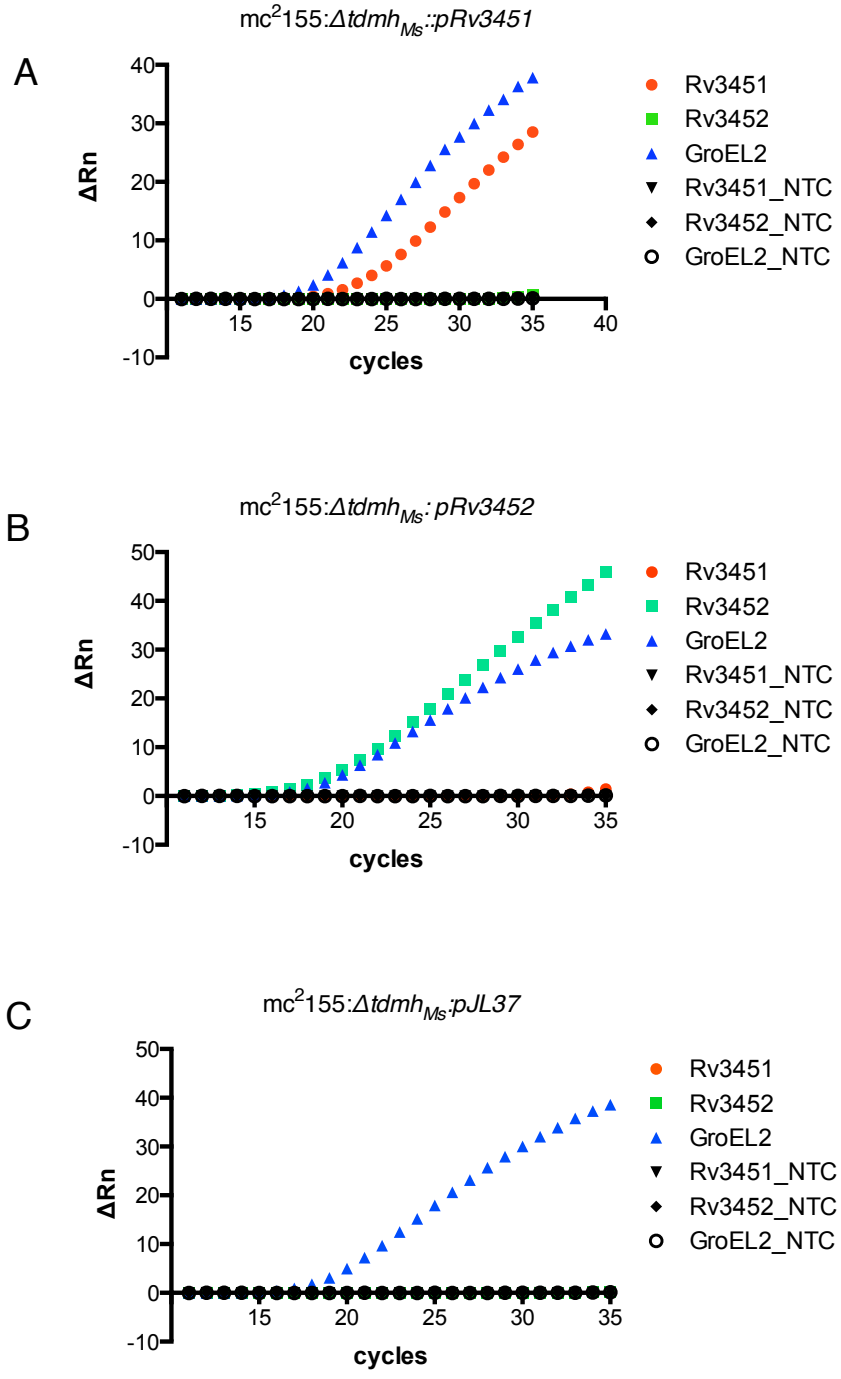

A
mc²155:Δtdmh_Ms::pRv3451
ΔRn
cycles
Rv3451
Rv3452
GroEL2
Rv3451_NTC
Rv3452_NTC
GroEL2_NTC
B
mc²155:Δtdmh_Ms:pRv3452
ΔRn
cycles
Rv3451
Rv3452
GroEL2
Rv3451_NTC
Rv3452_NTC
GroEL2_NTC
C
mc²155:Δtdmh_Ms:pJL37
ΔRn
cycles
Rv3451
Rv3452
GroEL2
Rv3451_NTC
Rv3452_NTC
GroEL2_NTC

Figure S1

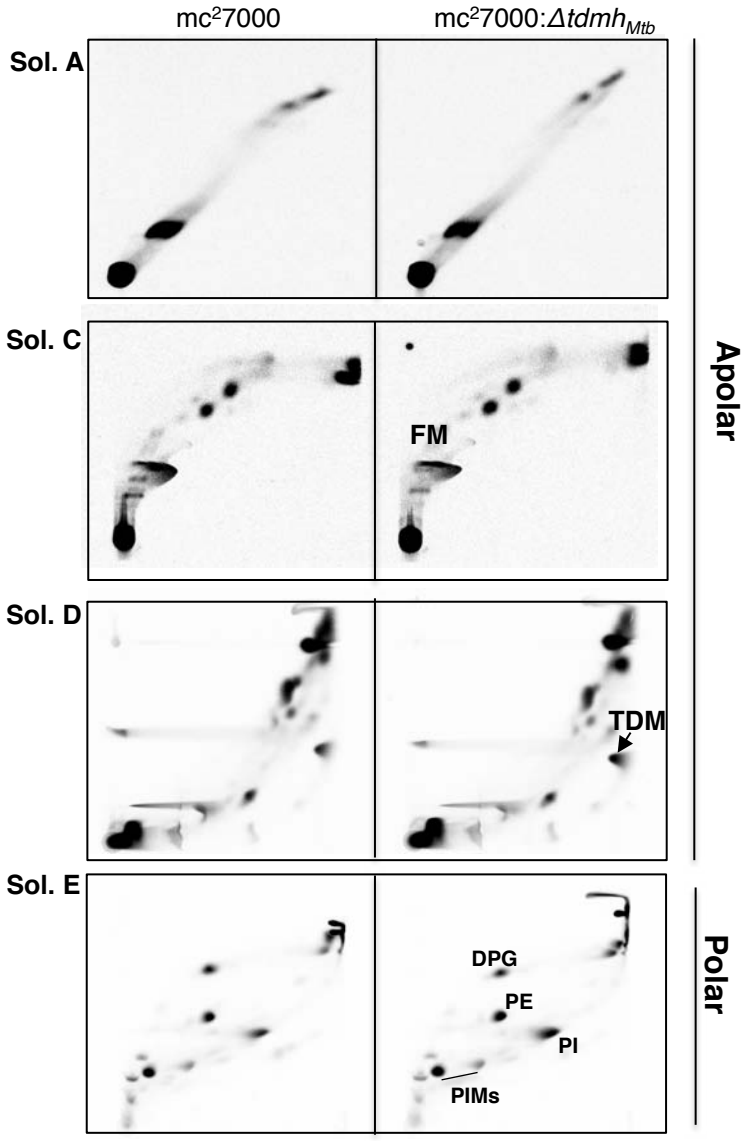

mc²7000
mc²7000:Δtdmh_Mtb
Sol. A
Sol. C
FM
Sol. D
TDM
Sol. E
DPG
PE
PI
PIMs
Apolar
Polar

Figure S2

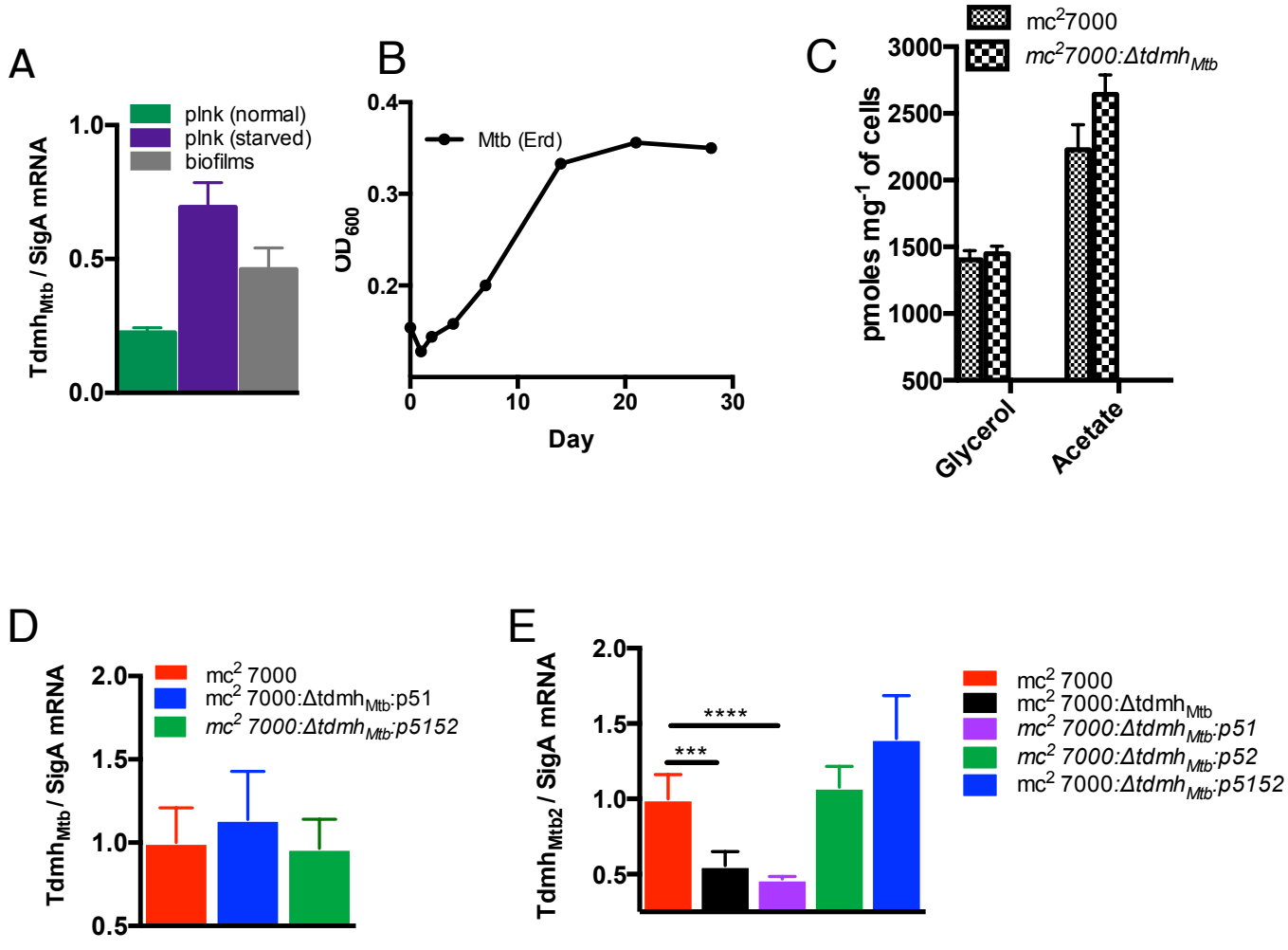

A
plnk (normal)
plnk (starved)
biofilms
Tdmh$_{Mtb}$ / SigA mRNA
1.0
0.5
0.0
B
Mtb (Erd)
OD$_{600}$
0.4
0.3
0.2
0
10
20
30
Day
C
mc^{2}7000
mc^{2}7000:Δtdmh$_{Mtb}$
pmoles mg^{-1} of cells
3000
2500
2000
1500
1000
500
Glycerol
Acetate
D
mc^2 7000
mc^2 7000:Δtdmh$_{Mtb}$:p51
mc^2 7000:Δtdmh$_{Mtb}$:p5152
Tdmh$_{Mtb}$ / SigA mRNA
2.0
1.5
1.0
0.5
E
****
***
Tdmh$_{Mtb2}$ / SigA mRNA
2.0
1.5
1.0
0.5
mc^2 7000
mc^2 7000:Δtdmh$_{Mtb}$
mc^2 7000:Δtdmh$_{Mtb}$:p51
mc^2 7000:Δtdmh$_{Mtb}$:p52
mc^2 7000:Δtdmh$_{Mtb}$:p5152

Figure S3

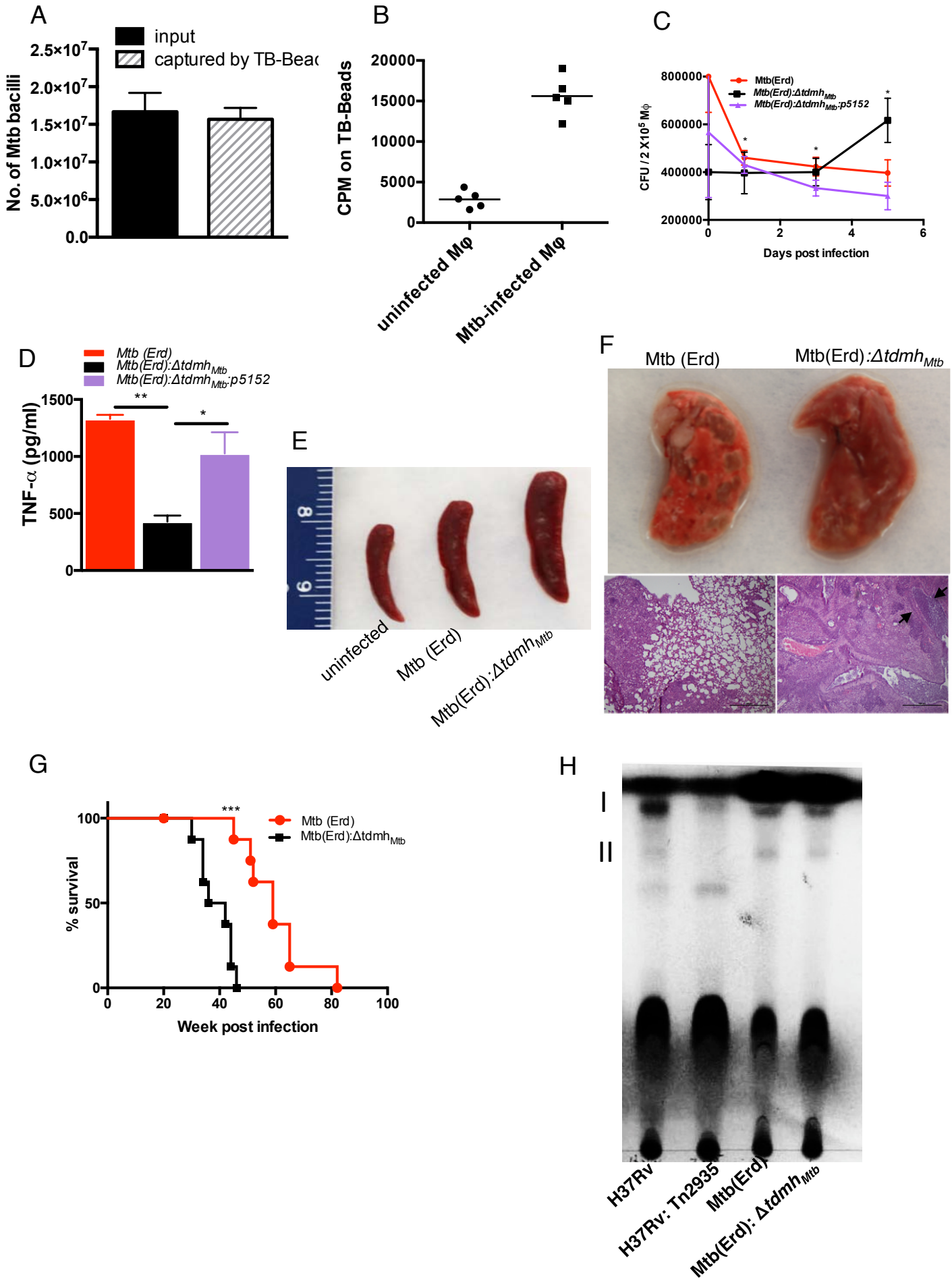
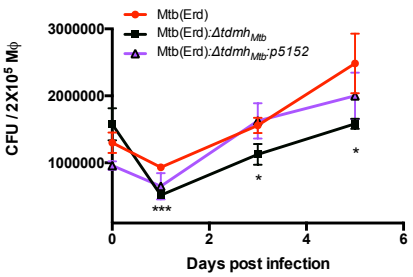
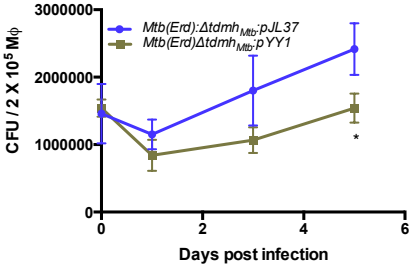

A
input
captured by TB-Bead
No. of Mtb bacilli
2.5×10^7
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
B
CPM on TB-Beads
20000
15000
10000
5000
0
uninfected Mφ
Mtb-infected Mφ
C
Mtb(Erd)
Mtb(Erd):Δtdmh_Mtb
Mtb(Erd):Δtdmh_Mtb:p5152
CFU / 2 X10^5 Mφ
800000
600000
400000
200000
0
2
4
6
Days post infection
D
Mtb (Erd)
Mtb(Erd):Δtdmh_Mtb
Mtb(Erd):Δtdmh_Mtb:p5152
TNF-α (pg/ml)
1500
1000
500
0
**
*
E
uninfected
Mtb (Erd)
Mtb(Erd):Δtdmh_Mtb
F
Mtb (Erd)
Mtb(Erd):Δtdmh_Mtb
G
% survival
100
50
0
***
Mtb (Erd)
Mtb(Erd):Δtdmh_Mtb
0
20
40
60
80
100
Week post infection
H
I
II
H37Rv
H37Rv: Tn2935
Mtb(Erd)
Mtb(Erd): Δtdmh_Mtb

Figure S4

A



B



C

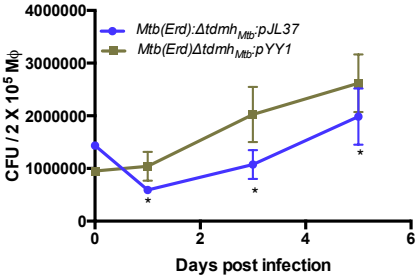


Figure S5

Supplemental figure legends:

Figure S1 (related to figure 1). Real time PCR analysis to determine the expression of Mttdmh (Rv3451) and Mttdmh2 (Rv3452) from extrachromosomal plasmids pRV3451 (A), and pRv3452 (B), respectively, in mc^{2}155:*Δmstdmh*. Panel C shows expression of the two genes in an isogenic strain carrying empty vector, pJL37. GroEL2 was used as endogenous control. Each of the gene expression analysis also included a corresponding no template control (NTC).

Figure S2 (related to figure 2). Two dimensional radio-TLC of polar and apolar lipids from 4-week biofilms of mc^{2}7000 and mc^{2}7000:*Δmttdmh* in solvent systems A, C, D and E as described previously (Ojha et al., 2010). The position of TDM and FM were determined by the mobility of pure lipids, simultaneously run on separate plate in the same solvent system (data not shown). DPG, PIM, PE and PI indicate diphosphatidylglycerol, phosphatidylinositol mannoside, phosphatidylethanolamine and phosphatidylinositol, respectively. The positions are marked as per the published reference (Besra, 1998).

Figure S3 (related to figure 3). **(A)** Relative abundance of Rv3451 RNA with respect to SigA, an unresponsive gene under starvation (Betts et al., 2002), in planktonic (plnk) cells either in normal media (7H9OADC) or pre-exposed for 96 hours in limiting nutrient media (7H9 base without supplement), as well as biofilm cells of mc^{2}7000 (mean ± SE, n=3). **(B)** Influx of glycerol and acetate over two

hours in planktonic cells of mc^{2}7000 and mc^{2}7000:*Δmttdmh* cultured in nutrient-rich (7H9OADC) media. **(C)** Influx of glycerol over 6 hours in 4-week biofilms of mc^{2}7000, mc^{2}7000:*Δmttdmh* and mc^{2}7000:*Δmttdmh:p5152.*

Figure S4 (related to figure 5). **(A)** Quantitative estimation of Mtb (mc^{2}7000) bacilli that could be captured by TB-beads from a lysate 10^7 macrophages (RAW264.7) infected at an MOI of 10. Input denotes bacteria in the unprocessed lysates. **(B)** A comparative analysis of radioactivity associate with TB beads when mixed with the lysates of either uninfected or Mtb (mc^{2}7000)-infected macrophages**.** Before counting the radioactivity TB-Beads were washed with excess of cold PBS at least three times. **(C)** The colony forming units (CFU) of various strains of Mtb in primary activated BMMs from C57BL/6 mice for specified time period. Percentage survival of a particular strain for a specified time is shown in Fig 5B, and was determined from the corresponding 0-day value, set as 100%. **(D)**. Release of TNF-α by primary resting C57BL/6 BMM infected with Mtb (Erd), Mtb (Erd):*Δtdmh*$_{Mtb}$ or Mtb (Erd):*Δtdmh*$_{Mtb}$*:p5152* after 48 hours of infection. **(E)** Increased splenomegaly in infection with Mtb(Erd):*Δtdmh*$_{Mtb}$ mutant as compared to the parent wild-type after 14 weeks of infection. **(F)** H&E stain of lung tissue sections from mice after 14 weeks of infection with either Mtb (Erd) or Mtb (Erd):*Δtdmh*$_{Mtb}$ (scale bar, 500μm). The arrows indicate epithelial linings of a conducting airway congested with host cells. **(G)** Survival plot of C57BL/6 mice infected with either Mtb(Erd) or

Mtb(Erd):*Δtdmh*$_{Mtb}$ (n= 8). **(H)** Levels of Phthiocerol dimycoserosate (I) and Phthiodiolone dimycocerosate (II) in H37Rv, Mtb(Erd) and Mtb(Erd):*Δmttdmh.* A H37Rv:Tn2937, with transposon insertion in a PDIM biosynthetic gene, ppsE, was used as a negative control. Lipids were extracted in petroleum ether and developed in petroleum ether:diethyl ether (9:1).

Figure S5 (related to figure 6). (A-C) The colony forming units (CFU) of various strains of Mtb in primary activated BMMs from either MyD88-/- (A and C), or C57BL/6 BMMs (B). Percentage survival of a particular strain for a specified time in A- C, are shown in Fig 6A, 6C and 6D, respectively, and was determined using the corresponding 0-day value, set as 100%.

Table S1: List of plasmids used in this study

Name	Remarks	References
pJL37	*P_{hsp60}-based expression vector, kan^r*	*(van Kessel and Hatfull, 2007)*
pMH94	*L5-attp-based integrative vector, kan^r*	*(Lee et al., 1991)*
pLAM12	*Acetamide-inducible expression vector, kan^r*	*(Payne et al., 2009)*
pRv3451	*pJL37 + Rv3451@NdeI & XhoI*	*This study*
pRv3452	*pJL37 + Rv3452 @NdeI & XhoI*	*This study*
pRv0646c	*pJL37 + Rv0646c @NdeI & XhoI*	*This study*
pRv1984	*pJL37 + Rv1984 @NdeI & XhoI*	*This study*
pRv2301	*pJL37 + Rv2301 @NdeI & XhoI*	*This study*
pRv3724	*pJL37 + Rv3724 @NdeI & XhoI*	*This study*
pRv1758	*pJL37 + Rv1758 @NdeI & XhoI*	*This study*
pYY1	*pJL37 + $tdmh_{Ms}$ @NdeI & XhoI*	*This study*
pAO10	*pLAM12 + $tdmh_{Ms}$ @NdeI & EcoRI*	*This study*
p51	*pMH94 + Rv3451+470bp ups @SacI and XbaI*	*This study*
p52	*pMH94 + Rv3452 +503bp ups @SacI and XbaI*	*This study*
p5152	*pMH94 + Rv3451-52 + 470bp ups @ SacI and XbaI*	*This study*

Table S2: List of mycobacterial strains used in this study

Name	Remarks	References
mc^{2}7000	*M. tuberculosis H37Rv:ΔRD1:ΔpanCD*	*(Ojha et al., 2008)*
mc^{2}7000:Δ$tdmh_{Mtb}$	*Isogenic deletion of $tdmh_{Mtb}$ in mc^{2}7000, hyg^r*	*This study*
mc^{2}7000:Δ$tdmh_{Mtb}$:p51	*mc^{2}7000:Δ$tdmh_{Mtb}$ with p51 @ L5 attB site, hyg^r, kan^r*	*This study*
mc^{2}7000:Δ$tdmh_{Mtb}$: p52	*mc^{2}7000:Δ$tdmh_{Mtb}$ with p52 @ L5 attB site, hyg^r, kan^r*	*This study*
mc^{2}7000:Δ$tdmh_{Mtb}$: p5152	*mc^{2}7000: Δmttdmh with p5152 @ attB site, hyg^r, kan^r*	*This study*
mc^{2}7000: pLAM12	*mc^{2}7000 harboring pLAM12, kan^r*	*This study*
mc^{2}7000: pAO10	*mc^{2}7000 harboring pAO10, kan^r*	*This study*
mc^{2}7000: Δ$tdmh_{Mtb}$: pLAM12	*mc^{2}7000: Δ$tdmh_{Mtb}$ harboring pLAM12, hyg^r, kan^r*	*This study*
mc^{2}7000: Δ$tdmh_{Mtb}$: pAO10	*mc^{2}7000: Δ$tdmh_{Mtb}$ harboring pAO10, hyg^r, kan^r*	*This study*
mc^{2}7000: Δ$tdmh_{Mtb}$: pJL37	*mc^{2}7000: Δ$tdmh_{Mtb}$ harboring pJL37, hyg^r, kan^r*	*This study*
mc^{2}7000: Δ$tdmh_{Mtb}$: pYY1	*mc^{2}7000: Δ$tdmh_{Mtb}$ harboring pYY1, hyg^r, kan^r*	*This study*
Mtb(Erd)	*M. tuberculosis Erdman, ATCC 35801*	*(Miyoshi-Akiyama et al., 2012)*
Mtb(Erd):Δ$tdmh_{Mtb}$	*Isogenic deletion of $tdmh_{Mtb}$ in Mtb(Erd), hyg^r*	*This study*
Mtb(Erd): Δ$tdmh_{Mtb}$: p5152	*Mtb(Erd): Δ$tdmh_{Mtb}$ with p5152 @ attB site, hyg^r, kan^r*	*This study*
Mtb(Erd): Δ$tdmh_{Mtb}$: pLAM12	*Mtb(Erd): Δ$tdmh_{Mtb}$* harboring pLAM12, *hyg^r kan^r*	*This study*
Mtb(Erd): Δ$tdmh_{Mtb}$: pAO10	*Mtb(Erd): Δ$tdmh_{Mtb}$* harboring pAO10, *hyg^r kan^r*	*This study*
Mtb(Erd): Δ$tdmh_{Mtb}$: pJL37	*Mtb(Erd): Δ$tdmh_{Mtb}$* harboring pJL37, *hyg^r kan^r*	*This study*
Mtb(Erd): Δ$tdmh_{Mtb}$: pYY1	*Mtb(Erd): Δ$tdmh_{Mtb}$* harboring pYY1, *hyg^r, kan^r*	*This study*
mc^{2}155	*High-frequency transformation strain of M. smegmatis*	*(Snapper et al., 1990)*
mc^{2}155: Δ$tdmh_{Mtb}$	*Isogenic deletion of $tdmh_{Mtb}$ in mc^{2}155, hyg^r*	*(Ojha et al., 2010)*
mc^{2}155: Δ$tdmh_{Mtb}$: pJL37	*mc^{2}155: Δ$tdmh_{Mtb}$ harboring pJL37, hyg^r kan^r*	*This study*
mc^{2}155: Δ$tdmh_{Mtb}$: pRv3451	*mc^{2}155: Δ$tdmh_{Mtb}$ harboring pRv3451, hyg^r kan^r*	*This study*
mc^{2}155: Δ$tdmh_{Mtb}$: pRv3452	*mc^{2}155: Δ$tdmh_{Mtb}$ pRv3452, hyg^r kan^r*	*This study*
mc^{2}155: Δ$tdmh_{Mtb}$: pRv0646c	*mc^{2}155: Δ$tdmh_{Mtb}$ harboring pRv0646c, hyg^r kan^r*	*This study*
mc^{2}155: Δ$tdmh_{Mtb}$: pRv1984	*mc^{2}155: Δ$tdmh_{Mtb}$ harboring pRv1984, hyg^r kan^r*	*This study*
mc^{2}155: Δ$tdmh_{Mtb}$: pRv2301	*mc^{2}155: Δ$tdmh_{Mtb}$ harboring pRv2301, hyg^r kan^r*	*This study*
mc^{2}155: Δ$tdmh_{Mtb}$: pRv3724	*mc^{2}155: Δ$tdmh_{Mtb}$ harboring pRv3724, hyg^r kan^r*	*This study*
mc^{2}155: Δ$tdmh_{Mtb}$: pRv1758	*mc^{2}155: Δ$tdmh_{Mtb}$ harboring pRv1758, hyg^r kan^r*	*This study*

Supplementary experimental procedures:

Construction of mutants and plasmids:

Construction of mc^{2}155:*Δmstdmh* is described previously (Ojha et al., 2010). Deletion mutant of *mttdmh* (Rv3451) was constructed using the gene replacement technique described earlier (Bardarov et al., 2002). The left arm and the right arm of the allelic exchange substrates (AES) were amplified using the primers: AGCAGTGGACCGCTTAAGGTGTGGCCCAATTCG, CGGGCATCCATTCTAGAAGGCAACCGGGGTCCAAA, and CCGCGCCGGGTCGCTAGCACATCTGCCTGGA, AACAGGGCGATCAGATCTATGTGATCGTCCGCTGCG, respectively. The AES were cloned in AflII – XbaI (left arm) and NheI-BglII (right arm) of pYUB854, and subsequently packaged in TM4 derivative, phAE87. The recombinant transducing particles were transduced in the target Mtb strains. At least two recombinants *hyg*^{r} colonies were confirmed by Southern blot for the expected genotype. The mutants were complemented by a derivative of L5attP-based integrative plasmid, pMH94, carrying either full length Rv3451 (p51), or Rv3451-52 (p5152), with 500bp upstream region. The complementing fragments were amplified using the primers, AGCAGTGGACCGGAGCTCGTGTGGCCCAATTCG, AACCTCATGACGCTCTAGAATGGGCTTACTGACAAA and CCGGAATCTATGTCTAGACTCGCGTT, and cloned into SacI and XbaI sites of pMH94. The plasmids, pRv3451 and pRv3452, were constructed by cloning the

full-length ORFs into the NdeI and SalI sites of an extrachromosomal plasmid, pJL37(Lewis and Hatfull, 2000). pAO10 was constructed by subcloning NdeI-XhoI fragment containing Mstdmh from pmsmeg1529 (Ojha et al., 2010) into NdeI- BamHI sites of an extrachromosomal plasmid pLAM12 (Ojha et al., 2005), downstream of acetamide-inducible promoter.

Real-time PCR:

Real-time PCR was performed on a StepOnePlus RTPCR System (Applied Biosystems) with SYBR Green mastermix following the manufacturer's instructions. RNA extraction from the respective bacterial suspension were extracted using Ribopure kit and concentration determined by Nanodrop. First strand synthesis from 200ng of total RNA was performed using Maxima reverse transcriptase (RT) (Fermentas) at room temperature for 10 minutes, followed by 30 minutes at 55°C and 5 mintues at 85°C. The 40ng of reverse transcriptase product was used for a RTPCR reaction containing 5µM of forward and reverse primers and 1X SYBR green RTPCR master mix. The amplification conditions were as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s. the forward and reverse primer sequences were 5'-CCAACGACGCCATATCGCACATTA-3' and 5'-AAAGCTGATGCTGCCCAACGGAA-3' for Rv3451; 5'-GCTTTCGTCAGTTCATTGCG-3' and 5'-GGCCAAGAAATCACCGTTG-3' for Rv3452; 5'-TGCAGTCGGTGCTGGACAC-3' and 5'-

CGCGCAGGACCTGTGAGCGG-3' for sigA; 5'-GAACGTGCGATCCCCAATC-3' and 5'-TCGTCATACGCAATTGTCTTAGC-3' for GroEL2.

Stress susceptibility of Mtb *in vitro*:

For peroxide sensitivity, Mtb(Erd), Mtb(Erd):*Δmttdmh*, Mtb(Erd):*Δmttdmh:p5152* were cultured in biofilms for four weeks. The media beneath the pellicles were replaced with either normal or modified Sauton's media *(*pH 5.0 and 40mM H_2O_2). After 4 hours exposure at 37 °C, pellicles were disrupted, as described previously (Ojha et al., 2008), and plated on 7H11agar to determine the number of viable cells. Similarly, acetamide induced Mtb(Erd):pAO10, Mtb(Erd):pLAM12 were, washed and exposed to the normal or modified Sauton's media for 4 hours prior to determining the number of viable cells by plating. For lysozyme sensitivity, homogenous suspension of cells grown in respective conditions was plated directly on 7H11OADC agar with or without 6µg/mL of lysozyme. For sensitivity to antimicrobial peptide LL37, acetamide induced Mtb(Erd):*pAO10* and Mtb(Erd):*pLAM12* were washed, resuspended in PBSTween-80 with or without 20µM of the peptide and incubated at 37°C for five days on rotary shaker before plating to determine the number of viability. For isoniazid sensitivity assay, 10µg/mL of the drug was added to the cultures and after the three days of exposure cells were diluted and plated as described for other experiments.

Supplementary reference:

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