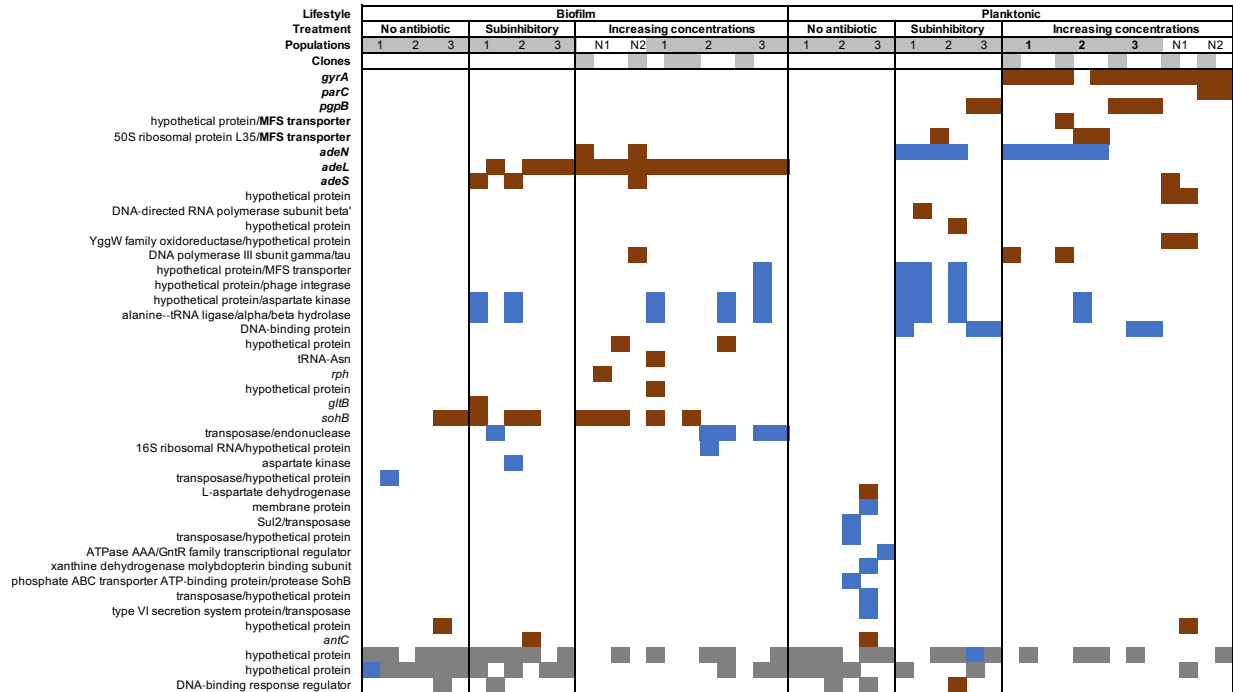
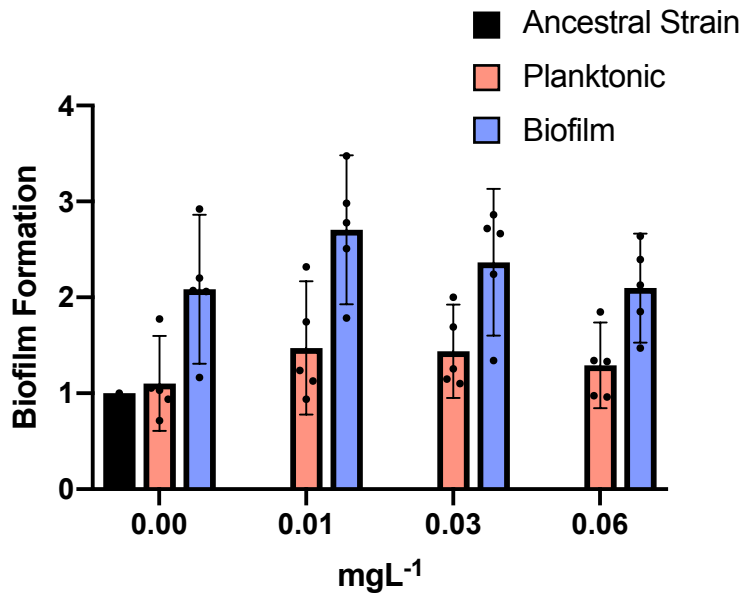


600

601 **Figure S1. Genetic diversity of samples at subinhibitory concentrations of**
 602 **ciprofloxacin (A) or during the evolutionary rescue experiment with increasing**
 603 **concentrations of ciprofloxacin (B). Biofilm populations in blue and planktonic**
 604 **populations in red.**



605 **Figure S2. Mutated genes in the sequenced clones differ between treatments.** Each
606 column represents one clone. Grey shaded clones were used for MIC and fitness
607 estimations. Red color indicates SNPs or small indels, blue color indicates new junction
608 evidences and grey indicates missing coverage indicative of a deletion.
609



610

611 **Figure S3. Biofilm production under subinhibitory concentrations of CIP.** Blue and
 612 red bars show biofilm and planktonic clones, respectively. The ancestral strain is
 613 represented by the black bar. Individual clone results are shown as points. The averages
 614 are shown by bars. 95% CI are indicated by the error bars. Biofilm clones produced more
 615 biofilm than planktonic clones: two tailed t-test of biofilm formation with 0.00 mg/L of
 616 CIP: $p = 0.006$, $t = 3.008$, $df = 32$; with 0.01 mg/L of CIP: $p = 0.0006$, $t = 3.780$, $df = 32$;
 617 with 0.03 mg/L of CIP: $p = 0.0077$, $t = 2.841$, $df = 32$ and with 0.0625 mg/L of CIP: $p =$
 618 0.018 , $t = 2.471$, $df = 32$.

619

Treatment and Populations		MIC (mg/L)	Fold MIC increase
No Antibiotic			
Planktonic			
	P1	0.25 ± 0.00	2
	P2	0.25 ± 0	2
	P3	0.21 ± 0.03	1.68
Biofilm			
	B1	0.25 ± 0	2
	B2	0.25 ± 0	2
	B3	0.25 ± 0	2
Subinhibitory			
Planktonic			
	P1	2.33 ± 0.72	18.64
	P2	4 ± 0	32
	P3	1 ± 0	8
Biofilm			
	B1	0.41 ± 0.07	3.28
	B2	0.5 ± 0	4
	B3	0.5 ± 0	4
Evolutionary rescue			
Planktonic			
	P1	26.67 ± 5.34	213.36
	P2	32 ± 0	256
	P3	6.67 ± 1.34	53.36
Biofilm			
	B1	1 ± 0	8
	B2	0.5 ± 0	4
	B3	0.83 ± 0.17	6.64

620

621 **Table S1. Antibiotic susceptibility of the populations propagated in the absence, in**
622 **subinhibitory concentrations or increasing concentrations of CIP at the end of the**
623 **experiment (day 12). MICs are expressed in mg/L and standard errors of the mean are**
624 **indicated. Fold increase in MIC compared to the ancestral strain are also indicated.**

Treatment and Populations	MIC measured in biofilms (mg/L)	Fold MIC increase
Evolutionary rescue		
Planktonic		
P1	> 256 ± 0	>2048
P2	> 256 ± 0	>2048
P3	> 256 ± 0	>2048
Biofilm		
B1	32 ± 0	256
B2	32 ± 0	256
B3	32 ± 0	256

625

626 **Table S2. Antibiotic susceptibility of the populations propagated in increasing**
627 **concentrations of CIP at the end of the experiment (day 12).** MICs were measured in
628 biofilms and are expressed in mg/L and standard errors of the mean are indicated. Fold
629 increase in MIC compared to the ancestral strain are also indicated.

	Days	Increasing concentrations				Subinhibitory concentrations			
		Nonsynonymous/Synonymous us	Intergenic mutations	Structural variants	Total mutations	Nonsynonymous/Synonymous us	Intergenic mutations	Structural variants	Total mutations
Biofilm	1	4/0	0	4	8	4/0	0	4	8
	3	5/0	0	8	13	5/0	0	8	13
	4	2/0	0	2	4	6/1	2	8	17
	6	3/1	0	4	8	-	-	-	-
	7	6/0	3	4	10	-	-	-	-
	9	5/0	2	6	11	4/0	1	6	11
	10	7/0	2	5	12	-	-	-	-
	12	7/0	1	6	13	5/0	1	7	12
Planktonic	1	1/0	0	3	4	1/0	0	3	4
	3	2/0	0	2	4	2/0	0	2	4
	4	1/0	0	2	3	1/0	0	3	4
	6	5/0	2	7	12	-	-	-	-
	7	6/0	3	4	10	-	-	-	-
	9	3/1	3	6	10	2/0	1	4	7
	10	5/2	5	6	13	-	-	-	-
	12	7/1	1	5	13	2/0	2	7	11

632 **Table S4. Attributes of the contending mutations each day in the experimental**
633 **evolution.**

Locus Tag	Mutation	Annotation	Description
ACX60_RS01385	A→G	E292G (GAG→GGG)	energy-dependent translational throttle protein EttA
ACX60_RS04575	C→T	R226H (CGT→CAT)	fructose 1,6-bisphosphatase
ACX60_RS18160	(ATGGTG) ⁹ → ⁸	coding (454-459/987 nt)	cation transporter

634

635 **Table S5. Mutated genes in the ancestral strain compared to the *A. baumannii***

636 **ATCC 17978-mff complete genome (GCF_001077675.1) after 10 days of evolution**

637 **in M9+.**

638

Transporter Family	Regulator	Efflux pump	Substrates^a
RND	AdeSR	AdeABC	AG, BL , CHL, ERY, FQ , NAL, TET, TGC
RND	AdeL	AdeFGH	CHL, ERY, FQ , NAL, SUL, TET, TGC, TMP
RND	AdeN	AdeIJK	AZI, BL , CHL, ERY, FQ , FUA, MIN, NAL, RIF, SA, SUL, TET, TMP
MATE	-	AbeM	FQ , GEN

640

641 **Table S7. Efflux pumps and their regulators in *A. baumannii* 17978 targeted under**

642 **CIP pressure.** Table adapted from (Li, Elkins et al. 2016). AG aminoglycosides, AZI

643 azithromycin, BL β -lactams, CHL chloramphenicol, CIP CIP, CL clindamycin, ERY

644 erythromycin, FLO florfenicol, FUA fusidic acid, GEN gentamicin, MIN minocycline,

645 NAL nalidixic acid, NOR norfloxacin, RIF rifampicin, SUL sulfonamides, TET

646 tetracycline, TGC tigecycline, TMP trimethoprim. ^a References in (57).