Supplementary table 1a. MIC-values and susceptibility of the population and sequencing results for the start and ciprofloxacin-treated populations of PAO1, PAOΔmutS (*mutS*) and PAOMY-Mgm (*mutMY*).

	MIC and susceptibility of population (μg/ml)	**MIC of resistant colonies (μg/mL)**	***nfxB*** **NfxB**	***gyrA*** **GyrA**	***gyrB*** **GyrB**	***parC*** **ParC**	***parE*** **ParE**
PAO1 start	0.19, dz[a] -, (-)	-[b]	nc[c]	nc	nc	nc	nc
PAO1 A, Cip, day 3	1.75, dz -, (-)	-	-	-	-	-	-
PAO1 B, Cip, day 3	1.5, dz -, (-)	-	-	-	-	-	-
PAO1 C, Cip, day 3	1.5, dz -, (-)	-	-	-	-	-	-
PAO1 A, Cip, day 10	1.5, dz -, (+++)	32	*del C pos. 6* stop	*C248T* T83I	nc	nc	Nc
PAO1 B, Cip, day 10	1, dz 3, (+)	8	nc	*G259T* D87Y	nc	nc	Nc
PAO1 C, Cip, day 10	1, dz -, (++)	16	*336 ins G* Frameshift	nc	*C1397A* S466Y	nc	Nc
PAO1 A, Cip, day 17	1.5, dz -, (+++)	32	nc	*C248T* T83I	nc	nc	Nc
PAO1 B, Cip, day 17	0.5, dz 4, (+)	16	*del C pos. 6* stop	*G259T* D87Y	nc	nc	Nc
PAO1 C, Cip, day 17	1.5, dz -, (+++)	16	*G461A* G154D	nc	*C1397A* S466Y	nc	Nc
PAO1 A, Cip, day 24*	1.5, dz -, (+++)	32	nc	*C248T* T83I	nc	nc	Nc
PAO1 B, Cip, day 24	0.75, dz 4, (++)	16	*G436A* E146K	*G259T* D87Y	nc	nc	Nc
PAO1 C, Cip, day 24*	1.5, dz 12, (+)	8	*G461A* G154D	nc	*C1397A* S466Y	nc	Nc
PAO1 A, Cip, day 94	1.5, dz 8, (+)	32	nc	*C248T* T83I	nc	nc	Nc
PAO1 B, Cip, day 94	1.5, dz 4, (++)	16	nc	*G259T* D87Y	nc	nc	Nc
PAO1 C Cip, day 94	4, dz 6, (++)	32	nc	*G259T* D87Y	*C1397A* S466Y	nc	Nc

	MIC and susceptibility of population (μg/ml)	MIC of resistant colonies (μg/mL)	*nfxB* NfxB	*gyrA* GyrA	*gyrB* GyrB	*parC* ParC	*parE* ParE
mutS start	0.19, dz -, (+++) [d]	-	nc	nc	nc	nc	Nc
mutS A, Cip, day 3	1.5, dz 8, (+)	64	*C434T* T145I	*C248T* T83I	nc	nc	Nc
mutS B, Cip, day 3	1, dz 8, (++)	64	nc	*C248T* T83I	nc	nc	Nc
mutS C, Cip, day 3	1, dz 4, (++)	64	nc	*C248T* T83I	nc	nc	Nc
mutS A, Cip, day 10	1.5, dz 12, (+)	64	nc	*C248T* T83I	nc	nc	Nc
mutS B, Cip, day 10	1.5, dz 12, (+)	64-128	*G538A* G180S	*C248T* T83I	nc	nc	Nc
mutS C, Cip, day 10	1.5, dz 16, (++)	64	*G461A* G154D	*C248T* T83I	nc	nc	Nc
mutS A, Cip, day 17	1.5, dz 12, (++)	64	nc	*C248T* T83I	nc	nc	Nc
mutS B, Cip, day 17	4, dz 16, (+)	64	*G538A* G180S	*C248T* T83I	nc	nc	Nc
mutS C, Cip, day 17	3, dz 12, (++)	64	nc	*C248T* T83I	nc	nc	Nc
mutS A, Cip, day 24	3, dz 24, (+)	64	*G461A* G154D	*C248T* T83I	nc	nc	Nc
mutS B, Cip, day 24	4, dz 16, (+)	64	nc	*C248T* T83I	nc	nc	Nc
mutS C, Cip, day 24	3, dz 8, (++)	128	nc	*C248T* T83I	nc	nc	Nc
mutS A, Cip day 94	8, dz 8, (++)	128	nc	*C248T* T83I	nc	*C260T* S87L	Nc
mutS B, Cip day 94	4, dz -, (+++)	128	nc	*C248T* T83I	nc	nc	Nc
mutS C, Cip day 94	4, dz 16, (++)	128	*T499C* S167P	*C248T* T83I	nc	*C260T* S87L	Nc

	MIC and susceptibility of population (μg/ml)	MIC of resistant colonies (μg/mL)	*nfxB* NfxB	*gyrA* GyrA	*gyrB* GyrB	*parC* ParE	*parE* ParE
mutMY start	0.19, dz -, (+++) [d]	-	nc	nc	nc	nc	Nc
mutMY A, Cip, day 3	1.5, dz -, (++)	16	*C473A* A158E	nc	*C1397A* S466Y	nc	Nc
mutMY B, Cip, day 3	1, dz -, (++)	16	*G538T* G180C	*G259T* D87Y	nc	nc	Nc
mutMY C, Cip, day 3	1.5 , dz -, (+++)	16	nc	*G259T* D87Y	nc	nc	Nc
mutMY A, Cip, day 10	1, dz 6, (++)	16	nc	nc	*C1397A* S466Y	nc	Nc
mutMY B, Cip, day 10	1.5, dz 6, (++)	16	*G538T* G180C	*G259T* D87Y	nc	nc	Nc
mutMY C, Cip, day 10	1.5, dz 8, (+)	16	nc	*G259T* D87Y	nc	nc	Nc
mutMY A, Cip, day 17	3, dz 12, (+)	16	*G538T* G180C	nc	*C1397A* S466Y	nc	Nc
mutMY B, Cip, day 17	1.5, dz 12, (++)	16	*G538T* G180C	*G259T* D87Y	nc	nc	Nc
mutMY C, Cip, day 17	6, dz 16, (+)	16	nc	*G259T* D87Y	nc	nc	Nc
mutMY A, Cip, day 24	6, dz 16, (+)	16	*G539T* G180V	*G259T* D87Y	nc	nc	Nc
mutMY B, Cip, day 24	4, dz 12, (++)	16	nc	*G259T* D87Y	nc	nc	Nc
mutMY C, Cip, day 24	6, dz 12, (+)	16	nc	*G259T* D87Y	nc	nc	Nc
mutMY A, Cip, day 94	2, dz -, (+++)	8	*G140T* R47L	nc	*C1397A* S466Y	nc	Nc
mutMY B, Cip, day 94	2, dz -, (+++)	32	nc	*G259T* D87Y	nc	nc	Nc
mutMY C, Cip, day 94	6, dz -, (++)	32	*C495A* A165D	*G259T* D87Y	nc	nc	Nc

[a]double zone, [b]not determined, [c]no change, [d]nfxB, gyrA and gyrB sequences are shown in supplementary table 2. *Interpretation of bacterial population in figure 1.

Supplementary table 1b. MIC-values and susceptibility of the population and sequencing results for the start and control populations of PAO1, PAOΔmutS (*mutS*) and PAOMY-Mgm (*mutMY*).

	MIC and susceptibility of population (μg/ml)	**MIC of resistant colonies (μg/mL)**	***nfxB*** **NfxB**	***gyrA*** **GyrA**	***gyrB*** **GyrB**
PAO1 start	0.19, dz -, (-)	-	nc	nc	nc
PAO1 A, day 3	0.19 dz - (-)	-	-	-	-
PAO1 B, day 3	0.094 dz - (+) 16	0.19 dz 6	nc	nc	nc
PAO1 C, day 3	0.125 dz - (-)	-	-	-	-
PAO1 A, day 10	0.19 dz - (+) 0.75	0.25	nc	nc	nc
PAO1 B, day 10	0.19 dz - (+) 24	0.25	nc	nc	nc
PAO1 C, day 10	0,19 dz - (-)	-	-	-	-
PAO1 A, day 17	0.19 dz - (++) 4	0.25	nc	nc	nc
PAO1 B, day 17	0.19 dz - (++) 6	0.25 (+)0.5	nc	nc	nc
PAO1 C, day 17	0.19 dz - (+) 0.38	0.75 dz 6	nc	nc	nc
PAO1 A, day 24	0.19 dz - (++) 32	1.5	nc	nc	nc
PAO1 B, day 24	0.19 dz - (++) 3	1 dz 8	nc	nc	nc
PAO1 C, day 24	1.5 dz - (+++) 24	0.25	nc	nc	nc
PAO1 A, day 94	0.094 dz - (++) 0.5	0.19 dz 6	nc	nc	nc
PAO1 B, day 94	0.125 dz - (+) 0.38	0.75 dz 6	nc	nc	nc
PAO1 C, day 94	0.125 dz - (+) 0.19	0.25	nc	nc	nc

	MIC and susceptibility of population (μg/ml)	**MIC of resistant colonies (μg/mL)**	***nfxB*** **NfxB**	***gyrA*** **GyrA**	***gyrB*** **GyrB**
mutS start	0.19, dz -, (+++)	-	nc	nc	nc
mutS A, day 3	0.19 dz - (+++) 3	8	*T518C* Leu/Pro	nc	nc
mutS B, day 3	0.19 dz - (+++) 2	8	*T86C* Leu/Pro	nc	nc
mutS C, day 3	0.125 dz - (++) 1	12	*T530C* Phe/Ser	-	nc
mutS A, day 10	0.25 dz - (+++) 2	8	*T41C* Leu/Pro	-	nc
mutS B, day 10	0.19 dz - (+++) 6	24	nc	nc	nc
mutS C, day 10	0.19 dz - (+++) 2	>32	-	nc	nc
mutS A, day 17	0.19 dz - (+++) 3	12	*T77C* Leu/Pro	nc	nc
mutS B, day 17	0.19 dz - (+++) 2	8	-	nc	nc
mutS C, day 17	0.19 dz - (+++) 4	8-24	nc	nc	nc
mutS A, day 24	0.25 dz - (+++) 2	32	nc	C248T Thr/Ile	nc
mutS B, day 24	0.25 dz - (+++) 4	32	-	C248T Thr/Ile	nc
mutS C, day 24	0.19 dz - (+++) 2	24	nc	C248T Thr/Ile	nc
mutS A, day 94	0.94 dz - (+++) 1	32	nc	C248T Thr/Ile	nc
mutS B, day 94	0.125 dz - (+++) 0.75	8	-	C248T Thr/Ile	nc
mutS C, day 94	0.125 dz - (+++) 1.5	8	nc	C248T Thr/Ile	nc

	MIC and susceptibility of population (μg/ml)	MIC of resistant colonies (μg/mL)	*nfxB* NfxB	*gyrA* GyrA	*gyrB* GyrB
mutMY start	0,19 dz - (+++) 2	2 dz - (+) 3	nc	nc	nc
mutMY A, day 3	0.19 dz - (+++) 6	8	*G475T* Stop codon	nc	nc
mutMY B, day 3	0.19 dz - (++) 1.5	12	*G214T* Stop codon	nc	nc
mutMY C, day 3	0.19 dz - (++) 1.5	8	ND	nc	nc
mutMY A, day 10	0.19 dz - (+++) 1.5	8	*C124A* Arg/Ser	nc	nc
mutMY B, day 10	0.19 dz - (+++) 2	8	ND	nc	nc
mutMY C, day 10	0.19 dz - (++) 1.5	8	nc	nc	nc
mutMY A, day 17	0.19 dz - (+++) 4	8	ND	nc	nc
mutMY B, day 17	0.19 dz - (+++) 2	8	*C124A* Arg/Ser	nc	nc
mutMY C, day 17	0.19 dz - (++) 1	8	*G175T* Stop codon	nc	nc
mutMY A, day 24	0.19 dz - (+++) 12	8-12	nc	nc	nc
mutMY B, day 24	0.19 dz - (+++) 2	8	G91T Stop codon	nc	nc
mutMY C, day 24	0.19 dz - (+++) 2	8	ND	nc	nc
mutMY A, day 94	0.064 dz - (++) 0.75	12	nc	*G259T* D87Y	nc
mutMY B, day 94	0.047 dz - (++) 0.75	4 (++)12	nc	nc	nc
mutMY C, day 94	0.047 dz - (++) 0.5	0.75 (+++)8	nc	*G259T* D87Y	nc

dz = double zone, - = not determined, nc = no change.

Supplementary table 2. MIC-values and sequencing results for individual ciprofloxacin resistant colonies isolated from the populations of PAO1, PAOΔmutS (*mutS*) and PAOMY-Mgm (*mutMY*) after 94 days evolution in the presence of 0,05µg/mL ciprofloxacin and from the start population of PAOΔmutS (*mutS*) and PAOMY-Mgm (*mutMY*) (pre-existant resistant subpopulations) (lineage A). nc= no change

	MIC (µg/mL)	***nfxB*** **NfxB**	***gyrA*** **GyrA**	***gyrB*** **GyrB**	***parC*** **ParC**	***parE*** **ParE**
PAO1 A-1, Cip, day 94	16	-	*C248T* T83I	nc	-	-
PAO1 A-2, Cip, day 94	8	-	*C248T* T83I	nc	-	-
PAO1 A-3, Cip, day 94	8	-	*C248T* T83I	nc	-	-
PAO1 A-4, Cip, day 94	8	-	*C248T* T83I	nc	-	-
PAO1 A-5, Cip, day 94	16	-	*C248T* T83I	nc	-	-
PAO1 A-6, Cip, day 94	16	-	*C248T* T83I	nc	-	-
PAO1 A-7, Cip, day 94	16	-	*C248T* T83I	nc	-	-

	MIC (µg/mL)	***nfxB*** **NfxB**	***gyrA*** **GyrA**	***gyrB*** **GyrB**	***parC*** **ParC**	***parE*** **ParE**
mutS start-1	3	nc	*C248T* T83I	nc	-	-
mutS start-2	3	nc	*C248T* T83I	nc	-	-
mutS start-3	3	nc	*C248T* T83I	-	-	-
mutS start-4	2	nc	*C248T* T83I	nc	-	-
mutS start-5	4	nc	*C248T* T83I	nc	-	-
mutS start-6	3	nc	*C248T* T83I	nc	-	-
mutS A-1, Cip, day 94	64-128	nc	*C248T* T83I	nc	C260T S87L	-
mutS A-2, Cip, day 94	64-128	nc	*C248T* T83I	nc	C260T S87L	-
mutS A-3, Cip, day 94	64-128	nc	*C248T* T83I	nc	C260T S87L	-
mutS A-4, Cip, day 94	64-128	nc	*C248T* T83I	nc	nc	-
mutS A-5, Cip, day 94	64-128	nc	*C248T* T83I	nc	nc	-
mutS A-6, Cip, day 94	64-128	nc	*C248T* T83I	nc	nc	-

	MIC (μg/mL)	***nfxB*** **NfxB**	***gyrA*** **GyrA**	***gyrB*** **GyrB**	***parC*** **ParC**	***parE*** **ParE**
mutMY start-1	-	nc	nc	nc	-	-
mutMY start-2	4	*G125T* R42L	nc	nc	-	-
mutMY start-3	4	*G539T* G180V	nc	nc	-	-
mutMY start-4	4	*G539T* G180V	nc	nc	-	-
mutMY start-5	4	nc	nc	*C2246A* P749H	-	-
mutMY start-6	6	*G172T* stop	nc	nc	-	-
mutMY A-1, Cip, day 94	16	-	nc	*C1397A* S466Y	-	-
mutMY A-2, Cip, day 94	8	-	nc	*C1397A* S466Y	-	-
mutMY A-3, Cip, day 94	< 4	-	nc	*C1397A* S466Y	-	-
mutMY A-4, Cip, day 94	8	-	nc	*C1397A* S466Y	-	-
mutMY A-5, Cip, day 94	8-16	-	nc	*C1397A* S466Y	-	-
mutMY A-6, Cip, day 94	16	-	nc	*C1397A* S466Y	-	-
mutMY A-7, Cip, day 94	16	-	nc	*C1397A* S466Y	-	-
mutMY A-8, Cip, day 94	8	-	nc	*C1397A* S466Y	-	-
mutMY A-9, Cip, day 94	8	-	nc	*C1397A* S466Y	-	-

- = not determined, nc = no change

Supplementary table 3. Susceptibility to ceftazidime, meropenem and tobramycin of the start strains and C-lines of PAO1, PAOΔmutS (*mutS*) and PAOMY-Mgm (*mutMY*) populations that evolved in the presence or absence of 0.05 μg/mL ciprofloxacin.

	Ceftazidime (μg/mL)		**Meropenem (μg/mL)**		**Tobramycin (μg/mL)**	
	with ciprofloxacin (0.05 μg/mL)	**without ciprofloxacin**	**with ciprofloxacin (0.05 μg/mL)**	**without ciprofloxacin**	**with ciprofloxacin (0.05 μg/mL)**	**without ciprofloxacin**
PAO1 start	-	1.5, dz[a] -, (-)	-	0.094, dz -, (-)	-	1.5, dz -, (-)
PAO1 C, day 3	1.5, dz 8, (+) 12	2, dz -, (-)	0.094, dz -, (++) 0.38	0.094, dz -, (-)	1.5, dz -, (-)	1.5, dz -, (-)
PAO1 C, cip, day 10	2, dz -, (-)	2, dz -, (-)	0.125, dz -, (+) 0.25	0.094, dz -, (-)	1.5, dz -, (-)	1.5, dz -, (-)
PAO1 C, cip, day 17	2, dz -, (++) 4	2, dz -, (-)	0.094, dz -, (+) 0.25	0.094, dz -, (-)	1.5, dz -, (-)	1.5, dz -, (-)
PAO1 C, cip, day 24	2, dz -, (++) 4	2, dz -, (-)	0.094, dz -, (++) 0.5	0.094, dz -, (-)	1.5, dz - (-)	1.5, dz -, (-)
PAO1 C, cip, day 94	4, dz -, (-)	2.5, dz -, (-)	0.064, dz 0.25, (+) 8	0.094, dz -, (-)	1.5, dz -, (-)	1.5, dz -, (-)
mutS start	-	2, dz -, (++) 3	-	0.094, dz -, (+++) 0.25	-	1.5, dz -, (-)
mutS C, Cip, day 3	2, dz -, (++) 6	1.5. dz -, (-)	0.094, dz -, (+++) 0.5	0.125, dz -, (-)	1.5, dz -, (-)	1.5, dz -, (-)
mutS C, Cip, day 10	2, dz -, (+++) 8	3, dz -, (+) 8	0.094, dz -, (+++) 1.5	0.125, dz -, (++) 0.25	1.5, dz -, (+) 2	1.5, dz -, (-)
mutS C, Cip, day 17	2, dz -, (+++) 16	3, dz -, (+) 6	0.094, dz -, (+++) 0.75	0.094, dz -, (++) 1.5	1.5, dz -, (+) 2	1.5, dz -, (-)
mutS C, Cip, day 24	2, dz -, (+++) 8	3, dz -, (++) 4	0.094, dz -, (+++) 8	0.094, dz -, (++) 0.38	1.5, dz -, (-)	1.5, dz -, (-)
mutS C, Cip day 94	1, dz -, (+++) 12	1.5, dz -, (++) 4	0.094, dz -, (+++) 3	0.094, dz -, (++) 0.75	1.5, dz -, (-)	2, dz -, (-)
mutMY start	-	1.5, dz 2, (+) 4	-	0.094, dz -, (++) 0.5	-	1.5, dz -, (-)
mutMY C, Cip, day 3	1.5, dz 8, (-)	2, dz -, (-)	0.094, dz 0.19, (++) 2	0.094, dz -, (++) 0.25	2, dz -, (-)	1.5, dz -, (-)
mutMY C, Cip, day 10	2, dz -, (++) 6	2, dz -, (+) 3	0.094, dz -, (++) 0.75	0.094, dz -, (+) 0.19	1.5, dz -, (-)	1.5, dz -, (-)
mutMY C, Cip, day 17	2, dz -, (++) 6	3, dz -, (+) 4	0.094, dz -, (++) 1.0	0.094, dz -, (++) 0.25	1.5, dz -, (-)	1.5, dz -, (-)
mutMY C, Cip, day 24	2, dz -, (++) 6	2, dz -, (+) 3	0.094, dz -, (+++) 1.0	0.094, dz -, (++) 1	1.5, dz -, (-)	1.5, dz -, (-)
mutMY C, Cip, day 94	2, dz 4, (+) 6	1.5, dz -, (+) 4	0.094, dz 0.125, (++) 1.5	0.094, dz -, (++) 0.75	1.5, dz -, (+) 3	1.5, dz -, (-)

[a]dz = double zone

Supplementary table 4. Determination of colonies with lack of growth on glucose minimal media(possible auxotrophs) in PAO1, PAOΔmutS (*mutS*) and PAOMY-Mgm (*mutMY*) bacterial populations that have evolved in the presence and absence of ciprofloxacin for 24 and 94 days.

	Number of colonies on M9 plates	**Number of colonies on LB plates**	**% possible auxotrophs**
PAO1 start	50	50	0
mutS start	50	50	0
mutMY start	50	50	0
PAO1 A Cip, day 24	50	50	0
PAO1 B Cip, day 24	50	50	0
PAO1 C Cip, day 24	49	50	2
mutS A Cip, day 24	49	50	2
mutS B Cip, day 24	50	50	0
mutMY A Cip, day 24	50	50	0
mutMY B Cip, day 24	50	50	0
mutMY C Cip, day 24	48	50	4
PAO1 A, day 24	50	50	0
PAO1 B, day 24	50	50	0
PAO1 C, day 24	50	50	0
mutS A, day 24	49	50	2
mutS B, day 24	41	50	18
mutS C, day 24	50	50	0
mutMY A, day 24	50	50	0
mutMY B, day 24	50	50	0
mutMY C, day 24	50	50	0
PAO1 A Cip, day 94	50	50	0
PAO1 B Cip, day 94	47	50	6
PAO1 C Cip, day 94	50	50	0
mutS A Cip, day 94	48	50	4
mutS B Cip, day 94	48	50	4
mutS C Cip, day 94	50	50	0
mutMY A Cip, day 94	50	50	0
mutMY B Cip, day 94	46	50	8
mutMY C Cip, day 94	50	50	0
mutS A, day 94	50	50	0
mutS B, day 94	50	50	0
mutS C, day 94	29	50	42
mutMY A, day 94	9	50	72
mutMY B, day 94	50	50	0
mutMY C, day 94	49	50	2