Supplementary Information

Search-and-replace genome editing without double-strand breaks or donor DNA

Andrew V. Anzalone, Peyton B. Randolph, Jessie R. Davis, Alexander A. Sousa, Luke W. Koblan, Jonathan M. Levy, Peter J. Chen, Christopher Wilson, Gregory A. Newby, Aditya Raguram, and David R. Liu

Supplementary Discussion	Page # 2-5
Supplementary Table 1 . Activities of prime editors, Cas9 nuclease, Cas9 H840A nickase, and PE2-dRT at <i>HEK3</i> , <i>HEK4</i> , <i>EMX1</i> , and <i>FANCF</i> on-target and off-target sites	6
Supplementary Table 2 . Sequences of DNA oligonucleotides, pegRNAs and sgRNAs used for <i>in vitro</i> experiments	7
Supplementary Table 3 . Sequences of pegRNAs and sgRNAs used in mammalian cell experiments	8-16
Supplementary Table 4 . Sequences of primers used for mammalian cell genomic DNA amplification and HTS	17
Supplementary Table 5 . Sequences of 100-mer single-stranded DNA oligonucleotide donor templates used in HDR experiments	18
Supplementary Sequences 1 . Sequences of yeast dual fluorescent reporter plasmids used in this study	19-22
Supplementary Sequences 2 . DNA sequences of mammalian prime editor plasmids, example pegRNA plasmid, and lentivirus plasmids	23-29
Supplementary Sequences 3 . Amino acid sequences of prime editors and M-MLV reverse transcriptase mutants used in this study	30
Supplementary Note 1 . FACS gating examples for GFP-positive cell sorting and cell line genotypes	31-33
Supplementary Note 2. FACS sorting of GFP-positive neuronal nuclei	34
Supplementary Note 3. Method for cloning pegRNAs	35-37
Supplementary Note 4. Python script for quantifying pegRNA scaffold integration	38-39
Supplementary Figure 1.	40
Supplementary References	41

The Supplementary Information file contains:

- Supplementary Discussion that provides additional background and descriptions of the data,
- Supplementary Tables 1-5 that list activity data and short DNA and RNA sequences,
- Supplementary Sequences 1-3 that contain DNA and protein sequences of key constructs,
- · Supplementary Notes 1-4 that contain FACS information, cloning methods, and scripts,
- Supplementary Figure 1 that contains original gel image data, and
- Supplementary References.