CLAIMS

1. A vector system for use in the treatment of ~~a method of treating~~ a disease, the vector system comprising

   (a) a first vector comprising a nucleic acid sequence encoding:

      (i) a first fragment of an endonuclease,

      (ii) a first fragment of an intein, and

      (ii) a first guide RNA (gRNA); and

   (b) a second vector comprising a nucleic acid sequence encoding:

      (i) a second fragment of the endonuclease,

      (ii) a second fragment of the intein, and

      (ii) a second guide RNA (gRNA);

   wherein the first gRNA binds to a region, which is located 5' to a sequence of interest comprised in a nucleic acid sequence in the genome, preferably DNA, of a target cell,

   wherein the second gRNA binds to a region located 3' to the sequence of interest comprised in the nucleic acid sequence in the genome, preferably DNA, of a target cell;

   wherein the first fragment and the second fragment of the intein are capable of associating into a functional intein,

   wherein the functional intein is capable of ligating the first and the second fragment of the endonuclease to form a functional endonuclease;

   wherein the functional endonuclease is capable of excising the sequence of interest.

2. The vector system for use of any of the preceding claims, wherein the endonuclease is Cas9, preferably *Streptococcus pyogenes* Cas9 (SpCas9), more preferably the Cas9 comprises an amino acid sequence as set forth in SEQ ID NO: 1 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID 1.

3. The vector system for use of any of the preceding claims, wherein i) the first fragment of the endonuclease comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 3 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2 and 3, and/or ii) the second fragment of the endonuclease comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 5 or an amino acid sequence having at least

60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 4 and 5.

~~4. The vector system for use of any of the preceding claims, wherein the second fragment of the endonuclease comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 5 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 4 and 5.~~

45. The vector system for use of any of the preceding claims, wherein the intein is selected from the group consisting of i) Npu of SEQ ID 6 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID 6, ii) NrdJ-1 of SEQ ID 7 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID 7 and iii) gp-41 as shown in SEQ ID 8 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID 8.

56. The vector system for use of any of the preceding claims, wherein i) the first fragment of the intein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-11 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 9-11, and/or ii) wherein the second fragment of the intein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12-14 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 12-14.

~~7. The vector system for use of any of the preceding claims, wherein the second fragment of the intein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12-14 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 12-14.~~

68. The vector system for use of any of the preceding claims, wherein the first and/or the second vector is a viral vector, preferably wherein the viral vector is an adeno-associated virus (AAV) or lentivirus.

~~9. The vector system for use of any of the preceding claims, wherein the viral vector is an adeno-associated virus or lentivirus.~~

~~10. The vector system for use of any of the preceding claims, wherein the viral vector is an adeno-associated virus (AAV).~~

7~~11~~. The vector system for use of ~~any of the preceding~~ claim~~s~~ 6, wherein the AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, and AAV11 or any combination thereof.

~~12. The vector system for use of any of the preceding claims, wherein the AAV is AAV1, AAV5, AAV6 or AAV9, preferably AAV9.~~

8~~13~~. The vector system for use of any of the preceding claims, wherein the viral vector is coated with a dendrimer, preferably wherein the dendrimer is a PAMAM (Poly(amidoamine)), more preferably wherein the dendrimer is a 2nd generation PAMAM.

~~14. The vector system for use of any of the preceding claims, wherein the dendrimer is a PAMAM (Poly(amidoamine)).~~

~~15. The vector system for use of any of the preceding claims, wherein the dendrimer is a 2nd generation PAMAM.~~

9~~16~~. The vector system for use of any of the preceding claims, wherein the nucleic acid of the first and/or the second vector further comprises:

(iv) a nuclear localization signal, preferably comprising a sequence selected from the list consisting of SEQ ID NOs: 15-20 or an amino acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence selected from the list consisting of SEQ ID NOs: 15-20.

10~~7~~. The vector system for use of any of the preceding claims, wherein the first fragment of the nuclease and/or the second fragment of the intein and the nucleic acid(s) encoding the first and/or the second gRNA are operatively coupled to a promoter, wherein the promoter(s) ~~preferably~~ is/are inducible, preferably wherein i) the promoter that is operatively coupled to the first fragment of the nuclease and/or the second

fragment of the intein is selected from the group consisting of CBH, B29 promoter, CD14 promoter, CD43 promoter, CD45 promoter, CD68 promoter, desmin promoter, elastase-1 promoter, endoglin promoter, fibronectin promoter, Flt-1 promoter, GFAP promoter, GPIIb promoter, ICAM-2 promoter, Mb promoter, NphsI promoter, SP-B promoter, SYN1 promoter and WASP promoter; and/or ii) wherein the promotor that is operatively coupled to the first and/or the second gRNA is an RNA polymerase III promoter, further optionally selected from the group consisting of U6, H1 and 7SK.

~~18. The vector system for use of any of the preceding claims, wherein the promoter that is operatively coupled to the first fragment of the nuclease and/or the second fragment of the intein is selected from the group consisting of CBH, preferably as depicted in SEQ ID NO: 22, B29 promoter, CD14 promoter, CD43 promoter, CD45 promoter, CD68 promoter, desmin promoter, elastase-1 promoter, endoglin promoter, fibronectin promoter, Flt-1 promoter, GFAP promoter, GPIIb promoter, ICAM-2 promoter, Mb promoter, NphsI promoter, SP-B promoter, SYN1 promoter or WASP promoter.~~

~~19. The vector system for use of any of the preceding claims, wherein the promotor that is operatively coupled to the first and/or the second gRNA is an RNA polymerase III promoter, preferably selected from the list consisting of U6, H1 and 7SK, more preferably the promoter is U6.~~

11~~20~~. The vector system for use of any of the preceding claims, wherein ~~the method further comprises:~~

- the first vector is to be administered~~ing~~ to the subject~~ the first vector~~; ~~and~~
- the second vector is to be administered~~ring~~ to the subject~~ the second vector~~; and/or
- ~~.~~ the sequence of interest is excised.

~~21. The vector system for use of any of the preceding claims, wherein the method further comprises:~~

~~- excising the sequence of interest~~.

12~~22~~. The vector system for use of any of the preceding claims, wherein the first and the second vector are to be administered to the patient simultaneously or sequentially, preferably sequentially with a time delay of at least 5 minutes, at least 10 minutes, at least 15 minutes, at least 30 minutes, of at least 1 hour, at least 2 hours, at least 3

hours, at least 4 hours, at least 8 hours, at least 12 hours, at least 24 hours, at least 48 hours, at least 72 hours, at least 1 week or at least 2 weeks.

~~23. The vector system for use of any of the preceding claims, wherein the first and the second vector are administered to the patient sequentially, preferably with a time delay of at least 5 minutes, at least 10 minutes, at least 15 minutes, at least 30 minutes, of at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 8 hours, at least 12 hours, at least 24 hours, at least 48 hours, at least 72 hours, at least 1 week or at least 2 weeks.~~

13~~24~~. The vector system for use of any of the preceding claims, wherein the subject is a mammal, preferably a human or a pig~~, more preferably a human~~.

14~~25~~. The vector system for use of any of the preceding claims, wherein the first and the second vectors are to be administered systemically, enterally, parenterally, intravenously, intra-arterially, topically, intraperitoneally, intramuscularly, intradermally, intrathecally, intravitreally, subcutaneously, transdermally and/or transmucosally.

15~~26~~. The vector system for use of any of the preceding claims, wherein the disease is selected from the group consisting of Duchenne muscular dystrophy, hereditary myopathy with early respiratory failure, early-onset myopathy with fatal cardiomyopathy, core myopathy with heart disease, centronuclear myopathy, limb-girdle muscular dystrophy type 2J, familial dilated cardiomyopathy 9, hypertrophic cardiomyopathy and tibial muscular dystrophy, preferably Duchenne muscular dystrophy, more preferably characterized by a deletion of exon 52 of the dystrophin gene.

~~27. The vector system for use of any of the preceding claims, wherein the disease is a muscular disease, preferably the disease is Duchenne muscular dystrophy, preferably characterized by a deletion of exon 52 of the dystrophin gene.~~

16~~28~~. The vector system for use of any of the preceding claims, wherein i) the nucleic acid sequence of interest is exon 51 of the dystrophin gene, preferably the exon 51 comprises a sequence of 23 or 24 or a nucleic acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID 23 or 24, and/or ii) wherein the first gRNA comprises a nucleic acid

sequence as set forth in any of SEQ ID NOs: 25 or 26 or a nucleic acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID NOs: 25 or 26 and/or the second gRNA comprises a nucleic acid sequence as set forth in any of SEQ ID NOs: 27 or 28 or a nucleic acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID NOs: 27 or 28.

~~29. The vector system for use of any of the preceding claims, wherein the first gRNA comprises a nucleic acid sequence as set forth in any of SEQ ID NOs: 25 or 26 or a nucleic acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID NOs: 25 or 26 and/or the second gRNA comprises a nucleic acid sequence as set forth in any of SEQ ID NOs: 27 or 28 or a nucleic acid sequence having at least 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or 100% sequence identity to a sequence as shown in SEQ ID NOs: 27 or 28.~~

~~30. The vector system for use of any of the preceding claims, wherein the deletion of exon 51 of DMD restores the reading frame of the DMD, thereby enabling the translation of a truncated but functional DMD.~~

17~~31~~. A vector system as defined in any of the preceding claims.

18~~32~~. A first and/or a second vector as defined in any one of claims 1-16~~30~~.

~~33. A second vector as defined in any one of claims 1-30.~~

~~34. A combination of the first vector of claim 32 and the second vector of claim 33.~~

19~~35~~. A pharmaceutical composition comprising the vector system as defined in any one of claims 1-16~~30 or comprising the combination of claim 34~~.

20~~36~~. ~~A method for excising a sequence of interest from the genome, preferably DNA, of a subject, comprising the administration of t~~The vector system of claim 17~~31, the combination of claim 34~~ or the pharmaceutical composition of claim 19~~35~~ for use in the treatment of a disease, the treatment comprising ~~and thereby~~ excising ~~the~~ a sequence of interest from the genome, preferably DNA, of a subject.