

COMPOSITIONS COMPRISING A CAS12i2 VARIANT POLYPEPTIDE AND USES THEREOF

SEQUENCE LISTING

5 The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 31, 2021, is named 51451-008WO5_Sequence_Listing_03_30_2021_ST25, and is 1,589,401 bytes in size.

BACKGROUND

10 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) genes, collectively known as CRISPR-Cas or CRISPR/Cas systems, are adaptive immune systems in archaea and bacteria that defend particular species against foreign genetic elements.

SUMMARY

15 It is against the above background that the present disclosure provides certain advantages and advancements over the prior art.

 Although the disclosure disclosed herein is not limited to specific advantages or functionalities, in one example, the present disclosure provides a variant Cas12i2 polypeptide comprising a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3 to 146 or any one of SEQ ID
20 NOs: 495 to 512.

 In one aspect, the present disclosure refers to a variant Cas12i2 polypeptide comprising a sequence having at least 95% identity to a sequence set forth in SEQ ID NO: 4, wherein the variant Cas12i2 polypeptide comprises a D581R, I926R, and/or V1030G substitution of the amino acid sequence of SEQ ID NO: 2.

25 In another aspect, the present disclosure refers to a composition comprising a variant Cas12i2 polypeptide as disclosed herein, or a nucleic acid encoding the Cas12i2 polypeptide, 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.

 In another aspect, the present disclosure refers to a nucleic acid molecule encoding a variant
30 Cas12i2 polypeptide as disclosed herein.

 In another aspect, the present disclosure refers to an mRNA molecule encoding a variant Cas12i2 polypeptide as disclosed herein.

 In another aspect, the present disclosure refers to a cell comprising the composition or variant Cas12i2 polypeptide as disclosed herein.

35 In another aspect, the present disclosure refers to a composition or formulation comprising the variant Cas12i2 polypeptide, the nucleic acid, or the mRNA as disclosed herein.

In another aspect, the present disclosure refers to an *ex vivo* method of obtaining a deletion in DNA in a cell, wherein the method comprises contacting the composition as disclosed herein, with DNA in the cell.

5 In another aspect, the present disclosure refers to a composition comprising the variant Cas12i2 polypeptide, the nucleic acid, the mRNA molecule, or the composition as disclosed herein, for use in obtaining a deletion in DNA in a cell.

In another aspect, the present disclosure refers to a method of producing the composition as disclosed herein.

10 In another aspect, the present disclosure refers to a method of complexing a variant Cas12i2 polypeptide as disclosed herein with an RNA guide.

In another aspect, the present disclosure refers to a method of complexing a variant binary complex prepared according to the method as disclosed herein with a target nucleic acid.

In one example of the variant Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 4.

15 In another example of the variant Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 5.

In another example of the variant Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 495.

20 In another example of the variant Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 496.

In another example of the variant Cas12i2 polypeptide, the variant Cas12i2 polypeptide further comprises one or more substitution of Table 2.

In another example of the variant Cas12i2 polypeptide, the variant Cas12i2 polypeptide is a variant of a Cas12i2 polypeptide comprising the sequence set forth in SEQ ID NO: 2.

25 In one example, the present disclosure yet further provides a composition comprising a variant Cas12i2 polypeptide as described herein, 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.

In one example of the composition, the direct repeat sequence comprises a nucleotide sequence with at least 95% sequence identity to any one of SEQ ID NO: 492-494.

In another example of the composition, the direct repeat sequence comprises a nucleotide sequence set forth in any one of SEQ ID NO: 492-494.

5 In another example of the composition, the spacer sequence comprises between about 11 and about 50 nucleotides.

In another example of the composition, the spacer sequence comprises between about 15 and about 35 nucleotides.

10 In another example of the composition, the spacer sequence binds to a target nucleic acid sequence, wherein the target nucleic acid sequence is adjacent to a 5'-NTTN-3' sequence.

In another example of the variant Cas12i2 polypeptide or the composition, the variant Cas12i2 polypeptide further comprises at least one nuclear localization signal (NLS), at least one nuclear export signal (NES), or at least one NLS and at least one NES.

15 In another example of the variant Cas12i2 polypeptide or the composition, the variant Cas12i2 polypeptide further comprises 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.

20 In another example of the composition, the composition is present in a delivery system comprising a nanoparticle, a liposome, an exosome, a microvesicle, or a gene-gun.

In one example, the present disclosure yet further provides a nucleic acid molecule encoding a variant Cas12i2 polypeptide as described herein.

In one example, the present disclosure yet further provides a cell comprising a composition or variant Cas12i2 polypeptide as described herein.

25 In one example of the cell, the cell is a eukaryotic cell or a prokaryotic cell.

In another example of the cell, the cell is a mammalian cell or a plant cell.

In another example of the cell, the cell is a human cell.

In one example, the present disclosure yet further provides a composition or formulation comprising a variant Cas12i2 polypeptide as described herein, and optionally an RNA guide and/or a cell.

30 In one example, the present disclosure yet further provides a composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a complex, and wherein the variant Cas12i2 polypeptide exhibits increased complex formation with the RNA guide as compared to a parent polypeptide.

35 In one example, the present disclosure yet further provides a composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a complex, and wherein the Cas12i2 variant polypeptide exhibits increased binding affinity to the RNA guide, as compared to a parent polypeptide.

In one example, the present disclosure yet further provides a composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a complex, and the Cas12i2 variant polypeptide and the RNA guide exhibit increased protein-RNA interactions, as compared to a parent polypeptide and the RNA guide.

5 In one example of the composition, the variant Cas12i2 polypeptide exhibits increased complex formation, increased binding affinity to the RNA guide and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.

In another example of the composition, the variant Cas12i2 polypeptide exhibits increased complex formation, increased binding affinity to the RNA guide and/or increased stability over a range of
10 incubation times.

In another example of the composition, the variant Cas12i2 polypeptide exhibits increased complex formation, increased binding affinity to the RNA guide and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.

15 In another example of the composition, the variant Cas12i2 polypeptide exhibits increased complex formation, increased binding affinity to the RNA guide and/or increased stability when a T_m value of the variant binary complex is at least 8°C greater than the T_m value of the parent complex.

In another example of the composition, the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.

20 In another example of the composition, the variant Cas12i2 polypeptide exhibits equivalent to or greater enzymatic activity than the parent polypeptide.

In another example of the composition, the equivalent to or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.

In another example of the composition, the variant Cas12i2 polypeptide exhibits increased stability and/or protein-RNA interactions.

25 In another example of the composition, the variant Cas12i2 polypeptide further lacks enzymatic activity.

In another example of the composition, the variant Cas12i2 polypeptide further exhibits increased enzymatic activity.

30 In another example of the composition, the variant Cas12i2 polypeptide exhibits increased RNA guide complex formation, RNA guide binding activity and/or RNA guide binding specificity.

In another example of the composition, the variant Cas12i2 polypeptide exhibits altered on-target binding.

In another example of the composition, the variant Cas12i2 polypeptide exhibits altered off-target binding.

35 The disclosure yet further provides a composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary

CLAIMS

WHAT IS CLAIMED IS:

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1. A variant Cas12i2 polypeptide comprising a sequence having at least 95% identity to a sequence set forth in SEQ ID NO: 4, wherein the variant Cas12i2 polypeptide comprises a D581R, I926R, and/or V1030G substitution of the amino acid sequence of SEQ ID NO: 2.
- 10 2. The variant Cas12i2 polypeptide of claim 1, wherein the variant Cas12i2 polypeptide comprises a D581R, I926R, and V1030G substitution of the amino acid sequence of SEQ ID NO: 2.
3. The variant Cas12i2 polypeptide of claim 1 or 2, comprising a sequence having at least 99% identity to a sequence set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 495, or SEQ ID NO: 496.
- 15 4. The variant Cas12i2 polypeptide of any one of claims 1 to 3, comprising the sequence set forth in SEQ ID NO: 4.
5. The variant Cas12i2 polypeptide of any one of claims 1 to 3, comprising the sequence set forth in SEQ ID NO: 5.
- 20 6. The variant Cas12i2 polypeptide of any one of claims 1 to 3, which further comprises a D911R substitution of the amino acid sequence of SEQ ID NO: 2.
7. The variant Cas12i2 polypeptide of any one of claims 1 to 3, comprising the sequence set forth in SEQ ID NO: 3.
8. The variant Csa12i2 polypeptide of any one of claims 1 to 3, which further comprises a G624R, F626R, P868T, E1035R, or S1046G substitution of the amino acid sequence of SEQ ID NO: 2.
- 25 9. The variant Cas12i2 polypeptide of any one of claims 1 to 3, comprising the sequence set forth in SEQ ID NO: 495.
10. The variant Cas12i2 polypeptide of any one of claims 1 to 3, comprising the sequence set forth in SEQ ID NO: 496.
- 30 11. The variant Cas12i2 polypeptide of any one of claims 1 to 3, further comprising a substitution of Table 2.
12. A composition comprising a variant Cas12i2 polypeptide of any one of claims 1 to 11, or a nucleic acid encoding the Cas12i2 polypeptide, 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.

13. The composition of claim 12, wherein the direct repeat sequence comprises a nucleotide sequence with at least 95% sequence identity to any one of SEQ ID NO: 492-494.
14. The composition of claim 13, wherein the direct repeat sequence comprises a nucleotide sequence set forth in any one of SEQ ID NO: 492-494.
- 5 15. The composition of any one of claims 12 to 14, wherein the spacer sequence comprises between 11 and 50 nucleotides.
16. The composition of claim 15, wherein the spacer sequence comprises between 15 and 35 nucleotides.
- 10 17. The composition of any one of claims 12 to 16, wherein the spacer sequence binds to a target nucleic acid sequence, and wherein the target nucleic acid sequence is adjacent to a 5'-NTTN-3' sequence.
18. The variant Cas12i2 polypeptide of any one of claims 1 to 11, or the composition of any one of claims 12 to 17, wherein the variant Cas12i2 polypeptide further comprises at least one nuclear localization signal (NLS), at least one nuclear export signal (NES), or at least one NLS and at least one NES.
- 15 19. The variant Cas12i2 polypeptide of any one of claims 1 to 11 or 18, or the composition of any one of claims 12 to 18, wherein the variant Cas12i2 polypeptide further comprises 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.
- 20 20. The composition of any one of claims 12 to 19, wherein the composition is present in a delivery system comprising a nanoparticle, a liposome, an exosome, a microvesicle, or a gene-gun.
21. A nucleic acid molecule encoding a variant Cas12i2 polypeptide of any one of claims 1 to 11, or 18, or 19.
- 25 22. An mRNA molecule encoding a variant Cas12i2 polypeptide of any one of claims 1 to 11, 18, or 19.
23. The mRNA molecule of claim 22, wherein the mRNA molecule comprises:
 - (i) a pseudouridine;
 - (ii) a cap; and/or
 - 30 (iii) a poly-A sequence.
24. A cell comprising the composition or variant Cas12i2 polypeptide of any previous claim.
25. The cell of claim 24, wherein the cell is a eukaryotic cell or a prokaryotic cell.

26. The cell of claim 24 or 25, wherein the cell is a mammalian cell or a plant cell.
27. The cell of any one of claims 24 to 26, wherein the cell is a human cell.
28. A composition or formulation comprising the variant Cas12i2 polypeptide of any one of claims 1 to 11 or 15 to 20, the nucleic acid of claim 21, or the mRNA of claim 22 or 23.
- 5 29. The composition or formulation of claim 28, comprising an RNA guide and/or a cell.
30. The variant Cas12i2 polypeptide of any one of claims 1 to 3, 11, 18, or 19, or the composition of any one of claims 12 to 20 or 28, wherein the variant Cas12i2 polypeptide comprises at least one of a G624R, F626G, F626R, D835G, D835R, L836G, L836R, P868G, P868R, P868T, S879G, S879R, D911G, D911R, V1020G, V1020R, E1035G, E1035R, S1046G, and S1046R substitution
10 of amino acid sequence of SEQ ID NO: 2.
31. An *ex vivo* method of obtaining a deletion in DNA in a cell, wherein the method comprises contacting the composition of any one of claims 12 to 20 or 28 to 30, with DNA in the cell.
32. A composition comprising the variant Cas12i2 polypeptide of any one of claims 1 to 11, 18, 19, or 30, the nucleic acid of claim 21, the mRNA molecule of claims 22 or 23, or the composition of any
15 one of claims 12 to 20 or 28 to 30, for use in obtaining a deletion in DNA in a cell.
33. A method of producing the composition of any one of claims 12 to 20, 28 to 30, or 32.
34. A method of complexing a variant Cas12i2 polypeptide of any one of claims 1 to 11, 18, 19, or 30 with an RNA guide.
35. A method of complexing a variant binary complex prepared according to the method of claim 31
20 with a target nucleic acid.