

CLAIMS

WHAT IS CLAIMED IS:

1. A variant Cas12i4 polypeptide comprising a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-59.
2. The variant Cas12i4 polypeptide of claim 1, wherein the variant Cas12i4 polypeptide:
 - i) is a variant of a parent polypeptide of SEQ ID NO: 2;
 - ii) ~~3. The variant Cas12i4 polypeptide of claim 1 or 2, wherein the variant Cas12i4 polypeptide~~ comprises a substitution of Table 2;
 - iii) ~~4. The variant Cas12i4 polypeptide of any one of claims 1 to 3, comprising~~ the sequence set forth in any one of SEQ ID NOs: 3-59; and/or
 - iv) the variant Cas12i4 polypeptide comprises a RuvC domain or a split RuvC domain.
- ~~5. The variant Cas12i4 polypeptide of any one of claims 1 to 4, comprising the sequence set forth in SEQ ID NO: 3.~~
- ~~6~~3. The variant Cas12i4 polypeptide of claim 1 or 2 ~~any one of claims 1 to 4~~, comprising the sequence set forth in SEQ ID NO: 4.
- ~~7~~4. The variant Cas12i4 polypeptide of any one of claims 1 to ~~3~~6, wherein the variant Cas12i4 polypeptide exhibits increased binary complex formation with an RNA guide or increased nuclease activity, relative to a parent polypeptide, optionally wherein a binary complex comprising the variant Cas12i4 polypeptide exhibits increased stability, relative to a parent binary complex.
- ~~8. The variant Cas12i4 polypeptide of any one of claims 1 to 7, wherein a binary complex comprising the variant Cas12i4 polypeptide exhibits increased stability, relative to a parent binary complex.~~
- ~~9. The variant Cas12i4 polypeptide of any one of claims 1 to 8, wherein the variant Cas12i4 polypeptide exhibits increased nuclease activity, relative to a parent polypeptide.~~
- ~~10~~5. The variant Cas12i4 polypeptide of any one of claims 1 to ~~4~~9, wherein the variant Cas12i4 polypeptide further comprises a substitution of Table 4, optionally wherein the substitution of Table 4 increases binary complex formation with an RNA guide or increases stability of a binary complex comprising the variant Cas12i4 polypeptide, relative to a parent polypeptide.

- ~~11. The variant Cas12i4 polypeptide of claim 10, wherein the substitution of Table 4 increases binary complex formation with an RNA guide, relative to a parent polypeptide.~~
- ~~12. The variant Cas12i4 polypeptide of claim 10 or 11, wherein the substitution of Table 4 increases stability of a binary complex comprising the variant Cas12i4 polypeptide, relative to a parent binary complex.~~
- ~~13. The variant Cas12i4 polypeptide of any one of claims 1 to 12, wherein the variant Cas12i4 polypeptide further comprises a substitution that increases ternary complex formation with an RNA guide and a target nucleic acid, relative to a parent polypeptide.~~
- ~~14. The variant Cas12i4 polypeptide of any one of claims 1 to 13, wherein the variant Cas12i4 polypeptide further comprises a substitution that increases ternary complex stability, relative to a parent polypeptide.~~
- ~~15. The variant Cas12i4 polypeptide of claim 13 or 14, wherein the substitution is a substitution of Table 5, Table 6, Table 7, Table 8, Table 9, and/or Table 10.~~
- ~~16. The variant Cas12i4 polypeptide of any one of claims 1 to 15, wherein the variant Cas12i4 polypeptide further comprises a substitution that increases on-target binding to a target nucleic acid, relative to a parent polypeptide.~~
- ~~176.~~ The variant Cas12i4 polypeptide of any one of claims 1 to ~~510~~, wherein the substitution is a substitution of [Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, and/or Table 11](#).
- ~~187.~~ A composition comprising the variant Cas12i4 polypeptide of any one of claims 1 to ~~617~~, 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.
- ~~19. The composition of claim 18, wherein the direct repeat sequence comprises:~~
- ~~a. nucleotide 1 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~b. nucleotide 2 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~

- ~~e. nucleotide 3 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~d. nucleotide 4 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~e. nucleotide 5 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~f. nucleotide 6 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~g. nucleotide 7 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~h. nucleotide 8 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~i. nucleotide 9 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~j. nucleotide 10 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~k. nucleotide 11 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~l. nucleotide 12 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~m. nucleotide 13 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~

- ~~n. nucleotide 14 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or~~
 - ~~o. a sequence that is at least 90% identical to a sequence of SEQ ID NO: 61 or a portion thereof.~~
20. ~~The composition of claim 19, wherein the direct repeat sequence comprises:~~
- ~~a. nucleotide 1 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~b. nucleotide 2 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~c. nucleotide 3 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~d. nucleotide 4 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~e. nucleotide 5 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~f. nucleotide 6 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~g. nucleotide 7 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~h. nucleotide 8 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~i. nucleotide 9 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~

- ~~j. nucleotide 10 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~k. nucleotide 11 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~l. nucleotide 12 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~m. nucleotide 13 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~n. nucleotide 14 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or~~
 - ~~o. a sequence that is at least 95% identical to a sequence of SEQ ID NO: 61 or a portion thereof.~~
- ~~21. The composition of claim 20, wherein the direct repeat comprises:~~
- ~~a. nucleotide 1 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~b. nucleotide 2 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~c. nucleotide 3 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~d. nucleotide 4 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~e. nucleotide 5 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~f. nucleotide 6 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~g. nucleotide 7 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~h. nucleotide 8 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~

- i. ~~nucleotide 9 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- j. ~~nucleotide 10 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- k. ~~nucleotide 11 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- l. ~~nucleotide 12 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- m. ~~nucleotide 13 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- n. ~~nucleotide 14 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or~~
- o. ~~SEQ ID NO: 61 or a portion thereof.~~
- 22. ~~The composition of any one of claims 18 to 21, wherein the direct repeat sequence comprises AGN₁N₂N₃N₄GUGUN₅N₆N₇CAGN₈GACN₉C (SEQ ID NO: 125), wherein N₁ is A or G, N₂ is C or U, N₃ is A or G, N₄ is U or C, N₅ is C or U, N₆ is C or U, N₇ is U, A, C, or G, N₈ is U or C, and N₉ is A or C.~~
- 23. ~~The composition of any one of claims 18 to 22, wherein the spacer sequence comprises about 15 nucleotides to about 35 nucleotides in length.~~
- 24. ~~The composition of any one of claims 18 to 23, wherein the spacer sequence binds to a target strand sequence of a target nucleic acid, and wherein a non-target strand sequence of the target nucleic acid sequence is adjacent to a protospacer adjacent motif (PAM) sequence.~~
- 25. ~~The composition of claim 24, wherein the PAM sequence is 5'-TTN-3', 5'-NTTN-3', 5'-NTN'-3', 5'-NNTN-3', 5'-VTN-3', or 5'-NVTN-3', wherein N is any nucleotide and V is A, G, or C.~~
- 268. The variant Cas12i4 polypeptide [of any one of claims 1-7](#) or the composition of [claim 7](#)~~any previous claim~~, wherein the variant Cas12i4 polypeptide further comprises a nuclear localization signal (NLS).~~7~~
- 27. ~~The variant Cas12i4 polypeptide or the composition of any previous claim, wherein the variant Cas12i4 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.

- ~~289.~~ A composition comprising a nucleic acid that encodes the Cas12i4 polypeptide of any one of claims 1-7 or 8~~or the composition of any previous claim.~~
- ~~2910.~~ The composition of claim ~~928~~, wherein the nucleic acid is:
- ~~i)~~ codon-optimized for expression in a cell;
 - ~~ii)30.~~ ~~The composition of claim 28 or 29, wherein the nucleic acid is~~ operably linked to a promoter;
 - ~~iii)31.~~ ~~The composition of any one of claims 28 to 30, wherein the nucleic acid is~~ in a vector, optionally;
 - ~~32.~~ ~~The composition of claim 31,~~ wherein the vector comprises a retroviral vector, a lentiviral vector, a phage vector, an adenoviral vector, an adeno-associated vector, or a herpes simplex vector.
- ~~3311.~~ The variant Cas12i4 polypeptide of any one of claims 1-8 or the composition of claim 9 or 10~~any previous claim~~, wherein the variant Cas12i4 polypeptide or the nucleic acid encoding the variant Cas12i4 polypeptide is present in a delivery system comprising a nanoparticle (e.g., a lipid nanoparticle), a liposome, an exosome, a microvesicle, or a gene-gun.
- ~~34.~~ ~~A cell comprising the variant Cas12i4 polypeptide or the composition of any previous claim.~~
- ~~35.~~ ~~The cell of claim 34, wherein the cell is a eukaryotic cell.~~
- ~~36.~~ ~~The cell of claim 34 or 35, wherein the cell is a mammalian cell or a plant cell.~~
- ~~37.~~ ~~The cell of any one of claims 34 to 36, wherein the cell is a human cell.~~
- ~~38.~~ ~~A composition comprising a variant Cas12i4 polypeptide or a complex comprising the variant Cas12i4 polypeptide, wherein the variant Cas12i4 polypeptide comprises a sequence having at least 95% identity to a sequence set forth in any one of SEQ ID NOs: 3-59, and wherein the variant Cas12i4 polypeptide or the complex exhibits enhanced enzymatic activity, enhanced binding activity, enhanced binding specificity, and/or enhanced stability, relative to a parent polypeptide or a complex comprising the parent polypeptide.~~
- ~~39.~~ ~~The composition of claim 38, wherein the variant Cas12i4 polypeptide comprises a substitution of Table 2, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, and/or Table 11.~~
- ~~40.~~ ~~The composition of claim 38 or 39, wherein the variant Cas12i4 polypeptide comprises the sequence set forth in any one of SEQ ID NOs: 3-59.~~
- ~~41.~~ ~~The composition of any one of claims 38 to 40, wherein the variant Cas12i4 polypeptide comprises the sequence set forth in SEQ ID NO: 3.~~
- ~~42.~~ ~~The composition of any one of claims 38 to 41, wherein the variant Cas12i4 polypeptide comprises the sequence set forth in SEQ ID NO: 4.~~

- ~~43. The composition of any one of claims 38 to 42, wherein the enhanced enzymatic activity is enhanced nuclease activity.~~
- ~~44. The composition of any one of claims 38 to 43, wherein the variant Cas12i4 polypeptide exhibits enhanced binding activity to an RNA guide, relative to the parent polypeptide.~~
- ~~45. The composition of any one of claims 38 to 44, wherein the variant Cas12i4 polypeptide exhibits enhanced binding specificity to an RNA guide, relative to the parent polypeptide.~~
- ~~46. The composition of any one of claims 38 to 45, wherein the complex comprising the variant Cas12i4 polypeptide is a variant binary complex that further comprises an RNA guide, and the variant binary complex exhibits enhanced binding activity to a target nucleic acid (e.g., on-target binding activity), relative to a parent binary complex.~~
- ~~47. The composition of any one of claims 38 to 46, wherein the complex comprising the variant Cas12i4 polypeptide is a variant binary complex that further comprises an RNA guide, and the variant binary complex exhibits enhanced binding specificity to a target nucleic acid (e.g., on-target binding specificity), relative to a parent binary complex.~~
- ~~48. The composition of any one of claims 38 to 47, wherein the complex comprising the variant Cas12i4 polypeptide is a variant binary complex that further comprises an RNA guide, and the variant binary complex exhibits enhanced stability, relative to a parent binary complex.~~
- ~~49. The composition of any one of claims 38 to 48, wherein the variant binary complex and a target nucleic acid form a variant ternary complex, and the variant ternary complex exhibits increased stability, relative to a parent ternary complex.~~
- ~~50. The composition of any one of claims 38 to 49, wherein the variant Cas12i4 polypeptide further exhibits enhanced binary complex formation, enhanced protein-RNA interactions, and/or decreased dissociation from an RNA guide, relative to the parent polypeptide.~~
- ~~51. The composition of any one of claims 38 to 50, wherein the variant binary complex further exhibits decreased dissociation from a target nucleic acid, and/or decreased off-target binding to a non-target nucleic acid, relative to the parent binary complex.~~
- ~~52. The composition of any one of claims 38 to 51, wherein the enhanced enzymatic activity, enhanced binding activity, enhanced binding specificity, and/or enhanced stability occur over a range of temperatures, e.g., 20°C to 65°C.~~
- ~~53. The composition of any one of claims 38 to 52, wherein the enhanced enzymatic activity, enhanced binding activity, enhanced binding specificity, and/or enhanced stability occur over a range of incubation times.~~

- ~~54. The composition of any one of claims 38 to 53, wherein the enhanced enzymatic activity, enhanced binding activity, enhanced binding specificity, and/or enhanced stability occur in a buffer having a pH in a range of about 7.3 to about 8.6.~~
- ~~55. The composition of any one of claims 38 to 54, wherein the enhanced enzymatic activity, enhanced binding activity, enhanced binding specificity, and/or enhanced stability occurs when a T_m value of the variant Cas12i4 polypeptide, variant binary complex, or variant ternary complex is at least 8°C greater than the T_m value of the parent polypeptide, parent binary complex, or parent ternary complex.~~
- ~~56. The composition of any one of claims 38 to 55, wherein the variant Cas12i4 polypeptide comprises a RuvC domain or a split RuvC domain.~~
- ~~57. The composition any one of claims 38 to 56, wherein the parent polypeptide comprises the sequence of SEQ ID NO: 2.~~
- ~~58. The composition of any one of claims 38 to 57, wherein the RNA guide comprises a direct repeat sequence and a spacer sequence.~~
- 59~~12~~. The composition of claim 7~~58~~, wherein the direct repeat comprises:
- nucleotide 1 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
 - nucleotide 2 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
 - nucleotide 3 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
 - nucleotide 4 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
 - nucleotide 5 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
 - nucleotide 6 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;

- g. nucleotide 7 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- h. nucleotide 8 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- i. nucleotide 9 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- j. nucleotide 10 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- k. nucleotide 11 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- l. nucleotide 12 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- m. nucleotide 13 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- n. nucleotide 14 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or
- o. a sequence that is at least 90% identical to a sequence of SEQ ID NO: 61 or a portion thereof.

~~60. The composition of claim 58 or 59, wherein the direct repeat comprises:~~

- ~~a. nucleotide 1 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~b. nucleotide 2 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~

- ~~e. nucleotide 3 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~d. nucleotide 4 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~e. nucleotide 5 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~f. nucleotide 6 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~g. nucleotide 7 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~h. nucleotide 8 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~i. nucleotide 9 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~j. nucleotide 10 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~k. nucleotide 11 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~l. nucleotide 12 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~m. nucleotide 13 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~

- ~~n. nucleotide 14 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or~~
- ~~o. a sequence that is at least 95% identical to a sequence of SEQ ID NO: 61 or a portion thereof.~~
61. The composition of any one of claims 58 to 60, wherein the direct repeat comprises:
- ~~a. nucleotide 1 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~b. nucleotide 2 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~c. nucleotide 3 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~d. nucleotide 4 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~e. nucleotide 5 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~f. nucleotide 6 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~g. nucleotide 7 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~h. nucleotide 8 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~i. nucleotide 9 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~j. nucleotide 10 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~k. nucleotide 11 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~l. nucleotide 12 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~m. nucleotide 13 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~n. nucleotide 14 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or~~

- ~~o. SEQ ID NO: 61 or a portion thereof.~~
- ~~62. The composition of any one of claims 58 to 61, wherein the direct repeat sequence comprises AGN₁N₂N₃N₄GUGUN₅N₆N₇CAGN₈GACN₉C (SEQ ID NO: 125), wherein N₁ is A or G, N₂ is C or U, N₃ is A or G, N₄ is U or C, N₅ is C or U, N₆ is C or U, N₇ is U, A, C, or G, N₈ is U or C, and N₉ is A or C.~~
- ~~63. The composition of any one of claims 58 to 62, wherein the spacer sequence comprises between 15 and 35 nucleotides in length.~~
- ~~64. The composition of any one of claims 58 to 63, wherein the spacer sequence comprises complementarity to a target strand sequence of a target nucleic acid.~~
- ~~65. The composition of claim 64, wherein the target nucleic acid comprises a non-target strand sequence adjacent to a protospacer adjacent motif (PAM) sequence.~~
- ~~66. The composition of claim 65, wherein the PAM sequence is a 5'-TTN-3', 5'-NTTN-3', 5'-NTN'-3', 5'-NNTN-3', 5'-VTN-3', or 5'-NVTN-3', wherein N is any nucleotide (e.g., A, G, T, or C) and V is A, G, or C.~~
- ~~67. The composition of any one of claims 38 to 66, wherein the variant Cas12i4 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.~~
- ~~68. A composition comprising a nucleic acid that encodes a Cas12i4 polypeptide of any one of claims 38 to 67, wherein optionally the nucleic acid is codon-optimized for expression in a cell.~~
- ~~69. The composition of claim 68, wherein the cell is a eukaryotic cell.~~
- ~~70. The composition of claim 68 or 69, wherein the cell is a mammalian cell or a plant cell.~~
- ~~71. The composition of any one of claims 68 to 70, wherein the cell is a human cell.~~
- ~~72. The composition of claim 68, wherein the nucleic acid encoding the variant Cas12i4 polypeptide is operably linked to a promoter.~~
- ~~73. The composition of claim 68 or 72, wherein the nucleic acid encoding the variant Cas12i4 polypeptide is in a vector.~~
- ~~74. The composition of claim 73, wherein the vector comprises a retroviral vector, a lentiviral vector, a phage vector, an adenoviral vector, an adeno-associated vector, or a herpes simplex vector.~~

~~75. The composition of any one of claims 38 to 74, wherein the composition is present in a delivery composition comprising a nanoparticle (e.g., a lipid nanoparticle), a liposome, an exosome, a microvesicle, or a gene gun.~~

~~76~~13. An RNA guide or a nucleic acid encoding the RNA guide, wherein the RNA guide comprises a direct repeat sequence comprising:

- a. nucleotide 1 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- b. nucleotide 2 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- c. nucleotide 3 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- d. nucleotide 4 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- e. nucleotide 5 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- f. nucleotide 6 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- g. nucleotide 7 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- h. nucleotide 8 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- i. nucleotide 9 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;

- j. nucleotide 10 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- k. nucleotide 11 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- l. nucleotide 12 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- m. nucleotide 13 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;
- n. nucleotide 14 through nucleotide 36 of a sequence that is at least 90% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or
- o. a sequence that is at least 90% identical to a sequence of SEQ ID NO: 61 or a portion thereof.

~~77. The RNA guide or the nucleic acid encoding the RNA guide of claim 76, wherein the direct repeat comprises:~~

- ~~a. nucleotide 1 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~b. nucleotide 2 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~c. nucleotide 3 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~d. nucleotide 4 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~e. nucleotide 5 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~

- ~~f. nucleotide 6 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~g. nucleotide 7 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~h. nucleotide 8 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~i. nucleotide 9 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~j. nucleotide 10 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~k. nucleotide 11 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~l. nucleotide 12 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~m. nucleotide 13 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
 - ~~n. nucleotide 14 through nucleotide 36 of a sequence that is at least 95% identical to a sequence of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or~~
 - ~~o. a sequence that is at least 95% identical to a sequence of SEQ ID NO: 61 or a portion thereof.~~
78. The RNA guide or the nucleic acid encoding the RNA guide of claim 76 or 77, wherein the direct repeat comprises:
- ~~a. nucleotide 1 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~

- ~~b. nucleotide 2 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~c. nucleotide 3 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~d. nucleotide 4 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~e. nucleotide 5 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~f. nucleotide 6 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~g. nucleotide 7 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~h. nucleotide 8 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~i. nucleotide 9 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~j. nucleotide 10 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~k. nucleotide 11 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~l. nucleotide 12 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~m. nucleotide 13 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124;~~
- ~~n. nucleotide 14 through nucleotide 36 of any one of SEQ ID NOs: 60, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124; or~~
- ~~o. SEQ ID NO: 61 or a portion thereof.~~

~~79~~14. The composition of any one of claims 7, 8, or 12 or the RNA guide or the nucleic acid encoding the RNA guide of claim 13~~any one of claims 76 to 78~~, wherein the direct repeat sequence comprises AGN₁N₂N₃N₄GUGUN₅N₆N₇CAGN₈GACN₉C (SEQ ID NO: 125), wherein N₁ is A or G, N₂ is C or U, N₃ is A or G, N₄ is U or C, N₅ is C or U, N₆ is C or U, N₇ is U, A, C, or G, N₈ is U or C, and N₉ is A or C.

~~80~~15. The RNA guide or the nucleic acid encoding the RNA guide of claim 13 or 14~~any one of claims 76 to 79~~, wherein the RNA guide further comprises a spacer sequence.

~~84~~16. The composition of any one of claims 7, 8, 12, 16, or 15 or the RNA guide or the nucleic acid encoding the RNA guide of claim 15~~80~~, wherein the spacer sequence:
i) comprises about 15 to about 35 nucleotides in length;
ii) recognizes a target nucleic acid; and/or
iii) comprises sequence complementarity to a target strand sequence of a target nucleic acid.

~~82. The RNA guide or the nucleic acid encoding the RNA guide of claim 80 or claim 81, wherein the spacer sequence recognizes a target nucleic acid.~~

~~83~~17. The composition of claim 16 or the RNA guide or the nucleic acid encoding the RNA guide of claim 16~~82~~, wherein the target nucleic acid comprises a target sequence adjacent to a protospacer adjacent motif (PAM) sequence, optionally wherein the PAM sequence comprises a nucleotide sequence set forth as 5'-TTN-3', 5'-NTTN-3', 5'-NTN'-3', 5'-NNTN-3', 5'-VTN-3', or 5'-NVTN-3', wherein N is any nucleotide (e.g., A, G, T, or C) and V is A, G, or C.

~~84~~18. A composition comprising the RNA guide or the nucleic acid encoding the RNA guide of any one of claims 13 to 17~~76 to 83~~.

~~85~~19. The composition of claim 18~~84~~, wherein the composition is a delivery composition comprising a nanoparticle (e.g., a lipid nanoparticle), a liposome, an exosome, a microvesicle, or a gene-gun.

~~86. The RNA guide or the nucleic acid encoding the RNA guide of any one of claims 76 to 83, wherein the nucleic acid encoding the RNA guide is operably linked to a promoter.~~

~~87. The RNA guide or the nucleic acid encoding the RNA guide of any one of claims 76 to 83 or 86, wherein the nucleic acid encoding the RNA guide is in a vector.~~

~~88. The RNA guide or the nucleic acid encoding the RNA guide of any one of claims 76 to 83 or 86 to 87, wherein the vector comprises a retroviral vector, a lentiviral vector, a phage vector, an adenoviral vector, an adeno-associated vector, or a herpes simplex vector.~~

~~89~~20. A cell comprising variant Cas12i4 polypeptide of any one of claims 1 to 6, 8 or 11, the composition of any one of claims 7 to 12, 14, or 16 to 19, or the RNA guide or the nucleic acid encoding the RNA guide of any one of claims 13 to 17~~76 to 83 or 86 to 88~~.

~~90~~21. The cell of claim 20~~89~~, wherein the cell is a eukaryotic cell, optionally wherein the eukaryotic-

~~91. The cell of claim 89 or 90, wherein the~~ cell is a mammalian cell (e.g., a human cell) or a plant cell.

~~92. The cell of any one of claims 89 to 91, wherein the cell is a human cell.~~

- ~~93~~22. A method for editing a gene in a cell, the method comprising contacting the cell with a variant of any one of claims [1 to 6, 8 or 11](#)~~1-17, 26, 27, or 33~~; a composition of any one of claims [7 to 10, 12, 18, or 19](#)~~18-32, 38-75, 84, or 85~~; or an RNA guide of any one of claims [13 to 17](#)~~76-83 or 86-88~~.
- ~~94~~23. A nucleic acid molecule encoding a Cas12i4 variant of SEQ ID NO: 4, wherein the sequence of the nucleic acid molecule is 95% identical to [any one](#) ~~the selected from the group consisting of~~ SEQ ID NOs: 222-228.
- ~~95.~~ ~~The nucleic acid molecule of claim 94, wherein the sequence of the nucleic acid molecule comprises a sequence selected from the group consisting of SEQ ID NOs: 222-228.~~