CLAIMS

1. An isolated and purified nucleotide sequence, comprising:

(a) a micro-dystrophin gene encoding a protein comprising:

an amino-terminal actin-binding domain;

a β-dystroglycan binding domain; and

a spectrin-like repeat domain, comprising at least four spectrin-like repeats, in which two of the at least four spectrin-like repeats comprise a neuronal nitric oxide synthase binding domain; and

(b) a regulatory cassette.

2. An isolated and purified nucleotide sequence according to Claim 1, in which the at least four spectrin-like repeats include spectrin-like repeat 1 (SR1), spectrin-like repeat 16 (SR16), spectrin-like repeat 17 (SR17), and spectrin-like repeat 24 (SR24); and/or

in which the protein encoded by the micro-dystrophin gene further comprises at least a portion of a hinge domain;

optionally in which the hinge domain is selected from at least one of a Hinge 1 domain, a Hinge 2 domain, a Hinge 3 domain, a Hinge 4 domain, and a hinge-like domain.

3. An isolated and purified nucleotide sequence according to Claim 1 or 2, in which the protein encoded by the micro-dystrophin gene has between five spectrin-like repeats and eight spectrin-like repeats; and/or

in which the protein encoded by the micro-dystrophin gene has at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 4, or

in which the protein encoded by the micro-dystrophin gene has at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 4, or

in which the protein encoded by the micro-dystrophin gene has at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 5, or

in which the protein encoded by the micro-dystrophin gene has at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 5, or

in which the regulatory cassette is the CK8 promoter, and in which the CK8 promoter has at least 80% sequence identity to the nucleic acid sequence of SEQ ID NO: 19, or

in which the regulatory cassette is the CK8 promoter, and in which the CK8 promoter has at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 19, or

in which the regulatory cassette is the cTnT promoter, and in which the cTnT promoter has at least 80% sequence identity to the nucleic acid sequence of SEQ ID NO: 1, or

in which the regulatory cassette is the cTnT promoter, and in which the cTnT promoter has at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 1.

4. An isolated and purified nucleotide sequence, comprising:

a micro-dystrophin gene encoding a protein comprising:

an amino-terminal actin-binding domain; and

at least two spectrin-like repeats that are directly coupled to each other, in which the at least two spectrin-like repeats that are directly coupled to each other are selected from at least one of spectrin-like repeat 1 directly coupled to spectrin-like repeat 2, spectrin-like repeat 2 directly coupled to spectrin-like repeat 3, spectrin-like repeat 1 directly coupled to spectrin-like repeat 16, spectrin-like repeat 17 directly coupled to spectrin-like repeat 23, spectrin-like repeat 17 directly coupled to spectrin-like repeat 24, and spectrin-like repeat 23 directly coupled to spectrin-like repeat 24; or

an isolated and purified nucleotide sequence, comprising:

a micro-dystrophin gene encoding a protein comprising, in order:

a Hinge 1 domain (H1);

a spectrin-like repeat 1 (SR1);

a spectrin-like repeat 16 (SR16);

a spectrin-like repeat 17 (SR17);

a spectrin-like repeat 24 (SR24); and

a Hinge 4 domain (H4); and optionally

in which the H1 is directly coupled to the SR1; or

in which the SR 1 is directly coupled to the SR16; or

in which the SR16 is directly coupled to the SR17; or

in which the SR 17 is directly coupled to the SR24; or

in which the SR24 is directly coupled to the H4; or

in which the protein encoded by the micro-dystrophin gene further comprises between the SR1 and the SR16, in order, a spectrin-like repeat 2 (SR2) and a spectrin-like repeat 3 (SR3), optionally in which the SR1 is directly coupled to the SR2 and the SR2 is further coupled to the SR3; or

in which the H1 is directly coupled to the SR1, the SR1 is directly coupled to the SR16, the SR16 is directly coupled to the SR17, the SR17 is directly coupled to the SR23, the SR23 is directly coupled to the SR24, and the SR24 is directly coupled to the H4.

5. A pharmaceutical composition, comprising:

an isolated and purified nucleotide sequence according to any of Claims 1 to 4; and

a delivery vehicle; or comprising:

a micro-dystrophin gene comprising the nucleic acid sequence of SEQ ID NO: 16; and

an adeno-associated virus (AAV) vector or a recombinant adeno-associated virus (rAAV) vector; or comprising:

a micro-dystrophin gene encoding a protein, in which the protein comprises the amino acid sequence of SEQ ID NO: 4; and

an adeno-associated virus (AAV) vector or a recombinant adeno-associated virus (rAAV) vector; or comprising:

a micro-dystrophin gene comprising the nucleic acid sequence of SEQ ID NO: 18; and

an adeno-associated virus (AAV) vector or a recombinant adeno-associated virus (rAAV) vector; or comprising:

a micro-dystrophin gene encoding a protein, in which the protein comprises the amino acid sequence of SEQ ID NO: 5; and

an adeno-associated virus (AAV) vector or a recombinant adeno-associated virus (rAAV) vector; or

a pharmaceutical composition for the treatment or prophylactic treatment of muscular dystrophy, comprising:

a micro-dystrophin gene comprising the nucleic acid sequence of SEQ ID NO: 16 or SEQ ID NO: 18; and

an adeno-associated virus (AAV) vector or a recombinant adeno-associated virus (rAAV) vector, in which a serotype of the AAV vector or the rAAV vector is selected from at least one of serotype 6, serotype 8, and serotype 9.

6. A pharmaceutical composition according to Claim 5, in which a serotype of the AAV vector or the rAAV vector is selected from at least one of serotype 6, serotype 8, and serotype 9.

7. A pharmaceutical composition according to Claim 5, in which the delivery vehicle comprises a recombinant adeno-associated virus vector and/or

in which the delivery vehicle expresses the micro-dystrophin gene, and in which the protein encoded by the micro-dystrophin gene has at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 4, or

in which the delivery vehicle expresses the micro-dystrophin gene, and in which the protein encoded by the micro-dystrophin gene has at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 4, or

in which the delivery vehicle expresses the micro-dystrophin gene, and in which the protein encoded by the micro-dystrophin gene has at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 5, or

in which the delivery vehicle expresses the micro-dystrophin gene, and in which the protein encoded by the micro-dystrophin gene has at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 5, or

in which the regulatory cassette is the CK8 promoter, and in which the CK8 promoter has at least 80% sequence identity to the nucleic acid sequence of SEQ ID NO: 19, or

in which the regulatory cassette is the CK8 promoter, and in which the CK8 promoter has at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 19, or

in which the regulatory cassette is the cTnT promoter, and in which the cTnT promoter has at least 80% sequence identity to the nucleic acid sequence of SEQ ID NO: 1, or

in which the regulatory cassette is the cTnT promoter, and in which the cTnT promoter has at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 1.

8. A pharmaceutical composition according to Claim 5, 6 or 7, in which the pharmaceutical composition is configured to reduce a pathological effect or symptom of a muscular dystrophy selected from at least one of myotonic muscular dystrophy, Duchenne muscular dystrophy, Becker muscular dystrophy, limb-girdle muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, oculopharyngeal muscular dystrophy, distal muscular dystrophy, and Emery-Dreifuss muscular dystrophy; or

in which the pharmaceutical composition is configured to reduce a pathological effect or symptom of a muscular dystrophy selected from at least one of Duchenne muscular dystrophy and Becker muscular dystrophy; and/or

in which the pharmaceutical composition is configured to reduce a pathological effect or symptom of at least one of sarcopenia, heart disease, and cachexia.

9. A pharmaceutical composition for use in treating or prophylactically treating a subject having muscular dystrophy comprising a micro-dystrophin gene operably coupled to a regulatory cassette; or comprising:

a micro-dystrophin gene comprising the nucleic acid sequence of SEQ ID NO: 16 or SEQ ID NO: 18; and

an adeno-associated virus (AAV) vector or a recombinant adeno-associated virus (rAAV) vector, in which a serotype of the AAV vector or the rAAV vector is selected from at least one of serotype 6, serotype 8, and serotype 9; or

a pharmaceutical composition for use in the treatment or prophylactic treatment of muscular dystrophy, comprising:

a micro-dystrophin gene comprising the nucleic acid sequence of SEQ ID NO: 16 or SEQ ID NO: 18; and

an adeno-associated virus (AAV) vector or a recombinant adeno-associated virus (rAAV) vector, in which a serotype of the AAV vector or the rAAV vector is selected from at least one of serotype 6, serotype 8, and serotype 9.

10. An isolated and purified nucleotide sequence according to Claim 1 or 2, or a pharmaceutical composition for use according to Claim 9, in which the regulatory cassette is selected from the group consisting of a CK8 promoter and a cardiac troponin T (cTnT) promoter; and/or

in which the regulatory cassette is configured to express the micro-dystrophin gene such that a level of expression of the micro-dystrophin gene is at least 100-fold higher in striated muscle cells than the level of expression of the micro-dystrophin gene in non-muscle cells.

11. A pharmaceutical composition for use according to Claim 9 or 10, in which the pharmaceutical composition further comprises a recombinant adeno-associated virus vector configured to express the micro-dystrophin gene in the subject; and/or

in which the micro-dystrophin gene encodes a protein having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 4, or

in which the micro-dystrophin gene encodes a protein having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 4, or

in which the micro-dystrophin gene encodes a protein having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 5, or

in which the micro-dystrophin gene encodes a protein having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 5, or

in which the regulatory cassette is the CK8 promoter, and in which the CK8 promoter has at least 80% sequence identity to the nucleic acid sequence of SEQ ID NO: 19, or

in which the regulatory cassette is the CK8 promoter, and in which the CK8 promoter has at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 19, or

in which the regulatory cassette is the cTnT promoter, and in which the cTnT promoter has at least 80% sequence identity to the nucleic acid sequence of SEQ ID NO: 1, or

in which the regulatory cassette is the cTnT promoter, and in which the cTnT promoter has at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 1.

12. A pharmaceutical composition for use according to Claim 9, 10 or 11, in which the micro-dystrophin gene expresses a micro-dystrophin protein in one or more muscles of the subject such that contractility of the one or more muscles is enhanced; and/or

in which the micro-dystrophin gene expresses a micro-dystrophin protein in one or more skeletal muscles of the subject such that a specific-force generating capacity of at least one of the one or more skeletal muscles is increased to within at least 40% of a normal specific-force generating capacity; and/or

in which the micro-dystrophin gene expresses a micro-dystrophin protein in one or more cardiac muscles of the subject such that a baseline end-diastolic volume defect is restored to within at least 40% of a normal end-diastolic volume; and/or

in which the micro-dystrophin gene expresses a micro-dystrophin protein such that localization of the neuronal nitric oxide synthase to the dystrophin-glycoprotein complex is enhanced in the subject.

13. A pharmaceutical composition for use according to any of Claims 9 to 12, in which the muscular dystrophy is selected from at least one of myotonic muscular dystrophy, Duchenne muscular dystrophy, Becker muscular dystrophy, limb-girdle muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, oculopharyngeal muscular dystrophy, distal muscular dystrophy, and Emery-Dreifuss muscular dystrophy; or

in which the muscular dystrophy is selected from at least one of Duchenne muscular dystrophy and Becker muscular dystrophy; or

in which the pharmaceutical composition reduces a pathological effect or symptom of the muscular dystrophy; and optionally

in which the pathological effect or symptom of the muscular dystrophy is selected from at least one of muscle pain, muscle weakness, muscle fatigue, muscle atrophy, fibrosis, inflammation, increase in average myofiber diameter in skeletal muscle, cardiomyopathy, reduced 6-minute walk test time, loss of ambulation, and cardiac pump failure.

14. A pharmaceutical composition for use according to any of Claims 9 to 13, in which the treatment or prophylactic treatment further comprises identifying the subject having the muscular dystrophy.

15. A pharmaceutical composition for use according to any of Claims 9 to 14, in which the subject is a mammal; or in which the subject is a human.