

Figure S1

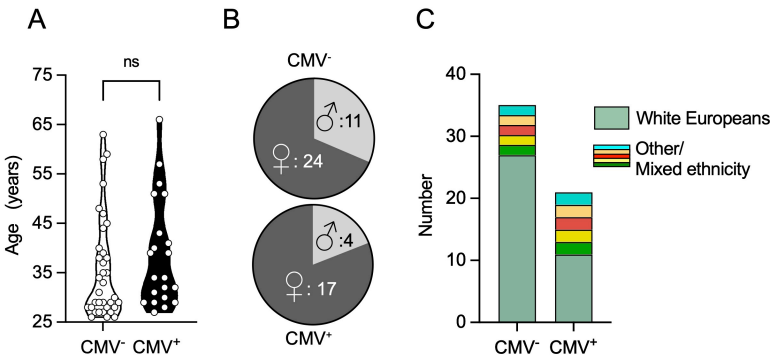


Figure S1. Cohort of healthy adult blood donors.

A. Violin plots compare the ages of healthy adult participants in the CMV⁻ and CMV⁺ groups.

B. Pie charts illustrate the sex distribution (♀/♂) within the CMV⁻ and CMV⁺ groups. **C.** Bar graphs show the distribution of self-reported ethnic origins across the two groups.

The cohort includes 56 individuals in total: 35 CMV⁻ and 21 CMV⁺.

Statistical significance of the age difference between CMV⁻ and CMV⁺ was assessed using the paired two-tailed t test (ns = nonsignificant).

Figure S2

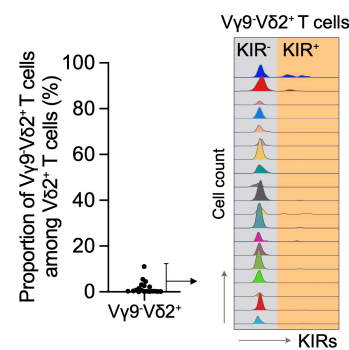


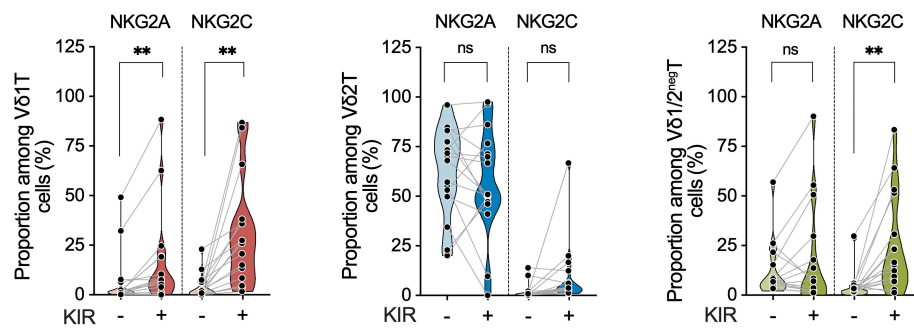
Figure S2. $V\gamma 9-V\delta 2^+$ T cells are a minor population in CMV^+ healthy individuals and exhibit minimal KIR expression.

A. Graph shows the proportion of $V\gamma 9-V\delta 2^+$ T cells within the $V\delta 2$ T cell population in CMV^+ individuals.

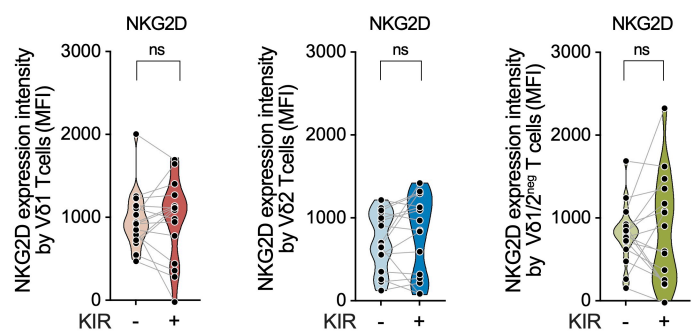
B. Histogram overlay depicts KIR expression on $V\gamma 9-V\delta 2^+$ T cells from each CMV^+ individual.

Figure S3

A



B



C

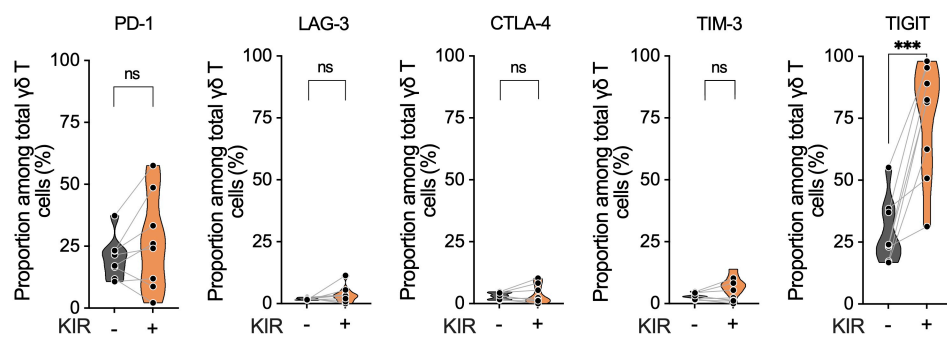


Figure S3. Differential expression of additional NK cell markers and checkpoint inhibitors.

A. Violin plots compare the proportions of KIR⁻ and KIR⁺ cells expressing the indicated markers within Vδ1 (pink, left panel), Vδ2 (blue, middle panel) and Vδ1/2^{neg} (green, right panel) T cells.

B. Violin plots compare the expression intensity of NKG2D on KIR⁻ and KIR⁺ cells within Vδ1 (pink, left panel), Vδ2 (blue, middle panel) and Vδ1/2^{neg} (green, right panel) T cell subsets.

C. Violin plots compare the proportions of KIR⁻ and KIR⁺ cells expressing the indicated checkpoint inhibitors within total γδ T cells.

Each dot represents an individual donor (N=16, **A, B**; N=8, **C**). Statistical significance of the difference between KIR⁻ and KIR⁺ cells was assessed using the paired two-tailed t test (**= p<0.005, ns = nonsignificant).

Figure S4

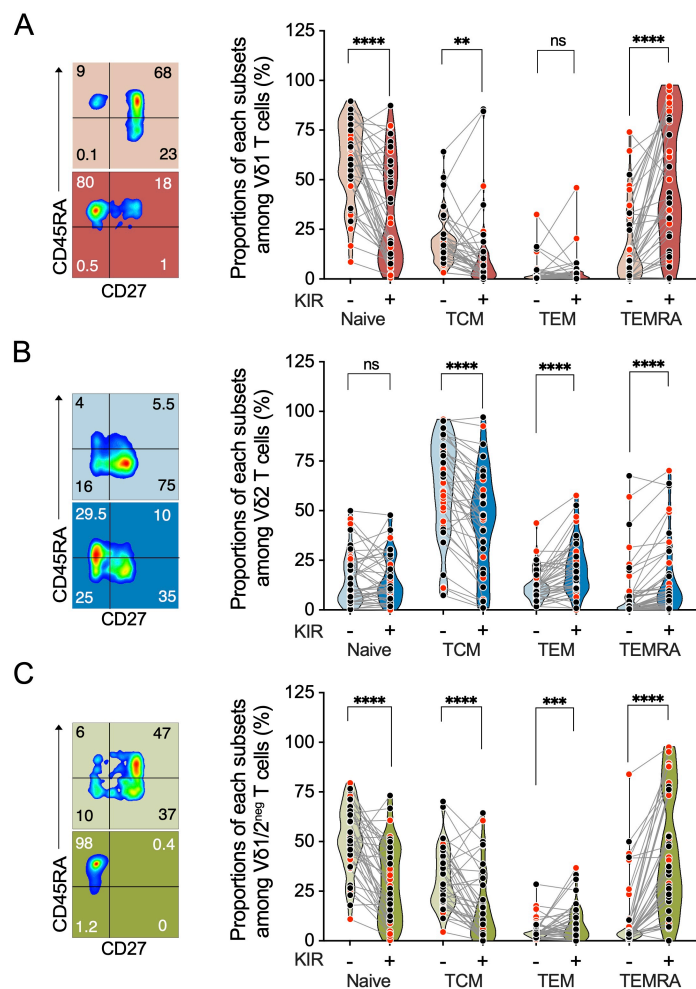


Figure S4. All KIR⁺ γδ T cell subsets predominantly exhibit a TEMRA-like phenotype.

A-C. Representative pseudocolor plots (left panels) and corresponding violin plots (right panels) illustrate the distribution of CD27/CD45RA-defined subsets among KIR⁻ (lighter tones) and KIR⁺ γδ T cells (darker tones). These subsets include naïve, central memory (TCM), effector memory (TEM), and terminally differentiated effector memory (TEMRA) populations across Vδ1 (**A**), Vδ2 (**B**), and Vδ1/2^{neg} (**C**) subsets. Each dot represents an individual donor (n = 40). Samples from CMV⁺ individuals are shown in red symbols. Statistical significance of the difference between KIR⁻ and KIR⁺ cells was assessed using the paired two-tailed t test (**p≤0.005, ***= p≤0.001, ****p≤0.0001, ns = nonsignificant).

Figure S5

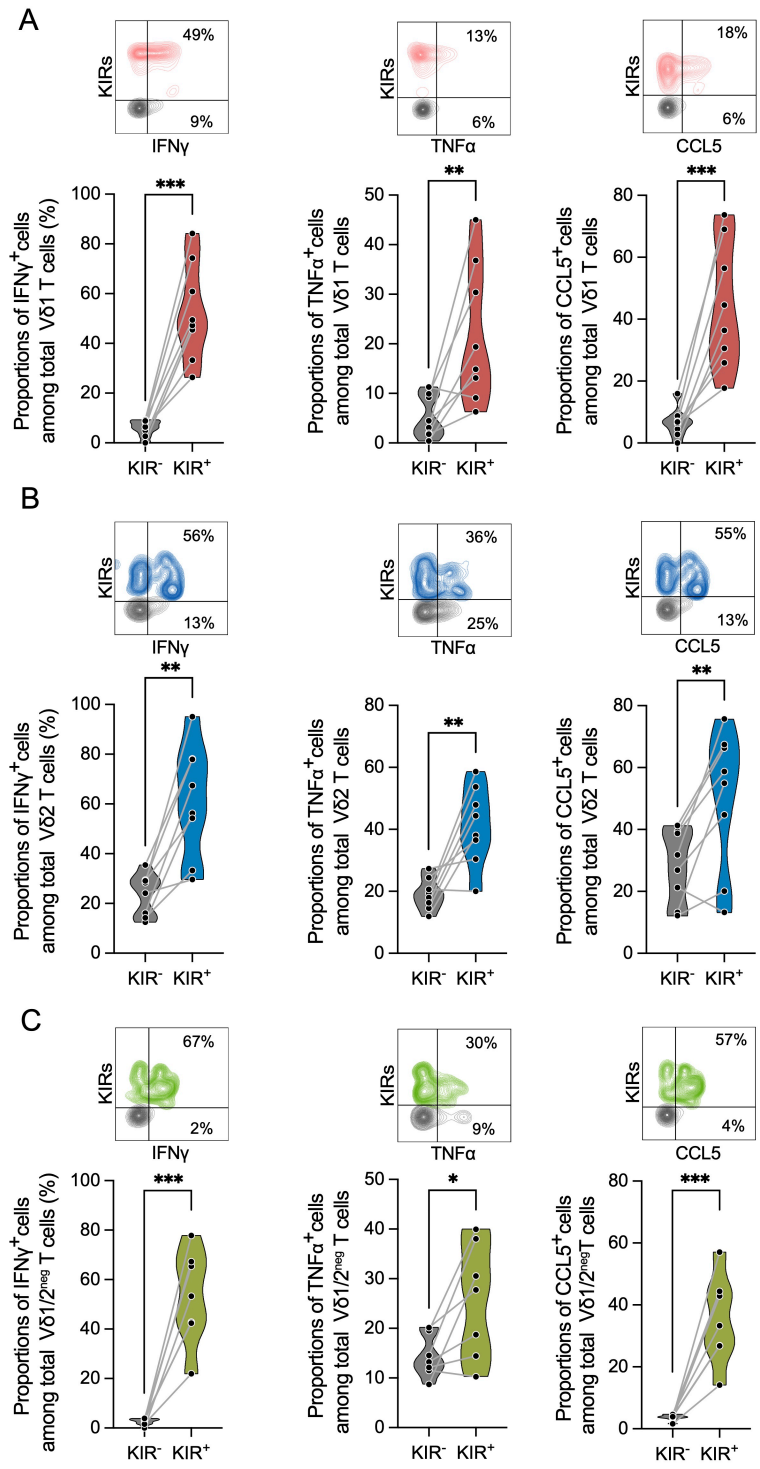


Figure S5: KIR⁺ $\gamma\delta$ T cells exhibit elevated levels of cytokine production following activation across $\gamma\delta$ T cell subsets.

A–C. Representative overlaid contour plots (top panels) and violin plots (bottom panels) compare proportions of KIR⁻ (grey) and KIR⁺ (pink for V δ 1 (**A**), blue for V δ 2 (**B**), green for V δ 1/2^{neg} (**C**)) T cells producing IFN γ , TNF α and CCL5, after 4 hours of PMA/ionomycin stimulation.

Each dot represents an individual donor (N=8 for V δ 1 and V δ 2; N=7 for V δ 1/2^{neg}, as one donor lacks KIR expression in this subset). Statistical significance of the difference between KIR⁻ and KIR⁺ subsets was assessed using the paired two-tailed t test (*p=0.02, **p $\leq$ 0.009, ***p $\leq$ 0.0006, ****p<0.0001).

Figure S6

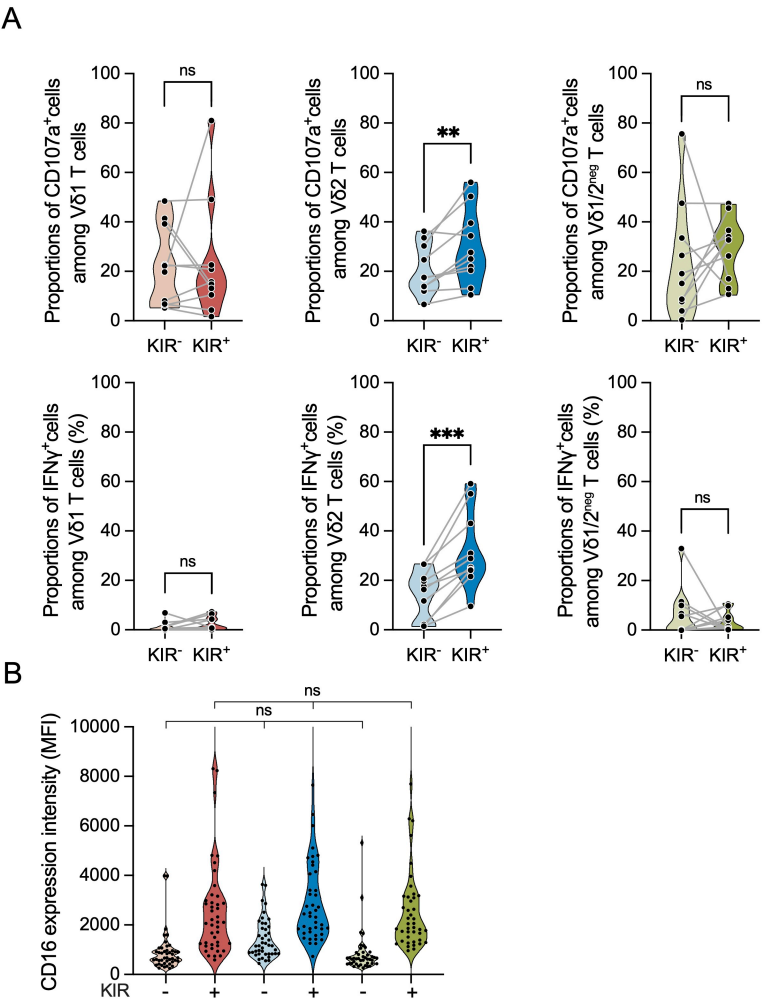


Figure S6: KIR expression is associated with enhanced ADCC in V δ 2 T cells but not in other $\gamma\delta$ T cell subsets.

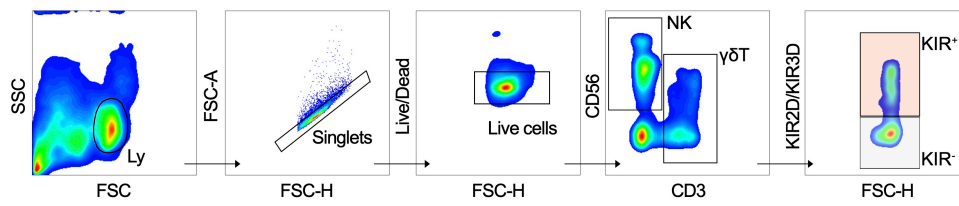
A. Violin plots illustrate degranulation (top panels) and IFN γ production (bottom panel) in KIR $^{-}$ and KIR $^{+}$ populations of V δ 1 (pink), V δ 2 (blue) and V δ 1/2 neg (green) $\gamma\delta$ T cells following an ADCC assay. Cells were co-cultured for 5 hours with Rituximab-coated Raji cells. Data represent the percentage of CD107a $^{+}$ or IFN γ $^{+}$ cells within each indicated subset. Each dot represents an individual donor (N=10).

B. Violin plots depict CD16 surface expression levels, quantified as median fluorescence intensity (MFI), in the same $\gamma\delta$ T cell subsets, color-coded as in panel A and stratified by KIR expression. Each dot represents an individual donor (N=44).

Statistical significance between KIR $^{-}$ and KIR $^{+}$ populations within each subset was assessed using the paired two-tailed t test (**p $\leq$ 0.007, ***p $\leq$ 0.0002, ns = nonsignificant).

Figure S7

A



B

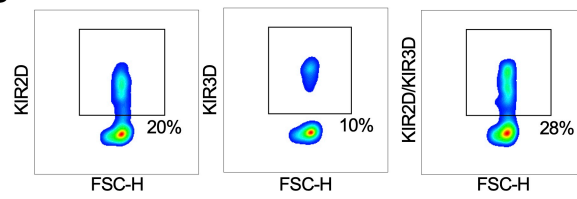


Figure S7: Gating strategy.

A. Representative pseudocolor plots illustrate sequential gating strategy for KIR⁻ and KIR⁺ populations within $\alpha\beta$ T cell-depleted PBMCs. KIR⁻ and KIR⁺ populations were defined using KIR2D/KIR3D antibodies conjugated to the same fluorochrome.

B. Representative pseudocolor plots showing single stain controls and the combined staining.

Supplementary Table 1: List of monoclonal antibodies used for flow cytometry analysis

Specificity	Clone	Vendor; Catalog number
CD3	OKT3	BioLegend; 317323
CD56	5.1H11	BioLegend; 362549
CD14	M5E2	BioLegend; 301833
CD19	HIB19	BioLegend; 302227
TCR $\alpha\beta$	IP26	BioLegend; 306705
TCR V γ 9	B3	BioLegend; 331328
TCR V δ 1	REA173	Miltenyi Biotec; 130-120-583
TCR V δ 2	123R3	Miltenyi Biotec; 130-125-878
CD27	L128	BD Biosciences; 563816
CD45RA	HI100	BioLegend; 304141
CD244	C1.7	BioLegend; 329519
CD57	REA769	Miltenyi Biotec; 130-111-968
CD16	REA423	Miltenyi Biotec; 130-113-395
LILRB1	HP-F1	Thermo Fisher Scientific; 367-5129-41
NKG2A	REA1161	Miltenyi Biotec; 130-120-377
NKG2C	REA205	Miltenyi Biotec; 130-119-776
NKG2D	1D11	BioLegend; 285147
KIR2D	REA1042	Miltenyi Biotec; 130-117-479
KIR2DL1	REA284	Miltenyi Biotec; 130-118-484
KIR2DL1/S1	REA1010/11PB6	Miltenyi Biotec; 130-118-973
KIR2DL2/L3	REA1006/DX27	Miltenyi Biotec; 130-116-954
KIR2DL2/L3/S2	DX27	BioLegend; 312623
KIR2DS4	REA860	Miltenyi Biotec; 130-114-773
KIR3DL1/S1	REA168	Miltenyi Biotec; 130-128-302
KIR3DL1	DX9	BioLegend; 312729
PD-1	A17188B	BioLegend; 621619
TIM-3	F38-2E2	BioLegend; 345008
CTLA-4	L3D10	BioLegend; 349905
TIGIT	A15153G	BioLegend; 372704
LAG-3	3DS223H	Thermo Fisher Scientific; 17-2239-42
CD107a	LAMP-1	BioLegend; 328656
Granzyme B	REA226	Miltenyi Biotec; 130-116-486
Perforin	dG9	BioLegend; 308129
IFN γ	REA600	Miltenyi Biotec; 130-132-901
TNF α	REA656	Miltenyi Biotec; 130-127-550
CCL5	VL1	BioLegend; 515507
IL-17A	BL168	BioLegend; 512305
ROR γ t	REA278	Miltenyi Biotec; 130-124-035

Surface markers

Intracellular markers