PBMC-derived SMRTs showed > 90% predicted efficacy for the brain sequences. Furthermore, the Multiple Lentiviral Expression System (MULE) is currently being used to engineer lentiviral constructs to deliver Sa or Sp Cas9 and targeted gRNAs to cells. These lentiviruses will be produced in a library approach to simultaneously measure the ability of thousands of gRNAs to silence HIV-1 expression.

Conclusions: Accumulation of this data as well as delivery of a library of gRNAs will allow analysis of Sa and Sp Cas9 enzyme efficiency, gRNA specificity – including consideration of the effect of a leading G nucleotide in the protospacer, and on- and off-target cleavage efficacy.

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Construct a series of universal gRNAs targeting various regions within HIV

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Background: The persistence of proviral DNA in infected CD4+ leukocytes presents a barrier to HIV-1 treatment. HIV is one of the most genetically diverse pathogens due to its high mutation and recombination rates, large population size, and rapid replication rate. Coinfection and superinfection by divergent HIV strains have become more common. While CRISPR-saCas9 can eliminate latent proviral DNA, its efficacy is limited by HIV strain diversity. Thus, it's critical to design a global patient coverage gRNA.

Methods: We initially use pNl43 as a reference strain to list all potential gRNAs that target LTR, Gag, Pol, and Tat. Bioinformatics analysis was performed to check gRNA conservation with 4725 complete sequences from the Los Alamos database. Fifteen potential gRNA were selected, and the efficacy of those gRNAs was affirmed by electroporation with through Lonza electroporation system in Myeloid cells and T cells. Treated cells were evaluated for viral DNA excision spanning target for gRNA by monitoring HIV-1 DNA, protein, and progeny virus levels.

Results: The virus was reduced by up to 93% after single-gRNA CRISPR RNP treatments, respectively. No recorded off-target cleavages were detected. gRNA-LTR-508, gRNA-ψ-796, gRNA-Gag-1842, and gRNA-Pol-2396 as best candidate gRNA due to their high conservation (up to 79%), low off-target, and high efficacy were selected to do single gRNA and combination dual gRNA screen in ex vivo with primary CD4+ T cells. Up to 22% indel mutation was detected with single gRNA treatment and around 50% HIV p24 decrease in dual treatment primary CD4+ T cells.

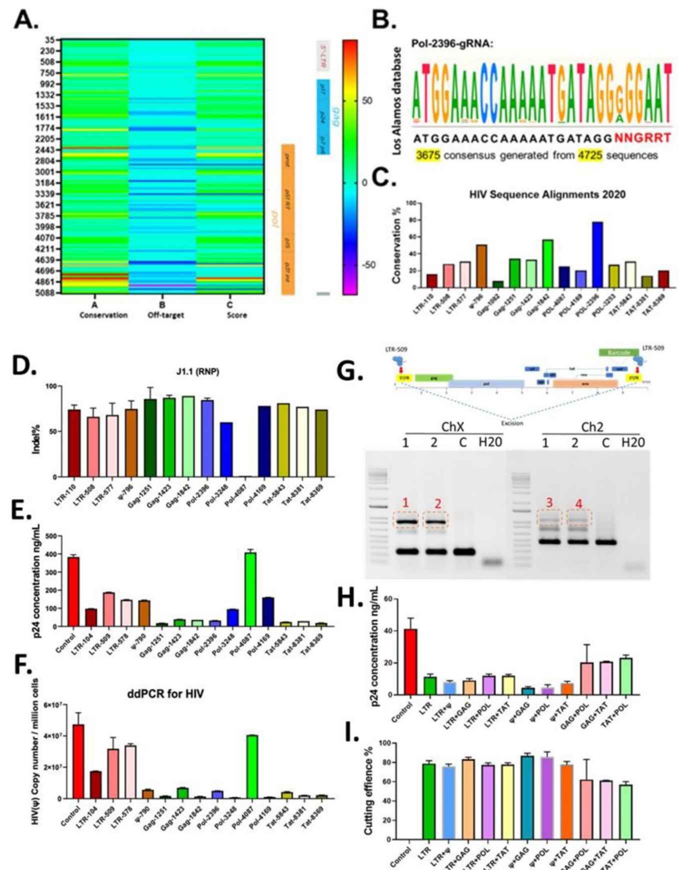

Figure. Single gRNA and combination dual gRNA screen.

Conclusions: Our results demonstrated that our gRNAs afford broader antiviral coverage with low off-target, and high efficacy providing a promising step in the direction of eliminating HIV-1 infection through gene editing approaches.

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