Supplementary information

Supplementary Table 1 Data collection and refinement statistics

	Vβ8.2 TCR-mCD1d-α-GalCer	**Vβ7 TCR-mCD1d-α-GalCer**	**Vβ11 TCR-hCD1d-α-GalCer**
Data collection			
Temperature	100K	100K	100K
Resolution limits (Å)	50-2.9 (3.06-2.90)	50-2.8 (2.95-2.80)	50-2.5 (2.59-2.50)
Space Group	$P2_12_12_1$	$P2_12_12_1$	P2
Cell dimensions (Å)	*a*=61.113, *b*=81.506, *c*= 233.451, α=β=γ=90.00°	*a*=58.229, *b*=81.913, *c*=243.253, α=β=γ=90.00°	*a=112.11, b=82.36 c=117.18,* α=90.00, β=101.3, γ=90.00°
Total N°. observations	149997	205387	195771
N°. unique observations	26743	29526	66054
Multiplicity	5.6 (6.0)	7.0 (5.8)	3.0 (2.7)
Data completeness	100 (100)	99.7 (99.1)	91.1 (74.3)
I/σ_1	11.1 (2.1)	13.7 (2.2)	10.5 (1.7)
$R_{p.i.m}$ [1] (%)	7.6 (31.5)	5.3 (30.4)	-
R_{merge} [2] (%)	-	-	8.8 (34.3)
Refinement statistics			
R_{factor} [3] (%)	23.4	22.4	21.6
R_{free} [4] (%)	29.8	27.1	27.9
Non hydrogen atoms			
- protein	6559	6528	12995
- lipid	60	60	120
- water	28	47	494
- other	56	56	93
Ramachandran plot			
- Most favoured	88.9	91.6	89.4
- Allowed region (%)	11.1	8.3	10.1
- Generously allowed region (%)	0.0	0.1	0.5
B-factors (Å^2)			
- Average main chain	48.8	44.0	42.8
- Average side chain	49.0	44.3	42.5
- α-GalCer	52.1	49.0	29.7
- water	38.7	33.1	38.9
rmsd bonds (Å)	0.007	0.006	0.007
rmsd angles (°)	1.076	1.029	1.119

[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] $R_{merge} = \Sigma_{hkl} \Sigma_i | I_{hkl,i} - \langle I_{hkl} \rangle | / \Sigma_{hkl} \langle I_{hkl} \rangle$

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

[4] 5% of data was used for the R_{free} calculation

Values in parentheses refer to the highest resolution bin.

Supplementary Table 2 SPR data for NKT TCR interactions

Interaction	K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	$t_{1/2}$ (s)	K_D Calc. (nm)	Chi^2	K_D obs. (nm)
Vβ8.2 NKT-mCD1d-α-GalCer	6.40E+05	0.04	17.32	54.9	16.36	69.7
Vβ8.2 NKT-hCD1d-α-GalCer	1.03E+06	0.09	7.7	84.9	7.21	93.9
Vβ7 NKT- mCD1d-α-GalCer	4.26E+05	0.1	6.93	224	2.14	295
Vβ7 NKT-hCD1d-α-GalCer	1.28E+05	0.49	1.41	3810	4.09	3420
hNKT TCR-mCD1d-α-GalCer	3.15E+05	0.33	2.1	1060	9.15	910
hNKT TCR-hCD1d-α-GalCer	6.27E+05	0.15	4.62	236	9.95	219

Supplementary Table 3 SPR data for mutant NKT TCR interactions

Mutant	K_D (nM)
Vβ7 wt	134
Vβ7 D26A	171
Vβ7 S54A	32.6
Vβ7 S56A	189
Vβ7 Y50F	520
Vβ7 E57A	225
Vβ8.2 wt	29.4
Vβ8.2 N28A	33.7
Vβ8.2 Y48F	> 1690
Vβ8.2 Y50F	377
Vβ8.2 E56A	165

Supplementary Figures

Suppl. Fig. 1. Characterisation of recombinant NKT TCRs

(A) Purified bacterially-expressed TCRs were analysed by SDS-PAGE under reducing conditions (lanes 1-4) and non-reducing conditions (lanes 5-8). Lanes 1&5 control TCR LC13, lanes 2&6 human Vα24Vβ11 (NKT-15), lanes 3&7 mouse Vα14-Vβ7 and lanes 4&8 mouse Vα14-Vβ8.2.

(B) The structural integrity of the constant domains of the recombinant soluble TCRs were assessed by using the conformationally-specific mAb, 12H8 in an ELISA. Plate bound TCRs, LC13 (positive control), human Vα24Vβ11 NKT-15 TCR, mouse Vα14Vβ7 TCR, mouse Vα14Vβ8.2 TCR or irrelevant HLA B8-FLR (negative control) were treated with graded amounts of 12H8. 12H8-reactivity is shown as absorbance at 492nm on the vertical axis and concentration of 12H8 (μg/ml) is indicated on the horizontal axis.

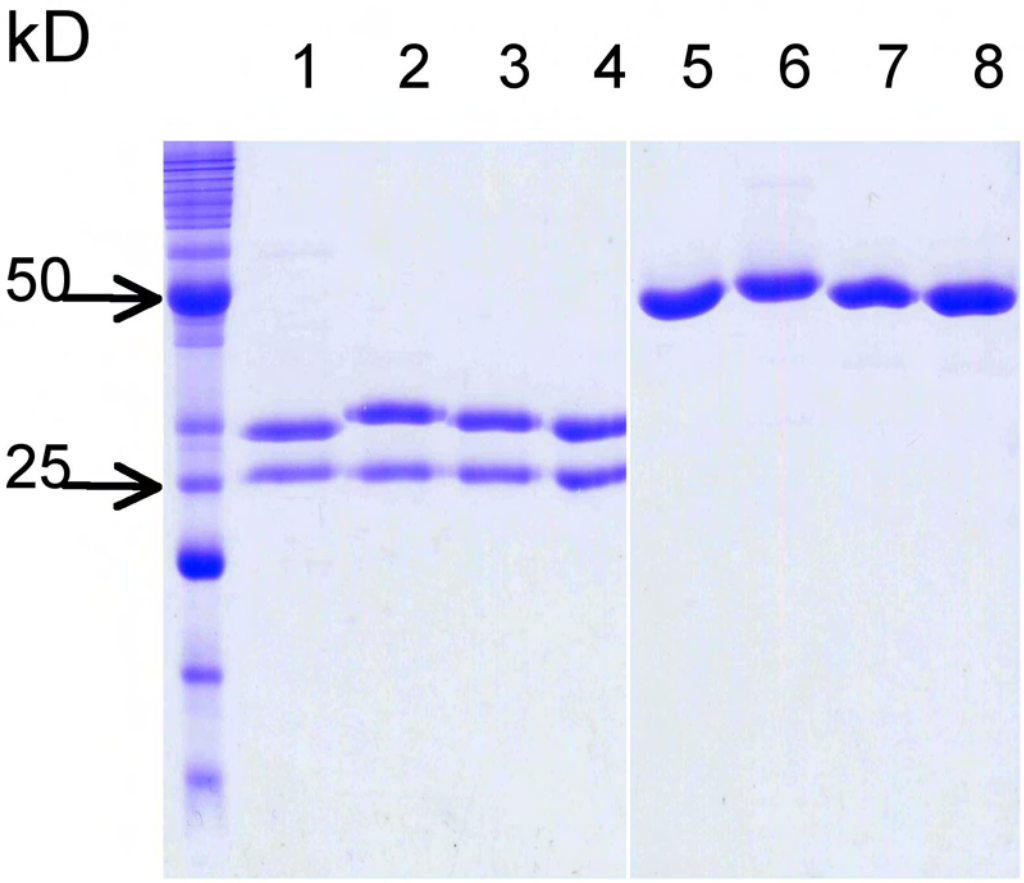
Suppl. Fig. 2 Shift in the position of the galactose head group of α-GalCer in mCD1d when ligated to the mouse NKT TCR. Mouse NKT TCR CDR1α, purple; CDR3α, yellow; mCD1d in ternary complex, grey; α-GalCer in ternary complex, magenta; mCD1d in binary complex, pink; α-GalCer in binary complex, green.

Suppl. Fig. 3 Sequence alignments between mouse and human NKT TCRs

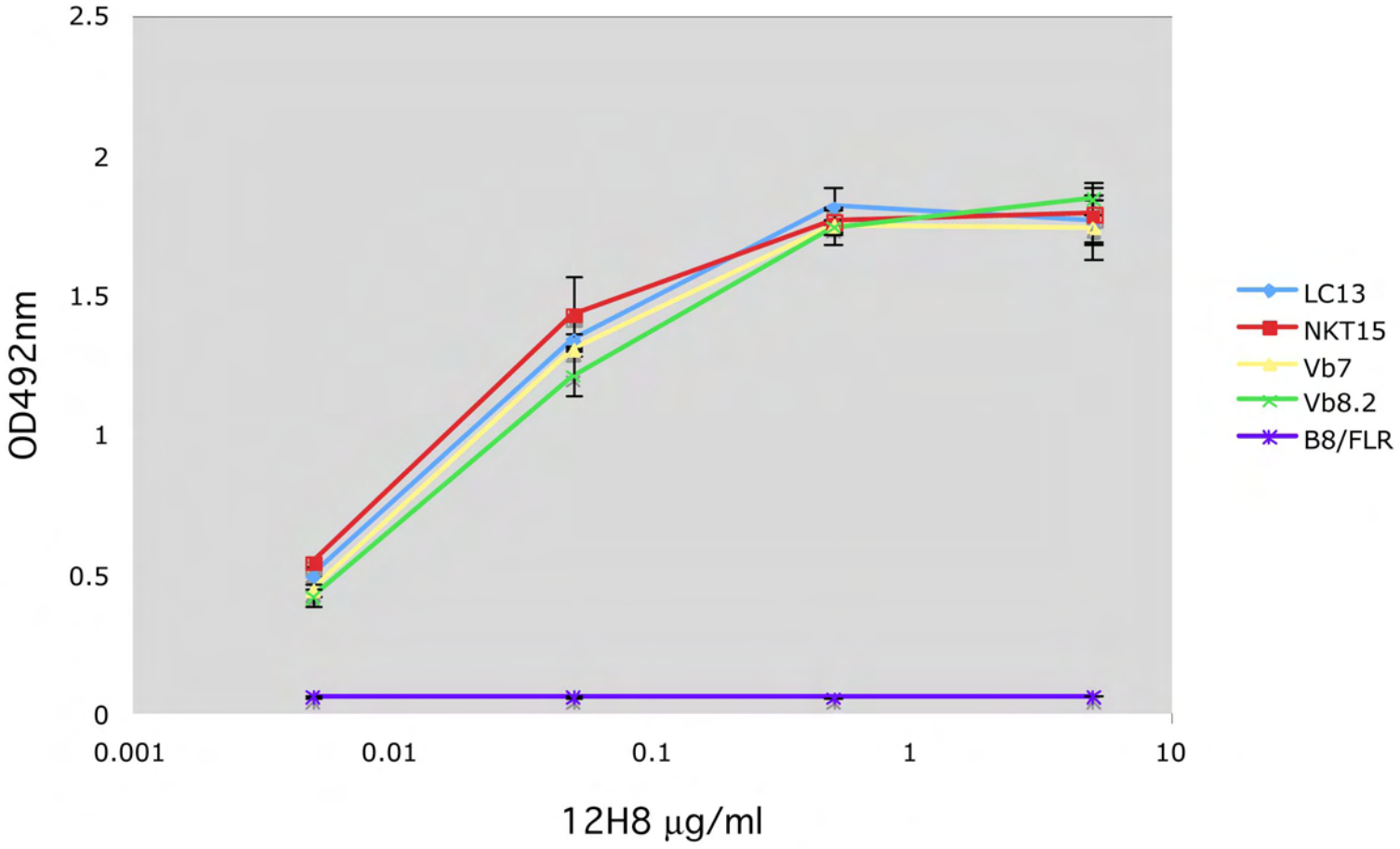
Suppl. Fig. 4 Comparison of mouse NKT TCR-mCD1d-α-GalCer with human NKT TCR-hCD1d-α-GalCer. Parallel docking mode of the human and mouse NKT-TCR onto CD1d-α-GalCer. **(A)** Superposition of mouse Vα14-Vβ8.2 NKT TCR-mCD1d-α-GalCer and human Vα24-Vβ11 NKT TCR-hCD1d-α-GalCer. mTCR α-chain, cyan; mTCR β-chain, green; α-GalCer in mouse complex, magenta; mCD1d heterodimer, grey; hTCR α-chain, salmon; hTCR β-chain, yellow; α-GalCer in human complex, marine; hCD1d heterodimer, palegreen; **(B)** Superposition of mouse Vα14-Vβ7 NKT TCR-mCD1d-α-GalCer and human Vα24-Vβ11 NKT TCR-hCD1d-α-GalCer. mTCR β-chain, blue. mTCR α-chain, α-GalCer in mouse and human complex, mCD1d, hTCR and hCD1d colour coding as in **A**.

Supplementary Fig 1

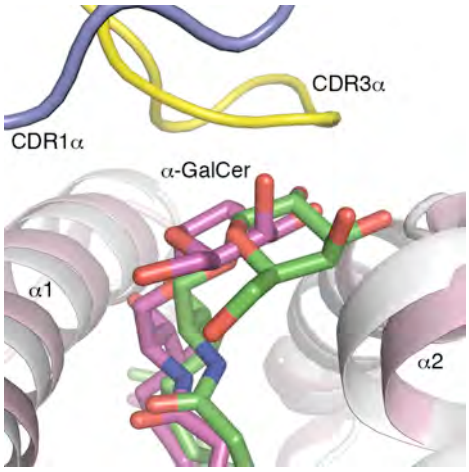
a)



b)



Supplementary Figure 2



Supplementary Figure 3

	CDR1b						CDR2b								
	26	27	28	29	30	31	48	49	50	51	52	53	54	55	56
Mouse Vb8.2	T	N	N	H	N	N	Y	S	Y	G	A	G	S	T	E
Mouse Vb7	D	M	S	H	E	T	I	S	Y	D	V	D	S	N	S
Human Vb11	T	M	G	H	D	K	Y	S	Y	G	V	N	S	T	E

	CDR3b																										
	92	93	94	95	96	97	98	99	100	101	102	103															117
Mouse Vb8.2	C	A	S	G	D	A	G	G	N	Y	A	E	Q	F	F	G	P	G	T	R	L	T	V	L			
Mouse Vb7	C	A	S	S	S	T	G	L	D	T	Q	Y	F	G	P	G	T	R	L	L	V	L					
Human Vb11	C	A	S	S	G	L	R	D	R	G	L	Y	E	Q	Y	F	G	P	G	T	R	L	T	V	T		

	CDR1a						CDR2a					
	26	27	28	29	30	31	49	50	51	52	53	54
Mouse Va14	V	T	P	D	N	H	L	V	D	Q	K	D
Human Va24	V	S	P	F	S	N	M	T	F	S	E	N

	CDR3a																										
	90	91	92	93	94	95	96	97	98	99	100	103	104	105	106	107	108	109	110	111	112	113	114	115	116		
Mouse Va14	C	V	V	G	D	R	G	S	A	L	G	R	L	H	F	G	A	G	T	Q	L	I	V	I	P		
Human Va24	C	V	V	S	D	R	G	S	T	L	G	R	L	Y	F	G	R	G	T	Q	L	T	V	W	P		

Supplementary Figure 4