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Abstract:

New genetic tools are needed to understand the functional interactions between HIV and human host factors in primary cells. We recently developed a method to edit the genome of primary CD4+ T cells by electroporation of CRISPR/Cas9 ribonucleoproteins (RNPs). Here, we adapted this methodology to a high-throughput platform for the efficient, arrayed editing of candidate host factors. CXCR4 or CCR5 knockout cells generated with this method are resistant to HIV infection in a tropism-dependent manner, whereas knockout of LEDGF or TNPO3 results in a tropism-independent reduction in infection. CRISPR/Cas9 RNPs can furthermore edit multiple genes simultaneously, enabling studies of interactions among multiple host and viral factors. Finally, in an arrayed screen of 45 genes associated with HIV integrase, we identified several candidate dependency/restriction factors, demonstrating the
power of this approach as a discovery platform. This technology should accelerate target validation for pharmaceutical and cell-based therapies to cure HIV infection.