

# Xynapse™-T Reagent for Antigen-Specific Stimulation of T cells

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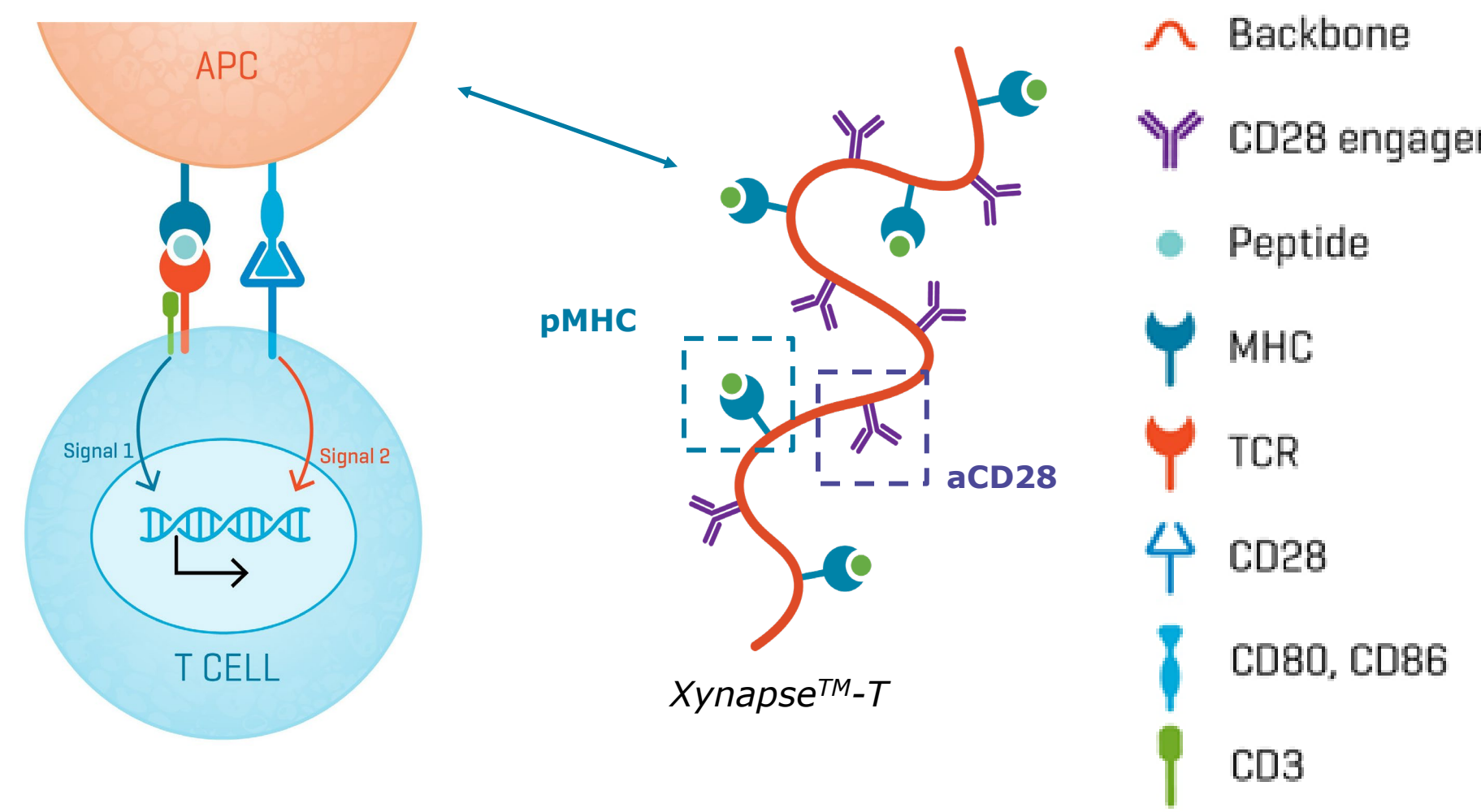
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## The Xynapse™-T reagent – artificial Antigen presenting molecule

## Conclusion

### Introduction

We have developed an artificial antigen-presenting scaffold technology called Xynapse™ (previously referred to as PAX). We have used this platform to make artificial antigen presenting molecules that can serve as a substitute for antigen-presenting cells (APCs) in various applications such as expansion of rare T cells from biological samples and potency testing of cellular immunotherapy drug products. Xynapse™-T is decorated with MHCp monomers and CD28-engagers, providing TCR-specific and CD28 co-stimulatory signals that trigger T cell activation. This study evaluates Xynapse™-T reagents ability to stimulate antigen-specific T cells by assessing activation marker expression, cytokine production, and expansion.



### Conclusions

We have made a novel type of reagent (Xynapse™-T) based on a scaffold presenting MHCp complexes and agonistic anti-CD28 engagers. We show that these reagents are capable of mimicking APCs by inducing expression of activation markers, cytokine secretion and stimulating expansion of specific T cells.

Xynapse™-T technology – New simple solution for activation antigen specific T cells that can be used for

- Potency testing of engineered T cells
- Functional characterization of engineered T cells
- Enrichment of TCR engineered T cells
- Expansion of rare antigen-specific T cells

## Activation and expansion virus specific and rare MART-1 specific CD8+ T cells from healthy donor PBMCs with Xynapse™-T reagent

### PBMCs stimulated with Xynapse™-T, MHC<sub>EBV</sub> reagents and subsequently stained with MHC<sub>EBV</sub> Dextramer®

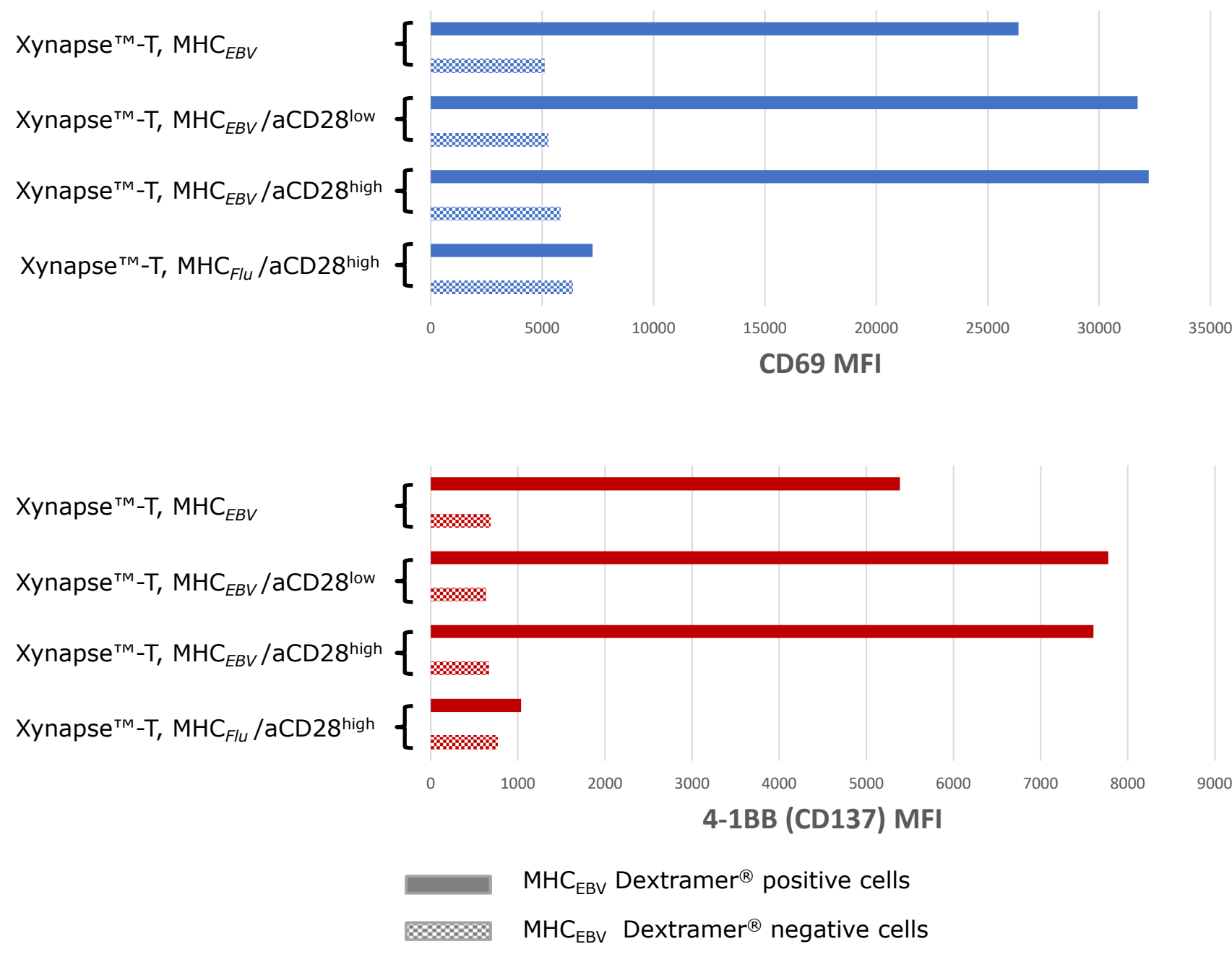


Figure 1. Xynapse™-T reagents efficiently stimulate upregulation of activation markers on antigen specific CD8+ T cells.

### PBMCs stimulated with Xynapse™-T, MHC<sub>CMV1</sub> reagents (w/o aCD28) and subsequently stained with two MHC<sub>CMV1</sub> Dextramer®

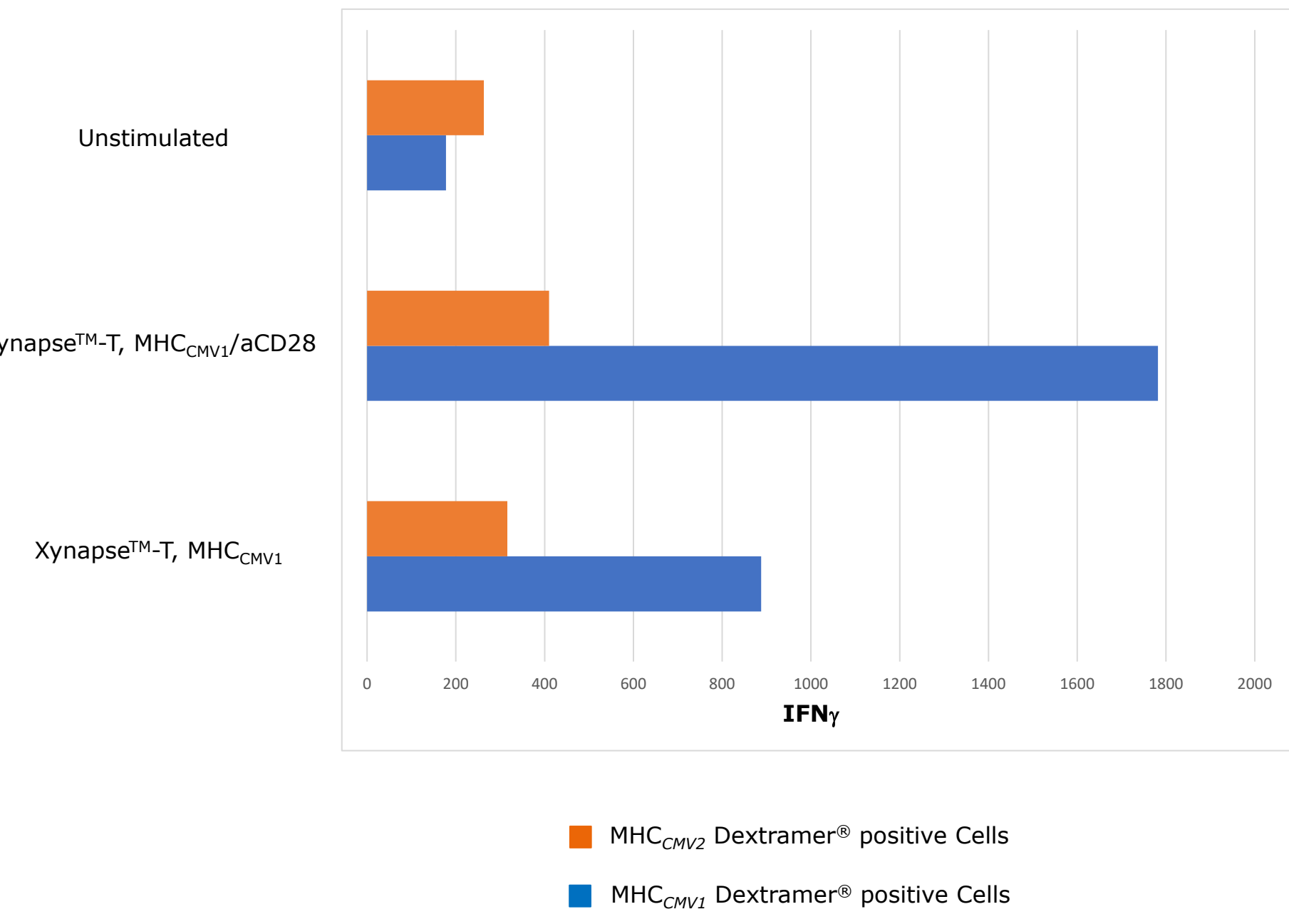
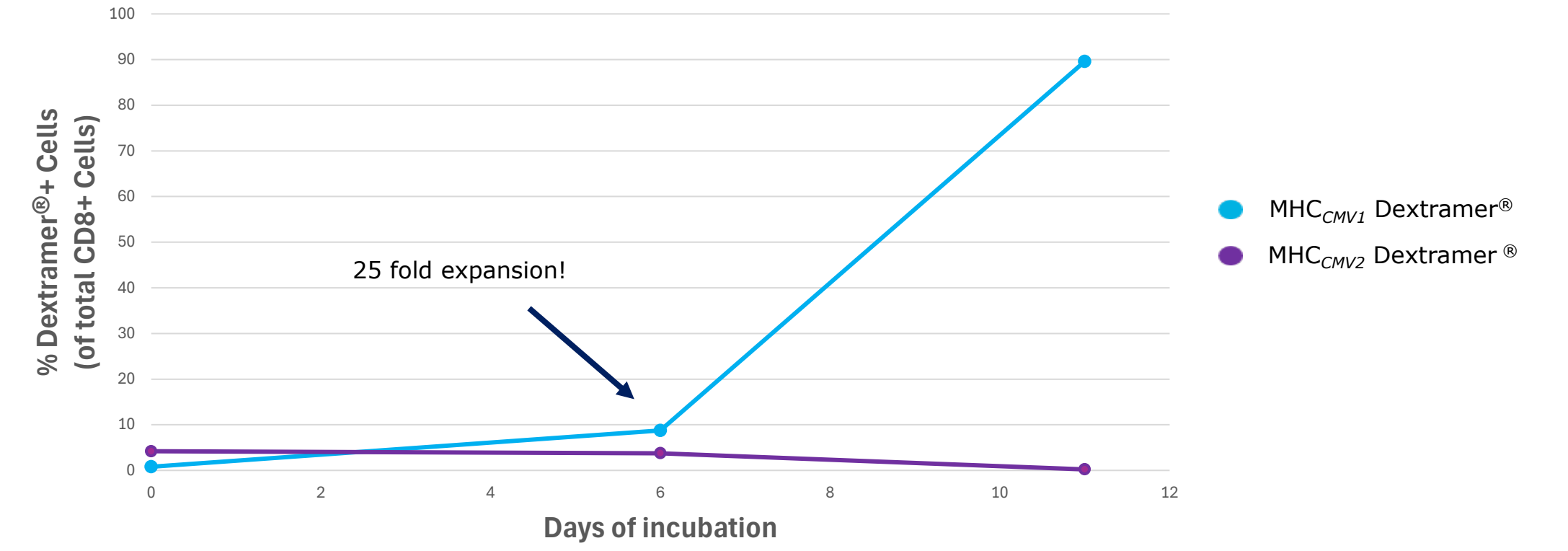


Figure 2. Xynapse™-T reagents induce cytokine secretion in epitope-specific CD8+ T cells.

### Stimulation of PBMCs with Xynapse™-T, MHC<sub>CMV1</sub>/aCD28 reagent



### Stimulation of PBMCs with Xynapse™-T, MHC<sub>CMV1</sub> reagent (no aCD28)

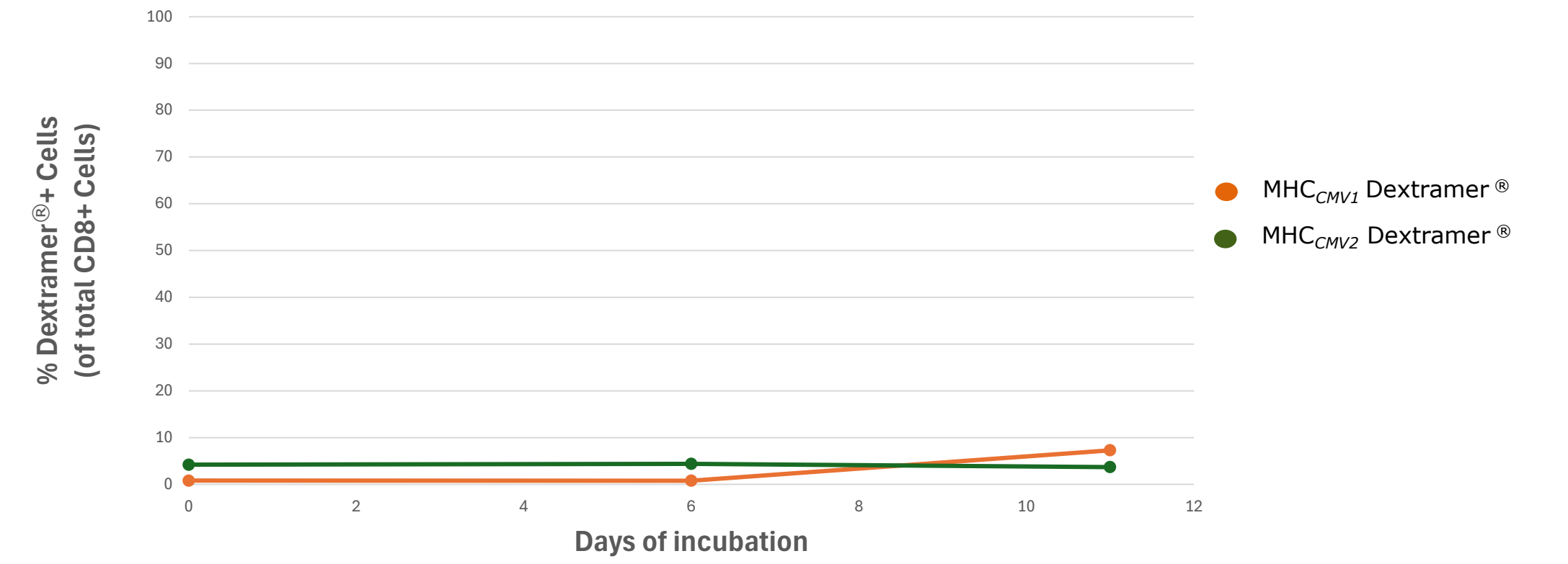


Figure 3. CD28 dependent expansion of epitope-specific CD8+ T cells with Xynapse™-T reagent.

### PBMCs stimulated with Xynapse™-T, MHC<sub>MART-1</sub> reagents and subsequently stained with MHC<sub>MART-1</sub> Dextramer®

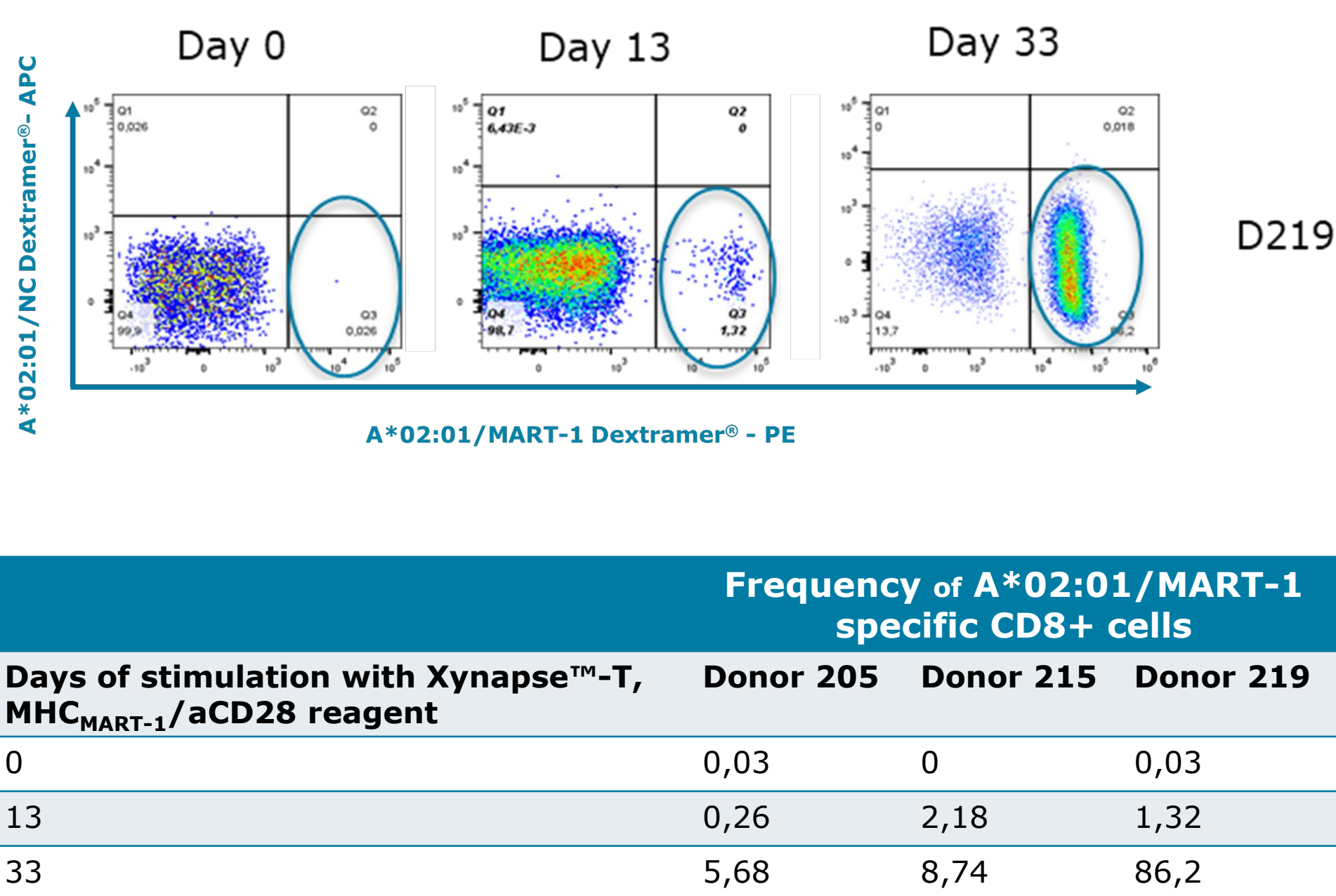


Figure 4. Xynapse™-T reagents efficiently expand rare HLA-A\*0201/MART-1 specific CD8+ T cells.

### Untouched CD3+ T cell enriched PBMCs using Pan T Cell Isolation Kit (Miltenyi) were stimulated with Xynapse™-T, MHC<sub>MART-1</sub> or MHC<sub>Flu</sub> specific reagents for 6-12 days and subsequently expanded T cells were analyzed for proliferation and CCR7/CD45RA phenotype distribution.

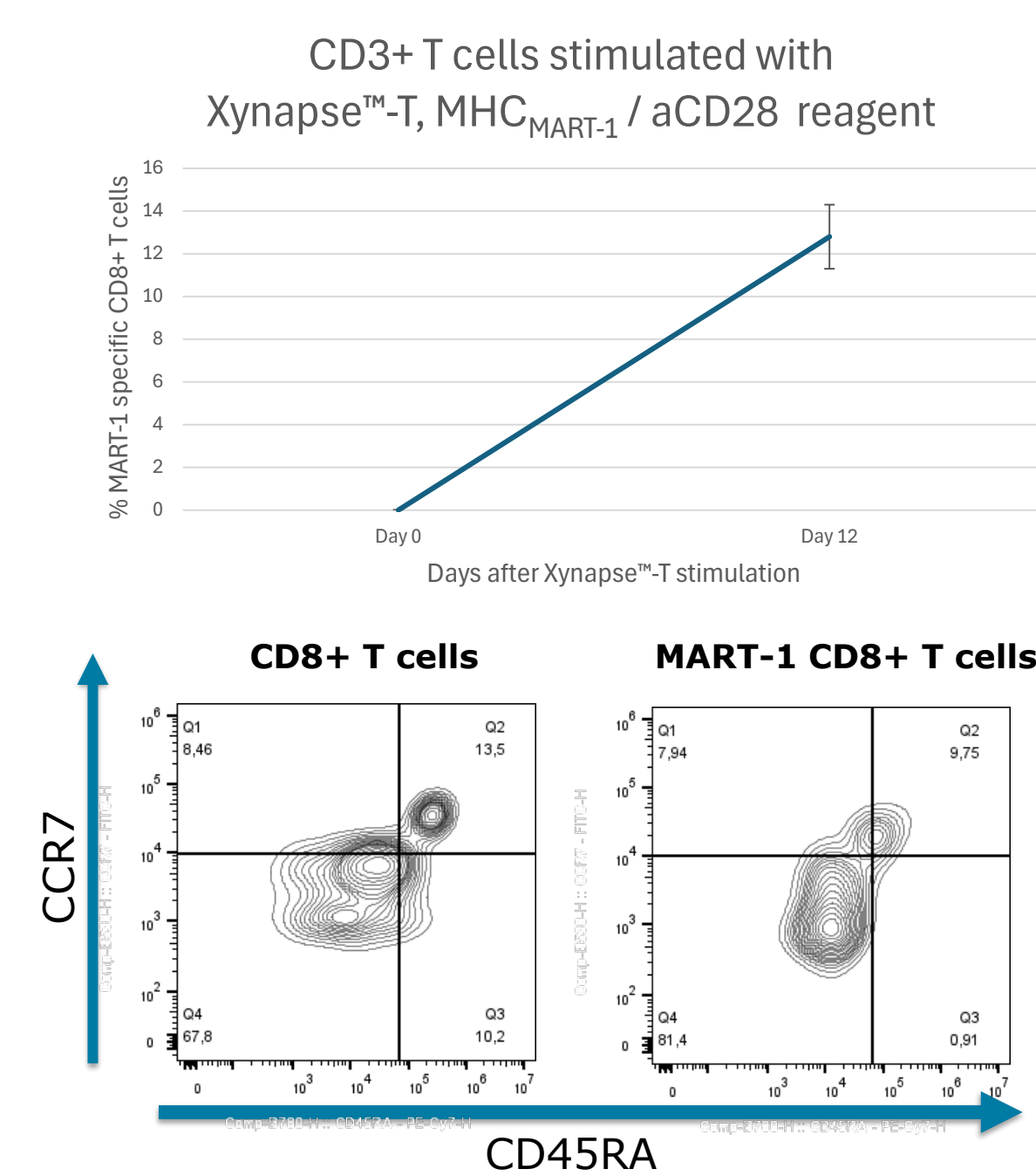


Figure 5a. Expanded MART-1 CD8+ T cell phenotype closely resemble the average CD8+ T cell population.

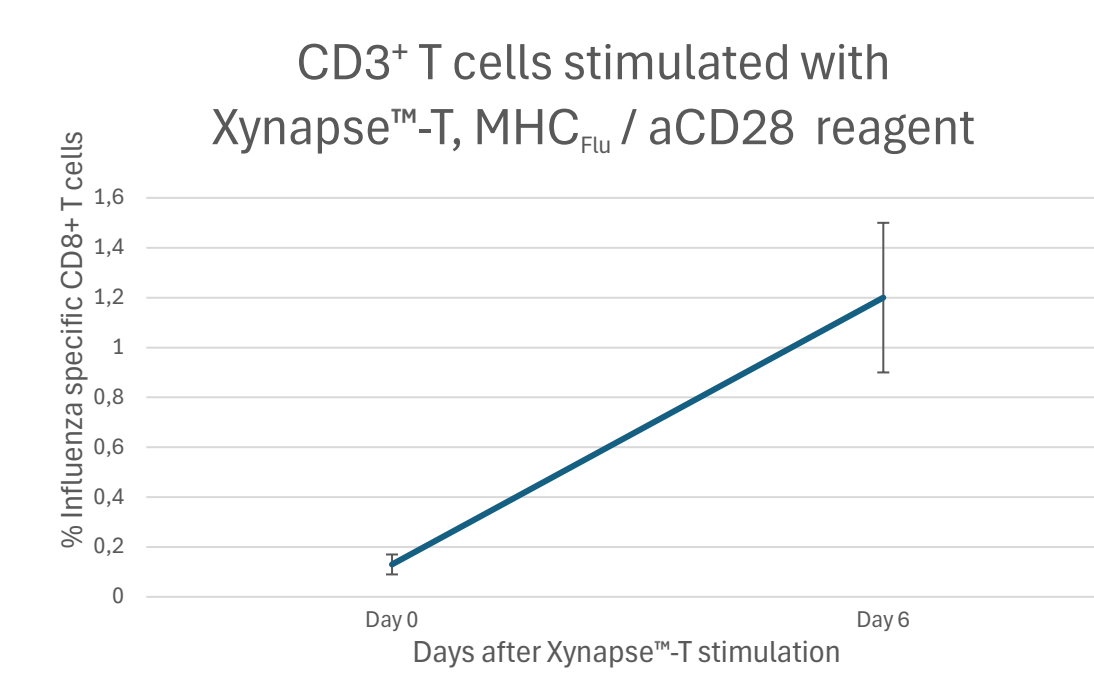
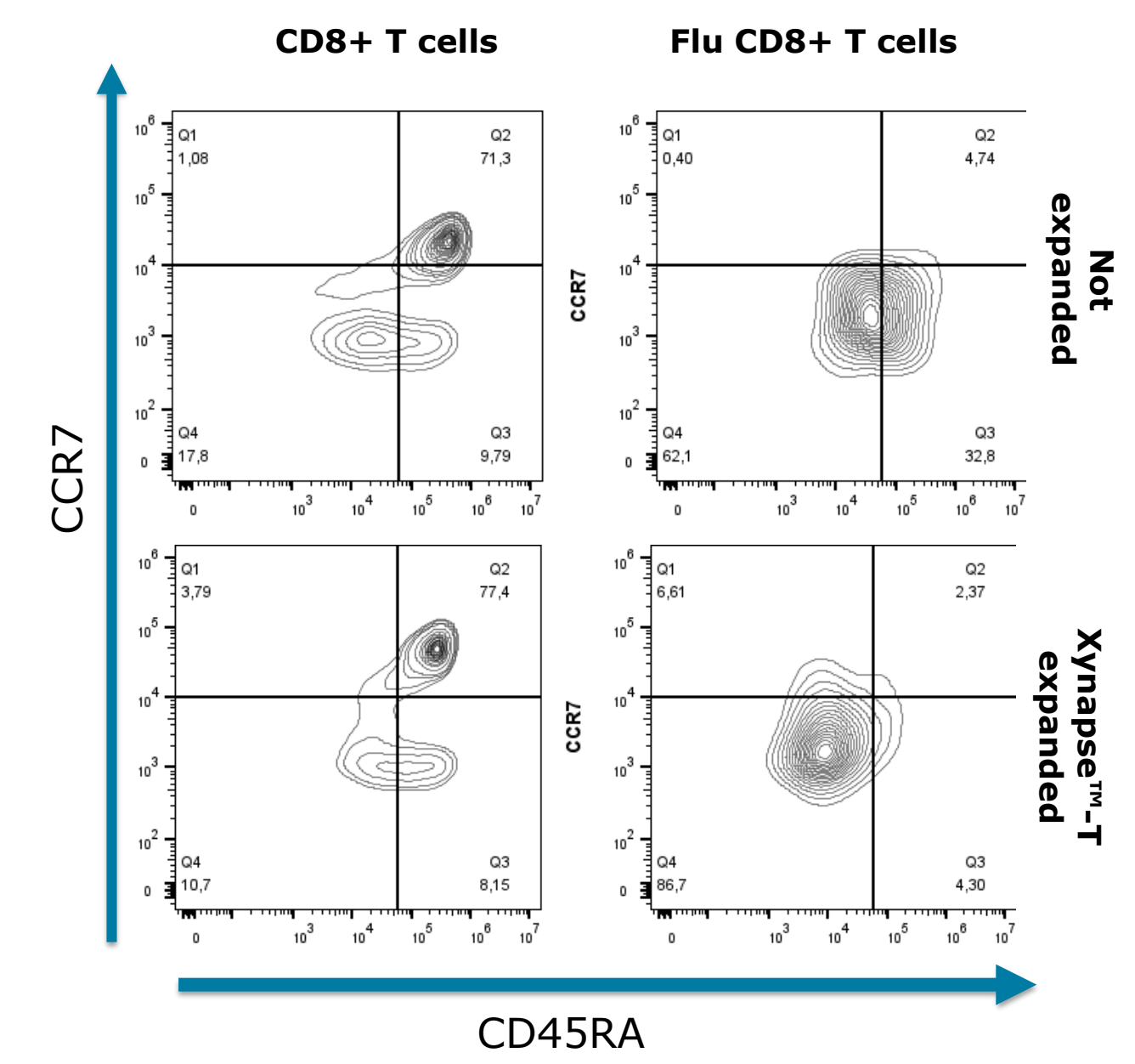


Figure 5b. Expansion of Influenza-specific T cells results in downregulation of CD45RA, and phenotype mobility from CCR7-CD45RA+ to CCR7-CD45RA-, mimicking a natural antigen-specific stimulation.



## Enrichment and phenotype of engineered TCRt cells with Xynapse™-T reagent

TCRt engineered cells were stimulated and enriched using Xynapse™-T reagent compared to traditional methods using peptide loaded APC or aCD3 & aCD28 based activation. After 7 days the TCRt cells were evaluated for proliferation and specific enrichment.

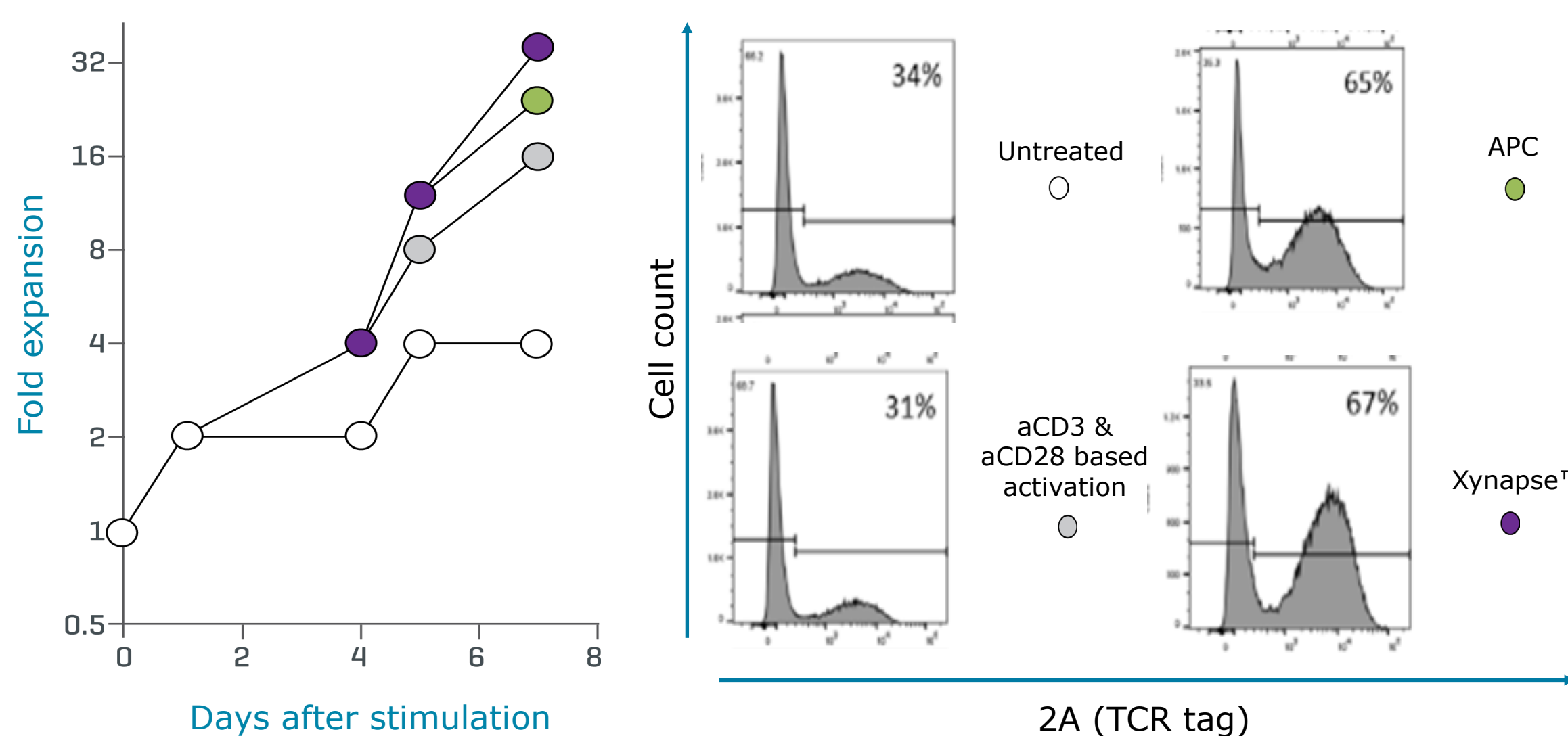


Figure 6: TCRt engineered cells stimulated with Xynapse™-T showed a specific enrichment as efficient as with peptide loaded APC, and superior to aCD3 & aCD28 based activation.

Engineered TCRt cells were expanded for 7 days with Xynapse™-T or peptide pulsed APC and subsequently compared for expression of phenotypic biomarkers in flowcytometry.

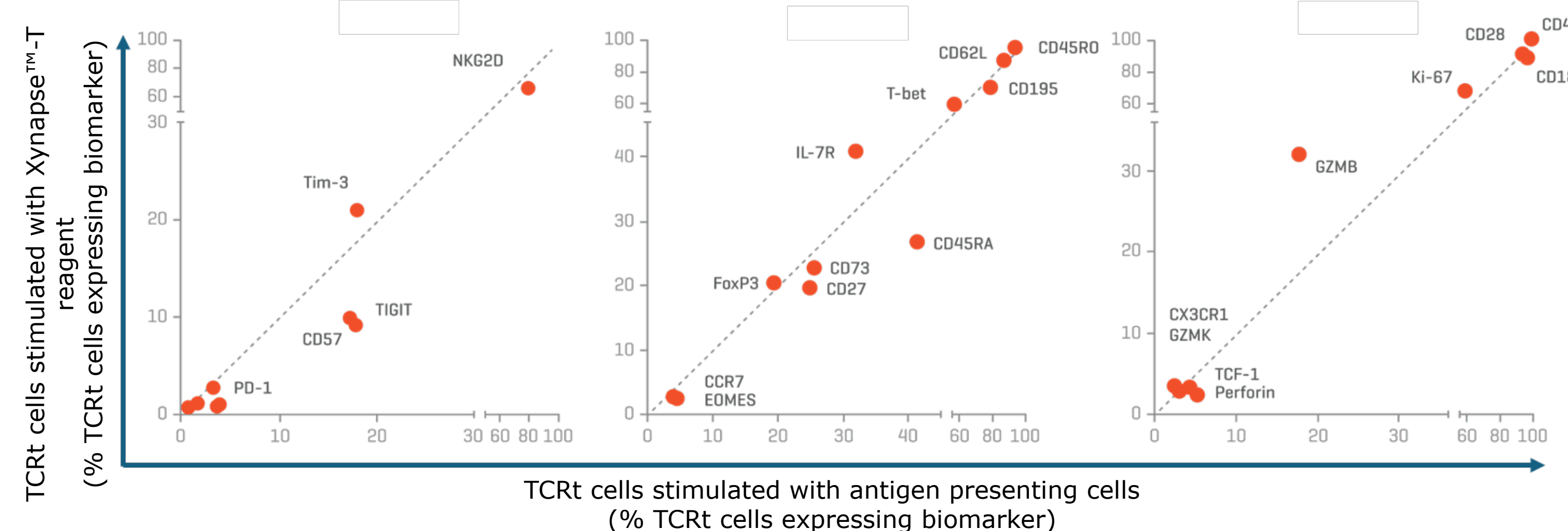


Figure 7. The phenotype of TCRt engineered cells stimulated with Xynapse™-T reagent closely mimics that of cells exposed to APCs.

Data kindly provided by collaborators