Supplementary material:

Table S1. The sizes of the biofilm populations (CFU/ml) of WT PAO1 and *ΔkatA* (katA) colony biofilm in the two lineages (lineage -1 and -2) evolved under exposure to 0.1mg/L CIP in (A) anaerobic conditions with 1 mM KNO3 and (B) aerobic conditions. ND not determined

(A)

Passage number	Pao1-1 Cip Anaerobic	Pao1-2 Cip Anaerobic	katA-1 Cip Anaerobic	katA-2 Cip Anaerobic
P0	3,35E+07	ND	2,21E+07	ND
P1	2,55E+04	1,08E+03	9,50E+02	2,07E+04
P2	4,59E+05	9,80E+03	2,42E+05	6,38E+03
P3	2,67E+05	5,22E+04	7,80E+04	2,02E+05
P4	2,90E+05	8,00E+02	2,70E+03	5,10E+03
P5	1,21E+06	1,02E+05	5,02E+05	6,00E+05
P6	5,16E+06	2,29E+06	1,36E+06	9,93E+05

(B)

Passage number	Pao1-1 Cip Aerobic	Pao1-2 Cip Aerobic	KatA-1 Cip Aerobic	KatA-2 Cip Aerobic
P0	3,36E+08	ND	3,00E+08	ND
P1	4,18E+09	2,75E+09	1,42E+09	2,61E+09
P2	1,24E+10	9,40E+07	6,25E+07	5,39E+09
P3	4,41E+09	3,90E+07	1,77E+09	1,76E+09
P4	7,60E+09	1,68E+08	1,38E+09	5,90E+09
P5	4,73E+09	1,53E+09	1,83E+08	8,30E+09
P6	5,41E+09	1,03E+08	1,45E+08	5,70E+09

Table S2. The frequency of the different types of mutations presented as % of total number of mutations in the evolved populations of WT PAO1 and *ΔkatA P. aeruginosa* (KatA). CIP: evolved with 0.1 mg/L ciprofloxacin, CTRL: control, BIO: biofilm, PLA: planktonic

	Transition		Transversion			
	A:T-G:C	G:C-A:T	G:C-T:A	G:C-C:G	A:T-T:A	A:T-C:G
PAO1 CTRL BIO	19%	5%	5%	14%	19%	7%
PAO1 CTRL PLA	12%	5%	7%	13%	16%	4%
PAO1 CIP BIO	9%	8%	7%	16%	13%	12%
PAO1 CIP PLA	18%	15%	6%	19%	16%	8%
KatA CTRL BIO	6%	3%	5%	14%	10%	41%
KatA CTRL PLA	8%	6%	7%	18%	12%	30%
KatA CIP BIO	15%	7%	6%	13%	11%	25%
KatA CIP PLA	7%	3%	8%	17%	11%	33%

Table S3.

List of genes mutated after evolution of *ΔkatA P. aeruginosa* in the absence (CTRL) or presence (CIP) of ciprofloxacin (0.1 mg/L) for seven passages in four CIP biofilm lineages (AI; DI; BII and DII), three CIP planktonic (PLA) lineages (A;B;C), four CTRL biofilm lineages (A;B;C;D) and four CTRL planktonic (PLA) lineages (A;B;C;D). The frequency of mutation, the sequence coverage/mutation, the change in the coding region, the amino acid changes and the names and functions of the respective genes are presented.

Figure S1. The development of CIP resistance in *ΔkatA* (katA*)* and WT PAO1 biofilm (biofilm) and planktonic (PLA) populations recovered from 1 mg/L CIP during passages (P0 to P6). Under exposure to ciprofloxacin, *ΔkatA* CIP-biofilm populations developed a significantly higher resistant subpopulation compared to WT PAO1 CIP-biofilm populations at P3(p= 0.01), P4(p=0.02), P5(p=0.02) and P6(p=0.03). The values represent the mean (SEM) of the replicates in each condition.

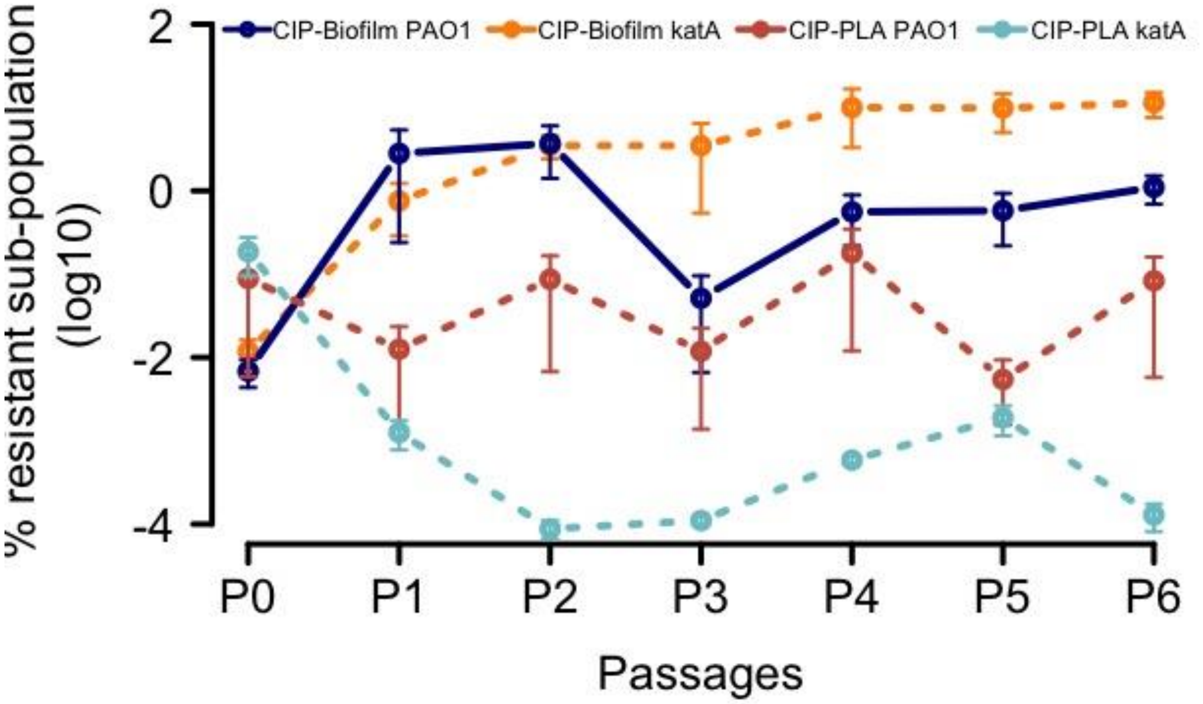


Figure S2. The number of nonsynonymous mutations by gene function category (mean +SEM) documented for *ΔkatA* biofilm (BIO) and planktonic (PLA) populations evolved in the absence (CTRL) or presence of CIP(CIP). * means that the CIP BIO *ΔkatA* and CIP PLA *ΔkatA* is significantly different from CTRL BIO *ΔkatA* and CTRL PLA *ΔkatA*, respectively.

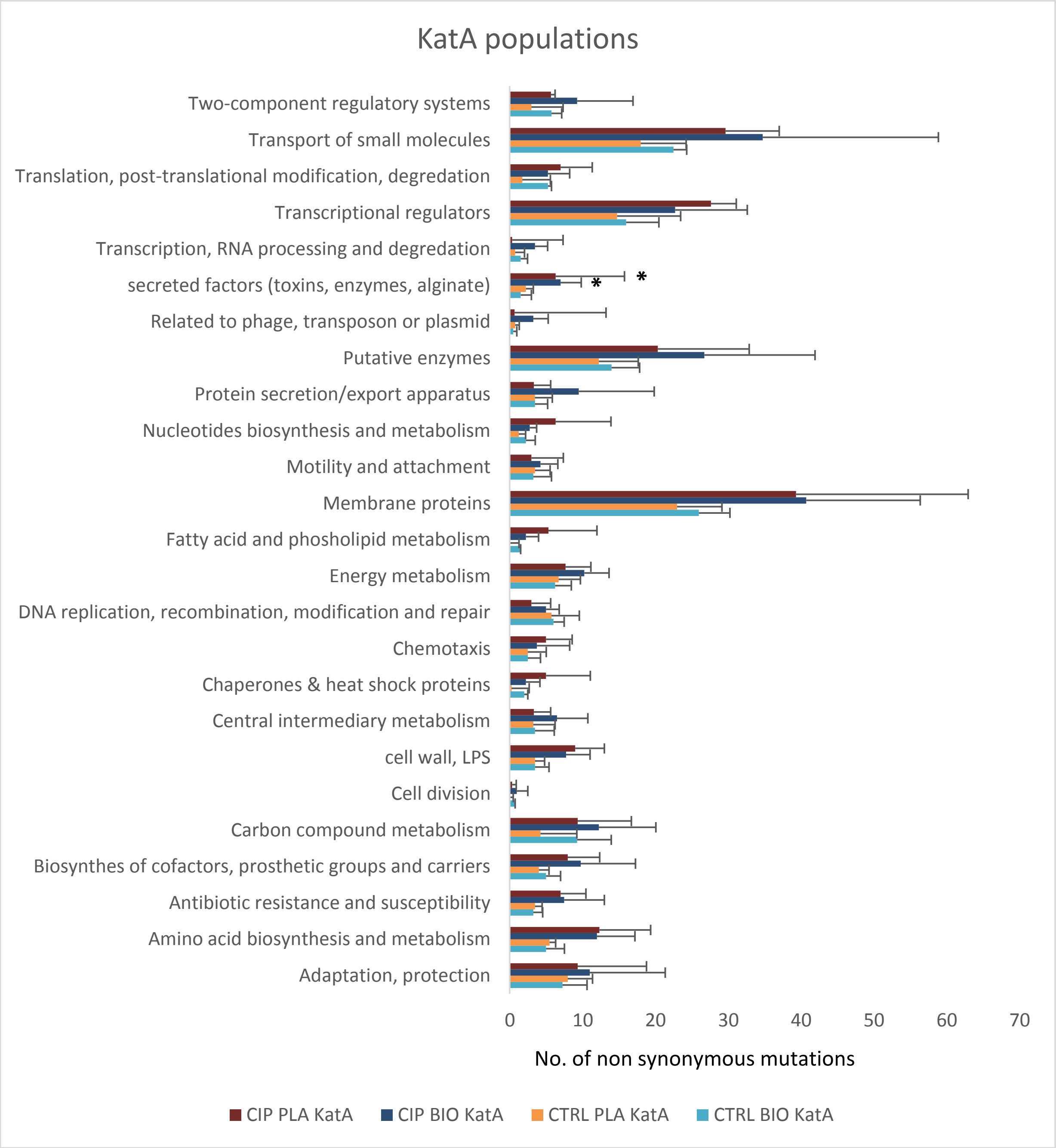
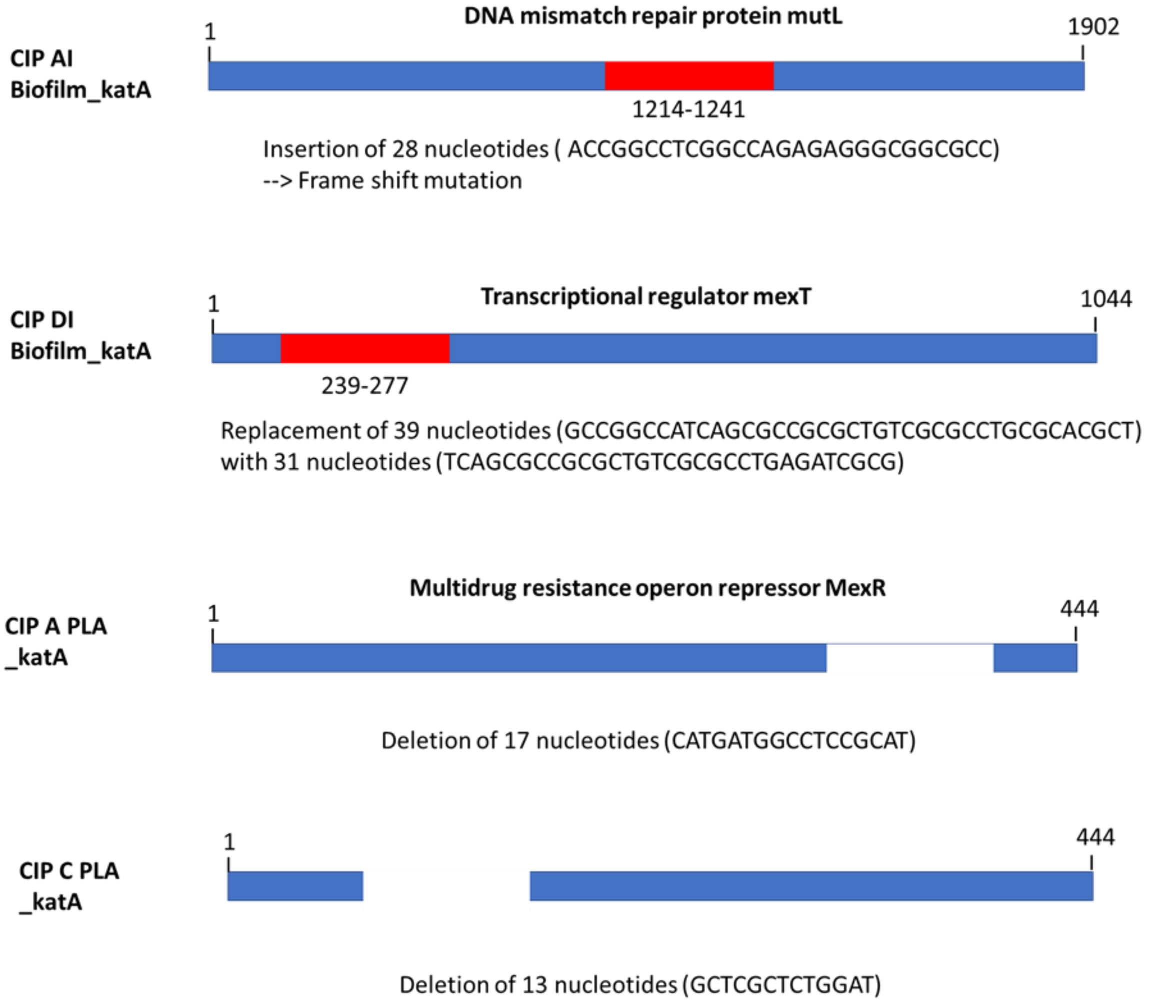


Figure S3. Large insertion in *mutL*, replacement in *mexT* and deletion in *mexR*, respectively in lineages AI and DI of CIP-evolved *ΔkatA* biofilm and in lineages A and C of CIP evolved *ΔkatA* planktonic culture.



Insertion of 28 nucleotides (ACCGGCCTCGGCCAGAGAGGGCGGCGCC)
--> Frame shift mutation

Replacement of 39 nucleotides (GCCGGCCATCAGCGCCGCGCTGTCGCGCCTGCGCACGCT)
with 31 nucleotides (TCAGCGCCGCGCTGTCGCGCCTGAGATCGCG)

Deletion of 17 nucleotides (CATGATGGCCTCCGCAT)

Deletion of 13 nucleotides (GCTCGCTCTGGAT)