

# Discovery of TSC-200-A02: A natural HPV16 E7-specific TCR-T cell therapy candidate for the treatment of HPV-positive solid tumors

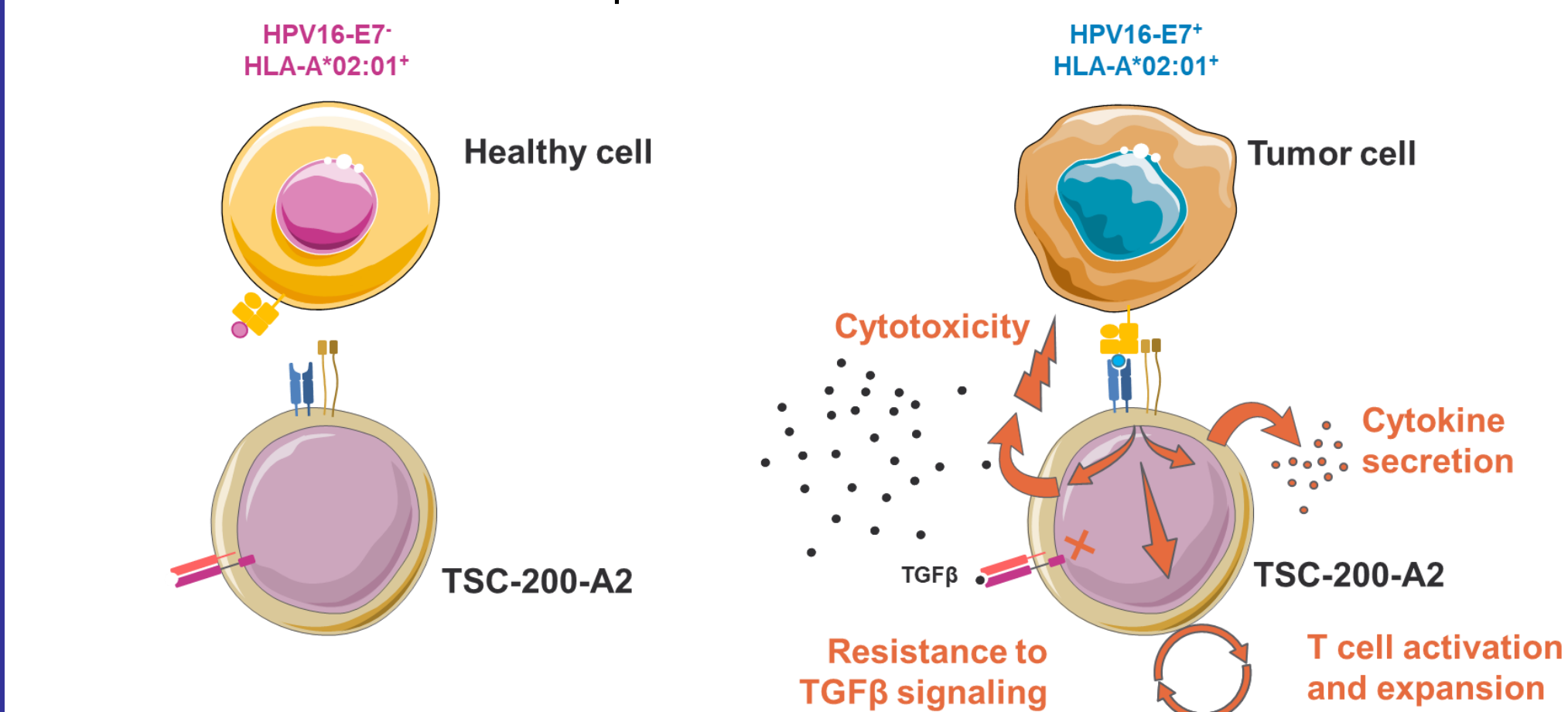
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## Introduction

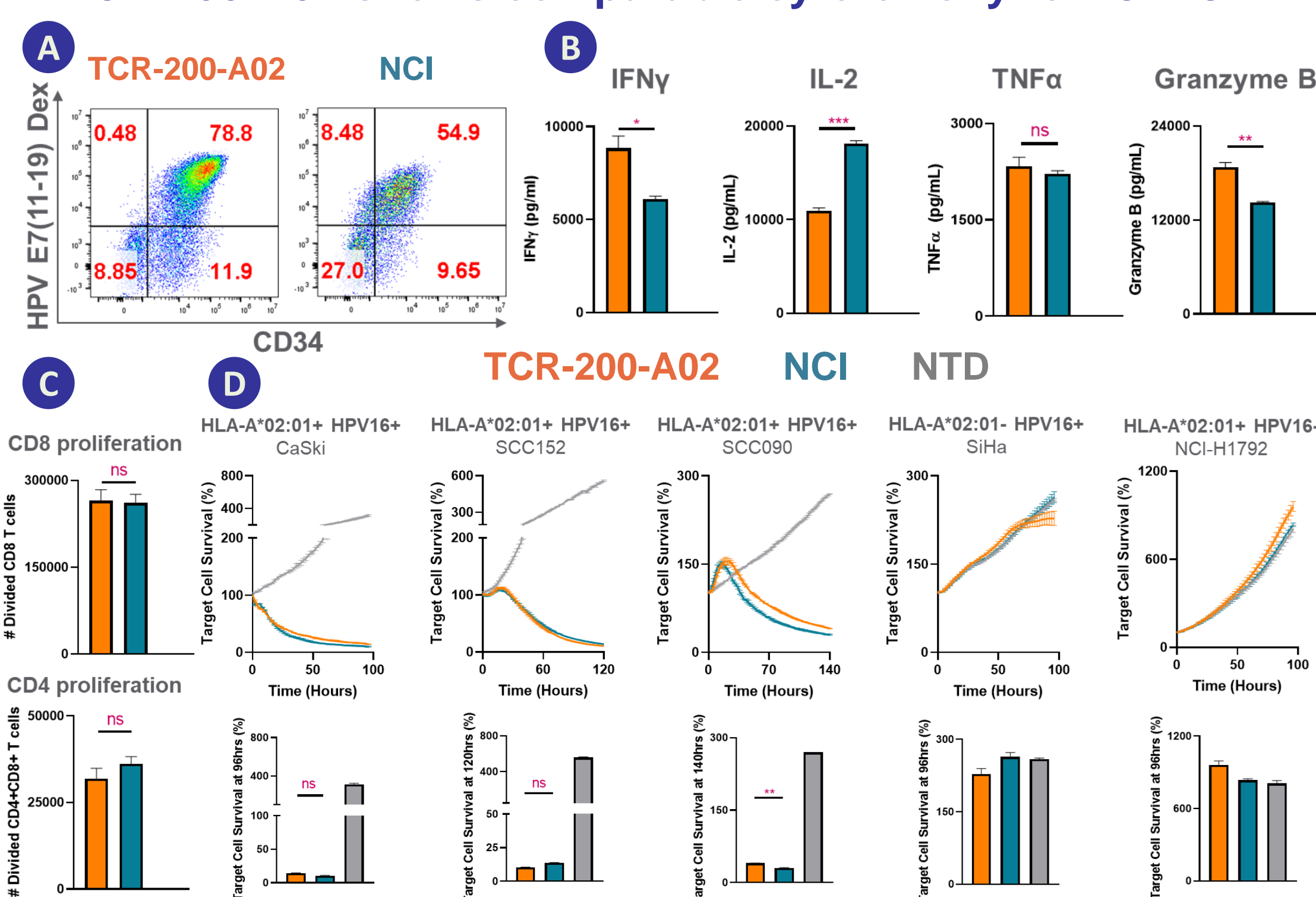
Human papilloma virus (HPV) is an oncogenic virus responsible for over 90% of cervical and anal cancers and over 25% of head and neck cancers, which are typically incurable upon metastasis. HPV E7 oncoprotein is a compelling target for TCR-engineered T cell therapy as it is homogenously expressed in every tumor cell, essential for tumor cell survival, and not expressed by healthy tissues. Notably, a recent clinical trial of an E7-directed TCR-T cell therapy conducted at the National Cancer Institute (NCI) showed a 50% objective response rate in heavily pre-treated patients with HPV+ cancers (1).

Using TScan's proprietary *ReceptorScan* platform, we discovered 453 putative HPV16 E7<sub>11-19</sub>-specific TCRs by screening 681 million naïve CD8+ T cells from 15 unique healthy donors. We tested each TCR for expression in primary T cells and for its ability to kill T2 cells pulsed with the E7<sub>11-19</sub> peptide, using the NCI TCR as a benchmark for these studies (2). The top 3 TCRs from this screen were evaluated in depth for cytotoxicity, cytokine production, and T cell proliferation in response to a panel of HPV16+ cancer cell lines expressing varying levels of HLA-A\*02:01 and E7. A lead TCR was identified that showed comparable activity relative to the NCI TCR. The lead TCR was evaluated for allo-reactivity using an array-based screen and for off-target reactivity using our proprietary *SafetyScan* platform, which is a highly sensitive screen for off-target recognition based on supraphysiologic expression of protein fragments that span the entire human proteome. No alloreactivity was observed to 108/110 HLAs tested, and only a few putative off-targets were identified. The TCR-T cells showed no reactivity to a panel of normal primary human cells, including cells that naturally express the putative off-targets identified in the *SafetyScan* screen. In vivo studies in immunocompromised mice showed efficient control of xenogeneic tumor cell growth by TCR-200-A02 cells in two independent tumor models.

To further enhance the activity of our T cells, we designed a transposon-based vector that delivers the TCR gene, along with the genes for CD8  $\alpha/\beta$  and a dominant-negative form of TGF $\beta$ RII, into both CD4+ and CD8+ T cells. We have advanced the resulting autologous TCR-T cell therapy candidate, TSC-200-A02, to IND-enabling studies. These results validate the use of *ReceptorScan*, in conjunction with *SafetyScan*, as a way to rapidly identify naturally occurring, high affinity TCRs that are suitable for clinical development.

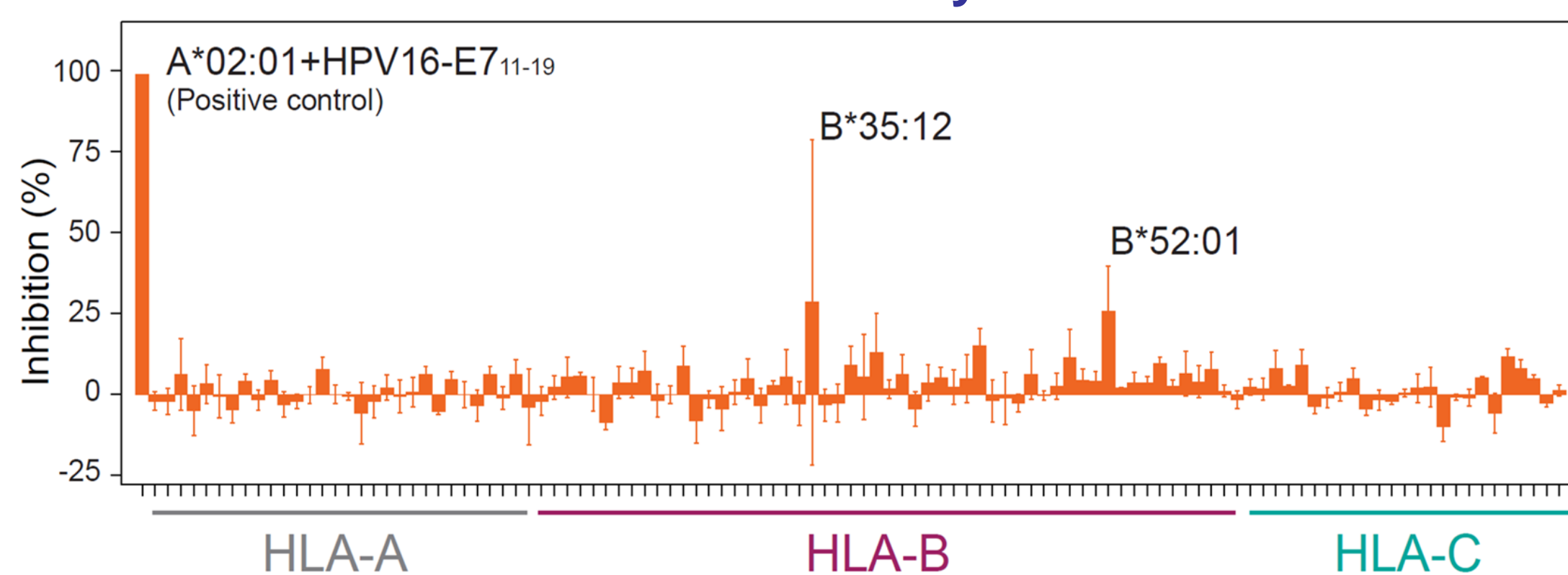


## TCR-200-A02 shows comparable cytotoxicity to NCI TCR



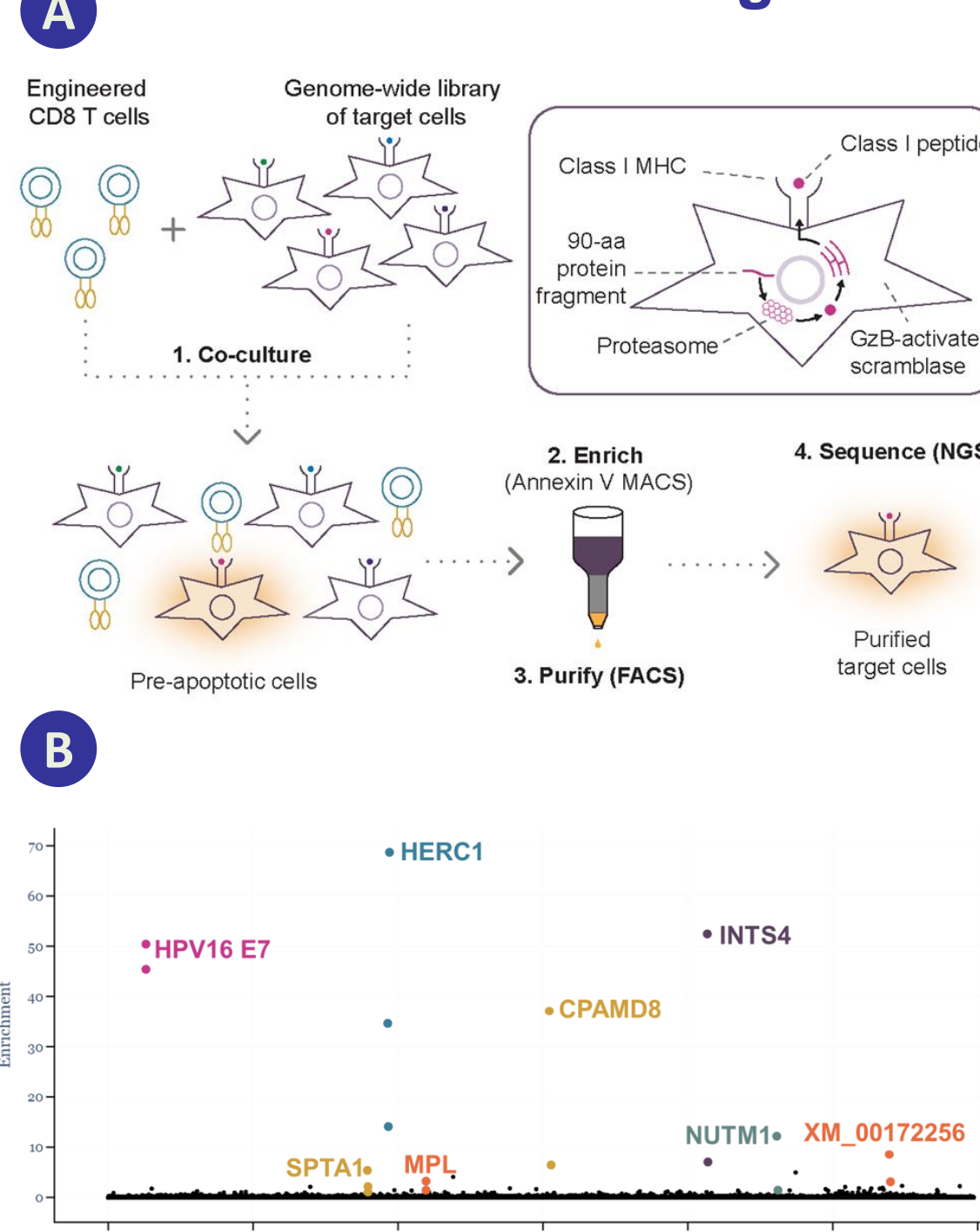
Pan T cells (natural mixture of CD4+ and CD8+ T cells) were transduced to express HPV16 E7<sub>11-19</sub>-specific TCRs and assessed for functional responses to target cells. **(A)** TCR-200-A02 shows efficient surface expression of TCR-200 (HPV16 E7<sub>11-19</sub>-specific dextramer) and Q-tagged CD8 $\alpha$  (QBEnd/10). **(B)** TCR-200-A02 efficiently secretes cytokines when co-cultured with SCC152 cells. **(C)** TCR-200-A02 exhibits HPV16-specific CD8+ and CD4+ T cell proliferation when co-cultured with SCC152 cells. **(D)** TCR-200-A02 shows strong cytotoxicity in HLA-A\*02:01+ HPV16+ target cell lines CaSki, SCC152, SCC090, and no reactivity to HLA-A\*02:01- HPV16+ cell line SiHa or the HLA-A\*02:01+ HPV16- cell line NCI-H1792. TCRs were compared by one-way ANOVA followed by Dunnett's multiple comparison test. Differences that were non-significant (ns) are shown; all other differences were significant with  $P < 0.05$ . Data are representative of 3 unique donors.

## TCR-200-A02 shows no alloreactivity to 108/110 HLAs tested



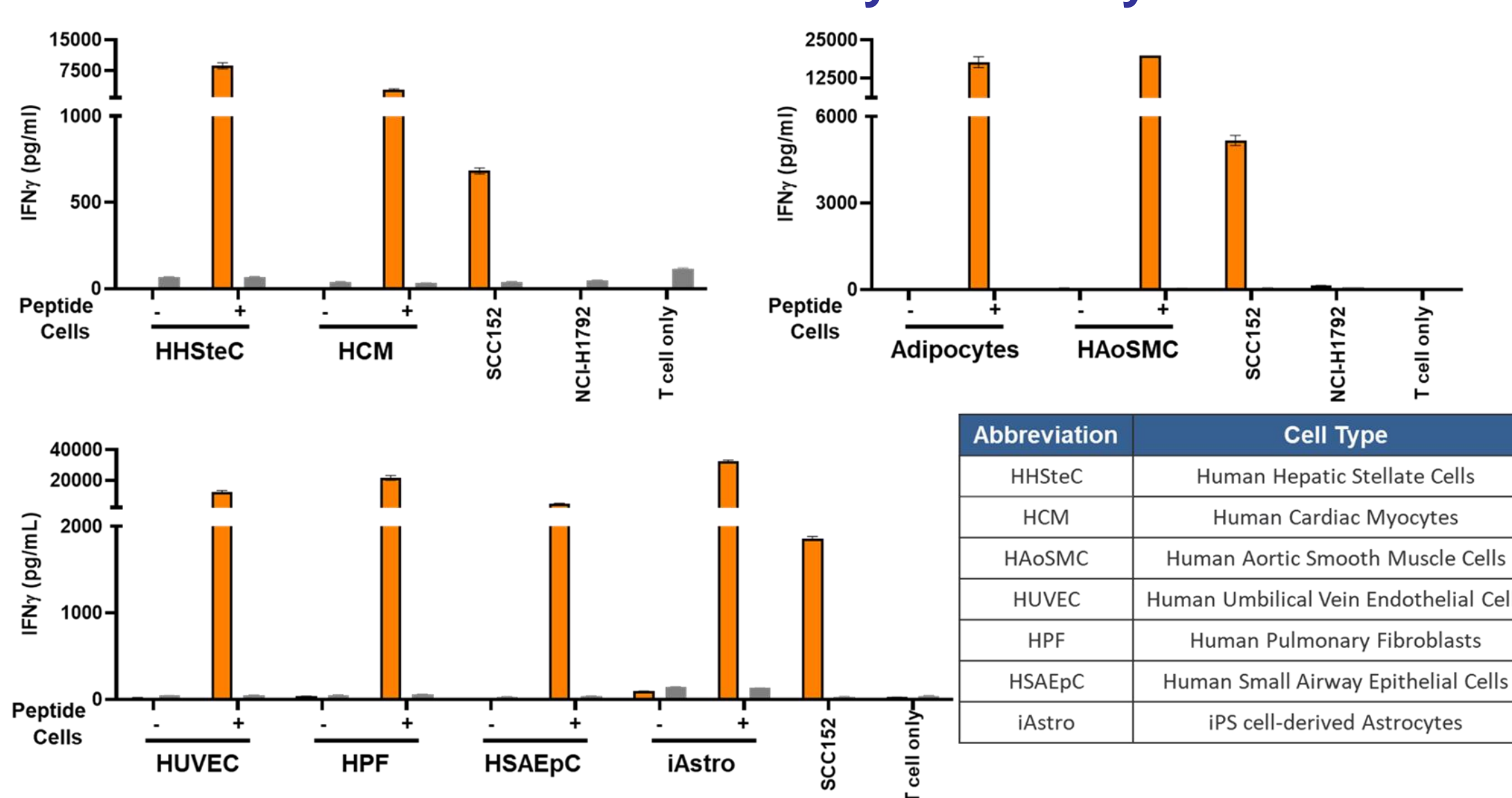
TCR-200-A02-expressing CD3+ T cells showed no alloreactivity to 108/110 of the most frequent class I MHCs in the US population.

## TScan's genome-wide *SafetyScan* screen identifies putative off-targets for TCR-200-A02



**(A)** Overview of TScan's proprietary genome-wide *SafetyScan* screen. **(B)** *SafetyScan* screen of TCR-200-A02 identifies seven potential off-targets in a screen of >600,000 protein fragments spanning every w.t. human protein. The screen is designed to overpredict off-targets by overexpressing 90-aa protein fragments, which are more efficiently processed than full-length proteins. Putative off-targets are identified by gene names. XM\_0017722256 maps to the heterochromatic centromere region of chromosome 20 and has been removed from the RefSeq annotation indicating a lack of evidence for its expression. No expression of this gene was detected using RNA-seq analysis of 51 samples including normal tissue samples, cancer cell lines and tumor samples.

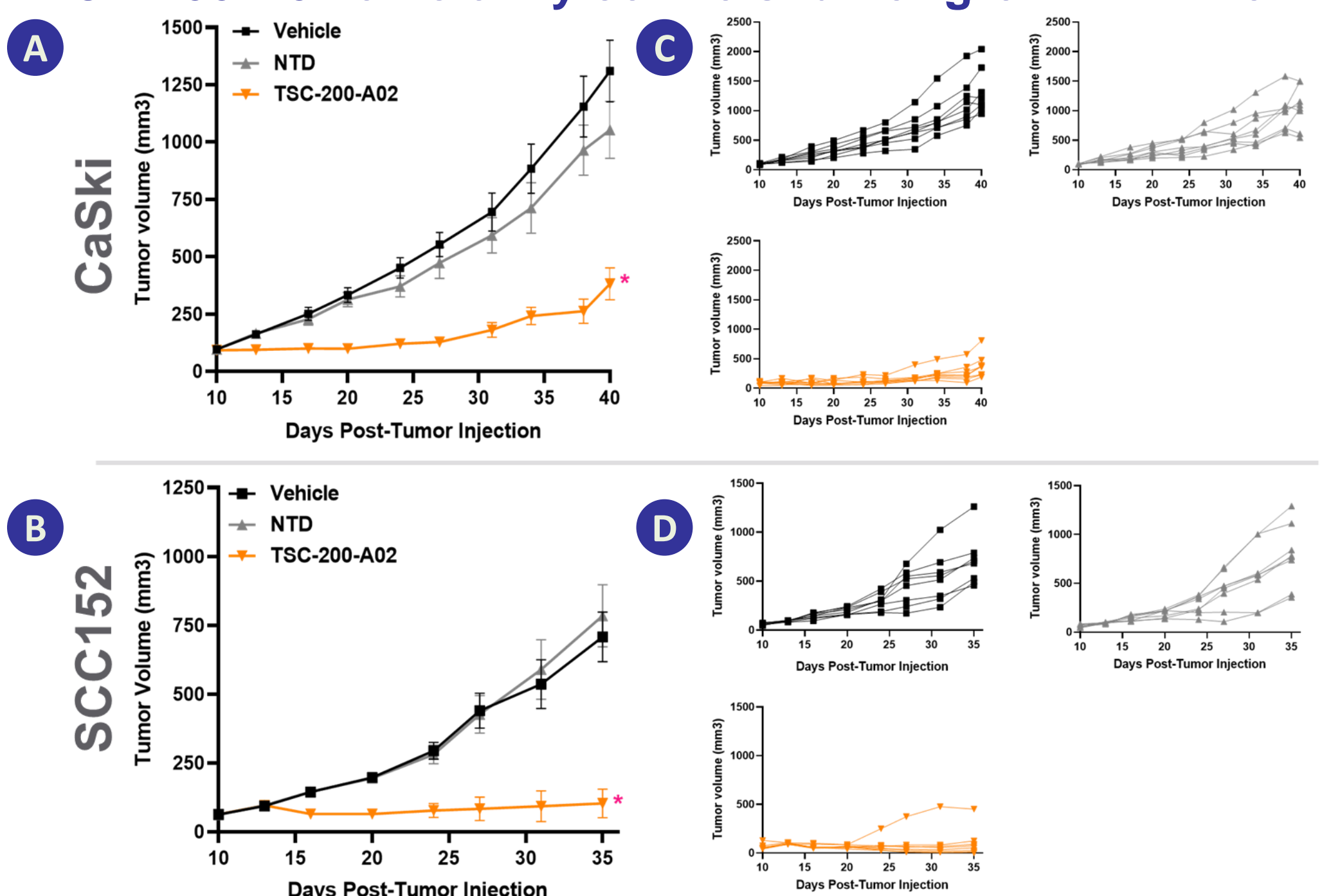
## TCR-200-A02 shows no reactivity to healthy human cells



Primary cells or iPSC-derived cells from healthy HLA-A\*02:01+ human donors naturally expressing off-targets identified in the genome wide *SafetyScan* screen were co-cultured with TCR-200-A02 or nontransduced (NTD) cells. Cell pulsed with E7 peptide served as positive controls. IFN $\gamma$  secretion in culture supernatants was used as a read-out of TCR-200-A02 reactivity to target cells. SCC152 cells were used as a positive control and NCI-H1792 cells were used as a negative control.

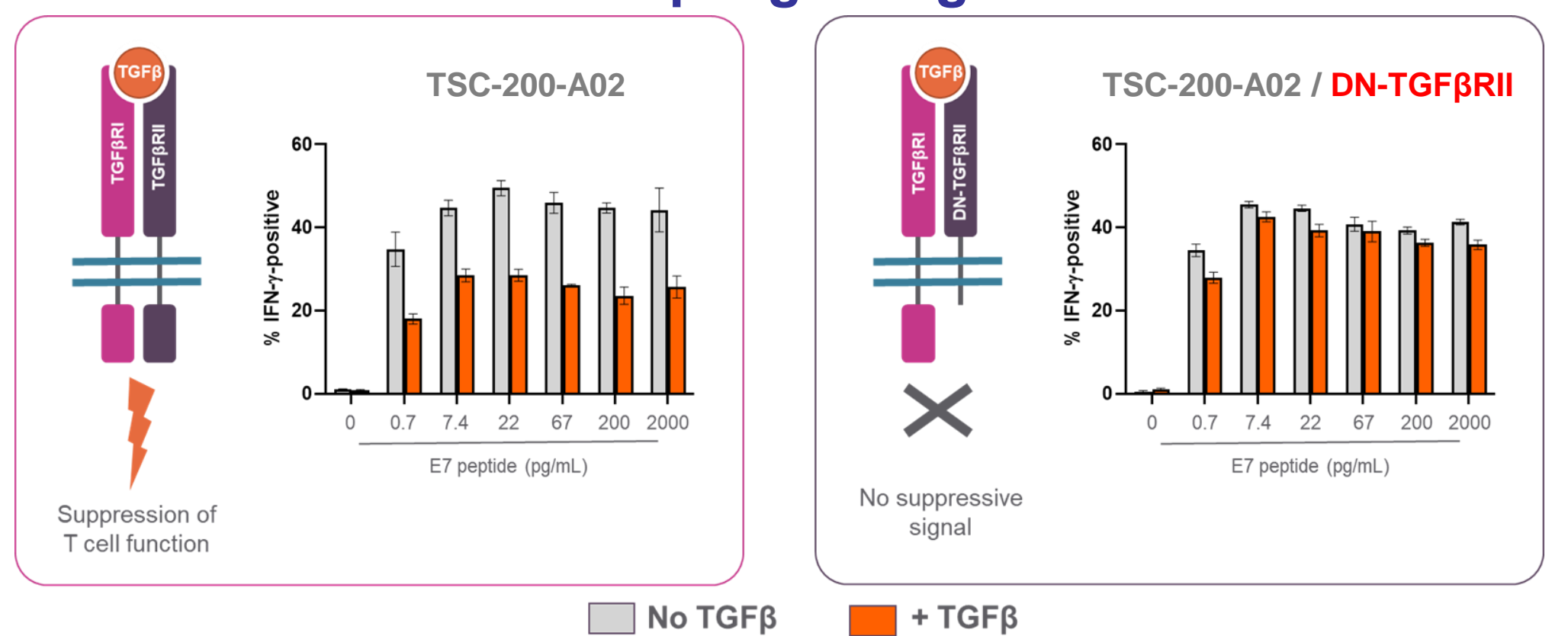
- Nagarsheth NB, Norberg SM, Sinkoe AL, et al. TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. *Nat Med.* 2021 Mar;27(3):419-425.
- Jin BY, Campbell TE, Draper LM, et al. Engineered T cells targeting E7 mediate regression of human papillomavirus cancers in a murine model. *JCI Insight.* 2018;3(8): e99488

## TCR-200-A02 efficiently controls tumor growth in vivo



NCG mice were subcutaneously inoculated with either  $1 \times 10^6$  CaSki or SCC152. When tumors reached  $95 \pm 15 \text{ mm}^3$  on Day 10, the mice were randomized and treated on Day 11 with  $20 \times 10^6$  cells of TCR-200-A02, non-transduced (NTD) cells or vehicle. **(A & B)** Treatment with TCR-200-A02 showed significant inhibition of tumor growth in vivo. **(C & D)** Individual mouse tumor growth per group over time. \* $p < 0.05$ , One-way ANOVA, Holms-Šidák correction for multiple comparisons test in vivo.

## TGF $\beta$ RII-DN provides resistance to the suppressive effect of TGF $\beta$ signaling



Dominant negative TGF $\beta$  Receptor II (DN-TGF $\beta$ RII) renders TSC-200-A02 resistant to TGF $\beta$ -mediated suppression. T cells were co-transduced with lentivirus encoding TCR-200-A02 and DN-TGF $\beta$ RII and were FACS sorted into DN-TGF $\beta$ RII positive and DN-TGF $\beta$ RII negative fractions. Intracellular IFN $\gamma$  within the TSC-200-A02 expressing T cells was quantified after 24 h co-culture with peptide-pulsed T2 cells in the presence or absence of 5 ng/mL TGF $\beta$ .