SUPPLEMENTARY INFORMATION

Table S1: Strains and Plasmids used in this study

Name	Description	Source/ Reference
Plasmids		
p2NIL	Cloning vector, kan^r	(1)
p2NILegtD	p2NIL vector carrying 1955bp region downstream *egtD* and 1923bp region upstream *egtD*	This study
pGOAL17	Plasmid carrying *lacZ* and *sacB* genes as a *Pac*I cassette; Ap^r	(1)
pGOAL19	Plasmid carrying *hyg, lacZ, and sacB* genes as a *Pac*I cassette; Ap^r, Hyg^r	(1)
p2NILegtD17	p2NILegtD containing the pGOAL17 *Pac*I cassette	This study
p2NILegtD19	p2NILegtD containing the pGOAL19 *Pac*I cassette	This study
pMV306	*E.coli- Mycobacterium* integrating shuttle vector, Hyg^r	(2)
pMV306D	pMV306 carrying the region from 2398bp upstream of *egtD* to 1169bp downstream of *egtD*	This study
Strains		
mc^{2}155	*ept*-1(efficient plasmid transformation) mutant *mc^{2}6*	Laboratory stock
Δ*mshA*	Derivative of *mc^{2}155* carrying a transposon insertion in *mshA*, kan^r	(3)
Δ*egtD*	Derivative of *mc^{2}155* carrying an unmarked deletion of *egtD*	This study
Δ*mshA/*Δ*egtD*	Derivative of Δ*mshA* carrying an unmarked deletion of *egtD*	This study
Δ*egtD attB:: pMV306D*	Derivative of Δ*egtD* carrying pMV306D integrated at the attB locus; Hyg^r	This study
Δ*mshA/*Δ*egtD attB::pMV306D*	Derivative of *mshA/*Δ*egtD* carrying pMV306D integrated at the attB locus*;* Hyg^r	This study
DHα	*E.coli* for routine cloning experiments	Laboratory stock
H37Rv	*Mycobacterium tuberculosis* laboratory strain	(4)

Table S2: Primers used to generate the suicide plasmid and the complementation vector in this study

Name	Sequences (5' to 3' direction)	Restriction enzymes	PCR product
6247 USF	GCATATGGTAGTTGGCCAGTGAGAG	**NdeI**	1923bp upstream MSMEG_*6247* including 18bp of its 5'end
6247 USR	AAGCTTGAACTGCATCGCCAGTACA	**HindIII**	
6247 DSF	GATGCATGGCGATACCGACAACAG	**Nsil I**	1955bp downstream MSMEG_6247 including 129bp of its 3' end
6247 DSR	GCATATGCTCACCGAGGTGTCC	**NdeI**	
6247 CF	AAGCTTCTCGACATGACGCGAACAT	**HindIII**	4528bp product that includes 2398bp upstream MSMEG_6247 to 1169bp downstream MSMEG_6247
6247 CR	AAGCTTGAGGCCGACCTGGACTATA	**HindIII**	

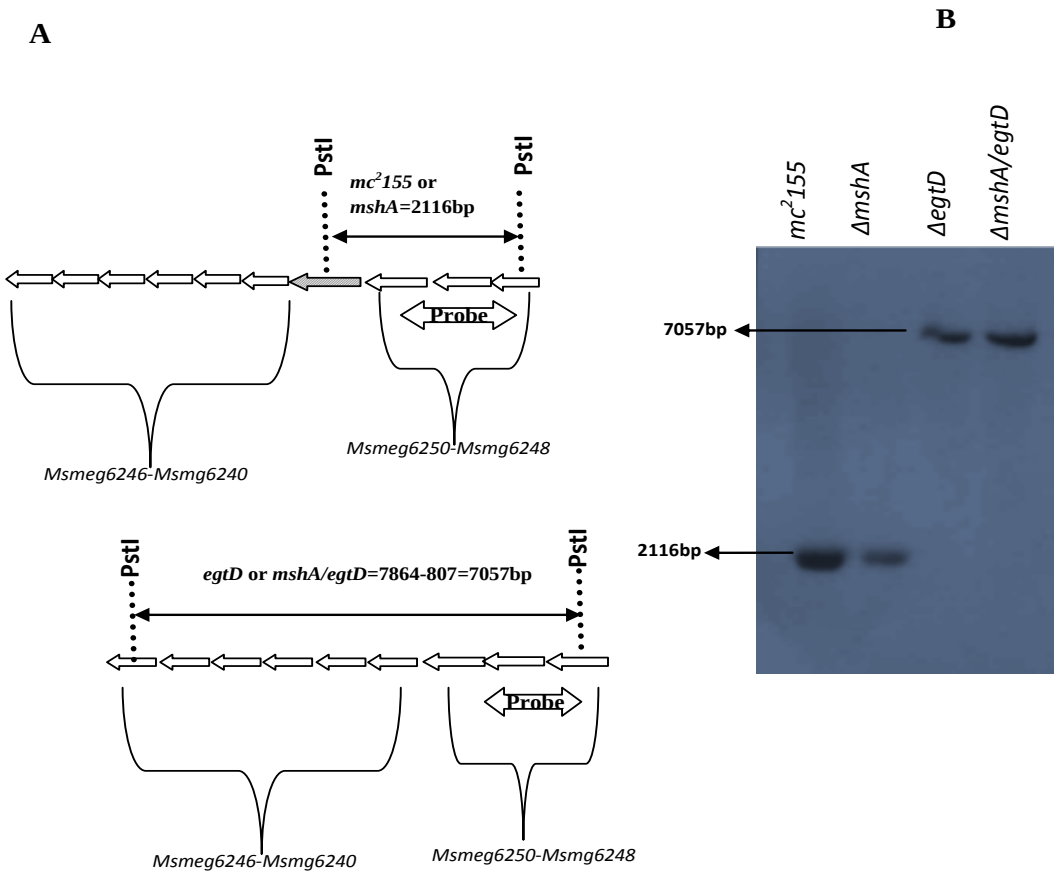

Figure S1: Genotyping of *M. smegmatis* Δ*egtD* and Δ*mshA*/Δ*egtD* mutants

Southern blotting of digested genomic DNA of the different strain was performed as previously described (5)

A: Restriction map of the wild-type and mutant strains.

B: Southern blotting of the fragment restricted by *Pst*I, the band (2116bp) in the first and second lanes represents the wild type and the Δ*mshA* mutant which have an intact *egtD* gene, while the 7057bp for Δ*mshA*/Δ*egtD* and Δ*egtD* strains indicates the deletion of *egtD* and the loss of the *Pst*I site.

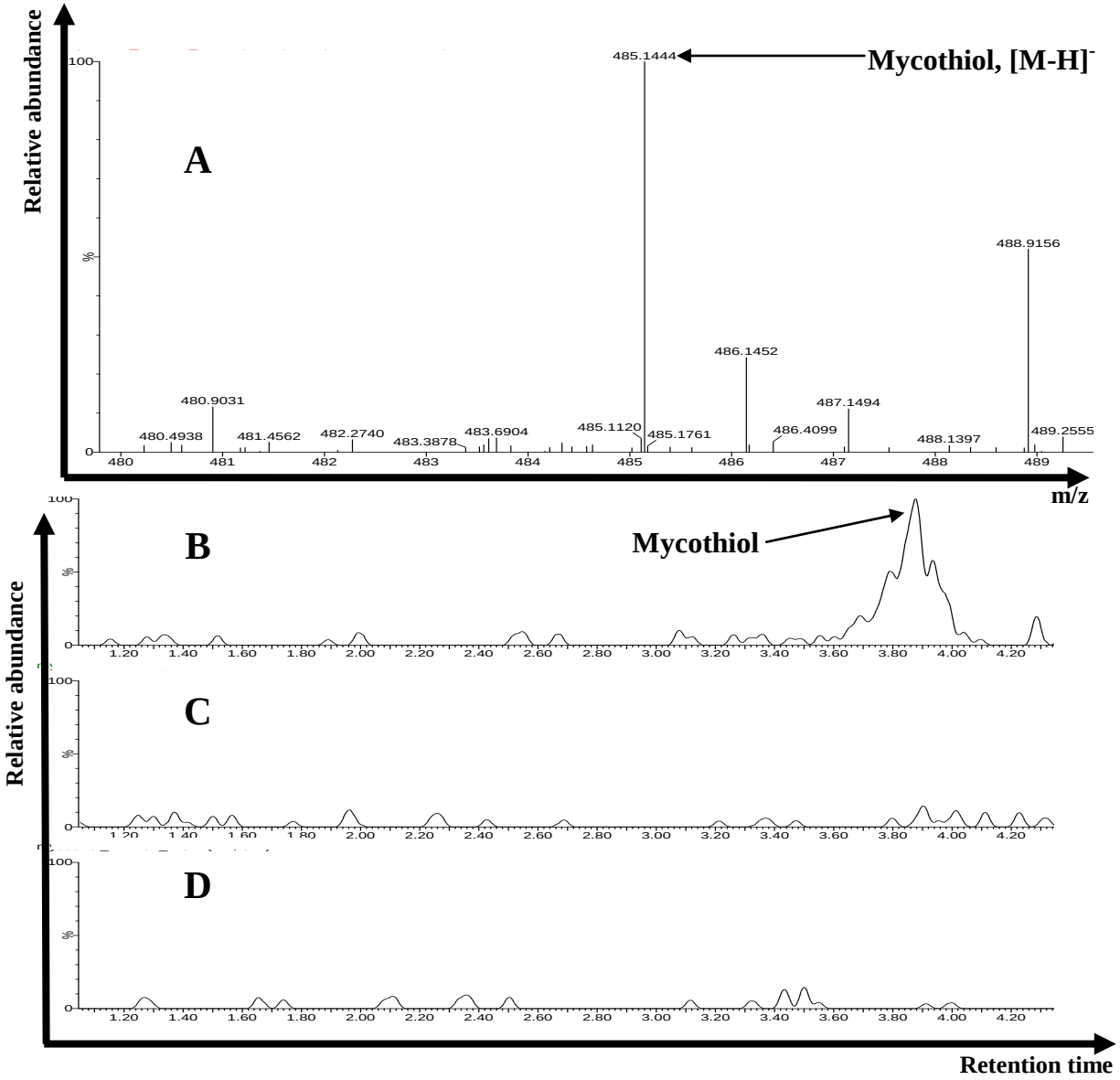

Figure S2: Detection of mycothiol in *M. smegmatis*

Liquid chromatography-electrospray ionization-high resolution mass spectrometry (LC-ESI-HRMS) was performed with a Waters Synapt G2 MS (Waters Corporation, Milford, MA, USA). Compounds were separated on a Phenomenex Synergi Fusion column (250 x 2 mm, 4 μm) at 25 °C using a 0.1% formic acid (in water) (solvent A) / acetonitrile (solvent B) gradient: Starting with 100% solvent A for 6 min at a flow rate of 0.2 ml/min. The acetonitrile was increased linearly to 95% over 21 min and maintained for 3 min. The column was re-equilibrated for 7 min (total run time was 32 min). Mycothiol was analyzed

in ESI negative and the MS instrument was operated in scan mode (cone voltage = 15 V). The source capillary was at 2.5 kV. The source and desolvation temperatures were 120 ºC and 275 ºC, respectively. The desolvation and cone gas flows were 650 and 50 L/h, respectively.

Figure S2A: The ESI negative mass spectrum (of the peak at 3.87 min; **Figure S2B**) of the cell lysate of the wild type. Analysis was performed using LC-ESI-HRMS. Accurate mass measurement (m/z 485.1444) was performed within 0.6 ppm of the calculated exact mass (m/z 485.1441) of the deprotonated mycothiol ion (6), which strongly indicates the presence of mycothiol.

Figure S2B, S2C, and S2D: The extracted ion chromatograms at m/z 485.144. Mycothiol was detected (retention time of 3.87 min) of the cell lysate of the wild type (**Figure S2B**), but not in its culture media (**Figure S2C**). As a negative control the cell lysate of the Δ*mshA* mutant was analysed (**figure S2D**).

Table S3 Quantification of ERG in *M.tb* (*H37Rv)* at OD_{600} of 0.5 (pg/10^5 CFU)

Intracellular	**Extracellular**
0.803 ±0.17	8.43 ±2.24

Data is representative of 3 separate experiments expressed as mean ± standard deviation

Table S4: Relative abundance of MSH expressed by the peak area of the extracted ion chromatogram m/z 485.

$mc^2$155	398.55 ± 19.36
Δ*egtD*	458.86 ± 23.04

Data is representative of 3 separate experiments expressed as mean ± standard deviation

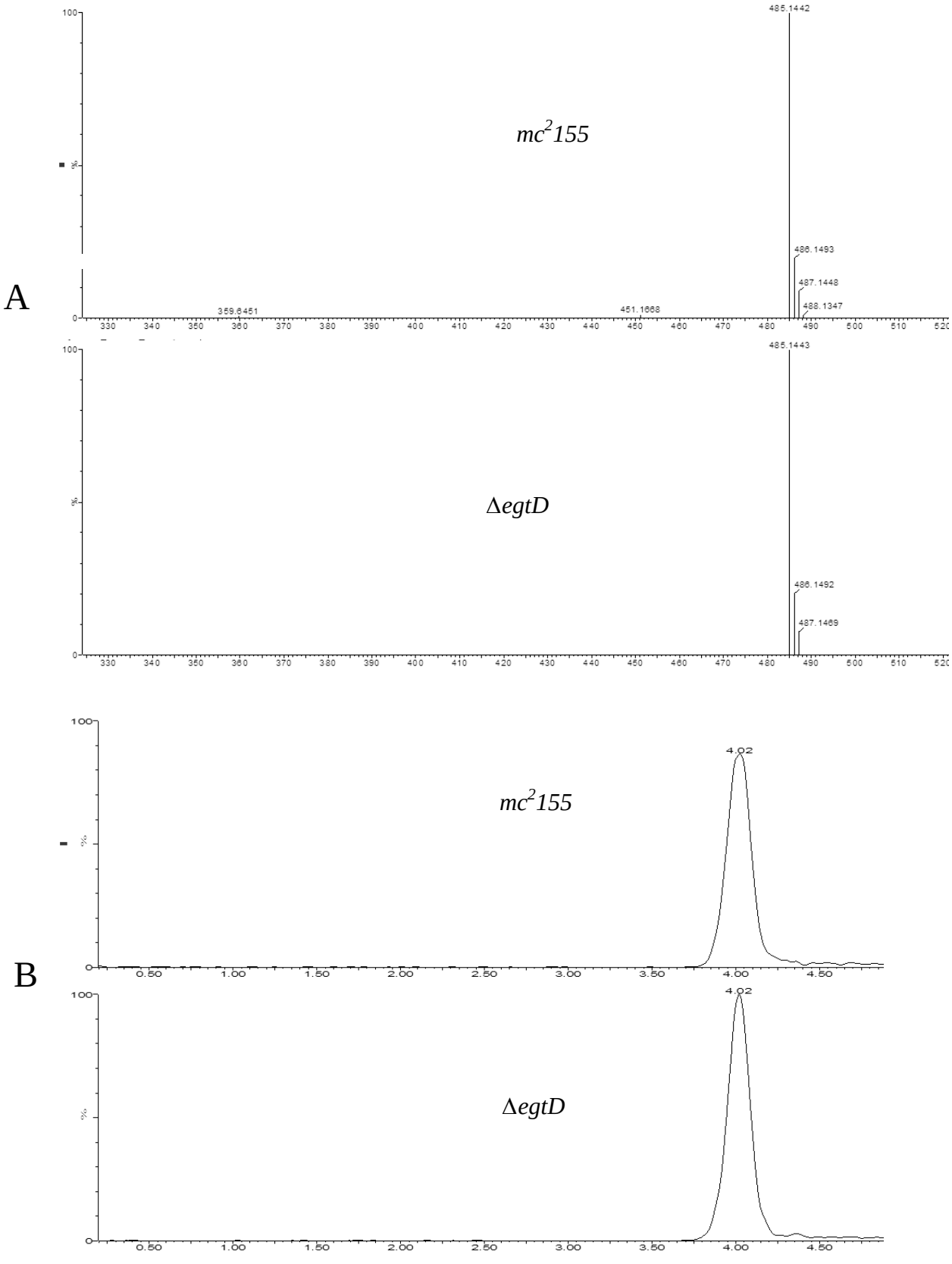

Figure S3: Relative quantification of mycothiol by LC-ESI-HRMS

Figure S3A: The ESI negative mass spectrum (of the peak at 3.87 min) of the cell lysate of the wild type and of the ERG-deficient single mutant.

Figure S3B: The extracted ion chromatograms at m/z 485.144.of the cell lysate of the wild type and of the ERG-deficient single mutant

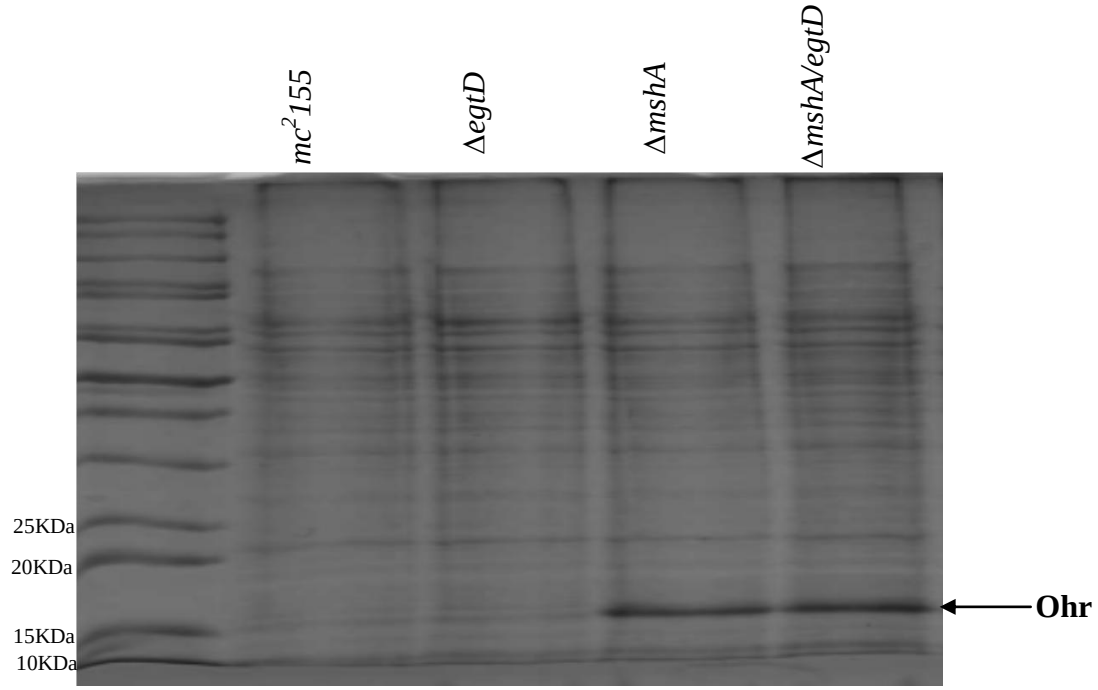

Figure S4: SDS PAGE of *M.smegmatis* cell extract

The different *M.smegmatis* strains were grown on 7H11 solid culture for 11 days, and proteins were extracted and analysed as previously described (7) (8).

Relative intensities of protein fragment bands at 15KDa between the different strains were determined using the UN-SCAN-IT automated digitizing system (V 5.1, Silk Scientific Corporation) and showed at least a 7-fold increase in band intensity in the Δ*mshA* and Δ*mshA/egtD* mutants. A similar banding pattern was observed when mycobacteria were grown in liquid culture to an OD_{600}~0.8 (figure not shown). The identity of the 15KDa fragment was revealed to be *MSMEG_0447* (Ohr) by mass spectrometry using the Thermo Scientific EASY-nLC II connected to a LTQ Orbitrap Velos mass spectrometer (Thermo

Scientific, Bremen, Germany). Quantification revealed a slight increase in the expression of Ohr in the MSH/ERG-deficient double mutant relative to the MSH-deficient single mutant. As was shown previously, Ohr is over-expressed in the *mshA M.smegmatis* mutant, but Ohr remained over-expressed in this same strain even when *egtD* was deleted. The wild type and Δ*egtD* mutant do not exhibit an over-expression of Ohr.

References

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