

High Efficiency T Cell Editing

Utilizing Synthego's CRISPRRevolution™ Synthetic sgRNA

Edit Valuable Primary T Cells with Confidence

T cells are a key component of the adaptive immune system. Both Chimeric Antigen Receptor and T Cell Receptor immunotherapies have been clinically approved for the treatment of various blood cancers such as lymphomas and multiple myeloma, providing exciting avenues for the future of cancer therapeutics. These cells are primarily from donated human tissue and are therefore an incredibly valuable and scarce resource. Efficient editing and sustained viability of these cells following editing remain critical challenges for researchers to advance new immunotherapies into the clinic. Ensure the success of your T cell therapeutic programs with Synthego's modified synthetic sgRNA.

Efficient Multigene Knockout in Primary Human Resting CD4+ T Cells

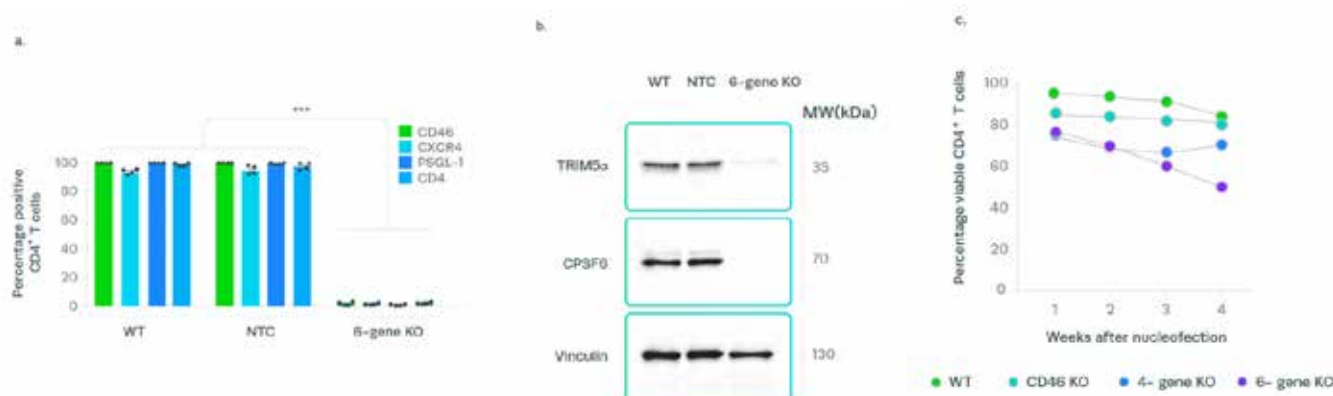


Fig. 1. **Resting CD4+ T Cells Retain High Viability Following Multigene Gene Knockouts.** Simultaneous six-gene knockout following a single RNP nucleofection with Synthego's modified synthetic sgRNAs resulted in the depletion of 4 cell surface markers via flow cytometry (a) and depletion of cytoplasmic proteins via immunoblotting (b). Viability of multigene (4- and 6-gene) knockout demonstrated high cell survival 4 weeks following nucleofection as compared to wild-type (WT) controls (c). (Figure adapted from Albanese et al, Nat Methods 19, 81–89 (2022)).



"We started experimenting with guides from other companies. However, Synthego's modified sgRNAs always worked the best for us."

Manuel Albanese, Ph.D.

Postdoctoral Researcher, Max von Pettenkofer Institute and Gene Center, Virology, National Reference Center for Retroviruses, LMU München



High Editing Efficiency and Viability with Synthego's Modified sgRNA in Activated CD4+/CD8+ T Cells

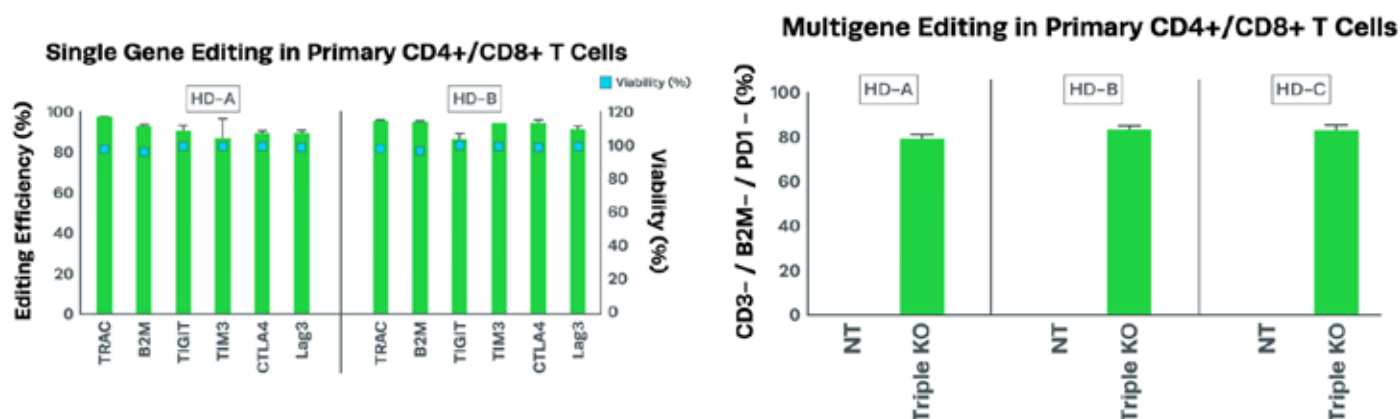


Fig 2. **High editing efficiency and viability observed in activated human primary T cells.** Robust gene knockout is demonstrated in human primary T cell knockout using Synthego's modified synthetic sgRNA following CRISPR/Cas9 RNP nucleofection. High editing efficiency (>70%) of various loci were observed in activated human primary CD4+/CD8+ T cells, across two independent donors (HD-A, HD-B) as assessed by ICE analysis (a). Additionally, high cell viability (>95%) were consistently achieved, irrespective of editing locus as assessed by flow cytometry (a). High multigene editing efficiency on human primary T cells was achieved using Synthego's synthetic sgRNA. Three sgRNA targeting TRAC, B2M, and PD1 were used to perform multigene editing via RNP nucleofection. Post nucleofection, greater than 75% triple knockout efficiency for CD3, B2M, and PD1 was observed across three independent T cell donors as assessed by flow cytometry (b).

Synthego sgRNAs Outperform Other Vendors in Editing CD4+ T Cells

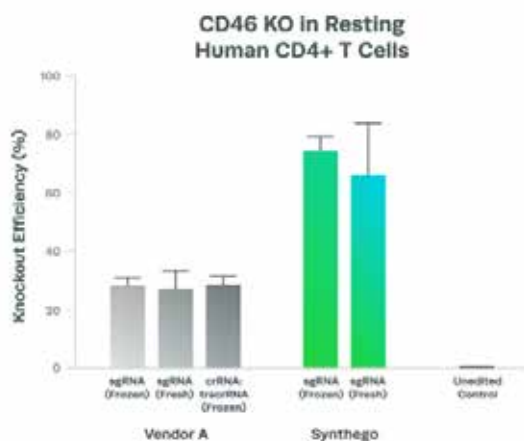


Fig 3. **Synthego's modified synthetic sgRNAs demonstrate superior editing efficiency compared to other vendors at the CD46 in resting human CD4+ T cells.** Synthego synthetic sgRNAs were compared against another vendor's sgRNA and 2-part (crRNA:trRNA), demonstrating consistently high (60%+) knockout efficiency regardless of preparation condition. (Unpublished data, Albanese et al.)

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