

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 OF THE INVENTION

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

Although the ~~invention~~disclosure disclosed herein is not limited to specific advantages or functionalities, in one aspect, the ~~invention~~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 NOs: 495 to 512.

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

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

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

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

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

In another ~~aspect~~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.

In one aspect, ~~the invention~~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 ~~aspect~~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 ~~aspect~~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 ~~aspect~~example of the composition, the spacer sequence comprises between about 11 and about 50 nucleotides.

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

10 In another ~~aspect~~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 ~~aspect~~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 ~~aspect~~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 ~~aspect~~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 aspect, the invention~~present disclosure yet further provides a nucleic acid molecule encoding a variant Cas12i2 polypeptide as described herein.

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

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

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

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

~~In one aspect, the invention~~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 aspect, the invention~~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 aspect, the invention~~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 aspect, the invention ~~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 aspectexample 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 aspectexample 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 aspectexample 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 aspectexample 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 aspectexample of the composition, the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.

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

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

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

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

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

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

35 The inventiondisclosure 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

complex, and wherein the variant binary complex exhibits decreased complex dissociation than a complex formed by a parent polypeptide and the RNA guide.

The ~~invention~~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 complex, and wherein the RNA guide exhibits decreased dissociation from the variant Cas12i2 polypeptide than an RNA guide of a parent complex.

The ~~invention~~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 complex, and wherein the variant binary complex exhibits increased stability than a complex formed by a parent polypeptide and the RNA guide.

In one ~~aspect~~example of the composition, the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased stability over a range of incubation times.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid 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 ~~aspect~~example of the composition, the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.

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

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

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

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

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

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

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

The inventiondisclosure yet further provides a method of complexing a variant Cas12i2 polypeptide as described herein with an RNA guide, e.g., RNA guide, as described herein.

5 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, an RNA guide and a target nucleic acid, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased ternary complex formation with the target nucleic acid as compared to a parent binary complex.

10 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, an RNA guide and a target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased binding affinity to the target nucleic acid, as compared to a parent binary complex.

15 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, an RNA guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibit increased protein-RNA interactions as compared to a parent binary complex.

20 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, an RNA guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibit increased protein-DNA interactions as compared to a parent binary complex.

In one aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.

25 In another aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability over a range of incubation times.

In another aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.

30 In another aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.

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

In another aspectexample of the composition, the variant binary complex exhibits equivalent to or greater enzymatic activity than the parent binary complex.

In another aspectexample 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 aspectexample of the composition, the variant binary complex exhibits increased stability and/or protein-RNA interactions.

5 In another aspectexample of the composition, the variant binary complex exhibits increased stability and/or protein-DNA interactions.

In another aspectexample of the composition, the variant binary complex further lacks enzymatic activity.

10 In another aspectexample of the composition, the variant binary complex further exhibits increased enzymatic activity.

In another aspectexample 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 aspectexample of the composition, the variant binary complex exhibits increased target nucleic acid complex formation, target nucleic acid activity and/or target nucleic acid specificity.

15 In another aspectexample of the composition, the variant binary complex exhibits altered on-target binding.

In another aspectexample of the composition, the variant binary complex exhibits altered off-target binding

20 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, an RNA guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, wherein the variant binary complex and target nucleic acid form a variant ternary complex, and wherein the variant ternary complex exhibits decreased complex dissociation than a parent ternary complex.

25 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, an RNA guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, wherein the variant binary complex and target nucleic acid form a variant ternary complex, and wherein the target nucleic acid exhibits decreased dissociation from the variant ternary complex than a parent ternary complex.

30 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, an RNA guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, wherein the variant binary complex and target nucleic acid form a variant ternary complex, and wherein the variant ternary complex exhibits increased stability than a parent ternary complex.

35 In one aspectexample of the composition, the variant ternary complex exhibits increased stability over a range of temperatures, e.g., 20°C to 65°C.

In another aspectexample of the composition, the variant ternary complex exhibits increased stability over a range of incubation times.

In another aspectexample of the composition, the variant ternary complex exhibits increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.

In another aspectexample of the composition, the variant ternary complex exhibits increased stability when a T_m value of the variant ternary complex is at least 8°C greater than the T_m value of the parent ternary complex.

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

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

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

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

In another aspectexample of the composition, the variant binary complex exhibits increased stability and/or protein-DNA interactions.

In another aspectexample of the composition, the variant ternary complex exhibits increased stability.

In another aspectexample of the composition, the variant binary complex further lacks enzymatic activity.

In another aspectexample of the composition, the variant binary complex further exhibits increased enzymatic activity.

In another aspectexample 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 aspectexample of the composition, the variant binary complex exhibits increased target nucleic acid complex formation, target nucleic acid binding activity and/or target nucleic acid binding specificity.

In another aspectexample of the composition, the variant binary complex exhibits altered on-target binding.

In another aspectexample of the composition, the variant binary complex exhibits altered off-target binding.

The inventiondisclosure yet further provides a method of complexing a variant binary complex as described herein with a target nucleic acid, e.g., DNA, as described herein.

The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased binding affinity to a target nucleic acid as compared to a parent binary complex.

The inventiondisclosure 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 complex, and wherein the variant binary complex exhibits increased target binding affinity to a target locus of a target nucleic acid as compared to a parent binary complex.

In one aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.

In another aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation and/or increased stability over a range of incubation times.

5 In another aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.

In another aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation and/or increased stability when a T_m value of the binary complex is at least
10 8°C greater than the T_m value of the parent binary complex.

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

In another aspectexample of the composition, the variant binary complex exhibits equivalent to or greater enzymatic activity than the parent binary complex.

15 In another aspectexample 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 aspectexample of the composition, the variant binary complex exhibits increased stability and/or protein-RNA interactions.

In another aspectexample of the composition, the variant binary complex exhibits increased
20 stability and/or protein-DNA interactions.

In another aspectexample of the composition, the variant binary complex further lacks enzymatic activity.

In another aspectexample of the composition, the variant binary complex further exhibits increased enzymatic activity.

25 In another aspectexample 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 aspectexample of the composition, the variant binary complex exhibits increased target nucleic acid complex formation, target nucleic acid activity and/or target nucleic acid specificity.

In another aspectexample of the composition, the variant binary complex exhibits altered on-
30 target binding.

In another aspectexample of the composition, the variant binary complex exhibits altered off-target binding.

The inventiondisclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides
35 individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes specifically bind with two or more target loci of a target nucleic acid.

The inventiondisclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides
40 individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary

complexes exhibit increased on-target binding of two or more target loci of a target nucleic acid as compared to parent binary complexes.

The ~~invention~~disclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes exhibit increased on-target binding with two or more target loci of a target nucleic acid as compared to parent binary complexes.

The ~~invention~~disclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes exhibit on-target ternary complex formation with two or more target loci of a target nucleic acid.

The ~~invention~~disclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes exhibit increased ternary complex formation with two or more target loci of a target nucleic acid as compared to parent binary complexes.

In one ~~aspect~~example of the composition, the variant binary complex exhibits increased ternary complex formation with the target nucleic acid and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased ternary complex formation with the target nucleic acid and/or increased stability over a range of incubation times.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased ternary complex formation with the target nucleic acid and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased ternary complex formation with the target nucleic acid and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.

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

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

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

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

In another aspectexample of the composition, the variant binary complex exhibits increased stability and/or protein-DNA interactions.

In another aspectexample of the composition, the variant binary complex further lacks enzymatic activity.

5 In another aspectexample of the composition, the variant binary complex further exhibits increased enzymatic activity.

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

10 In another aspectexample of the composition, the variant binary complex exhibits increased target nucleic acid ternary complex formation, target nucleic acid binding affinity and/or target nucleic acid binding specificity.

In another aspectexample of the composition, the variant binary complex exhibits altered on-target binding.

15 In another aspectexample of the composition, the variant binary complex exhibits altered off-target binding.

The inventiondisclosure yet further provides a method of complexing a variant binary complex described herein with a target nucleic acid, e.g., DNA, as described herein.

20 The inventiondisclosure 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 complex, and wherein the variant binary complex exhibits increased on-target binding affinity to a target locus of a target nucleic acid as compared to a parent binary complex.

25 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits decreased binding affinity to a non-target locus of a target nucleic acid as compared to a parent binary complex.

The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased activity at an on-target locus of a target nucleic acid as compared to a parent binary complex.

30 The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits decreased activity at a non-target locus of a target nucleic acid as compared to a parent binary complex.

35 In one aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation at the target locus and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.

In another aspectexample of the composition, the variant binary complex exhibits increased ternary complex formation at the target locus and/or increased stability over a range of incubation times.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased ternary complex formation at the target locus and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased ternary complex formation at the target locus and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.

In another ~~aspect~~example of the composition, the parent binary complex comprises a parent polypeptide that comprises the amino acid sequence of SEQ ID NO: 2.

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

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

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased stability and/or protein-RNA interactions.

In another ~~aspect~~example of the composition, the variant binary complex exhibits increased stability and/or protein-DNA interactions.

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

The ~~invention~~disclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form a plurality of variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the plurality of variant binary complexes exhibit increased on-target binding to two or more target loci of a target nucleic acid as compared to a plurality of parent binary complexes.

The ~~invention~~disclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form a plurality of variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the plurality of variant binary complexes exhibit decreased off-target binding to two or more non-target loci of a target nucleic acid as compared to a plurality of parent binary complexes.

The ~~invention~~disclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form a plurality of variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the plurality of variant binary complexes exhibit increased on-target activity at two or more target loci of a target nucleic acid as compared to a plurality of parent binary complexes.

The ~~invention~~disclosure yet further provides a composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form a plurality of variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the

plurality of variant binary complexes exhibit decreased off-target activity at two or more non-target loci of a target nucleic acid as compared to a plurality of parent binary complexes.

In one ~~aspect~~example of the composition, the plurality of variant binary complexes exhibit increased ternary complex formation at the target loci of the target nucleic acid and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.

In another ~~aspect~~example of the composition, the plurality of variant binary complexes exhibit increased ternary complex formation at the target loci of the target nucleic acid and/or increased stability over a range of incubation times.

In another ~~aspect~~example of the composition, the plurality of variant binary complexes exhibit increased ternary complex formation at the target loci the target nucleic acid and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.

In another ~~aspect~~example of the composition, the plurality of variant binary complexes exhibit increased ternary complex formation at the target loci of the target nucleic acid and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.

In another ~~aspect~~example of the composition, the plurality of parent binary complexes comprise a parent polypeptide that comprises the amino acid sequence of SEQ ID NO: 2.

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

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

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

In another ~~aspect~~example of the composition, the plurality of variant binary complexes exhibit increased stability and/or protein-DNA interactions.

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

The ~~invention~~disclosure yet further provides a method of complexing a variant binary complex as described herein with a target nucleic acid, e.g., DNA, as described herein.

The ~~invention~~disclosure yet further provides a method of complexing a plurality of variant binary complexes described herein with a target nucleic acid, e.g., DNA, as described herein.

In one ~~aspect~~example of a composition or Cas12i2 polypeptide described herein, the variant Cas12i2 polypeptide comprises at least one of a D581, G624, F626, D835, L836, P868, S879, D911, I926, V1020, V1030, E1035, and S1046 substitution of amino acid sequence of SEQ ID NO: 2.

In another ~~aspect~~example of the composition or Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises at least one of a D581G, D581R, G624R, F626G, F626R, D835G, D835R, L836G, L836R, P868G, P868R, P868T, S879G, S879R, D911G, D911R, I926G, I926R, V1020

G, V1020R, V1030G, V1030R, E1035G, E1035R, S1046G, and S1046R substitution of amino acid sequence of SEQ ID NO: 2.

In another [aspectexample](#) of the composition or Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises at least one of a D581R, G624R, F626R, P868T, D911R, I926R, V1030G, E1035R, and S1046G substitution of amino acid sequence of SEQ ID NO: 2.

In another [aspectexample](#) of the composition or Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises at least one substitution listed in Table 1.

In another [aspectexample](#) of the composition or Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises any one amino acid sequence of SEQ ID NO: 3 to 146 or any one amino acid sequence of SEQ ID NOs: 495 to 512.

In another [aspectexample](#) of the composition or Cas12i2 polypeptide, the variant Cas12i2 polypeptide comprises at least one of an epitope peptide, nuclear localization signal, and nuclear export signal.

In another [aspectexample](#) of the composition, the RNA guide comprises a DNA targeting sequence.

In another [aspectexample](#) of the composition, the DNA targeting sequence is an RNA guide.

In another [aspectexample](#) of the composition, the DNA targeting sequence is between 13 to 30 nucleotides.

In another [aspectexample](#) of the composition, the RNA guide comprises a direct repeat sequence linked to a DNA targeting sequence.

In another [aspectexample](#) of the composition, the composition further comprises a target nucleic acid.

In another [aspectexample](#) of the composition, the target nucleic acid is present in a cell.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide and RNA guide are encoded in a vector, e.g., expression vector.

The [inventiondisclosure](#) yet further provides a cell comprising a composition as described herein.

The [inventiondisclosure](#) yet further provides a method of expressing a vector as described herein.

The [inventiondisclosure](#) yet further provides a method of producing a composition as described herein.

The [inventiondisclosure](#) yet further provides a method of delivering a composition as described herein.

The [inventiondisclosure](#) yet further provides a kit or system comprising a composition as described herein or one or more component thereof.

In one [aspectexample](#) of a composition described herein, the RNA guide comprises or consists of, or about, 43 nucleotides.

In another [aspectexample](#) of the composition, the RNA guide is a tracr-less RNA guide.

In another [aspectexample](#) of the composition or Cas12i2 polypeptide, the variant Cas12i2 polypeptide further exhibits about 40x greater enzymatic activity than parent polypeptide.

In another [aspectexample](#) of the composition or Cas12i2 polypeptide, the variant Cas12i2 polypeptide exhibits increased on-target specificity as compared to the parent polypeptide.

In another [aspectexample](#) of the composition or Cas12i2 polypeptide, the variant Cas12i2 polypeptide exhibits decreased off-target specificity as compared to the parent polypeptide.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide selectively induces a deletion adjacent to a 5'-NTTN-3' sequence, wherein N is any nucleotide.

In another [aspectexample](#) of the composition, the deletion is downstream of the 5'-NTTN-3' sequence.

5 In another [aspectexample](#) of the composition, the parent polypeptide does not induce the deletion.

In another [aspectexample](#) of the composition, the length of the deletion is greater than the length of a Cas9 polypeptide-induced deletion.

In another [aspectexample](#) of the composition, the deletion is in a gene of a cell.

In another [aspectexample](#) of the composition, the deletion is up to about 40 nucleotides in length.

10 In another [aspectexample](#) of the composition, the deletion is from about 4 nucleotides to 40 nucleotides in length.

In another [aspectexample](#) of the composition, the deletion is from about 4 nucleotides to 25 nucleotides in length.

15 In another [aspectexample](#) of the composition, the deletion is from about 10 nucleotides to 25 nucleotides in length.

In another [aspectexample](#) of the composition, the deletion is from about 10 nucleotides to 15 nucleotides in length.

In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 15 nucleotides of the 5'-NTTN-3' sequence.

20 In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 10 nucleotides of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion starts within about 10 nucleotides to about 15 nucleotides of the 5'-NTTN-3' sequence.

25 In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 10 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion starts within about 10 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence.

30 In another [aspectexample](#) of the composition, the deletion ends within about 20 nucleotides to about 30 nucleotides of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion ends within about 20 nucleotides to about 25 nucleotides of the 5'-NTTN-3' sequence.

35 In another [aspectexample](#) of the composition, the deletion ends within about 25 nucleotides to about 30 nucleotides of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion ends within about 20 nucleotides to about 30 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion ends within about 20 nucleotides to about 25 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion ends within about 25 nucleotides to about 30 nucleotides downstream of the 5'-NTTN-3' sequence.

5 In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 20 nucleotides to about 30 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 20 nucleotides to about 25 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 25 nucleotides to about 30 nucleotides downstream of the 5'-NTTN-3' sequence.

15 In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 10 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 20 nucleotides to about 30 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 10 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 20 nucleotides to about 25 nucleotides downstream of the 5'-NTTN-3' sequence.

20 In another [aspectexample](#) of the composition, the deletion starts within about 5 nucleotides to about 10 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 25 nucleotides to about 30 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion starts within about 10 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 20 nucleotides to about 30 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the deletion starts within about 10 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 20 nucleotides to about 25 nucleotides downstream of the 5'-NTTN-3' sequence.

30 In another [aspectexample](#) of the composition, the deletion starts within about 10 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence and ends within about 25 nucleotides to about 30 nucleotides downstream of the 5'-NTTN-3' sequence.

In another [aspectexample](#) of the composition, the 5'-NTTN-3' sequence is 5'-NTTY-3', 5'-NTTC-3', 5'-NTTT-3', 5'-NTTA-3', 5'-NTTB-3', 5'-NTTG-3', 5'-CTTY-3', 5'-DTTR-3', 5'-CTTR-3', 5'-DTTT-3', 5'-ATTN-3', or 5'-GTTN-3', wherein Y is C or T, B is any nucleotide except for A, D is any nucleotide except for C, and R is A or G.

In another [aspectexample](#) of the composition, the 5'-NTTN-3' sequence is 5'-CTTT-3', 5'-CTTC-3', 5'-GTTT-3', 5'-GTTC-3', 5'-TTTC-3', 5'-GTTA-3', or 5'-GTTG-3'.

In another ~~aspectexample~~ of the composition, the deletion is in an exon of the gene, e.g., B2M, TRAC, PDCD1.

In another ~~aspectexample~~ of the composition, the deletion overlaps with a mutation in the gene.

In another ~~aspectexample~~ of the composition, the deletion overlaps with an insertion in the gene.

In another ~~aspectexample~~ of the composition, the deletion removes a repeat expansion of the gene.

In another ~~aspectexample~~ of the composition, the deletion disrupts one or both alleles of the gene.

In another ~~aspectexample~~ of the composition, the deletion is induced in a eukaryotic cell or a prokaryotic cell.

In another ~~aspectexample~~ of the composition, the deletion is induced in an animal cell, a plant cell, or a fungal cell or the cell is derived from an animal cell, a plant cell, or a fungal cell.

In another ~~aspectexample~~ of the composition, the deletion is induced in a mammalian cell or derived from a mammalian cell.

In another ~~aspectexample~~ of the composition, the deletion is induced in a human cell or derived from a human cell.

In another ~~aspectexample~~ of the composition, the deletion is induced in a primary cell.

In another ~~aspectexample~~ of the composition, the deletion is induced in a cell line.

In another ~~aspectexample~~ of the composition, the deletion is induced in a T cell.

In another ~~aspectexample~~ of the composition, the deletion is induced in a stem cell (e.g., a totipotent/omnipotent stem cell, a pluripotent stem cell, a multipotent stem cell, an oligopotent stem cell, or an unipotent stem cell), a differentiated cell, or a terminally differentiated cell.

In another ~~aspectexample~~ of the composition, 2 or more (e.g., multiplexed targeted deletions) deletions are induced.

The ~~inventiondisclosure~~ yet further provides a method of obtaining a deletion in a cell, wherein the method comprises contacting a variant Cas12i2 polypeptide or complex as described herein with DNA in the cell.

The ~~inventiondisclosure~~ yet further provides a composition or formulation comprising a variant Cas12i2 polypeptide as described herein, an RNA guide, and a cell.

The ~~inventiondisclosure~~ yet further provides a method of producing a composition as described herein.

The ~~inventiondisclosure~~ yet further provides a method of complexing a variant Cas12i2 polypeptide as described herein with an RNA guide, such as an RNA guide described herein.

The ~~inventiondisclosure~~ yet further provides a method of complexing a variant binary complex as described herein with a target nucleic acid.

The ~~inventiondisclosure~~ yet further provides a method of delivering a composition as described herein.

The ~~inventiondisclosure~~ yet further provides a composition comprising a variant Cas12i2 polypeptide, wherein the variant Cas12i2 polypeptide comprises a substitution that increases interactions between the variant Cas12i2 polypeptide and a nucleic acid, as compared to a parent polypeptide.

In one [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 4.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.

5 In another [aspectexample](#) of the composition, the interactions are electrostatic interactions.

In another [aspectexample](#) of the composition, the interactions are non-specific interactions.

In another [aspectexample](#) of the composition, the interactions are aromatic, hydrophobic, Van der Waals, and/or cation-pi interactions.

10 In another [aspectexample](#) of the composition, the substitution is in or adjacent to a nucleic acid interface.

In another [aspectexample](#) of the composition, the nucleic acid is an RNA guide comprising a direct repeat sequence and a spacer sequence.

In another [aspectexample](#) 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.

15 In another [aspectexample](#) of the composition, the direct repeat sequence comprises a nucleotide sequence set forth in any one of SEQ ID NO: 492-494.

In another [aspectexample](#) of the composition, the substitution increases interactions between the variant Cas12i2 polypeptide and the direct repeat sequence.

20 In another [aspectexample](#) of the composition, the substitution increases binary complex formation, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, a binary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent binary complex.

In another [aspectexample](#) of the composition, the substitution is an arginine, lysine, glutamine, asparagine, histidine, tyrosine, or serine substitution.

25 In another [aspectexample](#) of the composition, the substitution is in an RNA binding interface.

In another [aspectexample](#) of the composition, the substitution is a substitution in the Wedge domain or Rec2 domain.

In another [aspectexample](#) of the composition, the substitution is a substitution listed in Table 4.

30 In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3 to 146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3 to 146 or in any one of SEQ ID NOs: 495 to 512 and further having a substitution listed in Table 4.

35 In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3 to 146 or in any one of SEQ ID NOs: 495 to 512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3 to 146 or in any one of SEQ ID NOs: 495 to 512 and further has a substitution listed in Table 4.

In another [aspectexample](#) of the composition, the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises a sequence set forth in SEQ ID NO: 4.

In another [aspectexample](#) of the composition, the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.

In another [aspectexample](#) of the composition, the nucleic acid is a target nucleic acid.

In another [aspectexample](#) of the composition, the nucleic acid is double-stranded DNA.

In another [aspectexample](#) of the composition, the substitution increases interactions between the variant Cas12i2 polypeptide and double-stranded DNA.

In another [aspectexample](#) of the composition, the double-stranded DNA comprises a PAM sequence.

In another [aspectexample](#) of the composition, the substitution increases ternary complex formation, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.

In another [aspectexample](#) of the composition, the substitution is an arginine, lysine, glutamine, asparagine, histidine, or serine substitution.

In another [aspectexample](#) of the composition, the substitution is in a double-stranded DNA binding interface.

In another [aspectexample](#) of the composition, the substitution is a substitution in the Rec1 domain, PI domain, or Wedge domain.

In another [aspectexample](#) of the composition, the substitution is a substitution listed in Table 5.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 5.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 5.

In another [aspectexample](#) of the composition, the nucleic acid is single-stranded DNA.

In another [aspectexample](#) of the composition, the single-stranded DNA comprises a non-target strand.

In another [aspectexample](#) of the composition, the single-stranded DNA comprises a target strand.
In another [aspectexample](#) of the composition, the substitution increases ternary complex formation, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.

In another [aspectexample](#) of the composition, the substitution is an arginine, lysine, glutamine, asparagine, histidine, or alanine substitution.

In another [aspectexample](#) of the composition, the substitution is in a single-stranded DNA binding interface.

In another [aspectexample](#) of the composition, the substitution is a substitution in the PI domain, Rec1 domain, Wedge domain, RuvC domain, Rec2 domain, or Nuc domain.

In another [aspectexample](#) of the composition, the substitution is a substitution listed in Table 6.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3 to 146 or in any one of SEQ ID NOs: 495 to 512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3 to 146 or in any one of SEQ ID NOs: 495 to 512 and further having a substitution listed in Table 6.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3 to 146 or in any one of SEQ ID NOs: 495 to 512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3 to 146 or in any one of SEQ ID NOs: 495 to 512 and further has a substitution listed in Table 6.

In another [aspectexample](#) of the composition, the substitution increases interactions between the variant Cas12i2 polypeptide and a DNA/RNA hybrid molecule.

In another [aspectexample](#) of the composition, the DNA/RNA hybrid molecule is a heteroduplex comprising a spacer sequence of an RNA guide and a target strand.

In another [aspectexample](#) of the composition, the substitution stabilizes the heteroduplex.

In another [aspectexample](#) of the composition, the substitution increases ternary complex formation, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.

In another [aspectexample](#) of the composition, the substitution is an arginine, lysine, glutamine, asparagine, histidine, or serine substitution.

In another [aspectexample](#) of the composition, the substitution is a substitution in the Rec1 domain, PI domain, Rec2 domain, or RuvC2 motif.

In another [aspectexample](#) of the composition, the substitution is a substitution listed in Table 7.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 7.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 7.

In another [aspectexample](#) of the composition, the substitution increases interactions between the variant Cas12i2 polypeptide and bases of (a) a double-stranded DNA duplex and/or (b) a heteroduplex comprising a spacer sequence of an RNA guide and a target strand.

In another [aspectexample](#) of the composition, the double-stranded DNA duplex comprises a PAM sequence.

In another [aspectexample](#) of the composition, the interactions are aromatic, hydrophobic, Van der Waals, and/or cation-pi interactions.

In another [aspectexample](#) of the composition, the substitution stabilizes the R-loop.

In another [aspectexample](#) of the composition, the substitution increases ternary complex formation, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.

In another [aspectexample](#) of the composition, the substitution is an arginine, lysine, tryptophan, phenylalanine, tyrosine, methionine, histidine, glutamine, threonine, or valine substitution.

In another [aspectexample](#) of the composition, the substitution is a substitution in the Wedge domain, Rec1 domain, or RuvC domain.

In another [aspectexample](#) of the composition, the substitution is a substitution listed in Table 8.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 8.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another ~~aspect~~example of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 8.

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

In another ~~aspect~~example of the composition, the variant Cas12i2 polypeptide exhibits increased enzymatic activity, as compared to a parent polypeptide.

In another ~~aspect~~example of the composition, the composition further comprises an RNA guide comprising a direct repeat sequence and a spacer sequence.

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

In another ~~aspect~~example of the composition, the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises a sequence set forth in SEQ ID NO: 4. 239.

In another ~~aspect~~example of the composition, the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.

The ~~invention~~disclosure yet further provides a cell comprising a composition described herein.

In one ~~aspect~~example of the cell, the composition does not substantially affect viability of the cell.

The ~~invention~~disclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, wherein the variant Cas12i2 polypeptide comprises a substitution that increases flexibility of the variant Cas12i2 polypeptide during DNA binding, as compared to a parent polypeptide.

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

In another ~~aspect~~example of the composition, the Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.

In another ~~aspect~~example of the composition, the substitution increases binding of the variant Cas12i2 polypeptide to DNA.

In another ~~aspect~~example of the composition, the substitution increases binding of the variant Cas12i2 polypeptide to double-stranded DNA.

In another ~~aspect~~example of the composition, the substitution increases binding of the variant Cas12i2 polypeptide to single-stranded DNA.

In another ~~aspect~~example of the composition, the substitution increases ternary complex formation, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.

In another [aspectexample](#) of the composition, the substitution is a substitution of a bulky amino acid to an amino acid with a smaller side chain.

5 In another [aspectexample](#) of the composition, the substitution is an alanine, valine, glycine, or serine substitution.

In another [aspectexample](#) of the composition, the substitution is in the Helical II domain of the variant Cas12i2 polypeptide.

In another [aspectexample](#) of the composition, the substitution is a substitution listed in Table 9.

10 In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 9.

15 In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 9.

20 The [inventiondisclosure](#) yet further provides a composition comprising a variant Cas12i2 polypeptide, wherein the variant Cas12i2 polypeptide comprises a substitution that stabilizes a domain-domain interface that forms during ternary complex formation, as compared to a parent polypeptide.

In one [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 4.

25 In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.

In another [aspectexample](#) of the composition, the domain-domain interface forms when single-stranded DNA contacts an active site of the variant Cas12i2 polypeptide.

30 In another [aspectexample](#) of the composition, the domain-domain interface is a Helical II domain-Nuc domain interface.

In another [aspectexample](#) of the composition, the substitution increases ternary complex formation, as compared to a parent polypeptide.

35 In another [aspectexample](#) of the composition, a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.

In another [aspectexample](#) of the composition, the substitution is an aspartic acid, glutamic acid, arginine, or lysine substitution.

In another aspectexample of the composition, the substitution is a substitution listed in Table 10.

In another aspectexample of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

5 In another aspectexample of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 10.

In another aspectexample of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

10 In another aspectexample of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 10.

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

15 In another aspectexample of the composition, the variant Cas12i2 polypeptide exhibits increased enzymatic activity, as compared to a parent polypeptide.

In another aspectexample of the composition, the composition further comprises an RNA guide comprising a direct repeat sequence and a spacer sequence.

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

In another aspectexample of the composition, the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises a sequence set forth in SEQ ID NO: 4.

25 In another aspectexample of the composition, the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.

The inventiondisclosure yet further provides a cell comprising the composition as described herein.

30 In one aspectexample of the cell, the composition does not substantially affect viability of the cell.

The inventiondisclosure yet further provides a composition comprising a variant Cas12i2 polypeptide, wherein the variant Cas12i2 polypeptide comprises a substitution that increases on-target specificity of the variant Cas12i2 polypeptide, as compared to a parent polypeptide.

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

In another aspectexample of the composition, the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.

In another aspectexample of the composition, the substitution increases on-target DNA binding.

In another [aspectexample](#) of the composition, the substitution decreases off-target DNA binding.

In another [aspectexample](#) of the composition, the substitution increases on-target ternary complex formation, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, an on-target ternary complex comprising the
5 variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.

In another [aspectexample](#) of the composition, the substitution is a substitution of an amino acid contacting the spacer sequence of an RNA guide.

In another [aspectexample](#) of the composition, the substitution is a substitution of a bulky amino acid to an amino acid with a smaller side chain.

10 In another [aspectexample](#) of the composition, the substitution is an alanine, serine, valine, glutamine, or asparagine substitution.

In another [aspectexample](#) of the composition, the substitution is a substitution in the Wedge domain, Rec1 domain, Rec2 domain, or RuvC domain.

In another [aspectexample](#) of the composition, the substitution is a substitution in the Helical II
15 domain.

In another [aspectexample](#) of the composition, the substitution is a substitution listed in Table 11.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

20 In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 11.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

25 In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 11.

In another [aspectexample](#) of the composition, the substitution decreases a catalysis rate (Kcat) of the variant Cas12i2 polypeptide.

30 In another [aspectexample](#) of the composition, the substitution is an alanine, serine, threonine, valine, leucine, methionine, asparagine, or isoleucine substitution.

In another [aspectexample](#) of the composition, the substitution is a substitution in the Wedge domain, Rec1 domain, Rec2 domain, or RuvC domain.

In another [aspectexample](#) of the composition, the substitution is a substitution in the RuvC
35 domain.

In another [aspectexample](#) of the composition, the substitution is a substitution listed in Table 12.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 12.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 12.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide exhibits increased on-target enzymatic activity, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide exhibits decreased off-target enzymatic activity, as compared to a parent polypeptide.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide exhibits off-target editing that is no more than 10% of the on-target editing.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide exhibits off-target editing that is no more than 5% of the on-target editing.

In another [aspectexample](#) of the composition, the composition further comprises an RNA guide comprising a direct repeat sequence and a spacer sequence.

In another [aspectexample](#) 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 [aspectexample](#) of the composition, the direct repeat sequence comprises a nucleotide sequence set forth in any one of SEQ ID NO: 492-494.

In another [aspectexample](#) of the composition, the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises a sequence set forth in SEQ ID NO: 4.

In another [aspectexample](#) of the composition, the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.

The ~~inventiondisclosure~~ yet further provides a cell comprising the composition as described herein.

In one [aspectexample](#) of the cell, the composition does not substantially affect viability of the cell.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide exhibits a higher ratio of on-target binding to off-target binding, as compared to a Cas9 polypeptide.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide exhibits a higher ratio of on-target activity to off-target activity, as compared to a Cas9 polypeptide.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide exhibits a higher ratio of on-target editing to off-target editing, as compared to a Cas9 polypeptide.

In another [aspectexample](#) of the composition, the variant Cas12i2 polypeptide exhibits less off-target binding, as compared to a Cas9 polypeptide.

In another aspectexample of the composition, the variant Cas12i2 polypeptide exhibits less off-target activity, as compared to a Cas9 polypeptide.

In another aspectexample of the composition, the variant Cas12i2 polypeptide exhibits less off-target editing, as compared to a Cas9 polypeptide.

In another aspectexample of the composition, off-target binding, off-target activity, and/or off-target editing by the variant Cas12i2 polypeptide is at least 10% less than off-target binding, off-target activity, and/or off-target editing by a Cas9 polypeptide.

In another aspectexample of the composition, off-target binding, off-target activity, and/or off-target editing by the variant Cas12i2 polypeptide is at least 20% less than off-target binding, off-target activity, and/or off-target editing by a Cas9 polypeptide.

In another aspectexample of the composition, off-target binding, off-target activity, and/or off-target editing by the variant Cas12i2 polypeptide is at least 30% less than off-target binding, off-target activity, and/or off-target editing by a Cas9 polypeptide.

In another aspectexample of the composition, off-target binding, off-target activity, and/or off-target editing by the variant Cas12i2 polypeptide is at least 40% less than off-target binding, off-target activity, and/or off-target editing by a Cas9 polypeptide.

In another aspectexample of the composition, off-target binding, off-target activity, and/or off-target editing by the variant Cas12i2 polypeptide is at least 50% less than off-target binding, off-target activity, and/or off-target editing by a Cas9 polypeptide.

In yet another aspectexample, the inventiondisclosure provides a cell comprising a composition described herein.

In one aspectexample of the cell, the composition does not substantially affect viability of the cell.

Definitions

The present inventiondisclosure will be described with respect to particular embodimentexamples and with reference to certain Figures, but the inventiondisclosure is not limited thereto but only by the claims. Terms as set forth hereinafter are generally to be understood in their common sense unless indicated otherwise.

As used herein, the term “activity” refers to a biological activity. In some embodimentexamples, the activity refers to effector activity. In some embodimentexamples, activity includes enzymatic activity, e.g., catalytic ability of an effector. For example, activity can include nuclease activity. In some embodimentexamples, activity includes binding activity, e.g., binding activity of an effector to an RNA guide and/or target nucleic acid.

As used herein, the term “adjacent to” refers to a nucleotide or amino acid sequence in close proximity to another nucleotide or amino acid sequence. In some embodimentexamples, a nucleotide sequence is adjacent to another nucleotide sequence if no nucleotides separate the two sequences. In some embodimentexamples, a nucleotide sequence is adjacent to another nucleotide sequence if a small number of nucleotides separate the two sequences (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides). In some embodimentexamples, a first sequence is adjacent to a second sequence if the two sequences are separated by about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In some embodimentexamples,

the term “adjacent to” is used to refer a protein residue that interacts with another protein residue. In some [embodimentexamples](#), the term “adjacent to” is used to refer a protein residue that interacts with a nucleotide or nucleic acid. In some [embodimentexamples](#), the term “adjacent to” is used to refer to a protein domain or motif that interacts with another protein domain or motif. In some [embodimentexamples](#), the term “adjacent to” is used to refer to a protein domain or motif that interacts with a nucleotide or nucleic acid sequence. As used herein, the term “adjacent to” is used to refer to the positioning of an indel (insertion/deletion) in a modified cell of the disclosure.

As used herein, the term “complex” refers to a grouping of two or more molecules. In some [embodimentexamples](#), the complex comprises a polypeptide and a nucleic acid molecule interacting with (e.g. binding to, coming into contact with, adhering to) one another.

As used herein, the term “binary complex” refers to a grouping of two molecules (e.g., a polypeptide and a nucleic acid molecule). In some [embodimentexamples](#), a binary complex refers to a grouping of a polypeptide and a targeting moiety (e.g., an RNA guide). In some [embodimentexamples](#), a binary complex refers to a ribonucleoprotein (RNP). As used herein, the term “variant binary complex” refers to the grouping of a variant Cas12i2 polypeptide and RNA guide. As used herein, the term “parent binary complex” refers to the grouping of a parent polypeptide and RNA guide or a reference polypeptide and RNA guide.

As used herein, the term “ternary complex” refers to a grouping of three molecules (e.g., a polypeptide and two nucleic acid molecules). In some [embodimentexamples](#), a “ternary complex” refers to a grouping of a polypeptide, an RNA molecule, and a DNA molecule. In some [embodimentexamples](#), a ternary complex refers to a grouping of a polypeptide, a targeting moiety (e.g., an RNA guide), and a target nucleic acid (e.g., a target DNA molecule). In some [embodimentexamples](#), a “ternary complex” refers to a grouping of a binary complex (e.g., a ribonucleoprotein) and a third molecule (e.g., a target nucleic acid). As used herein, the terms “variant ternary complex,” “variant Cas12i2 ternary complex,” and “Cas12i2 variant ternary complex” refer to the grouping of a variant Cas12i2 polypeptide, RNA guide, and target nucleic acid (e.g., a variant Cas12i2 ribonucleoprotein and target nucleic acid). As used herein, the terms “parent ternary complex,” “parent Cas12i2 ternary complex,” and “Cas12i2 parent ternary complex” refer to the grouping of a parent polypeptide, RNA guide, and target nucleic acid (e.g., a parent ribonucleoprotein and target nucleic acid) or a reference polypeptide, RNA guide, and target nucleic acid (e.g., a reference ribonucleoprotein and target nucleic acid).

As used herein, the term “deletion” refers to a loss or removal of nucleotides in a nucleic acid sequence. The deletion can be in a genome of an organism. The deletion can be in a cell. The deletion can be a DNA sequence. The deletion can be an RNA sequence. The deletion can be a frameshift mutation or a non-frameshift mutation. A Cas12i2-induced deletion described herein can refer to a deletion of up to about 100 nucleotides, such as from about 4 nucleotides and 100 nucleotides, from about 4 nucleotides and 50 nucleotides, from about 4 nucleotides and 40 nucleotides, from about 4 nucleotides and 25 nucleotides, from about 10 nucleotides and 25 nucleotides, from about 10 nucleotides and 15 nucleotides, from a nucleic acid molecule. In some [embodimentexamples](#), a Cas12i2-induced deletion described herein occurs downstream of

a 5'-NTTN-3' sequence. In some [embodimentexamples](#), the term "Cas12i2-induced" refers to a deletion that results from a DNA break induced by a Cas12i2 polypeptide. In some [embodimentexamples](#), the term "Cas12i2-induced" refers to a deletion that results from a DNA break induced by a Cas12i2 polypeptide and repaired by a cell's DNA repair machinery.

As used herein, the term "domain" refers to a distinct functional and/or structural unit of a polypeptide. In some [embodimentexamples](#), a domain may comprise a conserved amino acid sequence.

As used herein, the terms "editing efficiency" and "indel activity" refer to the ability of an enzyme (e.g., a variant Cas12i2 polypeptide) to introduce an indel (insertion/deletion) into a sequence. For example, in some [embodimentexamples](#), an enzyme that introduces an indel into each of ten target loci exhibits an editing efficiency of 100%. An enzyme that introduces an indel into five out of ten target loci exhibits an editing efficiency of 50%. In another example, an enzyme that introduces an indel at a target locus in 50% of a plurality of cells exhibits an editing efficiency of 50%. As used herein, the terms "editing efficiency," "indel activity," and "on-target editing" refer to the ability of an enzyme (e.g., a variant Cas12i2 polypeptide) to selectively introduce an indel at a target locus. In some [embodimentexamples](#), editing efficiency at a target locus is compared to editing efficiency at a non-target locus. In some [embodimentexamples](#), editing at a target locus is compared to editing at a non-target locus.

As used herein, the term "effector activity" refers to a biological activity. In some [embodimentexamples](#), effector activity includes enzymatic activity, e.g., catalytic ability of an effector. For example, effector activity can include nuclease activity.

As used herein, the term "interface" refers to one or more residues of a variant Cas12i2 polypeptide (e.g., a domain/motif or a portion of a domain/motif) in contact with (e.g., that interact with or are adjacent to) a nucleic acid molecule or a distinct domain/motif or a portion of a distinct domain/motif of the variant Cas12i2 polypeptide. In some [aspectexamples](#), an interface is a buried surface area between adjacent domains or motifs. In some [aspectexamples](#), an interface is a surface area between the a polypeptide and a ligand (e.g., DNA or RNA) where the polypeptide and ligand make contact. As used herein, the term "nucleic acid interface" refers to residues of the variant Cas12i2 polypeptide that are in close proximity to (e.g., are adjacent to) or interact with a nucleic acid sequence (e.g., a DNA sequence or an RNA sequence). As used herein, the term "RNA binding interface" refers to the residues of the variant Cas12i2 polypeptide that are in close proximity to (e.g., are adjacent to) or interact with an RNA guide (e.g., the direct repeat of the RNA guide). As used herein, the term "double-stranded DNA binding interface" refers to the residues of the variant Cas12i2 polypeptide that are in close proximity to (e.g., are adjacent to) and/or interact with double-stranded DNA. As used herein, the term "single-stranded DNA binding interface" refers to the residues of the variant Cas12i2 polypeptide that are in close proximity to (e.g., are adjacent to) and/or interact with single-stranded DNA. As used herein, the term "domain-domain interface" refers to a domain in close-proximity to (e.g., adjacent to) a separate domain. In some [embodimentexamples](#), a domain-domain interface (e.g., a Helical II domain-Nuc domain interface) forms upon complex formation (e.g., ternary complex formation).

As used herein, the terms “parent,” “parent polypeptide,” and “parent sequence” refer to an original polypeptide (e.g., starting polypeptide) to which an alteration is made to produce a variant Cas12i2 polypeptide of the present ~~invention~~disclosure. In some ~~embodiment~~examples, the parent is a polypeptide having an identical amino acid sequence of the variant with one or more variations at one or more specified positions. In exemplary ~~embodiment~~examples, variations refer to amino acid changes within the polypeptide sequence. The parent may be a naturally occurring (wild-type) polypeptide. In a particular ~~embodiment~~example, the parent is a polypeptide with at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 70%, at least 72%, at least 73%, at least 74%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to a polypeptide of SEQ ID NO: 2. In some ~~embodiment~~examples, variations may include structural changes such as linkages, fusions, or other changes that do not alter the original amino acid sequence of the parent. In some ~~embodiment~~examples, the parent polypeptide sequence includes amino acid variations and structural changes.

As used herein, a “plurality of variant binary complexes” refers to a plurality of binary complexes comprising a plurality of variant Cas12i2 polypeptides. A plurality of binary complexes comprising a plurality of parent polypeptides (e.g., wild-type Cas12i2 polypeptides parent of SEQ ID NO: 2) are referred to herein as a “plurality of parent binary complexes.” A plurality of Cas12i2 polypeptides, e.g., a plurality of variant Cas12i2 polypeptides, and two or more distinct targeting moieties, e.g., two or more distinct RNA guides, individually form two or more binary complexes (e.g., a plurality of binary complexes). In such ~~embodiment~~examples, complex formation may be accomplished simultaneously in a single composition or independently in separate compositions.

As used herein, the term “protospacer adjacent motif” or “PAM” refers to a DNA sequence adjacent to a target sequence to which a binary complex comprising a polypeptide (e.g., an enzyme such as Cas12i2 or a variant thereof) and a targeting moiety (e.g., an RNA guide) binds. In some ~~embodiment~~examples, a PAM is required for enzyme activity. In the case of a double-stranded target, the targeting moiety (e.g., the RNA guide) binds to a first strand of the target, and a PAM sequence as described herein is present in the second, complementary strand. For example, in some ~~embodiment~~examples, the RNA guide binds to the target strand (e.g., the spacer-complementary strand), and the PAM sequence as described herein is present in the non-target strand (i.e., the non-spacer-complementary strand). In some ~~embodiment~~examples, the target strand (i.e., the spacer-complementary strand) comprises a 5’-NAAN-3’ sequence.

As used herein, the terms “reference composition,” “reference molecule,” “reference sequence,” “reference,” and “reference complex” refer to a control, such as a negative control or a parent (e.g., a parent sequence, a parent protein, a wild-type protein, or a complex comprising a parent sequence). For example, a reference molecule refers to a Cas12i2 polypeptide to which a variant Cas12i2 polypeptide is compared. Likewise, a reference RNA guide refers to a targeting moiety to which a modified RNA guide is compared. The variant or modified molecule may be compared to the reference molecule on the basis of sequence (e.g., the variant or modified molecule may have X% sequence identity or homology with the reference

molecule), thermostability, or activity (e.g., the variant or modified molecule may have X% of the activity of the reference molecule). For example, where the reference molecule is a Cas12i2 polypeptide, a variant or modified molecule may be characterized as having no more than 10% of an activity of the reference Cas12i2 polypeptide or may be characterized as having at least 10% greater of an activity of the reference Cas12i2 polypeptide. Examples of reference Cas12i2 polypeptides include naturally occurring unmodified Cas12i2 polypeptides, e.g., naturally occurring Cas12i2 polypeptide from archaea or other bacterial species. In certain embodimentexamples, the reference Cas12i2 polypeptide is a naturally occurring Cas12i2 polypeptide having the closest sequence identity or homology with the variant Cas12i2 polypeptide to which it is being compared. In certain embodimentexamples, the reference Cas12i2 polypeptide is a parental molecule having a naturally occurring or known sequence on which a mutation has been made to arrive at the variant Cas12i2 polypeptide.

As used herein, the terms “RNA guide” or “RNA guide sequence” refer to any RNA molecule that facilitates the targeting of a polypeptide described herein to a target nucleic acid. For example, an RNA guide can be a molecule that recognizes (e.g., binds to) a target nucleic acid. An RNA guide may be designed to be complementary to a specific nucleic acid sequence. An RNA guide comprises a DNA targeting sequence and a direct repeat (DR) sequence. The terms CRISPR RNA (crRNA), pre-crRNA and mature crRNA are also used herein to refer to an RNA guide.

As used herein, the term “substantially identical” refers to a sequence, polynucleotide, or polypeptide, that has a certain degree of identity to a reference sequence.

As used herein, the term “targeting moiety” refers to a molecule or component (e.g., nucleic acid and/or RNA guide) that facilitates the targeting of another molecule or component to a target nucleic acid. In some embodimentexamples, the targeting moiety specifically interacts or associates with the target nucleic acid.

As used herein, the terms “target nucleic acid,” “target sequence,” “target substrate,” and “on-target locus” refer to a nucleic acid sequence to which a targeting moiety (e.g., RNA guide) specifically binds. In some embodimentexamples, the DNA targeting sequence of an RNA guide binds to a target nucleic acid. Binding of a binary complex to a target locus is referred to herein as “on-target binding.”

As used herein, the terms “non-target” and “off-target” refer to a nucleic acid sequence other than the sequence to which a targeting moiety specifically binds or is intended to specifically bind. A non-target locus is an unintended target of a targeting moiety (e.g., an RNA guide). Binding of a binary complex to a non-target locus is referred to herein as “off-target binding.” In some embodimentexamples, a non-target locus is a locus on a target nucleic acid. In some embodimentexamples, a non-target locus is a locus on a nucleic acid other than the target nucleic acid (e.g., a non-target nucleic acid).

As used herein, the terms “upstream” and “downstream” refer to relative positions within a single nucleic acid (e.g., DNA) sequence in a nucleic acid molecule. As used herein, the terms “upstream” and “downstream” refer to the positioning of two sequences relative to one another. “Upstream” and “downstream” relate to the 5’ to 3’ direction, respectively, in which RNA transcription occurs. A first sequence is upstream of a second sequence when the 3’ end of the first sequence occurs before the 5’ end

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 any one of SEQ ID NOs: 3 to 146 or any one of SEQ ID NOs: 495 to 512.
2. The variant Cas12i2 polypeptide of claim 1, comprising the sequence set forth in SEQ ID NO: 4.
- 10 3. The variant Cas12i2 polypeptide of claim 1 or 2, comprising the sequence set forth in SEQ ID NO: 5.
4. The variant Cas12i2 polypeptide of claim 1, comprising the sequence set forth in SEQ ID NO: 495.
5. The variant Cas12i2 polypeptide of claim 1, comprising the sequence set forth in SEQ ID NO: 496.
6. The variant Cas12i2 polypeptide of any one of claims 1 to 5, further comprising a substitution of
15 Table 2.
7. The variant Cas12i2 polypeptide of any one of claims 1 to 6, wherein the variant Cas12i2 polypeptide is a variant of a Cas12i2 polypeptide comprising the sequence set forth in SEQ ID NO: 2.
8. A composition comprising a variant Cas12i2 polypeptide of any one of claims 1 to 7, wherein the
20 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.
9. The composition of claim 8, wherein the direct repeat sequence comprises a nucleotide sequence with at least 95% sequence identity to any one of SEQ ID NO: 492-494.
10. The composition of claim 9, wherein the direct repeat sequence comprises a nucleotide sequence
25 set forth in any one of SEQ ID NO: 492-494.
11. The composition of any one of claims 8 to 10, wherein the spacer sequence comprises between about 11 and about 50 nucleotides.
12. The composition of claim 11, wherein the spacer sequence comprises between about 15 and about 35 nucleotides.
- 30 13. The composition of any one of claims 8 to 12, 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.
14. The variant Cas12i2 polypeptide of any one of claims 1 to 7, or the composition of any one of any one of claims 8 to 13, 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. The variant Cas12i2 polypeptide of any one of claims 1 to 7 or 14, or the composition of any one of claims 8 to 14, 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.
16. The composition of any one of claims 8 to 15, wherein the composition is present in a delivery system comprising a nanoparticle, a liposome, an exosome, a microvesicle, or a gene-gun.
17. A nucleic acid molecule encoding a variant Cas12i2 polypeptide of any one of claims 1 to 7, 14, or 15.
18. A cell comprising the composition or variant Cas12i2 polypeptide of any previous claim.
19. The cell of claim 18, wherein the cell is a eukaryotic cell or a prokaryotic cell.
20. The cell of claim 18 or 19, wherein the cell is a mammalian cell or a plant cell.
21. The cell of any one of claims 18 to 20, wherein the cell is a human cell.
22. A composition or formulation comprising the variant Cas12i2 polypeptide of any one of claims 1 to 7, 14, or 15 and optionally an RNA guide and/or a cell.
23. 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.
24. 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.
25. 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.

26. ~~The composition of any one of claims 23 to 25, wherein 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.~~
- 5 27. ~~The composition of any one of claims 23 to 26, wherein the variant Cas12i2 polypeptide exhibits increased complex formation, increased binding affinity to the RNA guide and/or increased stability over a range of incubation times.~~
28. ~~The composition of any one of claims 23 to 27, wherein 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.~~
- 10 29. ~~The composition of any one of claims 23 to 28, wherein 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.~~
- 15 30. ~~The composition of any one of claims 23 to 29, wherein the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.~~
31. ~~The composition of any one of claims 23 to 30, wherein the variant Cas12i2 polypeptide exhibits equivalent to or greater enzymatic activity than the parent polypeptide.~~
32. ~~The composition of claim 31, wherein the equivalent to or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.~~
- 20 33. ~~The composition of any one of claims 23 to 32, wherein the variant Cas12i2 polypeptide exhibits increased stability and/or protein-RNA interactions.~~
34. ~~The composition of any one of claims 23 to 33, wherein the variant Cas12i2 polypeptide further lacks enzymatic activity.~~
- 25 35. ~~The composition of claim 34, wherein the variant Cas12i2 polypeptide further exhibits increased enzymatic activity.~~
36. ~~The composition of any one of claims 23 to 35, wherein the variant Cas12i2 polypeptide exhibits increased RNA guide complex formation, RNA guide binding activity and/or RNA guide binding specificity.~~
- 30 37. ~~The composition of any one of claims 23 to 36, wherein the variant Cas12i2 polypeptide exhibits altered on-target binding.~~

38. ~~The composition of any one of claims 23 to 37, wherein the variant Cas12i2 polypeptide exhibits altered off target binding.~~
39. ~~A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits decreased complex dissociation than a complex formed by a parent polypeptide and the RNA guide.~~
40. ~~A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, and wherein the RNA guide exhibits decreased dissociation from the variant Cas12i2 polypeptide than an RNA guide of a parent complex.~~
41. ~~A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased stability than a complex formed by a parent polypeptide and the RNA guide.~~
42. ~~The composition of any one of claims 39 to 41, wherein the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.~~
43. ~~The composition of any one of claims 39 to 42, wherein the variant binary complex exhibits increased stability over a range of incubation times.~~
44. ~~The composition of any one of claims 39 to 43, wherein the variant binary complex exhibits increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.~~
45. ~~The composition of any one of claims 39 to 44, wherein the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid 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.~~
46. ~~The composition of any one of claims 39 to 45, wherein the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.~~
47. ~~The composition of any one of claims 39 to 46, wherein the variant Cas12i2 polypeptide exhibits equivalent or greater enzymatic activity than the parent polypeptide.~~
48. ~~The composition of claim 47, wherein the equivalent or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.~~

49. ~~The composition of any one of claims 39 to 48, wherein the variant Cas12i2 polypeptide exhibits increased stability and/or protein-RNA interactions.~~
50. ~~The composition of any one of claims 39 to 49, wherein the variant Cas12i2 polypeptide further lacks enzymatic activity.~~
- 5 51. ~~The composition of any one of claims 39 to 49, wherein the variant Cas12i2 polypeptide further exhibits increased enzymatic activity.~~
52. ~~The composition of any one of claims 39 to 51, wherein the variant Cas12i2 polypeptide exhibits increased RNA-guide complex formation, RNA-guide binding activity and/or RNA-guide binding specificity.~~
- 10 53. ~~The composition of any one of claims 39 to 52, wherein the variant Cas12i2 polypeptide exhibits altered on-target binding.~~
54. ~~The composition of any one of claims 39 to 53, wherein the variant Cas12i2 polypeptide exhibits altered off-target binding.~~
- 15 55. ~~A method of complexing the variant Cas12i2 polypeptide with the RNA-guide, e.g., RNA-guide, of any one of claims 39 to 54.~~
56. ~~A composition comprising a variant Cas12i2 polypeptide, an RNA-guide and a target nucleic acid, wherein the variant Cas12i2 polypeptide and the RNA-guide form a variant binary complex, and wherein the variant binary complex exhibits increased ternary complex formation with the target nucleic acid as compared to a parent binary complex.~~
- 20 57. ~~A composition comprising a variant Cas12i2 polypeptide, an RNA-guide and a target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA-guide form a variant binary complex, and wherein the variant binary complex exhibits increased binding affinity to the target nucleic acid, as compared to a parent binary complex.~~
- 25 58. ~~A composition comprising a variant Cas12i2 polypeptide, an RNA-guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA-guide form a variant binary complex, and wherein the variant binary complex exhibit increased protein-RNA interactions as compared to a parent binary complex.~~
- 30 59. ~~A composition comprising a variant Cas12i2 polypeptide, an RNA-guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA-guide form a variant binary complex, and wherein the variant binary complex exhibit increased protein-DNA interactions as compared to a parent binary complex.~~

60. ~~The composition of any one of claims 56 to 59, wherein the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.~~
- 5 61. ~~The composition of any one of claims 56 to 60, wherein the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability over a range of incubation times.~~
62. ~~The composition of any one of claims 56 to 61, wherein the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.~~
- 10 63. ~~The composition of any one of claims 56 to 62, wherein the variant binary complex exhibits increased ternary complex formation, increased binding affinity to the target nucleic acid and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.~~
- 15 64. ~~The composition of any one of claims 56 to 63, wherein the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.~~
65. ~~The composition of any one of claims 56 to 64, wherein the variant binary complex exhibits equivalent to or greater enzymatic activity than the parent binary complex.~~
66. ~~The composition of claim 65, wherein the equivalent to or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.~~
- 20 67. ~~The composition of any one of claims 56 to 66, wherein the variant binary complex exhibits increased stability and/or protein-RNA interactions.~~
68. ~~The composition of any one of claims 56 to 67, wherein the variant binary complex exhibits increased stability and/or protein-DNA interactions.~~
- 25 69. ~~The composition of any one of claims 56 to 68, wherein the variant binary complex further lacks enzymatic activity.~~
70. ~~The composition of any one of claims 56 to 68, wherein the variant binary complex further exhibits increased enzymatic activity.~~
- 30 71. ~~The composition of any one of claims 56 to 70, wherein the variant Cas12i2 polypeptide exhibits increased RNA-guide complex formation, RNA-guide binding activity and/or RNA-guide binding specificity.~~

72. ~~The composition of any one of claims 56 to 71, wherein the variant binary complex exhibits increased target nucleic acid complex formation, target nucleic acid activity and/or target nucleic acid specificity.~~
- 5 73. ~~The composition of any one of claims 56 to 72, wherein the variant binary complex exhibits altered on target binding.~~
74. ~~The composition of any one of claims 56 to 73, wherein the variant binary complex exhibits altered off target binding~~
- 10 75. ~~A composition comprising a variant Cas12i2 polypeptide, an RNA guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, wherein the variant binary complex and target nucleic acid form a variant ternary complex, and wherein the variant ternary complex exhibits decreased complex dissociation than a parent ternary complex.~~
- 15 76. ~~A composition comprising a variant Cas12i2 polypeptide, an RNA guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, wherein the variant binary complex and target nucleic acid form a variant ternary complex, and wherein the target nucleic acid exhibits decreased dissociation from the variant ternary complex than a parent ternary complex.~~
- 20 77. ~~A composition comprising a variant Cas12i2 polypeptide, an RNA guide and target nucleic acid, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, wherein the variant binary complex and target nucleic acid form a variant ternary complex, and wherein the variant ternary complex exhibits increased stability than a parent ternary complex.~~
- 25 78. ~~The composition of any one of claims 75 to 77, wherein the variant ternary complex exhibits increased stability over a range of temperatures, e.g., 20°C to 65°C.~~
79. ~~The composition of any one of claims 75 to 78, wherein the variant ternary complex exhibits increased stability over a range of incubation times.~~
80. ~~The composition of any one of claims 75 to 79, wherein the variant ternary complex exhibits increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.~~
- 30 81. ~~The composition of any one of claims 75 to 80, wherein the variant ternary complex exhibits increased stability hen a T_m value of the variant ternary complex is at least 8°C greater than the T_m value of the parent ternary complex.~~
82. ~~The composition of any one of claims 75 to 81, wherein the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.~~

- ~~83. The composition of any one of claims 75 to 82, wherein the variant Cas12i2 polypeptide exhibits equivalent or greater enzymatic activity than the parent polypeptide.~~
- ~~84. The composition of claim 83, wherein the equivalent or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.~~
- 5 ~~85. The composition of any one of claims 75 to 84, wherein the variant Cas12i2 polypeptide exhibits increased stability and/or protein-RNA interactions.~~
- ~~86. The composition of any one of claims 75 to 85, wherein the variant binary complex exhibits increased stability and/or protein-DNA interactions.~~
- 10 ~~87. The composition of any one of claims 75 to 86, wherein the variant ternary complex exhibits increased stability.~~
- ~~88. The composition of any one of claims 75 to 87, wherein the variant binary complex further lacks enzymatic activity.~~
- ~~89. The composition of any one of claims 75 to 87, wherein the variant binary complex further exhibits increased enzymatic activity.~~
- 15 ~~90. The composition of any one of claims 75 to 89, wherein the variant Cas12i2 polypeptide exhibits increased RNA-guide complex formation, RNA-guide binding activity and/or RNA-guide binding specificity.~~
- ~~91. The composition of any one of claims 75 to 90, wherein the variant binary complex exhibits increased target nucleic acid complex formation, target nucleic acid binding activity and/or target nucleic acid binding specificity.~~
- 20 ~~92. The composition of any one of claims 75 to 91, wherein the variant binary complex exhibits altered on-target binding.~~
- ~~93. The composition of any one of claims 75 to 92, wherein the variant binary complex exhibits altered off-target binding.~~
- 25 ~~94. A method of complexing the variant binary complex with the target nucleic acid, e.g., DNA, of any one of claims 75 to 93.~~
- ~~95. A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased binding affinity to a target nucleic acid as compared to a parent binary complex.~~
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96. ~~A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased target binding affinity to a target locus of a target nucleic acid as compared to a parent binary complex.~~
- 5 97. ~~The composition of any one of claims 95 to 96, wherein the variant binary complex exhibits increased ternary complex formation and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.~~
98. ~~The composition of any one of claims 95 to 97, wherein the variant binary complex exhibits increased ternary complex formation and/or increased stability over a range of incubation times.~~
- 10 99. ~~The composition of any one of claims 95 to 98, wherein the variant binary complex exhibits increased ternary complex formation and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.~~
100. ~~The composition of any one of claims 95 to 99, wherein the variant binary complex exhibits increased ternary complex formation and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.~~
- 15 101. ~~The composition of any one of claims 95 to 100, wherein the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.~~
102. ~~The composition of any one of claims 95 to 101, wherein the variant binary complex exhibits equivalent to or greater enzymatic activity than the parent binary complex.~~
- 20 103. ~~The composition of claim 102, wherein the equivalent to or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.~~
104. ~~The composition of any one of claims 95 to 103, wherein the variant binary complex exhibits increased stability and/or protein-RNA interactions.~~
105. ~~The composition of any one of claims 95 to 104, wherein the variant binary complex exhibits increased stability and/or protein-DNA interactions.~~
- 25 106. ~~The composition of any one of claims 95 to 105, wherein the variant binary complex further lacks enzymatic activity.~~
107. ~~The composition of any one of claims 95 to 105, wherein the variant binary complex further exhibits increased enzymatic activity.~~
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108. ~~The composition of any one of claims 95 to 107, wherein the variant Cas12i2 polypeptide exhibits increased RNA guide complex formation, RNA guide binding activity and/or RNA guide binding specificity.~~
- 5 109. ~~The composition of any one of claims 95 to 108, wherein the variant binary complex exhibits increased target nucleic acid complex formation, target nucleic acid activity and/or target nucleic acid specificity.~~
110. ~~The composition of any one of claims 95 to 109, wherein the variant binary complex exhibits altered on target binding.~~
- 10 111. ~~The composition of any one of claims 95 to 110, wherein the variant binary complex exhibits altered off target binding.~~
112. ~~A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes specifically bind with two or more target loci of a target nucleic acid.~~
- 15 113. ~~A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes exhibit increased on target binding of two or more target loci of a target nucleic acid as compared to parent binary complexes.~~
- 20 114. ~~A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes exhibit increased on target binding with two or more target loci of a target nucleic acid as compared to parent binary complexes.~~
- 25 115. ~~A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes exhibit on target ternary complex formation with two or more target loci of a target nucleic acid.~~
- 30 116. ~~A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the variant binary complexes exhibit increased ternary complex formation with two or more target loci of a target nucleic acid as compared to parent binary complexes.~~

117. ~~The composition of any one of claims 112 to 116, wherein the variant binary complex exhibits increased ternary complex formation with the target nucleic acid and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.~~
- 5 118. ~~The composition of any one of claims 112 to 117, wherein the variant binary complex exhibits increased ternary complex formation with the target nucleic acid and/or increased stability over a range of incubation times.~~
119. ~~The composition of any one of claims 112 to 118, wherein the variant binary complex exhibits increased ternary complex formation with the target nucleic acid and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.~~
- 10 120. ~~The composition of any one of claims 112 to 119, wherein the variant binary complex exhibits increased ternary complex formation with the target nucleic acid and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.~~
- 15 121. ~~The composition of any one of claims 112 to 120, wherein the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.~~
122. ~~The composition of any one of claims 112 to 121, wherein the variant Cas12i2 polypeptide exhibits equivalent or greater enzymatic activity than the parent polypeptide.~~
123. ~~The composition of claim 122, wherein the equivalent or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.~~
- 20 124. ~~The composition of any one of claims 112 to 123, wherein the variant Cas12i2 polypeptide exhibits increased stability and/or protein-RNA interactions.~~
125. ~~The composition of any one of claims 112 to 124, wherein the variant binary complex exhibits increased stability and/or protein-DNA interactions.~~
- 25 126. ~~The composition of any one of claims 112 to 125, wherein the variant binary complex further lacks enzymatic activity.~~
127. ~~The composition of any one of claims 112 to 125, wherein the variant binary complex further exhibits increased enzymatic activity.~~
- 30 128. ~~The composition of any one of claims 112 to 127, wherein the variant Cas12i2 polypeptide exhibits increased RNA-guide complex formation, RNA-guide binding activity and/or RNA-guide binding specificity.~~

129. ~~The composition of any one of claims 112 to 128, wherein the variant binary complex exhibits increased target nucleic acid ternary complex formation, target nucleic acid binding affinity and/or target nucleic acid binding specificity.~~
- 5 130. ~~The composition of any one of claims 112 to 129, wherein the variant binary complex exhibits altered on-target binding.~~
131. ~~The composition of any one of claims 112 to 130, wherein the variant binary complex exhibits altered off-target binding.~~
132. ~~A method of complexing the variant binary complex with the target nucleic acid, e.g., DNA, of any one of claims 112 to 131.~~
- 10 133. ~~A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the Cas12i2 variant polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased on-target binding affinity to a target locus of a target nucleic acid as compared to a parent binary complex.~~
- 15 134. ~~A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits decreased binding affinity to a non-target locus of a target nucleic acid as compared to a parent binary complex.~~
- 20 135. ~~A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits increased activity at an on-target locus of a target nucleic acid as compared to a parent binary complex.~~
- 25 136. ~~A composition comprising a variant Cas12i2 polypeptide and an RNA guide, wherein the variant Cas12i2 polypeptide and the RNA guide form a variant binary complex, and wherein the variant binary complex exhibits decreased activity at a non-target locus of a target nucleic acid as compared to a parent binary complex.~~
- 30 137. ~~The composition of any one of claims 133 to 136, wherein the variant binary complex exhibits increased ternary complex formation at the target locus and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.~~
138. ~~The composition of any one of claims 133 to 137, wherein the variant binary complex exhibits increased ternary complex formation at the target locus and/or increased stability over a range of incubation times.~~

- ~~139. The composition of any one of claims 133 to 138, wherein the variant binary complex exhibits increased ternary complex formation at the target locus and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.~~
- 5 ~~140. The composition of any one of claims 133 to 139, wherein the variant binary complex exhibits increased ternary complex formation at the target locus and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.~~
- ~~141. The composition of any one of claims 133 to 140, wherein the parent binary complex comprises a parent polypeptide that comprises the amino acid sequence of SEQ ID NO: 2.~~
- 10 ~~142. The composition of claim 141, wherein the variant Cas12i2 polypeptide exhibits equivalent or greater enzymatic activity than the parent polypeptide.~~
- ~~143. The composition of claim 142, wherein the equivalent or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.~~
- ~~144. The composition of any one of claims 133 to 143, wherein the variant binary complex exhibits increased stability and/or protein-RNA interactions.~~
- 15 ~~145. The composition of any one of claims 133 to 144, wherein the variant binary complex exhibits increased stability and/or protein-DNA interactions.~~
- ~~146. The composition of any one of claims 133 to 145, wherein the variant Cas12i2 polypeptide exhibits increased binary complex formation, RNA guide binding activity and/or RNA guide binding specificity.~~
- 20 ~~147. A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form a plurality of variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the plurality of variant binary complexes exhibit increased on-target binding to two or more target loci of a target nucleic acid as compared to a plurality of parent binary complexes.~~
- 25 ~~148. A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form a plurality of variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the plurality of variant binary complexes exhibit decreased off-target binding to two or more non-target loci of a target nucleic acid as compared to a plurality of parent binary complexes.~~
- 30 ~~149. A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form a plurality of variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the plurality of variant binary~~

- complexes exhibit increased on-target activity at two or more target loci of a target nucleic acid as compared to a plurality of parent binary complexes.
- 5 ~~150. — A composition comprising a plurality of variant Cas12i2 polypeptides and two or more distinct RNA guides, wherein the distinct RNA guides individually form a plurality of variant binary complexes with separate variant Cas12i2 polypeptides, and wherein the plurality of variant binary complexes exhibit decreased off-target activity at two or more non-target loci of a target nucleic acid as compared to a plurality of parent binary complexes.~~
- 10 ~~151. — The composition of any one of claims 147 to 150, wherein the plurality of variant binary complexes exhibit increased ternary complex formation at the target loci of the target nucleic acid and/or increased stability over a range of temperatures, e.g., 20°C to 65°C.~~
- 15 ~~152. — The composition of any one of claims 147 to 151, wherein the plurality of variant binary complexes exhibit increased ternary complex formation at the target loci of the target nucleic acid and/or increased stability over a range of incubation times.~~
- 20 ~~153. — The composition of any one of claims 147 to 152, wherein the plurality of variant binary complexes exhibit increased ternary complex formation at the target loci the target nucleic acid and/or increased stability in a buffer having a pH in a range of about 7.3 to about 8.6.~~
- 25 ~~154. — The composition of any one of claims 147 to 153, wherein the plurality of variant binary complexes exhibit increased ternary complex formation at the target loci of the target nucleic acid and/or increased stability when a T_m value of the binary complex is at least 8°C greater than the T_m value of the parent binary complex.~~
- 30 ~~155. — The composition of any one of claims 147 to 154, wherein the plurality of parent binary complexes comprise a parent polypeptide that comprises the amino acid sequence of SEQ ID NO: 2.~~
- ~~156. — The composition of any one of claims 147 to 155, wherein the variant Cas12i2 polypeptide exhibits equivalent or greater enzymatic activity than the parent polypeptide.~~
- ~~157. — The composition of claim 156, wherein the equivalent or greater enzymatic activity occurs at a temperature range from about 20°C to about 90°C.~~
- ~~158. — The composition of any one of claims 147 to 157, wherein the variant Cas12i2 polypeptide exhibits increased stability and/or protein-RNA interactions.~~
- ~~159. — The composition of any one of claims 147 to 158, wherein the plurality of variant binary complexes exhibit increased stability and/or protein-DNA interactions.~~

	160. The composition of any one of claims 147 to 159, wherein the variant Cas12i2 polypeptide exhibits increased binary complex formation, RNA guide binding activity and/or RNA guide binding specificity.
5	161. A method of complexing the variant binary complex with the target nucleic acid, e.g., DNA, of any one of claims 147 to 160.
	162. A method of complexing the plurality of variant binary complexes with the target nucleic acid, e.g., DNA, of any one of claims 147 to 161.
10	163. The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, and 133 to 160, wherein the variant Cas12i2 polypeptide comprises at least one of a D581, G624, F626, D835, L836, P868, S879, D911, I926, V1020, V1030, E1035, and S1046 substitution of amino acid sequence of SEQ ID NO: 2.
15	164.26. The composition of any one of claims 23 to 25 54, 56 to 93, 95 to 131, 133 to 160, and 163 , wherein the variant Cas12i2 polypeptide comprises at least one of a D581G, D581R, G624R, F626G, F626R, D835G, D835R, L836G, L836R, P868G, P868R, P868T, S879G, S879R, D911G, D911R, I926G, I926R, V1020G, V1020R, V1030G, V1030R, E1035G, E1035R, S1046G, and S1046R substitution of amino acid sequence of SEQ ID NO: 2.
20	165.27. The composition of any one of claims 23 to 26 54, 56 to 93, 95 to 131, 133 to 160, 163, and 164 , wherein the variant Cas12i2 polypeptide comprises at least one of a D581R, G624R, F626R, P868T, D911R, I926R, V1030G, E1035R, and S1046G substitution of amino acid sequence of SEQ ID NO: 2.
	166. The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 165, wherein the variant Cas12i2 polypeptide comprises at least one substitution listed in Table 1.
25	167. The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 166, wherein the variant Cas12i2 polypeptide comprises any one amino acid sequence of SEQ ID NO: 3 to 146 or any one amino acid sequence of SEQ ID NOs: 495 to 512.
	168. The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 167, wherein the variant Cas12i2 polypeptide comprises at least one of an epitope peptide, nuclear localization signal, and nuclear export signal.
30	169. The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 168, wherein the RNA guide comprises a DNA targeting sequence.
	170. The composition of claim 169, wherein the DNA targeting sequence is an RNA guide.

171. ~~The composition of claim 169 or 170, wherein the DNA targeting sequence is between 13 to 30 nucleotides.~~
172. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 171, wherein the RNA guide comprises a direct repeat sequence linked to a DNA targeting sequence.~~
- 5 173. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 172, further comprising a target nucleic acid.~~
174. ~~The composition of claim 173, wherein the target nucleic acid is present in a cell.~~
175. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 174, wherein the variant Cas12i2 polypeptide and RNA guide are encoded in a vector, e.g., expression~~
- 10 ~~vector.~~
176. ~~A cell comprising the composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 175.~~
177. ~~A method of expressing the vector of claim 175.~~
178. ~~A method of producing the composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to~~
- 15 ~~160, and 163 to 175.~~
179. ~~A method of delivering the composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 175.~~
180. ~~A kit or system comprising a composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 175 or one or more component thereof.~~
- 20 181. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, and 163 to 175, wherein the RNA guide comprises or consists of, or about, 43 nucleotides.~~
182. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, 163 to 175, and 181, wherein the RNA guide is a tracer-less RNA guide.~~
- 25 183. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, 163 to 175, 181, and 182, wherein the variant Cas12i2 polypeptide further exhibits about 40x greater enzymatic activity than parent polypeptide.~~
184. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, 163 to 175, and 181 to 183, wherein the variant Cas12i2 polypeptide exhibits increased on target specificity as compared to the parent polypeptide.~~

185. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, 163 to 175, and 181 to 184, wherein the variant Cas12i2 polypeptide exhibits decreased off target specificity as compared to the parent polypeptide.~~
- 5 186. ~~The composition of any one of claims 23 to 54, 56 to 93, 95 to 131, 133 to 160, 163 to 175, and 181 to 185, wherein the variant Cas12i2 polypeptide selectively induces a deletion adjacent to a 5'-NTTN-3' sequence, wherein N is any nucleotide.~~
187. ~~The composition of claim 186, wherein the deletion is downstream of the 5'-NTTN-3' sequence.~~
188. ~~The composition of claim 186 or 187, wherein the parent polypeptide does not induce the deletion.~~
- 10 189. ~~The composition of any one of claims 186 to 188, wherein the length of the deletion is greater than the length of a Cas9 polypeptide induced deletion.~~
190. ~~The composition of any one of claims 186 to 189, wherein the deletion is in a gene of a cell.~~
191. ~~The composition of any one of claims 186 to 190, wherein the deletion is up to about 40 nucleotides in length.~~
- 15 192. ~~The composition of any one of claims 186 to 191, wherein the deletion is from about 4 nucleotides to 40 nucleotides in length.~~
193. ~~The composition of any one of claims 186 to 192, wherein the deletion is from about 4 nucleotides to 25 nucleotides in length.~~
194. ~~The composition of any one of claims 186 to 193, wherein the deletion is from about 10 nucleotides to 25 nucleotides in length.~~
- 20 195. ~~The composition of any one of claims 186 to 194, wherein the deletion is from about 10 nucleotides to 15 nucleotides in length.~~
196. ~~The composition of any one of claims 186 to 195, wherein the deletion starts within about 5 nucleotides to about 15 nucleotides of the 5'-NTTN-3' sequence.~~
- 25 197. ~~The composition of any one of claims 186 to 196, wherein the deletion starts within about 5 nucleotides to about 10 nucleotides of the 5'-NTTN-3' sequence.~~
198. ~~The composition of any one of claims 186 to 197, wherein the deletion starts within about 10 nucleotides to about 15 nucleotides of the 5'-NTTN-3' sequence.~~
199. ~~The composition of any one of claims 186 to 198, wherein the deletion starts within about 5 nucleotides to about 15 nucleotides downstream of the 5'-NTTN-3' sequence.~~
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200. ~~The composition of any one of claims 186 to 199, wherein the deletion starts within about 5 nucleotides to about 10 nucleotides downstream of the 5' NTTN 3' sequence.~~
201. ~~The composition of any one of claims 186 to 200, wherein the deletion starts within about 10 nucleotides to about 15 nucleotides downstream of the 5' NTTN 3' sequence.~~
- 5 202. ~~The composition of any one of claims 186 to 201, wherein the deletion ends within about 20 nucleotides to about 30 nucleotides of the 5' NTTN 3' sequence.~~
203. ~~The composition of any one of claims 186 to 202, wherein the deletion ends within about 20 nucleotides to about 25 nucleotides of the 5' NTTN 3' sequence.~~
- 10 204. ~~The composition of any one of claims 186 to 203, wherein the deletion ends within about 25 nucleotides to about 30 nucleotides of the 5' NTTN 3' sequence.~~
205. ~~The composition of any one of claims 186 to 204, wherein the deletion ends within about 20 nucleotides to about 30 nucleotides downstream of the 5' NTTN 3' sequence.~~
206. ~~The composition of any one of claims 186 to 205, wherein the deletion ends within about 20 nucleotides to about 25 nucleotides downstream of the 5' NTTN 3' sequence.~~
- 15 207. ~~The composition of any one of claims 186 to 206, wherein the deletion ends within about 25 nucleotides to about 30 nucleotides downstream of the 5' NTTN 3' sequence.~~
208. ~~The composition of any one of claims 186 to 207, wherein the deletion starts within about 5 nucleotides to about 15 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 20 nucleotides to about 30 nucleotides downstream of the 5' NTTN 3' sequence.~~
- 20 209. ~~The composition of any one of claims 186 to 208, wherein the deletion starts within about 5 nucleotides to about 15 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 20 nucleotides to about 25 nucleotides downstream of the 5' NTTN 3' sequence.~~
210. ~~The composition of any one of claims 186 to 209, wherein the deletion starts within about 5 nucleotides to about 15 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 25 nucleotides to about 30 nucleotides downstream of the 5' NTTN 3' sequence.~~
- 25 211. ~~The composition of any one of claims 186 to 210, wherein the deletion starts within about 5 nucleotides to about 10 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 20 nucleotides to about 30 nucleotides downstream of the 5' NTTN 3' sequence.~~
- 30 212. ~~The composition of any one of claims 186 to 211, wherein the deletion starts within about 5 nucleotides to about 10 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 20 nucleotides to about 25 nucleotides downstream of the 5' NTTN 3' sequence.~~

213. ~~The composition of any one of claims 186 to 212, wherein the deletion starts within about 5 nucleotides to about 10 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 25 nucleotides to about 30 nucleotides downstream of the 5' NTTN 3' sequence.~~
- 5 214. ~~The composition of any one of claims 186 to 213, wherein the deletion starts within about 10 nucleotides to about 15 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 20 nucleotides to about 30 nucleotides downstream of the 5' NTTN 3' sequence.~~
215. ~~The composition of any one of claims 186 to 214, wherein the deletion starts within about 10 nucleotides to about 15 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 20 nucleotides to about 25 nucleotides downstream of the 5' NTTN 3' sequence.~~
- 10 216. ~~The composition of any one of claims 186 to 215, wherein the deletion starts within about 10 nucleotides to about 15 nucleotides downstream of the 5' NTTN 3' sequence and ends within about 25 nucleotides to about 30 nucleotides downstream of the 5' NTTN 3' sequence.~~
- 15 217. ~~The composition of any one of claims 186 to 216, wherein the 5' NTTN 3' sequence is 5' NTTY-3', 5' NTTC 3', 5' NTTT 3', 5' NTTA 3', 5' NTTB 3', 5' NTTG 3', 5' CTTY 3', 5' DTTR 3', 5' CTTR 3', 5' DTTT 3', 5' ATTN 3', or 5' GTTN 3', wherein Y is C or T, B is any nucleotide except for A, D is any nucleotide except for C, and R is A or G.~~
218. ~~The composition of any one of claims 186 to 217, wherein the 5' NTTN 3' sequence is 5' CTTT-3', 5' CTTC 3', 5' GTTT 3', 5' GTTC 3', 5' TTTC 3', 5' GTTA 3', or 5' GTTG 3'.~~
- 20 219. ~~The composition of any one of claims 186 to 218, wherein the deletion is in an exon of the gene; e.g., B2M, TRAC, PDCD1.~~
220. ~~The composition of any one of claims 186 to 219, wherein the deletion overlaps with a mutation in the gene.~~
221. ~~The composition of any one of claims 186 to 220, wherein the deletion overlaps with an insertion in the gene.~~
- 25 222. ~~The composition of any one of claims 186 to 221, wherein the deletion removes a repeat expansion of the gene.~~
223. ~~The composition of any one of claims 186 to 222, wherein the deletion disrupts one or both alleles of the gene.~~
- 30 224. ~~The composition of any one of claims 186 to 223, wherein the deletion is induced in a eukaryotic cell or a prokaryotic cell.~~

- ~~225. The composition of any one of claims 186 to 224, wherein the deletion is induced in an animal cell, a plant cell, or a fungal cell or the cell is derived from an animal cell, a plant cell, or a fungal cell.~~
- ~~226. The composition of any one of claims 186 to 225, wherein the deletion is induced in a mammalian cell or derived from a mammalian cell.~~
- 5 ~~227. The composition of any one of claims 186 to 226, wherein the deletion is induced in a human cell or derived from a human cell.~~
- ~~228. The composition of any one of claims 186 to 227, wherein the deletion is induced in a primary cell.~~
- ~~229. The composition of any one of claims 186 to 228, wherein the deletion is induced in a cell line.~~
- ~~230. The composition of any one of claims 186 to 229, wherein the deletion is induced in a T cell.~~
- 10 ~~231. The composition of any one of claims 186 to 230, wherein the deletion is induced in a stem cell (e.g., a totipotent/omnipotent stem cell, a pluripotent stem cell, a multipotent stem cell, an oligopotent stem cell, or an unipotent stem cell), a differentiated cell, or a terminally differentiated cell.~~
- ~~232. The composition of any one of claims 186 to 231, wherein 2 or more (e.g., multiplexed targeted deletions) deletions are induced.~~
- 15 ~~233. A method of obtaining a deletion in a cell, wherein the method comprises contacting the variant Cas12i2 polypeptide or complex of any one of claims 1 to 16, 22 to 54, 56 to 93, 95 to 131, 133 to 160, 163 to 175, and 181 to 232 with DNA in the cell.~~
- ~~234. A composition or formulation comprising the variant Cas12i2 polypeptide of any one of claims 1 to 16, 22 to 54, 56 to 93, 95 to 131, 133 to 160, 163 to 175, and 181 to 232, an RNA guide, and a cell.~~
- 20 ~~235. A method of producing the composition of any previous claim.~~
- ~~236. A method of complexing a variant Cas12i2 polypeptide of any previous claim with an RNA guide, such as an RNA guide of any previous claim.~~
- 25 ~~237. A method of complexing a variant binary complex of any previous claim with a target nucleic acid.~~
- ~~238. A method of delivering the composition of any previous claim.~~
- ~~239. A composition comprising a variant Cas12i2 polypeptide, wherein the variant Cas12i2 polypeptide comprises a substitution that increases interactions between the variant Cas12i2 polypeptide and a nucleic acid, as compared to a parent polypeptide.~~
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240. ~~The composition of claim 239, wherein the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 4.~~
241. ~~The composition of claim 239, wherein the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.~~
- 5 242. ~~The composition of claim 239, wherein the interactions are electrostatic interactions.~~
243. ~~The composition of claim 239, wherein the interactions are non-specific interactions.~~
244. ~~The composition of claim 239, wherein the interactions are aromatic, hydrophobic, Van der Waals, and/or cation-pi interactions.~~
245. ~~The composition of claim 239, wherein the substitution is in or adjacent to a nucleic acid interface.~~
- 10 246. ~~The composition of any one of claims 239 to 245, wherein the nucleic acid is an RNA guide comprising a direct repeat sequence and a spacer sequence.~~
247. ~~The composition of claim 246, wherein the direct repeat sequence comprises a nucleotide sequence with at least 95% sequence identity to any one of SEQ ID NO: 492-494.~~
248. ~~The composition of claim 246, wherein the direct repeat sequence comprises a nucleotide sequence set forth in any one of SEQ ID NO: 492-494.~~
- 15 249. ~~The composition of any one of claims 246 to 248, wherein the substitution increases interactions between the variant Cas12i2 polypeptide and the direct repeat sequence.~~
250. ~~The composition of any one of claims 239 to 249, wherein the substitution increases binary complex formation, as compared to a parent polypeptide.~~
- 20 251. ~~The composition of any one of claims 239 to 250, wherein a binary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent binary complex.~~
252. ~~The composition of any one of claims 239 to 251, wherein the substitution is an arginine, lysine, glutamine, asparagine, histidine, tyrosine, or serine substitution.~~
- 25 253. ~~The composition of any one of claims 239 to 252, wherein the substitution is in an RNA binding interface.~~
254. ~~The composition of any one of claims 239 to 253, wherein the substitution is a substitution in the Wedge domain or Rec2 domain.~~
255. ~~The composition of any one of claims 239 to 254, wherein the substitution is a substitution listed in Table 4.~~

256. ~~The composition of any one of claims 239 to 254, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 5 257. ~~The composition of any one of claims 239 to 254, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 4.~~
- 10 258. ~~The composition of any one of claims 239 to 254, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 15 259. ~~The composition of any one of claims 239 to 254, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 4.~~
- 20 260. ~~The composition of any one of claims 246 and 249 to 254, wherein the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises a sequence set forth in SEQ ID NO: 4.~~
- 25 261. ~~The composition of any one of claims 246 and 249 to 254, wherein the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.~~
- 30 262. ~~The composition of any one of claims 239 to 245, wherein the nucleic acid is a target nucleic acid.~~
263. ~~The composition of any one of claims 239 to 245 and 262, wherein the nucleic acid is double-stranded DNA.~~
264. ~~The composition of claim 263, wherein the substitution increases interactions between the variant Cas12i2 polypeptide and double stranded DNA.~~
265. ~~The composition of claim 263 or claim 264 wherein the double stranded DNA comprises a PAM sequence.~~
266. ~~The composition of any one of claims 239 to 245 and 262 to 265, wherein the substitution increases ternary complex formation, as compared to a parent polypeptide.~~
267. ~~The composition of any one of claims 239 to 245 and 262 to 266, wherein a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.~~

268. ~~The composition of any one of claims 239 to 245 and 262 to 267, wherein the substitution is an arginine, lysine, glutamine, asparagine, histidine, or serine substitution.~~
269. ~~The composition of any one of claims 239 to 245 and 262 to 268, wherein the substitution is in a double-stranded DNA binding interface.~~
- 5 270. ~~The composition of any one of claims 239 to 245 and 262 to 269, wherein the substitution is a substitution in the Rec1 domain, PI domain, or Wedge domain.~~
271. ~~The composition of any one of claims 239 to 245 and 262 to 270, wherein the substitution is a substitution listed in Table 5.~~
- 10 272. ~~The composition of any one of claims 239 to 245 and 262 to 270, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
273. ~~The composition of any one of claims 239 to 245 and 262 to 270, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 5.~~
- 15 274. ~~The composition of any one of claims 239 to 245 and 262 to 270, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
275. ~~The composition of any one of claims 239 to 245 and 262 to 270, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 5.~~
- 20 276. ~~The composition of any one of claims 239 to 245, wherein the nucleic acid is single-stranded DNA.~~
277. ~~The composition of claim 276, wherein the single-stranded DNA comprises a non-target strand.~~
278. ~~The composition of claim 276, wherein the single-stranded DNA comprises a target strand.~~
- 25 279. ~~The composition of any one of claims 239 to 245 and 276 to 278, wherein the substitution increases ternary complex formation, as compared to a parent polypeptide.~~
280. ~~The composition of any one of claims 239 to 245 and 276 to 279, wherein a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.~~
- 30 281. ~~The composition of any one of claims 239 to 245 and 276 to 280, wherein the substitution is an arginine, lysine, glutamine, asparagine, histidine, or alanine substitution.~~

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- ~~282. The composition of any one of claims 239 to 245 and 276 to 281, wherein the substitution is in a single stranded DNA binding interface.~~
- ~~283. The composition of any one of claims 239 to 245 and 276 to 282, wherein the substitution is a substitution in the PI domain, Rec1 domain, Wedge domain, RuvC domain, Rec2 domain, or Nue domain.~~
- ~~284. The composition of any one of claims 239 to 245 and 276 to 283, wherein the substitution is a substitution listed in Table 6.~~
- ~~285. The composition of any one of claims 239 to 245 and 276 to 283, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- ~~286. The composition of any one of claims 239 to 245 and 276 to 283, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 6.~~
- ~~287. The composition of any one of claims 239 to 245 and 276 to 283, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- ~~288. The composition of any one of claims 239 to 245 and 276 to 283, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 6.~~
- ~~289. The composition of any one of claims 239 to 245, wherein the substitution increases interactions between the variant Cas12i2 polypeptide and a DNA/RNA hybrid molecule.~~
- ~~290. The composition of claim 289, wherein the DNA/RNA hybrid molecule is a heteroduplex comprising a spacer sequence of an RNA guide and a target strand.~~
- ~~291. The composition of any one of claims 239 to 245 and 289 to 290, wherein the substitution stabilizes the heteroduplex.~~
- ~~292. The composition of any one of claims 239 to 245 and 289 to 291, wherein the substitution increases ternary complex formation, as compared to a parent polypeptide.~~
- ~~293. The composition of any one of claims 239 to 245 and 289 to 292, wherein a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.~~

- ~~294. The composition of any one of claims 239 to 245 and 289 to 293, wherein the substitution is an arginine, lysine, glutamine, asparagine, histidine, or serine substitution.~~
- ~~295. The composition of any one of claims 239 to 245 and 289 to 294, wherein the substitution is a substitution in the Rec1 domain, PI domain, Rec2 domain, or RuvC2 motif.~~
- 5 ~~296. The composition of any one of claims 239 to 245 and 289 to 295, wherein the substitution is a substitution listed in Table 7.~~
- ~~297. The composition of any one of claims 239 to 245 and 289 to 295, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 10 ~~298. The composition of any one of claims 239 to 245 and 289 to 295, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 7.~~
- ~~299. The composition of any one of claims 239 to 245 and 289 to 295, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 15 ~~300. The composition of any one of claims 239 to 245 and 289 to 295, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 7.~~
- ~~301. The composition of any one of claims 239 to 245, wherein the substitution increases interactions between the variant Cas12i2 polypeptide and bases of (a) a double-stranded DNA duplex and/or (b) a heteroduplex comprising a spacer sequence of an RNA guide and a target strand.~~
- 20 ~~302. The composition of claim 301, wherein the double-stranded DNA duplex comprises a PAM sequence.~~
- ~~303. The composition of any one of claims 239 to 245 and 301 to 302, wherein the interactions are aromatic, hydrophobic, Van der Waals, and/or cation-pi interactions.~~
- 25 ~~304. The composition of any one of claims 239 to 245 and 301 to 303, wherein the substitution stabilizes the R-loop.~~
- ~~305. The composition of any one of claims 239 to 245 and 301 to 304, wherein the substitution increases ternary complex formation, as compared to a parent polypeptide.~~
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306. ~~The composition of any one of claims 239 to 245 and 301 to 305, wherein a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.~~
- 5 307. ~~The composition of any one of claims 239 to 245 and 301 to 306, wherein the substitution is an arginine, lysine, tryptophan, phenylalanine, tyrosine, methionine, histidine, glutamine, threonine, or valine substitution.~~
308. ~~The composition of any one of claims 239 to 245 and 301 to 307, wherein the substitution is a substitution in the Wedge domain, Rec1 domain, or RuvC domain.~~
- 10 309. ~~The composition of any one of claims 239 to 245 and 301 to 308, wherein the substitution is a substitution listed in Table 8.~~
310. ~~The composition of any one of claims 239 to 245 and 301 to 308, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 15 311. ~~The composition of any one of claims 239 to 245 and 301 to 308, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 8.~~
312. ~~The composition of any one of claims 239 to 245 and 301 to 308, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 20 313. ~~The composition of any one of claims 239 to 245 and 301 to 308, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 8.~~
314. ~~The composition of any one of claims 239 to 313, wherein the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.~~
- 25 315. ~~The composition of any one of claims 239 to 314, wherein the variant Cas12i2 polypeptide exhibits increased enzymatic activity, as compared to a parent polypeptide.~~
316. ~~The composition of any one of claims 239 to 315, wherein the composition further comprises an RNA guide comprising a direct repeat sequence and a spacer sequence.~~
- 30 317. ~~The composition of claim 316, wherein the direct repeat sequence comprises a nucleotide sequence with at least 95% sequence identity to any one of SEQ ID NO: 492-494.~~

318. ~~The composition of claim 316, wherein the direct repeat sequence comprises a nucleotide sequence set forth in any one of SEQ ID NO: 492-494.~~
319. ~~The composition of claim 316, wherein the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises a sequence set forth in SEQ ID NO: 4.~~
- 5 320. ~~The composition of claim 316, wherein the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.~~
321. ~~A cell comprising the composition of any one of claims 239 to 320.~~
322. ~~The cell of claim 321, wherein the composition does not substantially affect viability of the cell.~~
- 10 323. ~~A composition comprising a variant Cas12i2 polypeptide, wherein the variant Cas12i2 polypeptide comprises a substitution that increases flexibility of the variant Cas12i2 polypeptide during DNA binding, as compared to a parent polypeptide.~~
324. ~~The composition of claim 323, wherein the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 4.~~
- 15 325. ~~The composition of claim 323, wherein the Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.~~
326. ~~The composition of any one of claims 323 to 325, wherein the substitution increases binding of the variant Cas12i2 polypeptide to DNA.~~
327. ~~The composition of any one of claims 323 to 326, wherein the substitution increases binding of the~~
20 ~~variant Cas12i2 polypeptide to double-stranded DNA.~~
328. ~~The composition of any one of claims 323 to 327, wherein the substitution increases binding of the variant Cas12i2 polypeptide to single-strand DNA.~~
329. ~~The composition of any one of claims 323 to 328, wherein the substitution increases ternary complex formation, as compared to a parent polypeptide.~~
- 25 330. ~~The composition of any one of claims 323 to 329, wherein a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.~~
331. ~~The composition of any one of claims 323 to 330, wherein the substitution is a substitution of a bulky amino acid to an amino acid with a smaller side chain.~~
332. ~~The composition of any one of claims 323 to 331, wherein the substitution is an alanine, valine,~~
30 ~~glycine, or serine substitution.~~

333. ~~The composition of any one of claims 323 to 332, wherein the substitution is in the Helical H domain of the variant Cas12i2 polypeptide.~~
334. ~~The composition of any one of claims 323 to 333, wherein the substitution is a substitution listed in Table 9.~~
- 5 335. ~~The composition of any one of claims 323 to 332, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 10 336. ~~The composition of any one of claims 323 to 332, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 9.~~
337. ~~The composition of any one of claims 323 to 332, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 15 338. ~~The composition of any one of claims 323 to 332, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 9.~~
- 20 339. ~~A composition comprising a variant Cas12i2 polypeptide, wherein the variant Cas12i2 polypeptide comprises a substitution that stabilizes a domain-domain interface that forms during ternary complex formation, as compared to a parent polypeptide.~~
340. ~~The composition of claim 339, wherein the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 4.~~
341. ~~The composition of claim 339, wherein the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.~~
- 25 342. ~~The composition of any one of claims 339 to 341, wherein the domain-domain interface forms when single-stranded DNA contacts an active site of the variant Cas12i2 polypeptide.~~
343. ~~The composition of any one of claims 339 to 342, wherein the domain-domain interface is a Helical H domain-Nuc domain interface.~~
- 30 344. ~~The composition of any one of claims 339 to 343, wherein the substitution increases ternary complex formation, as compared to a parent polypeptide.~~

345. ~~The composition of any one of claims 339 to 344, wherein a ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.~~
346. ~~The composition of any one of claims 339 to 345, wherein the substitution is an aspartic acid, glutamic acid, arginine, or lysine substitution.~~
- 5 347. ~~The composition of any one of claims 339 to 346, wherein the substitution is a substitution listed in Table 10.~~
348. ~~The composition of any one of claims 339 to 346, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 10 349. ~~The composition of any one of claims 339 to 346, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 10.~~
- 15 350. ~~The composition of any one of claims 339 to 346, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
351. ~~The composition of one of claims 339 to 346, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 10.~~
- 20 352. ~~The composition of any one of claims 323 to 351, wherein the parent polypeptide comprises the amino acid sequence of SEQ ID NO: 2.~~
353. ~~The composition of any one of claims 323 to 352, wherein the variant Cas12i2 polypeptide exhibits increased enzymatic activity, as compared to a parent polypeptide.~~
- 25 354. ~~The composition of any one of claims 323 to 353, wherein the composition further comprises an RNA guide comprising a direct repeat sequence and a spacer sequence.~~
355. ~~The composition of claim 354, wherein the direct repeat sequence comprises a nucleotide sequence with at least 95% sequence identity to any one of SEQ ID NO: 492-494.~~
356. ~~The composition of claim 354, wherein the direct repeat sequence comprises a nucleotide sequence set forth in any one of SEQ ID NO: 492-494.~~
- 30 357. ~~The composition of claim 354, wherein the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises a sequence set forth in SEQ ID NO: 4.~~

358. ~~The composition of claim 354, wherein the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.~~
359. ~~A cell comprising the composition of any one of claims 323 to 358.~~
- 5 360. ~~The cell of claim 359, wherein the composition does not substantially affect viability of the cell.~~
361. ~~A composition comprising a variant Cas12i2 polypeptide, wherein the variant Cas12i2 polypeptide comprises a substitution that increases on-target specificity of the variant Cas12i2 polypeptide, as compared to a parent polypeptide.~~
- 10 362. ~~The composition of claim 361, wherein the variant Cas12i2 polypeptide comprises the sequence set forth in SEQ ID NO: 4.~~
363. ~~The composition of claim 361, wherein the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.~~
364. ~~The composition of any of claims 361 to 363, wherein the substitution increases on target DNA binding.~~
- 15 365. ~~The composition of any one of claims 361 to 364, wherein the substitution decreases off target DNA binding.~~
366. ~~The composition of any one of claims 361 to 365, wherein the substitution increases on target ternary complex formation, as compared to a parent polypeptide.~~
- 20 367. ~~The composition of any one of claims 361 to 366, wherein an on-target ternary complex comprising the variant Cas12i2 polypeptide exhibits increased stability, as compared to a parent ternary complex.~~
368. ~~The composition of any one of claims 361 to 367, wherein the substitution is a substitution of an amino acid contacting the spacer sequence of an RNA guide.~~
- 25 369. ~~The composition of any one of claims 361 to 368, wherein the substitution is a substitution of a bulky amino acid to an amino acid with a smaller side chain.~~
370. ~~The composition of any one of claims 361 to 369, wherein the substitution is an alanine, serine, valine, glutamine, or asparagine substitution.~~
371. ~~The composition of any one of claims 361 to 370, wherein the substitution is a substitution in the Wedge domain, Rec1 domain, Rec2 domain, or RuvC domain.~~
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372. ~~The composition of any one of claims 361 to 371, wherein the substitution is a substitution in the Helical II domain.~~
373. ~~The composition of any one of claims 361 to 372, wherein the substitution is a substitution listed in Table 11.~~
- 5 374. ~~The composition of any one of claims 361 to 372, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 10 375. ~~The composition of any one of claims 361 to 372, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 11.~~
376. ~~The composition of any one of claims 361 to 372, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
- 15 377. ~~The composition of any one of claims 361 to 372, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 11.~~
378. ~~The composition of any one of claims 361 to 367, wherein the substitution decreases a catalysis rate (K_{cat}) of the variant Cas12i2 polypeptide.~~
- 20 379. ~~The composition of any one of claims 361 to 367 and 378, wherein the substitution is an alanine, serine, threonine, valine, leucine, methionine, asparagine, or isoleucine substitution.~~
380. ~~The composition of any one of claims 361 to 367 and 378 to 379, wherein the substitution is a substitution in the Wedge domain, Rec1 domain, Rec2 domain, or RuvC domain.~~
- 25 381. ~~The composition of any one of claims 361 to 367 and 378 to 380, wherein the substitution is a substitution in the RuvC domain.~~
382. ~~The composition of any one of claims 361 to 367 and 378 to 381, wherein the substitution is a substitution listed in Table 12.~~
- 30 383. ~~The composition of any one of claims 361 to 367 and 378 to 381, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~

384. ~~The composition of any one of claims 361 to 367 and 378 to 381, wherein the variant Cas12i2 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further having a substitution listed in Table 12.~~
- 5 385. ~~The composition of any one of claims 361 to 367 and 378 to 381, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512.~~
386. ~~The composition of any one of claims 361 to 367 and 378 to 381, wherein the variant Cas12i2 polypeptide comprises a sequence set forth in any one of SEQ ID NOs: 3-146 or in any one of SEQ ID NOs: 495-512 and further has a substitution listed in Table 12.~~
- 10 387. ~~The composition of one of claims 361 to 386, wherein the variant Cas12i2 polypeptide exhibits increased on-target enzymatic activity, as compared to a parent polypeptide.~~
388. ~~The composition of any one of claims 361 to 387, wherein the variant Cas12i2 polypeptide exhibits decreased off-target enzymatic activity, as compared to a parent polypeptide.~~
- 15 389. ~~The composition of any one of claims 361 to 388, wherein the variant Cas12i2 polypeptide exhibits off-target editing that is no more than 10% of the on-target editing.~~
390. ~~The composition of any one of claims 361 to 388, wherein the variant Cas12i2 polypeptide exhibits off-target editing that is no more than 5% of the on-target editing.~~
391. ~~The composition of any one of claims 361 to 390, wherein the composition further comprises an RNA guide comprising a direct repeat sequence and a spacer sequence.~~
- 20 392. ~~The composition of claim 391, wherein the direct repeat sequence comprises a nucleotide sequence with at least 95% sequence identity to any one of SEQ ID NO: 492-494.~~
393. ~~The composition of claim 391, wherein the direct repeat sequence comprises a nucleotide sequence set forth in any one of SEQ ID NO: 492-494.~~
- 25 394. ~~The composition of claim 391, wherein the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises a sequence set forth in SEQ ID NO: 4.~~
395. ~~The composition of claim 391, wherein the direct repeat sequence is set forth in SEQ ID NO: 492 and the variant Cas12i2 polypeptide comprises the sequence set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 495, and SEQ ID NO: 496.~~
- 30 396. ~~The composition of any one of claims 365 to 395, wherein the variant Cas12i2 polypeptide exhibits a higher ratio of on-target binding to off-target binding, as compared to a Cas9 polypeptide.~~

397. ~~The composition of any one of claims 365 to 396, wherein the variant Cas12i2 polypeptide exhibits a higher ratio of on target activity to off target activity, as compared to a Cas9 polypeptide.~~
398. ~~The composition of any one of claims 365 to 397, wherein the variant Cas12i2 polypeptide exhibits a higher ratio of on target editing to off target editing, as compared to a Cas9 polypeptide.~~
- 5 399. ~~The composition of any one of claims 365 to 395, wherein the variant Cas12i2 polypeptide exhibits less off target binding, as compared to a Cas9 polypeptide.~~
400. ~~The composition of any one of claims 365 to 396, wherein the variant Cas12i2 polypeptide exhibits less off target activity, as compared to a Cas9 polypeptide.~~
- 10 401. ~~The composition of any one of claims 365 to 397, wherein the variant Cas12i2 polypeptide exhibits less off target editing, as compared to a Cas9 polypeptide.~~
402. ~~The composition of any one of claims 365 to 401, wherein off target binding, off target activity, and/or off target editing by the variant Cas12i2 polypeptide is at least 10% less than off target binding, off target activity, and/or off target editing by a Cas9 polypeptide.~~
- 15 403. ~~The composition of any one of claims 365 to 402, wherein off target binding, off target activity, and/or off target editing by the variant Cas12i2 polypeptide is at least 20% less than off target binding, off target activity, and/or off target editing by a Cas9 polypeptide.~~
404. ~~The composition of any one of claims 365 to 403, wherein off target binding, off target activity, and/or off target editing by the variant Cas12i2 polypeptide is at least 30% less than off target binding, off target activity, and/or off target editing by a Cas9 polypeptide.~~
- 20 405. ~~The composition of any one of claims 365 to 404, wherein off target binding, off target activity, and/or off target editing by the variant Cas12i2 polypeptide is at least 40% less than off target binding, off target activity, and/or off target editing by a Cas9 polypeptide.~~
406. ~~The composition of any one of claims 365 to 405, wherein off target binding, off target activity, and/or off target editing by the variant Cas12i2 polypeptide is at least 50% less than off target binding, off target activity, and/or off target editing by a Cas9 polypeptide.~~
- 25 407. ~~A cell comprising the composition of any one of claims 365 to 406.~~
408. ~~The cell of claim 407, wherein the composition does not substantially affect viability of the cell.~~