Supplementary Information

Modulation of host cell membrane nanoenvironment by mycobacterial glycolipids: Involvement of $PI(4,5)P_2$ signaling lipid?

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The supporting information contains two supplementary figures.

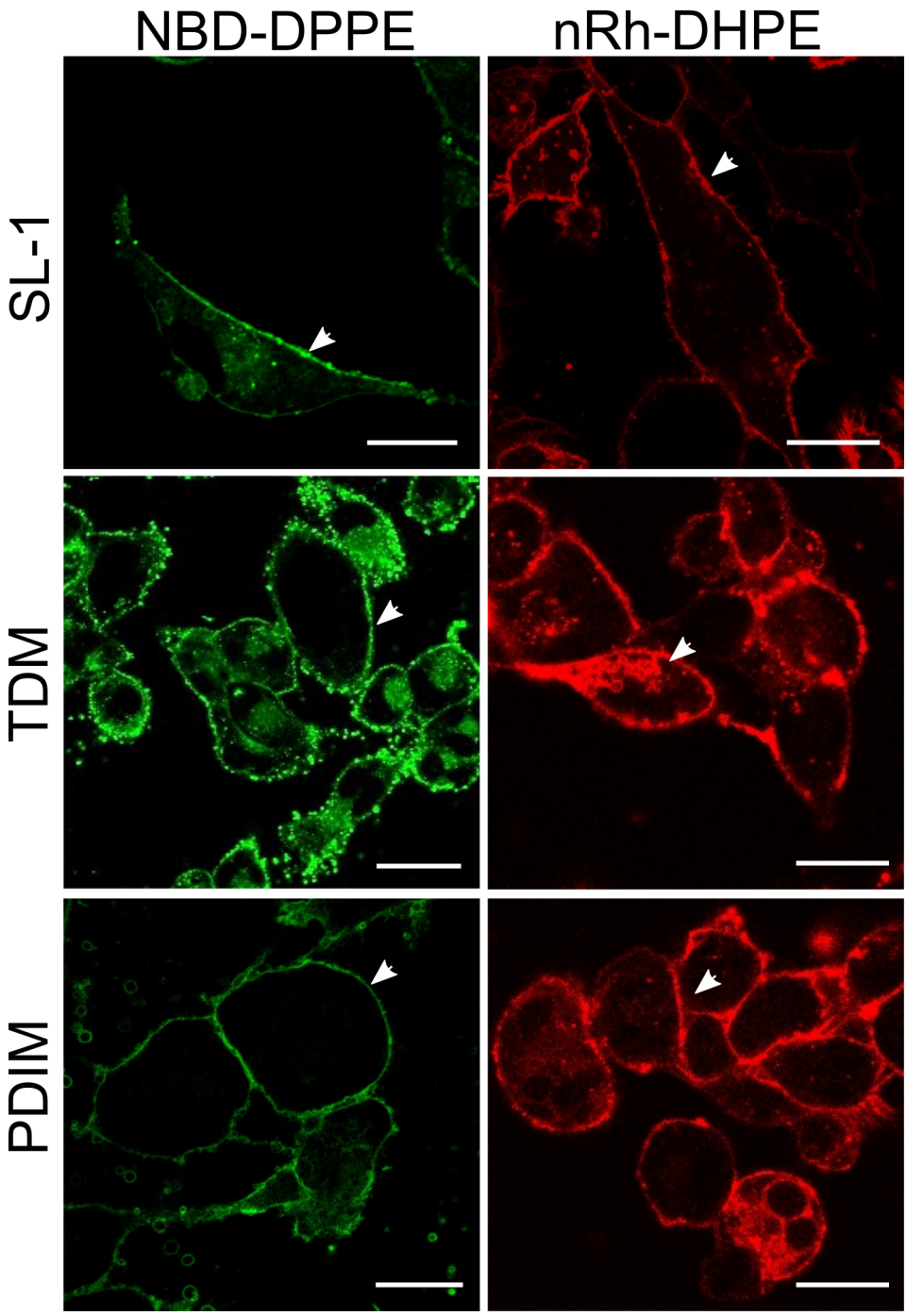

Fig. S1: Plasma membrane of THP-1 macrophages labelled with fluorescently labeled *Mtb* lipid suspensions: *N-Rh* DHPE (5 μg/mL) and NBD-DPPE (10 μg/mL) were added to the *Mtb* lipid suspensions and incubated with cells. Confocal images were acquired after 1 h and 4 h. Images shown are representative of 1 h, (plasma membrane stained with *N-Rh*-DHPE and NBD-DPPE have been marked with a white arrow). Scale bar: 10 μm.

Fluorescently labeled *Mtb* lipid vesicles for TDM, SL-1 and PDIM (i.e, *Mtb* lipid suspensions with *N-Rh* DHPE) were incubated THP-1 (**Fig. S1**). Observed fluorescence was limited to cell periphery indicating fusion and subsequent incorporation of *Mtb* lipid liposomes into the THP-1 cell membrane. The plasma membrane distribution pattern of the dye varied for distinct *Mtb* lipids. For SL-1 and PDIM the fluorescence was homogeneously distributed on the plasma membrane, but for TDM, fluorescent patches were observed. This could be due to the arrangement of lipid domains on the cell surface (in presence of *Mtb* lipids) leading to localized enrichment of the fluorescent lipid probe.

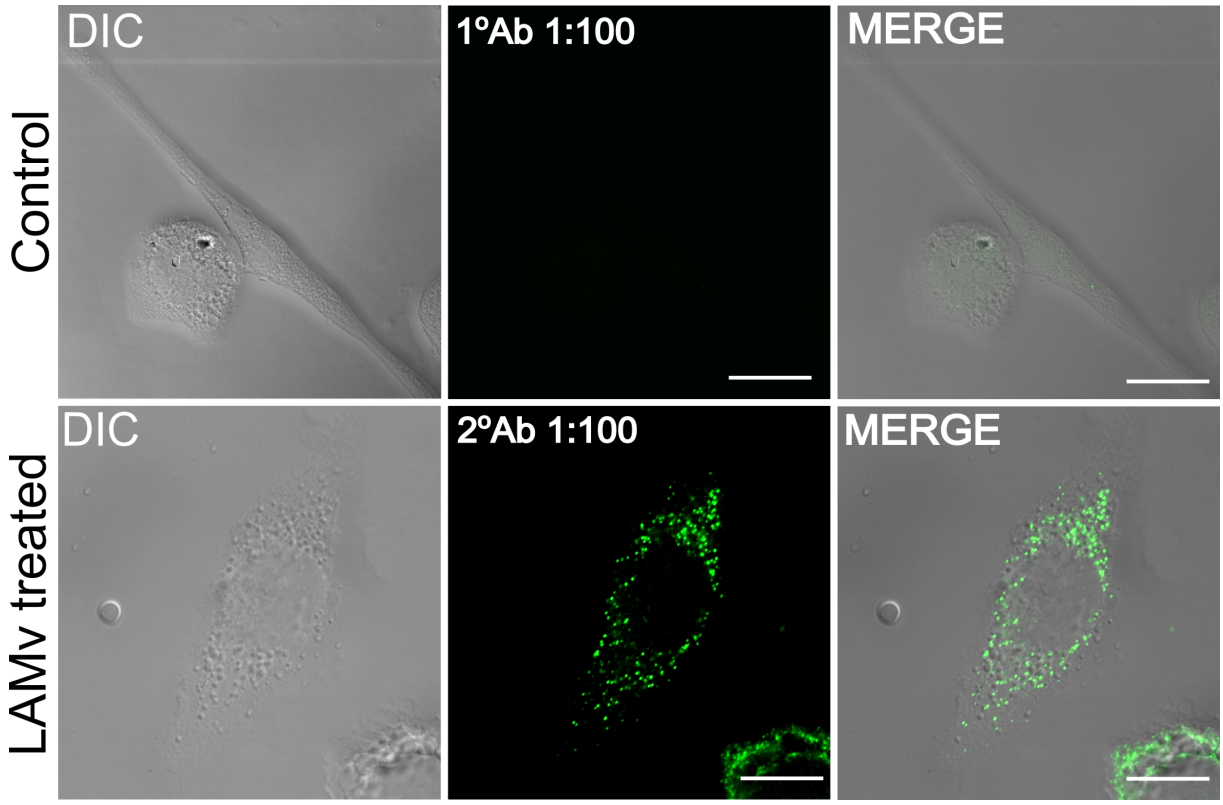

Fig. S2: Insertion of exogenously added purified Mtb (H37Rv) Lipoarabinomannan (LAMv) in THP-1 macrophages: Cells were (A) untreated and (B) treated with purified LAMv for 4 h at a given concentration of 10 μg/mL and then incubated with monoclonal anti Mycobacterium tuberculosis LAM, Clone CS-35 (primary antibody; 1:100-50) for 2 h at 4 °C, followed by secondary antibody incubation with CF 488A, goat anti-mouse IgG (H+L) (1:100) for 50 min at RT. Confocal images were acquired using LSCM (63x oil objective), confirming the insertion of LAM (shown with a white arrow) into THP-1 membrane surface. Scale 10 μm.

LAM insertion in THP-1 macrophages was observed using immunofluorescence microscopy with anti-LAM specific antibody. A punctate-like appearance of LAM was observed at the cell periphery and center, confirming the presence of exogenously added *Mtb* LAM-v in THP-1 cells. LAM staining was not just limited to the cell surface, indicating its plausible internalization due to active cellular processes such as endocytosis and its contribution cannot be ruled out at present.