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Supplemental information

Reduced off-target effect of NG-BE4max

by using NG-HiFi system

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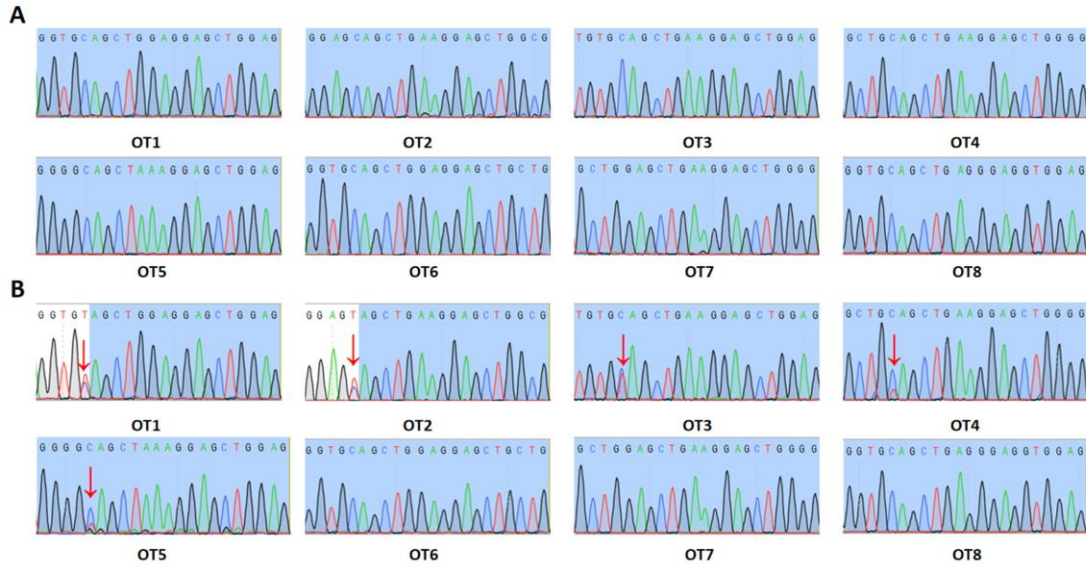


Figure S1. The significantly increased off-target effect in *Hoxc13* (Q87Stop) rabbits generated by SpCas9-NG. Representative sequencing chromatograms of off-target detection in WT (A) and *Hoxc13* (Q87Stop) (B) rabbits. The 20 bp of the POTS and the PAM are represented in shadow. Red arrows, the off-target effect.

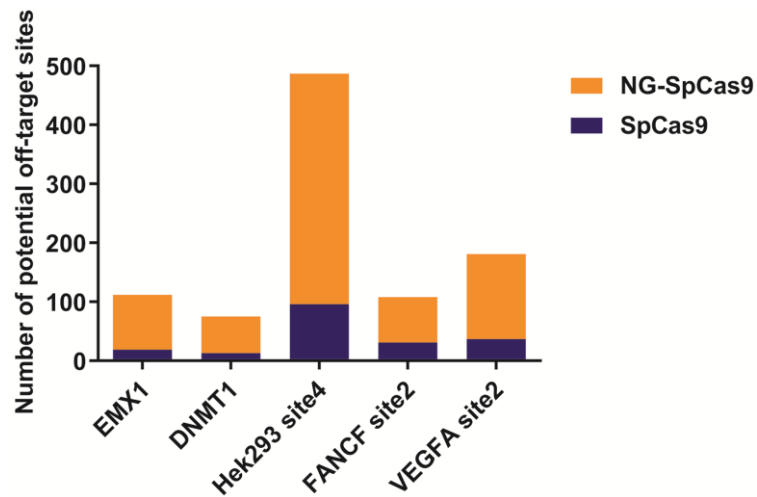


Figure S2. The significantly increased potential off-target sites (POTs) by SpCas9-NG compared with wild-type SpCas9. The potential off-target sites (POTs) were predicted by using Cas-OFFinder.

A

Wildtype p. 225 -S--N--V--R--H--A--L--G--S--S--E--Y--P--L--R--R--R--Q--C--E-
c. 675 ·TCCAACGTCCGCCACGCCCTGGGCTCTAGCGAGTACCCTCTGCGGGCGGCCAGTGTGAA
p. 245 -E--V--A--R--A--L--G--K--E--S--L--R--E--V--Q--L--E--E--L--E-
c. 735 ·GAGGTGGCCCGGGCGCTCGGCAAGGAGACCTTCGGGAGGTGCAGCTGGAGGAGCTGGAG

Mutant p. 225 -S--N--V--R--H--A--L--G--S--S--E--Y--P--L--R--R--R--Q--C--E-
c. 675 ·TCCAACGTCCGCCACGCCCTGGGCTCTAGCGAGTACCCTCTGCGGGCGGCCAGTGTGAA
p. 245 -E--V--A--R--A--L--G--K--E--S--L--R--E--V--*
c. 735 ·GAGGTGGCCCGGGCGCTCGGCAAGGAGACCTTCGGGAGGTGTAG

B

Wildtype p. 693 -L--E--Q--I--F--R--E--A--E--S--M--V--A--R--K--Q--F--G--H--P-
c. 2079 ·CTGGAGCAGATCTTCCGCGAGGCGGAGAGCATGGTTGCGCGCAAGCAGTTCGGGCACCCG
p. 713 -Q--I--E--A--R--I--K--E--V--S--A--Q--W--E--Q--L--K--E--L--A-
c. 2139 ·CAGATCGAGGCCCGCATCAAGGAGGTGTCCGCCAGTGGGAGCAGCTGAAGGAGCTGGCG

Mutant p. 693 -L--E--Q--I--F--R--E--A--E--S--M--V--A--R--K--Q--F--G--H--P-
c. 2079 ·CTGGAGCAGATCTTCCGCGAGGCGGAGAGCATGGTTGCGCGCAAGCAGTTCGGGCACCCG
p. 713 -Q--I--E--A--R--I--K--E--V--S--A--Q--W--E--*
c. 2139 ·CAGATCGAGGCCCGCATCAAGGAGGTGTCCGCCAGTGGGAGTAG

Figure S3. The premature termination codon (PTC) mutation was determined in the OT1 (GALK1, p.Q258stop) and OT2 (SPTB, p.Q727stop) sites. Asterisk, PTC mutation. (A) OT1 (GALK1, p.Q258stop), (B) OT2 (SPTB, p.Q727stop).

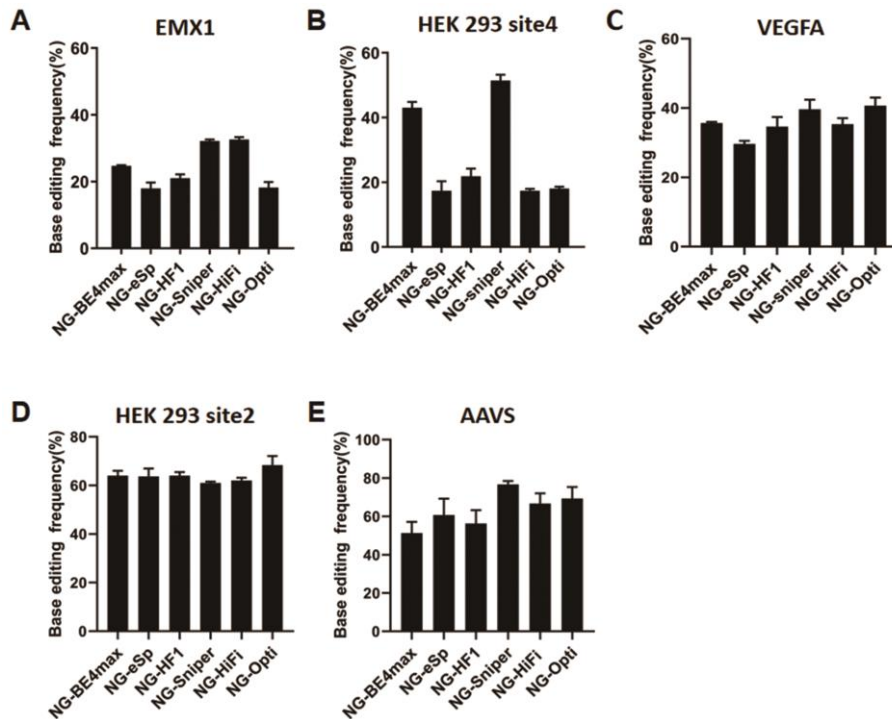


Figure S4. On-target base editing efficiency of five endogenous genomic loci using NG-BE4max and high-fidelity base editors in HEK 293T cells.

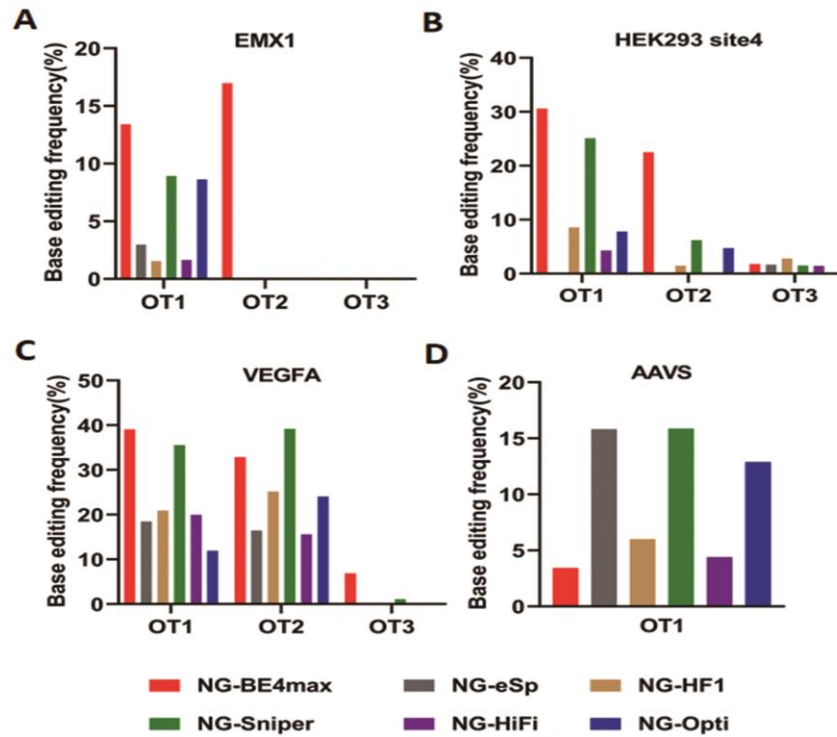


Figure S5. The off-target effect of NG-BE4max and five high-fidelity base editors were determined by deep sequencing.

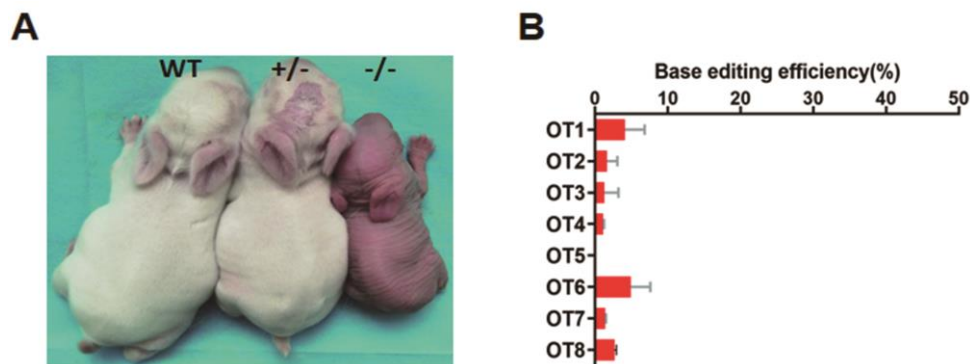


Figure S6. (A) Photographs of the F1 *Hoxc13* (Q87Stop) rabbits generated by the NG-HiFi system. (B) The significantly reduced off-target effect for F1 generation *Hoxc13* rabbits generated by the NG-HiFi system.

Table S1. Generation of *Hoxc13* (Q87Stop) rabbits by NG-BE4max and NG-HiFi base editors.

Table S2. The predicted phenotype of off-target mutation.

Table S3. The primers used for identifying potential off-target sites of *Hoxc13* (Q87Stop) rabbits.

The mismatched nucleotides are shown in lower case.

Table S4. Primers used for genotyping of mutation sites in this study.

Table S5. The primers used for identifying potential off-target sites of NG-BE4max and five high-fidelity base editors in 293T cells. The mismatched nucleotides are shown in lower case.

Table S6. Primers of the site mutation of high-fidelity CRISPR/Cas9 systems.