

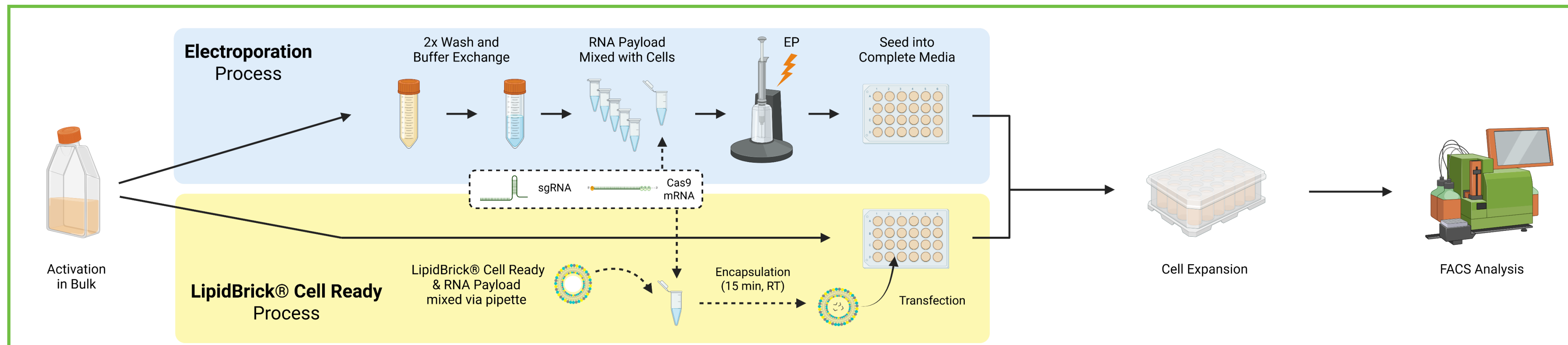
Streamlining CRISPR-Cas9 gene editing with LipidBrick® Cell Ready

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Introduction

Viral transduction, the standard for cell engineering, is increasingly being challenged by alternative gene transfer methods due to limitations in manufacturing timelines, cost, and safety. Non-viral delivery of CRISPR-based genome editing components offers a precise and efficient approach for targeted gene modification, revolutionizing the development of therapies for genetic disorders. In this study we used Sartorius' LipidBrick® Cell Ready, an off-the-shelf lipid-based solution, to deliver CRISPR editing components for T-cell receptor (TCR) knockout in activated T-cells. Compared to electroporation, a current standard for non-viral gene editing, an analysis of transfection efficiency, viability effects, and cell expansion following treatment shows that LipidBrick® Cell Ready offers an efficient and viable alternative gene editing workflow.

Materials and Methods



Frozen CD4/CD8 T-cells were thawed, washed in media, activated, and seeded at 1.0e6 Total Viable Cells (TVC)/mL on Day 0. On Day 3, T-cells were harvested, washed with PBS, and divided for parallel treatment with either Sartorius' LipidBrick® Cell Ready or standard electroporation. LipidBrick® Cell Ready formulations were prepared with varying Cas9 mRNA sgRNA ratios, total RNA payload amounts, and reagent concentrations for cargo delivery. Cell count and viability (CCV) measurements were taken 4 hours post-treatment for LipidBrick®-treated cells and both pre- and post-transfection for electroporated cells, with all samples incubated overnight in 24-well tissue culture plates. On Day 4, CCV was assessed for all treatment groups before transfer to G-rex 24-well plates for expansion, and at harvest, samples underwent final CCV analysis, flow cytometry for T-cell phenotype evaluation, and TRAC knockout efficiency determination.

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LipidBrick® Cell Ready delivery system enhances T-cell growth kinetics compared to standard methods

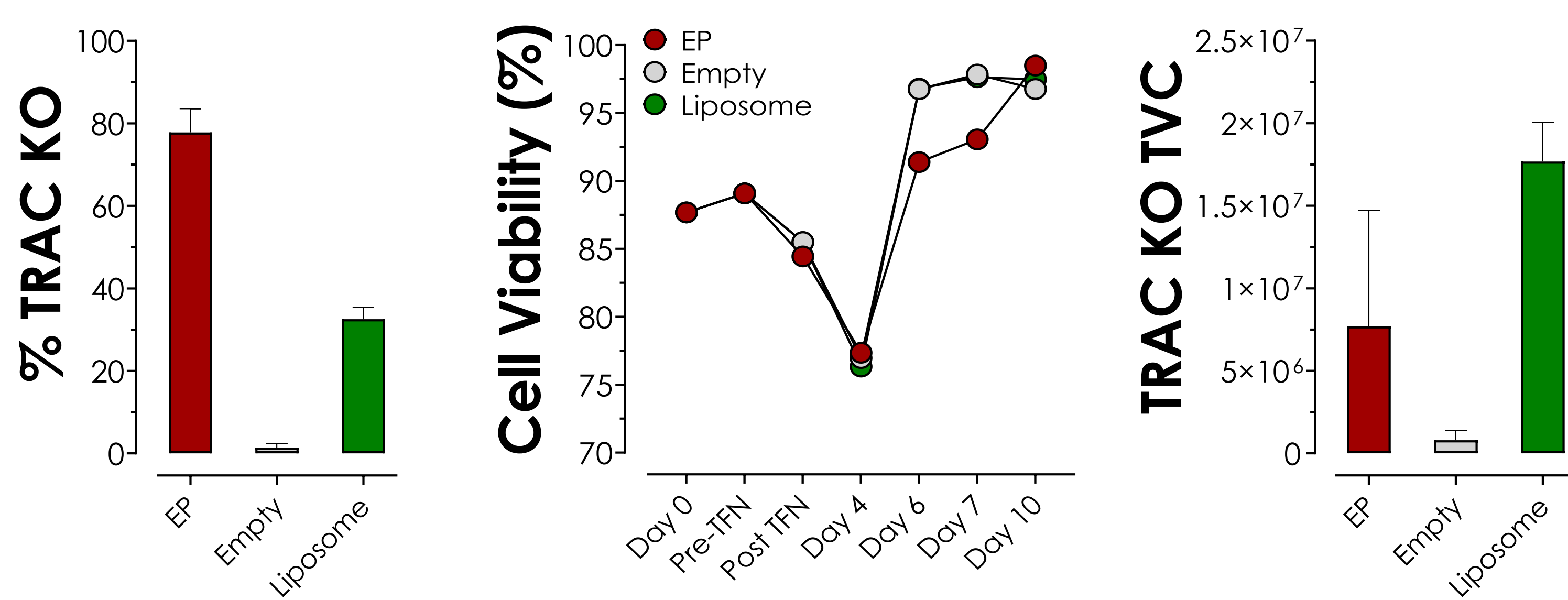


Figure 1. Delivery method impacts TRAC knockout efficiency and T cell growth kinetics. Activated T cells were transfected with a CRISPR/Cas9 system targeting TRAC for depletion. Electroporated T cells demonstrated a higher TRAC knockout efficiency compared to cells treated with LipidBrick® Cell Ready (left) with and without cargo ("Empty" control group). All conditions showed a drop in cell viability (middle), with liposome transfection showing quicker recovery for T cell expansion. LipidBrick® Cell Ready delivery method demonstrated minimal impact to T cell growth kinetics, resulting in more successfully edited viable cells (right).

LipidBrick® Cell Ready with less payload per liposome demonstrates better knockout efficiency

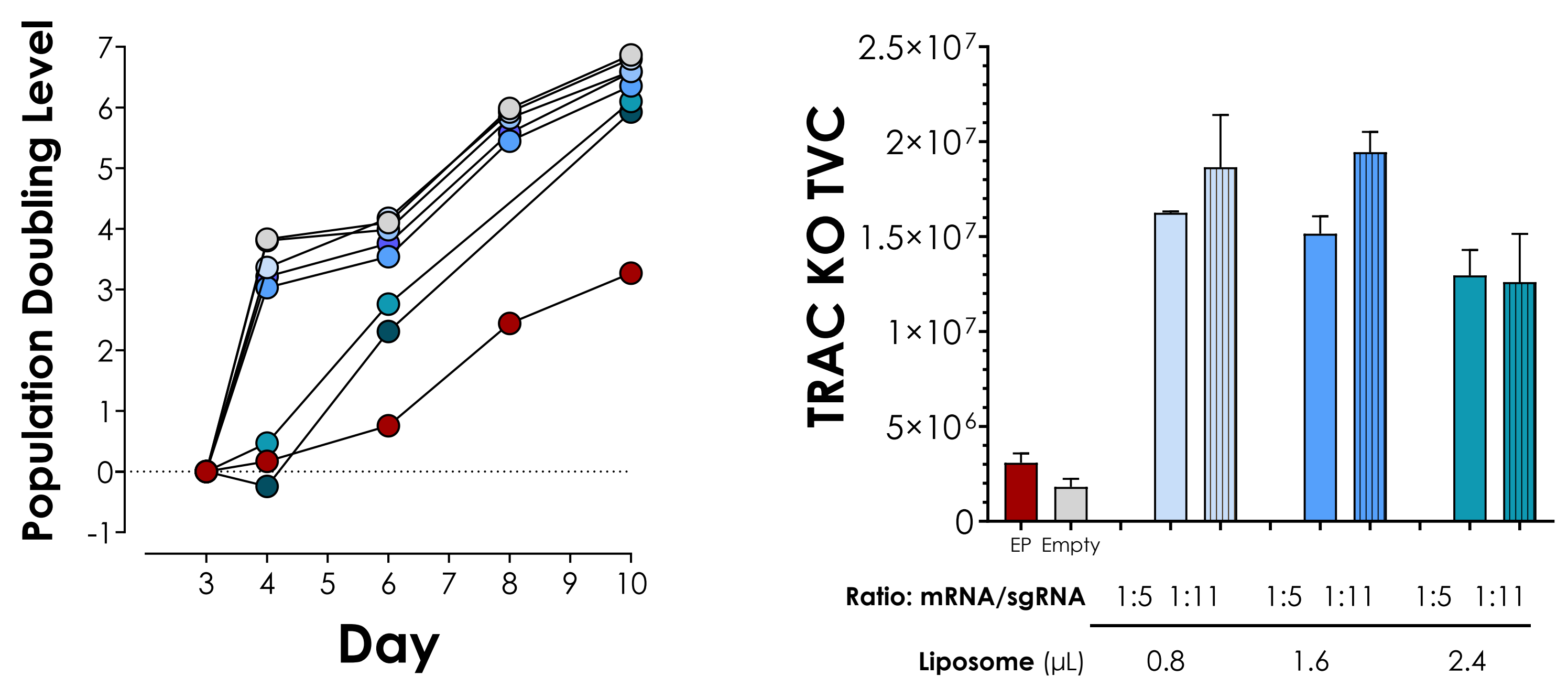
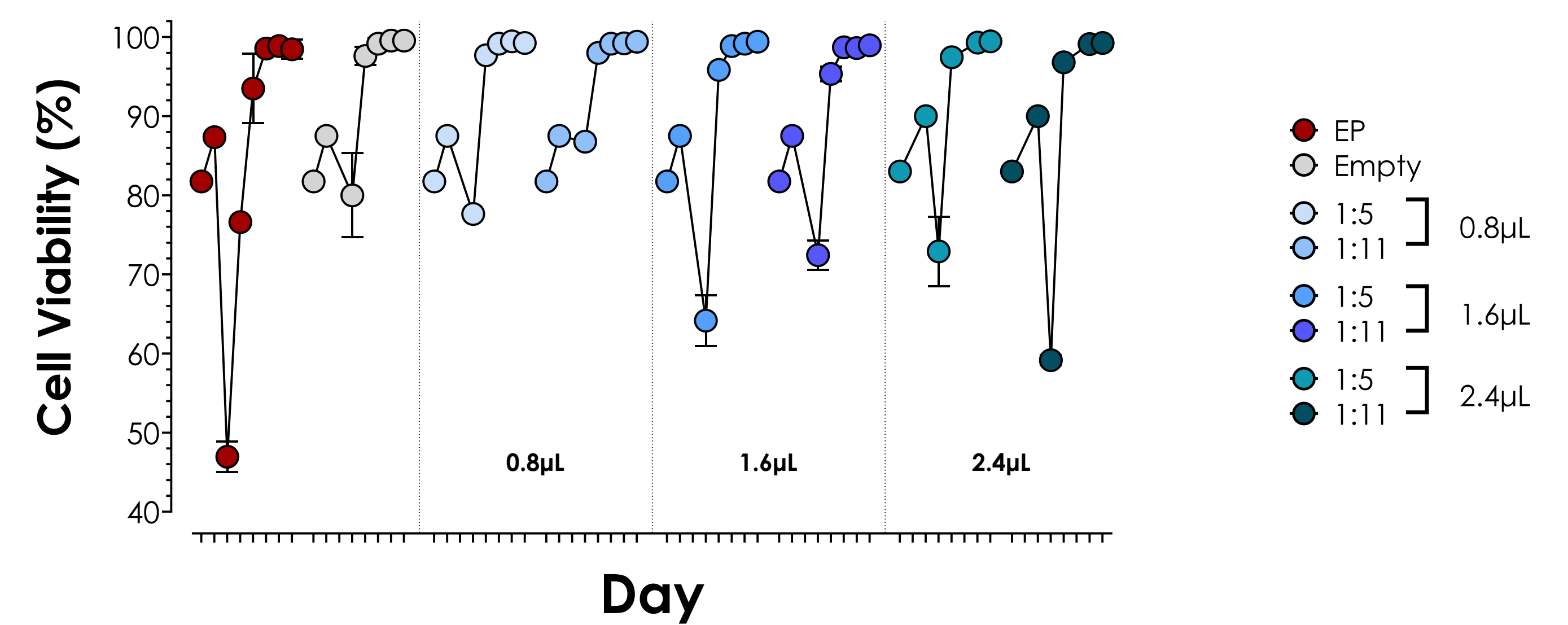
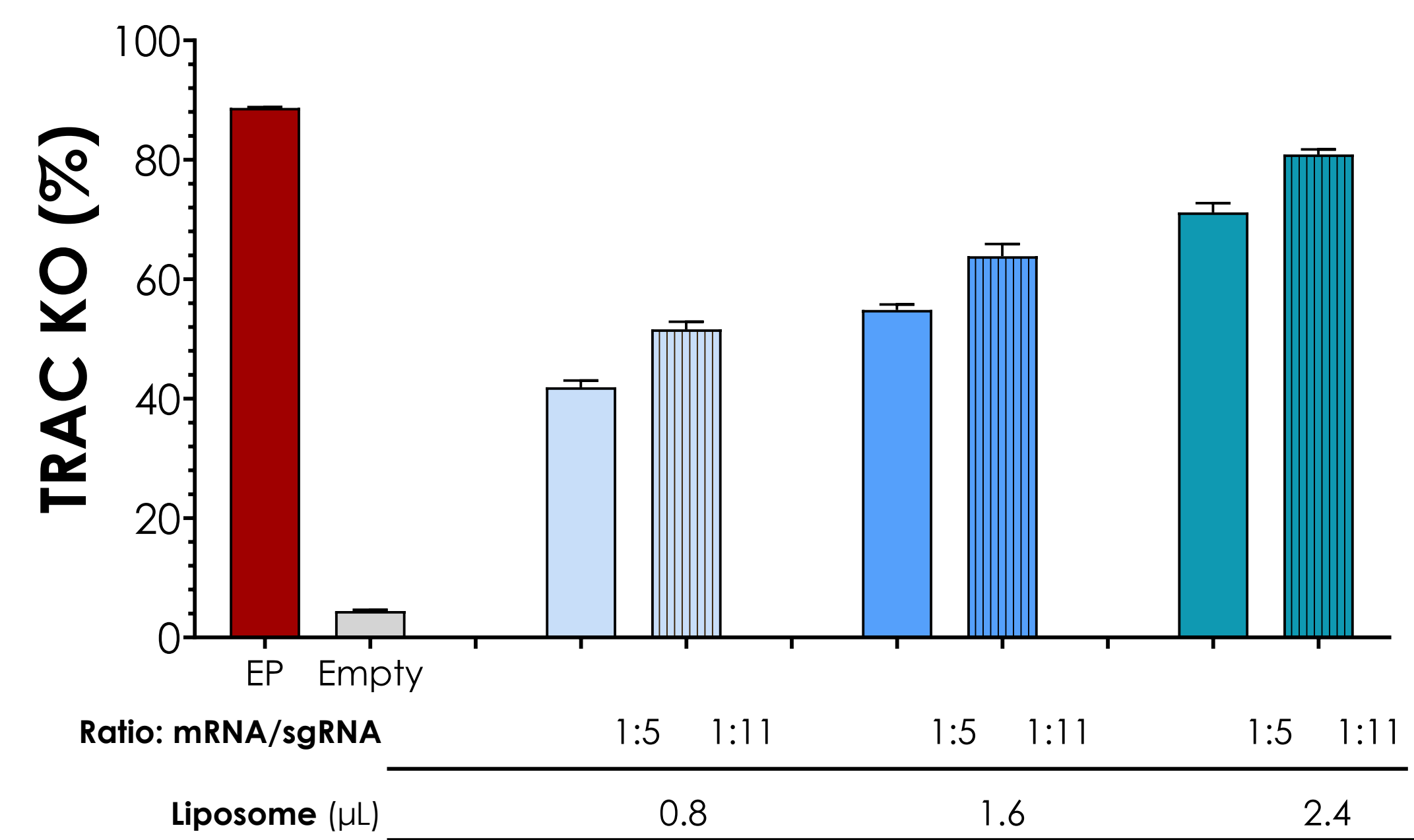


Figure 5. A balanced nucleic acid ratio, total RNA and liposome volume achieved TRAC knockout efficiency comparable to electroporation. Liposomes have a capacity threshold for encapsulating nucleic acids and can impact cell viability through several mechanisms. To optimize editing efficiency on par with delivery by the industry standard, electroporation, we incrementally increased LipidBrick® Cell Ready reagent for payload encapsulation and T cell delivery. Cas9 mRNA and sgRNA maintained at a constant amount and ratio demonstrated a liposome-volume-dependent increase in TRAC knockout efficiency following delivery (top). While both empty and loaded liposomes affected cell viability (middle), the acute toxicity observed with LipidBrick® Cell Ready quickly recovered. The rapid recovery resulted in minimal impact on T cell expansion compared to T cells engineered using electroporation (bottom).

Payload specificity determines gene modification efficiency with LipidBrick® Cell Ready delivery system

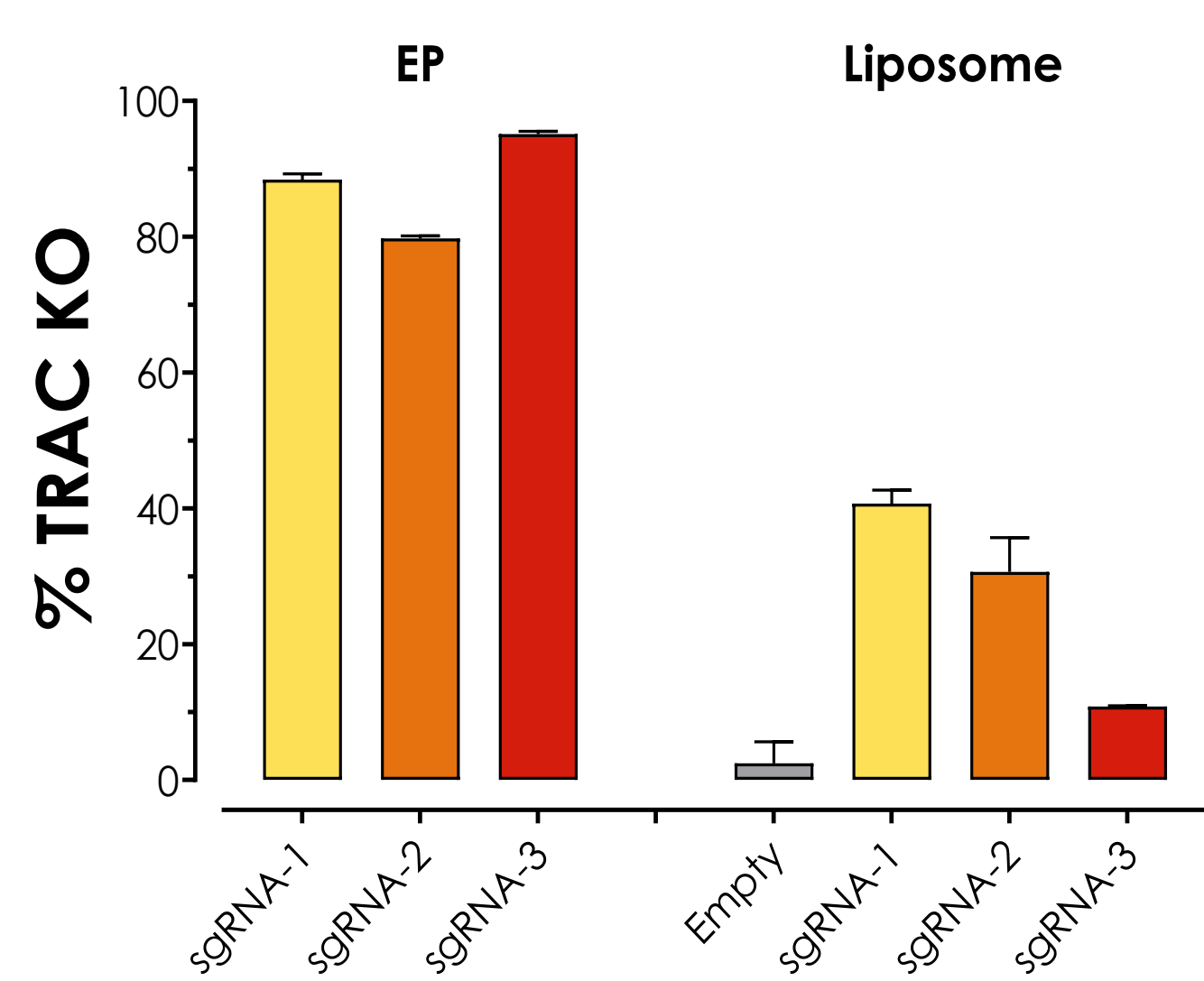


Figure 2. Payload specificity in combination with non-viral transfection method influence gene knockout efficiency. To demonstrate payload compatibility with delivery method, three sgRNA sequences were generated targeting different regions on the TRAC loci. Each TRAC-guide sequence paired with Cas9 mRNA at a 1:1 ratio were delivered to T cells by electroporation and LipidBrick® Cell Ready, respectively. TRAC knockout efficiency was markedly greater when delivered by EP, independent of the sgRNA sequence, while delivery by liposome demonstrated variable results. These findings suggest that further optimization of payload sequence compatibility and nucleic acid ratio (Electroporation study, data not shown) are critical factors for encapsulation and delivery by LipidBrick® Cell Ready.

Optimal mRNA to sgRNA ratio determines targeted knockout efficiency in LipidBrick® Cell Ready delivery system

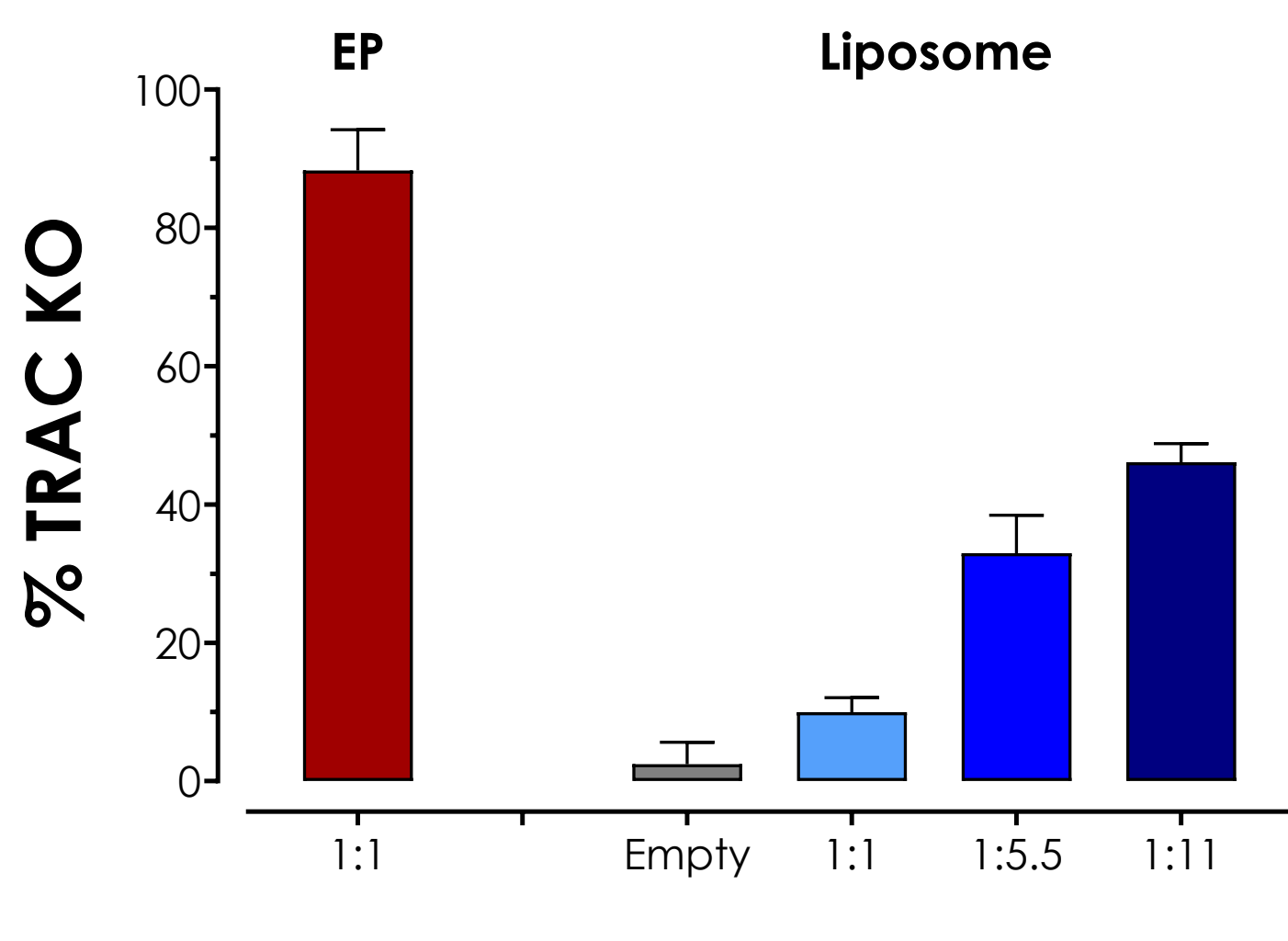


Figure 3. An optimal mRNA to sgRNA ratio increases TRAC KO efficiency. The method of delivery can impact the cellular uptake and availability of mRNA and sgRNA, thus affecting the optimal ratio. To identify the optimal ratio of mRNA to sgRNA for delivery by LipidBrick® Cell Ready, we evaluated three ratios. Transfected T-cells demonstrated increasing gene editing efficiency which correlated with an excess in sgRNA reaching optimal TRAC knockout efficiency at a ratio of 1 mRNA to 11 sgRNA molecules.

Increased total RNA at a constant mRNA to sgRNA ratio does not correlate with enhanced targeted knockout efficiency

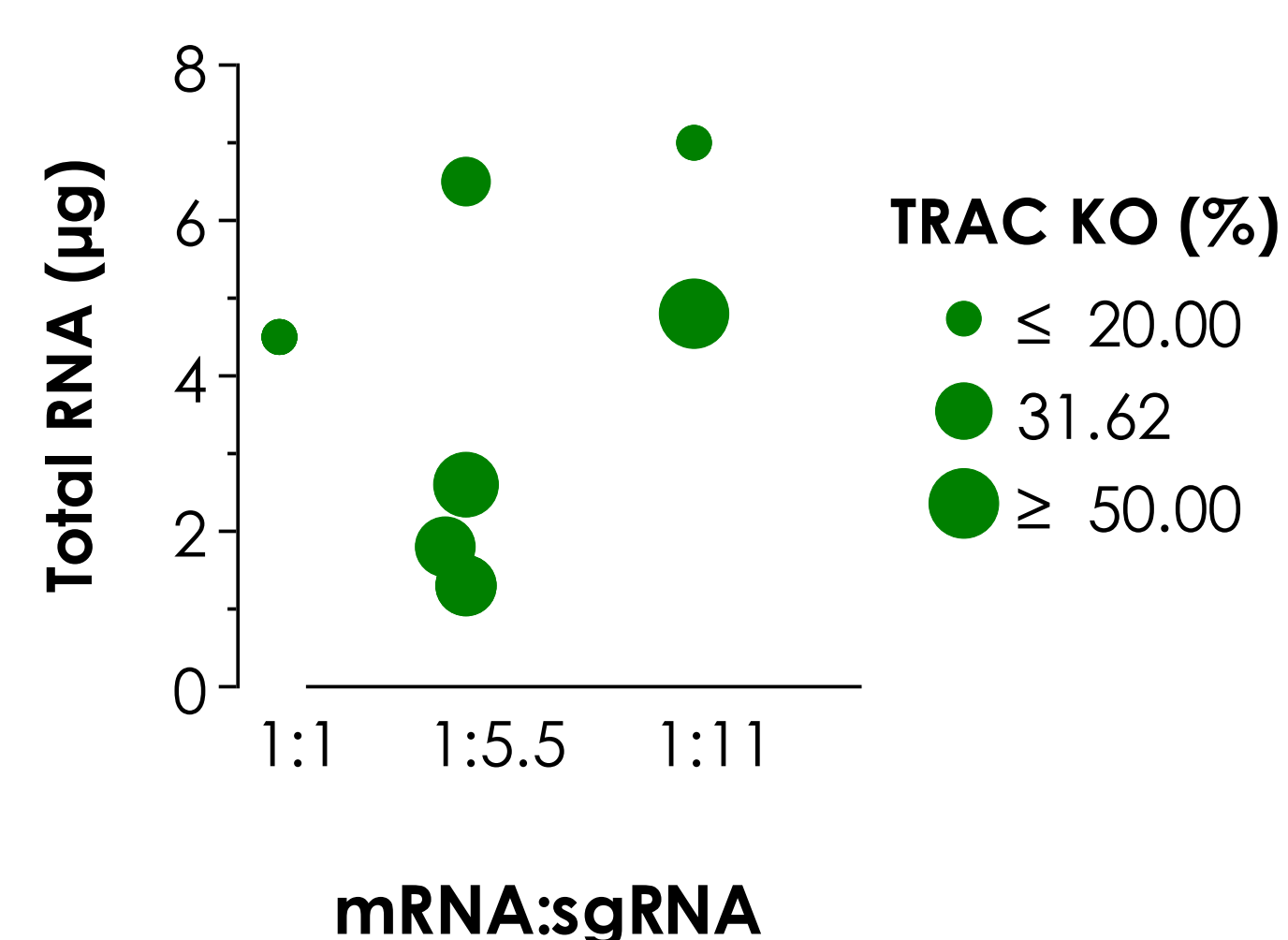


Figure 4. Less RNA at a constant ratio translated to an increase in gene editing. Having established the lipid-mediated delivery ratio of mRNA/sgRNA for gene editing, we next aimed to understand whether more total RNA, while maintaining a constant ratio, increases TRAC knockout. Increasing the total amount of RNA had a limiting effect on engineering T cells demonstrating that lower amounts of total RNA resulted in greater gene editing efficiency. Delivery by LipidBrick® Cell Ready required less nucleic acid payload to efficiently knock out TRAC.

Conclusion

- Gene editing tools delivered via Sartorius' LipidBrick® Cell Ready achieved an 80% TRAC knockout efficiency in T cells following process optimization.
- TRAC sgRNA sequences effective for gene knockout through electroporation did not perform similarly with LipidBrick® Cell Ready delivery, suggesting the necessity for prior screening of the mRNA/sgRNA payload.
- Optimizing the ratio of nuclease mRNA to sgRNA and adjusting the LipidBrick® Cell Ready volume are critical factors in improving knockout efficiency.
- Increasing the total RNA amount (mRNA and sgRNA) did not further enhance knockout efficiency with the same mRNA/sgRNA ratio and liposome volume.
- Electroporation achieved high TCR knockout rates but adversely affected T-cell health, restricting expansion. Transfection initiated through delivery of CRISPR editing components by LipidBrick® Cell Ready maintained better cell viability and expansion, resulting in a higher yield of viable TCR-depleted T cells.
- Sartorius' LipidBrick® Cell Ready provides a streamlined and efficient method for delivering RNA-guide nucleases to engineer T cells, offering a viable alternative to electroporation for the generation of larger doses of healthier TCR-depleted drug product.