

Molecular Cell, Volume 73

Supplemental Information

Inhibiting the Evolution of Antibiotic Resistance

Mark N. Ragheb, Maureen K. Thomason, Chris Hsu, Patrick Nugent, John Gage, Ariana N. Samadpour, Ankunda Kariisa, Christopher N. Merrikh, Samuel I. Miller, David R. Sherman, and Houra Merrikh

Figure S1

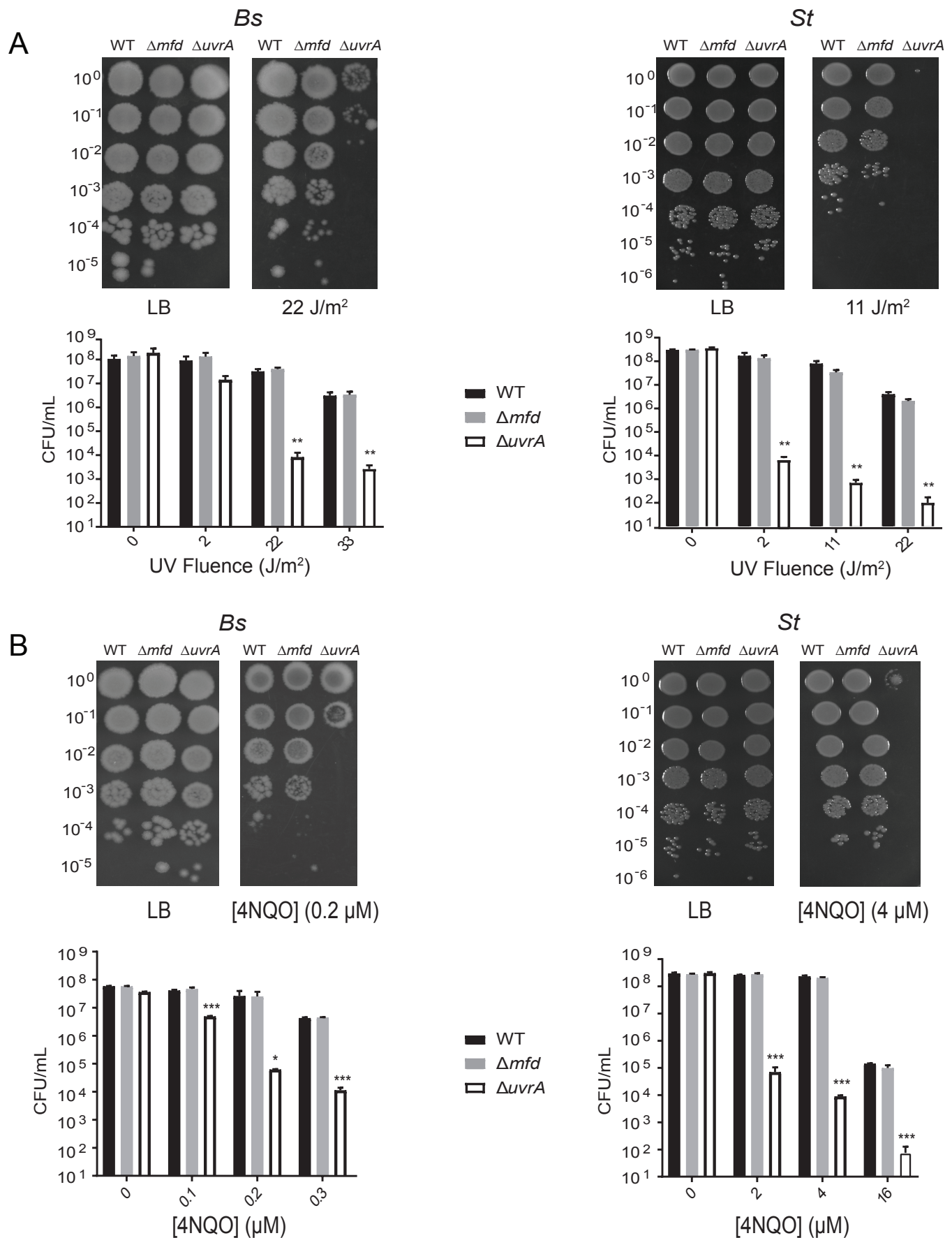


Figure S1. Cells lacking Mfd are not significantly sensitive to DNA damaging agents, Related to Figure 1 Survival assays to (A) UV damage and (B) 4NQO for WT, Δmfd , and $\Delta uvrA$ strains of *B. subtilis* HM1 (*Bs*) and *S. typhimurium* ST19 (*St*). (*uvrA* knockouts, known to be sensitive to DNA damage, were included for comparison to *mfd* knockouts). Data represents at least two independent experiments with duplicates for each experiment. Errors bars indicate s.e.m. Statistical significance was determined using two-tailed Student's t-test (*p-value <0.05, **p-value <0.01, ***p-value <0.001).

Figure S2

A

Rifampicin

Rifampicin

No mutations					
No mutations					
			I572S	I572S	
G536V	G536V	G536V	G536V	G536V	
S574F	S574F	S574F	S574F	S574F	
I572F	I572F	I572F	I572F	I572F	I572F
I572F	I572F	I572F	I572F	I572F	I572F
S512F	S512F	S512F	S512F	S512F	S512F
		H526Y	H526Y	H526Y	H526Y
I572F	I572F	I572F	I572F	I572F	I572F
		D516E	D516E	D516E	D516E
S512P	S512P	S512P	S512P	S512P	S512P
	L511Q	L511Q	L511Q	L511Q	L511Q
Q148L	Q148L	Q148L	Q148L	Q148L	Q148L
	I572S	I572S	I572S	I572S	I572S
L511Q	L511Q	L511Q	L511Q	L511Q	L511Q
D516G	D516G	D516G	D516G	D516G	D516G
Q513H	Q513H	Q513H	Q513H	Q513H	Q513H
D516G	D516G	D516G	D516G	D516G	D516G

WT replicates

Δmfd replicates

No mutations						*	
						L511P	*
						D516G	*
			I572F	I572F		*	
			L538P	L538P	L538P	*	
			H526Y	H526Y	H526Y	*	
			I572N	I572N	I572N	*	
			Q148L	Q148L	Q148L	*	
			Q148L	Q148L	Q148L	*	
			H526Y	H526Y	H526Y	*	
			S509R	S509R	S509R	*	
			D516G	D516G	D516G	*	
			D516G	D516G	D516G	*	
			Q513L	Q513L	Q513L	*	
			Q148L	Q148L	Q148L	*	
			Q148L	Q148L	Q148L	*	
			Q148L	Q148L	Q148L	*	
			Q148L	Q148L	Q148L	*	
			I572N	I572N	I572N	*	
			I572N	I572N	I572N	*	
			I572N	I572N	I572N	*	
			I572N	I572N	I572N	*	
			I572N	I572N	I572N	*	
			I572N	I572N	I572N	*	

24 48 72 96 120 144
Time (h)

24 48 72 96 120 144
Time (h)

B

Trimethoprim

Trimethoprim

						D27E	*			
						F153S	*			
			M20I	M20I	M20I	M20I	D27E	F153S	*	
			W30G	W30G	W30G	W30G	W30G			*
			M20I	M20I	M20I	M20I	M20I			*
			F153S	F153S	F153S	F153S	F153S	F153S	D27E	*
			M20I	M20I	M20I	M20I	M20I	M20I	F153S	*
			W30G	W30G	W30G	W30G	W30G	W30G	D27E	*
			M20I	M20I	M20I	M20I	M20I	M20I	D27E	*
			F153S	F153S	F153S	F153S	F153S	F153S	D27E	*
			M20I	M20I	M20I	M20I	M20I	M20I	F153S	*
			F153S	F153S	F153S	F153S	F153S	F153S	D27E	*
			F153S	F153S	F153S	F153S	F153S	F153S	D27E	*

WT replicates

Δmfd replicates

No mutations						*						
No mutations											*	
No mutations												*
						P21L	*					
						M20I	*					
						W30C	W30C	*				
						L28R	L28R	L28R	*			
						F153S	F153S	F153S	*			
						D27E	D27E	D27E	D27E	*		
						F153S	F153S	F153S	F153S	*		
						W30G	W30G	W30G	W30G	*		
						D27E	D27E	D27E	D27E	F153S	*	
						D27E	D27E	D27E	D27E	F153S	*	

24 48 72 96 120 144 168
Time (h)

24 48 72 96 120 144 168
Time (h)

Figure S2. Cells lacking Mfd show fewer and delayed resistance-conferring mutations, Related to Figure 2, Figure S3, and Table S1

(A) Sequencing of *rpoB* was performed at each time point from rifampicin evolution assays to identify mutations that confer resistance in WT and Δmfd strains of *S. typhimurium* (12 replicates per strain). (B) Sequencing of *folA* was performed at each time point from trimethoprim evolution assays to identify mutations that confer resistance in WT and Δmfd strains of *S. typhimurium* (12 replicates per strain). Shown are the position and corresponding amino acid changes. *Indicates replicates sequenced by whole genome sequencing.

Figure S3

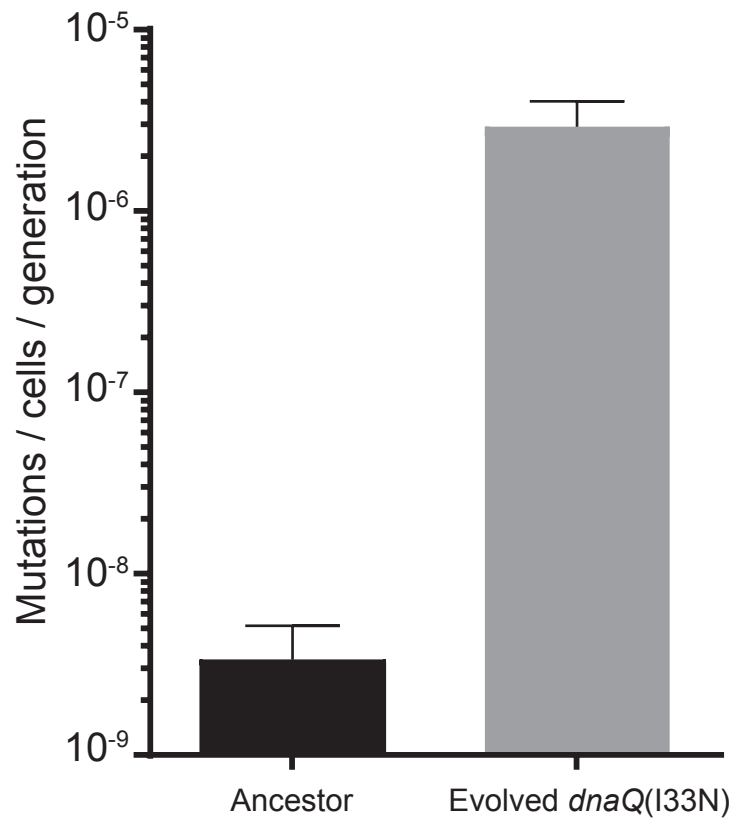
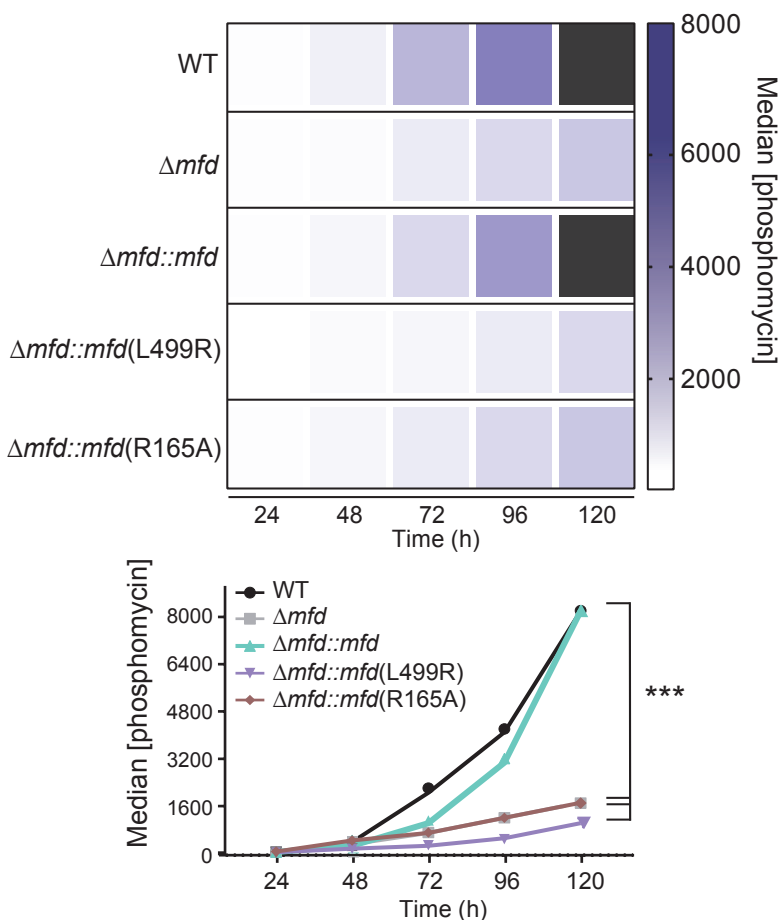


Figure S3. Development of hypermutation in evolved WT strains of *S. typhimurium*, Related to Figure 2, Figure S2, and Table S1

Mutation rate analysis of *S. typhimurium* strains evolved to trimethoprim. Assays were performed on rifampicin plates as described in Figure 1. The individual ancestor and evolved WT (containing a *dnaQ*133N mutation) isolates used in this experiment are indicated in Table S1. The number of replicates per isolate is 12.

Figure S4

A



B

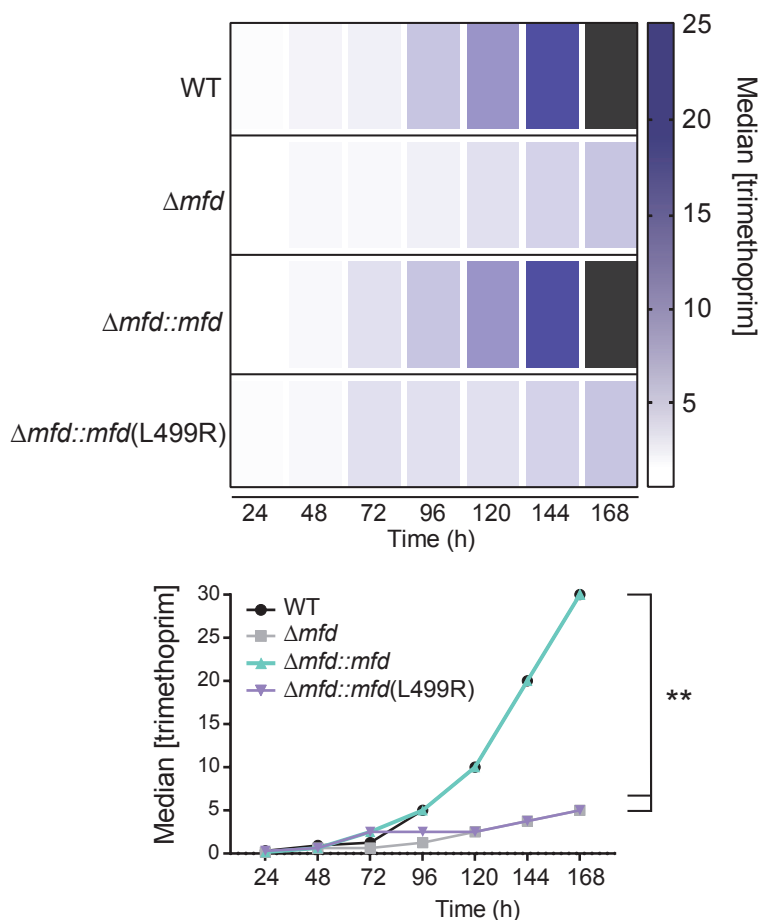


Figure S4. Mfd requires interaction with RNAP and UvrA to promote evolution to antibiotics, Related to Figure 4

Evolution of indicated *S. typhimurium* ST19 strains to phosphomycin (A) and trimethoprim (B). Plots and statistical testing for evolution assays were performed as described in Figure 2. ***p-value < 0.001 between WT and Δmfd , between WT and $\Delta mfd::mfd(L499R)$, and between WT and $\Delta mfd::mfd(R165A)$ strains for evolution to phosphomycin. **p-value < 0.01 between WT and Δmfd and between WT and $\Delta mfd::mfd(L499R)$ strains for evolution to trimethoprim. n = 12-24 replicates per strain.

Figure S5

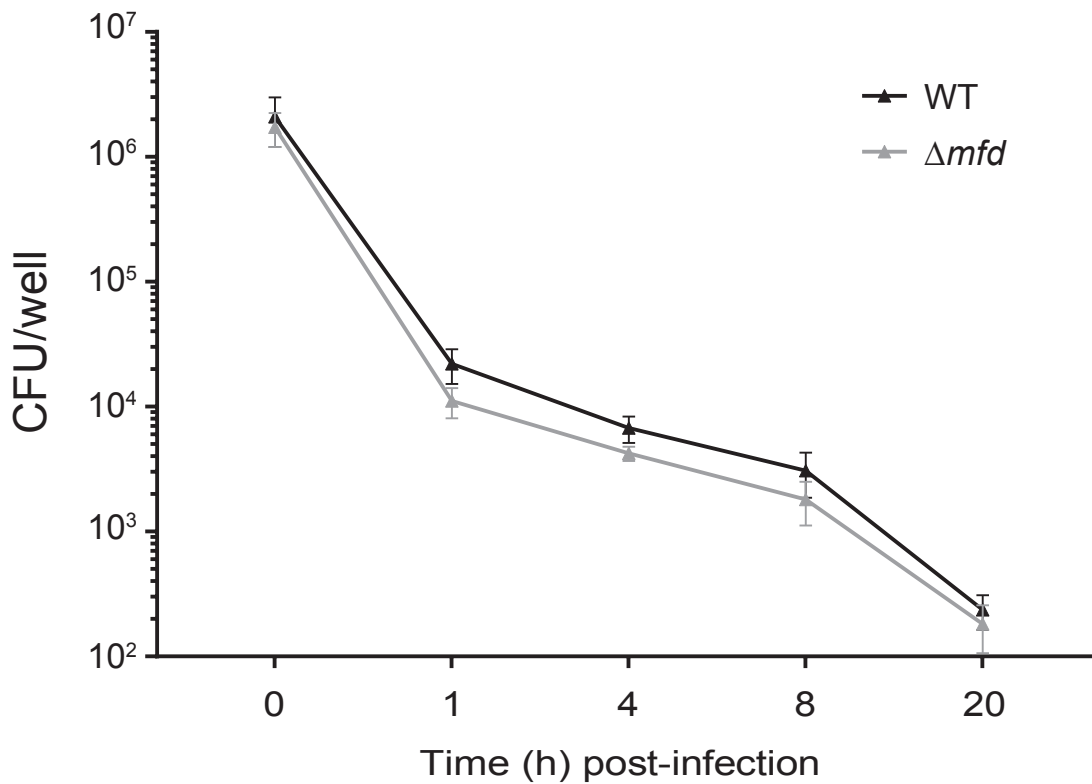


Figure S5. Strains lacking Mfd show no survival defects in bone marrow macrophages, Related to Figure 1

Murine-derived bone marrow macrophages (BMMs) were infected with WT and Δmfd strains of *S. typhimurium* ST19 and harvested for CFU enumeration at indicated times points. Data represents two independent experiments with triplicate samples for each given experiment. Error bars indicate s.e.m.