[0006] The present disclosure provides guide RNAs (referred to herein as "CasX guide RNAs") that bind to and provide sequence specificity to the CasX proteins; nucleic acids encoding the CasX guide RNAs; and modified host cells comprising the CasX guide RNAs and/or nucleic acids encoding same. CasX guide RNAs are useful in a variety of applications, which are provided.

[0007] The present disclosure provides archaeal Cas9 polypeptides and nucleic acids encoding same, as well as their associated guide RNAs (archaeal Cas9 guide RNAs) and nucleic acids encoding same.

[0007a] In one aspect, the present disclosure refers to a composition comprising:

a) a CasX polypeptide, or a nucleic acid molecule encoding the CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a CasX guide RNA, or one or more DNA molecules encoding the CasX guide RNA, wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasX polypeptide is capable of forming a complex with the CasX guide RNA, and wherein the complex is capable of binding the target nucleic acid.

[0007b] In another aspect, the present disclosure refers to a CasX single guide RNA molecule, or a DNA molecule comprising a nucleotide sequence encoding the CasX single guide RNA molecule, the CasX single guide RNA molecule comprising:

a) a targeter sequence comprising a guide sequence that hybridizes to a target nucleic acid, and a duplex-forming segment; and

b) an activator sequence that hybridizes with the duplex-forming segment of the targeter sequence to form a double stranded RNA (dsRNA) duplex that can bind a CasX polypeptide.

[0007c] In another aspect, the present disclosure refers to a CasX fusion polypeptide comprising:

a) a CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a heterologous polypeptide.

[0007d] In another aspect, the present disclosure refers to a nucleic acid molecule comprising a nucleotide sequence encoding any one of the CasX fusion polypeptide disclosed herein.

[0007e] In another aspect, the present disclosure refers to one or more recombinant expression vectors comprising nucleotide sequences encoding:

(a) a non-naturally occurring CasX guide RNA comprising an activator RNA and a targeter RNA, wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid; and

(b) a CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain.

[0007f] In another aspect, the present disclosure refers to a eukaryotic cell comprising one or more of:

a) a CasX polypeptide, or a nucleic acid molecule encoding the CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain;

b) a CasX fusion polypeptide, or a nucleic acid molecule encoding the CasX fusion polypeptide, wherein the CasX fusion polypeptide comprises: i) a CasX polypeptide; and ii) a heterologous polypeptide, and wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

c) a CasX guide RNA, or a nucleic acid molecule encoding the CasX guide RNA, wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid; and

wherein the eukaryotic cell is not a human embryo or human germ cell.

[0007g] In another aspect, the present disclosure refers to a cell comprising any one of the CasX fusion polypeptide disclosed herein, or a nucleic acid molecule encoding the CasX fusion polypeptide.

[0007h] In another aspect, the present disclosure refers to an *in vitro* or *ex vivo* method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a CasX guide RNA comprising a nucleotide sequence that hybridizes to a target sequence of the target nucleic acid,

wherein said contacting results in modification of the target nucleic acid by the CasX polypeptide and wherein the method is not performed on a human germ line cell.

[0007i] In another aspect, the present disclosure refers to an in vitro or ex vivo method of modulating transcription from a target DNA, modifying a target nucleic acid, or modifying a protein associated with a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) any one of the CasX fusion polypeptide disclosed herein; and

b) a CasX guide RNA comprising a nucleotide sequence that hybridizes to a target sequence of the target nucleic acid,

wherein the method is not performed on a human germ line cell.

[0007j] In another aspect, the present disclosure refers to a transgenic, multicellular, non-human organism whose genome comprises a transgene comprising a nucleotide sequence encoding one or more of:

a) a CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain;

b) any one of the CasX fusion polypeptide disclosed herein, and

c) a CasX guide RNA, wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid.

[0007k] In another aspect, the present disclosure refers to a kit comprising:

a) a CasX polypeptide and a CasX single guide RNA;

b) a CasX polypeptide, a CasX guide RNA, and a DNA donor template;

c) a CasX fusion polypeptide and a CasX guide RNA;

d) a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

e) an mRNA encoding a CasX polypeptide, and a CasX single guide RNA;

f) an mRNA encoding a CasX polypeptide; a CasX guide RNA, and a DNA donor template;

g) an mRNA encoding a CasX fusion polypeptide, and a CasX guide RNA;

h) an mRNA encoding a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and a DNA donor template, wherein the CasX polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasX polypeptide is capable of forming a complex with the CasX guide RNA, and wherein the complex is capable of binding the target nucleic acid; and

wherein the CasX fusion polypeptide is of any one of the CasX fusion polypeptide disclosed herein.

[0007l] In another aspect, the present disclosure refers to a sterile container comprising a CasX system comprising:

a) a CasX polypeptide and a CasX single guide RNA;

b) a CasX polypeptide, a CasX guide RNA, and a DNA donor template;

c) a CasX fusion polypeptide and a CasX guide RNA;

d) a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

e) an mRNA encoding a CasX polypeptide, and a CasX single guide RNA;

f) an mRNA encoding a CasX polypeptide; a CasX guide RNA, and a DNA donor template;

g) an mRNA encoding a CasX fusion polypeptide, and a CasX guide RNA;

h) an mRNA encoding a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and a DNA donor template, wherein the CasX polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasX polypeptide is capable of forming a complex with the CasX guide RNA, and wherein the complex is capable of binding the target nucleic acid; and
wherein the CasX fusion polypeptide is any one of the CasX fusion polypeptide disclosed herein.

[0007m] In yet another aspect, the present disclosure refers to an implantable device comprising a CasX system comprising:

a) a CasX polypeptide and a CasX single guide RNA;

b) a CasX polypeptide, a CasX guide RNA, and a DNA donor template;

c) a CasX fusion polypeptide and a CasX guide RNA;

d) a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

e) an mRNA encoding a CasX polypeptide, and a CasX single guide RNA;

f) an mRNA encoding a CasX polypeptide; a CasX guide RNA, and a DNA donor template;

g) an mRNA encoding a CasX fusion polypeptide, and a CasX guide RNA;

h) an mRNA encoding a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and a DNA donor template,

wherein the CasX polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasX polypeptide is capable of forming a complex with the CasX guide RNA, and wherein the complex is capable of binding the target nucleic acid; and
wherein the CasX fusion polypeptide is any one of the CasX fusion polypeptide disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] **Figure 1** depicts two naturally occurring CasX protein sequences.

[0009] **Figure 2** depicts an alignment of the two identified naturally occurring CasX protein sequences.

[0010] **Figure 3 (panels a-b)** depicts a schematic domain representation for CasX. Also shown are results from various searches attempting to identify homologs of CasX. Also depicted are portions of the CasX-containing CRISPR loci there were identified from two different species.

[0011] **Figure 4 (panels a-c)** depicts experiments performed to demonstrate plasmid interference by CasX expressed in *Escherichia coli*.

[0012] **Figure 5 (panels a-b)** depicts experiments performed (PAM dependent plasmid interference by CasX) to determine a PAM sequence for CasX**.**

[0013] **Figure 6 (panels a-c)** depicts experiments performed to determine that CasX is a dual-guided CRISPR-Cas effector complex.

[0014] **Figure 7** presents a schematic of CasX RNA guided DNA interference.

[0015] **Figure 8** presents a schematic of experimental design for one embodiment to demonstrate editing in human cells using CasX.

[0016] **Figure 9** presents data showing recombinant expression and purification of CasX.

[0017] **Figure 10** presents data using various different tracrRNA sequences (different sequence lengths) for cleavage activity.

[0018] **Figure 11** presents data related to CasX functioning at room temperature versus 37°C.

[0019] **Figure 12 (panels a-e)** presents a information related to an archaeal Cas9 CRISPR system (the ARMAN-1 type II CRISPR-cas system).

[0020] **Figure 13** presents example archaeal Cas9 proteins (ARMAN-1 and ARMAN-4, SEQ ID NOs: 71 and 72, respectively). Catalytic residues that correspond to D10 and H840 of *S. pyogenes* are bold and underlined.

CLAIMS

What is claimed is:

1. A composition comprising:

a) a CasX polypeptide, or a nucleic acid molecule encoding the CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a CasX guide RNA, or one or more DNA molecules encoding the CasX guide RNA, wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasX polypeptide is capable of forming a complex with the CasX guide RNA, and wherein the complex is capable of binding the target nucleic acid.

2. The composition of claim 1, wherein the CasX polypeptide comprises an amino acid sequence having 70% or 85% or more identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3.

3. The composition of claim 1 or claim 2, wherein the CasX guide RNA is a single guide RNA.

4. The composition of claim 1 or claim 2, wherein the CasX guide RNA is a dual-guide RNA.

5. The composition of any one of claims 1-4, wherein
the composition comprises a lipid, or one or more of: a buffer, a nuclease inhibitor, and a protease inhibitor, or
wherein a) and b) are within a liposome or a) and b) are within a particle.

6. The composition of any one of claims 1-5, wherein the CasX polypeptide is a) a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule; b) a catalytically inactive CasX polypeptide (dCasX); c) a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule and comprises one or more mutations at a position corresponding to those selected from: D672, E769, and D935 of SEQ ID NO: 1; or d) a catalytically inactive CasX polypeptide (dCasX) and comprises one or more mutations at a position corresponding to those selected from: D672, E769, and D935 of SEQ ID NO: 1.

7. The composition of any one of claims 1-6, further comprising a DNA donor template.

8. A CasX single guide RNA molecule, or a DNA molecule comprising a nucleotide sequence encoding the CasX single guide RNA molecule, the CasX single guide RNA molecule comprising:
a) a targeter sequence comprising a guide sequence that hybridizes to a target nucleic acid, and a duplex-forming segment; and
b) an activator sequence that hybridizes with the duplex-forming segment of the targeter sequence to form a double stranded RNA (dsRNA) duplex that can bind a CasX polypeptide.

9. The CasX single guide RNA molecule of claim 8, wherein the guide sequence has a length of from 19 to 30 nucleotides.

10. The CasX single guide RNA molecule or the DNA molecule of Claim 8 or 9, wherein the nucleotide sequence encoding the CasX single guide RNA is operably linked to: a) a promoter, b) a promoter functional in a eukaryotic cell, c) a promoter functional in a eukaryotic cell and functional in one or more of: a plant cell, a fungal cell, an animal cell, cell of an invertebrate, a fly cell, a cell of a vertebrate, a mammalian cell, a primate cell, a non-human primate cell, and a human cell, or d) the promoter of a), b), or c) and wherein the promoter is one or more of: a constitutive promoter, an inducible promoter, a cell type-specific promoter, and a tissue-specific promoter.

11. The CasX single guide RNA molecule or the DNA molecule of any one of Claims 8-10, wherein the DNA molecule is a recombinant expression vector or wherein the DNA molecule is a recombinant expression vector that is a recombinant adeno-associated viral vector, a recombinant retroviral vector, or a recombinant lentiviral vector.

12. The DNA molecule of Claim 10, wherein the promoter is functional in a prokaryotic cell.

13. A CasX fusion polypeptide comprising:
a) a CasX polypeptide, wherein the CasX polypeptide:
i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and
ii) comprises 3 partial RuvC domains that form a single RuvC domain; and
b) a heterologous polypeptide.

14. The CasX fusion polypeptide of Claim 13, wherein the CasX polypeptide comprises an amino acid sequence having 70% or 85% or more identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3.

15. The CasX fusion polypeptide of Claim 13 or 14, wherein the CasX polypeptide is a) a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule, b) a catalytically inactive CasX polypeptide (dCasX), c) a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule and comprises one or more mutations at a position corresponding to those selected from: D672, E769, and D935 of SEQ ID NO: 1, or d) a catalytically inactive CasX polypeptide (dCasX) and comprises one or more mutations at a position corresponding to those selected from: D672, E769, and D935 of SEQ ID NO: 1.

16. The CasX fusion polypeptide of any one of Claims 13-15, wherein the heterologous polypeptide is fused to the N-terminus and/or the C-terminus of the CasX polypeptide and/or comprises a nuclear localization signal.

17. The CasX fusion polypeptide of any one of Claims 13-16, wherein the heterologous polypeptide:
a) is a targeting polypeptide that provides for binding to a cell surface moiety on a target cell or target cell type,
b) exhibits an enzymatic activity that modifies target DNA,
c) exhibits an enzymatic activity that modifies target DNA and wherein the heterologous polypeptide exhibits one or more enzymatic activities selected from: nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity and glycosylase activity,
d) exhibits an enzymatic activity that modifies a target polypeptide associated with a target nucleic acid,
e) exhibits an enzymatic activity that modifies a target polypeptide associated with a target nucleic acid and wherein the heterologous polypeptide exhibits histone modification activity, or
f) the heterologous polypeptide exhibits the enzymatic activity as in d) or e) and one or more enzymatic activities selected from: methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity,

demyristoylation activity, glycosylation activity (e.g., from O-GlcNAc transferase) and deglycosylation activity.

18. The CasX fusion polypeptide of any one of Claims 13-17, wherein the heterologous polypeptide is:

a) an endosomal escape polypeptide,

b) an endosomal escape polypeptide comprising an amino acid sequence selected from: GLFXALLXLLXSLWXLLLXA (SEQ ID NO:94), and GLFHALLHLLHSLWHLLLHA (SEQ ID NO:95), wherein each X is independently selected from lysine, histidine, and arginine,

c) a chloroplast transit peptide,

d) a chloroplast transit peptide comprising an amino acid sequence selected from MASMISSSAVTTVSRASRGQSAAMAPFGGLKSMTGFPVRKVNTDITSITSNGGRVKCMQVWPPIGKKKFETLSYLPPLTRDSRA (SEQ ID NO:83);
MASMISSSAVTTVSRASRGQSAAMAPFGGLKSMTGFPVRKVNTDITSITSNGGRVKS (SEQ ID NO:84);
MASSMLSSATMVASPAQATMVAPFNGLKSSAAFPATRKANNDITSITSNGGRVNCMQVWPPIEKKKFETLSYLPDLTDSGGRVNC (SEQ ID NO:85);
MAQVSRICNGVQNPSLISNLSKSSQRKSPLSVSLKTQQHPRAYPISSSWGLKKSGMTLIGSELRPLKVMSSVSTAC (SEQ ID NO:86);
MAQVSRICNGVWNPSLISNLSKSSQRKSPLSVSLKTQQHPRAYPISSSWGLKKSGMTLIGSELRPLKVMSSVSTAC (SEQ ID NO:87);
MAQINNMAQGIQTLNPNSNFHKPQVPKSSSFLVFGSKKLKNSANSMLVLKKDSIFMQLFCSFRISASVATAC (SEQ ID NO:88);
MAALVTSQLATSGTVLSVTDRFRRPGFQGLRPRNPADAALGMRTVGASAAPKQSRKPHRFDRRCLSMVV (SEQ ID NO:89);
MAALTTSQLATSATGFGIADRSAPSSLLRHGFQGLKPRSPAGGDATSLSVTTSARATPKQQRSVQRGSRRFPSVVVC (SEQ ID NO:90);
MASSVLSSAAVATRSNVAQANMVAPFTGLKSAASFPVSRKQNLDITSIASNGGRVQC (SEQ ID NO:91);
MESLAATSVFAPSRVAVPAARALVRAGTVVPTRRTSSTSGTSGVKCSAAVTPQASPVISRSAAAA (SEQ ID NO:92); and
MGAAATSMQSLKFSNRLVPPSRRLSPVPNNVTCNNLPKSAAPVRTVKCCASSWNSTINGAAATTNGASAASS (SEQ ID NO:93),

e) a chloroplast transit peptide,

f) a chloroplast transit peptide comprising an amino acid sequence selected from MASMISSSAVTTVSRASRGQSAAMAPFGGLKSMTGFPVRKVNTDITSITSNGGRVKCMQVWPPIGKKKFETLSYLPPLTRDSRA (SEQ ID NO:83);

MASMISSSAVTTVSRASRGQSAAMAPFGGLKSMTGFPVRKVNTDITSITSNGGRVKS (SEQ ID NO:84);
MASSMLSSATMVASPAQATMVAPFNGLKSSAAFPATRKANNDITSITSNGGRVNCMQVWPP IEKKKFETLSYLPDLTDSGGRVNC (SEQ ID NO:85);
MAQVSRICNGVQNPSLISNLSKSSQRKSPLSVSLKTQQHPRAYPISSSWGLKKSGMTLIGSELR PLKVMSSVSTAC (SEQ ID NO:86);
MAQVSRICNGVWNPSLISNLSKSSQRKSPLSVSLKTQQHPRAYPISSSWGLKKSGMTLIGSEL RPLKVMSSVSTAC (SEQ ID NO:87);
MAQINNMAQGIQTLNPNSNFHKPQVPKSSSFLVFGSKKLKNSANSMLVLKKDSIFMQLFCSF RISASVATAC (SEQ ID NO:88);
MAALVTSQLATSGTVLSVTDRFRRPGFQGLRPRNPADAALGMRTVGASAAPKQSRKPHRFD RRCLSMVV (SEQ ID NO:89);
MAALTTSQLATSATGFGIADRSAPSSLLRHGFQGLKPRSPAGGDATSLSVTTSARATPKQQRS VQRGSRRFPSVVVC (SEQ ID NO:90);
MASSVLSSAAVATRSNVAQANMVAPFTGLKSAASFPVSRKQNLDITSIASNGGRVQC (SEQ ID NO:91);
MESLAATSVFAPSRVAVPAARALVRAGTVVPTRRTSSTSGTSGVKCSAAVTPQASPVISRSAA AA (SEQ ID NO:92); and
MGAAATSMQSLKFSNRLVPPSRRLSPVPNNVTCNNLPKSAAPVRTVKCCASSWNSTINGAAA TTNGASAASS (SEQ ID NO:93),
g) a protein that increases or decreases transcription,
h) a protein that increases or decreases transcription and is a transcriptional repressor domain,
i) a protein that increases or decreases transcription and is a activation domain, or
j) a protein binding domain.

19. A nucleic acid molecule comprising a nucleotide sequence encoding the CasX fusion polypeptide of any one of Claims 13-18.

20. The nucleic acid molecule of Claim 19, wherein the nucleotide sequence encoding the CasX fusion polypeptide is operably linked to a) a promoter, b) a promoter functional in a eukaryotic cell, c) a promoter is functional in a eukaryotic cell and wherein the promoter is functional in one or more of: a plant cell, a fungal cell, an animal cell, cell of an invertebrate, a fly cell, a cell of a vertebrate, a mammalian cell, a primate cell, a non-human primate cell, and a human cell, d) a promoter as in a), b), or c) and wherein the promoter is one or more of: a constitutive promoter, an inducible promoter, a cell type-specific promoter, and a tissue-specific promoter.

21. The nucleic acid molecule of Claim 19 or 20, wherein the DNA molecule is a recombinant expression vector or wherein the DNA molecule is a recombinant expression vector that is a recombinant adeno-associated viral vector, a recombinant retroviral vector, or a recombinant lentiviral vector.

22. The nucleic acid molecule of Claim 20, wherein the promoter is functional in a prokaryotic cell.

23. The nucleic acid molecule of Claim 19, wherein the nucleic acid molecule is an mRNA.

24. One or more recombinant expression vectors comprising nucleotide sequences encoding:

(a) a non-naturally occurring CasX guide RNA comprising an activator RNA and a targeter RNA, wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid; and

(b) a CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain.

25. The one or more recombinant expression vectors of Claim 24, wherein the CasX polypeptide comprises an amino acid sequence having 70% or 85% or more identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3.

26. The one or more recombinant expression vectors of Claim 24 or 25, wherein the CasX guide RNA is a single guide RNA.

27. The one or more recombinant expression vectors of Claim 24 or 25, wherein the CasX guide RNA is a dual-guide RNA.

28. The one or more recombinant expression vectors of Claim 27, wherein said one or more recombinant expression vectors comprises a first nucleotide sequence encoding the activator and a second nucleotide sequence encoding the targeter, and wherein said first and second nucleotide sequences are present on different DNA molecules.

29. The one or more recombinant expression vectors of any one of Claims 24-28, wherein said one or more recombinant expression vectors comprises a nucleotide sequence encoding the CasX

polypeptide that is operably linked to a) a promoter, b) a promoter that is functional in a eukaryotic cell, c) a promoter that is functional in a eukaryotic cell and is functional in one or more of: a plant cell, a fungal cell, an animal cell, cell of an invertebrate, a fly cell, a cell of a vertebrate, a mammalian cell, a primate cell, a non-human primate cell, and a human cell, or d) a promoter as in a), b), or c) and is one or more of: a constitutive promoter, an inducible promoter, a cell type-specific promoter, and a tissue-specific promoter.

30. The one or more recombinant expression vectors of Claim 29, wherein the promoter is functional in a prokaryotic cell.

31. A eukaryotic cell comprising one or more of:

a) a CasX polypeptide, or a nucleic acid molecule encoding the CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain;

b) a CasX fusion polypeptide, or a nucleic acid molecule encoding the CasX fusion polypeptide, wherein the CasX fusion polypeptide comprises: i) a CasX polypeptide; and ii) a heterologous polypeptide, and wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

c) a CasX guide RNA, or a nucleic acid molecule encoding the CasX guide RNA, wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid; and

wherein the eukaryotic cell is not a human embryo or human germ cell.

32. The eukaryotic cell of claim 31, comprising the nucleic acid molecule encoding the CasX polypeptide, wherein said nucleic acid molecule is integrated into the genomic DNA of the cell and/or the eukaryotic cell is a plant cell, a mammalian cell, an insect cell, an arachnid cell, a fungal cell, a bird cell, a reptile cell, an amphibian cell, an invertebrate cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, or a human cell.

33. A cell comprising a CasX fusion polypeptide of any one of Claims 15-18, or a nucleic acid molecule encoding the CasX fusion polypeptide.

34. The cell of claim 33, wherein the cell is a prokaryotic cell and/or comprises the nucleic acid molecule encoding the CasX fusion polypeptide, wherein said nucleic acid molecule is integrated into the genomic DNA of the cell.

35. An *in vitro* or *ex vivo* method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a CasX polypeptide, wherein the CasX polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a CasX guide RNA comprising a nucleotide sequence that hybridizes to a target sequence of the target nucleic acid,

wherein said contacting results in modification of the target nucleic acid by the CasX polypeptide and wherein the method is not performed on a human germ line cell.

36. The method of claim 35, wherein said modification is cleavage of the target nucleic acid, wherein

a) the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA;

b) said contacting takes place *in vitro* outside of a cell,

c) said contacting takes place inside of a cell in culture,

d) said contacting takes place inside of a cell in culture and the cell is a eukaryotic cell,

e) said contacting takes place inside of a cell in culture and the cell is a eukaryotic cell selected from: a plant cell, a fungal cell, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell,

f) said contacting takes place inside of a cell in culture and the cell is a prokaryotic cell, or

g) said contacting results in genome editing.

37. The method of Claim 35 or 36, wherein the CasX guide RNA is a single guide RNA.

38. The method of Claim 35 or 36, wherein the CasX guide RNA is a dual guide RNA.

39. An *in vitro* or *ex vivo* method of modulating transcription from a target DNA, modifying a target nucleic acid, or modifying a protein associated with a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a CasX fusion polypeptide of any one of Claims 13-18; and

b) a CasX guide RNA comprising a nucleotide sequence that hybridizes to a target sequence of the target nucleic acid,
wherein the method is not performed on a human germ line cell.

40. The method of Claim 39, wherein the CasX guide RNA is a single guide RNA.

41. The method of Claim 39, wherein the CasX guide RNA is a dual guide RNA.

42. The method of any of Claims 39-41, wherein said modification is not cleavage of the target nucleic acid.

43. The method of any of claims 39-42, wherein:
a) the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA,
b) said contacting takes place *in vitro* outside of a cell,
c) said contacting takes place inside of a cell in culture,
d) the cell is a eukaryotic cell,
e) the cell is a eukaryotic cell selected from: a plant cell, a fungal cell, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell, or
f) the cell is a prokaryotic cell.

44. The method of Claim 43, wherein the CasX polypeptide is a catalytically inactive CasX polypeptide (dCasX).

45. The method of Claim 43 or 44, wherein the CasX polypeptide comprises one or more mutations at a position corresponding to those selected from: D672, E769, and D935 of SEQ ID NO: 1.

46. A transgenic, multicellular, non-human organism whose genome comprises a transgene comprising a nucleotide sequence encoding one or more of:
a) a CasX polypeptide, wherein the CasX polypeptide:
    i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and
    ii) comprises 3 partial RuvC domains that form a single RuvC domain;
b) a CasX fusion polypeptide of any one of Claims 13-18, and

c) a CasX guide RNA, wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid.

47. The transgenic, multicellular, non-human organism of claim 46, wherein the CasX polypeptide comprises an amino acid sequence having 70% or 85% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3.

48. A kit comprising:
a) a CasX polypeptide and a CasX single guide RNA;
b) a CasX polypeptide, a CasX guide RNA, and a DNA donor template;
c) a CasX fusion polypeptide and a CasX guide RNA;
d) a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;
e) an mRNA encoding a CasX polypeptide, and a CasX single guide RNA;
f) an mRNA encoding a CasX polypeptide; a CasX guide RNA, and a DNA donor template;
g) an mRNA encoding a CasX fusion polypeptide, and a CasX guide RNA;
h) an mRNA encoding a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;
i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA;
j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and iii) a DNA donor template;
k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA; or
l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and a DNA donor template,
wherein the CasX polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;
wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasX polypeptide is capable of forming a complex with the CasX guide RNA, and wherein the complex is capable of binding the target nucleic acid; and
wherein the CasX fusion polypeptide is a CasX fusion polypeptide of any one of claims 13-18.

49. The kit of claim 48, wherein the CasX polypeptide comprises an amino acid sequence having 70% or 85% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3.

50. A sterile container comprising a CasX system comprising:

a) a CasX polypeptide and a CasX single guide RNA;

b) a CasX polypeptide, a CasX guide RNA, and a DNA donor template;

c) a CasX fusion polypeptide and a CasX guide RNA;

d) a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

e) an mRNA encoding a CasX polypeptide, and a CasX single guide RNA;

f) an mRNA encoding a CasX polypeptide; a CasX guide RNA, and a DNA donor template;

g) an mRNA encoding a CasX fusion polypeptide, and a CasX guide RNA;

h) an mRNA encoding a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and a DNA donor template,

wherein the CasX polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasX polypeptide is capable of forming a complex with the CasX guide RNA, and wherein the complex is capable of binding the target nucleic acid; and

wherein the CasX fusion polypeptide is a CasX fusion polypeptide of any one of claims 13-18.

51. An implantable device comprising a CasX system comprising:

a) a CasX polypeptide and a CasX single guide RNA;

b) a CasX polypeptide, a CasX guide RNA, and a DNA donor template;

c) a CasX fusion polypeptide and a CasX guide RNA;

d) a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

e) an mRNA encoding a CasX polypeptide, and a CasX single guide RNA;

f) an mRNA encoding a CasX polypeptide; a CasX guide RNA, and a DNA donor template;

g) an mRNA encoding a CasX fusion polypeptide, and a CasX guide RNA;

h) an mRNA encoding a CasX fusion polypeptide, a CasX guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and iii) a DNA donor template;
k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; and ii) a nucleotide sequence encoding a CasX guide RNA; or
l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasX fusion polypeptide; ii) a nucleotide sequence encoding a CasX guide RNA; and a DNA donor template,
wherein the CasX polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;
wherein the CasX guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasX polypeptide is capable of forming a complex with the CasX guide RNA, and wherein the complex is capable of binding the target nucleic acid; and
wherein the CasX fusion polypeptide is a CasX fusion polypeptide of any one of claims 13-18.