Supplemental Material

Novel Insights into the Mechanism of Inhibition of MmpL3, a Target of Multiple Pharmacophores in *Mycobacterium tuberculosis*

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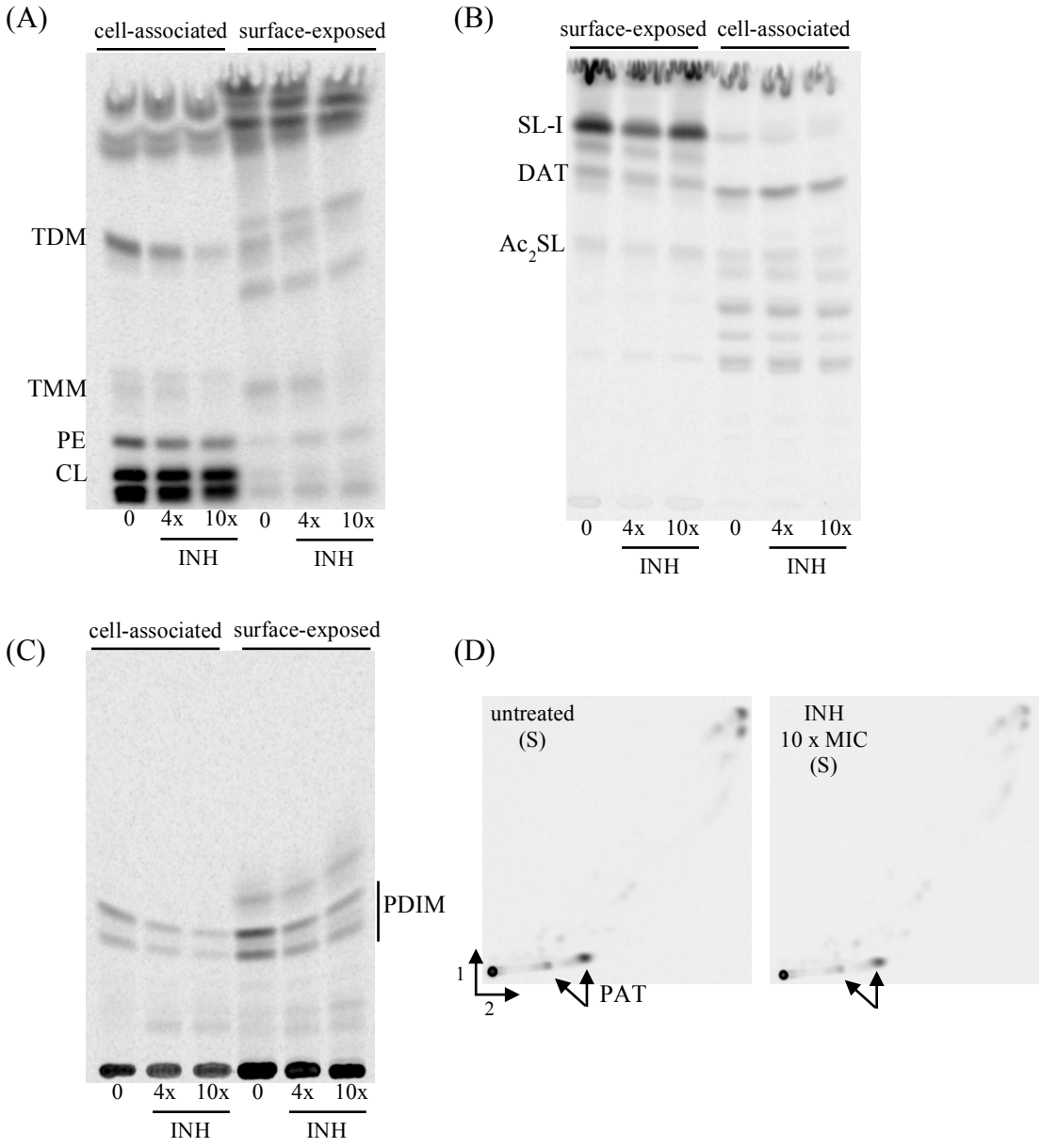
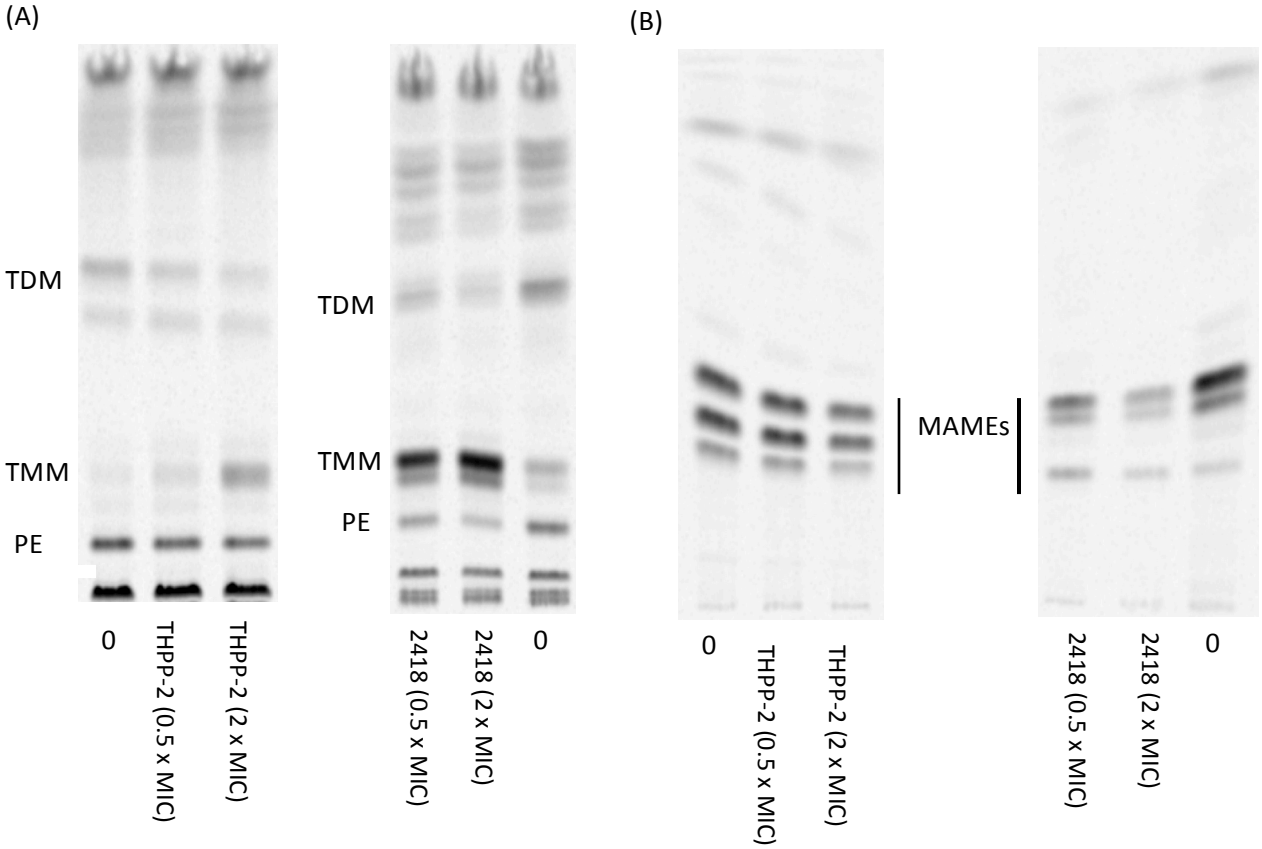
Figure S1: Effects of INH on the biosynthesis of trehalose mono- and di-mycolates, sulfolipids, di- and poly-acyltrehaloses and phthiocerol dimycocerosates in *Mtb*.

Surface exposed and cell-associated [^{14}C]-acetate-labeled and [1-^{14}C]propionate-labeled lipids from untreated and INH-treated *Mtb* H37Rv mc^{2}6206 cells were analyzed by TLC as described in Fig. 4.
The solvent systems used are: **(A)** [$CHCl_3$:CH_3OH:H_2O, 20:4:0.5] ([^{14}C]-acetate-labeled lipids); **(B)** [$CHCl_3$:CH_3OH:H_2O, 60:30:6] ([1-^{14}C]propionate-labeled lipids); **(C)** [petroleum ether (60/80°C):ethyl acetate, 98:2; three developments] ([1-^{14}C]propionate-labeled lipids); **(D)** first dimension: [petroleum ether (60/80°C):acetone, 92:8; three developments]; second dimension: [acetone:toluene, 95:5] ([1-^{14}C]propionate-labeled lipids), and revealed by autoradiography. The same volume of samples was loaded per lane. CL, cardiolipin; PE, phosphatidylethanolamine.



<u>Figure S2</u>: Effect of THPP-2 and 2418 on the transfer of mycolic acids to their cell envelope acceptors.

Mtb H37Rv mc^{2}6206 cultures were either untreated or treated with THPP-2 (1.3 µg/ml [0.5 x MIC] or 5.3 µg/ml [2 x MIC]) or 2418 (1.56 µg/ml [0.5 x MIC] or 6.2 µg/ml [2 x MIC]) and labeled with [^{14}C]-acetate as described under Materials and Methods.
Analyses of lipids (A) and cell wall-bound mycolic acid methyl esters (MAMEs) (B) from untreated and treated cells were as described in Fig. 3.



<u>Figure S3</u>: Predicted topology of MmpL3 and mapping of the mutations reported to confer resistance to SQ109, THPP compounds, adamantyl ureas, BM212 and indolcarboxamides.

The topology of MmpL3 from *Mtb* was predicted using the TOPPRED software (http://mobyle.pasteur.fr/cgi-bin/portal.py?#forms::toppred). The amino acids found to be mutated in the various spontaneous resistant mutants are according to references # 9-11, 13-14 and 17-19 and the data presented in Table 4.

Black stars indicate residues thought to participate in the transmembrane electrochemical proton gradient of MmpL3 (D251, R259, D640, Y641, D710; R715). Red hexagons indicate residues mutated in SQ109 (and analogs) resistant mutants; blue hexagons are residues mutated in BM212 (and analogs) resistant mutants of *Mtb* or *M. smegmatis*; brown hexagons are residues mutated in indolcarboxamide resistant mutants; magenta hexagons are residues mutated in THPP resistant mutants; the green residue (G253) is the mutated residue in adamantyl urea as well as some BM212 and indolcarboxamide resistant mutants.