

Supplementary information

Table S1: Primers used for RT-PCR analysis

<i>Ifng</i> forward	5'-GCCAAGCGGCTGACTGA-3'
<i>Ifng</i> reverse	5'-TCAGTGAAGTAAAGGTACAAGCTACAATCT-3'
<i>Il17a</i> forward	5'-CCACGTCACCCTGGACTCTC-3'
<i>Il17a</i> reverse	5'-CTCCGCATTGACACAGCG-3'
<i>iNOS</i> forward	5'-GGAGCAGGTGGAAGACTATTTCTT-3'
<i>iNOS</i> reverse	5'-CATGATAACGTTTCTGGCTCTTGA-3'
<i>Tnfa</i> forward	5'-CATCTTCTCAA AATTCGAGTGACAA-3'
<i>Tnfa</i> reverse	5'-TGGGAGTAGACAAGGTACAACCC-3'
<i>Il12p35</i> forward	5'-GATGACATGGTGAAGACGGCC-3'
<i>Il12p35</i> reverse	5'-GGAGGTTTCTGGCGCAGAGT-3'
<i>Hmbs</i> forward	5'-GAAACTCTGCTTCGCTGCATT-3'
<i>Hmbs</i> reverse	5'-TGCCCATCTTTCATCACTGTATG-3'

Table S2

Pulmonary IFN- γ response of C57BL/6 mice infected intratracheally with *Mtb* WT, MGM1990 or MGM1991

A	IFN-γ production (pg/ml)				
	Group^a	rAg85A	85A₂₄₁₋₂₆₀	ESAT6₁₋₂₀	Medium
WT		4,550	3,770	15,590	<5
MGM1990		8,690	10,790	13,450	<5
MGM1991		2,130	2,170	5,370	<5
Naive		30	<5	<5	<5

B	IFN-γ (SFC/10⁶ cells)				
	Group^a	rAg85A	85A₂₄₁₋₂₆₀	ESAT6₁₋₂₀	Medium
WT		16	98	436	2
MGM1990		168	254	494	6
MGM1991		4	22	62	6
Naive		ND	ND	ND	ND

a Mice were infected with 10⁴ CFU of *Mtb* WT, MGM1990 or MGM1991 by the intratracheal route. Lungs were recovered 3 weeks after the infection, homogenized and pulmonary leucocytes were cultured with 5 μ g/ml Ag85A, 10 μ g/ml 85A₂₄₁₋₂₆₀, 10 μ g/ml ESAT6₁₋₂₀ or cultured in the absence of antigen (medium). (A) IFN- γ secretion detected by ELISA in 72h culture supernatant. (B) IFN- γ detected by ELISPOT assay. ND: no spots detected

Table S3

Spleen cell cytokine response of C57BL/6 mice infected intratracheally with 10^4 CFU of *Mtb* WT, MGM1990 or MGM1991

A		IFN-γ secretion pg/ml		
Group^a	PPD	ESAT6₁₋₂₀	Medium	
WT	41,890 \pm 1,757	19,706 \pm 8,182	<5	
MGM1990	26,888 \pm 13,276*	5,127 \pm 4,381**	<5	
MGM1991	32,737 \pm 1,758*	7,407 \pm 3,729**	213 \pm 112	
Naive	3,453 \pm 5,097	1,340 \pm 2,996	27 \pm 38	
B		IL-17A secretion pg/ml		
Group^a	PPD	ESAT6₁₋₂₀	Medium	
MGM1985	35,2 \pm 3,9	17,4 \pm 12	<4	
MGM1990	42,5 \pm 2,8**	6,6 \pm 5	<4	
MGM1991	26,0 \pm 14,2	11,5 \pm 6,2	<4	
Naive	<4	11,5 \pm 10	<4	

a Mice were infected with 10^4 CFU of *Mtb* WT, MGM1990 or MGM1991 by the intratracheal route. Splenocytes were recovered 3 weeks after infection and cultured with 25 μ g/ml PPD, 10 μ g/ml ESAT6₁₋₂₀ or cultured in the absence of antigen (medium). Results expressed as mean \pm SD cytokine levels (pg/ml) of four (MGM1991 /naive) or five (WT/1990) mice tested individually in each group. (A) IFN- γ secretion, (B) IL-17A secretion. * $P < 0,05$ ** $P < 0,01$ as compared to cytokine levels in mice infected with *Mtb* WT, (Mann-Whitney test)

Table S4

Spleen cell cytokine response of C57BL/6 mice infected intratracheally with 10⁴ CFU of *Mtb* WT, MGM1990 or MGM1991

A			
IFN-γ secretion pg/ml (mean\pmSD)			
Group^a	PPD	ESAT₁₋₂₀	Medium
WT	79,820 \pm 26,214	39,916 \pm 10,474	<5
MGM1990	49,930 \pm 34,27	10,493 \pm 9,188*	139 \pm 311
MGM1991	54,209 \pm 1,020	14,960 \pm 8,584*	104 \pm 208
Naive	2,790 \pm 2,475	<5	<5
B			
IL-17A secretion pg/ml (mean\pmSD)			
Group^a	PPD	ESAT_{6 1-20}	Medium
WT	70 \pm 26	31 \pm 20	6 \pm 5
MGM1990	57 \pm 28	39 \pm 17	34 \pm 23
MGM1991	85 \pm 21	33 \pm 15	17 \pm 6
Naive	38 \pm 29	10 \pm 5	15 \pm 7

a Mice were infected by 10⁴ CFU of WT, MGM1990, or MGM1991 by the intratracheal route. Splenocytes were recovered 10 weeks after infection and cultured with 25 μ g/ml PPD, 10 μ g/ml ESAT_{6 1-20} or cultured in the absence of antigen (medium). Cytokine production detected by ELISA. (A) IFN- γ secretion, (B) IL-17A secretion. * P <0,05 MGM1990 or MGM1991 vs WT, (Mann-Whitney test).

Fig S1: Confirmation of PDIM biosynthesis in mycolic acid methyltransferases knockouts.

Wild type, MGM1990, or MGM1991 were labeled with C¹⁴ propionic acid and chloroform:methanol extracts prepared. Shown are thin layer chromatography analysis of wt, MGM1990 and MGM1991, confirming the presence of PDIM.

Fig S2: Mycolic acid analysis of *in vivo* revertants of MGM1990.

After passage in a mouse, many colonies of MGM1990 assumed the morphology of MGM1991. Shown is TLC analysis of mycolic acid methyl esters from MGM1991, MGM1990, or 6 colony morphology variants that arose during mouse passage of MGM1990. To confirm loss of the inserted *mmaA3-mmaA4* cassette, mycolic acids were extracted from 6 colonies. Left: MGM1991. The rightmost strain was named MGM1995.

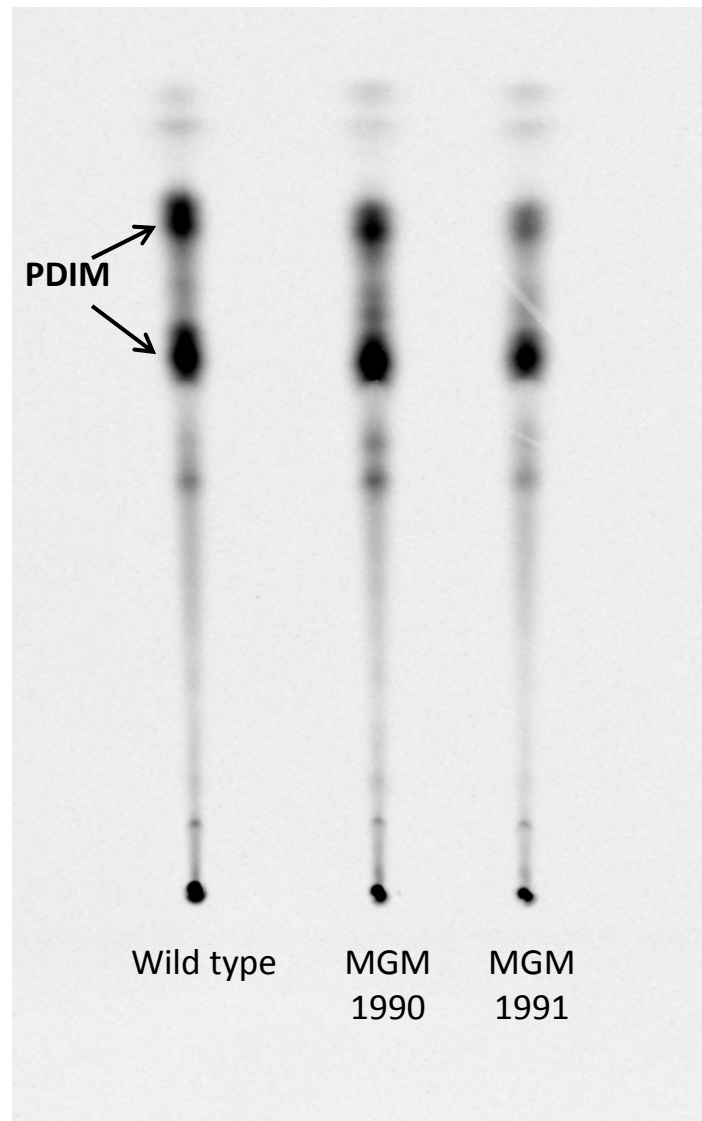


Figure S1

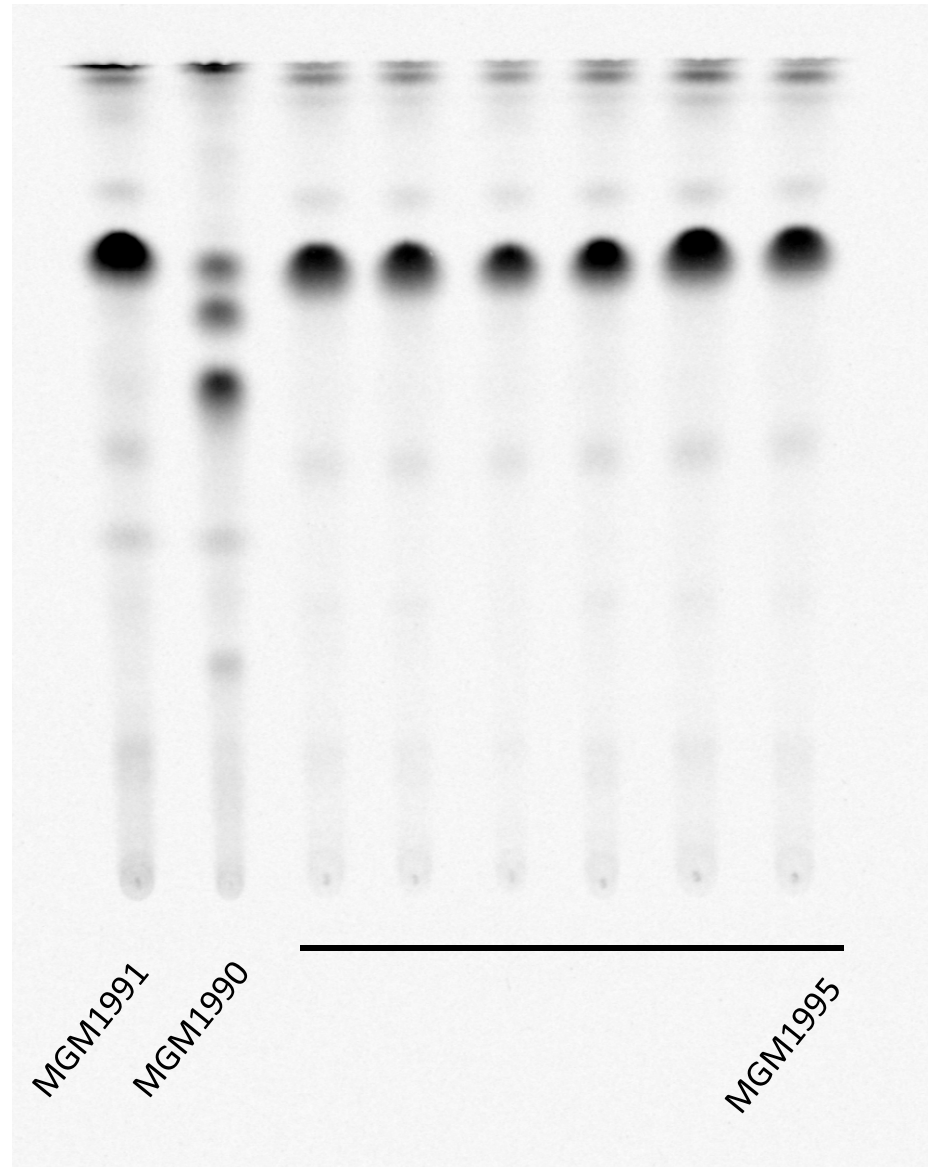


Figure S2