

## Supplementary Materials for

# The PDIM paradox of *Mycobacterium tuberculosis*: new solutions to a persistent problem

Claire V. Mulholland, Thomas J. Wiggins†, Jinhua Cui†, Catherine Vilchèze, Saranathan Rajagopalan,  
Michael W. Shultis, Esmeralda Z. Reyes-Fernández, William R. Jacobs Jr., Michael Berney\*

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA.

†These authors contributed equally to this work

\*Correspondence to: [michael.berney@einsteinmed.edu](mailto:michael.berney@einsteinmed.edu)

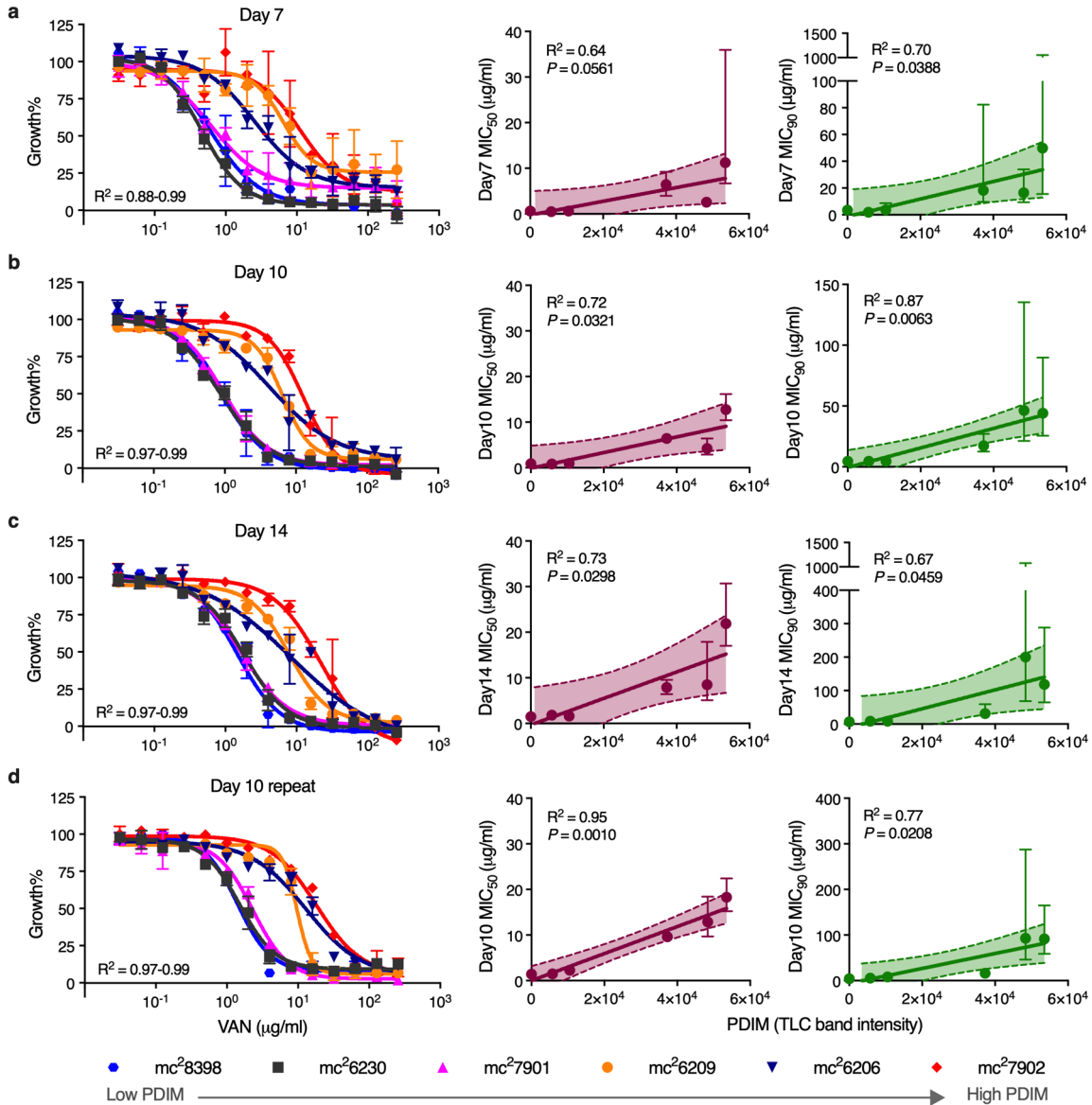
### This PDF file includes:

#### Supplementary Figures 1–6

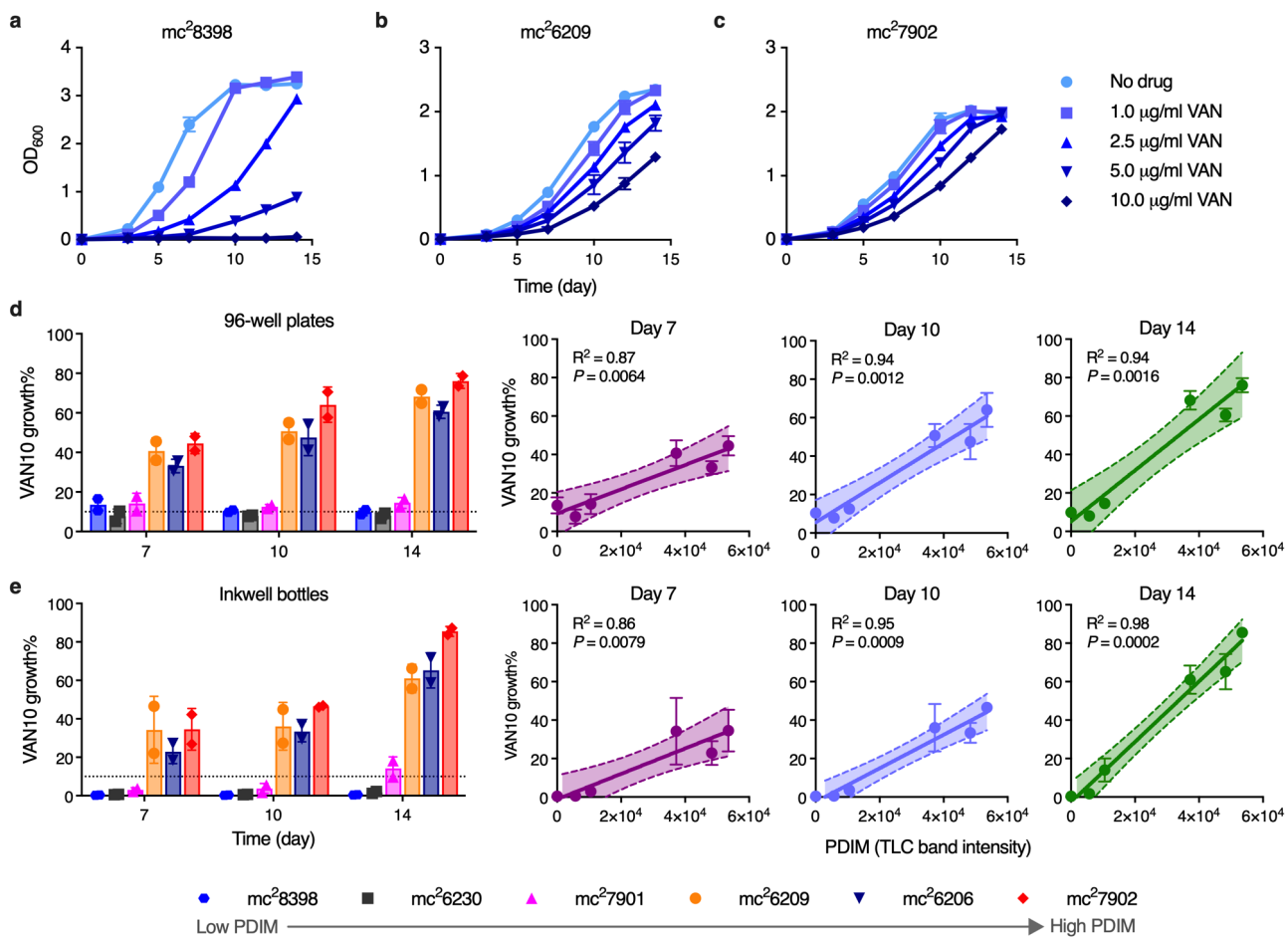
- Supplementary Fig. 1. Vancomycin resistance is predictive of PDIM production in *Mtb*.
- Supplementary Fig. 2. VAN10 assay development.
- Supplementary Fig. 3. VAN-P MIC assay validation in *Mtb* CDC1551.
- Supplementary Fig. 4. Effect of leucine supplementation on vancomycin sensitivity and growth of PDIM(+) and PDIM(-) *Mtb*
- Supplementary Fig. 5. Effect of propionate supplementation on the sensitivity of PDIM(+) and PDIM(-) *Mtb* to compounds with different modes of action and molecular weights
- Supplementary Fig. 6. Propionate increases the resistance of *Mtb* CDC1551 to rifampicin, but not isoniazid, in a PDIM-dependent manner.

#### Supplementary Tables 1–8

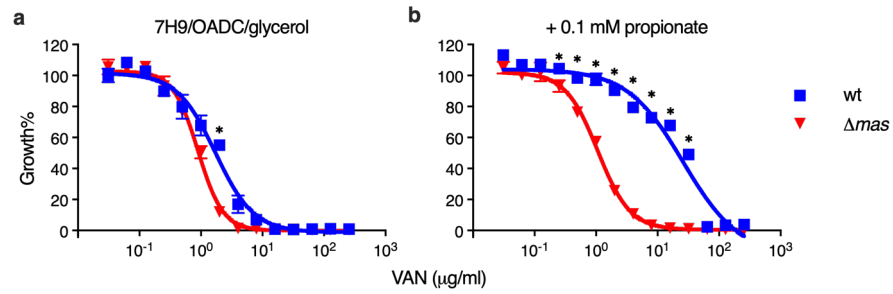
- Supplementary Table 1. BSL2-approved attenuated *Mtb* strains included in this study.
- Supplementary Table 2. Virulent *Mtb* strains included in this study.
- Supplementary Table 3. Culture media reagents and supplements used in this study.
- Supplementary Table 4. Primers used in this study.
- Supplementary Table 5. Inhibitors used in this study.
- Supplementary Table 6. Chemical standards used for LC-MS.
- Supplementary Table 7. Putative metabolites used for LC-MS data normalization.
- Supplementary Table 8. Whole genome sequencing accession numbers.



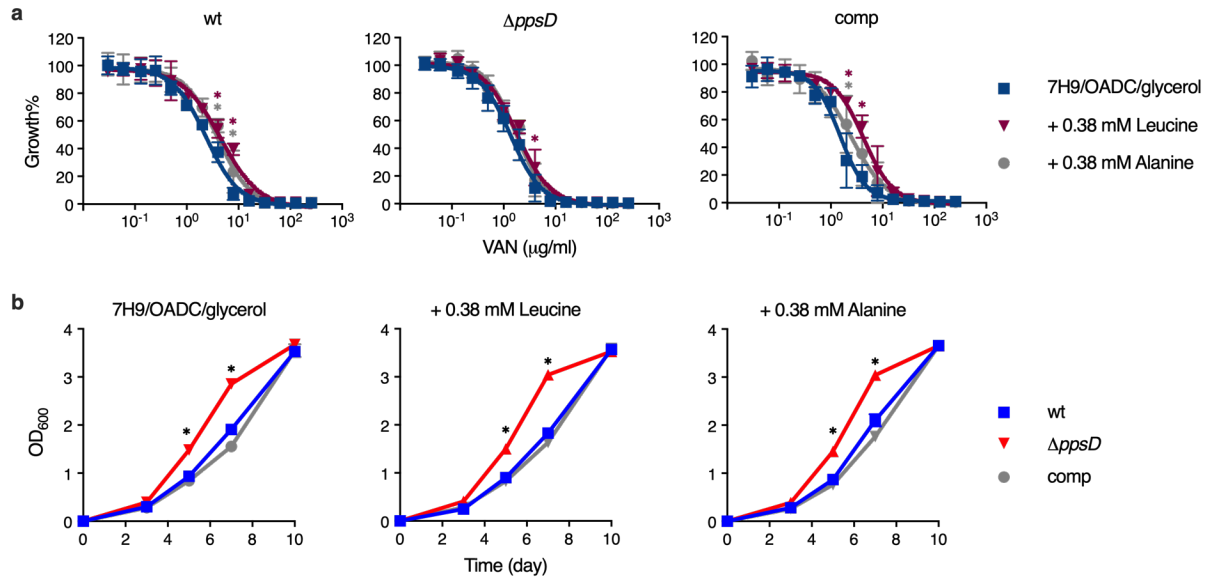
**Supplementary Fig. 1 | Vancomycin resistance is predictive of PDIM production in *Mtb*.** **a–c**, Vancomycin resistance of six BSL2-approved attenuated *Mtb* H37Rv strains (Supplementary Table 1) and correlation with PDIM lipid levels determined by TLC (Fig. 1a). These strains comprise our PDIM reference strain set. Minimum inhibitory concentration (MIC) assays were performed using the broth microdilution method in 7H9/OADC/glycerol/tyloxapol media with pantothenate, arginine, leucine, and methionine (PALM) supplements. Bacterial growth was measured after (a) 7, (b) 10, and (c) 14 days of incubation, and normalized to drug-free control wells. Simple linear regression was performed to assess the correlation between vancomycin MIC<sub>50</sub> (maroon) and MIC<sub>90</sub> (green) with PDIM levels as determined by TLC densitometric analysis of Fig. 1a. **d**, Experimental repeat measured after 10 days of incubation. MIC plots show mean  $\pm$  SD for  $n = 2$  technical replicates from a single experiment. Bands on regression plots show the 95% CI and error bars show the 95% CI for MIC values calculated from the curve fit.



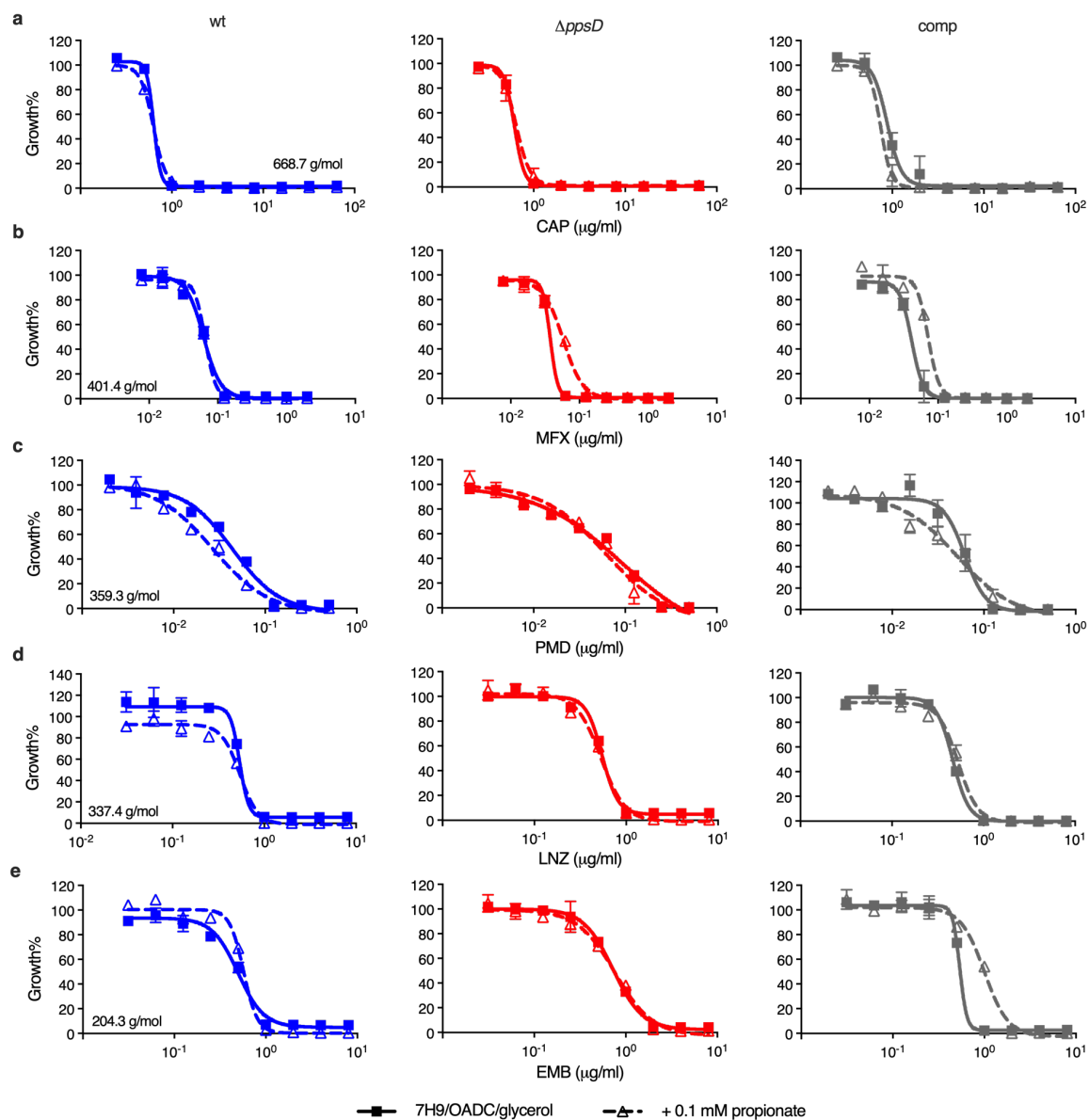
**Supplementary Fig. 2 | VAN10 assay development.** **a–c**, Growth curves of (a) *Mtb mc*<sup>2</sup>8398 [PDIM(-)], (b) *mc*<sup>2</sup>6209 [PDIM(+)], and (c) *mc*<sup>2</sup>7902 [PDIM(+)] (see Fig. 1a) in standard 7H9/OADC/glycerol/tyloxapol + PALM media with increasing concentrations of vancomycin. Mean  $\pm$  SD for  $n = 3$  biological replicates. For some data points the SD is smaller than the data symbols. This experiment was performed once. **d,e**, ‘VAN10 assay’ showing relative growth of the PDIM reference strain set in standard 7H9/OADC/glycerol/tyloxapol + PALM media with 10  $\mu$ g/ml vancomycin compared to drug-free controls in (d) 96-well plates and (e) inkwell bottles. Bacterial growth was assessed by measuring optical density after 7, 10, and 14 days of incubation as indicated.  $\text{VAN10 OD} / \text{VAN0 OD} \times 100 = \text{VAN10 growth\%}$ . Simple linear regression was performed to assess the correlation between VAN10 growth% at each time point and PDIM lipid levels as determined by TLC densitometric analysis of Fig. 1a. Mean  $\pm$  SD for  $n = 2$  independent experiments, each performed in triplicate. Bands on regression plots show the 95% CI.



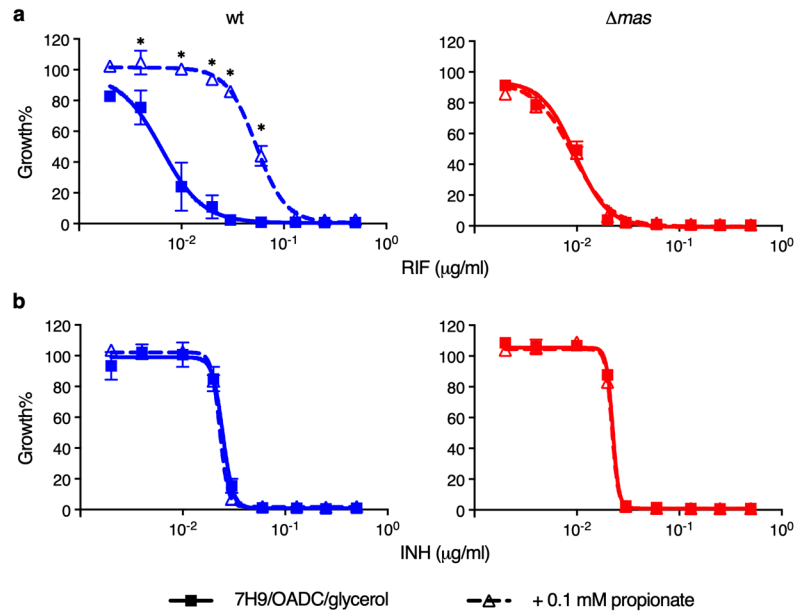
**Supplementary Fig. 3 | VAN-P MIC assay validation in *Mtb* CDC1551.** Vancomycin MIC assays of isogenic PDIM(+) (wildtype) and PDIM(-) ( $\Delta mas$ ) CDC1551 in **a**, standard 7H9/OADC/glycerol/tyloxapol media and **b**, supplemented with 0.1 mM propionate. Mean  $\pm$  SD for  $n = 2$  technical replicates from a single experiment. This experiment was performed once.  $P < 0.001$ ; two-way ANOVA with Šidák's multiple comparison test. For some data points the SD is smaller than the data symbols.



**Supplementary Fig. 4 | Effect of leucine supplementation on vancomycin sensitivity and growth of PDIM(+) and PDIM(-) *Mtb*.** **a**, Vancomycin MIC assays of isogenic PDIM(+) and PDIM(-) *Mtb* H37Rv in standard 7H9/OADC/glycerol/tyloxapol media and supplemented with either 0.38 mM L-leucine (50 mg/l, same concentration provided in PALM supplemented media) or 0.38 mM L-alanine as a non-branched-chain amino acid control. Mean  $\pm$  SD for  $n = 4$  biological replicates from two independent experiments.  $P < 0.001$  compared to unsupplemented media; two-way ANOVA with Tukey's multiple comparison test. **b**, Growth of PDIM(+) and PDIM(-) H37Rv in standard 7H9/OADC/glycerol/tyloxapol media and supplemented with either L-leucine or L-alanine. Mean  $\pm$  SD for  $n = 3$  biological replicates.  $*P < 0.001$  for both wt and comp versus  $\Delta ppsD$ ; two-way ANOVA with Tukey's multiple comparison test. This experiment was repeated once with similar results. For some data points the SD is smaller than the data symbols.



**Supplementary Fig. 5 | Effect of propionate supplementation on the sensitivity of PDIM(+) and PDIM(-) *Mtb* to compounds with different modes of action and molecular weights.** MIC assays of isogenic PDIM(+) and PDIM(-) *Mtb* H37Rv in 7H9/OADC/glycerol/tyloxapol media  $\pm$  0.1 mM propionate for **a**, capreomycin (CAP); **b**, moxifloxacin (MFX); **c**, pretomanid (PMD); **d**, linezolid (LNZ); and **e**, ethambutol (EMB). The molecular weight of each compound is shown on the corresponding MIC plot. Mean  $\pm$  SD for  $n = 2$  technical duplicates from a single experiment. This experiment was performed once. For some data points the SD is smaller than the data symbols.



**Supplementary Fig. 6 | Propionate increases the resistance of *Mtb* CDC1551 to rifampicin, but not isoniazid, in a PDIM-dependent manner.** Sensitivity of isogenic PDIM(+) and PDIM(-) CDC1551 in 7H9/OADC/glycerol/tyloxapol media  $\pm$  0.1 mM propionate to **a**, rifampicin (RIF), and **b**, isoniazid (INH). Mean  $\pm$  SD for  $n = 2$  technical replicates from a single experiment. This experiment was performed once. \* $P < 0.001$ ; two-way ANOVA with Šidák's multiple comparison test. For some data points the SD is smaller than the data symbols.

**Supplementary Table 1 | BSL2-approved attenuated *Mtb* strains included in this study.** These strains comprise our PDIM reference strain set, with the exception of mc<sup>2</sup>6230 AE1601 and AE1611, which are PDIM(+) and PDIM(-) clones isolated from our mc<sup>2</sup>6230 stock.

Strain name	Genotype	Reference
mc <sup>2</sup> 6206	H37Rv $\Delta$ panCD $\Delta$ leuCD	Jain <i>et al.</i> (2014) <sup>28</sup>
mc <sup>2</sup> 6209	H37Rv $\Delta$ panCD $\Delta$ leuCD $\Delta$ secA2	Jain <i>et al.</i> (2014) <sup>28</sup>
mc <sup>2</sup> 6230	H37Rv $\Delta$ RD1 $\Delta$ panCD	Sambandamurthy <i>et al.</i> (2006) <sup>65</sup> ; Jain <i>et al.</i> (2012) <sup>66</sup>
mc <sup>2</sup> 7901	H37Rv $\Delta$ panCD $\Delta$ leuCD $\Delta$ metA	Vilchèze <i>et al.</i> (2018) <sup>67</sup>
mc <sup>2</sup> 7902	H37Rv $\Delta$ panCD $\Delta$ leuCD $\Delta$ argB	Vilchèze <i>et al.</i> (2018) <sup>67</sup>
mc <sup>2</sup> 8398	H37Rv $\Delta$ metA $\Delta$ argB	This study
mc <sup>2</sup> 6230 AE1601*	H37Rv $\Delta$ RD1 $\Delta$ panCD [PDIM(+)]	This study
mc <sup>2</sup> 6230 AE1611†	H37Rv $\Delta$ RD1 $\Delta$ panCD [PDIM(-)]	This study

\*PDIM(+) and †PDIM(-) clones identified by VAN10-P screening of single colonies (see Extended Data Fig. 4).

**Supplementary Table 2 | Virulent *Mtb* strains included in this study.** Single PDIM(+) clones isolated in this study have been assigned unique 'AE' reference numbers as indicated.

Strain name	Genotype	Source/Construction
H37Rv-A	H37Rv	In-house culture collection
H37Rv-B	H37Rv	C57BL/6 mouse passage of H37Rv-A
H37Rv-SC (AE1028)*	H37Rv	PDIM(+) clone isolated from H37Rv-B
H37Rv $\Delta$ ppsD	H37Rv $\Delta$ Rv2934 hyg <sup>R</sup>	Specialized transduction of H37Rv-SC
H37Rv $\Delta$ ppsD::comp	H37Rv $\Delta$ Rv2934::comp hyg <sup>R</sup> kan <sup>R</sup>	Transformation of H37Rv $\Delta$ ppsD with pMV361-ppsD
H37Rv $\Delta$ tgs1-1	H37Rv $\Delta$ Rv3130c hyg <sup>R</sup>	Specialized transduction of H37Rv-B
H37Rv $\Delta$ tgs1-2	H37Rv $\Delta$ Rv3130c hyg <sup>R</sup>	Specialized transduction of H37Rv-B
H37Rv $\Delta$ tgs1-3	H37Rv $\Delta$ Rv3130c hyg <sup>R</sup>	Specialized transduction of H37Rv-B
H37Rv $\Delta$ tgs1-4	H37Rv $\Delta$ Rv3130c hyg <sup>R</sup>	Specialized transduction of H37Rv-B
H37Rv $\Delta$ tgs1-5	H37Rv $\Delta$ Rv3130c hyg <sup>R</sup>	Specialized transduction of H37Rv-B
H37Rv $\Delta$ tgs1-7	H37Rv $\Delta$ Rv3130c hyg <sup>R</sup>	Specialized transduction of H37Rv-B
H37Rv $\Delta$ tgs1-8	H37Rv $\Delta$ Rv3130c hyg <sup>R</sup>	Specialized transduction of H37Rv-B
H37Rv $\Delta$ tgs1-9	H37Rv $\Delta$ Rv3130c hyg <sup>R</sup>	Specialized transduction of H37Rv-B
CDC1551-A	CDC1551	In-house culture collection
CDC1551-B	CDC1551	In-house culture collection
CDC1551 $\Delta$ mas	CDC1551 $\Delta$ Rv2940c hyg <sup>R</sup>	Specialized transduction of CDC1551-B
Erdman	Erdman	In-house culture collection
HN878	HN878	In-house culture collection
KZN 4207	KZN 4207	In-house culture collection
CDC1551-SC (AE5005)*	CDC1551	PDIM(+) clone isolated from a Rag <sup>-/-</sup> mouse
Erdman-SC (AE3003)*	Erdman	PDIM(+) clone isolated from a Rag <sup>-/-</sup> mouse
HN878-SC (AE8005)*	HN878	PDIM(+) clone isolated from a Rag <sup>-/-</sup> mouse

\*PDIM(+) clone identified by VAN10-P screening of single colonies (see Extended Data Fig. 4).



**Supplementary Table 3 | Culture media reagents and supplements used in this study.**

<b>Name</b>	<b>Catalog #</b>	<b>Supplier</b>
Middlebrook 7H9	271310	BD Difco
Middlebrook 7H10	262710	BD Difco
Glycerol	G33	Fisher Chemical
Dextrose (D-glucose)	D16	Fisher Chemical
Tyloxapol	T8761	Sigma-Aldrich
Tween 80	P1754	Sigma-Aldrich
Sodium oleate	11-1280	Strem Chemicals
Bovine serum albumin fraction V	A-420-1	GoldBio
Catalase	C1345	Sigma-Aldrich
D-Calcium pantothenate	243305000	Acros Organics
L-Arginine	A5006	Sigma-Aldrich
L-Leucine	L8000	Sigma-Aldrich
L-Methionine	166165000	Acros Organics
Hygromycin B	10687010	Invitrogen
Kanamycin	BP906	Fisher BioReagents
Sodium propionate	P1880	Sigma-Aldrich
Vitamin B <sub>12</sub>	V2876	Sigma-Aldrich
Sodium pyruvate	BP356	Fisher BioReagents
Sodium acetate	A13184.30	Thermo Scientific Chemicals
Sodium butyrate	A11079.06	Thermo Scientific Chemicals
Valeric acid	149570050	Acros Organics
Cholesterol	C3045	Sigma-Aldrich

**Supplementary Table 4 | Primers used in this study.**

<b>Primer name</b>	<b>Sequence</b>
<b>ppsC homopolymeric tract region</b>	
ppsC_homopoly_F	CTGCTGACCCACTCGATCA
ppsC_homopoly_R	TGTCGGGCCTGTGGTAAG
<b>Knockout strain confirmation</b>	
Universal_uptag	GATGTCTCACTGAGGTCTCT
Rv2940c_LL	TCATGGGCATCACGTTAC
Rv2940c_LR	AGAACTCAGCATCGAAACC
Rv2934_LL	CATGCAGGCGGTACTCAC
Rv2934_LR	GGATCGGGATCGTAGAAC
Rv3130c_LL	CACAATGGCTTTCATTCCG
Rv3130c_LL	GGGTACAGGGACGTAGGC
<b>pMV361-ppsD vector construction and confirmation</b>	
pMV361_ppsD_Hsp60_for	GATCCAGCTGCAGAATTCATGACAAGTCTGGCGGAGCG
pMV361_ppsD_rev	CTACGTCGACATCGATAAGCTTCTTATCCTCCCTGACTTCAGGTTTAGGT
pMV361_vector_for	GAATTCTGCAGCTGGATCCCG
complement_vector_rev	GAAGCTTATCGATGTCGACGTAG
pMV361-LL	GGAATCACTTCGCAATGGC
pMV361-LR	ATCGTACGCTAGTTAACTACG
ppsD_sequencing_2	CGCCAGCGCTCGCAAC
ppsD_sequencing_3	CGGTGCTGGCCGACTGGATG
ppsD_sequencing_4	GGCATAAAAATCGGCCGGCG
ppsD_sequencing_5	TAATTCGCGGCGGGGACAGC
ppsD_sequencing_6	CGAAGCGGCGGAAGAGAATCC
ppsD_sequencing_7	TCTGGGGGCCAATTGCGCGG

**Supplementary Table 5 | Inhibitors used in this study.**

<b>Name</b>	<b>Abbreviation</b>	<b>Catalog #</b>	<b>Supplier</b>
Azithromycin dihydrate	AZM	A2076	TCI America
Bedaquiline	BDQ	Gift	Kevin Pethe, Nanyang Technological University, Singapore
Capreomycin sulfate	CAP	J66684	Alfa Aesar
Ethambutol dihydrochloride	EMB	J60695	Alfa Aesar
Erythromycin	ERY	E0751	TCI America
Isoniazid	INH	I3377	Sigma
Linezolid	LNZ	460592500	Acros Organics
Moxifloxacin hydrochloride	MFX	457960010	Acros Organics
Pretomanid	PMD	HY-10844	MedChemExpress
Ramoplanin	RAM	sc-253424	Santa Cruz Biotechnology
Rifampicin	RIF	R3501	Sigma
Teicoplanin (A2)	TEC	RS048	BIOTANG Inc.
Vancomycin hydrochloride	VAN	J62790	Alfa Aesar

**Supplementary Table 6 | Chemical standards used for LC-MS.**

<b>Name</b>	<b>Catalog #</b>	<b>Supplier</b>
n-Propionyl coenzyme A lithium salt	P5397	Sigma-Aldrich
Methylmalonyl coenzyme A tetralithium salt	sc-215385	ChemCruz
Succinic acid	33272	Alfa Aesar
Sodium pyruvate	BP356	Fisher BioReagents

**Supplementary Table 7 | Putative metabolites used for LC-MS data normalization.**

<b>Compound</b>	<b>m/z</b>	<b>Pathway</b>
Glycine	74.0248	Amino acids and amino acid metabolism
Pyruvic acid	87.0088	Central carbon
L-Alanine	88.0404	Amino acids and amino acid metabolism
L-Serine	104.0353	Amino acids and amino acid metabolism
Uracil	111.0200	Nucleotide metabolism
L-Proline	114.0561	Amino acids and amino acid metabolism
L-Valine	116.0717	Amino acids and amino acid metabolism
Succinic acid	117.0193	Central carbon
L-Threonine	118.0510	Amino acids and amino acid metabolism
Nicotinic acid	122.0248	Nicotinate and nicotinamide metabolism
L-Leucine	130.0874	Amino acids and amino acid metabolism
L-Isoleucine	130.0874	Amino acids and amino acid metabolism
L-Aspartic Acid	132.0302	Amino acids and amino acid metabolism
L-Malic acid	133.0142	Central carbon
Adenine	134.0472	Nucleotide metabolism
Salicylic acid	137.0244	Other
4-Hydroxybenzoic acid	137.0244	Folate biosynthesis
alpha-Ketoglutaric acid	145.0142	Central carbon
L-Glutamine	145.0619	Amino acids and amino acid metabolism
L-Lysine	145.0983	Amino acids and amino acid metabolism
L-Glutamic acid	146.0459	Amino acids and amino acid metabolism
L-Methionine	148.0438	Amino acids and amino acid metabolism
Guanine	150.0421	Nucleotide metabolism
L-Histidine	154.0622	Amino acids and amino acid metabolism
Orotic acid	155.0098	Nucleotide metabolism
L-Phenylalanine	164.0717	Amino acids and amino acid metabolism
DL-Glyceraldehyde 3-phosphate	168.9907	Central carbon
Dihydroxyacetone phosphate	168.9907	Central carbon
D-Glycerol 3-phosphate	171.0064	Central carbon
Shikimic acid	173.0455	Amino acids and amino acid metabolism
N2-Acetyl-L-ornithine	173.0932	Amino acids and amino acid metabolism
L-Arginine	173.1044	Amino acids and amino acid metabolism
a-D-Glucose	179.0561	Central carbon
L-Tyrosine	180.0666	Amino acids and amino acid metabolism
L-Tryptophan	203.0826	Amino acids and amino acid metabolism
Thymidine	241.0830	Nucleotide metabolism
D-Fructose 6-P/a-D-Glucose 6-P	259.0224	Central carbon
Adenosine	266.0895	Nucleotide metabolism
Inosine	267.0735	Nucleotide metabolism
dTMP	321.0493	Nucleotide metabolism
cAMP	328.0452	Nucleotide metabolism
Trehalose/Maltose	341.1089	Central carbon
5'-AMP	346.0558	Nucleotide metabolism
dTDP	401.0157	Nucleotide metabolism
Trehalose 6-phosphate	421.0753	Central carbon
ADP	426.0221	Nucleotide metabolism
ATP	505.9885	Nucleotide metabolism
Acetyl-CoA	808.1185	Central carbon
Propionyl-CoA	822.1341	Central carbon
Methylmalonyl-CoA	866.1240	Central carbon

**Supplementary Table 8 | Whole genome sequencing accession numbers. BioProject PRJNA923717.**

Strain	BioSample accession	SRA accession number/s		
mc <sup>2</sup> 6206	SAMN32734011	SRR23080350		
mc <sup>2</sup> 6209	SAMN32734012	SRR23080349		
mc <sup>2</sup> 6230	SAMN32734013	SRR23080327	SRR23080338*	SRR23080318†
mc <sup>2</sup> 6230 AE1601	SAMN35027541	SRR24495990†		
mc <sup>2</sup> 6230 AE1611	SAMN35027542	SRR24495989		
mc <sup>2</sup> 7901	SAMN32734014	SRR23080317		
mc <sup>2</sup> 7902	SAMN32734015	SRR23080316		
mc <sup>2</sup> 8398	SAMN32734016	SRR23080315		
H37Rv-A	SAMN32734017	SRR23080314		
H37Rv-B	SAMN32734018	SRR23080313		
H37Rv-SC (AE1028)	SAMN32734019	SRR23080348		
H37Rv $\Delta$ <i>ppsD</i>	SAMN32734020	SRR23080347		
H37Rv $\Delta$ <i>tgs1-1</i>	SAMN32734021	SRR23080346		
H37Rv $\Delta$ <i>tgs1-2</i>	SAMN32734022	SRR23080345		
H37Rv $\Delta$ <i>tgs1-3</i>	SAMN32734023	SRR23080344		
H37Rv $\Delta$ <i>tgs1-4</i>	SAMN32734024	SRR23080343		
H37Rv $\Delta$ <i>tgs1-5</i>	SAMN32734025	SRR23080342	SRR23080341*	
H37Rv $\Delta$ <i>tgs1-7</i>	SAMN32734026	SRR23080340	SRR23080339*	
H37Rv $\Delta$ <i>tgs1-8</i>	SAMN32734027	SRR23080337		
H37Rv $\Delta$ <i>tgs1-9</i>	SAMN32734028	SRR23080336		
CDC1551-A	SAMN32734029	SRR23080335		
CDC1551-B	SAMN32734030	SRR23080334		
CDC1551 $\Delta$ <i>mas</i>	SAMN32734031	SRR23080333*		
Erdman	SAMN32734032	SRR23080332		
HN878	SAMN32734033	SRR23080331		
KZN 4207	SAMN32734034	SRR23080330		
CDC1551-SC (AE5005)	SAMN32734035	SRR23080329		
Erdman-SC (AE3003)	SAMN32734036	SRR23080328		
HN878-SC (AE8005)	SAMN32734037	SRR23080326		
H37Rv-B col5	SAMN32734038	SRR23080325		
H37Rv-B col6	SAMN32734039	SRR23080324		
H37Rv-B col16	SAMN32734040	SRR23080323		
H37Rv-B col17	SAMN32734041	SRR23080322		
H37Rv-B col19	SAMN32734042	SRR23080321		
H37Rv-B col23	SAMN32734043	SRR23080320		
H37Rv-B col36	SAMN32734044	SRR23080319		

\*Data acquired using Illumina MiSeq with a 300-cycle kit (76 bp reads). †Data acquired on the Illumina NextSeq system (151 bp reads). All remaining data was acquired using Illumina MiSeq with a 600-cycle kit (301 bp reads).