

Description of Additional Supplementary Files

Supplementary Data 1: Mutations >5% in populations of *E. coli*, evolved under recurrent aminoglycoside treatment to high persistence.

¹Naming is as follows: the first abbreviation denotes the antibiotic that was used during the evolution experiments and the number indicates the population.

²Location with regards to the used reference for *E. coli* K12 MG1655 NC_000913.

Supplementary Data 2: Mutations <5% that target the main targets identified before in populations of *E. coli*, evolved under recurrent aminoglycoside treatment to high persistence.

Here, only the target genes identified in Supplementary Data 1 were analyzed for low frequency mutations without any cut-off frequency (thereby potentially containing false positives), supplemented with genes that locate in the same operon if a clear operon structure was available on EcoCyc2. The full list of genes grouped in operons: *nuoABCDCDEFGHIJKLMN*, *mscL*, *yaaU*, *yqhA*, *kdpDE*, *astABCDE*, *oppABCD*, *gadBC*, *yhiD*, *dctA*, *kch*, *yobD*, *accD*, *ybbW-glxK-ybbY-allB-glxR-hyi-gcl*, *hycABCDEFGH*, *yrhB*, *mdtABCD-baeSR*, *mdtEF*, *crp*, *manXYZ*, *mgtA*.

¹Naming is as follows: the first abbreviation denotes the antibiotic that was used during the evolution experiments and the number indicates the population.

²Location with regards to the used reference for *E. coli* K12 MG1655 NC_000913.3.

Supplementary Data 3: Key resources produced and/or used in this study.

Supplementary Data 4: Primers used in this study.