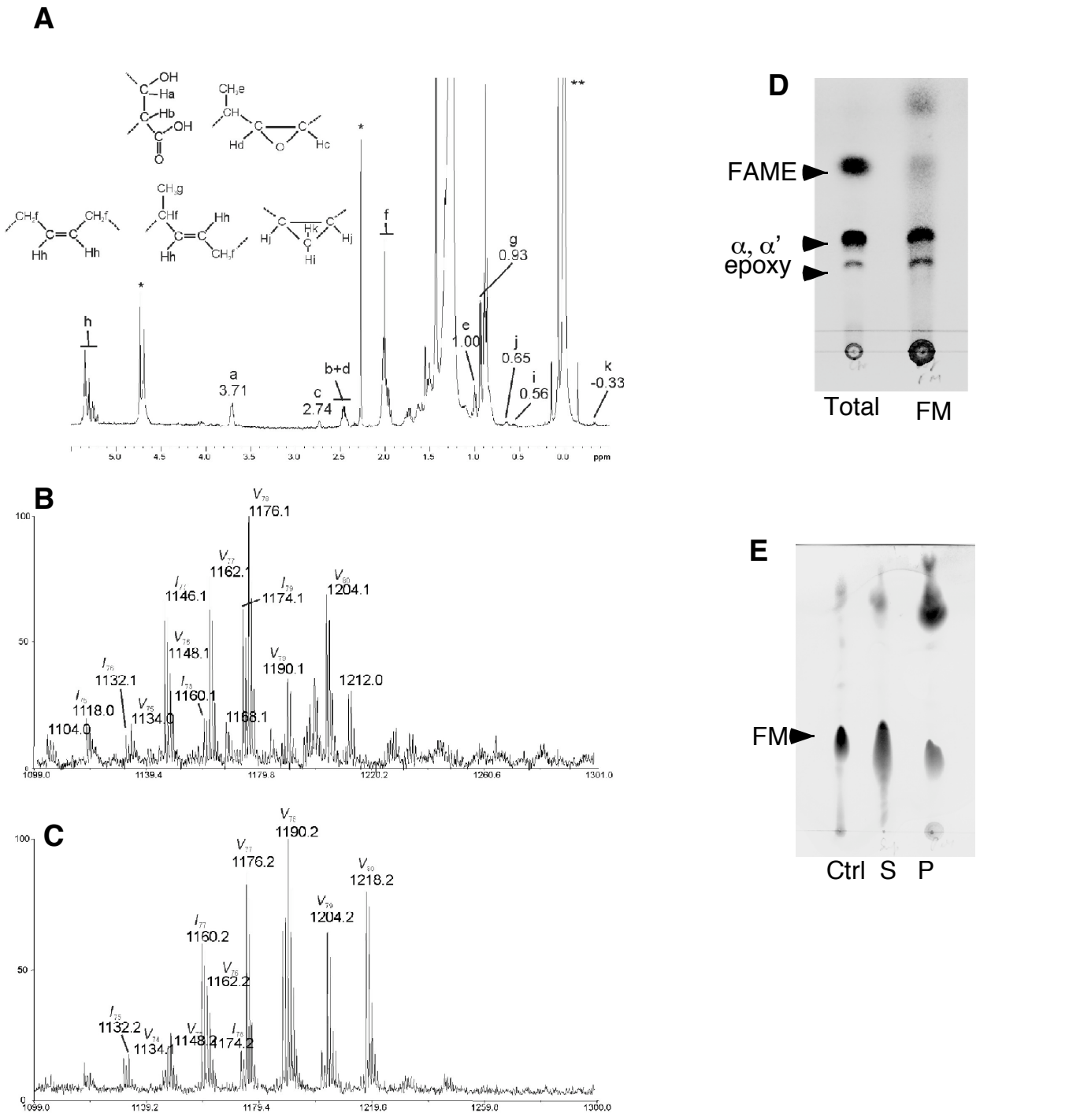

FIg S1: **(A)**- 1H-NMR spectrum of purified Spot 1. Proton signals associated to functional groups, including insaturations, ≤-hydroxy and epoxy groups were labeled accordingly from (a) to (h). **(B)**. MALDI-TOF-MS spectrum of native Spot1. **(C)**. MALDI-TOF-MS spectrum of methyl esterified Spot 1. Individual signals were associated to [M+Na]+ adducts of ±-mycolates (I) and epoxy-mycolates (V) according to previously published data (Alahari et al., 2007, Laval et al., 2001). **(D)**- TLC of methyl esters of Spot1 (FM) compared with the methyl ester of total cellular mycolates of M. smegmatis (Total) containing alpha, alpha', epoxy mycolates as well as fatty acyl methyl esters (FAME). **(E)**- TLC of apolar lipids extracted from the supernatant (S) and cell pellet (P) after solubilization of M.smegmatis

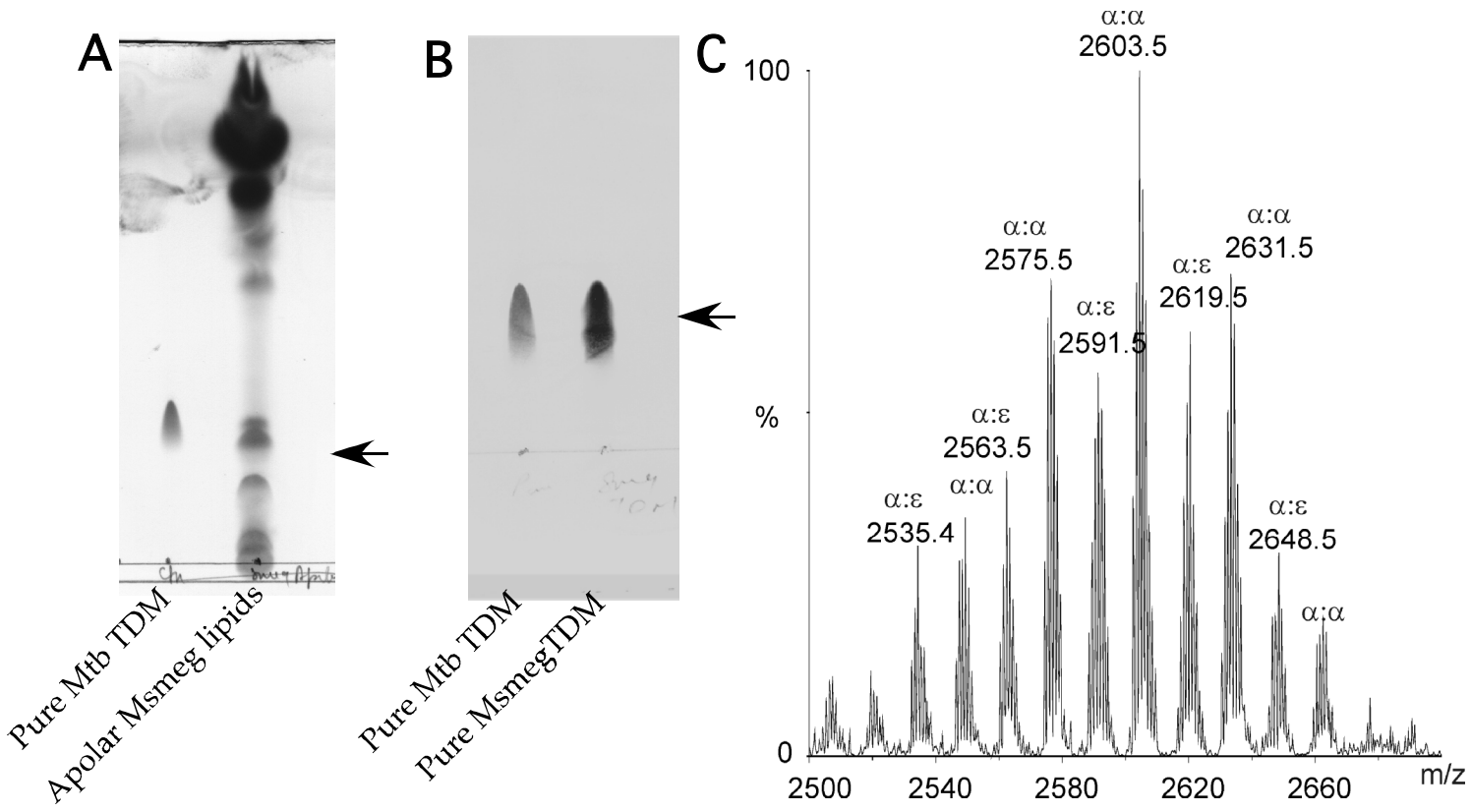

Figure S2- A- Purification and characterization of Trehalose dimycolate (TDM) from *M. smegmatis*. Thin layer chromatography (TLC) of TDM containing apolar lipids from *M. smegmatis* biofilms extracted in petroleum ether. The TLC was developed in chloroform:methanol:water::90:10:1, sprayed with 0.5% molybdophosphoric acid and charred for visualization. Purified Trehalose Dimycolate from *M. tuberculosis* (Sigma) was loaded as reference. **B**- TLC of the purified lipid co-migrating with Mtb TDM in panel A (marked with arrow). The lipid was extracted from TLC in chloroform:methanol::2:1. **C**- MALDI-TOF-MS analysis of purified *M. smegmatis* TDM as shown in panel B (marked with arrow). Spectrum shows complex pattern of signals attributed to [M+Na]+ adducts of a mixture of TDM differentially substituted by alpha-mycolates and epoxy mycolates. The two main families that can be observed are either exclusively substituted by alpha-mycolates (a:a) or by a mixture of epoxy and alpha-mycolates (e:a). Presence of both types of mycolates was also confirmed by 1 H-NMR analysis of intact TDM (not shown).

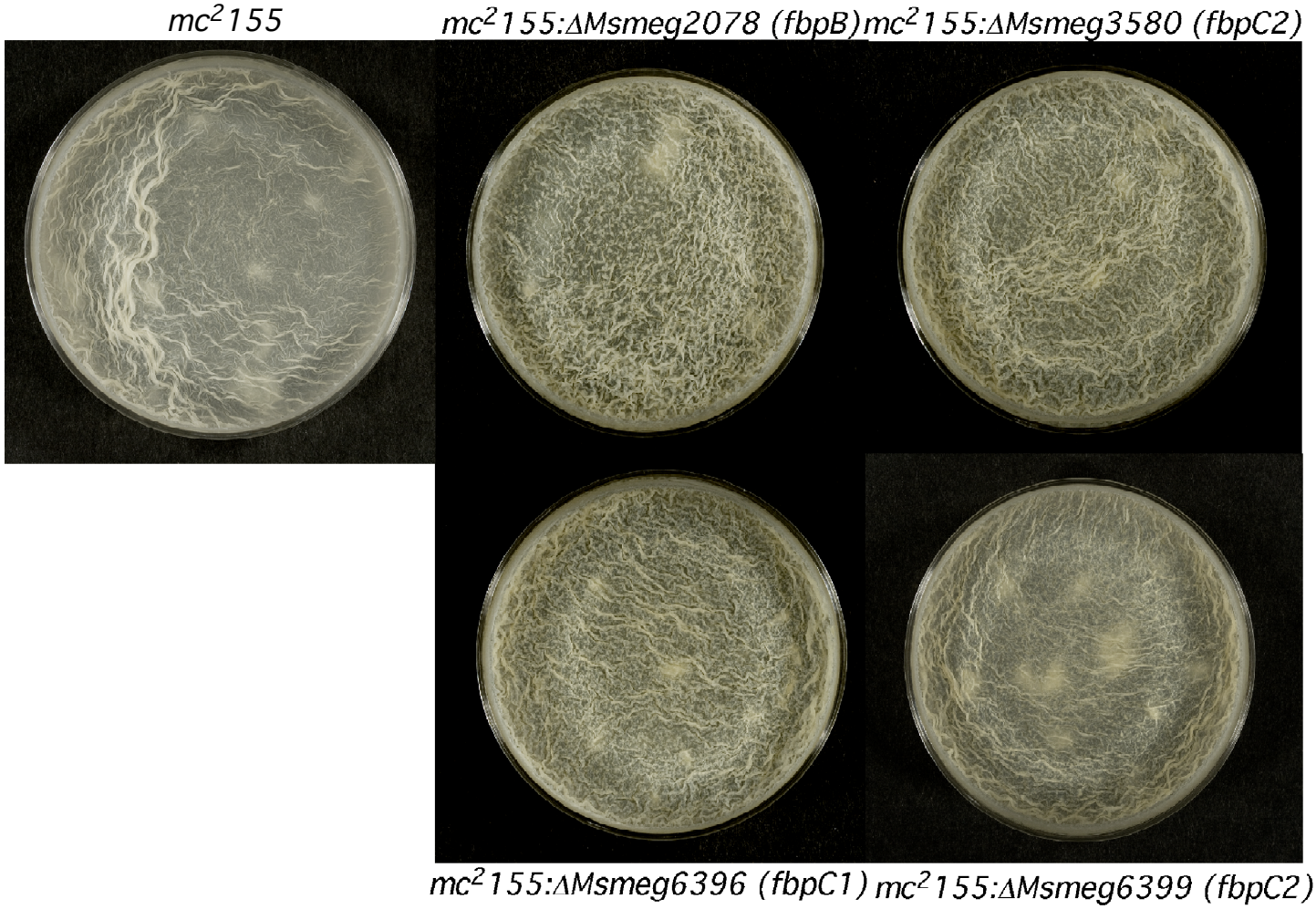

Figure S3- 5-day biofilms of *M. smegmatis* mutants each with deletions in one of the four mycolyltransferase homologues. The genotype of the mutant strain against each panel denotes the gene deleted in the mutant.

Sequence Name	Sequence
1529kontF	CCC AGC GCG GGG ACC TTA AGC GGC GTC GCG CTG GCC GT
1529kontR	CAA CCG GGC CGG CTT CTA GAG GGA AAT CAC GAT CGC C
1529koctF	CCG CAG GCA GCA GGG CTA GCT CGC GGG TCT GGT TTA GGG
1529koctR	GGT CAA CGG CAG GCC AAG CTT CGC CGA CGG GTT ACC
2078kontAfl	AGT TGT GCA GTG CGC TTA AGC CGA TGG TCC ACG CGC
2078kontXba	CAA TGG CCA CGA ATC TAG ACA ATG AAG GTC ATA CTG C
2078koctNhe	GCA GCG GGT GCT CGG CTA GCC CCG TAT CAC ACG AAA TC
2078koctBglII	CGG CTC GGT GGG TGT AGA TCT GCT CTC CGC GCT GGG C
3580kontAfl	CCG ACT CGC ATG AGC TTA AGC GTC TCG GGC CGG GTC GG
3580kontXba	GTG CGG TCC GCC GCT CTA GAA CTG CAC ACG GAT GTC GC
3580koctNheI	GTG GAA CTA CTG GGG CTA GCA GCT GCT GGA GAT GA
3580koctBglII	CCG CAT CGT CGA GGA GAT CTG CTC AAC CGG CGG GGC C
6396kontAfl	TCT ACG CGG CAT CGC TTA AGG GCA CGT TGA ACC CGT C
6396kontXbaI	CAC GTC TCA TCC TCT AGA CCT TCC TGC CG
6396koctNheI	CTT CCC GGC CTC GGG CTA GCA CGA CTG GGG CAG CTG
6396koctBglII	CGG ATG TAG TCG GAG ATC TCC GAG TCG ACC TCC CCG G
6398kontAfl	CTG ATC CTG TCG GCC TTA AGC CCA GGT CAG TTC CGG TAT
6398kontXba	GCG ACC GCA ACC GTC TAG ACG GCG CGA CAG ACC TGC CGC
6398koctNheI	CTG GGC GTA CTG GGG CTA GCA GCT GCA GGC GAT GAA G
6398koctBglII	CTC GGC GGT GAG AAA GAT CTC CCA CTG CTT GCT GCC GT
6399kontAfl	CCC CAC ACA GTG TTT CTT AAG AAC ATG GGC ATG CTG GGC
6399kontXba	AAT GTG GTG AAC ATT CTA GAA ATG ACT GTC GCC ACC
6399koctnhe	GCG GGC AAC CAC GGC TAG CCG TAC TGG GGC GCT CAG C
6399koctbglII	GGT CTC CCA CTT GTA GAT CTT GCA GCC GCC GTC CTT G
6583kontbgl	CGA CAT CCG CGC CCA GAT CTA ATC CCA CTT CTT CGG C
6583kontnhe	CGC GAT CAG CCC CGC TAG CAT GAG CAC CGT CGC GGC
6583koctxbaI	GCG ACG CGG CCG GTC TAG ACC GGA TCC CGT AAA CC
6583koctaflII	GAA TCA CCC AGC GGC TTA AGG CCC TCG GCG TCG ACG TG

Table S1: List of primers used for constructing allelic exchange substrate (AES) for the deletion of mycolyltransferases and Msmeg_1529 (TDMH).

Sequence Name	Sequence
hygprimerF	AGC GGC TCC CAG AAT TCC TGG TCG TTC
hygprimerR	GCG AAC TGC TCG CCT TCA CCT TCC TGC AC
1529scrnF	GGC GCC CAG TTC TTC TAC TAC AAC G
1529scrnR	GCC ACG AAA GCG GCG GCC TGA TTC G
6396scrnF	GCC AAC CGC GAC GTC AAG CCG A
6396scrnR	CTT ACG CCG AGC GTT CTT TGC C
6398scrnF	CAA CAA TCA GAC CTA CAC CTA TAA G
6398scrnR	CGG CGT CAG GAA ACC CGA CAG
6399scrnF	CAA CTA CGA CGT GTG GGA TGT G
6399scrnR	CGG CCA TCG ACA GAC CGA CAA C

Table S2 : List of primers used to screen the *DfbpA* Msmeg_1529 mutants of *M. smegmatis*. Hygromycin resistant colonies of each mutant were screened for the presence of 5′ and 3′ junction sequence between of hyg^r and flanking region using two primer pairs- (hygrprimerF, scrnF) and (hygrprimerR, scrnR).

Sequence Name	Sequence
smeg0194expF	GAA GGG GGA CGT TCC ATA TGA AAT GGA TTA GAG CTC T
smeg0194expR	ATC AGT TGG TCG CCT CGA GAG GCC CGG TGT TGA TGC
smeg1184expF	CAT GAG CTA TGG GGC ATA TGA GAC TCG CCG GTC TCG T
smeg1184expR	GCG CCA CTA GGT CAC TCG AGC GGC GCC GTC GGG GGC
smeg1529expF	GTG ACG ATG GCG CAT ATG ATT TCC CTC CGG AAG CCG
smeg1529expR	ATT CGA ATC CCT ACT CGA GAC CCG CGA TCA GCC CTG
smeg2095expF	CCG GTT AGA TTC ACA TAT GCT TTC TCG CCG GAT CGG
smeg2095expR	CAC TCA ACA TCA CTC GAG CGG TAG GTC AGC CGA C
smeg5878expF	ATG CGC TTG GAG TCC ATA TGA ACG TTC TCA AAT T
smeg5878expR	GGG ATC ACA ACC GCT CGA GCA CGT ACT GCG CGG CCT GG
smeg6354expF	TTT GGG GGT CGG GCA TAT GTT CAA GAG CAC GCT TTC
smeg6354expR	CCT CCC GCA CTC ACT CGA GCA GGA CTC GCG CGG CGA C
smeg6398expF	TCA GAT AGG GAG CCA TAT GAA GTT CGT TGG GAG AAT G
smeg6398expR	TCG CCG GTC CCC GAA AGC TTC CCG GAA ATG ATC GAG CG
smeg6398compF	TCA GAT AGG GAG CCA TAT GAA GTT CGT TGG GAG AAT G
smeg1529comF	GTG ACG ATG GCG CAT ATG ATT TCC CTC CGG AAG CCG
smeg1529compR	ATT CGA ATC CCT ACT CGA GAC CCG CGA TCA GCC CTG

Table S3- List of primers used to express six serine esterases and Msmeg_6398 in *E. coli* in *M. smegmatis*. The primers annotated as "comp" were used for making complementation construct for the respective mutants. smeg6398expR was also used for making complementation construct.