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## Introduction

**Background:** While T cell receptor (TCR)-engineered T cell (TCR-T) therapy has transformed the landscape of cancer immunotherapy, efficacy and durability of response are often limited by tumor heterogeneity, antigenic escape, and loss of HLA heterozygosity. Treatment of solid tumors with multiple TCR-Ts specific for different antigens and restricted to several human leukocyte antigens (HLAs) is a promising strategy to overcome cancer immune evasion, and potentially to increase the efficacy of TCR-T therapy. MAGE-A4 is expressed in multiple malignancies, including non-small cell lung cancer, colon cancer, and melanoma, with expression in healthy tissue limited to immune-privileged sites [1]. Expression of MAGE-A4 has been additionally associated with poor prognosis in multiple indications [2,3]. In combination with TCR-T candidates currently in TScan's clinical pipeline, development of MAGE-A4-specific TCR-Ts is expected to further advance the effort of treating patients with multiplex TCR-T therapy.

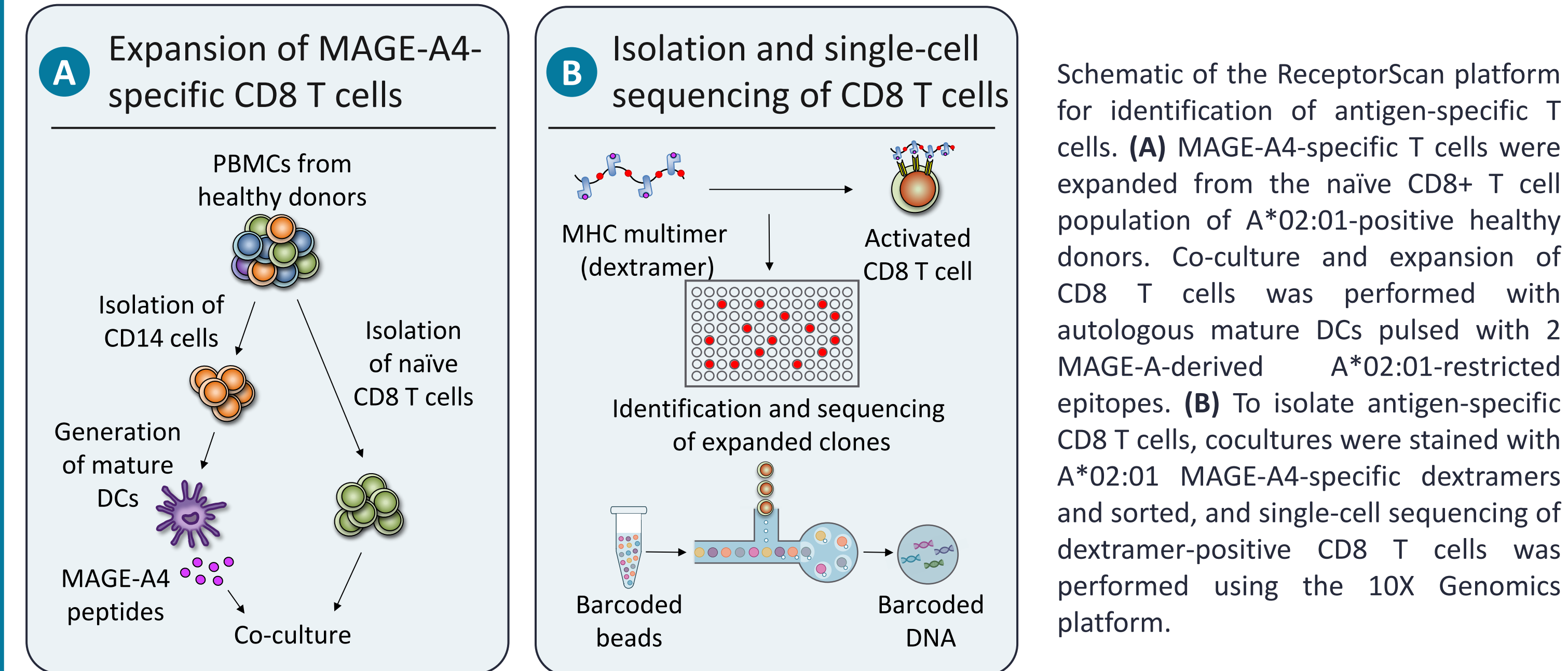
**Methods:** Using TScan's ReceptorScan platform, we discovered TCRs specific for two A\*02:01-restricted MAGE-A4-derived epitopes. The high-throughput screening assay ActivScan was used to select high-expressing and functional TCRs from libraries of MAGE-A4-specific clonotypes, and TCRs were then comprehensively examined for their cytotoxic function using cancer cell lines expressing varying levels of MAGE-A4. Alloreactivity was evaluated by examining reactivity to 110 HLA class I allotypes, and off-target reactivity of lead TCRs was evaluated using our proprietary SafetyScan platform to assess TCR peptide specificity. Safety was additionally confirmed by co-culturing engineered T cells with normal primary human cells. Finally, TCR efficacy was also assessed by transferring engineered T cells into mice implanted with MAGE-A4-expressing xenografts.

**Results:** ReceptorScan identified 2100+ TCRs specific for two MAGE-A4 epitopes by screening naïve CD8<sup>+</sup> T cells from 10 unique healthy donors. Functionally potent TCRs with high avidity were selected using the ActivScan platform. Multiple TCRs displayed efficacy and potency in cytotoxicity assays, as well as in their ability to release cytokine and proliferate in response to target peptide specificity by the lead TCR, and no alloreactivity was observed to the 110 allotypes tested or to normal primary human cells. MAGE-A4-specific TCR-T cells also displayed *in vivo* efficacy in reducing tumor burden in xenograft mouse models.

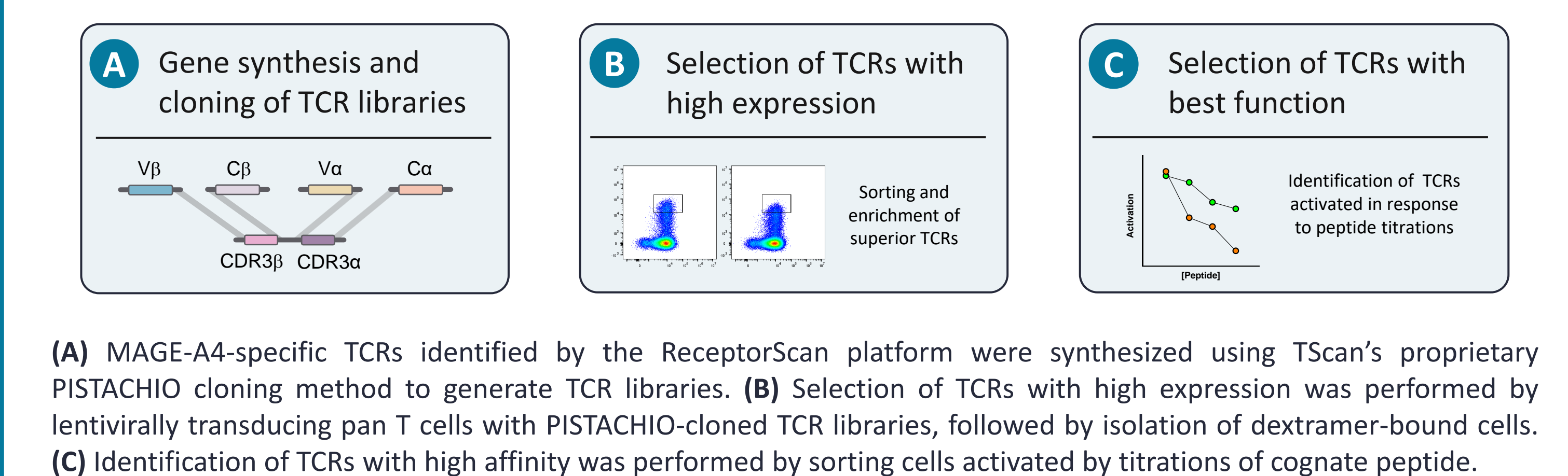
**Conclusions:** The autologous MAGE-A4-specific TCR-T therapy candidate TSC-202-A0201 has been advanced to IND-enabling studies to be added to TScan's Immunobank of TCRs, with the ultimate goal of being used in a multiplex approach known as T-Plex as a best-in-class immunotherapy strategy for treating patients with solid tumors.

- Weon JL and Potts PR. The MAGE protein family and cancer. *Curr Opin Cell Biol.* 2015; 37: 1-8.
- Yoshida N, Abe H, Ohkuri T, Wakita D, Sato M, Noguchi D, et al. Expression of the MAGE-A4 and NY-ESO-1 cancer-testis antigens and T cell infiltration in non-small cell lung carcinoma and their prognostic significance. *Int J Oncol.* 2006; 28(5): 1089-98.
- Poojary M, Jishnu PV, Kabekkodu SP. Prognostic Value of Melanoma-Associated Antigen-A (MAGE-A) Gene Expression in Various Human Cancers: A Systematic Review and Meta-analysis of 7428 Patients and 44 Studies. *Mol Diagn Ther.* 2020; 24(5): 537-555.

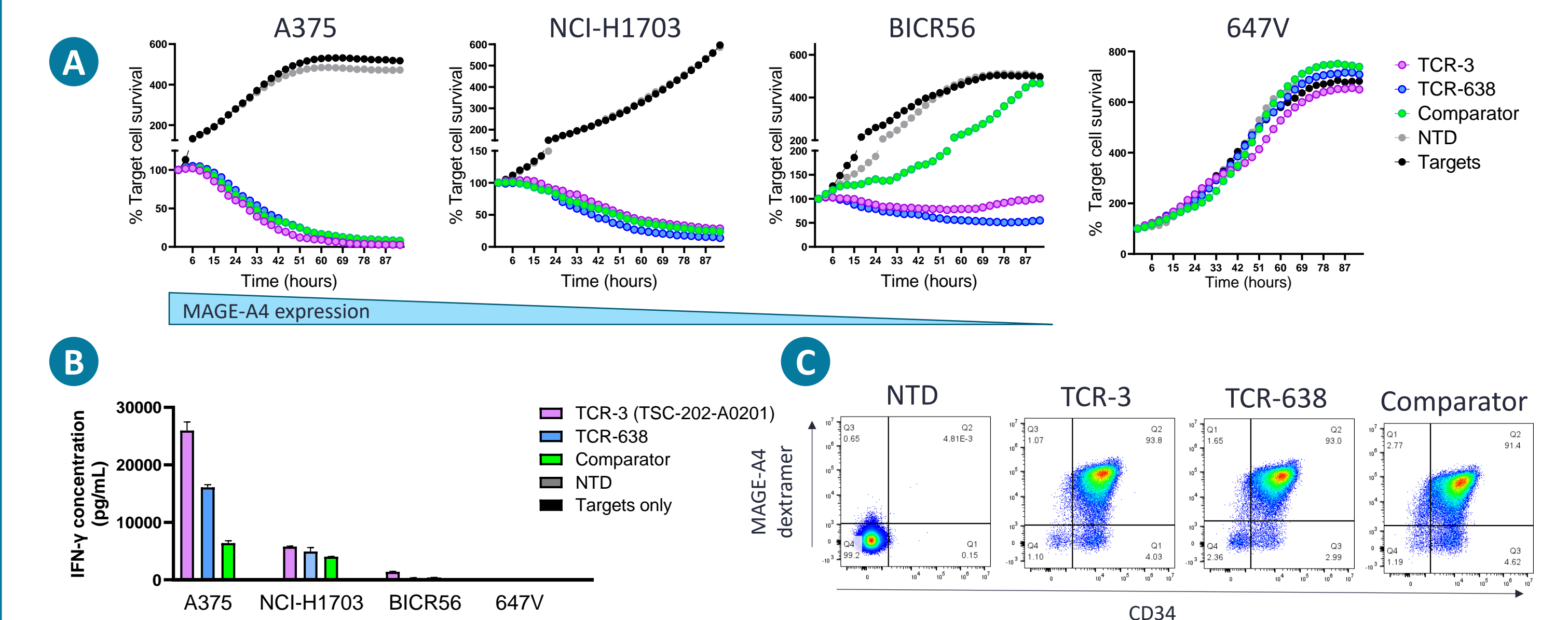
## ReceptorScan platform identifies over 2100 novel MAGE-A4-specific TCRs



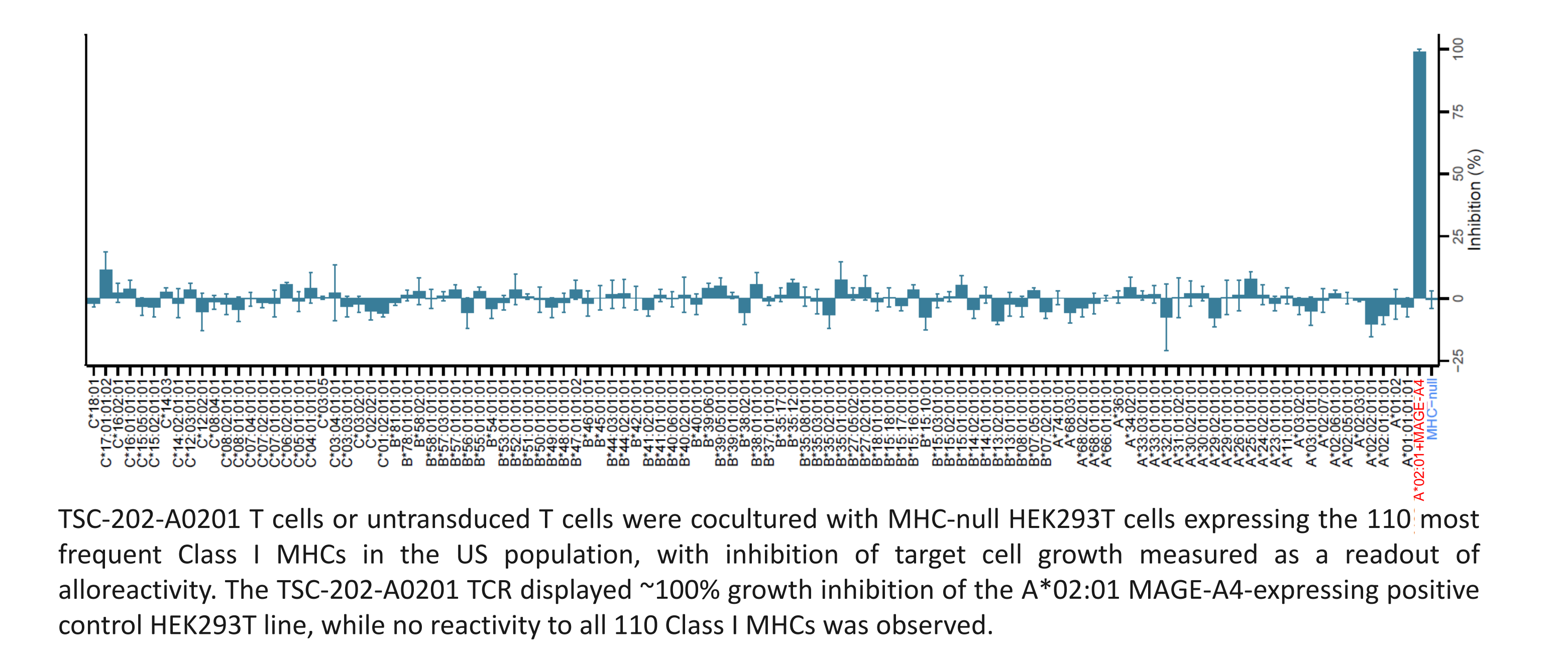
## ActivScan platform identifies 98 TCRs with high expression and affinity



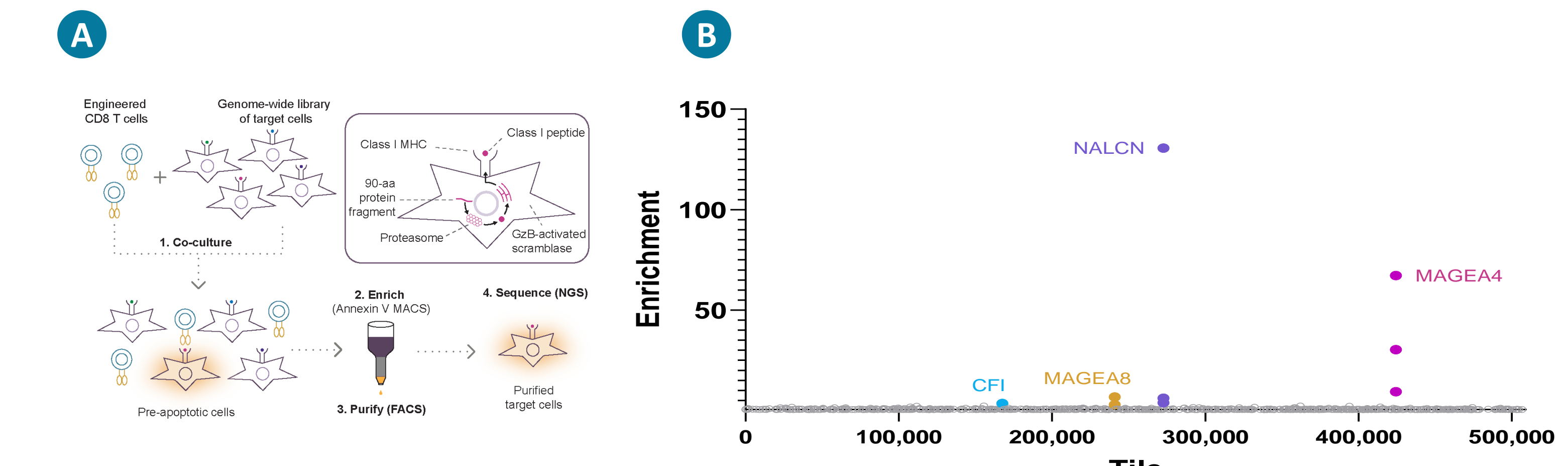
## MAGE-A4 TCRs identified by ReceptorScan are specific and potent



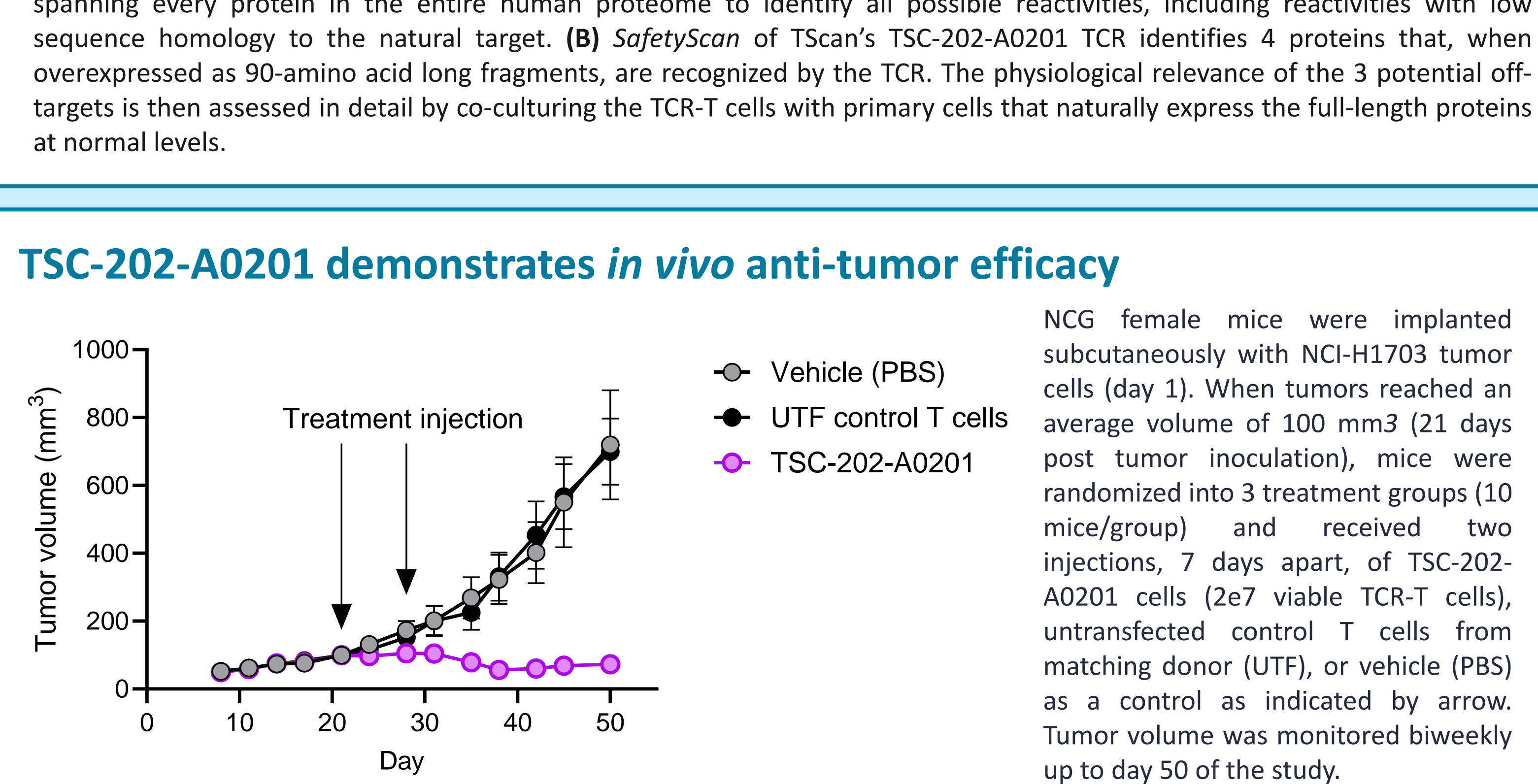
## No alloreactivity of TSC-202-A0201 TCR to 110 HLA-I allotypes was observed



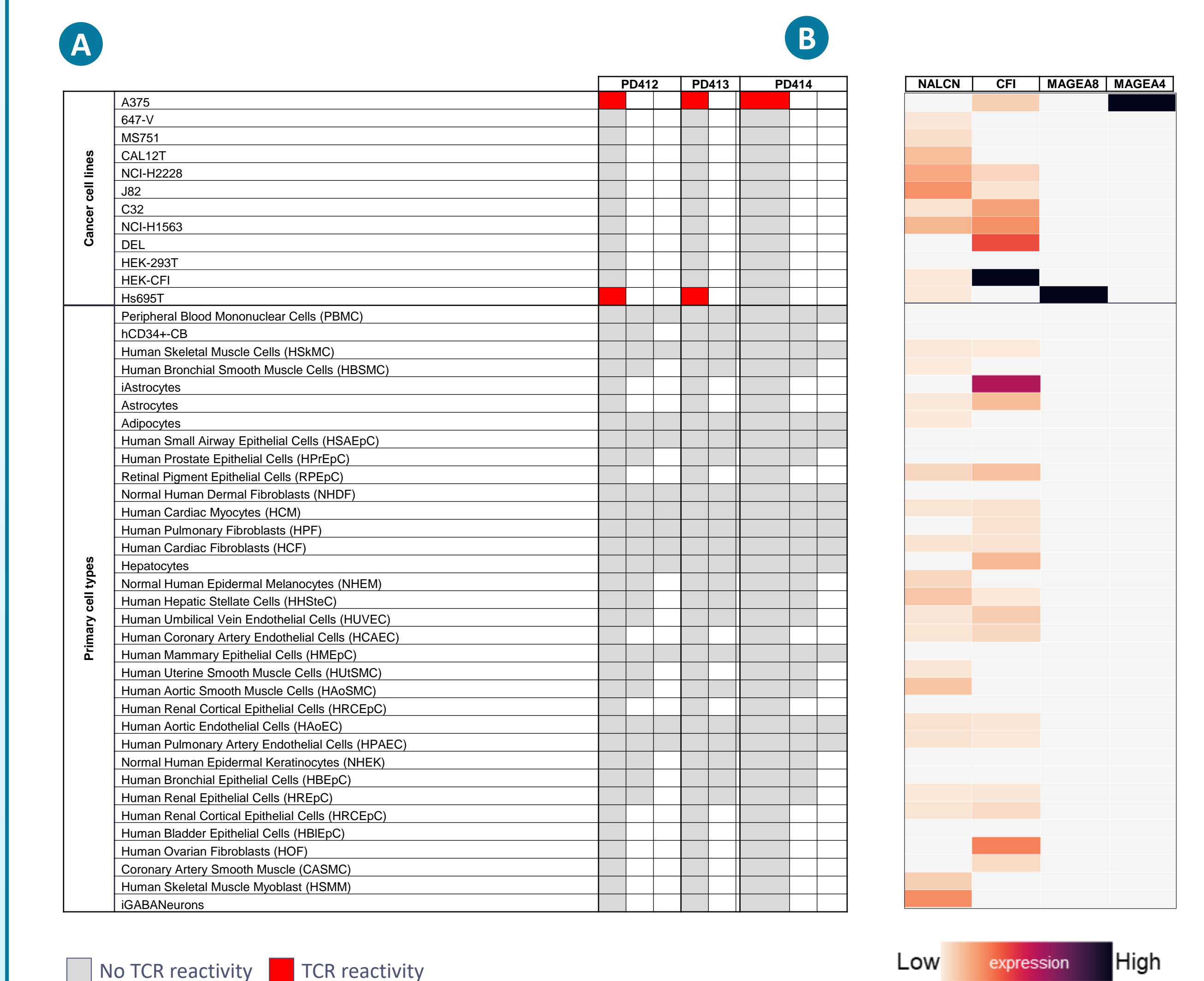
## Genome-wide safety screen identifies putative off-targets of TSC-202-A0201 TCR



## TSC-202-A0201 demonstrates *in vivo* anti-tumor efficacy



## TSC-202-A0201 TCR-T cells display no risk of off-tumor reactivity



Three batches of process-representative TSC-202-A0201 TCR-T cells (PD412, PD413, and PD414) were assessed for risk of off-tumor reactivity. (A) Top: TSC-202-A0201 TCR-T cells showed no reactivity to a panel of HLA-A\*02:01-positive cancer cell lines naturally expressing off-targets, or to HEK293T cells transduced to express physiologically relevant levels of CFI, while reactivity to the MAGE-A8-positive (MAGE-A4-negative) cancer cell line H695T was observed for two TCR-T cell batches. Bottom: A panel of 70 HLA-A\*02:01-positive normal primary or iPSC-derived human cell samples derived from 34 tissues was tested as targets for TSC-202-A0201 to test off-tumor reactivity. Normal primary human cells included epithelial cells, mesenchymal cells, endothelial cells, fibroblasts, and muscle cells derived from multiple vital and non-vital organs, reproductive and non-reproductive organs, and male and female donors. The data is presented as a tabulated summary of the reactivity of the TCR-T cells as assessed by IFN- $\gamma$  measurement; each colored cell in the table illustrates a single lot of cells for the indicated cell type. For each cell type, 1-3 lots of cells (i.e. donors) were tested, depending on the availability of the primary cells. Reactivity is indicated in red, lack of reactivity by gray. (B) Expression of the putative off-targets of the TSC-202-A0201 therapeutic TCR was determined by RNAseq in the various cell types tested; the average expression of the off-target for each cell type is presented as a heatmap to indicate the range of expression across samples. The color scale used in RNAseq heat maps has TPM values of zero set to white, and values above zero follow a continuous color scale up to 100 TPM.

## Additional Posters from TScan Therapeutics

# 359: Preclinical Models for T-Plex, a Customized Multiplexed TCR-T Cell Therapy Addressing Intra-Tumor Antigen and HLA Heterogeneity

# 384: Development of a Target Agnostic Platform to Assess the Reactivity of T Cell Receptor (TCR)-Engineered T Cell (TCR-T) Therapies to Primary Human Tissues