Supplementary Fig 1. Mass spectra profiles of mycolic acids from corynebacteria. *C. hofmanii*, peaks at m/z 495 and 521 for C32:0 and C34:1 acids; *C. matruchotii*, peaks at m/z 495, 521, 547 for C32:0, C34:1, C36:2 acids; *C. ovis*, one major homologue at m/z 495 for C32:0 corynomycolic acids; *C. vitarumen*, peaks at m/z 493, 495, 521, and 523 corresponding to C32:1, C32:0, C34:1, and C34:0 acids respectively; *C. diphtheriae*, one major homologue at m/z 495 for C32:0 corynomycolic acid C32H64O3.
**Supplementary Fig 2.** Mass spectra profiles of mycolic acids from nocardia. *N. brasiliensis*, C54, C56, C58 with 2 and 3 double bonds; *N. asteroides*, C54:2, C56:2. The value m/z 828 corresponds to homologue C56H108O3 with 2 double bonds; *N. rubra*, C40 to C48 with 1 and 2 double bonds; *N. corallina*, C40 to 46 with 1 and 2 double bonds.
Supplementary Fig 3. Mass spectra profiles of mycolic acids from various Mycobacterium tuberculosis (MTB) strains. The major MA species in M. tuberculosis are alpha and methoxy-MA, such as m/z 1136 for alpha-MA homologue C78H152O3 and m/z 1252 for methoxy-MA C85H168O4; while M. bovis BCG contains alpha-MA as well as keto-MA, such as m/z 1236 for keto-MA C84H164O4.
Supplementary Fig 4. Mass spectra profiles of mycolic acids of non-tuberculous mycobacteria: (A) Absence of oxygenated MA; α MA only: M. fallax, M. triviale; α and α'MA: M. abscessus, M. chelonae.
Supplementary Fig 4. Mass spectra profiles of mycolic acids of non-tuberculous mycobacteria: (B) α, keto, methoxy: *M. kansasii, M. ulcerans, M.gordonae.*
Supplementary Fig 4. Mass spectra profiles of mycolic acids of non-tuberculous mycobacteria: (C) α, (α'), epoxy: M. fortuitum, M. senegalense, M. chitae, M. smegmatis, M. porcinum, M. farginogenes.
Supplementary Fig 4. Mass spectra profiles of mycolic acids of non tuberculous mycobacteria: (D) α, (keto), wax : M. phlei, M. xenopi, M. avium complex (MAC). The presence of wax ester is evoked by the existence of dicarboxylic acids, at m/z 916 for the homologue C60H116O5.
Supplementary Fig 4. Mass spectra profiles of mycolic acids of non-tuberculous mycobacteria: (E) \( \alpha, \alpha', \) keto: *M. simiae*

Supplementary Fig 4. Mass spectra profiles of mycolic acids of non-tuberculous mycobacteria: (F) \( \alpha, \omega-1 \) methoxy: *M. alvei*
Supplementary Fig. 5. Tandem Mass Spectrometry of Mycolic Acids (MAs). Corresponding ESI/MS and MS/MS results for MAs derived from *Nocadia asteroids* and *Corynebacterium diphtheriae* are shown in A and B, respectively. Product ion analysis (MS/MS) of m/z 814 indicating the presence of C14:0 (m/z 227), C16:0 (m/z 255), and C18:0 (m/z 283) fatty acyls in the α-branch (A, inset). Product ion analysis (MS/MS) of m/z 523 indicating the presence of C16:1 (m/z 253), C18:0 (m/z 283) fatty acyls in the alpha-branch (B, inset).
Supplementary Fig. 6. Comparison of mycolic acid (MA) profiles of extracts from cultured mycobacterial strains with MA extracts from *Mycobacterium tuberculosis* (MTB)-infected mice and mycobacteria-infected human patients. MA were isolated from cultured mycobacteria (black font), lung tissue of mice infected with MTB (green), sputum of TB patients (red), and sputum (NTM sputum) and corresponding cultured strains (NTM isolate) of patients infected with non-tuberculous mycobacteria (NTM) (blue). To determine which mycobacterial strains the patients’ MA profiles are most similar to, MA profiles were subject to hierarchical cluster analysis with Euclidean distance as the distance metric. All sputum samples from TB patients clearly grouped with the MA profiles derived from MTB strains (box), possibly by the presence of abundant MA species with C26:0 fatty acyl alpha-branch in both α- and methoxy-MAs (arrow). The same is true for MA isolated from the lungs of TB infected mice. The MA profile of NTM isolate-1, genetically characterized as *Mycobacterium avium* complex (MAC), aligned with MAC in our analysis. NTM sputum-2 and NTM isolate-2 (from the same patient) aligned closest with the MA profile of *M. chelonae*. Interestingly, strain classification of NTM-2 identified it as *M. massiliense*, which is indistinguishable from *M. chelonae / M. abscessus* by partial 16S rRNA gene sequencing.

670 MRM transitions

<table>
<thead>
<tr>
<th>Non-oxygenated mycolic acids (α, α’, α-2)</th>
<th>Oxygenated mycolic acids</th>
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**Supplementary Table**

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<th>Strain</th>
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<td>MTB H37Ra</td>
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<td>MTB mouse lung</td>
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<td>Patient S5</td>
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<td>Patient S4</td>
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<td>MTB CDC1551</td>
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**Supplementary Figure**

- **Non-oxygenated mycolic acids (α, α’, α-2)**
  - M. xenopi
  - M. simiae
  - M. chelonea
  - M. abscessus
  - NTM isolate-2
  - NTM sputum-2
  - M. phlei
  - M. gordonae
  - M. fallax
  - M. ulcerans
  - M. triviale
  - M. kansasii
  - MAC Q14
  - MAC M151
  - NTM isolate-1
  - M. smegmatis
  - M. senegalense
  - M. fortuitum
  - M. porcinum
  - M. chltae
  - M. farcinogenenes
  - M. alvei
  - M. bovis BCG

- **Oxygenated mycolic acids**
  - C26-α-MAs
  - C24-α-MAs
  - C24-keto-MAs
  - C26-keto-MAs
  - C24-meo-MAs
  - C26-meo-MAs

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*Normalized intensity*