



# T-Therapeutics

## OpTiMus<sup>®</sup> Platform for Human TCR Discovery

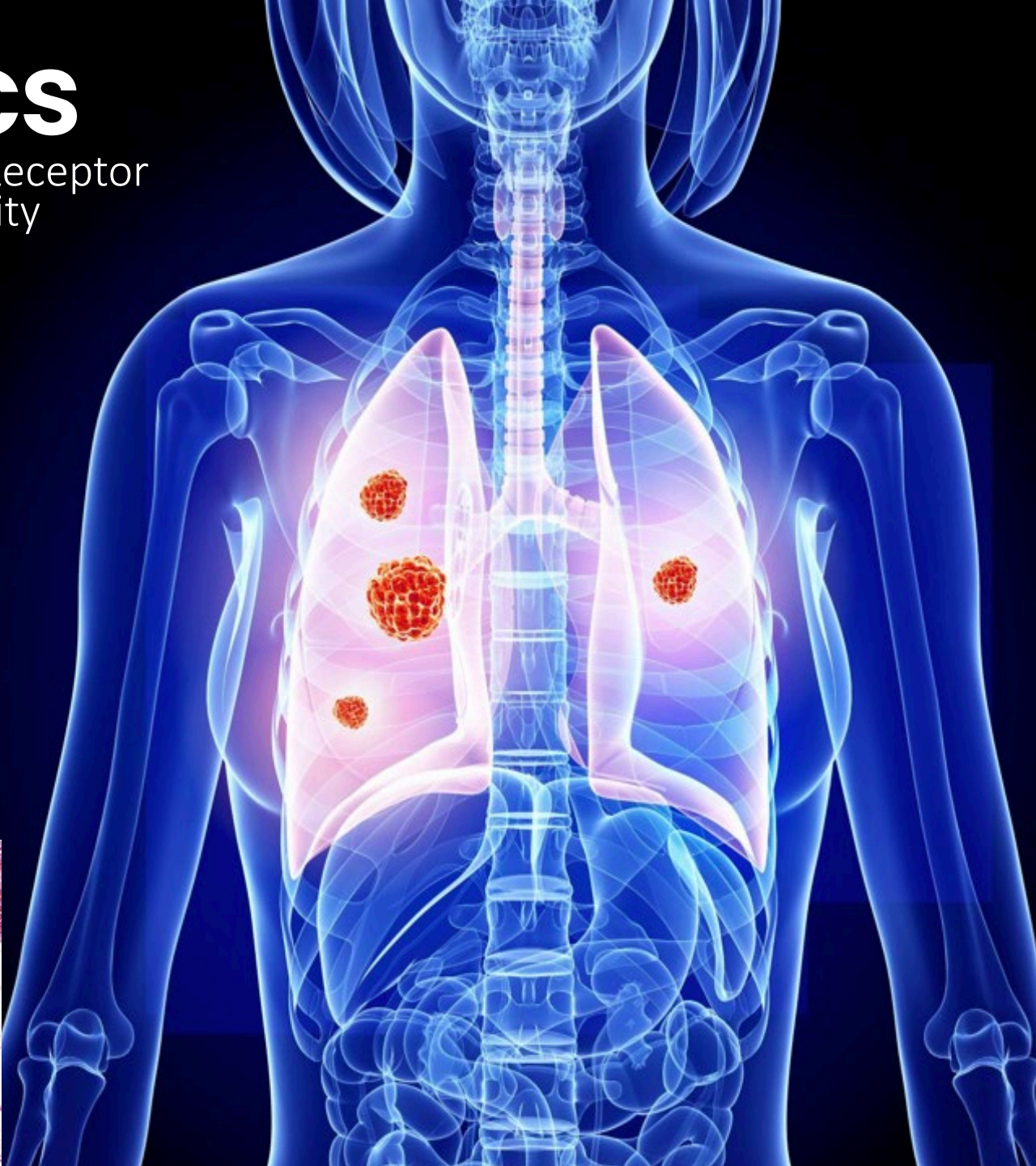
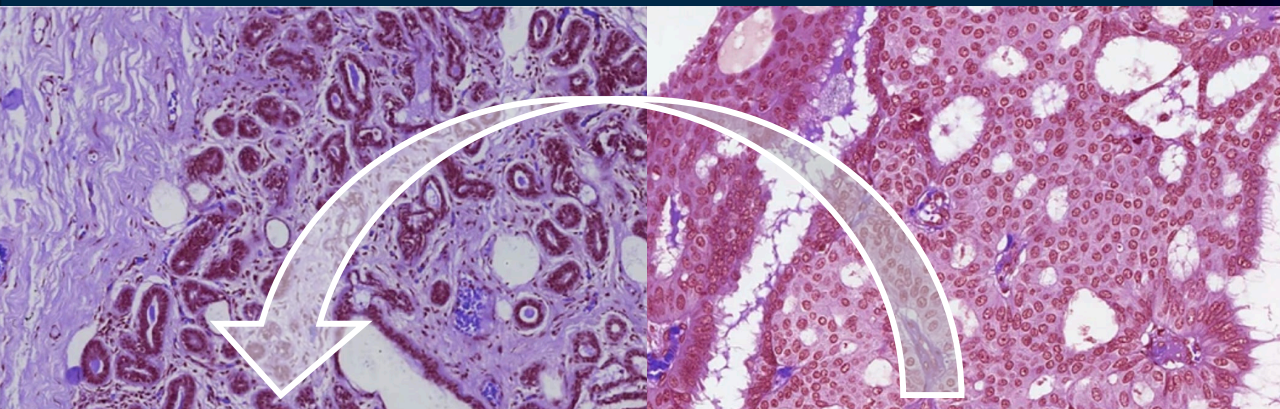
[T-Therapeutics.com](https://www.t-therapeutics.com)



# T-Therapeutics

Making T Cell Receptor  
biologics a reality

*We intend to discover, develop and  
bring into the clinic transformative  
soluble TCR-biologics that will reshape  
the clinical landscape for cancer*



Cambridge University and Sanger Institute – spin out



**UNIVERSITY OF  
CAMBRIDGE**  
School of Clinical Medicine

We were founded to discover and develop soluble TCR bi-specifics:

- Derived from T cells
- Recruit T cells to cancerous, inflamed or infected tissues
- Activate T cells to kill diseased cells or moderate T cell activity to suppress inflammation

We are funded by a blue-chip VC syndicate:

- F Prime Capital, Sofinnova Partners, Cambridge Innovation Capital, Digitalis and Sanofi Ventures

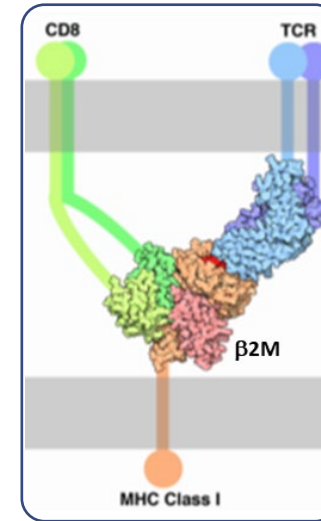
We are building a pipeline of soluble TCR biologics [pMHC-T cell engagers]:

# OpTiMus<sup>®</sup> proprietary human-TCR mice

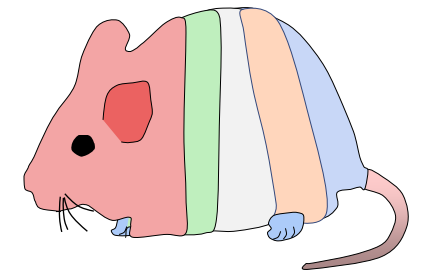
## An unlimited source of human TCRs against human self-peptides and neo-antigens

- 10 years of work - major barrier to entry
- > 40 genetic engineering steps at 7 loci
- 1.6 million bases of human DNA transferred
- Mouse genes silenced
- Human TCR V, D and J genes intact, rearrange normally and assemble into functional TCRs

The mouse has 1.6 million base pairs of human DNA - equivalent to the number of letters in two volumes of Lord of the Rings : 1.6m



Sextuple transgenic  
*TCR $\alpha$ /TCR $\beta$ /CD8/MHC I/ $\beta$ 2m*

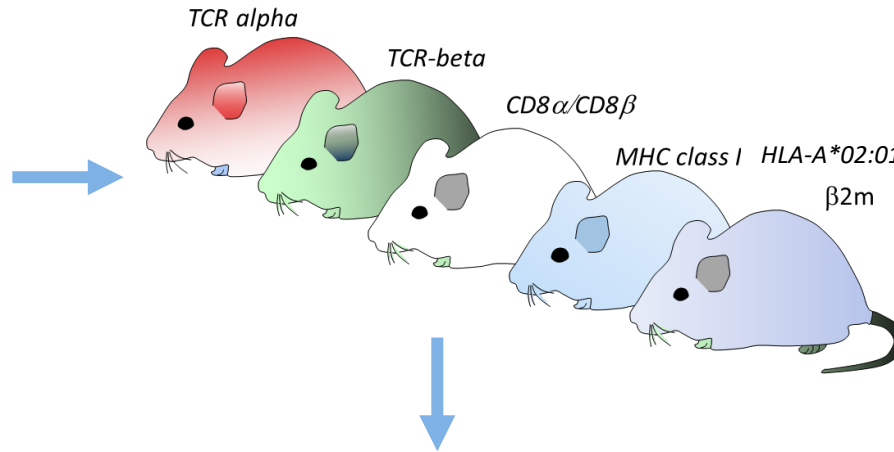


# OpTiMus<sup>®</sup> humanized mouse platform is enabled to generate human TCRs selected on human MHC class I

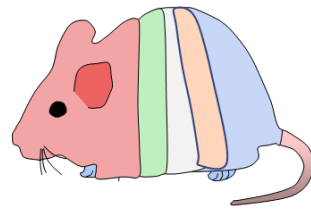
CTL/MHC Class I immune synapse



Individual genes were humanized using gene targeting in ES cells

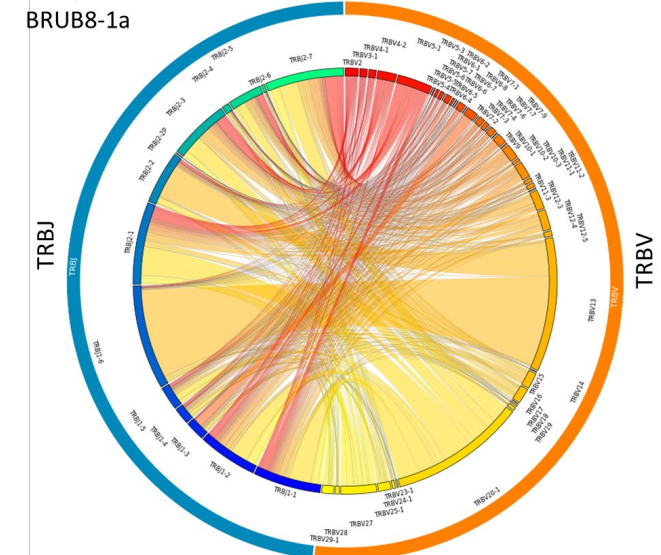
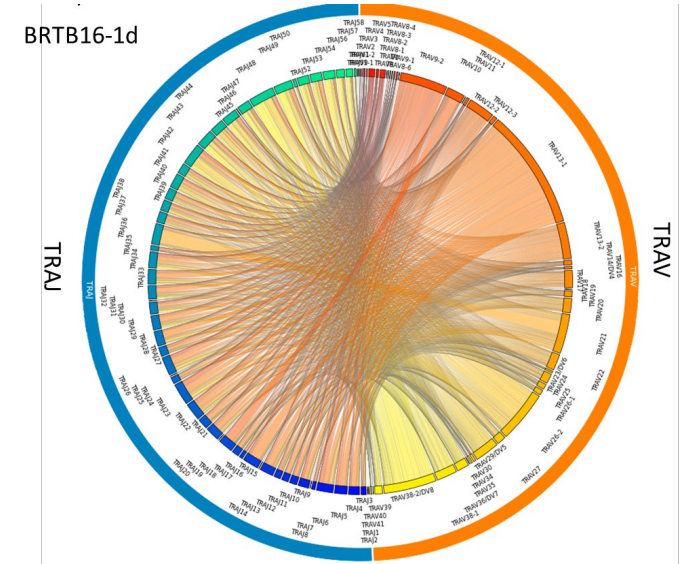


Humanized alleles were bred together to create a compound transgenic mouse

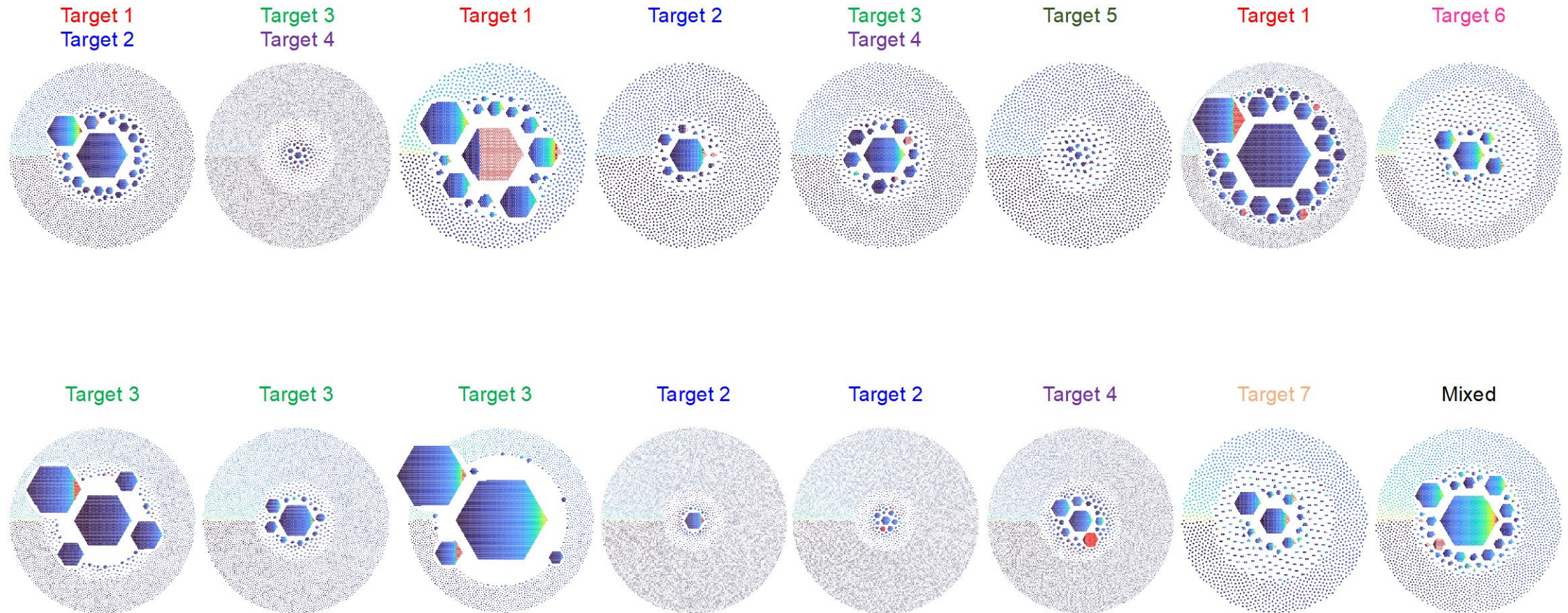


**Sextuple transgenic**  
TCR $\alpha$ /TCR $\beta$ /CD8/MHC I/ $\beta 2m$

Broad repertoire of TCR $\alpha$  and TCR $\beta$  V&J usage

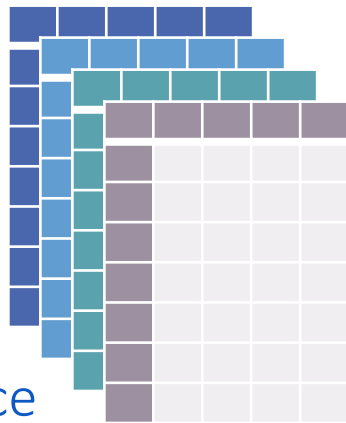
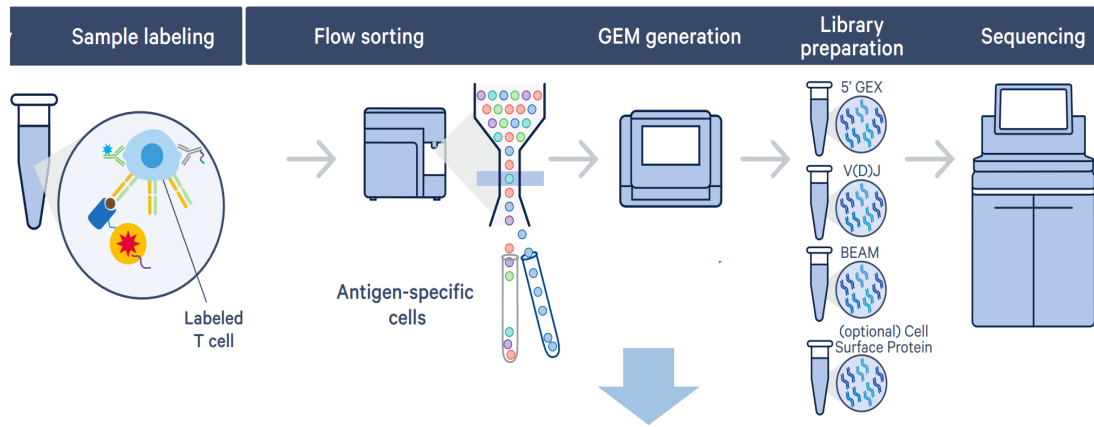


# Millions of human TCR sequences from immunization of humanized mice

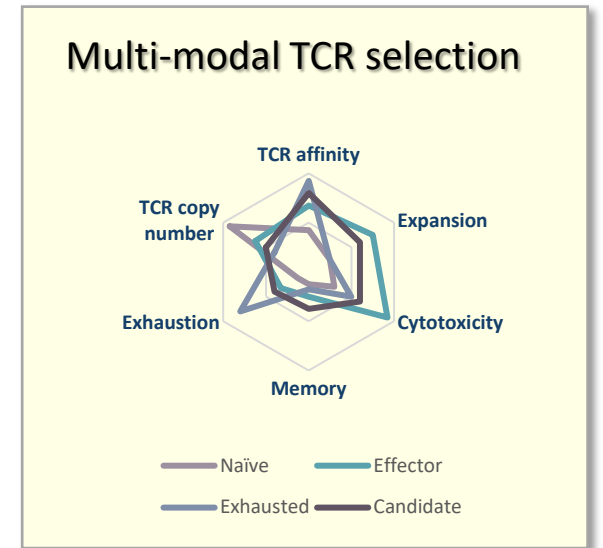
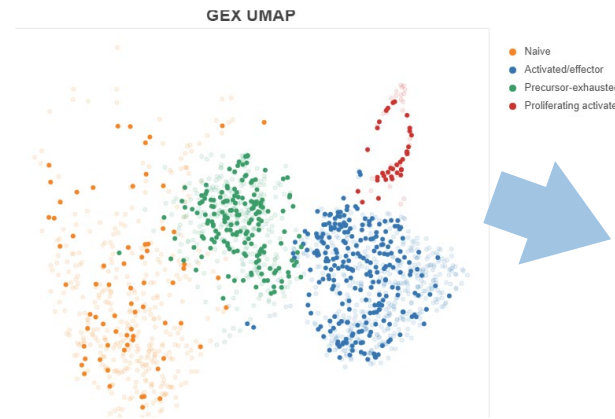
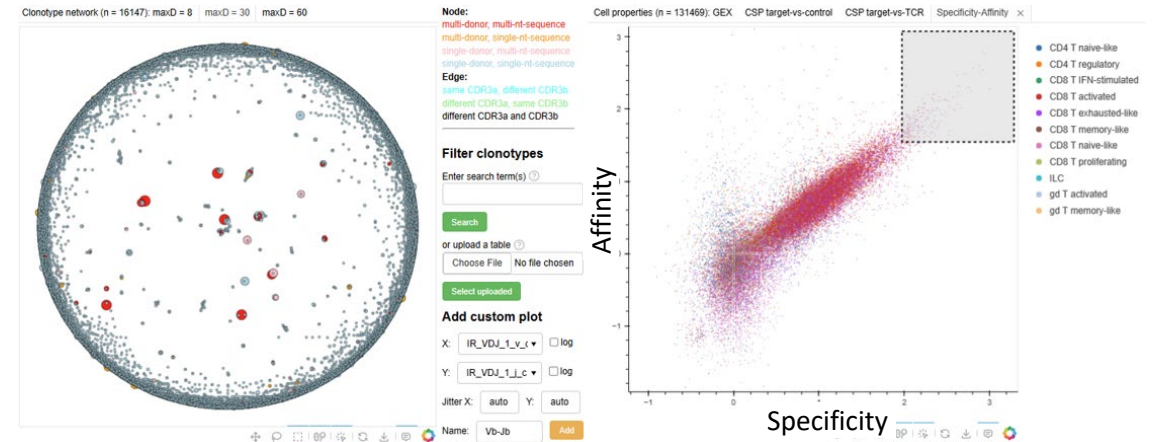


- *Great immune response from transgenic mice for a variety of targets*
- *Plenty of diversity and clonal expansion, which ones to choose?*

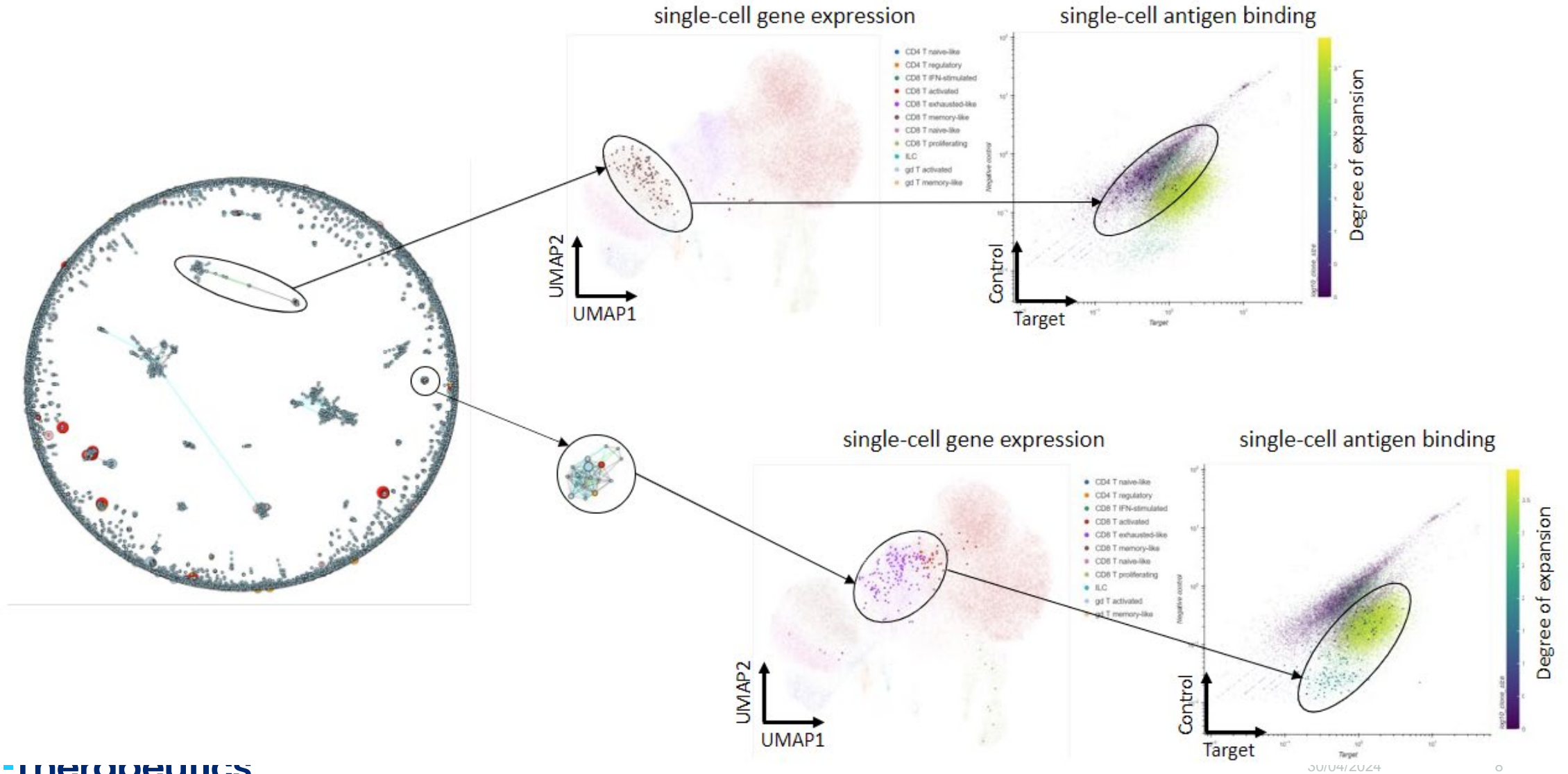
# TCR discovery through multi-modal phenotyping



- TCR sequence
  - Whole transcriptome
  - Cell surface protein
  - Target pMHC binding

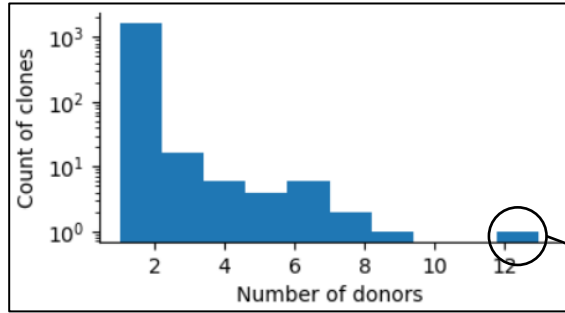


# TCR Sequence similarity correlate with T cell phenotype similarity

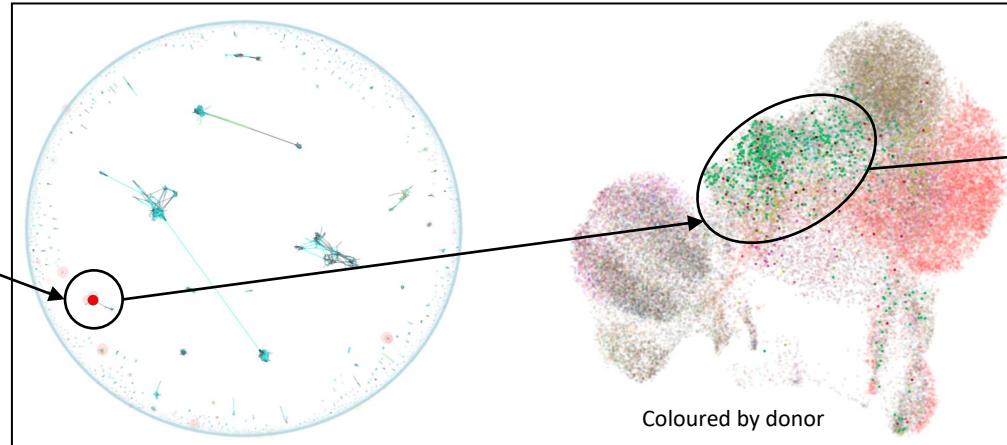




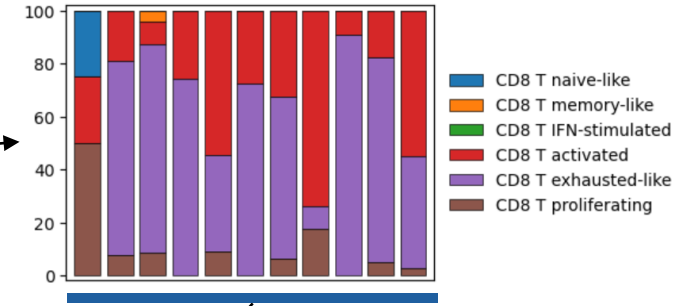
# Cells of the same clonotype share expression phenotypes across donors



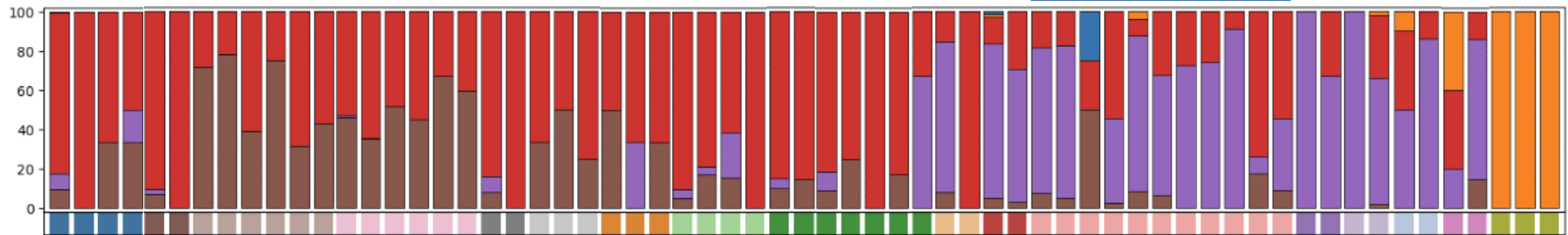
A large number of TCR clonotypes were found in multiple mice.



Cells of a multi-donor clonotype localise in similar positions in the gene expression space, regardless of the donor they are from.

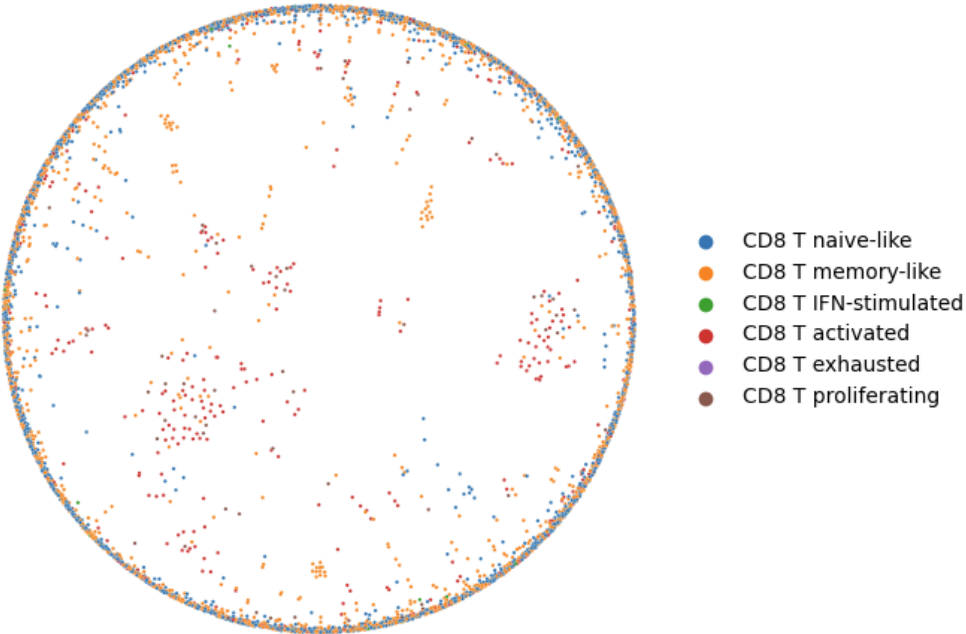


Similar cell type composition across donors.

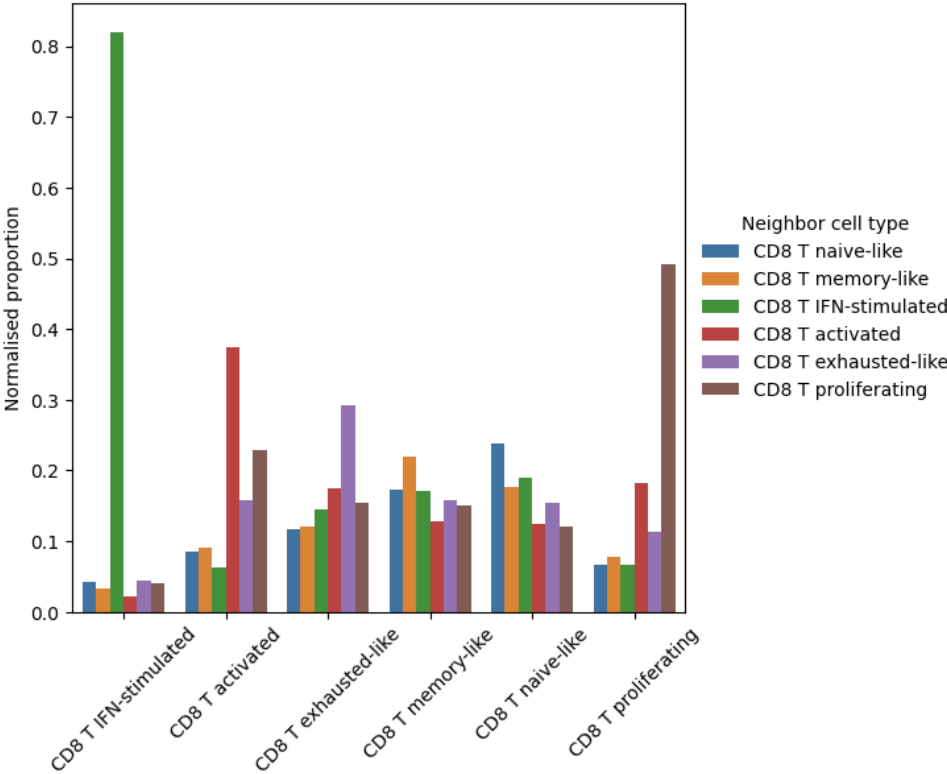


# Clonotypes with similar expression phenotypes cluster together in TCR sequence space

Clonotypes clustered by sequence similarity

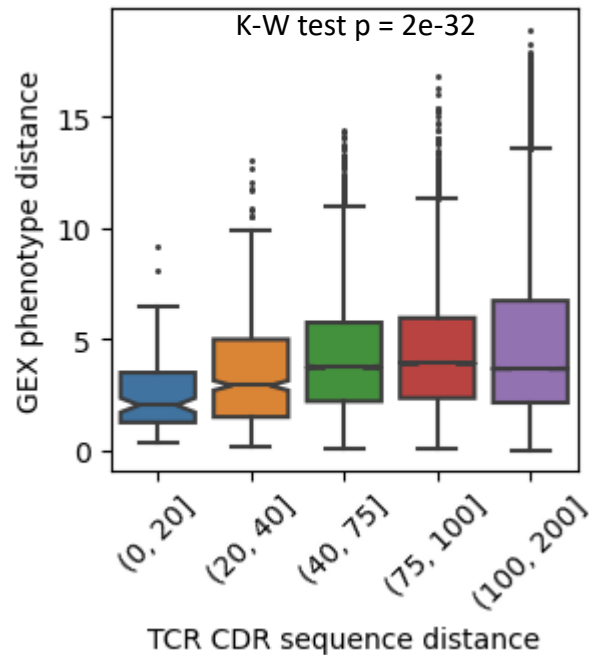


Cell type proportion of top10 nearest neighbours in TCR sequence space

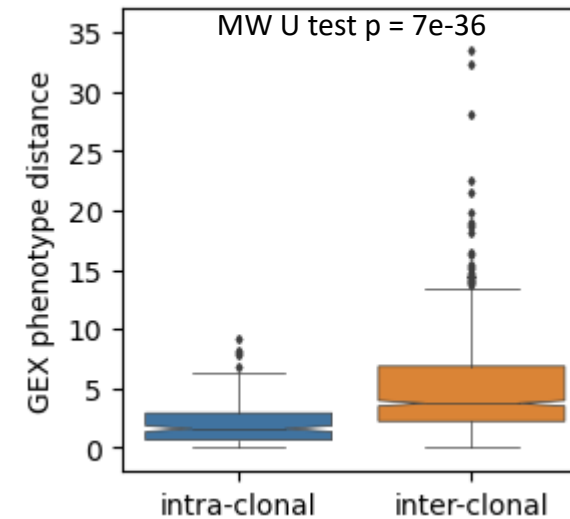


# Quantitative comparison of expression phenotype similarity between same or different clonotypes

Clonotypes with more similar CDR sequences have more similar expression phenotype

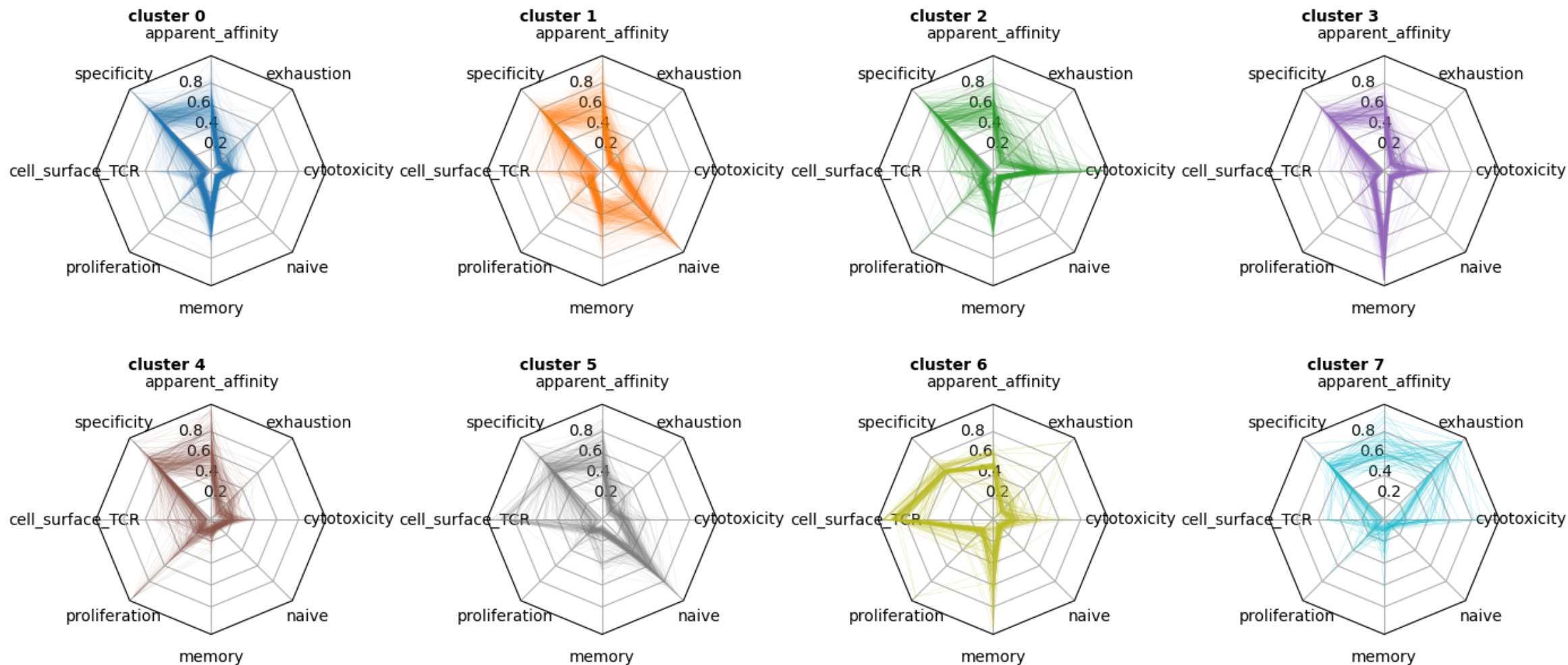


T cells carrying the same TCR are more similar in expression phenotype than those carry different TCR sequences



*The sequence and the intrinsic properties of TCR likely drives the cellular phenotype of the T cells.*

# Mouse platform yields TCRs that drive a diverse range of phenotypes

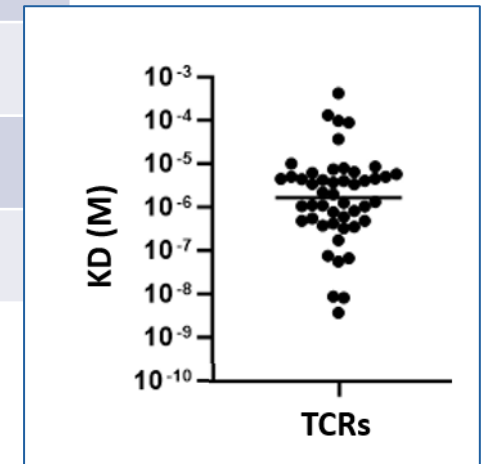


# Summary: platform and multi-modal analysis enable optimal TCR choices

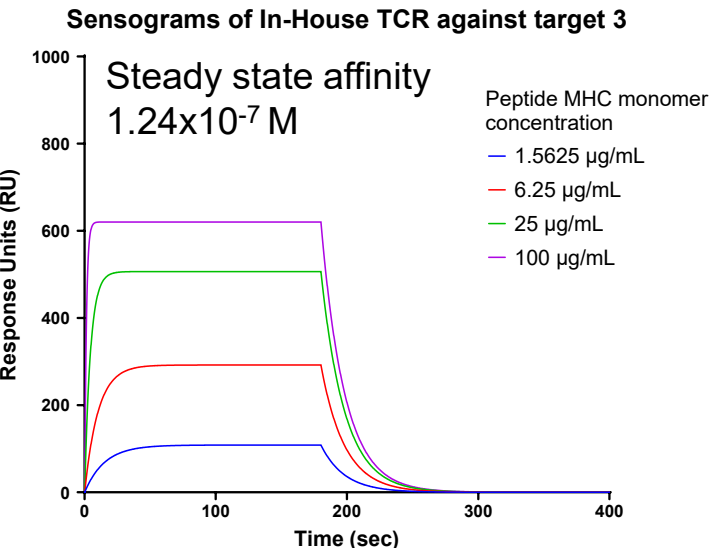
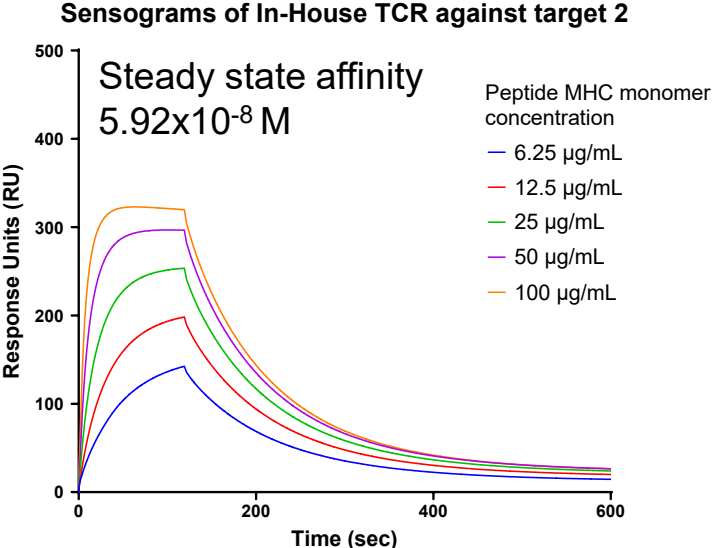
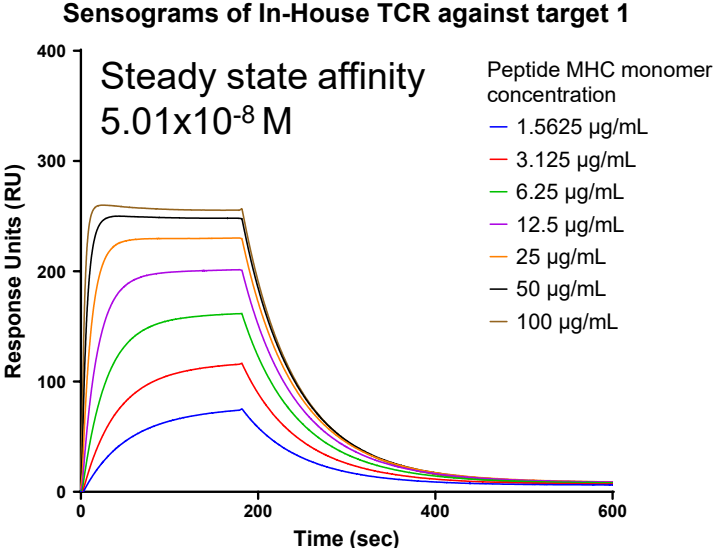
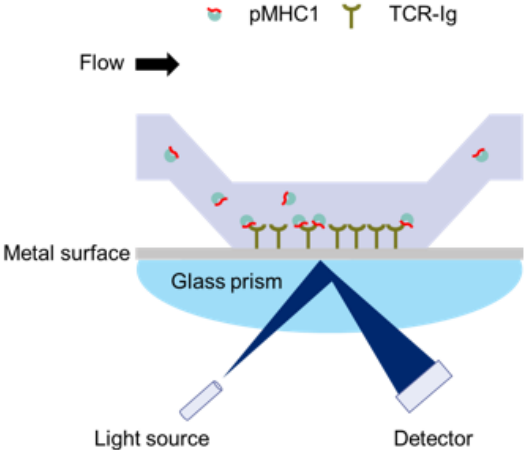
- Integrating sc-TCR sequence, GEX and CSP abundance to characterise and select the optimal TCR
- TCR sequence similarity correlates with T cell phenotype similarity
  - The sequence and the intrinsic properties of TCR likely drives the cellular phenotype of the T cells
- Our mouse platform yields TCRs that drive a diverse range of phenotypes - *select to suit different clinical applications*

# Competitive advantages of OpTiMus<sup>®</sup> platform over others

	TCR discovery from human blood	TCR discovery from OpTiMus <sup>®</sup> mouse
Numbers of CD8 <sup>+</sup> T cells	10 <sup>9</sup> cells / 5L blood	10 <sup>7</sup> cells/spleen
Diversity	++	++++
Human Ag negative selection	+++	+/-
Binders to human antigen	<10	100s
Affinity	10 <sup>-4</sup> - 10 <sup>-6</sup> M	10 <sup>-4</sup> - 10 <sup>-8</sup> M
Phenotype association	No	Yes
Clonotype clustering	No	Yes
Rapid throughput	No	Yes

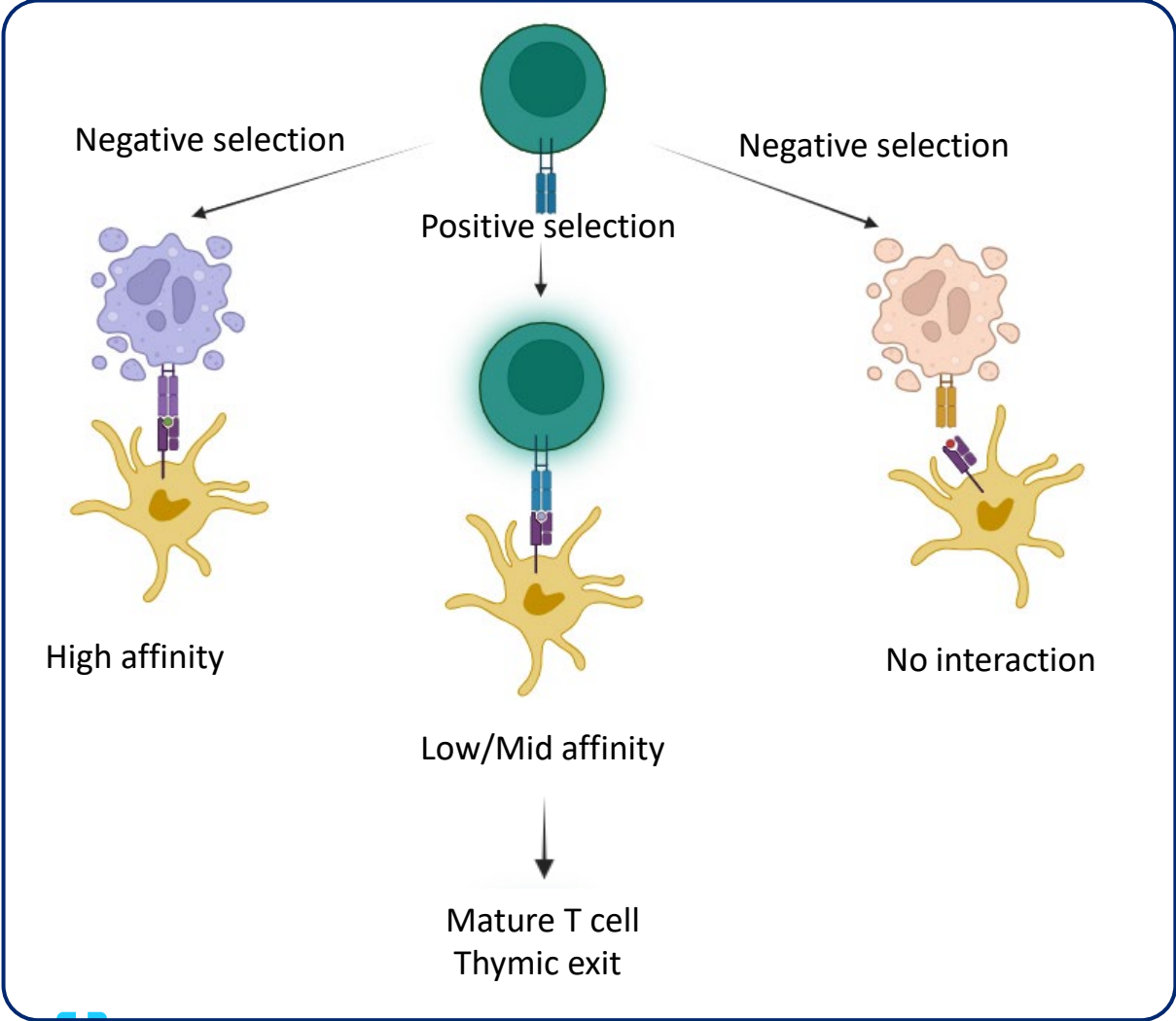


# Identification of high affinity TCR from OpTiMus<sup>®</sup> platform

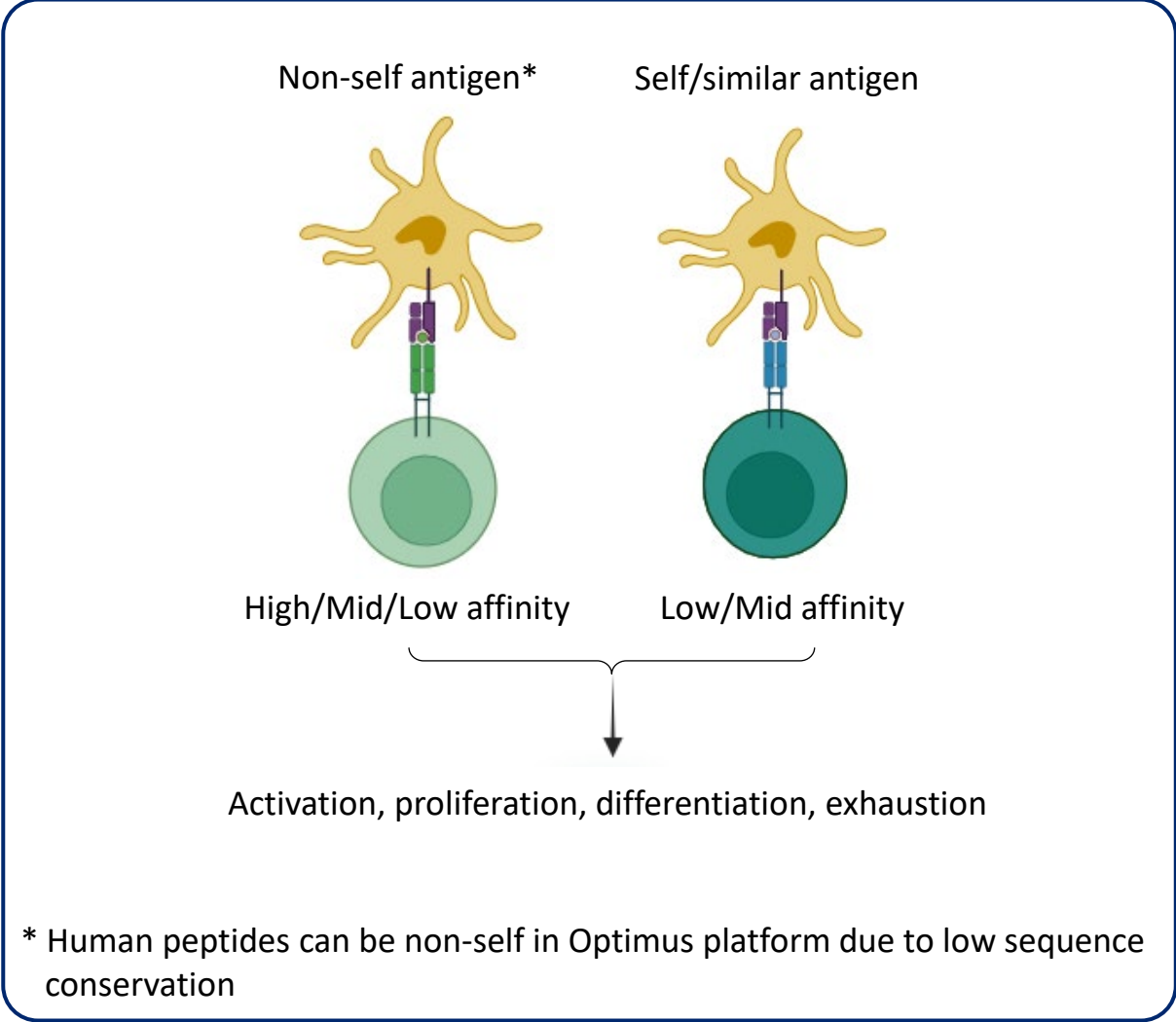


# Thymic negative selection eliminates high affinity TCRs against self antigen

## MHCI constrained thymic T cell selection against self-antigen



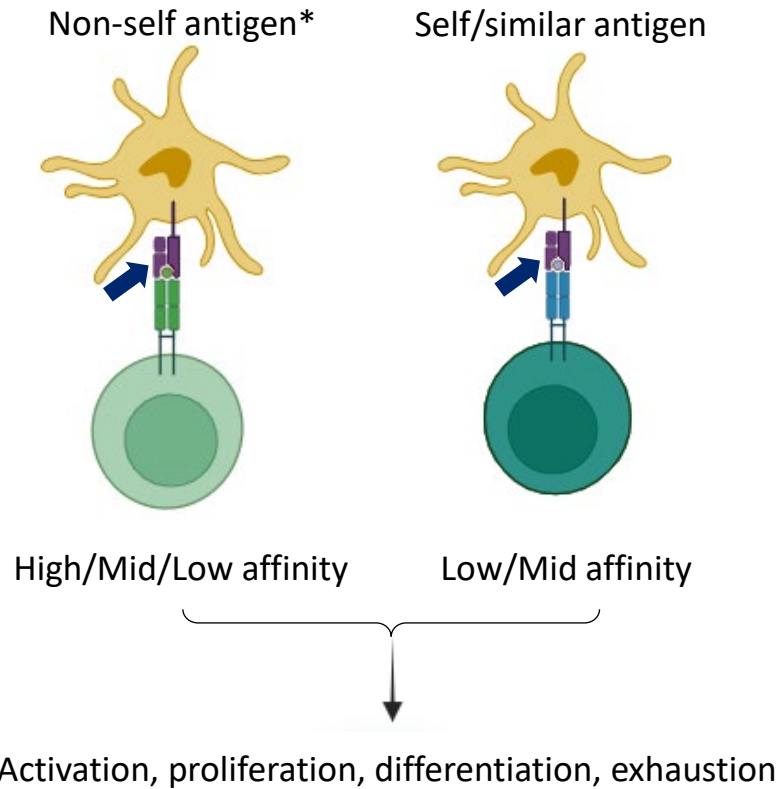
## Mature T cell antigen interaction – syngeneic MHC priming





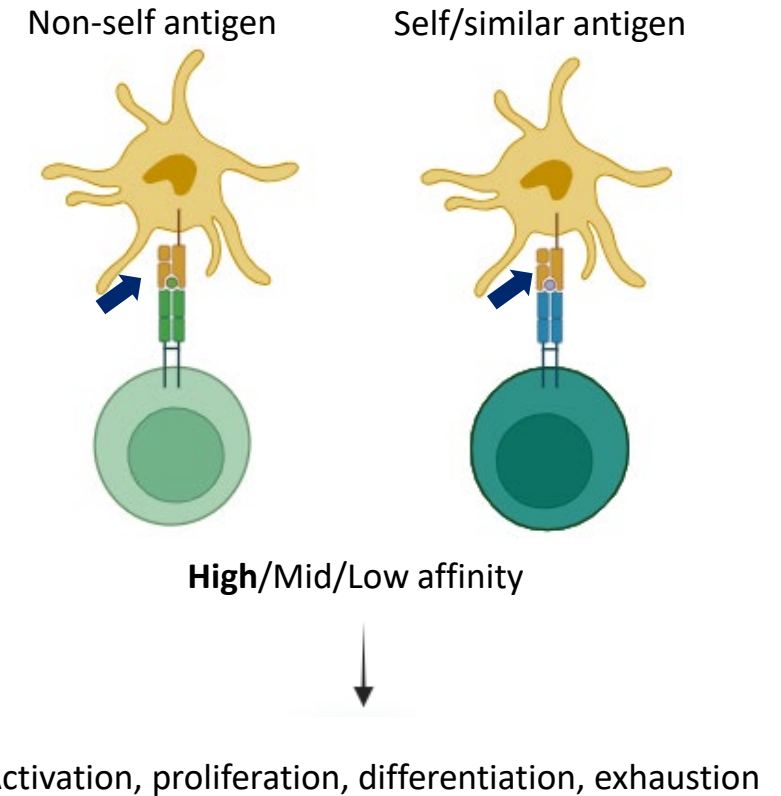
# Thymic negative selection eliminates high affinity TCRs against self antigen

## Mature T cell antigen interaction – syngeneic MHC priming



\* Human peptides can be non-self in Optimus platform due to low sequence conservation

## Mature T cell antigen interaction – allogenic MHC priming



Allogenic MHC priming allows the generation of high affinity TCRs despite sequence conservation

# In vivo allo MHC priming has been observed in GvHD patients, but not attempted as a TCR discovery method using transgenic mouse platform

> Clin Cancer Res. 2011 Sep 1;17(17):5615-25. doi: 10.1158/1078-0432.CCR-11-1066. Epub 2011 Jul 19.

## PRAME-specific Allo-HLA-restricted T cells with potent antitumor reactivity useful for therapeutic T-cell receptor gene transfer

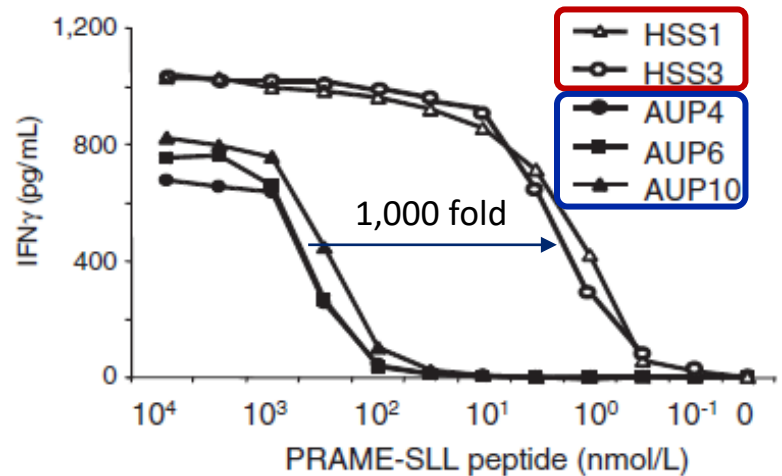
Avital L Amir<sup>1</sup>, Dirk M van der Steen, Marleen M van Loenen, Renate S Hagedoorn, Renate de Boer, Michel D G Kester, Arnoud H de Ru, Gert-Jan Lugthart, Cees van Kooten, Pieter S Hiemstra, Inge Jedema, Marieke Griffioen, Peter A van Veelen, J H Frederik Falkenburg, Mirjam H M Heemskerck

Affiliations – collapse

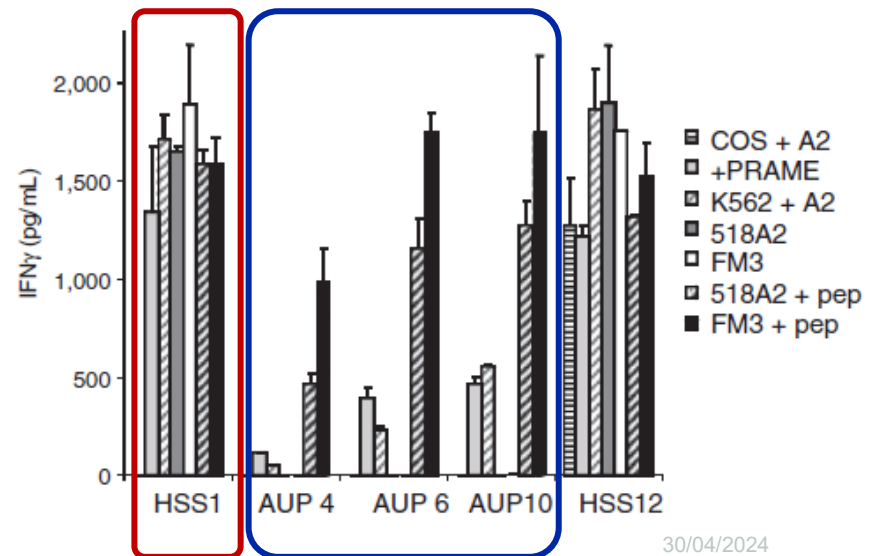
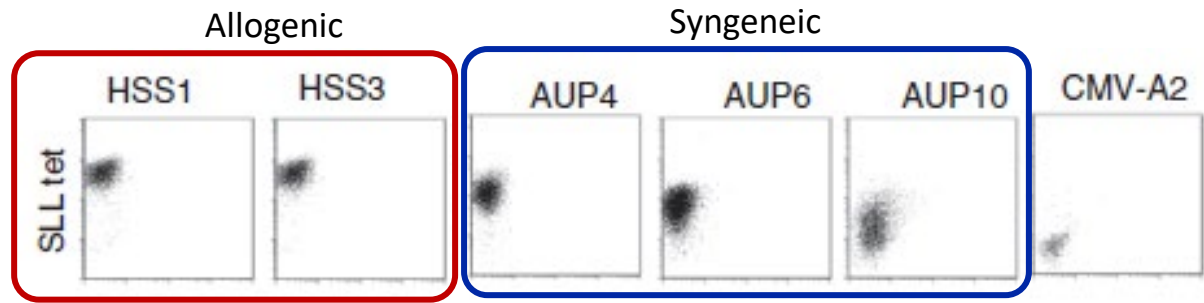
### Affiliation

<sup>1</sup> Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands.

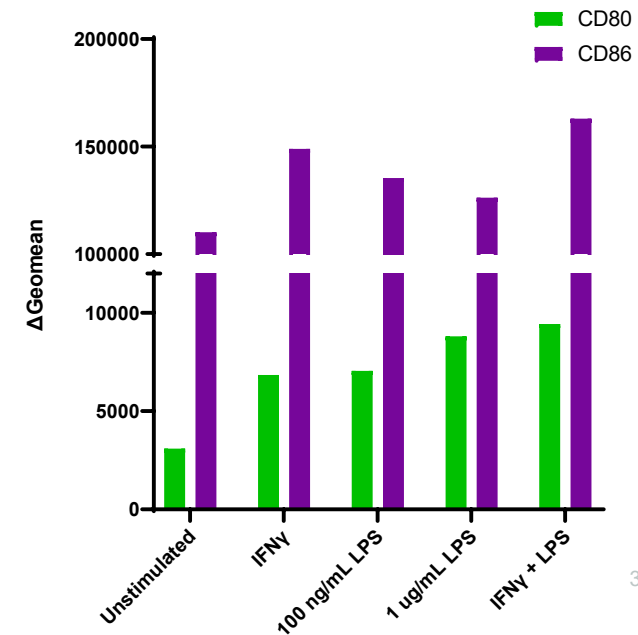
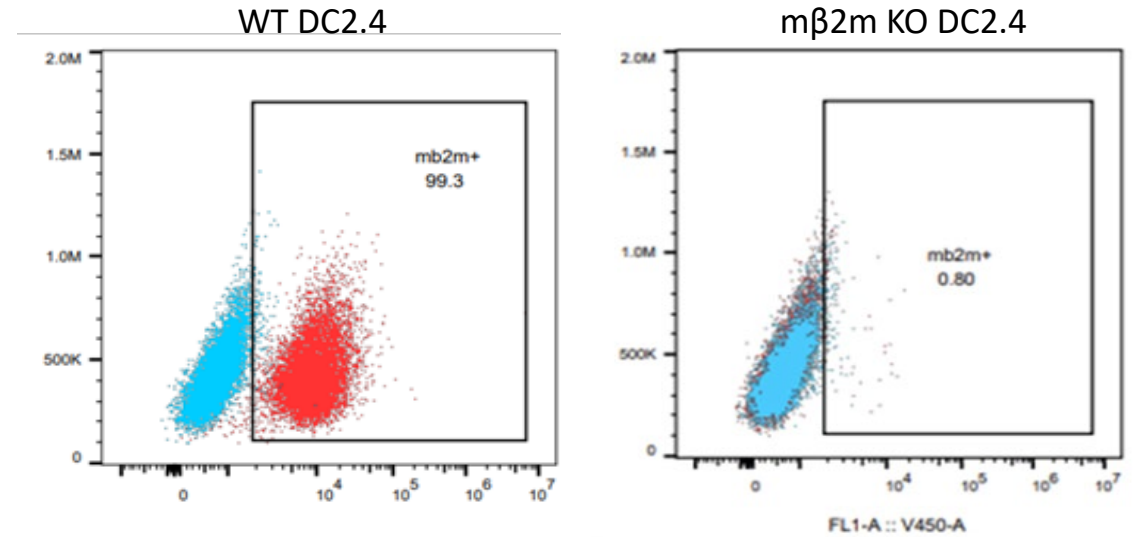
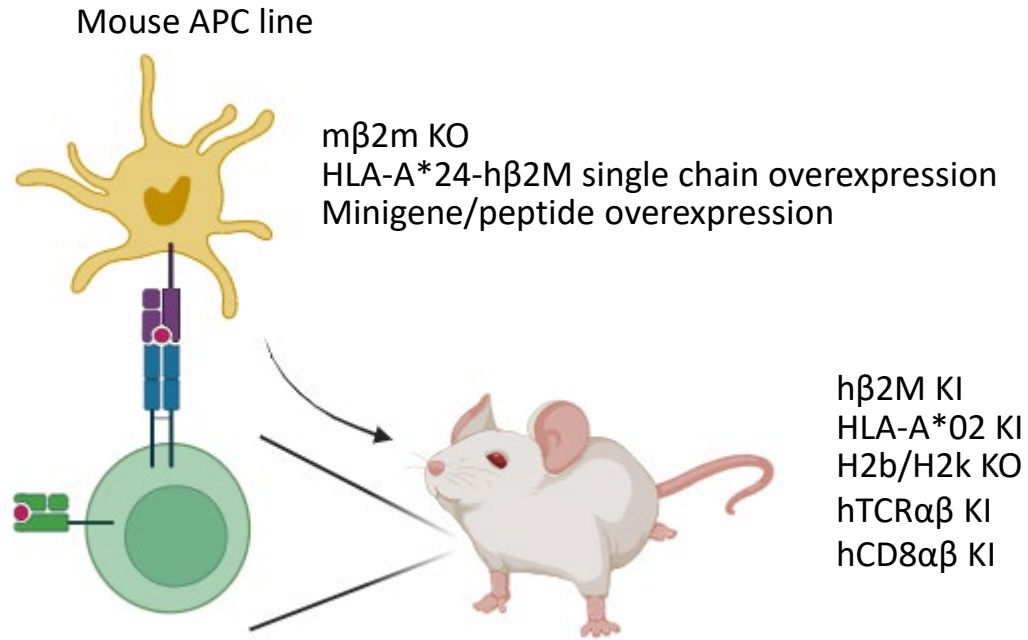
PMID: 21771875 DOI: 10.1158/1078-0432.CCR-11-1066



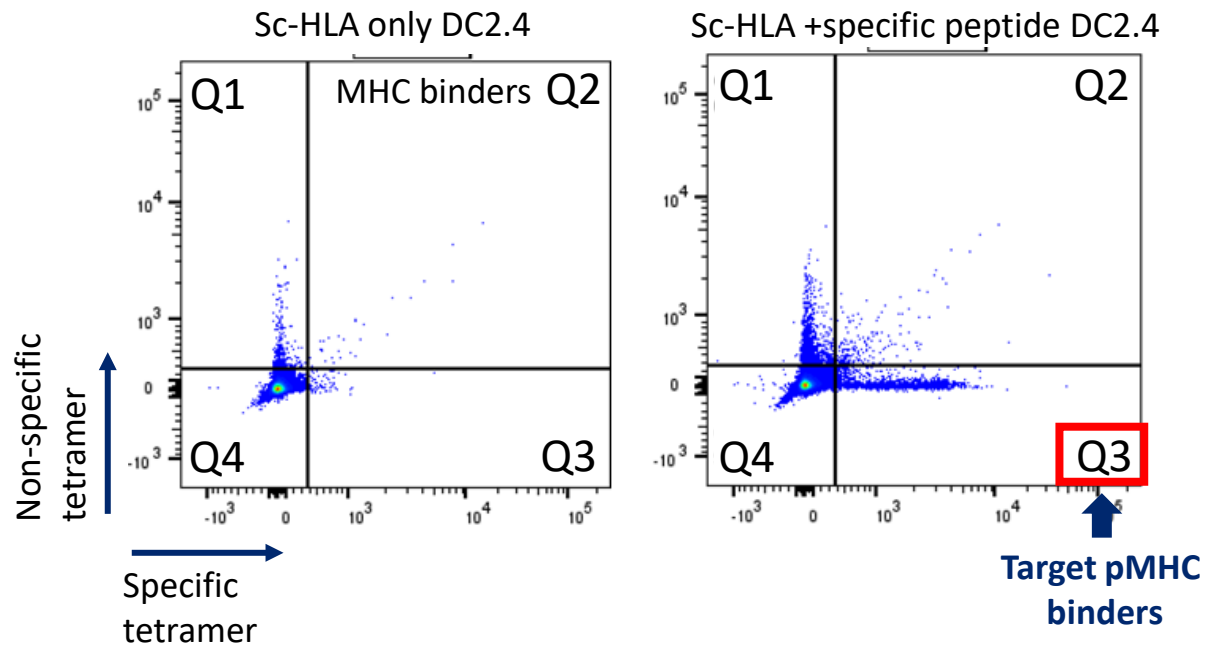
### Allogenic vs syngeneic priming derived CTL clones



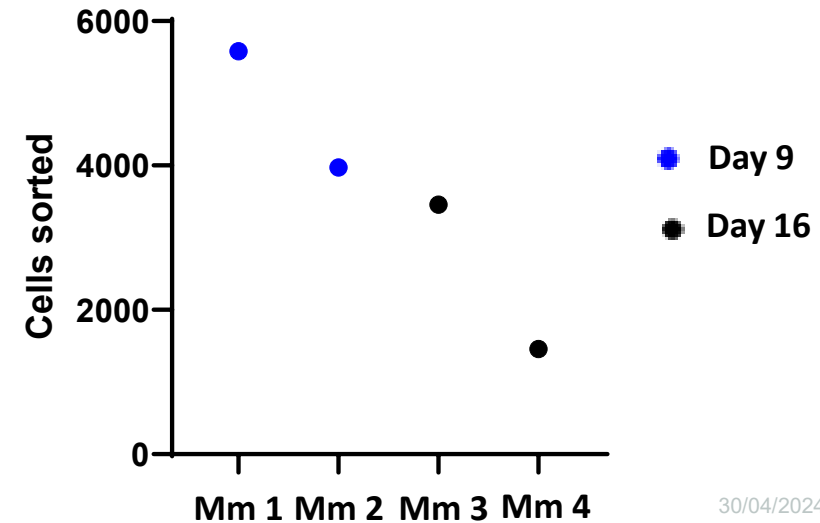
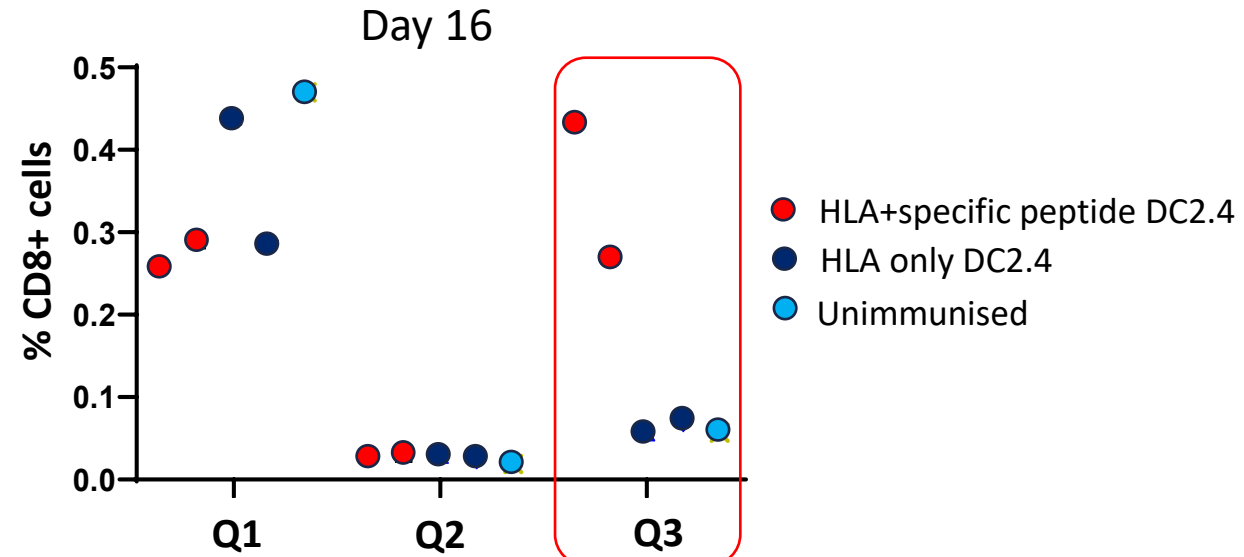
# Allo-MHC priming based TCR discovery strategy using Optimus<sup>®</sup> mice



# Peptide-MHC specific reactive CD8 T cell response can be captured by dual tetramer staining

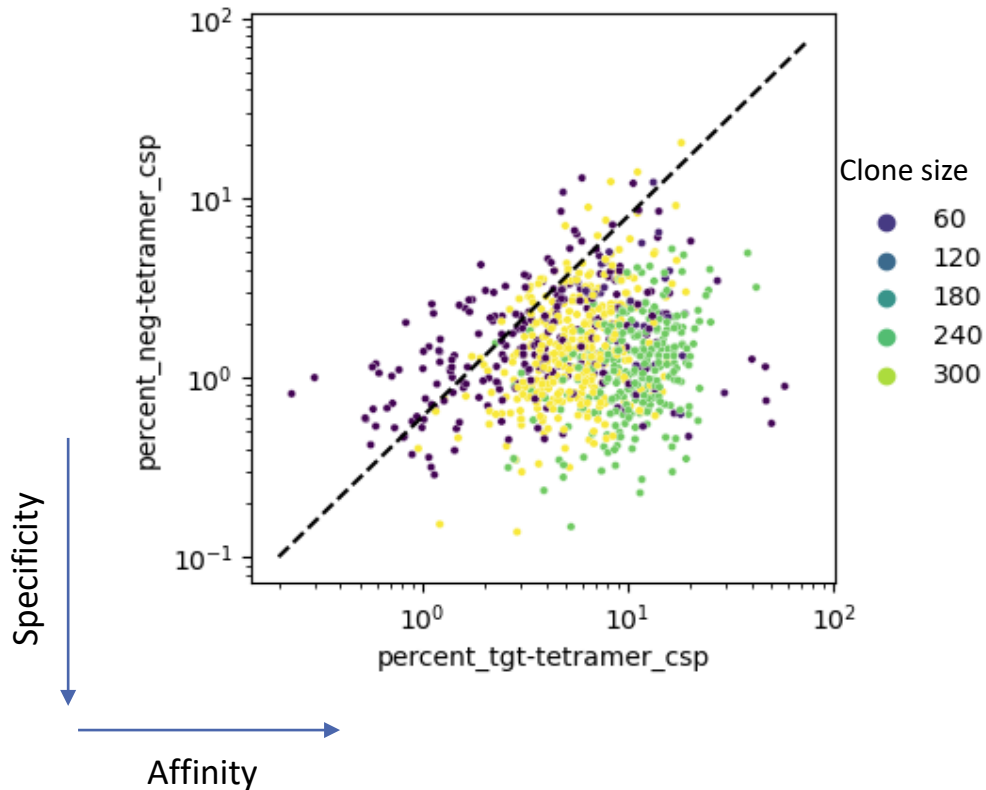


- CD8b based positive sorting to enhance specificity
- Dual tetramer (peptide specific and non-specific) staining to enrich for pMHC specific TCRs

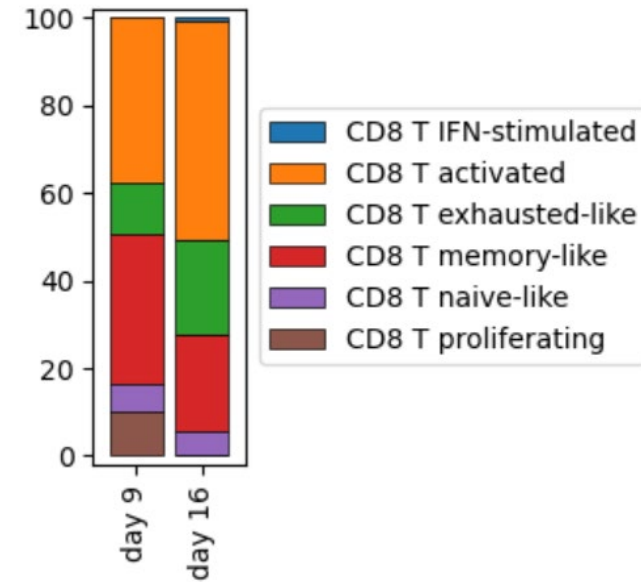


# *In silico* analysis confirms tetramer positive T cell specificity, phenotype, clonal expansion

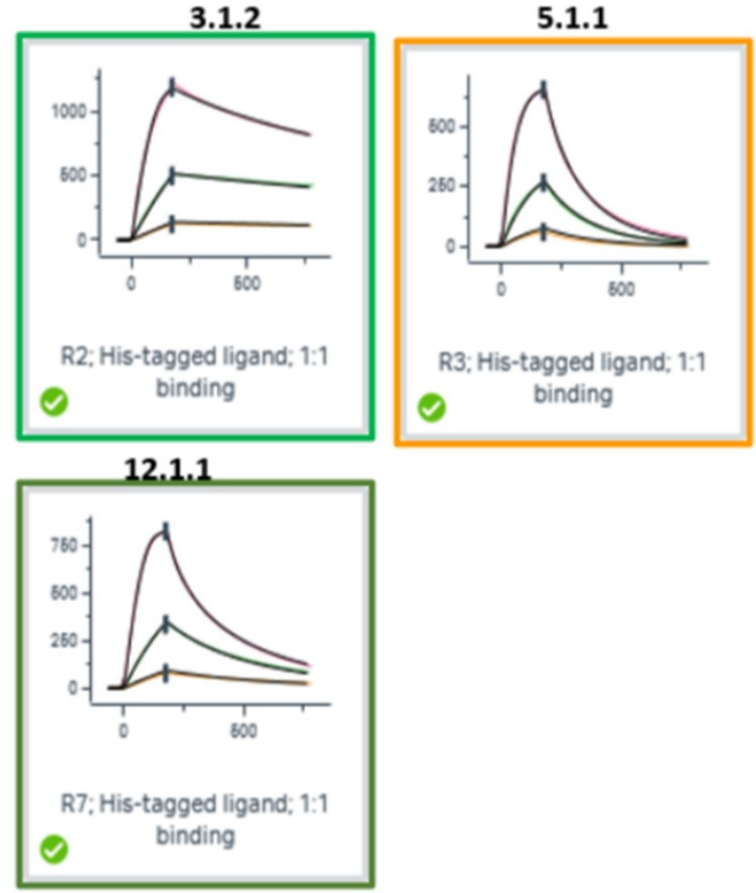
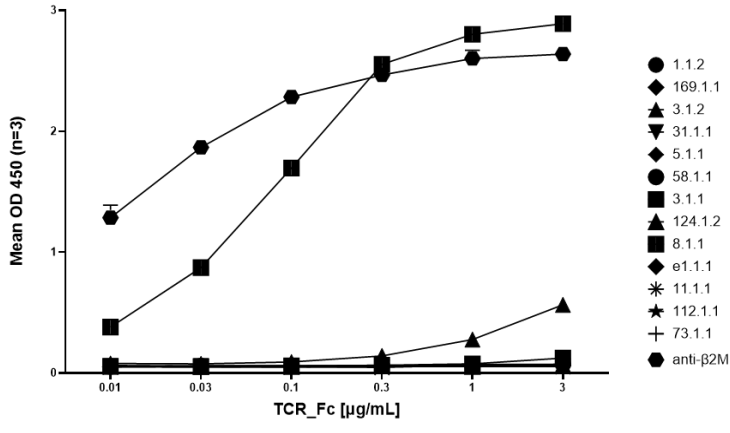
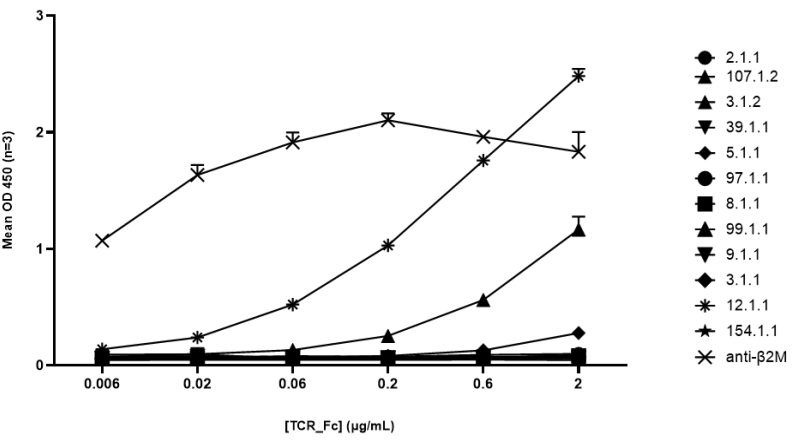
## T cell specificity and clonal expansion



## Tetramer specific T cell phenotype



# In vivo allo-priming generates high affinity TCRs



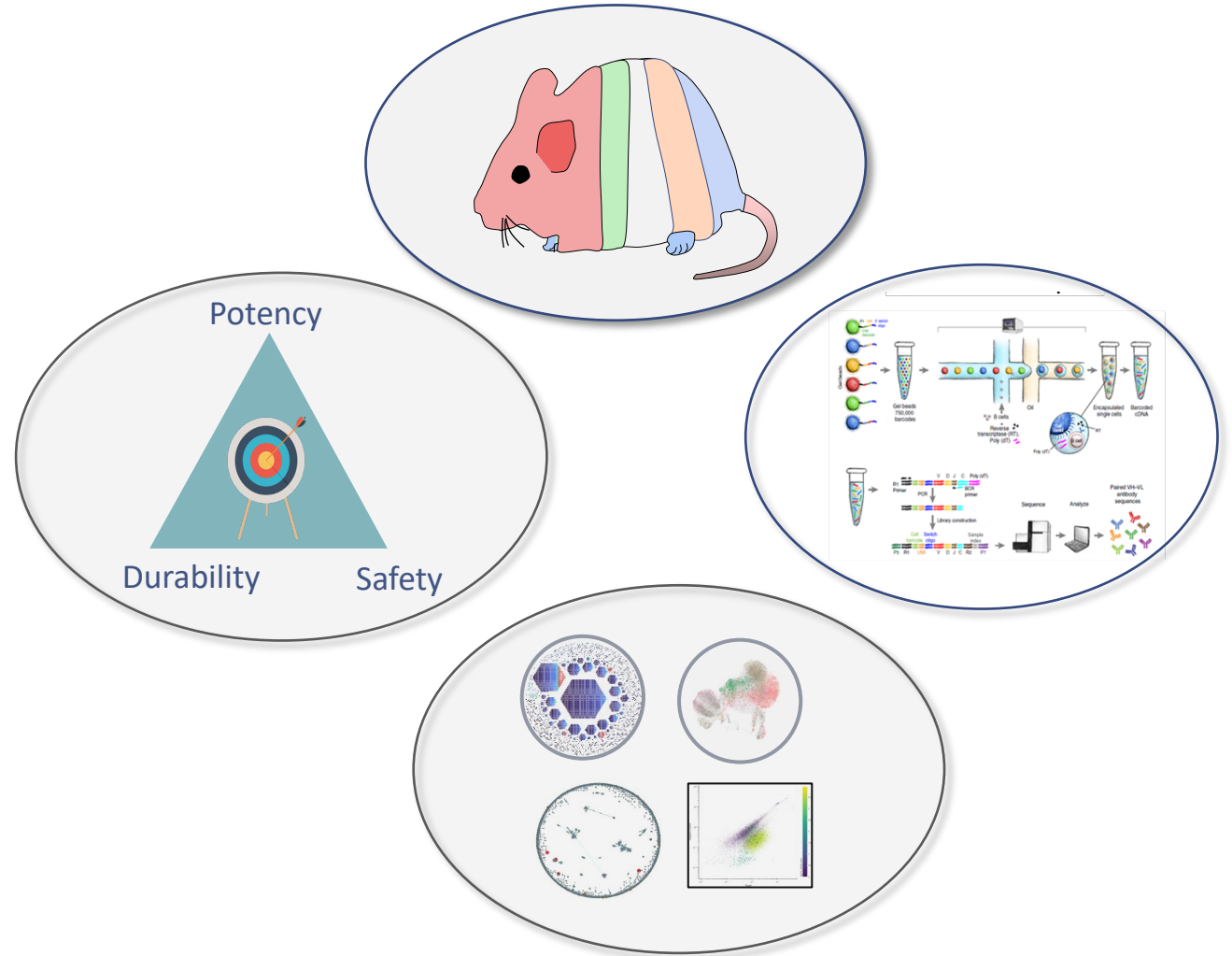
# Opportunity: Early partnership in human TCR-T<sub>eff</sub> or TCR-T<sub>reg</sub>

- **Today : Discovery partnership**

- Access to hTCRs against any pMHC target for TCR-T<sub>eff</sub>/TCR-NK or TCR-T<sub>reg</sub> applications

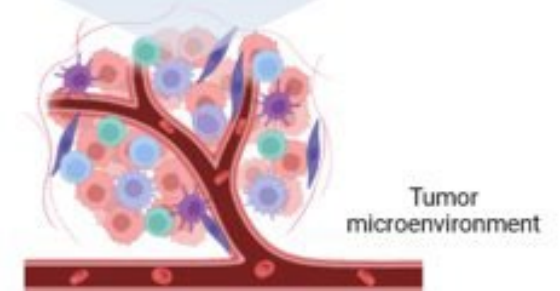
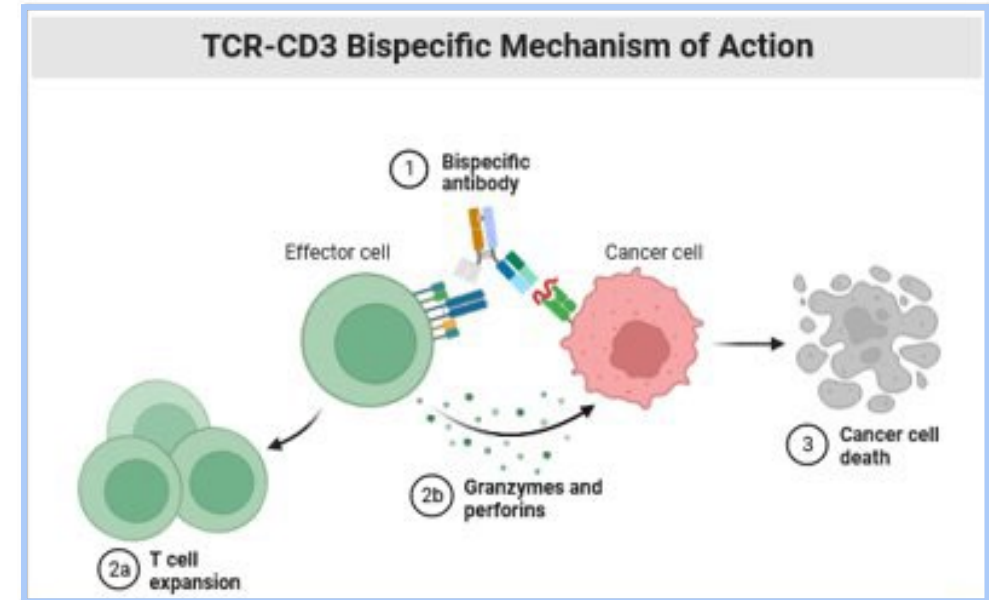
- **Future : Discovery and development partnerships**

- Soluble TCR biologics



# Development of T-cell engagers for cancer treatment

- Single cell analysis of tumour tissues
  - Cancer-specific genes and predicted peptides on cancer targeting
- Validation of cancer targets
  - Reactivity of T cells on cancer cells
  - Assessment of pHLA stability
  - Confirmation of predicted peptide via Immunopeptidomics of targeted cancer tissues
- Development of bi-specific molecules for induction of killing on cancer cells *in vitro* and *in vivo*







# T-Therapeutics : Making T Cell Receptor biologics a reality

We are : An experienced team : Of Drug developers and innovators

We have : A Best-in-Class TCR discovery platform: Proprietary highly engineered hTCR mouse

We have : A World leading hTCR discovery pipeline : Delivers 1,000s of target specific hTCRs

We have : A first in class molecular format : Soluble TCR, natural structure & manufacturable

We are currently : Oncology focused : De-risked best-in-class & novel first-in-class opportunities

Our goal : To bring our first drug into the clinic within four years

We have : Secured significant Series A investment : From World Class investors to support this ambition

We are assembling : Internal capability for discovery, development, preclinical assessment and CMC

Opportunity : Early partnerships in human TCR-T or TCR-NK space

# Thank you

## Contact

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