

The Development of ‘Off-the-shelf’ Manufacturing Strategies for iPSC-based Gamma-Delta T Cells



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Introduction

$\gamma\delta$ T cells are ideal for development of allogeneic cell therapies for cancer, principally because they broadly recognize and kill tumor cells without initiating a graft-versus-host disease (GvHD) response. $\gamma\delta$ T cells can also differentiate between healthy and tumor tissues, making them particularly attractive for adoptive cellular therapies against solid tumor cancers.

Early clinical trials have revealed that adoptively transferred $\gamma\delta$ T cells are well tolerated and, so far, appear to be safe. However, expansion of $\gamma\delta$ T cells can be challenging. A minor lymphocyte population in blood, initial $\gamma\delta$ T cell counts are low, their frequency and expansion capacity vary among donors, and in some cases - *ex vivo* activation and expansion can reduce their durability and lead to exhaustion.

These limitations can be overcome using induced pluripotent stem cells (iPSCs). iPSCs possess properties of unlimited self-renewal and multi-lineage differentiation potential. Additionally, genetic modification of iPSC-derived $\gamma\delta$ T cells (i- $\gamma\delta$ T cells) as well as selection and propagation of specific effector clones represent a source of near limitless and rejuvenated immune cells.

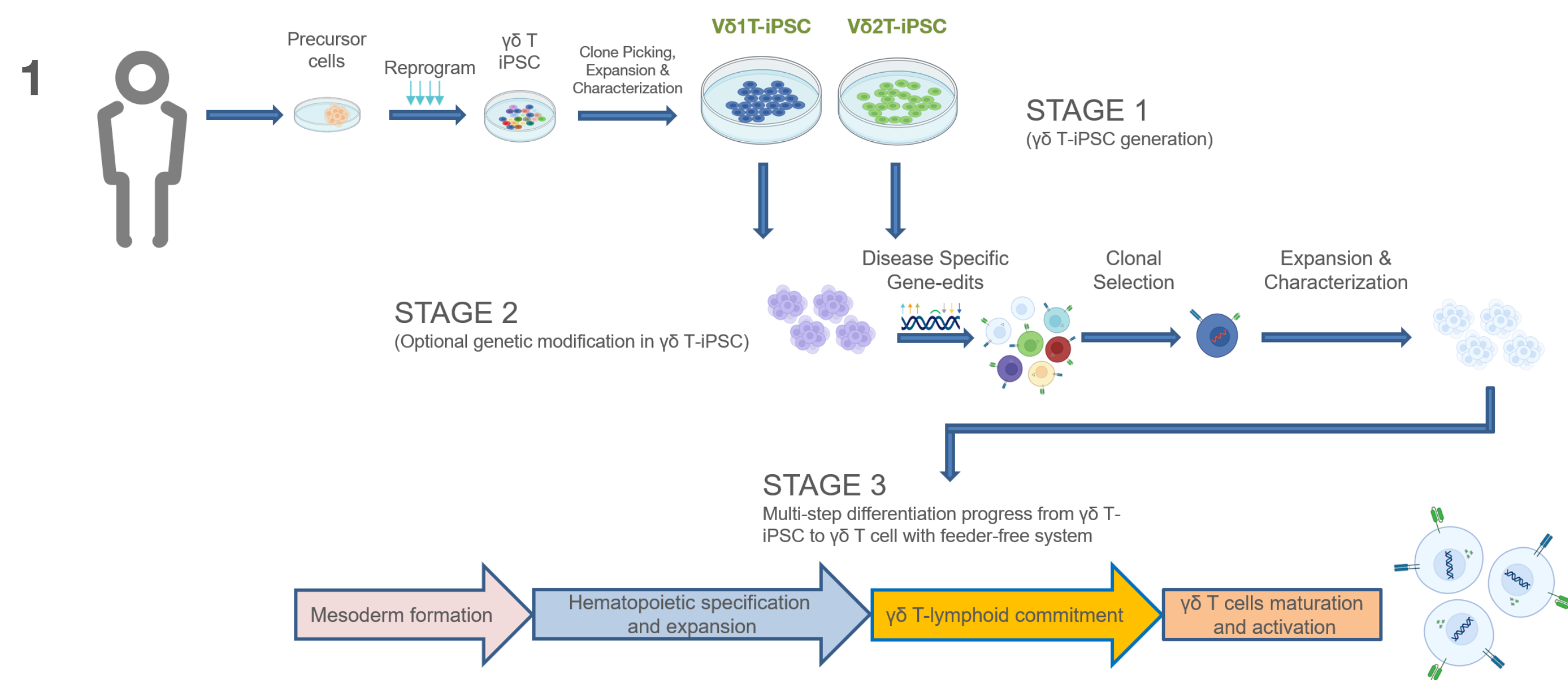


Diagram of Manufacturing Strategies for iPSC-derived Vδ1+ and Vδ2+ $\gamma\delta$ T cells (Fig.1). Precursor cells from healthy donors were obtained and reprogrammed into $\gamma\delta$ T-iPSCs. After genotyping and characterization, we obtained both Vδ1T- and Vδ2T-iPSC clones, which were expanded and underwent a feeder-free multi-step differentiation process to generate functional i- $\gamma\delta$ T cells. Additional cell and disease specific gene-edits to the Vδ1T- and Vδ2T-iPSC clones are underway.

Materials and Methods

Obtaining parent cells: Using IN8bio's protocol, precursor cells were obtained from healthy donors.

iPSC reprogramming: $\gamma\delta$ T cells were reprogrammed into iPSCs using non-integrating vectors encoding Oct3/4, Sox2, Klf4, and c-Myc.

Genotyping & Karyotyping: Multiplex genomic PCR assays and Sanger sequencing were performed to examine rearrangements at TCRG and TCRD gene loci. iPSC lines were evaluated with G-band karyotype analysis to detect chromosome abnormalities.

Multi-step differentiation: Proprietary feeder-free multi-step differentiation strategies were established and applied to generate multiple effector cell populations. Cells were thereby differentiated from iPSC via hematopoietic progenitor cells (HPCs) to progenitor T cells and further to $\gamma\delta$ T cell subsets.

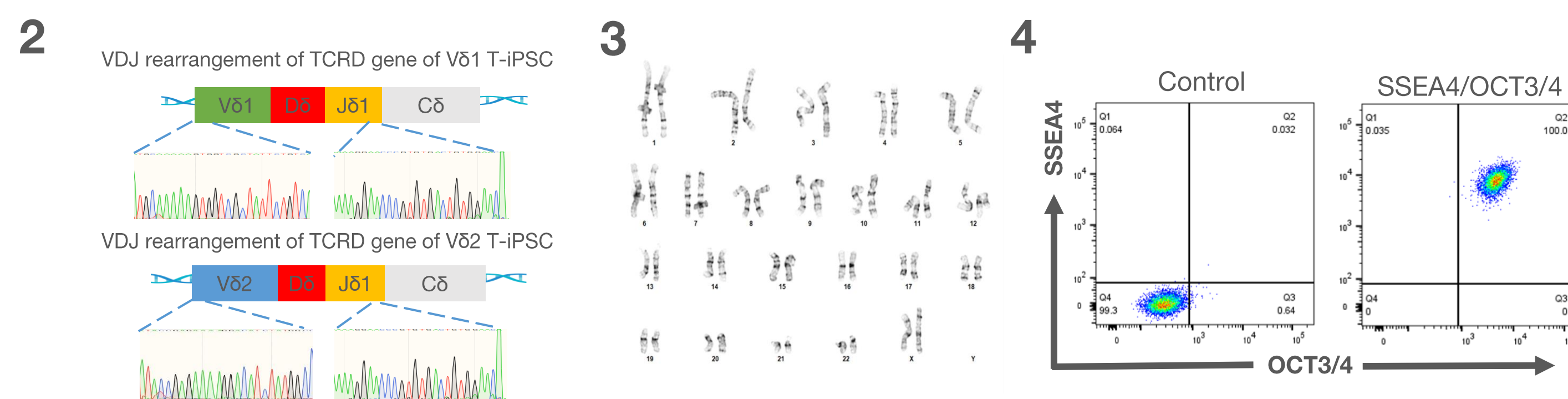
Cell marker detection: Flow cytometry was used to identify cell surface markers for iPSCs (Tra-1-60, OCT3/4, SSEA4), HPCs (CD34, CD43), progenitor T cells (CD5, CD7), and i- $\gamma\delta$ T cells (CD3, $\gamma\delta$ TCR, Vδ1 TCR).

Cytotoxicity: GBM cell (U87-GFP) Were co-cultured with i- $\gamma\delta$ T cells at the indicated Effector: Target (E:T) ratios for 5 hours in vitro, stained with 7-AAD and acquired by flow cytometry. GFP+/7AAD+ cell percentages indicate cytotoxicity.

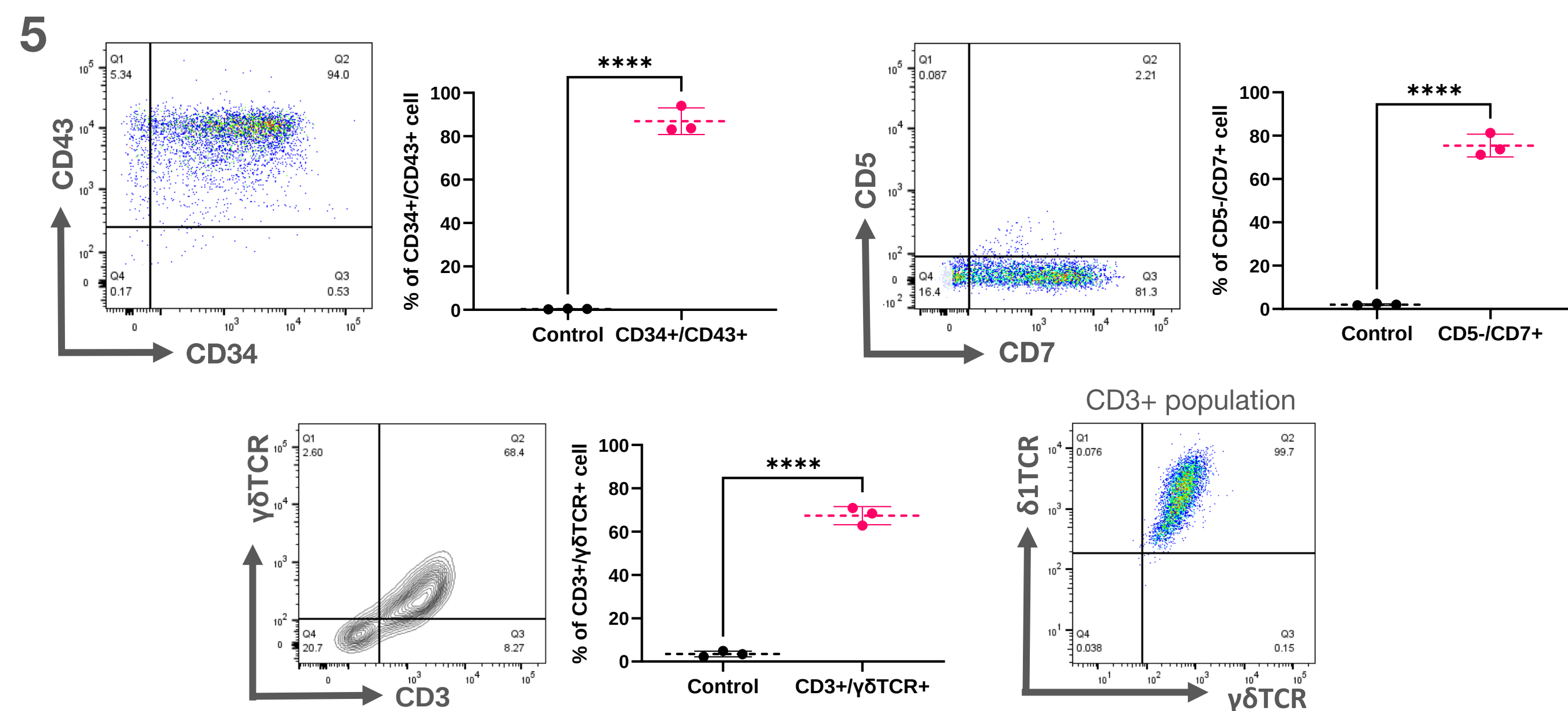
Statistical Analysis: Statistical significance of changes observed between groups (3 biological replicates per group) was determined by Student's t-test utilizing GraphPad Prism v9.

Results

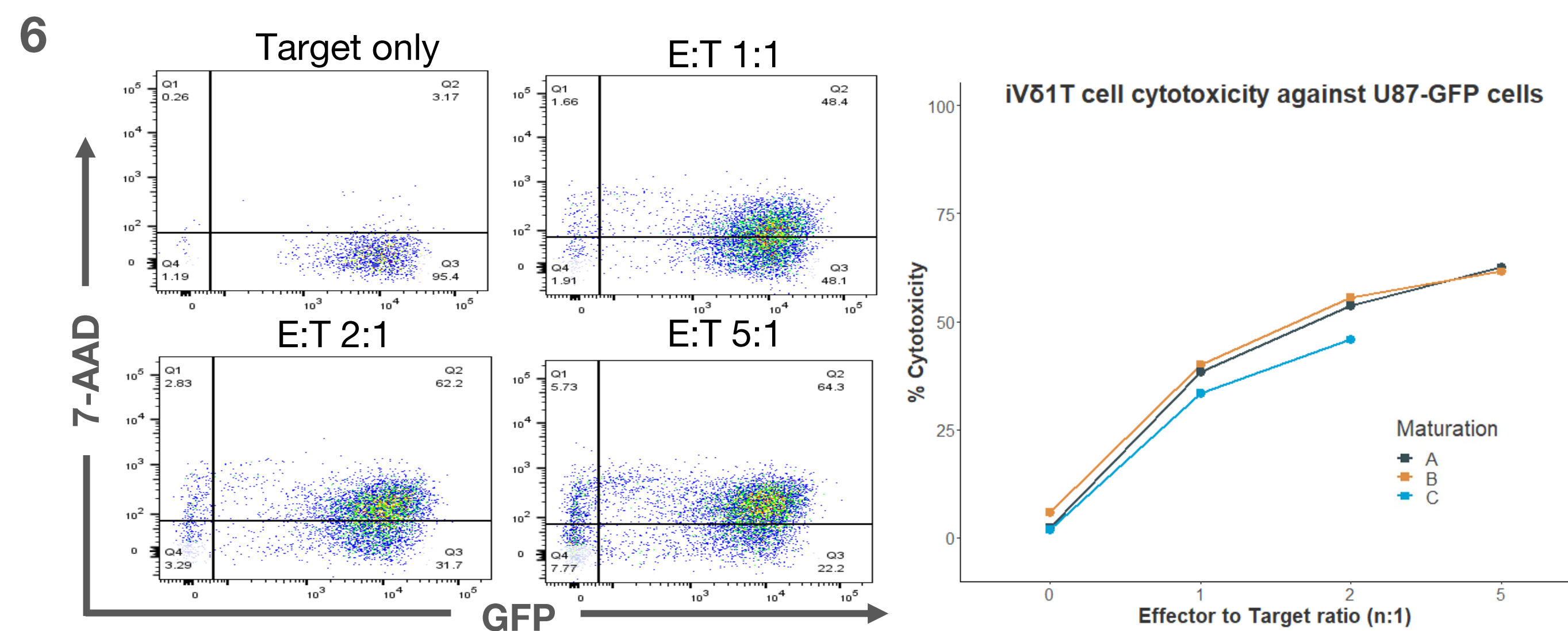
The selected Vδ1 T-iPSC clone was identified as V γ 4-to-J γ 1/2 and Vδ1-to-Jδ1 recombination, and the selected Vδ2 T-iPSC clones were V γ 2-to-J γ 1/2 and Vδ2-to-Jδ1. All selected $\gamma\delta$ T-iPSC clones have normal karyotypes (Fig 3). The $\gamma\delta$ T-iPSC clones also highly express the pluripotency markers SSEA4 and OCT3/4 (Fig. 4).



Vδ1 T-iPSCs were expanded and differentiated with a feeder free culture system. At different stages, high yields of CD34+/CD43+ HPCs (86.9%), CD5-/CD7+ progenitor T cells (75.4%) and CD3+/ $\gamma\delta$ TCR+ i- $\gamma\delta$ T cells were obtained. All the iPSC differentiated Vδ1 T cells have Vδ1 TCRs (Fig. 5). **** P<0.0001.

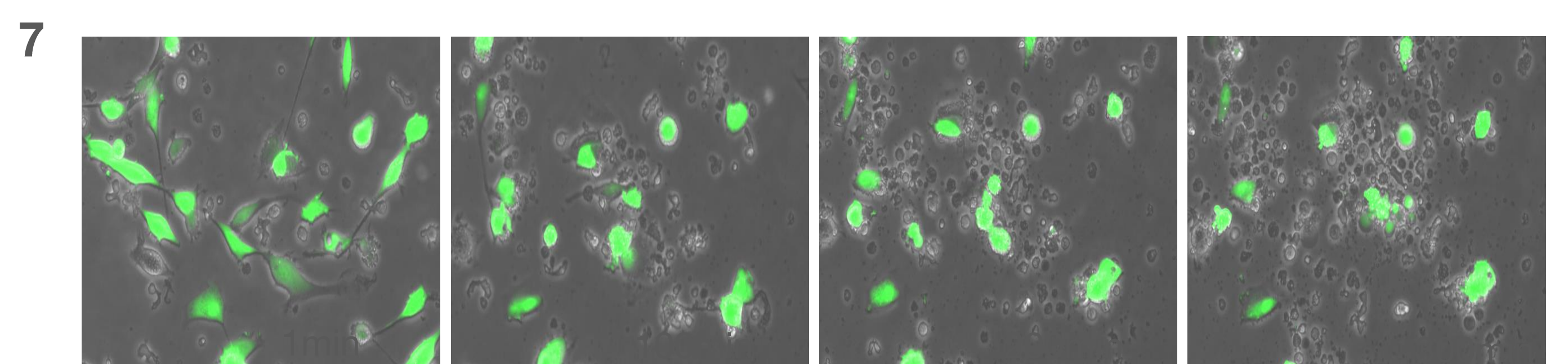


i-Vδ1 T Cells are highly cytotoxic against U87-GFP cells (glioblastoma cell line) in a dose-dependent manner. GFP+/7-AAD+ cell percentages indicate cytotoxicity. (Fig. 7).

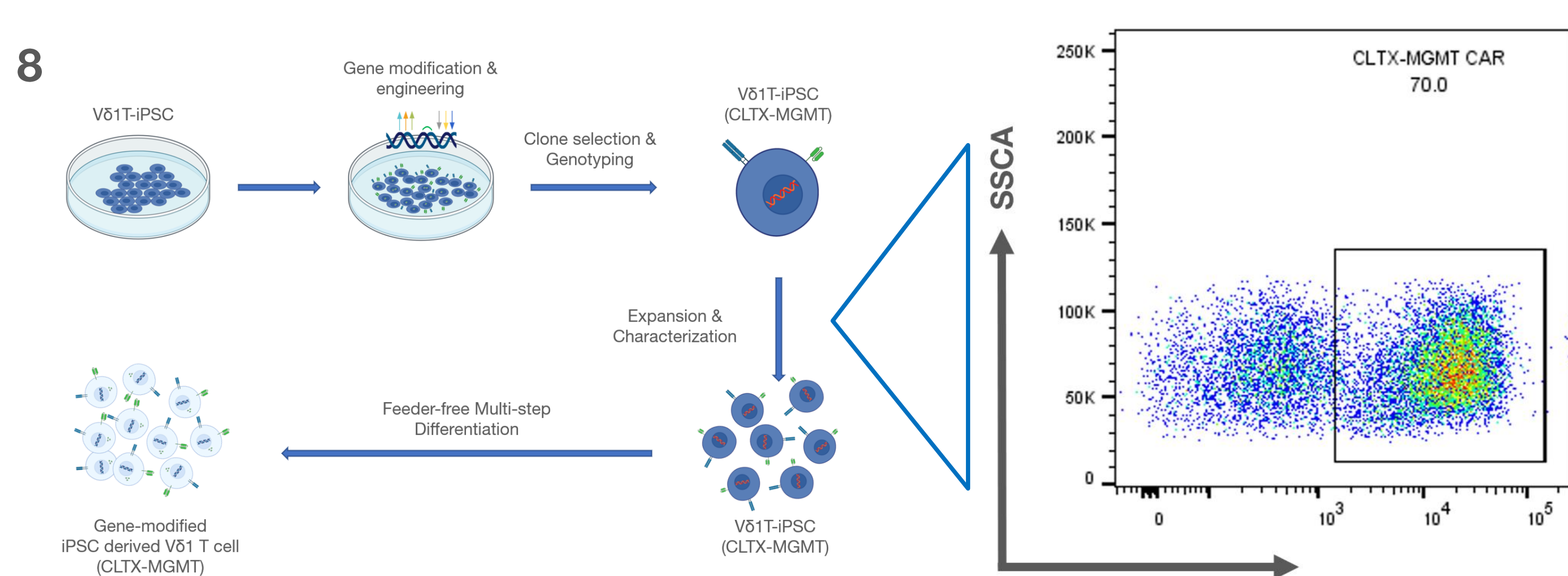


Results

U87-GFP cells were co-cultured with i-Vδ1 T cells at E:T= 5:1. Images were taken every minute (min) for a total of 300 min. Panel images taken at different time points show U87-GFP cells were killed by i-Vδ1 T cells during coculture (Fig 7).



Vδ1 T-iPSCs were transduced with 2xCLTX-noZ-MGMT CAR lentiviral vector with high efficiency (70%). (Fig. 8) These genome modified Vδ1 T-iPSC will be cloned and characterized before going through the multi-step differentiation process to further enhance the targeting and cytotoxicity of i-Vδ1 T cells against GBM and other solid tumors.



Conclusions

- From healthy donor precursor cells, IN8bio has successfully generated Vδ1-T and Vδ2-T iPSC lines with normal karyotypes that highly express pluripotency markers.
- IN8bio has established a proprietary feeder-free multi-step differentiation process, through which $\gamma\delta$ T-iPSCs can be differentiated into hematopoietic progenitor cells (HPCs), progenitor T cells and cytotoxic $\gamma\delta$ T lymphocytes in a stepwise manner.
- The iPSC-derived Vδ1 T lymphocytes show high levels of dose-dependent cytotoxicity against U-87 glioblastoma cell-lines.
- Genome modifications including the addition of chemotherapy resistance, CARs and other edits on i- $\gamma\delta$ T cells may be necessary to successfully target solid tumor cancers. iPSC derived $\gamma\delta$ T cells offer a promising future for scaling manufacturing, enhancing targeting and killing efficacy, T cell fitness and overcoming the immunosuppressive tumor microenvironment (TME) for cellular immunotherapies against solid tumor cancers.
- References
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 - 3. Iriguchi, S. *et al. Nat Commun* 12, 430 (2021).