

Fig. S1. *M. tuberculosis* utilizes [4-¹⁴C]cholesterol as energy and carbon source. A) *M. tuberculosis* (1.6 OD/ml, live (L) or heat inactivated (HI) at 100°C/15 min) was incubated with 1 μCi/ml [4-¹⁴C]Cholesterol (50 mCi/mmol) for 24 and 72 h at 37 °C. Labeled CO₂ trapped on strips was determined and expressed in DPM. Data are representative of two independent experiments duplicate. B) *M. tuberculosis* cells were washed, extracted with chloroform/methanol 2:1 and analyzed by TLC using chloroform/ammonium acetate (97:3) as solvent system. Radiolabeled lipids were detected by PhosphorImager. B, bacteria cells; M, spent medium; SF, solvent front; Chon, cholestenone; Cho, cholesterol; AD, androstenedione; SF, solvent front.

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1B4V --GYVPAVVIGTGYGAAVSALRLGEAGVQTLMLEMGQLWNQPGPDGNIFCGMLNPKRSS
ML MKPDYDVLII GSGFGGSVSALRLTEKGYRVGVLEAGRRFADEDFA-----
TB MKPDYDVLII GSGFGGSVTALRLTEKGYRVGVLEAGRRFSDEEFA-----
      .:.*:.*:.*:.*:.* * * .: .:* * : :

1B4V WFKNRTEAPLGSFLWLDVVRNIDPYAGVLDNRVNDQMSVYVGRGVGGSLVNGGMAVEP
ML ----KTSWDLRKFLWAPKLG-----YGIQRIHLLRNVMILAGAGVGGGSLNYANTLYVP
TB ----KTSWDLRKFLWAPRLG-----YGIQRIHPLRNVMILAGAGVGGGSLNYANTLYVP
      :. * .*** : * : : . * ***** . *

1B4V KRSYFEEILP-RVD-SSEMYDRYFPRANSMLRVNHIDTKWFEDTEWYKFARVSREQAGKA
ML PEPFFANQQWAHITDWHSELAPHYDQAQRMLGVV-----CNPTFTDADRILKEVVDEM
TB PEPFFADQQWSHITDWRGELMPHYQQAQRMLGVV-----QNPTFTDADRIVKEVADEM
      .:* : : : : : * * * : : . * : * . :

1B4V GLGTVFVFNVDYFGYMQR--EAAGE-----VPKSALA-TEVIYGNHNG-KQSL
ML GFGDTFVPTPVGVVFFGPDGTQTPGRTVADPYFGGVGPVRTGCLECGCCMTGCRHGAKNTL
TB GFGDTWVPTPVGVVFFGPDGKTPGKTVDPYFGGAGPARTGCLECGCCMTGCRHGAKNTL
      *:* .:*. . : : : * . . . . * : * .** *::*

1B4V DKTYLAAALGTGKVTIQTTLHQVKTIRQTKDGGYALTVEQKDTDGKLL--ATKEISCRYLF
ML VKNYLGLAESAGAQVI-PMTTVKGFELRSEGLWEVHTV---RTGSWLRRRRTFTAHL
TB VKNYLGLAESAGAQVI-PMTTVKGFERRSDGLWEVRTV---RTGSWLRDRRTFTAHL
      *.*. * .:* . * : * * . . : * : : . * . * : : . * .

1B4V LGAGSLGSTEELLVRARDTGTLPNLNSEVAGWGPNGNIMTARANHMWNPTGAH-----Q
ML LAAGTWGTQRLFRMRDQGLPRLSQRLGVLTRTNSSEIVGATLNVMPDRDLTHGVAIT
TB LAAGTWGTQHLLFKMRDRGRPLGLSKRLGVLTRTNSSEIVGAATLKVNPDDLTHGVAIT
      *.*. * : .*. : * * * * * . . . : * : : . . . *

1B4V SSI-----PAL----GIDAWDNSDSSVFAIAPMPAGLETWVSLYLAIKPNQRGTF
ML SSIHPTSDTHIEPIRYGKGSNAMGLLQTLMTDGGPEGTDVPRWRQLLHQASEDFRMLR
TB SSIHPTADTHIEPVRYGKGSNAMGLLQTLMTDGGPQGTDPVPRWRQLLQATASQDFRGTIR
      *** : * : * : : : . * : : * . * : : * :

1B4V VYDA-----ATDRAKLNWTRDQNAFA-----VN----AAK
ML LINPRRWSERTVIALVMQHLDNSITFTTKRGLGIRWYSSKQNGEPNPSWIPIGNEVTR
TB MLNPRQWSERTVIALVMQHLDNSITFTTKRGLGIRWYSSKQGHGEPNPTWIPIGNQVTR
      : : : : * . . . . : : :

1B4V ALFDRINKANGTIYRYDLFGTQLKAFADDFCYHPLGGCVLGA-----TDDYGRVAGYKN
ML RLAAKIDGVAG-----GTWGELEFNIPLTAHFLGGAVIGDNAEHGVIDPYHRVYGYPT
TB RIAAKIDGVAG-----GTWGELEFNIPLTAHFLGGAVIGDDPEHGVIDPYHRVYGYPT
      : : * . * ** : * : * * * . * . * * * * .

1B4V LYVTDGSLIPGSVGNPFVITIALAERNVERIKQDVTAS-----
ML LYVVDGAAISANLGNVPSLSIAAQAERAASLWPNKGQHDQRPRQGESYRRLAPIADHPV
TB LYVVDGAAISANLGNVPSLSIAAQAERAASLWPNKGETDRRPPQGEPIYRRLAPIQPAHPV
      ***.*. * .:.*. * : * * * * . . :

1B4V -----
ML VPAAELGALRWL-----
TB VPADAPGALRWLPIDPVSNAG

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S2: Putative features of *M. leprae* ChoD protein.

Alignment of primary sequences of a functional cholesterol oxidase (1B4V, *Streptomyces* sp.) and putative ChoD proteins from *M. leprae* (ML) and *M. tuberculosis* (TB). Asterisks (*) indicate positions, which have a single, fully conserved amino acid residue. Colons (:) indicate conservation between groups of strongly similar properties and periods (.) indicate conservation between groups of amino acids with weakly similar properties, according to Clustal software. Amino acids and sequences shaded in grey are not fully conserved in ML and TB proteins. FAD binding motifs are indicated in yellow, active site residues in highlighted in green and the loop regions important for substrate specificity are coloured in blue.

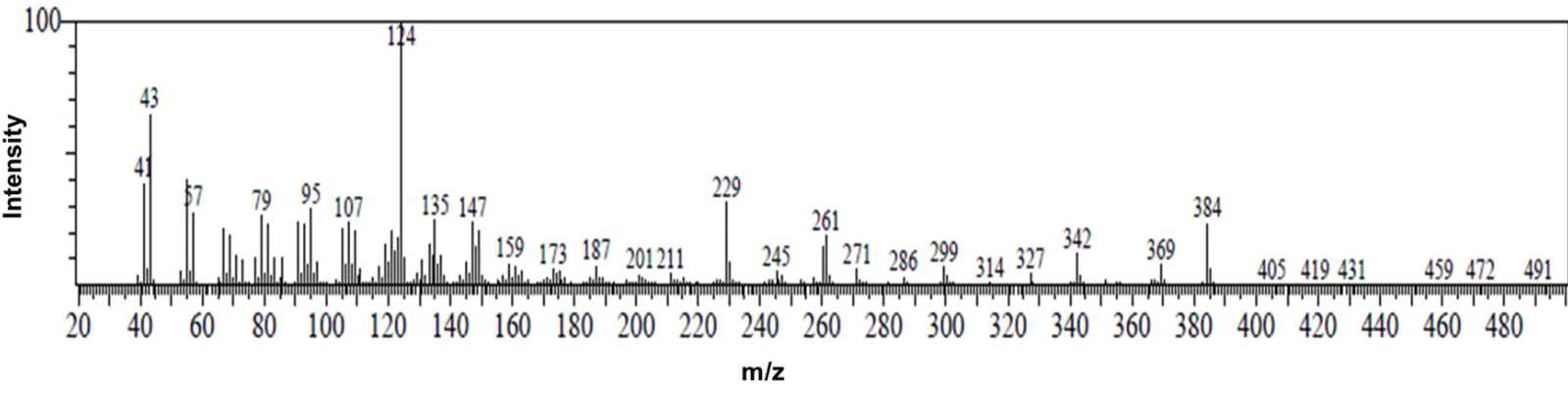


Fig. S3. Mass spectrum of the peak correspondent to cholesterol present on lipid extract of *nude* mouse derived-*M. leprae*.

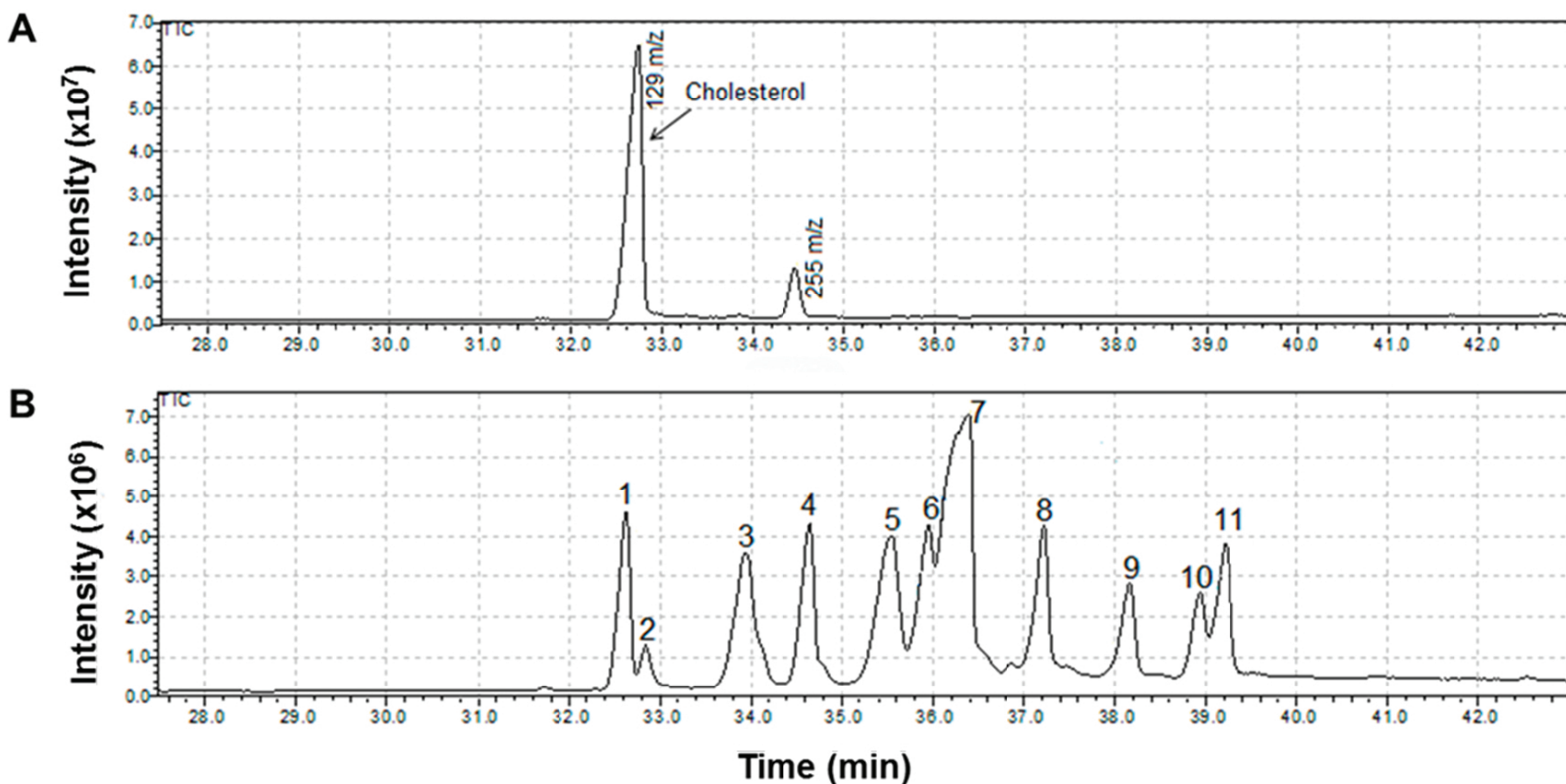


Fig. S4. GC-MS analysis of non-infected *nude* mouse footpad lipid extract **A)** GC-MS chromatogram of Bis(trimethylsilyl)trifluoroacetamide (BSTFA) - trimethylchlorosilane (TMCS)-derived extracts of non-infected *nude* mouse footpad and **B)** steroid standards mix. Standards: 1, Cholesterol; 2, ergosta-7,22-dien-3-ol; 3, cholesta-5,7-dien-3-ol; 4, cholesta-8,24-dien-3-ol; 5, ergosta-5,7,22-trien-3-ol; 6, cholest-4-en-3-one; 7, ergost-8(14)-en-3-ol; 8, stigmasterol; 9, ergost-7-ene; 10, lanosta-8,24-dien-3-ol; 11, β -Sitosterol