Aptamer Functionalized Lipid Nanoparticles (Apt-LNPs) Enable Specific *ex vivo* Gene Editing of Primary Human T cells

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Abstract

Chimeric antigen receptor (CAR) T cells have transformed hematologic cancer treatment, yet manufacturing is often hindered by the high costs of viral vectors and the cellular stress caused by electroporation (EP). Lipid nanoparticles (LNPs) offer a promising non-viral alternative, utilizing natural endocytic pathways to deliver genetic cargo while minimizing toxicity. While traditional targeted LNPs rely on passive serum proteins or bulky, immunogenic antibodies, this project introduces a modular platform using synthetic aptamers for cell-specific delivery. Because aptamers are small, chemically stable, and non-immunogenic compared to antibodies, they allow for more robust, precise targeting of markers like CD8, CD62L, and CD71—creating a highly controlled, "plug-and-play" platform for manufacturing.

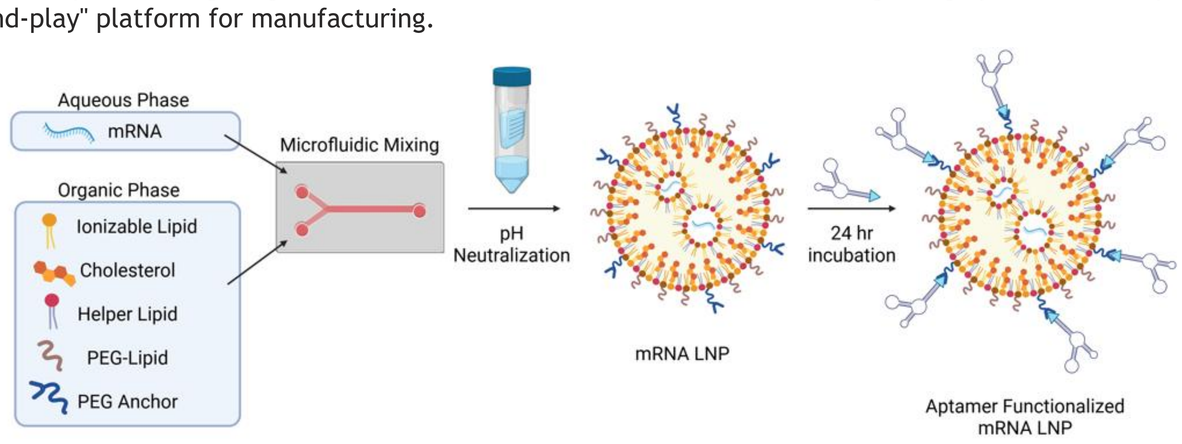

Fig 1. | Formulation and functionalization of aptamer LNPs. Microfluidic mixing of aqueous payload and organic lipid phases. Post formulation, aptamers are conjugated and purified to generate functionalized LNPs.

Methods

- **Aptamer Selection:** Discovered via iterative Cell-SELEX, NGS, and sequence analysis (Fig 2).[1-3]
- **LNP formulation:** Ignite Microfluidic Device; Aptamers were conjugated post-formulation (Fig 1).
- **LNP Characterization:** Size, polydispersity, and zeta potential were analyzed via Zetasizer. RNA concentration and encapsulation efficiency were determined by Quant-iT RNA BR kit (Fig 3).
- **Ex Vivo Transfection:** Primary T cells and unfractionated PBMCs were isolated from healthy donors. Cells were activated with anti-CD3/anti-CD28 beads and cultured in supplemented media. Flow cytometry was utilized to quantify protein expression and cellular phenotyping (Fig 4-5).
- **Data Analysis:** Data processing and visualization were performed using GraphPad Prism.

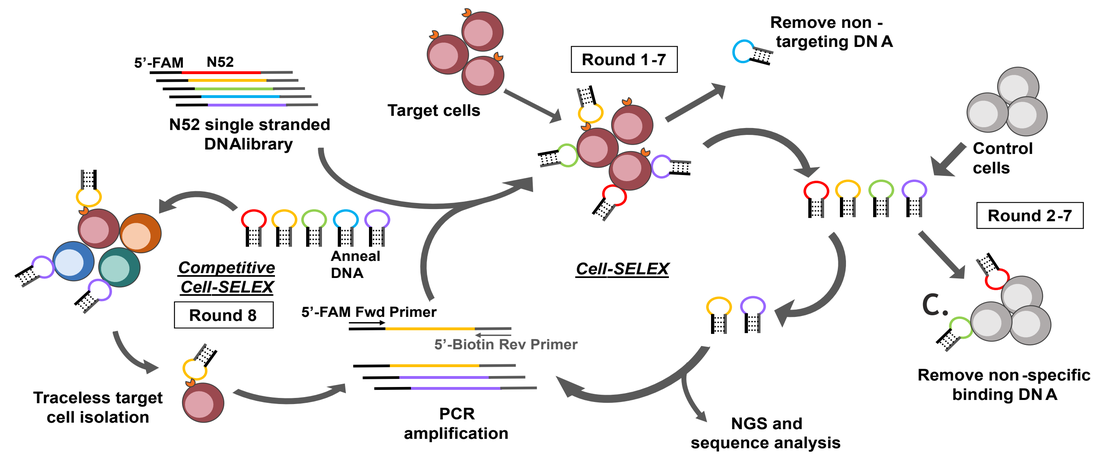

Fig 2. | Schematic of Cell-SELEX. ssDNA library undergoes iterative rounds of target cell binding, counter-selection, PCR amplification, NGS, and competitive selection to isolate target-specific aptamers.

Results

Characterization of T cell-Targeted Aptamers and Formulated LNPs

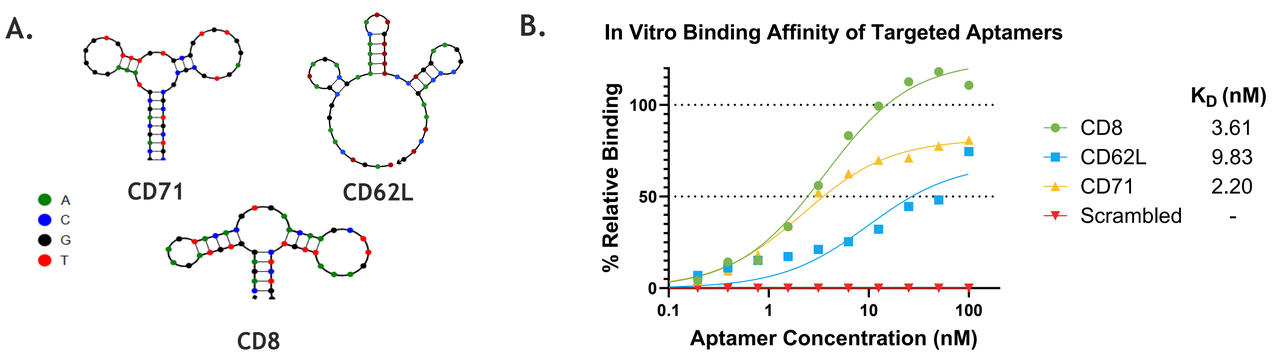

Figure 3. | A.) Predicted secondary structures of aptamer targets: CD71 (activated/proliferating), CD62L (naïve/central memory), and CD8 (cytotoxic). **B.)** Equilibrium binding curves and dissociation constants (K_D) for aptamers and scrambled control in Jurkats and T cells; normalized to 100 nM. **C.)** Size distribution by intensity measured by Dynamic Light Scattering (DLS). **D.)** Zeta potential of LNP formulations. Mean ± SD (n > 3 independent batches).

Targeting Specificity and Cellular Selectively

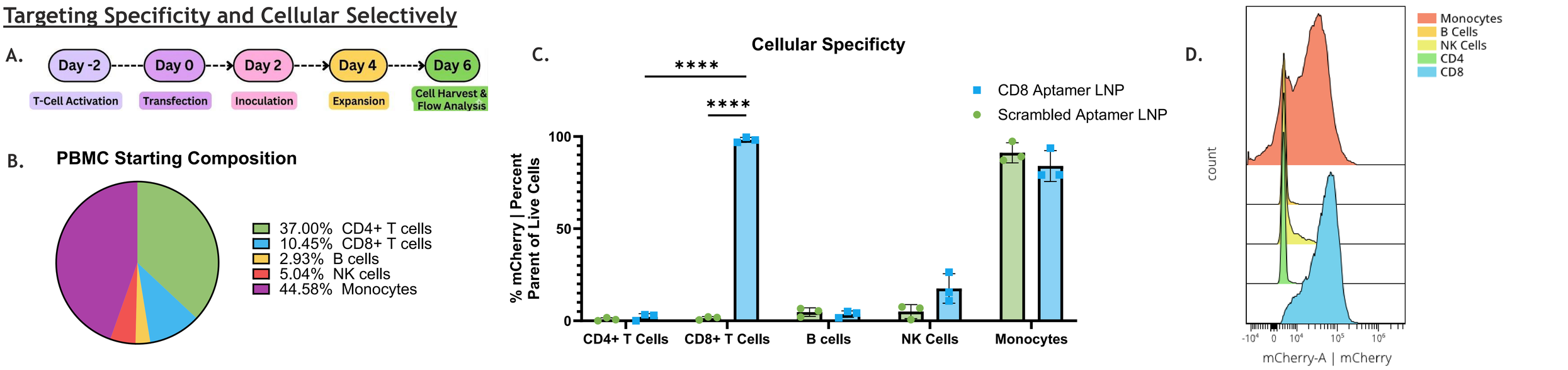

Figure 4. | A.) Experimental timeline of T cell engineering. B.) Baseline cellular composition of human PBMCs. **C.)** Cellular specificity of mCherry expression across major immune cell subsets following treatment with CD8-targeted vs. scrambled LNPs; Two-way ANOVA with Šídák's multiple comparisons (****p < 0.0001**). D.)** Representative flow cytometry histograms demonstrating selective mCherry expression in CD8+ T cells. Mean ± SD (n=3 biological replicates).

Functional Knockout and Phenotypic Fitness

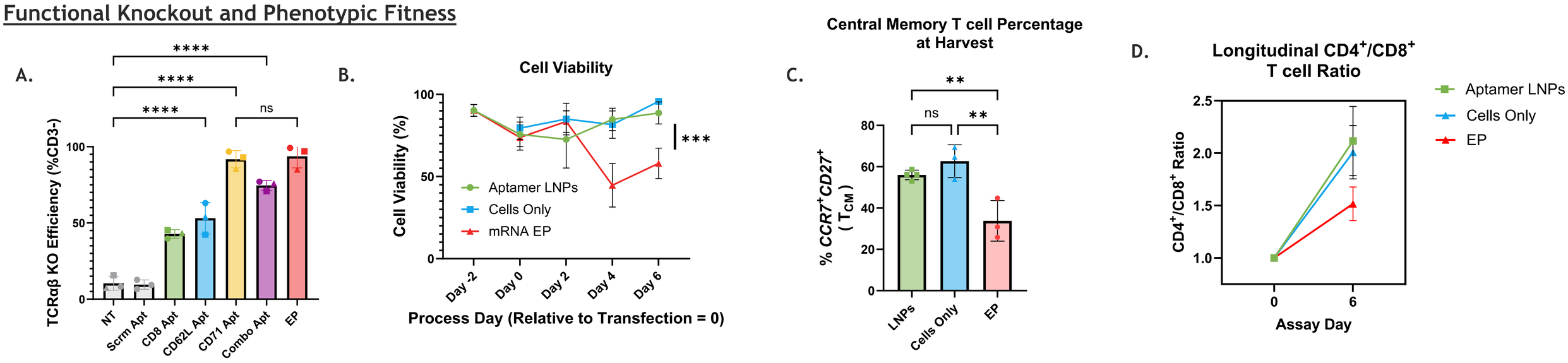

Figure 5. | A.) Comparison of $TCR\alpha\beta$ CRISPR knockout efficiency across various aptamer-functionalized LNP formulations without ApoE or serum proteins compared to electroporation. **B.)** Longitudinal T cell viability across the engineering process from activation (Day -2) through harvest (Day 6). **C.)** Percentage of high-fitness central memory T cells (T_{CM}; $CCR7^+CD27^+$) remaining after treatment. **D.)** CD4+/CD8+ T cell ratio T cell ratio tracking over time. Data represent mean ± SD (N= 3 biological replicates). Statistical significance determined by one or two-way ANOVA with Tukey's post-hoc test (**p < 0.01, ***p < 0.001, ****p < 0.0001).

Discussions & Conclusions

- **Precision Targeting:** Aptamer-functionalized LNPs enable high-efficiency delivery of CRISPR-Cas9 and mRNA payloads to specific T cell subsets within complex primary cell mixtures, achieving ~100% delivery in targeted populations.
- **Superior T Cell Fitness:** Unlike electroporation, LNP delivery preserves high cell viability and maintains critical T_{CM} and naïve phenotypes
- **Versatile Payload Delivery:** The modular "plug-and-play" nature of the platform supports diverse genetic cargoes.
- **Scalable Manufacturing Alternative:** By eliminating the toxicity of physical delivery methods and the complexity of viral vectors, this non-viral system offers a streamlined pathway for the rapid, cost-effective production of next-generation cell therapies.
- **Future Directions:** Evaluate therapeutic efficacy of aptamer-LNPs in functional CAR-T assays (antigen-specific cytotoxicity, cytokine release, and proliferation) and optimize aptamer stability for *in vivo* translation.

Acknowledgments & References

1. Wu, A. Y. et al. *Adv Healthc Mater* **15**, e2501234 (2026)
2. Cheng, E. L. et al. *JACS* **144**, 13851-13864 (2022)
3. Kacherovsky, N. et al. *Nat Biomed Eng* **3**, 783-795 (2019)

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