Figure 1

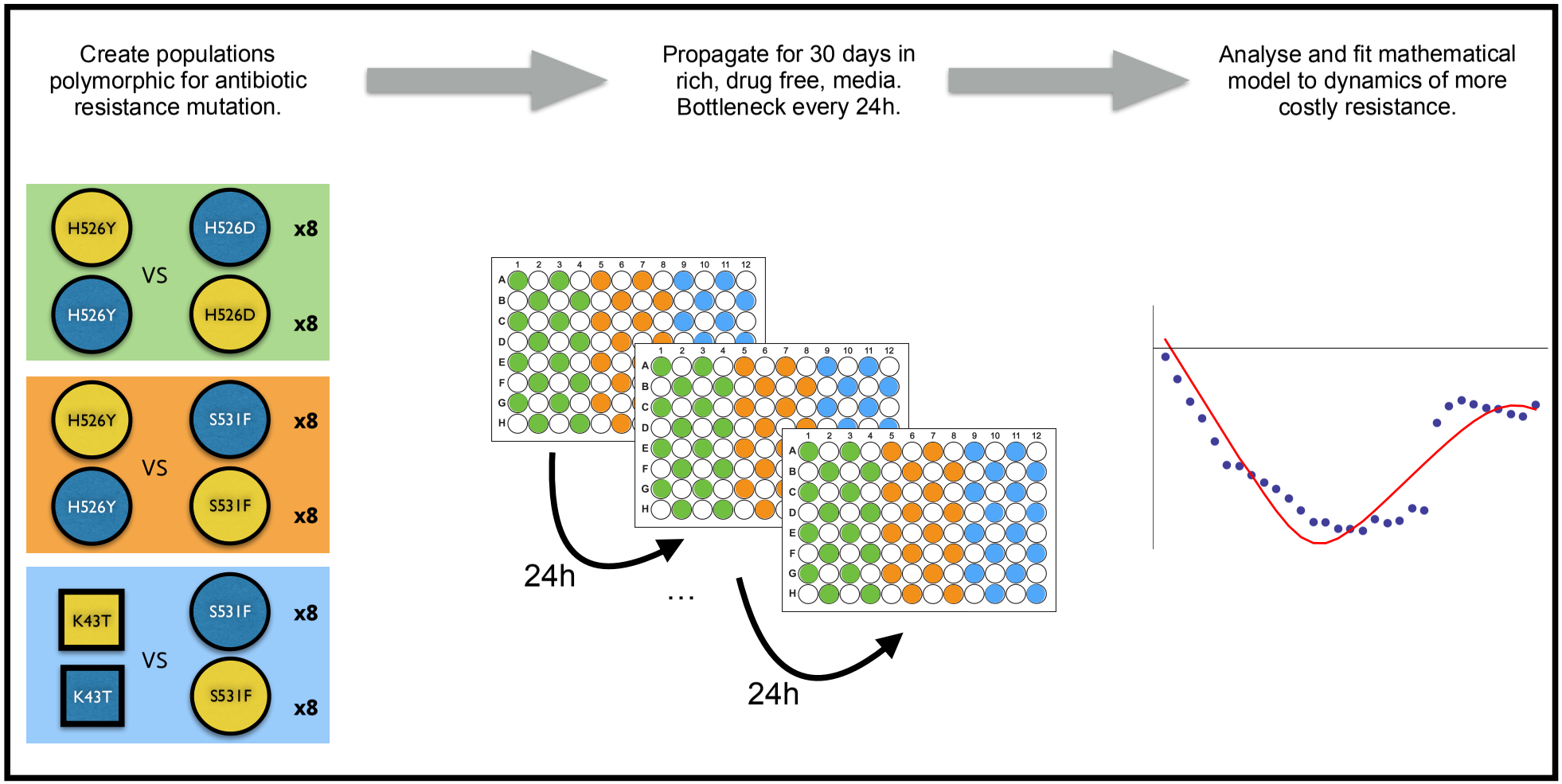

Figure 1. Testing the evolvability of different antibiotic resistances.

Figure 2

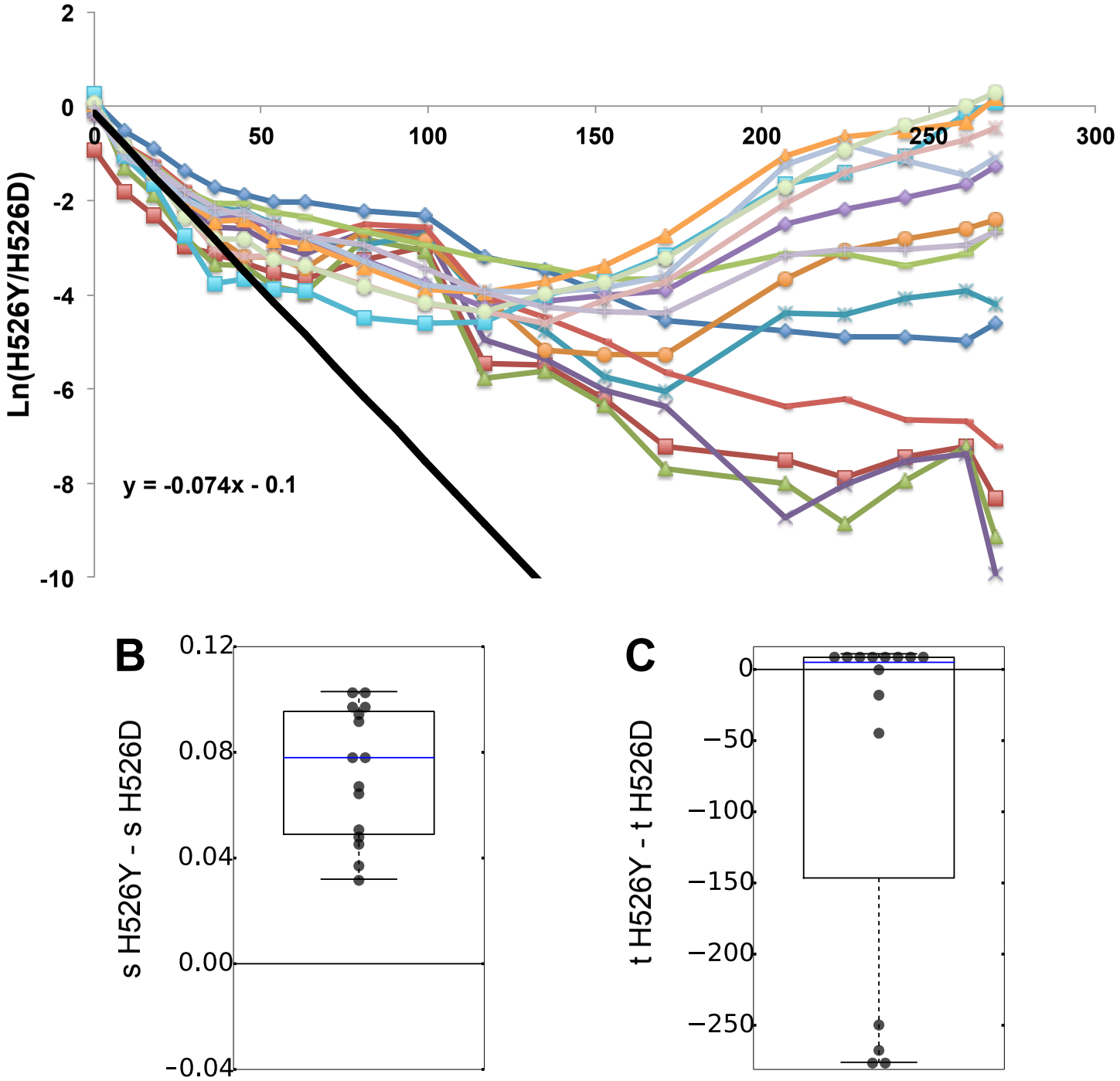

Figure 2. High evolvability of costly *H526Y* allows its long-term maintenance

Figure 3

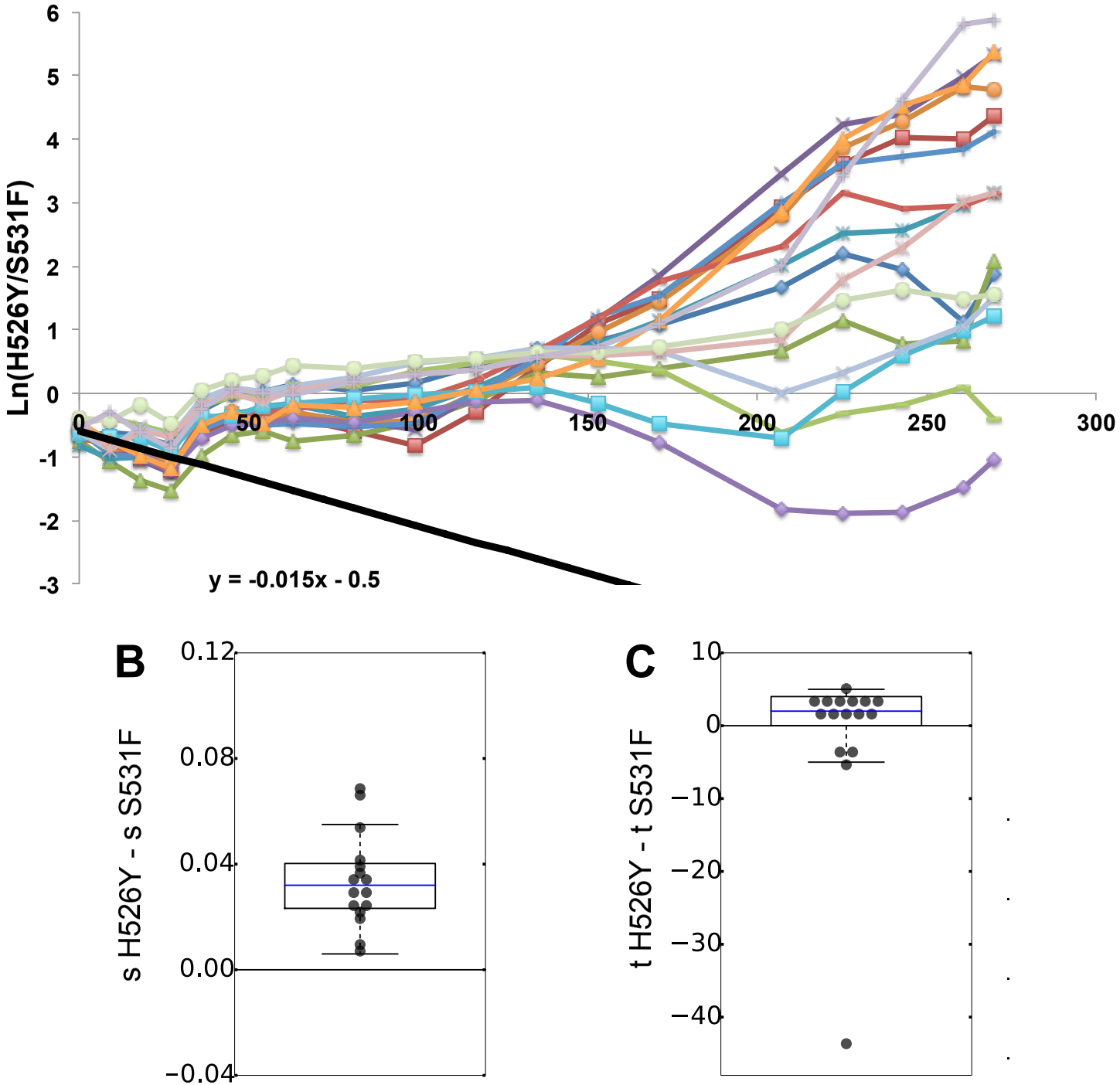

Figure 3. Different evolvabilities between Rifampicin resistance alleles

Figure 4

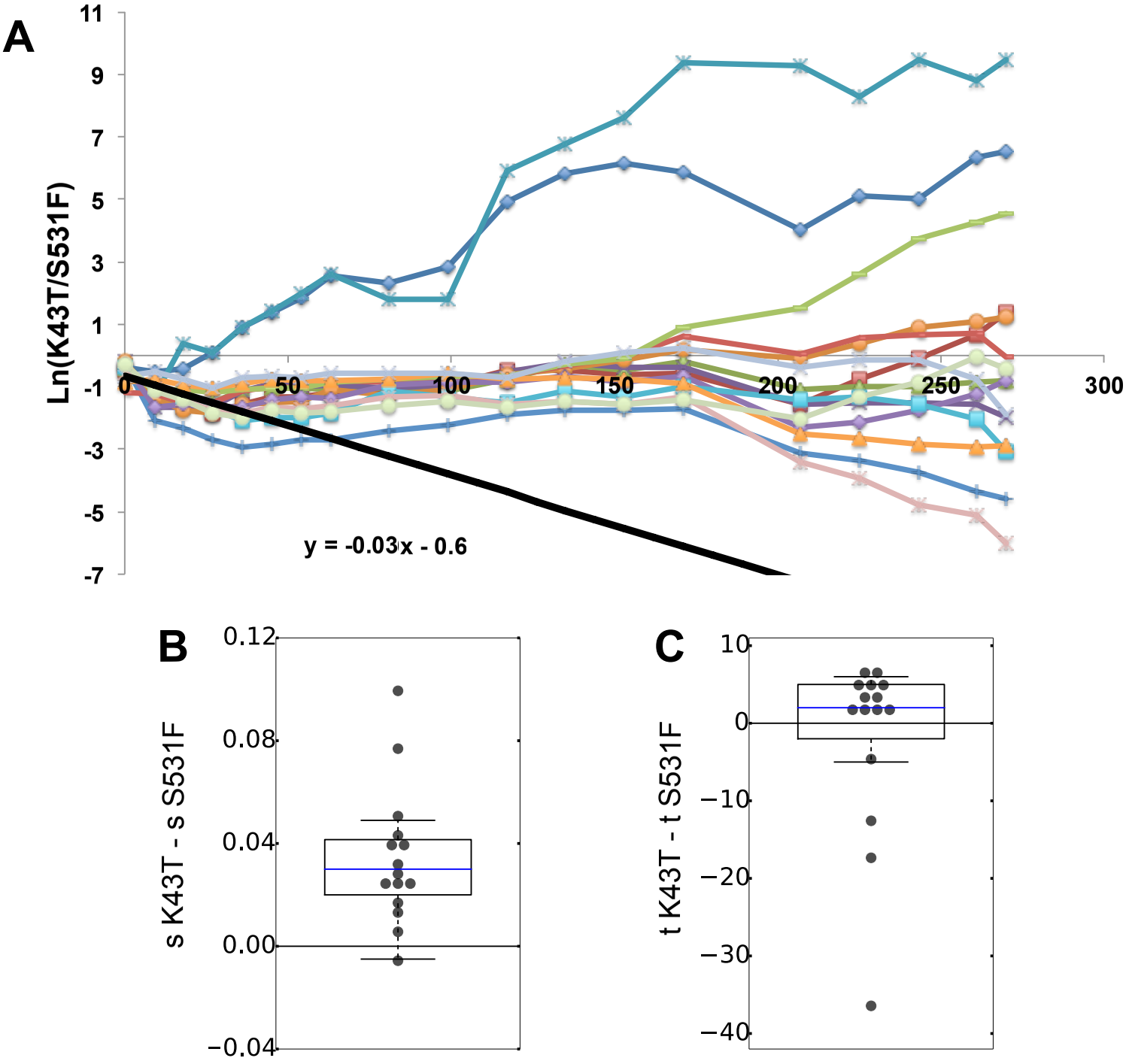

Figure 4. Differences in evolvability between Rifampicin and Streptomycin resistance alleles

Figure 5

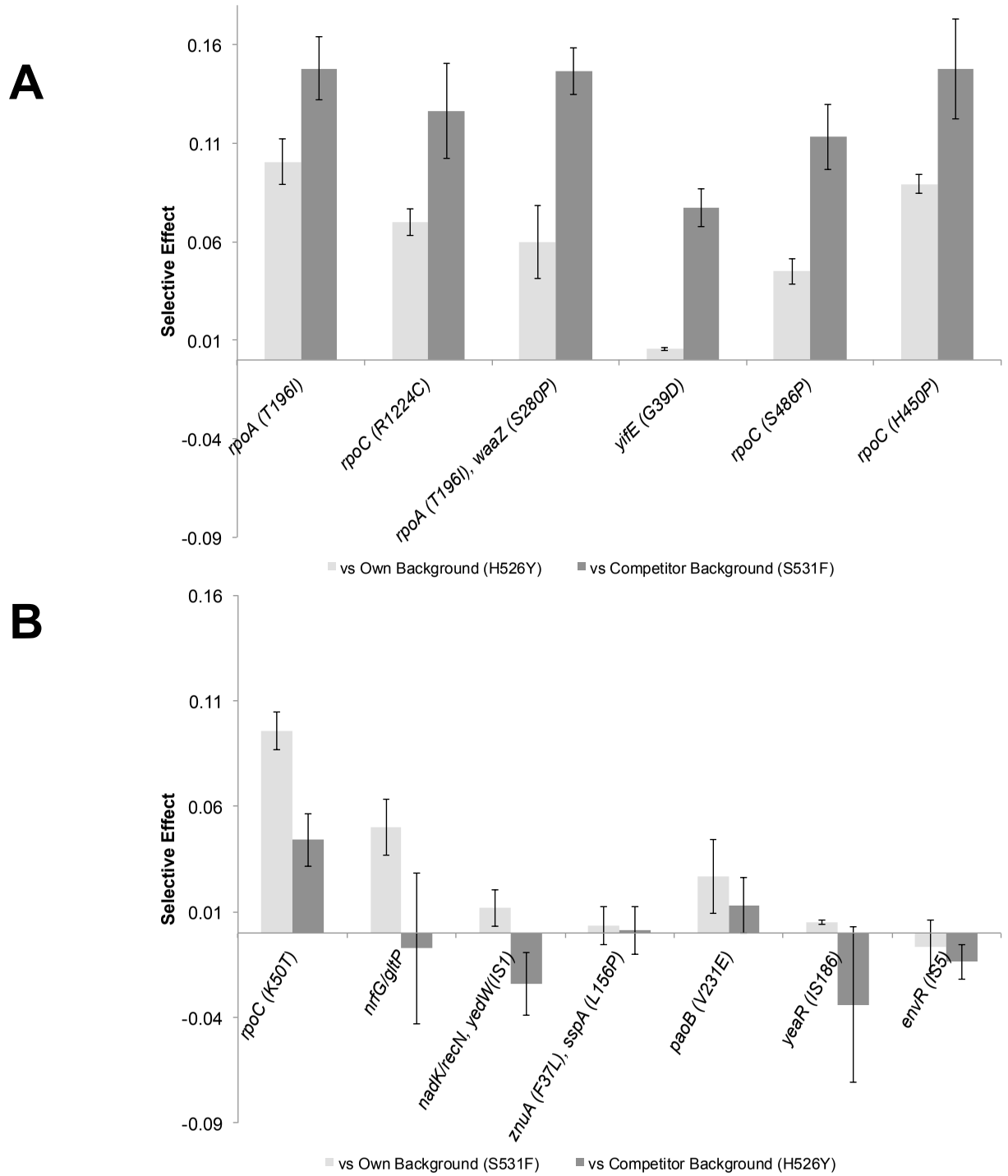

Figure 5. Fitness effects of the evolved mutants in the competition between H526Y and S531F

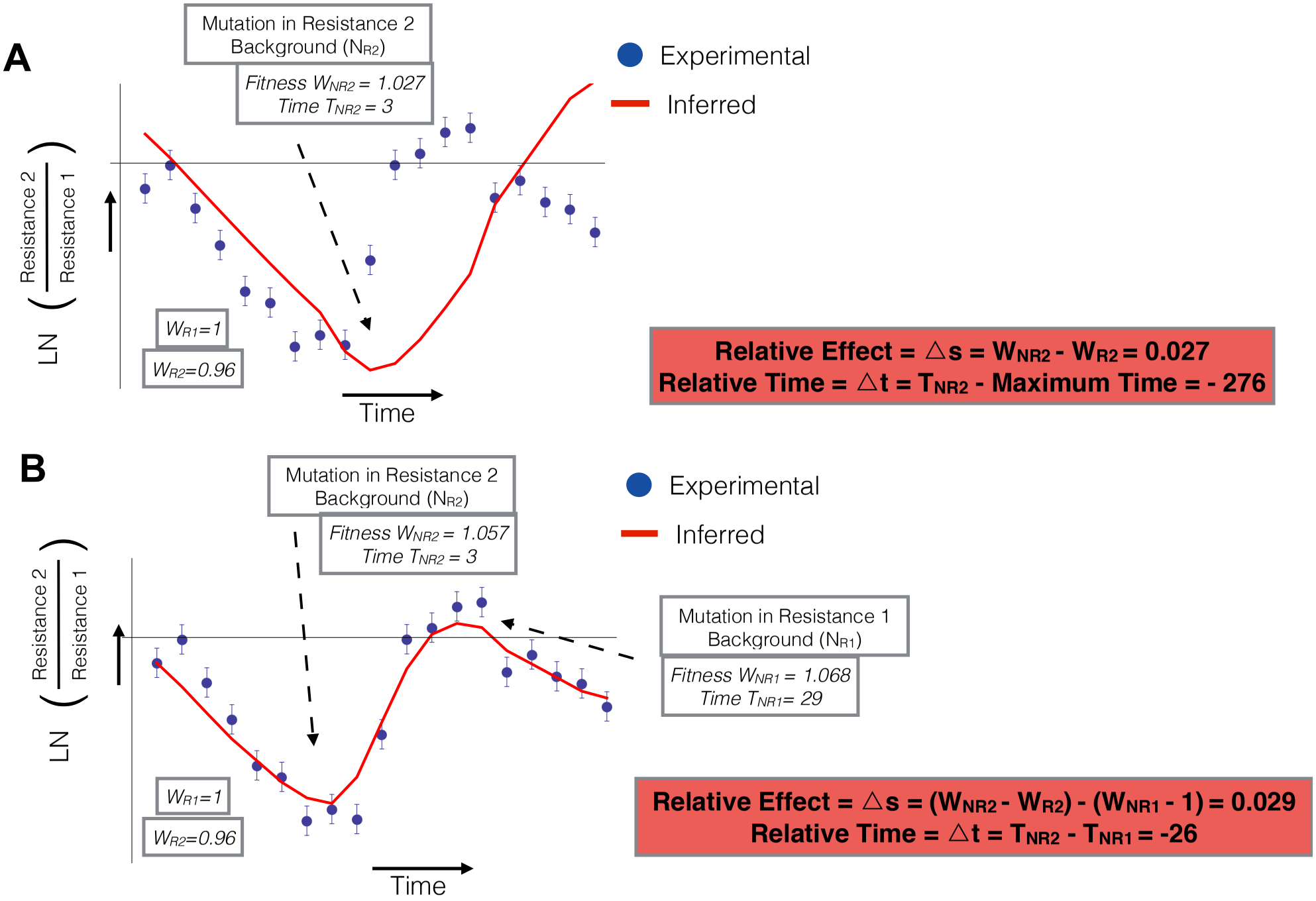

Figure S1. Example of the fitting process for a simulated experimental population.

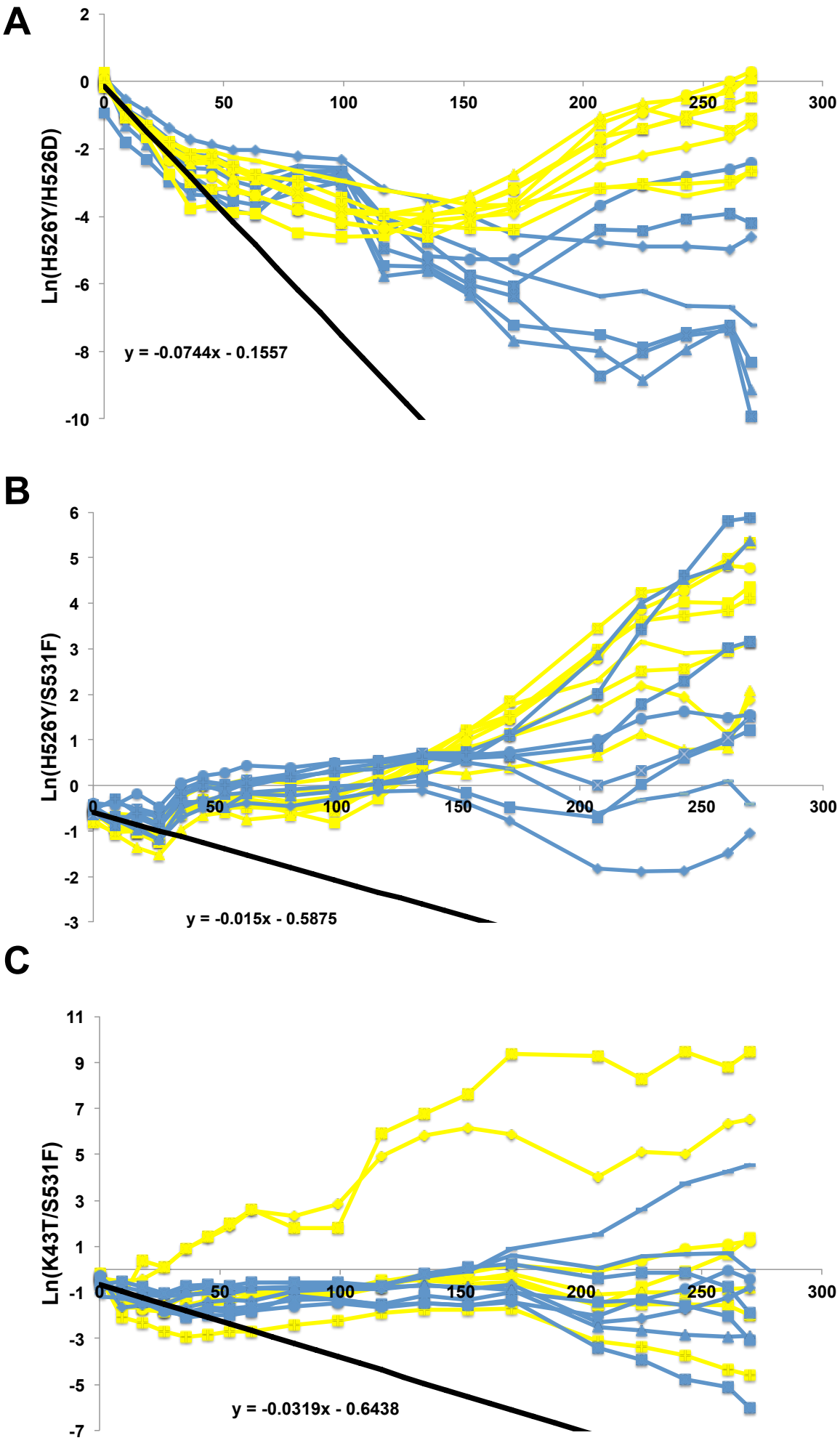

Figure S2. Long-term dynamics with the identification of the fluorescent backgrounds

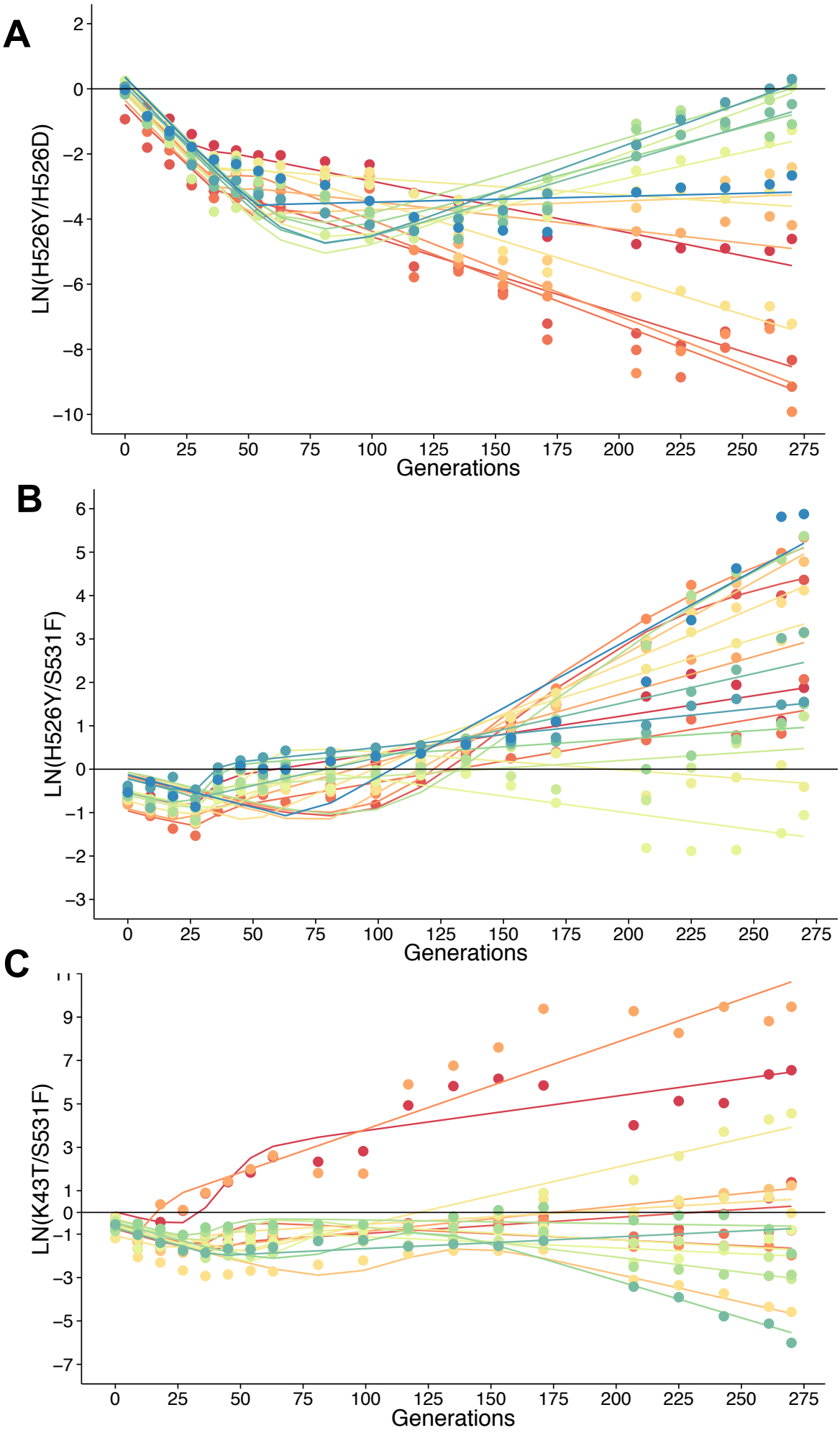

Figure S3. Long-term dynamics from the inferred parameters for each replicate population

Figure S4

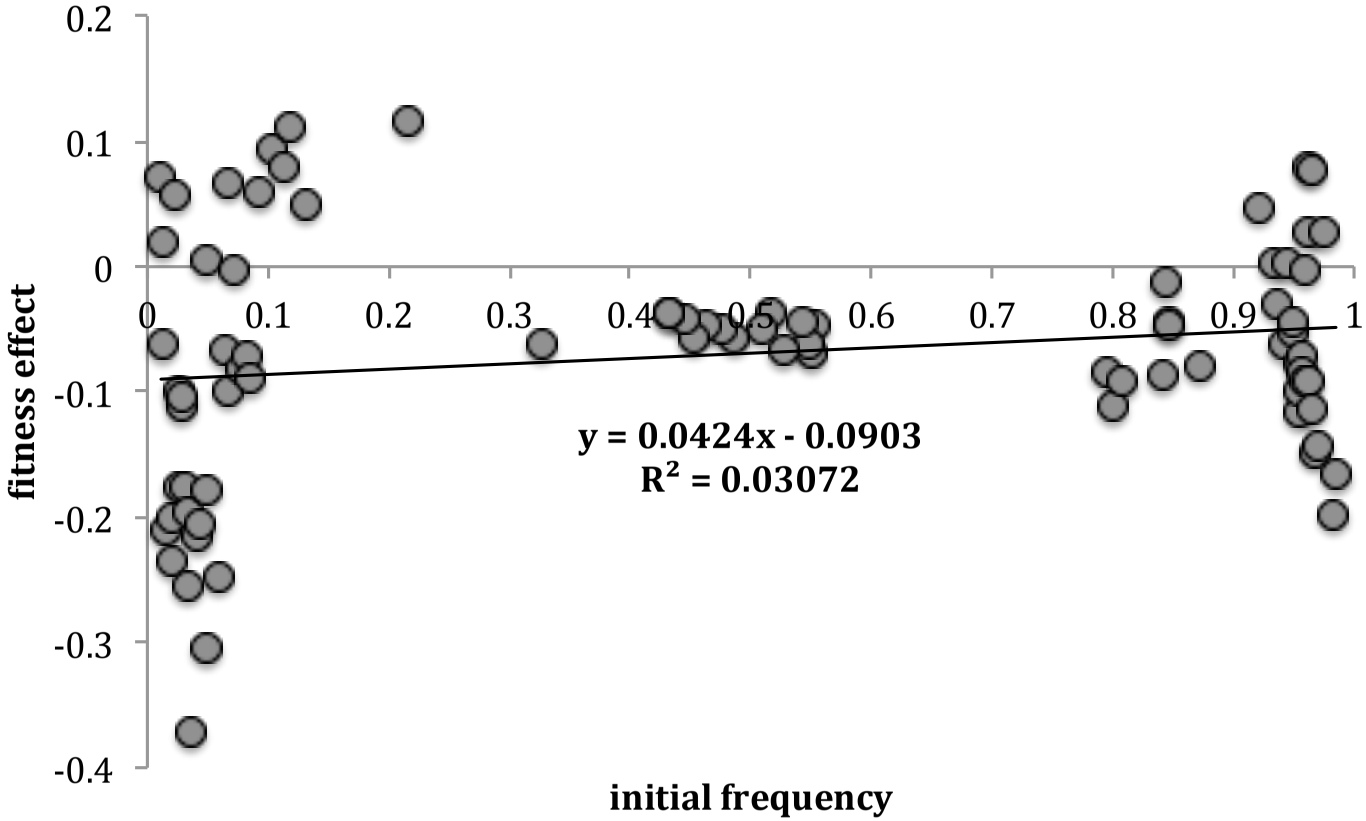

Figure S4. Test for negative frequency dependence selection in competitions between resistance strains *H526Y* and *H526D*

		H526D (R1)		H526Y (R2)	
	Initial H526Y Frequency	**W**	**T**	**W**	**T**
Population 1	0.538	1.576	11	1.552	15
Population 2	0.382	1.293	11	1.263	22
Population 3	0.488	1.387	41	1.348	51
Population 4	0.468	1.483	26	1.44	32
Population 5	0.413	1.421	21	1.409	30
Population 6	0.476	1.474	31	1.478	42
Population 7	0.513	1.218	11	1.19	19
Population 8	0.493	1.776	18	1.767	23
Population 9	0.557			1.017	3
Population 10	0.461			1.028	29
Population 11	0.581			1.023	3
Population 12	0.58	1.002	46	1.02	3
Population 13	0.523			1.023	14
Population 14	0.55	1.001	38	1.028	23
Population 15	0.59	1.668	37	1.671	45

Table 1. Evolutionary Parameters estimated for the competition between strains *H526Y* and *H526D*. W stands for the fitness of the emerging haplotype and T its time of appearance. Initial Freq stands for the inferred initial frequency of the *H526Y* background. In the cases where only one of the backgrounds has acquired a mutation, the inferred parameters are in bold for the background where no mutation was inferred.

		S531F (R1)		H526Y (R2)	
	Initial H526Y Frequency	W	T	W	T
Population 1	0.369	1.459	15	1.472	17
Population 2	0.458	1.041	46	1.051	3
Population 3	0.277	1.436	15	1.45	19
Population 4	0.472	1.034	7	1.054	4
Population 5	0.286	1.529	8	1.554	12
Population 6	0.454	1.585	55	1.637	60
Population 7	0.381	1.615	36	1.655	40
Population 8	0.325	1.465	11	1.491	15
Population 9	0.473	1.199	19	1.194	14
Population 10	0.378	1.163	14	1.154	10
Population 11	0.356	1.595	17	1.601	19
Population 12	0.483	1.028	3	1.049	4
Population 13	0.37	1.344	11	1.349	12
Population 14	0.444	1.542	9	1.562	12
Population 15	0.446	1.505	16	1.514	17
Population 16	0.468	1.647	55	1.7	59

Table 2. Evolutionary Parameters estimated for the competition between strains *H526Y* and *S531F*. The meaning of the parameters is as in Table 1.

		S531F (R1)		K43T (R2)	
	Initial K43T Frequency	W	T	W	T
Population 1	0.505	1.207	16	1.188	3
Population 2	0.319	1.349	20	1.359	26
Population 3	0.299	1.546	18	1.547	21
Population 4	0.349	1.26	16	1.253	18
Population 5	0.319	1.669	4	1.737	5
Population 6	0.359	1.457	4	1.474	10
Population 7	0.333	1.088	52	1.06	34
Population 8	0.256	1.325	7	1.334	12
Population 9	0.43	1.687	37	1.732	42
Population 10	0.335	1.097	3	1.09	4
Population 11	0.357	1.537	50	1.529	53
Population 12	0.388	1.16	11	1.144	6
Population 13	0.41	1.195	5	1.193	7
Population 14	0.379	1.095	43	1.058	6
Population 15	0.329	1.663	25	1.672	30

Table 3. Evolutionary Parameters estimated for the competition between strains *K43T* and *S531F*. The meaning of the parameters is as in Table 1.

Table 4. Potential compensatory mutations identified in the genomes of the clones evolved in the competition between resistances H526Y and S531F.

Background	Genome Position	Gene(s)	Mutation	Annotation	Frequency	Function
H526Y	3,438,465	*rpoA*	C→T	T196I	51.5%	*RNA polymerase, alpha subunit*
	4,184,828	*rpoC*	T→C	S486P	25.5%	*RNA polymerase, beta prime subunit*
	4,187,042	*rpoC*	C→T	R1224C	6.1%	
	4,184,721	*rpoC*	A→C	H450P	2.9%	
	3,797,382	*waaZ*	T→C	S280P	19.7%	*Lipopolysaccharide core biosynthesis protein*
	3,946,224	*yifE*	G→A	G39D	12.0%	*Conserved protein, UPF0438 family, unknown function*
	4,292,389	*nrfG/gltP*	A→T	Intergenic	5.0%	*[**nrfG**] Heme lyase (NrfEFG) for insertion of heme into c552, subunit NrfG/[**gltP**] glutamate/aspartate: proton symporte*
	3,637,091	*pitA*	C→T	A476V	2.6%	*Phosphate transporter, low-affinity; tellurite importer*
	374,196	*mhpE/mhpT*	T→C	Intergenic	2.4%	*4-hyroxy-2-oxovalerate/4-hydroxy-2-oxopentanoic acid aldolase, class I/putative 3-hydroxyphenylpropionic transporter*
	3,464,375	*bfr*	C→T	R125C	2.0%	*Bacterioferritin, iron storage and detoxification protein*
	1,743,180	*ydhQ*	T→C	S324S	3.1%	*Hypothetical protein*
	3,898,043	*yieL*	C→A	T186T	2.5%	*Putative xylanase*
S531F	4,183,521	*rpoC*	A→C	K50T	14.8%	*RNA polymerase, beta prime subunit*
	4,292,389	*nrfG/gltP*	A→T	Intergenic	19.6%	*[**nrfG**] Heme lyase (NrfEFG) for insertion of heme into c552, subunit NrfG/ [**gltP**] Glutamate/aspartate:proton symporte*
	2,749,808	*nadK/recN*	Δ1 bp	Intergenic	18.1%	*[**nadK**] NAD kinase/ [**recN**] Recombination and repair protein*
	1,940,499	*znuA*	T→C	F37L	17.7%	*Zinc transporter subunit: periplasmic-binding component of ABC superfamily*
	3,374,976	*sspA*	T→C	L156P	12.2%	*Stringent starvation protein A*
	300,420	*paoB*	T→A	V231E	11.8%	*PaoABC aldehyde oxidoreductase, FAD-containing subunit*
	2,036,728	*yedW*	IS1 Ins	Coding	9.9%	*Putative DNA-binding response regulator in two-component system with YedV*
	1,877,853	*yeaR*	IS186 Ins	Coding	8.4%	*Hypothetical protein*
	3,410,893	*envR*	IS5 Ins	Coding	6.8%	*DNA-binding transcriptional regulator*
	2,361,326	*yfaY*	A→G	V110V	9.4%	*Hypothetical protein*

Table S1. Fitness costs imposed by the antibiotic resistance alleles (*K43T*, *S531F*, *H526Y*, *H526D*) measured in competition against the sensitive reference strain.

	Antibiotic Resistance	Target Genes	Aminoacid change (location)	Fitness cost (relative to wild-type)	Standard Err
K43T	Streptomycin	*rpsL*	AAA - ACA (128)	**0.092**	0.034
S531F	Rifampicin	*rpoB*	TCC - TTC (1592)	**0.096**	0.011
H526Y	Rifampicin	*rpoB*	CAC - TAC (1376)	**0.073**	0.014
H526D	Rifampicin	*rpoB*	CAC - GAC (1576)	**0.064**	0.017

Table S2. Genotypes of the evolved clones from competition between *H526Y*^{Rif} and *S531F*^{Rif}

Genotype of H526Y Evolved Clones

galK:YFP, rpoB (H526Y), rpoA (T196I), waaZ (S280P)
galK:YFP, rpoB (H526Y), rpoA (T196I)
galK:YFP, rpoB (H526Y), rpoA (T196I)
galK:YFP, rpoB (H526Y), rpoA (T196I)
galK:YFP, rpoB (H526Y), rpoA (T196I)
galK:YFP, rpoB (H526Y), rpoA (T196I)
galK:YFP, rpoB (H526Y), rpoA (T196I)
galK:YFP, rpoB (H526Y), rpoA (T196I)
galK:CFP, rpoB (H526Y), rpoC (Unknown)
galK:CFP, rpoB (H526Y), yifE (G39D)
galK:CFP, rpoB (H526Y), rpoC (R1224C)
galK:CFP, rpoB (H526Y), rpoC (S486P)
galK:CFP, rpoB (H526Y), rpoC (S486P)
galK:CFP, rpoB (H526Y), rpoC (S486P)
galK:CFP, rpoB (H526Y), rpoC (H540P)

Genotype of S531F Evolved Clones

galK:YFP, rpoB (S531F), envR (IS5)
galK:YFP, rpoB (S531F), znuA (F37L), sspA (L156P)
galK:YFP, rpoB (S531F), znuA (F37L), sspA (L156P)
galK:YFP, rpoB (S531F), yeaR (IS186)
galK:YFP, rpoB (S531F), nadK/recN (Intergenic), yedW (IS1)
galK:YFP, rpoB (S531F), rpoC (K50T)
galK:YFP, rpoB (S531F), paoB (V231E)
galK:YFP, rpoB (S531F), nrfG/gltP (Intergenic)