

Jacob E. Bridge, Patricia N. Claudio-Vazquez, Emily J. Pomeroy, Sophia J. Wenthe, Branden S. Moriarity, and Beau R. Webber

Background

Adoptive T cell therapies, in which patient T cells are isolated, engineered, expanded, and re-infused, have shown great success in the treatment of cancer. Because of their diverse functionality and MHC-independent cytolytic activity, $\gamma\delta$ T cells, which recognize non-peptide antigens via canonical $\gamma\delta$ T cell receptors (TCRs), have garnered increasing interest as a potential allogeneic immunotherapy. However, their relative infrequency within peripheral blood, numbering around 1-5% of circulating lymphocytes, has limited their clinical application. Current methods of *in vitro* $\gamma\delta$ T cell expansion suffer from numerous drawbacks, including the requirement for feeder cells and failure to expand populations bearing polyclonal $\gamma\delta$ TCR repertoires. Here we describe a novel antibody-based procedure for the large-scale production of non-virally engineered $\gamma\delta$ T cells, which possess increased TCR heterogeneity and potent anti-cancer activity.

Antibody-Based Expansion

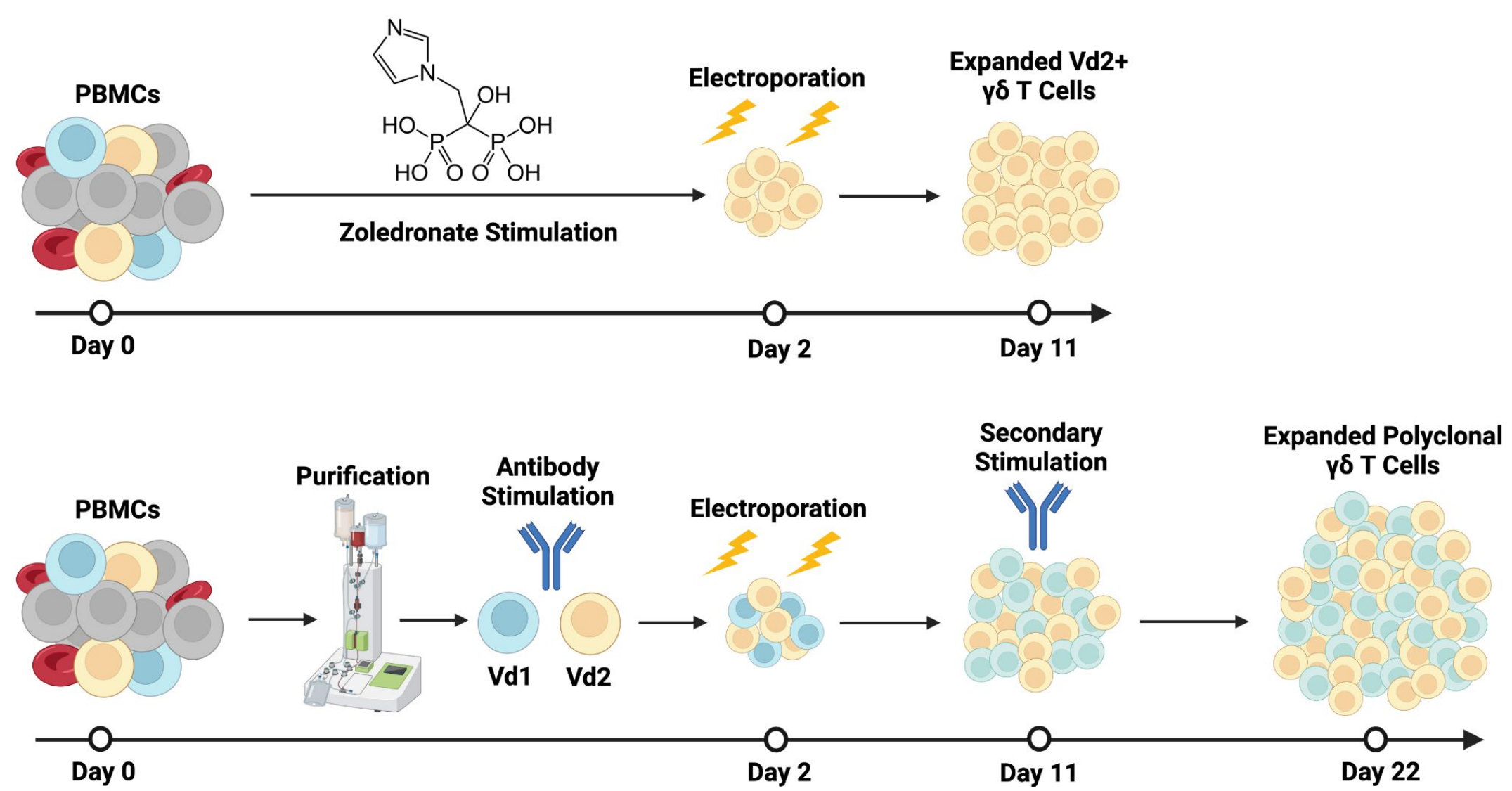


Figure 1. Methods of generating engineered $\gamma\delta$ T cells. (Top) PBMCs are stimulated with zoledronate in the presence of IL2, IL7, and IL15. (Bottom) $\gamma\delta$ T cells are purified from PBMCs, then stimulated with plate-bound anti-pan-GDTCR antibody and soluble CD28 in the presence of IL2, IL7, and IL15. Cells are electroporated on day 2, expanded for nine days, and re-stimulated on day 11.

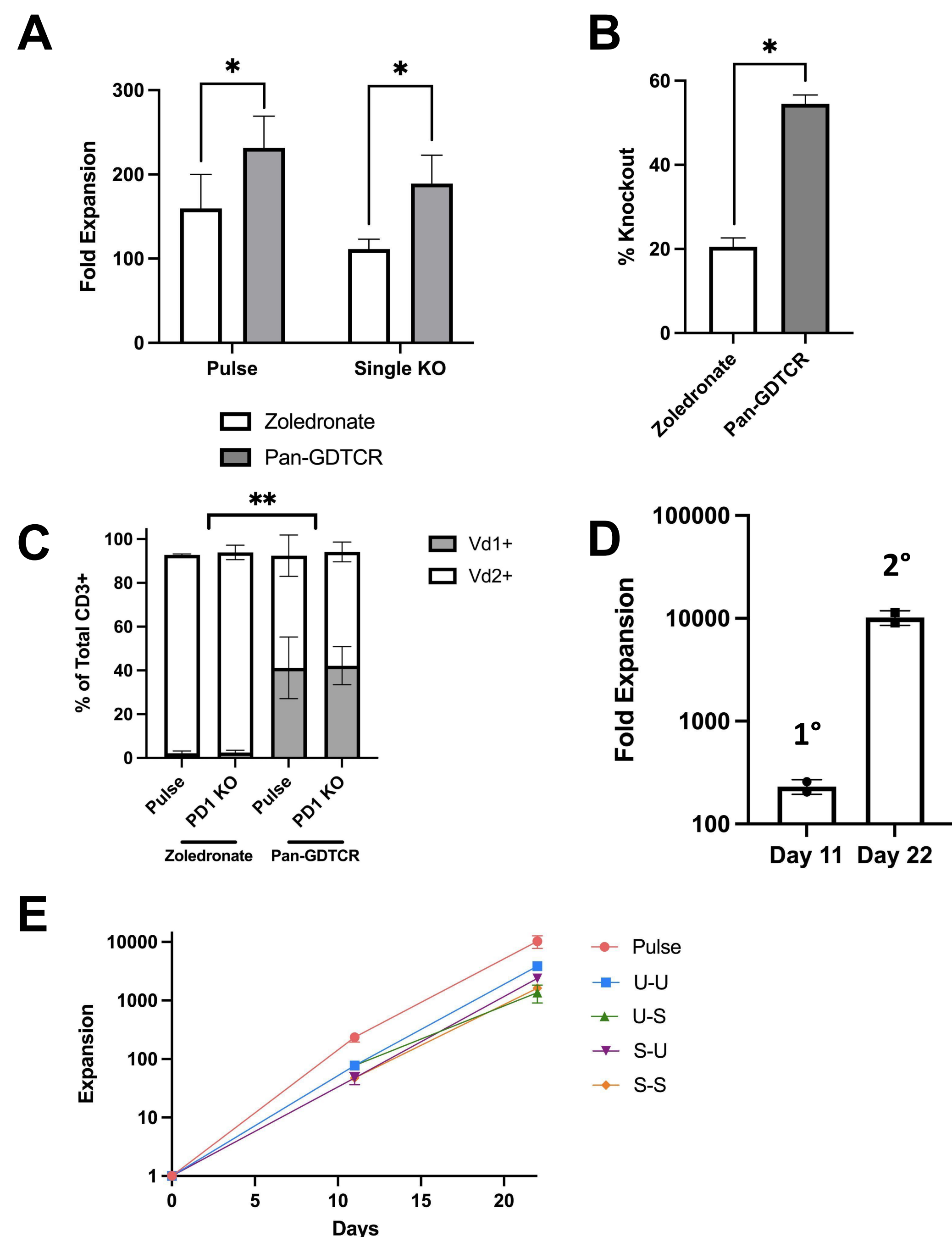
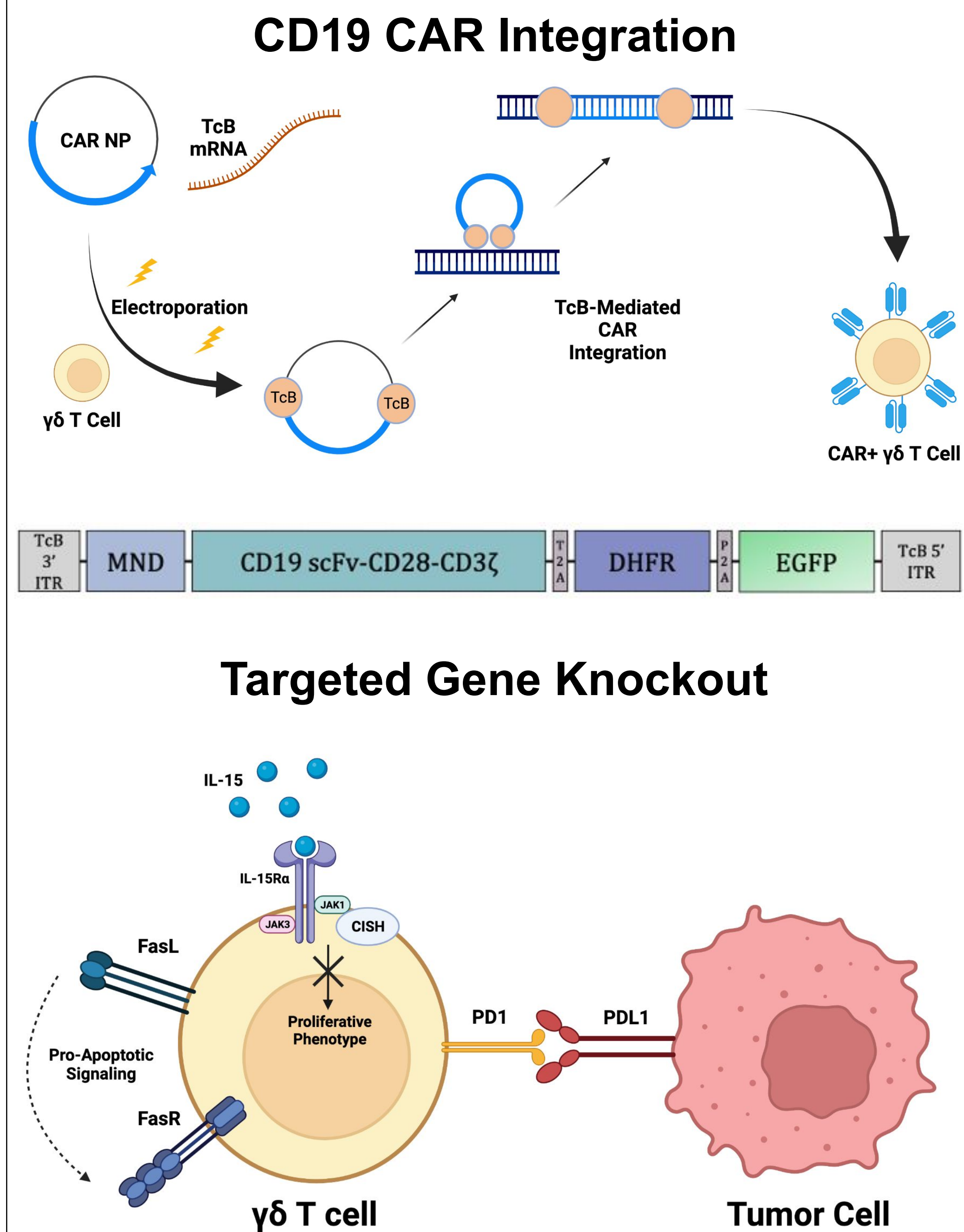


Figure 2. Comparing zoledronate and Ab-based $\gamma\delta$ T cell stimulation. Peripheral blood $\gamma\delta$ T cells were either cultured with Zo or isolated and stimulated with pan-GDTCR Ab + soluble CD28. Cells were then electroporated with Cas9 mRNA, PD1 sgRNA, or CARs on day 2.

Engineering Strategy



In Vitro Cytotoxicity

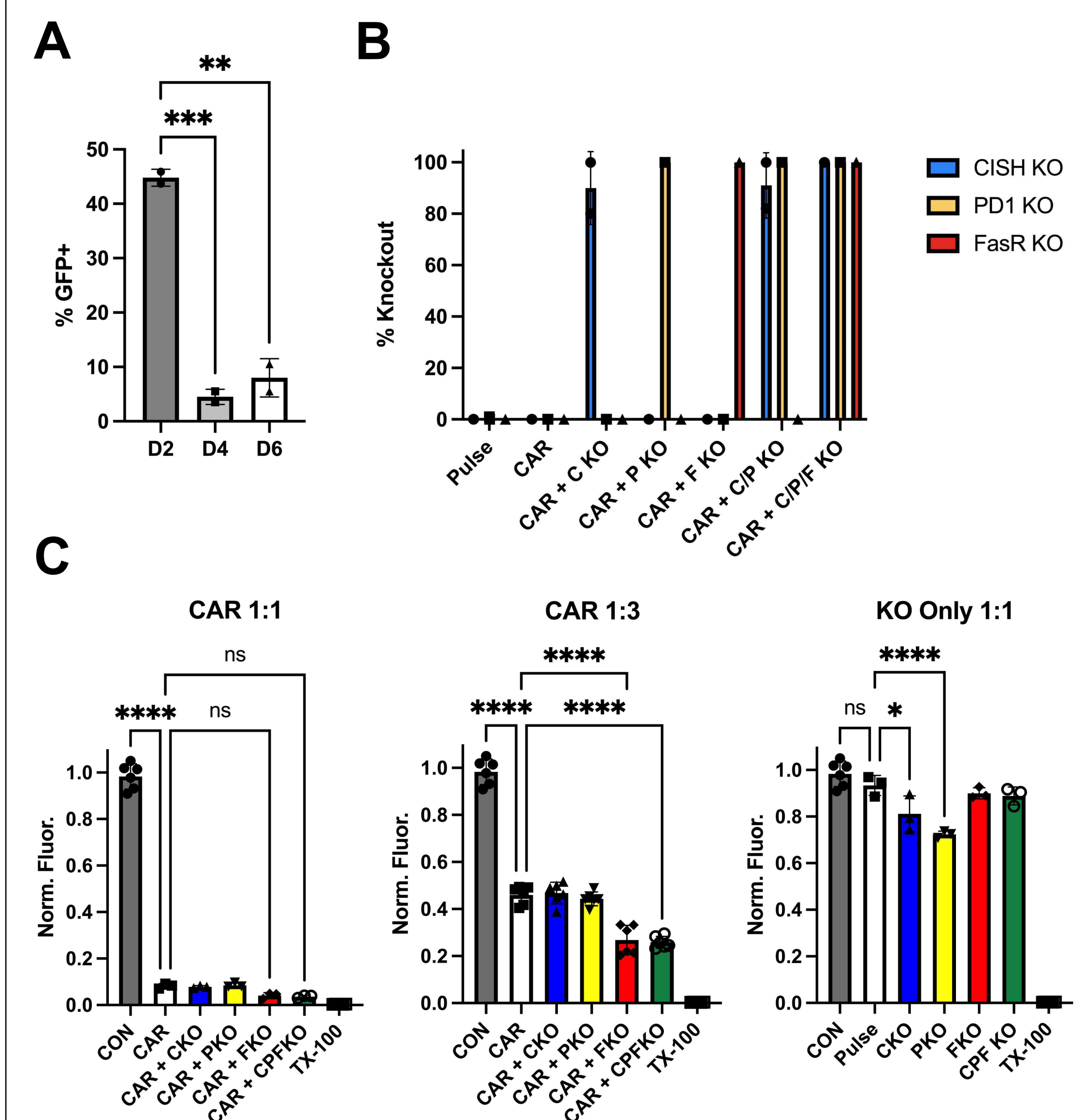


Figure 3. Optimization, gene knockout, and functional activity of CAR $\gamma\delta$ T cells. (A) CAR expression of $\gamma\delta$ T cells at day 22. Cells were electroporated with CD19 CAR NP and TcB mRNA on day 2, 4, or 6. (B) Base editor knockout at day 22. (C) *In vitro* co-culture of day 22 $\gamma\delta$ T cells with Raji-Luc cancer cells. Cytolytic activity was quantified by reduction of luminosity at 48 hours. Effector:Target ratios are displayed for each graph.

In Vivo Anti-Tumor Activity

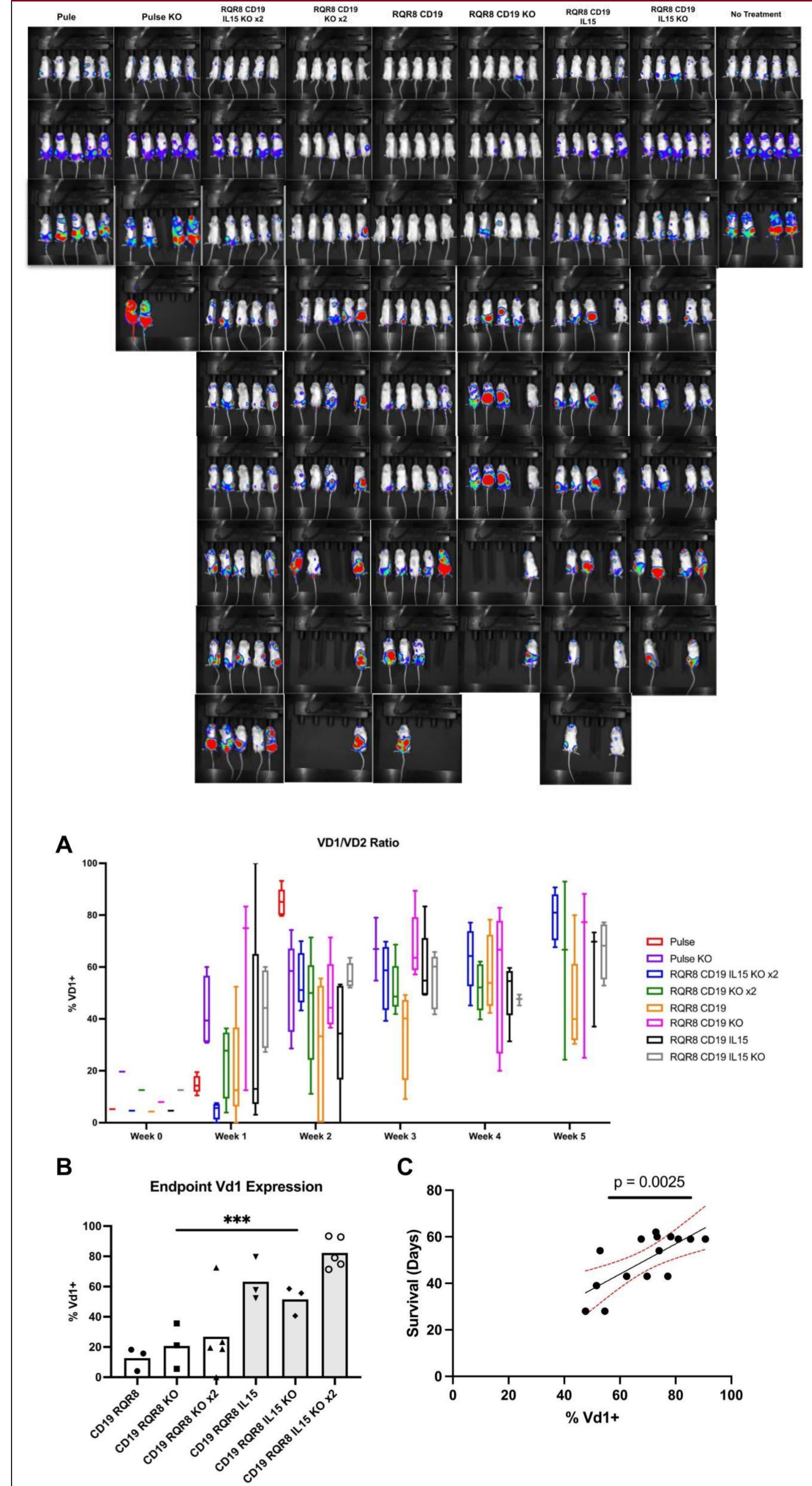


Figure 4. *In vivo* imaging of mice challenged with Burkitt's Lymphoma and treated with CAR $\gamma\delta$ T cells. (Top) Live imaging. (A-C) Graphical representations of overall survival and Vd1 expression.

Conclusions / Future Directions

Conclusions:

- Serial antibody-based stimulation yields greater engineering efficiency, outgrowth, and TCR heterogeneity than Zo-based expansion
- CAR integration and multiplex knockout of regulatory genes enhances $\gamma\delta$ T cell cytotoxicity
- CAR+ $\gamma\delta$ T cells display potent anti-tumor activity *in vivo*

Future Directions:

- Test additional targets for gene knockout, including PDL1 and IL-17
- Determine the relative functional activity of the Vd1 and Vd2 subsets
- Therapeutic potential of cord blood and iPSC $\gamma\delta$ T cells

This work was supported by:

Children's Cancer Research Fund
NIH T32 AI007313
UMN Center for Genome Engineering
NIH R21 AI163731-01