CLAIMS

WHAT IS CLAIMED IS

1. A variant polypeptide comprising an alteration relative to a parent polypeptide of SEQ ID NO: 3, and wherein the alteration is a substitution of Table 2.

2. The variant polypeptide of claim 1, wherein:

i) the substitution is a P14R substitution, an E311R substitution, a D32R substitution, an I61R substitution, a G223R substitution, an N109R substitution, and/or a D719R substitution;

ii) the variant polypeptide comprises:

a) a P14R substitution, an E311R substitution, and a D32R substitution;

b) a P14R substitution, an E311R substitution, and a G223R substitution;

c) a P14R substitution, an E311R substitution, a D32R substitution, and an I61R substitution; or

d) a D32R substitution, an N109R substitution, an E311R substitution, and a D719R substitution; and/or

iii) the variant polypeptide further comprises a K208G substitution, a D302G substitution, a D590G substitution, an E154G substitution, a D567G substitution, an L38G substitution, a D145G substitution, a C13G substitution, a T338G substitution, a P14G substitution, a D55G substitution, a K221G substitution, a K35G substitution, and an E736G substitution.

3. A variant polypeptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO: 3 and comprising:

i) a substitution at one or more of positions P14, E311, D32, I61, G223, N109, and D719 relative to SEQ ID NO: 3;

ii) one or more of the following substitutions: P14R, E311R, D32R, I61R, G223R, N109R, and D719R;

iii) a substitution at one or more of positions K208, D302, D590, E154, D567, L38, D145, C13, T338, P14, D55, K221, K35, and E736 relative to SEQ ID NO: 3; or

iv) one or more of the following substitutions: K208G, D302G, D590G, E154G, D567G, L38G, D145G, C13G, T338G, P14G, D55G, K221G, K35G, and E736G.

4. The variant polypeptide of any one of claims 1-3, wherein the variant polypeptide comprises a substitution at:

i) P14 (e.g., a P14R substitution);

ii) E311 (e.g., an E311R substitution);

iii) D32 (e.g., a D32R substitution);

iv) I61 (e.g., a I61R substitution);

v) G223 (e.g., a G223 substitution);

vi) N109 (e.g., a N109R substitution); and/or

vii) D719 (e.g., a D719R substitution).

5. The variant polypeptide of claim 3, wherein the variant polypeptide comprises a substitution at position P14 (e.g., a P14R substitution), an E311 (e.g., an E311R substitution), a D32 (e.g., a D32R substitution), an I61 (e.g., a I61R substitution), a G223 (e.g., a G223 substitution), an N109 (e.g., a N109R substitution), a D719 (e.g., a D719R substitution), or any combination thereof, optionally wherein the variant polypeptide comprises a substitution at position:

i) P14 (e.g., a P14R substitution), E311 (e.g., an E311R), and D32 (e.g., a D32R substitution) relative to SEQ ID NO: 3 (e.g., a P14R, E311R, D32R variant polypeptide);

ii) P14 (e.g., a P14R substitution), E311 (e.g., an E311R), and G223 (e.g., a G223R substitution) relative to SEQ ID NO: 3 (e.g., a P14R, E311R, G223R variant polypeptide);

iii) P14 (e.g., a P14R substitution), E311 (e.g., an E311R), D32 (e.g., a D32R substitution), and I61 (e.g., an I61R substitution) relative to SEQ ID NO: 3 (e.g., a P14R, E311R, D32R and I61R variant polypeptide); or

iv) D32R (e.g., a D32R substitution), N109 (e.g., an N109R), E311 (e.g., an E311R substitution), and D719 (e.g., a D719R substitution) relative to SEQ ID NO: 3 (e.g., a D32R, N109R, E311R and D719R variant polypeptide).

6. The variant polypeptide of any one of claims 1-5, wherein:
i) the variant polypeptide exhibits increased binary complex formation with an RNA guide, relative to the parent polypeptide;

ii) a binary complex comprising the variant polypeptide exhibits increased stability, relative to the parent binary complex; and/or

iii) the variant polypeptide exhibits increased nuclease activity, relative to the parent polypeptide.

7. A composition comprising the variant polypeptide of any one of claims 1-6, wherein the composition further comprises an RNA guide or a nucleic acid encoding the RNA guide, wherein the RNA guide comprises a direct repeat sequence and a spacer sequence.

8. The variant polypeptide or the composition of any previous claim, wherein the variant polypeptide further comprises a nuclear localization signal (NLS), a peptide tag, a fluorescent protein, a base-editing domain, a DNA methylation domain, a histone residue modification domain, a localization factor, a transcription modification factor, a light-gated control factor, a chemically inducible factor, or a chromatin visualization factor.

9. A composition comprising a nucleic acid that encodes the variant polypeptide and/or the RNA guide of any previous claim.

10. The composition of claim 9, wherein the nucleic acid is:
i) codon-optimized for expression in a cell;

ii) operably linked to a promoter; and/or

iii) in a vector, optionally wherein the vector comprises a retroviral vector, a lentiviral vector, a phage vector, an adenoviral vector, an adeno-associated vector, or a herpes simplex vector.

11. The composition of claim 9 or 10, wherein the sequence of the nucleic acid molecule is 95% identical to a sequence selected from the group consisting of SEQ ID NOs: 1, 2, or 21-27.

12. The variant polypeptide or the composition of any previous claim, wherein the variant polypeptide is present in a delivery system comprising a nanoparticle (e.g., a lipid nanoparticle), a liposome, an exosome, a microvesicle, or a gene-gun.

13. The variant polypeptide of any one of claims 1-6, 8, or 12, or the composition of any one of claims 7-12, wherein the variant polypeptide comprises a RuvC domain or a split RuvC domain.

14. The composition of any one of claims 7-13, wherein the direct repeat sequence is at least 90% identical to any one of SEQ ID NOs: 4-13 or comprises a sequence having at least 90% identity to SEQ ID NO: 14 or SEQ ID NO: 15.

15. The composition of any one of claims 7-14, wherein the spacer:
i) sequence comprises between 15 and 35 nucleotides in length; and/or

ii) comprises complementarity to a target strand sequence of a target nucleic acid.

16. The composition of claim 15, wherein the target nucleic acid comprises a non-target strand sequence adjacent to a protospacer adjacent motif (PAM) sequence, optionally wherein the PAM

sequence is 5’-TTR-3’, 5’-NTTR-3’, 5’-NTTN-3’, 5’-RTTR-3’, 5’-ATTR-3’, or 5’-RTTG-3’, wherein N is any nucleotide, Y is C or T, and R is A or G, or wherein the PAM sequence is 5’-TTG-3’, 5’-TTA-3’, 5’-ATTG-3’, 5’-TTTA-3’, or 5’-TTTG-3’.

17. A cell comprising the variant polypeptide of any one of claims 1-6, 8, 12, or 13, or the composition of any one of claims 7-16.

18. The composition of claim 17, wherein the cell is a eukaryotic cell, optionally wherein the eukaryotic cell is a mammalian cell (e.g., a human cell) or a plant cell.

19. The composition of any one of claims 7-18, wherein the composition is present in a delivery composition comprising a nanoparticle (e.g., a lipid nanoparticle), a liposome, an exosome, a microvesicle, or a gene-gun.

20. A method for editing a gene in a cell, the method comprising contacting the cell with the variant polypeptide or composition of any one of the previous claims.