Supplemental materials

The *Mycobacterium tuberculosis* MmpL11 cell wall lipid transporter is important for biofilm formation, intracellular growth and non-replicating persistence

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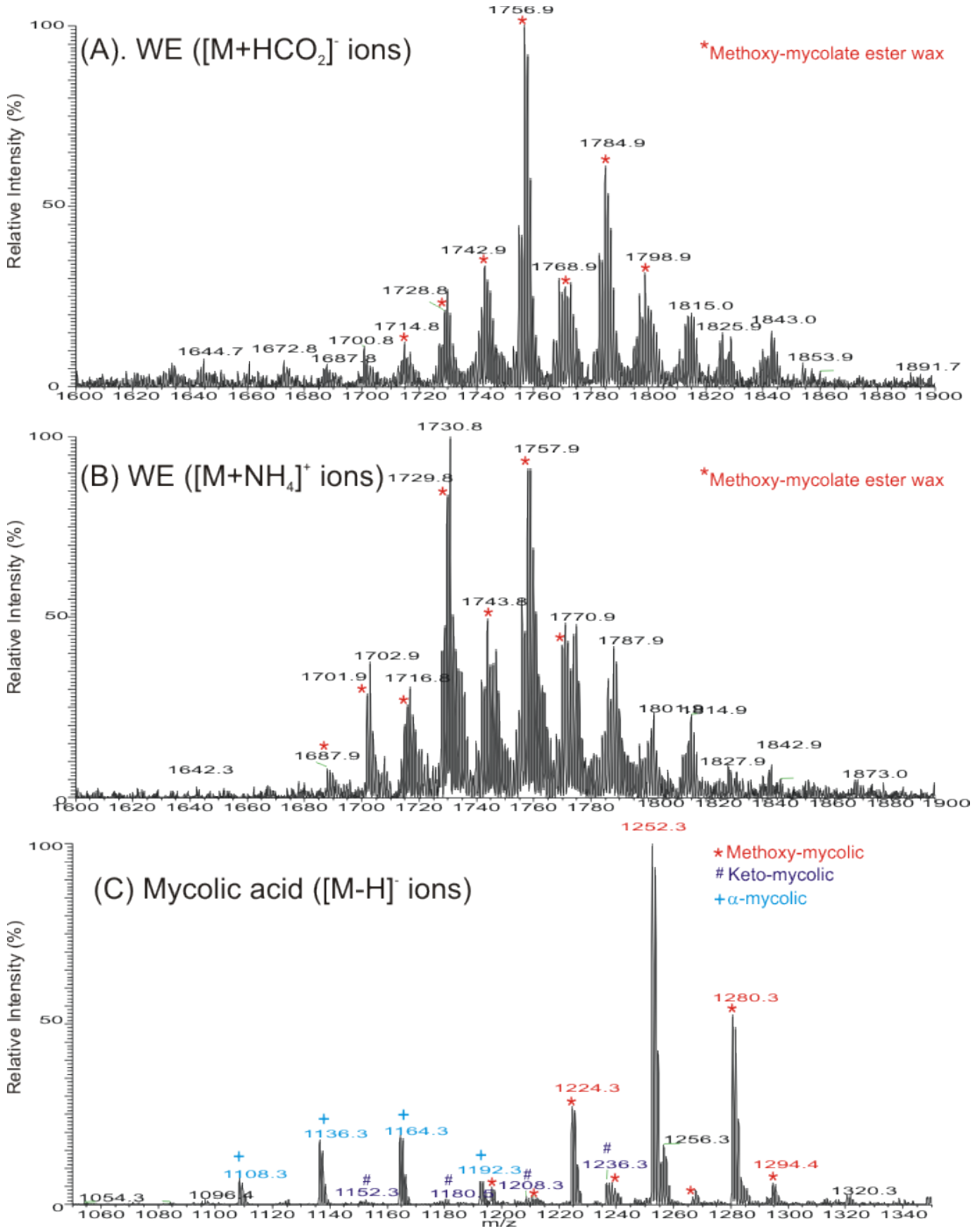

Figure S1. The ESI mass spectra of the mycolate ester wax seen as (A) the $[M + HCO_2]^-$ ions in the negative-ion mode, and (B) the $[M + NH_4]^+$ ions in the positive-ion mode isolated from *M. tuberculosis* H3Rv. Panel (C) is the ESI mass spectrum of the $[M – H]^-$ ions of mycolic acid isolated from the same lipid extract. The profiles of the spectra (Panels A and B), which are dominated by the methoxy wax ester ion species (marked with "*") are similar to that of mycolic acids (Panel C), of which the methoxy mycolic acids (marked with "*") are also predominant. The results point to the notion that the wax ester molecules (Panels A and B) consists of a

tritriacontadienol (33:2) linked to the mycolic acid via an ester bond to form a tritriacontadienyl mycolate ester, consistent with results reported previously (1).

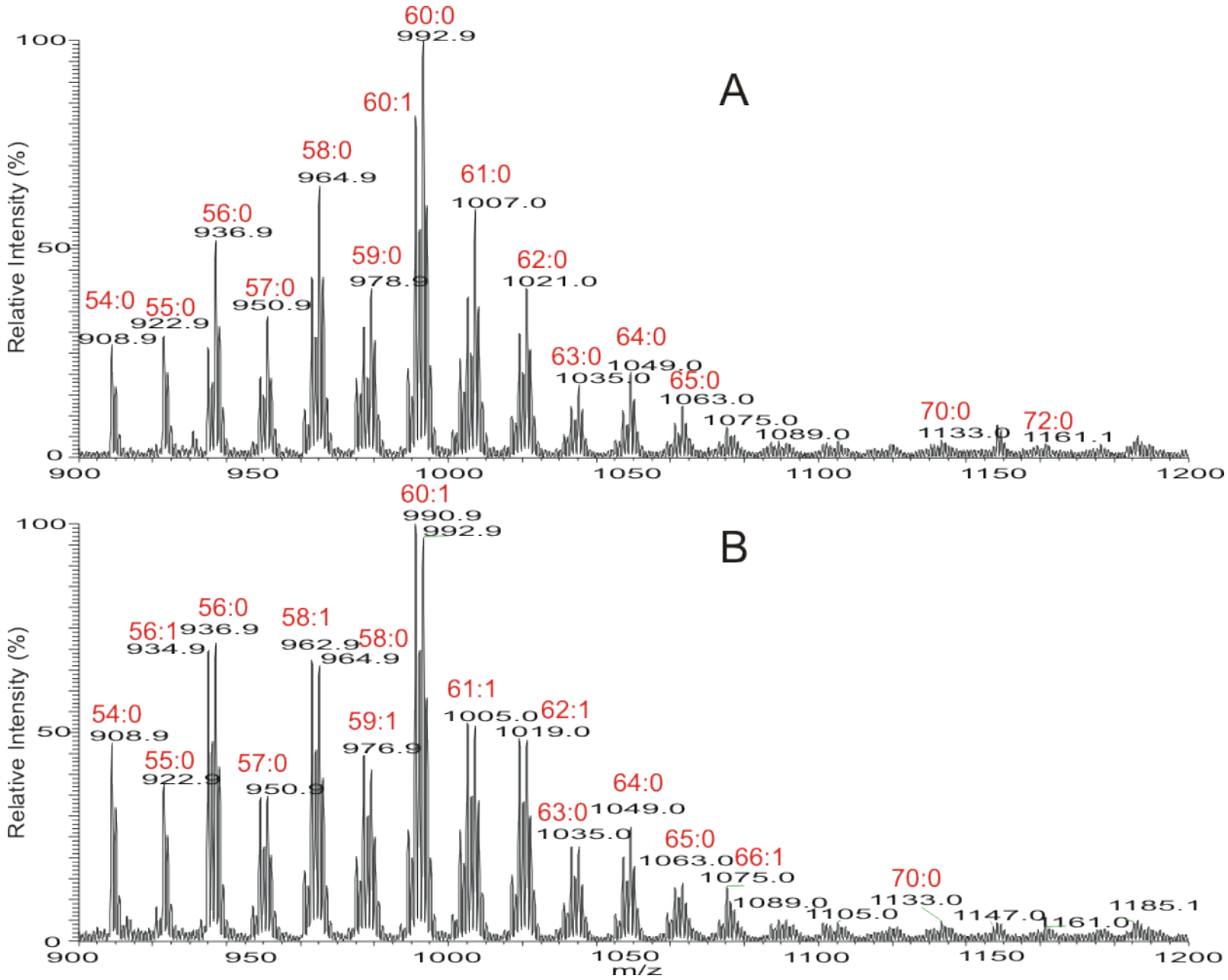

Figure S2. The ESI mass spectra of the $[M + NH_4]^+$ ions of TAGs consisting of a very long chain fatty acyl substituent (>20:0) from the lipid extracts isolated from (A) WT H37Rv, and (B) *mmpL11* mutant. Designations in red represent triacylglycerols in the forms of "Total Number of Carbon chain:number of unsaturation".

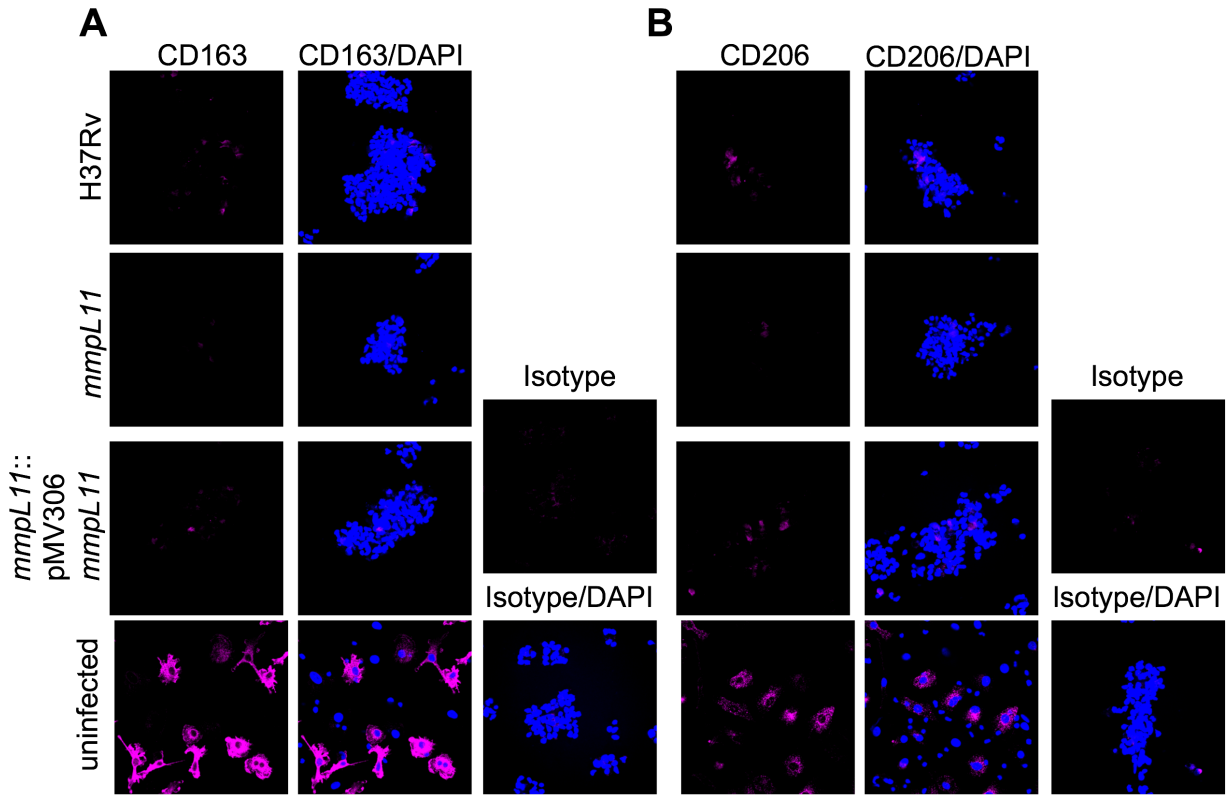

Figure S3. In vitro granuloma structures have low expression of CD163 and CD206. In vitro granuloma structures were fixed at t=7 and labeled for CD163 (A, n=2) and CD206 (B, n=1).

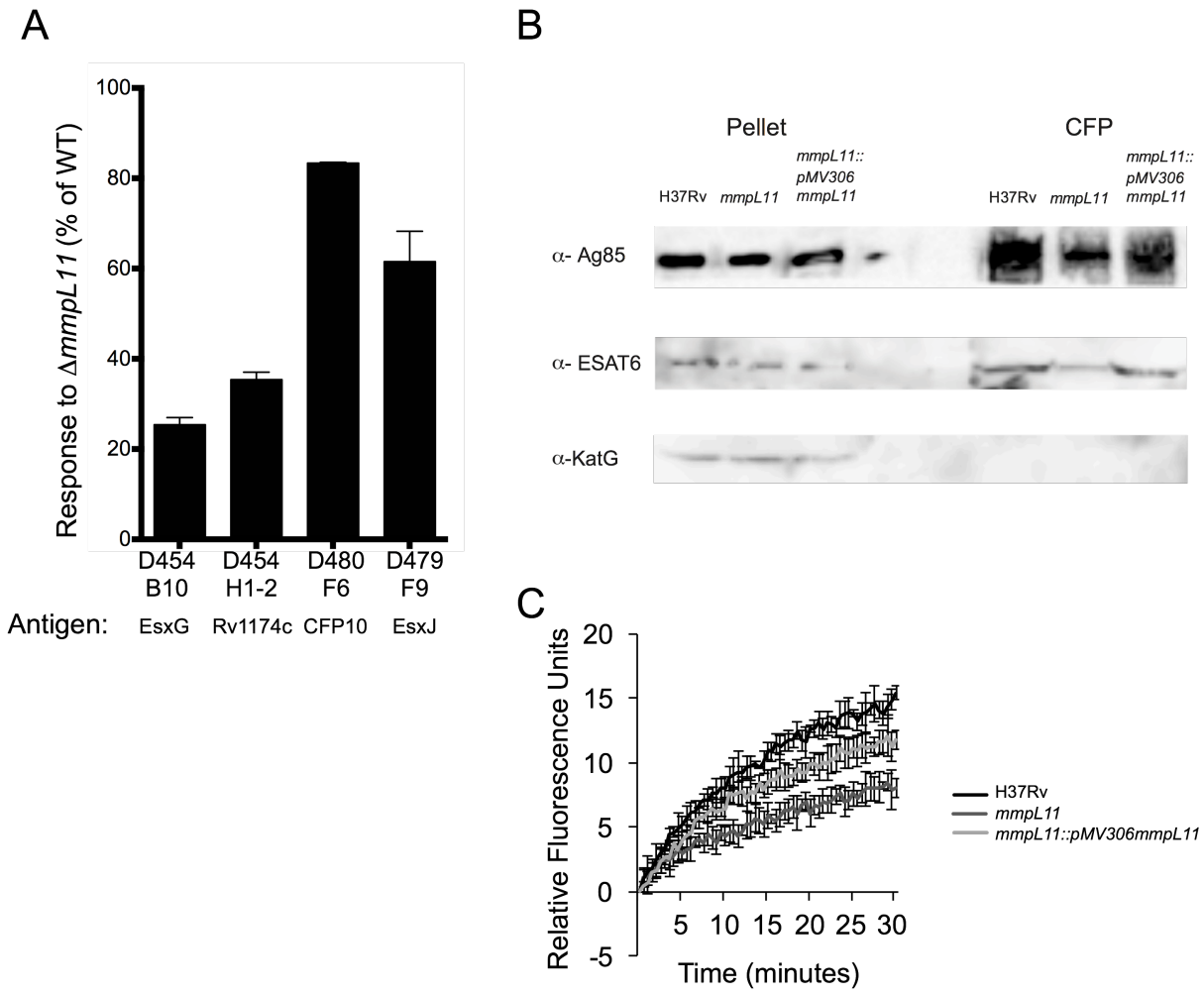

Figure S4 A. Monocyte-derived dendritic cells (DC) isolated as previously described (2) were infected with wild-type *M. tuberculosis* or the *mmpL11* mutant (MOI:5) and used as antigen presenting cells in an IFN-γ ELISPOT assay. 10,000 infected DC were plated in each well and the IFN- γ response of 20,000 CD8+ T cell clones was measured. The antigen recognized by each clone has previously been determined (3-4) and is indicated below the graph. The response of the T cell clones to DC infected with the *mmpL11* mutant strain is shown as a percent of the response to DC infected with wild-type *M. tuberculosis*. Results are the mean and SD from two independent experiments performed in duplicate. B. Western analysis of total lysates and culture filtrate proteins (CFP) of wild-type *M. tuberculosis* or the *mmpL11* mutant. Logarithmic-phase mycobacterial cultures grown in 7H9 medium were normalized to OD_{600}, washed once in PBS, and resuspended in Sauton's medium without Tween 80 for five days. Following centrifugation, the CFP were obtained by TCA precipitating culture supernatants and total lysates obtained by bead-beating cell pellets. Proteins were resolved by SDS-15%PAGE, transferred to nitrocellulose and probed with antibody against secreted proteins Ag85 (BEI resources, NR13800) and ESAT-6 (BEI Resources, NR13803), and cytosolic protein KatG (BEI Resources, NR13793). C. *M. tuberculosis* strains were incubated in the presence of 20 μM ethidium bromide. Accumulation of ethidium bromide was followed over time by monitoring

emission at 590 nm upon excitation at 530 nm and expressed as relative fluorescence units. A representative experiment is shown with the average and standard deviation of 4 technical replicates.

Supplemental References

1. **Pacheco SA**, **Hsu F-F**, **Powers KM**, **Purdy GE**. 2013. MmpL11 protein transports mycolic acid-containing lipids to the mycobacterial cell wall and contributes to biofilm formation in Mycobacterium smegmatis. J Biol Chem **288**:24213–24222.

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