Supplementary information

A conserved human population of TRAV26^{+} type II Natural Killer T cells solely recognise CD1d

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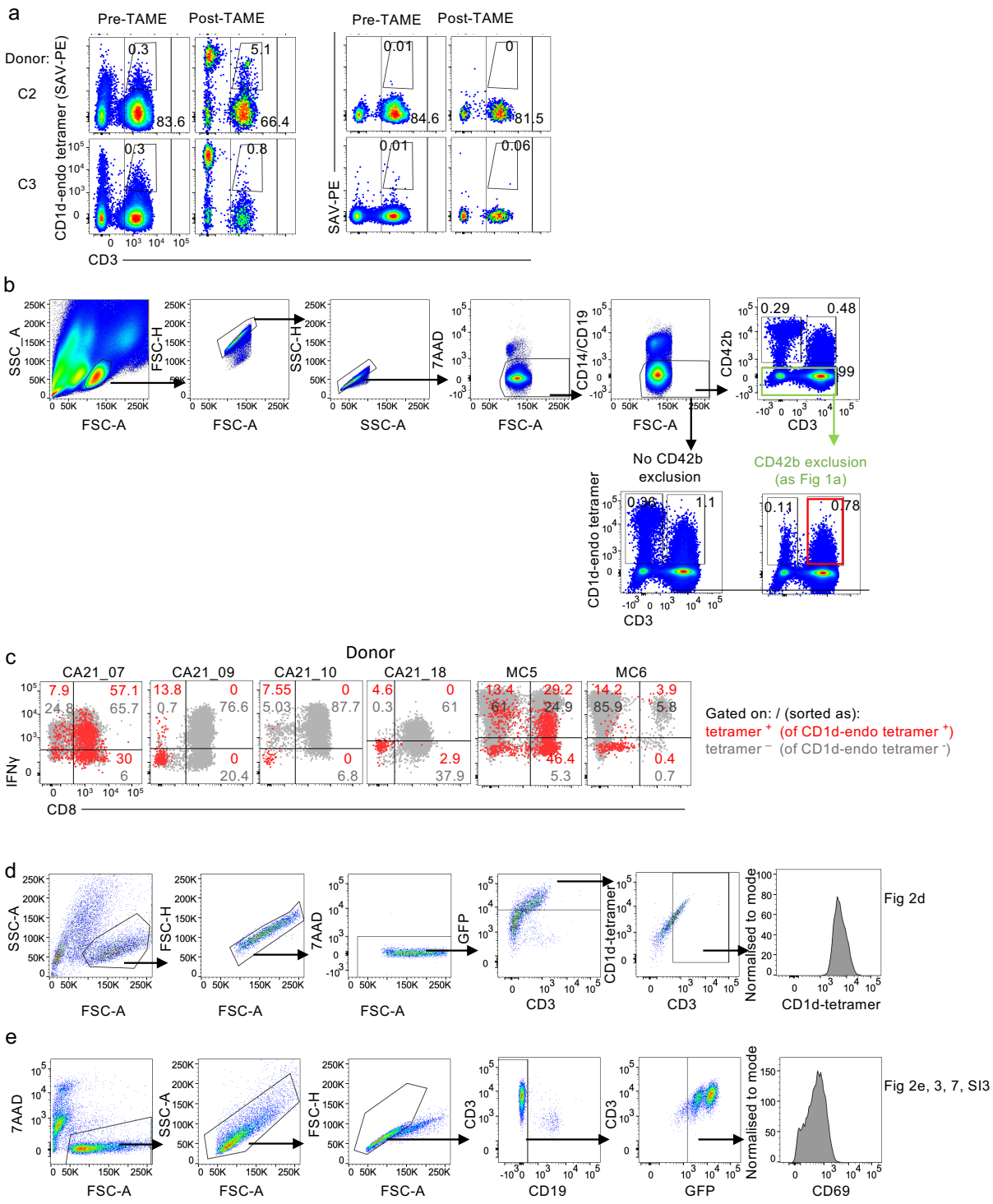

Fig. S1. (a) Flow cytometry plots of three donors showing CD3 *vs* CD1d-endo tetramer and streptavidin (SAV)-PE control staining pre- and post-CD1d-endo tetramer associated magnetic enrichment (TAME), gated on 7AAD^{-}CD14^{-}CD19^{-} single lymphocytes. Data shown is from one experiment, representative of 5 donors acquired over 2 experiments where SAV-PE enrichments were performed alongside CD1d-endo TAME. **(b)** Gating strategy for flow cytometry analysis of CD1d-endo tetramer staining of PBMCs with and without CD42b exclusion gate applied. Data is from donor CH7 and representative of the data graphed in Figures 1 and 2c. **(c)** Overlayed dot plots show CD8 *versus* IFNγ on stimulated CD1d-endo tetramer $^{+}$ cells (in red, gated as per the red gate in (1d)), stimulated (grey) or unstimulated (black) CD1d-endo tetramer $^{-}$ sorted cells. Data shown is from 6 donors, analysed across 4 experiments. **(d)** Gating strategy for flow cytometry analysis of CD1d-tetramer stain TCR reporter lines HEK293T or SKW3.β2m $^{-/-}$. The plot shown is from T26A stained with CD1d-endo tetramer and representative of data in figure 2d. **(e)** Gating strategy for flow cytometry analysis of TCR reporter lines HEK293T or SKW3.β2m $^{-/-}$ co-cultures with C1R lines. The plot shown is from NKT15:C1R.wt co-culture in the presence of SM and is representative of a datapoint in Figure 2e.

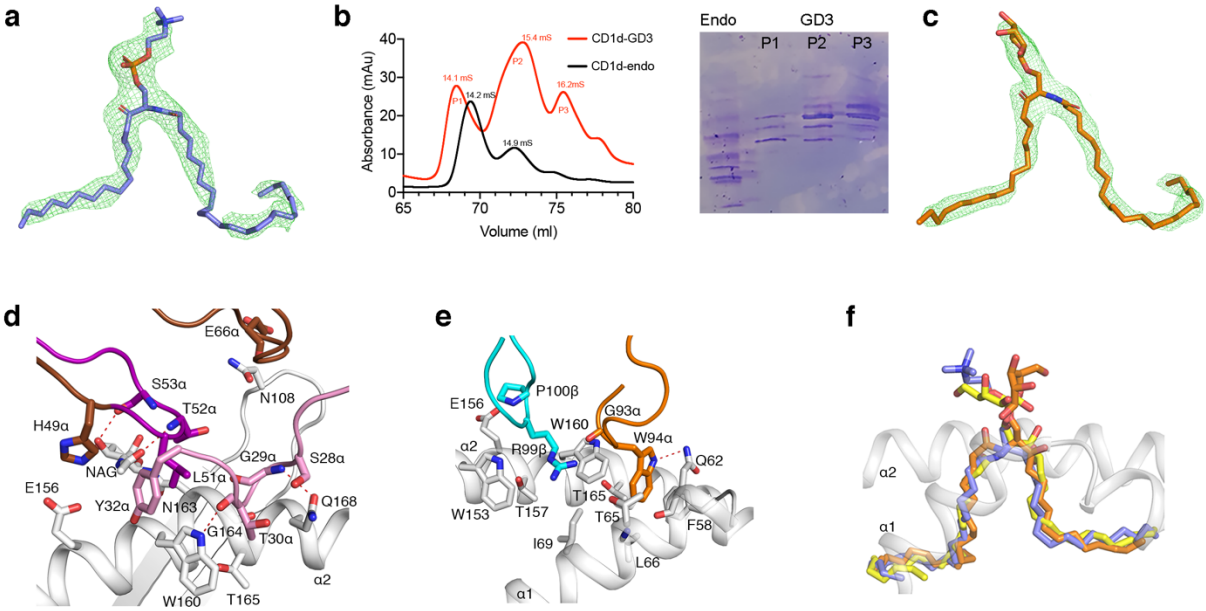

Fig. S2. (a) Fo-Fc electron density map of SM contoured at 2.2σ level. (**b**) Anion exchange chromatography profiles of CD1d-endo and CD1d-GD3 and the isoelectric focussing (IEF) gel of CD1d-endo with peaks P1, P2 and P3 of CD1d-GD3 isolated from the anion exchange purification. (**c**) Fo-Fc electron density map of GD3 (partially modelled) contoured at 2.2σ level. (**d-e**) Molecular interactions of the T26A CDR1α (pink), CDR2α (purple), CDR3α (orange), CDR3β (cyan) and FWα (brown) with CD1d-GD3. (**f**) Superposition of the CD1d-SM (in light blue, PDB code:8SGB), the partially modelled CD1d-GD3 (in orange, PDB code: 8SGM) and α-GalCer (in yellow, PDB code: 1ZT4).

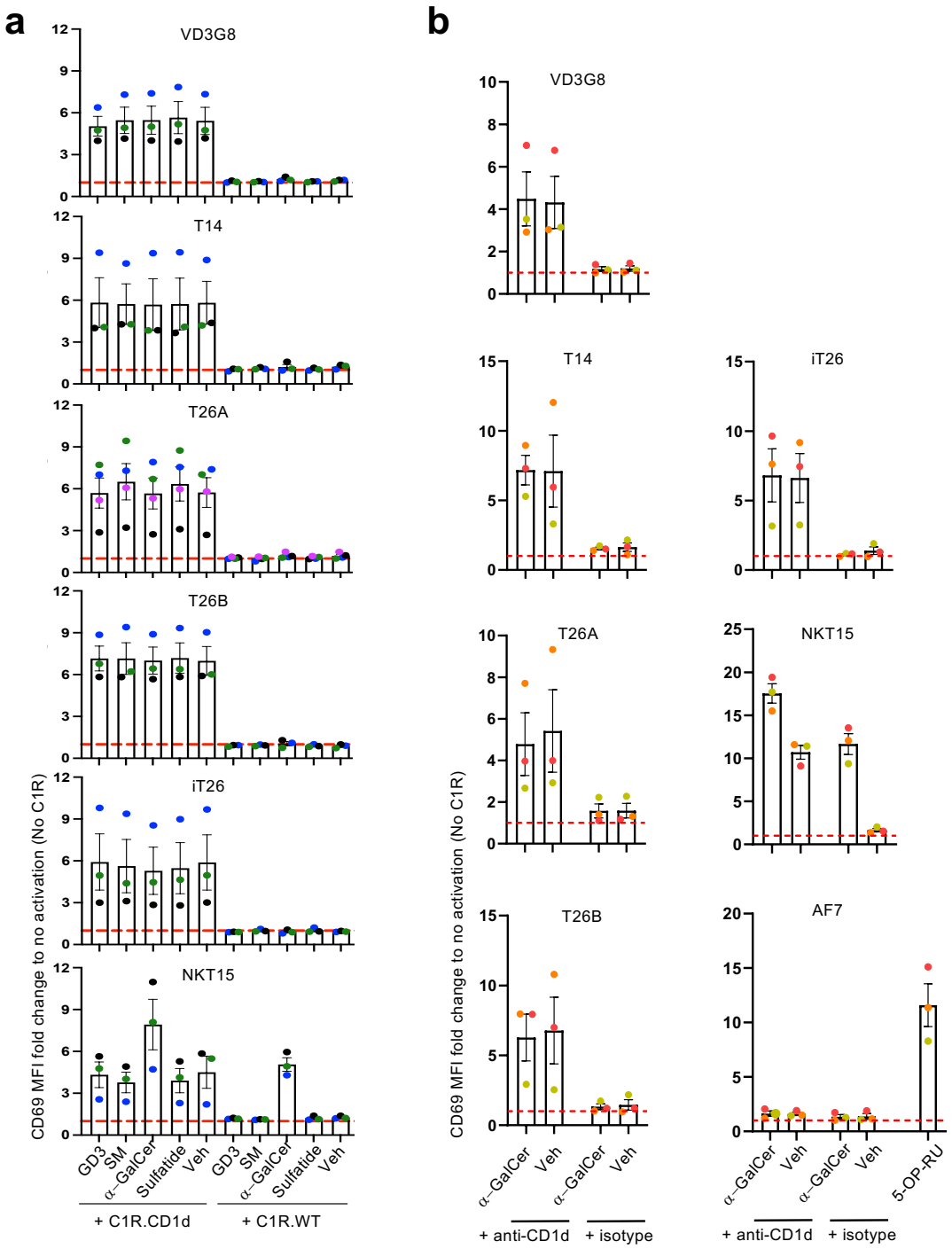

Fig. S3. **(a)** SKW3.β2m$^{-/-}$ TCR-transduced cell lines were co-cultured with wild type C1R cells (C1R.WT) or C1R transduced to express high levels of CD1d (C1R.CD1d) in the presence or absence of GD3, sphingomyelin (SM), sulfatide (all at 20 μg/mL), α-GalCer (1 μg/mL) or vehicle control (Veh). Graphs show fold increase in CD69 mean fluorescence intensity (MFI) relative to co-cultures with C1R.WT in the absence of exogenous Ag+/- SEM. Red line depicts fold-increase = 1 (baseline). Data shown is from *n* = *3* independent experiments, *n* = 4 for T26A, each represented by a different coloured dot. **(b)** SKW3.β2m$^{-/-}$ TCR-transduced cell lines were co-cultured with wild type C1R C1R.CD1d in the presence or absence of α-GalCer (1μg/mL), or vehicle control (Veh) together with either anti-CD1d (25μg/mL) or isotype control (isotype). The AF7 MAIT SKW3.β2m$^{-/-}$ TCR-transduced cell line and 5-OP-RU (1nM) were also included as controls. Graphs show fold increase in CD69 MFI relative to SKW3.β2m$^{-/-}$ TCR-transduced cell lines in the absence of C1R.CD1d cells, exogenous antigen or antibody +/- SEM. Red line depicts fold-increase = 1 (baseline). Data shown is from *n* = 3 independent experiments, each represented by a different coloured dot. The conditions pertaining co-cultures with α-GalCer and no lipid are also shown in Fig. 2e retaining the same colour-coding.

Table S1. Data collection and refinement statistics.

	T26A NKT TCR-CD1d-SM	T26A NKT TCR-CD1d-GD3
Data collection		
Temperature	100K	100K
Resolution limits (Å)	38.2-2.8 (2.9-2.8)	45.1-2.5 (2.6-2.5)
Space Group	$P4_1$	$P4_1$
Cell dimensions (Å)	*a*=136.79, *b*=136.79, *c*=69.80	*a*=133.79, *b*=133.79, *c*=68.57
Total N°. observations	443095 (56686)	298286 (31367)
N°. unique observations	32086 (4639)	42283 (4426)
Multiplicity	13.8 (12.2)	7.1 (7.1)
Data completeness	100 (100)	100 (100)
Wilson B-factors (Å^2)	66.5	49.2
I/σ_I	7.0 (2.3)	11.5 (3.70)
$R_{p.i.m}$ [1] (%)	6.4 (25.7)	3.9 (18.7)
Refinement statistics		
R_{factor} [2] (%)	21.7	18.6
R_{free} [3] (%)	25.6	23.3
Non hydrogen atoms		
- Protein	6296	6306
- Water	32	413
- Heterogen	124	152
Ramachandran plot (%)		
- Most favoured	91	95
- Allowed	7.8	4.8
B-factors (Å^2)		
- protein	67.7	51.4
- ligands	77	64.3
rmsd bonds (Å)	0.004	0.008
rmsd angles (°)	0.76	0.99

[1] $R_{p.i.m} = \Sigma_{hkl} [1/(N-1)]^{1/2} \Sigma_i | I_{hkl,i} - \langle I_{hkl} \rangle | / \Sigma_{hkl} \langle I_{hkl} \rangle$.

[2] $R_{factor} = (\Sigma | |F_o| - |F_c| |) / (\Sigma |F_o|)$ - for all data except as indicated in footnote 3.

[3] 5% of data was used for the R_{free} calculation.

Values in parentheses refer to the highest resolution bin.

Table S2. T26A NKT TCR molecular contacts with and CD1d-SM.

TCR gene	TCR residues	CD1d residues	Bond type
CDR1α	Ser^{28}	Gln^{168}	VDW
CDR1α	Gly^{29}	Gly^{164}, Gln^{168}	VDW
CDR1α	$Gly^{29\text{-}N}$	$Gln^{168\text{-}O\varepsilon1}$	HB
CDR1α	Thr^{30}	Trp^{160}, Gly^{164}, Thr^{165}, Gln^{168}	VDW
CDR1α	Tyr^{32}	Glu^{156}, Trp^{160}	VDW
FWα	$His^{49\text{-}N\varepsilon2}$	$Glu^{156\text{-}O\varepsilon2}$	HB
CDR2α	Leu^{51}	Asn^{163}, NAG^{278}	VDW
CDR2α	Thr^{52}	Asn^{163}, NAG^{278}	VDW
CDR2α	$Thr^{52\text{-}N}$	$NAG^{278\text{-}O}$	HB
CDR2α	Ser^{53}	NAG^{278}	VDW
CDR2α	$Ser^{53\text{-}O\gamma}$	$NAG^{278\text{-}O}$	HB
CDR3α	Gly^{93}	Trp^{160}	VDW
CDR3α	Trp^{94}	Gln^{62}, Leu^{66}, Trp^{160}	VDW
CDR3β	Arg^{99}	Ile^{69}, Trp^{153}, Thr^{157}, Trp^{160}	VDW
CDR3β	$Arg^{99\text{-}N\eta1}$	$Thr^{157\text{-}O\gamma1}$	HB
CDR3β	Pro^{100}	Glu^{156}	VDW

HB: Hydrogen bond, VDW: van der Waals,

Cut-off at 4 Å for VDW interactions and 3.5 Å for HB, NAG: N-acetyl-glucosamine.

Table S3. T26A NKT TCR molecular contacts with and CD1d-GD3.

TCR gene	TCR residues	CD1d residues	Bond type
CDR1α	Ser^{28}	Gln^{168}	VDW
CDR1α	Gly^{29}	Gly^{164}, Gln^{168}	VDW
CDR1α	$Gly^{29\text{-}N}$	$Gln^{168\text{-}O\varepsilon1}$	HB
CDR1α	Thr^{30}	Trp^{160}, Gly^{164}, Thr^{165}	VDW
CDR1α	$Thr^{30\text{-}O}$	$Trp^{160\text{-}N\varepsilon1}$	HB
CDR1α	Tyr^{32}	Glu^{156}, Trp^{160}	VDW
FWα	His^{49}	Glu^{156}	VDW
CDR2α	Leu^{51}	Trp^{160}, Asn^{163}, NAG^{278}	VDW
CDR2α	Thr^{52}	Asn^{163}, NAG^{278}	VDW
CDR2α	$Thr^{52\text{-}N}$	$NAG^{278\text{-}O}$	HB
CDR2α	Ser^{53}	NAG^{278}	VDW
CDR2α	$Ser^{53\text{-}O\gamma}$	$NAG^{278\text{-}O}$	HB
FWα	Glu^{66}	Asn^{108}	VDW
CDR3α	Gly^{93}	Trp^{160}	VDW
CDR3α	Trp^{94}	Phe^{58}, Gln^{62}, Thr^{65}, Leu^{66}, Trp^{160}, Thr^{165}	VDW
CDR3α	$Trp^{94\text{-}N\varepsilon1}$	$Gln^{62\text{-}N\varepsilon2}$	HB
CDR3β	Arg^{99}	Ile^{69}, Trp^{153}, Thr^{157}, Trp^{160}	VDW
CDR3β	Pro^{100}	Glu^{156}	VDW

HB: Hydrogen bond, VDW: van der Waals,

Cut-off at 4 Å for VDW interactions and 3.5 Å for HB, NAG: N-acetyl-glucosamine.