CLAIMS

What is claimed is:

1. A fusion protein comprising:

a first fragment comprising a nucleobase deaminase or a catalytic domain thereof,

a second fragment comprising a nucleobase deaminase inhibitor, and

a protease cleavage site between the first fragment and the second fragment.

2. The fusion protein of claim 1, wherein the nucleobase deaminase is an adenosine deaminase, which is preferably selected from the group consisting of tRNA-specific adenosine deaminase (TadA), adenosine deaminase tRNA specific 1 (ADAT1), adenosine deaminase tRNA specific 2 (ADAT2), adenosine deaminase tRNA specific 3 (ADAT3), adenosine deaminase RNA specific B1 (ADARB1), adenosine deaminase RNA specific B2 (ADARB2), adenosine monophosphate deaminase 1 (AMPD1), adenosine monophosphate deaminase 2 (AMPD2), adenosine monophosphate deaminase 3 (AMPD3), adenosine deaminase (ADA), adenosine deaminase 2 (ADA2), adenosine deaminase like (ADAL), adenosine deaminase domain containing 1 (ADAD1), adenosine deaminase domain containing 2 (ADAD2), adenosine deaminase RNA specific (ADAR) and adenosine deaminase RNA specific B1 (ADARB1).

3. The fusion protein of claim 1, wherein the nucleobase deaminase is a cytidine deaminase, which is preferably selected from the group consisting of APOBEC3B (A3B), APOBEC3C (A3C), APOBEC3D (A3D), APOBEC3F (A3F), APOBEC3G (A3G), APOBEC3H (A3H), APOBEC1 (A1), APOBEC3 (A3), APOBEC2 (A2), APOBEC4 (A4) and AICDA (AID).

4. The fusion protein of claim 3, wherein the catalytic domain is mouse A3 cytidine deaminase domain 1 (CDA1) or human A3B cytidine deaminase domain 2 (CDA2).

5. The fusion protein of any one of claims 1-4, wherein the nucleobase deaminase inhibitor is an inhibitory domain of a nucleobase deaminase, preferably an inhibitory domain of a cytidine deaminase, wherein the nucleobase deaminase inhibitor preferably comprises an amino acid sequence selected from SEQ ID NO: 1-2 and 48-135, or an amino acid sequence having at least 85% sequence identity to any of the amino acid sequence selected from SEQ ID NO: 1-2 and 48-135, and wherein more preferably the nucleobase

deaminase inhibitor comprises the amino acid sequence of SEQ ID NO:1, amino acids residues AA128-AA223 of SEQ ID NO:1 or the amino acid sequence of SEQ ID NO:2.

6. A fusion protein comprising:

a first fragment comprising first nucleobase deaminase or a catalytic domain thereof, and

a second fragment comprising an inhibitory domain of a second nucleobase deaminase,

wherein the first nucleobase deaminase is same as or different from the second nucleobase deaminase.

7. The fusion protein of claim 5, wherein the first and second nucleobase deaminases are different, and wherein preferably each is independently selected from the group of human and mouse APOBEC3B (A3B), APOBEC3C (A3C), APOBEC3D (A3D), APOBEC3F (A3F), APOBEC3G (A3G), APOBEC3H (A3H), APOBEC1 (A1), APOBEC3 (A3), APOBEC2 (A2), APOBEC4 (A4) and AICDA (AID).

8. A fusion protein comprising a first fragment comprising:

a nucleobase deaminase or a catalytic domain thereof,

a nucleobase deaminase inhibitor,

a first RNA recognition peptide, and

a TEV protease cleavage site between the nucleobase deaminase or a catalytic domain thereof and the nucleobase deaminase inhibitor.

9. A dual guide RNA system, comprising:

a target single guide RNA comprising a first spacer having sequence complementarity to a target nucleic acid sequence proximate to a first PAM site,

a helper single guide RNA comprising a second spacer having sequence complementarity to a second nucleic acid sequence proximate to a second PAM site,

a clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) protein, and

a nucleobase deaminase,

wherein the second PAM site is from 34 to 91 bases from the first PAM site.

10. The dual guide RNA system of claim 8, wherein the second spacer is 7-23 bases in length, preferably 8-15 bases in length, and more preferably is 9-12 bases in length.

11. The dual guide RNA system of claim 8 or 9, wherein the nucleobase deaminase is provided as a fusion protein which comprises nucleobase deaminase, a nucleobase deaminase inhibitor, and a protease cleavage site between the nucleobase deaminase and the nucleobase deaminase inhibitor.

12. The dual guide RNA system of claim 11, wherein the protease cleavage site is a TEV protease cleavage site.

13. The dual guide RNA system of claim 12, further comprising a C-terminal part of TEV protease (TEVc) and an N-terminal part of TEV protease (TEVn) which, in combination, can cleave the TEV protease cleavage site.

14. The dual guide RNA system of any one of claims 10-13 for use in conducting genome editing in a cell.

15. A guide RNA comprising a scaffold which comprises, from the 5’ to 3’ direction,
a first stem loop portion,
a second stem loop portion,
a third stem loop portion, and
a fourth stem loop portion,
wherein the third stem loop comprises five base pairings within.