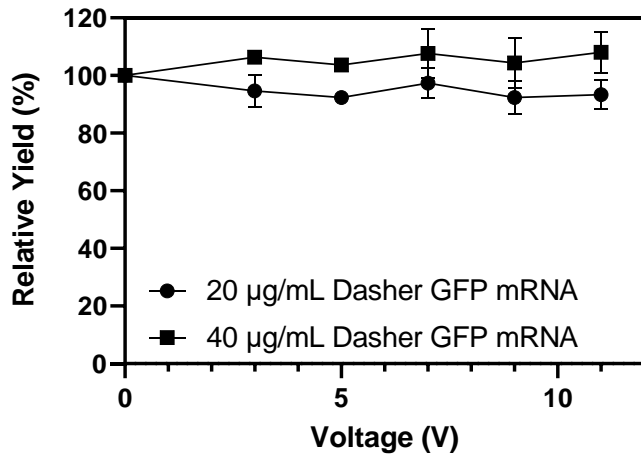


Scalable continuous-flow electroporation platform enabling T cell transfection for cellular therapy manufacturing

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A Jurkat cells



B Primary Pan T cells

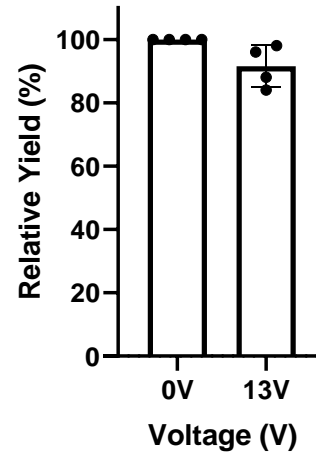


Figure S1: Delivery of mRNA encoding GFP to Jurkat and primary T cells. (A) Impact of varying waveform voltage amplitude on relative yield during delivery of mRNA to Jurkat cells ($n = 3$) or (B) primary T cells from four healthy donors ($n = 4$). Data shown as mean \pm standard deviation. Some error bars are too small to be visible (A).

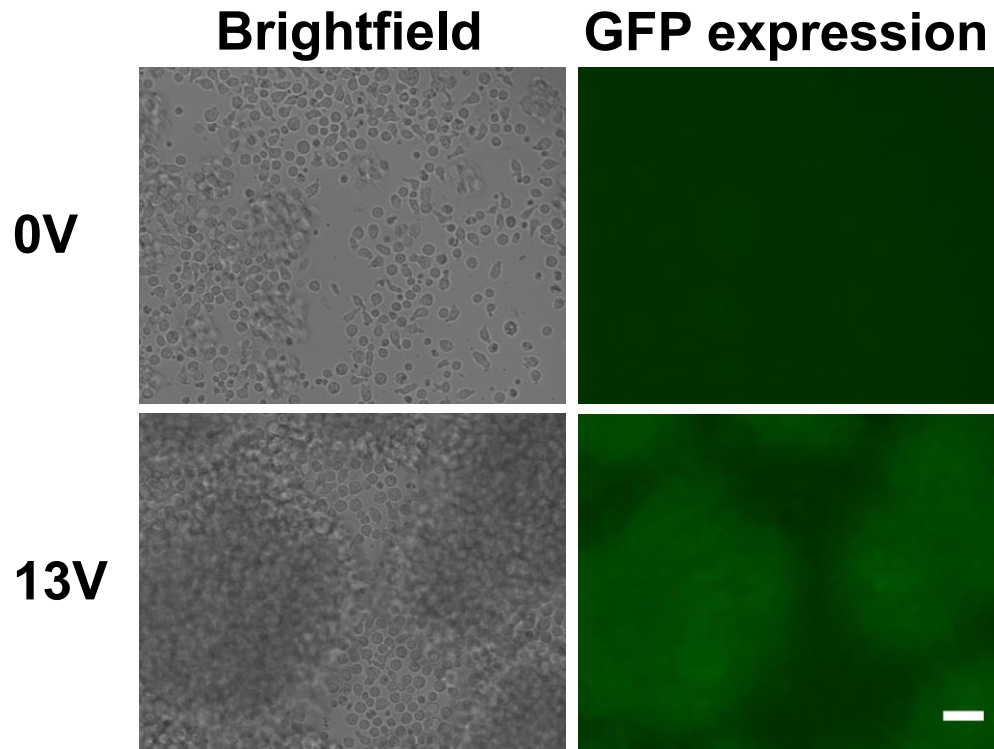


Figure S2: Representative microscopic images from primary T cells 24-h post-transfection with mRNA encoding for GFP (40 $\mu\text{g}/\text{mL}$). Waveform: Bipolar rectangular wave with $f = 100$ Hz, $V = 13\text{V}$, $t = 100$ μs . Scale bar, 50 μm .

Gene Pulser: Delivery of mRNA or plasmid DNA encoding GFP

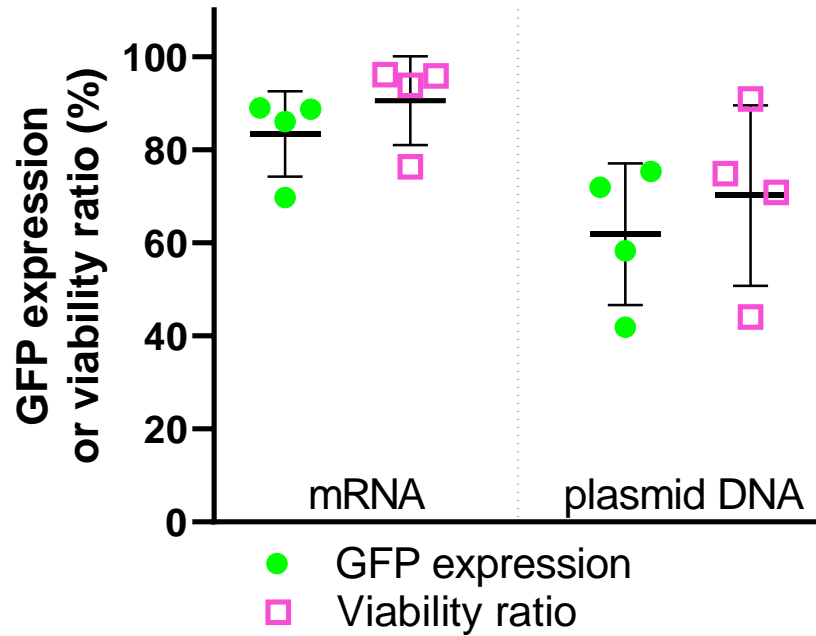


Figure S3: Transfection of primary T cells with mRNA (40 $\mu\text{g}/\text{mL}$) or plasmid DNA (75 $\mu\text{g}/\text{mL}$) encoding GFP using the Bio-Rad Gene Pulser. $n = 4$. Data shown as mean \pm standard deviation.

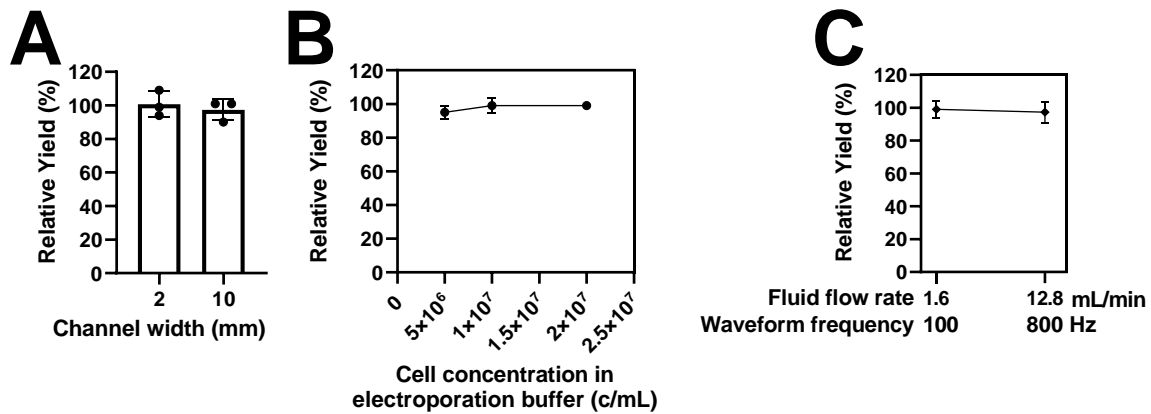


Figure S4: Increasing cell processing throughput for clinical-scale volumes. (A) Plot of relative yield from Jurkat cells transfected with mRNA encoding GFP in either the 2- or 10-mm channels ($n = 3$). (B) Plot of relative yield from Jurkat cells transfected with mRNA encoding GFP in the 2-mm channel at varying cell concentrations ($n = 3$). (C) Plot of relative yield from Jurkat cells transfected with mRNA encoding GFP in the 10-mm channel at varying flow rates and waveform frequencies ($n = 3$). Data shown as mean \pm standard deviation. Some error bars are too small to be visible (B).

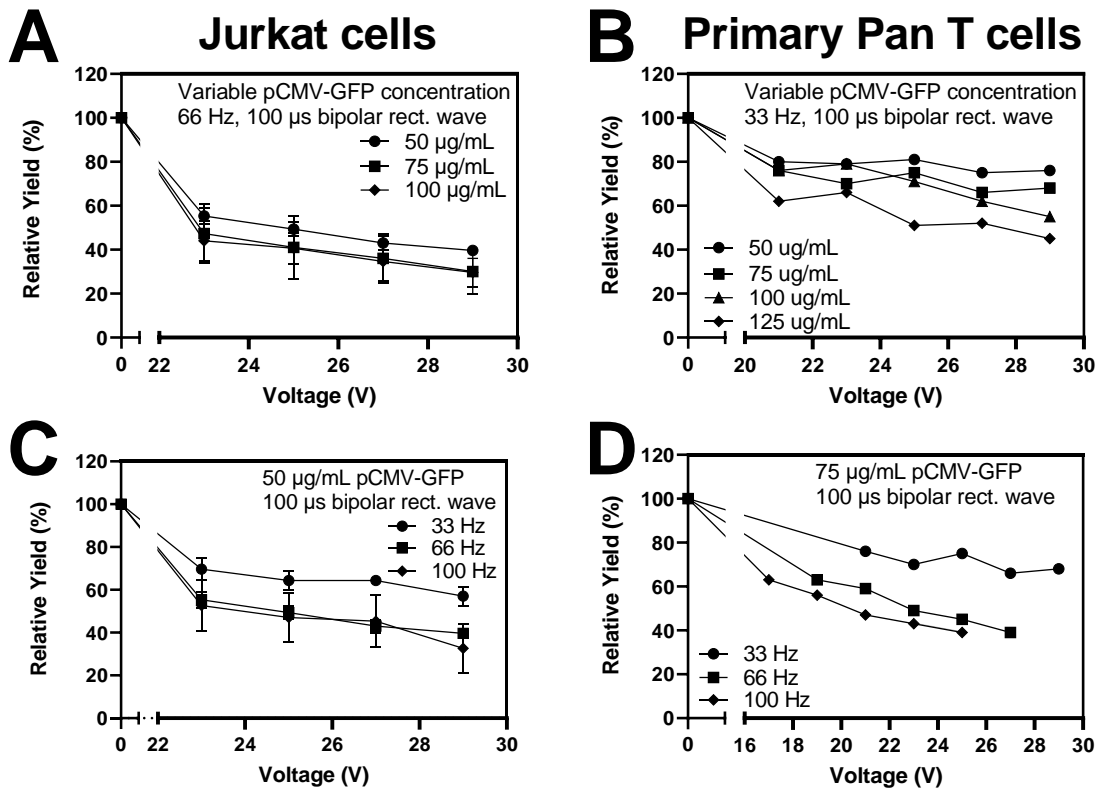


Figure S5: Relative yield data for various transfection parameters for delivering plasmid DNA to Jurkat and primary T cells. (A) Impact of varying plasmid concentration on relative yield for delivering plasmid DNA to Jurkat and (B) primary T cells. (C) Impact of varying waveform frequency on relative yield for delivering plasmid DNA to Jurkat and (D) primary T cells. Data shown as mean \pm standard deviation; $n = 3$ (A, C). Data shown as values from a representative donor; $n = 1$ (B, D).

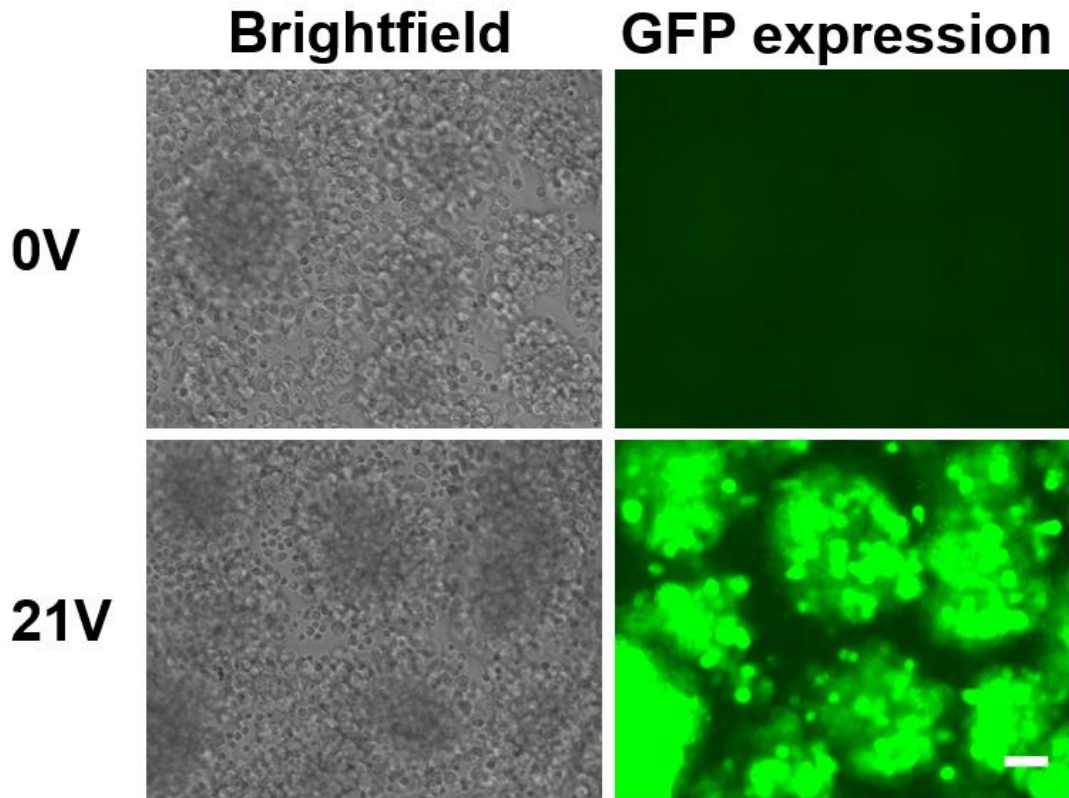


Figure S6: Representative microscopic images from primary T cells 24-h post-transfection with plasmid DNA encoding for GFP (75 $\mu\text{g}/\text{mL}$). Waveform: Bipolar rectangular wave with $f = 66$ Hz, $V = 21$ V, $t = 100$ μs . Scale bar, 50 μm .

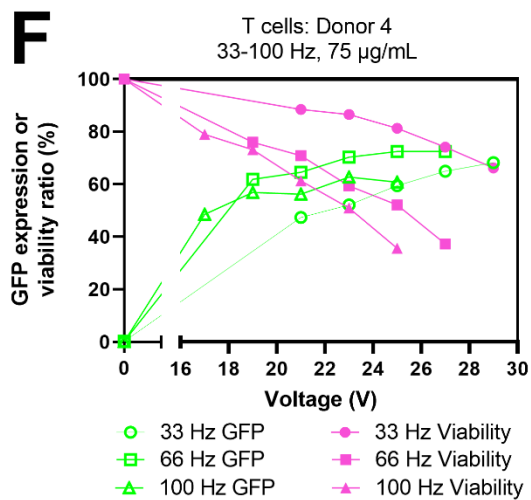
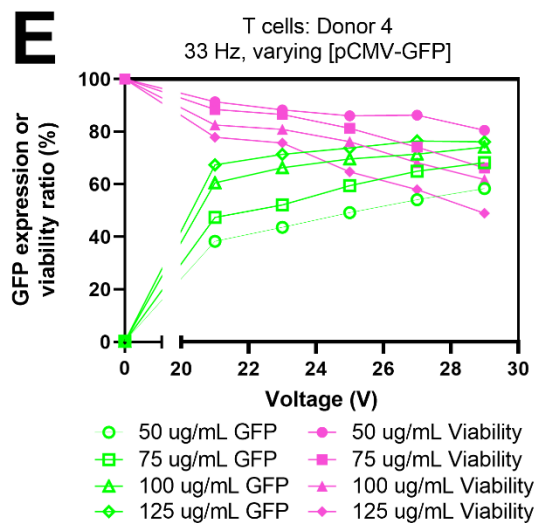
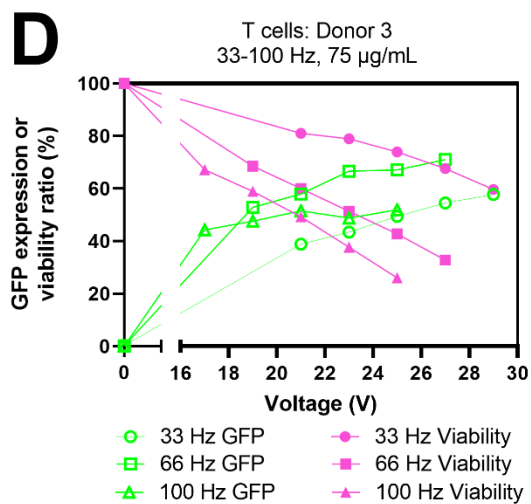
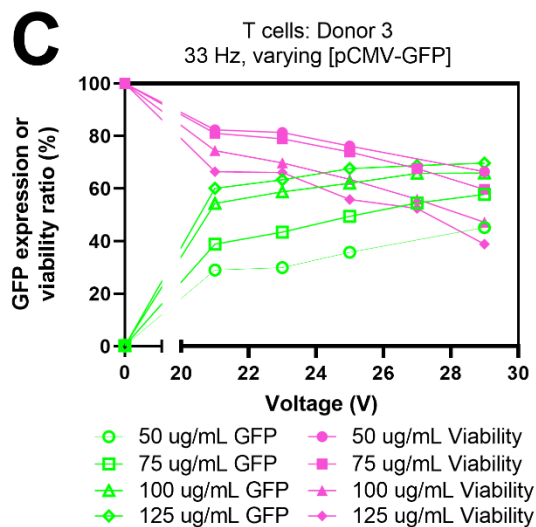
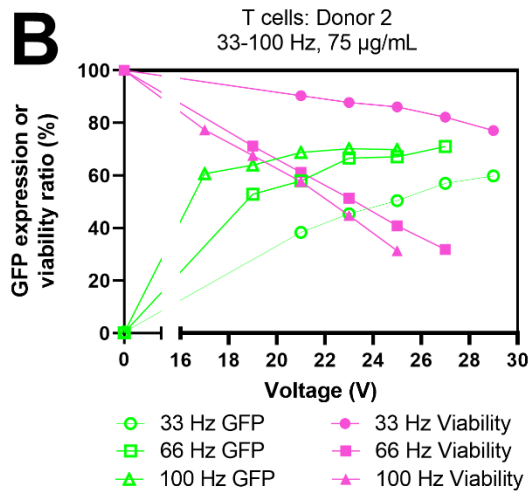
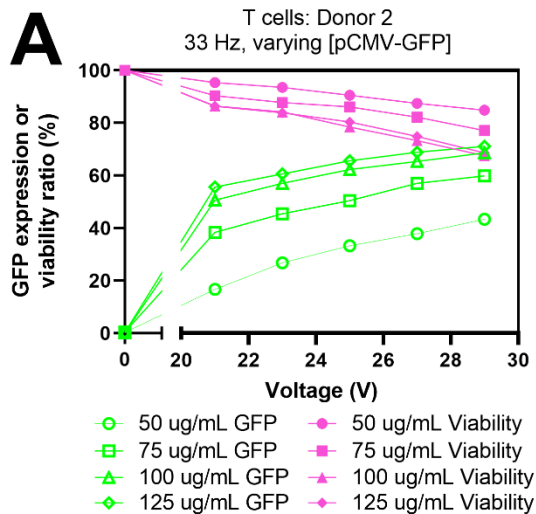


Figure S7: Results for various transfection parameters for delivering plasmid DNA to primary T cells from additional donors. (A) Impact of varying plasmid concentration and (B) waveform frequency for primary T cell donor 2. (C) Impact of varying plasmid concentration and (D) waveform frequency for primary T cell donor 3. (E) Impact of varying plasmid concentration and (F) waveform frequency for primary T cell donor 4.

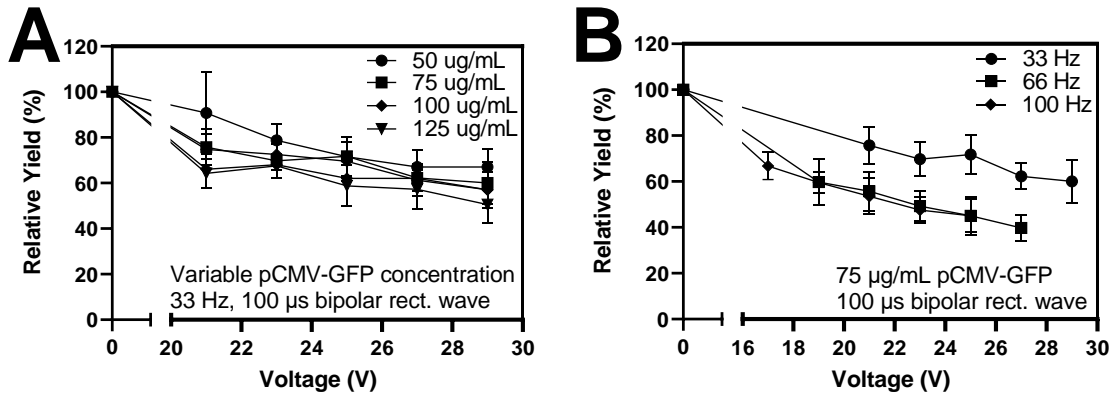


Figure S8: Relative yield data for delivering plasmid DNA primary T cells from four healthy donors. (A) Impact of varying plasmid concentration on relative yield for delivering plasmid DNA to primary T cells. (B) Impact of varying waveform frequency on relative yield for delivering plasmid DNA to primary T cells. Data shown as mean \pm standard deviation; $n = 4$. Data shown here include relative yield data presented in Figure S5B and S5D.

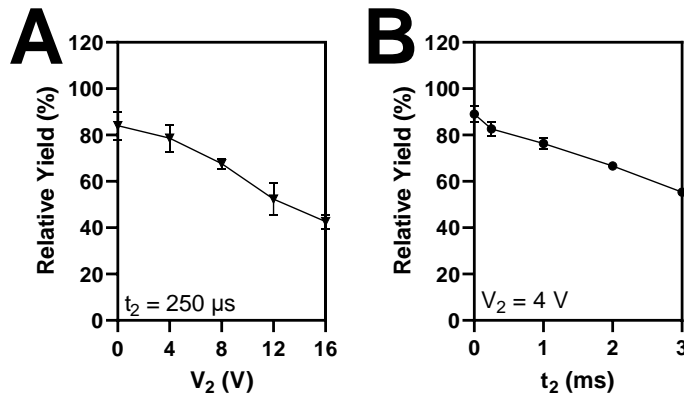


Figure S9: Relative yield data from delivery of an arbitrary electrical waveform to Jurkat cells. (A) Impact of varying V_2 while $t_2 = 250 \mu\text{s}$ ($n = 3$). (B) Impact of varying t_2 while $V_2 = 4 \text{ V}$ ($n = 3$). In both (A) and (B), we fix $f = 66 \text{ Hz}$, $V_1 = 21 \text{ V}$, and $t_1 = 75 \mu\text{s}$. Data shown as mean \pm standard deviation. Some error bars are too small to be visible.

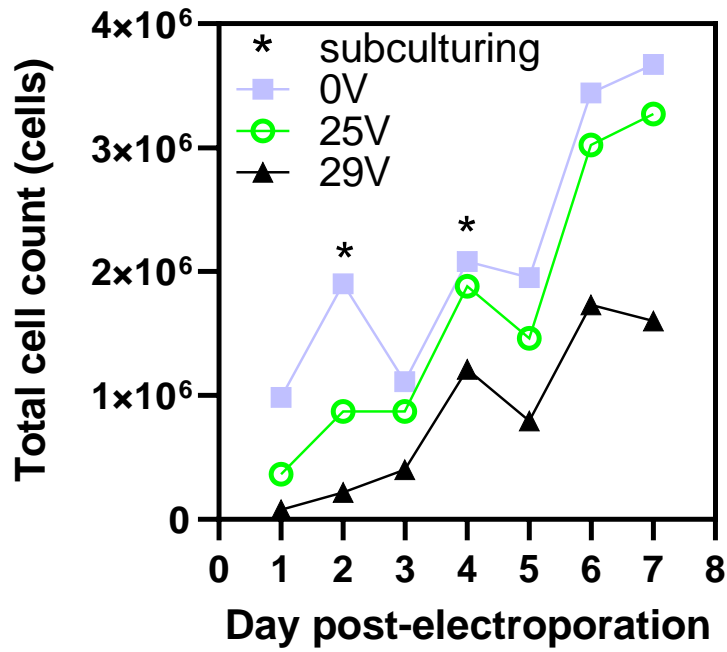


Figure S10: Proliferation of primary T cells transfected with ribonucleoproteins (RNPs) targeting TRAC/TRBC. Plot of total cell count as a function of days post-electroporation from a representative donor ($n = 1$). T cells were subcultured on days 2 and 4 (indicated by *) using fresh media supplemented with IL-2 (100 IU/mL).