

# Potent T cell-engaging (TCE) single-domain antibodies that cross-react with non-human primate (NHP) T cell receptor (TCR) constant region $\alpha\beta$ as an alternative to CD3 engagement

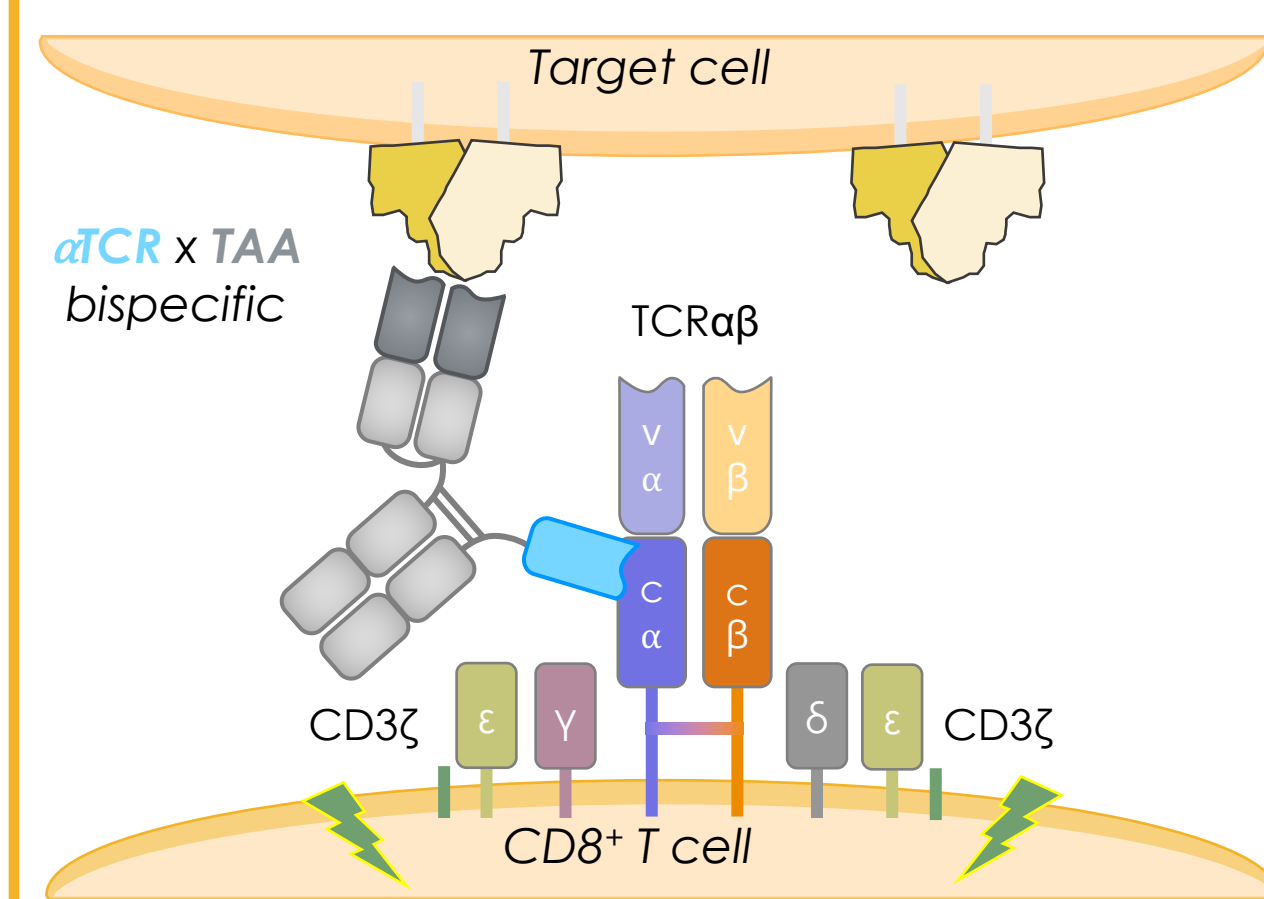
Ana Carranco-Prescott, Kitty Sompiyachoke, Anna Ebacher, Cole Townsend, Rohan Katpally, Paul Khalifé, Andrew Avery, Jonas Spaulding, Heather Lynaugh, Elizabeth Parker, Nidhi Goyani, Morgan Morrill, Srijita Paul, Lauryn Taylor, Nicole Yarbrough, Lauren Audi, Karen Stemm, Patricia Sackett, Kaleigh Canfield, Beth Sharkey, C. Garrett Rappazzo, Cory Ahonen, Kevin Schutz, Michael B. Battles

# ADIMAB

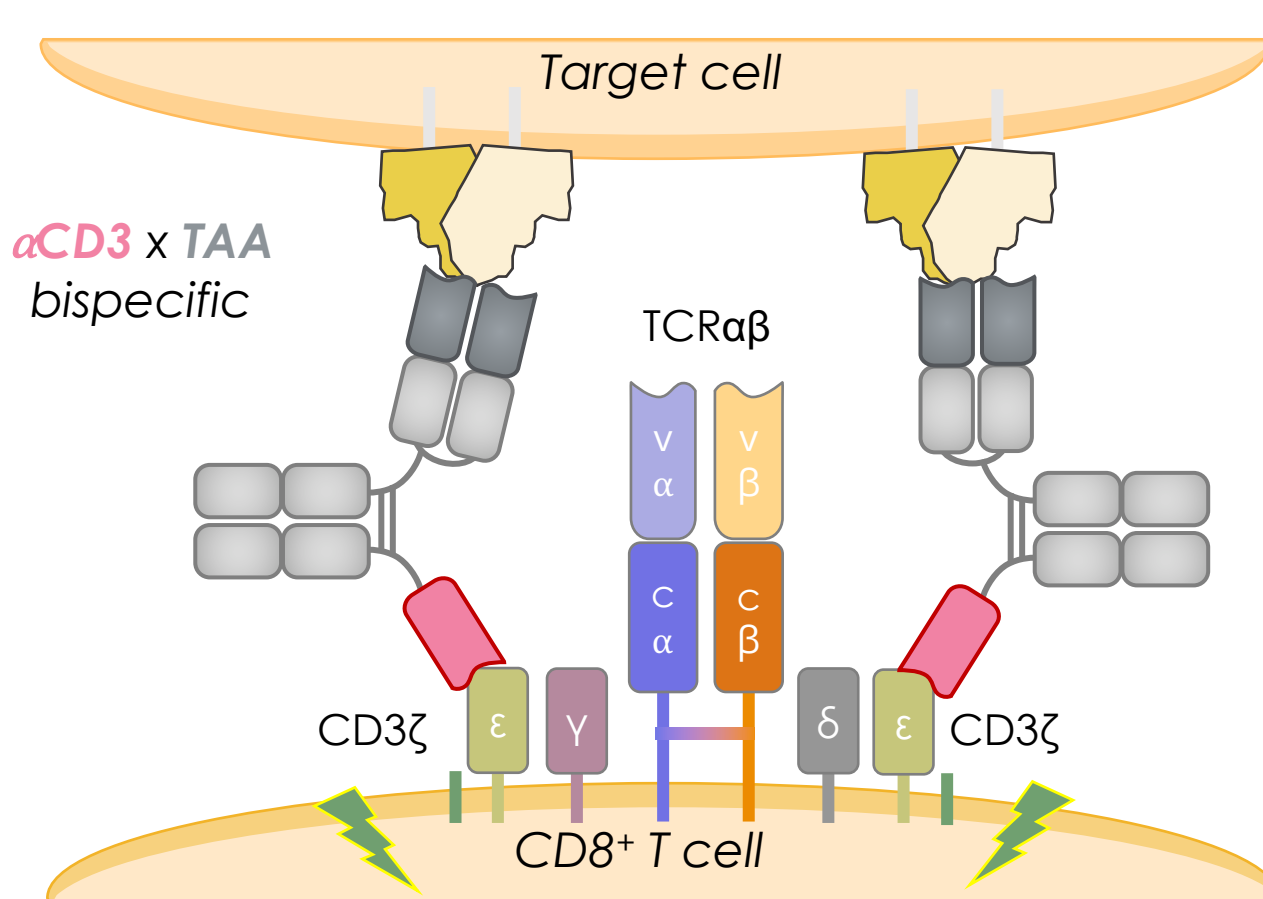
## Background – Targeting the TCR constant domain

- T cell engagers (TCE) primarily use CD3 for T cell engagement
- TCR-based redirection is clinically validated (e.g. AZD5492)
- The TCR constant domain presents an alternative engagement node

### Anti-TCR-based T Cell Engagers

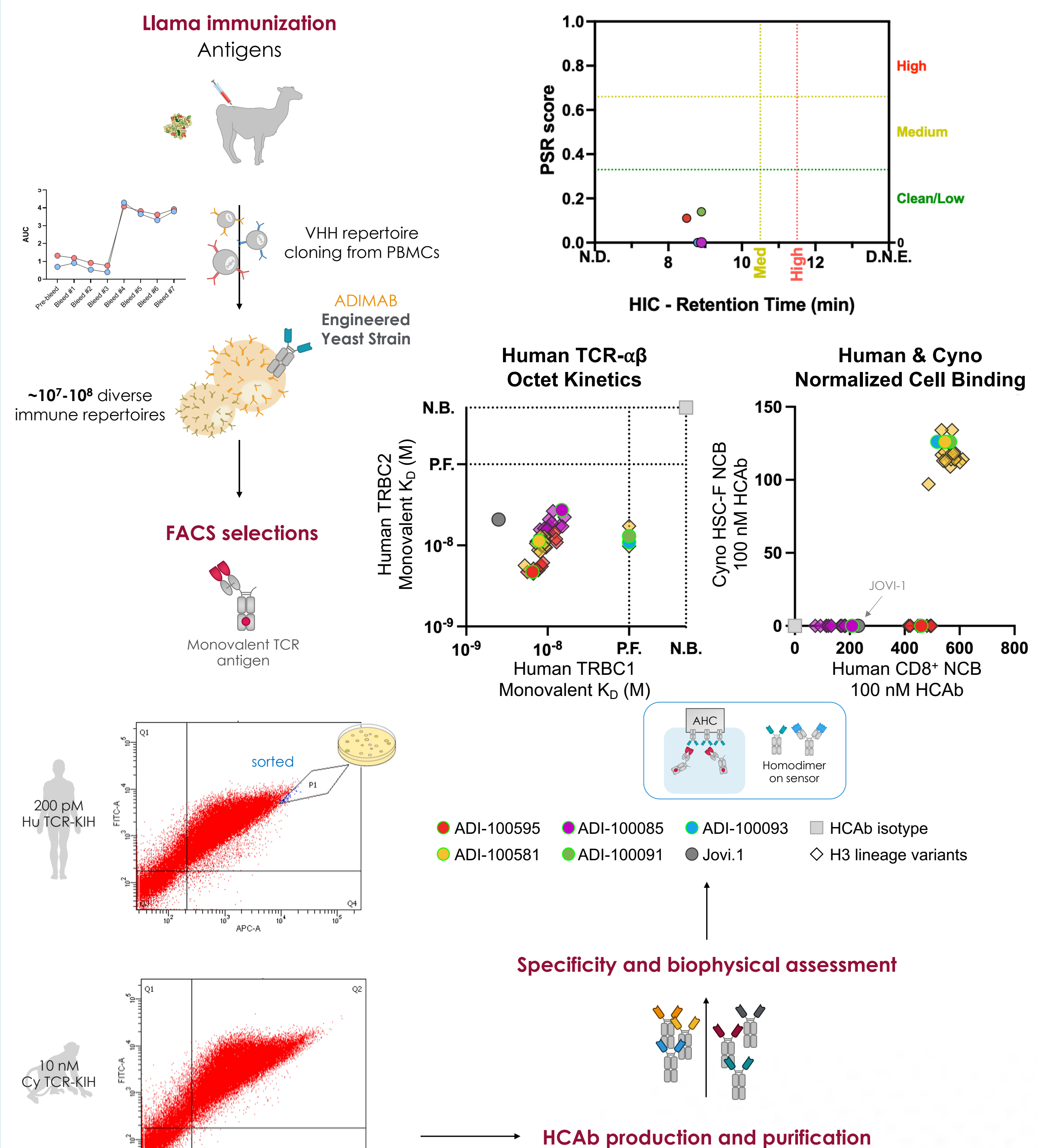


### Anti-CD3-based T Cell Engagers



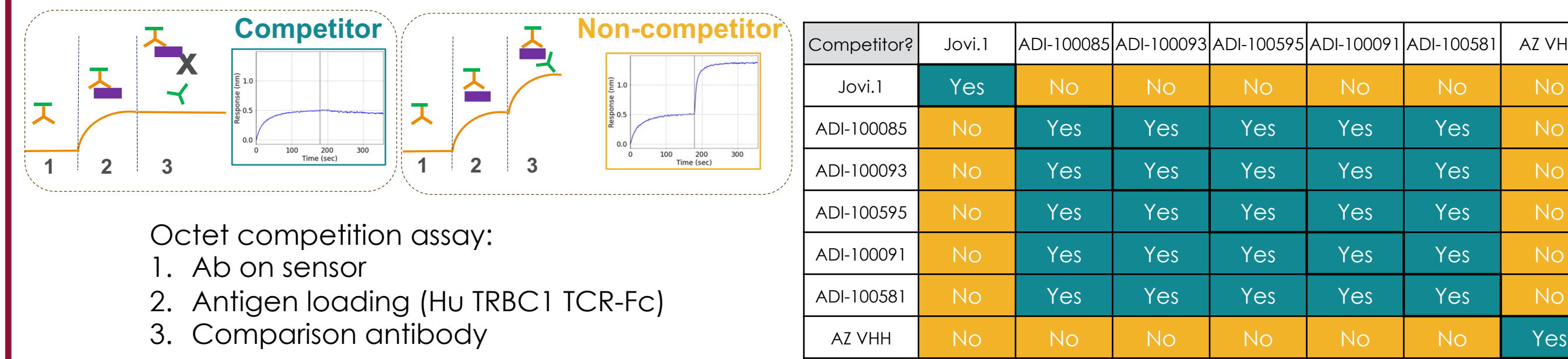
## Methods – Discovery and optimization of $\alpha$ TCR VHHS

- Five lead lineages were identified via FACS selection
- Three of the five lineages are cross reactive, binding to both recombinant cynomolgus TCR and cynomolgus T cells



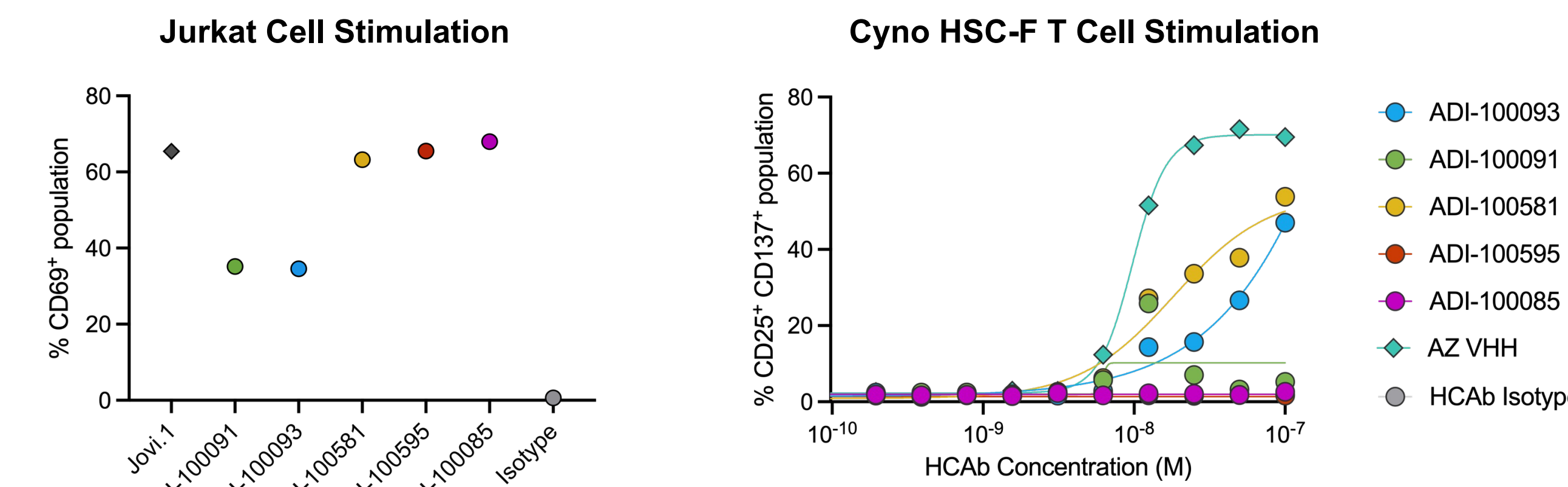
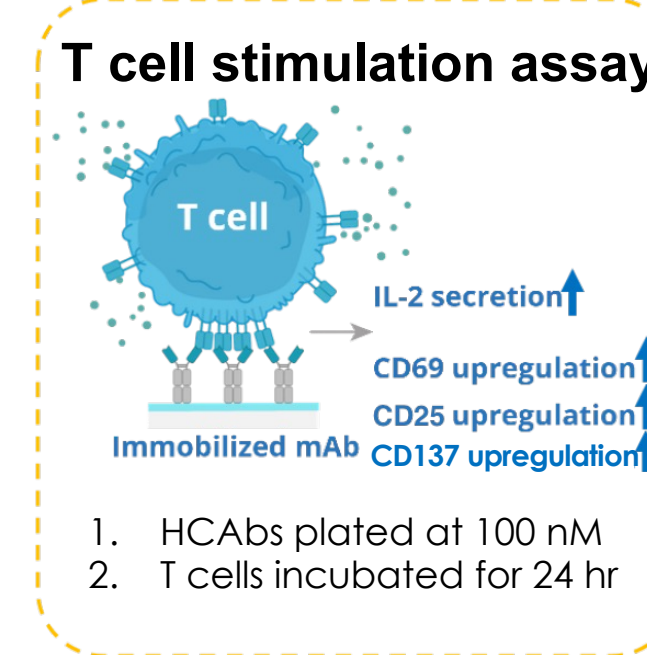
## Results - The five $\alpha$ TCR VHH lineages are in a shared bin

- Matrix binning reveals that the five identified  $\alpha$ TCR VHH lineages are in a shared bin, distinct from Jovi.1 and AZD5492  $\alpha$ TCR antibodies



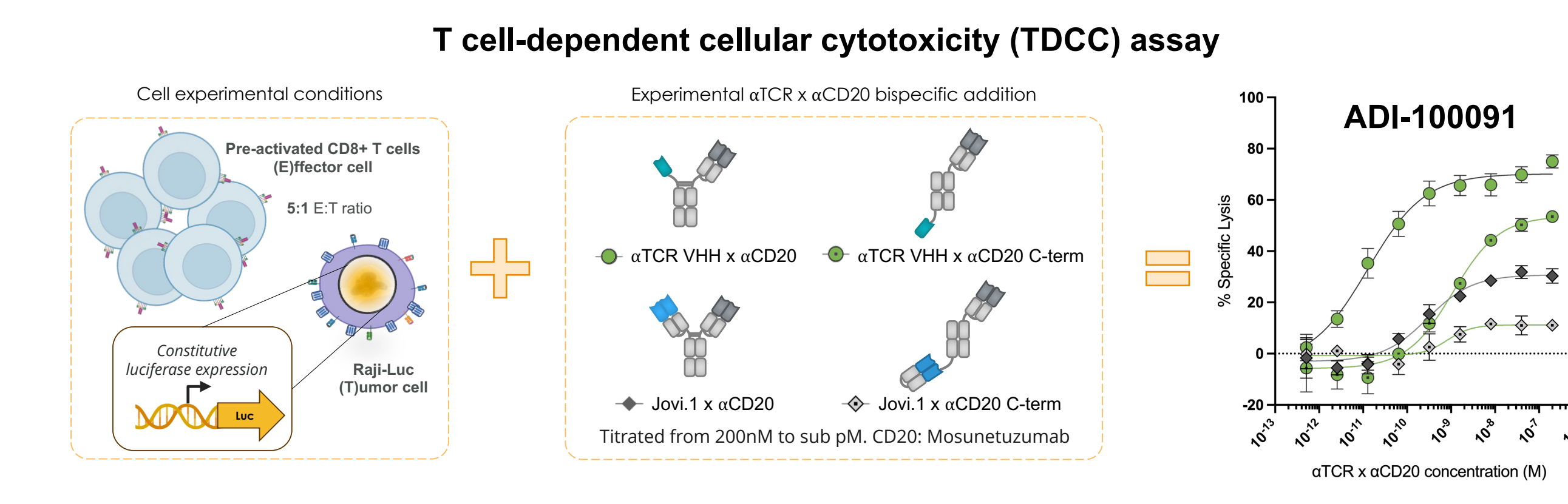
## Results - ADI $\alpha$ TCR VHHs stimulate human and cyno T cells

- $\alpha$ TCR VHH lineages were immobilized on plates for T cell stimulation assays
- All five lineages stimulate human Jurkat cells at 100 nM HcAb concentration, as measured by CD69 upregulation
- Three lineages stimulate cyno HSC-F cells as measured by double CD25 and CD137 upregulation



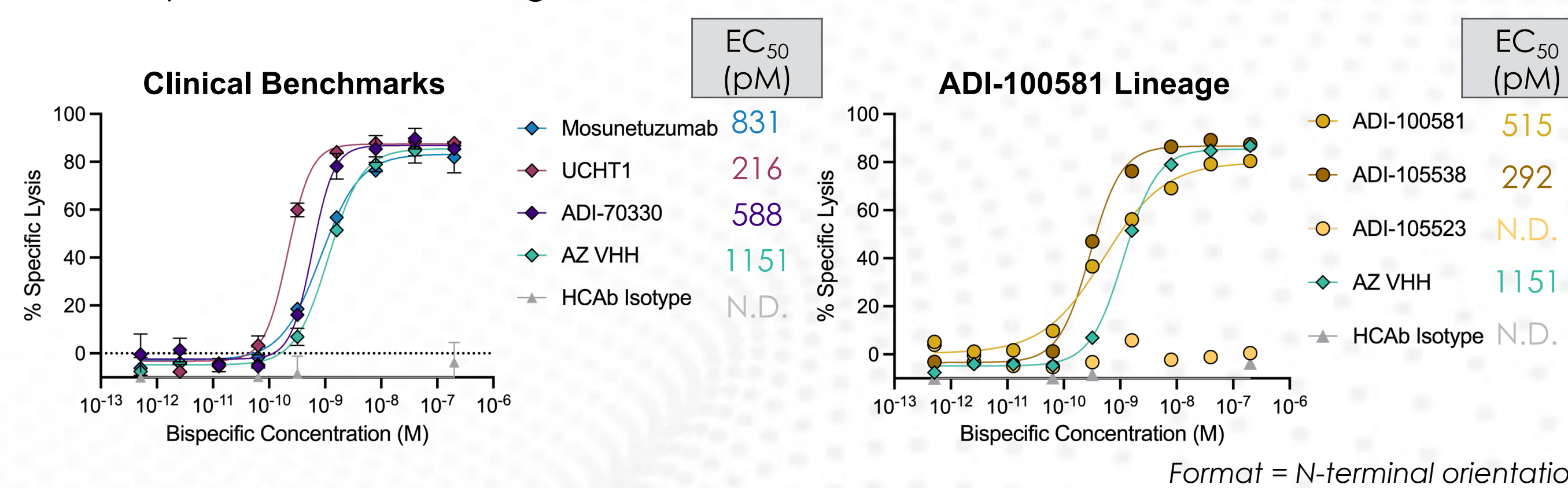
## Results – Geometry of T cell-engaging domain is important for potency of $\alpha$ TCR bispecifics

- We investigated N-terminal and C-terminal fusions for CD20 bispecifics that include an  $\alpha$ TCR arm as a T cell engager
- N-terminal fusions had higher potency than C-terminal fusions



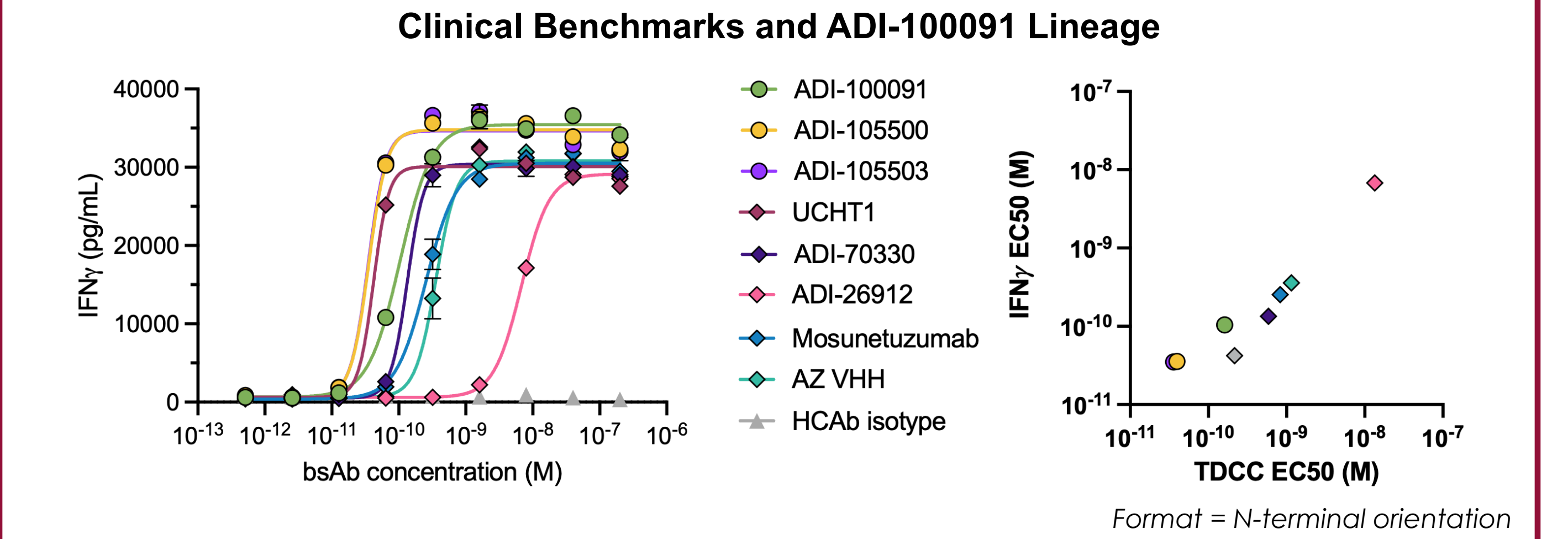
## Results – ADI-100581 lineage drives potent cytotoxicity

- The five  $\alpha$ TCR VHH lineages were optimized, and higher- and lower-affinity progeny were selected for functional characterization as CD20 bispecifics
- Higher-affinity ADI-100581 progeny (ADI-105538) has improved cytotoxicity compared to clinical-stage  $\alpha$ CD3 and  $\alpha$ TCR benchmarks



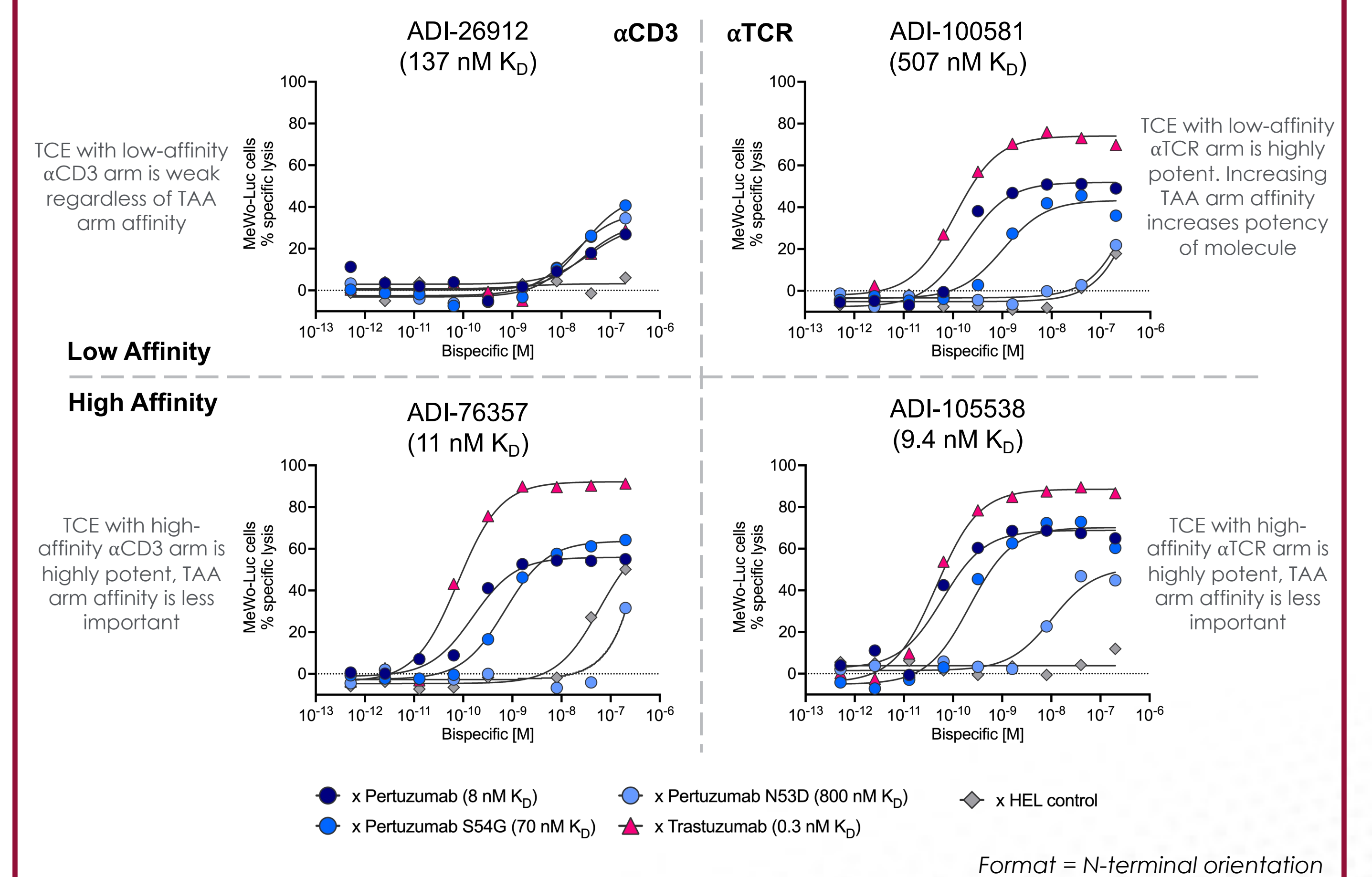
## Results – Potency and cytokine production are correlated

- Cytokines (TNF $\alpha$ , IL-2, and IFN $\gamma$ ) were collected from TDCC supernatants from all lineages for analysis; only IFN $\gamma$  data shown
- Increased potency of  $\alpha$ TCRxCD20 bispecifics in TDCC resulted in increased cytokine secretion



## Results – Affinity of T cell-engaging arm is less important for potency in $\alpha$ TCR bispecifics than $\alpha$ CD3 bispecifics

- We paired affinity variants of  $\alpha$ HER2 (Pertuzumab, 8 nM- 800 nM; Trastuzumab, 0.3 nM) with high- and low-affinity TCEs
- TAA-arm affinity drives potency of  $\alpha$ CD3 and  $\alpha$ TCR TCEs
- TCE-arm affinity strongly influences potency for  $\alpha$ CD3 bispecifics but has limited effect for  $\alpha$ TCR bispecifics



## Conclusions

- The VHHs described here target the TCR constant domain and occupy a distinct epitope bin, engaging both TRBC1 and TRBC2 for pan- $\alpha\beta$  T cell redirection.
- Bispecific constructs containing  $\alpha$ TCR VHHs drive potent cytotoxicity, with potency governed by engagement geometry and TAA-arm affinity. This data supports a broad therapeutic window.
- TDCC assays paired with cytokine evaluation suggest a correlation between potency of CD20 bispecifics, regardless of the epitope on the TCR-CD3 complex.
- Cross-species reactivity with cynomolgus TCR was confirmed in biochemical and functional studies. This establishes TCR constant domain targeting as an alternative to CD3 $\epsilon$  engagement with translatability to non-human primate studies.

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