

Supplementary materials

Supplementary Figure legends

Figure S1. Flow cytometry gating strategy and inhibitory receptor expression on $\alpha\beta$ T cell subsets.

(A) Representative flow cytometry gating strategy used to identify V δ 1, V δ 2, CD8, and CD4 T cells. After exclusion of debris and dead cells, CD3 T lymphocytes were gated and subsequently subdivided based on the expression of CD4, CD8, and $\gamma\delta$ T-cell receptor chains.

(B) Representative histograms showing the expression of inhibitory receptors LAG-3, TIM-3, PD-1, TIGIT, and CTLA-4 on CD8 T cells at the indicated time points. Negative controls are shown for comparison.

(C) Representative histograms showing the expression of inhibitory receptors LAG-3, TIM-3, PD-1, TIGIT, and CTLA-4 on CD4 T cells at the indicated time points. Negative controls are shown for comparison.

(D) Representative flow cytometry plots showing the expansion of V δ 2 T cells following stimulation with zoledronate at the indicated time points.

Figure S2. Longitudinal expression of immune checkpoint receptors on T-cell subsets in healthy donors.

Kinetics of TIM-3, LAG-3, PD-1, CTLA-4, and TIGIT expression were evaluated by flow cytometry in individual donors (ID01–ID08) at day 0, 3, 5, 7 and 9. For each donor, the percentage of positive cells (upper panels) and the median fluorescence intensity (MFI; lower panels) are shown for CD4, CD8, V δ 1, and V δ 2 T cells. Each line represents one cellular subset within the same donor across time. Data are displayed without averaging to highlight inter-donor variability in checkpoint modulation during culture.

Figure S3. Longitudinal expression of immune checkpoint receptors on V δ 2 T cells after stimulation with zoledronic acid.

Kinetics of TIM-3, LAG-3, PD-1, CTLA-4, and TIGIT expression were evaluated by flow cytometry in individual donors (ID01–ID08) at day 0, 3, 6, 9 and 12 after stimulation with zoledronic acid. For each donor, the percentage of positive cells (upper panels) and the median fluorescence intensity (MFI; lower panels) are shown for V δ 2 T cells. Data are displayed without averaging to highlight inter-donor variability in checkpoint modulation during culture.

Figure S4. Donor-level trajectories of differentiation-state composition within ICR⁺ T-cell populations.

Spaghetti plots showing the distribution of differentiation states (naïve [T_n], central memory [T_{cm}], effector memory [T_{em}], and terminally-differentiated effector memory [T_{emra}]) within checkpoint-positive (ICR⁺) CD4, CD8, V δ 1, and V δ 2 T-cell populations across time (day 0, day 3, and day 6). Each colored line represents an individual donor (n = 8). The dashed black line indicates the group's mean at each time point. Values represent the percentage of each differentiation subset within the corresponding ICR⁺ population (raw percentages).

Figure S5. Functional activity and receptor expression in T-cell subsets

(A) Expression of Ki67, IFN- γ , and TNF- α in V δ 1, V δ 2, CD8, and CD4 T-cell subsets following stimulation with IL-15+IPP. Data are displayed as box-and-whisker plots with individual data points overlaid.

(B) Expression of TIM-3 and PD-1 in V δ 1, V δ 2, CD8, and CD4 T-cell subsets under different stimulation conditions: no stimulation (NS), IL-15, IL-15+IPP, and bead stimulation. Data are displayed as box-and-whisker plots with individual data points overlaid.

Abbreviations: IPP, isopentenyl pyrophosphate.

Figure S1

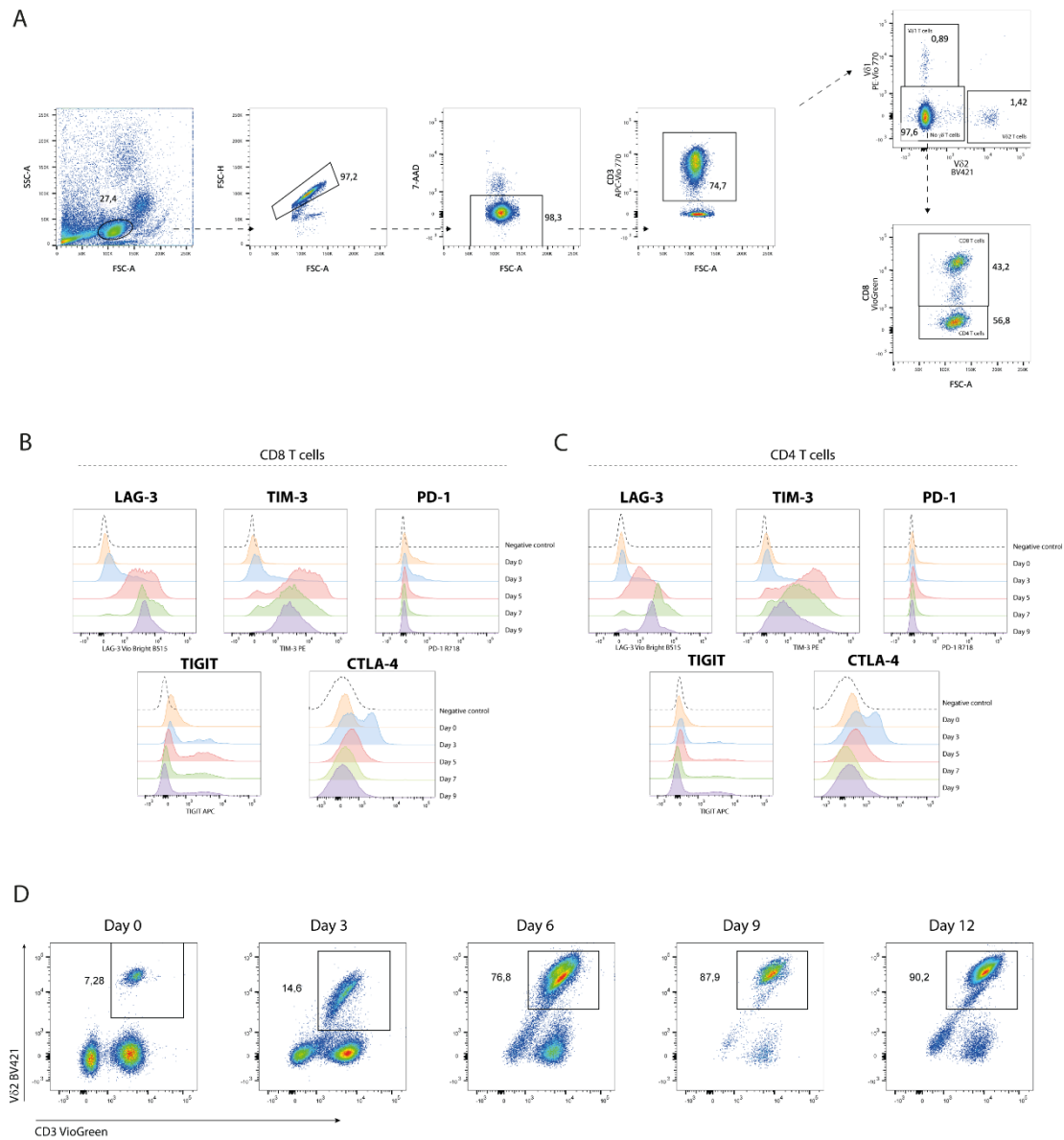


Figure S2

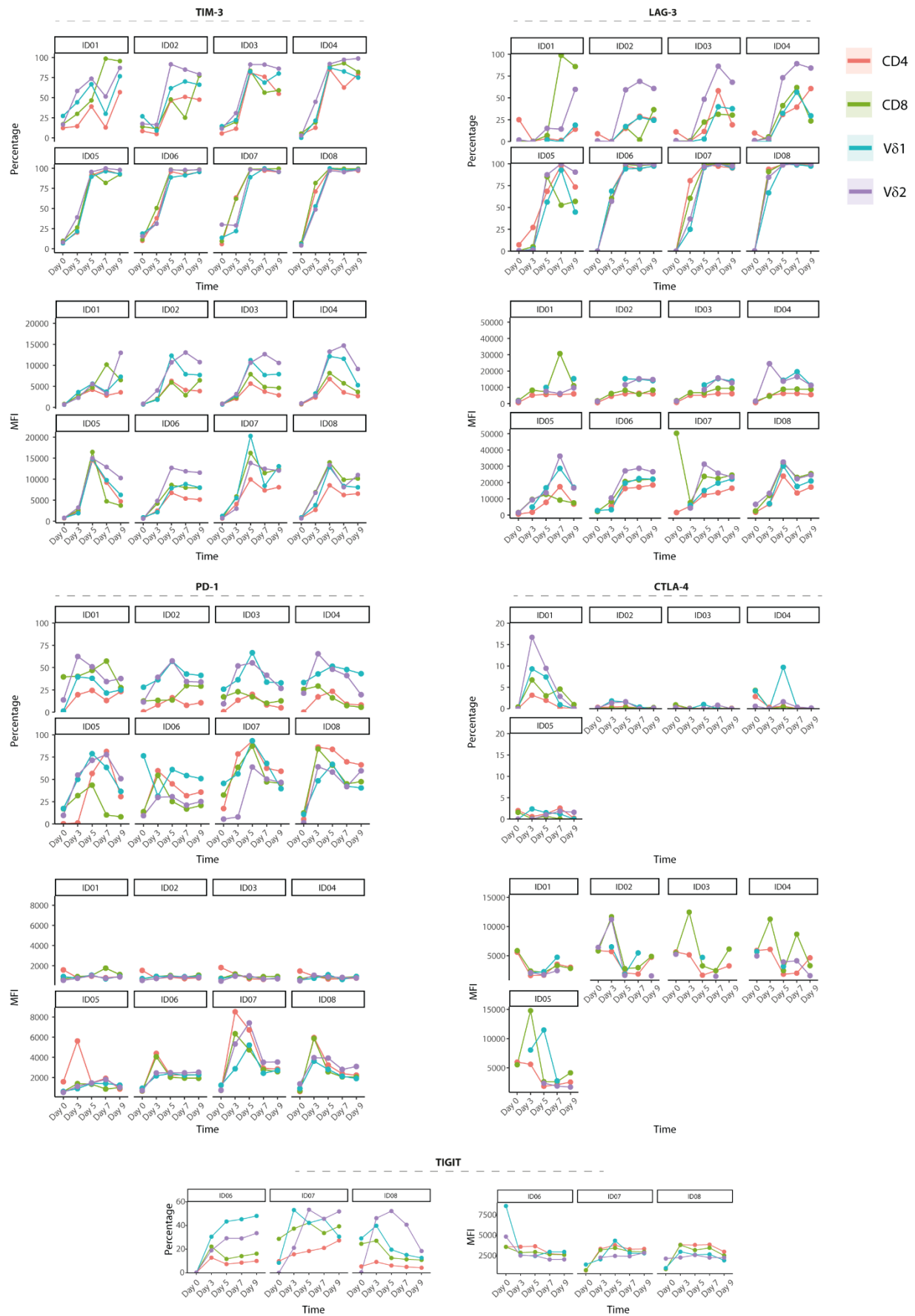


Figure S3

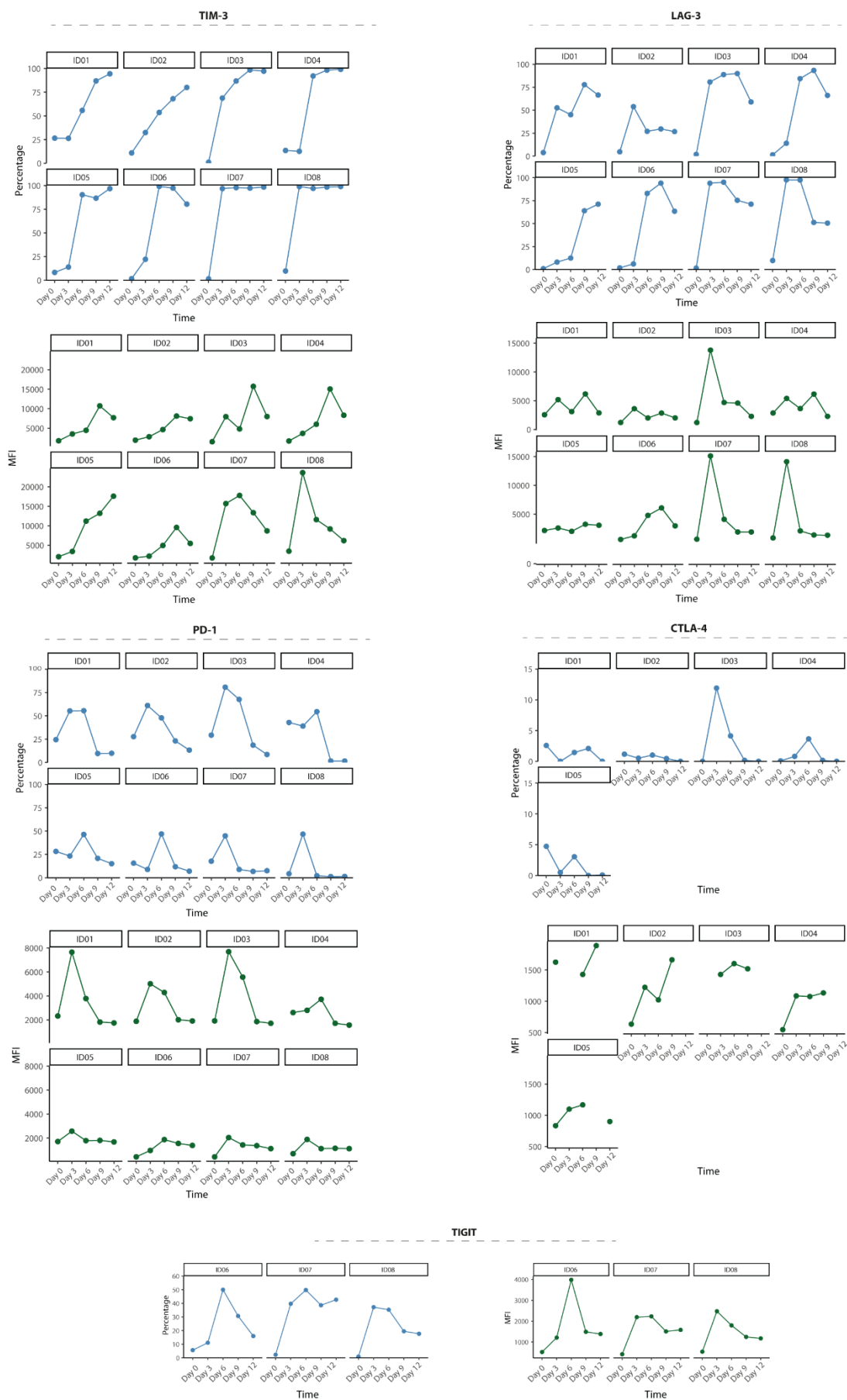


Figure S4

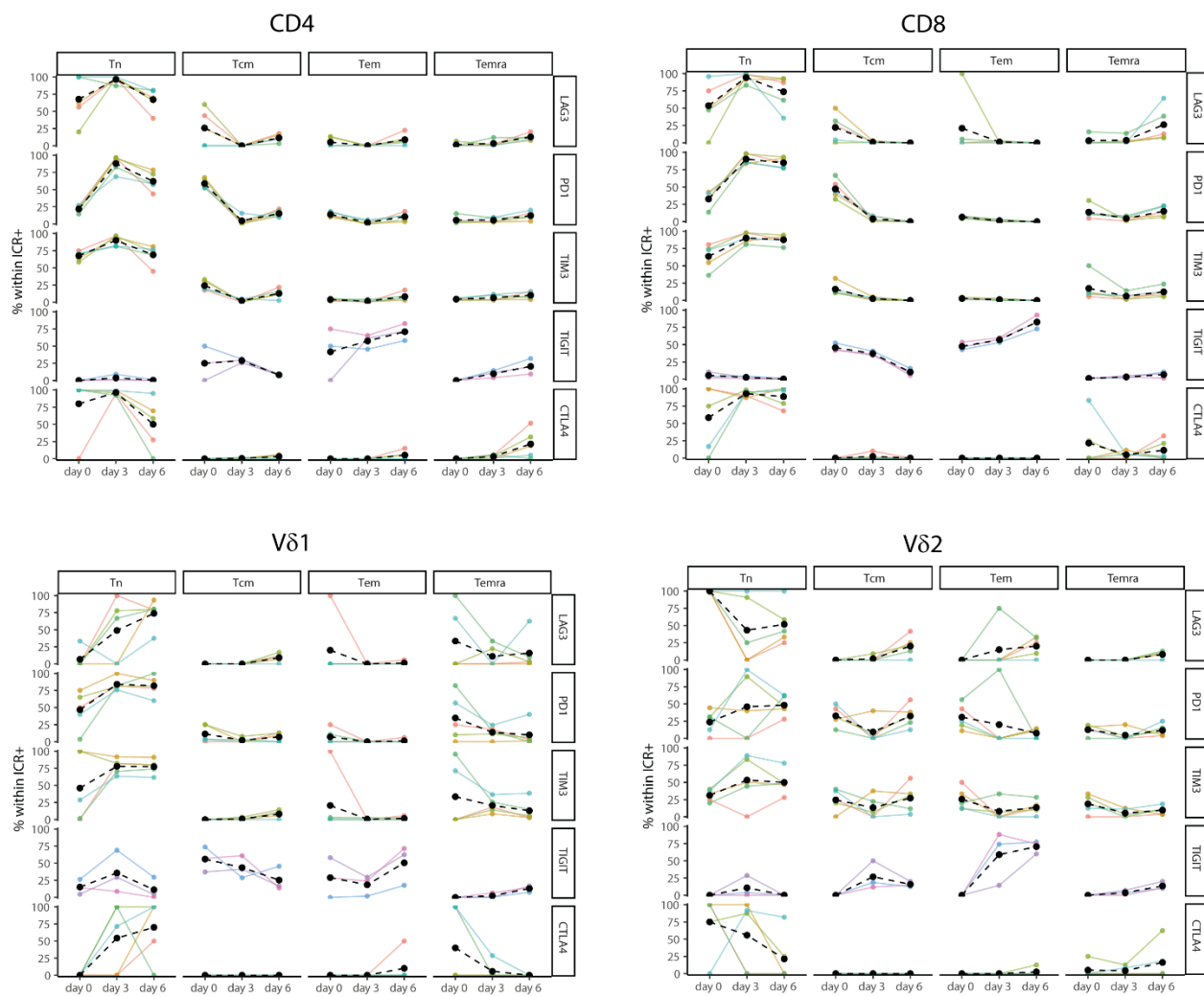
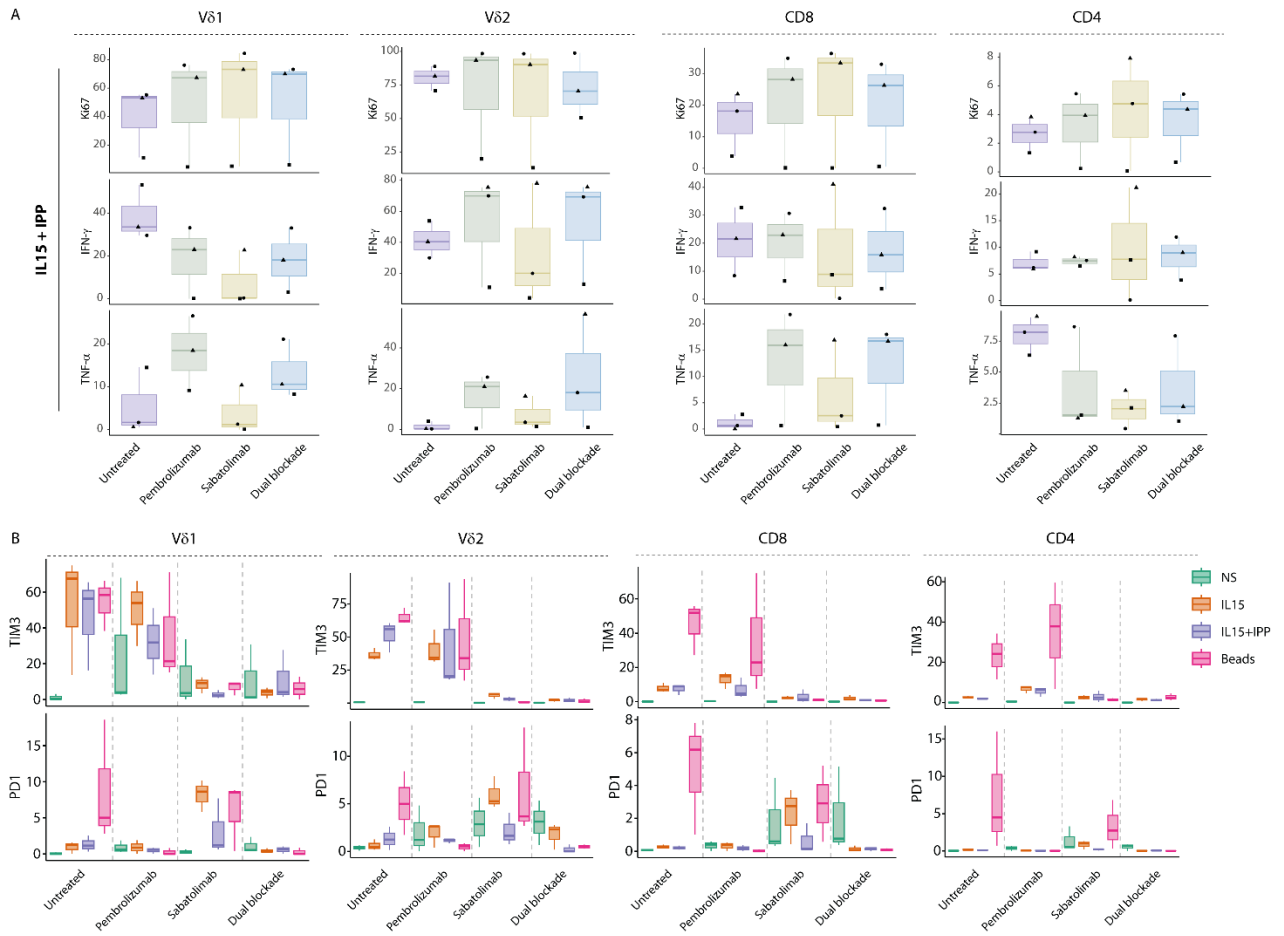


Figure S5



Supplementary Table 1. List of monoclonal antibodies

Monoclonal antibody			Supplier		Catalog number
<i>Antibody</i>	<i>Fluorochrome</i>	<i>Clone</i>		<i>Dilution</i>	
Anti-human CD223 (LAG-3)	VioBright B515	REA351	Miltenyi	1:50	130-120-012
Anti-human CD223 (LAG-3)	APC-Vio770	REA351	Miltenyi	1:50	130-130-284
Anti-human CD366 (TIM-3)	VioBrightFITC	F38-2E2	Miltenyi	1:50	130-125-979
Anti-human CD366 (TIM-3)	PE	REA635	Miltenyi	1:50	130-119-785
Anti-human CD279 (PD-1)	R718	EH12.1	BD Biosciences	1:2	566974
Anti-human TIGIT	APC	REA1004	Miltenyi	1:50	130-116-815
Anti-human CD152 (CTLA-4)	APC	REA1003	Miltenyi	1:50	130-116-811
Anti-human CD3	APC-Vio770	REA613	Miltenyi	1:50	130-113-136
Anti-human CD3	VioGreen	REA613	Miltenyi	1:50	130-113-142
Anti-human TCR V δ 1	PE-Vio770	REA173	Miltenyi	1:50	130-117-697
Anti-human TCR V δ 2	BV421	B6	BD Biosciences	1:5	743749
Anti-human CD4	VioGreen	REA623	Miltenyi	1:50	130-113-230
Anti-human CD8	VioGreen	REA734	Miltenyi	1:50	130-110-684
Anti-human CD8	Vio Bright V600	REA7341	Miltenyi	1:50	130-131-282
Anti-human CD45RA	BV605	5H9	BD Biosciences	1:5	740424
Anti-human CD27	BV786	L128	BD Biosciences	1:10	563327
Anti-human CD27	PE-Vio770	REA499	Miltenyi	1:50	130-113-641
Anti-human IFN- γ	BB700	B27	BD Biosciences	1:5	566394
Anti-human TNF- α	APC	REA656	Miltenyi	1:50	130-120-063
7-AAD Staining solution	-	-	Miltenyi	1:100	130-111-568
Viability 488/520 Fixable Dye	-	-	Miltenyi	1:100	130-137-055

Supplementary Table 2. List of reagents and tools

Reagent	Supplier	Catalog number
Ficoll-Paque PLUS	Cytiva	17144003
StableCell™ RPMI-1640 containing stable glutamine and sodium bicarbonate	Sigma-Aldrich	R2405-500ML
25 mM HEPES buffer	Euroclone	ECM0180
Penicillin-Streptomycin Solution 100X	Euroclone	ECB3001D
Fetal Bovine Serum (South America), Heat Inactivated	Dominique Dutscher	S181H-500
Dulbecco's PBS (1x), with Ca&Mg, w/o Phenol Red	Sial	SIAL-PBS-1A
Dynabeads™ Human T-Activator CD3/CD28	Thermo Fisher Scientific	11131D
Human IL-2 Recombinant Protein	Peptotech	200-02
Trypan Blue Solution	Cytiva	SV30084.01
Turk's Reagent for leukocytes counting	TITOLCHIMICA	TC75700QQ
Human IL-15 Recombinant Protein	Miltenyi	130-095-762
Isopentenyl pyrophosphate (IPP) triammonium salt solution	Sigma-Aldrich	I0503
Monensin sodium salt	Sigma-Aldrich	M5273
Sabatolimab (anti-TIM-3)	Selleck Chemicals	A2037
Pembrolizumab (anti-PD-1)	Selleck Chemicals	A2005
Dead Cell Removal Kit	Miltenyi	130-090-101
Inside Stain Kit	Miltenyi	130-090-477
BD™ BD IntraSure™ Kit	BD Biosciences	641776
Chromium Next GEM Single Cell 3' Reagent Kit v3.1 (Dual Index)		
Tool and instrument	Supplier	Identifier
FlowJo	FlowJo LLC, BD Biosciences	Version 10.10.0
R Studio	-	Version 2026.01.0+392
R	-	Version 4.5.2
BD FACSLytic™	BD Biosciences	
Chromium X	10x Genomics	