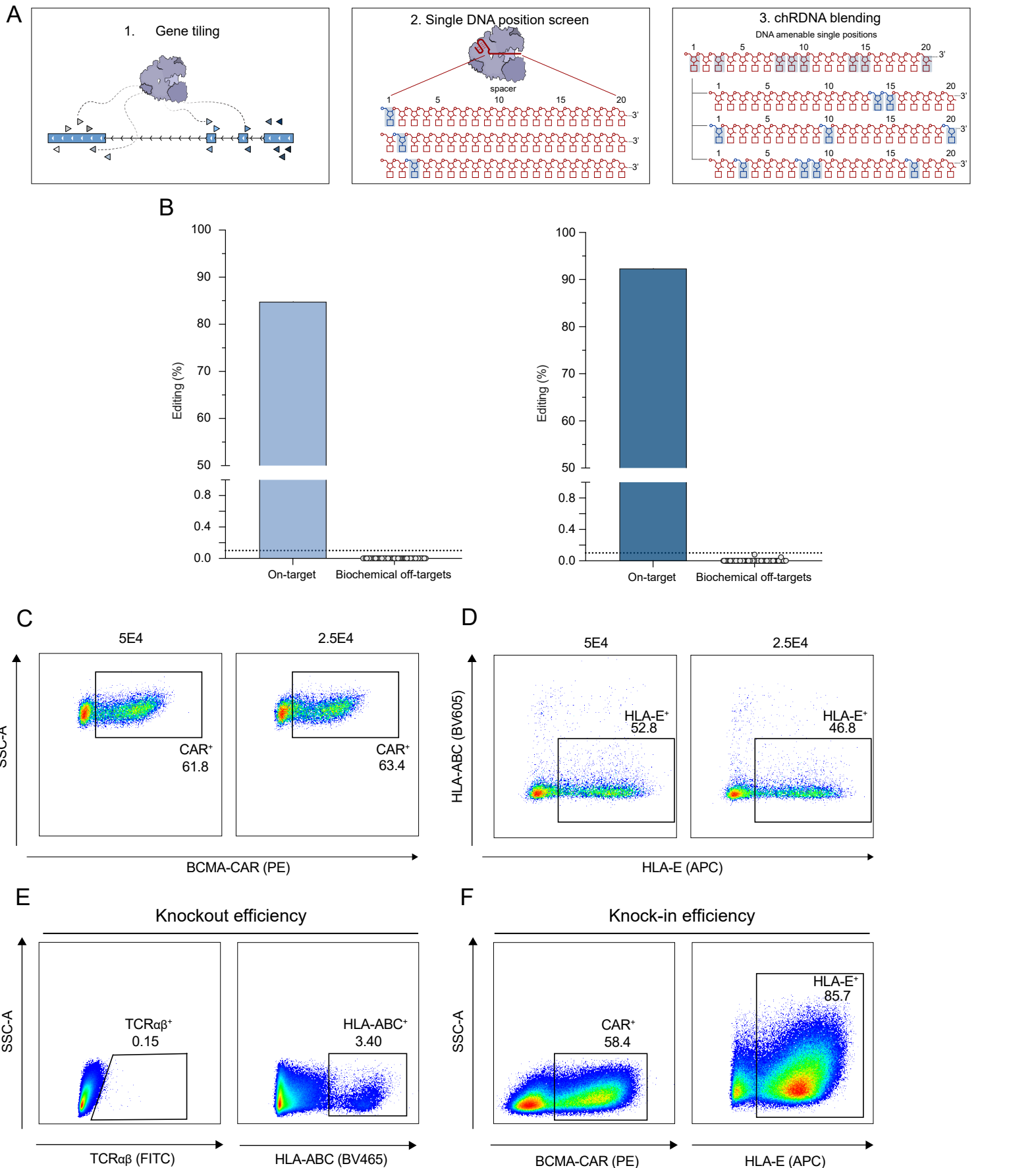


Figure S1. chRDNA-mediated gene editing did not result in any off-target editing above the limit of detection.



(A) Schematic representation of the chRDNA design process. (B, left panel) Editing rates of the optimized *TRAC* chRDNA at the *TRAC* locus on-target site and off-target sites detected in the biochemical SITE-Seq[®] assay, each point is the mean of 3 biological replicates, lower limit of detection 0.1% (dashed line). (B, right panel) Editing rates of optimized *B2M* chRDNA for the *B2M* on-target site and off-target sites detected in the biochemical SITE-Seq[®] assay, each point is the mean of 3 biological replicates, lower limit of detection 0.1% (dashed line). (C) Dot plots of BCMA-CAR transgene insertion with AAV MOI titration. (D) Dot plots of B2M-HLA-E fusion protein transgene insertion with AAV MOI titration. (E) Representative dot plots of the TCR $\alpha\beta$ and HLA-ABC⁻ knockout efficiencies of the drug product. (F) Representative dot plots of the BCMA-CAR transgene and B2M-HLA-E fusion protein knock-in efficiencies.

AAV, adeno-associated virus; B2M, beta-2 microglobulin; BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; chRDNA, CRISPR hybrid RNA-DNA; HLA, human leukocyte antigen; MOI, multiplicity of infection; TRAC, T cell receptor alpha constant.