T-Cell Receptor signatures of T cell subsets in disease revealed by robust high-throughput TCR sequencing.

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TCR repertoire in AIDs rationale

- T cell receptor (TCR) is a **hallmark characteristics** of T cell **specificity**
- **Tissues** are specifically **targeted** in AIDs
- TCR **repertoire** has been shown to be **altered** in **AIDs** compared to healthy volunteers (HV)
- AIDs are for many of them due to the Teff/Treg **imbalance**

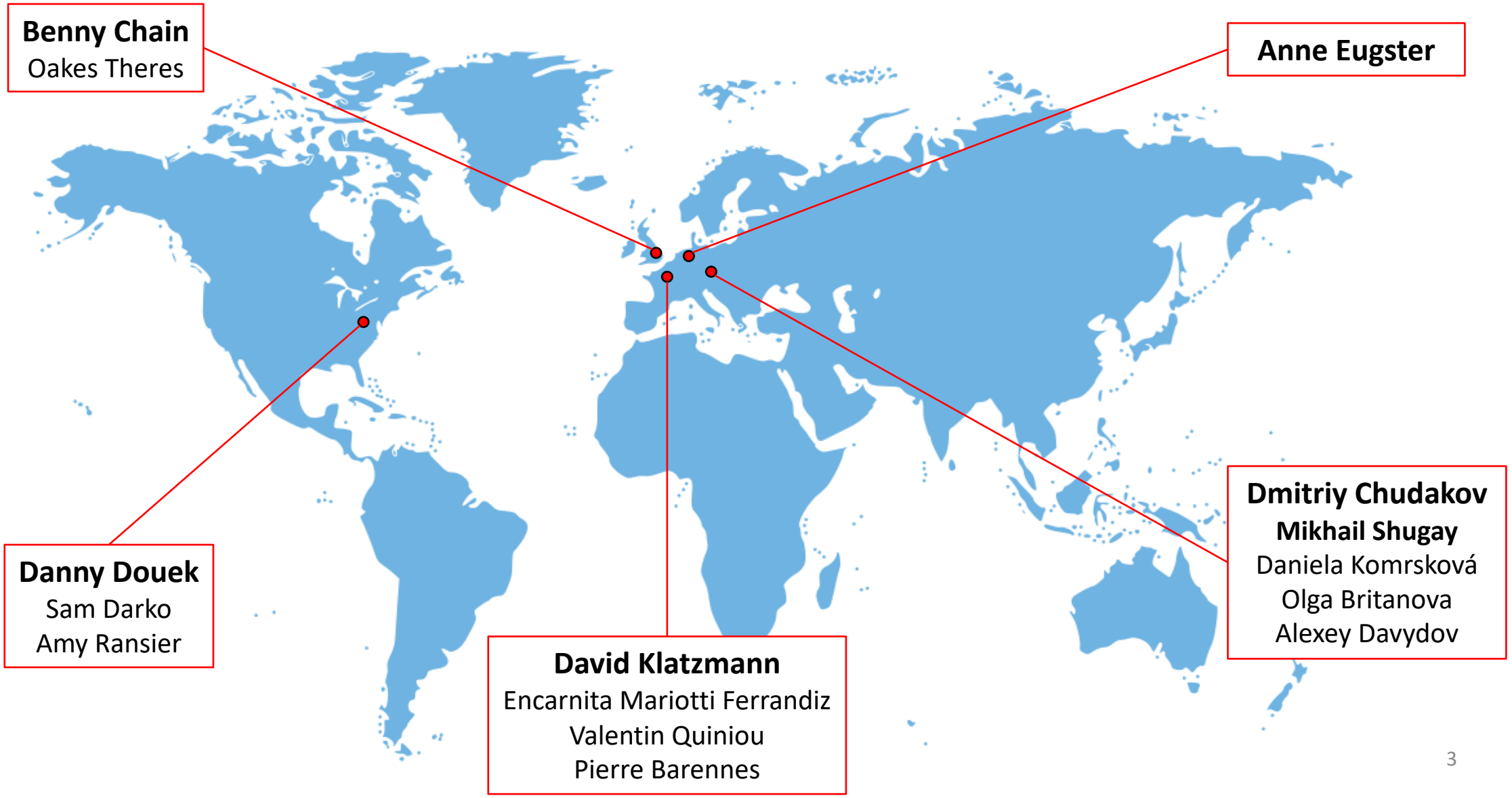
Hypothesis :

→ **Antigen-specific protective Treg vs. pathogenic Teff balance may be altered**

→ **T cell repertoires may provide biomarkers of disease vs. healthiness**

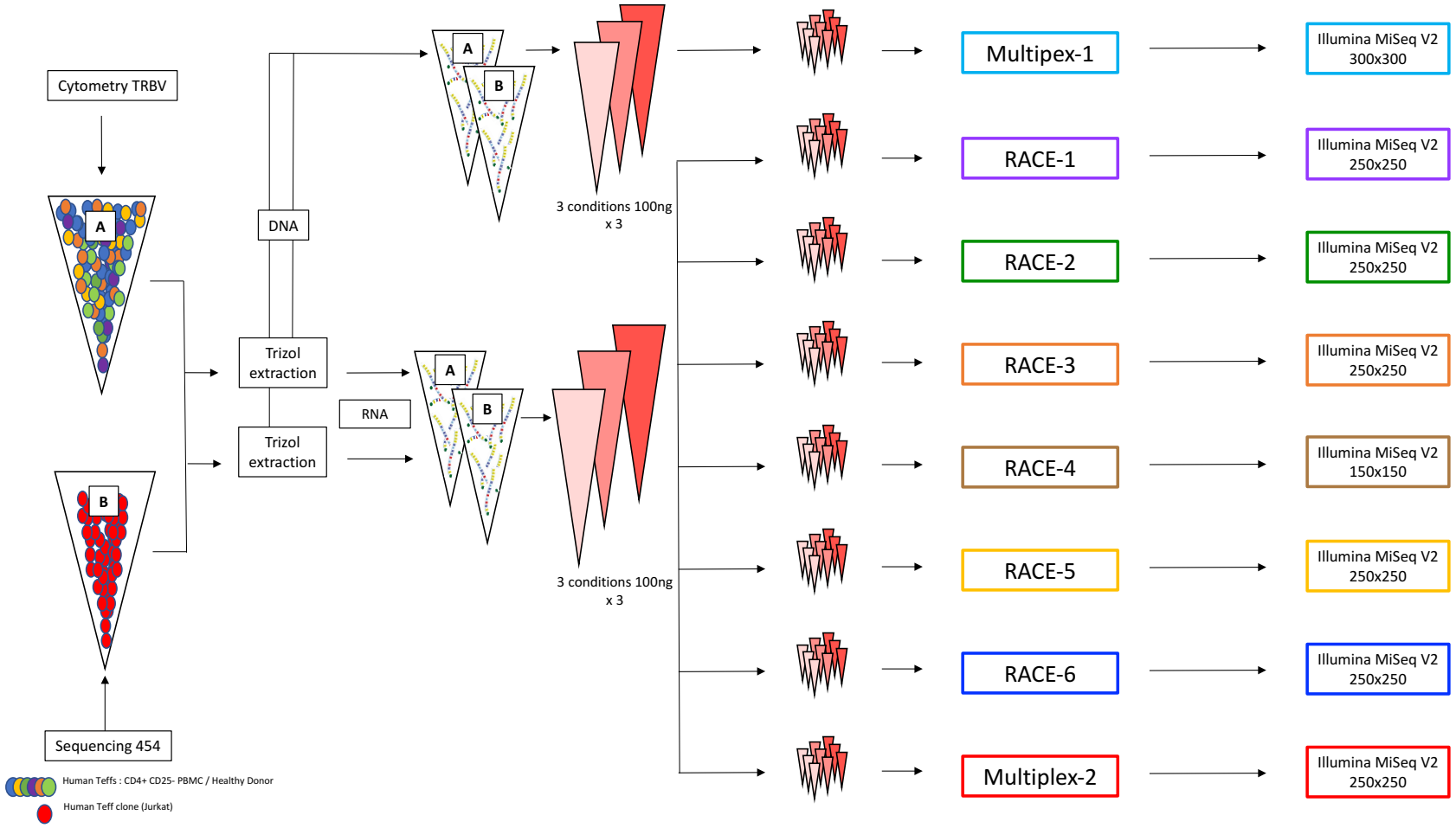
Methodology validation

International collaboration



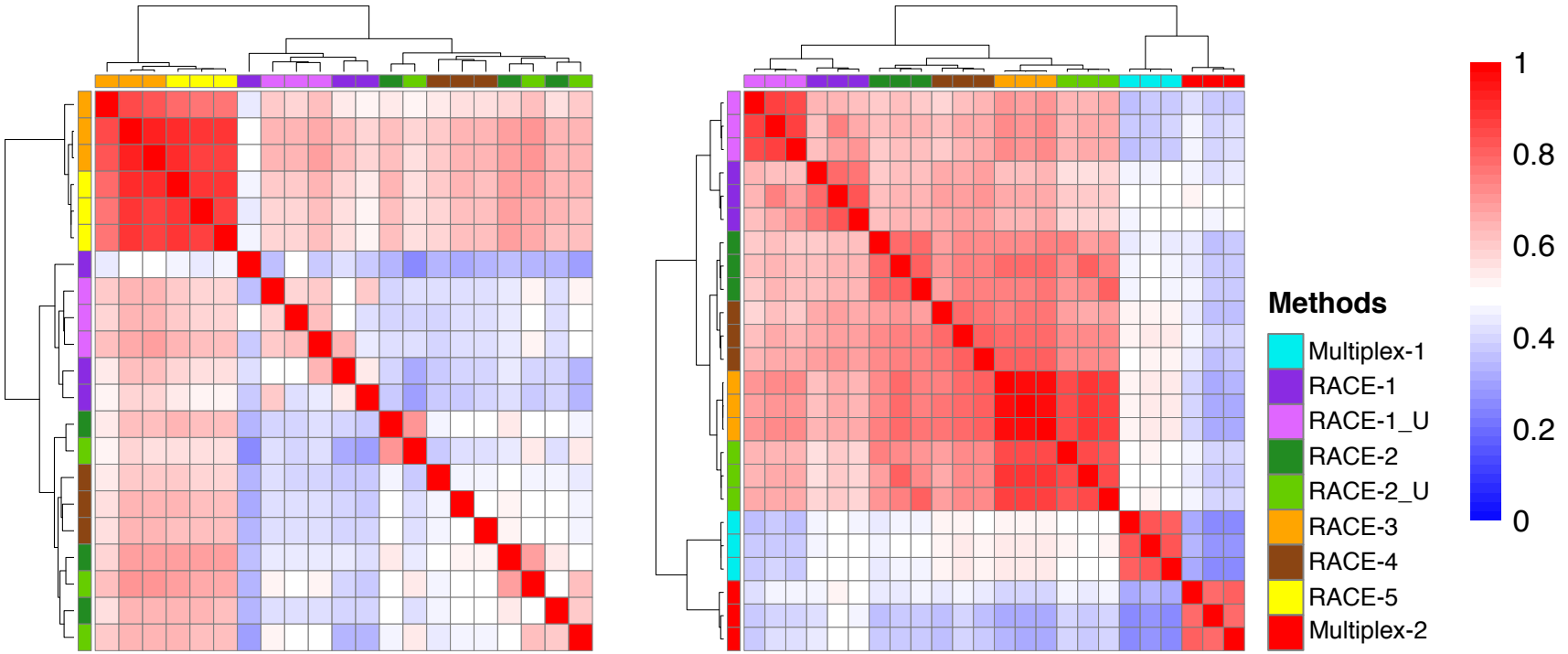
Methodology validation

We compared the same sample using 8 protocols



Goal : to evaluate the reproducibility between replicates and between methodology

Methodology validation

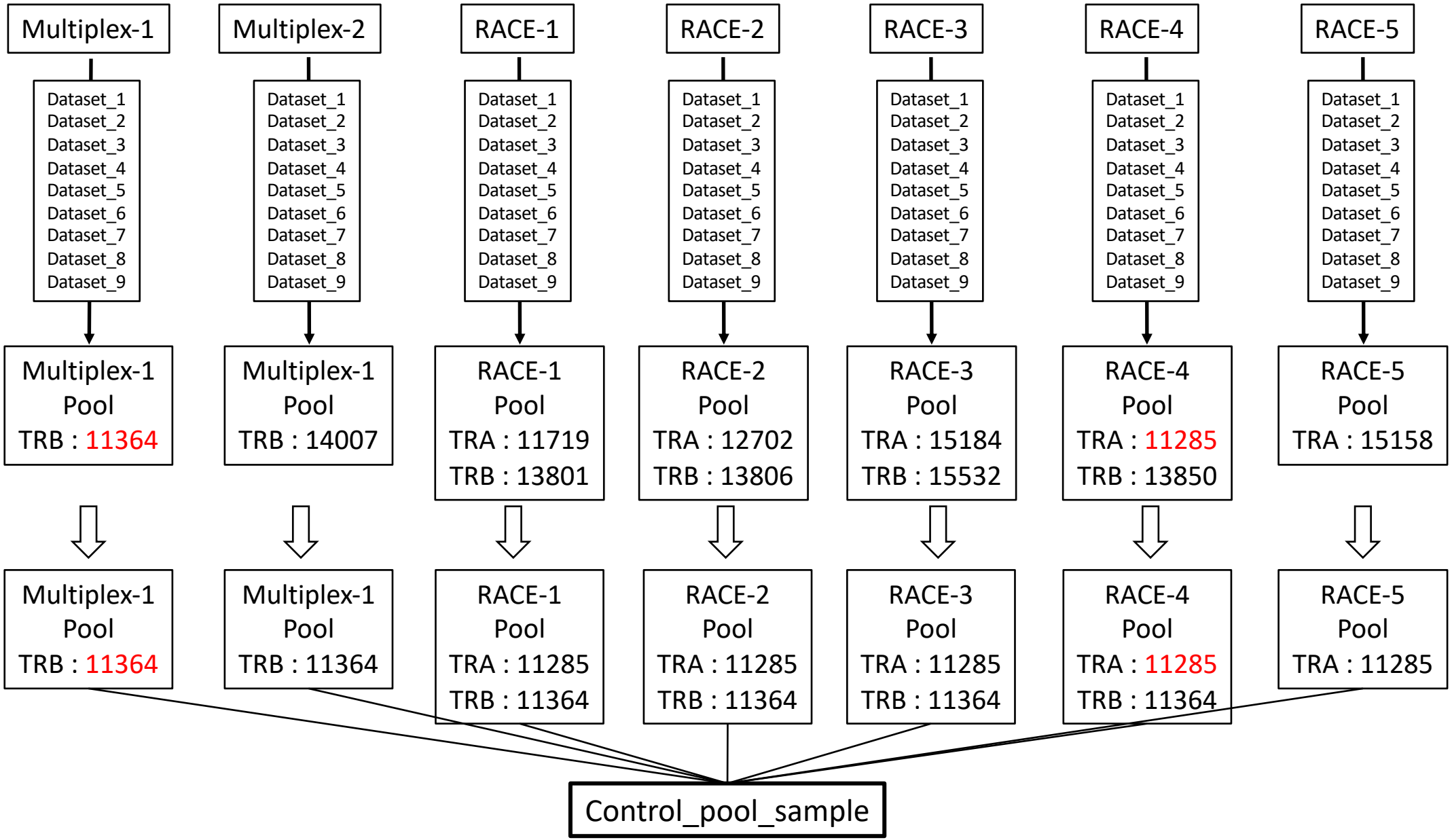


Barennes et al. In review

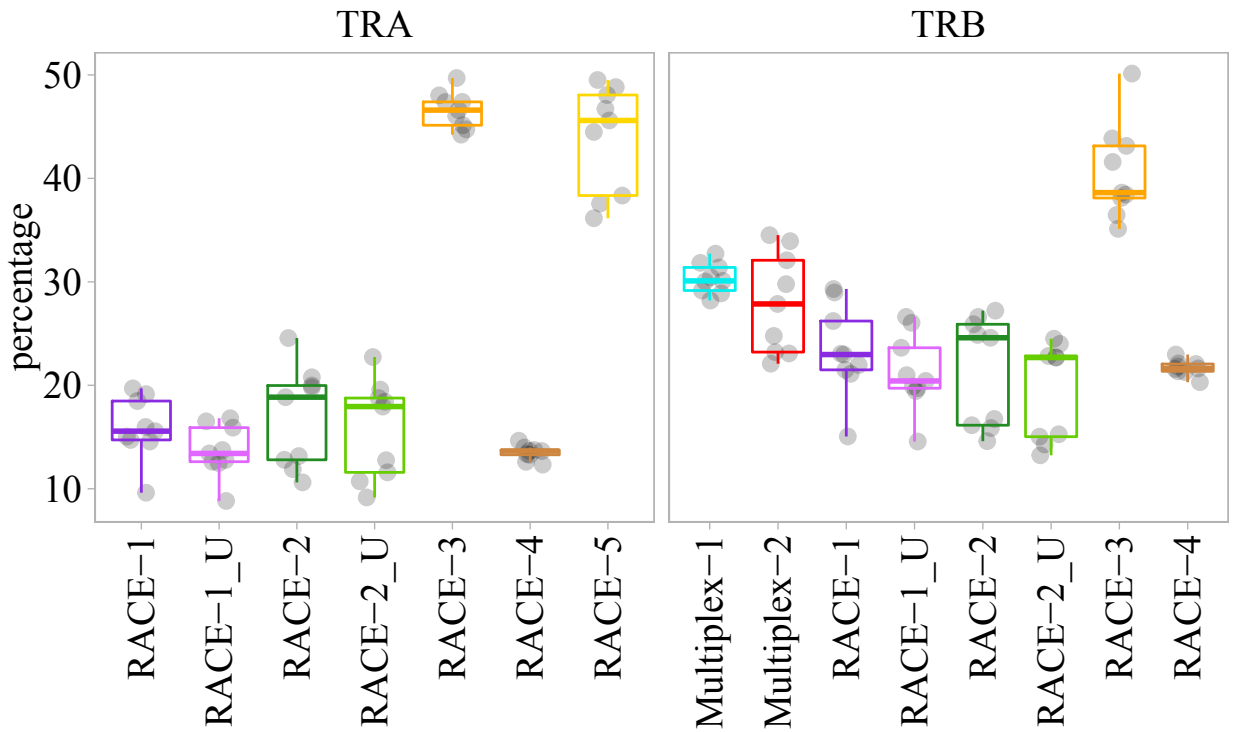
Similarity score from same sample reveal :

RACE-3 has the highest reproducibility score (intra- & inter-methods)

Methodology validation



Methodology validation



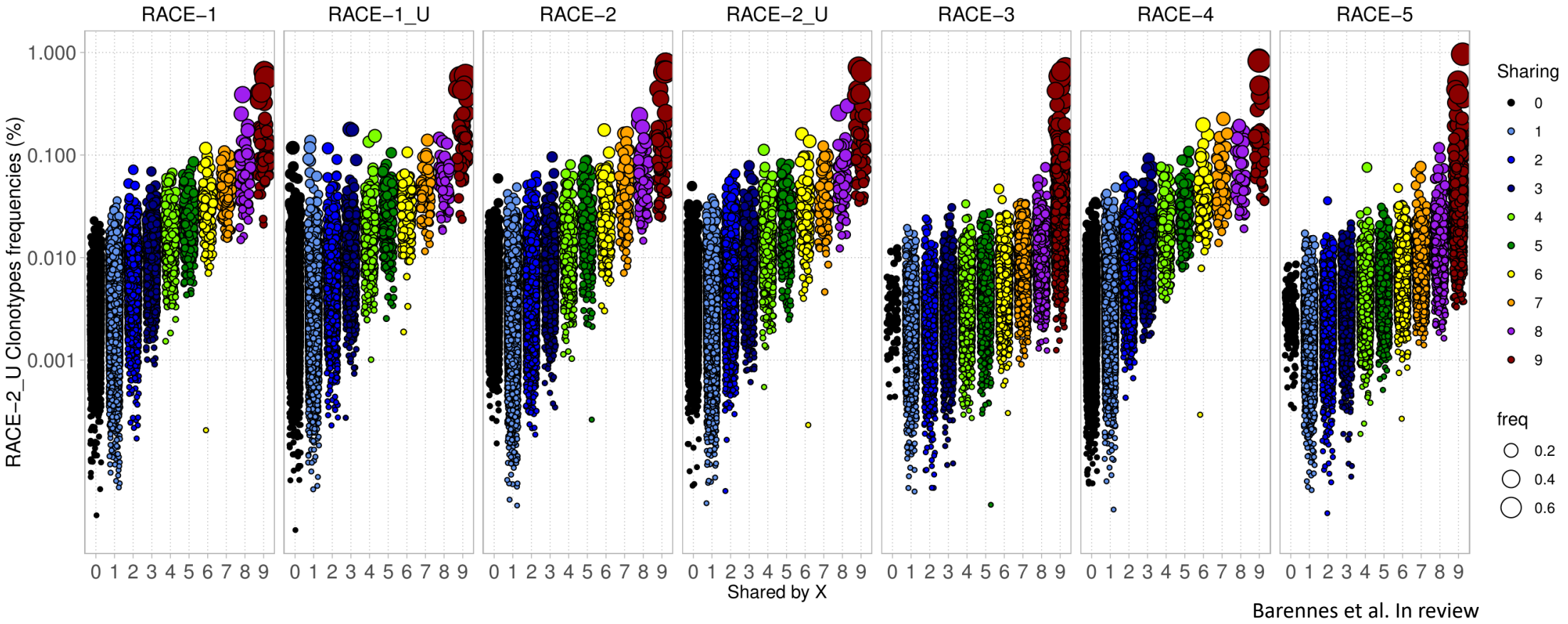
Barennes et al. In review

Replicates fraction in control :

RACE-3 shares the highest fraction of control's clonotypes for both chains

Methodology validation

TRA



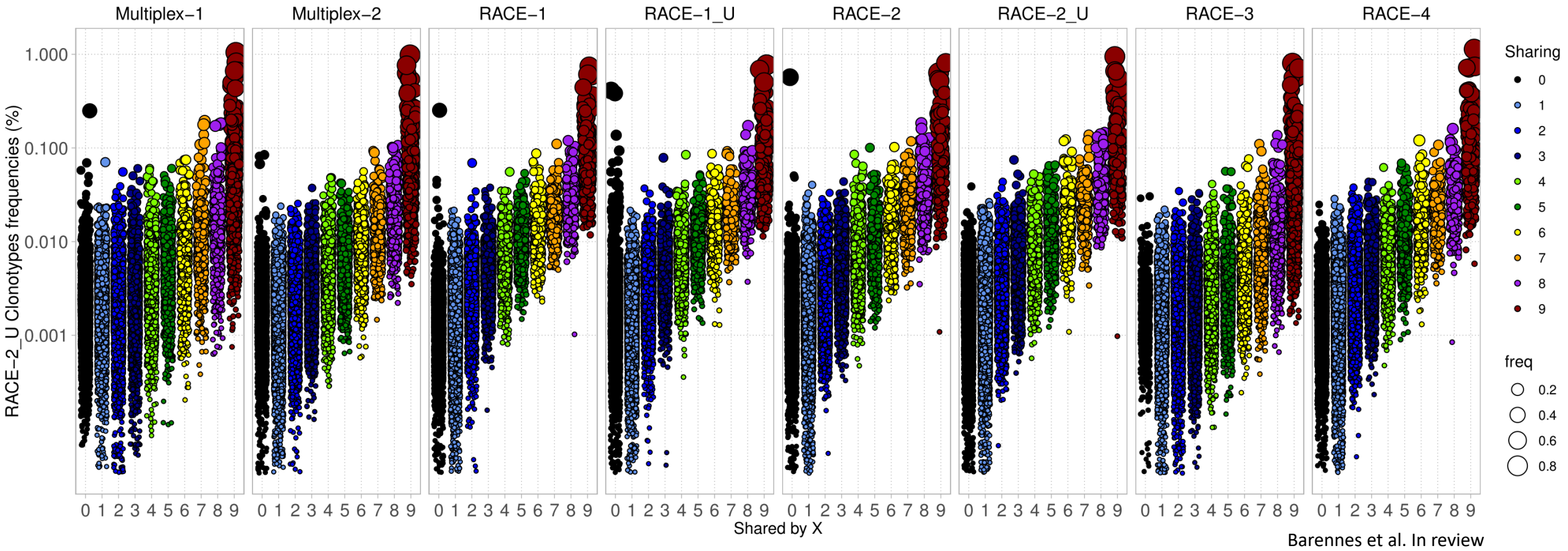
Barennes et al. In review

Distribution of control's clonotypes by replicates

RACE-3 captures the most number of clonotypes, with the greatest sensitivity and reliability

Methodology validation

TRB



Barennes et al. In review

Replicates fraction in control :

RACE-3 captures the most number of clonotypes, with the greatest sensitivity and reliability

⇒ Selection of RACE-3 methodology (TakaraBio® SMARTer Human TCR a/b Profiling Kit)

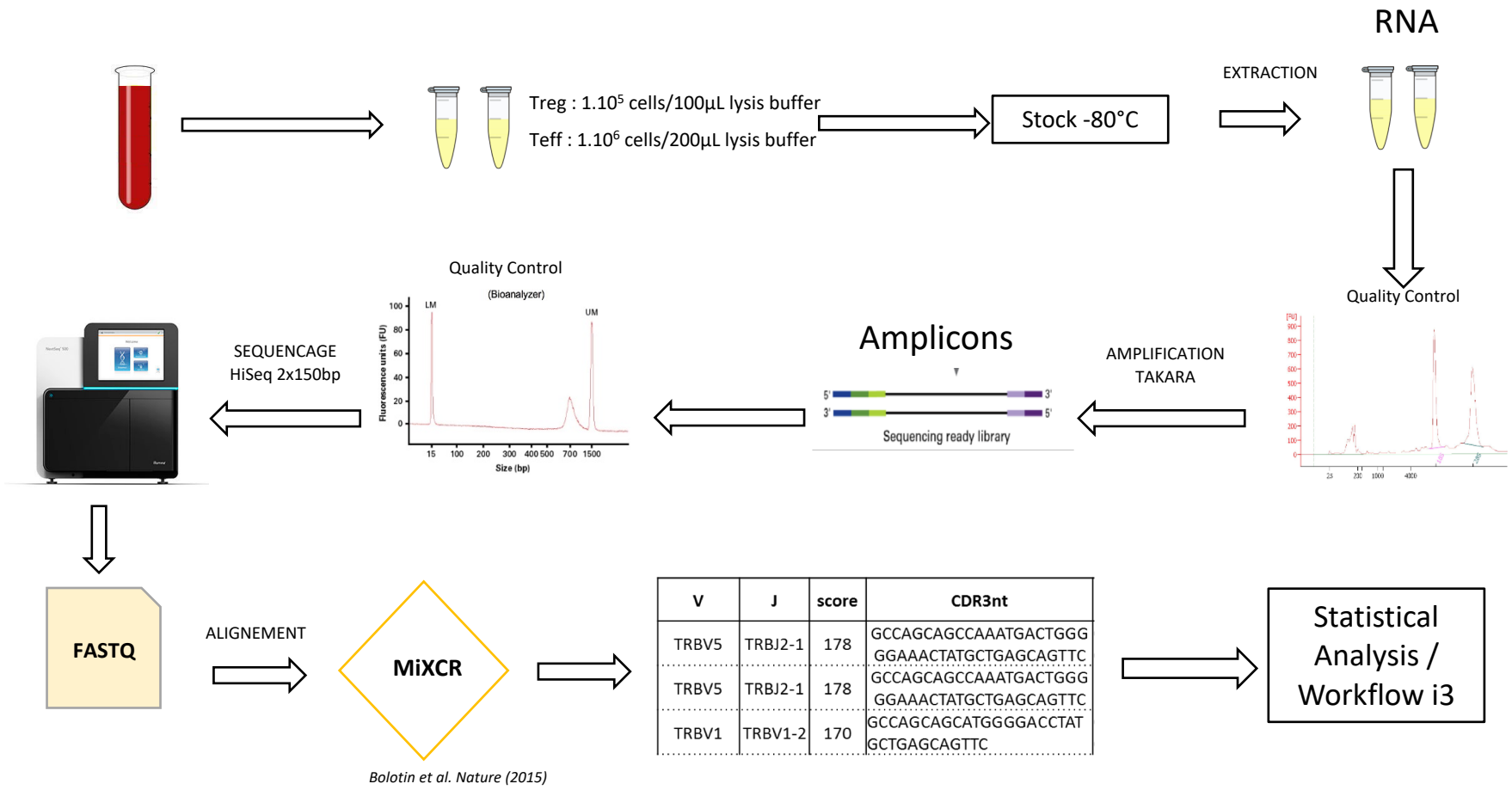
TCR repertoire in T1D

T1D is :

- a T cell **mediated** disease
- characterized largely by :
  - T cell-mediated **destruction** of insulin-producing **pancreatic β cells**
  - **defect** in **IL-2 production**, possibly explaining Treg **quantitative** and/or **qualitative** dysfunctions

→ **Pilot TCR** study on **T1D** patients compared to **healthy volunteers**

Material and Method

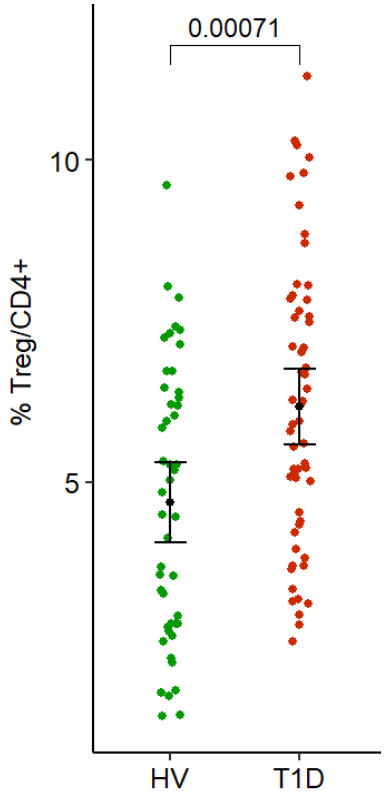


V	J	score	CDR3nt
TRBV5	TRBJ2-1	178	GCCAGCAGCCAAATGACTGGG GGAAACTATGCTGAGCAGTTC
TRBV5	TRBJ2-1	178	GCCAGCAGCCAAATGACTGGG GGAAACTATGCTGAGCAGTTC
TRBV1	TRBV1-2	170	GCCAGCAGCATGGGGACCTAT GCTGAGCAGTTC

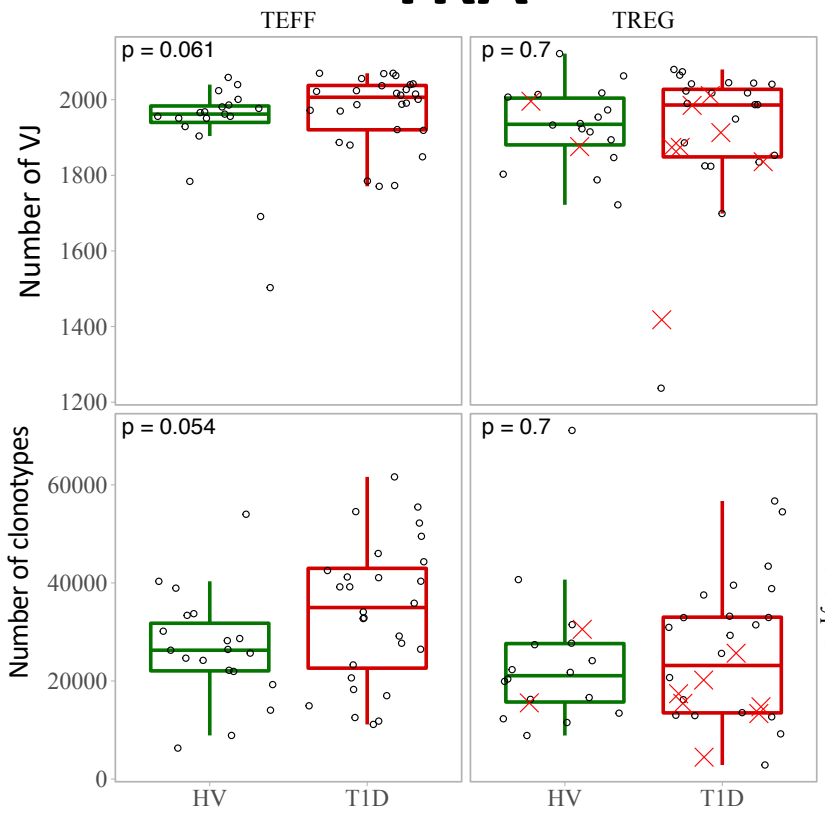
Bolotin et al. Nature (2015)

Clinical groups	Subject Number	F / H
T1D	28	10/16
HV	19	14/5

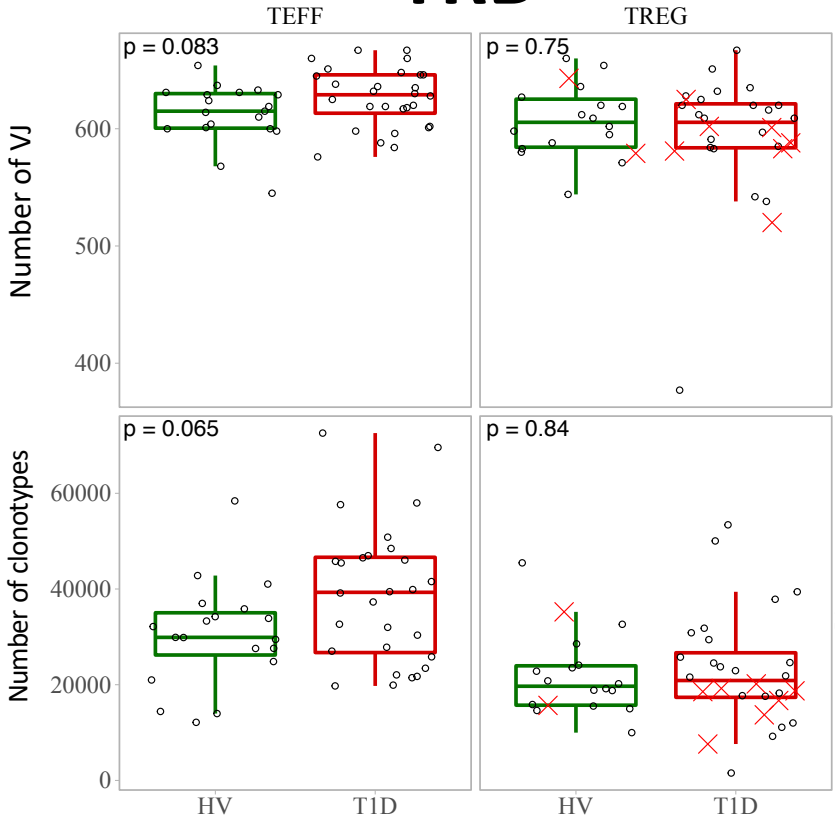
αβTCRs are not quantitatively altered



TRA

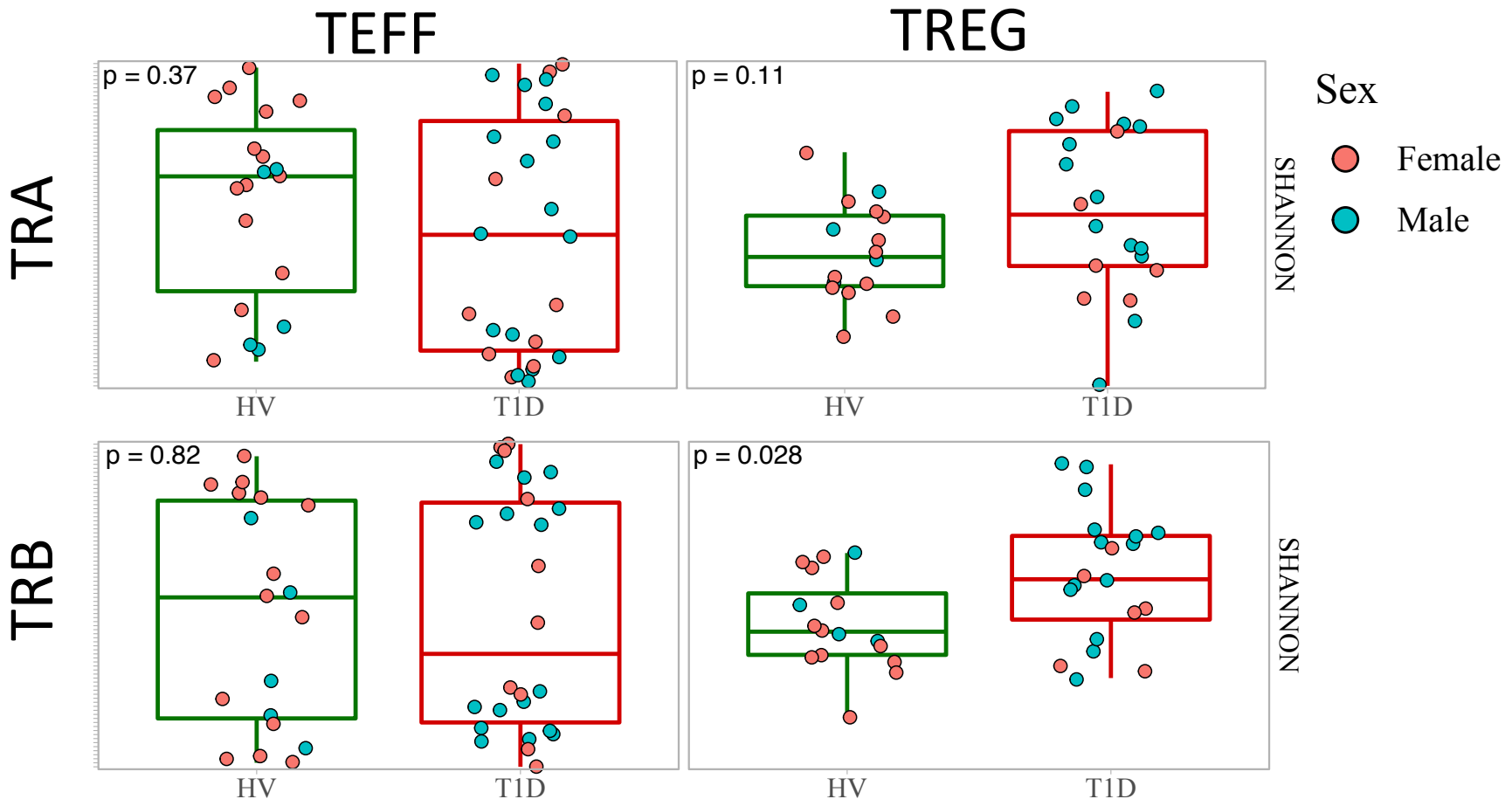


TRB



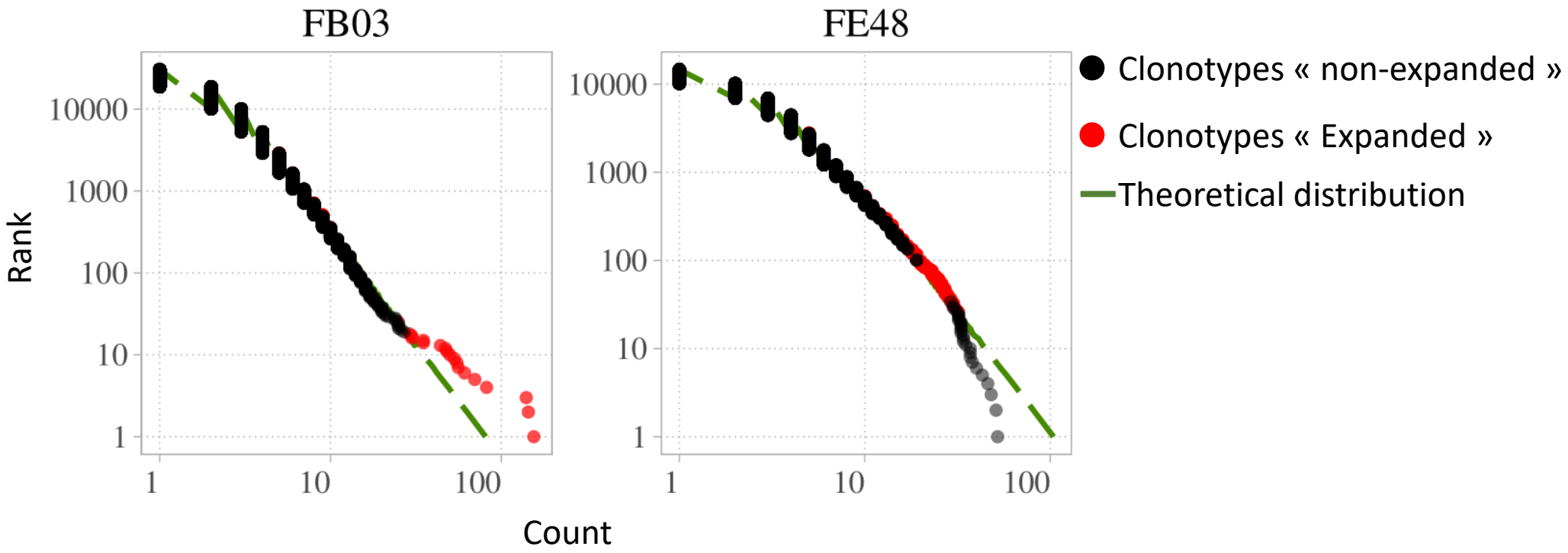
⇒ **need to explore if Tregs in T1D have a qualitative defect**

Higher diversity in Treg from T1D



⇒ **Shannon index reveals significant higher diversity for Treg-T1D (TRB)**

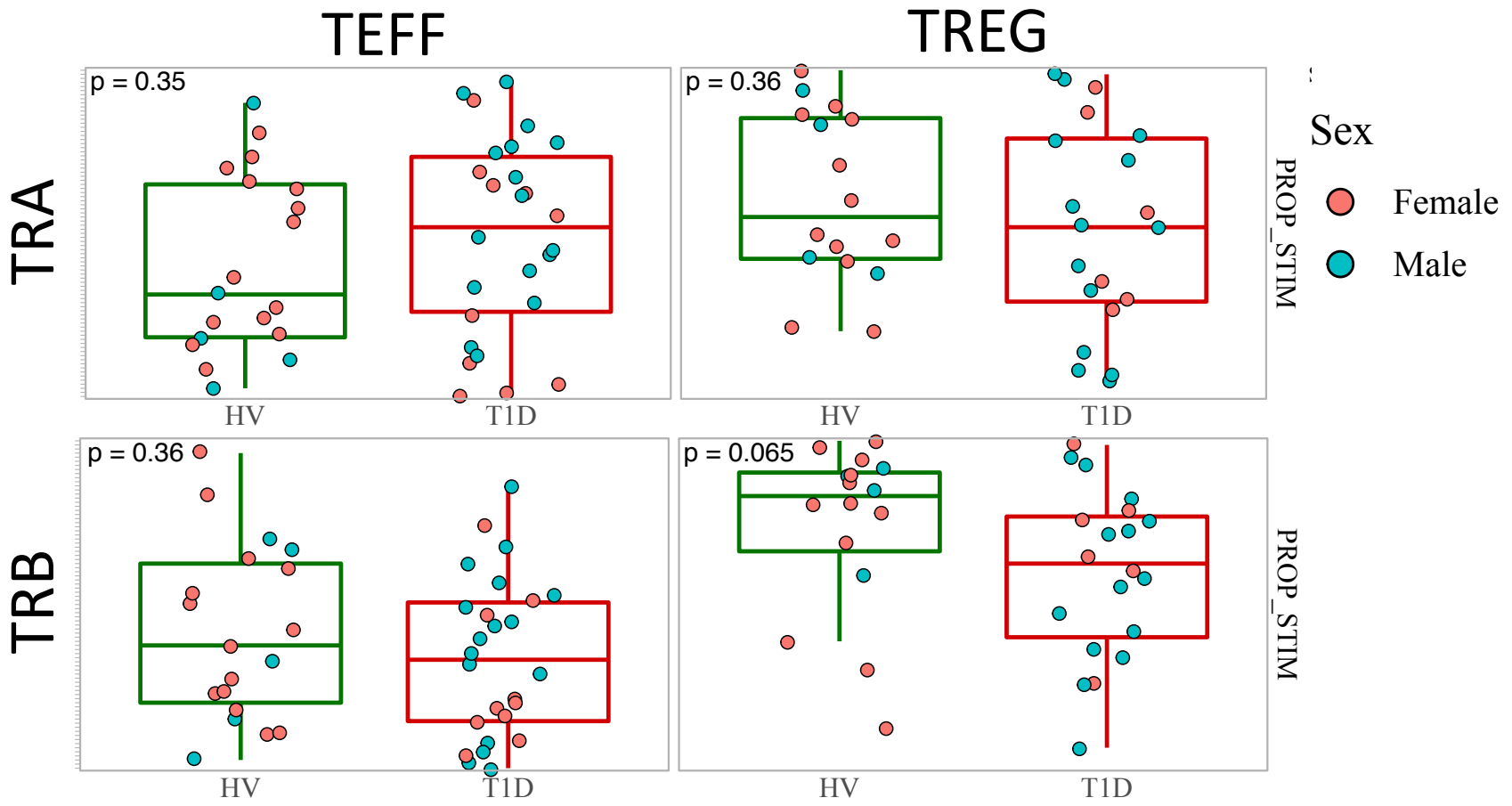
Expanded clonotypes can be tracked



Prop_stim : **Proportion of highly stimulated cells** (total number of reads at/or above the threshold, normalized by the total number of reads in the entire repertoire)

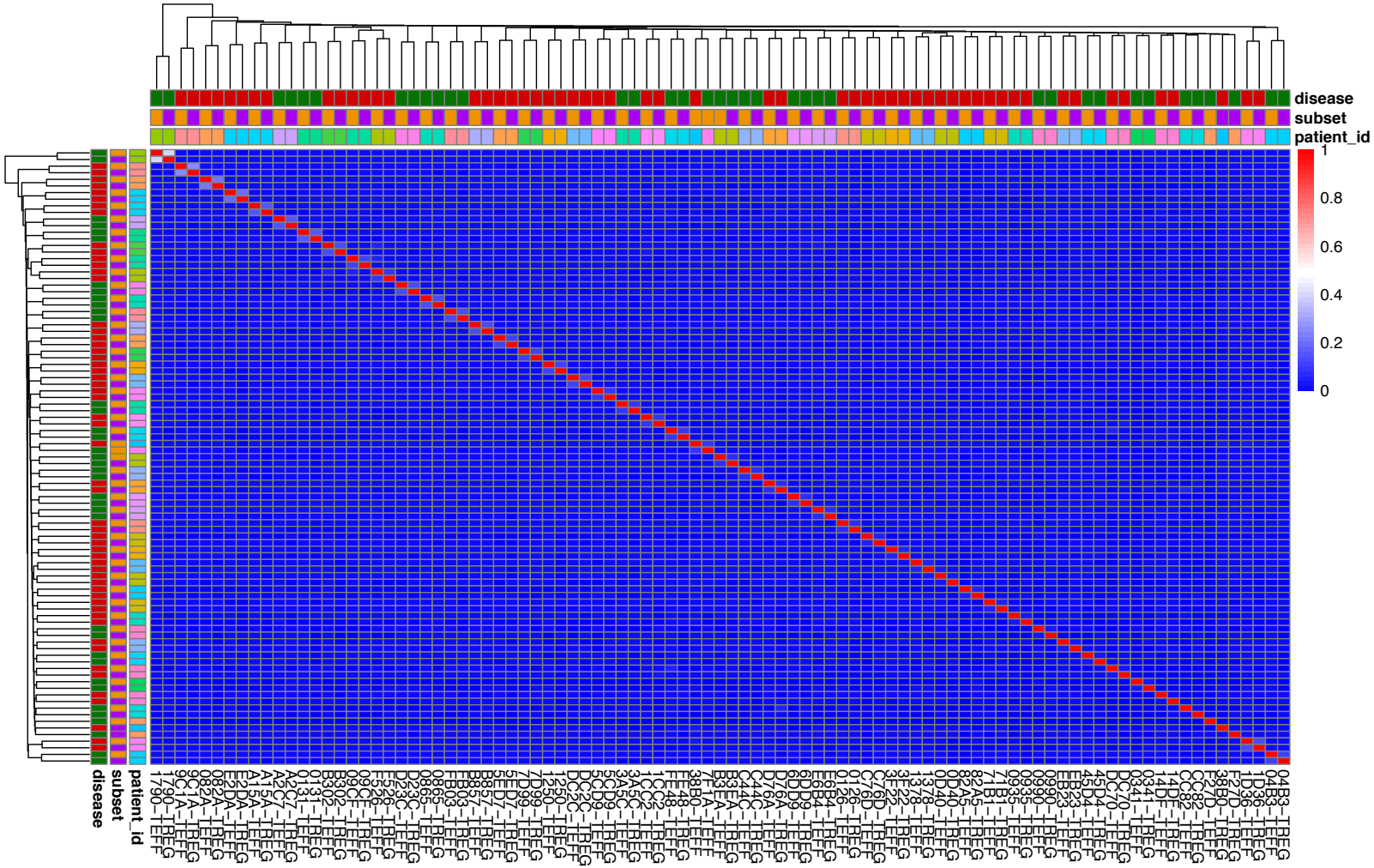
powerTCR : Koch H et al. PLOS Computational Biology (2018)

Expanded clonotypes can be tracked



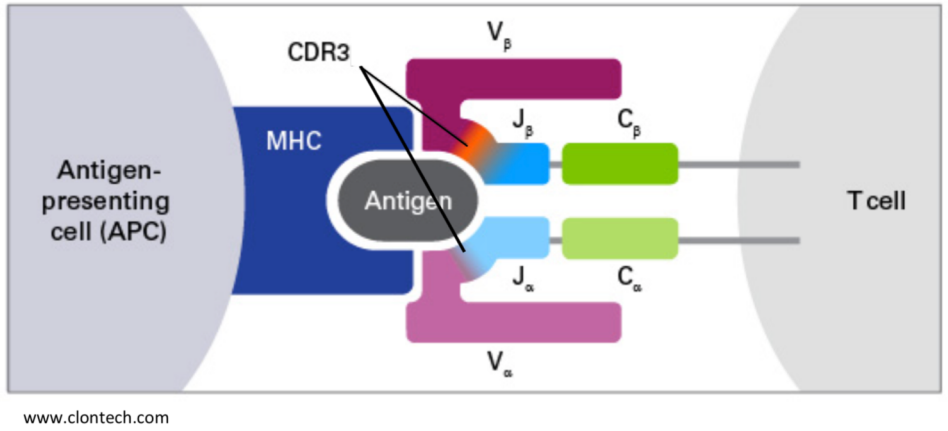
=> Higher diversity in Treg-T1D (TRB) could be explained by the lower proportion of "Highly stimulated cells" => Defect of IL-2 production

βTCRs cluster patient but not groups

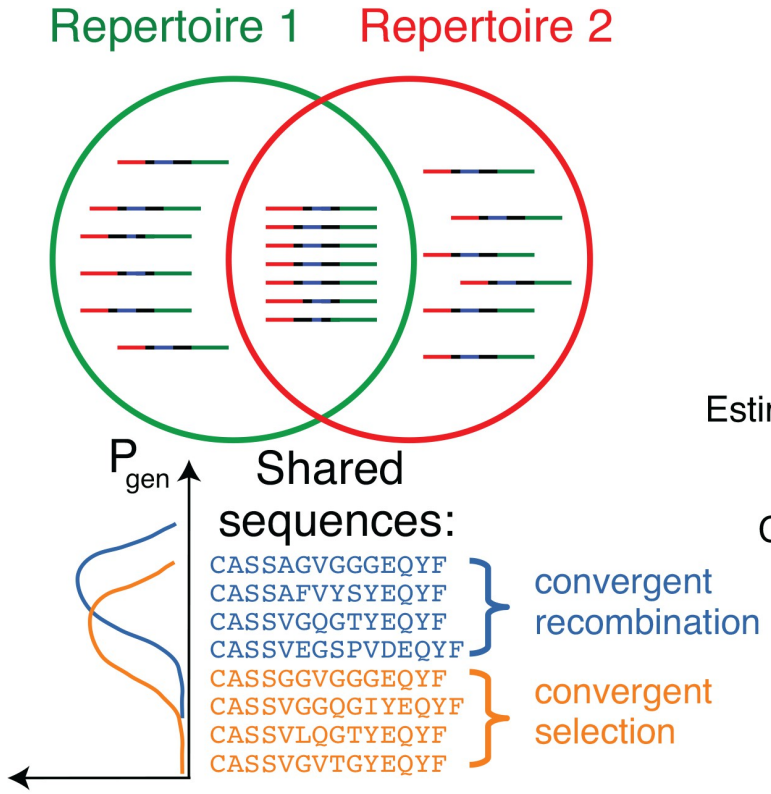


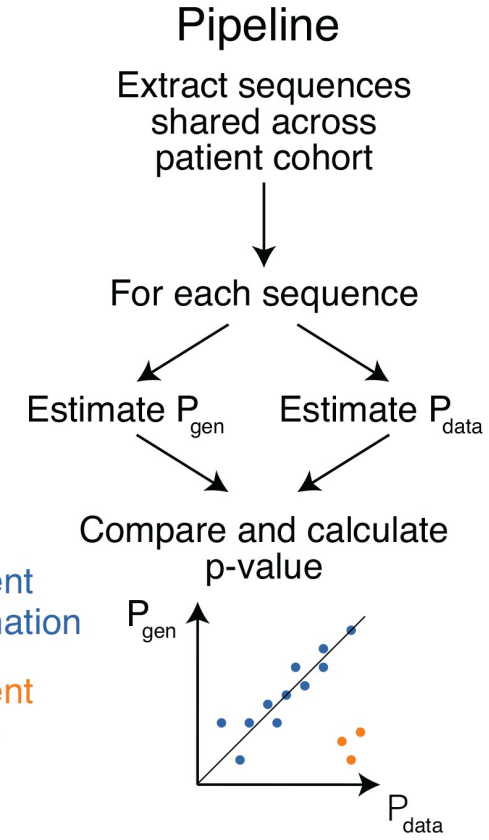
To explore more precisely and find clonotypes associated with disease, we used probabilistic model

Probability distribution model



CDR3aa : Antigen Binding specific Region

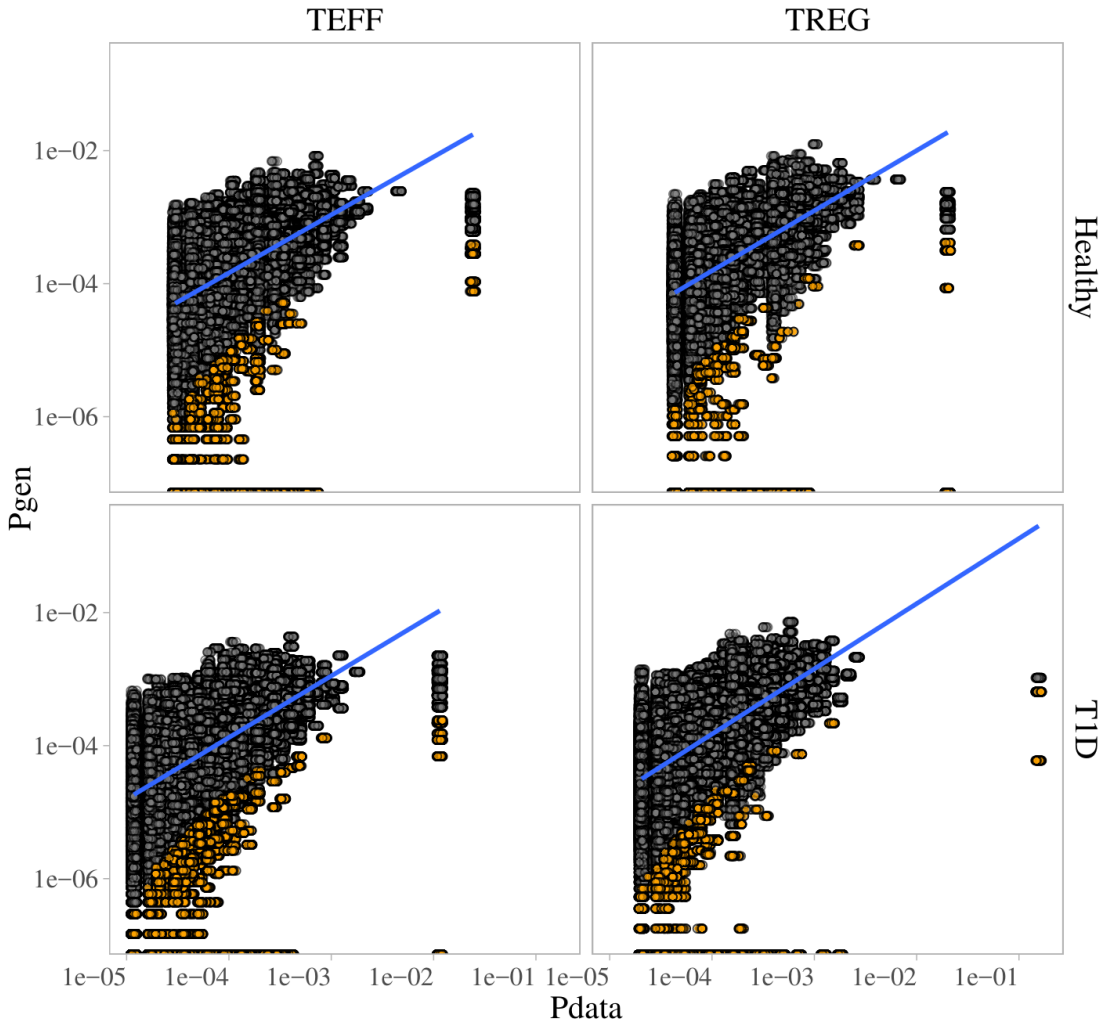




Pogorely et al. eLIFE (2018)

Orange dots indicates CDR3aa that are **significantly over-observed** in samples in comparison with the probability of generation => “Immune Involved response”

Probability distribution reveals higher over-observed CDR3aa in T1D



- CDR3aa p.adjusted > 0.05 → Non-Enriched CDR3aa
- CDR3aa p.adjusted < 0.05 → Enriched CDR3aa

Two-sided Fisher's Exact Test
HV vs T1D

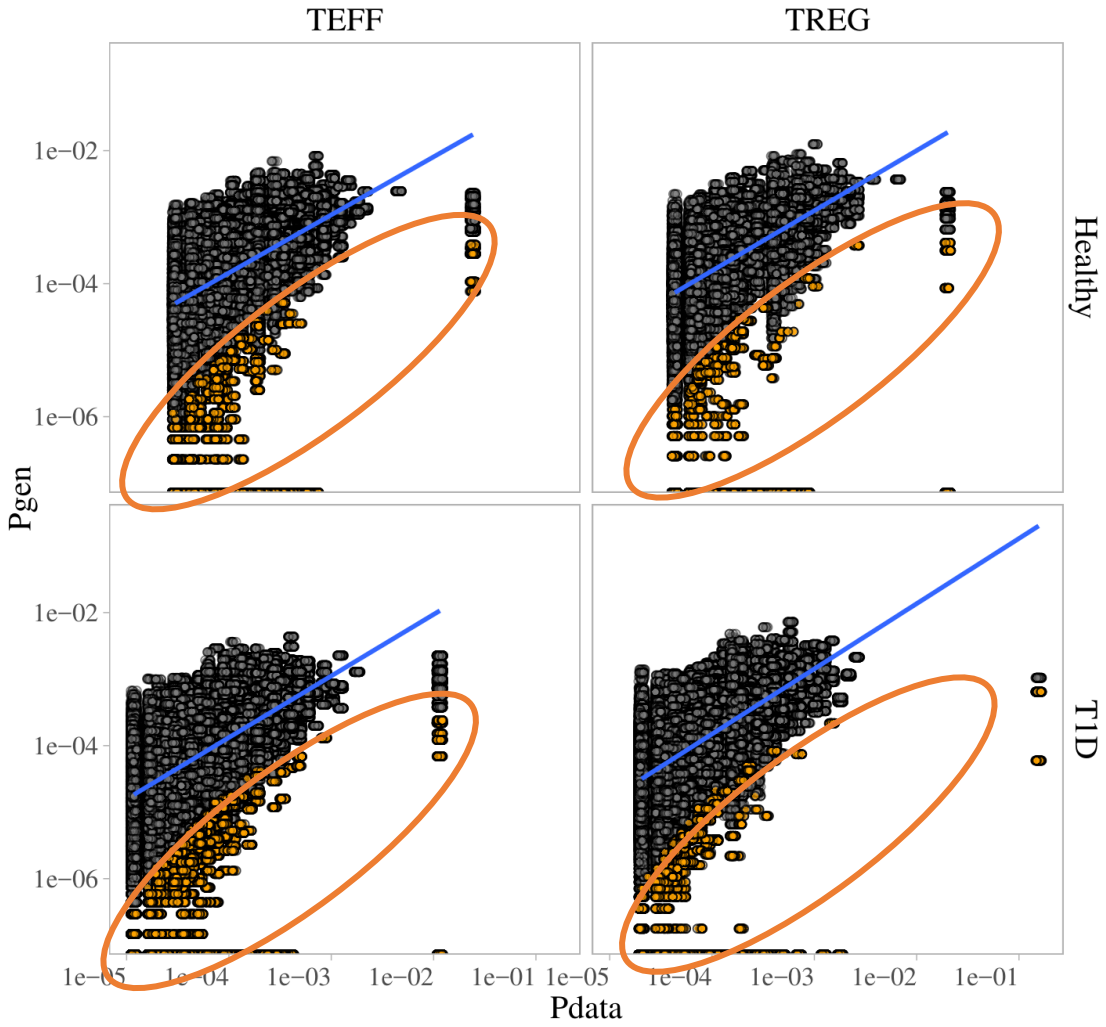
	TEFF		TREG	
	Enrich	Non-Enrich	Enrich	Non-Enrich
HV	11 568 (1.02%)	1 120 308 (98.98%)	8 238 (0.95%)	855 492 (99.05%)
T1D	29 291 (1.37%)	2 102 723 (98.63%)	15 978 (1.15%)	1 376 895 (98.85%)

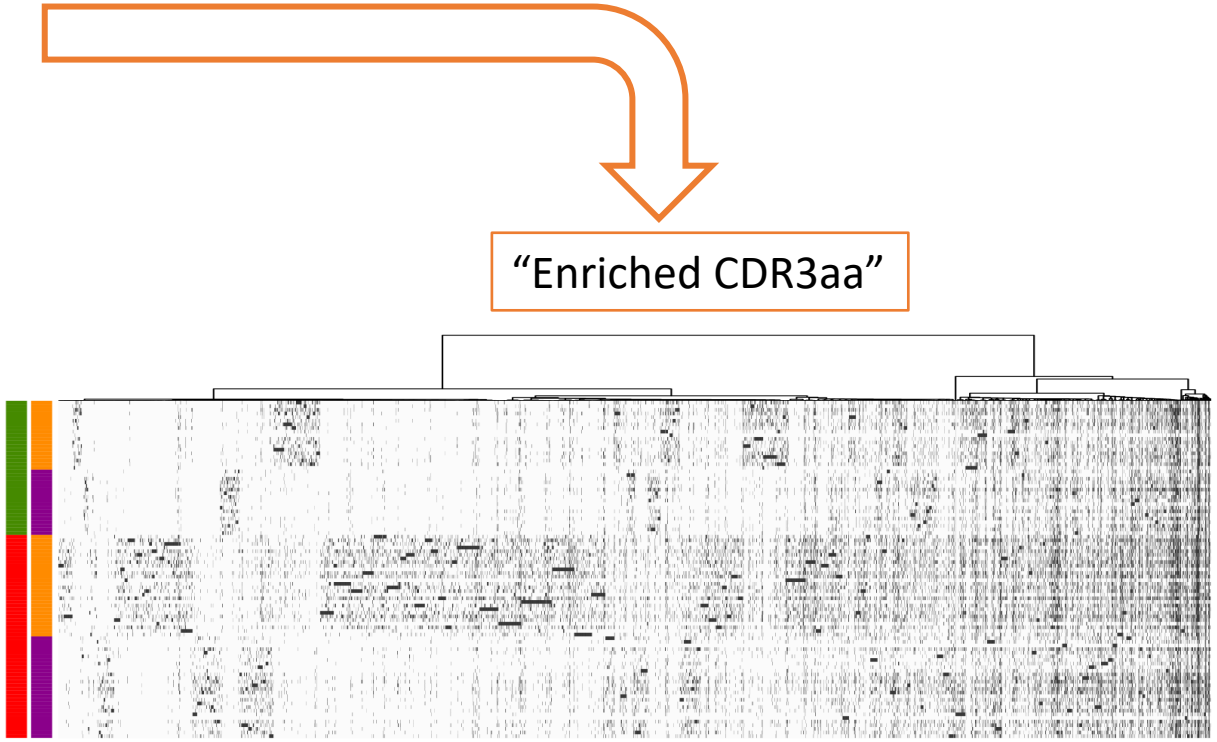
TEFF
p-value :
< 2.2e-16

TREG
p-value :
< 2.2e-16

⇒ **Significant higher proportion of CDR3aa that could be involved in an Immune response in T1D**

Probability distribution reveals higher over-observed CDR3aa in T1D





Are these CDR3aa shared between patients? What about cell subset sharing?

Identification of cell type/diagnosis TCR signatures

Presence (black) or absence (light grey) of the CDR3aa p.adj < 0.05

CDR3aa that are not T1D associated

=> We observed "private" clusters in each condition but also high proportion of public CDR3aa

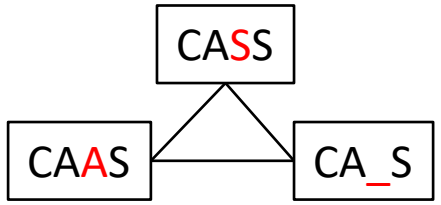
Are these clusters T1D associated?

Clustering of “Immune Involved” CDR3aa

1

Link CDR3aa: Compute Levenshtein distance

Filter CDR3aa differ by 1 aa

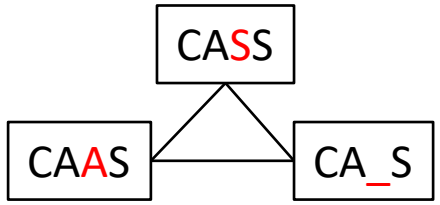


Insertion – Deletion - Substitution

Clustering of “Immune Involved” CDR3aa

1 **Link CDR3aa: Compute Levenshtein distance**

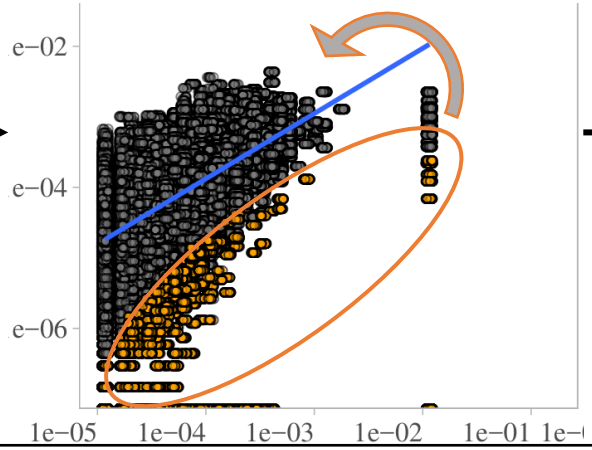
Filter CDR3aa differ by 1 aa



Insertion – Deletion - Substitution

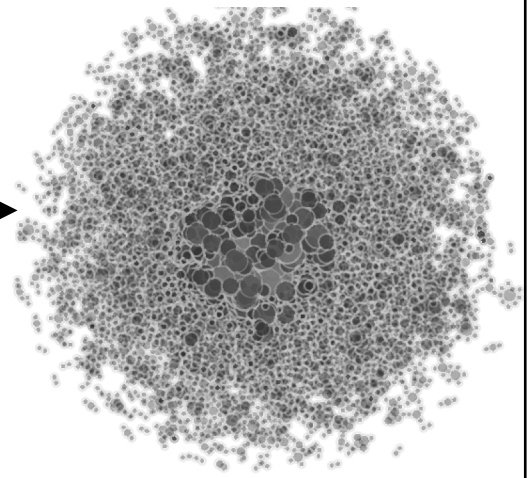
2 **Link CDR3aa Immune Involved with the rest of the repertoire**

BY Diagnosis-Subset Repertoire



$Enrich_{CDR3aa}$	$Non\ Enrich_{CDR3aa}$
CASSGEYQF →	CASSGGEYQF
CASSQGEYQF X	CASSQTTEYQF
CASSGGEYQF →	CASSGGEAQF
CASSTEFEYQF X	CASTTE_EYQF

Enrich+ Network

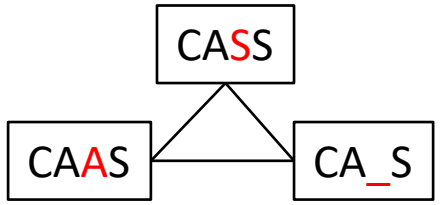


Clustering of “Immune Involved” CDR3aa

1

Link CDR3aa: Compute Levenshtein distance

Filter CDR3aa differ by 1 aa

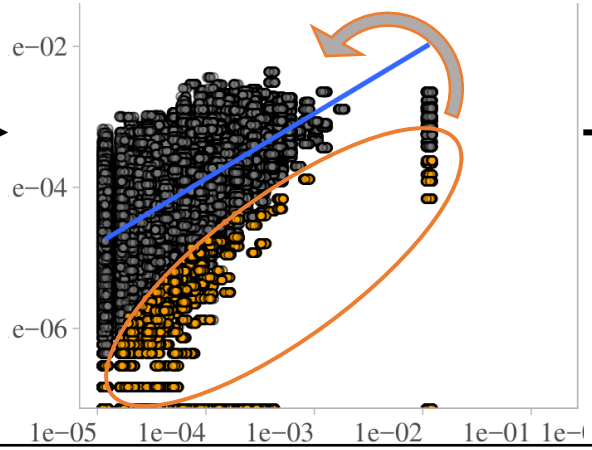


Insertion – Deletion - Substitution

2

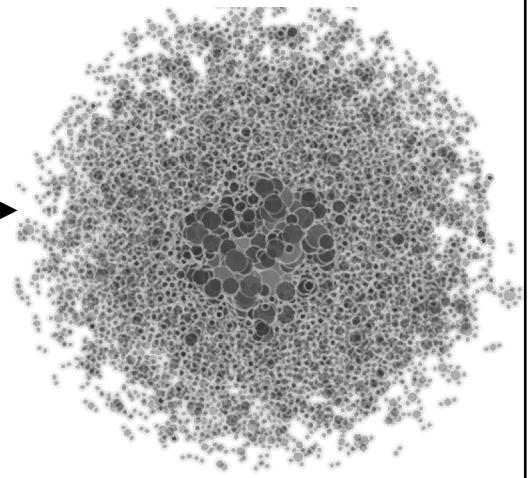
Link CDR3aa “Immune Involved” with the rest of the repertoire

BY Diagnosis-Subset Repertoire



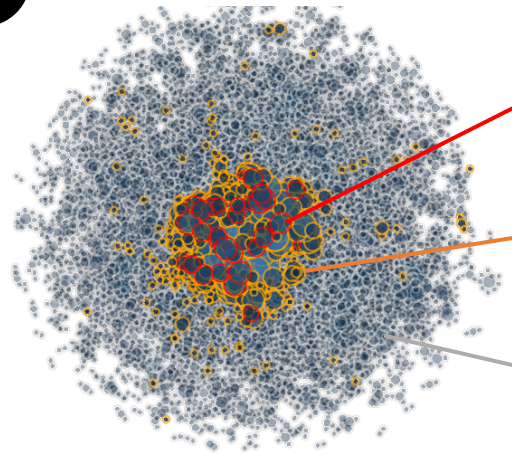
$Enrich_{CDR3aa}$	$Non\ Enrich_{CDR3aa}$
CASSGEYQF →	CASSGGEYQF
CASSQGEYQF X	CASSQTTEYQF
CASSGGEYQF →	CASSGGEAQF
CASSTEFEYQF X	CASTTE_EYQF

Enrich+ Network



3

Enrich+ T1D Network



Attribute T1D specificity from Database on Enrich+ CDR3aa

Red : Perfect Match T1D CDR3aa specific

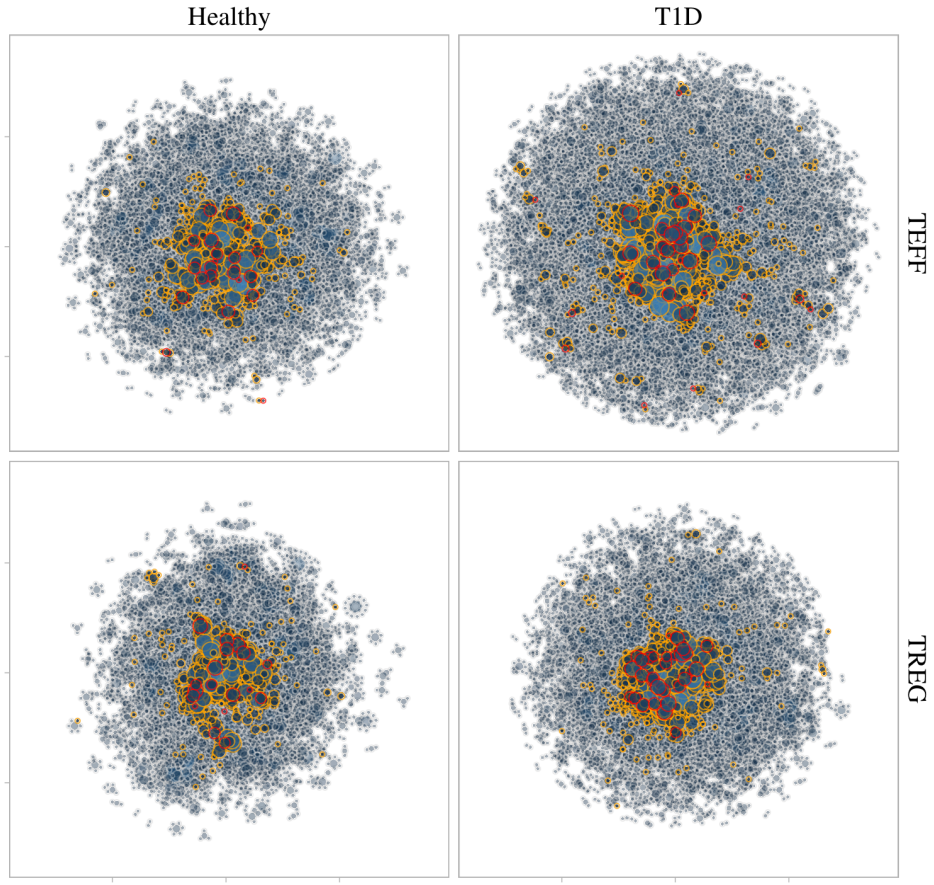
Orange : Lev Distance with T1D specific CDR3aa = 1

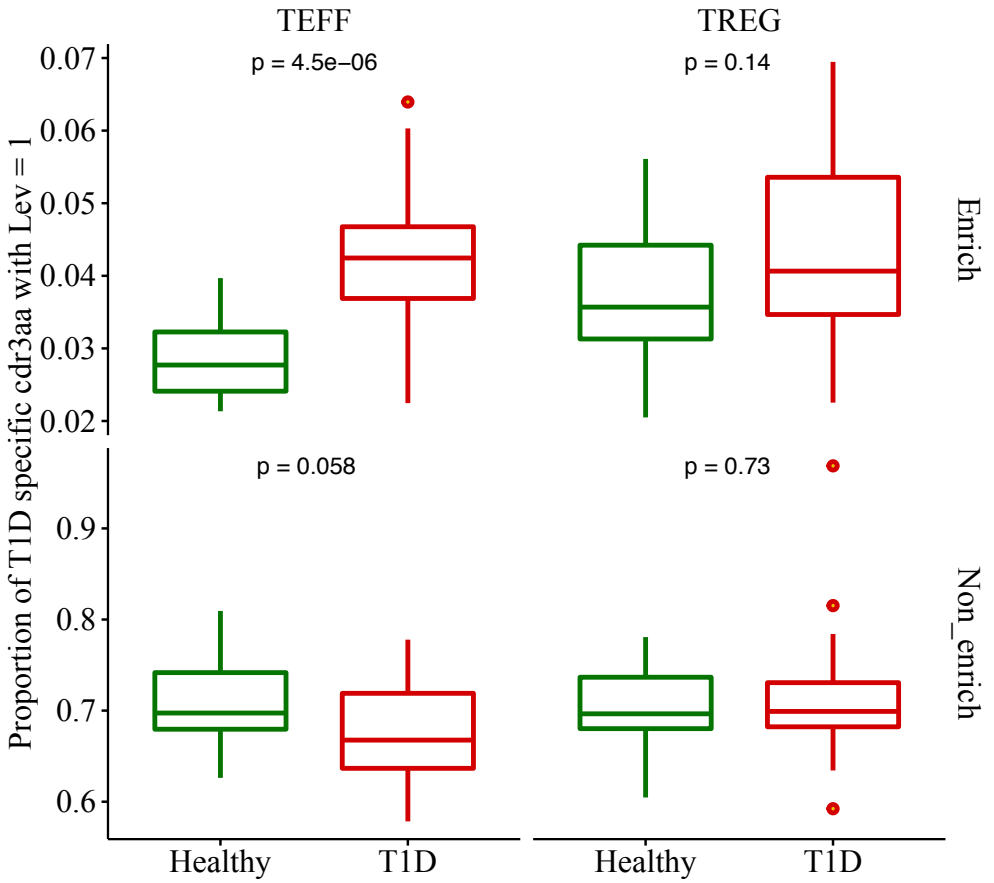
Grey : Unknown Specificity

TCR ID	TR GENE	cdr3aa	Species	Category	Pathology	Epitope_peptide
2619	TRB	CSVEATRADTQYF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQCCTSISSL
975	TRA	CAVGALAGTASKLTF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQCCTSISSL
1746	TRB	CASSLATSGGGSDTQYF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL
3000	TRB	CASSLATSGGGSDTQYF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL
3195	TRB	CASSLATSGGGSDTQYF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL
3549	TRB	CASRNLGLNTEAF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL
3562	TRB	CASSFRRVTDTQYF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL
3573	TRB	CASSLAVIRTDTQYF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL
3600	TRB	CASSLGQGETEAF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL
3637	TRB	CASSQVRLAGGGEQF	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL
102	TRA	CADAGGTSYKLFGQGTIL	HomoSapiens	Autoimmune	Diabetes Type 1	KRGIVEQSSTSISSL

$Enrich+_{CDR3aa}$
CASSGEYQF
CASSQGEYQF
CASSGGEYQF
CASSTEFEYQF
CASSGGEYQF
CASSGGEAQF

TEFF T1D Enrich CDR3aa are more T1D specific than HV





⇒ **Highest proportion of "specific CDR3aa-Lev1" in "Enriched CDR3aa" from T1D-Teff**

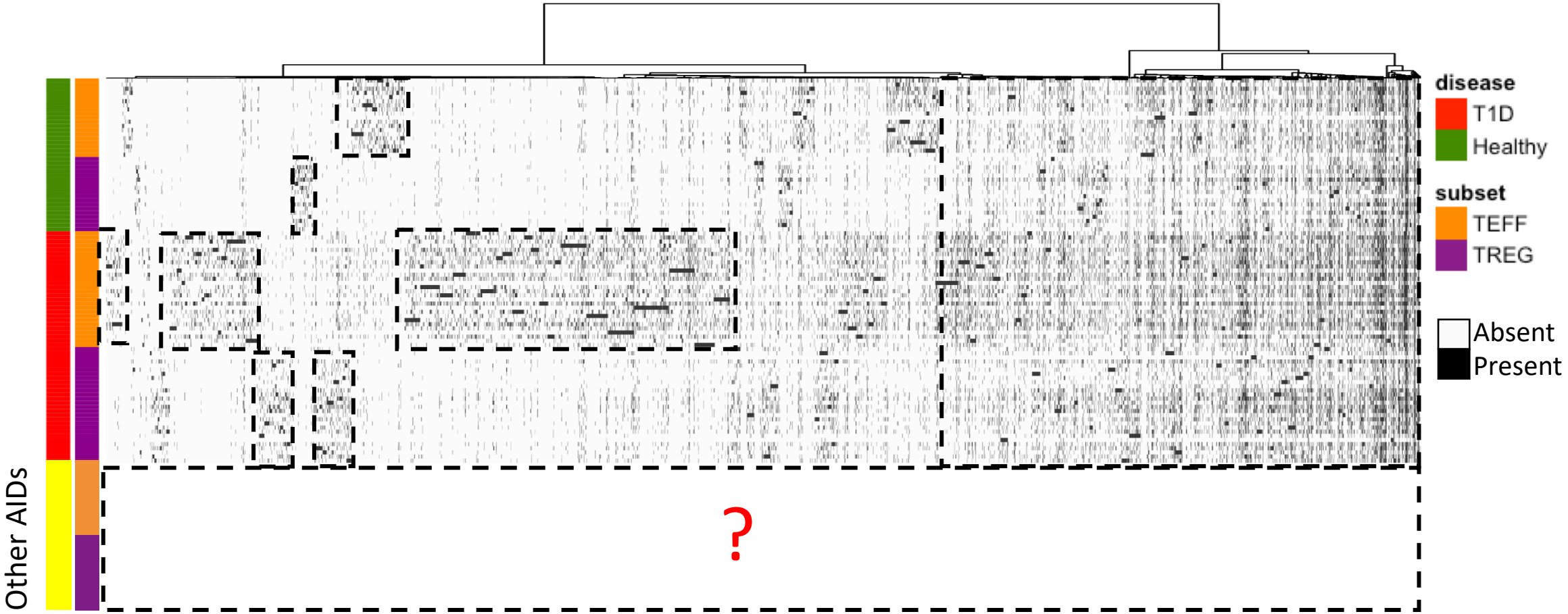
Conclusions

TCR repertoire in **T1D** patients **revealed**

- a **higher diversity** of **Treg** repertoire in T1D compared with HV
- a tendency toward **lower expansions** in **Treg** T1D
- **Higher** proportion of **enriched** CDR3aa in T1D compared with HV
- Some of these **CDR3aa** are **private** in T1D with **higher** proportion of **T1D disease association**
- Go further by **comparing** specific **clusters** with **Logo**, **Connection** Stats, higher number of **neighbours** (Lev1) than expected, add other AIDs, …

Shared clusters with other AIDs ?

Presence (black) or absence (light grey) of the CDR3aa p.adj < 0.05



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THANK YOU FOR YOUR ATTENTION