[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. 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.

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.

8. A cell comprising the one or more expression vectors of any one of claims 1-6, or a descendant of the cell wherein the cell comprises an edited base.

9. The one or more expression vectors of any one of claims 1-6 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.

10. The one or more expression vectors for use of claim 9, wherein

(A) the editing comprises:

(i) editing a cytosine base to a uracil base, optionally wherein the uracil base is replaced with a thymine base during DNA replication, or

(ii) editing an adenine base to a hypoxanthine base, optionally wherein the hypoxanthine base is replaced with a guanine base during replication; or

(B) the editing in the cell results in:

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

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

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

(iv) editing or creating a splicing sequence.

11. The one or more expression vectors for use of claim 9 or 10, wherein the editing corrects a disease mutation, optionally wherein an exon is edited.

12. The one or more expression vectors for use of any one of claims 9-11, wherein the base 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.

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.

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