Supplementary materials – Rapid and consistent evolution of colistin resistance in XDR *Pseudomonas aeruginosa* during morbidostat culture

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Table S1 ResFinder results for Strain PA77

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
$bla_{\text{OXA}-50}$	100	789/789	c2	1012098..1012886	Beta-lactam resistance	AY306132
aph(3’)-IIb	100	807/807	c2	2010995..2011801	Aminoglycoside resistance	X90856
bla_{PAO}	99.25	1194/1194	c2	2024047..2025240	Beta-lactam resistance	AY083592
fosA	99.75	408/408	c1	2556300..2556707	Fosfomycin resistance	NZ_ACWU01000146
$bla_{\text{OXA}-10}$	100	801/801	plsm	17857..18657	Beta-lactam resistance	EU886981
aacA4	99.82	555/555	plsm	18689..19243	KM278199	
aac(6’)Ib-cr	99.42	519/519	plsm	18725..19243	Fluoroquinolone and aminoglycoside resistance	EF636461
$bla_{\text{IMP}-8}$	99.87	741/741	plsm	19320..20060	Beta-lactam resistance	EU042136
aacA4	99.82	555/555	plsm	20670..21224	KM278199	
aac(6’)Ib-cr	99.42	519/519	plsm	20706..21224	Fluoroquinolone and aminoglycoside resistance	EF636461
aph(3’)-XV	99.87	795/795	plsm	21295..22089	Aminoglycoside resistance	Y18050
aadA10	99.88	834/806	plsm	22206..23011	Aminoglycoside resistance	U37105
$bla_{\text{OXA}-2}$	100	828/828	plsm	23041..23868	Beta-lactam resistance	DQ310703
sul1	100	927/927	plsm	24258..25184	Sulphonamide resistance	CP002151

Table S2 ResFinder results for Strain PA83

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
sul1	100	840/840	chromosome	2792489..2793328	Sulphonamide resistance	AY224185
tet(G)	100	1176/1176	chromosome	2805670..2806845	Tetracycline resistance	AF133140
$bla_{\text{VIM}-2}$	100	801/801	chromosome	3161692..3162492	Beta-lactam resistance	AF302086
dfrB5	100	237/237	chromosome	3162641..3162877	Trimethoprim resistance	AY943084
aac(3)-Id	99.79	477/477	chromosome	3163003..3163479	Aminoglycoside resistance	AB114632
aac(3)-Id	99.79	477/477	chromosome	381826..382302	Aminoglycoside resistance	AB114632
dfrB5	100	237/237	chromosome	382428..382664	Trimethoprim resistance	AY943084
fosA	99.26	408/408	chromosome	4627680..4628087	Fosfomycin resistance	NZ_ACWU01000146
catB7	98.75	639/639	chromosome	5178919..5179557	Phenicol resistance	AF036933
dfrB5	100	237/237	chromosome	6206269..6206505	Trimethoprim resistance	AY943084
aac(3)-Id	99.79	477/477	chromosome	6206631..6207107	Aminoglycoside resistance	AB114632
$bla_{\text{OXA}-4}$	100	831/831	chromosome	6218833..6219663	Beta-lactam resistance	AY162283
aadA2	99.87	792/792	chromosome	6219776..6220567	Aminoglycoside resistance	JQ364967
cmlA1	99.05	1260/1260	chromosome	6220829..6222088	Phenicol resistance	AB212941
sul1	100	927/616	chromosome	6222534..6223149	Sulphonamide resistance	CP002151
tet(G)	100	1176/1176	chromosome	6225960..6227135	Tetracycline resistance	AF133140
sul1	100	852/852	chromosome	6230553..6231404	Sulphonamide resistance	AY963803
$bla_{\text{OXA}-50}$	99.62	789/789	chromosome	6757336..6758124	Beta-lactam resistance	AY306135
aph(3’)-IIb	98.02	807/807	chromosome	951985..952791	Aminoglycoside resistance	X90856
bla_{PAO}	99.5	1194/1194	chromosome	965134..966327	Beta-lactam resistance	FJ666065

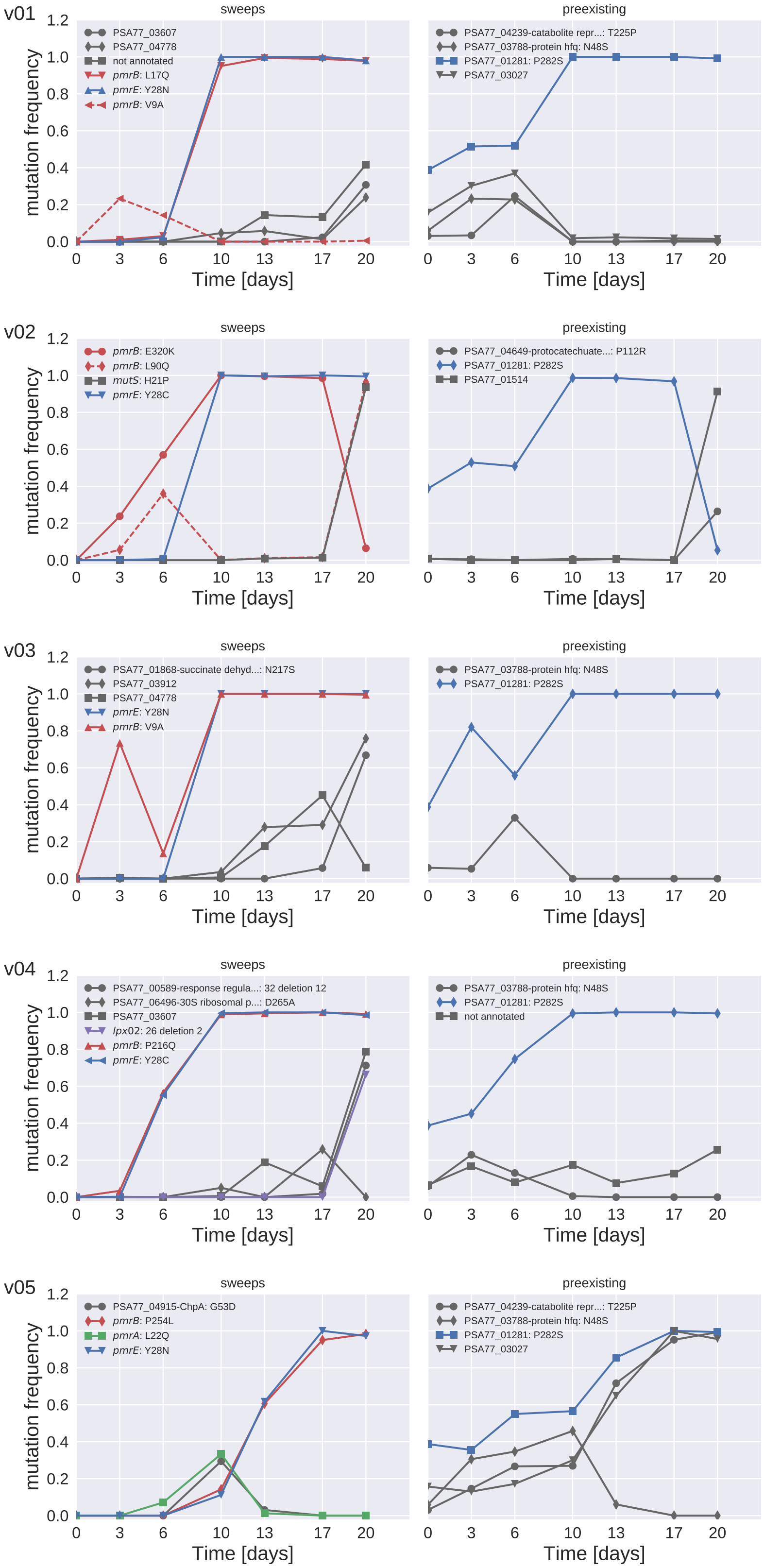

Figure S1 **SNP trajectories in cultures of PA77 (three week experiment).** The left panels contain trajectories of SNPs not observed in the initial sample (sweeps), the right panels contains those already present in the initial sample (preexisting). Mutations that follow the trajectory of the mutator allele are omitted due to their large number. Trajectories of these mutations can be found in Table S7.

TWO WEEK EXPERIMENT WITH STRAIN PA77

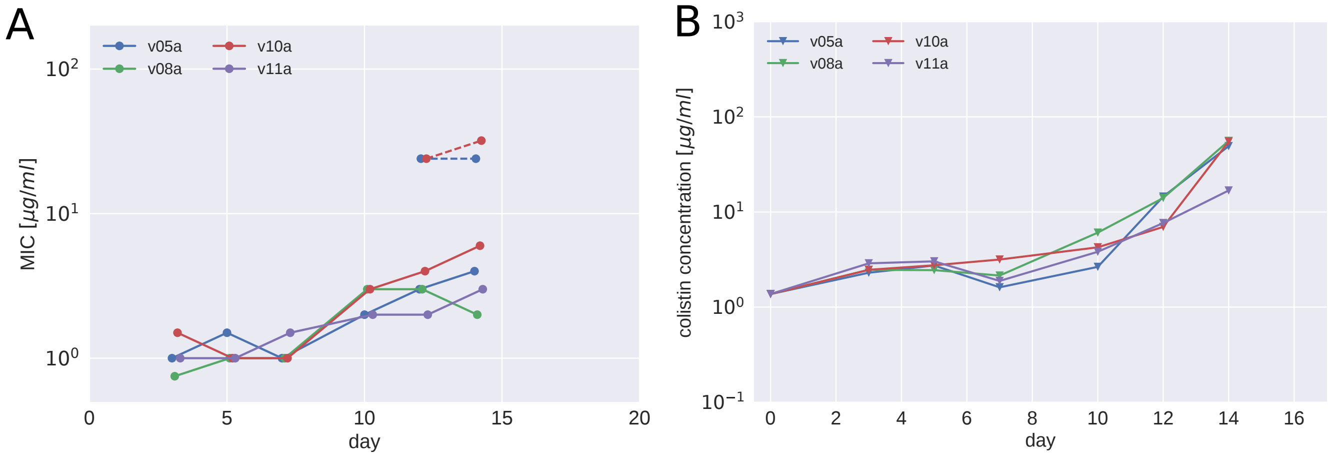

Figure S2 **Colistin resistance evolution in PA77 (two week experiment)**. (A)The time course of MIC in E-tests for each vial in preliminary experiments for strain PA77. Sub-populations showing a higher MIC than the main population were observed after 14 days, respectively. (B) Colistin concentration in each vial in units of the MIC of the initial cultures.

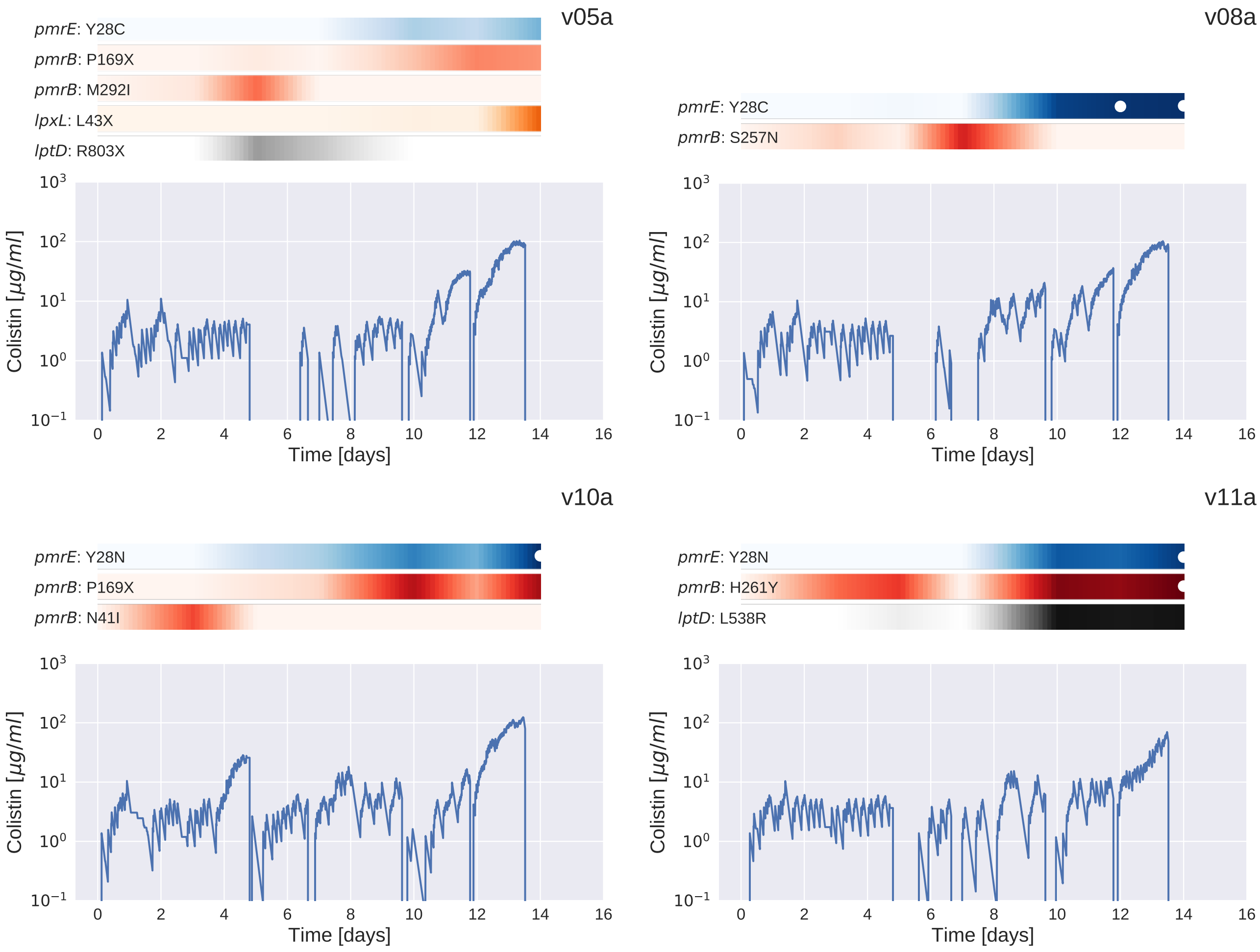

Figure S3 **The dynamics of mutations in PA77 (two week experiment).** For each culture vial, the plot shows the dynamics of colistin concentration in liquid culture. This concentration is inferred from the cycles of colistin addition and waste removal in 10 minute intervals. The shaded bars above the plots show the abundance of different mutations during the experiment. Time points at which a mutation reached a frequency above 95% are highlighted with a white circle. The frequencies of *pmrE* and *pmrB* mutations correlate well with the initial rise in colistin tolerance. The deep dips in colistin concentration every 2-3 days correspond to transfers to fresh culture vials and mark the time points at which samples were taken.

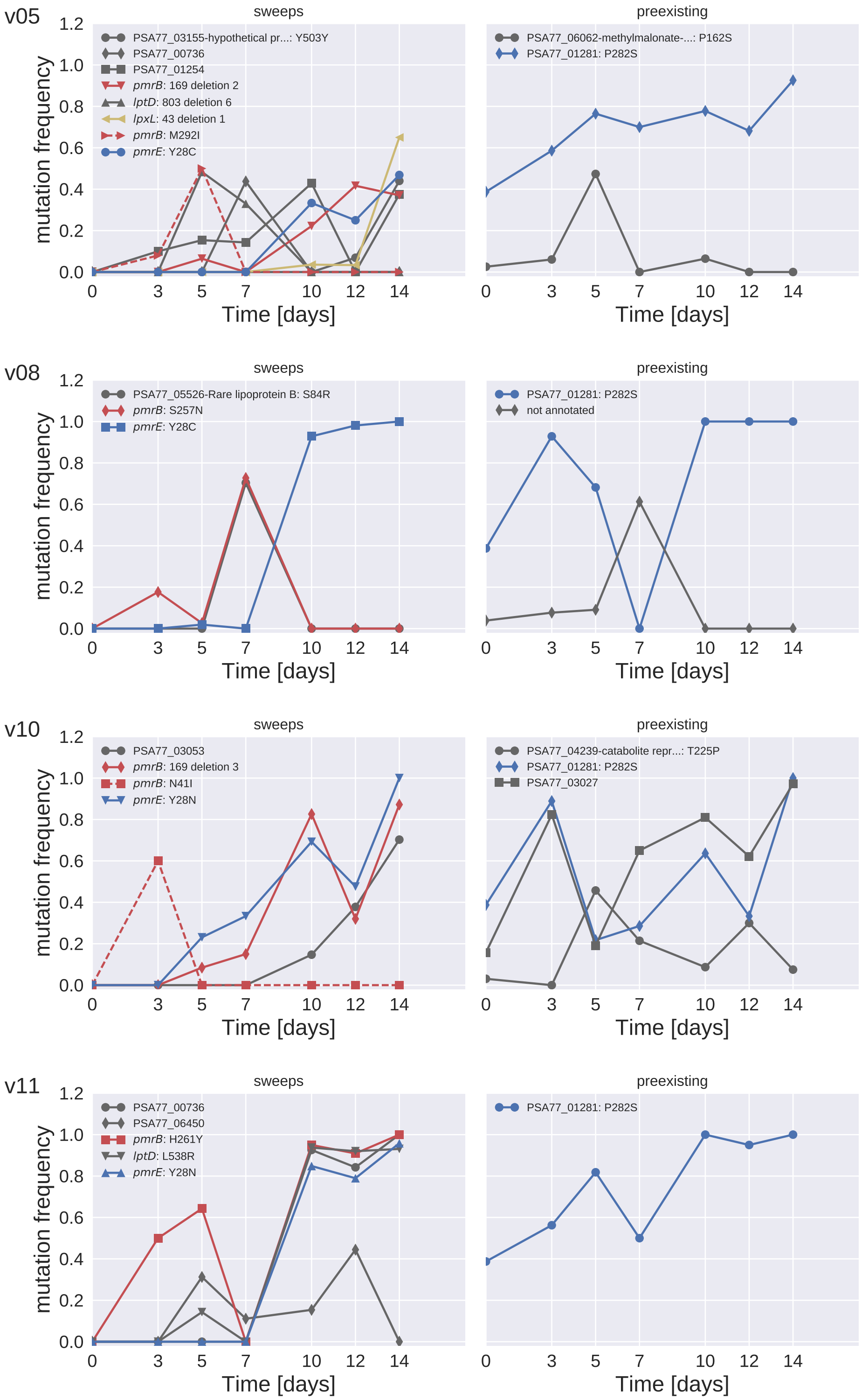

Figure S4 **Mutation trajectories in cultures of PA77 (two week experiment).** The left panels contain trajectories of SNPs not observed in the initial sample (sweeps), the right panels contains those already present in the initial sample (preexisting). This experiment was sequenced at substantially lower coverage than the other two, hence frequency estimates of SNPs are less precise.

EXPERIMENT WITH STRAIN PA83

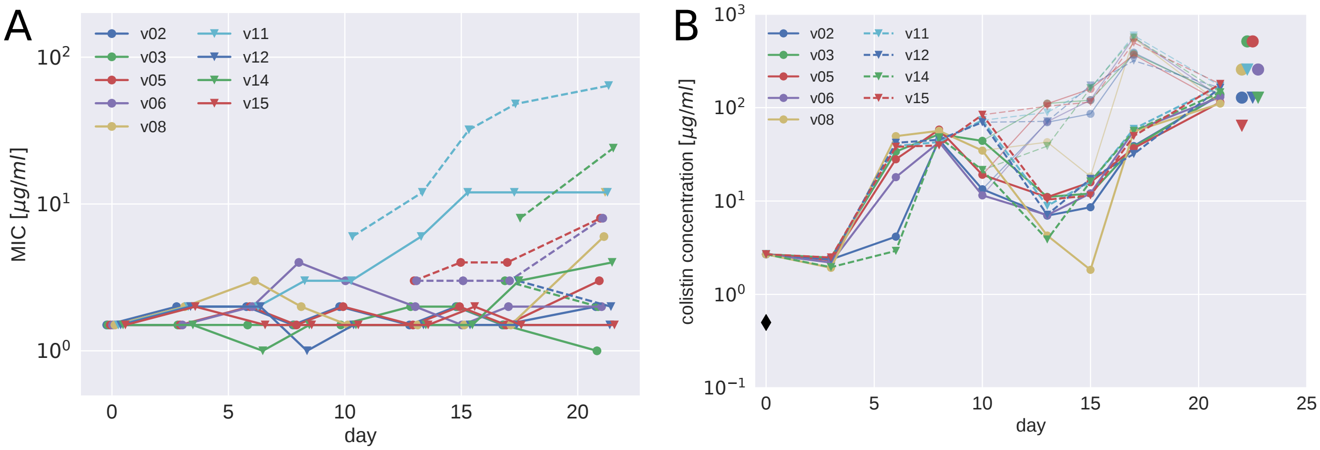

Figure S5 **Colistin resistance evolution in PA83**. (A) The time course of MIC in E-tests for each vial in preliminary experiments for strain PA83. Sub-populations showing a higher MIC than the main population were observed after 9 and 14 days, respectively. (B) Colistin concentration in each vial in units of the MIC of the initial cultures. Concentrations for PA83 have at day 14 and 18 have been corrected for an error during the preparation of the colistin stock solution, which had a 10-fold lower concentration than intended.

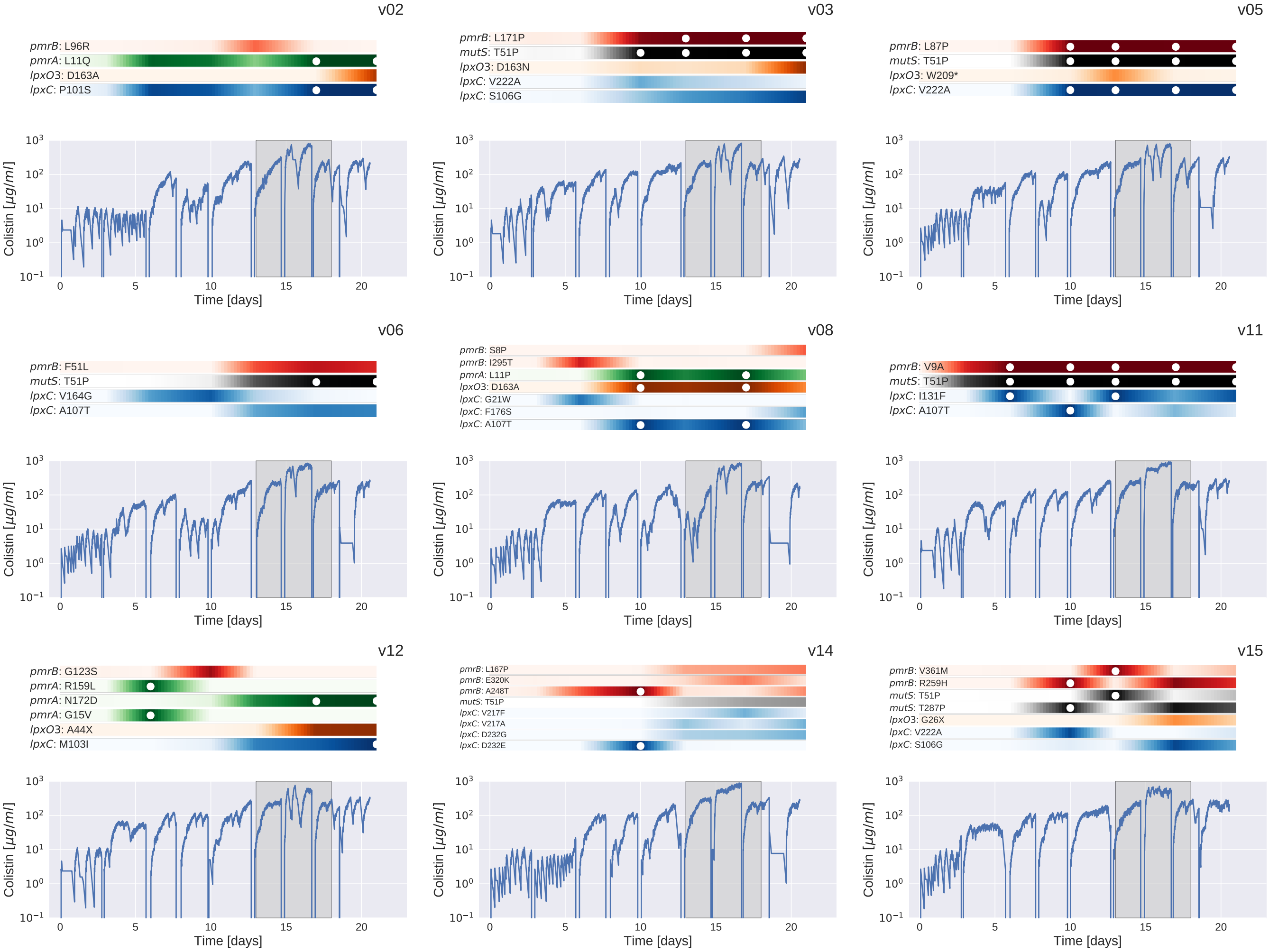

Figure S6 **The dynamics of mutations in cultures of PA83.** For each culture vial, the plot shows the dynamics of colistin concentration in liquid culture. This concentration is inferred from the cycles of colistin addition and waste removal in 10 minute intervals. The shaded bars above the plots show the abundance of different mutations during the experiment. Time points at which a mutation reached a frequency above 95% are highlighted with a white circle. The deep dips in colistin concentration every 2-3 days correspond to transfers to fresh culture vials and mark the time points at which samples were taken. The time interval during which a colistin stock solution with 10-fold lower concentration was used is indicated by a grey box. During these days, the colistin solution in the culture vials was about 10-fold lower than indicated.

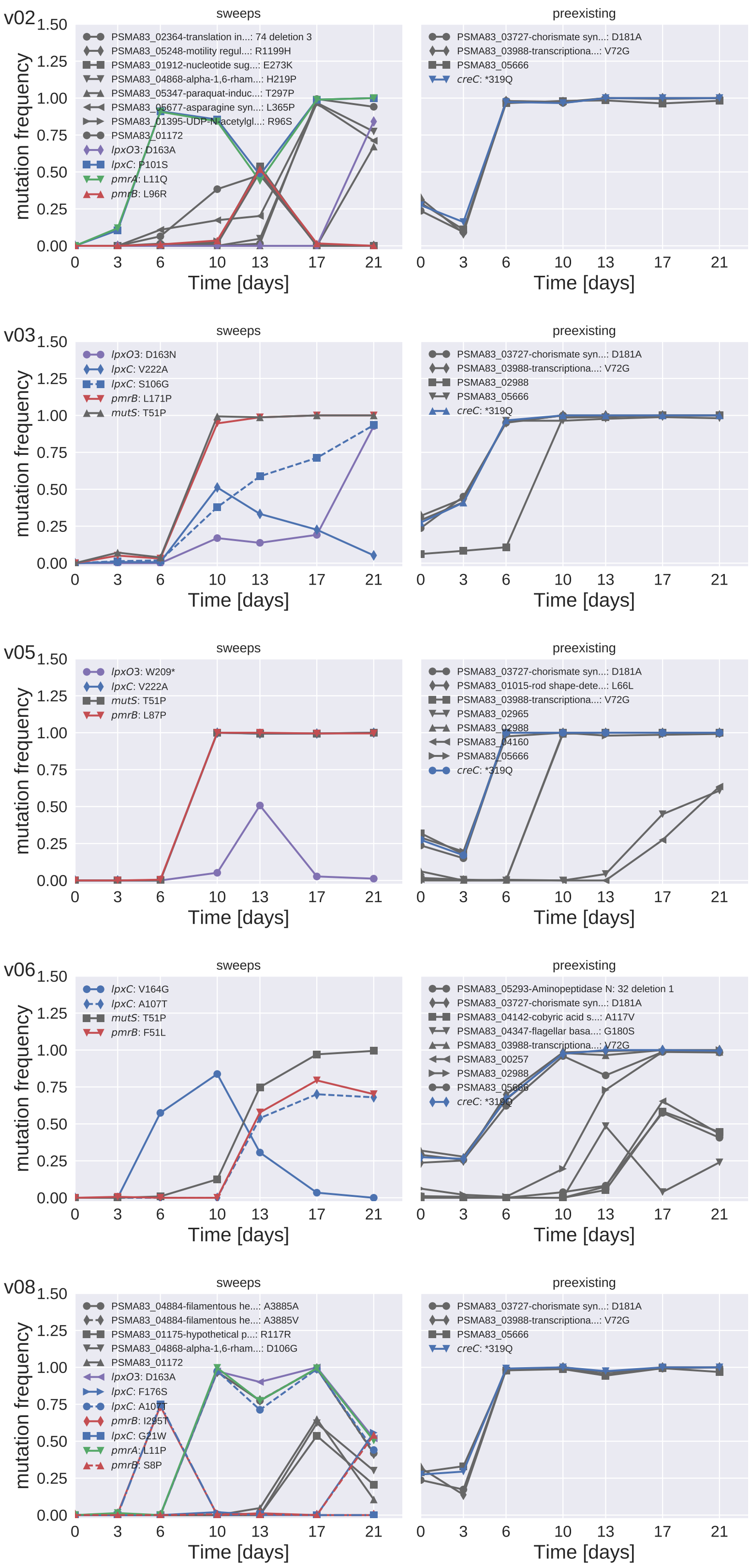

Figure S7 **SNP trajectories in cultures of PA83.** The left panels contain trajectories of SNPs not observed in the initial sample (sweeps), the right panels contains those already present in the initial sample (preexisting). Mutations that follow the trajectory of the mutator allele are omitted due to their large number. Trajectories of these mutations can be found in Table S7.

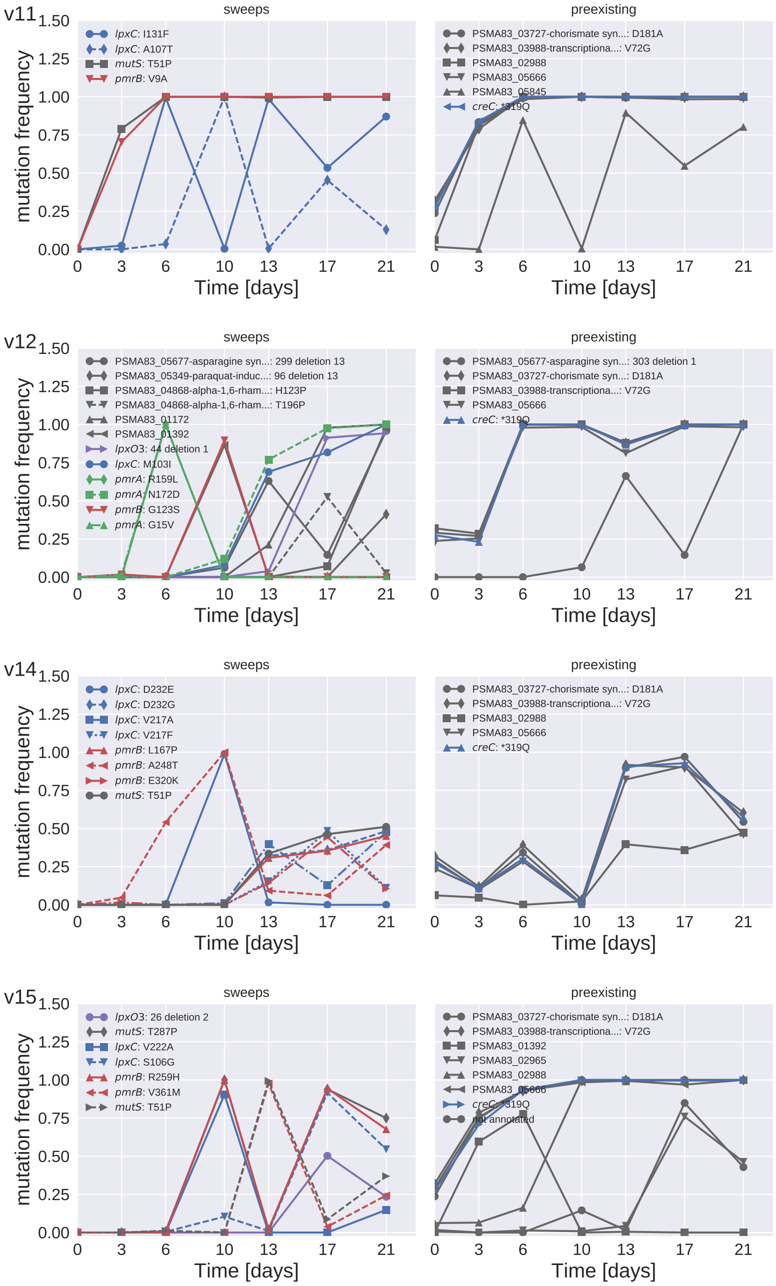

Figure S8 **SNP trajectories in cultures of PA83 (contd.).** The left panels contain trajectories of SNPs not observed in the initial sample (sweeps), the right panels contains those already present in the initial sample (preexisting). Mutations that follow the trajectory of the mutator allele are omitted due to their large number. Trajectories of these mutations can be found in Table S7.

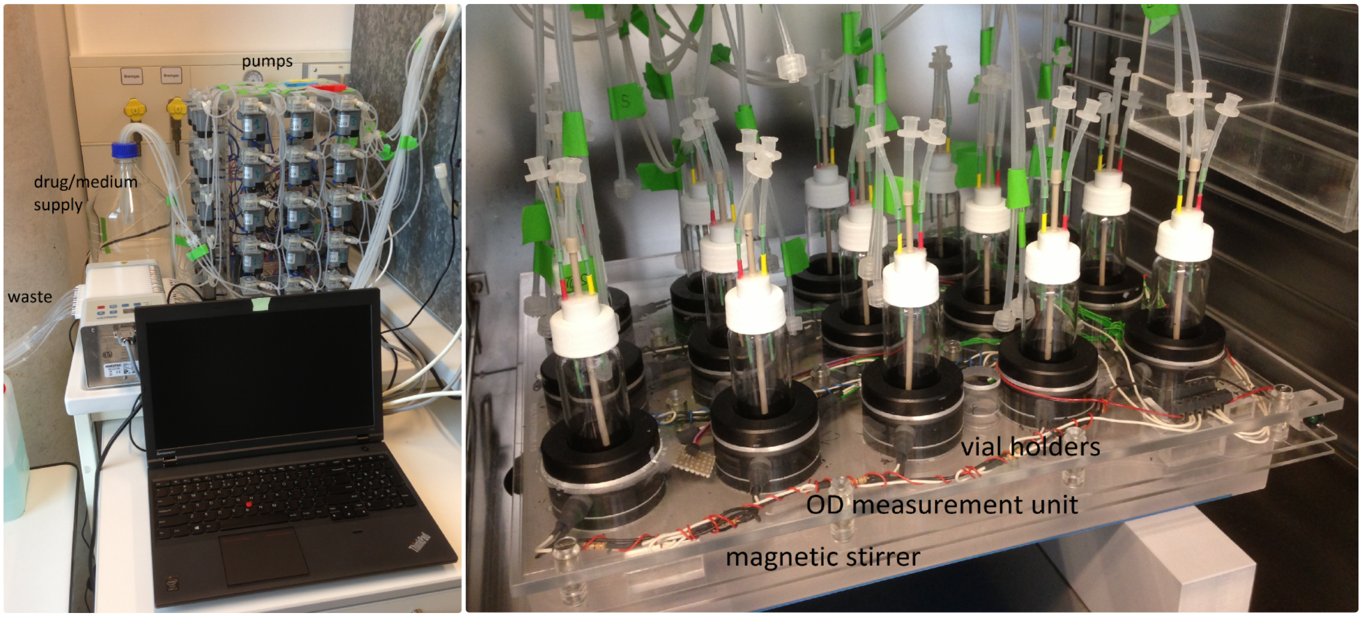

Figure S9 **The morbidostat.** The left picture shows the pump array, the waste pump, and the inlet in the back of the incubator. The right picture shows the sample holder on a magnetic stirrer and the OD measurement electronics.