[0005] In some instances, engineered nucleases could possibly result in unwanted consequences (e.g. translocations, inversions and deletions) that may occur due to the induction of multiple DSB in the chromosome of a genetically-edited cell. For example, some evidence of chromosomal rearrangements including translocations, inversions and deletions have been observed following nuclease treatment (Kosicki, *et al.* (2018) *Nat Biotechnol* 36:765 and Shin, *et al.* (2017) *Nat Comm* doi:10.1038/ncomms15646), and more recently, there has been concern about induction of the p53 pathway following cleavage in some cells leading to apoptosis using the CRISPR/Cas system (Ihry, *et al.* (2018) *Nat Med* 24:939-946 and Haapaniemi, *et al.* (2018) *Nat Med* 24:927-930). Also, HDR typically is very inefficient in most eukaryotes, making gene correction difficult (Eid, *et al.* (2018) *Biochem J* 475:1955-1964).

[0006] In addition, Cas9 base editors such as AID-dCas9, APOBEC-dCas9 (e.g. APOBEC3G or APOBEC1), BE2, BE3 and BE4 (*see, e.g.,* Komor, *et al.* (2016) *Nature* 533:420-424; Komor, *et al.* (2017) *Science Advances* 3(8), eaao4774; Kim, *et al.* (2017) *Nat Biotechnol* 35(4):371-376) can exhibit a lack of specificity (see Kim, *et al.* (2019) *Nat Biotechnol* 10.1038/s41587-019-0050-1; Zuo, *et al.* (2019) *Science* DOI:10.1126/science.aav9973), rendering them unsuitable for a variety of purposes, including *in vivo* and *ex vivo* therapeutic applications.

[0007] Thus, there remains a need to accomplish genome (base) editing without inducing a double strand break and with high specificity.

SUMMARY

[0007a] In one aspect, provided herein is one or more expression vectors for expressing a system for editing an adenine or cytosine base in DNA, the system comprising: (i) an adenosine or cytidine deaminase, wherein the deaminase is a dimer comprising a first fusion protein comprising a first inactive domain of the deaminase and a first ZFP DNA-binding domain, and a second fusion protein comprising a second inactive domain of the deaminase and a second ZFP DNA-binding domain, wherein the first and second inactive domains dimerize to form an active deaminase; and (ii) a nickase fused to one or more ZFP DNA-binding domains.

[0007b] In another aspect, provided herein is one or more expression vectors for expressing a system for editing an adenine or cytosine base in DNA, the system comprising: (i) an adenosine or cytidine deaminase fused to one or more zinc finger

protein (ZFP) DNA-binding domains, and (ii) a zinc finger nuclease (ZFN) nickase, wherein the ZFN nickase is a dimer comprising a first polypeptide comprising a catalytically active nickase domain fused to a ZFP DNA-binding domain and a second polypeptide comprising a catalytically inactive nickase domain fused to another ZFP DNA-binding domain, wherein the first and second polypeptides dimerize to form an active ZFN nickase.

[0007c] In another aspect, provided herein is a kit for use in editing a base in DNA, the kit comprising the one or more expression vectors as described herein, optionally wherein the one or more expression vectors are one or more plasmid, viral, or mRNA vectors.

[0007d] In another aspect, provided herein is a cell comprising the one or more expression vectors as described herein, or a descendant of the cell wherein the cell comprises an edited base.

[0007e] In another aspect, provided herein is the one or more expression vectors as described herein for use in a method of editing a base in a target DNA in a cell, the method comprising providing to the cell said one or more expression vectors.

[0007f] In yet another aspect, provided herein is use of the cell as described herein or a descendant thereof in the manufacture of a medicament for treating a disease in a subject in need thereof, wherein said cell or descendant thereof is to be administered to the subject.

[0007g] In yet another aspect, provided herein is the cell as described herein or a descendant thereof for use in therapy.

[0008] The present disclosure provides methods and compositions to selectively edit DNA in a cell (for example, a base editor), including editing (*e.g.*, of a single base) without making a double-stranded cut in the target DNA (*e.g.*, the edited genome). Such base editors can be cytosine base editors (CBEs) which change a C:G to a T:A or adenine base editors (ABEs) which change A:T to G:C. Furthermore, because no double-stranded break is induced, there are no free DNA ends in the endogenous target and no translocations occur. Base editors as described herein can be used for gene knock out (*e.g.*, changing a regular codon into a stop codon, for instance using a cytosine base editor and/or mutating a splice acceptor site using either cytosine or adenine base editors); introducing mutations (*e.g.*, activating or repressing mutations) into a control element (*e.g.*, promoter region) of a gene;

CLAIMS

What is claimed is:

1. ~~A composition~~ One or more expression vectors for expressing a system for editing an adenine ~~(A)~~ or cytos~~id~~ine ~~(C)~~ base in DNA, the system~~composition~~ comprising:

(i) an adenosine or cytidine deaminase, ~~at least one zinc finger protein (ZFP) DNA-binding domain;~~

~~at least one DNA-destabilizing molecule;~~

~~and at least one adenine or cytosine deaminase;~~

~~wherein the composition does not make a double-stranded cut in the DNA.~~

wherein the deaminase is a dimer comprising

a first fusion protein comprising a first inactive domain of the deaminase and a first ZFP DNA-binding domain, and

a second fusion protein comprising a second inactive domain of the deaminase and a second ZFP DNA-binding domain,

wherein the first and second inactive domains dimerize to form an active deaminase; and

(ii) a nickase fused to one or more ZFP DNA-binding domains.

2. One or more expression vectors for expressing a system for editing an adenine or cytosine base in DNA, the system comprising:

(i) an adenosine or cytidine deaminase fused to one or more zinc finger protein (ZFP) DNA-binding domains, and

(ii) a zinc finger nuclease (ZFN) nickase, wherein the ZFN nickase is a dimer comprising

a first polypeptide comprising a catalytically active nickase domain fused to a ZFP DNA-binding domain and

a second polypeptide comprising a catalytically inactive nickase domain fused to another ZFP DNA-binding domain,

wherein the first and second polypeptides dimerize to form an active ZFN nickase.

3. The one or more expression vectors of claim 1, wherein

the second fusion protein comprises a catalytically active or inactive ZFN nickase domain, and

the system further comprises a third fusion protein comprising a catalytically inactive or active ZFN nickase domain and a third ZFP DNA-binding domain,

wherein the second and third fusion proteins form an active ZFN nickase through dimerization of the catalytically active and inactive ZFN nickase domains.

4. The one or more expression vectors of any one of claims 1-3, wherein the system further comprises a uracil DNA glycosylase inhibitor (UGI), optionally wherein the UGI is at the N- or C-terminal end of the fusion protein(s).

5. The one or more expression vectors of any one of claims 1-4, wherein the expression vector(s) are viral vectors, plasmid vectors(s), or mRNA vector(s).

6. The one or more expression vectors of claim 5, wherein the expression vector(s) is an adeno-associated viral (AAV) vector.

7. A kit for use in editing a base in DNA, the kit comprising the one or more expression vectors of any one of claims 1-6, optionally wherein the one or more expression vectors are one or more plasmid, viral, or mRNA vectors.

~~2. The composition of claim 1, wherein the DNA-destabilizing molecule is a Cas9 nickase or a Cas9 protein operably linked to a single guide RNA (sgRNA).~~

~~3. The composition of claim 1, wherein the composition does not comprise a Cas9 protein.~~

~~4. The composition of any of the preceding claims, wherein the DNA-destabilizing molecule is a zinc finger nuclease (ZFN) nickase.~~

~~5. The composition of any of the preceding claims, wherein the at least one ZFP DNA-binding domain is operably linked to the Cas9 nickase or an inactivated Cas9 protein (dCas9).~~

~~6. The composition of any of the preceding claims, comprising first and second ZFP DNA-binding domains, wherein the first ZFP DNA-binding domain is operably linked to the Cas9 nickase.~~

~~7. The composition of any of the preceding claims, wherein the adenine or cytosine deaminase is comprised of first and second inactive domains that dimerize to form an active adenine or cytosine deaminase.~~

~~8. The composition of any of the preceding claims, wherein the first inactive domain of the adenine or cytosine deaminase is operably linked to the Cas9 nickase and the second inactive domain of the adenine or cytosine deaminase is operably linked to the second ZFP DNA-binding domain.~~

~~9. The composition of claim 2, wherein the adenine or cytosine deaminase and the ZFP DNA-binding domain are operably linked to the Cas9 nickase.~~

~~10. The composition of claim 2, comprising first and second ZFP DNA-domains, the first ZFP operably linked to the Cas9 nickase and the second ZFP DNA-binding domain operably linked to the adenine or cytosine deaminase.~~

~~11. The composition of any of the preceding claims, wherein the at least one ZFP DNA-binding domain is operably linked to the adenine or cytosine deaminase.~~

~~12. The composition of any of the preceding claims, further comprising a ZFN nickase.~~

~~13. The composition of any of the preceding claims, wherein the DNA destabilizing factor comprises at least one protein and/or at least one nucleotide.~~

~~14. The composition of claim 13, wherein the DNA-destabilizing factor comprises a protein.~~

~~15. The composition of claim 14, wherein the DNA-destabilizing factor comprises a Cas protein and/or a protein as shown in Table A.~~

~~16. The composition of claim 13, wherein the DNA-destabilizing nucleotide comprises an oligonucleotide.~~

~~17. The composition of claim 16, wherein the oligonucleotide comprises a peptide nucleic acid (PNA); a locked nucleic acid (LNA) and/or a bridged nucleic acid (BNA).~~

~~18. The composition of claim 17, wherein the PNA comprises: N-Lys-Lys-Lys-NNNNNNNNNNN-OOO-NNNNNNNNNNN-Lys-Lys-Lys-C; N-Lys-Lys-Lys-NNNNNNNNNNN-OOO-NNNNNNNNNNNNNNN-Lys-Lys-Lys-C; N-Lys-Lys-Lys-NNNNNNNNNNN-OOO-NNNNNNNNNNN-Lys-Lys-Lys-C; N-Lys-Lys-Lys-NNNNNNNNNNN-OOO-NNNNNNNNNNN-Lys-Lys-Lys-C; and/or N-Lys-Lys-Lys-NNNNNNNNNNNNNNN-Lys-Lys-Lys-C, where O indicates 8-amino-2,6-dioxaoctanoic acid linkers; C indicates cytosine and the Lys residues are optional.~~

~~19. The composition of claim 17, wherein the LNA comprises: 5'-NnNnNnNnNnNnNnNtctctnNnNnNnNnNnNnNnNnnNnnNnnNnnNn-3' (SEQ ID NO:1); 5'-N*n*NnNnNnNnNnNnNtctctnNnNnNnNnNnNnNnNnnNnnNnnNnn*N*n-3' (SEQ~~

~~ID NO:69); and/or 5'-~~

~~NnNnNnNnNnNnNnNtctctnNnNnNnNnNnNnNnNnnNnnNnnNnnNn-Chol-TEG-3' (SEQ ID NO:70), where LNA nucleotides are shown in uppercase; DNA nucleotides are in lower case; "*" indicates phosphorothioate bonds.~~

~~20. One or more polynucleotides encoding one or more compositions according to any of the preceding claims.~~

821. A cell comprising the one or more expression vectors ~~compositions according to any~~ of any one of claims 1-6 ~~to 19 or the one or more polynucleotides according to claim 20~~, or a descendant of the cell wherein the cell comprises an edited base.

922. The one or more expression vectors of any one of claims 1-6 for use in a~~A~~ method of editing a base in a target DNA in a cell, the method comprising providing to the cell said one or more expression vectors~~a composition according to any of claims 1 to 19 or the one or more polynucleotides of claim 20~~.

1023. The one or more expression vectors for use ~~method~~ of claim 922, wherein

(A) the editing comprises:

(i) editing a cytoidsine base ~~("C")~~ to a uracil base ~~("U")~~, optionally wherein the uracil base~~U~~ is replaced with a thym~~id~~ine base ~~("T")~~ during DNA replication, or~~;~~

~~(ii)~~ editing an adenine base ~~("A")~~ to a~~n~~ hypoxanthine base~~inosine ("I")~~, optionally wherein the hypoxanthine base~~I~~ is replaced with a guanine base ~~("G")~~ during replication; ~~and/~~or

~~(iii)~~(ii) ~~(iii) editing a CA or AC dinucleotide to a UI or an IU.~~

~~24.~~ ~~(B)~~ ~~The method of claim 22, wherein~~ the editing in the cell results in:

(i) changing a C:G base pair to a~~n~~ T:A base pair~~,~~;

~~(ii) changing a C:G base pair to a G:C base pair;~~

~~(iii)~~(ii) changing an A:T base pair to a G:C base pair~~,~~;

~~(iv)~~(iii)introduction of a stop codon~~,~~; and/or

~~(v)~~(iv) editing or creating a splicing sequence.

11~~25~~. The one or more expression vectors for use of claim 9 or 10~~method of claim 24~~, wherein the editing corrects a disease mutation~~, .~~optionally

~~26. The method of claim 22,~~ wherein an exon is edited.

~~27. A kit for use in editing a base in a target DNA, the kit comprising one or more polynucleotides of claim 20.~~

12~~28~~. The one or more expression vectors for use of any one of claims 9-11, ~~Use of one or more compositions according to any of claims 1 to 19 or the one or more polynucleotides of claim 20 for editing a target DNA molecule in an isolated cell or a subject, optionally~~ wherein the base ~~DNA~~ is in a chromosome or an extrachromosomal episome in the cell, optionally wherein the cell is a cell in or obtained from a subject in need of the editing~~ or the subject is edited~~.

13. Use of the cell of claim 8 or a descendant thereof in the manufacture of a medicament for treating a disease in a subject in need thereof, wherein said cell or descendant thereof is to be administered to the subject.

~~29. The use of claim 28, wherein the editing comprises:~~

~~(iv) editing a cytidine base ("C") to a uracil base ("U"), optionally wherein the U is replaced with a thymidine base ("T") during DNA replication;~~

~~(v) editing an adenine base ("A") to an inosine ("I"), optionally wherein the I replaced with a guanine base ("G") during replication; and/or~~

~~(vi) editing a CA or AC dinucleotide to a UI or an IU.~~

~~30. The use of claim 28 or claim 29, wherein the editing in the cell or subject results in:~~

~~(vi) changing a C:G base pair to an T:A base pair;~~

~~(vii) changing a C:G base pair to a G:C base pair;~~

~~(viii) changing an A:T base pair to a G:C base pair;~~

~~(ix) introduction of a stop codon; and/or~~

~~(x) editing or creating a splicing sequence.~~

~~31. The use of any of claims 28 to 30, wherein an exon is edited.~~

14. The cell of claim 8 or a descendant thereof for use in therapy.