

Supplemental Data for

Pathway Profiling in *Mycobacterium tuberculosis*: Elucidation of a Cholesterol-Derived Catabolite
and the Enzymes that Catalyze its Metabolism *

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Running title: *Cholesterol metabolic profile of the M. tb Δigr mutant*

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Table S1. Cholesterol-derived metabolite parent ions (MH⁺ or MNa⁺) identified using LC/MS/MS are listed. Data were analyzed with XCMS. The exact masses were utilized to determine molecular formulae. Unnatural ¹³C isotopomers were used to identify cholesterol-derived compounds. Isotopomers and fragmentation were used to determine likely structures. Compound numbers correspond to the structures shown in Figure S2.

A. WT H37Rv and <i>igr</i> complement ions						
Exact Mass	Molecular Formula	Calculated Mass	Retention Time (min)	Number of ¹³C labels	Compound	Integrated Peak Intensity
299.1638	C ₁₉ H ₂₃ O ₃	299.1647	61.9	3	4 + O - 2H	1.3E+07
303.1950	C ₁₉ H ₂₇ O ₃	303.1960	66.5	3	5 + O	7.2E+06
285.1845	C ₁₉ H ₂₅ O ₂	285.1855	69.9	3	4	3.0E+08
287.2001	C ₁₉ H ₂₇ O ₂	287.2011	71.0	3	5	1.8E+08
B. <i>Δigr</i> ions						
Exact Mass	Molecular Formula	Calculated Mass	Retention Time (min)	Number of ¹³C labels	Compound	Integrated Peak Intensity
355.1746	C ₁₈ H ₂₇ O ₇	355.1757	57.0	3		9.7E+06
297.1691	C ₁₆ H ₂₅ O ₅	297.1702	57.8	3	6	1.9E+08
295.1534	C ₁₆ H ₂₃ O ₅	295.1545	58.0	3	7	1.2E+08
283.1897	C ₁₆ H ₂₇ O ₄	283.1909	59.1	3		1.3E+08
281.1742	C ₁₆ H ₂₅ O ₄	281.1753	62.8	3		6.7E+07
355.2109	C ₁₉ H ₃₁ O ₆	355.2121	62.8	3		5.6E+07
398.1989	C ₁₃ H ₃₀ O ₇	298.1992	62.9	3		6.1E+07
709.4133	-		62.9	3		3.0E+06
295.1899	C ₁₇ H ₂₇ O ₄	295.1909	63.4	3		4.1E+08
277.1431	C ₁₆ H ₂₁ O ₄	277.1440	65.8	3		2.8E+07
323.1850	C ₁₈ H ₂₇ O ₅	323.1859	66.2	3		6.9E+07
311.1846	C ₁₇ H ₂₇ O ₅	311.1858	67.4	3	1	2.6E+09
309.1692	C ₁₇ H ₂₅ O ₅	309.1702	68.4	3	8	8.7E+08
279.1950	C ₁₇ H ₂₇ O ₃	279.1960	70.4	3		2.9E+08
337.2006	C ₁₉ H ₂₉ O ₅	337.2015	70.8	3		8.9E+07
744.5028	-		70.9	3		3.7E+06
293.1745	C ₁₇ H ₂₅ O ₄	293.1753	71.2	3		2.3E+07
325.2004	C ₁₈ H ₂₉ O ₅	325.2015	71.5	3	9	6.6E+07
305.2107	C ₁₉ H ₂₉ O ₃	305.2117	71.9	3		1.5E+07
343.2265	C ₂₂ H ₃₁ O ₃	343.2273	72.3	4	10	2.7E+07
345.2420	C ₂₂ H ₃₃ O ₃	345.2430	72.3	4	11	1.6E+07
351.2161	C ₂₀ H ₃₁ O ₅	351.2172	72.7	3		8.6E+06
371.2574	C ₂₄ H ₃₅ O ₃	371.2586	73.6	4	12	5.4E+07
429.2991	C ₂₇ H ₄₁ O ₄	429.3005	73.7	5	13	4.9E+06

Figure S1. TLC of *M. tuberculosis* extracts cultured with LDL-¹⁴C-[1,7,15,22,26]-cholesterol. 10,000 cpm were loaded per lane. TLC was resolved with 75:25 (toluene:acetone v/v) and visualized by autoradiography. **A.** *M. tuberculosis* H37Rv cell pellet. **B.** *M. tuberculosis* H37Rv culture medium. **C.** *M. tuberculosis* Δ *igr* cell pellet. **D.** *M. tuberculosis* Δ *igr* culture medium. **E.** *igr* complement cell pellet. **F.** *igr* complement culture medium.

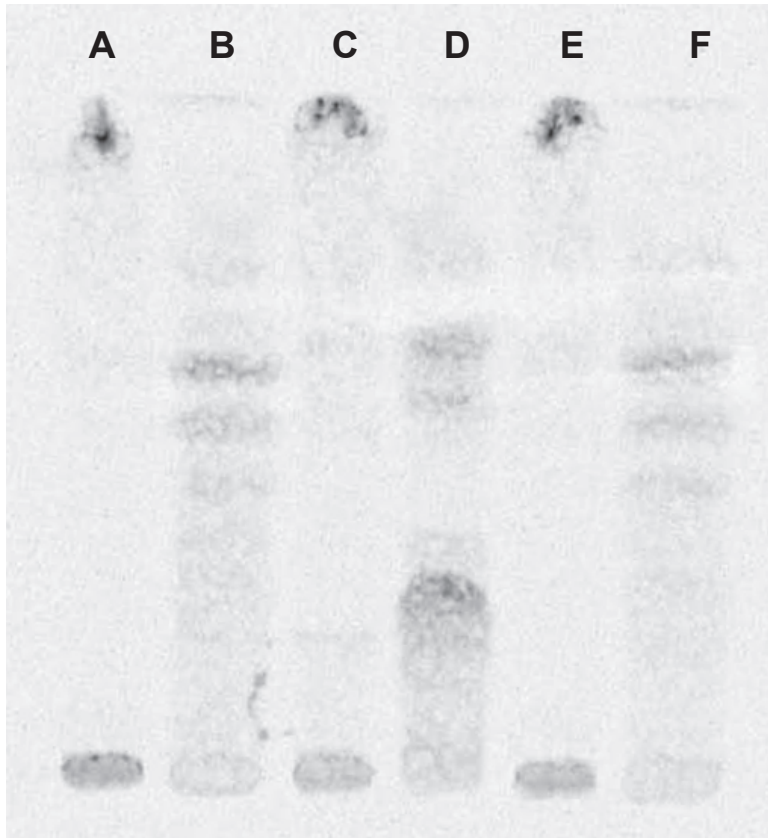


Figure S2. Assigned structures of *Δigr* metabolite ions identified by LC/MS/MS.

