SUPPORTING INFORMATION

In vivo-selected pyrazinoic acid-resistant *M. tuberculosis* strains harbor missense mutations in the aspartate decarboxylase PanD and the unfoldase ClpC1

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CONTENTS:

Supplemental data:

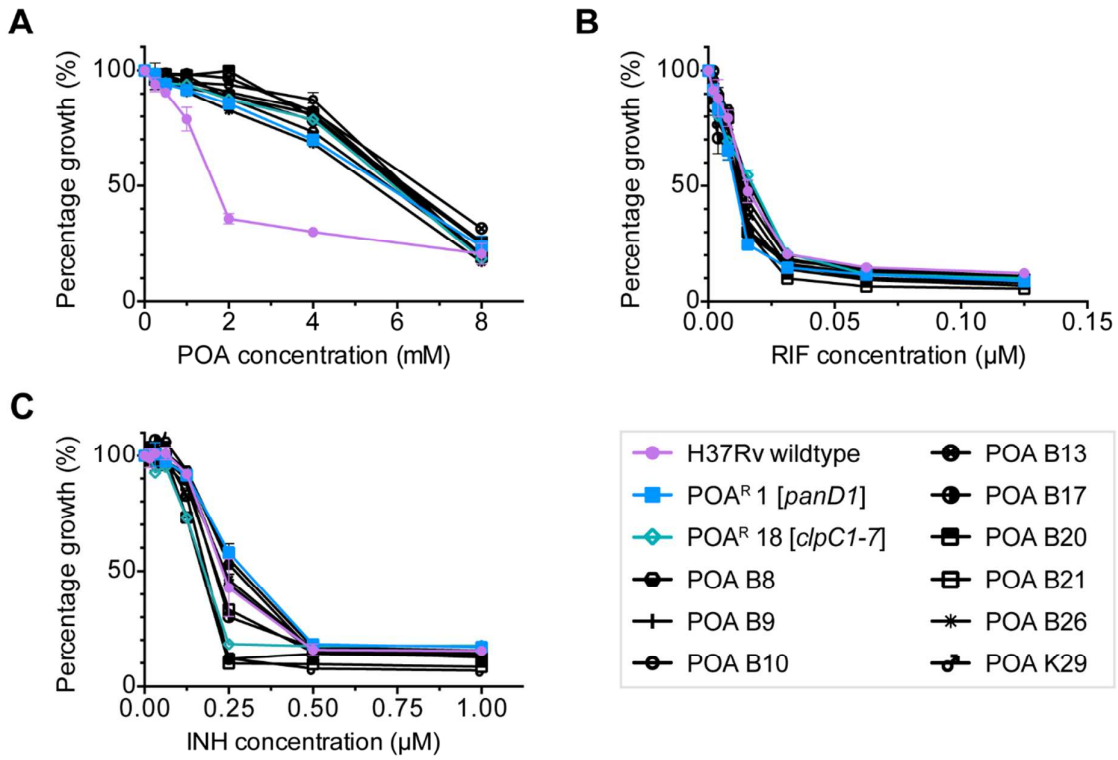

FIGURE S1 Growth inhibition dose-response curves for nine POA-resistant *M. tuberculosis* strains selected in mice, representative POA-resistant strains selected in vitro*:* POA^R 1 [*panD1*] described previously in (*1*) and POA^R 18 [*clpC1-7*] described previously in (*2*), and the wild-type parent *M. tuberculosis* H37Rv strain for (A) POA, (B) rifampicin, RIF, and (C) isoniazid, INH. Experiments were carried out 3 times independently with technical replicates. Mean values and standard deviations from representative experiments are shown.

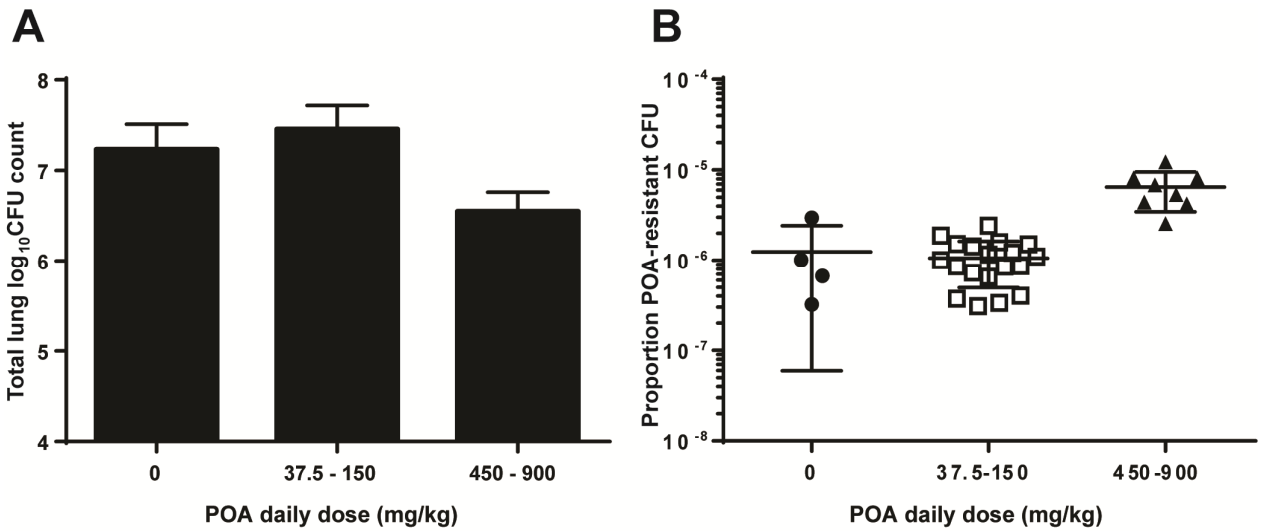

FIGURE S2 Selection of POA-resistant mutants in vivo as a function of POA dose. (A) Mean (±SD) total lung CFU counts and (B) proportion of total lung CFU that are POA-resistant in individual mice after 8 weeks of treatment with the indicated dose of POA. In panel B, horizontal lines represent the mean and SD.

TABLE S1 Abundance of acid-fast bacilli within lesions after infection with POA-resistant mutants versus wild-type *M. tuberculosis* H37Rv

	M. tuberculosis H37Rv strain			
	Wild-type	POA^R 1 [*panD1*]	POA^R 7 [*ppsC1*]	POA^R 18 [*clpC1-7*]
Lesion 1	24 clusters (5 - 20 bacilli per cluster)	37 clusters (5 - 20 bacilli per cluster)	0	8 single bacilli
Lesion 2	23 clusters (5 - 20 bacilli per cluster)	5 clusters (1 to 5 bacilli per cluster)	28 single bacilli	21 clusters (1 to 5 bacilli per cluster)
Lesion 3	22 clusters (5 - 20 bacilli per cluster)	21 clusters (5 - 20 bacilli per cluster)	5 clusters (1 to 6 bacilli per cluster)	15 single bacilli
Lesion 4	36 clusters (5 - 20 bacilli per cluster)	10 clusters (5 - 20 bacilli per cluster)	0	13 clusters (2 to 20 bacilli per cluster)
Lesion 5	28 clusters (5 - 20 bacilli per cluster)	20 clusters (5 - 20 bacilli per cluster)	45 single bacilli	8 clusters (2 to 5 bacilli per cluster)

TABLE S2 Phenotypic and genotypic characteristics of POA-resistant *M. tuberculosis* strains selected in mice.

M. tuberculosis Strain name	Drug, dose (mg/kg) and dosing frequency of treatment regimen	Frequency of POA (300 µg/mL) resistant CFU after treatment	Mutations in *panD*	POA		PZA	
				Broth MIC_{50}[a] (mM)	Agar MIC[b] (mM)	S/R[c]	Agar MIC[b] (mM)
JHU WT[d]	-	-	None	1.5	1	S	2
POA B1[e]	POA 450 twice daily Lung 1	0.0008163	G351A/ Met117Ile	6.0	>4	R	>4
POA B2[e]	POA 450 twice daily Lung 2	0.0004233	G382T/ Ala128Ser	5.5	>4	R	>4
POA B3[e]	POA 450 twice daily Lung 3	0.0006829	A349G/ Met117Val	6.0	>4	R	>4
POA B4[e]	POA 450 twice daily Lung 4	0.0008130	C61A/ His21Asn	5.5	>4	R	>4
POA B5[e]	POA 450 once daily Lung 1	0.0004412	C63A/ His21Gln	5.5	>4	R	>4
POA B7[e]	POA 450 once daily Lung 4	0.0012222	C383A/ Ala128Glu	6.0	>4	R	>4
POA B8[d, e]	POA 450 once daily Lung 5	0.0002564	None	5.5	>4	R	>4
POA B9[d, e]	POA 150 once daily Lung 1	0.0000308	A389G/ Glu130Gly	6.0	>4	R	>4
POA B10[d, e]	POA 150 once daily Lung 2	0.0000374	Δ 418T	6.0	>4	R	>4
POA B11[e]	POA 150 once daily Lung 3	0.0000652	T407C/ Leu136Pro	6.0	>4	R	>4
POA B12[e]	POA 150 once daily Lung 4	0.0001104	G346T/ Asp116Tyr	5.5	>4	R	>4
POA B13[d, e]	POA 150 once daily Lung 5	0.0000730	None	6.5	>4	R	>4
POA B14[e]	POA 75 twice daily Lung 1	0.0000336	G412A/ Val138Met	6.0	>4	R	>4
POA B15[e]	POA 75 twice daily Lung 2	0.0001429	T321A/ Phe107Leu	6.0	>4	R	>4
POA B16[e]	POA 75 twice daily Lung 3	0.0001165	T392C/ Leu131Pro	6.0	>4	R	>4
POA B17[d, e]	POA 75 twice daily Lung 4	0.0000403	G351T/ Met117Ile	6.0	>4	R	>4
POA B18[e]	POA 75 twice daily Lung 5	0.0001000	T407G/ Leu136Arg	6.0	>4	R	>4
POA B19[e]	POA 75 once daily Lung 1	0.0001212	G376T/ Glu126Stop	6.0	>4	R	>4

POA B20[d, e]	POA 75 once daily Lung 2	0.0000851	None	6.0	>4	R	>4
POA B21[d, e]	POA 75 once daily Lung 3	0.0001512	None	6.0	>4	R	>4
POA B22 [e]	POA 75 once daily Lung 5	0.0002418	T413C/ Val138Ala	5.5	>4	R	>4
POA B23 [e]	POA 37.5 once daily Lung 1	0.0001593	G382T/ Ala128Ser	6.0	>4	R	>4
POA B24 [e]	POA 37.5 once daily Lung 2	0.0000857	A343G/ Ile115Val	6.0	>4	R	>4
POA B25 [e]	POA 37.5 once daily Lung 3	0.0001515	G351C/ Met117Ile	6.0	>4	R	>4
POA B26[d, e]	POA 37.5 once daily Lung 4	0.0000859	T49C/ Cys17Arg	5.5	>4	R	>4
POA B27 [e]	POA 37.5 once daily Lung 5	0.0001882	T413C/ Val138Ala	6.0	>4	R	>4
POA B28 [e]	PZA 150 once daily Lung 3	0.0001709	C381A/ Asn127Lys	6.0	>4	R	>4
POA K29[d, f]	POA 450 twice daily Lung 9	Not available	None	6.0	>4	R	>4

[a] MIC_{50}, POA concentration that inhibits 50% of growth compared to drug free control. Drug susceptibility tests were carried out 3 times independently and mean values are shown.

[b] Maximum concentration of drug tested was 4 mM.

[c] BACTEC MGIT 960 test for susceptibility (S) or resistance (R) to 100 µg/mL PZA.

[d] Strains were subjected to whole genome sequencing. Polymorphisms detected in these strains are described in Table 1.

[e] Strains were isolated from BALB/c mice as described (*3*).

[f] Strain was isolated from a C3HeB/FeJ mouse as described (*3*).

1. Gopal, P., Yee, M., Sarathy, J., Low, J. L., Sarathy, J. P., Kaya, F., Dartois, V., Gengenbacher, M., and Dick, T. (2016) Pyrazinamide Resistance Is Caused by Two Distinct Mechanisms: Prevention of Coenzyme A Depletion and Loss of Virulence Factor Synthesis. *ACS Infect. Dis. 2*, 616-626. DOI: 10.1021/acsinfecdis.6b00070.
2. Yee, M., Gopal, P., and Dick, T. (2016) Missense mutations in the unfoldase ClpC1 of the caseinolytic protease complex are associated with pyrazinamide resistance in Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* DOI: 10.1128/aac.02342-16.
3. Lanoix, J.-P., Tasneen, R., O'Brien, P., Sarathy, J., Safi, H., Pinn, M., Alland, D., Dartois, V., and Nuermberger, E. (2016) High systemic exposure of pyrazinoic acid has limited anti-tuberculosis activity in murine and rabbit models of tuberculosis. *Antimicrob. Agents Chemother.* DOI: 10.1128/aac.03085-15.