estimate Mutation Rates from Fluctuation Analysis. G3 (*Bethesda*) **5**, 2323-2327 (2015).