mediated by cis acting elements in the 3' UTR (Mignone *et al* (2002) *Genome Biol* 3(3): PMCID: PMC139023).

**[0009]** However, there remains a need for additional methods and compositions to engineered nuclease cleavage systems to provide enhanced transcription/translation efficiency and to increase nuclease activity and/or specificity.

## SUMMARY

**[0010]** The present disclosure provides methods and compositions to increase expression of an artificial nuclease, as well as increase the efficiency (activity) and/or specificity of a nuclease (*e.g.,* nuclease pair) for its intended target. Thus, described herein are polynucleotides (*e.g.,* DNA expression vector or mRNA) for expressing artificial nucleases (*e.g.,* zinc finger nucleases (ZFNs), TALENs, CRISPR/Cas nucleases) comprising engineered promoters comprising elements in the 5' and/or 3' untranslated sequences that enhance expression of the artificial nucleases. Optionally, the nuclease-encoding polynucleotides further comprise sequences encoding small peptides (including but not limited to polycationic peptides such as peptide tags and/or nuclear localization sequences), and/or comprise mutations in one or more of the DNA binding domain regions (*e.g.,* the backbone of a zinc finger protein or TALE) and/or one or more mutations in a *FokI* nuclease cleavage domain or cleavage half domain. When these polynucleotide components are used individually or in any combination (*e.g.,* peptide sequence such as FLAG (*e,g,,* 3X FLAG), NLS, WPRE and/or poly A signal in any combination), the methods and compositions of the disclosure provide surprising and unexpected increases in expression of artificial nucleases with increased efficiency of cleavage and/or targeted integration of a transgene (*e.g.,* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more fold cleavage as compared to nucleases without the sequences/modifications described herein) *in vitro* or *in vivo* and/or targeting specificity (reduced off-target effects). The disclosure also provides methods of using these compositions for targeted cleavage of cellular chromatin in a region of interest and/or integration of a transgene via targeted integration at a predetermined region of interest in cells.

**[0010a]** In one aspect, the present disclosure refers to a zinc finger nuclease (ZFN) comprising first and second ZFNs, the first ZFN comprising the ZFN designated 71557 comprising a zinc finger protein (ZFP) comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|
| QSGNLAR (SEQ ID NO: 19) | LKQNLCM (SEQ ID NO: 15) | WQSNLQN (SEQ ID NO: 20) | TSGNLTR (SEQ ID NO: 17) | RRSHLTS (SEQ ID NO: 21) |

And the second ZFN comprising the ZFN designated 71728 comprising a ZFP comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| QSSDLSR (SEQ ID NO: 30) | LKHNLLT (SEQ ID NO: 25) | DQSNLRA (SEQ ID NO: 31) | RNFSLTM (SEQ ID NO: 27) | LRHDLER (SEQ ID NO: 32) | HRSNLNK (SEQ ID NO: 29) |

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**[0010b]**In another aspect, the present disclosure refers to one or more polynucleotides encoding the first and second ZFNs as disclosed herein, optionally wherein the one or more polynucleotides are carried on AAV vectors.

**[0010c]** In another aspect, the present disclosure refers to one or more polynucleotides encoding the first and second ZFNs as disclosed herein, wherein the one or more polynucleotides are carried on AAV vectors, wherein a first polynucleotide encoding the first ZFN comprises an AAV vector comprising the sequences as shown in Table 4 and a second polynucleotide encoding the second ZFN comprises an AAV vector comprising the sequences as shown in Table 5.

**[~~0010c~~0010d]** In another aspect, the present disclosure refers to an AAV vector as disclosed herein, wherein the AAV vector encoding the first ZFN comprises the sequence shown in SEQ ID NO:43.

**[~~0010d~~0010e]** In another aspect, the present disclosure refers to a cell comprising one or more ZFNs, one or more polynucleotides, and/or one or more AAV vectors as disclosed herein, optionally a stem cell or precursor cell.

**[~~0010e~~0010f]** In another aspect, the present disclosure refers to a pharmaceutical composition comprising one or more ZFNs, one or more polynucleotides, one or more AAV vectors and/or one or more cells as disclosed herein.

**[~~0010f~~0010g]** In another aspect, the present disclosure refers to the one or more ZFNs, one or more polynucleotides, one or more AAV vectors, one or more cells and/or one or more pharmaceutical composition as disclosed herein for use in therapy, wherein the one or more ZFNs, one or more polynucleotides, one or more AAV vectors, one or more cells and/or one or more pharmaceutical composition as disclosed herein cleaves an endogenous albumin gene in a subject.

**[~~0010g~~0010h]** In another aspect, the present disclosure refers to use of one or more ZFNs, one or more polynucleotides, one or more AAV vectors, one or more cells and/or one or more pharmaceutical composition as disclosed herein in the manufacture of a medicament for treatment or prevention of a genetic disease~~cleaving an endogenous albumin gene in a subject~~.

**[~~0010h~~0010i]** In another aspect, the present disclosure refers to a kit comprising one or more ZFNs, one or more polynucleotides, one or more AAV vectors, one or more cells and/or one or more pharmaceutical compositions as disclosed herein.

**[~~0010i~~0010j]** In another aspect, the present disclosure refers to a composition comprising: (a) a first polynucleotide encoding a first ZFN designated 71557 comprising a zinc finger protein (ZFP) comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|
| QSGNLAR (SEQ ID NO: 19) | LKQNLCM (SEQ ID NO: 15) | WQSNLQN (SEQ ID NO: 20) | TSGNLTR (SEQ ID NO: 17) | RRSHLTS (SEQ ID NO: 21) |

, the first polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 4 or SEQ ID NO:43; (b) a second polynucleotide encoding a second ZFN designated 71728 comprising a ZFP comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| QSSDLSR (SEQ ID NO: 30) | LKHNLLT (SEQ ID NO: 25) | DQSNLRA (SEQ ID NO: 31) | RNFSLTM (SEQ ID NO: 27) | LRHDLER (SEQ ID NO: 32) | HRSNLNK (SEQ ID NO: 29) |

, the second polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 5 or SEQ ID NO:56; and (c) a donor polynucleotide comprising a sequence encoding a Factor IX (FIX) sequence.

**[~~0010j~~0010k]** In another aspect, the present disclosure refers to use of the composition as disclosed herein in the manufacture of a medicament for treating

hemophilia in a subject in need thereof, wherein the composition is to be administered to the subject such that the ZFN cleaves an endogenous albumin gene in the subject, the FIX sequence is integrated into the cleaved albumin gene and a FIX protein is expressed in the subject.

**[~~0010k~~0010l]** In another aspect, the present disclosure refers to use of one or more compositions as disclosed herein in the manufacture of a medicament for treating and/or preventing hemophilia in a subject in need thereof, wherein the composition is to be administered to the subject such that that a FIX protein is expressed in a cell of the subject.

**[~~0010l~~0010m]** In another aspect, the present disclosure refers to a composition comprising: (a) a first polynucleotide encoding a first ZFN designated 71557 comprising a zinc finger protein (ZFP) comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|
| QSGNLAR (SEQ ID NO: 19) | LKQNLCM (SEQ ID NO: 15) | WQSNLQN (SEQ ID NO: 20) | TSGNLTR (SEQ ID NO: 17) | RRSHLTS (SEQ ID NO: 21) |

, the first polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 4 or SEQ ID NO:43; (b) a second polynucleotide encoding a second ZFN designated 71728 comprising a ZFP comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| QSSDLSR (SEQ ID NO: 30) | LKHNLLT (SEQ ID NO: 25) | DQSNLRA (SEQ ID NO: 31) | RNFSLTM (SEQ ID NO: 27) | LRHDLER (SEQ ID NO: 32) | HRSNLNK (SEQ ID NO: 29) |

, the second polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 5 or SEQ ID NO:56; and (c) a donor polynucleotide comprising a sequence encoding an iduronate-2-sulfatase (IDS) sequence.

**[~~0010m~~0010n]** In another aspect, the present disclosure refers to use of the composition as disclosed herein in the manufacture of a medicament for treating Mucopolysaccharidosis II (MPS II) disease in a subject in need thereof, wherein the composition is to be administered to the subject such that the ZFN cleaves an endogenous albumin gene in the subject, the IDS sequence is integrated into the cleaved albumin gene and an IDS protein is expressed in the subject.

**[~~0010n~~0010o]** In another aspect, the present disclosure refers to use of one or more compositions as disclosed herein in the manufacture of a medicament for treating and/or preventing MPS II disease in a subject in need thereof, wherein the composition is to be such that that an IDS protein is expressed in a cell of the subject.

**[0010p]** In another aspect, the present disclosure refers to a composition comprising: (a) a first polynucleotide encoding a first ZFN designated 71557 comprising a zinc finger protein (ZFP) comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|
| QSGNLAR (SEQ ID NO: 19) | LKQNLCM (SEQ ID NO: 15) | WQSNLQN (SEQ ID NO: 20) | TSGNLTR (SEQ ID NO: 17) | RRSHLTS (SEQ ID NO: 21) |

, the first polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 4 or SEQ ID NO:43; (b) a second polynucleotide encoding a second ZFN

designated 71728 comprising a ZFP comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| QSSDLSR (SEQ ID NO: 30) | LKHNLLT (SEQ ID NO: 25) | DQSNLRA (SEQ ID NO: 31) | RNFSLTM (SEQ ID NO: 27) | LRHDLER (SEQ ID NO: 32) | HRSNLNK (SEQ ID NO: 29) |

, the second polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 5 or SEQ ID NO:56; and (c) a donor polynucleotide comprising a sequence encoding an alpha-L iduronidase (IDUA) sequence.

**[0010q]** In another aspect, the present disclosure refers to use of the composition as disclosed herein in the manufacture of a medicament for treating Mucopolysaccharidosis type I (MPS I) disease, wherein the composition is to be administered to a subject in need thereof such that the ZFN cleaves an endogenous albumin gene in the subject, the IDUA sequence is integrated into the cleaved albumin gene and an IDUA protein is expressed in the subject.

**[0010r]** In another aspect, the present disclosure refers to use of one or more compositions as disclosed herein in the manufacture of a medicament for treating and/or preventing MPS I disease in a subject in need thereof, wherein the composition is to be administered to the subject such that that a IDUA protein is expressed in a cell of the subject.

**[~~0010o~~0010s]** In another aspect, the present disclosure refers to a kit comprising one or more compositions as disclosed herein.

**[0011]** Thus, described herein is a polynucleotide (mRNA, plasmid, viral vector such as AAV) encoding a nuclease (*e.g.,* ZFN, TALEN, CRISPR/Cas nuclease, etc.), the polynucleotide further comprising at least one, two, three, four, five, six, seven or eight of any combination of the following elements: (i) a sequence encoding

## CLAIMS

What is claimed is:

1. A zinc finger nuclease (ZFN) comprising a first and a second ZFN~~s~~, the first ZFN comprising the ZFN designated 71557 comprising a zinc finger protein (ZFP) comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|
| QSGNLAR (SEQ ID NO: 19) | LKQNLCM (SEQ ID NO: 15) | WQSNLQN (SEQ ID NO: 20) | TSGNLTR (SEQ ID NO: 17) | RRSHLTS (SEQ ID NO: 21) |

and the second ZFN comprising the ZFN designated 71728 comprising a ZFP comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| QSSDLSR (SEQ ID NO: 30) | LKHNLLT (SEQ ID NO: 25) | DQSNLRA (SEQ ID NO: 31) | RNFSLTM (SEQ ID NO: 27) | LRHDLER (SEQ ID NO: 32) | HRSNLNK (SEQ ID NO: 29) |

.

2. One or more polynucleotides encoding the first and second ZFNs of claim 1, optionally wherein the one or more polynucleotides are carried on AAV vectors.

3. ~~The o~~One or more polynucleotides encoding the first and second ZFNs of claim 1 ~~2~~, wherein the one or more polynucleotides are carried on AAV vectors, wherein a first polynucleotide encoding the first ZFN comprises an AAV vector comprising the sequences as shown in Table 4 and a second polynucleotide encoding the second ZFN comprises an AAV vector comprising the sequences as shown in Table 5.

4. An AAV vector of claim ~~2 or~~ 3, wherein the AAV vector encoding the first ZFN comprises the sequence shown in SEQ ID NO:43.

5. The AAV vector of claim ~~2 or~~ 3, wherein the AAV vector encoding the second ZFN comprises the sequence shown in SEQ ID NO:56.

6. A cell comprising one or more ZFNs of claim 1, one or more polynucleotides of any of claims 2 to 3, and/or one or more AAV vectors of claims 4 and 5, optionally a stem cell or precursor cell.

7. A pharmaceutical composition comprising one or more ZFNs of claim 1, one or more polynucleotides of any of claims 2 to 3, one or more AAV vectors of claims 4 and 5 and/or one or more cells of claim 6.

8. The one or more ZFNs, one or more polynucleotides, one or more AAV vectors, one or more cells and/or one or more pharmaceutical composition of any of the preceding claims for use in therapy, wherein the one or more ZFNs, one or more polynucleotides, one or more AAV vectors, one or more cells and/or one or more pharmaceutical composition of any of the preceding claims cleaves an endogenous albumin gene in a subject.

9. Use of one or more ZFNs, one or more polynucleotides, one or more AAV vectors, one or more cells and/or one or more pharmaceutical composition of any of the preceding claims in the manufacture of a medicament for treatment or prevention of a genetic disease~~cleaving an endogenous albumin gene in a subject~~.

10. The one or more ZFNs, one or more polynucleotides, one or more AAV vectors, one or more cells and/or one or more pharmaceutical composition for use of claim 8, further comprising that a donor is to be administered, optionally carried on an AAV vector, to the subject such that the donor is integrated into the cleaved albumin gene in the subject.

11. A kit comprising one or more ZFNs of claim 1, one or more polynucleotides of any of claims 2 to 3, one or more AAV vectors of claims 4 and 5, one or more cells of claim 6 and/or one or more pharmaceutical compositions according to claim 7.

12. A composition comprising:

(a) a first polynucleotide encoding a first ZFN designated 71557 comprising a zinc finger protein (ZFP) comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| QSGNLAR (SEQ ID NO: 19) | LKQNLCM (SEQ ID NO: 15) | WQSNLQN (SEQ ID NO: 20) | TSGNLTR (SEQ ID NO: 17) | RRSHLTS (SEQ ID NO: 21) |

, the first polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 4 or SEQ ID NO:43;

(b) a second polynucleotide encoding a second ZFN designated 71728 comprising a ZFP comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| QSSDLSR (SEQ ID NO: 30) | LKHNLLT (SEQ ID NO: 25) | DQSNLRA (SEQ ID NO: 31) | RNFSLTM (SEQ ID NO: 27) | LRHDLER (SEQ ID NO: 32) | HRSNLNK (SEQ ID NO: 29) |

, the second polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 5 or SEQ ID NO:56; and

(c) a donor polynucleotide comprising a sequence encoding a Factor IX (FIX) sequence.

13. The composition of claim 12, wherein the donor polynucleotide is an AAV vector comprising the sequences as shown in Table 6 or ~~, optionally~~ the sequence as shown in SEQ ID NO:59.

14. The composition of claim 12 or 13, wherein the first, second and donor polynucleotides are carried on separate AAV vectors.

15. Use of the composition of any of claims 12 to 14 in the manufacture of a medicament for treating hemophilia in a subject in need thereof, wherein the composition is to be administered to the subject such that the ZFN cleaves an endogenous albumin gene in the subject, the FIX sequence is integrated into the cleaved albumin gene and a FIX protein is expressed in the subject.

16. Use of one or more compositions of any of claims 12 to 14 in the manufacture of a medicament for treating and/or preventing hemophilia in a subject in need thereof, wherein the composition is to be administered to the subject such that that a FIX protein is expressed in a cell of the subject.

17. A kit comprising one or more compositions of any of claims 12-14.

18. A composition comprising:

(a) a first polynucleotide encoding a first ZFN designated 71557 comprising a zinc finger protein (ZFP) comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|
| QSGNLAR (SEQ ID NO: 19) | LKQNLCM (SEQ ID NO: 15) | WQSNLQN (SEQ ID NO: 20) | TSGNLTR (SEQ ID NO: 17) | RRSHLTS (SEQ ID NO: 21) |

, the first polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 4 or SEQ ID NO:43;

(b) a second polynucleotide encoding a second ZFN designated 71728 comprising a ZFP comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| QSSDLSR (SEQ ID NO: 30) | LKHNLLT (SEQ ID NO: 25) | DQSNLRA (SEQ ID NO: 31) | RNFSLTM (SEQ ID NO: 27) | LRHDLER (SEQ ID NO: 32) | HRSNLNK (SEQ ID NO: 29) |

, the second polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 5 or SEQ ID NO:56; and

(c) a donor polynucleotide comprising a sequence encoding an iduronate-2-sulfatase (IDS) sequence.

19. The composition of claim 17, wherein the donor polynucleotide is an AAV vector comprising the sequences as shown in Table 7, optionally the sequence as shown in SEQ ID NO:65.

20. The composition of claim 18 or 19, wherein the first, second and donor polynucleotides are carried on separate AAV vectors.

21. Use of the composition of any of claims 18 to 20 in the manufacture of a medicament for treating Mucopolysaccharidosis II (MPS II) disease in a subject in need thereof, wherein the composition is to be administered to the subject such that the ZFN

cleaves an endogenous albumin gene in the subject, the IDS sequence is integrated into the cleaved albumin gene and an IDS protein is expressed in the subject.

22. Use of one or more compositions of any of claims 18 to 20 in the manufacture of a medicament for treating and/or preventing MPS II disease in a subject in need thereof, wherein the composition is to be such that that an IDS protein is expressed in a cell of the subject.

23. A kit comprising one or more compositions of any of claims 18 to 20.

24. A composition comprising:

(a) a first polynucleotide encoding a first ZFN designated 71557 comprising a zinc finger protein (ZFP) comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|
| QSGNLAR (SEQ ID NO: 19) | LKQNLCM (SEQ ID NO: 15) | WQSNLQN (SEQ ID NO: 20) | TSGNLTR (SEQ ID NO: 17) | RRSHLTS (SEQ ID NO: 21) |

, the first polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 4 or SEQ ID NO:43;

(b) a second polynucleotide encoding a second ZFN designated 71728 comprising a ZFP comprising recognition helix regions as shown in the table below:

| F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| QSSDLSR (SEQ ID NO: 30) | LKHNLLT (SEQ ID NO: 25) | DQSNLRA (SEQ ID NO: 31) | RNFSLTM (SEQ ID NO: 27) | LRHDLER (SEQ ID NO: 32) | HRSNLNK (SEQ ID NO: 29) |

, the second polynucleotide optionally comprising an AAV vector comprising the sequences as shown in Table 5 or SEQ ID NO:56; and

(c) a donor polynucleotide comprising a sequence encoding an alpha-L iduronidase (IDUA) sequence.

25. The composition of claim 24, wherein the donor comprises the sequences as shown in Table 8, optionally the sequence as shown in SEQ ID NO:72.

26. The composition of claim 24 or claim 25, wherein the first, second and donor polynucleotides are carried on separate AAV vectors.

27. Use of the composition of any of claims 24 to 26 in the manufacture of a medicament for treating Mucopolysaccharidosis type I (MPS I) disease, wherein the composition is to be administered to a subject in need thereof such that the ZFN cleaves an endogenous albumin gene in the subject, the IDUA sequence is integrated into the cleaved albumin gene and an IDUA protein is expressed in the subject.

28. Use of one or more compositions of any of claims 24 to 26 in the manufacture of a medicament for treating and/or preventing MPS I disease in a subject in need thereof, wherein the composition is to be administered to the subject such that that a IDUA protein is expressed in a cell of the subject.

29. A kit comprising one or more compositions of any of claims 24 to 26.