Supporting information

Structural and functional evidence that lipoprotein LpqN supports cell envelope biogenesis in *M. tuberculosis*

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Supplemental Materials

ESI HPLC /MS analysis of lipid extracts

ESI HPLC/MS analysis was performed using an Agilent 6550 A QTOF instrument with an Agilent 1290 HPLC, operated by Agilent Masshunter software as previously described[1]. Separation of lipids was achieved by a Supelco 100 × 2.1 mm (2.7 μm particle size) Ascentis Express C-8 column at a flow rate of 250 μl/min. The mobile phase contained 5 mM ammonium formate (pH 5.0) both in solvent A, acetonitrile:water (60:40, v/v), and solvent B, isopropanol:acetonitrile (90:10, v/v). A gradient elution in the following manner was applied: 0 min; 68 % A, 0–3 min; 70% A, 3–8 min; 50% A, 8–13 min; 35% A, 13–18 min; 25% A, 18–28 min; 15% A, 28–33 min; 5% A, 33–40 min; 0% A, 40–50 min; 0% A, 50–51 min; 68% A, 51–60 min; 68% A.

1. Howard, N.C., Marin, N.D., Ahmed, M., Rosa, B.A., Martin, J., Bambouskova, M., Sergushichev, A., Loginicheva, E., Kurepina, N., Rangel-Moreno, J., Chen, L., Kreiswirth, B.N., Klein, R.S., Balada-Llasat, J.M., Torrelles, J.B., Amarasinghe, G.K., Mitreva, M., Artyomov, M.N., Hsu, F.F., Mathema, B., Khader, S.A.: Mycobacterium tuberculosis carrying a rifampicin drug resistance mutation reprograms macrophage metabolism through cell wall lipid changes. *Nat Microbiol* **3**, 1099-1108

Table S1. Trimethoprim minimal inhibitory concentrations (MICs), determined via M-PFC, to assess interactions between MmpL3/11$_{TB}$ D2 domains and LpqN-family proteins.

Insert in pUAB200	Insert in pUAB300	Trim MIC (µg/mL)
-	Rv2763 (dfr) positive control	>200
-	LpqT	25
-	LprG	25
-	Mtc28	12.5
MmpL3 D2	-	<6.25
MmpL3 D2	LpqT	<6.25
MmpL3 D2	LprG	<6.25
MmpL3 D2	Mtc28	<6.25
MmpL11 D2	-	<6.25
MmpL11 D2	LpqT	50
MmpL11 D2	LprG	50
MmpL11 D2	Mtc28	25

Table S2 Primers used in this study

Name	Primer (5'-3')
ΔlpqN 5' F	tataagatcttagtcgtagccggcgtagtt
ΔlpqN 5' R	tataaagcttgctgtcggtcttgatgttga
ΔlpqN 3' F	tatatctagagcagaagacggtggtgattc
ΔlpqN 3' R	agctggtaccatgtggtagcggaactcgac
lpqN -865	aggtgccatacgagctgaac
lpqN +1637	tcaagggaatcgagaagtgc
lpqN +367	gcgatcctctccaaactcac
hyg primer 22	tggctaaaatgtatcctaaatcag
hyg primer 3500	tgttataacagacactgcttg
mmpL3D1.102	atcaattggcaagcacgtcacgcagagc
mmpL3D1.573	atatcgatcaacggcagcgccagcacttc
mmpL3D2.990	tacaattgcaatcctgggcaaacacgt
mmpL3D2.1185	taatcgatcatcacccggttaaccagcttg
mmpL11D1.90	atcaattgcgatgacgcagtcggggaatc
mmpL11D1.349	atatcgatgcgttcggcgttggcaatatc
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mmpL11D2.1587	ataggatcctcacggttgcgtcgcggacac
lpqN.61	atatcatatgagtttcaacatcaagaccgacag
lpqN.687	atatggatccttagggcgtgatggtcgtctc
lpqN qRT.Forward	gcgatcctctccaaactcac
lpqN qRT.Reverse	ggaatcaccaccgtcttctg
lpqN.pUAB300.F	aaggatccagtttcaacatcaagaccgacag
lpqN.pUAB300.R	ttatcgatttagggcgtgatggtcgtctg
lpqT.pUAB300.F	taggatcctgcggaccgaaatcgcctg
lpqT.pUAB300.R	ataagctttactttgccgcgacgacg
lprG.pUAB300.F	cggtggaggtggtgggtccggatcctgctcgtcgggctcgaag

lprG.pUAB300.R	tacgtcgacatcgataagcttcagctcaccgggggcttc
mtc28.pUAB300.F	taggatccgatcccctgctgccaccg
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lpqN -570.F	atgatatcgacctcggtgtcgtcgtg
lpqN +HA.R	taaagcttttaagcgtaatctggaacatcgtatgggtagggcgtgatggtcgtctg
lpqN TAP.F	atggatccggcaacatcgagatgtcgccga
lpqN TAP.R	tcaatgatgatgatgatgatgtcctcctcctcccttgtcgtcatcgtctttgtagtcggatccgggcgtgatggtcgtctgct
mmpL11 TAP.F	atggatcccgcctcgaaatgggccttca
mmpL11 TAP.R	atggatcccctcgcctcctccaacatcg
Ag85A.F	agtggatcccccgggctgcagcgcaagccgaagcggccctg
Ag85A.R	agtggtggtggtggtggctagcggcgccctggggcgcggg

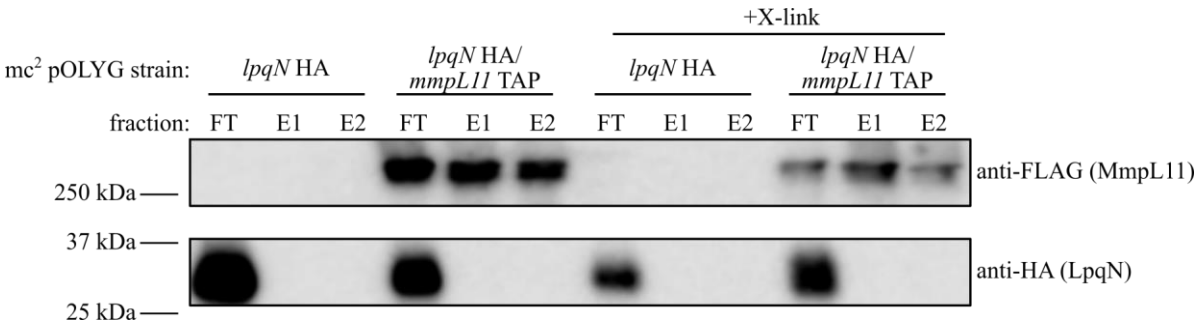


Figure S1. $LpqN_{TB}$ does not co-purify with $MmpL11_{TB}$ when co-expressed in *M. smegmatis*. HA-tagged LpqN and tandem (FLAG + HIS) affinity purification (TAP)-tagged MmpL11 were co-expressed in *M. smegmatis* mc^2155 in the presence/absence of protein cross-linking agent (1% formaldehyde, +X-link). MmpL11 TAP was purified via HisPur affinity resin. Resin flow through (FT) and elutions 1 and 2 (E1/E2) were analyzed for the presence of MmpL11/LpqN protein via Western blot with anti-FLAG/anti-HA antibodies. *M. smegmatis* solely expressing HA-tagged LpqN serves as a negative control for non-specific binding.

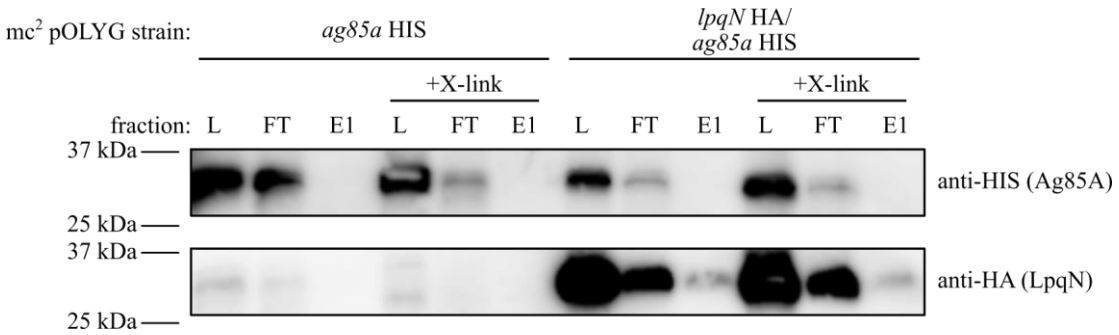


Figure S2. $LpqN_{TB}$ does not co-purify with $Ag85A_{TB}$ when co-expressed in *M. smegmatis*. HA-tagged LpqN and HIS-tagged Ag85A were co-expressed in *M. smegmatis* mc^2155 in the presence/absence of protein cross-linking agent (1% formaldehyde, +X-link). LpqN HA was purified via anti-HA affinity resin. Crude lysate (L), resin flow through (FT), and elution 1 (E1) were analyzed for the presence of Ag85A/LpqN protein via Western blot with anti-HIS/anti-HA antibodies. *M. smegmatis* solely expressing HIS-tagged Ag85A serves as a negative control for non-specific binding.

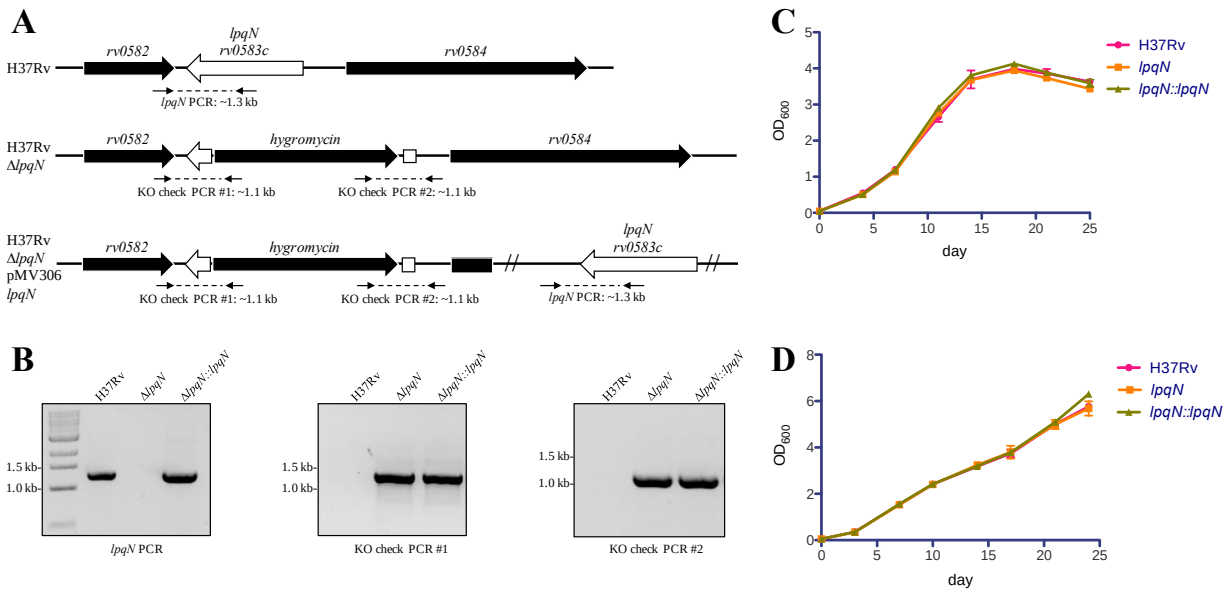
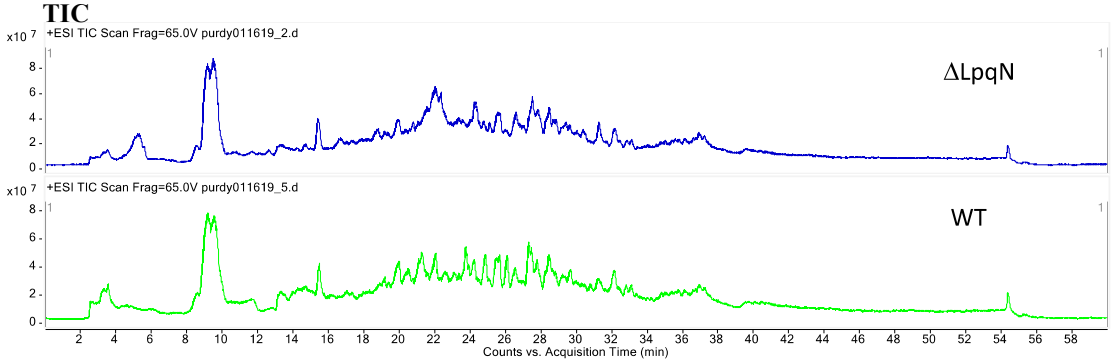
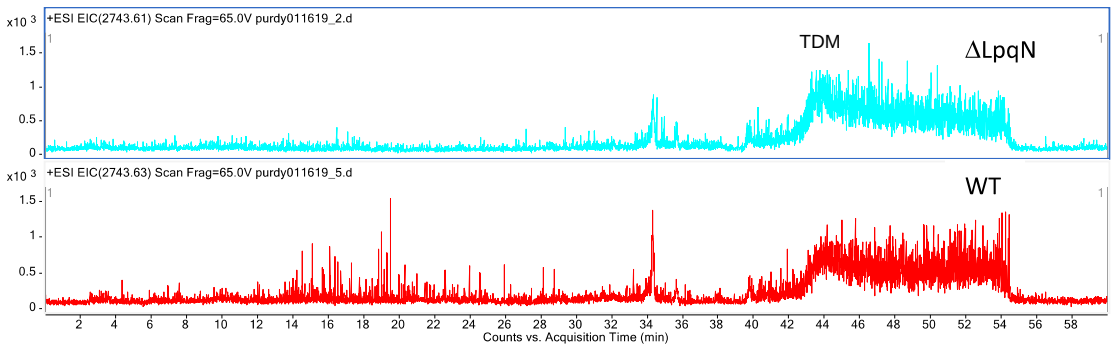


Figure S3. Generation of H37Rv Δ*lpqN*. (A) Genomic organization of *rv0583c*/*lpqN* in *Mtb* H37Rv, *hyg* resistance cassette allelic exchange strategy (Δ*lpqN*), and chromosomal complementation of *lpqN* using plasmid pMV306 (Δ*lpqN::lpqN*). Diagnostic PCR products are indicated with dashed lines. (B) Diagnostic PCRs performed with genomic template DNA isolated from H37Rv, Δ*lpqN*, and Δ*lpqN::lpqN Mtb*. Primers used = *lpqN* PCR: lpqN +367/lpqN +1637; KO check PCR #1: hy*g* primer 3500/lpqN +1637; KO check PCR #2: hy*g* primer 22/lpqN -865. (C) Growth of *Mtb* strains in 7H9 medium. (D) Growth of *Mtb* strains in Sauton's medium.

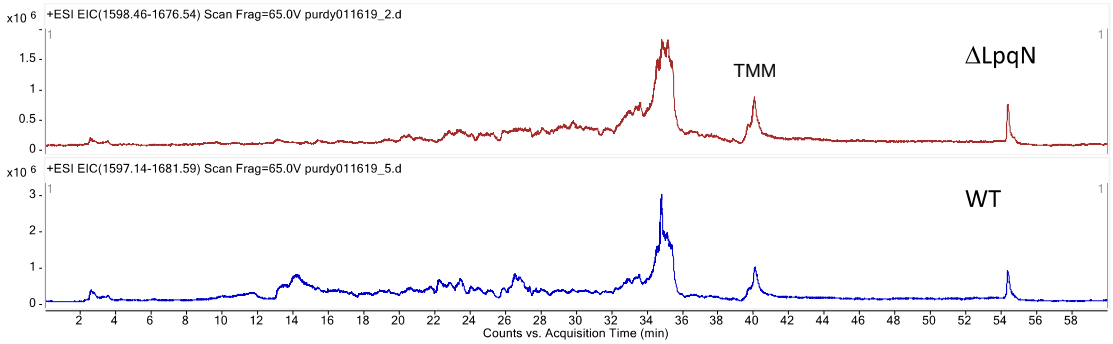
A **TIC**



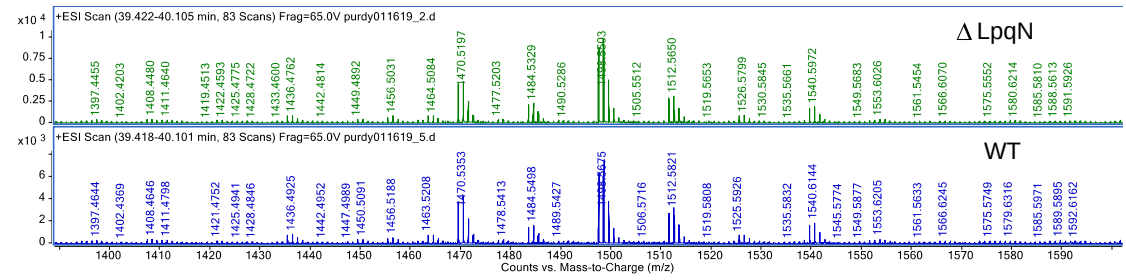
B **TDM**



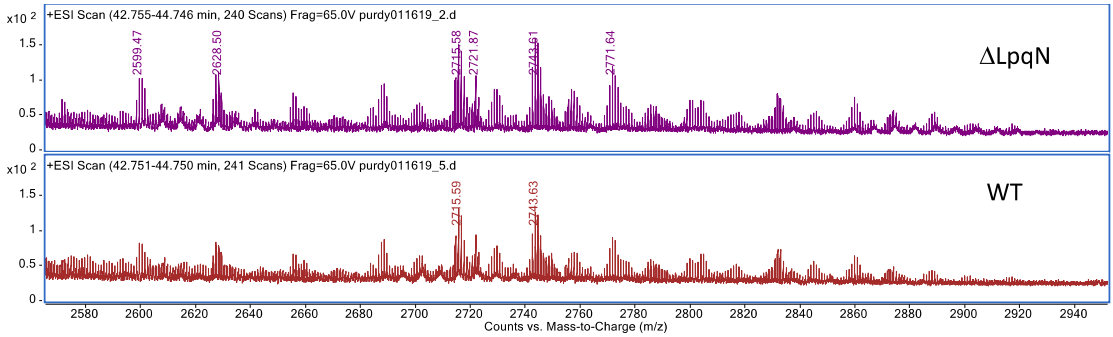
C **TMM**



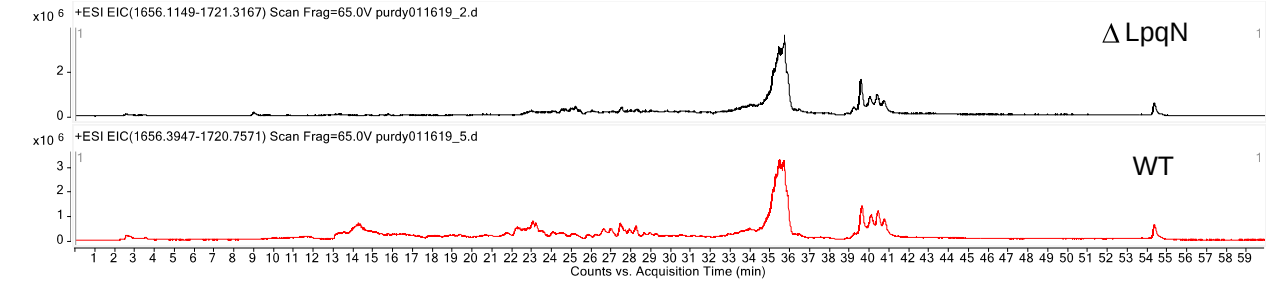
D **TMM** ($[M+NH_4]^+$)



TDM ($[M+NH_4]^+$)



E Wax ester



F Wax ester ($[M+NH_4]^+$)

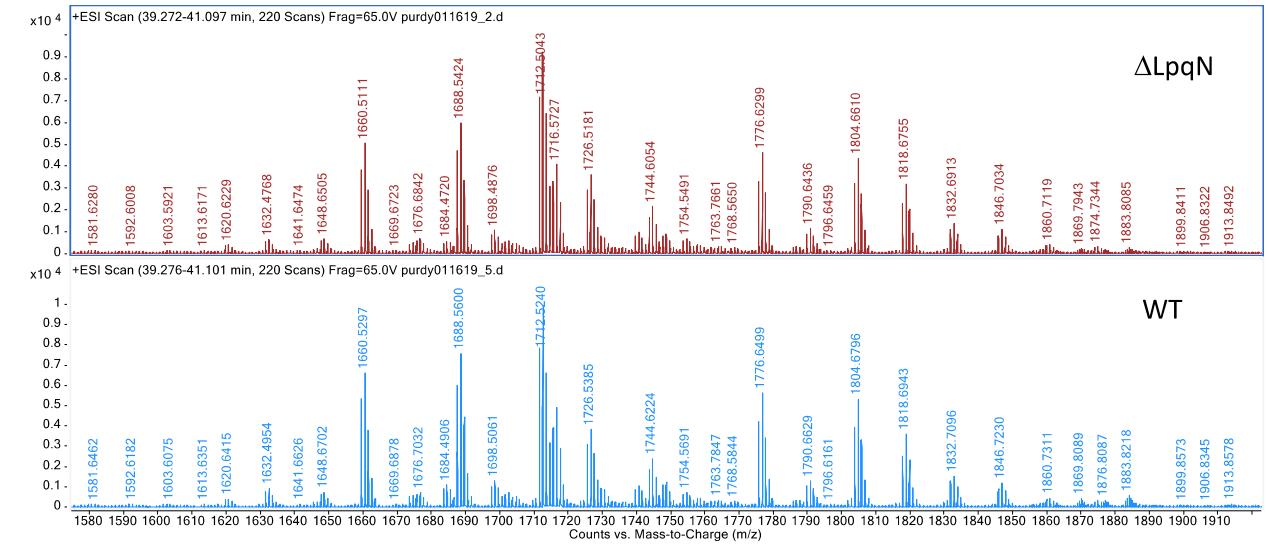
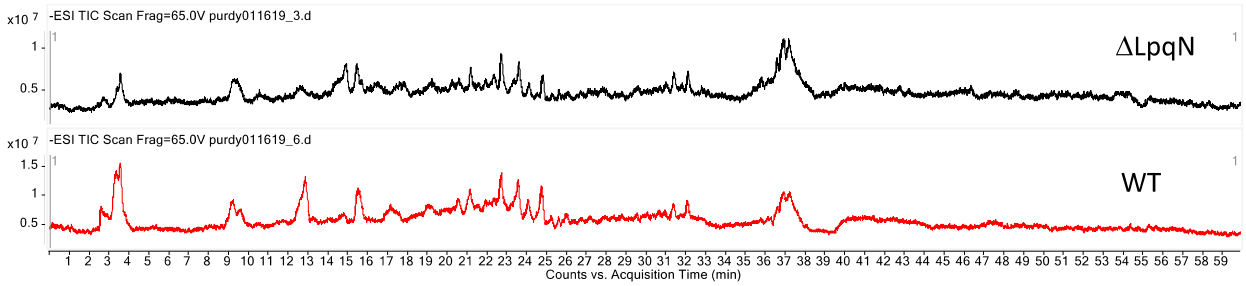
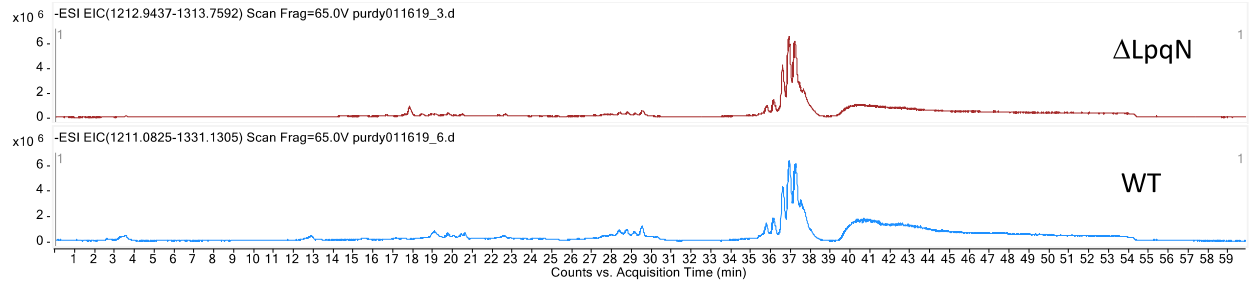


Figure S4. Positive-ion ESI HPLC/MS analysis of lipid extracts from wild type *M. tuberculosis* H37Rv and the *lpqN* mutant. (A) ESI HPLC/MS Total ion chromatogram (TIC) in the positive ion mode, (B) Selected ion chromatogram of TDM (elution time: 43-45.3 min), (C) Selected ion chromatogram of TMM (elution time: 39.5-40.5 min), (D) The ESI mass spectra of TMM $[M + NH_4]^+$ ions (top panels) and TDM $[M + NH_4]^+$ ions (bottom panels), (E) Selected ion chromatograms of wax ester (elution time: 39.5-41 min), (F) ESI MS spectra of the $[M+ NH_4]^+$ ions of wax esters.

A TIC



B mycolic acids



C mycolic acids [M - H]$^-$

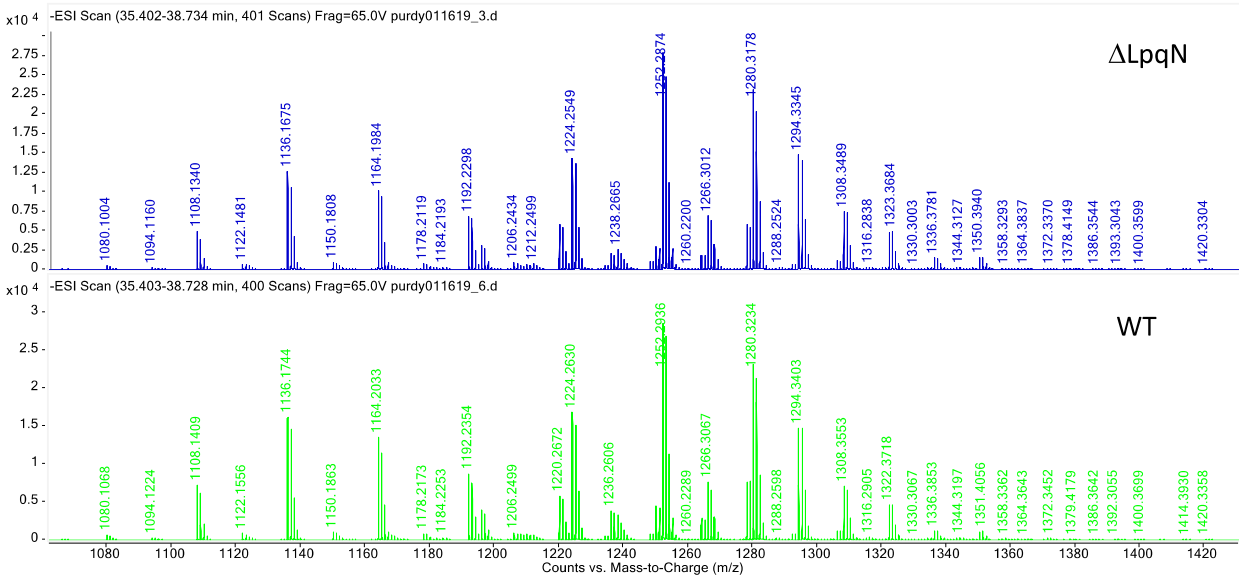


Figure S5. Negative-ion ESI HPLC/MS analysis of lipid extracts from wild type *M. tuberculosis* H37Rv and the *lpqN* mutant. (A) ESI HPLC/MS Total ion chromatogram (TIC) in the negative ion mode, (B) Selected ion chromatogram of mycolic acids (elution time: 35.5-38 min), (C) ESI MS spectra of the [M – H]$^-$ ions of mycolic acids.