

Supplementary information

$\gamma\delta$ T cell receptor recognition of CD1d in a lipid-independent manner

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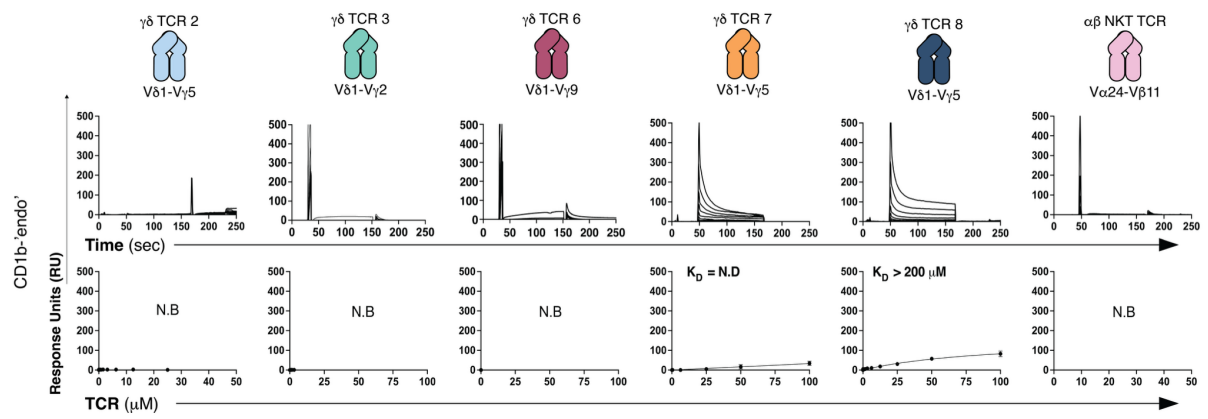
Supplemental Data

Supplemental Figures 1-11

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Supplementary Table 1 : $\gamma\delta$ TCRs used within this study. $\gamma\delta$ TCRs: 2, 3, 6, 7 & 8 were previously identified from PBMC derived CD3⁺ $\gamma\delta$ T cells from ref 19.

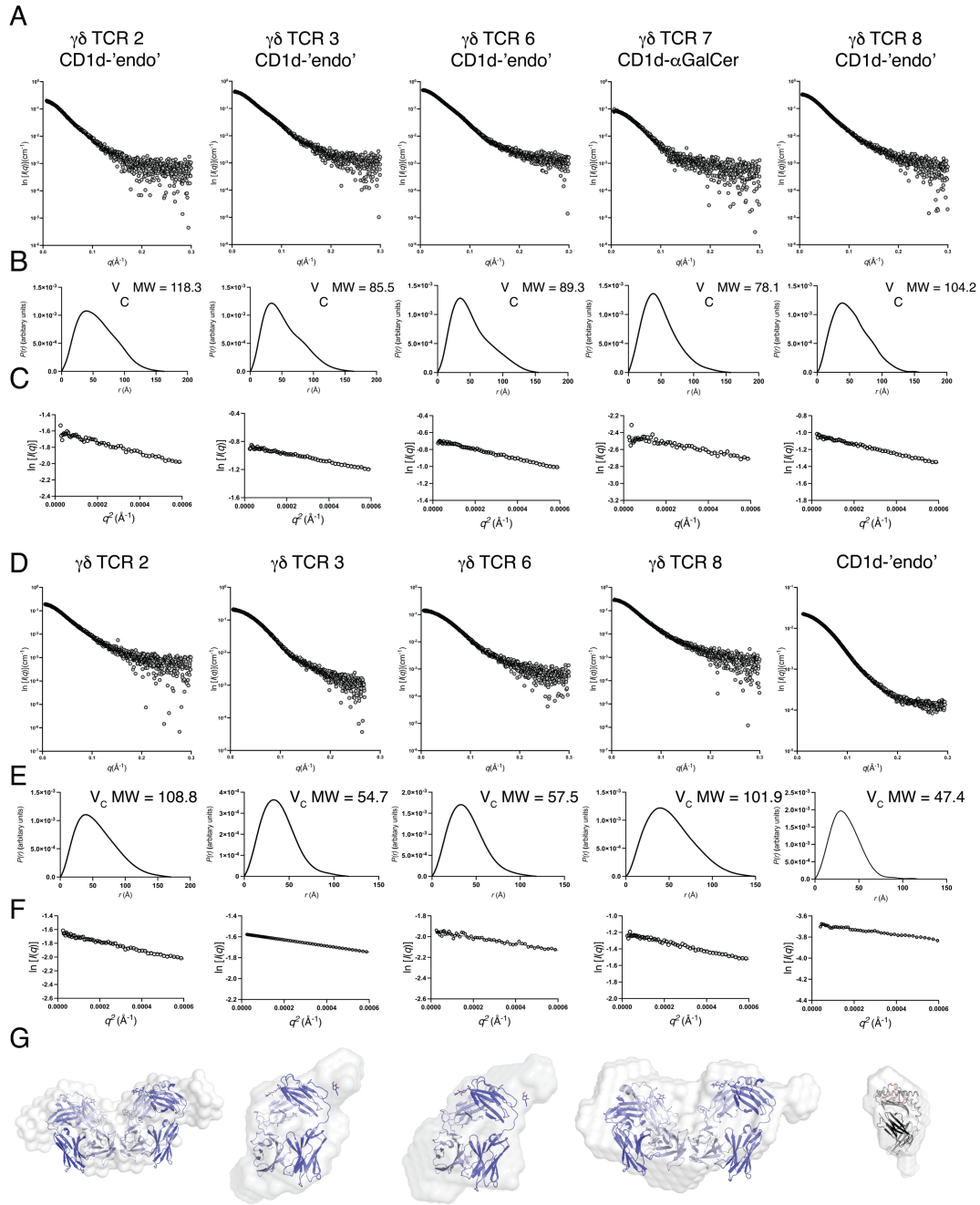
TCR	Donor	Chain Usage	CDR3 γ	CDR3 δ
2	3	V δ 1-V γ 5	CATWDRLYYKKLF	CALGVWGDKLIF
3	3	V δ 1-V γ 2	CATWDGLSYKKLF	CALGELLVRSSLTAQLFF
6	5	V δ 1-V γ 9	CALWEARPFYYKKLF	CALGELWGFPNRDKLIF
7	6	V δ 1-V γ 5	CATWDRPEANYKKLF	CALGEPFLRSLIWEYTDKLIF
8	6	V δ 1-V γ 5	CATWDALAKLF	CALGETFLPSLGGWTDKLIF
9C2		V δ 1-V γ 5	CATWDRGNPKTHYYKKLF	CALGDPGGLNTDKLIF
DP10.7		V δ 1-V γ 4	CATWDEKYYKKLF	CALGEPsyWGFPRTTRVIF



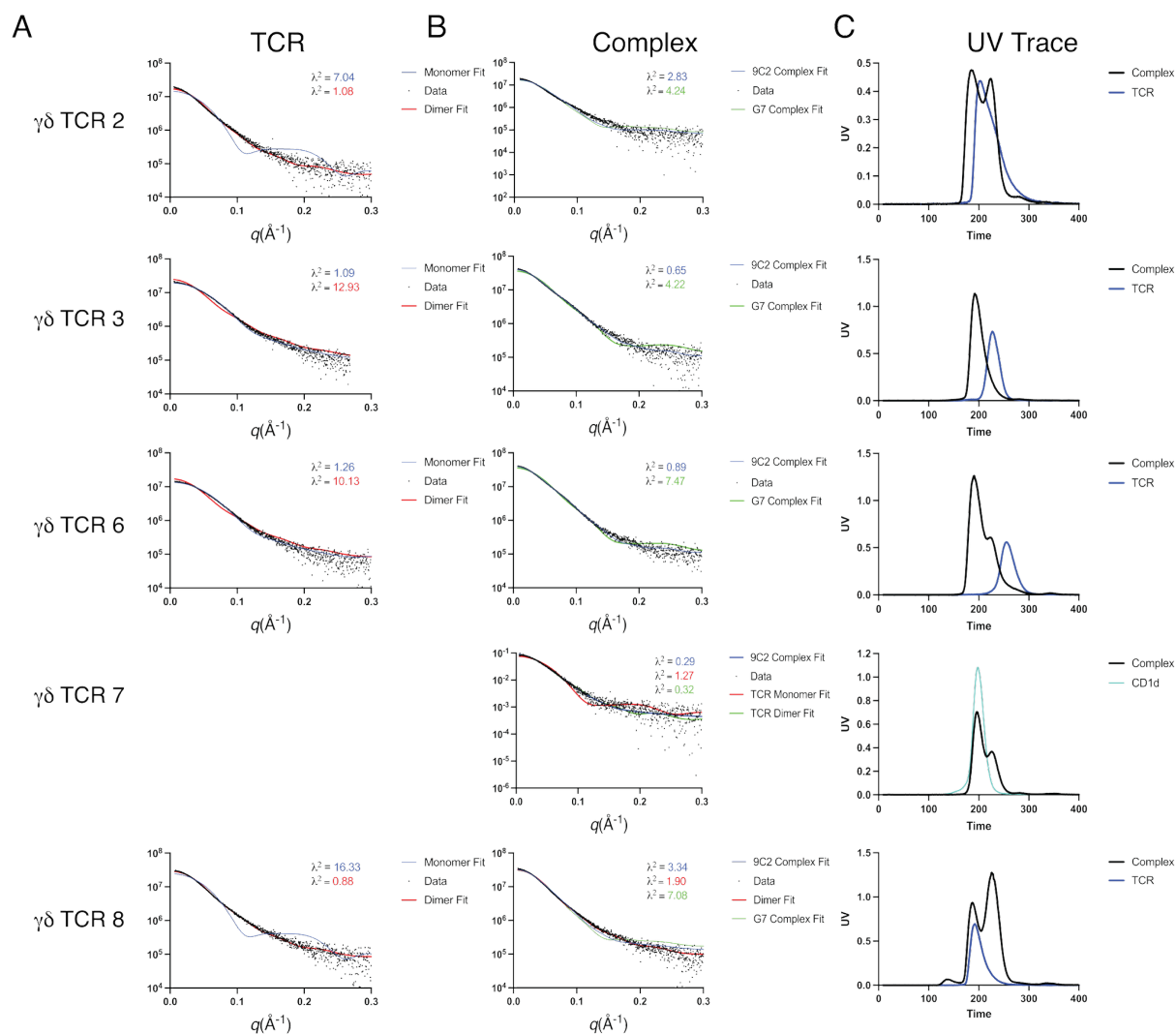
Supplementary Figure 1: $\gamma\delta$ TCR Affinity Measurements. Affinity measurement analysis of $\gamma\delta$ TCRs 2, 3 & 6, 7, 8 and the $\alpha\beta$ NKT TCR determined by SPR against CD1b-'endo'. Sensograms are representative of a single dilution series. Error bars denote S.E.M. N=2 independent experiments, performed in duplicate. N.D = not determined.

Supplementary Table 2. SEC-SAXS Data analysis.

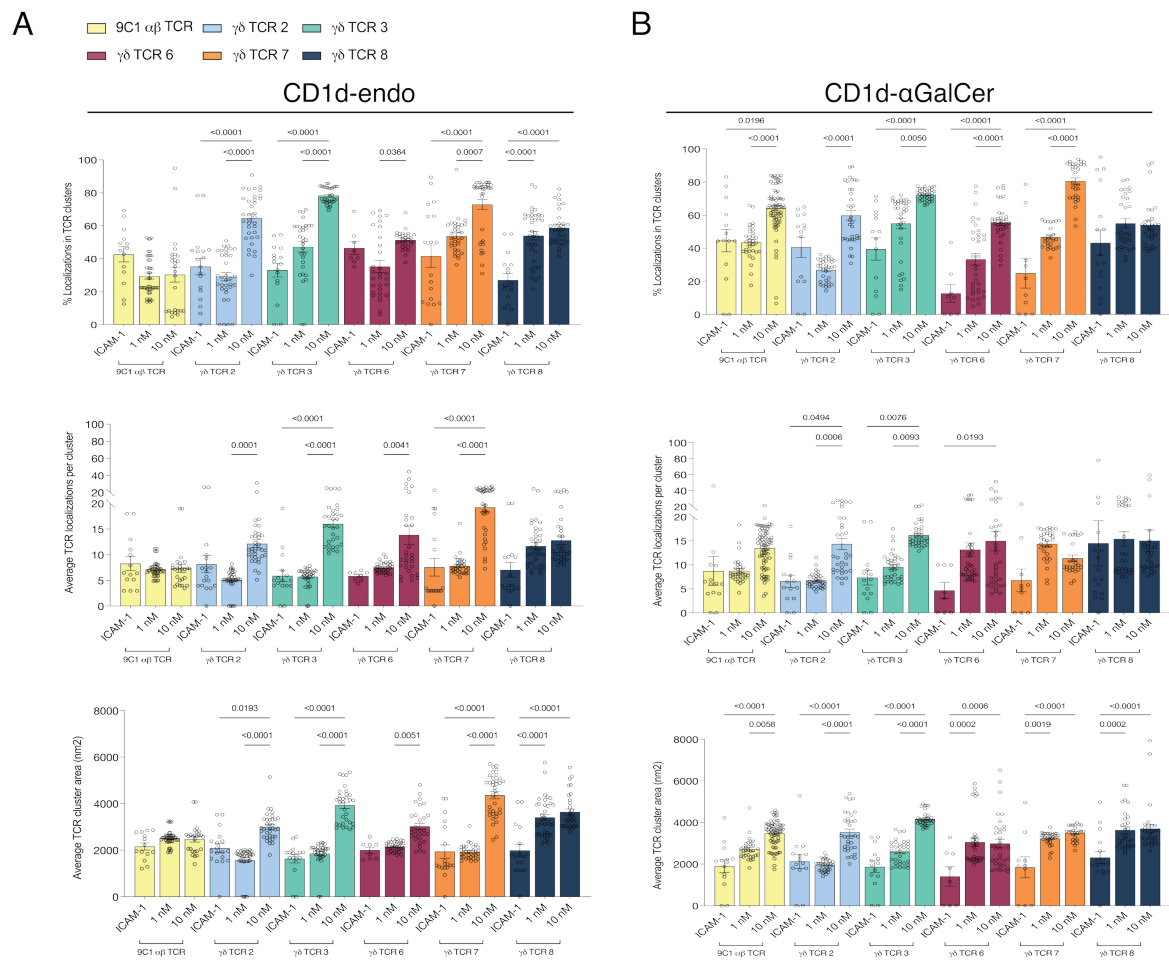
Sample	Concentration (mg/mL)	Radius of Gyration, R_g (Å)	Maximum Dimension, D_{max} (Å)	Molecular Mass, from V_p (kDa)	Oligomeric state	Observed Stoichiometry (TCR:CD1d)	Calculated Molecular Mass (kDa)
$\gamma\delta$ TCR 2	8.55	43.4 ± 0.36	170	95.9	1.8	2:0	52.08
$\gamma\delta$ TCR 3	8	29.84 ± 0.19	114	43.6	0.83	1:0	52.37
$\gamma\delta$ TCR 6	7.85	30.3 ± 0.20	118	46.2	0.8	1:0	54.27
$\gamma\delta$ TCR 8	7.25	40.05 ± 0.24	149	85.3	1.5	2:0	52.79
CD1- endo	7	27.14 ± 0.02	118	51.8	0.84	0:1	43.73
$\gamma\delta$ TCR 2 CD1d- 'endo'	8	43.58 ± 0.36	163	104	1.09	1:1	95.81
$\gamma\delta$ TCR 3 CD1d- 'endo'	5	40.81 ± 0.31	164	76.5	0.79	1:1	96.09
$\gamma\delta$ TCR 6 CD1d- 'endo'	7.85	40.47 ± 0.18	155	80	0.83	1:1	96.52
$\gamma\delta$ TCR 7 CD1d- α -GalCer	4	37.07 ± 0.52	157	67.1	0.69	Un- complexed	97.30
$\gamma\delta$ TCR 8 CD1d- 'endo'	8	40.86 ± 0.19	157	88.2	0.88	Un- complexed	100.60



Supplementary Figure 2: $\gamma\delta$ TCR SEC-SAXS data collection. SEC-SAXS scattering profile (A), P(r) distribution plot (B), Guinier analysis (C) and *ab initio* model (D) for $\gamma\delta$ TCR 2, $\gamma\delta$ TCR 3, $\gamma\delta$ TCR 6 and $\gamma\delta$ TCR 8 complexed with CD1d-'endo' or CD1d- α -GalCer. (D-G) SEC-SAXS monomer samples, scattering profile (D), P(r) plot (E), Guinier analysis (F) and *ab initio* reconstruction (G). Data was processed in BioXTSAS RAW software suite and GraphPad Prism 10.3.1. The 9C2 $\gamma\delta$ was aligned to the *ab initio* model, colored white, via DAMMIF, with γ/δ chains in light and dark blue respectively. CD1d shown in dark grey and β_2 M in black. V_C denotes volume of correlation, MW denotes molecular weight.

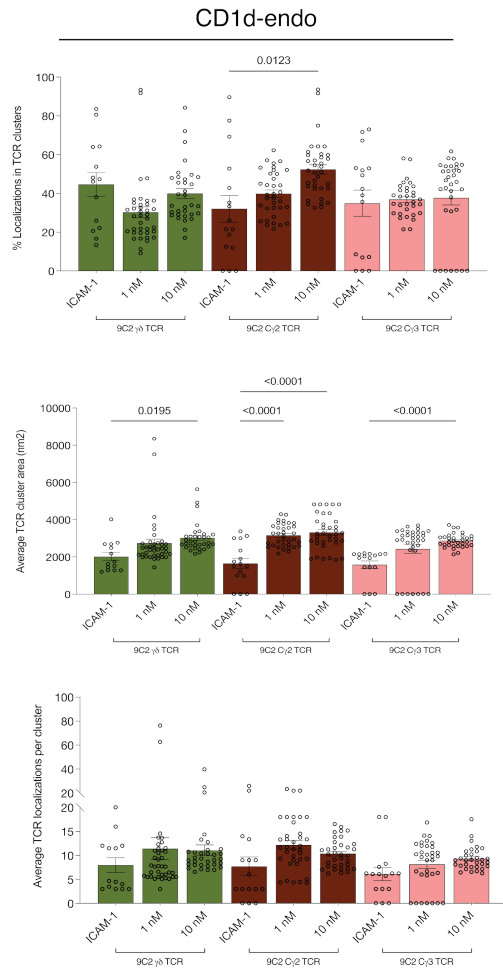


Supplementary Figure 3: SEC-SAXS CRYSOLOG analysis. CRYSOLOG analysis of $\gamma\delta$ TCR 2, $\gamma\delta$ TCR 3, $\gamma\delta$ TCR 6 and $\gamma\delta$ TCR 8, for TCR alone (A) or complexed to CD1d-‘endo’ (B). SEC UV Trace overlay comparing TCR alone and TCR-CD1d-‘endo’ was used to assess TCR co-complexation (C).

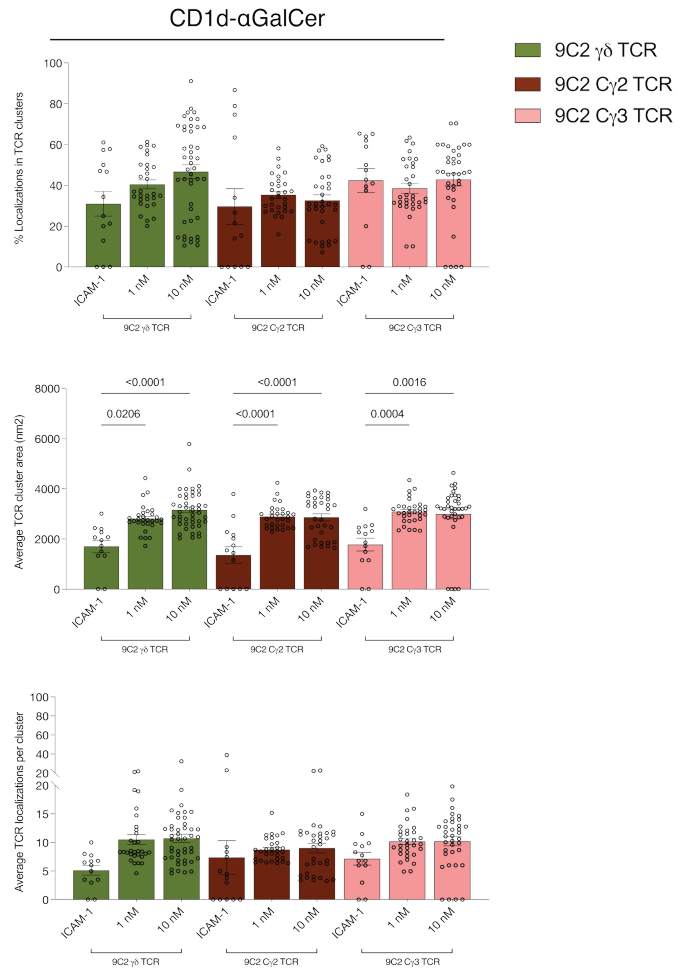


Supplementary Figure 4: Analysis of dSTORM images from Figure 3. DBSCAN was used to analyse CD1d-endo (**A**) and CD1d- α GalCer (**B**) induced clustering. Data was processed in GraphPad Prism 10.3.1. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test against ICAM-1 controls. Error bars represent S.E.M.

A



B

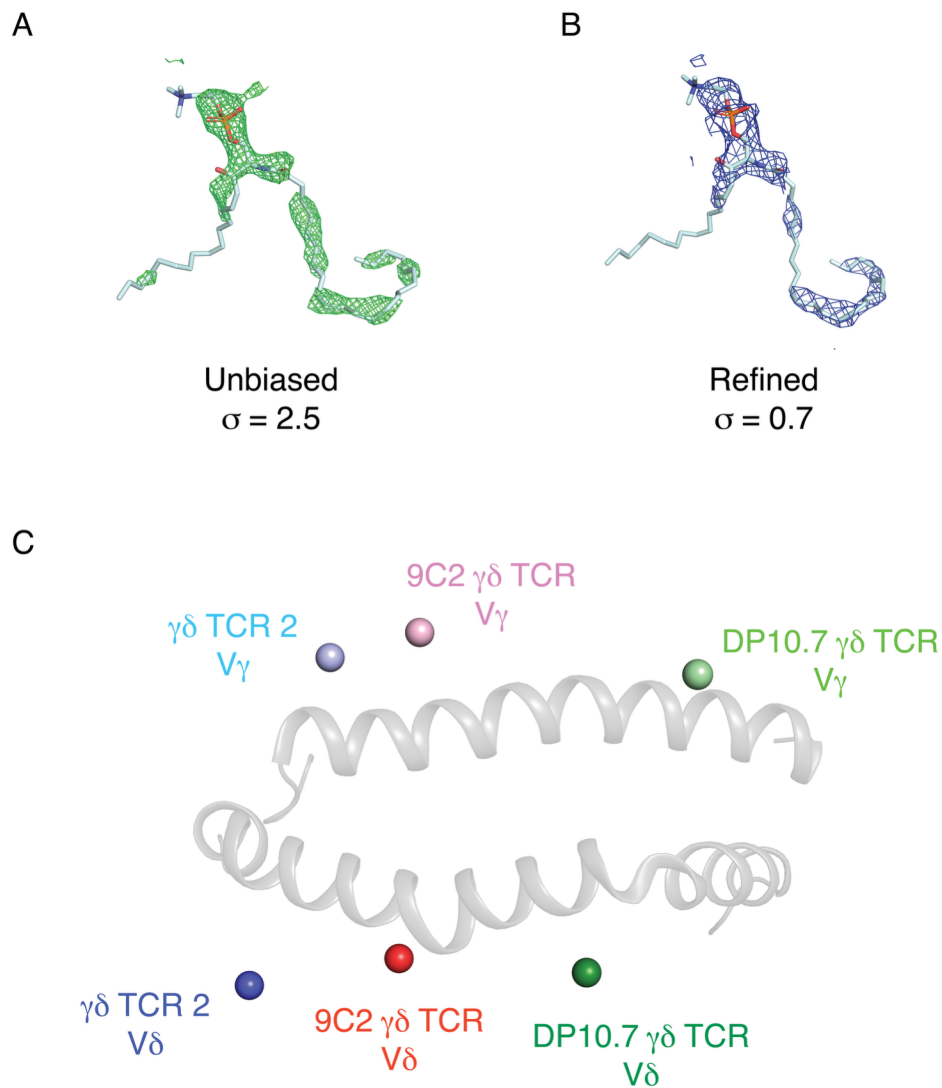


Supplementary Figure 5: Analysis of *d*STORM images from Figure 4. DBSCAN was used to analyse CD1d-endo (A) and CD1d- α GalCer (B) induced clustering. Data was processed in GraphPad Prism 10.3.1. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test against ICAM-1 controls. Error bars represent S.E.M.

Supplementary Table 3. Data collection and refinement statistics

$\gamma\delta$ TCR 2-CD1d-'endo'	
Data collection	
Temperature	100K
Resolution (Å)	46.65-2.86 (3.02-2.86)
Space group	P 43 21 2
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	178.09, 178.09, 75.43
	$\alpha=\beta=\gamma=90^\circ$
No. of observations	296948 (43221)
No. of unique observations	28516 (4072)
<i>I</i> / σ <i>I</i>	16.4 (2.3)
Completeness (%)	99.8 (98.7)
Multiplicity	10.4 (10.6)
<i>R</i> _{merge}	0.106 (1.058)
<i>R</i> _{pim}	0.049 (0.491)
CC 1/2	0.999(0.750)
Refinement	
<i>R</i> _{work} / <i>R</i> _{free}	20.65/24.97
No. atoms	
Protein	6400
Ligand/ion	142
<i>B</i> -factors	
Protein	84.08
Ligand/ion	108.61
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	0.938
Ramachandran (%)	
Favoured	97.20
Allowed	2.8
Outliers	0

*Values in parentheses are for highest-resolution shell.



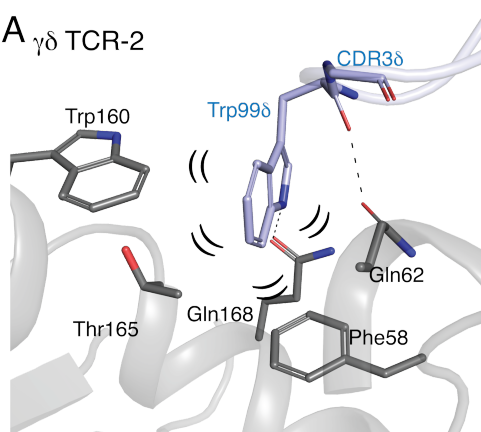
Supplementary Figure 6: Electron density for $\gamma\delta$ TCR-CD1d-endo lipid antigen. Unbiased (A) and refined (B) density for the lipid antigen built into the antigen presenting cleft of CD1d-endo. The sphingomyelin lipid antigen built into the density is shown as sticks. (C) Variable domain COM for $\gamma\delta$ TCR 2, 9C2 $\gamma\delta$ TCR and DP10.7 $\gamma\delta$ TCR. V_γ/V_δ shown in light and dark variants of blue, red and green. CD1d shown in grey.

Supplementary Table 4. $\gamma\delta$ TCR 2 contacts with CD1d-‘endo’.

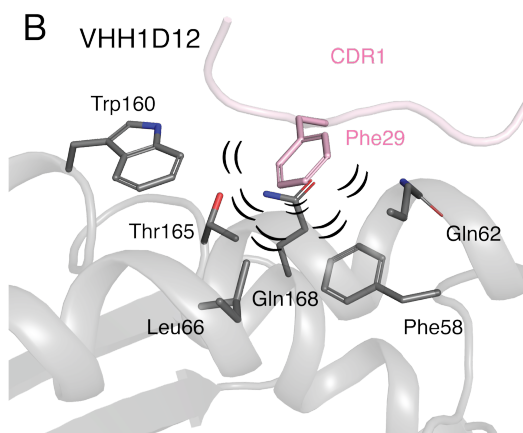
TCR Gene	TCR Residues	CD1d Residues	Bond Type
CDR1 γ	Ile31	Trp153	VDW
	Phe34	His68, Ile69	VDW
	Tyr35	Gln61, Glu64, Thr65, His68	VDW
FW γ	His37	Gln61	
	Arg49 ^{NH1}	Gln61 ^{Oϵ1}	HB
	Arg49	Gln61	VDW
	Tyr52	Gln61	VDW
CDR2 γ	Asp54 ^{Oδ1,2}	His68 ^{Nϵ2}	HB
	Ser56	His68	VDW
	Asn57	Arg71	VDW
CDR3 γ	Arg103	Trp153, Glu156	VDW
	Leu104	Glu156, Trp160	VDW
	Tyr105	Thr65, Trp160	VDW
CDR1 δ	Trp33	Glu175	VDW
	Ser34	Gln168	VDW
CDR3 δ	Val98	Gln62	VDW
	Trp99	Phe58, Gln62, Trp160, Thr165, Gln168	VDW
	Trp99 ^{Nϵ1}	Gln168 ^{Oϵ1}	HB
	Trp99 ^O	Gln62 ^{Nϵ2}	HB
	Gly100 ^O	Gln61 ^{Nϵ2}	HB
	Gly100	Gln61	VDW
	Asp101	Ser59, Gln61	VDW

HB: Hydrogen bond, VDW: Van der Waals, SB: salt bridge. Cut-off at 4 Å for VDW interactions and SB, 3.5 Å for HB.

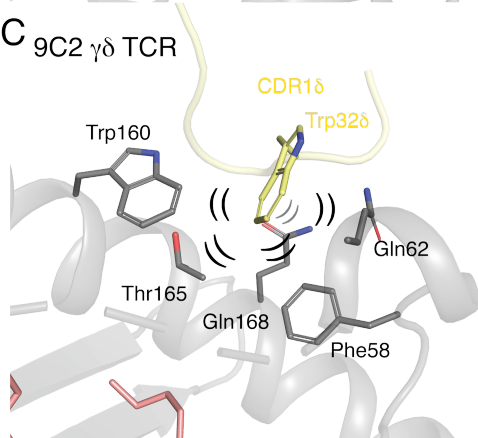
A $\gamma\delta$ TCR-2



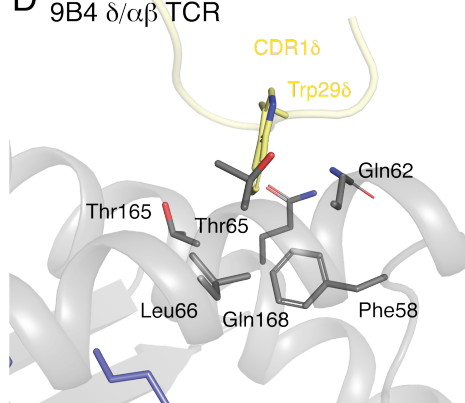
B VHH1D12



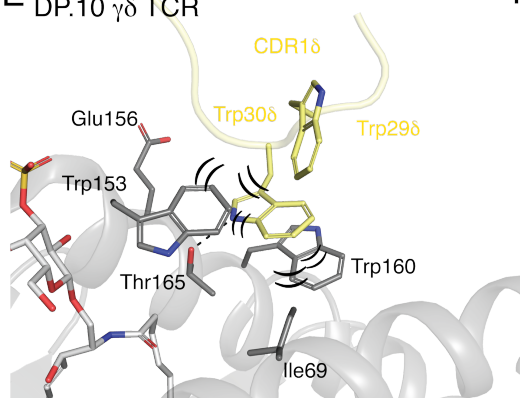
C 9C2 $\gamma\delta$ TCR



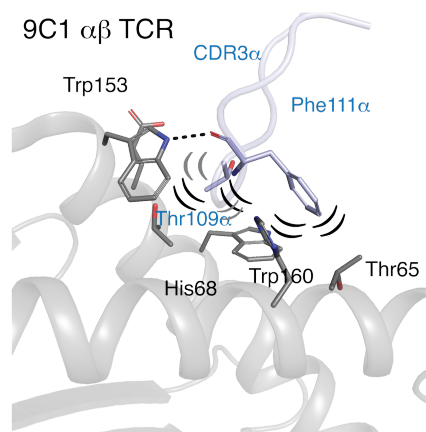
D 9B4 $\delta/\alpha\beta$ TCR



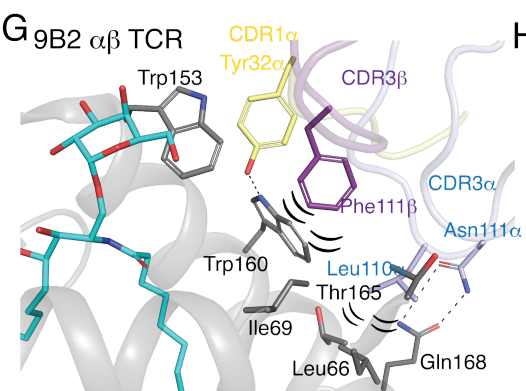
E DP.10 $\gamma\delta$ TCR



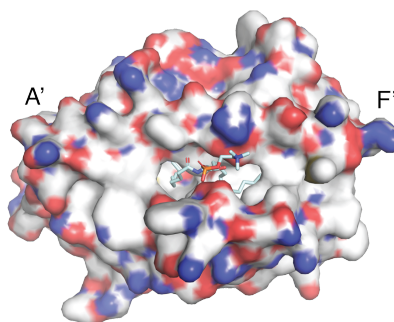
F 9C1 $\alpha\beta$ TCR



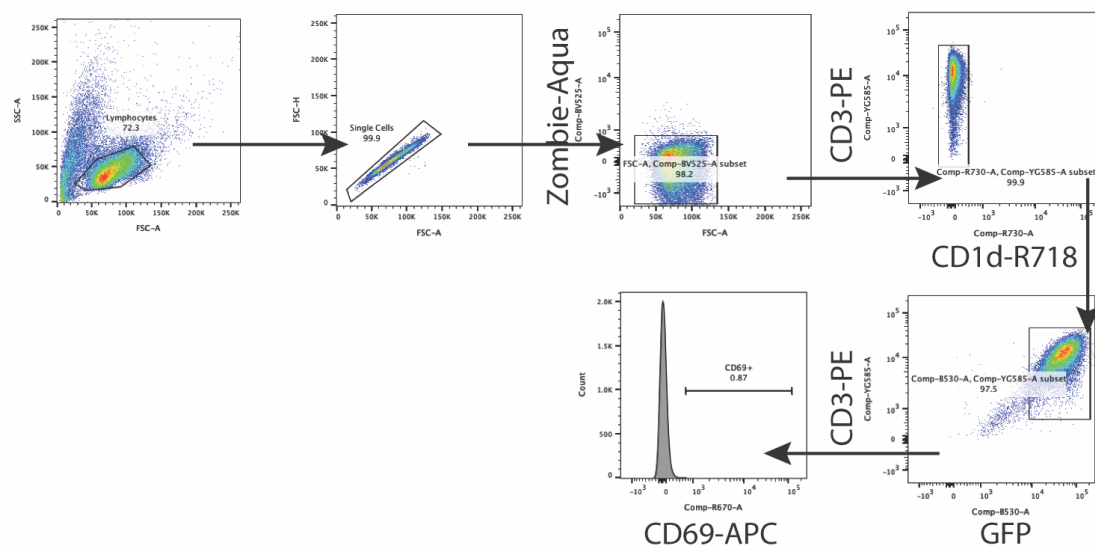
G 9B2 $\alpha\beta$ TCR



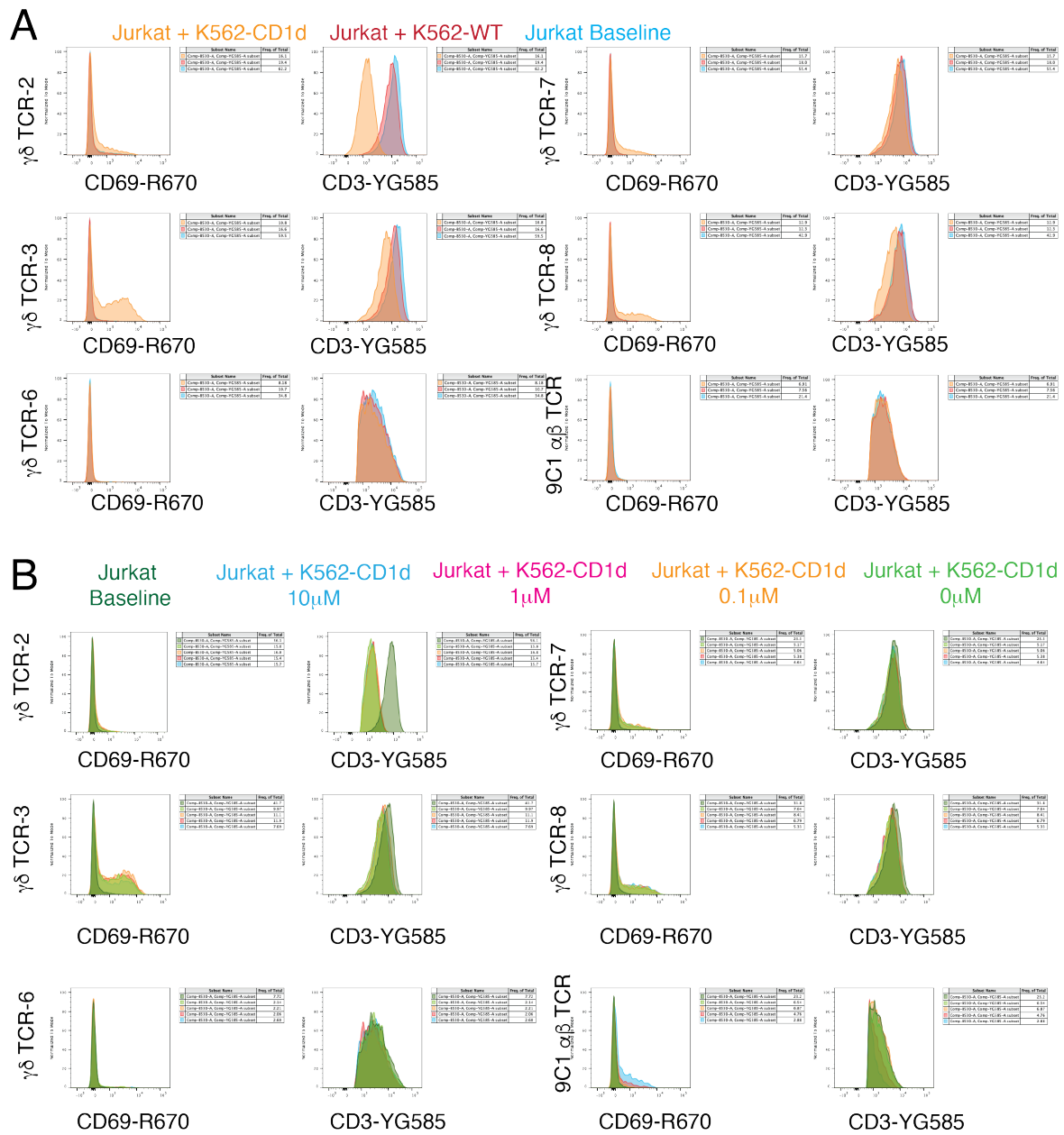
H



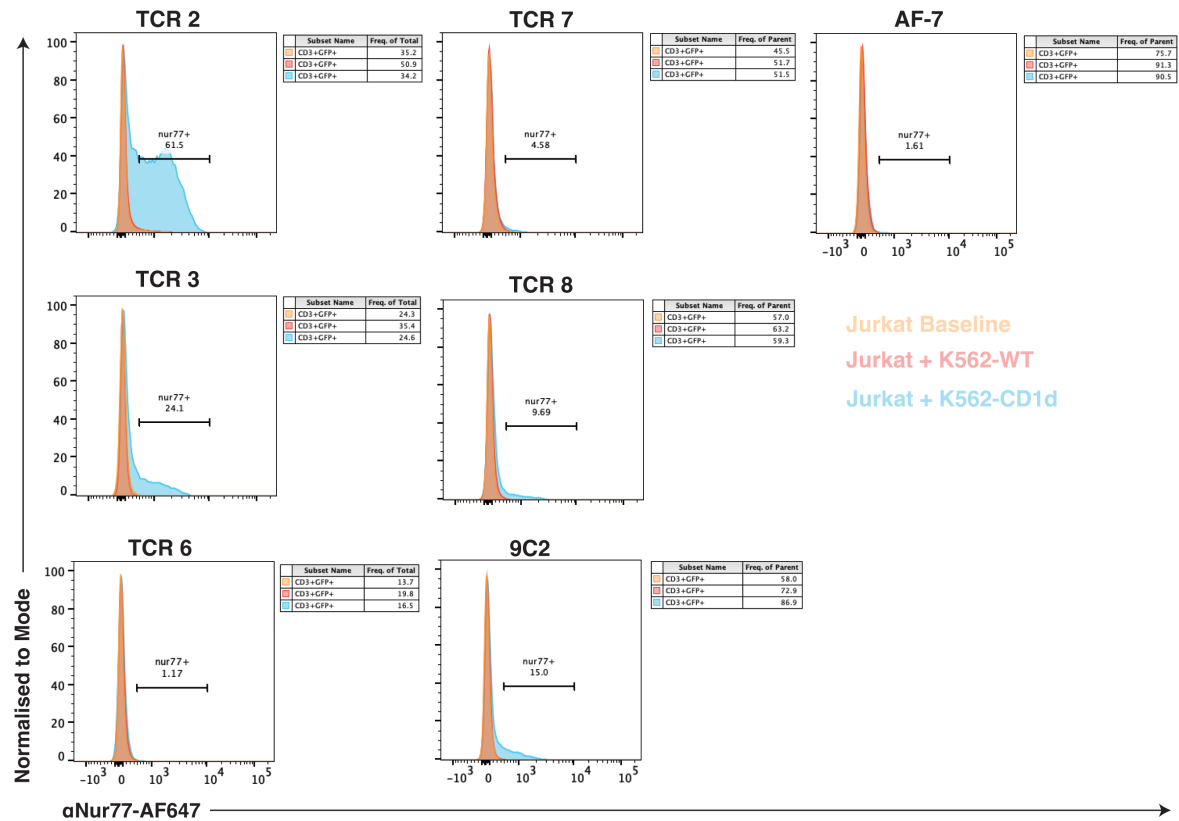
Supplementary Figure 7: A conserved CD1d binding motif. Comparison of interactions between the A' of CD1d and $\gamma\delta$ TCR 2 (A), VHH1D12 (B), 9C2 (C), DP.10 (D), 9B4 (E), 9C1 (F) and 9B2 (G) . Surface charge of CD1d (F). CD1d is shown in dark grey, CDR1 α/δ in yellow, CDR3 α/δ in light blue and CDR3 γ in purple.



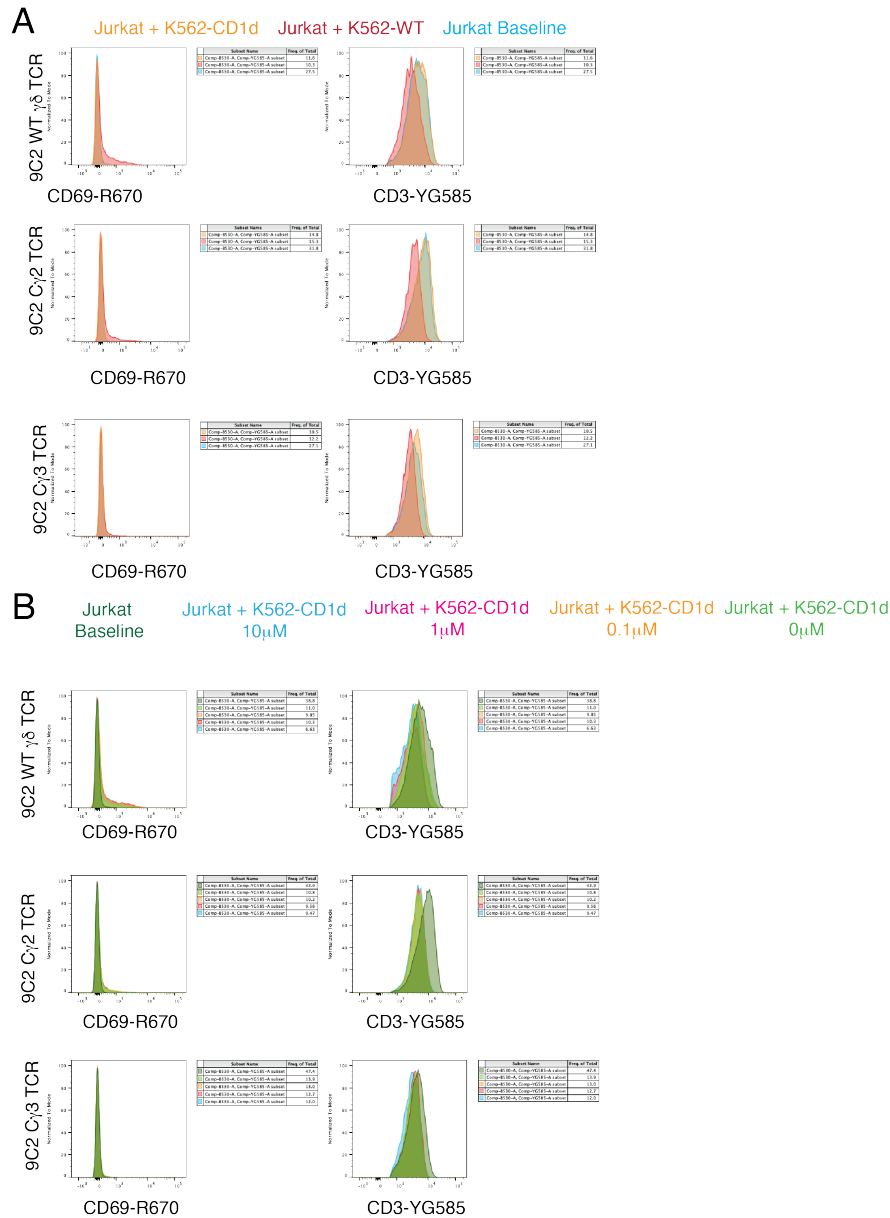
Supplementary Figure 8: T cell activation gating strategy. Gating strategy utilised to identify Jurkat.76 TCR activation.



Supplementary Figure 9: Representative Histograms from CD1d induced activation. (A) CD69 upregulation and CD3 downregulation from Figure 2A following $\gamma\delta$ TCR Jurkat cells activation against K562-WT or K562-CD1d cells following 16 hours. (B) CD69 upregulation and CD3 downregulation after 16 hours from Figure 2B following $\gamma\delta$ TCR Jurkat activation against serial dilution of α -GalCer (μ M) with K562-CD1d cells. Analysis was performed utilising the gating strategy seen in Supplementary Figure 8; lymphocytes, single cells, live cells, CD1d⁺CD3⁺, CD3⁺GFP⁺. Histograms are representative of a single condition, data was generated from two independent experiments performed in duplicate.



Supplementary Figure 10: Representative histograms of Nur77 upregulation from Figure 2C. Nur77 upregulation after 2 hours from Figure 2B following $\gamma\delta$ TCR Jurkat activation against K562-WT or K562-CD1d cells. Analysis was performed utilising the gating strategy seen in Supplementary Figure 8; lymphocytes, single cells, live cells, CD1d⁻CD3⁺, CD3⁺GFP⁺. Gates for Nur77 are depicted on the histogram within the bracket. Histograms are representative of a single condition, data was generated from at least two independent experiments performed in duplicate.



Supplementary Figure 11: Representative histograms of CD1d activation on C γ alleles.

(A) CD69 upregulation and CD3 downregulation for Figure 4 A ‘endo’ activation following 16 hours for 9C2 C γ allele transduced Jurkat cells. Activation was measured against K562-WT and K562-CD1d cells. (B) CD69 upregulation and CD3 downregulation for Figure 4 B for 9C2 C γ allele transduced Jurkat cells following serial dilution of α -GalCer (μ M) with K562-CD1d cells after 16 hours. Analysis was performed utilising the gating strategy seen in Supplementary Figure 8; lymphocytes, single cells, live cells, CD1d $^+$ CD3 $^+$, CD3 $^+$ GFP $^+$. Histograms are representative of a single condition, data was generated from two independent experiments performed in duplicate wells.