Postgraduate University Interdisciplinary
Doctoral Study of Molecular Biosciences

Doctoral thesis defence

Characterisation of the phenotype, transcriptional markers and T cell receptor repertoire of circulating MAIT and γδ T lymphocytes in psoriasis vulgaris

Karakterizacija fenotipa, transkripcijskih biljega i repertoara T staničnih receptora cirkulirajućih limfocita MAIT i γδ T u vulgarnoj psorijazi

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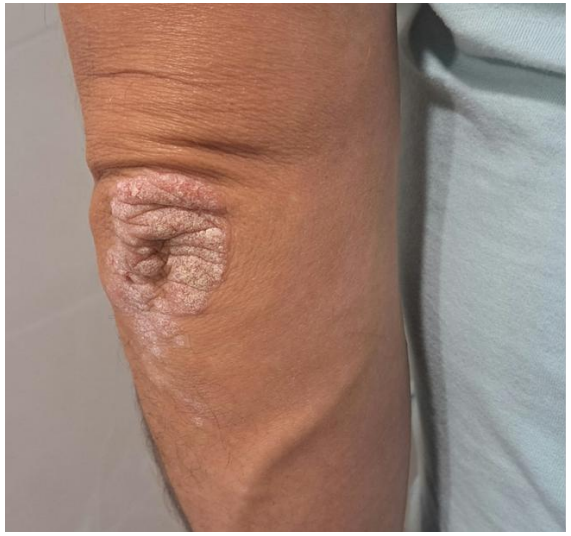
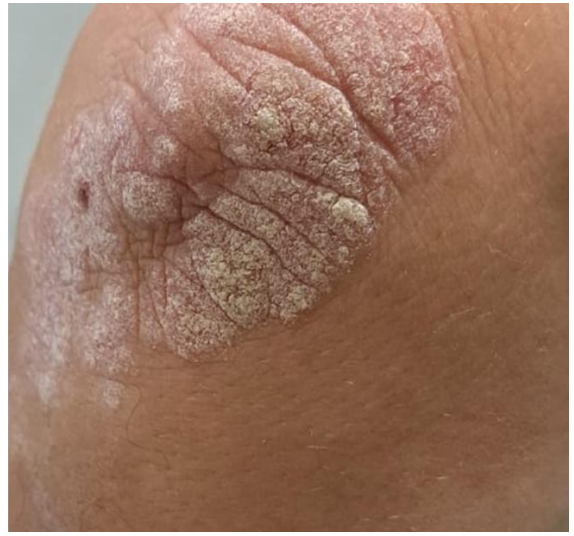
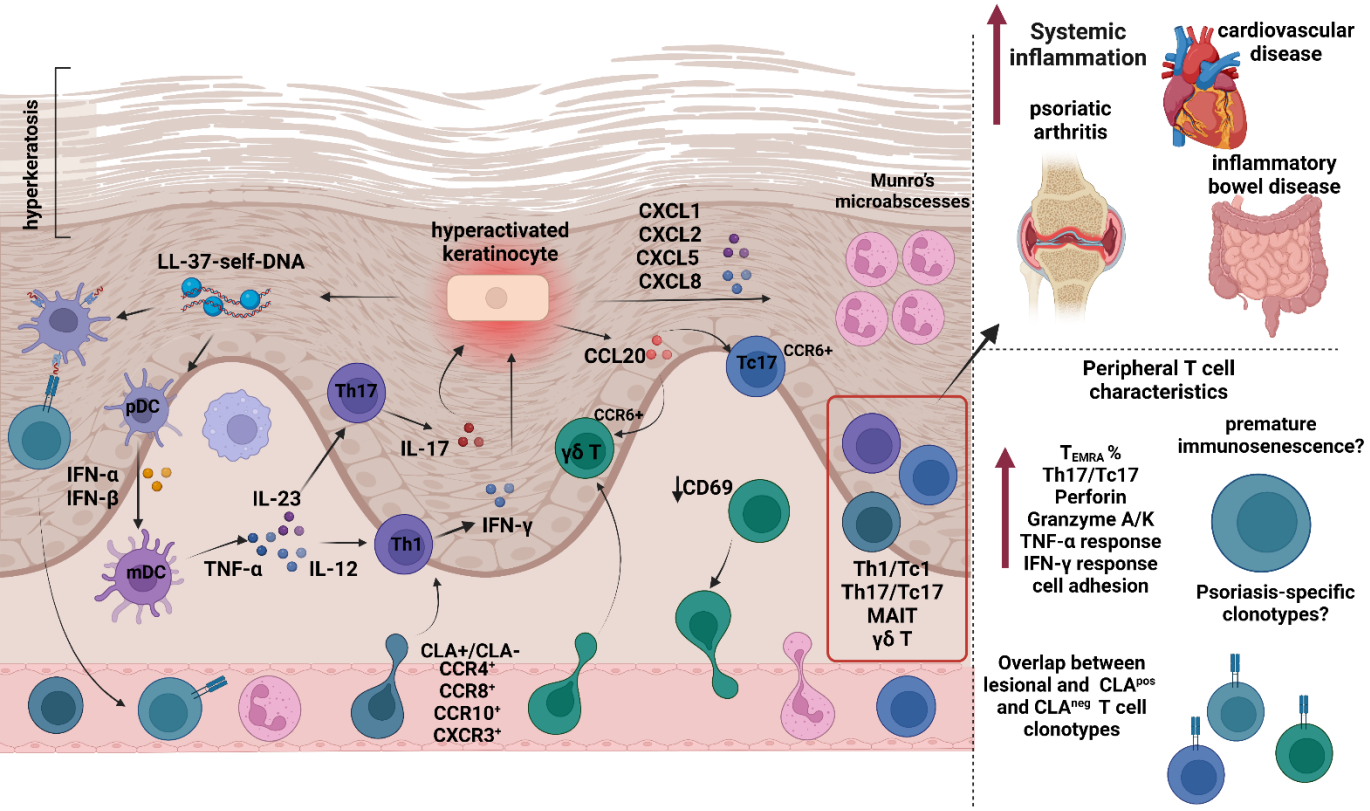
Osijek, 22nd April, 2025.

Psoriasis vulgaris

- chronic autoinflammatory skin disease
- well-defined, erythematous plaques covered with silvery-white scales
- associated comorbidities and signs of systemic inflammation





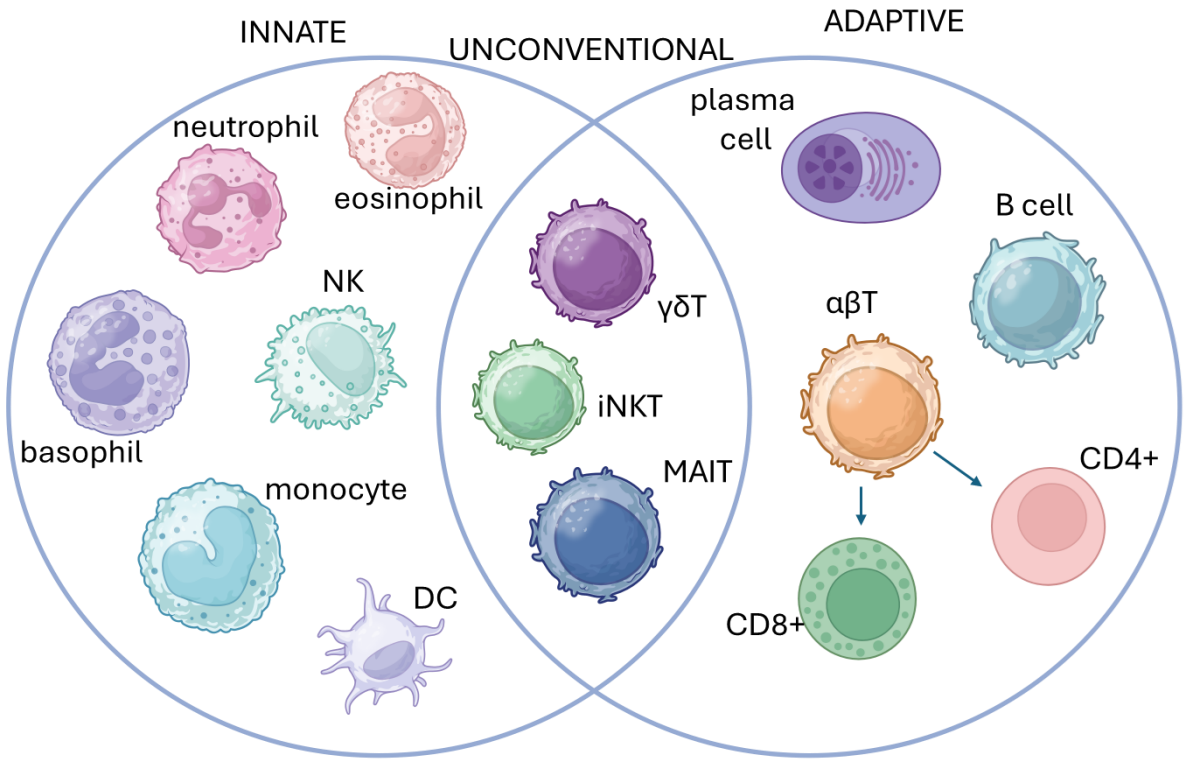


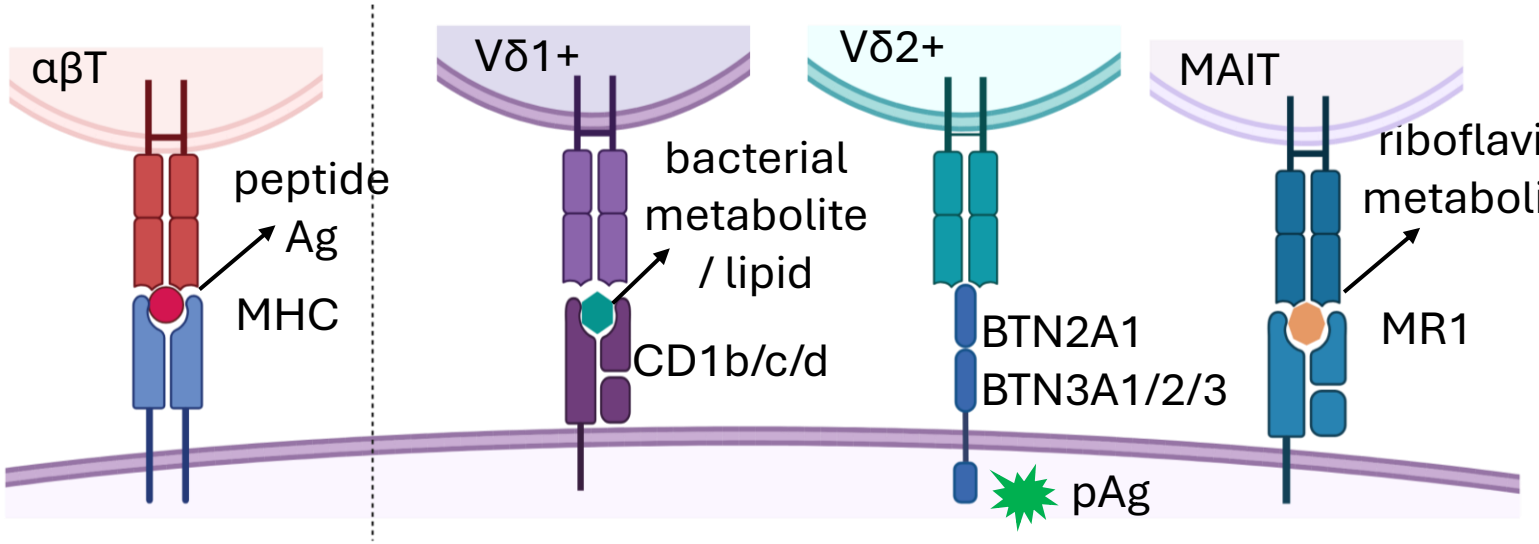
- T cell-mediated pathology
- complex network of interactions between resident and infiltrating cells
- (re)circulating T cells contribute to spreading inflammation beyond the skin

How unconventional T cells contribute to the immunopathology of psoriasis vulgaris?

Unconventional T cells

- ~10–30% of the T cell compartment
- enriched in mucosal and epithelial sites → skin, respiratory, digestive, and reproductive tracts
- rapid effector functions
- recognition of non-peptide ligands presented by non-polymorphic antigen presenting molecules
- TCR-independent activation



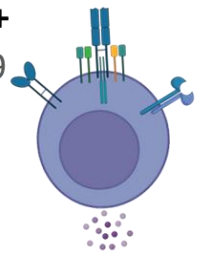


γδ T cells

- 1-10% of peripheral T cells

Vδ2+

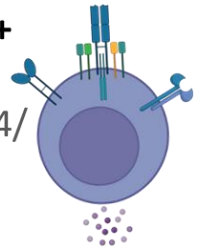
Vγ9



- 50%–90% of the peripheral γδ T
- Vγ9+Vδ2+ / Vγ9-Vδ2 +
- innate-like effector profile

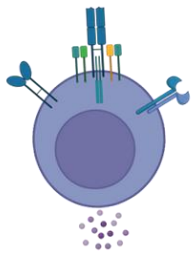
Vδ1+

Vγ2/3/4/ 5/8/9



- up to ~ 30% of peripheral γδ T
- epithelial and mucosal sites → gut, skin, spleen, and liver
- adaptive immunobiology

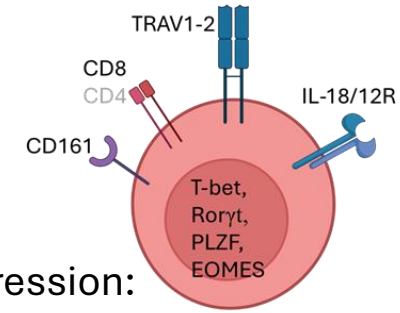
Vδ3+



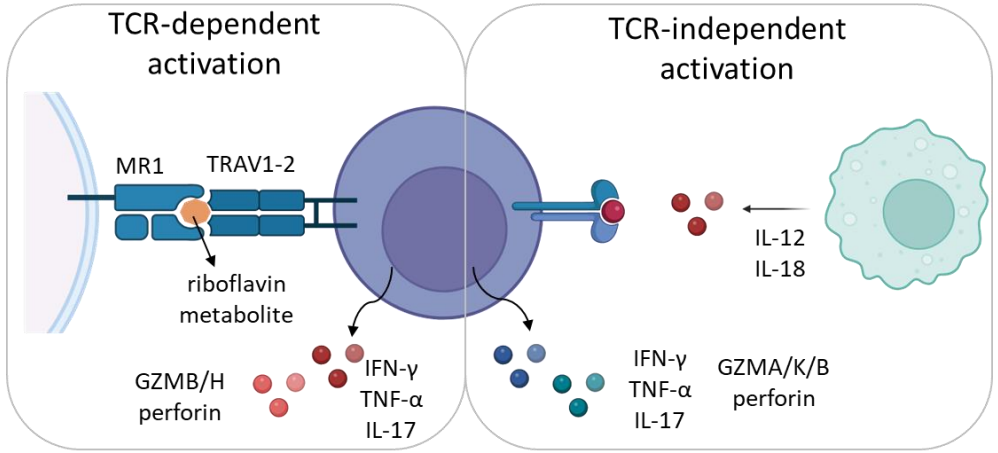
- <1% peripheral γδ T
- enriched in the liver and gut
- anti-microbial immunity
- ??

MAIT cells

- Up to 5% of peripheral T cells



- subsets based on the CD4 /CD8 expression:
  - CD4−CD8+ → ~80% of circulating MAIT cells
  - CD4−CD8− → ~ 15% of the total MAIT
  - CD4+CD8− / CD4+CD8+ → < 5%
- variation in phenotype, function and TCR repertoire



MAIT and γδ T cells in psoriasis – current knowledge and gaps

MAIT

- frequencies within psoriatic plaques comparable to those found in healthy skin (Teunissen et al. 2014)
- produce **IL-17** (rarely in normal skin)
- **male patients** exhibited significantly **lower proportions of CD4⁺ and DP MAIT** cells (Plužarić et al. 2020)
- **TRB repertoires** in psoriasis patients undergo **significant age-related reshaping** (Jirouš et al. 2023)

γδ T

- **Vγ9Vδ2 reduced in peripheral blood** (Laggner et al. 2011)
- **higher proportions of Vγ9Vδ2 in psoriatic lesions** (Laggner et al. 2011)
- **heightened activation** linked to a substantial **up-regulation of BTN3A1** on monocytes (Zhou et al. 2022)
- produce **IFN-γ and TNF-α**, while IL-17 production was minimal

Limitations of current studies

- few human studies
- small sample size
- narrow range of phenotyping markers
- lack of TCR analysis

Aims

- Quantify **the frequency and subset composition**
- Investigate **immunotranscriptome** to identify **differentially expressed genes and pathways**
- Assess **immunophenotype and functional diversity**
- Characterise **the diversity and features** of **TCR repertoires**
- **Correlate** alterations in immunotranscriptome, TCR repertoire, and phenotype with anthropometric, biochemical, and clinical features of PV

Hypotheses

The contribution of circulating MAIT and γδ T cells to psoriatic inflammation will be observed through:

- **Altered composition, phenotypes and gene expression profiles** (changes in activation, differentiation, migration and effector profiles)
- **Differential TCR repertoire characteristics** (changes in clonality, diversity, and the presence of disease-associated patterns)
- **Disease-associated changes** that **correlate with anthropometric and biochemical characteristics**, as well **as clinical indicators of disease severity**

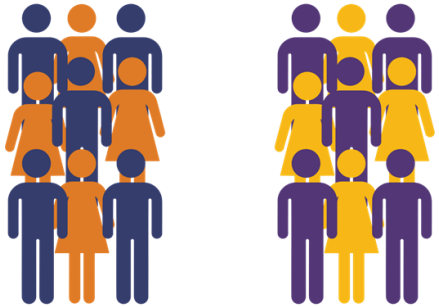
Materials and methods

Sample collection

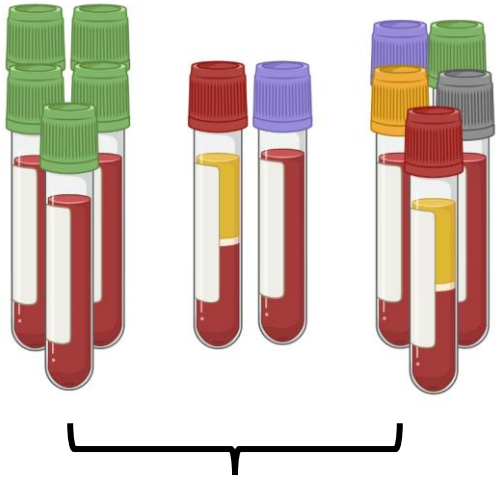
Department of Dermatology and Venereology Clinical Hospital Centre Osijek

Therapy-naive PV patients

Healthy control participants



- Psoriasis Area and Severity Index (PASI)
- Dermatology Life Quality Index (DLQI)
- anthropometric and demographic characteristics
- Exclusion criteria:
  - autoimmune or inflammatory diseases
  - malignancies
  - allergic reactions (6 weeks prior to sampling)



PBMC isolation

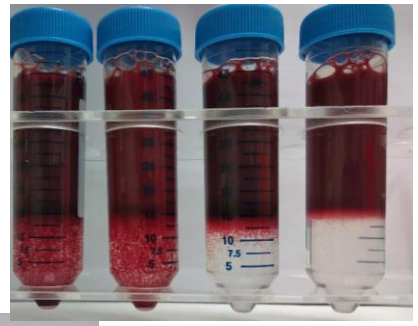
haematological and biochemical lab tests

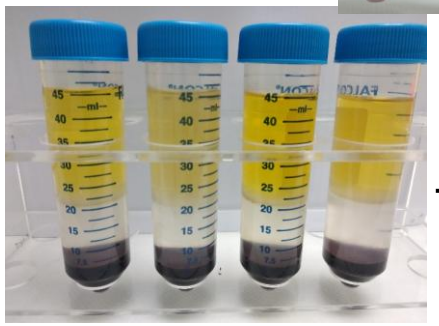
anti-CMV IgM/IgG, HBsAg, anti-HBs, anti-HCV, Mtb markers

PBMC isolation

20 ml heparinised blood

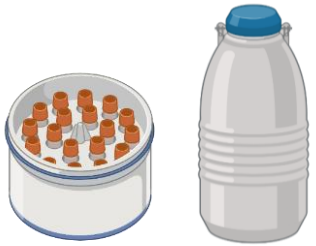
Lymphoprep medium density gradient centrifugation





Cell counting

Cryopreservation (10% DMSO in FBS) -80, 48h, Mr. Frosty liquid nitrogen



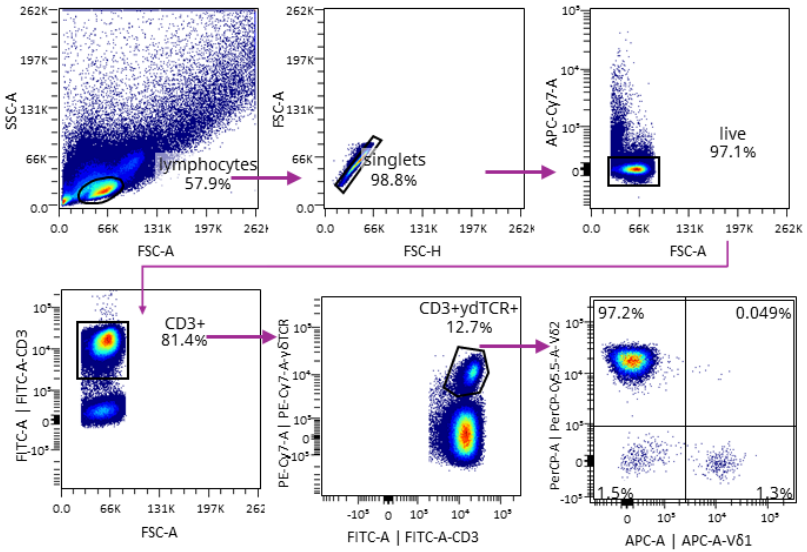
Flow cytometry

- 93 subjects (60 PV + 30 HC)

γδ T

↓

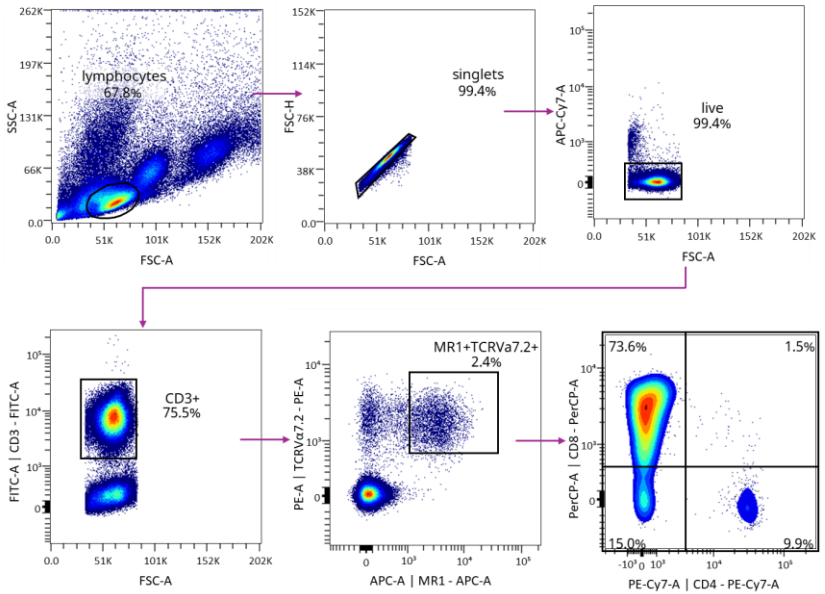
- CD3
- TCRγδ
- Vδ1
- Vδ2



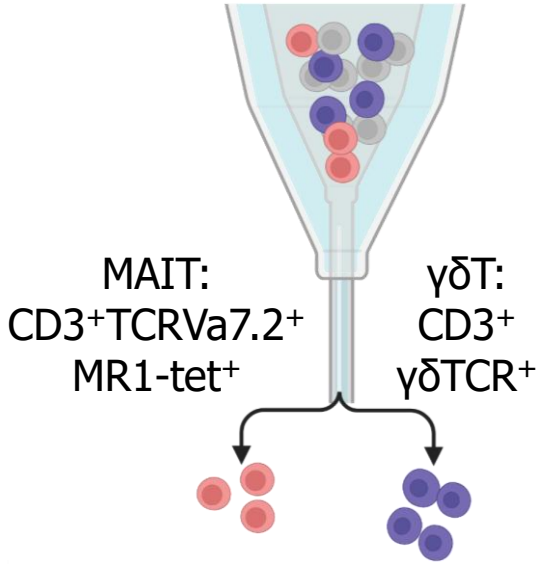
MAIT

↓

- CD3
- TCRVα7.2
- 5-OP-RU-MR1 tetramer
- CD4
- CD8



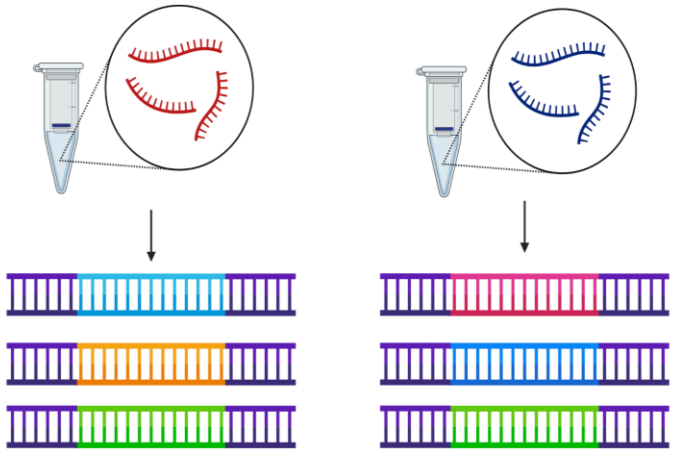
FACS sorting



- Cells sorted into TRIzol™ reagent
- total RNA extraction - Direct-zol commercial kit

TCR repertoire analysis

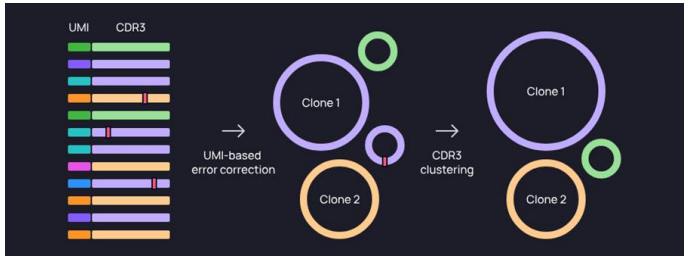
- Archer® Immunoverse™-HS TCR kit
- simultaneous sequencing of the CDR3 regions of α-, β-, γ-, and δ-chains
- 40 sequencing libraries (25 PV + 15 HC)



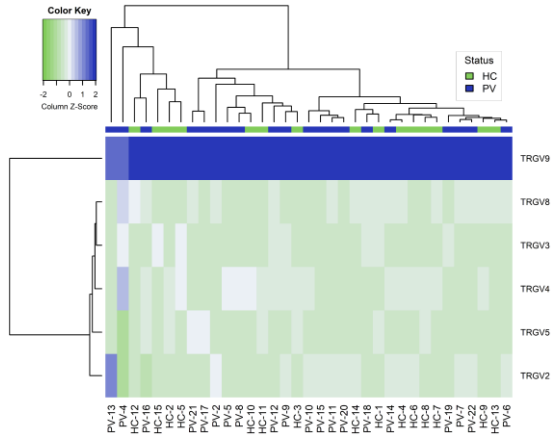
NextSeq 550
Illumina platform

bioinformatics pipeline:

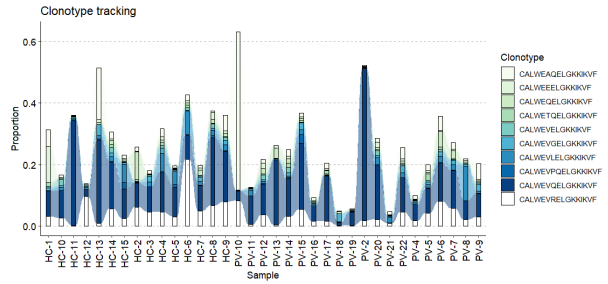
MiXCR



VDJTools



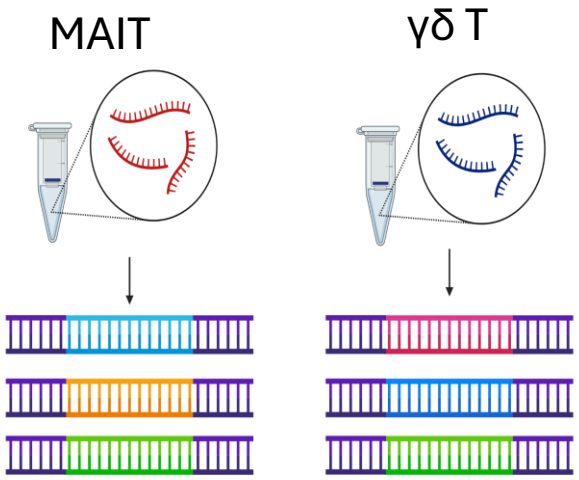
immunarch



Transcriptomic profiling

- Immune Response Panel (Illumina)
- 395 genes: activation, effector functions, adhesion, migration, TCR signalling,etc.
- 24 sequencing libraries (12 PV + 12 HC)

Miniseq Illumina sequencer



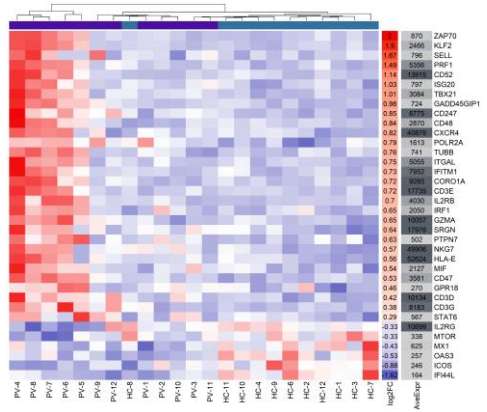
bioinformatics pipeline:

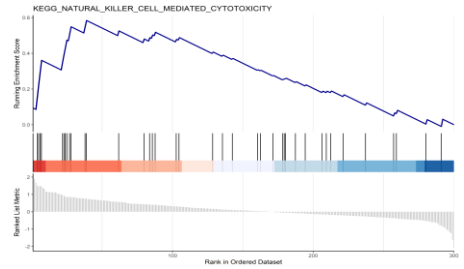
STAR

featureCounts

DEseq2

clusterProfiler





Spectral flow cytometry

Surface panel - 34 markers:

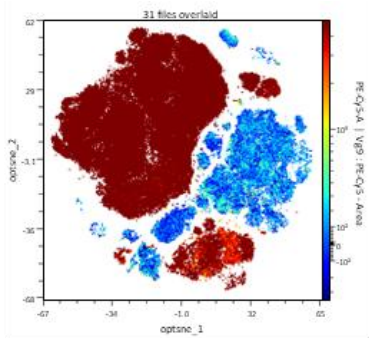
Laser	Flourophore	Antigen	Clone
UV - 355nm	Spark UV387	CD4	SK3
	BUV395	CD45	HI30
	BUV496	CD16	3G8
	BUV563	CD56	NCAM16.2
	BUV615	CD25	2A3
	BUV661	CD11a	G-25.2
	BUV805	CD3	UCHT1
V – 405 nm	BV421	PD-1	EH12.1
	BV480	CD94	HP-3D9
	VioBlue	αβTCR	REA652
	BV510	CD57	QA17A04
	BV570	HLA-DR	L243
	BV605	Va7.2	3C10
	BV650	CCR7	G043H7
	BV711	CD45RA	HI100
	BV750	CD69	FN50
	BV786	CXCR3	G025H7
B – 488 nm	FITC	Vδ1	REA173
	RB545	CXCR5	RF8B2
	BB700	CD103	Ber-ACT8
	PerCP Vio700	CLA	REA1101
	AF555	Vδ3	P11.5B
YG – 561 nm	PE	MR1-tet	
	Spark YG 581	CD127 (IL-7Rα)	A019D5
	PE-Dazzle594	CD27	M-T271
	PE-Cy5	Vγ9	IMMU 360
	PE-Cy7/vio770	CX3CR1	2A9-1
	Pe-Fire 700	CD8a	SK1
	PE-Fire 810	CD28	CD28.2
Red - 637 nm	APC	CD161	W18070C
	Alexa Fluor 647	CCR6	11A9
	VioBright R720	Vδ2	REA771
	APC-Vio 770	γδTCR	REA591
	Zombie NIR	Live/Dead	free amines
	APC-Fire 810	CD38	HIT2

Intracellular panel – 17 surface + 10 IC markers :

Laser	Flourophore	Antigen	Clone
UV - 355nm	Spark UV387	CD4	SK3
	BUV496	CD16	3G8
	BUV563	CD56	NCAM16.2
	BUV661	CD11a	G-25.2
	BUV805	CD3	UCHT1
V - 405nm	VioBlue	αβTCR	REA652
	BV605	CD27	M-T271
	BV711	CD45RA	HI100
	BV750	CX3CR1	2A9-1
	BV786	PD-1	EH12.1
B – 488 nm	FITC	Vδ1	REA173
	PerCP Vio700	Vδ2	REA771
	AF555	Vδ3	P11.5B
YG - 561nm	Spark YG 581	CD127 (IL-7Rα)	A019D5
	Pe-Fire 700	CD8a	SK1
	PE-Fire 810	CD28	28.2
Red - 637nm	APC-Vio770	γδTCR	REA591

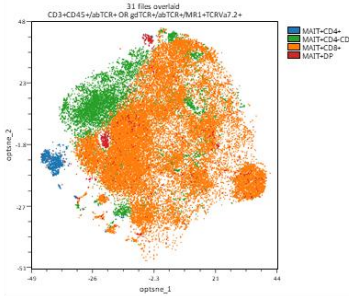
Laser	Flourophore	Antigen	Clone
UV - 355nm	BUV395	Ki67	SolA15
B – 488 nm	RB545	Granzyme B	GB11
	RB780	Granzyme K	G3H69
	PeCP Cy5.5	Perforin	dG9
YG - 561nm	Pe-eFlour610	EOMES	WD1928
	PE-Cy5	T bet	4B10
	PE-Cy7	Granzyme A	CB9
	PE	TOX	TXRX10
Red-637nm	APC	Granulysin	DH2
	R718	BLIMP-1	6D3

- 34 samples (20 PV + 14 HC)



- OMIQ platform for analysis

Dotmatics OMIQ

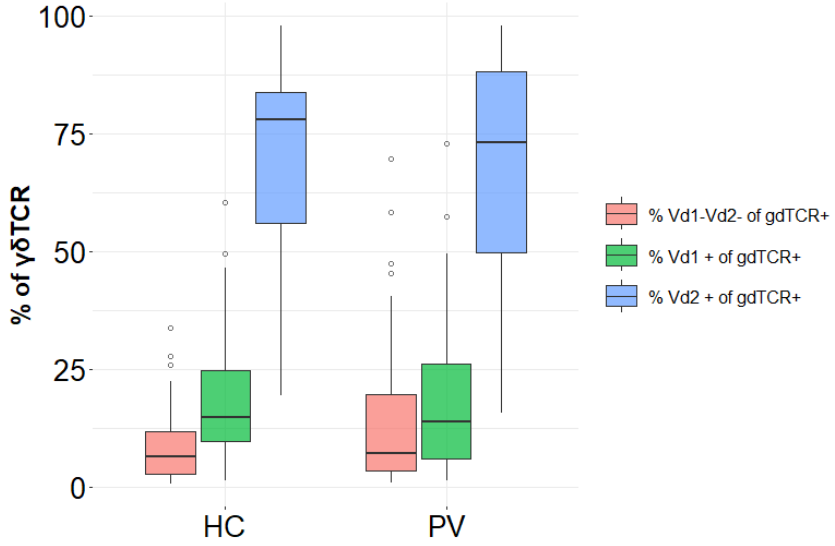


Spectral analyser Sony ID7000

Results

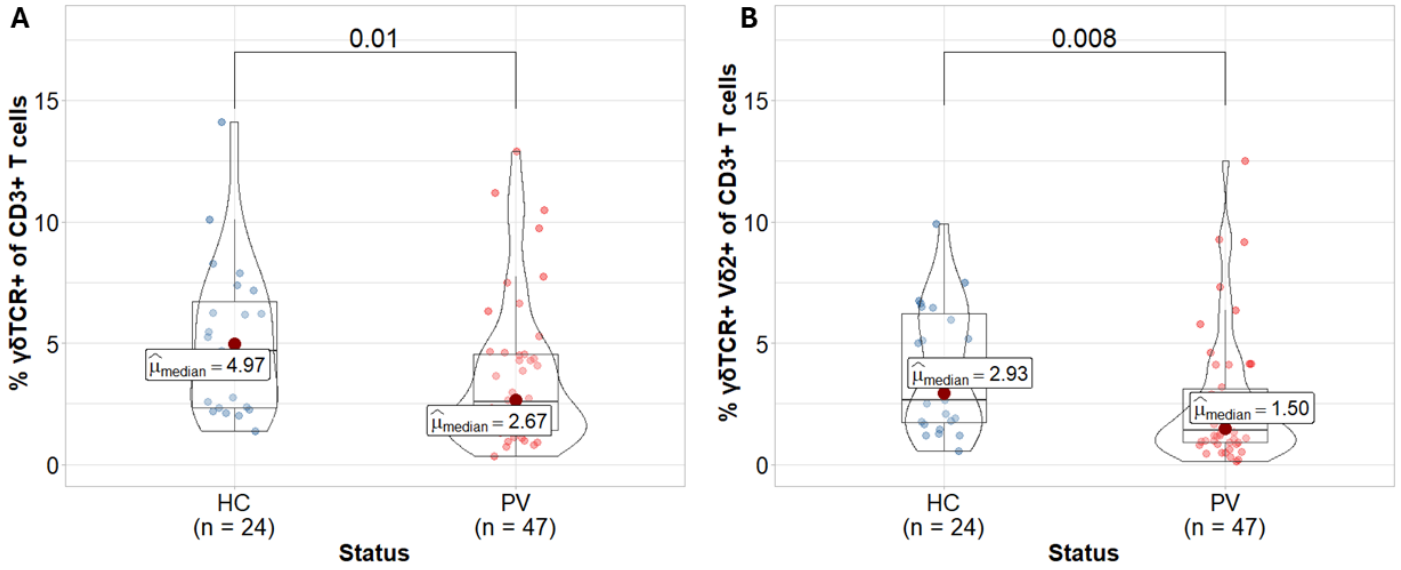
Investigation of the composition of circulating γδ T cell compartment

- The composition of peripheral γδ T compartment is not altered in psoriasis



Distribution of γδ T cell subsets in psoriasis patients and healthy controls. *Vδ2+ cells dominate the peripheral γδ T cell population, followed by Vδ1+ and Vδ1-Vδ2- subsets, with no significant differences in case-control comparison. N (PV) = 63, N(HC) = 33.*

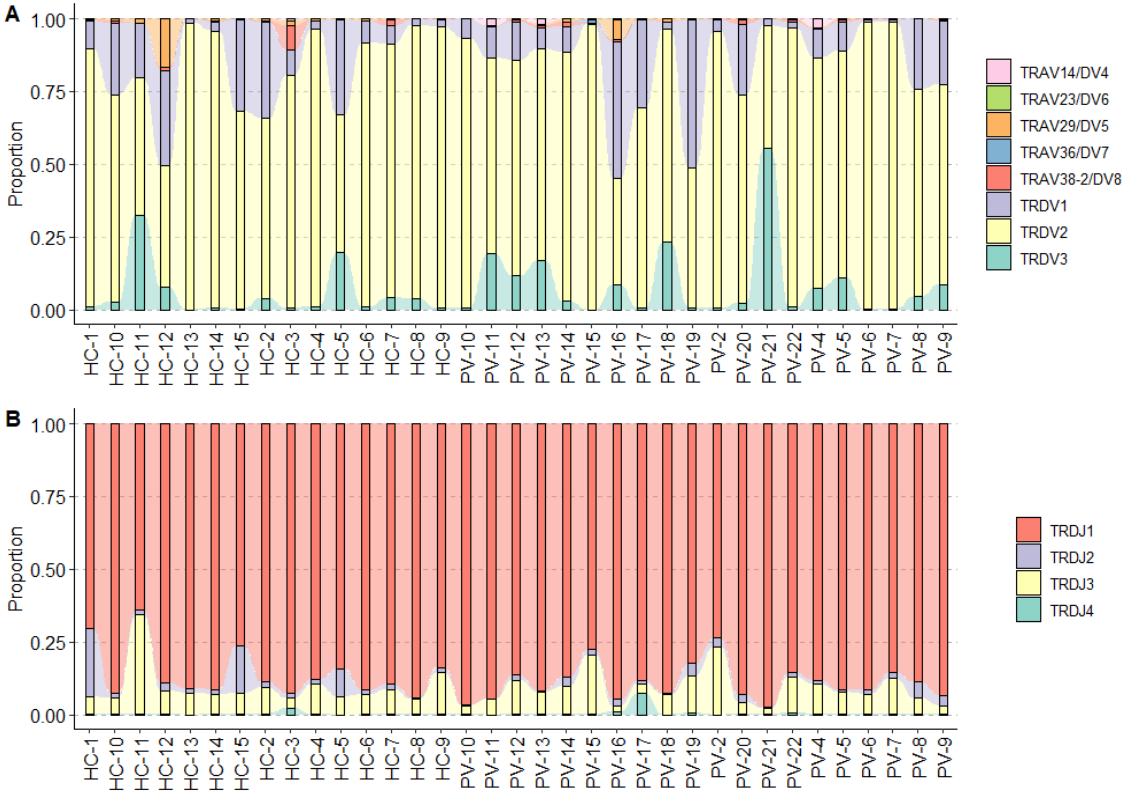
➢ A significant **reduction in γδTCR⁺ and Vδ2⁺** cells within the CD3⁺ T cell population in **male PV patients**



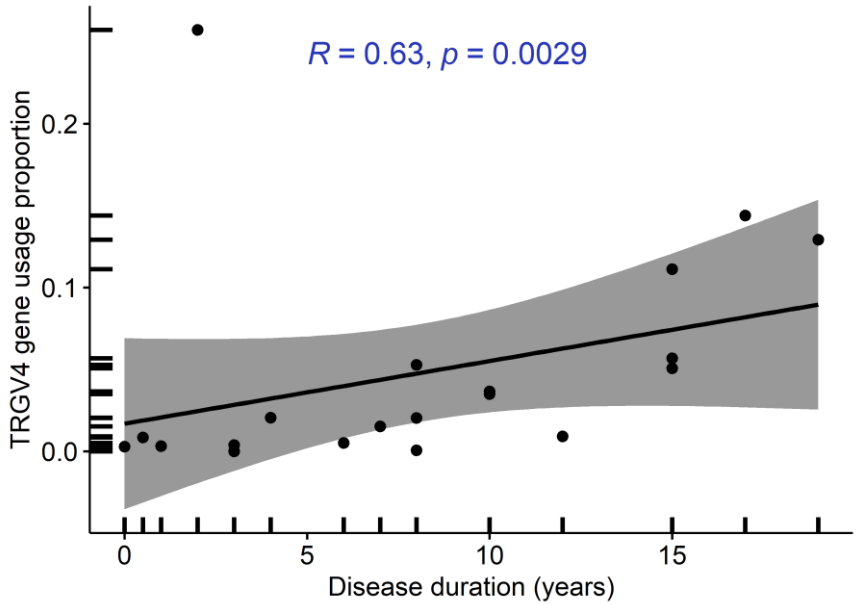
Male psoriasis patients show reduced γδ T and *Vδ2+* cell proportions compared to healthy men. *Proportions of A) total γδ TCR+ and B) Vδ2+ cells within CD3+ T cells are significantly lower in male psoriasis patients (PV) compared to healthy male controls (HC). Mann-Whitney U test*

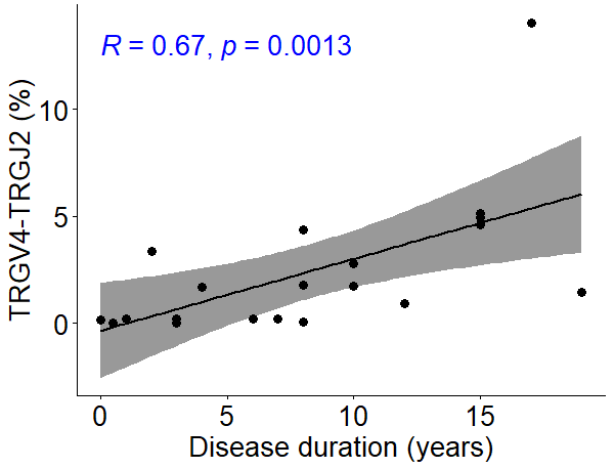
Profiling of γδ TCR repertoires

- Circulating **γδ** TCR repertoires are composed primarily of **TRGV9-TRGJP** and **TRDV2-TRDJ1** rearranged clonotypes, with no significant differences in case-control comparison

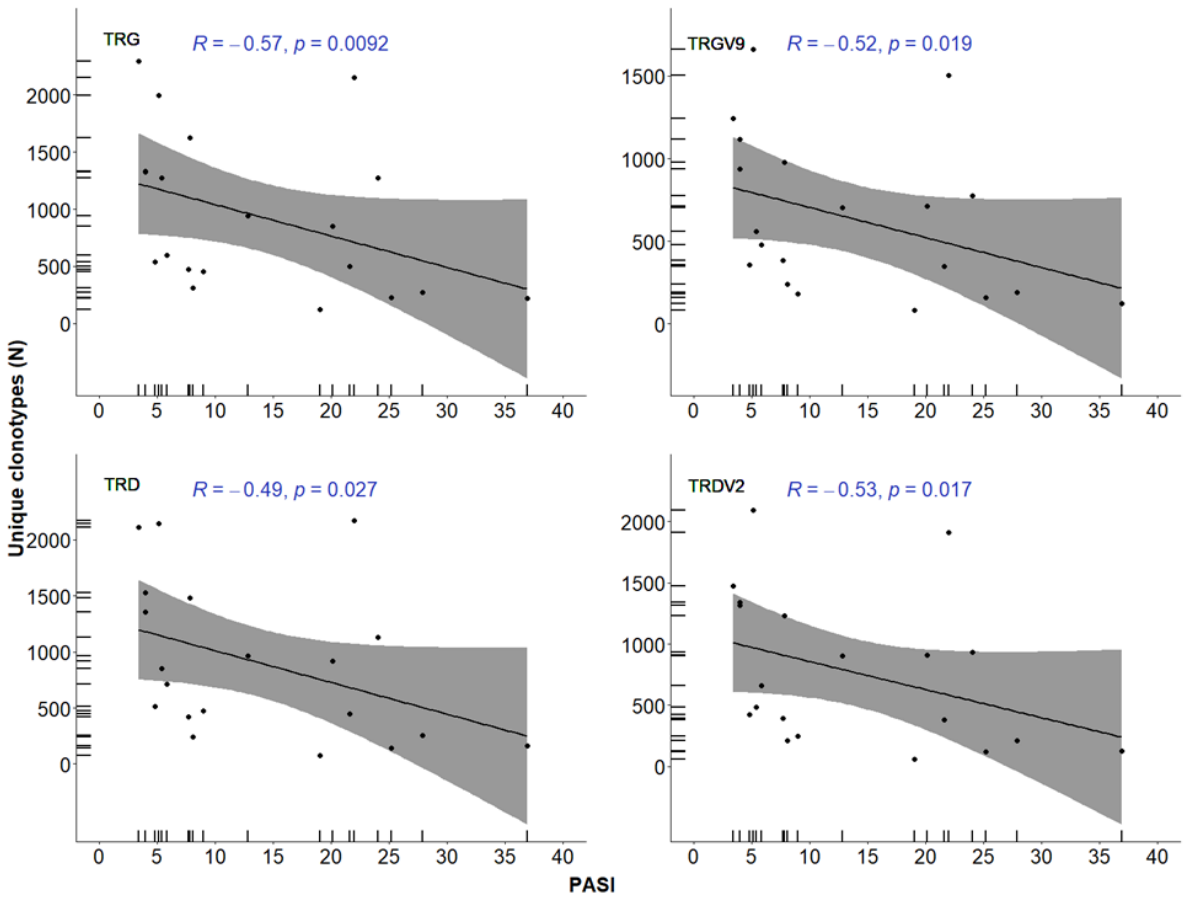


- the proportion of **TRGV4 clonotypes** increases with the **duration of psoriasis**



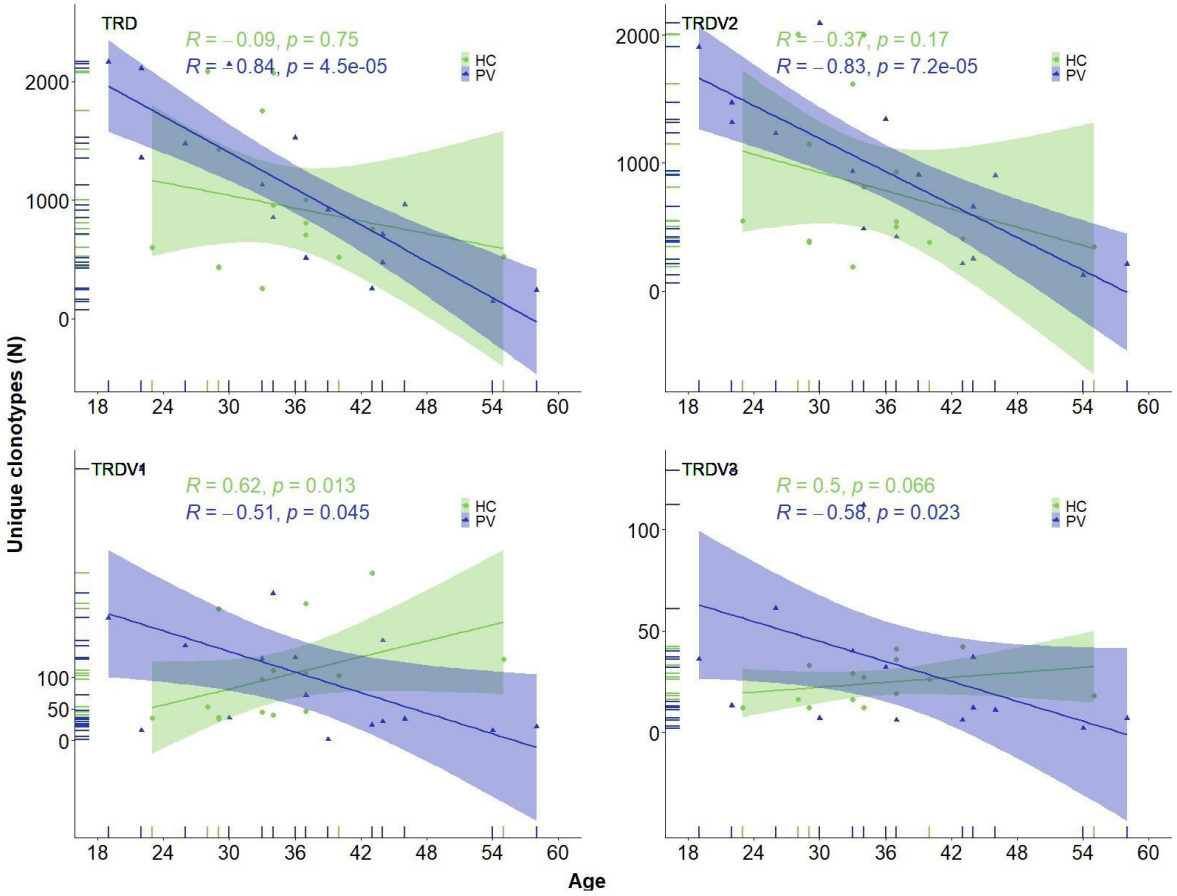


➢ TRG and TRD repertoire diversity declines with disease severity



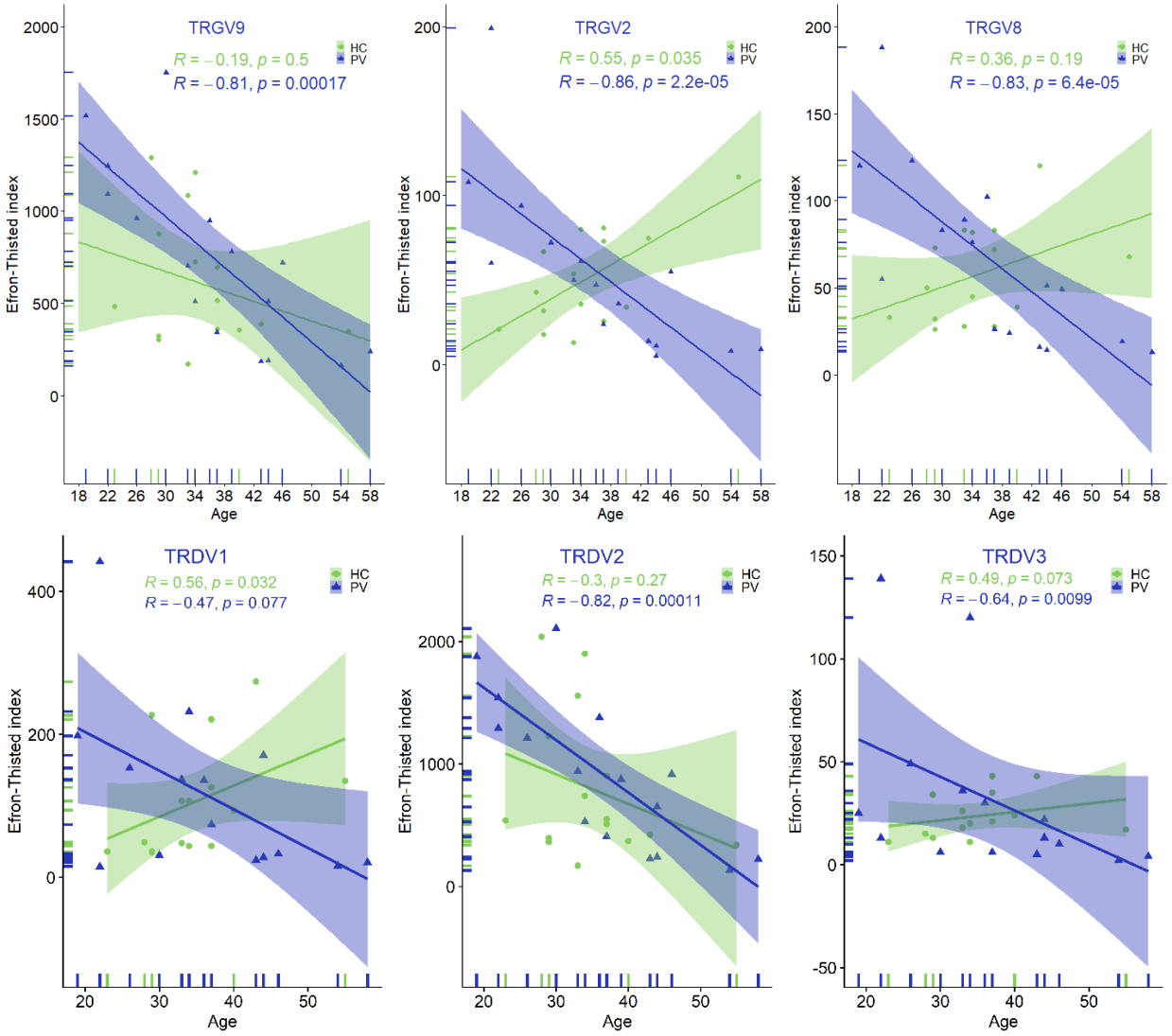
The number of unique TCRγ and TCRδ clonotypes decreases significantly with increasing PASI score. *Spearman's correlation analysis between PASI score and A) total TRG, B) TRGV9, C) total TRD, and D) TRDV2 clonotypes*

➢ Ageing is linked to a loss of unique TRG and TRD clonotypes

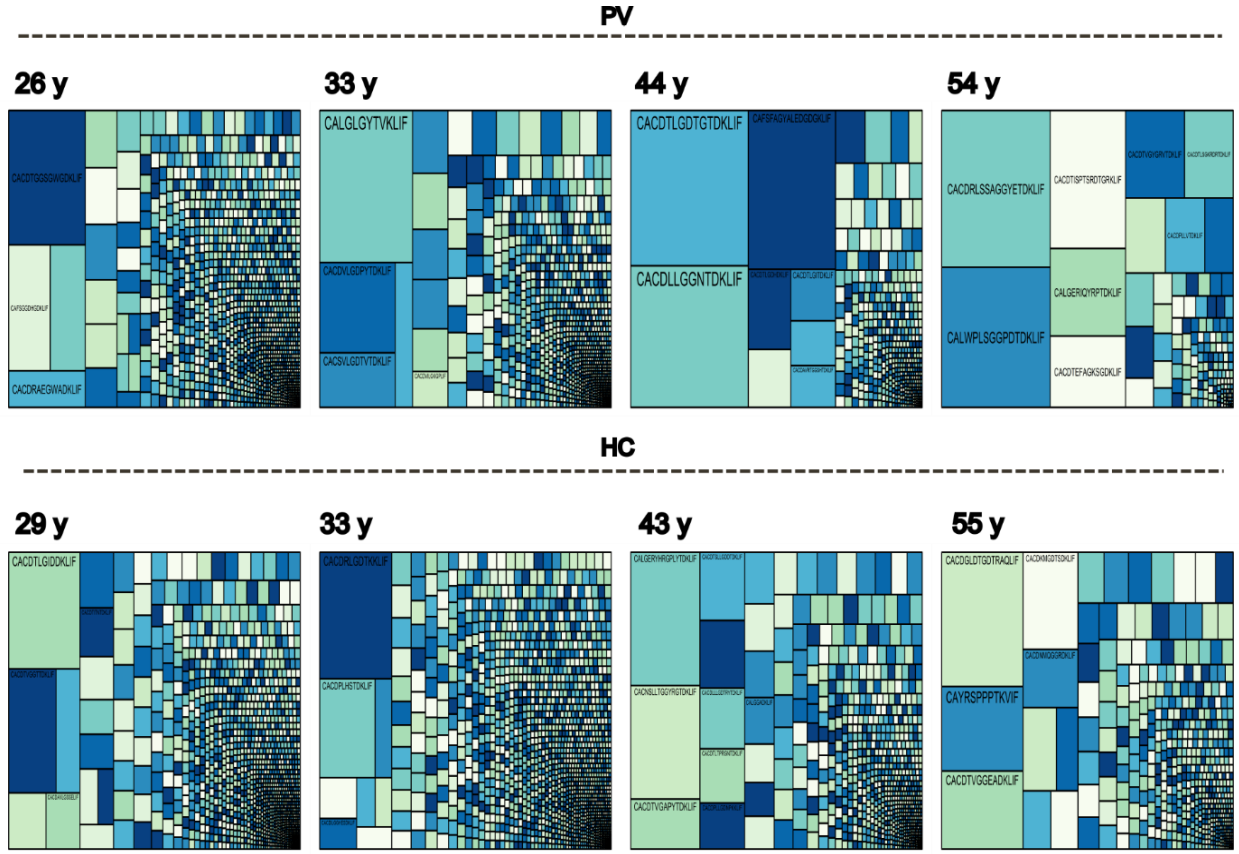


Decline in unique TRD clonotype number with age in psoriasis patients. **Age related decline in unique TRG clonotype number in psoriasis patients.** *The number of unique TRD clonotypes, including TRDV-2/1/3 subgroups, declines significantly with age in psoriasis patients, whereas this trend is absent or opposite in healthy controls. Spearman's correlation analysis. N(PV) = 16, N(HC)=15.*

➢ **Ageing-associated** reduction **in rare clonotypic variants** accompanied by an **increased proportion of hyperexpanded clonotypes**



Spearman's correlation analysis. N(PV) = 16, N(HC)=15.



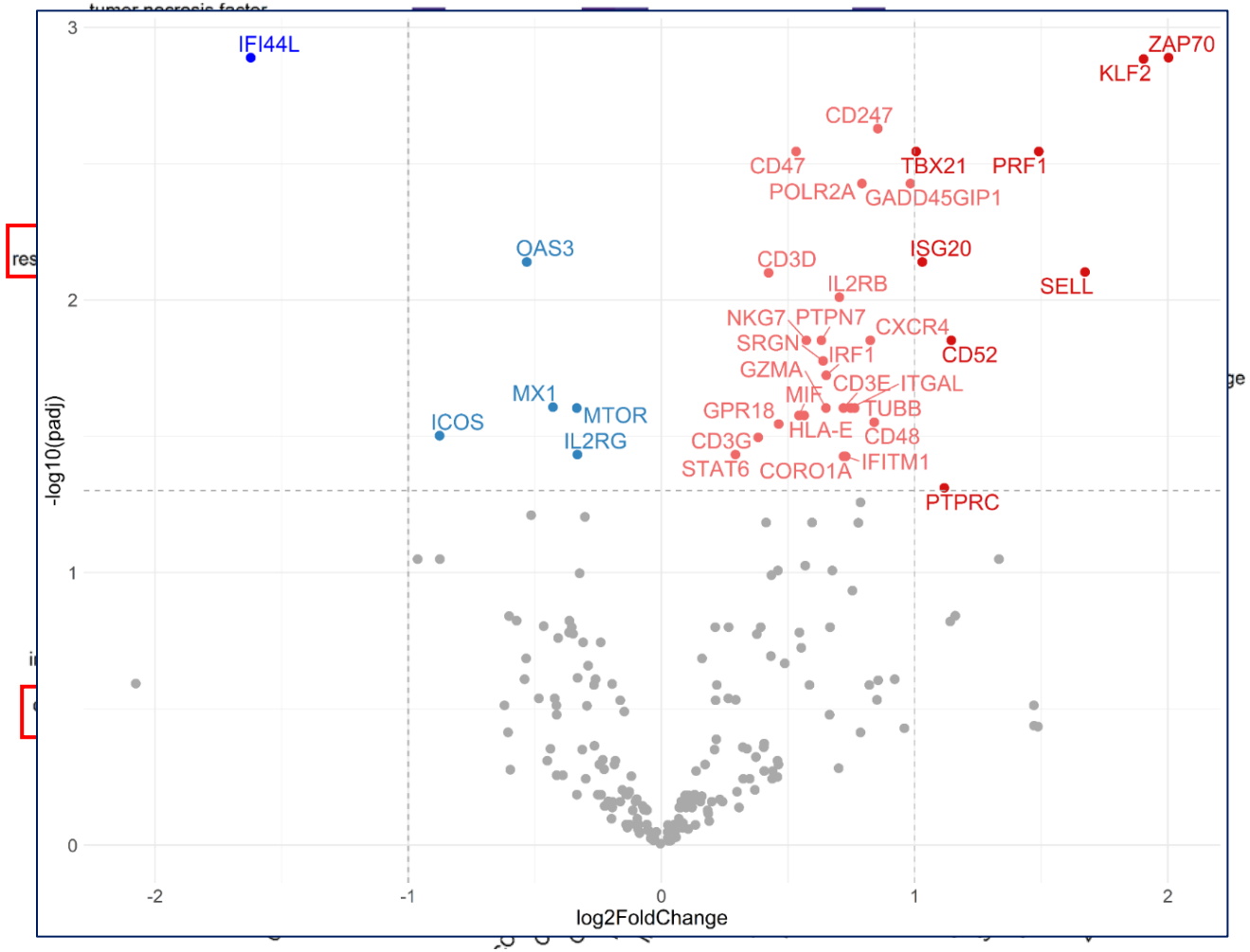
Representative treemaps illustrating greater TCRδ clonal focusing in psoriasis vulgaris patients compared to age-matched healthy controls. *Each rectangle represents a clonotype, with size corresponding to its relative frequency in the repertoire. "y" denotes age in years.*

Transcriptomic signatures of γδ T cells

➢ **altered in psoriasis (36 DEG)**

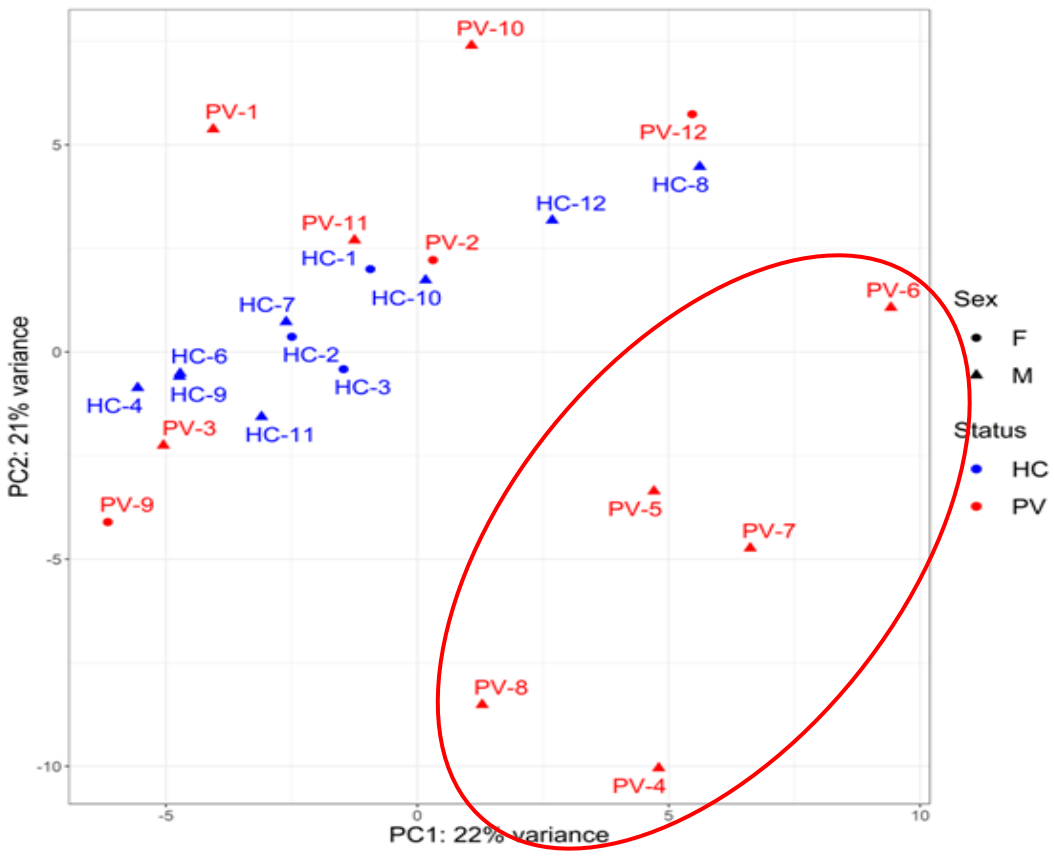
- **Upregulated genes:**
  - **activation** (*CD3D/E/G, CD247, ZAP70, CD48, IL2R*B)
  - **cytotoxicity** (*PRF1, GZMA, SRGN, NKG7*)
  - **IFN-γ production and response** (*TBX21, IRF1, IL2RB, IFITM1, ISG20, GADD45G*)
  - **tissue migration** (*KLF2, SELL, CXCR4*)
  - **survival** (*CD47, HLA-E*)

- **Downregulated genes:**
  - **antiviral response** (*MX1, OAS3, IFI44L)*

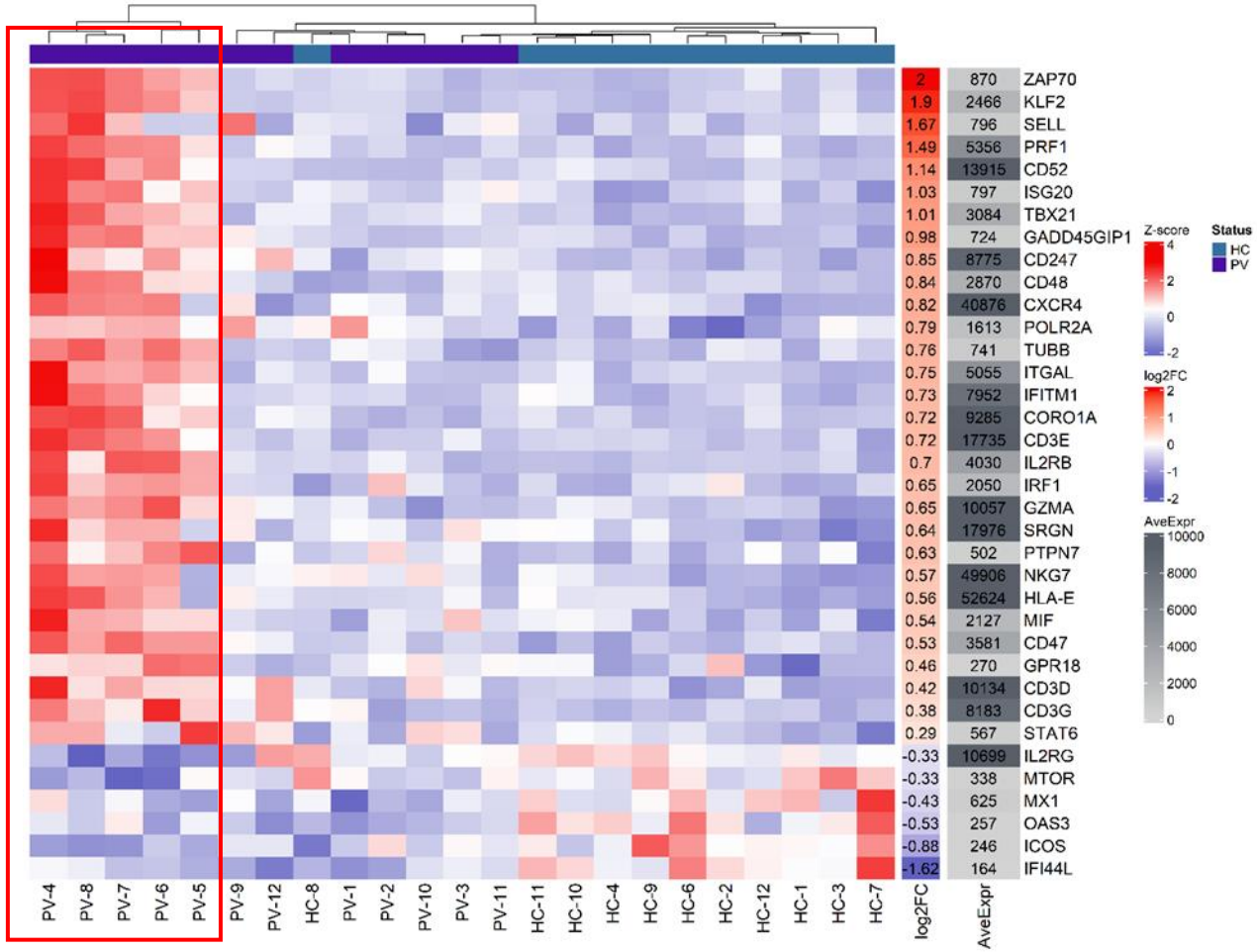


GO-BP enrichment analysis of upregulated genes in psoriasis. *Rows represent enriched Gene Ontology Biological Process (GO-BP) terms, while columns correspond to upregulated genes associated with these terms. The colour intensity reflects the fold change of each gene.*

➢ The **upregulated gene signature** is **particularly pronounced** in a **subset of patients**

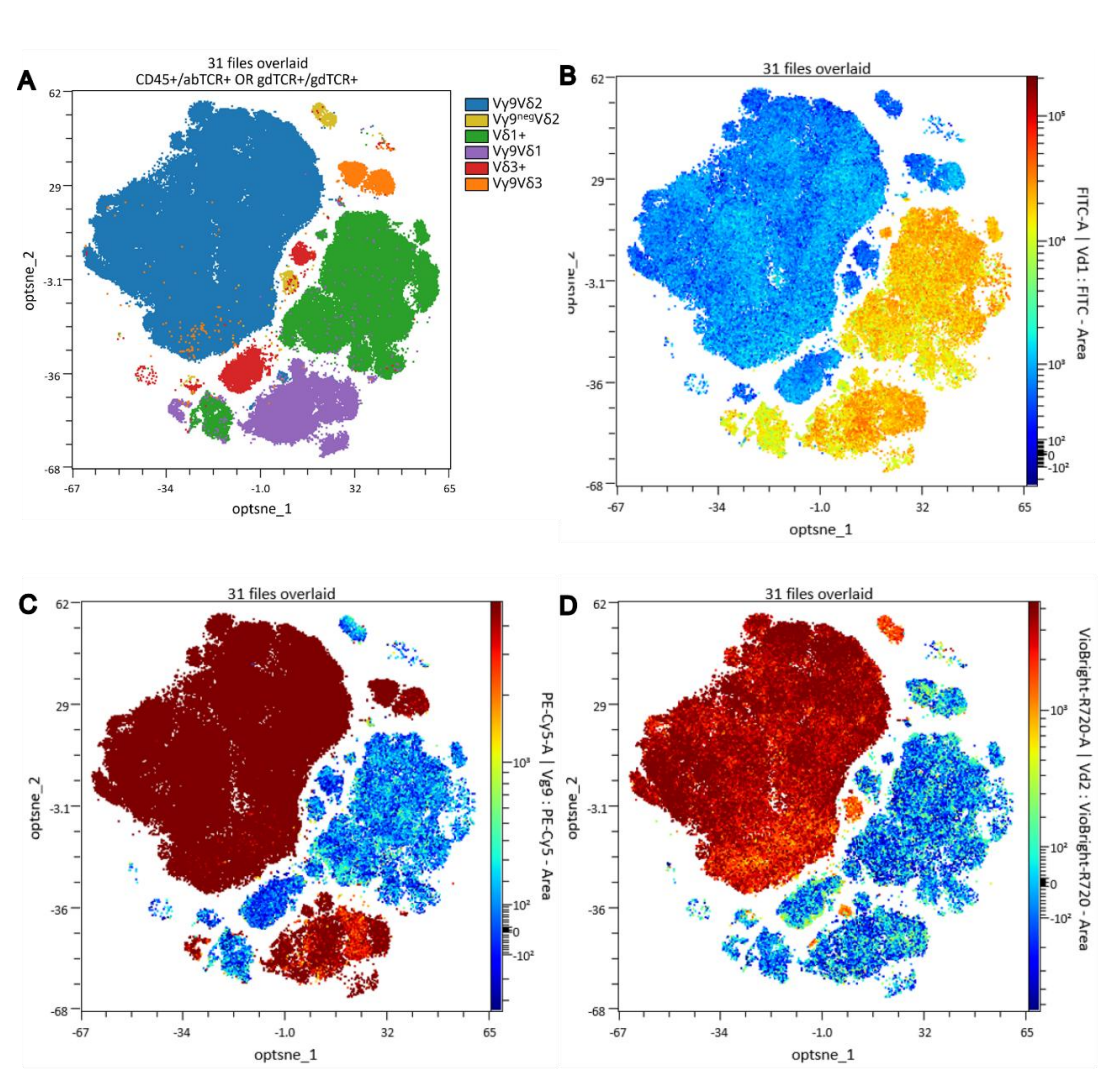


Principal component analysis (PCA) of psoriasis and healthy control samples. *PCA plot showing the distribution of psoriasis vulgaris (PV) and healthy control (HC) samples based on gene expression profiles.*



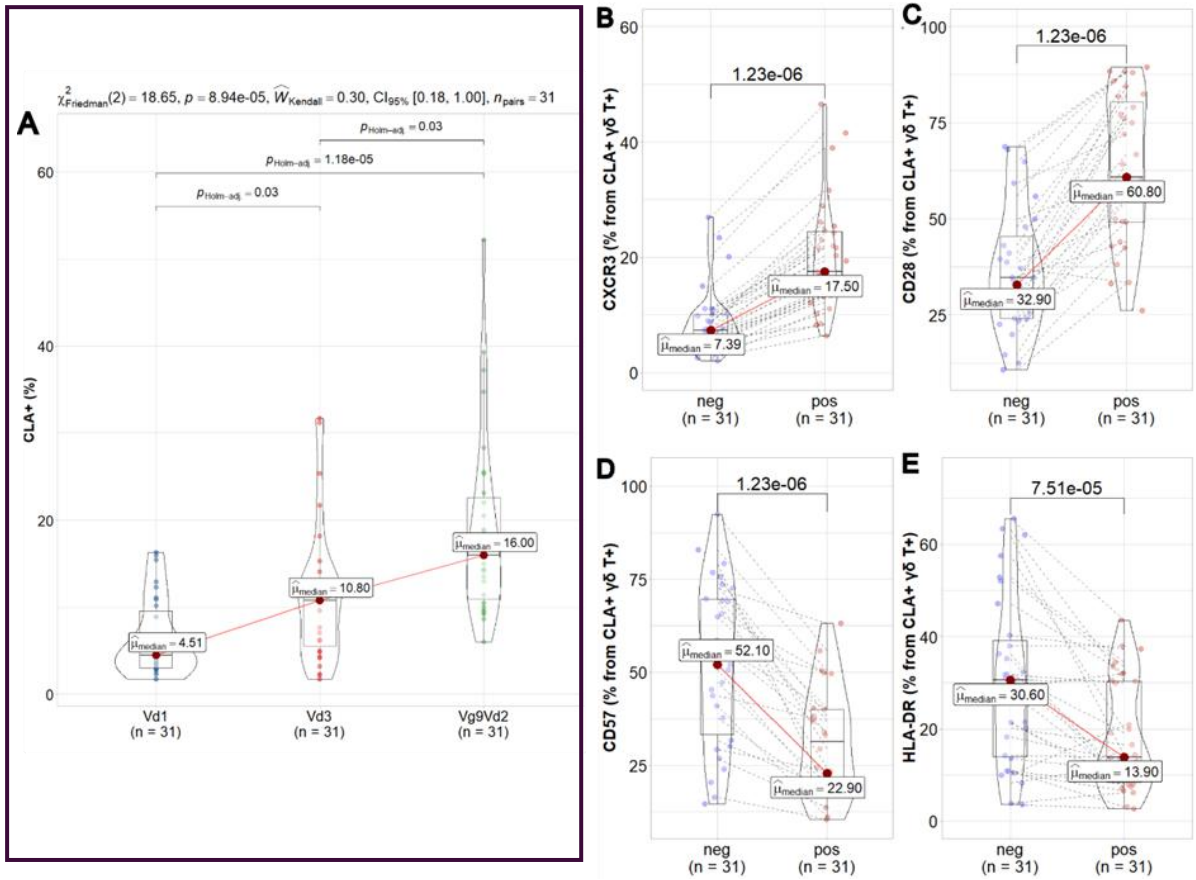
Differentially expressed genes in psoriasis patients and healthy controls. *Rows show genes ranked by log2 fold change; columns represent individual samples. Colour intensity indicates Z-scored expression.Top dendrograms show sample clustering; colour bar indicates group (PV: psoriasis vulgaris, HC: healthy controls). "AveExpr" = average gene expression.*

High-throughput profiling of γδ T cells



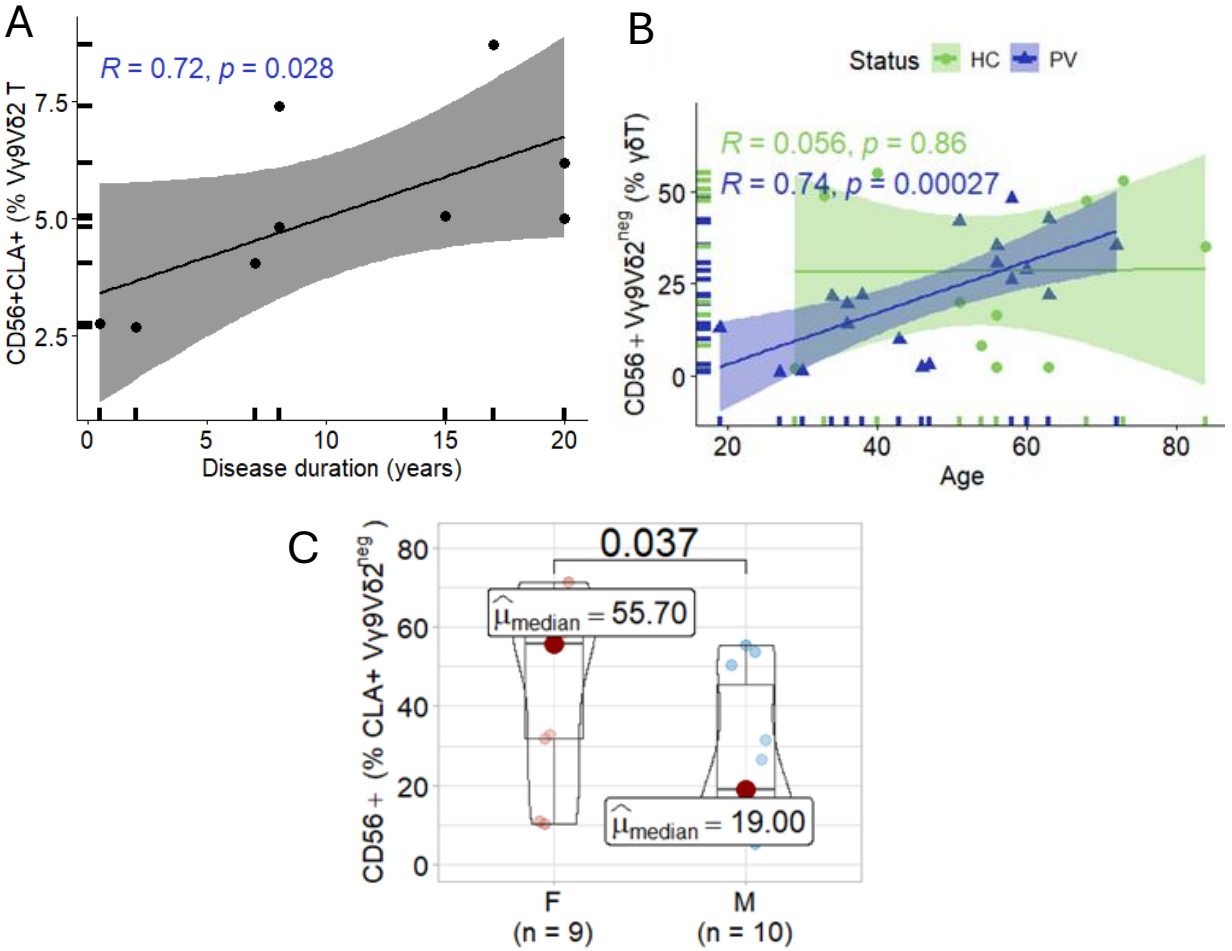
γδ T cells cluster by Vγ9 and δ chain expression. *A) Opt-SNE plot shows clustering driven by δ and* Vγ9 *chain expression. Fluoresecence signal intensity for B) Vδ1, C)* Vγ9, and D) *Vδ2 populations. The data represents a combined overlay analysis of all samples.*

➢ **Vγ9Vδ2** cells have **the highest skin-homing potential**

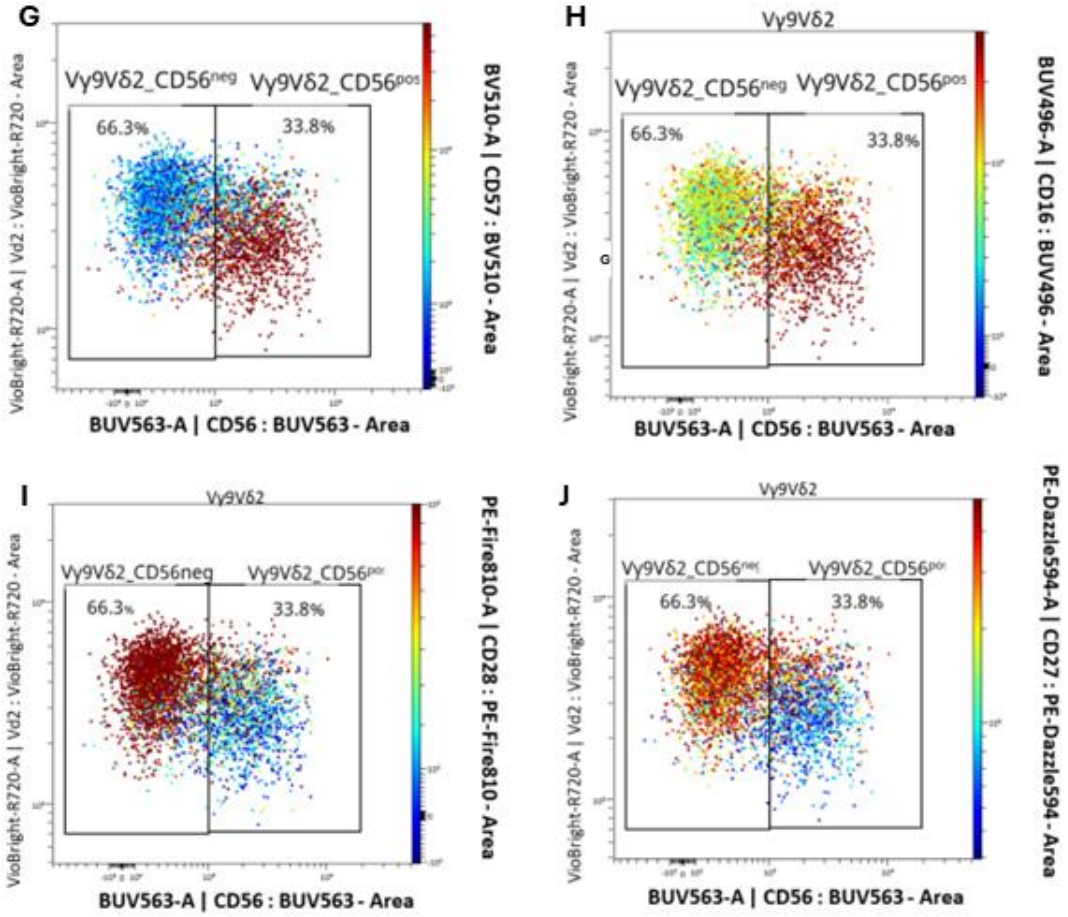


CLA expression on γδ T cells is enriched in Vγ9Vδ2 cells and associated with tissue-homing and naïve-like phenotypes. *Friedman's test (A) and Wilcoxon signed-rank test (B–E).*

➢ CD56 as marker of disease-driven alterations



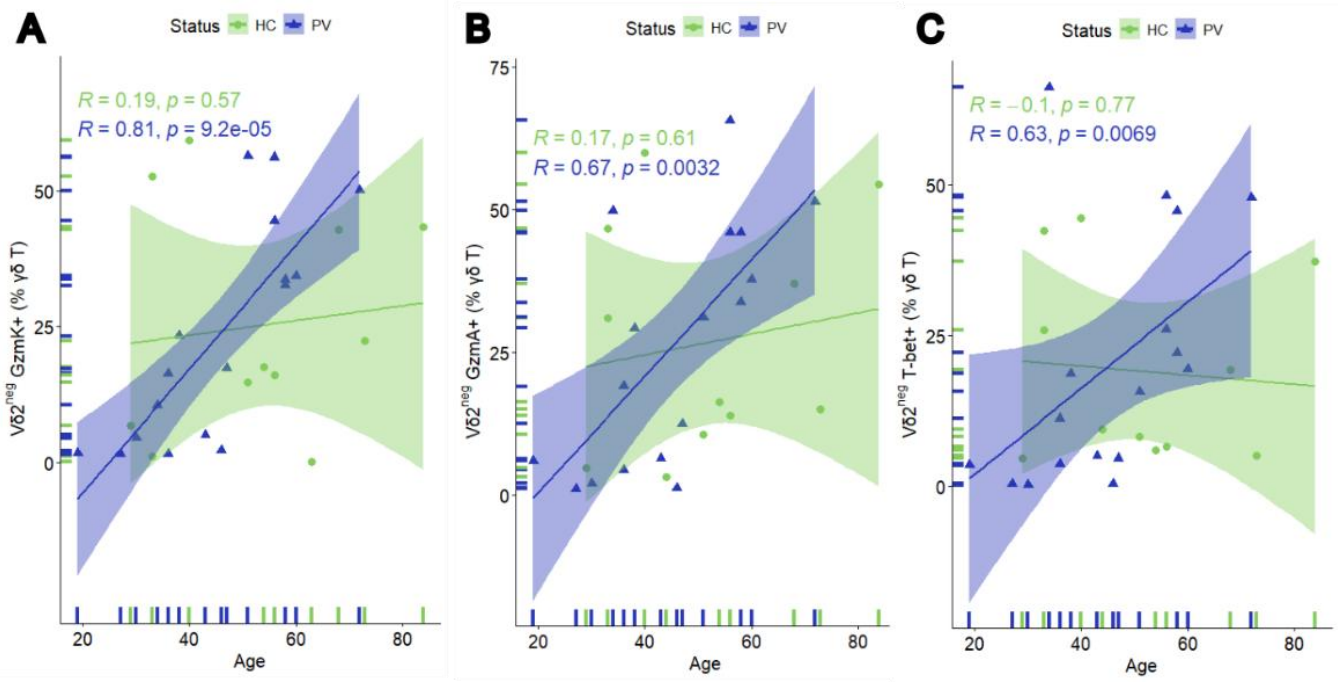
CD56 expression in γδ T cell subsets is altered in psoriasis. *A) CD56 expression in Vγ9Vδ2 cells increases with disease duration, B) In Vγ9Vδ2⁻ cells, CD56 expression correlates with age in PV patients, C) Female patients show a higher frequency of CD56+ γδ cells*

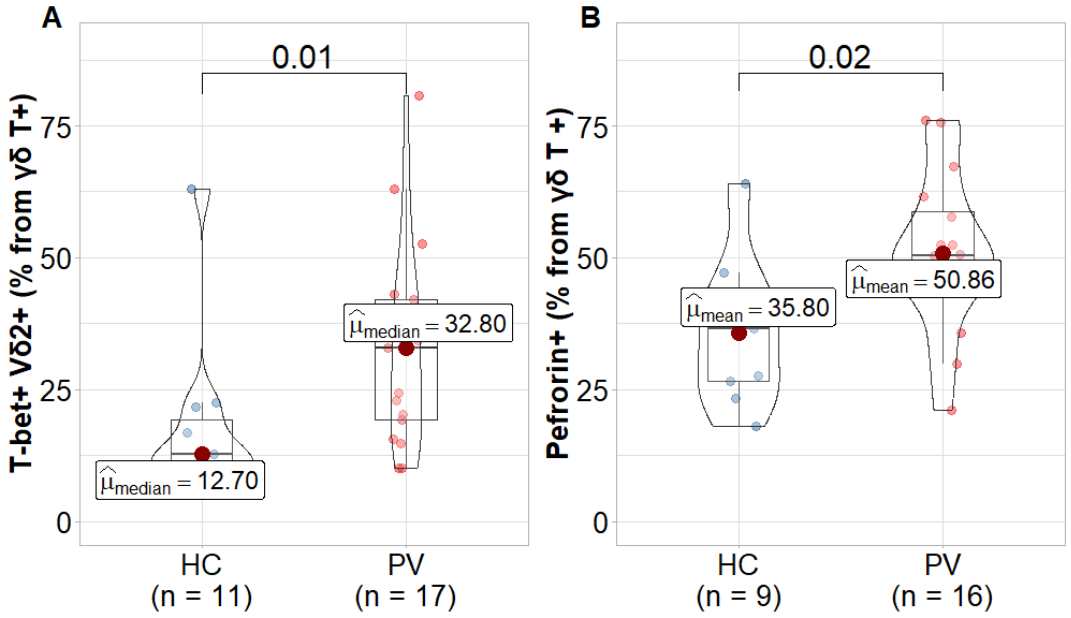


CD56+ Vγ9Vδ2 cells exhibit elevated expression of cytotoxic effector markers and reduced expression of naïve-like markers. *Representative fluorescence intensity plots illustrate the distribution of G) CD57, H) CD16, I) CD28, and J) CD27 expression on CD56+ and CD56⁻ Vγ9Vδ2 T cells.*

➢ **Cytotoxic/effector profile** of Vγ9Vδ2^{-} cells increases with **age in PV**

➢ **Transcriptomic alterations confirmed at protein level**



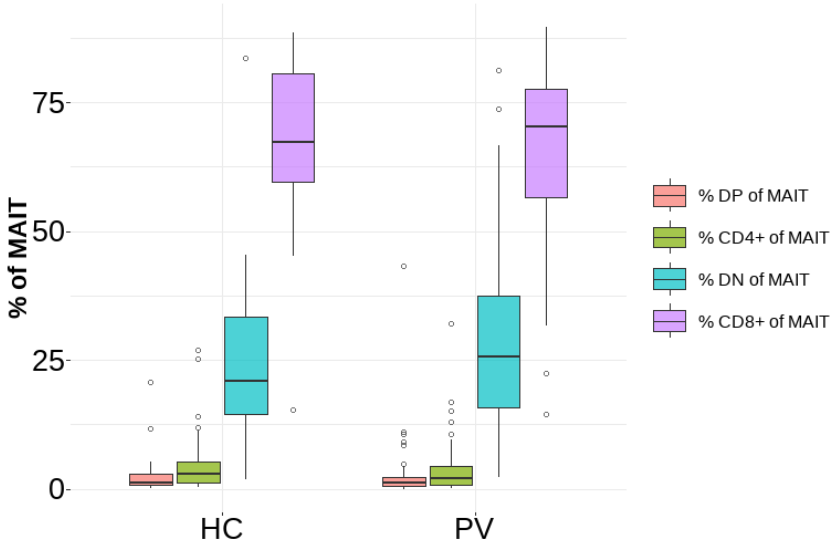


Age-associated increase in cytotoxic granule expression and effector markers in Vγ9Vδ2^{-} cells of psoriasis patients. *The proportion of Vγ9Vδ2^{-} cells expressing A) GzmK, B) GzmA, and C) T-bet within the γδ T cell compartment increases significantly with age in psoriasis patients, but not healthy controls. Sperman's correlation coefficient*

Elevated T-bet and perforin expression in γδ T cells in psoriasis patients. *Violin plots depict the percentages of A) T-bet^{+}, B) perforin^{+}, γδ T cells in healthy controls (HC) and psoriasis patients (PV). Mann-Whitney U test (A, C), Student-t test (B).*

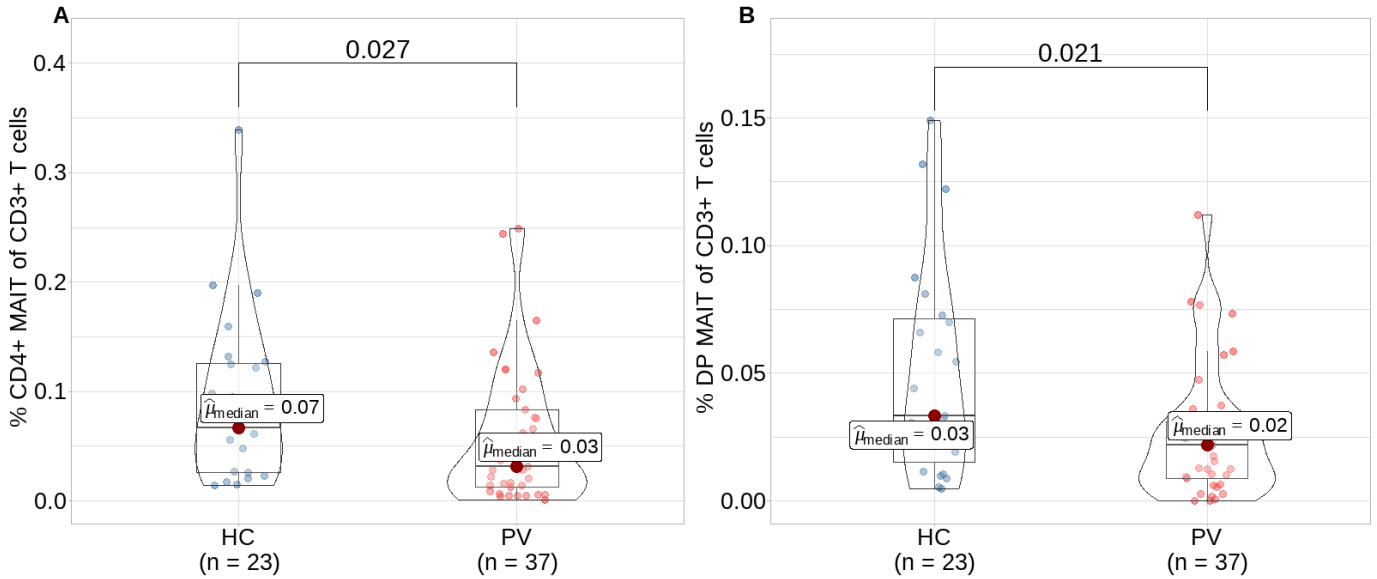
Investigation of the composition of circulating MAIT cell compartment

- The composition of circulating MAIT compartment remains stable in psoriasis



Distribution of MAIT cell subsets in psoriasis patients and healthy controls. *$CD8^+$ MAIT cells are the most abundant, followed by DN ($CD4^-CD8^-$), and less represented CD4+ and DP ($CD4^+CD8^+$) subsets, with no significant differences between healthy controls (HC) and psoriasis patients (PV). N (PV) = 63, N (HC) = 32.*

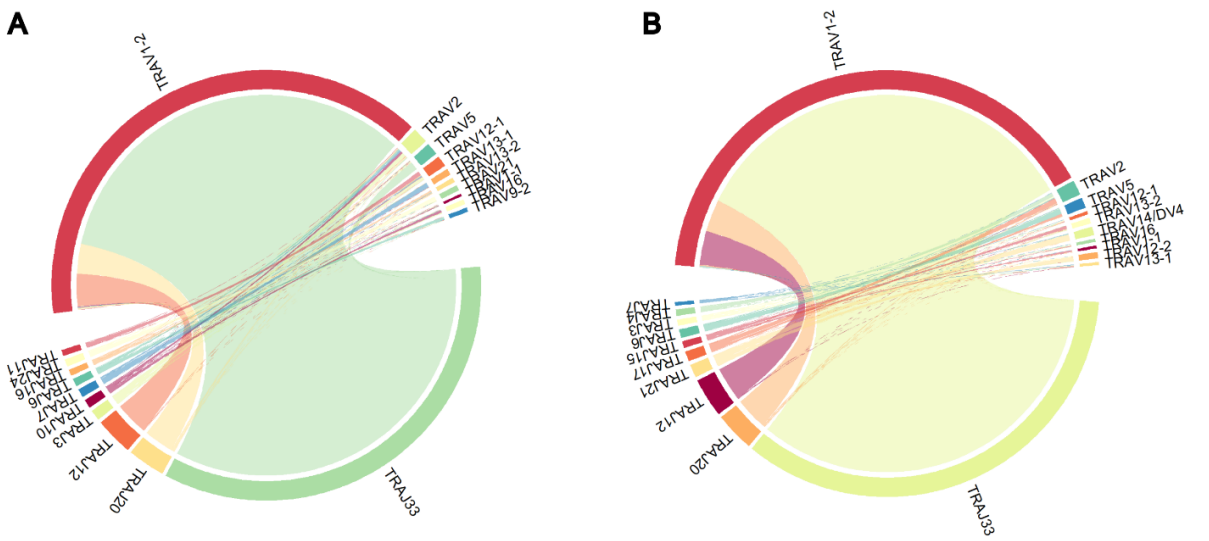
➢ **Male psoriasis patients** show **reduced $CD4^+$ and DP ($CD4^+CD8^+$)** MAIT subsets proportions compared to healthy men



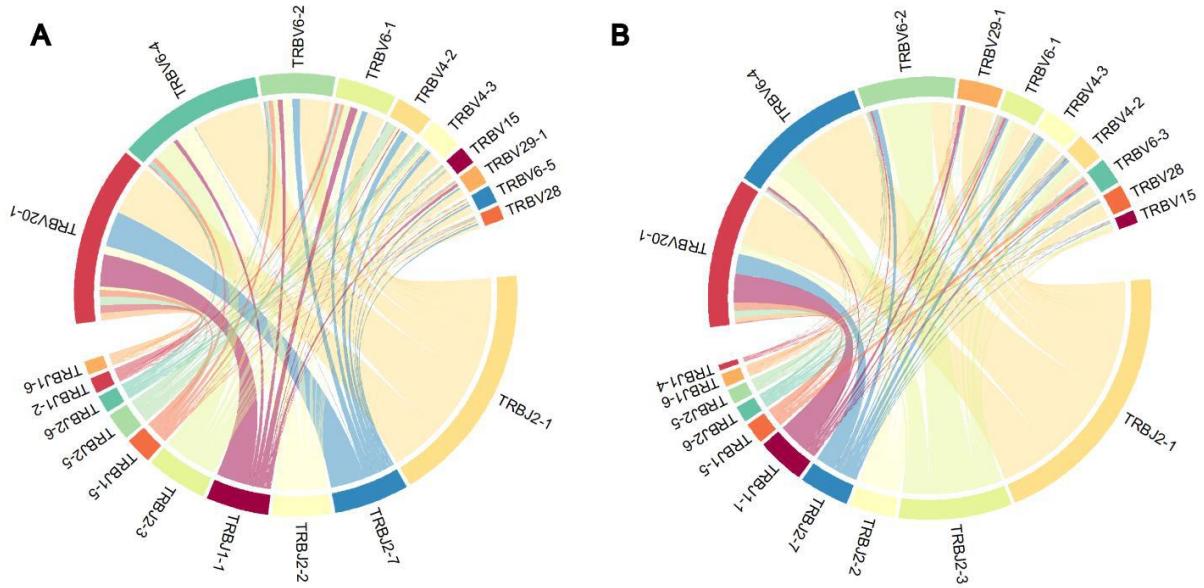
Male psoriasis patients show reduced CD4+ and DP MAIT subsets proportions compared to healthy men. *Proportions of A) CD4+ and B) DP (CD4+CD8+) subsets within CD3+ T in male psoriasis patients (PV) versus healthy male controls (HC). Based on an age-matched cohort of participants under 60 years old. Mann-Whitney U test.*

Profiling of MAIT TCR repertoires

- Circulating **MAIT** TCR repertoires mainly consist of **TRAV1-2-TRAJ33/20/12** and **TRBV20-1, TRBV6-4, TRBV6-2, TRBV6-1, and TRBV4-2** clonotypes, with slight skewing in TCRβ transcripts in psoriasis



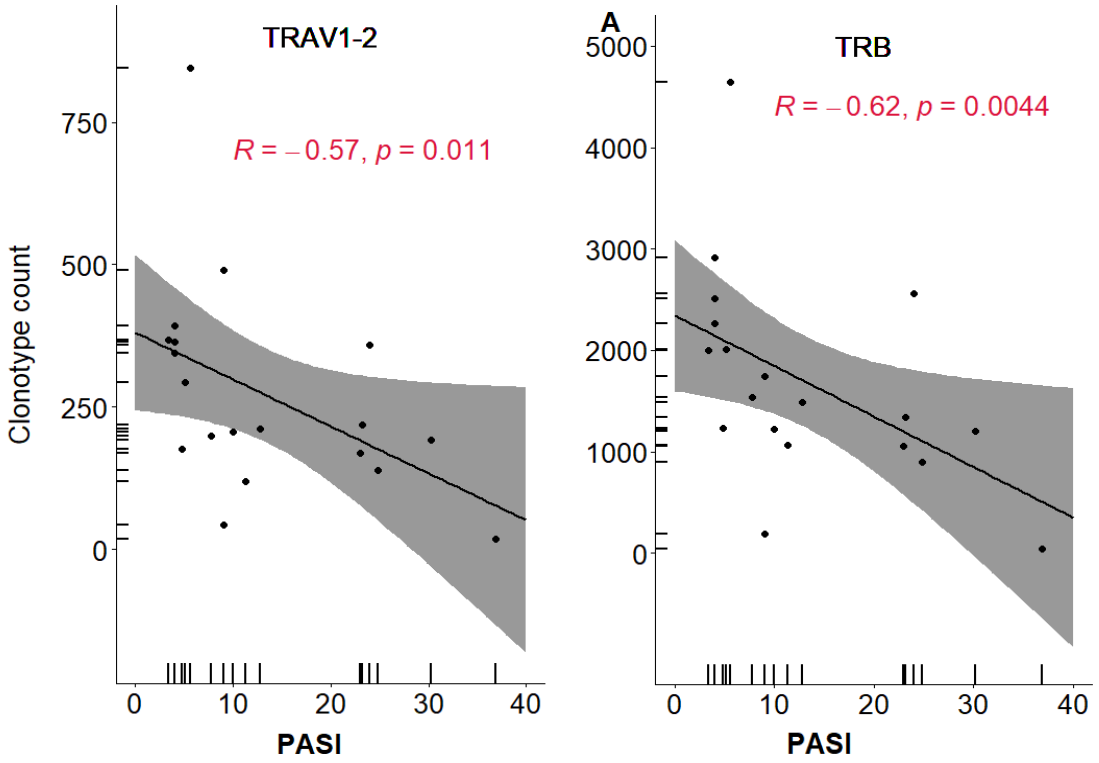
TRAV-TRAJ pairing frequencies in psoriasis and healthy group reveals overall similarity. *Chord diagram of pairings between the top 10 TRAV and TRAJ variants in A) pooled PV (N=26) , B) pooled HC (N=12) samples.*



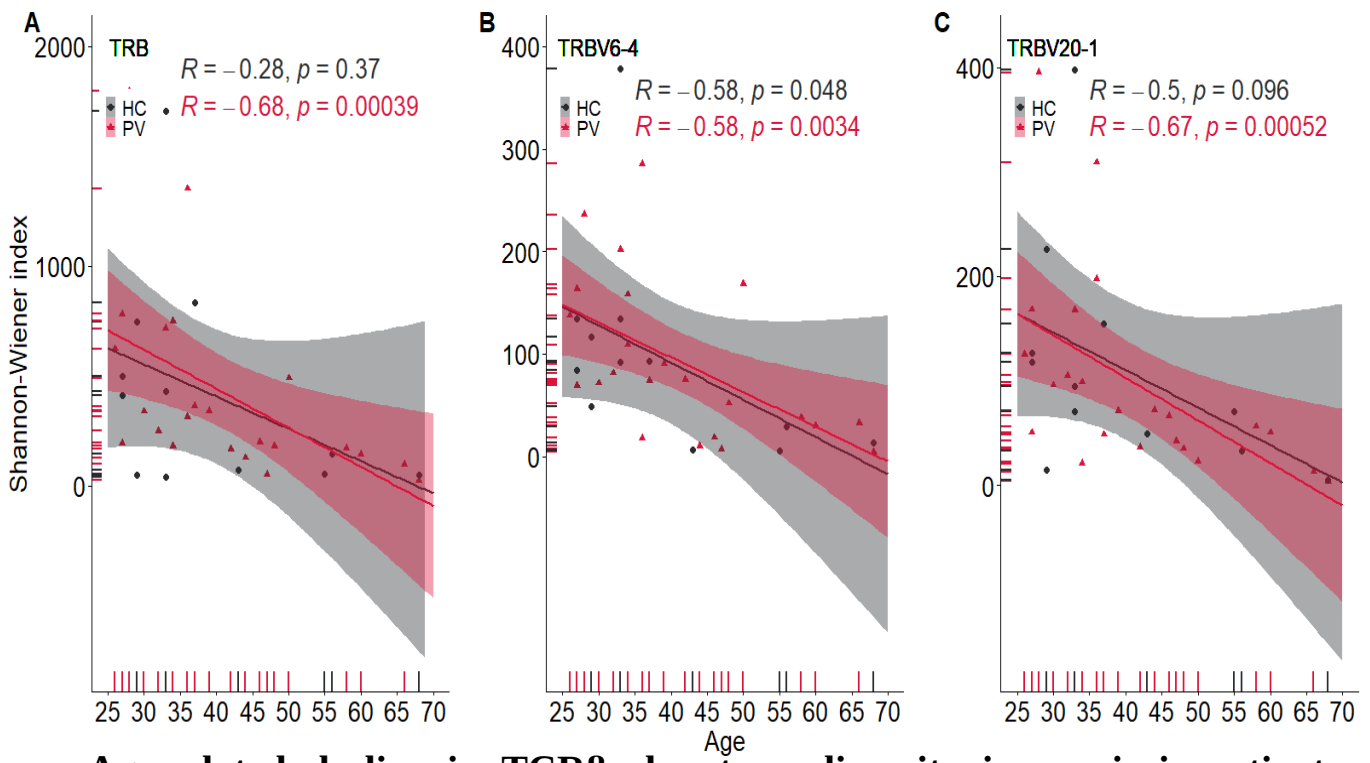
TRBV-TRBJ pairing frequencies in psoriasis and healthy repertoires reveals overall similarity with several psoriasis-specific alterations. *Chord diagram of pairings between the top 10 TRBV and top 10 TRBJ variants in A) pooled PV (N=26) and B) pooled HC (N=12) samples.*

- **MAIT TCR repertoire diversity** declines significantly with **disease severity** in **male psoriasis patients**

- **Repertoire diversity declines** significantly with **age** of psoriasis patients, with similar, but less prominent trends in healthy examinees



TRAV1-2 and TRB clonotype count declines with disease severity in male patients. *A) Unique TRAV1-2 and B) TRB clonotype count decreases significantly with PASI scores. Spearman's correlation analysis.*



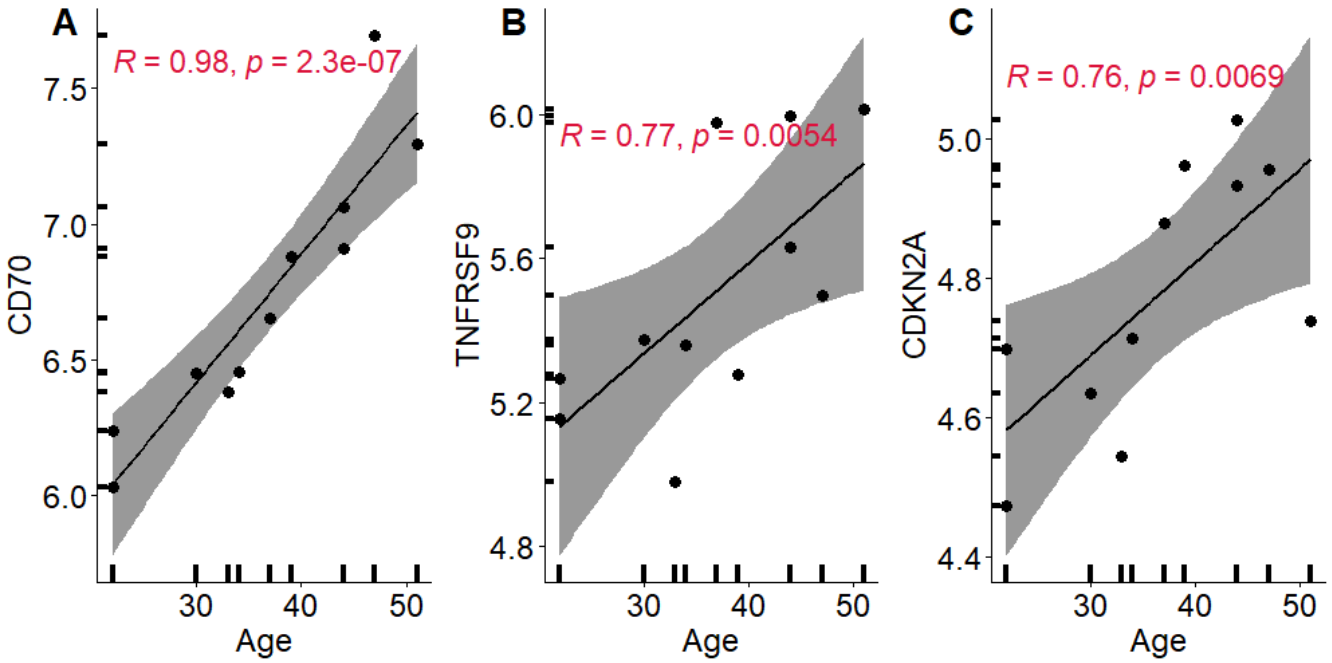
Age-related decline in TCRβ clonotype diversity in psoriasis patients. *Significant negative association between age and Shannon-Wiener diversity index in psoriasis (PV) and weaker or non-significant correlations in healthy controls (HC) across A) the complete TCRβ repertoire, B) TRBV6-4, and C) TRBV20-1 clonotypes. Spearman's correlation analysis.*

Transcriptomic signatures of MAIT cells

- **remained unaltered in psoriasis**

* the cohort primarily consisted of patients with mild disease

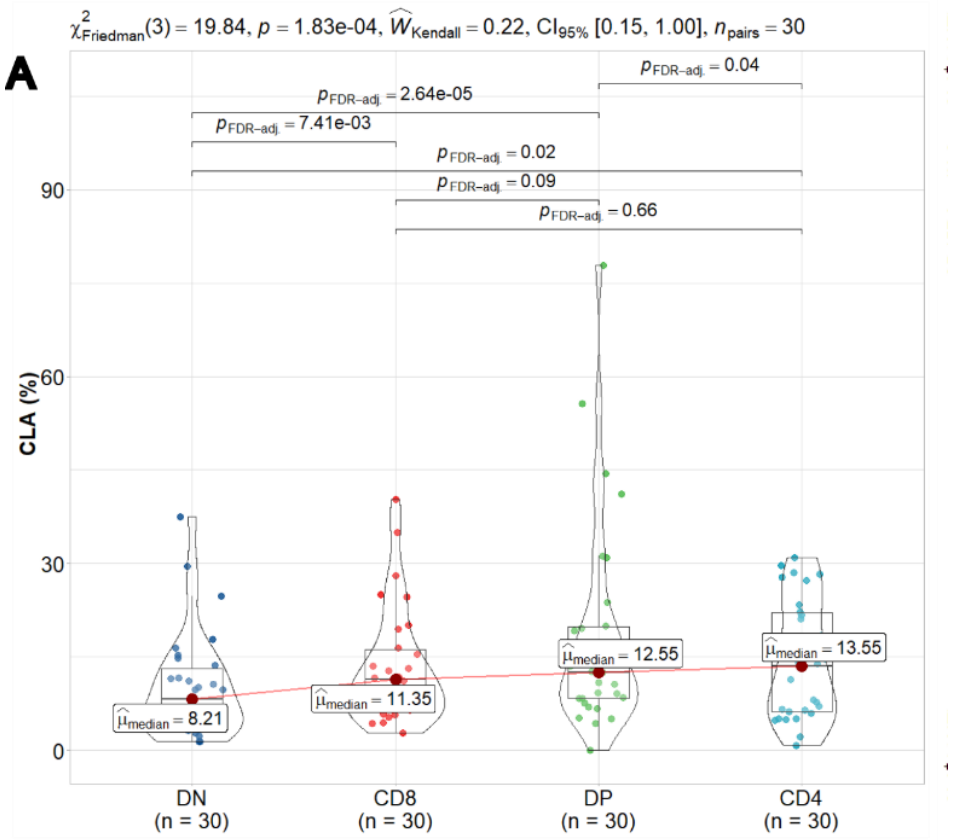
… but reveal perturbations linked to age



- **CD70** → expressed on activated immune cells
  → overexpression linked to autoimmune conditions
- **TNFRSF9 (4-1BB)** → strongly induced by TCR activation MAIT cells
- **CDKN2A** → biomarker for cellular aging
  → upregulation associated with repeated stimulation

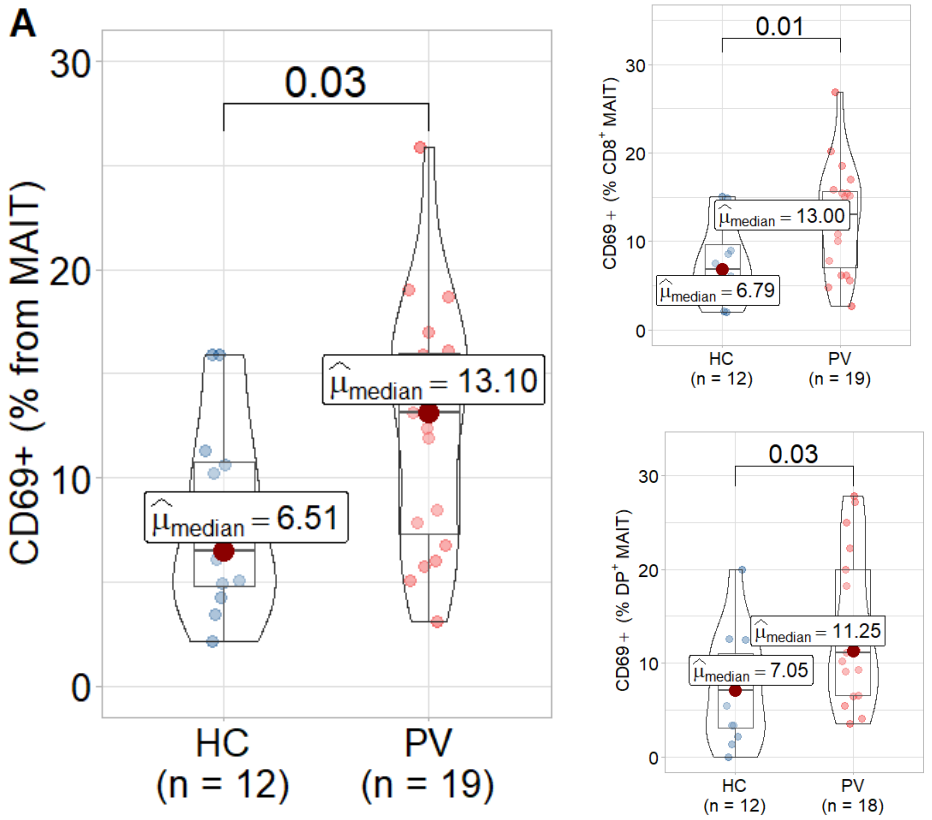
High-throughput profiling of MAIT cells

➢ Rare subsets **(CD4⁺, DP) have the highest skin-homing potential**



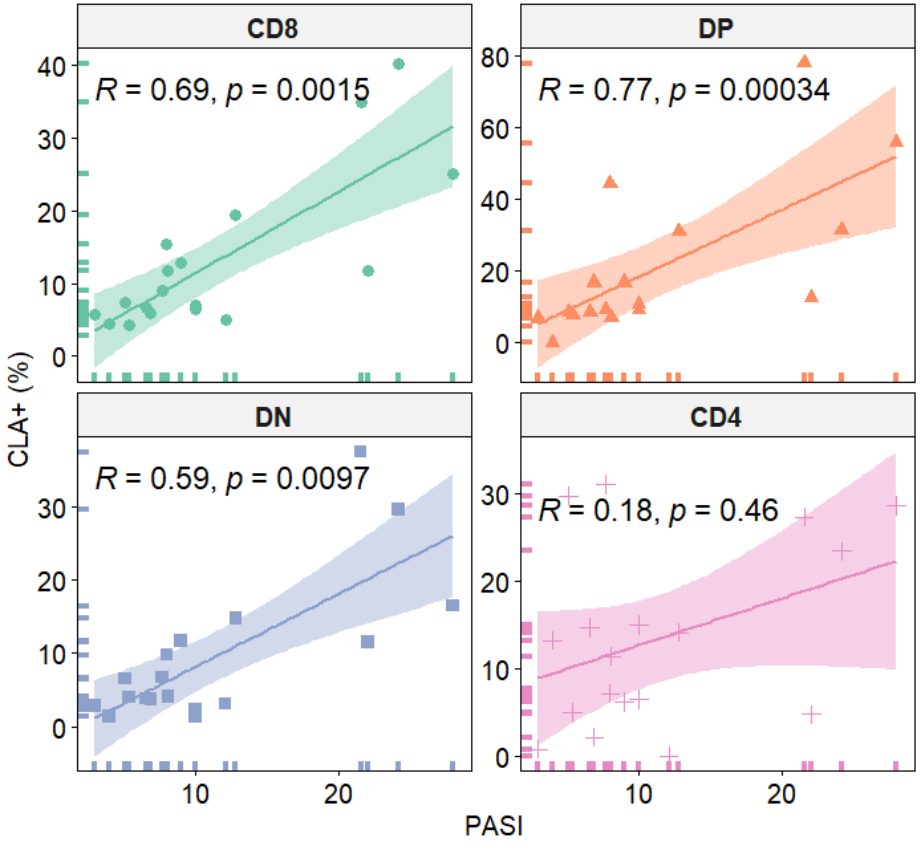
CLA expression on MAIT cells is the highest in CD4⁺ and DP subsets. *Friedman's test*

➢ **Increased** expression of **activation marker CD69** on MAIT cells in PV

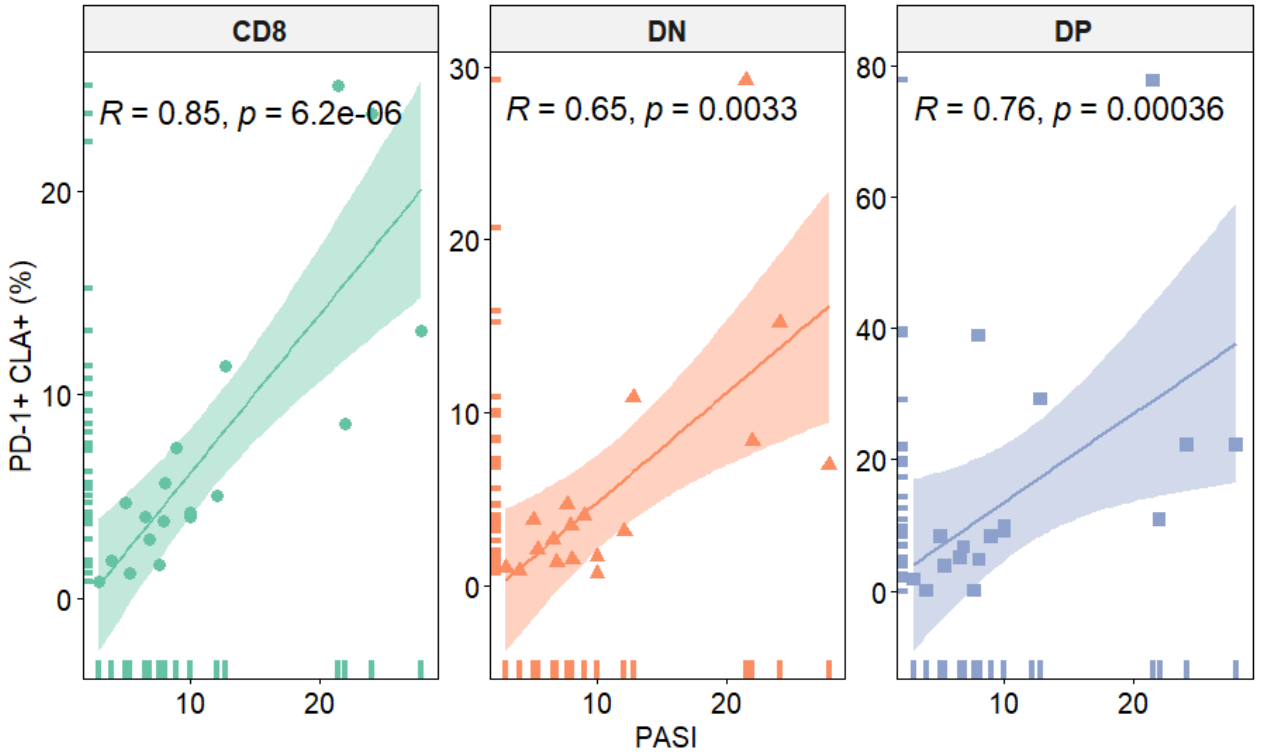


CD69 expression is elevated in MAIT cells of psoriasis patients. *Mann-Whitney U test*

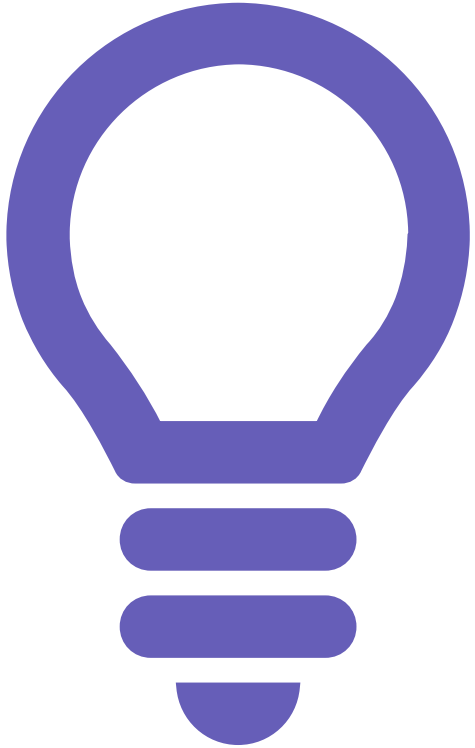
➢ the **frequency of CLA+ MAIT** cells and their expression of **immune checkpoint receptor PD-1** positively **correlate with disease severity**



The percentage of CLA⁺ cells positively correlate with PASI score in all MAIT subsets, without statistical significance for CD4⁺. *Spearman's correlation coefficient*

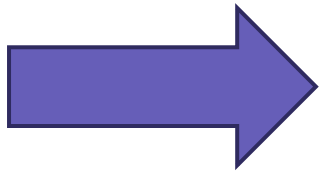


The percentage of PD-1⁺ CLA⁺ cells correlates with PASI scores in CD8, DN, and DP subsets. *Spearman's correlation coefficient*



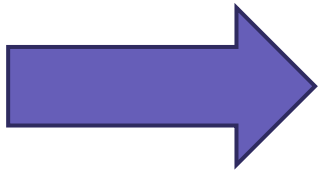
Conclusions and future directions

- disease severity- and age-associated **reshaping of TCR repertoires**
- **heightened activation states**
- **increased cytotoxic capacities**



significant contributions of MAIT and γδ T cells to disease pathogenesis

- **Heterogeneity among patients**
- **sex-associated differences**



should be acknowledged and further investigated in future studies

Remaining questions

- Does disease progression (and ageing) drive γδ T cells toward a cytotoxic phenotype with restricted TCR diversity?

- What is the response of MAIT and γδ T cells to current therapeutic interventions?
- How does disease progression influence their function and phenotype over time?

- What are the characteristics and roles of tissue-resident/infiltrating cell populations?

Future directions

CITE-seq analysis
(targeted transcriptome + TCRseq+protein markers)

Longitudinal studies

Paired blood-skin analysis

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