

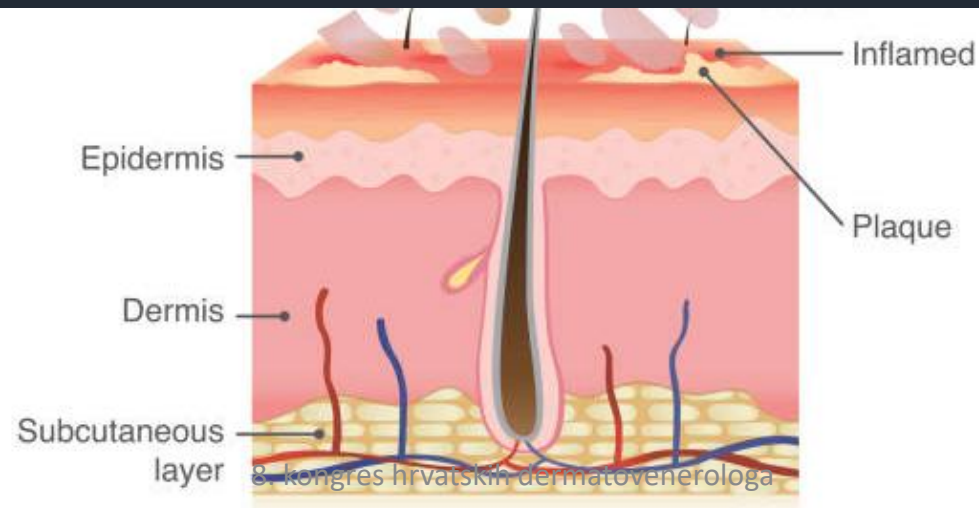
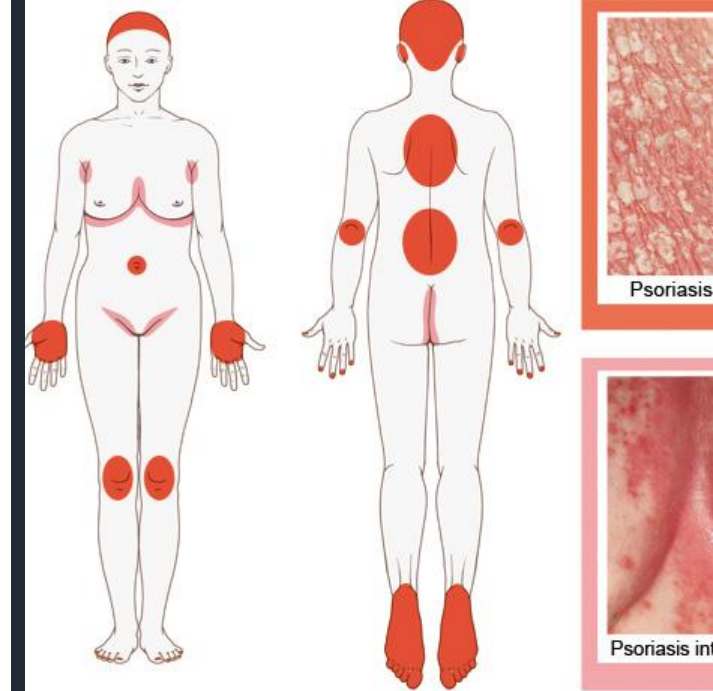


MAIT and $\gamma\delta$ T cells in psoriasis: types, roles and therapeutic potential

Stana Tokić¹, Maja Jirouš¹, Vera Plužarić^{1,2}, Marija Šola², Maja Tolušić-Levak^{1,2}, Martina Mihalj^{1,2}, Mario Štefanić¹

Psoriasis vulgaris

- a common, hard to treat autoinflammatory dermatosis that usually persists for life.
- reported prevalence ranges between 0.09% and 11.4% making psoriasis a serious global problem.
- characterized by sharply demarcated, red papules and plaques covered with silvery-white scales
- preferentially appears on elbows, knees, scalp, and the lumbar area
- considered to have autoimmune etiology but no autoantigen defined yet



PV pathogenesis



Trigger?

susceptible genetic background,
impaired skin barrier and uncontrolled
immune response



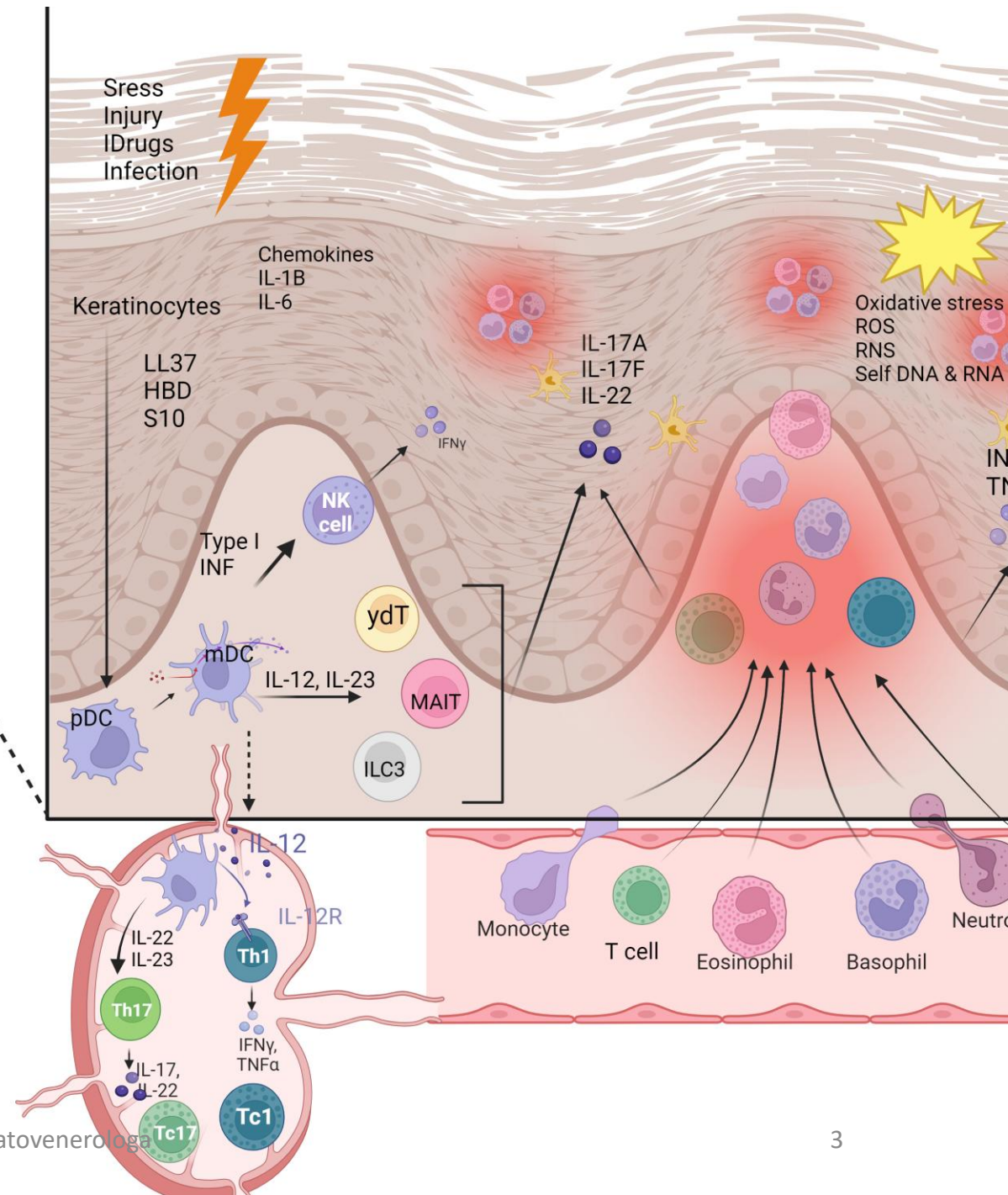
Inflammatory cascade is mounted by
innate immune cells



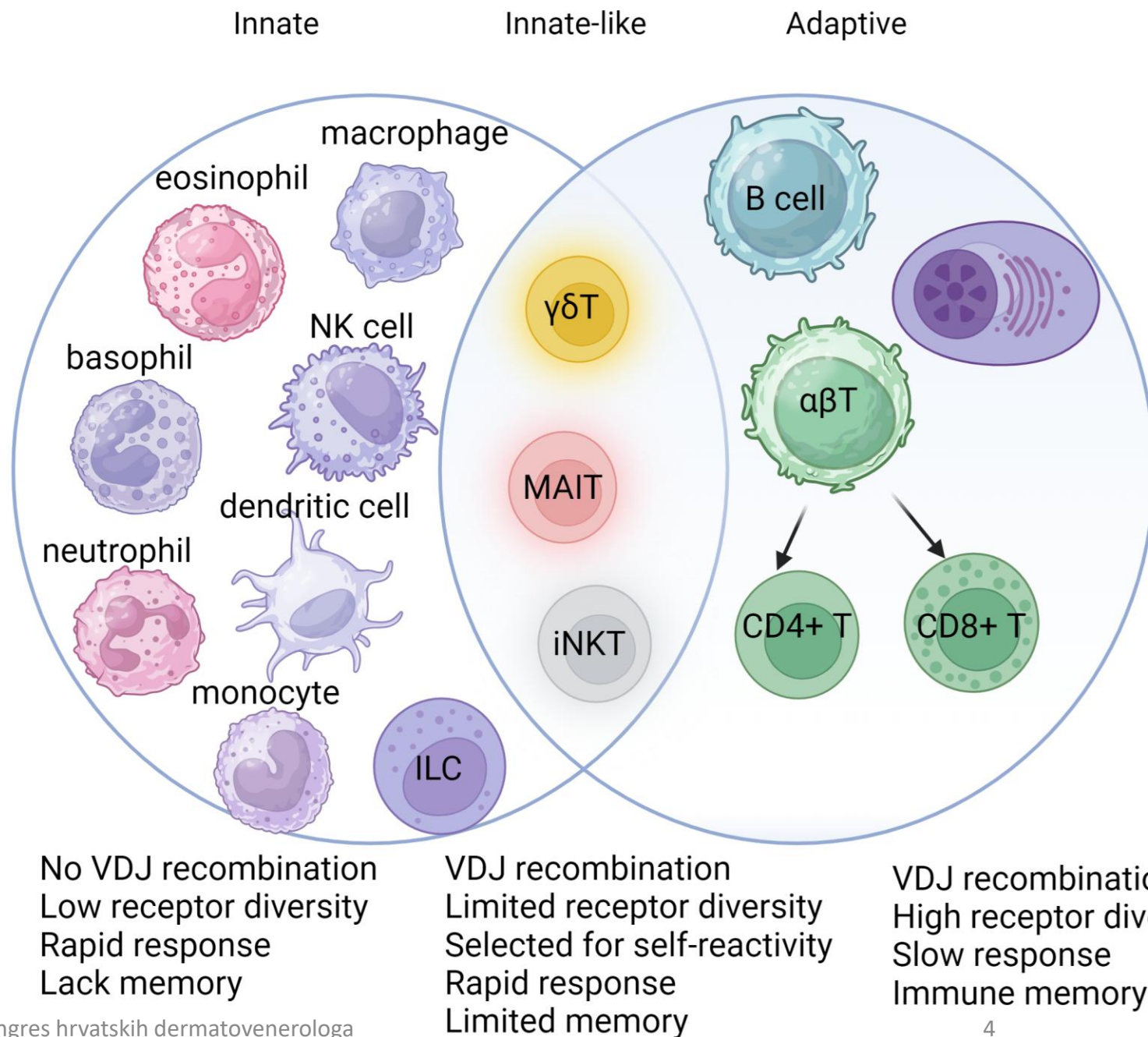
Inflammatory cytokines
mediate keratinocyte
proliferation, angiogenesis and
chemokine production

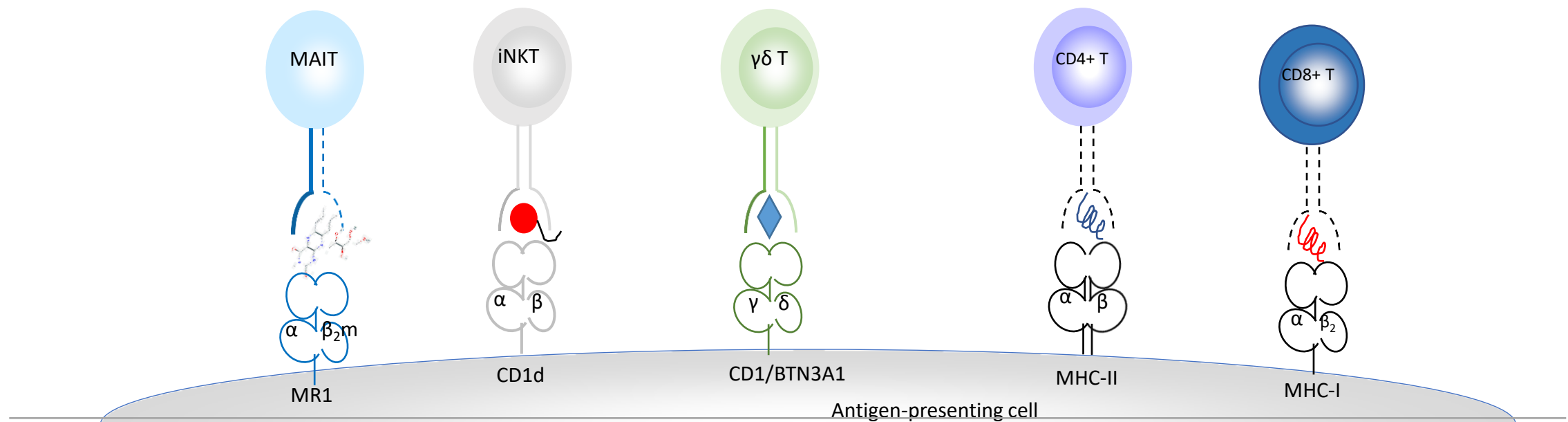


Inflammation escalates into self-
amplifying loop mediated by
adaptive immune cells



Innate-like T cells – bridge between innate and adaptive immunity

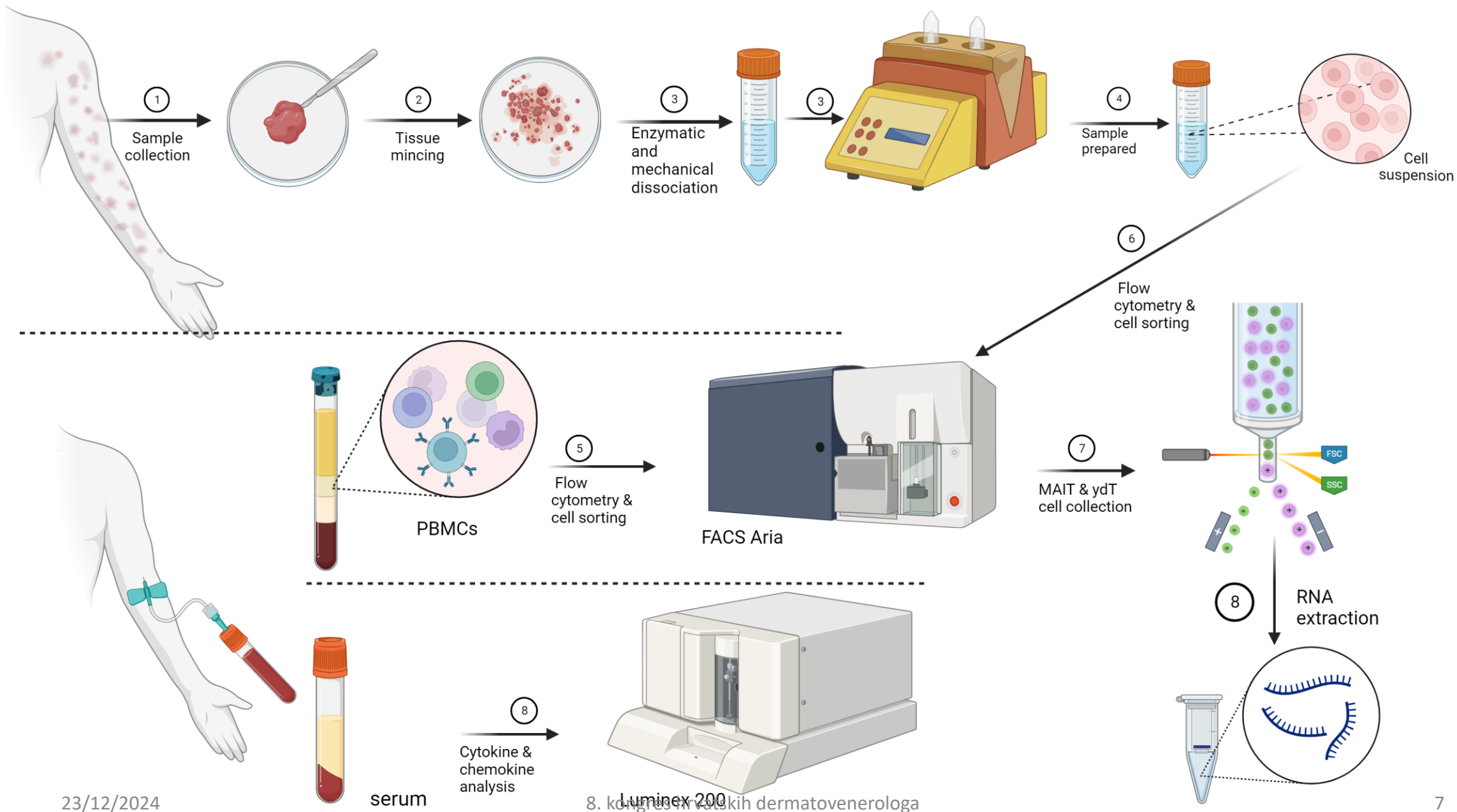




	MAIT	iNKT	$\gamma\delta$ T	CD4+ $\alpha\beta$	CD8+ $\alpha\beta$
Blood frequency	0.1-15%	0-1.1%	V δ 1: 0.25-6.2% V δ 2: 0.08-22%	10-20%	3-10%
Surface markers	CD161+++ IL-18R+CCR6	CD161+	CD161++IL-18R	Lineage dependent	Lineage dependent
Antigen (Ag)	Vitamin B metabolites	Glycolipids	Phosphorylated isoprenoids	Peptide antigens	Peptide antigens
Effector molecules	INF γ , IL-17, IL-22, TNF α , GZMB, PRF		INF γ , IL-17, IL-22, TNF α , GZMB, PRF	Lineage dependent	Lineage dependent
Ag presentation	MR-1	CD1	BTN3A1, CD1c, CD1d	MHC II	MHC I

TCR repertoire	Invariant	Invariant	Variable (V γ & V δ chain)	Variable	Variable
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How do peripheral and tissue proportions of MAIT and $\gamma\delta$ T lymphocytes change in psoriasis?



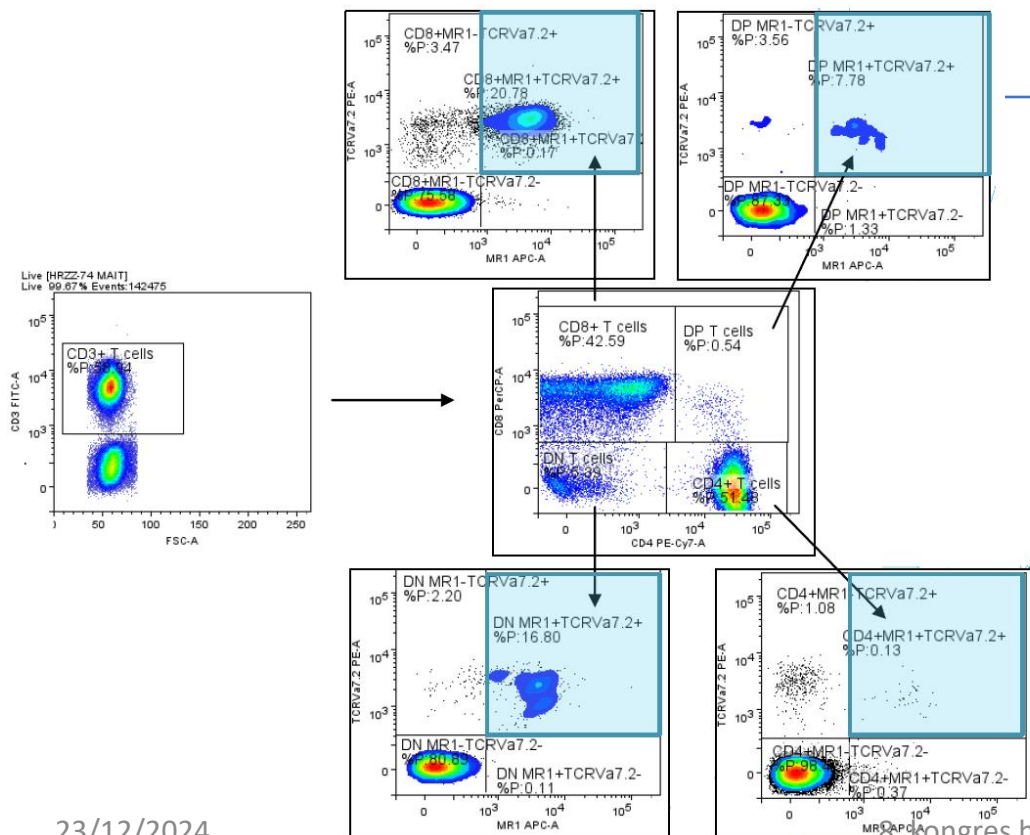


MAIT cells – frequency in blood of PV?

Differential Skewing of Circulating MR1-Restricted and $\gamma\delta$ T Cells in Human Psoriasis Vulgaris

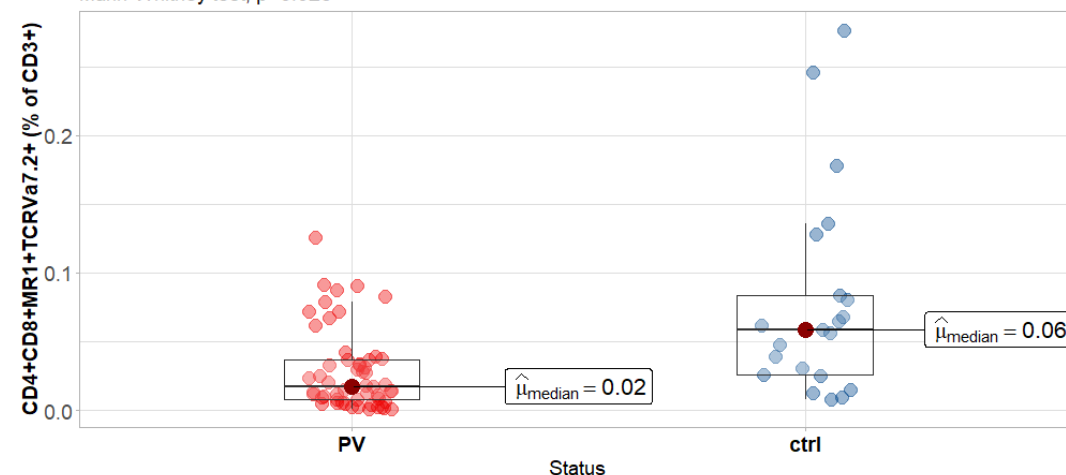
Vera Plužarić^{1,2†}, Mario Štefanić^{3†}, Martina Mihalj^{2,4}, Maja Tolušić Levak^{2,5}, Ivanka Muršić², Ljubica Glavaš-Obrovac¹, Martin Petrek⁶, Peter Balogh⁷ and Stana Tokić^{1*}

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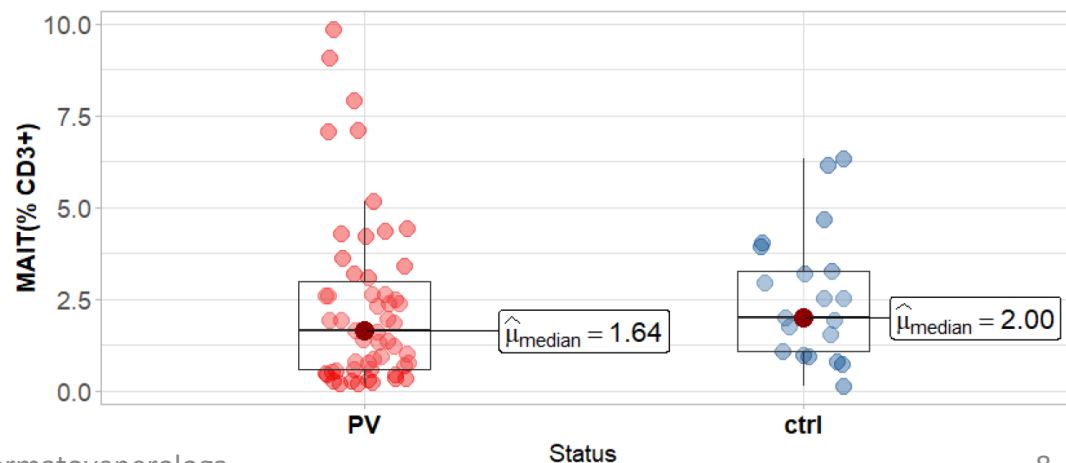
CD4+CD8+MR1+TCR γ 7.2+ MAIT cells frequencies in the blood

Mann-Whitney test; $p=0.025$



Peripheral MAIT frequencies

$W_{\text{Mann-Whitney}} = 674.00$, $p = 0.21$, $\hat{r}_{\text{biserial}}^{\text{rank}} = 0.19$, $CI_{95\%} [-0.10, 0.45]$, $n_{\text{obs}} = 75$



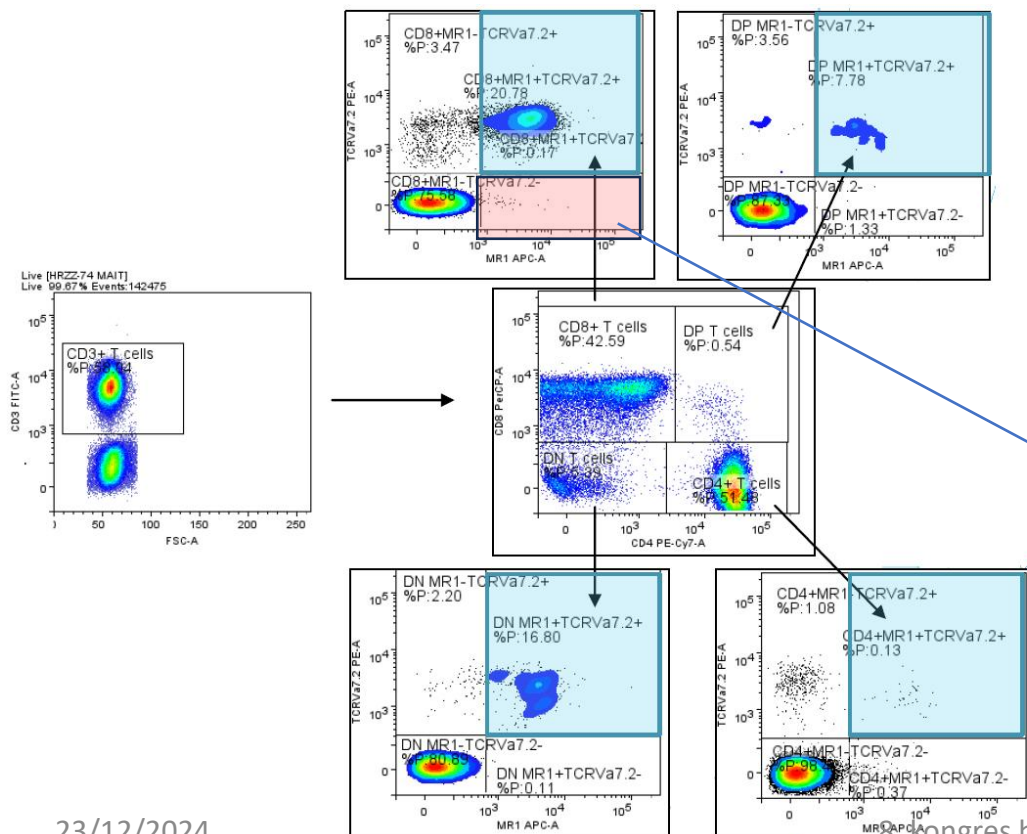


MAIT cells – frequency in skin of PV?

Differential Skewing of Circulating MR1-Restricted and $\gamma\delta$ T Cells in Human Psoriasis Vulgaris

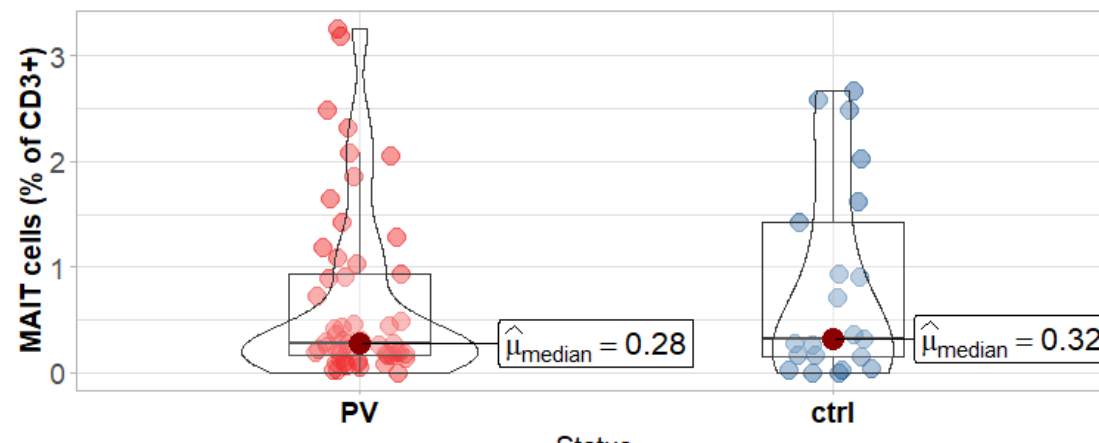
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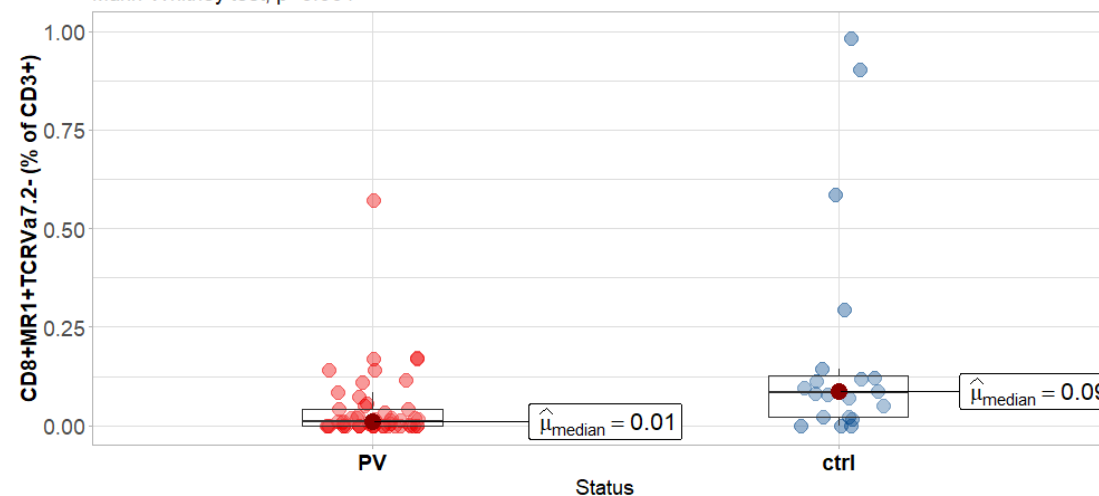
MAIT cells frequencies in the skin of PV patients and healthy controls

$W_{\text{Mann-Whitney}} = 574.50$, $p = 0.93$, $\hat{r}_{\text{rank biserial}}^{\text{rank}} = 0.01$, $CI_{95\%} [-0.27, 0.30]$, $n_{\text{obs}} = 75$



CD8+MR1+TCRVa7.2- atypical MAIT cells frequencies in the skin

Mann-Whitney test; $p = 0.001$



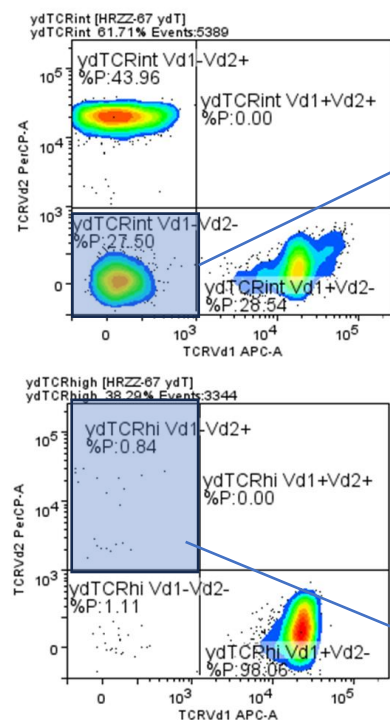
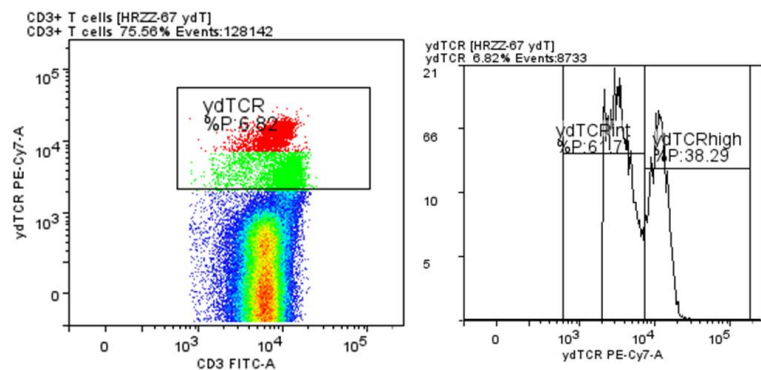


$\gamma\delta$ T cells – frequency in blood of PV?

Differential Skewing of Circulating MR1-Restricted and $\gamma\delta$ T Cells in Human Psoriasis Vulgaris

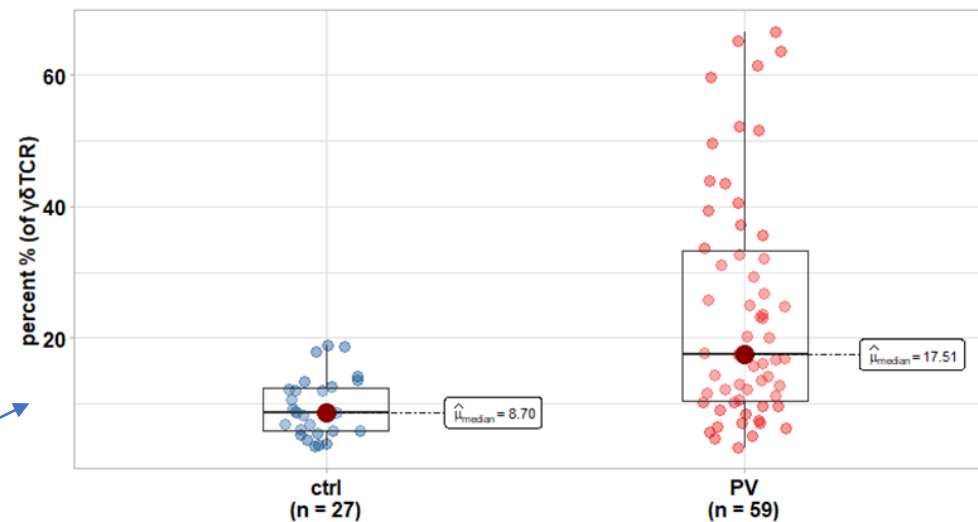
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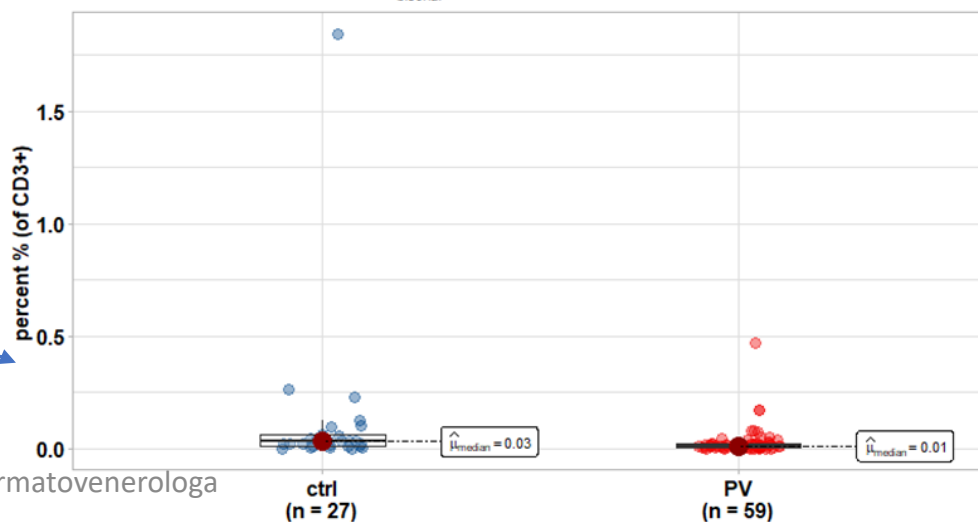
Vd1-Vd2- $\gamma\delta$ int

$W_{\text{Mann-Whitney}} = 334.00$, $p = 1.72e-05$, $\hat{r}_{\text{rank biserial}} = -0.58$, $CI_{95\%} [-0.73, -0.38]$, $n_{\text{obs}} = 86$



Vd1-Vd2+ $\gamma\delta$ hi

$W_{\text{Mann-Whitney}} = 1.1e+03$, $p = 0.005$, $\hat{r}_{\text{rank biserial}} = 0.38$, $CI_{95\%} [0.13, 0.58]$, $n_{\text{obs}} = 86$

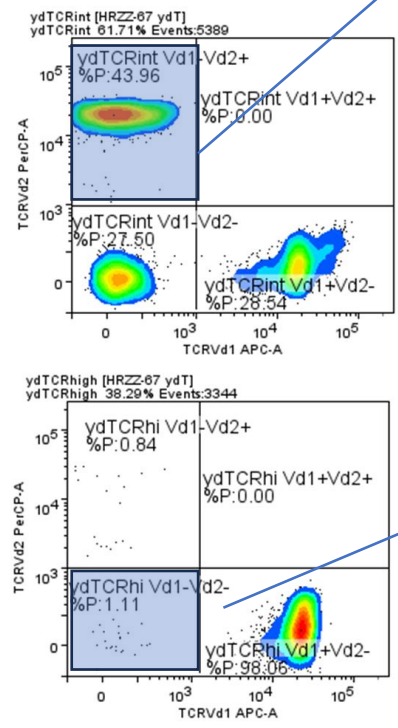
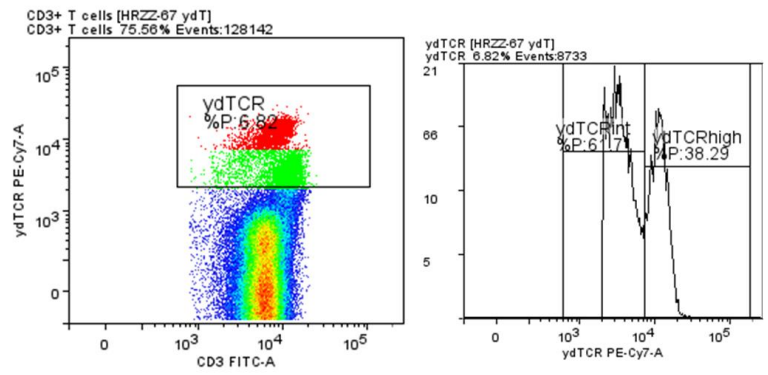




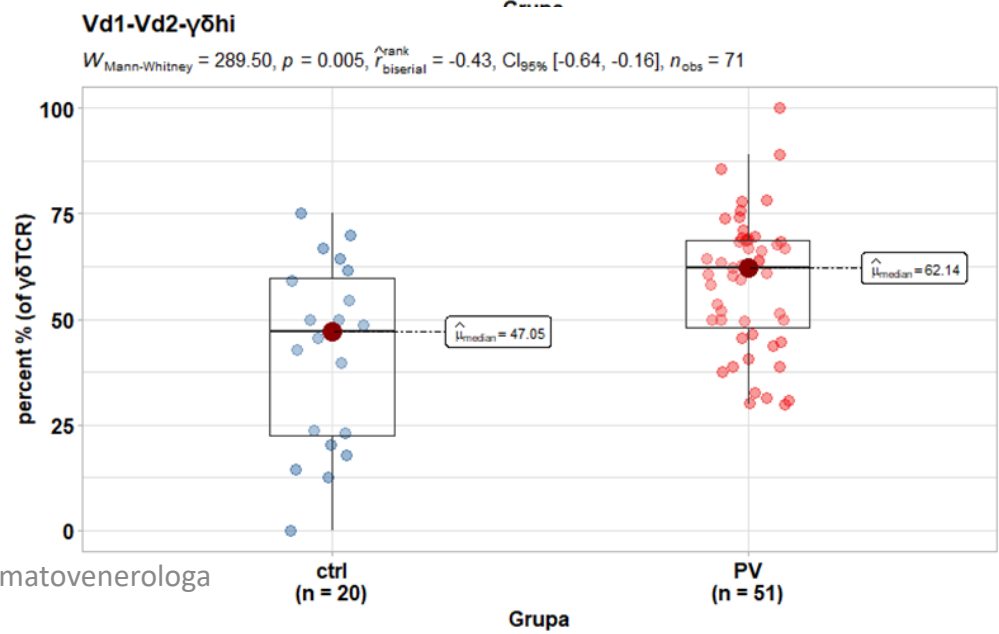
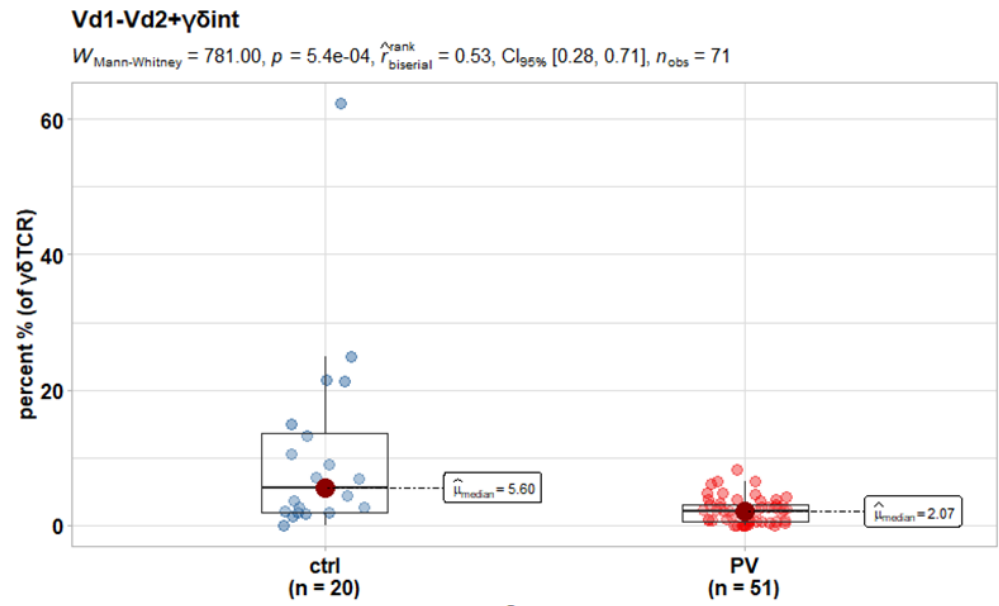
Differential Skewing of Circulating MR1-Restricted and $\gamma\delta$ T Cells in Human Psoriasis Vulgaris

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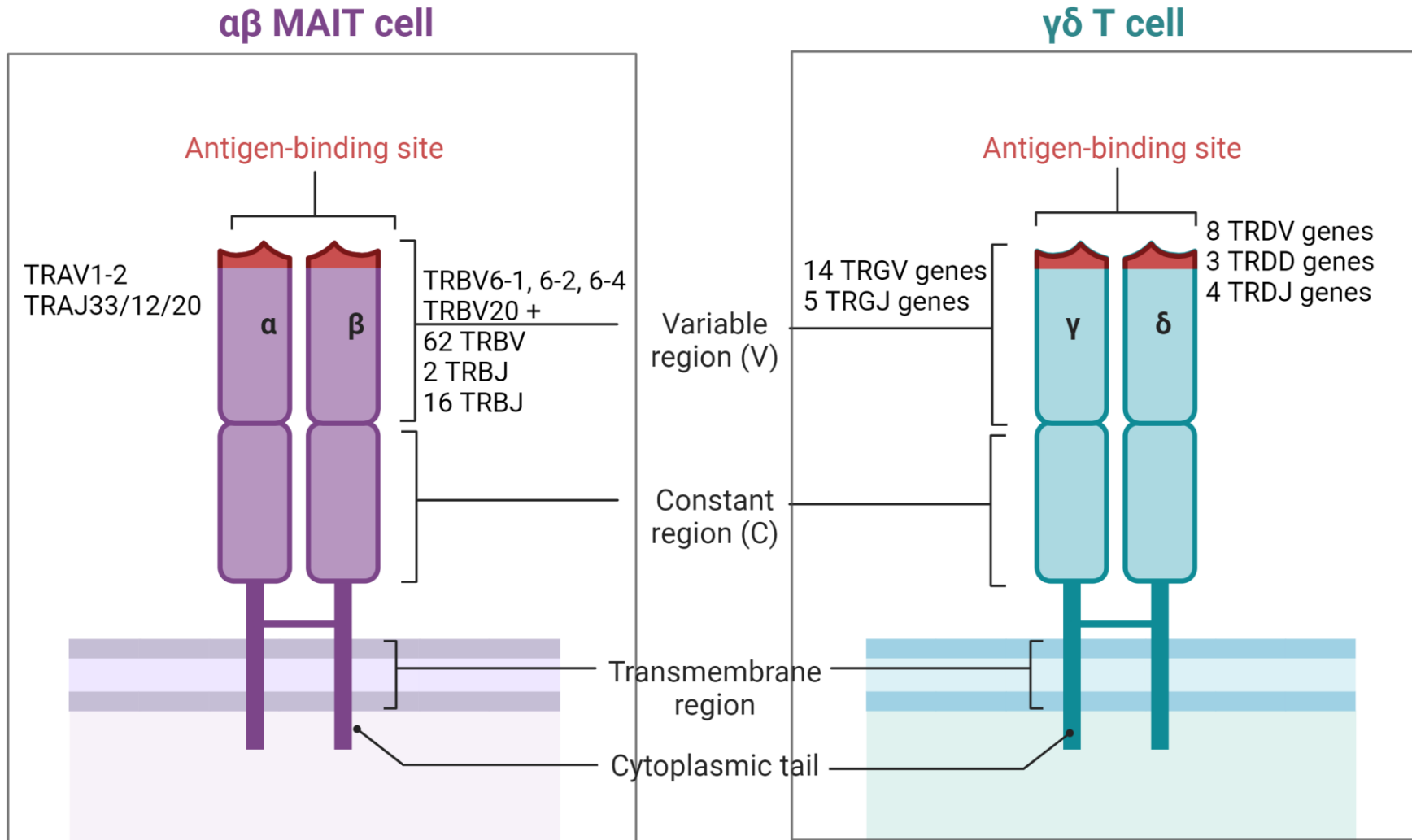


$\Gamma\delta$ T cells – frequency in skin of PV?

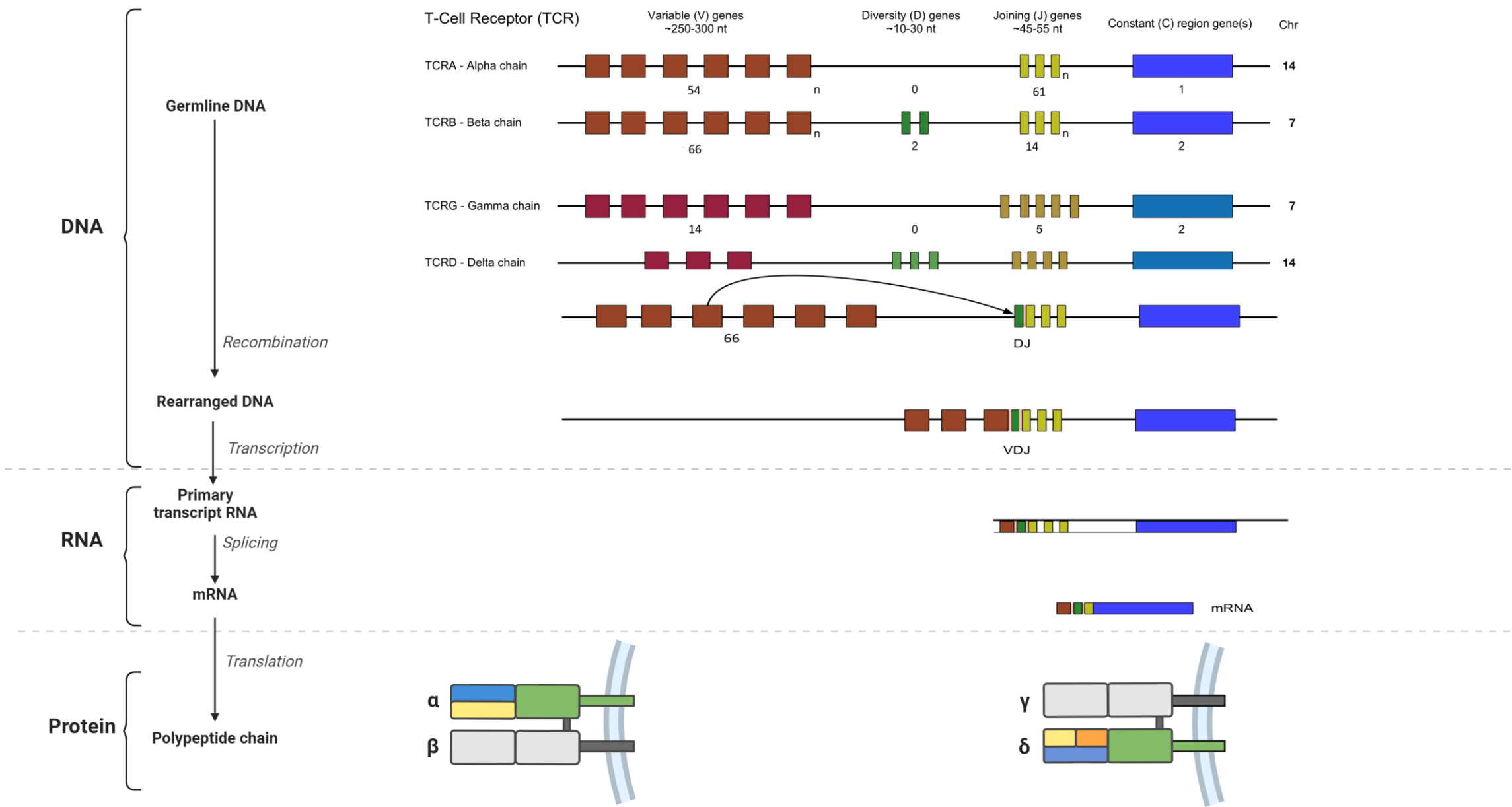


What are the molecular events that guide MALT and $\gamma\delta$ T cell compositional, functional and clonotypic rearrangement in PV?

MAIT and $\gamma\delta$ T cell receptors



TCR receptor formation



Applied NGS pipelines

TCR Seq

Immune Repertoire Plus TCR β Panel - MiniSeq

Archer Immuniverse TCR Panel - NextSeq

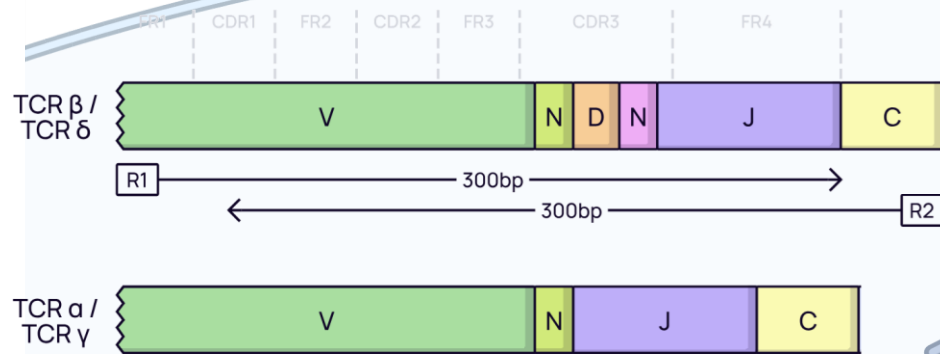
MiXCR, VDJTools, Immunarch

RNA Seq

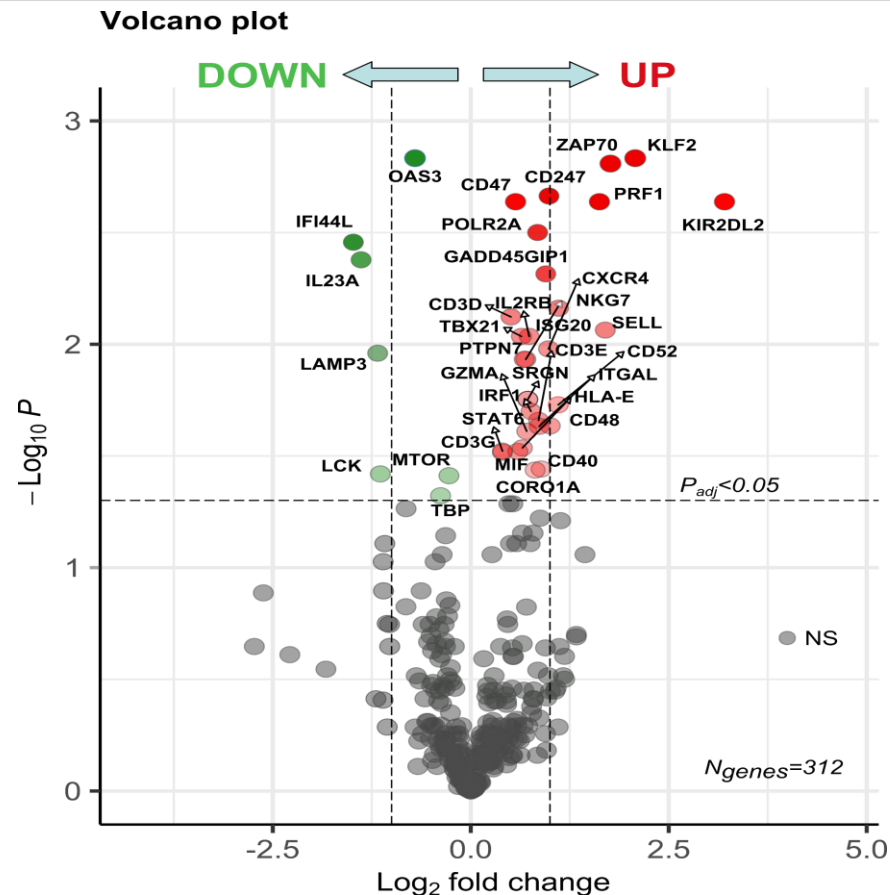
AmpliSeq Immune Response Panel - MiniSeq

BaseSpace RNA Amplicon, DeSeq2

KEGG, GSEA, STRING-db



Type I/II immunity and cytotoxicity signature genes mark transcriptional programs of peripheral $\gamma\delta$ T



Upregulated

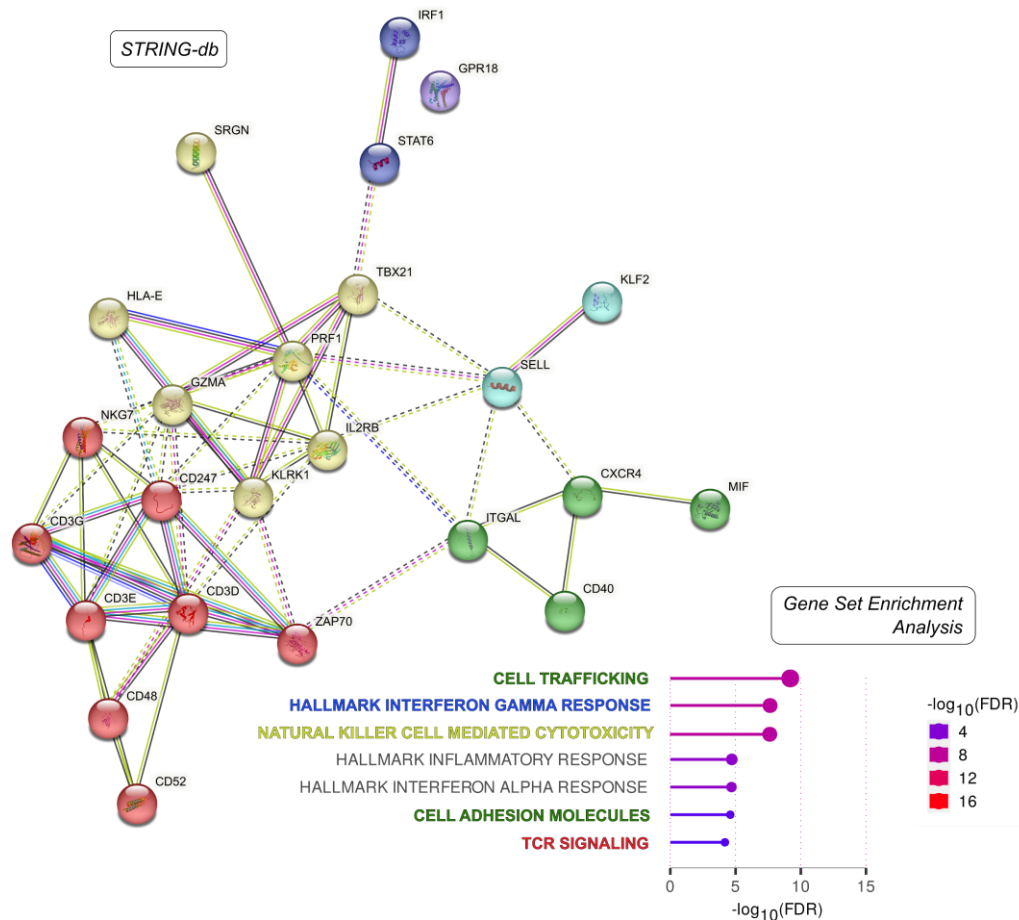
- STAT6 & TBX21 Th1/2 lineage transcription factors
- IRF1 - master regulator of interferon/IFN signalling
- IFN γ -inducible targets *ISG20*, *CD40*, *KLRK1*, *GPR18*, *IL2RB*
- genes related to cytotoxicity PRF1, GZMA, NKG7, SRGN, HLA-E
- Cell trafficking *KLF2*, *CXCR4*, *GPR18*, *MIF*, *CORO1A*
- Cell adhesion SELL, CD47, ITGAL
- TCR signalosome *CD3D/E/G*, *ZAP70*, *CD247*

Downregulated

- IFI44L - feedback regulator of IFN response
- MTOR - major nutrient-sensitive regulator of metabolism & stress
- IL23

A) The volcano plot displaying differentially expressed genes in flow sorted CD3+ $\gamma\delta$ TCR+ $\gamma\delta$ T cells of PV patients vs. healthy controls ($p < 0.05$; the log-transformed adjusted p-Value are plotted against the log-2 fold change for each tested gene). sets.

Type I/II immunity and cytotoxicity signature genes mark transcriptional programs of peripheral $\gamma\delta$ T



FDR and GSEA in PV vs control

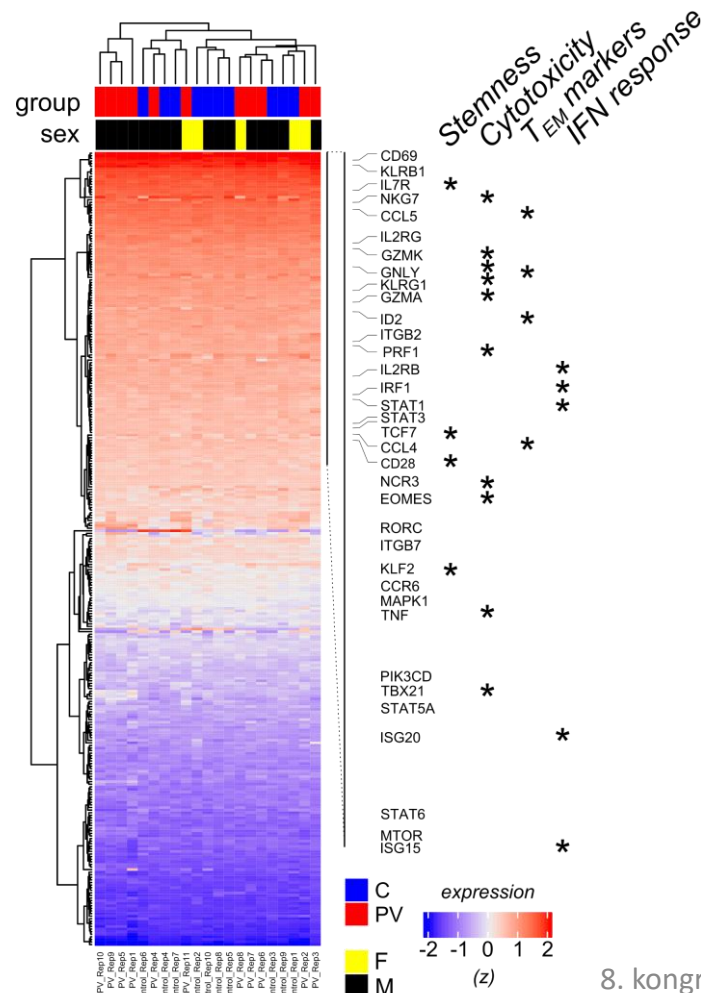
- The FDR was highest for genes related to cell trafficking and INF gamma response
- Members of the TCR signalosome coincided well with hallmark genes of NK cytotoxicity, type I immunity and INF γ response

The STRING-db output illustrating protein-protein associations between distinct functional groups of upregulated genes/proteins (solid lines connect genes/proteins within the same functional group, and dotted lines genes/proteins from different functional groups) and GSEA identifying

AGE	PASI	DLQI	CRP	BMI	GD%t	GDINT%t	GDINTVD2	GDINTVD1	GDINTDN	GDHI%t	GDHIVD2	GDHIVD1	GDHIDN
IKZF3	TP63	BST2	PMEL	ISG15	GZMB	CD247	SIT1	OAS1	PGF	ITGAX	TARP	HLA-DRA	IL2RB
FAS	FASLG	SLAMF7	HLA-DQA	PTPN6	GZMH	TBX21	IL7R	KIAA0101	LMNA	OAS1	SIT1	IL1B	CD47
CRTAM	CCNB2	ISG15	PDCD1	CD3D	G6PD	CD3D	GRAP2	IL10	FCGR3B	KLRF1	CD226	TNFSF13B	PTPN7
CDKN2A	CD70	TCF7	AMICA1	FAS	IFIT2	GZMB	CD6	MKI67	TIGIT	G6PD	GRAP2	TNFSF13B	CD3E
FCGR2B	IFIH2	MYC		CD19	CD63	CD3G	CD40LG	TLR7	KIR2DL1	CD244	LAMP1	CIITA	IRF1
FCRLA	HLA-C	LCK		CRTAM	LEXM	TARP	BTLA	CD38	IKZF2	B3GAT1	CD79B	LST1	IRF1
CD70	CBLB	CD27		CIITA	ITGAX	KIR2DL2	CD27	HLA-DRA	TYROBP	IKZF2	KLRD1	AIF1	KLRG1
KLRF1	IFI27	HLA-C		FCRLA	ITGB1	ITGAM	ITGAE	TNFSF13B	IL7	TIGIT	HAVCR2	LRG1	CXCR4
TIGIT	ZEB1	HLA-G		IL2RA	B3GAT1	NKG7	CXCR6	TNFSF13B	KIR2DL2	CD8A	PTPRCAP	IRF1	MIF
IKZF2	ID3			CD79A	CXCR2	GUSB	NT5E	IL1B	CD63	GZMH	CD160	IRF1	KLF2
IL7R				ENTPD1	CXCR2	AKT1	KLRB1	LRG1	SH2D1B	ITGB1	TLR7	ABCF1	ITGAL
SH2D1B				CD4	HAVCR2	IFIT2	SDHA	VEGFA	PTPN7	IL7	IKZF2	CD48	GATA3
HLA-G				TIGIT	TNFRSF18	SRGN	GZMK	VEGFA	PDCD1LG2	KRT5	HLA-DMA	MIF	GADD45
ISG15				IKZF2	TBX21	ITGAL	CD28	FOXM1	CRTAM	CD63	HLA-DMA	HLA-E	ZAP70
TNFRSF14				TNRRSF14	KRT5	PRF1	MYC	IL1A	NCR1	LILRB1	FCRLA	CD3E	CD40
CD6				ITGAE	AKT1	PTPN7	RORC	CD244	TP63	M6PR	CRTAM	SIT1	CD48
CTLA4				IKZF1	NCR1	HAVCR2	RORC	PTGS2	ITGAE	SKAP2		LAPTM5	LAPTM5
PDCD1					GNLY	MIF	CCR2	C1QB	LST1	FCGR2B		HMBS	C10ORF54
IL7R					ISG15	TNFRSF14	ZEB1	TNFRSF9		ITGB7		KLRB1	MS4A1
LCK					CD244	KLRD1	HLA-F-AS1	IL6		GZMB		CD6	TYROBP
CD27					ITGB2	TGFB1	PIK3CD	CD68		CXCR2		KLRG1	HMBS
					BATF	IRF1	CD226	CD1D		CXCR2		ITGAL	ISG20
					KLRF1	IRF1	IFITM2	CDKN3		IRF4		IL2RB	PTPRC
					OAS1	GZMH	CD79B	CCNB2		IFNG		CD70	GZMK
					IFI35	KIR2DL3	KLRG1	SKAP2		LEXM		CD70	EOMES
					CD97	GNLY	HLA-G	CSF1R		CMKLR1		CD247	HLA-E
					ITGB7	KLRK1	HLA-G	FCGR1A		ICOS		CD47	CORO1A
					IKZF2	LAPTM5	LY9	CD40LG		TARP		CD3D	HLA-F
					KIR2DL2	CD52	CCR5	CD48		CD27		GRAP2	NCR3
					LILRB1	CD52	LRG1	CD27		TNFAIP8		CD79B	CD27
					CD3D	HLA-E	IFIT3	MYC		CTLA4		PTPN7	CCL5
					CD47	FASLG	FASLG	C10ORF54		MMP9			IL10RA
					CD8A	IL2RB	FASLG	SDHA		GRAP2			IRF9
					HLA-DOB	IFIT2	IFIT2	CD28		GZMK			SRGN
					LCK	GZMH	GZMH	MIF		IGF1R			PRF1
					KLRD1	IL23A	FCRLA	NT5E		CXCR6			IFIH1
					SIT1	CXCL1	EGR2	CXCR6		PDCD1			MAPK14
					AIF1	CCR7	TLR7	LAPTM5		CD40LG			NKG7
					IRS1	HLA-DMB	MELK	MS4A1		CCR2			ADORA2A

Different $\gamma\delta$ T cell subsets and clinical features associate with different gene profiles

MAIT cell transcriptome is enriched in cytotoxic and memory-effector markers



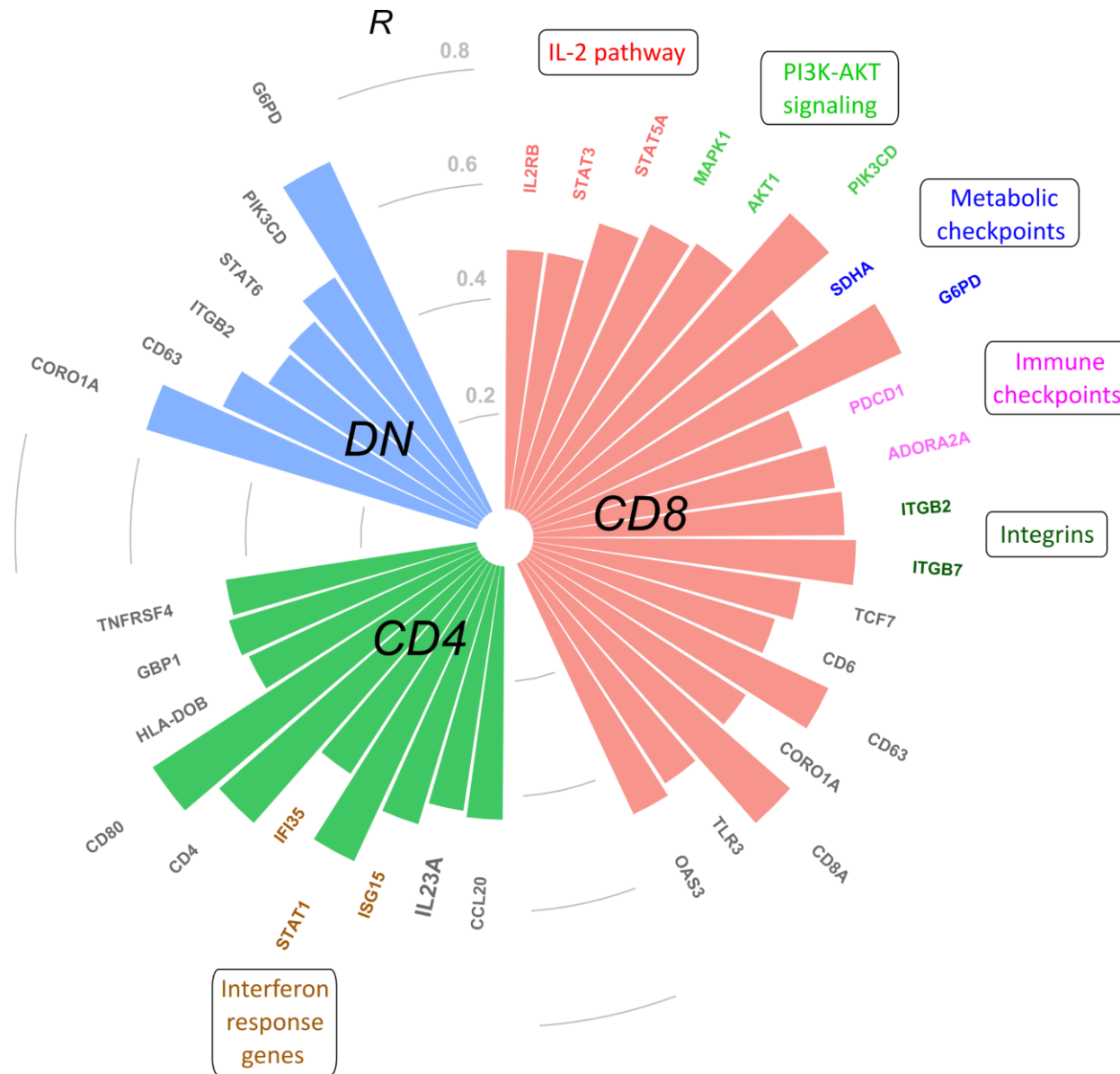
Upregulated

- KLRB1 (CD161)
- stem-like genes (TCF7, IL7R, CD28)
- cytotoxicity (NKG7, GZMK, GNLY, KLRG1, GZMA, PRF1, EOMES)
- effector-memory markers (ID2, GNLY, GZMK/A, CCL4/5)
- IFN response (IL2RB, IRF1, STAT1)

Downregulated

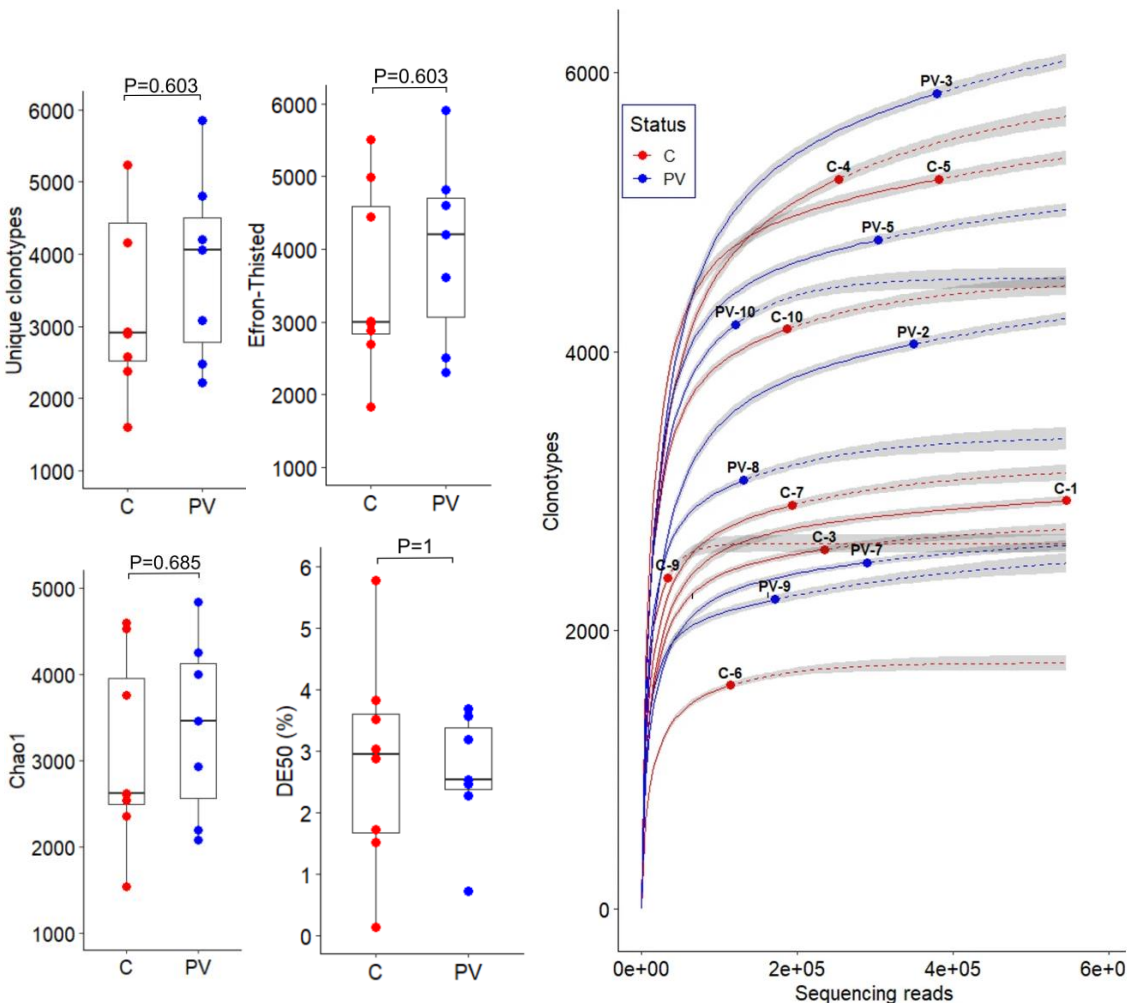
- Th1 lineage transcription factor – TBX21
- IFN response (ISG20, ISG15)

CD4+ and CD8+/DN MAIT cells exhibit different gene expression profiles



Characterization of the TCR β repertoire of peripheral MR1-restricted MAIT cells in psoriasis vulgaris patients

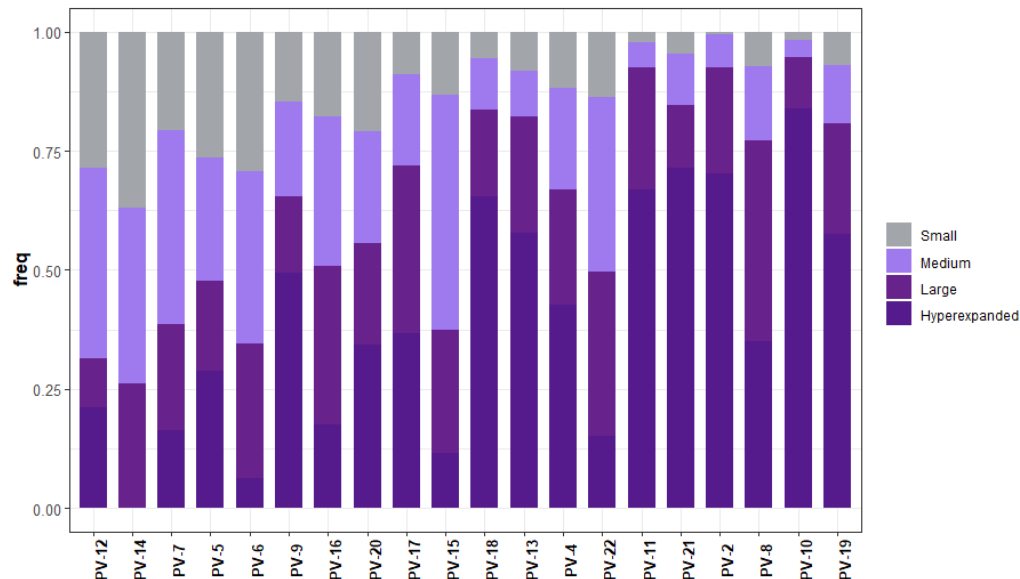
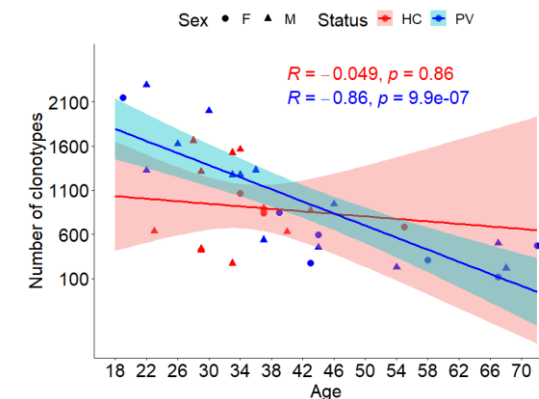
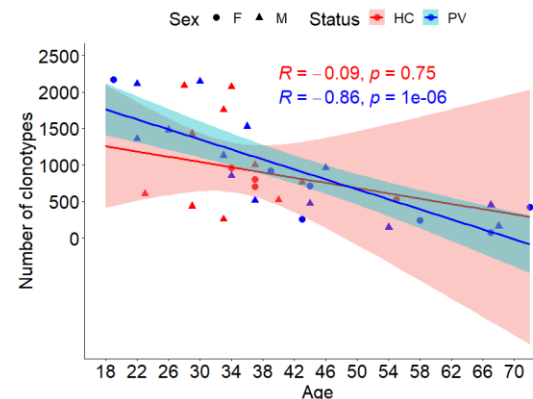
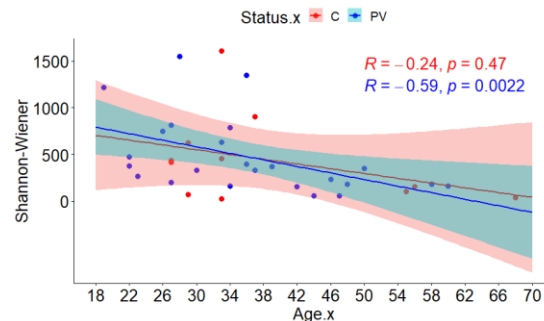
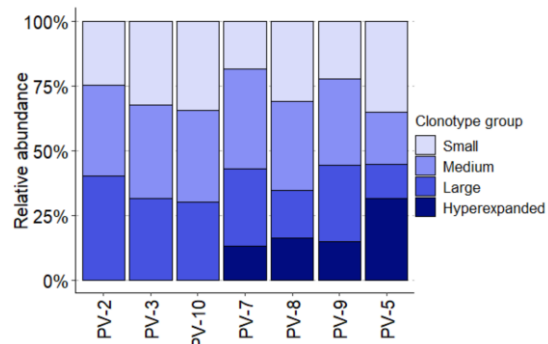
Maja Jirouš Drulak^{1*}, Zvonimir Grgić², Vera Plužarić^{2,3}, Marija Šola³, Teuta Opačak-Bernardi¹, Barbara Viljetić¹, Kristina Glavaš⁴, Maja Tolušić-Levak^{3,5}, Vlatka Periša⁶, Martina Mihalj^{3,7}, Mario Štefanić^{8*}, Stana Tokić^{2*}



The peripheral MAIT and $\gamma\delta$ TCR repertoire of PV patients is highly diverse and individually unique

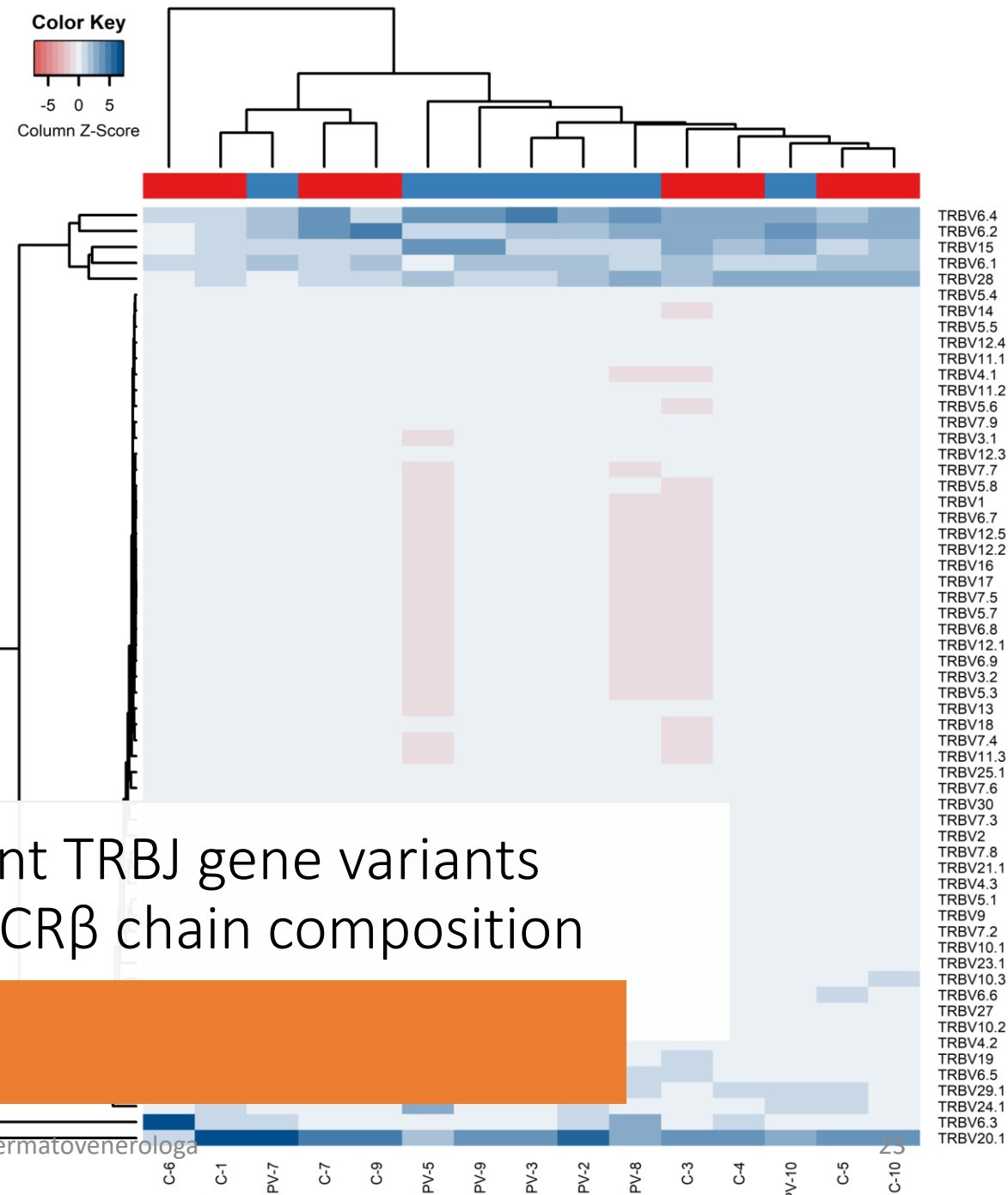
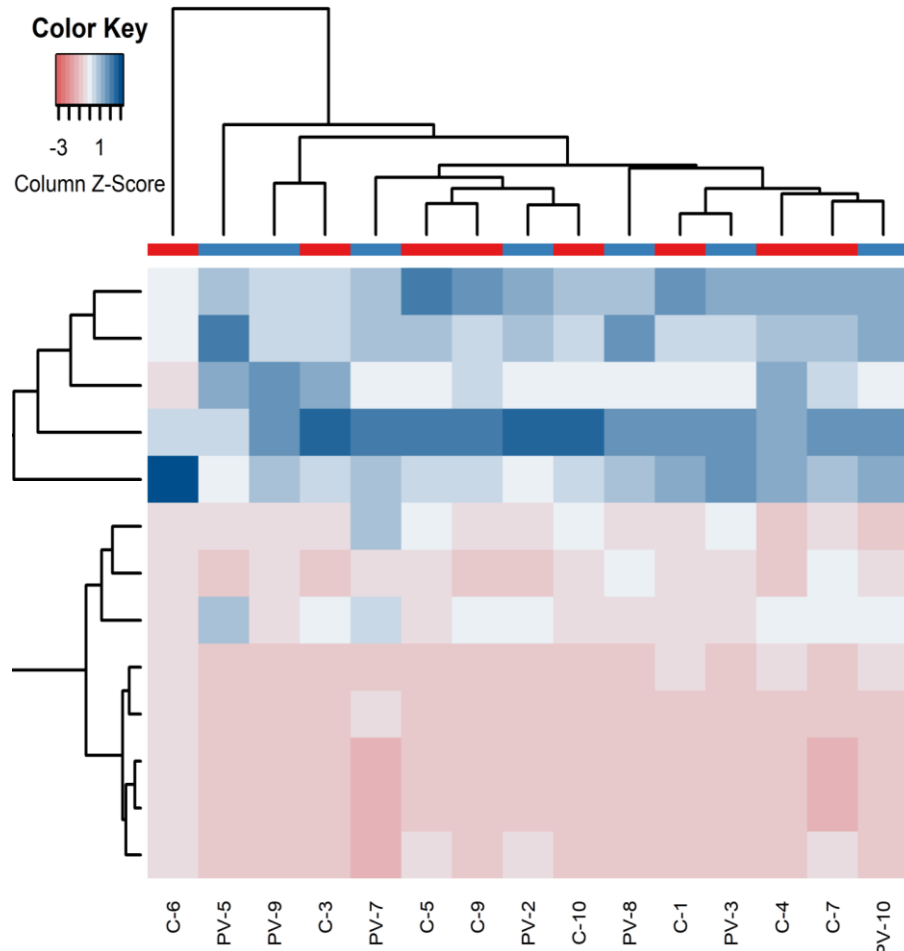
Low-frequency clones in both PV patients and healthy controls are similarly distributed (Efron-Thisted, Chao1).

The α -, β -, γ -, and δ -TCR repertoires were highly private and individually unique, with small public clonotype content, and high CDR3 amino acid length variability in both subject groups.



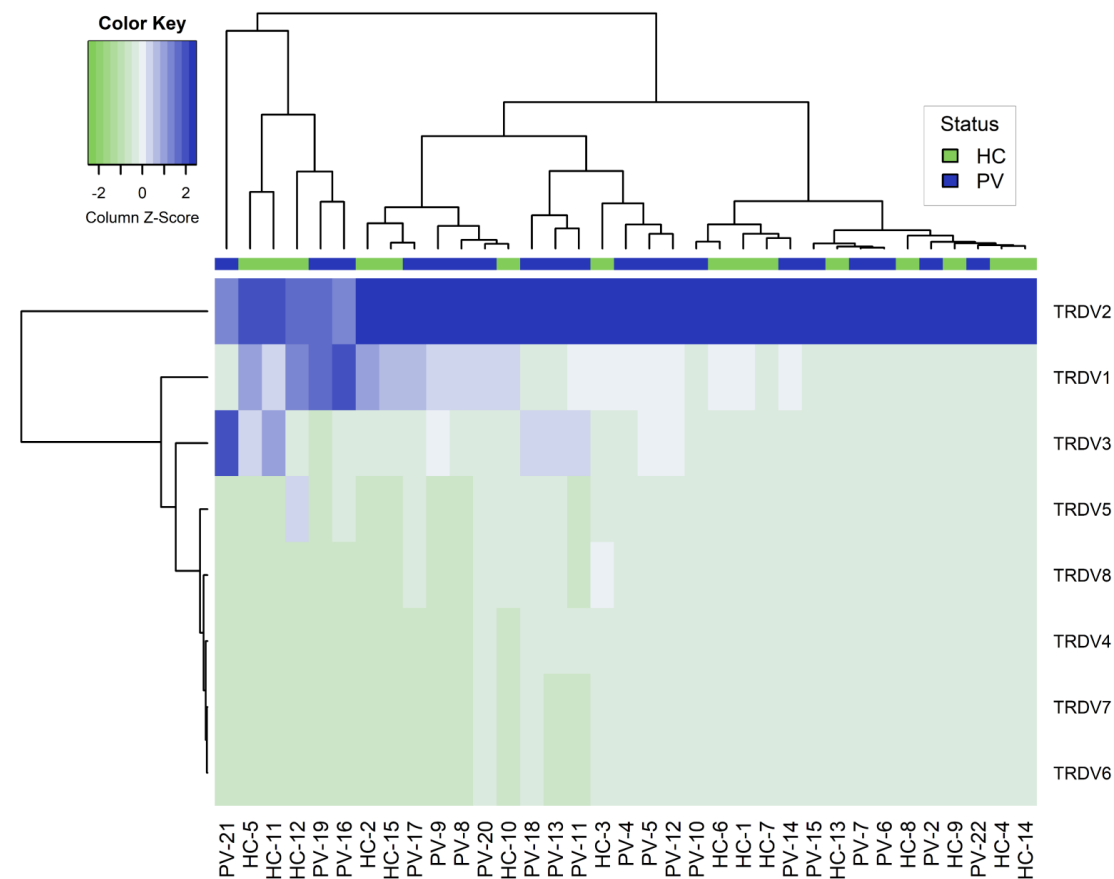
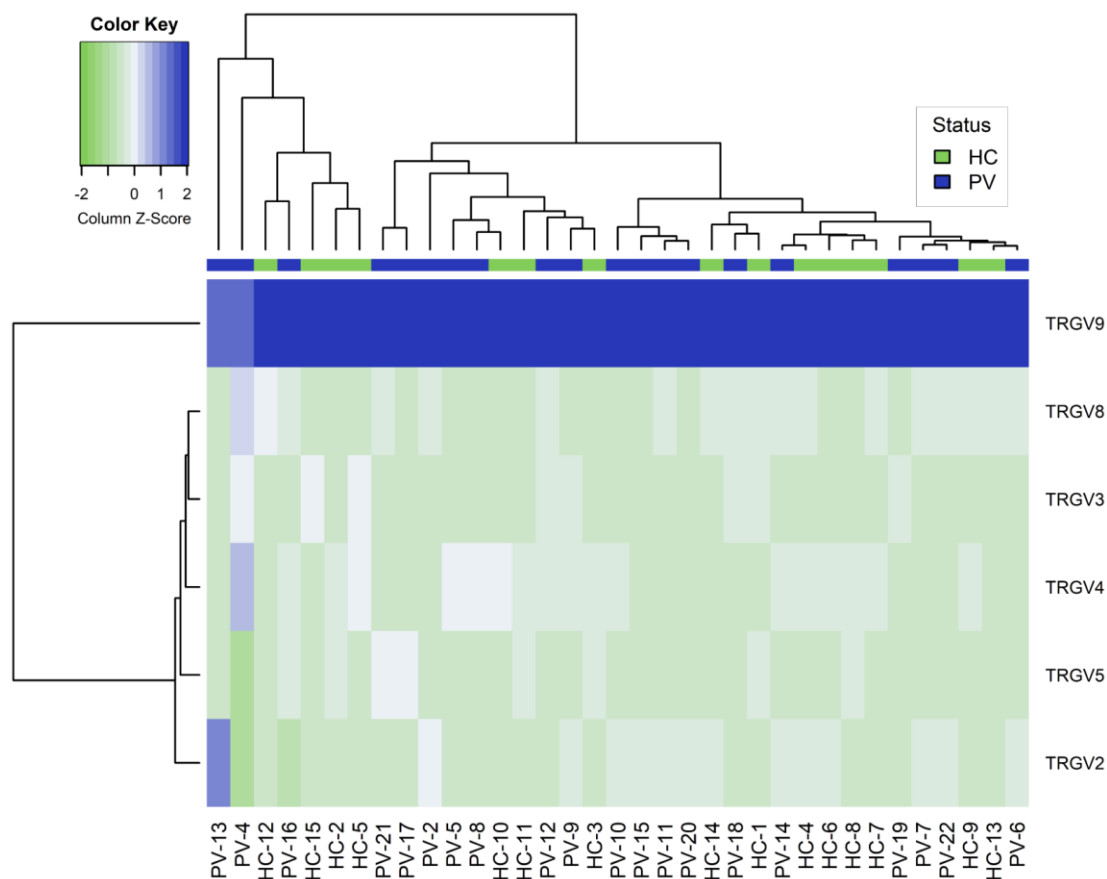
Age and PASI negatively correlate with TCR repertoire diversity of peripheral MAIT and $\gamma\delta$ T cells

- Increasing age and PASI were identified as negative predictors of TRB, TRD and TRG clonotype number and diversity in PV, but not healthy controls, an effect dominantly driven by an increase in hyperexpanded clonotype compartment.



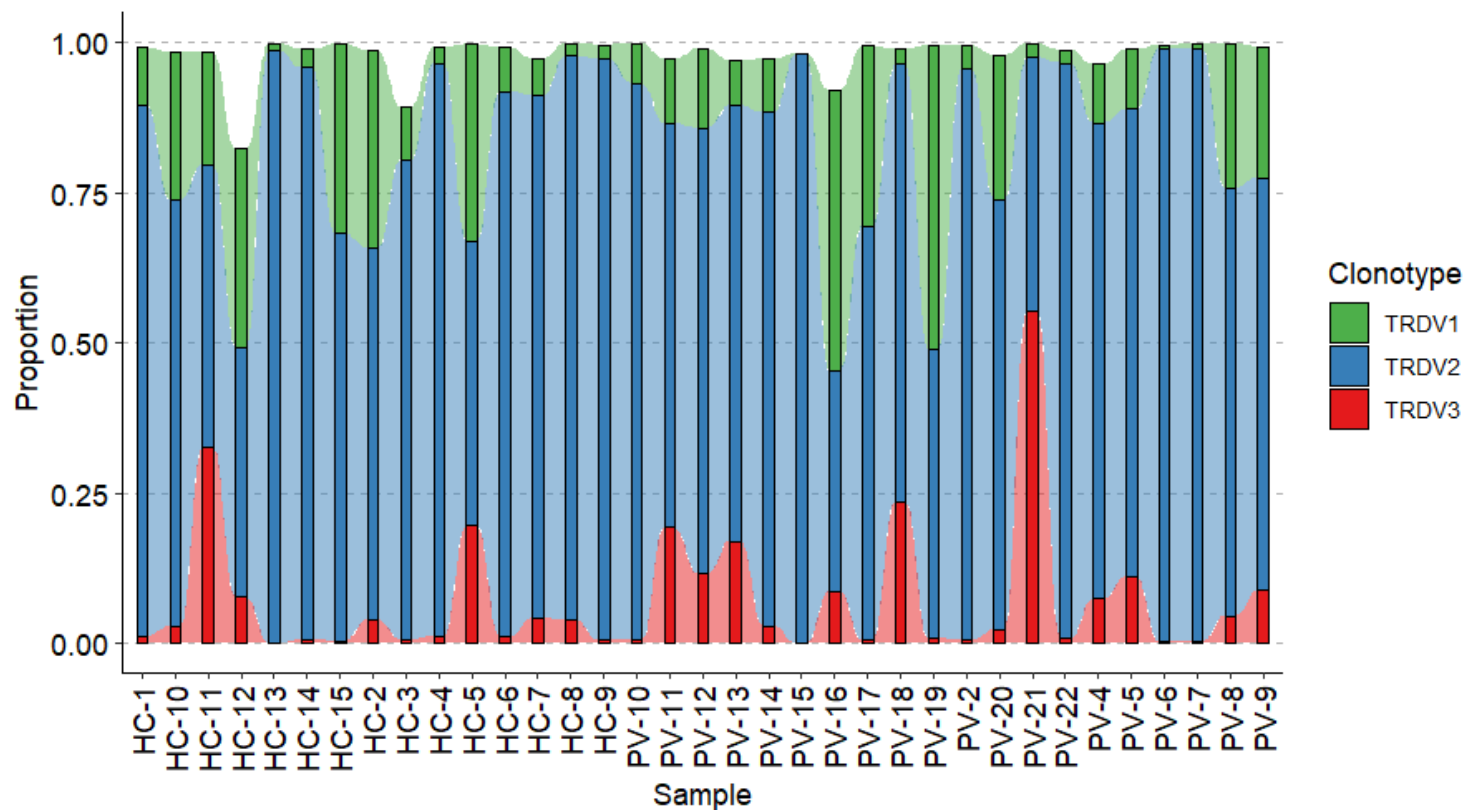
59 TRBV and 13 different TRBJ gene variants participate in the MAIT TCR β chain composition

8 TRDV and 6 different TRGV gene variants participate in the $\gamma\delta$ TCR chain composition

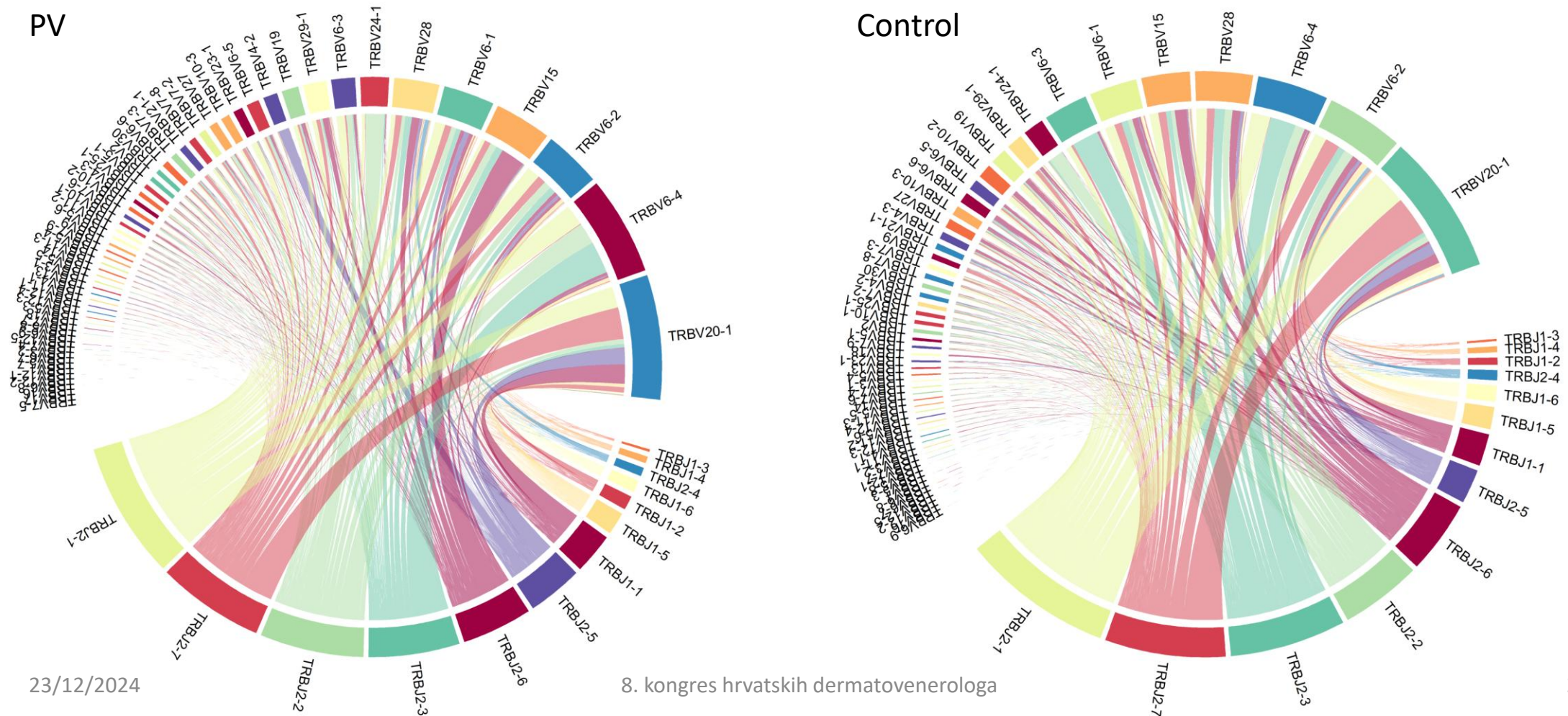


TRDV1 and TRDV3 clonotype compartments are expanded in PV

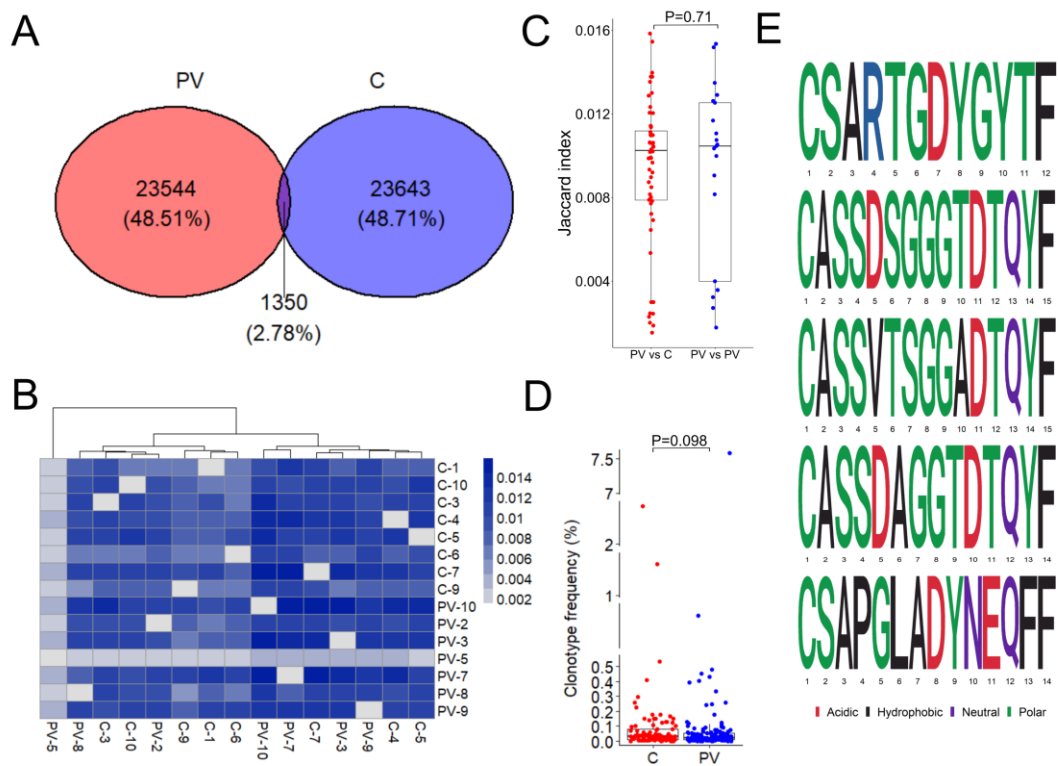
- TRDV1, TRDV2 and TRDV3 gene variants accounted for the majority of the TRD repertoire, with no significant differences in case-control comparisons
- The variety of TRDV1 and TRDV3 clonotype compartments is altered in PV, reflecting TRDV1 clonotype expansion (Inverse Simpson Index; median (Me(PV vs. CTRL): 3.71 vs. 7.20, $p=0.037$, Mann-Whitney test) and reduced TRDV3 clonotype heterogeneity (Shannon-Wiener Index; 3.91 vs 9.85, $p=0.036$).



The MAIT TCR β repertoire of PV patients is skewed toward TRBV6-4/TRBJ2-3, TRBV20-1/TRBJ1-1 and TRBV15/TRBJ2-6 gene associations



The public repertoire of MAIT cells comprises unique clonotypes corresponding to blood and skin TCR β collections of PV patients

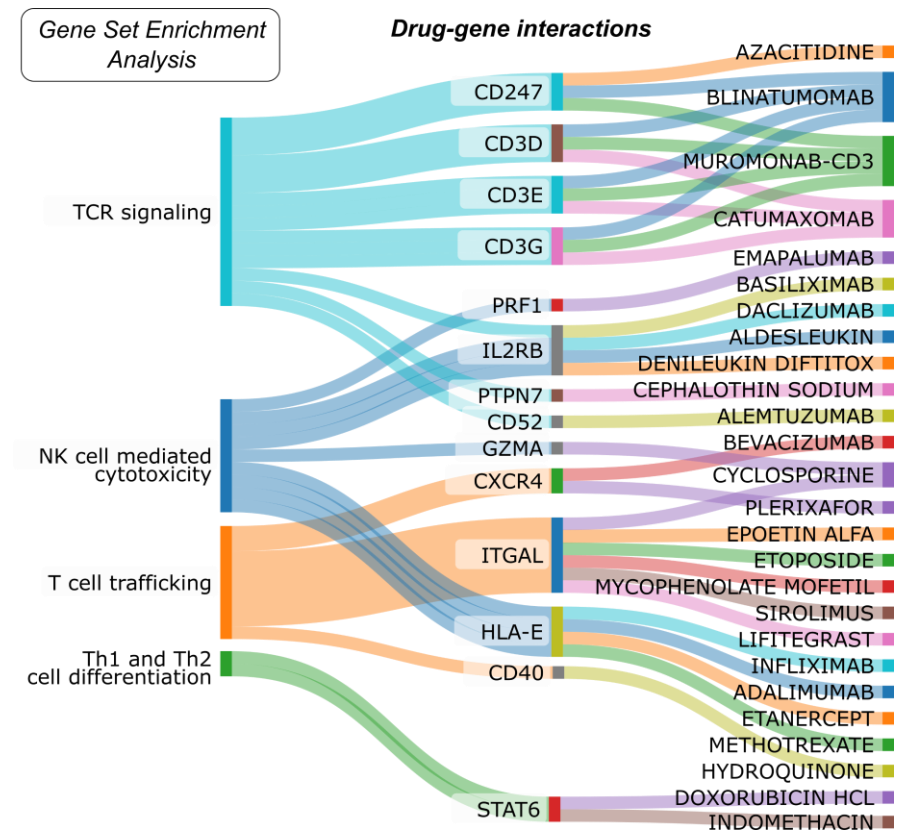
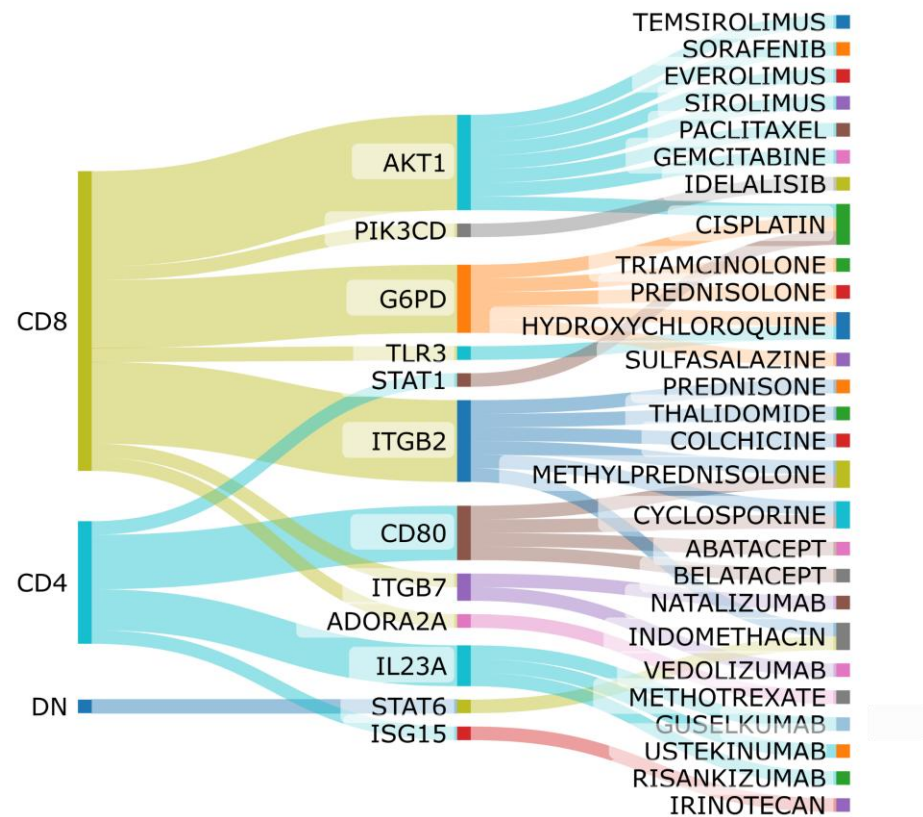


In PV vs. healthy comparison of public MAIT TCR β clones a total of 420 healthy- and 336 PV-unique clonotypes were shared within, but not between groups, with eight being frequently shared ($\geq 4/7$) in healthy, and five in PV.

PV-unique clonotypes were matched with previously published blood, non-lesional and lesional skin TCR β repertoires of PV patients, supporting their diagnostic and therapeutic relevance.

What is the therapeutic relevance of identified genetic targets?

MAIT and $\gamma\delta$ T cells are enriched in druggable molecules increasing the likelihood of off-target effects in patients receiving biological agents.





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