

Non-viral knock-in efficiency is enhanced by priming cells with SoluPrime

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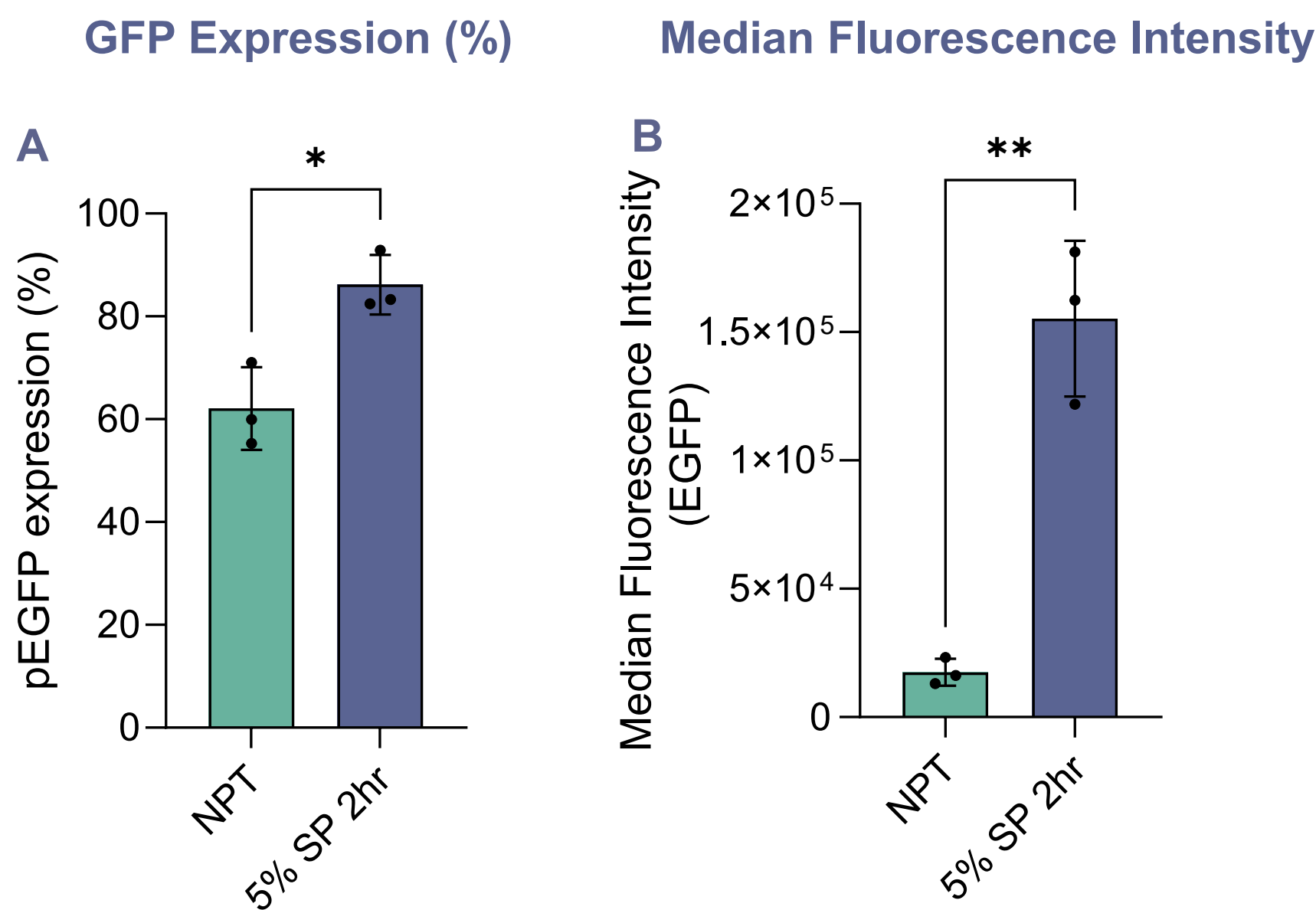
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Introduction

Non-viral CAR-T manufacturing aims to reduce the cost and complexity associated with viral vectors. CRISPR-based editing enables targeted gene insertion, offering key advantages such as precise integration of the gene of interest alongside knockout of constitutive gene expression. However, significant manufacturing challenges remain, including lower CAR expression, reduced cell viability and expansion, and complex process development requirements. Both dsDNA delivery and electroporation negatively impact T cell health, and many knock-in enhancers further reduce the yield of CAR-positive cells by compromising viability and expansion capacity. SoluPrime is a chemically defined reagent that doesn't contain peptides, nucleotides, or small molecules. It can be simply added to cell culture media 30 minutes prior to electroporation, enhancing knock-in efficiency without further compromising cell health.

SoluPrime transfection enhancer

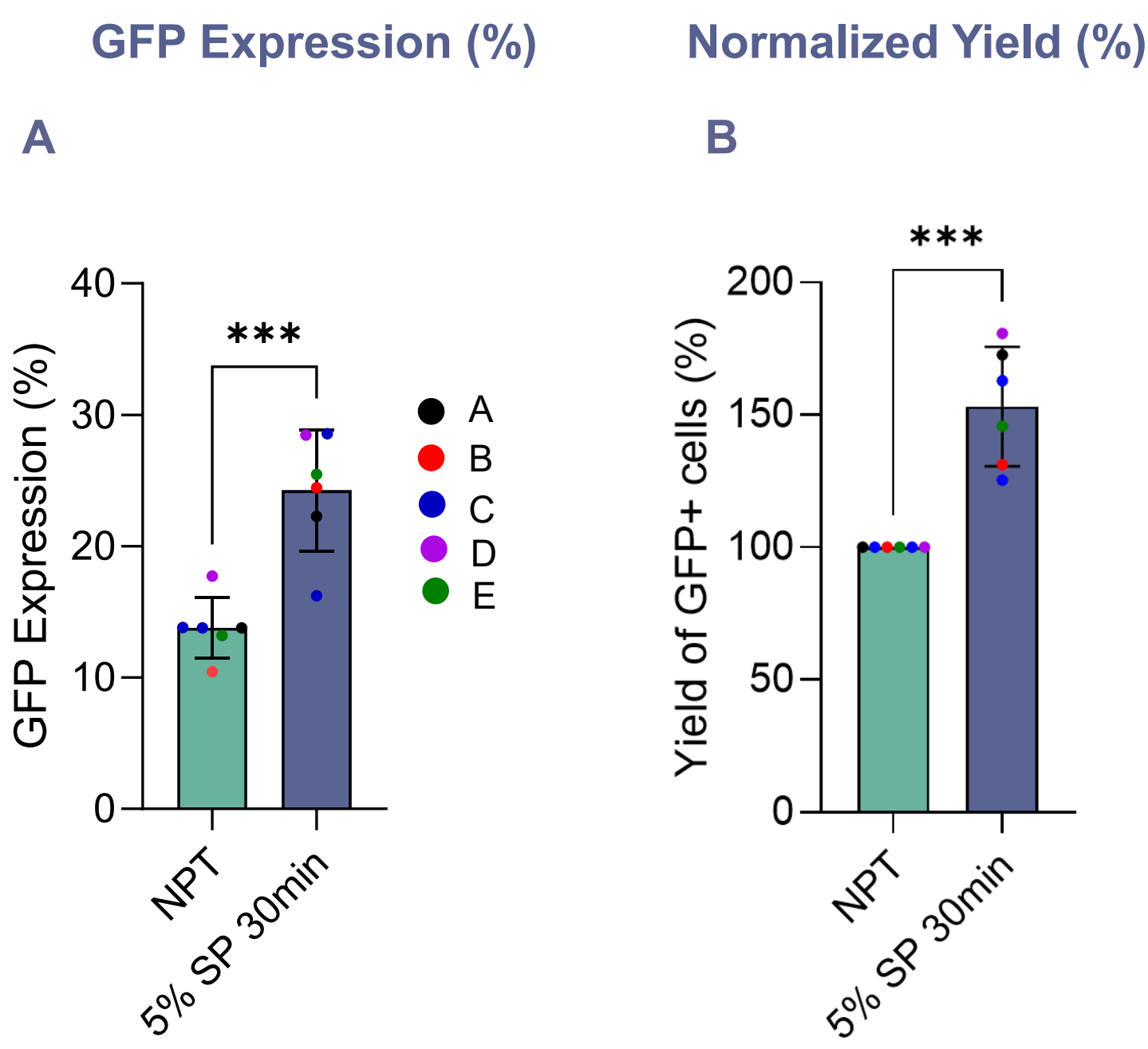
Figure 1 – Enhanced plasmid expression



39% increase in GFP Expression & ~800% increase in MFI

Figure 1: Activated T cells (72hr) were pretreated with SoluPrime (SP; 5% v/v) for 2hr prior to electroporation. GFP plasmid (5.8kb) was delivered using pulse code EO115. Cells were cultured for 1 day in TexMACS media post-electroporation and EGFP expression (A) was assessed by flow cytometry. Median fluorescence intensity is also depicted (B) Data is depicted as the mean ± SD from 3 technical replicates. NPT = No Pretreatment. *p<0.05; **p<0.01, t-test.

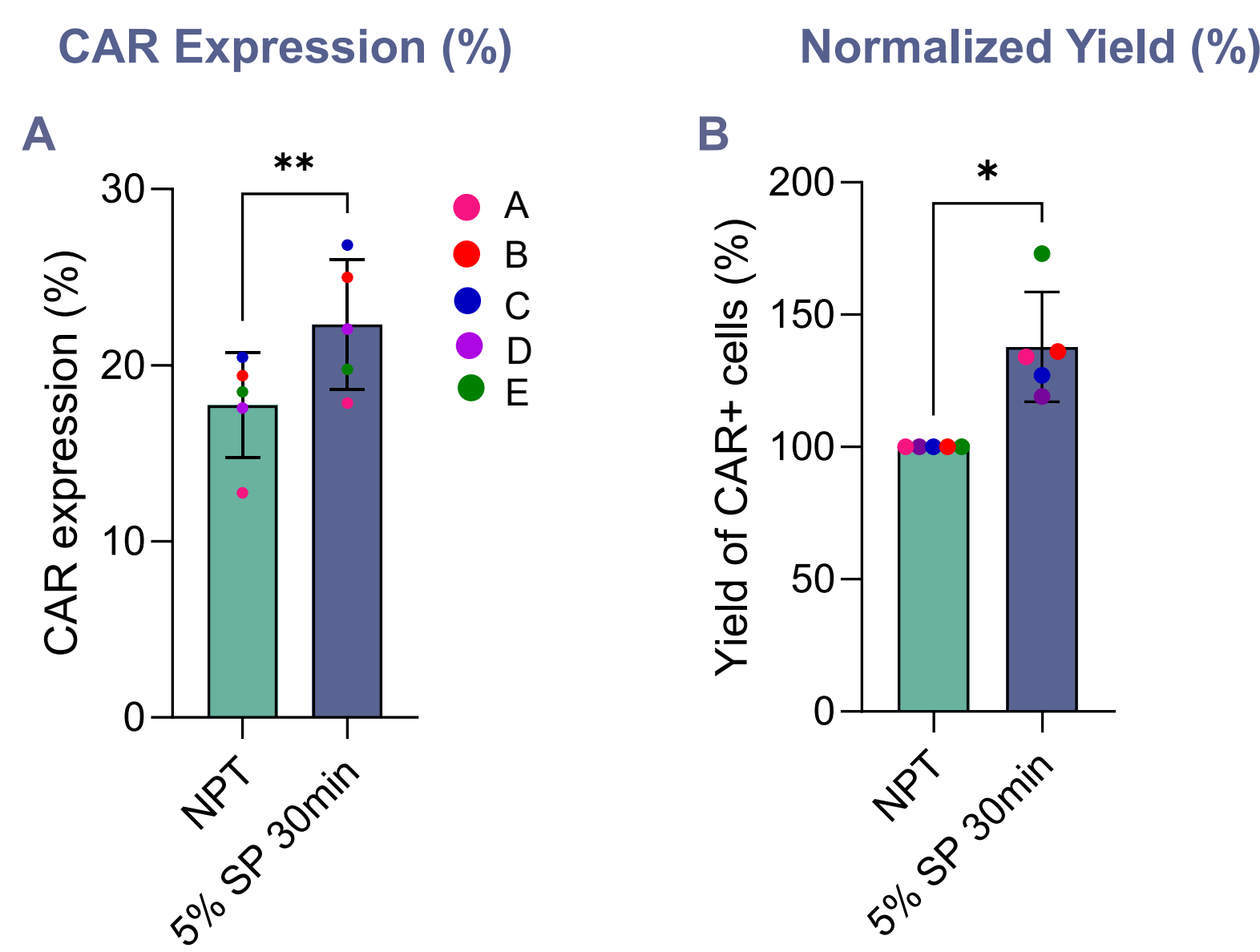
Figure 2 – Increased knock in and yield of ssDNA-GFP



75% increase in GFP Expression & 53% increase in Yield

Figure 2: Activated T cells (72hr; 5 donors) were pretreated with SoluPrime (5% v/v) for 30min prior to electroporation. The knock-in cargo (Cas9 RNP (TRAC; 36pmol) + ssDNA_TRAC_EGFP_CTS (8pmol)) was delivered using pulse code EO115. Cells were cultured for days 7 in TexMACS media post-electroporation. GFP expression (A) was assessed by flow cytometry. Yield of GFP positive cells (B; normalised to NPT) assessed at 7 days is also shown. Data is depicted as the mean ± SD from 5 donors (2-4 technical replicates per donor). NPT = No Pretreatment. **p<0.01; ***p<0.001, t-test.

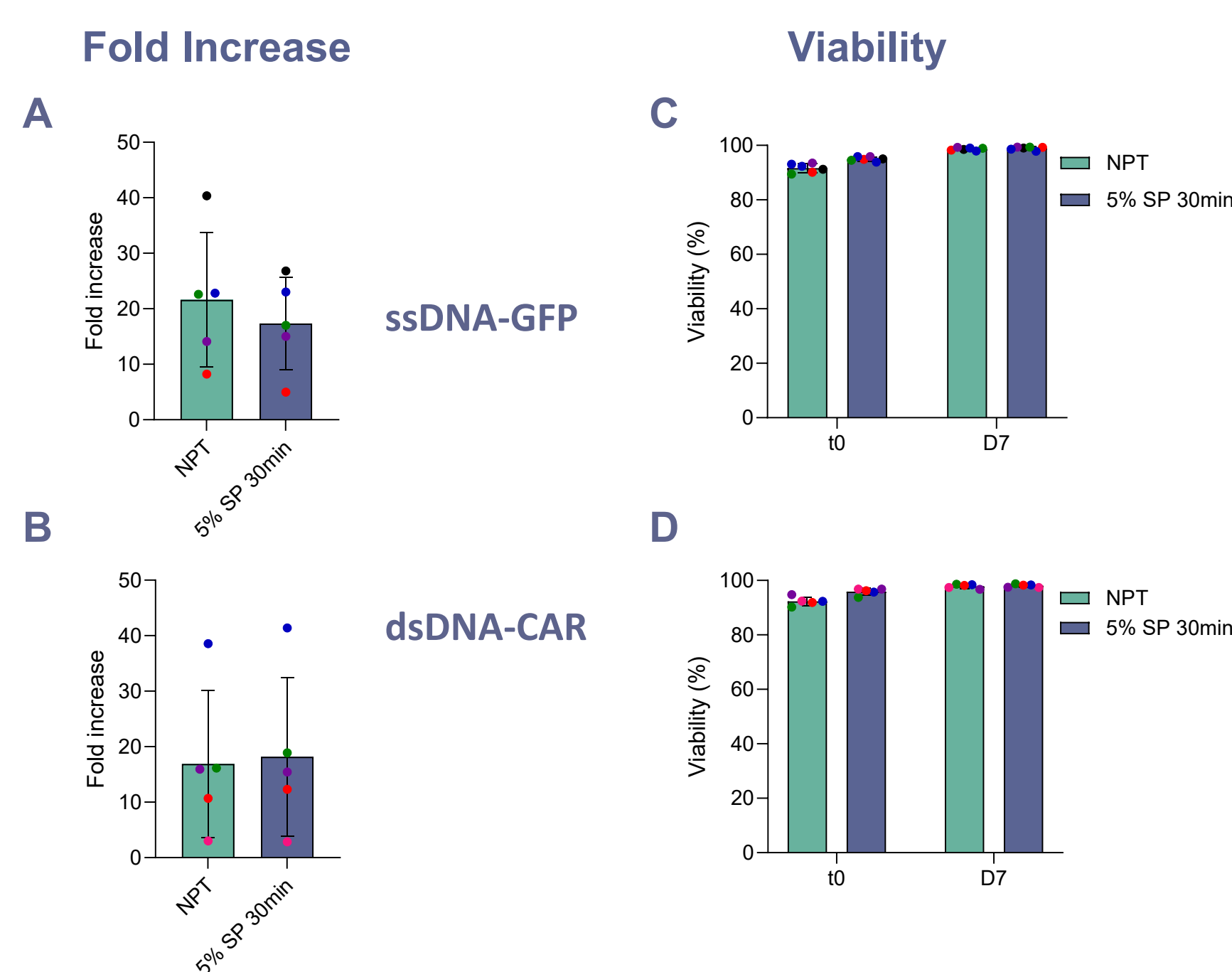
Figure 3 – Increased knock in and yield of dsDNA CD19-CAR



26% increase in CAR Expression & 38% increase in Yield

Figure 3: Activated T cells (72hr; 5 donors) were pretreated with SoluPrime (SP; 5% v/v) for 30min prior to electroporation. The knock-in cargo (Cas9 RNP (TRAC; 36pmol) + npCD19 CAR (8pmol)) was delivered using pulse code EO115. Cells were cultured for 7 days in TexMACS media post-electroporation. CAR expression (A) was assessed by flow cytometry. Yield of CAR positive cells (B; normalised to NPT) assessed at 7 days is also shown. Data is depicted as the mean ± SD from 5 donors (2 technical replicates per donor). NPT = No Pretreatment. *p<0.05; **p<0.01, t-test

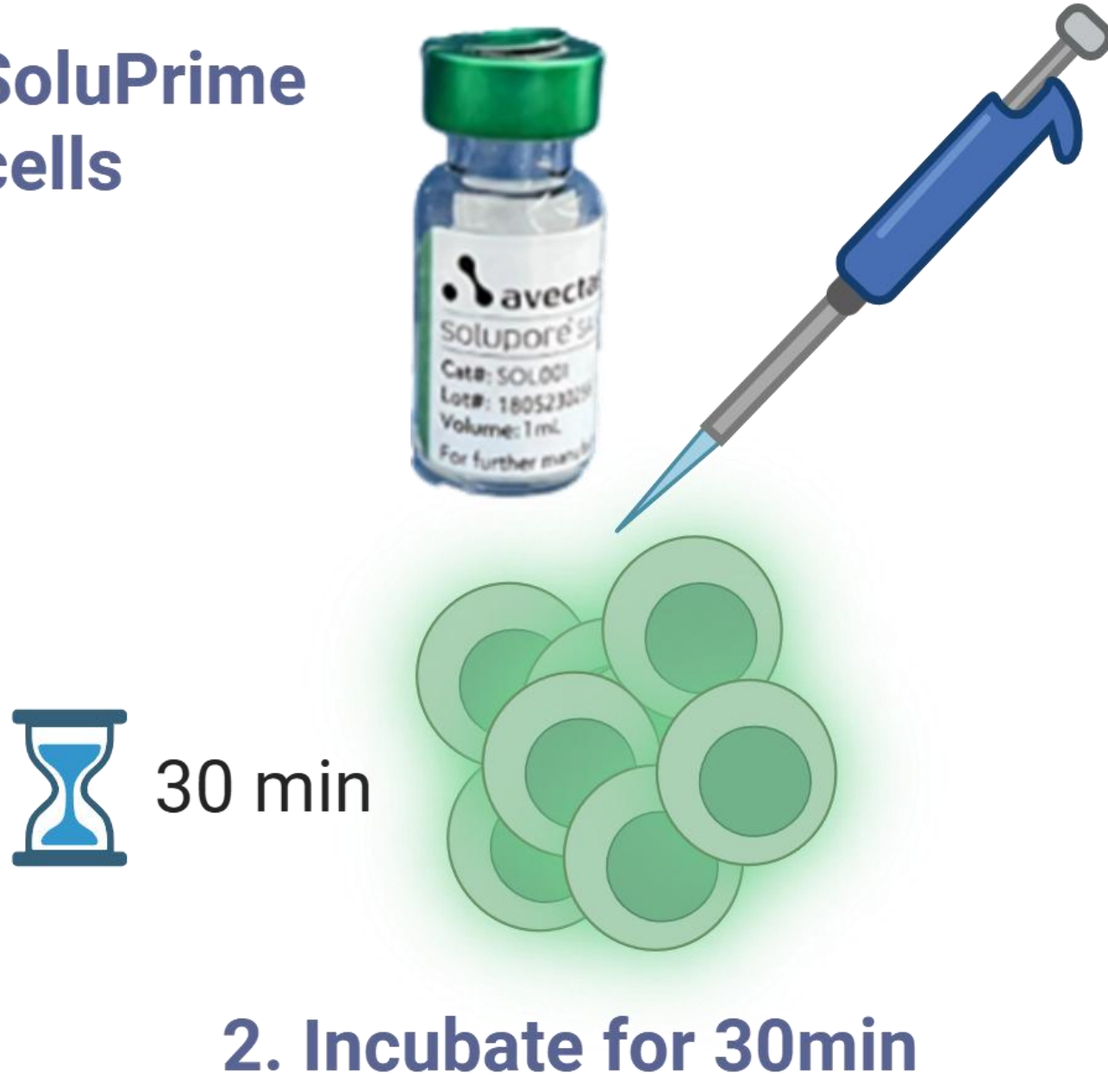
Figure 4 – No change in cell expansion



Cell expansion unaffected by SoluPrime

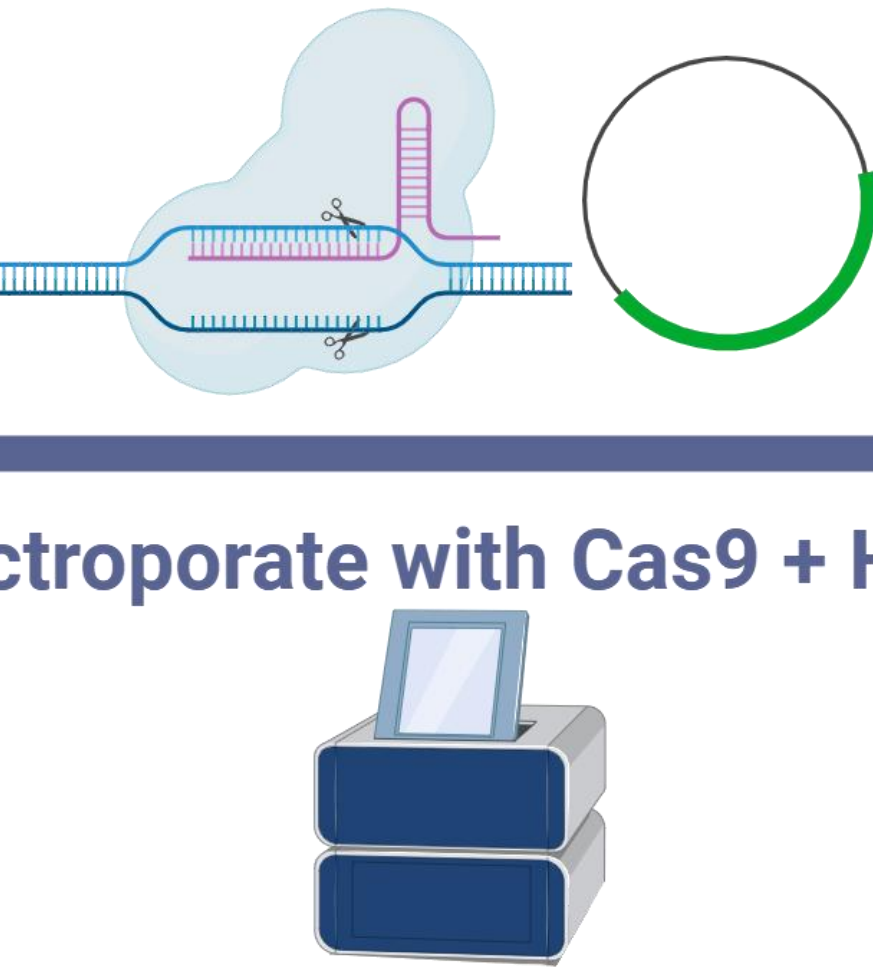
Figure 4: Activated T cells (72hr; 5 donors) were pretreated with SA (SP; 5% v/v) for 30min prior to electroporation. The knock-in cargo (Cas9 RNP (TRAC; 36pmol) + npCD19 CAR (8pmol); A&C) or (Cas9 RNP (TRAC; 36pmol) + npCD19 CAR (8pmol); B&D) was delivered using pulse code EO115. Cell counts and viability (C&D) were assessed immediately post-transfection(t0) and after 7 days in culture in TexMACS media and fold increase (A&B) calculated from seeding number. Data is depicted as the mean ± SD from 5 donors (2 technical replicates per donor).

1. Simply add SoluPrime to activated T cells

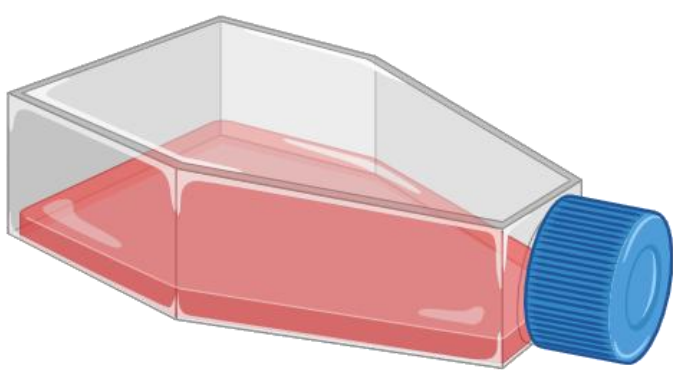


2. Incubate for 30min

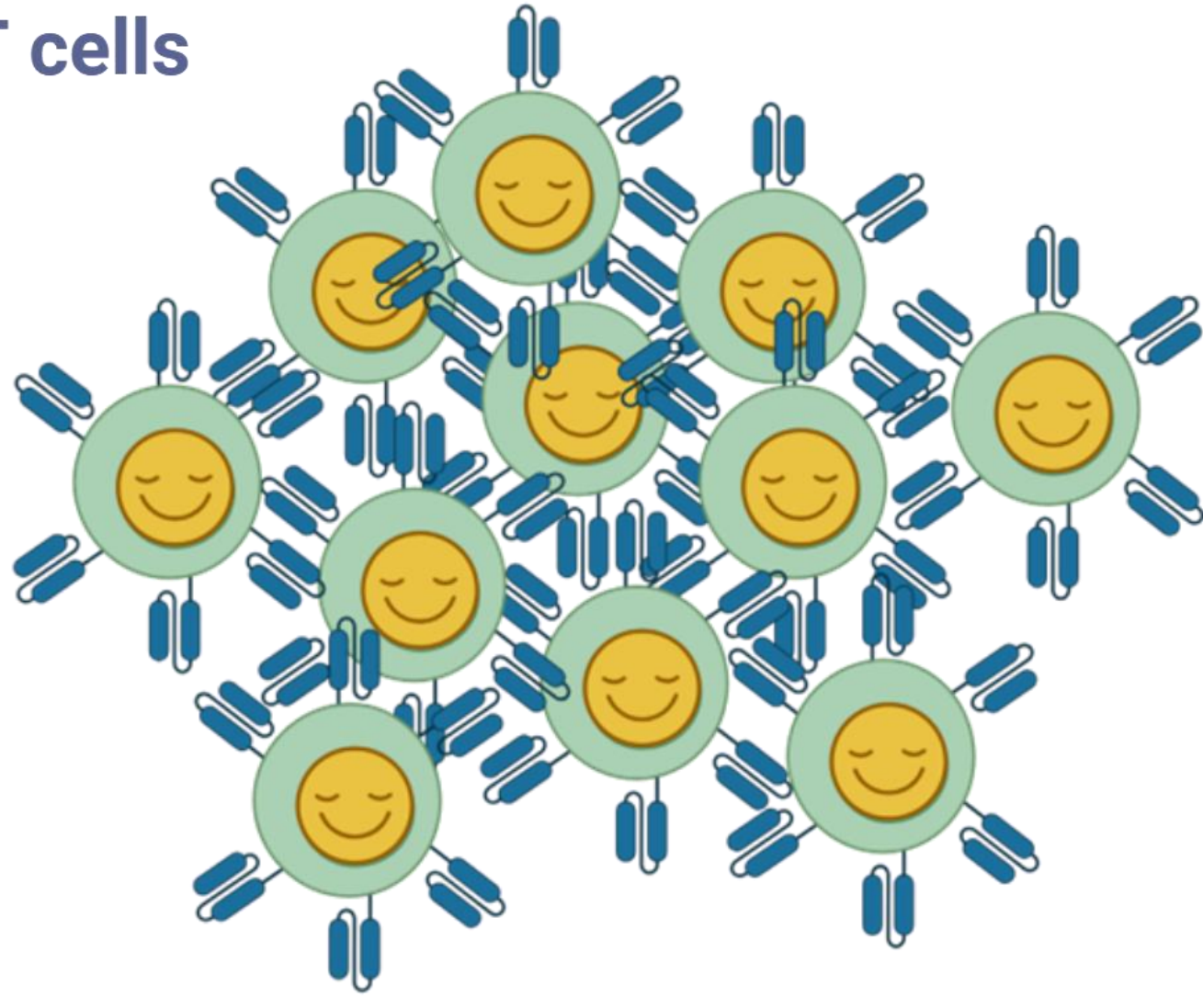
3. Electroporate with Cas9 + HDRT



4. Culture cells

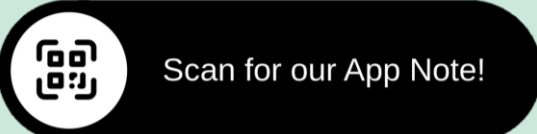


5. Harvest healthy CAR expressing T cells



Summary

- A simple process change 30min prior to electroporation with SoluPrime results in:
- Increased plasmid expression and MFI
 - Increased knock-in of ssDNA GFP construct
 - Increased yield of CAR-T cells



SoluPrime
by Avecitas