

Supplementary Information

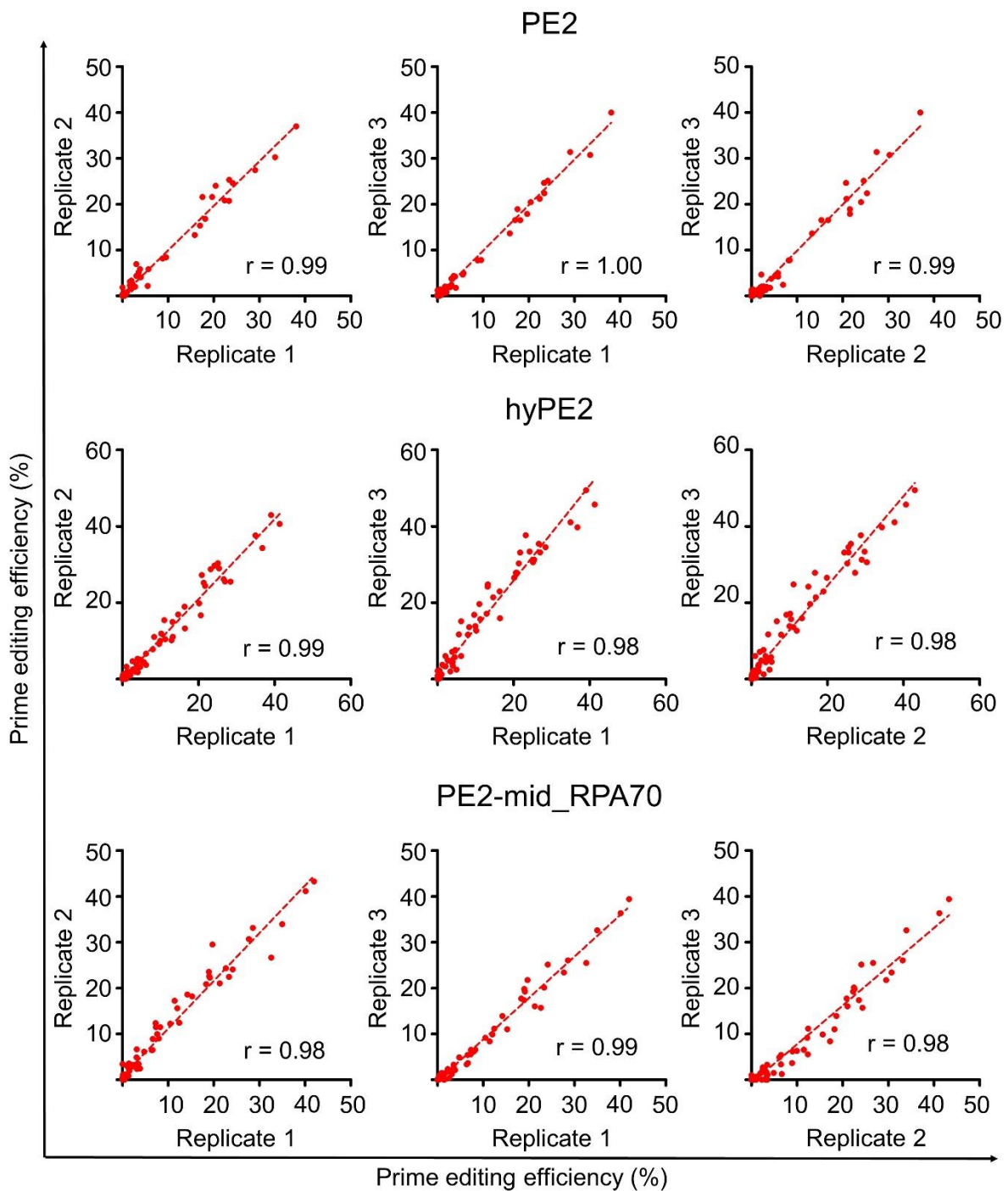
Generation of a more efficient prime editor 2 by addition of the Rad51 DNA-binding domain

Contents:

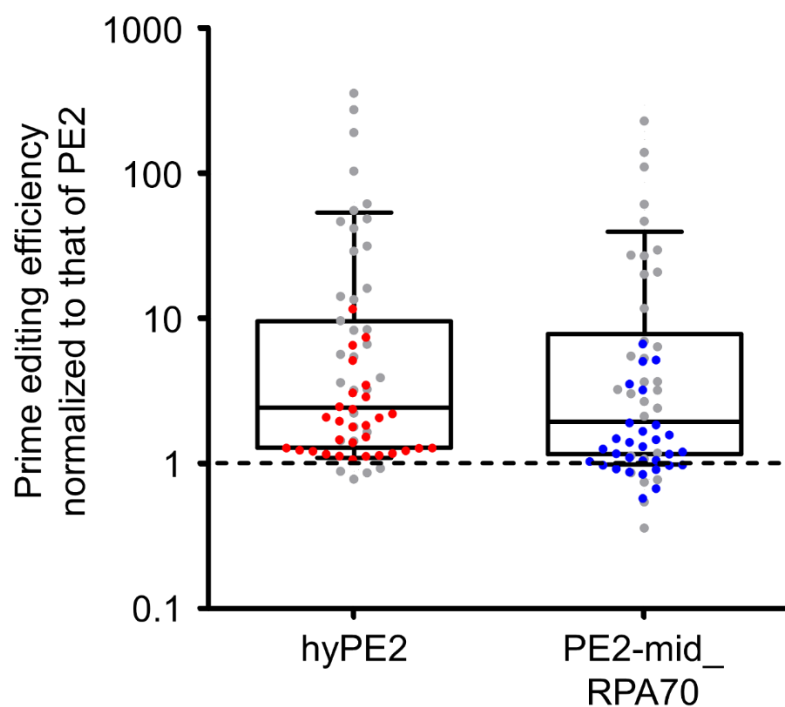
Supplementary Figures 1 – 5

Supplementary Tables 1 – 4

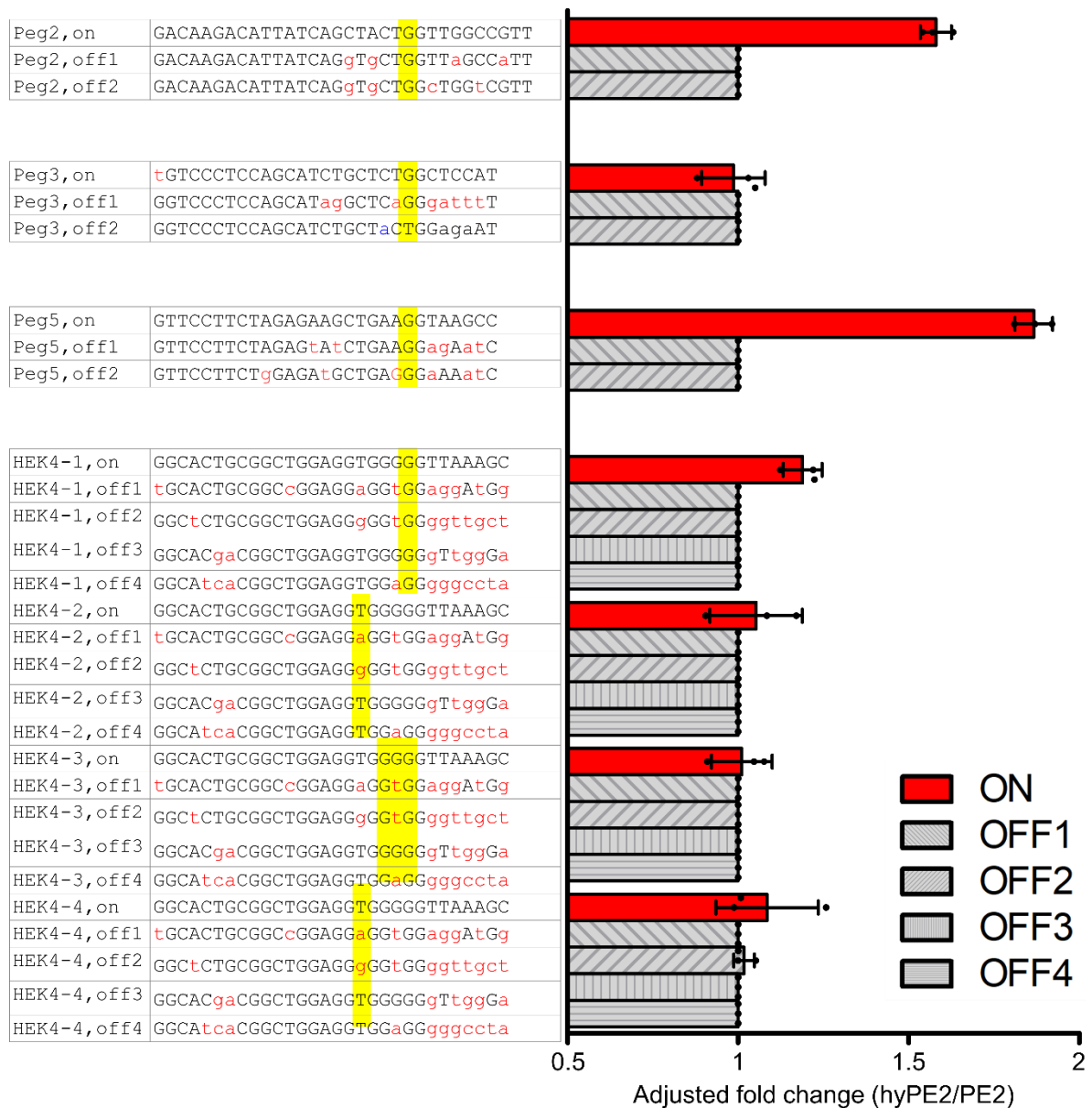
Supplementary Note



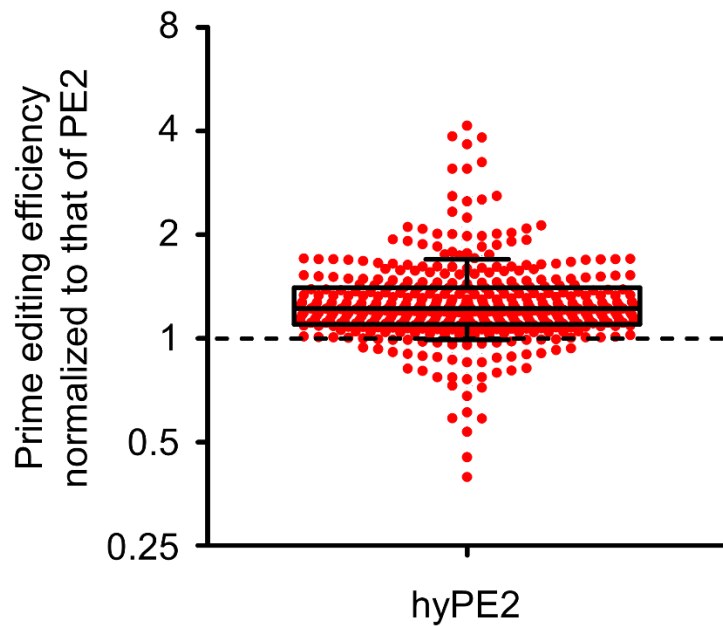
Supplementary Figure 1. Correlations between prime editing efficiencies from biological replicates for high-throughput evaluations of prime editing activities. The biological replicates were independently cultured, transfected with plasmids encoding prime editor 2 (variants), and analyzed. The Pearson correlation coefficients (r) and trend lines are shown. The number of analyzed target sequences $n = 83$. Source data are provided as a Source Data file.



Supplementary Figure 2. Prime editing efficiencies of PE2 variants normalized to the efficiency of PE2 at the same target sequences, which had been lentivirally integrated in HEK293T cells. Target sequences with PE2-induced prime editing efficiencies less than 1% are shown as gray dots. The number of target sequences $n = 64$. Data of minimum to maximum values are presented. For the boxes, the top, middle, and bottom lines represent the 25th, 50th, and 75th percentiles, respectively. The whiskers indicate the 10th and 90th percentile values. Source data are provided as a Source Data file.

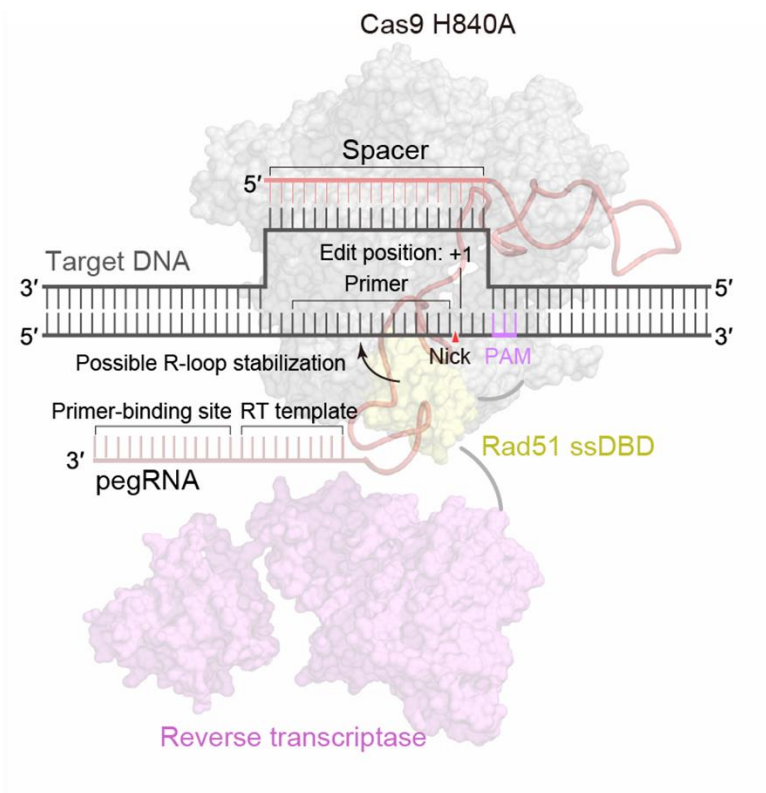


Supplementary Figure 3. Off-target effects of hyPE2 and PE2 at the potential off-target sites for pegRNAs 2, 3, and 5 and for four different pegRNAs targeting HEK4 in HEK293T cells. The intended editing positions are highlighted in yellow; mismatched and bulged nucleotides are indicated in red and blue lower-case fonts, respectively. Data points are overlaid. Data are means \pm S.D. for three independent biological replicates. on, on-target site; off, off-target site. Source data are provided as a Source Data file.

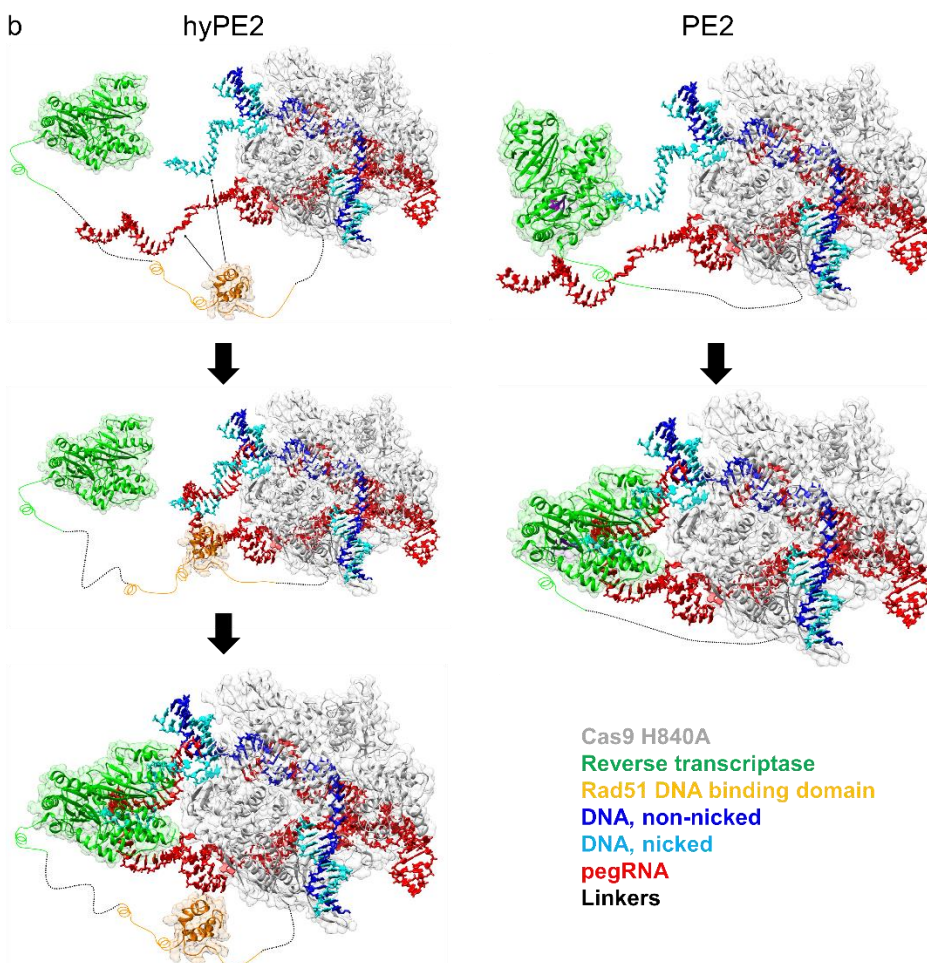


Supplementary Figure 4. Prime editing efficiencies of PE2 variants normalized to the efficiency of PE2 at the same target sequences, which had been lentivirally integrated in HEK293T cells. The number of target sequences $n = 423$. PegRNAs that resulted in PE2-directed prime editing efficiencies higher than 1% are shown. Data of minimum to maximum values are presented. For the boxes, the top, middle, and bottom lines represent the 25th, 50th, and 75th percentiles, respectively. The whiskers indicate the 10th and 90th percentile values. Source data are provided as a Source Data file.

a



b



Supplementary Figure 5. Structure of hyPE2. **a**, Schematic representation of hyPE2. **b**, Three-dimensional structures predicted for hyPE2 (left) and PE2 (right) before and after binding of the reverse transcriptase domain to the nicked target ssDNA/pegRNA hybrid. Modeling of a hypothetical interaction between Rad51, the nicked target ssDNA, and the pegRNA primer binding site is shown for hyPE2. Gray, Cas9 H840A; Green, Reverse transcriptase; Yellow, Rad51 DNA binding domain; Blue, DNA, non-nicked; Light blue, DNA, nicked; Red, pegRNA; Black, Linkers.

Supplementary Table 1. Characteristics (sequences of target, pegRNA spacer, and extension) of pegRNAs used in this study and relevant editing efficiencies (provided as a separate file).

Supplementary Table 2. Information about endogenous target sites and associated potential off-target sites (provided as a separate file). Mismatched and bulged nucleotides in the off-target sites are indicated in red and blue lower-case fonts, respectively. Mismatched nucleotides at the 5' end of target sequences that are 20-nt away from the PAM were not counted as mismatches.

Supplementary Table 3. Training and test datasets used for the development of PEselector (provided as a separate file).

Supplementary Table 4. Oligonucleotides used in this study (provided as a separate file).

Supplementary Note

PE2 (addgene # 132775)

NLS

Cas9 H840A

Linker A

Reverse transcriptase

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PE2-mid_RPA70

NLS

Cas9 H840A

Linker A

Linker B

RPA70 ssDBD

Reverse transcriptase

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hyPE2 = PE2-mid_Rad51

NLS

Cas9 H840A

Linker A

Linker B

Rad51 ssDBD

Reverse transcriptase

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PE2-N_Rad51

NLS

Cas9 H840A

Linker A

Linker B

Rad51 ssDBD

Reverse transcriptase

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PE2-C_Rad51

NLS

Cas9 H840A

Linker A

Linker B

Rad51 ssDBD

Reverse transcriptase

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