

GENE THERAPY WITH DYSFERLIN DUAL VECTORS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 63/024,338, filed May 13, 2020, the contents of which are hereby incorporated by reference into the present application.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] This application contains, as a separate part of the disclosure, a Sequence Listing in computer-readable form which is incorporated by reference in its entirety and identified as follows: Filename: 106887_8070_SL.txt; Size: 129,123 bytes, created; May 11, 2021.

TECHNICAL FIELD

[0003] This disclosure provides polynucleotides comprising fragments of the human dysferlin gene and plasmids, viral vectors, cells, and compositions comprising such polynucleotides, and methods of using such polynucleotides, plasmids, viral vectors, and compositions to treat subjects with dysferlin deficiency, such as limb girdle muscular dystrophy type 2B, Miyoshi Myopathy, and distal anterior compartment myopathy.

BACKGROUND

[0004] Dysferlinopathies are autosomal recessive disorders including limb girdle muscular dystrophy type 2B (LGMD2B), Miyoshi myopathy, and distal anterior compartment myopathy, collectively known as the dysferlinopathies. Limb Girdle Muscular Dystrophy type 2B (LGMD2B) represents one of the most common LGMDs in the United States with worldwide reports of incidence of 1/100,000-1/200,000. Miyoshi Myopathy is more limited distal lower extremity form of dysferlinopathy. In fact, in considering the disease spectrum, LGMD2B often begins distal with atrophy of gastrocnemius muscle and then spreads over time to affect proximal muscles. Loss of dysferlin leads to a progressive form of dystrophy with chronic muscle fiber loss, inflammation, fat replacement and fibrosis all leading to deteriorating muscle weakness.

[0005] The dysferlin gene is large, with 55 exons so far identified spanning at least 150 kb of genomic DNA. These exons predict a cDNA of approximately 6.5 kb and a protein of 2,088 amino acids. Dysferlin is a 237 kDa protein composed of a C-terminal hydrophobic

transmembrane domain and a longer cytoplasmic oriented hydrophilic region with multiple C2 domains. A growing body of work has shown that loss of dysferlin compromises Ca^{2+} -dependent membrane repair in skeletal muscle (Song et al., Proc. Natl. Acad. Sci USA 98: 4084-4088, 2001; Schnepf et al., J. Virol. 77:3495-3504, 2003). In addition dysferlin has been shown to interact with other proteins involved in membrane repair including annexins A1 and A2, AHNK, and caveolins-3. The importance of this system is emphasized when considering that skeletal muscle is mechanically active and predisposed to injury; thus, a robust membrane resealing mechanism must be present. Absent or mutant dysferlin leads to impaired membrane repair and a cascade of events starting with muscle fiber necrosis resulting in muscle fiber loss and progressive limb weakness. The loss of muscle fiber regenerative capacity is thought to be a contributory consequence of dysferlin deficiency. Dysferlin has also been associated with vesicle trafficking and endocytosis, T tubule formation and others.

[0006] Mutations in the dysferlin gene cause allelic autosomal recessive disorders including limb girdle muscular dystrophy type 2B (LGMD2B), Miyoshi myopathy and distal anterior compartment myopathy, collectively known as the dysferlinopathies (*see, e.g.,* Grose *et al.*, PLoS one 7:e39233, 2012, Bansal *et al.*, Nature 423, 168-172, 2003, Moore, S.A., *et al.*, J. Neuropathol. Exp. Neurol 65: 995-1003, 2006, Rosales *et al.*, Muscle Nerv 42:14-21, 2010, Sondergaard et al., Anns of Clin. Trans. Neurol. 2:256-270, 2015, Evesson et al., J. Biol. Chem. 285: 28529-28539, 2010, and Klinge et al., Soc. Exp. Biol. 21: 1768-1776, 2007, each of which are incorporated by reference in their entirety). A less common phenotype of dysferlin deficiency presents with rigid spine syndrome (Klinge et al., Muscle Nerve 41: 166-173, 2010, which is incorporated by reference in its entirety). Typically patients present in their early twenties with slowly progressive weakness and high serum creatine kinase (CK). Approximately one-third of patients become wheelchair-dependent within 15 years of onset. Clinically the heart is spared and cognitive function is not affected. The phenotypic variants with a relatively restricted distribution of muscle weakness set the stage for potential regional gene replacement therapy that could greatly impact quality of life for this disorder (Grose *et al.*, PLoS One 7:e39233, 2012, Barton *et al.*, Muscle Nerve 42: 22-29, 2010). Single nucleotide changes are the typical DYSF gene mutation, which also favor success in gene transfer serving to protect the transgene product from immunorejection (Rodine-Klapac *et al.*, Mol. Ther. 18: 109-117, 2010, Mendell *et al.*, N. Eng. J. Med. 363: 1429-1437, 2010,

Mendell *et al.*, Ann. Neurol. 66:290-297, 2009, each of which are incorporated by reference in their entireties).

[0007] There is no cure or treatment for dysferlinopathies. Collectively, pre-clinical studies that have assessed gene replacement or surrogate gene replacement have shown that multiple strategies exhibit some efficacy in restoring membrane repair. The dysferlin gene includes 55 exons encompassing 150 kb of genomic DNA with its associated cDNA at 6.5 kb. However, for gene replacement, the packaging limit of AAV is 4.7kb, which is below dysferlin's cDNA sequence at 6.5 kb. Thus, there is a need for a treatment for LGMD2B, necessitating a new mechanism that is capable of delivering the functional full-length dysferlin protein to a subject in need.

SUMMARY

[0008] Disclosed herein is a recombinant polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide comprises a first nucleotide sequence, wherein the first nucleotide sequence consists of: (a) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (b) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (c) the nucleotide sequence of SEQ ID NO: 13 or 15; (d) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (e) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment consists of the amino acid sequence of SEQ ID NO: 9; or (f) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (e) across the full length of the nucleotide sequence of (e).

[0009] In some embodiments, the recombinant polynucleotide further comprises one or more additional nucleotide sequences selected from an inverted terminal repeat (ITR), a promoter, an intron, a selection marker, or an origin of replication (ORI).

[0010] In some embodiments, the recombinant polynucleotide further comprises an additional nucleotide sequence comprising an ITR. In some embodiments, the ITR is an AAV ITR. In some embodiments, the AAV ITR is an AAV2 ITR or an AAV3 ITR. In some embodiments, the recombinant polynucleotide comprises two ITRs. In some embodiments, the ITR comprises the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 17.

[0011] In some embodiments, the recombinant polynucleotide further comprises an additional nucleotide sequence comprising a promoter. In some embodiments, the promoter is a muscle-specific promoter. In some embodiments, the muscle-specific promoter is selected from a human skeletal actin gene element, a cardiac actin gene element, a desmin promoter, a skeletal alpha-actin (ASKA) promoter, a troponin I (TNNI2) promoter, a myocytespecific enhancer binding factor mef binding element, a muscle creatine kinase (MCK) promoter, a truncated MCK (tMCK) promoter, a myosin heavy chain (MHC) promoter, a hybrid a-myosin heavy chain enhancer-/MCK enhancer-promoter (MHCK7) promoter, a C5-12 promoter, a murine creatine kinase enhancer element, a skeletal fast-twitch troponin c gene element, a slow-twitch cardiac troponin c gene element, a slow-twitch troponin i gene element, hypoxia- inducible nuclear factor. In some embodiments, muscle-specific promoter is a MHCK7 promoter. In some embodiments, the promoter is a recombinant promoter. In some embodiments, the recombinant promoter is a recombinant muscle-specific promoter. In some embodiments, the recombinant-muscle specific promoter is a recombinant myosin heavy chain-creatine kinase muscle-specific promoter. In some embodiments, the promoter comprises the nucleotide sequence of SEQ ID NO: 4.

[0012] In some embodiments, the recombinant polynucleotide further comprises an additional nucleotide sequence comprising an intron. In some embodiments, the intron comprises a 5' donor site, branch point, and/or 3' splice site. In some embodiments, the intron is a chimeric intron. In some embodiments, the intron comprises a 5' donor site from a human β -globin gene. In some embodiments, the intron comprises a branch point from an immunoglobulin G (IgG) heavy chain. In some embodiments, the intron comprises a 3' splice acceptor site from an immunoglobulin G (IgG) heavy chain. In some embodiments, the intron comprises the nucleotide sequence of SEQ ID NO: 5.

[0013] In some embodiments, the recombinant polynucleotide further comprises an additional nucleotide sequence comprising a selection marker. In some embodiments, the selection marker is an antibiotic resistance gene. In some embodiments, the antibiotic resistance gene is a β -lactamase gene or kanamycin resistance gene. In some embodiments, the recombinant polynucleotide comprises the nucleotide sequence of SEQ ID NO: 6 or SEQ ID NO: 18. In some embodiments, the recombinant nucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0014] In some embodiments, the recombinant nucleotide does not comprise an AAV sequence other than one or more ITRs.

[0015] In some embodiments, the recombinant nucleotide does not comprise a viral sequence other than one or more ITRs.

[0016] Disclosed herein is a recombinant polynucleotide sequence encoding a fragment of a human dysferlin protein, wherein the recombinant polynucleotide comprises a second nucleotide sequence, wherein the second nucleotide sequence consists of: (a) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (b) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8, or 19 across its respective full length of SEQ ID NO: 2, 8, or 19; (c) the nucleotide sequence of SEQ ID NO: 14 or 16; (d) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (e) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (f) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (e) across the full length of the nucleotide sequence of (d).

[0017] In some embodiments, the recombinant polynucleotide further comprises one or more additional nucleotide sequences comprising an inverted terminal repeat (ITR), a selection marker, an origin of replication (ORI), an untranslated region (UTR), or a polyadenylation (polyA) signal.

[0018] In some embodiments, the recombinant polynucleotide further comprises an additional nucleotide sequence comprising an ITR. In some embodiments, the ITR is an AAV ITR. In some embodiments, the AAV ITR is an AAV2 ITR or an AAV3 ITR. In some embodiments, the recombinant nucleotide comprises two ITRs. In some embodiments, the ITR comprises the nucleotide sequence of SEQ ID NO: 3 or 17.

[0019] In some embodiments, the recombinant polynucleotide further comprises a nucleotide sequence comprising a polyA signal. In some embodiments, the polyA signal is an artificial polyA signal. In some embodiments, the polyA signal comprises the nucleotide sequence of SEQ ID NO: 7.

[0020] In some embodiments, the recombinant polynucleotide further comprises an additional nucleotide sequence comprising a selection marker. In some embodiments, the selection marker is an antibiotic resistance gene. In some embodiments, the antibiotic

resistance gene is a β -lactamase gene or kanamycin resistance gene. In some embodiments, the recombinant polynucleotide comprises the nucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 19.

[0021] In some embodiments, the recombinant nucleotide does not further comprise a first polynucleotide sequence encoding a first fragment of the hDYSF protein.

[0022] In some embodiments, the recombinant nucleotide does not comprise an AAV sequence other than one or more ITRs.

[0023] In some embodiments, the recombinant nucleotide does not comprise a viral sequence other than one or more ITRs.

[0024] Further disclosed herein is a dual adeno-associated viral (AAV) vector system comprising: (a) a first AAV vector, wherein the first AAV vector comprises a first recombinant polynucleotide encoding a N-terminal fragment of a human dysferlin (hDYSF) protein, wherein the first recombinant polynucleotide comprises a first nucleotide sequence, wherein the first nucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) a second AAV vector, wherein the second AAV vector comprises a second recombinant polynucleotide encoding a C-terminal fragment of a human dysferlin protein, wherein the second recombinant polynucleotide comprises a second nucleotide sequence, wherein the second nucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (v) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v).

[0025] Further disclosed herein is adeno-associated viral (AAV) vector comprising any of the recombinant polynucleotides disclosed herein. In some embodiments, the recombinant polynucleotide encodes an N-terminal fragment of a human dysferlin protein. In some embodiments, the recombinant polynucleotide encodes a C-terminal fragment of a human dysferlin protein. In some embodiments, the AAV vector is AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAVrh.10, AAVrh.20, or AAVrh.74. In some embodiments, the AAV vector is AAVrh.74.

[0026] Further disclosed herein is a composition comprising any of the AAV vectors disclosed herein.

[0027] Further disclosed herein is a composition comprising (a) a first recombinant adeno-associated viral (rAAV) vector, wherein the first rAAV vector comprises a first recombinant polynucleotide encoding a N-terminal fragment of a human dysferlin (hDYSF) protein, wherein the first recombinant polynucleotide comprises a first nucleotide sequence, wherein the first nucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) a second rAAV vector, wherein the second rAAV vector comprises a second recombinant polynucleotide encoding a C-terminal fragment of a human dysferlin protein, wherein the second recombinant polynucleotide comprises a second nucleotide sequence, wherein the second nucleotide

sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (v) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v).

[0028] In some embodiments, the molar ratio of first and second rAAV vectors is between about 100:1-1:100, about 10:1-1:10, about 2:1-1:2, or about 1:1.

[0029] Further disclosed herein is an adeno-associated viral (AAV) vector comprising: (a) a first inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 1 or 6; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across the full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide is flanked by the first and second ITRs.

[0030] In some embodiments, the ITR is an AAV ITR. In some embodiments, the AAV ITR is an AAV2 ITR or an AAV3 ITR. In some embodiments, the first and/or second ITR comprise the nucleotide sequence of SEQ ID NO: 3 or 17.

[0031] In some embodiments, the AAV vector further comprises one or more additional polynucleotide sequences comprising a promoter, an intron, a selection marker, or an origin of replication (ORI).

[0032] In some embodiments, the AAV vector further comprises an additional polynucleotide sequence comprising a promoter. In some embodiments, the promoter is a muscle-specific promoter. In some embodiments, the muscle-specific promoter is a myosin heavy chain complex—E box muscle creatine kinase fusion enhancer/promoter. In some embodiments, the promoter is a recombinant promoter. In some embodiments, the recombinant promoter is a recombinant muscle-specific promoter. In some embodiments, the recombinant-muscle specific promoter is aMHCK7 promoter. In some embodiments, the promoter comprises the nucleotide sequence of SEQ ID NO: 4.

[0033] In some embodiments, the AAV vector further comprises an additional polynucleotide sequence comprising an intron. In some embodiments, the intron comprises a 5' donor site, branch point, and/or 3' splice site. In some embodiments, the intron is a chimeric intron. In some embodiments, the intron comprises a 5' donor site from a human β - globin gene. In some embodiments, the intron comprises a branch point from an immunoglobulin G (IgG) heavy chain. In some embodiments, the intron comprises a 3' splice acceptor site from an immunoglobulin G (IgG) heavy chain. In some embodiments, the intron comprises the nucleotide sequence of SEQ ID NO: 5.

[0034] In some embodiments, the AAV vector further comprises an additional polynucleotide sequence comprising a selection marker. In some embodiments, the selection marker is an antibiotic resistance gene. In some embodiments, the antibiotic resistance gene is a β -lactamase gene or kanamycin resistance gene. In some embodiments, the AAV vector comprises the nucleotide sequence of SEQ ID NO: 6 or SEQ ID NO: 15.

[0035] In some embodiments, the AAV vector does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0036] In some embodiments, the AAV vector does not comprise an AAV sequence other than one or more ITRs.

[0037] In some embodiments, the AAV vector does not comprise a viral sequence other than one or more ITRs.

[0038] Further disclosed herein is an adeno-associated viral (AAV) vector comprising: (a) a first inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human

dysferlin protein, wherein the polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2 or 8; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ ID NO: 2 or 8; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16; (v) a polynucleotide encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide is flanked by the first and second ITRs.

[0039] In some embodiments, the AAV vector further comprises one or more polynucleotide sequences comprising a selection marker, an origin of replication (ORI), an untranslated region (UTR), or a polyadenylation (polyA) signal.

[0040] In some embodiments, the ITR is an AAV ITR. In some embodiments, the AAV ITR is an AAV2 ITR or an AAV3 ITR. In some embodiments, the ITR comprises the nucleotide sequence of SEQ ID NO: 3 or 17.

[0041] In some embodiments, the AAV vector further comprises an additional polynucleotide sequence comprising a polyA signal. In some embodiments, the polyA signal is an artificial polyA signal. In some embodiments, the polyA signal comprises the nucleotide sequence of SEQ ID NO: 7.

[0042] In some embodiments, the AAV vector further comprises an additional polynucleotide sequence comprising a selection marker. In some embodiments, the selection marker is an antibiotic resistance gene. In some embodiments, the antibiotic resistance gene is a β -lactamase gene or kanamycin resistance gene.

[0043] In some embodiments, the AAV vector comprises the nucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 16.

[0044] In some embodiments, the AAV vector does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0045] In some embodiments, the AAV vector does not comprise an AAV sequence other than one or more ITRs.

[0046] In some embodiments, the AAV vector does not comprise a viral sequence other than one or more ITRs.

[0047] Disclosed herein is a dual adeno-associated viral (AAV) vector system comprising: (I) a first AAV vector, wherein the first AAV vector comprises (a) a first inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 1 or 6; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across the full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide is flanked by the first and second ITRs; and (II) a second AAV vector, wherein the second AAV vector comprises (a) a third inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 2 or 8; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ ID NO: 2 or 8; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16; (v) a polynucleotide encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a fourth ITR, wherein the polynucleotide is flanked by the third and fourth ITRs.

[0048] Further disclosed herein is an adeno-associated viral (AAV) packaging system comprising: (a) a plasmid comprising the recombinant polynucleotide disclosed herein; (b) an

adenovirus helper plasmid; and (c) a rep-cap plasmid. In some instances, the the adenovirus helper plasmid comprises pHELP plasmid.

[0049] Further disclosed herein is an adeno-associated viral packaging system comprising: (a) a plasmid comprising the recombinant polynucleotide disclosed herein; and (b) an adenovirus helper plasmid. In some instances, the the adenovirus helper plasmid comprises pHELP plasmid.

[0050] Further disclosed herein is a method for producing an adeno-associated viral (AAV) vector, comprising contacting a cell with an AAV packaging system, wherein the AAV packaging system comprises: (a) a plasmid comprising the recombinant polynucleotide disclosed herein; (b) an adenovirus helper plasmid; and (c) a rep-cap plasmid. In some instances, the cell is a host cell, optionally a mammalian host cell, further optionally HEK293.

[0051] Further disclosed herein is a method for producing an adeno-associated viral (AAV) vector, comprising transducing a packaging cell line with an AAV packaging system, wherein the AAV packaging system comprises (a) a plasmid comprising an AAV expression cassette comprising any of the recombinant polynucleotides disclosed herein; and (b) an adenovirus helper plasmid, and wherein the packaging cell line expresses an adeno-associated viral rep and cap genes. In some instances, the AAV rep gene is Rep78. In some instances, the AAV cap gene is Rh74 *cap* gene.

[0052] Further disclosed herein is a cell comprising any of the recombinant polynucleotides disclosed herein.

[0053] Further disclosed herein is a cell comprising an AAV expression cassette, wherein the AAV expression cassette comprises any of the recombinant polynucleotides disclosed herein. In some instances, the plasmid comprising a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 18 or 19. In some instances, the plasmid comprising a polynucleotide of SEQ ID NO: 18 or 19.

[0054] Further disclosed herein is a method of treating a dysferlinopathy, comprising administering to a subject in need thereof: (a) an effective amount of a first recombinant polynucleotide comprising a first polynucleotide sequence encoding an N-terminal of a human dysferlin (hDYSF) protein, wherein the first polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide

sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) an effective amount of a second recombinant polynucleotide comprising a second polynucleotide sequence encoding a C-terminal fragment of a human dysferlin protein, wherein the second polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (v) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v).

[0055] In some embodiments, the first polynucleotide is administered intramuscularly or intravenously. In some embodiments, the second polynucleotide is administered intramuscularly or intravenously.

[0056] In some embodiments, the first and second polynucleotides are administered simultaneously or sequentially.

[0057] In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0058] Further disclosed herein is a method of treating a dysferlinopathy, comprising administering to a subject in need thereof (a) an effective amount of a first adeno-associated viral (AAV) vector, wherein the first AAV vector comprises a first polynucleotide encoding

an N-terminal of a human dysferlin (hDYSF) protein, wherein the first polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 1 or 6; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across the full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) an effective amount of a second AAV vector, wherein the second AAV vector comprises a second polynucleotide encoding a C-terminal fragment of a human dysferlin protein, wherein the second polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 2 or 8; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ ID NO: 2 or 8; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16; (v) a polynucleotide encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide of (v) across the full length of the nucleotide sequence of (v).

[0059] In some embodiments, the first AAV vector is administered intramuscularly or intravenously. In some embodiments, the second AAV vector is administered intramuscularly or intravenously. In some embodiments, the first and second AAV vectors are administered simultaneously.

[0060] In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0061] In some embodiments, a method of treating a dysferlinopathy comprises administering to a subject in need thereof an effective amount of any of the AAV dual vector systems disclosed herein.

[0062] In some embodiments, the AAV dual vector system is administered intramuscularly or intravenously.

[0063] In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0064] In some embodiments, a method of treating a dysferlinopathy comprises administering to a subject in need thereof an effective amount of any of the compositions disclosed herein.

[0065] In some embodiments, the composition is administered intramuscularly or intravenously.

[0066] In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0067] Further disclosed herein is use of a composition in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof, wherein the composition comprises (a) a first recombinant polynucleotide comprising a first polynucleotide sequence encoding an N-terminal of a human dysferlin (hDYSF) protein, wherein the first polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) a second recombinant polynucleotide comprising a second polynucleotide sequence encoding a C-terminal fragment of a human dysferlin protein, wherein the second polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at

least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (v) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v).

[0068] In some embodiments, the first polynucleotide is administered intramuscularly or intravenously. In some embodiments, the second polynucleotide is administered intramuscularly or intravenously. In some embodiments, the first and second polynucleotides are administered simultaneously.

[0069] In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0070] Disclosed herein is use of a composition in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof, wherein the composition comprises: (a) an effective amount of a first adeno-associated viral (AAV) vector, wherein the first AAV vector comprises a first polynucleotide sequence encoding an N-terminal of a human dysferlin (hDYSF) protein, wherein the first polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 1 or 6; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across the full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) an effective amount of a second adeno-associated viral (AAV) vector, wherein the second AAV vector comprises a second polynucleotide encoding a C-terminal fragment of a human dysferlin protein, wherein the second polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 2 or 8; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ

ID NO: 2 or 8; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16; (v) a polynucleotide encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide of (v) across the full length of the nucleotide sequence of (v).

[0071] In some embodiments, the first AAV vector is administered intramuscularly or intravenously. In some embodiments, the second AAV vector is administered intramuscularly or intravenously. In some embodiments, the first and second AAV vectors are administered simultaneously.

[0072] In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0073] Disclosed herein is use of a composition in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof, wherein the composition comprises any of the AAV dual vector systems disclosed herein.

[0074] In some embodiments, the composition is administered intramuscularly or intravenously.

[0075] In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0076] Disclosed herein is use of any of the compositions disclosed in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof.

[0077] In some embodiments, the composition is administered intramuscularly or intravenously.

[0078] In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0079] In some embodiments, the effective amount of the first AAV vector is between about 1×10^6 - 1×10^{16} vg/kg, about 1×10^8 - 1×10^{15} vg/kg, or about 1×10^{10} - 1×10^{14} vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard.

[0080] In some embodiments, the effective amount of the second AAV vector is between about 1×10^6 - 1×10^{16} vg/kg, about 1×10^8 - 1×10^{15} vg/kg, or about 1×10^{10} - 1×10^{14} vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard.

[0081] In some embodiments, the first AAV vector is administered at least 1, 2, 3, 4, or 5 times.

[0082] In some embodiments, the second AAV vector is administered at least 1, 2, 3, 4, or 5 times.

[0083] In some embodiments, the effective amount of the AAV dual vector system is between about 1×10^{10} - 1×10^{13} vector genomes (vg), about 1×10^{11} - 1×10^{13} vg, 1×10^{12} - 1×10^{13} vg.

[0084] In some embodiments, the AAV dual vector system is administered at least 1, 2, 3, 4, or 5 times.

[0085] In some embodiments, the effective amount of the composition is between about 1×10^{10} - 1×10^{13} vector genomes (vg), about 1×10^{11} - 1×10^{13} vg, 1×10^{12} - 1×10^{13} vg.

[0086] In some embodiments, the composition is administered at least 1, 2, 3, 4, or 5 times.

[0087] Disclosed herein is a recombinant polynucleotide encoding a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide sequence comprising a nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20. In some embodiments, the recombinant polynucleotide sequence comprising a nucleotide sequence of SEQ ID NO: 20.

[0088] Disclosed herein is a method of making the recombinant polynucleotide encoding a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide sequence comprising a nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20, in which the method comprises contacting a cell with the recombinant polynucleotide comprising a first polynucleotide sequence encoding an N-terminal of a human dysferlin (hDYSF) protein, wherein the first polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (iii) the nucleotide sequence of SEQ ID

NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) a second recombinant polynucleotide comprising a second polynucleotide sequence encoding a C-terminal fragment of a human dysferlin protein, wherein the second polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (v) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v).

[0089] Disclosed herein is a method of making the recombinant polynucleotide encoding a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide comprising a nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20, in which the method comprises contacting a cell with the dual AAV vector system comprising: (I) a first AAV vector, wherein the first AAV vector comprises (a) a first inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 1 or 6; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across

the full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide is flanked by the first and second ITRs; and (II) a second AAV vector, wherein the second AAV vector comprises (a) a third inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin protein, wherein the polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2 or 8; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ ID NO: 2 or 8; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16; (v) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide of (v) across the full length of the nucleotide sequence of (v); and (c) a fourth ITR, wherein the polynucleotide is flanked by the third and fourth ITRs.

[0090] In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a muscle cell, a heart cell, a stem cell, a satellite cell, and/or a liver cell.

[0091] Disclosed herein is a method of making the recombinant polynucleotide encoding a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide sequence comprising a nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20, in which the method comprises administering to a subject with the recombinant polynucleotide comprising a first polynucleotide sequence encoding an N-terminal of a human dysferlin (hDYSF) protein, wherein the first polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) a second recombinant polynucleotide comprising a second polynucleotide sequence encoding a C-terminal fragment of a human dysferlin protein, wherein the second polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (v) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v).

[0092] Disclosed herein is a method of making the recombinant polynucleotide encoding a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide comprising a nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20, in which the method comprises administering to a subject with the dual AAV vector system comprising: (I) a first AAV vector, wherein the first AAV vector comprises (a) a first inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 1 or 6; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across the full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of

the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide is flanked by the first and second ITRs; and (II) a second AAV vector, wherein the second AAV vector comprises (a) a third inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 2 or 8; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ ID NO: 2 or 8; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16; (v) a polynucleotide encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a fourth ITR, wherein the polynucleotide is flanked by the third and fourth ITRs.

[0093] Disclosed herein is a method of treating muscular dystrophy of a subject, comprising expression of the recombinant polynucleotide encoding a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide sequence comprising a nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20 in the subject.

[0094] In some embodiments, the method comprises administering to a subject with the recombinant polynucleotide comprising a first polynucleotide sequence encoding an N-terminal of a human dysferlin (hDYSF) protein, wherein the first polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (b) a second recombinant polynucleotide comprising a second polynucleotide sequence encoding a C-terminal fragment of a human dysferlin protein, wherein the second polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (v) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v).

[0095] In some embodiments, the method comprises administering to a subject with the dual AAV vector system comprising: (I) a first AAV vector, wherein the first AAV vector comprises (a) a first inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 1 or 6; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across the full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the

polynucleotide is flanked by the first and second ITRs; and (II) a second AAV vector, wherein the second AAV vector comprises (a) a third inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 2 or 8; (ii) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ ID NO: 2 or 8; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16; (v) a polynucleotide encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a fourth ITR, wherein the polynucleotide is flanked by the third and fourth ITRs.

[0096] In some embodiments, the subject is a mammal selected from human, a non-human primate, a canine, an ovine, a horse, a porcine, a murine, a rat, a rabbit, a bovine, or a feline.

[0097] In some embodiments, the subject suffers from dysferlinopathy. In some embodiments, dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0098] FIG. 1 provides the schematic of the dual AAV vector system for treating dysferlinopathies. The 5' vector (*e.g.*, 5' hDYSF AAV vector), pAAV.MHCK7.DYSF5'.PTG (PTG=promoter/transgene) contains the muscle specific MHCK7 promoter, chimeric intron, consensus Kozak sequence and 5' portion of the DYSF cDNA corresponding to amino acids 1-1113 of SEQ ID NO: 12. The 3' vector (*e.g.*, 3' hDYSF AAV vector), pAAV.DYSF3'.POLYA, contains a 3' portion of the DYSF cDNA corresponding to amino acids 794-2080 of SEQ ID NO: 12 and DYSF 3'UTR harboring a polyadenylation signal.

[0099] FIG. 2 provides the pAAV.MHCK7.DYSF5'.PTG DNA Vector Plasmid Map.

[0100] FIG. 3 provides the pAAV.DYSF3'.POLYA Vector DNA Plasmid Map.

[0101] FIGS. 4A-4D show dysferlin expression following delivery of a dual vector system. Robust full-length dysferlin expression was seen following delivery of both vectors by immune staining (FIG. 4A) and western blot (FIG. 4C). Delivery of either vector alone had no aberrant dysferlin expression (FIG. 4B: immune staining, FIG. 4D: western blot). 3222 is the full-length control.

[0102] FIGS. 5A-5C show results of timecourse of dysferlin expression following rAAVrh74.MHCK7.DYSF.DV delivery. FIG. 5A demonstrates full-length dysferlin expression by dysferlin immunolabeling (top panels) seen following delivery of dual vectors to left tibialis anterior (LTA). Dysferlin expression persisted through 1, 3, and 6 months post-treatment with no aberrant response in pathology (H&E, lower panels). Scale bar, 100 μ m. N=4 per timepoint. FIG. 5B shows western blot for 1, 3, 6 month samples demonstrating expression of full-length dysferlin in injected LTAs (2 per group). γ -tubulin used as loading control. FIG. 5C shows a biodistribution plot of vector genomes per μ g genomic DNA at 3 and 6 months post-injection for various tissues. Note: the LTA was treated; logarithmic axis.

[0103] FIG. 6 shows Western blot analysis of target muscle (LTA) and non-target tissues from 4 individual animals treated by intramuscular injection with rAAVrh.74.MHCK7.DYSF.DV at 3 or 12 month endpoints.

[0104] FIGS. 7A-7C show dysferlin expression following systemic delivery of AAVrh.74.MHCK7.DYSF.DV. FIG. 7A shows dysferlin immunolabeling of tissues after systemic delivery of 6×10^{12} vg (2.4×10^{13} vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard) AAVrh.74.MHCK7.DYSF.DV (n=6 per dose). Muscles shown are heart, gastrocnemius, diaphragm and quadriceps for Dysf^{-/-}, treated (AAV.DV) and wild-type (WT) tissues. FIG. 7B shows quantification of centralized nuclei in the tibialis anterior (LTA), gastrocnemius (RGAS), quadriceps (LQD), triceps (RTri) and diaphragm. *p<0.05 significant difference between sample and wild-type, # no significant difference between sample and wildtype. FIG. 7C shows a western blot of tissue lysates (H: heart, G: gastrocnemius, Q: quadriceps, D: diaphragm) demonstrating full length dysferlin band at 237 kD, γ -tubulin included as a loading control.

[0105] FIG. 8 shows dose-dependent membrane resealing activity following AAVrh.74.DYSF.DV delivery.

[0106] FIG. 9 shows a reversal of fibrosis and inflammation following systemic delivery of AAVrh.74.MHCK7.DYSF.DV. BlaJ mice were treated with 6×10^{12} vg (2.4×10^{13} vg/kg),

based on a supercoiled DNA or plasmid as the quantitation standard.

AAVrh.74.MHCK7.DYSF.DV (n=6). The psoas muscle was removed and analyzed for the presence of fibrosis (middle column) and CD8 mononuclear cells. There was a significant reduction in both parameters following gene delivery.

[0107] FIGS. 10A-10B demonstrate that systemic delivery of rAAVrh.74.MHCK7.DYSF.DV restores functional deficits in Dysf^{-/-} mice. **FIG. 10A:** Diaphragm muscle strips were harvested and subjected to a protocol to assess specific force. Treated diaphragms demonstrated significant improvement in force (**P> 0.01, ANOVA) which was not different from wild-type force at both doses [2e12 vg total AAV.DYSF DV (8e13 vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard), or 6e12 vg total AAV.DYSF.DV (2.4e13 vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard)]. **FIG. 10B** shows there was a dose dependent response in membrane resealing. There was no significant improvement at low dose.

[0108] FIGS. 11A-11D shows results from monitoring of T cell responses to AAV capsid and dysferlin. Peripheral blood mononuclear cells were isolated and exposed to peptides comprising the AAV5 and AAVrh.74 capsid (blue bars) as well as human dysferlin (green). T cell responses to AAV5 capsid and dysferlin were monitored at 3 months (**FIG. 11A**) and 6 months (**FIG. 11B**). T cell responses to AAVrh.74 capsids and dysferlin were monitored at 3 months (**FIG. 11C**) and 6 months (**FIG. 11D**).

[0109] FIGS. 12A-12C show dysferlin expression in non-human primates. **FIG. 12A** shows histology (H&E) and dysferlin immunofluorescence (IF) images of NHP tissue at 3 and 6 months post-injection of either AAV5.DYSF or AAVrh.74.DYSF.DV. H&E stained sections show lack of immune infiltration and necrosis of fibers. IF sections show overexpression of dysferlin in injected tissues as compared to native (sham). **FIG. 12B** shows western blot image of tissues from 3 and 6 months post-injection for both AAV5.DYSF and AAVrh.74.DYSF.DV. Importantly, injected tissues demonstrate an overexpression of dysferlin as compared to sham control. Positive (+) control is wild-type mouse tissue and the negative (-) control is 129-Dysf^{-/-} uninjected tissue. **FIG. 12C** shows biodistribution of vector genomes following IM injection with AAVrh.74.DYSF.DV into the left TA, note logarithmic scale.

[0110] **FIG. 13** demonstrates the use of anti-FLAG to confirm vector derived dysferlin expression. An N-terminal FLAG tag was used to discriminate between endogenous and AAV derived dysferlin.

DETAILED DESCRIPTION

[0111] Definitions

[0112] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. While not explicitly defined below, such terms should be interpreted according to their common meaning.

[0113] The terminology used in the description herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety.

[0114] The practice of the present technology will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, microbiology, cell biology, and recombinant DNA, which are within the skill of the art.

[0115] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the disclosure also contemplates that in some embodiments, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0116] Unless explicitly indicated otherwise, all specified embodiments, features, and terms intend to include both the recited embodiment, feature, or term and biological equivalents thereof.

[0117] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1.0 or 0.1, as appropriate, or alternatively by a variation of +/- 15 %, or

alternatively 10%, or alternatively 5%, or alternatively 2% and such ranges are included. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term “about”. It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0118] The practice of the present technology will employ, unless otherwise indicated, conventional techniques of organic chemistry, pharmacology, immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual*, 2nd edition (1989); *Current Protocols In Molecular Biology* (F. M. Ausubel, *et al.* eds., (1987)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, a Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)).

[0119] As used herein, the terms “increased”, “decreased”, “high”, “low” or any grammatical variation thereof refer to a variation of about 90%, 80%, 50%, 20%, 10%, 5%, 1%, 0.5%, or even 0.1% of the reference composition, polypeptide, protein, etc.

[0120] The terms or “acceptable,” “effective,” or “sufficient” when used to describe the selection of any components, ranges, dose forms, etc. disclosed herein intend that said component, range, dose form, etc. is suitable for the disclosed purpose.

[0121] Also as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0122] It is to be inferred without explicit recitation and unless otherwise intended, that when the present disclosure relates to a polypeptide, protein, polynucleotide or antibody, an equivalent or a biologically equivalent of such is intended within the scope of this disclosure. As used herein, the term “biological equivalent thereof” is intended to be synonymous with “equivalent thereof” when referring to a reference protein, antibody, polypeptide or nucleic acid, intends those having minimal sequence identity while still maintaining desired structure or functionality. Unless specifically recited herein, it is contemplated that any polynucleotide, polypeptide or protein mentioned herein also includes equivalents thereof. For example, an equivalent intends at least about 70% homology or identity, or at least 80 % homology or identity and alternatively, or at least about 85 %, or alternatively at least about 90 %, or alternatively at least about 95 %, or alternatively 98 % percent homology or identity across the length of the reference sequence and exhibits substantially equivalent biological activity

to the reference protein, polypeptide or nucleic acid. Alternatively, when referring to polynucleotides, an equivalent thereof is in one aspect, a polynucleotide that hybridizes under stringent conditions to the reference polynucleotide or its complement that in a further aspect, has the same or similar activity or function as the reference polynucleotide or its complement

[0123] An equivalent of a protein or a polypeptide (referred to herein as the reference) shares at least 50% (or at least 60%, or at least 70%, or at least 80%, or at least 90%) identity to the reference and retains the reference's function and manufacturability.

[0124] As used herein, the terms "function," "activity," and "enzymatic activity" are used interchangeably. Loss of dysferlin has been shown to compromise Ca²⁺-dependent membrane repair in skeletal muscle (Song *et al.*, *Proc. Natl. Acad. Sci. USA* 98:4084-4088, 2001 and Schnepf *et al.*, *J. Virol.* 77:3495-3504, 2003). In addition, dysferlin has been shown to interact with other proteins involved in membrane repair, including annexins A1 and A2, AHNK, and caveolin-3 (Schnepf *et al.*, *J. Virol.* 77:3495-3504, 2003, Duan *et al.*, *J. Virol.* 72:8568-8577, 1998, Donsante *et al.*, *Gene Ther.* 8:1343-1346, 2001, and Monahan *et al.*, *Expert Opin. Drug. Saf.* 1:79-91, 2002). The loss of muscle fiber regenerative capacity is thought to be a contributory consequence of dysferlin deficiency (Song *et al.*, *Gene Ther.* 8:1299-1306, 2001). Dysferlin has also been associated with vesicle trafficking and endocytosis and T tubule formation (Eveson *et al.*, *The Journal of Biological Chemistry* 285:28529-28539, 2010, Klinge *et al.*, *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 21:1768-1776, 2007, and Klinge *et al.*, *Muscle & Nerve* 41:166-173, 2010). Accordingly, examples of activities of dysferlin include, but are not limited to, membrane repair in skeletal muscle, such as membrane resealing, prevention or restoration of muscle fiber regenerative capacity, vesicle trafficking, endocytosis, and transverse (T-) tubule formation. Membrane repair assays, vesicle trafficking assays, and tube formation assays are known in the art and can be used to measure dysferlin activity *in vitro*. See, e.g., Carmeille *et al.*, *Methods Mol. Biol.* 1668:195-207, 2017, Vassilieva and Nusrat, *Methods Mol. Biol.* 440:3-14, 2008, Demonbreun *et al.*, *Am. J. Pathol.* 184(1):248-59, 2014, each of which are incorporated by reference in their entireties. Additional methods for measuring the activity of dysferlin is found, for example, in Grose *et al.*, *PLoS One* 7:e39233, 2012 and Sondergaard *et al.*, *Annals of Clin. Trans. Neurol.* 2:256-270, 2015.

[0125] An equivalent of a polynucleotide (referred to herein as the reference) shares at least 50% (or at least 60%, or at least 70%, or at least 80%, or at least 90%) identity to the

reference, and encodes the same polypeptide as the one encoded by the reference, or encodes an equivalent of the polypeptide encoded by the reference.

[0126] To arrive at a position or a consecutive segment of a test sequence equivalent to (or corresponding to) an/a amino acid/nucleotide residue or a consecutive segment of a reference sequence, a sequence alignment is performed between the test and reference sequences. The positions or segments aligned to each other are determined as equivalents.

[0127] The term “affinity tag” refers to a polypeptide that may be included within a fusion protein to allow detection of the fusion protein and/or purification of the fusion protein from the cellular milieu using a ligand that is able to bind to, i.e., has affinity for, the affinity tag. The ligand may be, but is not limited to, an antibody, a resin, or a complementary polypeptide. An affinity tag may comprise a small peptide, commonly a peptide of approximately 4 to 16 amino acids in length, or it may comprise a larger polypeptide. Commonly used affinity tags include polyarginine, FLAG, V5, polyhistidine, c-Myc, Strep II, maltose binding protein (MBP), N-utilization substance protein A (NusA), thioredoxin (Trx), and glutathione *S*-transferase (GST), among others (for examples, see GST Gene Fusion System Handbook - Sigma-Aldrich). In an embodiment the affinity tag is a polyhistidine tag, for example a His₆ tag (SEQ ID NO: 21). The inclusion of an affinity tag in a fusion protein allows the fusion protein to be purified from the cellular milieu by affinity purification, using an affinity medium that is able to tightly and specifically bind the affinity tag. The affinity medium may comprise, for example, a metal-charged resin or a ligand covalently linked to a stationary phase (matrix) such as agarose or metal beads. For example, polyhistidine tagged fusion proteins (also referred to as His tagged fusion proteins) can be recovered by immobilized metal ion chromatography using Ni²⁺ or Co²⁺ loaded resins, anti-FLAG affinity gels may be used to capture FLAG tagged fusion proteins, and glutathione cross-linked to a solid support such as agarose may be used to capture GST tagged fusion proteins. In one aspect, an affinity tag is a purification tag or marker.

[0128] As used herein the terms “purification”, “purifying”, or “separating” refer to the process of isolating one or more biomaterials (*e.g.*, polynucleotides, polypeptides, or viral vectors) from a complex mixture, such as a cell lysate or a mixture of polypeptides. The purification, separation, or isolation need not be complete, i.e., some components of the complex mixture may remain with the one or more biomaterials (*e.g.*, polynucleotides, polypeptides, or viral vectors) after the purification process. However, the product of purification should be enriched for the one or more biomaterials (*e.g.*, polynucleotides, polypeptides, or viral vectors) relative to the complex mixture before purification and a

significant portion of the other components initially present within the complex mixture should be removed by the purification process.

[0129] The term “cell” as used herein may refer to either a prokaryotic or eukaryotic cell, optionally obtained from a subject or a commercially available source. In some instances, the cell is a host cell, for example, a mammalian cell or a mammalian host cell. In some instances, the host cell is also referred to herein as a production cell or a packaging cell. In some cases, the cell line is a packaging cell line.

[0130] “Eukaryotic cells” comprise all of the life kingdoms except monera. They can be easily distinguished through a membrane-bound nucleus. Animals, plants, fungi, and protists are eukaryotes or organisms whose cells are organized into complex structures by internal membranes and a cytoskeleton. The most characteristic membrane-bound structure is the nucleus. Unless specifically recited, the term “host” includes a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Non-limiting examples of eukaryotic cells or hosts include simian, bovine, porcine, murine, rat, avian, reptilian and human, e.g., HEK293 cells, Chinese Hamster Ovary (CHO) cells, CHO-S cells, CHO-K1 cells, 293T cells, HeLa cells, Baby hamster kidney (BHK) cells, Sf9 cells, stem cells, satellite cells, and muscle cells. Examples of muscle cells include, but are not limited to, skeletal muscle cells, cardiac muscle cells, and smooth muscle cells.

[0131] “Prokaryotic cells” that usually lack a nucleus or any other membrane-bound organelles and are divided into two domains, bacteria and archaea. In addition to chromosomal DNA, these cells can also contain genetic information in a circular loop called an episome. Bacterial cells are very small, roughly the size of an animal mitochondrion (about 1-2 μm in diameter and 10 μm long). Prokaryotic cells feature three major shapes: rod shaped, spherical, and spiral. Instead of going through elaborate replication processes like eukaryotes, bacterial cells divide by binary fission. Examples include but are not limited to *Bacillus* bacteria, *E. coli* bacterium, and *Salmonella* bacterium.

[0132] The term “encode” as it is applied to nucleic acid sequences refers to a polynucleotide which is said to “encode” a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

[0133] The terms “equivalent” or “biological equivalent” are used interchangeably when referring to a particular molecule, biological, or cellular material and intend those having

minimal homology while still maintaining desired structure or functionality (for example, having a similar function or activity). It should be understood, without being explicitly stated that when referring to an equivalent or biological equivalent to a reference polypeptide, protein, or polynucleotide, that an equivalent or biological equivalent has the recited structural relationship to the reference polypeptide, protein, or polynucleotide and equivalent or substantially equivalent biological activity. For example, non-limiting examples of equivalent polypeptides, proteins, or polynucleotides include a polypeptide, protein or polynucleotide having at least 60%, or alternatively at least 65%, or alternatively at least 70%, or alternatively at least 75%, or alternatively 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95% identity thereto or for polypeptide, polynucleotide or protein sequences across the length of the reference polypeptide, polynucleotide, or protein. Alternatively, an equivalent polypeptide is one that is encoded by a polynucleotide or its complement that hybridizes under conditions of high stringency to a polynucleotide encoding such reference polypeptide sequences and that have substantially equivalent or equivalent biological activity. Conditions of high stringency are described herein and incorporated herein by reference. Alternatively, an equivalent thereof is a polypeptide encoded by a polynucleotide or a complement thereto, having at least 70%, or alternatively at least 75%, or alternatively 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95% identity, or at least 97% sequence identity across the length of the reference polynucleotide to the reference polynucleotide, e.g., the wild-type polynucleotide. Such equivalent polypeptides have the same biological activity as the polypeptide encoded by the reference polynucleotide.

[0134] Non-limiting examples of equivalent polynucleotides, include a polynucleotide having at least 60%, or alternatively at least 65%, or alternatively at least 70%, or alternatively at least 75%, or alternatively 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95%, or alternatively at least 97%, identity to a reference polynucleotide. An equivalent also intends a polynucleotide or its complement that hybridizes under conditions of high stringency to a reference polynucleotide. Such equivalent polynucleotides have the same biological activity as the reference polynucleotide.

[0135] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) having a certain percentage (for example, 80%, 85%, 90%, or 95%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences across the length of the reference polynucleotide. The alignment and the percent homology or sequence identity can be determined using software

programs known in the art, for example those described in Current Protocols in Molecular Biology (Ausubel et al., eds. 1987) Supplement 30, section 7.7.18, Table 7.7.1. In certain embodiments, default parameters are used for alignment. A non-limiting exemplary alignment program is BLAST, using default parameters. In particular, exemplary programs include BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the following Internet address:

ncbi.nlm.nih.gov/cgi-bin/BLAST. Sequence identity and percent identity can be determined by incorporating them into clustalW (available at the web address: genome.jp/tools/clustalw/, last accessed on Jan. 13, 2017) or Clustal Omega (available at ebi.ac.uk/Tools/msa/clustalo/).

[0136] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence that may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present disclosure.

[0137] As used herein, the term “at least 90% identical” refers to an identity of two compared sequences (polynucleotides or polypeptides) of about 90% to about 100%. It also include an identity of at least at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, about 91% to about 100%, about 92% to about 100%, about 93% to about 100%, about 94% to about 100%, about 95% to about 100%, about 96% to about 100%, about 97% to about 100%, about 98% to about 100%, or about 99% to about 100%.

[0138] As used herein, the terms “retain” “similar” and “same” are used interchangeably while describing a function, an activity or an functional activity of a polynucleotide, a protein and/or a peptide, referring to a functional activity of at least about 20% (including but not limited to: at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or about 100%) of the activity of the reference protein, polynucleotide and/or peptide.

[0139] “Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0140] Examples of stringent hybridization conditions include: incubation temperatures of about 25° C. to about 37° C.; hybridization buffer concentrations of about 6×SSC to about 10×SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4×SSC to about 8×SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40° C. to about 50° C.; buffer concentrations of about 9×SSC to about 2×SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5×SSC to about 2×SSC. Examples of high stringency conditions include: incubation temperatures of about 55° C. to about 68° C.; buffer concentrations of about 1×SSC to about 0.1×SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1×SSC, 0.1×SSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed. In one aspect, an equivalent polynucleotide is one that hybridizes under stringent conditions to a reference polynucleotide or its complement. In another aspect, an equivalent polypeptide is a polypeptide that is encoded by a polynucleotide is one that hybridizes under stringent conditions to a reference polynucleotide or its complement.

[0141] As used herein, “expression” refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[0142] As used herein, the term “functional” may be used to modify any molecule, biological, or cellular material to intend that it accomplishes a particular, specified effect.

[0143] As used herein, the terms “nucleic acid sequence” and “polynucleotide” are used interchangeably to refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to,

single-, double-, or multi-stranded DNA or RNA, genomic DNA, complementary DNA (cDNA), DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. In certain embodiments, the polynucleotide comprises and/or encodes a messenger RNA (mRNA), a short hairpin RNA, and/or small hairpin RNA. In one embodiment, the polynucleotide is or encodes an mRNA. In certain embodiments, the polynucleotide is a double-strand (ds) DNA, such as an engineered ds DNA or a ds cDNA synthesized from a single-stranded RNA.

[0144] The term “protein”, “peptide” and “polypeptide” are used interchangeably and in their broadest sense to refer to a compound of two or more subunits of amino acids, amino acid analogs or peptidomimetics. The subunits may be linked by peptide bonds. In another aspect, the subunit may be linked by other bonds, e.g., ester, ether, etc. A protein or peptide must contain at least two amino acids and no limitation is placed on the maximum number of amino acids which may comprise a protein’s or peptide’s sequence. As used herein the term “amino acid” refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, amino acid analogs and peptidomimetics.

[0145] As used herein, a consecutive amino acid sequence refers to a sequence having at least two amino acids. However, it is noted that a consecutive amino acid sequence of a first part and a second part does not limit the amino acid sequence to have the first part directly conjugated to the second part. It is also possible that the first part is linked to the second part via a third part, such as a link, thus forming one consecutive amino acid sequence.

[0146] As used herein, the terms “conjugate,” “conjugated,” “conjugating,” and “conjugation” refer to the formation of a bond between molecules, and in particular between two amino acid sequences and/or two polypeptides. Conjugation can be direct (i.e. a bond) or indirect (i.e. via a further molecule). The conjugation can be covalent or non-covalent.

[0147] As used herein a consecutive amino acid sequence may comprise two or more polypeptides conjugated with each other directly or indirectly (for example via a linker).

[0148] As used herein, the term “recombinant expression system” refers to a genetic construct or constructs for the expression of certain genetic material formed by recombination.

[0149] A “gene delivery vehicle” is defined as any molecule that can carry inserted polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, micelles biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; lipid nanoparticles; polypeptides; polysaccharides; lipopolysaccharides; artificial viral

envelopes; metal particles; and bacteria, or viruses, such as rabies virus, flavivirus, lentivirus, baculovirus, adenovirus and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

[0150] A polynucleotide disclosed herein can be delivered to a cell or tissue using a gene delivery vehicle. “Gene delivery,” “gene transfer” “mRNA-based delivery”, “transducing,” and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to as a “transgene”) into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes, including for example protamine complexes, lipid nanoparticles, polymeric nanoparticles, lipid-polymer hybrid nanoparticles, and inorganic nanoparticles, or combinations thereof) as well as techniques facilitating the delivery of “naked” polynucleotides (such as electroporation, “gene gun” delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide can be unmodified or can comprise one or more modifications; for example, a modified mRNA may comprise ARCA capping; enzymatic polyadenylation to add a tail of 100-250 adenosine residues (SEQ ID NO: 22); and substitution of one or both of cytidine with 5-methylcytidine and/or uridine with pseudouridine. The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art and described herein.

[0151] A “plasmid” is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded. Plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively the proteins produced may act as toxins under similar circumstances.

[0152] “Plasmids” used in genetic engineering are called “plasmid vectors”. Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics and a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene.

[0153] A “yeast artificial chromosome” or “YAC” refers to a vector used to clone large DNA fragments (larger than 100 kb and up to 3000 kb). It is an artificially constructed chromosome and contains the telomeric, centromeric, and replication origin sequences needed for replication and preservation in yeast cells. Built using an initial circular plasmid, they are linearized by using restriction enzymes, and then DNA ligase can add a sequence or gene of interest within the linear molecule by the use of cohesive ends. Yeast expression vectors, such as YACs, YIps (yeast integrating plasmid), and YEps (yeast episomal plasmid), are extremely useful as one can get eukaryotic protein products with posttranslational modifications as yeasts are themselves eukaryotic cells, however YACs have been found to be more unstable than BACs, producing chimeric effects.

[0154] As used herein, the term “viral capsid” or “capsid” refers to the proteinaceous shell or coat of a viral particle. Capsids function to encapsidate, protect, transport, and release into host cell a viral genome. Capsids are generally comprised of oligomeric structural subunits of protein (“capsid proteins”). As used herein, the term “encapsidated” means enclosed within a viral capsid.

[0155] As used herein, the term “helper” in reference to a virus or plasmid refers to a virus or plasmid used to provide the additional components necessary for replication and packaging of a viral particle or recombinant viral particle, such as the modified AAV disclosed herein. The components encoded by a helper virus may include any genes required for virion assembly, encapsidation, genome replication, and/or packaging. For example, the helper virus may encode necessary enzymes for the replication of the viral genome. Non-limiting examples of helper viruses and plasmids suitable for use with AAV constructs include pHELP (plasmid), adenovirus (virus), or herpesvirus (virus).

[0156] As used herein, a biological sample, or a sample, can be obtained from a subject, cell line or cultured cell or tissue. Exemplary samples include, but are not limited to, cell

sample, tissue sample, liquid samples such as blood and other liquid samples of biological origin (including, but not limited to, ocular fluids (aqueous and vitreous humor), peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, bronchoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, ascites, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions/flushing, synovial fluid, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, or umbilical cord blood.

[0157] As used herein, the term “detectable marker” refers to at least one marker capable of directly or indirectly, producing a detectable signal. A non-exhaustive list of this marker includes enzymes which produce a detectable signal, for example by colorimetry, fluorescence, luminescence, such as horseradish peroxidase, alkaline phosphatase, β -galactosidase, glucose6 phosphate dehydrogenase, chromophores such as fluorescent, luminescent dyes, groups with electron density detected by electron microscopy or by their electrical property such as conductivity, amperometry, voltammetry, impedance, detectable groups, for example whose molecules are of sufficient size to induce detectable modifications in their physical and/or chemical properties, such detection may be accomplished by optical methods such as diffraction, surface plasmon resonance, surface variation, the contact angle change or physical methods such as atomic force spectroscopy, tunnel effect, or radioactive molecules such as ^{32}P , ^{35}S , ^{89}Zr or ^{125}I .

[0158] As used herein, the term “purification marker” refers to at least one marker useful for purification or identification. A non-exhaustive list of this marker includes His, lacZ, GST, maltose-binding protein, NusA, BCCP, c-myc, CaM, FLAG, GFP, YFP, cherry, thioredoxin, poly(NANP), V5, Snap, HA, chitin-binding protein, Softag 1, Softag 3, Strep, or S-protein. Suitable direct or indirect fluorescence marker comprise FLAG, GFP, YFP, RFP, dTomato, cherry, Cy3, Cy 5, Cy 5.5, Cy 7, DNP, AMCA, Biotin, Digoxigenin, Tamra, Texas Red, rhodamine, Alexa fluors, FITC, TRITC or any other fluorescent dye or hapten.

[0159] As used herein, an epitope tag is a biological structure or sequence, such as a protein or carbohydrate, which acts as an antigen that is recognized by an antibody. In certain embodiments, an epitope tag is used interchangeably with a purification marker and/or an affinity tag.

[0160] A “composition” is intended to mean a combination of two or more compounds, such as a combination of an active polypeptide, polynucleotide, viral vector, or antibody and another compound or composition, inert (e.g., a detectable label) or active (e.g., a gene delivery vehicle).

[0161] A “pharmaceutical composition” is intended to include the combination of an active polypeptide, polynucleotide or antibody with a carrier, inert or active such as a solid support, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

[0162] As used herein, the term “pharmaceutically acceptable carrier” encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin (1975) Remington’s Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton).

[0163] A “subject,” “individual” or “patient” is used interchangeably herein, and refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, non-human primates, murines, rats, rabbit, simians, bovines, ovine, porcine, canines, feline, farm animals, sport animals, pets, equine, and primates, particularly human. Besides being useful for human treatment, the present invention is also useful for veterinary treatment of companion mammals, exotic animals and domesticated animals, including mammals, rodents, and the like. In one embodiment, the mammals include horses, dogs, and cats. In another embodiment of the present invention, the human is an adolescent or infant under the age of eighteen years of age.

[0164] “Treating” or “treatment” of a disease includes: (1) preventing the disease, i.e., causing the clinical symptoms of the disease not to develop in a patient that may be predisposed to the disease but does not yet experience or display symptoms of the disease; (2) inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms; or (3) relieving the disease, i.e., causing regression of the disease or its clinical symptoms. In one aspect, the term “treatment” excludes prevention or prophylaxis.

[0165] The term “suffering” as it related to the term “treatment” refers to a patient or individual who has been diagnosed with or is predisposed to a disease.

[0166] An “effective amount” is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the bioavailability of the therapeutic agent, the

route of administration, etc. It is understood, however, that specific dose levels of the therapeutic agents of the present invention for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, and diet of the subject, the time of administration, the rate of excretion, the drug combination, and the severity of the particular disorder being treated and form of administration. Treatment dosages generally may be titrated to optimize safety and efficacy. In one aspect, an effective amount is a therapeutically effective amount. Typically, dosage-effect relationships from *in vitro* and/or *in vivo* tests initially can provide useful guidance on the proper doses for patient administration. In general, one will desire to administer an amount of the compound that is effective to achieve a serum level commensurate with the concentrations found to be effective *in vitro*. Determination of these parameters is well within the skill of the art. These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks. Consistent with this definition, as used herein, the term “therapeutically effective amount” is an amount sufficient to inhibit RNA virus replication *ex vivo*, *in vitro* or *in vivo*.

[0167] The term administration shall include without limitation, administration by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.) and can be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants, excipients, and vehicles appropriate for each route of administration. The invention is not limited by the route of administration, the formulation or dosing schedule.

[0168] As used herein, the term “AAV” is a standard abbreviation for adeno-associated virus. Adeno-associated virus is a single-stranded DNA parvovirus that grows only in cells in which certain functions are provided by a co-infecting helper virus. There are currently thirteen serotypes of AAV that have been characterized. General information and reviews of AAV can be found in, for example, Carter, *Handbook of Parvoviruses* 1:169-228, 1989, and Berns, *Virology* 1743-1764, 1999. However, it is fully expected that these same principles will be applicable to additional AAV serotypes since it is well known that the various serotypes are quite closely related, both structurally and functionally, even at the genetic level. (See, for example, Blacklowe, *Parvoviruses and Human Disease* 165-174, 1988, J. R. Pattison, ed.; and Rose, *Comprehensive Virology* 3:1-61, 1974). For example, all AAV serotypes apparently exhibit very similar replication properties mediated by homologous rep

genes; and all bear three related capsid proteins such as those expressed in AAV2. The degree of relatedness is further suggested by heteroduplex analysis which reveals extensive cross-hybridization between serotypes along the length of the genome; and the presence of analogous self-annealing segments at the termini that correspond to “inverted terminal repeat sequences” (ITRs). The similar infectivity patterns also suggest that the replication functions in each serotype are under similar regulatory control.

[0169] An “AAV expression cassette” as used herein refers to a nucleotide sequence comprising one or more polynucleotides of interest (or transgenes) that are flanked by AAV terminal repeat sequences (ITRs). Such AAV expression cassette can be replicated and packaged into infectious viral particles (*e.g.*, AAV vectors) when present in a host cell that has been transfected with a vector encoding and expressing rep and cap gene products.

[0170] An “AAV virion” or “AAV vector” or “AAV viral particle” or “AAV vector particle” refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide AAV expression cassette. If the particle comprises a heterologous polynucleotide (*i.e.* a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an “AAV vector particle” or simply an “AAV vector”. Thus, production of AAV vector particle necessarily includes production of AAV expression cassette, as such a plasmid is contained within an AAV vector particle.

[0171] Adeno-associated virus (AAV) is a replication-deficient parvovirus, the single-stranded DNA genome of which is about 4.7 kb in length including 145 nucleotide inverted terminal repeat (ITRs). There are multiple serotypes of AAV. The nucleotide sequences of the genomes of the AAV serotypes are known. For example, the nucleotide sequence of the AAV serotype 2 (AAV2) genome is presented in Srivastava *et al.*, *J Virol*, 45: 555-564 (1983) as corrected by Ruffing *et al.*, *J Gen Virol*, 75: 3385-3392 (1994). As other examples, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-3 is provided in GenBank Accession No. NC_1829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_001862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively (see also U.S. Patent Nos. 7,282,199 and 7,790,449 relating to AAV-8); the AAV-9 genome is provided in Gao *et al.*, *J. Virol.*, 78: 6381-6388 (2004); the AAV-10 genome is provided in *Mol. Ther.*, 13(1): 67-76 (2006); and the AAV-11 genome is provided in *Virology*, 330(2):

375-383 (2004). Cloning of the AAVrh.74 serotype is described in Rodino-Klapac., *et al. Journal of translational medicine* **5**, 45 (2007). *Cis*-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the ITRs. Three AAV promoters (named p5, p19, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding rep and cap genes. The two rep promoters (p5 and p19), coupled with the differential splicing of the single AAV intron (*e.g.*, at AAV2 nucleotides 2107 and 2227), result in the production of four rep proteins (rep 78, rep 68, rep 52, and rep 40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins. A single consensus polyadenylation site is located at map position 95 of the AAV genome. The life cycle and genetics of AAV are reviewed in Muzyczka, *Current Topics in Microbiology and Immunology*, **158**: 97-129 (1992).

[0172] Recombinant AAV genomes of the disclosure comprise nucleic acid molecule of the invention and one or more AAV ITRs flanking a nucleic acid molecule. AAV DNA in the rAAV genomes may be from any AAV serotype for which a recombinant virus can be derived including, but not limited to, AAV serotypes AAVrh.74, AAVrh.10, AAVrh.20, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12 and AAV-13. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, for example, Marsic *et al.*, *Molecular Therapy*, **22**(11): 1900-1909 (2014). As noted in the Background section above, the nucleotide sequences of the genomes of various AAV serotypes are known in the art. In some embodiments, to promote skeletal muscle specific expression, AAV1, AAV6, AAV8 or AAVrh.74 is used.

[0173] As used in the specification and claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells, including mixtures thereof.

[0174] As used herein, the term “comprising” or “comprises” is intended to mean that the compositions and methods include the recited elements, but not excluding others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the stated

purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention or process steps to produce a composition or achieve an intended result. Embodiments defined by each of these transition terms are within the scope of this invention.

[0175] The term “isolated” as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively that are present in the natural source of the macromolecule. The term “isolated nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments. The term “isolated” is also used herein to refer to polypeptides, proteins and/or host cells that are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides. In other embodiments, the term “isolated” means separated from constituents, cellular and otherwise, in which the cell, tissue, polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, which are normally associated in nature. For example, an isolated cell is a cell that is separated from tissue or cells of dissimilar phenotype or genotype. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, does not require “isolation” to distinguish it from its naturally occurring counterpart.

[0176] The term “recombinant” as used herein with respect to polypeptides or polynucleotides, such as DNA or RNA, refers to molecules formed by laboratory methods of recombination, such as molecular cloning. Molecular cloning techniques are known in the art and may include, but is not limited to, PCR amplification of a polynucleotide, enzymatic digestion of a polynucleotide, ligation of a polynucleotide into an expression cassette (*e.g.*, mammalian expression cassette), transformation, transfection or transduction of a cell with the polynucleotide, and expression of the polynucleotide to produce the polypeptide. *See e.g.*, Green and Sambrook, *Molecular Cloning: A Laboratory Manual*, 2012. The term “recombinant polynucleotide” is meant to include fragments of protein-encoding polynucleotides. For instance, a recombinant polynucleotide may include a fragment of the polynucleotide that encodes for a human dysferlin protein. A recombinant polynucleotide may be produced by PCR amplification of a fragment of a protein-encoding polynucleotide. A recombinant polypeptide may be produced by expression of one or more recombinant polynucleotides.

[0177] Disclosed herein are polynucleotides encoding fragments of a human dysferlin (hDSYSF) protein. Further disclosed herein are plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions comprising polynucleotides encoding fragments of a human dysferlin (hDSYSF) protein. Also disclosed herein are methods of making and using such polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions.

[0178] Disclosed herein is a recombinant polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide comprises a first nucleotide sequence, wherein the first nucleotide sequence consists of: (a) the nucleotide sequence of SEQ ID NO: 1, 6, or 18; (b) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18; (c) the nucleotide sequence of SEQ ID NO: 13 or 15; (d) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15; (e) a nucleotide sequence encoding the hDYSF protein, wherein the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (f) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of (e) across the full length of the nucleotide sequence of (e).

[0179] Further disclosed herein is a recombinant polynucleotide sequence encoding a fragment of a human dysferlin protein, wherein the recombinant polynucleotide comprises a first nucleotide sequence, wherein the first nucleotide sequence consists of: (a) the nucleotide sequence of SEQ ID NO: 2, 8, or 19; (b) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19; (c) the nucleotide sequence of SEQ ID NO: 14 or 16; (d) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16; (e) a polynucleotide sequence encoding the hDYSF protein, wherein the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (f) a polynucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, or 100% identical to the polynucleotide sequence of (e) across the full length of the nucleotide sequence of (e).

[0180] Further disclosed herein are adeno-associated viral (AAV) vectors. In some embodiments, an adeno-associated viral (AAV) vector comprises: (a) a first inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 1 or 6; (ii) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6; (iii) the nucleotide sequence of SEQ ID NO: 13 or 15; (iv) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across the full length of SEQ ID NO: 13 or 15; (v) a nucleotide sequence encoding the hDYSF protein, wherein the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide is flanked by the first and second ITRs.

[0181] In some embodiments, an adeno-associated viral (AAV) vector comprises: (a) a first inverted terminal repeat (ITR); (b) a polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide consists of: (i) the nucleotide sequence of SEQ ID NO: 2 or 8; (ii) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ ID NO: 2 or 8; (iii) the nucleotide sequence of SEQ ID NO: 14 or 16; (iv) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16; (v) a nucleotide sequence encoding the hDYSF protein, wherein the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide is flanked by the first and second ITRs.

[0182] Further disclosed herein are adeno-associated viral (AAV) expression cassettes. In some embodiments, the AAV expression cassette comprises: (a) a first inverted terminal repeat (ITR), wherein the first ITR comprises any of the ITRs disclosed herein; (b) any of the 5' hDYSF polynucleotides disclosed herein; and (c) a second ITR, wherein the second ITR comprises any of the ITRs disclosed herein, wherein the 5' hDYSF polynucleotide of (b) is flanked by the first and second ITRs of (a) and (c).

[0183] In some embodiments, the AAV expression cassette comprises: (a) a first inverted terminal repeat (ITR), wherein the first ITR comprises any of the ITRs disclosed herein; (b) any of the 3' hDYSF polynucleotides disclosed herein; and (c) a second ITR, wherein the second ITR comprises any of the ITRs disclosed herein, wherein the 3' hDYSF polynucleotide of (b) is flanked by the first and second ITRs of (a) and (c).

[0184] Further disclosed herein are adeno-associated viral (AAV) vectors. In some embodiments, the AAV vector comprises: (a) a first inverted terminal repeat (ITR), wherein the first ITR comprises any of the ITRs disclosed herein; (b) any of the 5' hDYSF polynucleotides disclosed herein; and (c) a second ITR, wherein the second ITR comprises any of the ITRs disclosed herein, wherein the 5' hDYSF polynucleotide of (b) is flanked by the first and second ITRs of (a) and (c).

[0185] In some embodiments, the AAV vector comprises: (a) a first inverted terminal repeat (ITR), wherein the first ITR comprises any of the ITRs disclosed herein; (b) any of the 3' hDYSF polynucleotides disclosed herein; and (c) a second ITR, wherein the second ITR comprises any of the ITRs disclosed herein, wherein the 3' hDYSF polynucleotide of (b) is flanked by the first and second ITRs of (a) and (c).

[0186] Further disclosed herein are dual adeno-associated viral (AAV) vector systems. In some embodiments, the dual AAV vector system comprises: (a) a first AAV vector, wherein the first AAV vector comprises any of the 5' hDYSF polynucleotides disclosed herein; and (b) a second AAV vector, wherein the second AAV vector comprises any of the 3' hDYSF polynucleotides disclosed herein.

[0187] Further disclosed herein are dual adeno-associated viral (AAV) vector systems. In some embodiments, the dual AAV vector system comprises, consists of, or consists essentially of: (a) a first AAV vector, wherein the first AAV vector comprises, consists of, or consists essentially of any of the 5' hDYSF AAV vectors disclosed herein; and (b) a second AAV vector, wherein the second AAV vector comprises, consists of, or consists essentially of any of the 3' hDYSF AAV vectors disclosed herein.

[0188] Further disclosed herein are adeno-associated viral (AAV) vectors. In some embodiments, the AAV vectors comprise any of the 5' hDYSF polynucleotides disclosed herein.

[0189] In some embodiments, the AAV vectors comprise any of the 3' hDYSF polynucleotides disclosed herein.

[0190] In some embodiments, the polynucleotides, plasmids, viral vectors (e.g., viruses or viral particles), vector systems, viral packaging systems, cells, and compositions further comprise one or more nucleotide sequences comprising, consisting of, or consisting essentially of an inverted terminal repeat (ITR), promoter, intron, selection marker, or origin of replication (ORI).

[0191] In some embodiments, the polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions further comprise one or more additional nucleotide sequences comprising an inverted terminal repeat (ITR), selection marker, origin of replication (ORI), untranslated region (UTR), or polyadenylation (polyA) signal.

[0192] Further disclosed herein are methods of treating a dysferlinopathy. In some embodiments, a method of treating a dysferlinopathy comprises administering to a subject in need thereof any of polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions disclosed herein.

[0193] Further disclosed herein are uses of any of the polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions disclosed herein in the manufacture of a medicament for the treatment of a dysferlinopathy.

[0194] Homologous Recombination and hDYSF Fragments

[0195] AAV-mediated gene therapy presents a desirable treatment strategy for multiple diseases; however, it is hindered by the restrictive 4.7 kb packaging limit of the AAV virion. Of particular interest are diseases with no current cure or effective therapy, such as dysferlinopathies. Disclosed herein is a method of making or producing a full length of dysferlin gene by homologous recombination of two partially genomes. In one example, the two partially packaged genomes is shown in FIG 1, as pAAV.MHCK7.DYSF5'.PTG and pAAV.DYSF3'.POLYA. Once the two genomes, whether through viral delivery by being packaged into AAV vector or non-viral methods (e.g., LNP), are delivered to a cell (e.g., myocytes), they generate a transcript comprising the full length dysferlin coding region, leading to expression of a functional dysferlin protein. The overlap region between the two polynucleotides facilitates the homologous recombination that leads to the transcript

containing the full-length dysferlin gene. By separating the full length dysferlin gene to two partially packaged genomes, this method successfully bypasses the AAV packaging limitation and produce a functional, full-length dysferlin gene. In one embodiment, the transcript is an expression cassette comprising the sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20. In one embodiment, the transcript is an expression cassette comprising the sequence of SEQ ID NO: 20.

[0196] In some embodiments, disclosed herein is a recombinant polynucleotide encoding a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide sequence comprising a nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20. In some instances, the recombinant polynucleotide comprising a nucleotide sequence of SEQ ID NO: 20. In some instances, disclosed herein is a method of making the recombinant polypeptide. In some cases, the method comprises contacting a cell with the recombinant polynucleotide encoding a 5' fragment of the hDYSF protein and a second recombinant polynucleotide encoding a 3' fragment of the hDYSF protein. In some cases, the method comprises cotacting a cell with a dual AAV vector system described herein. In some cases, the cell is a eukaryotic cell, optionally a muscle cell, a heart cell, and/or a liver cell. In some cases, the method comprises administering to a subject with the recombinant polynucleotide encoding a 5' fragment of the hDYSF protein and a second recombinant polynucleotide encoding a 3' fragment of the hDYSF protein. In some cases, the method comprises administering to a subject a dual AAV vector system described herein. In some cases, the method comprises treating muscular dystrophy of a subject, by expressing the recombinant polynucleotide comprising SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20. In some cases, the subject suffers from dysferlinopathy, optionally selected from LGMD2B or Miyoshi myopathy.

[0197] Also disclosed herein are two recombinant polynucleotides, each encoding a fragment of human dysferlin (hDYSF) protein, that can lead to the production of the full length dysferlin gene by homologous recombination as described above. In some embodiments, a recombinant polynucleotide encodes a 5' fragment of the hDYSF protein. In some embodiments, the recombinant polynucleotide encoding the 5' fragment of the hDYSF protein is referred to as the 5' hDYSF polynucleotide. In some embodiments, a recombinant

polynucleotide encodes a 3' fragment of the hDYSF protein. In some embodiments, the recombinant polynucleotide encoding the 3' fragment of the hDYSF protein is referred to as the 3' hDYSF polynucleotide.

[0198] *5' hDYSF polynucleotide*

[0199] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of between 3330-3365, 3330-3360, 3330-3355, 3335-3365, 3335-3350, 3340-3365, 3340-3360, or 3340-3355 consecutive nucleotides of a region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of 3360, 3359, 3358, 3357, 3356, 3355, 3354, 3353, 3352, 3351, 3350, 3349, 3348, 3347, 3346, 3345, 3344, 3343, 3342, 3341, or 3340 or fewer consecutive nucleotides of a region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0200] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of between 3330-3365, 3330-3360, 3330-3355, 3335-3365, 3335-3350, 3340-3365, 3340-3360, or 3340-3355 consecutive nucleotides of a region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide is at least 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% to the nucleotide sequence of SEQ ID NO: 11 across

the full length of the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of 3360, 3359, 3358, 3357, 3356, 3355, 3354, 3353, 3352, 3351, 3350, 3349, 3348, 3347, 3346, 3345, 3344, 3343, 3342, 3341, or 3340 or fewer consecutive nucleotides of a region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide is at least 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide is at least 85% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide is at least 90% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11.

3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide is at least 95% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0201] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of between 3330-3365, 3330-3360, 3330-3355, 3335-3365, 3335-3350, 3340-3365, 3340-3360, or 3340-3355 consecutive nucleotides of a region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide comprises 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or fewer nucleotide mismatches in the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of 3360, 3359, 3358, 3357, 3356, 3355, 3354, 3353, 3352, 3351, 3350, 3349, 3348, 3347, 3346, 3345, 3344, 3343, 3342, 3341, or 3340 or fewer consecutive nucleotides of a region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide comprises 30, 25, 20, 19, 18,

17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or fewer nucleotide mismatches in the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises 15 or fewer nucleotide mismatches in the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises 10 or fewer nucleotide mismatches in the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises 5 or fewer nucleotide mismatches in the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises 1 nucleotide mismatch in the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises at least 1 nucleotide mismatch in the region between nucleotides 100-4000, 100-3900, 100-3800, 100-3750, 100-3716, 150-4000, 150-3900, 150-3800, 150-

3750, 150-3716, 200-4000, 200-3900, 200-3800, 200-3750, 200-3716, 250-4000, 250-3900, 250-3800, 250-3750, 250-3716, 300-4000, 300-3900, 300-3800, 300-3750, 300-3716, 350-4000, 350-3900, 350-3800, 350-3750, 350-3716, 370-4000, 370-3900, 370-3800, 370-3750, 370-3716, 377-4000, 377-3900, 377-3800, 377-3750, or 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises at least 1 nucleotide mismatch in the region between nucleotides 377-3716 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0202] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of 3330-3365, 3330-3360, 3330-3355, 3335-3365, 3335-3350, 3340-3365, 3340-3360, or 3340-3355 consecutive nucleotides of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide comprises a region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of 3360, 3359, 3358, 3357, 3356, 3355, 3354, 3353, 3352, 3351, 3350, 3349, 3348, 3347, 3346, 3345, 3344, 3343, 3342, 3341, or 3340 or fewer consecutive nucleotides of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide comprises a region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11 across the full length of the region. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 90% identical to the nucleotide sequence of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11 across the

full length of the region. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 95% identical to the nucleotide sequence of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11 across the full length of the region. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 99% identical to the nucleotide sequence of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11 across the full length of the region. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0203] In some embodiments, the 5' hDYSF polynucleotide comprises 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or fewer nucleotide mismatches to the nucleotide sequence of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide comprises 5 or fewer nucleotide mismatches to the nucleotide sequence of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide comprises 2 or fewer nucleotide mismatches to the nucleotide sequence of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide comprises 1 or fewer nucleotide mismatches to the nucleotide sequence of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-

3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0204] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of 3360, 3359, 3358, 3357, 3356, 3355, 3354, 3353, 3352, 3351, 3350, 3349, 3348, 3347, 3346, 3345, 3344, 3343, 3342, 3341, or 3340 or fewer consecutive nucleotides of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide comprises a region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide is at least 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region comprising nucleotide positions 3400-3716, 3400-3700, 3400-3650, 3400-3600, 3400-3550, 3400-3500, 3390-3716, 3390-3700, 3390-3650, 3390-3600, 3390-3550, 3390-3500, 3380-3716, 3380-3700, 3380-3650, 3380-3500, 3371-3716, 3371-3700, 3371-3650, 3371-3600, 3371-3550, or 3371-3500 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0205] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of between 3330-3365, 3330-3360, 3330-3355, 3335-3365, 3335-3350, 3340-3365, 3340-3360, or 3340-3355 consecutive nucleotides of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide does not comprise a region consisting of nucleotide positions 1-100, 1-200, 1-300, 1-350, 1-375, 1-376, 100-200, 100-300, 100-350, 100-375, 100-376, 200-300, 200-350, 200-375, or 200-376 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not comprise a region consisting of nucleotide positions 1-376 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0206] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of 3360, 3359, 3358, 3357, 3356, 3355, 3354, 3353, 3352, 3351, 3350, 3349, 3348, 3347, 3346, 3345, 3344, 3343, 3342, 3341, or 3340 or fewer consecutive nucleotides of SEQ ID NO: 11, wherein the 5' hDYSF polynucleotide does not comprise a region consisting of nucleotide positions 1-100, 1-200, 1-300, 1-350, 1-375, 100-200, 100-300, 100-350, 100-375, 200-300, 200-350, 200-375 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not comprise a region consisting of

nucleotide positions 1-376 of SEQ ID NO: 11. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0207] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence of SEQ ID NO: 1. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 across the full length of SEQ ID NO: 1. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 1 across the full length of SEQ ID NO: 1. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 92% identical to the nucleotide sequence of SEQ ID NO: 1 across the full length of SEQ ID NO: 1. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO: 1 across the full length of SEQ ID NO: 1. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0208] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence of SEQ ID NO: 13. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 across the full length of SEQ ID NO: 13. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 13 across the full length of SEQ ID NO: 13. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 92% identical to the nucleotide sequence of SEQ ID NO: 13 across the full length of SEQ ID NO: 13. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO: 13 across the full length of SEQ ID NO: 13. In some embodiments, the 5' hDYSF polynucleotide does not further

comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0209] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding a fragment of an hDYSF protein comprising the N-terminal region of a wild-type hDYSF protein. In some embodiments, the fragment of the hDYSF protein comprising the N-terminal region of a wild-type hDYSF protein is referred to as an N-terminal hDYSF protein. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein comprises a region comprising, consisting of, or consisting essentially of amino acid residues 1-1113, 200-1113, 400-1113, 500-1113, 600-1113, 650-1113, 650-1100, 700-1100, 700-1113, 700-1050, 700-1000, 800-1113, 800-1100, 800-1050, 900-1113, 900-1100, 1000-1113, or 1000-1100 of SEQ ID NO: 12. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of encoding an N-terminal hDYSF protein across the full length of the nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein comprises a region comprising, consisting of, or consisting essentially of amino acid residues 1-1113, 200-1113, 400-1113, 500-1113, 600-1113, 650-1113, 650-1100, 700-1100, 700-1113, 700-1050, 700-1000, 800-1113, 800-1100, 800-1050, 900-1113, 900-1100, 1000-1113, or 1000-1100 of SEQ ID NO: 12. In some embodiments, the N-terminal hDYSF protein comprises a region comprising, consisting of, or consisting essentially of amino acid residues 999-1113, 999-1100, 1000-1113, or 1000-1100. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0210] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein is at least 1000 amino acids in length, and wherein the N-terminal hDYSF protein comprises a region comprising amino acid residues 999-1113, 999-1100, 1000-1113, or 1000-1100 of SEQ ID NO: 12. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of encoding an N-terminal hDYSF protein across the full length of the nucleotide sequence encoding the N-terminal hDYSF

protein, wherein the N-terminal hDYSF protein is at least 1000 amino acids in length, and wherein the N-terminal hDYSF protein comprises a region comprising amino acid residues 999-1113, 999-1100, 1000-1113, or 1000-1100 of SEQ ID NO: 12. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0211] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 9. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0212] In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of encoding an N-terminal hDYSF protein across the full length of the nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 9. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 90% identical to the nucleotide sequence of encoding an N-terminal hDYSF protein across the full length of the nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 9. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 92% identical to the nucleotide sequence of encoding an N-terminal hDYSF protein across the full length of the nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 9. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 95% identical to the nucleotide sequence of encoding an N-terminal hDYSF protein across the full length of the nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 9. In some embodiments, the 5' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 97% identical to the nucleotide sequence of encoding an N-terminal hDYSF protein

across the full length of the nucleotide sequence encoding the N-terminal hDYSF protein, wherein the N-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 9. In some embodiments, the N-terminal hDYSF protein comprises an amino acid sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 9 across the full length of SEQ ID NO: 9. In some embodiments, the N-terminal hDYSF protein comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 9 across the full length of SEQ ID NO: 9. In some embodiments, the N-terminal hDYSF protein comprises an amino acid sequence that is at least 92% identical to the amino acid sequence of SEQ ID NO: 9 across the full length of SEQ ID NO: 9. In some embodiments, the N-terminal hDYSF protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9 across the full length of SEQ ID NO: 9. In some embodiments, the N-terminal hDYSF protein comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 9 across the full length of SEQ ID NO: 9. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0213] In some embodiments, the 5' hDYSF polynucleotide comprises the nucleotide sequence of SEQ ID NO: 6. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 6 across the full length of SEQ ID NO: 6. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 85% identical to the nucleotide sequence of SEQ ID NO: 6 across the full length of SEQ ID NO: 6. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 6 across the full length of SEQ ID NO: 6. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0214] In some embodiments, the 5' hDYSF polynucleotide comprises the nucleotide sequence of SEQ ID NO: 15. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 6 across the full length of SEQ ID NO: 15. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 85% identical

to the nucleotide sequence of SEQ ID NO: 15 across the full length of SEQ ID NO: 15. In some embodiments, the 5' hDYSF polynucleotide comprises a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 15 across the full length of SEQ ID NO: 15. In some embodiments, the 5' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0215] *3' hDYSF polynucleotide*

[0216] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of between 3500-4100, 3500-4000, 3500-3900, 3500-3880, 3500-3870, 3600-4100, 3600-4000, 3600-3900, 3600-3880, 3600-3870, 3700-4100, 3700-4000, 3700-3900, 3700-3880, 3700-3870, 3800-4100, 3800-4000, or 3800-3900 consecutive nucleotides of a region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of 4100, 4000, 3900, 3880, or 3870 or fewer consecutive nucleotides of a region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0217] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of between 3500-4100, 3500-4000, 3500-3900, 3500-3880, 3500-3870, 3600-4100, 3600-4000, 3600-3900, 3600-3880, 3600-3870, 3700-4100, 3700-4000, 3700-3900, 3700-3880, 3700-3870, 3800-4100, 3800-4000, or 3800-3900 consecutive nucleotides of a region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-

6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11, wherein the 3' hDYSF polynucleotide is at least 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of 4100, 4000, 3900, 3880, or 3870 or fewer consecutive nucleotides of a region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11, wherein the 3' hDYSF polynucleotide is at least 80%, 82%, 85%, 87%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide is at least 85% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11.

6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide is at least 90% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide is at least 95% to the nucleotide sequence of SEQ ID NO: 11 across the full length of the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0218] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of between 3500-4100, 3500-4000, 3500-3900, 3500-3880, 3500-3870, 3600-4100, 3600-4000, 3600-3900, 3600-3880, 3600-3870, 3700-4100, 3700-4000, 3700-3900, 3700-3880, 3700-3870, 3800-4100, 3800-4000, or 3800-3900 consecutive nucleotides of a region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11, wherein the 3' hDYSF polynucleotide comprises 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or fewer nucleotide mismatches in the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680,

2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of 4100, 4000, 3900, 3880, or 3870 or fewer consecutive nucleotides of a region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11, wherein the 3' hDYSF polynucleotide comprises 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or fewer nucleotide mismatches in the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises 15 or fewer nucleotide mismatches in the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises 10 or fewer nucleotide mismatches in the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises 5 or fewer nucleotide mismatches in the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-

6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises 1 nucleotide mismatch in the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises at least 1 nucleotide mismatch in the region between nucleotides 2600-6850, 2600-6800, 2600-6780, 2600-6750, 2600-6725, 2600-6700, 2700-6850, 2700-6800, 2700-6780, 2700-6750, 2700-6725, 2700-6700, 2700-6680, 2700-6650, 2700-6625, 2700-6620, 2700-6619, 2750-6850, 2750-6800, 2750-6780, 2750-6750, 2750-6725, 2750-6700, 2750-6680, 2750-6650, 2750-6625, 2750-6620, 2750-6619, 2754-6850, 2754-6800, 2754-6780, 2754-6750, 2754-6725, 2754-6700, 2754-6680, 2754-6650, 2754-6625, 2754-6620, or 2754-6619 of SEQ ID NO: 11. In some embodiments, the 5' polynucleotide comprises at least 1 nucleotide mismatch in the region between nucleotides 2754-6619 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0219] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of between 3500-4100, 3500-4000, 3500-3900, 3500-3880, 3500-3870, 3600-4100, 3600-4000, 3600-3900, 3600-3880, 3600-3870, 3700-4100, 3700-4000, 3700-3900, 3700-3880, 3700-3870, 3800-4100, 3800-4000, or 3800-3900 consecutive nucleotides of SEQ ID NO: 11, wherein the 5' hDSYF polynucleotide does not comprise a region consisting of nucleotide positions 6620-6914, 6620-6900, 6620-6800, 6620-6700, 6700-6914, 6700-6800, 6800-6914, or 6800-6900 of SEQ ID NO: 11. In some embodiments, the 3' hDSYF polynucleotide does not comprise a region consisting of nucleotide positions 6620-6914 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0220] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of 4100, 4000, 3900, 3880, or 3870 or fewer consecutive nucleotides of SEQ ID NO: 11, wherein the 3' hDYSF polynucleotide does not comprise a region consisting of nucleotide positions 6620-6914, 6620-6900, 6620-6800, 6620-6700, 6700-6914, 6700-6800, 6800-6914, or 6800-6900 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide does not comprise a region consisting of nucleotide positions 6620-6914 of SEQ ID NO: 11. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0221] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence of SEQ ID NO: 2. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 across the full length of SEQ ID NO: 2. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 2 across the full length of SEQ ID NO: 2. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 92% identical to the nucleotide sequence of SEQ ID NO: 2 across the full length of SEQ ID NO: 2. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO: 2 across the full length of SEQ ID NO: 2. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0222] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence of SEQ ID NO: 14. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 across the full length of SEQ ID NO: 14. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 14 across the full length of SEQ ID NO: 14. In some

embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 92% identical to the nucleotide sequence of SEQ ID NO: 14 across the full length of SEQ ID NO: 14. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO: 14 across the full length of SEQ ID NO: 14. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0223] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding a fragment of an hDYSF protein comprising the C-terminal region of a wild-type hDYSF protein. In some embodiments, the fragment of the hDYSF protein comprising the C-terminal region of a wild-type hDYSF protein is referred to as a C-terminal hDYSF protein. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises a region comprising, consisting of, or consisting essentially of amino acid residues 750-2080, 750-2000, 750-1900, 775-2080, 775-2000, 775-1900, 794-2080, 794-2000, or 794-1900 of SEQ ID NO: 12. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of encoding a C-terminal hDYSF protein across the full length of the nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises a region comprising, consisting of, or consisting essentially of amino acid residues 750-2080, 750-2000, 750-1900, 775-2080, 775-2000, 775-1900, 794-2080, 794-2000, or 794-1900 of SEQ ID NO: 12. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0224] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises, consists of, or consists essentially of 1400, 1350, 1325, 1300, 1290, or 1287 or fewer amino acids in length, and wherein the C-terminal hDYSF protein comprises a region comprising amino acid residues 750-2080, 750-2000, 750-1900, 775-2080, 775-2000, 775-1900, 794-2080, 794-2000, or 794-1900 of SEQ ID NO:

12. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of encoding a C-terminal hDYSF protein across the full length of the nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein is 1400, 1350, 1325, 1300, 1290, or 1287 or fewer amino acids in length, and wherein the C-terminal hDYSF protein comprises a region comprising amino acid residues 750-2080, 750-2000, 750-1900, 775-2080, 775-2000, 775-1900, 794-2080, 794-2000, or 794-1900 of SEQ ID NO: 12. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0225] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises, consists of, or consists essentially of 1400, 1350, 1325, 1300, 1290, or 1287 or fewer amino acids in length, wherein the C-terminal hDYSF protein does not comprise a region comprising amino acid residues 678-793, 678-750, 678-725, or 678-700 of SEQ ID NO: 12. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0226] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 10. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0227] In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of a nucleotide sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of encoding a C-terminal hDYSF protein across the full length of the nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 10. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 90% identical to the nucleotide sequence of encoding a C-terminal hDYSF protein across the full length of the nucleotide sequence encoding the C-

terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 10. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 92% identical to the nucleotide sequence of encoding a C-terminal hDYSF protein across the full length of the nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 10. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 95% identical to the nucleotide sequence of encoding a C-terminal hDYSF protein across the full length of the nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 10. In some embodiments, the 3' hDYSF polynucleotide comprises, consists of, or consists essentially of nucleotide sequence that is at least 97% identical to the nucleotide sequence of encoding a C-terminal hDYSF protein across the full length of the nucleotide sequence encoding the C-terminal hDYSF protein, wherein the C-terminal hDYSF protein comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NO: 10. In some embodiments, the C-terminal hDYSF protein comprises an amino acid sequence that is at least 80%, 82%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 10 across the full length of SEQ ID NO: 10. In some embodiments, the C-terminal hDYSF protein comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 10 across the full length of SEQ ID NO: 10. In some embodiments, the C-terminal hDYSF protein comprises an amino acid sequence that is at least 92% identical to the amino acid sequence of SEQ ID NO: 10 across the full length of SEQ ID NO: 10. In some embodiments, the C-terminal hDYSF protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10 across the full length of SEQ ID NO: 10. In some embodiments, the C-terminal hDYSF protein comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 10 across the full length of SEQ ID NO: 10. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0228] In some embodiments, the 3' hDYSF polynucleotide comprises the nucleotide sequence of SEQ ID NO: 8. In some embodiments, the 3' hDYSF polynucleotide comprises a

nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 8 across the full length of SEQ ID NO: 8. In some embodiments, the 3' hDYSF polynucleotide comprises a nucleotide sequence that is at least 85% identical to the nucleotide sequence of SEQ ID NO: 8 across the full length of SEQ ID NO: 8. In some embodiments, the 3' hDYSF polynucleotide comprises a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 8 across the full length of SEQ ID NO: 8. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0229] In some embodiments, the 3' hDYSF polynucleotide comprises the nucleotide sequence of SEQ ID NO: 16. In some embodiments, the 3' hDYSF polynucleotide comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 16 across the full length of SEQ ID NO: 16. In some embodiments, the 3' hDYSF polynucleotide comprises a nucleotide sequence that is at least 85% identical to the nucleotide sequence of SEQ ID NO: 16 across the full length of SEQ ID NO: 16. In some embodiments, the 3' hDYSF polynucleotide comprises a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 16 across the full length of SEQ ID NO: 16. In some embodiments, the 3' hDYSF polynucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

[0230] In some embodiments, the sequences of the 5' hDSYF polynucleotide and the 3' hDYSF polynucleotide comprise an overlap of at least 500, 600, 700, 800, 900, 950, 960, or 963 nucleotides. In some embodiments, the sequences of the N-terminal hDSYF protein and of the C-terminal hDSYF protein comprise an overlap of at least 50, 100, 150, 200, 250, 300, or 320 amino acids.

[0231] Inverted Terminal Repeats

[0232] In some embodiments, the polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions further comprise a nucleotide sequence comprising, consisting of, or consisting essentially of one or more inverted terminal repeats (ITRS). In some embodiments, the polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions further comprise two, three, four, five, or six or more nucleotide sequences comprising, consisting of, or consisting essentially of two,

three, four, five, or six or more ITRs. In some embodiments, the two or more ITRs are the same. In some embodiments, the two or more ITRs are different.

[0233] In some embodiments, the recombinant polynucleotide is flanked by the two or more ITRs. In some embodiments, the 5' hDYSF polynucleotide is flanked by a first pair of ITRs. In some embodiments, the 3' hDYSF polynucleotide is flanked by a second pair of ITRs. In some embodiments, the ITRs in the first pair of ITRs are the same. In some embodiments, the ITRs in the first pair of ITRs are different. In some embodiments, the ITRs in the second pair of ITRs are the same. In some embodiments, the ITRs in the second pair of ITRs are different. In some embodiments, the ITRs in the first pair of ITRs are the same as the ITRs in the second pair of ITRs. In some embodiments, at least one ITR in the first pair of ITRs is the same as at least one ITR in the second pair of ITRs. In some embodiments, the ITRs in the first pair of ITRs are different from the ITRs in the second pair of ITRs. In some embodiments, at least one ITR in the first pair of ITRs is different from at least one ITR in the second pair of ITRs.

[0234] In some embodiments, the ITR is a viral ITR. In some embodiments, the ITR is an AAV ITR. In some embodiments, the AAV ITR is selected from an ITR from at least one of AAV serotypes AAVrh.20, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAVrh.74, AAV-8, AAV-9, AAV-10, AAVrh.10, AAV-11, AAV-12 and AAV-13. In some embodiments, the AAV ITR is an AAV2 ITR. In some embodiments, the AAV ITR is an AAV5 ITR. The ITR sequences for AAV1-6 can be found, for example, in Grimm *et al.*, J. Virol.80(1):426-39, 2006, which is incorporated by reference in its entirety.

[0235] In some embodiments, the recombinant polynucleotide does not comprise an AAV sequence other than an inverted terminal repeat (ITR).

[0236] In some embodiments, the recombinant polynucleotide does not comprise a viral sequence other than an inverted terminal repeat (ITR).

[0237] In some embodiments, the ITR comprises the nucleotide sequence of SEQ ID NO: 3. In some embodiments, the ITR comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 3 across the full length of SEQ ID NO: 3. In some embodiments, the ITR comprises the nucleotide sequence of SEQ ID NO: 3. In some embodiments, the ITR comprises a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 3 across the full length of SEQ ID NO: 3. In some embodiments, the ITR comprises a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO: 3 across the full length of SEQ ID NO:

3. In some embodiments, the ITR comprises a nucleotide sequence comprising 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 or fewer nucleotide mismatches to the nucleotide sequence of SEQ ID NO: 3. In some embodiments, the ITR comprises a nucleotide sequence comprising 5 or fewer nucleotide mismatches to the nucleotide sequence of SEQ ID NO: 3.

[0238] Promoters

[0239] In some embodiments, the polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions further comprise a nucleotide sequence comprising, consisting of, or consisting essentially of one or more promoters. In some embodiments, the promoter is a eukaryotic promoter. Examples of eukaryotic promoters include, but are not limited to, a cytomegalovirus (CMV) promoter, elongation factor 1 alpha (EF1a) promoter, CAG promoter, phosphoglycerate kinase gene (PGK) promoter, tetracycline response element (TRE) promoter, human U6 nuclear (U6) promoter, and UAS promoter. In some embodiments, the promoter is a mammalian promoter. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the promoter is an inducible promoter.

[0240] In some embodiments, the promoter is a tissue-specific promoter. Examples of tissues include, but are not limited to, muscle, epithelial, connective, and nervous tissue. Examples of tissue-specific promoters include, but are not limited to, B29 promoter, CD14 promoter, CD43 promoter, CD45 promoter, CD68 promoter, desmin promoter, elastase-1 promoter, endoglin promoter, fibronectin promoter, Flt-1 promoter, GFAP promoter, ICAM-2 promoter, INF- β promoter, Mb promoter, Nphs1 promoter, OG-2 promoter, SP-B promoter, SYN1 promoter, WASP promoter, SV40/bAlb promoter, SV40/hAlb promoter, SV40/CD43 promoter, SV40/CD45 promoter, and NSE/RU5' promoter.

[0241] In some embodiments, the promoter is a muscle-specific promoter. In some embodiments, the muscle-specific promoter is a myosin heavy chain complex—E box muscle creatine kinase fusion enhancer/promoter.

[0242] In some embodiments, the promoter is a recombinant promoter. In some embodiments, the recombinant promoter is a recombinant muscle-specific promoter. In some embodiments, the recombinant-muscle specific promoter is a recombinant myosin heavy chain-creatine kinase muscle-specific promoter. In another embodiment, the muscle-specific promoter comprises a human skeletal actin gene element, a cardiac actin gene element, a desmin promoter, a skeletal alpha-actin (ASKA) promoter, a troponin I (TNNI2) promoter, a

myocytespecific enhancer binding factor mef binding element, a muscle creatine kinase (MCK) promoter, a truncated MCK (tMCK) promoter, a myosin heavy chain (MHC) promoter, a hybrid α -myosin heavy chain enhancer-/MCK enhancer-promoter (MHCK7) promoter, a C5-12 promoter, a murine creatine kinase enhancer element, a skeletal fast-twitch troponin c gene element, a slow-twitch cardiac troponin c gene element, a slow-twitch troponin i gene element, hypoxia- inducible nuclear factor.

[0243] In some embodiments, the promoter comprises the nucleotide sequence of SEQ ID NO: 4. In some embodiments, the promoter comprises a nucleotide sequence that is at least 80%, 82%, 85%, 87%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 4 across the full length of SEQ ID NO: 4.

[0244] Introns

[0245] In some embodiments, the polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions further comprise a nucleotide sequence comprising, consisting of, or consisting essentially of one or more introns. In some embodiments, the intron is a eukaryotic intron. In some embodiments, the intron is a mammalian intron. In some embodiments, the intron is a synthetic intron. In some embodiments, the intron is a chimeric intron. In some embodiments, the intron is from a non-coding exon. In some embodiments, the intron is upstream of or 5' to the 5' hDYSF polynucleotide.

[0246] In some embodiments, the intron comprises at least one of a 5' donor site, branch point, or 3' splice site. In some embodiments, the intron comprises two or more of a 5' donor site, branch point, or 3' splice site. In some embodiments, the intron comprises a 5' donor site, branch point, and 3' splice site.

[0247] In some embodiments, the intron comprises a 5' donor site from a human β -globin gene.

[0248] In some embodiments, the intron comprises a branch point from an immunoglobulin G (IgG) heavy chain.

[0249] In some embodiments, intron comprises a 3' splice acceptor site from an immunoglobulin G (IgG) heavy chain

[0250] In some embodiments, the intron comprises the nucleotide sequence of SEQ ID NO: 5. In some embodiments, the intron comprises a nucleotide sequence that is at least 80%, 82%, 85%, 87%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 5 across the full length of SEQ ID NO: 5.

[0251] Selection Marker

[0252] In some embodiments, the polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions further comprise a nucleotide sequence comprising, consisting of, or consisting essentially of one or more selection markers. In some embodiments, the selection marker is a bacterial selectable marker. In some embodiments, the selection marker is an antibiotic resistance gene. Examples of antibiotic resistance genes include, but are not limited to, β -lactamase, kanamycin resistance gene, neo gene from Tn5, mutant FabI gene from *E.coli* genome, and *URA3* (an orotidine-5' phosphate decarboxylase from yeast). In some embodiments, the antibiotic resistance gene is a β -lactamase gene. In some embodiments, the antibiotic resistance gene is a kanamycin resistance gene.

[0253] Polyadenylation signal

[0254] In some embodiments, the polynucleotides, plasmids, viral vectors, vector systems, viral packaging systems, cells, and compositions further comprise a nucleotide sequence comprising, consisting of, or consisting essentially of one or more polyadenylation (polyA) signals. In some embodiments, the polyA signal is an artificial polyA signal.

[0255] In some embodiments, the polyA signal comprises the nucleotide sequence of SEQ ID NO: 7. In some embodiments, the polyA signal comprises a nucleotide sequence that is at least 80%, 82%, 85%, 87%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 7 across the full length of SEQ ID NO: 7.

[0256] Expression Cassettes and Packaging Systems

[0257] Further disclosed herein are adeno-associated viral (AAV) expression cassettes. In some embodiments, the AAV expression cassette comprises: (a) a first inverted terminal repeat (ITR), wherein the first ITR comprises any of the ITRs disclosed herein; (b) any of the 5' hDYSF polynucleotides disclosed herein; and (c) a second ITR, wherein the second ITR comprises any of the ITRs disclosed herein, wherein the 5' hDYSF polynucleotide of (b) is flanked by the first and second ITRs of (a) and (c). In some embodiments, an AAV expression cassette comprising any of the 5' hDYSF polynucleotides disclosed herein is referred to as a 5' hDYSF AAV expression cassette.

[0258] Further disclosed herein are adeno-associated viral (AAV) plasmids. In some embodiments, the AAV expression cassette comprises: (a) a first inverted terminal repeat (ITR), wherein the first ITR comprises any of the ITRs disclosed herein; (b) any of the 3' hDYSF polynucleotides disclosed herein; and (c) a second ITR, wherein the second ITR

comprises any of the ITRs disclosed herein, wherein the 3' hDYSF polynucleotide of (b) is flanked by the first and second ITRs of (a) and (c). In some embodiments, an AAV expression cassette comprising any of the 3' hDYSF polynucleotides disclosed herein is referred to as a 3' hDYSF AAV expression cassette.

[0259] In some embodiments, an adeno-associated viral (AAV) expression cassette comprises: (a) a first inverted terminal repeat (ITR); (b) a polynucleotide sequence encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 1; (ii) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 1 across the full length of SEQ ID NO: 1; (iii) the nucleotide sequence of SEQ ID NO: 13; (iv) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 13 across the full length of SEQ ID NO: 13; (v) a nucleotide sequence encoding the hDYSF protein, wherein the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or (vi) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide sequence is flanked by the first and second ITRs. In some embodiments, any of the AAV expression cassettes disclosed herein further comprise one or more additional polynucleotide sequences comprising a promoter, intron, selection marker, or origin of replication (ORI). In some embodiments, the AAV expression cassette comprises the nucleotide sequence of SEQ ID NO: 6. In some embodiments, the AAV expression cassette comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 6 across the full length of SEQ ID NO: 6. In some embodiments, the AAV expression cassette comprises the nucleotide sequence of SEQ ID NO: 15. In some embodiments, the AAV expression cassette comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 15 across the full length of SEQ ID NO: 15. In some embodiments, the AAV expression cassette does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein. In some embodiments, the AAV expression cassette does not comprise

an AAV sequence other than an inverted terminal repeat (ITR). In some embodiments, the AAV expression cassette does not comprise a viral sequence other than an inverted terminal repeat (ITR).

[0260] In some embodiments, an adeno-associated viral (AAV) expression cassettes comprises: (a) a first inverted terminal repeat (ITR); (b) a polynucleotide sequence encoding a fragment of a human dysferlin protein, wherein the polynucleotide sequence consists of: (i) the nucleotide sequence of SEQ ID NO: 2; (ii) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 2 across the full length of SEQ ID NO: 2; (iii) the nucleotide sequence of SEQ ID NO: 14; (ii) a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 14 across the full length of SEQ ID NO: 14; (v) a polynucleotide sequence encoding the hDYSF protein, wherein the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or (vi) a polynucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and (c) a second ITR, wherein the polynucleotide sequence is flanked by the first and second ITRs. In some embodiments, the AAV expression cassette comprises the nucleotide sequence of SEQ ID NO: 8. In some embodiments, the AAV expression cassette comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 8 across the full length of SEQ ID NO: 8. In some embodiments, the AAV expression cassette comprises the nucleotide sequence of SEQ ID NO: 16. In some embodiments, the AAV expression cassette comprises a nucleotide sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 16 across the full length of SEQ ID NO: 16. In some embodiments, the AAV expression cassette further comprises one or more polynucleotide sequences comprising a selection marker, origin of replication (ORI), untranslated region (UTR), or polyadenylation (polyA) signal.

[0261] Further disclosed herein are adeno-associated viral (AAV) packaging systems. In some embodiments, the AAV packaging systems comprise: (a) any of the 5' hDYSF AAV expression cassettes disclosed herein; (b) an adenovirus helper plasmid; and (c) a rep-cap

plasmid. In some embodiments, the adenovirus helper plasmid comprises one or more genes from an adenovirus. In some embodiments, the one or more genes from the adenovirus mediate AAV replication. In some embodiments, the one or more genes from the adenovirus are selected from E4, E2a, and VA. In some embodiments, the rep-cap plasmid comprises one or more polynucleotides encoding the adeno-associated virus rep and cap genes. In some embodiments, the rep gene encodes for one or more of life cycle proteins selected from Rep78, Rep68, Rep62, and Rep40. In some embodiments, the cap gene encodes for one or more of capsid proteins selected from VP1, VP2, and VP3. In some embodiments, the 5' hDYSF AAV expression cassette comprises one or more ITRs. In some embodiments, the ITRs are AAV ITRs. In some embodiments, the serotype of the AAV ITRs is the same as the serotype of the AAV capsid protein. In some embodiments, the serotype of the AAV ITRs is different from the serotype of the AAV capsid protein. In some embodiments, the serotype of the AAV rep gene is the same as the serotype of the AAV capsid protein. In some embodiments, the serotype of the AAV rep gene is different from the serotype of the AAV capsid protein. In some embodiments, an AAV packaging system comprising any of the 5' hDYSF AAV expression cassettes disclosed herein is referred to as a 5' hDYSF AAV packaging system.

[0262] In some embodiments, the AAV packaging systems comprise: (a) any of the 3' hDYSF AAV expression cassettes disclosed herein; (b) an adenovirus helper plasmid; and (c) a rep-cap plasmid. In some embodiments, the adenovirus helper plasmid comprises one or more genes from an adenovirus. In some embodiments, the one or more genes from the adenovirus mediate AAV replication. In some embodiments, the one or more genes from the adenovirus are selected from E4, E2a, and VA. In some embodiments, the rep-cap plasmid comprises one or more polynucleotides encoding the adeno-associated virus rep and cap genes. In some embodiments, the rep gene encodes for one or more of life cycle proteins selected from Rep78, Rep68, Rep62, and Rep40. In some embodiments, the cap gene encodes for one or more of capsid proteins selected from VP1, VP2, and VP3. In some embodiments, the 3' hDYSF AAV expression cassette comprises one or more ITRs. In some embodiments, the ITRs are AAV ITRs. In some embodiments, the serotype of the AAV ITRs is the same as the serotype of the AAV capsid protein. In some embodiments, the serotype of the AAV ITRs is different from the serotype of the AAV capsid protein. In some embodiments, the serotype of the AAV rep gene is the same as the serotype of the AAV capsid protein. In some embodiments, the serotype of the AAV rep gene is different from the serotype of the AAV capsid protein. In some embodiments, an AAV packaging system comprising any of the 3'

hDYSF AAV expression cassettes disclosed herein is referred to as a 3' hDYSF AAV packaging system.

[0263] In some embodiments, the adeno-associated viral packaging system comprises: (a) any of the 5' hDYSF AAV expression cassettes disclosed herein; and (b) an adenovirus helper plasmid. In some embodiments, the adenovirus helper plasmid comprises one or more genes from an adenovirus. In some embodiments, the one or more genes from the adenovirus mediate AAV replication. In some embodiments, the one or more genes from the adenovirus are selected from E4, E2a, and VA.

[0264] In some embodiments, the adeno-associated viral packaging system comprises: (a) any of the 3' hDYSF AAV expression cassettes disclosed herein; and (b) an adenovirus helper plasmid. In some embodiments, the adenovirus helper plasmid comprises one or more genes from an adenovirus. In some embodiments, the one or more genes from the adenovirus mediate AAV replication. In some embodiments, the one or more genes from the adenovirus are selected from E4, E2a, and VA.

[0265] Viral Vectors

[0266] Further disclosed herein are adeno-associated viral (AAV) vectors (*e.g.*, AAV viruses or AAV particles). In some embodiments, the AAV vectors comprise, consist of, or consist essentially of any of the 5' hDYSF polynucleotides disclosed herein. In some embodiments, an AAV vector comprising any of the 5' hDYSF polynucleotides disclosed herein is referred to as a 5' hDYSF AAV vector.

[0267] In some embodiments, a 5' hDYSF AAV vector comprises any of the 5' hDYSF AAV expression cassettes disclosed herein.

[0268] In some embodiments, the AAV vectors comprise, consist of, or consist essentially of any of the 3' hDYSF polynucleotides disclosed herein. In some embodiments, an AAV vector comprising any of the 3' hDYSF polynucleotides disclosed herein is referred to as a 3' hDYSF AAV vector.

[0269] In some embodiments, a 3' hDYSF AAV vector comprises any of the 3' hDYSF AAV expression cassettes disclosed herein.

[0270] In some embodiments, the AAV vector is an AAV of serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, rh.10, rh.20, or rh.74. In some embodiments, the AAV vector is an AAV of serotype rh.74. In some embodiments, the AAV vector is not an AAV of serotype 5.

[0271] Further disclosed herein are dual adeno-associated viral (AAV) vector systems comprising two or more of the AAV vectors disclosed herein. In some embodiments, the dual AAV vector system comprises: (a) a first AAV vector, wherein the first AAV vector

comprises any of the 5' hDYSF polynucleotides disclosed herein; and (b) a second AAV vector, wherein the second AAV vector comprises any of the 3' hDYSF polynucleotides disclosed herein.

[0272] In some embodiments, the dual AAV vector system comprises, consists of, or consists essentially of: (a) a first AAV vector, wherein the first AAV vector comprises, consists of, or consists essentially of any of the 5' hDYSF AAV vectors disclosed herein; and (b) a second AAV vector, wherein the second AAV vector comprises, consists of, or consists essentially of any of the 3' hDYSF AAV vectors disclosed herein.

[0273] Compositions

[0274] Further disclosed herein are compositions comprising, consisting of, or consisting essentially of any of the 5' hDYSF polynucleotides disclosed herein. Further disclosed herein are compositions comprising, consisting of, or consisting essentially of any of the 3' hDYSF polynucleotides disclosed herein. Further disclosed herein are compositions comprising, consisting of, or consisting essentially of any of the 5' hDYSF plasmids disclosed herein. Further disclosed herein are compositions comprising, consisting of, or consisting essentially of any of the 3' hDYSF plasmids disclosed herein. Further disclosed herein are compositions comprising, consisting of, or consisting essentially of any of the dual AAV vector systems disclosed herein. Further disclosed herein are compositions comprising, consisting of, or consisting essentially of any of the AAV vectors disclosed herein.

[0275] Further disclosed herein is a composition comprising, consisting of, or consisting essentially of: (a) a recombinant adeno-associated virus (rAAV) vector, wherein the rAAV vector comprises, consists of, or consists essentially of any of the 5' hDYSF polynucleotides disclosed herein; and (b) a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant.

[0276] Further disclosed herein is a composition comprising, consisting of, or consisting essentially of: (a) a recombinant adeno-associated virus (rAAV) vector, wherein the rAAV comprises, consists of, or consists essentially of any of the 3' hDYSF polynucleotides disclosed herein; and (b) a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant.

[0277] Further disclosed herein is a composition comprising, consisting of, or consisting essentially of: (a) a first recombinant adeno-associated virus (rAAV), wherein the first rAAV comprises, consists of, or consists essentially of any of the 5' hDYSF polynucleotides disclosed herein; and (b) a second recombinant adeno-associated virus (rAAV), wherein the

second rAAV comprises, consists of, or consists essentially of any of the 3' hDYSF polynucleotides disclosed herein.

[0278] Further disclosed herein is a composition comprising, consisting of, or consisting essentially of: (a) a first adeno-associated viral (AAV) particle, wherein the first AAV particle comprises, consists of, or consists essentially of any of the 5' hDYSF AAV vectors disclosed herein; and (b) a second adeno-associated viral (AAV) particle, wherein the second AAV particle comprises, consists of, or consists essentially of any of the 3' hDYSF AAV vectors disclosed herein.

[0279] In some embodiments, any of the compositions disclosed herein further comprise at least one of a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant. Acceptable carriers, diluents and adjuvants are nontoxic to recipients and are preferably inert at the dosages and concentrations employed and include buffers and surfactants such as pluronics. Examples of acceptable carriers include, but are not limited to, phosphate buffered saline, preservatives and the like.

[0280] The pharmaceutically acceptable carrier, diluent, or excipient may be suitable for injectable use. Examples of pharmaceutically acceptable carriers, diluents or excipients suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating actions of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0281] Sterile injectable solutions are prepared by incorporating the polynucleotides, plasmids, viral vectors, or dual vector systems disclosed herein in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed

by filter sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying technique that yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

[0282] Methods for Producing AAV Vectors

[0283] Disclosed herein are methods of producing an adeno-associated viral (AAV) vector (e.g., virus or viral particle). Methods of producing AAV vectors are known in the art. For instance, such methods are disclosed in, for example, WO 01/83692, which is incorporated by reference herein in its entirety. General principles of AAV production are reviewed in, for example, Carter, *Current Opinions in Biotechnology* 1533-1539, 1992; and Muzyczka, *Curr. Topics in Microbial. and Immunol.* 158:97-129, 1992, each of which are incorporated by reference in their entirety. Various approaches for producing AAVs are described in Ratschin *et al.*, *Mol. Cell. Biol.* 4:2072, 1984; Hermonat *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6466, 1984; Tratschin *et al.*, *Mol. Cell. Biol.* 5:3251, 1985; McLaughlin *et al.*, *J. Virol.*, 62:1963, 1988; and Lebkowski *et al.*, *Mol. Cell. Biol.*, 7:349, 1988; Samulski *et al.*, *J. Virol.*, 63:3822-3828, 1989; U.S. Patent No. 5,173,414; WO 95/13365 and corresponding U.S. Patent No. 5,658,776 ; WO 95/13392; WO 96/17947; PCT/US98/18600; WO 97/09441 (PCT/US96/14423); WO 97/08298 (PCT/US96/13872); WO 97/21825 (PCT/US96/20777); WO 97/06243 (PCT/FR96/01064); WO 99/11764; Perrin *et al.*, *Vaccine* 13:1244-1250, 1995; Paul *et al.*, *Human Gene Therapy* 4:609-615, 1993; Clark *et al.*, *Gene Therapy* 3:1124-1132, 1996; U.S. Patent. No. 5,786,211; U.S. Patent No. 5,871,982; and U.S. Patent. No. 6,258,595, each of which are incorporated by reference in their entirety.

[0284] In some embodiments, the method for producing an adeno-associated viral (AAV) vector comprises transducing a cell with any of the AAV packaging systems disclosed herein. In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a recombinant cell that stably expresses the adeno-associated virus rep and cap genes. In some embodiments, the method further comprises culturing the cell to produce a population of transduced cells. In some embodiments, the method further comprises collecting the supernatant from the population of transduced cells. In some embodiments, the method further comprises subjecting the supernatant to one or more purification steps to produce a purified AAV vector sample, wherein the AAV vector sample is substantially free from cellular debris and proteins.

Alternatively, or additionally, the method further comprises lysing the population of transduced cells to produce a cellular lysate. In some embodiments, the method further comprises subjecting the cellular lysate to one or more purification steps to produce a purified AAV vector sample, wherein the AAV vector sample is substantially free from cellular debris and proteins. In some embodiments, the purity of the purified AAV vector sample is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% pure.

[0285] In some embodiments, the method for producing an adeno-associated viral (AAV) vector comprises transducing a cell with any of the 5' hDYSF AAV packaging systems disclosed herein. In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a recombinant cell that stably expresses the adeno-associated virus rep and cap genes. In some embodiments, the method further comprises culturing the cell to produce a population of transduced cells. In some embodiments, the method further comprises collecting the supernatant from the population of transduced cells. In some embodiments, the method further comprises subjecting the supernatant to one or more purification steps to produce a purified AAV vector sample, wherein the AAV vector sample is substantially free from cellular debris and proteins. Alternatively, or additionally, the method further comprises lysing the population of transduced cells to produce a cellular lysate. In some embodiments, the method further comprises subjecting the cellular lysate to one or more purification steps to produce a purified AAV vector sample, wherein the AAV vector sample is substantially free from cellular debris and proteins. In some embodiments, the purity of the purified AAV vector sample is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% pure.

[0286] In some embodiments, the method for producing an adeno-associated viral (AAV) vector comprises transducing a cell with any of the 3' hDYSF AAV packaging systems disclosed herein. In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a recombinant cell that stably expresses the adeno-associated virus rep and cap genes. In some embodiments, the method further comprises culturing the cell to produce a population of transduced cells. In some embodiments, the method further comprises collecting the supernatant from the population of transduced cells. In some embodiments, the method further comprises subjecting the supernatant to one or more purification steps to produce a purified AAV vector sample, wherein the AAV vector sample is substantially free from cellular debris and proteins.

Alternatively, or additionally, the method further comprises lysing the population of transduced cells to produce a cellular lysate. In some embodiments, the method further comprises subjecting the cellular lysate to one or more purification steps to produce a purified AAV vector sample, wherein the AAV vector sample is substantially free from cellular debris and proteins. In some embodiments, the purity of the purified AAV vector sample is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% pure.

[0287] Cells

[0288] Further disclosed herein are cells comprising any of the 5' hDYSF polynucleotides disclosed herein. The cells can be prokaryotic or eukaryotic cells. Non-limiting examples of eukaryotic cells include mammalian, e.g., hamster, murine, rat, canine, ovine or human cells. In some embodiments, the cells are transfected with a plasmid comprising any of the 5' hDYSF polynucleotides disclosed herein. In some embodiments, the cells are transduced with any of the 5' hDYSF AAV expression cassettes disclosed herein. In some embodiments, the cells are infected with any of the 5' hDYSF AAV vectors disclosed herein.

[0289] Further disclosed herein are cells comprising any of the 3' hDYSF polynucleotides disclosed herein. In some embodiments, the cells are transfected with a plasmid comprising any of the 3' hDYSF polynucleotides disclosed herein. In some embodiments, the cells are transduced with any of the 3' hDYSF AAV expression cassettes disclosed herein. In some embodiments, the cells are infected with any of the 3' hDYSF AAV vectors disclosed herein.

[0290] Any of the cells disclosed herein may be packaging cells that produce infectious rAAV. In some embodiments, the packaging cells are stably transformed cancer cells such as HeLa cells, 293 cells and PerC.6 cells (a cognate 293 line). In another embodiment, packaging cells are cells that are not transformed cancer cells, such as low passage 293 cells (human fetal kidney cells transformed with E1 of adenovirus), MRC-5 cells (human fetal fibroblasts), WI-38 cells (human fetal fibroblasts), Vero cells (monkey kidney cells) and FRhL-2 cells (rhesus fetal lung cells). Non-limiting examples of prokaryotic cells comprise bacterial cells (e.g., *Escherichia coli*) and archaeal cells. The cells of the disclosure can be used to produce a cell bank, e.g., an Accession Cell Banks (ACB) for non-GMP purpose or GMP Master Cell Bank (MCB). The aliquote of the cells, in one embodiment, are expanded from an original inoculum to a larger volume before culture in the bioreactor for the production.

[0291] Methods of Treatment

[0292] Further disclosed herein are methods of treating a dysferlinopathy. In some embodiments, a method of treating a dysferlinopathy comprises, consists of, or consists essentially of administering to a subject in need thereof: (a) an effective amount of a first polynucleotide, wherein the first polynucleotide comprises any of the 5' hDYSF polynucleotides disclosed herein; and (b) an effective amount of a second polynucleotide, wherein the second polynucleotide comprises any of the 3' hDYSF polynucleotides disclosed herein. In some embodiments, the first polynucleotide is administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.). In some embodiments, the first polynucleotide is administered intramuscularly or intravenously. In some embodiments, the second polynucleotide is administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.). In some embodiments, the second polynucleotide is administered intramuscularly or intravenously. In some embodiments, the first and second polynucleotides are administered simultaneously. In some embodiments, the first and second polynucleotides are administered sequentially. In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0293] In some embodiments, a method of treating a dysferlinopathy comprises, consists of, or consists essentially of administering to a subject in need thereof: (a) an effective amount of a first adeno-associated viral (AAV) vector, wherein the first AAV vector comprises any of the 5' hDYSF AAV vectors disclosed herein; and (b) an effective amount of a second adeno-associated viral (AAV) vector, wherein the second AAV vector comprises any of the 3' hDYSF AAV vectors disclosed herein. In some embodiments, the first AAV vector is administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.). In some embodiments, the first AAV vector is administered intramuscularly or intravenously. In some embodiments, the second AAV vector is administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or

topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.). In some embodiments, the second AAV vector is administered intramuscularly or intravenously. In some embodiments, the first and second AAV vectors are administered simultaneously. In some embodiments, the first and second AAV vectors are administered sequentially. In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0294] In some embodiments, a method of treating a dysferlinopathy comprises, consists of, or consists essentially of administering to a subject in need thereof: (a) an effective amount of a first AAV expression cassette, wherein the first AAV expression cassette comprises any of the 5' hDYSF AAV expression cassettes disclosed herein; and (b) an effective amount of a second AAV expression cassette, wherein the second AAV expression cassette comprises any of the 3' hDYSF AAV expression cassettes disclosed herein. In some embodiments, the first AAV expression cassette is administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.). In some embodiments, the first AAV expression cassette is administered intramuscularly or intravenously. In some embodiments, the second AAV expression cassette is administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.). In some embodiments, the second AAV expression cassette is administered intramuscularly or intravenously. In some embodiments, the first and second AAV expression cassettes are administered simultaneously. In some embodiments, the first and second AAV expression cassettes are administered sequentially. In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0295] In some embodiments, a method of treating a dysferlinopathy comprises, consists of, or consists essentially of administering to a subject in need thereof an effective amount of a composition comprising (a) any of the 5' hDYSF polynucleotides disclosed herein and any of the 3' hDYSF polynucleotides disclosed herein; (b) any of the 5' hDYSF AAV vectors disclosed herein and any of the 3' hDYSF AAV vectors disclosed herein; (c) any of the 5' hDYSF AAV expression cassettes disclosed herein and any of the 3' hDYSF AAV expression cassettes disclosed herein; or (d) any of the dual AAV vector systems disclosed

herein. In some embodiments, the composition is administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.). In some embodiments, the composition is administered intramuscularly or intravenously. In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0296] Further disclosed herein are uses of any of the recombinant polynucleotides, plasmids, viral vectors, vector systems, and compositions in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof.

Disclosed herein is use of a composition comprising (a) any of the 5' hDYSF polynucleotides disclosed herein and any of the 3' hDYSF polynucleotides disclosed herein; (b) any of the 5' hDYSF AAV vectors disclosed herein and any of the 3' hDYSF AAV vectors disclosed herein; (c) any of the 5' hDYSF AAV expression cassettes disclosed herein and any of the 3' hDYSF AAV expression cassettes disclosed herein; or (d) (e) any of the dual AAV vector systems disclosed herein in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof. In some embodiments, the composition is administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.). In some embodiments, the composition is administered intramuscularly or intravenously. In some embodiments, the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.

[0297] Titers of AAV vectors to be administered in methods of the invention will vary depending, for example, on the particular AAV, the mode of administration, the treatment goal, the individual, and the cell type(s) being targeted, and may be determined by methods standard in the art. Titers of AAV may range from at least about 1×10^6 , about 1×10^7 , about 1×10^8 , about 1×10^9 , about 1×10^{10} , about 1×10^{11} , about 1×10^{12} , about 1×10^{13} to about 1×10^{14} or more DNase resistant particles (DRP) per ml. Dosages may also be expressed in units of viral genomes (vg). For instance, dosages of AAV may range from at least about 1×10^6 , about 1×10^7 , about 1×10^8 , about 1×10^9 , about 1×10^{10} , about 1×10^{11} , about 1×10^{12} , about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , about 8×10^{12} , about 9×10^{12} , about 1×10^{13} to about 1×10^{14} viral genomes.

[0298] AAV dosage can be determined by multiple methods, which include but are not limited to ELISA, assessment of the reverse transcriptase activity, FACS, transduction assays northern blotting (e.g., semi-quantitative northern), dot blot analysis or PCR (e.g., qPCR). It is well known that the AAV doses can be determined by measuring AAV vector genomes with quantitative real-time PCR (qPCR). Such qPCR methods overcome the inconsistency or arbitrary results from conventional transduction assays. In one embodiment of PCR dosage determination, plasmid DNA is used as a calibration standard. The forms of the plasmids can impact the dosage results from the qPCR methods. In one embodiment, the circular or supercoiled DNA or plasmids are used as a quantification standard.

[0299] In some embodiment, dosages may be expressed in the units of vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard. For example, dosages of AAV is about 1×10^6 - 1×10^{16} vg/kg, about 1×10^8 - 1×10^{15} vg/kg, or about 1×10^{10} - 1×10^{14} vg/kg,), based on a supercoiled DNA or plasmid as the quantitation standard. In another embodiment, the dosages is about at least 1×10^6 , about 1×10^7 , about 1×10^8 , about 1×10^9 , about 1×10^{10} , about 1×10^{11} , about 1×10^{12} , about 2×10^{12} , about 4×10^{12} , about 6×10^{12} , about 8×10^{12} , about 1×10^{13} , about 2×10^{13} , about 2.4×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , about 8×10^{13} , about 9×10^{13} , about 1×10^{14} , about 1×10^{15} , or at least about 1×10^{16} vg/kg. In one embodiment, the dosage is at least 2×10^{12} , 4×10^{12} , 6×10^{12} , 8×10^{12} , 1×10^{13} , 2×10^{13} , 2.4×10^{13} , 3×10^{13} , 4×10^{13} , 5×10^{13} , 6×10^{13} , 7×10^{13} , or 8×10^{13} vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard.

[0300] In some embodiments, the methods disclosed herein comprise administering at least about 1×10^6 , about 1×10^7 , about 1×10^8 , about 1×10^9 , about 1×10^{10} , about 1×10^{11} , about 1×10^{12} , about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , about 8×10^{12} , about 9×10^{12} , about 1×10^{13} vg in a total volume of 1.5 ml per injection. In some embodiments, the methods disclosed herein comprise administering a total daily dose of at least about 1×10^6 , about 1×10^7 , about 1×10^8 , about 1×10^9 , about 1×10^{10} , about 1×10^{11} , about 1×10^{12} , about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , about 8×10^{12} , about 9×10^{12} , about 1×10^{13} , about 2×10^{13} , about 5×10^{13} , about 7×10^{13} , about 1×10^{14} vg. One exemplary method of determining encapsidated vector genome titer uses quantitative PCR, such as the methods described in Pozsgai *et al.*, *Mol. Ther.* 25(4): 855-869, 2017, which is incorporated by reference in its entirety.

[0301] In some embodiments, any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times a day. In some embodiments, any of the polynucleotides,

plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 times a week. In some embodiments, any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 times a month. In some embodiments, any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject at least every 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days. In some embodiments, any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject at least every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 weeks. In some embodiments, any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject for a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days. In some embodiments, any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject for a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 weeks. In some embodiments, any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject for a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, or 20 months.

[0302] In some embodiments, the methods disclosed herein comprise administering any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein systemically. For example, systemic administration is administration into the circulatory system so that the entire body is affected. Systemic administration includes enteral administration such as absorption through the gastrointestinal tract and parenteral administration through injection, infusion or implantation.

[0303] In some embodiments, the methods disclosed herein comprise administering any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein locally. In some embodiments, the methods disclosed herein comprise administering any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein to one or more tissues. In some embodiments, the tissue is selected from muscle, epithelial, connective, and nervous tissue. In some embodiments, the tissue is a muscle tissue.

[0304] In some embodiments, the methods disclosed herein comprise administering any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed

herein to the subject's foot. In some embodiments, the methods disclosed herein comprise administering any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein to the subject's extensor digitorum brevis (EDB) muscle.

[0305] Combination therapies are also contemplated by the invention. Combination as used herein includes both simultaneous treatment and sequential treatments. Combinations of methods of the invention with standard medical treatments (*e.g.*, corticosteroids) are specifically contemplated, as are combinations with novel therapies.

[0306] In some embodiments, the methods disclosed herein further comprise detecting the presence or absence of a mutation in a dysferlin gene in the subject prior to or subsequent to administering any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein to the subject. In some embodiments, any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein are administered to the subject upon detection of the presence of the mutation in the dysferlin gene. Exemplary dysferlin mutations include, but are not limited to, c.1392dupA, c.3035G>A (p.W1012X), c.2858dupT, c.2779del G, c.5594delG, c.4201dupA, c.1795_1799dupTACT, c.3832C>T (p.Q1278X), c.757C>T (p.R253W), c.855+1delG, c.3126G>A (p.W1042X), c.1663C>T (p.R555W), c.610C>T (p.R204X), c.3112C>T (p.R1038X), c.1368C>G (p.C456W), c.5713C>T (p.R1905X), c.3826C>G (p.I1276V), c.3843 +1G>A, c.4167+1G>C, c.2643+1G>A, c.797T>C (p.I266P), c.4876delG, c.3477C>A (p.Y1159X), c.3137G>A (p.R1046H), c.509C>A (p.A170E), c.3967C>T (p.Q1323X), 3191_3196dupGAGGCG, c.3992G>T (p.R1331L), c.3516_3517delTT, c.247delG, c.1180+11C>T, c.896G>A (p.G299E), c.5078G>A (p.R1693Q), c.5979dupA, c.3348+1_3348+4delGTAT, c.5314_5318delAGCCC, and c565C>G (p.L189V). In some instances, the dysferlin gene comprises one or more mutations including, but not limited to, c.1392dupA, c.3035G>A (p.W1012X), c.2858dupT, c.2779del G, c.5594delG, c.4201dupA, c.1795_1799dupTACT, c.3832C>T (p.Q1278X), c.757C>T (p.R253W), c.855+1delG, c.3126G>A (p.W1042X), c.1663C>T (p.R555W), c.610C>T (p.R204X), c.3112C>T (p.R1038X), c.1368C>G (p.C456W), c.5713C>T (p.R1905X), c.3826C>G (p.I1276V), c.3843 +1G>A, c.4167+1G>C, c.2643+1G>A, c.797T>C (p.I266P), c.4876delG, c.3477C>A (p.Y1159X), c.3137G>A (p.R1046H), c.509C>A (p.A170E), c.3967C>T (p.Q1323X), 3191_3196dupGAGGCG, c.3992G>T (p.R1331L), c.3516_3517delTT, c.247delG, c.1180+11C>T, c.896G>A (p.G299E), c.5078G>A (p.R1693Q), c.5979dupA, c.3348+1_3348+4delGTAT, c.5314_5318delAGCCC, and c565C>G (p.L189V).

[0307] In some embodiments, the methods disclosed herein further comprise detecting levels of dysferlin protein in the subject prior to administering or subsequent to any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein to the subject. In some embodiments, the methods disclosed herein further comprise detecting levels of dysferlin protein in the subject after administering any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions disclosed herein to the subject. In some embodiments, detecting the levels of dysferlin comprises detecting expression of the dysferlin gene. Detecting expression of the dysferlin gene may comprise quantifying dysferlin DNA or RNA levels. Alternatively, or additionally, detecting the levels of dysferlin protein comprises quantifying the levels of dysferlin protein. In some embodiments, the levels of dysferlin protein are detected in a sample from the subject. In some embodiments, the sample is a body fluid sample. Examples of body fluid samples include, but are not limited to, blood, urine, sweat, saliva, stool, and synovial fluid. In some embodiments, the blood sample is a plasma or serum sample. In some instances, the method further comprises a dysferlin DNA sequencing test, e.g., from Athena Diagnostics (CPT: 81408(1)).

[0308] In some embodiments, the methods disclosed herein further comprise modifying the dose or dosing frequency of any of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions that is administered to the subject. In some embodiments, modifying the dose or dosing frequency is based on the detection of dysferlin protein levels. In some embodiments, the dose or dosing frequency is reduced when dysferlin protein levels in the subject increase as compared to the dysferlin protein levels in the subject from an earlier time point (*e.g.*, prior to administering the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions, or after administering an initial dose of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions, but prior to administering a subsequent dose of the polynucleotides, plasmids, viral vectors, dual vector systems, or compositions).

[0309] Kits

[0310] In a yet further aspect, a kit is provided that comprises, or alternatively consists essentially of, or yet further consisting of, any of one or more of the polynucleotides, polypeptides, vectors, cells and systems, or the compositions, and instructions for use. In one aspect, any of one or more of the polynucleotides, polypeptides, vectors, cells and systems, or the compositions are detectably labeled or further comprise a purification or detectable marker. In some instances, the kit comprises a) a first polynucleotide, wherein the first

polynucleotide is the recombinant polynucleotide described herein, and a second polynucleotide, wherein the second polynucleotide is the recombinant polynucleotide described herein; or b) a first adeno-associated viral (AAV) vector, wherein the first AAV vector is the AAV vector described herein, and a second adeno-associated viral (AAV) vector, wherein the second AAV vector is the AAV vector described herein; or c) an AAV dual vector system described herein; or d) a composition described herein; or e) a cell (e.g., a host cell, optionally mammalian cell) described herein; and optionally an instruction for use.

EXAMPLES

[0311] Example 1: Generation of a dual AAV vector system

[0312] This example provides an exemplary method for producing the dual AAV vector systems disclosed herein. In this example, the dual AAV vector, rAAVrh.74.MHCK7.DYSF.DV is produced. The rAAVrh.74.MHCK7.DYSF.DV is a non-replicating, recombinant AAV, serotype rh74 (AAVrh74) expressing human dysferlin from dual vectors (DV) under the control of the muscle specific MHCK7 promoter. The dual vectors contain either the 5' portion or the 3' portion of the dysferlin cDNA sequence, and these portions are overlapping by ~1 kb to facilitate recombination to produce a full length human dysferlin gene. The expression cassette containing a portion of the human dysferlin cDNA is flanked by AAV2 inverted terminal repeat sequences (ITR) (FIG. 1).

[0313] To construct rAAVrh.74.MHCK7.DYSF.DV, the human dysferlin cDNA was split into two constructs that adhered to the packaging capacity of AAV (<4.7kb). The 5' vector (e.g., 5' hDYSF AAV vector), pAAV.MHCK7.DYSF5'.PTG (PTG=promoter/transgene) contains a muscle specific MHCK7 promoter, chimeric intron, consensus Kozak sequence and 5' portion of the DYSF cDNA corresponding to amino acids 1-1113 of the Dysferlin amino acid sequence. The 3' vector (e.g., 3' hDYSF AAV vector), pAAV.DYSF3'.POLYA, contains a 3' portion of the DYSF cDNA corresponding to amino acids 794-2080 of the Dysferlin amino acid sequence and DYSF 3'UTR harboring a polyadenylation signal. Sequences of the expression cassettes of the 5' hDYSF AAV vector and 3' hDYSF AAV vector are disclosed as SEQ ID NOs: 6 and 8, respectively.

[0314] Previous studies have validated cardiac expression using MHCK7 promoter (Salva *et al. Mol Ther* **15**, 320-329 (2007), which is incorporated by reference in its entirety) and AAVrh74 achieving skeletal, diaphragm, and cardiac muscle expression (Sondergaard *et al. Annals of clinical and Transl Neurology* **2**, 256-270 (2015), which is incorporated by reference in its entirety). The 5' hDYSF AAV vector and 3' hDYSF AAV vector were

encapsidated into separate AAVrh.74 virions. The molecular clone of the AAVrh.74 serotype was cloned from a rhesus macaque lymph node and is discussed in in Rodino-Klapac *et al. Journal of Translational medicine* **5**, 45 (2007), which is incorporated by reference in its entirety.

[0315] 5' *hDYSF AAV Vector (AAV vector plasmid pAAV.MHCK7.DYSF5'.PTG)*

[0316] The first recombinant single-stranded AAV vector was produced using the AAV vector DNA plasmid pAAV.MHCK7.DYSF5'.PTG. The plasmid was constructed by inserting the MHCK7 expression cassette driving a 5' portion of the human dysferlin partial cDNA sequence (human cDNA, Genbank Accession # NM_003494.3) into the vector backbone pAAV-CMV (Clontech) (see FIG. 2 for plasmid map and Table 1 for specific sequence information). A chimeric intron was present and composed of the 5' donor site from the first intron of the human β -globin gene and the branch point and 3' splice acceptor site from the intron that is between the leader and the body of an immunoglobulin gene heavy chain variable region. The only viral sequences included in this vector are the inverted terminal repeats of AAV2, which are required for both viral DNA replication and packaging of the rAAV vector genome. The sequence between the two ITRs is the portion of DNA that is encapsidated into AAVrh74 virions.

Table 1. Molecular Features of one exemplary plasmid pAAV.MHCK7.DYSF5'.PTG

TYPE	START	END	NAME	DESCRIPTION
REGION	8229	8373	5' ITR	Wild-type AAV2 inverted terminal repeat
REGION	22	813	MHCK7	Mouse myosin heavy chain complex—E box muscle creatine kinase fusion enhancer/promoter
REGION	823	970	Chimeric intron	5' donor site from human β -globin gene and the branch point and 3' splice acceptor site from IgG heavy chain
GENE	993	4329	hDYSF cDNA	Human dysferlin cDNA (transcript variant 8; 377-3716) aa1-1113
REGION	4440	4584	3' ITR	Wild-type AAV2 inverted terminal repeat
GENE	6370	7230	AmpR	β -lactamase gene
REGION	7378	8045	ori	Plasmid origin of replication

[0317] 3' *hDYSF AAV vector (AAV vector plasmid pAAV.DYSF3'.POLY)*

[0318] The second recombinant single-stranded AAV vector was produced using the AAV vector DNA plasmid pAAV.DYSF3'.POLYA. The plasmid was constructed by inserting the human dysferlin partial cDNA sequence (human cDNA, Genbank Accession # NM_003494.3) into the vector backbone pAAV-CMV (Clontech) (see FIG. 3 for plasmid

map and Table 2 for specific sequence information). The endogenous dysferlin 3' untranslated region and polyA signal sequences were used for efficient transcription termination. The only viral sequences included in this vector are the inverted terminal repeats of AAV2, which are required for both viral DNA replication and packaging of the rAAV vector genome. The sequence between the two ITRs is the portion of DNA that is encapsidated into AAVrh74 virions.

Table 2. Molecular Features of one exemplary plasmid pAAV.DYSF3'.POLYA				
TYPE	START	END	NAME	DESCRIPTION
REGION	1	145	5' ITR	Wild-type AAV2 inverted terminal repeat
GENE	204	3866	hDYSF cDNA	Human dysferlin cDNA (transcript variant 8; 2754-6619) aa794-2080
REGION	4070	4364	hDYSF 3'UTR	3' untranslated region of the human dysferlin gene
REGION	4378	4427	pA	Artificial polyadenylation signal
REGION	4481	4625	3' ITR	Wild-type AAV2 inverted terminal repeat
GENE	6411	7271	AmpR	β -lactamase gene
REGION	7419	8086	ori	Plasmid origin of replication

[0319] *AAV Helper Plasmid (pNLRep2-Caprh74)*

[0320] The parent plasmid, pNLrep, was constructed from p5E18 and pCLR3K. (See Bansal, D., *et al.* Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature* **423**, 168-172 (2003), which is incorporated by reference in its entirety). p5E18 is based on pAAV/Ad. It contains the AAV2 *rep* and *cap* genes, with the p5 promoter removed from the 5' end of *rep* and placed at the 3' end of *cap*, which results in the presence of a 3 kb spacer sequence between p5 and *rep* (see Table 3 for specific sequence information). To generate pCLR3K, the human collagen intron was amplified by PCR and then cloned into pAd/AAV at position 1,052. To construct pNLrep, the BamHI/XbaI fragment of p5E18 was replaced with the BamHI/XbaI fragment containing the 3 kb collagen intron from pCLR3K. The rh74 *cap* gene was PCR amplified and cloned in place of the AAV2 *cap* gene in pNLrep using Swa I/Not I restriction sites to yield pNLRep2-Caprh74. The identity of the AAV rh74 capsid gene was confirmed by DNA plasmid sequencing.

Table 3. Molecular Features of plasmid pNLRep2-Caprh.74				
TYPE	START	END	NAME	DESCRIPTION
GENE	84	815	5' end of Rep78	5' end of Rep78 ORF
REGION	816	3886	Col Intron	3 kb human collagen intron

GENE	3887	5017	3' end of Rep78	3' end of Rep78 ORF
GENE	5037	7253	rh74 Cap	rh74 <i>cap</i> gene
REGION	7428	7507	p5 promoter	AAV2 p5 promoter region

[0321] *Adenovirus Helper plasmid (pHELP)*

[0322] Plasmid pHELP was obtained from Applied Viromics (Fremont, CA 94538) and is 11,635 bp in size (see Table 4 for specific sequence information). The plasmid contains the regions of adenovirus genome that are important for AAV replication, namely *E2A*, *E4*, and VA RNA (the adenovirus E1 functions are provided by 293 cells). The plasmid was based on a pBluescript backbone and also contains, the *bla* gene encoding the TEM-1 β -lactamase gene conferring resistance to ampicillin (10,182-11,042 bp), a bacterial ColE1 origin of replication (9,315- 10,167 bp) and f1 single-strand DNA replication origin (11,172 – 11,627 bp). The adenovirus sequences present in this plasmid represent only ~28% (9,280 / 35,938) of the adenovirus genome, and does not contain *cis* elements critical for replication such as the inverted terminal repeats. The identity of these 3 adenovirus genes were confirmed by DNA plasmid sequencing performed. DNA Analysis revealed 100% homology with the 3 Adenovirus type 5 gene regions (GenBank Accession number AF369965, which is incorporated by reference in its entirety).

Table 4. Molecular Features of plasmid pHELP				
TYPE	START	END	NAME	DESCRIPTION
REGION	1	5336	<i>E2A</i>	DNA binding protein, required for AAV helper functions.
REGION	5337	8537	<i>E4</i>	E4 ORF6 required for AAV helper functions.
REGION	8537	9280	VA RNA	Viral Associated RNA is non-coding RNA that regulates viral translation and is required for AAV helper functions.
GENE	11,042	10,182	Ampr	Ampicillin resistance gene

[0323] **Example 2: Manufacturing of Viral Products Using a Dual AAV Vector System**

[0324] HEK 293 cells were transfected with the 3 production plasmids ((i) AAV vector plasmid, e.g., 5' hDYSF AAV vector or 3' hDYSF AAV vector; (ii) adenovirus (Ad) helper plasmid; and (iii) AAV helper plasmid) using an optimized calcium phosphate co-precipitation method. Transfecting the cells comprises preparing a DNA/calcium solution containing the AAV vector plasmid, Ad helper plasmid, AAV helper plasmid and CaCl₂ and mixing with an equal volume of 2X HEPES buffered saline to obtain an optimal precipitate.

The precipitate was then added to the HEK 293 cells and incubated. The precipitate was then added to the HEK 293 cells and incubated. Post incubation the medium was exchanged at which time nuclease is added.

[0325] Example 3: Determination of Efficacy of rAAVrh.74.MHCK7.DYSF.DV Intramuscular Delivery

[0326] The two AAV expression cassettes were generated containing 5' and 3' portions of the MHCK7.DYSF cassette with ~1kb of overlapping sequence (see FIG. 1). The plasmids were packaging into AAVrh.74 vectors. 4 week old *Dysf*^{-/-} mice were treated with 1×10^{11} vg of each vector by intramuscular injection into the tibialis anterior muscle and necropsied at 1 month. Robust full-length dysferlin expression was seen following delivery of both vectors by immune staining (FIG. 4A) and western blot (FIG. 4C). Delivery of either vector alone had no aberrant dysferlin expression (FIG. 4B, immune staining, and FIG. 4D, western blot). 3222 is the full-length control. The number of muscle fibers expressing dysferlin was quantified and shown in Table 5.

Table 5. Dysferlin expression following IM delivery of rAAVrh.74.MHCK7.DYSF.DV					
Test Article (vector)	Animal ID (eartag #)	Animal Strain	Endpoint (months)	Dose	% Fibers expressing Dysf*
rAAVrh.74.MHCK7.DYSF.DV	3266	Dysf ^{-/-}	1	2x10 ¹¹ vg	75%
	3267				68%
	3268				81%
	674				63%
	675				83%
* Four 20x fields were counted per muscle (~550-600 fibers per animal)					

[0327] A time course study to assess safety following intramuscular injection to the tibialis anterior muscle was initiated (Table 6). Protein expression and vector biodistribution were also assessed. At 1, 3, 6, 9, and 12 month endpoints animals were fully necropsied and assessed for dysferlin expression (FIGs. 5A-5C (1, 3, and 6 months shown)), vector biodistribution (Table 7) and histopathology on muscle and non-target organs. The tibialis muscle (TA) was injected. Tissues analyzed for histopathology for each animal included: Gonad, liver, heart, lung, spleen, kidney, diaphragm, left (treated) and right tibialis anterior muscles. No findings were identified.

Table 6. rAAVrh.74.MHCK7.DYSF.DV Long-Term Safety Study					
Test Article (vector)	Animal ID (eartag #)	Animal Strain	Endpoint (months)	Dose	% Fibers expressing Dyst*
rAAVrh.74.MHCK7.DYSF.DV	832	Dysf ^{±/-}	3	2x10 ¹¹ vg	70%
	833				78%
	834				79%
	835		6		90%
	836				91%
	815				68%
	816		9		70%
	817				79%
	818				68%
	819		12		89%
	820				87%
	821				89%
* Four 20x fields were counted per muscle (~550-600 fibers per animal)					

Table 7. Vector biodistribution in rAAVrh.74.MHCK7.DYSF.DV treated animals at three months			
Tissue	Vector genome copies/μg		
	Animal 832	Animal 833	Animal 834
LTA (treated muscle)	1.12E+05	1.12E+05	2.61E+05
RTA (contralateral muscle)	Undet.	1.87E+02	1.02E+02
Heart	4.54E+03	1.96E+03	9.11E+02
Lung	2.62E+03	1.00E+04	6.86E+02
Liver	2.42E+05	9.19E+04	2.24E+05
Kidney	6.71E+03	2.76E+03	1.86E+02
Spleen	4.13E+03	3.43E+03	4.49E+02
Gonad	2.30E+02	3.32E+02	4.15E+01

[0328] Following intramuscular injection, expression of dysferlin was found in the injected tibialis anterior muscle (FIG. 6). Following intravenous delivery, expression of dysferlin was found in skeletal and heart muscle (FIG. 7A).

[0329] Additional cohorts of mice were treated to determine the minimum effective dose for membrane repair. Three doses of AAV vectors were injected into the FDB of 129Dysf^{-/-} mice at 8 weeks of age (n=6 per group). A control Dysf^{-/-} group received saline and a group of 129WT mice served as strain specific normal controls. As shown in FIG. 8, AAVrh.74.DYSF.DV treatment revealed dose dependent membrane resealing. Parallel expression studies show that high dose results in expression >50% of fiber transduction. This dose is equivalent to what was given to the tibialis anterior muscle for the expression and safety studies when normalized for muscle weight (FIGs. 4A-5C).

Table 8. AAVrh.74.MHCK7.Dysferlin.DV Dose Response			
Mouse Strain	Treatment	Total Dose (vg)	12 weeks
129-Dysf ^{tm1Kcam/J}	AAVrh.74.MHCK7.Dysf.DV	6x10 ⁹	n = 6
129-Dysf ^{tm1Kcam/J}	AAVrh.74.MHCK7.Dysf.DV	2x10 ¹⁰	n = 6
129-Dysf ^{tm1Kcam/J}	AAVrh.74.MHCK7.Dysf.DV	6x10 ¹⁰	n = 6
129-Dysf ^{tm1Kcam/J}	PBS	N/A	n = 6
129S1/SvImJ Normal Controls	PBS	N/A	n = 6

[0330] *Systemic Delivery of rAAVrh.74.MHCK7.DYSF.DV*

[0331] A dose finding study was conducted to test the feasibility/effectiveness of systemic delivery of dual vector delivery. BlaJ mice (AJ dysferlin deficient mice backcrossed onto BL6 mice) were used for the study based on an established functional MRI/MRS outcome in this strain. 3 groups of mice (n=6 per group) were treated at 6 weeks of age by tail vein injection with either saline, 2e12 vg total AAV.DYSF DV (8e13 vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard), or 6e12 vg total AAV.DYSF.DV (2.4e13 vg/kg, based on a supercoiled DNA or plasmid as the quantitation standard). Endpoint analysis occurred at 3 months and included diaphragm physiology, membrane repair assay in the FDB, and full necropsies to quantify dysferlin expression and assess histopathology.⁴

[0332] At study endpoint of 4 months, full necropsies were performed. The diaphragm was subjected to force measurements, the FDB muscle was tested for restoration of membrane repair, and muscles and organs were harvested for expression, vector biodistribution, and histopathology. At high dose, dysferlin expression was widespread in all muscles (FIGs. 7A and 7B), while low dose had low level variable expression. At 20 weeks, the BlaJ have a very mild phenotype histologically. There is a significant increase in central nuclei as a marker of regeneration compared to controls. Treated mice showed a significant decrease in central nucleation at high dose (FIG. 7B). The most affected muscle, the psoas demonstrated a reduction in fibrosis and inflammation upon treatment at high dose (6e12 vg) (FIG. 9). The force deficits in the diaphragm were restored at both high and low dose (FIG. 10A) and there was a dose dependent response in membrane repair in the FDB muscle (FIG. 10B).

[0333] **Example 4: Safety and Efficacy of Full-Length Dysferlin Expression by AAV5 and AAVrh.74.Mhck7.Dysf.Dv Delivery in NHPs**

[0334] **Methods:** We treated 4 NHPs with AAV.MHCK7.DYSF.FLAG by intramuscular injection. 2 NHPs were treated with AAV5.MHCK7.DYSF and 2 were treated with AAVrh.74. MHCK7.DYSF.FLAG. Mirroring our clinical trial design, one animal was

analyzed at 3 months and two at 6 months. Animals received baseline chemistries and immunological studies including ELISpot analysis to measure T cells against AAV5 and rh.74 capsid and dysferlin (FIGs. 11A-11D) and anti-AAV Ab titers (Table 9).

[0335] Peptide pools used to stimulate the PBMCs were designed to be 15 amino acids long, overlapping by 10 amino acids so as to capture all possible antigenic epitopes. Cells reacting to the peptides release interferon- γ , quantified as spots through an ELISpot assay. Spots per million cells were counted with 50 spots/ 1×10^6 cells as the positive reaction threshold. No sustained immune response was observed. All animals had expression at study endpoint (FIG. 16A). These studies were repeated every two weeks for the entire study. At the study endpoint full necropsies were performed on the animals that in addition to gene expression studies included histopathology and biodistribution studies on vital organ tissues.

[0336] Results: No observable toxicity was found. Applicants used anti-dysferlin antibody that does not distinguish between rhesus and human dysferlin to demonstrate overexpression of dysferlin (FIGs. 12A-12C). Anti-FLAG immune staining was also done to confirm vector derived dysferlin expression (FIG. 13). For AAV5.DYSF injected TAs, the muscles demonstrated 104.9% (3mo) and 122.6% (6mo) overexpression of dysferlin while AAVrh.74.DYSF.DV injected TAs had 122.0% (3mo) and 115.2% (6mo) overexpression as compared to the uninjected control. No toxicity was observed at the tissue level in the NHPs with a lack of inflammation or muscle fiber necrosis. Immunological assays did not show any aberrant responses to the capsid or transgene by ELISpot (FIGs. 11A-11D). In addition full complete blood count and chemistry panels showed no abnormal values in any of the macaques. As expected, anti-AAV antibody titers were elevated following gene transfer. Endpoint anti-AAVrh.74 titers were lower than those for anti-AAV5.

Table 9. Anti-AAV5 and Anti-AAVrh.74 antibodies following intramuscular injection in NHPs.

Weeks Post Injection	AAV5.hDYSF			AAVrh.74.DYSF.DV	
	06C011	06C029	07C019	10-158	10-172
0	<5	<5	<5	<5	<5
2	1,600	6,400	6,400	400	400
4	51,200	51,200	51,200	800	800
6	51,200	102,400	102,400	800	200
8	51,200	204,800	102,400	800	800
10	51,200	102,400	51,200	1,600	1,600
12	51,200	204,800	102,400	1,600	800
14	51,200	204,800	51,200		800
16	51,200	102,400			800
18	51,200	51,200			1,600

20	51,200	25,600			1,600
22	51,200	25,600			1,600
24	51,200	51,200			3,200

[0337] Equivalents

[0338] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs.

[0339] The present technology illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising,” “including,” “containing,” *etc.* shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present technology claimed.

[0340] Thus, it should be understood that the materials, methods, and examples provided here are representative of preferred aspects, are exemplary, and are not intended as limitations on the scope of the present technology.

[0341] The present technology has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the present technology. This includes the generic description of the present technology with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0342] In addition, where features or aspects of the present technology are described in terms of Markush groups, those skilled in the art will recognize that the present technology is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0343] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[0344] Other aspects are set forth within the following claims.

WHAT IS CLAIMED IS:

1. A recombinant polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide comprises a first nucleotide sequence, wherein the first nucleotide sequence consists of:
 - (a) the nucleotide sequence of SEQ ID NO: 1, 6, or 18;
 - (b) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6, or SEQ ID NO: 18 across its respective full length of SEQ ID NO: 1, 6, or 18;
 - (c) the nucleotide sequence of SEQ ID NO: 13 or 15;
 - (d) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across its respective full length of SEQ ID NO: 13 or 15;
 - (e) a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment consists of the amino acid sequence of SEQ ID NO: 9; or
 - (f) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (e) across the full length of the nucleotide sequence of (e).
2. The recombinant polynucleotide of claim 1, further comprising one or more additional nucleotide sequences selected from an inverted terminal repeat (ITR), a promoter, an intron, a selection marker, or an origin of replication (ORI).
3. The recombinant polynucleotide of claim 2, wherein the ITR is an AAV ITR.
4. The recombinant polynucleotide of claim 3, wherein the AAV ITR is an AAV2 ITR or an AAV3 ITR.
5. The recombinant polynucleotide of any one of claims 2-4, wherein the recombinant polynucleotide comprises two ITRs.
6. The recombinant polynucleotide of any one of claims 2-5, wherein the ITR comprises the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 17.
7. The recombinant polynucleotide of claims 2-6, wherein the promoter comprises a muscle-specific promoter.

8. The recombinant polynucleotide of claim 7, wherein the muscle-specific promoter is selected from a human skeletal actin gene element, a cardiac actin gene element, a desmin promoter, a skeletal alpha-actin (ASKA) promoter, a troponin I (TNNI2) promoter, a myocytespecific enhancer binding factor mef binding element, a muscle creatine kinase (MCK) promoter, a truncated MCK (tMCK) promoter, a myosin heavy chain (MHC) promoter, a hybrid a-myosin heavy chain enhancer-/MCK enhancer-promoter (MHCK7) promoter, a C5-12 promoter, a murine creatine kinase enhancer element, a skeletal fast-twitch troponin c gene element, a slow-twitch cardiac troponin c gene element, a slow-twitch troponin i gene element, hypoxia- inducible nuclear factor.
9. The recombinant polynucleotide of claim 7, wherein the muscle-specific promoter comprises a MHCK7 promoter.
10. The recombinant polynucleotide of any one of claims 2-6, wherein the promoter comprises a recombinant promoter.
11. The recombinant polynucleotide of claim 10 wherein the recombinant promoter comprises a recombinant muscle-specific promoter.
12. The recombinant polynucleotide of any one of claims 2-11, wherein the promoter comprises the nucleotide sequence of SEQ ID NO: 4.
13. The recombinant polynucleotide of any one of claims 2-12, wherein the intron comprises a 5' donor site, branch point, and/or 3' splice site.
14. The recombinant polynucleotide of any one of claims 2-13, wherein the intron comprises a chimeric intron.
15. The recombinant polynucleotide of any one of claims 2-14, wherein the intron comprises a 5' donor site from a human β -globin gene.
16. The recombinant polynucleotide of any one of claims 2-15, wherein the intron comprises a branch point from an immunoglobulin G (IgG) heavy chain.
17. The recombinant polynucleotide of any one of claims 2-16, wherein the intron comprises a 3' splice acceptor site from an immunoglobulin G (IgG) heavy chain.

18. The recombinant polynucleotide of any one of claims 2-17, wherein the intron comprises the nucleotide sequence of SEQ ID NO: 5.
19. The recombinant polynucleotide of any one of claims 2-18, wherein the selection marker is an antibiotic resistance gene.
20. The recombinant polynucleotide of claim 19, wherein the antibiotic resistance gene is a β -lactamase gene or kanamycin resistance gene.
21. The recombinant polynucleotide of claim 1, wherein the recombinant polynucleotide comprises the nucleotide sequence of SEQ ID NO: 6 or SEQ ID NO: 18.
22. The recombinant polynucleotide of any one of claims 1-21, wherein the recombinant nucleotide does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.
23. The recombinant polynucleotide of any one of claims 1-22, wherein the recombinant nucleotide does not comprise an AAV sequence other than one or more ITRs.
24. The recombinant polynucleotide of any one of claims 1-22, wherein the recombinant nucleotide does not comprise a viral sequence other than one or more ITRs.
25. A recombinant polynucleotide sequence encoding a fragment of a human dysferlin protein, wherein the recombinant polynucleotide comprises a second nucleotide sequence, wherein the second nucleotide sequence consists of:
 - (a) the nucleotide sequence of SEQ ID NO: 2, 8 or 19;
 - (b) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2, 8 or 19 across its respective full length of SEQ ID NO: 2, 8 or 19;
 - (c) the nucleotide sequence of SEQ ID NO: 14 or 16;
 - (d) a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across its respective full length of SEQ ID NO: 14 or 16;
 - (e) a polynucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or

- (f) a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (e) across the full length of the nucleotide sequence of (d).

26. The recombinant polynucleotide of claim 25, further comprising one or more additional nucleotides comprising an inverted terminal repeat (ITR), a selection marker, an origin of replication (ORI), an untranslated region (UTR), or a polyadenylation (polyA) signal.
27. The recombinant polynucleotide of claim 26, wherein the ITR is an AAV ITR.
28. The recombinant polynucleotide of claim 27, wherein the AAV ITR is an AAV2 ITR or an AAV3 ITR.
29. The recombinant nucleotide of any one of claims 25, 27 or 28, wherein the recombinant nucleotide further comprises two ITRs.
30. The recombinant polynucleotide of any one of claims 26-29, wherein the ITR comprises the nucleotide sequence of SEQ ID NO: 3 or 17.
31. The recombinant polynucleotide of any one of claims 26-30, wherein the polyA signal is an artificial polyA signal.
32. The recombinant polynucleotide of any one of claims 26-31, wherein the polyA signal comprises the nucleotide sequence of SEQ ID NO: 7.
33. The recombinant polynucleotide of any one of claims 26-32, wherein the selection marker comprises an antibiotic resistance gene.
34. The recombinant polynucleotide of claim 33, wherein the antibiotic resistance gene comprises a β -lactamase gene or kanamycin resistance gene.
35. The recombinant polynucleotide of claim 25, wherein the recombinant polynucleotide comprises the nucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 19.
36. The recombinant polynucleotide of any one of claims 25-35, wherein the recombinant nucleotide does not further comprise a first polynucleotide sequence encoding a first fragment of the hDYSF protein.

37. The recombinant polynucleotide of any one of claims 25-36, wherein the recombinant nucleotide does not comprise an AAV sequence other than one or more ITRs.
38. The recombinant polynucleotide of any one of claims 25-36, wherein the recombinant nucleotide does not comprise a viral sequence other than one or more ITRs.
39. A dual adeno-associated viral (AAV) vector system comprising:
- (a) a first AAV vector, wherein the first AAV vector comprises the recombinant polynucleotide of any one of claims 1-24; and
 - (b) a second AAV vector, wherein the second AAV vector comprises the recombinant polynucleotide of any one of claims 25-38.
40. An adeno-associated viral (AAV) vector comprising the recombinant polynucleotide of any one of claims 1-24.
41. The AAV vector of claim 40, wherein the AAV vector is AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAVrh.10, AAVrh.20, or AAVrh.74.
42. The AAV vector of claim 40, wherein the AAV vector is AAVrh.74.
43. An adeno-associated viral (AAV) vector comprising the recombinant polynucleotide of any one of claims 25-38.
44. The AAV vector of claim 43, wherein the AAV vector is AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAVrh.10, AAVrh.20, or AAVrh.74.
45. The AAV vector of claim 43, wherein the AAV vector is AAVrh.74.
46. A composition comprising the recombinant polynucleotide of any one of claims 1-38 or the AAV vector of any one of claims 40-45.
47. A composition comprising:
- (a) a first recombinant adeno-associated viral (rAAV) vector, wherein the first rAAV vector comprises the AAV vector according to any one of claims 40-42; and

- (b) a second rAAV vector, wherein the second rAAV vector comprises the AAV vector according to any of claims 43-45.

48. The composition of claim 47, wherein the molar ratio of first and second rAAV vectors is between about 100:1-1:100, about 10:1-1:10, about 2:1-1:2, or about 1:1.

49. An adeno-associated viral (AAV) vector comprising:

- (a) a first inverted terminal repeat (ITR);
 - (b) a polynucleotide encoding a fragment of a human dysferlin (hDYSF) protein, wherein the polynucleotide consists of:
 - i. the nucleotide sequence of SEQ ID NO: 1 or 6;
 - ii. a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 1 or 6 across the full length of SEQ ID NO: 1 or 6;
 - iii. the nucleotide sequence of SEQ ID NO: 13 or 15;
 - iv. a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 13 or 15 across the full length of SEQ ID NO: 13 or 15;
 - v. a nucleotide sequence encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 9; or
 - vi. a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and
 - (c) a second ITR,
- wherein the polynucleotide is flanked by the first and second ITRs.

50. The AAV vector according to claim 49, further comprising one or more additional polynucleotides selected from a promoter, an intron, a selection marker, or an origin of replication (ORI).

51. The AAV vector according to claims 49 or 50, wherein the ITR is an AAV ITR.

52. The AAV vector according to claim 51, wherein the AAV ITR is an AAV2 ITR or an AAV3 ITR.

53. The AAV vector according to any one of claims 49-52, wherein the first and/or second ITR comprise the nucleotide sequence of SEQ ID NO: 3 or 17.
54. The AAV vector according to any one of claims 50-53, wherein the promoter is a muscle-specific promoter.
55. The AAV vector of claim 54, wherein the muscle-specific promoter is a myosin heavy chain complex—E box muscle creatine kinase fusion enhancer/promoter.
56. The AAV vector according to any one of claims 50-53, wherein the promoter is a recombinant promoter.
57. The AAV vector according to claim 56, wherein the recombinant promoter is a recombinant muscle-specific promoter.
58. The AAV vector according to claim 57, wherein the recombinant-muscle specific promoter is a MHCK7 promoter.
59. The AAV vector according to any one of claims 50-58, wherein the promoter comprises the nucleotide sequence of SEQ ID NO: 4.
60. The AAV vector according to any one of claims 50-59, wherein the intron comprises a 5' donor site, branch point, and/or 3' splice site.
61. The AAV vector according to any one of claims 50-60, wherein the intron is a chimeric intron.
62. The AAV vector according to any one of claims 50-61, wherein the intron comprises a 5' donor site from a human β -globin gene.
63. The AAV vector according to any one of claims 50-62, wherein the intron comprises a branch point from an immunoglobulin G (IgG) heavy chain.
64. The AAV vector according to any one of claims 50-63, wherein the intron comprises a 3' splice acceptor site from an immunoglobulin G (IgG) heavy chain.
65. The AAV vector according to any one of claims 50-64, wherein the intron comprises the nucleotide sequence of SEQ ID NO: 5.

66. The AAV vector according to any one of claims 50-65, wherein the selection marker is an antibiotic resistance gene.
67. The AAV vector of claim 66, wherein the antibiotic resistance gene is a β -lactamase gene or kanamycin resistance gene.
68. The AAV vector according to any one of claims 49-67, wherein the AAV vector comprises the nucleotide sequence of SEQ ID NO: 6 or SEQ ID NO: 15.
69. The AAV vector according to any one of claims 49-68, wherein the AAV vector does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.
70. The AAV vector according to any one of claims 49-69, wherein the AAV vector does not comprise an AAV sequence other than one or more ITRs.
71. The AAV vector according to any one of claims 49-69, wherein the AAV vector does not comprise a viral sequence other than one or more ITRs.
72. An adeno-associated viral (AAV) vector comprising:
- (a) a first inverted terminal repeat (ITR);
 - (b) a polynucleotide encoding a fragment of a human dysferlin protein, wherein the polynucleotide consists of:
 - i. the nucleotide sequence of SEQ ID NO: 2 or 8;
 - ii. a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 2 or 8 across the full length of SEQ ID NO: 2 or 8;
 - iii. the nucleotide sequence of SEQ ID NO: 14 or 16;
 - iv. a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 14 or 16 across the full length of SEQ ID NO: 14 or 16 ;
 - v. a polynucleotide encoding the fragment of the hDYSF protein, wherein the fragment of the hDYSF protein consists of the amino acid sequence of SEQ ID NO: 10; or

- vi. a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of (v) across the full length of the nucleotide sequence of (v); and
 - (c) a second ITR,
wherein the polynucleotide is flanked by the first and second ITRs.
73. The AAV vector according to claim 72, further comprising one or more polynucleotides selected from a selection marker, an origin of replication (ORI), an untranslated region (UTR), or a polyadenylation (polyA) signal.
74. The AAV vector according to claims 72 or 73, wherein the ITR is an AAV ITR.
75. The AAV vector according to claim 74, wherein the AAV ITR is an AAV2 ITR or an AAV3 ITR.
76. The AAV vector according to any one of claims 72-75, wherein the ITR comprises the nucleotide sequence of SEQ ID NO: 3 or 17.
77. The AAV vector according to any one of claims 73-76, wherein the polyA signal is an artificial polyA signal.
78. The AAV vector according to any one of claims 73-77, wherein the polyA signal comprises the nucleotide sequence of SEQ ID NO: 7.
79. The AAV vector according to any one of claims 73-78, wherein the selection marker is an antibiotic resistance gene.
80. The AAV vector according to claim 79, wherein the antibiotic resistance gene is a β -lactamase gene or kanamycin resistance gene.
81. The AAV vector according to any one of claims 72-80, wherein the AAV vector comprises the nucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 16.
82. The AAV vector according to any one of claims 72-81, wherein the AAV vector does not further comprise a second polynucleotide sequence encoding a second fragment of the hDYSF protein.

83. The AAV vector according to any one of claims 72-82, wherein the AAV vector does not comprise an AAV sequence other than one or more ITRs.
84. The AAV vector according to any one of claims 72-82, wherein the AAV vector does not comprise a viral sequence other than one or more ITRs.
85. A dual adeno-associated viral (AAV) vector system comprising:
- (a) a first AAV vector, wherein the first AAV vector comprises the AAV vector according to any one of claims 40-42 and 49-71; and
 - (b) a second AAV vector, wherein the second AAV vector comprises the AAV vector according to any one of claims 43-45 and 72-84.
86. The dual adeno-associated viral (AAV) vector system of claim 85, wherein the AAV vector is AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAVrh.10, AAVrh.20, or AAVrh.74.
87. The dual adeno-associated viral (AAV) vector system of claim 85, the AAV vector is AAVrh.74.
88. An adeno-associated viral (AAV) packaging system comprising:
- (a) a plasmid comprising the recombinant polynucleotide of any one of claims 1-38;
 - (b) an adenovirus helper plasmid; and
 - (c) a rep-cap plasmid.
89. An adeno-associated viral packaging system comprising:
- (a) a plasmid comprising the recombinant polynucleotide of any one of claims 1-38; and
 - (b) an adenovirus helper plasmid.
90. The adeno-associated viral packaging system of claim 88 or 89, wherein the adenovirus helper plasmid comprises pHELP plasmid.
91. A method for producing an adeno-associated viral (AAV) vector, comprising contacting a cell with the plasmid comprising the recombinant polynucleotide of any one of claims 1-38, or the AAV packaging system of claim 88.

92. The method of claim 91, wherein the cell is a host cell, optionally a mammalian host cell, further optionally HEK293.
93. A method for producing an adeno-associated viral (AAV) vector, comprising transducing a packaging cell line with the plasmid comprising the recombinant polynucleotide of any one of claims 1-38, wherein the packaging cell line expresses an adeno-associated viral rep and cap genes.
94. The method of claim 93, wherein the AAV rep gene is Rep78.
95. The method of claim 93, wherein the AAV cap gene is Rh74 *cap* gene.
96. A cell comprising the recombinant polynucleotide of any one of claims 1-38.
97. A cell comprising a plasmid that comprising the recombinant polynucleotide of any one of claims 1-38.
98. The AAV packaging system of any one of claims 88-90, the method of any one of claims 91-95, or the cell of claim 96 or 97, wherein the plasmid comprising a polynucleotide that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 18 or 19.
99. The AAV packaging system of any one of claims 88-90, the method of any one of claims 91-95, or the cell of claim 96 or 97, wherein the plasmid comprising a polynucleotide of SEQ ID NO: 18 or 19.
100. A method of treating a dysferlinopathy, comprising administering to a subject in need thereof:
- (a) an effective amount of a first polynucleotide, wherein the first polynucleotide is the recombinant polynucleotide of any one of claims 1-24; and
 - (b) an effective amount of a second polynucleotide, wherein the second polynucleotide is the recombinant polynucleotide of any one of claims 25-38.
101. The method of claim 100, wherein the first polynucleotide and/or second polynucleotide is administered intramuscularly or intravenously.
102. The method of claim 100 or 101, wherein the first and second polynucleotides are administered simultaneously or sequentially.

103. A method of treating a dysferlinopathy, comprising administering to a subject in need thereof:
- (a) an effective amount of a first adeno-associated viral (AAV) vector, wherein the first AAV vector is the AAV vector of any one of claims 40-42 and 49-71; and
 - (b) an effective amount of a second AAV vector, wherein the second AAV vector is the AAV vector of any one of claims 43-45 and 72-84.
104. The method of claim 103, wherein the first AAV vector and/or second AAV vector is administered intramuscularly or intravenously.
105. The method of claim 103 or 104, wherein the first and second AAV vectors are administered simultaneously or sequentially.
106. A method of treating a dysferlinopathy, comprising administering to a subject in need thereof an effective amount of the AAV dual vector system of any one of claim 39 or 85-87.
107. The method of claim 106, wherein the AAV dual vector system is administered intramuscularly or intravenously.
108. A method of treating a dysferlinopathy, comprising administering to a subject in need thereof an effective amount of the composition of any one of claims 46-48.
109. The method of claim 108, wherein the composition is administered intramuscularly or intravenously.
110. The method of any one of claims 100-109, wherein the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.
111. Use of a composition in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof, wherein the composition comprises:
- (a) a first polynucleotide, wherein the first polynucleotide is the recombinant polynucleotide of any one of claims 1-24; and
 - (b) a second polynucleotide, wherein the second polynucleotide is the recombinant polynucleotide of any one of claims 25-38.

112. Use of a composition in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof, wherein the composition comprises:
- (a) an effective amount of a first adeno-associated viral (AAV) vector, wherein the first AAV vector is the AAV vector of any one of claims 40-42 and 49-71; and
 - (b) an effective amount of a second adeno-associated viral (AAV) vector, wherein the second AAV vector is the AAV vector of any one of claims 43-45 and 72-84.
113. Use of a composition in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof, wherein the composition comprises the AAV dual vector system of claim 39 or 85-87.
114. Use of a composition according to any one of claims 46-48 in the manufacture of a medicament to treat a dysferlinopathy in a subject in need thereof.
115. The use of any one of claims 111-114, wherein the composition is administered intramuscularly or intravenously.
116. The use of any one of claims 111-114, wherein the dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.
117. The method of any one of claims 100-110, wherein the effective amount of the first AAV vector is between about 1×10^6 - 1×10^{16} vg/kg, about 1×10^8 - 1×10^{15} vg/kg, or about 1×10^{10} - 1×10^{14} vg/kg, wherein the dosage is measured by a supercoiled PCR quantification standard.
118. The method of claim 117, wherein the effective amount of the first AAV vector is 1.2×10^{13} vg/kg, wherein the dosage is measured by a supercoiled PCR quantification standard.
119. The method of any of claims 100-110 and 117, wherein the effective amount of the second AAV vector is between about 1×10^6 - 1×10^{16} vg/kg, about 1×10^8 - 1×10^{15} vg/kg, or about 1×10^{10} - 1×10^{14} vg/kg, wherein the dosage is measured by a supercoiled PCR standard.

120. The method of claim 119, wherein the effective amount of the second AAV vector is 1.2×10^{13} vg/kg, wherein the dosage is measured by a supercoiled PCR quantification standard.
121. The method of any one of claims 117-120, wherein the total AAV vector dosage is 2.4×10^{13} vg/kg, wherein the dosage is measured by a supercoiled PCR standard.
122. A recombinant polynucleotide encoding a human dysferlin (hDYSF) protein, wherein the recombinant polynucleotide sequence comprising a nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 20.
123. The recombinant polynucleotide of claim 122, wherein the recombinant polynucleotide sequence comprising a nucleotide sequence of SEQ ID NO: 20.
124. A method of making the recombinant polynucleotide of claim 122, comprising contacting a cell with or expressing in a cell the recombinant polynucleotide of any one of claims 1-24 and the recombinant polynucleotide of any one of claims 25-38.
125. A method of making the recombinant polynucleotide of claim 122, comprising contacting a cell with the dual AAV vector system of any one of 39 and 85-87.
126. A method of making the recombinant polynucleotide of claim 124 or 125, wherein the cell is a eukaryotic cell.
127. A method of making the recombinant polynucleotide of claim 124 or 125, wherein the cell is a muscle cell, a heart cell, a stem cell, a satellite cell, and/or a liver cell.
128. A method of making the recombinant polynucleotide of claim 122, comprising administering to a subject with the recombinant polynucleotide of any one of claims 1-24 and the recombinant polynucleotide of any one of claims 25-38.
129. A method of making the recombinant polynucleotide of claim 122, comprising administering to a subject with the dual AAV vector system of any one of claims 39 and 85-87 or the composition of claim 47 or 48.
130. A method of treating muscular dystrophy of a subject, comprising expression of the recombinant polynucleotide of claim 122 in the subject.

131. The method of claim 130, comprising administering to a subject with the recombinant polynucleotide of any one of claims 1-24 and the recombinant polynucleotide of any one of claims 25-38 or the composition of claim 47 or 48.
132. The method of claim 130, comprising administering to a subject with the dual AAV vector system of any one of claims 39 and 85-87.
133. The method of any one of claims 128-132, wherein the subject is a mammal selected from human, a non-human primate, a canine, an ovine, a horse, a porcine, a murine, a rat, a rabbit, a bovine, or a feline.
134. The method of any one of claims 128-133, wherein the subject suffers from dysferlinopathy.
135. The method of claim 134, wherein dysferlinopathy is limb girdle muscular dystrophy type 2B (LGMD2B) or Miyoshi myopathy.