Supporting Information

Marrero et al. 10.1073/pnas.1000715107

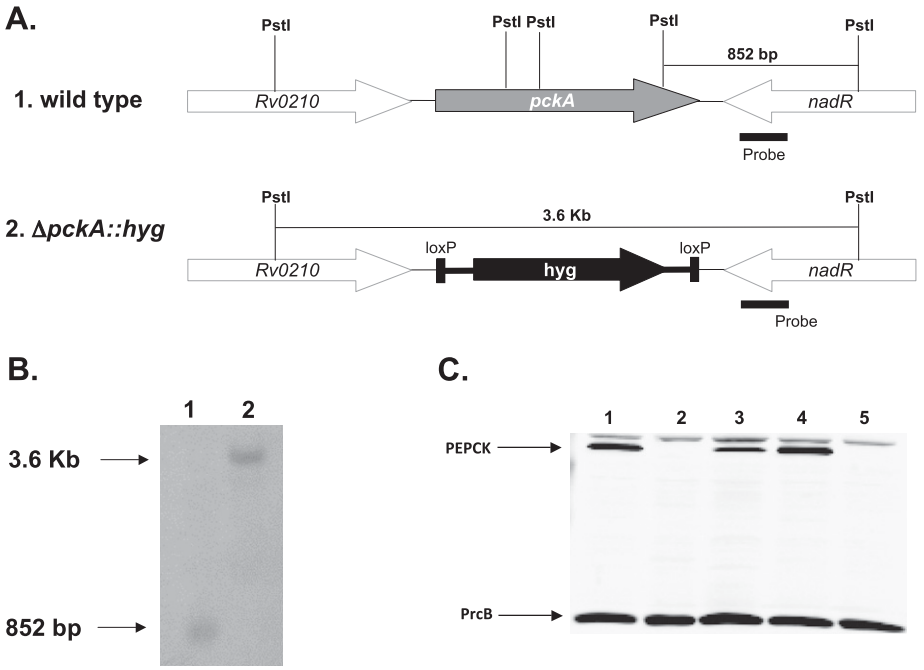

Fig. S1. Analysis of *pckA* knockout (Δ*pckA*) and complemented strains. (*A*) Genomic region of *pckA* in WT and Δ*pckA*. Location of PstI sites and hybridization site of probe used for Southern blot are depicted. (*B*) Southern blot of PstI digested chromosomal DNA from WT and Δ*pckA* probed with the DNA fragment indicated in *A*. Southern blot analysis was preformed using ECL Direct Nucleic Acid Labeling and Detection System (GE Healthcare), following the manufacturer's directions, except for the detection reagent, which was TMB Substrate Reagent Set (BD OptEIA). (*C*) Immunoblot of *Mtb* cell lysates from WT (lane 1), Δ*pckA* (lane 2), complemented mutant (lance 3), *pckA*-TetON grown with atc (lane 4), *pckA*-TetON grown without atc (lane 5). PEPCK (60 kDa), and PrcB (29 kDa) proteins were detected using anti-PEPCK rabbit serum and anti-PrcB rabbit serum (1). PrcB served as loading control. Immunoblots were developed using the Odyssey Infrared Imaging System (LI-COR Biosciences).

1. Gandotra S, Schnappinger D, Monteleone M, Hillen W, Ehrt S (2007) *In vivo* gene silencing identifies the *Mycobacterium tuberculosis* proteasome as essential for the bacteria to persist in mice. *Nat Med* 13:1515–1520.

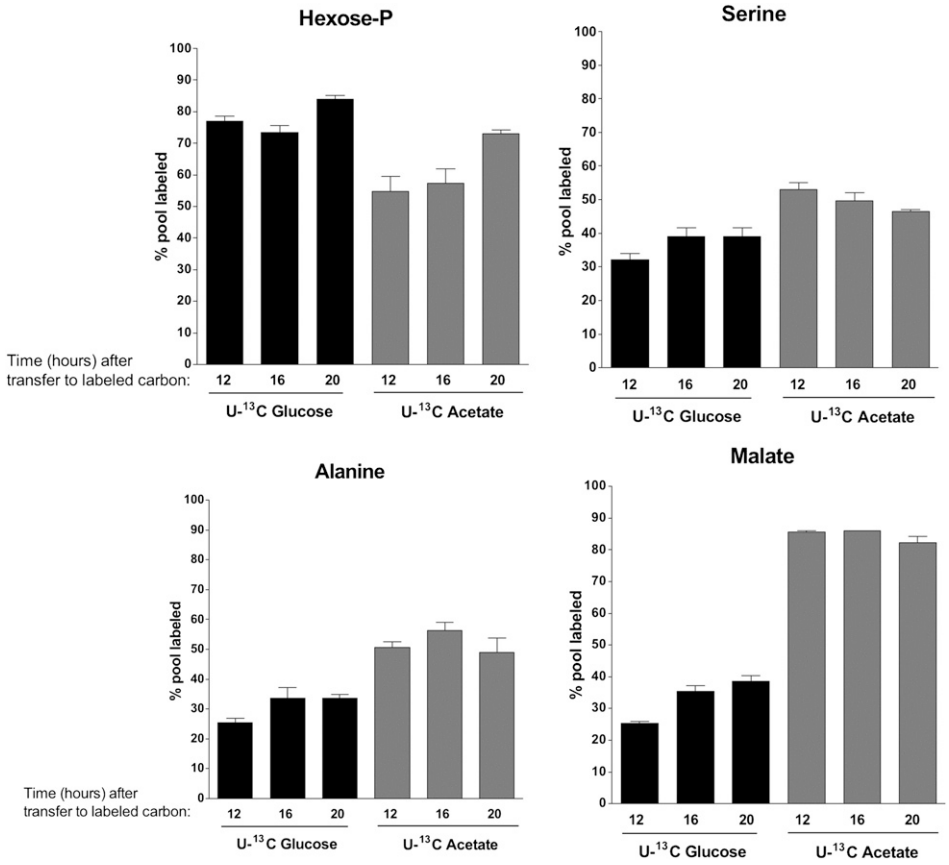

Fig. S2. Kinetics of ^{13}C incorporation into intracellular metabolite pools. *Mtb* was seeded on 0.22-μM nitrocellulose filters and grown on 7H10 agar plates containing 10% OADC supplement and 0.5% glycerol for 5 days. Filters were then transferred to 7H10 plates containing 0.5% BSA, 0.085% NaCl, and 0.2% U-^{13}C acetate or 0.2% U-^{13}C glucose. Incorporation of U-^{13}C acetate or U-^{13}Cglucose–derived ^{13}C into the intracellular pool of selected metabolites after 12, 16, or 20 h incubation is shown. Each bar represents the mean of three sample replicates; error bars indicate SD.

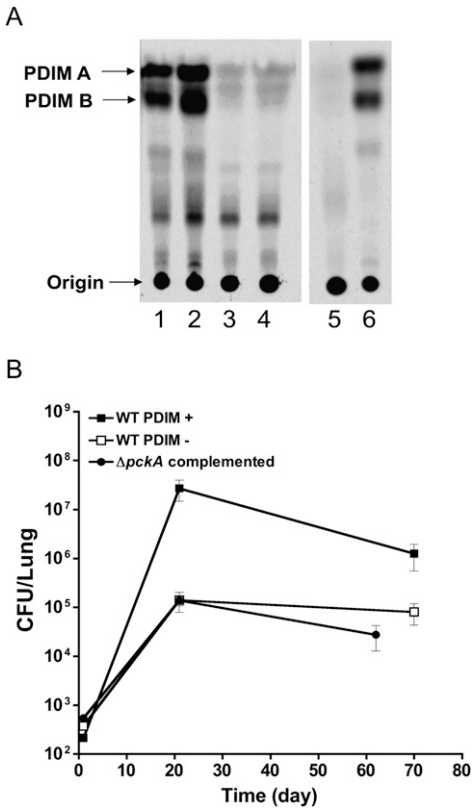

Fig. S3. Loss of PDIM in *Mtb* Erdman causes a 2.5 $\log_{10}$ attenuation in mouse lungs. (*A*) PDIM analysis of WT *Mtb* (lane 1) Δ*pckA* (lane 2), complemented mutant (lane 3), and *pckA*-TetON (lane 4). Lanes 5 and 6 show lipids from two colony isolates from WT transformed with the empty plasmid pTC-MCS (1). *Mtb* lipid profile analysis using [1-^{14}C] propionic acid was performed as described elsewhere (2), and TLC plates were visualized using a Typhoon Trio+ Variable Mode Imager (Amersham Biosciences). (*B*) Bacterial loads of PDIM+ WT, PDIM− WT, and complemented Δ*pckA* in mouse lungs. Each data point represents the mean of data from four mice; error bar indicates SD.

1. Klotzsche M, Ehrt S, Schnappinger D (2009) Improved tetracycline repressors for gene silencing in mycobacteria. *Nucleic Acids Res* 37:1778–1788.
2. Domenech P, Reed MB (2009) Rapid and spontaneous loss of phthiocerol dimycocerosate (PDIM) from *Mycobacterium tuberculosis* grown in vitro: Implications for virulence studies. *Microbiology* 155:3532–3543.

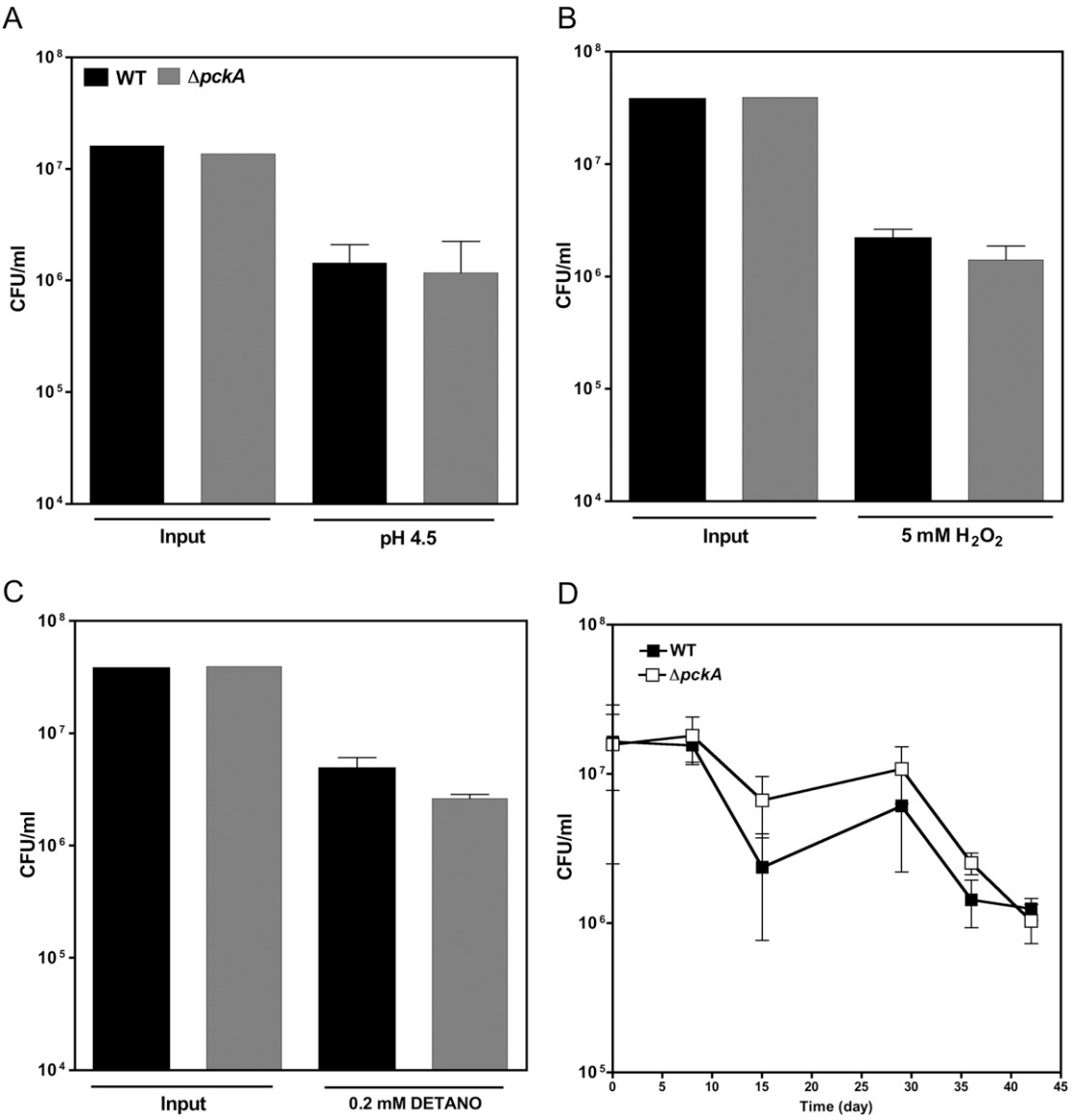

Fig. S4. Δ*pckA* is not significantly more sensitive than WT to low pH, oxidative, and nitrosative stress or carbon starvation. WT (black bars) and Δ*pckA* (gray bars) were exposed to (*A*) pH 4.5 for 6 days in complete 7H9 medium, (*B*) 5 mM H_2O_2 for 4 h in complete 7H9 medium, or (*C*) 0.2 mM DETANO for 3 days in complete 7H9 medium. (*D*) Log phase grown WT and mutant cultures were subcultured into PBS + 0.05% Tyloxapol to a final OD of 0.02. CFU were determined at the indicated time points by plating serial dilutions on 7H10 plates. Each data point represents the mean of three culture replicates; error bars indicate SD. Data are representative of two independent experiments.

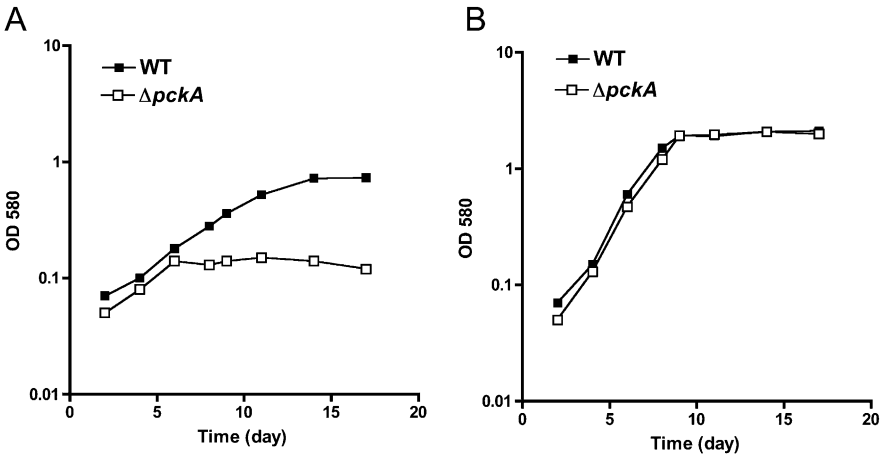

Fig. S5. Propionate does not inhibit growth of Δ*pckA* in the presence of glycerol. Growth of WT (■) and Δ*pckA* (□) in carbon-defined media containing (*A*) 0.1% propionate or (*B*) 0.1% propionate and 0.1% glycerol.

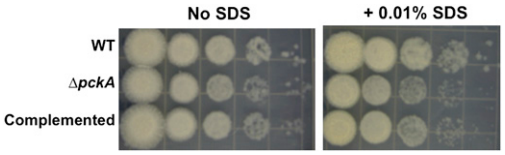

Fig. S6. Δ*pckA* is not significantly more sensitive than WT to detergent. Serial dilutions of WT, Δ*pckA*, and the complemented mutant were spotted on 7H10 plates containing either no SDS or 0.01% SDS. Plates were incubated at 37 °C for 2 weeks.

Table S1. LC-MS masses, natural abundance, and retention time measurements of metabolites detected in unlabeled *Mtb* cell lysate

	Metabolite	Calculated *m/z*	Observed *m/z*	Natural abundance M+1(%)	Retention time (min)
[M-H] ions					
	Hexose-P	259.0224	259.0238	7.9	3.6
	PEP	166.9751	166.9765	0.1	2.2
	Pyruvate	87.0088	87.0098	2.9	1.0
	Malate	133.0142	133.0150	4.1	1.8
[M+H] ions					
	Serine	106.0499	106.0502	4.0	9.4
	Alanine	90.0550	90.0560	3.8	10.5
	Aspartate	134.0448	134.0450	6.6	7.8