

CLAIMS

1. An engineered variant Cas12a endonuclease comprising a polypeptide sequence comprising a mutation at an amino acid position corresponding to position E95, E125, N256, R747, H759, N813, K932, N933, S934, V936, S982, or K984 with reference to amino acid position numbering of LbCas12a ND2006, optionally wherein the endonuclease exhibits hyperactivity.
2. The engineered variant Cas12a endonuclease of claim 1, wherein the mutation is E95R, E95Y, E125A, E125W, N256A, R747Y, H759V, H759D, N813R, N813H, K932L, N933E, N933V, S934Q, V936E, V936M, V936K, S982N, or K984R.
3. An engineered variant Cas12a endonuclease comprising a polypeptide sequence comprising a mutation at an amino acid position corresponding to position N256, I831, K932, N933, S934, V936, Q944, S982, F983, K984, M986, or T988 with reference to amino acid position numbering of LbCas12a ND2006, optionally wherein the endonuclease exhibits hypoactivity.
4. The engineered variant Cas12a endonuclease of claim 3, wherein the mutation is N256K, I831A, I831Y, K932A, K932F, K932H, K932M, K932N, K932Q, K932R, K932S, K932T, K932W, K932Y, N933L, S934W, V936G, Q944D, Q944E, Q944K, Q944M, S982T, S982W, F983G, F983L, K984F, M986G, M986L, M986S, or T988F.

5. The engineered variant Cas12a endonuclease of claim 3 or 4, comprising a mutation at an amino acid position corresponding to position Q944, optionally wherein the mutation is Q944D, Q944E, Q944K, Q944M, further optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 86, 87, 88, or 89.

6. The engineered variant Cas12a endonuclease of any one of claims 3-5, wherein the polypeptide sequence comprises mutations selected from: K932F and F983L; K932F and T988F; K932R and Q944D; K932R and F983L; K932R and T988F; K932Y and F983L; K932Y and T988F; N933L and Q944M; V936G and Q944D; V936G and S982W; V936G and M986G; V936G and T988F; Q944D and S982W; Q944D and F983L; Q944D and T988F; S982W and F983L; S982W and T988F; or F983G and M986G.

7. The engineered variant Cas12a endonuclease of claim 6, comprising any of the following mutations:

(i) K932R and Q944D, optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 104;

(ii) N933L and Q944M, optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 109;

(iii) V936G and Q944D, optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 110;

(iv) Q944D and S982W, optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 114;

(v) Q944D and F983L, optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 115; or

(vi) Q944D and T988F, optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 116.

8. An engineered variant Cas12a endonuclease comprising a polypeptide sequence comprising a mutation at an amino acid position corresponding to position N813, I831, K932, N933, S934, V936, Q944, S982, F983, K984, M986, or T988 with reference to amino acid position numbering of LbCas12a ND2006, optionally wherein the endonuclease exhibits low indiscriminate ssDNase activity.

9. The engineered variant Cas12a endonuclease of claim 8, wherein the mutation is N813H, N813R, N813W, I831A, I831Y, K932A, K932F, K932H, K932M, K932N, K932Q, K932R, K932S, K932T, K932W, K932Y, N933E, N933L, S934K, S934Q, V936E, V936G, Q944D, Q944E, Q944K, S982W, F983G, F983L, K984F, M986F, M986G, or T988F.

10. The engineered variant Cas12a endonuclease of claim 8 or 9, comprising a mutation at an amino acid position corresponding to position Q944, optionally wherein the mutation is Q944D, Q944E, or Q944K, further optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 86, 87, or 88.

11. The engineered variant Cas12a endonuclease of claim 10, wherein the mutations are (i) N933L and Q944M or (ii) F983G and M986G.

12. The engineered variant Cas12a endonuclease of claim 11, comprising the mutations N933L and Q944M, optionally wherein the polypeptide sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the amino acid sequence of SEQ ID NO: 109.

13. An engineered variant Cas12a endonuclease comprising a polypeptide sequence comprising one or more mutations at amino acid positions corresponding to positions R833, E835, R836, F931, R935, K940, Q941, Y943, and/or Q944K, with reference to amino acid position numbering of LbCas12a ND2006.

14. The engineered variant Cas12a endonuclease of claim 13, wherein the one or more mutations are selected from R833L, R833K, R833M, E835D, R836G, R935G, K940G, Q941K, Y943T, Y943F, and Q944K.

15. The engineered variant Cas12a endonuclease of claim 13 or 14, wherein the mutations are K940G and Q944K; R836G and Q944K; R833M, E835D, and Y943T; R836G, Q944K, and R935G; R833M, E835D, Y943T, and R935G; or R833M, E835D, Y943T, and Q941K.
16. A fusion protein comprising an engineered variant Cas12a endonuclease of any one of claims 1-15 and a base editing enzyme, optionally wherein the base editing enzyme comprises a deaminase, a guanine oxidase, or a guanine methyltransferase.
17. The fusion protein of claim 16, wherein the deaminase is a cytidine deaminase or an adenosine deaminase, optionally wherein the deaminase comprises a rAPOBEC1 polypeptide, an evoAPOBEC1 polypeptide, a hAPOBEC3A polypeptide, an evoCDA polypeptide, an evoFERNY polypeptide, or a TadA polypeptide.
18. The fusion protein of claim 16 or 17, further comprising:
- (i) a uracil glycosylase inhibitor (UGI);
 - (ii) one or more nuclear localization signal (NLS), optionally selected from an SV40 NLS, a nucleoprotein (NP) NLS, and a bipartite (BP) NLS;
 - (iii) a uracil DNA glycosylase (UNG), optionally a human UNG (hUNG) or an Escherichia coli UNG (eUNG);
 - (iv) a N-methyl purine glycosylase (MPG), optionally wherein the MPG is positioned at or near the N-terminal or C-terminal ends of the fusion protein;

(v) one or more linker, optionally wherein the linker comprises the sequence of SGSETPGTSESATPES (SEQ ID NO: 203) or SGGSSGGSSGSETPGTSESATPESGGSSGGS (SEQ ID NO: 204); and/or

(vi) a DNA binding domain (DBD), optionally wherein the DBD is a Rad51 DBD.

19. A polynucleotide encoding an engineered variant Cas12a endonuclease of any one of claims 1-15 or a fusion protein of any one of claims 16-18.

20. A cell comprising (a) an engineered variant Cas12a endonuclease of any one of claims 1-15 or a polynucleotide of claim 19 and (b) a guide RNA or a polynucleotide encoding a guide RNA, optionally wherein the cell is a human cell.

21. A method of gene editing comprising

- (i) contacting a target nucleic acid sequence with the fusion protein of any one of claims 16-18 and a guide RNA, wherein the target nucleic acid comprises a target nucleobase; and
- (ii) modifying the target nucleobase.

22. The method of claim 21, wherein the method is performed *in vitro*, *ex vivo*, or *in vivo*.