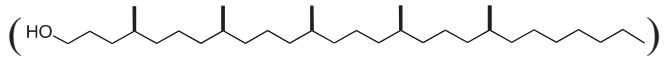
Supporting Information

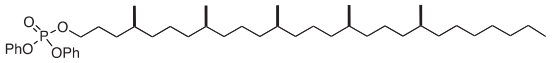
Roy et al. 10.1073/pnas.1408549111

SI Materials and Methods

The synthesis of phosphomycoketide (PM) was accomplished from C_{32} mycoketide (1)

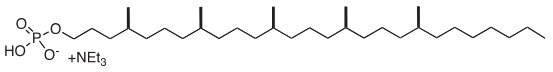


in two steps.



[1]

(4*S*,8*S*,12*S*,16*S*,20*S*)-4,8,12,16,20-Pentamethylheptacosyl diphenyl phosphate (**1**): Diphenyl chlorophosphate (59 mg, 46 μL, 0.22 mmol) was added to a solution of C_{32} mycoketide (1) (44 mg, 94 μmol) and 4-dimethylaminopyridine (27 mg, 0.22 mmol) in dichloromethane (1.0 mL) under argon The reaction mixture was stirred at room temperature overnight. TLC showed the reaction was complete. The reaction mixture was diluted with dichloromethane and washed with aqueous 1.0 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 5% ethyl acetate in hexane to give **1** as a colorless oil: 53 mg (81% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 7.34 (m, 4H), 7.22 (m, 4H), 7.18 (m, 2H), 4.24 (m, 2H), 1.74 (m, 2H), 1.40–1.00 (m, 43H), 0.92–0.80 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 150.8, 150.7, 129.9, 125.38, 125.37, 120.21, 120.17, 69.9 (d, $J_{C\text{-}P}$ = 6.5 Hz), 37.66, 37.55, 37.5, 37.3, 37.2, 32.94, 32.93, 32.90, 32.6, 32.5, 32.1, 30.1, 29.5, 28.0, 27.9, 27.2, 24.61, 24.60, 24.5, 22.8, 19.93, 19.92, 19.90, 19.89, 19.6, 14.3; ^{31}P NMR ($CDCl_3$) δ −11.8.



[2]

Triethylammonium (4*S*,8*S*,12*S*,16*S*,20*S*)-4,8,12,16,20-pentamethylheptacosylphosphate (**2**): To a solution of **1** (52 mg, 74 μmol) in a mixed solvent of ethanol/ethyl acetate [3.0 mL (vol/vol: 1:1)], PtO_2 (8.4 mg, 37 μmol) was added. The resulting suspension was degassed with three vacuum-argon cycles and then saturated with hydrogen by five vacuum-hydrogen cycles. After stirring the mixture under the hydrogen atmosphere for 60 h, TLC showed the reaction was complete. The catalyst was removed by filtration over a pad of celite, and the solution was neutralized by triethylamine and evaporated to give the PM molecule **2** as a yellow oil: 43 mg (90% yield). ^{1}H NMR ($CDCl_3$/TMS) δ 3.89 (m, 2H), 3.12 (m, 4H), 1.61 (m, 2H), 1.40–1.00 (m, 49H), 0.95–0.80 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 66.2 (m), 45.7, 37.66, 37.6, 37.2, 32.97, 32.94, 32.92, 32.88, 32.0, 30.1, 29.5, 27.2, 24.63, 24.59, 22.8, 19.9, 19.8, 19.7, 14.2, 8.6; ^{31}P NMR ($CDCl_3$) δ 2.34; API-ES MS calcd for $C_{32}H_{66}O_4P^-$ (M^-): 545.4, found: 545.5.

1. Li NS, Scharf L, Adams EJ, Piccirilli JA (2013) Highly stereocontrolled total synthesis of beta-D-mannosyl phosphomycoketide: A natural product from Mycobacterium tuberculosis. *The Journal of Organic Chemistry* 78(12):5970–5986.

A

TCR	α chain	CDR1	CDR2	CDR3	β chain	CDR1	CDR2	CDR3
DN6	TRAV36	LTSSGIE	VTNFRS	V–TRAJ52*01 CAWAGGTSYGKLTFGQGTILTVHP	TRBV6-6*01	MNHNY	SVGAGI	V–TRBD2*01–TRBJ2-7*01 CASRHGLASYEQYFGPGTRLTVT
22.5	TRAV26-2*01	TISGTDY	GLTSN	V–TRAJ47*02 CILRGYGNKLVFGAGTILRVKS	TRBV7-9*03	SEHNR	FQNEAQ	V–TRBD2*02–TRBJ2-3*01 CASRTKSGRGADTQYFGPGTRLTVL
1.6	TRAV9-2*03	ATGYPS	TKADDK	V–TRAJ36*01 CALSGYPGANNLFFGTGTRLTV	TRBV20-1*01	DFQATT	SNEGSKA	V–TRBD2*01–TRBJ2-5*01 CSARGRRVGGETQYFGPGTRLLVL
22.2	TRAV17*01	TSINN	IRSNERE	V–TRAJ52*01 CATRTSYGKLTFGQGTILTVHP	TRBV7-9*03	SEHNR	FQNEAQ	V-TRBD2*01-TRBJ2-7*01 CASSFRRRGGPSYEQYFGPGTRLTVT
1.22	TRAV22*01	DSVNN	IPSGT	V–TRAJ35*01 CAVRIGFGNVLHCGSGTQVIVLP	TRBV7-9*03	SEHNR	FQNEAQ	V–TRBD2*01–TRBJ2-4*01 CASSLPPRLAGAKNIQYFGAGTRLSVL
2.4	TRAV26-1*01	TISGNEY	GLKNN	V–TRAJ49 CIVRAWNQFYFGTGTSLTVIP	TRBV7-9*03	SEHNR	FQNEAQ	V–TRBD2*02–TRBJ1-4*01 CASRGGGGLARSNEKLFFGSGTQLSVL
CD8-1	TRAV8-6*02	SSVSVY	YLSGSTLV	V–TRAJ17*01 CAVRRAAGNKLTFGGGTRVLVKP	TRBV20-1*01	DFQATT	SNEGSKA	V–TRBD1*01–TRBJ2-7*01 CSARTYPGTGFYEQYFGPGTRLTVT

B

TRBV7-9 CDR3 loop sequences

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22.5  CASRTK-SGRG-ADTQYFGPGTRLTVL
22.2  CASSFR-RRGGPSYEQYFGPGTRLTVT
1.22  CASSLPPRLAGAKNIQYFGAGTRLSVL
2.4   CASRGGGGLARSNEKLFFGSGTQLSVL
      ***              :**.**:*:**
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Fig. S1. T-cell receptor (TCR) amino acid sequences. (*A*) Amino acid sequences of the CDR1, CDR2, and CDR3 loops and gene segments used in the six TCRs evaluated in this study and that of the CD1c-mannosyl-β1-PM (MPM)-specific TCR CD8-1. (*B*) Comparison of the CDR3β loop sequences of the TRVB7-9 TCRs. -, gaps in the sequence introduced for alignment purposes; *sequence identities; : and ., sequence similarities.

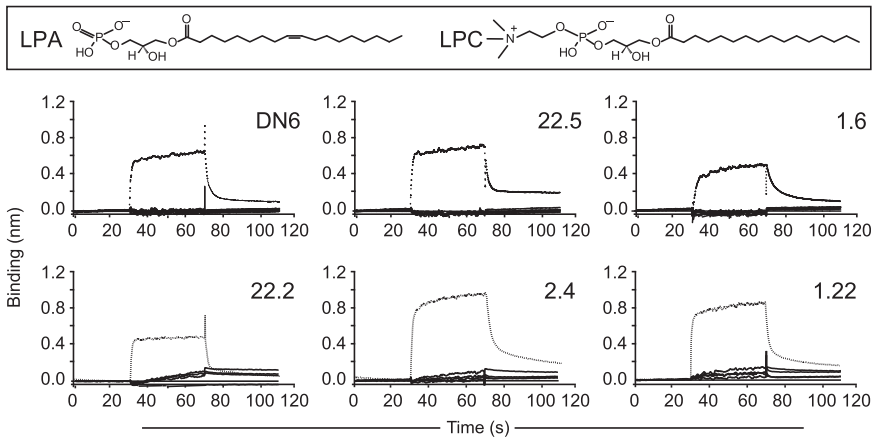

Fig. S2. Binding analysis of six αβ TCRs to CD1c loaded with lysophosphatidic acid (LPA) and lysophosphatidylcholine (LPC). The structures of LPA and LPC are shown in the top box. Top sensograms show reference-subtracted binding of the DN6, 22.5, and 1.6 TCRs with increasing concentrations of CD1c loaded with LPA (0.8–26 μM, solid lines). Bottom sensograms show reference-subtracted binding of the 22.2, 2.4, and 1.22 TCRs with increasing concentrations of CD1c loaded with LPA and LPC (0.8–26 μM, solid lines). Binding to CD1c-PM (dotted lines) is shown in all sensograms as a positive control (18 μM, dotted lines).

TCR	TRA/TRB	Coreceptor	Specificity	Affinity (K_D)	Function
DN6*	36/6-6	DN	PM	7.1	IFN-γ
22.5*	26-2/7-9	CD8+	PM MPM	9.5 27.3	IFN-γ
1.6*	9-2/20-1	CD4+	PM	24.7	IFN-γ
CD8-1	8-6/20-1	CD8+	MPM	N.D.	IFN-γ
22.2*	17/7-9	CD4+	PM	16.0	IFN-γ
22.48	8-6/12.3	CD4+	PM	N.D.	IL-4,IL-13
2.4*	26-1/7-9	CD4+	PM	6.5	IFN-γ
2.25	29-1/7-8	CD4+	PM	N.D.	IL-4
2.30	19/4-1	CD4+	PM	N.D.	IFN-γ
1.22*	22/7-9	CD4+	PM	7.3	IFN-γ
CS52.1	21-1/18	CD4+	PM	N.D.	IFN-γ

Fig. S3. CD1c-reactive TCR composition, coreceptor expression, specificity, affinity, and effector function. Summary table showing the Vα and Vβ gene segments used in CD1c reactive T-cell clones with TCR V domain composition, coreceptor expression, mycolipid specificity, measured affinity of the TCR for CD1c-lipid, and effector function. DN, double negative. *Clones analyzed further for binding to CD1c.

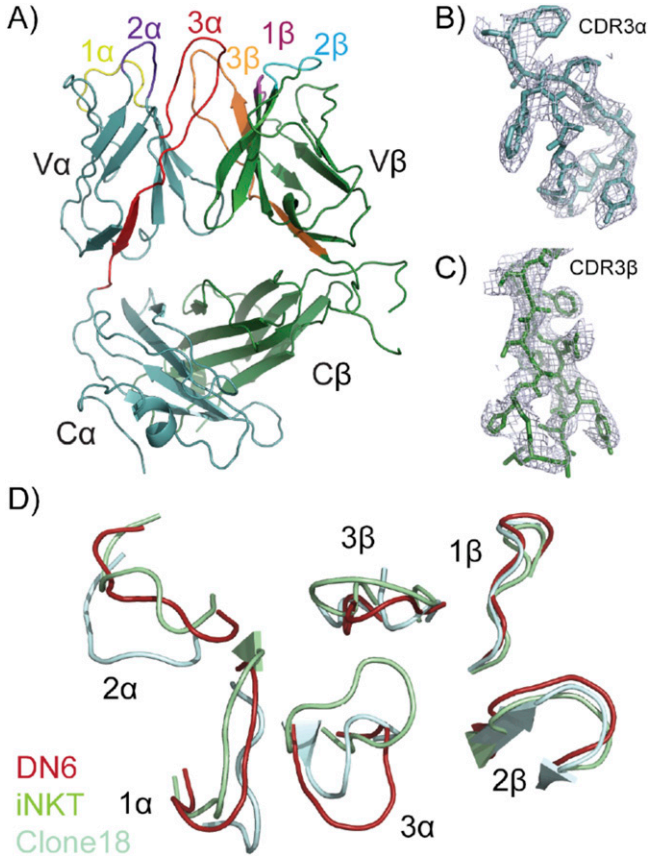

Fig. S4. Structure of DN6 TCR. (*A*) Structure of the DN6 TCR; α and β chains are colored as light blue and dark green, respectively. CDR loops are colored as such: CDR1α; yellow, CDR2α; violet, CDR3α; red, CDR1β; magenta, CDR2β; blue, CDR3β; orange. 2Fo-Fc electron density maps of CDR3α (*B*) and CDR3β (*C*) loops are shown as blue mesh contoured at 1σ. Alignment of uncomplexed CDR loops from DN6, iNKT (PDB ID code 2CDE) and clone 18 (PDB ID code 4G8E) TCRs are shown and colored as dark red, light green, and light cyan, respectively (*D*).

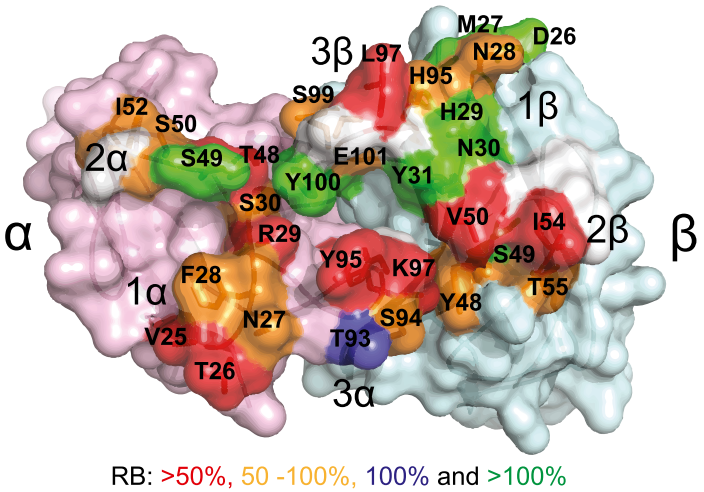

Fig. S5. Structural representation of TCR CDR loop mutants. Surface representation of the DN6 TCR structure showing the CDR loops. The α chain is colored as light pink and β chain is colored as light blue. Mutations are mapped and labeled onto the structure. Mutant residues are colored based on the relative binding and color schemes shown in Fig. 4 *A* and *B*; gray color indicates alanine or glycine residue in the CDR loops.

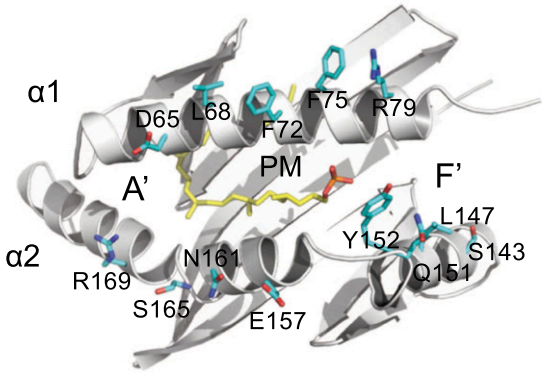

Fig. S6. Structural representation of CD1c mutational positions. Ribbon diagram of the CD1c platform domain showing the α1 and α2 helices, shown in white. PM is colored yellow. Side chains of residues mutated to alanine are shown, labeled and colored as cyan in the diagram.

Table S1. Potential hydrogen bonds between PM and CD1c

PM contact atom	CD1c contact atom	Distance	Significance
O1	Tyr-73A O	3.99	*
O1	Leu-77A N	4.20	*
OAL	Tyr-73A O	3.26	†
OAL	Leu-77A N	3.38	*

*Weakly possible hydrogen bond (distance > 3.3 Å).
†Strong possibility of a hydrogen bond (distance < 3.3 Å).