

Postgraduate University Interdisciplinary  
Doctoral Study of Molecular Biosciences

**Doctoral thesis topic defence**

Characterisation of the phenotype, transcriptional markers  
and T cell receptor repertoire of circulating MAIT and  $\gamma\delta$  T  
lymphocytes in psoriasis vulgaris

Karakterizacija fenotipa, transkripcijskih biljega i repertoara T staničnih  
receptora cirkulirajućih MAIT i  $\gamma\delta$  T limfocita u vulgarnoj psorijazi

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Doctoral candidate: Maja Jirouš Drulak

Supervisor 1: Assoc. Prof. Stana Tokić

Supervisor 2: Assoc. Prof. Martin Davey

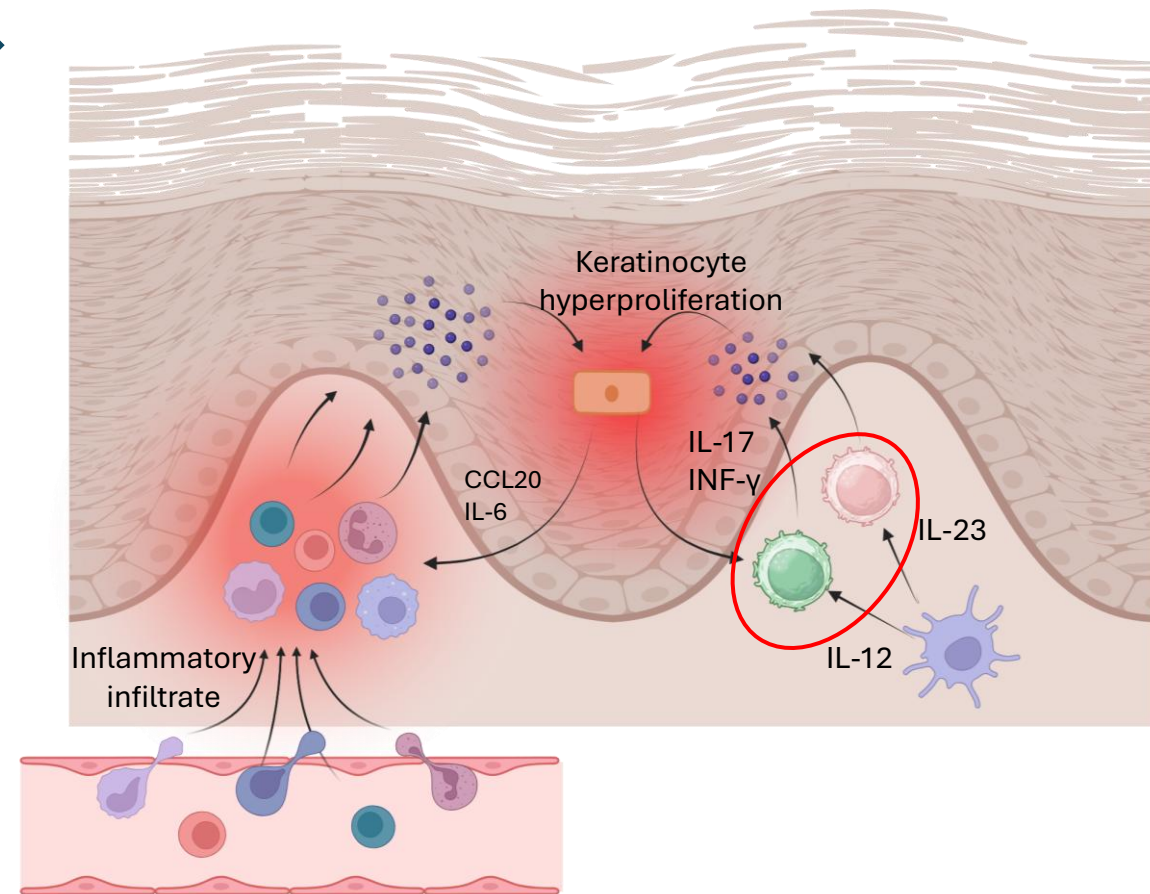
Osijek, 21<sup>st</sup> May 2024.

# Psoriasis vulgaris

- chronic autoimmune disease
- well-defined, raised, erythematous lesions with silvery-white scales
- global prevalence: 2–3%
- genetics and environmental triggers



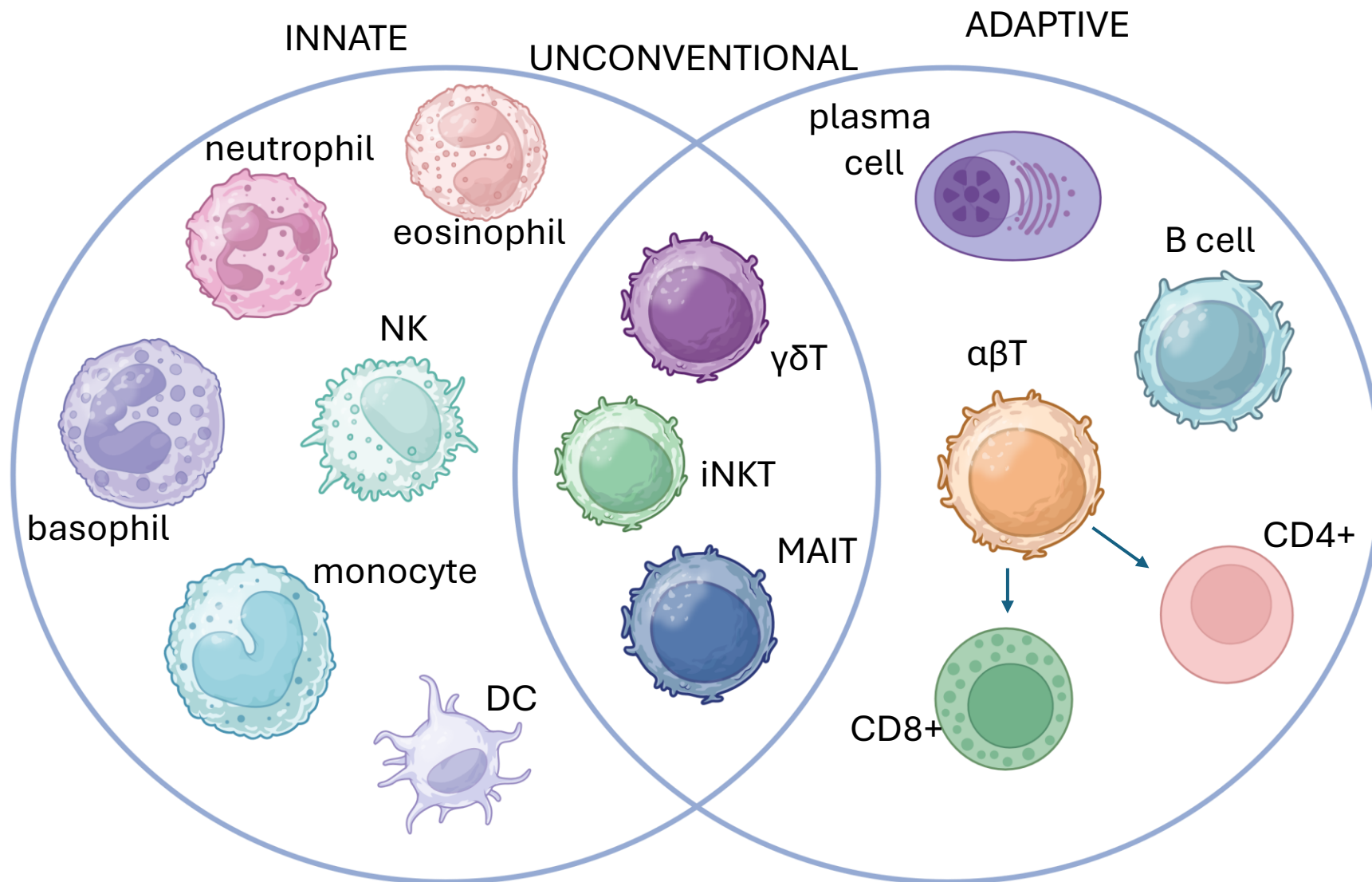
<https://saguaroderm.com/services/medical-dermatology/psoriasis/>



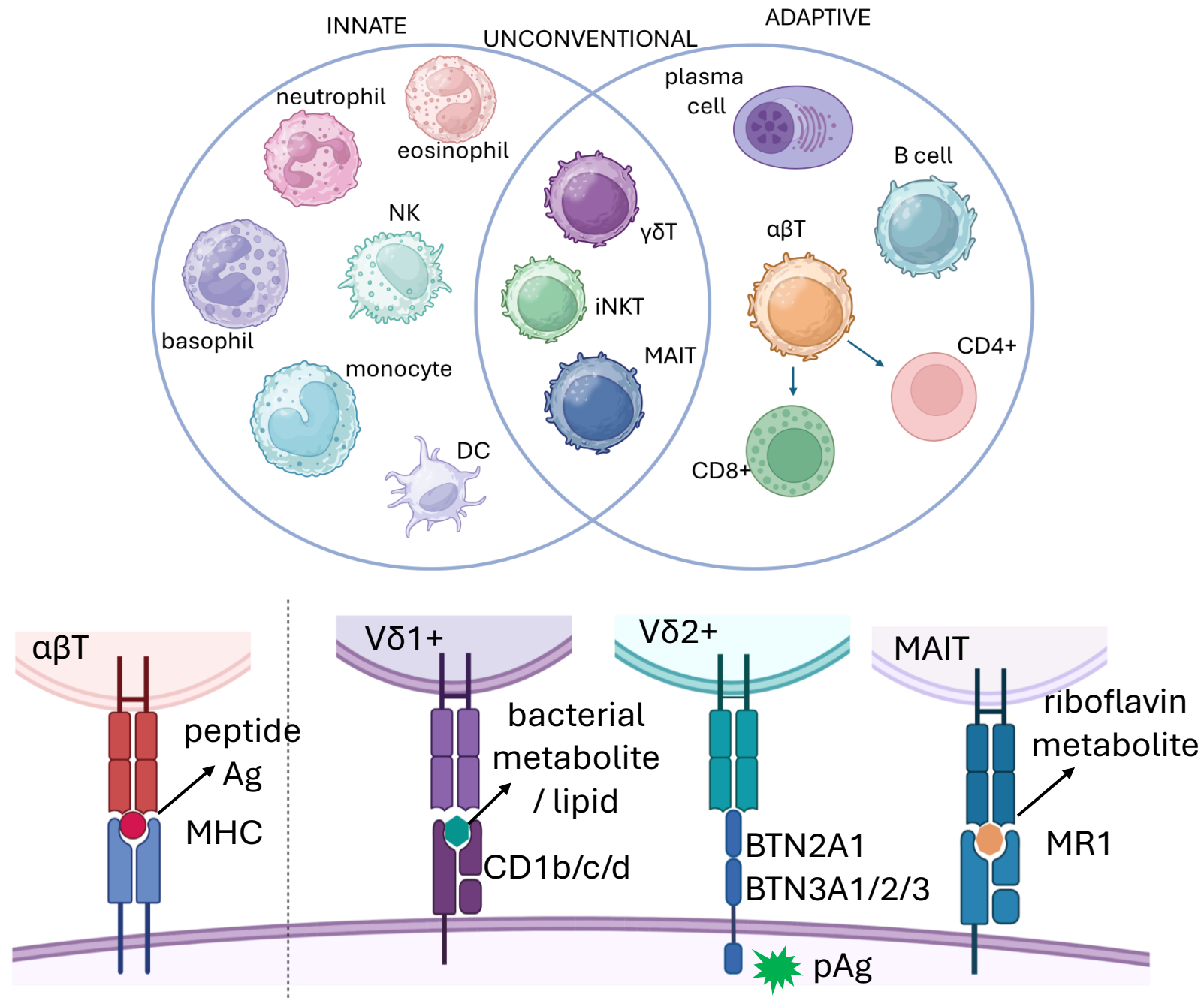
- dysregulation of the immune system and aberrant T cell activation
- multiple comorbidities and other autoimmune disorders
- autoantigens?



What is the role of  
unconventional T cells  
in psoriasis vulgaris?

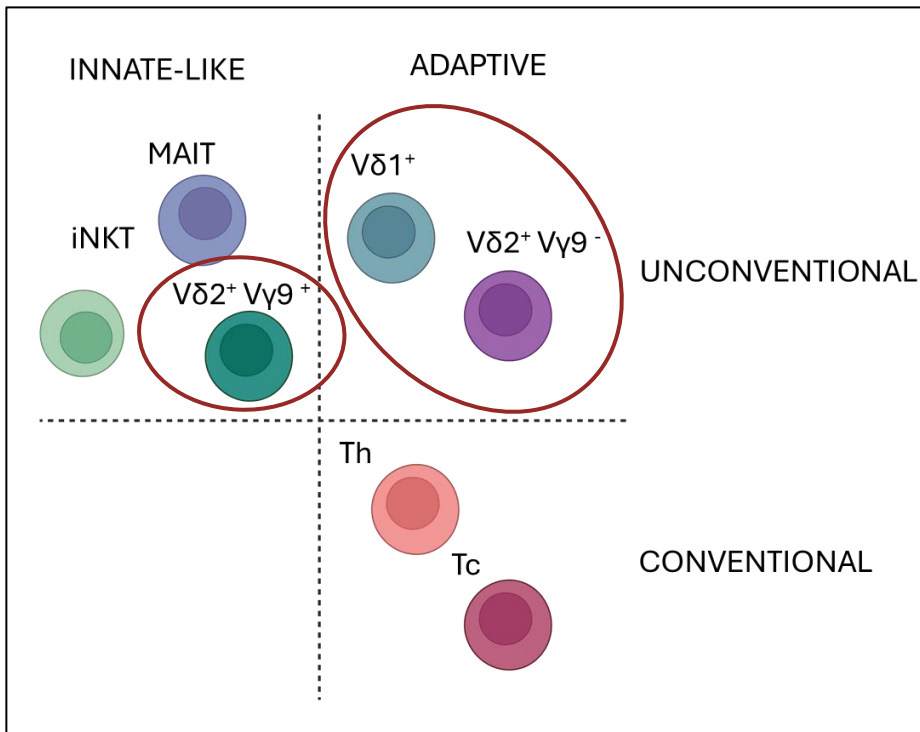


- ~10–30% of the T cell compartment
- enriched in mucosal and epithelial sites → skin, respiratory, digestive, and reproductive tracts
- rapid effector functions
- recognition of non-peptide ligands presented by non-polymorphic antigen presenting molecules
- TCR-independent activation



# $\gamma\delta$ T cells

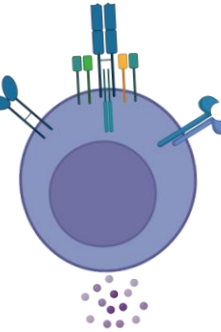
- 1-10% of peripheral T cells
- tissue homeostasis, anti-microbial response, stress surveillance, anti-tumor activity, autoimmunity



Human  $\gamma\delta$  T cells can be distinguished by  $\delta$  chain usage:

**$V\delta 2^+$**

$V\gamma 9$



- 50%–90% of the peripheral  $\gamma\delta$  T
- $V\gamma 9^+ V\delta 2^+$  /  $V\gamma 9^- V\delta 2^+$
- innate-like effector profile

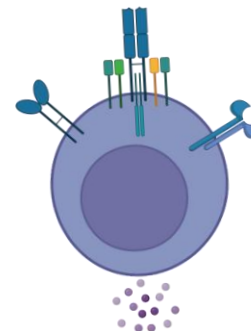
**$V\delta 1^+$**

$V\gamma 2/3/4/5/8/9$



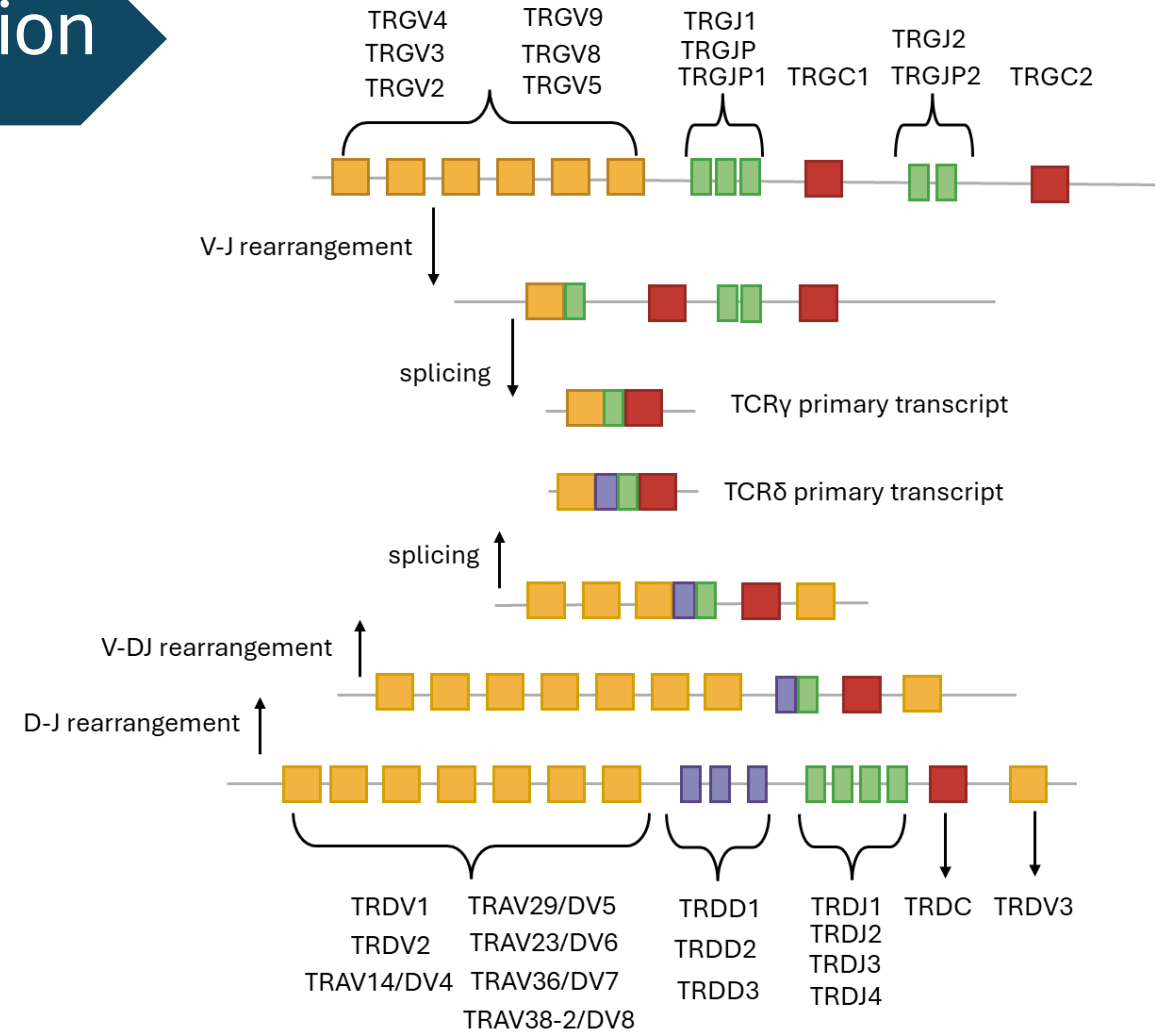
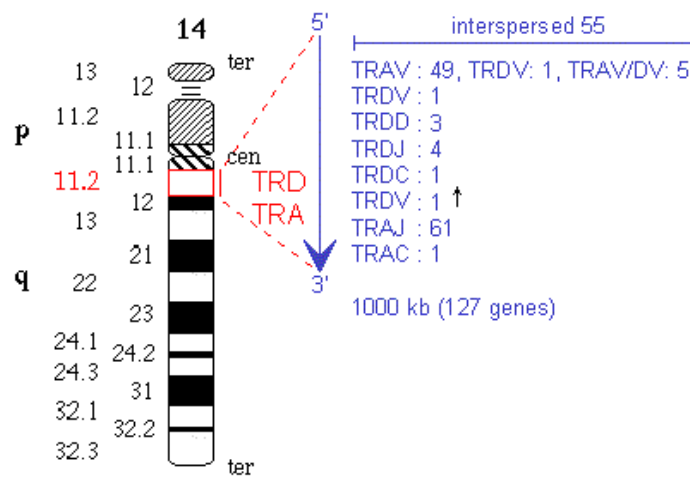
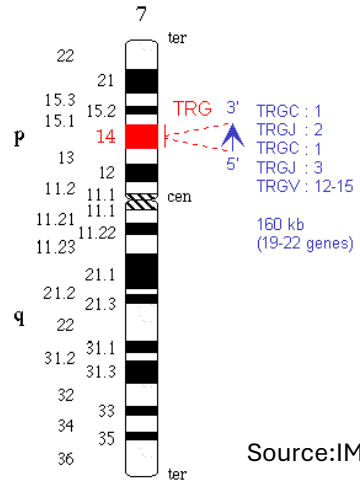
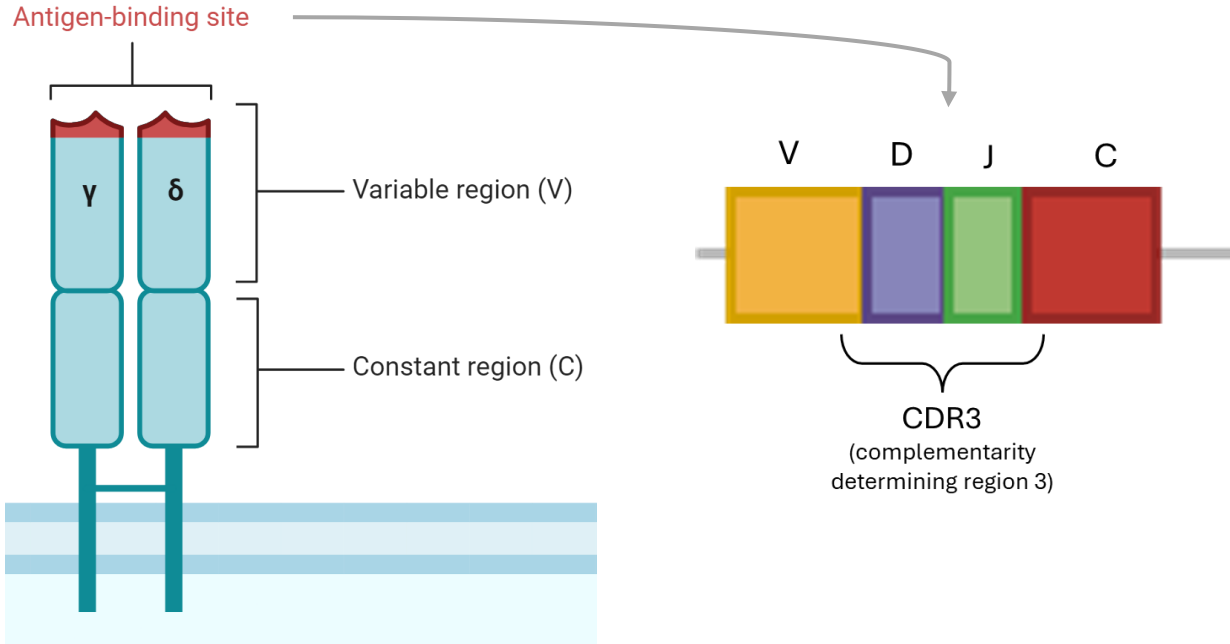
- up to ~ 30% of peripheral  $\gamma\delta$  T
- epithelial and mucosal sites → gut, skin, spleen, and liver
- adaptive immunobiology

**$V\delta 3^+$**



- <1% peripheral  $\gamma\delta$  T
- enriched in the liver and gut
- anti-microbial immunity
- ??

## $\gamma\delta$ TCR structure and V(D)J recombination

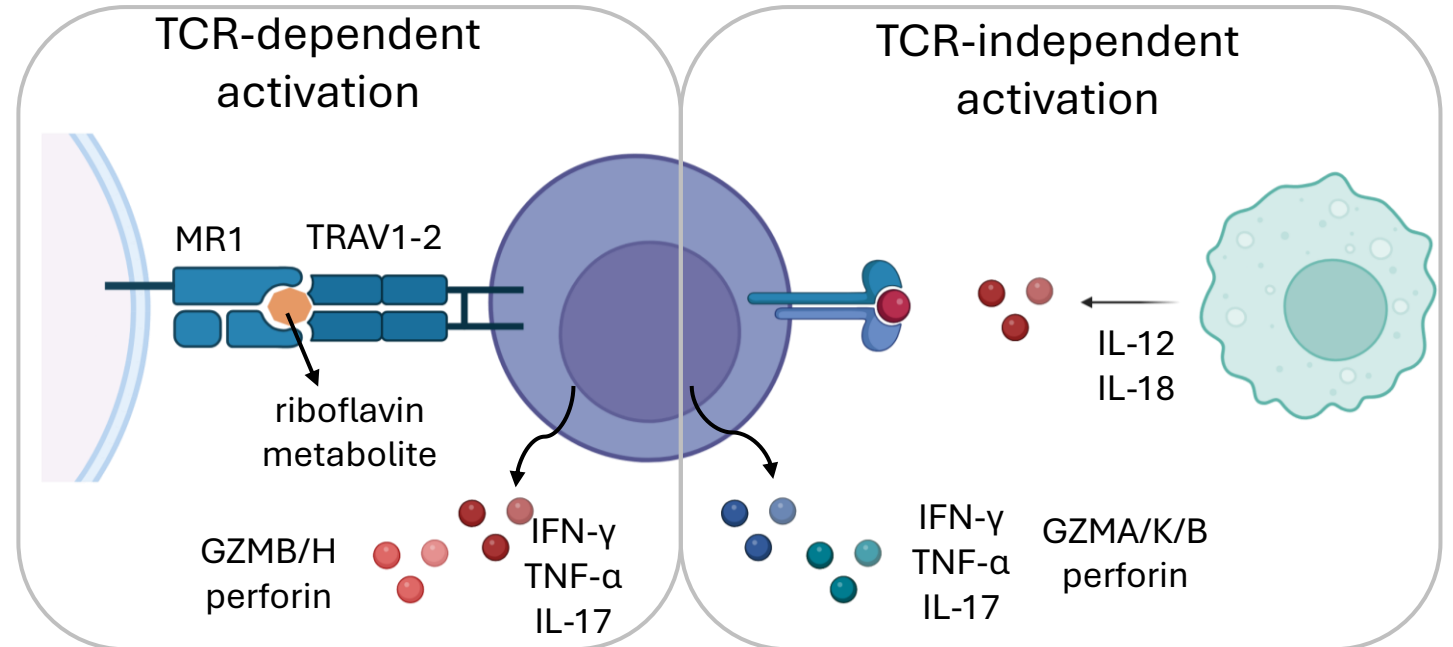
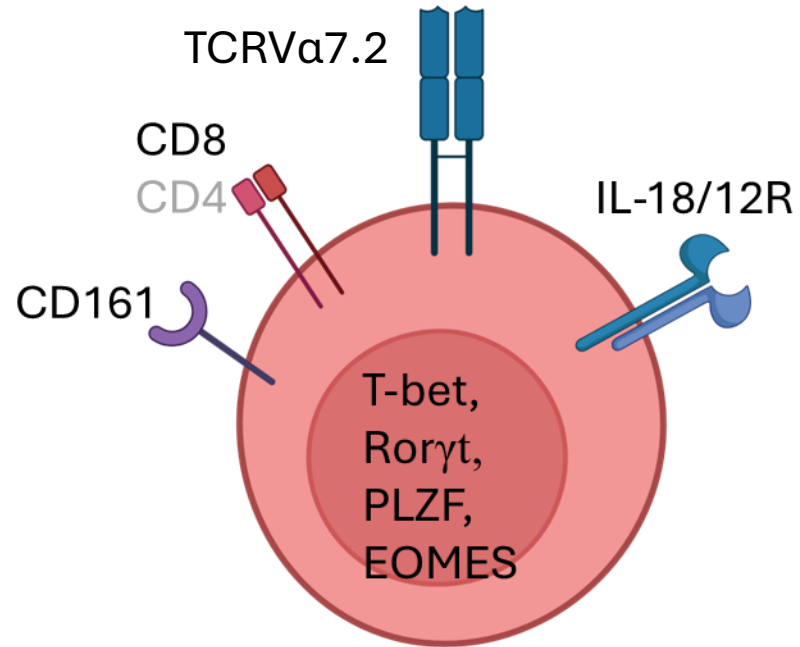


- diversity amplified by junctional diversity → insertion of palindromic sequences and of non-templated nucleotides

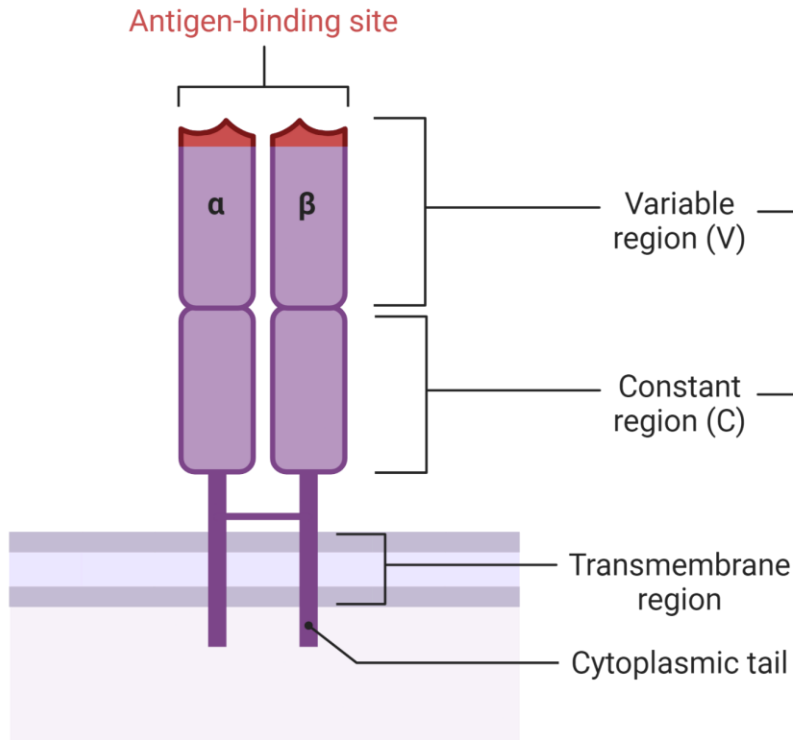
# MAIT cells

(mucosal-associated invariant T)

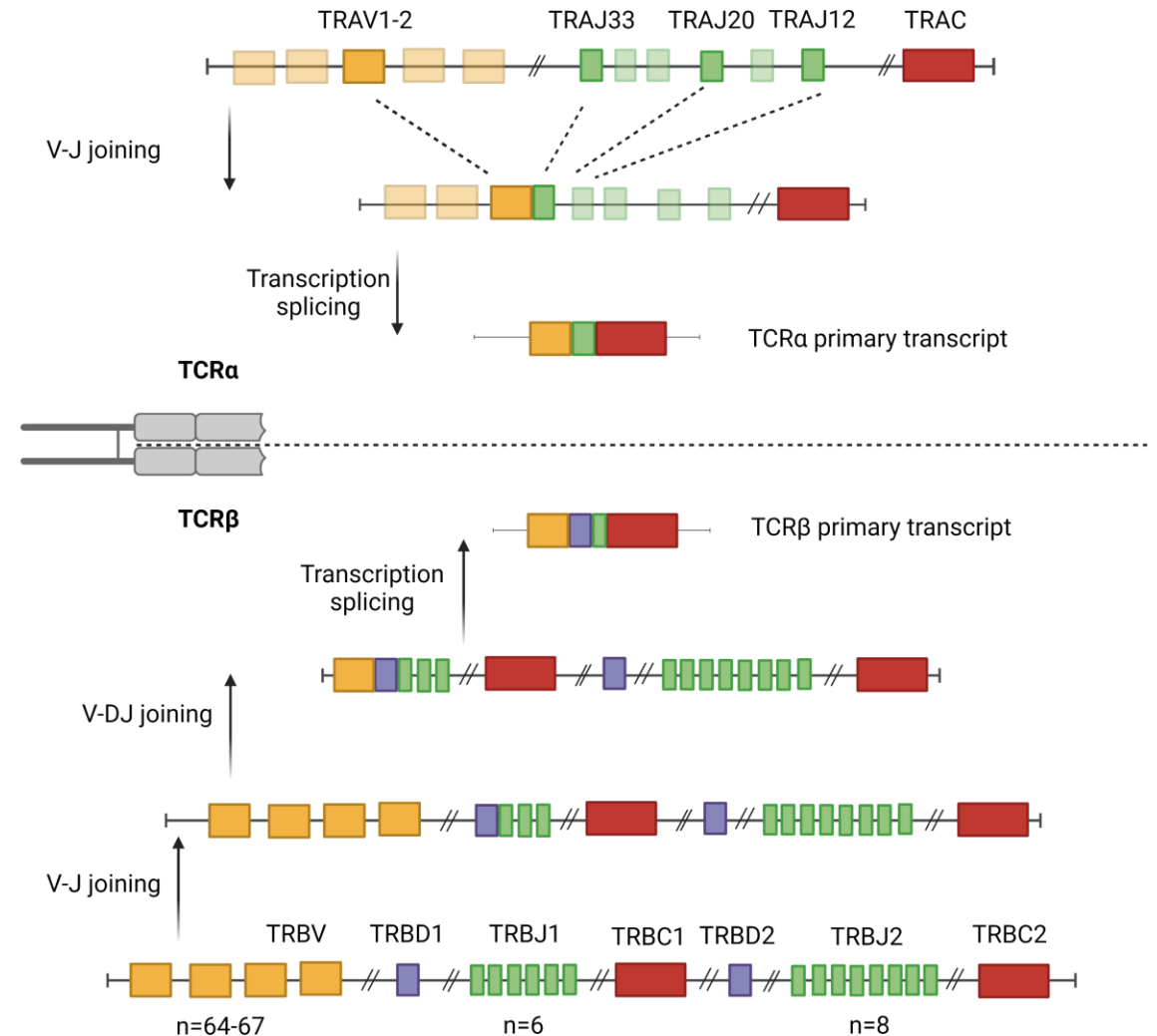
- Up to 5% of peripheral T cells
- subsets based on the CD4 /CD8 expression:
  - CD4-CD8+ → ~80% of total circulating MAIT cells
  - CD4-CD8- → ~ 15% of the total MAIT
  - CD4+CD8- / CD4+CD8+ → < 5%
- identified using MR1 tetramers loaded with the bacterial ligand 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU)
- variation in phenotype, function and TCR repertoire



# MAIT $\alpha\beta$ TCR structure and recombination



- semi-invariant TCR consisting of an invariantly rearranged TCR  $\alpha$  chain (**TRAV1-2** – TRAJ33/20/12), combined with diversely rearranged TCR  $\beta$  chain

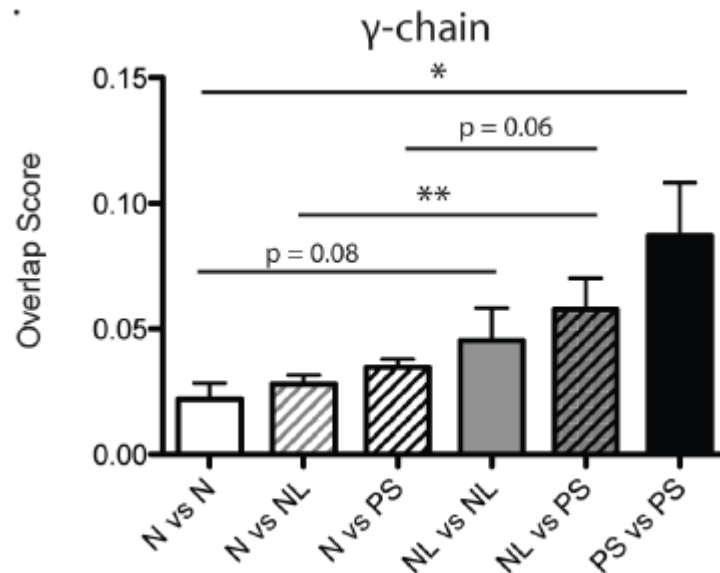


# T cells in psoriasis

## Deep Sequencing of the T-cell Receptor Repertoire Demonstrates Polyclonal T-cell Infiltrates in Psoriasis [version 1; referees: 2 approved]

Jamie L. Harden<sup>1</sup>, David Hamm<sup>2</sup>, Nicholas Gulati<sup>1</sup>, Michelle A. Lowes<sup>3</sup>,  
James G. Krueger<sup>1</sup>

- non-specific polyclonal infiltrate in affected skin and a higher level of shared, public TCR $\gamma$  clonotypes in patients with psoriasis



## Clinically resolved psoriatic lesions contain psoriasis-specific IL-17-producing $\alpha\beta$ T cell clones

Tiago R. Matos,<sup>1,2,3</sup> John T. O'Malley,<sup>1</sup> Elizabeth L. Lowry,<sup>1</sup> David Hamm,<sup>4</sup> Ilan R. Kirsch,<sup>4</sup> Harlan S. Robins,<sup>4</sup> Thomas S. Kupper,<sup>1</sup> James G. Krueger,<sup>5</sup> and Rachael A. Clark<sup>1</sup>

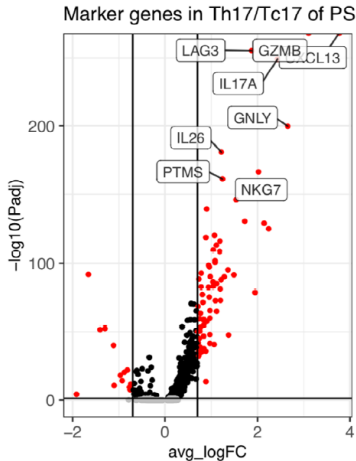
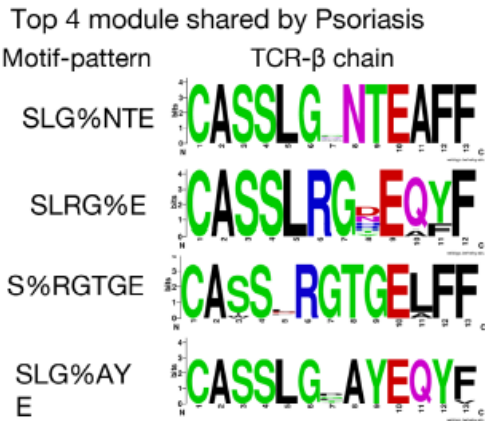
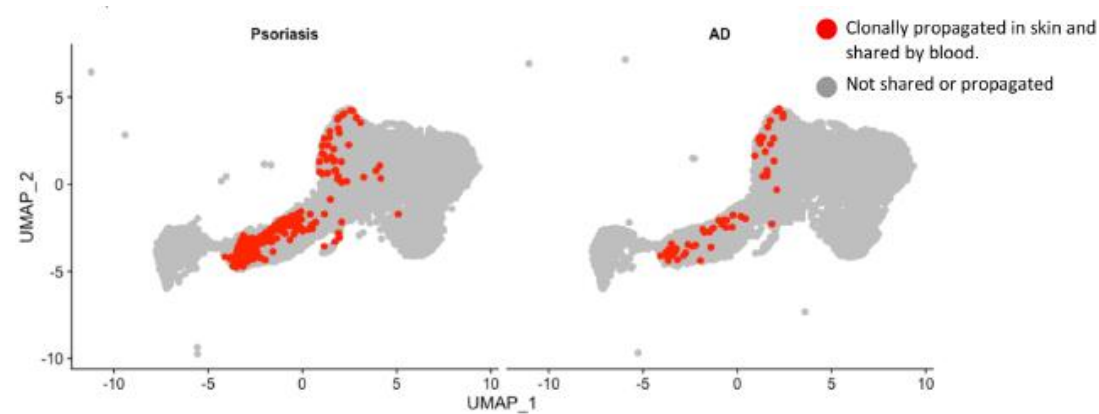
- clinically resolved psoriatic lesions contained oligoclonal populations of T cells that produced IL-17A in both resolved and active psoriatic lesions

|                                           | Shared in patients | Nucleotide sequences identical? | Present in healthy controls? |
|-------------------------------------------|--------------------|---------------------------------|------------------------------|
| <b>Shared TCR<math>\beta</math> CDR3</b>  |                    |                                 |                              |
| CASSQDLAGGPDQYF                           | 3, 13              | Yes                             | No                           |
| CSARGGNTIYF                               | 1, 9               | Yes                             | No                           |
| CASSISATGDEAFF                            | 3, 5               | Yes                             | No                           |
| CASSDRYLGGSNPLHF                          | 12, 14             | Yes                             | No                           |
| CASSPQETQYF                               | 3, 4               | No                              | No                           |
| CASSLVGQAYEQYF                            | 4, 13              | No                              | No                           |
| CASSESNQPHF                               | 11, 13             | No                              | No                           |
| CASSFYNEQFF                               | 3, 13              | No                              | No                           |
| CASSLGEKLFF                               | 9, 13              | No                              | No                           |
| CASSLGAGQPQHF                             | 8, 13              | No                              | No                           |
| CASSEGEQYF                                | 6, 8               | No                              | No                           |
| CASSPGPSYEQYF                             | 10, 12             | No                              | No                           |
| CASSPLAGSYNEQFF                           | 3, 10              | No                              | No                           |
| CASSATGYGYTF                              | 5, 11              | No                              | No                           |
| CASSLGTGELFF                              | 3, 11              | No                              | No                           |
| <b>Shared TCR<math>\alpha</math> CDR3</b> |                    |                                 |                              |
| CALSKAAGNKLTF                             | 1, 3               | Similar <sup>a</sup>            | No                           |
| CALRNTGGFKTIF                             | 1, 3               | Similar <sup>a</sup>            | No                           |
| CIVRVYGGATNKLIF                           | 7, 3               | Similar <sup>a</sup>            | No                           |
| CAVDSGGSNYKLTF                            | 9, 10              | No                              | No                           |

# Single-cell profiles reveal distinctive immune response in atopic dermatitis in contrast to psoriasis

Bowen Zhang<sup>1,2</sup> | Lennart M. Roesner<sup>3,4</sup> | Stephan Traidl<sup>3,4</sup> |  
Valerie A. C. M. Koeken<sup>1,2,5</sup> | Cheng-Jian Xu<sup>1,2,5</sup> | Thomas Werfel<sup>3,4</sup> | Yang Li<sup>1,2,4,5</sup>

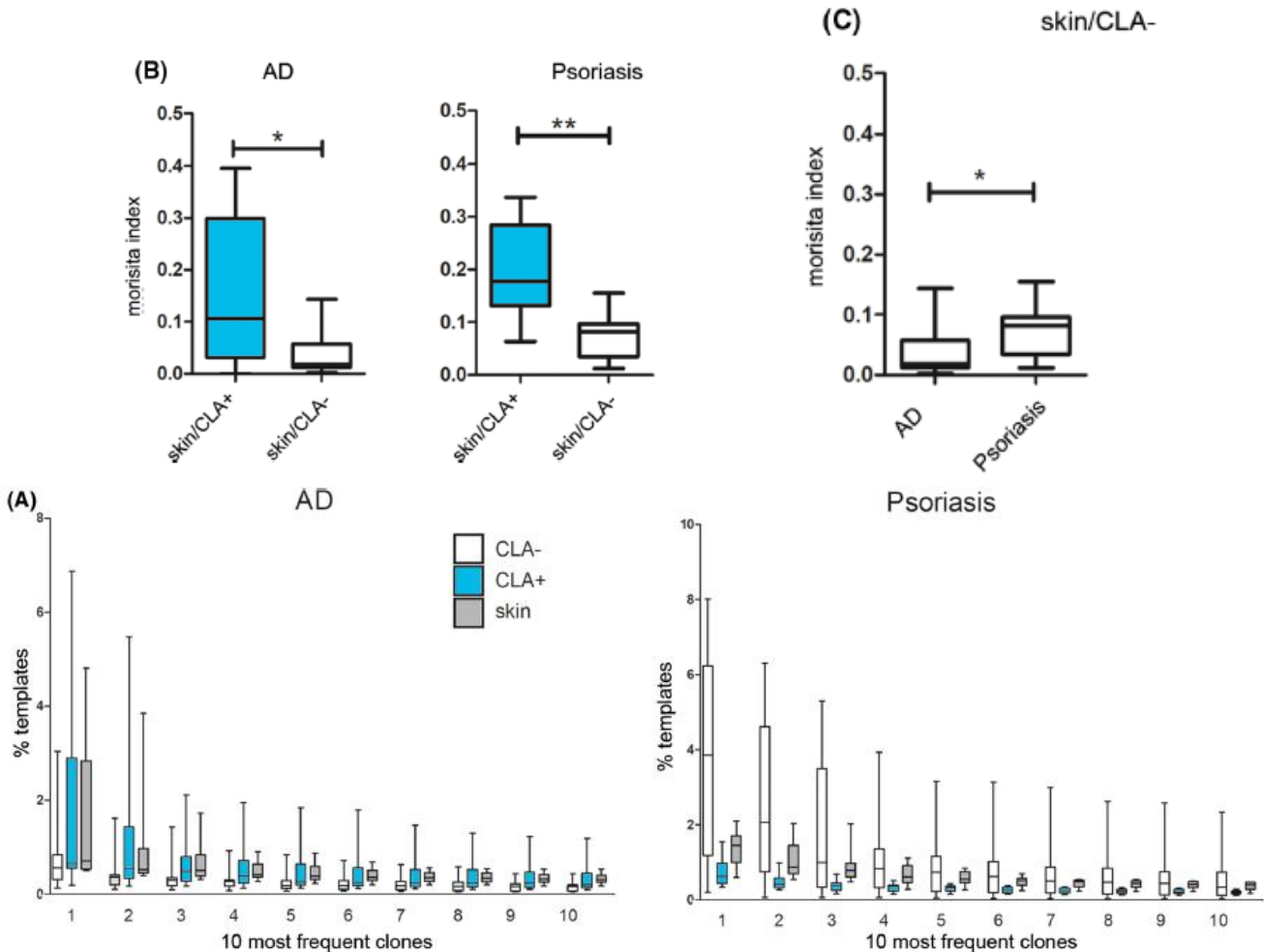
- a systemic involvement of type-3 inflammation in PV circulating cells



# T-cell receptor sequencing specifies psoriasis as a systemic and atopic dermatitis as a skin-focused, allergen-driven disease

Lennart M. Roesner<sup>1,2</sup> | Ahmed K. Farag<sup>1</sup> | Rebecca Pospich<sup>1</sup> | Stephan Traidl<sup>1,2</sup> |  
Thomas Werfel<sup>1,2</sup>

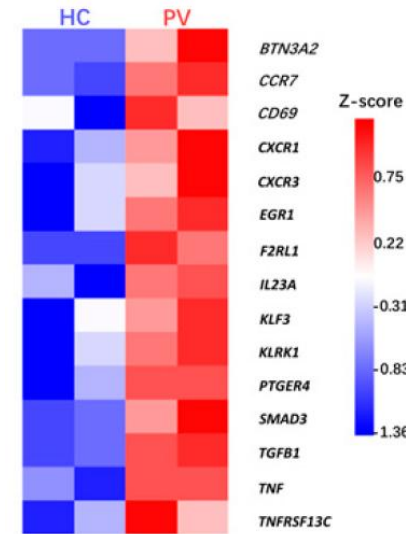
- T-cell clones that infiltrate psoriatic skin lesions do not exclusively possess skin-homing ability



# $\gamma\delta$ T cells in PV

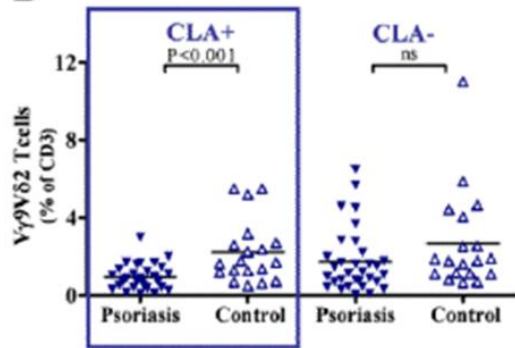
## Up-regulation of *BTN3A1* on $CD14^+$ cells promotes $V\gamma9V\delta2$ T cell activation in psoriasis

Jiaqing Zhou<sup>A1</sup>, Jingjing Zhang<sup>B,A,1</sup>, Lu Tao<sup>2</sup>, Kexin Peng<sup>2</sup>, Qiaoran Zhang<sup>2</sup>, Kexiang Yan<sup>2</sup>, Jing Luan<sup>2</sup>, Jiewen Pan<sup>2</sup>, Xiaohui Su<sup>B,C,D</sup>, Jiping Sun<sup>B,C,D</sup>, Zhenghua Zhang<sup>A,2</sup>, and Lei Shen<sup>B,C,D,2</sup>



- more than 2000 differentially expressed genes (DEGs) in peripheral  $V\gamma9V\delta2$  cells of affected individuals compared to healthy controls

B



## Identification of a novel pro-inflammatory human skin-homing $V\gamma9V\delta2$ T cell subset with a potential role in psoriasis

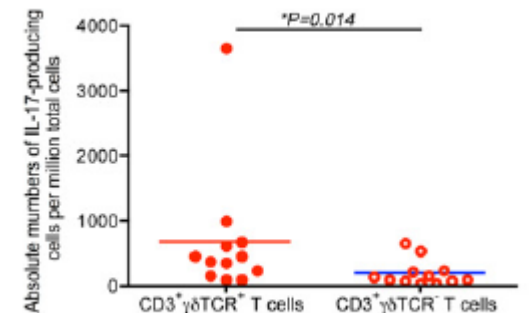
Ute LAGGNER<sup>\*</sup>, Paola DI MEGLIO<sup>\*</sup>, Gayathri K. PERERA<sup>\*</sup>, Christian HUNDHAUSEN<sup>\*</sup>, Katie E. LACY<sup>\*</sup>, Niwa ALI<sup>\*</sup>, Catherine H. SMITH<sup>†</sup>, Adrian C. HAYDAY<sup>‡</sup>, Brian J. NICKOLOFF<sup>§</sup>, and Frank O. NESTLE<sup>\*</sup>

- changes in the distribution of the skin-homing CLA+CCR6+  $V\gamma9V\delta2$
- infiltration into the affected skin

## Pivotal Role of Dermal IL-17-Producing $\gamma\delta$ T Cells in Skin Inflammation

Yihua Cai,<sup>1,2</sup> Xiaoyan Shen,<sup>1</sup> Chuanlin Ding,<sup>2</sup> Chunjian Qi,<sup>2</sup> Kejia Li,<sup>1</sup> Xia Li,<sup>1</sup> Venkatakrishna R. Jala,<sup>2</sup> Huang-ge Zhang,<sup>2</sup> Tian Wang,<sup>3</sup> Jie Zheng,<sup>1,\*</sup> and Jun Yan<sup>2,\*</sup>

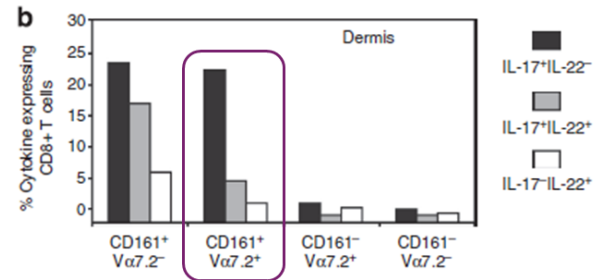
- increased prevalence of  $\gamma\delta$  T cells in the dermis of psoriatic plaques that expressed higher levels of IL-17



# MAIT cells in PV

## The IL-17A-Producing CD8<sup>+</sup> T-Cell Population in Psoriatic Lesional Skin Comprises Mucosa-Associated Invariant T Cells and Conventional T Cells

Marcel B.M. Teunissen<sup>1</sup>, Nataliya G. Yermenko<sup>2</sup>, Dominique L.P. Baeten<sup>2</sup>, Saskia Chielie<sup>1</sup>, Phyllis I. Spuls<sup>1</sup>, Menno A. de Rie<sup>1</sup>, Olivier Lantz<sup>3</sup> and Pieter C.M. Res<sup>1</sup>

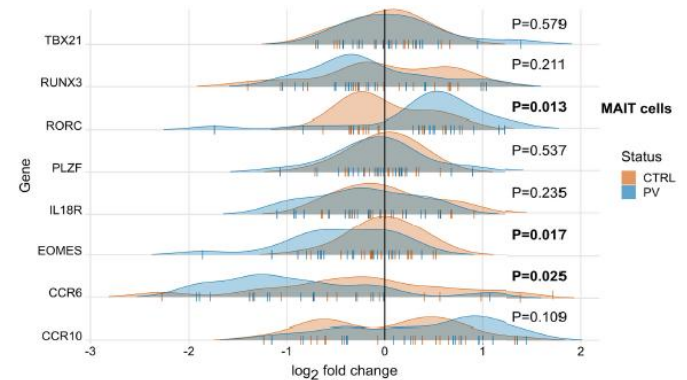
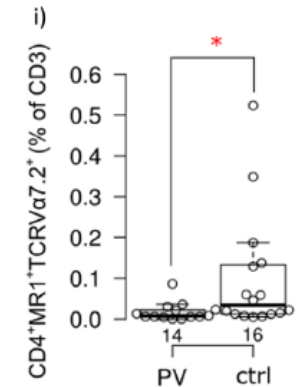


- IL-17A expression in TCR-Va7.2+CD161+ cells within the psoriatic plaque-derived CD8+ lymphocytes

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

Vera Plužarić<sup>1,2†</sup>, Mario Štefanić<sup>3,4†</sup>, Martina Mihalj<sup>2,4</sup>, Maja Tolušić Levak<sup>2,5</sup>, Ivanka Muršić<sup>2</sup>, Ljubica Glavaš-Obrovac<sup>1</sup>, Martin Petrek<sup>6</sup>, Peter Balogh<sup>7</sup> and Stana Tokić<sup>1\*</sup>

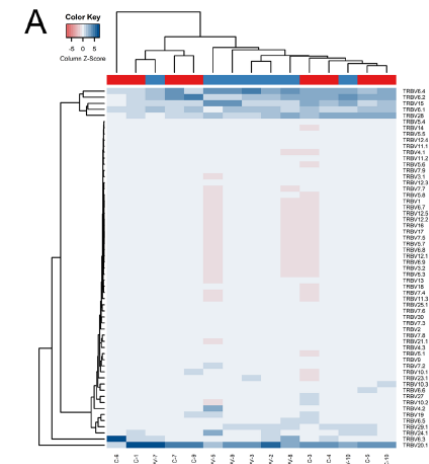
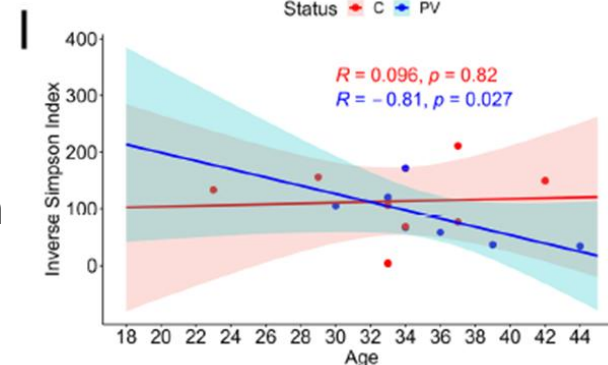
- decrease in the proportion of CD4<sup>+</sup> MR1-tet+TCRVa7.2<sup>+</sup> cells
- altered expression of the EOMES, CCR6, and ROR $\gamma$ t



## Characterization of the TCR $\beta$ repertoire of peripheral MR1-restricted MAIT cells in psoriasis vulgaris patients

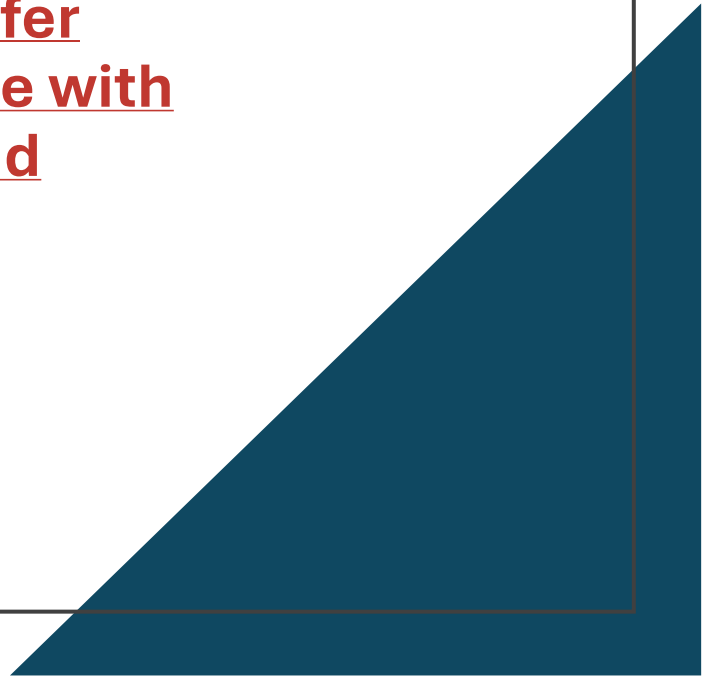
Maja Jirouš Drulak<sup>1,2,3</sup>, Zvonimir Grgić<sup>2</sup>, Vera Plužarić<sup>2,3</sup>, Marija Šola<sup>3</sup>, Teuta Opačak-Bernardi<sup>1</sup>, Barbara Viljetić<sup>1</sup>, Kristina Glavaš<sup>4</sup>, Maja Tolušić-Levak<sup>3,5</sup>, Vlatka Periša<sup>6,7</sup>, Martina Mihalj<sup>3,8</sup>, Mario Štefanić<sup>2,3,5</sup> & Stana Tokić<sup>1,2,3,5</sup>

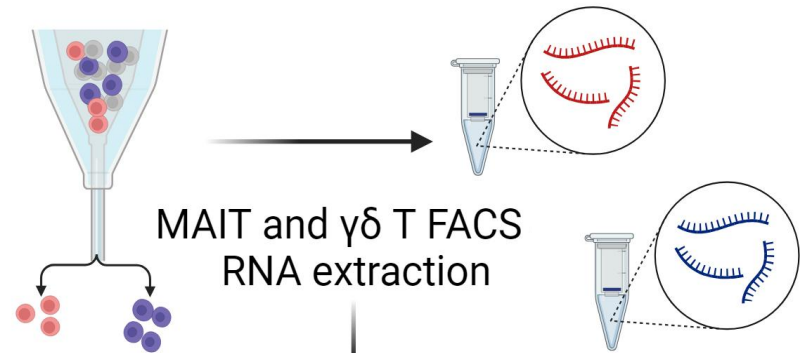
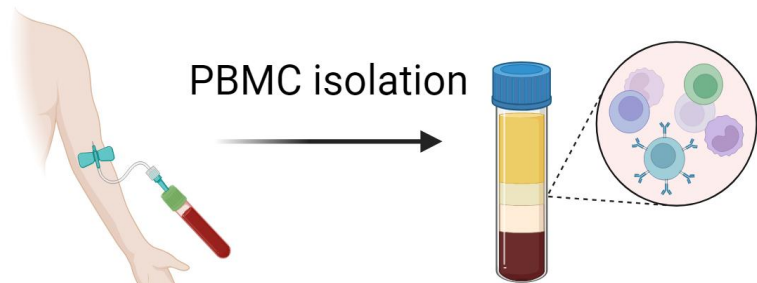
- high diversity in TCR $\beta$  clonotypic variants
- hyperexpanded clonotypes increasing with age of PV but not healthy individuals



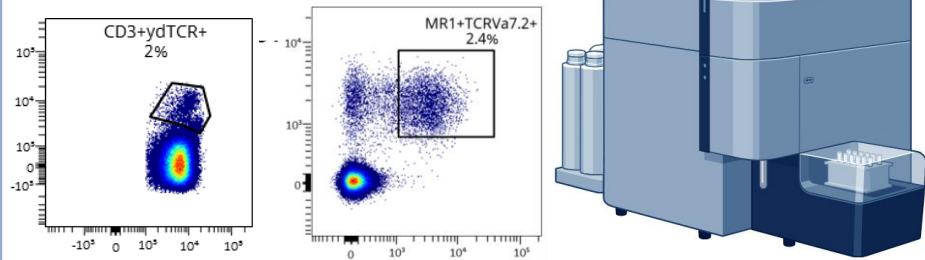
# Hypothesis

- The **peripheral proportions, phenotype and gene expression** of immune markers related to activation, differentiation, migration and effector capacity, as well as **the clonal repertoire of T cell receptors** of circulating MALT and  $\gamma\delta$  T lymphocytes in patients with psoriasis vulgaris **differ significantly from the healthy controls, and correlate with anthropometric and biochemical characteristics and indicators of disease severity.**

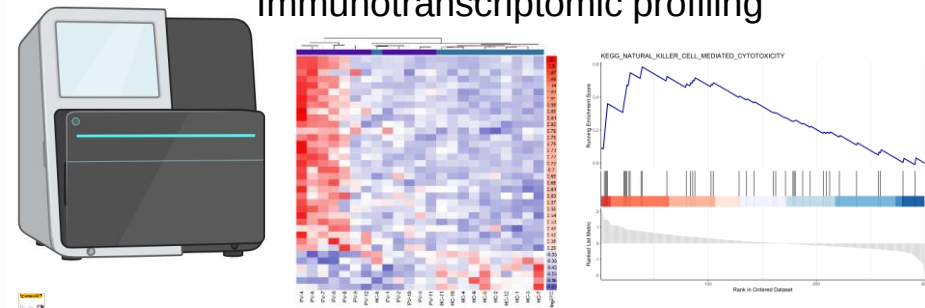




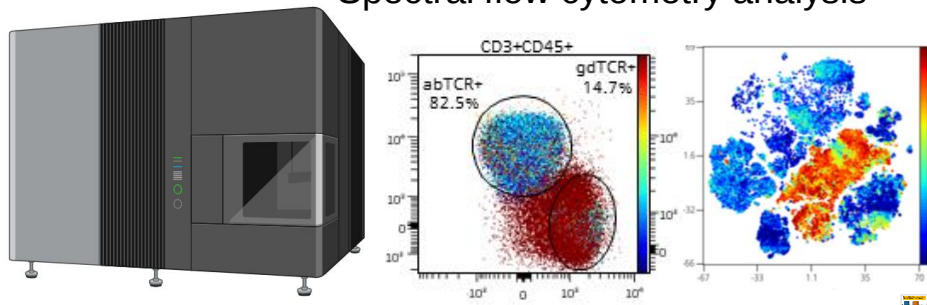
Flow cytometry  
immunophenotyping



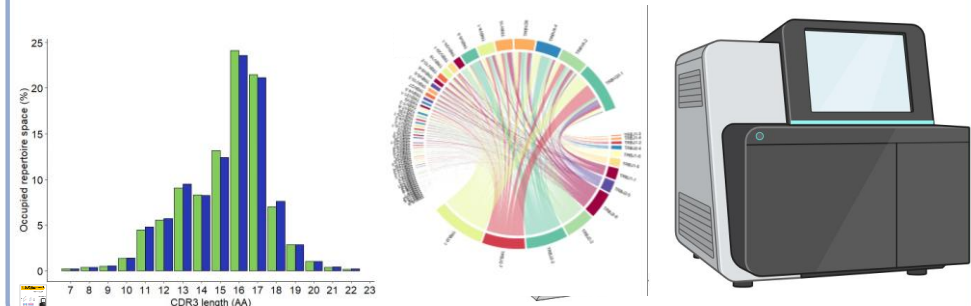
Immunotranscriptomic profiling



Spectral flow cytometry analysis



TCR repertoire analysis



# Sample collection

## PBMC isolation

Department of Dermatology and  
Venereology Clinical Hospital Centre Osijek

Therapy-naïve 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)



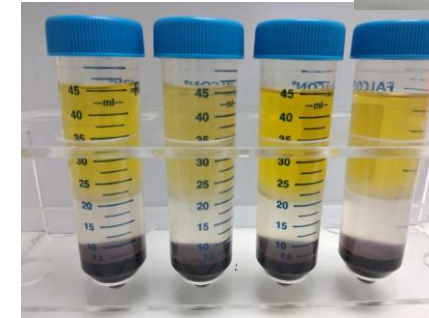
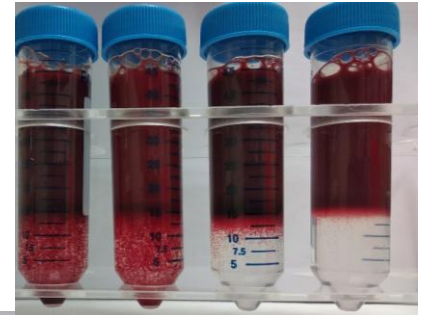
PBMC isolation

haematological  
and biochemical  
lab tests

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

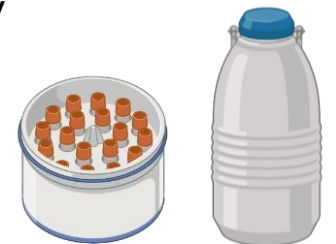
20 ml heparinised blood

Lymphoprep  
medium density  
gradient  
centrifugation



Cell  
counting

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



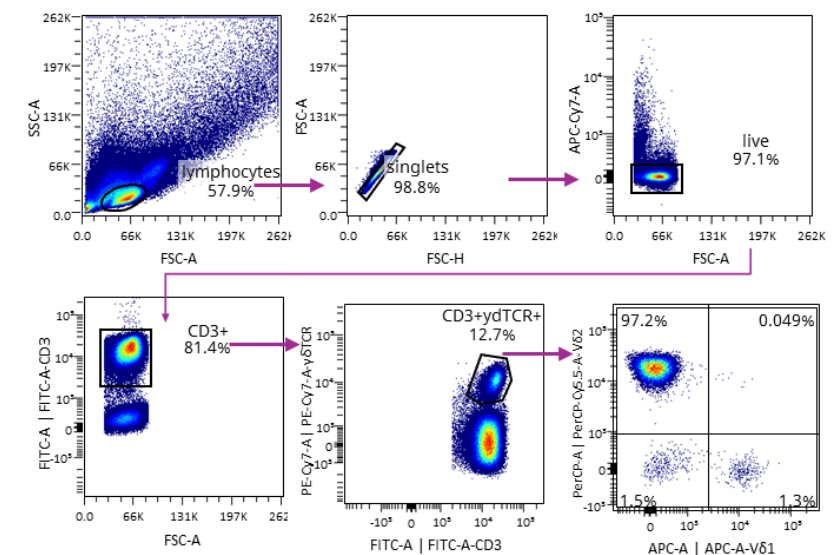
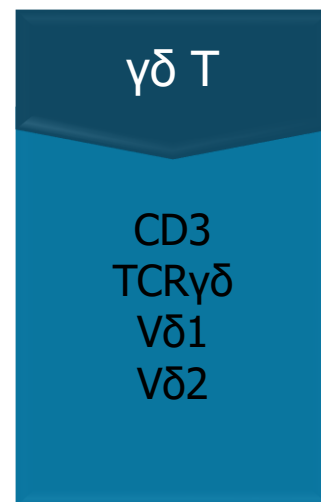
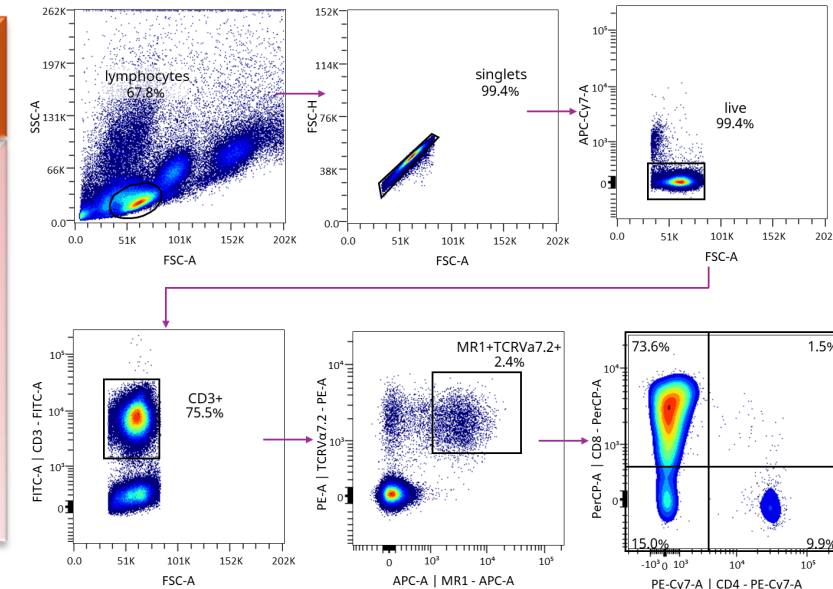
# Analysis of the frequencies of MAIT and $\gamma\delta$ T lymphocyte populations and subpopulations by flow cytometry

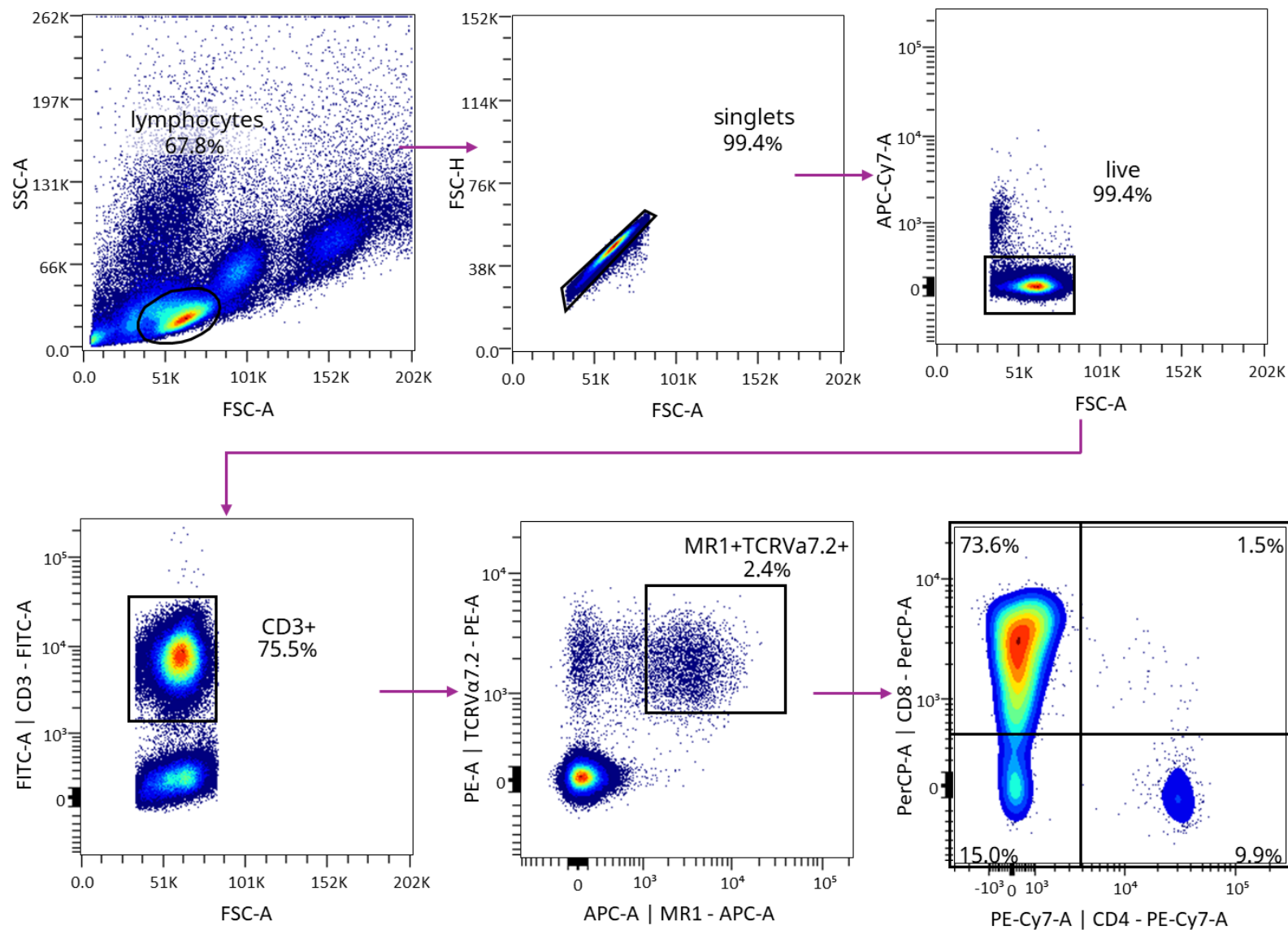
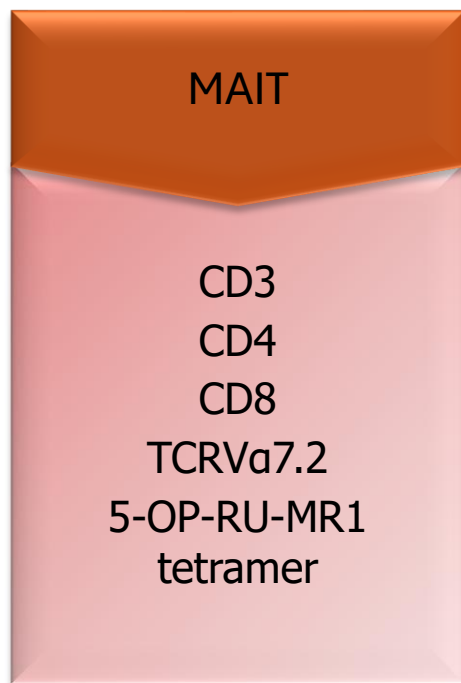
- to determine the relative frequency of peripheral MAIT and  $\gamma\delta$  T lymphocyte subpopulations
- a minimum of 90 subjects (60 PV + 30 HC)

BD FACS Canto II flow cytometer



Szentágothai Research Centre in Pécs





# Analysis of the frequencies of MAIT and $\gamma\delta$ T lymphocyte populations and subpopulations by flow cytometry

- to determine the relative frequency of peripheral MAIT and  $\gamma\delta$  T lymphocyte subpopulations
- 90 subjects (60 PV + 30 HC)

BD FACS Canto II flow cytometer

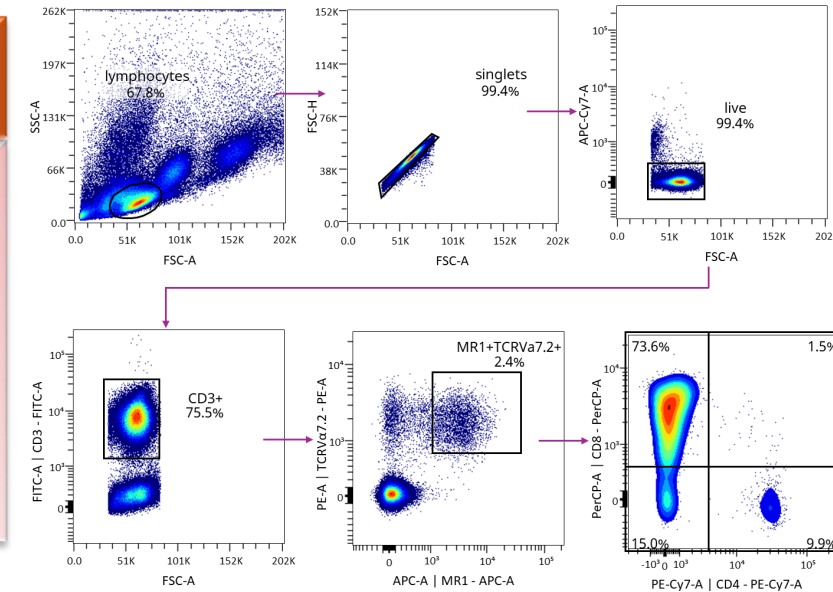


Szentágotthai Research Centre in Pécs



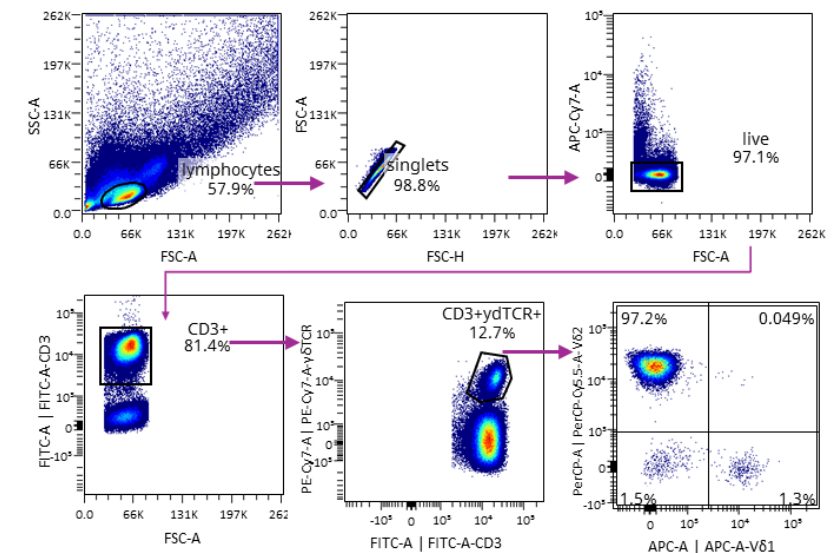
MAIT

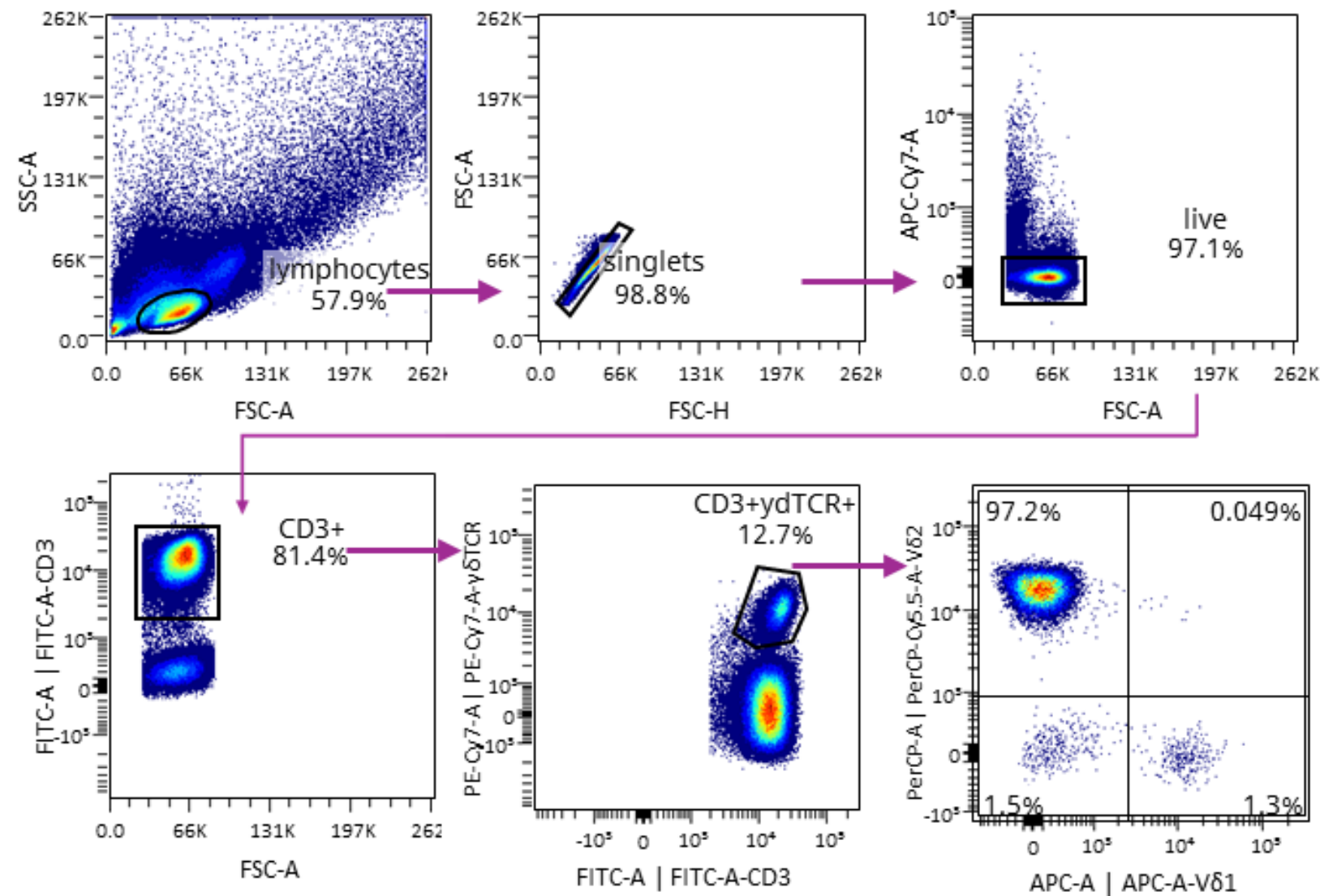
CD3  
CD4  
CD8  
TCRVa7.2  
5-OP-RU-MR1  
tetramer



$\gamma\delta$  T

CD3  
TCR $\gamma\delta$   
V $\delta$ 1  
V $\delta$ 2





# Single-cell analysis of surface and intracellular markers of MAIT and $\gamma\delta$ T lymphocytes by spectral flow cytometry

## Surface panel

- live/dead, CD3, CD45, CD4, CD8
- $\alpha\beta$ TCR, TCRVa7.2, MR1-tet, CD161
  - $\gamma\delta$ TCR, V $\delta$ 1, V $\delta$ 2, V $\delta$ 3, V $\gamma$ 9
- CD25, CD69, HLA-DR, CD38, CD28
  - CD127, CD27, CD45RA
  - CD11a, CD103, CCR7, CCR6
- CXCR3, CXCR5, CX3CR1, CD57, PD-1
  - CD16, CD56, CD94
  - CLA

## Intracellular panel

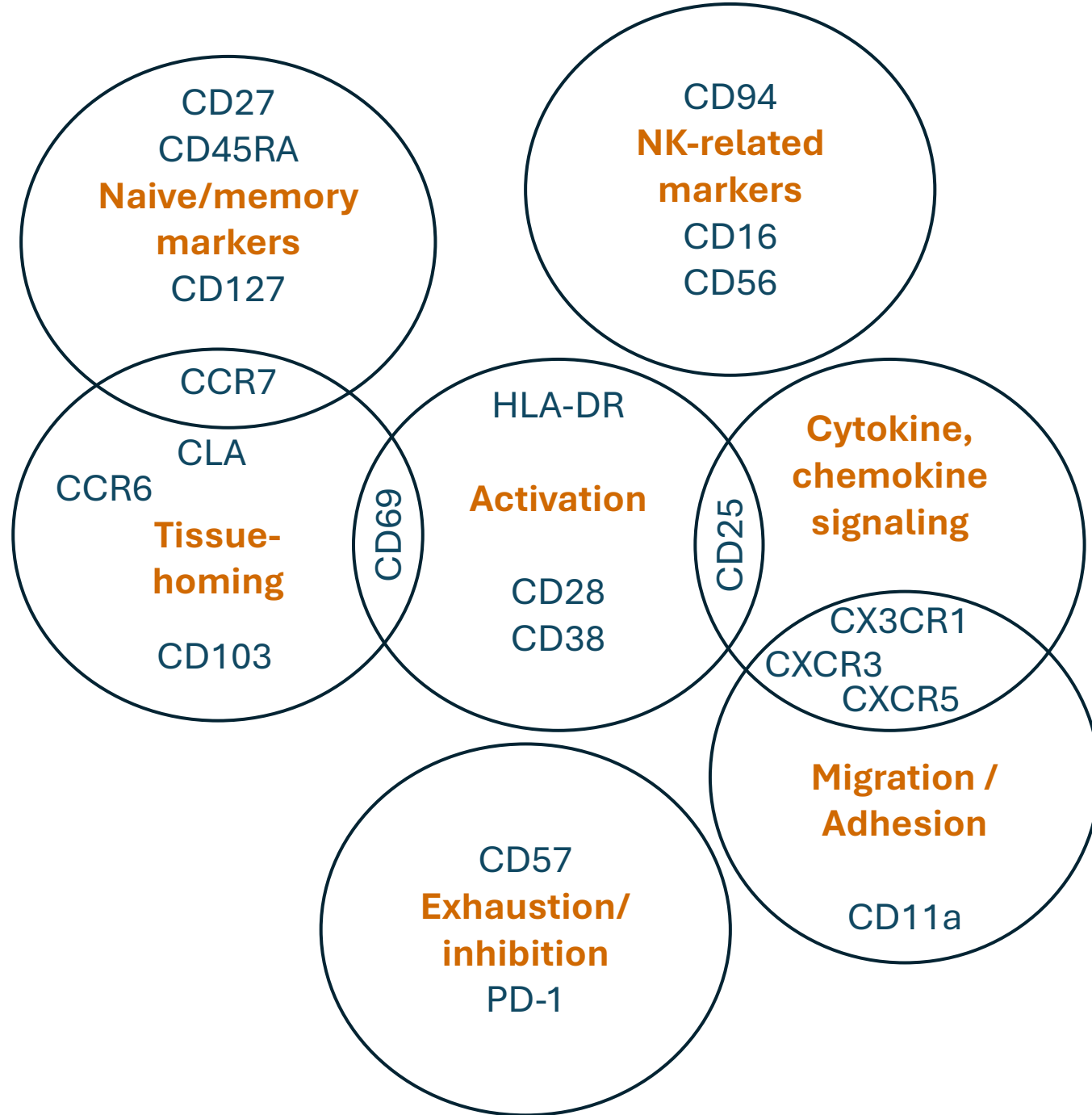
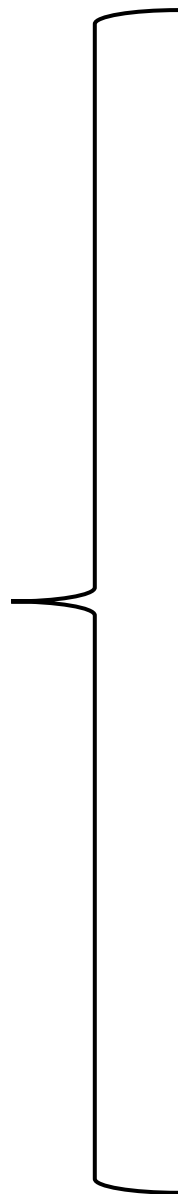
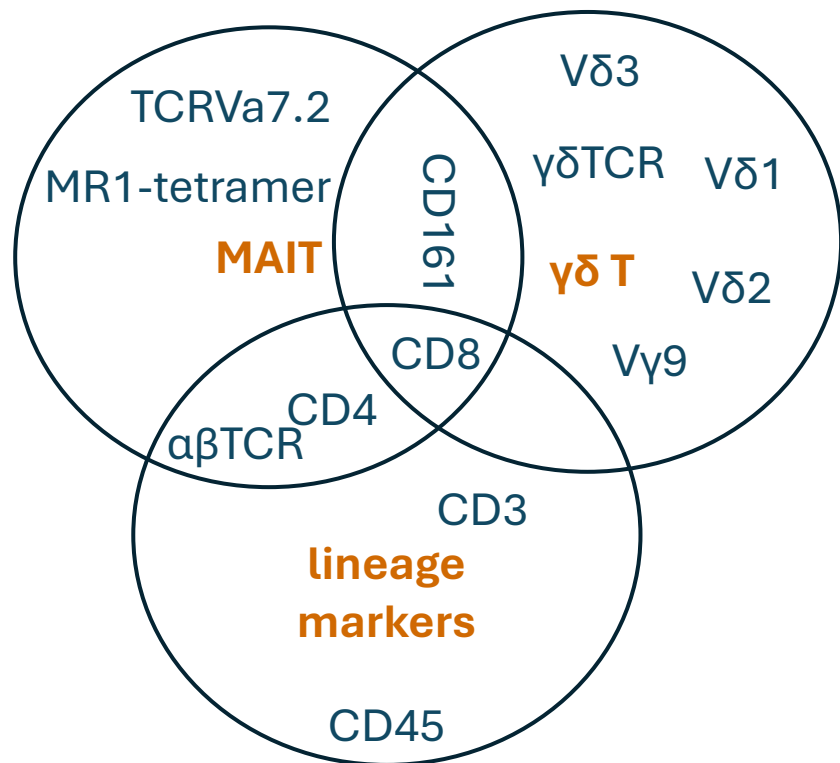
- CD3, CD4, CD8a,  $\alpha\beta$ TCR
  - $\gamma\delta$ TCR, V $\delta$ 1, V $\delta$ 2, V $\delta$ 3
  - CD16, CD56,
  - CD11a, CX3CR1, PD-1
- CD27, CD28, CD45RA, CD127
  - T-bet, EOMES, TOX, BLIMP-1
- GzmA, GzmB, GzmK, granulysin, perforin
  - Ki67

- ▶ 25 samples (15 PV + 10 HC)
- ▶ Spectral analyser Sony ID7000 at University of Warwick Medical School



- ▶ OMIQ platform for analysis

# Surface panel



# Single-cell analysis of surface and intracellular markers of MAIT and $\gamma\delta$ T lymphocytes by spectral flow cytometry

## Surface panel

- live/dead, CD3, CD45, CD4, CD8
- $\alpha\beta$ TCR, TCRVa7.2, MR1-tet, CD161
  - $\gamma\delta$ TCR, V $\delta$ 1, V $\delta$ 2, V $\delta$ 3, V $\gamma$ 9
- CD25, CD69, HLA-DR, CD38, CD28
  - CD127, CD27, CD45RA
  - CD11a, CD103, CCR7, CCR6
- CXCR3, CXCR5, CX3CR1, CD57, PD-1
  - CD16, CD56, CD94
  - CLA

## Intracellular panel

- CD3, CD4, CD8a,  $\alpha\beta$ TCR
  - $\gamma\delta$ TCR, V $\delta$ 1, V $\delta$ 2, V $\delta$ 3
  - CD16, CD56,
  - CD11a, CX3CR1, PD-1
- CD27, CD28, CD45RA, CD127
  - T-bet, EOMES, TOX, BLIMP-1
- GzmA, GzmB, GzmK, granulysin, perforin
  - Ki67

- ▶ 25 samples (15 PV + 10 HC)
- ▶ Spectral analyser Sony ID7000 at University of Warwick Medical School



- ▶ OMIQ platform for analysis

## Intracellular panel

### Surface markers

### Intracellular markers

CD3  
CD8a  
**lineage markers**  
CD4  
 $\alpha\beta$ TCR

$\gamma\delta$ TCR  
V $\delta$ 1  
 **$\gamma\delta$  T**  
V $\delta$ 2  
V $\delta$ 3

CD56  
**NK-related markers**  
CD16

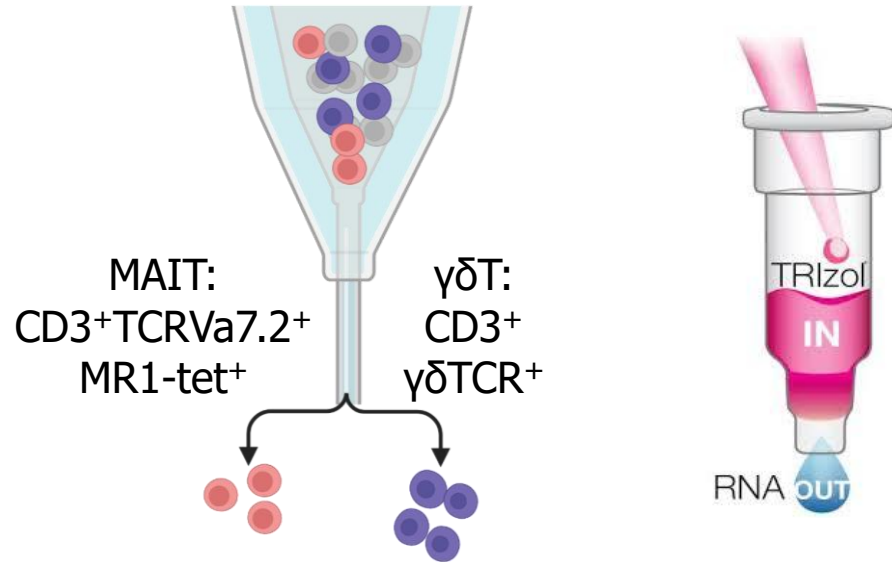
CD11a PD-1  
**Adhesion, activation, differentiation**  
CD28 CX3CR1

CD27 CD127  
**Naive/memory markers**  
CD45RA

BLIMP-1  
T-bet EOMES  
**Differentiation & proliferation**  
TOX  
Ki67

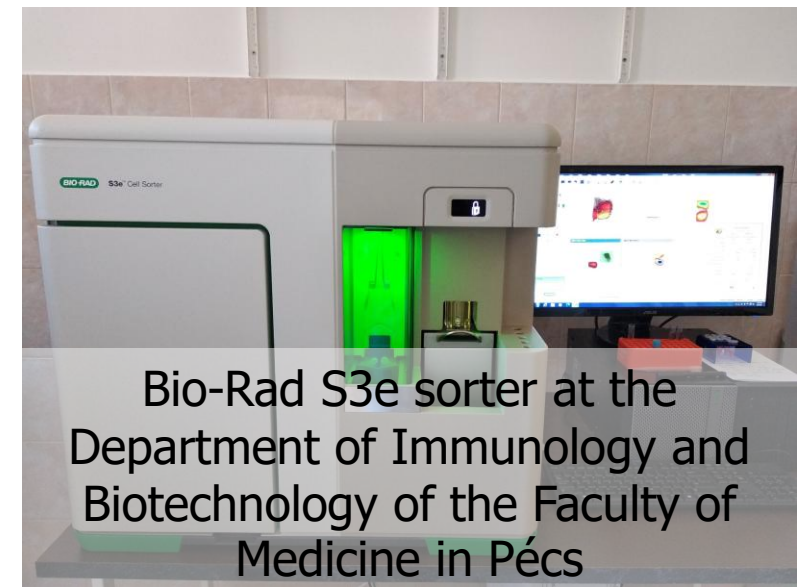
GzmA GzmB  
**Cytotoxic granules**  
GzmK perforin  
granulysin

# Fluorescence-activated cell sorting of peripheral MAIT and $\gamma\delta$ T lymphocytes and total RNA extraction



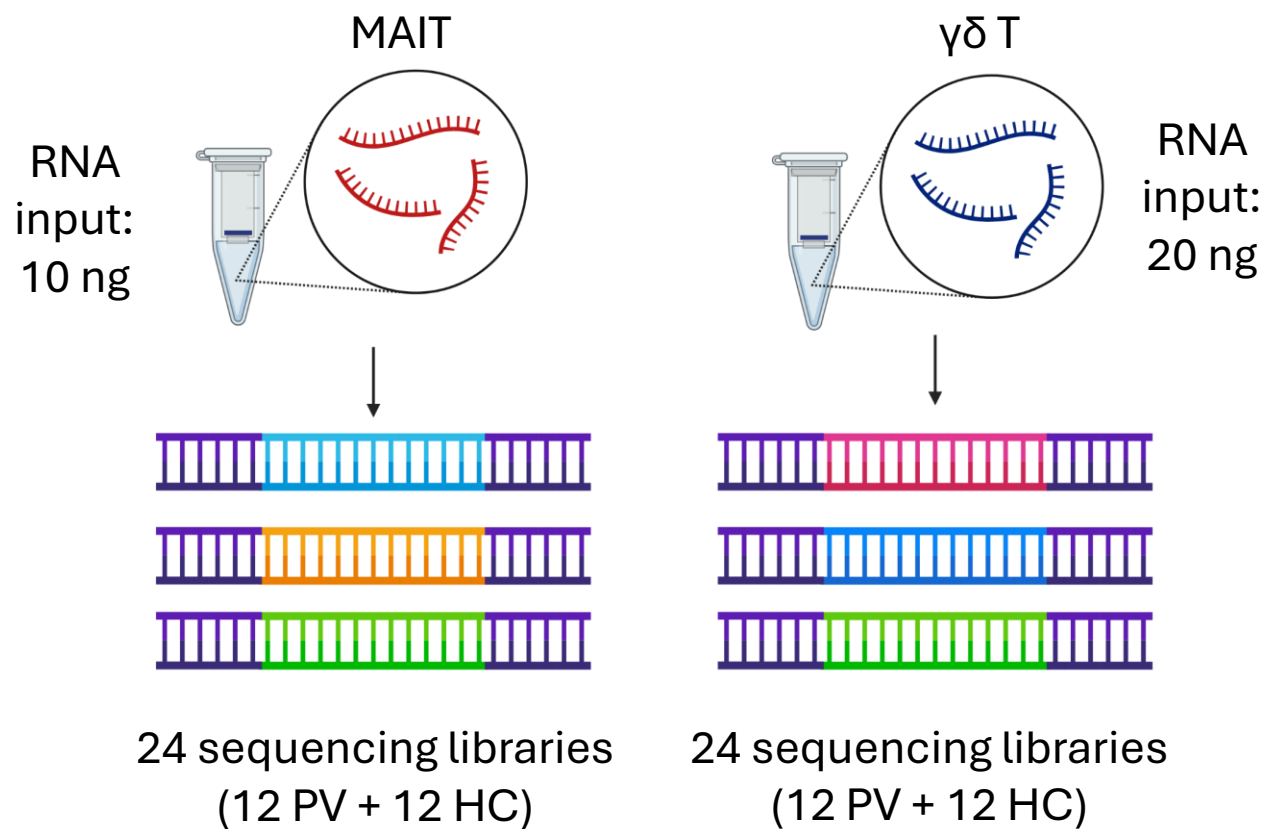
- Cells directly sorted into TRIzol™ reagent
- total RNA extraction - Direct-zol commercial kit

|                                     | Me (Q1, Q3)           |
|-------------------------------------|-----------------------|
| <b>PBMC input (x10<sup>6</sup>)</b> | 12.9 (10.6, 15.9)     |
| Sorted $\gamma\delta$ T             | 140760 (71220,249827) |
| $\gamma\delta$ T extracted RNA (ng) | 106 (51,197)          |
| Sorted MAIT                         | 37198 (12166, 69199)  |
| MAIT extracted RNA (ng)             | 45 (27-75)            |



# Immunotranscriptomic profiling analysis of MAIT and $\gamma\delta$ T lymphocytes by RNA sequencing

- Immune Response Panel (Illumina)
- 395 immunologically relevant genes



Miniseq Illumina sequencer,  
Faculty of Medicine Osijek



# Bioinformatic analysis

 **Galaxy**



STAR

RNA-seq aligner  
hg38 genome  
reference



featureCounts

Measure gene  
expression in  
RNA-Seq  
experiments from  
SAM or BAM files



DEseq2

Test for differential  
expression by use  
of negative  
binomial  
generalized linear  
models

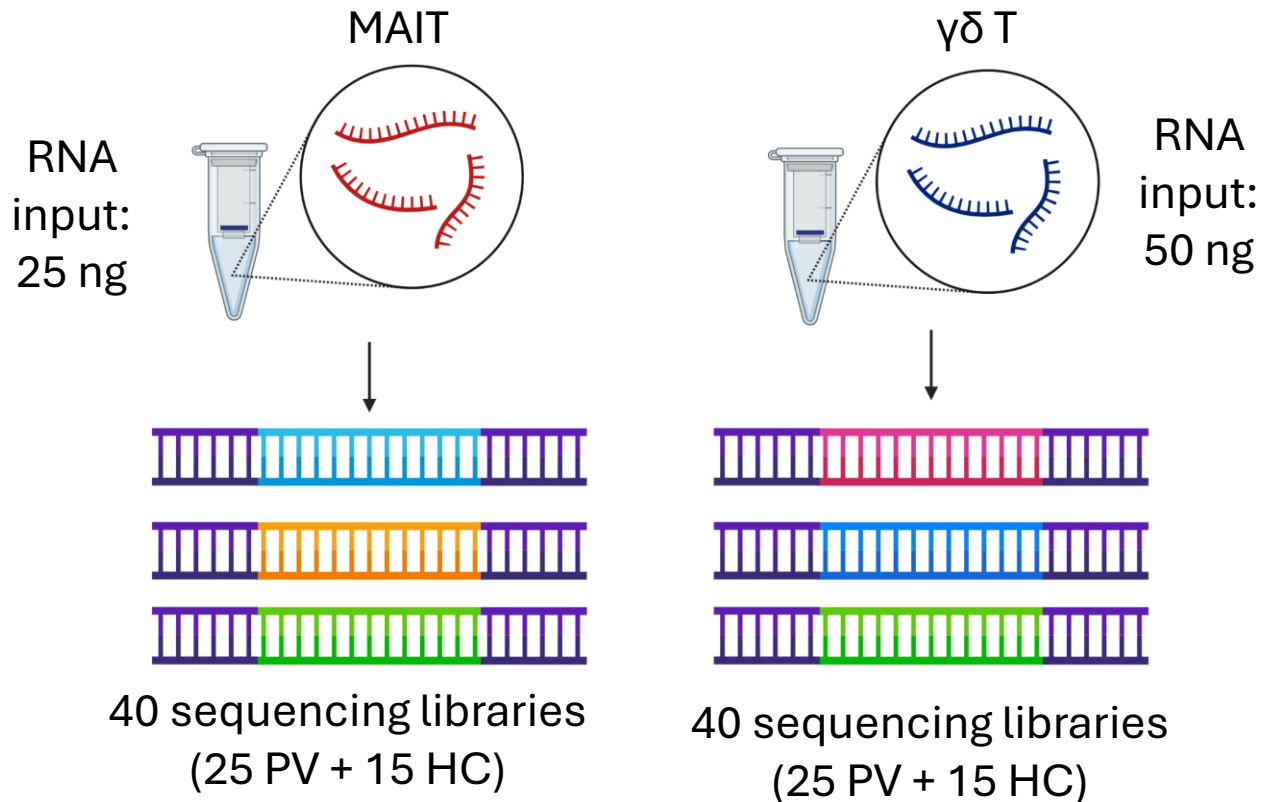


clusterProfiler

Statistical analysis  
and visualisation of  
functional profiles for  
genes and gene  
clusters

# The analysis of T-cell receptor repertoires of MAIT and $\gamma\delta$ T lymphocytes

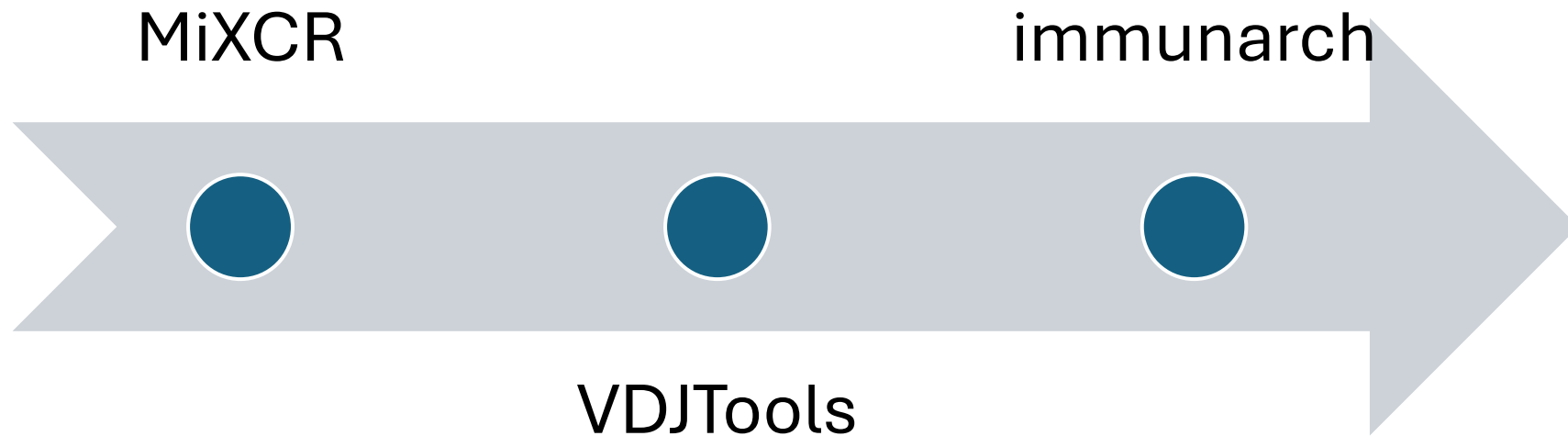
- Archer® Immunoverse™-HS TCR kit
- simultaneous sequencing of the CDR3 regions of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -chains



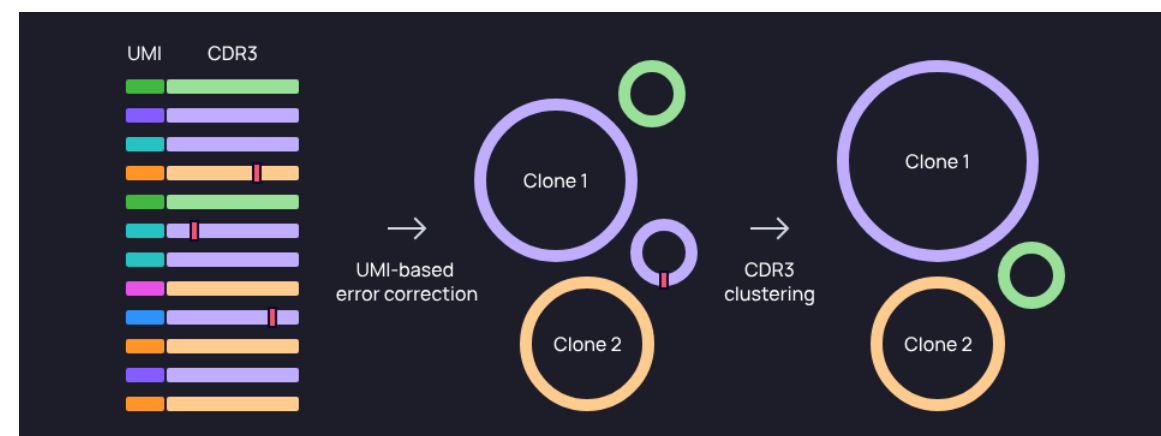
NextSeq 550 Illumina platform



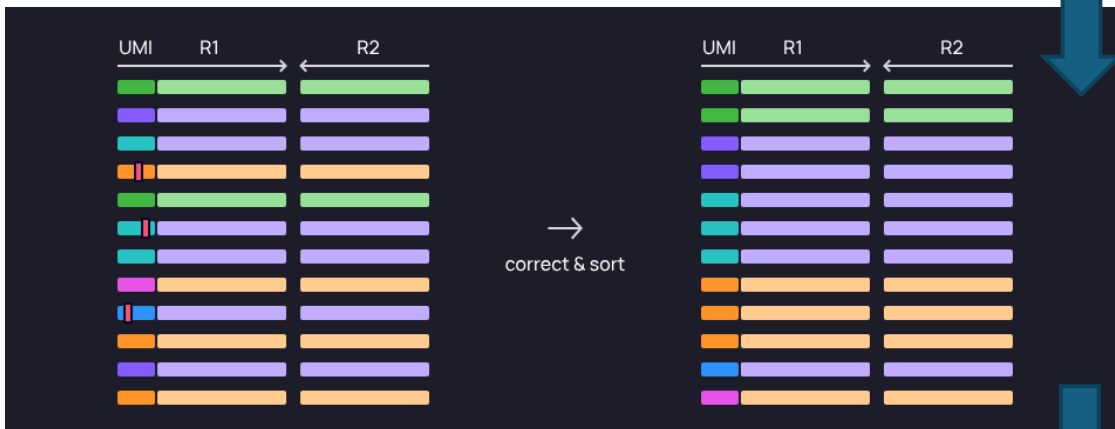
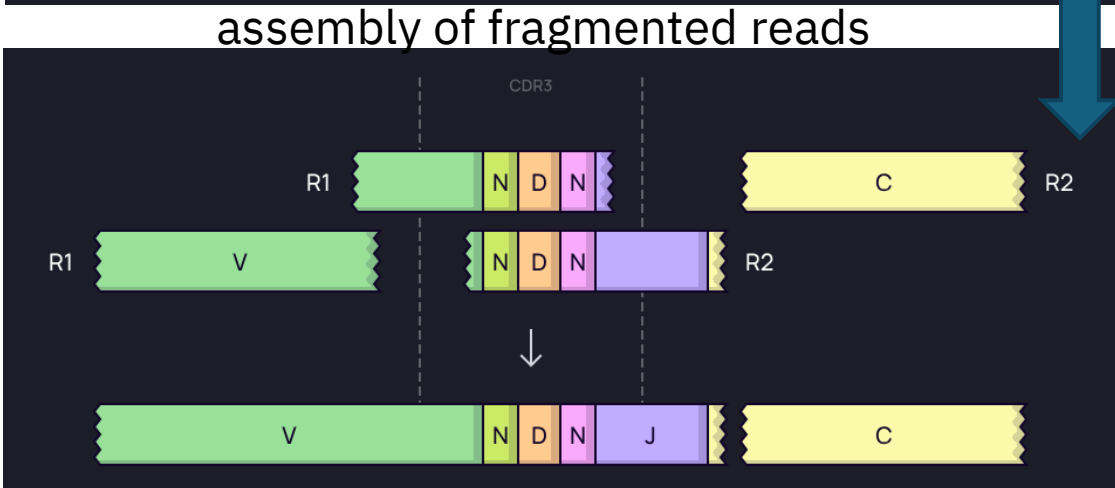
# Bioinformatic pipeline



## clonotype assembly



|    | cloneId | cloneCount           | clonelfraction                                | clonalsequence                                           | clonalsequenceQuality                                    | allHwitswithScore | allDhtswithScore | allDhtswithScore | allHwitswithScore | allHwitswithScore | allAlignments  | allAlignments  | allAlignments |
|----|---------|----------------------|-----------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|-------------------|------------------|------------------|-------------------|-------------------|----------------|----------------|---------------|
|    | 0       | 25802                | 0.1420619408112187                            | TGTCACGACGACGAGGATGGAGGGGTTTGGGCGCAAGCTCGACTTCT          | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)  | TRB2*00(38)      | TRB2-6*00(248)   | TRB2*00(38)       | TRB2-6*00(248)    | TRB2*00(38)    | TRB2-6*00(248) | TRB2*00(38)   |
| 1  | 4568    | 0.02515072642887984  | TGCAGTCTCTACGAGGATGGGCGCATCTCTTTT             | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 2  | 2796    | 0.01530435658978272  | TGTCGACGATGAGCGGAGGGGGGCTGGGACCTCTCT          | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 3  | 2555    | 0.014638831658637383 | TGTCGACGATCTGATCTGGGTGGCTTGGGCGCAGCTCTGACTTCT | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 4  | 2922    | 0.01388573561212484  | TGTCGACGATGAGGCTGACGGGACGACCTGAGCTCTTTT       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 5  | 2456    | 0.014891332415091072 | TGCAGTCTGAGGCTGAGGATCTGACGACGATCTCTCT         | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 6  | 2174    | 0.013909717825189264 | TGTCGACGATGAGCTGACGGGCGGAGCTGACGATCTTTT       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 7  | 2180    | 0.01705437920841018  | TGTCGAGTCTCCCGGTGACGGGACGACCTGACGATCTTTT      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 8  | 2185    | 0.01589914756356362  | TGCAGTCTGCTACAGGCGGACCTCTGACGACGATCTCT        | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 9  | 1891    | 0.010411552384907735 | TGTCGACGATCTACTGACGGAGGGGACGAGTACGACTATT      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 10 | 1864    | 0.01026029433585647  | TGTCGACGATCTGAGCTGGGCGGACCTGACGAGCAGCTCTTT    | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 11 | 1847    | 0.01016938488441844  | TGTCAGTCTGAGGACGAGCGGGGGGGGCTACGACGATCTCT     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 12 | 1824    | 0.010076337723331    | TGTCAGTCTGAGGAGGAGGACGGGGGGGCACTACCGACATCTT   | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 13 | 1817    | 0.010004125387474352 | TGTCGACGATGAGCTGACGGGAGGACGATCACTGACATCTTT    | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 14 | 1799    | 0.0099050240888936   | TGTCAGTCTGAGCTCTGACGATCTCTACATGACGATCTCT      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 15 | 1587    | 0.0087377839538885   | TGTCAGCAGCTGGGACGAGCTATGAGAACTCTTTT           | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 16 | 1385    | 0.00762500280339986  | TGTCGACGATGAGCTGACGGGAGGCGGACGATCAGCATATT     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*00(38)       | TRB2-6*00(248) | TRB2*00(38)    | TRB2*00(38)   |
| 17 | 1352    | 0.00744390153475568  | TGTCGACGAGTCTTTAGGAGGACGATCACTGACGATCTCT      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRB15*00(1669.5)                                         | TRB2*00(38)       | TRB2-6*00(248)   | TRB2*00(38)      | TRB2-6*00(248)    | TRB2*0            |                |                |               |

[illegible]

| cloneId | cloneCount | cloneFraction         | clonalSequence                                  | clonalSequenceQuality                            | allVHitsWithScore   | allDHitsWithScore  | allJHitsWithScore | allCHitsWithScore             | allVAlignments                | allDAlignments         | allJAlignments | allCAlignments |
|---------|------------|-----------------------|-------------------------------------------------|--------------------------------------------------|---------------------|--------------------|-------------------|-------------------------------|-------------------------------|------------------------|----------------|----------------|
| 0       | 25802      | 0.14206194081211287   | TGTGCCACAGCAGAGATGGAGGGTTTGGGGCCAACTGCTGACTTTC  | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV15*00(1069.5)   | TRBD2*00(30)       | TRBJ2-6*00(248)   | TRBC2*00(106.5)               | 327 345 364 6                 |                        |                |                |
| 1       | 4568       | 0.025150722642807984  | TGCAGTGTATCAGAGGTAGGGCACTGAAGCTTCTTT            | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(967.8)  | TRBD2*00(25)       | TRBJ1-1*00(217.4) | TRBC1*00(105.6)               | 321 331 355 0 10 150.0        | 27 32 48 12            |                |                |
| 2       | 2796       | 0.015394356503785272  | TGTGCCACAGTACGGCGGAGGGGGCGTGAAGCAGTCTTC         | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1089.3)  | TRBD1*00(40)       | TRBJ2-1*00(202.5) | TRBC2*00(104.1)               | 327 342 364 0 15 175.0        |                        |                |                |
| 3       | 2655       | 0.014618031658637303  | TGTGCCACAGTGCCTGGTGGGTCTGGGGCCAACTGCTGACTTTC    | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV2*00(1050.2)    | TRBD2*00(35)       | TRBJ2-6*00(258.1) | TRBC2*00(106.3)               | 330 343 367 6                 |                        |                |                |
| 4       | 2522       | 0.01388575361321404   | TGTGCCACAGTGAAGCTAGCGGGAACACGGGAGCTGTTTTT       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1083.7)  | TRBD2*00(35)       | TRBJ2-2*00(246.3) | TRBC2*00(102.7)               | 327 341 364 0 14 170.0        |                        |                |                |
| 6       | 2450       | 0.013489332415691672  | TGCAGTGTAGGGGACAGCTAGCTTCTACGACGAGTCTTC         | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(977.5)  | TRBD2*00(50)       | TRBJ2-7*00(217.6) | TRBC2*00(105.4)               | 321 332 355 0 11 155.0        |                        |                |                |
| 7       | 2174       | 0.011969717825189264  | TGTGCCACAGTGAAGCTAGCGGGGGGAGATGACGAGTATTTT      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-1*00(1092.6)  | TRBD2*00(39)       | TRBJ2-3*00(222.3) | TRBC2*00(103.2)               | 327 344 364 0 17 185.0        |                        |                |                |
| 8       | 2126       | 0.011705437026841018  | TGTGCCAGTAGTCCCGTGGGACGGGAGCCCAATGAGCAGTCTTC    | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV28*00(1051.8)   | TRBD2*00(30)       | TRBJ2-1*00(218)   | TRBC2*00(106)                 | 327 339 364 0 12 153535T 44.0 |                        |                |                |
| 9       | 2105       | 0.011589814177563662  | TGCAGTGTCTACAGGGGGGCTTCTACGAGCAGTACTTC          | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(975.2)  | TRBD1*00(50)       | TRBJ2-7*00(227.2) | TRBC2*00(104.5)               | 321 330 355 0 9 145.0         |                        |                |                |
| 10      | 1891       | 0.010411562284927735  | TGTGCCACAGTTATACTAGCGGAGAGGGGACACGATACGATATTT   | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-2*00(1063.7)  | TRBV6-3*00(1063.7) | TRBD2*00(40)      | TRBJ2-3*00(237.7)             | TRBC2*00(103.1)               | 327 342 364 0 15 175.0 |                |                |
| 11      | 1864       | 0.010262904335856847  | TGTGCCACAGTGAATTGGCGGAGCTTACGACGAGCAGTACTTC     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-1*00(1077.4)  | TRBD2*00(35)       | TRBJ2-7*00(206.9) | TRBC2*00(105.5)               | 327 342 364 0 15 175.0        |                        |                |                |
| 12      | 1847       | 0.010169304886441844  | TGCAGCGTTGAAGAGCAGCGGGGGGGGCTACGAGCAGTACTTC     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV29-1*00(1065.4) | TRBD2*00(29)       | TRBD1*00(25)      | TRBJ2-7*00(221.9)             | TRBC2*00(103.3)               | 321 335 355 0 14 170.0 |                |                |
| 13      | 1824       | 0.01004267033723331   | TGCAGCGTTGAAGAGGACAGCGGGGCAATCAGCCCGAGCATTTT    | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV29-1*00(1080)   | TRBD1*00(50)       | TRBJ1-5*00(237.7) | TRBC1*00(106.7)               | 321 335 355 0 14 170.0        |                        |                |                |
| 14      | 1817       | 0.010004129387474192  | TGTGCCACAGTGAATAGCGGGAGGGTACAATGAGCAGTCTTC      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-1*00(1083)    | TRBD2*00(60)       | TRBJ2-1*00(227.7) | TRBC2*00(105.1)               | 327 342 364 0 15 175.0        |                        |                |                |
| 15      | 1799       | 0.0099050240880936    | TGCAGTGTAGCTCTCAGCGATTCTACAATGAGCAGTCTTC        | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(953.7)  | TRBJ2-1*00(242.6)  | TRBC2*00(105.4)   | 321 339 355 0 18 1533235A334C |                               |                        |                |                |
| 16      | 1587       | 0.00873778389538885   | TGCAGCAGCTGGGACAGAAATAGAAAAGCTGTTTTT            | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV10-2*00(1072.4) | TRBD1*00(30)       | TRBJ1-4*00(222.5) | TRBC1*00(105.9)               | 327 340 364 0 13 165.0        | 13 19 36 13            |                |                |
| 17      | 1385       | 0.007625602202339986  | TGTGCCACAGTGAATAGCGGGAGGGGACAGATACGAGTATTTT     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1111.9)  | TRBD2*00(45)       | TRBJ2-3*00(222.3) | TRBC2*00(103.5)               | 327 346 364 0 19 195.0        |                        |                |                |
| 18      | 1352       | 0.007443909153475568  | TGTGCCACAGTTTTAGGGACGGATTACAATGAGCAGTCTTC       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV28*00(1080.7)   | TRBD1*00(25)       | TRBD2*00(25)      | TRBJ2-1*00(227.8)             | TRBC2*00(106)                 | 327 342 364 0 15 175.0 |                |                |
| 19      | 1346       | 0.007410874053682038  | TGTGCCACAGCTTGGGGGGGAGCTTATCTAGACAGATACGAGATTTT | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV7-8*00(942.5)   | TRBD1*00(35)       | TRBJ2-3*00(251.8) | TRBC2*00(103.1)               | 327 342 364 0 15 175.0        |                        |                |                |
| 20      | 1313       | 0.007229181004817619  | TGTGCCACAGTGAATCACTAGCGGGGAGATACGAGTATTTT       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1102.6)  | TRBD2*00(45)       | TRBJ2-3*00(221.9) | TRBC2*00(102.3)               | 327 344 364 0 17 185.0        |                        |                |                |
| 21      | 1298       | 0.00714659325533792   | TGTGCCACAGTGAATCACTAGCGGGAGGACATGAGCAGTCTTC     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1113.7)  | TRBD2*00(50)       | TRBJ2-1*00(217.6) | TRBC2*00(103.4)               | 327 346 364 0 19 195.0        |                        |                |                |
| 22      | 1269       | 0.00698692360631728   | TGTGCCAGTAGCGGTGGGAGGCTTACCCCTCCACTT            | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV19*00(1066.1)   | TRBJ1-6*00(201)    | TRBC1*00(105.6)   | 327 338 364 0 11 155.0        | 29 45                         |                        |                |                |
| 23      | 1263       | 0.006953888506538197  | TGTGCCACAGTTTTGGGACAGCTCTCTGGGGCCAACTGCTGACTTTC | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV28*00(1067)     | TRBD1*00(35)       | TRBJ2-6*00(260.9) | TRBC2*00(107)                 | 327 341 364 6                 |                        |                |                |
| 24      | 1215       | 0.006689607708189952  | TGCAGCGTAGGGAGAGACGGGATACCGGGAGCTGTTTTT         | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV29-1*00(1059.3) | TRBD1*00(40)       | TRBJ2-2*00(226.5) | TRBC2*00(102.8)               | 321 329 355 0 8 140.0         |                        |                |                |
| 25      | 1175       | 0.006469373709566414  | TGTGCCACAGGAGACTAGTGAACACGGGAGCTGTTTTT          | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV2*00(1053.9)    | TRBD1*00(25)       | TRBD2*00(25)      | TRBJ2-2*00(226.5)             | TRBC2*00(103.9)               | 330 338                |                |                |
| 27      | 958        | 0.005274604267033724  | TGTGCCACAGTGAAGGAGGAGCGGGAGCTAGGGAGCAGTCTTC     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV10-3*00(1060.8) | TRBD2*00(40)       | TRBJ2-1*00(196.8) | TRBC2*00(103.4)               | 327 342 364 0 15 175.0        |                        |                |                |
| 28      | 958        | 0.005274604267033724  | TGTGCCACAGTGAAGGAGGAGCGGGAGCTAGGGAGCAGTCTTC     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-3*00(1063.8)  | TRBV6-2*00(1063.4) | TRBD2*00(60)      | TRBJ2-5*00(236.9)             | TRBC2*00(104.8)               | 327 342 364 0 15 175.0 |                |                |
| 29      | 953        | 0.005247075017205781  | TGTGCCACAGTGAAGGAGGAGCTCTGGGGGCAACGCTGACTTTC    | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-1*00(1087.1)  | TRBJ2-6*00(272.8)  | TRBC2*00(106.2)   | 327 343 364 0 16 180.0        |                               |                        |                |                |
| 31      | 874        | 0.004812112869924294  | TGCAGCAGCTAGCAGAGACGGGAGCTGTTTTT                | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV10-1*00(1109.4) | TRBD2*00(30)       | TRBJ2-2*00(226.6) | TRBC2*00(104.6)               | 327 339 364 0 12 160.0        | 20 26 48 13            |                |                |
| 32      | 869        | 0.004784583620096352  | TGTGCCACAGCCAGAAAGCTCTGGGGCCAACTGCTGACTTTC      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV15*00(1045.5)   | TRBJ2-6*00(273.4)  | TRBC2*00(106.2)   | 327 339 364 0 12 160.0        |                               |                        |                |                |
| 33      | 834        | 0.004591878871300757  | TGTGCCACAGCAGAGATTCTCGGGGCAACGCTGACTTTC         | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV15*00(1071.3)   | TRBJ2-6*00(243.3)  | TRBC2*00(105.9)   | 327 345 364 0 18 190.0        |                               |                        |                |                |
| 34      | 789        | 0.004344115622849277  | TGTGCCAAGCGAGGGTGCATATACTAGCAGTCTTC             | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV5-3*00(1095.9)  | TRBD1*00(25)       | TRBD2*00(25)      | TRBJ2-1*00(228.4)             | TRBC2*00(106.6)               | 327 342 364 0 15 175.0 |                |                |
| 35      | 767        | 0.004222986923606332  | TGTGCCACAGTGAACAGGGGAACTGAAGCTTCTTC             | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1114.9)  | TRBD1*00(25)       | TRBJ1-1*00(232.1) | TRBC1*00(104.7)               | 327 340 364 0 13 165.0        | 15 20 36 15            |                |                |
| 36      | 763        | 0.004200963523743978  | TGCAGTGTAGAGATGGACAGGGGTCTTACGAGCAGTCTTC        | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(984)    | TRBD1*00(50)       | TRBJ2-7*00(212.3) | TRBC2*00(104.2)               | 321 336 355 0 15 175.0        |                        |                |                |
| 37      | 749        | 0.00412388162422574   | TGTGCCACAGTTACCAAAACAGGCTTGGGGGCAACGAGCAGTCTTC  | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-5*00(1073.8)  | TRBJ2-6*00(267.9)  | TRBC2*00(105.7)   | 327 342 364 6                 |                               |                        |                |                |
| 38      | 739        | 0.004068823124569855  | TGTGCCACAGTGAAGCTAGGCGGTAGCAGATACGAGTATTTT      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1085.3)  | TRBD2*00(45)       | TRBJ2-3*00(247.1) | TRBC2*00(102.8)               | 327 341 364 0 14 170.0        |                        |                |                |
| 39      | 664        | 0.00365588437150723   | TGCAGCGTTGAATTGAGGAGGGTGTAGCAGTACTTC            | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV29-1*00(1093.8) | TRBD2*00(50)       | TRBJ2-7*00(194.6) | TRBC2*00(101.9)               | 321 333 355 0 12 160.0        | 23 33 48 14            |                |                |
| 40      | 655        | 0.003606331727460427  | TGTGCCACAGTGTGCTAGCGGGGTGAGCAGTGAAGCAGTCTTC     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1075.5)  | TRBD2*00(40)       | TRBJ2-1*00(207.2) | TRBC2*00(103.8)               | 327 340 364 0 13 165.0        |                        |                |                |
| 41      | 642        | 0.003534755677907777  | TGTGCCACAGCAGGGTAGCAGGGGACACAATGAGCAGTCTTC      | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV15*00(1053.3)   | TRBD1*00(30)       | TRBJ2-1*00(233)   | TRBC2*00(105.3)               | 327 341 364 0 14 170.0        | 16 12                  |                |                |
| 42      | 624        | 0.003435650378527185  | TGTGCCACAGTGAAGGAGGAGACCGGGGAGCTGTTTTT          | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-4*00(1081.3)  | TRBD1*00(35)       | TRBJ2-2*00(237.1) | TRBC2*00(103.2)               | 327 342 364 0 15 175.0        |                        |                |                |
| 43      | 608        | 0.003345650378527185  | TGTGCCACAGTGAAGGAGGAGGAGGAGGAGCTGTTTTT          | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV24-1*00(1096.1) | TRBD1*00(25)       | TRBJ2-2*00(236.9) | TRBC2*00(105.6)               | 327 342 365 0 15 175.0        |                        |                |                |
| 44      | 588        | 0.0032374397797660013 | TGCAGTGTAGGACCGGGAGGAGCAGTCTTC                  | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(974.6)  | TRBD1*00(30)       | TRBD2*00(30)      | TRBJ2-1*00(197.3)             | TRBC2*00(105.1)               | 321 332 355 0 11 155.0 |                |                |
| 45      | 585        | 0.0032209222298692363 | TGTGCCACAGCAGAGCTCGGGAGGTACAATGAGCAGTCTTC       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV15*00(1062.1)   | TRBD2*00(30)       | TRBJ2-1*00(228.2) | TRBC2*00(104.5)               | 327 343 364 0 16 180.0        |                        |                |                |
| 46      | 584        | 0.0032154163799036476 | TGCAGTGTAGCTCGGGGAGGAGGAGGAGGAGGAGTCTTC         | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(984)    | TRBD1*00(30)       | TRBJ2-5*00(222.8) | TRBC2*00(106.2)               | 321 334 355 0 13 165.0        |                        |                |                |
| 47      | 569        | 0.003132828630419821  | TGCAGTGTAGAGATGGACAGGGGTGAGCAGTGAAGCAGTCTTC     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(939.7)  | TRBD2*00(35)       | TRBJ2-3*00(231.1) | TRBC2*00(103.2)               | 321 332 355 0 11 155.0        |                        |                |                |
| 48      | 563        | 0.0030997935306262903 | TGCAGTGTAGAGATGGACAGGGGTGAGCAGTGAAGCAGTCTTC     | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-2*00(1054.8)  | TRBV6-3*00(1054.8) | TRBD1*00(35)      | TRBJ2-2*00(245.6)             | TRBC2*00(102.2)               | 327 342 364 0 15 175.0 |                |                |
| 49      | 560        | 0.0030832759807295253 | TGTGCCACAGTGTAGGAGGAGGAGGAGGAGGAGGAGTCTTC       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV20-1*00(818)    | TRBD1*00(30)       | TRBJ2-1*00(243.7) | TRBC2*00(105.9)               | 321 332 355 0 11 155.0        |                        |                |                |
| 50      | 556        | 0.003061252580867171  | TGTGCCACAGTGTAGGAGGAGGAGGAGGAGGAGGAGTCTTC       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV11-2*00(1058.1) | TRBD2*00(30)       | TRBJ2-3*00(242.5) | TRBC2*00(103.7)               | 330 338                       |                        |                |                |
| 51      | 517        | 0.0028465244322092224 | TGTGCCACAGCAGTGAAGGAGGAGGAGGAGGAGGAGTCTTC       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-2*00(1059.1)  | TRBV6-3*00(1059.1) | TRBD1*00(30)      | TRBJ2-1*00(237.8)             | TRBC2*00(104.6)               | 327 342 364 0 15 175.0 |                |                |
| 52      | 517        | 0.0028465244322092224 | TGTGCCACAGCAGTGAAGGAGGAGGAGGAGGAGGAGTCTTC       | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-2*00(1061)    | TRBV6-3*00(1061)   | TRBD2*00(30)      | TRBJ2-1*00(233.5)             | TRBC2*00(104.8)               | 327 342 364 0 15 175.0 |                |                |
| 53      | 506        | 0.0027859600825877497 | TGTGCCACAGCAGCAGTCTATCCCTGAAAAAGCTGTTTTT        | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV15*00(1056.6)   | TRBJ1-4*00(217.5)  | TRBC1*00(105.1)   | 327 341 364 0 14 170.0        |                               |                        |                |                |
| 54      | 498        | 0.002741913282863042  | TGTGCCACAGCAGCAGTCTATCCCTGAAAAAGCTGTTTTT        | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV6-1*00(1096.8)  | TRBJ2-6*00(262.6)  | TRBC2*00(106.7)   | 327 342 364 0 15 175.0        |                               |                        |                |                |
| 55      | 494        | 0.0027198898830006883 | TGTGCCACAGCAGTGAATCTCTGGGGCAACGCTGACTTTC        | FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF | TRBV15*00(1053.3)   | TRBJ1-4*00(217.5)  | TRBC1*00(105.1)   | 327 341 364 0 14 170.0        |                               |                        |                |                |

# VDJTools

## Pre-processing

- frequency-based correction to eliminate erroneous clonotypes
  - filtering non-functional clonotypes

## Basic repertoire characteristics

- read counts
- number of clonotypes
  - CDR3 length
- NDN size, convergence

## Diversity estimation

- diversity indices: Chao1, Efron-Thisted, D50, Shannon-Wiener, Simpson

## V(D)J segment usage

- the frequency of associated reads for each of V/J segments present in sample
  - spectratype

## Overlap analysis

- clonotype sharing for a pair of samples
- Jaccard index, Morisita-Horn index

## Shared (public) clonotypes analysis

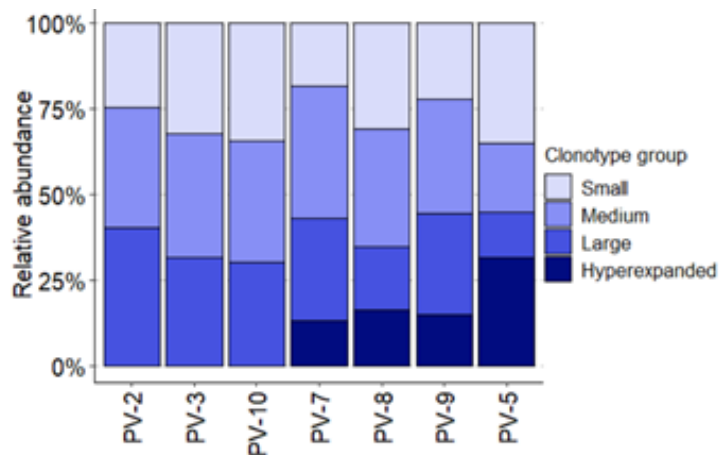
- Clonotypes with the same AA sequences

# immunarch



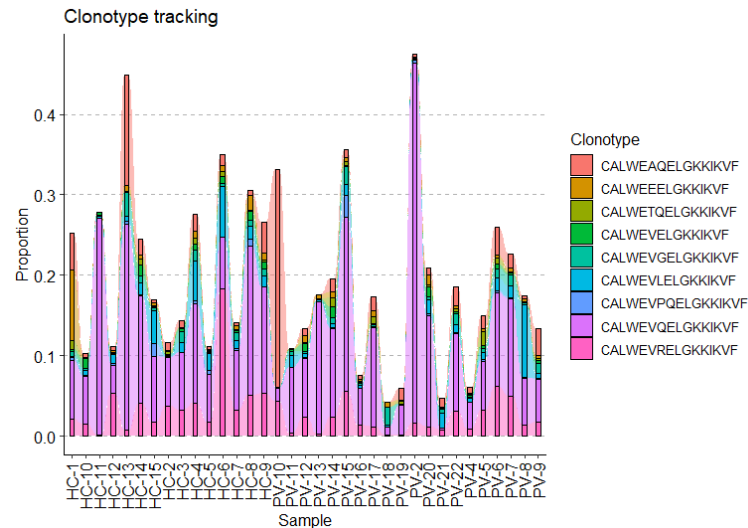
## Relative abundance analysis

- the proportion of the repertoire occupied by the clones of a given size



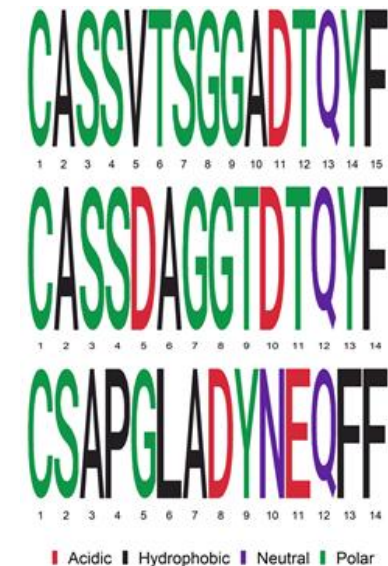
## Tracking of clonotypes

- segment usage
- The most frequent
  - Public



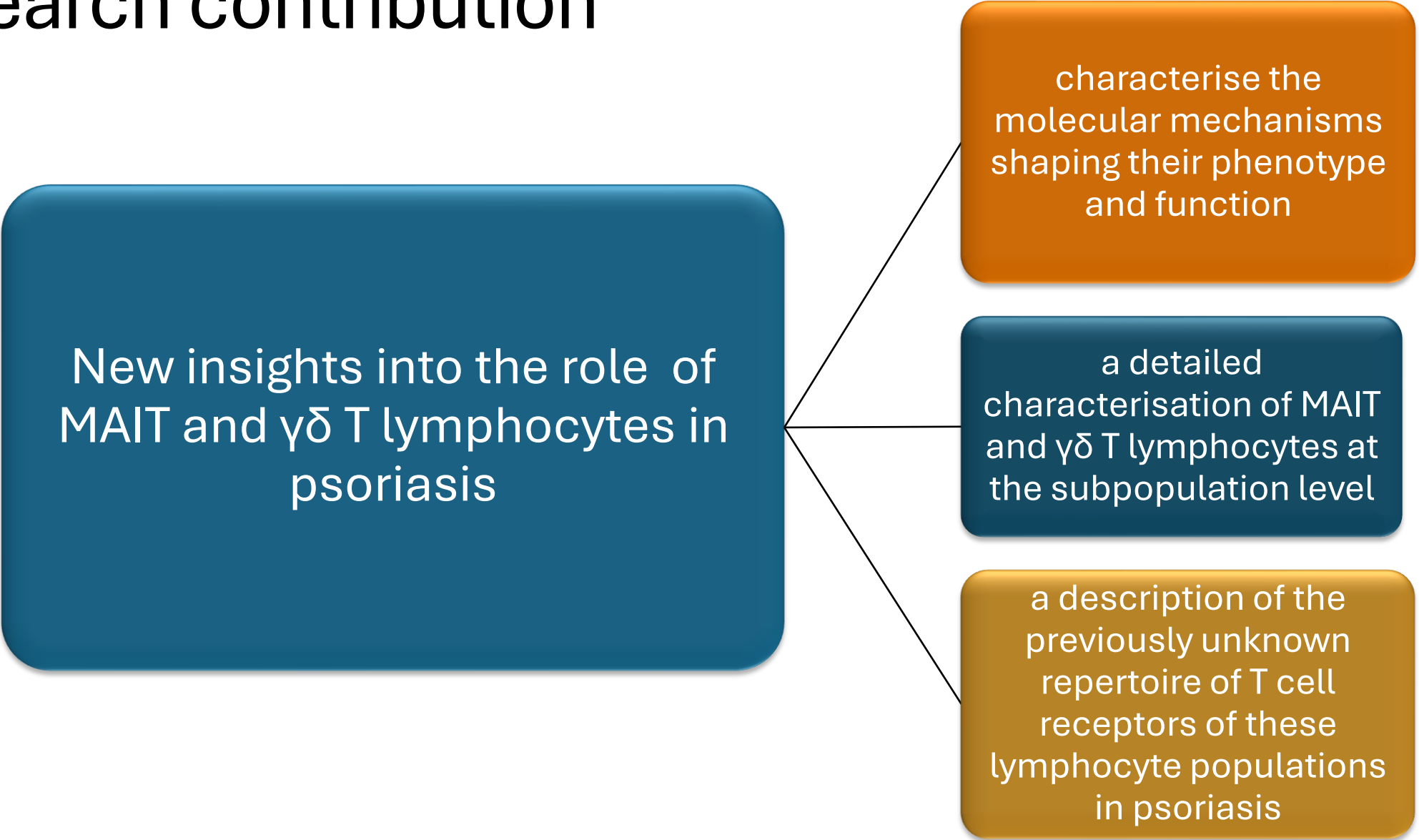
## Sequence motif visualisation

- Position probability matrix



# Research contribution

New insights into the role of  
MAIT and  $\gamma\delta$  T lymphocytes in  
psoriasis



```
graph LR; A[New insights into the role of MAIT and γδ T lymphocytes in psoriasis] --- B[characterise the molecular mechanisms shaping their phenotype and function]; A --- C[a detailed characterisation of MAIT and γδ T lymphocytes at the subpopulation level]; A --- D[a description of the previously unknown repertoire of T cell receptors of these lymphocyte populations in psoriasis];
```

characterise the  
molecular mechanisms  
shaping their phenotype  
and function

a detailed  
characterisation of MAIT  
and  $\gamma\delta$  T lymphocytes at  
the subpopulation level

a description of the  
previously unknown  
repertoire of T cell  
receptors of these  
lymphocyte populations  
in psoriasis

Thank you for your  
attention!

## Discussion

Funding:

Croatian Science Foundation, „NGS analysis of MAIT and  $\gamma\delta$  T cell transcriptome: phenotype, function and TCR repertoire in the etiology of Psoriasis vulgaris” (UIP-2019-04-3494, PI: Assoc. Prof. Stana Tokić).