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Supplementary Materials for

Prediction of resistance development against drug combinations by collateral responses to component drugs

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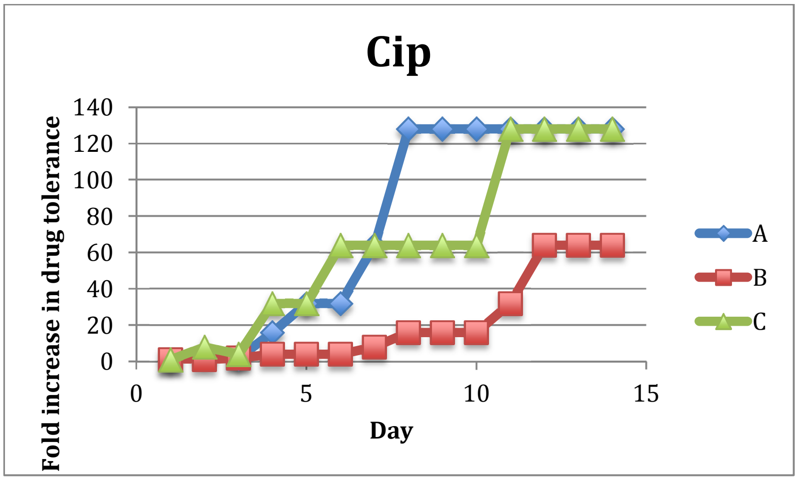
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Fold increase in drug tolerance
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80
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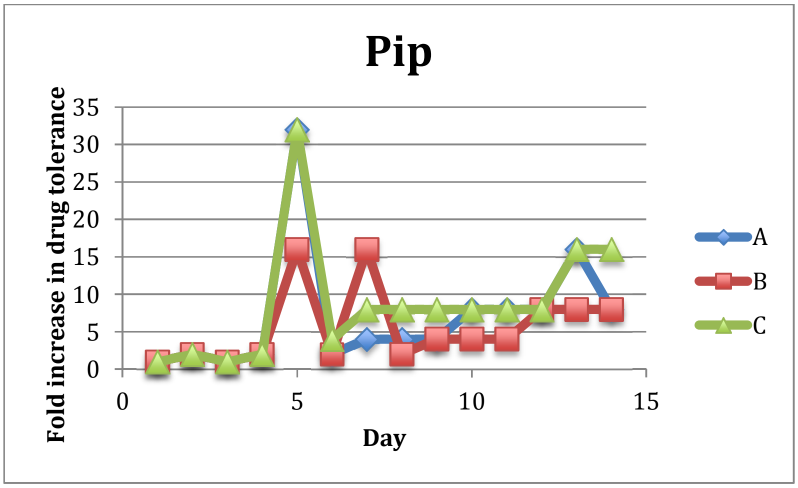
5
10
15
Day
A
B
C

B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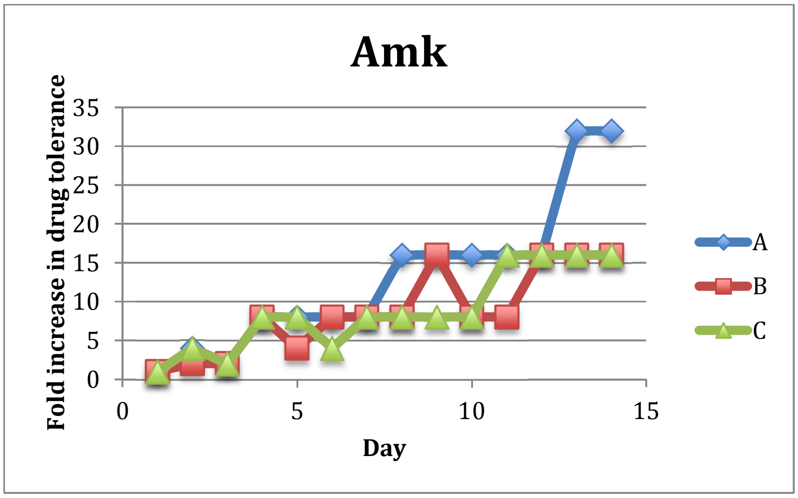
0
5
10
15
Day
A
B
C

C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Fold increase in drug tolerance
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10
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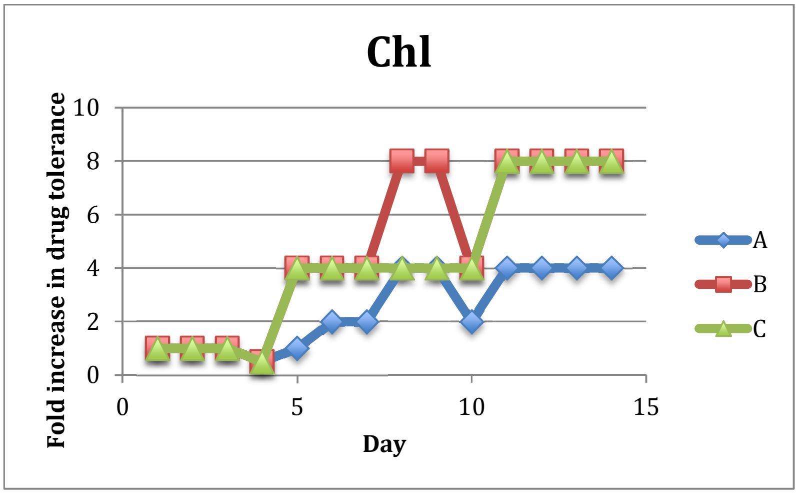
0
5
10
15
Day
A
B
C

D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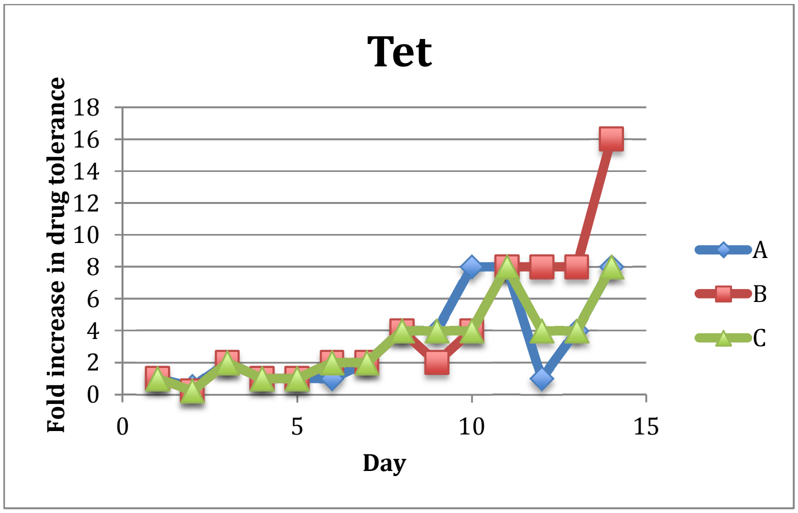
2
0
0
5
10
15
Day
A
B
C

E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Fold increase in drug tolerance
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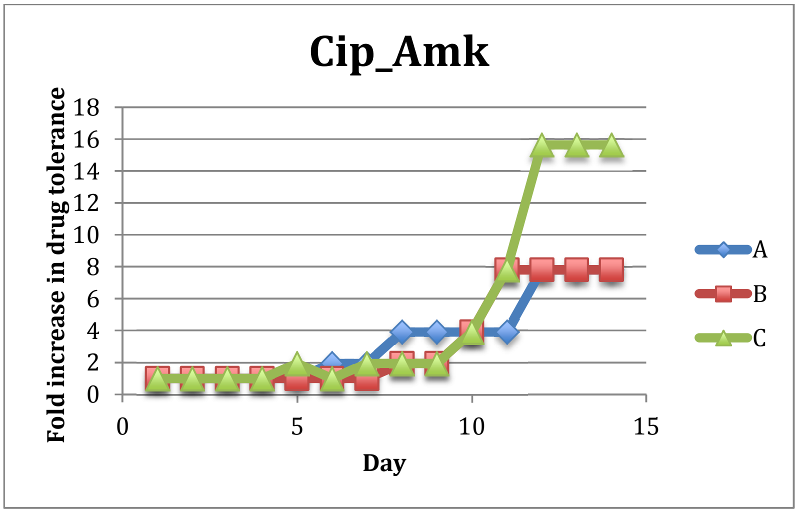
16
14
12
10
8
6
4
2
0
0
5
10
15
Day
A
B
C

F


Cip_Amk
Fold increase in drug tolerance
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16
14
12
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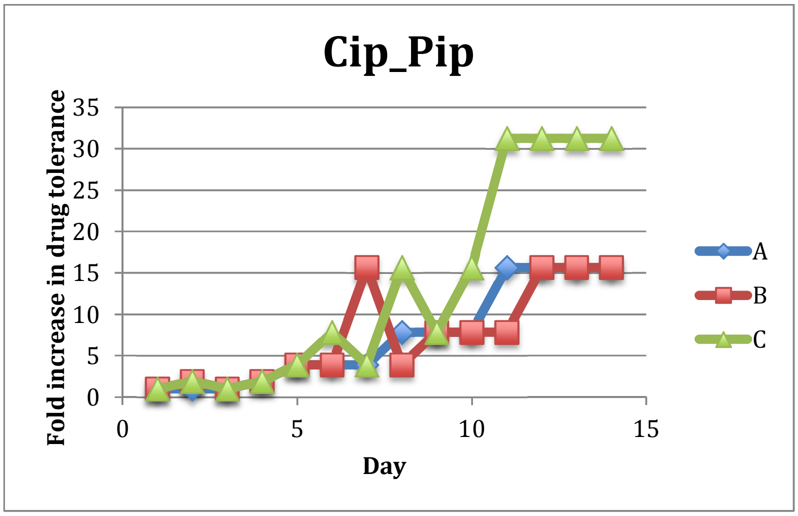
8
6
4
2
0
0
5
10
15
Day
A
B
C

G


Cip_Pip
Fold increase in drug tolerance
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30
25
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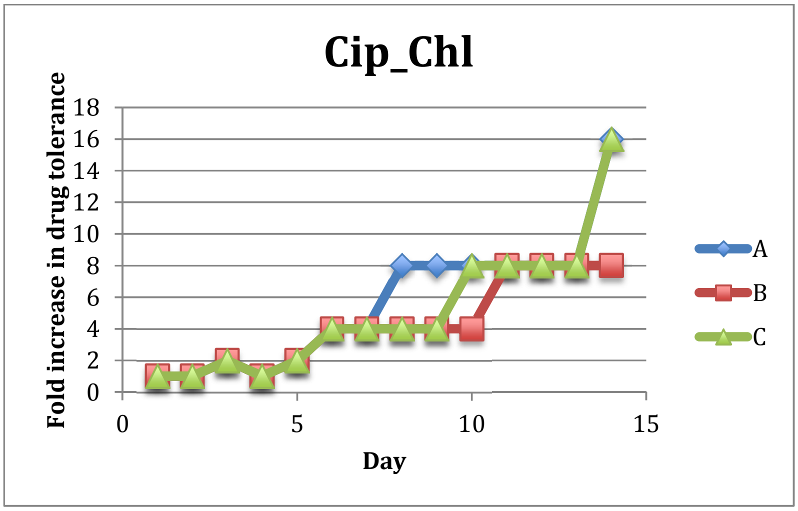
10
5
0
0
5
10
15
Day
A
B
C

H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Fold increase in drug tolerance
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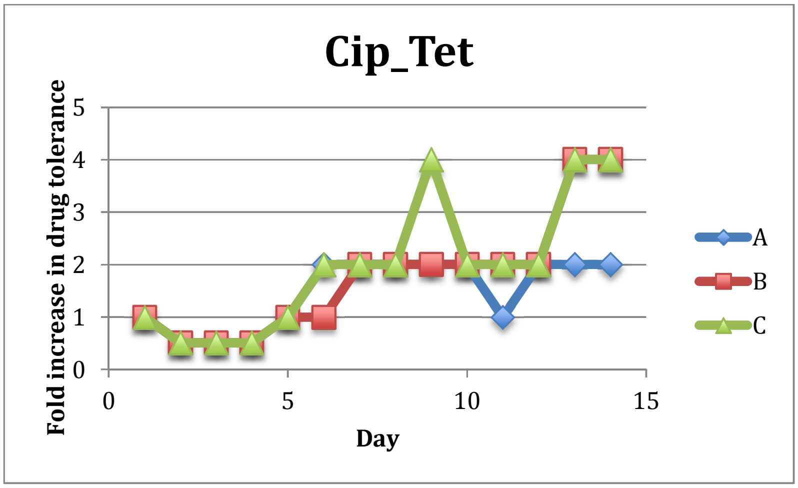
16
14
12
10
8
6
4
2
0
0
5
10
15
Day
A
B
C

I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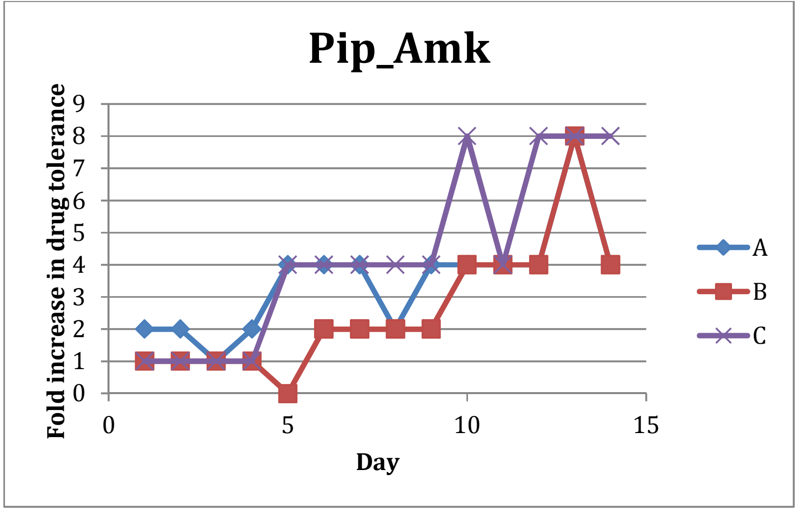
4
3
2
1
0
0
5
10
15
Day
A
B
C

J


Pip_Amk
Fold increase in drug tolerance
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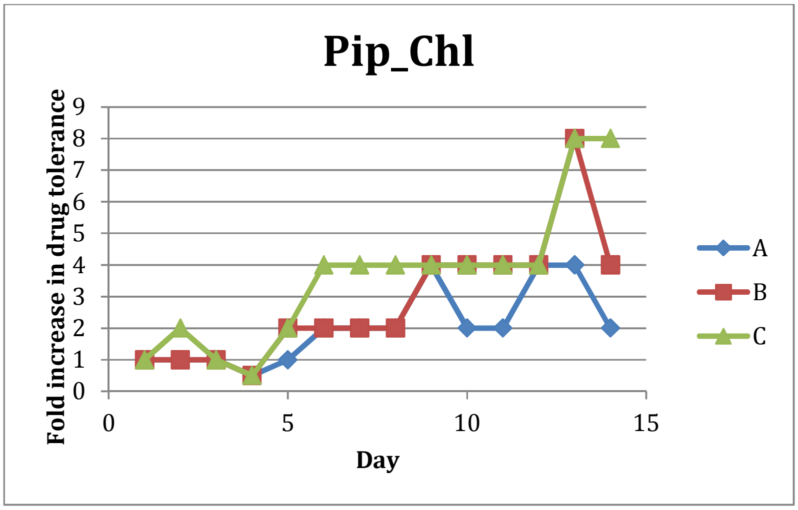
3
4
5
6
7
8
9
0
5
10
15
Day
A
B
C

K


Pip_Chl
Fold increase in drug tolerance
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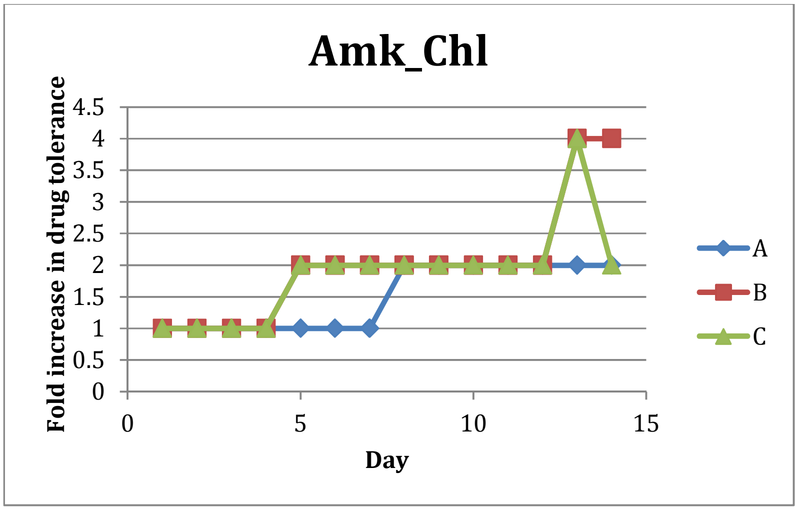
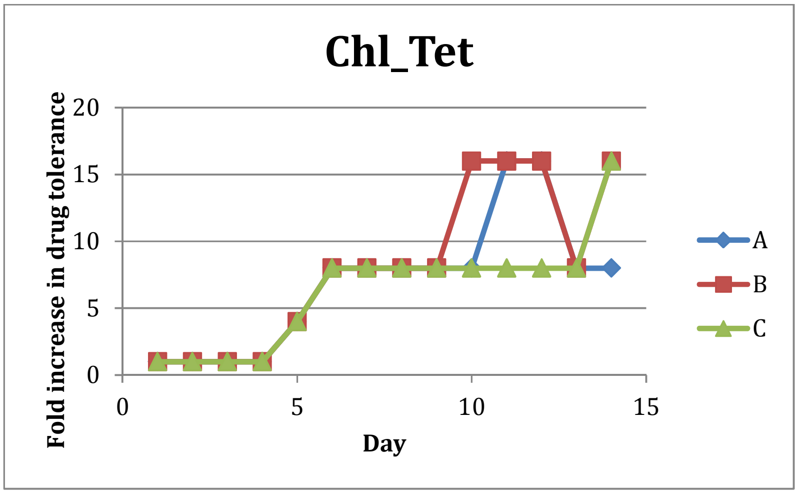
1
2
3
4
5
6
7
8
9
0
5
10
15
Day
A
B
C

L


Amk_Chl
Fold increase in drug tolerance
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
5
10
15
Day
A
B
C

M



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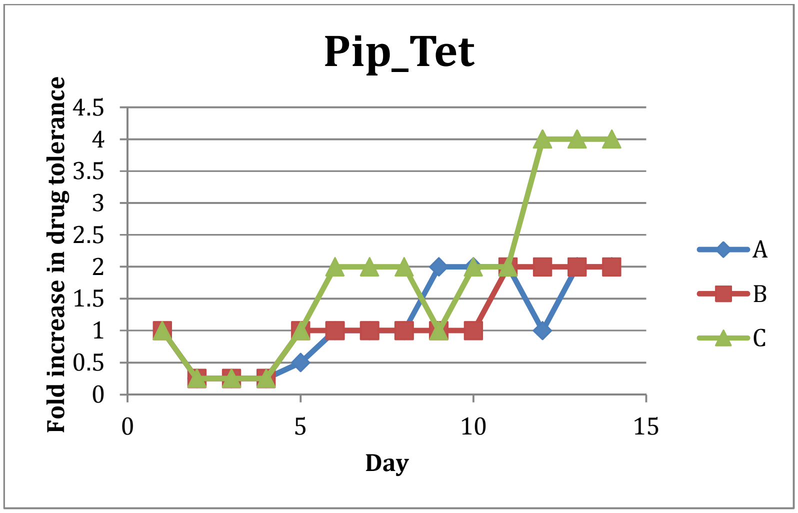

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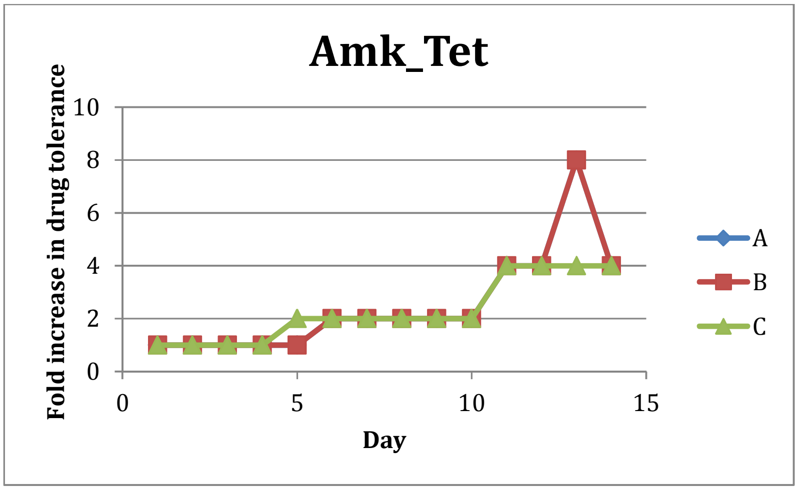

Fig. S1. Fold increase in drug tolerance during evolution experiment.

The figures shows the fold increase in the drug tolerance as the strains are passaged into higher drug concentrations (A- O). Due to the large inoculum, some variability is observed.

Fig. S2. IC$_{90}$ dose-response curves.
Dose response curves of all lineages (Available from dropbox
https://dl.dropboxusercontent.com/u/24306487/Munck-Supplment.zip).
The title indicates the drug(s) that the strain is evolved to, and the x-axis legend denotes the antibiotic(s) tested against. Fitting is performed with the R package drc() using the default four-parameter logistic model (Materials and Methods). Error bars depict SD.

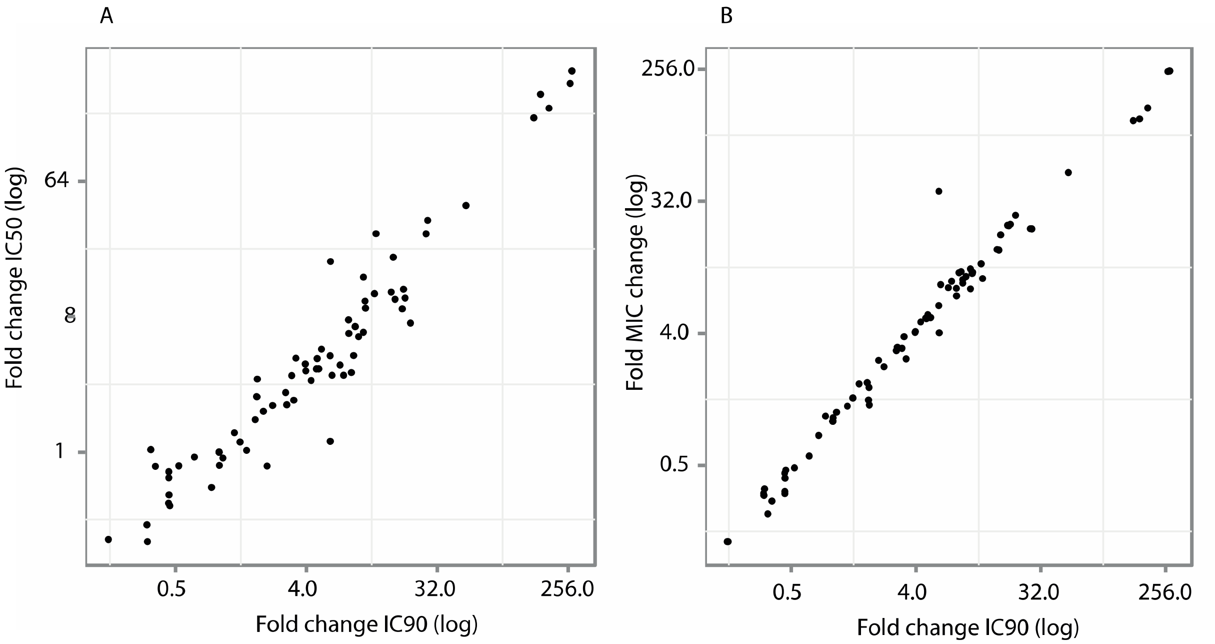

Fig. S3. Correlation between IC$_{50}$ or MIC, and IC$_{90}$-based measurements.
Correlation between the fold change in inhibitory concentration (IC) of the single drug evolved lineages, measured as IC50 vs. IC90 (A) and between the fold change of the MIC (defined as IC95) vs. IC90 (B).

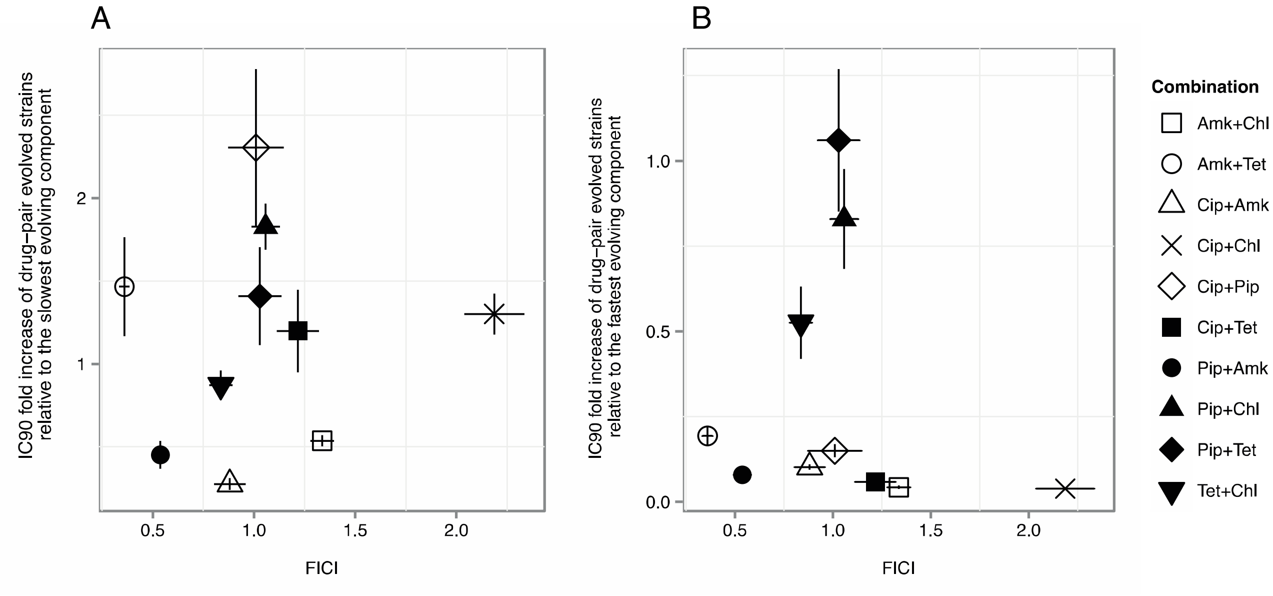

Fig. S4. Alternative correlation between FICI and evolution of resistance.
A) Correlation between FICI and the fold IC_{90} increase for of the drug-pair evolved lineages relative to the slowest evolving single-drug component: $\Delta IC_{90}[AB]_{AB}/ \min(\Delta IC_{90}[A]_{A},\ \Delta IC_{90}[B]_{B})$.
B) Correlation between FICI and the fold IC_{90} increase for of the drug-pair evolved lineages relative to the fastest evolving single-drug component: $\Delta IC_{90}[AB]_{AB}/ \max(\Delta IC_{90}[A]_{A},\ \Delta IC_{90}[B]_{B})$.
Error-bars depict SD of FICI and SEM of evolution measure across the three parallel-evolved lineages. No significant correlation was found ($P > 0.05$, Bonferroni corrected Spearman correlation).

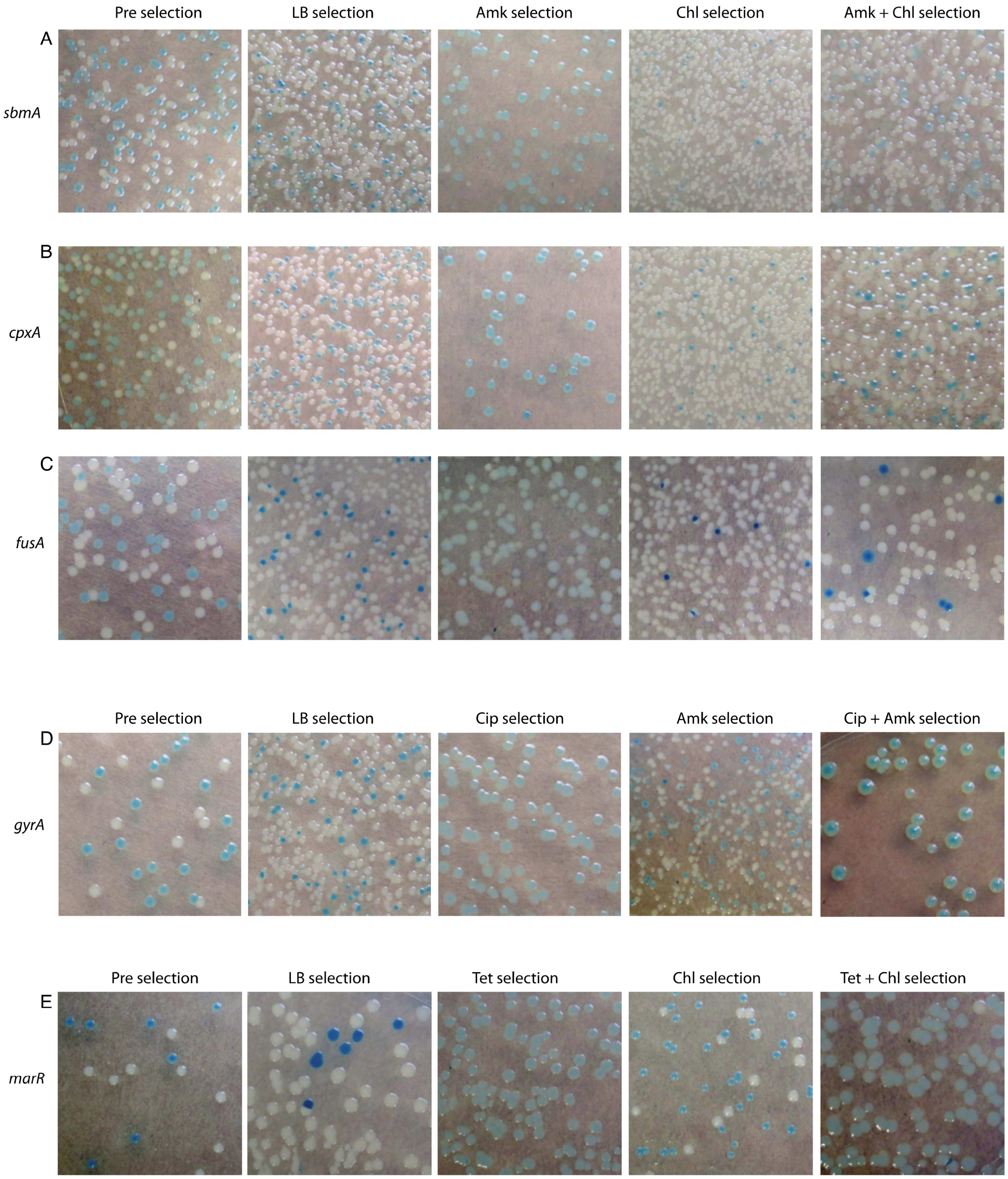

Fig. S5. Competition between wild-type ΔlacZ and mutant.
WT and mutant alleles were mixed 1:1 and grown overnight in sub-inhibitory concentrations of AB and plated on LB+IPTG+Xgal. The presence of chloramphenicol selects against the three mutations that confers amikacin resistance (*sbmA*, *cpxA* and *fusA*). In contrast the *gyrA* mutation is always selected when ciprofloxacin is present and it is not counter selected by amikacin. *marR* confers resistance to both tetracycline and chloramphenicol and is hence selected by both drugs.

Table S1. Raw OD_{600} readings.
Xcel file containing the OD600 measurements used for the dose response curves (Available from dropbox https://dl.dropboxusercontent.com/u/24306487/Munck-Supplment.zip).
The drug and strain column denotes the drug tested against and the strain tested, respectively. The alternating colors for the first two columns define experiments performed with the same AB solutions on the same day. AB01-AB11 is the drug concentrations from high to low in µM. C1-C11 is the raw OD600 reads. Control contains the positive (green) and negative (red) controls per plate.

Sample name	# reads	# reads aligned	% aligned	% genome >= 3X coverage
Amk_A	7685868	5368728	69.85194125	99.880
Amk_B	7362134	5270482	71.58905285	99.879
Amk_C	6373603	4729217	74.20005608	99.826
Amk_Chl_A	7756344	5274450	68.00175443	99.894
Amk_Chl_B	8423816	5783829	68.66043845	99.890
Amk_Chl_C	6139952	4400827	71.67526717	99.843
Amk_Tet_A	6161495	4182408	67.8797597	99.788
Amk_Tet_B	6085215	3919626	64.41228453	99.588
Amk_Tet_C	4689621	3060365	65.25825861	99.553
Chl_A	9225854	6611896	71.66703483	99.908
Chl_B	8007126	5907936	73.78347737	99.882
Chl_C	7453420	5546359	74.41361147	99.894
Chl_Tet_A	10788928	7375354	68.36039688	99.904
Chl_Tet_B	5938086	4207339	70.85345345	99.832
Chl_Tet_C	11031762	7576840	68.68204735	99.920
Cip_Amk_A	8240061	5745627	69.72796682	99.886
Cip_Amk_B	9038514	6491753	71.82323333	99.901
Cip_Amk_C	7846062	5563908	70.91338304	99.860
Cip_A	7376758	5479379	74.27895832	99.886
Cip_B	12740551	8709252	68.35851919	99.671
Cip_C	9313738	6650894	71.40950282	99.921
Cip_Chl_A	11938429	8371154	70.11939343	99.563
Cip_Chl_B	9645647	6937157	71.9200796	99.886
Cip_Chl_C	3052136	2143958	70.24451073	98.044
Cip_Pip_A	5809372	4045512	69.63768201	99.806
Cip_Pip_B	7511248	5532805	73.66026258	99.869
Cip_Pip_C	7251959	5092565	70.22330104	99.874
Cip_Tet_A	5853771	4109034	70.19464889	99.613
Cip_Tet_B	7874764	5277459	67.01736077	99.873
Cip_Tet_C	6488866	4447375	68.53855512	99.457
Pip_Amk_A	5214046	3718643	71.31971985	99.811
Pip_Amk_B	5591230	4106716	73.44924104	99.642
Pip_Amk_C	7464970	5652272	75.71727683	99.808
Pip_A	8772610	6209919	70.78758773	99.895
Pip_B	8003544	5843302	73.00893204	99.890
Pip_C	9623410	7193111	74.74596842	99.933
Pip_Chl_A	8544433	5796615	67.84083859	99.919
Pip_Chl_B	6621964	4842826	73.13277451	99.710
Pip_Chl_C	9850064	6651940	67.531947	99.861
Pip_Tet_A	9984949	7114928	71.2565282	99.931
Pip_Tet_B	7268505	4962485	68.27380596	99.750
Pip_Tet_C	10015645	6906844	68.96055122	99.921
Tet_A	8432649	5792993	68.69719112	99.917
Tet_B	8864063	6440958	72.66372091	99.897
Tet_C	13861024	9727194	70.17659013	99.953
WT_MG1655	13973666	9645699	69.02769109	99.941

Table S2. Sequencing coverage.
The number of SOLiD reads generated, the number and percentage of reads that aligned to the *E. coli* MG1655 genome, and the percentage of the genome that had at least 3 times or greater coverage per sample is reported.

A. SNPs Identified in the starting *E. coli* MG1655 Strain

Position	Mutation	Genbank ID	Gene	Product	Conservative
547694	A->G		ylbE		Yes
802885	C->A	NP_415292.2	ybhJ	predicted hydratase	No
1903785	G->A	NP_416335.4	yebN	conserved inner membrane protein	No
3957957	C->T				

B. INDELs Identified in the starting *E. coli* MG1655 Strain

From Position	Type	INDEL	Genbank ID	Gene	Product
547832	insertion	G->GG			

C. SNPs Identified in the evolved MG1655 Strain

Strain/AB_selection	Position	Mutation	Genbank ID	Gene	Product	Conservative
Amk_A	396442	G->A	NP_414911.1	sbmA	microcin B17 transporter	No
Amk_A	1363139	G->C	NP_415817.1	puuB	gamma-Glu-putrescine oxidase; FAD/NAD(P)-binding	No
Amk_A	1976527	G->T				
Amk_A	1976528	G->A				
Amk_A	3469498	T->C	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
Amk_A	3469708	G->T	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
Amk_A	3875481	A->C	NP_418153.4	yidB	conserved protein; DUF937 family	No
Amk_A	4102908	C->A	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
Amk_B	396368	C->A	NP_414911.1	sbmA	microcin B17 transporter	No
Amk_B	2021141	G->A	NP_416460.1	fliR	flagellar export pore protein	Yes
Amk_B	3469714	G->A	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
Amk_B	4102763	G->A	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
Amk_C	396611	G->A	NP_414911.1	sbmA	microcin B17 transporter	No
Amk_C	1976527	G->T				
Amk_C	1976528	G->A				
Amk_C	1990891	A->T				
Amk_C	2690454	C->T	YP_026170.1	purL	phosphoribosylformyl-glycineamide synthetase	No
Amk_C	3469709	G->T	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
Amk_C	4025094	T->A	NP_418286.1	fre	NAD(P)H-flavin reductase	No

Amk_C	4102449	A->T	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
AmkChl_A	4184421	C->G	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
AmkChl_B	1617423	C->T	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance	No
AmkChl_B	1976527	G->T				
AmkTet_A	810513	G->T	NP_415297.1	bioF	8-amino-7-oxononanoate synthase	No
AmkTet_A	4101770	C->T	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
AmkTet_B	3469759	A->C	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
AmkTet_C	1285929	G->A				
AmkTet_C	3451124	C->G	NP_417780.1	rpsJ	30S ribosomal subunit protein S10	No
AmkTet_C	3470824	A->T	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
AmkTet_C	4092723	G->A				
AmkTet_C	4092726	C->T				
AmkTet_C	4092728	C->T				
AmkTet_C	4102680	T->A	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
AmlChl_C	1150959	T->C	NP_415612.1	acpP	acyl carrier protein (ACP)	No
AmlChl_C	1977294	C->A				
AmlChl_C	1998241	G->T	NP_416430.1	fliY	cystine transporter subunit	No
AmlChl_C	4102440	C->T	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
Chl_A	1976527	G->T				
Chl_A	1976528	G->A				
Chl_A	2089530	C->T	NP_416524.1	hisD	bifunctional histidinal dehydrogenase/ histidinol dehydrogenase	No
Chl_A	4632601	C->T	NP_418813.1	rob	right oriC-binding transcriptional activator; AraC family	No
Chl_B	1500383	G->T				
Chl_B	1617399	A->T	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance	No
Chl_C	848200	G->A				
Chl_C	882884	C->A				
Chl_C	1977294	C->A				
Chl_C	3370896	G->A	NP_417692.1	nanA	N-acetylneuraminate lyase	Yes
Chl_C	4632867	C->T	NP_418813.1	rob	right oriC-binding transcriptional activator; AraC family	No
ChlTet_A	1977294	C->A				
ChlTet_B	1617492	C->T	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance	No
ChlTet_B	1977291	C->T				
ChlTet_B	1977294	C->A				
ChlTet_B	3212876	G->A	NP_417539.1	rpoD	RNA polymerase; sigma 70 (sigma D) factor	No
ChlTet_B	4184369	G->T	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
ChlTet_C	1617492	C->T	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance	No

ChlTet_C	3350464	A->C	YP_026207.1	arcB	aerobic respiration control sensor histidine protein kinase; cognate to two-component response regulators ArcA and RssB	No
Cip_A	1976527	G->T				
Cip_A	2337184	C->A	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
Cip_A	2337220	C->T	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
Cip_A	4187448	C->G	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
Cip_A	4187449	A->T	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	Yes
Cip_A	4187450	G->C	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
Cip_B	1195443	C->T	NP_415654.1	icd	e14 prophage	Yes
Cip_B	1195455	C->T	NP_415654.1	icd	e14 prophage	Yes
Cip_B	1195468	T->C	NP_415654.1	icd	e14 prophage	Yes
Cip_B	1195470	A->G	NP_415654.1	icd	e14 prophage	Yes
Cip_B	1210633	C->A				
Cip_B	1210635	A->G				
Cip_B	1976527	G->T				
Cip_B	1976528	G->A				
Cip_B	2337183	T->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
Cip_B	3876753	A->C	YP_026241.1	gyrB	DNA gyrase; subunit B	No
Cip_B	4632867	C->T	NP_418813.1	rob	right oriC-binding transcriptional activator; AraC family	No
Cip_C	485058	C->A	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
Cip_C	2337183	T->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
Cip_C	2337202	C->A	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
Cip_C	3256446	A->C	YP_026204.1	tdcG	L-serine dehydratase 3	No
CipAmk_A	442247	T->C				
CipAmk_A	1990685	C->T	NP_416422.1	pgsA	phosphatidylglycerophosphate synthetase	No
CipAmk_A	4092726	C->T				
CipAmk_A	4092728	C->T				
CipAmk_A	4102470	G->A	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
CipAmk_B	485144	G->T	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
CipAmk_B	1976527	G->T				
CipAmk_B	1976528	G->A				
CipAmk_B	3469714	G->T	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
CipAmk_C	1977294	C->A				
CipAmk_C	2069371	T->A		isrC		No
CipAmk_C	2337183	T->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
CipAmk_C	2398061	C->T	NP_416786.4	nuoG	NADH:ubiquinone oxidoreductase; chain G	No
CipAmk_C	3469714	G->T	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
CipAmk_C	4031406	T->A	YP_026273.1	trkH	potassium transporter	No

CipAmk_C	4275937	T->A	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing	No
CipChl_A	1195443	C->T	NP_415654.1	icd	e14 prophage	Yes
CipChl_A	1195455	C->T	NP_415654.1	icd	e14 prophage	Yes
CipChl_A	1210633	C->A				
CipChl_A	1210635	A->G				
CipChl_A	1617394	T->A	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance	No
CipChl_A	1718801	C->T	NP_416159.2	slyA	DNA-binding transcriptional activator	No
CipChl_A	1976528	G->A				
CipChl_A	2040948	A->G				
CipChl_B	4187354	A->C	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
CipChl_B	4275549	C->T	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing	No
CipChl_C	111493	G->T				
CipChl_C	882884	C->A				
CipPip_A	156256	T->G				
CipPip_A	2337183	T->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
CipPip_A	4183254	G->T	NP_418414.1	rpoB	RNA polymerase; beta subunit	No
CipPip_A	4632867	C->T	NP_418813.1	rob	right oriC-binding transcriptional activator; AraC family	No
CipPip_B	1622859	A->C	NP_416055.1	ydeJ	conserved protein	Yes
CipPip_B	1976527	G->T				
CipPip_B	1976528	G->A				
CipPip_B	3534356	G->A	NP_417864.1	ompR	DNA-binding response regulator in two-component regulatory system with EnvZ	No
CipPip_B	4187442	T->G	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
CipPip_B	4275914	A->G	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing	Yes
CipPip_C	92851	G->T	NP_414626.1	ftsI	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	No
CipPip_C	92852	C->T	NP_414626.1	ftsI	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	Yes
CipPip_C	2337202	C->A	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
CipPip_C	3534290	G->T	NP_417864.1	ompR	DNA-binding response regulator in two-component regulatory system with EnvZ	No
CipTet_B	1976527	G->T				
CipTet_B	1976528	G->A				
CipTet_B	3048873	T->C				
CipTet_B	3533133	C->T	NP_417863.1	envZ	sensory histidine kinase in two-component regulatory system with OmpR	No
CipTet_C	1363138	C->G	NP_415817.1	puuB	gamma-Glu-putrescine oxidase; FAD/NAD(P)-binding	No
CipTet_C	1363139	G->A	NP_415817.1	puuB	gamma-Glu-putrescine oxidase; FAD/NAD(P)-binding	No
CipTet_C	1617483	T->G	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance	No

CipTet_C	2337201	C->T	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A	No
Pip_A	92851	G->T	NP_414626.1	ftsI	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	No
Pip_A	485070	T->G	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
Pip_A	1363140	C->G	NP_415817.1	puuB	gamma-Glu-putrescine oxidase; FAD/NAD(P)-binding	No
Pip_A	1617132	A->C				
Pip_A	1617133	G->C				
Pip_A	1617134	G->T				
Pip_B	1617133	G->C				
Pip_B	1617134	G->T				
Pip_B	4183082	A->G	NP_418414.1	rpoB	RNA polymerase; beta subunit	No
Pip_C	92326	C->T	NP_414626.1	ftsI	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	No
Pip_C	99209	G->T	NP_414631.1	ftsW	Lipid II flippase	No
Pip_C	1976527	G->T				
Pip_C	1977294	C->A				
Pip_C	3533169	A->C	NP_417863.1	envZ	sensory histidine kinase in two-component regulatory system with OmpR	No
PipAmk_A	485598	G->A	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
PipAmk_A	4102886	A->G	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
PipAmk_B	3470123	G->A	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
PipAmk_C	2623803	G->C	NP_416997.1	ppx	exopolyphosphatase	No
PipAmk_C	3471188	C->A	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding	No
PipAmk_C	4102452	C->T	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR	No
PipAmk_C	4632747	A->C	NP_418813.1	rob	right oriC-binding transcriptional activator; AraC family	No
PipChl_A	485024	C->T	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
PipChl_A	1976527	G->T				
PipChl_A	1976528	G->A				
PipChl_A	4187340	C->T	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
PipChl_A	4275549	C->T	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing	No
PipChl_B	1976527	G->T				
PipChl_B	1976528	G->A				
PipChl_B	4184379	G->C	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
PipChl_B	4632867	C->T	NP_418813.1	rob	right oriC-binding transcriptional activator; AraC family	No
PipChl_C	484997	A->G	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
PipChl_C	1976527	G->T				
PipChl_C	3214645	T->C				
PipChl_C	4632867	C->T	NP_418813.1	rob	right oriC-binding transcriptional activator; AraC family	No
PipTet_A	485076	C->A	NP_414997.1	acrR	DNA-binding transcriptional repressor	No

PipTet_A	1906727	G->A	NP_416340.1	mgrB	regulatory peptide for PhoPQ; feedback inhibition	No
PipTet_A	1976527	G->T				
PipTet_A	1976528	G->A				
PipTet_A	3533586	G->A	NP_417863.1	envZ	sensory histidine kinase in two-component regulatory system with OmpR	No
PipTet_B	484998	C->T	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
PipTet_B	2729754	T->A	NP_417083.1	clpB	protein disaggregation chaperone	Yes
PipTet_B	4187339	G->A	NP_418415.1	rpoC	RNA polymerase; beta prime subunit	No
PipTet_C	484997	A->G	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
PipTet_C	1976527	G->T				
PipTet_C	1976528	G->A				
PipTet_C	3533368	C->T	NP_417863.1	envZ	sensory histidine kinase in two-component regulatory system with OmpR	No
PipTet_C	4183090	G->T	NP_418414.1	rpoB	RNA polymerase; beta subunit	No
Tet_A	484987	G->A	NP_414997.1	acrR	DNA-binding transcriptional repressor	No
Tet_A	1617480	C->T	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance	No
Tet_A	3438262	C->T	NP_417754.1	rpoA	RNA polymerase; alpha subunit	No
Tet_B	483223	G->T	NP_414995.1	acrB	multidrug efflux system protein	No
Tet_B	985121	A->C	NP_415449.1	ompF	outer membrane porin 1a (Ia	No
Tet_C	985350	G->A	NP_415449.1	ompF	outer membrane porin 1a (Ia	No

D. INDELs Identified in the evolved MG1655 Strain

Strain/AB_selection	From Position	Type	INDEL	Genbank ID	Gene	Product
Amk_B	753063	insertion	C->CTACGGTTCGCAC	NP_415248.1	gltA	citrate synthase
Amk_B	2925947	insertion	T->TTATAT			
Amk_B	4081530	deletion	GCCATGC->G	NP_418330.1	fdoG	formate dehydrogenase-O; large subunit
AmkTet_A	1617207	insertion	T->TAATC	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance
AmkTet_A	3469704	insertion	C->CAAC	NP_417799.1	fusA	protein chain elongation factor EF-G; GTP-binding
AmkTet_A	4468534	deletion	CCG->C			
AmkTet_B	484953	deletion	AT->A			
Chl_A	882788	insertion	A->AGTAATAAT			
Chl_B	4254115	deletion	ACACTGG->A	NP_418465.4	plsB	glycerol-3-phosphate O-acyltransferase
ChlTet_A	1617515	deletion	CA->C	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance
Cip_A	4275869	deletion	CGCG->C	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing
Cip_C	987835	insertion	G->GGCG	NP_415450.1	asnS	asparaginyl tRNA synthetase

Cip_C	1142060	insertion	A->ACCATCGC	NP_415602.1	rne	fused ribonucleaseE: endoribonuclease/RNA-binding protein/RNA degradosome binding protein
Cip_C	3445977	deletion	TGG->T	NP_417769.1	rplN	50S ribosomal subunit protein L14
Cip_C	4275871	deletion	CGCA->C	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing
CipAmk_A	485481	deletion	AT->A	NP_414997.1	acrR	DNA-binding transcriptional repressor
CipAmk_A	2337194	deletion	CCGA->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A
CipAmk_B	2337194	deletion	CCGA->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A
CipChl_A	458212	deletion	GA->G	NP_414973.1	lon	DNA-binding ATP-dependent protease La
CipChl_A	2337194	deletion	CCGA->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A
CipChl_B	485598	insertion	C->CGCC	NP_414997.1	acrR	DNA-binding transcriptional repressor
CipChl_B	986403	deletion	TAAC->T			
CipChl_C	2337194	deletion	CCGA->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A
CipChl_C	4275869	deletion	CGCG->C	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing
CipPip_B	485036	insertion	T->TG	NP_414997.1	acrR	DNA-binding transcriptional repressor
CipPip_B	2337194	deletion	CCGA->C	NP_416734.1	gyrA	DNA gyrase (type II topoisomerase); subunit A
CipPip_C	485136	deletion	CA->C	NP_414997.1	acrR	DNA-binding transcriptional repressor
CipPip_C	4275889	insertion	A->AGTA	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing
CipTet_A	986138	deletion	TGCAG->T	NP_415449.1	ompF	outer membrane porin 1a (Ia
CipTet_A	4184028	insertion	C->CAGC	NP_418415.1	rpoC	RNA polymerase; beta prime subunit
CipTet_A	4275869	deletion	CGCG->C	NP_418487.1	soxR	DNA-binding transcriptional dual regulator; Fe-S center for redox-sensing
CipTet_B	2105	deletion	CATCA->C	NP_414543.1	thrA	fused aspartokinase I and homoserine dehydrogenase I
CipTet_B	484937	deletion	AT->A			
CipTet_C	4632753	deletion	TTGC->T	NP_418813.1	rob	right oriC-binding transcriptional activator; AraC family
Pip_A	1617115	deletion	TGCAACTAATTACTTGCCAGG->T			

Pip_B	1712342	deletion	CT->C			
PipAmk_B	4102928	insertion	C->CACCAACATCAAC	NP_418347.1	cpxA	sensory histidine kinase in two-component regulatory system with CpxR
PipTet_B	1617324	insertion	A->AA	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance
PipTet_B	3533825	deletion	GCAAGGTGACGAT->G	NP_417863.1	envZ	sensory histidine kinase in two-component regulatory system with OmpR
PipTet_C	1617318	deletion	TG->T	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance
Tet_B	1617384	insertion	A->AAAAGGCTGGGTGG	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance
Tet_C	1617384	insertion	A->AAAAGGCTGGGTGG	NP_416047.4	marR	DNA-binding transcriptional repressor of multiple antibiotic resistance

Table S3. Individual SNPs and INDELs.
A) SNPs that were identified in the WT non-evolved strain of MG1655 when compared to the deposited Genbank sequence of MG1655 (NC_000913). Annotations were obtained from the deposited Genbank record.
B) INDELs that were identified in the WT non-evolved strain of MG1655 when compared to the deposited Genbank sequence of MG1655 (NC_000913). Annotations were also obtained from the deposited Genbank record.
C) SNPs that were identified in the evolved lineages based on comparison to the deposited Genbank sequence of MG1655 (NC_000913) with SNPs found in the non-evolved strain masked (A). Annotations were also obtained from the deposited Genbank record.
D) INDELs that were identified in the evolved lineages based on comparison to the deposited Genbank sequence of MG1655 with INDELs found in the non-evolved strain masked (B). Annotations were also obtained from the deposited Genbank record.

A)

	MAGE-Oligo
*cpxA*_G4102763A-	TTAATGTGGTGGCGGCGTCTGTTCCGGGCGATTGATAAGTGGGTACCGCCAGGACAGCGTTTGTTATTGGTGACCACCGAAGGCCGCGTG
*fusA*_G3469714A+	GAGTTTCTACTTCAACCTTCATGATCGGCTCAAGCAGAACTGGTTTCACTTTCTTAAAGCCTTCTTTAAAGGCGATAGAAGCAGCCAGTT
*sbmA*_C396368A-	ATATGACGCAGTTGTTGCCAGTTCGCCATGTAATATTCGTTCATCTCTGTACGCCAGCGGAACACGTAGTGACTGACAAAGAAGTTGTTC
*gyrA*_T2337183C+	ACCGAAGTTACCCTGACCGTCTACCAGCATATAACGCAGCGAGAATGGCTGCGCCATGCGGACGATCGTGCCATAGACCGCCGAGTCACC
*marR*_C1617480T+	GTAAAACTTACCACCGGCGGCGCGGCAATATGTGAACAATGCCATTAATTAGTTGGCCAGGACCTGCACCAAGAATTAACAAAAAACCTG

B)

qPCR	**WT forward primer**	**Mutant forward primer**	**Reverse primer**
cpxA	ACGCTGTCCTGGCGGTG	ACGCTGTCCTGGCGGTA	CACGCCAGATGACCGAGC
fusA	GCTCAAGCAGAACTGGTTTCG	GCTCAAGCAGAACTGGTTTCA	TAATCCCTGGCGAATACATCC
sbmA	GTTCCGCTGGCGTACAGC	GTTCCGCTGGCGTACAGA	GCGGAGAGCGTTACCAGC
gyrA	CCATGCGGACGATCGTGT	CCATGCGGACGATCGTGC	GCGATGTCGGTCATTGTTGG
marR	GCAATATGTGAACAATGCCATC	GCAATATGTGAACAATGCCATT	TGGCGATTCCAGGTTGTCC

Table S4. Oligos for MAGE and qPCR primers.
A) MAGE oligos: The MAGE oligos were designed according to *(76)*. The oligo name reflects the SNP change at the position number according to MG1655 (Accession no. NC_000913). The + and – denote whether the oligo is targeting the plus or the minus strand.
B) qPCR primers: Forward qPCR primers are identical except for the 3’ nucleotide being altered according to the introduced SNP. The reverse primer is common for both forward primers.