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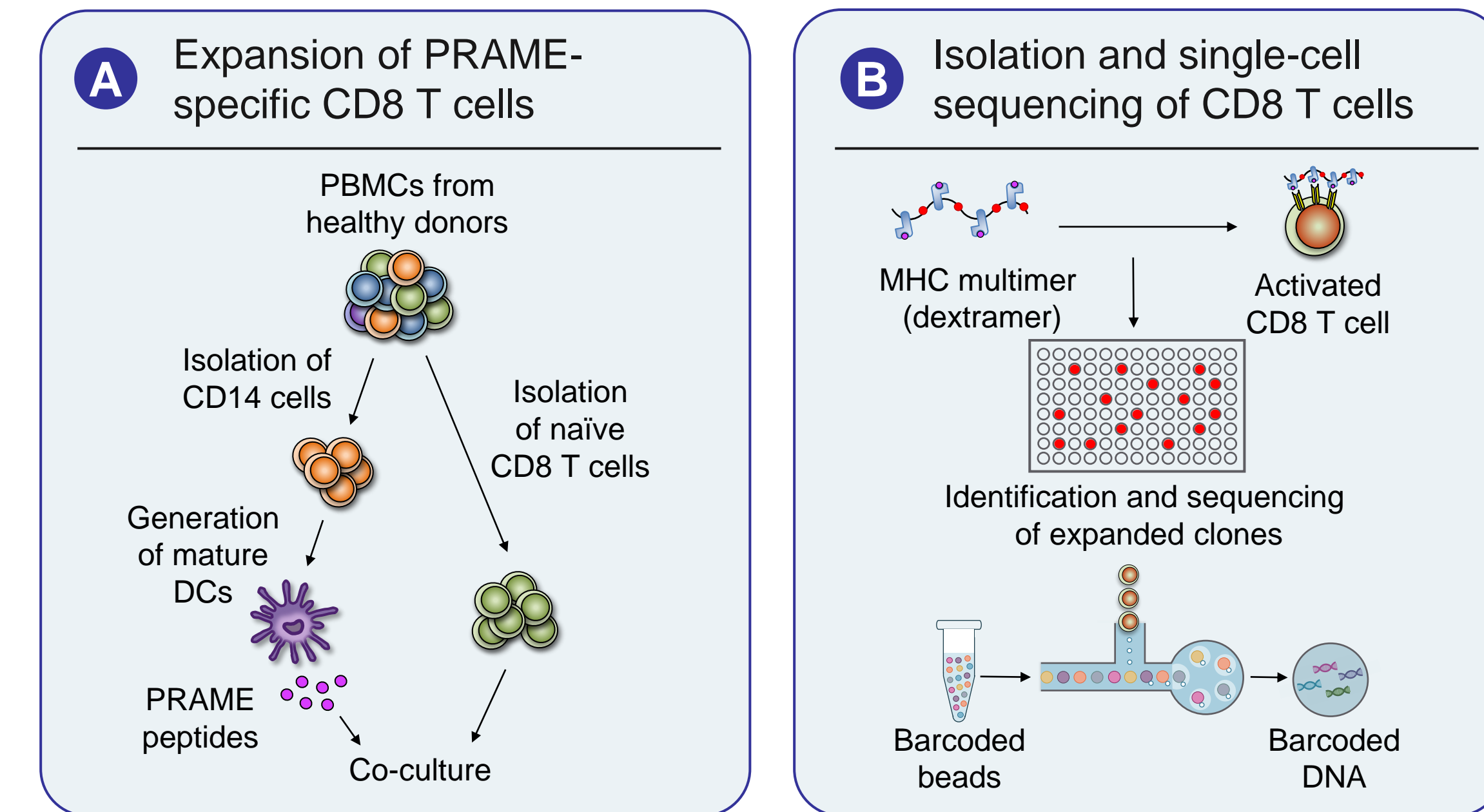
Background: The cancer/testis antigen PRAME exemplifies an ideal TCR-T cell therapy target due to its high expression in multiple malignancies and its absence in normal tissues. Initially identified in metastatic cutaneous melanoma¹, PRAME is highly expressed in various additional solid tumors including lung, head & neck, and ovarian cancers. PRAME plays a pivotal role in multiple cellular processes and has been demonstrated to exhibit protumorigenic function primarily through inhibition of retinoic acid receptor signaling². Targeting of PRAME in solid tumors, particularly when performed as part of a TCR-T multiplexing strategy, represents a promising therapeutic approach in the treatment of many cancer indications.

Methods: We discovered TCRs specific for 5 different A*02:01-restricted PRAME-derived epitopes using TScan's proprietary ReceptorScan platform. Using an activation-based screening technology termed ActivScan, we identified the most functional TCRs from a library of 1300 PRAME-specific TCRs to select for TCRs with greatest avidity and expression. These highly active TCRs were examined for their cytotoxic function using a panel of PRAME-expressing A*02:01-positive cell lines. Lead TCRs were assessed for potential off-target reactivity using our proprietary SafetyScan platform, in which recognition of antigens derived from all proteins that comprise the human proteome is evaluated. Safety was further confirmed by examining alloreactivity to high-frequency Class I HLAs and by testing TCR reactivity to a panel of normal primary human cells. Lastly, we tested TCR-T cells for their ability to control tumor growth *in vivo* using a PRAME-expressing xenograft mouse model.

Results: We screened 871 million naïve CD8⁺ T cells from 16 unique healthy donors in ReceptorScan to identify 5706 TCRs specific for 5 PRAME epitopes. PRAME₄₂₅₋₄₃₃-specific TCRs demonstrated superior recognition of a PRAME-expressing cell line compared to all other PRAME epitopes tested. Following selection of high-expressing and high avidity PRAME₄₂₅₋₄₃₃-specific TCRs in ActivScan, TCRs were evaluated for their cytotoxic function, and two TCRs compared favorably to a clinical-stage benchmark TCR with respect to cytotoxicity, cytokine release, and T cell proliferation. Safety assessment demonstrated that few off-target peptides were recognized by lead TCRs, minimal alloreactivity was observed to 110 allotypes tested, and no reactivity to normal primary human cells was found. PRAME₄₂₅₋₄₃₃-specific TCR-T cells were also able to control tumor growth *in vivo* following infusion into immunodeficient mice implanted with PRAME-expressing xenografts.

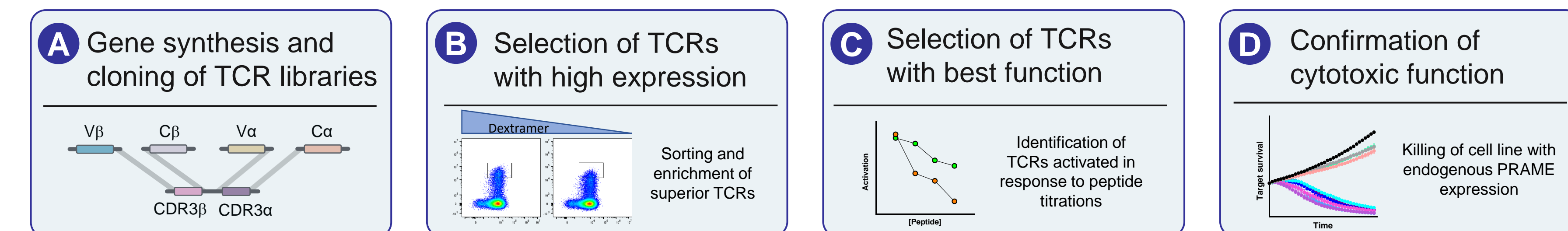
Conclusions: Based on its demonstrated activity, this autologous TCR-T cell therapy candidate, along with additional candidates, has been advanced to IND-enabling studies, one of which will be included in TScan's ImmunoBank to enable multiplexed TCR-T therapy for solid tumors.

ReceptorScan platform identifies over 5000 novel PRAME TCRs



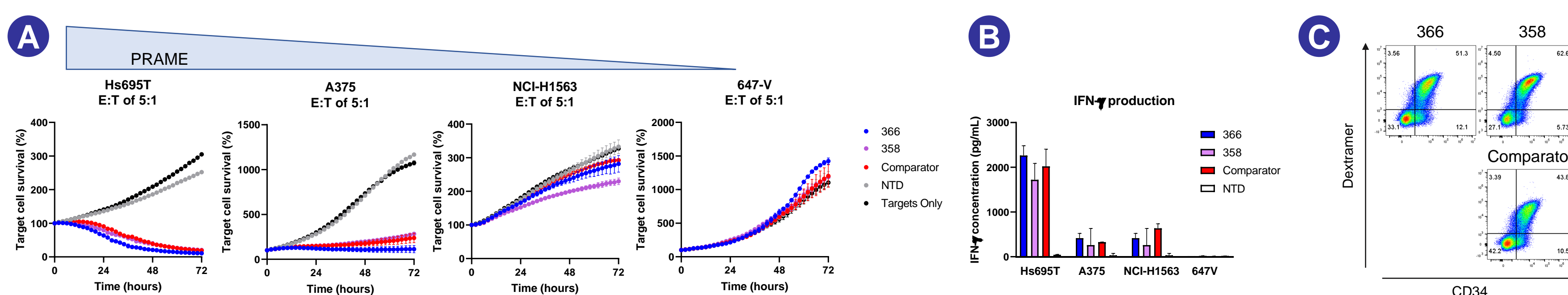
Schematic representation of the ReceptorScan platform for identification and selection of antigen-specific T cells. **(A)** PRAME-specific T cells were expanded from the naïve CD8⁺ T cell population of A*02:01-positive healthy donors. Co-culture and expansion of naïve CD8 T cells was performed with autologous mature DCs pulsed with 5 PRAME-derived A*02:01-restricted epitopes. **(B)** To isolate antigen-specific CD8 T cells, co-cultures were stained with DNA-barcoded A*02:01 PRAME-specific dextramers and sorted, and single-cell sequencing of dextramer-positive CD8 T cells was performed using the 10X Genomics platform.

ActivScan platform identifies 7 TCRs with high expression and affinity



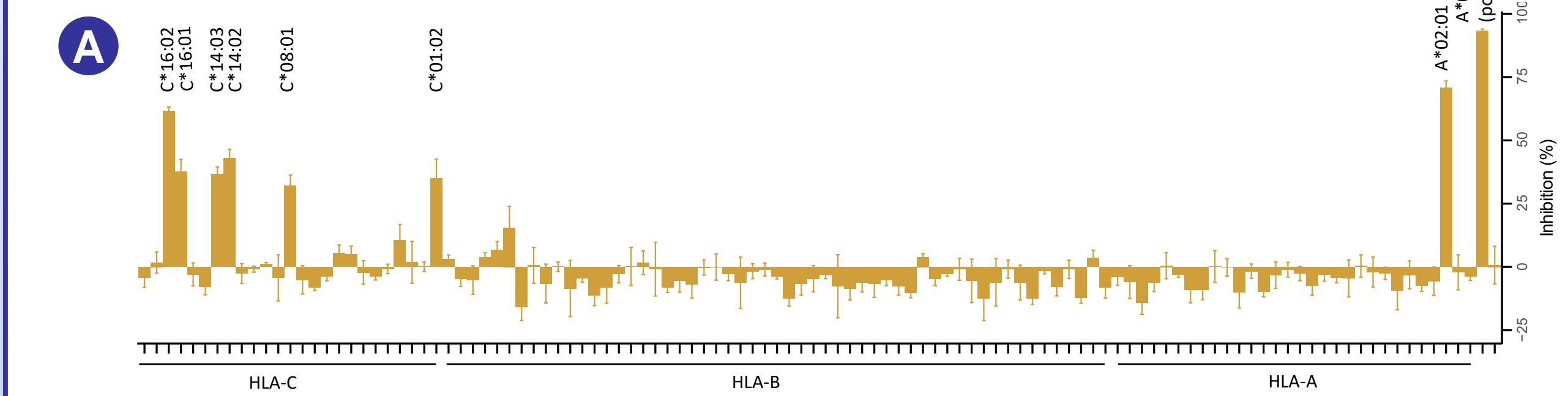
(A) Libraries for PRAME-specific TCRs identified by the ReceptorScan platform were synthesized using TScan's proprietary PISTACHIO cloning method. **(B)** Selection of TCRs with high expression was performed by transducing pan T cells with viruses encoding PISTACHIO-cloned TCR libraries, followed by isolation of dextramer-bound cells. **(C)** Identification of TCRs with high affinity was performed using ActivScan by sorting cells that responded to titrations of peptide. **(D)** 5 out of 7 selected TCRs displayed cytotoxicity towards a cell line endogenously expressing PRAME, similar to a comparator TCR.

PRAME TCRs show specificity and functionality against target cell lines



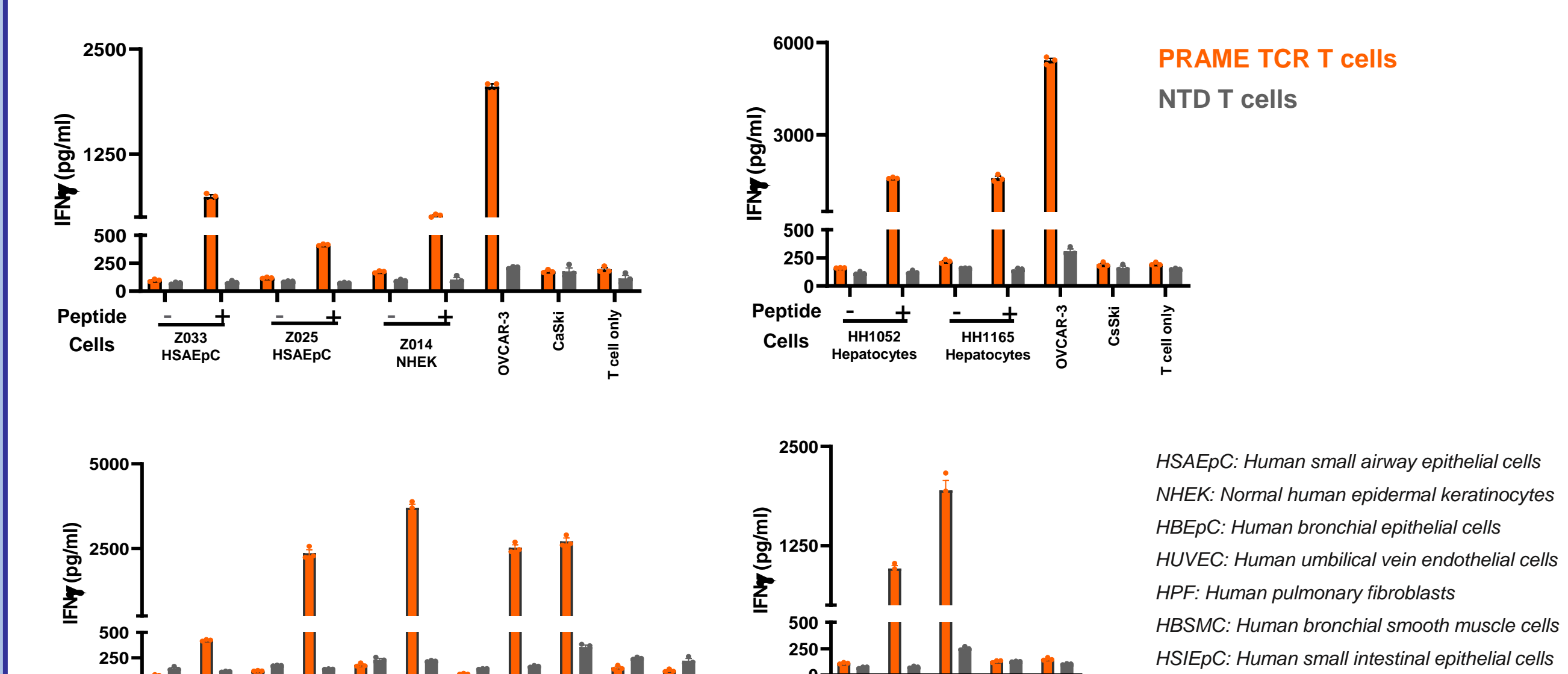
Pan T cells isolated from three HLA-A*02:01-positive healthy donor PBMCs were transduced to express PRAME₄₂₅₋₄₃₃-specific TCRs, as well as the comparator TCR, and T cells were assessed for functional responses against target cells. **(A)** Cytotoxicity of PRAME TCRs to HLA-A*02:01⁺ PRAME⁺ target cell lines Hs695T, A375, NCI-H1563, and to the HLA-A*02:01⁻ PRAME-negative control cell line 647V are shown. **(B)** Production of IFN-γ was measured in co-culture supernatants at 24 h (E:T 1:1). **(C)** Dot plots depict TCR expression, as assessed by A*02:01-restricted PRAME₄₂₅₋₄₃₃ dextramer staining.

PRAME TCR shows no alloreactivity to 104/110 HLA types



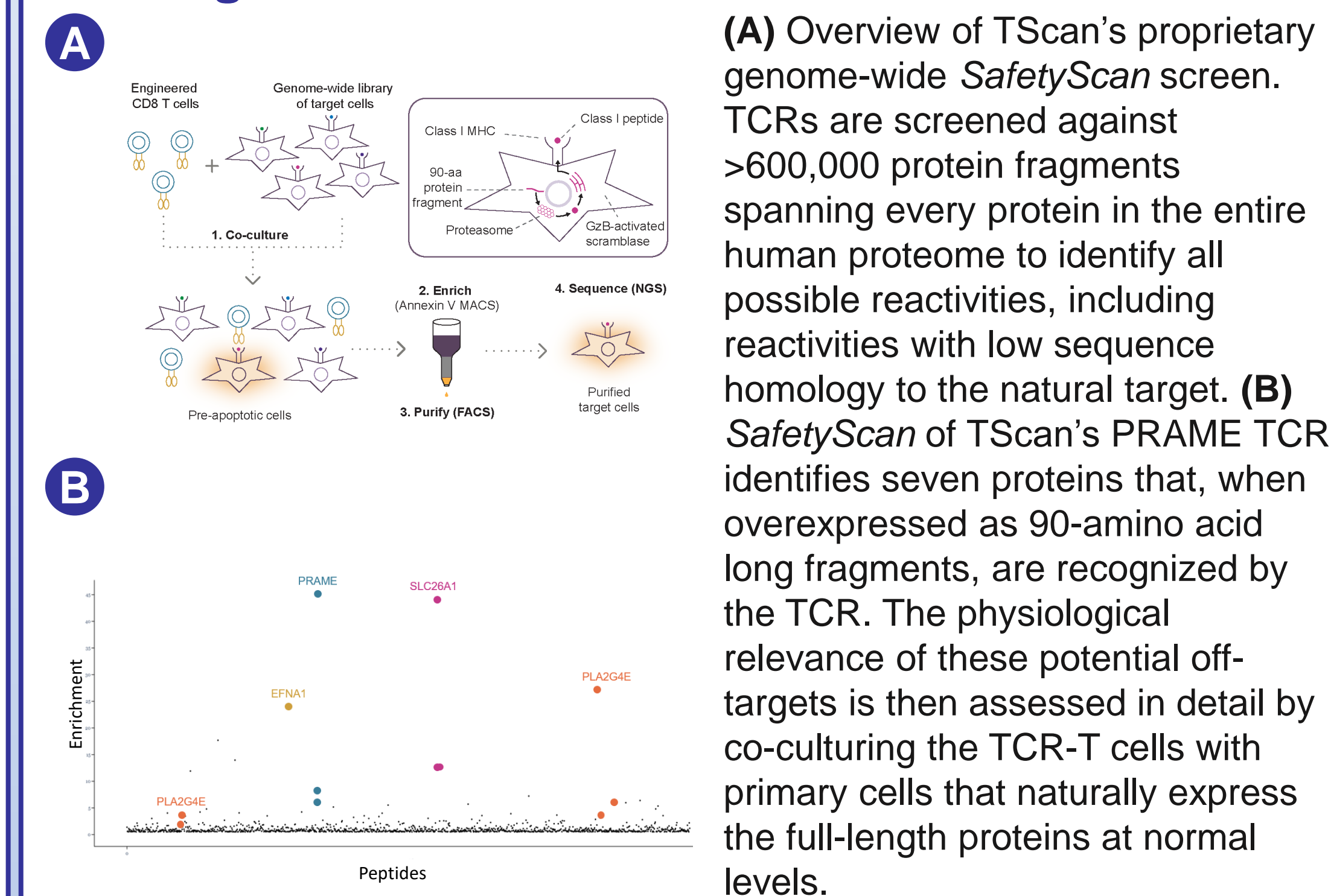
(A) PRAME TCR-expressing T cells or untransduced T cells were cocultured with MHC-null HEK293T cells expressing the 110 most frequently-encountered Class I MHCs in the US population, with inhibition of target cell growth measured as a readout of alloreactivity. Though HEK293T cells express low-level PRAME, a positive control A*02:01 HEK293T line expressing a fragment containing the PRAME₄₂₅₋₄₃₃ epitope was included in the screen. **(B)** PRAME TCR-expressing T cells were cocultured with MHC-null or A*02:01 Ramos cells, which are PRAME-null, in the presence or absence of PRAME₄₂₅₋₄₃₃ peptide. Lack of response in the absence of peptide confirms that PRAME TCR does not display alloreactivity to A*02:01.

PRAME TCR shows no reactivity to healthy human primary cells

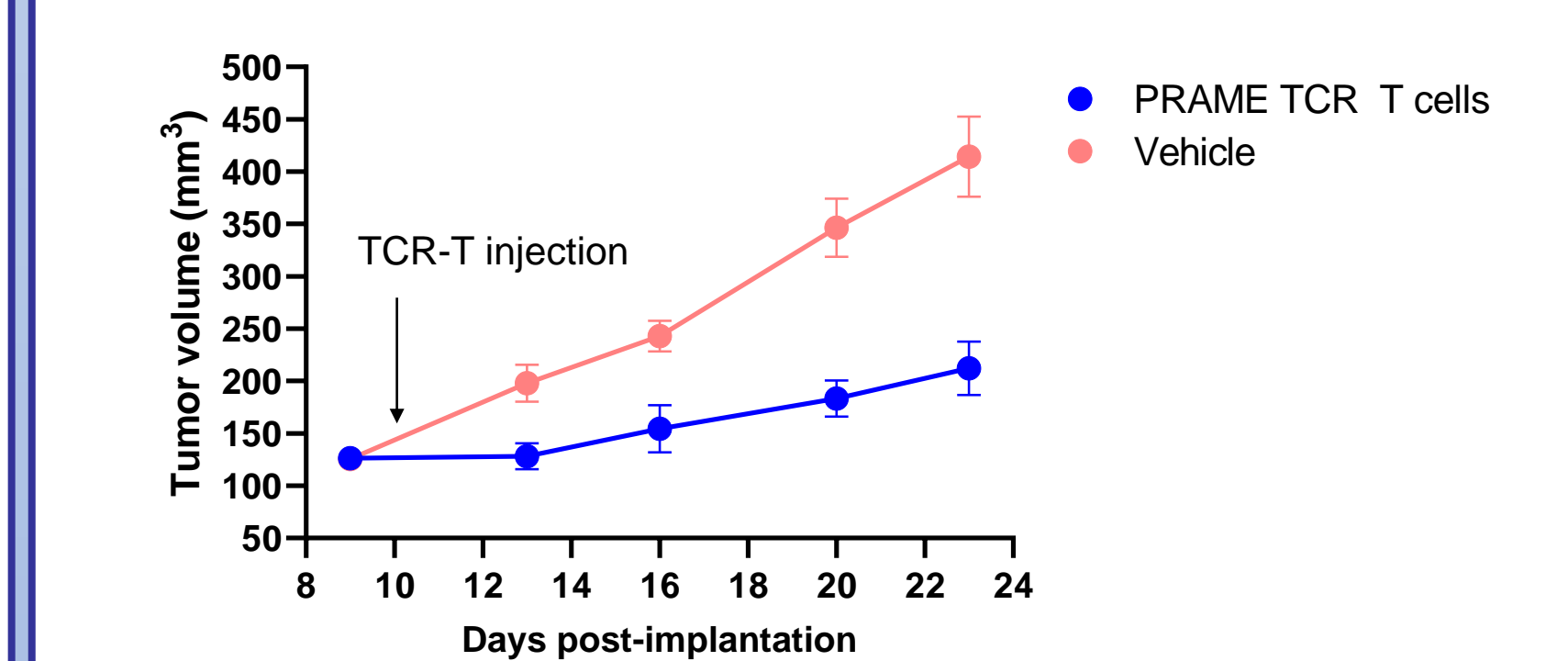


PRAME TCR-expressing pan T cells or NTD cells were tested for reactivity to primary cells derived from healthy HLA-A*02:01+ human donors naturally expressing off-targets identified in the genome-wide safety screen. Target cells were pulsed with the PRAME₄₂₅₋₄₃₃ peptide or were left unpulsed, and then were co-cultured with TCR 366-expressing T cells or NTD T cells. IFN-γ secretion was measured as a readout of reactivity. OVCAR-3 cells were used as a positive control, and CaSki or Lucey cells were used as negative controls.

Genome-wide safety screen identifies putative off-targets of PRAME TCR



PRAME TCR demonstrates anti-tumor efficacy in vivo



NCG mice were implanted s.c. with 1.5e7 PRAME-expressing Hs695T cells in the right flank (n=7 mice/group). On day 10, mice were injected i.v. with 2e7 PRAME TCR-expressing T cells or with vehicle (PBS) as a control (shown by arrow). Tumor volume was monitored from day 9 to day 23 post-implantation.

References

- Ikeda H, Lethe B, Lehmann F, van Baren N, Baurain JF, de Smet C, et al. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. *Immunity*. 1997;6(2):199-208.
- Epping MT, Wang L, Edel MJ, Carlee L, Hernandez M, Bernards R. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. *Cell*. 2005;122(6):835-47.