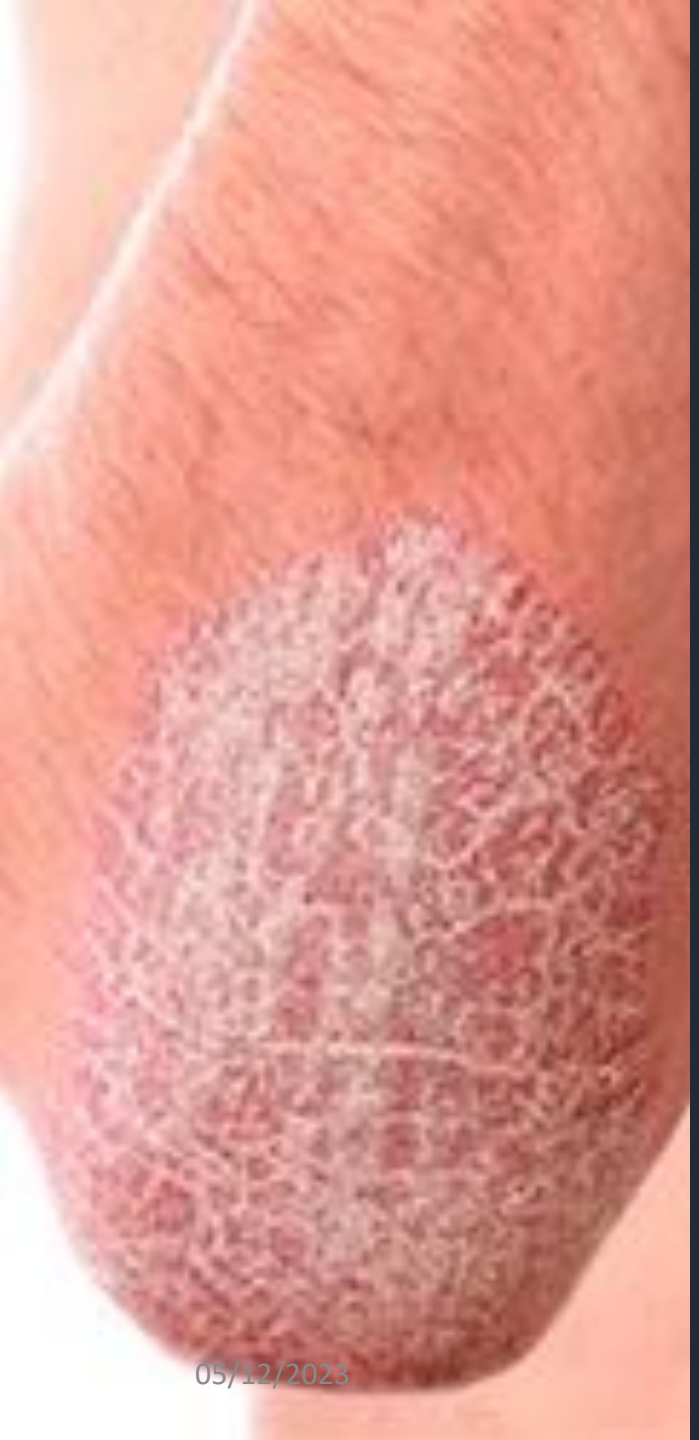
# Assessment of T-cell Receptor Repertoire and Immunotranscriptome of Peripheral Innate-like T cells for the Advancement of Psoriasis Diagnostics and Immunotheraphy.

**Stana Tokić**[1], Maja Jirouš[1], Zvonimir Grgić[1], Vera Plužarić[1,2], Marija Šola[2], Teuta Opačak-Bernardi[1], Barbara Viljetić[1], Kristina Glavaš[3], Maja Tolušić-Levak[1,2], Martina Mihalj[1,2], Mario Štefanić[1]



## Psoriasis vulgaris

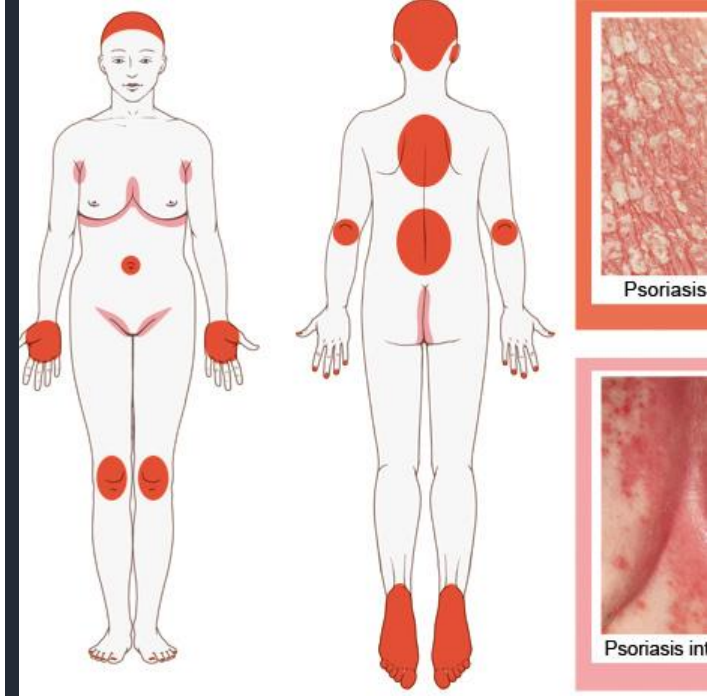
- a common, hard to treat autoinflammatory dermatosis that usually persists for life.

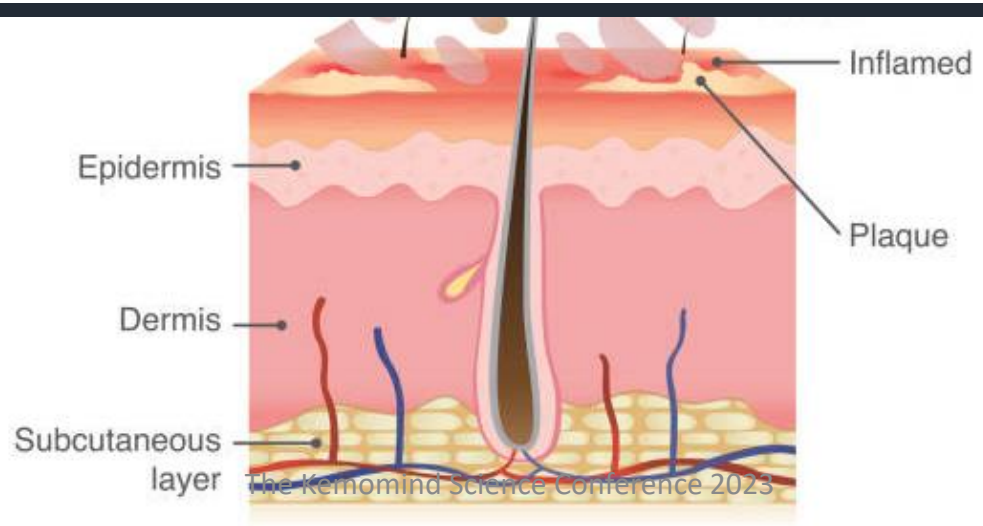
- reported prevalence ranges between 0.09% and 11.4% making psoriasis a serious global problem.

- 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



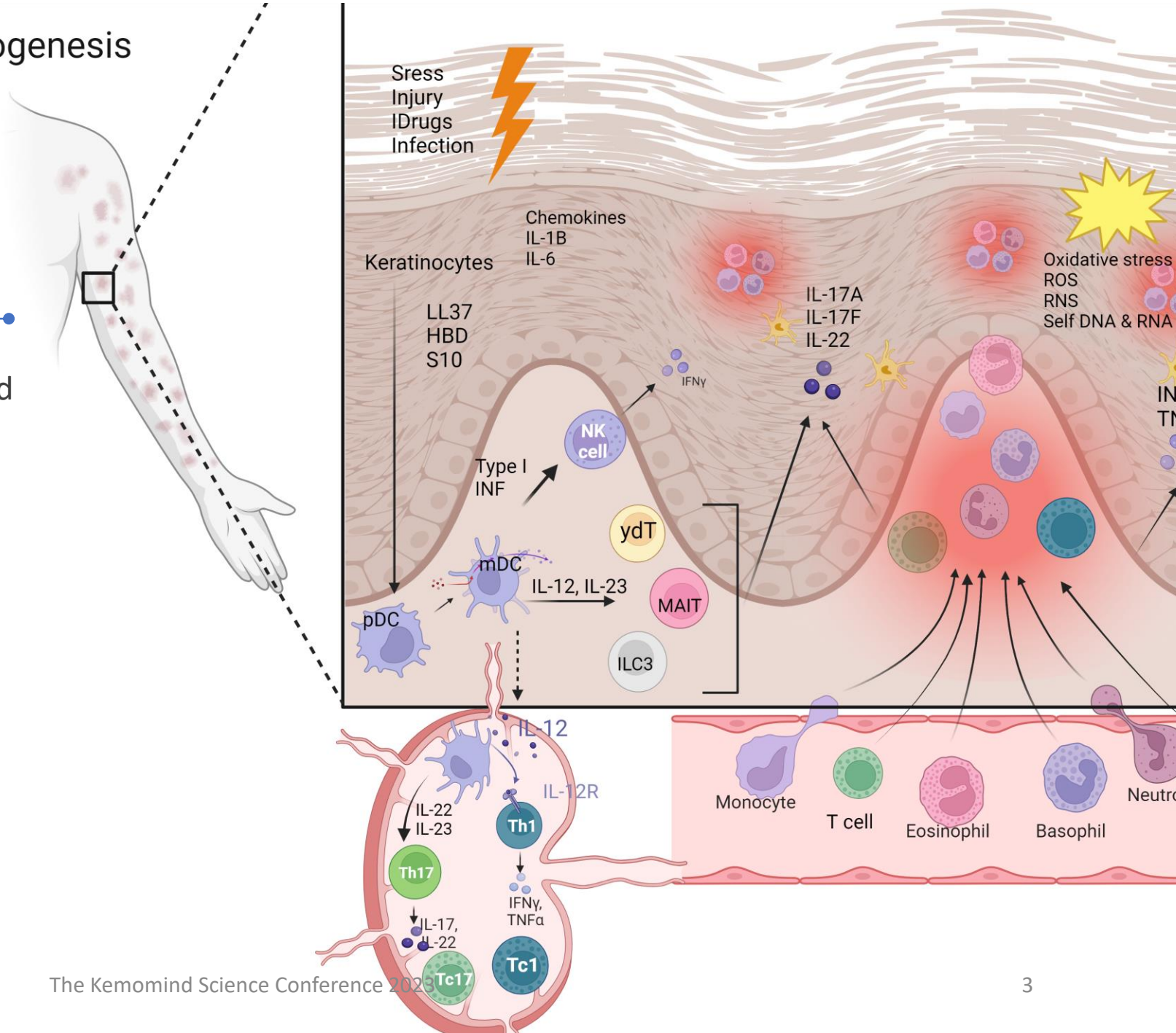
# PV pathogenesis

Trigger?
susceptible genetic background, impaired skin barrier and uncontrolled immune response

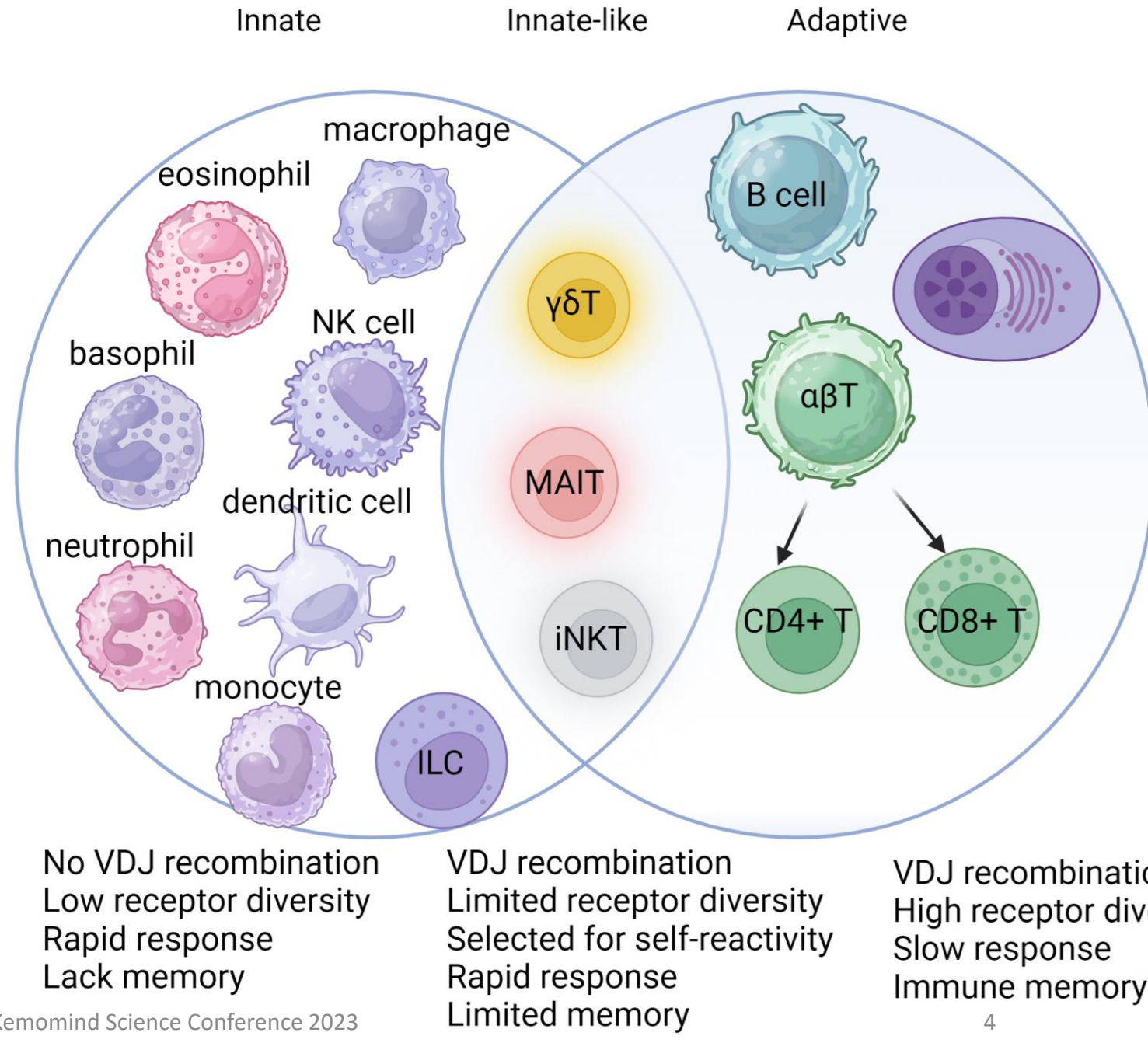
Inflamatory cascade is mounted by innate immune cells

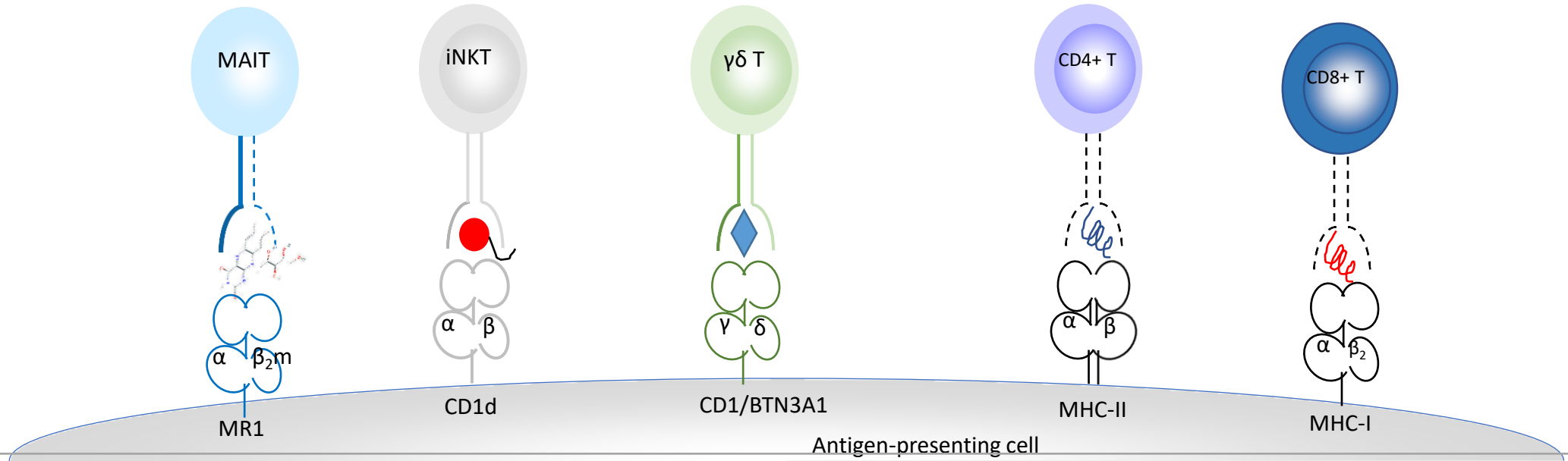
Inflammatory cytokines mediate keratinocyte proliferation, angiogenesis and chemokine production

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



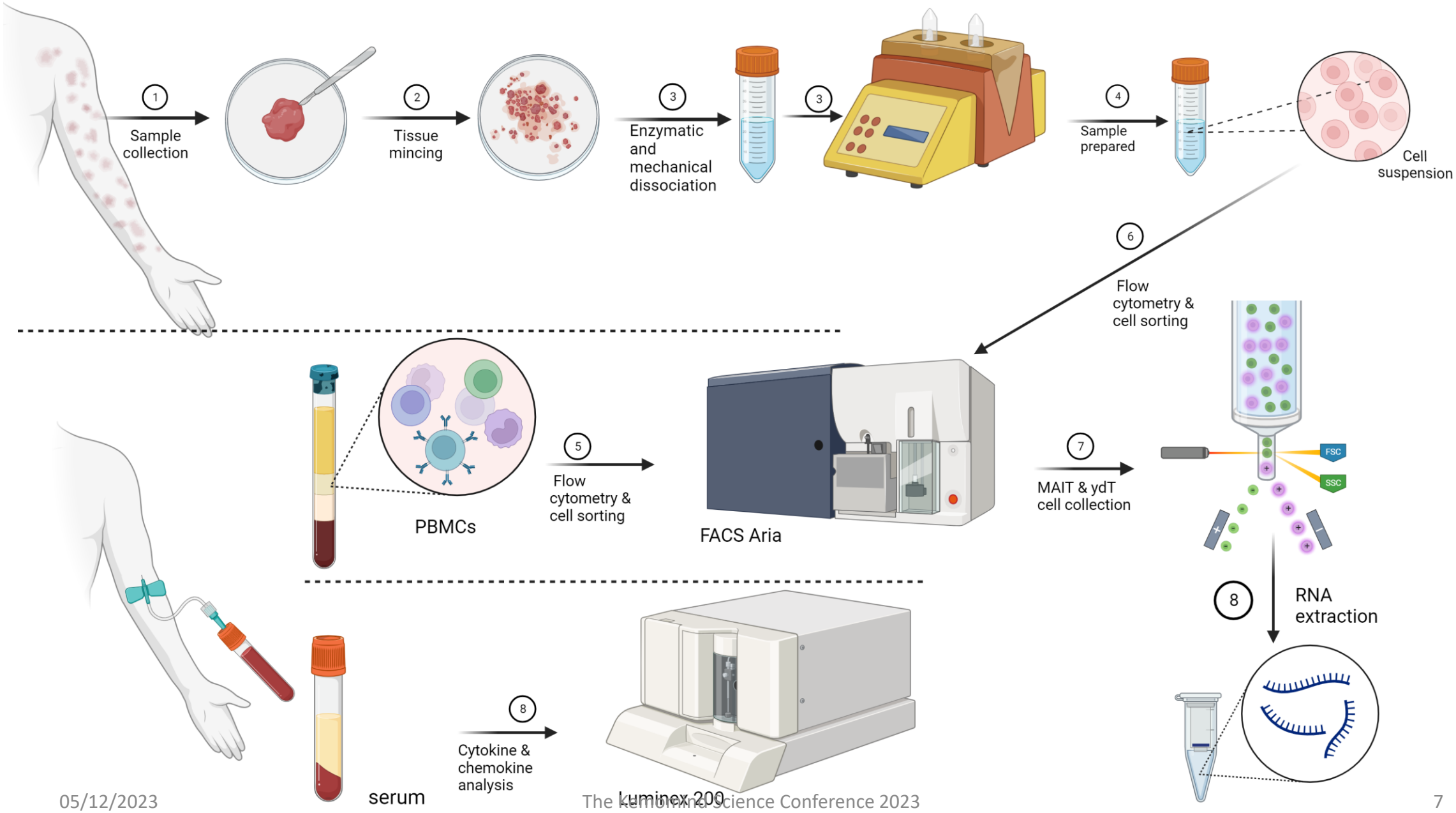
# Innate-like T cells – bridge between innate and adaptive immunity






| | MAIT | iNKT | γδT | CD4+αβ | CD8+αβ |
|---|---|---|---|---|---|
| **Blood frequency** | 0.1-15% | 0-1.1% | Vδ1: 0.25-6.2%<br>Vδ2: 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** | INFγ, IL-17, IL-22, TNFα, GZMB, PRF | | INFγ, IL-17, IL-22, TNFα,<br>GZMB, PRF | Lineage dependent | Lineage dependent |
| **Ag presentation** | MR-1 | CD1 | BTN3A1, CD1c, CD1d | MHC II | MHC I |
| **TCR repertoire** | Invariant | Invariant | Variable (γ & δ chain) | Variable | Variable |

# How do peripheral and tissue proportions of MAIT and γδ T lymphocytes change in psoriasis?


1
Sample collection
2
Tissue mincing
3
Enzymatic and mechanical dissociation
3
4
Sample prepared
Cell suspension
6
Flow cytometry & cell sorting
PBMCs
5
Flow cytometry & cell sorting
FACS Aria
7
MAIT & ydT cell collection
FSC
SSC
8
RNA extraction
serum
8
Cytokine & chemokine analysis
Luminex 200

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# MAIT cells – frequency in blood of PV?

## Differential Skewing of Circulating MR1-Restricted and γδ T Cells in Human Psoriasis Vulgaris

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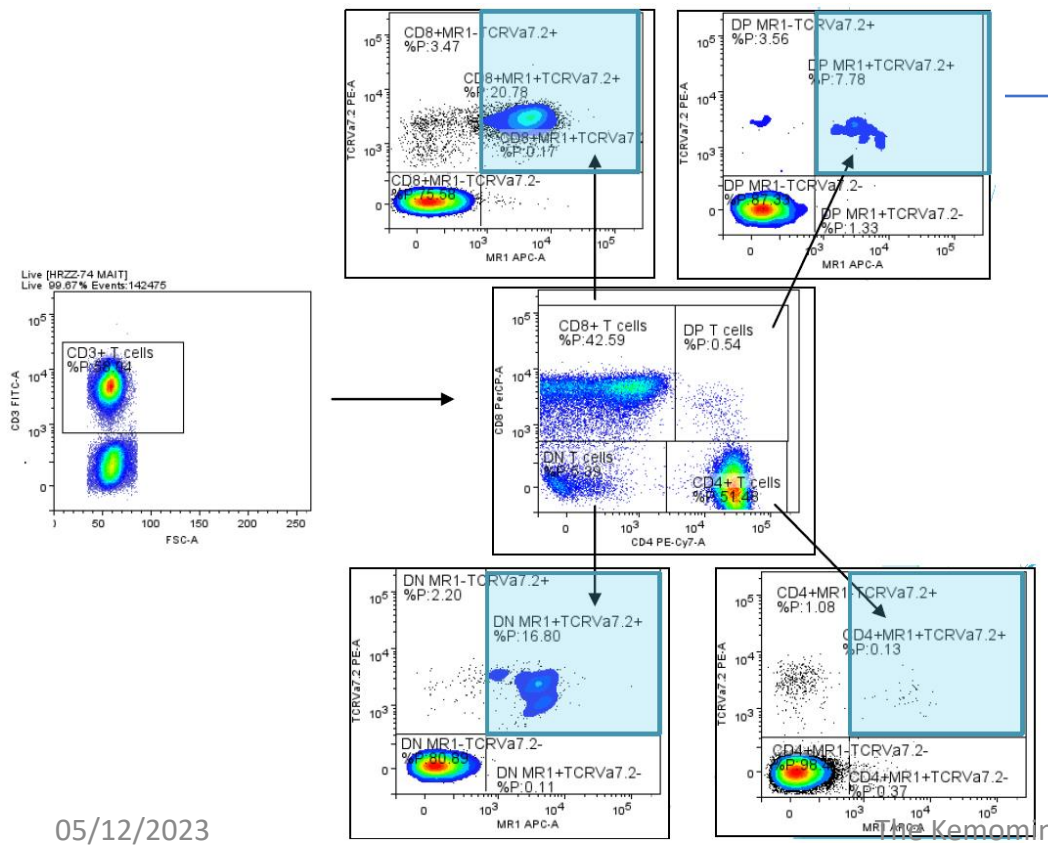


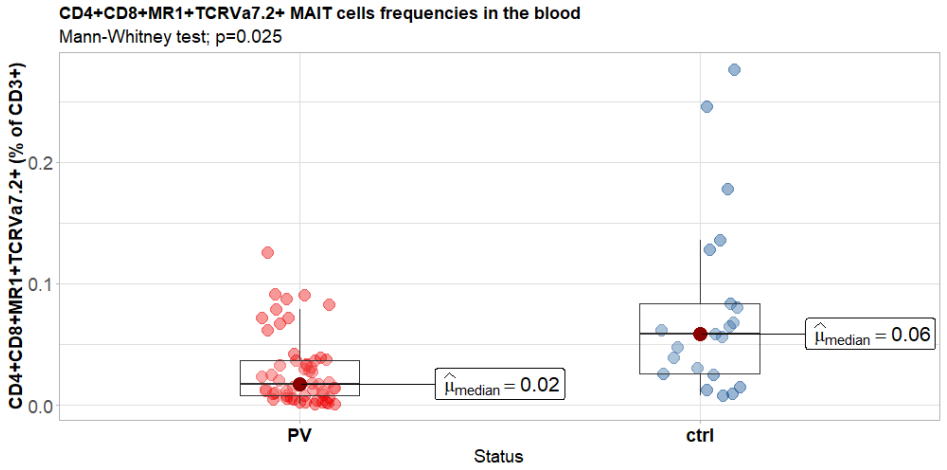


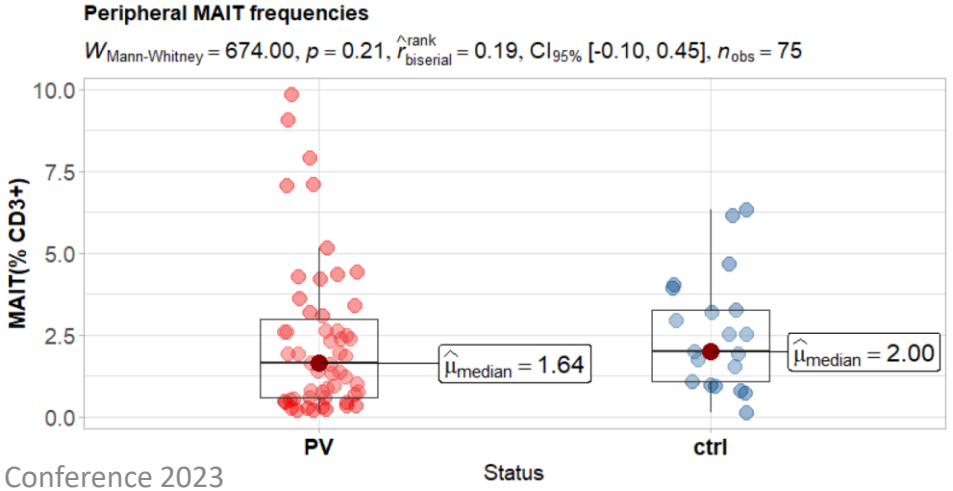

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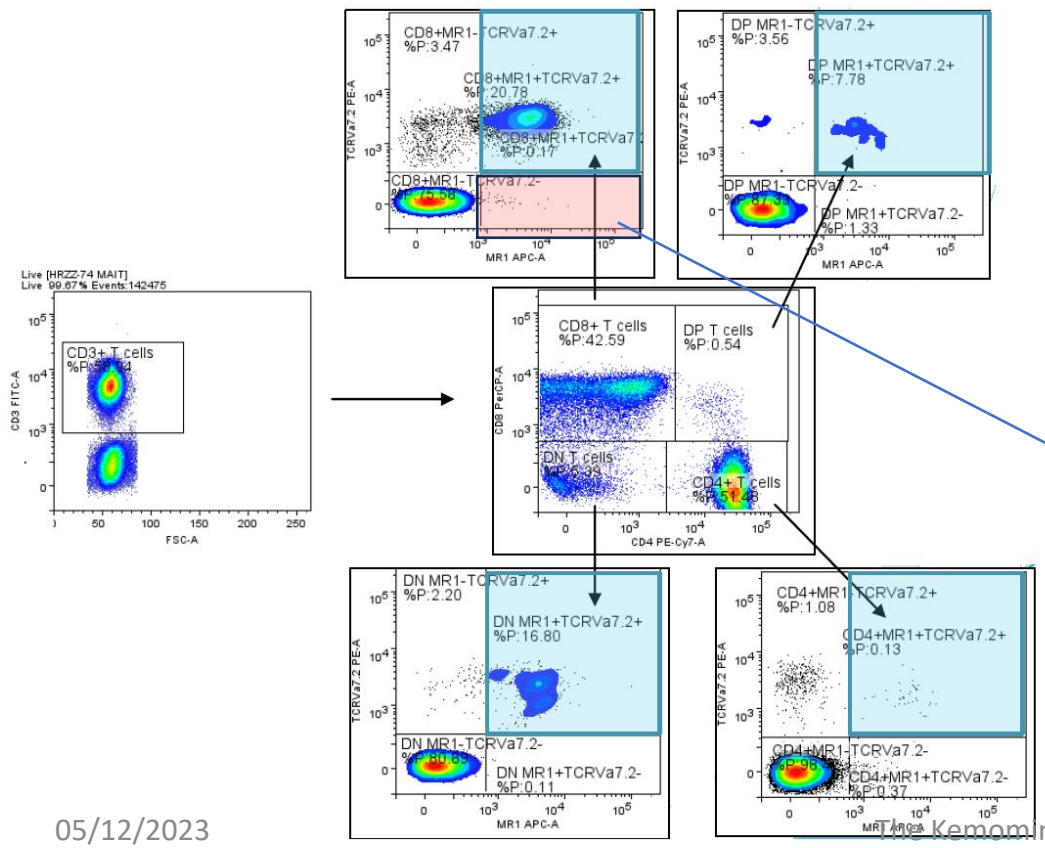


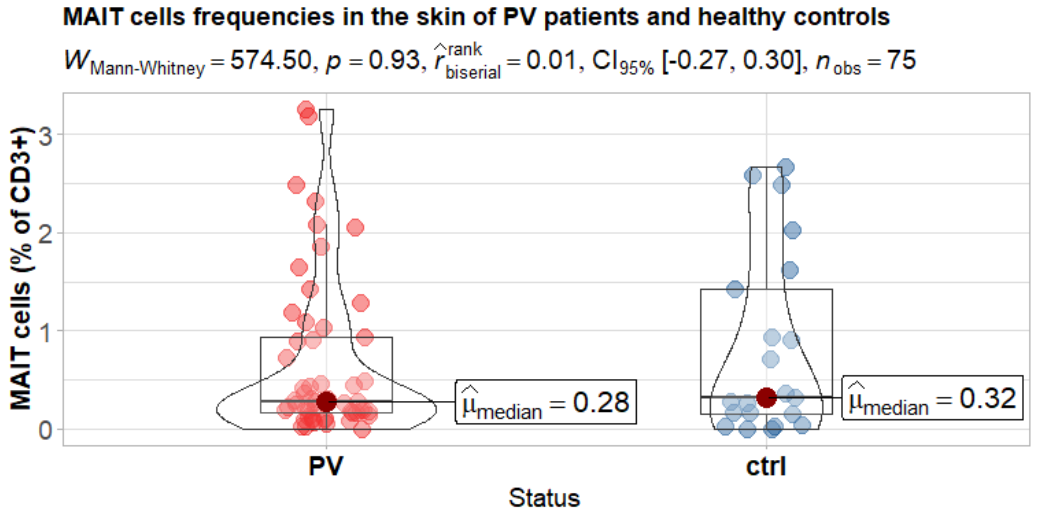


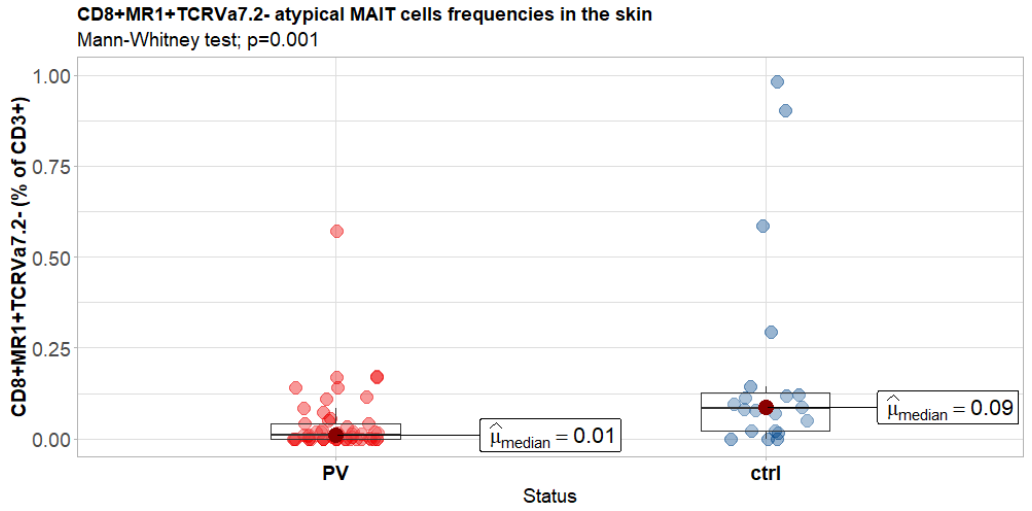

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# γδ T cells – frequency in blood of PV?

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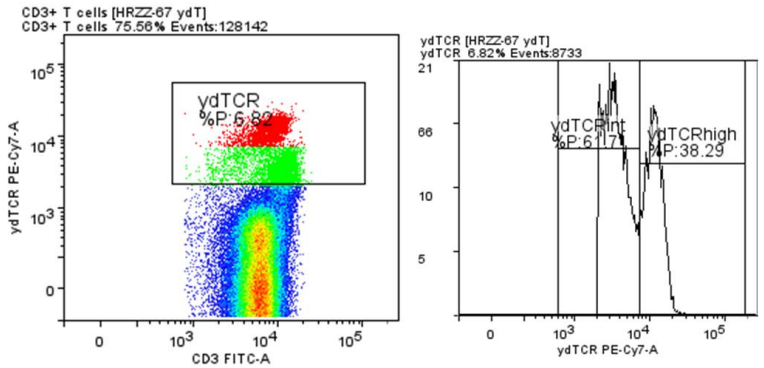


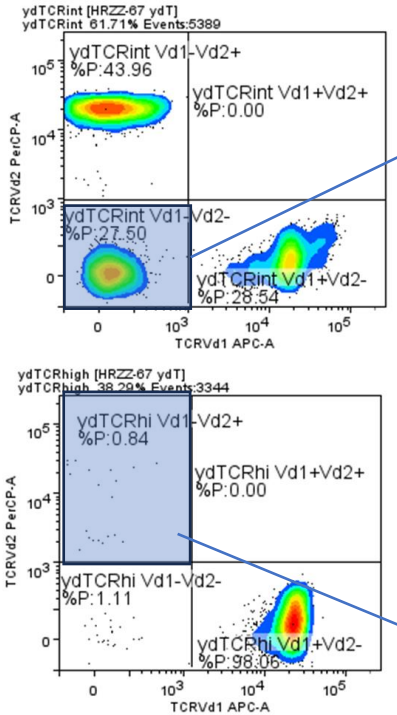


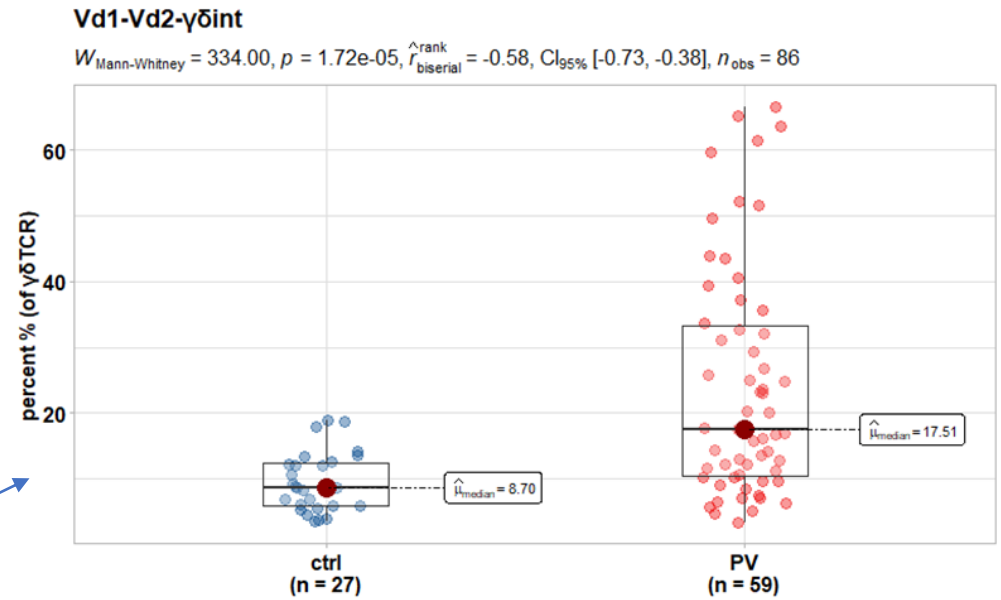


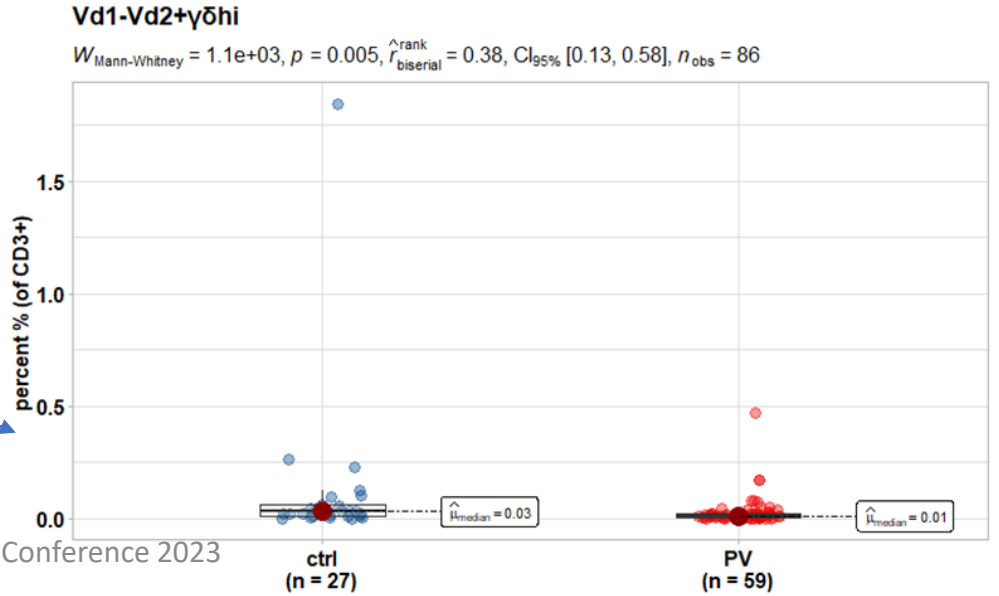

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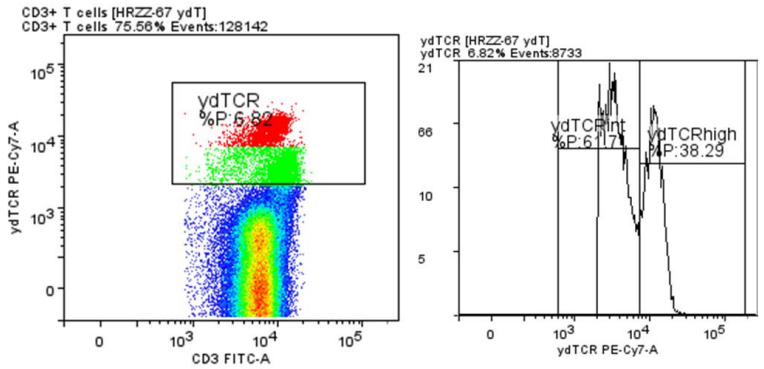
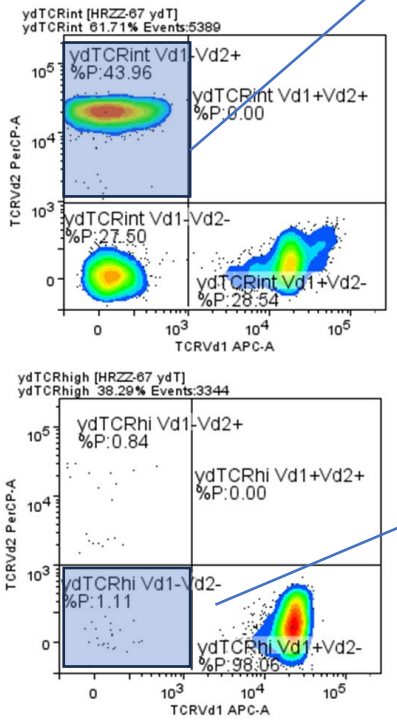
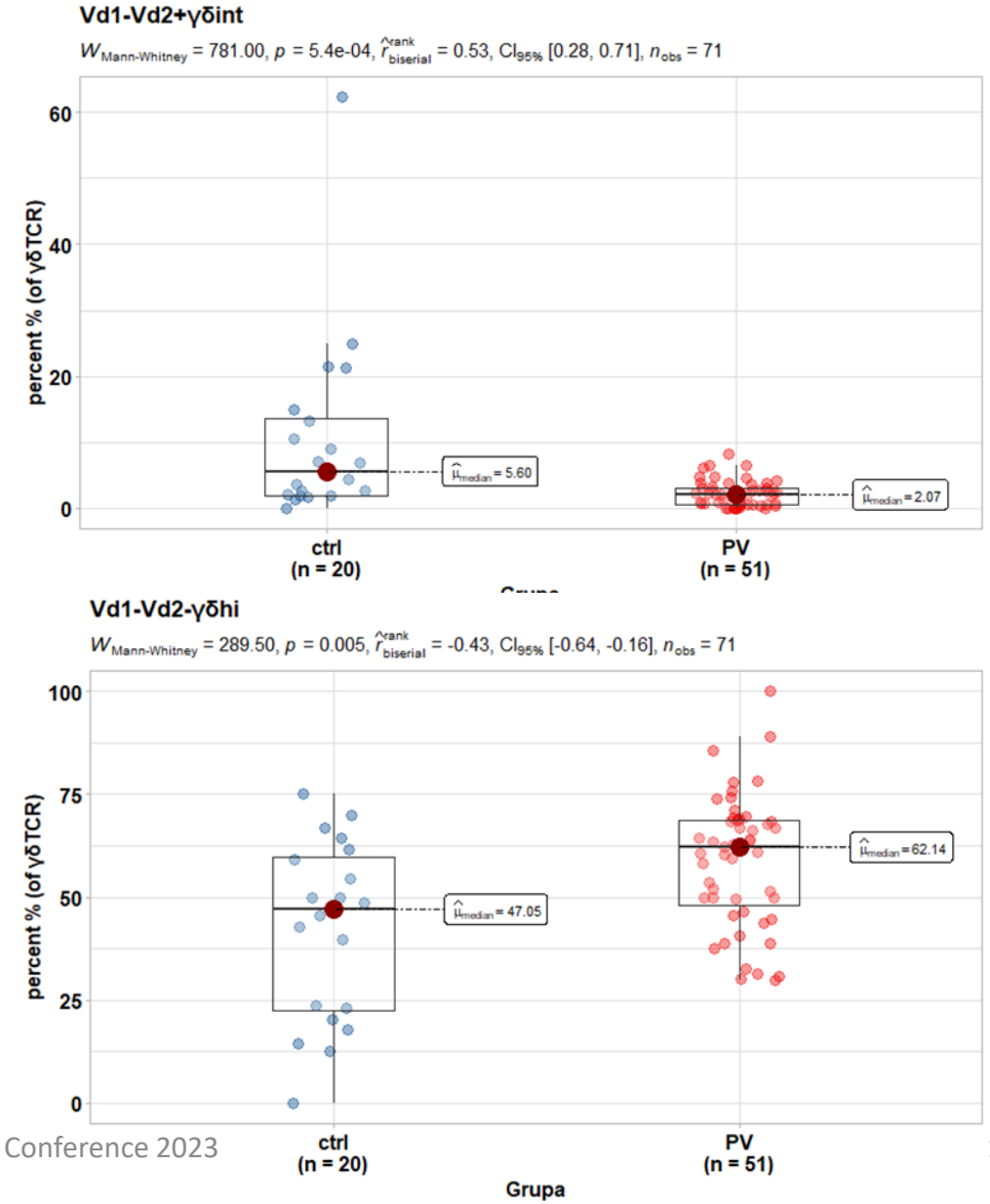
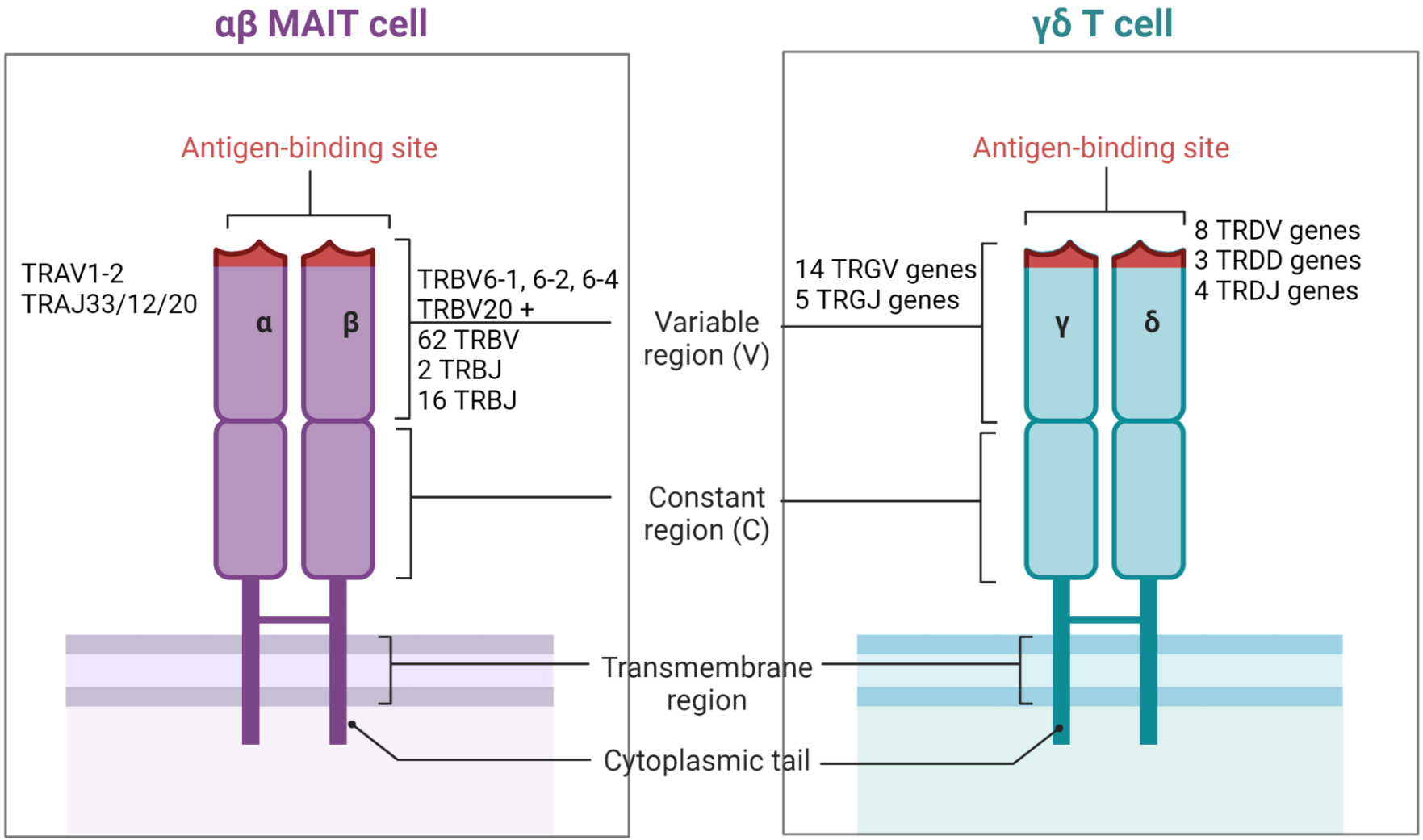


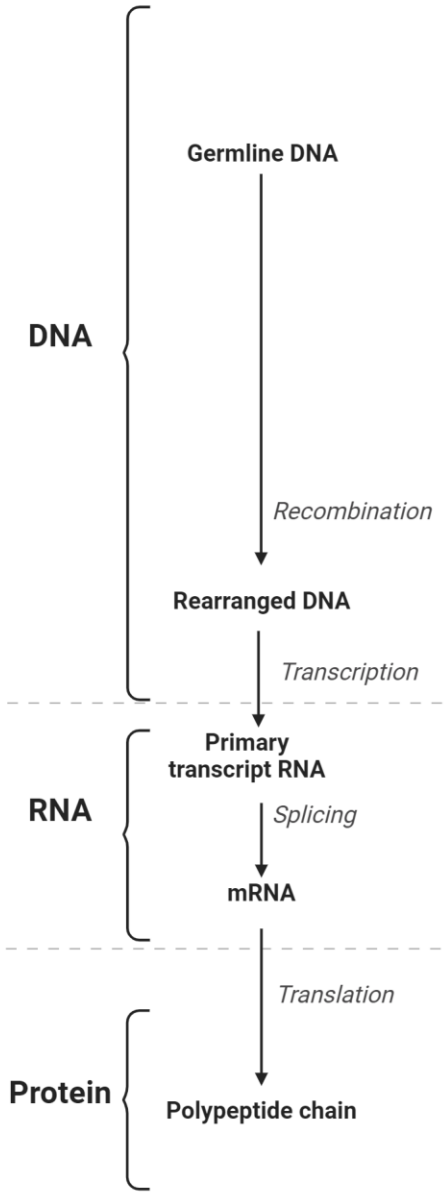



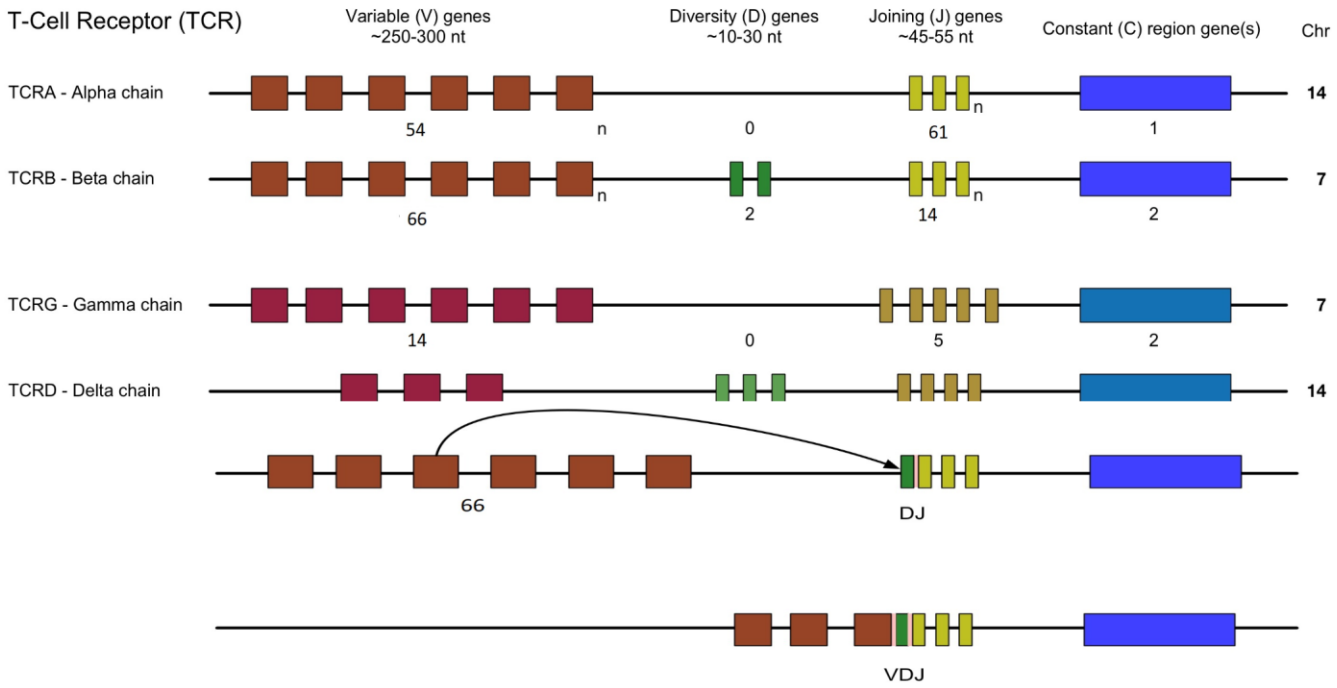
# What are the molecular events that guide MAIT and γδ T cell compositional, functional and clonotypic rearrangements in PV?

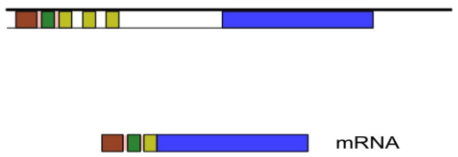
# MAIT and γδ T cell receptors

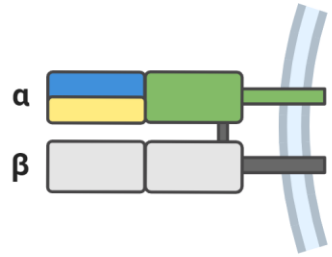


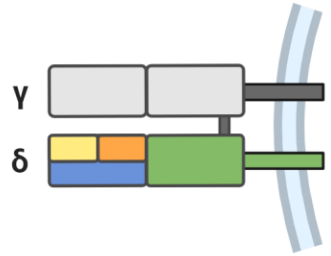
# TCR receptor formation



# Applied NGS pipelines

TCR Seq

Immune Repertoire Plus TCRβ Panel - MiniSeq

Archer Immuniverse TCR Panel - NextSeq

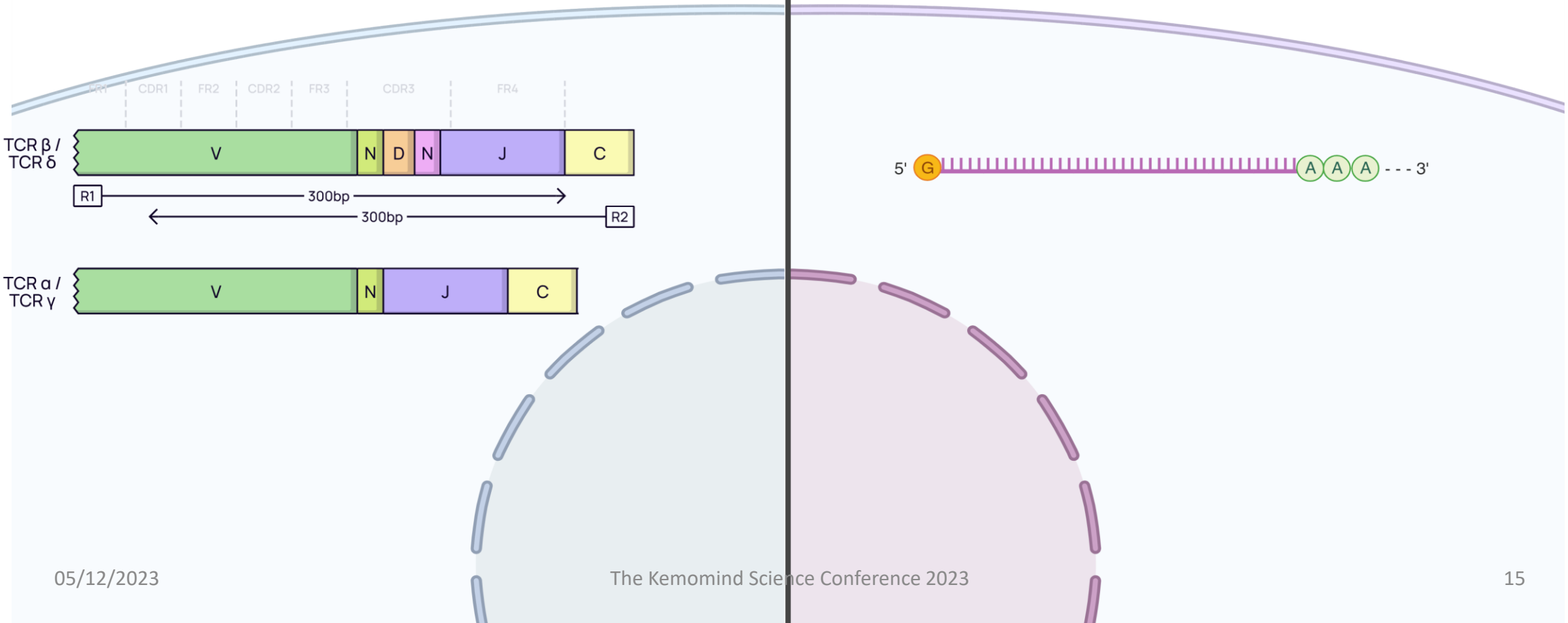
MiXCR, VDJTools, Immunarch

RNA Seq

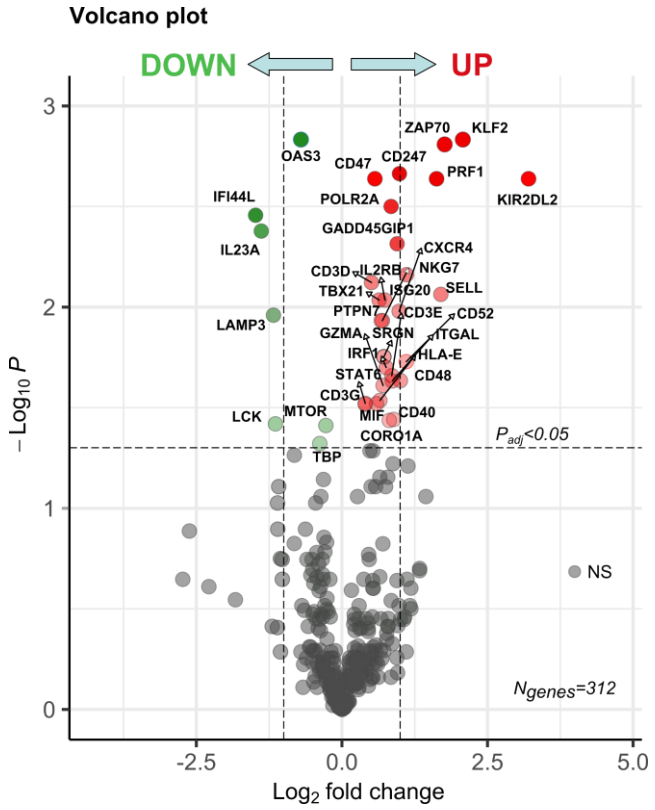
AmpliSeq Immune Response Panel - MiniSeq

BaseSpace RNA Amplicon, DeSeq2

KEGG, GSEA, STRING-db



# Type I/II immunity and cytotoxicity signature genes mark transcriptional programs of peripheral γδ T




## Upregulated

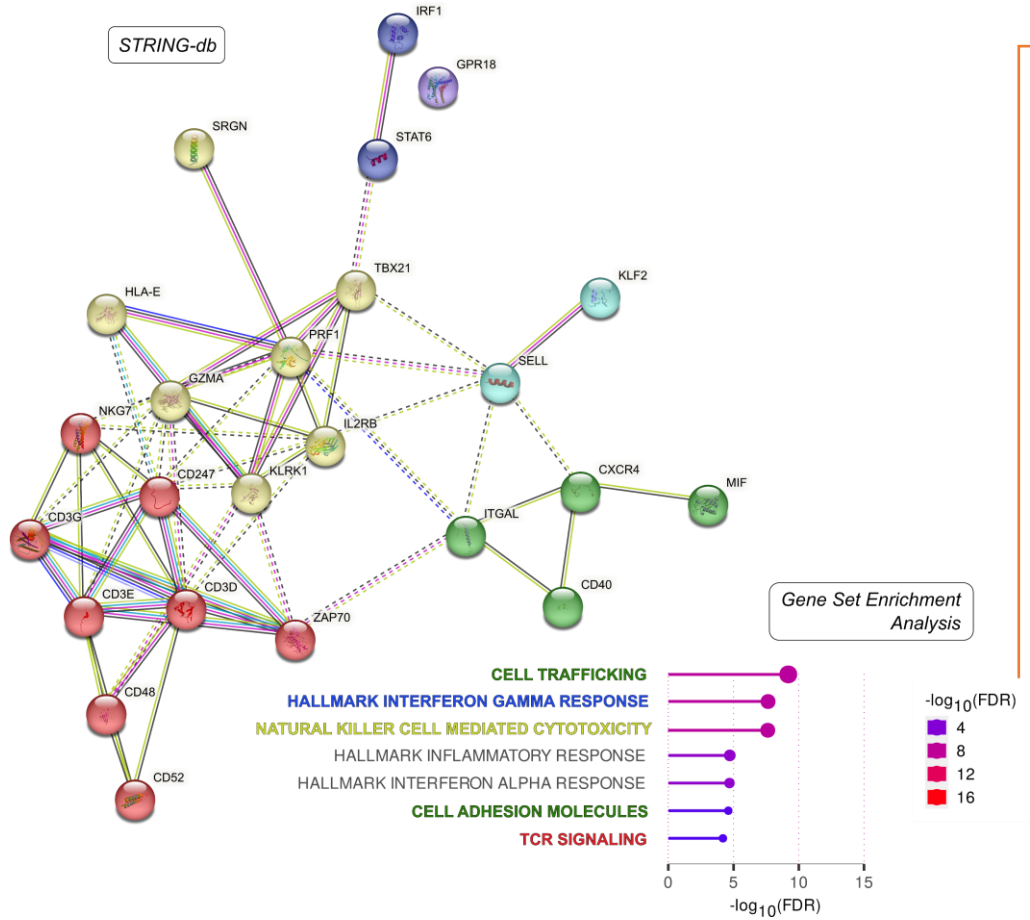
- STAT6 & TBX21 Th1/2 lineage transcription factors
- IRF1 - master regulator of interferon/IFN signalling
- IFNγ-inducible targets *ISG20, CD40, KLRK1, GPR18, IL2RB*
- genes related to cytotoxicity PRF1, GZMA, NKG7, SRGN, HLA-E
- Cell trafficking *KLF2, CXCR4, GPR18, MIF, CORO1A*
- Cell adhesion (SELL, 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+γδTCR+ γδT 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 γδ T




## FDR and GSEA in PV vs control

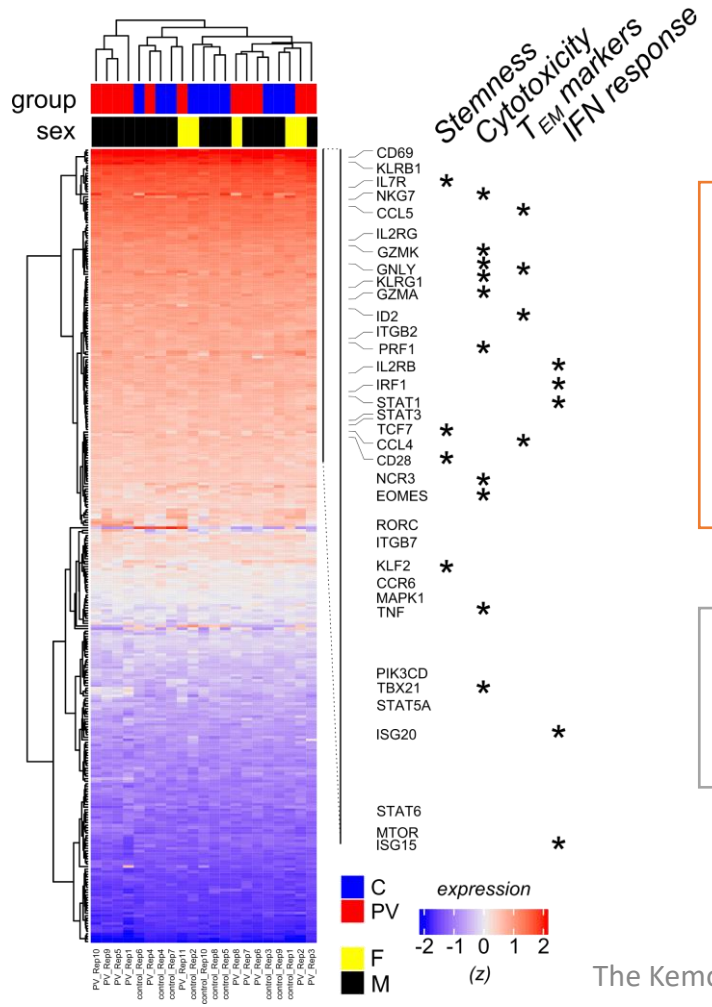
- The FDR was highest for genes relted to cell trafficking and INF gamma response
- Members of the TCR signalosome coincided well with hallmark genes of NK cytotoxicity, type I immunity and INFy response

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

| 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 |
| | | | | | CD3G | CD47 | FASLG | C10ORF54 | | MMP9 | | | IL10RA |
| | | | | | CD8A | IL2RB | FASLG | SDHA | | GRAP2 | | | IRF9 |
| | | | | | LAG3 | HLA-DOB | IFIT2 | CD28 | | GZMK | | | SRGN |
| | | | | | CTSW | LCK | 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 γδT cell subsets and clinical features associate with different gene profiles

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



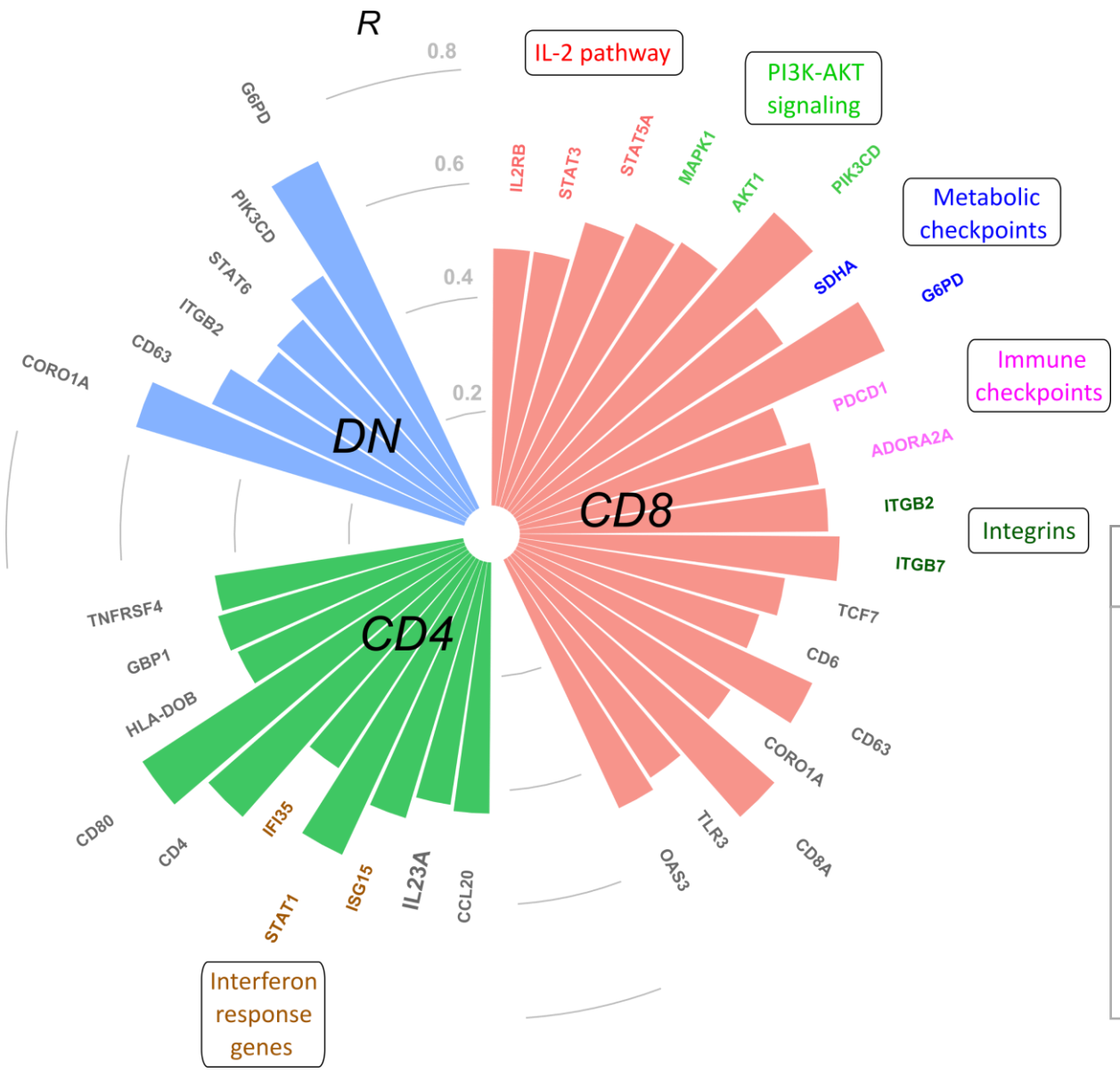

## Upregulated

- KLRB1 (CD161), and).
- 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




| CD8+ MAIT | DN MAIT | CD4+ MAIT |
|---|---|---|
| • PI3K-AKT signaling<br>• IL-2 pathway<br>• immunoregulatory molecules<br>• gut-homing integrins | • PI3K-AKT signaling<br>• Th2 lineage differentiation<br>• integrins | • IL23A<br>• interferon response genes<br>• co-stimulatory receptors from the TNFRSF family<br>• skin-homing cytokines such as CCL20 |

**Characterization of the TCRβ repertoire of peripheral MR1-restricted MAIT cells in psoriasis vulgaris patients**

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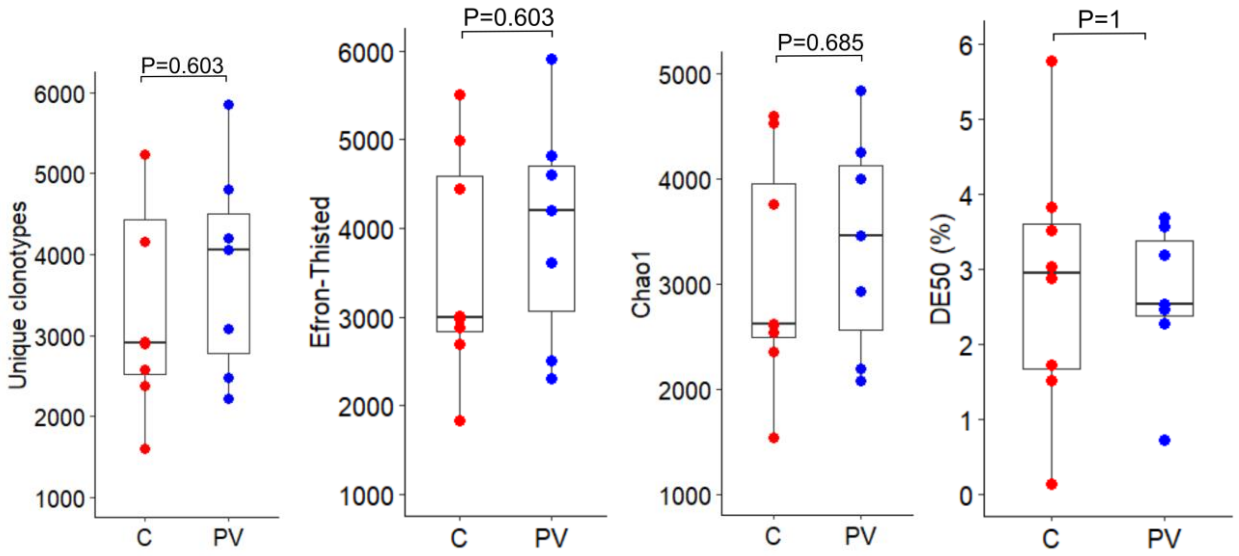
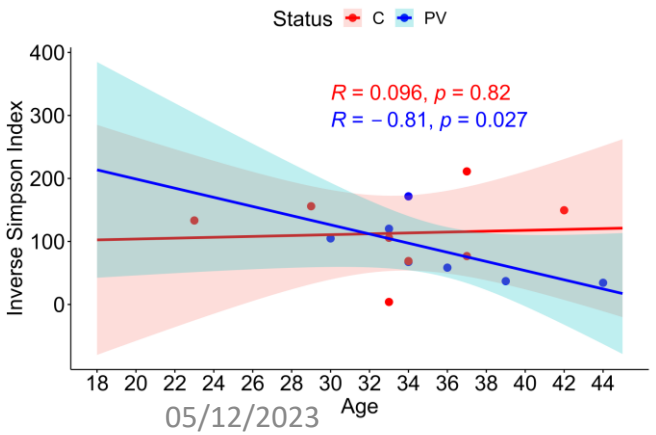
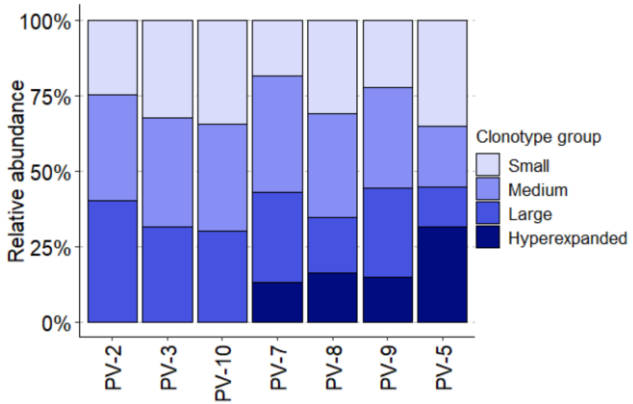


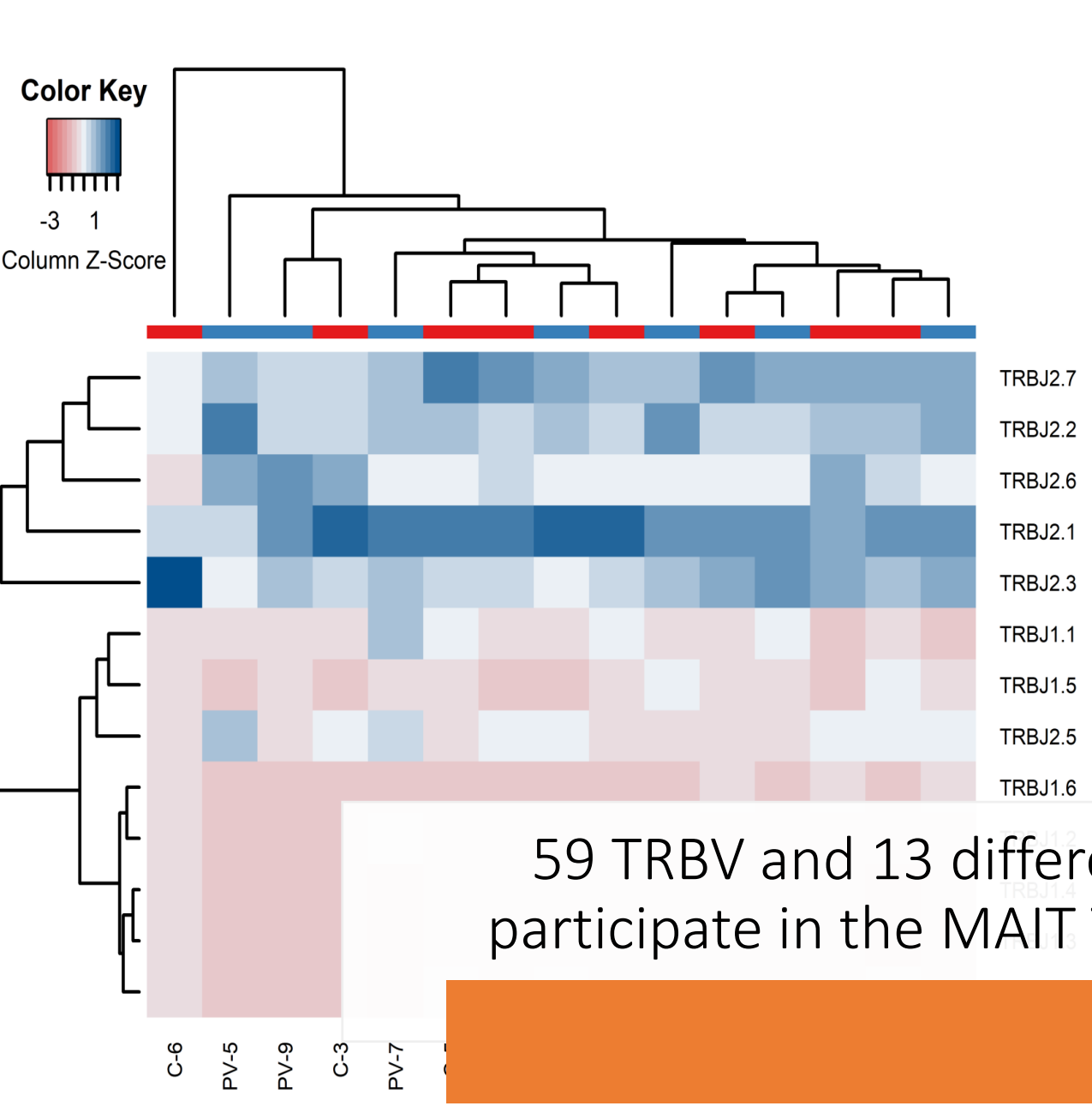



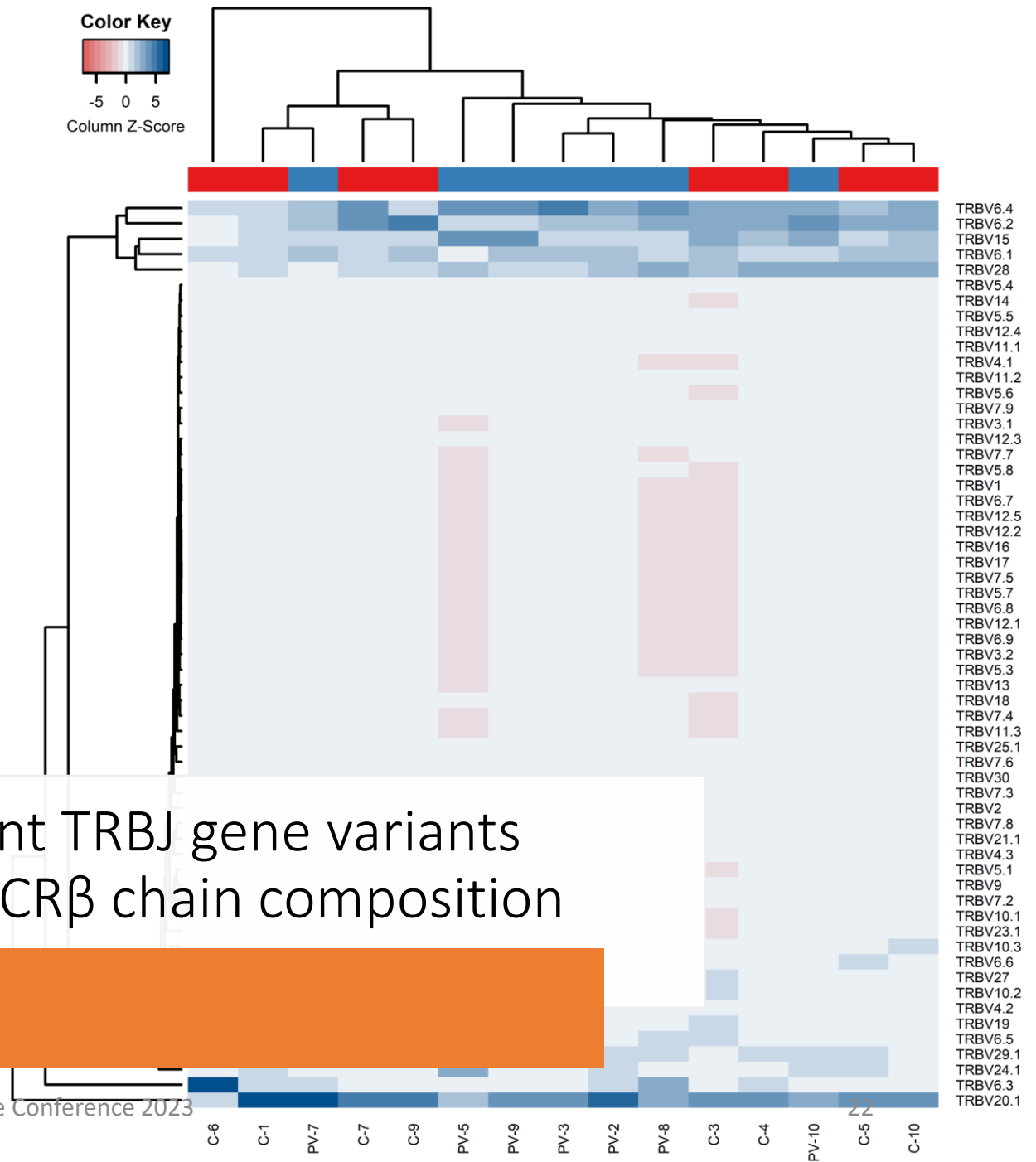




# The peripheral MAIT TCRβ repertoire of PV patients is highly diverse and expands with age

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

High-frequent clonotypes, defined by the DE50 index as species occupying at least 50% of all sequencing reads, accounted for less than 3% of all clonotypic variants in both groups, suggesting presence of only few expanded clonotypes in MAIT TCRβ repertoires of PV patients and healthy controls.

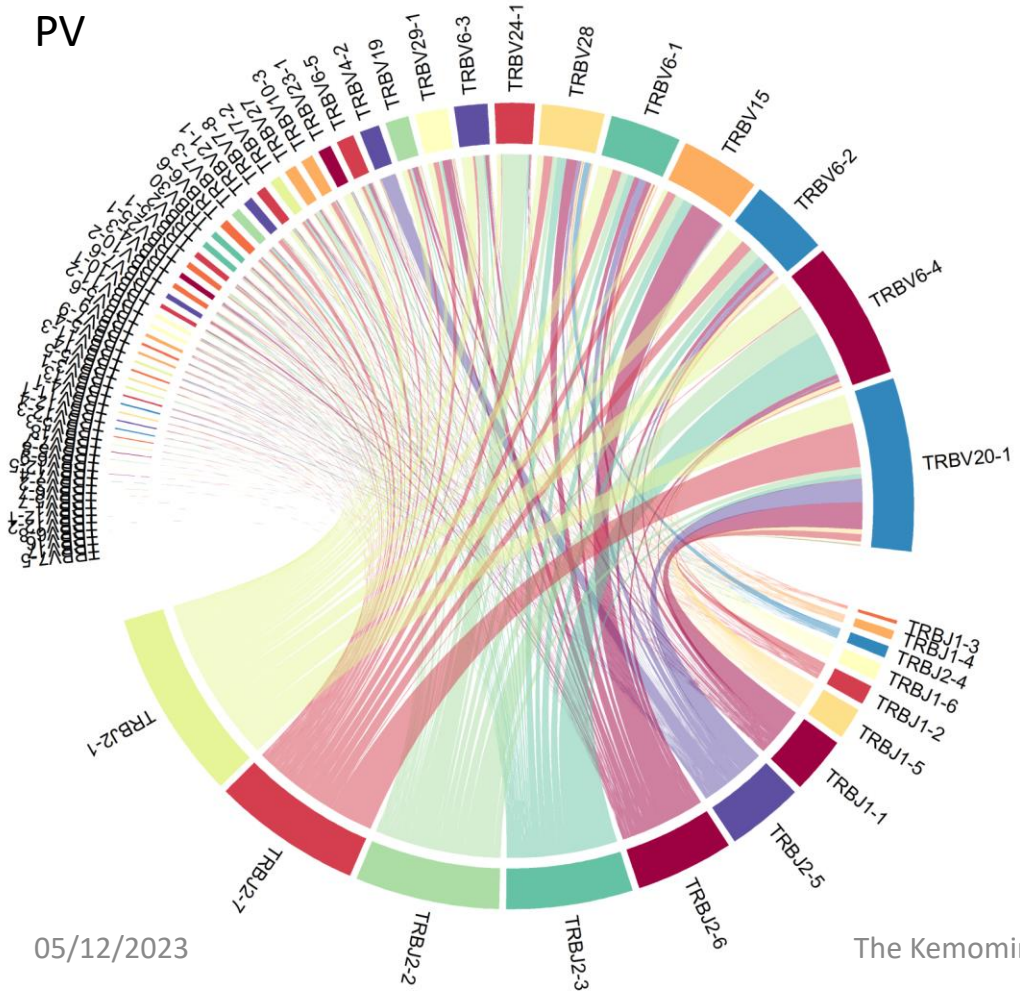


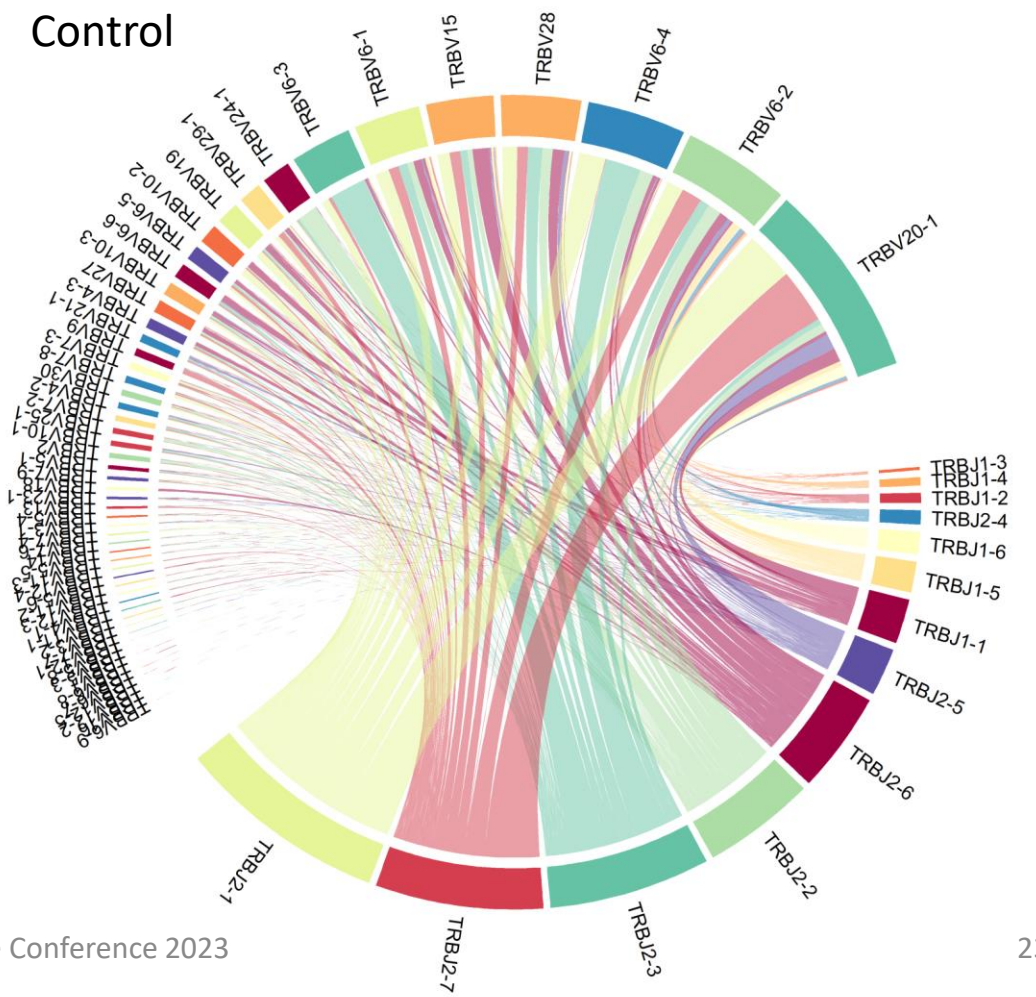

# 59 TRBV and 13 different TRBJ gene variants participate in the MAIT TCRβ chain composition

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

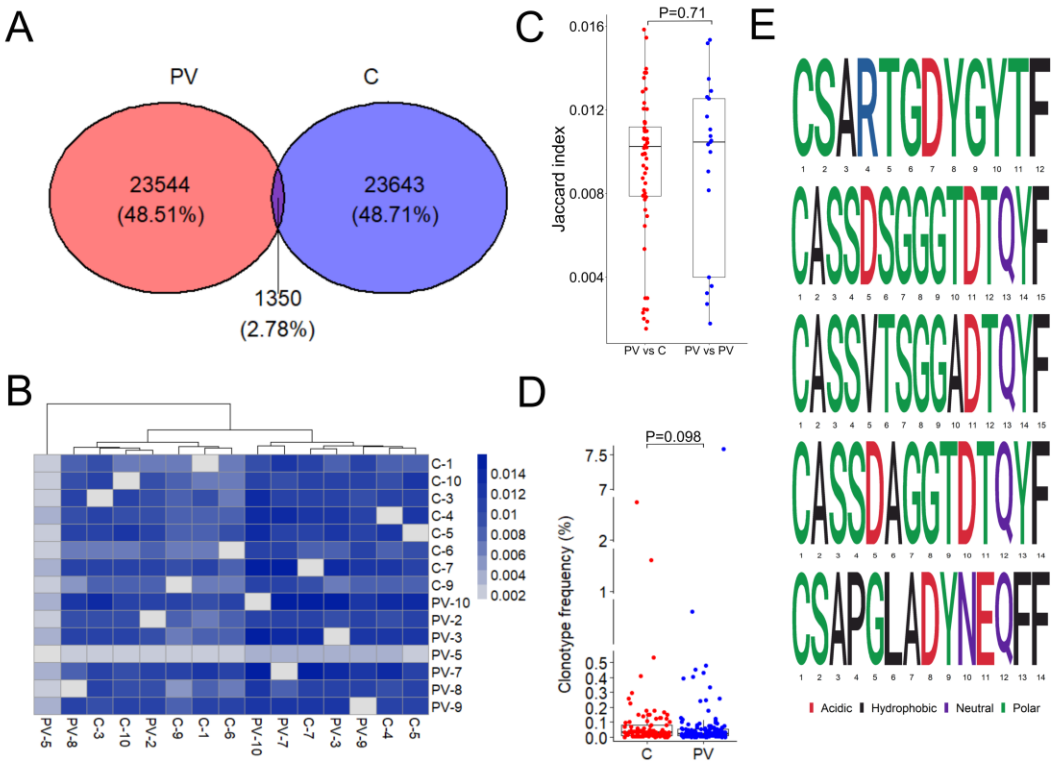
PV




Control



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

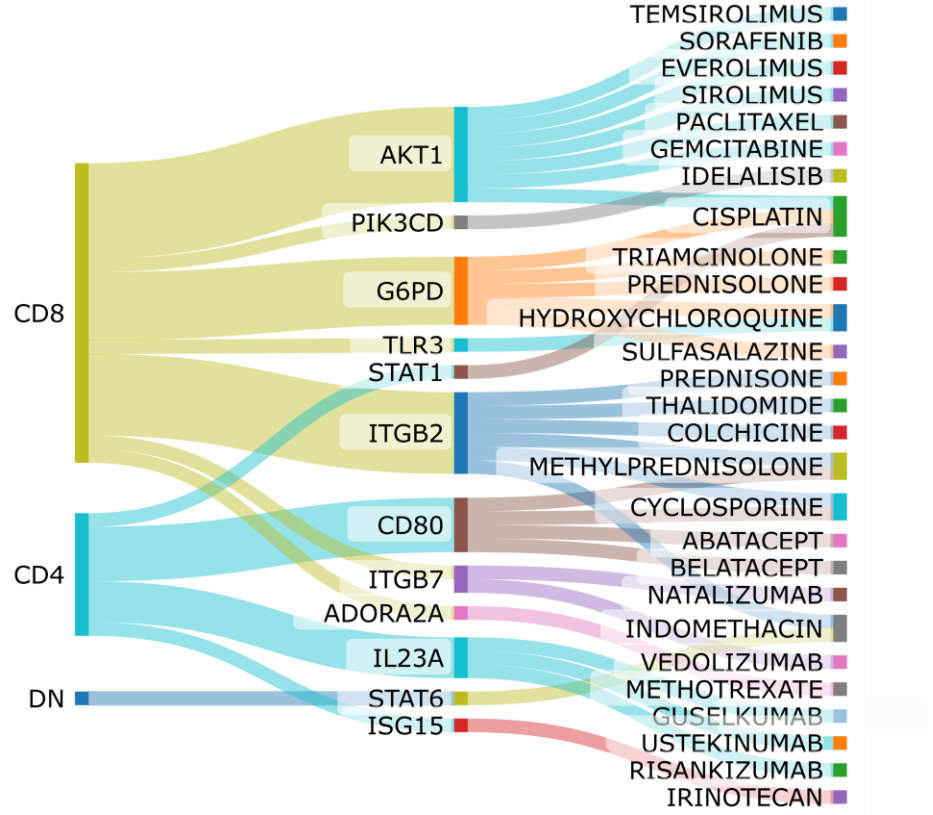



In PV vs. healthy comparison of public MAIT TCRβ clones a total of 420 healthy- and 336 PV-unique clonotypes were shared within, but not between groups, with eight being frequently shared (≥ 4/7) in healthy, and five in PV.

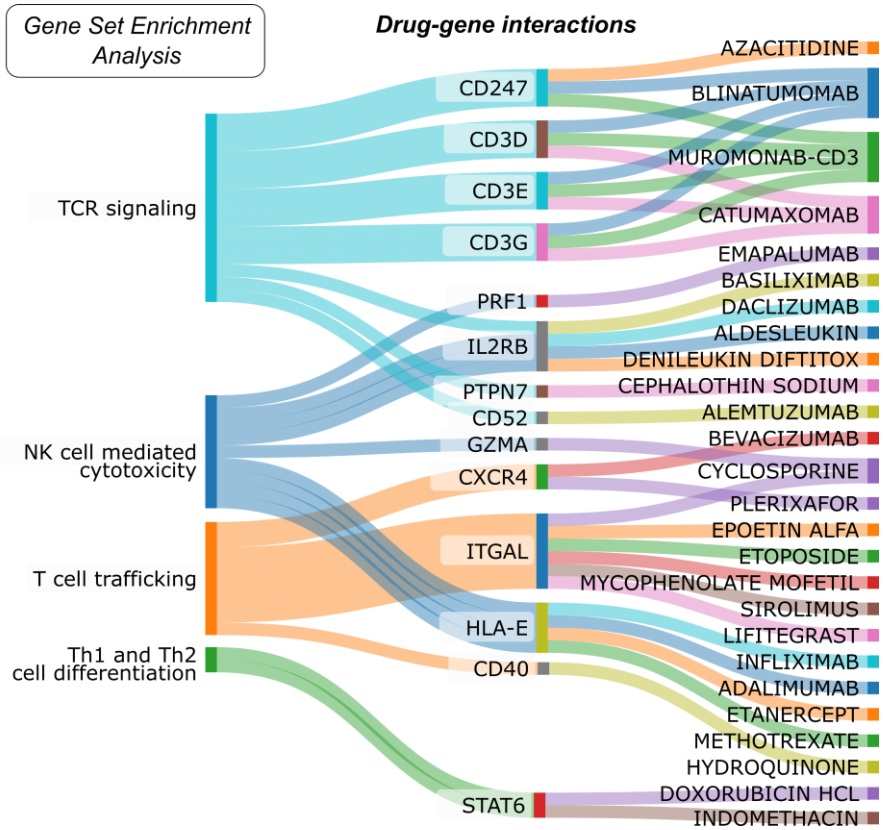
PV-unique clonotypes were matched withpreviously published blood, non-lesional and lesional skin TCRβ repertoires of PV patients, supporting their diagnostic and therapeutic relevance.

# What is the therapeutic relevance of identified genetic targets?

# MAIT and γδ T cells are enriched in druggable molecules increasing the likelihood of off-target effects in patients receiving biological agents.




MAIT cell targets




γδ T cell targets

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THANK YOU FOR YOUR ATTENTION