Supplemental Information

Conformation of functional r-langerin.

A truncated form of recombinant human langerin (r-langerin) consisting of the ECD (neck domain and CRD) was produced in *E. coli* as a fusion protein with N-terminal His_6 and FLAG domains and purified by IMAC. Functional r-langerin was obtained by refolding and subsequent mannan-agarose affinity chromatography (1). To assess functionality, Ca^{2+} dependent binding of r-langerin to a known ligand (soybean agglutinin) was evaluated in an ELISA based assay. The r-langerin efficiently bound various concentration of agglutinin, and EDTA chelation of Ca^{2+}in this assay inhibited the r-langerin-agglutinin interaction (Figure S1A). Additionally, r-langerin binding of agglutinin was abolished by preincubation with mannan, thus demonstrating that the carbohydrate dependence of the r-langerin activity. Langerin was previously shown to bind mannose, and glucose residues of oligosaccharide chains, but not galactose (2). Sugar specificity of the r-langerin was evaluated with an inhibition assay by pretreating the lectin with different sugar moieties. As expected mannan, mannose and glucose reduced the binding of r-langerin to agglutinin by 71.14 ± 3.8%, 70.2 ± 2.4%, and 54.0 ± 4.4%, respectively. Galactose, however, only weakly inhibited the r-langerin binding activity (13.0 ± 0.83% inhibition) (Figure S1B). These data are consistent with previous sugar specificity studies and demonstrated a functional Ca^{2+}dependent trimeric r-langerin construct.

A liquid phase SPR assay was exploited to establish the affinity of agglutinin and a second ligand (yeast invertase) to r-langerin. The r-langerin was immobilized on a sensor chip and exposed to a consistent flow of agglutinin or yeast-invertase at various concentrations (5 to 160 nM). The sensograms shown in Figure S2 demonstrate concentration dependent binding of the ligands. The analysis of SPR data with a steady state affinity model established the equilibrium dissociation constant (K_D) of yeast invertase and agglutinin as 0.0345 μM and 0.187 μM, respectively. The r-langerin not only demonstrated a greater affinity for yeast invertase, but also increased avidity as demonstrated by the slow dissociation of this ligand compared to agglutinin. It should be noted that r-langerin interactions measured by SPR were completely abolished in the presence of 300 mM methylmannoside and 50 mM EDTA. These data provided a baseline of langerin reactivity that can be used in comparison to langerin binding of potential mycobacterial ligands.

1) Stambach, N. S., and Taylor, M. E. (2003) Characterization of carbohydrate recognition by langerin, a C-type lectin of Langerhans cells. *Glycobiology* **13**, 401-410

2) Tada, Y., Riedl, E., Lowenthal, M. S., Liotta, L. a., Briner, D. M., Crouch, E. C., and Udey, M. C. (2006) Identification and characterization of endogenous Langerin ligands in murine extracellular matrix. *J. Invest. Dermatol.* **126**, 1549-1558

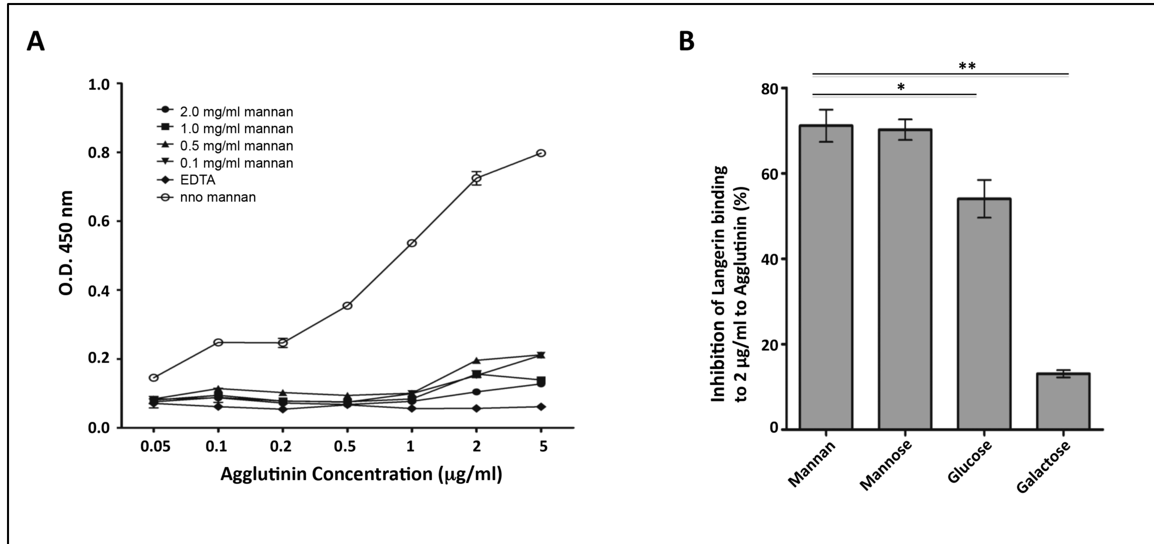

Figure S1. Confirmation of functionality and specificity of r-langerin.
A) ELISA based r-langerin binding/inhibition assay. The r-langerin (in the presence of $CaCl_2$), or r-langerin pretreated with mannan (0.1, 0.5, 1 and 2 mg/ml) or 2mM EDTA was assessed against various concentrations of agglutinin (0.05, 0.1, 0.2, 0.5, 1, 2, and 5 μg/ml). B) Sugar specificity of r-langerin was measured based on binding to agglutinin in the presence of $CaCl_2$ after pretreating the ligand with 100 mM monosaccharide (galactose, glucose or mannose) or 100 μg/ml mannan. A one-way ANOVA combined with Tukey's multiple comparison analysis was performed to evaluate the statistical difference between sugar pretreatments. Error bar indicates SD of three independent r-langerin assays. **; $p<0.01$, *; $p<0.05$.

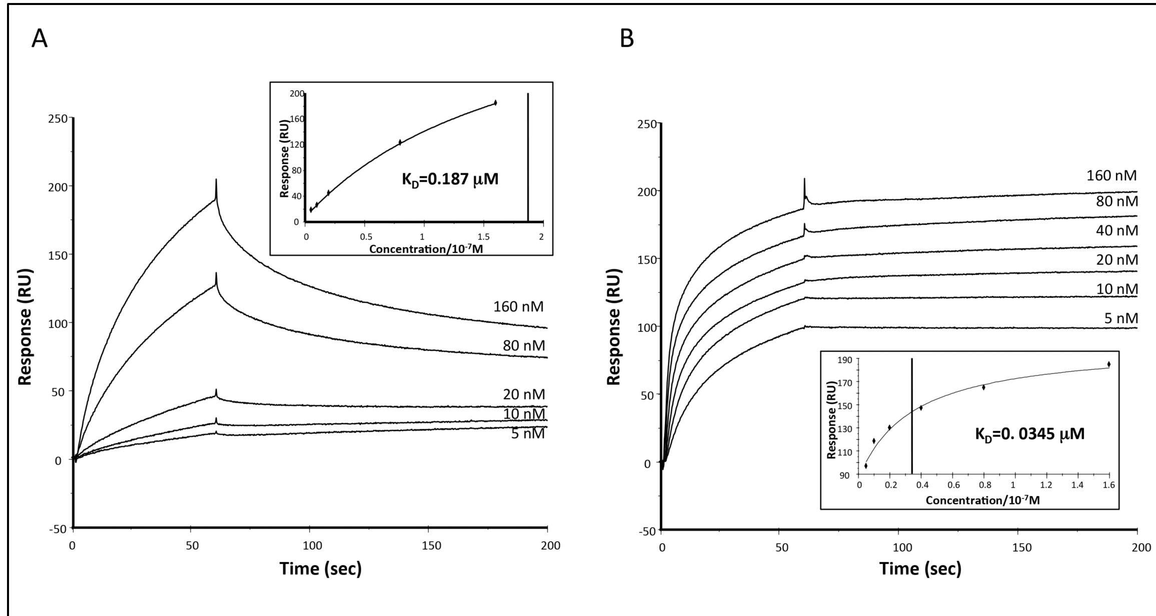

Figure S2. r-Langerin affinity to agglutinin and yeast invertase. Sensograms of immobilized r-langerin binding to agglutinin (C) and yeast invertase (D) as measured by SPR. The insets depicted the K_D values obtained from steady-state affinity measurements.

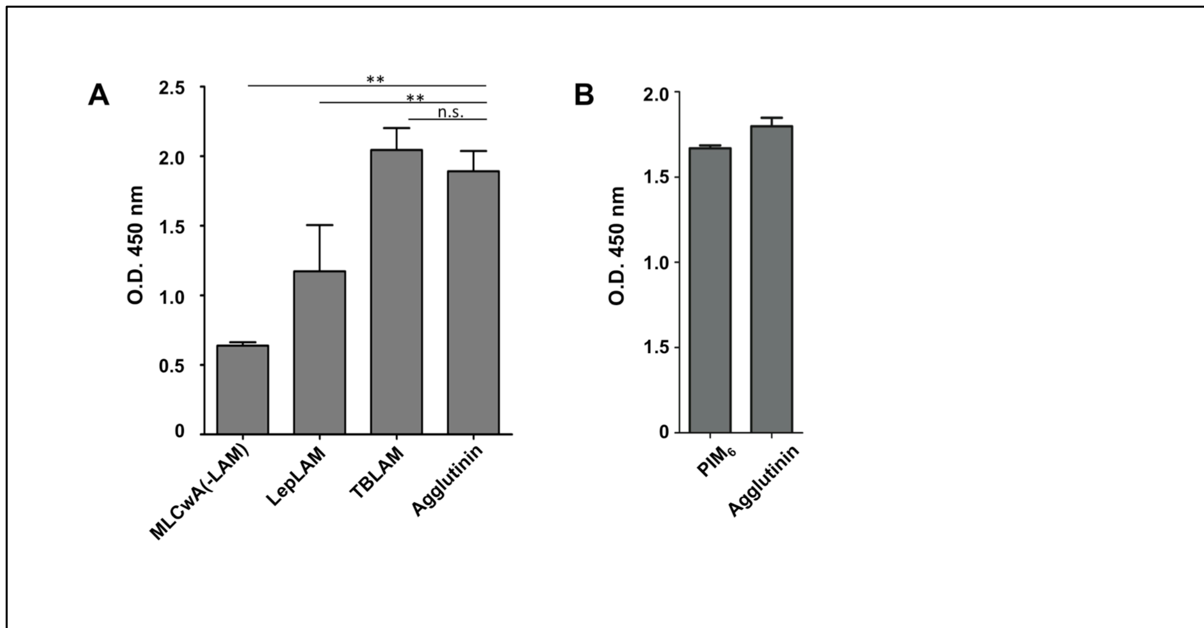

Figure S3. Concanavaline A reactivity to mycobacterial ligands on microtiter plates.
A) ConA reactivity to mycobacterial protein preparations and ManLAM by ligand ELISA. The ligands (0.05 μg per well) with ConA (0.5 μg/ml) (n=8 independent assays). A one-way ANOVA combined with Tukey's multiple comparison analysis was performed to determine the statistical difference between each mycobacterial ligand and agglutinin. **; $p<0.01$, *; $p<0.05$.
B) ConA reactivity of PIM_6 by ligand ELISA (n=4 independent assays).

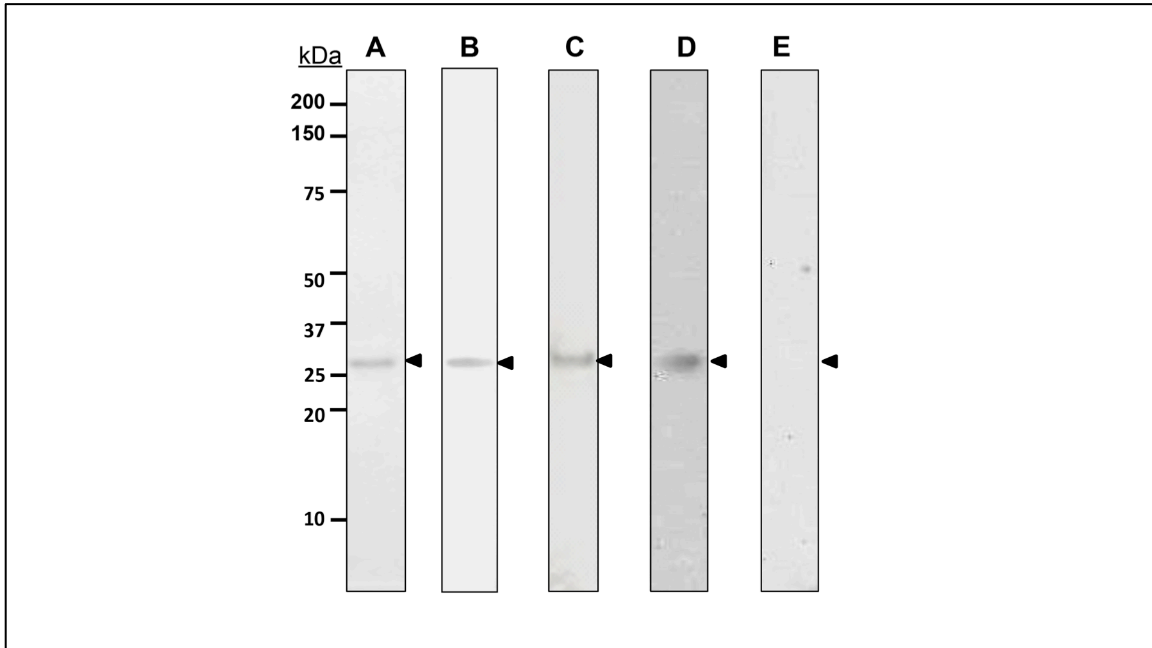

Figure S4.Verification of lectin binding of glycosylated rSodC purified by preparative SDS-PAGE elution. Final purified rSodC was resolved by SDS-PAGE and stained with Coomassie brilliant blue (A), transferred to nitrocellulose membrane and hybridized to anti His antibody (B) or PVDF probed ConA (C), r-langerin (D), or r-langerin in the presence of 300 mM methylmannoside (F). The arrowhead indicated the glycosylated rSodC. The data were reproduced in three individual experiments.