

What is Claimed is:

1. A nucleic acid molecule comprising a nucleotide sequence, which encodes a miniaturized dystrophin polypeptide comprising a modified spectrin repeat 16 (R16) domain, wherein a part of the spectrin repeat 16 (R16) domain is replaced by a
5 corresponding part of a ~~different spectrin repeat domain~~.

2. ~~The nucleic acid molecule of claim 1, wherein the different spectrin repeat domain is spectrin repeat 2 (R2) domain.~~

10 3. ~~The nucleic acid molecule of claim 1 or 2, wherein the modified R16 domain comprises an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a sequence selected from the group consisting of SEQ ID NO: 68, 69, 70 and 71.~~

15 4. ~~The nucleic acid molecule of any one of claims 1 to 3, wherein the miniaturized dystrophin polypeptide comprises from N terminus to C terminus a hinge 1 (H1) domain, a spectrin repeat 1 (R1) domain, the modified R16 domain, a spectrin repeat 17 (R17) domain, a hinge 3 (H3) domain, a spectrin repeat 23 (R23) domain, a spectrin repeat 24
20 (R24) domain, and a hinge 4 (H4) domain of dystrophin.~~

5. ~~The nucleic acid molecule of claim 4, wherein (i) the H1 domain and the R1 domain are fused directly, (ii) the R1 domain and the modified R16 domain are fused directly, (iii) the modified R16 domain and the R17 domain are fused directly, (iv) the R17 domain and
25 the H3 domain are fused directly, (v) the H3 domain and the R23 domain are fused directly, (vi) the R23 domain and the R24 domain are fused directly, or (vii) the R24 domain and the H4 domain are fused directly, or (vii) any combination thereof.~~

30 6. ~~The nucleic acid molecule of any one of claims 1 to 5, wherein the miniaturized dystrophin polypeptide does not comprise a spectrin repeat 2 (R2) domain, spectrin repeat 3 (R3) domain, spectrin repeat 4 (R4) domain, spectrin repeat 5 (R5) domain, spectrin~~

repeat 6 (R6) domain, spectrin repeat 7 (R7) domain, spectrin repeat 8 (R8) domain, spectrin repeat 9 (R9) domain, spectrin repeat 10 (R10) domain, spectrin repeat 11 (R11) domain, spectrin repeat 12 (R12) domain, spectrin repeat 13 (R13) domain, spectrin repeat 14 (R14) domain, spectrin repeat 15 (R15) domain, spectrin repeat 18 (R18) domain, spectrin repeat 19 (R19) domain, spectrin repeat 20 (R20) domain, spectrin repeat 21 (R21) domain, and/or spectrin repeat 22 (R22) domain.

7. — The nucleic acid molecule of any one of claims 1 to 6, wherein the miniaturized dystrophin polypeptide further comprises an ABD1 domain and/or a CR domain.

8. — The nucleic acid molecule of claim 7, wherein the miniaturized dystrophin polypeptide consists essentially of or consists of, from N terminus to C terminus, the ABD1 domain, the H1 domain, the R1 domain, the modified R16 domain, the R17 domain, the H3 domain, the R23 domain, the R24 domain, the H4 domain, and the CR domain of dystrophin.

9. — The nucleic acid molecule of any one of claims 4 to 8, wherein the H1 domain is an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 74.

10. — The nucleic acid molecule of any one of claims 4 to 9, wherein the R1 domain is an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 75.

11. — The nucleic acid molecule of any one of claims 4 to 10, wherein the modified R16 domain is an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 76.

12. — The nucleic acid molecule of any one of claims 4 to 11, wherein the R17 domain is an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 77.

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13. — The nucleic acid molecule of any one of claims 4 to 12, wherein the H3 domain is an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 78.

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14. — The nucleic acid molecule of any one of claims 4 to 13, wherein the R23 domain is an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 79.

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15. — The nucleic acid molecule of any one of claims 4 to 14, wherein the R24 domain is an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 80.

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16. — The nucleic acid molecule of any one of claims 4 to 15, wherein the H4 domain is an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 81.

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17. — The nucleic acid molecule of any one of claims 1 to 16, wherein the miniaturized dystrophin polypeptide further comprises at the N terminus an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 73.

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18. — ~~The nucleic acid molecule of any one of claims 1 to 17, wherein the miniaturized dystrophin polypeptide further comprises at the C terminus an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 82.~~

19. — ~~The nucleic acid molecule of any one of claims 1 to 18, and wherein the miniaturized dystrophin polypeptide comprises an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence set forth as SEQ ID NO: 83.~~

20. — ~~The nucleic acid molecule of any one of claims 1 to 18, wherein the miniaturized dystrophin polypeptide comprises an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 84.~~

21. — ~~The nucleic acid molecule of any one of claims 1 to 18, wherein the miniaturized dystrophin polypeptide comprises an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 85.~~

22. — ~~The nucleic acid molecule of any one of claims 1 to 18, wherein the miniaturized dystrophin polypeptide comprises an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 86.~~

23. — ~~The nucleic acid molecule of any one of claims 1 to 18, wherein the miniaturized dystrophin polypeptide comprises an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 87.~~

24. — The nucleic acid molecule of any one of claims 1 to 23, wherein the miniaturized dystrophin polypeptide exhibits a higher expression of the miniaturized dystrophin polypeptide than BXA-212372 (SEQ ID NO: 88).

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25. — The nucleic acid molecule of claim 24, wherein the miniaturized dystrophin polypeptide expression is at least about 1.5 fold, at least about 1.6 fold, at least about 1.7 fold, at least about 1.8 fold, at least about 1.9 fold, at least about 2 fold, at least about 2.1 fold, at least about 2.2 fold, at least about 2.3 fold, at least about 2.4 fold, at least about 2.5 fold, at least about 2.6 fold, at least about 2.7 fold, at least about 2.8 fold, at least about 2.9 fold or at least about 3 fold higher than the BXA-212372 polypeptide (SEQ ID NO: 88) expression.

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26. — The nucleic acid molecule of any one of claims 1 to 25, which further comprises a promoter.

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27. — The nucleic acid molecule of claim 26, wherein the promoter is a tissue specific promoter.

28. — The nucleic acid molecule of claim 26 or 27, wherein the promoter drives expression of the therapeutic protein in muscle cells, hepatocytes, endothelial cells, neuronal cells, sinusoidal cells, or any combination thereof.

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29. — The nucleic acid molecule of any one of claims 26 to 28, wherein the promoter is selected from the group consisting of a C5-12(T) promoter, an MLC2v-cTNT455 promoter, a mouse thyretin promoter (mTTR), an endogenous human factor VIII promoter (F8), a human alpha-1 antitrypsin promoter (hAAT), a human albumin minimal promoter, a mouse albumin promoter, a tristetraprolin (TTP) promoter, a CASI promoter, a synapsin 1 gene promoter, a CAG promoter, a cytomegalovirus (CMV) promoter, α 1-antitrypsin (AAT), muscle creatine kinase (MCK), myosin heavy chain alpha (α MHC), myoglobin (MB),

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desmin (DES), SPc5-12, 2R5Sc5-12, dMCK, tMCK, and a phosphoglycerate kinase (PGK) promoter.

30. — The nucleic acid molecule of any one of claims 1 to 29, which further comprises an intronic sequence.

31. — The nucleic acid molecule of claim 30, wherein the intronic sequence is positioned 5' to the nucleotide sequence encoding the miniaturized dystrophin polypeptide.

32. — The nucleic acid molecule of claim 30, wherein the intronic sequence is positioned 3' to the promoter.

33. — The nucleic acid molecule of any one of claims 30 to 32, wherein the intronic sequence comprises a synthetic intronic sequence.

34. — The nucleic acid molecule of any one of claims 1 to 33, which further comprises a post-transcriptional regulatory element.

35. — The nucleic acid molecule of claim 34, wherein the post-transcriptional regulatory element is positioned 3' to the nucleotide sequence encoding the miniaturized dystrophin polypeptide.

36. — The nucleic acid molecule of claim 34 or 35, wherein the post-transcriptional regulatory element comprises a mutated woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), a microRNA binding site, or a DNA nuclear targeting sequence, or any combination thereof.

37. — The nucleic acid molecule of any one of claims 1 to 36, which further comprises a 3'UTR poly(A) tail sequence.

38. — The nucleic acid molecule of claim 37, wherein the 3'UTR poly(A) tail sequence is selected from the group consisting of dystrophin poly(A), bGH poly(A), actin poly(A), hemoglobin poly(A), and any combination thereof.

5 39. — The nucleic acid molecule of claim 37 or 38, wherein the 3'UTR poly(A) tail sequence comprises dystrophin poly(A).

40. — The nucleic acid molecule of any one of claims 1 to 39, which further comprises an enhancer sequence.

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~~541.~~ The nucleic acid molecule of claim ~~440~~, which further comprises a first inverted terminal repeat (ITR) and/or a second ITR.

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42. — The nucleic acid molecule of claim 41, wherein the first ITR and the second ITR are identical.

43. — The nucleic acid molecule of claim 41 or 42, wherein the first ITR and/or the second ITR are derived both from adeno-associated virus (AAV).

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44. — The nucleic acid molecule of any one of claims 1 to 43, which comprises a sequence encoding a heterologous moiety.

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45. — The nucleic acid molecule of claim 44, wherein the heterologous moiety is selected from the group consisting of albumin or a fragment thereof, an immunoglobulin Fc region, the C-terminal peptide (CTP) of the β -subunit of human chorionic gonadotropin, a PAS sequence, a HAP sequence, a transferrin or a fragment thereof, an albumin-binding moiety or a derivative thereof, and any combination thereof.

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~~746.~~ A vector comprising the nucleic acid molecule of ~~any one of claims 51 to 45.~~

47. ~~The vector of claim 46, which is selected from the group consisting of a adenoviral vector, a retroviral vector, poxvirus vector, a baculovirus vector, a herpes viral vector.~~

948. A The vector of claim 46, which is an recombinant adeno-associated virus (rAAV) vector, particle comprising an AAV capsid and a nucleic acid molecule comprising a nucleotide sequence, which encodes a miniaturized dystrophin polypeptide comprising a modified R16 domain, wherein a part of the R16 domain is replaced by a corresponding part of a R2 domain, and wherein the miniaturized dystrophin polypeptide comprises an amino acid sequences at least about 98% identical to the amino acid sequence set forth as SEQ ID NO: 83.

10. The rAAV vector particle of claim 9, wherein the miniaturized dystrophin polypeptide comprises an amino acid sequence set forth as SEQ ID NO: 83.

11. The rAAV vector particle of claim 9, wherein the nucleotide sequence comprises in order a C5-12(T) promoter, an SV40 intron, a coding sequence for the miniaturized dystrophin polypeptide comprising the amino acid sequence set forth as SEQ ID NO: 83, a 3' UTR, and a polyA sequence.

12. The rAAV vector particle of claim 9, wherein the nucleotide sequence comprises in order a C5-12(T) promoter having the nucleotide sequence set forth as of SEQ ID NO: 109, an SV40 intron having the nucleotide sequence set forth as SEQ ID NO: 110, a coding sequence for the miniaturized dystrophin polypeptide having the nucleotide sequence set forth as SEQ ID NO: 111, a 3' UTR having the nucleotide sequence set forth as SEQ ID NO: 112, and a polyA sequence having the nucleotide sequence set forth as SEQ ID NO: 113.

13. The rAAV vector particle of claim 12, wherein the nucleic acid molecule further comprises a first ITR and a second ITR both from AAV.

~~1449. The vector of claim 48, wherein the rAAV vector particle of claim 13, wherein the first ITR and the second ITR are is selected from the genome of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, and AAV11.~~

5 15. The rAAV vector particle of claim 14, wherein the AAV capsid is from AAV8.

16. The rAAV vector particle of claim 14, wherein the AAV capsid is from AAV9.

10 ~~1750. The vector of claim 48, wherein the rAAV vector particle of claim 12, wherein the AAV capsid is from AAV8 or AAV9.~~

18. The rAAV vector particle of claim 12, wherein the AAV capsid is from AAV8.

15 19. The rAAV vector particle of claim 12, wherein the AAV capsid is from AAV9.

20. A rAAV vector particle comprising an AAV capsid and a nucleic acid molecule comprising a nucleotide sequence comprising in order a C5-12(T) promoter having the nucleotide sequence set forth as of SEQ ID NO: 109, an SV40 intron having the nucleotide sequence set forth as SEQ ID NO: 110, a coding sequence for a miniaturized dystrophin polypeptide having the nucleotide sequence set forth as SEQ ID NO: 111, a 3' UTR having the nucleotide sequence set forth as SEQ ID NO: 112, and a polyA sequence having the nucleotide sequence set forth as SEQ ID NO: 113, wherein the nucleic acid molecule further comprises a first ITR and a second ITR both from the genome of AAV2 flanking the nucleotide sequence, and wherein the AAV capsid is from AAV8 or AAV9.

25 21. The rAAV vector particle of claim 20, wherein the AAV capsid is from AAV8.

30 ~~51. The nucleic acid molecule of any one of claims 1 to 45 or vector of any one of claims 46 to 50, wherein the nucleic acid molecule or the vector is formulated with a delivery agent.~~

52. ~~The nucleic acid molecule or vector of claim 51, wherein the delivery agent comprises a lipid nanoparticle.~~

53. ~~The nucleic acid molecule or vector of claim 51, wherein the delivery agent is selected from the group consisting of liposomes, non-lipid polymeric molecules, endosomes, and any combination thereof.~~

54. ~~The nucleic acid molecule of any one of claims 1 to 45, the vector of any one of claims 46 to 50, or the nucleic acid molecule or vector of any one of claims 51 to 53, which is formulated for intravenous, transdermal, intradermal, subcutaneous, pulmonary, or oral delivery, or any combination thereof.~~

55. ~~The nucleic acid molecule or vector of claim 54, which is formulated for intravenous delivery.~~

56. ~~A polypeptide encoded by the nucleic acid molecule of any one of claims 1 to 45, the vector of any one of claims 46 to 50, or the nucleic acid molecule or the vector of any one of claims 51 to 55.~~

57. A host cell comprising the nucleic acid molecule of ~~any one of claims 51 to 45 or~~ vector of any one of claims 46 to 50.

58. ~~The host cell of claim 57, wherein the cell is a CHO cell, a HEK293 cell, a HBK cell, a COS cell, a NSO cell, or a HT1080 cell.~~

59. A pharmaceutical composition comprising (a) ~~the nucleic acid of any one of claims 1 to 45, the vector of any one of claims 46 to 50, the nucleic acid molecule or the vector of any one of claims 51 to 55, the polypeptide of claim 56, or the host cell of claim 57 or 58;~~ and (b) a pharmaceutically acceptable excipient.

22. A pharmaceutical composition comprising (a) the rAAV vector particle of claim 21; and (b) a pharmaceutically acceptable excipient.

23. A kit comprising the pharmaceutical composition of claim 22, and instructions for administering the pharmaceutical composition to a subject in need thereof.

24. The rAAV vector particle of claim 20, wherein the AAV capsid is from AAV9.

25. A pharmaceutical composition comprising (a) the rAAV vector particle of claim 24; and (b) a pharmaceutically acceptable excipient.

~~2660. A kit, comprising the nucleic acid of any one of claims 1 to 45, the vector of any one of claims 46 to 50, the nucleic acid molecule or the vector of any one of claims 51 to 55, the polypeptide of claim 56, the host cell of claim 57 or 58, or the pharmaceutical composition of claim 2559, and instructions for administering the nucleic acid molecule, the vector, the polypeptide, the host cell, or the pharmaceutical composition to a subject in need thereof.~~

~~61. A method of producing a miniaturized dystrophin polypeptide, comprising: culturing the host cell of claim 57 or 58 under suitable conditions and recovering the miniaturized dystrophin polypeptide.~~

~~62. A method of expressing a miniaturized dystrophin polypeptide in a subject in need thereof, comprising administering to the subject the nucleic acid of any one of claims 1 to 45, the vector of any one of claims 46 to 50, the nucleic acid molecule or the vector of any one of claims 51 to 55, the host cell of claim 57 or 58, or the pharmaceutical composition of claim 59.~~

~~63. A method of treating a subject having a disease or condition comprising administering to the subject the nucleic acid of any one of claims 1 to 45, the vector of any one of claims 46 to 50, the nucleic acid molecule or the vector of any one of claims 51 to~~

55, the polypeptide of claim 56, the host cell of claim 57 or 58, or the pharmaceutical composition of claim 59.

64. — The method of claim 63, wherein the disease or condition is a disease caused by dystrophin deficiency.

65. — The method of claim 64, wherein the disease is Sarcopenia, a heart disease, cachexia, Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), X-linked dilated cardiomyopathy (XLDC), facioscapulohumeral muscular dystrophy, myotonic muscular dystrophy, limb girdle muscular dystrophy, oculopharyngeal muscular dystrophy, Emery Dreifuss muscular dystrophy, distal muscular dystrophy, and/or congenital muscular dystrophy.

66. — The method of any one of claims 62 to 65, wherein the nucleic acid molecule, the vector, the polypeptide, the host cell, or the pharmaceutical composition is administered intravenously, transdermally, intradermally, subcutaneously, orally, or pulmonarily, or any combination thereof.

67. — The method of any one of claims 63 to 66, further comprising administering to the subject a second agent.

68. — The method of any one of claims 62 to 67, wherein the subject is a human.

69. — The method of any one of claims 62 to 68, wherein the administration of the nucleic acid molecule, the vector, the polypeptide, the host cell, or the pharmaceutical composition to the subject results in increased dystrophin protein expression, relative to dystrophin protein expression in the subject prior to the administration, wherein the dystrophin protein expression is increased by at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 11 fold, at least about 12 fold, at least about 13 fold, at least about 14 fold, at least about 15 fold, at least about 20 fold, at least

about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold, at least about 90-fold, or at least about 100-fold.

5 270. The ~~A~~ nucleic acid molecule of claim 1 ~~comprising a nucleotide sequence, wherein which encodes a~~ the miniaturized dystrophin polypeptide ~~comprisesing~~ an amino acid sequence set forth as ~~of~~ SEQ ID NO: 83.

10 ~~71. The nucleic acid molecule of any one of claims 1 to 45, the vector of any one of claims 46 to 50, the nucleic acid molecule or vector of any one of claims 51 to 55, the polypeptide of claim 56, the host cell of claim 57 or 58, the pharmaceutical composition of claim 59, the kit of claim 60, or the method of any one of claims 61 to 69, comprising a nucleotide sequence encoding a miniaturized dystrophin polypeptide comprising an amino acid sequence of SEQ ID NO: 83.~~

15 372. The ~~A~~ nucleic acid molecule of claim 1, comprising a nucleotide sequence comprising in order a C5-12(T) promoter ~~of SEQ ID NO: 109~~, an SV40 intron ~~of SEQ ID NO: 110~~, a coding sequence for the miniaturized dystrophin polypeptide comprising the amino acid sequence set forth as SEQ ID NO: 83 ~~BXA-220931 of SEQ ID NO: 111~~, a 3' UTR ~~of SEQ ID NO: 112~~, and a polyA sequence ~~of SEQ ID NO: 113~~.

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25 473. The nucleic acid molecule of ~~any one of claims 1 to 45, the vector of any one of claims 46 to 50, the nucleic acid molecule or vector of any one of claims 51 to 55, the polypeptide of claim 56, the host cell of claim 57 or 58, the pharmaceutical composition of claim 59, the kit of claim 60, or the method of any one of claims 61 to 69, comprising a~~ nucleotide sequence comprising in order a C5-12(T) promoter having the nucleotide sequence set forth as ~~of~~ SEQ ID NO: 109, an SV40 intron having the nucleotide sequence set forth as ~~of~~ SEQ ID NO: 110, a coding sequence for the miniaturized dystrophin polypeptide having the nucleotide sequence set forth as ~~BXA-220931 of SEQ ID NO: 111~~,
30 a 3' UTR having the nucleotide sequence set forth as ~~of~~ SEQ ID NO: 112, and a polyA sequence having the nucleotide sequence set forth as ~~of~~ SEQ ID NO: 113.