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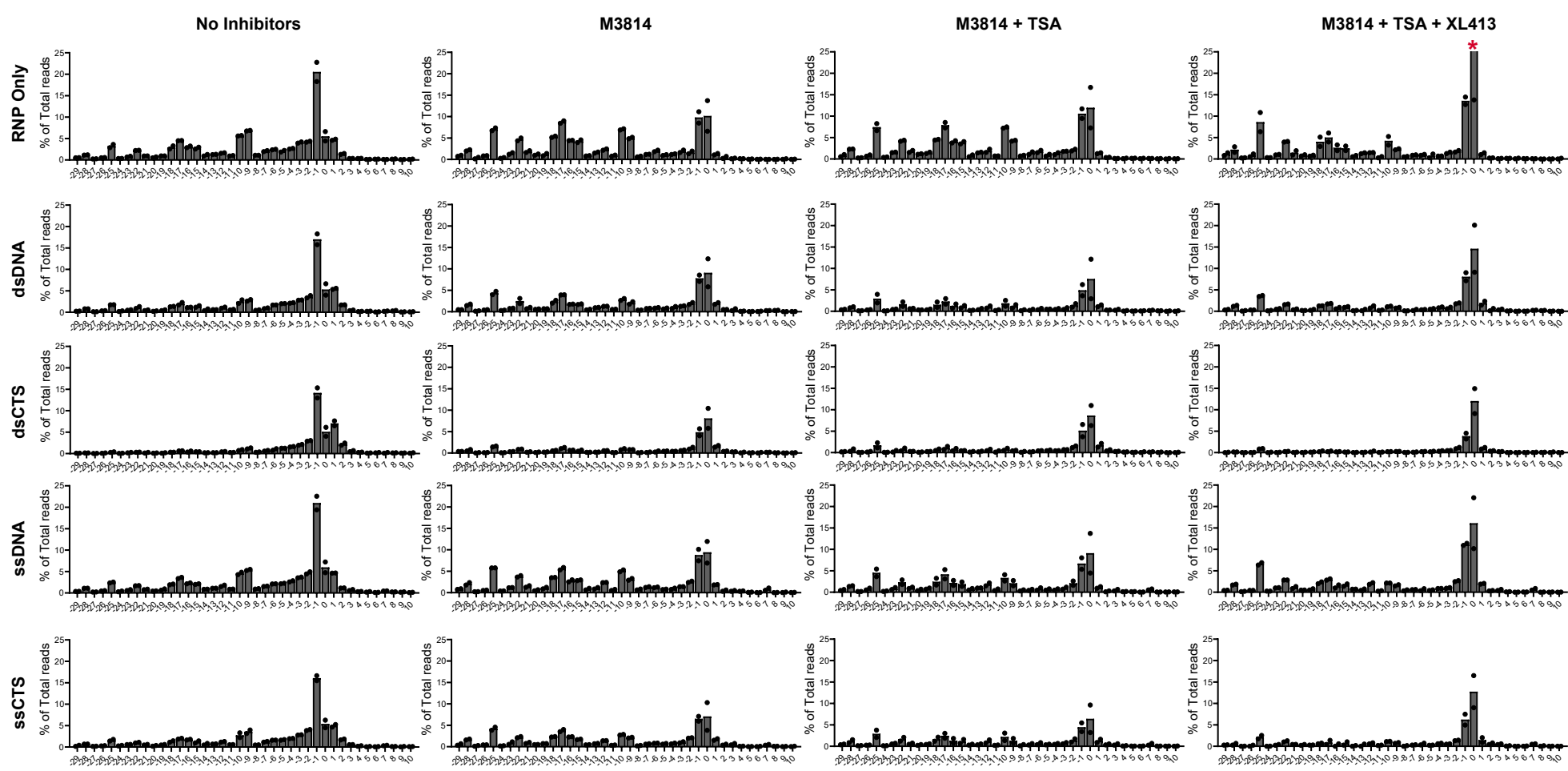
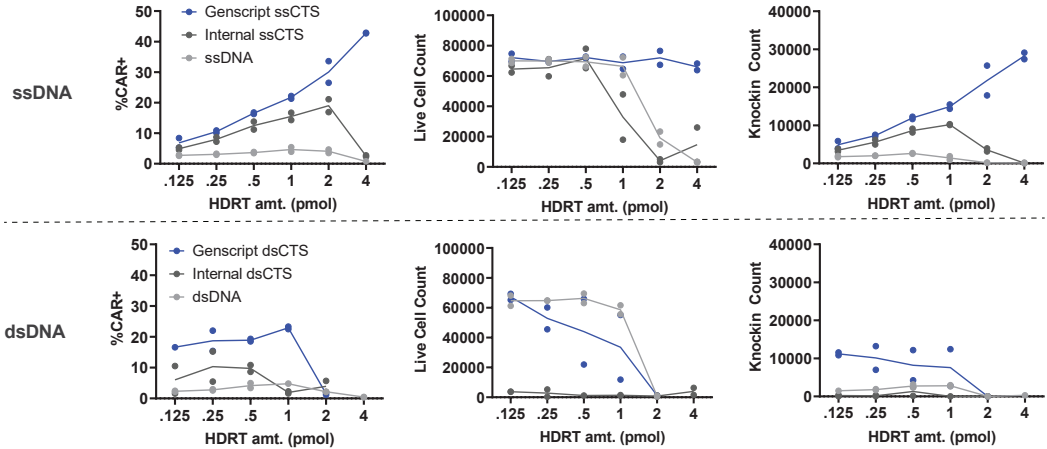
Supplementary information

High-yield genome engineering in primary cells using a hybrid ssDNA repair template and small-molecule cocktails

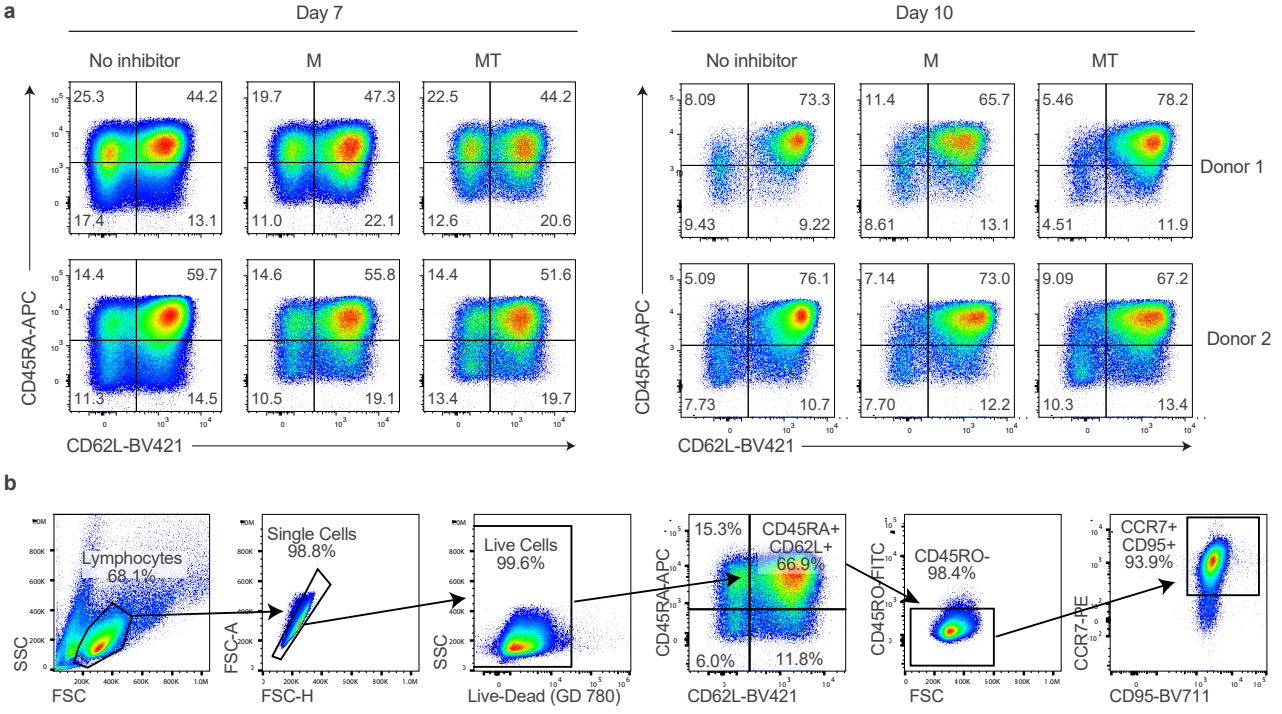
In the format provided by the authors and unedited



Supplementary Figure 1 – Comparison of indels generated in the presence of HDRT and inhibitor combinations. Comparison of insertions and deletions (indels) generated in the presence of a CD5 Cas9 RNP +/- small dsDNA, dsCTS, ssDNA, or ssCTS CD5-HA HDRTs at non-toxic concentrations (800nM) with and without M3814, M3814 + TSA, and M3814 + TSA + XL413 inhibitor combinations. 0 = unmodified sequence. Representative plots from a single independent healthy human blood donor are shown. *% of total reads for "RNP Only M3814 + TSA + XL413" Donor 2 sample (36.5%) could not be displayed within Y axis. RNP = ribonucleoprotein, HDRT = homology-directed repair template, dsCTS = dsDNA + CTS HDRT, ssCTS = ssDNA + CTS HDRT.



Supplementary Figure 2 – Comparison of Genscript versus in-house HDRT production
Comparison of Genscript HDRTs with internally generated HDRTs for both ssCTS (top) and dsCTS templates (bottom). Shown for each are %knock-in, live cell counts, and knock-in cell counts in comparison to internally generated ssDNA or dsDNA controls, respectively. Each experiment was performed with T cells from 2 independent healthy human blood donors represented by individual dots + mean. RNP = Ribonucleoprotein, CTS = Cas9 Target Site, dsCTS = dsDNA HDRT + CTS sites, ssCTS = ssDNA HDRT + CTS sites, HDRT = homology-directed-repair template.



Supplementary Figure 3 – Cellular Phenotyping of large-scale CAR-T production
(a) Representative flow plots for T cell immunophenotype analysis based on CD45RA and CD62L expression at Day 7 and Day 10 post-activation. (b) Gating strategy for extended flow cytometric analysis demonstrating CD45RA+CD62L+CD45RO-CCR7+CD95+ population immunophenotypically consistent with a Tscm population.