

# Identification of a T cell receptor recognizing shared HLA-A\*02:01 presented epitope from TP53 frameshift

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## BACKGROUND AND AIMS

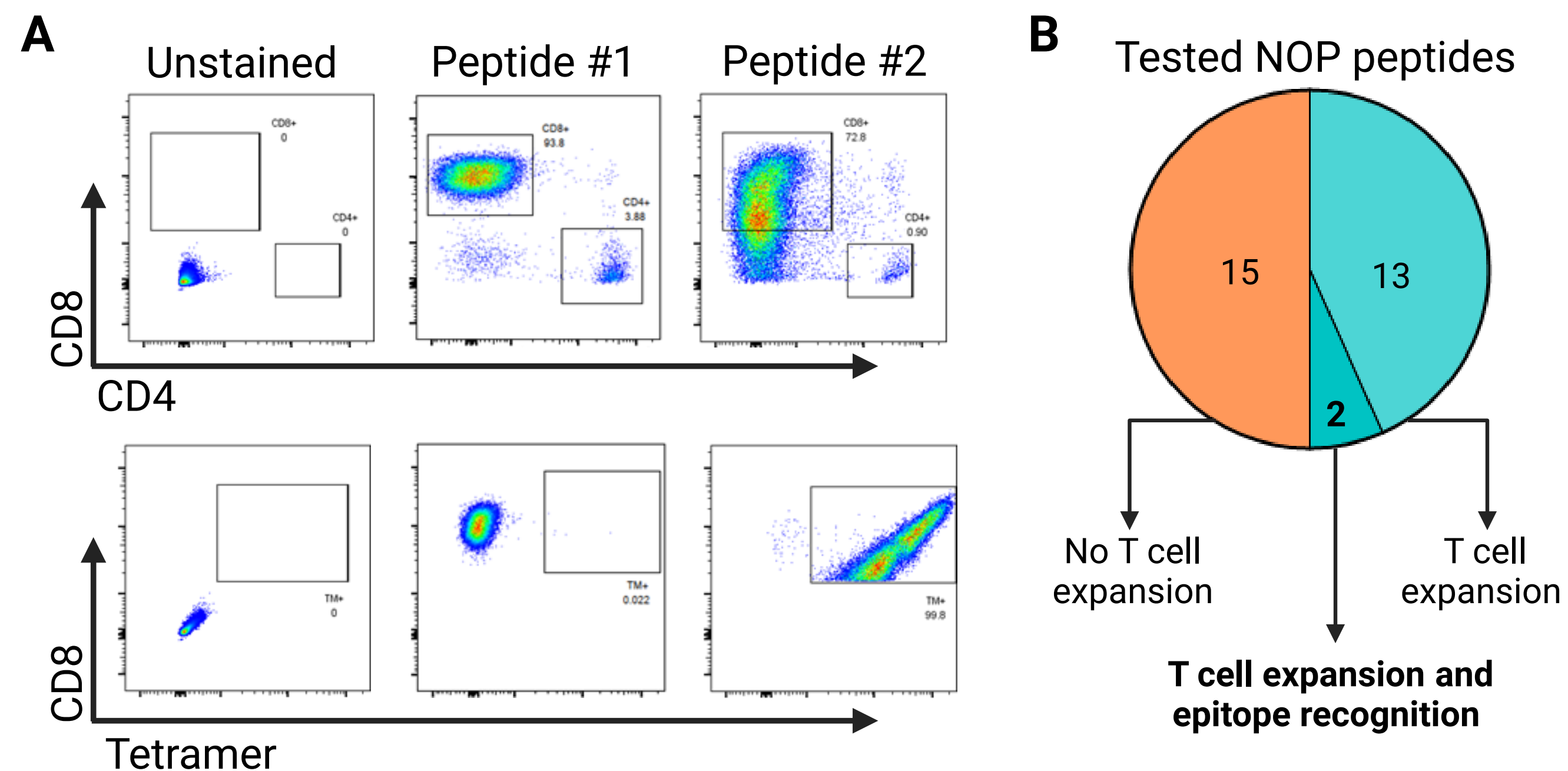
- Tumor cell killing is mediated via recognition of peptide-MHC complexes on the tumor cell surface by their cognate T cell receptors (TCR) on cytotoxic T cells
- The relative lack of shared tumor specific neoantigens limits effective T-cell mediated immunotherapy
- Neo-open reading frame peptides (NOPs) arising from insertions and deletions are a shared class of neoantigens, providing opportunity to design shared T cell therapies
- We aimed to identify and validate a TCR that can recognize a shared epitope derived from a NOP

## METHODS

- NOP prediction was performed for all patients in The Cancer Genome Atlas (TCGA)
- Binding prediction of NOP-derived epitopes for the HLA-A\*02:01 allele was used to rank the top 30 epitopes
- Peripheral blood mononuclear cells of healthy donors were stained with fluorescently labeled tetramers to expand and enrich for NOP-specific CD8<sup>+</sup> T cells recognizing their cognate epitope presented on the cell surface of the HLA-A\*02:01 HEK293 cell line
- Alpha and beta chains of TCRs were sequenced from single cells recognizing their presented cognate epitope
- In vitro* expanded or TCR engineered CD8<sup>+</sup> T cells were used in co-culture assays with a tumor cell line naturally expressing NOPs of interest
- A mRNA construct encoding a NOP was used to transfect human monocyte-derived dendritic cells (moDC) and the epitope presentation was detected by co-culture with *in vitro* expanded antigen specific CD8<sup>+</sup> T cells
- The activation of antigen specific CD8<sup>+</sup> T cells was detected by increased CD137 and CD107a activation marker expression
- The engineered T cells were tested in a dose titration experiment to assess the TCR affinity

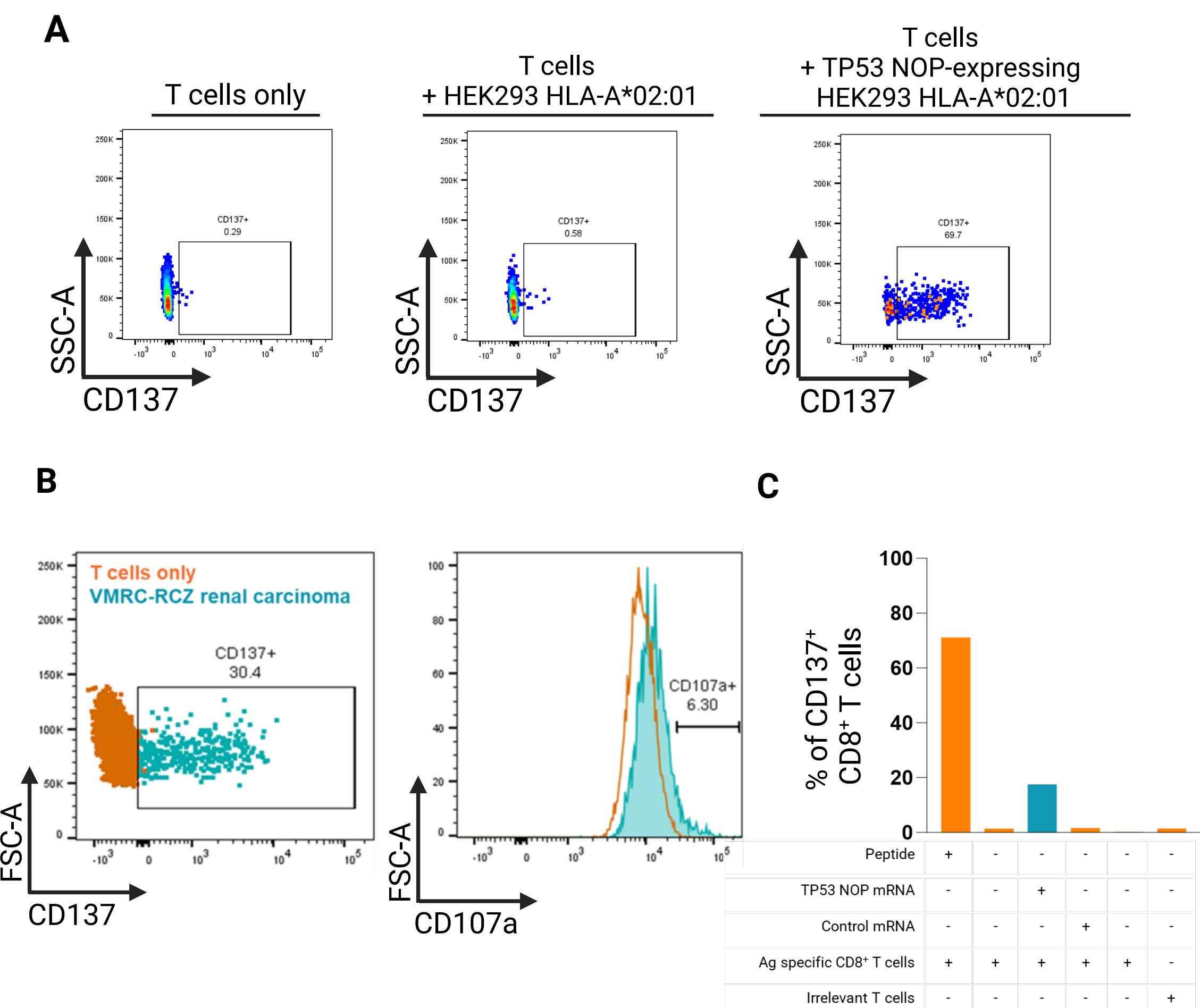
## RESULTS

Figure 1: Screening and *in vitro* expansion of antigen specific CD8<sup>+</sup> T cells against NOPs



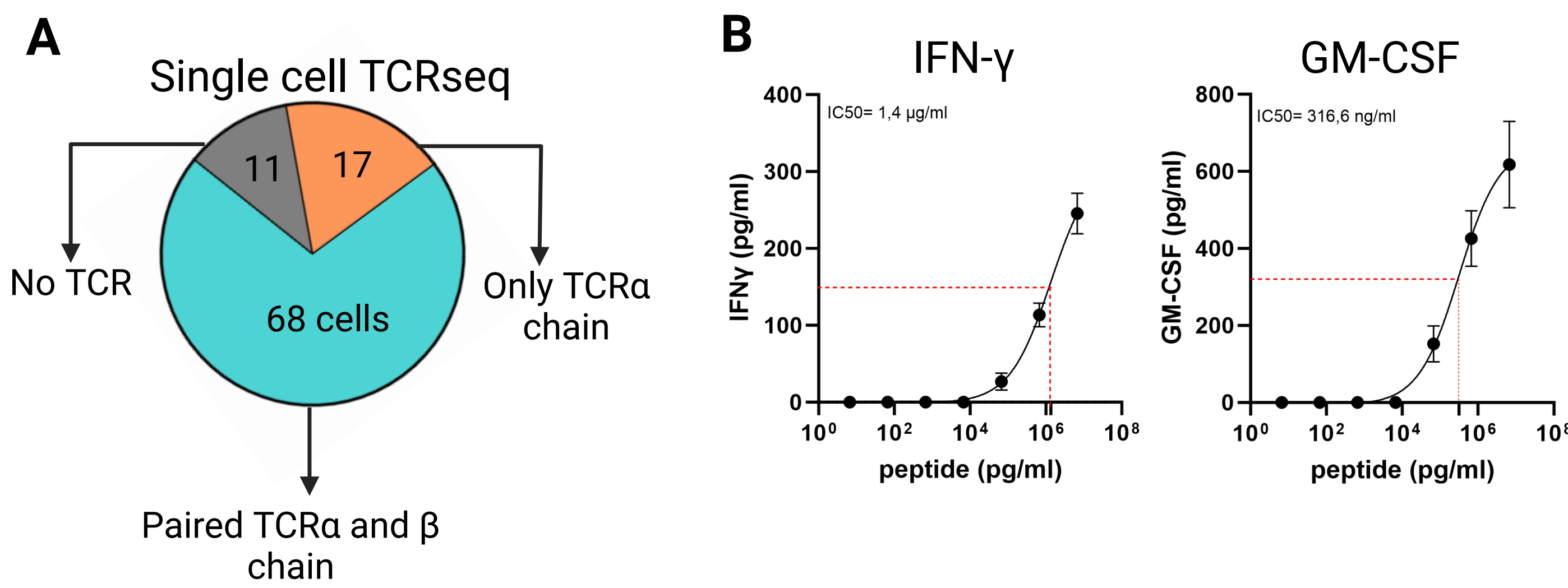
**A)** *In vitro* expanded and enriched NOP-derived peptide specific CD8<sup>+</sup> T cells were analyzed by CD8/tetramer staining (representative data). **B)** Fifteen out of 30 screened peptides induced *in vitro* antigen-specific CD8<sup>+</sup> T cell expansion. Recognition of two different NOP epitopes presented on the overexpressed HEK cell line model by respectively expanded antigen specific CD8<sup>+</sup> T cells was observed. One of the recognized epitopes was derived from TP53 NOP.

Figure 2: *In vitro* expanded antigen specific CD8<sup>+</sup> T cells recognize a TP53 NOP-derived HLA-A\*02:01-restricted epitope presented on (tumor) cell lines and on mRNA transfected antigen-presenting cells



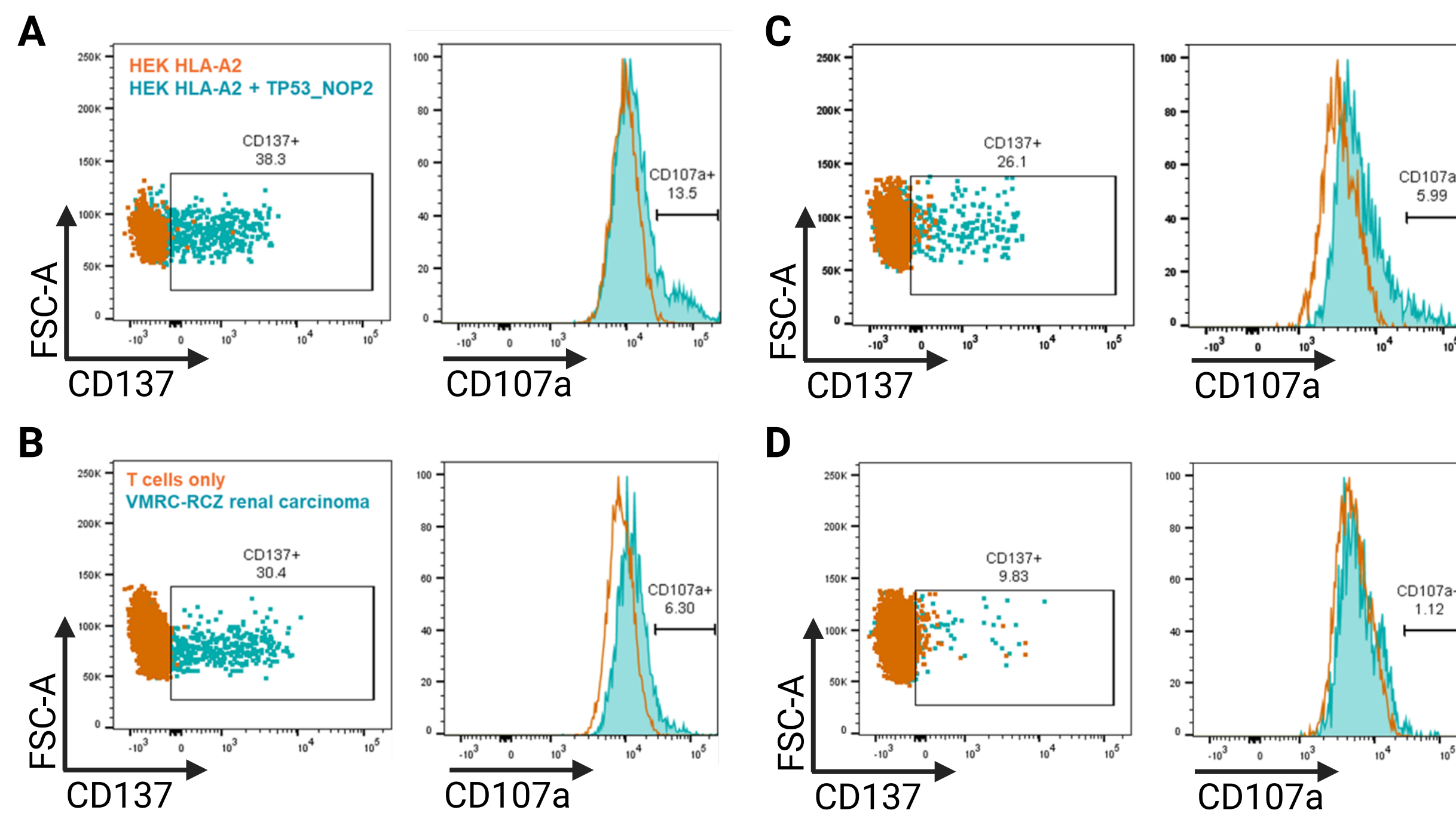
**A)** Upregulation of CD137 on *in vitro* expanded CD8<sup>+</sup> T cells upon recognition of TP53-derived, HLA-A\*02:01-restricted epitope presented on cell surface of HEK293 cell line overexpressing TP53 NOP. **B)** *In vitro* expanded TP53 NOP-specific CD8<sup>+</sup> T cells upregulate CD137 and CD107a activation markers upon co-culture with renal cell carcinoma tumor cell line VMRC-RCZ naturally expressing TP53 NOP and with **C)** monocyte-derived dendritic cells transfected with mRNA encoding TP53 NOP sequence.

Figure 3: Identification of a TCR sequence recognizing a TP53 NOP-derived epitope



**A)** Sequencing and identification of a functional population of a TCR recognizing TP53 NOP-derived epitope. **B)** The recognition pattern of TCR-transduced CD8<sup>+</sup> T cells was tested by stimulating them peptide (6.7 pg – 6.7 ug/ mL). The amount of IFN-γ and GM-CSF produced at each dose of peptide is shown.

Figure 4: TCR engineered T cells recognize a TP53 NOP-derived HLA-A\*02:01 restricted epitope presented on (tumor) cell lines



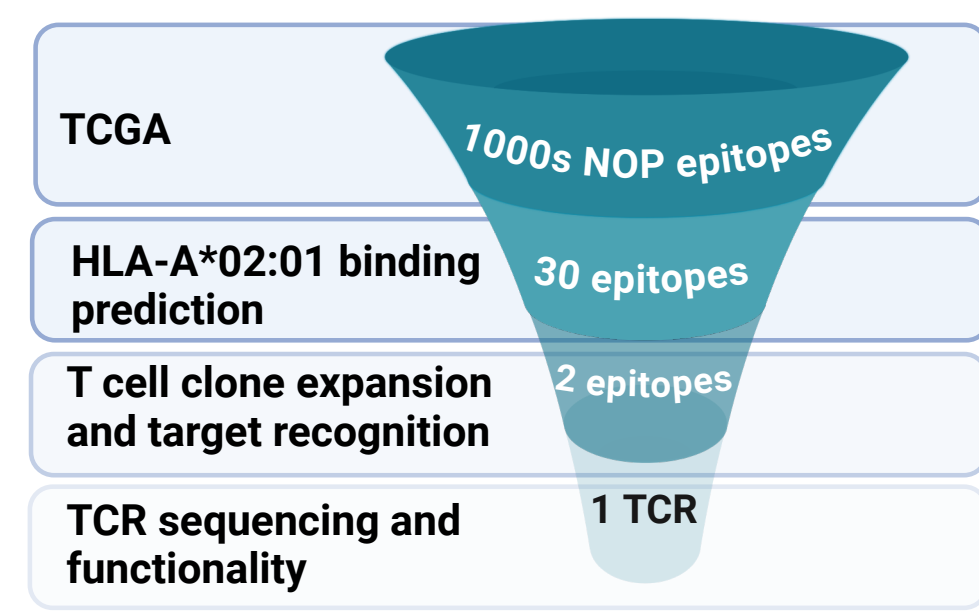
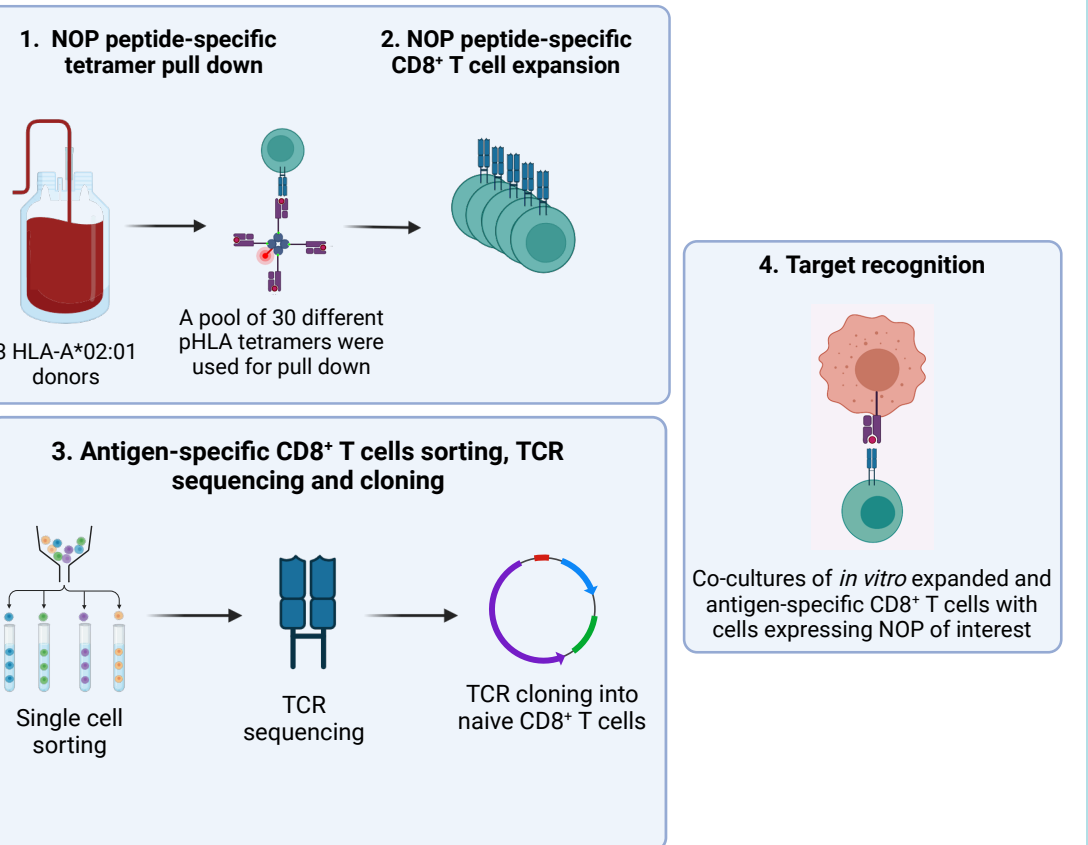
The recognition of presented TP53 NOP epitope by HEK cells overexpressed with full length TP53 NOP or VMRC-RCZ renal carcinoma cell line naturally expressing the same NOP by TCR engineered CD8<sup>+</sup> T cells (**A**, **B**). The extent of antigen recognition and TCR functionality was measured as upregulation of CD137 and CD107a on the engineered CD8 T cells.

## CONCLUSIONS

- We identified and validated a TCR that recognizes a HLA-A\*02:01- restricted TP53 NOP-derived epitope.
- TP53 NOP is a target expressed in 1-3% of patients across multiple cancer types and thus this study shows the feasibility of identifying shared targets for TCR- mediated therapies.

## KEY TAKEAWAYS

- Problem:** Relative lack of tumor specific and shared neoantigen targets
- Aim:** Identify and validate tumor specific and shared targets for T cell mediated therapy



- Key finding:** Identification and validation of a TCR targeting a shared TP53 NOP.