Appendix

Dual inhibition of the terminal oxidases eradicates antibiotic-tolerant *Mycobacterium tuberculosis*

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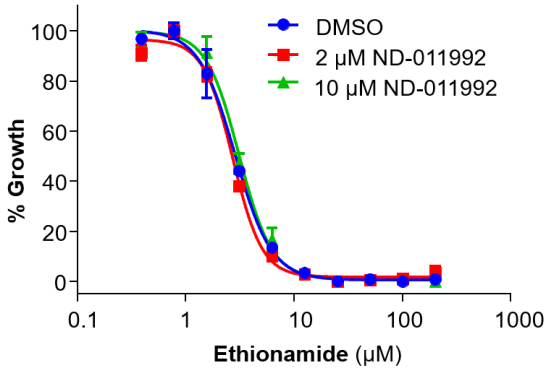
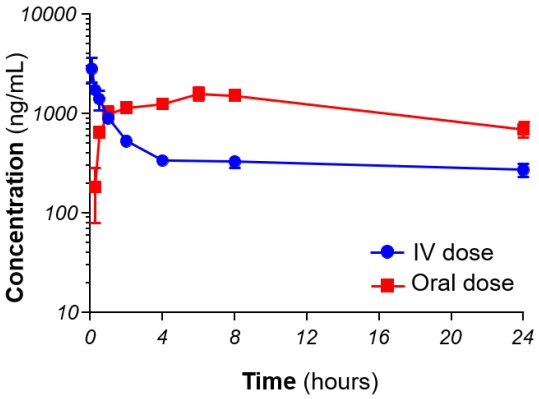
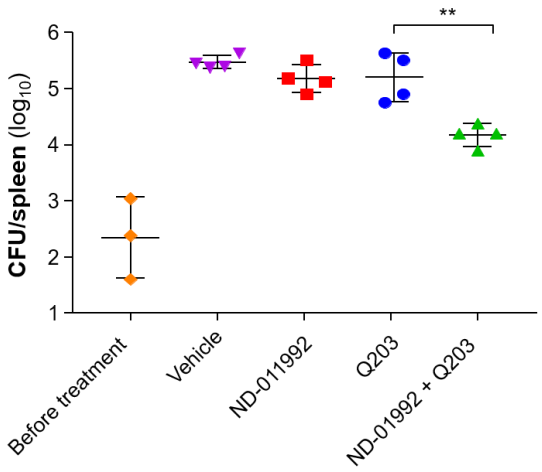


APPENDIX FIGURE S1. Effect of ND-011992 treatment in Ethionamide minimum inhibitory concentration (MIC). *M. tuberculosis* H37Rv cultures were treated with varying concentrations of ethionamide in the presence of DMSO (blue circles), 2 µM ND-011992 (red squares), or 10 µM ND-011992 (green triangles). Data are expressed as the mean ± S.D. of triplicates for each condition.



APPENDIX FIGURE S2. Plasma concentration of ND-011992 after intravenous and oral dosing in male Swiss albino mice. Plasma concentration of ND-011992 was quantified over a 24-hour period after an intravenous (IV) dose of 2 mg/kg (blue circles) or oral administration of 10 mg/kg (red squares).



APPENDIX FIGURE S3. The drug combination ND-011992/Q203 reduces bacterial load in the spleen of *M. tuberculosis*-infected mice. Efficacy of ND-011992-Q203 combination treatment in a mouse model of acute tuberculosis. Bacterial load (CFU) were enumerated in the spleen of mice before treatment (orange diamonds), or after 5 daily oral administration of vehicle control (purple triangles), 5 mg/kg Q203 (blue circles), 25 mg/kg ND-011992 (red squares), or 25 mg/kg ND-011992 + 5 mg/kg Q203 (green triangles). **P < 0.01, unpaired Student's t-test, two-tailed; n = 4.

APPENDIX TABLE S1. Cyt-*bd* over-expression reduced the potency of ND-011992. *M. bovis* BCG (transformed with empty pMV262 vector or pMV262-*cydABDC*) strains were treated with a dose-range of ND-011992, Q203, or BDQ for 15 hours before quantification of intracellular ATP levels.

	ATP IC_{50} (μM)		
	ND-011992	Q203	BDQ
pMV262	0.52 ± 0.014	0.0010 ± 0.000045	0.16 ± 0.0069
pMV262-*cydABDC*	17.2 ± 3.64	0.0012 ± 0.000206	0.19 ± 0.014

APPENDIX TABLE S2. Minimum Inhibitory Concentration 50% (MIC_{50}) of ND-011992 in *M. tuberculosis* H37Rv ΔctaE-qcrCAB.

MIC_{50} (μM)	Repeat 1		Repeat 2	
	ND-011992	BDQ	ND-011992	BDQ
WT H37Rv	25.2	1.02	25.5	0.50
ΔctaE-qcrCAB	0.76	0.56	0.23	0.33
ΔctaE-qcrCAB::comp.	3.54	0.63	20.0	0.65

APPENDIX TABLE S3. Frequency of spontaneous resistance to ND-011992 + Q203 in *M. tuberculosis* H37Rv and *M. bovis* BCG.

Drug concentration (μM)		**Frequency of Resistance**	
Q203	ND-011992	H37Rv	BCG
0.1	0	2.4×10^{-8}	1.0×10^{-8}
	6	2.1×10^{-9}	6.6×10^{-9}
	10	$< 2.3\times10^{-10}$	3.7×10^{-10}
0.5	0	N.D.	6.7×10^{-9}
	6	N.D.	7.3×10^{-10}
	10	N.D.	$< 3.7\times10^{-10}$

APPENDIX TABLE S4. *In vitro* pharmacokinetic and toxicity of ND-011992

Metabolic stability	Stability in mouse plasma (% remaining after 2h)			110.3% (Propantheline bromide: 12.1%)
	Stability in simulated gastric fluid (% remaining after 24h)			99.02% (omeprazole: 0.00%)
	Microsomal stability	Human	CL_{int}, μL min^{-1} mg^{-1}	15.7
			% remaining after 60 min	58.1% (Propafenone: 0.4%)
		Mouse	CL_{int}, μL min^{-1} mg^{-1}	<9.6
			% remaining after 60 min	87.6% (Propafenone: 0.2%)
Drug permeability	Caco2 assay (mean P_{app}, 10^{-6} cm s^{-1})			<0.48
Cytotoxicity	HepG2 (IC_{50})			>20 μM

APPENDIX TABLE S5. Pharmacokinetic properties of ND-011992 in mice after intravenous (IV) and oral (PO) administration

		IV	PO
Dose	mg/kg	2.00	10.00
C_{max}	ng/mL	3583.45	1601.80
T_{max}	H	-	6.00
V_d	L/kg	5.35	-
Cl	mL/min/kg	0.96	-
$T_{1/2}$	h	64.11	>18
$AUC_{0\text{-last}}$	ng· h/mL	9298.20	26954.80
$AUC_{0\text{-}\infty}$	ng· h/mL	34603.48	41694.93
AUC_{extra}	%	73.13	35.35
$MRT_{0\text{-last}}$	h	9.46	11.10
F	%	-	57.98