Claims

1. A protein having an amino acid sequence selected from:

(i) as shown in any one of SEQ ID NO: 1, 2, 3 ;

~~(ii) compared with the sequence shown in any one of SEQ ID NOs: 1, 2, 3, a sequence having one or more amino acid substitutions, deletions or additions~~

(iii) a sequence having an amino acid sequence which has ~~at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 80%, 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%, or at least 99% sequence identity compared to any one of SEQ ID NOs: 1, 2, and 3;

~~for example,~~ the protein is an effector protein in the CRISPR/Cas system.

2. The protein of claim 1, comprising a sequence selected from the following, or consists of a sequence selected from the following:

(A) (i) as shown in SEQ ID NO: 1;

~~(ii) compared with the sequence shown in SEQ ID NOs: 1, a sequence having one or more amino acid substitutions, deletions or additions~~

(iii) a sequence having an amino acid sequence which has ~~at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 80%, 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%, or at least 99% sequence identity compared to SEQ ID NOs: 1;

for example, the protein has an amino acid sequence as shown in SEQ ID NO:1; or

(B) (i) as shown in SEQ ID NO: 2;

~~(ii) compared with the sequence shown in SEQ ID NOs: 2, a sequence having one or more amino acid substitutions, deletions or additions~~

(iii) a sequence having an amino acid sequence which has ~~at least 20%, at least~~

~~30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 80%, 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%, or at least 99% sequence identity compared to SEQ ID NOs: 2;

for example, the protein has an amino acid sequence as shown in SEQ ID NO:2; or

(C) (i) as shown in SEQ ID NO: 3;

~~(ii) compared with the sequence shown in SEQ ID NOs: 3, a sequence having one or more amino acid substitutions, deletions or additions (for example, 1, 2, 3, 4, 5 , 6, 7, 8, 9 or 10 amino acid substitutions, deletions or additions)~~

(iii) a sequence having an amino acid sequence which has at least ~~20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 80%, 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%, or at least 99% sequence identity compared to SEQ ID NOs: 3;

for example, the protein has an amino acid sequence as shown in SEQ ID NO:3.

3. A conjugate comprising the protein of claim 1 and a modified portion,

wherein

(1) the modified portion is optionally selected from an additional protein or polypeptide, a detectable label, and any combinations thereof, wherein the additional protein or polypeptide is optionally selected from an epitope tag, a reporter gene sequence, a nuclear localization signal (NLS) sequence, a targeting moiety, a transcription activation domain (for example, VP64), a transcription repression domain (for example, KRAB domain or SID domain), a nuclease domain (for example, Fok1), a domain having an activity selected from: nucleotide deaminase, methylase activity, demethylase, transcription activation activity, transcription inhibition activity, transcription release factor activity, histone modification activity, nuclease activity, single-stranded RNA cleavage activity, double-stranded RNA

cleavage activity, single-stranded DNA cleavage activity, double-stranded DNA cleavage activity, and nucleic acid binding activity; and any combinations thereof; and/or

(2) the modified portion is optionally connected to the N-terminus or C-terminus of the protein through a linker; and/or

(3) the conjugate optionally comprises an epitope tag; and/or

(4) the conjugate comprises an NLS sequence;

for example, the NLS sequence is shown in SEQ ID NO: 19;

for example, the NLS sequence is located at, near, or close to the end of the protein (e.g., N-terminal or C-terminal).

4. A fusion protein comprising the protein of claim 1 and an additional protein or polypeptide.

5. The fusion protein of claim 4, wherein

(1) the additional protein or polypeptide is linked to the N-terminus or C-terminus of the protein through a linker; and/or

(2) the additional protein or polypeptide is selected from an epitope tag, a reporter gene sequence, a nuclear localization signal (NLS) sequence, a targeting moiety, a transcription activation domain (for example, VP64), a transcription repression domain (for example, KRAB domain or SID domain), a nuclease domain (for example, Fok1), a domain having an activity selected from: a nucleotide deaminase, methylase activity, a demethylase, transcription activation activity, transcription inhibition activity, transcription release factor activity, histone modification activity, nuclease activity, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, single-stranded DNA cleavage activity, double-stranded DNA cleavage activity, and nucleic acid binding activity ; and any combinations thereof; and/or

(3) the fusion protein comprises an epitope tag; and/or

(4) the fusion protein comprises an NLS sequence;

for example, the NLS sequence is shown in SEQ ID NO: 19;

for example, the NLS sequence is located at, near, or close to the end of the protein (e.g., N-terminal or C-terminal); and/or

(5) the fusion protein has an amino acid sequence as shown in SEQ ID NO:20.

6. An isolated nucleic acid molecule comprising a sequence selected from the following or consisting of a sequence selected from the following:

(i) a sequence as shown in SEQ ID NO: 7 or 13;

~~(ii) compared with the sequence as shown in SEQ ID NO: 7 or 13, a sequence having one or more base substitutions, deletions or additions (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 base substitutions, deletions or additions);~~

(ii~~i~~) a sequence having ~~at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%,~~ at least 90%, at least 95% sequence identity with the sequence as shown in SEQ ID NO:7 or 13;

(~~iv~~iii) a sequence that hybridizes to the sequence as described in any one of (i) to (ii~~i~~) under stringent conditions; or

(iv) a complementary sequence of the sequence as described in any one of (i)-(ii~~i~~);

in addition, the sequence as described in any one of (ii)-(iv) substantially retains the biological function of the sequence from which it is derived; the isolated nucleic acid molecule is a direct repeat sequence in the CRISPR/Cas system, which forms a complex with the protein of claim 1;

for example, the isolated nucleic acid molecule is RNA~~;~~

~~for example, the isolated nucleic acid molecule is a direct repeat sequence in the CRISPR/Cas system~~.

7. The isolated nucleic acid molecule of claim 6, wherein

(1) the nucleic acid molecule comprises one or more stem loops or optimized

secondary structures;

for example, the sequence as described in any one of (ii) to (v) retains the secondary structure of the sequence from which it is derived; and/or

(2) the nucleic acid molecule comprises a sequence selected from the following, or consists of a sequence selected from the following:

(a) a nucleotide sequence as shown in SEQ ID NOs: 7 or 13;

(b) a sequence that hybridizes to the sequence as described in (a) under stringent conditions; or

(c) a complementary sequence of the nucleotide sequence as shown in SEQ ID NO: 7 or 13.

8. A complex comprising:

(i) a protein component, which is selected from: the protein of claim 1, the conjugate of claim 3, the fusion protein of any one of claims 4-5, and any combinations thereof; and

(ii) a nucleic acid component, which comprises a direct repeat sequence ~~the isolated nucleic acid molecule of any one of claims 6-7~~ and a targeting sequence capable of hybridizing to the target sequence from 5' to 3' direction, the direct repeat sequence can form a complex with the protein of claim 1;

wherein the protein component and the nucleic acid component combine with each other to form a complex;

for example, the nucleic acid component is a guide RNA in the CRISPR/Cas system;

for example, the nucleic acid molecule is RNA;

for example, the complex does not contain trans-activating crRNA (tracrRNA),

wherein the targeting sequence is optionally attached to the 3' end of the nucleic acid molecule and/or the targeting sequence optionally comprises a complementary sequence of the target sequence.

9. An isolated nucleic acid molecule comprising:

(i) a nucleotide sequence encoding the protein of claim 1 or the fusion protein of any one of claims 4-5;

(ii) a nucleotide sequence encoding a direct repeat sequence in the CRISPR/Cas system, which can form a complex with the protein of claim 1~~the isolated nucleic acid molecule of any one of claims 6-7~~; and/or

(iii) a nucleotide sequence containing (i) and (ii);

for example, the nucleotide sequence described in any one of (i) to (iii) is codon-optimized for expression in a prokaryotic cell or an eukaryotic cell.

10. A vector comprising the isolated nucleic acid molecule of claim 9.

11. A host cell comprising the isolated nucleic acid molecule of claim ~~23~~ 9 or the vector of claim 10.

12. A composition comprising:

(i) a first component, which is selected from: the protein of claim 1, the conjugate of claim 3, the fusion protein of any one of claims 4-5, a nucleotide sequence encoding the protein or fusion protein, and any combinations thereof; and

(ii) a second component, which is a nucleotide sequence containing a guide RNA, or a nucleotide sequence encoding the nucleotide sequence containing a guide RNA;

wherein the guide RNA includes a direct repeat sequence and a targeting sequence from the 5' to 3', and the targeting sequence can hybridize with the target sequence;

the targeting RNA can form a complex with the protein, conjugate or fusion protein as described in (i);

for example, the direct repeat sequence is an isolated nucleic acid molecule as defined in any one of claims 6-7;

for example, the composition does not contain a trans-activating crRNA (tracrRNA).

13. A composition comprising one or more vectors comprising:

(i) a first nucleic acid, which is a nucleotide sequence encoding a protein of claim 1 or the fusion protein any one of claims ~~6-7~~4-5; optionally, the first nucleic acid is operationally linked to a first regulatory element; and

(ii) a second nucleic acid, which encodes a nucleotide sequence comprising a guide RNA; optionally the second nucleic acid is operationally linked to a second regulatory element;

wherein:

the first nucleic acid and the second nucleic acid are present on the same or different vectors;

the guide RNA includes a direct repeat sequence and a targeting sequence from the 5' to 3', and the targeting sequence can hybridize with the target sequence;

the guide RNA can form a complex with the effector protein of claim 1 or fusion protein as described in (i~~).~~);

for example, the direct repeat sequence is an isolated nucleic acid molecule as defined in any one of claims 6-7;

for example, the composition does not contain a trans-activating crRNA (tracrRNA),

wherein the first regulatory element and/or the second regulatory element is optionally a promoter, such as an inducible promoter.

14. The composition of any one of claims 12-13, wherein

(1) at least one component of the composition is non-naturally occurring or modified; and/or

(2) the targeting sequence is connected to the 3' end of the direct repeat sequence; and/or

(3) the targeting sequence comprises a complementary sequence of the target sequence; and/or

(4) when the target sequence is DNA, the target sequence is located at the 3' end of the protospacer adjacent motif (PAM), and the PAM has a sequence shown by 5'-TTN, wherein N is selected from A, G, T, C; when the target sequence is RNA, the target sequence does not have PAM domain restrictions; and/or

(5) the target sequence is a DNA or RNA sequence derived from a prokaryotic cell or an eukaryotic cell; or, the target sequence is a non-naturally occurring DNA or RNA sequence; and/or

(6) the target sequence is present in a cell;

for example, the target sequence is present in a nucleus or in a cytoplasm (e.g., organelle);

for example, the cell is an eukaryotic cell;

for example, the cell is a prokaryotic cell; and/or

(7) the protein is linked to one or more NLS sequences, or the conjugate or fusion protein comprises one or more NLS sequences;

for example, the NLS sequence is connected to the N-terminus or C-terminus of the protein;

for example, the NLS sequence is fused to the N-terminus or C-terminus of the protein.

15. A kit comprising one or more components selected from: the protein of claim 1, the conjugate of claim 3, the fusion protein of any one of claims 4-5, the isolated nucleic acid molecule of any one of claims 6-7, the complex of claim 8, the isolated nucleic acid molecule of claim 9, the vector of claim 10, the composition of any one of claims 12-14;

for example, the kit comprises the composition of claim 17, and instructions for using the composition;

for example, the kit includes the composition of claim 18, and instructions for

using the composition.

16. A delivery composition comprising a delivery vehicle and one or more selected from the group consisting of: the protein of claim 1, the conjugate of claim 3, the fusion protein of any one of claims 4-5, the isolated nucleic acid molecule of any one of claims 6-7, the complex of claim 8, the isolated nucleic acid molecule of claim 9, the vector of claim 10, the composition of any one of claims 12-14;

for example, the delivery vehicle is a particle;

for example, the delivery vehicle is selected from a lipid particle, sugar particle, metal particle, protein particle, liposome, exosome, microvesicle, gene gun, or viral vector (e.g., replication defective retrovirus, lentivirus, adenovirus or adeno-associated virus).

17. A non-diagnostic and non-therapeutic method for modifying a target gene, comprising: contacting the complex of claim 8 or the composition of any one of claims 12-14 with the target gene, or delivering that to a cell containing the target gene; the target sequence is present in the target gene.

18. The method of claim 17, wherein

the modification refers to a break in the target sequence, such as a double-strand break in DNA or a single-strand break in RNA;

for example, the modification also includes the insertion of an exogenous nucleic acid into the break.

19. A non-diagnostic and non-therapeutic method for altering the expression of a gene product, comprising: combining the complex of claim 8 or the composition of any one of claims 12-14 with a nucleic acid molecule encoding the gene product, or delivering that to a cell containing the nucleic acid molecule in which the target sequence is present, wherein the expression of the gene product is optionally altered,

preferably the gene product is a protein.

20. The method of any one of claims 17-19,

(1) wherein the nucleic acid molecule is present in a cell;

for example, the cell is a prokaryotic cell;

for example, the cell is an eukaryotic cell, such as a mammalian cell (such as a human cell) or a plant cell; or.

the nucleic acid molecule is present in a nucleic acid molecule (e.g., a plasmid) *in vitro*; and/or

(2) wherein the protein, conjugate, fusion protein, isolated nucleic acid molecule, complex, vector or composition is contained in a delivery vehicle;

for example, the delivery vehicle is selected from a lipid particle, sugar particle, metal particle, protein particle, liposome, exosome, viral vector (such as replication-defective retrovirus, lentivirus, adenovirus or adeno-associated virus); and/or

(3) which is used to change one or more target sequences in a target gene or a nucleic acid molecule encoding a target gene product to modify a cell, cell line, or organism.

~~21. A cell or progeny thereof obtained by the method of any one of claims 17-20, wherein the cell contains a modification that is not present in its wild type.~~

~~22. A cell product of the cell or progeny thereof of claim 21.~~

~~23~~21. An in vitro, isolated or *in vivo* cell or cell line or progeny thereof, the cell or cell line or the progeny thereof comprises: the protein of claim 1, the conjugate of claim 3, the fusion protein of any one of claims 4-5, the isolated nucleic acid molecule of any one of claims 6-7, the complex of claim 8, the isolated nucleic acid molecule of claim 9, the vector of claim 10, the composition of any one of claims

12-14;

for example, the cell is an eukaryotic cell;

for example, the cell is an animal cell (for example, a mammalian cell, such as a human cell) or a plant cell;

for example, the cell is a stem cell or stem cell line.

~~24~~22. Use of the protein of claim 1, the conjugate of claim 3, the fusion protein of any one of claims 4-5, the isolated nucleic acid molecule of any one of claims 6-7, the complex of claim 8, the isolated nucleic acid molecule of claim 9, the vector of claim 10, the composition of any one of claims 12-14 or the kit of claim 15 in

~~(1) a nucleic acid editing (for example, gene or genome editing);~~

~~for example, the gene or genome editing includes modifying genes, knocking out genes, altering the expression of gene products, repairing mutations, and/or inserting polynucleotides; or~~

~~(2)~~ the preparation of a preparation for:

(i) the isolated gene or genome editing;

(ii) the detection of an isolated single-stranded DNA;

(iii) editing the target sequence in the target locus to modify a biological or non-human organism;

(iv) treating the disease caused by defects in the target sequence in the target locus.