

Clinical translation of non-viral gene editing: Efficient electroporation of CD34+, NK, and T cells in large volume

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Introduction

Non-viral gene editing technologies have become attractive alternatives to viral transduction for cell therapy manufacturing, offering cargo flexibility and reduced safety concerns. Among these, electroporation is recognized as the gold standard and increasingly adopted in clinical applications, including the first approved CRISPR-based cell therapy product, Casgevy® Therapy. However, electroporation approaches have historically faced challenges in scalability and process optimization, often resulting in lengthy development cycles and reduced efficiency at large volumes. To overcome these limitations, we developed an improved cartridge design for our large-volume electroporation platform, enabling efficient delivery of complex cargos into up to 20 mL of cell suspension.



With our **4D-Nucleofector® LV Unit PRO** we provide:

- An optimized cartridge design for improved performance
- Efficient electroporation of complex cargos like CRISPR/Cas9 or Transposon/Transposase
- Easy scale up from 100 µl to up to 20 mL
- Electroporation of large volumes with up to 2E⁹ cells
- Flexible volumes in fixed volume cartridge (0.5, 1.0, 1.5 or 2.0 mL)
- Easily compatible with cell densities from 2.5E7 to 1E8/mL

Material and methods

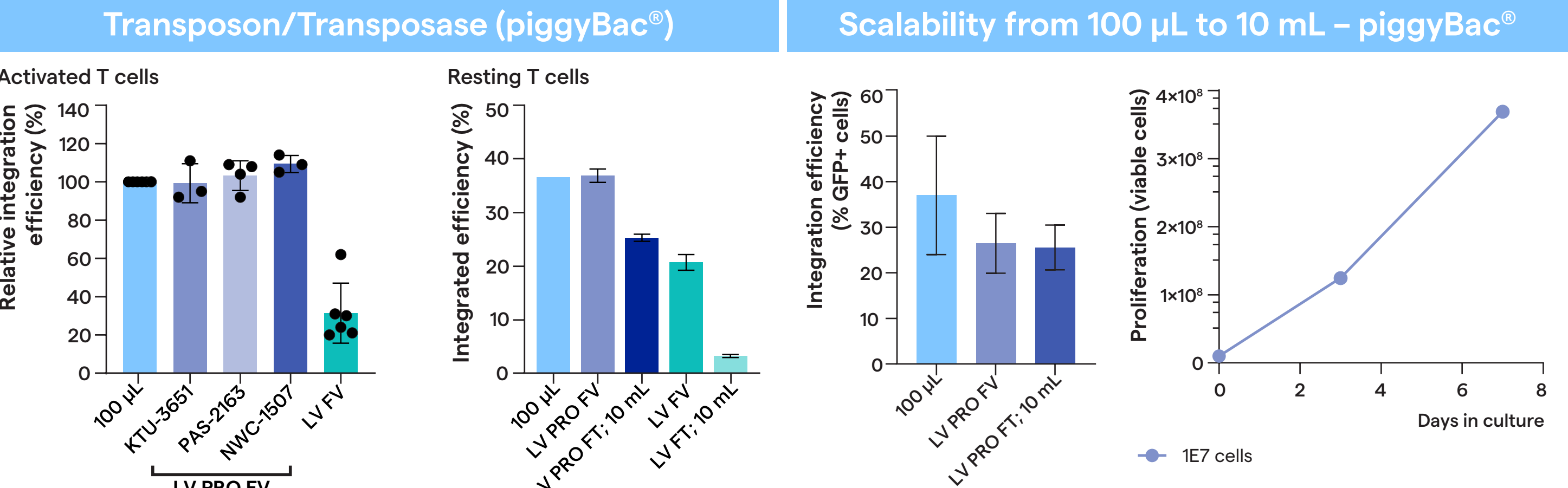
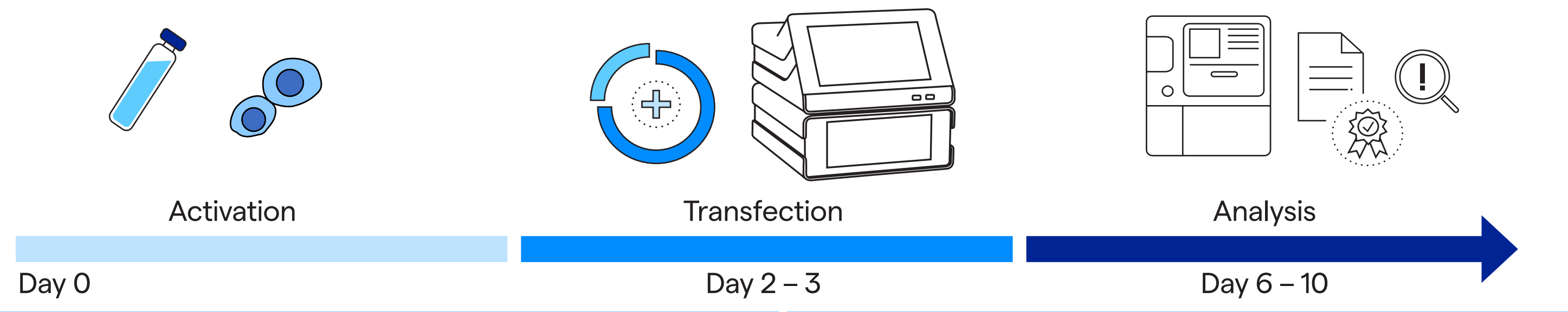
Cell material: **T cells** – Cryopreserved human PBMC (Lonza) or CD3+ Pan T Cells (Lonza), activated with TransAct™ (Miltenyi; 3 days) or Dynabeads (Thermo Fisher Scientific®; 2 days); **CD34+** – Cells were isolated from a fresh mobilized leukopak and used fresh or cryopreserved; **NK cells** – Isolation from CD34-depleted leukopak using negative isolation (Miltenyi), expansion w/ or w/o LCL-WEL for 12 – 14 days. **Analysis:** **T/NK cells** – TCR alpha (TRAC KO), CD45 KO and/or GFP (CRISPR KI or piggyBac®) expression was evaluated by flow cytometry (NovoCyte, Agilent). **NK/CD34+ cells** – gDNA was isolated and **BCL11A** or **CD45** locus amplified using PCR, editing detection via Sanger sequencing.

Transfection: Cells were resuspended in P3 Nucleofector® Solution, cargo was added, the mixture transferred into the required Nucleofector® Vessel and transfected.

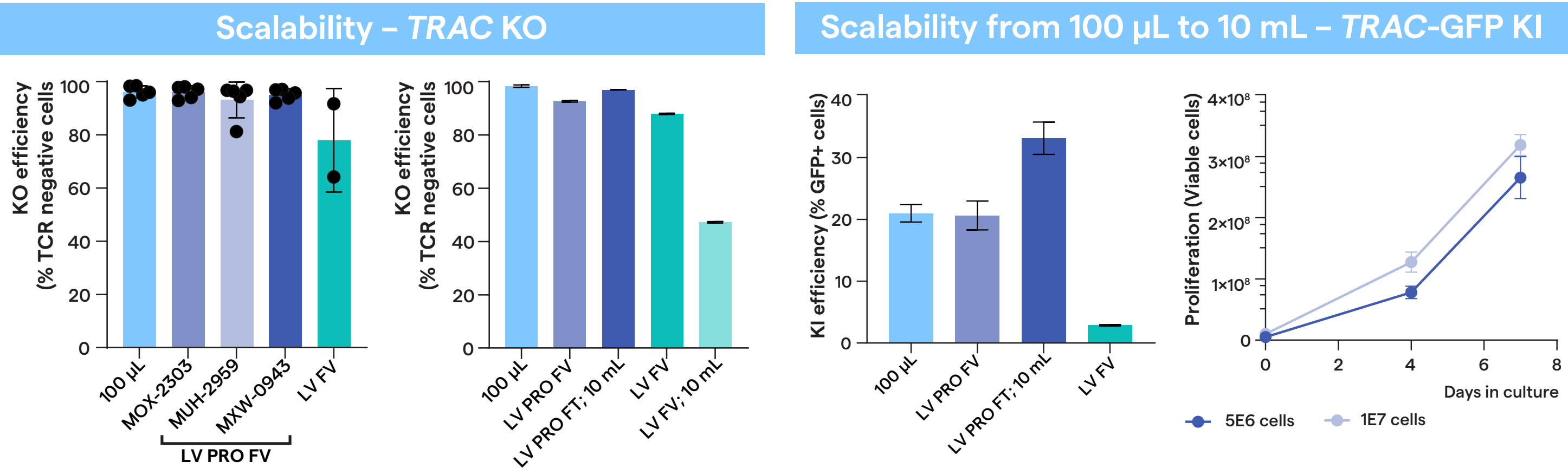
Abbreviations:

- 100 µL** – 100 µL Nucleocuvette® Vessel
- LV PRO FV** – Fixed volume 2 mL Nucleocuvette® Cartridge PRO
- LV PRO FT** – Flow-through LV Nucleocuvette® Cartridge PRO
- LV FV** – Fixed volume 1 mL Nucleocuvette® Cartridge (1st generation)

T cells



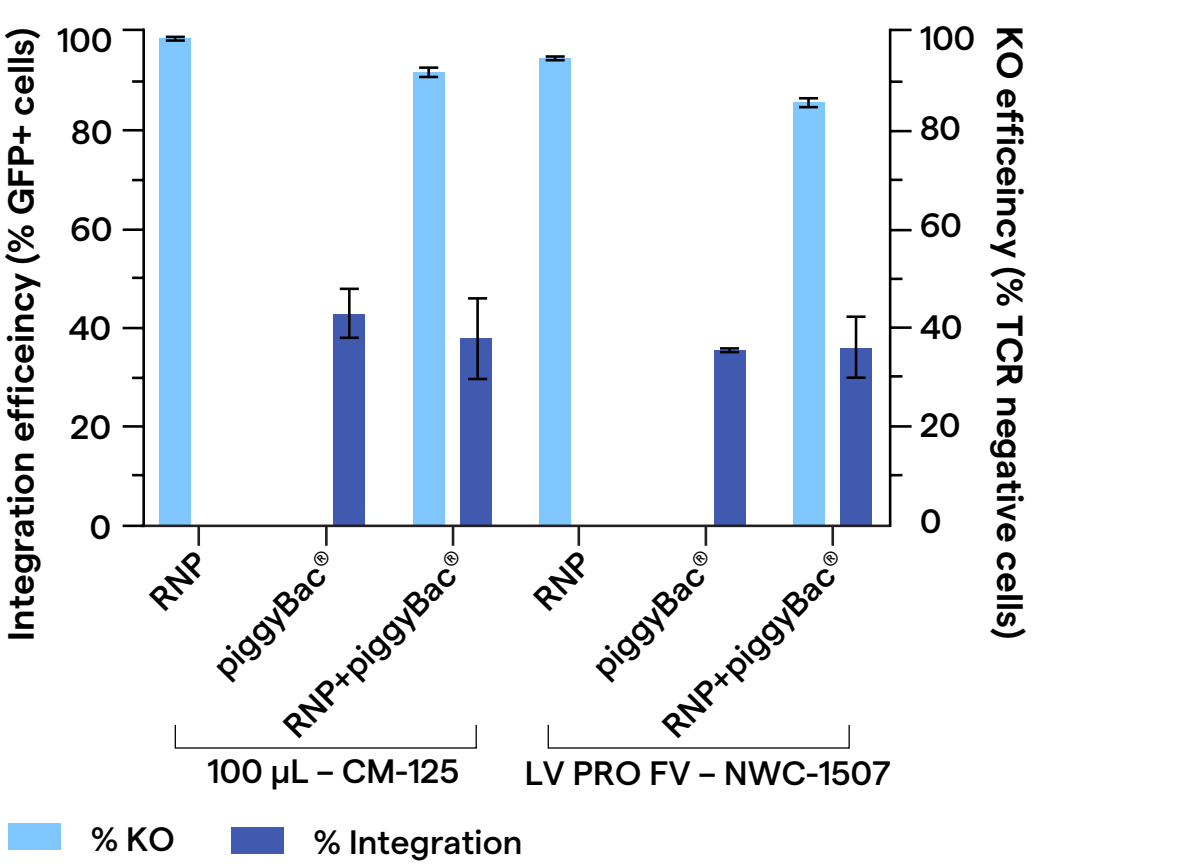
Relative efficiency of LV PRO FV for Transposase mRNA (43 µg/mL) + GenCircle™ Transposon (100 µg/mL; n = 2 donors)



KO in the constant region of TCRα (TRAC) with 0.77 µM Cas9 and sgRNA in ratio 1:2 (for FV n = 4 donors; for FT n = 1 donor)

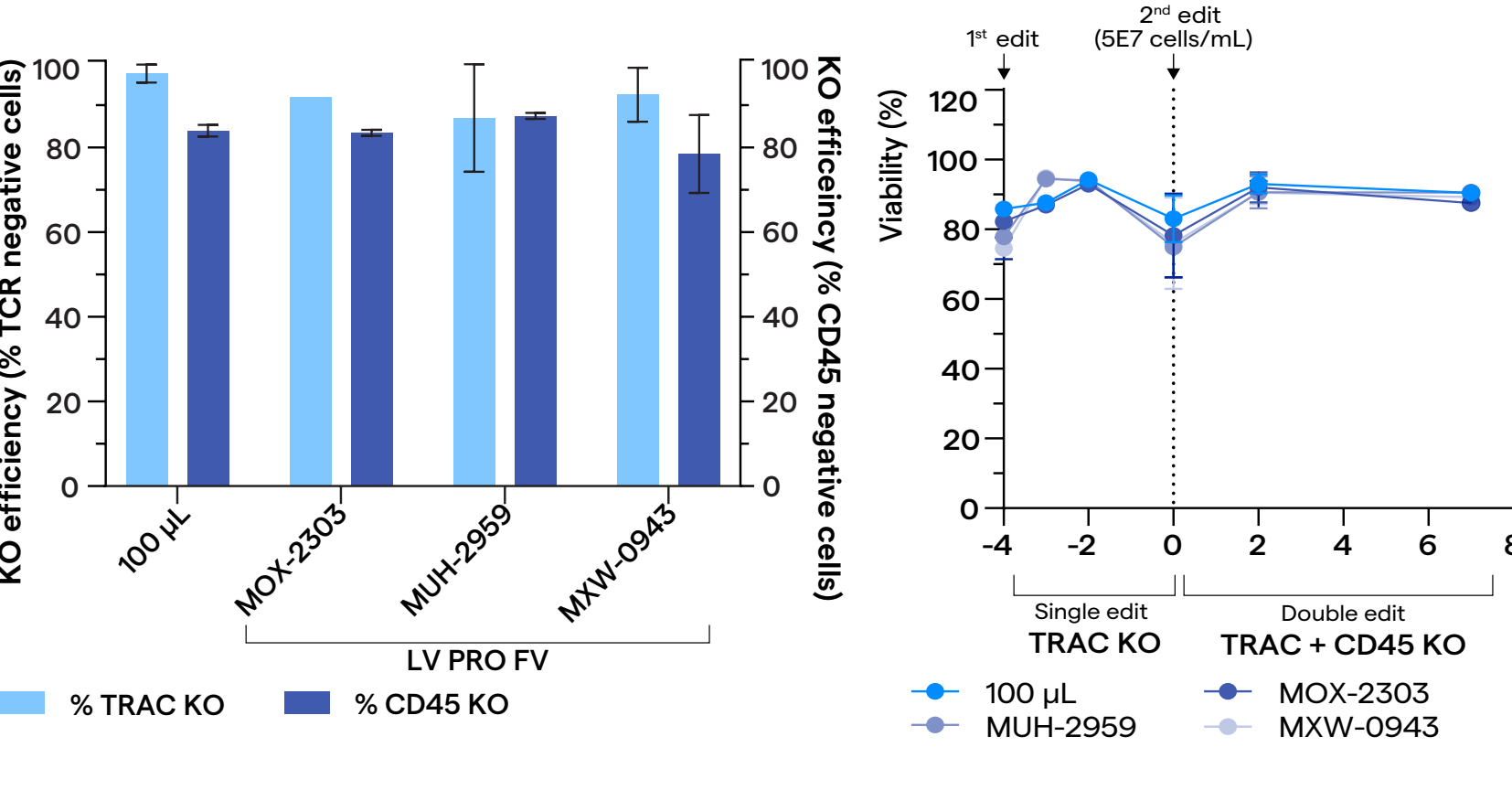
Editing with 0.77 µM Cas9 and sgRNA in ratio 1:2 plus 50 nM dsDNA HDRT (n = 2 donors)

TRAC KO + piggyBac® Transposon System



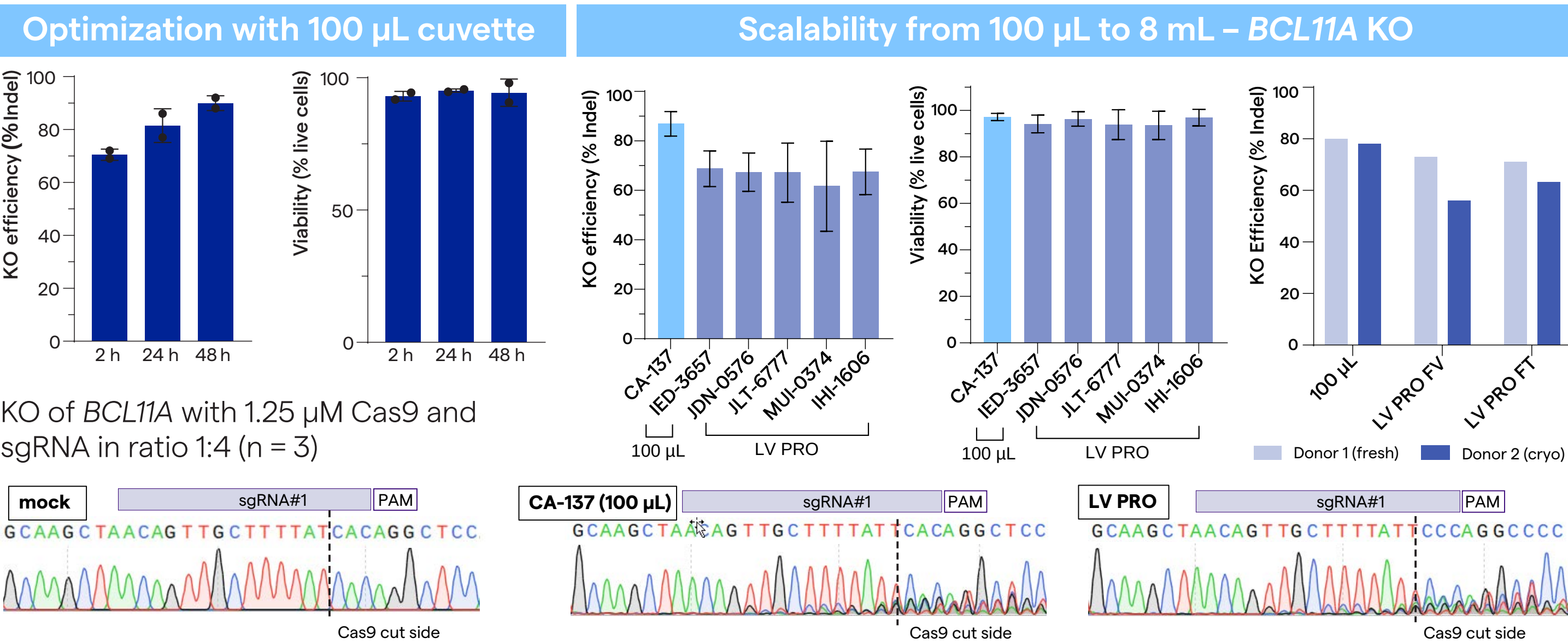
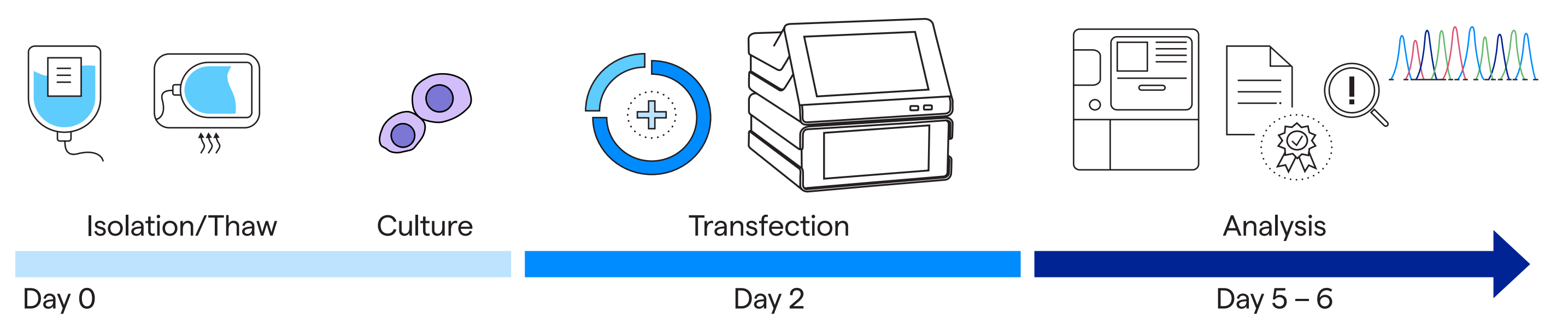
Editing efficiency with Transposase mRNA (86 µg/mL) + GenCircle™ Transposon (200 µg/mL) and TRAC KO (0.77 µM RNP) (n = 2)

CRISPR/Cas9 Double KO of TRAC and CD45

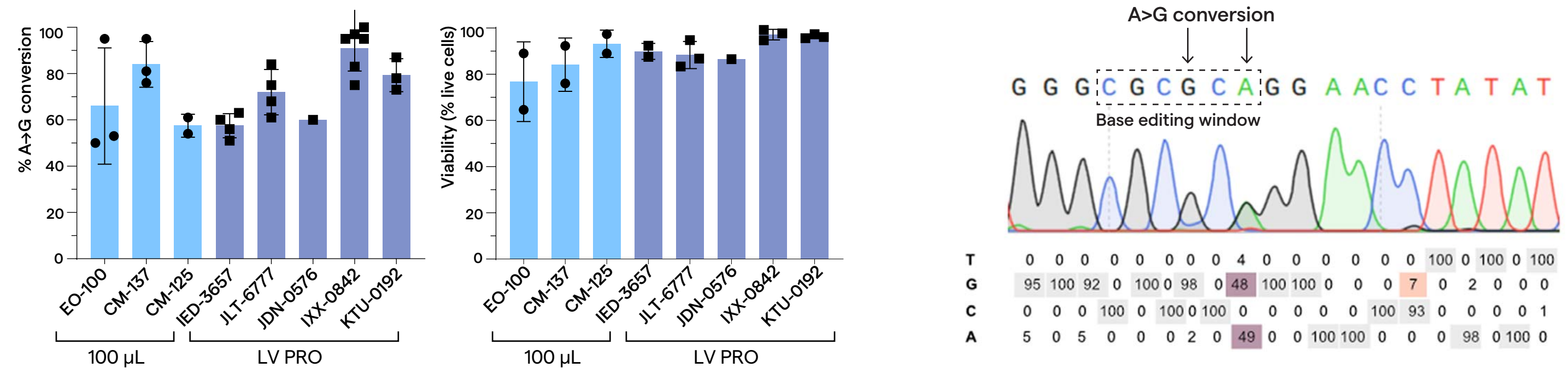


Efficiency with TRAC KO + CD45 KO for 0.77 µM Cas9 + sgRNA in ratio 1:2 each (n = 2 donors)

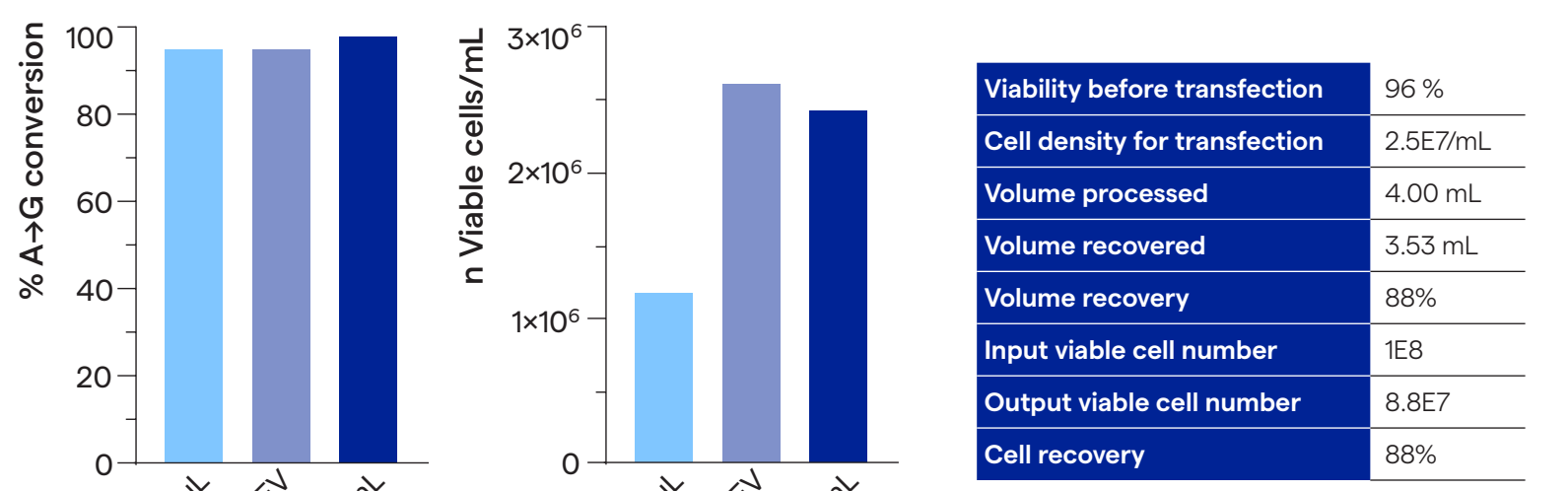
CD34+ HSCs



Base editing with ABE mRNA – CD45

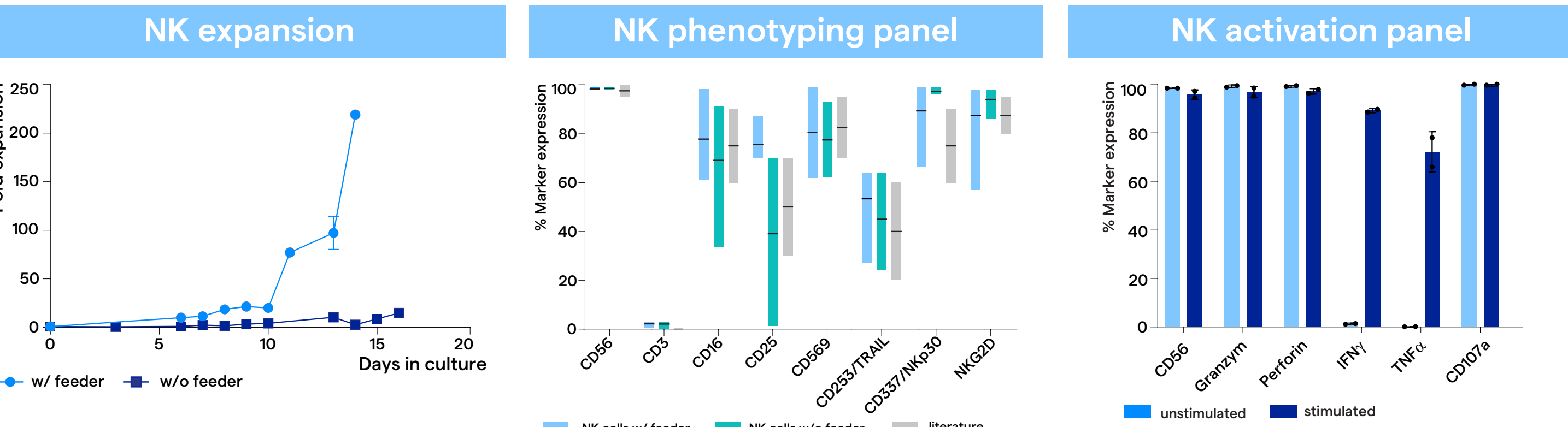
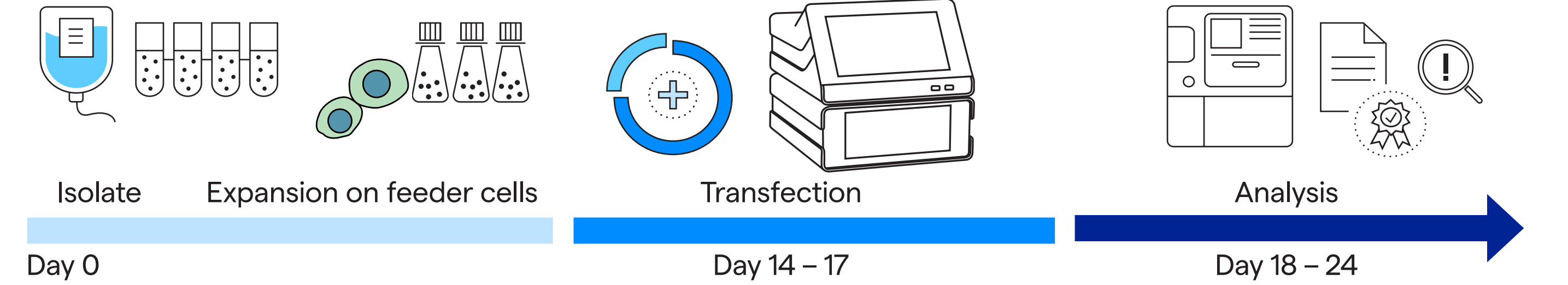


Scalability from 100 µL to 4 mL – Base editing of CD45

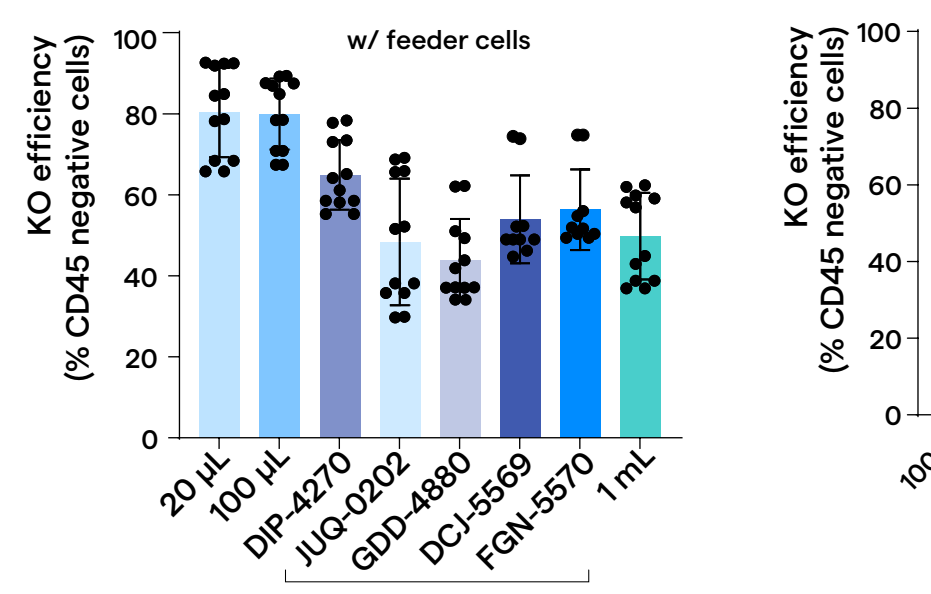


A>G conversion in LV PRO FV for ABE mRNA (100 µg/mL) + CD45 sgRNA (150 µg/mL; n = 3 donors)

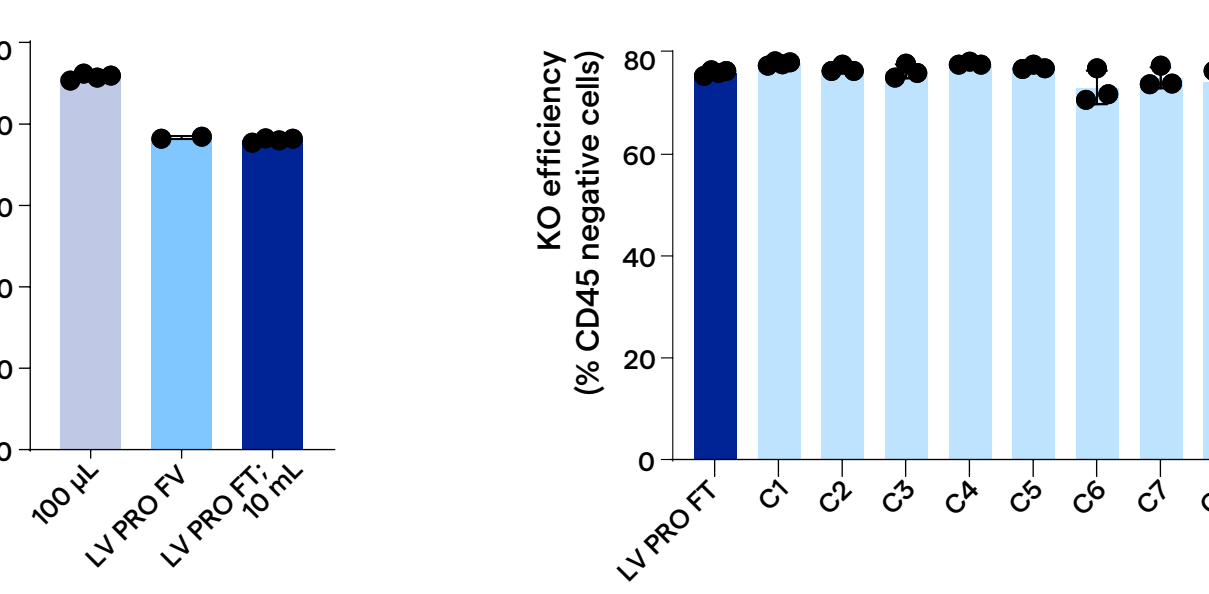
NK cells



Scalability – CD45 KO

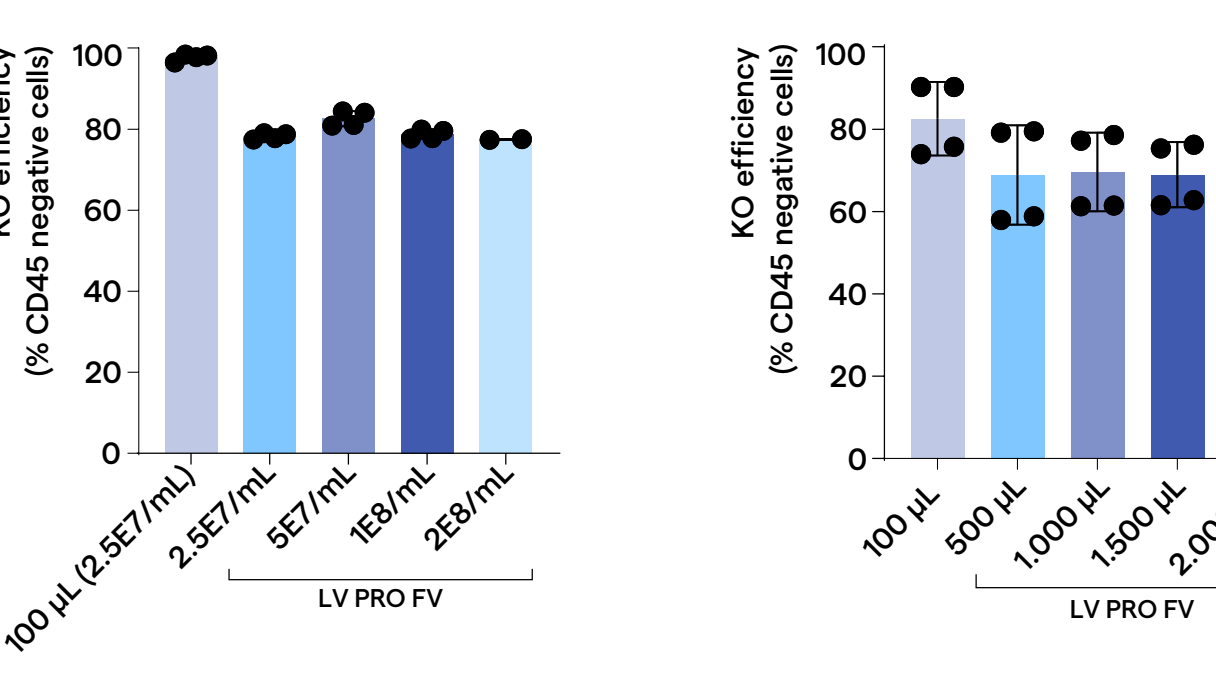


Scalability from 100 µL to 10 mL – CD45 KO

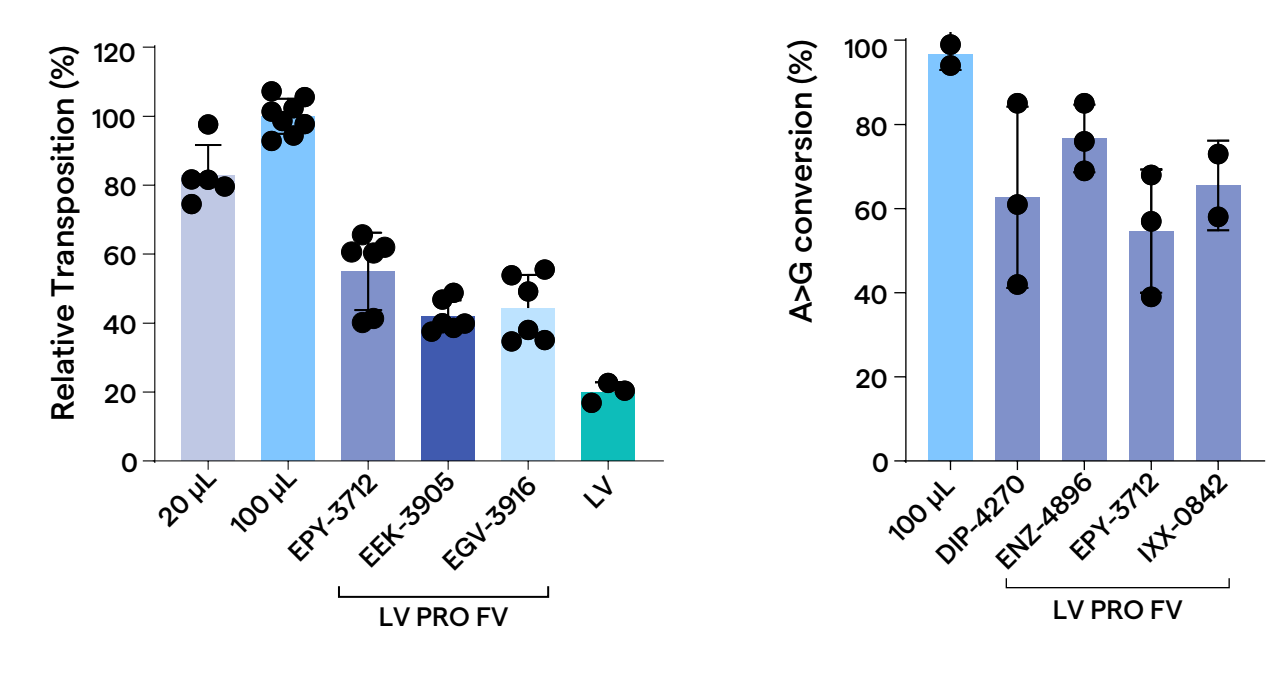


KO of CD45 with 1.54 µM Cas9 and sgRNA in ratio 1:2 (n = 3 donors)

Performance is independent of volume or cell number



Transposon/Transposase (piggyBac®) and Base editing



Summary

- The next generation 4D-Nucleofector® LV Unit PRO offers
- Reliable, robust and efficient delivery of complex, clinically relevant cargos
- Easy scale up of cell engineering up to 4 billion cells
- Applications protocols for T cells, NK cells and CD34+ HSC

Thus enabling non-viral manufacturing of cell therapy products

Learn more.

