CLAIMS

~~What is claimed is:~~

1. A CRISPR-associated protein comprising a polypeptide ~~encoding~~comprising a variant of ~~AsCpf1~~Cas12a, wherein the variant of ~~AsCpf1~~Cas12a is selected from the group consisting of ~~M537R (~~SEQ ID NOs.: 472~~)~~, ~~F870L (SEQ ID NO.:~~ 473~~),~~ and ~~M537R/F870L (SEQ ID NO.:~~ 465~~)~~.

2. The CRISPR-associated protein according to claim 1, wherein the variant of ~~AsCpf1~~Cas12a is ~~M537R (~~SEQ ID NO.: 472~~)~~.

3. The CRISPR-associated protein according to claim 1, wherein the variant of ~~AsCpf1~~Cas12a is ~~F870L (~~SEQ ID NO.: 473~~)~~.

4. The CRISPR-associated protein according to claim 1, wherein the variant of ~~AsCpf1~~Cas12a is ~~M537R/F870L (~~SEQ ID NO.: 465~~)~~.

5. A CRISPR ribonucleoprotein complex, comprising:
a guide RNA; and
a CRISPR-associated protein comprising a polypeptide ~~encoding~~comprising a variant of ~~AsCpf1~~Cas12a, wherein the variant of ~~AsCpf1~~Cas12a is selected from the group consisting of ~~M537R (~~SEQ ID NOs.: 472~~), F870L (SEQ ID NO.:~~ 473~~),~~ and ~~M537R/F870L (SEQ ID NO.:~~ 465~~)~~.

6. The CRISPR ribonucleoprotein complex of claim 5, wherein the variant of ~~AsCpf1~~Cas12a is ~~M537R (~~SEQ ID NO.: 472~~)~~.

7. The CRISPR ribonucleoprotein complex of claim 5, wherein the variant of ~~AsCpf1~~Cas12a is ~~F870L (~~SEQ ID NO.: 473~~)~~.

8. The CRISPR ribonucleoprotein complex of claim 5, wherein the variant of ~~AsCpf1~~Cas12a is ~~M537R/F870L (~~SEQ ID NO.: 465~~)~~.

9. An *in vitro* method of increasing efficiency of gene editing at TTT**N** PAM sites in a cell with a CRISPR ribonucleoprotein complex, comprising:
contacting a cell with the CRISPR ribonucleoprotein complex according to any one of

claims 5-8.

10. The method according to claim 9, wherein the TTT**N** PAM sites consists of one selected form the group of TTTA, TTTT and TTTC PAM sites.

11. A kit comprising a guide RNA and a CRISPR-associated protein comprising a polypeptide ~~encoding~~comprising a variant of ~~AsCpf1~~Cas12a, wherein the variant of ~~AsCpf1~~Cas12a is selected from the group consisting of ~~M537R (~~SEQ ID NOs.: 472~~)~~, ~~F870L (SEQ ID NO.:~~ 473~~)~~, and ~~M537R/F870L (SEQ ID NO.:~~ 465~~)~~.

12. The kit according to claim 11, wherein the variant of ~~AsCpf1~~Cas12a is ~~M537R (~~SEQ ID NO.: 472~~)~~.

13. The kit according to claim 11, wherein the variant of ~~AsCpf1~~Cas12a is ~~F870L (~~SEQ ID NO.: 473~~)~~.

14. The kit according to claim 11, wherein the variant of ~~AsCpf1~~Cas12a is ~~M537R/F870L (~~SEQ ID NO.: 465~~)~~.

15. A CRISPR-associated protein comprising a polypeptide ~~encoding~~comprising a variant of ~~As~~Cas12a, wherein the variant of ~~As~~Cas12a is selected from the group consisting of ~~at least one variant amino acid selected from amino acid positions 499-640 and 840-913, provided that the variant AsCas12a provides an improvement in CRISPR/AsCas12a-associated nuclease activity at non-canonical TTTT PAM sites.~~

~~16. The~~A ~~CRISPR-associated protein according to claim 15, wherein the variant of AsCas12a is selected from the group consisting of~~ SEQ ID NOs.: 59-245, provided that the variant Cas12a provides an improvement in CRISPR/Cas12a-associated nuclease activity at non-canonical TTTT PAM sites.

1~~7~~6. The CRISPR-associated protein according to claims 15 ~~or 16~~, wherein the variant of ~~As~~Cas12a further comprises mutations of ~~M537R/F870L (~~SEQ ID NO.: 465~~)~~.

1~~8~~7. A CRISPR ribonucleoprotein complex, comprising:

a guide RNA; and

a CRISPR-associated protein comprising a polypeptide ~~encoding~~comprising a variant of ~~As~~Cas12a, wherein the variant of ~~As~~Cas12a is selected from the group consisting of ~~at least one variant amino acid selected from amino acid positions 499-640 and 840-913, provided that the variant AsCas12a provides an improvement in CRISPR/AsCas12a-associated nuclease activity at non-canonical TTTT PAM sites.~~

~~19. The CRISPR ribonucleoprotein complex of claim 18, wherein the variant of AsCas12a is selected from the group consisting of~~ SEQ ID NOs.: 59-245, provided that the variant Cas12a provides an improvement in CRISPR/Cas12a-associated nuclease activity at non-canonical TTTT PAM sites.

~~20~~18. The CRISPR ribonucleoprotein complex of claim~~s~~ 1~~8~~7 ~~or 19~~, wherein the variant of ~~As~~Cas12a further comprises mutations of ~~M537R/F870L (~~SEQ ID NO.: 465~~)~~.

~~2~~19. An *in vitro* method of increasing efficiency of gene editing at non-canonical TTTT PAM sites in a cell with a CRISPR ribonucleoprotein complex, comprising:

contacting a cell with the CRISPR ribonucleoprotein complex according to ~~any of~~ claim~~s~~ 17 or 18 ~~20~~.

2~~2~~0. A kit comprising a guide RNA and a CRISPR-associated protein comprising a polypeptide ~~encoding~~comprising a variant of ~~As~~Cas12a, wherein the variant of ~~As~~Cas12a is selected from the group consisting of ~~at least one variant amino acid selected from amino acid positions 499-640 and 840-913, provided that the variant AsCas12a provides an improvement in CRISPR/AsCas12a-associated nuclease activity at non-canonical TTTT PAM sites.~~

~~23. The kit according to claim 22, wherein the variant of AsCas12a is selected from the group consisting of~~ SEQ ID NOs.: 59-245, provided that the variant Cas12a provides an improvement in CRISPR/Cas12a-associated nuclease activity at non-canonical TTTT PAM sites.

2~~4~~1. The kit according to claims 20~~2 or 23~~, wherein the variant of ~~As~~Cas12a further comprises mutations of ~~M537R/F870L (~~SEQ ID NO.: 465~~)~~.

2~~5~~2. A nucleic acid encoding a CRISPR-associated protein comprising a polypeptide ~~encoding~~comprising a variant of ~~As~~Cas12a, wherein the variant of ~~As~~Cas12a

is selected from the group consisting of ~~at least one variant amino acid selected from amino acid positions 499-640 and 840-913, provided that the variant AsCas12a provides an improvement in CRISPR/AsCas12a-associated nuclease activity at non-canonical TTTT PAM sites.~~

~~26. The nucleic acid of claim 25, wherein the variant of AsCas12a is selected from the group consisting of~~ SEQ ID NOs.: 59-245, provided that the variant Cas12a provides an improvement in CRISPR/Cas12a-associated nuclease activity at non-canonical TTTT PAM sites.

2~~7~~3. The nucleic acid of claim~~s 25 or~~ 26, wherein the variant of ~~As~~Cas12a further comprises mutations of ~~M537R/F870L (~~SEQ ID NO.: 465~~)~~.

2~~8~~4. The nucleic acid of claim~~s~~ 2~~2~~5~~-~~ or 2~~7~~3, wherein the nucleic acid is operably linked to suitable transcription elements to express the nucleic acid.

2~~9~~5. The nucleic acid of any one of claims 2~~5~~2-2~~8~~4, wherein the nucleic acid is DNA or RNA.

~~30~~26. A polynucleotide ~~sequence~~ encoding a Cas12a polypeptide, comprising one member selected from the group consisting of SEQ ID NOs~~.~~: 5-17.

~~31~~27. A~~n amino acid sequence encoding a~~ Cas12a polypeptide, comprising one member selected from the group consisting of SEQ ID NOs.: 18-30.

~~32~~8. A CAS endonuclease system comprising an expression cassette ~~encoding~~comprising a polynucleotide ~~sequence~~ encoding a Cas12a polypeptide, comprising one member selected from the group consisting of SEQ ID NOs~~.~~: 5-17.

~~33~~29. A CAS endonuclease system comprising an amino acid sequence ~~encoding~~comprising a Cas12a polypeptide, comprising one member selected from the group consisting of SEQ ID NOs.: 18-30.

34~~0~~. An *in vitro* method of performing genome editing in a eukaryotic cell, comprising:

introducing an CAS endonuclease system into the eukaryotic cell, said CAS endonuclease system comprising an expression cassette ~~encoding~~comprising a

polynucleotide ~~sequence~~ encoding a Cas12a polypeptide~~, comprising one member~~ selected from the group consisting of SEQ ID NOs.: 5-17.

3~~5~~1. A*n in vitro* method of performing genome editing in a eukaryotic cell, comprising:

introducing an CAS endonuclease system into the eukaryotic cell, said CAS endonuclease system comprising ~~an amino acid sequence encoding~~ a Cas12a polypeptide ~~comprising one member~~ selected from the group consisting of SEQ ID NOs.: 18-30.

3~~6~~2. A CRISPR-associated protein comprising a fusion polypeptide ~~encoding~~comprising ~~an~~ ~~As~~Cas12a open reading frame, a nuclear localization signal, optionally an amino acid linker and optionally an affinity tag~~.~~

~~37. The CRISPR-associated protein of claim 36~~, wherein the ~~As~~Cas12a open reading frame is selected from the group consisting of SEQ ID NOs.: 59-245.

3~~8~~3. ~~The~~A CRISPR-associated protein ~~of claim 36~~comprising a fusion polypeptide comprising a Cas12a open reading frame, a nuclear localization signal, optionally an amino acid linker and optionally an affinity tag, wherein the Cas12a open reading frame is selected from the group consisting of SEQ ID Nos.: 59-245 and wherein the nuclear localization signal is selected from SEQ ID NOs.: 475, 477, 479, 481 and 483.

3~~9~~4. ~~The~~A CRISPR-associated protein ~~of claim 36~~comprising a fusion polypeptide comprising a Cas12a open reading frame, a nuclear localization signal, optionally an amino acid linker and optionally an affinity tag, wherein the CRISPR-associated protein is ~~encoded by~~selected from SEQ ID NOs.: 488-491.

~~40~~35. ~~The~~A CRISPR-associated protein ~~of claim 36~~comprising a fusion polypeptide comprising a Cas12a open reading frame, a nuclear localization signal, optionally an amino acid linker and optionally an affinity tag, wherein the CRISPR-associated protein is selected from SEQ ID NOs.: 492 and 493.

~~41~~36. A*n in vitro* method of performing genome editing in a eukaryotic cell, comprising:

introducing an CAS endonuclease system into the eukaryotic cell, said CAS endonuclease system comprising a CRISPR-associated protein of any one of claims 362-4035.