

Scaled from [research scale](#) to [GMP-ready production](#)

INTEGRATED END-TO-END MANUFACTURING WORKFLOW



INTRODUCTION & RATIONALE

The clinical translation of gene-edited allogeneic CAR-T therapies demands manufacturing platforms that combine effective alloreactivity control, TCRαβ knockout, with scalable, reproducible expansion, while preserving critical quality attributes. Prior work by our group ([Pavlovic et al., 2025](#)) established the G-Rex bioreactor as a valid expansion system at research scale, sustaining high yields, editing efficiency and a therapeutically relevant memory phenotype. The **CERTERA** project integrates G-Rex as the central expansion step of a GMP-ready, end-to-end platform for universal anti-CD19 TCRαβ-KO CAR-T cells (ARI-0001 vector, licensed from Hospital Clínic Barcelona).

HYPOTHESIS

Incorporating the G-Rex bioreactor as the central expansion step of an integrated Prodigy + MaxCyte + G-Rex + GatheRex platform enables clinical-scale yields while preserving the product's critical quality attributes, validating process transferability to a GMP environment.

1 CRISPR/CAS9 OPTIMIZATION — LOCKED CONDITION FOR DOWNSTREAM RUNS

Three RNP conditions tested in primary T cells (n=4 donors); TRAC knockout assessed by ICE at D6 post-electroporation.

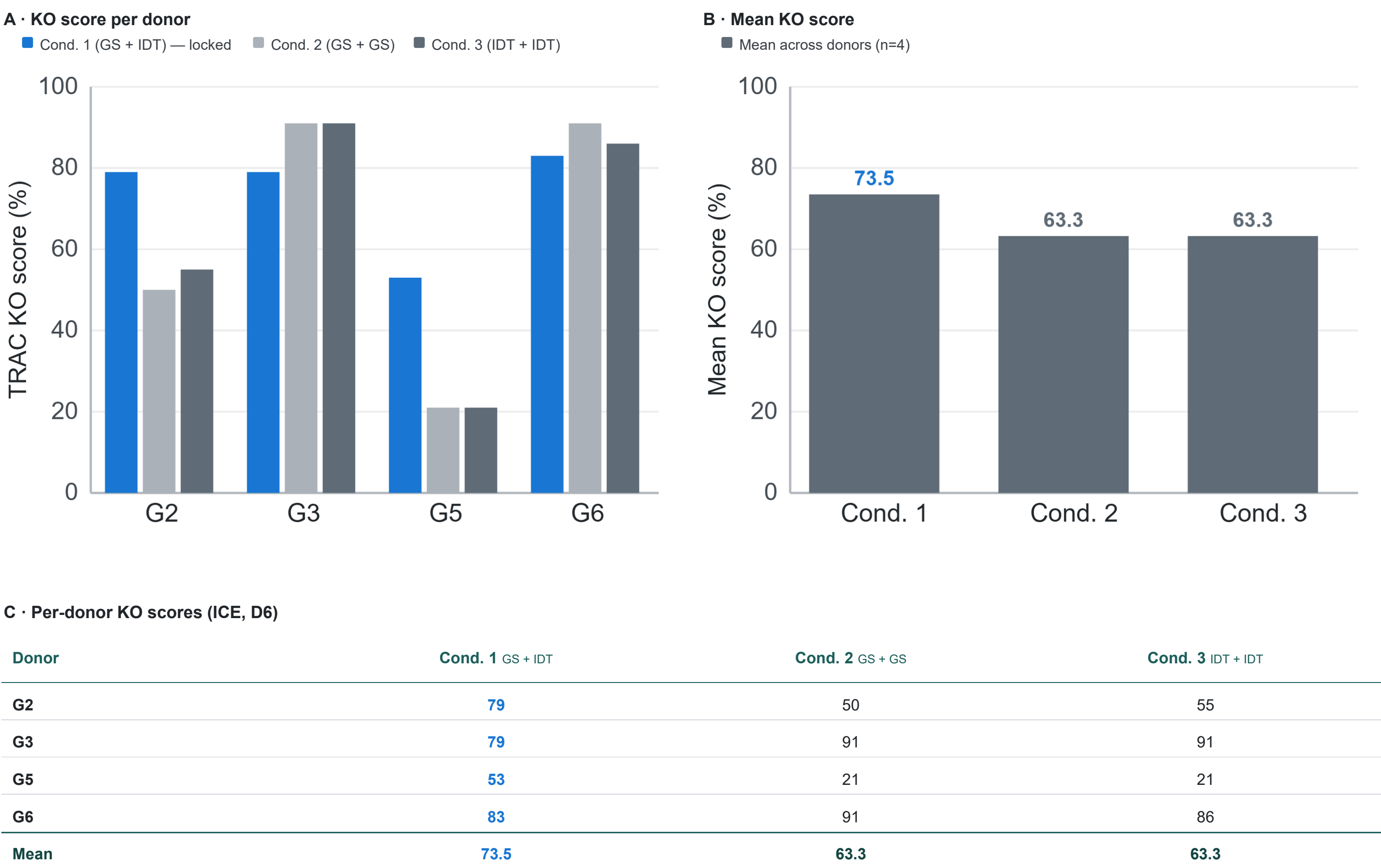


Figure 1. CRISPR/Cas9 RNP optimization in primary T cells (n=4 donors). **(A)** Per-donor TRAC KO score by ICE analysis at D6 across three RNP conditions. **(B)** Mean KO score per condition. **(C)** Per-donor values. Condition 1 (sgRNA Genscript + Cas9 IDT) delivered the most consistent knockout across donors (mean 73.5%) and was locked in for CERTERA-01 onward. Mann–Whitney (two-tailed).

3 RUN-TO-RUN REPRODUCIBILITY ACROSS CERTERA PRODUCTIONS

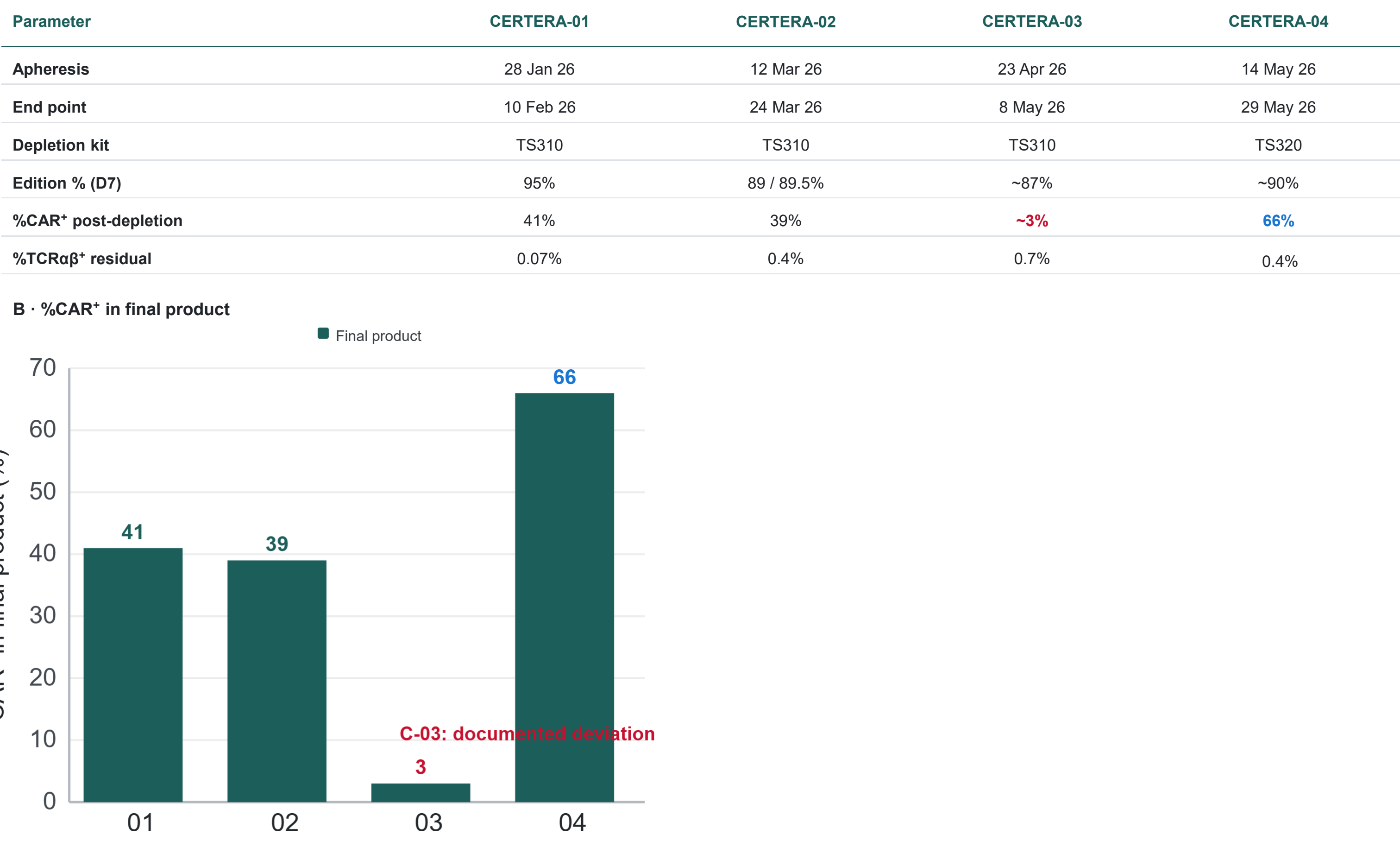


Figure 3. Run-to-run reproducibility across four production runs from healthy-donor apheresis. **(B)** %CAR+ in the final drug product; CERTERA-03 showed a lower yield linked to a documented, under-investigation process deviation, while 01/02/04 fall within comparable ranges (41/39/66%) — supporting process consistency.

CONCLUSIONS

- CRISPR/Cas9 optimization:** the locked condition (sgRNA GS + Cas9 IDT) delivers consistent TRAC knockout across donors (mean ≥73%).
- G-Rex as scale-up enabler:** at production scale, G-Rex delivers ≈22× higher yields than Prodigy expansion under identical biological conditions, while preserving memory phenotype and cytolytic function.
- Process reproducibility:** four consecutive engineering runs show consistent edition (≥89% at D7); TCRαβ+ residual was ≤0.7% with TS310 (runs 01–03) and 0.4% with TS320 (CERTERA-04)
- Functional integrity:** TCRαβ knockout does not impair cytolytic activity of the final product against CD19+ tumor cells.
- Translational readiness:** the integrated Prodigy + MaxCyte GTX + G-Rex + GatheRex platform is ready for transfer to a dedicated GMP cleanroom for formal validation prior to AEMPS submission.

2 PRODIGY VS G-REX EXPANSION COMPARISON ★

In CERTERA-01 the CRISPR-edited pool was split between Prodigy and G-Rex expansion under identical biological conditions (D0–D7 post-edition).

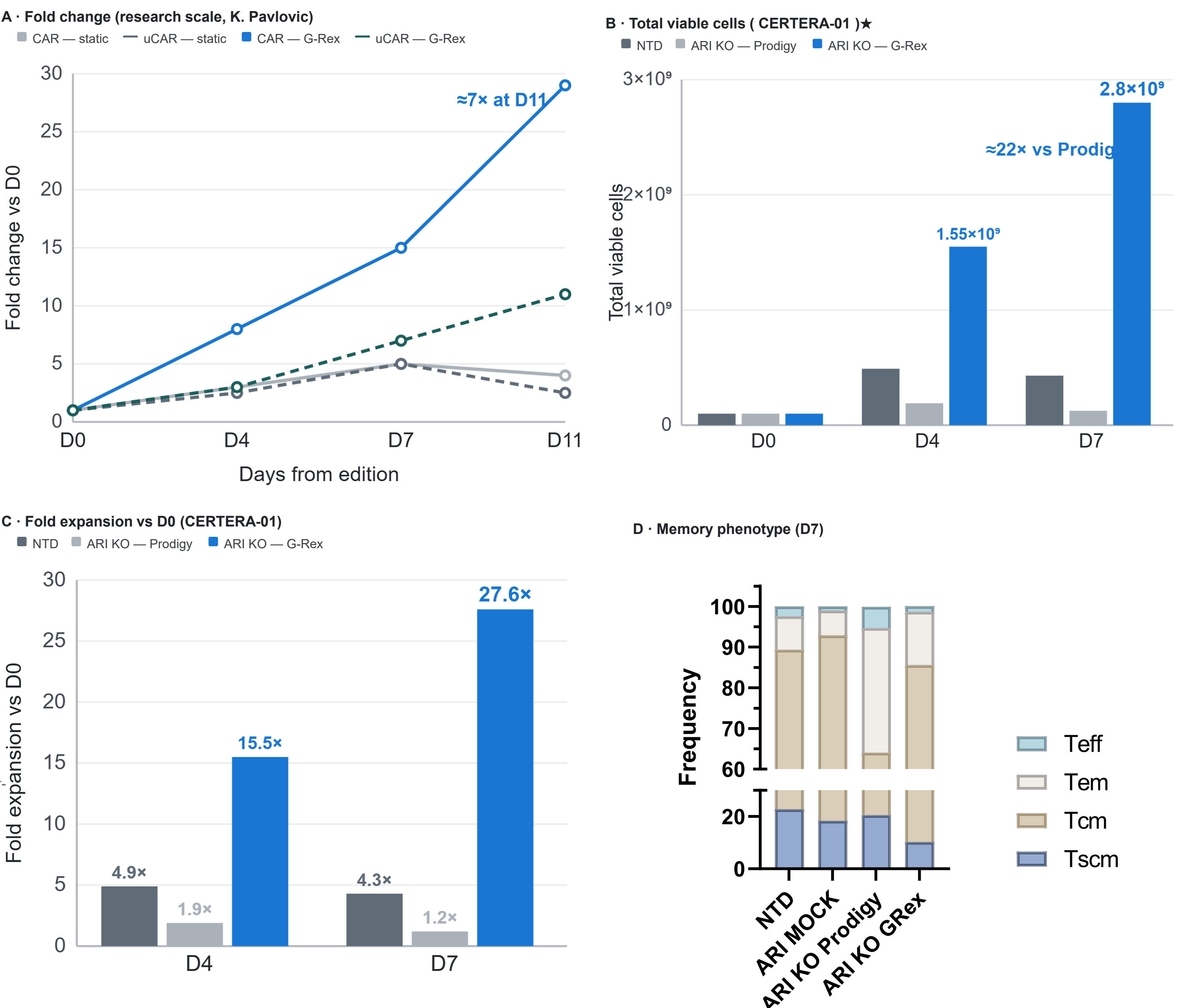


Figure 2. G-Rex enables production-scale expansion. **(A)** Research-scale foundation: G-Rex reaches ≈7× higher CAR-T fold change at D11 vs static culture, preserving phenotype and cytolytic function (adapted from Pavlovic et al., 2025). **(B)** At production scale (CERTERA-01), under identical biological conditions, G-Rex yields ≈22× more total viable cells than Prodigy at D7 (2.8×10⁹ vs 1.25×10⁹). **(C)** Fold expansion 27.6× (G-Rex) vs 1.2× (Prodigy) at D7. Mann–Whitney (two-tailed).

4 POST-AB-DEPLETION PRODUCT: FUNCTION & PHENOTYPE

Final drug product after TS310/TS320 depletion across CERTERA-01–04. Potency by 48-h co-culture vs CD19+ Namalwa (5 E:T ratios).

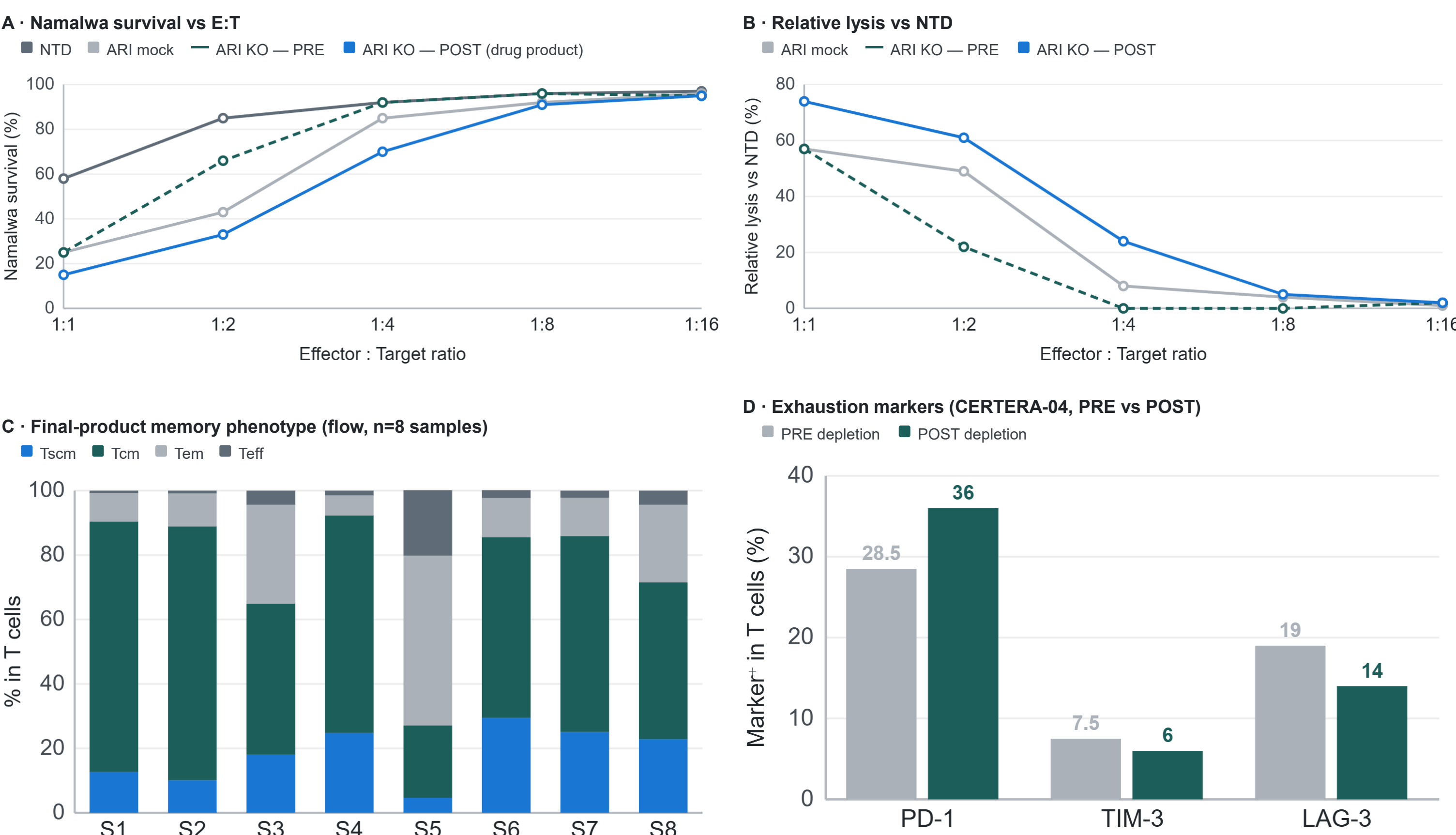


Figure 4. Post-ab-depletion drug-product characterization (TS310/TS320). **(A)** Namalwa survival across five E:T ratios (CERTERA-02, fresh cells); **(B)** relative lysis vs NTD [derived: 100 (1 – survival_{cond}/survival_{NTD})].. ARI KO POST-depletion CAR-T cells show cytolytic activity comparable to non-edited ARI mock, confirming TCRαβ knockout does not impair effector function. **(C)** Memory phenotype by flow cytometry (CD45RA/CD62L gating) across 8 analysed samples: Tscm+Tcm (central/stem-memory) predominates (mean ≈76%), indicating a memory-rich product. **(D)** Exhaustion markers and residual TCRαβ+TCRγδ+ — *paste GraphPad export*. Mann–Whitney (two-tailed).