Supplemental Material

Supplemental Fig. 1. ***M.tb*** **HN885 opsonization by complement component C3 and association with CR3. (A) C3 deposition on the surface of Erdman and HN885** ***M.tb***. Bacilli ($2x10^7$) were incubated in the presence or absence of 25% non-immune human serum, washed, reduced, denatured and resolved by 7.5% SDS-PAGE followed by Western blotting for C3. Results show the reduced and denatured fragments of C3bi (75 kDa β-chain and 68 kDa α-chain) and C3bi2 (43 kDa α^1-chain). Representative experiment of n=2. **(B) Binding of Erdman and HN885** ***M.tb*** **strains to CHO-CR3 cells.** CHO CR3 cells ($1x10^5$) were adhered to glass coverslips in 24 well tissue culture plates overnight at 37°C and 5% CO_2. Washed monolayers were incubated with either *M.tb* strain Erdman or HN885 in either RH containing 2.5% human serum or RHH at 37°C for 2h. Infected monolayers were washed, fixed, stained and bacilli/cell was assessed as described for the MDM association assay. Results are mean values of bacilli per cell ± SEM (counting a minimum of 300 consecutive cells/coverslip by phase contrast and fluorescence microscopy, n=3, by triplicate).

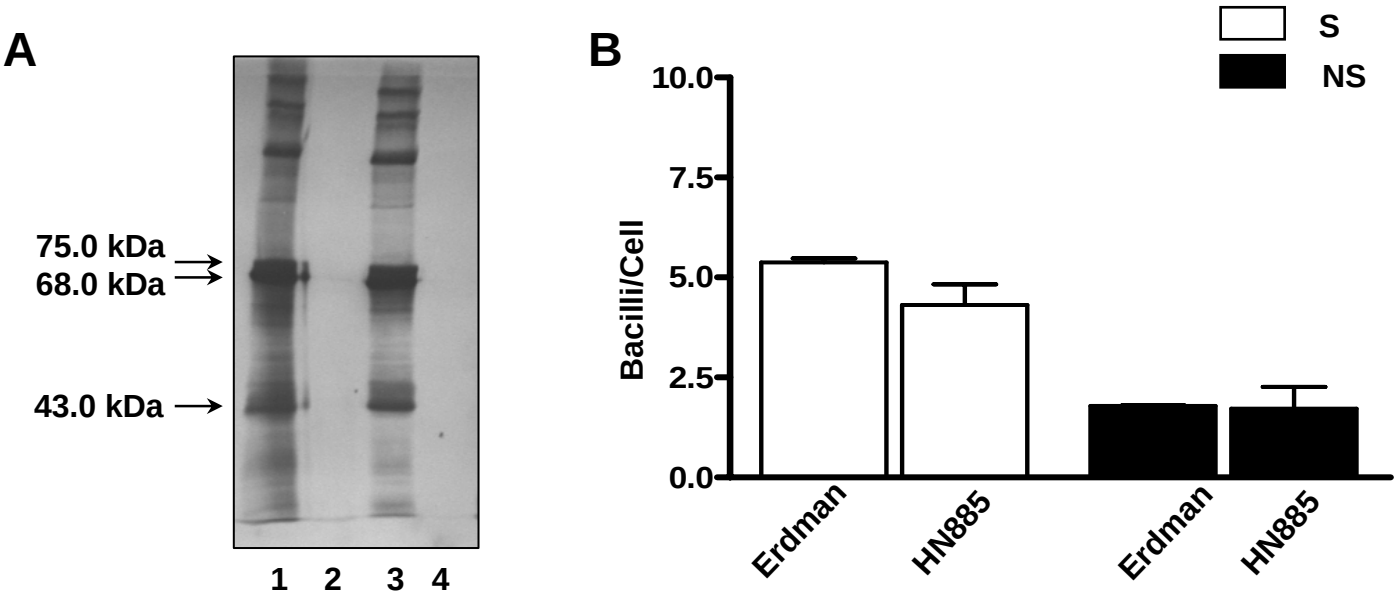
Supplemental Fig. 2. Differences in the presence of ManLAM on the surface of ***M.tb*** **strains.** Live *M.tb* single cell suspensions ($1x10^6$) were blocked with 2% BSA in PBS, washed with PBS followed by staining with mouse IgG or anti-LAM CS-35 or CS-40 in 2% BSA in PBS for 20 min at 4°C, washed again and stained with IgG (H+L) Alexa 647 in 2% BSA in PBS for 20 min at 4°C. After further washing, samples were analyzed by flow cytometry. Mean fluorescence intensity was measured for each test group and mean values of 3 samples were obtained. Shown is a representative experiment (n=3). Results are similar to those in Fig. 2.

Supplemental Fig. 3. Total PIM analysis of ***M.tb*** **strains. (A)** Comparative 2D TLC analysis of PIMs extracted from *M.tb* strains $H_{37}R_v$, Erdman, and HN885. Total lipid was extracted with chloroform:methanol (2:1, v/v) for 24 h followed by chloroform:methanol (1:2, v/v) for an additional 24 h. PIMs (by total weight) were run in a 2D-TLC using silica gel 60 with a 1st dimension in chloroform:methanol:water (60:30:6, v/v/v) and 2nd dimension in chloroform:acetic acid:methanol:water (40:25:3:6, v/v/v/v). TLC plates were sprayed with 10 % sulfuric acid in ethanol or alpha-naphthol (data not shown) and heated at 110°C for 3 min. Major differences in lower- and higher-order PIM content were observed between *M.tb* strains, where strains $H_{37}R_v$ and Erdman contain significantly more higher-order PIMs than strain HN885. A representative experiment of 2-4 experiments is shown. **(B)** Bar graph shows the results derived from the PIM densitometry analysis performed using the ImageJ program from the NIH (http://rsb.info.nih.gov/ij/UT). Data are presented as mean ± SEM, **P*<0.05 and ***P*<0.005 One-way ANOVA followed by a Tukey post-test.

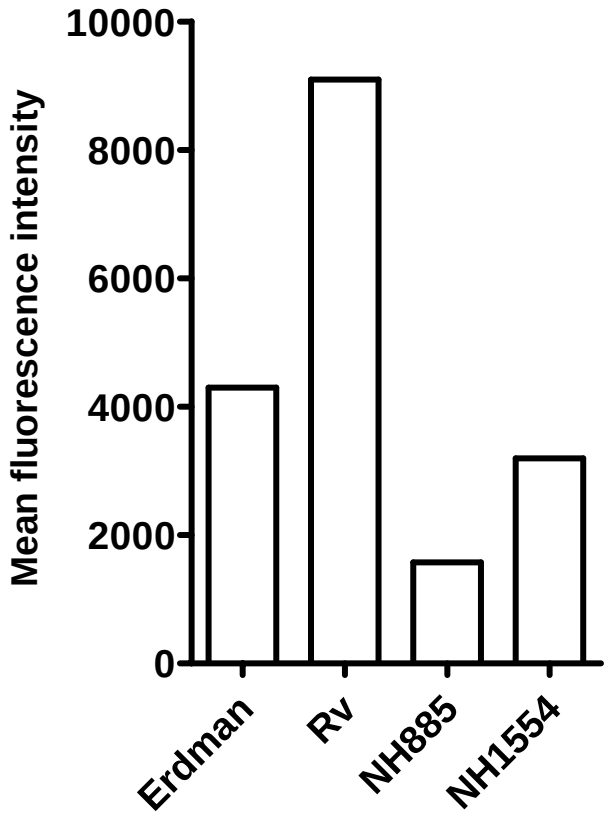
Supplemental Fig. 4. Triglycerides and PGL content in ***M.tb*** **strains. (A)** Triglycerides as well as phthiocerol dimycocerosates (PDIMs) were visualized by 1-D TLC using the total lipid (TL) and the solvent system petroleum ether:acetone (96:4, v/v) and sprayed with 5% phosphomolybdate acid in ethanol or 10% sulfuric acid in ethanol (data not shown) and heated to detect lipids. **(B)** PGL-tb content in strains HN885 and HN1554 was visualized by 1-D TLC using TL and the solvent system chloroform:methanol (95:5, v/v) and sprayed with 10% sulfuric acid in ethanol or α-naphthol or 5% phosphomolybdate acid in ethanol. Spots were identified as PGL-tb when positive using all developers. As a positive control for PGL, total lipid from *M. bovis* BCG (BCG) was used. Arrows indicate PGL-tb present in strains HN885 and HN1554 and mycoside B in *M. bovis* BCG. **C.** Presence of PGL-tb was visualized by 2D-TLC using TL and the solvent system as a 1st-D: chloroform:methanol (96:4, v/v) and as the 2nd-D: toluene:acetone (80:20, v/v). TLCs were developed as described above. Presence of PGL (arrows) was confirmed

by a 2D-TLC comparison with PGL content from strain *M. bovis* BCG. Sample 1, TL *M.t*b $H_{37}R_v$; 2, TL *M.tb* $H_{37}R_a$; 3. TL *M.tb* Erdman; 4, TL *M.tb* HN885; 5, TL *M.tb* HN1554; and 6, TL *M. bovis* BCG.

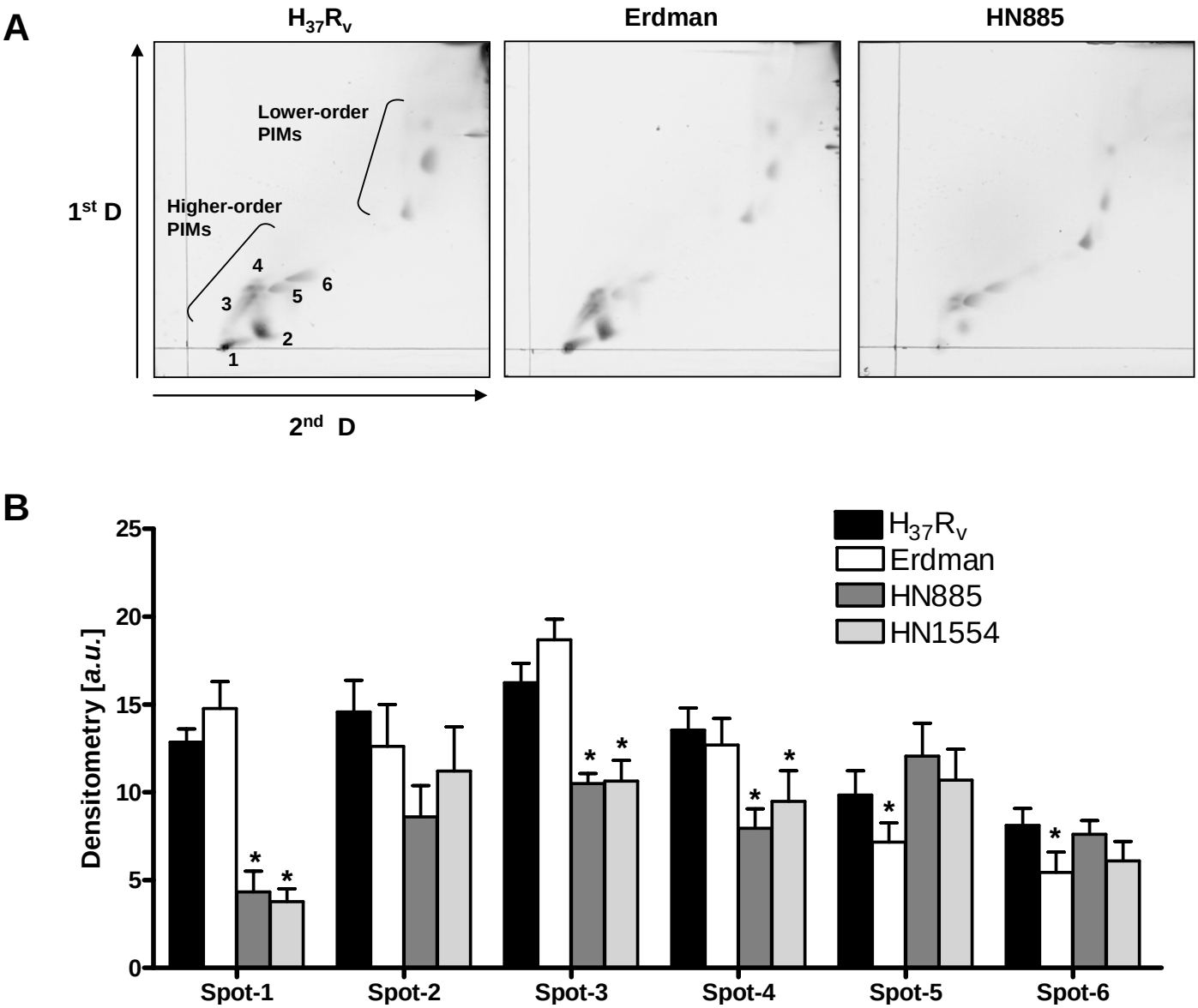
Supplemental Figure 1, Torrelles *et al.*



Supplemental Figure 2, Torrelles *et al.*



Supplemental Figure 3, Torrelles *et al.*



Supplemental Figure 4, Torrelles *et al.*