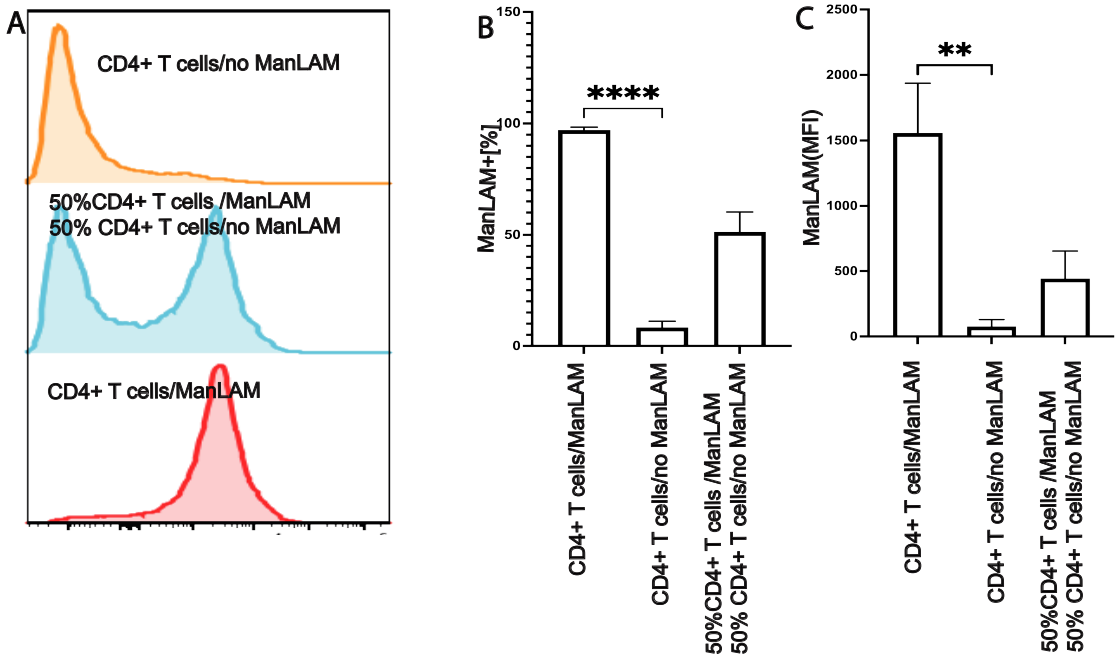

Fig. S1 Flow cytometric analysis of ManLAM on the surface of CD4+ T cells
Purified human CD4+ T cells were incubated with or without ManLAM (40 µg/mL) for 1h. In one experimental condition ManLAM exposed and unexposed cells were mixed in a 1:1 ratio. CD4+ T cells were then washed and incubated with live-dead stain, human anti-LAM mAb, and rabbit anti-CD3, followed by AF647-goat anti-human, and AF594-goat anti-rabbit. Incorporation of ManLAM into the CD4+ T cell membranes was then measured by flow cytometry.
A. Flow cytometry histograms of a representative experiment of three from three donors showing incorporation of ManLAM into T cell membranes.
B. Summary results of three experiments from three donors of CD4+ T cells showing percentages of CD4+ T cells that incorporate ManLAM into their membranes after exposure to the glycolipid.
C. Summary results of three experiments from three donors of CD4+ T cells showing MFI of ManLAM when CD4+ T cells are exposed to the glycolipid. Data in **B** and **C** are represented as mean ± SEM, (**p≤0.01, ****p≤0.0001).

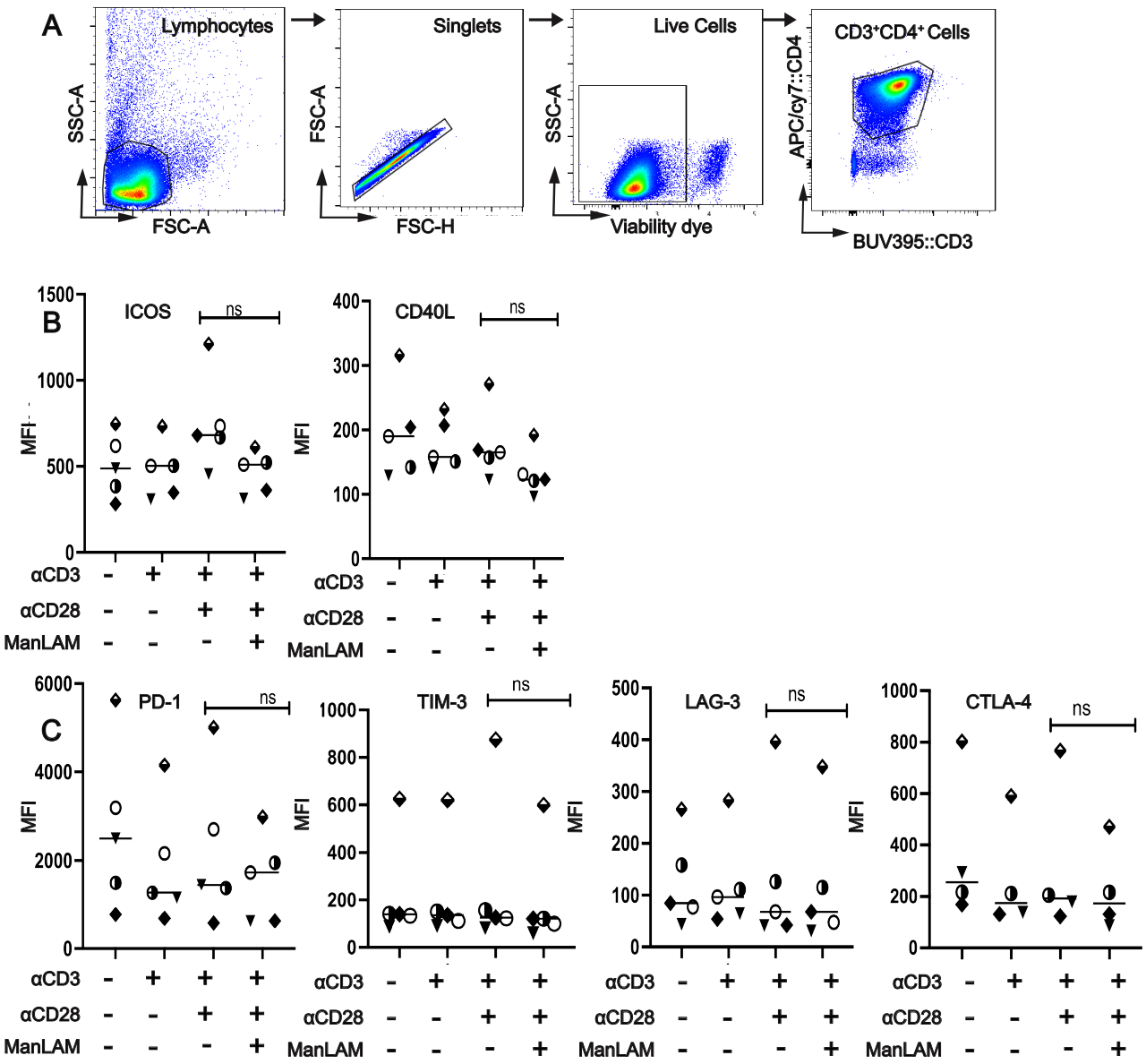

Fig. S2 Inhibition of human CD4+ T cells by ManLAM is not associated with up-regulation of CTLA-4, PD-1, TIM-3 or Lag-3 or down-regulation of CD40L or ICOS.
Purified human CD4+ T cells were incubated with or without ManLAM (40 µg/mL) for 1h. CD4+ T cells were cultured in medium or stimulated with either (1µg/mL) plate bound anti-CD3 alone or together with soluble anti-CD28 (1µg/mL) for 48 h. Levels of expression of the indicated markers were measured by flow cytometry.

A. Gating strategy for a representative experiment of five from five donors for panels **B** and **C**. The median fluorescence intensity (MFI) was measured on CD3+ and CD4+ double positive events.
B. Summary of ICOS and CD40L expression measured as MFI is shown for five experiments from five donors of CD4+ T cells.
C. Summary of PD-1, TIM-3, LAG-3 and CTLA-4 expression measured as MFI is shown for five experiments from five donors of CD4+ T cells (for CTLA-4 are four experiments from four donors). Each data point represents a single donor, and each symbol corresponds to the same donor. Results shown as mean ± SEM and analyzed by Wilcoxon matched pairs signed rank test, ns = not significant.

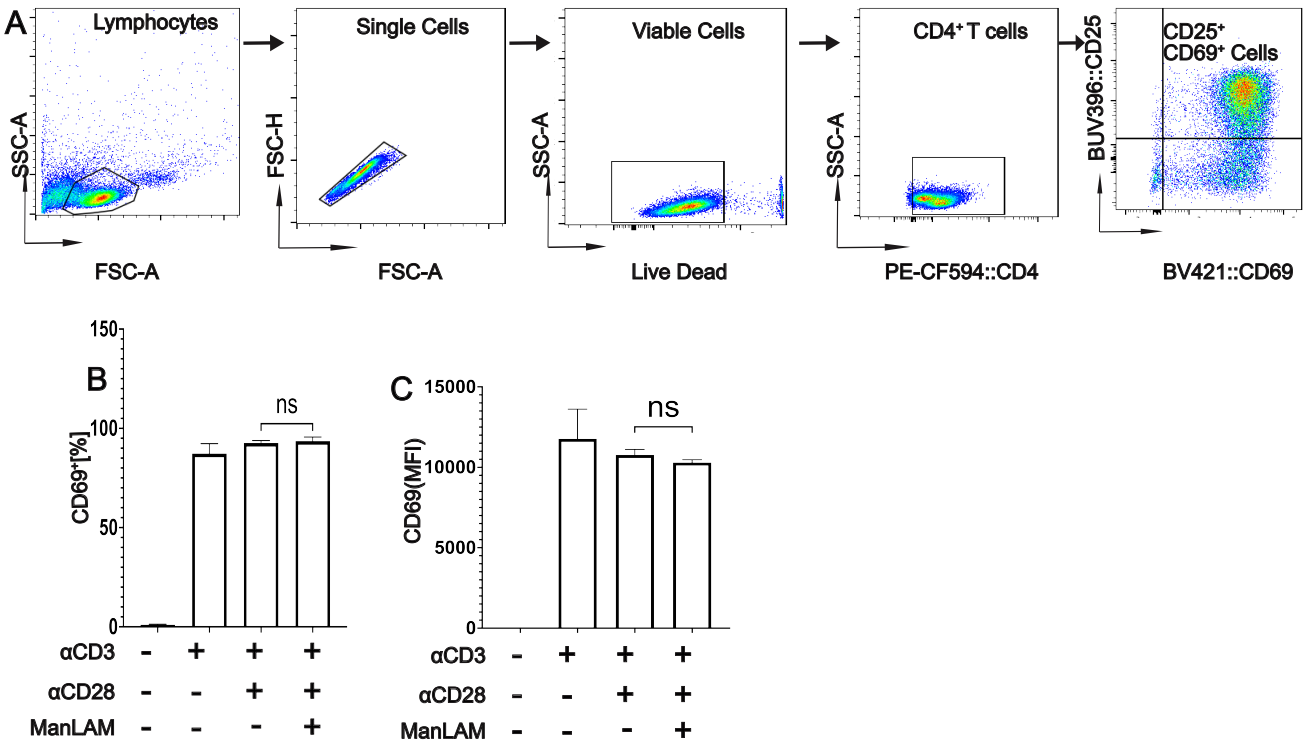

Fig. S3 CD69 expression by human CD4+ T cells is not affected by ManLAM. Purified human CD4+ T cells were incubated with or without ManLAM (40 µg/mL) for 1 h. CD4+ T cells then were cultured in medium or stimulated with either plate bound anti-CD3 alone or together with soluble anti-CD28 (both at 1µg/mL) for 24 h. T cells were then stained for CD4, CD69, and CD25.

A. Gating strategy for a representative experiment of four from three donors for Fig. 2A-D, and supplementary Fig. S3B-C

B. CD69 expression as percentage by human CD4+ T cells when exposed to ManLAM, compared to unexposed cells (summary results of four experiments from three donors of CD4+T cells).

C. CD69 expression as MFI by human CD4+ T cells when exposed to ManLAM, compared to unexposed cells (summary results of four experiments from three donors of CD4+ T cells). Data in **B** and **C** are represented as mean ± SEM (ns=Not significant).

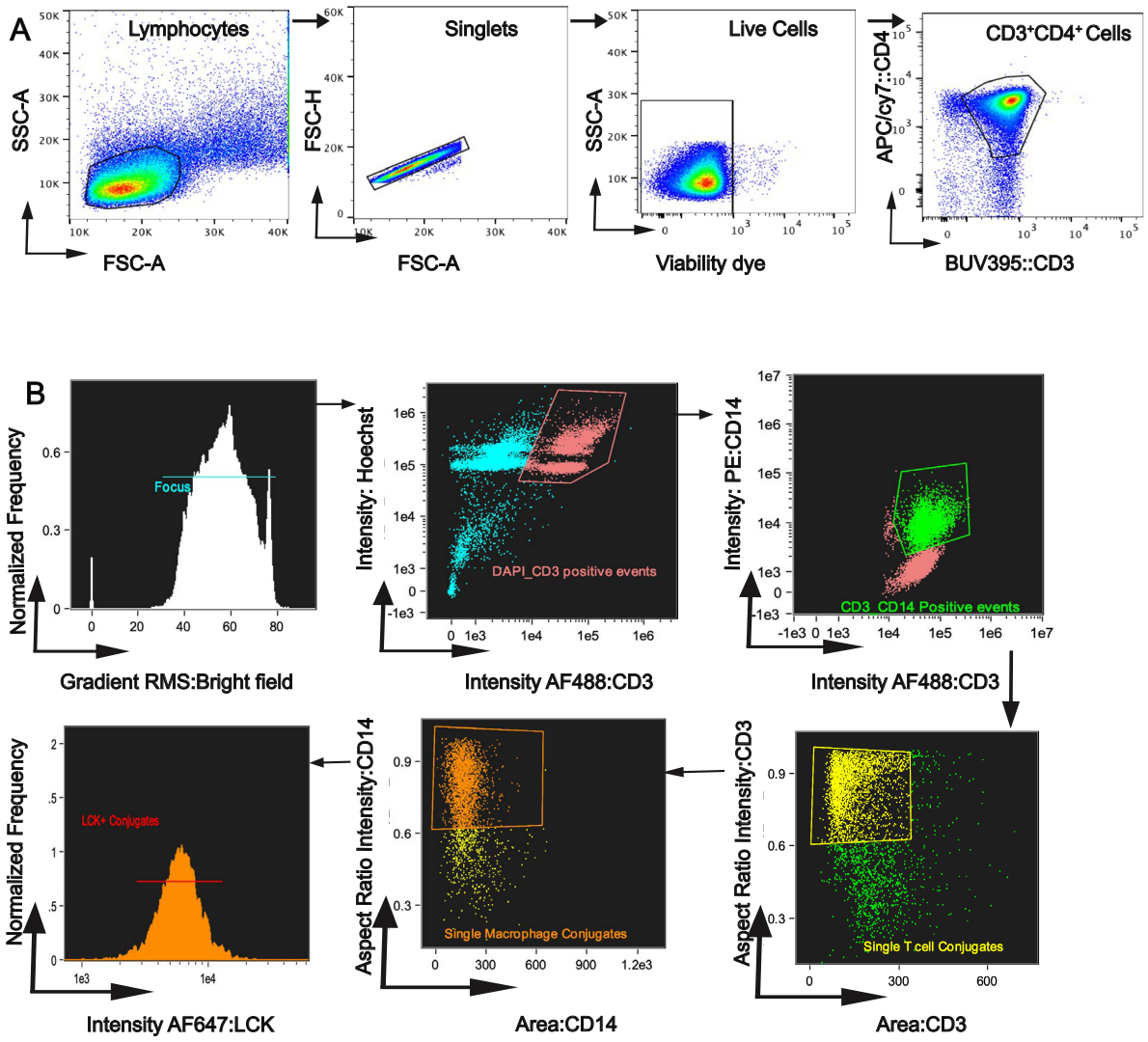

Fig. S4 Gating strategies for intracellular cytokine staining (ICS, Fig.1B-D) and Imaging flow cytometry (IFC, Fig.4A-C, Fig.5A-E)

A. Gating strategy for a representative experiment of six from six donors for ICS. Percentage of cells expressing cytokines were gated on CD3+ and CD4+ double positive cells.

B. Imaging flow cytometry graphs of a representative experiment of four from two donors showing the gating strategy for IFC. The gating was initially done on events that were in focus (about 30,000 events were acquired per sample), followed by the gate for all CD3+ events. CD3+ events positive for CD14+ were considered conjugates. Aspect ratio of 0.6 for CD3+ and CD14+ was used to include only conjugates with one T cell and one macrophage. LCK redistribution analysis was done on LCK+ conjugates.