

7 **Supplementary Table 3.**

8 Verification of hits. To ensure the phenotype was mediated by the encoded peptide, the level
9 of resistance conferred after re-coding, induction by IPTG, removal of the start codon and
10 introduction of a frame shift was determined. Growth on plates containing 6 mg/L kanamycin
11 is indicated with '+' , weak growth with '(+)' and no growth with '-' .

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	recloned		recoded	no ATG	frame shift
	1mM IPTG	no IPTG			
<i>arp1</i>	+	-	(+)	-	-
<i>arp2</i>	+	-	ND	ND	ND
<i>arp3</i>	+	-	(+)	-	-

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17 **Supplementary Table 4.**

18 Whole genome sequencing results of six chromosomal mutants selected on 6 mg/L
 19 kanamycin (DA54853, DA54847, DA54850) or amikacin (DA54862, DA54869, DA54871).
 20 Insertions of IS5 (*insH*), IS1 (*insB*) and adenine (A) occurred upstream (us) of the
 21 corresponding gene.

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DA-number	gene	substitution	insertion/deletion
DA54853	<i>cydA</i>		152us:IS5
DA54847	<i>abrB</i>	T293A	
	<i>cydA</i>	G266T	
	<i>yffS</i>		36us:A
DA54850	<i>potD</i>	A445T	
	<i>mdtB</i>	G1088A	
	<i>ubiF</i>		31_45del
DA54862	<i>ubiJ</i>		G270del
DA54869	<i>ubiF</i>		31_45del
DA54871	<i>cydA</i>		175us:IS1

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25 **Supplementary Table 5.** List of transmembrane helices tested for increased aminoglycoside
 26 resistance.

Gene	Helix #	Sequence	Length (aa)	Increasing resistance
<i>envZ</i>	1	MLLLIVTLLFASLVTTYLVVL	21	No
	2	MPLFRYTLAIMLLAIGGAWLFI	22	No
<i>phoQ</i>	1	MFLATAAVVLVLSLAYGMVAL	22	No
	2	MFIYVLSANLLLVIPLLVAAW	22	No
<i>pstC</i>	1	MLAALIVLLMLGGIIVSLIIS	21	No
	2	MIYGTLVTSFIALLIAPVVSF	21	No
<i>murP</i>	1	MLIPGFIAAGLLGLIATLIATV	22	No
	2	MALNFMKVFSKGLFTFLVILVG	22	No
<i>pitA</i>	1	MFAGLDLHTGLLLLLALAFVLF	22	No
	2	MLAVVMAAVFNFLGVLLGGLSV	22	No

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