Supplemental Material:
Lipoarabinomannan localization and abundance during *Mycobacterium smegmatis* growth.
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Fig. S1. Percoll density gradient centrifugation of spheroplast preparation. Spheroplast preparations centrifuged on a self forming gradient of 60% Percoll separate into a low density band of material containing spheroplasts, debris and intact cells at the surface of the gradient and a diffuse high density band containing pure spheroplasts. The diagram at the right indicates how fractions were collected for subsequent analysis.

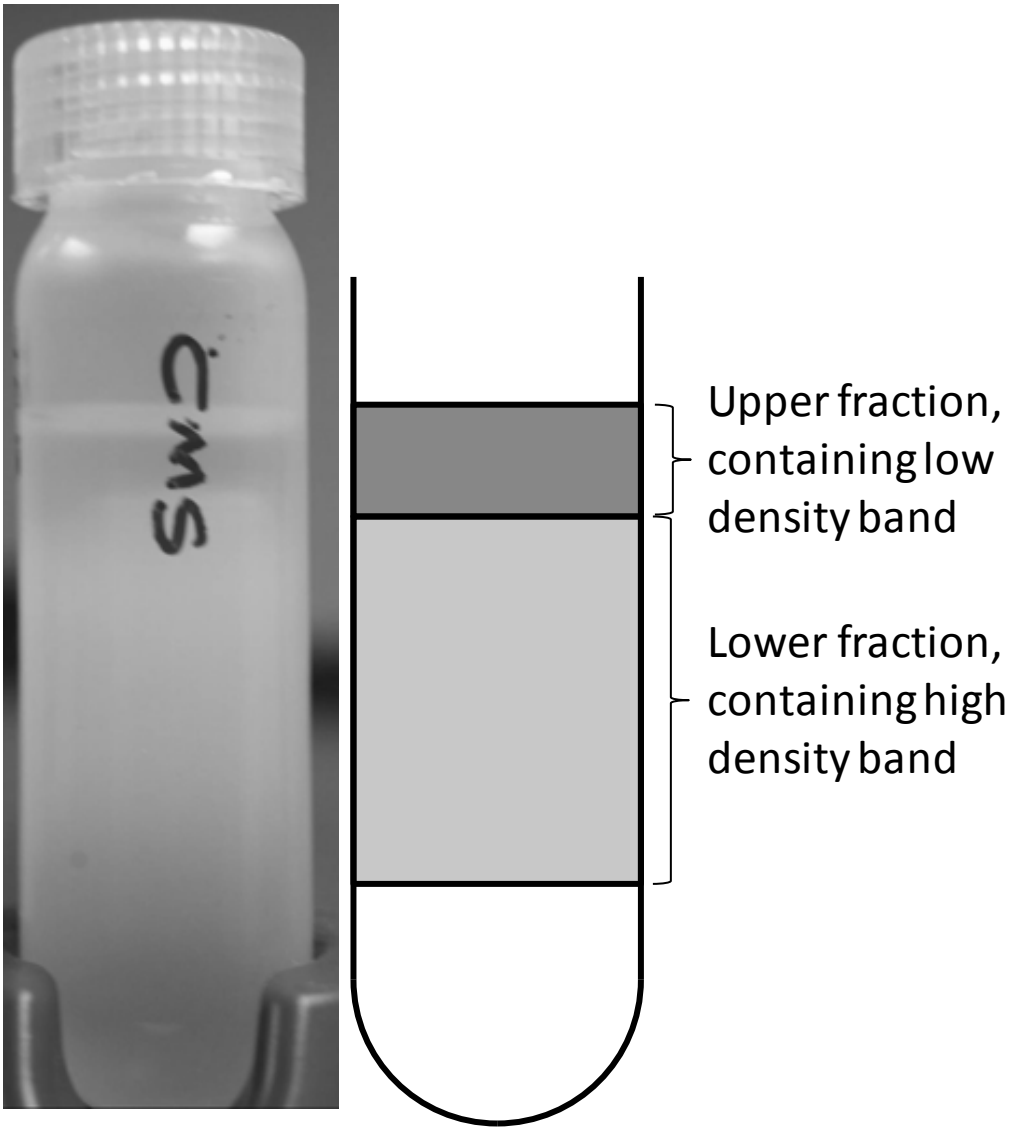

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Fig. S2. **SDS-PAGE analysis of LAM content of subcellular fractions from *Mycobacterium smegmatis***. Equal amounts protein from each fraction were extracted and loaded on 10-20% Tricine gels. Panel A – Tricine gel silver stained. Panel B - Electroblot of 10-20% Tricine gel visualized with CS-35. Lane 1 – total cell lysate. Lane 2 – 27,000 X g pellet (cell-wall enriched). Lane 3 – 27,000 X g supernatant (containing cytosol and membranes). Lane 4 – cytosol enriched fraction. Lane 5 – membrane enriched fraction. *M. smegmatis* were grown in Middlebrook 7H9 medium supplemented with OADC, harvested, washed with phosphate-buffered saline, and stored at -80°C until used. The cells (10 g wet weight) were resuspended in 30 ml of buffer, containing 50 mM MOPS (pH 8.0), 5 mM β-mercaptoethanol, 10% glycerol and 10 mM $MgCl_2$ at 4°C and subjected to probe sonication (Soniprep 150, MSE Sanyo; 1-cm probe) for a total 10 cycles of 60 second pulses with 90 seconds of cooling between pulses. The lysates were centrifuged at 27,000 x *g* for 30 min at 4°C. The resulting mycobacterial cell-wall enriched pellets were resuspended in buffer A. Membrane enriched fractions were obtained by centrifugation of the 27,000 x *g* supernatant at 100,000 x *g* for 1 h at 4°C. The supernatant was carefully removed and used as the cytosol enriched fraction. The 100,000xg pellet was gently resuspended in buffer A by homogenization and recentrifuged at 100,000xg for 1 h at 4°C. Washed membrane enriched fraction was resuspended in buffer A. Protein concentrations were determined using the BCA protein assay reagent kit (Pierce).

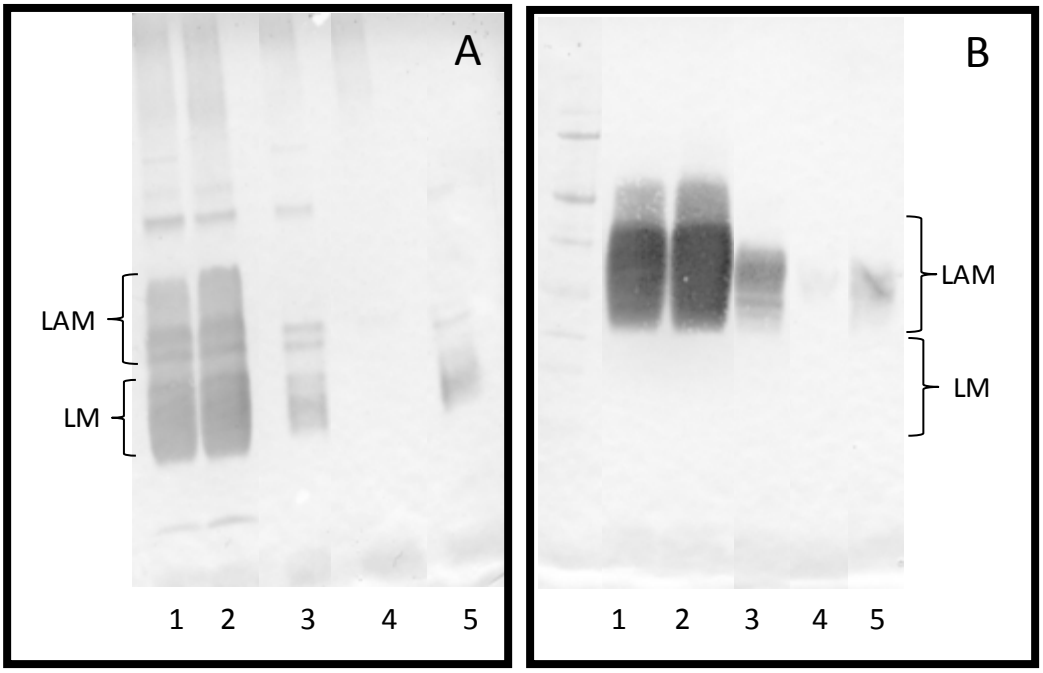

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Fig. S3. **Regrowth of *Mycobacterium smegmatis* from a spheroplast preparation**. Panel A – DIC image of bacteria after 96 hrs of regrowth in 7H9 medium supplemented with OADC at 37°C showing the regeneration of the bacilli form. Panel B – Image of a typical colony generated by plating a spheroplast preparation on 7H11 agar.

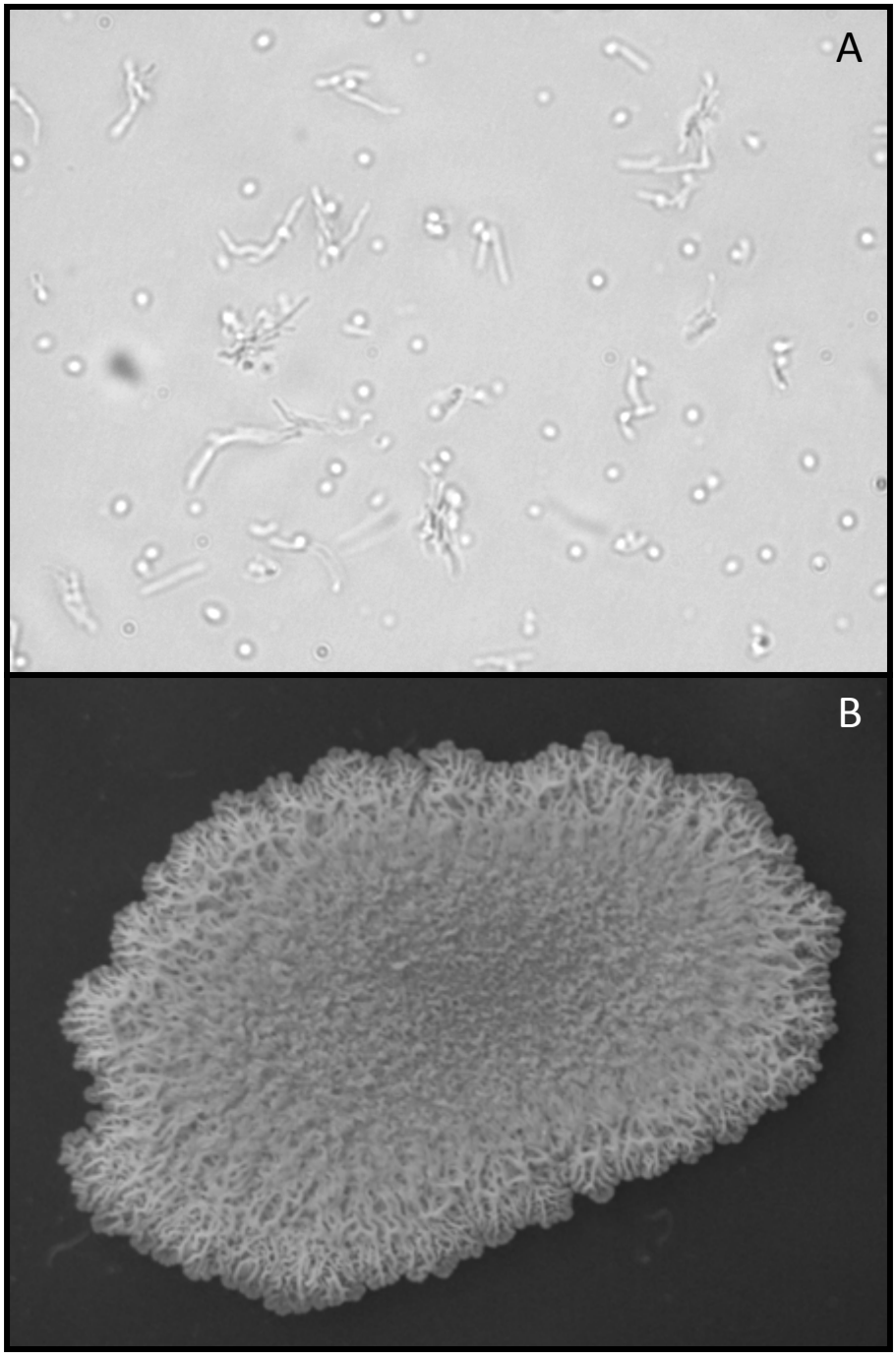

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Fig. S4. Comparison of colony forming units (CFU) and culture OD in a representative *Mycobacterium smegmatis* culture over 140 hrs. Bacteria were grown in Middlebrook 7H9 broth supplemented with OADC and 0.05% Tween 80, aliquots of the culture were taken and plated on Middlebrook 7H11 agar or diluted for OD readings.

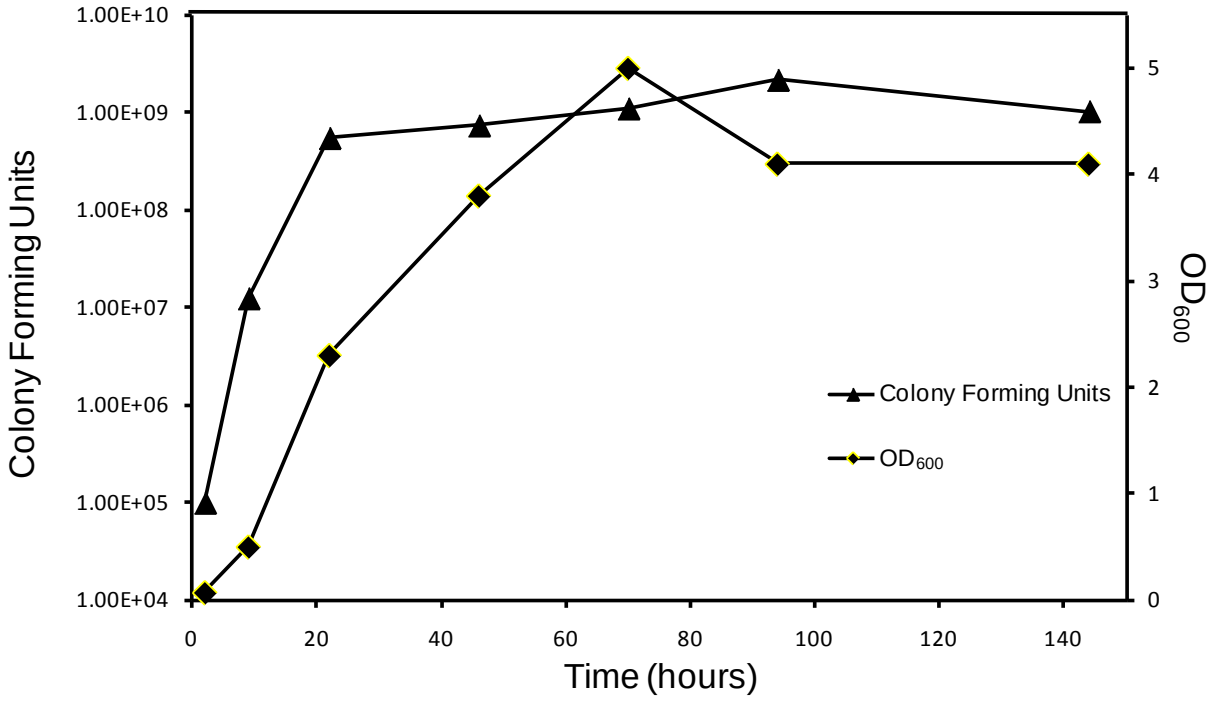

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Fig. S5. Growth phase associated changes in the morphology and acid fast staining of *M. smegmatis*. *M. smegmatis* mc^2 155 cells were grown in 7H9 medium supplemented with OADC and 0.05% Tween 80 at 37°C. Cells were grown for 24 h (Panel A) or 144 h (Panel B) subjected to Ziehl-Neelsen acid-fast staining, counterstaining with methylene blue and light microscopy. Images are 400 X.